Regulation of Interparticle Interactions Enables the Formation of Functional Nanoparticle Assemblies

विद्या वाचस्पति की
उपाधि की अपेक्षाओं की आंशिक पूर्ति में प्रस्तुत शोध प्रबंध

A thesis submitted in partial fulfillment of the requirements of the degree of Doctor of Philosophy

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2023

Dedicated to,
Ma, Baba, and ৺Boro Jethu

Certificate

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Thesis Abstract

Self-assembly is undoubtedly one of the most efficient ways to create complex materials with distinct functions. The ability to control the interparticle interactions between the building blocks is the key to achieving the desired outcomes from a self-assembly process. This requires the smart choice of building blocks that respond to stimuli in a precise fashion. Nanoparticles (NPs) are a unique class of building blocks, as they offer both their surface and core to install the desired interactions and functions. In the proposed thesis work, the surface of plasmonic NPs has been appropriately functionalized with the 'ligand of choice' to regulate their interaction with biomolecules, leading to the formation of *'functional'* bio-nano hybrids. Further, the impact of such finely tuned interactions in NP self-assembly processes under equilibrium as well as non-equilibrium domains was studied.

First, we have prepared a multifunctional bioplasmonic network via a long-range electrostatically directed self-assembly process between positively charged NPs and negatively charged ATP molecules in the presence of $CaCl_2$. The screening of charges by Ca^{2+} ions and the coordination between ATP- Ca^{2+} helped in regulating the strength of electrostatic attraction between oppositely charged ATP molecules and AuNPs. This turned out to be decisive in transforming an uncontrolled aggregation (instant precipitation) into a kinetically controlled self-assembly process (bioplasmonic network). ATP and AuNP retained their inherent properties in the bioplasmonic network, thereby enabling their use for various catalytic, photocatalytic, and SERS-based applications. In another study, we observed that the 'dead' ATP-AuNP precipitated state slowly transformed into the 'live' bioplasmonic network. Detailed microscopic and spectroscopic studies revealed mechanistic insight into the role of the self-association property of ATP molecules in transforming the kinetically trapped precipitate into an active bioplasmonic network. This motivated us to explore the dynamic nature of the ATP-AuNP precipitate to construct non-equilibrium self-assembled systems through the sequential and tandem action of two competing phosphoryl transferase enzymes (hexokinase and creatine phosphokinase that consume and generate the aggregating trigger ATP, respectively). The enzymatically driven dynamic conversion between ATP and ADP molecules helped in regulating the electrostatic interactions between AuNP and aggregating trigger. After establishing the decisive role of ligand chemistry and interplay of forces, our next focus was to incorporate the thermoplasmonic property of core-NPs in achieving multi-

responsive dynamic self-assembled system. The assembly step was triggered by the autonomous dissolution of moisture in the hygroscopic mixture of thymine-capped AuNPs in DMSO medium, leading to the formation of controlled aggregates. The sunlight-triggered plasmonic heat generated from the thymine-AuNPs in the controlled aggregates was used as the thermal energy source for the evaporation of water from the solvent mixture, thereby triggering the disassembly process. The use of thermoplasmonic property enabled the coupling of light into the solvent-mediated dynamic self-assembly process.

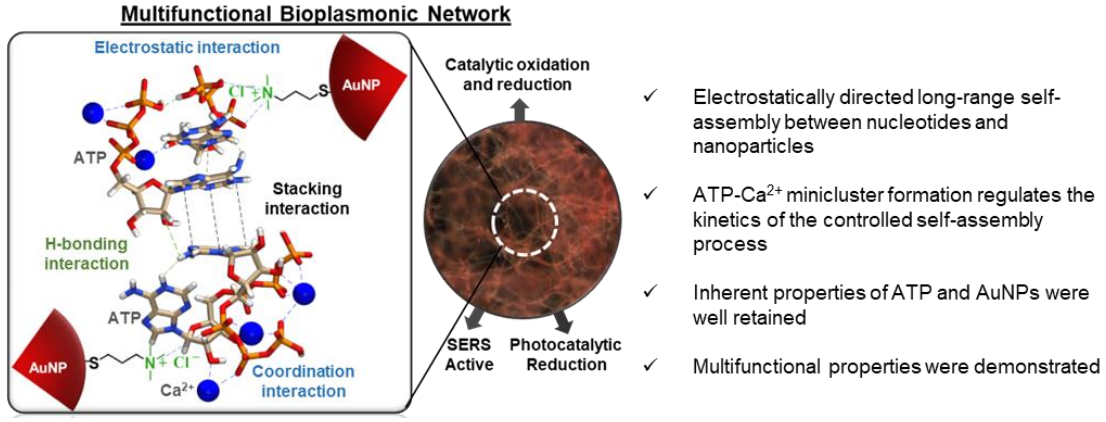
Thus, the use of both surface and core properties of NPs helped in imparting desirable functions of dynamicity, adaptability, multi-stimuli-responsiveness, and multifunctionality in the NP self-assembly process. In short, fine control over the assembly pathways enabled us to achieve various static and dynamic self-assembled structures from a limited set of NP building blocks.

Thesis Synopsis

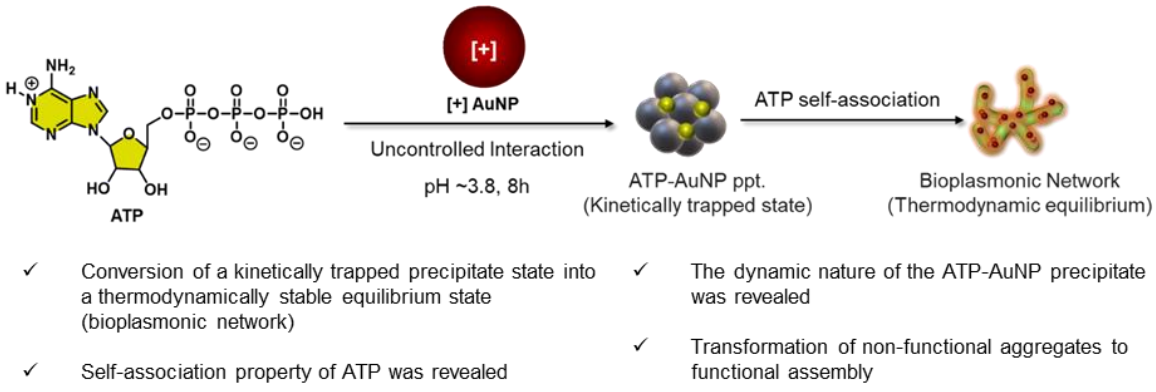
One of the ultimate goals of self-assembly is to create complex matter with desired functionalities. The ability to control the interparticle interactions between the building blocks is the key to achieving desired outcomes from a self-assembled state. This requires the smart choice of building blocks that respond to stimuli in a precise fashion. Nanoparticles (NPs) are a unique class of building blocks, as they offer both their surface and core to install the desired interactions and functions. In my thesis work, the surface of plasmonic NPs has been appropriately functionalized with a *'ligand of choice'* to regulate their interaction with biomolecules, leading to the formation of *'functional'* bio-nano hybrids. The functional assemblies were further utilized for various catalytic, photocatalytic, and sensing applications. Further, the impact of such finely tuned interactions in NP self-assembly processes was studied under equilibrium as well as non-equilibrium domains. The surface ligand-directed dynamic self-assembly process also helped us to realize transient functions from a self-assembled state. Similarly, the core property of plasmonic nanoparticles was also coupled with the self-assembly process. For instance, the plasmonic heat was explored to install dynamicity and multi-stimuli-responsiveness in solvent-mediated dynamic self-assembly of NPs.

In Chapter 1, the fundamental design principles, as well as the importance of both surface ligands and core, were discussed to create complex functional NP assemblies. In the subsequent Chapters, various static and dynamic functional NP assemblies were created from a limited set of NP building blocks, where both the surface and core properties of nanoparticles were used. Chapter 2 describes the various methods and techniques involved in the thesis. In Chapter 3, we have prepared a multifunctional bioplasmonic network via a long-range electrostatically directed self-assembly process between positively charged NPs and negatively charged ATP molecules in the presence of $CaCl_2$. The screening of charges by Ca^{2+} ions and the coordination between ATP- Ca^{2+} helped in regulating the strength of electrostatic attraction between oppositely charged ATP molecules and AuNPs. This turned out to be decisive in transforming an uncontrolled aggregation (instant precipitation) into a kinetically controlled self-assembly process (bioplasmonic network). ATP and AuNP retained their inherent properties in the bioplasmonic network, thereby enabling their use for various model catalytic, photocatalytic, and SERS-based applications. The electrostatically directed self-assembly process was further

extended to different nucleotide-nanoparticle hybrid systems, confirming the generality of our protocol.

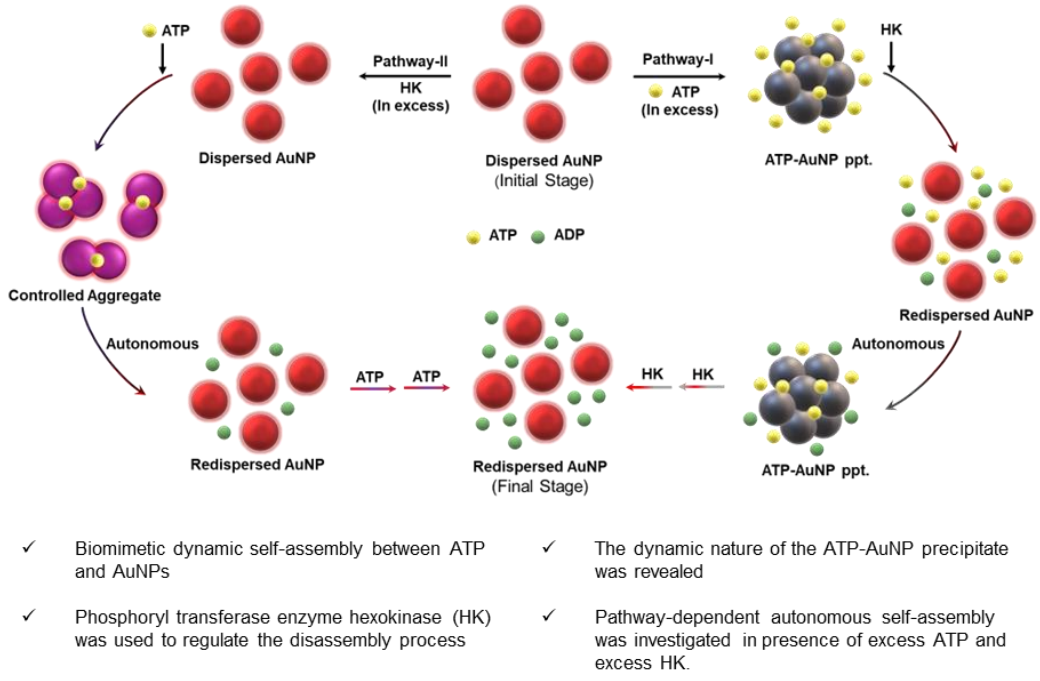


In another interesting study, we observed that in the absence of metal ions, oppositely charged ATP and AuNPs undergo an uncontrolled aggregation, leading to the complete precipitation of NPs. Unprecedentedly, the *'dead'* ATP-AuNP precipitate slowly transformed into the *'live'* bioplasmonic network, where AuNPs regained their plasmonic property. Detailed microscopic and spectroscopic studies were carried out to understand the mechanism. NMR and theoretical studies revealed that the self-association property of ATP molecules played a major role in transforming the kinetically trapped precipitate state into an active bioplasmonic network (thermodynamically more stable state).



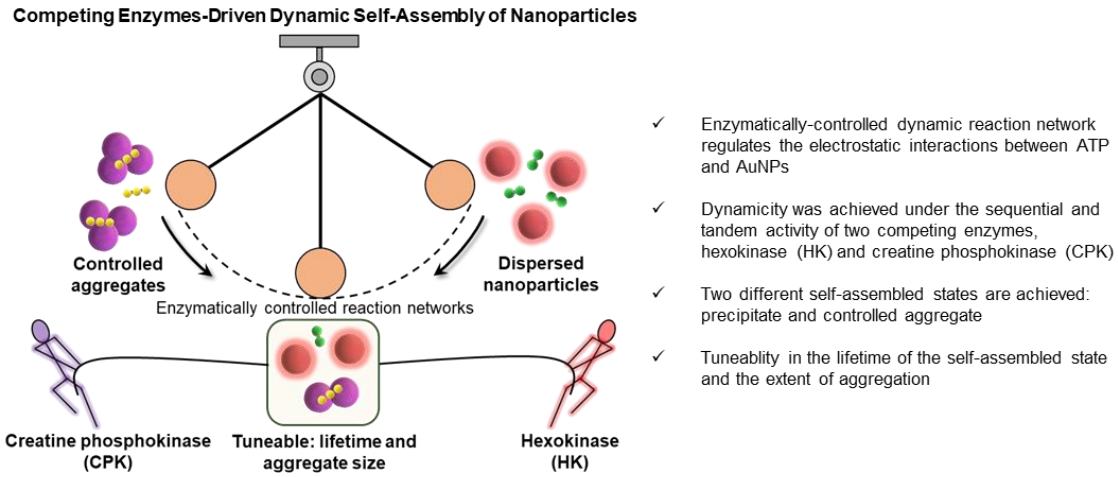
In Chapter 4a, the dynamic nature of the ATP-AuNP precipitate to construct non-equilibrium self-assembled systems was explored. Here, the kinetics of the assembly-disassembly process were controlled via the interparticle interactions to achieve pathway-dependent autonomous reversibility in the dynamic self-assembly process. In this context, we have achieved autonomous reversibility in assembling as well as disassembling steps, by controlling the

respective pathways. Here, negatively charged ATP molecules act as an aggregating trigger to drive the self-assembly of [+] AuNPs, and the hexokinase (HK) enzyme plays the role of the disassembling trigger. HK resists the self-assembly process by converting ATP into adenosine diphosphate (ADP) in the AuNP aggregates, leading to a decrease in the electrostatic attraction and disassembly of the aggregates. The autonomous self-assembly process was realized by achieving dominance of the assembly step over disassembly by using excess ATP as the aggregating trigger. In another pathway, the dominance of the disassembly step over assembly was achieved by using an excess of HK as the disassembling trigger to realize the autonomous self-assembly process. Along with achieving the autonomous process, assembling-disassembling pathways had a significant impact on the nature of the aggregated state. The excess-ATP-driven pathway led to the autonomous shuttling between completely dispersed and completely precipitated states, whereas the excess-HK-driven pathway resulted in the shuttling between completely dispersed and controlled aggregated states. Kinetic control of the competing reactions between ATP and HK also helped in controlling the lifetime of the aggregate states.

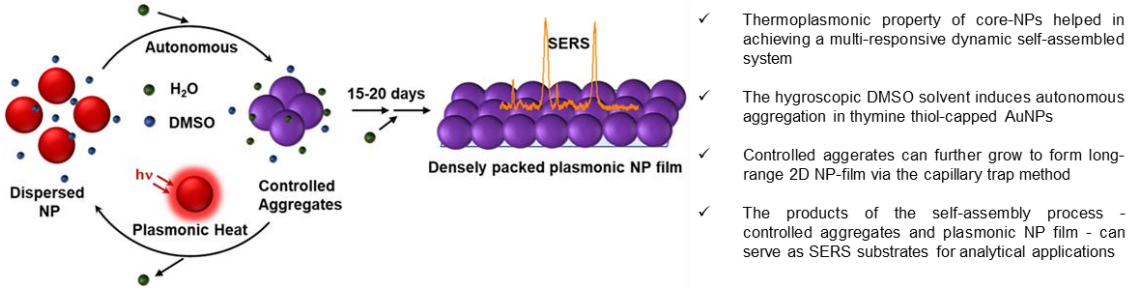


In Chapter 4b, we demonstrated the sequential and tandem action of two competing phosphoryl transferase enzymes (HK and creatine phosphokinase) to choreograph a dynamic self-assembled state. The enzymatically driven dynamic conversion between ATP and ADP molecules helped in regulating the electrostatic interactions between oppositely charged

AuNPs and nucleotides. Interestingly, the in-situ generation of ATP molecules through enzymatically controlled reaction networks helped in overcoming multiple additions of co-factor molecules in each cycle. Even the lifetime of the transient self-assembled state (controlled aggregates) and the extent of aggregation were successfully tuned by changing the amounts of enzymes and substrates. This study further confirms the power of surface chemistry and interparticle interactions in designing adaptive materials with temporal functions.



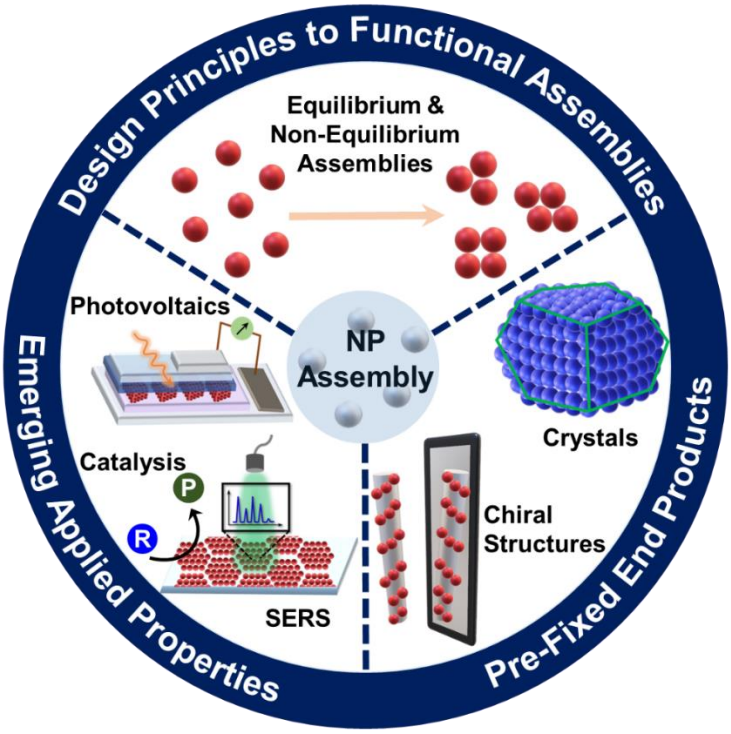
After establishing the decisive role of ligand chemistry and the interplay of forces, In Chapter 5, we incorporated the thermoplasmonic property of core-NPs in achieving a multi-responsive dynamic self-assembled system. The assembly step was triggered by the autonomous dissolution of moisture in the hygroscopic mixture of thymine-capped AuNPs in the DMSO medium, leading to the formation of controlled aggregates. The sunlight-triggered plasmonic heat generated from the thymine-AuNPs in the controlled aggregates was used as the thermal energy source for the evaporation of water from the solvent mixture, thereby triggering the disassembly process. The use of thermoplasmonic property enabled the coupling of light into the solvent-mediated dynamic self-assembly process. Moreover, the kinetically trapped, controlled aggregates underwent a long-range self-assembly process to create a densely packed plasmonic NP film at the liquid-air interface. Raman studies further proved that the products of the self-assembly process - controlled aggregates and densely packed plasmonic NP film - can serve as effective surface-enhanced Raman scattering (SERS) substrates for analytical applications.



Thus, through my thesis, we have shown that the use of both surface and core properties of NPs can help in imparting desirable functions of dynamicity, adaptability, multi-stimuli-responsiveness, and multifunctionality in the NP self-assembly process. In short, fine control over the assembly pathways by regulating interparticle interactions enabled us to achieve various static and dynamic self-assembled structures from a limited set of NP building blocks.

Chapter 1

Design Principles for Creating Functional Nanoparticle Assemblies



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Rao, A.;* **Roy, S.**; Jain, V.; Pillai, P. P.* Nanoparticle Self-Assembly: From Design Principles to Complex Matter to Functional Materials. *ACS Appl. Mater. Interfaces* **2023**, *15*, 25248−25274.* Corresponding author.

1.1 Introduction

The creation of complex matter with varying degrees of functionality is the current goal of material science to meet the ever-growing demands in energy as well as medical sectors.[1-7] There are mainly two different ways by which new materials can be synthesized: (i) top-down and (ii) bottom-up approaches.[8-13] Several top-down approaches have been explored over the years, for example, etching, grinding, ball milling, etc.[8,10-12] Among them, lithography techniques are noteworthy.[11,12] It has shown several advantages, such as great consistency and controllability in macroscopic pattern formations, which can be attributed to its extensive usage in the device fabrication sector throughout the years.[11,12] Similarly, the bottom-up approaches, such as the self-assembly processes, are equally important, which is, interestingly, nature's preferred way of creating complex materials.[1,14-20] One of the key advantages of the self-assembly process is that a series of materials can be synthesized from a limited set of starting materials. For instance, various DNA/RNA molecules are formed through the combination of only four/five nucleobases. Also, due to its dynamic nature, self-assembly provides the much-needed error correction ability during the synthesis of complex materials.[21] These facts have motivated researchers to create complex materials using the principles of self-assembly, which spurred extensive research around the 1960s. Considering the prospects and future of the emerging field, the 1987 Nobel Prize in Chemistry has been awarded to Prof. Cram, Prof. Lehn, and Prof. Pedersen *"for their development and use of molecules with structure-specific interactions of high selectivity."*[22] For the very first time, they introduced molecules that can *'recognize'* each other, which became the foundation of *'host-guest'* and *'supramolecular chemistry.'*[22] Over the years, extensive research has been carried out in this direction, and the area has grown a lot from simple host-guest-based recognition systems to complex supramolecular architectures.[19,20] After that, a significant expansion has occurred in all the possible directions of self-assembly, with a greater aim of achieving one target: mimicking nature's ability to create materials with desired and structure-specific functions.[14-17] This next era of self-assembly and supramolecular chemistry was pioneered by Prof. Meijer, Prof. Aida, Prof. Stupp, and many others, where the focus was beyond creating only fascinating self-assembled architectures. In a pioneering work, Prof. Meijer and co-workers have first time synthesized amphiphilic dendrimer molecules that exert generation-dependent structure formation in an aqueous medium.[23] Prof. Stupp has pioneered another important area, which is called bioinspired structural materials. In a pathbreaking study, the authors have co-assembled an amphiphilic peptide with hydroxyapatite nanocrystals to form extremely robust artificial

bone-like materials.[24] In another landmark study, Prof. Aida and co-workers have synthesized a photoactive coaxial nanotubular structure where the electron-accepting trinitrofluorenone group laminates over an electron-donating layer of hexabenzocoronene, which allows the generation of spatially separated charge carriers with a quick photoconductive response in a light on-off cycle. [25]

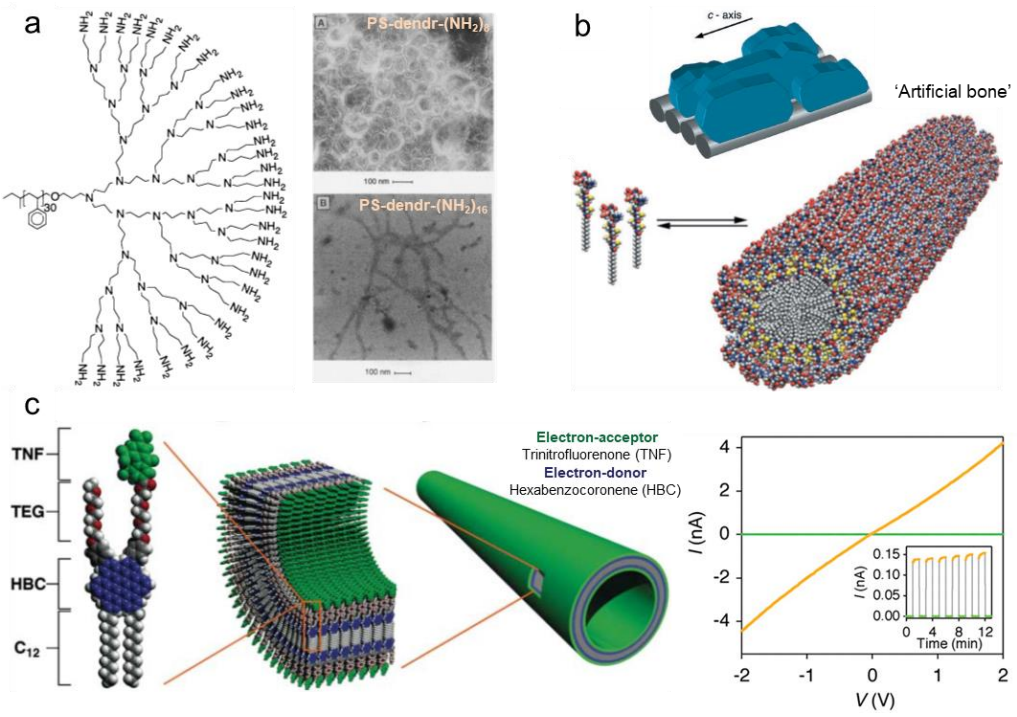

Figure 1.1. (a) A representative example of an amphiphilic dendrimer molecule that exerts a generation-dependent self-assembled structure formation. Reproduced with permission from ref. 23. Copyright 1995, American Association for the Advancement of Science. (b) Schematics of the co-assembly process between an amphiphilic peptide and hydroxyapatite nanocrystal to form extremely robust artificial bone-like material. Reproduced with permission from ref. 24. Copyright 2001, American Association for the Advancement of Science. (c) Schematics showing a photoactive coaxial nanotubular structure made through the self-assembly process between an electron donor-acceptor moiety that depicts a quick photoconductive response in a light-on-off cycle. Reproduced with permission from ref. 25. Copyright 2006, American Association for the Advancement of Science.

Over the years, extensive research has led to the generation of several new fields, for example, chiral luminescence and chiral plasmon, aggregation-induced emission (AIE), drug delivery, colloidal assembly, out-of-equilibrium self-assembly, and so on. However, to get desired functions from a self-assembled state, one needs to understand the fundamental principles of the self-assembly process, which mainly depends on two factors: (a) interparticle interactions and (b) building blocks.[3,26] In a typical self-assembly process, the complex organized structures are formed by vigorously shaking the individual components, either by thermal energies or by

an external shaker/stirrer.[3] This picture is deceptively simple and depicts the need for appropriately *'programmed'* building blocks. Therefore, one needs to control different chemical and physical interactions for coding the positional information onto the building blocks to realize the next generation of complex matter. There are numerous ways of coding the positional information on individual building blocks in the self-assembled structure. This kind of information can be imparted onto the building blocks by using appropriate interactions, such as complementary shapes, entropy, external fields, surface ligands, etc.[3,26] Therefore, in short, fine control over the interparticle interactions and a judicious choice of building blocks are the keys to creating active materials with distinct functions. Among various self-assembly processes, nanoparticle-based self-assembly is quite fascinating because it can create complex matter with exceptional properties that are very difficult to achieve with molecular self-assembly. Interestingly, both the core and surface ligands of nanoparticles have a decisive role in driving the self-assembly process under various chemical (metal ions, solvent, acid/base, gases, etc) and physical stimuli (light, magnetic field, temperature, and electric field) to create functional assemblies.[5] The initial era of research in NP self-assembly was focused more on developing materials with robust and permanent properties since the chemists were accustomed to making stable end-products.[3] Over the last few decades, scientists have also achieved significant progress in the field of NP self-assembly and supramolecular chemistry to create complex materials.[8,19,20] Still, the diversity and complexity of structures realized by nature remain elusive. One of the ways to reveal its secrets is mimicking nature. This realization has forced the supramolecular chemists to think beyond the domain of static self-assembly and create complex materials with more life-like properties (including adaptability, self-replication, self-healing, and autocorrections), leading to the establishment of a new domain named dynamic NP self-assembly.[8,14,27,28] Here again, fine control over the kinetics and thermodynamics is required, which can be achieved by careful utilization of both surface ligands and core properties of NPs. This can lead to the formation of static and dynamic self-assembled NP structures with desired functionalities. This Chapter describes the fundamental design principles as well as the importance of both surface ligands and core to create complex functional NP assemblies. In the subsequent Chapters, *'both'* surface and core properties of nanoparticles were utilized during the self-assembly process to create functional static and dynamic materials from a limited set of NP building blocks.

1.2 Creating Complex Matter Using Nanoparticles

It has become evident from the last few decades that to tackle and overcome modern-day challenges and deliver real-life solutions for medical purposes, electronic devices, or emerging materials of exclusive properties, the assembly of materials is more reliable and effective than its individual counterparts.[3,4,26] Over the years, several building blocks have been utilized exclusively for this purpose, for example, organic molecules, inorganic clusters or coordination complexes, polymers, colloids, etc.[3,8,19,20,26] Among them, nanoparticle-based self-assembly is quite fascinating because it can create complex matter with properties incorporated into it, such as plasmonic, luminescence, magnetism, etc., that are very difficult to achieve with other atomic and molecular building blocks.[26] This chapter is focused on important interactions and guiding principles of self-assembly processes in nanomaterials, mostly focused on metal NPs. A nanoparticle mainly consists of two parts: core and surface ligand. The core part can exert various properties, such as plasmonic, luminescence, magnetism, etc., while the surface ligands stabilize the NPs in the colloidal state as well as control the interparticle interactions to preserve the core properties in the self-assembled states.[27] Therefore, core and surface ligands are complementary to each other to obtain functional assemblies of NPs. Surface ligands consist of three parts: binding group, spacer, and terminal group.[29] The binding group or atom functionalizes the ligand on the surface of the inorganic nanoparticle, and it also modulates the core property through effective bonding interactions. The spacer group provides the colloidal stability of the NPs and tunes the effective rate and efficiency of the self-assembly process as well as optoelectronic properties. The terminal/head group is the most important part of the surface ligand, which governs the interparticle interaction through different covalent and noncovalent bonding during the self-assembly process and controls various functions in the assembled states, such as catalysis, photophysics, and electronic properties.[27] Therefore, both the core and surface ligands of nanoparticles have a decisive role in driving the self-assembly process under various chemical and physical stimuli, which have been discussed in the following sections.

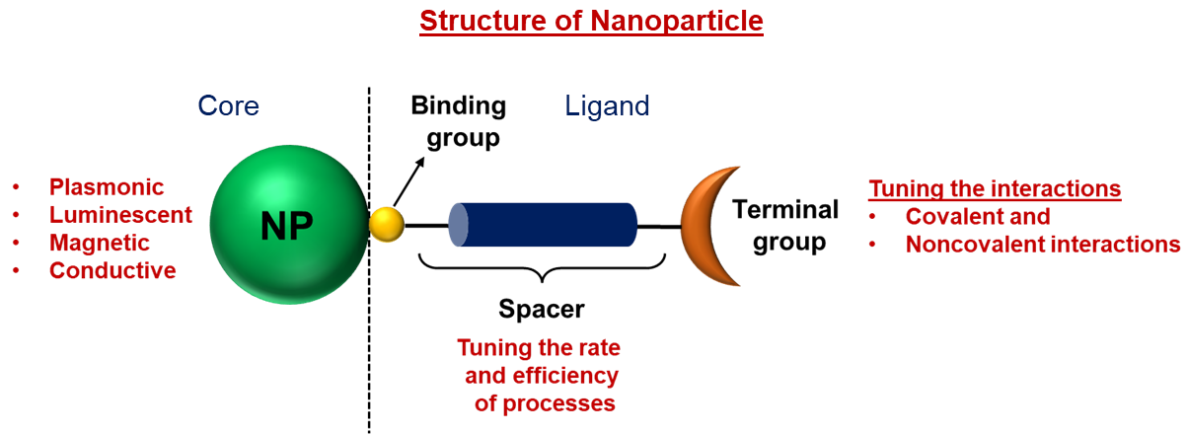

Figure 1.2. A schematic representation of a nanoparticle structure showing different properties and interactions emanating from the core and from surface ligands of NPs. Reproduced with permission from ref. 29. Copyright 2021 The Royal Society of Chemistry.

1.3 Encoding Programmable Nanoparticle Self-Assembly

1.3.1 Programmable nanoparticle self-assembly using intermolecular interactions

Ligands are an essential component of NP architecture, as they not only impart stability to the NPs but dictate their interactions with the environment as well.[29-32] Because of the ubiquitous nature of ligands, researchers have employed the interactions originating from the surface ligands to create different ordered NP self-assemblies. In this direction, several studies have been undertaken to understand the design principles to form self-assemblies at different length scales.[14,32-34] In a recent example, Nie and co-workers utilized controlled electrostatic interactions to govern the high-yield synthesis of colloidal molecules (Figure 1.3.a, b).[35] The authors utilized NPs, uniformly coated with charged polymers, for the directional assembly of colloidal molecules. Here, the polymers were designed to provide the much-needed electrostatic complementarity, steric stability, and electrostatic repulsions so as to guide the formation of directional contacts without the need for site-specific or directional interactions. Later, the coordination number of the colloidal molecules was varied by changing the polymer chain length, grafting density, and NP size so as to change the ratio of interacting moieties.

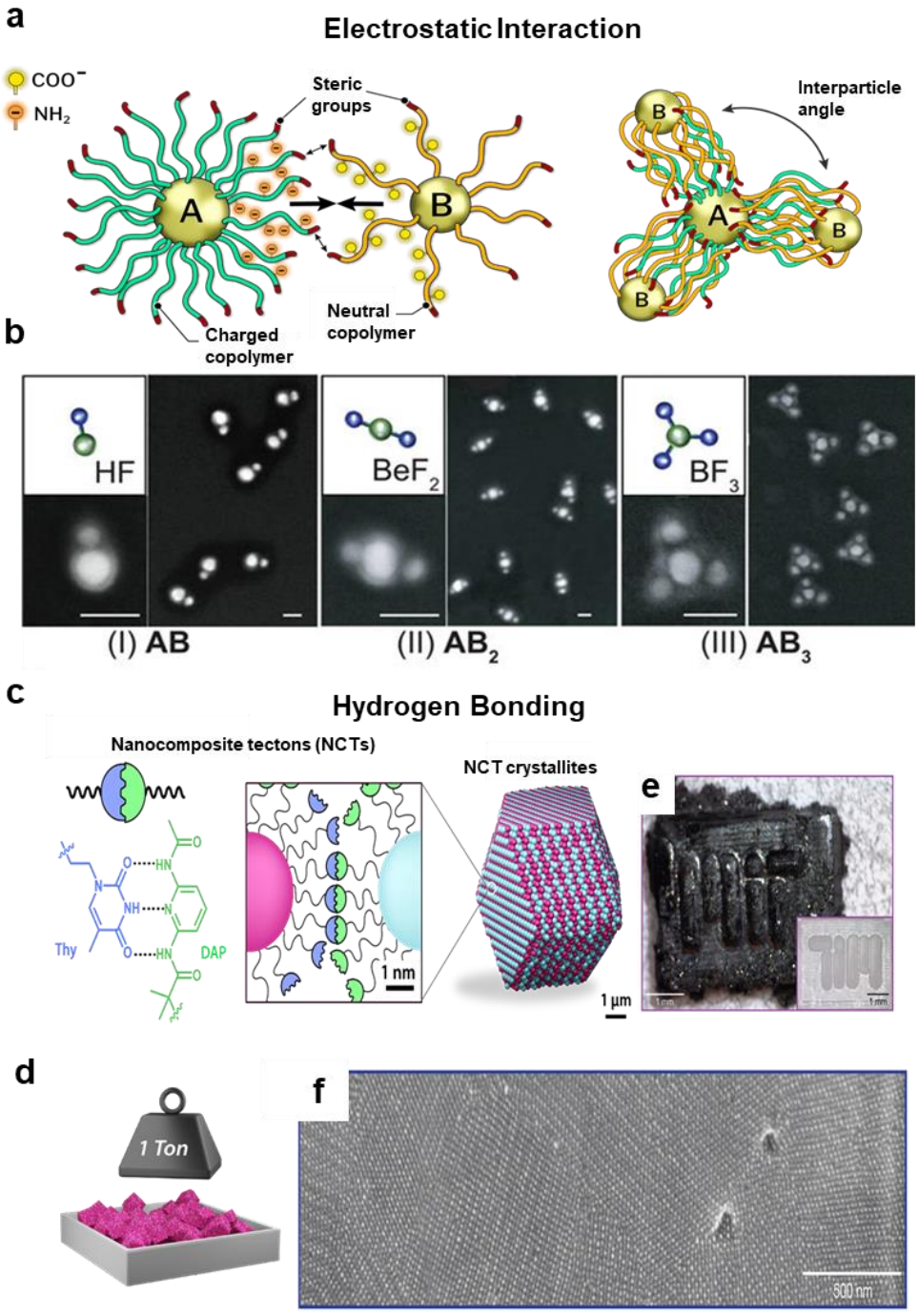

Figure 1.3. Molecular codes for guiding self-assembly. (a) Schematic illustration of electrostatically driven self-limiting assembly of polymer grafted NP into an AB3 structure. (b) SEM image of different ABx (x = 1-3) structures, assembled from 36 nm AuNP-As and 20 nm AuNP-Bs, along with insets showing their corresponding molecular equivalents. Reproduced with permission from ref. 35. Copyright 2020, American Association for the Advancement of Science. Schematics of H-bonding assisted assembly of nanocomposite tectons (NCTs) (c) into 3D and (d) their sintering into a macroscopic material. (e), (f) corresponds to the optical and SEM images of the bulk solid prepared by sintering the 3D supraparticles, respectively. Reproduced with permission from ref. 38. Copyright 2021, Springer Nature.

In another class of NP superstructures, hydrogen-bonding interactions were utilized to self-assemble structures with arbitrary shape and complexity.[36,37] One of the key promises of self-assembly is the bottom-up fabrication of macroscopic materials. In this direction, MacFarlane and co-workers have utilized the complementary hydrogen-bonding interactions for the rapid assembly of faceted 3D NP superstructures (Figure 1.3.c-f).[38] These superstructures were then *'sintered'* into arbitrary shapes for the fabrication of macroscopic materials. This approach of sintering the assembled NP superstructures into bulk materials allowed the authors to exhibit nano as well as microstructural control. Here, size, crystallographic symmetry, as well as the chemical composition of the superstructures were used to tune the properties of the hierarchically fabricated free-standing macrostructures.

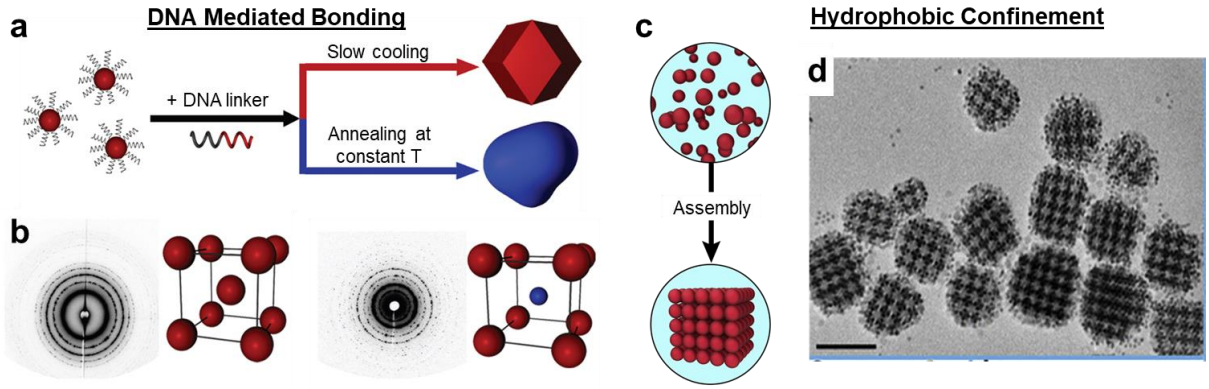

Figure 1.4. Molecular codes for guiding self-assembly. (a) Schematics showing the two different ways of DNA-mediated NP-crystallization leading to either single or polycrystalline assemblies. (b) Representative 2- and 3-D SAXS data for b.c.c. and CsCl superlattices were prepared by following the slow-cooling approach. Reproduced with permission from ref. 46. Copyright 2014, Springer Nature. (c) Schematics showing the preparation of 3D supracrystals in an oil-in-water emulsion. (d) TEM images of the colloidal supracrystals (scale bar 100 nm). Reproduced with permission from ref. 49. Copyright 2018, American Chemical Society.

In addition to the use of complementary H-bonding units, thiolated DNA can also be used as a ligand for the fabrication of a wide variety of self-assembled structures.[39-41] The use of DNA as a ligand is very attractive as it can be used to design and control the thermodynamics as well as kinetics of NP self-assembly. Here, the bases present at the end of the DNA sequence are unpaired and, therefore, act as sticky ends, which can be used to install sequence-specific interactions between the NP building blocks and drive the formation of an ordered assembly. Such DNA-mediated H-bonding interactions can be switched '*off*' and '*on*' by conveniently changing the temperature of the solution, above and below the DNA melting temperature, respectively. In their seminal works, groups of Mirkin and Alivisatos have independently demonstrated the potential of DNA in guiding the assembly of NPs into higher-order aggregates.[42,43] Later, Mirkin and co-workers showed the formation of polycrystalline

superlattice by annealing the aggregates just below the DNA melting temperature. Although interesting, the organized structure lacked a well-defined shape.[39,44,45] Later, the authors dissolved the NP aggregates by heating the solution above the DNA melting temperature, and slow-cooling over the course of days resulted in the formation of Wulff equilibrium crystal structures (Figure 1.4.a, b).[46] From then on, the field has developed at a rapid pace, where one can use the principle of maximizing DNA hybridization events (complementary contact model) to predict, as well as realize the formation of 3D superlattices having arbitrary shapes and crystal structures. In an alternate approach, binary self-assembled structures were prepared using an oil-in-water emulsion process.[47,48] Here, Ouyang and co-workers prepared oil-in-water droplets containing a mixture of two distinct hydrophobic NPs with pre-determined concentrations (Figure 1.4.c, d).[49] Later, the slow and gradual evaporation of the *'good solvent'* (organic phase) initiated the assembly of binary supracrystals. More importantly, the stoichiometry, size, as well as lattice structure were easily modulated by controlling the concentration ratio between the NPs, surfactant concentration, and shear rates.

1.3.2 External fields driven nanoparticle self-assembly

The use of external fields to drive the formation of self-assembled structures is another attractive strategy for the manipulation of a wide variety of NPs.[5,50,51] Of particular interest are light, magnetic field, and electric field, which can be instantaneously delivered as well as removed from the system. In such cases, these external fields act as a non-chemical template for the organization of NPs in various complex structures. For instance, one can use magnetic forces emanating from the NP core for their self-assembly under the influence of a magnetic field.[52,53] In one recent example, Klajn and co-workers evaporated a monodisperse solution of superparamagnetic magnetite nanocubes at the liquid-air interface in the presence of a magnetic field (Figure 1.5.a).[54] Under the influence of the applied field, the magnetic dipoles partially aligned with the field, resulting in 1-dimensional (1D) helical NP chains (Figure 1.5.b) with high yields. These observations were rationalized on the basis of a clever interplay between magnetic dipole-dipole interactions, van der Waals interactions, entropic forces, and Zeeman coupling resulting in the formation and growth of chiral nanocube clusters.[54] Later, in order to maximize their packing, neighbouring helices adopted the same handedness, thereby revealing a novel mechanism for the symmetry breaking and chiral amplification process.

Similar to the magnetic field, light can also be used as an external stimulus to direct the formation of appropriately functionalized NPs. Here, the use of light presents several advantages over the use of magnetic fields, such as site-specific delivery and wavelength dependence. Due to these reasons, light-driven chemistries such as cis-trans isomerization,[55,56] use of photoacids, [57,58] photochemical cycloadditions,[59-61] and photothermal effects[62,63] have been extensively exploited to design reversible self-assembly of NPs. In this direction, Grzybowski and co-workers used light-induced dipole-dipole and covalent interactions for the assembly of colloidal crystals and supraspheres[55](Figure 1.5.c and vide-infra). AuNPs were decorated with a mixture of dodecylamine (DDA) and a photo-isomerizable azobenzene dithiol ligand, which reversibly self-assembled under the action of UV light because of dipole-dipole and crosslinking interactions. Here, the degree of reversibility depended on the strength of dipole-dipole and covalent interactions. For instance, at low dithiol surface coverage, reversible (metastable) crystalline assemblies were formed, whereas permanent cross-links resulted in the formation of irreversible supraspheres at high dithiol surface coverages.

In another set of examples, the thermoplasmonic properties of metal NPs have been utilized to trigger the self-assembly process at the nanoscale.[62,63] The heat dissipated from a photoexcited NP will create a thermal gradient resulting in the preferential movement of particles (thermophoresis), and thereby initiating the self-assembly process. In this direction, Zheng and co-workers have used plasmon-mediated thermophoresis to demonstrate a reversible self-assembly of Au nanotriangles with excellent control over the aggregate size and morphology (Figure 1.5.d).[62] The highlight of the work was the manipulation and assembly of the particles with low-power light, which makes this approach attractive over the commonly used optical tweezers.

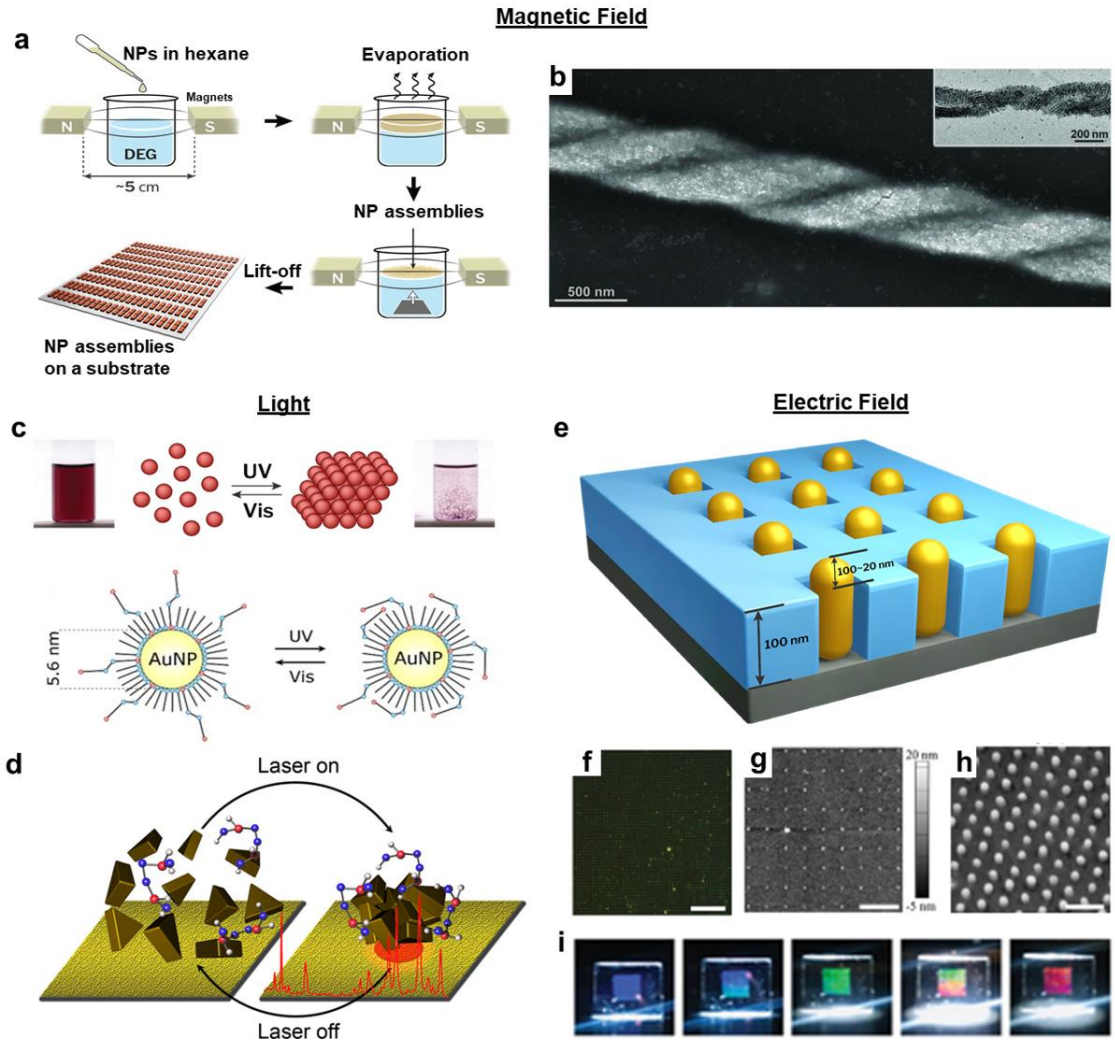

Figure 1.5. Guiding self-assembly using external fields. (a) Schematics illustrating the experimental setup for the self-assembly of helical 1D nanowires from cubic iron oxide NPs. (b) SEM images showing a well-defined double helix structure of an individual single-stranded 1D nanowire. Reproduced with permission from ref. 54. Copyright 2014, American Association for the Advancement of Science. (c) Schematics and optical photographs showing the light induced self-assembly of azobenzene functionalized AuNPs. The azobenzene groups on AuNPs undergo cis-trans isomerization under visible and UV light irradiation. Reproduced with permission from ref. 55. Copyright 2007, Proceedings of the National Academy of Sciences of the United States of America. (d) Schematic for plasmon enhanced thermophoresis driven reversible self-assembly of nanoparticles with enhanced SERS sensing activity. Reproduced with permission from ref.62. Copyright 2016, American Chemical Society. (e) Schematic illustration of electrophoretic deposition based self-assembly of vertically aligned AuNRs. (f) AFM images (scale bar = 10 μm) and a 3D colored map showing the vertically aligned AuNR array. (g) Dark-field microscopy images (scale bar = 50 μm) and (h) SEM images (scale bar = 500 nm) of the hexagonal array of vertically aligned AuNRs. (i) Digital photographs of the vertically aligned gold nanorod arrays, observed at different viewing angles from 110° (left) to 70° (right). Reproduced with permission from ref. 64. Copyright 2021, John Wiley and Sons.

In order for the NPs to be incorporated into a wide variety of functional optical, electrical, or chemical devices, their precise assembly, along with long-range order, remains a roadblock. In

this direction, Mulvaney and co-workers utilized electrophoresis to direct the deposition of NPs with nanometer resolution over large length scales.[64] Later, the protocol was extended for the vertically oriented assembly of gold nanorods on a conductive ITO substrate coated with a patterned PMMA resist layer (Figure 1.5.e-i).[64] Here, the applied electric field induced an electric dipole moment along the nanorod axis, thereby resulting in the vertical alignment of the nanorods during the assembly process. More importantly, the strength of the electric dipole was controlled by controlling the polarizability of the electric double layer around the nanorod. This allows for the electrophoretic deposition of materials possessing weak inherent polarizability by enhancing their surface charge and resulting in electrical double layers.

1.3.3 Shape and entropy guided nanoparticle self-assembly

As opposed to the other methods of controlling NP organization, where the interactions between the NPs are coded with the help of suitable ligands, the shape of the NP core can also initiate their self-assembly. Here, one of the key interactions between the particles is depletion interaction, where it becomes entropically more favorable for the smaller solute particles to stay in the bulk solution as compared to staying in between two colloids.[3,65] This results in a net osmotic pressure acting to push the particles together. This *'depletion force'* driven assembly of NPs has been pioneered by Pine and co-workers for the formation of a wide variety of colloidal architectures.[66-69] An analogous recognition mechanism uses the shape-complementarity of particles for the formation of self-assembled structures. Here, instead of slow evaporation of shape uniform nanocrystals, mixtures of nanocrystals with varying shapes are co-crystallized into 2D superlattices. In a series of studies, Murray and co-workers pioneered the co-crystallization of NPs with anisotropic shapes.[70,71] For instance, the authors demonstrated the successful co-crystallization between rhombic GdF_3 and tripodal Gd_2O_3 nanoplates over micrometer length scales (Figure 1.6.a-d). [69] Here, the size and shape of the component NPs provided the necessary complementary *'lock-and-key'* type interactions. Building on this work, the same group demonstrated the co-crystallization between nanorods and nanospheres into three intriguing phases over centimetre length scales (Figure 1.6.e-g).[70] Additionally, the authors revealed the necessity of short-range attractive depletion, as well as ligand-induced interactions, for the successful realization of the co-crystal. Here, the presence of asymmetric interaction strengths between different nanocrystals was crucial for obtaining the co-crystallization instead of phase separation of the NPs.

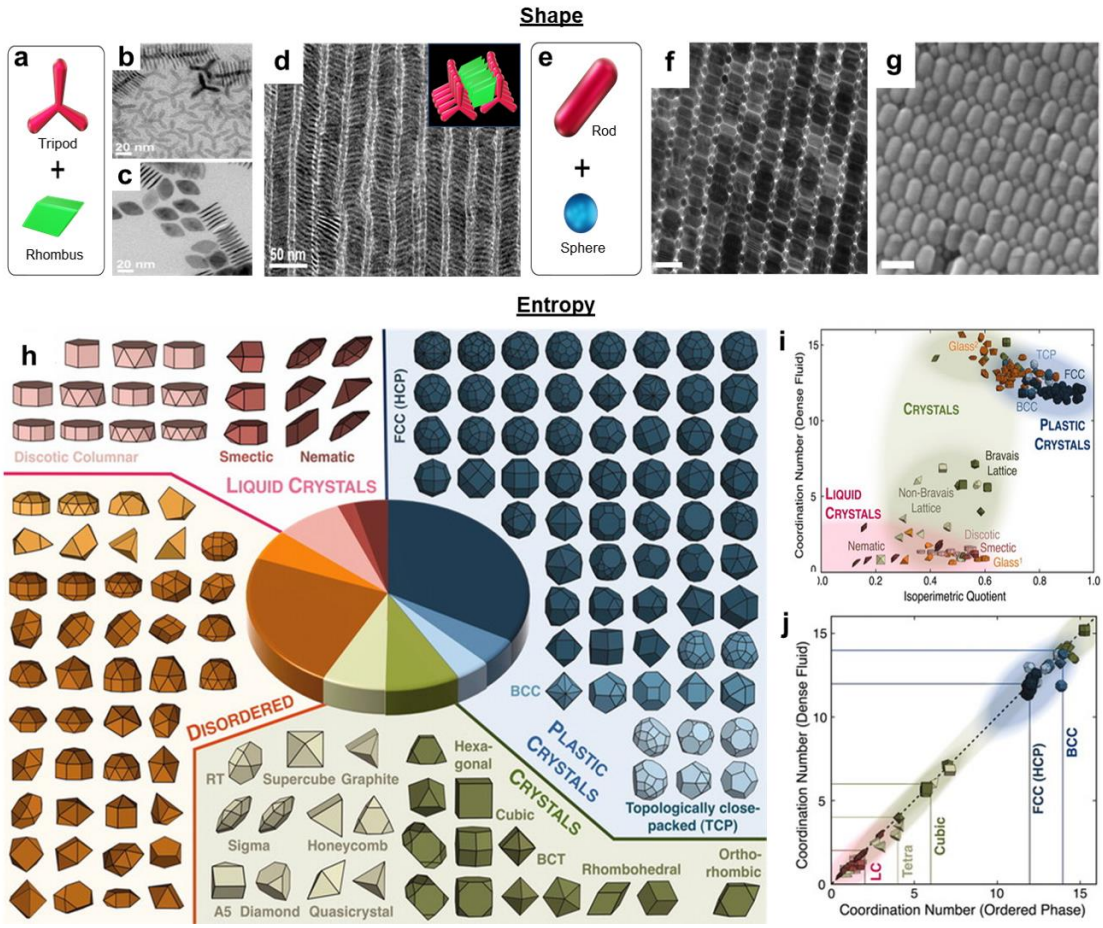

Figure 1.6. Guiding self-assembly using shape and entropy. (a) Schematics of rhombic and tripodal nanoplates. (b), (c) and (d) are TEM images of Gd_2O_3 tripodal nanoplates, GdF_3 rhombic nanoplates, and binary self-assembled superlattice, respectively. Reproduced with permission from ref. 69. Copyright 2013, American Chemical Society. (e) Schematics of $NaYF_4$ rod and Fe_3O_4 spheres. (f) TEM image (scale bar = 50 nm) shows the self-assembled superlattice where spheres are positioned between two layers of NRs. (g) SEM image (scale bar is 50 nm) depicts the binary NP self-assembly (BNSA). Reproduced with permission from ref. 70. Copyright 2013, American Chemical Society. (h) Systematic classification of different polyhedral particles into four different assembled organizations. (i) Graph showing the coordination number (CN) and isoperimetric quotient (IQ) corresponding to different families of assemblies formed by polyhedral particles. (j) Polyhedra have almost identical coordination numbers in the ordered phase, and the fluid state is close to the ordering transition. Reproduced with permission from ref. 71. Copyright 2012, American Association for the Advancement of Science.

In another landmark study, Glotzer and co-workers demonstrated the formation of ordered self-assembled structures under the influence of entropy.[71,72] Here, the authors performed systematic and exhaustive modeling of the assembly of polyhedral hard particles upon evaporation (Figure 1.6.h-j).[71] Note that for hard particles, all the possible conformations are similar due to the lack of enthalpic contribution and, therefore, are governed only by the maximization of orientational and positional entropy. This entropy maximization rule, for a

polyhedral particle, favors organization along the facets, resulting in the formation of an *'entropic bond.'* It was observed that the systems were assembled in either of the following four categories: crystals, plastic crystals, liquid crystals, or completely disordered structures (Figure 1.6.h). A design rule was then established depending on the high correlation between the *'coordination number'* of the polyhedron in the fluid phase and the *'isoperimetric quotient'* (IQ), which measures the deviation of a particle's shape from a sphere. According to this rule, (i) highly faceted particles with high coordination numbers formed plastic crystals, (ii) low faceted particles having low coordination numbers formed liquid crystals, and (iii) polyhedral with intermediate coordination numbers formed crystals. This study, therefore, provided a comprehensive framework allowing one to predict the assembly of NPs by simply determining its shape parameter and coordination number in the fluid phase. As can be clearly seen from the above examples, NPs can either assemble into large amorphous or crystalline structures or discrete assemblies. We would like to not only reiterate the importance of this point, but would like to highlight the need to find ways of fabricating discrete assemblies as well. Here, the formation of discrete assemblies, arguably the more challenging task, can be likened to the synthesis of different molecules in the field of organic chemistry. These discrete assemblies could then be appropriately crystallized or assembled in subsequent steps for the fabrication of useful macroscopic entities that would have been difficult to prepare under a single macroscale self-assembly approach. These aspects are discussed next.

1.4 Types of Complex Structures: A Thermodynamic Point of View

Having understood the important guiding principles that can be used to govern and dictate the outcomes of a self-assembly process, a natural subsequent question is: what kinds of complex NP assemblies can one realize? Herein, we have defined systems with varying interactions between their components as more complex than ones that have monotonous interactions, as opposed to the hierarchical nature of the final self-assembled state. The complexity that is associated with a self-assembled state can be attributed to its position in the Gibbs free energy landscape (Figure 1.7). [17,73-75] Although there is no universal definition for complexity that is associated with a system, it is agreeable that cells (following non-equilibrium self-assembly) are more complex than crystals (following mostly equilibrium self-assembly).[14,18] Several distinctions can then be drawn between the two. For instance, NP assembly, once formed, does not change with time, while living cells have the ability to evolve. Furthermore, most NP assemblies do not need a constant supply of energy to stay in an organized state. On the other

hand, cells do need a continuous influx of energy to stay *alive* and function as suitable building blocks for life. Although distinctions between different kinds of self-assembled states are still a matter of inquiry, some broad distinctions can still be made. For instance, assemblies that can be reconfigured are more complex than the ones that do not. The system's ability to reconfigure originates from the depth of the interaction potential that the state occupies. Here, states occupying a global minimum are too tightly wound and cannot be reconfigured easily, while the ones occupying local minima can be modified under the action of a suitable stimulus.

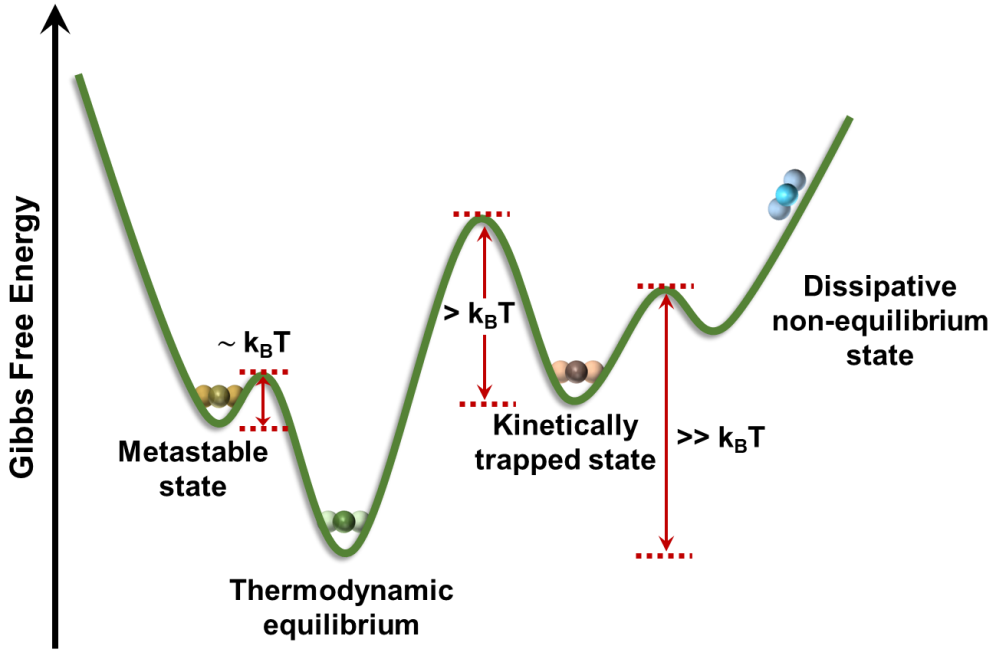

Figure 1.7. The free energy landscape is occupied by different classes of thermodynamic self-assembled states. Schematic illustration of Gibb's free energy diagram showing different thermodynamic states associated with the outcomes of a self-assembly process. Reproduced with permission from ref. 75. Copyright 2023, American Chemical Society.

Broadly speaking, one can classify the self-assembled states into the following three categories:[74,75]

(a) Thermodynamic equilibrium state- Here, the self-assembled state occupies a global minimum. Such structures, once formed, do not need any energy to sustain themselves. Most of the research in the field of artificial self-assembly has been focused on generating desired outcomes from equilibrium self-assembly. A key feature of such self-assemblies is the reproducibility that one gets by having the desired structure occupy a global minimum.

(b) Kinetically trapped state- Here, the self-assembled state occupies a local minimum instead of a global minimum. These states can either be metastable or a kinetic trap, depending on whether the energy barrier (marked in red) is comparable to or higher than the thermal energy (k_BT), respectively. These states, under the influence of a suitable trigger, can be modified or re-configured and can install the much-needed property of reversibility and adaptation to a system.

(c) Dissipative non-equilibrium state- Here, the self-assembled state comes into existence only under a constant supply of energy and/or matter. In this class of self-assembly, the interactions that hold the building blocks together persist only if the system continuously dissipates energy.

1.4.1. Illustrations of equilibrium self-assembly

Although in our and most of the other classification schemes, equilibrium or thermodynamic self-assembly is assigned to be least complex, it does not mean such assemblies are easy to prepare or are of less utility. Nature frequently utilizes equilibrium self-assembly mechanisms to reproducibly create a wide variety of functional materials such as lipid bilayers, DNA, proteins, virus, enzymes, etc.[76-78] Furthermore, most of the observed self-assembly processes such as the formation of micelles, protein assembly, crystallization, etc. are thermodynamically favored processes, where the final state lies at a global minimum.[14,79-84] In order to effectively design such equilibrium self-assemblies, the interactions between the building blocks need to drive the self-assembled state to a thermodynamic minimum so as to reproducibly form the desirable structure.

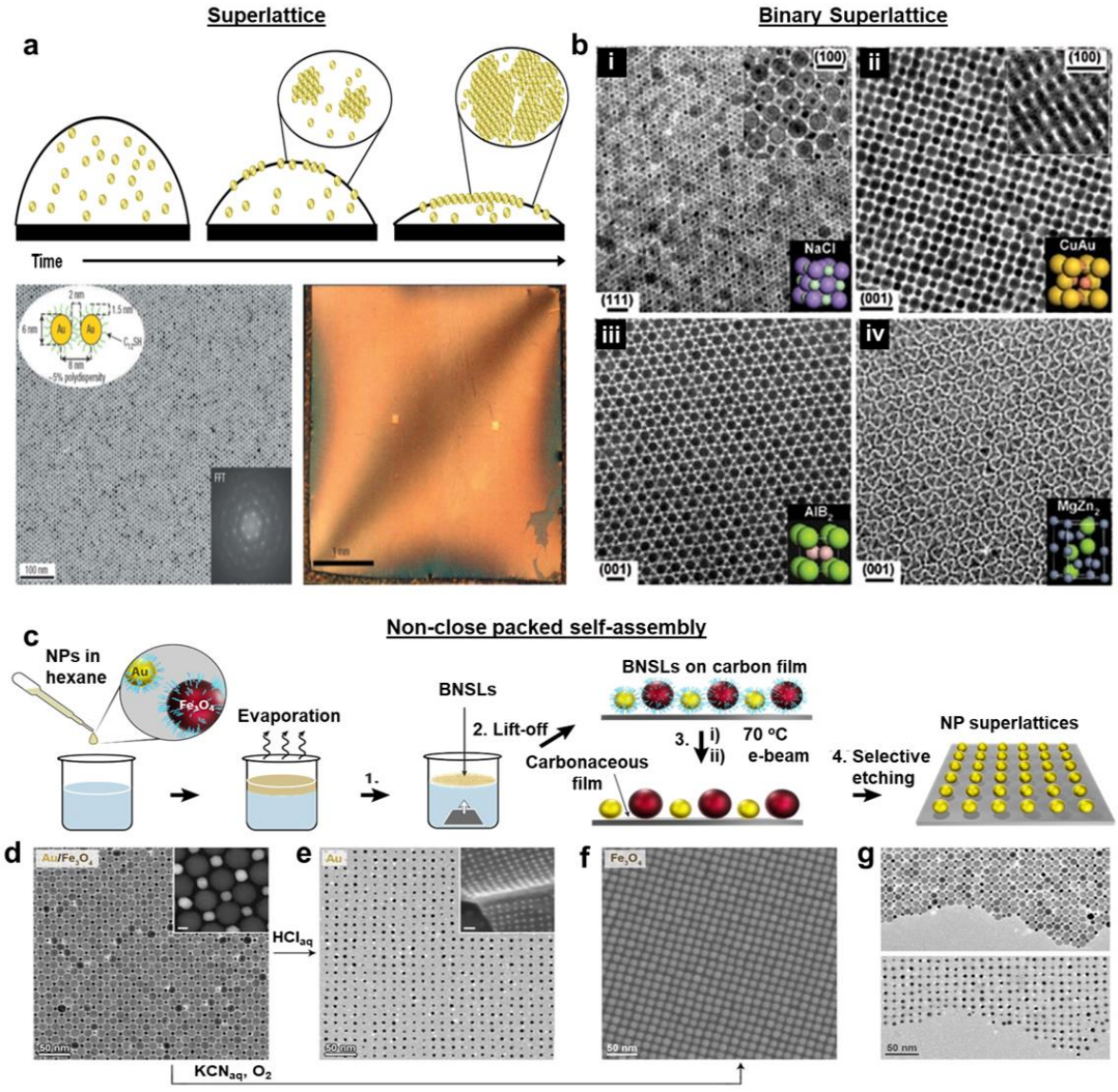

Figure 1.8. Assemblies occupying a global minimum. (a) Schematic diagram showing the self-assembly of dodecanethiol (DDT) capped AuNPs to form large domains of 2D superlattice at the liquid-air interface. The bottom panels show TEM and optical photographs of a typical NP monolayer produced. Reproduced with permission from ref. 80. Copyright 2006, Springer Nature. (b) TEM images of binary NP superlattices, self-assembled from different NPs, and their modeled unit cells. Reproduced with permission from ref. 82. Copyright 2006, Springer Nature. (c) Schematic diagram showing the method of formation of a non-closed packed superlattice. (d) TEM image showing the formation of an AB-type superlattice between Au and Fe_3O_4 NPs. The scale bar of the inset is 5 nm. (e) TEM image of a non-closed packed AuNP array formed after selective etching of Fe_3O_4. The scale bar of the inset is 5 nm. (f) SEM image of a non-closed packed Fe_3O_4 NP array formed after selective etching of AuNPs. (g) TEM image of a close packed AB-type superlattice before (top) and after (bottom) etching of Fe_3O_4 NPs. Reproduced with permission from ref. 85. Copyright 2017, American Association for the Advancement of Science.

One of the most prominent examples of NP self-assembly involves two dimensional (2D) superlattices, where NPs are neatly arranged in a crystalline order. Such ordered 2D crystals are commonly prepared by evaporating the dispersions of NPs over the liquid-gas interface. In

order to have a maximal domain of such 2D crystals, interactions between the NPs and the support need to be finely tuned. In this direction, Heinrich and co-workers prepared long-range hexagonal monolayers of dodecanethiol (DDT) capped AuNPs by evaporating a toluene solution of the NP (Figure 1.8a).[80] In this approach, the early-stage evaporation traps the AuNPs at the liquid-air interface, from which NP islands nucleate and grow to form mm sized AuNP films (Figure 1.8a). This new drop-drying regime was a robust, simple, and scalable way of creating high-quality and long-range 2D crystals. Similar experiments have been performed with mixtures of NPs of various compositions and sizes to create a wealth of 2D close packed crystal lattices (Figure 1.8b). In their seminal work, Murray and co-workers utilized a similar evaporation driven assembly protocol involving two sizes of spherical NPs to prepare more than 15 unique binary nanocrystal superlattices (BNSLs).[81,82] In an alternate approach, Klajn and co-workers built on an evaporation driven strategy to realize non-close packed self-assemblies (Figure 1.8c-g).[85] Here, the authors used a mixture of monodisperse Au and iron oxide (Fe_3O_4) NPs, which were assembled at the diethylene glycol-air interface, to initially form a close-packed superlattice. These 2D superlattices were then transferred to a suitable substrate like carbon coated TEM grids. The authors then selectively etched either AuNPs or Fe_3O_4 NPs, resulting in non-close-packed arrays with vacancies stabilized by the underlying carbon substrate. Such non-closed packed assemblies are useful for a wide range of optical, mechanical as well as catalytic properties (*vide infra*).

1.4.2. Illustrations of kinetically trapped self-assembly

A key characteristic of kinetically trapped self-assembled states is their reversible nature. To create such assemblies, the interparticle interactions should guide the assembled state to a local minimum as opposed to a global minimum. A key design principle here, therefore, is to use relatively weak or moldable/tunable interactions. This will enable the reversal of the self-assembly process under the influence of a suitable trigger. With this principle in mind, several systems have been designed that show reversible transformations in response to a wide variety of signals such as solvents, metal ions, temperature, light, magnetic field, biomolecules, etc.[52,86,87] In a pioneering study, Liz-Marzan and co-workers employed solvent as a trigger to assemble a dispersion of polystyrene (PS) stabilized hydrophobic AuNPs. [87] Here, water was used to trigger the assembly of hydrophobic PS-AuNPs dispersed in tetrahydrofuran (THF). A key challenge in the use of solvent as a trigger is the inevitable dilution of the solution, which affects the extent as well as the kinetics of the self-assembly process, resulting in irreproducible

aggregation kinetics. In order to resolve this challenge, the authors used a permeable silica nanocapsule to confine the hydrophobic AuNPs so as to limit the number of particles participating in the aggregation process.[88] Here, water being permeable to the silica nanocapsule could efficiently trigger the aggregation of PS-AuNPs, resulting in a bathochromic shift in the absorption spectrum (Figure 1.9a). Upon confining these hydrophobic AuNPs, the number of NPs participating in the aggregation process could be controlled and preserved, resulting in highly reproducible plasmon band shifts (Figure 1.9b, c) despite changes in the solvent compositions.

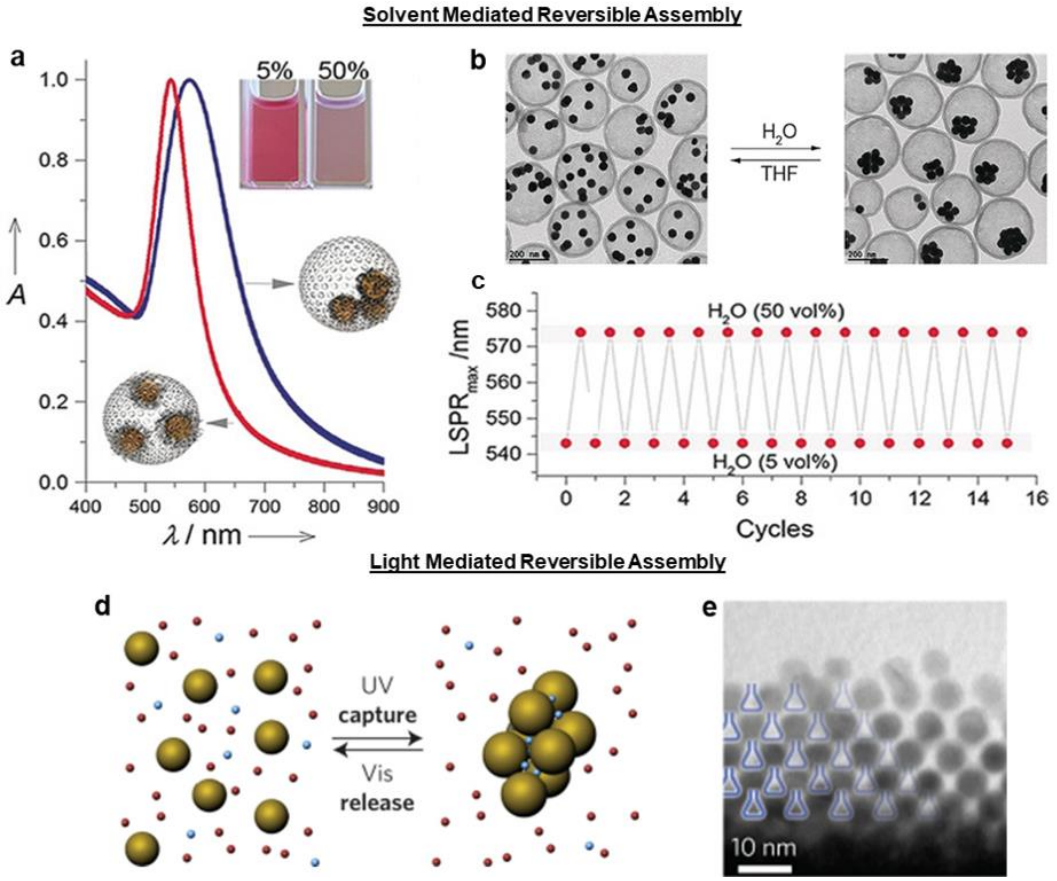

Figure 1.9. Assemblies occupying a local minimum. (a) UV-Vis absorption spectra of the nanocapsules containing hydrophobic AuNPs under low (shown in red), and high (shown in blue) water content. (b) TEM images showing the reversible clustering of AuNPs. (c) Variation of SPR maximum for ~15 cycles. Reproduced with permission from ref. 88. Copyright 2018, John Wiley & Sons. (d) Schematics for the light-induced self-assembly of photo-responsive AuNPs, along with the reversible trapping of polar molecules. (e) A representative SEM image of the nanoflasks. Reproduced with permission from ref. 91. Copyright 2016, Springer Nature.

In another class of stimuli responsive self-assembly, NP systems were constructed that could show reversible transformations upon the addition of suitable chemical stimuli. The reversible nature of such kinetically trapped self-assemblies is key in the design and fabrication of

systems with reusable chemical functionalities. For instance, one can create reusable sensors for a wide range of chemical and biological stimuli.[88,89] In a classic example, Grzybowski and co-workers used light as a trigger to assemble AuNPs functionalized with azobenzene thiols in a reversible fashion.[90] The photoswitchable aggregation of AuNPs was later extended to control their catalytic activity towards a hydrosilylation reaction (photo-switchable catalysis). In their study, AuNP catalysts were decorated with a mixed self-assembled monolayer (m-SAM) of azobenzene terminated thiols and dodecylamine (DDA). When dispersed, AuNPs could readily catalyze a hydrosilylation reaction, while irradiation with UV light aggregated the NPs, resulting in a switching-off of the reaction. Later, Klajn and co-workers smartly utilized azobenzene-functionalized NPs and the voids between the NPs in the aggregates as '*nanoreactors*' for accelerating various reactions (Figure 1.9d).[91] These voids, formed between azobenzene functionalized AuNPs, contain a high number of polar cis-azobenzene moieties and, therefore, can be used to trap a variety of polar guest molecules during the assembly process. The authors, using this strategy, could trap a range of polar reactant molecules inside these cavities and demonstrate accelerated reaction rates (Figure 1.9e).

1.4.3. Illustrations of dissipative non-equilibrium self-assembly

Dissipative self-assembled systems differ noticeably from all the other classes of self-assembly that have been discussed so far, as they come into existence only under a constant influx of matter and/or energy.[92-94] Because of this constant need for energy and/or matter to sustain the assembled state, these systems are oftentimes classified as non-equilibrium or out-of-equilibrium systems.[92-94] Interestingly, these structures are transient in nature and form only when their function is required. A key challenge in the design and realization of such systems is the ability to vary the interparticle interactions in a temporal fashion. In this direction, Grzybowski and co-workers used light as a trigger to vary the interparticle interactions and create a dissipative self-assembled NP state.[55] Here, the AuNP and AgNP were functionalized with mixed self-assembled monolayers (m-SAM) of dodecylamine (DDA) and a photoswitchable thiol containing azobenzene headgroup. These NPs, because of the low dipole moment of trans-azobenzene (μ= 0 D), are readily dispersed in hydrophobic solvents like toluene or hexane. Upon exposure to UV light, the thermodynamically more stable trans-azobenzene isomerized to its metastable cis-configuration with a higher dipole moment (μ= 4.4 D), resulting in aggregation induced color change.[55] Due to the dissipative nature of the aggregates, they persisted only under the continuous irradiation of UV light, and disassembled

once the irradiation was discontinued. This inspired researchers to routinely employ groups like azobenzene[55] and spiropyran[58] to skillfully create a range of dissipative self-assembled systems. In a recent study, Klajn and co-workers introduced a novel strategy of assembling non-photo responsive NPs under the action of light using a photo responsive medium.[58] Here, light-driven reversible isomerization of Spiropyran was used to modulate the pH of the solution, ultimately driving the aggregation of NPs. This light driven acidification and de-acidification of the solution was coupled to the protonation and de-protonation of carboxylate functionalized NPs in both solution and gel states (Figure 1.10a, b).[58] Such light powered systems are very attractive because of '*zero-waste*' production and almost instantaneous deactivation. The dissipative systems discussed so far differ markedly from the natural systems, which use chemical fuels to drive the self-assembly process.[93,94] Inspired by biological systems, researchers have used chemical fuels for creating dissipative self-assembled systems. In a pioneering example, van Esch and co-workers developed a strategy to create a dissipative self-assembled molecular system by using triggered esterification and a spontaneous de-esterification reaction.[95,96] The authors were successful in establishing control over reaction rates and amounts of chemical fuel to not only choreograph the formation of a transient gel but also govern properties like lifetime, stiffness, and self-regeneration capability of the gel state. Later, this idea of using two competing and opposite reactions was utilized to create a wide variety of dissipative self-assembly in molecular systems,[97-101] with limited examples involving NPs.[102-107] In one such report in NPs, Boekhoven and co-workers have shown the transient assembly of silicon nanocrystals (SiNCs) through triggered esterification and autonomous de-esterification reactions (Figure 1.10c).[102] A carboxylate functionalized SiNC was used, which readily solubilizes in water at basic pH and shows no tendency for assembly because of the strong electrostatic repulsions. Upon addition of the chemical fuel (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, EDC), the nanocrystals lose its charge and gets activated for the self-assembly process. After the complete consumption of EDC molecules, this NC-assembly eventually transforms into individual monomers through autonomous hydrolysis of NHS-esters. The authors have further shown that the kinetics of cellular uptake can be regulated using such dissipative self-assembled NP systems.

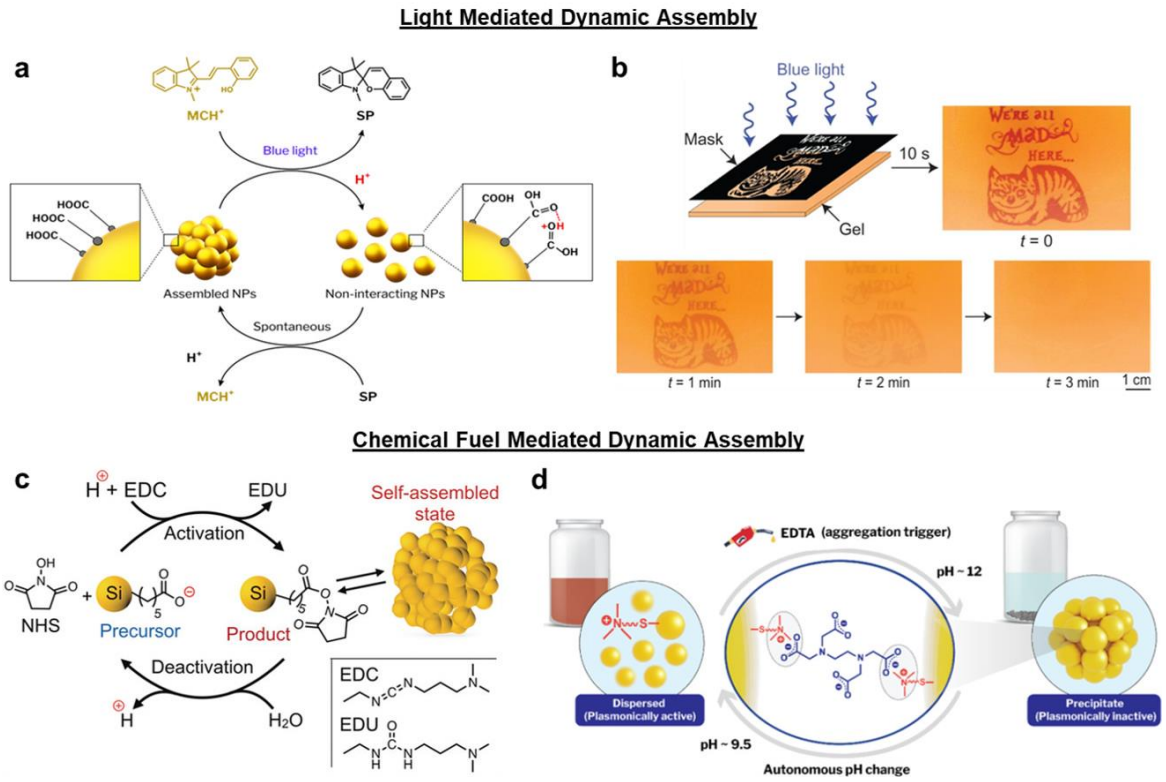

Figure 1.10. Assemblies formed under a constant influx of energy. (a) Schematics of light induced dynamic self-assembly of non-photo-responsive AuNPs in the presence of Spiropyran. (b) Schematic representation, as well as optical photographs of the self-erasing photopatterns. Reproduced with permission from ref. 58. Copyright 2015, Springer Nature (c) Reaction cycle for the chemical fuel driven dissipative self-assembly in SiNCs. Reproduced with permission from ref. 102. Copyright 2018, John Wiley & Sons. (d) Scheme for the formation of transiently stable AuNP precipitates, where the assembling step was triggered by EDTA, and the autonomous pH change initiates the disassembly step. Reproduced with permission from ref. 106. Copyright 2021, American Chemical Society.

It is not trivial to replicate a chemically fueled transient self-assembly in metal NPs by following the common strategies reported for molecular systems because of the strong van der Waals forces experienced by NPs during the aggregation. As a result, achieving a transient switching between completely dispersed and completely precipitated states of metal NPs is a challenging task. Pillai and co-workers have accomplished this task by exploiting the pH-dependent temporal fluctuations in the strength of electrostatic interactions between AuNPs.[106] The addition of ethylenediaminetetraacetic acid (EDTA) triggered the assembling step in [+] AuNPs, leading to complete precipitation (Figure 1.10d). In the presence of atmospheric CO_2, the EDTA loses its aggregating ability due to the autonomous changes in the ionic strength and pH of the solution. This results in the complete re-dispersal of [+] AuNPs from the electrostatically bonded AuNP-EDTA precipitates. The unique and non-destructive way of disassembly led to easy waste removal (a simple decantation of the supernatant was sufficient), negligible plasmon dampness, and, remarkably, a transiently stable precipitate state of NP. In

all these examples, the addition of a trigger results in the spontaneous assembly of systems into higher-order structures that are inherently unstable in the reaction conditions. These structures, therefore, disassemble autonomously once the trigger is removed. This strategy results in the construction of systems with tunable lifetimes and properties, which can have far reaching impacts. For instance, the design and realization of polymers with tunable lifetime, that can spontaneously degrade after the completion of their pre-designed lifetime is attractive to potentially combat the challenge of plastic pollution. Furthermore, systems with self-healing and self-replication capabilities can be used to design products that can self-repair when damaged. The possibilities and promises of these classes of materials are endless.

1.5. Products of Nanoparticle Self-Assembly

As discussed in previous sections, a fine-tuning of interparticle interactions and forces can create NP assemblies with different complexities. Now, it is important to understand what kind of products are typically formed upon the completion of NP self-assembly. The key purpose of the present section is to summarize a few of the recent but prominent products of the NP self-assembly process that have high prospects in applied studies. Accordingly, this section identifies and covers examples based on 3D crystals, chiral assemblies, and bio-nano supramolecular structures as the key products of the NP self-assembly process.

1.5.1. Three-dimensional (3D) nanoparticle crystals

The creation of NP assemblies with long-range order is important because of their potential application in optoelectronics, sensing, catalysis, etc. As a result, different methods have been developed to prepare NP crystals, which involve electrostatics, H-bonding, van der Waals, hydrophobic, dipole-dipole, and covalent interactions.[26] In this section, we have restricted our discussion to recent strategies where ionic and covalent interactions were used for the creation of 3D NP crystals (a few examples of 2-and 3-D crystal formation have already been covered in the previous section). In one of the first examples of 3D NP crystals, Grzybowski and co-workers showed the formation of a non-close packed diamond-like crystal lattice using oppositely charged metal NPs.[108] Here, oppositely charged Au and Ag NPs precipitated out of the solution at the point of overall electroneutrality (i.e., when $\sum Q_+ + \sum Q_- = 0$), resulting in the formation of *nanoionic* precipitates. These precipitates were then converted into micrometer-sized and regularly faceted crystals by first re-dispersing the precipitates in the

DMSO-water mixture, followed by the slow evaporation of water. This slow evaporation of the *good solvent* (water) resulted in controlled destabilization of the charged particles, ultimately resulting in the formation of diamond-like crystals. Likewise, a series of 3D crystals were prepared by employing complementary interactions between two different types of surface-functionalized NPs. In another seminal work, Grzybowski and co-workers designed a system incorporating two distinct functionalities on the surface of the same NP for the fabrication of micrometer-sized 3D crystals.[109] The NPs were functionalized with a mixed monolayer of oppositely charged ligands containing quaternary ammonium and carboxylic acid groups (Figure 1.11a). Here, the carboxylic acid ligands provided H-bonding attractions, while the quaternary ammonium group imparted the much-needed electrostatic repulsions to the NP system, thereby decelerating the assembly kinetics and ultimately resulting in the formation of micrometer-sized, well-ordered 3D crystals. Interestingly, the end product was decided by the pH of the reaction mixture, based on which the NPs choose to form 3D crystals (with like-charged or oppositely-charged NPs) or amorphous aggregates or remain unaggregated. In this way, the presence of a mixed charge monolayer helped in flexibly guiding the NP assembly by modifying the interparticle potentials.

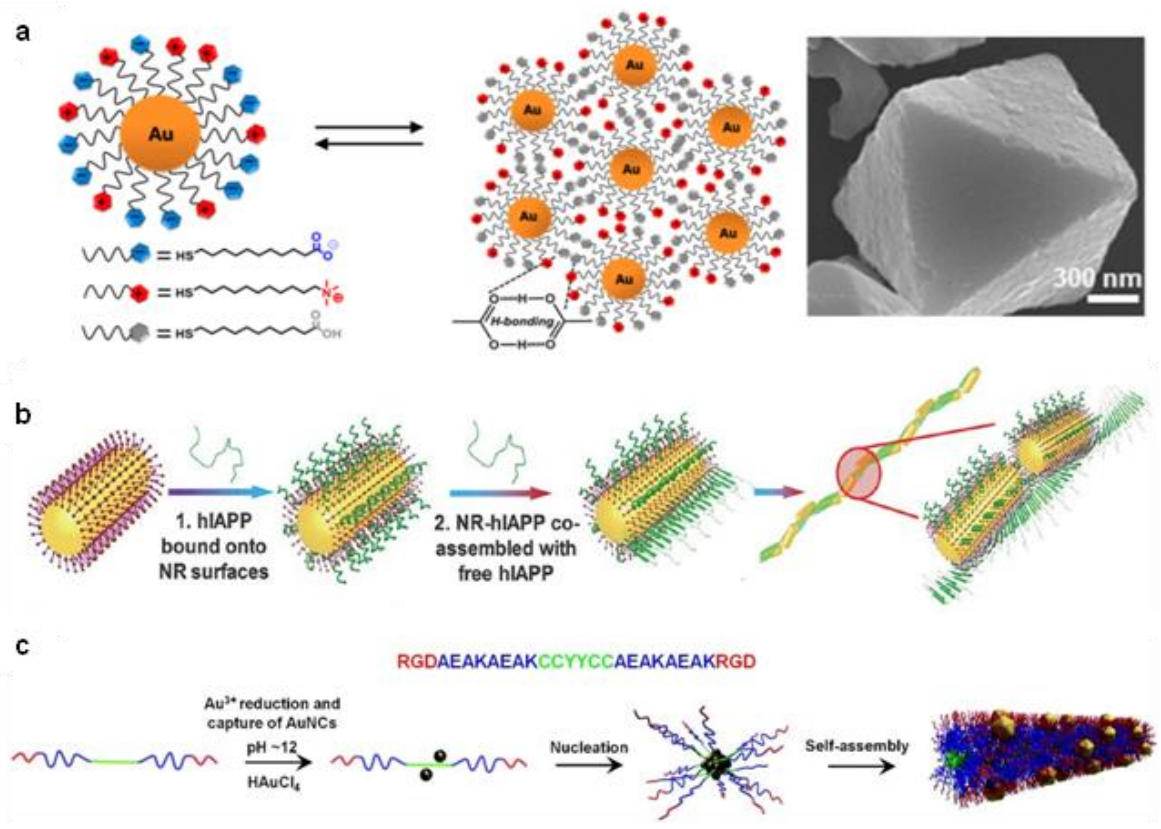

Figure 1.11. Products of NP Self-Assembly. (a) Schematics of self-assembly between like-charged AuNPs to form 3D supercrystals. The perfect balance between attractive (H-bonding) and repulsive

(electrostatic) interactions decelerates the self-assembly kinetics, ultimately resulting in micrometer-sized, well-ordered 3D crystals. Reproduced with permission from ref 109. Copyright 2015 The Royal Society of Chemistry. (b) Scheme showing the chiral assembly of AuNRs with human islet amyloid polypeptides (hIAPPs). Reproduced with permission from ref 114. Copyright 2021 American Association for the Advancement of Science. (c) Schematics showing the self-assembled nanofiber formation through the self-assembly between peptides and Au nanoclusters. Reproduced with permission from ref 118. Copyright 2017 American Chemical Society and Copyright 2020 The Royal Society of Chemistry.

1.5.2. Chiral nanoparticle assembly

The realization of chiroptical activity in nanoscale architecture has started a renaissance in the field of light-matter interaction, which exerts a direct impact on various prominent applied studies such as catalysis, device fabrication, biosensing, etc.[110-113] For instance, the chiroptical activity of plasmonic NRs was used as a tool to monitor the formation of amyloid fibrils in real samples from patients diagnosed with Parkinson's disease.[112] The optical properties of the NPs can be fine-tuned as a function of size, shape, and composition during the self-assembly process. Therefore, self-assembly is one of the most promising phenomena for building chiral macroscopic materials. The most natural way to make chiral NP assemblies is in the presence of chiral templates such as DNA, amino acids, liquid crystals, amyloid fibrils, or different helical supramolecular structures.[110-113] In such cases, NP assemblies show chiroptical activity through photonically driven dipole-dipole interaction between NP and chiral adsorbates. In a seminal work, Moura, Kotov, Liu, and co-workers have prepared long-range helical assemblies of gold nanorods with human islet amyloid polypeptides (hIAPPs) (Figure 1.11b).[114] The authors were successful in overcoming the challenge of 'low optical asymmetry' in chiral plasmonic NP assemblies by achieving a long-range ordering, as observed in the case of liquid crystals. The Kuhn dissymmetry factor (g-factor) was estimated to be 0.12 at 661 nm, which is the highest reported so far in any chiral plasmonic assemblies. The authors have also envisioned that the long-range ordering of NPs can be further improved to build materials with high optical asymmetry, useful for bioanalytical applications.

1.5.3. Bio-nano architectures

Coupling the properties of biomolecules with inorganic NPs to create hybrid materials with superior functions is an emerging area of research. However, it is often challenging to retain the inherent functional properties of both biomolecules and NPs in the hybrid state.[192-195] Therefore, a well-controlled self-assembly process is the key for preserving the inherent properties of individual components in bio-nano hybrids. Such accomplishments have resulted

in several exciting applications of bio-nano self-assembled structures in solar driven CO_2 reduction, photocatalytic H_2 evolution, efficient solar cell fabrication etc.[115-117] Peptides are one of the most used scaffolds for the creation of bio-nano hybrid materials. Wei, Shang and co-workers have prepared nanofibers from the self-assembly process between peptides and Au nanoclusters (Figure 1.11c).[118] The luminescence of Au nanoclusters was enhanced 70-fold in the self-assembled nanofibers due to the electron confinement in nanoclusters induced by 1D nanostructure formation. The authors have further utilized these self-assembled bio-nano hybrids for fluorescent thermometry and live-cell imaging.

1.6. Emerging Applied Properties of Nanoparticle Self-Assembly

It is a dream of every researcher working in self-assembly to create complex matter with specific and distinct functionalities, thereby mimicking the naturally occurring self-assembled architectures. For this, it is essential to achieve a controlled aggregation between the monomer NPs to realize the superior properties of their self-assembly structures. As a result, the choice of monomer NPs is important, which essentially is dependent on the applied property of interest. For example, noble metal and iron-based NPs should be the choice if one is interested in the plasmonic and magnetic properties in the self-assembled structures, respectively. Self-assembled architectures obtained from such NP can exhibit superior sensing, catalysis, photovoltaics, cargo release, display, and light-harvesting properties compared to their monomer counterparts, and a few of the recent reports are summarized in this Section.

1.6.1. Creation of SERS substrates from nanoparticle self-assembly

Self-assembled structures containing plasmonic NPs are often used in sensing studies because of their large molar absorption cross section (10^8-10^{10} M^{-1} cm^{-1}).[119] The proximity between NPs in the assembled state will result in strong plasmonic coupling and generation of hot spots,[120] which, in principle, can lower the detection limit of analytes trapped between the NPs. Thus, extensive research has been carried out to create and use stable self-assembled NP structures as substrates for various sensing applications.[121-123] In a landmark study, Mihi, Liz-Marzan, and co-workers have prepared plasmonic architectures of hexagonally packed AuNP supercrystals of uniform periodicity over a large area of 0.5 cm^2 (Figure 1.12a).[123] The absorption of the plasmonic architectures was tuned from visible to near-infrared region by varying the NP ordering and lattice parameters of the AuNP supercrystals. This is a remarkable

result as it gives high flexibility in using different probe lasers as per the requirement. The authors explored the effect of NP ordering and lattice parameters (L = 400, 500, 600, 740, and 1600 nm) on the SERS activity of the plasmonic architectures. The maximum SERS activity was obtained for L = 500 nm because of the good overlap between the wavelengths of lattice plasmon and probe laser (785 nm). Moreover, an enhancement in the SERS signal was observed with an increase in the number of NPs in the clusters, which finally reached a 3-fold increase when compared to isolated NPs or random assemblies.

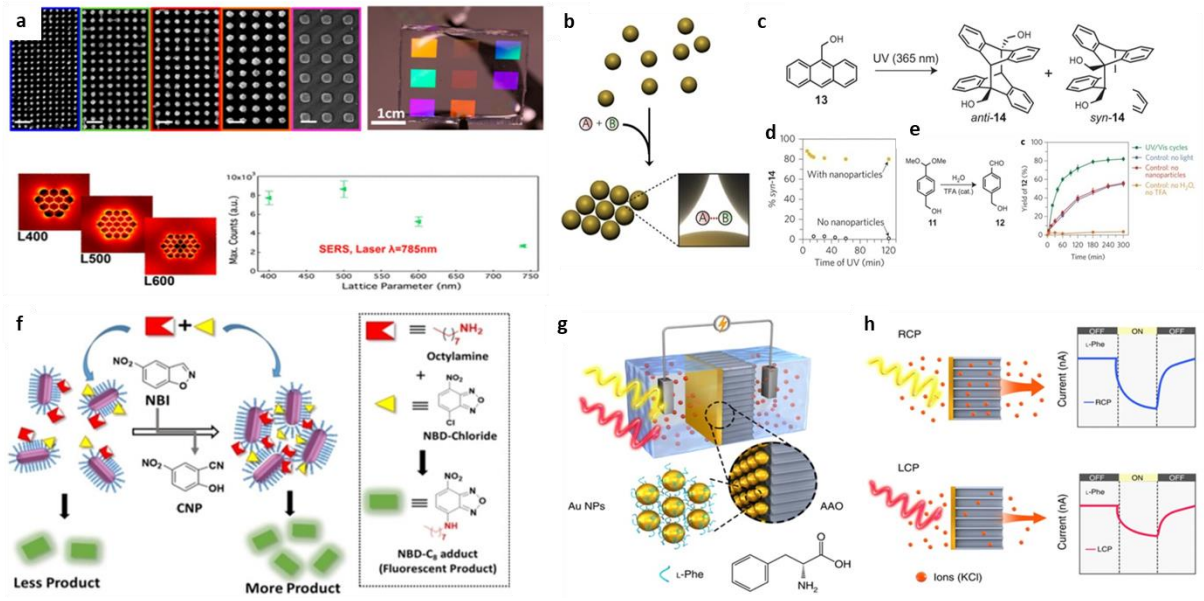

Figure 1.12. Applied properties of NP self-assembly. As a robust SERS substrate for sensing applications. (a) SEM images of AuNP 3D assemblies with varying lattice parameters, *L* = 400, 500, 600, 740, and 1600 nm (from left to right). The scale bar is 1 μm. The optical photograph of AuNP assemblies with varying lattice parameters in the PDMS mold is shown. Snapshots of unit clusters of AuNP superlattice, showing varying electric fields as a function of different *L* values = 400, 500, and 600 nm. The maximum SERS activity was obtained for *L* = 500 nm because of the good overlap between the wavelengths of lattice plasmon and probe laser (785 nm). Reproduced with permission from ref 123. Copyright 2018 American Chemical Society. Application of NP self-assembly in catalysis. (b) Schematics representing chemical reactions inside nano flasks formed in a dynamic AuNP self-assembled system. (c, d) UV-induced dimerization of anthracene in the presence and absence of AuNP, showing the stereoselectivity of the reaction to preferentially form *syn*-isomer. (e) Light accelerated hydrolysis of acetal in nanoflasks. Reproduced with permission from ref 91. Copyright 2016 Springer Nature. NP self-assembly for devices. (f) Schematics of AuNR assembly during catalytic Kemp elimination with concurrent aromatic nucleophilic substitution reaction within hydrophobic cavities formed upon NR aggregation. Reproduced with permission from ref 126. Copyright 2021 John Wiley & Sons. (g) Schematic illustration of the electrochemical setup incorporated with self-assembled AuNPs functionalized with l-phenylalanine. (h) Photocurrent generated was higher for the l-phenylalanine functionalized AuNP film when irradiated with right-handed circularly polarized light. Reproduced with permission from ref 110. Copyright 2022 Springer Nature.

1.6.2. Catalysis with nanoparticle assembly

NPs render their surface and active sites for the chemical reactions to proceed along the altered energy landscape.[124,125] The size-dependent catalytic activity of NPs, arising from the large surface-to-volume ratio, can be coupled to optical properties of metal NPs, such as surface plasmon resonance, to carry chemical transformations in the presence of light. Often, the analogy between NP catalysts and natural catalysts, enzymes, is made in terms of reaction rate, selectivity, and yield. Controlling these parameters is a holy grail in the area of NP catalysis, and doing catalysis with self-assembled nanostructures is emerging as an effective tool to achieve this target. Self-assembled nanostructures often bring out confinement effects that can effectively manipulate the chemical and physical properties of the molecules. Along with this, the design of tandem catalysts is at ease by virtue of self-assembled systems to precisely control and engineer the structure-shape-morphology aspects. For instance, Klajn and co-workers observed an acceleration in chemical reactivity inside the nano flasks because of the nanoconfinement effect in a dynamically self-assembled system (Figure 1.12b).[91] The authors constructed light triggered assembly of azobenzene functionalized AuNPs and utilized the voids between the NPs in the aggregated state as nanoreactors to promote chemical reactions. The change from trans to cis-azobenzene functionalized AuNPs, upon UV light excitation, was used to reversibly trap polar molecules within the voids and accelerate the reaction. Once the reaction was over, the nano flasks were easily disintegrated by shining visible light so as to get the trans-azobenzene functionalized AuNPs, thereby releasing the products into the bulk solution.

Along with accelerating the chemical reactions, the use of nano flasks helped in altering the stereoselectivity of the product as well (Figure 1.12c-e). While dimerization of 9-(hydroxymethyl)anthracene in toluene favors the formation of an *anti*-product in the presence of light, the same reaction in the presence of the nano flasks favored *syn*-isomer. The observed acceleration and stereoselectivity in the chemical reactions arise possibly because of the increased effective molarity as well as the pre-organization of the reactants within the nanocavities. Such on-demand control of catalytic activity also overcame the diffusion limitations of confined space catalysis. In a recent example, Maiti and co-workers demonstrated the enhanced assembly of catalysts driven by the formation of a transition state during catalytic conversion (Figure 1.12f).[126] The assembled state can be used to carry another reaction completely unrelated to the original catalytic reaction as well. CTAB-functionalized AuNRs catalyzed the Kemp elimination reaction, during which NRs flocked together. The

aggregation was induced by lowering of surface charge of AuNRs because of the formation of a negatively charged transition state on the NR surface. The aggregated AuNRs possess hydrophobic cavities and thus efficiently accelerate the rate of aromatic nucleophilic substitution reaction. The results obtained potentially lead to the emergence of complex multifunctional dynamic systems.

1.6.3. Self-assembled nanoparticle based electronic devices

The creation of nanoscale devices for the development of very large scale integrated (VLSI) electronic architectures is essential in the current scenario. However, *'top-down'* approaches such as lithography have a certain lower limit, and reaching below the limit is practically impossible. This limitation of conventional 'top-down' approaches can be overcome through the *'bottom-up'* self-assembly process. Exploiting properties of NP self-assembly for new technologies requires a correct functional form and more often needs processing at the macroscale. In this regard, the field of NP self-assembly is realizing its potential for the development and manufacturing of materials and devices with important properties that otherwise are not available or limited by cost. The present status of the field suggests that self-assembled materials are gaining ground in chemical separations, electronics, photonics, and energy storage and conversion.[127-129] In a recent study, Xu, Klajn, Kotov, Kuang, and co-workers designed polarization-sensitive optoionic membranes from self-assembled chiral plasmonic NPs (Figure 1.12f, g).[110] It is quite challenging to achieve optoelectronic effects that can differentiate between absorption of circularly polarized lights, and hence, it often necessitates the use of chiral meta surfaces having out-of-plane architecture. The self-assembled AuNPs modified with L-phenylalanine can generate 1-2 orders higher photocurrent under right-handed circularly polarized light as compared to left-handed circularly polarized light. The polarization dependent photocurrent originates because of the enhanced trapping of electrons by the coupling of plasmon and chiral modes, which further transduces the optoionic effect at the NP membrane.

1.7 Conclusions and Outline

This chapter summarizes the journey of NP self-assembly and supramolecular systems, starting from the simple host-guest-based recognition systems to complex static and dynamic NP architectures. The examples discussed in this chapter outline the design principles to create functional NP assemblies. This clearly depicts the need for tuneable interparticle interactions encoded in the surface chemistry of NPs. The programmable assemblies can also be guided by the careful utilization of the core NPs. All these different approaches, essential to obtaining functional NP assemblies, have been thoroughly discussed in this chapter. This chapter also showcases the importance of both surface ligands and the core part in NP assembly to get desired functions from a limited set of NP building blocks. The varying complexities of NP self-assembly were also discussed, and a thermodynamic point of view to regulate NP assemblies to obtain complex assemblies was demonstrated. A set of recent examples was also discussed where NP assemblies were utilized to obtain emerging properties and practical applications.

There is still a lack of appropriate approaches or information to get predictable assemblies and desired functions (for example, nanoscopic to macroscopic property, hybrid, and new property, retention of inherent property, etc.) from NP assemblies, which requires precise control over the kinetics and thermodynamics of the self-assembly process. This can be achieved by the controlled regulation of interparticle interactions, which is governed by the surface ligands. In this process, the core properties can also be preserved, which can be further utilized to get desired functions from the NP assemblies. In light of this, the underlying design principles have been utilized in this thesis to get functional NP assemblies using both the surface ligands and core parts of NPs. In chapters 3 and 4, interparticle interactions were regulated to control the assembly pathways between NPs and biomolecules to obtain functional spatiotemporal assemblies of NPs. In Chapter 5, both surface ligand and core properties of NPs were used to obtain static and dynamic functional NP assemblies.

Thus, the use of both surface and core properties of NPs helped in imparting desirable functions of dynamicity, adaptability, multi-stimuli-responsiveness, and multifunctionality in the NP self-assembly process. In short, fine control over the assembly pathways enabled us to achieve various static and dynamic self-assembled structures from a limited set of NP building blocks.

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Chapter 2

Methods and Techniques

2.1 Materials and Reagents

Tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$), hydrogen peroxide (H_2O_2) (30% w/w) in H_2O, tetramethylammonium hydroxide (TMAOH) 25 % wt. in water, 11-mercaptoundecanoic acid (MUA), Hydrazine monohydrate ($N_2H_4.H_2O$ 50-60%), tetrabutylammonium borohydride (TBAB), p-nitrophenol, benzyl mercaptan, 1,10-dibromodecane, hexamethyldisilazane, chlorotrimethylsilane, potassium iodide, decanoic acid, silver acetate, thiourea, acetonitrile, β-nicotinamide adenine dinucleotide sodium salt (NAD^+), β-nicotinamide adenine dinucleotide reduced disodium salt (NADH), 1,4-benzene dithiol (BDT), 3,3′,5,5′-tetramethylbenzidine (TMB), hexokinase (HK), D-(+)-glucose, creatine phosphokinase, phosphocreatine disodium salt hydrate, and potassium ferricyanide were purchased from Sigma-Aldrich. Adenosine-5′-triphosphate disodium salt hydrate (ATP), Adenosine-5′-diphosphate disodium salt hydrate (ADP), Adenosine-5′-monophosphate disodium salt hydrate (AMP), (Di-n-dodecyl)-dimethyl-ammonium bromide (DDAB), thymine, sodium hydroxide, and dodecylamine (DDA) were purchased from Alfa Aesar. Dimethyl sulfoxide (DMSO) and ethanol were purchased from Rankem Analytical Reagent Laboratory Chemicals and TCI Chemicals (India) Pvt. Ltd., respectively. All the reagents were used as received without any further purification. The positively charged N,N,N-trimethyl(11-mercaptoundecyl) ammonium ion (TMA) was synthesized according to the reported procedure.[1]

2.2 Synthesis of Organic Ligands and Their Characterization

2.2.1 Synthesis of thiolated thymine ligand:(1-(10-mercaptodecyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione)

The thiolated thymine ligand (1-(10-mercaptodecyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione) was synthesized using a modified reported procedure.[2,3] First, 25 mmol of thymine (1), 25 mmol of 1,10-dibromodecane, 17.5 mmol of hexamethyl di-silazane, 25mmol of trimethylchlorosilane, 25 mmol of potassium iodide, and 25 mL of dry acetonitrile were added in a 100 mL round-bottomed flask. The mixture was then refluxed at about 100 °C for 24 h under a nitrogen atmosphere. Next, the resulting mixture was cooled to room temperature. After that, the solvent and the volatile reagents were removed under reduced pressure. The resulting solid residue was dissolved in chloroform, and gravity filtration was used to remove the precipitating salt. The chloroform solution was then washed 3−4 times with water using a separating funnel and was dried with anhydrous Na_2SO_4. Finally, after evaporating the chloroform, a crude product was obtained. The crude product was purified using column

chromatography (Eluent methanol/chloroform, 1:25) to obtain 2 (1-(10-bromodecyl)-5-methyl pyrimidine-2,4(1*H*,3*H*)-dione). Next, 2.8 g 1-(10-bromodecyl)-5-methylpyrimidine-2,4-(1*H*,3*H*)-dione (8.1 mmol) and 0.8 g thiourea (10.5mmol) were taken in a 100 mL two-necked flask and dissolved in 50 mL ethanol (99%, degassed) and refluxed for 20 h under N2 atmosphere to prevent disulfide formation. Then, the solvent was removed by rotary evaporation. Following this, a 70 mL aqueous solution of 0.7 g NaOH was added, and the mixture was refluxed for 3 h. After that, the reaction mixture was cooled to room temperature and extracted in three portions with chloroform solution. Then, the combined organic fractions were dried over Na2SO4. The crude product was purified using column chromatography (eluent methanol/chloroform, 1:25) to get the final product 3 (1-(10-mercaptodecyl)-5-methyl pyrimidine-2,4(1*H*,3*H*)-dione) which yielded 0.9 g (3 mmol, ~40%) (Figure 2.1-2.3).

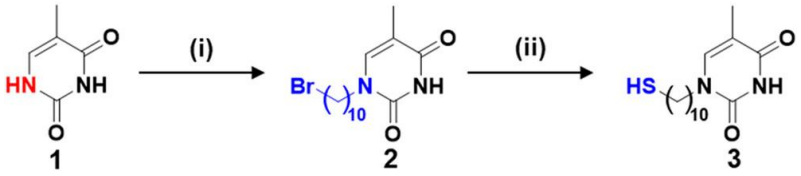

Figure 2.1. Synthesis of thymine thiol (1-(10-mercaptodecyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione) (**3**). (i) $(Me_3Si)_2NH$, Me_3SiCl, KI, CH_3CN, reflux 24 h,100 °C, N_2 atmosphere and 1,10-dibromodecane, (ii) thiourea, ethanol, reflux, 20 h; NaOH (aq), reflux, 3 h.

2.2.2 Characterization of thymine thiol ligand: (1-(10-mercaptodecyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione) ligand

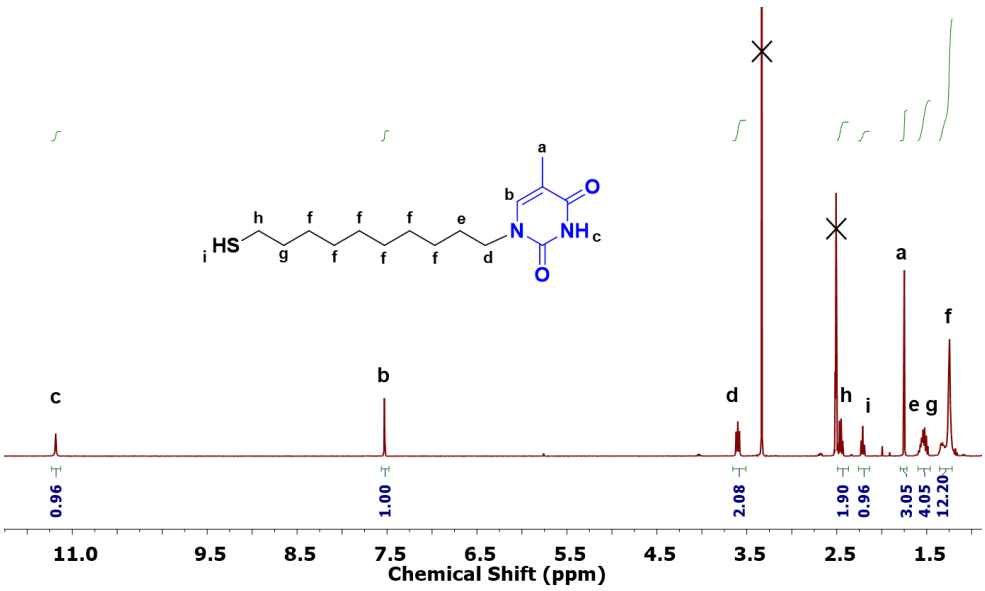

Figure 2.2. ^{1}HNMR spectrum of 1-(10-mercaptodecyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione in DMSO-d6 solvent (x corresponds to solvent peaks).

^{1}H NMR Data: ^{1}H NMR (400 MHz, DMSO) δ 11.18 (s, 1H), 7.53 (d, J = 1.2 Hz, 1H), 3.66 – 3.51 (m, 2H), 2.46 (q, J = 7.3 Hz, 2H), 2.21 (t, J = 7.6 Hz, 1H), 1.76 (t, J = 5.3 Hz, 3H), 1.54 (dq, J = 22.1, 7.5 Hz, 4H), 1.36 – 1.22 (m, 12H).

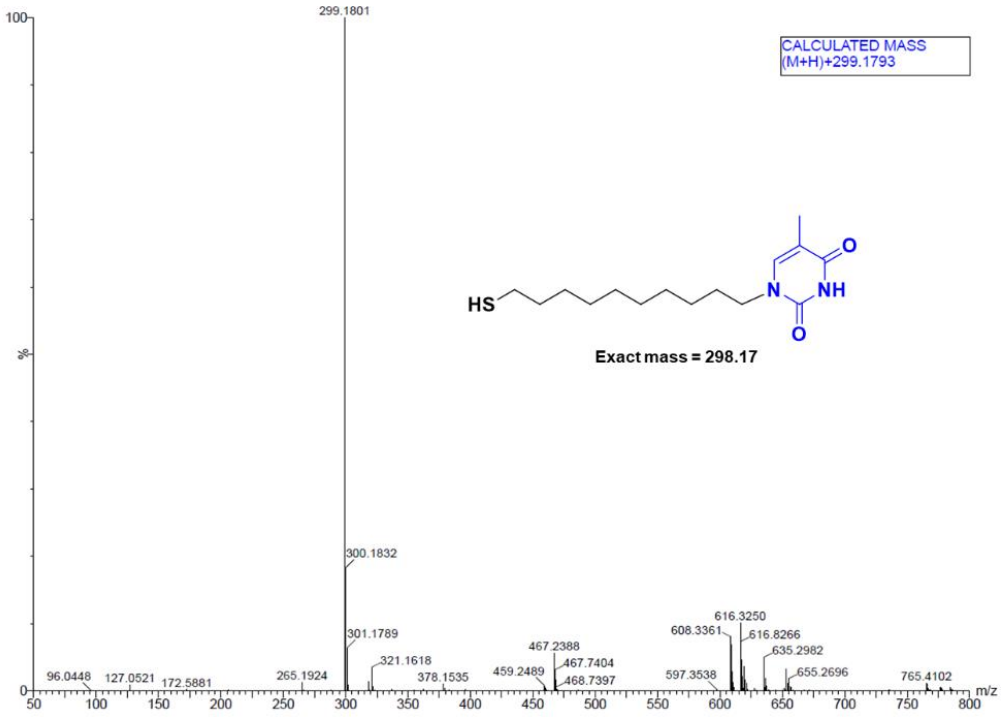

Figure 2.3. High resolution mass spectroscopy (HRMS) data of 1-(10-mercaptodecyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

2.2.3 Synthesis and characterization of C5-thymine thiol ligand: 1-(5-mercaptopentyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione

The C5-thymine thiol ligand was prepared using a similar procedure adopted for the C10-thymine thiol ligand (1,5-dibromopentane was used instead of 1,10-dibromodecane, Figure 2.4.)

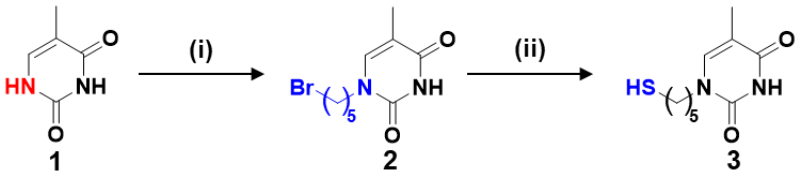

Figure 2.4. Synthesis of C5-thymine thiol, (1-(5-mercaptopentyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione) (**3**): (i) $(Me_3Si)_2NH$, Me_3SiCl, KI, CH_3CN, reflux 24 h,100 ^{0}C, N_2 atmosphere and 1,5-dibromopentane (ii) Thiourea, ethanol, reflux, 20h; NaOH (aq), reflux, 3h.

The C5-thymine thiol ligand was thoroughly characterized through ^{1}H NMR and HRMS study (Figure 2.5, 2.6).

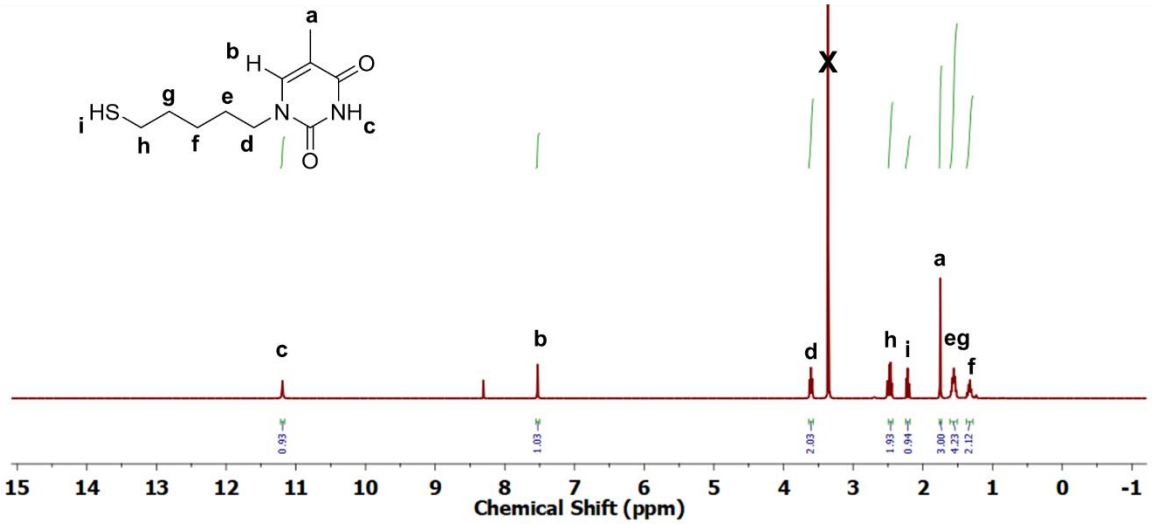

Figure 2.5. ^{1}HNMR spectrum of 1-(5-mercaptopentyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione in DMSO-d6 solvent (x corresponds to solvent peaks).

^{1}H NMR Data: ^{1}H NMR (400 MHz, DMSO) δ 11.2 (s, 1H), 7.5 (d, J = 1.2 Hz, 1H), 3.6 (m, 2H), 2.5 (q, J = 7.3 Hz, 2H), 2.2 (t, J = 7.6 Hz, 1H), 1.8 (t, J = 5.3 Hz, 3H), 1.5 (dq, J = 22.1, 7.5 Hz, 4H), 1.3 (m, 2H).

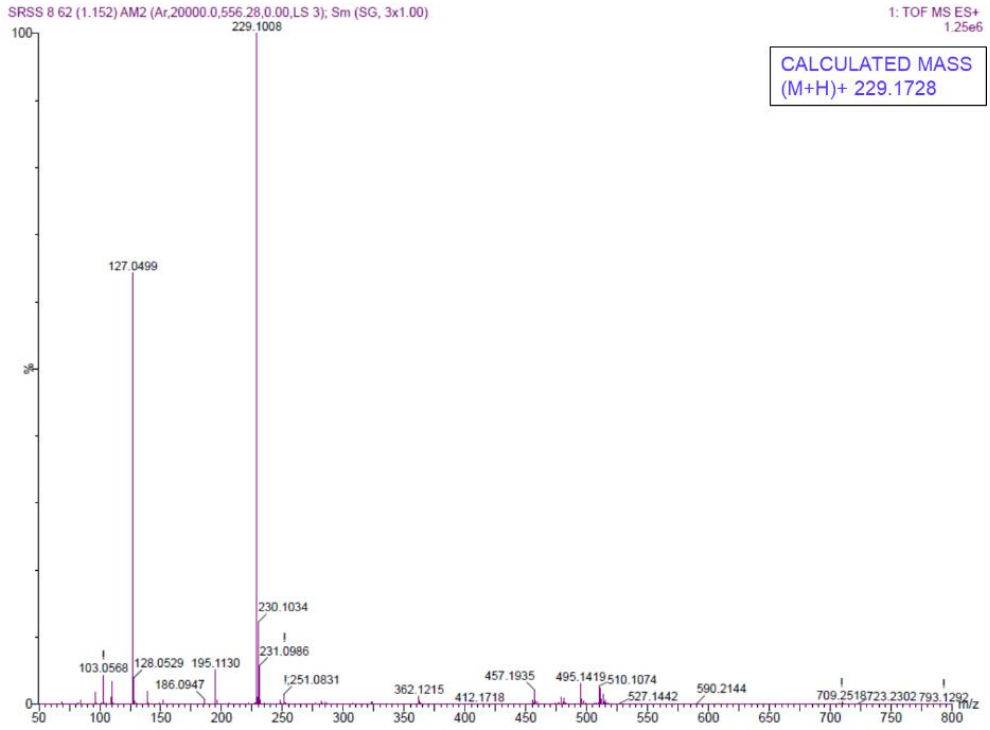

Figure 2.6. High resolution mass spectroscopy (HRMS) spectrum of 1-(5-mercaptopentyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

2.3 Synthesis of Dodecylamine (DDA) Capped AuNPs with Different Sizes

Various thiolated ligands functionalized gold nanoparticles were synthesized using a place exchange protocol.[4,5] First, dodecylamine (DDA) capped AuNPs were synthesized in toluene by a modified previous literature.[4,5]$HAuCl_4.3H_2O$ and hydrazine monohydrate ($N_2H_4.H_2O$) were used as the gold precursor and the reducing agent. In a typical experiment, $HAuCl_4.3H_2O$ (7.5 mg), DDA (71 mg), and DDAB (88 mg) were mixed together in toluene (2.1 mL) and sonicated for ~10 min. For the complete reduction of Au (III) salt, another toluene solution (1 mL) of 18.5 mg of TBAB and 35.5 mg of DDAB was rapidly injected into the solution. Then, the resulting seed solution was aged overnight. The seed particles were grown to 5.5 ± 0.2 nm DDA-AuNPs. For this, a growth solution was prepared by mixing 355 mg of DDAB, 890 mg of DDA, 75.5 mg of $HAuCl_4.3H_2O$, and 3 mL of seed solution in 19 mL toluene. Then, another toluene solution (7.5 mL) of 95 μL of $N_2H_4.H_2O$ and 500 mg of DDAB was added dropwise to reduce the growth solution. The solution was then kept overnight under stirring to complete the growth of ~5.5 nm of DDA-AuNPs (Figure 2.8, 2.9a-d).

These ~5.5 nm DDA-AuNPs were further grown to ~10 nm DDA-AuNPs. Then, a growth solution was prepared by adding 395 mg of DDAB, 795 mg of DDA, 74.7 mg of $HAuCl_4.3H_2O$, and a seed solution (2.95 mL of ~5.5 nm DDA-AuNPs) in 14 mL toluene. The growth solution was further reduced by adding another toluene solution of 80 μL of $N_2H_4.H_2O$ and 298 mg of DDAB dropwise. The solution was stirred overnight to complete the growth, yielding a monodisperse ~10.3 ± 0.7 nm of DDA-AuNPs, as confirmed through TEM analysis (Figure 2.9e-f).

2.4. Place Exchange of DDA Capped AuNPs with [+]/[-]/Thymine Thiol Ligands

In a typical synthesis of thiolated-AuNPs, 15 mL of DDA-AuNPs were first purified by precipitating them in 50 mL methanol, yielding a black precipitate. The supernatant was carefully removed, and the precipitate was then re-dispersed in 20 mL toluene. Then, a thiol ligand solution in 10 mL dichloromethane (DCM) was added. A ~40-fold molar excess of ligand was added during the place exchange. The solution was left overnight for complete ligand exchange. After that, the supernatant was decanted, and the precipitate was washed with

DCM (3×50 mL) and acetone (50 mL) to remove the excess ligand. The precipitate was then dried and redispersed in H_2O or DMSO based on the polarity of the ligand.

A representative place exchange protocol is given below, where DDA-capped AuNPs are place-exchanged with C-10 thymine thiol ligands (Figure 2.7).

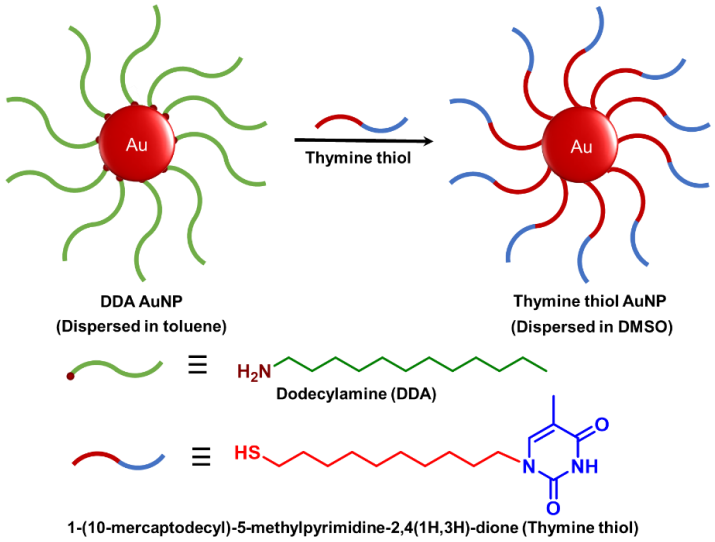

Figure 2.7. Schematics for the preparation of Thy-AuNPs. DDA-capped AuNPs were place exchanged with thymine thiol ligands to prepare AuNPs, dispersed in a DMSO medium.

2.5. Characterization of [+] and [-] AuNPs

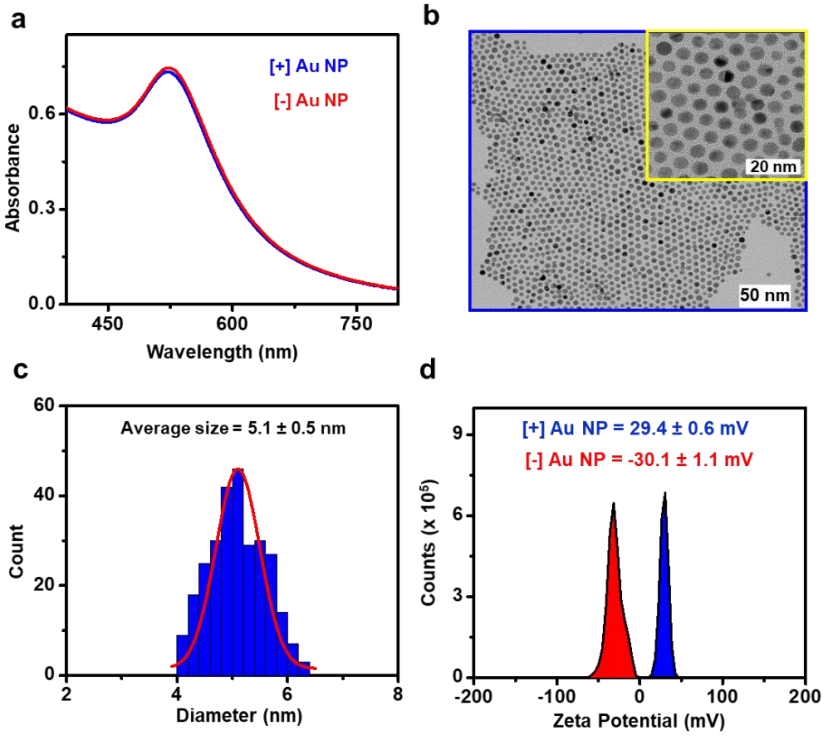

Figure 2.8. Absorption, TEM and surface characterization of AuNPs. (a) UV-vis absorption of [+] and [-] AuNPs. (b) Representative TEM image of [+] AuNPs, and (c) the corresponding size distribution histogram (from ~400 NPs). (d) Typical zeta potential plots for [+] and [-] AuNPs. The error was estimated from three independent measurements.

2.6. Characterization of ~5.5 nm and ~10.3 nm Thy-AuNPs

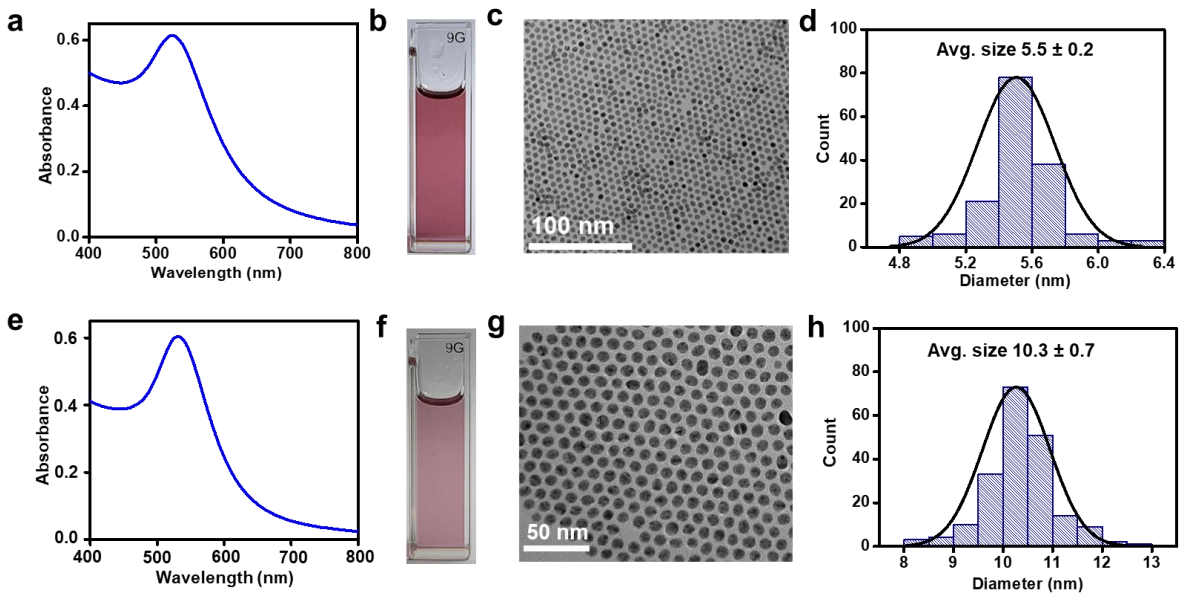

Figure 2.9. Characterization of ~5.5 nm and ~10.3 nm Thy-AuNPs. (a) and (e) are the UV-vis absorption spectra of ~5.5 nm and ~10.3 nm Thy-AuNPs, respectively. (b) and (f) are the optical photographs of ~5.5 nm and ~10.3 nm Thy-AuNPs, respectively. (c) and (g) Representative TEM images of ~5.5 nm and ~10.3 nm Thy-AuNPs, respectively. (d) and (h) are the corresponding size distribution histograms of ~5.5 nm and ~10.3 nm Thy-AuNPs, respectively. The average size was estimated to be 5.5 ± 0.2 nm and 10.3 ± 0.7 nm.

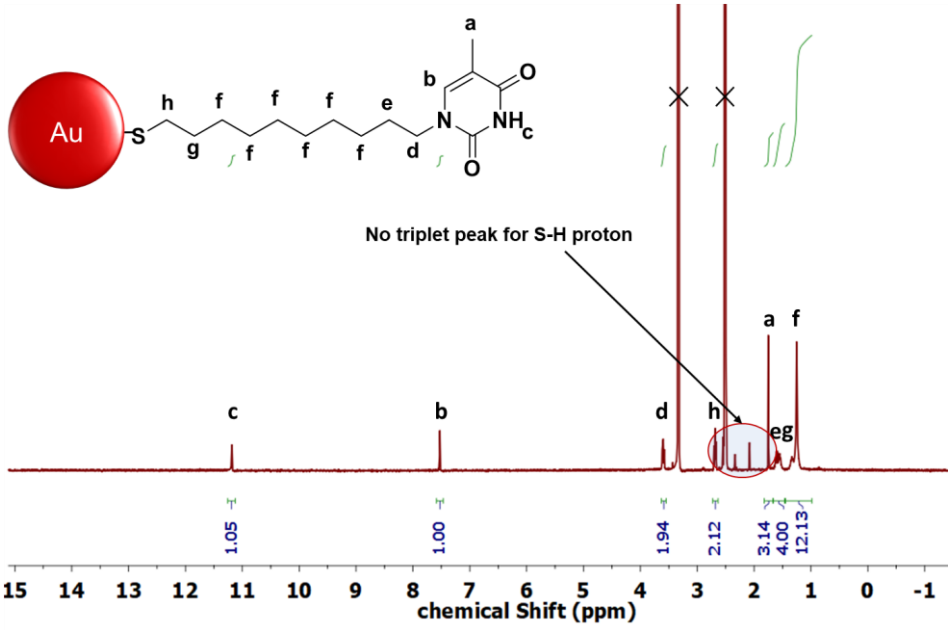

Figure 2.10. [1]HNMR spectrum of 1-(10-mercaptodecyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine thiol) functionalized AuNP in DMSO-d6 solvent (x corresponds to solvent peaks). The

absence of a triplet peak of the S-H proton (at ~2.25 ppm) confirms the functionalization of thymine ligands on the AuNP surface via the thiol end.

2.7. Synthesis and Characterization of [+]/Thy-AgNPs

AgNPs were synthesized following a slightly modified reported procedure.[4] At first, 1.72 g of decanoic acid (DA) was dissolved in 100 mL toluene. Then, 64 µL of hydrazine hydrate was dissolved by sonication for 2-3 min. After that, the solution was mixed with 1 mL of 1 mM tetrabutylammonium borohydride (TBAB). Finally, a 10 mL toluene solution of silver acetate (167 mg) and dodecylamine (DDA) (371 mg) was mixed with the already prepared solution under stirring. The color proceeds from milky white to yellow, then orange, and finally to deep reddish brown. The solution was kept for ~4 h with stirring and further for 2 days without stirring for complete growth. Finally, the toluene solution of AgNPs was filtered through a 0.2 µm syringe filter to remove the precipitate of large particles. The thiolated ligand functionalized AgNP was prepared through a place exchange reaction. In a typical synthesis, 30 mL toluene solution of AgNPs was first purified by precipitating them in 50 mL methanol. The supernatant was carefully removed, and the precipitate was then re-dispersed in 20 mL toluene. Next, the thiolated ligand solution in 10 mL dichloromethane (DCM) was added. A ~40-fold molar excess of the thiol ligand (~50 mg) was added during the place exchange. The solution was left overnight to complete the ligand exchange process. Next, the supernatant was decanted, and the precipitate was washed with DCM (3×50 mL) and acetone (50 mL) to remove all the excess ligands. The precipitate was then dried and redispersed in milli-Q water or DMSO, based on the polarity of the ligand. The AgNPs showed a surface plasmon band at ~420 nm (Figure 2.11).

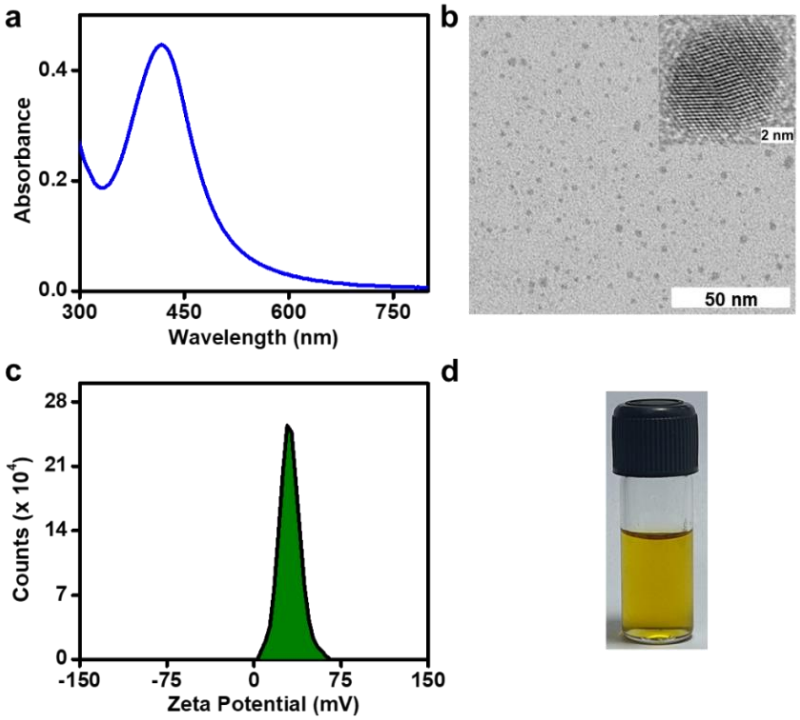

Figure 2.11. Characterization of [+] AgNP. (a) UV-visible absorption spectra of [+] AgNPs. (b) A representative TEM image of [+] AgNP. The inset shows the HRTEM image of a single [+] AgNP. (c) Zeta potential curve confirming the positive charge of the surface of AgNP. (d) An optical photograph of [+] AgNP.

2.8. Synthesis and Characterization of [+] InP/ZnS QDs

InP/ZnS quantum dots (QDs) were prepared following a reported one-pot synthesis procedure.[6] In a typical synthesis, indium acetate (0.2 mmol, 58 mg), myristic acid (0.7 mmol, 157 mg), and 6 mL octadecene were taken in a three-neck RB and heated to 110 ^{0}C, under constant N_2 flow with continuous stirring, till a clear solution was obtained. The reaction mixture was degassed for 1h at 110 ^{0}C, followed by cooling down to room temperature. Now a mixture of tris(trimethylsilyl phosphine) ($P(TMS)_3$) (0.2 mmol, 580 µL), zinc stearate (0.2 mmol, 125 mg), and 1-dodecanethiol (DDT, 0.2 mmol, 48 µL) was prepared under inert atmosphere and was injected into the reaction mixture, followed by heating at 270 ^{0}C for 1h. The color slowly changes from light yellow to dark orange with the increase in temperature, indicating the growth of QDs. When the reaction temperature reached ~230 ^{0}C, DDT started to decompose, leading to the formation of a ZnS shell. After keeping for one hour at 270 ^{0}C, the reaction was quenched rapidly using a cold-water bath, followed by the addition of 2 mL of cold toluene to arrest the growth of InP/ZnS QDs. The resultant InP/ZnS QDs were purified two times by precipitation using excess ethanol and re-dispersed back in chloroform, and used for further

studies. The water-stable [+] InP/ZnS QDs were prepared through a place exchange reaction. In a typical place exchange reaction, 5 mL of myristic acid capped InP/ZnS QD solution in chloroform (1.5 µM) was mixed with 2 mL of aqueous N,N,N-trimethyl(11-mercaptoundecyl) ammonium chloride (TMA) solution (25 mg/mL). The mixture was stirred for about 4 h, leading to a complete phase transfer of QDs from the organic to the aqueous phase. The phase transfer can be easily monitored by the color change of chloroform (yellowish orange to colorless) and water (colorless to yellowish orange) layers. The bifunctional TMA ligand helped in both QD surface functionalization (via the thiol group) as well as in the phase transfer process (via the quaternary ammonium group). The aqueous phase was carefully separated and precipitated with isopropyl alcohol (IPA) to remove the unbound TMA ligands. The precipitate was then redispersed in deionized water for further studies (Figure 2.12).

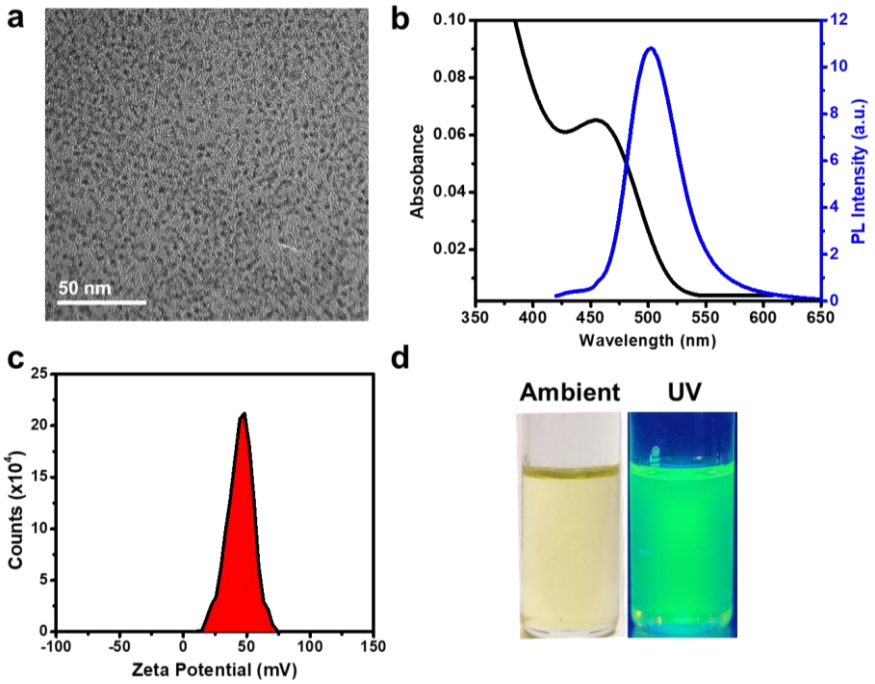

Figure 2.12. Characterization of [+] InP/ZnS QD. (a) Steady-state absorption and emission spectra of [+] InP/ZnS QDs. (b) A representative TEM image of [+] InP/ZnS QD. (c) Zeta potential curve confirming the positive charge of the surface of InP/ZnS QDs. (d) Optical photographs of [+] InP/ZnS QD solution under normal and UV irradiation.

2.9. Synthesis and Characterization of [+] AuNRs

Cetyltrimethylammonium bromide (CTAB) functionalized positively charged gold nanorods (AuNR) were prepared using a literature protocol.[7] In a typical synthesis, 250 μL of 10 mM $HAuCl_4$ was added to 10 mL of 0.1M CTAB solution and kept under stirring at room temperature. After that, 600 μL of 10 mM $NaBH_4$ was added to the solution and stirred for 5 min. Then, the solution was kept undisturbed for ~1 h for the complete formation of Au seeds. Separately, 10 mM $AgNO_3$ and 0.1M ascorbic acid were prepared. After that, in a 15 mL test tube, 10 mL of 0.1M CTAB, 500 μL of $HAuCl_4$, 100 μL of 10 mM $AgNO_3$, 200 μL 1M HCl, 80 μL of 0.1 M ascorbic acid, and 24 μL of Au seed solution were added sequentially. After that, the solution was kept in the water bath at 30 ^{0}C for 4 h for complete growth. Finally, the solution was centrifuged at 6000 rpm for 5 min to remove the excess CTAB and concentrated to 0.5 mL (Figure 2.13).

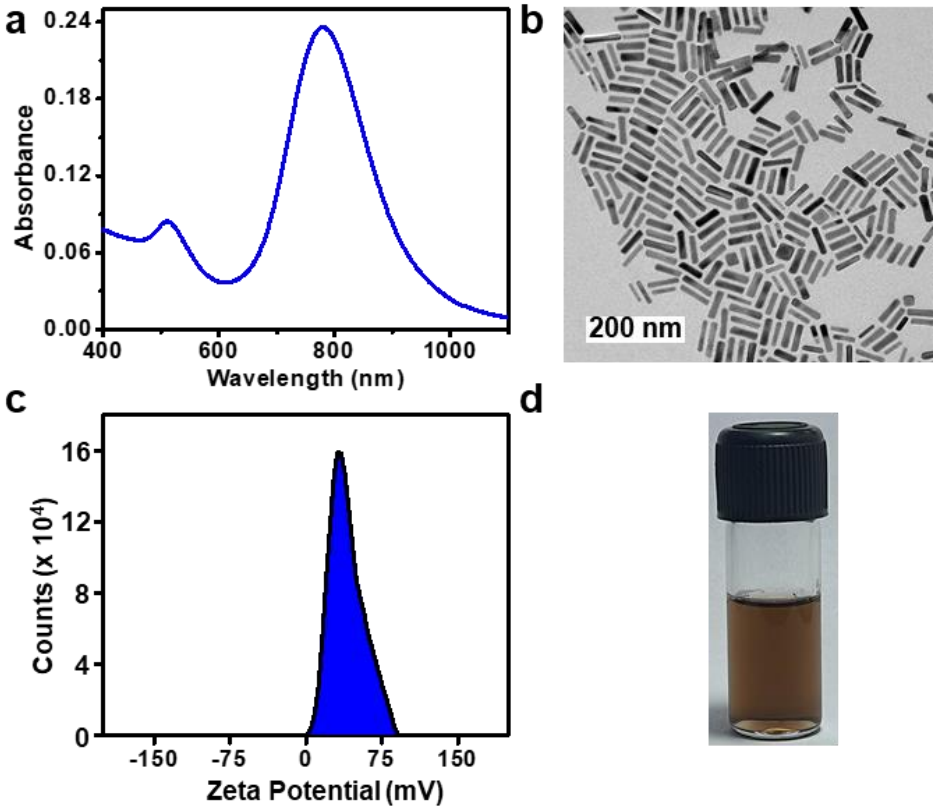

Figure 2.13. Characterization of [+] AuNR. (a) Absorption spectra and (b) representative TEM image of CTAB functionalized [+] AuNR. (c) Zeta potential curve confirming the positive charge on the surface of AuNR. (d) An optical photograph of [+] AuNR.

2.10. Microscopy Studies

Detailed microscopy (TEM, SEM, AFM, and Dark field microscopy) studies were performed in order to characterize nanoparticles, different self-assembled states such as precipitate, controlled aggregates, white ATP network, and bioplasmonic network and also to monitor the network formation and dynamic self-assembly process.

2.10.1 Scanning electron microscopy (SEM) study

SEM was carried out to image different self-assembled states. For the bioplasmonic network, a few threads were carefully lifted off on the Si wafer substrate and kept for drying. For time-dependent microscopy studies, the samples were prepared by drop-casting the reaction mixture at different stages of the network formation process. Then the drop was subsequently removed by means of a tissue paper after ~10 min so as to minimize the '*drying effect*'. The samples were dried at room temperature before imaging. Finally, FE-SEM was performed on the ZEISS Ultra Plus FESEM instrument. SEM was also used to characterize the self-assembled NP-film as well as to monitor the ATP-triggered dynamic self-assembly processes.

2.10.2 Transmission electron microscopy (TEM) study

Like SEM, TEM was utilized to characterize nanoparticles as well as to image and study different aggregated states, For the bioplasmonic network, a few threads were carefully placed on 400 mesh Formvar coated Cu grid (Tedpella Inc.) and dried before imaging. For time-dependent microscopy studies, the samples were prepared by drop-casting the reaction mixture at different stages of the network formation process. In order to minimize the '*drying effect*', the drop was subsequently removed by a tissue paper after ~10 min. After that, the samples were dried, and TEM was performed on the JEOL JEM-2200FS HRTEM instrument. TEM was also utilized to characterize AuNRs, QDs, and AgNPs as well as to study different static and dynamic self-assembled states such as controlled aggregates, precipitate, redispersed nanoparticles, etc.

2.10.3 Dark field microscopy study

A Dark Field Microscopy Study was performed to monitor the time-dependent formation of the bioplasmonic network. In a typical experiment, 10 µL [+] AuNP (~6 nM in terms of AuNPs) and 1 mM ATP were homogeneously mixed in a 3 mL 7.5 mM $CaCl_2$ solution to make the stock solution. Darkfield microscopy was performed in the liquid state. To make the samples for dark field microscopy, a few drops of the stock solution were drop cast on a cleaned

(with ethanol) glass slide. Next, a clean cover slip was placed on the solution and fixed on the glass slide with a double-sided tape for imaging. Images were captured using the Carl Zeiss microscope (model no. axioimager z1) instrument. For time-dependent microscopy studies, the samples were prepared by drop-casting the reaction mixture at different stages of the network formation process.

2.10.4 Atomic force microscopy (AFM) study

The AFM was carried out to characterize the bioplasmonic network. The sample for the bioplasmonic network was prepared by drop-casting the network on a mica substrate. The mica substrate was then left overnight at room temperature for complete drying. After that, AFM imaging was performed on Key Sight 5500 instrument (Agilent Technology) under tapping mode with a silicon nitride tip.

2.11. UV-vis Study

Extinction spectra of NP solution were recorded in SHIMADZU UV-3600 plus UV-VIS-NIR spectrophotometer using an optical quartz cuvette (10 mm path length) or optical glass cuvette (10 mm path length). The optical density of the solution in most of the studies was ~0.3 (~6 nM concentration in terms of AuNPs). The aggregation of NPs in the presence of nucleotides or antisolvent, as well as the enzyme and light-driven dynamic self-assembly of NPs, were monitored through absorption studies.

In order to measure the absorbance of the fully-grown bioplasmonic ATP network and white ATP network in the solid state, %T was recorded by placing a few threads of both networks on a quartz plate. Then, %T was converted to Absorbance as per the equation, Absorbance = 2 – log(%T).

2.12. Zeta Potential Studies

The zeta potential (ζ) of charged AuNPs was measured in milli-Q water (pH~ 7.0) on the Nano ZS90 (Malvern) Zetasizer instrument. The optical density of the nanoparticle solution was maintained at ~0.3 during all the measurements. The error was calculated from three different experiments. ζ was determined by measuring the electrophoretic mobility and using Henry's equation.[8]

$$U_E = \frac{2\epsilon\zeta f(K_a)}{3\eta}$$

U_E: Electrophoretic mobility

ζ: Zeta potential

ε: Dielectric constant

η: Viscosity

$f(K_a)$: Henry's Function

2.13. Powder X-ray Diffraction

All the powder X-ray diffractions were recorded using a Bruker D8 Advanced X-Ray Diffractometer with a Cu K_α (λ = 1.541 Å) source and processed using PDXL software. Samples for PXRD analysis were prepared by drop casting the bioplasmonic ATP network and white ATP network on a glass substrate and kept under reduced pressure at room temperature for several days for complete drying.

2.14. SERS Studies

In a typical experiment, a bioplasmonic ATP network was placed on a glass slide. Next, 1 mM 1,4-benzene dithiol (1,4-BDT) was added to the bioplasmonic ATP network and kept undisturbed for ~6h. The functionalized bioplasmonic ATP network was washed several times with ethanol to remove the unadsorbed 1,4-BDT molecules. A similar procedure was used for the white ATP network (as SERS substrate) and Nile Blue A (another Raman marker). The Raman spectra were collected using a laser source having an excitation wavelength of 632.8 nm and with an acquisition time of 10 seconds using a 50x objective at intensity I = 10%. Finally, the spectra were presented after correcting the baseline.

Raman and surface enhanced Raman scattering (SERS) studies were carried out with standard Raman marker molecules 1,4-BDT and benzyl mercaptan in a Renishaw inVia Raman microscope using a laser source having an excitation wavelength of 785 nm, and with an acquisition time of 10 s using a 50x objective at an intensity I= 10%. Finally, the spectra were presented after correcting the baseline.

2.15. Simulation Protocol: Details

In order to understand the mechanism of bioplasmonic network formation, a detailed MD simulation was performed.

ATP^{2-} molecules, whose 3D structures were constructed from SMILES[9] code using OpenBabel 3.1.0,[10] were parametrized using antechamber 19.0,[11] with partial atomic charges calculated

using Gaussian 09.[12] These parameters were put together into GROMACS[13] supported format by the use of the script acpype.py (available at https://github.com/t-/acpype). The gold nanoparticle was manually constructed by placing individual "gold" atoms as described in the manuscript.

2.15.1 System details (and setup protocol) for all cases:

All computer simulations were performed using the GROMACS 2019.4 with double precision. All simulations used explicit water (TIP4P water model[14]) to simulate local water interactions accurately.[15] In each case, the box dimensions were first determined by roughly back-calculating the size required for obtaining the required ATP concentration. Then, an empty box was filled with pure water (TIP4P) using GROMACS' *solvate* command. Then, the required number of ATP molecules (25 in the presence of Nanoparticle, 10 in its absence) were randomly placed in the box using GROMACS command *insert-molecules*, allowing replacement of overlapping solvent molecules. The system was then neutralized using Cl^- ions when the nanoparticle was present and Na^+ ions when it was absent. The AMBER99SB[16] forcefield parameters were used for ions and water. To this neutral system, the required concentration of $CaCl_2$ was added where required using GROMACS' *genion* feature. Finally, the system was energy-minimized with a force tolerance of 300 kJ/mol/nm in the presence of nanoparticles and 150 kJ/mol/nm in its absence, with an upper limit of 12500 steps.

All the simulations were performed under NVT conditions, PBCs (Periodic Boundary Conditions) were applied in all 3 directions, and a time-step of 1fs was used. No constraints were applied. A cut-off of 1 nm was used for electrostatic and van der Waals interactions. In all cases, the PME (Particle Mesh Ewald)[17] method was used to compute electrostatic interactions with order 4 (cubic interpolation) and Fourier spacing of 0.16 nm. All simulations were performed at a fixed temperature of 300K using the modified Berendsen thermostat[18] option available in GROMACS.

2.15.2 Setup with nanoparticle: A cubic simulation box with a side length of 11.9 nm was used when AuNP was present. ATP concentration was ~10 mM (scaled 10x from the experimentally used 1 mM). 25 ATP molecules were used per simulation. Various concentrations of $CaCl_2$ were used (0 mM, 5 mM, 20 mM, 40 mM,80 mM). For each concentration, 3-4 simulations were performed, lasting up to 25 ns (except in the case of 0 mM, where the aggregation reached near (95%) completion within 20 ns).

2.15.3 Setup without Nanoparticle: A smaller cubic box with a side length of 7.12 nm was used when AuNP was not present. Aggregation was found to be extremely slow in the absence of AuNP. Hence, an ATP concentration of 50 mM (instead of 10 mM) was used to obtain a 3D structure of aggregate within computationally tangible timescales. However, to calculate the timescale of aggregation for 10 mM ATP (scaled from experimental 1 mM), 3 additional simulations were performed, where the aggregate was not obtained, but the rate of aggregation (by comparing with the results from 50 mM simulations) was estimated. Simulation lengths were between 250-400 ns, but for consistency in comparisons, all trajectories were truncated at 250 ns.

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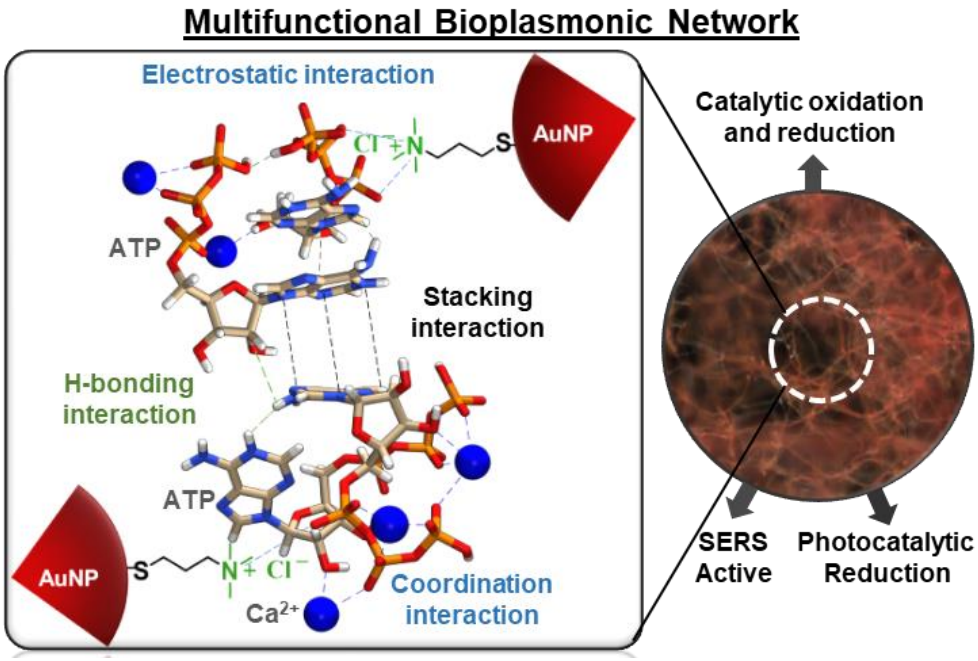
Chapter 3

Kinetically Regulated Self-Assembly between Nucleotides and Nanoparticles to Form Multifunctional Bioplasmonic Network



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Roy, S.; Adury, V. S. S.; Rao, A.; Roy, S.; Mukherjee, A.; Pillai, P. P. Electrostatically Directed Long-Range Self-Assembly of Nucleotides with Cationic Nanoparticles to Form Multifunctional Bioplasmonic Networks. *Angew. Chem., Int. Ed.* **2022**, *61*, e202203924.

3.1 Abstract

Self-assembly is undoubtedly one of the most efficient ways to create complex materials such as bio-nano hybrids. However, it is challenging to retain the inherent properties of the individual components in the hybrid state because of (i) the sensitive nature of both biomolecules and nanoparticles and (ii) the inherent complication arising from the lack of control over the bio-nano interactions. Therefore, a well-controlled self-assembly process is required to preserve the inherent properties of biomolecules and nanoparticles in a hybrid state. In this direction, we have prepared a multifunctional bioplasmonic network through long-range controlled self-assembly between positively charged gold nanoparticles ([+] AuNPs) and negatively charged adenosine 5'-triphosphate (ATP) molecules in the presence of $CaCl_2$. The present study unveils the ability of ATP to undergo a long-range self-assembly in the presence of cations and AuNPs. Conventionally, ATP is used as an aggregating trigger to initiate the self-assembly process in various organic/inorganic building blocks, whereas the formation of long-range ATP superstructures is not reported so far, to the best of our knowledge. The strong electrostatic repulsion arising from the triphosphate groups prevents the ATP from undergoing a long-range aggregation process within themselves. We have played around the same electrostatic interactions for choreographing a long-range self-assembly process in ATP molecules in the presence of [+] AuNPs. Modeling studies provided qualitative insight into the major interactions driving the bioplasmonic network formation. The presence of Ca^{2+} ions helped in screening the charges to regulate the electrostatic interaction and formed ATP-Ca^{2+} miniclusters, which were decisive in transforming an uncontrolled aggregation process into a kinetically controlled thermodynamic equilibrium state. The bioplasmonic network was found to be extremely robust under various stimuli (such as sonication, heat, etc.), as opposed to the commonly found bio-nano structures. The generality of the protocol was further demonstrated in a wide range of nucleotides and nanoparticles. More interestingly, in the absence of Ca^{2+} ions, the uncontrolled aggregation between ATP and AuNPs forms ATP-AuNP ppt., which further transforms into a bioplasmonic network, driven by the self-association property of ATP molecules. The regulation of interparticle forces helped to retain the inherent properties of ATP (biocatalysis) and AuNPs (plasmonics and catalysis) in the self-assembled bioplasmonic network. More interestingly, the bioplasmonic network showed a synergistic effect of both ATP and AuNP in the catalytic oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) molecule. In short, our work unveils a fundamentally new discovery of ATP to undergo a long-range self-assembly process, which will definitely change the perspective of chemists toward

biomolecules and encourage its use beyond the conventional role of aggregating triggers in Supramolecular Chemistry.

3.2 Introduction

An ordered arrangement of biomolecules and inorganic materials is often observed in natural systems for attaining the desired stability and functionality.[1-4] Examples of such kinds of natural structures are bone, dentine, mollusk shells, sponge spicules, etc. These robust natural composites accomplish their properties through a mind-boggling, million-year-development enhanced hierarchical assembly. For instance, in bone, self-assembled collagen triple helices are sorted out with nanocrystalline hydroxyapatite, imparting extreme robustness (Figure 3.1).[5-7] Also, a prime case of a few various types of molluscan shell material is nacre, which is composed of calcium carbonate (as calcite or aragonite) with an organic framework containing glycine and alanine-rich protein and polysaccharide.[5-8] Another class of fascinating composites can be found in the spicules of hexactinellid sponges. This adaptable bio-nano hybrid is made out of layers of hydrated silica, isolated by many slenderer organic layers.[6,9-12]

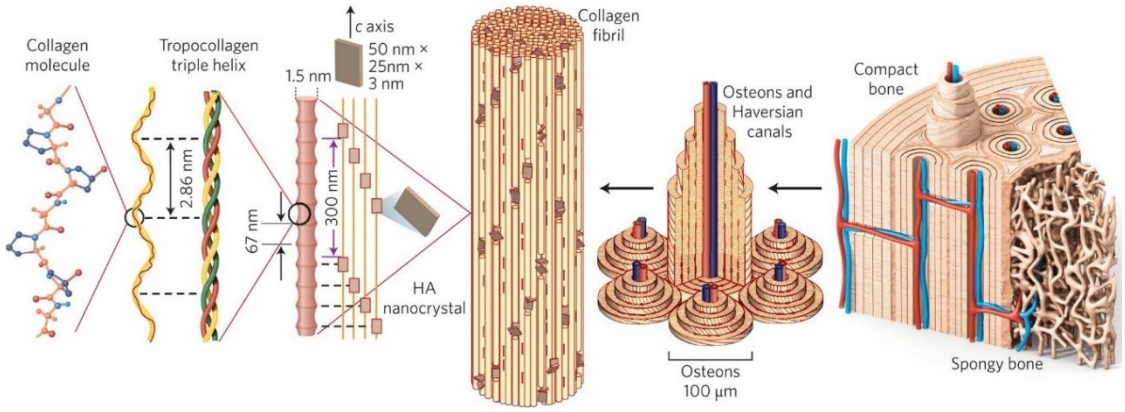

Figure 3.1. Schematic shows the macroscale arrangements in natural bio-nano hybrid, bone. Bone is made of osteons and Haversian canals, which surround blood vessels. Osteons have a lamellar structural morphology, where individual lamella consist of fibers arranged in certain patterns. The fibers are composed of several mineralized collagen fibrils, which are products of directed self-assembly between collagen triple helices and hydroxyapatite nanocrystals (HA). Reproduced with permission from ref. 6. Copyright 2015, Springer Nature.

Replication of such ordered arrangements in artificial systems is essential for the creation of complex matter with advanced properties,[8-18] and such studies lie at the intersection of biological and material sciences. Thus, bio-nano hybrid materials are the closest replicas of naturally occurring self-assembled structures.[19-27] In a self-assembly process, ideally, the functions of the individual components should be retained along with the emergence of

inherently absent properties. Some of the classic examples include the introduction of inherently absent photocatalytic properties to biological systems by forming assemblies with nanoparticles (NP),[28-30] transfer of chiral properties from biomolecules to nanomaterials,[31-36] targeted delivery and controlled release using DNA-NP hybrids,[37,38] etc. In a seminal study, King and co-workers have shown that CdS nanorod can impart and improve the inherently absent photocatalytic N_2 fixation efficiency of MoFe protein (Figure 3.2a).[28] Similarly, Kotlyar and co-workers have shown that chiroptical properties can emerge in achiral nanoparticle through chiral induction of biomolecules such as DNA (Figure 3.2b).[31] Even though there are a few successful examples, in general, even the task of retaining the inherent properties of individual components in a bio-nano hybrid is often challenging,[39] because of the (i) sensitive nature of biomolecules and nanomaterials, and (ii) lack of control over interparticle interactions in a self-assembly process.

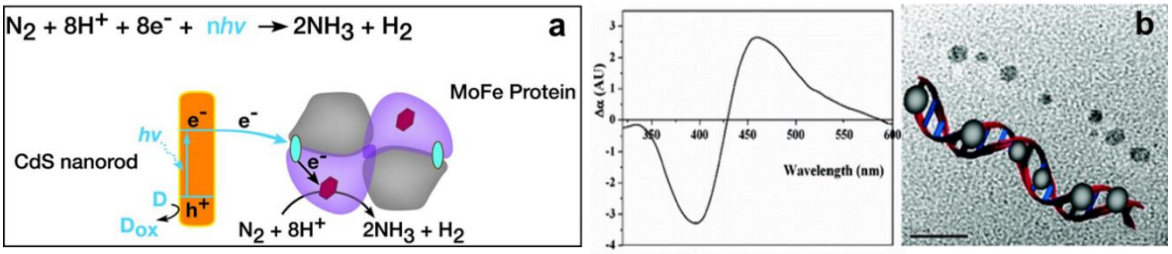

Figure 3.2. Artificial bio-nano hybrid systems. (a) Nanoparticle improves bio-property: CdS nanorod is improving the N_2 fixation efficiency of MoFe protein under light illumination. Reproduced with permission from ref. 28. Copyright 2016, American Association for the Advancement of Science. (b) Biomolecule improves nano-property: DNA molecule is inducing chirality in achiral AgNPs. Reproduced with permission from ref. 31. Copyright 2006, American Chemical Society.

The present study reports a directed and controllable self-assembly process between ATP and plasmonic AuNPs, resulting in the formation of a multifunctional bioplasmonic network. Conventionally, ATP is used as a trigger to initiate the self-assembly process in various organic/inorganic building blocks.[40-48] Even though the short-order self-association of ATP molecules has been studied in acidic pH or in the presence of metal ions,[49-52] their ability to form supramolecular structures is scarcely reported. The strong electrostatic repulsion arising from the triphosphate groups prevents its participation in a long-range self-assembly process (whereas, monophosphates such as AMP and GMP are well-known to form superstructures).[53-55] In the present work, a fine-control over the same electrostatic interaction was achieved to unveil the long-range self-assembling capability of ATP biomolecules in the presence of cationic AuNPs and divalent ions. The self-assembly process was computationally modelled

that not only supported the experimental observations, but also provided a qualitative insight into the major interactions driving the formation of ATP-based superstructures (electrostatics, π-π stacking, and H-bonding). A precise regulation of various interactions was found to be crucial in controlling the formation of ATP-based robust bioplasmonic networks. Specifically, the screening of charges by Ca^{2+} ions and the coordination between ATP-Ca^{2+} helped in regulating the strength of electrostatic attraction between oppositely charged ATP molecules and AuNPs. This turned out to be decisive in transforming an uncontrolled aggregation (instant precipitation) into a kinetically controlled self-assembly process (bioplasmonic network). Intriguingly, it was also observed that the ATP-AuNP precipitate can further transform into functional bioplasmonic network. The self-association property of ATP molecules was the driving force. The highlight was the retention of inherent properties of ATP (bio-catalysis) and AuNPs (catalysis and plasmonics) in the self-assembled superstructures. The electrostatically directed self-assembly was generalized by preparing different bio-nano networks (containing various nucleotides and nanomaterials) leading to the emergence of a novel class of multifunctional bio-plasmonic network. The present work calls for the use of biomolecules beyond their conventional role of aggregating triggers in supramolecular chemistry.

3.3 Experimental Section

The synthesis and characterization of different nanoparticles, as well as the absorption, MD simulation, and microscopy studies to monitor the bioplasmonic network formation, were already discussed in Chapter 2.

3.4 Results and Discussion

3.4.1 Formation of bioplasmonic network

It is clear from previous reports that the strong electrostatic repulsions emanating from the triphosphate groups can retard or even prevent the metal ion-triggered self-assembly process in ATP molecules.[53-55] To understand the self-assembly process better, titration of nucleotides (ATP and AMP) with salt solutions was performed following a reported procedure (Figure 3.3). [53,55] In a typical experiment, 1 mL of 1 mM nucleotide solution (AMP or ATP) was taken in a quartz cuvette and titrated with a 10 mM stock solution of $ZnCl_2$ and $CaCl_2$. The aggregation process was followed by monitoring the absorption change at 550 nm. As none of

the products are colored, the variation in the optical density at 550 nm corresponds to light scattering due to aggregation. There was a gradual increase in the absorbance at 550 nm for AMP was observed with the increasing addition of $ZnCl_2$. At ~2 mM Zn^{2+} concentration, visible turbidity appeared in the solution, confirming the formation of aggregated AMP (Figure 3.3). On the contrary, no such absorption changes (at 550 nm) or turbidity were observed in the case of ATP upon the addition of 7.5 mM $ZnCl_2$ or $CaCl_2$, which further confirms the strong electrostatic repulsion between ATP molecules arising from the tri-phosphate groups (Figure 3.3).

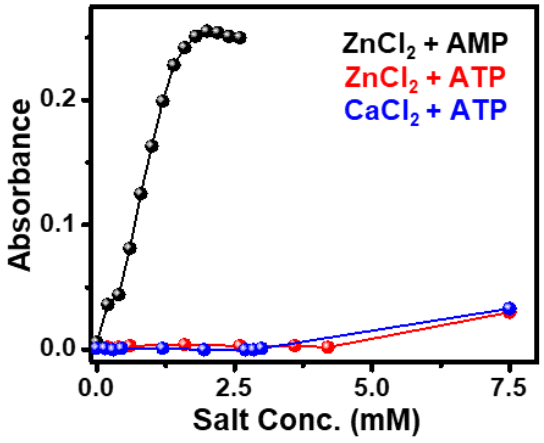

Figure 3.3. Variation in the absorbance of 1 mM nucleotide solution (AMP or ATP), monitored at 550 nm as a function of M^{2+} concentration.

We envisaged the possibility of playing around the same electrostatic interactions for choreographing a self-assembly process in ATP molecules. For this, the self-assembly of ATP molecules in the presence of various positively charged aggregating triggers was studied. AuNP with permanent positive charges (arising from the quaternary groups of N,N,N-trimethyl(11-mercaptoundecyl)ammonium chloride on the surface) was our first choice as aggregating trigger, as it can impart plasmonic properties to the ATP based self-assembled superstructures. The addition of 5.1 ± 0.5 nm cationic AuNPs (~6 nM in terms of NPs) to a 1 mM aqueous solution of ATP in a glass vial led to the instant aggregation, followed by a complete precipitation within ~8h (Figure 3.4). This confirms that the cationic AuNPs can overcome the strong electrostatic repulsions between ATP molecules, leading to an uncontrolled aggregation (the precipitate contains both AuNP and ATP; Figure 3.4).

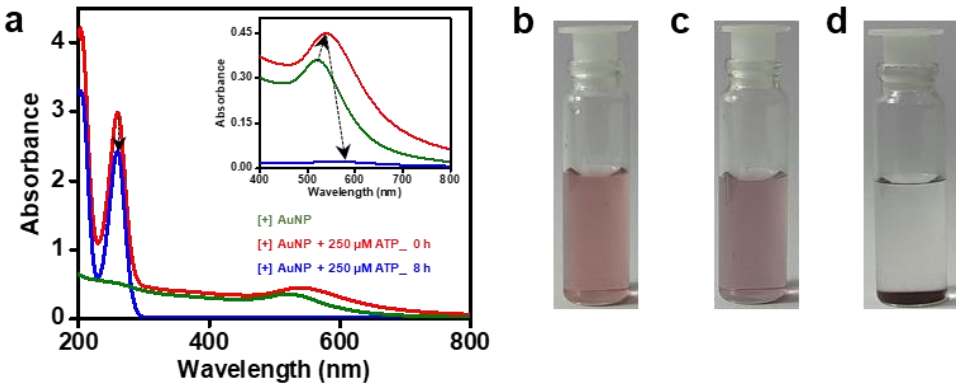

Figure 3.4. Uncontrolled aggregation of AuNP in the presence of ATP. (a) Absorption spectra depict the aggregation of cationic AuNP in the presence of 250 µM ATP. (b) Wine-red color of AuNP solution in water. (c) Instant aggregation of AuNP in the presence of 250 µM ATP leads to a bluish-purple-colored solution. (d) Complete precipitation of cationic AuNP within ~8h in the presence of 250 µM ATP.

This observation was crucial in the present study, as it indicated that a fine-tuning of electrostatic attraction between cationic AuNPs and anionic ATP molecules can result in a kinetically controlled self-assembly process. Accordingly, efforts were dedicated to decrease the strength of electrostatic attractions in ATP-AuNP bio-nano hybrids by the addition of divalent metal ions. The primary task was to find an optimum concentration of divalent ions that can screen the electrostatic attraction while retaining the prospects of a controlled self-assembly process. The addition of ~7.5 mM Ca^{2+} ions resulted in a stable ATP-AuNP bio-nano hybrid solution with minimum aggregation in the initial days, confirming the effective screening of charges. ATP-AuNP bio-nano hybrid started to aggregate from day ~4, and the formation of long-range superstructures was completed within 15 ± 2 (Std. dev. ~1.6) days (Figures 3.5, 3.28-3.31).

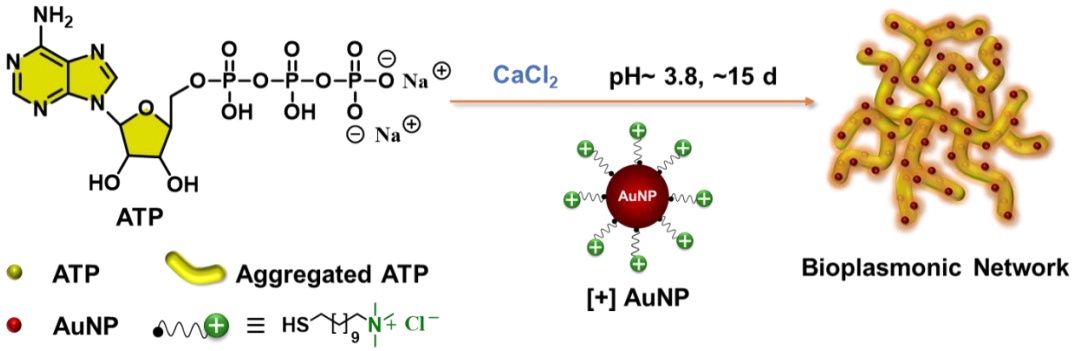

Figure 3.5. Schematics showing the kinetically controlled bioplasmonic network formation.

Impressively, the plasmonic wine-red color of AuNPs was preserved in the ATP-based bio-nano superstructures (Figure 3.6a), indicating the retention of the inherent properties of AuNPs (vide infra). HRTEM studies revealed the uniform presence of AuNPs in the ATP-based superstructures (Figure 3.6b), which was further confirmed using solid-state UV-vis absorption studies (Figure 3.6c). A shift in the plasmon band from ~519 nm to ~524 nm indicated a mild aggregation between the AuNPs.

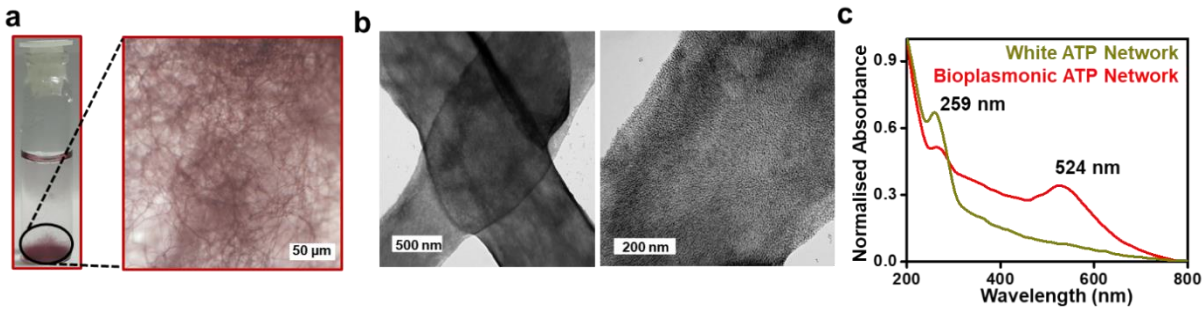

Figure 3.6. Characterization of bioplasmonic network. (a) Optical photograph of bioplasmonic network, clearly showing a wine-red color. (b) HRTEM images of bioplasmonic network at different magnifications. (c) Solid-state UV-vis. absorption spectra of white ATP and bioplasmonic network. Characteristic peaks of the ATP molecule (~259 nm) and the plasmonic band of AuNP (~524 nm) confirm the presence of an ATP-AuNP hybrid in the bioplasmonic network.

Additional analytical studies confirmed the presence of both ATP and AuNPs in the bioplasmonic network. Characteristic diffraction peaks corresponding to ATP and AuNPs were observed in powder X-ray diffraction studies (Figure 3.32 in Annexure). A broad diffraction peak, at 2θ ~23^{o}, confirms the stacking of ATP molecules in the self-assembled superstructures.[56] The presence of signature peaks of ɣ-PO_3 and adenine moieties in FTIR and Raman spectra (Figures 3.33, 3.34) confirm that ATP is intact in the self-assembled network.[57,58] Similar ATP-AuNP bio-nano superstructures were formed in the presence of other metal ions as well, even though the kinetics were different (Table 3.1).

Table 3.1. Bioplasmonic network formation in the presence of different M^{2+} ions

Composition	Required Days	Final Structure
1 mM ATP + 15 mM NaCl + 6 nM [+] AuNP	~30± 4	Bioplasmonic network
1 mM ATP + 7.5 mM $CaCl_2$ + 6 nM [+] AuNP	~15 ± 2	Bioplasmonic network
1 mM ATP + 7.5 mM $MgCl_2$ + 6 nM [+] AuNP	~13 ± 1	Bioplasmonic network

1 mM ATP + 2.5 mM $AlCl_3$ + 6 nM [+] AuNP	~35± 3	Bioplasmonic network

In a separate experiment, it was observed that ~7.5 mM Ca^{2+} ions could itself trigger the self-assembly process in ATP molecules (Figure 3.7), even though the kinetics was much slower compared to cationic AuNP triggered ATP-aggregation ($t_{Ca^{2+}}$ 25 ± 3 (Std. dev. ~2.2) days and $t_{(AuNP + Ca^{2+})}$ 15 ± 2 (Std. dev. ~1.6) days). ATP molecules were self-assembled to form a well-organized one-dimensional white network in the presence of Ca^{2+} ions, which turned to a wine-red color upon the addition of cationic AuNPs (post-functionalization, Figure 3.7b-d). Such divalent cation-triggered long-range self-assembly process in ATP molecules itself is unprecedented in literature. In another control experiment performed to prove the role of electrostatics, the anionic AuNPs (arising from the deprotonated 11-mercaptoundecanoic acid ligand on the NP surface) failed to interact with ATP molecules both under in-situ and post-functionalization conditions (Figures 3.7b, d).

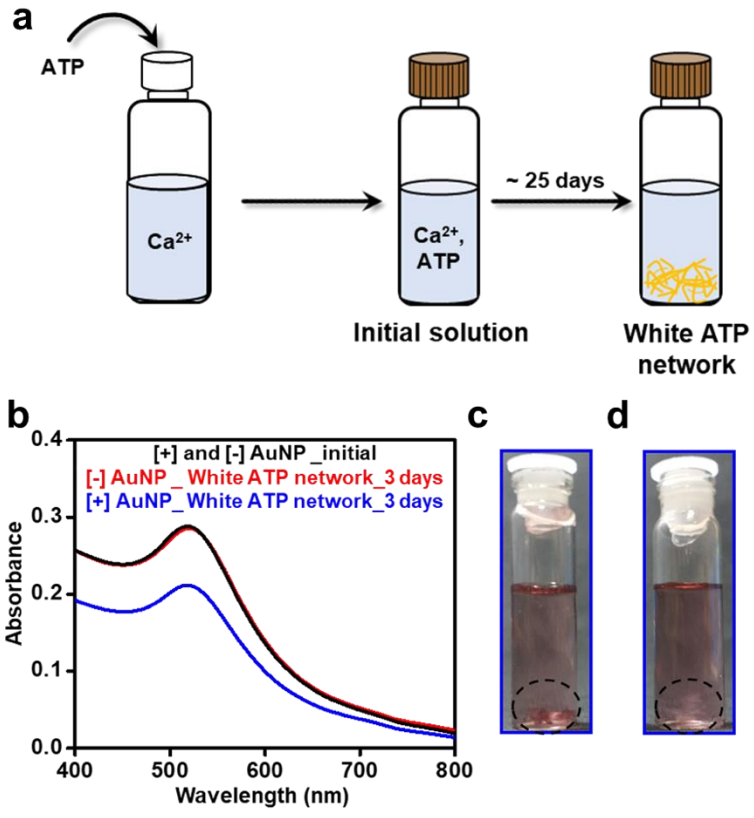

Figure 3.7. Preparation and post-functionalization of white ATP network. (a) Schematic representation for the synthetic protocol of white ATP network. 1 mM ATP was added to 7.5 mM $CaCl_2$ solution and kept undisturbed for ~25 days to complete the formation of a white ATP network. (b) Post-functionalization of white ATP network. UV-vis absorption of [+] and [-] AuNPs in the presence and absence of white ATP network. Optical photographs of vial containing white ATP networks in the presence of (c) [+] and (d) [-] AuNPs. A prominent wine-red color on the white ATP network was only

observed in the case of [+] AuNP (black circle), confirming the successful adsorption of [+] AuNPs on the ATP network.

All these experiments conclusively prove the necessity of finely-tuned electrostatic interactions in directing the formation of bioplasmonic networks. The bioplasmonic network could be prepared in bulk as well, demonstrating the scalability of the self-assembly protocol (Figure 3.8).

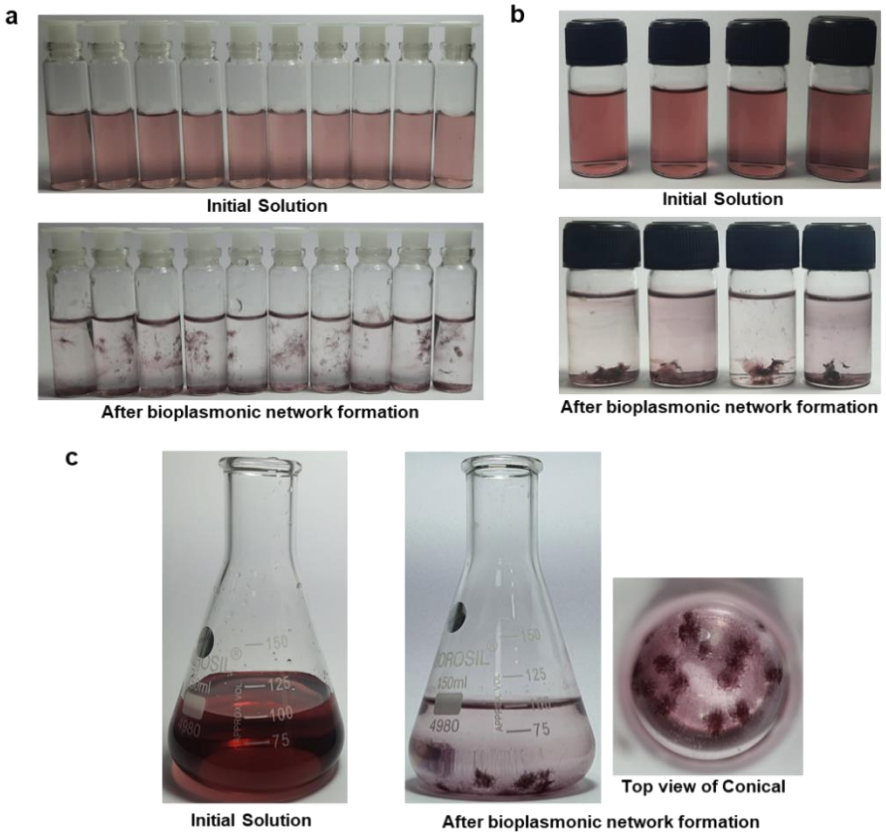

Figure 3.8. Bulk synthesis of bioplasmonic network. (a) Photographs showing the formation of the bioplasmonic network in (a) 3 mL, (b) 12 mL, and (c) 100 mL solutions. The amounts of ATP, Ca^{2+}, and [+] AuNPs were scaled up as per the volume of the solution.

3.4.2. Mechanistic understanding of bioplasmonic network formation

Detailed experimental and molecular modeling studies were performed to gain a better insight into the mechanism involved in the formation of a bioplasmonic network. First, the process of self-assembly in ATP molecules was monitored using time-dependent absorption and microscopy studies. The working solution contained 1 mM ATP molecules, ~6 nM [+] AuNP, and 7.5 mM $CaCl_2$ (pH of the mixture was ~3.8). The time-dependent variation in the surface plasmon band of AuNPs (~519 nm) was monitored to study the progress of the self-assembly

process (all the UV-vis. studies were performed in a glass cuvette to monitor the absorption spectra without disturbing the system). The plasmon color of the supernatant constantly decreased as a function of the self-assembly process, along with a slight bathochromic shift of ~5 nm. The kinetic plots shown in Figures 3.9a and b reveals the presence of three distinct stages in the formation of a bioplasmonic network. In the first stage, no noticeable aggregation between ATP and cationic AuNPs was observed for ~16 days because of the high ionic strength (~2.55 x 10^{-2} M) created by 7.5 mM Ca^{2+} ions (labeled as the '*lag phase*'). The intensity of the plasmon band in the supernatant continuously decreased from day ~17, indicating the onset of aggregation between ATP and AuNPs. This stage corresponds to the '*elongation phase*' that lasted till ~25 days, finally leading to the long-range self-assembled bioplasmonic network. No appreciable spectral changes were observed after ~25 days, which corresponds to the '*saturation phase*' (it is worth recollecting that the self-assembly process in a glass vial was over in 15±2 days. It is well known that the kinetics of the self-assembly process strongly depends on the nature of surfaces, such as roughness, corners, and edges[59]). A similar trend in the kinetics of the self-assembly process was observed when the absorption corresponding to ATP was monitored as a function of time (Figure 3.35). Further, pH-dependent aggregation studies were performed to ascertain the active role of electrostatic interactions in the self-assembly process. For this, the pH of the reaction mixture (1 mM ATP + 6 nM AuNP + 7.5 mM Ca^{2+} in glass cuvette) was increased from ~3.8 to ~7. The rationale being that the complete neutralization of the positive charge on the adenosine-N1 atom in ATP will favor stronger electrostatic attraction at pH ~7[52] as compared to pH ~3.8. To our delight, the kinetics of the self-assembly process at pH ~7 was much faster than at pH ~3.8, and the bioplasmonic network formation was completed within 10 days (Figure 3.9b). Interestingly, there was no '*lag phase'* at pH ~7 (Figure 3.9b), and an instant aggregation process was observed between oppositely charged ATP and AuNPs. Thus, the disappearance of the lag phase and completion of bioplasmonic network formation in a shorter time, compared to the white ATP network, clearly confirms that AuNP is actively participating in the network formation (8±1 vs 15±2 days for bioplasmonic and white ATP network formations, respectively).

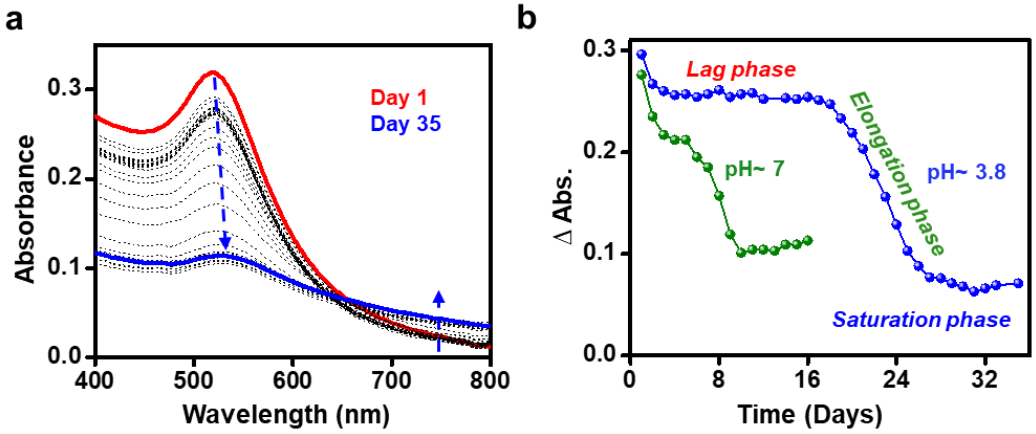

Figure 3.9. Kinetic study of bioplasmonic network formation. (a) Time-dependent absorption spectra showing a continuous decrease in the absorption peak of AuNPs from day ~17 of the self-assembly process at pH ~3.8. The increase in the absorbance ~750 nm confirms the aggregation process. (b) The corresponding kinetic plot showing the variation in the absorption of AuNP, which can be labeled into three stages: '*lag phase'*, '*elongation phase'*, and *saturation phase*. Comparison of kinetic plots for the self-assembly between ATP and AuNP, in the presence of Ca^{2+} ion, at pH ~3.8 and ~7.0.

To understand the kinetic process involved in the bioplasmonic network formation, the absorbance values at ~750 nm (scattering) were monitored with respect to time. The kinetic data fit well with the Finke-Watzky (F-W) model,[60] which is generally used to study nucleation and agglomeration processes in neurological protein aggregation, transition metal nanoclusters formation, solid-state photochemical transformation, organometallic catalyst formation, etc[60]. F-W model is a 2-step process comprised of (i) slow continuous nucleation A→B (rate constant = k_n), followed by (ii) fast autocatalytic surface growth A + B→2B (rate constant = k_e) (Figure 3.10). Here, 'A' denotes the monomeric form of the '*ready-to-aggregate*' ATP, AuNP, and Ca^{2+} ions in the working solution, while B represents the concentration of aggregated species (scattering values at 750 nm, which confirms the formation of different sizes of aggregates). Initiation of the assembling process increases the surface area of aggregated species B, which further accelerates the self-assembly process, yielding higher 'n' polymeric species as $A+B_n \rightarrow B_{n+1}$ (here, bioplasmonic network). The fitting using the F-W model allowed us to gain quantitative information on various kinetic parameters such as nucleation rate constant (k_n), elongation rate constant (k_e), lag phase, inflection point, etc. The equation used for the kinetic analysis[60] is given below:

$$B = \frac{A_0 - \dfrac{\dfrac{k_n + k_e A_0}{k_e}}{1 + \dfrac{k_n}{k_e A_0}\exp\left(k_n + k_e A_0\right)}}{A_0}$$

where,

A_0 = Total concentration of monomeric species (ATP + [+] AuNP + Ca^{2+} ions)

B = Total concentration of aggregated species (scattering values at 750 nm, which confirms the formation of different sizes of aggregates)

Important rate constants and other parameters obtained from the *F-W fitting*,

Nucleation rate constant, $k_n = 3.33 \times 10^{-10}$ day^{-1} {Obtained from the *sigmoidal fitting*}

Elongation rate constant, $k_e = 1061.46$ $M^{-1}day^{-1}$

Maximum rate (v_{max}) = $\left(\frac{dB_t}{dt}\right)_{max} = 0.048$ day^{-1}

Inflection point, $t_m = \frac{\ln(\frac{k_e A_0}{k_n})}{k_e A_0} = 20.63$ days

Lag phase (t_{lag}/ $t_{induction}$) = $t_m - \frac{2}{k_e A_0} = 18.41$ days

Half-time, $t_{50} = \frac{A_0}{2} = 20.56$ days

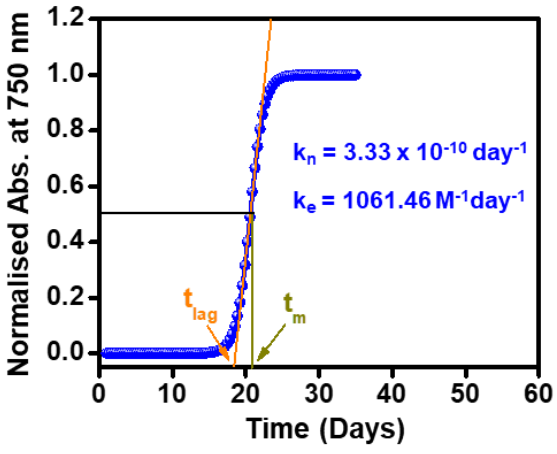

Figure 3.10. Time-dependent variation in the scattering intensity (750 nm) was fit with the Finke-Watzky (F-W) model to obtain various kinetic parameters such as nucleation K_n & elongation K_e rate constants, lag phase t_{lag}, and inflection point t_m.

Next, time-dependent TEM, SEM and dark-field microscopy studies were performed to visualize the self-assembly process between ATP and cationic AuNP. The presence of small ATP-AuNP aggregates (100-500 nm) was observed in the initial days (stage-1, 2-4 days) of the self-assembly process, which kept on aggregating to form small nanotubes (stage-2, 6-8 days), and finally to long inter-twined plasmonic nanowires extending up to hundreds of micrometers (9-17 days, stages-3 and 4 in Figure 3.11).

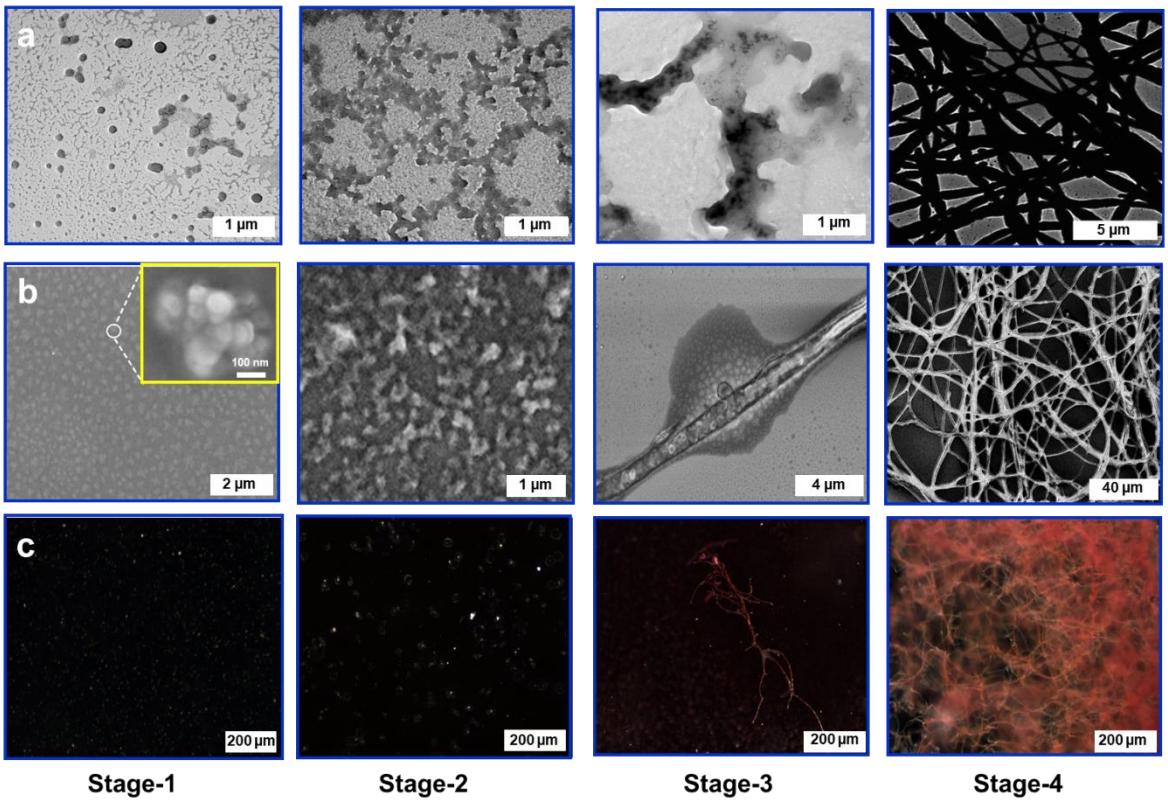

Figure 3.11. Time-dependent (a) HRTEM, (b) SEM, and (c) dark field microscopy images showing the temporal formation of the bioplasmonic network.

3.4.3 Molecular picture

Multiple all-atom, explicit water molecular dynamics simulations were performed to obtain a molecular-level understanding of the kinetics and the nature of bioplasmonic network formation. Computational modeling of the self-assembly process between ATP and AuNPs, at a quantitative level, was challenging primarily because of the large system and timescales of the process. Hence, the concentrations of all the components were scaled by ten times for modeling the self-assembling process, which enabled us to study the aggregation within nanoseconds. To understand the timescale of the aggregation, we have performed three

simulations for each case, starting from a random distribution of the ATP and ions in the simulation box. When AuNP was present, we computed the fraction of aggregated ATP at each time point ($n_a(t)$) of the simulation by counting the fraction of the total number of ATP molecules within a cut-off (4 nm) from the AuNP. The rate of aggregation was determined from the exponential fit of $n_a(t)$. This also gave an idea of the timescale required for the 100% aggregation process (approximately 3 times of the time constant). Figure 3.12a shows the fraction of ATP aggregation with time in the presence and absence of AuNP at various $CaCl_2$ concentrations. As aggregation increases with time, the complementary function of non-aggregated function (1- $n_a(t)$) was fitted with an exponential function, $\exp[(t-t_0)/\tau]$, to obtain the time constant τ. Figure 3.12a shows the resulting exponential fit for different systems: ATP + AuNP, ATP + AuNP + varying concentrations of Ca^{2+} ions (5–80 mM), and ATP + Ca^{2+} ions (80 mM).

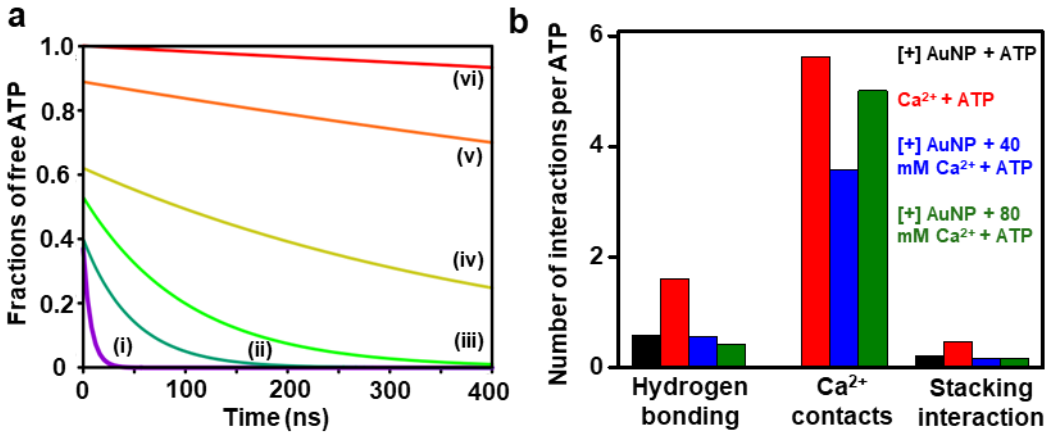

Figure 3.12. Molecular modeling studies for the formation of a bioplasmonic network. (a) Timescales of all different cases (i) ATP + AuNP (No Ca^{2+}), (ii) ATP + AuNP + 5 mM Ca^{2+}, (iii) ATP + AuNP + 20 mM Ca^{2+}, (iv) ATP + AuNP + 40 mM Ca^{2+}, (v) ATP + AuNP + 80 mM Ca^{2+}, and (vi) ATP + 80 mM Ca^{2+} (No AuNP), plotted together to compare the rate of aggregation. (b) Bar chart showing the relative frequency of different stabilizing interactions.

As found by experiments, the rate of aggregation followed the trend ATP + AuNP > ATP + AuNP + Ca^{2+} ions > ATP + Ca^{2+} ions. A semi-quantitative knowledge of the structure of the aggregates and different interactions participating in the assembling process was achieved as well. Specifically, the average number of hydrogen bonding, π-π stacking, and Ca^{2+}-ATP^{2-} coordination (per ATP molecule) contributing to a stable aggregate were computed (Figure 3.13). The average number of Ca^{2+} ions interacting with ATP molecules in aggregates decreases in the presence of AuNPs, showing that a part of Ca^{2+}-ATP coordination is now replaced by AuNP-ATP interactions (Figure 3.12b). Noticeably, the cationic AuNP provides a

strong motive force to form the final aggregate by offering multiple interaction points for ATP molecules. Thus, the strong electrostatic attraction between ATP and AuNP compensates for the loss of other favorable interactions during the assembling process. The snapshots, along with the simulation trajectories, during the bioplasmonic network formation, show the formation of small coordination polymers of ATP molecules and Ca^{2+} ions (Figure 3.14). These sometimes interact with each other to form larger coordination polymers or occasionally break off as well. The Ca^{2+} ions interacting with the ATP molecules provide a dragging effect, reducing the effective rate of migration of ATP molecules towards the AuNPs by many orders of magnitude (50-100 times slower). A greater retardation of aggregation rate was observed upon increasing the concentration of Ca^{2+} in the system (Figure 3.12a).

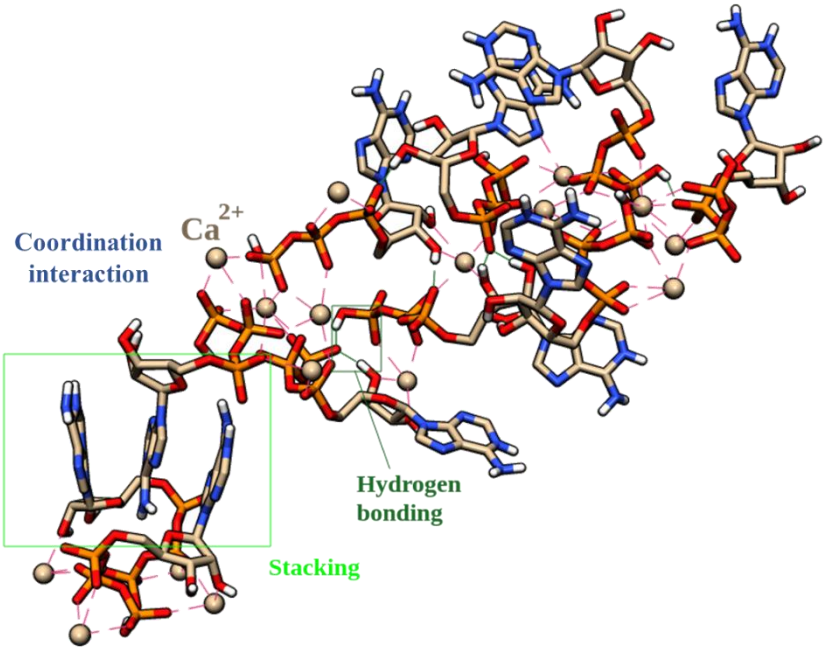

Figure 3.13. 3D structure of the aggregate obtained with only ATP and $CaCl_2$ in solution, and pictorial description of key interactions.

The timescales of analysis (Figures 3.12a) clearly reproduce the uncontrolled aggregation between ATP and AuNP in the absence of Ca^{2+} ions. A key insight from the interaction data obtained is that the organized structure of the bioplasmonic network in the final aggregate (Figure 3.12a) is a result of interaction with the Ca^{2+} in solution, rather than the inter-ATP interactions such as hydrogen-bonding or stacking. Thus, simulations show that the ATP molecules organize themselves around the Ca^{2+} ions first, which then interact with [+] AuNPs

to form the final network. The projected rate of aggregation for ATP in the presence of both NP and Ca^{2+} is much higher than in the absence of AuNP.

Based on the molecular modeling and time-dependent experimental studies, a plausible mechanism for the formation of an ATP-based bioplasmonic network was deduced (Figure 3.14). The role of Ca^{2+} ions was crucial in the formation of the bioplasmonic network. Both screening of charges and ATP-Ca^{2+} coordination led to a kinetically controlled electrostatically directed self-assembly process between ATP molecules and cationic AuNPs, resulting in the formation of small aggregates. Other non-covalent interactions, such as H-bonding and π-π stacking too assisted in the subsequent self-assembly process, finally leading to the bioplasmonic network formation.

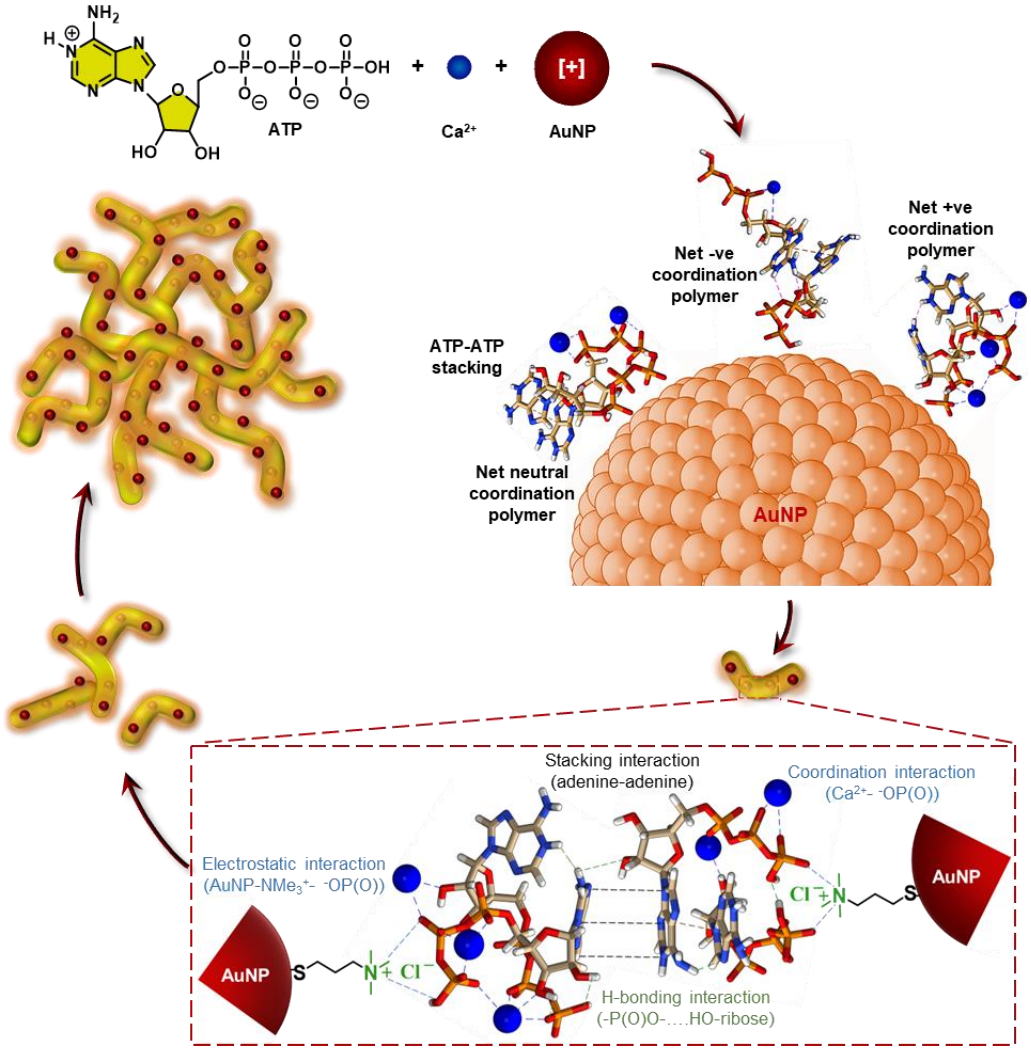

Figure 3.14. Schematics of the plausible mechanism for bioplasmonic network formation. 3D structure obtained near AuNP after ~25 ns simulation with 40 mM $CaCl_2$. ATP-Ca^{2+} coordination polymers electrostatically bound on the surface of AuNPs, thereby triggering the self-assembly process. Next,

other non-covalent interactions such as H-bonding and π-π stacking assist in the propagation of the self-assembly process, finally leading to the formation of long inter-twined bioplasmonic nanowires.

3.4.4 ATP-AuNP precipitate to bioplasmonic network

From the previous study, it was quite evident that in the absence of Ca^{2+} ions, [+] AuNPs immediately undergo an uncontrolled aggregation followed by complete precipitation upon addition of ATP molecules. Interestingly, the plasmonically inactive black precipitate was found to transform into plasmonically active aggregates, after 35 days (Figure 3.15). We believe that the plasmonically inactive black precipitate may be in a kinetically trapped state, whereas the bioplasmonic network is in a thermodynamically stable state.

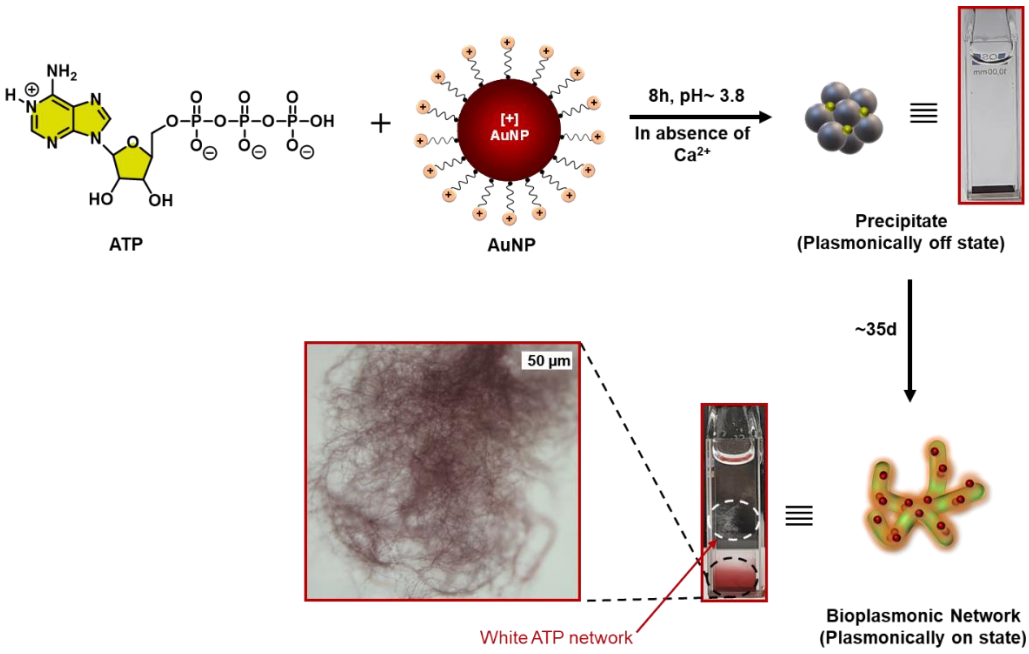

Figure 3.15. Schematics and optical photographs of the cuvettes showing the transformation of plasmonically inactive ATP-AuNP precipitate into the bioplasmonic network.

To better understand this observation, a series of controlled experiments and detailed microscopy (TEM) studies have been performed. All these experiments revealed that dispersed AuNPs in the presence of ATP undergo uncontrolled aggregation, leading to the formation of a kinetically trapped precipitate state (stage 2) (Shown in Figure 3.16). However, with time, the excess ATP present in the supernatant solution undergoes a self-association process to form white ATP fibers (as shown in stage 3 of Figure 3.16). Slowly, the precipitate state got broken

and AuNPs started post-functionalized over the ATP fibers, leading to the formation of a bioplasmonic network (stage 4 of Figure 3.16).

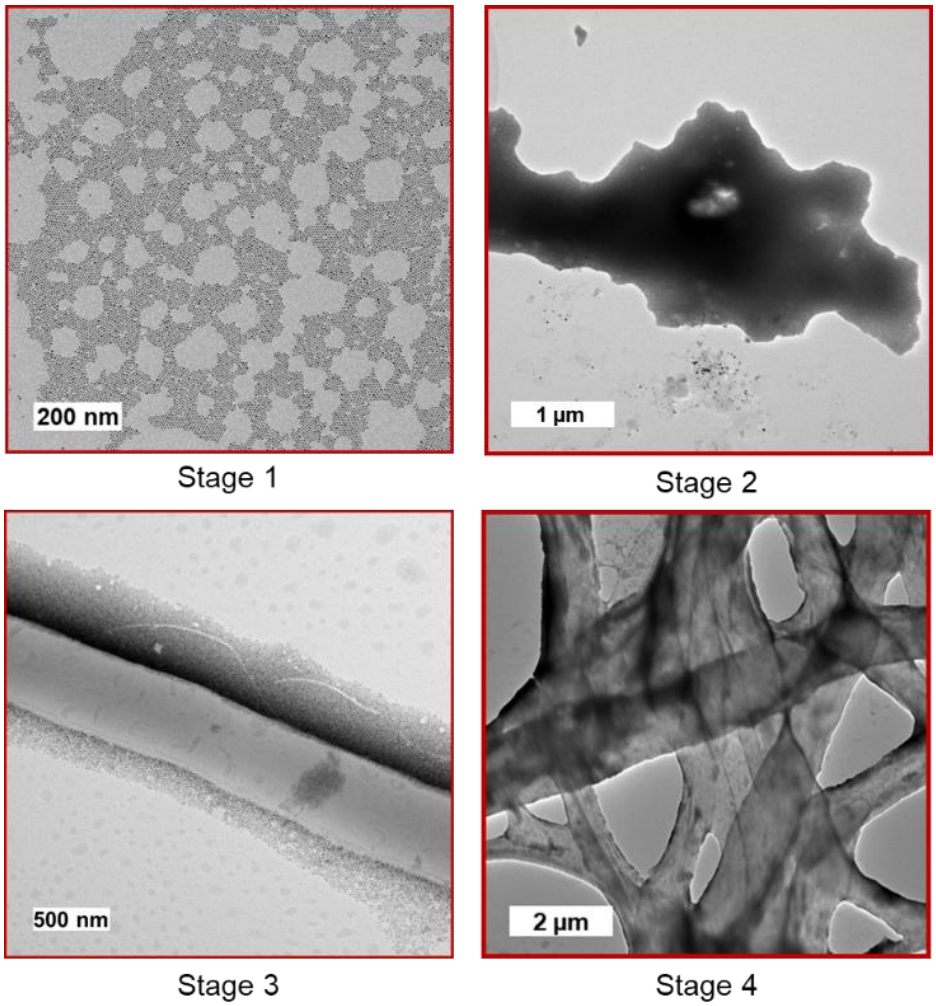

Figure 3.16. Time-dependent TEM images show the transformation of kinetically trapped precipitate (stage 2) into a bioplasmonic network (stage 4).

To study the self-association of ATP molecules in the supernatant solution, we have performed a time-dependent ^{1}H NMR experiment. we observed that the chemical shift values for H-2 and H-8 protons were in the up-field region (from 8.53 to 8.52 ppm and from 8.32 to 8.29 ppm in 37 days for H-2 and H-8 protons, respectively; Figure 3.17). These changes in chemical shift values ($\Delta\delta$ = 0.01 and 0.03 ppm for H-2 and H-8 protons, respectively) are comparable with the previous report[17], which indicates that ATP molecules undergo π-π stacking during the formation of a bioplasmonic network.

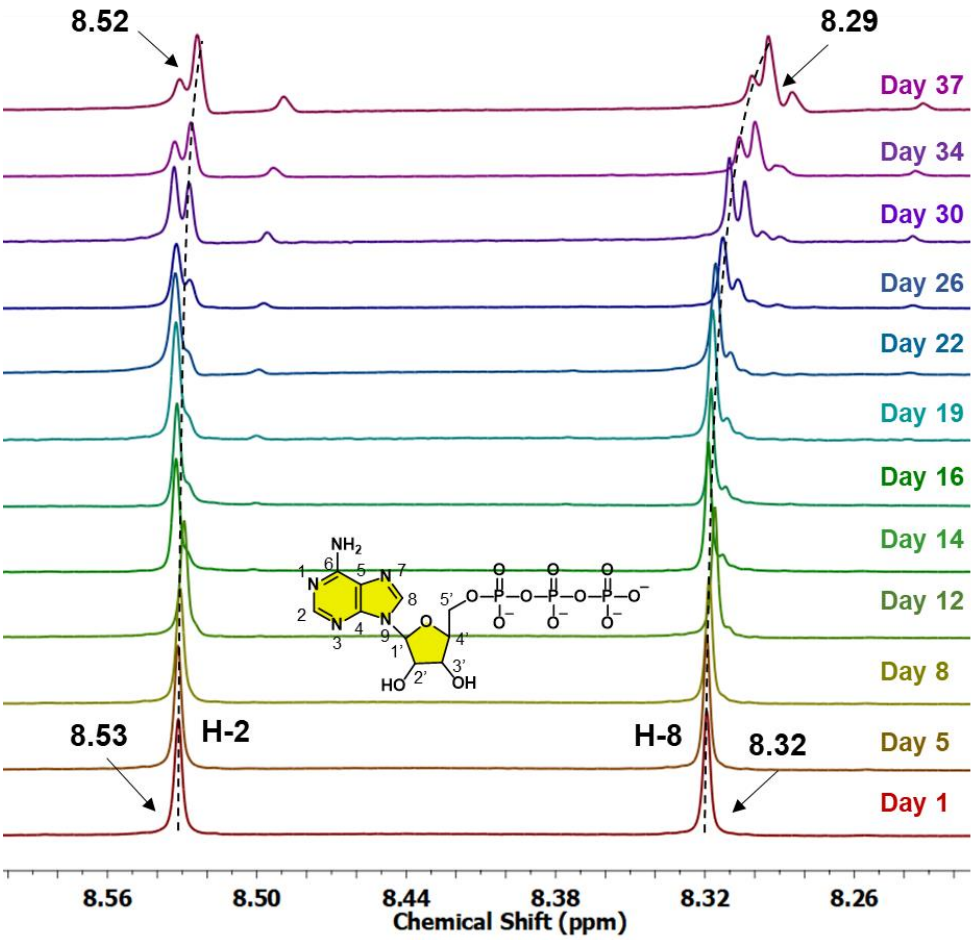

Figure 3.17. Time-dependent ^{1}H NMR study shows self-association of ATP molecules.

In order to obtain deeper insights, we have examined the intermediate aggregated state (on day 20) through 2D NMR studies. π-π stacking during the aggregation in ATP molecules can result in interactions between different sets of protons. Some of the important interacting proton pairs include H-2↔H-1' and H-2↔H-8. We too observed a strong NOEs of H-2↔H-1' and H-2↔H-8 in the corresponding ROESY spectra (Figure 3.18). This conclusively proved that the ATP molecules undergo π-π stacking interaction through head-to-tail arrangement of the adenine moieties. In this head-to-tail stacked arrangement, there can be H-bonding between (i) the phosphate groups and the protons of the adenine residue, and (ii) the ring nitrogen and the protons of the adenine residue. All these interactions, collectively, are responsible for the NOEs observed of H-2↔H-1' and H-2↔H-8. This also gives a directional nature during the self-assembly process.

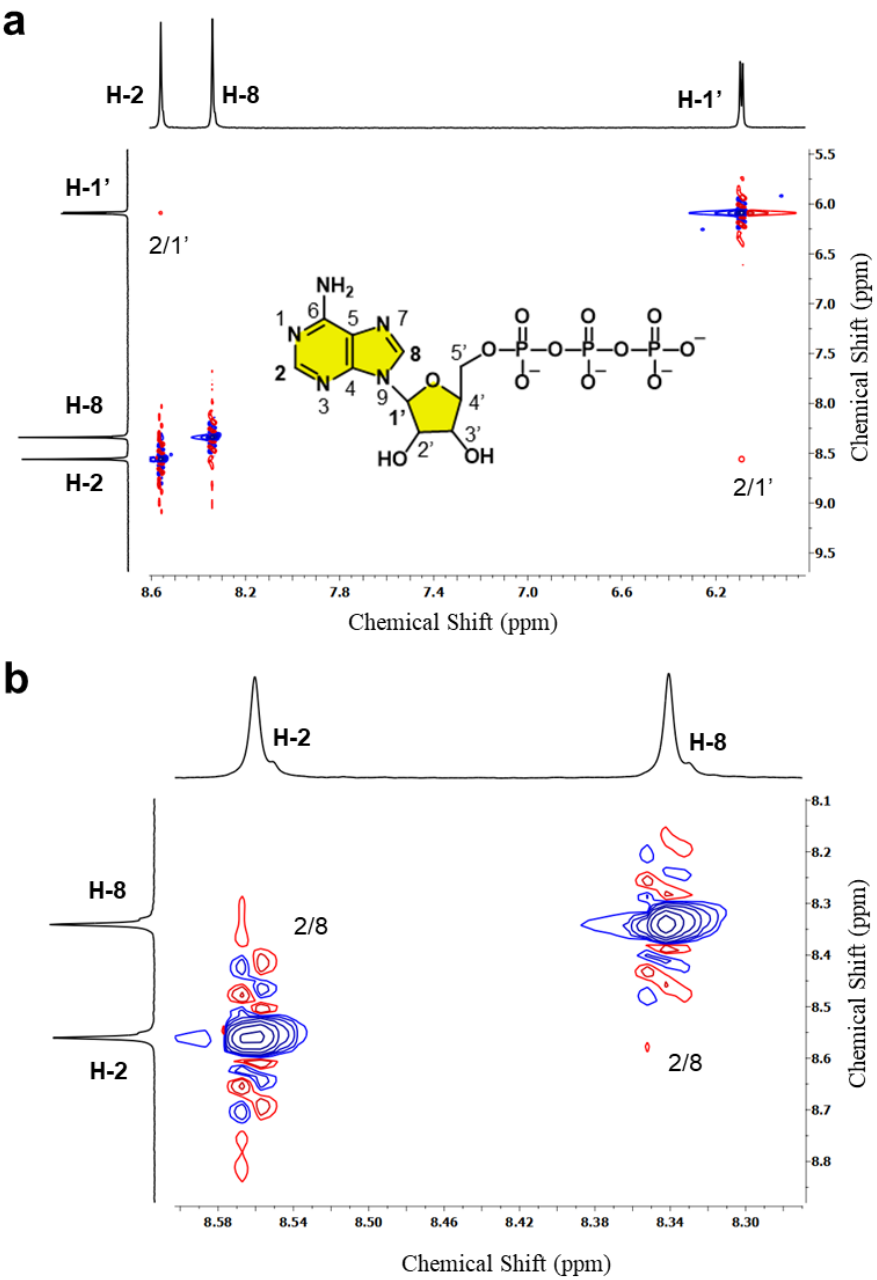

Figure 3.18. Partial ROESY spectra of intermediate aggregated state of bioplasmonic network showing NOEs of (a) H-2↔H-1' and (b) H-2↔H-8 pairs of protons.

From NMR studies, it is clear that ATP molecules aggregate in a head-to-tail fashion because of the π-π stacking and H-bonding interactions (Figure 3.19). The phosphate groups and sugar groups impart the hydrophilic nature, which can undergo electrostatic and H-bonding interactions. Whereas, the hydrophobic adenine moiety undergoes stacking in an alternate head-to-tail fashion, to from long-range 1D structures.

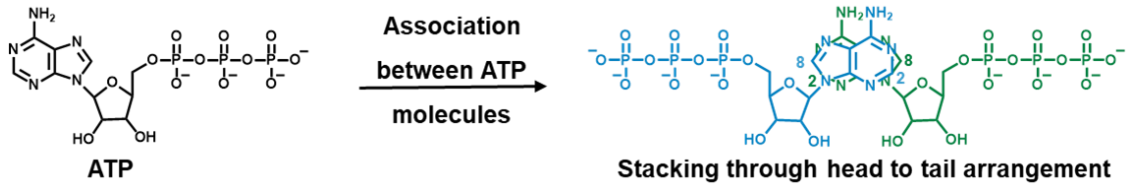

Figure 3.19. Schematics showing the stacking between ATP molecules leading to 1D assembly in the bioplasmonic network.

Therefore, from all these experiments, a plausible mechanism can be drawn (Figure 3.20). ATP molecules, in very high concentration by itself can undergo the self-assembly process to form the white ATP network. While, in the presence of [+] AuNPs, it forms the precipitate. The excess ATP present in the supernatant solution then undergoes the self-assembly process to form the white ATP network. This triggers the disassembly of ATP-AuNP precipitate, leading to the post-functionalization of ATP network, to form bioplasmonic network.

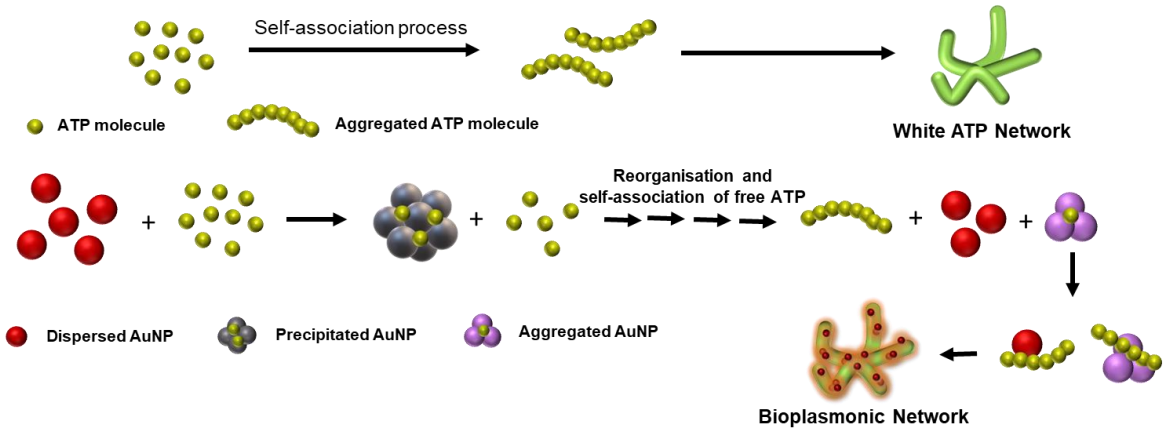

Figure 3.20. Schematics showing the probable mechanism of precipitate to bioplasmonic network transformation.

3.4.5 Multifunctional bioplasmonic network

The concept of the cation-directed self-assembly approach was extended to a wide range of small biomolecules and nanomaterials. From the nanomaterial side, a change in the core (cationic silver nanoparticles, AgNP and indium phosphide quantum dots, InP QDs) and shape (cationic gold nanorods, AuNR) had a negligible influence on the mode of the self-assembly process (Figure 3.21). From the biomolecule side, a series of nucleotides were able to form

long-range self-assembled bioplasmonic networks with [+] AuNP (nucleotides studied include adenosine monophosphate AMP, adenosine diphosphate ADP, guanosine tri-phosphate GTP, oxidized and reduced forms of nicotinamide adenine dinucleotide phosphate NAD^+ and NADH).

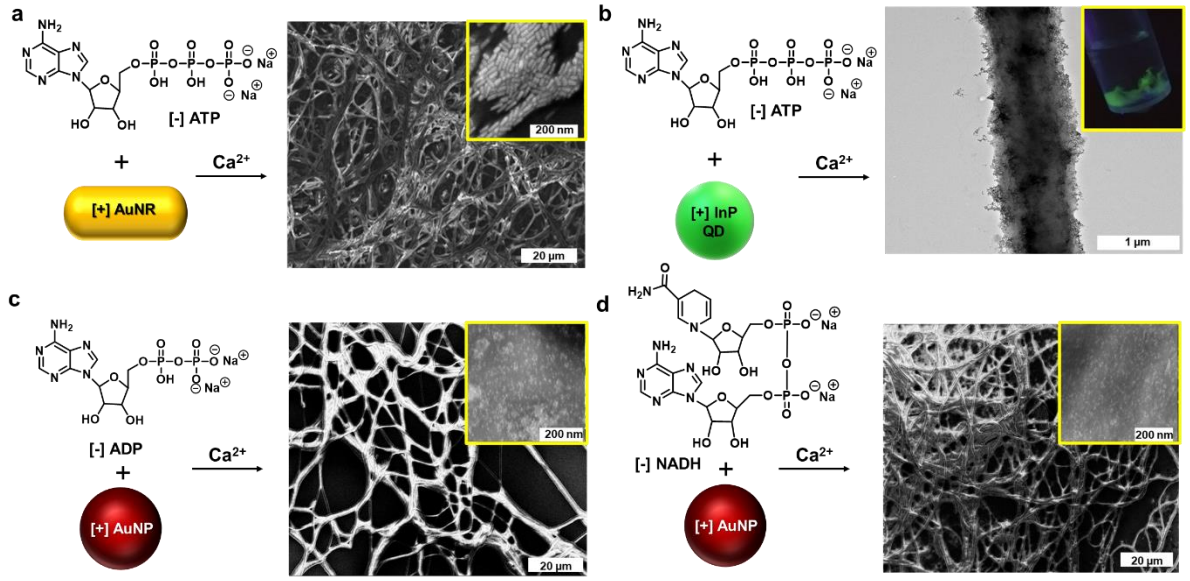

Figure 3.21. Generality of electrostatically directed long-range self-assembly in the formation of bio-nano assemblies. ATP-based network formation in the presence of (a) [+] AuNR and (b) [+] InP QD. The inset of 'a' shows the presence of AuNRs on ATP networks. The inset of 'b' shows the green luminescent ATP-InP QD network under UV light, confirming the retention of photoluminescence of InP QDs in the self-assembled network. AuNP-based bioplasmonic network formation in the presence of (c) ADP and (d) NADH. The presence of AuNPs on self-assembled nucleotides can be clearly seen in the respective insets.

Retention of the individual properties, along with the emergence of inherently absent properties, is the desired outcome of a self-assembly process. Often, the self-assembled structures formed from bio-nano hybrids even fail to retain their stability under mild to harsh conditions that are routinely used in many applied studies. Remarkably, the ATP-AuNP-based supramolecular networks were stable under harsh conditions such as sonication and heating (~4 h @80 ^oC). Next, the catalytic property of ATP molecules in the bioplasmonic network was tested by performing the model 3,3',5,5'-tetramethylbenzidine (TMB) oxidation reaction (Figure 3.22).[61] This catalytic reaction was a perfect choice for the present study since both ATP and AuNP can, independently, catalyze the TMB oxidation. In a typical ATP-catalyzed TMB oxidation reaction, the parent diamine molecule gets oxidized to form a radical cation (λ_{max} ~370 nm) upon treatment with H_2O_2. This radical cation forms a blue-colored charge transfer complex (λ_{max} ~652 nm) with the parent TMB molecule. The typical roles of ATP in

this reaction are to (i) mimic the distal histidine residue of the biological peroxidase enzyme, (ii) activate the H_2O_2, and (iii) stabilize the oxidized product via single electron transfer reaction. Likewise, AuNPs is reported to catalyze the degradation of H_2O_2 into hydroxide radicals (·OH), which then triggers the oxidization of TMB molecules. We hypothesized that a close proximity of AuNP and ATP in the self-assembled bio-nano networks can lead to a synergistic effect.

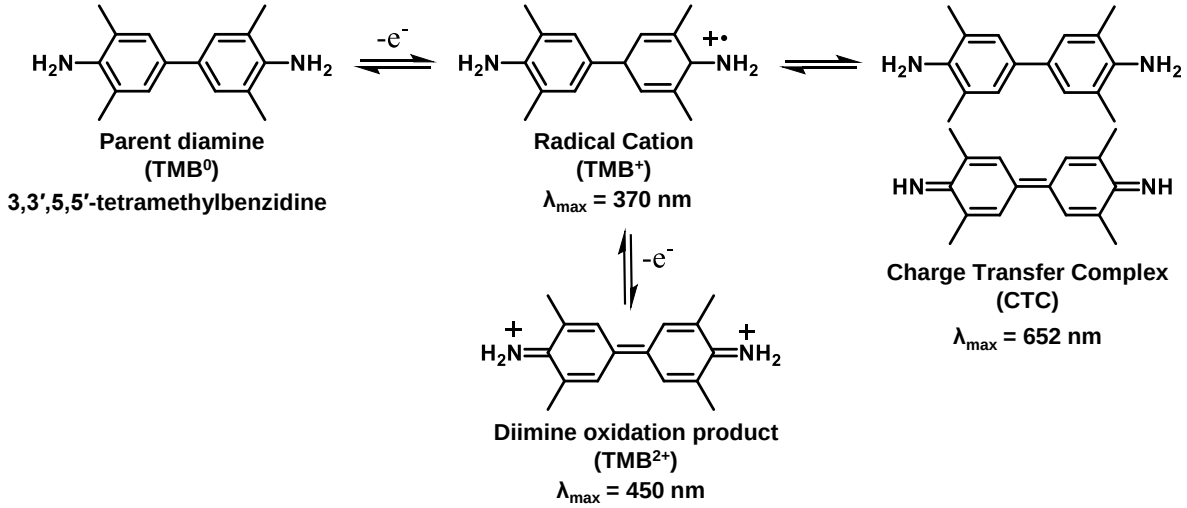

Figure 3.22. Reaction scheme and mechanism of TMB oxidation. On oxidation, TMB molecule forms a radical cation that forms a charge transfer complex with the parent TMB molecule, which has a characteristic absorption peak at λ_{max} ~652 nm.

In a typical TMB oxidation reaction, a bioplasmonic network was added to the working solution containing 2 mL of acetate buffer (0.2 M, pH~ 3.6), 100 µL TMB solution (2 mM), and 215 µL H_2O_2 (1 M). A gradual increase in the absorption at ~652 nm was observed as the reaction proceeded, and the solution color turned to blue from colorless (Figure 3.23a, b), confirming the formation of the oxidized product of TMB (~60 µM). Control experiments confirm that a synergistic effect was indeed observed in bioplasmonic networks (Figure 3.23c). Interestingly, the catalytic activity of bioplasmonic network was better than the corresponding physical mixture of AuNP and ATP-Ca^{2+} white network. This confirms the importance of the proximity of ATP and AuNP in the bioplasmonic networks for achieving the enhanced catalytic performance.

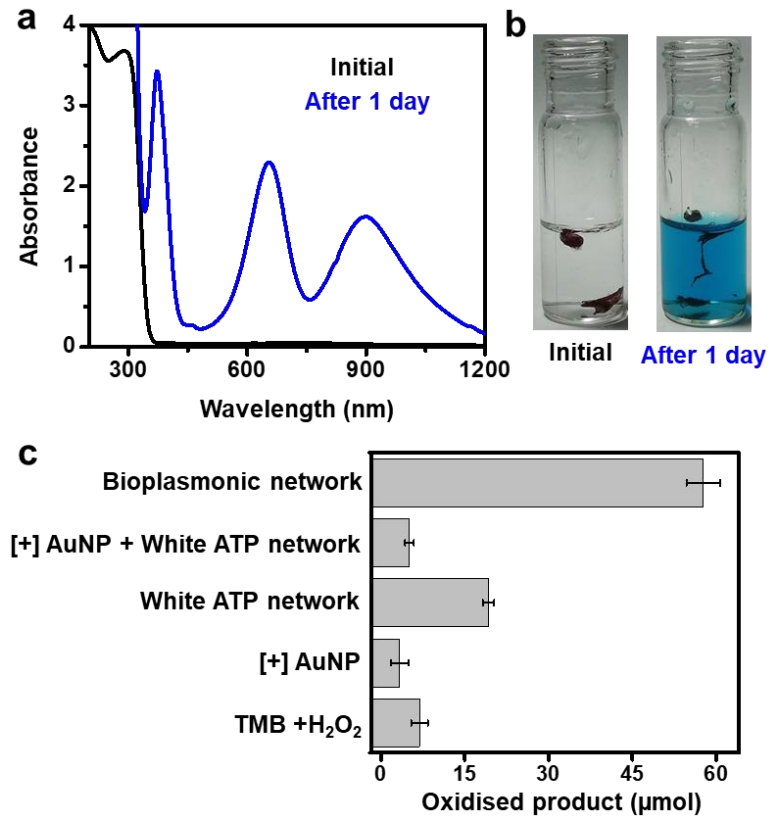

Figure 3.23. TMB oxidation reaction with bioplasmonic network. (a) The appearance of peaks at λ_{max} ~370 nm and λ_{max} ~652 nm confirms the formation of the oxidized product of the TMB molecule. (b) Photographs of the vials at the initial and final stages of the TMB oxidation reaction. The appearance of a blue color after 1 day corresponds to the oxidized charge-transfer complex. (c) Summary of product formation for TMB oxidation under different reaction conditions. The error bars indicate the standard deviation obtained from 3 different experiments from three different batches of catalysts.

Further, the plasmonic properties of AuNPs in the self-assembled bio-nano networks were tested. For this, model catalytic, photocatalytic, and scattering properties of AuNPs were systematically investigated. A quantitative reduction of p-nitrophenol (PNP) to p-aminophenol (PAP) by the borohydride ions (BH_4^-) was achieved[62] when the bioplasmonic network was used as the nanocatalyst (Figure 3.24a). AuNPs assist in overcoming the kinetic barrier for the reduction process by offering its surface for the adsorption of reactants, and mediating the transfer of electrons between the reactants.[62] The addition of a bioplasmonic network to a solution of PNP and BH_4^- showed a gradual decrease in the absorption of the 4-nitrophenolate ion at ~400 nm, along with the appearance of a new band ~300 nm corresponding to PAP (Figure 3.24b). The PNP reduction followed a pseudo-first order kinetics with a rate constant of ~2.1 x 10^{-2} min^{-1}, comparable to standard NP catalysts (Figure 3.24d). The heterogeneous nature of bioplasmonic networks helped in the easy product separation, as well as retention of their catalytic properties for at least 5 cycles (Figure 3.24c). In a separate experiment, the

catalytic properties of the AuNP post-functionalized white ATP network were found to be superior to bioplasmonic networks (Figure 3.24d). This again proves that AuNP forms an active part of the self-assembly process during the bioplasmonic network formation. As a result, not all the surface of AuNP is available for catalysis, unlike with post-functionalized network.

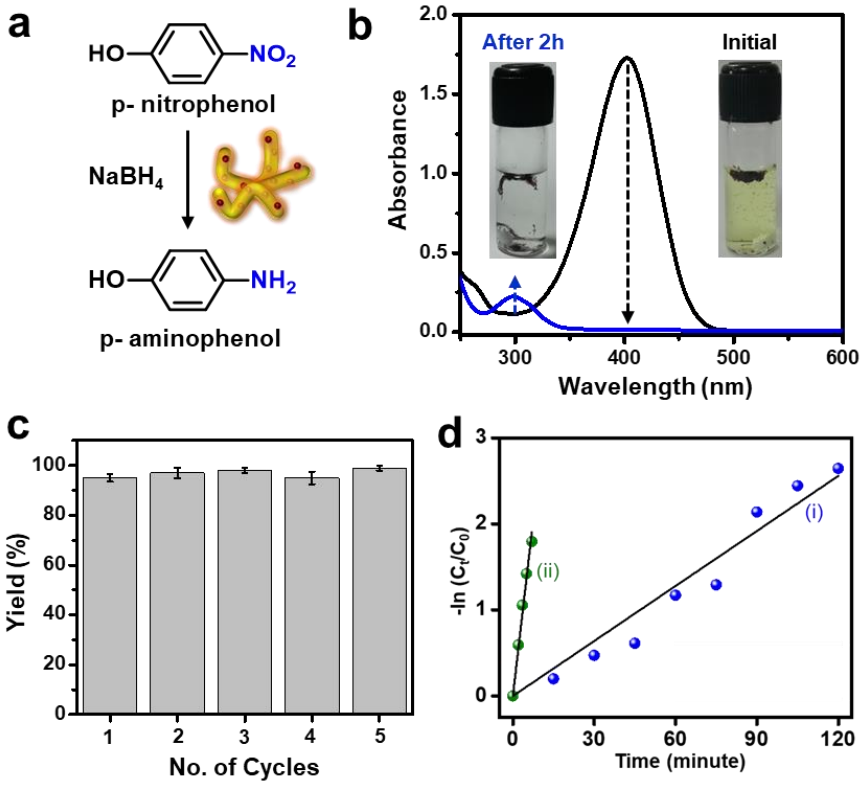

Figure 3.24. (a) A model nano-catalytic reaction for the reduction of PNP to PAP by BH_4^- ions in the presence of a bioplasmonic network. (b) Absorption spectra confirm the reduction of PNP (~400 nm) to PAP (~300 nm). Optical photographs of vials correspond to the reaction mixture before and after the reduction. (c) The bar plot shows the retention of the catalytic properties of the bioplasmonic network for at least 5 cycles. (d) Comparison of catalytic activities of bioplasmonic and white ATP network. Corresponding linearized plots for the first-order analysis by tracking the absorption changes of PNP peak at 400 nm in the presence of (i) bioplasmonic network and (ii) white ATP network, post-functionalized with AuNP.

Further, the retention of the photocatalytic property of AuNPs in the bioplasmonic network was tested in the model one-electron reduction of ferricyanide to ferrocyanide (Figure 3.25a).[63] The reaction mixture containing ferricyanide and bioplasmonic-network in ~1.5 M ethanol was irradiated with a 3W blue LED (intensity measured at the cuvette wall was ~35 mW/cm^2). The hot electrons generated in the photoexcited AuNPs in the bio-nano network reduced the ferricyanide to ferrocyanide, whereas the hot holes were scavenged by ethanol to replenish the photocatalyst. The bio-nano network catalyzed photo-redox reaction resulted in a decrease in the ferricyanide absorption at ~420 nm, along with a gradual increase in ferrocyanide

absorption at ~240 nm (Figure 3.25b). The formation of ferrocyanide was confirmed by the Prussian blue test as well (Figure 3.25b). The plasmonically photocatalyzed reduction of ferricyanide followed a pseudo-first order kinetics with a rate constant of ~7.27 x 10^{-3} min^{-1} (Figure 3.36). Photocatalytic activity of the bioplasmonic network was retained for at least 5 cycles, with a marginal decrease in the conversion yield (~60% to ~40%, Figure 3.25c. The decrease in the yield can be attributed to the etching of AuNP by ferricyanide.[64]).

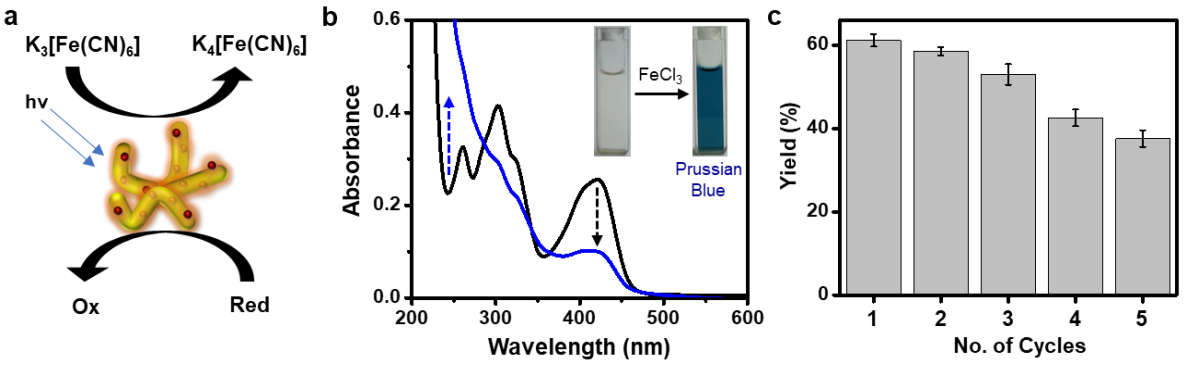

Figure 3.25. (a) A model photocatalytic reaction for the reduction of ferricyanide to ferrocyanide by bioplasmonic network. (b) Absorption spectra showing a decrease in the ferricyanide peak (~420 nm), along with a concomitant increase in the ferrocyanide peak (~240 nm), confirm the retention of photocatalytic property of AuNPs in the bioplasmonic network. The inset shows the confirmatory Prussian blue test for the product formation. (c) The bar plot shows the retention of the photocatalytic properties of the bioplasmonic network for at least 5 cycles.

Finally, surface enhanced Raman spectroscopy (SERS) studies were performed to prove the multi-functionality of the bioplasmonic network.[65] Standard Raman marker, 1,4-benzene dithiol (BDT), was adsorbed on the surface of the bioplasmonic network, as well as on the white ATP network. Characteristic Raman signals were obtained only in the case of bioplasmonic network, which again confirms the retention of plasmonic properties of AuNPs in the self-assembled structure (Figure 3.27). An increase in the loading of AuNPs led to an appreciable increment in the SERS activity of the bioplasmonic networks (Figure 3.26).

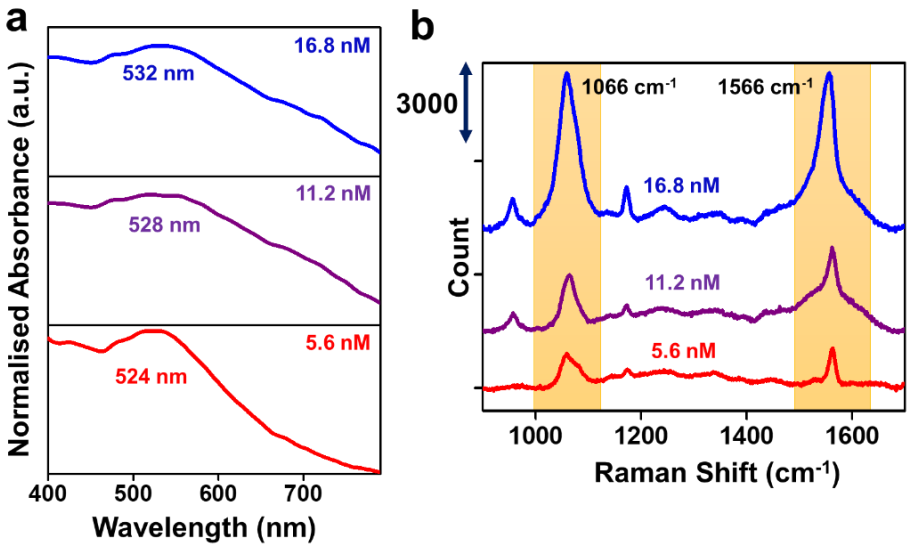

Figure 3.26. Effect of AuNP loading on plasmon coupling and SERS. (a) Absorption spectra of bioplasmonic ATP networks with different loading of AuNPs, as mentioned in the panels. An increase in the AuNP loading led to a stronger plasmon coupling between the NPs, as evident from the absorption studies (surface plasmon bands shifted from 524 nm to 532 nm, as the loading was increased from 5.56 nM to 16.68 nM). (b) Raman spectra of 1,4-BDT molecule adsorbed on bioplasmonic ATP networks, with different loading of AuNPs, as mentioned in the panels. This increase in the peak intensities of both the characteristic peaks of 1,4-BDT molecule (1066 cm^{-1} and 1566 cm^{-1}) as a function of AuNP loading, confirms the role of '*hotspots*' present on bioplasmonic ATP network in SERS enhancement.

The high SERS enhancement factors (EF) of ~5 x 10^4, prove the suitability of ATP based bioplasmonic networks to emerge as a robust SERS substrate for future bioanalytical studies (Figure 3.37 and Table 3.2). All these experiments confirm the versatility and robustness of the electrostatically directed self-assembly approach in the formation of ATP-AuNP based long-range superstructures, while retaining the inherent properties of the individual components.

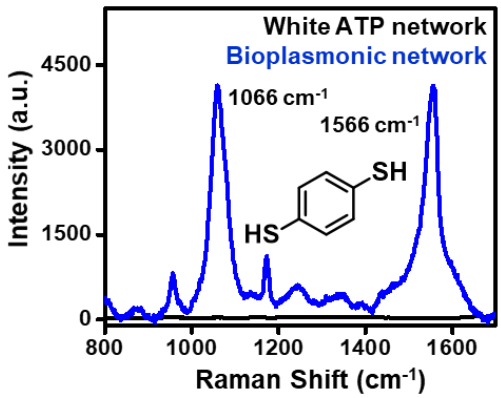

Figure 3.27. Raman spectra of 1,4-BDT in the presence of bioplasmonic and white ATP networks as the SERS substrates.

3.5 Conclusions

The unprecedented long-range self-assembling ability of ATP biomolecules was demonstrated. Divalent ions and cationic AuNPs were able to trigger the aggregation process in ATP (and in other nucleotides), by participating in favorable electrostatic interactions with the triphosphate groups. Detailed kinetic, NMR, and modeling studies revealed the involvement of various non-covalent interactions in the self-assembly process. A precise regulation of electrostatic attraction between ATP and AuNPs was achieved in the presence of Ca^{2+} ions, which turned out to be crucial in the formation of ATP-based superstructures. The screening of charges by Ca^{2+} ions and the ATP-Ca^{2+} coordination helped in transforming an uncontrolled precipitation process into a kinetically controlled self-assembly process. Remarkably, the inherent properties of ATP and AuNPs were well retained in the bioplasmonic network, thereby imparting multifunctionality to the ATP-based superstructures. The generality of the electrostatically directed self-assembly process was demonstrated in different a of bio-nano systems, which can find suitability in future bio-analytical applications. Imparting a dynamic behavior to such self-assembled bio-nano hybrids, via a further fine-tuning of interparticle interactions and incorporation of stimuli-responsive components, can be a future direction for realizing structure-specific functions similar to those observed in natural superstructures.

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3.6 Appendix

3.6.1 Characterization of bioplasmonic network

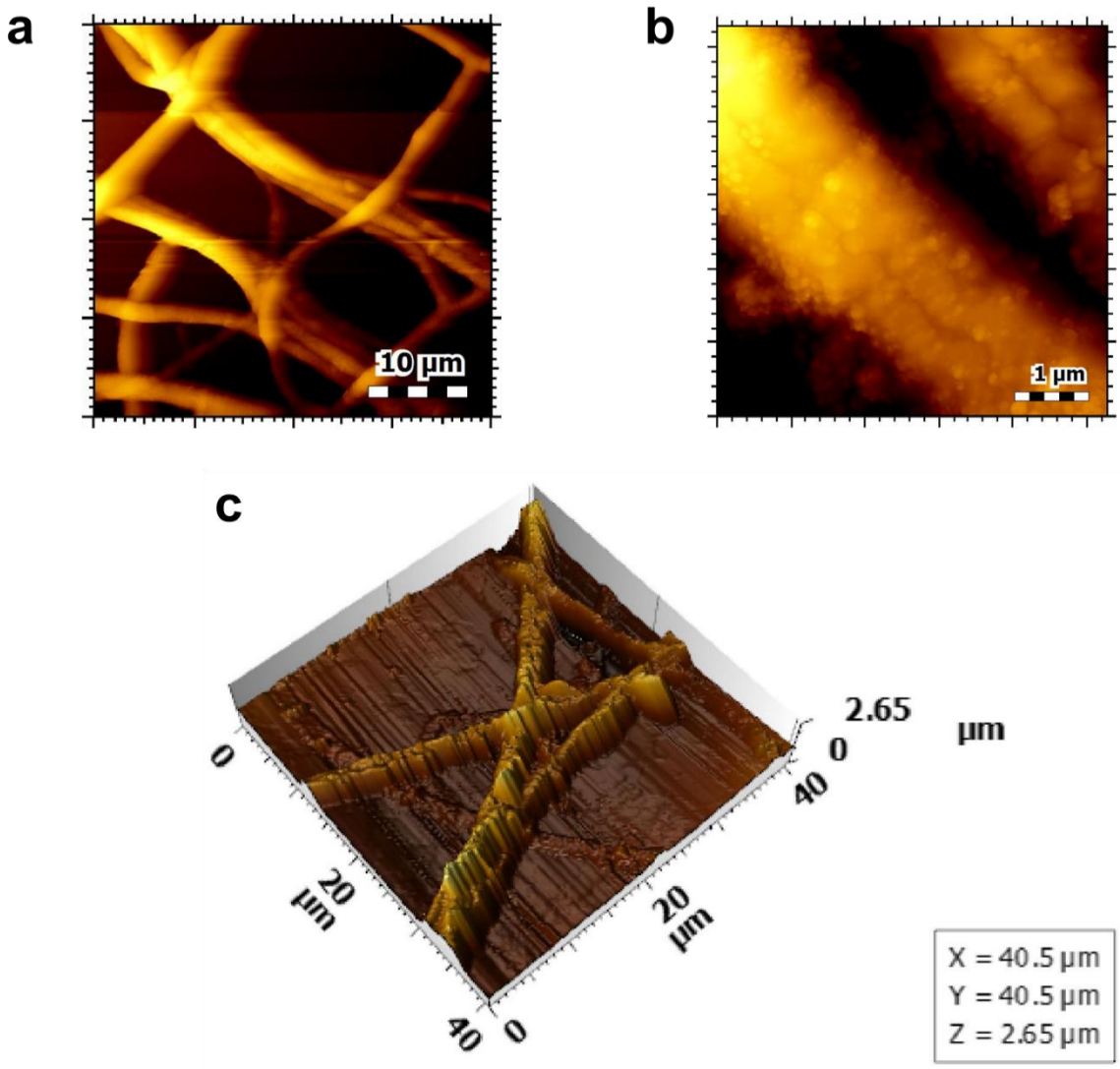


Figure 3.28. Characterization of bioplasmonic network through AFM. Tapping mode AFM images of bioplasmonic network. (a) and (b) are the 2D height images of bioplasmonic network, at different magnifications. (c) A representative 3D height image of the bioplasmonic network.

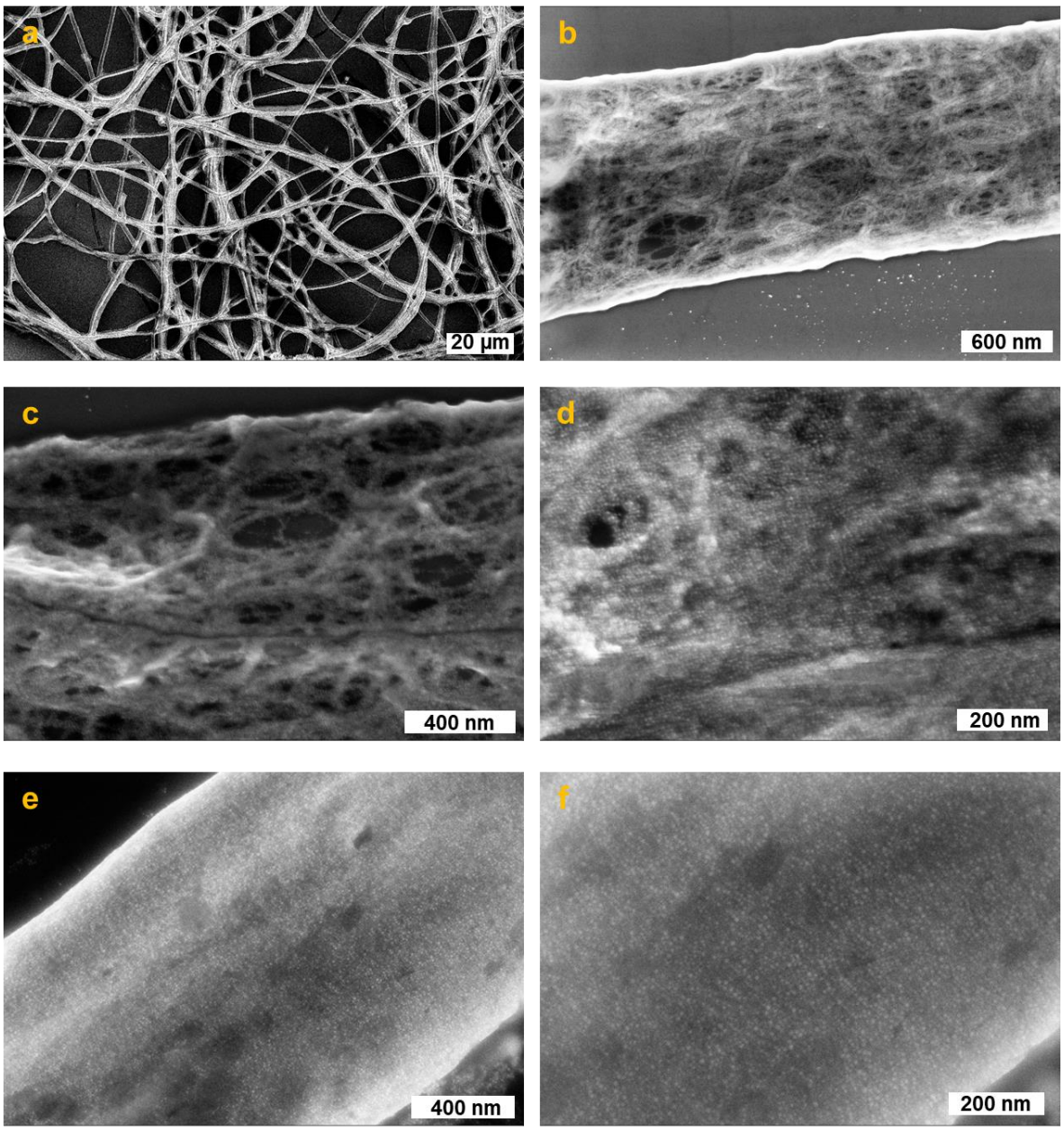


Figure 3.29. SEM characterization of bioplasmonic network. (a-f) SEM images of ATP-AuNP bioplasmonic networks confirm the formation of long-range superstructures. The higher magnification images confirm the decoration of AuNPs on the surface of self-assembled ATP structures.

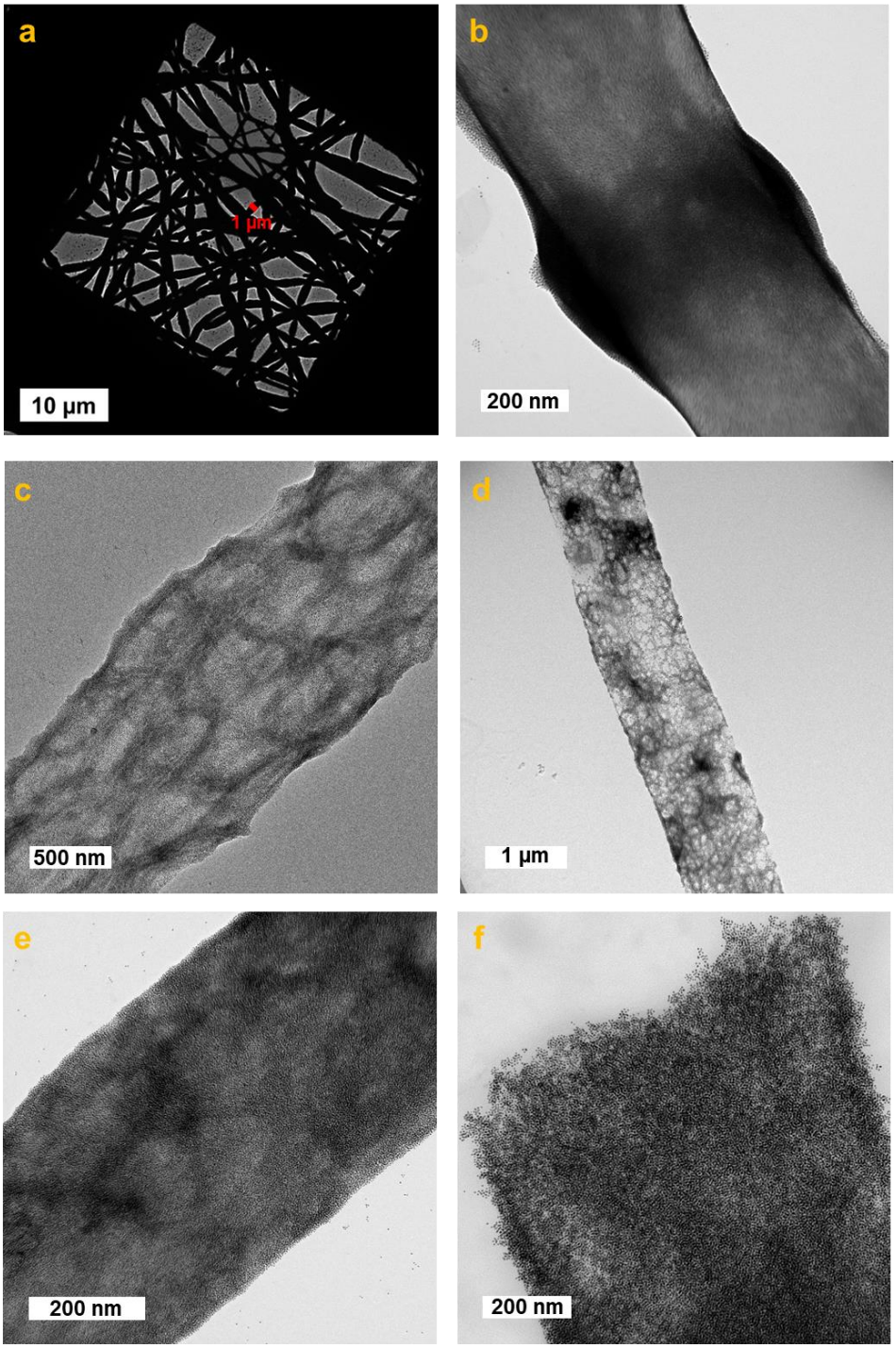
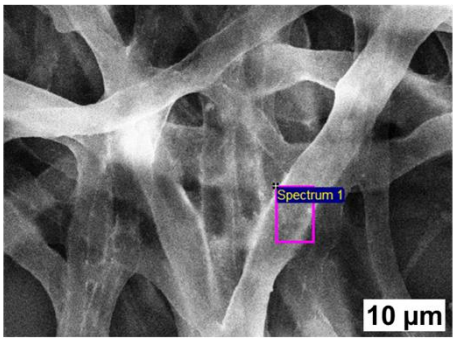


Figure 3.30. TEM characterization of bioplasmonic network. (a-f) TEM images of ATP-AuNP bioplasmonic networks confirm the formation of long-range superstructures. From the TEM analysis, the average diameter of the fibres was estimated to be 1.04 µm (Std. dev. 0.311). The higher magnification images confirm the decoration of AuNPs on the surface of self-assembled ATP structures.

a



b

Element	Weight%	Atomic%
C K	35.68	53.24
N K	12.35	15.80
O K	23.26	26.05
P K	4.34	2.51
Ca K	0.50	0.22
Au M	23.86	2.17
Totals	100.00	

c

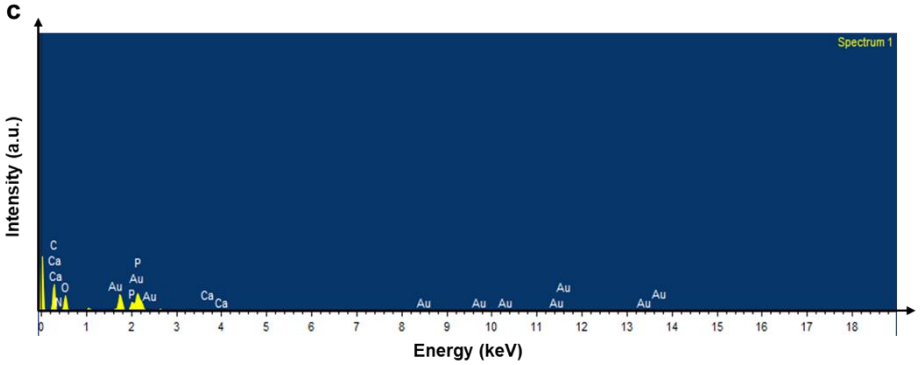


Figure 3.31. Elemental analysis of bioplasmonic network through EDAX. (a) SEM image of ATP-AuNP bioplasmonic network on which the EDAX analysis was performed (the magenta-coloured region) (b) Table summarizing the elemental composition of ATP-AuNP bioplasmonic network. (c) EDAX spectrum confirming the presence of carbon, nitrogen, oxygen, phosphorus, calcium, and gold in ATP-AuNP bioplasmonic network.

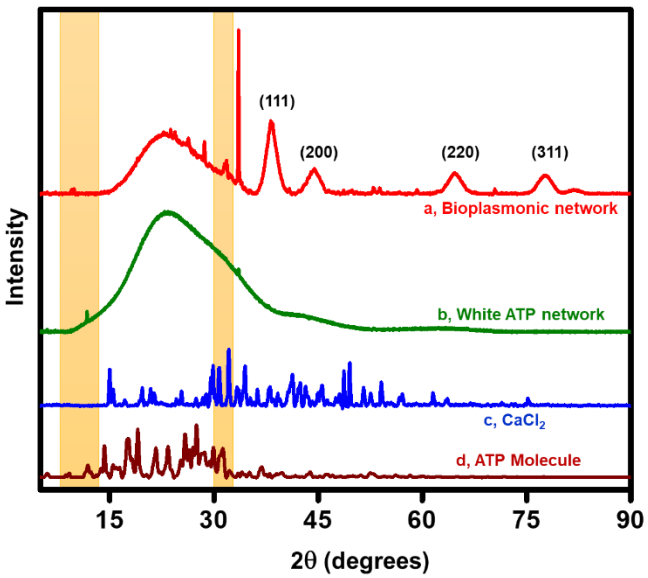


Figure 3.32. Powder X-ray diffraction (PXRD) studies. Diffraction patterns of (a) bioplasmonic network, (b) white ATP network, (c) $CaCl_2$, and (d) ATP molecule. Characteristic peaks for ATP (2θ values: 9.53, 9.88 and 31.8) and AuNPs (2θ values: 38.25 (111), 44.38 (200), 64.82 (220) and 77.76 (311)) were observed in bioplasmonic network. The presence of three broad peaks (2θ values of 23.35, 42.53 and 62.35 degree) in bioplasmonic and white ATP networks confirm the short-range ordering of ?. Further, the broad peak around ~23 degree confirms the stacking of ATP molecules in the self-assembled networks.

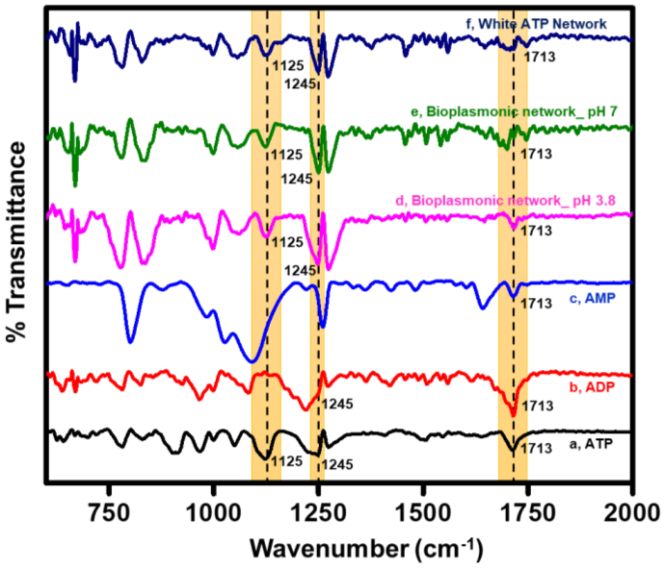


Figure 3.33. Confirmation of ATP molecules in self-assembled structures through FTIR studies. FTIR spectra of (a) ATP, (b) ADP, (c) AMP, bioplasmonic networks formed at (d) pH~3.8 and (e) pH~7.0, and (f) white-ATP network. Peaks at ~1713 cm^{-1} and ~1245 cm^{-1} correspond to the N-H deformation of the amine group of adenine and asymmetric stretching vibration of β-PO_2^{2-}, respectively. Another important peak at ~1125 cm^{-1} (asymmetric stretching frequency of γ-PO_3; present in ATP but absent in ADP and AMP) confirms the presence of ATP molecule in both plasmonic and white ATP network.

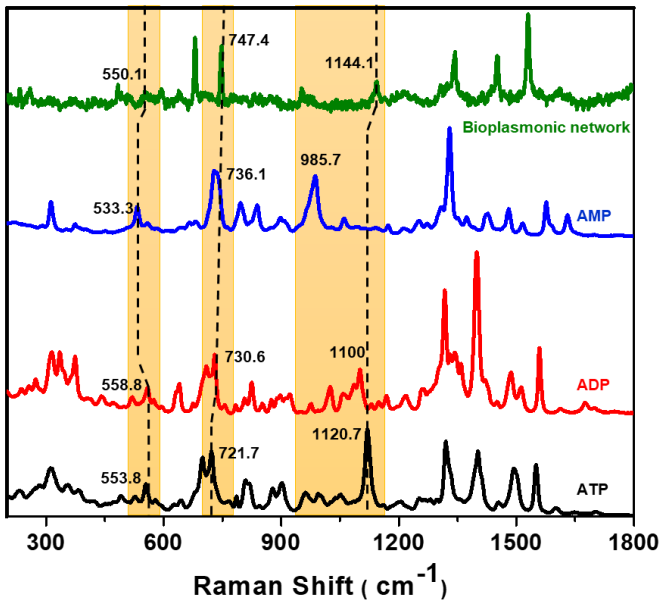


Figure 3.34. Confirmation of ATP molecules in bioplasmonic network through Raman studies. Raman peak at 1120.7 cm^{-1} (symmetric stretching vibration of the triphosphate group in ATP molecule) is shifted to 1144.1 cm^{-1} in bioplasmonic ATP network (formed at acidic pH~ 3.8), confirming the presence of ATP molecules in the network.

3.6.2 Kinetics of bioplasmonic network formation

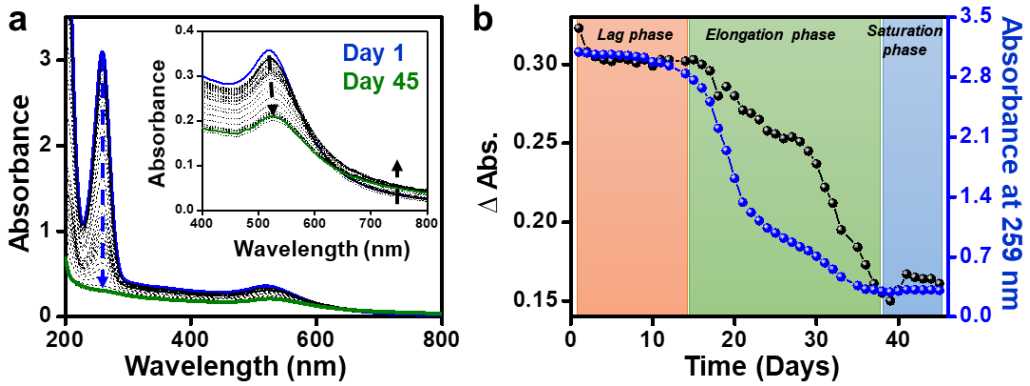


Figure 3.35. Kinetics of bioplasmonic network formation at pH ~3.8. (a) Time-dependent absorption spectra showing a continuous decrease in the absorption peak of ATP (l_{max} ~259 nm) and AuNP (Δ Abs. = Abs. at 520 nm – Abs. at 750 nm), from day ~10 of the self-assembly process at pH ~3.8. The increase in the absorbance ~750 nm confirms the aggregation process. (b) The corresponding kinetic plot showing the variation in absorption of ATP and AuNP, which can be labelled into three stages: lag phase, elongation phase, and saturation phase.

3.6.3 Kinetics of photocatalytic reduction of ferricyanide with bioplasmonic ATP network.

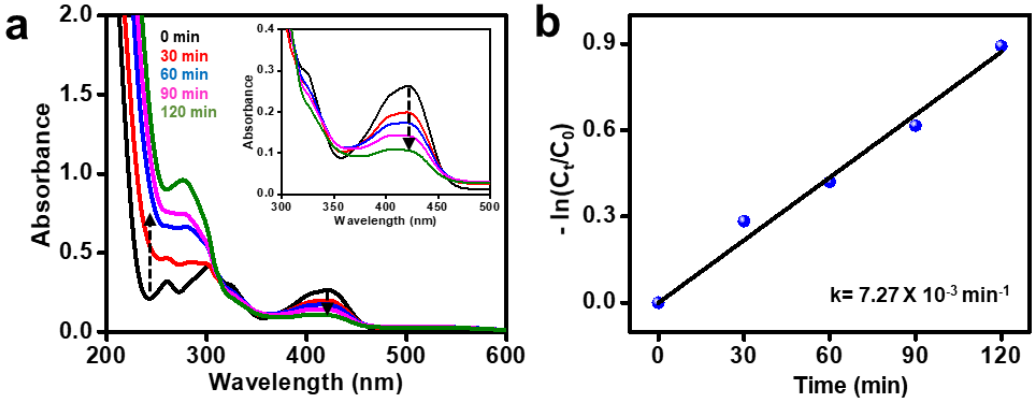


Figure 3.36. Kinetics of photocatalytic reduction of ferricyanide with bioplasmonic network. (a) Spectral changes in the absorption of ferricyanide in the presence of bioplasmonic network, upon irradiation with 3W blue LED. Inset shows a steady decrease in the absorbance of ferricyanide peak. (b) A linearized plot for the first-order analysis by tracking the absorption changes of ferricyanide peak at 420 nm.

3.6.4 SERS enhancement factor (EF) calculation

In a typical experiment, the bioplasmonic ATP network was spread on a glass slide over an area of 0.5 x 0.5 cm^2. Then, 20 µL 1 mM 1,4-BDT (in ethanol solution) was added to it, and kept undisturbed for ~6h. Raman spectra were recorded after washing the functionalized bioplasmonic ATP network several times with ethanol, to remove the unabsorbed BDT molecules. As 1,4-BDT molecule can chemisorb on the AuNP surface and form a monolayer, it is hypothesized that only the chemisorbed molecules will be functionalised on the bioplasmonic ATP network after 3-4 times washing. Control experiments were performed with white ATP network (having no AuNPs) as SERS substrate, to calculate the enhancement factor. White ATP network was drop cast over the area of 0.5 x 0.5 cm^2 and 20 µL of 50 mM 1,4-BDT was added on it. Raman spectra were recorded without any further washing of the white ATP network, in order to retain the BDT molecules on the substrate (all the 1,4-BDT molecules will be physiosorbed on the white ATP network, as there is no strong mode of interaction between white ATP network and BDT).

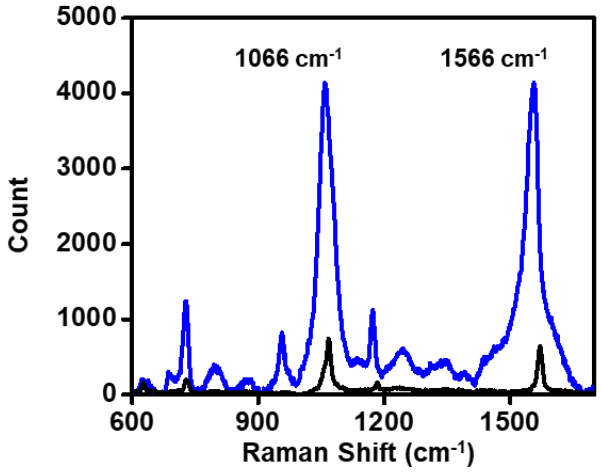


Figure 3.37. SERS enhancement factor calculation. Raman spectra of 1,4-BDT molecule on the surface of bioplasmonic ATP network (blue spectrum) and white ATP network (black spectrum).

Table 3.2: SERS EF Calculation

SERS substrate	**Probe (1,4-BDT) concentration**	**Peak position used for EF calculation**	**Area under the peak**
Bioplasmonic ATP network	1 mM	1066 cm^{-1}	173338.50292
White ATP network	50 mM	1066 cm^{-1}	16363.72674

Calculation:

SERS Enhancement Factor (EF) was calculated using the formula,[ref]

$$EF = \left(\frac{I_{SERS}}{I_{Raman}}\right) \times \left(\frac{N_{Raman}}{N_{SERS}}\right)$$

N_{Raman} and N_{SERS} denotes the number of probe molecules (here 1,4-BDT) for normal Raman and SERS signals, respectively.

I_{Raman} and I_{SERS} represents the normal Raman and SERS intensities (here intensity of 1066 cm^{-1} peak for 1,4-BDT molecule was considered), respectively.

Calculated value, from the area under the 1066 cm^{-1} peak of the Raman spectrum,

I_{Raman}= 16363.72674 and **I_{SERS}= 173338.50292**

$$N_{Raman} = L\ nm^2 \times \frac{(N_A \times p\ \mu L \times q\ mM)}{1000\ mL \times a\ nm^2}$$

Where,

L= LASER spot Area (nm^2)
N_A= Avogadro's number = $6.022 \times 10^{23}\ mol^{-1}$
a= Area (nm^2), where substrate is drop casted= (0.5 cm × 0.5 cm) = $2.5\times 10^{13}\ nm^2$
q= Concentration of the stock solution (mM) of Raman active molecule, 1,4-BDT =50 mM
p= Volume (µL) of stock Raman active molecule, 1,4-BDT drop casted in 'a' nm^2 area= 20 µL

Substituting all the values,

$$N_{Raman} = L\ nm^2 \times \frac{6.022\times 10^{23}\times 20\ \mu L \times 50\ mM)}{1000\ mL \times (2.5\times 10^{13})\ nm^2} = 24088\ L$$

Radius (r) of AuNP is 2.55 nm
So, surface area of a single AuNP= $4 \times \pi \times (2.55)^2\ nm^2$ = ~82 nm^2

The number AuNPs in the Laser spot area (L nm^2) = $\frac{L\ nm^2}{82\ nm^2} = \frac{L}{82}$

Thiol footprint = 0.214 nm^2.[24]

Number of 1,4-BDT molecules on a single AuNP = $\frac{82\ nm^2}{0.214\ nm^2} =$ ~382

Total number of 1,4-BDT molecules in the LASER spot area = $\frac{L}{82} \times 382 = \frac{382\ L}{82}$

N_{SERS} = $\frac{382\ L}{82}$ and **N_{Raman} = 24088 L**

Now, $EF = \left(\frac{I_{SERS}}{I_{Raman}}\right) \times \left(\frac{N_{Raman}}{N_{SERS}}\right)$

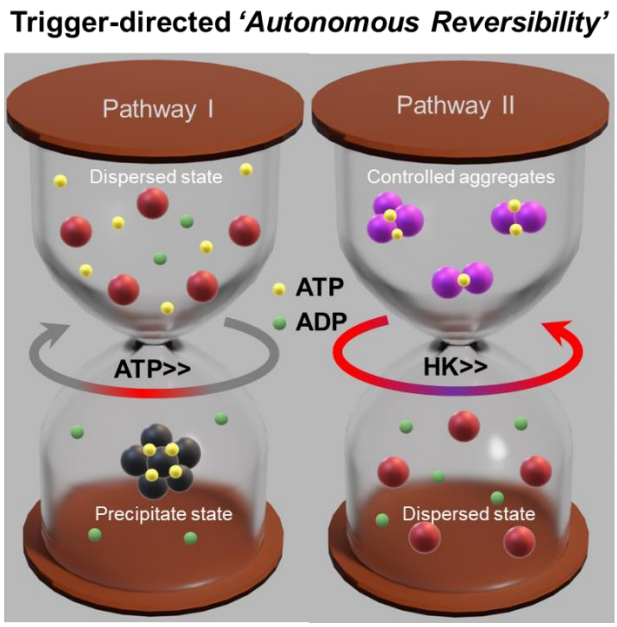
Substituting all the values,

$$EF = \left(\frac{173338.50292}{16363.72674}\right) \times \left(\frac{24088\ L}{\frac{382\ L}{82}}\right)$$

EF = 5.477×10^4

Chapter 4a

Autonomous-Reversibility Brings Temporal Control in Biomimetic Nanoparticle Self-Assembly Process



4a.1 Abstract

The majority of biological assemblies exhibit spatiotemporal behavior, which nature employs to accomplish autonomous reversibility to carry out diverse biological activities. To achieve such functionality in an artificial biomimetic system, the assembly-disassembly kinetics must be precisely controlled. In the current study, we tried to shed light on how controlling the assembly-disassembly kinetics can lead to different pathways to achieve autonomous reversibility. In this direction, we constructed an adenosine triphosphate (ATP) triggered dynamic self-assembled system based on gold nanoparticles (AuNPs) through the controlled regulation of electrostatic interactions. The addition of ATP molecules induces aggregation of oppositely charged AuNPs, leading to the precipitation of NPs. The incorporation of phosphoryl transferase enzyme (Hexokinase, HK) converts the ATP in the AuNP aggregates into adenosine diphosphate (ADP). As a result, the electrostatic attraction in the AuNP aggregates decreases, leading to the redispersion of AuNPs. ATP acts as the aggregating trigger, and HK plays the role of the disassembling agent. Importantly, we have observed a complete shuttling between a precipitate state and a dispersed state, which is a scarcely observed phenomenon in AuNP-based self-assembly systems. The autonomous self-assembly process was achieved by adding an excess aggregating trigger (ATP) or excess disassembling agent (HK) into the system. In this way, multiple assembly-disassembly cycles were choreographed. Further, the pathways used for achieving the autonomous self-assembly process dictate the nature of the assembled state. Specifically, excess-ATP trigged pathways enabled the autonomous shuttling between completely dispersed and completely precipitated states. On the other hand, the excess HK led to the shuttling between completely dispersed and controlled aggregate states. Therefore, this study also unveils the importance of the triggers in dictating the autonomous shuttling between assembled and disassembled states, demonstrating the pathway dependence in the AuNP self-assembly process. More interestingly, the kinetic control also helped us to obtain the tuneable lifetime of the transient self-assembled state.

4a.2 Introduction

Non-equilibrium self-assembly plays a significant role in biological systems to generate complex matter with *'life-like functions'* such as adaptivity, self-healing, homeostasis, etc.[1-14] For instance, the guanosine triphosphate (GTP) and adenosine triphosphate (ATP) triggered dynamic self-assembly of microtubules, and actin filaments are involved in the programmed

cell death (*apoptosis*),.[15,16] Installing such functions in artificial systems has led to the creation of active materials. However, mimicking biological functions, such as autonomous reversibility in biomimetic self-assembled systems, is not a trivial task as it requires precise control over the assembly and disassembly kinetics of the self-assembly process, which the nature has achieved through the fine control over the enzymatic cascade reaction networks.[3,4] An overall strategy to create artificial transient self-assembled systems adopted so far is through controlling the kinetics of two competing processes: (i) a quick self-assembling step and (ii) a slow disassembling step.[17] To install the dynamicity into the system, the assembly is generally performed under an unstable environment or in the presence of a dormant deactivator, leading to the autonomous disassembly process.[18-22] In this direction, one of the pioneering works was reported by Grzybowski and co-workers, where the authors used light as a fuel to study the dynamic self-assembly of gold nanoparticles (AuNPs) (Figure 4a.1).[19] AuNPs were decorated with photoisomerizable azobenzene dithiol ligands. The nonpolar nature of the trans-form of azobenzene (because of a zero-dipole moment) makes the NPs disperse in nonpolar solvents (such as toluene and hexane). Under UV light irradiation, the azobenzene ligands undergo a trans-to-cis isomerization, accompanied by an increase in the dipole moment and polarity of the NP system. As a result, the NP system loses its stability in the nonpolar medium and starts to aggregate to form the transiently stable self-assembled state. Upon turning OFF the UV light (or under visible light irradiation), the photoisomerizable azobenzene ligands revert to the transform, leading to the breaking of the aggregates and complete redispersion of NPs in the nonpolar medium (initial state). Therefore, an autonomous dynamicity was installed in the system.

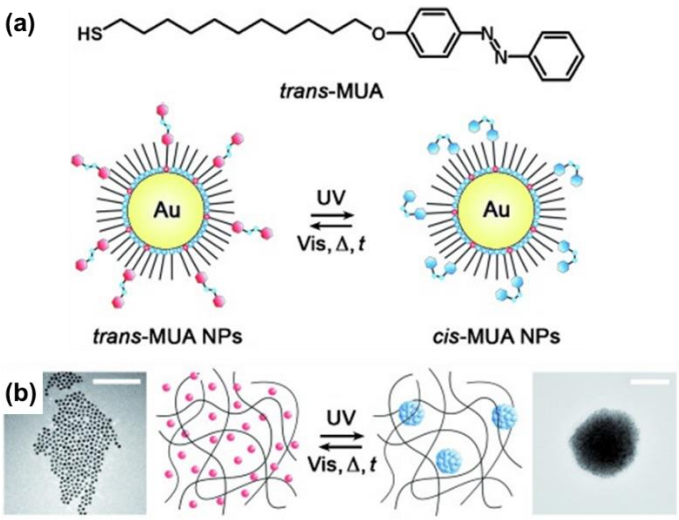


Figure 4a.1. (a) Schematics showing the light-driven cis-trans isomerization of azobenzene ligands functionalized on the AuNPs. (b) Photoisomerization under UV and visible light led to a reversible

polarity change, resulting in the dynamic self-assembly of AuNPs. The TEM images corresponding to the dispersed and aggregated stages of AuNPs are also shown. Reproduced with permission from ref 19. Copyright 2007, Proceedings of the National Academy of Sciences of the United States of America.

Later, because of more resemblances with biological assemblies, chemical trigger-driven dissipative self-assembled systems have achieved a growing interest in the area of dynamic self-assembly.[23-44] One of the pioneering works in this area was performed by van Esch and co-workers, where the authors demonstrated the dissipative self-assembly of a molecular gelator through triggered esterification and autonomous de-esterification reactions (Figure 4a.2).[27,28] Assembling trigger was required after each self-assembly cycle to carry out the next cycle of aggregation, which followed an autonomous disassembly cycle. In this way, multiple triggered assembly and autonomous disassembly cycles were demonstrated.

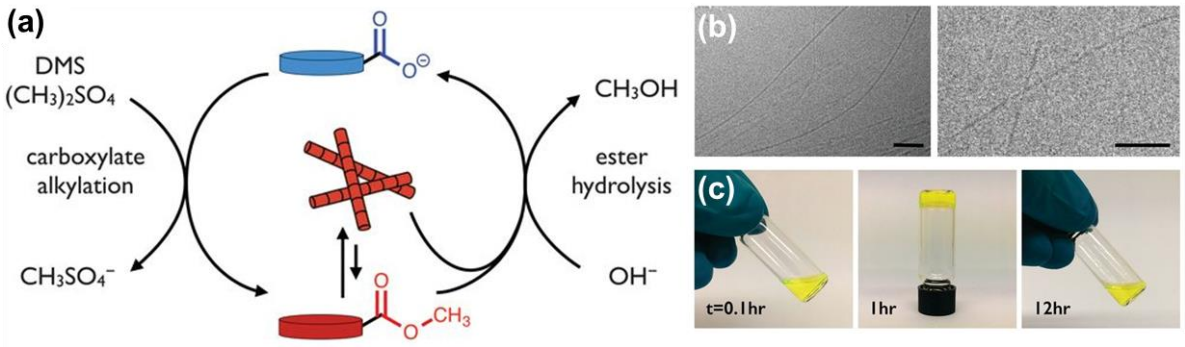


Figure 4a.2. (a) Schematics showing the use of chemically triggered esterification and autonomous de-esterification of the building blocks, resulting in the formation of transient gels. (b) Cryo-TEM images correspond to the transiently stable gel (scale bar is 100 nm), and (c) optical photographs show a transient self-assembly process, typically a sol-gel transformation. Reproduced with permission from ref 28. Copyright 2015, American Association for the Advancement of Science.

In principle, one can achieve the autonomous self-assembly process by designing appropriate assembly and disassembly pathways. The trick here is to achieve the dominance of an assembling step over the disassembly step, or vice-versa. For example, a self-assembly process performed in the presence of an excess assembly agent will ensure an autonomous assembly step for multiple cycles. Likewise, the autonomous disassembly step can be achieved by performing the self-assembly process in the presence of an excess disassembly trigger. Such dominance of assembly or disassembly steps can lead to fine control over the lifetime of assembled/disassembled states. For instance, the hirudin enzymes (here, the disassembly

trigger) secreted by the blood-sucking leeches prevent the effective assembly of fibrin to heal the wound in the prey. [45] Replicating similar chemistries in artificial systems is essential to control the autonomous process as well as the lifetime of the self-assembly process. In this context, we have achieved autonomous dynamicity in both assembling as well as disassembling steps in AuNP self-assembly by controlling the respective pathways. Here, negatively charged ATP molecules acted as an aggregating trigger to drive the self-assembly of [+] AuNPs, and the Hexokinase (HK) enzyme plays the role of the disassembling trigger. HK resists the self-assembly process by converting ATP into adenosine diphosphate (ADP) in the AuNP aggregates, leading to a decrease in the electrostatic attraction and disassembly of the aggregates (Figure 4a.3). The autonomous self-assembly process was realized by achieving dominance of the assembly step over disassembly by using excess ATP as the aggregating trigger. In another pathway, the dominance of the disassembly step over assembly was achieved by using an excess of HK as the disassembling trigger to relieve the autonomous self-assembly process. Along with achieving the autonomous process, assembling-disassembling pathways had a significant impact on the nature of the aggregated state. Excess-ATP driven pathway led to the autonomous shuttling between completely dispersed and completely precipitated states, whereas the excess-HK driven pathway resulted in the shuttling between completely dispersed and controlled aggregated states. Kinetic control of the competing reactions between ATP and HK also helped in controlling the lifetime of the aggregate states.

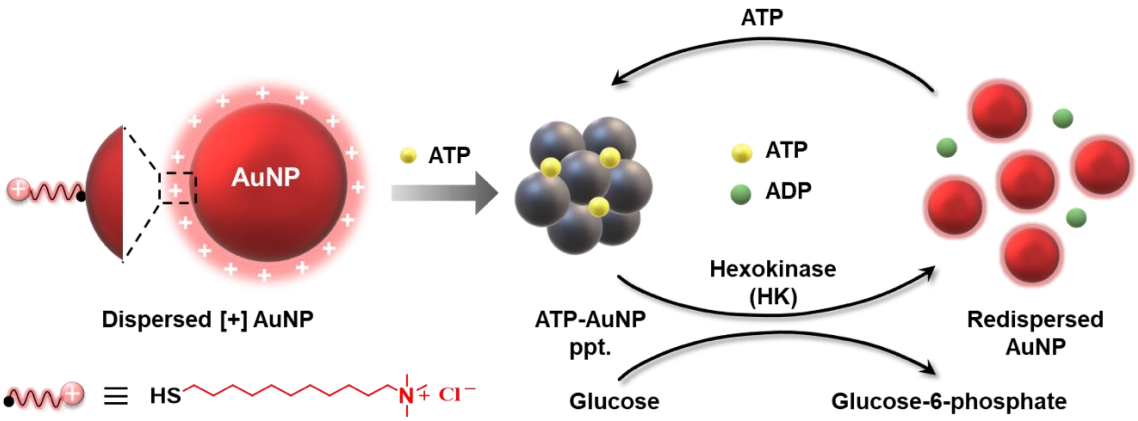


Figure 4a.3. Biomimetic dynamic self-assembly of AuNPs. The electrostatic attraction between oppositely charged ATP and AuNPs triggers the assembly step, leading to the complete precipitation. The addition of the Hexokinase (HK) enzyme initiates the ATP to ADP conversion, resulting in a decrease in the electrostatic attraction in AuNP precipitates. This triggers the disassembly process, leading to the complete re-dispersal of AuNPs. In this way, shuttling between completely dispersed and completely precipitated states of AuNPs was achieved. Here, ATP acts as the assembling trigger, and the HK enzyme plays the role of the disassembling agent.

4a.3 Experimental Section

The synthesis and characterization of nanoparticles, as well as the absorption and microscopy studies to monitor the dynamic self-assembly process, were already discussed in Chapter 2.

4a.4 Results and Discussion

4a.4.1 ATP triggered dynamic self-assembly of AuNPs

Previous studies have confirmed that the co-assembly of negatively charged ATP and positively charged AuNPs is predominantly guided by electrostatic attraction.[46,47] Therefore, in order to study the self-assembly between ATP and AuNPs, we have first synthesized [+] AuNPs, functionalized with N,N,N-trimethyl(11-mercaptoundecyl)ammonium chloride ligand, which imparts a permanent positive charge on NPs. To an aqueous dispersion of [+] AuNPs (~6 nM in terms of NPs) in a quartz cuvette, ~50 μM ATP (here, aggregating trigger) was added. This triggered an immediate self-assembly process in AuNPs, resulting in an instant aggregation, followed by the complete precipitation of NPs (plasmonically off state) within ~8 h (Figure 4a.4). The absorption data shows an instant ~10 nm red shift, along with an increased scattering intensity after the addition of ~50 μM ATP, confirming the aggregation process. This uncontrolled self-assembly process leads to complete precipitation, which is evident from the absorption studies (Figures 4a.4a, b). After precipitation, a substantial decrease (ΔAbs ~0.37) in the absorbance value at ~259 nm (corresponds to the characteristic peak of the adenine moiety of the ATP molecule) was also observed. This confirms that the precipitate contains both the ATP and AuNPs. To install dynamicity in the self-assembly process, a phosphotransferase enzyme (HK) was introduced, which converts ATP into ADP in AuNP aggregates, and, at the same time, glucose (G) gets converted into glucose-6-phosphate (G-6-P) (Figure 4a.3). Because of the decrease in the electrostatic attraction, one can expect the redispersion of the precipitated AuNPs. With this in mind, 5.25 units of HK and ~250 equivalents of G were added to the solution containing precipitated AuNP and incubated at 37 °C. This resulted in an immediate redispersion of NPs, accompanied by a blue shift of ~58 nm in the absorbance (Figure 4a.4b). Interestingly, a complete transition from a *'plasmonically off'* precipitate state to a *'plasmonically on'* dispersed NP state was observed, with a remarkable revival of the plasmon band. The dynamic light scattering (DLS) study also corroborates well with the absorption data, confirming the reversible self-assembly process (Figure 4a.4c). It is

worth mentioning that the task of converting AuNP precipitates back to a completely dispersed state is not a trivial task in NP self-assembly.[43]

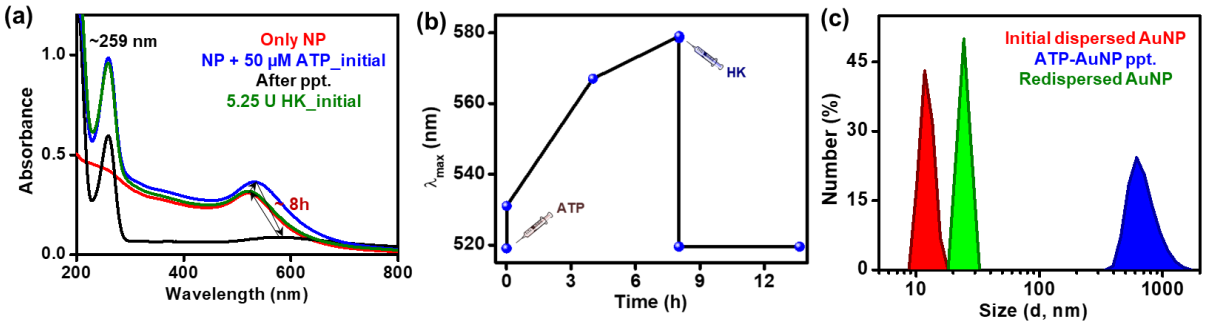


Figure 4a.4. ATP-triggered dynamic self-assembly of AuNPs. (a) The absorption data depicts an instant aggregation of [+] AuNPs, followed by precipitation upon the addition of ~50 µM ATP (red to blue to black). The addition of 5.25 units of HK enzyme breaks the AuNP-ATP precipitate instantly to completely redisperse the NPs (green trace). The corresponding (b) variation in the λ_{max} with time and (c) DLS data for one assembly-disassembly cycle.

We further carried out detailed microscopy studies (TEM) to confirm the ATP-triggered NP-aggregation step, followed by HK-driven breaking of precipitates into the dispersed AuNPs (Figure 4a.5). The dynamic self-assembly process was also evident from the optical photographs of the cuvettes (Figure 4a.5).

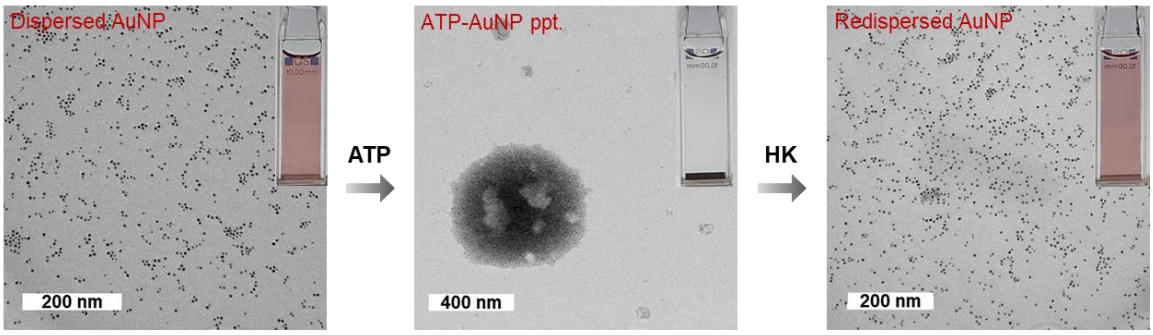


Figure 4a.5. Optical and microscopic characterization of ATP-triggered dynamic self-assembly of AuNPs. TEM images and optical photographs of the three different stages (dispersed, precipitate, and redispersed) involved in the ATP and HK enzyme-triggered self-assembly process of AuNPs. Impressively, a complete shuttling between plasmonically *'off'* and *'on'* states of AuNPs was achieved.

The phosphoryl transferase activity of the HK enzyme was monitored through a time-dependent ^{31}P NMR study (Figure 4a.6). To visualize the changes, all the components were taken in ~40 times excess (2×10^{-3} M ATP, 0.5 M G, and 70 units of HK/ mL). At the initial stage of the HK assay, there was a peak of around 23 ppm, corresponding to the β-phosphate

of the ATP molecules. With time, the β-phosphate peak started diminishing, and a new peak appeared at ~1 ppm, corresponding to G-6-P (ATP + G + HK_5h). After ~24 h, the intensity of the β-phosphate peak decreased substantially with the appearance of one more new peak at ~0.05 ppm, corresponding to free phosphate. This detailed ^{31}P NMR study essentially proved the transformation of ATP into ADP and simultaneous conversion of G to G-6-P, leading to the redispersion of precipitated NPs because of the reduction of electrostatic attraction between [+] AuNPs and negatively charged nucleotides in the NP precipitates.

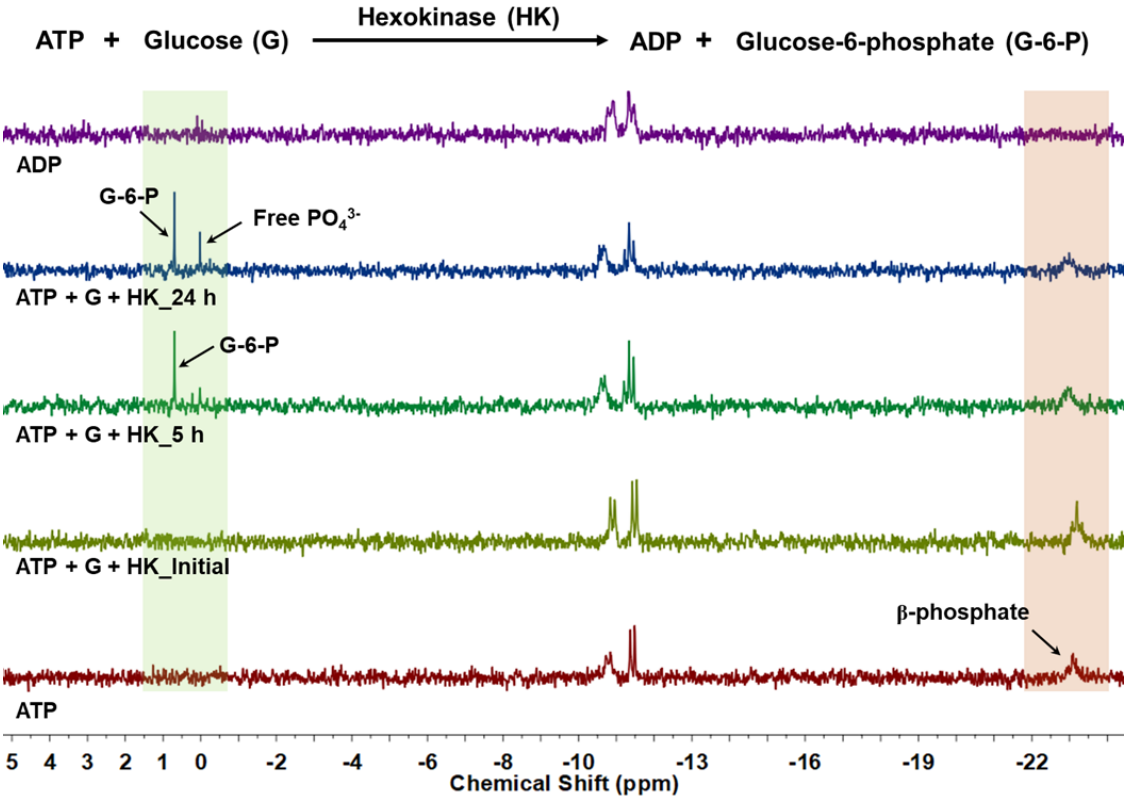


Figure 4a.6. Time-dependent ^{31}P NMR was performed to monitor the phosphoryl transferase activity of the HK enzyme during the dynamic self-assembly process. The conversion of ATP and glucose (G) into ADP and G-6-P, respectively, were monitored with time.

4a.4.2 Pathways to obtain autonomous reversibility in the dynamic self-assembly process

Irrespective of the system, the common trend in dissipative self-assembly is to take excess enzyme (disassembling trigger) to achieve an autonomous disassembly process – pathway 2. As mentioned before, autonomous reversibility can also be obtained in the presence of an excess aggregating trigger – pathway 1, which has been scarcely studied (Figure 4a.7). In the

present study, both assembly pathways have been investigated to obtain a better idea of pathway dependence on autonomous reversibility.

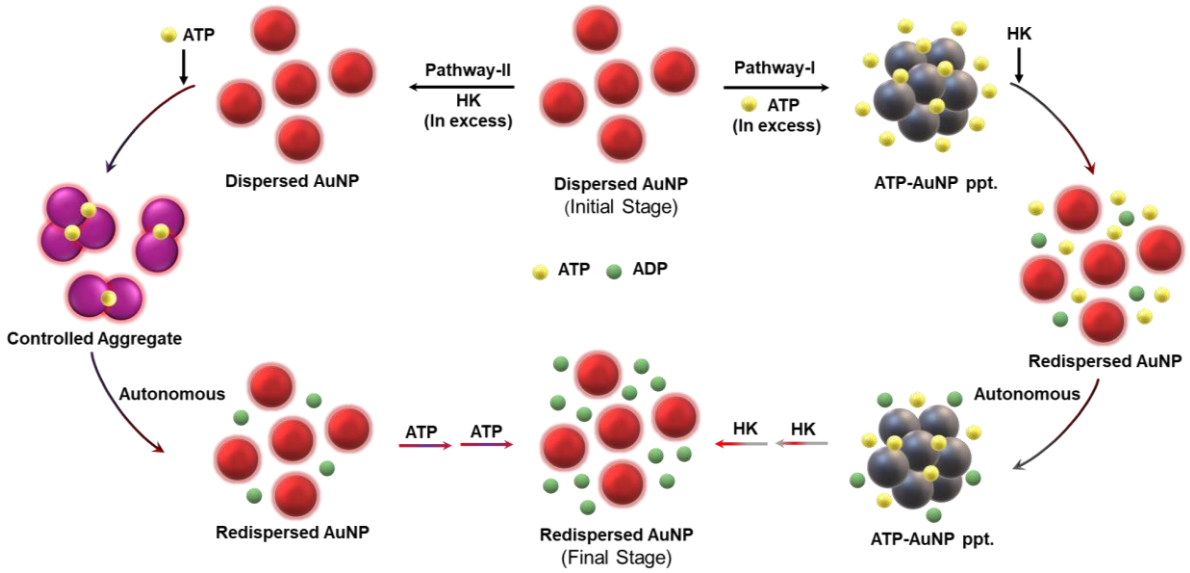


Figure 4a.7. Attaining autonomous reversibility in dynamic self-assembly process through two different pathways: Pathway 1 is in the presence of excess aggregating trigger (ATP), and pathway 2 is in the presence of excess disassembly agent (HK enzyme).

4a.4.3 Pathway 1: Excess ATP-driven autonomous reversibility

In pathway I, an excess aggregating ATP trigger was added in the initial stage to dominate the opposing action of the disassembling HK trigger. This will ensure autonomous reversibility in the self-assembly process. Specifically, the addition of HK enzyme will degrade ATP into ADP, leading to the disassembly process. However, the excess free ATP present in the solution will spontaneously initiate the next cycle of aggregation step. This dynamic cycle will continue until all the ATP is consumed or reaches below the limiting concentration required to obtain the aggregation. As the assembly process is the dominating step, the aggregation process leads to a complete precipitation of AuNPs (plasmonically off-state). Thus, shuttling between a completely dispersed and a completely precipitated state is achieved in an autonomous fashion in pathway 1. In a typical experiment, the dynamic self-assembly was performed in the presence of an excess ATP concentration (250 μM), followed by the addition of 3 units of HK to the completely precipitated state (Note: ~50 μM is the minimum concentration of ATP required to precipitate ~6 nM AuNP). This led to an instant aggregation, followed by the complete precipitation of NPs. The self-assembly process was monitored through UV-vis absorption and dynamic light scattering (DLS studies. The purplish color of the redispersed

NPs in the optical photograph and an instant redshift (~18 nm shift) in the plasmon band, along with increased scattering intensity (Δabs at 750nm is ~0.06), confirms the immediate aggregation process (Figure 4a.9d). Eventually, all the AuNP aggregates precipitated from the solution within 8 h. The DLS study also confirms the instant aggregation process, where the hydrodynamic size increased from ~154 to ~1080 nm (Figure 4a.9b). Now, it is important to estimate the amount of HK enzyme required to dephosphorylate 250 µM ATP and redisperse the NP-precipitates completely (Figure 4a.8). It was observed that 9 units of HK enzyme were sufficient to completely redisperse the AuNP precipitates (~584 to ~520 nm). Thus, logically, the amount of HK enzyme (disassembling trigger) should be much lower than 9 units to achieve autonomous aggregation.

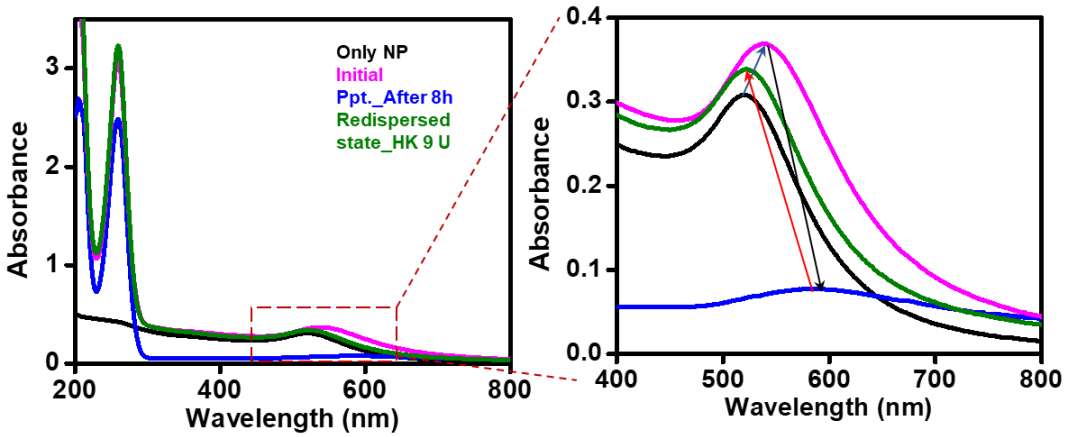


Figure 4a.8. Dynamic self-assembly of [+] AuNPs in the presence of 250 µM ATP. The absorption data depicts an instant aggregation, followed by complete precipitation of [+] AuNPs after adding ~250 µM ATP. The addition of 9U HK/3 mL led to complete redispersion of AuNPs.

Accordingly, 3 units of HK enzyme were used in each disassembly step (Figure 4a.9d). After the addition of 3 units of HK to the ATP-AuNP precipitate in cycle-1, NPs instantly got redispersed. However, the NPs immediately re-aggregated because of the excess ATP present in the supernatant solution, as evident from the optical photographs, DLS, and absorption studies (Figure 4a.9a, b, and c). DLS studies showed that the ATP-AuNP precipitates didn't redisperse completely (1080 ± 13.2 nm to 154 ± 1.4 nm), which can be attributed to the excess ATP present in the solution. This assembly-disassembly cycle continued till all the ATP was consumed by the HK enzyme. After the complete consumption of ATP, AuNPs stay redispersed (Figure 4a.8d), and a complete revival of the wine-red color was obtained. In short, the dynamic self-assembly process achieved through pathway 1 consists of autonomous

assembly and triggered disassembly steps. In the presence of 250 µM ATP, three cycles of assembly and disassembly process were achieved.

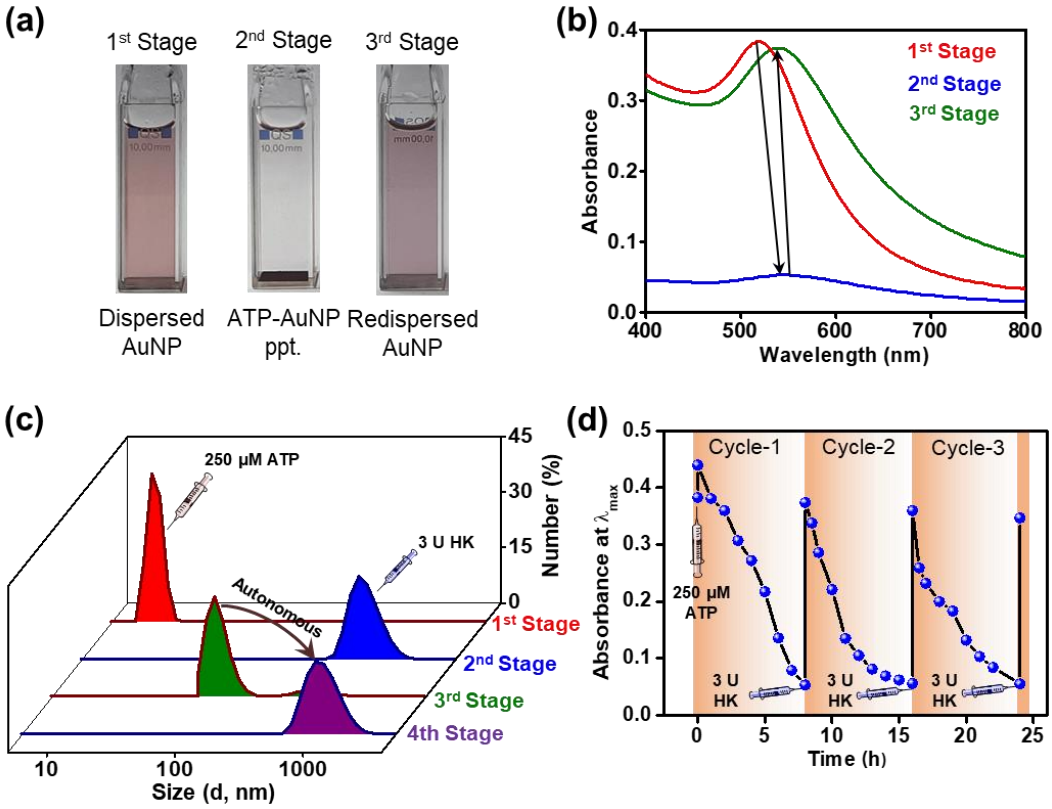


Figure 4a.9. Autonomous self-assembly of AuNPs through pathway 1. (a) Optical photographs show the dispersed (1st stage), self-assembled (2nd stage), and redispersed (3rd stage) stages of autonomous self-assembly of AuNPs. The assembly step was triggered by ATP (250 mM), and the disassembly step was initiated by the HK enzyme (3 units). The corresponding changes in the (b) UV-vs absorption and (c) hydrodynamic diameter (DLS data) are shown. (d) Three cycles of autonomous self-assembly process were achieved with 250 mM ATP.

In principle, the number of dynamic cycles can be increased by enhancing the concentration of the aggregating trigger ATP. Accordingly, the dynamic self-assembly of AuNPs was performed with varying concentrations of ATP (350 µM - 1 mM) (Figure 4a.10). Here, as well, the disassembly process was triggered by adding 3 units of HK enzyme in each step to achieve autonomous reversibility in the self-assembly process. The number of assembly-disassembly cycles was increased from 3 to 8, as the ATP concentration increased from 250 µM to 1 mM (Figure 4a.10). Interestingly, we observed a complete shuttling between plasmonically *'off'* and *'on'* states, along with negligible damping in the NP absorbance even after 8 cycles. The pH of the solution was also monitored throughout the assembly-disassembly cycles. A negligible change in pH was observed. In the case of 1 mM ATP concentration, the pH was

found to be maintained at ~3.6. Towards the end of the cycles, it was noticed that the assembly time was longer. As a result, the extent of aggregation decreased, leading to a partial precipitation of AuNPs (Figure 4a.10). This can be attributed to the decrease in the ATP concentration after each cycle, which in turn will slow down the assembling kinetics. Therefore, new knowledge of the dynamic self-assembly process was acquired, where the disassembly process was faster than the assembly step, but still, the autonomous self-assembly dominated because of the excess concentration of assembling triggers.

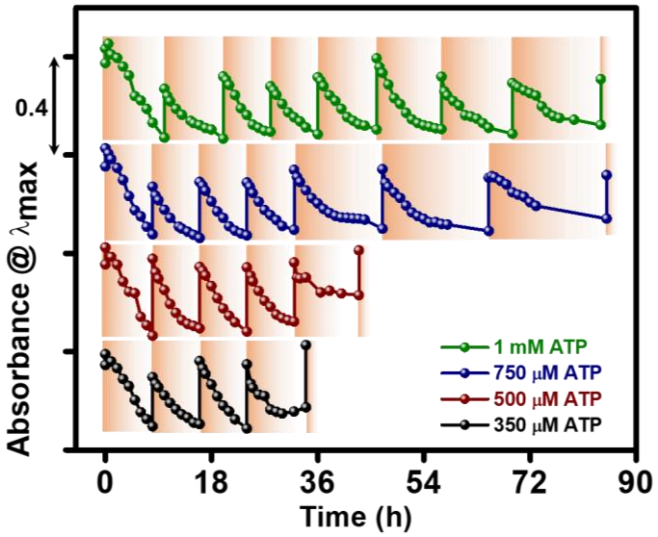


Figure 4a.10. A plot showing the increase in the number of dynamic self-assembly cycles, with an increase in the concentration of ATP-trigger.

4a.4.4 Pathway 2: Excess HK-driven autonomous reversibility

In pathway II, autonomous reversibility was attained in the presence of excess disassembling HK trigger. Specifically, the ATP-triggered assembly between the AuNPs was carried out in the presence of an excess disassembling HK trigger. Here, it must be noted that the construction of a dynamic self-assembly process requires faster assembly kinetics than the disassembly process, along with the disassembly process being autonomous (Figure 4a.11a). To meet the requirement, the self-assembly study was performed in 3 mL 10 mM HEPES buffer, having a pH of ~7.4. At this pH, ATP will be fully deprotonated, leading to faster assembly kinetics. This is because of the stronger electrostatic attraction between the oppositely charged ATP and AuNPs, as compared to the self-assembly process at pH ~3.6 (pH in pathway I was ~3.6). This

led to the aggregation of ~6 nM [+] AuNPs even in much lower ATP concentration (~5 μM) (Figure 4a.11b). Now, to obtain autonomous reversibility, dynamic self-assembly was performed in the presence of excess HK (~0.36 units of HK, ~6 times higher than required) with ~250 equivalents of G at 37 ^{o}C. Upon addition of 5 μM ATP to the solution, the [+] AuNPs underwent an instant aggregation, accompanied by a ~10 nm red-shift in the λ_{max} value and a simultaneous increase in the scattering intensity (Δabs. @ 750 nm ~0.051) (Figure 4a.11b). Unlike in pathway 1, the excess disassembling HK trigger in solution resists the complete precipitation of the AuNPs aggregates. This led to the formation of a plasmonically active-controlled aggregate state. However, because of the excess HK enzyme present in the system, NPs started disassembling within ~15 min. The controlled aggregates eventually redispersed completely within 35-40 min (Figure 4a.11b). After the 1st cycle, a fresh aliquot of 5 μM ATP was added to start the next cycle of the assembly step. The HK is already in excess in the solution, which results in the autonomous disassembly process. Thus, the domination of the disassembly process in pathway 2 leads to the shuttling between the completely dispersed and controlled aggregate states of AuNPs. In this way, we were successful in extending the triggered assembly and autonomous disassembly steps for 5 cycles (Figure 4a.11c). It must be noted that the autonomous disassembly step was found to be comparatively slower after each cycle. This slow disassembly kinetics can be attributed to the electrostatic attraction between [+] AuNPs and negatively charged ADP molecules in the system, which increased with each cycle. As the disassembly kinetics highly depend upon the activity of the HK enzyme, one can tune the lifetime of the transient self-assembled state (here, the controlled aggregates) by varying HK amounts. Interestingly, varying the amounts of HK enzymes had a significant effect on the disassembly kinetics, which allowed us to tune the lifetime of the controlled aggregated state. The lifetime of the self-assembled state was monitored with respect to the temporal change in the λ_{max} value (Figure 4a.11d). In the presence of 0.06 units of HK enzyme, the self-assembled state '*lived'* for ~130 min, after which the disassembly process dominated, and the controlled aggregates completely redispersed in ~270 min. Thus, the total time took to complete one assembly-disassembly cycle was ~270 min. With 0.18 and 0.36 units of HK enzyme, two and three cycles of triggered assembly and autonomous disassembly were observed in ~270 min (Figure 4a.11d). In this way, we can successfully tune the lifetime of the transient controlled aggregated state by regulating the disassembly kinetics, which is a desired feat to realize temporal functions from the self-assembled products.

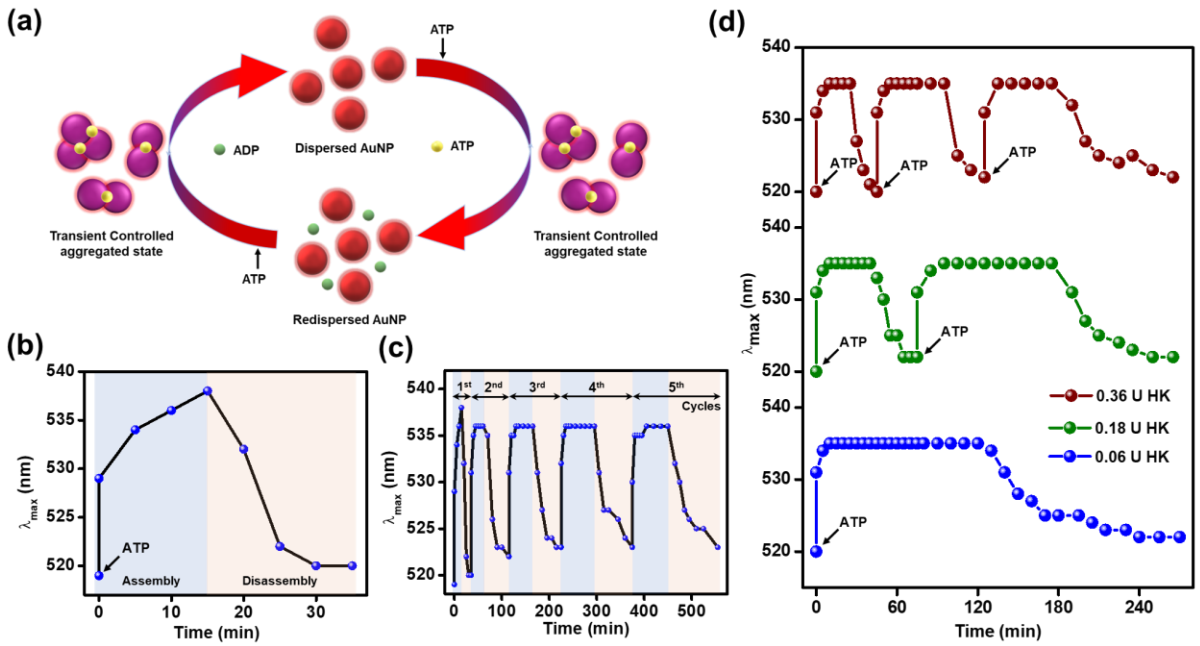


Figure 4a.11. Autonomous self-assembly of AuNPs through pathway 2. (a) Schematics showing the excess HK-driven autonomous reversibility in ATP-triggered dynamic self-assembly process. The reaction mixture contained 6 nM AuNPs, 5 μM of ATP, and 0.36 units of HK enzyme (excess). (b) A plot showing the temporal change in the λ_{max} of AuNPs during one cycle of the self-assembly process. (c) With 0.36 units of HK enzyme, five cycles of ATP-triggered dynamic self-assembly process was achieved. (d) A plot showing the increase in the number of dynamic self-assembly cycles, with an increase in the concentration of HK enzyme, within a time of ~270 min.

Even though autonomous reversibility was achieved in both pathways, the nature of the aggregated states was different (Figure 4a.7). Another important aspect is the tunability in the lifetime of the aggregated sates achieved in pathways 1 and 2. Based on the amount, moreover, one can also tune the extent of aggregation and the lifetime of the transient state by regulating the competing kinetics in the assembly and disassembly steps. The recyclability will be obtained by adding HK enzyme/ATP molecule at each cycle, which will trigger the autonomous activity in pathways I and II, respectively.

4a.5 Conclusions

The current study reveals the importance of different assembly pathways in obtaining autonomous reversibility in biomimetic self-assembly based on AuNP and ATP systems. ATP-triggered dynamic self-assembly of AuNPs was achieved through the controlled regulation of electrostatic interactions by coupling with a phosphoryl transferase enzyme HK as the

disassembling agent. The HK-driven conversion of ATP into ADP lowered the electrostatic attraction that glued the AuNPs in the self-assembled state, leading to the complete redispersion of NPs. Interestingly, in the present study, we observed a complete transition between a dispersed and a precipitated state, which is extremely challenging in NP-based systems. Further, the autonomous reversibility function was installed into the system in two ways. In pathway 1, the dominance of the aggregating trigger (excess ATP was present) over the disassembling trigger (HK enzyme) ensured an autonomous assembly process. This led to the autonomous reversible shuttling between dispersed and completely precipitate states. The number of autonomous-assembly and triggered-disassembly cycles was increased from 3 to 8, as the ATP concertation was increased from 250 μM to 1 mM. In pathway 2, the dominance of the disassembling trigger (excess HK was present) over the assembling trigger (ATP) ensured an autonomous disassembly process. Unlike in pathway 1, the excess disassembling trigger in solution resists the complete precipitation of the AuNPs aggregates. This led to the formation of a plasmonically active-controlled aggregate state. As a result, a reversible shuttling between a completely dispersed state and a controlled aggregate state was achieved. Three cycles of triggered assembly and autonomous disassembly processes were achieved in pathway 2. Even though autonomous reversibility was achieved through both pathways, the nature of the aggregated states was different: complete precipitate state (pathway 1) vs controlled aggregated state (pathway 2). This study helped us to understand the fundamental dissimilarity between the two pathways, which has not been addressed so far. More interestingly, precise control of the competing kinetics involved in the assembly and disassembly steps allowed us to achieve the much-needed tunability in the lifetime of the self-assembled state, which can be beneficial to realize temporal functions. Therefore, the current study can be considered as a model system for understanding pathway-dependent autonomous reversibility in various synthetic, biological, and biomimetic self-assembled systems.

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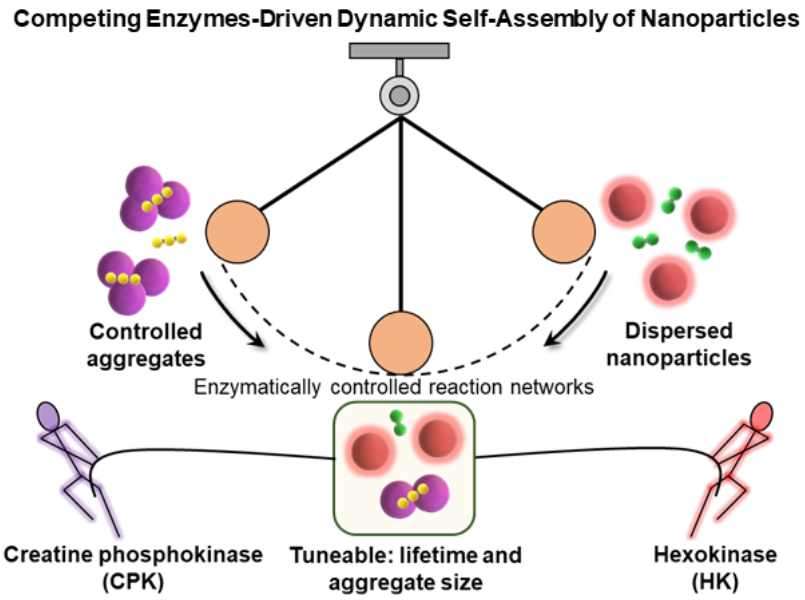
Chapter 4b

Sequential and Tandem Activity of Antagonistic Enzymes Regulate the Dynamic Self-Assembly of Gold Nanoparticles



4b.1 Abstract

Biological systems function through non-equilibrium processes. Therefore, designing artificial non-equilibrium systems, such as oscillation, self-healing materials, etc., is essential in understanding biological systems better. This has motivated researchers to design adaptive materials with spatiotemporal behavior. In this direction, we have designed a gold nanoparticle (AuNP)-based dynamic self-assembled system, by regulating the electrostatic interaction through the sequential and tandem activity of two antagonistic enzymes, hexokinase (HK) and creatine phosphokinase (CPK), that consume and generate adenosine triphosphate (ATP), to trigger the disassembly and assembly processes, respectively. The sequential addition of these two competing enzymes showed a complete shuttling between a dispersed state and a precipitated state of AuNPs, which is scarcely observed in NP-based systems. More importantly, our study reveals that both these antagonistic enzymes can work under the same reaction conditions. Here, multiple dynamic self-assembly cycles were achieved under a tandem condition, when both the enzymes were simultaneously present in excess in the system. This was achieved by the in-situ generation and consumption of ATP through enzymatically controlled reaction networks. This also resulted in the formation of a distinctly different self-assembled state - the controlled aggregate - which is plasmonically active. Furthermore, the lifetime of the controlled aggregated state and the degree of aggregation were successfully modulated under the tandem condition by tuning the amounts of enzymes and substrates, which will be helpful in future in developing active materials with temporal functions.

4b.2 Introduction

Life operates under nonequilibrium.[1-5] Therefore, it requires a continuous influx of energy to - sustain and maintain its functions.[6-10] For this, nature has selected a variety of highly energetic biomolecules, which undergo a chemical change to release the energy needed to maintain a steady state.[5] The most common ones are guanosine triphosphate (GTP) and adenosine triphosphate (ATP), which triggers the dissipative self-assemblies of microtubules and actin filaments, respectively.[11,12] For the better understanding of how life functions, researchers have now started replicating the biological self-assemblies in artificial systems.[13-21] This provides a unique opportunity to create active matter with life-like properties, such as self-healing, oscillation, repair, self-replication, etc.[22-25] In this direction, the ATP molecule has shown promising results in creating artificial complex systems having spatiotemporal behavior,

similar to the ones observed in biological assemblies.[26-37] The highly energetic ATP molecules generally trigger the monomer building blocks to undergo a self-assembly process to form a higher-order architecture. However, the self-assembled system dissipates back to the monomeric state in the presence of a dephosphorylating enzyme.[38,39] A suitable dephosphorylating enzyme can be used to hydrolyse the ATP into adenosine diphosphate (ADP) or adenosine monophosphate (AMP) or adenosine and phosphate ions (Pi). Once the ATP is completely consumed, a fresh addition of the ATP molecule eventually triggers the next dissipative cycle. Therefore, the addition of co-factor ATP molecules becomes essential at each cycle to maintain the steady state. On top of that, the enzymatic cycles keep on accumulating waste, for example, ADP molecules in the system. This limitation of waste accumulation, can leads to dampness in the reversibility study. This could be overcome by converting the waste ADP molecules back into ATP (co-factor regeneration), which in turn will trigger the next dynamic cycle. This approach essentially requires another complementary enzyme that can convert the ADP molecules into ATP. This fascinating chemistry was employed by Whitesides's group for the large-scale enzymatic synthesis of glucose-6-phosphate.[40] Later, the groups of George and Hermans employed analogous chemistry to introduce transient helicity into the supramolecular systems.[29,30] In a pioneering study, George and co-workers have demonstrated conformational changes in self-assembled naphthalene diimide derivative, at the expense of ATP molecules through tandem enzymatic reactions. These conformational changes were accompanied with transient helicity, mimicking the biological system (Figure 4b.1).[29]

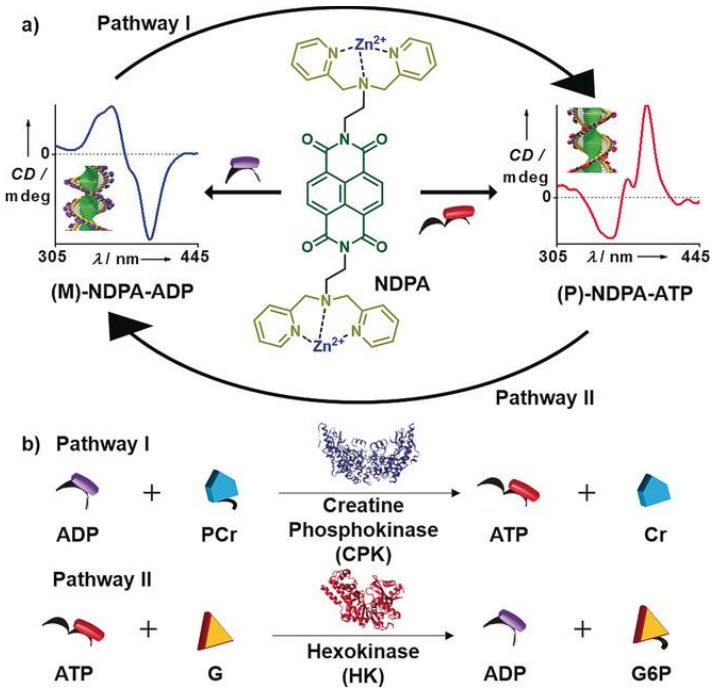


Figure 4b.1. Schematic shows the ATP-triggered temporal control over supramolecular helicity through tandem actions of HK and CPK enzymes. Reproduced with permission from ref 29. Copyright 2017, John Wiley & Sons.

Further, Spruijt and Landfester's groups have used this chemistry to demonstrate reversible coacervation.[32,41] Very recently, Prins and co-workers have also shown an elegant approach to create transient complex fluorescence pattern formation in agarose gel by controlling the ATP-concentration gradient through the competing actions of the two antagonistic enzymes (Figure 4b.2).[37]

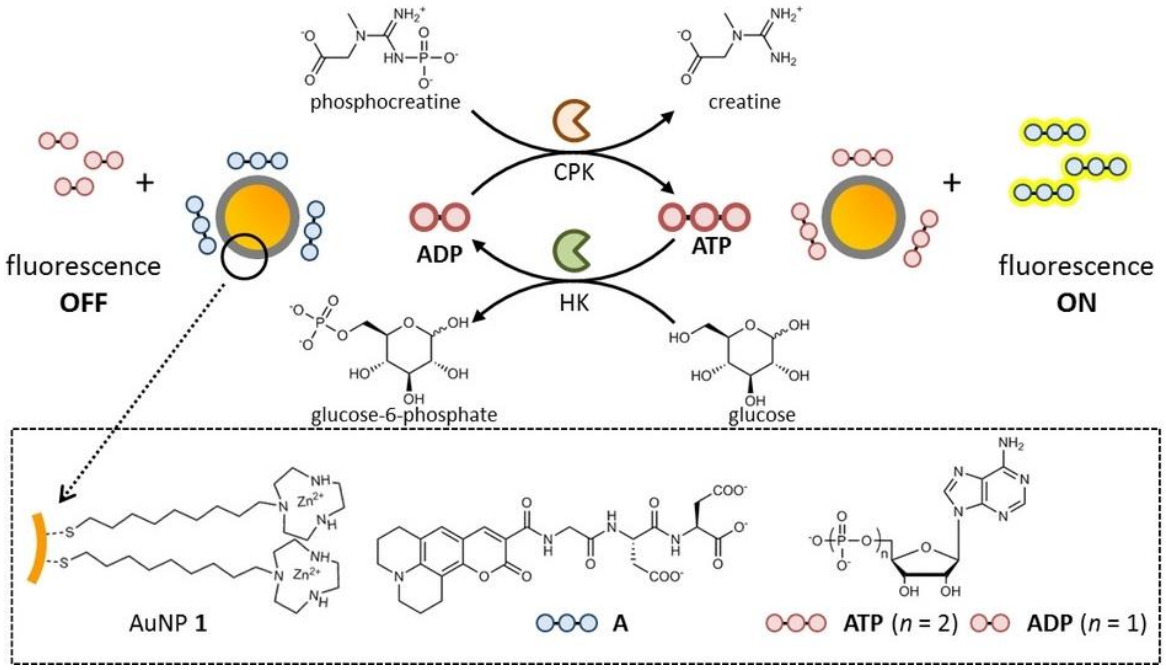


Figure 4b.2. Schematic shows that the potential of enzymatic reaction networks in temporally controlling the ATP concentration, forming a transient fluorescence response. Reproduced with permission from ref 37. Copyright 2023, John Wiley & Sons.

In order to check the suitability of this captivating chemistry of enzymatically controlled reaction networks beyond molecular systems, we selected positively charged AuNPs as our building blocks. The choice of plasmonic AuNPs will enable us to visualize the dynamic self-assembly process through visual color changes as well. On top of that, an NP-based dynamic system is quite challenging as it needs to overcome the strong van der Waals attraction force in the disassembly step. In the current study, we explored the role of two antagonistic enzymes in regulating the electrostatic interaction between nucleotides and [+] AuNPs, and choreograph a NP-based dynamic self-assembled system. [+] AuNPs tend to undergo aggregation through electrostatic self-assembly with negatively charged nucleotide molecules.[36,42] Addition of the phosphoryl transferase enzyme (hexokinase, HK) led to the dephosphorylation of ATP into

ADP. This will reduce the attraction between the electrostatically bounded AuNPs, triggering the redispersion of the self-assembled state. The addition of fresh ATP would be the most intuitive way to initiate the second cycle of self-assembly, and such an approach would require the constant addition of ATP fuel to realise multiple cycles. However, our idea was to mimic the biological self-assembly process to trigger the next cycle of assembly by phosphorylating ADP back to ATP using creatine phosphokinase (CPK) enzyme. This enzymatic regeneration of ATP will reinstate the electrostatic attraction between the AuNP monomers, thereby triggering the assembling process (Figure 4b.3). The sequential addition of these two competing enzymes led to a complete transition from a plasmonically-off precipitate state to a plasmonically-on dispersed state, which is scarcely reported in the literature. Additionally, these two competing enzymes were found to act in tandem when present together in the system. In this case, the shuttling was observed between a plasmonically-on controlled aggregate state and a plasmonically-on dispersed state (Figure 4b.3). Thus, the composition of the assembled state participating in the assembly-disassembly process was tuned by controlling the pathway of the self-assembly process. More interestingly, the lifetime of the transient self-assembled state, as well as the extent of aggregation were regulated by varying the amounts of enzymes and substrates (glucose, G and phosphocreatine, PCr). Thus, the present study calls for the need of controlling the pathways of the self-assembly process in nanoparticles to mimic the reaction networks observed in biological systems.

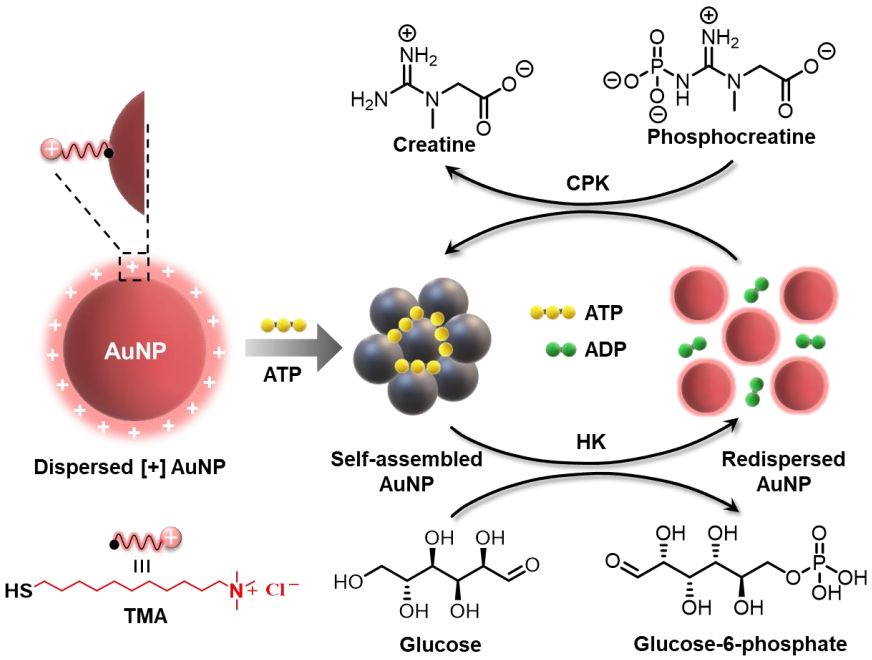


Figure 4b.3. Design of a dynamic self-assembly process in AuNPs by exploring the tandem activity of antagonistic enzymes, HK and CPK.

4b.3 Experimental Section

The synthesis and characterization of AuNPs, as well as the absorption and microscopy studies to monitor the dynamic self-assembly process, were already discussed in Chapter 2.

4b.4 Results and Discussion

4b.4.1 Self-assembly of [+] AuNPs with negatively charged nucleotides

It has been clear from previous studies that positively charged AuNPs can undergo a self-assembly process with negatively charged ATP molecules through electrostatic interaction.[36,42,43] Our target was to install dynamicity into the self-assembly system, that would be controlled by the enzymes as seen in biological systems. In this context, we envisaged to introduce two competing phosphoryl transferase enzymes - HK and CPK. HK will dephosphorylate the ATP to ADP; whereas CPK will phosphorylate ADP back to ATP. An optimised condition was figured out, where a minimum concentration of ATP could trigger the electrostatic aggregation between the [+] AuNPs; whereas a similar concentration of ADP would fail to aggregate the [+] AuNPs. Under such an optimised condition, the assembly and disassembly processes between the [+] AuNPs can be enzymatically regulated by controlling the ATP-to-ADP conversation and vice-versa. To check this hypothesis, we first synthesized permanently positively charged AuNPs functionalized with N,N,N-trimethyl(11-mercaptoundecyl)ammonium chloride ligand and dispersed in 3 mL 10 mM HEPES buffer. The pH of the solution was maintained at ~7.4. The addition of ~5 µM ATP solution to ~6 nM [+] AuNPs led to an instant aggregation followed by a complete precipitation of the NPs (Figure 4b.4b,c). In a separate control experiment, it was observed that ~5 µM ADP failed to aggregate ~6 nM [+] AuNPs (Figure 4b.4b,c). Time-dependent UV-vis absorption studies corroborated well with the visual observation. Significant spectral changes corresponding to aggregation and precipitation were observed in the presence of ~5 µM ATP (~0.3 decrease in the optical density @λ_{max}, along with ~30 nm red-shift in the λ_{max}) (Figure 4b.4b,c). On the other hand, negligible spectral changes were observed in the presence of ~5 µM ADP (Figure 4b.4b,c). This confirms that the ATP and ADP can serve as the assembling and disassembling triggers, respectively. Thus, in principle, an enzymatic conversion of ATP-to-ADP and ADP-to-ATP can regulate the self-assembly process in [+] AuNPs, which is described in the next sections.

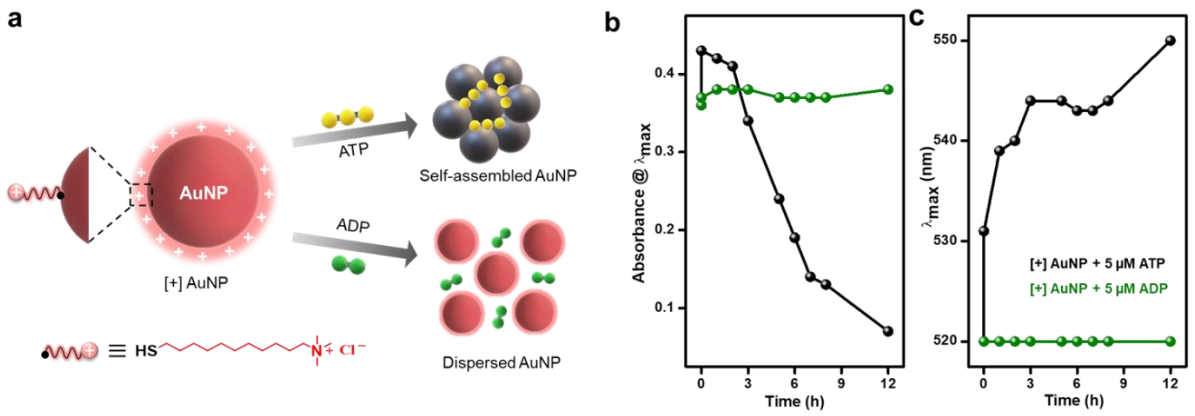


Figure 4b.4. Interaction between [+] AuNPs and negatively charged ATP and ADP molecules. (a) Schematic shows the selective aggregation of [+] AuNPs in the presence of ATP molecules, while NPs stay dispersed in the presence of ADP molecules. Plots show the temporal changes in the (b) absorbance @ λ_{max} and (c) λ_{max} of ~6 nM [+] AuNPs in the presence of ~5 μM ATP and ~5 μM ADP.

Our first task was to optimise and confirm the activity of HK and CPK enzymes to disassemble and assemble [+] AuNPs, by converting ATP-to-ADP and ADP-to-ATP, respectively. The following sections summarise the independent self-assembly studies in the presence of HK and CPK enzymes.

4b.4.2 HK-driven disassembly of [+] AuNPs

As mentioned earlier, the addition of ~5 μM ATP solution to ~6 nM [+] AuNPs led to an instant aggregation followed by a complete precipitation of the NPs. As per our hypothesis, the conversation of ATP into ADP in the electrostatically bonded [+] AuNPs assemblies will weaken the electrostatic attraction between the [+] AuNPs, leading to the disassembly process. Now, HK enzyme is reported to dephosphorylate ATP into ADP in the presence of glucose (during this process glucose is converted into glucose-6-phosphate, G-6-P). In a typical experiment, ~5 μM ATP was added into ~6 nM [+] AuNPs dispersed into 3 mL 10 mM HEPES buffer (pH ~7.4). The self-assembly process was monitored through absorption, TEM, and ^{31}P NMR techniques. [+] AuNPs underwent an instant aggregation with a ~10 nm red-shift in the λ_{max} value, followed by the complete precipitation within ~8h (Δabs. @ λ_{max} ~0.3) (Figure 4b.5b). Next, 4 μL of ~0.06 units of HK and ~250 equiv. of glucose was added to the electrostatically bonded [+] AuNPs – [-] ATP precipitates. There was an instant enhancement in the absorbance value, confirming the breaking and disassembly of the AuNP precipitates (Figure 4b.5b). A complete redispersion of AuNPs was observed within ~4h, with ~100%

revival in the absorption intensity (Figure 4b.5b). The next cycle of assembly process was initiated by a fresh addition of ~5 µM ATP, which resulted in the instant aggregation and re-precipitation in ~8h, with a ~30 nm red-shift in the λ_{max} (Figure 4b.5b). Optical photographs and TEM studies also confirm the shuttling between the dispersed and completely precipitated states of AuNPs (Figure 4b.5c and Figure 4b.6). It is not a trivial task to visualize the shuttling between a dispersed and a completely precipitated states of AuNP, as the strong van der Waals attraction between the NPs would resist the complete re-dispersal.[25] The effective activity of HK enzymes in dephosphorylating ATP-to-ADP could be the key in completely redispersing AuNP precipitates.

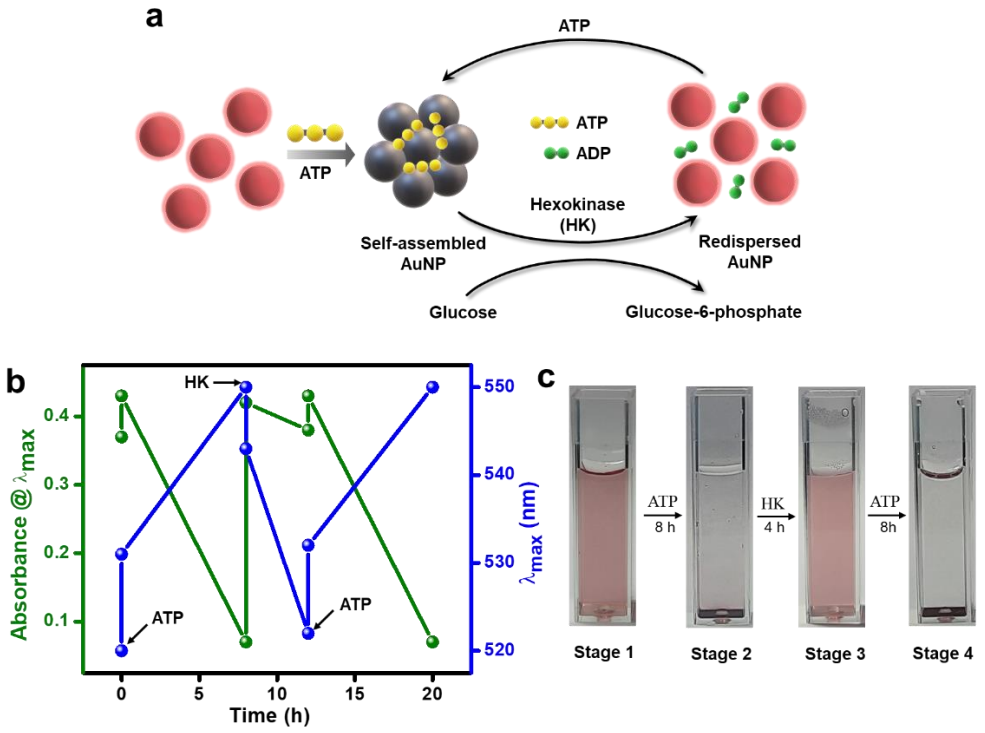


Figure 4b.5. ATP-triggered dynamic self-assembly of AuNPs. (a) Schematic shows the ATP-triggered dynamic self-assembly of AuNPs coupled with HK enzyme. (b) Plot shows the simultaneous changes in absorbance @ λ_{max} and λ_{max} with time during the dynamic self-assembly process. (c) Optical photographs show a complete shuttling between dispersed AuNPs and ATP-AuNP precipitates.

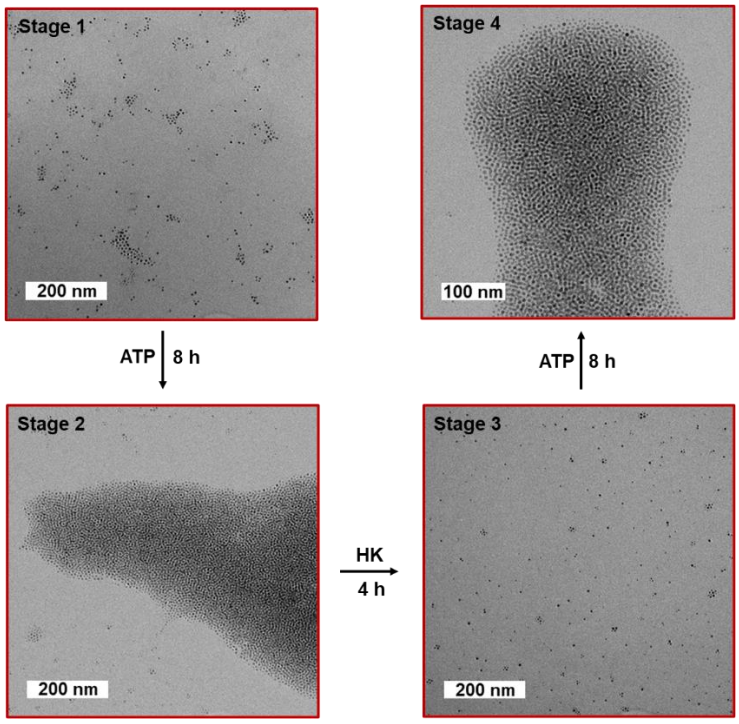


Figure 4b.6. TEM images corresponding to different stages of ATP-triggered assembly and HK-driven disassembly processes.

To confirm the HK-driven conversion of ATP-to-ADP and glucose to G-6-P, the disassembly process was monitored through time-dependent ^{31}P NMR spectroscopy (~400 times excess ATP, HK, and glucose were used in for the NMR studies: 2 x 10^{-3} M ATP, 24 units of HK, 250 equiv. glucose) (Figure 4b.7a). ATP molecules have a characteristic β-phosphate peak at ~-23 ppm, which is absent in the case of ADP. During the disassembly process, the β-phosphate peak started diminishing with the appearance of a new peak at ~3 ppm (Figure 4b.7a). This new peak corresponds to the G-6-P, which was formed as a by-product during the HK assay (a slight shift in the peak position was observed when compared to the pure G-6-P, which could be attributed to the influence of buffer and AuNPs). A clear disappearance of the β-phosphate peak conclusively confirms the HK-driven dephosphorylation of ATP-to-ADP, which further weakens the electrostatic attraction between the AuNPs causing the complete disassembly.

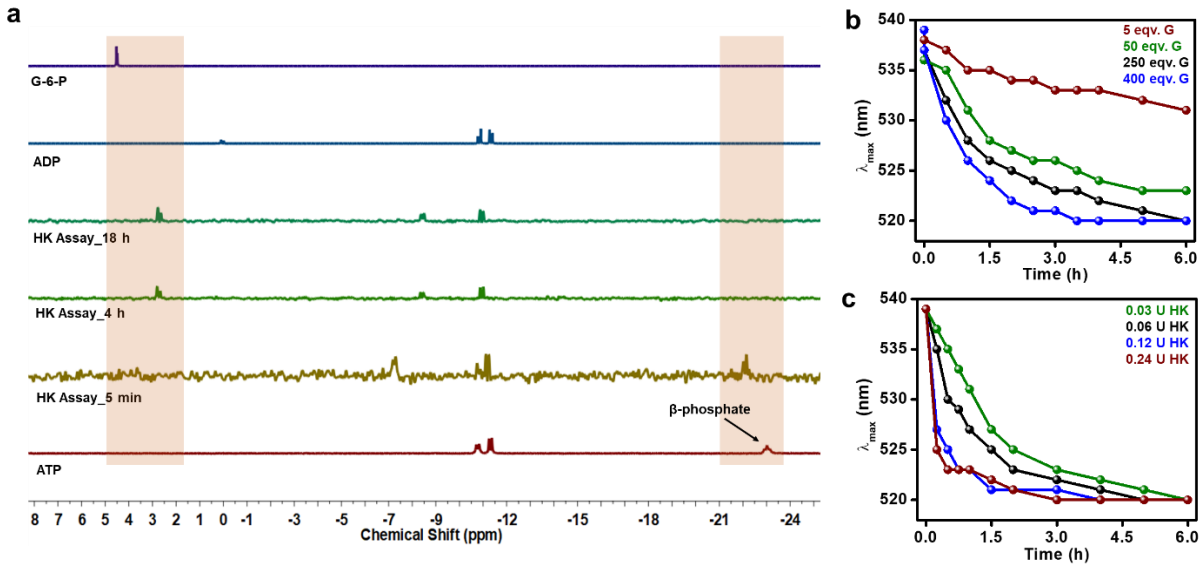


Figure 4b.7. HK-driven disassembly of [+] AuNPs. (a) A time-dependent ^{31}P NMR study confirms the phosphoryl transferase activity of the HK enzyme, i.e., ATP to ADP conversion during the disassembly of AuNPs. Kinetics of AuNPs-disassembly and the lifetime of the assembled state were controlled by varying the concentrations of (b) glucose and (c) HK in the system.

Further, the extend of the disassembly process, and thereby the lifetime of the aggregated states can be regulated by controlling the concentration of HK enzyme and glucose. A complete redispersal of AuNPs was observed in the presence of 400 equiv. concentration of glucose ($\Delta\lambda$ ~0); whereas an incomplete redispersal was observed at lower glucose concentrations ($\Delta\lambda$ ~12, 4, 0 nm for 5, 50, 250 equiv. of glucose) (Figure 4b.7b). Likewise, variations in the HK concentration influenced the lifetime (τ) of the aggregated state: τ increased from 1.5 h to 6 h, as the concentration of HK was decreased from 0.24 units to 0.03 units (Figure 4b.7c). All these studies once again confirm the active role of HK and glucose in dephosphorylating ATP-to-ADP, and thereby triggering the disassembly process.

4b.4.3 CPK-driven assembly of [+] AuNPs

In the previous section, the action of HK in dephosphorylating ATP to ADP and, thus, disassembling the AuNP aggregates was optimised. Now, the next cycle of assembly should be optimised to achieve the dynamic and multiple self-assembly process. The addition of a fresh batch of ATP will obviously trigger the next cycle of assembly; however, it would also lead to the accumulation of waste in the form of ADP. Also, nucleotides are considered as fuels in self-assembly, and thus, their accumulation as waste and ineffective use is not desirable. Our

idea was to convert the waste accumulated in the form of ADP back to ATP using a phosphoryl transferase enzyme, and then use this regenerated ATP for the next assembling process (Figure 4b.8a). This will also prevent the multiple addition of ATP fuels into the system. In this context, we have selected creatine phosphokinase (CPK) enzyme CPK as the phosphoryl transferase enzyme, which will convert ADP back to ATP in the presence of phosphocreatine (PCr). During this process PCr will be converted to creatine (Cr). CPK-triggered assembly of [+] AuNPs in the presence of ADP and PCr was optimised as follows. In a typical reaction, ~5 µM ADP was added to ~6 nM [+] AuNPs dispersed into 3 mL 10 mM HEPES buffer (pH ~7.4). As expected, [+] AuNPs retained their stability, without undergoing any aggregation (ADP is a weak aggregating agent at this concentration) (Figure 4b.8b). This reaction mixture was incubated at 37 ^{0}C, and 2 µL of 2 units of CPK and 3 equiv. PCr were added. An immediate aggregation of [+] AuNPs was observed, leading to complete precipitation within ~12h (Figure 4b.8b). Optical photographs and TEM images corroborate well with the aggregation results obtained from UV-Vis absorption studies (Figure 4b.8c).

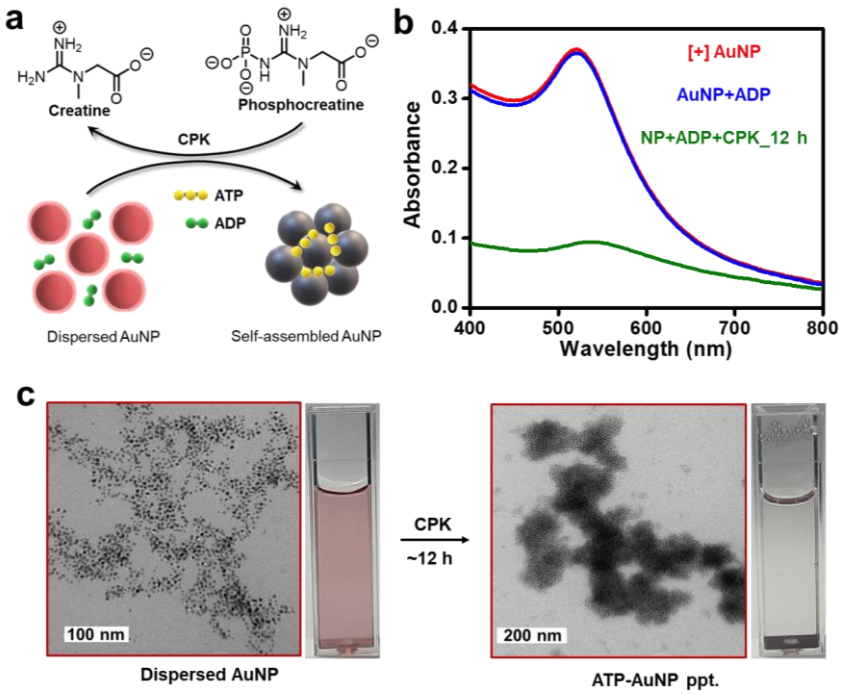


Figure 4b.8. CPK-driven self-assembly of AuNPs. (a) Schematic shows the CPK-driven ADP to ATP conversion, resulting in the self-assembly of AuNPs. (b) Absorption study proves the CPK-driven ADP to ATP conversion leading to the NP-aggregation, followed by a complete precipitation of AuNPs in ~12 h. (c) TEM images and optical photographs confirm the NP-aggregation because of the CPK-driven ADP to ATP conversion.

CPK-triggered assembling process in [+] AuNPs involves the phosphorylation of ADP to ATP, which was confirmed using ^{31}P NMR spectroscopy (Figure 4b.9a) (~400 times excess ADP, CPK, and PCr were used for the NMR studies: 2 x 10^{-3} M ADP, 80 units of CPK, 2 equiv. PCr). After the addition of CPK and PCr into a mixture of ADP and [+] AuNPs, n emergence of a new peak at ~ -23 ppm was observed which corresponds to the β-phosphate peak of ATP molecules (Figure 4b.9a). Alongside, a new peak at ~1.5 ppm was emerging with time which corresponds to the free-phosphate group (Figure 4b.9a). As time proceeded, the intensity of peaks corresponding to β-phosphate and free-phosphate groups kept on increasing; whereas the peak corresponding to the PCr was decreasing (Figure 4b.9a). Thus, the NMR studies conclusively prove that the CPK transfers the phosphates from the PCr to the ADP to from ATP molecules during the self-assembly process.

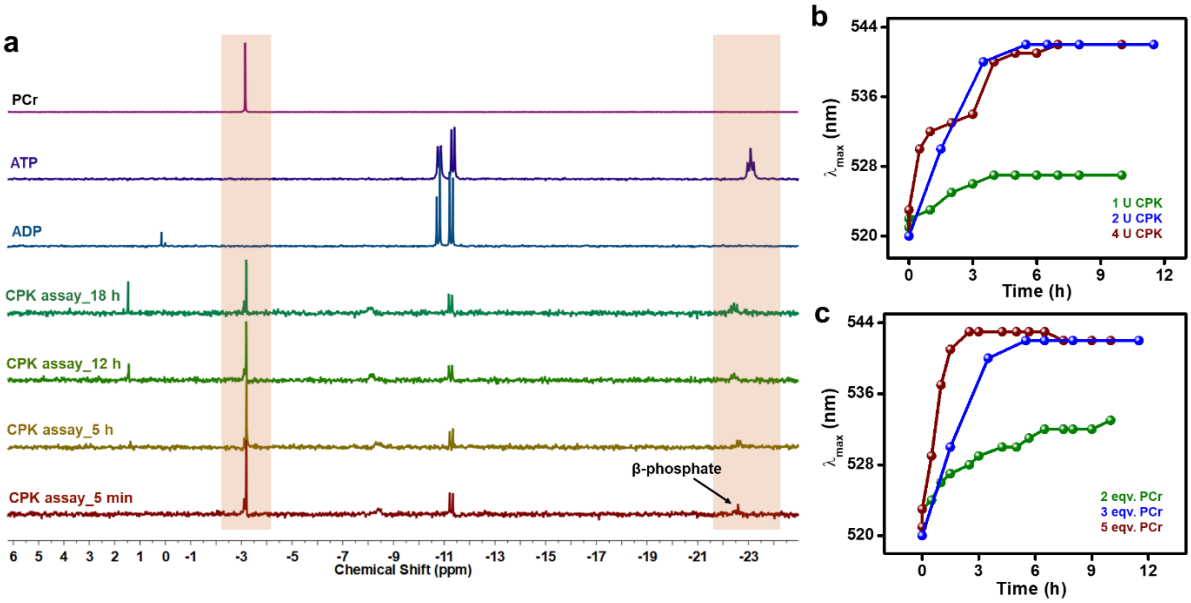


Figure 4b.9. CPK-driven self-assembly of [+] AuNPs. (a) A time-dependent ^{31}P NMR study proves the phosphoryl transferase activity of the CPK enzyme, i.e., ADP to ATP conversion during the self-assembly process. Kinetics of AuNPs-disassembly and the lifetime of the assembled state were controlled by varying the concentrations of (b) CPK and (c) PCr.

In this case as well, the assembly kinetics was highly dependent on the amount of CPK (Figure 4b.9b) and PCr added (Figure 4b.9c). The extend of assembly process was more at higher CPK concentrations, as expected: 2-4 units of CPK yielded bigger aggregates with a ~22 nm red-shift in λ_{max}; whereas 1 unit of CPK resulted in the formation of much smaller aggregates with a ~7 nm red-shift in the λ_{max} (Figure 4b.9b). Apart from the lifetime of the self-assembled state,

the extent of the self-assembly also varied significantly. A similar trend was observed in the assembling process by increasing the concentration of PCr (Figure 4b.9c).

4b.4.4 Dynamic self-assembly of AuNPs by the sequential addition of two competing enzymes, HK and CPK

Next objective was to couple both the enzymatically driven assembly and disassembly cycles. This will ensure a dynamic self-assembly without the need of multiple addition of the co-factor ATP molecules in the system (Figure 4b.10a). This is not a trivial task, because a common reaction condition has to be optimised wherein both the enzymes will be active. The entire process of self-assembly was monitored using UV-vis absorption spectroscopy and TEM studies. In a typical experiment, ~5 µM ATP was added to ~6 nM [+] AuNPs dispersed into 3 mL 10 mM HEPES (pH ~7.4). This triggered the self-assembly process with a ~22 nm red shift in the λ_{max}, eventually leading to the complete precipitation of AuNPs (Figure 4b.10c). Addition of 0.06 units of HK / and ~250 equiv. glucose to the solution led to the disassembly of AuNPs (ATP is converted into ADP by HK, which decreases the electrostatic attraction between the AuNP aggregates) (Figure 4b.10b). Further, 2 units of CPK and 3 equiv. PCr was added to the redispersed AuNPs to regenerate the ATP aggregating trigger from ADP. To our delight, an immediate aggregation of AuNPs, followed by the complete precipitation of the system was observed (Figure 4b.10b). This confirms that the CPK is able to regenerate ATP from ADP under the experimental conditions, and trigger the electrostatic attraction between the [+] AuNPs. Each assembly-disassembly stage was monitored through visual colour inspection and TEM studies as well, which confirmed that the step-by-step addition of these two complementary enzymes led to the shuttling between a dispersed state and a precipitated state (Figure 4b.10c,d). To achieve the reversibility in the dynamic self-assembly process, we repeated the assembly-disassembly cycle four times by the sequential addition of these two complementary enzymes (Figure 4b.10b). The dynamic self-assembled cycles were monitored through the temporal changes in the λ_{max}, where we observed a significant ~20 nm shift in the assembly cycle, and > 90% absorption revival in the disassembly step. Thus, the current study ensures that the two complementary enzymes – HK and CPK - can work in a single system to achieve a dynamic self-assembly process between [+] AuNPs and nucleotide molecules.

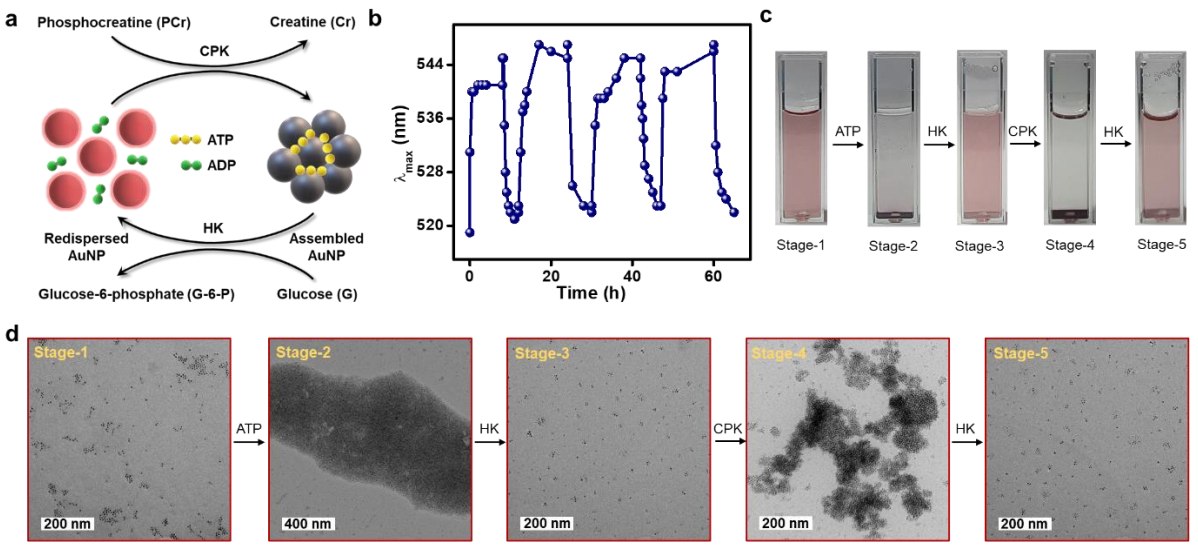


Figure 4b.10. Dynamic self-assembly of AuNPs by the sequential addition of two competing enzymes. (a) Schematic shows the sequential addition of HK and CPK to [+] AuNPs to trigger the disassembly and assembly steps, respectively. The corresponding assembly-disassembly process was monitored using (a) absorption, (c) visual color change and (d) TEM studies. (b) Temporal variations in the absorption λ_{max} as a function of HK and CPK addition, which confirms that the dynamic self-assembly process can be repeated for at least four cycles without any damping. (c) Optical photographs of cuvettes showing the shuttling between a dispersed state (wine-red plasmonically-on state) and a precipitated state (plasmonically-off state), upon the sequential addition of HK and CPK. (d) TEM images confirm the HK-driven redispersion of AuNPs (stages 3 & 5) and CPK-driven aggregation of AuNPs (stage 4) during the dynamic self-assembly process.

4b.4.5 Dynamic self-assembly of AuNPs by enzymatically controlled reaction networks

It is quite evident from previous experiments that a reversible dynamic self-assembly process can be achieved in by controlling the opposing kinetics of (i) assembly process (CPK-driven ADP to ATP conversion) and (ii) disassembly process (HK-driven ATP to ADP conversion). The basic idea here is the variation in the ATP concentration that is regulated by the two complementary enzymes. Next, we were curious to know whether both these enzymes can function in tandem. One of the ways to achieve this is by controlling the addition of the substrate molecules (PCr and glucose) that are essential for the functioning of these enzymes (Figure 4b.11a). In a typical experiment, ~6 nM [+] AuNPs were dispersed into 3 mL 10 mM HEPES buffer, having a pH of ~7.4. Then ~5 μM ADP, 0.36 units of HK, 250 equiv. glucose, 12 units of CPK were added. As 5 μM ADP is not sufficient to trigger the self-assembly process, [+] AuNPs remained in the dispersed state. The addition of 2 equiv. of PCr resulted in an immediate aggregation of the AuNPs. This is because PCr will stimulate the action of CPK to convert ADP to ATP, thereby triggering the assembly process. One important point to be

noted here is that HK and glucose (disassembling trigger) are already present in the system. This will ensure that the assembly step triggered by the CPK is in a controlled fashion. As a result, a controlled aggregate stage is expected instead of a complete precipitate state. The temporal changes in the λ_{max} value proved the formation of a transiently stable controlled aggregates with a ~21 nm red-shift, which persisted for ~10 min (Figure 4b.11b, c). After ~10 min, the activity of HK dominated, initiating the disassembly step. It took ~30 min to redisperse the AuNP aggregates back into individual particles (Figure 4b.11c), with a complete revival of the absorption peak ($\Delta\lambda_{max} = 0$). This entire process of assembly-disassembly cycle, tiggered by the tandem action of the competing enzymes, was monitored using visual colour change as well (Figure 4b.11d). The reaction mixture (without PCr) showed a typical wine-red colour corresponding to the dispersed AuNPs. Addition of 2 equiv. of PCr led to a colour change from wine-red to blueish-red because of the plasmon coupling effect,[44] arising from the aggregation of AuNPs. After ~40 min, the wine-red colour was revived because of the HK-driven ATP to ADP conversion, leading to the redispersion of AuNPs (Figure 4b.11d).

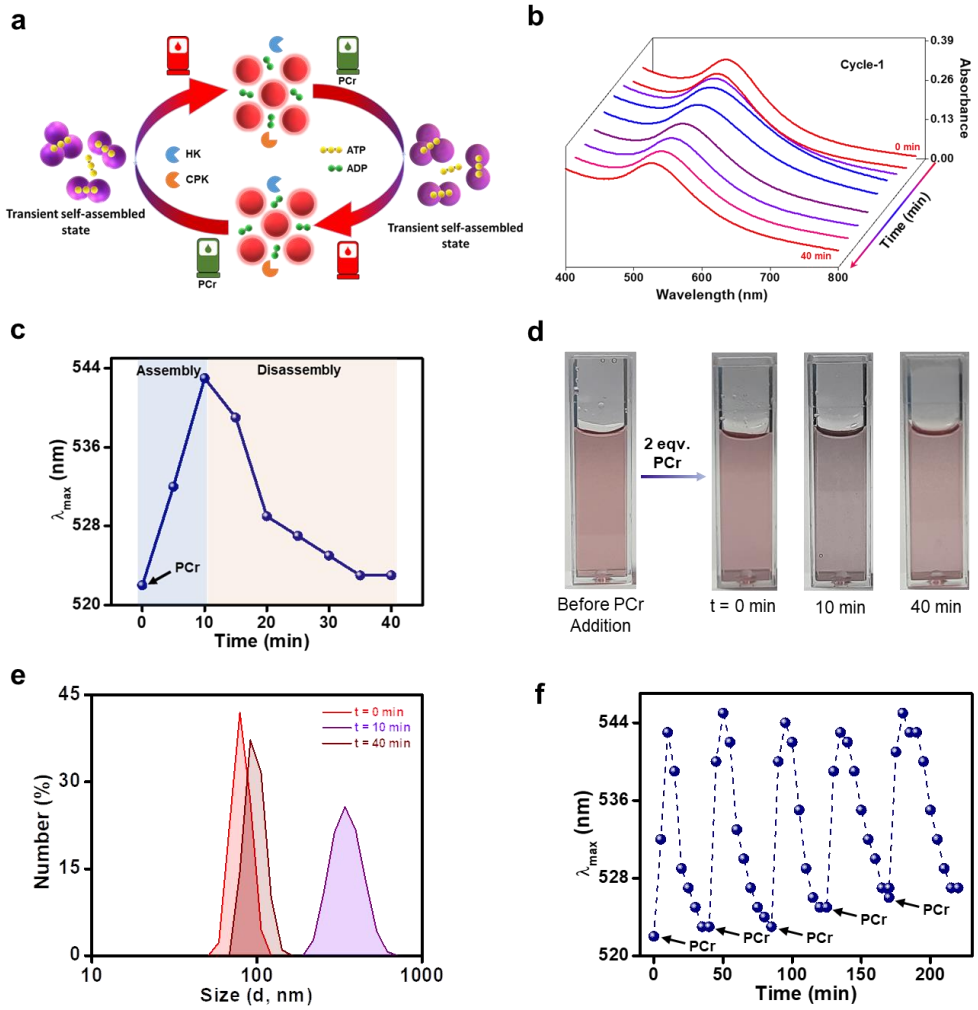


Figure 4b.11. Dynamic self-assembly of AuNPs by the tandem action of two competing enzymes. (a) Schematic shows the PCr-triggered dynamic self-assembly of AuNPs, coupled with two competing enzymes - HK and CP - that consume and regenerate ATP, respectively. (b) The temporal evolution of the absorbance data shows the change in the absorption profile during the dynamic self-assembly process. (c) A plot showing the time-dependent changes in the λ_{max} during the dynamic self-assembly process. (d) Time-dependent optical photographs of the cuvettes confirm the dynamic self-assembly process. The blueish color of the NP-solution at t= 10 min and revival of wine-red color at t= 40 min confirm the PCr-driven aggregation and autonomous redispersion of AuNPs, respectively. (e) DLS study shows the shuttling between dispersed and aggregates states of AuNPs, again confirming the dynamic nature of NP-aggregation. (f) Temporal variations in the absorption λ_{max} as a function of PCr addition, which confirms that the dynamic self-assembly process can be repeated for at least five cycles.

The dynamic self-assembly process, under the tandem action of the enzymes, was further monitored using the DLS study (Figure 4b.11e), which corroborated well with the absorption studies. Here, as well, we observed a temporal change in the size of the NP-aggregates, which increased till ~10 min after the addition of 2 equiv. of PCr (Figure 4b.11e). After ~40 min, the size of the aggregates decreased to the size of individual NPs confirming the complete redispersal process (Figure 4b.11e). At this stage, a fresh addition of 2 equiv. of PCr can restart the dynamic self-assembly process. In this way, we were successful in extending the dynamic self-assembly process for multiple cycles, by adding a fresh batch of PCr at every dispersed stage (Figure 4b.11f). The temporal changes monitored in the λ_{max} shows a fast assembly kinetics, followed by a slow but autonomous disassembly kinetics. In each cycle, the lifetime of the transiently stable controlled aggregate state was approximately ~10 min, and the disassembly step occurred within ~30-40 min (Figure 4b.11f). With each cycle, the disassembly step was found to be slightly slower. The accumulation of G-6-P and Cr can be attributed to the time delay for disassembly in the latter cycles. We could observe 5 dissipative self-assembled cycles within a short span of ~220 min (Figure 4b.11f). A significant and consistent shift in the λ_{max} (~21-23 nm) was observed in each assembling cycle; whereas a slight damping effect[7,28,45] was observed in the revival of λ_{max} ($\Delta\lambda_{max}$ ~ 5 nm) during the disassembly step after two cycles (Figure 4b.11f). In short, a reversible dynamic self-assembly process was achieved between the dispersed and controlled aggregate states of AuNPs, by the tandem activity of two antagonistic enzymes.

4b.5 Conclusions

It has been demonstrated that enzymatically controlled reaction networks can be an effective strategy to choreograph a reversible dynamic self-assembly process in AuNP-nucleotide nano-bio hybrid system. This was achieved by coordinating the antagonistic action of the two

phosphoryl transferase enzymes: HK will trigger the disassembly process by dephosphorylating ATP to ADP; whereas CPK will trigger the assembly process by regenerating ATP from ADP. The dynamic self-assembly process as realised under sequential as well as random action of these competing enzymes. Both these approaches ensured that a dynamic self-assembly process can be achieved without the need of multiple addition of the co-factor ATP molecules in the system (Figure 4b.10a). Under the sequential condition (where HK and CPK were added to the system sequentially), a reversible shuttling between a dispersed and a completely precipitated state was achieved. On the other hand, a reversible shuttling between a dispersed and a controlled-aggregate state was achieved under the tandem condition (where both HK and CPK enzymes were present simultaneously). Additionally, the lifetime of the transiently self-assembled state and the extent of aggregation were tuned by varying the amount of HK, CPK, glucose and PCr, which will be beneficial to install temporal functions into the system. In short, the potential of enzymatically controlled reaction networks has been demonstrated beyond the molecular system, which enabled us design a bio-mimetic dynamic self-assembly process in plasmonic AuNPs.

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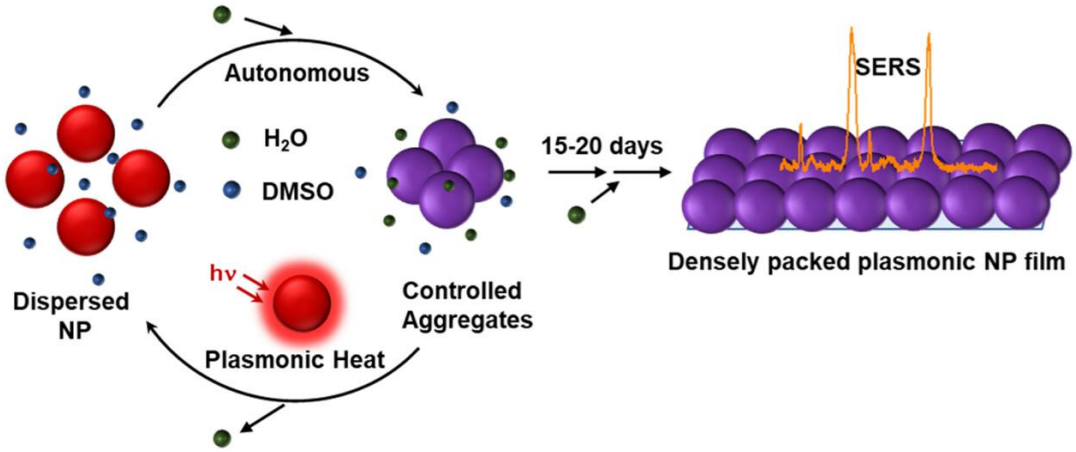
Chapter 5

Coupling of Light into the Solvent-Mediated Self-Assembly of Gold Nanoparticles Using Thermoplasmonics



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Roy, S.; Kashyap, R. K.; Pillai, P. P. Thermoplasmonics Enable the Coupling of Light into the Solvent-Mediated Self-Assembly of Gold Nanoparticles. *J. Phys. Chem. C* **2023**, *127*, 10355−10365.

5.1 Abstract

Nanoparticles (NPs) can offer their core as well as surface for manifesting various optoelectronic properties, making them one of the prominent classes of materials in modern science. In this direction, we have used plasmonic NPs as the building blocks to design a multi-stimuli-responsive, dynamic solvent-mediated self-assembly process. We selected plasmonic gold nanoparticles functionalized with hydrophobic thymine thiol (Thy-AuNPs) (dispersed in dimethyl sulfoxide, DMSO) as our NP building blocks. The hygroscopic nature of DMSO led to the autonomous dissolution of atmospheric moisture into the DMSO dispersion of Thy-AuNPs, thereby triggering the assembly process. This led to the formation of long-term stable (for weeks) controlled aggregates of Thy-AuNPs, wherein the inherent plasmonic properties of Thy-AuNPs were well retained. This enabled the use of core-thermoplasmonic properties of Thy-AuNPs in realizing the disassembly process. The sunlight-triggered plasmonic heat dissipated from the Thy-AuNPs in controlled aggregates was used as the thermal energy source for the evaporation of water, which further helped in the disassembly step. In this way, sunlight was coupled as fuel into the solvent-mediated dynamic self-assembly process of plasmonic NPs. Additionally, the plasmon heat-driven disassembly process occurred within ~10 min compared with the electrical heating at 65 ^{0}C, which took ~5 h for the complete re-dispersion. The concept of light-coupled solvent-mediated dynamic self-assembly was generalized by extending it to plasmonic NPs of different sizes and cores. Further, the controlled aggregate state was exposed to an open atmosphere for ~15-20 days, which led to the formation of densely packed plasmonic NP film at the liquid-air interface through the *'Capillary trap'* phenomena. Therefore, by controlling the assembly pathways, the same NP building block was forced to form two thermodynamically different aggregated states. Raman studies proved that the products of the self-assembly process-controlled aggregates and densely packed plasmonic NP film can serve as effective surface-enhanced Raman scattering (SERS) substrates for analytical applications. More interestingly, in the case of the dynamically controlled aggregation process, a temporal variation in the SERS signal was observed. In short, the ability to preserve the plasmonic properties of Thy-AuNPs in the aggregated state enabled the use of the core properties of NPs in achieving the disassembly step, which in turn led to the realization of dynamicity, multi-stimuli-responsiveness, and sustainability in the self-assembly process of NPs.

5.2 Introduction

Installing dynamicity to the self-assembly process is the key to achieve adaptability in supramolecular architectures, which in turn is essential to create materials that could mimic the complex functions seen in living systems.[1-4] The stimuli-responsive self-assembly is one of the promising strategies to achieve this task.[5-7] This requires the smart choice of building blocks that can respond to external changes in a precise fashion. Nanoparticles (NPs) are a unique class of building blocks, as both their core and surface properties can be utilized to impart responsiveness to external stimuli.[4,8,9] Moreover, the versatile properties emanating from the NP core can be translated into self-assembled structures to achieve the desired functions.[9–11] Chemical (metal ions, molecules, pH, solvent, gases, etc.) and physical (light, magnetic or electric field, temperature, sound waves, etc.) stimuli have been used to trigger the self-assembly processes in NPs.[12–27] It is worth mentioning that most of the dynamic self-assembly processes in NPs have been achieved either with chemical or physical triggers. In a seminal work, Liz-Marzán and co-workers have demonstrated solvent (chemical trigger) as an effective stimulus for the reversible controlled aggregation of AuNPs (Figure 5.1).[14] Similarly, physical trigger such as light has been utilized by Kimizuka and co-workers to generate plasmonic heat to drive the self-assembly of AuNRs (Figure 5.2).[20] The design of NP building blocks that are responsive to multiple triggers has been the target in recent years to expand the scope and complexity of the stimuli-responsive self-assembly process. In this direction, we designed a dynamic self-assembled system based on plasmonic NPs that respond to both chemical (solvent) and physical (light) stimuli, thereby triggering the assembly and disassembly steps, respectively.

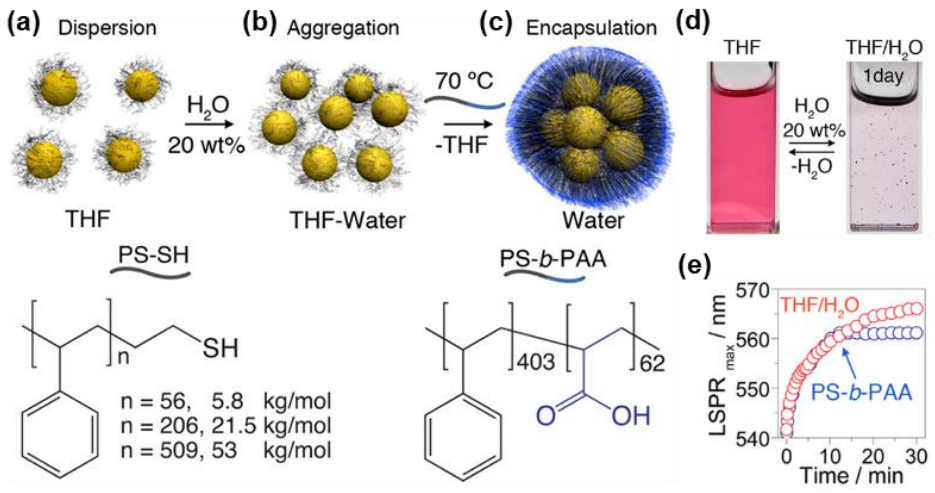


Figure 5.1. Solvent-mediated self-assembly of polystyrene-coated AuNPs. (a) Polystyrene-coated AuNPs were dispersed in THF. (b) The addition of ~20 wt% water (antisolvent) leads to the aggregation

of AuNPs. (c) The aggregation can be restricted by further addition of polymeric surfactant (PS-*b*-PAA) at any time, where the polyacrylic shell stabilizes the cluster in water. (d) Optical photographs ensure the hydrophobic interactions driven reversibility of $Au_{18}@PS_{509}$ NPs aggregation. (e) Temporal change in LSPR maxima shows a bathochromic shift after adding water (red dots). The addition of PS-b-PAA at 10 min restricts further aggregation. Reproduced with permission from ref. 14. Copyright 2012, American Chemical Society.

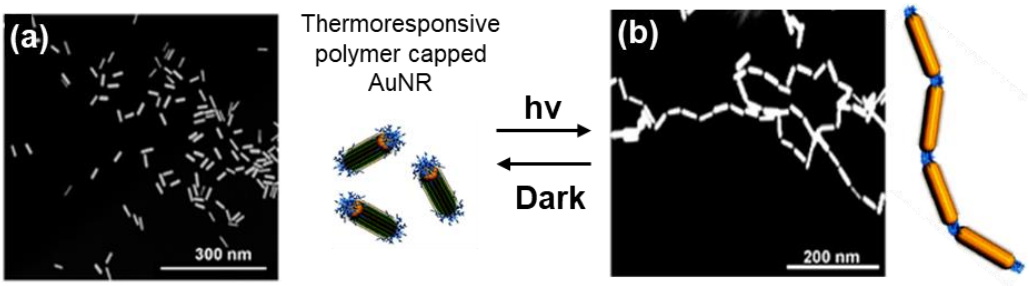


Figure 5.2. Plasmonic heat-driven self-assembly of gold nanorods (AuNRs). Upon light irradiation, thermoresponsive polymer, PNIPAm, functionalized dispersed AuNRs (a) undergo an end-to-end assembly (b), which can redisperse into individual NRs under dark. Reproduced with permission from ref. 20. Copyright 2009 The Royal Society of Chemistry.

The multi-stimuli-responsiveness was installed in the solvent-mediated self-assembly process by utilizing both the core as well as the surface properties of plasmonic gold nanoparticles. The solvent-mediated self-assembly process is based on the difference in the solubility of building blocks in solvent and anti-solvent systems, which in turn is dictated by the polarity of the ligands present on the NP surface.[14,28–37] In the current study, thymine thiol-capped gold nanoparticles (Thy-AuNPs) dispersed in dimethyl sulfoxide (DMSO) were used as the building blocks. The presence of hydrophobic thymine thiol led to the destabilization of AuNPs in water (anti-solvent), and this property was used to trigger the assembly process in Thy-AuNPs. When kept open, the hygroscopic DMSO allowed for the autonomous dissolution of atmospheric moisture, resulting in the self-assembly of Thy-AuNPs. It was observed that the Thy-AuNPs underwent a kinetically controlled aggregation process rather than the commonly observed uncontrolled precipitation process. The dissolution of water (H_2O) will destabilize the hydrophobic thymine ligands, thereby increasing the van der Waals attraction between the AuNPs, leading to the aggregation process. However, the strong H-bonding interaction between DMSO and H_2O will reduce the effect of H_2O in changing the hydrophobicity of the medium.[38] This will prevent the H_2O molecules from completely destabilizing the hydrophobic thymine thiol ligands, resulting in the controlled self-assembly of Thy-AuNPs. The ability to form the controlled aggregates is essential to retain the inherent properties of AuNP building

blocks in the self-assembled state. The thermoplasmonic property of the core-AuNPs was used for the disassembly process. Upon light irradiation, plasmonic AuNPs are known to dissipate a large amount of heat to the surroundings, which is called plasmonic heat (vide infra).[38,39] This results in an increase in the local temperature, which is sufficient to cause the evaporation of H_2O.[40] In the present study, the light irradiation on the controlled aggregates increased the solution temperature to ~80 °C, leading to the evaporation of H_2O, which resulted in the disassembly of Thy-AuNP controlled aggregates (Figure 5.3). The thermoplasmonic property of the controlled aggregates allowed us to couple light into the solvent-mediated self-assembly process, thereby achieving multi-stimuli-responsiveness. The coupling of light into a self-assembly process is highly desirable, as light is a neat fuel that generates zero waste. The concept of a light-coupled solvent-driven self-assembly process was then extended to plasmonic NP systems with different sizes and cores. Further, the kinetically trapped Thy-AuNP-controlled aggregates underwent a long-range self-assembly process to form a densely packed plasmonic NP film at the liquid-air interface when exposed to an open atmosphere. These plasmonic NP films can effectively coat the plastic surface, as well as serve as a large-area surface-enhanced Raman scattering (SERS) substrate for analytical applications. Our work demonstrates the full use of the core as well as the surface properties of NPs in achieving a dynamic self-assembled system, thereby installing multi-stimuli-responsiveness and sustainability to the system.

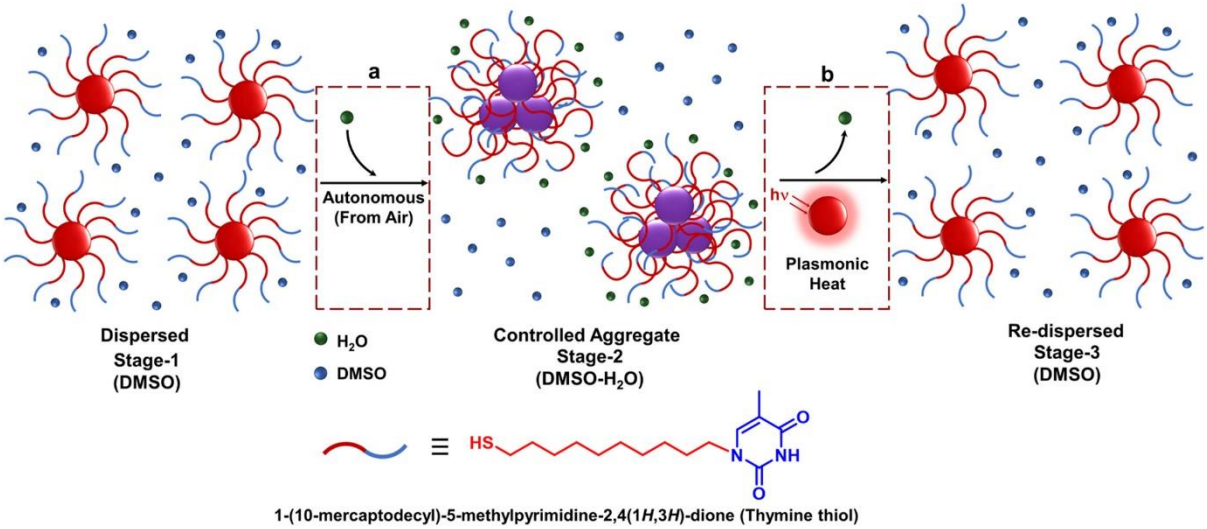


Figure 5.3. Schematic shows the multi-stimuli-responsive and dynamic solvent-mediated self-assembly of plasmonic NPs. (a) The hygroscopic nature of DMSO results in the autonomous dissolution of atmospheric H_2O, thereby destabilizing the Thy-AuNPs and triggering the assembly step to form the controlled aggregates. (b)The disassembly process was triggered by the plasmonic heat dissipated from the Thy-AuNPs upon sunlight irradiation, which evaporated the water.

5.3 Experimental Section

The synthesis and characterization of thymine molecules[41,42] and nanoparticles, as well as the absorption, DLS, and microscopy studies to monitor the dynamic self-assembly process and SERS studies, were already discussed in Chapter 2.

5.4 Results and Discussion

5.4.1 Solvent-mediated autonomous self-assembly of Thy-AuNPs

Solvent-mediated self-assembly is an effective strategy to create complex architectures from different building blocks, including biological (formation of lipid bilayers, gating of ion channels, dynamic protein folding processes, etc.), molecular (micelles, reverse micelles, vesicles, etc.), and nanoparticle (controlled aggerates, structural transformations, superlattices, etc.) systems.[14,28-37,43,44] The choice of building blocks in our study was plasmonic NPs due to their unique optoelectronic properties. Also, the flexibility in using the core and the surface properties of NPs provide an added advantage in achieving the desired feats in the self-assembly process. The solvent-mediated self-assembly relies on the difference in the dispersion of NPs building blocks in solvent and antisolvent systems, which in turn is dictated by the ligands present on the NP surface. The choice of the ligand as well as the solvent system was crucial in the current study, as one of the objectives was to design an autonomous solvent-mediated self-assembled system. The solvent system was selected to be DMSO-H_2O, wherein the hygroscopic nature of the DMSO solvent will cause the autonomous dissolution of moisture from the atmosphere. The target here was to create an NP system that would be completely dispersed in DMSO (good solvent) and unstable in H_2O (anti-solvent). Accordingly, the surface of AuNPs was functionalized with a hydrophobic thymine thiol (1-(10-mercaptodecyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione) ligand (Thy-AuNP), using a ligand exchange procedure. As expected, the Thy-AuNPs lost their colloidal stability in H_2O (Figure 5.4). This proves that DMSO and H_2O are good and antisolvent in our study, respectively, which is the basic criteria for designing a solvent-mediated self-assembled system. Next, control experiments were done by adding small amounts of H_2O into the DMSO solution of Thy-AuNPs (~12 nM in terms of NPs) to verify the solvent-driven aggregation of NPs (Figure 5.4). Upon addition of ~50 wt% of water, an immediate color change (wine-red to purple) was observed, which was

accompanied by a bathochromic shift in the LSPR band (~523 nm to ~536 nm; $\Delta\lambda$ ~ 13 nm) (Figure 5.4). The color change and redshift can be attributed to the plasmon coupling effect arising due to the aggregation of Thy-AuNPs.[45] This control experiment confirms that the DMSO-H_2O mixture is a good solvent system for constructing the solvent-mediated self-assembly process in Thy-AuNPs.

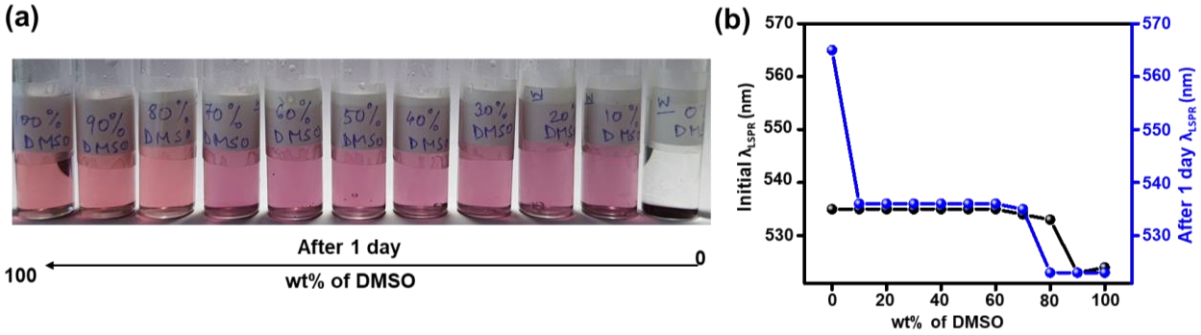


Figure 5.4. Self-assembly study of Thy-AuNP in varying compositions of DMSO-H_2O mixture. (a) Optical photographs of vials having Thy-AuNP in varying compositions of DMSO-H_2O mixture. A clear change in the plasmon color from wine-red to purple is seen in 10-70 wt% DMSO mixture, corresponding to the formation of stable, controlled aggregates. (b) The λ_{LSPR} vs. wt% of the DMSO plot confirms the formation of a controlled aggregated state in the regime of ~10-70 wt% of DMSO.

To get the autonomous assembly, the hygroscopic nature of DMSO was explored. For this, the Thy-AuNPs were dispersed in 3 mL DMSO and kept open to an ambient atmosphere. Within ~3 days, the color of the solution changed from wine-red to purple, along with a red shift in the LSPR band from ~523 nm to ~536 nm (the changes were similar to the control experiment where H_2O was added manually) (Figure 5.5). This confirms the autonomous self-assembly of Thy-AuNPs. All these changes were accompanied by a clear increase in the volume of Thy-AuNPs solution by 700 µL. This can be attributed to the dissolution of atmospheric H_2O in DMSO.

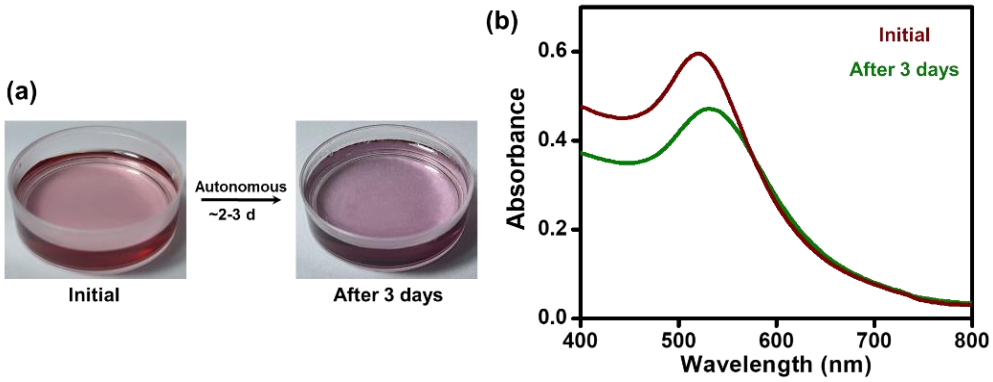


Figure 5.5. (a) Optical photographs showing the autonomous self-assembly of Thy-AuNP (dispersed in 3 mL DMSO) upon exposure to the atmosphere for 2-3 days (b) Absorption data shows the formation of controlled aggregates with a λ_{LSPR} shift of ~13 nm.

The time of aggregation step was reduced from 3 days to ~8 h by placing the DMSO solution of Thy-AuNPs in a hygroscopic chamber (*vide infra*). The controlled aggregates of Thy-AuNPs were stable for a long time (~20 days) (Figure 5.18), thereby retaining the inherent plasmonic properties of AuNPs during the self-assembly process (this is a key requirement for using the core properties for realizing the disassembly step, *vide infra*). The formation of the controlled aggregates was confirmed using dynamic light scattering (DLS) and transmission electron microscopy (TEM) studies (Figure 5.8), which proved that the Thy-AuNPs formed sub-micrometer-sized controlled aggregates.

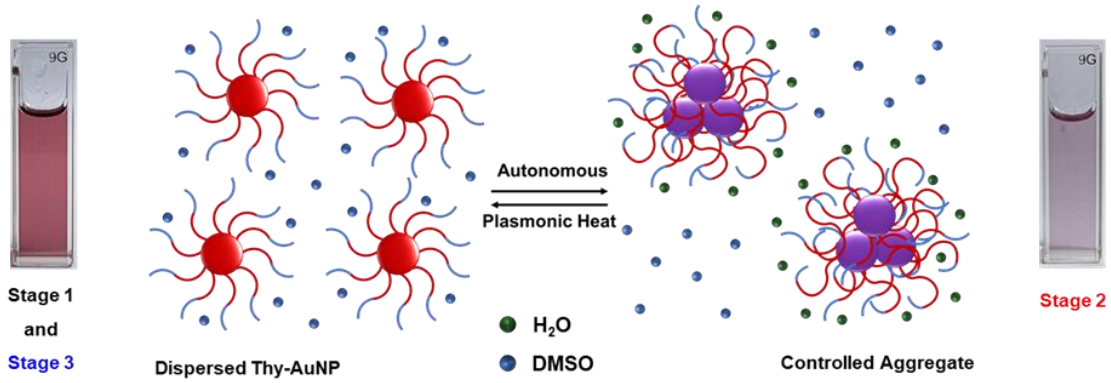


Figure 5.6. Light-coupled solvent-mediated self-assembly of Thy-AuNPs. Scheme showing the autonomous assembly of dispersed Thy-AuNPs (stage 1) to form controlled aggregates (stage 2), via the dissolution of atmospheric moisture. The optical photographs of the vials corresponding to stages 1 or 3 (dispersed or re-dispersed NPs) and stage 2 (controlled aggregates) are also shown.

5.4.2 Plasmon heat triggered disassembly-assembly of Thy-AuNPs

Our next goal was to find ways to disassemble the Thy-AuNPs controlled aggregates to achieve dynamicity in the self-assembly process. The cause for the assembly was the destabilization of the hydrophobic thymine ligands due to the dissolution of the DMSO solution with H_2O. Thus, the removal of H_2O from the system is the logical way to break the Thy-AuNPs-controlled aggregates. To begin with, the temperature of Thy-AuNPs controlled aggregates in the DMSO-H_2O mixture was increased to ~65 ^oC by normal electrical heating (Figure 5.19). The color of the solution changed from purple (assembled state) to wine-red (disassembled state) after ~5 h

of heating (Figure 5.19). This confirms the breaking of Thy-AuNPs-controlled aggregates. This proved that the thermal energy could serve as the stimulus to disassemble the Thy-AuNPs-controlled aggregates. Next, we searched for alternate sources of thermal energy (other than the normal electrical heating) as the stimulus to break the Thy-AuNPs-controlled aggregates. Our intention was to incorporate the core properties of plasmonic AuNPs in the assembly-disassembly process (Note that the formation of controlled aggregates helped to retain the plasmonic properties of Thy-AuNPs in the assembled state). This led us to explore the thermoplasmonic properties of the AuNPs. Upon light irradiation, plasmonic AuNPs eventually dissipate the excess energy in the form of heat to the surroundings within a few picoseconds via a series of non-radiative relaxation steps (Landau damping, electron-electron, electron-phonon, and phonon-phonon interactions, Figure 5.7a).[39,46,47]

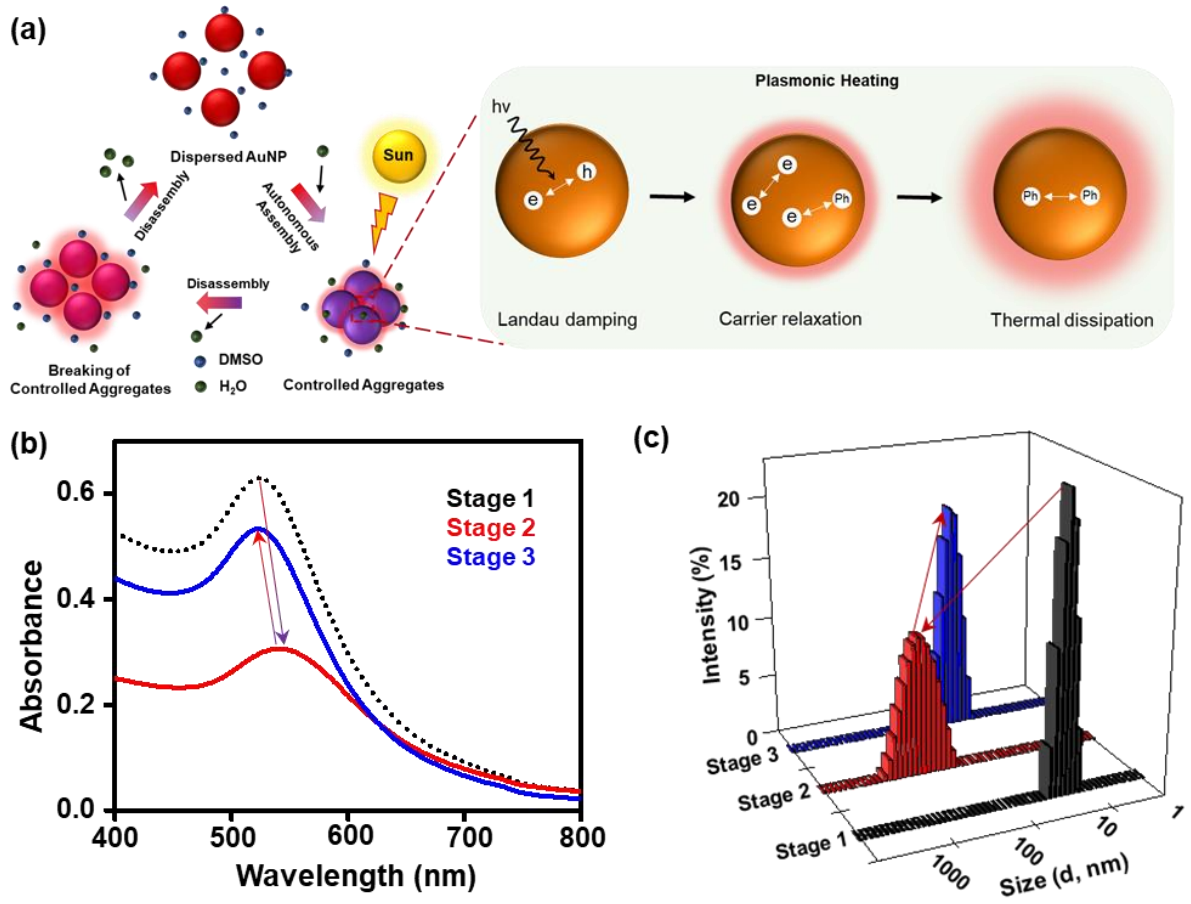


Figure 5.7. (b) Scheme showing the generation and dissipation of plasmonic heat from controlled aggregates of Thy-AuNP. The plasmonic heat acts as the thermal energy source for the evaporation of the anti-solvent (H_2O), thereby triggering the disassembly step. The process of light-coupled solvent-mediated self-assembly was monitored using (c) UV-vis absorption and (d) DLS studies. A clear shuttling of the λ_{LSPR} band and the hydrodynamic diameter (D_h) was observed, corresponding to dispersed (λ_{LSPR} ~523 nm; D_h ~12 nm) to controlled aggregates (λ_{LSPR} ~543 nm; D_h ~800 nm) to re-dispersed (λ_{LSPR} ~523 nm; D_h ~12 nm) stages.

The thermal energy dissipated is often termed plasmonic heat and forms the basis for the area of thermoplasmonics. The fundamental and applied aspects of thermoplasmonics have been extensively documented in the literature.[20,39,40,46-53] The importance of plasmonic heat lies in the huge amount of heat dissipated in a localized area. Our idea here was to use the plasmonic heat dissipated from Thy-AuNPs as the thermal energy source to evaporate the H_2O from the DMSO-H_2O mixture. For this, the controlled aggregates of Thy-AuNPs were irradiated with sunlight (Figures 5.6, 5.7). The disassembly of the controlled aggregates of Thy-AuNPs was complete within ~10 min of sunlight exposure, as it was evident from the reversal of color (purple to wine-red) and LSPR band (λ_{LSPR} shifted from ~543 nm to ~523 nm) (Figures 5.7b). Interestingly, the temperature of the solution increased to ~80 ^{0}C, and a weight loss of ~700 mg was observed within ~10 min of sunlight exposure, which was because of the evaporation of H_2O (note that ~0.7 mL of H_2O was absorbed by the DMSO during the autonomous assembly step). Both TEM and DLS studies further confirmed the breaking of controlled aggregates of Thy-AuNPs to dispersed NPs (Figures 5.7c and 5.8).

The ability to retain the plasmonic properties of AuNPs in the controlled aggregates enabled the use of thermoplasmonics in the present study. Control experiments overrule the role of direct heating from sunlight in the disassembly step (only a marginal weight loss of ~60 mg was observed after irradiating ~3.8 mL of DMSO-H_2O mixture in the absence of Thy-AuNPs). Our experiments conclusively proved the role of sunlight-triggered plasmon-heating in disassembling the controlled aggregates, thereby allowing the coupling of light in the dynamic self-assembly of Thy-AuNPs. Moreover, the idea of incorporating '*light-to-heat conversion*' helped in speeding up the disassembly step (from ~8h under normal heating to ~10 min under plasmonic heating), along with installing sustainability aspects to the self-assembly process. Thus, the appropriate use of both core and surface properties of AuNPs enabled us to design a light-enabled solvent-mediated dynamic self-assembly process in NPs.

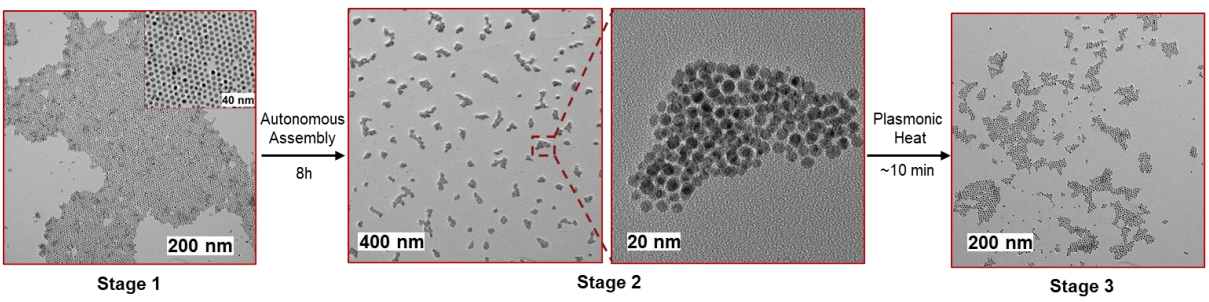


Figure 5.8. Microscopic characterization of light-coupled solvent-mediated self-assembly of Thy-AuNPs. TEM images correspond to dispersed (stage 1) to controlled aggregates (stage 2) to re-dispersed (stage 3) stages.

5.4.3 Effect of ligand composition

The impact of the ligand composition (DDA and Thy) on the dynamic self-assembly of AuNP was studied as well. Three different sets of AuNPs were prepared with a 5%, 10%, and 50% feed ratio of thymine thiol ligands (these percentages are with respect to the amount of thymine thiol added during the synthesis of Thy-AuNP). All these three mixed-ligand AuNPs were dispersed in DMSO solvent, with the LSPR peaks at 521, 517, and 518 nm, respectively. The assembly-disassembly behavior of 50% thy-DDA mixed-ligand AuNPs was similar to thy-AuNPs: 518 nm (stage-1, dispersed) → 550 nm (stage-2, assembled) → 518 nm (stage-3, redispersed) (Figure 5.9a).

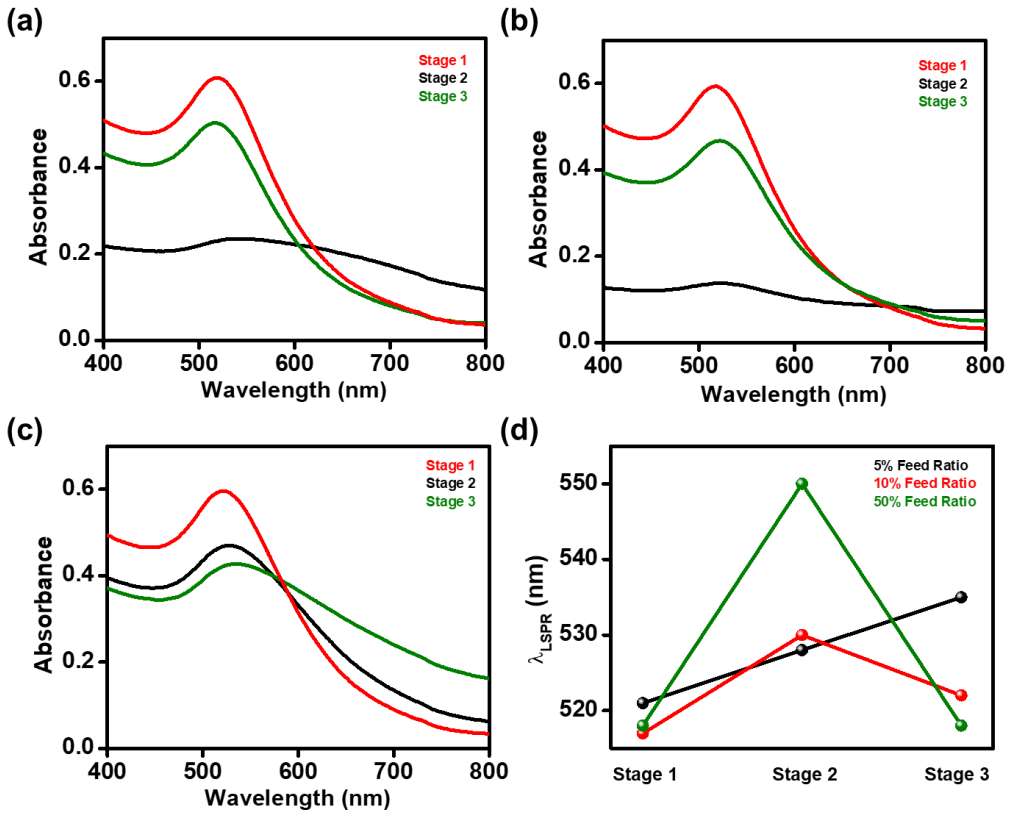


Figure 5.9. Impact of the ligand composition (DDA and Thy) on the dynamic solvent-mediated self-assembly of AuNP. UV-vis absorption spectra corresponding to the three stages of solvent-mediated self-assembly for (a) ~50%, (b) ~10%, and (c) ~5% thy-DDA mixed-ligand AuNPs (the percentages are with respect to the amount of thymine thiol used in the preparation of Thy-AuNP). (d) Corresponding variation in the change of LSPR peak for each stage in the solvent-mediated self-assembly process.

However, the extent of assembly and disassembly was reduced by 10% and ~5% thy-DDA mixed-ligand AuNPs. The assembly step (step-2) led to a bathochromic shift of ~13 and ~7 nm for 10% and ~5% thy-DDA mixed-ligand AuNPs, respectively (Figure 5.9). The aggregated 10% thy-DDA mixed-ligand AuNPs disassembled upon heating, with the LSPR returning to only 523 nm: 518 nm (stage-1, dispersed) → 531 nm (stage-2, assembly) → 523 nm (stage-3, redispersed) (Figure 5.9a). A difference of ~5 nm bathochromic shift was observed between dispersed (stage-1) and redispersed (stage-3) stages of 10% thy-DDA mixed-ligand AuNPs. This confirms the incomplete disassembly of 10% thy-DDA mixed-ligand AuNPs. On the other hand, the ~5% thy-DDA mixed-ligand AuNPs showed no signs of disassembly and kept on aggregating with time. This study confirmed that a minimum amount of thymine thiol should be available on the surface of AuNPs to achieve a complete disassembly of NPs (Figure 5.9).

5.4.4 Theoretical calculation

In the present system, there are 3 main non-covalent interactions: van der Waals (attractive), steric (repulsive), and hydrophobic. The dissolution of H_2O into the DMSO solution will increase the hydrophobic interactions between the Thy-AuNPs, which in turn will destabilize the NP system. However, a partial de-stabilization of Thy-AuNPs was observed in the present study, wherein the extent of aggregation of NPs remained constant till ~70 wt% of H_2O (Figure 5.4). One would expect a complete destabilization of the hydrophobic ligand (and thus the precipitation) at such a high wt% of H_2O in DMSO. However, the Thy-AuNPs remained as controlled aggregates under our experimental conditions. This essentially means that the increase in the hydrophobicity between the Thy-AuNPs can trigger the aggregation process; however, the hydrophobicity may not be strong enough to completely precipitate Thy-AuNPs. To get a better insight into the controlled aggregation process, the interaction potentials were theoretically calculated using the extended Derjaguin–Landau–Verwey–Overbeek (EDLVO) model.[50,54] The interaction potential was considered as a sum of van der Waals and steric forces upon considering the soft-sphere model proposed by Korgel et al.[55] Therefore, the total interaction energy (E_{total}) can be calculated as per equation (1):

$$E_{total} = E_{vdW} + E_{steric} \quad (1)$$

Here, E_{total} is the total potential energy, and E_{vdW} and E_{steric} are the van der Waals and the steric potential energies, respectively. The steric interaction is the main repulsive force that keeps the

Thy-AuNP dispersed in the DMSO medium, equation (2), while the van der Waals force allows the AuNPs to attract each other during the self-assembly process, equation (3).

$$E_{steric} = \frac{100R\delta_{SAM}^2 k_B T}{\pi\sigma_{thiol}^3} e^{-\frac{\pi D}{\delta_{SAM}}} \quad (2)$$

$$E_{vdW} = -\frac{A}{12}\left\{\frac{4R^2}{C^2-4R^2} + \frac{4R^2}{C^2} + 2\ln\left[\frac{C^2-4R^2}{C^2}\right]\right\} \quad (3)$$

Here, C= D+2R+2δ_{SAM} = center-to-center distance between two spherical NPs, R is the radius of the NP (~2.75 nm), δ_{SAM} is the thickness of the surface ligands, thymine thiols (~14 Å), and D is the distance between two NPs. σ_{thiol} and A are the diameter of the area occupied by the thymine thiol molecule on the NP surface (~4.3 Å) and Hamaker constant (~4 x 10^{-9} J for AuNP), respectively. k_B is the Boltzmann's constant, and T is the temperature. Figure 5.10b shows that the repulsive steric interaction gradually increases with a decrease in the interparticle distance, thereby stabilizing the NP system. On the other hand, the attractive van der Waals force increases with a decrease in the interparticle distance, destabilizing the NP system. The plot of total interaction energy (E_{total}) vs separation distance shows an overall weak attractive potential energy (~0.0021 eV) at ~6.1 nm, below which the total energy is dominated by the steric energy (Figure 5.10). This dominance of the repulsive interaction energy is crucial in providing the entropic freedom between the NPs during the self-assembly process, leading to the formation of controlled aggregates.

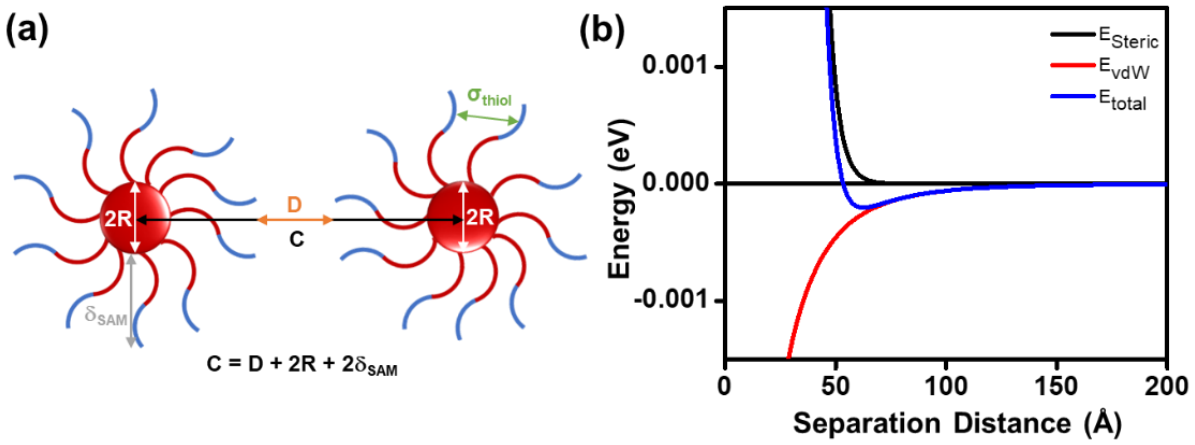


Figure 5.10. Role of interparticle interactions in the self-assembly of Thy-AuNPs. (a) Scheme showing the interaction between two NPs, spaced at a distance of D. The center-to-center distance between the

two NPs (having diameter 2R, ~5.5 nm) is C= D+2R+2δ_{SAM}, where δ_{SAM} is the thickness for the thymine thiol ligand (~14 Å) and σ_{thiol} is the diameter of the area occupied by the thymine thiol ligand on the AuNP surface (~4.3 Å). (b) A plot showing the variation of steric, van der Waals, and total interaction potential between the two Thy-AuNPs as a function of separation distance.

5.4.5 Reversibility and diversity of light-coupled dynamic self-assembly of thy-AuNPs

The aggregation step was triggered by the autonomous dissolution of moisture from the atmosphere into the DMSO solution of Thy-AuNPs, whereas the disassembly step occurred due to the evaporation of water triggered by the plasmonic heat. For testing the prospects of reversibility, the assembly step was speed-up by exposing the DMSO solution of Thy-AuNPs to an artificially created humid atmosphere, as shown in Figure 5.19. This modification helped in reducing the time of assembly from 2-3 days to ~8h. Each assembly-disassembly step was monitored through UV-vis absorption and dynamic light scattering (DLS) studies (Figure 5.11a, b). The optical photographs of the vials and λ_{LSPR} data prove a reversible shuttling between the dispersed and controlled aggregate states of Thy-AuNPs (524 nm → 544 nm → 523 nm). The DLS studies confirm that the aggregated Thy-AuNPs completely break back to dispersed individual NPs during the disassembly step. The solvent-driven assembly-disassembly process in Thy-AuNPs was successfully repeated for five cycles (Figure 5.11), with a complete reversal of the λ_{LSPR} to its initial position. DLS study also corroborates well with the absorption data. A slight dampening of the plasmon intensity was observed after each cycle, which could be because of the loss of NPs due to the irreversible sticking of NPs in micropipette tips and Petri dish.

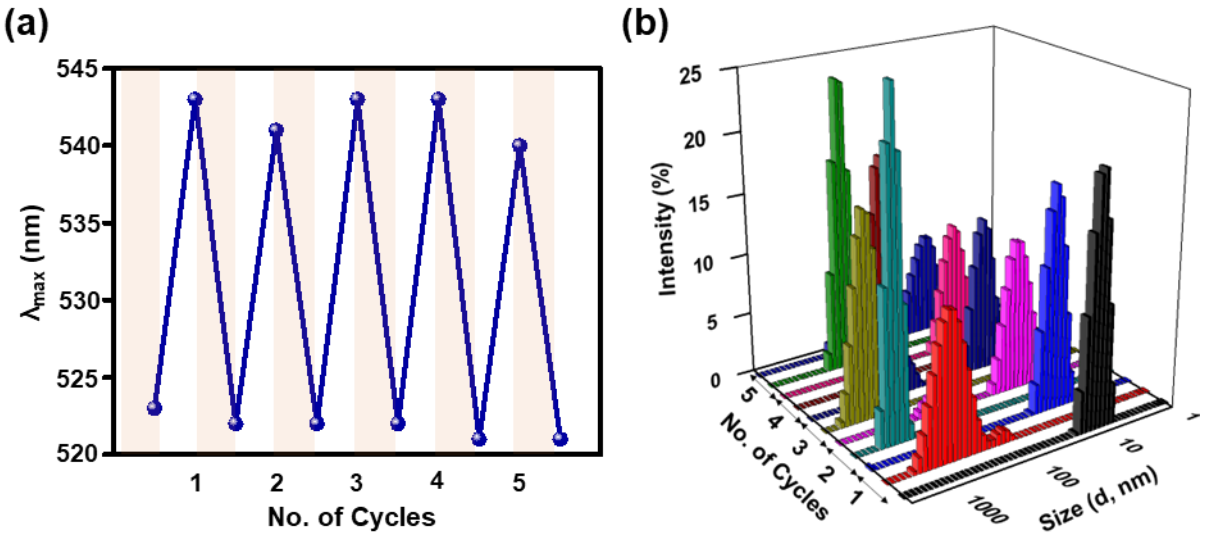


Figure 5.11. Reversibility and scope of light-coupled solvent-mediated self-assembly of Thy-AuNPs. (a) The reversibility in the light-coupled solvent-mediated self-assembly of Thy-AuNPs was monitored using UV-Vis absorption studies. A clear reversibility in the shuttling of the λ_{LSPR} band depicts the dynamicity in the controlled aggregation and disassembly steps. (b) DLS study further confirms the dynamicity in the controlled aggregation phenomena.

AuNPs functionalized with a shorter C5-thymine thiol was prepared to study the impact of carbon chain length of ligand on the self-assembly process (Figure 5.12). The extent of plasmon coupling was higher (LSPR shifted to 685 nm) in C5-thy-AuNP compared to C10-thy-AuNP (LSPR shifted to 544 nm) (Figure 5.12). This confirms that the C5-thy-AuNPs formed much closer aggregates because of the stronger van der Waals interaction between the NPs, arising from the shorter carbon chain length of the thymine-thiol ligand.

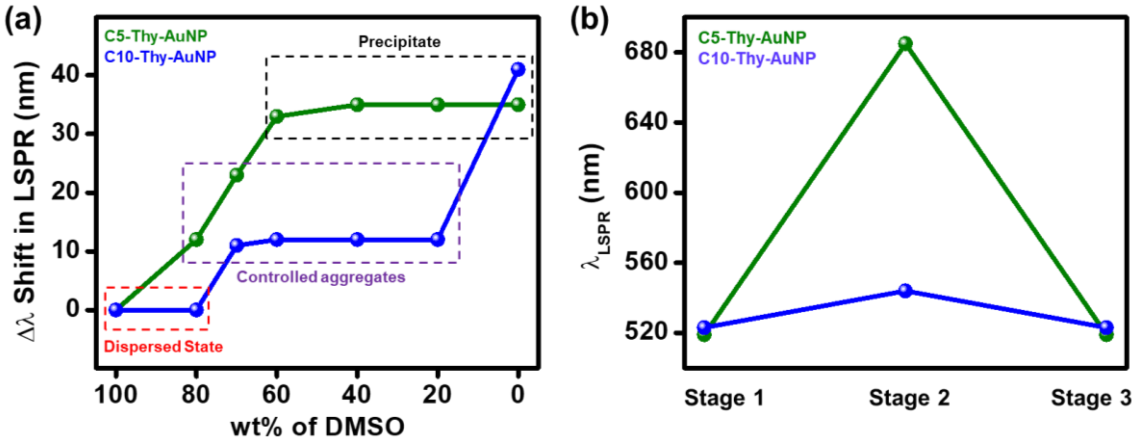


Figure 5.12. (a) A comparison of $\Delta\lambda_{LSPR}$ shift and dynamic self-assembly process in C5-thy-AuNP and C10-thy-AuNP in the DMSO-water medium summarizes the effect of ligand chain length in the self-assembly of Thy-AuNPs. (b) The shorter C5-thymine ligand brings the AuNPs much closer compared to the longer C10-thymine ligands, leading to a stronger van der Waals interaction and better plasmon coupling between the NPs.

The generality of the light-coupled solvent-mediated self-assembly process was proved by extending the system with respect to the NP size and core. The autonomous assembly and plasmonic-heat-driven disassembly steps were also demonstrated in bigger-sized Thy-AuNPs (~10.3 nm AuNP) (Figure 5.13a, b). The λ_{LSPR} shift (530 nm → 543 nm → 529 nm) and DLS data confirm the reversible self-assembly process between controlled aggregate state and dispersed state (Figure 5.13a, b). Likewise, the dynamic solvent-mediated self-assembly process was replicated in Thy-AgNP as well (Figure 5.13c, d).

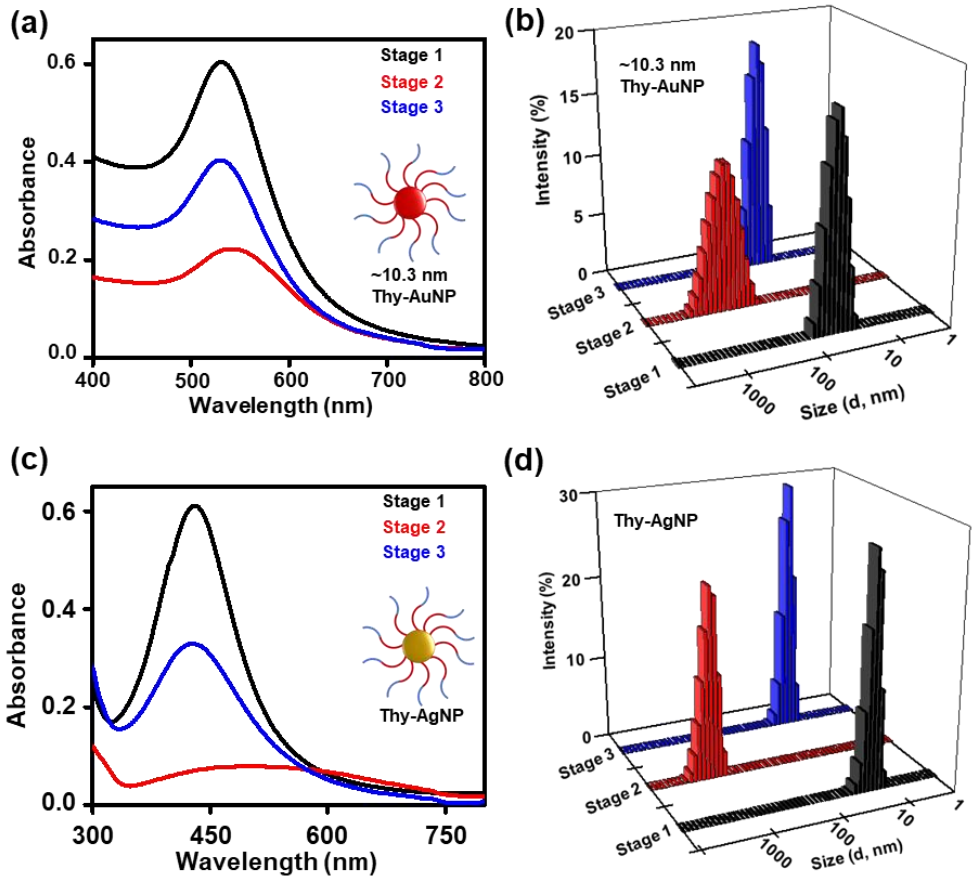


Figure 5.13. Scope of light-coupled solvent-mediated self-assembly of thymine thiol NPs. (a) UV-vis absorption and (b) DLS data corresponding to the light-coupled solvent-mediated self-assembly of ~10.3 nm sized Thy-AuNPs. (c) UV-vis absorption and (d) DLS data prove that the concept of light-coupled solvent-mediated self-assembly can be extended to Thy-AgNPs as well.

Similarly, the Thy-AuNPs also exhibited solvent-mediated dynamic self-assembly properties in the DMSO-methanol solvent-anti-solvent mixture (Figure 5.14). However, here the assembled state was a precipitate rather than a controlled aggregate, which was the case in DMSO-water mixture.

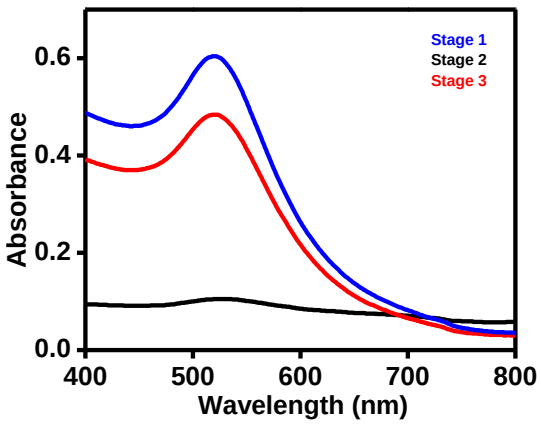


Figure 5.14. UV-vis absorption spectra of Thy-AuNP at different stages of the dynamic self-assembly in DMSO-methanol mixture: stage-1 (dispersed, λ_{LSPR} = 519 nm) → stage-2 (precipitated, λ_{LSPR} = 539 nm) → stage-3 (redispersed, λ_{LSPR} = 519 nm).

5.4.6 Products of light-coupled self-assembly process and their applied properties

The retention of plasmonic properties of Thy-AuNPs in the aggregated state allowed us to compare the optoelectronic properties of these plasmonic NPs in dispersed and aggregated states. The surface enhanced Raman scattering (SERS) activities of Thy-AuNPs in the initial dispersed stage, controlled aggregated stage, and the final redispersed stage was compared using benzyl mercaptan as the Raman marker molecule. Characteristic Raman signals of benzyl mercaptan were observed at ~1420 cm^{-1} (C–C–H ring bending), ~1360 cm^{-1} (C–C ring stretching), ~1315 cm^{-1} (CH_2 bending), and ~1240 cm^{-1} (CH_2 wagging and C–C(Ph) stretching)[56] (Figure 5.15). The variation in the SERS activity followed the different stages of self-assembly process in Thy-AuNPs, with controlled aggregated NPs showing the most SERS activity in the study. Specifically, a ~2.5 times enhancement in the SERS activity of Thy-AuNPs was observed in the aggregated state over the dispersed states. The formation of *'hot spots'* because of the plasmon coupling between the closely spaced AuNPs in the controlled aggregates is attributed to the enhanced SERS activity of assembled stage over initial and final re-dispersed states.[45] Thus, the dynamicity of the self-assembly process was reflected at the property level as well.

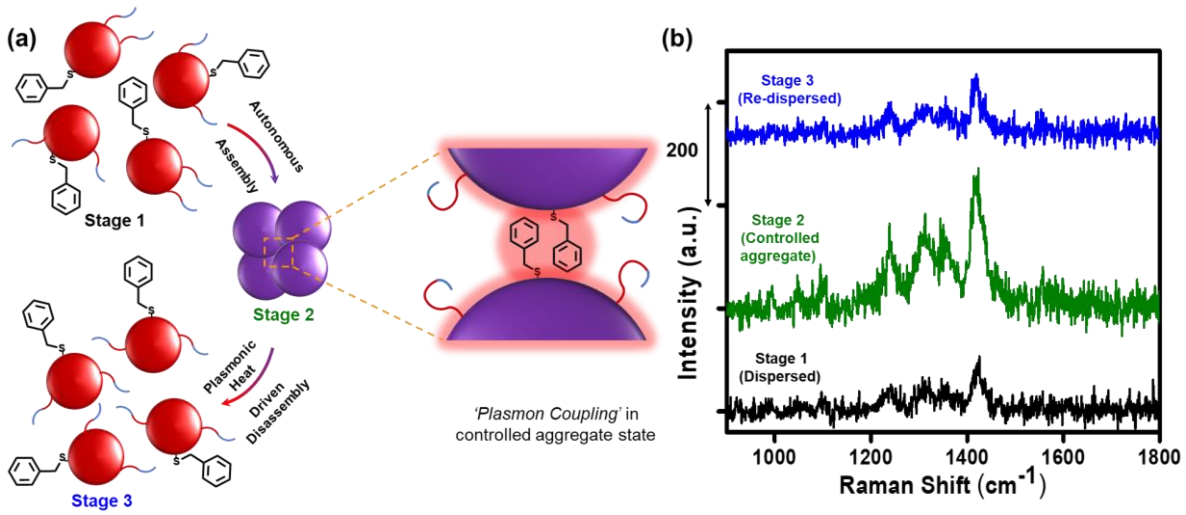


Figure 5.15. SERS activity follows the light-coupled solvent-mediated self-assembly of Thy-AuNPs. (a) Scheme showing the trapping of Raman marker (benzyl mercaptan) in the 'hot spots' created during the controlled aggregation process. (b) SERS data corresponding to different stages of self-assembly process in Thy-AuNPs. Approximately 2.5-fold enhancement in the Raman signals was observed for

the controlled aggerates (stage 2, green spectrum), which is attributed to the trapping of benzyl mercaptan in the 'hot spots' generated as a result of the assembling process.

Finally, the prospects of creating large area self-assembled NP film were studied. For this, the controlled aggregates of Thy-AuNPs were further left exposed to air. Within ~15 days, a densely-packed NP film of Thy-AuNPs was formed in a large area (~0.8 cm^2) at the liquid-air interface (Figure 5.16b). It is worth mentioning that the color of the NP film was still plasmonic (corresponding to the controlled aggregates) rather than black (corresponding to the precipitates). The formation of this densely-packed plasmonic NP film can be explained as follows. The exposure of controlled aggregates of Thy-AuNPs results in the higher dissolution of atmospheric H_2O into the DMSO solution. The H_2O occupies the top portion of the solution (densities of H_2O and DMSO are 997 $kg.m^{-3}$ and 1101.4 $kg.m^{-3}$ at 25 ^oC, respectively)[57], and constitute more toward the liquid-air interface. Also, a large gradient in the surface tension is created because of the dissolution of H_2O in DMSO solution (surface tension values of H_2O and DMSO are 43.53 dynes/cm and 72.75 dynes/cm at 20 ^oC, respectively)[58]. As a result, the capillary forces can trigger the migration of controlled aggregates of Thy-AuNPs towards the higher surface tension region. This process is referred to as *'capillary trap'* in the literature.[29] Now, the controlled aggregates of Thy-AuNPs is in direct contact with the air. The gradual and continuous dissolution of H_2O from the atmosphere can enhance the aggregation process, thereby forming the densely-packed plasmonic NP film at the liquid-air interface.

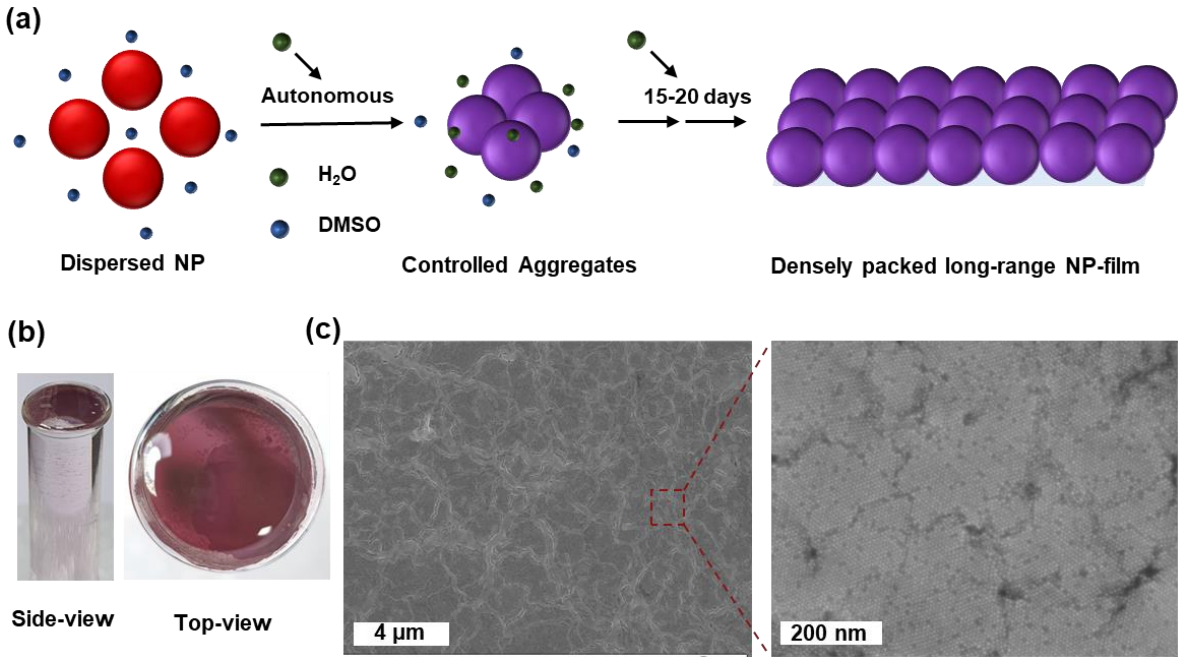
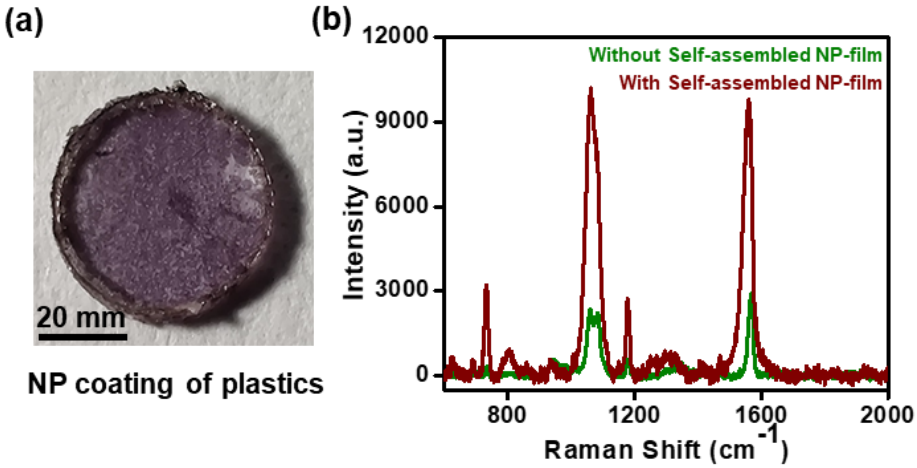


Figure 5.16. Formation of densely-packed plasmonic NP film. (a) Scheme showing the formation of long-range (a few cm^2) assembly of NPs as a result of prolonged exposure of controlled aggregates of Thy-AuNPs. (b) Optical photographs showing the formation of plasmonic NP film at the liquid-air interface. (c) Representative SEM images showing the long-range order of densely-packed plasmonic NP film.

The plasmonic NP film was utilized to effectively coat different surfaces, including plastic materials, as shown in Figure 5.17a. It is worth mentioning that no *'coffee-ring effect'* was observed during the coating.[59] Further, the plasmonic NP film served as an effective substrate for SERS studies (enhancement factor, EF, was 4.972×10^4), which proves their potential for future bioanalytic applications.[13,60,61] SERS studies were performed with a standard Raman marker molecule, 1,4-benzene dithiol (1,4-BDT) (Figure 5.17b). Here, we observed a significant enhancement of the characteristic Raman signals at ~1064 cm^{-1} (C–S stretching)[13] and ~1560 cm^{-1} (phenyl ring stretching)[13] in the NP film-coated silicon substrate, compared to the bare silicon substrate (in the absence of NPs) (Figure 5.17b). In all the above studies, the retention of inherent plasmonic properties of NPs in the aggregated state (because of the controlled aggregate formation) allowed us to showcase the applied properties associated with the light-coupled dynamic self-assembly process of Thy-AuNPs.



5.17. Properties of densely-packed plasmonic NP film. (a) Optical photograph of a plastic Petri dish coated with plasmonic NP film. The purple color (because of the aggregated NPs) confirms the retention of plasmonic properties in the thin film. (b) SERS activity of the plasmonic NP film, coated on a silicon substrate (1,4-BDT molecule was used as the Raman marker). The SERS enhancement factor (EF) was calculated to be 4.972×10^4.

5.5 Conclusions

A solvent-mediated dynamic self-assembled system was developed based on thymine-capped plasmonic AuNP building blocks. The assembly step was achieved through the autonomous dissolution of H_2O from the air, due to the hygroscopic nature of the DMSO medium. This led to the destabilization of Thy-AuNPs, resulting in the formation of controlled aggregates. Interestingly, the plasmonic property of the NP building blocks was preserved in the controlled aggregates, which enabled the use of the core-thermoplasmonic property of AuNPs in the disassembly step. The sunlight-triggered plasmonic heat generated from the Thy-AuNPs in the controlled aggregates was utilized as the thermal energy source for the evaporation of H_2O molecules from the solvent mixture, thereby triggering the disassembly process. By doing so, light was coupled into the solvent-mediated self-assembly of NPs. In short, the use of both surface and core properties of NP building blocks helped in installing dynamicity, muti-responsiveness, and sustainability in the NP self-assembly. The products of the self-assembly process, both controlled aggregates and densely-packed plasmonic NP film, showed excellent SERS activities, proving their potential for future bioanalytical applications.

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5.6 Appendix

5.6.1 Stability of controlled aggregates

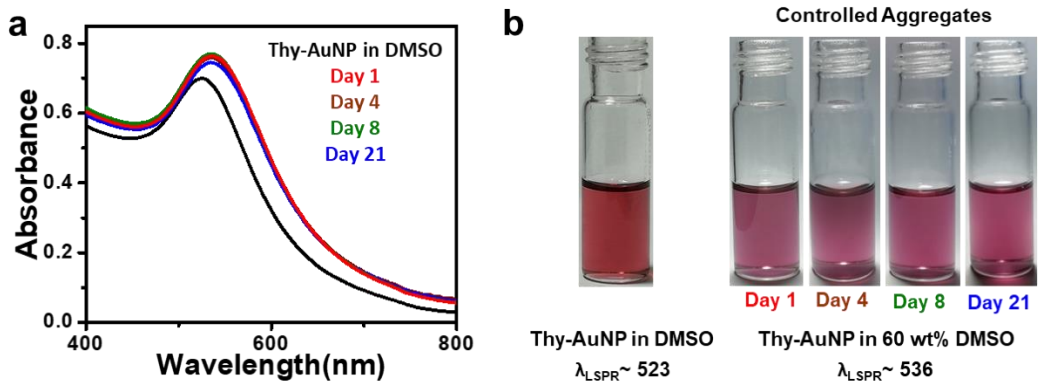

Figure 5.18. Stability of Thy-AuNP controlled aggregates. (a) UV-vis studies depict a negligible change in the absorbance of Thy-AuNP with time, confirming the stability of the controlled aggregates formed in 60:40 wt% of DMSO:H_2O. (b) Time-dependent optical photographs also prove the long-term stability of Thy-AuNP controlled aggregates.

5.6.2 Dynamic controlled aggregation study

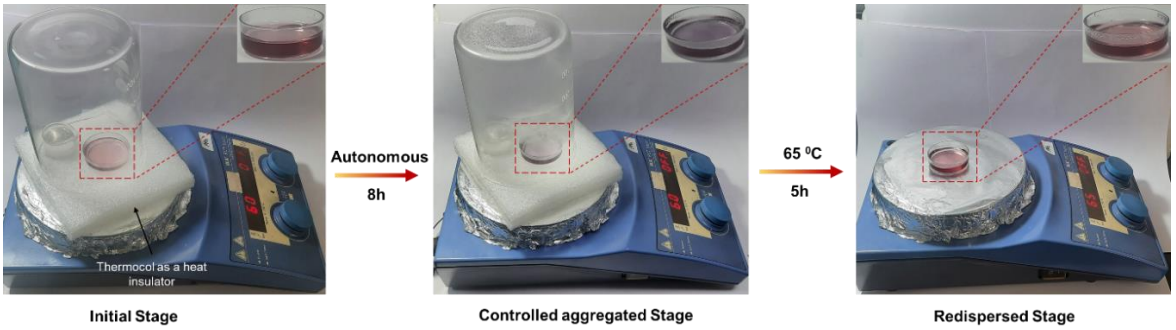

Figure 5.19. Speeding-up the controlled aggregation process. Optical photograph of the set-up used to accelerate the dynamic controlled aggregation process. In a typical experiment, a Petri dish containing Thy-AuNPs (d=~5.5 nm, O.D.=0.6) in DMSO was kept in a humid atmosphere for ~8h. The humid atmosphere was prepared by heating 10 mL of water (kept in the same chamber) at ~60 °C. An insulating material (thermocol) was kept between the hot plate and the Petri dish to reduce the heating effect (so that the heating should not disassemble the controlled aggregates). The disassembly of the controlled aggregates was achieved by directly heating the Petri dish at 65 °C for 5h.

Chapter 6

Conclusions and Future Directions

In conclusion, by regulating interparticle interactions, we were able to control the assembly pathways between gold nanoparticles (AuNPs) and biomolecules. In one example, [+] AuNPs participated in an uncontrolled aggregation with ATP molecules to form non-functional ATP-AuNP precipitates. Whereas, when the same assembly process was performed in the presence of Ca^{2+} ions, we obtained a multifunctional bioplasmonic network. Interestingly, in the presence of excess ATP, this uncontrolled ATP-AuNP ppt. transformed into the bioplasmonic network. Here, the self-association property of ATP was the driving force. This proves that the ATP-NP precipitate is dynamic in nature, which motivated us to couple enzymatic biocatalytic reactions to construct a dynamic self-assembled system. Further, a dynamic self-assembled system was constructed through the sequential and tandem action of two competing enzymes. This led to the formation of a transiently controlled aggregate state.

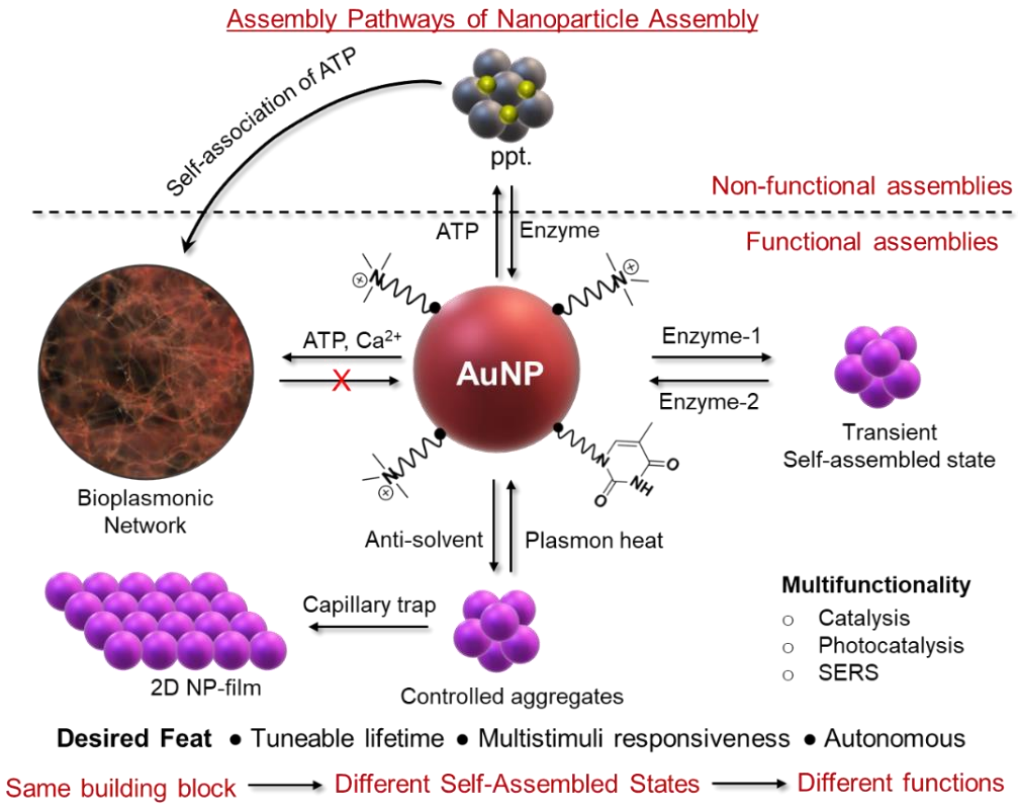

Figure 6.1. Regulation of interparticle interactions controls the assembly pathways of NP self-assembly, leading to the formation of various functional NP assemblies from the same NP building block. Surface ligands and core properties of AuNPs play a significant role in obtaining different static and dynamic self-assembled architectures.

In another set of studies, controlled aggregates were formed by exploring the solvent-mediated self-assembly process. The retention of plasmonic properties in the controlled aggregates allowed us to explore the core thermoplasmonic property of NPs in achieving the disassembly

cycle. In this way, light was coupled into the solvent-mediated NP self-assembly process. Finally, these controlled aggerates were grown to form long-range 2D NP-film via the capillary trap method. It is to be noted that most of the self-assembled states prepared in this thesis were functionally active, which allowed us to explore their catalytic, photocatalytic, and SERS properties. Along with achieving functional NP assemblies, we were also successful in achieving some of the desired feats in NP self-assembly, such as tunable lifetime, multi-stimuli responsiveness, and autonomous self-assembly. In short, we were able to generate different self-assembled states from the same NP building block, which showcases the power of interparticle interactions in the NP self-assembly process.

Therefore, it is quite evident from the thesis work that a minute change in the assembly pathway can lead to the formation of a different self-assembled product. Hence, it keeps a vast number of prospects for the future to investigate the system further to achieve pathway-dependent product formation. This idea of creating complex hierarchical materials can be brought by introducing a retro self-assembly approach, similar to retrosynthesis in synthetic organic chemistry. Hence, the required interactions can be encoded in the building blocks to achieve the ultimate target assembly, providing much-needed predictive power to the field of NP self-assembly. Even the machine learning approach with a vast data set can be one of the future directions to obtain predesigned complex architectures from the NP self-assembly, which has huge potential to bring revolution in the device fabrication industry. Here, the dynamic nature of the self-assembly process will further benefit the pattern formation over the conventional top-down approaches by providing the much-needed error correction abilities. Also, incorporating flow chemistry in the self-assembly process will be very useful to easily scale-up the synthesis of static self-assembled materials and incorporation into industries. Static self-assembled materials are useful to obtain distinct functions from the individual components. For instance, the bioplasmonic network we prepared was explored for catalytic, photocatalytic, and SERS studies. In the future, the conductivity and chirality aspects of the bioplasmonic network can also be explored. Similarly, the self-assembly process, when performed under a reversible domain, can form either kinetically trapped or metastable systems. These systems are essential in designing reversible and multi-stimuli-responsive systems to realize emergent functionalities. On the other hand, dynamic self-assembled systems provide the opportunity to obtain temporal functions similar to those observed in biological systems. These dynamic systems will be useful for creating adaptive and self-healing kind of materials. Therefore, various properties can be coupled to the transient self-assembled state to realize temporal

functions such as transient catalysis, FRET, SERS, etc. For example, antagonistic enzyme-coupled reaction networks that form the plasmonic controlled aggregates can be explored further for temporal plasmonic photocatalytic applications in the future. Therefore, the field of NP self-assembly can deliver plenty of future prospects and opportunities, for example, self-repairing systems, transient functions, and reconfigurable multifunctional devices, which can be highly relevant for technological breakthroughs.

List of Publications

Included in the thesis

1. **Roy, S.**; Kashyap, R. K.; Pillai, P. P. Thermoplasmonics Enable the Coupling of Light into the Solvent-Mediated Self-Assembly of Gold Nanoparticles. *J. Phys. Chem. C* **2023**, *127*, 10355−10365.
2. **Roy, S.**; Pillai, P. P. What Triggers the Dynamic Self-Assembly of Molecules and Materials? *Langmuir* **2023**, *39*, 12967-12974.
3. Rao, A.;* **Roy, S.**; Jain, V.; Pillai, P. P.* Nanoparticle Self-Assembly: From Design Principles to Complex Matter to Functional Materials. *ACS Appl. Mater. Interfaces* **2023**, *15*, 25248–25274. *Corresponding author
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5. **Roy, S.**; Tyagi, S.; Pillai, P. P. Pathway-dependent Autonomous Reversibility in Biomimetic Temporal Self-Assembly of Nanoparticles. (*Submitted*)
6. **Roy, S.**; Pillai, P. P. Tandem Activity of Competing Enzymes Regulate Electrostatic Interaction Leading to the Dynamic Self-Assembly of AuNPs. (*Manuscript under preparation*)
7. **Roy, S.**; Pillai, P. P. The Role of ATP-Self-Association in Transforming a Plasmonically-Off Precipitate State into a Bioplasmonic Network. (*Manuscript under preparation*)

Not included in the thesis

8. Kashyap, R. K.; Dwivedi, I.; Roy, S.; **Roy, S.**; Rao, A.; Subramaniam, C.; Pillai, P. P. Insights into the Utilization and Quantification of Thermoplasmonic Properties in Gold Nanorod Arrays. *Chem. Mater.* **2022**, *34*, 7369–7378.
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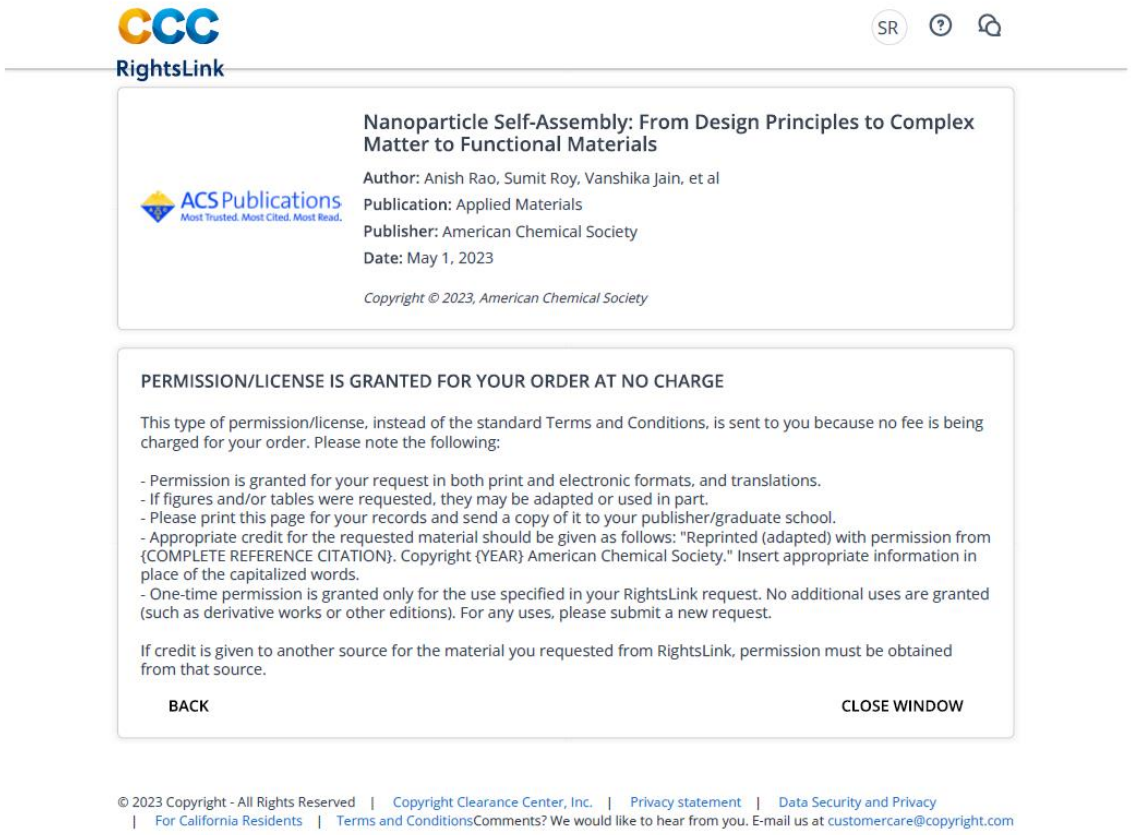

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Last updated October 2022

Figure 1.3 c,d,e,f

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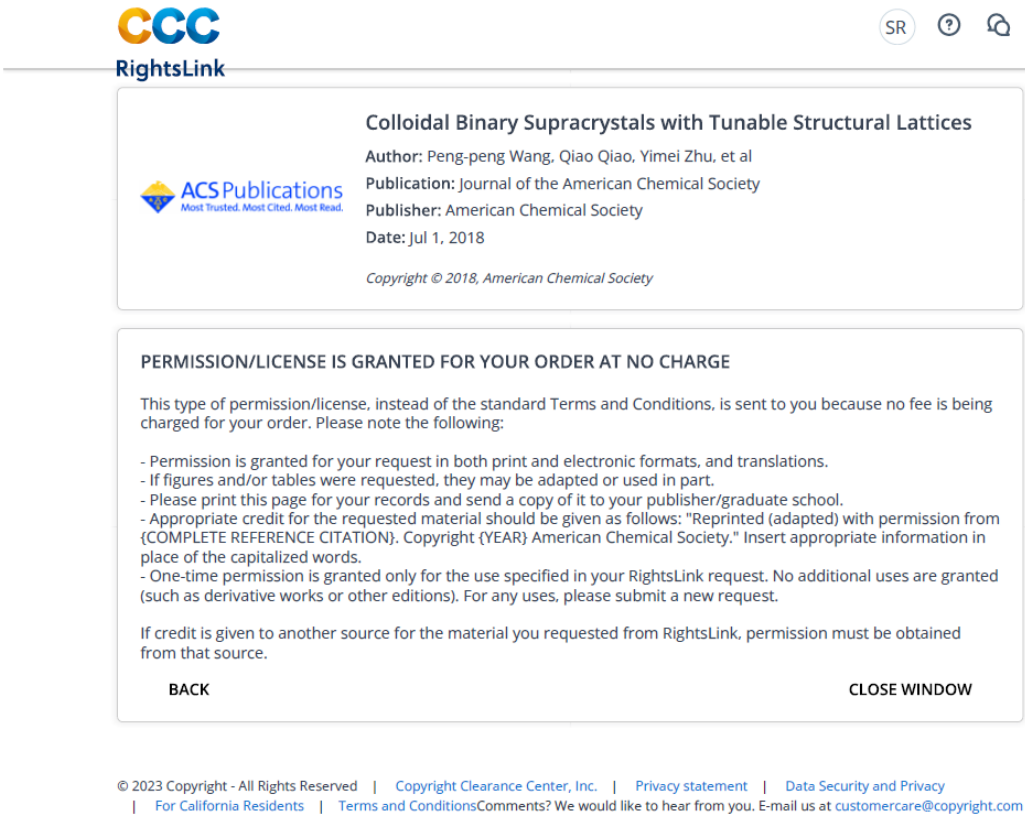

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Colloidal Binary Supracrystals with Tunable Structural Lattices

Author: Peng-peng Wang, Qiao Qiao, Yimei Zhu, et al

Publication: Journal of the American Chemical Society

Publisher: American Chemical Society

Date: Jul 1, 2018

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Figure 1.5 d

Light-Directed Reversible Assembly of Plasmonic Nanoparticles Using Plasmon-Enhanced Thermophoresis

Author: Linhan Lin, Xiaolei Peng, Mingsong Wang, et al

Publication: ACS Nano

Publisher: American Chemical Society

Date: Oct 1, 2016

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Figures 1.5 f-i

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Shape-Directed Binary Assembly of Anisotropic Nanoplates: A Nanocrystal Puzzle with Shape-Complementary Building Blocks

Author: Taejong Paik, Christopher B. Murray
Publication: Nano Letters
Publisher: American Chemical Society
Date: Jun 1, 2013

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Shape Alloys of Nanorods and Nanospheres from Self-Assembly

Author: Xingchen Ye, Jaime A. Millan, Michael Engel, et al

Publication: Nano Letters

Publisher: American Chemical Society

Date: Oct 1, 2013

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Figure 1.12a

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Publication: ACS Nano
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Date: Aug 1, 2018

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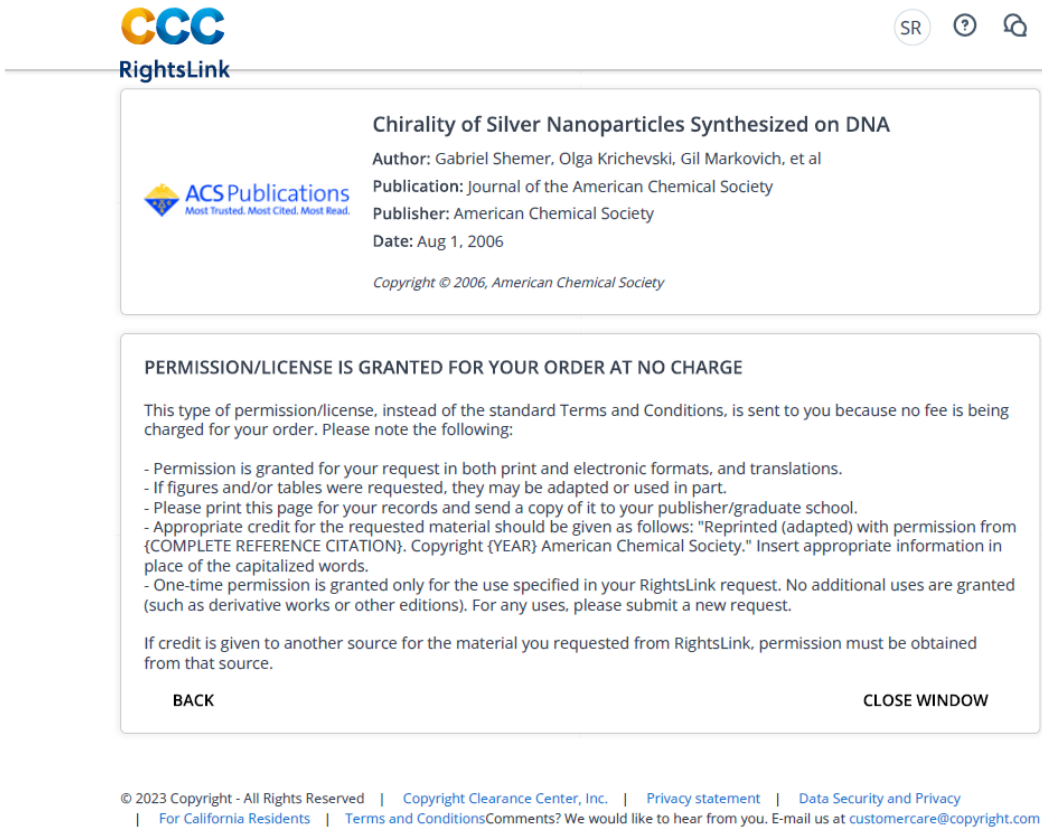

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Publication: Journal of the American Chemical Society
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Author: Sumit Roy, Radha Krishna Kashyap, Pramod P. Pillai
Publication: The Journal of Physical Chemistry C
Publisher: American Chemical Society
Date: Jun 1, 2023

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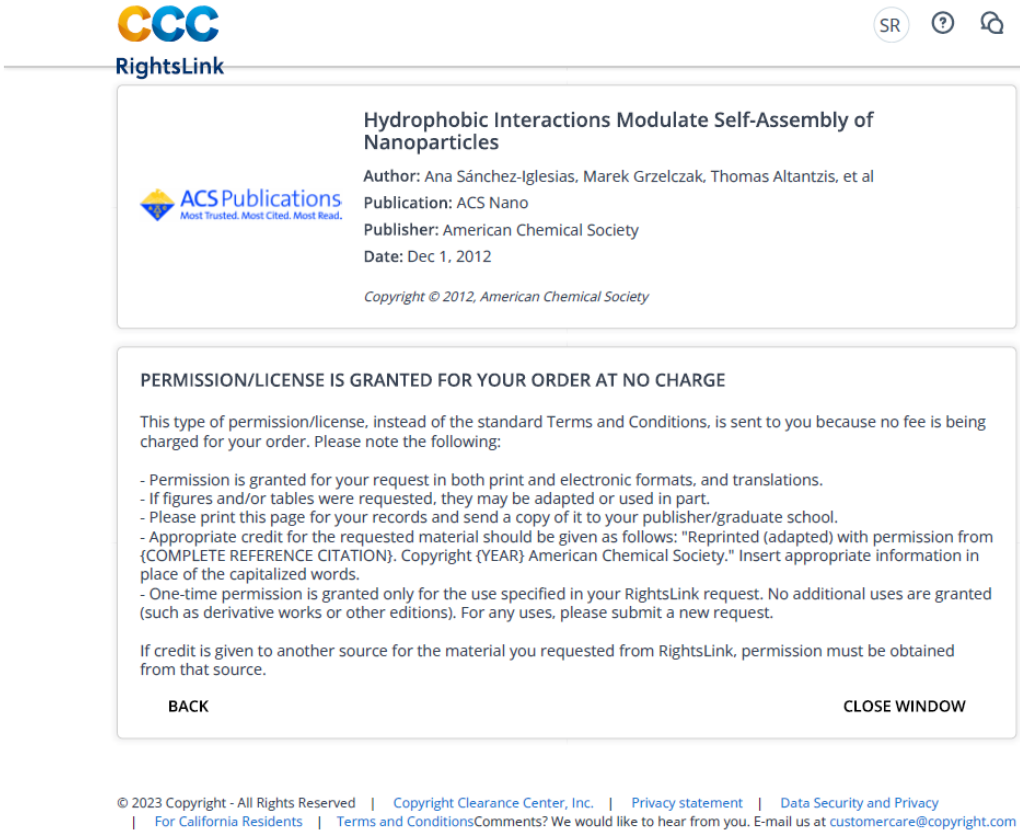

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Title, Description or Numeric Reference of the Portion(s)	Figure 1 and scheme 1	**Title of the Article / Chapter the Portion Is From**	Photothermally-triggered self-assembly of gold nanorods.
Editor of Portion(s)	Fava, Daniele; Winnik, Mitchell A.; Kumacheva, Eugenia	**Author of Portion(s)**	Fava, Daniele; Winnik, Mitchell A.; Kumacheva, Eugenia
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C) use is limited to no more than the greater of (a) 25% of the text of an issue of a journal or other periodical or (b) two articles from such an issue;

D) no User may sell or distribute any particular anthology, whether photocopied or electronic, at more than one institution of learning;

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F) any permission granted shall expire at the end of the class. No permission granted shall in any way include any right by User to create a substantively non-identical copy of the Work or to edit or in any other way modify the Work (except by means of deleting material immediately preceding or following the entire portion of the Work copied).

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C) use is limited to not more than the greater of (a) 25% of the text of an issue of a journal or other periodical or (b) two articles from such an issue;

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