

Theoretical models of emergent structure and organization of (bio-) polymeric systems

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by

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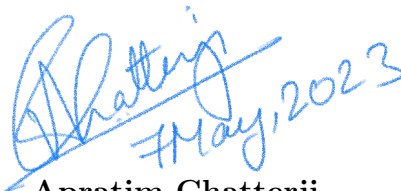
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Certified that the work incorporated in the thesis entitled '**Theoretical models of emergent structure and organization of (bio-) polymeric systems**' submitted by **Debarshi Mitra** was carried out by the candidate, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other University or institution.

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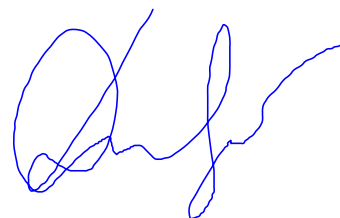
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ABSTRACT

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In the first part of the study, I discuss how free-standing helices may occur in semi-flexible polymeric systems without using torsional potentials. The helical motif is ubiquitous in macro-molecular systems and understanding the emergence of these structures is of interest to a broad community of researchers. Experiments have shown how a polymeric chain made of micron-sized colloidal monomers spontaneously forms a helical structure when the chain is heated above a threshold temperature. I outline how a minimal coarse-grained polymer model provides mechanistic insights into the formation of these structures [1]. Furthermore, in a separate study, I establish that switching on generic interactions e.g. the bare Coulomb potential or other long-ranged spherically symmetric repulsive interactions between monomers of the bead-spring model of a uniformly-charged, semi-flexible polymer, induce instabilities which results in the formation of transient helical structures [2]. The key factors which control the emergence of these structures is the persistence length and the charge density. The transient helices may also be induced by switching on screened Coulomb interactions, such that monomers have charges of differing polar-

ities and magnitudes. Moreover, the transient helices can be made long-lived when the polymer is confined to a cuboid of appropriate dimensions.

In the second part of the study, I elucidate key aspects of the organization of bacterial chromosomes at micron-length scales. The mechanism of chromosome organization of *E.coli* is one of the least understood aspects of its life cycle. In this thesis we rely on the principles of entropic repulsion to shed light on the organization of *E.coli* chromosome. The *E.coli* chromosome is often modeled as a bead spring ring polymer. We introduce cross-links in the DNA ring polymer, resulting in the formation of loops within each bacterial chromosome. I use simulations to show that the chosen polymer topology leads to an emergent organization of the chromosome-polymer along the cell long-axis, such that various segments get spatially localized as seen *in-vivo*, through FISH experiments. This organization relies on the mechanism of entropic repulsion between the constituent loops. Additionally, the contact map generated from our simulations reproduces the macro-domain-like organization, as seen in Hi-C maps of previous experiments. Thus, observations from complementary experimental techniques probing bacterial chromosome organization were reconciled [3]. In a different study, I outline the principles by which certain ring polymer architectures lead to higher entropic forces of segregation, in confinement. Furthermore, I show how the polymer organizes itself in different ways in cylindrical confinement depending on the polymer topology it adopts. The results were validated using an analytical blob-theory approach. Lastly, it was established that the same mechanism can also be invoked to explain the organization of *C.crescentus* chromosomes [4].

Contents

1	Introduction	1
1.1	Introduction to Soft Matter	1
1.2	Equilibrium properties of polymers	4
1.2.1	Mean squared end-to-end distance and Radius of Gyration . .	4
1.2.2	Ideal Polymers	5
1.2.3	Real polymer chains	7
1.2.4	Monomer-Solvent Interactions	7
1.2.5	Stiffness in polymers	9
1.3	Dynamics of polymers	10
1.3.1	The Rouse Model	10
1.3.2	The Zimm model	11
1.4	Helix formation in semiflexible polymers	11

1.5	Organization of Bacterial Chromosomes	16
1.5.1	Entropic Segregation of Bacterial Chromosomes	17
1.5.2	FISH experiments	17
1.5.3	Hi-C experiments	19
1.5.4	Role of polymer topology	19
1.5.5	Further questions	20
1.6	Outline of the thesis	21
2	Rigidity Mediates Helix Formation of Colloidal Chains in Poor Sol- vent.	22
2.1	Experimental Background	24
2.1.1	Model	28
2.1.2	Results	31
2.2	Discussions and Conclusions	39
3	Transient helix formation in charged semiflexible polymers without confinement effects.	41
3.1	Introduction	41
3.2	Model	44

3.3	Results and Analysis:	48
3.4	Conclusions and Discussions	79
3.5	Persistence length	82
4	Emergent Helicity in non-uniformly charged, semiflexible polymers.	84
4.1	Introduction	84
4.2	Model	86
4.2.1	Mapping Simulation units to real or SI units	88
4.3	Results	90
4.3.1	Helix Formation	90
4.3.2	Parameters controlling helix formation	97
4.4	Confinement stabilizes helices	104
4.5	Discussion	105
5	Polymer architecture orchestrates the segregation and spatial organization of replicating <i>E. coli</i> chromosomes in slow growth.	107
5.1	Introduction	107
5.2	The <i>E.coli</i> chromosome	113
5.2.1	Basic Information	113

5.2.2	Experimental observations yet to be reconciled	116
5.3	Model	117
5.3.1	The bead spring model of the chromosome	117
5.3.2	Are the modified architectures viable?	121
5.3.3	Modeling replication	123
5.3.4	Details of cross-linking process in DNA-2	124
5.3.5	Choice of Monte Carlo simulations	125
5.4	Results and Analysis	126
5.4.1	Enhanced propensity for DNA segregation	126
5.4.2	Localization of oriC to quarter positions	131
5.4.3	Enhanced entropic repulsion between daughter chromosomes due to the presence of loops:	131
5.4.4	Localisation of other genomic loci.	136
5.4.5	Mechanism of localization of genomic loci.	141
5.4.6	Macrodomain organisation: Hi-C Maps	143
5.4.7	Macrodomain organisation : Arc-2-2 architecture	150
5.5	Polymer Architecture influences Replisome dynamics	153
5.6	Conclusions and Discussion	155

6	Topology driven spatial organization of ring-polymers under confinement.	159
6.1	Introduction	159
6.2	Bacterial chromosomes	164
6.2.1	Basic Background	164
6.2.2	Unresolved questions:	167
6.3	Model	168
6.4	Results	179
6.4.1	Segregation propensity of polymer architectures	179
6.4.2	Propensity of segregated polymers to overlap	185
6.4.3	Spatial organization of polymer segments: tracked by localization of genomic loci	186
6.4.4	The blob theory approach	192
6.4.5	Limitations of the blob theory approach	193
6.4.6	Principles of segregation and localisation of polymer segments due to polymer architectures	196
6.4.7	Summary	199
6.5	Localization and organization in <i>C. crescentus</i> chromosome.	201

6.6	Conclusions and Discussions	206
6.7	Free Energy of polymers of different topologies in a cylinder of diameter D	209
6.7.1	Arc-0: Free energy difference δF between the overlapped and the segregated configurations.	209
6.7.2	δF between the overlapped and the segregated states of two polymers with Arc-2 architecture.	212
6.7.3	$\delta F/N$ corresponding to all polymer architectures	218
7	Conclusions and Future Directions	219
7.1	Helix formation in polymers	219
7.1.1	Handedness of helices	219
7.1.2	Formation of helices due to dipolar interactions	220
7.2	Organization of polymers in cylindrical confinement	221
7.2.1	Organization of polymers in spherical confinement	221
7.2.2	Polymer topologies due to transient cross-links	221
7.2.3	Organization of chromosomes in fast growth conditions	222
7.2.4	Incorporation of active processes	222
	Bibliography	223

List of Publications

1. *Transient helix formation in charged semiflexible polymers without confinement effects.*

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2. *Rigidity Dictates Spontaneous Helix Formation of Thermoresponsive Colloidal Chains in Poor Solvent.*

Bipul Biswas, Debarshi Mitra, Fayis KP, Suresh Bhat, Apratim Chatterji, and Guruswamy Kumaraswamy ACS Nano, 15, 12, 1970219711, 2021

3. *Polymer architecture orchestrates the segregation and spatial organization of replicating E. coli chromosomes in slow growth.*

Debarshi Mitra, Shreerang Pande, and Apratim Chatterji, Soft Matter, 18, 5615-5631, 2022

4. *Topology driven spatial organization of ring-polymers under confinement.*

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5. *Emergent Helicity in non-uniformly charged, semiflexible polymers*

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Chapter 1

Introduction

1.1 Introduction to Soft Matter

The term ‘Soft matter’ refers to a class of systems that share certain key characteristics, using which they can be differentiated from other classes of materials. For instance, soft materials are ‘soft’ i.e they are more easily deformable as compared to metals and other crystalline solids. In comparison to hard matter systems, which are held together by strong covalent bonds, soft matter systems are held together by weak van der Waals forces. These weak forces allow the constituent molecules to spatially re-organize and flow more easily in response to external stimuli (such as electric fields, magnetic fields or mechanical forces), leading to the observed deformability of soft matter systems. Additionally, the structural organization of soft matter systems can be amorphous, which can contribute to their ease of deformation. In soft matter systems, the sizes of the constituent particles range from a few nanometers to microns. At these length scales, the trajectories of the constituents can be treated

classically. However, thermal fluctuations play a crucial role in the dynamics and equilibrium organization of these systems. These systems, are therefore amenable to the framework of classical equilibrium and non-equilibrium statistical mechanics.

The constituents of these systems may interact with each other *via* various kinds of interactions, such as screened Coulomb interactions, dipole-dipole interactions, depletion interactions, hydrophobic interactions, etc. The equilibrium state of these systems is governed by the minimization of the Free energy function. This function has contributions both from the enthalpic term and the entropic term. At constant temperature and volume, the equilibrium state is decided by the global minimum of the Helmholtz free energy which is given by

$$F = U - TS \tag{1.1}$$

Here, ‘U’ represents the enthalpic term while ‘S’ represents the entropy of the system and ‘T’ represents the temperature. In most cases, for soft matter systems ‘U’ is comparable to ‘TS’ and hence the equilibrium ground state is decided by a competition between the entropic and the enthalpic terms.

Soft matter systems typically comprise assemblies of the following classes of constituent particles:

- Polymer solutions, gels and melts
- Colloidal suspensions and gels
- Liquid crystals

- Surfactants and block copolymers
- Emulsions suspensions and foam
- Biological systems such as chromosomes, cells and tissues

Soft matter systems may be amorphous i.e they do not possess long-range order such as that seen in crystalline systems. However, soft matter systems may often self-assemble to form higher-order structures spontaneously. Such self-assembled structures have found uses for a large number of technological applications (such as the fabrication of nano-devices and materials) and such instances of self-assembly can also be seen inside cells and tissues. These are crucial for life processes to function seamlessly. Thus, understanding the principles by which these self-assembled structures (with a well-defined organization) self-emerge is of significance from a fundamental science point of view.

In this thesis, I will discuss how organization or structure emerges in polymeric systems. In the first part of the study, I discuss how polymeric systems may spontaneously form helical structures. In the second part of the study, I shall discuss how bacterial chromosomes may be organized within the cell based on entropic interactions within different segments of the chromosomes. But before I elaborate on these, in the next section I will give a brief overview of the properties of polymers.

1.2 Equilibrium properties of polymers

Polymers are macromolecules that are chains of repeating chemical units termed monomers. In the bead spring model of a polymer, these monomers interact with other neighboring monomers *via* harmonic interactions (springs). In many cases, physicists often develop coarse-grained models using bead spring models.

In such cases, each ‘coarse-grained bead’ may subsume with it many groups of chemical units. For instance, when we shall discuss about coarse-grained models of chromosomes, we shall work with a coarse-grained model of the chromosome where each monomer subsumes $\sim 10kbp$ of DNA, refer Fig.1.1.

1.2.1 Mean squared end-to-end distance and Radius of Gyration

The end-to-end vector of a polymer N monomers is defined as:

$$\vec{R}_{ee} = \sum_{i=1}^{N-1} \vec{u}_i \quad (1.2)$$

where $\vec{u}_i = \vec{r}_{i+1} - \vec{r}_i$ denotes the bond-vectors along the chain contour.

A polymer continuously changes conformations due to thermal fluctuations and hence a polymer conformations can be mapped to a random walk or a self-avoiding random walk, where each bond corresponds to a random walk step in 3D. This will be expounded upon in the subsequent sections.

It is therefore easy to see that for polymers $\langle \vec{R}_{ee} \rangle = 0$. This average can be computed by performing an ensemble average or averaging over many different conformations of the polymers at different times. Therefore, one often looks at the mean squared end-to-end distance $\langle R_{ee}^2 \rangle$ to characterize the size of polymer chains.

So far we have described properties of linear polymers where the chain ends remain free. However, one may also find macro-molecules in nature (such as bacterial chromosomes) which are ring polymers. In such cases the ends of the macro-molecules are also linked to each other by a harmonic spring. This brings about a change in topology of the macro-molecule. For ring polymers $\langle R_{ee}^2 \rangle$ is not useful to characterise the sizes of these chains. Therefore, one may use a quantity called the radius of gyration R_g to characterize the size of ring polymers, branched polymers and polymers of various topologies. R_g of a single conformation is given by

$$R_g^2 = \frac{1}{N} \sum_{i=1}^N (\vec{R}_i - \vec{R}_{com})^2 \quad (1.3)$$

where R_{com} denotes the position vector of the center of mass of the polymer chain in a particular conformation. Note that R_g is an important measure of the size of linear polymers too.

1.2.2 Ideal Polymers

The simplest model of a polymer is that of an ideal chain (or a Gaussian) polymer. In the case of these polymers, it is assumed that the monomers interact only with their neighboring monomers by harmonic spring interactions and any two monomers

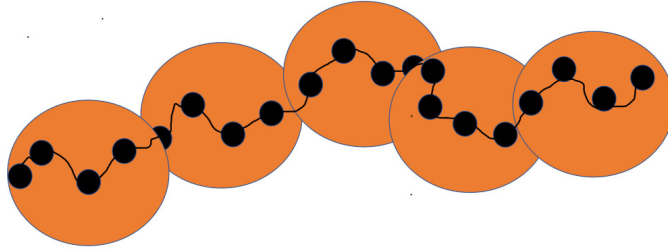


Figure 1.1: The schematic shows a coarse grained polymer model of DNA. In the bacterial cell bare DNA is organized (and “dressed”) by histone like proteins and various other proteins acting at different length scales. We have developed a coarse grained model where each monomer (shown in orange) subsume $\sim 10kbp$ of DNA.

can occupy the same position in 3D space. This implies that the monomers do not have any ‘excluded volume’. Thus the ideal polymer chain conformation can be visualized as a random walk, in 3D space. This is an idealized simplification and is often inadequate to model real polymers where ‘excluded volume’ interactions play a crucial role.

The mean squared end-to-end distance of these ideal polymer chains is given by

$$\langle R^2 \rangle = Na^2 \quad (1.4)$$

where a is the equilibrium bond length between any two monomers and N denotes the number of monomers [5, 6, 7]. The probability distribution of the end-to-end distances of these chains have a Gaussian distribution, hence these chains are also called Gaussian chains [5, 6, 7].

1.2.3 Real polymer chains

In the previous section we have discussed properties of ideal polymer chains. Polymers in reality are not ideal and each monomer has a certain ‘excluded volume’. Thus, monomers do not overlap spatially since each monomer is akin to a hard sphere. Therefore, in 3D space, no two monomers have the same coordinates at any instant of time. The bond vectors along the contour of such a polymer chain can be visualized as performing a self-avoiding random walk in 3D. In this case the root mean square end-to-end distance R scales as $N^{0.58}$ [5]. Note that the exponent changes from 0.50 for the ideal case to 0.58 for the case of real chains. This exponent can be found empirically by experiments done on linear polymers in dilute solutions. Analytically, one may use Flory theory to arrive at the exponent of 0.6 [8]. However, more involved analytical calculations using the renormalization group approach yield an exponent of 0.58 [9].

When a coarse-grained polymer model is developed for biological and synthetic polymers, each coarse-grained monomer subsumes aggregates of the macro-molecule. In this case the coarse-grained monomers may penetrate each other to a fair degree so the inter-monomer repulsion in this case may also be approximated by an effective “soft” potential which is repulsive at short distances [10].

1.2.4 Monomer-Solvent Interactions

The properties of polymer chains also depend on the solvent in which the polymer chain is immersed. These interactions can be divided into three sub-categories:

Good solvent

When a polymer chain is immersed in a ‘good solvent’ the polymer chain swells due to excluded volume interactions between the monomers and the root R scales as $N^{0.6}$ [5, 6]. This implies that the interactions between the monomers and the solvent is energetically favorable.

Bad solvent

When the polymer chain is immersed in a bad solvent the monomer-solvent interaction is unfavorable and hence the monomers try to reduce contact with the solvent thus inducing an effective attraction between the monomers. The polymer thus shrinks in size and forms a globule. In this case R scales as $N^{1/3}$ [5, 6].

Theta solvent

A theta solvent refers to a kind of solvent that is just about bad enough to nullify the swelling of the polymer chain. In this solvent the R scales as $N^{0.50}$, similar to that of ideal chains [5, 6].

It is also important to remember that the solvent quality can also be changed by changing the temperature of the system. At higher temperatures, the entropy of the system dominates over the enthalpic interactions, and hence the chain swells. The solvent may be treated as a good solvent in this case. At low temperatures the effective interactions between the monomers dominate, and hence the chain may collapse

and the solvent may be treated as a bad solvent. At an intermediate temperature the characteristic size of the chain may be similar to that of an ideal chain. In this case the solvent is treated as a theta solvent.

1.2.5 Stiffness in polymers

So far we have discussed the properties of flexible polymer chains. For these chains, if one looks at the bond angle subtended by two consecutive bond vectors, then these bond angles can take all possible values. However, in a semi-flexible polymer or a stiff polymer chain, there are constraints on the bond angles along the chain contour. Some examples of naturally occurring semi-flexible polymers are: Actin molecules, DNA and microtubules.

Persistence length

The degree of polymer stiffness is often quantified by the persistence length of the polymer. The persistence length is an estimate of the average length scale over which the orientation of the bond vectors in a polymer chain remains correlated [5, 6].

Consider two bond vectors r_0 and r_j . Let the angle between these vectors be θ , then $\langle \cos\theta \rangle$ can be expressed as

$$\langle \cos\theta \rangle = \exp(-j/P) \quad (1.5)$$

where P denotes the persistence length of the polymer chain.

If the persistence length of a polymer exceeds the contour length R_c (length of the polymer if it is stretched from end to end), then the polymer is said to be stiff or rigid.

Kuhn Length

Kuhn length of a polymer is an estimate of the length scale over which different segments of a polymer chain can be considered to be statistically independent [5, 6]. A semi-flexible polymer can be coarse grained into a flexible chain where each ‘monomer’ has a lengthscale equal to that of the Kuhn segment. Thus the polymer can be considered to be a ‘freely jointed chain’ of such Kuhn monomers. The kuhn length is equal to twice the persistence length of the chain, for Worm like Chains (WLC).

1.3 Dynamics of polymers

1.3.1 The Rouse Model

The Rouse model describes the dynamics of ideal polymer chains [11]. In this model each of the monomers are assumed to be performing random motion with additional constraints due to chain connectivity. No excluded volume interactions are considered in this model. Using the Rouse model one may estimate the conformational relaxation timescale of an ideal polymer chain. The relaxation timescale of the chain τ_c may be defined as the time by which the center of mass of the chain diffuses by a characteristic length R_g . This may be related to the timescale of diffusion of a single monomer τ_m where τ_m represents the timescale over which a free monomer (which is not bound to its neighbors by harmonic interactions) diffuses by unit distance.

The Rouse model predicts that:

$$\tau_c \approx N^2 \tau_m \tag{1.6}$$

where N denotes the number of monomers in the polymer chain.

1.3.2 The Zimm model

In the bead-spring polymer when each monomer moves through the solvent it drags solvent molecules with it and hence also interacts over long distances with other monomers along the chain, *via* the solvent. This is termed as hydrodynamic interaction. The Rouse model ignores such interactions in its framework and thus fails for the case of polymers in dilute solutions. Using the Zimm model [12], τ_c is estimated as

$$\tau_c \approx N^{3/2} \tau_m \tag{1.7}$$

Note that the Zimm model predicts a weaker dependence of the chain length to the relaxation time of the polymer chain, as compared to the Rouse model.

1.4 Helix formation in semiflexible polymers

The helical motif is ubiquitous in nature and in macromolecules. They span a variety of length scales and some commonly known examples include the secondary structures of proteins, the double-helical DNA, collagen molecules, microtubules, actin molecules, viral capsids and flagella of several bacterial species. Furthermore, design

of synthetic helical molecules are significant from a technological point of view as they are used to make functional optical materials and nanomechanical systems.

For these reasons researchers have delineated a number of principles by which such helical macromolecules may be designed. In this thesis I outline how helix formation may occur in stiff polymer chains due to a variety of non-torsional interactions between the monomers.

In Chapter 2, I discuss how I developed a computational model to provide insights into experiments conducted by my collaborators, on a polymeric chain. In the experiments, a semiflexible chain of micron-sized colloidal monomers was synthesized, refer Fig.1.2. This was done by ensuring that PNIPAM microgels are adsorbed on the surface of the colloids. These PNIPAM microgels were made to cross-link with each other. The microgels adsorbed on the surface of the same colloid as well as the ones adsorbed on different colloids get cross-linked to each other. Thus, polymeric chains of ‘micron-sized’ colloidal ‘monomers’ were synthesized. The degree of rigidity could also be controlled by varying the cross-linking times of the PNIPAM microgels.

When the system is heated above the LCST temperature of PNIPAM microgels, this semi-flexible polymeric chain of micron-sized colloidal ‘monomers’ spontaneously adopts a helical conformation when the chain is heated. However, this happens only for chains which has an intermediate value of rigidity. Very rigid and very flexible chains do not show helical conformations. So the question that we address in this chapter is: What is the mechanism by which these chains of rigidity spontaneously adopt helical conformations?

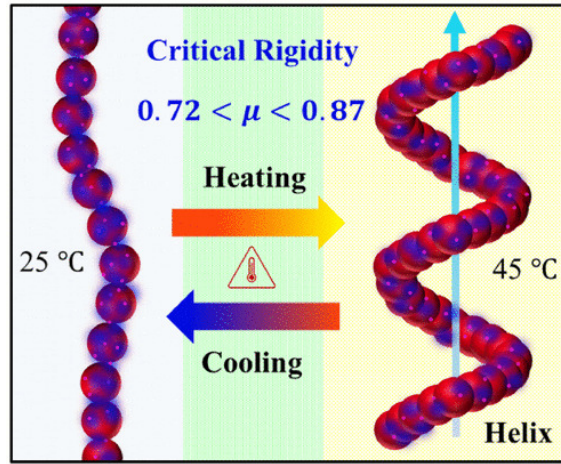


Figure 1.2: The schematic shows helix formation in a polymeric chain of micron-sized colloidal ‘monomers’. PNIPAM microgels (shown in violet) have been adsorbed on the surface of these colloids and then the PNIPAM microgels are made to cross-link to form the polymeric chain. When the chain is heated above a certain temperature, chains of an intermediate rigidity, assume a helical conformation. The schematic has been reproduced from [1].

In Chapter 3, I discuss how transient helix formation may occur in a rigid polymer chain due to the sudden induction of charge on the monomers, due to (say) a change in pH of a solvent. The mechanism of helix formation may be summarised as follows:

- The equilibrium configuration of a rigid chain is that of a linear chain. However, there are small deviations in the monomer positions from the axis due to thermal fluctuations.
- If the monomers suddenly then get charged and interact with each other through bare Coulomb interactions, then these deviations get accentuated due to repulsive Coulomb interactions.
- The polymer then assumes an angular conformation (with sharp bends along the contour) which is energetically unfavourable, since the polymer is stiff.

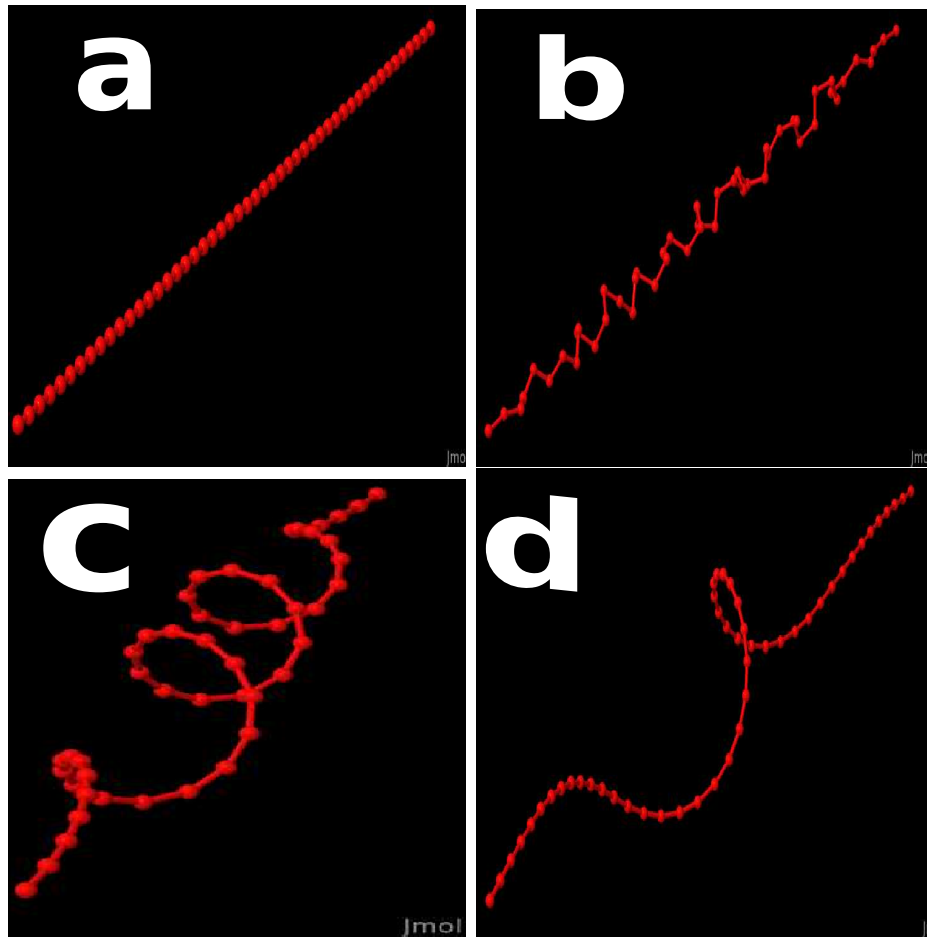


Figure 1.3: Subfigures (a-d) show various stages of the helical instability for a rigid polymeric chain where the monomers interact with each other through a long ranged repulsive interaction. The snapshots are (a) for the initial configuration with 49 monomers. The conformation appears to be that of a linear chain, however the monomer positions show a slight deviation from the axis due to thermal fluctuations. (b) The configuration showing the formation and accentuation of angular conformations due to sudden induction of charges on the monomers. (c) The configuration at a subsequent time when the helix is formed. (d) A configuration at an even later time showing the unwinding of the helix. The simulation snapshots have been reproduced from [2].

- Due to the stiffness of the polymer chain these sharp bends along the contour relax gradually and thus assume a helical conformation due to the relaxation process.
- Note that these transient helices keep unwinding axially, continuously due to the stiffness of the chain.
- The chain finally assumes a linearly extended configuration which is the equilibrium configuration of these charged, rigid rods.

The above aspects can also be visualized by referring to Fig.1.3.

In Chapter 4 of the thesis I modify the model presented in Chapter 3 in the following ways:

- When the stiff rod gets charged the monomers may assume charges of both polarities, i.e there are both positive and negative interactions induced.
- After the monomers are charged, the monomers interact with each other *via* the screened Coulomb interaction instead of a bare Coulomb interaction.
- We show that one obtains transient helices only when the fraction of charges of a certain polarity are more than its counterparts. This implies that repulsive interactions are key to forming these helices.
- In the last part of the work we show that if these transient helices are confined in a cuboid, then one obtains long-lived helices.
- Since transient helices unwind by extending axially, confinement by a cuboid prevents the polymer from stretching axially.

- Note that only for certain widths and lengths of the confining cylinder we obtain these long-lived helices.

1.5 Organization of Bacterial Chromosomes

Bacterial chromosomes such as the *E.coli* chromosome do not have a dedicated protein-based machinery to spatially segregate daughter chromosomes. This is unlike higher order eukaryotic chromosomes where self-assembled structures like mitotic spindles mechanically separate daughter chromosomes. When the DNA of a *E.coli* cell is stretched end-to-end it is of *mm* length, and it is packed into a micron sized cell. Therefore, the chromosome is compacted 1000 times inside the cell. Furthermore, the chromosome has to be organized in specific ways to perform biological functions. The organization of bacterial DNA is achieved by the action of proteins at all length scales. At smaller length scales the organization is achieved by histone-like proteins and other NAPs (Nucleoid Associated proteins). At larger length scales the organization is achieved by MukBEF proteins and others. Thus, the bacterial chromosome can be thought of as a ring polymer where each bead subsumes $\sim kbps$ of DNA.

Even at such large length scales the bacterial chromosome is organized in a manner that is different to that of a self-avoiding random walk polymer. This organization has been inferred from past experiments. We shall describe those experiments and then subsequently also discuss how our polymer model reconciles observations of complementary experiments pertaining to organization of bacterial chromosomes.

1.5.1 Entropic Segregation of Bacterial Chromosomes

The works of Suckjoon Jun et al [13, 14, 15, 16, 17, 18, 19] had suggested that bacterial daughter chromosomes segregate spontaneously in the cell. This is because in cylindrical cells ring polymers segregate to different halves of the cylinder to increase conformational entropy. This implies that the two ring polymers repel each other entropically. This was suggested as a viable mechanism by which *E.coli* chromosomes segregate. However, subsequently observations from complementary experiments suggested that the entropic model is incomplete. I shall now describe these experiments in greater detail.

1.5.2 FISH experiments

In FISH (Fluorescence *in-situ* Hybridization) techniques .chromosomal segments are labelled by attaching fluorescent probes. These probes can be tracked under a microscope and one can thereby extract information about the dynamics of the corresponding chromosomal segments.

In the FISH experiments conducted by Cass et al [20] on *E.coli* chromosomes it was found that certain segments of the DNA such as the *oriC* are preferentially localised in certain regions of the cell along the long-axis. Similarly, other segments are also localized in other regions of the cell. This localization of segments can be observed both before and after the replication of the chromosomal segments.

Such localization patterns cannot be reconciled using the entropy model of a

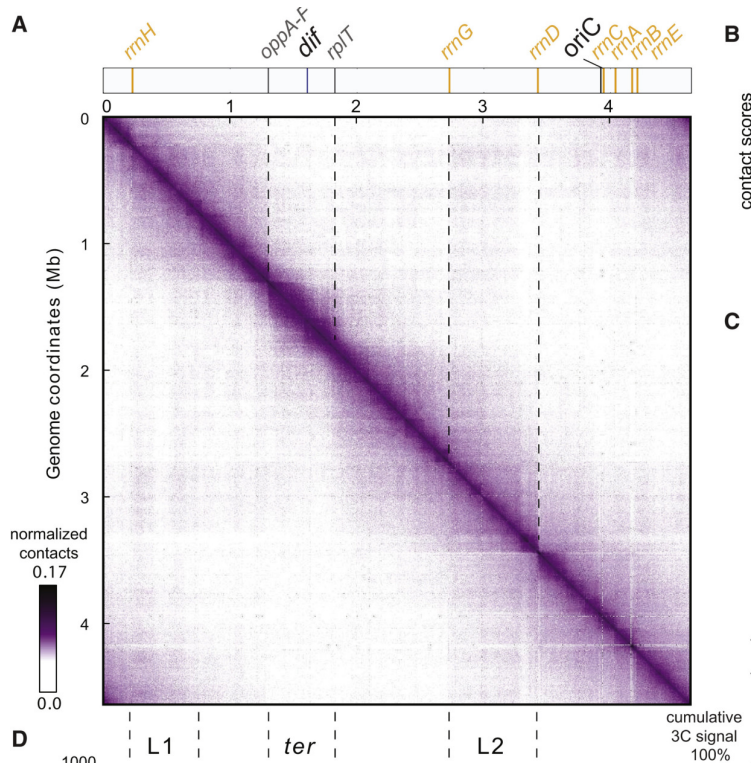


Figure 1.4: The figure shows the Hi-C map of the *E. coli* chromosomes. This data was published in [21] and the figure has been reproduced here after obtaining requisite permissions. The main diagonal is trivial, however one notes that there are block-like patches of high contact. Thus, certain segments of the chromosome interact preferentially with certain other segments and not certain other segments. These domains of high contact are termed Chromosomal Interaction Domains (CIDs). The relatively white regions indicate segments of low or no contact. This map indicates that the chromosome-polymer is not organized merely as a self-avoiding random walk polymer but has a specific organization.

polymer alone, since the chromosomal segments is likely to be found anywhere within the cell-half that each chromosome-ring-polymer occupies. So one of the questions we address in this thesis is : How do such localization patterns self-emerge in bacterial chromosomes?

1.5.3 Hi-C experiments

Hi-C experiments indicate the contact probabilities of different segments of the chromosome coming into contact with each other [22]. It indicates which segments of a chromosome are statistically close to a particular segment. Note that segments which are far away along the chain contour can also be proximal to a particular segment in 3D space. Such spatial correlations can be measured by Hi-C data information. It therefore gives insights about the organization of the chromosome-polymer in 3D space, refer Fig.1.4. Hi-C data is obtained by averaging over a large number of cells which may be in different stages of the life-cycle.

FISH experiments and Hi-C experiments are two complementary sets of experiments which together provide information on the organization of the genome in 3D space. The motivation for our study on bacterial chromosomes, is as follows: Can we develop a coarse grained polymer model such that observations of both Hi-C experiments are reconciled? In the next section I briefly discuss how that was achieved.

1.5.4 Role of polymer topology

The *E.coli* chromosome is a ring polymer. However, it is also known that binding proteins such as MukBEF bridge distant genomic regions along the contour. This may lead to the further formation of looped structures within the bacterial chromosome. In light of this, we assume that the *E.coli* chromosome adopts a polymer topology where the chromosome ring polymer has further internal loops.

This modification to the polymer topology, induces entropic repulsion between the different constituent loops within a single chromosome. Consequently, the segments belonging to the loops get spatially localized to certain regions of the cell-long axis. We show in Chapter-5 that a certain polymer topology called Arc-2-2 reconciles observations both from Hi-C and FISH experiments. We have also shown that there is biological evidence that the polymer topology we have assumed for our studies may arise in *E.coli* chromosomes due to the colocalization of *rrn* operons and other binding proteins.

1.5.5 Further questions

Having matched different experimental results for *E.coli* chromosomes, in Chapter 6 we further ask the following questions:

- Ring polymers segregate spontaneously inside a cylinder. Are the forces of entropic segregation higher for the case of polymers of different topologies?
- Can different polymer topologies be designed such that different localization patterns of the segments are obtained in a confining cylinder?
- Can a particular polymer topology reconcile the findings obtained from experiments conducted on the *C.crescentus* chromosome?

We answer these questions through our computational studies conducted in Chapter 6. We design looped architectures in various ways. We employ computational and analytical (blob-theory based) methods to show that the entropic forces between two

polymers is higher for certain classes of polymer topologies. Furthermore, we have also shown that a particular topology leads to the organization of the polymer such as that seen in *C.crescentus* chromosomes.

1.6 Outline of the thesis

The thesis primarily addresses two broad classes of problems:

- Helix formation in rigid polymers
- Role of polymer topology in the organization of confined polymers

In Chapters 2,3 and 4, I discuss various aspects of helix formation. The broad ideas that have been incorporated has also been summarised in the Introduction. In Chapters 5 and 6, I discuss how polymer topology leads to an organization of confined polymers. I also discuss its relevance to bacterial chromosomes and discuss avenues of future investigation.

Chapter 2

Rigidity Mediates Helix Formation of Colloidal Chains in Poor Solvent.

Introduction

¹.

The helical motif is ubiquitous in Nature. Typically, helix formation is attributed to specific, torsional interactions in natural [23] and in self-assembled systems[24]. Proteins form stable α -helical conformations [25, 26, 27] driven by hydrogen bonding interactions. The double-stranded helical structure of DNA [28] is stabilized by

¹This chapter has been adapted from the paper [1]

base pair interactions. Large scale self-assembled biological structures, such as microtubules and several viruses also show helical organization [29]. Here too, helicity arises from directional interactions.

Inspired by these, researchers have sought to obtain helical order in colloidal assemblies. For example, specific inter-particle interactions were engineered by grafting DNA onto colloidal particles, such that the colloids assembled into precisely controlled helical structures [30].

Helical chains have many technological implications, for example, in functional optical materials [31, 32, 33] and in nanomechanical systems[34, 35, 36, 37, 38]. Furthermore, colloids are model systems to examine the physics of self-assembly: micron-sized colloids show Brownian motion and are large enough to allow visualization using optical microscopy.

Helical assembly of colloids has also been effected by the presence of external fields [39]. Another strategy to cause helix formation is to use geometric confinement as colloids assemble[40, 41, 42]. Colloidal chains of magnetic particles have been shown to form helices by the application of a magnetic field [43].

Past theoretical works suggest that the lower energetic cost of bending compared to compression, of string-like systems results in helical [44, 45, 46] shapes. Snir and Kamien have demonstrated how entropy (depletion interactions) may drive helix formation in a tube [47]. These results suggest that helix formation results from a balance of chain rigidity and attractive interactions, even in unconfined systems.

Experiments performed by my collaborators demonstrate that colloidal chains with intermediate rigidity form helical structures on heating [1]. They have outlined a strategy for preparation of such chains by crosslinking of microgel PNIPAM

(Poly(N-isopropylacrylamide)) coated colloids. Heating above the PNIPAM lower critical solution temperature (LCST) induces solvophobic (“solvent-hating” or the propensity to minimize contact with the solvent) interactions in the microgels. The lower critical solution temperature (LCST) is the temperature above which the components of certain mixtures separate into distinct phases. As the microgels phase separate it is free-energetically favourable for them to minimize contact with the underlying solvent which leads to solvophobic interactions with the solvent. Chains of lower rigidity form compact structures on heating while very rigid chains do not show a change in shape. These temperature-induced transitions were reversible.

The experimental system can be modelled using a coarse-grained model that captures the essential physics of the experiments. This chapter will discuss this minimal model and how it validates the experimental results.

2.1 Experimental Background

A schematic of the chain preparation is shown in Fig.2.1 . Briefly, PNIPAM microgel particles are adsorbed on colloids (micron-sized) to form “monomers”. Upon imposition of an AC field, monomers experience dipolar interactions[48, 49, 50] and are aligned in the field direction. Monomers are then ”polymerized” by crosslinking of the PNIPAM microgels.

The resultant chains exhibit different levels of rigidity, depending on the preparation protocol. If the cross-linking time is increased then we get more and more rigid chains and vice-versa. Since the chains are not flexible, the decay in tangent-tangent

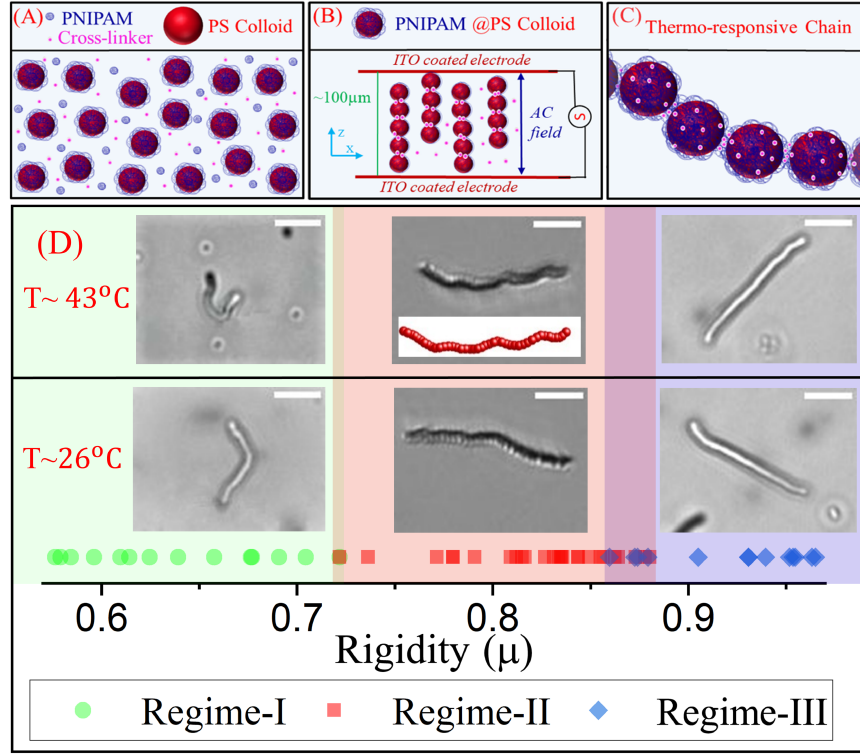


Figure 2.1: **Experimental results of my collaborators: Refer to [1] for more details.** 1st row: (A) Schematic of dispersion of PNIPAM coated PS colloids and cross-linker. (B) Linear chains of particles form on application of an AC electric field. (C) Schematic of thermo-responsive colloidal polymer. (D) Representative optical micrographs showing the structural changes of thermo-responsive colloidal polymers on heating from $\sim 26^\circ\text{C}$ (bottom panel) to $\sim 40^\circ\text{C}$ (top panel). The most rigid chains (D, right column, Regime-III) decrease in size marginally, but show no shape change. (D, middle column, Regime-II) Chains with intermediate rigidity form helical structures on heating. The inset shows the same chain, replotted by obtaining the spatial coordinates of points along the backbone of the chain for ease of visualization. These points do not correspond to the center of mass of individual colloids. (D, left column, Regime-I) The least rigid chains exhibit a large decrease in size on heating. All the scale bars are $10\mu\text{m}$. Each data point below the images in (D) represents an individual chain. We present data for about 50 independent chains whose rigidity parameter, μ , is indicated on the scale below. Blue diamonds represent rigid chains that showed no change in shape, while red squares are the intermediate rigidity chains that formed helices. The green circles represent chains that showed a large decrease in size.

correlation for the bond angles is weak for 15 to 40 monomer long chains. Thus, the persistence length cannot be estimated reliably in the experiments. Instead they define a dimensionless metric that was termed rigidity (μ) to characterize chain stiffness.

Chain rigidity, μ , is calculated as ratio of the mean square end-to-end distance ($\langle R^e(t)^2 \rangle$) for the chain to the square of its contour length (L) at $25C$. Thus, $\mu = \langle R^e(t)^2 \rangle / L^2$. Higher values of μ correspond to increasing chain rigidity.

At $25C$, the chains are swollen. On heating to $45C$, viz. above the LCST of the PNIPAM microgels ($32 - 33C$), the experiments show a transition in chain conformation. They investigate several chains with μ varying from 0.57 to 0.96. Each data point in Fig.2.1D represents an individual chain, indicating qualitative differences in the structural response of the chains on heating, depending on their rigidity (low rigidity Regime-I, $\mu < 0.72$; intermediate rigidity, Regime-II, $0.72 < \mu < 0.87$; high rigidity, Regime-III, $\mu > 0.87$). Chains in Regime-I ($\mu < 0.72$) exhibit a significant decrease in their spatial extent. The most rigid chains (Regime-III, $\mu > 0.87$) show a small decrease in chain size, but preserve their extended, linear conformation (Fig.2.1D, 3rd column). The most interesting change is observed for chains with intermediate rigidity (Regime-II, $0.72 < \mu < 0.87$, Fig.2.1D). At thermal equilibrium, these chains show helical conformations.

For more details pertaining to the experiments refer to [1].

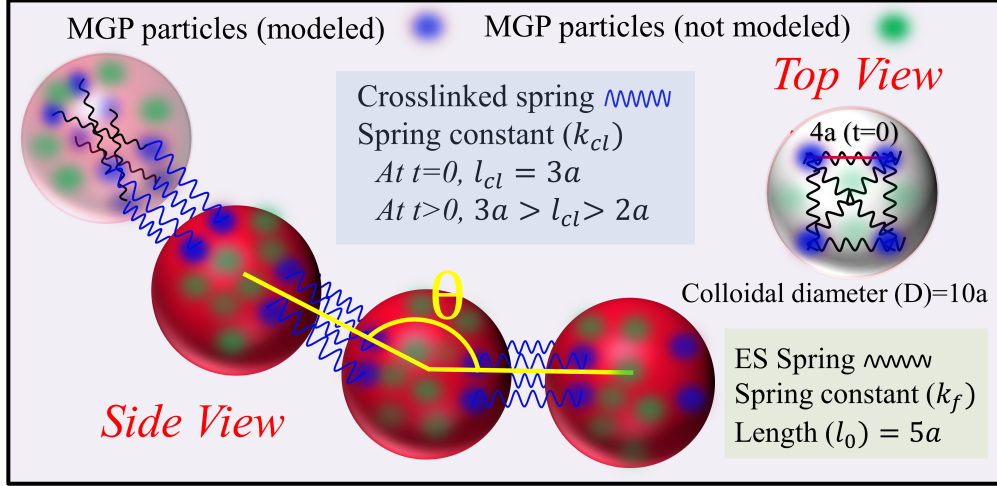


Figure 2.2: Schematic diagram of the simulation model. The polymer chain comprises colloids (red spheres) of diameter, $D = 10a$. One of the colloids is depicted as see-through, and is presented in side and top views, to clearly visualize the placement of the MGP point particles. Neighboring colloids are connected through 4 MGP point particles (blue fuzzy circles), interacting through springs (blue) with stiffness κ_{cl} , initially with equilibrium length $3a$. MGP point particles are held on the sphere surface using harmonic springs (black) with spring constant κ_f to represent electrostatic interactions (ES). Initially, the 4 MGP point particles on a hemisphere are positioned at the vertices of a square of side $4a$ and connected by springs (black) also of spring constant κ_f , as seen in the top view. The angle θ is the angle between 2 vectors joining the center of colloid to the centers of its neighbors.

2.1.1 Model

We simulate a minimal model that captures the essential physics of the experimental system. We consider a polymeric chain comprising of 40 colloids as a suitably modified bead spring model of a polymer. Each bead (monomer) represents one colloid in the polymeric chain. The beads interact with each other *via* the WCA potential that prevents overlap. Neighboring beads are bonded as described below as in the experimental system, where colloids are bonded through microgel (MGP) particles, refer Fig.2.2.

We performed Brownian dynamics simulations where the friction constant of a monomer is γ , and the unit of time τ is set by $\tau = a^2\gamma/k_BT$, the time taken for an isolated colloidal particle to diffuse a distance of a . The thermal energy $k_BT = 1$ sets the energy unit. If we set $a = 1, \gamma = 1$ such that $\tau = 1$, the over-damped stochastic Brownian dynamics equation is integrated with time step $dt = 10^{-5}\tau$.

In our minimal model, colloidal monomer connectivity through the MGP mesh is modeled through interacting point particles on the surface of the colloid. The diameter of each colloid is $10a$, where a is the unit of length in our simulations. There are 8 MGP point particles per colloid with 4 on each hemisphere along the axis of the polymer chain, refer Fig.2.2. MGP point particles on a hemisphere interact with each other through a harmonic potential with a stiff spring constant, κ_f . To mimic the effect of electrostatic interactions, the MGP particles are constrained to the surface *via* additional springs of length $5a$ and spring constant κ_f , connecting the center of the colloid to each of the point particles. At time $t = 0$, the 4 MGP point particles on a hemisphere form the vertices of a square of side $4a$ and lie on a plane. Each of these point particles are also linked to their counterparts on

the neighboring colloid through weaker harmonic spring interactions, with spring constant $\kappa_{cl} = 100; k_B T/a^2 = 10^{-2}\kappa_f$. The equilibrium spring length between an MGP particle and its counterpart on the surface of a neighboring colloid is $\ell_{cl} = 3a$. For lower values of κ_f , the polymeric chains do not attain helical conformations.

In the experimental realization, one typically expects a greater number of MGPs and hence a larger number of cross-links between the surfaces of two adjacent colloids. There also exist cross-links between MGPs located on the surface of the same colloid. These cross-links collectively would restrict the mobility of each individual MGP and, furthermore, would also give rise to asymmetry of forces between neighbouring colloids on heating. Our minimal model with 4 MGP particles in a square configuration and high values of κ_f , sufficiently restricts the mobility of the MGP particles. However, a different model incorporating a greater number of MGPs would also yield qualitatively similar results.

We also give the rationale for the choice of $\ell_{cl} = 3a$. At time $t = 0$, the closest surface-to-surface distance between two adjacent colloids is a . The normal to the plane of the formed by the 4 MGP particles (arranged in a square configuration) placed on the colloidal surface lies along the axis of the colloidal chain. Furthermore, the plane is at a perpendicular distance of $4a$ from the center of the colloid on which it is placed. Since the radius of the colloids is $5a$, a choice of $\ell_{cl} = 3a$ prevents overlap of colloidal surfaces and also leads to significant asymmetry in the direction of resultant forces experienced by each colloid as a consequence of the stochastic shrinkage of inter-colloid springs, above the LCST temperature.

At time $t = 0$, the center of the colloidal beads are placed linearly along the y-axis and at a distance of $11a$ from each other. In the experimental system, on heating

above the LCST, the MGP undergo a volume transition and their size decreases by a factor of ≈ 2 . The resultant shrinkage of the cross-linked mesh and hydrophobicity-induced attraction induces effective tension along the chain, pulling colloids towards each other. To mimic this in the simulations, we abruptly decrease the value of the equilibrium spring length ℓ_{cl} just after $t = 0$. Each connection between MGP point particles on neighboring colloids is decreased from $3a$ at $t = 0$ to a random value uniformly distributed between $2a$ and $3a$. This corresponds to a $\Delta\ell_{cl} = a$. In the subsequent ‘Results’ section we shall also discuss the case of different values of $\Delta\ell_{cl}$. For instance, $\Delta\ell_{cl} = 0.2a$ would correspond to a decrease in the equilibrium length of the springs from $3a$ to a random value uniformly distributed between $2.8a$ to $3a$. The decrease in ℓ_{cl} for a chosen $\Delta\ell_{cl}$, is different for each spring serves to mimic the complex stochastic variations in crosslinking between the physical MGP, and results in an asymmetry in contractile forces between colloids along the chain. This random variation in the decrease in ℓ_{cl} is critical for the formation of helices. If ℓ_{cl} is decreased uniformly for all springs, chains shrink in size, but no shape transitions are observed.

We use a 3 body bending interaction, parametrized by an energy, ϵ_b (that can be mapped to the experimental rigidity, μ), to model the chain stiffness. In our simulations, the measure of the rigidity of the polymeric chain is controlled by the value of parameter ϵ_b . However in the experiment, the rigidity of the chains are characterized using the dimensionless parameter μ as defined previously. The polymer chain in the simulations is described by introducing a 3 body bending interaction, with a bending energy $U_b = \epsilon_b \cos(\theta)$, where θ is the angle between vectors $(-\mathbf{r}_i, \mathbf{r}_{i+1})$. The vector $\mathbf{r}_i$ is the vector joining monomer $i - 1$ to its neighbouring monomer i along the chain contour. The value of ϵ_b can be mapped to the chain rigidity, μ to

match simulations with experiments. This model allows us to describe the behavior of chains with μ varying from 0.8 (corresponding to $\epsilon_b = 0k_BT$) to 0.9 (corresponding to $\epsilon_b = 12 \times 10^3 k_BT$), covering the range of μ corresponding to Regime-II and Regime-III in the experiments. It is not possible to access more flexible chains using this model due to the rigidity inherent to the MGP-mediated bonding between colloids. The high values of ϵ_b were chosen to obtain values of μ which match those obtained from experiments.

2.1.2 Results

Of specific interest to us is the emergence of helical motifs when colloidal chains of intermediate rigidity are heated to above the LCST of the MGP; this corresponds to abrupt shrinkage in ℓ_{cl} values and emergence of helices in our simulation model. Helicity of a chain can be quantified using two quantities, the global helical order parameter $H4$ and the local helical order parameter $H2$ where,

$$H4 = \frac{1}{N-2} \left(\sum_{i=2}^{i=N-1} \mathbf{u}_i \right)^2; H2 = \frac{1}{N-3} \left(\sum_{i=2}^{i=N-2} \mathbf{u}_i \cdot \mathbf{u}_{i+1} \right) \quad (2.1)$$

where $\mathbf{u}_i$ is the unit vector along $\mathbf{r}_i \times \mathbf{r}_{i+1}$. A compact tightly-wound perfect helix in the continuum limit of infinitesimal $\mathbf{r}_i, \mathbf{r}_{i+1}$ vectors will have vectors $\mathbf{u}_i$ pointing along the helix axis, and hence both $H4$ and $H2$ will have a values of ≈ 1 . However, if the handedness varies along the chain - for example, if half of the chain is right handed, and the other half is left-handed, $H4$ will have values close to zero but $H2$ will remain non-zero. Hence, we use $H2$ to identify local helical order[51, 2]. A semi-flexible polymeric chain, with $\Delta\ell_{cl} = 0$, is without helical order and has significantly

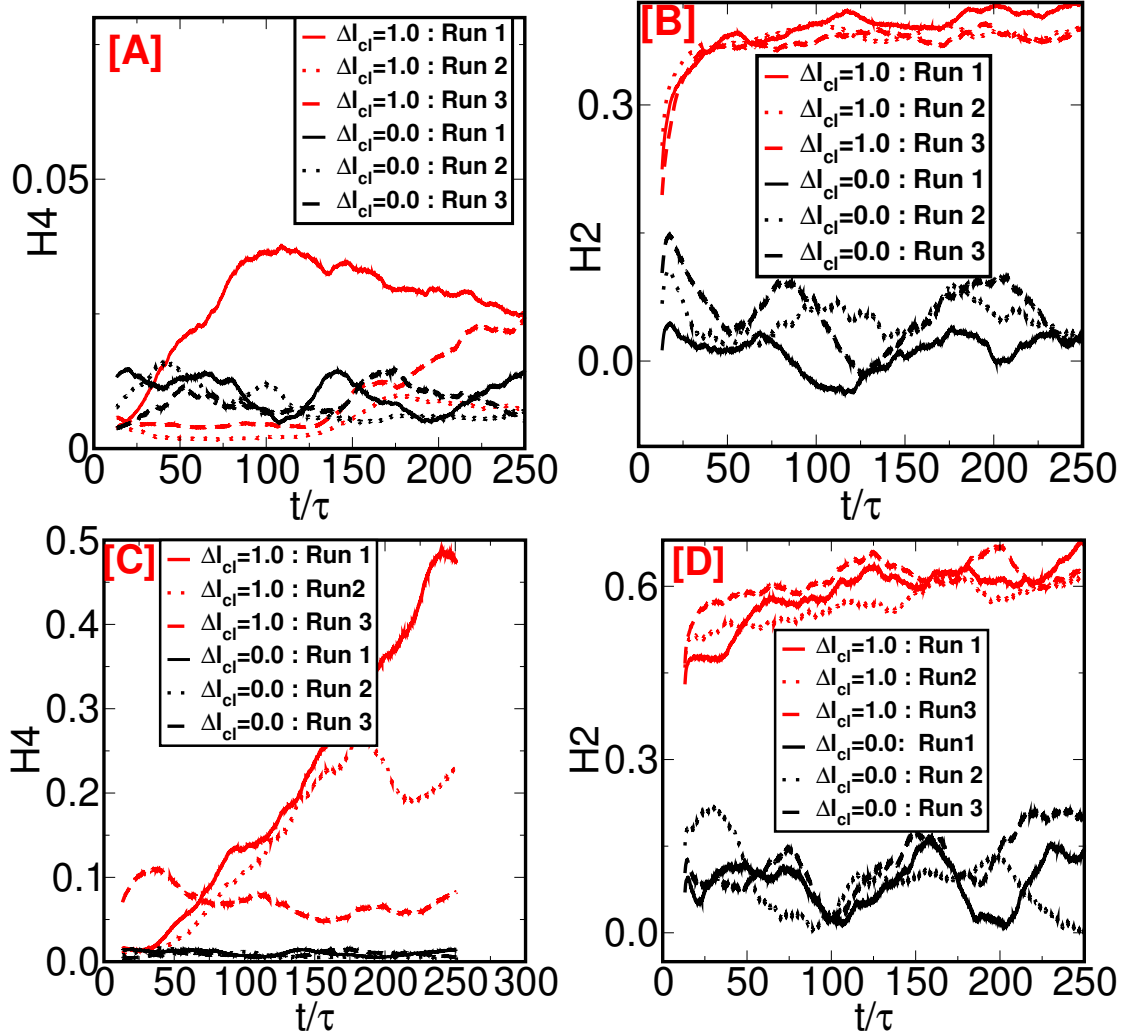


Figure 2.3: (A) $H4$ running averaged over 25τ and (B) $H2$ running averaged over 25τ , as a function of time for $\kappa_{cl} = 100k_B T/a^2$ and $\epsilon_b = 5000k_B T$ ($\mu \sim 0.8$) with 3 independent runs for chains of 40 colloidal monomer, under hydrophobic contractile forces with $\Delta\ell_{cl} = 1$ (measured in units of a). For comparison we also show 3 independent runs, corresponding to identical polymeric chains of colloids with identical parameters, but without hydrophobic attraction, i.e $\Delta\ell_{cl} = 0$. Subfigures (C) and (D) show $H2$, $H4$ data for the same parameters as in (A) and (B) but for a shorter chain with only 25 monomers.

lower values of both $H2$ and $H4$ as compared to a chain with helical order (refer Fig.2.3).

In our simulations, we observe that chains with $DP = 25$ and intermediate rigidity attain a helical conformation characterized by large values of $H4$ and $H2$ ($\approx 0.4 - 0.6$) simultaneously (FIG.2.3).

However, larger helical chains (with 40 monomers) with similar rigidity exhibit relatively small values of $H4 \approx 0.04$ and high values of $H2 \approx 0.4$ (comparable to those for the $DP=25$ chains). We attribute this to the lower probability of having different chain segments with opposing helical windings for a shorter chain with $DP < 25$, consistent with experimental observations. Thus, here we focus on $H2$ to quantify the formation of helicity in the chains.

We examine the structure of chains at steady state, for $t > 50\tau$ after the abrupt decrease in ℓ_d , corresponding to heating above the LCST (refer FIG.2.3). We track the steady state value of $\langle H2 \rangle$ for chains with different rigidity (FIG. 2.4C). $\langle H2 \rangle$ shows non-monotonic behavior with increase in ϵ_b . At low ϵ_b , chains are relatively less rigid due to the low bending energy, and show $\langle H2 \rangle$ close to zero. With increase in ϵ_b , $\langle H2 \rangle$ increases to over 0.4 for chains of intermediate rigidity. For further increase in ϵ_b , there is a decrease in $\langle H2 \rangle$ such that helices are no longer observed when $\epsilon_b = 16 \times 10^3 k_B T$ or more, corresponding to highly rigid chains with $\mu > 0.9$. These trends are in excellent accord with the experimental results: helices form only over a critical range of chain rigidity. Chains that are either more flexible or more rigid do not form helices.

We have also investigated the decrease in chain size, measured by the end-to-end distance $R_{ee}(t)$ normalized by the contour length R_c , when ℓ_d is decreased to mimic

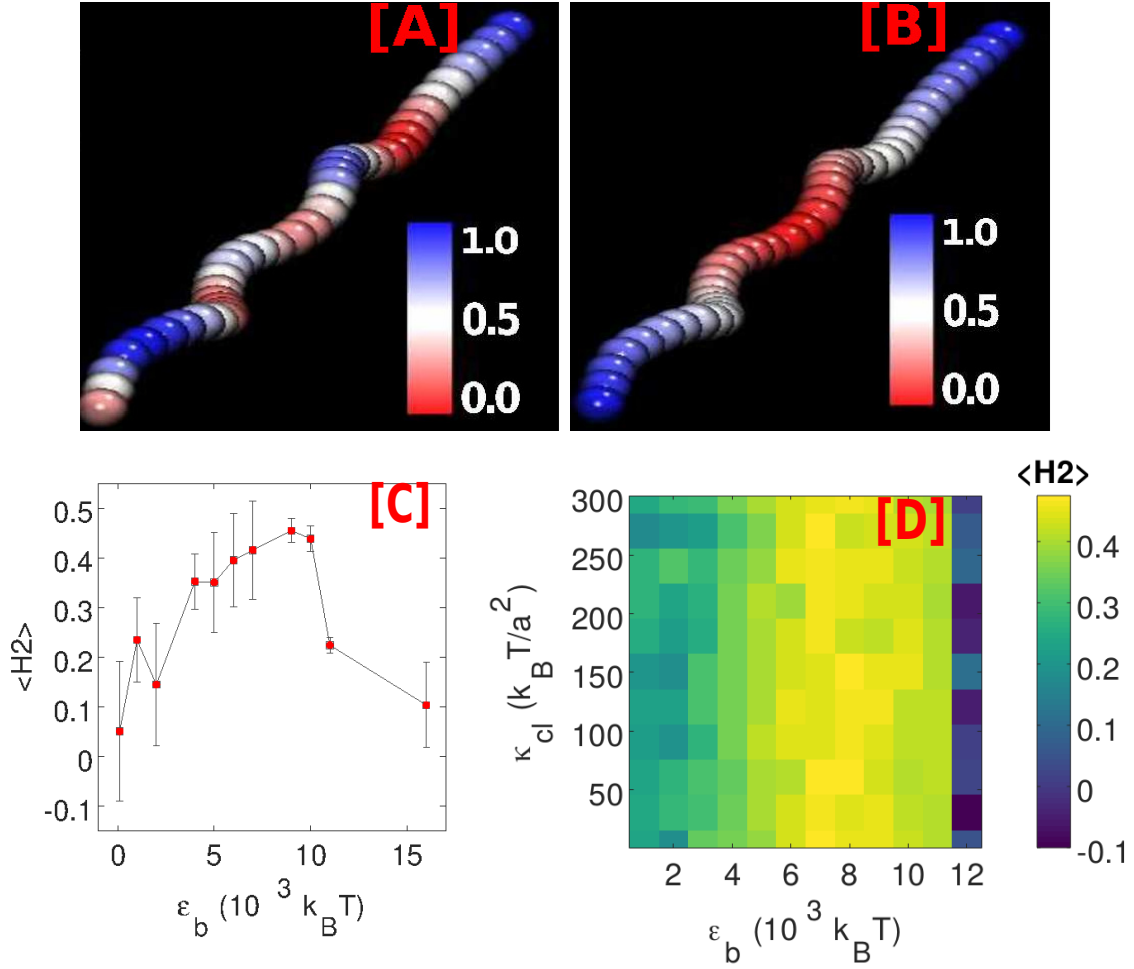


Figure 2.4: Subfigures (A) and (B) show a snapshot of the helical conformation of the polymeric chain at $t = 6.66\tau$ iteration. The colour gradient indicates the degree of deviation of the monomers from the helical axis along the two perpendicular directions, $\hat{x}$ and $\hat{z}$, respectively. The periodic variation in the colour gradient is indicative of the helical conformation attained by the polymeric chain. (C) Time averaged values of $H2$ (averaged over 200τ from 50τ to 250τ , for different values of ϵ_b with $\Delta\ell_{cl} = a$, for a fixed values of κ_{cl} , $\kappa_{cl} = 100k_bT/a^2$. There are 40 monomers in the chain. The error bars have been calculated from 10 independent runs. (D) Phase diagram showing the range of the parameters κ_{cl} and ϵ_b , which are conducive to helix formation as characterized by $\langle H2 \rangle$, for a chain of 40 monomers.

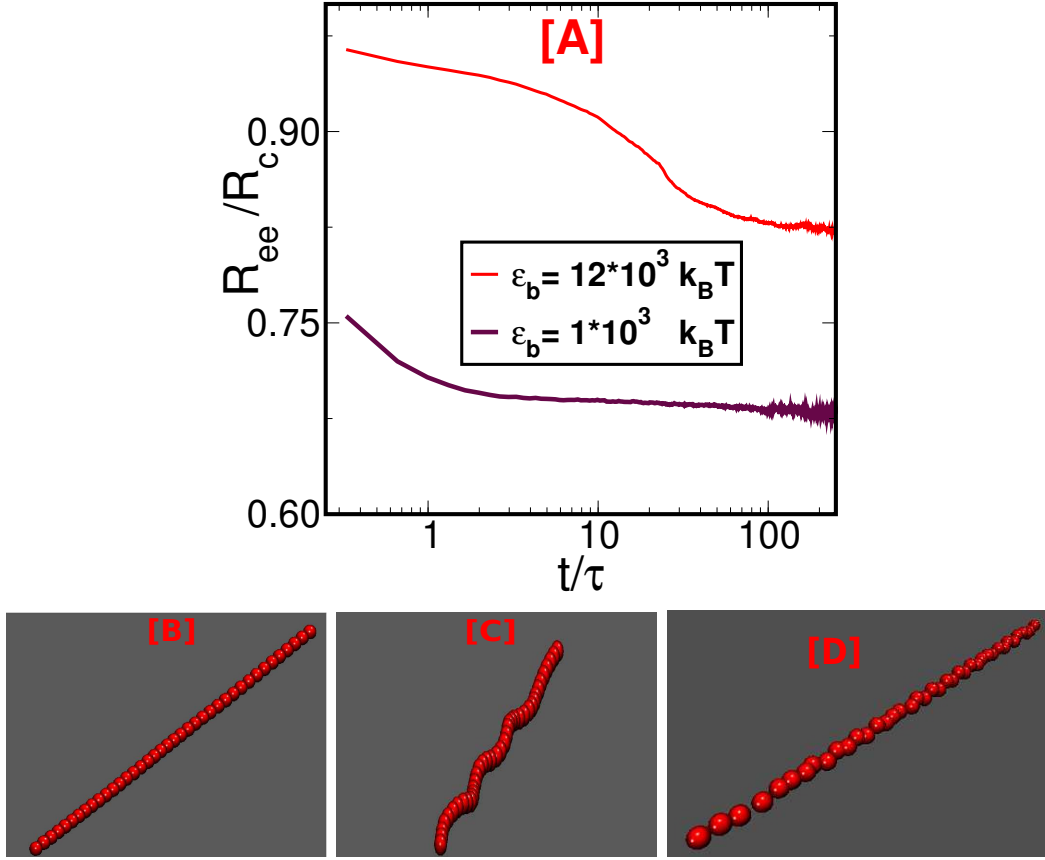


Figure 2.5: (A) The end to end distance R_{ee} (as a fraction of contour length R_c) is plotted as a function of time for chains of two different flexibilities of $\epsilon_b = 10^3 k_B T$ and $\epsilon_b = 16 \times 10^3 k_B T$, respectively, when hydrophobic compression is introduced at time $t = 0$. (B) Representative snapshots of the conformations of the polymeric chain for the stiff chain: $\epsilon_b = 12 \times 10^3 k_B T$, the (C) semi-flexible chain with $\epsilon_b = 5 \times 10^3 k_B T$, and (D) the relatively flexible chain with $\epsilon_b = 10^2 k_B T$ respectively. Only the semi-flexible chain attains a noticeable helical conformation.

hydrophobicity. On increase in ϵ_b , the decrease in R_{ee}/R_c is more modest as compared to the decrease observed in experiments. For relatively less rigid chains with $\epsilon_b = 10^3 k_B T$, R_{ee}/R_c decreases to about 0.7 at steady state; whereas for relatively more rigid chains, i.e. with $\epsilon_b = 12 \times 10^3 k_B T$, the steady state R_{ee}/R_c decreases to only about 0.82 (FIG.2.5).

Chain behavior is determined by the strength of bonding between monomers, given by κ_{cl} , and by the bending rigidity, given by ϵ_b . Most of the results presented here are for chains with constant $\kappa_{cl} = 100k_BT/a^2$. In Fig.2.4D we present a phase diagram based on the structure formation as chains are heated above their LCST, as a function of κ_{cl} and ϵ_b . We find that the trends observed, viz. helix formation for chains with intermediate rigidity (corresponding to intermediate ϵ_b), hold robustly over a wide range of κ_{cl} . There is no substantial influence of κ_{cl} over the range of ϵ_b where helix formation is observed above the LCST.

Finally, we examine the mechanism of helix formation in the simulations. In our experiments, there is an increase in the average helicity with temperature. To mimic this, we examine the change in $H2$ with increase in $\Delta\ell_{cl}$ for chains with $\epsilon_b = 5000k_BT$. With increase in $\Delta\ell_{cl}$ from $0.2a$ to a , there is an increase in the steady state $H2$ values (running averaged over 25τ), consistent with the experimental observations (FIG.2.6).

Now we focus on the dynamics of the polymer chain at relatively short time scales to gain an understanding of the mechanism of helix formation. We look at the evolution of mean $\langle\cos\theta\rangle$ per triplet of monomers along the chain, as a function of time as shown in FIG.2.7A, FIG.2.7B and FIG.2.7C for different values of ϵ_b . The mean is calculated over the angles subtended by all triplets of neighbouring monomers along the chain. Note that at time $t = 0$, the centers of the colloidal particles are placed along the y-axis, thus $\theta = \pi$ for all monomer triplets at $t = 0$. The conformation at $t = 0$ is as shown in Fig.2.7D. Soon after at $t \approx 0.01\tau$, $\langle\cos\theta\rangle$ reaches its maximum value(FIG.2.7A and FIG.2.7B) , which implies that asymmetric contractile forces lead to the formation of sharp angular conformations along the chain contour. This gives rise to the kinked conformation of the polymeric chain as

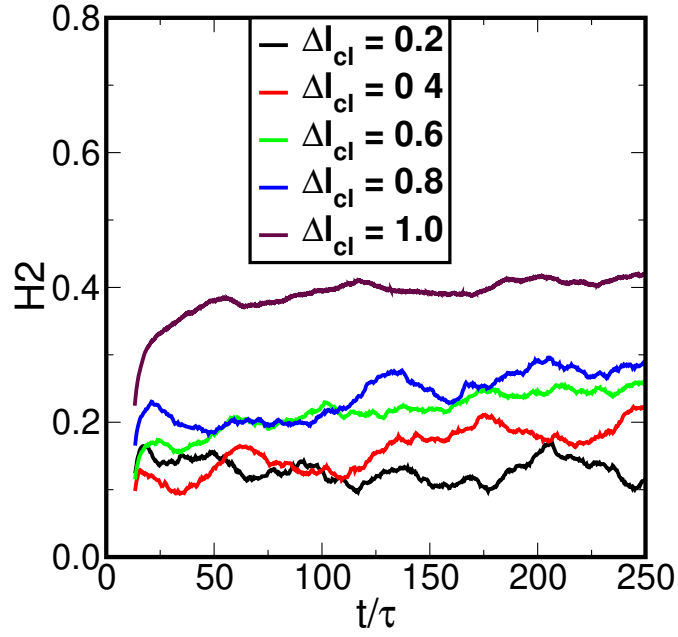


Figure 2.6: $H2$ running averaged over 25τ as a function of time for $\kappa_{cl} = 100k_B T/a^2$ and $\epsilon_b = 5000k_B T$ ($\mu \sim 0.8$) under contractile forces due to hydrophobicity for various values of $\Delta\ell_{cl}$ as indicated by the legends.

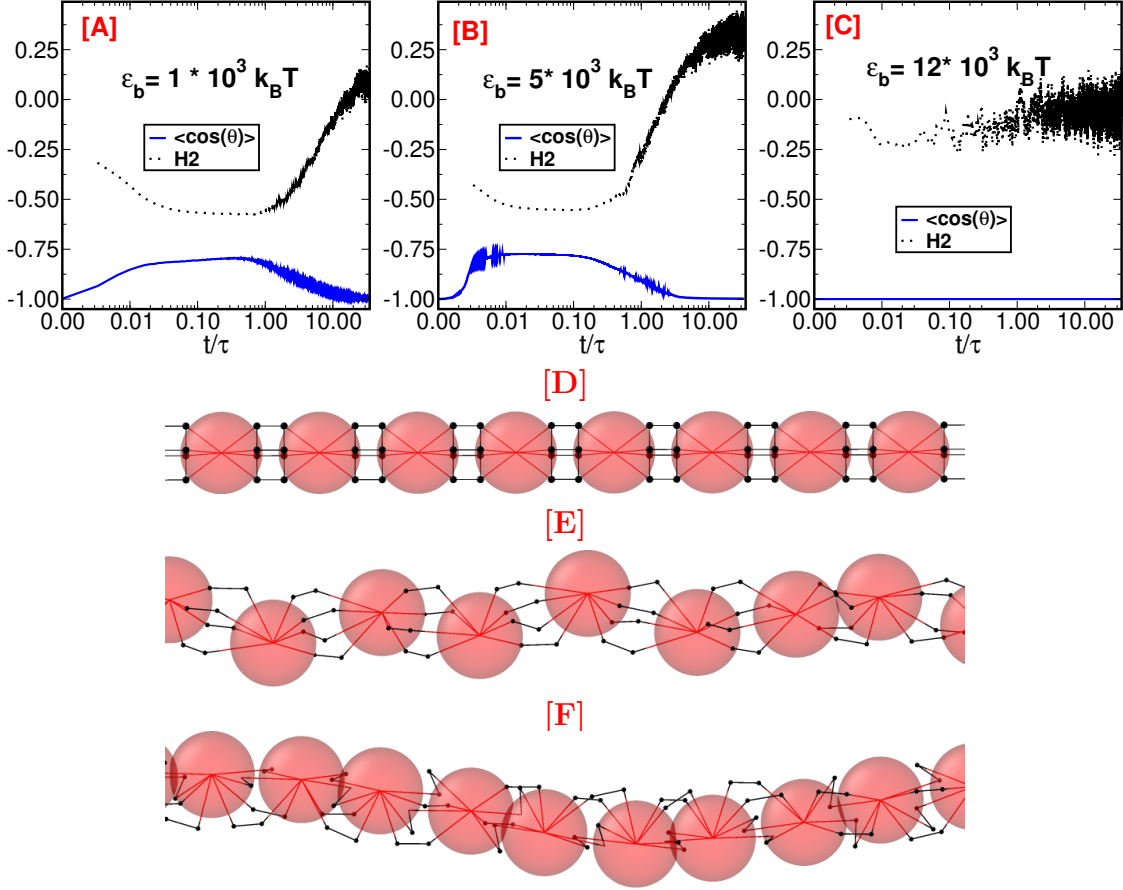


Figure 2.7: (A) The quantity $\langle \cos\theta \rangle$ (averaged over all the triplets of neighbouring colloidal-monomers) *versus* time t for $\epsilon_b = 1 \times 10^3 k_B T$, (B) for $\epsilon_b = 5 \times 10^3 k_B T$, and (C) for $\epsilon_b = 12 \times 10^3 k_B T$. Subfigures (D),(E) and (F) show the mechanism of helix formation corresponding to a chain with $\epsilon_b = 5 \times 10^3 k_B T$. (D) shows the initial configuration of the colloidal particles at time $t = 0$ and the cross-links between MGPs, all of equal length and lined up parallel to each other. Subfigures (E) and (F) show the different lengths and orientations of the cross-linked springs when the colloids attain an angular and a helical conformation respectively. For aid of visualization, the colloids have been made translucent and their size is scaled down. Thus the distortion of the cross-links gives rise to asymmetric forces between colloids which in turn gives rise to the helical conformation.

seen in the snapshot of FIG.2.7E for chains of low or intermediate rigidity. However these sharp angles (kinks) are energetically penalized by the rigidity of the polymeric chain. Thus the sharp angles gradually relax, as is indicated by the gradual decrease in $\langle \cos\theta \rangle$ of FIG.2.7A and (B), at $t \gg 0.33\tau$ and leads to a conformation such as that of FIG.2.7F for chains of intermediate rigidity. This leads to the formation of helical conformations as confirmed by the gradual increase of helical order parameter $H2$ beyond time $t/\tau = 2$, refer FIG.2.7B for chains of intermediate rigidity. At longer times $\langle \cos\theta \rangle$ as well as $H2$ fluctuates about a mean value. This is the mechanism by which helical conformations are attained by polymeric chains, as seen in FIG.2.4A and FIG.2.4B for chains of intermediate rigidity. The helical conformations formed are stable for $t \gg \tau$. We note that highly rigid chains ($\mu \approx 0.9$) do not allow the formation of such sharp angles (or kinks) that are crucial to helix formation (FIG.2.7C). Thus a highly rigid chain does not attain helical conformations on heating. Alternately a chain that is relatively less rigid ($\mu \approx 0.8$) forms a kinked structure, but these sharp angles do not relax in time as much, and therefore do not lead to appreciable values of $H2$ (FIG.2.7A). Refer to Fig.2.5[D] for a representative snapshot of the kinked conformation attained by the relatively less rigid chain.

2.2 Discussions and Conclusions

We present a simulation model by which we explain the mechanism underlying helix formation in thermo-responsive colloidal chains. PNIPAM micro-gel coated colloids are made to form linear chains of such colloidal monomers. Above the LCST of PNIPAM, these chains behave as if they are in a bad solvent and exhibit strong attractive interactions. There is a decrease in the size of the chains due to attractive

interactions. Rigid chains show a small decrease in size but show no other change in their shape. More flexible chains form compactify significantly. Chains with intermediate rigidity show the formation of helical structures on heating. We use simulations to show that the assymetric attractive interactions between monomers of a semiflexible chain lead to helical conformations.

The simulation model discussed in this chapter is non-standard and was developed to decipher the mechanism of helix formation in the experiment. We learn from the model that to induce helix formation, each monomer must experience asymmetric forces. A simple bead spring model would not lead to helix formation because the forces between two adjacent monomers are along the line joining their centers. In the model constructed for this study, we keep 4 springs on each side of the monomer to ensure that the forces acting on neighbouring monomers is not along the line joining their centers. This is a crucial ingredient which gives rise to helix formation

Chapter 3

Transient helix formation in charged semiflexible polymers without confinement effects.

3.1 Introduction

¹

Creating emergent structures through intelligent engineering of physical interactions between macro-molecules is a versatile method to self-assemble or self-organize structures with a target morphology. A particular macro-molecular morphology of great interest across disciplines is the helix, as it is a recurring motif across chemistry, biology[26, 52, 53] and physics [44, 54, 51, 55, 56, 47]. Forging helical struc-

¹This chapter closely follows the paper [2].

tures at the nm to 10μ length scales remain challenging, though helical springs are ubiquitous in NEMS/MEMS devices[36, 35], piezoelectric devices[38] and helical micro-swimmers[57, 58, 59, 60] are used for micro-rheology. These helices are produced primarily by various “bottom up approaches”, e.g. vapour deposition which is dependent on the detailed interactions of the constituent atoms/ molecules, or alternatively using helical templates[61, 62, 63, 37, 64, 65, 66]. Helices can also emerge due to suitable confinement effects [67, 68]. It would be of interest to devise alternate strategies to obtain spontaneously formed helical architectures at $nm-\mu$ length scales using physical forces by approaches which remain independent of chemical details of the monomer constituent.

There have been previous reports of extremely short lived helix formation in polymers in bad solvents undergoing collapse due to hydrophobic forces [51] which act at nm length scales. Others have observed helices on optimally packing tubular filaments at particular ratios of pitch and radius [44]. But in a more detailed paper, the authors comment that compaction of a chain of spheres gives very different results from compaction of a tube [26]. This is because the tube can be considered as a compact object made up of discs which has very different symmetry properties in terms of interaction potentials compared to those acting between spherical beads, say, of a polymeric chain. Another study shows that the ground state of a self attracting chain shows a variety of structural motifs including the helix, depending on the nature of the stiffness present in the chain (energetic or entropic) [69]. Our study reports the self-emergence of free standing helical structures using the most generic of repulsive potentials such as Coulomb repulsion which could have influence in understanding emergence of such structures at $nm-\mu$ length scales, in a variety of situations within the living cell or outside.

Here we show emergent structures with transient helical order in a free standing (unconfined) bead spring model of a semiflexible polymeric chain using generic interactions. Our computer simulations show that helical structures can be obtained by inducing instabilities with either Coulomb interactions or other long ranged power law repulsive interactions between the monomers if we start out from an initial configuration where the uncharged polymer chain is straight for a polymer chain whose persistence length is lesser than the contour length. At time $t = 0$ if a neutral polymer becomes charged, the chain adopts a helical configuration before the helical structure dissolves to adopt a stretched linear configuration at long times due to long ranged repulsion. We also show that a stiff polymer chain in thermal equilibrium with its bath can also result in a helical conformation if repulsive Coulomb interactions between the monomers is switched on. A helical structure may also be obtained if a semi-flexible polymer chain, with persistence length $\ell_p < N_c$ where N_c : the contour length) is pulled at both ends by a constant force, and released just as the repulsive Coulomb interaction is switched on between the monomers. Experimentally this may be accomplished by pulling the polymer chain with an AFM tip [70, 71] and the charges may be induced by changing the pH of the solution, [72, 73, 74, 75, 76].

Note that in all of the above we obtain transient helices without the use of torsion inducing potentials or hydrogen-bond mimicking potentials acting between monomers. In this paper, we also show how thermal fluctuations play an important role in the the formation of helical structures. In addition, we induce time dependent potentials where the charge of a semi-flexible polymer varies with time (say as pH changes with time) [77, 78] . As a consequence, helices are formed periodically in phase with the driving.

The chapter is organized as follows. The following section describes the model of

a semi-flexible polymer dressed with additional long range interactions which leads to helix formation. The additional interactions have the form $\sim 1/r$, or $\sim 1/r^3$. This implies that there is no screening of Coulomb charges, when we describe helix formation starting out from a straight line initial condition or of a stiff polymer in thermal equilibrium or from a stretched condition due to force applied to the end monomers. Next we discuss the mechanism of helix formation starting out from a straight line initial condition (for simplicity) with $1/r$ (Case A) and $1/r^3$ (Case B). At the end we discuss the range of values of semi-flexibility energies/spring constants/strength of Coulomb forces for which we obtain helices. We do this by plotting a suitable state-diagram. We finally conclude with Discussions and future outlook.

3.2 Model

We use the bead spring model of a polymer for our simulations. The model polymer could be a real polymer, or it can be string of colloids stitched together to form a semi-flexible polymeric chain as described in [79, 80]. Thereby, the monomer size and the number of beads N in the chain determine the length scale of helical configurations formed. The unit of length in our study is a , where $a = 1$ is the equilibrium length of the harmonic-springs between two adjacent monomers with energy $u_H = \kappa(r - a)^2$ between adjacent monomers; r is the distance between the monomers. The spring constant κ is $20k_B T/a^2$ for Case A, which has repulsive Coulomb interactions $u_c = \epsilon_c(a/r)$ acting between all monomer pairs of the chain. The parameter $\epsilon_c = 87.27k_B T$ is the measure of the Coulomb energy when a pair of charges are at a distance a from each other. Case B uses $\kappa = 10k_B T/a^2$, along with

the additional interaction u_d between all the monomers of the chain. The form of the potential u_d is $u_d = \epsilon_d(a/r)^3$ with $\epsilon_d = 107.70k_BT$ with cutoff at $r_c = 4a$. Diameter of each monomer is $\sigma = 0.727a$, and excluded volume (EV) of monomers are modeled by the WCA (Weeks Chandler Anderson) potential. This choice corresponds to the good solvent condition.

The polymeric chain is semi-flexible; the corresponding bending energy u_b is $u_b = \epsilon_b \cos(\theta)$, where θ is the angle between vectors $(-\mathbf{r}_i, \mathbf{r}_{i+1})$. The vector $\mathbf{r}_i$ is the vector joining monomer $i - 1$ to its neighbouring monomer i along the chain contour. The thermal energy $k_BT = 1$ sets the energy unit. We performed Brownian dynamics simulations where the friction constant is ζ , and the unit of time τ is set by $\tau = a^2\zeta/k_BT$, the time taken for a isolated monomer particle to diffuse a distance of a . If we set $\zeta = 1$ such that $\tau = 1$, since k_BT and a are already chosen to be one, the over-damped stochastic Brownian dynamics equation is integrated with time step $dt = 0.0001\tau$.

Unless clarified otherwise, we mostly observe the polymer dynamics by starting out from the same straight line initial condition for the above mentioned cases (A) and (B): a linear polymer chain of 49 monomers is placed along the y axis with adjacent monomers at a distance of a from each other. The fluctuation dissipation theorem determines the magnitude of the random force on each particle for all cases. For studies with cases (A) and (B), we choose $\epsilon_b = 10k_BT$ (corresponding to the persistence length $\ell_p = 11a$, as calculated by simulations) and $80k_BT$, respectively. A large difference in the values of ϵ_b was chosen to demonstrate that helix formation is robust for a range of parameter values. We use box-size $\gg 50a$ for a chain with 49 beads, such that periodic boundary conditions are never invoked. Hence we do not use Ewald technique to calculate Coulomb interactions as self interactions with

periodic images of the monomers are irrelevant. Moreover, no counterions were considered for our simulations, so the charges are not screened.

Subsequently, we study transient helix formation of chains in thermal equilibrium, *viz.*, we establish that a semiflexible polymer with 60 monomers in the chain and with persistence length ℓ_p greater than the contour length ($N_c = 60a$) and in thermal equilibrium with the bath, develops a local helical order once the repulsive Coulomb interactions ($\epsilon_c = 87.27k_B T$) between the monomers is switched on. The spring constant $\kappa = 200k_B T/a^2$. Furthermore, if a semi-flexible polymer, with 60 monomers in the chain but with $\ell_p < 60a$, is stretched by applying a constant force at both ends by a constant force $20k_B T/a$ and then released, and simultaneously the repulsive Coulomb interaction ($\epsilon_c = 87.27k_B T$) is switched on between the monomers, then the polymer again develops a transient helical order.

From the experimental perspective, it would be more instructive to specify the ℓ_p of a polymer rather than specify the simulation parameter ϵ_b , which we tune to fix ℓ_p . To that end, we calculate the relation between ϵ_b and ℓ_p for small angular deviations of bond-angles from angle π . The calculation details are given in the Sec.3.5. The relation between angle α (as shown in Sec.3.5) and ϵ_b is given by,

$$(\epsilon'_b - 1)/\epsilon'_b = \cos \alpha \quad (3.1)$$

where $\epsilon'_b = \epsilon_b/k_B T$ and $\alpha = \pi - \theta$. From polymer physics [5], we know that for WLC (worm like chain) model, for the small angles of bends, the persistence length ℓ_p is given by $\ell_p = 2a/\alpha^2$. Thereby,

$$\ell_p \approx a\epsilon_b/k_B T \quad (3.2)$$

Thus ℓ_p increases linearly with ϵ_b . As an example, a polymer with bending energy

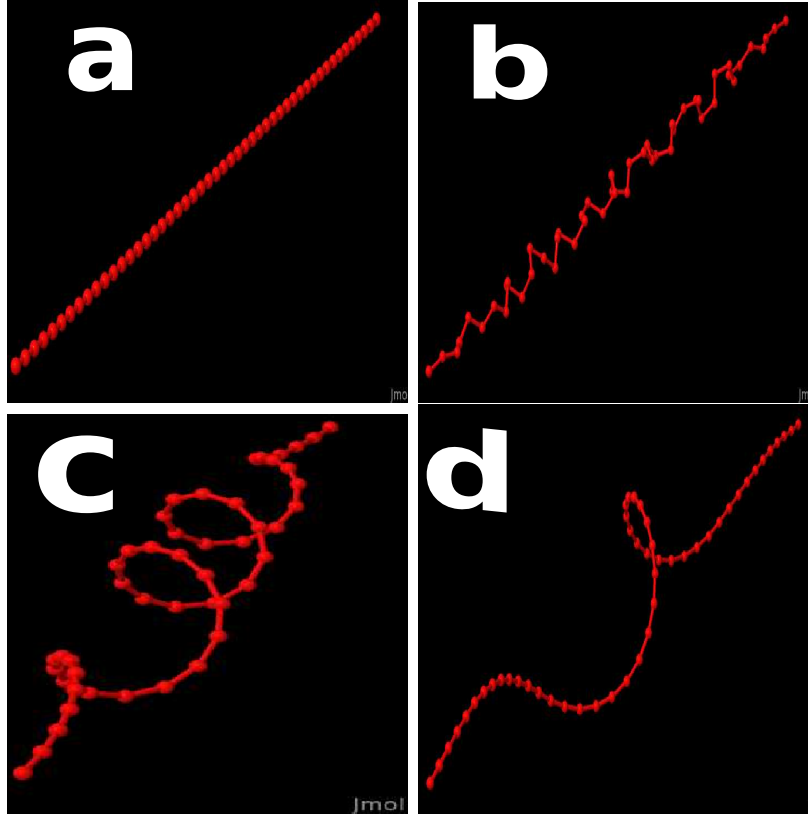


Figure 3.1: Figures 1(a-d) shows various stages of the helical instability for a semi-flexible polymeric chain starting from the straight initial configuration with potential u_d : case B. The snapshots are (a) for the straight line initial configuration at time $t = 0$ with 49 monomers (b) the configuration at time $t = 3.3 \times 10^{-2}\tau$ ($\mathbf{H2} = \mathbf{0.23}, \mathbf{H4} = \mathbf{0.006}$) (c) the configuration at a subsequent time $t = \tau$, when the helix is formed ($\mathbf{H2} = \mathbf{0.81}, \mathbf{H4} = \mathbf{0.43}$) (d) configuration showing the unwinding of the helix at time $t = 5\tau$ ($\mathbf{H2} = \mathbf{0.65}, \mathbf{H4} = \mathbf{0.29}$).

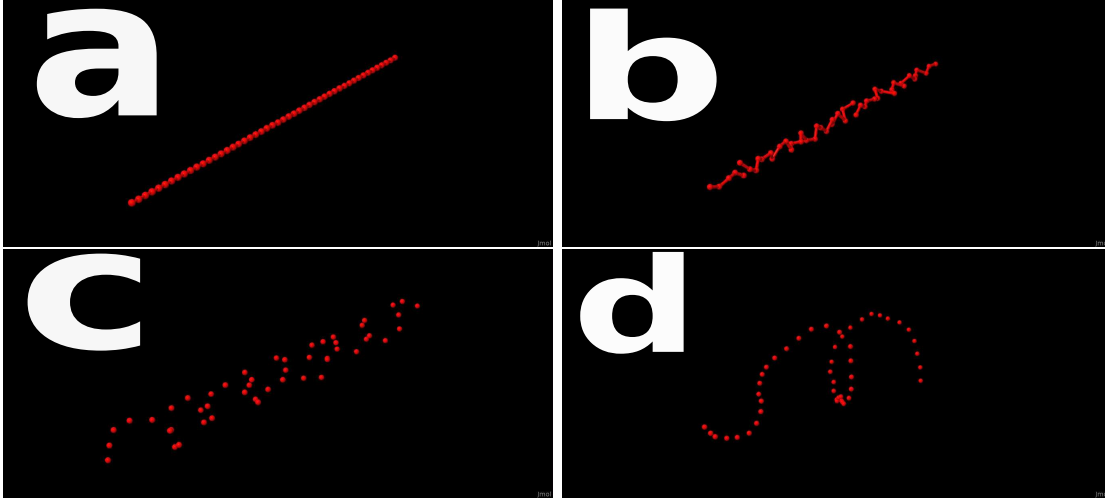


Figure 3.2: Figures 1(a-d) shows various stages of the helical instability for a semi-flexible polymeric chain starting from the initial condition for case A with potential u_c acting between monomer pairs. The snapshots are $t = 0$ (b) snapshot of configuration at time $t = 3.3 \times 10^{-2}\tau$ (c) Snapshot of the subsequent helix formed using 49 monomers at time $t = \tau$ and (d) unwinding of the helix formed by 49 monomers at time $t = 5\tau$.

$\epsilon_b = 10k_B T$ will have persistence length $\ell_p \approx 10a$ as per the above equation. This matches with the earlier mentioned value of $\ell_p = 11a$, where we explicitly calculated the $\ell_p = 11a$ from the decay of the correlation function of the end-to-end vector for a semi-flexible polymer (with u_c kept fixed at 0). At higher values of ϵ_b , the Eqn.2 will be more accurate.

3.3 Results and Analysis:

In Fig.3.1 we show representative snapshots from various stages of transient helix formation for a polymer with interaction energies corresponding to case-B starting out from a straight initial condition. As the bead-spring model of polymer chain starts

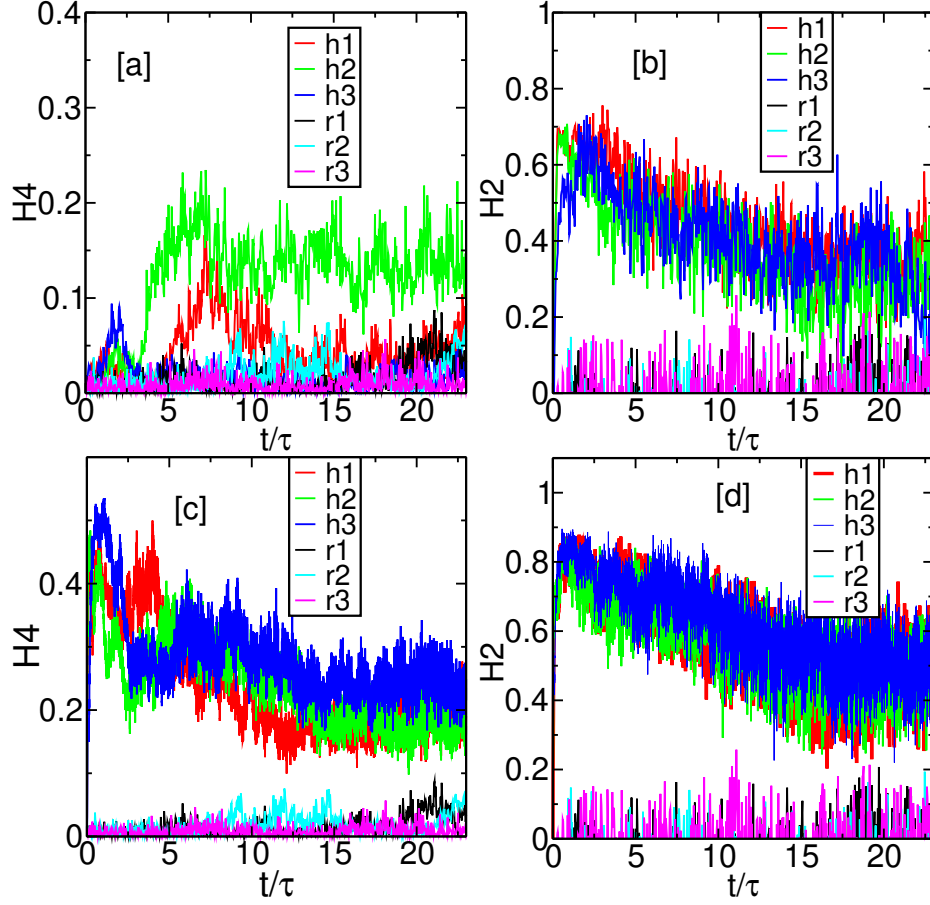


Figure 3.3: Subfigures (a),(b) shows $H4$ and $H2$ versus time t of a semi-flexible polymer chain of 49 monomers for three independent runs denoted by $h1, h2$ and $h3$ starting out from a straight initial configuration of the chain. Potential u_c acts between all monomers pairs. The interaction strengths correspond to Case A. For comparison, we also show $H4, H2$ values obtained for a semi-flexible polymer chain of 49 monomers with $u_c = 0$ starting from the same initial condition; these are denoted by $r1, r2$ and $r3$. Subplots (c),(d) shows $H4$ and $H2$ versus time of the semi-flexible polymer chain of 49 monomers for three independent runs denoted by $h1, h2$ and $h3$, such that potential u_d acts between all monomer pairs; the interaction energies correspond to case B. The initial configuration is a straight chain along y axis. The three independent runs for a chain of same length with $u_d = 0$ are denoted by $r1, r2$ and $r3$. Note that $H2$ is equal to average of the values of $\cos(\phi_i)$; ϕ_i are the torsion angles subtended along the chain contour. In each figure, data for $H2, H4$ is plotted every 1000 iterations, i.e. every 0.1τ .

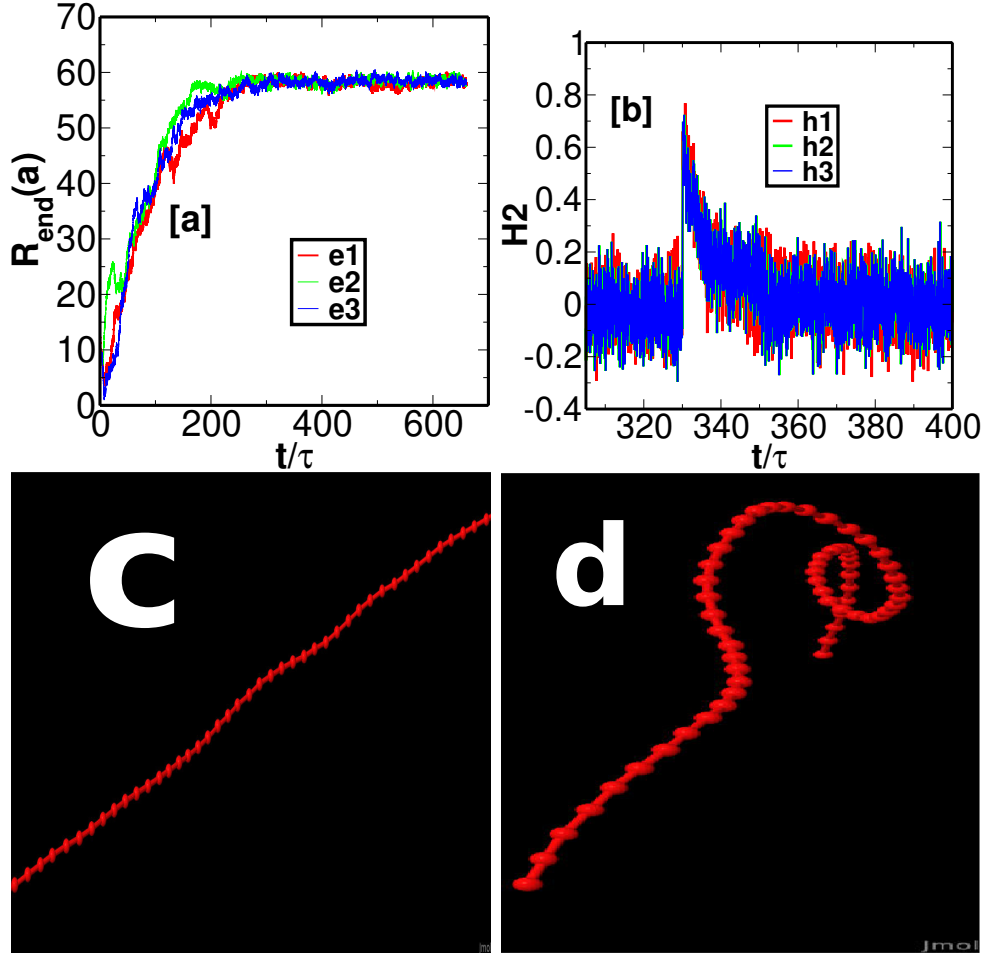


Figure 3.4: Subfigure (a) shows the end to end distance R_{end} versus time for an uncharged, semiflexible polymer chain of 60 monomers having $\epsilon_b = 400k_B T$ ($\ell_p = 400a$) and $\kappa = 200k_B T/a^2$ for three independent runs $e1, e2$ and $e3$. Initially the monomers were placed randomly, and we conclude that R_{end} takes about 200τ to reach its equilibrium value. Subfigure (b) shows $H2$ versus time for the 60 monomer polymer chain such that the repulsive Coulomb interaction ($\epsilon_c = 87.27k_B T$) is switched on at 333.33τ . This led to a increase in the value of $H2$ which later decreases as the helical order dissolves away. The data for three independent runs are labelled as $h1, h2$ and $h3$. Subfigure (c) shows the snapshot of the polymer configuration just before helix formation ($H2 = -0.09, H4 = 0.008$). Subfigure (d) shows the snapshot of the conformation of the polymer which has helical order ($H2 = 0.65, H4 = 0.09$)

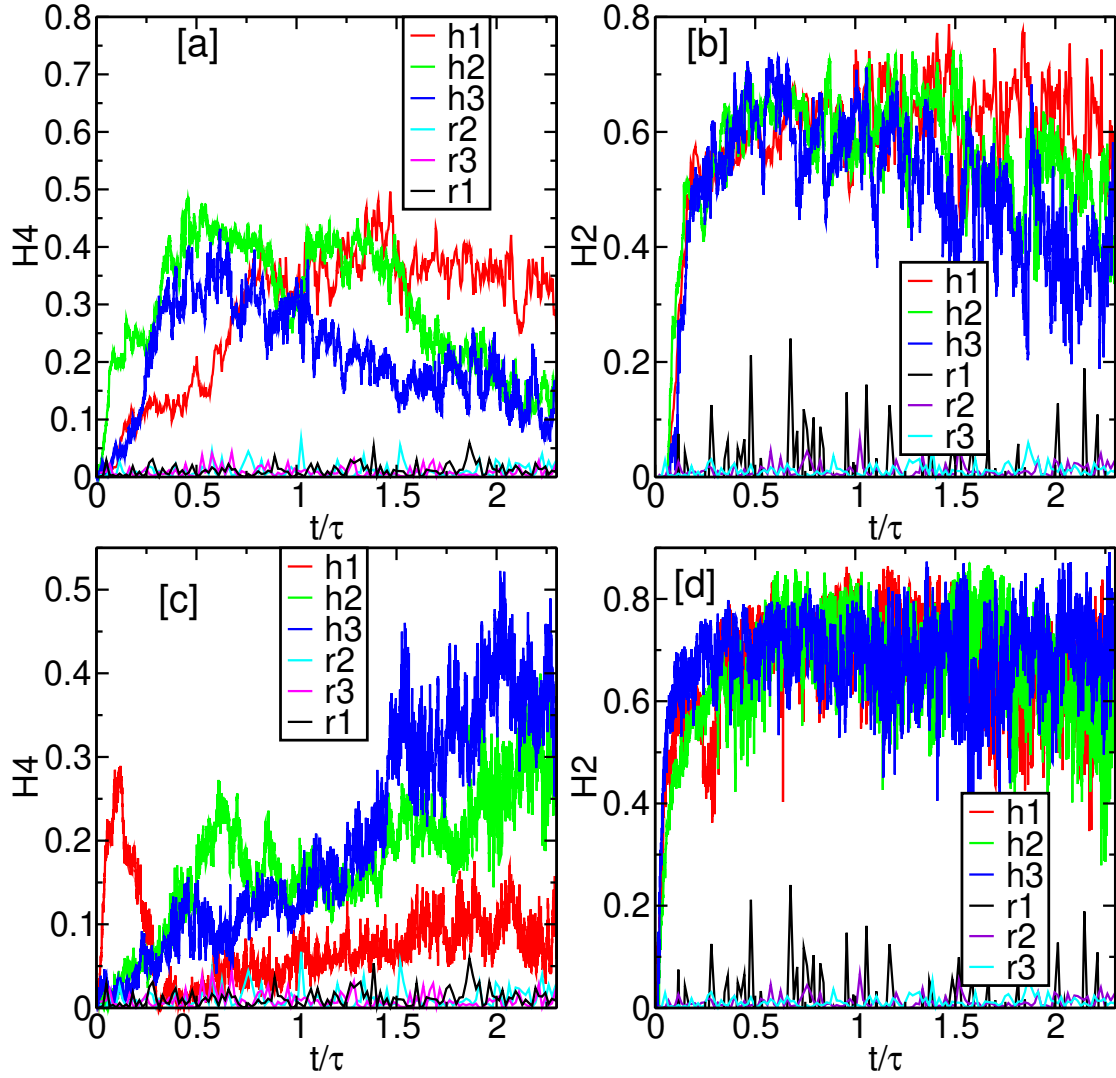


Figure 3.5: Subplots (a) and (b) shows the evolution of order parameter $H4$ and $H2$ versus time for a semi-flexible polymer chain of 25 monomers with forces due to potential u_c acting between all monomer pairs. Subplots (c) and (d) shows the evolution of $H4$ and $H2$ versus time for a semiflexible polymer chain of 25 monomers with forces due to potential u_d acting between all monomer pairs. The values of the parameters are the same as that of Fig.3.3.

out from the straight line initial configuration (refer Fig.3.1a), the thermal forces randomly displace the monomers from their initial positions. Furthermore, strong repulsive forces arising from u_d act along the line joining the centers of monomers make the monomers move away from each other, accentuating the angle between adjacent bonds and consequently the polymer forms a locally kinked structure as shown in Fig.3.1b which is penalized by the bending energy term. Thereby, the helical conformation of the polymer emerges at sections of the chain at a time $t \sim \tau$ to locally relax the the high bending energy costs due to kinks as seen in Fig.3.1c. But it dissolves away at times $t \gg \tau$ (refer Fig.3.1d). The unit of time of the problem is chosen as $\tau = (\zeta a^2/k_B T)$, the time taken for a isolated monomer particle to diffuse a distance of a . Similar snapshots for the helix formation process pertaining to case A have been provided in Fig.3.2.

Random fluctuations due to $k_B T$ displace the monomers just after time $t = 0$, which in turn leads to the development of the helical order, and thereby $k_B T$ plays a crucial role though ϵ_c, ϵ_d and κ, ϵ_b are all $\gg k_B T$. A perfectly straight polymer configuration at $T = 0$ stretches out but never gets to form helices as all the forces between monomers act along the line joining the centers.

We quantify the emergence of helicity as a function of time by calculating and plotting two quantities in Fig.3.3(a,b) and Fig.3.3(c,d) for cases A and B, respectively, viz., the global order parameter $H4$ and the local order parameter $H2$ where,

$$H4 = \frac{1}{N-2} \left(\sum_{i=2}^{i=N-1} \mathbf{u}_i \right)^2; H2 = \frac{1}{N-3} \left(\sum_{i=2}^{i=N-2} \mathbf{u}_i \cdot \mathbf{u}_{i+1} \right) \quad (3.3)$$

where $\mathbf{u}_i$ is the unit vector of $\mathbf{U}_i = \mathbf{r}_i \times \mathbf{r}_{i+1}$. A compact tightly wound perfect helix in the continuum picture with infinitesimal $\mathbf{r}_i, \mathbf{r}_{i+1}$ vectors will have vectors $\mathbf{u}_i$

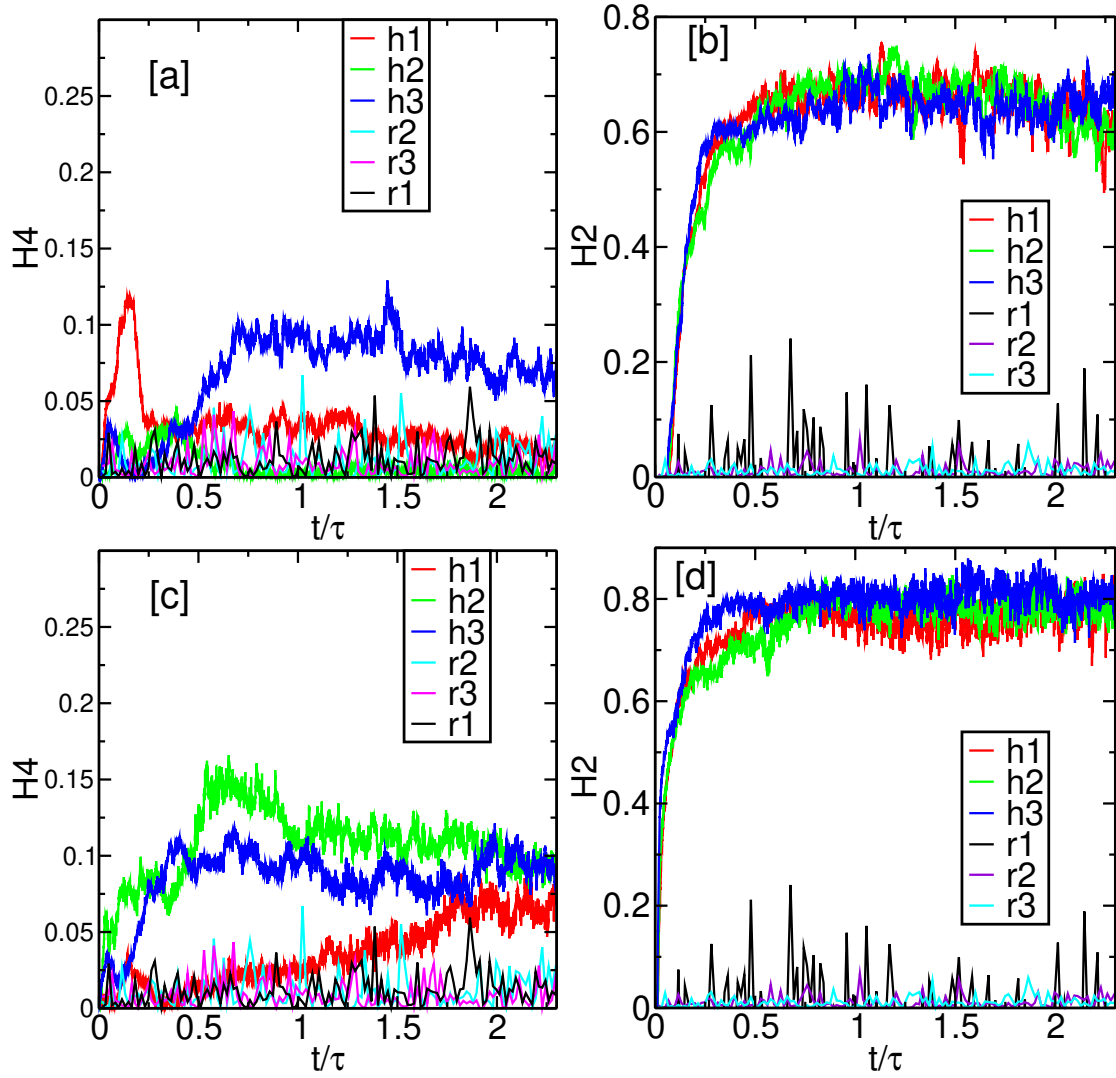


Figure 3.6: Subplots (a) and (b) shows the evolution of order parameter $H4$ and $H2$ versus time for a polymer chain for a semi-flexible polymer chain of 100 monomers, interacting with potential u_c acting between all monomer pairs. Subplots (c) and (d) shows the evolution of order parameter $H4$ and $H2$ versus time for a semiflexible polymer chain of 100 monomers, interacting with potential u_d acting between all monomer pairs. The values of the parameters are the same as that of Fig.3.3.

pointing along the helix axis, and hence $H4$ will have a value of ≈ 1 . However, if one obtains a helical structure where half of the chain is right handed, and the rest of it is left-handed, $H4$ will be zero. Hence, we need the other parameter $H2$ to identify local helical order [51, 81]. A simple semi-flexible polymer chain ($u_c = 0$ & $u_d = 0$) shows $H2, H4$ values ≈ 0 (or negative values of $H2$) as expected for a chain locally bent due to thermal fluctuations. But the polymer chains with additional interactions u_c or u_d lead to the formation of transient helices with distinctly non-zero positive values of $H2, H4$. The time taken for the helix to form is $\approx 0.5\tau$. Note that $H2$ is equal to $\langle \cos(\phi) \rangle$ where ϕ denotes the torsion angle, i.e. the angle between the planes formed by adjacent pair of the monomer-triplets along the length of the chain; the average is taken over the cosine of the various torsion angles formed along the length of the chain.

Furthermore, to establish that the helix formation is not just a consequence of the special straight line initial condition, we calculate $H2$ to establish the development of helical order in a semi-flexible polymer in thermal equilibrium. The Coulomb potential u_c and corresponding forces between monomer is switched on after ensuring that the polymer is in equilibrium. We choose a polymer whose persistence length greater than the contour length, place the monomers randomly and allow the polymer to relax and reach equilibrium such that the end to end vector fluctuates about an average value. Initially the end-to-end distance R_{end} increases as the bent polymer straightens itself. In Fig.3.4a we show R_{end} versus time t for the semiflexible polymer chain with 60 monomers, such that its length is $60a$ and $\ell_p = 400a$. Data is shown for three independent runs, e1, e2 and e3. We observe that it takes approximately 200τ for it to reach the equilibrium value, refer. For Fig.3.4b we again take the same semiflexible polymer with 60 monomers and switch on the repulsive Coulomb

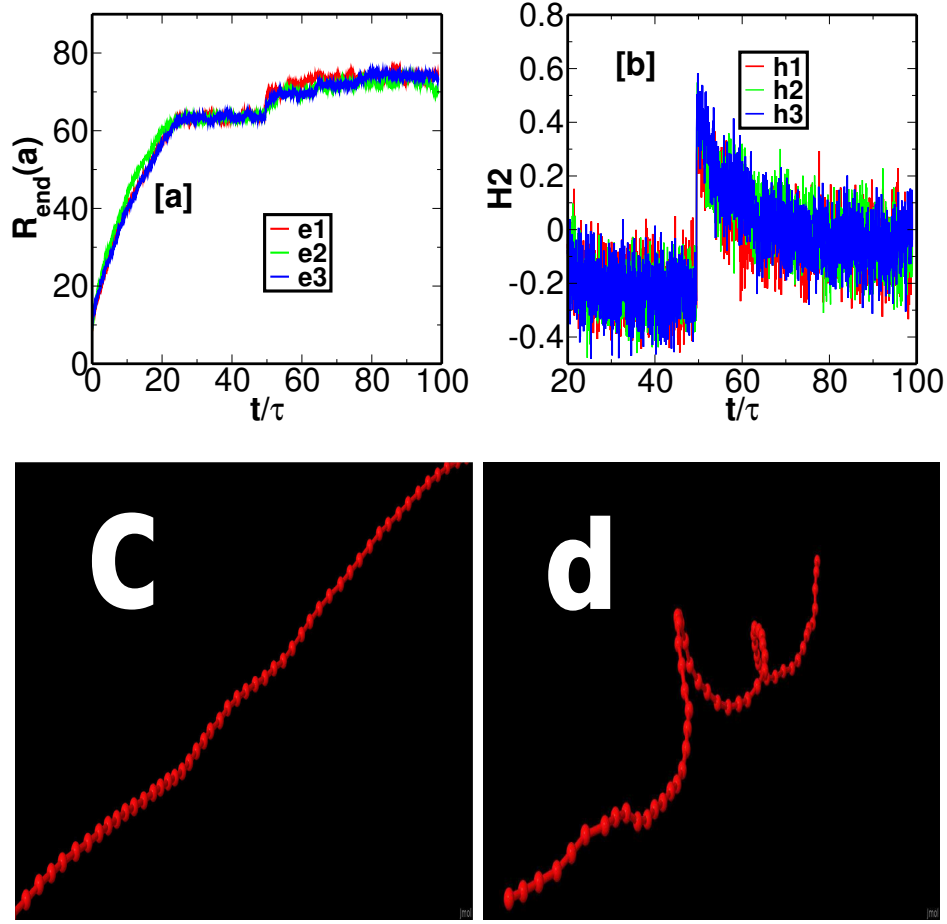


Figure 3.7: Subfigure (a) shows the end to end distance R_{end} versus time t for a polymer chain of 60 monomers, $\epsilon_b = 30k_B T$ ($\ell \approx 30a$) and $\kappa = 200k_B T/a^2$, where the end monomers are pulled outwards by the application of a constant force of $20k_B T/a$ in opposite directions. data is presented for 3 independent runs $e1, e2$ and $e3$. Note that the Coulomb repulsion ($\epsilon_c = 87.27k_B T$) between monomers was switched on at 50τ and the stretching force was set to 0, simultaneously. Subfigure (b) shows $H2$ versus time for the polymer chains having the same parameters values in (a). We once again see transient helix formation and its dissolution for three independent runs, $h1, h2$ and $h3$. Subfigure (c) shows the snapshot of the polymer configuration just before the Coulomb interaction is switched on ($H2 = -0.26, H4 = 0.012$). Subfigure (d) shows the snapshot of the helical conformation of the polymer ($H2 = 0.58, H4 = 0.10$)

interaction (u_c with $\epsilon_c = 87.27k_B T$) between the monomers at 333.33τ . We observe that there is a significant increase in the value of $H2$ immediately after $t = 333.33\tau$. The $H2$ value then gradually decreases, indicating that a transient helical structure dissolves away. Fig 3.4c and Fig3.4d show the snapshots of the helical conformation just before and after u_c was switched on.

We also investigated the emergence of helices in chains of $N = 25$ and $N = 100$ monomers, respectively (refer Fig.3.5 and Fig.3.6). A chain of 100 monomers has approximately 5 helical segments and thereby has relatively lower values of $H4$ in some of the independent runs, since different segments can form helices of opposing handedness. But the values of $H2$ obtained for $N = 25$ or $N = 100$ are comparable to that obtained for the $N = 49$ polymer chain at time $t = 2\tau$. Thus we establish that we indeed get helical conformations in our model semi-flexible polymer as long as we have long-ranged repulsive interactions between the monomers.

Suppose we have a polymer chain of length $60a$ with $\ell_p = 30a$ in thermal equilibrium, such that the persistence length is lower than the contour length. If we switch on u_c , we do not get any distinct helical order. However, if we stretch the polymer (say by a AFM-atomic force microscopy tip) and then switch on u_c , we again see emergence of a transient helical order. A semi-flexible chain can be stretched by applying a suitable value of a constant force ($\vec{F}_\pm = \pm(20k_B T/a) \hat{y}$) at both ends such that its end to end distance R_{end} becomes $\approx 60a$. We then allow the chain to explore different equilibrium conformations in the presence of the fixed stretching force acting on the end monomers.

Then the tension is released by switching off the force applied to the end monomers and simultaneously the repulsive Coulomb interaction (u_c with $\epsilon_c = 87.27k_B T$) is

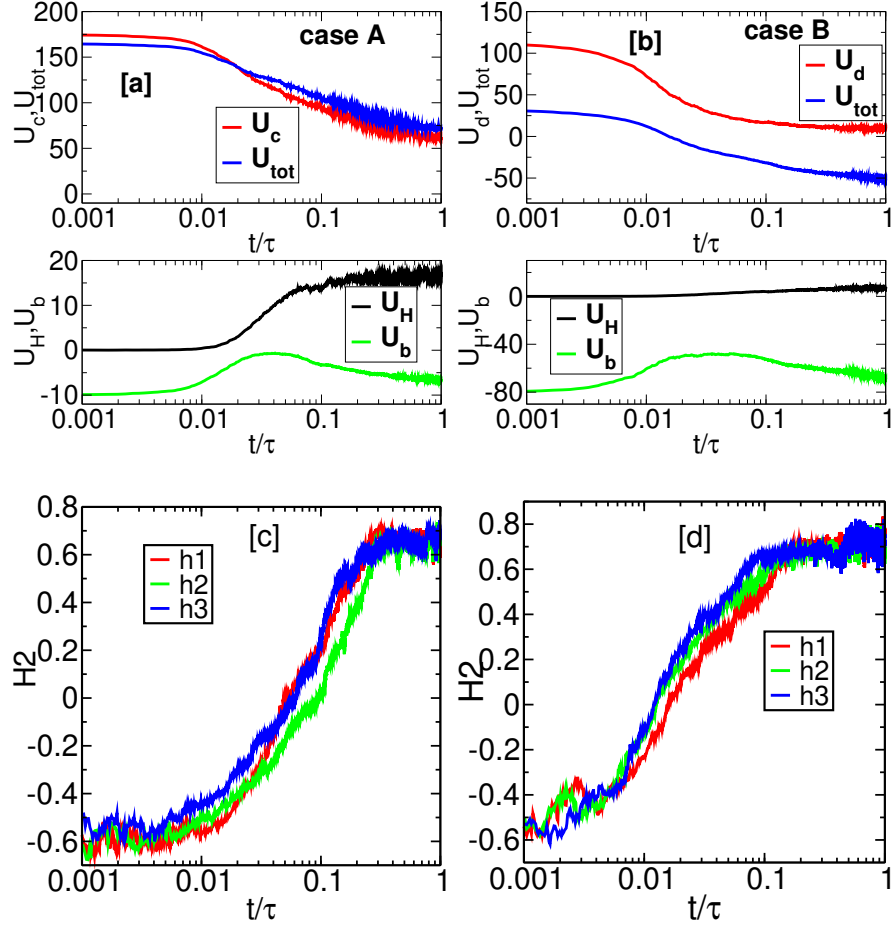


Figure 3.8: Subplots (a) and (b), corresponding to case A and case B, shows energies U_H , U_b , U_{tot} and U_c or U_d , respectively, where U_{tot} denotes the total energy per monomer and is the sum of U_H , U_b and U_c or U_d . The x-axis shows t/τ for relatively short times, i.e. $t < \tau$. Subfigures (c) and (d) show $H2 = \langle \cos \phi_i \rangle$ versus t/τ for three independent runs $h1, h2$ and $h3$ with u_c and u_d acting between the monomers, respectively. The angle ϕ_i also denotes the dihedral angle between the two planes formed by the monomers $(i, i+1, i+2)$ and $(i+1, i+2, i+3)$, respectively. The index i represents any monomer along the chain. The cosine of the angle ϕ_i was averaged along the length of the chain for all possible values of i to yield $H2 = \langle \cos \phi_i \rangle$ at time t .

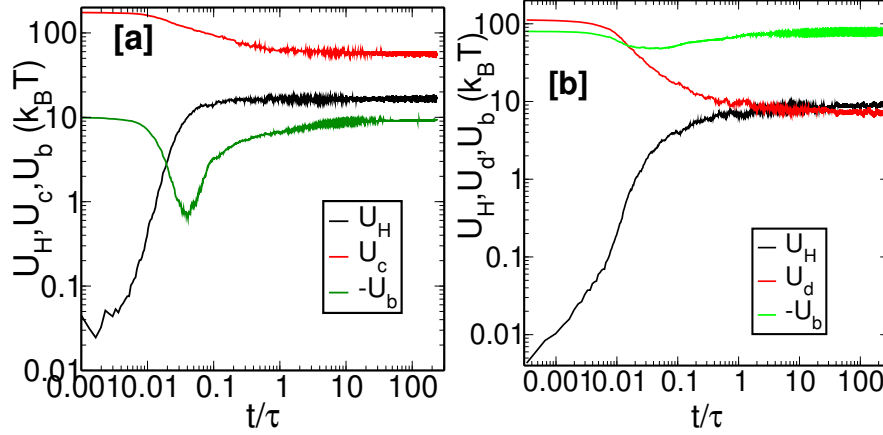


Figure 3.9: Subplots (a) and (b), corresponding to case A and case B, shows energies U_H , U_B and U_c and U_d , respectively. The x-axis shows t/τ . The values have been plotted in a log-log plot.

switched on between the monomers. In such a *in-silico* experiment, we do see the emergence of helical order by the measurement of $H2$. The relevant data is shown in Fig.3.7. In Fig.3.7a we show the evolution of R_{end} under the application of equal and opposite forces acting on the end monomers of the chain. The mean R_{end} reaches a mean value greater than the contour length in three independent runs within time 20τ . At 50τ there is an increase in the end to end distance because at this point the stretching force is released and the repulsive Coulomb interaction (with $\epsilon_c = 87.27k_B T$) is switched on. In Fig.3.7b we observe that there is a corresponding significant increase in the value of $H2$ which gradually decreases indicating that a transient helical structure was formed, which dissolves away. Fig.3.7c and Fig.3.7d show the snapshots of the helical conformation just before and soon after the repulsive Coulomb interaction was switched on between the monomers.

But what is the physics of helix formation in the semi-flexible polymer chains in the presence of spherically symmetric repulsive potentials u_c or u_d ? What role does

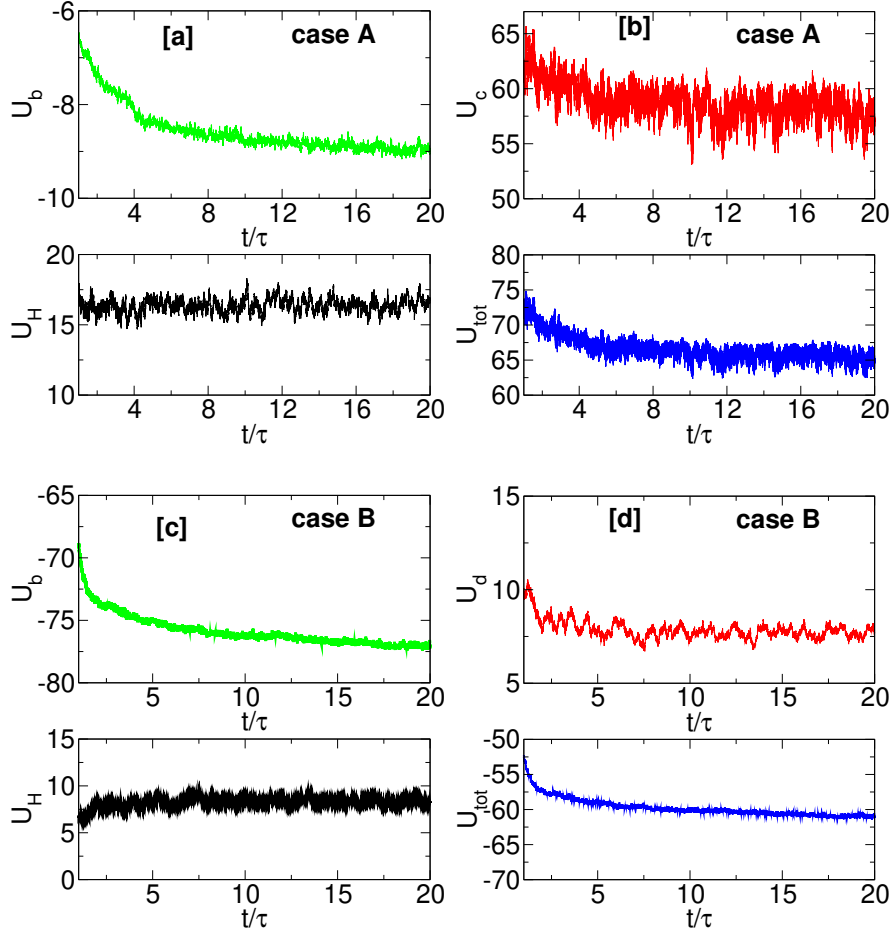


Figure 3.10: Subplots (a), (b), (c) and (d) show the same quantities as in Fig.3.8 (a,b) but over longer times $t > \tau$ and the x-axis is shown in linear scale. The parameters are the same as mentioned previously in Fig.3.8. Subplot (a) shows the values of spring energy U_H per monomer, and the bending energy U_b , per bend versus time t/τ corresponding to case-A. Subplot (b) shows the values of repulsive potential energy U_c , and the total energy U_{tot} , per monomer versus time corresponding to Case A. Subplot (c) shows the values of spring energy U_H , and the bending energy U_b , per monomer versus time corresponding to Case B. Subplot (d) shows the values of repulsive potential energy U_d , and the total energy U_{tot} , per monomer versus time corresponding to case-B.

temperature play? For the remainder of the manuscript, for simplicity, we report the dynamics of a polymer chain in a thermal bath starting out from a straight initial linear conformation.

To develop a detailed understanding of the mechanism of helix formation we note that just after time $t = 0$ the thermal kicks displace the monomers from a straight line initial condition. Thereafter, the magnitude of this random displacements gets accentuated by the repulsive u_c (or u_d) acting along the line joining the monomer centers, accompanied by an increase in the distances between monomers. This results in the lowering of the Coulomb energy per particle U_c (or U_d). However, sharp local kinks get created as is seen in Fig.3.1b and also results in increase of the contour length of the polymer. To release the bending energy due to sharp kinks, the kinked structure evolves to a structure with local helicity at different segments of the chain. We follow the values of the various contributions to the total energy U_{tot} as a function of time in Fig.3.8 to understand the development of structure of the polymer. Increase in the bond energy per spring, U_H with time t/τ indicates a corresponding stretching of bonds between adjacent monomers: we have independently checked that the bonds stretch and do not get compressed. Similarly an increase in the value of semi-flexible energy per triplet of monomers, U_b , would be indicative of sharp bends along the contour of the polymer chain.

We now discuss this in more detail. Just after time $t = 0$ the chain remains nearly straight with the bending energy per each bend nearly equal to $-\epsilon_b \cos \theta = -10k_B T$ since $\cos \theta \approx -1$ (case A). However, for time $t/\tau < 0.003$, the U_H increases slowly from value 0 due to random shifts in the monomer positions because of thermal fluctuations. But this increase is not discernible in the plots of the energy contributions versus time in Fig.3.8, but can be seen in the log-log plot of energy versus time given

in Fig.3.9.

Thereafter, formation of sharp bends/kinks resulting from the motion of monomers due to repulsive Coulomb forces (or from u_d) leads to the rapid increase of both U_H and U_b which is seen in 3.8(a,b) at times $t/\tau > 0.01$. This is accompanied by a decrease in the Coulomb energy per monomer U_c (and U_d), again refer Figs.3.8(a,b).

Following the rapid increase in U_b from time $0.01 < t/\tau < 0.03$, there is a sharp increase in forces trying to straighten the chain. The monomers still move apart from each other due to Coulomb repulsion, but simultaneously try to decrease the bending energy costs by radially spreading out the monomers locally in a manner such that the change in the bending angles along the chain contour becomes gradual. This dynamics can be deduced by observing the decrease of U_b after it reaches its peak at time $t/\tau \approx 0.03$. As a consequence of the local radial spreading out of the monomers, the chain develops helicity along the length of the chain, refer Figs.3.8(c,d). Note that the motion of a segment would also be constrained by the motion of adjacent segments along the chain. Thus, different segments of the chain could thus develop clock wise or anti-clock wise helicity since the initial deviations from the straight line configuration were in random directions due to thermal fluctuations.

The evolution of a straight chain into helical structures can also be followed by looking at the average (along the length of the contour) of the average of the cosine of the torsion angles along the length of the chain (as given by $H2$), as a function of time. This is plotted in Fig.3.8(c) and (d) for cases A and B, respectively. At time $t = 0$, when we have a straight chain, a plane between monomer triplets is undefined and so is the normal to the plane. But as soon as the monomers move due to thermal energy, planes can be defined using the positions of adjacent monomer-

triads and outward normals $\mathbf{u}_i$ to these planes can point in any direction but mostly normal to the $y = 0$ plane. At slightly longer times (i.e. when the sharp kinks get formed), since all values of $\cos(\phi)$ are possible, the average of $\cos(\phi)$ along the chain quickly goes to zero with time t for all the three independent runs. However, as the chain develops helical order beyond time $t/\tau > 0.05$, $\langle \cos(\phi) \rangle$ reaches a values in the range $0.4 - 0.6$, corresponding to an angle of around $\sim 60^\circ$.

At times beyond $t/\tau > 1$, i.e. after the the helix has already been formed, the value of u_H for the stretched springs starts fluctuating around an average value. However, uniform relatively uniform bends of a helical configuration are penalized by u_b and hence at times $t > 2\tau$ the uniform helical structures start to gradually locally unwind leading to a gradual increase in the pitch (data given later) of the helical structure. This can be understood by looking at the evolution of energies U_c, U_b and U_H with time in Fig.3.10(a,b) (case A) and in Fig.3.10(c,d) (case B). We also show U_{tot} which is the sum of U_c, U_b and U_H . There is a slight decrease in U_c or U_d with time and the values of the bending energy U_b also show a steady but slow decrease with time.

The next figure, Fig.3.10 shows the long time behaviour of the $U_c, U_b (U_d), U_H$ and U_{tot} as the helical conformations dissolve. From Fig.3.10b and Fig.3.10d it is evident that there is a crucial difference between Case A and Case B which arises from the difference in the rate of fall of the potential with increasing r . For Case A, U_c shows a decrease of about $\sim 8k_B T$ with time, whereas U_d shows a decrease of about $\sim 2.5k_B T$ over 20τ . Consequently the total energy per monomer, U_{tot} for the two cases also show a larger decrease for case A, as seen in Fig.3.10a and Fig.3.10c. This implies that the polymer chain in case A has a higher tendency to unwind and stretch itself out to a relatively more straight configuration due to the repulsive

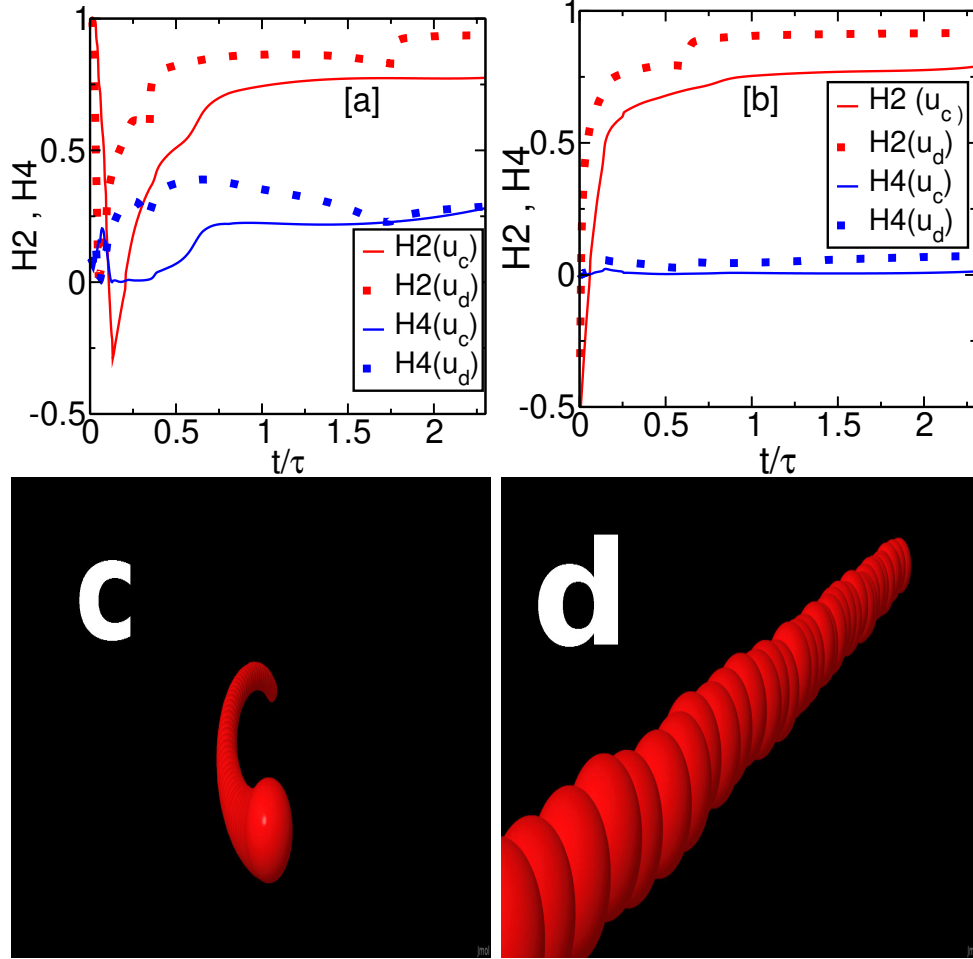


Figure 3.11: Subplot (a) shows $H4$ and $H2$ versus time a for polymer chain corresponding to u_c and u_d , respectively, at $k_B T = 0$ starting from a curved initial conformation. Subplot (b) shows $H4$ and $H2$ versus time for a polymer chain corresponding to u_c and u_d acting between the monomer pairs, respectively at $k_B T = 0$ starting from a initial condition in which the polymer aligned with $\hat{y}$ has small random displacements along $\hat{x}$ and $\hat{z}$. The starting configurations for (a) and (b) are given in subfigures (c) and (d), respectively.

forces of u_c as compared to polymer with potential u_d of case B. This is in spite of the value of ϵ_b , which is much higher for case B.

This is consistent with the data of $H2$ relaxation with time shown in Fig.3.3(b,d) and explains why $H2$ for case B shows relatively higher values as compared to $H2$ of case A at times $t > 1$.

Thermal fluctuations provide the initial random forces which leads to the slight displacement of the monomers away from its initial straight line configuration which makes the linear configuration unstable. At temperature $T = 0$, the polymer starting from a initial straight configuration along $\hat{y}$, stretches out to reach its minimum energy configuration in presence of U_c but never forms helices as forces arising from u_c (or u_d) and u_H act along the line joining the centres of the monomers. At temperature $T > 0$ and for time $t/\tau > 0$, the monomers move away from the straight line configuration, which leads to force components along the $\hat{x}$ and the $\hat{z}$ directions from u_c and u_b , and results in the emergence of helical conformations when $U_c \neq 0$ (or $U_d \neq 0$). To establish these conclusions, we ran a simulations to calculate $H2$ and $H4$ at temperature $T = 0$, however, starting from a uniformly curved initial condition such that the chain forms an arc in $x - y - z$ plane (refer Fig 3.11c). Such an initial conformation again leads to helical instabilities due to forces along the x axis and the z axes and therefore results in helices (as seen in data of Fig.3.11a). Alternatively, starting from an initial configuration of a relatively straight polymer chain along the y - axis but with small random displacements of all monomers along x and z coordinates maintaining temperature $k_B T = 0$ (refer Fig. 3.11d), we still obtain a helical conformation of the polymer as seen in the data of Fig.3.11. Details of the initial conditions are described in the following paragraph.

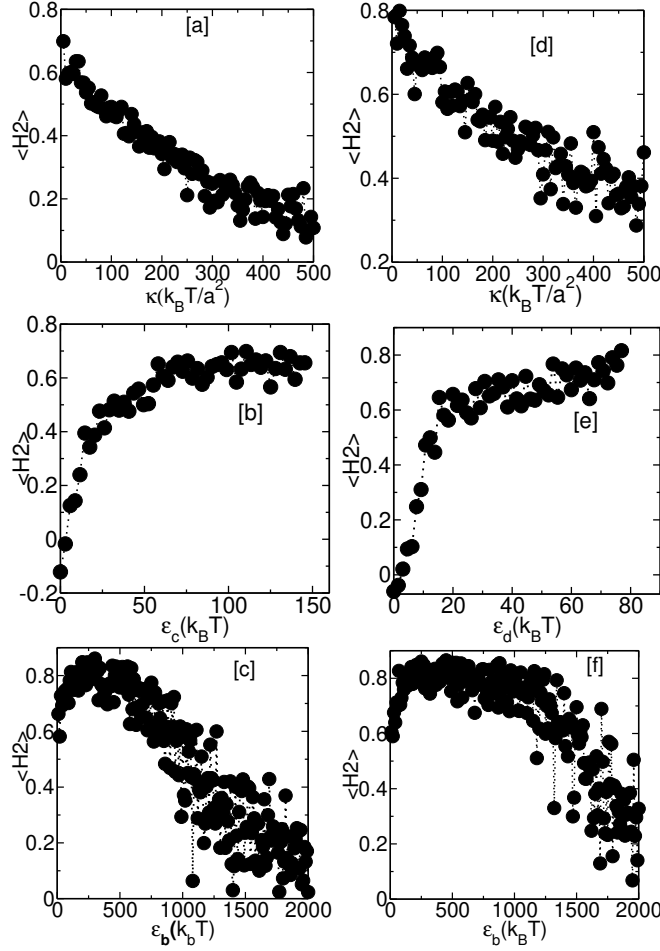


Figure 3.12: Semiflexible polymer with $\kappa = 20k_B T/a^2$, $\epsilon_c = 87.27k_B T$ & $\epsilon_b = 10k_B T$ corresponding to case-A : (a),(b),(c) show change in time averaged value of $\langle H2 \rangle$ with increase of κ , ϵ_c ϵ_b , respectively. We change one parameter at a time keeping the other two parameters fixed. Semiflexible polymer with $\kappa = 10k_B T/(a)^2$, $\epsilon_d = 107.70k_B T$ & $\epsilon_b = 80k_B T$ corresponding to case-B: (d),(e),(f) show change in time averaged value of $\langle H2 \rangle$ with increase of κ , ϵ_d ϵ_b , respectively, keeping two other parameters fixed. The time averaged $\langle H2 \rangle$ was calculated over 0.66τ , starting from $t = 0.66\tau$ to $t = 1.32\tau$.

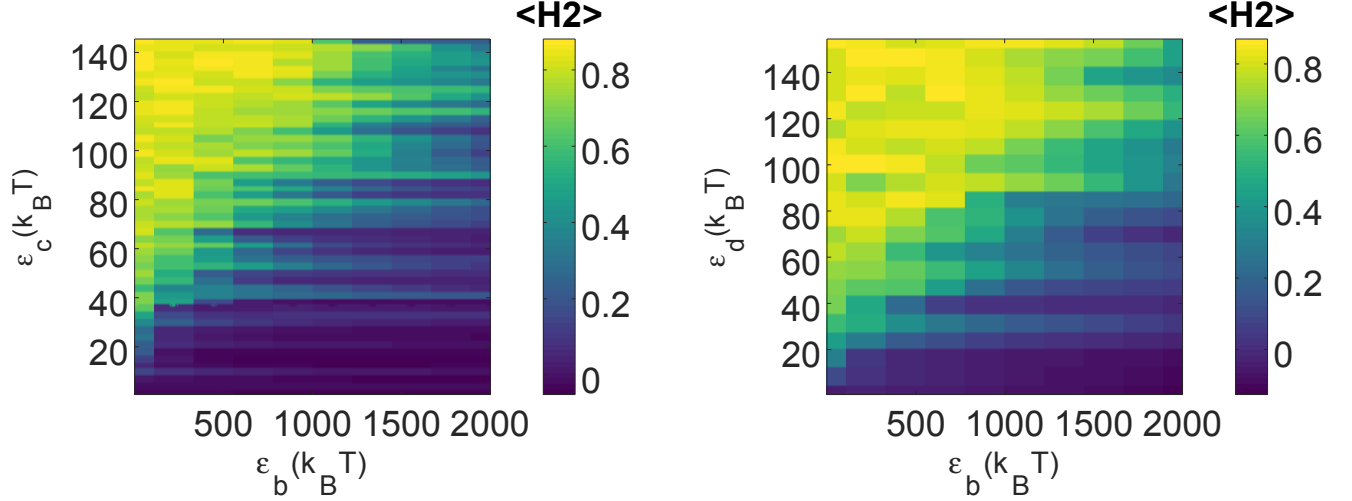


Figure 3.13: Left colormap: At $\kappa = 20k_B T/a^2$, the state diagram shows the range of ϵ_b and ϵ_c for which one obtains helices. Right colormap: At $\kappa = 10k_B T/a^2$, the state diagram shows the range of ϵ_b and ϵ_d for which one obtains helices. The average $\langle H2 \rangle$ was calculated from 0.66τ , to 1.32τ . This is because we expect the helix to have been formed by 0.66τ . When $H2 < 0.2$, one can hardly distinguish between a helical polymer and a semiflexible polymer with bends due to thermal fluctuations. The persistence length holds a linear relation with ϵ_b such $\ell_p = a\epsilon_b/k_B T$, and the charge q per unit length a is $q/a = \sqrt{4\pi\epsilon * \epsilon_c/a}$. The dielectric constant of the medium is ϵ .

Thus the role of temperature is to introduce deviations from the straight linear conformation, and this triggers the helical instability. Since the local helical instabilities are triggered by random fluctuations at finite $k_B T$, we do not have any control on the handedness of the chain at different segments of the chain. Furthermore, if a single or a couple of monomers are slightly displaced from a straight line configuration at $T = 0$, then the semi-flexibility drives the chain to become straight and it then stretches out along a straight line to reach its energy minimum configuration. Thereby it does not form a helix provided the magnitude of the displacement of the monomers from the straight linear conformation of the chain is lesser than a certain

value.

We emphasize that the helical configuration at $T > 0$ is not a energy minimum state but a configuration that the polymer accesses in its kinetic pathway to its (free) energy minimum state which is a stretched straight configuration(s) of monomers with local bends depending on the relative strengths of u_b and $k_B T$.

At non-zero $k_B T$, if the soft spring ($\kappa = 10k_B T/a^2$ and $\kappa = 20k_B T/a^2$ for cases A and B, respectively) joining the monomers becomes too stiff then the the position of monomers do not time-evolve to form a helix in response to forces arising from u_c and u_d . For high values of κ , stiff springs do not permit monomers to radially stretch out locally, thus preventing helix formation. We refer the reader to Figs.3.12 (a,d) which shows the decreasing values of the $\langle H2 \rangle$ order parameter with increasing values of κ . The angular brackets in $\langle H2 \rangle$ denote the average value of $H2$ calculated using data collected between 0.66τ to 1.32τ . On the other hand, increase in the value of ϵ_c in u_c (or ϵ_d in u_d) increases propensity of helix formation as observed in the increase in the value of $\langle H2 \rangle$ with ϵ_c (or ϵ_d) in Figs.3.12(b,e). For values of $\epsilon_c > 50k_B T$ and $\epsilon_d > 20k_B T$, $\langle H2 \rangle$ nearly saturates to values of 0.75. There is no helix formation when $u_c, u_d = 0$.

High values of ϵ_b in the expression for u_b hinder the formation of sharp kinks which subsequently stretch out radially to give rise to the helical structures, thereby, suppresses the instability: refer Figs.3.12(c,f) corresponding to cases with potentials u_c, u_d . We get finite values of $H2$ even when $\epsilon_b = 0$, as a charged polymer chain with the same sign of charge on the monomers behaves like a semi-flexible chain [82, 83, 84, 85] . Hence, an increase in the values of ϵ_b from zero leads to an initial increase of $\langle H2 \rangle$ as increased bending energy costs help in radially spreading out

the polymer as it leads to reduction of bending energy. Thereby, $\langle H2 \rangle$ reaches a peak value of 0.9 at intermediate ϵ_b values. But thereafter, $\langle H2 \rangle$ starts decreasing with further increase of ϵ_b as reasoned earlier.

Thus only in a certain range of these interactions of u_c (or u_d) and u_b do we obtain well formed helices. This is further illustrated by the two state diagrams shown in Figs.3.13 which map out the average values of $H2$ for various combinations of the values of ϵ_c (or ϵ_d) and ϵ_b . To obtain the colormaps shown in Figs.3.13(left) and 3.13(right), κ was fixed at the same values as given previously corresponding to case A and case B. The colormaps in Fig.3.13- indicate that for higher values of ϵ_c (or ϵ_d), helices can be obtained for relatively higher values of ϵ_b because the helix formation depends on the relative strengths of u_c (or u_d) and u_b .

A polymer with relatively very high values of ϵ_b is unable to form helices as formation of sharp kinks will be prevented by very high bending energies. Kinks, even if formed, will relax to form configurations which are stretched out resulting in lower values of $H2$ and higher values of pitch (as discussed later). The colour map in the subfigure on the right of Fig.3.13 also shows that case B leads to formation of helices even with higher values of ϵ_b as compared to that in Case A. This is because, the polymer with u_c (case A) would cause the polymer contour to stretch out more with relatively larger pitch during helix formation at times $t < \tau$ due to longer range of $1/r$ potential as compared to that of $1/r^3$. This results in lower values of $H2$ when ϵ_c is high as compared to a polymer with u_d potential with similar high values of ϵ_d . Similar arguments were discussed previously when discussing the long time relaxation of the helices in Figs.3.10(b,d). So when comparing helix formation in case A with case B with relatively large values of ϵ_c, ϵ_d (say, with the choice $\epsilon_c = \epsilon_d$), $H2$ values will be lower in case A, as helix formation will be suppressed more in case

A than in case B at identical high values of ϵ_b .

We have already discussed how ϵ_b is related to the persistence length. Similarly, we must express ϵ_c in terms of line charge density of a polymer. We remind the reader that for case-A the Coulomb repulsion between two similarly charged monomers of charge q placed at a distance of a from each other, is equal to ϵ_c . Therefore, $(q^2/4\pi\epsilon a) = \epsilon_c$, where ϵ denotes the dielectric constant. Hence,

$$q/a = \sqrt{4\pi\epsilon * \epsilon_c/a}. \quad (3.4)$$

As a reference, if the distance between monomers is $a = 10nm$, then at $T = 300K$, $\epsilon_c = 87.27k_B T$ corresponds to a charge of $\approx 36e$ on each monomer $10nm$ apart in water. This corresponds to a polymer chain having a charge density lower than the charge density of DNA. Bare DNA has around $22e$ charge in a 3.7 nm segment [86]. So such transient helical configurations can be seen in DNA or polymers with line-charge densities lower than that of the DNA, if they become charged from a neutral configuration.

What determines the pitch of the helix and how can we control it? To calculate the most frequently occurring pitch (at any given instant of time), P of the helical structures obtained in our simulations, we calculate the dot product of the unit vectors $\hat{r}_i$ along the chain with a vector perpendicular to the axis vector. This quantity is denoted as W . This shows periodic behaviour along the chain as is shown by Fig.3.14(a). The fourier transform of 'W', $W(k)$ (re-expressed in terms of the spatial variable $\sim 2\pi/k$ on the x-axis) is shown in Fig.3.14(b). It gives us one or more peaks. The monomer index corresponding to the peak with the highest amplitude denotes the quantity P or the most frequently occurring pitch of the helix

at any given instant of time.

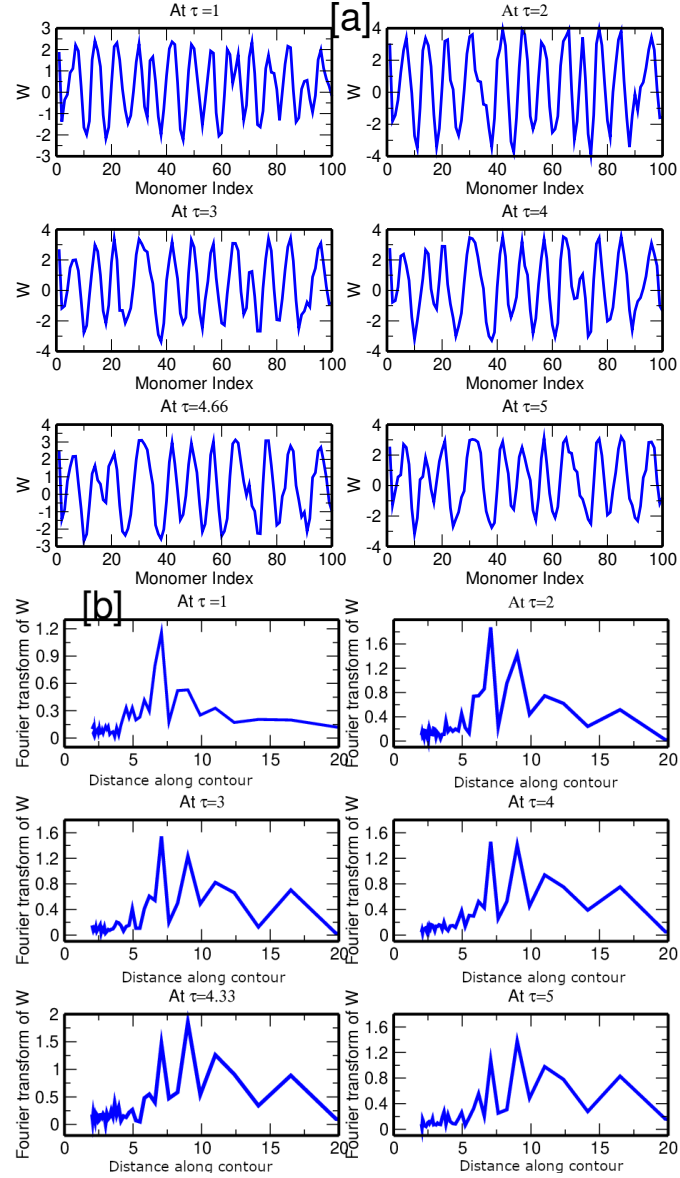


Figure 3.14: Subplot (a) shows the plot of W with respect to the monomer index for given times, corresponding to chain of length 100 with u_c acting between all monomer pairs. Subplot (b) shows the Plot of the Fourier transform of $W(k)$ (re-expressed in terms of the spatial variable $\sim 2\pi/k$ on the x-axis) for given times, corresponding to a chain of length 100 with u_c acting between all monomer pairs.

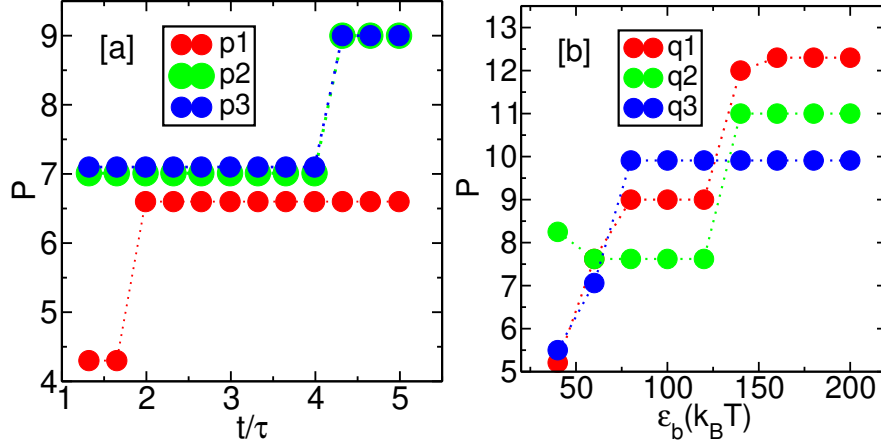


Figure 3.15: (a) Plot of the (most frequently occurring) pitch P versus time for a chain of 100 monomers for $\kappa = 20k_B T/a^2$, $\epsilon_b = 10k_B T$ & $\epsilon_c = 87.27k_B T$ (Case A) for 3 independent runs indicated by $p1$, $p2$ and $p3$. (b) At a fixed time $t = \tau$, we plot the (most frequently occurring) pitch P versus ϵ_b for 3 independent runs $q1$, $q2$ and $q3$. The other parameters correspond to that of Case A. The figures corresponding to Case B is given in Fig.3.16.

Once formed, the pitch of the helix increases with time as the helical structure gradually unwinds over time to decrease bending energy costs. To that end, we show the variation of the (most frequently occurring) pitch P versus time in Fig.3.15a for a chain length of $N = 100$ monomers for a semiflexible polymer with u_c acting between the monomers. A polymer with large N is chosen so that the fourier transform calculation yields more accurate results. The quantity P increases with increasing ϵ_b (refer Fig.3.15b) as a higher value of ϵ_b results in a higher energy cost associated with the local bends along the polymer chain. Thus a higher value of ϵ_b gives rise to fewer loops along the chain, or a higher average value of the pitch. We did not observe any significant dependence of the pitch on κ and ϵ_c (or ϵ_d). The corresponding data for P (versus time and ϵ_b) with u_d acting between all monomer pairs (Case B) are given in Fig.3.16.

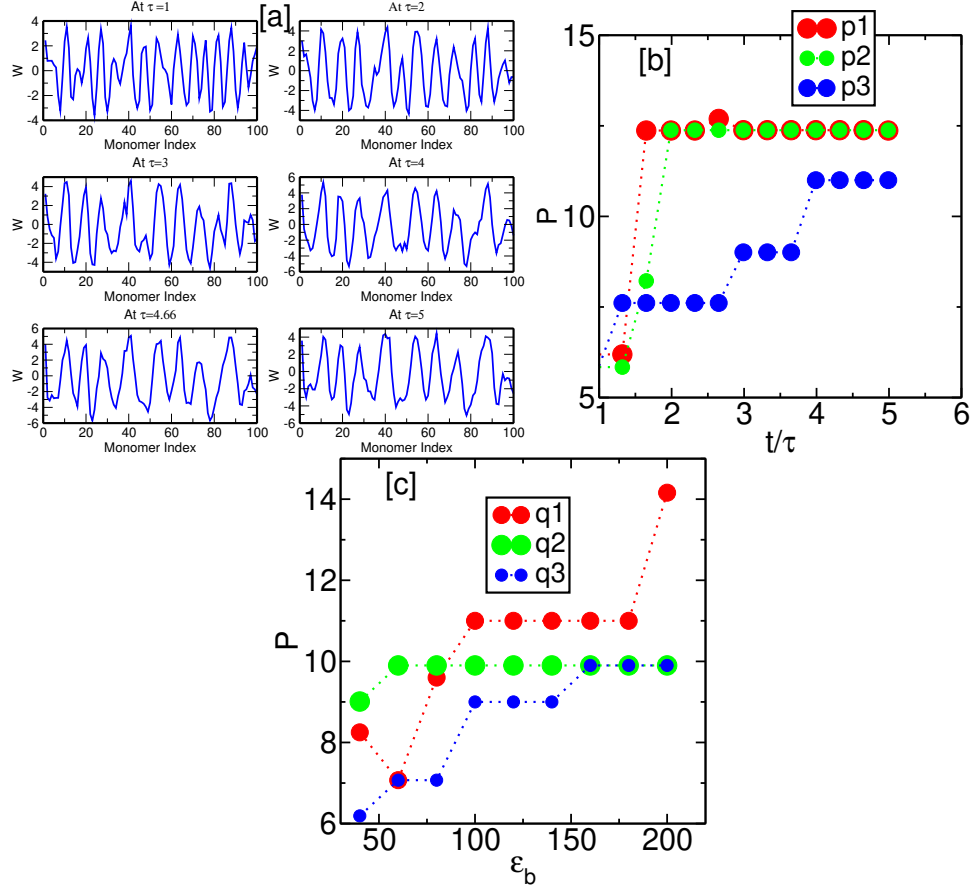


Figure 3.16: Investigating how (the most frequently occurring) P pitch changes over time and for various values of U_b with u_d acting between monomer pairs. (a) Plot of W with respect to the monomer index for given times, corresponding to corresponding with u_d acting between all monomer pairs. (b) Plot of the (most frequently occurring) pitch, P with respect to time for a chain of 100 monomers for 3 independent runs, p1, p2 and p3 keeping other parameters same as before (c) Plot of the (most frequently occurring) pitch with respect to various values of ϵ_b keeping other parameters same as before, for 3 independent runs q1, q2 and q3.

In Fig.3.15a we see that the quantity P is constant over some time before it abruptly shifts to a higher value. We explain how P is calculated to understand why that is the case. When we calculate the pitch, we take the Fourier transform of the quantity W , which is the dot product of bond vectors along the contour with

a vector perpendicular to the axis of the helical polymer chain. for the procedure of calculating P and also refer the figures which show the different values of the pitches obtained in the same helical configuration as it evolves with time. Thus, different segments of the chain form helical structures with slightly different values of the pitch. These in turn unwind at different rates. Hence there is more than one peak in the Fourier Spectrum of 'W' at any given instant of time. The monomer index corresponding to the peak with the highest amplitude at any given instant of time is denoted as P . Thus P represents the most frequently occurring pitch in the helical polymer chain. As the helical structure gradually unwinds segment by segment, the pitch corresponding to a particular segment increases and consequently the amplitude of the corresponding peak in the Fourier spectrum changes. However there is a change in the quantity P for the entire polymer chain, only when the position of the highest peak in the Fourier Spectrum of 'W' changes.

In Fig.3.15b, where we show the dependence of P with ϵ_b we choose not to calculate the ensemble mean, since there are large differences in the values of P at a fixed instant of time corresponding to different runs. To illustrate this point we show three independent runs, which show considerable differences in the value of pitch, at the same time and for the same value of ϵ_b . The difference in the values of P for independent runs arise due to the fact that initially the helix formation is triggered by the presence of thermal fluctuations.

As we saw earlier, that the formation of the helix depends on the strength of the Coulomb interaction ϵ_c or on the value of ϵ_d . The question is if the value of ϵ_c in the model polymer chain increases gradually with time, i.e. a neutral semi-flexible chain gradually becomes charged (e.g. say, due to change in pH), does the polymer still form a helix if it starts out from a relatively straight configuration in the presence of

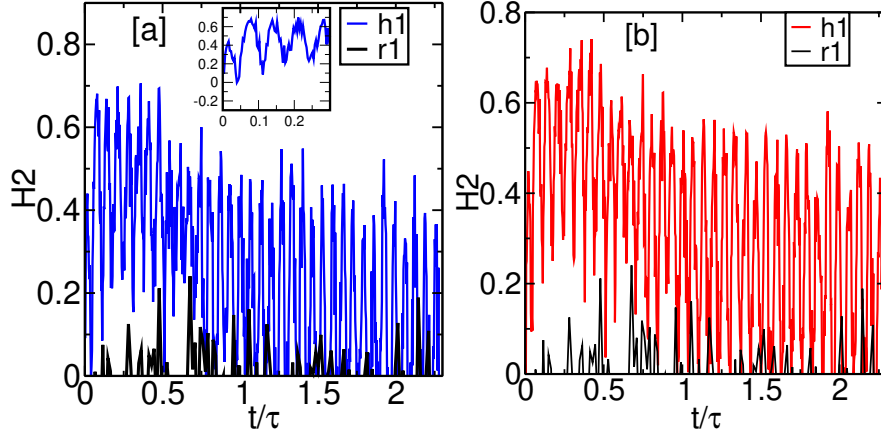


Figure 3.17: Subfigure (a) shows $H2$ versus time t , scaled by the relaxation time τ , for a chain of 49 monomers with a time dependent $U_c(t) = \epsilon_c^0 * (a/r) \cos^2(2\pi t/T_0)^2$ with $T_0 = 0.13\tau$ over many cycles. $H2$ varies periodically nearly in phase with the forcing. The inset shows that the periodicity is $T_0/2$. (b) Subfigure shows $H2$ versus time for $u_d(t)$ which has a similar time dependence as $u_c(t)$. We also give $H2$ data for when $u_c = 0$ and $u_d = 0$, denoted in the legend as $r1$ for comparison of response.

thermal fluctuations? Moreover, can the helix formation occur in a recurring manner as a response to a time dependent periodic repulsive interaction?

To that end, we choose a significantly more rigid polymer such that the persistence length is much larger than the contour length of the polymer chain with $N = 49$ monomers. We also choose a suitably higher charge density of the polymer chain. Moreover, we use a time dependent potential of the form $u_c(t) = \epsilon_c(t)(a/r)$ where $\epsilon_c(t) = \epsilon_c^0 * \cos^2(2\pi t/T_0)$ where we have chosen $T_0 = 0.13\tau$, $\epsilon_c^0 = 727.3k_B T$ and t denotes the simulation time. The values of ϵ_b and κ was changed to $300k_B T$ ($\ell_p = 300a$) & $1500k_B T/a^2$, respectively, to have a stiffer chain. We observe that we obtain helices, in a recurring manner. The helices form, then dissolve away as $\epsilon_c(t)$ becomes zero, such that the polymer becomes relatively straight in the thermal bath. The helical conformation forms again as the amplitude of the periodic forcing increases.

To substantiate that we show $H2$ versus time t in Fig.3.17a for a chain of 49 monomers under the influence of $u_c(t)$ and also for a chain of 49 monomers such that $u_c = 0$. We also show data for the same values of κ and ϵ_b but $u_d(t) = 769.34(a/r)^3 * \cos^2(2\pi t/T_0)$ in Fig.3.17b, where again we obtain helices in a recurring manner. The helices formed for these high values of κ and ϵ_b dissolve away significantly faster as compared to that of cases A and B, and quickly return to a relatively straight conformation. This again emphasizes that the helix formation does not critically depend upon the straight line initial condition provided ℓ_p is larger than the contour length; a factor of 6 in this case. Each time the polymer straightens up before reforming the helix, the configuration is slightly different due to the presence of $k_B T$. However, when $\ell_p \leq l_c$ where l_c is the contour length of the uncharged polymer, then helix formation is indeed initial condition dependent.

Thus for the value of T_0 chosen for this study, the helices can be made to form and dissolve away in a recurring, periodic fashion. The time scale τ is decided by the value of the friction constant ζ . Finally we want to put the relatively high value of ϵ_c in perspective. If the distance between monomers in our calculation is taken as $a = 10nm$, then at $T = 300K$, the Coulomb energy $\epsilon_c = 727k_B T$ used for the above study corresponds to a charge of $\approx 108e$ on each monomer in water, i.e. a line charge density of $\approx 11e$ per nm. As a reference, each base pair of DNA of size $\approx 3.4\text{\AA}$ has a charge of $\approx -2e$ at physiological pH [86]. Thus our choice of ϵ_c in this case leads to a line charge density twice than that of DNA.

To explore whether the helical structure becomes more long-lived when the two ends of the polymer chain are grafted (tethered) on to two parallel surfaces, we do not update the positions of the end monomers of a polymer chain while observing the dynamics of the chain. The distance between the fixed monomers is equal to the

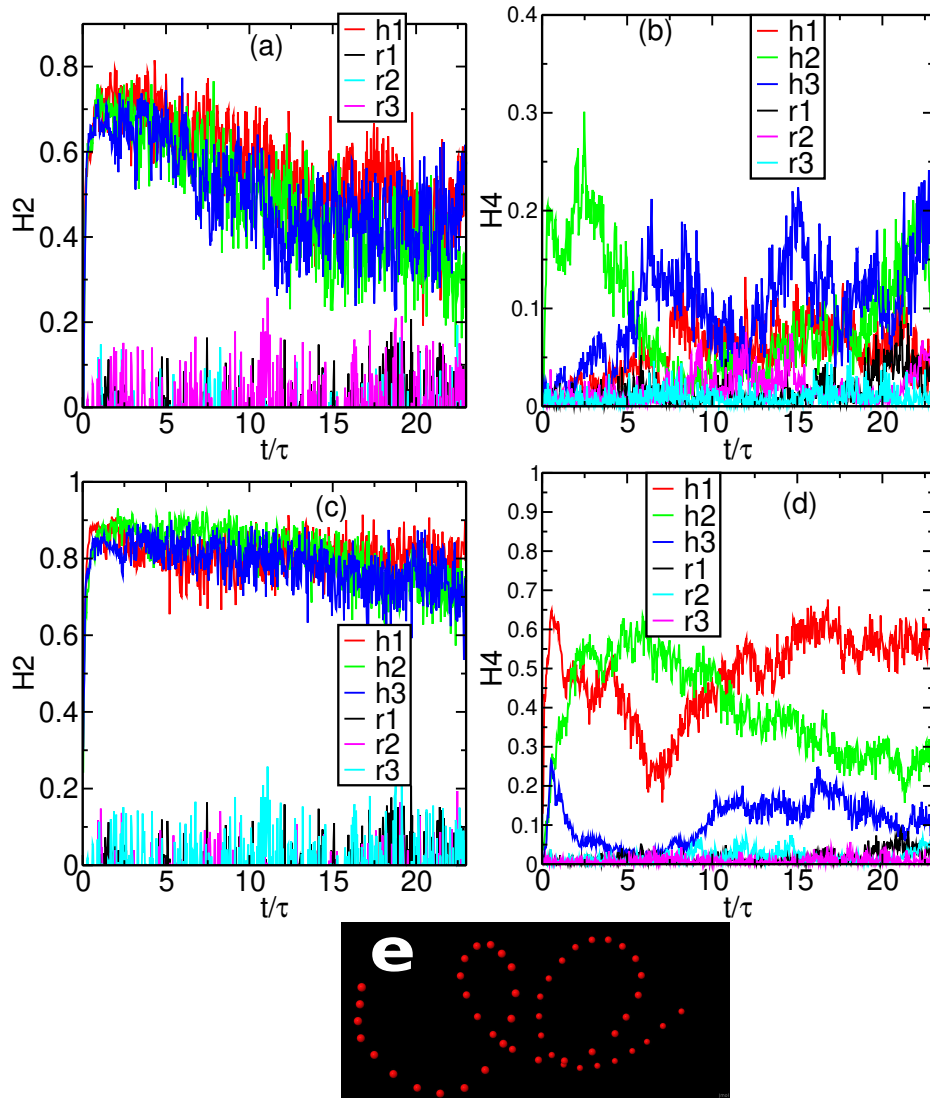


Figure 3.18: Subplots (a) and (b) shows the evolution of order parameter $H2$ and $H4$ versus time for a semi-flexible polymer chain of 49 monomers with its end monomers fixed and forces due to potential u_c acting between all monomer pairs, with all parameters being identical to that of Case A. Subplots (c) and (d) shows the evolution of $H2$ and $H4$ versus time for a semiflexible polymer chain of 49 monomers with its end monomers fixed and forces due to potential u_d acting between all monomer pairs, with all parameters being identical to that of Case B. Subplot (e) shows the snapshot of a polymer chain of 49 monomers at $t = 20\tau$, with end monomers fixed and u_d acting between all monomer pairs and other parameters pertaining to that of Case B ($H2 = 0.84$, $H4 = 0.50$).

contour length of the polymer chain (of 49 monomers) in the absence of charges. In this case the helical structures persist for a longer duration of time as compared to the helical structures resulting from a free standing polymer. This can be surmised from the data given in Fig.3.18. With u_c acting between the monomers, Figure 3.18a shows that there is a slight increase in the value of $H2$ for a polymer chain at longer times (e.g. at time $t = 20\tau$) as compared to a free standing polymer chain at similar times refer Fig.3.3b. Moreover, Fig.3.18c shows that there is a significant increase in the value of $H2$ at long times (at 20τ) as compared to an free standing polymer chain with u_d acting between the monomer pairs at similar long times, refer Fig.3.3d. Thus we conclude that the tethering hinders the relaxation of transient helical structure by preventing it to stretch axially. The effects are more pronounced with the interaction potential u_d . A snapshot of the helical conformation of a polymer chain of 49 monomers with end monomers fixed and u_d acting between the monomer pairs has also been provided in Fig.3.18e.

In our simulations so far we have implicitly assumed the solvent to be a good solvent. To investigate if the solvent quality affects helix formation, we present data for simulations with polymer in bad solvent conditions. To model bad solvent conditions, we apply an attractive Lennard Jones (LJ) interaction (of potential depth $= \epsilon_{lj}/4$). This is used in conjunction with the repulsive Coulomb interaction u_c with all parameters pertaining to that of Case A to study transient helix formation. A polymer in a bad solvent would lead to a collapse of the polymer, where as the Coulomb repulsion would keep the polymer in a stretched condition. We show that as long as strength of attractive interaction is relatively low as compared to the repulsive Coulomb interaction, we manage to obtain helices. If the ratio of the Lennard Jones interaction strength to the strength of the repulsive interaction i.e

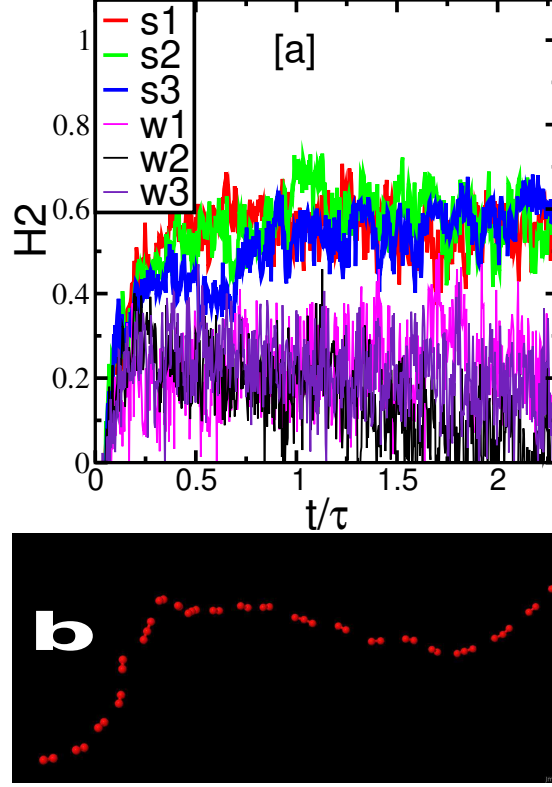


Figure 3.19: Subplot (a) shows $H2$ versus time for two different values of $k_{int} = \epsilon_{lj}/\epsilon_c$ (where $\epsilon_{lj}/4$ is the depth of the Lennard Jones potential) with three independent runs corresponding to each value. All parameters are identical to that of Case A. 's1', 's2' and 's3' denote three independent runs for $k_{int} = 2.29$, while 'w1', 'w2' and 'w3' denote three independent runs for $k_{int} = 3.43$. Subplot (b) shows the snapshot of the configuration of the polymer chain of 49 monomers for $k_{int} = 3.43$ at $t = 230\tau$.

($k_{int} = \epsilon_{lj}/\epsilon_c$), is greater than a certain critical value, then the helix formation is prevented. For a polymer chain of 49 monomers with u_c ($\epsilon_c = 87.27k_B T$) acting between all monomer pairs and other parameters kept identical to that of Case A, if k_{int} is lesser than $k_{int} = 3.43$, only then do we obtain helices. To substantiate the same, we have Fig.3.19 where we show $H2$ values versus time for a polymer chain of 49 monomers with $k_{int} = 2.29$ (‘s1’, ‘s2’ and ‘s3’ correspond to independent runs) while ‘w1’, ‘w2’ and ‘w3’ denote three independent runs with $k_{int} = 3.43$. We note that for all the three runs the value of $H2$ is significantly greater for $k_{int} = 2.29$. For $k_{int} = 3.43$ one obtains a long lived configuration with small clusters of monomers separated by stretched springs as shown in Fig.3.19b. A detailed study of the effect of unscreened Coulomb interaction and polymer collapse due to bad solvent conditions, and how the minimum value required for helix formation, k_{int}^c , depends on the chain length can be explored in a future study.

3.4 Conclusions and Discussions

In conclusion, we demonstrate that spherically symmetric long ranged *repulsion* can give rise to transient helices in a semi-flexible polymer. This is a consequence of the long range of the interactions used which helps to radially spread out the sharp kinks that are formed at short times by the polymer chain due to a combination of thermal forces and repulsive interactions between monomers. Importantly, we have considered the charges on the polymer chains to be unscreened by counterions. Our model is minimal by design and therefore doesn’t take into account atomistic chemical details of the monomers or the solvent particles. We find our findings to be non-intuitive apriori, because in previous studies emergent helices (in the absence of

torsional potentials) are observed typically as a consequence of packing effects due to confinement or energy minimization due to short ranged attractive interactions in filaments, where sharp kinks are explicitly prevented.

The transient helix formation that we observe cannot be analyzed using geometric or a energy minimization calculation as the minimum (free) energy configuration in the presence of Coulomb potential u_c (or u_d) is not a helix; it is a straight line configuration with deviations due to thermal fluctuations. However, a uncharged polymeric chain, which is slightly perturbed from a straight line initial condition or is in a bent configuration at $T = 0$, is put in conditions such that the charge on the monomer gets switched on at a time $t = 0$, it relaxes to equilibrium through a kinetically driven pathway where the intermediate stage is a helical configuration. This observation remains true even if we start out with a stiff polymer in thermal equilibrium with a solvent bath. The same phenomenon happens even if the monomer charge increases gradually from zero as shown in Fig.3.17. Interestingly, we can get the helix to form in a recursive fashion as has been demonstrated in Fig.3.17 as the charge is gradually increased and then decreased back to zero in a periodic manner.

Note that in this study we start with a straight initial condition when l_p is less than contour length. However, even in this case there are slight deviations from the axis that arise due to thermal fluctuations. These deviations are crucial to helix formation. If there are significant deviations from the straight line initial condition (when l_p is less than contour length) then one does not obtain helix formation. That is why one cannot start with an equilibrated configuration in this case. But as we have established in this chapter, if we have a stiff polymer chain (with persistence length much greater than contour length) and allow it to equilibrate and then switch on the interaction between the charged monomers, one indeed sees helix formation. The

straight line initial condition is imperative only in the case of semi-flexible polymers where the persistence length l_p is less than or equal to the contour length.

Since a free standing charged polymer chain tends to stretch out axially at long times, we can also use the charging and discharging of a (tethered) polymer chain to apply forces at the two surfaces to which the end monomers are kept attached. We obtain transient helices also on switching on a repulsive $1/r^3$ potentials for a stiff polymer in a thermal bath as long as the persistence length is greater than the contour length of the polymer chain. Since the charge densities required to see the transient formation is very much realizable in the laboratory, we hope that our study will spur future experiments.

Our proposed mechanism can be possibly used to design helical springs for NEMs/MEMs devices at $10nm - \mu$ length scales and using material of choice by arresting the relaxation process at a suitable time. As an example, we have shown that we obtain relatively long lived-helices by fixing both the ends of the chain and switching on the repulsive interactions between the monomers. In this case the helical structures persist for a longer duration of time as compared to the helical structures resulting from a free standing polymer, especially when we use $1/r^3$ interaction potentials. Since the relaxation time of the polymer chain depends on the friction constant ζ , a charged polymer can be made to relax slowly by placing it in a solvent of higher viscosity.

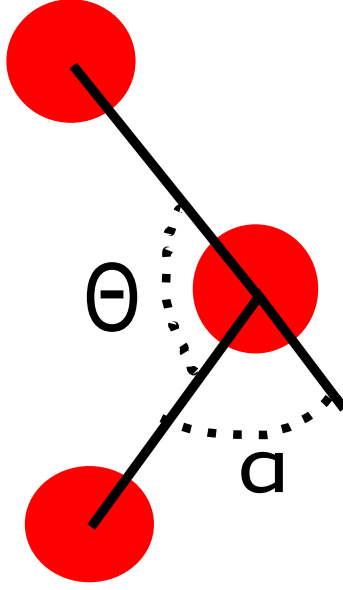


Figure 3.20: A schematic diagram showing a triplet of monomers in red and defining the angle θ and α for the convenience of the reader.

3.5 Persistence length

If we have a semi-flexible polymer chain with just the harmonic spring interaction u_H and the potential $u_b = \epsilon_b \cos \theta$ which introduces semi-flexibility along the chain contour then, the energy required to bend a triplet of monomers of semi-flexible chain from its straight line configuration (such that $\theta_0 = \pi$ and energy $u_b = -\epsilon_b$) to a configuration with $\theta < \pi$ is provided by the thermal energy. Therefore, if we equate the bending energy with the thermal energy and choose $k_B T = 1$ as we use $k_B T$ as the unit of energy:

$$\epsilon_b (\cos(\theta) - \cos(\pi)) \approx k_B T \quad (3.5)$$

$$\equiv \cos \pi - \cos \theta = \frac{-1}{\epsilon'_b}. \quad (3.6)$$

where,

$$\epsilon'_b = \epsilon_b/k_B T. \quad (3.7)$$

If we define $\alpha = (\pi - \theta)$, then

$$-\cos \theta = \cos \alpha = (\epsilon'_b - 1)/\epsilon'_b \quad (3.8)$$

For small values of η , one can write:

$$(1 - \frac{\alpha^2}{2}) = (\epsilon'_b - 1)/\epsilon'_b \equiv \alpha^2 = 2/\epsilon'_b \quad (3.9)$$

From polymer physics [5], we know that for WLC (worm like chain) model, for the small angles of bends, the persistence length ℓ_p is given by $\ell_p = 2a/\alpha^2$ Then using Eqn.3, the persistence length

$$\ell_p = a\epsilon_b/k_B T. \quad (3.10)$$

Chapter 4

Emergent Helicity in non-uniformly charged, semiflexible polymers.

4.1 Introduction

In the work discussed in the previous chapter we established that a bead-spring model of a semiflexible polymer chain with monomers all having the same value and polarity of charges and long range Coulomb interaction between monomers attains a transient helical conformation, when repulsive interactions are switched on between the monomers [2]. The monomers may become charged because of a change in the nature of the solvent, or if the ionic concentration of the solvent is suddenly changed or due to other changes in its environment. We obtained transient helices even when we replaced Coulomb interactions by long range repulsive power-law interactions.

Here we introduce a more generic model and establish that a semiflexible polymer chain with charged beads, each carrying a different value of charge and polarity, can also attain a transient helical conformation even due to the short range screened (spherically symmetric) Coulomb interactions between the monomers. But we show that asymmetry in the distribution of charges along the polymer chain plays a pivotal role in helix formation. A chain with an approximately equal number of positive and negative charges does not form helices. The helix formation takes place only for a certain range of parameters of the polymer, such as the persistence length, the spring constant, the Debye length etc. We further show that the helices formed by these screened electrostatic interactions can be made long-lived under geometric confinement, in a cuboid of appropriate dimensions.

In the case of bacterial nucleoids, this geometric confinement can be imposed by the bacterial cell leading to stable helical structures of nucleoids. These beads can be molecular units of a heteropolymer, or groups of amino acid sequences with length scale of the size of the beads, or even larger units of molecules such as nucleosome complex in the DNA dressed by suitable (charged) proteins, or even polymeric chains where the monomeric units are micron sized colloids. In this case, the colloidal surface can be dressed by microgel particles which have been cross-linked together as shown by [79, 1]. The colloidal particles then form a polymeric chain held together by cross-links with tunable semiflexibility (persistence length). If one uses colloids that are chemically different to each other, to form the chain, a change in the ionic concentration in the solution can result in a different value of charge on each colloid.

In the subsequent ‘Model’ section of the chapter, we first describe our simulation model of a charged bead spring polymer. There we outline all the interactions between the monomers and also explain how the charges are distributed among the

monomers. Then we describe our simulations in the ‘Results’ section where we first show that our results are robust for various initial conditions and then establish the mechanism of helix formation. In the same section, we also show the dependence of various parameters on helix formation and plot suitable state-diagrams. Finally, we show the effect of geometric confinement which stabilizes the formed helix. We finally end the chapter with a ‘Discussions’ section.

4.2 Model

We use Brownian dynamics simulations to study helix formation in a single semi-flexible, bead-spring model of a polymer chain, where the monomers have different values of charges along the chain contour as determined by a probability distribution. The center of each monomer is connected by harmonic springs of spring constant κ and of equilibrium length a to the center of two neighbouring monomers. We choose a to be the unit of length for our simulations. Furthermore, there is a bending energy cost per every triplet of monomers along the chain contour to incorporate effects of semiflexibility. The energy cost of having bends along the chain, is realized by the bending potential $V(\theta) = \epsilon_B \cos(\theta)$, where θ is the angle between the bond vectors $(-\mathbf{r}_i, \mathbf{r}_{i+1})$. The vector $\mathbf{r}_i$ is the vector joining monomer $i - 1$ to its neighbouring monomer i along the chain contour. The quantity ϵ_B can also be directly related to the persistence length ℓ_p ,

$$\ell_p \approx a\epsilon_B/k_B T \quad (4.1)$$

for small deviations of θ from π , refer appendix of [2]. In our simulations, energy is measured in units of the thermal energy $k_B T$. The excluded volume of the

monomers are modelled by the WCA (Weeks, Chandler, Andersen) potential and all the monomers have diameters $\sigma = 0.7a$.

Additionally, any two monomers i and j situated at a distance r_{ij} from each other, with charges q_i and q_j interact with each other through the screened Coulomb interaction.

$$V(r_{ij}) = \frac{q_i q_j}{4\pi\epsilon r_{ij}} \times \exp(-r_{ij}/\lambda_D) \dots \forall r_{ij} < r_c \quad (4.2)$$

where the Debye length λ_D is a parameter and sets the length scale over which Coulomb interactions get screened out. The permittivity ϵ of the background medium within which the chain is suspended, can be expressed in terms of the permittivity of vacuum ϵ_0 and the relative permittivity ϵ_r .

The strength of interactions is determined by the value of q_i and q_j , but for our calculation we will express charge in units of Z_0 , such that the interaction between any two charged monomers can be expressed as:

$$V(r_{ij}) = Z_i Z_j / r_{ij} \times \exp(-r_{ij}/\lambda_D) \dots \forall r_{ij} < r_c \quad (4.3)$$

where, Z_i and Z_j denote the value of charges and is expressed in units of Z_0 , on the interacting monomers in simulation units. In the subsequent section, we elaborate on how the values of Z can be related to charge in real units.

We assign charges to the monomers in the chain such that there is stochasticity in the distribution of charges along the polymer chain. The charge Z_i of each monomer i in simulations will be allocated in terms of the asymmetry parameter ψ and Z_0 such that $Z_i = Z_0 \times (r - \psi)$, where r is a random number whose values are chosen from

a uniform distribution of random numbers between 0 and 1. We vary ψ between 0 and 1 to estimate the net charge of the chain. A value of $\psi = 1$ implies that all monomers have random values of charges between $Z_1 = 0$ and $Z_2 = -Z_0$ s.u. A value of $\psi = 0$ implies that all monomers have random values of charges between $Z_1 = 0$ and $Z_2 = Z_0$ s.u. Correspondingly, for $\psi = 0.5$, the charge on the monomers can take random values between $Z_1 = -Z_0/2$ to $Z_2 = Z_0/2$ s.u. Thus, in general, the charge on a monomer i can vary between Z_1 and Z_2 .

We will establish later in the manuscript that if $\psi = 0.5$ such that there are statistically equal number of positive and negative charges, then we do not obtain helices. We need to introduce asymmetry in the distribution of charges using ψ , such that $Z_1 \neq Z_2$. The degree of asymmetry in the distribution of charges along the polymer chain is denoted by the quantity ψ . Thus, $\psi = 0.1$ corresponds to a chain that statistically has 90% of the monomers having a positive charge.

For our Brownian dynamics simulations where the friction constant is ζ , and the unit of time τ is set by $\tau = a^2\zeta/k_BT$, the time taken for an isolated monomer to diffuse a distance of a . If we set $\zeta = 1$ such that $\tau = 1$ with $k_BT = 1$ and $a = 1$ (units of energy and length, respectively, the over-damped stochastic Brownian dynamics equation is integrated with time step $dt = 0.0001\tau$.

4.2.1 Mapping Simulation units to real or SI units

We now establish a correspondence between the magnitudes of charges used in the simulation (expressed in s.u.), and the magnitudes of charges in real units. If two monomers having unit charges in the simulations, are at a unit distance away from

each other then they interact with Coulomb potential energy of strength $k_B T$. Let q_o be the charge in real units, corresponding to $Z = 1s.u$, where Z denotes the charge on a monomer in the simulations.

In real units two charges (q_0) of identical charges have potential energy given by

$$\frac{(q_0)^2}{4\pi\epsilon a} = \frac{Z^2}{a} = k_B T \quad (4.4)$$

Thus at room temperature of $T = 300K$, the value of q_o in water ($\epsilon_r = 80$) placed at unit distance (a) is given by,

$$q_o = \sqrt{4\pi\epsilon a k_B T} = \sqrt{37 \times 10^{-30} a} C. \quad (4.5)$$

where the charge q_0 is measured in Coulomb (C). If we choose $a = 1nm$, then $q_o = 1.9 \times 10^{-19}C$, or $1.2e$, where e denotes the electronic charge. If $a = 10nm$ then we obtain $q_o \approx 4e$. If $a = 100nm$ then, $q_o \approx 12e$ and if $a = 1\mu m$ then $q_o \approx 40e$. In this study we often use $Z_0 \sim 10s.u$ which corresponds to a charge of $40e$ for a monomer with size 10 nm. But that is the maximum charge on a monomer for $\psi = 1$. For lower values of ψ , the maximum charge that a monomer can take will be lower. Thus, the charges used in this study may feasibly occur in many macro-molecular systems.

4.3 Results

4.3.1 Helix Formation

We observe spontaneous emergence of helices if a uncharged semiflexible polymeric chain acquired charges at time $t = T_0$ due to change in external conditions, e.g. change in pH of the solvent in which it is immersed. The helices formed are transient but may be stabilized by other factors such as hydrogen bond and geometric confinement, as we show later. We establish that the stochasticity in the value or sign of the charges on the monomers along the chain contour do not affect helix formation as long as there is sufficient asymmetry (as determined by ψ) in the charge distribution. We have tried out different initial conditions, such that monomers $i - 1, i, i + 1, i + 2$ have different value of charges in different runs for a particular value of ψ and monitor the helicity developed as we switch on charges on the monomers.

We measure helicity using the order parameters $H2$ which is defined as

$$H2 = \frac{1}{N-3} \left(\sum_{i=2}^{i=N-2} \mathbf{u}_i \cdot \mathbf{u}_{i+1} \right) \quad (4.6)$$

where $\mathbf{u}_i$ is the unit vector of $\mathbf{U}_i = \mathbf{r}_i \times \mathbf{r}_{i+1}$. A simple uncharged, semi-flexible polymer chain shows $H2$ values ≈ 0 (or negative values of $H2$) as expected for a chain locally bent due to thermal fluctuations, while calculating $H2$ over helical segments of a polymer chain would lead to high positive values of $H2$ [1, 2].

Past studies [51, 2] have also used another order parameter which is given by,

$$H4 = \frac{1}{N-2} \left(\sum_{i=2}^{i=N-1} \mathbf{u}_i \right)^2; \quad (4.7)$$

A perfect helix with uniform handedness, will have vectors u_i pointing along the helix axis, and hence $H4$ will have a value of ≈ 1 . However, if one obtains a helical polymer where half of the chain is right-handed, and the rest of it is left-handed, $H4$ will be zero. In this study, we do not have any control over the handedness of the helical structures and thus for long chains, the chain is likely to have an equal number of right-handed and left-handed segments. Thus, we use the parameter $H2$ to quantify helicity, in the remainder of the manuscript. In the future, we will report how we can design left handed and right handed helices, made to order.

We start our simulations with a uncharged semiflexible polymer chain in a heat bath, allow the chain to reach equilibrium over $t = T_0 = 10\tau$ (10^5 iterations), and switch on charge on the monomers thereafter. We show the various stages of helix formation through representative snapshots give in Fig.4.1(a-c). We also show that the helical polymer chain can easily be distinguished from a non-helical, uncharged, semiflexible polymer chain using $H2$, which fluctuates near zero till $t = 10\tau$ and then show a finite non-zero value. In Fig.4.1d we show $H2$ as a function of time t for three independent runs. In Fig.4.1e, we plot the end to end distance R_{ee} , scaled by the contour length R_C ($R_c = 99a$) as a function of time. We see that the end to distances fluctuate at values ≈ 1 for three independent runs till $t = 10\tau$, and thereafter the R_{ee}/R_c shows a sudden increase. Thus we show that the helix formation is independent of the initial configuration of the monomers as well on the stochastic distribution of charges along the chain contour.

Mechanism of helix formation: The uncharged semiflexible polymer chain undergoes stochastic conformational fluctuations due to the thermal noise from $t = 0$ to $t = 10\tau$. When the electrostatic interactions are switched on, electrostatic forces leads to a reinforcement of the angular conformations with larger deviations in the

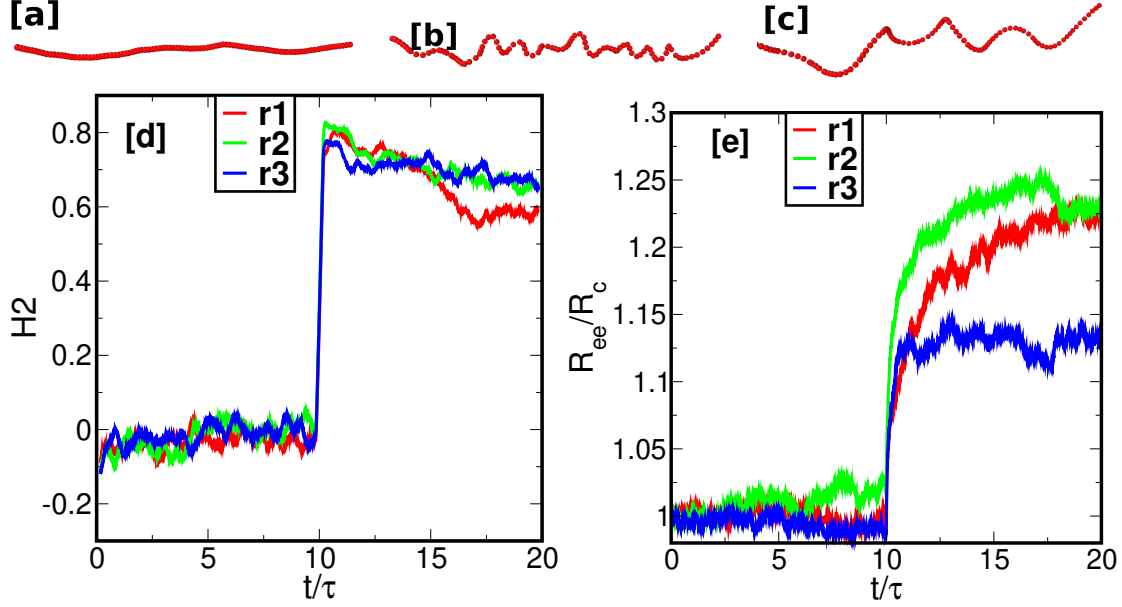


Figure 4.1: **Helix formation:** The snapshots show various stages of the helix formation in a bead-spring model of a semi-flexible polymeric chain with 100 monomers, with contour length $R_c = 99a$. Initially, the uncharged polymer is in equilibrium with a thermal bath: representative snapshot shown in [a] at time $t = 3\tau$. Snapshot [b] shows a representative conformation of the chain soon after the monomers acquire charge at time $t = 10\tau$, where we see formation of helices along the chain contour. At (c) $t = 20\tau$, we see that the helix gradually “unwinds” and relaxes to a helical conformation having a larger pitch. The charge on each monomer has a random value, between -4.0 to 36 s.u. (simulation unit). This corresponds to an asymmetry parameter of $\psi = 0.1$ and $Z_0 = 40$ s.u. The bending energy cost $\epsilon_B = 200k_B T$ (corresponding to a persistence length of $200a$ and spring constant $\kappa = 100k_B T/a^2$, and the Debye length $\lambda_d = 5a$). In (d) we show the helical order parameter $H2$ (running averaged over 0.33τ) as a function of simulation time t/τ , for 3 independent runs. We see a sharp rise in $H2$ at time $t = 10\tau$, which decays thereafter, indicating transient helix formation. Subfigure (e) shows the end-to-end distance R_{ee}/R_c versus t/τ .

angle θ from the chain axis. This is because neighbouring charges repel each other, and move neighbouring monomers along the line joining their centers, and away from the chain axis. These angular conformations (with large angular deviations) are penalised due to the semiflexibility. As the chain locally “straightens” out to reduce bending energy costs, the polymer consequently attains a helical conformation.

This can be surmised from Fig.4.2 where we plot the different energy contributions in the system, for parameters identical to that of Fig.4.1. In Fig.4.2a we plot the energy per spring, U_H versus time t . We see that at $t < T_0$, U_H fluctuates around $0.5k_B T$, as expected from the equipartition theorem. However at $t = 10\tau$, we see that U_H shows a drastic increase which indicates that the bonds get stretched severely as a consequence of the electrostatic (e.s) interactions. This is further corroborated by the data presented in Fig.4.2b where we plot the screened Coulomb energy per monomer U_{sc} as a function of time. We see that at $t = 10\tau$, U_{sc} shows a sudden spike which subsequently relaxes to lower values.

We also look at the bending energy U_b per triplet of monomers, in Fig.4.2c and we find that at $t = T_0 = 10\tau$, the bending energy shows a sudden spike, indicating a sharply kinked (angular) conformation. However, such a conformation is penalised by the bending energy which prevents such sharp angles. Thus the helix formation occurs as a relaxation of transiently formed sharp angles. We emphasize again that the helix formed is transient and eventually the helical conformation dissolves. Thereafter, the polymer chain adopts an extended linear configuration due to the electrostatic interactions. Furthermore, thermal fluctuations play a pivotal role in the helix formation in creating the initial small bends which get accentuated after switching on the charge on the monomers. The transient helices do not form, if we switch on charge at $T = 0$ where the minimum energy conformation of the chain is

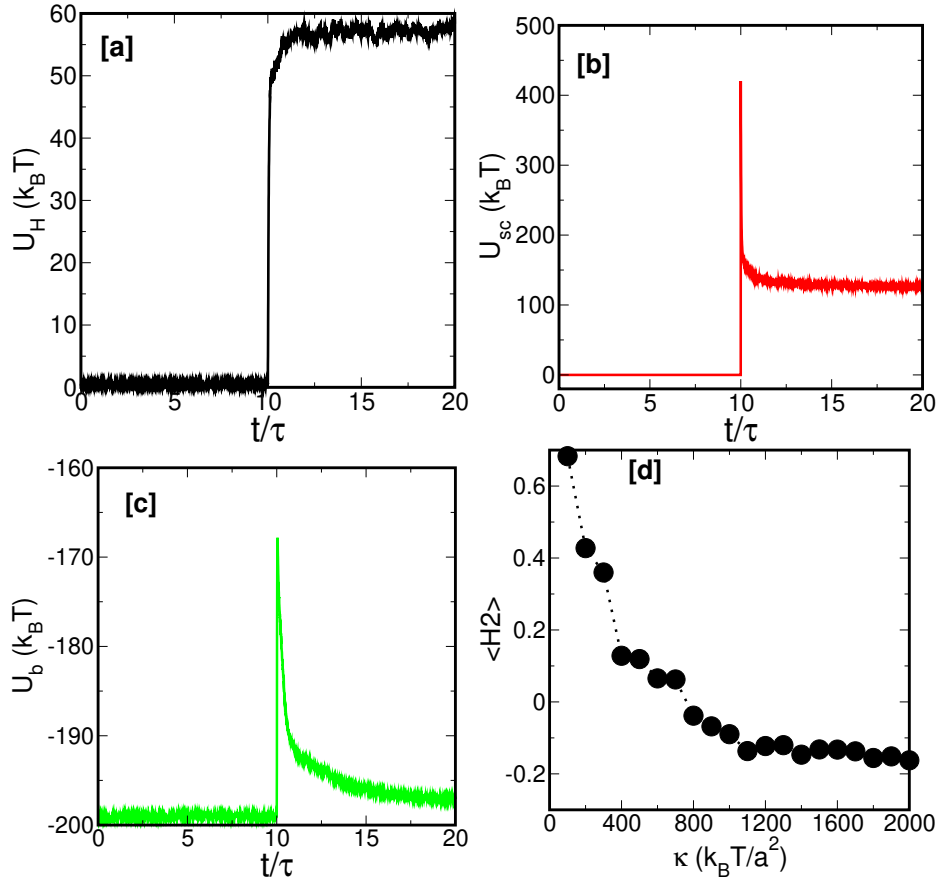


Figure 4.2: Mechanism of Helix formation In (a), (b) and (c) we show the harmonic spring energy (U_H), the screened Coulomb energy (U_{sc}) and the bending energy (U_b) per monomer. We see that just after the screened Coulomb interaction is switched on, the screened Coulomb energy and the bending energies peak, and subsequently relax leading to the “unwinding” of the helix. The springs in the polymer chains however continue to remain stretched due to the screened Coulomb interactions, as shown in (a). The sudden switching on of screened Coulomb interactions at $t = 10\tau$, leads to the formation of sharp angular conformations. This can be inferred by noting that U_b shows a peak at this point, in the plot given in (b). Subsequently, the transient helix formation takes place by the gradual relaxation of these sharp bends. Furthermore, if the springs are made too stiff, these sharp bends are not formed since a high value of κ prevents the monomer-bonds from stretching. Thus, in (d) we show that $\langle H^2 \rangle$ shows a monotonic decrease with increase in κ . We calculate the time averaged $\langle H^2 \rangle$ over 10τ to 20τ .

a perfect straight line. This has been explored in detail in [2], previously.

The importance of the formation of kinks in the subsequent emergence of helicity can also be gauged by the increasing the simulation κ parameter to increasingly high values, keeping $k_B T = 1$. We monitor the time averaged value of $H2$ with increasing κ in Fig.4.2d, and observe that $\langle H2 \rangle$ decreases with increasing κ . An increase in the spring constant prevents the bonds from stretching as much, and thereby formation of kinks on switching on of charges, and that in turn suppresses subsequent helix formation.

Unwinding of the transient helices

The emergent structure of helices “unwind” with time. There are two factors that contribute to the unwinding: the bending energy and the screened coulomb interaction. The high energy cost of bending leads to the gradual relaxation of “kinks” (sharp angles) along the chain contour and thus leads to a straightening of the chain, such that $\theta \approx \pi$. This results in a gradual dissolution of the helical structures. The repulsive electrostatic interaction also leads to an “effective” additional bending energy cost [87] and causes the polymer chain to adopt an extended configuration. Thus the helix formation occurs only as a kinetic pathway to an extended linear equilibrium conformation.

To quantify this unwinding process, we look at the periodicity parameter ‘W’ as a function of time. ‘W’ is essentially the dot product of bond vectors along the contour with a vector perpendicular to the axis of the helical polymer chain. We initialize the chain along the $\hat{y}$ direction. We assume that the helical axis is also

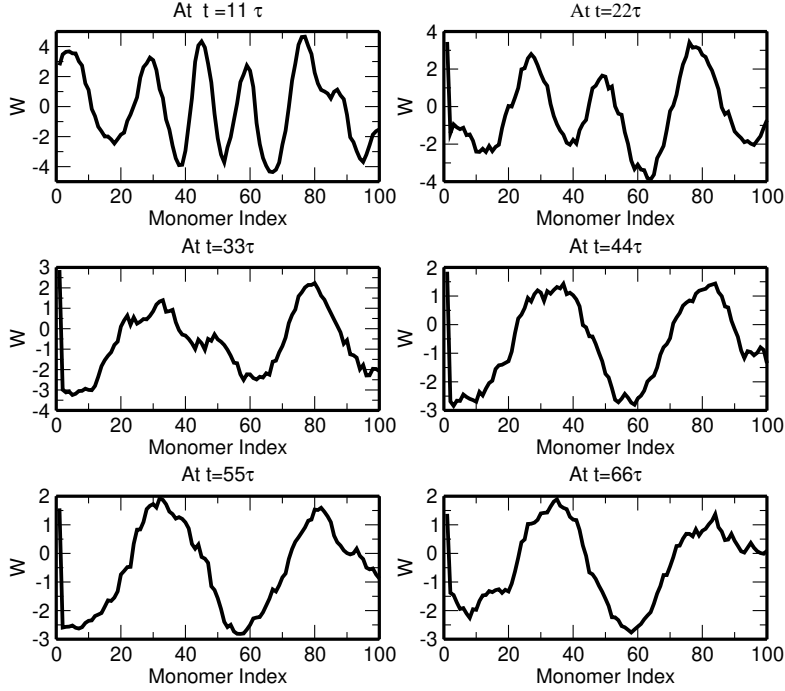


Figure 4.3: Unwinding of the helix: Change in helical pitch To quantitatively establish the unwinding of the helical polymer we look at the periodicity parameter 'W' as a function of time. 'W' is a scalar quantity resulting from the dot product of bond vectors along the chain contour with a vector perpendicular to the axis of the helical polymer chain. The periodicity in 'W' indicates the pitch of the helix. In subfigure (a) we show W for different times for a chain of 100 monomers, where we have switched on electrostatic interactions at $t = 10\tau$. The other parameters are also identical to that of the data presented in Fig.1 We note that due to the gradual unwinding of the helical polymer the periodicity in 'W' decreases.

along $\hat{y}$. Thus, we calculate 'W' as

$$W(i) = \hat{x} \cdot r_i \quad (4.8)$$

where r_i denotes the bond vectors along the chain contour. We now quantitatively establish that the helical conformations of the polymer relax by gradual unwinding.

To that end we plot in Fig.4.3 the periodicity parameter ‘W’ at various times, during the simulation run. We note that the periodicity in ‘W’ decreases as simulation time elapses. This indicates that the helical polymer gradually unwinds and the pitch keeps on increasing. At any given time there may be helical segments of varying pitch, which gradually increase. Note that the pitch of the helix can be found from the data presented in Fig.4.3 by conducting a Fourier transform. In that case, the x-axis value corresponding to the peak with the highest amplitude would correspond to the most frequently occurring pitch ‘P’ along the chain contour [2].

The most frequently occurring pitch of the helix ‘P’ at a particular time also depends on the value of the bending energy ϵ_B . A high value of ϵ_B leads to larger values of ‘P’ at a particular instant of simulation time. The other parameters do not affect the pitch of the helix.

4.3.2 Parameters controlling helix formation

There are 4 system parameters which can be tuned to obtain the helical conformations in polymers. They are:

1. The magnitude of the charge on a monomer which is proportional to Z_0 .
2. The Debye length of the screened Coulomb interaction: λ_D .
3. The bending energy ϵ_B , which is directly proportional to the persistence length [2].
4. The degree of asymmetry in the charge distribution along the polymer chain.

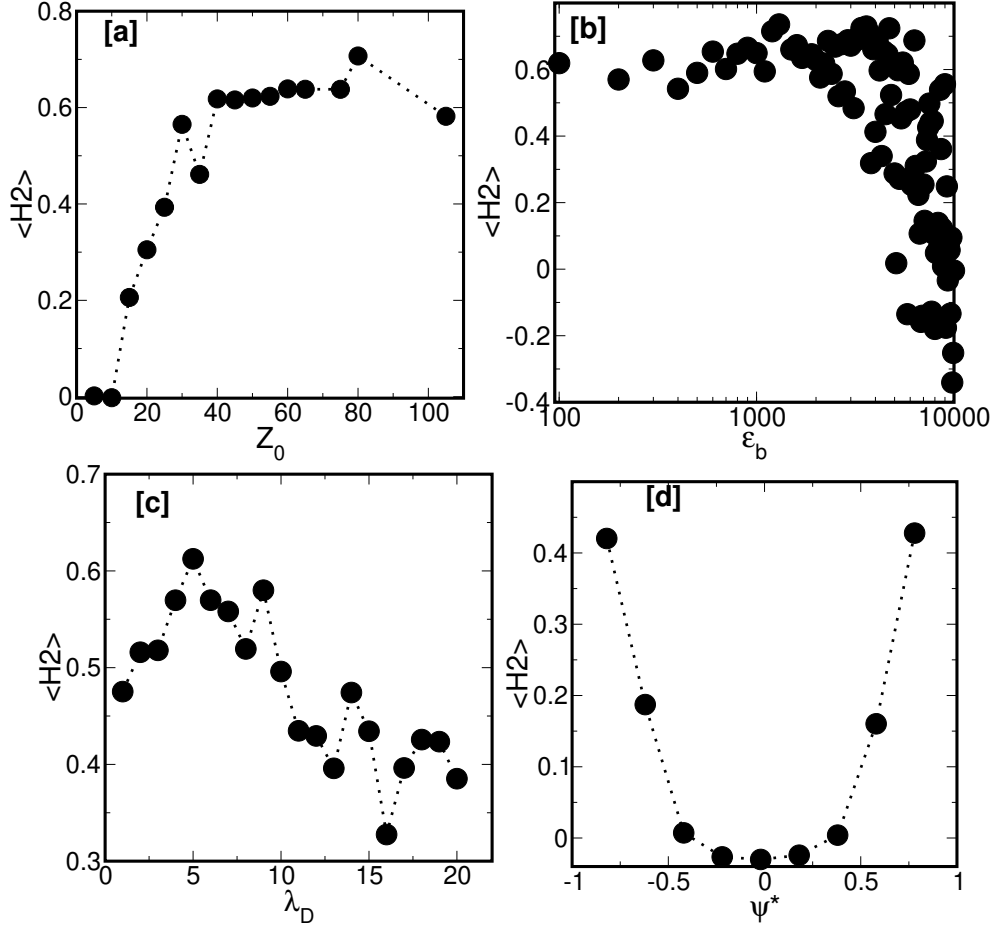


Figure 4.4: **Range of parameters conducive to helix formation:** For this set of subfigures (a-d), we keep the parameters Z_0 , ϵ_B , λ_D , $(\kappa,)$ and the asymmetry parameter identical to that in Fig.6.1, but then vary one parameter at a time keeping the others fixed. We plot the time averaged helicity order parameter $\langle H2 \rangle$ to analyze the helix formation as we modify different system parameters. We calculate the time averaged $\langle H2 \rangle$ over 10τ to 20τ . Subfigure (d) shows the dependence of $\langle H2 \rangle$ on the charge asymmetry parameter $\psi^* = 1 - 2\psi$. The quantity ψ^* indicates the asymmetry in the charge distribution in the polymer chain. A value of $\psi^* = -1$ indicates that all the charges in the polymer chain are negative while $\psi = 1$ indicates that all the charges in the polymer chain are positive. If $\psi^* \sim 0$ then the number of positive charges are statistically equal to the number of negative charges in the polymer chain. The data in (c) and (d) has been produced by averaging over 10 independent runs.

In Fig.4.4, we investigate how helix formation depends on a balance of system parameters, and thereby plot the time averaged $\langle H2 \rangle$ as we vary the parameters one at a time. Thereby, we keep the simulation parameters identical to those used to obtain data of Fig.4.1, and vary one parameter at a time. We compute the time averaged $\langle H2 \rangle$ over a duration of 10τ from $t = 10\tau$ to $t = 20\tau$.

In Fig.4.4a, we show that an increase in Z_0 , i.e. maximum value and range of charges of the monomers along the chain, leads to an increase in helical order obtained, as $\langle H1 \rangle$ increase with increase in Z_0 before it saturates. However $\langle H2 \rangle$ or helicity shows a decrease with increase in ϵ_B as shown in Fig.4.4b, and the trend reminiscent of increasing spring constant κ as shown in Fig.4.2d. This is because an increase in the bending energy (or equivalently the persistence length ℓ_p) prevents the formation of sharp angular conformations as a response to the electrostatic forces. As mentioned before, the formation of sharp angular conformations (kinks) at time $t = T_0$ are crucial to the subsequent formation of helices. Furthermore a higher chain rigidity also leads to the quicker “unwinding” and dissolution of the transient helices.

An increase in the Debye length λ_D leads to more electrostatic interactions and thus leads to a greater propensity of helix formation leading to high values of $\langle H2 \rangle$ for λ_D in the range of $5 - 10a$. Interestingly we see in Fig.4.4c that $\langle H2 \rangle$ shows a non-monotonic dependence on the Debye length, λ_D . Note that here we have $\psi = 0.1$ which implies that the majority of the charges along the chain have a positive value. When λ_D is increased then the strength of repulsive interactions increase leading to an increase in the helicity. However when λ_D is increased further then there are also attractive interactions along the chain (due to monomers having charges of opposite polarity) that each monomer experiences. This leads to a decrease in the propensity for helix formation and consequently lower values for $\langle H2 \rangle$ as there

are strong attractions between different chain segments which in turn suppresses the effects of bending energy costs which led to helix formation. We discuss the consequence of increasing λ_D for a lower value of ψ in a couple of paragraphs.

The asymmetry in the distribution of charges along the polymer chain plays a crucial role in helix formation. In Fig.4.4e we show how helicity as measured by $\langle H2 \rangle$ depends on the asymmetry parameter ψ for a constant value of Z_0 which is set at $Z_0 = 40\text{s.u.}$ For convenience, we now define a new asymmetry parameter ψ^* which depends on ψ as $\psi^* = 1 - 2\psi$. Note that $\psi^* = +1$ implies that all the charges along the polymer chain are negative while $\psi = -1$ denotes that all the charges are positive. If $\psi^* = 0$ then there are statistically equal number of positive and negative charges in the polymer chain. Thus greater the value of $|\psi^*|$ greater is the asymmetry in the distribution of charges.

We see that for $\psi^* \sim 0$ helicity goes down, which indicates that when the strength of attractive interactions become comparable to the strength of repulsive interactions then helicity goes down. Thus helicity crucially relies on the presence of strong repulsive interactions which may arise from both positive and negative charges. This symmetry gives rise to the ‘U’ shape seen in Fig.4.4d.

Finally we plot a phase diagram using these parameters, using $\kappa = 200k_B T/a^2$ (to obtain the parameter space which is conducive to helix formation. In Fig.4.5a we show the phase diagram with respect to the Debye length λ_D and the bending energy ϵ_B , keeping Z_0 and the charge distribution asymmetry parameter ψ the same as in Fig.4.1. We note that for $\lambda_D < 6a$, $\langle H2 \rangle$ shows a decrease with increase in ϵ_B beyond $1200k_B T$. This indicates that for relatively more rigid chains the helix formation is suppressed as has already been shown in Fig.4.4b. However, for intermediate values

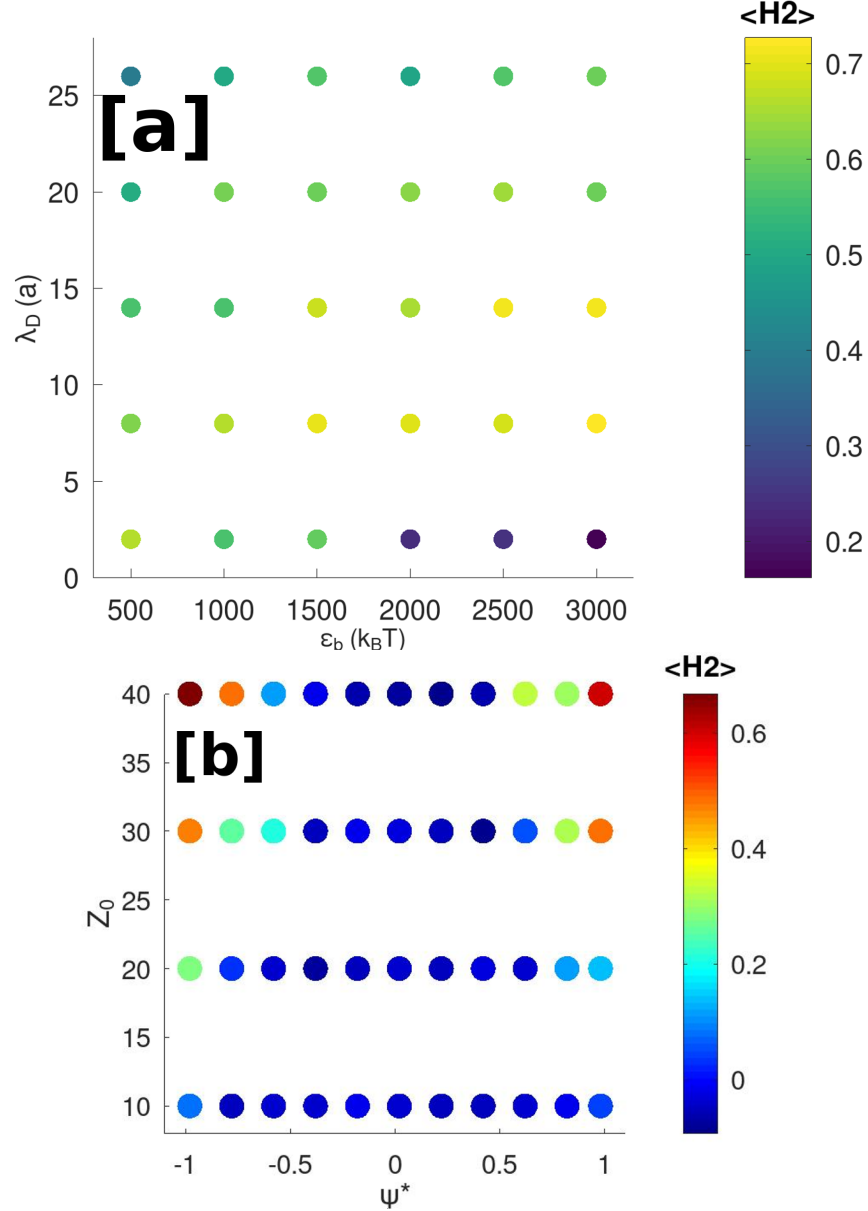


Figure 4.5: **Phase diagram of helix formation** Figure shows the parameter space conducive to helix formation. In (a) we show the phase diagram with respect to the Debye length λ_d and the bending energy ϵ_B . The maximum value of the charge in the polymer chain is $Z_0 = 40$ s.u and the asymmetry parameter $\psi = 0.1$ or $\psi^* = 0.8$. In (b) we show the phase diagram with respect to the charge Z_0 and the asymmetry parameter ψ^* which is related to ψ as $\psi^* = -2(\psi - 0.5)$. We keep the Debye length $\lambda_d = 5a$ and the bending energy $\epsilon_B = 200k_B T$. In both (a) and (b) we switch on charges at $t = 10\tau$ and the average is computed over an interval of 10τ .

of λ_D (i.e. $\lambda_D \approx 10a$, this suppression in propensity to form helices is not observed. This is because each monomer interacts electrostatically with a larger number of monomers along the polymer chain. However, for higher values of λ_D ($\lambda_D > 15a$), the propensity to form helices again decreases. This is consistent with the non-monotonic behaviour of $\langle H2 \rangle$ with increase in λ_D as established in Fig.4.4c and is for the same reason as explained.

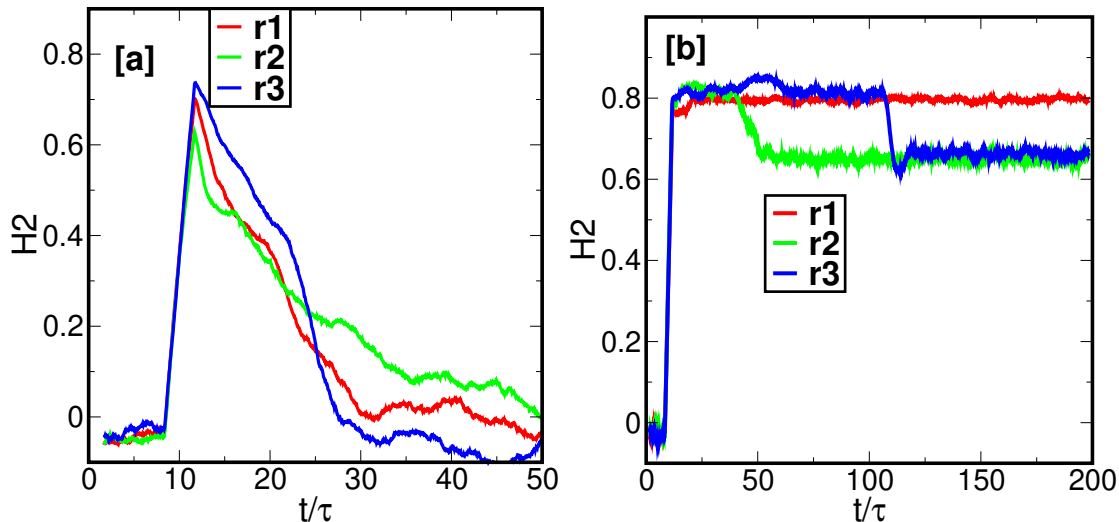


Figure 4.6: Stabilization of transient helices To examine whether geometric confinement is able to stabilize the transient helices we induce helix formation in a cuboid with aspect ratio 1 : 12. The length of the cuboidal box $L_c = 1.1R_c$, where R_c is the contour length of the polymer chain. We implement walls of the cuboid by WCA interactions and we keep the parameters identical to that in Fig.6.1 and vary one parameter at a time keeping the others constant. We switch on electrostatic interactions due to charges at 10τ . The initial configuration was that of a linear straight line along the y-axis. In (a) we show $H2$ (running averaged over 0.33τ) as a function of simulation time for an unconfined chain of 50 monomers for 3 independent runs. We see that the helix formed is transient and gradually dissolves with time. In (b) we show again a chain of 50 monomers similar to that of (a) but under confinement. We see that $H2$ (running averaged over 0.33τ) shows a high value even at long times for 3 independent runs. Thus geometric confinement lead to the stabilization of helices.

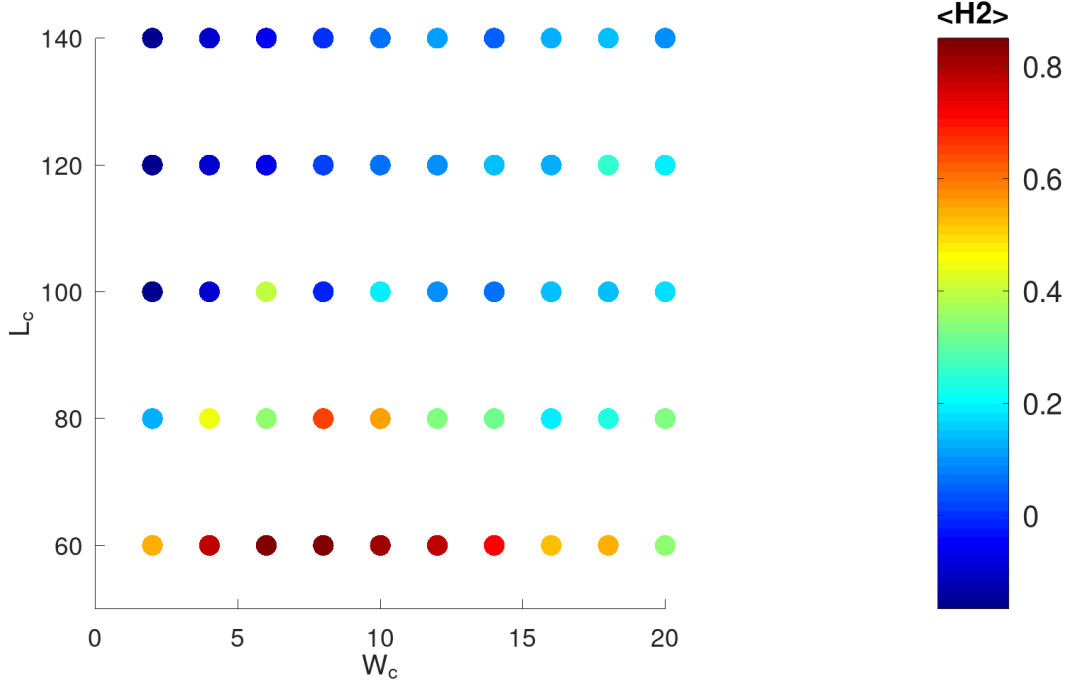


Figure 4.7: To examine the ranges of W_c (width of the confining cuboid) and L_c (length of the confining cuboid) in units of a , for which we obtain stable helix formation. We switch on electrostatic interactions due to charges at 10τ and measure $\langle H2 \rangle$ over the next 190τ . If $\langle H2 \rangle$ is found to be greater than 0.3 we conclude that helix formation takes place. The symbol 'H' denotes that the chain attains a helical conformation for the corresponding values of L_c and W_c , and the symbol 'X' denotes that no long-lived helicity was measured for the corresponding values of L_c and W_c . The value of contour length of the uncharged polymer R_c is $49a$ where a is the unit of length in our simulations. We find that helix formation occurs only when $L_c \sim R_c$ and does not depend on the value of W_c .

In Fig.4.5b we plot another phase diagram keeping the λ_D and ϵ_B fixed with the values being the same as that of Fig.4.1. We vary Z_0 and ψ^* to plot the phase diagram. We note that for small values of Z_0 , $\langle H2 \rangle \approx 0$ while for higher values of Z_0 , we obtain high values of $\langle H2 \rangle$ only when $\psi^* \approx \pm 1$. This once again emphasises that asymmetry in charge distribution of monomers along the chain is crucial to helix formation.

4.4 Confinement stabilizes helices

Will confinement of the polymeric chain to prevent stretching of the end-to-end distance lead to the stabilization of the helical conformations? To investigate this, we simulate the same system confined within a cuboid, where the rigid walls which are modelled by inducing repulsive WCA interactions to prevent the monomers crossing the walls. We show that for a particular aspect ratio 1 : 12 we get relatively longer lived helices with the length of the cuboid being L_c being $L_c = 1.1R_c$. Thus we show that the transient helices formed due to electrostatic interactions between charged monomers may be stabilized or made long lived under confinement. In Fig.4.6(a) we show that $H2$ or helicity for an unconfined chain dissolves away by 50τ whereas the helicity ($H2$) for a chain under confinement shows that $H2$ fluctuates about a high value even at long times, refer Fig.4.6(b). Thus geometric confinement leads to stable helices due to electrostatic interactions.

How does geometric confinement lead to the formation of stable helices? As mentioned before the transient helices dissolves as the pitch of the helix increases and the polymer chain stretches out axially due to the electrostatic interactions and the semiflexibility. However, the cuboidal confinement with length $L_c = 1.1R_c$ where R_c is the initial contour length of the chain, prevents the stretching of the polymer chain axially and thus stops the unwinding process. This leads to the formation of long lived stable helices. Thus a geometric confinement with the axial dimension $L_c \sim R_c$ successfully leads to long lived helical structures.

This again leads to the question, what are the aspect ratios of the cuboidal confinement imposed, that are conducive to helix formation? To investigate this we systematically vary the width of the cuboid W_c and the length of the cuboid L_c and measure $\langle H2 \rangle$. We switch on charges at $t = 10\tau$ and compute $\langle H2 \rangle$ over the next 190τ . We plot a colour map showing the range of W_c and L_c conducive to stable helix formation in Fig.4.7. We see that we obtain helix formation only when $L_c \sim R_c$ and does not depend on the value of W_c . Thus one may attain long-lived, helical structures due to electrostatic interactions between charged monomers in a polymer under confinement with the aspect ratio chosen suitably. These helical structures may then be employed for a variety of technological applications briefly mentioned in the ‘Introduction’.

4.5 Discussion

Through this study, we have established a generic mechanism by which the helical motif may spontaneously self-emerge in several bio-molecules. We posit that such emergent structures may arise due to the induction of charges along the bio-polymer chain contour due to change in the external conditions of the underlying solvent. These structures though have to rely on other mechanisms for being long-lived since, the helices due to the screened Coulomb interactions are transient. However, in the presence of confinement, these helices become long-lived. The helices we observe in our simulations do not have a preferential handedness and may on average have an equal number of left-handed and right-handed segments. For most, bio-molecules however, there is a preferred handedness when the molecules attain helical conformations. Future avenues of investigation may shed light on this aspect and elucidate

how a preferred handedness self-emerges, by incorporating specific constraints above and beyond the mechanism of helix formation, outlined here.

In this study when we have explored the case of long-lived helix formation under cuboidal confinement, we have not explored the case where R_c (contour length of polymer) is lesser than L_c (length of the polymer). This is because it is challenging to equilibrate a stiff polymer in a cuboid whose length is lesser than the contour length. However, if one has a very long stiff polymer (with a persistence length lesser than contour length) in a cuboid of length approximately equal to the persistence length, then different segments may be locally arranged in a rod-like fashion., then different segments may be locally arranged in a rod-like fashion. When charged interactions are induced in such a case, there may be helix formation locally along the chain contour. Note that the pitch of these helices in this case will not be affected by the dimensions of the cuboid. As already shown in Chapter-3 the pitch of the helix depends on the bending energy. These aspects may be explored in future studies.

Chapter 5

Polymer architecture orchestrates the segregation and spatial organization of replicating *E. coli* chromosomes in slow growth.

5.1 Introduction

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The replication and segregation of chromosomes into two halves of the cell before the start of cell division is an important step to maintain the life cycle of a living cell [88, 89]. Nature has devised advanced strategies to segregate the chromosomes of

¹This chapter closely follows the paper [3]

eukaryotes, but interestingly, the mechanism of chromosome segregation of a much simpler single-celled bacterial organism such as the *E. coli* is unknown till date [90, 91, 92, 93, 94, 95]. Investigators have identified mechanisms with contributions from proteins which leads to the chromosomal segregation in bacteria *B. subtilis* [95, 96] and *C. crescentus* [95, 97, 98], but any such mechanism could not be identified for *E. coli*.

The chromosome of *E. coli* can be thought of as having ring polymer architecture, and it has been proposed [13, 19] that the physical principles related to entropy maximization (or equivalently free energy minimization) might be used by nature to achieve chromosome segregation while replication is in progress. It has been shown that entropic repulsion between 2 ring polymers confined within a cylinder lead to their gradual segregation [13, 14, 15, 16, 17, 18, 19]. But then it has to be aided by some other hitherto unknown processes driven by proteins, as entropic repulsion is too weak [95] to have segregation within a time scale of 60 minutes, as seen *in-vivo* [99, 100, 101]. This has spawned a series of experiments over the last two decades.

Detailed *in-vivo* experiments, with DNA-segments (loci) labelled with fluorescent markers, have helped us visualize their spatio-temporal organization as the *E. coli* DNA replicates and simultaneously segregates [20, 102]. This led to the observation that certain segments of the contour (genomic loci) get localized to specific regions along the cell-long axis. A certain locus, viz. the *oriC* which is a particular biologically relevant segment of the DNA-polymer, is found to be localized at the mid-cell position before replication and to the quarter positions after replication. The standard deviation of its mean position, is less than one tenth of the length of the cell. Upto 7 other fluorescently labelled loci which are distributed along the chain contour are found to be well localized in ‘Fluorescence *in situ* hybridization

(FISH)’ experiments at certain positions along the cell long-axis [20]. In other experiments conducted in fast growth conditions, with multi-fork replication, the *oriC*s are found in positions $(1/8, 3/8)$, $(5/8, 7/8)$ position along the cell long axis. Though the mechanism of the localization of the *oriC* and other loci remain unexplained, researchers have observed the increase in protein MukBEF at the positions of the *oriC* during segregation. Thus, it has been suggested that the positions of the *oriC*s are correlated with the increase of MukBEF concentrations [103]. Other aspects of the segregation process remain unexplained (or controversial) as well, as detailed later, after providing relevant biological phenomenological background for the readers.

The experiments, which mark the spatio-temporal distribution of the DNA molecule in individual cells during the segregation process, however lack sufficient resolution. One typically follows just 8 – 13 loci on the DNA molecule. Complementary experiments, which provide more detailed global organization of the DNA-polymer by 3C (Chromosome Conformation Capture) and Hi-C contact maps [21, 104, 105, 106], lose out on the temporal information. The positional correlations presented in Hi-C maps is from data collected from a population of many *E. coli* cells which are at different stages of the cell cycle. These show the existence of regions with high contact probabilities known as topologically associated domains (TADs) and also point to the existence of 4 macro-domains (large scale genomic regions of relatively high contact probabilities having size $\sim 1\text{Mbp}$) in the DNA-organization of the *E. coli* cell [107, 108, 109, 110]. Since the replication and segregation constitute of 80% of the cell cycle, any model of segregation should also be consistent with data presented in Hi-C maps. Researchers have several theoretical models (e.g. the loop extrusion and the SBS model) to study the organisation and segregation of chromosomes, both eukaryotic and prokaryotic, which are optimised to obtain a match with the exper-

imental Hi-C map [111, 112, 113, 114, 115, 116, 117, 118]. Consensus has not been reached about the origin of the four macro-domains in *E.coli*, though SMC (Structural Maintenance of Chromosomes) proteins are expected to play an important role [110, 119, 114].

Many studies using coarse-grained polymer models have primarily focused on the organization of chromosome in bacterial or eukaryotic cells [120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 67, 130] but relatively fewer on the *organization of chromosomal loci* while replication and segregation are in progress [131, 103, 132]. A more detailed framework which reconciles complementary experimental results pertaining to both the spatial and temporal organization of bacterial chromosomes during its life cycle in slow [20] and fast growth conditions [133], is in order [134]. This work aspires to propose a coarse grained, unifying framework for a replicating chromosome in slow growth conditions.

To provide an understanding of the observed phenomenon, we rely on the concepts of enhanced entropic repulsion provided by the presence of loops within each of the two daughter DNA-polymers. These loops are created by two cross-links (referred to as contacts or links in the bacterial chromosome literature), which bridge specific chromosomal regions in each daughter chromosome. We further the idea of entropic repulsion between ring polymers (daughter chromosomes) [13], and thus induce entropic repulsive interactions between internal loops of each polymer. We achieve this by having a modified polymer architecture, which leads to additional entropic repulsion between two daughter DNA ring-polymers. We establish that this results in the localization of genomic loci as seen *in-vivo*. Thereby, we propose that the daughter DNAs adopt a slightly more evolved polymer architecture than just a simple ring polymer, as replication proceeds. We name the modified polymer ar-

chitecture as Arc-2, refer Fig.5.1a for a schematic. We later further modify Arc-2 architecture to Arc-2-2 architecture to obtain a better match with the Hi-C map of *E.coli* chromosomes.

It is already known that a branched/loop topology expedites the segregation process [13, 14, 15]. However a causal link between polymer topology and localisation of chromosomal loci had not been established till now. This simple but pivotal idea of modifying the polymer architecture provides a consistent picture of the segregation of *E. coli* daughter DNAs after replication, and quantitatively integrates disparate experimental measurements on (a) localization of loci along cell long-axis [20], (b) observation of four macro-domains in Hi-C maps[21].

In the subsequent paragraphs, we describe the minimal experimental background of bacterial DNA-replication and segregation for *E.coli* chromosomes. This allows us to list some open questions which have arisen due to differing interpretations of experimental observations. It will also help us give relevant phenomenological perspective as we suitably adapt a bead spring polymer model of the DNA to simulate a replicating and segregating chromosome. We further discuss whether our model and the proposed architecture is plausible to be adopted by the *E.coli* chromosome.

We present our simulation results obtained with the Arc-2 and Arc-2-2 architectures in the ‘Results’ section of the chapter. We thereby establish a mechanism of chromosome organization along the cell long-axis which is consistent with the FISH and Hi-C experiments on *E.coli* DNA. Our proposed model will also aim to resolve some other hitherto irreconcilable experimental observations. We end the chapter with a discussion on the proposed model and future avenues of investigation.

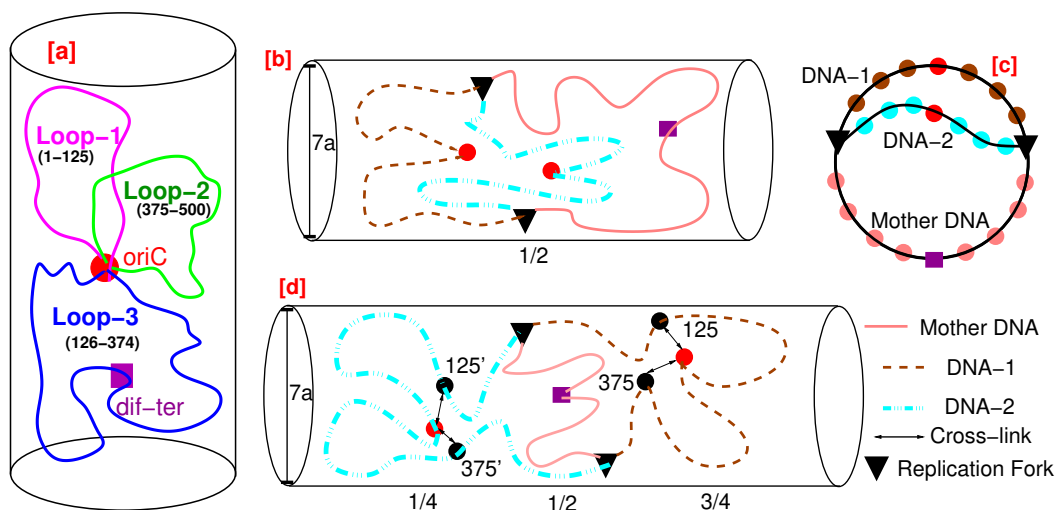


Figure 5.1: **Schematic showing the mother DNA in polymer architecture Arc-2 and the replication process.** (a) We show a coarse-grained model of the *E. coli* DNA within the cell. We use a bead spring ring-polymer with 500 monomers confined within a cylinder. We propose that the *E. coli* chromosome adopts the modified ring-polymer architecture Arc-2 as shown in the figure. We label monomer-1 as *oriC*, monomer-250 as *dif-ter* and monomer-1 and 500 are connected by a spring due to ring architecture. In Arc-2 we cross-link monomer-1 and monomer-125 (creating Loop1), monomer-375 and monomer-1 (creating Loop-2). Thereby, monomers 126 to 374 form Loop-3. The simulation starts with an equilibrated configuration of the mother DNA having the Arc-2 architecture within the cylindrical cell. As a consequence of the entropic repulsion between loops, the *oriC* gets localized spontaneously to mid-cell position. We start replication from this equilibrated configuration of the mother DNA. (b) Soon after replication begins at the *oriC*, there are two *oriC* (red circle) loci near the center of the long axis of the cylinder. The daughter-DNA polymers (DNA-1 and DNA-2) are linked to the mother-DNA at the two replication forks (RFs) as shown. The two replication forks (RFs) move along the two arms of the to-be-replicated mother-DNA to meet at *dif-ter* (purple square): also refer subfigure (c). Soon after the onset of replication in our model, we observe spontaneous emergence of the *ori-ori-ter* configuration in our simulations as seen *in-vivo*; refer schematic (b). (d) As replication proceeds, the chain length of DNA-1 and DNA-2 increases, while the mother-DNA chain length decreases. The cylinder (bacterial cell) elongates as replication proceeds. Once the 125-th and 375-th monomers of mother-DNA are replicated, the replicated monomers (125' and 375') are cross-linked to the *oriC* of DNA-2 as shown. As replication progresses, the configuration spontaneously evolves to the *ori-ter-ori* configuration as shown, with *oriCs* localized at the quarter positions. This is a consequence of daughters DNA-1 and DNA-2 adopting the Arc-2 architecture.

5.2 The *E.coli* chromosome

5.2.1 Basic Information

The *E.coli* chromosome is a single chain, has 4.6 million base pairs (BPs) and has a ring polymer architecture. It is known that there are topologically distinct domains of size $\sim 10kbp$ in *E.coli* due to gene expression [135, 110]. Various histone-like HU-proteins and other NAPs (nucloid associating proteins) also contribute to compacting the DNA at various length scales [15, 110].

The *E.coli* bacterial life cell cycle has three stages. The first stage is the B-period, which begins as soon as a new cell is formed after cell division, and lasts till the chromosome starts replication. In the B period, the average *E.coli* bacterial cell is $\approx 2.5\mu m$ long, though there can be size variations. It is roughly cylindrical with an aspect ratio of 2.5 (length to width ratio). The C-period of its life cycle begins with the start of the replication of the mother DNA at a certain loci (segment of the DNA) called the *oriC*. The C-period ends with the end of the replication process. Thereafter, the bacterial life cycle enters the D-period, which lasts till cell division occurs.

At the start of C-period, the *oriC* is found mid-cell along the long-axis and is replicated first. Thus immediately after the start of replication, two *oriC* loci belonging to two daughter strands are found near the middle of the cell. These daughter strands join with the mother DNA at the two replication forks (RFs); also refer Fig.5.1(b,c). The two daughter strands between the two RFs thus form a replication bubble. The two daughter strands and the replication bubble may not be distinguishable in FISH experiments immediately after the start of replication.

Thereafter, replication proceeds bidirectionally from the *oriC* to end at a loci called *dif-ter* in the *ter* macro-domain.

The *dif-ter* is located diametrically opposite to the *oriC*, if the chain contour was stretched out to form a circle: refer Fig.5.1c for a schematic. Thus the polymer segments between *oriC* and the *dif-ter* loci form two nearly equal arms of the ring polymer. The mother DNA-double helix is opened up in segments in a controlled manner by enzymes to form two single stranded chains, and thereafter suitable base pairs (BPs) are added on each strand to form replicated double stranded daughter DNAs. The RFs on the two arms, i.e. the segments on the two arms where replication is actively in progress, moves along the chain contour towards the *dif-ter*. They finally meet at *dif-ter*, at the end of replication. The *E.coli* cell is represented as a cylinder in the schematics.

The *oriC* of the mother is localized at the mid-cell at the start of replication. The organization soon after the onset of replication is that of the *ori-ori-ter* configuration. This implies that *oriCs* corresponding to the daughter DNAs and the yet to be replicated *dif-ter* of the mother-DNA are arranged linearly along the cell long axis such that the *dif-ter* does not lie between the two *oriCs*. This configuration has a much higher probability than the others [102]. After 15 – 20 minutes into the C-period, the FISH experiments show that the two *oriCs* branch out from the middle to occupy the so called quarter-quarter positions along the cell long-axis with the *dif-ter* in the middle, to form the so called *ori-ter-ori* configuration as shown in Fig.5.1d. This reorganization is known as the *ter-transition* [133, 20]. The cell long axis elongates while replication is in progress, to double its length as the cell readies itself for cell division. In slow growth conditions considered in this study, there are only two replication forks in a single cell cycle. But in fast growth conditions there

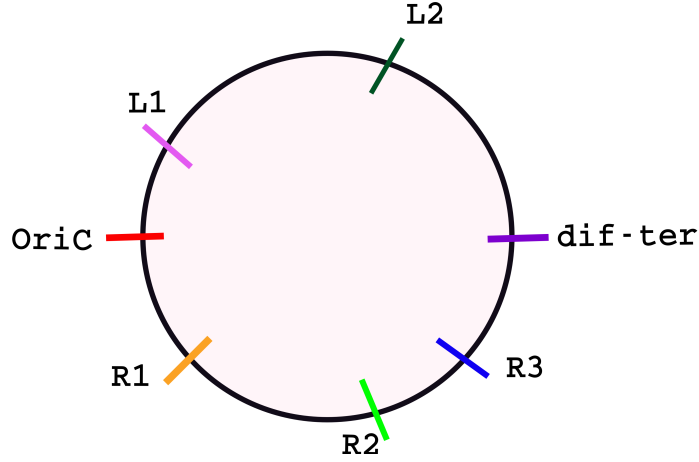


Figure 5.2: The schematic shows the positions of the loci along the chromosome-polymer contour, which are fluorescently tagged in [20]. These loci are *oriC*, L1, L2, R1, R2, R3 and *ter1*. We follow the monomers corresponding to the same loci in our simulations to infer the spatial organization of the chromosome-polymer. The monomers corresponding to these loci are 1, 22, 141, 443, 369, 304 and 228, respectively, in the 500 monomer ring polymer. The loci (monomers) are well-separated along the chain contour and therefore provide information on the spatial organization of the chromosome-polymer.

can be overlapping rounds of replication with multiple replication forks in a single cell cycle.

5.2.2 Experimental observations yet to be reconciled

We list various open questions, some of which arise due to differing interpretations of experiments. Our model for replication, segregation and localization of *E.coli* chromosomal loci tries to address or resolve the following open questions.

1. The mechanism of localization of *oriC* to the middle of the cell along the long axis before the start of replication is incompletely understood.
2. Post replication, the mechanism of localization of *oriCs* to the quarter positions along the long axis of the cell is incompletely understood.
3. The two *oriCs* of daughter DNA is observed to remain at the mid-cell for 15-20 minutes, before branching out to the positions along the cell long axis. This is referred to as the time of cohesion. Any mechanistic model that tries to explain the dynamics of chromosomal segregation should account for the delay in the spatial segregation of loci after replication of the corresponding loci.
4. There exist two opposing views on the spatial organization of replication forks:
 - (i) The ‘Replication factory’ model of chromosome replication suggests that the replication of the chromosome occurs only at a certain region (or factory) in the cell. This factory is said to be located in the mid-cell region of the cell along the cell long axis. The replication forks are assumed to be colocalized and relatively stationary in this region, while the chromosomal segments get replicated as

they get translocated to the mid-cell region [136]. (ii) The ‘Train track’ model of replication suggests that replication takes place at the replication forks as they move bi-directionally along the DNA-polymer contour before eventually meeting at the *dif-ter*. The DNA-polymer contour pervades the nucleoid and thus the RFs can remain spatially separated.

It has been observed by a recent experiment that the RFs move along the two arms of the chain contour [92]. The question is how do the replication forks remain at the mid-cell position for extended periods in the cell cycle, despite their movement along the contour track ?

5.3 Model

5.3.1 The bead spring model of the chromosome

We use a bead spring model of polymers to represent the bacterial chromosome. In the model of the modified-ring polymer we consider a chain of spherical beads, where each bead is connected to a pair of neighbouring beads by harmonic spring interactions given by $V_{spring} = \kappa(r - a)^2$, where $\kappa = 100k_B T/a^2$ is the spring constant of the springs. The quantity r denotes the distance between two neighbouring monomers. The unit of length in our simulations is a , which is the equilibrium length of springs between adjacent monomers along chain contour. Excluded volume interactions between monomers are modelled by the WCA potential. Each monomer has diameter $0.8a$ and the expression of the WCA potential is :

$$V_{WCA} = 4\epsilon[(\sigma/r)^{12} - (\sigma/r)^6] + \epsilon_0, \quad \forall r < 2^{1/6}\sigma \quad (5.1)$$

where $\epsilon = k_B T$ is chosen. The quantity ϵ_0 is needed to ensure that the potential goes smoothly to zero at the cutoff, and $V_{WCA} = 0$ for values of r greater than the cutoff. If the potential is cut-off at $r = 2^{1/6}\sigma$ as has been done here, then $\epsilon_0 = \epsilon$.

The unreplicated DNA of *E. coli* having 4.6 million bp (base pairs), is represented as a bead-spring ring polymer with 500 beads in our model, modified with additional CLs. Thus each bead represents around 9200 bp. For the ring architecture with 500 monomers, the last (500-th) monomer is connected by a spring to the first monomer, as well as with the 499-th monomer in the chain. We perform Monte Carlo (MC) simulations starting with a modified ring-polymer architecture, and equilibrate the polymer within a cylinder which mimics the confinement imposed by the cell, refer Fig.5.1a. We implement cylindrical confinement in our simulations by rejecting trial displacements which lead to monomers moving outside the confining cylinder. Therefore, we implement a potential which is infinite at the walls of the cylinder and beyond. We do not incorporate the effects of supercoiling in our coarse-grained model since the mean size of supercoiled plectoneme is $\approx 10kbp$ which is roughly the size of a single monomer in our model.

The first monomer in the ring is labelled as the *oriC* and monomer number 250 is assumed to be the *dif*-locus in *ter* domain, hereafter referred to as *dif-ter*. In the the Arc-2 architecture the 125-th and the 375-th monomer are cross-linked to monomer-1, i.e. the *oriC*. These cross-links (CLs) lead to the formation of loops, refer Fig.5.1a. This Arc-2 polymer spontaneously gives rise to the specific localisation of *oriCs* and other loci along the cell long axis, even as it adopts different configurations (statistical micro-states) in equilibrium.

We first equilibrate a single Arc-2 polymer which represents the mother DNA

confined within a cylinder of diameter $D_c = 7a$ and length $L_i = 17.5a$. From this equilibrated configuration of the mother DNA, we initiate the replication process in our model. This marks the beginning of the C period in the cell cycle. At this stage we start collecting data pertaining to the segregation process, which we present in the ‘Results’ sections.

We now explain how the dimensions of the confining cylinder are chosen. Note that the bead size is chosen to be $0.8a$ and there are 500 monomers in a cylinder (at the start of the simulation) with an aspect ratio of 2.5 and a volume fraction (ϕ) of 0.2 as seen *in-vivo*. Then we know that:-

$$\phi = 0.2 = N_i \times \frac{4}{3}\pi r_b^3 / \pi R_c^2 L_i \quad (5.2)$$

where $R_c = D_c/2$, $r_b = \sigma/2$ and N_i denotes the initial number of beads in the cylinder. Since $L_i/D_c = 2.5$, we obtain $L_i = 17.5a$ and $D_c = 7a$. The diameter of the *E.coli* cell is $\sim 1\mu m$, then the characteristic length scale in our simulations, $a \approx 150nm$. As the the DNA gets replicated and monomers get added as a consequence, the length of the cylinder doubles itself to be $L_f = 35a$, through an incremental increase every 2×10^5 MCS (Monte Carlo steps). This increase in the length of the cylinder is implemented simultaneous to the replication step where we add two monomers at every step.

After equilibration, we perform 150 million MCS in each simulation run. We do not model the cell division process in our minimal model, which occurs at ≈ 60 minutes *in-vivo*. Instead, after the completion of replication at 5×10^7 MCS, the two model daughter chromosomes remain attached at *dif-ter* loci by a spring of equilibrium length a . We follow the positions of different loci of the daughter

chromosomes, labelled as L1, L2, R1, R2, R3 and *ter1* in [20]. The positions of these loci correspond to monomer indices 22, 141, 443, 369, 304 and 228 in our ring polymer. Note that the positions of these loci correspond to well separated points along the chain contour and thus provide information on the organization of the chromosome-polymer, refer Fig.5.2.

After the equilibration of the mother DNA having Arc-2 architecture in the cylinder, we start replication of the mother DNA from monomer-1 i.e *oriC*. In this stage-II of simulation, we also ensure that chain crossing and release of topological constraints is allowed at regular but infrequent intervals, which is ensured by the presence of the topoisomerase enzyme *in-vivo*. The effects of topoisomerase is switched on every 10000 Monte Carlo steps (MCS) for the next 900 MCS. For these 900 MCS, we reduce the excluded volume between monomers by choosing $\sigma = 0.1a$; this allows chains to cross each other during Monte Carlo updates. We have also checked that if the rate of switching on the effect of topoisomerase, is chosen to be much lower than the rate chosen for this study, then the segregation process is slowed down as a consequence of increased topological constraints. However if the rate is chosen to be much higher than the value chosen here, then the effects of excluded volume are diminished and consequently entropic organization of loci is adversely affected. Note that this step renders the simulation-scheme to be inherently non-equilibrium. The small random displacement of a monomer in each MCS (with a maximum displacement $\delta = \pm 0.1a$ along $\hat{x}$, $\hat{y}$ and $\hat{z}$) models local diffusive motion of the chain.

5.3.2 Are the modified architectures viable?

We use CLs to bridge specific segments which are at a distance of about $\sim 1Mbp$ away from each other to create the Arc-2 architecture. The sites of the chosen monomers which form CLs, are consistent with the experimental observation pertaining to the aggregation of specific ribosomal RNA operons located at distant chromosomal loci along the chain contour [137]. Thus the aggregation of ribosomal RNA operons (rRNAP) located at distant chromosomal loci may induce ‘effective’ cross-links along the chromosome-polymer contour [137]. The sites of crosslinks in Arc-2 (monomer indices 125, 375 and the *oriC*) roughly correspond to the positions of the rRNA operons *rrnG*, *rrnH*, *rrnA* respectively, which are spatially colocalized *in-vivo* [137].

Alternatively, the CLs may arise due to SMC proteins such as MukBEF which are known to create contacts between segments separated by $\sim 900kbp$ [138, 110]. Previous studies already suggest that the *E.coli* chromosome is organized into ‘loop domains’ [139, 140, 141]. It has also been shown previously that depletion of some SMC proteins like MukBEF leads to defects in the segregation of the *E.coli* chromosome, thus underlining their importance [142, 21, 110]. Moreover, it has been shown that the MukBEF concentrations are higher in the locations within the cell where the *oriC*(s) are found [103].

In the Arc-2-2 architecture (discussed subsequently), we introduce two additional CLs at specific sites. The CLs link segments which are 82 monomers apart. Specifically, we crosslink monomers pairs (136, 218) and (282, 364) in addition to the CLs introduced for Arc-2. This is done specifically to obtain a contact map similar to the experimentally obtained Hi-C map for *E.coli*, while also localizing genomic loci as seen in FISH experiments.

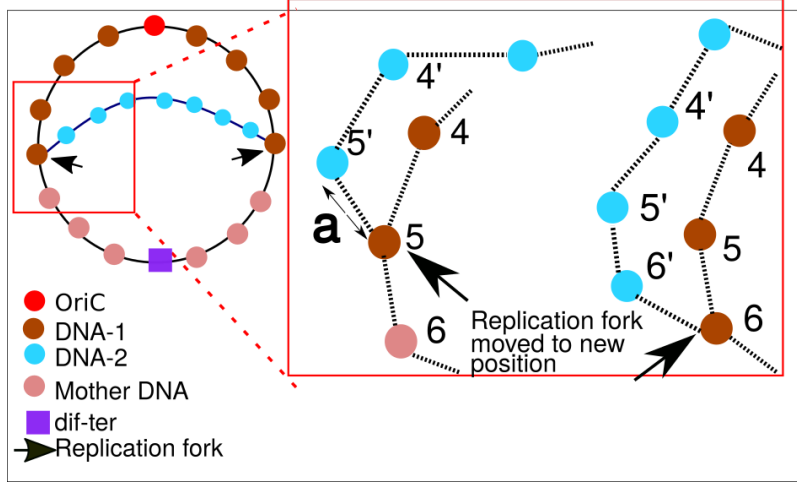


Figure 5.3: **A schematic detailing the method by which we mimic replication to create DNA-2 at the RFs in our simulations.** Suppose the two RFs are at position i and $500 - i$. For instance if $i = 5$, the RFs are at the position of the 5-th monomer and the 495-th monomer. The 5-th monomer is connected by springs to the 4-th and the 6-th monomer. During replication of the 5-th (and 495-th) monomer, a monomer will be introduced at a random position but maintaining distance a , from the 5-th monomer (and 495-th monomer). Thus, the 5-th (and the 495-th) monomer gets connected to a third monomer, which we label as 5' (and 495') belonging to DNA-2. The 5'-th (and 495'-th) monomer is in turn also connected by another spring to the 4'-th (and 496'-th) monomer. The RF then moves to the monomer number 6 and 494, respectively, where the above process is repeated after 2×10^5 iterations. After the parent DNA has been completely replicated, we have two 500-monomer chains, each with its own *oriC* and *dif-ter*. The two *dif-ter* remain connected by a spring till the end of simulations.

We also show subsequently that the sites of the CLs used in Arc-2 and Arc-2-2 show higher contact probabilities in the experimentally obtained contact map of [21]. Furthermore, we emphasize that our results are also reasonably robust to variations (by 5 monomers on either side) in the sites of cross-linking.

5.3.3 Modeling replication

The complex machinery of opening of the mother chromosomal strands by helicase which results in two single strands of DNA, and thereafter creating two daughter ds-DNA (double stranded DNA) is significantly simplified in our model. We model the replication forks only implicitly. The two replication forks (RFs) start moving bidirectionally from one monomer to the next starting from the *oriC* towards *dif-ter* as the simulation proceeds, refer Fig.5.1(b,c). Once the RF crosses a particular monomer along the chain contour of the mother DNA, we re-label that monomer as a monomer belonging to daughter DNA-1. This is equivalent to the addition of base pairs to the single stranded DNA to create the double stranded daughter-DNA. The creation of the other daughter, DNA-2 by replication is carried out in a different manner in our simulations. We create the other daughter DNA-2 by explicitly adding two monomers, representing 9200bp each, every 2×10^5 Monte Carlo steps (MCS). The newly added monomers are added in random directions at a distance a from the monomers of the parent DNA where the RFs are currently located.

Thus, we have assumed that blocks of 9.2 kbp of compacted ds-DNA is added at regular intervals to create daughter DNAs at the length scale a ($\approx 150nm$). Any of the processes occurring at lower length scales involving proteins cannot be resolved and are beyond the scope of our modeling. Thereby, in our description we cannot distinguish ss-DNA (single stranded) versus ds-DNA, nor can we comment anything about which is the leading strand or the lagging strand.

We discuss in detail the mechanism of creating DNA-2 near the RFs in Fig.5.3. Our modelling is in accordance with the train track model of replication, though we will see later that RFs will be located near the cell-center for extended periods

in its life cycle. Thus, consistency with the replication-factory model will emerge spontaneously from our simulations.

5.3.4 Details of cross-linking process in DNA-2

We introduce cross-links (CLs) between monomer 1' and 125' immediately after 125'-th monomer is introduced in DNA-2; also refer Fig.5.1d. Simultaneously, we also cross-link monomer 1' to 375' of DNA-2. Note that the 375'-th monomer gets introduced due to replication at the same time as the 125'-th monomer. These additional CLs introduced in DNA-2 are realised by harmonic springs of length a . Thereby, DNA-2 also adopts the Arc-2 architecture. We remind the reader that the corresponding CLs for DNA-1 are already present since the monomers of the mother DNA have been relabelled as DNA-1. The mother DNA was already in the Arc-2 architecture since the start of replication in the simulation.

The introduction of the CLs leads to the subsequent movement of the monomer pairs towards each other, unless prevented by topological constraints. Attempts to release Topological constraints are made at regular intervals as specified in Section III-A. We appreciate that the directed movement of cross-linked monomers towards each other, as modelled by us through the introduction of springs, is unlikely to occur *in-vivo*. In reality the spatial proximity of genomic segments is likely to be realised by other mechanisms (e.g. diffusion or directed transport by proteins) before they get cross-linked. Thus, it is possible that the “effective crosslinks” proposed herein, is achieved in reality over longer timescales than those implemented here. However in our coarse grained simulation model we do not take such mechanisms into account. Instead we investigate the consequences of the bacterial chromosomes adopting a

modified polymer architecture.

5.3.5 Choice of Monte Carlo simulations

We conduct our studies on the evolution of the spatial organization of replicating chromosomes using Monte Carlo (MC) simulations. MC has not usually been the simulation technique of choice for the study of dynamical aspects, but it has been established previously [143, 144] that Monte Carlo simulations effectively reproduce diffusive dynamics of particles, although at a coarse grained level. Thus Monte Carlo simulations have been used extensively on subjects ranging from phase separation kinetics [145] to polymer dynamics [146, 147]. We also ensure that we keep low volume fractions so as to obtain diffusive dynamics of chain segments.

Using Monte Carlo (MC) for the study also allows us to reduce the bead size of the beads in our bead spring model at already specified rates. This is done to mimic the effect of the Topoisomerase enzyme which is present *in-vivo* to release topological constraints. This cannot be done using conventional Langevin (or Brownian Dynamics) simulations as it would imply introducing discontinuities in potentials and tremendously high forces as a consequence. Note that the incorporation of topological constraint release implies that the study is being conducted on an intrinsically non-equilibrium system. Here, the MC simulation achieves the random displacement of monomers which mimics the local diffusive dynamics of chromosomal segments confined in a cylinder (cell).

5.4 Results and Analysis

5.4.1 Enhanced propensity for DNA segregation

In Fig.5.4a, we show a representative snapshot of our simulations at the end of replication with two daughter DNAs. We can identify the positions of the two *oriC* (red circles) and *dif-ter* (blue circle). The color gradient indicates the position of a monomer along the chain contour, and the color code is identical for the two DNA-polymers. Thus the reader can see the positional organization of the two polymers as one moves from monomer-1 to 500 (and monomers 1' to 500') along the chain contour. Monomers 125 and 375 as well as monomers 125' and 375' are close to their respective *oriCs* due to the presence of CLs. The *oriCs* correspond to monomer indices 1 and 1'.

In Fig.5.4b, we show data for the distance between the center of mass ($D_{C.O.M}$) of the two daughter DNA-polymers for 4 independent runs, during and after the process of replication of the mother DNA. We see the C.O.M. of the two polymers are initially close to each other, but they move apart such that their final values are $17.5a$, which is half of the length of the cylinder at the end of replication. Thus the segregation of the two daughter DNA-polymers to two halves of the cylinder is typically complete in $\approx 6 \times 10^7$ iterations as seen in runs 1, 2 and 3 of Fig.5.4b. This is 10^7 MCS after the end of replication of the mother DNA. We intentionally also show data for run4, to emphasize that there are infrequent cases where segregation remains incomplete.

In Fig.5.4c, we also compare the number of MCS required (τ_S) to accomplish entropic segregation of two overlapping ring-polymers with 500 monomers without

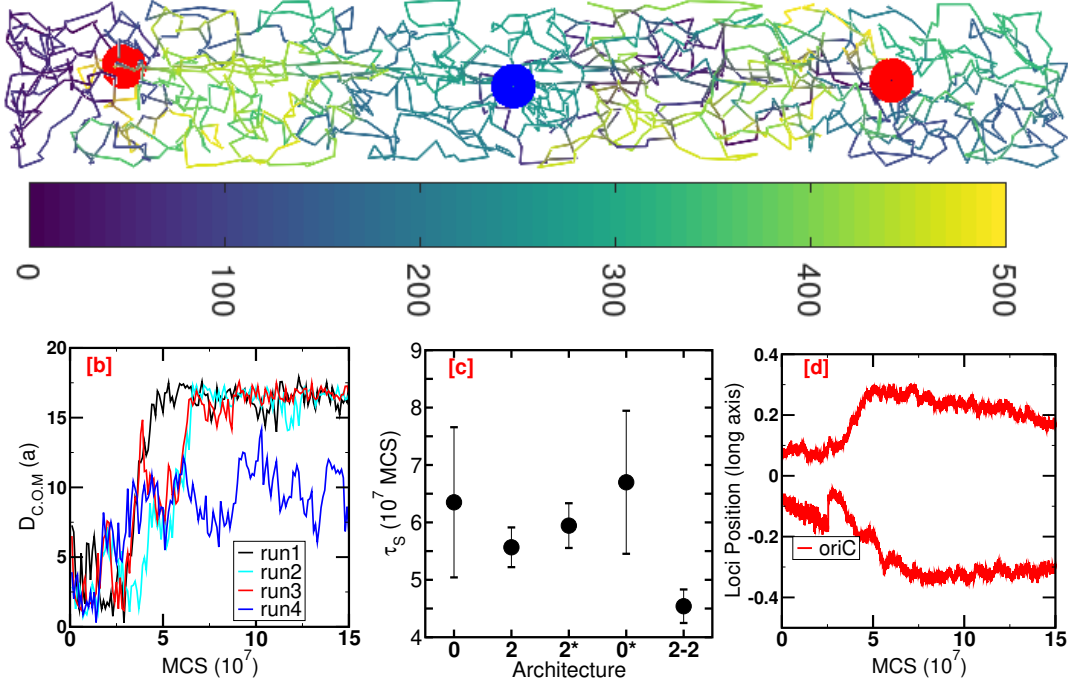


Figure 5.4: Effect of modified polymer architectures on chromosome-polymer segregation (a) A representative snapshot of the segregated polymers from our simulation at the end of the replication process at 5×10^7 MCS is shown. The length of the cylinder (not shown) is $35a$, diameter remains $7a$ ($a \equiv 150\text{nm}$). The two red circles represent the *oriC* close to the two quarter positions and the blue circle is the *dif-ter* locus of DNA-1 located at the mid-cell position. The *dif-ter* of DNA-2 is at a distance of a from the *dif-ter* of DNA-1 as they remain crosslinked till the end of the simulation. The colour changes as we move along the contour from monomer 1 to 500 (and $1'$ to $500'$): refer colour bar. We see that specific segments of the polymers get localized within the cylinder. This will also be quantitatively established later. (b) The distance $D_{C.O.M}$ between the two centers of mass (C.O.M) of each daughter DNA is plotted versus MCS, from the start of replication process. Data for 4 independent runs ($\equiv$ cell cycles for 4 individual cells) are shown. (c) The number of MCS (τ_s) required for entropic segregation calculated for different polymer architectures (refer main text) is shown in this subfigure. The quantity τ_s has been computed by averaging over 10 independent runs for each architecture. (d) The trajectories of the two *oriC*s are shown from the start of the replication. The trajectories shown are calculated by averaging over the trajectories of the *oriC*s from 20 independent runs. The cylinder doubles its length from $L_i = 17.5a$ to $L_f = 35a$ over 5×10^7 MCS, and the *oriC* trajectories shown are normalized by L_f . The trajectory corresponding to the negative half of the cylinder represents the trajectory of DNA-2, following convention of [20].

loops (Arc-0 architecture) and the Arc-2 architecture. When $D_{C.O.M}$ exceeds $11a$ ($\approx 1.5D_c$) at say at the I_{MCS} iteration, and furthermore, the $D_{C.O.M}$ remains greater than $11a$ for the next 4×10^7 MCS, we deem the daughter chromosomes to have been segregated at $\tau_S = I_{MCS}$. We observe that segregation takes about $\tau_S \approx 6 \times 10^7$ MCS with Arc-2 and even smaller τ_S with Arc-2-2, refer Fig.5.4(c). There are large variations in the value of τ_S for the Arc-0 architecture, but the average value is greater than the values of τ_S with Arc-2 architecture. Note that we implement the process of replication for all the cases shown in Fig.5.4c with identical replication rates.

The mechanism of segregation of the two polymers to two halves of the cylinder is purely entropic in all the cases. If the two ring-DNA-polymers (Arc-0) occupy different halves of the cylinder they can explore more phase space conformations, and therefore segregate to minimise free energy as has been shown previously by studies of Jun and Mulder [13, 14]. In the case of architecture Arc-2 (or Arc-2-2), the internal loops labelled Loop-1, Loop-2 and Loop-3 (refer Fig.5.1a) of both DNA-1 and DNA-2 repel each other, i.e. it gives rise to intra-chromosomal entropic repulsion. Moreover, the ‘internal’ loops also repel loops belonging to the other daughter DNA, i.e. it also gives rise to enhanced inter-chromosomal entropic repulsion, as discussed later. This ‘internally’ looped architecture of the chromosomes facilitates the segregation process, and as we show later, also leads to the localisation of genomic loci. Note that when the daughter chromosomes are *completely* segregated once $D_{C.O.M} \approx L_f/2 \equiv 17.5a$, which might take a bit longer. We avoid this criteria for identifying segregation, because fluctuations in $D_{C.O.M}$ often leads to $D_{C.O.M} < 17.5a$ over the next 4×10^7 MCS.

Moreover, if we introduce the additional CLs of Arc-2 architecture in both daugh-

ter DNAs after the replication of a ring polymer (Arc-0 architecture) is complete, i.e. after 5×10^7 MCS instead of our usual protocol, then the number of MCS required for segregation increases. We refer to this system as Arc-2* and show the corresponding τ_S in Fig.5.4c. If we prevent topological constraint release by not allowing chain crossing for a simple ring polymer architecture (Arc-0* with 500 monomers) they often get entangled and can only segregate in 40% of the runs. However, we checked that shorter ring polymers with 100 monomers can easily segregate even without incorporating the effects of topoisomerase, as seen previously [17]. The value of τ_S is calculated by averaging over the runs that lead to successful chromosome segregation from a pool of 10 independent runs for each architecture. For Arc-0, Arc2, Arc2* and Arc-2-2, we observe complete segregation of the two daughter DNA-polymers in 8 out of 10 independent runs during the length of the simulation (15×10^7 MCS). Thus we infer that the presence of internal loops in each ring-polymer for Arc-2 polymer architecture leads to lower τ_S , i.e. fewer MC steps, required for segregation as compared to that for a ring polymer which we term as Arc-0.

For Arc-0, Arc2, Arc2* and Arc-2-2, we observe complete segregation of the two daughter DNA-polymers in 8 out of 10 independent runs during the length of the simulation (15×10^7 MCS). Thus the error bars have been computed using those “successful” 8 runs. However, for the simulations with Arc0* architecture, we observe segregation in only 4 out of 10 independent runs. Therefore, the error bar has been calculated from those 4 “successful” runs for Arc0*.

Using the blob picture we have also analytically established in [4] that the difference in the free energy between the spatially segregated and the non-segregated states for cylindrically-confined polymers, is higher for Arc-2 (and Arc-2-2) than for Arc-0.

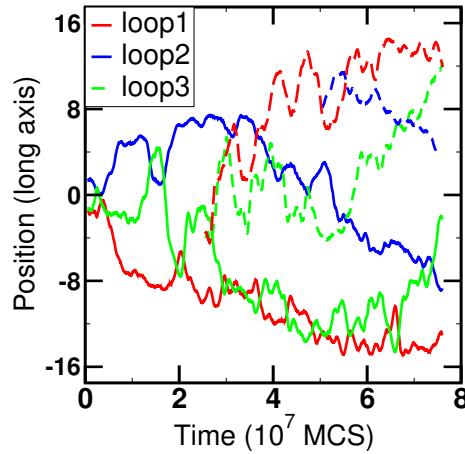


Figure 5.5: The figure shows the trajectories of the z coordinates of the C.O.M.s of the internal loops of DNA-1 and DNA-2, as a function of MCS. Data is shown for one representative simulation run. The position of C.O.M. of each loop has been calculated every 10000 MCS and is subsequently running averaged over the next 10^6 iterations. The process of calculating the running average reduces the fluctuations in the positions, and thereby represents the local position in a particular interval of the simulation run. We follow the same color scheme for the loops as in Fig.5.1. The solid line and the dashed line of a particular colour represent the loops of two daughter chromosomes DNA-1 (or equivalently mother DNA) and DNA-2, respectively. Note that the dashed blue curve corresponding to Loop-3 is seen only after 5×10^7 MCS, as all the constituent monomers have been replicated only by 5×10^7 MCS. The dashed pink and the green curves bifurcate after 2.5×10^7 MCS, i.e. when we introduce the CLs for DNA-2.

5.4.2 Localization of *oriC* to quarter positions

At the start of the replication process in our simulations, a new *oriC* monomer is created and is named the *oriC* of the DNA-2-chain. We see in Fig.5.4d, that the two *oriC*s are close to the middle of the cylinder at the start of the simulation. The two additional CLs are created after 2.5×10^7 MCS. Once the CLs are introduced between the newly added 125'-th and the 375'-th monomer and *oriC* of the DNA-2-chain at 2.5×10^7 MCS, we see a sharp change in *oriC* position of the DNA-2 polymer chain (bottom red line) of Fig.5.4d. For the monomers on the other chain, we only change the monomer status from mother to daughter as RF position moves along the contour and rename it monomers of DNA-1, and hence the CL between *oriC* & 125 and *oriC* & 375 monomers existed even at the start of replication. After the loops of DNA-2 are created, we observe that the *oriC*-s start migrating out to occupy the two quarter ($1/4$ & $-1/4$) positions on the elongated cylinder axis as measured from the mid-cell.

However this migration of the *oriC*s occurs only after $\sim 3 \times 10^7$ MCS. This is also in qualitative agreement with the experimental observation of a ‘cohesion time’ of ~ 15 mins. The two *oriC*s remain proximal near the mid cell position for ~ 15 mins and thereafter migrate to different cell halves [20].

5.4.3 Enhanced entropic repulsion between daughter chromosomes due to the presence of loops:

We establish the mechanism by which the addition of two CLs enhances the propensity of entropic segregation between DNA-1 and DNA-2 as discussed earlier. We note

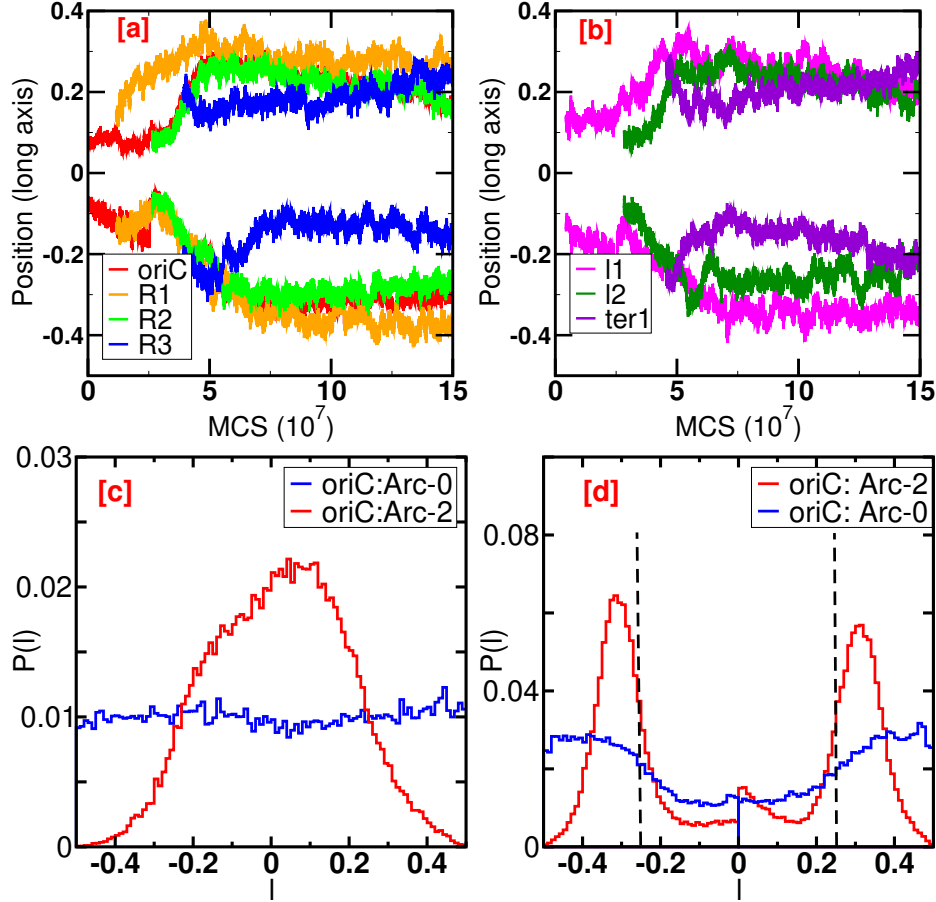


Figure 5.6: The first two subfigures show the trajectory of the (a) *oriC*, R1, R2 and R3 loci and (b) L1, L2, and *ter1* loci along the cylinder long axis after the replication of the corresponding monomer of the mother-DNA. The z axis lies along the cylinder long-axis. To plot the trajectories of the loci, we averaged the z coordinate over 20 independent runs and scale it by the final length L_f of the confining cylinder. The loci of DNA-2 are assigned the negative axis. (c) Comparison of the probability distribution of the *oriC* position along the cylinder long-axis for the mother-DNA for Arc-0 and Arc-2 polymer architectures. The x-axis has been scaled by L_i . Data is collected from 20 independent runs over 5×10^7 MCS after equilibration of a single polymer chain in a cylinder of length $17.5a$. Here we have switched off the replication process. We note that Arc-2 shows a peaked distribution around the midcell while for Arc-0 *oriC* shows a uniform distribution. (d) Comparison of the probability distribution of the position of *oriC* of daughter DNAs, post replication, for Arc-0 and Arc-2. The x-axis has been scaled by L_f . The black dashed lines in (d) represent the quarter positions and have been added for aid of visualization. We note that Arc-2 shows a narrower distribution approximately around the quarter positions, as compared to Arc-0. Data is collected from 20 independent runs post replication, from $10 - 15 \times 10^7$ MCS for the distributions shown in (d.)

that the additional CLs lead to the formation of internal loops within each daughter chromosome, refer Fig.5.1a. We follow the z-coordinates of the C.O.M of each of the internal loops for both DNA-1 and DNA-2, as a function of the Monte Carlo steps: *MCS*. These are shown in Fig.5.5. The long-axis of the cylinder is considered parallel to $\hat{z}$. Each color represents a particular loop. Note that there are two lines of the same colour corresponding to loops belonging to different polymers (daughter chromosomes). The solid line represents the position of the C.O.Ms of the loops in the mother DNA initially. After the bifurcation of the solid line into two lines, the solid and the dashed lines represent C.O.Ms of the loops of DNA-1 and DNA-2, respectively.

The C.O.Ms of the loops of DNA-2, are calculated after all the monomers belonging to a particular loop have been replicated, and CLs have been introduced at the appropriate positions. The bifurcation of the solid lines corresponding to Loops 1 and 2 occur at 2.5×10^7 MCS in Fig.5.5, because it is only then that the CLs are introduced which result in the formation of these loops for DNA-2. Loop-3 in DNA-2 is formed only after all the constituent monomers have been replicated. Thus the dashed blue line corresponding to Loop-3 of DNA-2 is shown only after 5×10^7 MCS in Fig.5.5.

We note that the two lines of the same color, corresponding to a particular loop belonging to DNA-1 and DNA-2, drift away from each other as the simulation evolves. This is indicative of entropic repulsion between the loops belonging to different DNAs. Furthermore, the presence of these loops thus enhances the propensity for entropic repulsion between the two daughter chromosomes. The loops belonging to a particular daughter-chromosome also occupy different regions along the cell long axis and minimize overlaps between them. We have shown data for only one simulation run

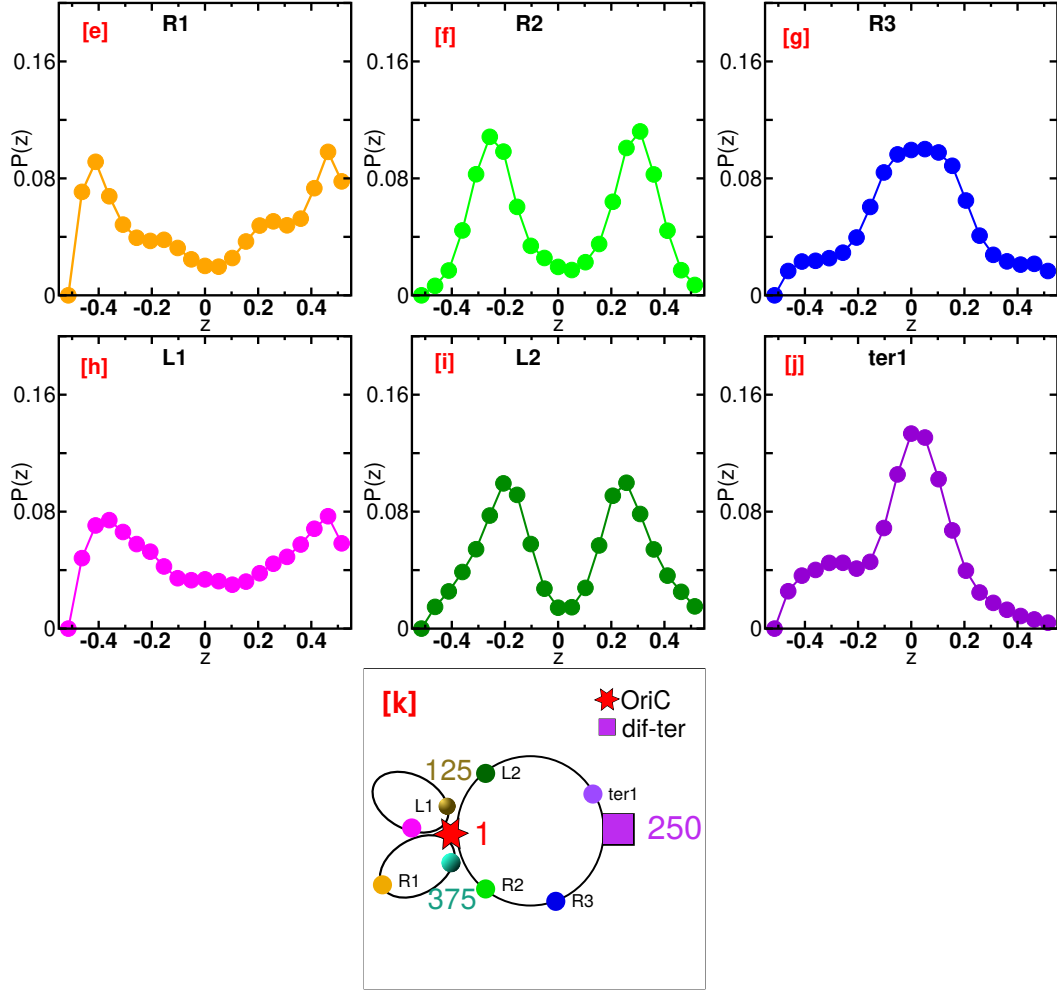


Figure 5.7: We show the probability distributions of the (a) R1 (b) R2 (c) R3 (d) L1 (e) L2 and (f) *ter1* loci for Arc-2 architecture, with the x-axis scaled by L_f . Data is collected from 20 independent runs post replication, from $10 - 15 \times 10^7$ MCS for the distributions shown in (a), (b), (c), (d), (e), and (f). In subfigure (g) we show the positions of the various loci along the Arc-2 polymer contour. Since the loci L2 and R2 are proximal to the sites of the CLs (monomers 125 and 375 respectively) they get localized close to the *oriCs*.

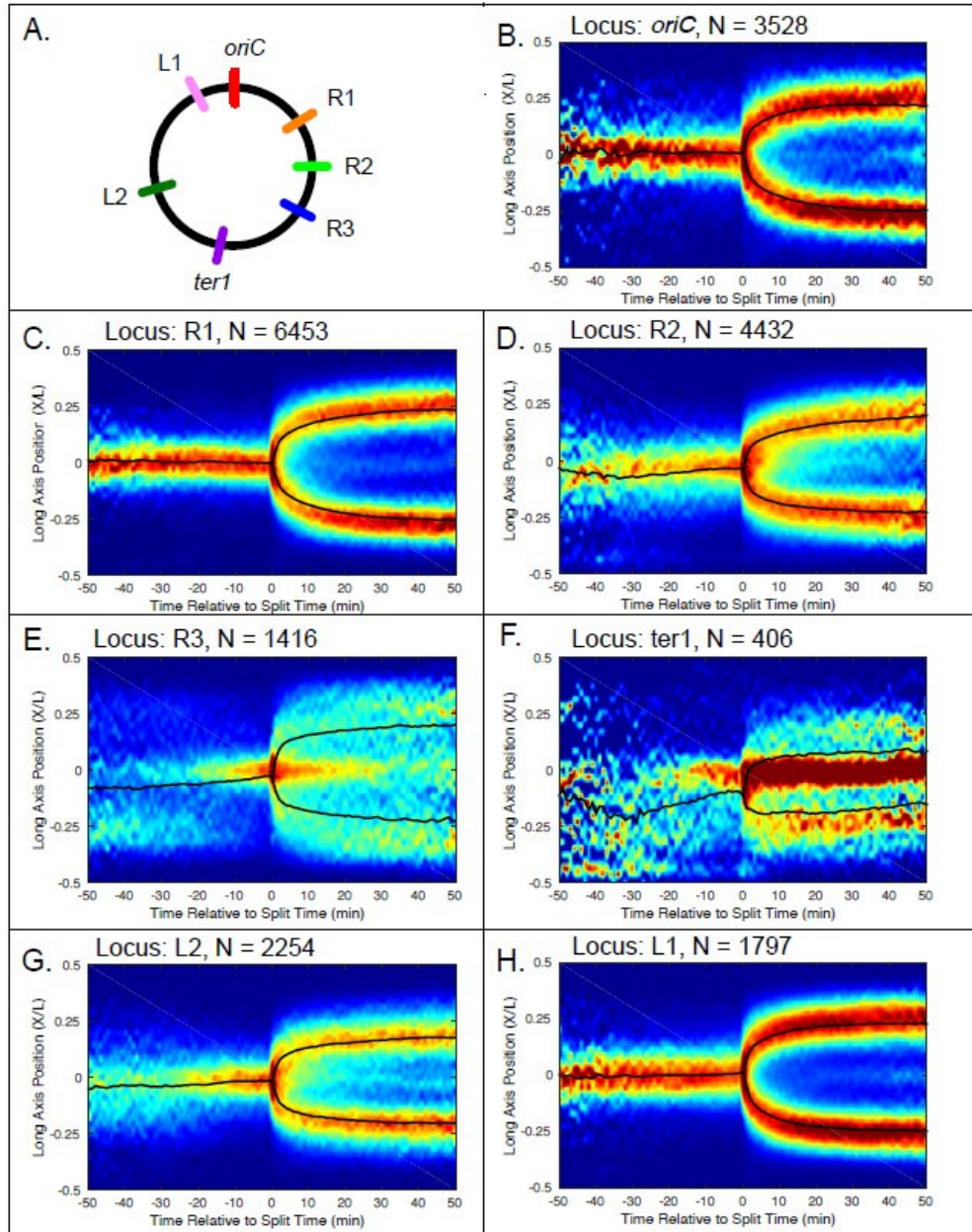


Figure 5.8: We reproduce here the experimentally obtained data of [20] after having obtained requisite permissions. The data shows the average trajectories of different loci. The color indicates the probability with red being the highest and blue being the lowest respectively. The data is presented here for aid of comparison with the results obtained from our simulations.

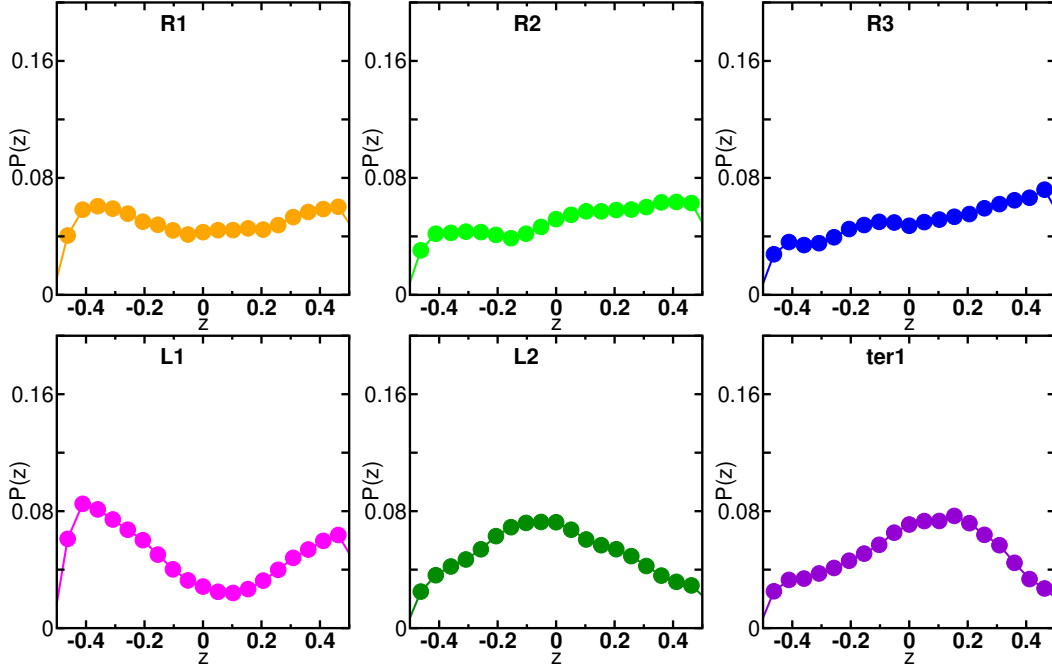


Figure 5.9: The data shows the probability distribution of the monomers corresponding to the (a) R1, (b) R2, (c) R3, (d) L1, (e) L2 and (f) ter1 loci along the cylinder long axis for architecture Arc-0. The probability distributions in (a), (b), (c), (d), (e) and (f)) have been calculated from 20 independent runs post replication, from $10 - 15 \times 10^7$ MCS.

but we have checked that other independent runs show different dynamics but share similar generic features in that the the overlap between loops remain minimal.

5.4.4 Localisation of other genomic loci.

In Fig.5.6(a,b) we follow the positions of the monomers corresponding to loci labelled as *oriC*, R1, R2, R3, L1, L2 and *dif-ter* [20] as the simulation proceeds. The reader can also refer to Fig.5.2) to get an idea of the position of the loci along the chain contour.

The trajectories of the different loci can be seen in Fig.5.6. We see that different loci segregate out to two different halves of the cylinder after their replication. We have plotted the z coordinates of the monomers from the stage that the RFs reach the individual monomer locus of the mother-DNA, and thereafter get replicated to form monomers of DNA-1 and DNA-2. We observe that though the two *oriC*s are formed at the start of the simulation, they branch out to the two halves of the cylinder only after the Loop-1 and Loop-2 of DNA-2 are created. This is consistent with the observation of cohesion time in experiments, where newly replicated loci spatially drift apart from the cell center after an interval of time [20]. Except for *oriC* and *R1* loci, all other loci have low cohesion times in our simulations. The trajectories of the loci are averaged over 20 independent runs using z -positions collected every 10^4 MCS. Each run starts from a statistically independent initial equilibrated configuration of the mother DNA. The trajectories of loci shown in Fig.5.6(a,b) is in agreement with the experimental data of loci trajectories as given in Cass et.al [20]. The loci reach specific locations along the cell long axis in our simulations. These locations are in agreement with those reported in experiments by [20]. However, we have larger fluctuations in the trajectories as compared to the data of Cass et.al. as their data is averaged over the trajectories of loci in approximately ten thousand cells.

The mutual repulsion between internal loops in an (unreplicated) mother-DNA-polymer with Arc-2 architecture also localizes the *oriC* to the middle of the cylinder at the beginning of replication process. To establish this, we create an equilibrium structure of a single Arc-2 DNA-polymer to simulate an unreplicated chromosome with the replication process switched off. We observe that the *oriC* localizes to the middle of the cylinder with $L_i = 17.5a$ which is as seen *in-vivo* [102, 20]: refer

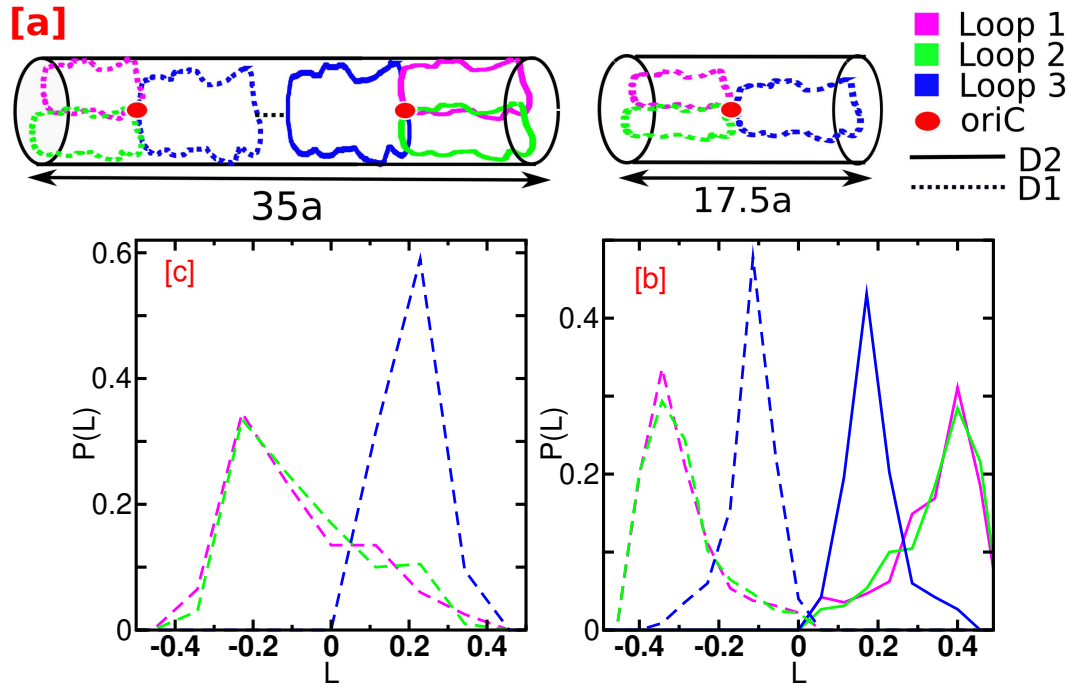


Figure 5.10: **Mechanisms of loci localisation due to entropic repulsion between loops.** Subfigure (a) shows a schematic of a single unreplicating mother chromosome having Arc-2 architecture (left). Subfigure (a) also shows a schematic of two daughter chromosomes linked at the *dif-ter* having Arc-2 architecture (right). Subfigure (b) shows the probability distributions $P(z)$ of the C.O.M.s of the three loops for the mother-DNA. The $P(z)$ have been computed from equilibrated (un-replicating) conformations of a single Arc-2 polymer confined in cylinder. The x-axis has been scaled by L_f . There is minimal overlap in the $P(z)$ of loops-1 & -2 with loop 3. The locus *oriC* is by design the junction of these 3 loops and hence gets localised to the mid-cell position. Subfigure (c) shows the $P(z)$ of the C.O.M.s of the three loops of DNA-1 and DNA-2 having the Arc-2 architecture. To plot the distributions we adopt a convention where we plot $P(z)$ of C.O.M of the loops of DNA-2 on the negative x -axis while those of DNA-1 are plotted on the positive x -axis. The x-axis has been scaled by L_f . The *oriC* locus is again at the junction of the three loops, which leads to its quarter positions for each DNA polymer. The distribution in (c) has been computed over 50 independent runs and over 10×10^7 MCS after the process of replication is over.

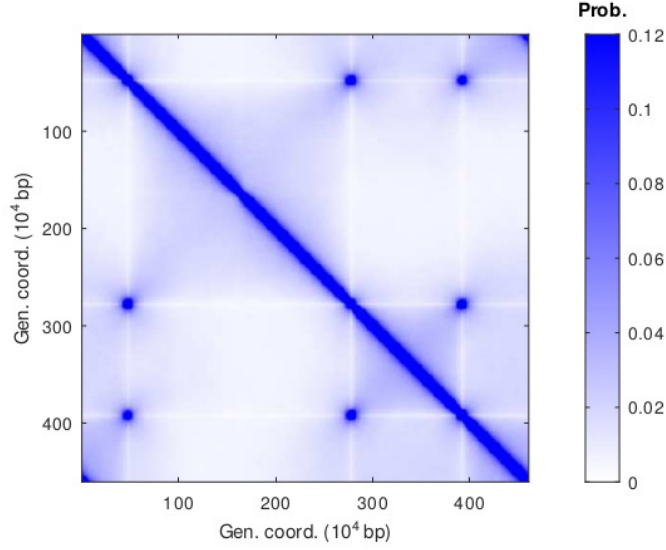


Figure 5.11: We show the simulated contact map obtained with Arc-2. The organisation obtained is not similar to that obtained by [21].

Fig.5.6c. Note that the effect of topoisomerase has been incorporated as described in the ‘Model’ section. In contrast, the *oriC* of a ring polymer with 500 monomers (Arc-0) is found to be non-localized along the cylinder axis under equilibrium conditions.

If replication is switched on, the two *oriC*s move to the two quarter positions along the cell axis if the daughter DNAs have Arc-2 architecture. To that end, we plot the probability distribution $P(z)$ of the positions of the two *oriC*s of DNA-1 and DNA-2 post replication, refer Fig.5.6d. The distribution is sharply peaked around the quarter positions for Arc-2 architecture as compared to the corresponding distributions for the Arc-0 architecture. Similar data for the other loci is given in Fig.5.7. Thus the presence of loops lead to the localization of different loci in agreement with experiments [20], as can be inferred from the experimentally obtained trajectory curves of Fig.5.8.

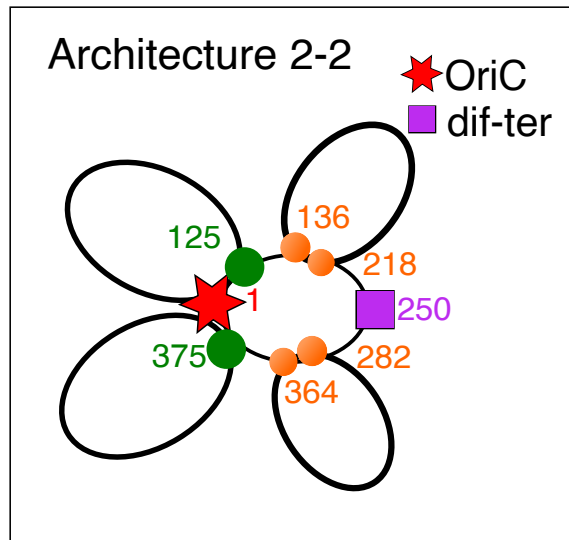


Figure 5.12: Schematic showing the cross-linking sites in Arc-2-2 architecture. The green spheres denote the monomers which were cross-linked to the *oriC* in the Arc-2 architecture. The orange monomers are additionally cross-linked in the Arc-2-2 architecture. We have also indicated the monomer indices corresponding to the cross-linked monomers.

However, Arc-0 fails to localize the other loci as well, refer Fig.5.9. As we see in Fig.5.6a,b all loci reach their home positions after segregation by the end of 8×10^7 MCS. But we start collecting statistical data to plot $P(z)$ in Fig.5.6, only after 10×10^7 MCS from the start of simulation. Thus each locus reaches its home position before we collect statistical data for the next 5×10^7 MCS to calculate $P(z)$. We do not model the division of the cell, hence each locus continues to fluctuate around its home position till 15×10^7 MCS. We can unambiguously conclude from the width of each distribution that each loci is localized at specific cellular regions, and the two *oriCs*, specifically are at the two quarter positions, respectively.

5.4.5 Mechanism of localization of genomic loci.

How does the architecture Arc-2 lead to the localisation of *oriC* to the mid-cell position of an unreplicated mother-chromosome and to the quarter positions for two daughter chromosomes? We examine architecture Arc-2 in detail in Fig.5.10(a) to investigate how it localizes polymer segments (genomic loci). Arc-2 has two side loops of length 125 monomers each and a bigger loop of 250 monomers, shown in pink, green and blue respectively, refer Fig.5.10a. In Fig.5.10b, we plot the probability distributions $P(z)$ of the positions of the C.O.Ms of the individual loops of the daughter chromosomes along the cylindrical axis for a single Arc-2 polymer. Due to the modified polymer architecture with internal loops, the two smaller loops, viz. Loop-1 and Loop-2 further repel the bigger Loop-3 entropically. We also show that the $P(z)$ of Loop-1 & Loop-2 overlap, and peak in the same cell-half, while $P(z)$ of Loop-3 is peaked in a different cell-half. There is minimal overlap in the distributions of Loop-1 and Loop-2 with that of Loop-3. Since the locus *oriC* is the junction of the

three loops by design, the *oriC* locus gets localised to the cell-center. Furthermore the other loci (belonging to particular loops) also get localised to specific regions since they are entropically restricted to specific regions in the cylinder (cell). A similar scenario holds for DNA-1 and DNA-2 which pervade the cylinder of length L_f , refer Fig.5.10c. Each daughter DNA now occupies a different half of the cell, and the *oriCs* get localized to the two quarter positions. Using the blob picture for polymers, we have analytically checked that an overlap of Loop-1 with Loop-3 (or Loop-2 with Loop-3) has a higher entropic cost, as compared to the configuration of the loops shown in Fig.5.10a. We report this in [4].

Note that though the $P(z)$ for Loop-1 and Loop-2 show nearly perfect overlap in Fig.5.10b, the C.O.Ms of Loop-1 and Loop-2 are more likely not to overlap in a particular microstate as can be confirmed from Fig.5.5 and Fig.5.4a. The local arrangement of the loops in a particular microstate along the z -axis from one pole can be that of Loop-1 $\leftrightarrow$ Loop-2 $\leftrightarrow$ Loop-3 configuration or the Loop-2 $\leftrightarrow$ Loop-1 $\leftrightarrow$ Loop-3 configuration due to entropic repulsion between the loops. In a particular run Loop-1 and Loop-2 can interchange positions, as can be confirmed from Fig.5.5. However the distributions plotted in Fig.5.10b and Fig.5.10c have been calculated over many microstates, as obtained from 50 independent runs. The configurations Loop-1 $\leftrightarrow$ Loop-2 $\leftrightarrow$ Loop-3 and the configuration Loop-2 $\leftrightarrow$ Loop-1 $\leftrightarrow$ Loop-3 are equally probable. Thereby, as a consequence of averaging over independent runs, the probability distributions nearly perfectly overlap.

From Fig.5.10c we note that there is minimal overlap between the C.O.Ms of Loop-3 of DNA-1 and DNA-2. However there is significant overlap between Loops-1 & 2 of each daughter DNA with that of Loop-3. This is because the entropic repulsion between Loop-1 and Loop-3 (or Loop-2 and Loop-3) is lesser as compared

to the repulsion between the two *bigger* loops denoted as Loop-3 corresponding to the two daughter DNAs.

5.4.6 Macrodomain organisation: Hi-C Maps

Modeling using Arc-2 architecture could reproduce experimental results related to spatial organization of DNA loci in the *E. coli* cell cycle. But it does not produce a coarse-grained Hi-C map similar to those obtained by experiments [21]. The Hi-C data is experimentally obtained for a large ensemble of cells. In Fig.5.11, we show a simulated contact map using data for the coordinates of different monomers over 7×10^7 MCS plotted in genomic coordinates with the Arc-2 architecture.

To generate the contact map we generate a 500×500 matrix indicating whether any two monomers (genomic segments) are in close proximity to each other. The matrix elements are either 0 for non-proximal segments, and 1 for proximal segments for a particular microstate, and we average over multiple microstates. We collect data from 10 independent runs, for every 10000 iterations, over a total interval of 7×10^7 iterations, after the replication process is complete. If the distance between any two monomers is less than $2a$, we consider them to be in close proximity or in contact. A different choice would lead to contact map with similar bright regions but with lower probability values. Thus it would not change the spatial organisation of macro-domains. We convert the simulation contact map to a contact map in genomic coordinates using the genomic coordinates of loci given in the Supplementary of [20]. Note that the *oriC*, viz. monomer-1 of our simulations, corresponds to 3.9 Mbp in genomic coordinates.

We note that we obtain three bright patches in Fig.5.11, with a large patch

of size 2.5 Mbp between 0.4 and 2.9Mbp. This calls for a further modification of the polymer architecture to obtain smaller substructures within the bigger patch to obtain agreement with the experimental Hi-C map.

We now introduce two *additional* CLs at specific sites to bridge segments which are $\sim 700\text{kb}$ apart. We cross-link monomers 136 & 218 and monomers 282 & 364 as seen in Fig.5.12. Thus we have two smaller loops of 80 monomers each, in addition to Loop-1 and Loop-2. We name this the Arc-2-2 architecture. The architecture was devised to obtain macrodomain like organization ensuring the localization of genomic loci is also maintained. In Fig.5.12 we show the experimentally obtained contact map obtained from [21] and our simulated contact map.

The sites of the CLs chosen for Arc-2-2 (and Arc-2) also have higher contact probabilities in the experimentally obtained contact map in [21]. This can be confirmed from Figs.5.12, 5.14 and 5.16, where we have shown zoomed-in images of the simulation and experimental contact maps, and have explicitly marked the sites of the CLs.

Our choice of the cross-linking monomers raises the following issues which we address here:

1. Although the sites of Arc-2 and Arc-2-2 CLs have high probabilities in the experimental Hi-C map, other regions also have high contact probabilities. A valid question for the reader to ask is: why have we not introduced other possible cross-links (CLs) in our model. We posit that there can be many other possible long-lived CLs. However we show that only a minimal number of CLs is required to obtain a match with the FISH data and to obtain a contact map

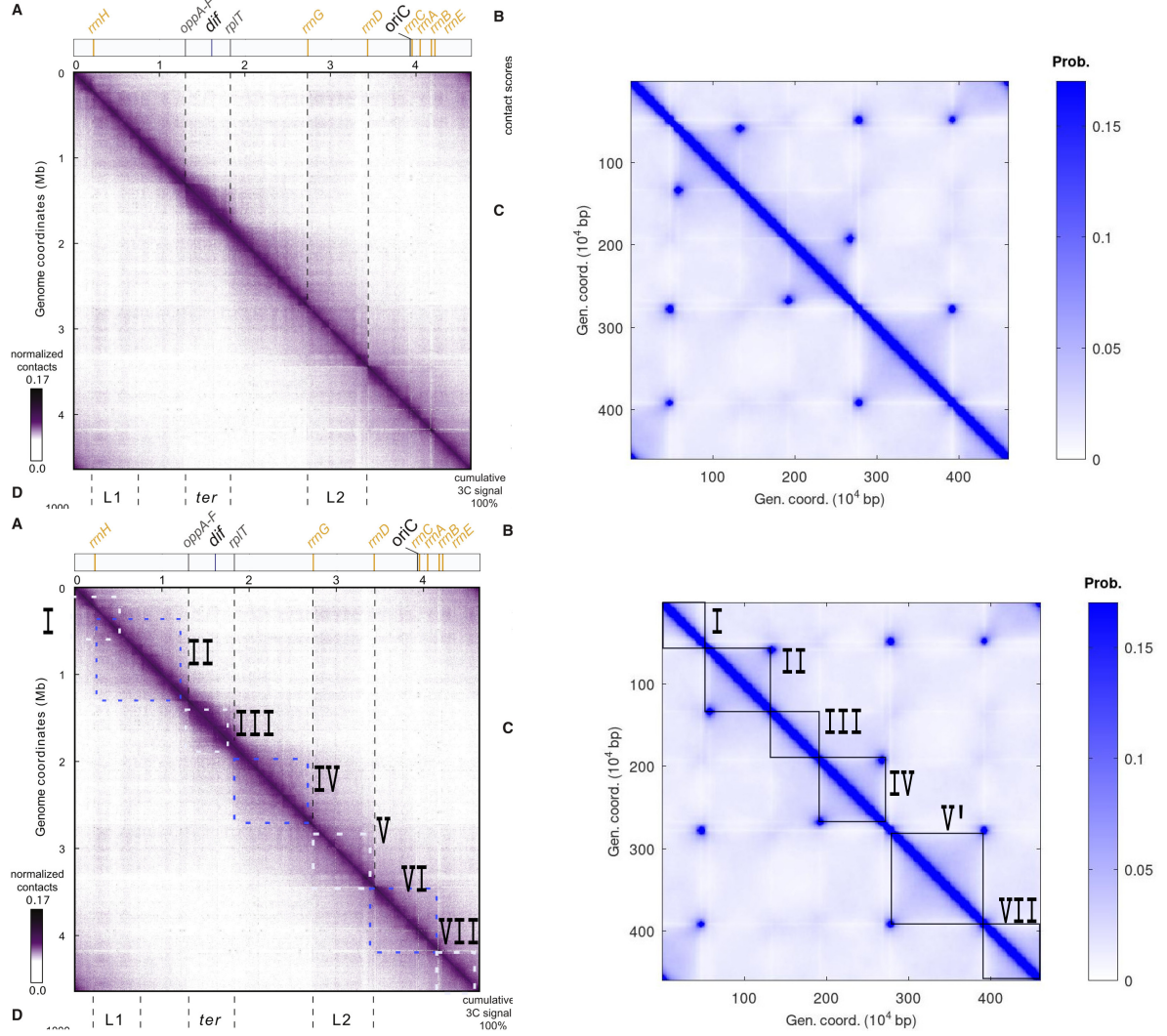


Figure 5.13: **Macrodomain organisation obtained with polymer architecture: Arc-2-2.** The top row shows the experimental contact map for *E.coli* chromosomes as published in [21] (requisite permissions obtained), and the simulated contact map. The simulated contact map has been obtained by averaging over 20 independent runs. The bottom row shows the experimental and the simulated contact map with regions of high contact probabilities explicitly marked. We note that all regions except the region marked ‘V’ shows a fair agreement with the corresponding regions in the experimentally obtained Hi-C map.

with similar macrodomain organization as seen in the experimental contact map.

2. We discuss why the CL sites in the simulated contact map have bright, broad patches, while experimental Hi-C map shows relatively tiny illuminated spots at the CL sites. Note that in the simulations we have a resolution only up to the length scale of one bead ~ 9.2 kbp. Thus we obtain big illuminated patches, as monomers proximal to the CL sites also contribute to the size of the bright spots. In the cell, the chromosome may have CLs (binding sites) localized at specific sites over a much smaller length scale (mediated by a suitable protein having a size of $\sim nm$), which in turn would change the topology over a much larger length scale of the DNA-polymer as considered in this study. Note that the experimentally obtained Hi-C contact map has a resolution of 5 kbp, which is much higher than the length scale of a “cross-link”. Therefore, these “cross-links” and contributions from its proximal segments would show up in the experimental Hi-C map as grey/purple spots. Thus, in the experimentally obtained Hi-C maps, the size of the bright spot corresponding to a “cross-link” in the chromosome would be relatively tiny.
3. We also note from Fig.5.12 that the simulated contact map shows the highest probabilities of contacts at the sites of the additional cross-links. However, this is not the case for the experimental contact map although the sites of the cross-links do show relatively high probabilities, as established in Fig.5.15. This is because in our minimal model we assume that the additional cross-links are permanent throughout the simulation run, whereas in the *E.coli* chromosome these may form transiently at various points during the cell-cycle. These additional “cross-links” in the *E.coli* chromosome may be long-lived despite

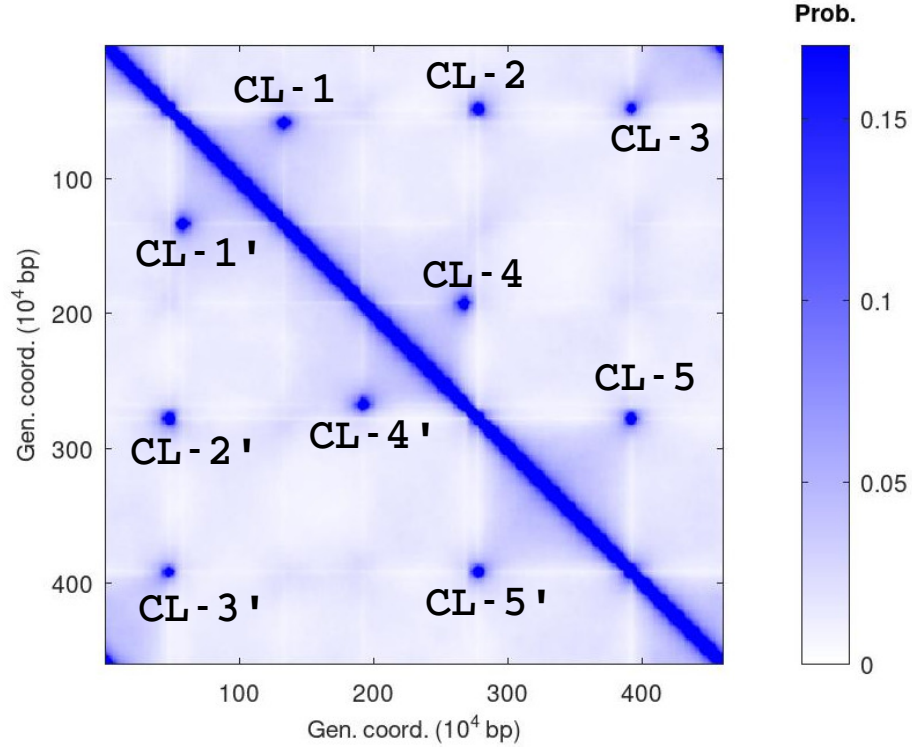


Figure 5.14: Contact map generated from simulations with CLs marked. The CLs with the primed indices are the "mirror" CLs present due to the symmetry of the contact map, i.e a CL with coordinates (x,y) has another corresponding bright spot with coordinate (y,x). We indicate these via the primed indices. Note that while we use only 4 CLs, we show here 5 CLs. This is because due to the CLs between monomer pairs (1,125) and (1,375), there is an "effective" CL created between monomer pairs (125,375).

forming and dissolving due to various biological processes. Furthermore, the experimental Hi-C map is calculated as an average over a large number of cells in different stages of their life cycle, where the CLs could be dissolved in some of the chromosomes.

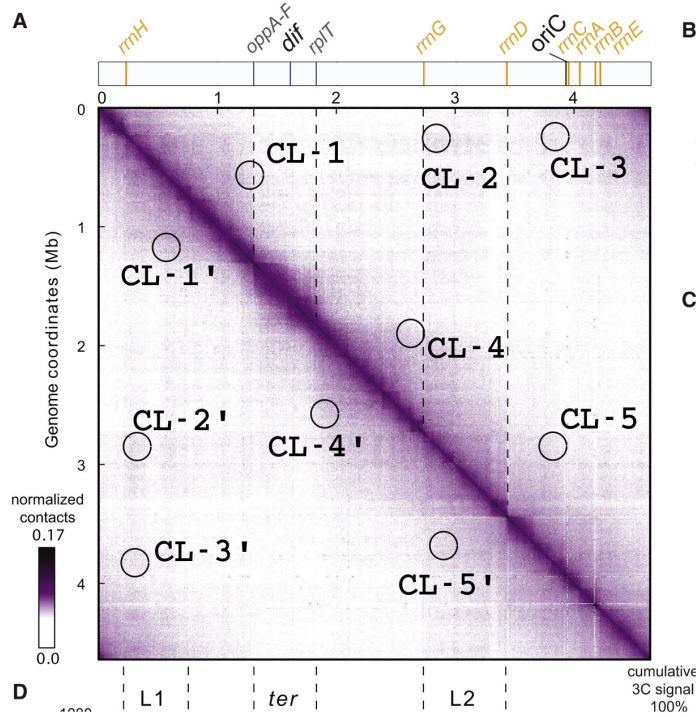


Figure 5.15: Contact map generated from Hi-C experiments with our additional cross-links (CLs) used in simulations, marked as CL-1,2,3 4,5. One can see that the sites of cross-linking have purple dots indicating high contact frequencies. Subsequently we provide the zoomed images of these CL-sites.

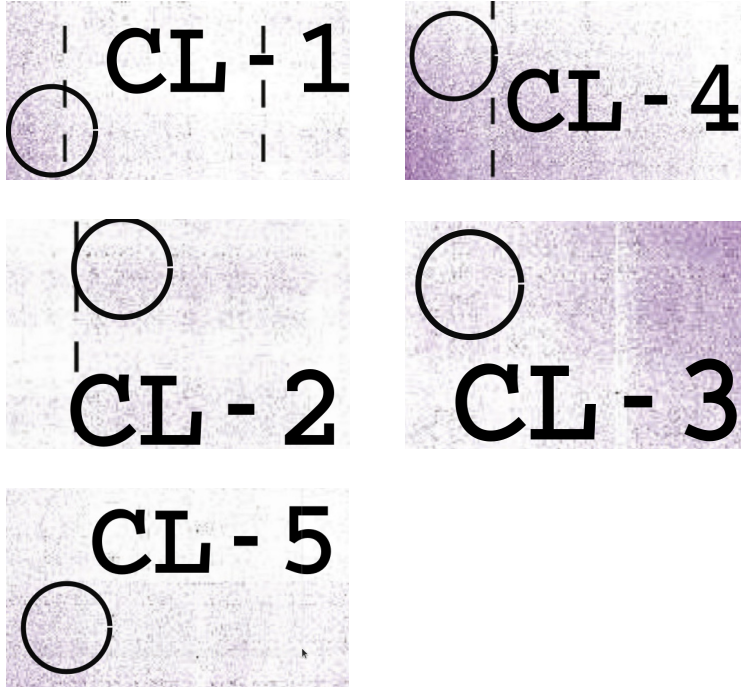


Figure 5.16: Zoomed images at the positions of the CLs (used in simulations) in the experimental HI-C map. These images have been generated by screen capture after zooming in to Fig. 5.15. We find that at the sites of the CLs (enclosed within open circles) there are grey/purple spots indicating high contact probabilities. We do not look at the zoomed images of the primed CLs indicated in Fig. 5.15 since due to the symmetry of the contact map they are equivalent to the unprimed CLs.

5.4.7 Macrodomain organisation : Arc-2-2 architecture

We now discuss the similarities of our simulated contact map obtained with the Arc-2-2 architecture with the experimentally obtained contact map in Fig.5.12. We show that there is a reasonably good agreement with the experimental contact map. The regions marked as I, II, III, IV and VII in the simulated contact map are in fair agreement with the corresponding regions marked in the experimentally obtained contact map. In the experimentally found contact map we have two small bright regions marked as regions V and VI, while in the simulated map we find relatively larger bright patch at that location which has been labelled as ‘V’.

We do not obtain a quantitative match with the experimental contact map due to the minimal nature of our model. We have used a maximum of 4 additional CLs, and we aim to reconcile observations from several complementary experiments using a minimal, unified framework. Thus in our opinion our minimal model which provides a qualitative match with the experimental HI-C data provides insights on the mechanism governing the formation of macrodomains *in-vivo*. A model with several parameters optimised to obtain an exact match with the experimentally obtained contact map may not be useful to understand the generic principles governing macrodomain organization and localization of genomic loci. Moreover, the segregation of daughter DNAs having architecture Arc-2-2 requires fewer MCS (τ_S) as compared to that of Arc-2, refer Fig.5.4c. The Arc-2-2 architecture also localizes the *oriC* and other loci as well as the Arc-2 architecture with very similar migration dynamics: refer data presented in Fig.5.17 and Fig.5.18. It was already hypothesised earlier that formation of ‘topological domains’ crucially depends on entropic repulsion between loops[148]. We hereby show that this mechanism can reconcile the

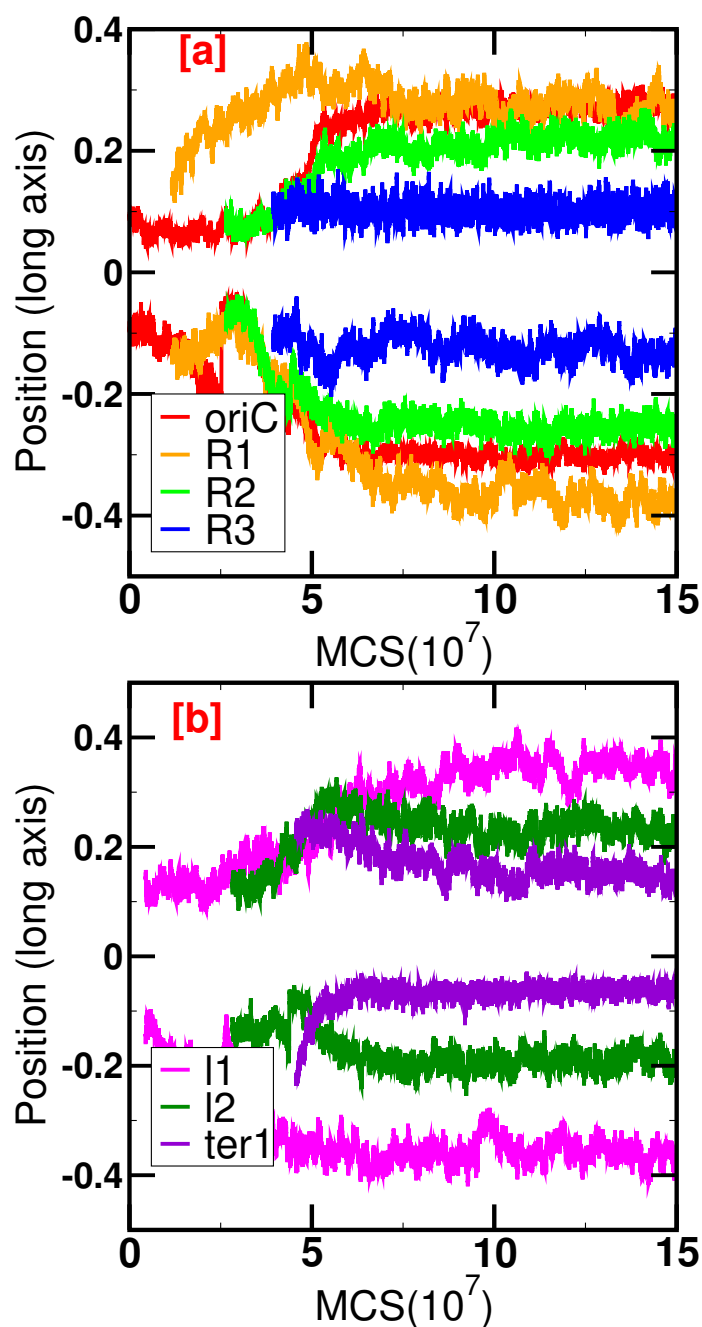


Figure 5.17: The data shows the trajectories of the (a) *oriC*, R1, R2 and R3 loci and (b) L1, L2, and *ter1* loci post replication, along the cylinder long axis for loop architecture Arc-2-2. To plot the locus trajectories we average over 20 independent runs and scale it by the final length of the confining cylinder. The newly replicated locus of DNA-2 is assigned the negative axis.

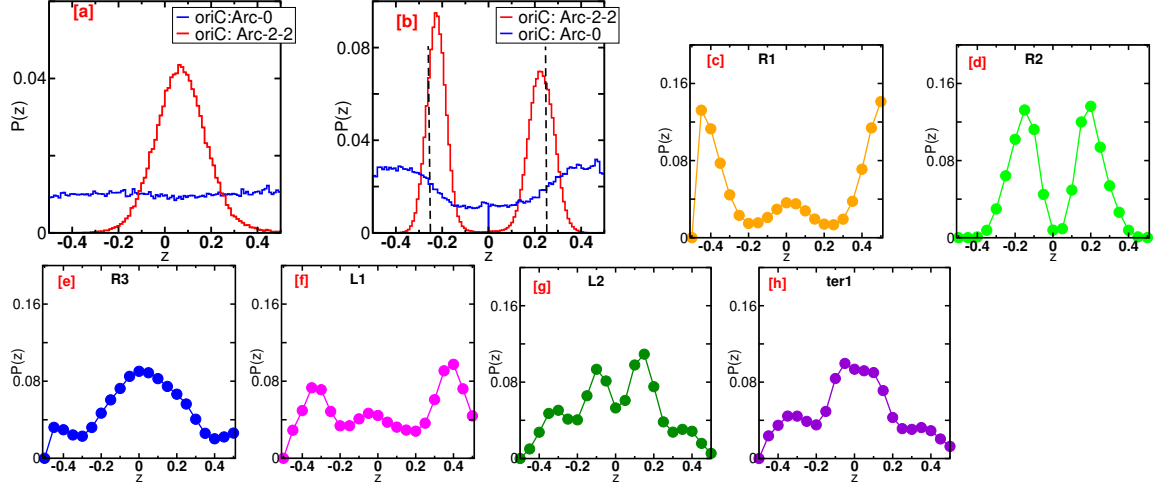


Figure 5.18: **Localisation of loci: Arc-2-2** (a) Comparison of the probability distribution of the *oriC* position along the cylinder long-axis for the parent DNA (before replication) for Arc-0 and Arc-2-2 polymer architectures. The x-axis has been scaled by L_i . Data is collected from 20 independent runs (where we do not model replication) over 5×10^7 MCS after equilibration of a single polymer chain in a cylinder of length $17.5a$. We note that the z -positions of *oriC* shows a narrow distribution around the midcell for Arc-2-2 architecture, while for Arc-0 *oriC* shows a uniform distribution. (b) The probability distribution of the *oriC* position post replication, for Arc-2-2 and Arc-0. The x-axis has been scaled by L_f . The black dashed lines in (b) represent the quarter positions and have been added for aid of visualization. We note that *oriC* shows a narrower distribution approximately around the quarter positions for Arc-2-2, as compared to Arc-0. We also show the probability distributions of the (e) R1 (f) R2 (g) R3 (h) L1 (i) L2 and (j) *ter1* loci for Arc-2-2 architecture. The x-axis has been scaled by L_f . Data is collected from 20 independent runs post replication, from $10 - 15 \times 10^7$ MCS to plot the distributions shown in (d), (e), (f), (g), (h), (i) and (j). Arc-0 fails to localize these loci, refer Fig.5.9.

observations from Hi-C experiments done on *E.coli* chromosomes using our proposed polymer architecture Arc-2-2.

5.5 Polymer Architecture influences Replisome dynamics

We plot the trajectory of the RFs for Arc-2 and Arc-2-2 architectures as a function of MCS to examine if we can resolve a controversy regarding the spatial positions of the RFs as they move along the chain contour. To that end we plot the z -coordinates of the monomer where the R.F is located at a particular iteration in the simulation run. We find that the trajectory shows an ‘M’ like pattern, which is directly indicative of the underlying polymer architecture, refer Fig.5.19.

To understand the origin of the ‘M’ shaped trajectory we note that the RFs move out bi-directionally from the *oriC*, which is initially localised close to the mid-cell position. This holds true for both Arc-2 and Arc-2-2 architectures. Thus in Fig.5.19a and (b) we note that the position of the RFs are relatively closer to the mid-cell position at the beginning of the simulation. The position of the two RFs then deviate significantly away from the mid-cell position as it moves along Loop-1 and Loop-2 of the mother-DNA. As the RFs complete their journey along Loop-1 and Loop-2 respectively, they are again found closer to the mid-cell position at 2.5×10^7 MCS.

It is at this point in the simulation that the 125-th and the 375-th monomers are replicated and thereafter the *oriCs* start moving away from the mid-cell position. Thereafter, the RFs start moving towards the *dif-ter* and thus the trajectory curves

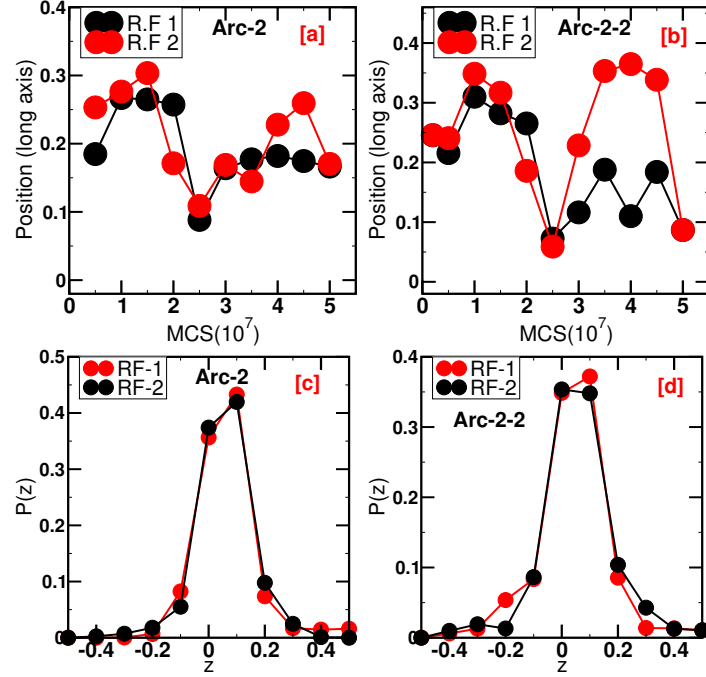


Figure 5.19: The position of the two replication forks RF-1 and RF-2 as a function of MCS averaged over 20 independent runs, for (a) Arc-2 architecture and (b) Arc-2-2 architecture. The position is scaled by the length of the cylinder $L(n)$ at that particular n -th Monte Carlo step. The values on the y -axis range from 0 to 0.5 because we plot the absolute value of the distance of RF-positions from the center of the cylinder, along the long axis. In subfigures (c) and (d) we plot the probability distributions of the z -coordinate of the two replication forks for Arc-2 and Arc-2-2, respectively. We scale the value of the z -coordinate by $L(n)$. The distributions have been plotted using coordinates of RFs collected every 2×10^5 MCS from 20 independent runs.

of the RFs again deviate from the mid-cell positions. The two RFs meet at the *dif-ter* locus which is localised close to the mid-cell position at the end of the replication process at 5×10^7 MCS. Thus the ‘M’ pattern arises solely from the polymer architecture adopted by the chromosomes. Although we model the replication process as per the ‘Train track model’ we show that the RFs have a higher probability of being located close to the mid-cell positions. We show this in subfigures (c) and (d) of Fig.5.19. We show that both the RFs have a higher probability of staying close to the mid-cell position for Arc-2 and Arc-2-2. Thus it is plausible that the ‘Replication factory model’ and the ‘Train track model’, which are seemingly in contradiction to each other, may be reconciled by experimentally validating the underlying polymer architecture as proposed by us.

5.6 Conclusions and Discussion

Past studies have indicated that loops or domains play an important role in the organization of chromosomes into macrodomains [149, 150]. However to the best of our knowledge no previous study, has investigated the role of loops/domains on the linear ordering of genomic loci. We propose that a certain looped architecture leads to the linear ordering of genomic loci. Furthermore the modified polymer architecture also leads to the macrodomain organisation of the *E.coli* chromosome. Our study also suggests a potential reconciliation mechanism of the train track model and the replication factory model.

We further establish in this work that a modification of the polymer architecture by adding 4 loops (or 2 loops) to the ring-polymer to form the Arc-2-2 architecture

(or Arc-2 architecture) leads to efficient segregation during replication of daughter DNAs. The reason for the localization of the two *oriCs* (to the quarter positions), and other loci to their respective home positions, is the entropic repulsion between the loops within each individual daughter chromosome. The positions of the loops and their sizes have been judiciously chosen to obtain a similarity with the Hi-C contact map while also obtaining the localization of genomic loci.

A previous simulation study [128] has highlighted the role of macrodomain organization in the enhancement of entropic repulsion between daughter chromosomes. In the study, certain genomic loci such as the *oriC* and the *ter* were constrained with the imposition of the condensation of macrodomains and their role on the genome organization was studied. We however take a different approach and do not assume any such organisation of loci *a priori*. We just introduce two or four crosslinks and show that there is a spontaneous emergence of loci organisation, with the organisational patterns being similar to those seen *in-vivo*. Our approach is markedly different from that of previous works such as that of [103] and [128], where a MukBEF gradient or other constraints are imposed to achieve the organisational patterns of loci seen *in-vivo*.

In an earlier simulation study [13], the unreplicated DNA polymer was externally constrained within a smaller cylinder in a concentric shell arrangement. However, we did not have to use the concentric shell model of [13], as compaction of the mother DNA-polymer was achieved to some degree, by the Arc-2-2 (or Arc-2) architecture of the unreplicated chromosome at the beginning of replication. There have been conflicting experimental reports with regards to the organization of *E.coli* chromosomes in slow growth. For instance [102] proposes a ‘Sausage’ model of organisation of the chromosome whereas [20] supports the ‘doughnut’ organisation. In our work

we obtain an organisation of the chromosome similar to that of [20] by employing a modified ring polymer architecture.

Several previous studies have adopted the route of optimising multiple simulation parameters to match their modeling results with experimental Hi-C contact maps, and thereby study the resultant organisation [151, 113]. These either use the ideas of transient loops or incorporate a multitude of cross-links modelled with springs of different spring constants for different cross-links correlated with the experimental time-averaged probability of finding two loci in proximity of each other. We however take an orthogonal approach and develop a minimal polymer based framework to try and obtain the localization of loci along the chain axis. In our opinion, such a simplified representation helps us to unearth key insights pertaining to the role of loops in the entropic organisation of the chromosome, which might otherwise be missed with more detailed models.

While the match of the calculated contact map with the experimental map is a consequence of the presence of the long-lived links between very specific monomers, our prediction of the location of CLs along the chain contour can now be explicitly verified. We had checked that change in position of CLs by 4-5 monomers on either side of the chain contour does not significantly alter results.

If one looks at the contact probability as a function of genomic separation for our model with permanent cross-links one will not obtain the power law inferred from experimental data and will also not obtain the observed characteristics. For instance, due to looping one would observe a polymer model with permanent cross-links that the contact probability within a loop increases with genomic distance, for all genomic segments at distances greater than half the length of the loop. This is

because the loop structure ensures that even if two segments are far apart in genomic distance, may be closer to each other along the contour of the polymer loop. This has been explored in detail in [148]. The authors suggest the model of a polymer with permanent loops may be further refined such that the looping happens *via* “transient” cross-linking with certain probabilities. The authors have termed this as the “dynamic looping” model. The looping probabilities may be fitted to obtain a match with the contact probability profiles obtained from experiments.

The principles outlined in this study may also extend to chromosomes of other bacterial species. To that end, in a complementary study [4] we have analyzed a large number of polymer architectures including Arc-2 and Arc-2-2 and compared their segregation dynamics. There we have also shown that a particular polymer architecture results in the localization of loci as seen in FISH experiments conducted on the *C.crescentus* chromosome.

Chapter 6

Topology driven spatial organization of ring-polymers under confinement.

6.1 Introduction

1

Bacterial chromosomes are ring-polymers, and just at the end of replication process one observes two partially segregated ring polymers conjoined at the specific point along their chain contour. The spatial segregation of these bacterial chro-

¹This chapter closely follows the paper [4]. This work was done in collaboration with Shreerang Pande (S.P). Therefore, some of the data presented in this chapter has been generated by him and might also appear in his thesis. Wherever relevant, in the figure captions, S.P has been duly credited.

mosomes occur due to the entropic repulsion between them [14]. The hitherto established paradigm of bacterial chromosomes being considered as amorphous self-avoiding random walk polymers without any internal organization within the cell, has now been deemed obsolete [151]. In this vein, we have previously proposed that the *E.coli* chromosome can adopt particular polymer architectures aided by bridging proteins (and colocalization of *rrn* operons) to enhance propensity for segregation due to entropic forces [3], as compared to the propensity of segregation in simple ring polymers under cylindrical confinement [14]. The proteins bridge different segments of the chain contour to create internal loops within the ring architecture of the *E.coli* chromosome [103, 110, 93]. These loops repel each other entropically and furthermore lead to an organization of genomic loci along the cell-long axis as seen in experiments [3].

These findings motivate us to do a systematic study to identify the pool of polymer architectures which could increase entropic forces which drive segregation of the polymers in cylindrical confinement. Some of these polymer architectures may be adopted by bacterial chromosomes. We show that these modified polymer architectures could achieve localization of polymer segments as has been observed *in-vivo* for chromosomal loci of two different bacterial species using Fluorescence *in-situ* hybridisation (FISH) experiments [20, 133]. Thus a detailed understanding of generic principles pertaining to the role of modified-ring polymer architectures in the entropy-driven segregation, and consequently localization of polymer segments within cylindrical confinement is imperative. This will help unearth the mechanisms of bacterial chromosome segregation in general.

It has been previously shown that the entropic repulsion between side-loops situated along the backbone of a bottle-brush polymer leads to a spontaneous emergence

of helical order under confinement [67]. Previous studies have also shown that two conjoined ring polymers spatially segregate spontaneously, to maximize conformational entropy [13, 14, 16]. Flexible polymers are considered amorphous with ability to adopt all possible conformations to maximize entropy. But we establish here that one can get segments of ring-polymers with modified topologies to organize themselves along the long axis in cylindrical confinement. We create different polymer architectures with just 2 to 4 added cross-links (CLs) in ring polymers. This gives rise to internal loops (rings) within the ring polymer. This, in turn, results in mutual avoidance of loops within the cylinder and thus lead to localization of segments along the cylindrical axis. As a consequence of this, one observes the self emergence of internal organization of polymer segments within cylindrical confinement.

Furthermore the presence of these internal loops within each modified ring polymers leads to the enhancement of entropic repulsion between two conjoined ring polymers. We also show that certain classes of looped architectures lead to greater enhancement of entropic forces than others. We also outline the principles governing the same. This mechanism of localization of polymer segments could lead to the localization of genomic loci in bacterial chromosomes, with relatively small fluctuations in positions along the cylinder long axis, as seen *in vivo*.

In this study we model two replicated bacterial chromosomes as two bead spring ring-polymers having 500 monomers each. We confine the polymers in a cylinder of appropriate dimensions to mimic the confinement imposed by the cell. Note that we do not model the process of replication and start our simulations with two overlapping ring polymers. In our model with 500 monomers, we label monomer-1 of each polymer as the *oricC*, and the 250-th monomer in the ring polymer(s) is called the *dif-ter*. The two *dif-ters* of the two ring polymers (with internal loops)

remain cross-linked to each other by a spring throughout the simulation run. This is inspired by the case of bacterial chromosomes where the newly replicated daughter chromosomes remain conjoined at the *dif-ter* locus prior to cell division [13, 14], refer Fig.6.1 for more details. Later, we also modify the architecture of these ring polymers to further introduce loops within each ring polymer.

Thus, we present here a systematic analysis to elucidate general principles concerning the propensity of segregation and subsequent organization of two initially overlapping polymers, for various architectures, which are under cylindrical confinement. We justify our computationally obtained observations for different architectures, with an analytic treatment of the Helmholtz free energy difference between the overlapped and the segregated states, and find that blob-theory-based calculations successfully explain our computational results. In addition, we also propose that a particularly designed polymer architecture is likely adopted by bacterial species *C.crescentus*. We posit that the cross-links that we introduce to modify the architectures may “effectively” arise in the *C.crescentus* chromosome due to the presence of proteins *in-vivo* [151].

We have compared experimental Hi-C maps and FISH data with corresponding data obtained from our simulations. The Hi-C contact maps gives the probability of any two segments (monomers) of a DNA-polymer being in spatial proximity to each other. Hi-C data is obtained by averaging over many cells and does not give the 3D organization of loci within the cell, though it can be reconstructed from Hi-C maps [113, 152, 153, 154].

On the other hand, FISH experiments are complementary in spirit to Hi-C experiments, in that it gives spatio-temporal distribution of certain tagged locus (or a

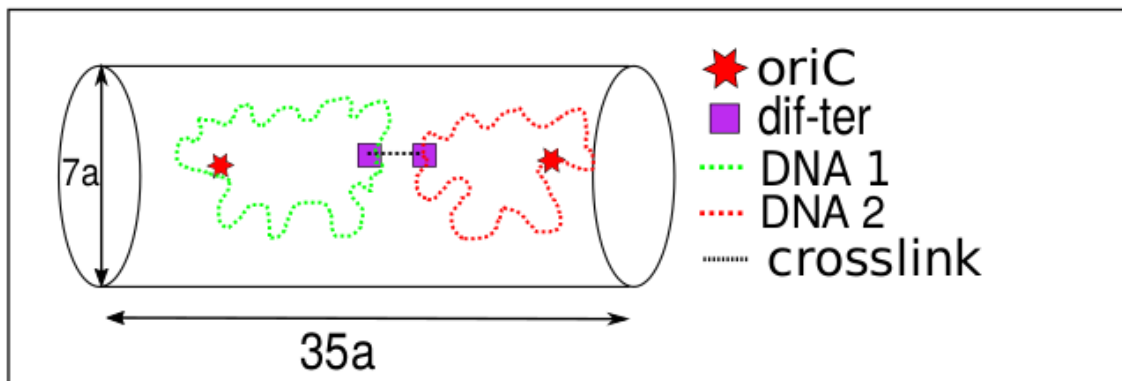


Figure 6.1: **A schematic representation of our simulation model:** Two ring polymers (modified suitably with additional crosslinks) of 500 monomers are confined within a cylinder. The two segments of each polymer chain labelled as *dif-ter* (following the convention for *E.coli* chromosomes) and represented by monomer index 250, have been crosslinked. This is inspired by the fact that newly replicated chromosomes in bacterial cells remained conjoined at the ‘ter’ prior to cell division.

small number of tagged loci) within a particular cell.

Previous studies, which have tried to reproduce contact maps of bacterial DNA, optimise a large number of constraints, to find a detailed match with the experimental contact map[113, 152, 153, 154, 140, 114, 122]. We take an orthogonal approach and focus more on the mechanism underlying the spatial localization of loci in our study and try to match large scale structures only. A detailed match would require a more detailed simulation model which might not be particularly insightful to unearth general principles. Thereby a detailed match is out of scope of this work.

We first give a brief overview of phenomenology of bacterial DNA segregation to provide context when we present our ‘Model’ and ‘Results’. At the end of the ‘Results’ section we describe the key insights we extract from our *in-silico* studies. Subsequently in a separate subsection, titled ‘Relevance to *C.crescentus*’, we establish the relevance of some of our results for the bacterial species *C.crescentus*. We end the

manuscript with the ‘Discussion’ section where we summarise our work and mention avenues of further investigation.

6.2 Bacterial chromosomes

6.2.1 Basic Background

The bacterium *C.crescentus* is a crescent shaped cell, and the bacterium *E.coli* cell is a spherocylinder. We consider the cells of these bacteria to be cylindrical in shape, with diameter $\approx 1\mu\text{m}$ and length of $\approx 2\mu\text{m}$. The chromosomes of *E.coli* and *C.crescentus* have 4.6 mega-base-pairs and 4 mega-base pairs, respectively. Moreover, both are considered as model organisms to study chromosome organization.

In the *E.coli* and *C.crescentus* bacterial cells, certain genomic segments of the chromosome are localised around certain positions along the cell long axis, as observed in experiments [20, 155]. Such localisation patterns cannot be explained from entropic models of segregating ring polymers. We remind the reader that bacterial chromosomes are ring polymers, and the replication of mother DNA to create two daughter DNAs starts at a point called the *oriC*. The replication then proceeds bidirectionally along the two arms of the ring polymer and ends at the *dif-ter* loci. At the end of replication, the cell has two daughter DNA-ring-polymer chains.

For the *E.coli* cell, at the start of replication the *oriC* is at the mid-cell position [103, 156, 157, 95, 158]. Post replication, the DNA-polymer organization changes so that the *dif-ter* locus moves to the middle with the two *oriC* loci occupying the 1/4-th and the 3/4-th positions along the cell longitudinal axis. The two *dif-ter* loci

of the two daughter DNA-polymer remain cross-linked till the time of cell division, which occurs a few minutes after replication is complete.

To ensure efficient segregation of initially overlapping DNA-polymer chains in cylindrical confinement, it is important that topological constraints are released. This is of utmost importance since newly replicated chromosomes may be hindered from segregating due to topological constraints. The release of topological constraints in the case of bacterial chromosomes is accomplished by the presence of the enzyme DNA Topoisomerase (Topo II) which allows newly replicated daughter chromosomes to pass through each other akin to crossing of segments in phantom polymer chains [159, 160, 161]. Thus a comparative study investigating the role topological constraint release on the segregation mechanism of initially overlapping polymers under confinement, is also in order.

Furthermore, detailed chromosomal contact maps obtained from Hi-C experiments [21] unambiguously have established the presence topologically associated domains (TAD) for several bacterial chromosomes. The presence of macrodomains (spanning sections of $\sim Mbp$) within bacterial chromosomes have also been observed [162, 21, 108]. These may arise as a consequence of loops at specific positions in the genome [3]. These loops may be crucial to the formation of macrodomains and other characteristic features seen *in-vivo* [3, 139, 110, 120].

Previous FISH experiments on bacterial chromosomes as reported in [3, 20, 155], involved the tagging of certain specific loci with fluorescent markers. The aim was to tag loci positions which were distributed over the entire ring contour, such that spatio-temporal information of different segments of the chain could be obtained. In our study, we plot the spatial distribution of the monomers corresponding to the

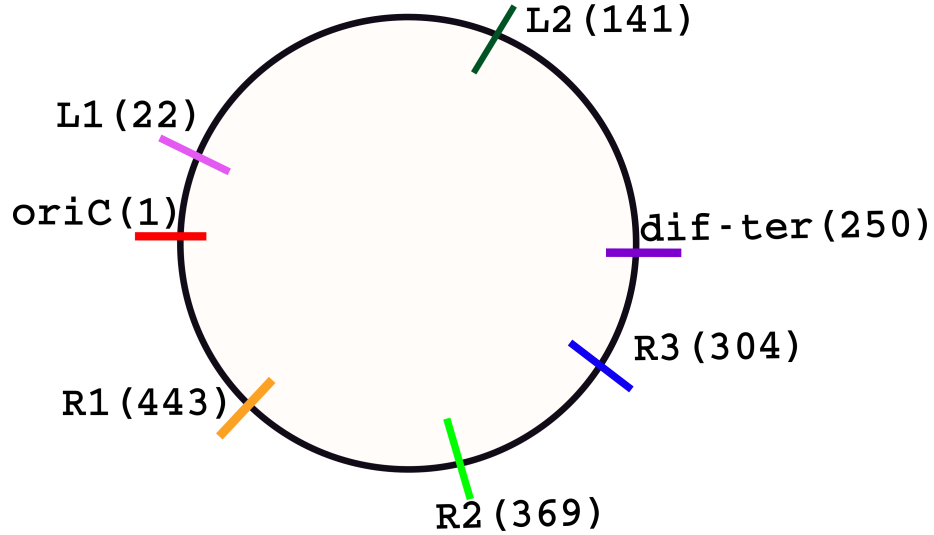


Figure 6.2: The schematic shows the positions of the loci along the chromosome-polymer contour, which were fluorescently tagged in the experiments of [20]. These loci are *oriC*, L1, L2, R1, R2, R3 and *ter1*. The monomers corresponding to these loci are 1, 22, 141, 443, 369, 304 and 228, respectively, in our model of ring polymer with 500 monomers. The spatio-temporal organization of the chromosome was inferred from the organization of these tagged loci. We thereby follow the monomers corresponding to the same loci (except *ter1*) in our simulations to infer the spatial organization of the chromosome-polymer. Instead of *ter1*, we follow the *dif-ter* locus, which is the 250-th monomer and is close to *ter1*.

same loci which were chosen experimentally in [20] for *E.coli* chromosome, though we have access to the position distributions of all the 500 monomers. The positions of these loci along the chain contour of a ring-polymer can be seen in Fig.6.2. The loci (monomers) are well-separated along the chain contour and therefore provide information on the spatial organization of the chromosome-polymer. The monomer indices are shown in the schematic. We follow and present data for the spatial distribution for these same loci for all the 12 polymer architectures. These polymer architectures have been classified into 3 different classes based on the orientation of the constituent loops with respect to the *dif-ter* cross-link.

When we map a particular architecture to the DNA of a particular bacteria, we can analyze the spatial distribution of any monomer corresponding to any particular locus that we need to. Thus, we chose to track different monomers from what is shown in Fig.6.2, when we compare our data from simulations to spatial distributions of loci of *C.crescentus*, as provided by experiments.

6.2.2 Unresolved questions:

Through this work we aspire to address the following unresolved questions:

1. It is already known that a branched topology leads to a higher propensity of the spatial segregation of two initially overlapping, confined polymers [13]. Do some classes of topologies (architectures) induce higher entropic forces of segregation than others?
2. Once the two initially overlapping polymers (daughter chromosomes) demix spatially, it is imperative that they stay demixed before cell division occurs *in-*

vivo. Are there certain classes of architecture which have a greater propensity of remaining demixed after they have spatially segregated?

3. In an earlier work [3] we have shown that a particular polymer architecture leads to the localization of certain polymer segments as seen *in-vivo* in the *E.coli* chromosome. The localization of these segments arise due to the entropic repulsion between the constituent loops. Can other architectures lead to the localization of these polymer segments to other specific regions along the cylindrical long axis?

Thereby can we design loops *a priori* along the chain contour using intuition to obtain localization of specific segments, which can then be matched to experimental data for other bacterial chromosomes?

4. Can the induced forces of entropic segregation in the case of different architectures, be calculated and justified analytically? Are these calculations (done for idealised systems) consistent with simulation data which measures propensity of polymer segregation ?
5. In the different classes of architectures that we study, what is the organization of the constituent loops along the cylindrical axis? How does the organization of the constituent loops vary for architectures of different classes?

6.3 Model

We perform Monte Carlo simulations using a bead spring model of two flexible ring polymers with 500 monomers in each chain. We introduce additional crosslinks to

obtain polymers of different architectures. If there are N monomers in the simulation, each Monte Carlo step (MCS) involves N attempts to displace a monomer in a random direction. The attempt may or may not be accepted as per the Metropolis criterion. When attempting a random displacement, the monomer should be chosen at random from N monomers.

Two neighbouring monomers (beads) along the chain contour interact *via* the harmonic spring potential with energy $V_H = \kappa(r - a)^2$, where r is the distance between the monomers. The unit of length in our study is a , where $a = 1$ is the equilibrium length of the harmonic-springs between two neighbouring monomers. The spring constant κ is $100k_B T/a^2$. The excluded volume (EV) interactions between monomers are modelled by the WCA (Weeks Chandler Anderson) potential and the diameter of each monomer is given by, $\sigma = 0.8a$. The form of the WCA potential is

$$V_{WCA} = 4\epsilon[(\sigma/r)^{12} - (\sigma/r)^6] + \epsilon_0, \forall r < 2^{1/6}\sigma. \quad (6.1)$$

The quantity ϵ_0 is needed to ensure that the potential goes smoothly to zero at the cutoff, and $V_{WCA} = 0$ for values of r greater than the cutoff. Note that the 250th monomer of the two polymers have a cross-link (CL) between them. This is inspired by the case of bacterial chromosomes where the newly replicated daughter chromosomes remain conjoined at the *dif-ter* locus prior to cell division [13, 14], refer Fig.6.1 for more details. The polymers are confined to a cylinder with appropriately chosen dimensions.

Dimensions of Cylindrical confinement: We confine two ring polymers within a cylinder of radius $3.5a$ and a length of $L = 35a$, refer Fig.6.1. This ensures an aspect ratio of 1 : 5. This aspect ratio is observed *in-vivo* for an *E.coli*

cell in slow growth conditions with two daughter chromosomes at the end of the replication process and just before cell division. This particular choice of parameters results in a colloidal volume fraction of monomers to be 0.2. We consider the walls of the cylinder to be hard (with infinite potential) such that we reject any Monte Carlo trial move in which a monomer-center attempts to occupy a position located outside the cylinder. The dimensions of the confining cylinder correspond to the volume accessible to the center of the monomers.

If we think that the polymer chains represent replicated *E.coli* daughter chromosomes and since the *E.coli* chromosome has 4.6Mbp of DNA, each monomer corresponds to 9.2kbp of DNA. Note that the Kuhn length of bare ds-DNA is known to be 100nm with approximately 300 base pairs (bp). Thus each monomer corresponds to a length scale much greater than the Kuhn length bare DNA. However, it is known that ds-DNA is compacted by histone like HU proteins in bacteria. Since the *E.coli* cell has diameter 1 μ m which is 7 a in our simulations, we may consider $a = 150$ nm in real units. We do not consider larger cylinders as it is established that segregation occurs only when the radius of the confining cylinder is less than the radius of gyration R_g of the polymer [14].

We confine the polymers in a cylinder instead of a spherocylinder, though it is known that the shape of an *E.coli* cell is closer to that of a sphero-cylinder. However, in this manuscript we just want to establish the principles of organization of polymers having different architectures subjected to cylindrical confinement, without any loss of generality of whether the polymer is a synthetic polymer or the chromosomes of other rod-shaped bacterial cells.

Initialising and Equilibrating the system: As polymers spontaneously seg-

regate due to excluded volume (EV) interactions, we equilibrate the system of two ring polymers in an initial mixed state by the following procedure. We introduce the monomers at random positions in the cylinder and allow the chain to diffuse freely *without* excluded volume interactions between monomers. However the harmonic spring interactions are present (including the additional crosslinks). We run the simulation for 10^6 Monte Carlo steps (MCS) to equilibrate. Since we initialize monomers randomly within the cylinder, neighbouring monomers along the chain contour can be initially far away. Thus at the start of the simulation, the springs between such monomers may be highly stretched. But with the start of the simulation, the monomers rapidly move towards each other, and the springs reach lengths close to their equilibrium values much before 10^5 MCS. Once the chains get completely ‘mixed’ spatially, we switch on the excluded volume interactions and study the segregation dynamics of the two polymers.

Due to the initialization procedure, concatenations between loops (topological entanglements) can get introduced. When we incorporate the effect of topoisomerase (i.e periodic shrinking of monomers) then these concatenations have suitable opportunities to get released over the length of the simulation runs. The procedure for the same is discussed in the subsequent paragraphs. However, for the case where we do not incorporate this topological constraint release mechanism (relevant for synthetic polymers), these topological links could prevent segregation and thus reduce success rates of segregation. From a biological point of view, if the two polymers are considered to be newly replicated chromosomes then it is not unlikely that the two DNA-polymers would be topologically linked just after replication. Hence, for studying such scenarios the initialization procedure employed by us is apt. For future studies of segregation properties with synthetic polymers, it will be more apt to ini-

tialize the system such that there are no concatenations between the two polymers.

Furthermore, in this study we use WCA potential instead of a hard-sphere potential, to ensure that there are no spatial overlaps between monomers. The reason for this has been elaborated upon in the next paragraph.

In this work we have incorporated excluded volume interactions using WCA interactions. Note that, we could have used hard-sphere interactions as well. But, it could lead to a slight complication due to the initialization procedure that we employ. We remind the reader that we initialize the system by introducing monomers randomly in the cylinder. As a consequence of this initialization procedure, the monomers can often have spatial overlaps. If we had hard-sphere interactions then the monomers which are spatially overlapped would not be displaced due to the Monte Carlo scheme, unless the trial displacement is large enough to overcome the spatial overlap. This is because the hard-sphere interaction implies that whenever there is a spatial overlap the interaction potential energy is infinity, and therefore as long there is spatial overlap the trial displacement steps will keep getting rejected. Note that this problem does not arise in the case of WCA interactions.

The same problem arises even if we start out with an initial condition without any spatial overlaps. In this study topological constraints are released by frequent shrinking of monomer sizes. After a certain number of MCS these monomers regain their original sizes. When the monomers regain their original sizes there could be spatial overlaps introduced. In this case too if we had hard-sphere interactions then the monomers which are spatially overlapped would not be displaced due to the

Monte Carlo scheme, unless the trial displacement is large enough to overcome the spatial overlap.

These problems do not arise when we use WCA interactions.

Release of Topological constraints: In addition to studying non-living polymers in which chains do not cross each other, we also consider polymer chains which can pass through each other at regular intervals to release topological constraints. This is done to mimic the effect of Topoisomerase in the *E.coli* cells (and other bacterial cells) which allows release of topological constraints by facilitating chain crossing. To implement this, we reduce the σ to $\sigma' = 0.2a$ after every 10000 MCS. We let the system evolve using Monte Carlo trial moves with $\sigma = 0.2a$ for the subsequent 900 MCS, to allow the release of topological constraints, before reverting to $\sigma = 0.8a$. Note that we allow chain crossing infrequently for only about 10% of the total duration of the simulation, as excluded volume interactions are important for entropic repulsion between loops which drives segregation. If the duration of topological constraint release is increased significantly then the entropic repulsion is significantly reduced, and if the duration is significantly reduced then segregation of overlapping polymers is prevented by the presence of topological constraints.

Though this is a convenient simulation strategy to release topological constraints all at once for all chain segments and is also computationally cheap, this is unlikely to mimic the real dynamics within the cell. However, the ‘equilibrium organization’ of chromosomes and the localization of loci (which will be established subsequently in this study) is likely to be unaffected by this choice since it only affects the dynamical aspects. Secondly, even though the action of Topoisomerase might produce

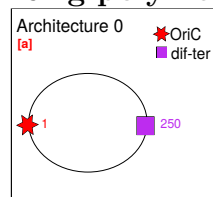
Architecture	Pairs of crosslinked monomers
Arc-0	no additional CL
Arc-2	(125,1), (375,1)
Arc-2-2	(125,1) (375,1) (156,218) (282,344)
Arc-3	(125,1)
Arc-4	(200,50)
Arc-6	(150,230) (350,270)
Arc-7	(20,100) (400,480)
Arc-8	(100,1) (125,200) (200,300) (375,300)
Arc-9	(125,1) (125,375) (187,312)
Arc-10	(20,100) (480,400) (187,313)
Arc-11	(125,1), (187,313)

Table 6.1: The table lists the monomer indices which have been cross-linked to obtain architectures as shown in Fig.6.3. The monomer pairs which are cross-linked are given in brackets.

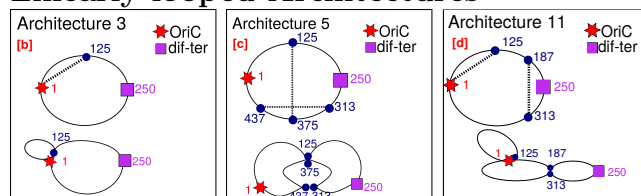
qualitatively different results with regards to the dynamics, here we choose to focus on whether a particular architecture leads to higher success rate of segregation than others, in the presence of a topological constraint release mechanism. Incorporating a more detailed model of the action of the Topoisomerase enzyme is outside the scope of our current work.

Modifying the ring-polymer architecture: We modify a ring polymer suitably to obtain different polymer architectures by introducing harmonic spring interactions between monomer pairs at specific sites along the chain contour. Thus we cross-link specific monomers along chain contour. The spring constants corresponding to the additional crosslinks are given by $\kappa = 100k_B T/a^2$ and the springs have equilibrium length of a . This results in a variety of looped structure of the polymers. We keep a minimal set of additional crosslinks and ensure that we do not introduce more than 4 additional crosslinks (CLs). In the table 6.1 we list the sites of crosslinks by which we generate different polymer architectures, as listed in Fig.6.3.

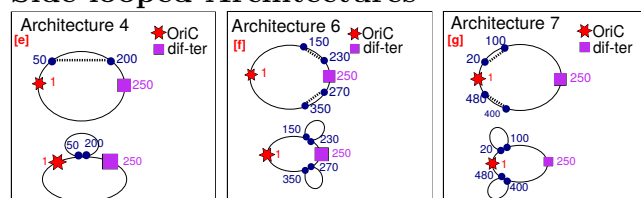
Ring polymer architecture



Linearly looped Architectures



Side looped Architectures



Hybrid looped Architectures

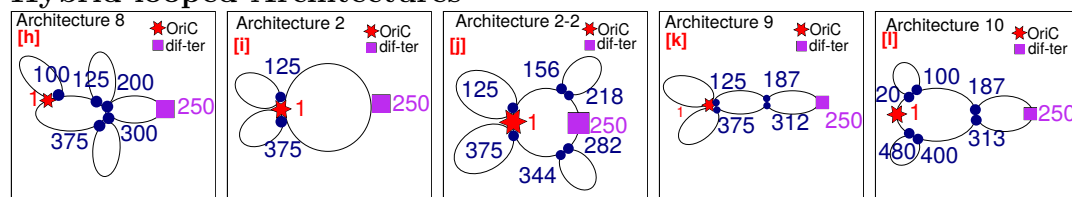


Figure 6.3: We have classified all the polymer architectures according as per our design of loops along the chain contour, refer to the ‘Model’ section. Each polymer of a particular architecture is cross-linked at *dif-ter* to another identical polymer (refer Fig.6.1). The dashed lines refer to the introduced springs between specific monomers (indices marked in blue) in the ring to create additional loops. With respect to the *dif-ter* position, the Arc-3 polymer has loops placed along a line along the cell long axis, and are called ‘linearly looped’ architecture. We see later that the monomers of loops occupy different sections of the cylinder along the long axis for the Arc-3 polymer. On the other hand, the Arc-4 architecture has a small ‘side loop’; we see that the monomers of the big loop and side loop overlap significantly. We introduce additional loops, with different orientations of loops to create Arc-5, Arc-11, Arc-6, Arc-7. We also have architectures having a combination of both ‘side loops’ and ‘linear loops’, referred as ‘hybrid’ architectures.

The considered polymer architectures represent a variety of looped structures, and the sizes of the loops vary from 60 monomers to 130 monomers.

Classification of looped architectures: We start with a ring polymer, which we label Arc-0. We then introduce a single crosslink and create two internal loops to create Arc-3, and Arc-4. In Arc-3 and Arc-4, the smaller loop (with 125 monomers) is placed differently with respect to the bigger loop with 375 monomers. In the two architectures the position of the smaller loop is at different contour lengths away from the *dif-ter* monomer, which is cross-linked to the *dif-ter* of the second ring polymer, refer Figs.6.1 and 6.3. .

We represent Arc-3 as having a ‘linear’ looped architecture where the two loops are schematically placed linearly along the cylindrical axis provided the axis is long enough (not shown). Arc-4 on the contrary has a ‘side loop’ architecture. This distinction is being made based on how the loops are arranged with respect to the *dif-ter* cross-link. This enables us to study the effect of loop orientation on the organization and segregation of spatially confined polymers. The nomenclature will be justified when we present data on the overlap of monomers from different loops.

In order to study the effect of additional loops we design architectures with 3 loops where these loops are oriented in different ways. In Arc-5 and Arc-11 the loops are placed linearly along the cylindrical axis, whereas in Arc-6 and Arc-7 we have two additional ‘side loops’. We have also designed Arc-6 and Arc-7 such that they have loops of the same size but in Arc-6 these loops are closer to the *dif-ter* cross-link whereas in Arc-7 the loops are located close to the *oriC* along the chain contour. We shall establish significantly that this leads to key differences in the propensity for entropic segregation, for two overlapping polymers having these architectures.

We also design a number of polymer architectures which employ both ‘linear’ as well as ‘side’ loops. Such architectures are termed as hybrid architectures. Such architectures may also have 2, 3 or 4 additional loops. This give rise to the architectures Arc-2, Arc-2-2, Arc-8, Arc-9 and Arc-10. We establish later that some of these hybrid architectures lead to distinct localization patterns of genomic loci while certain other architectures lead to high propensities of entropic segregation.

The nomenclature of Arc-0, Arc-2 and Arc-2-2 have been borrowed from the nomenclature used in [3], where we used these architectures to explain localization data of loci for the *E.coli* chromosome. In our previous work, we had explicitly modelled replication and the loop sizes for Arc-2-2 were slightly different from that presented in this work. That resulted in a better match with Hi-C data of *E.coli* chromosomes. The other architectures investigated in this work have been designed thereafter.

Importance of the ‘ter’ crosslink: The terminology ‘side loops’ and ‘linear loops’ can assume significance only because of the presence of the *dif-ter* cross-link between the two initially overlapping ring polymers. This restricts the number of conformations accessible to the two polymers. For instance Arc-3 and Arc-4 are not equivalent architectures only due to the presence of the *ter* crosslink.

Quantifying the organisation of specific polymer segments (tagged genomic loci): To establish the organization of the polymers along the cylindrical long axis we plot the probability distributions of the position along the cylindrical long axis of certain specific monomers. We calculate this distribution after the initially overlapping polymers have segregated to two halves of the cylinder. The chosen monomers are fairly equally spaced along the chain contour and hence reli-

ably indicate the degree of (linear) organization along the cylindrical axis. We study the spatial localization of monomers: 1, 22, 141, 443, 369, 304 and 250 in each of the two polymer chains. If the two polymer chains are thought of as being analogous to newly replicated chromosomes in *E.coli* cells, then the position of these monomers along the contour correspond to loci labelled as *oriC*, L1, L2, R1, R2, R3 and *dif-ter* in [20, 3]: refer schematic in Fig.6.2.

Modelling the *C.crescentus* bacterial species: We find that the confined polymer with a specially designed architecture successfully organises the polymer segments in a way that is particularly relevant for *C.crescentus*. Note that now each monomer represents 8 kbp of DNA, as the chromosome has 4Mbp. In the *C.crescentus* chromosome, as the *ori* remains tethered to the pole *in-vivo* [155]. In our simulation model for *C.crescentus*, the monomer index 22 corresponds to the *ori*, which we tether to one of the cylinder (cell) pole *via* a harmonic potential, with a spring constant $1k_B T/a^2$. We also look at the spatial distributions of other loci such as *pilA*, *pleC*, *podJ* and *ter* [151]. These loci correspond to the monomer indices 122, 182, 238 and 272, respectively. When we apply the results of our studies for a particular architecture to understand the chromosomal organization of *C.crescentus*, then we cross-link the 272-th monomer of each of the two chains, refer Fig.6.1, as that monomer represents the *dif-ter* position for *C.Crescentus*. We also look at the contact map obtained from our simulation model using the procedure outlined in [3].

Choice of Monte Carlo Simulation technique: We conduct our studies on effects of architecture on the success rates of segregation of initially overlapping polymers in confinement and subsequent localization using Monte Carlo (MC) simulations. It has been shown previously [143, 144] that Monte Carlo simulations successfully reproduces diffusive dynamics of particles, at a coarse grained level as

the polymers segregate. Thus Monte Carlo simulations have been used widely in studies on phase separation kinetics [145] and polymer dynamics [146, 147]. We emphasise that we have sufficiently low volume fractions to capture diffusive dynamics of polymers.

Furthermore we employ Monte Carlo (MC) for this study since it allows us to reduce the bead size of the beads in our bead spring model at infrequent intervals. We do this to mimic the effect of the Topoisomerase enzyme. Note that this cannot be done in the usual Langevin (or Brownian Dynamics) simulations as it would lead to discontinuities in potentials and infinite forces. Note that incorporation of topological constraint release implies that the study is intrinsically a non-equilibrium system where detailed balance is violated. In this case the Monte Carlo step displacement of monomers in the chain captures the local diffusive dynamics in the confined cylinder, and subsequent exploration of phase space.

6.4 Results

6.4.1 Segregation propensity of polymer architectures

We first present a comparison of segregation success rates of two spatially overlapping polymers, for each of the different polymer architectures. In each case, the two overlapping polymers have identical architectures. We study the phenomenon in the absence of topological constraint release as in synthetic polymers, as well as when we introduce topological constraint release to mimic DNA-polymers.

To estimate the segregation success rates for a particular architecture, we give 50

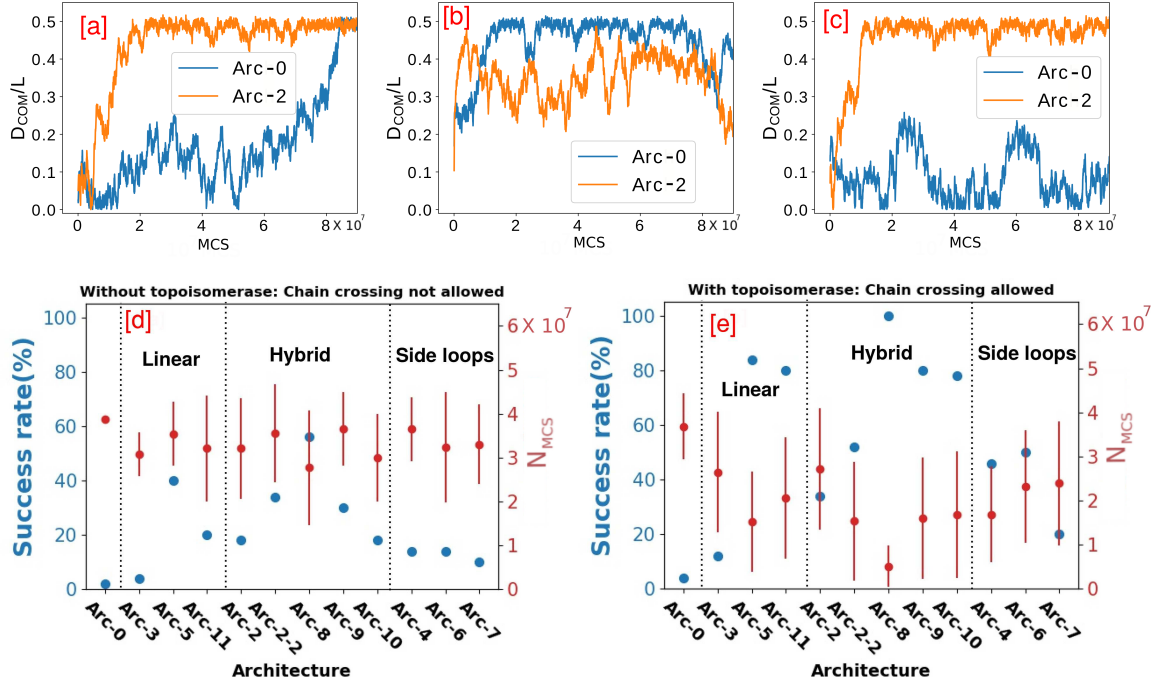


Figure 6.4: (*Data generated by S.P*) **Success rates of segregation of overlapping polymers for different architectures:** Subfigures (a,b,c) show the distance between the center of masses D_{com} versus MCS (Monte Carlo steps) of the two initially overlapping polymers as they undergo segregation in the Monte Carlo simulation for three independent runs for the Arc-0 (ring polymer) and Arc-2 architectures. Runs for which D_{com}/L fluctuates significantly below 0.5 are deemed as runs which did not lead to successful segregation. Chain crossing is allowed at designated rates (refer text) for the data presented in subfigures (a,b,c). The subfigures (d) and (e)) show a comparison of success rates of segregation for different architectures, which indicates the percentages of independent runs lead to segregation. We compute this by counting the number of runs which show successful segregation from a pool of 50 independent runs for each architecture. The right y-axis shows the mean number of MCS required for segregation (N_{MCS}). The error bars have been computed using the data from the successfully segregated cases from the entire set of 50 runs. We note that the architectures with additional cross-links lead to lower values of N_{MCS} and higher success rates of segregation, as compared to that of a ring polymer (Arc-0). The effect of topological constraint release has not been incorporated in (d), while release of topological constraints is allowed in (e).

independent runs each starting from a different initially overlapped configuration of polymers. In each run, we measure the distance D_{com} between the center of masses of the two polymers from start of simulations, i.e. when we switch on EV interactions between monomers after equilibration. If the mean distance $\langle D_{com} \rangle$ is $\geq d_T$, where d_T is designated threshold value, we deem the polymers to have segregated. We chose $d_T = 0.48L$, and $\langle D_{com} \rangle$ is calculated over 4×10^7 MCS post segregation. This implies that the two polymers occupy different halves of the cylinder.

In figure 6.4(a,b,c), we show the evolution of the values of D_{com} with Monte Carlo steps (MCS), starting from relatively low values (when polymers are in overlapped configuration) to higher values for three different independent runs, respectively. We compare data for Arc-0 architecture with that for Arc-2 architecture. We have intentionally shown data where the polymer is unable to fully segregate, so that the reader can directly envisage how D_{com} fluctuates for the cases with unsuccessful segregation.

Similar sets of 50 runs were given for each of the 11 architectures considered in this study. Thereafter, in Fig.6.4(d) we show the segregation success percentage and the mean number of MCS required for successful segregation (N_{MCS}) for different architectures, for the studies where chain crossing is disallowed. On the left y-axis we show the success rates of segregation, which indicates the percentage of independent runs that result in a segregated configuration of the polymers. The right axis shows variation of N_{MCS} for the different architectures.

We compare the data presented in Fig.6.4(d) with Fig.6.4(e), where we consider all architectures with chain crossing introduced. We observe that the ring polymer architecture Arc-0 has low segregation success rates both the studies. When chain

crossing is allowed, the rates of successful segregation slightly increases for the Arc-0 architecture as we see in Fig.6.4(e). We checked that for shorter polymers with 100 monomers, the polymers nearly always segregate even without release of topological constraints, as seen in previous studies [163]. Note that in this case the polymer segregates easily as we kept the cylinder dimensions unchanged *vis-a-vis* the $N = 500$ monomer model discussed in the previous chapter. This was done to explore the role of topological constraints, but this $N = 100$ monomer model in this case does not represent the *E.coli* chromosome

From Fig.6.4d (and Fig.6.4e) we further note that Arc-4 shows a higher success rate of segregation as compared to that of Arc-3. We remind the reader that both Arc-3 and Arc-4 have one additional loop each but the loops in the two cases are oriented differently with respect to the *ter* cross-link. However, the linearly looped architectures Arc-5 and Arc-11 (with two additional loops) have higher success rates of segregation, as compared to that of Arc-6 and Arc-7 which are side-looped architectures. Arc-6 and Arc-7 also have two additional loops each, of identical sizes but positioned differently with respect to the *ter*-CL. Interestingly, we note that Arc-6 shows a higher success rate of segregation as compared to Arc-7. Note that for both these architectures we have two additional side-loops but in Arc-6 the loops are closer to the *dif-ter* CL, whereas in Arc-7 the loops are located closer to the *oriC*. We reconcile these observations with further analysis presented subsequently in the text.

Effect of topological constraint release: We also observe that release of topological constraints leads to increased success rates of segregation and fewer MCS required for spatial segregation (N_{MCS}). For the data presented in Fig.6.4d, we do not allow the release of topological constraints by suitable decrease of the monomer

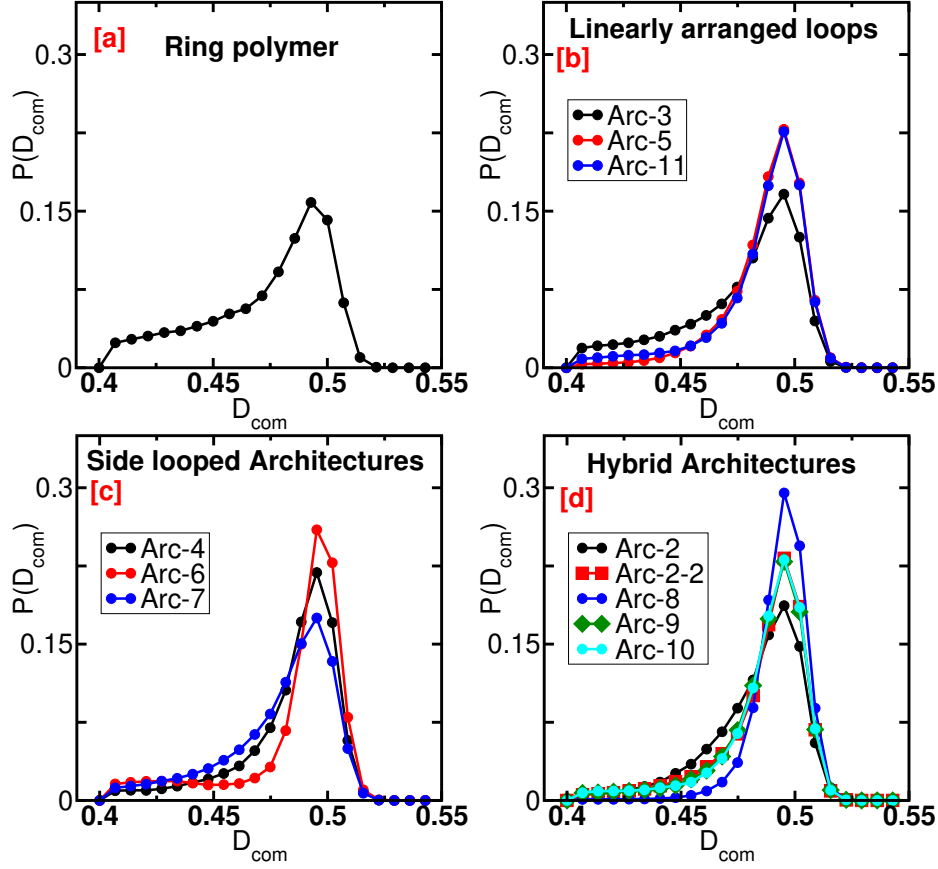
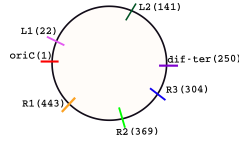
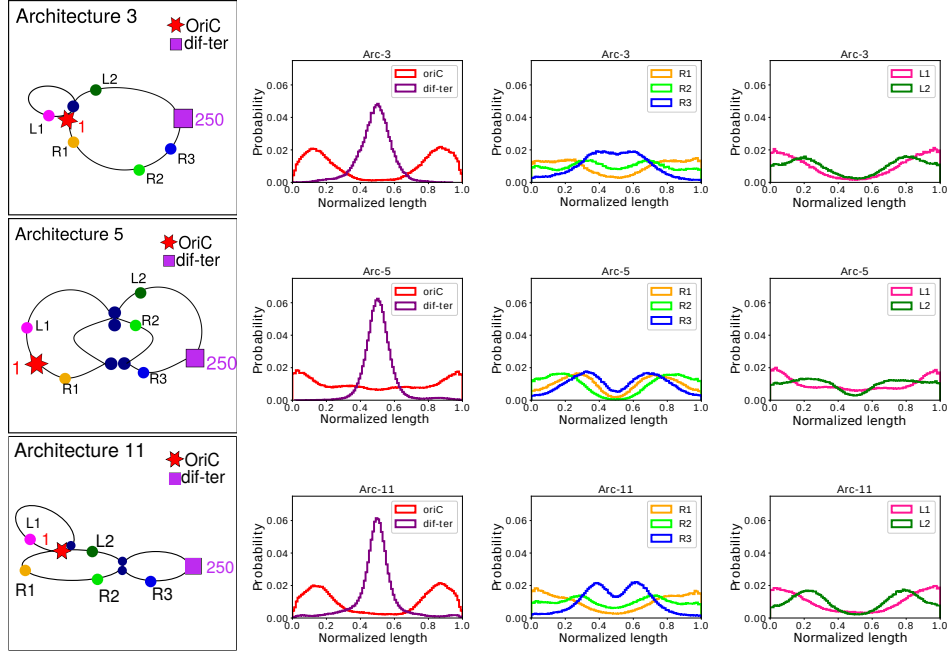


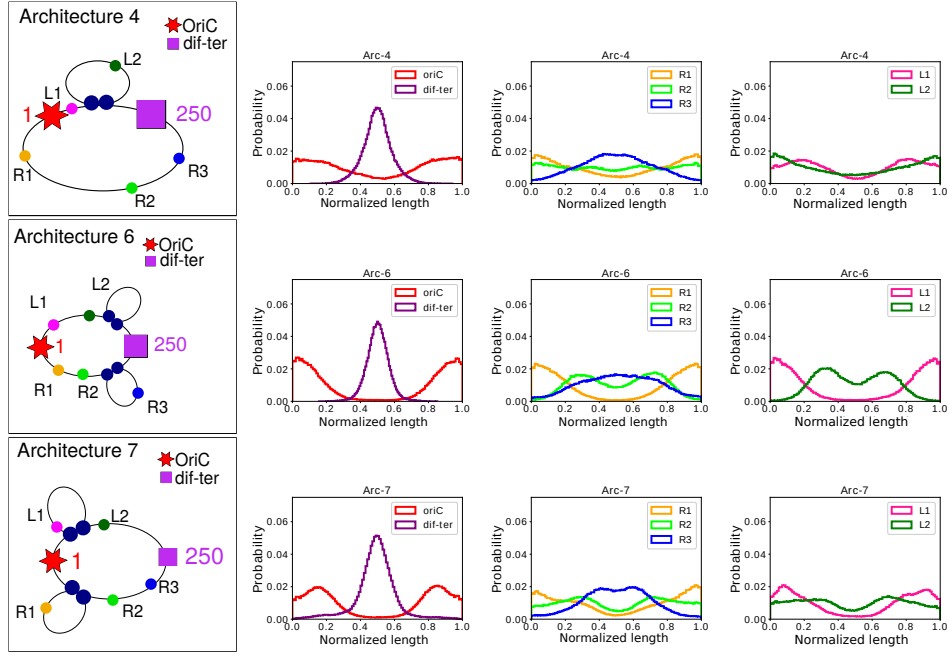
Figure 6.5: (*Data generated by S.P, analysed by D.M*) **Propensity of the two polymer to overlap post segregation:** A pair of segregated polymers might subsequently overlap spatially due to thermal fluctuations. We investigate whether particular polymer architectures disfavour spatial overlap between the pair of segregated polymers. We show the probability distribution $P(D_{com})$ of the distance between center of masses (D_{com}) of two polymers computed from 50 independent runs, for different architectures in the subfigures. We calculate these distributions from only those microstates for which $D_{com} \geq 0.4L$. Thereby, the number of microstates over which these distributions have been computed vary across architectures. For the ring polymer, the success rate of segregation is low, and hence fewer number of microstates have been used to calculate $P(D_{com})$. The x-axis has been scaled by the cylinder length L . Data is collected every 30000 MCS over 9×10^7 MCS. Since two polymers are deemed segregated if $\langle D_{com} \rangle \leq 0.48L$, non-zero $P(D_{com})$ at $D_{com} < 0.48L$ is indicative of the presence of overlaps. Chain crossing is allowed for the data presented.



Linearly looped architectures



Side looped architectures



184
Figure 6.6: (*Data produced by S.P*) The figure shows the spatial distribution of the *oriCs* and the other loci for linearly looped architectures and the side looped architectures. At the top we show a schematic of the chromosome contour with the loci and the corresponding monomer indices marked along the chain contour. Each row corresponds to the probability distribution of positions of tagged monomers for a particular architecture. There is also a schematic of the architecture with the loci marked, provided for aid of interpretation of corresponding localization data.

diameter at regular intervals. We also note that even for the architectures that successfully show segregation, N_{MCS} is much lower when topological constraints are released, as seen in Fig.6.4e. Thus the release of topological constraints is pivotal to accomplish timely and efficient segregation of spatially overlapping polymers, as in bacterial chromosomes.

6.4.2 Propensity of segregated polymers to overlap

After the initially overlapping polymers ‘demix’ due to entropic conditions, could some architectures again get spatially overlapped to some degree due to thermal fluctuations? This is crucial as segregated chromosomes need to remain well segregated with minimal overlap before cell division takes place. To investigate the same, we look at the probability distributions $P(D_{com})$ of the distance between the center of masses of the two polymers D_{com} for each of the architectures, refer Fig.6.5(a-d). The distribution has been computed using those microstates for which $D_{com} \geq 0.4L$. We observe that the Arc-0 (ring polymer) shows a relatively broad distribution as compared to other architectures. This indicates that a pair of polymers (chromosomes) with the Arc-0 architecture has significant overlap even after segregation. We also note that Arc-8 with 3 ‘side loops’ shows the highest propensity to avoid spatial overlap as $P(D_{com})$ is sharply peaked near $D_{com} = 0.5L$. We note that Arc-6 shows a narrower distribution as compared to Arc-7, while Arc-4 shows a narrower distribution as compared to Arc-3. We establish the underlying mechanism for these observations subsequently in the text.

The probability distributions shown in Fig.6.5 are indicative of the free energy of polymer configurations with different degrees of overlap (and corresponding D_{com})

for different architectures.

Thus, we show that for certain architectures the probability distribution of D_{com} is narrower, which only qualitatively indicates that the free-energy difference between the segregated and the unsegregated case may be higher for those architectures. This will later be corroborated by analytical calculations.

6.4.3 Spatial organization of polymer segments: tracked by localization of genomic loci

After the two polymers have segregated to two halves of the cylinder starting from a mixed configuration, we investigate specific organization in the polymers due to the presence of internal loops. We plot the spatial probability distribution of a few chosen monomers along the chain contour along the cylindrical long-axis for different architectures, refer Model section. In Fig.6.6 and Fig.6.7 we show the distribution of the position of loci along the cylinder long-axis. To plot these distributions, we consider microstates from only the successfully segregated cases. For this analysis, we have incorporated the effects of Topoisomerase. Note that we show the spatial distribution of only one of the *dif-ter* locus since the two *dif-ter* loci are crosslinked, and have nearly identical distributions. But for other loci, we plot the probability distributions such that it reflects the spatial distribution of the pair of loci from the pair of polymers.

Different monomers (genomic loci) get localized in some architectures due to the presence of internal loops. There the constituent loops of each architecture entropically repel each other and thus specific monomers or loci get localized to

specific regions along the cylinder long axis.

In data presented in Fig.6.6 and Fig.6.7, we can develop intuition to infer the positions where these loci get localized along the cylindrical long-axis due to the entropic repulsion between the constituent loops. In some architectures, such as Arc-0 we do not observe the localization of the loci. We discuss in detail the localization properties of all the loci for a few architectures. Similar intuition can be extended for the other architectures to understand the localization properties (or lack of localization).

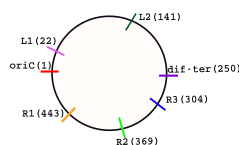
Arc-3: For a study with a pair of segregated polymers with Arc-3 architecture, the localization of *dif-ter* is automatically achieved due to the ter-cross-link coupled with the strong mutual entropic repulsion of the two large loops which span 126 – 250 – 375 – 499 of each ring-polymer. The smaller loop (spanning monomers from 1 – 125) of each ring polymer tries to avoid the larger loop and thereby is found near the poles. But the *oriC* forms the junction between the two loops, and hence the peak of its probability distribution is slightly shifted from the poles. When the *oriC* is located at the poles (in some microstates due to statistical fluctuation), then the loops of a polymers will spatially overlap with each other, which will be entropically disfavoured. Thus, the positions closest to the poles are preferentially occupied by locus L1 (monomer 22) which is part of the smaller loop. Locus R3 (monomer index 304) remains relatively close to the *dif-ter*-while avoiding the monomers of the smaller loop, thereby the probability of finding it is higher close to the center of the cylinder.

On the other hand R1 (monomer index 443) is part of the bigger loop and close to *oriC*. Thereby it remains relatively delocalized though it avoids polymer segments closer to *dif-ter*, while having a slightly higher propensity to be found near the *oriC*.

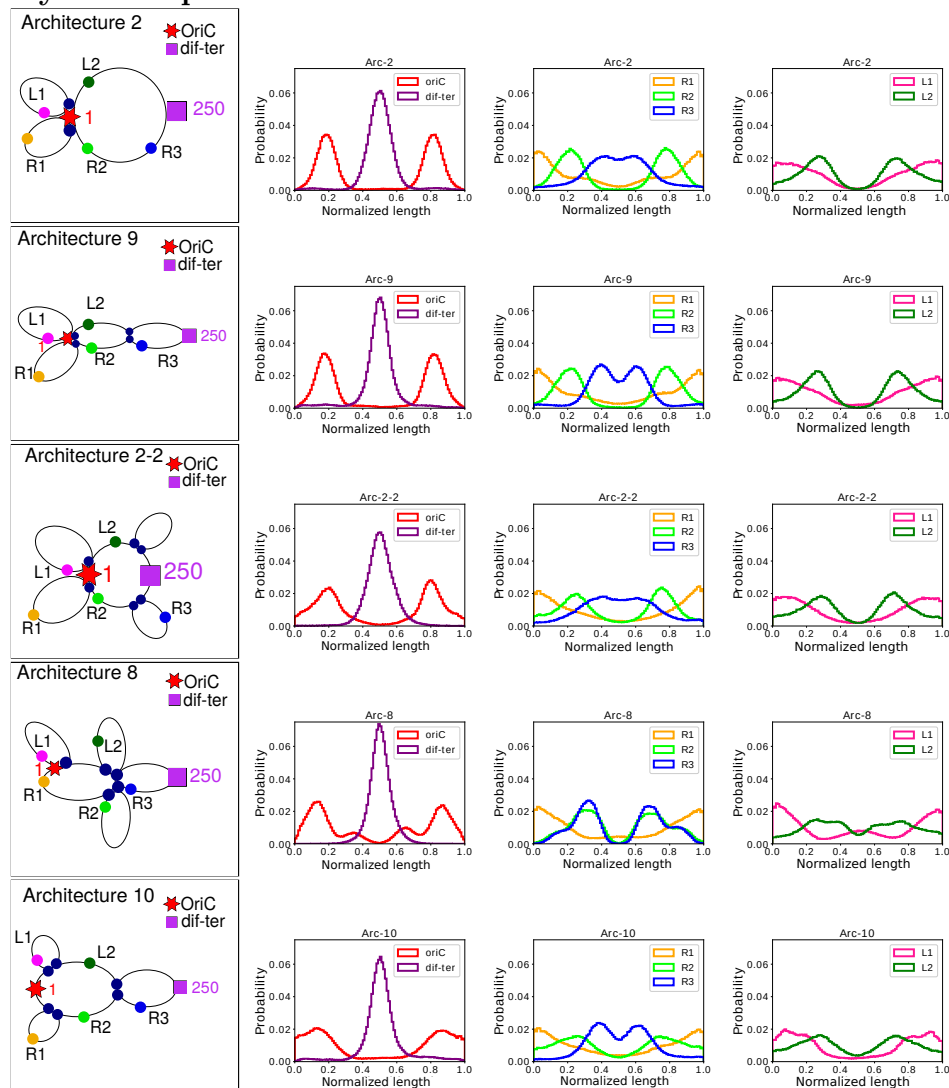
Locus R2 (monomer 369) is most delocalized and attempts to avoid the segments near *oriC* as well as segments near *dif-ter* and shows a small peak. Note that R2 is located at the mid point of the right arm of the ring polymer. Thereby, it is relatively unhindered from exploring different regions along the long-axis. Locus L2 (monomer 142) is near the cross-link between monomer-pairs 1 and 125, and has a distribution closer to the *oriC* distribution.

Arc-5: The Arc-5 architecture comprises of three loops placed linearly along the cell long axis. The middle loop has 124 monomers, while the two outer loops have 188 monomers each. Furthermore, the loci R1, R2, R3 and L2 are located close to the cross-links positions i.e. near the junction of the loops. For two segregated polymers with Arc-5 architecture, the monomers of the smaller loop in the middle get entropically repelled by monomers of the two larger loops on its two sides, and thereby occupies the regions around the quarter positions along the long axis. Hence, R1, R2, R3 and L2 are found near the quarter positions with a higher probability. The monomers around R2 and R3 avoid each other, thus the distribution peak for R3 is peaked closer to the location of *dif-ter*. As always, the *dif-ter* is localized near the center of the cylindrical long axis, due to the repulsion between loops from two different polymers. The loci *em oriC* and L1 are a part of the bigger loop with monomers 438 – 1 – 124. Due to the entropic repulsion between this loop and the other two, the *oriC* and L1 are found at the poles of the cylinder with higher probabilities.

Arc-4: On comparing the localization of Arc-3 (with linear loops) and Arc-4 (with side loops), the positions of L1 and L2 loci are more delocalized for the Arc-4 architecture. This is because the smaller side loop is forced to overlap with the bigger loop and is unable to localize itself at certain section of the cylinder. Even the data



Hybrid looped architectures



Ring polymer architectures

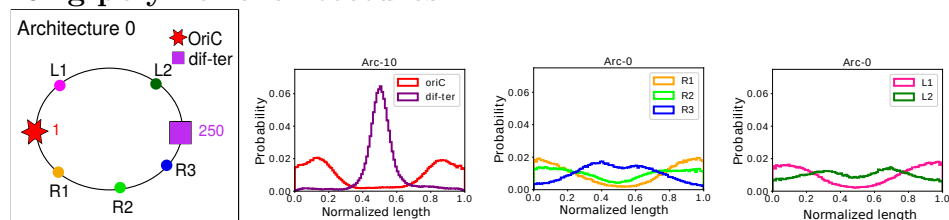


Figure 6.7: ¹⁸⁹ (Data produced by S.P) Localization of chosen monomers (genomic loci) for some architectures: The figure shows the spatial distribution of the *oriCs* and the other loci for hybrid architectures. At the top we show a schematic of the chromosome contour with the loci and the corresponding monomer indices marked along the chain contour. Other details are identical to that in the figure caption of Fig.6.6.

for loci R2 has a relatively flat distribution for Arc-4 as compared to the distribution for the Arc-3 architecture. Loci R3 is localized in both due to its proximity to *ter*.

Thus, we establish that we can get different polymer segments (genomic loci) to get localized in different parts of the cylinder (or the cell) by cleverly designing different polymer architectures and inducing the entropic repulsion between loops in cylindrical confinement, .

Hybrid architectures: Arc-2, Arc-2-2 & Arc-9: In Figure 6.7, we present the localization data for a pair of polymers with hybrid architectures, and in particular discuss the differences between Arc-2, Arc-9, and Arc-2-2 since they have similar side loops. In Arc-2, the *oriC* is localized at the junction of the two smaller loops (with 125 monomers each) and the bigger loop with 250 monomers. Loci R1 and L1, which are part of the two smaller loops, are found closer to the pole position, and have similar positional probability distribution. Meanwhile, the two smaller loops also try to avoid each while exchanging positions at the poles, and thereby have some probability of being found in the region of $0.25 - 0.75L$. Loci R2 and L2 are again closer to the point of cross-links with *oriC*, and thereby their distributions has peaks at positions close to *oriC*. Loci R3 is part of the bigger loop of the polymer and occupies the region closer to the center of cylinder.

The spatial distributions of loci for a pair of polymers with Arc-9 architectures are very similar to that for Arc-2, except that for the distribution of loci R3. The bigger 250-monomer loop of Arc-2 is now divided into two smaller loops of 125 monomers each, and R3 (monomer 304) is relatively closer to the added cross-link involving monomer 312. These additional loops again repel each other in addition to the repulsion between the two (smaller) loops already present for Arc-2. As a

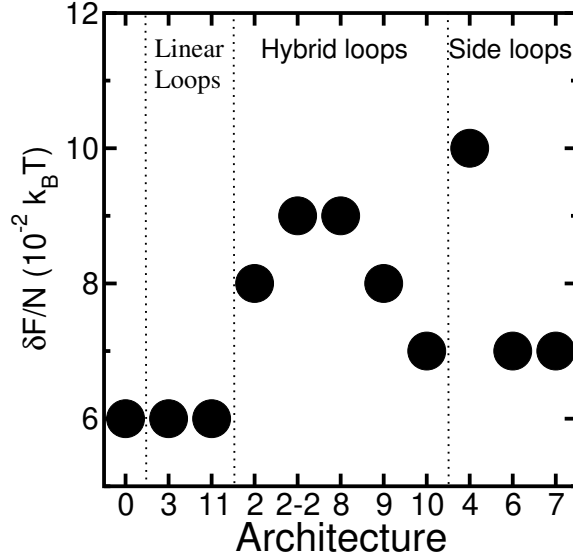


Figure 6.8: **Free energy calculations from the “blob theory approach”:** Fig. shows the analytically estimated Helmholtz free energy difference per monomer, $\delta F/N$ in units of $k_B T$ between the overlapped and segregated states of the two polymers, for several polymer architectures. We assume that the free energy associated with each blob is $k_B T$ and the diameter D of the confining (open) cylinder is $10a$.

consequence R3 has higher peaks at $0.4L$ & $0.6L$ for the Arc-9 architecture, and also prefers to remain in region between $0.25 - 0.75L$. The distribution for R3 is more spread out for Arc-2 architecture.

Arc-2-2 has positional distributions similar distributions to that for Arc-2 and Arc-9, but the presence of the two smaller side loops around the central loop leads R2 having higher probabilities near the poles, and broader distributions for R3 due to mutual repulsion between the two smaller loops near ter-cross-link. As a reference, we also provide the positional distributions for the ring-polymer Arc-0 architecture at the very end of Fig.6.7. The position of all the loci are highly delocalized resulting in very broad distributions, except for the *dif-ter* loci (and R3 loci) due to the presence of the ter-CL.

6.4.4 The blob theory approach

We next establish that our computational results are in agreement with an analytical treatment of free energy difference between the segregated and unsegregated states using a blob theory approach [164, 165, 166, 167, 168, 18]. Hence, we calculate the Helmholtz free energy difference per monomer $\delta F/N$, between the fully overlapped and the fully segregated states of the two polymers, for different architectures, refer Fig.6.8. We show that certain architectures have a larger $\delta F/N$ and thus lead to an enhancement of entropic repulsive forces. To incorporate the effect of different architectures in free energy calculations, we treat each loop (of each polymer) to be composed of two ‘effective linear segments’ (comprising of half the number of monomers in the loop) trapped in tubes of a suitable “effective” diameter D' . The value of D' is dependent on the architecture. We provide the details of these calculations in Sec-6.7.

The free energy difference between the overlapped and the segregated states of the polymers is proportional to f_{bl} . It is the free energy associated with each blob calculated with the ‘effective linear segment’, and is inversely proportional to $D^{5/3}$, where D is the diameter of cylinder of infinite length. In Fig.6.8 we compare the analytically estimated $\delta F/N$, for different architectures, for a choice of $D = 10a$ and $f_{bl} = k_B T$.

We note that Arc-3 and Arc-0, both with $\delta F/N = 3 \times 10^{-2} k_B T$, is the least favourable when it comes to segregation of polymers. In Fig.6.4, we note that Arc-3 and Arc-0 show similar low success rates of segregation, which is consistent with blob-theory calculations. We also note that Arc-4, Arc-2-2 and Arc-8 show the highest values of $\delta F/N$ among the investigated architectures.

However, $\delta F/N$ for Arc-4 exceeds that of Arc-8. This is not consistent with the success rates of segregation presented in Fig.6.4. In computational calculations, Arc-8 shows the highest success rate of segregation. Similarly, Arc-11 shows a high success rate of segregation, which is inconsistent with relatively low $\delta F/N$ values. In general side-looped architectures and architectures with hybrid loops have a higher $\delta F/N$ as compared to linear looped architectures and the ring polymer. We also note that Arc-4 shows a higher $\delta F/N$ as compared to Arc-3. Thus our conclusions about entropic costs arising from analytical free energy differences between mixed and overlapped state of two polymers are fairly consistent with previously presented simulation results in Fig.6.4, though a few cases show discrepancies. We attribute this to the limitations of the effective-tube-blob theory approach. We elaborate on these limitations in the subsequent section.

We emphasize here that we have no intention to establish a causal link between free energy calculations and the success rates of segregation. However, we indicate that there is consistency between free-energy differences between the fully overlapped and the segregated states, and the propensity to successfully segregate as obtained by computational measurements.

6.4.5 Limitations of the blob theory approach

The blob theory-based calculations that we present here rely on a few crucial approximations. Previous studies have indicated some shortcomings of this approach [169]. Our treatment considers polymers confined in an open cylinder. Moreover, the blob picture is valid only for long polymers and when the diameter D of the confining (open) cylinder is such that $D \geq 10\sigma$ [168]. Thus, the theoretical treatment

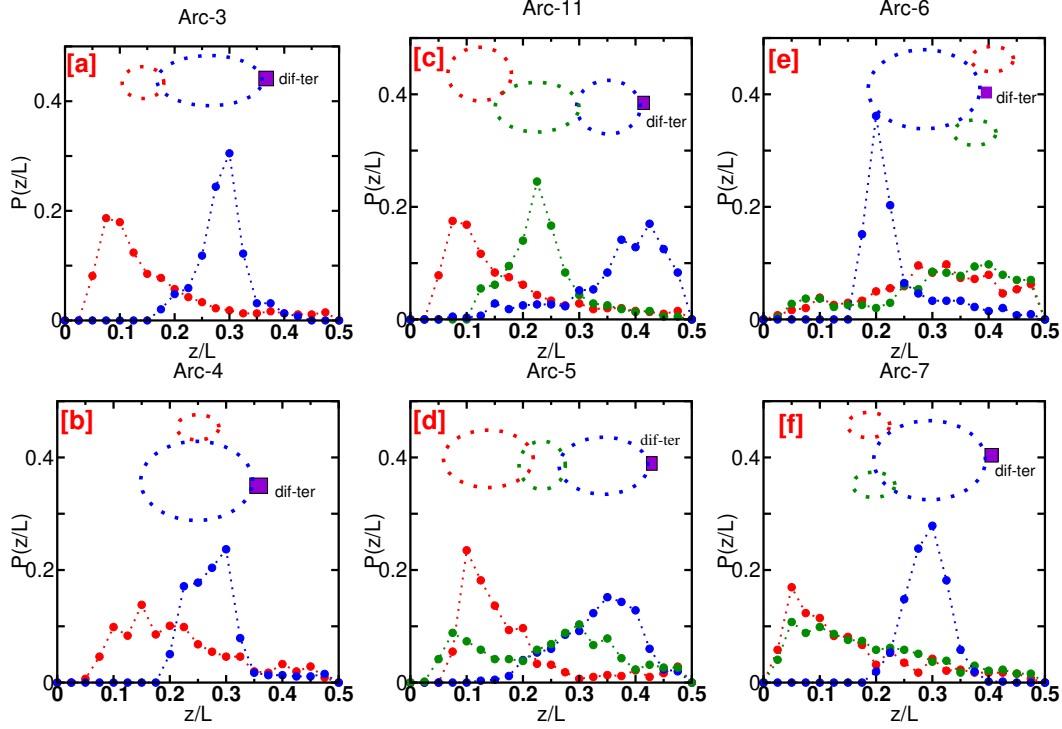


Figure 6.9: The subfigures show the spatial probability distributions $P(z/L)$ of the center of masses for loops corresponding to six architectures. The z axis is along the long axis of the cylinder and measured from one pole, and normalized by the length L of the cylinder. We also show schematic representations of the constituent loops in each subfigure. The colours of symbols in the plots correspond to the color of the particular constituent loop as shown in the schematic. Here we have two polymers conjoined at the *ter* loci. We show the data averaged over both polymers, but the range of the x – axis extends to only to $0.5L$, as the segregated polymers occupy different halves of the cylinder. The distributions have been computed from a pool of 50 independent runs for each architecture, considering only those runs which have successfully segregated.

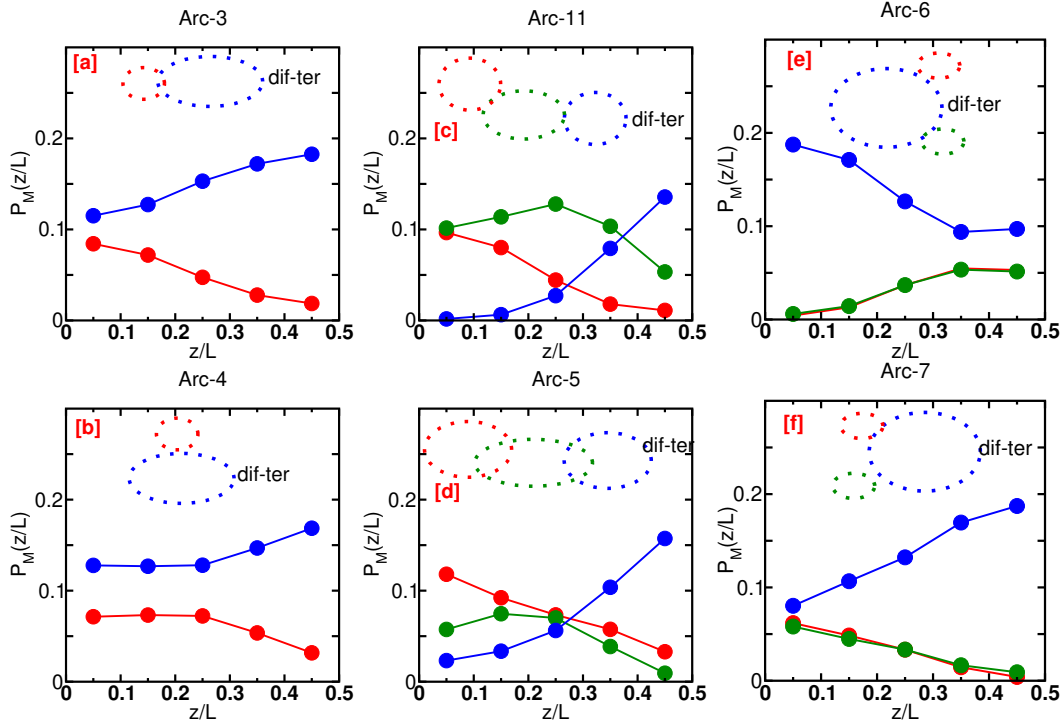


Figure 6.10: The subfigures show the normalized number density $P_M(z/L)$ of monomers of different loops along the long axis $\hat{z}$ of the cylinder, corresponding to the six architectures of Fig.6.9. To calculate the monomer density, we divide the long axis into 10 different sections, and calculate the mean number of monomers belonging to a particular loop in that section, and normalize it by the total number of monomer N ($N = 500$) in one polymer. The other figure specifications follow that of Fig.6.9.

assumes that the confining cylinder (and the “effective” sub-cylinders) to which the polymers are confined, are sufficiently big for blob theory to hold. We use a cylinder of diameter $7a$ and length $L = 35a$ in our simulations. This leads to a few discrepancies between our computationally obtained results and our analytical results as discussed. Be that as it may, the free energy analysis despite its limitations, is useful to decipher which polymer architectures are more amenable for efficient segregation starting from a mixed state.

6.4.6 Principles of segregation and localisation of polymer segments due to polymer architectures

We now establish how and why linear loops and side-loops differ in terms of the organization of the constituent loops along the cylindrical long-axis as shown in Fig.6.4 and Fig.6.5, and thereby further our understanding. To that end, for some selected architectures we plot (i) probability distributions of the positions of the center of mass (COM) of the loops and (ii) the number density of monomers for each loop along the cylindrical long-axis in Fig.6.9 and Fig.6.10, respectively.

We show in Fig.6.9 that in Arc-3 (with linear loop architecture) that the constituent loops show minimal spatial overlap, while for Arc-4 (with a small side loop) the two constituent loops overlap considerably as they explore different conformations. We find through our analytical calculations that a pair of polymers with Arc-4 architecture have a higher $\delta F/N$, as compared to a pair of polymers with the Arc-3 architecture.

This is also substantiated in Fig.6.10(a) and (b), where we see that monomers of the smaller loop (marked in red) effectively reduce spatial overlap with the monomers of the bigger loop (shown in blue) for Arc-3, while the effect is reduced for Arc-4. In Arc-3 and Arc-4, the monomer density of the bigger loop (blue) is higher near mid-cylinder, as the *ter* monomer in the bigger loop has cross-link with the *ter* of the other polymer. In Arc-4, the monomer densities of the smaller loop (red) have nearly the same values from the pole to the quarter position within the cylinder, whereas for Arc-3 the monomer densities decrease monotonically as one moves from the pole to mid-cylinder positions.

In side-looped architectures, constituent loops show more spatial overlap as compared to linearly-looped ones. This is because segments belonging to different loops of a polymer occupy “effective” cylinders of smaller radii. We illustrate this through the schematic diagrams for Arc-2 (which also has side-loops) presented in Sec-6.7. Thus there is a higher free energy cost of overlap of the two polymers having ‘side-looped’ architectures. We have also shown in Fig.6.4 that Arc-4 shows a higher success rate of segregation as compared to Arc-3. Moreover, Arc-3 also shows a broader distribution of D_{com} as compared to Arc-4 in Fig.6.5. This is because, in a cylinder with two polymers with Arc-4 architecture, the presence of the side-loops hinder the overlap of loops from two different polymers which have segregated to the two halves of the cylinder.

Furthermore, we note from Fig.6.4 that Arc-11 shows a high success rate of segregation. To investigate the underlying reason, we look at the probability distributions of the positions of the COMs of each constituent loop in Arc-11 in Fig.6.9. We show that all the constituent loops show significant overlap with its neighbouring loop(s), though the peaks of the distributions are at distinct locations along the long-axis. We attribute this to the finite length of the confining cylinder. This can also be surmised from Fig.6.10(c) where the monomers of the two loops (marked in red and blue) can be seen to largely occupy different regions of the cylinder, yet also show considerable spatial overlaps with the monomers of the central loop (marked in green). Thus, even though the configuration of the loops in Arc-11 is linear, the architecture is “effectively” a hybrid architecture having characteristics of both linear and side-looped architectures. This is the reason why Arc-11 shows high success rates of segregation as shown in Fig.6.4.

Note that in our analytical calculations we had assumed that the polymers are

in an open cylinder and thus we had obtained considerably lower values of $\delta F/N$ as compared to other hybrid (and side-looped) architectures. This effect is even more pronounced (as compared to that of Arc-11) in the case of the Arc-5 architecture, where the constituent loops show even greater spatial overlap with each other. This can be confirmed with the data presented in Fig.6.9(d) and Fig.6.10(d). We note that the COMs of the loops (and also the monomers of the loops) show considerable spatial overlap with each other. The degree of overlap is also higher than that of Arc-11, since the (larger) central loop in Arc-5 shows greater spatial overlap with the loops shown in red and blue, as compared to that of Arc-11. Thus, it also has a higher success rate of segregation as compared to that of Arc-11.

We had also previously mentioned that Arc-6 shows a much higher success rate of segregation as compared to Arc-7, although both these architectures have two side-loops of identical lengths (80 monomers each) attached to a bigger loop (340 monomers). Furthermore, Arc-6 shows a narrower distribution of D_{com} as compared to Arc-7 in Fig.6.5. This is because in Arc-6 the two smaller additional loops are close to the *dif-ter* cross-link while in Arc-7 the two additional loops are closer to the *oriC*.

As a consequence of their positions, in Arc-6 the two smaller loops have considerable overlap with each other and with the bigger loop, especially near the mid-cylinder positions. On the other hand, Arc-7 the side-loops of Arc-7 overlap considerably with each other but show relatively less overlap with the bigger loop. The monomer densities of the two smaller loops are very low near the mid-cell position, whereas the monomer densities of the bigger loop is relatively low near the pole positions. Thus the two side-loops in Arc-7 and the bigger loop mostly occupy different parts of the cylinder akin to the organization seen in linearly-looped architectures.

These can be confirmed from the distribution of the COMs of the constituent loops of Arc-6 and Arc-7, given in Fig.6.9(e) and (f), as well as from data in Fig.6.10 (e) and (f).

Note that in our analytical calculations we had calculated the same $\delta F/N$ for Arc-6 and Arc-7, however the actual $\delta F/N$ is likely to be higher for Arc-6, than that of Arc-7 since there are more overlaps between the loops in Arc-6. This is the reason why Arc-6 has a much higher success rate of segregation compared to that of Arc-7.

Thus, in light of the analysis presented in the preceeding paragraphs (and figures), we can redefine a side-looped architecture as the kind of architecture which leads to increased spatial overlaps between the constituent monomers of the internal loops. A “linearly looped” architecture on the other hand shows minimal spatial overlaps between the constituent monomers of the internal loops as they arrange themselves “linearly” along the long axis of the cylinder.

6.4.7 Summary

In summary, we conclude the following from our study on polymer architectures:

1. For two initially overlapping polymers confined in a cylinder, we show that side-looped architectures lead to larger entropic forces of segregation. This is established both analytically (as shown in Fig.6.8) and can also be inferred from our computational results presented in Fig.6.4 and Fig.6.5, for Arc-4 and Arc-3. Thus, Arc-4 which is a side-looped architecture leads to higher forces of segregation compared to Arc-3 which has a linear configuration of loops.

2. If the loops are linearly arranged in a cylinder of finite length, then due to the finite size of the cylinder the loops may overlap and act as “side-loops” in certain architectures, e.g. Arc-5 and Arc-11.
3. Some hybrid and side-looped architectures such as Arc-8 and Arc-6 lead to lesser fluctuations in D_{com} . Thus, spatially segregated polymers having such architectures would minimize partial overlaps due to thermal fluctuations. This is likely to be of importance for bacterial chromosomes where the two daughter chromosomes must segregate completely before cell-division.
4. Even for side-looped architectures, the segregation properties depend crucially on where the loops are located along the chain contour. This has been illustrated with the help of Arc-6 and Arc-7 in the preceding paragraphs. We have explained why Arc-6 induces higher forces of entropic segregation as compared to Arc-7.
5. Through the data presented in Fig.6.6 and Fig.6.7 we find that different architectures with additional loops lead to localization of genomic loci (monomers) at specific regions along the cylindrical axis with relatively lesser fluctuations about the mean position. However, Arc-0 (ring polymer) fails to show any such localization. Thus, one may design polymer architectures suitably to localize specific polymer segments in specific regions of the confining cylinder.
6. The polymer segments which are at the junction of two loops (i.e. near the cross-links) get more localized as compared to segments which are away from the junctions.

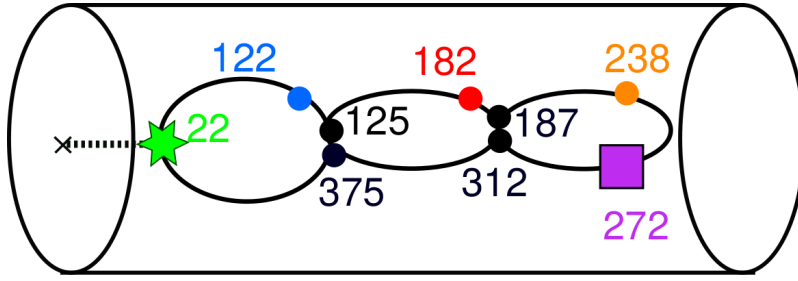


Figure 6.11: The figure shows a polymer having the Arc-C architecture which is confined in a cylinder. It is similar to Arc-11, and the monomer pairs which have been cross-linked are shown in black. These monomer pairs are 125, 375 and 187, 312. In this convention, the *oriC* is monomer 22 and is tethered to the pole by a spring of spring constant $1k_B T/a^2$. The other monomers which we have shown here correspond to specific genomic loci which have been studied using FISH experiments [155]. The monomer index 122 corresponds to the locus *pilA*. The monomer index 182 corresponds to the locus *pleC*. The monomer index 238 corresponds to the locus *podJ* and the monomer index 272 corresponds to the *ter*.

6.5 Localization and organization in *C. crescentus* chromosome.

Using the insights gained from the previous results on polymer architectures, we now design a polymer architecture called Arc-C, which is plausibly adopted by the *C. crescentus* chromosome. The polymer architecture Arc-C is shown in Fig.6.11. Note that Arc-C has a linearly looped configuration similar to Arc-11 and has just two additional CLs. To establish a correspondence with the *C. crescentus* chromosome, we first look at the spatial localisation patterns of loci as has been measured in experiments for the *C. crescentus* chromosome [170, 155, 151].

We present in Fig.6.12 (a) and (b) the spatial probability distributions of certain loci, as obtained from experiments [155] and our simulations respectively. We find that we reproduce the localisation patterns seen *in-vivo* to a fair degree, despite

the simplicity of our model. We find that the spatial distributions of the loci show distinct peaks. However, the position of the peaks do not exactly match those seen *in-vivo*. This in our opinion is to be expected since we employ a very minimal model. One may optimise the sizes of the loops in Arc-C to obtain an exact match with the FISH experiments, but here we only aim to establish the principles that reconciles complementary experiments conducted on the *C.crescentus* chromosome and hence we abstain from adopting the optimization route. A previous study has already established that such localisation patterns cannot be obtained from just a confined ring polymer with its *ori* tethered to the cell-pole [151].

Furthermore in Fig.6.12(c) and (d) we show the contact maps obtained from experiments and simulations respectively. We find that our simulated contact map is in qualitative agreement with experiments in that we obtain the other less prominent diagonal which is characteristic of *C.crescentus* Hi-C data [170]. Thus we reconcile to a far degree the experimental observations obtained from *two complementary* experiments, using our designed polymer architecture.

However there are also a few key differences between the experimentally obtained contact map and the one obtained from simulations. The differences and the reasons for the same are listed as follows:

1. The genomic regions corresponding to the sites of the cross-links (CLs) in the simulated contact map appear as bright blue spots along the less prominent diagonal, while such bright regions are not visible in the experimentally obtained contact map. However, we encourage the reader to zoom into these regions in the experimental contact map. On zooming in one finds that the sites of the CLs do correspond to points of high probability in the experimental contact

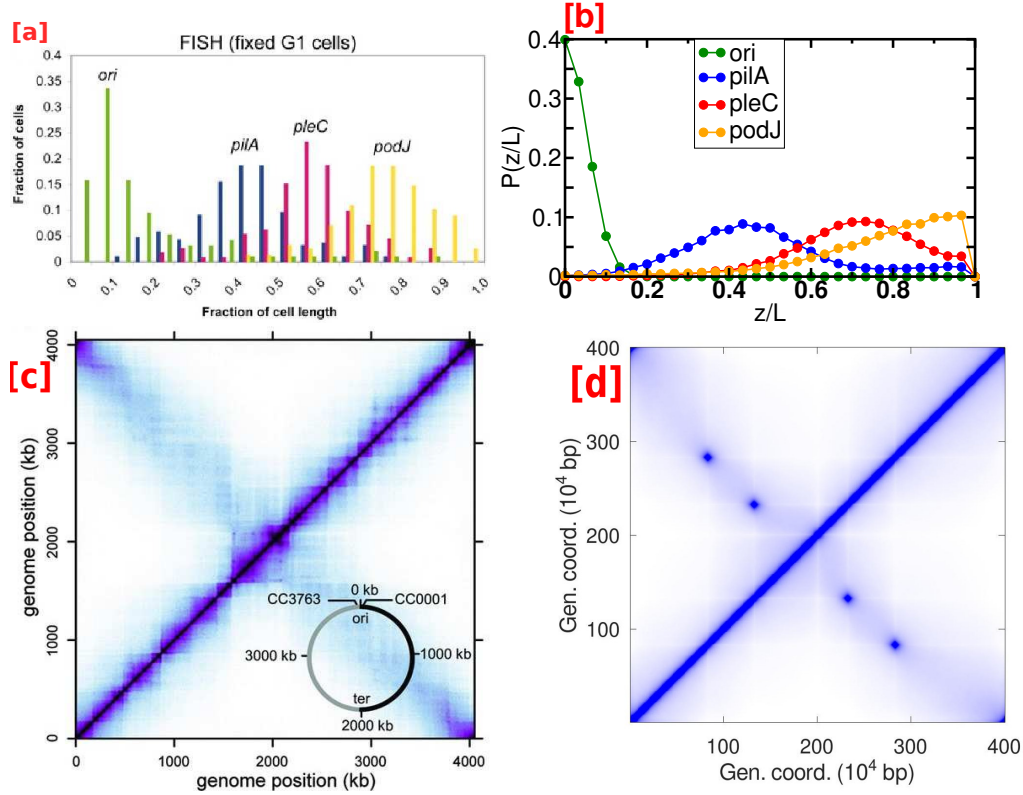


Figure 6.12: **Spatial organization of *C. crescentus* chromosome:** Subfigures (a) and (b) shows the spatial probability distributions of loci obtained from experiments and simulations respectively. In the simulations we use the polymer architecture Arc-C with the monomer corresponding to the *ori* tethered to the cell pole, as is the case *in-vivo*. The x-axis has been scaled by the length of the confining cylinder. The *ori* locus remains tethered to the cell-pole and therefore remains localised around the pole [170, 155, 151]. Furthermore we also compare the Hi-C contact map obtained from experiments shown in (c), which is in qualitative agreement with the contact map obtained from simulations in (d) and has the less prominent diagonal [170] characteristic of *C. crescentus* chromosome. Subfigures (a) and (c) have been reproduced from [155] (Copyright (2004) National Academy of Sciences, U.S.A.) and [170].

map, although they do not have the highest probabilities as in the simulated contact map.

2. The sites of Arc-C cross-links (CLs) have high probabilities in the experimental Hi-C map, however the other regions also have high contact probabilities. A question that the reader is likely to ask is: why have we not introduced other possible CLs in our model? We believe that there can be other possible CLs due to proteins which colocalize different segments of the DNA-polymer. However we establish here that only a minimal number of long-lived CLs are required to obtain a match with the FISH data and to obtain a contact map qualitatively similar to the experimentally obtained contact map.
3. Next, we elaborate on why the CL sites in the simulated contact map have bright, *broad* circular patches, while the experimental Hi-C map shows relatively tiny illuminated spots at the genomic sites corresponding to the sites of the CLs used in this study. Note that in the simulations we have a resolution up to the length scale of a single bead ~ 8 kbp. Thus we get large bright patches, as monomers proximal to the CL sites also contribute and enlarge the size of the blue spots.

In the cell, the chromosome may have CLs (binding sites) located at specific sites over a much smaller length scale, viz., CLs are realized by a suitable protein having a size of $\sim nm$. This will change the topology over a larger length scale of the DNA-polymer and comparable to what is considered in this study. However, the experimentally obtained Hi-C contact map has a much higher resolution of $\sim kbp$, and thus a protein sized experimental cross-link is much smaller than the length scale of a “cross-link” in simulations. Therefore, these “cross-links” and contributions from its proximal segments

would show up in the experimental Hi-C map as point-like blue spots. Thus, in the experimental Hi-C maps, the size of the bright spot corresponding to a “cross-link” in the chromosome would be relatively tiny.

4. As mentioned previously, we find that the simulated contact map shows the highest probabilities of contacts at the sites of the additional cross-links. However, this is not the case for the experimental contact map although the sites of the cross-links do show relatively high probabilities. This is because in our minimal model we assume that the additional cross-links are permanent throughout the simulation run, whereas in the *C.crescentus* chromosome these may form transiently at various points during the cell-cycle. These additional “cross-links” in the *C.crescentus* chromosome may be long-lived despite forming and dissolving due to various biological processes. Furthermore, the experimental Hi-C map is calculated as an average over a large number of cells in different stages of their life cycle, where the CLs could be dissolved in some of the chromosomes.

In the case of bacterial chromosomes such as *C.crescentus* it is likely that there exist many other “effective crosslinks” which may arise dynamically due to several proteins found *in-vivo* and plectoneme super-coiling [151]. However, we propose that the crosslinks we have introduced are relatively long-lived and is responsible for the organisation of the *C.crescentus* chromosome at the length scales considered in our study.

As mentioned before many past studies with more detailed models have used several cross-links to obtain a Hi-C map quantitatively similar to that of experiments [113, 152, 153, 154, 114, 122]. The position of these CLs were extracted from the Hi-

C map and the spring constants were optimised to obtain a contact map very similar to that obtained from experiments. Here we take an orthogonal approach and use just 2 additional CLs. Furthermore we also show that Arc-C reconciles observations from FISH experiments. Thus, we introduce as few constraints as possible in our model and avoid introducing a large number of constraints to achieve a quantitative match with the Hi-C experimental data.

We showed previously that a particular polymer architecture localises polymer segments (or genomic loci) of *E.coli* chromosomes [3]. Here we establish the mechanism by which this localization takes place in bacterial chromosomes and propose that this could be a generic method adopted by other bacterial species.

6.6 Conclusions and Discussions

We establish the topological mechanisms by which polymer segments of a polymer can be induced to get localised in a confined geometry by analyzing a pool of polymer architectures. This mechanism depends on the concept of tuning entropic repulsion between segments of polymers by modifying the topology and the excluded volume of monomers.

The two Arc-0 ring-polymers (each with 500 monomers) cross-linked at the *dif-ter* may also be considered as a single polymer (of 1000 monomers) with the monomers corresponding to the two *dif-ter* cross-linked. In this vein the spatial segregation of the two polymers may be interpreted as the spatial localization of the internal loops of a 1000 monomer chain within the cylinder.

For other architectures that we have considered with additional loops, one may correspondingly imagine the system as a single polymer of 1000 monomers having many internal loops. These internal loops organize themselves differently for different architectures, within the cylinder. Thus we show that by introducing strategically placed loops within a ring polymer, one can induce an “effective” repulsive interaction between particular polymer segments, which leads to specific spatial localization patterns, and thereby organization of the segments.

Such entropy-driven “effective” interactions in polymers may find a host of applications where one has to design polymer systems with specific organizational properties (as in block co-polymers), but without invoking explicit enthalpic interactions. We also establish the principle by which modified polymer architectures lead to an enhancement of the entropic repulsion between overlapping polymers, and thereby aid the segregation process. We show this by analytically estimating the free energy difference between overlapped and segregated states for several polymer architectures using the blob theory approach [168].

These modified architectures may arise in bacterial chromosomes to accomplish biological functions [140], but may also lead to the enhancement of the entropic forces of segregation. We also show that the release of topological constraints plays a crucial role in the enhancement of the segregation process and also results in a significant increase in the success rates of segregation. Furthermore several experimental results have shown previously that chromosomal loci show specific localisation patterns in bacterial chromosomes [20, 95]. Thus we show here that such localisation patterns observed for *C.crescentus* chromosomes, are consistent with those obtained with polymer architecture Arc-C. Previously, we established that architecture Arc-2-2 reconciles spatio-temporal localization patterns seen in experiments for the *E.coli*

bacterial chromosome [3]. Thereby, this current manuscript further strengthens the thesis that bacterial chromosomes employ this generic principle of entropy based organization of polymeric segments, to segregate and organize itself within the cell.

However, as we have established here, even for synthetic polymers in confinement, which do not have topological constraint release mechanism, the loops (and consequently polymer segments) will organize themselves using its internal loop topology for cases where segregation has been successful.

Note that it is possible (and likely) that if we run much longer simulations, then all the initially overlapping polymers would spatially segregate, for runs with a constraint release mechanism. This is also likely to be true for the case when we start out with non-concatenated polymers without a constraint release mechanism in place, though the dynamics will be slower because release of topological constraints may take more time. The mean value of N_{MCS} , the number of MCS required for segregation for particular architectures would increase if we wait for polymers in all independent runs to successfully segregate. Thus the mean N_{MCS} in that case, would show greater differences across architectures than what we have presented in this paper. In this manuscript, we focus instead on the success rates of segregation since the simulation times required for complete segregation for all architectures are computationally inaccessible. But, if we had 100% success rates of segregation, then N_{MCS} would show much larger variation in values. Thus these two quantities are complementary to each other in a sense, and either can be used for to reach the conclusions we have presented.

Future advances in nanotechnology may rely on the principles outlined here, of strategically designing polymer architectures to induce localisation of certain polymer

segments. One can envisage applications in nano-medicines which may require spatial localization of pharmaceutical cargo in confined environments [171, 172]. In the last decade there have been several experimental methods [173] developed to design well defined ring polymers, and moreover, derive more complex multicyclic topologies analogous to what we have used. This allows for exploration of their properties arising from their topology and thereby find new potential applications as they can be self assembled into hierarchical macromolecular architectures at larger scales [174] and more unique dynamical properties [175]. More fundamentally the approach of tuning entropic interactions between polymer segments *via* polymer architectures could also be employed by researchers from diverse backgrounds to obtain micro-phase separation of polymeric mixtures induced solely due to entropic considerations without having to invoke enthalpic interactions between the constituent polymers.

6.7 Free Energy of polymers of different topologies in a cylinder of diameter D

6.7.1 Arc-0: Free energy difference δF between the overlapped and the segregated configurations.

Free energy F_{seg} of two segregated ring-polymers in an infinite cylinder.

To calculate the Helmholtz free energy F_{seg} for two segregated ring polymers, conjoined at the ter and confined in a cylinder of diameter D , we use the blob theory approach which calculates the free energy of blobs of polymers in confinement in an

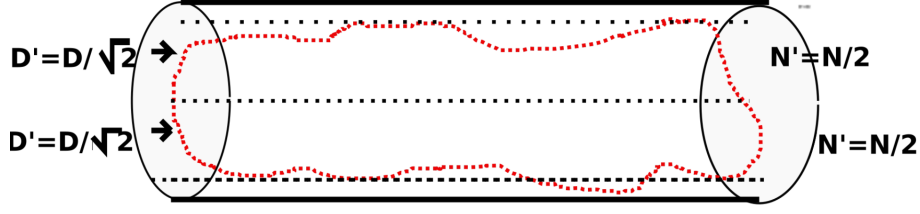


Figure 6.13: A schematic representation of single ring polymer with $N = 500$ monomers is shown. Each arm of the ring polymer from monomer number 1 to 250 with $N' = N/2 = 250$ monomers can be visualized as a linear segment constrained in an effective tube of diameter $D' \approx D/\sqrt{2}$. We assume that the two overlapping segments occupy equal volumes per unit length (L'), but the sum of the volumes of the two smaller cylinders is equal to the volume of the bigger cylinder, viz., $\pi(D')^2L' + \pi(D')^2L' = \pi D^2L'$, thus $D' = D/\sqrt{2}$.

infinitely long cylinder of diameter D . We calculate the free energy corresponding to just one confined ring polymer given by F_{conf} . The free energy of two segregated ring polymers is given by

$$F_{seg} = 2F_{conf} \quad (6.2)$$

To calculate F_{conf} , we first assume that each linear segment containing $N' = N/2$ monomers is constrained to a tube of an effective diameter of $D' \approx D/\sqrt{2}$ [168]. This choice of the diameter D' of the effective tubes ensures that the combined volume of the two tubes is equal to the volume of the cylinder of diameter D .

A segment (blob) of a self avoiding polymer chain with g monomers spans diameter D' such that $D' = a \times g^{3/5}$ monomers. Thus the total number of monomers in each 'blob' is given by

$$g = (D/\sqrt{2})^{5/3}. \quad (6.3)$$

and the total number of self-avoiding blobs within a cylinder, for two linear chain of

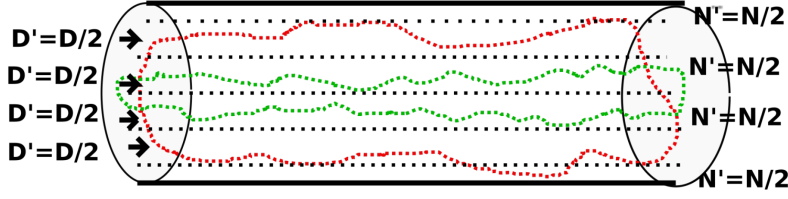


Figure 6.14: Fig. shows a schematic representation of two overlapping ring polymers (shown in red and green) where each arm can be visualized as a linear segment constrained in an effective tube of diameter $D' \approx D/2$.

N' monomers is therefore given by ,

$$n_{bl} = 2 \times (N'/g) = 2 \times \frac{N}{2} \times (\sqrt{2}/D)^{5/3} \quad (6.4)$$

If the free energy associated with each blob is given by f_{bl} (later taken $\approx k_B T$), then the free energy of two ring polymers is $2n_{bl}f_{bl}$ if they are not overlapping, i.e. in the segregated state. Thus the free energy of two ring polymers which are segregated in an infinite cylinder is F_{seg} is given by

$$F_{seg} = 2F_{conf} = 2 \times \frac{N}{D^{5/3}} \times 2^{5/6} \times f_{bl} \quad (6.5)$$

Free energy F_{ov} of 2 rings-polymers in overlapped state in an infinite cylinder.

To calculate the Helmholtz free energy F_{ov} for two overlapping ring polymers (conjoined at the ter) we assume that each arm of a single ring polymer containing $N' = N/2 = 250$ monomers occupies an effective tube of diameter $D' = D/2$. Note that there are 4 such linear arms since there are two overlapping ring polymers. The cross-sectional area of the four tubes (each containing a polymer arm) remains

$4 \times \pi(D')^2 = \pi D^2$. Thus the total number of monomers in each ‘blob’ is given by

$$g = (D/2)^{5/3}. \quad (6.6)$$

and the total number of blobs for four polymer-arms are therefore given by ,

$$n_{bl} = 4 \times (N'/g) = 4 \times \frac{N}{2} \times (2/D)^{5/3} \quad (6.7)$$

If the free energy associated with each blob is given by f_{bl} , then the free energy of the overlapped state F_{ov} is given by

$$F_{ov} = 2N \times (2/D)^{5/3} \times f_{bl} \quad (6.8)$$

Thus the **Free energy difference per monomer**, between the overlapped and the segregated state for two Arc-0 (ring polymers) in a cylinder of diameter D , is given by

$$\delta F/N = (F_{ov} - F_{seg})/N = 2.78 f_{bl}/D^{5/3} \quad (6.9)$$

6.7.2 δF between the overlapped and the segregated states of two polymers with Arc-2 architecture.

A polymer with the Arc-2 architecture has two loops of 125 monomers each in a chain of 500 monomers. Thus there are $N/4$ monomers in each of the smaller loops of a polymer having Arc-2 architecture, while the bigger loop has $N/2$ monomers, refer Fig.6.3 for the schematic.

Free energy F_{seg} of two Arc-2 polymers in segregated state in an infinite cylinder

In order to calculate the Helmholtz free energy for two segregated polymers (conjoined at the ter) of architecture Arc-2 using the blob theory approach, we calculate the free energy of just one confined polymer, F_{conf} in an infinitely long cylinder. The free energy of the two segregated ring polymers is $F_{seg} = 2F_{conf}$.

To calculate F_{conf} , we assume that F_{conf} is given by the sum of free energies in section-I and section-II: $F_{conf} = F_I + F_{II}$, where F_I and F_{II} denote the contributions to the free energy from sections I and II of the cylinder, refer the top subfigure of Fig.6.14.

- In polymer segments occupying section-I, each linear segment of $N' = N/8$ monomers (the number of monomers in one of the smaller loops is $N/4$) is constrained in a tube of an effective diameter of $D' \approx D/2$. The value of D' can be calculated considering that there are four cylinders of diameter D' . The hareview sum of the volume of the 4 cylinders is equal to that of a cylinder with diameter D . Thus the number of monomers in each ‘blob’ is given by,

$$g = (D/2)^{5/3}. \quad (6.10)$$

and the total number of blobs from 4 linear chains with $N/8$ monomers is given by,

$$n_{bl} = 4 \times \frac{N}{8} \times (2/D)^{5/3} \quad (6.11)$$

If the free energy associated with each blob is given by f_{bl} , then the free energy F_I

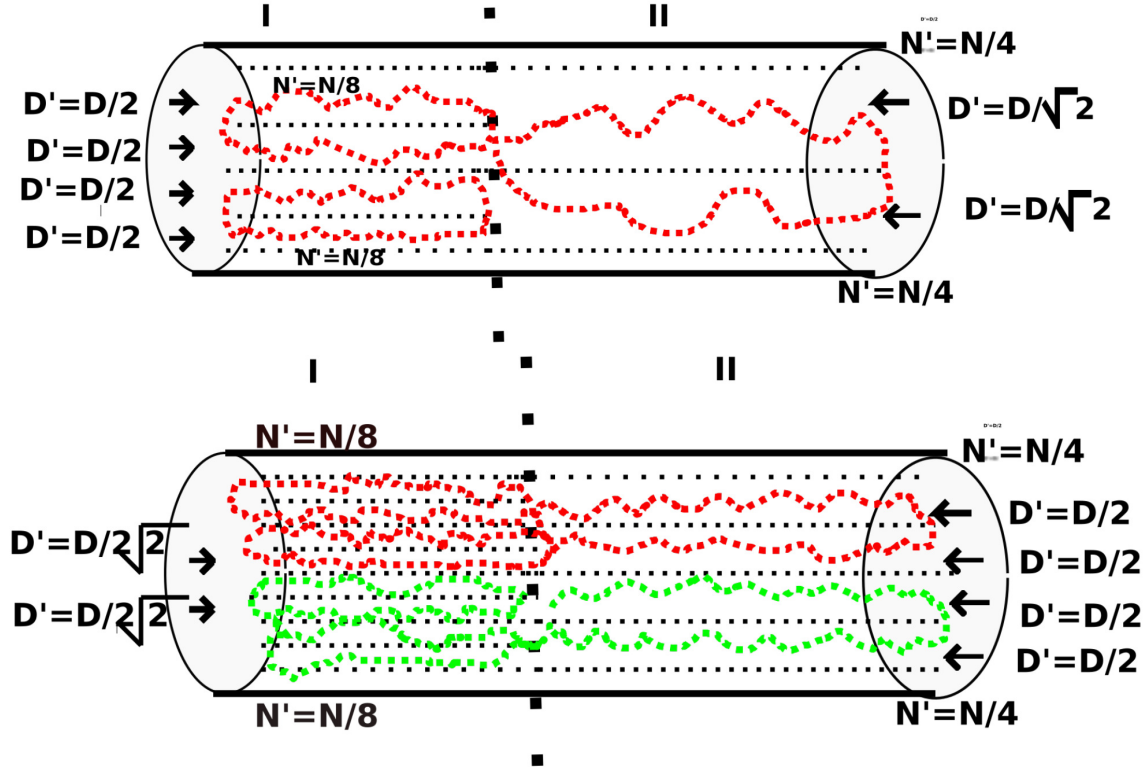


Figure 6.15: The top subfigure shows a schematic representation of single polymer in the Arc-2 configuration confined in a cylinder of diameter D . The cylinder can be divided into two sections: section-I containing the two smaller loops and Section-II containing the larger loop with $N/2$ monomers. The larger loop, which itself is a ring polymer with $N/2$ monomers, can be visualized as two linear overlapping linear chain segments with $N/4$ monomers in each segment. Each linear segment of $N' = N/4$ monomers is confined in an effective tube of diameter $D' \approx D/\sqrt{2}$. In section I, there are the two smaller loops (ring polymers) with $N/4$ monomers each. Each loop can be thought of as containing two linear segments with $N/8$ monomers, each occupying effective tubes of diameter $D' \approx D/2$.

The subfigure at the bottom shows a schematic representation of two overlapping Arc-2 polymers (shown in red and green) where the bigger loops of section-II can be conceptualized as four linear polymer segments, each constrained in an effective tube of diameter $D' \approx D/2$. In Section-I, there are 8 overlapping linear segments, each confined in cylindrical tubes of $D/(2\sqrt{2})$. Each smaller loop has $N/4$ monomers, hence each linear segment has $N/8$ monomers.

is given by

$$F_I = (N/2)/D^{5/3} \times 2^{5/3} f_{bl} \quad (6.12)$$

• In section II, each linear segment of $N/4$ monomers is constrained in a tube of an effective diameter of $D' \approx D/\sqrt{2}$. The number of monomers in each ‘blob’ is:

$$g = (D/\sqrt{2})^{5/3}. \quad (6.13)$$

and the total number of blobs from two chains each with $(N/4)$ monomers is:

$$n_{bl} = 2 \times \frac{N}{4} \times (\sqrt{2}/D)^{5/3} \quad (6.14)$$

If the free energy associated with each blob is given by f_{bl} , then $F_{II} = n_{bl}f_{bl}$:

$$F_{II} = 2 \times \frac{N}{2} \times (\sqrt{2}/D)^{5/3} \times f_{bl} \quad (6.15)$$

• So finally, for 2 segregated Arc-2 polymers which are next to each other in an infinite cylinder of diameter D ,

$$F_{seg} = 2F_{conf} = 2(F_I + F_{II}) \quad (6.16)$$

Free energy F_{ov} of two Arc-2 polymers in overlapped state in an infinite cylinder.

In order to calculate the Helmholtz free energy using the blob theory approach, for two overlapped polymers (conjoined at the ter) of architecture Arc-2, we once again

employ the same approach as before. Thus, To calculate F_{ov} we first assume that F_{ov} is given by, $F_{ov} = F_I + F_{II}$, where F_I and F_{II} denote the contributions to the free energy from sections I and II, as shown in the bottom subfigure of Fig.6.15.

- In section-I each linear segment of $N/8$ monomers is constrained in a tube of an effective diameter of $D' \approx D/2\sqrt{2}$. Thus the number of monomers in each ‘blob’ is given by,

$$g = (D/2\sqrt{2})^{5/3}. \quad (6.17)$$

and the total number of blobs are therefore is,

$$n_{bl} = 8 \times N/8 \times (2\sqrt{2})^{5/3}/D^{5/3} \quad (6.18)$$

If the free energy associated with each blob is given by f_{bl} , then the free energy F_I is given by

$$F_I = N \times (2\sqrt{2})^{5/3}/D^{5/3} f_{bl} \quad (6.19)$$

- Refer the subfigure at the bottom of Fig.6.15. In section-II of the cylinder in the bottom subfigure each linear segment of $N/4$ monomers is constrained in a tube of an effective diameter of $D' \approx D/2$. Thus the number of monomers in each ‘blob’ is given by,

$$g = (D/2)^{5/3}. \quad (6.20)$$

and the total number of blobs are therefore given by,

$$n_{bl} = 4 \times N/4 \times 2^{5/3}/D^{5/3} \quad (6.21)$$

If the free energy associated with each blob is given by f_{bl} , then the free energy F_{II}

is given by

$$F_{II} = N \times 2^{5/3} / D^{5/3} f_{bl} \quad (6.22)$$

Thus,

$$F_{ov} = F_I + F_{II} \quad (6.23)$$

Thus the free energy difference per monomer, δF , between the overlapped and the segregated states of a pair of polymers with architecture Arc-2 is:

$$\delta F/N = (F_{ov} - F_{seg})/N = 3.87 f_{bl} / D^{5/3} \quad (6.24)$$

Thus in this way we can calculate $\delta F/N$ can be calculated for each polymer architecture using the blob theory approach where each loop is treated as linear chains trapped in an effective tube.

Other possible overlapping arrangements of two Arc-2 polymers in confinement: We have assumed a particular configuration of the ‘Arc-2’ polymer where the two smaller loops occupy section-I and the bigger loop occupy section-II of cylinder, both in the overlapped and the segregated scenarios. To rule out the possibility that (i) one smaller loop occupies section-I, and the other smaller loop overlaps with the bigger loop in section-II of cylinder, we have calculated (using the method outlined above) that the free energy for confinement for such a configuration is higher than the configuration discussed in detail above. (ii) Other possible configurations, such as that of all loops occupying the same cylinder half, also have higher values of free energy. (iii) For one small loop occupying the same half as one of the bigger loops, the free energy of confinement (F_{conf}) is increased by $0.3N/D^{5/3}f_{bl}$. Similarly, we do this for all our calculations pertaining to different architectures.

Architecture	$\delta F/N$
Arc-0	$2.78 f_{bl}/D^{5/3}$
Arc-2	$3.87 f_{bl}/D^{5/3}$
Arc-2-2	$4.0 f_{bl}/D^{5/3}$
Arc-3	$2.78 f_{bl}/D^{5/3}$
Arc-4	$4.90 f_{bl}/D^{5/3}$
Arc-6	$3.30 f_{bl}/D^{5/3}$
Arc-7	$3.30 f_{bl}/D^{5/3}$
Arc-8	$4.36 f_{bl}/D^{5/3}$
Arc-9	$3.84 f_{bl}/D^{5/3}$
Arc-10	$3.23 f_{bl}/D^{5/3}$
Arc-11	$2.85 f_{bl}/D^{5/3}$

Table 6.2: The above table indicates the $\delta F/N$ corresponding to each polymer architecture, except Arc-5. This is because Arc-5 has a “nested loop” like architecture which cannot be treated by the simplistic blob theory approach.

In Fig.7 of the main manuscript we present $\delta F/N$ for each polymer architecture for an arbitrary set of parameters: $D = 10a$ and $f_{bl} = k_B T$.

6.7.3 $\delta F/N$ corresponding to all polymer architectures

In table 6.2 we present the analytically calculated $\delta F/N$ for different architectures, using the methods outlined in the previous sections.

Chapter 7

Conclusions and Future Directions

7.1 Helix formation in polymers

In this chapter we have discussed how helix formation may take place in a semiflexible or a rigid polymer, due to various interactions between the monomers. These strategies relies on the principle of inducing assymetric interactions between monomers of the semiflexible polymer. This leads to the formation of angular conformations of the polymer. These sharp angles subsequently relax due to the rigidity of the chain and a helical confomation emerges in the process.

7.1.1 Handedness of helices

In Chapters 3 and 4 we outline how Coulomb interactions between monomers lead to angular conformations. The formation of these angular conformations rely on the

presence of thermal fluctuations. These angular conformations evolve later to form helical structures. Thus, we do not have a control on the handedness of the helical structures that emerge.

In Chapter-2 we induce helix formation by stochastically decreasing the equilibrium length of the springs, connecting two adjacent monomers along the contour. Here too, we do not have a control on handedness of the helical conformation that emerges.

In nature, for most cases where macromolecules exhibit a helical conformation, there is a preferred handedness. So a possible future avenue of investigation is: Can our models be modified such that the helical polymers have a preferred handedness? The mechanism by which this is achieved may shed light on principles underlying helix formation in macromolecules.

7.1.2 Formation of helices due to dipolar interactions

In this thesis we have shown that helix formation may occur when the monomers of a rigid polymer interact *via* power law repulsive interactions or a screened Coulomb interaction. Can helices also self-emerge when the monomers interact with each other through dipolar interactions, where there is an explicit angle dependence? If the answer is yes, then this may be used as yet another strategy for designing synthetic helical molecules.

7.2 Organization of polymers in cylindrical confinement

In Chapters 5 and 6 of this thesis we show that polymers organize akin to bacterial chromosomes if they adopt specific ring-polymer topologies, under cylindrical confinement. In this context we have shown that the organization of a polymer having a particular topology named Arc-2-2, resembles the organization of *E.coli* chromosomes.

7.2.1 Organization of polymers in spherical confinement

It is also worth investigating if adoption of particular polymer topologies lead to a specific organization of the polymers in spheres. Can a particular topology result in the spatial segregation of the constituent loops of a polymer, under spherical confinement? This question is also of biological relevance since it is known that eukaryotic chromosomes are confined in spherical cells.

7.2.2 Polymer topologies due to transient cross-links

We have shown in this thesis that the topology Arc-2-2 successfully reconciles observations from complementary experiments, that probe *E.coli* chromosomal organization. We have chosen cross-links at specific sites along the contour, which give rise to the Arc-2-2 topology. These cross-links are present during the entire simulation run. However, in reality binding proteins keep getting attached and detached

stochastically, at various stages of the cell-cycle. If we refine our model such that the cross-links form transiently at different stages of the cell-cycle, and at varying sites, can we still match the FISH and the Hi-C experiments under some conditions? These conditions (or theoretical predictions) can then be tested against other experiments in the future.

7.2.3 Organization of chromosomes in fast growth conditions

In Chapter-5 of the thesis we conduct our studies on polymers having the Arc-2-2 topology, under cylindrical confinement. We show that the emergent organization is similar to *E.coli* chromosomes in slow growth conditions. This begs the question: Can the Arc-2-2 polymer topology also reconcile experiments done on fast growing *E.coli* chromosome, where there are multiple rounds of replication occurring in parallel? This is also a possible avenue of investigation.

7.2.4 Incorporation of active processes

In this thesis we have relied on the concepts of entropic repulsion between loops in a specific polymer topologies. We have shown that the resulting “equilibrium” organization is similar to that of *E.coli* chromosomes.

However, *E.coli* chromosomes and eukaryotic chromosomes are known to be out of equilibrium. The estimated relaxation times for these systems are greater than the cell-cycle timescales. It is worth investigating in a future study, if in nature certain active processes may aid the principles outlined in this thesis.

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