ENTROPY-DRIVEN SUPERCRYSTAL SELF-ASSEMBLY IN AQUEOUS
SOLVENT USING DNA-CAPPED NANOPARTICLES AS A MODEL SYSTEM

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Superlattices and crystals comprised of polymer-capped nanoparticles have generally been produced by the evaporation of organic solvent with the aid of optimized nonspecific forces between the polymer ligands. In contrast with these entropy-based strategies, recent efforts have exploited the enthalpically-favorable interactions between DNA molecules conjugated to nanoparticle surfaces to drive the formation of nanoparticle crystals in aqueous solvent. However, if treated as a pure polyelectrolyte without specific base-pairing interactions, control can still be exercised over the effective size and rigidity of the DNA corona by adjusting parameters such as surface density and ionic strength. Such structural control is difficult to achieve in organic solvent, making entropy-driven crystallization in aqueous solvent worthy of investigation. Since the integration of biomolecule-based components with solid-state devices is becoming a new direction in nanotechnology, it is critical to better understand factors at play in the evaporation process.

We produced nanoparticle superlattices by placing a droplet containing DNA-capped nanoparticles on a thin, micro-perforated film and allowing the droplet to evaporate. As the droplet shrank, smaller droplets were left behind in the perforations which packed the nanoparticles into free-standing, highly-ordered membranes upon evaporation. The membranes were of single-particle thickness and were fixed to the boundaries of the perforations. Real-time investigations of the crystallization process
were performed using small-angle X-ray scattering (SAXS). These studies revealed that crystallization occurs almost immediately when base-pairing forces are present, but could also be induced by volume restriction in the absence of DNA base-pairing forces. The behavior of nanoparticles at the air-liquid interface was probed using a technique in which the X-ray beam was oriented to skim the interface, revealing the formation of crystalline Gibbs monolayers under optimal ionic strength conditions. In all of these crystalline formations, DNA sequence length played a critical role in determining the interparticle spacing. In addition, thermodynamic models were developed to elucidate the spring-like behavior of the DNA corona during the assembly process, which could potentially be used to guide the assembly of nanoparticle crystals with unique optical properties.
BIOGRAPHICAL SKETCH

Michael J. Campolongo was born in St. Louis, MO, but grew up in Millville, NJ. After graduating from Millville Senior High School, Michael attended Rowan University where he earned a Bachelor of Science degree with highest honors in Electrical & Computer Engineering with a minor in Physics. He then attended Cornell University where he earned a Master of Engineering degree in Applied & Engineering Physics, and then a Master of Science degree while pursuing a Doctor of Philosophy degree in Biomedical Engineering. During this time, Michael shared a graduate student office with Tiffany, who he eventually married in 2009.

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To my parents, grandparents, and wife.
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CHAPTER ONE:
AN INTRODUCTION TO
SELF-ASSEMBLED NANOPARTICLE-BASED MATERIALS


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1.1 NANOPARTICLE ASSEMBLIES

Inorganic nanoparticles are of great interest in the development of optoelectronic devices, metamaterials, and molecular sensing applications due to the unique properties that arise directly from their nanoscale dimensions and symmetries. Substantial progress has been achieved in producing nanoparticles of numerous compositions, including gold, silver, iron oxide, and semiconducting quantum dots, while additional advances have afforded control over nanoparticle structure, giving rise to a variety of unique physical, chemical, and optical properties. For these reasons, it is has become common in the scientific community to refer to nanoparticles as “artificial atoms.” Moreover, the various morphologies, shapes, and symmetries achieved through a plethora of chemical synthesis techniques can form the basis of a nanoparticle “periodic table.”

With regard to the unique properties of artificial atoms, the question arises: what relationship should we expect between artificial atoms and their large-scale assemblies? The answer to this question has been one of central importance in the fields of nanotechnology and materials science over the past couple of decades. In general, highly-ordered nanoparticle assemblies exhibit unique properties that do not exist in disordered assemblies, bulk materials of the same composition, and isolated nanoparticles. For example, arrays of silver nanoparticles have been shown to exhibit vibrational coherence, while multi-component superlattices have demonstrated enhanced p-type conductivity. The unique optical properties that arise from crystalline assemblies are within the realm of plasmon hybridization theory, which describes the electromagnetic excitations between nanoparticles in a fashion that is analogous to the interactions between simple atomic and molecular orbitals. These theoretical insights can allow one to envision materials with customizable
optical properties through the controlled assembly of elementary nanoparticle subunits into well-defined macrostructures.\(^8\)

A critical step in designing stochiometric nanoparticle assemblies (“artificial molecules”) and extended arrays (“artificial solids”) is the ability to spatially coordinate nanoparticle interactions, thereby mimicking the vectorial bonds of molecules. While much progress has been made in the synthesis of a diverse assortment of nanoparticles, controlling the parameters necessary to assemble these particles into well-defined architectures in high yield remains a bottleneck in the design of nanoparticle-based materials.\(^1^6\) A key difficulty, for example, has been overcoming the nonspecific van der Waals forces that tend to dominate the interparticle potential, leading to disordered, irreversible aggregation.\(^1^7\) Mitigation of the nanoscale forces can be achieved by coating nanoparticles with a dense organic layer, which provides the necessary forces to counterbalance the strong attraction between the nanoparticle cores. In general, surfactant molecules and, more recently, biomolecules have been used towards this end.

The following section provides a more thorough introduction to the two classes of nanoscale interactions relevant to the field of optoelectronics, namely the plasmonic coupling phenomena that occur due to electromagnetic excitation, and the physical attractive and repulsive forces that govern self-assembly. In the latter parts of this chapter, these two seemingly unrelated types of interactions will be brought together to illustrate the controlled assembly of functional plasmonic nanostructures.

**1.2 CLASSES OF NANOPIRICLE INTERACTIONS**

*1.2.1 Plasmonic Interactions*

Plasmons are electron excitations that occur in metals and semiconductors in response to the alternating electric field component of visible light. The excitation of
conduction band electrons at one location can propagate through a medium, thus producing a collective oscillation known as plasmon resonance. The boundary conditions of the medium, however, are crucial to the behavior of plasmonic excitations. For example, while a continuous, “infinite” metal surface allows plasmons to freely propagate, nanoparticles restrict plasmons to finite dimensions producing unique plasmon modes. Consequently, nearby nanoparticles that are not in contact can experience strong near-field coupling and field enhancement effects, leading to propagation of these modes from one nanoparticle to another. This gives rise to optical properties within nanoparticle systems that are not attainable in continuous, bulk scale materials of the same composition.

From a quantum mechanical perspective, a plasmon is the quantization of oscillations within a plasma, analogous to how photons and phonons are quantizations of light and crystal lattice vibrations, respectively. In the case of nanoparticles (typically less than 200 nanometers in diameter), the nanoscale surface imposes a boundary condition on the polarizability of the metal, resulting in a shift of resonance frequencies to the optical regime. Consequently, the classical electrodynamics description of plasmons based on Mie theory can adequately serve as a theoretical foundation for predicting the plasmonic properties of metallic nanoparticles. Mie theory predicts that the key factors governing extinction spectra for spherical nanoparticles are the diameter, composition, and the surrounding dielectric environment. Owing to their simplicity in structure, however, isotropic spherical nanoparticles can only support individual plasmon modes. Rod-shaped nanoparticles, on the other hand, possess different surface energies and strains on their crystal faces, resulting in anisotropic light absorption and scattering. A few years after Mie’s work was published, Gans extended this theory to anisotropic nanoparticles, namely oblate and prolate spheroids. His analysis predicted that two distinct plasmon modes
(longitudinal and transverse) would arise directly from asymmetry of the boundary conditions. In general, the reduced symmetry of anisotropic nanoparticles allows for greater functionality by enabling multiple plasmonic modes that increase with the number of unique symmetries present.\textsuperscript{25}

Almost a century later, these same fundamental concepts have been applied to highly complex nanoparticle geometries using numerical modeling techniques, including the discrete dipole approximation (DDA), T-matrix, finite-difference time domain (FDTD), finite-element modeling (FEM), and the boundary element method (BEM). DDA simulations are among the most commonly used techniques, and have been applied to numerous geometries in order to predict how the shapes of nanoparticles affect their optical properties.\textsuperscript{26} In a typical DDA simulation, the particle and its dielectric environment are discretized into smaller subunits that are modeled as individual dipoles. The net polarization of the entire system is computed as the subunits are polarized by the incident light and then couple to each other. Using simulated results as a blueprint, controlling the sizes and shapes of nanoparticles affords a practical means for engineering the plasmonic resonance spectra for specific optical applications.

Plasmonic coupling between pairs and collections of nanoparticles is currently a burgeoning area of research, and spherical or simple anisotropic nanoparticles are often used as model systems for theoretical investigations. For spherical nanoparticles, electric field enhancement occurs in small regions between the nanoparticle surfaces, but decays exponentially with increasing separation. A general observation is that plasmonic coupling occurs when the separation-to-diameter ratio is less than 2.5.\textsuperscript{27,28} The absorption peak of a two-particle system appears as a red-shifted single-particle peak that, after sufficient separation, returns to the position corresponding to a single, isolated nanoparticle. Based on the experimentally measured absorption peaks of
several well-defined nanoparticle pairs, El-Sayed and co-workers derived a universal description of the fractional plasmon shift, given by the empirical equation

$$\frac{\Delta \lambda}{\lambda_0} \approx 0.18e^{-0.21 \frac{S}{D}},$$

(1.1)

where $\Delta \lambda$ is the plasmon peak shift, $\lambda_0$ is the plasmon peak of an isolated nanoparticle, and $S/D$ is the separation-to-diameter ratio. Systems comprising more than two nanoparticles are more complex, as multipolar modes arise from the interactions between each set individual modes within the system. Near-field coupling effects between nanoparticles in a chain have been shown to guide the propagation of plasmons down the chain – a phenomenon that is of considerable interest as a strategy for nanoscale energy transfer.\textsuperscript{29-33}

While lithographically patterned systems and small-scale collections of nanoparticles are useful for fundamental studies, high-throughput self-assembly methods will be needed to make the leap from academic interest to functional devices. Hence, it is important to address the factors that play a role in the self-assembly process.

1.2.2 Physical Interactions Between Nanoparticles

In addition to size and shape, interparticle separations are key parameters governing plasmonic coupling within nanoparticle networks. While the former are typically controlled \textit{a priori} through wet chemical synthesis techniques, the interactions that govern the equilibrium distances between nanoparticles are very difficult to control. An effective way to mitigate these forces and prevent unwanted aggregation is to anchor polymer chains to the surface, forming a labile corona around the particle core. The nanoparticle corona is a densely-packed layer of linear polymer chains that is relatively soft and compressible. In general, capping agents are used to
balance the core-core van der Waals attractive forces to prevent aggregation, and are paramount to the formation of highly-ordered nanoparticle assemblies. For example, citrate has often been used in the synthesis of gold nanoparticles as both a reducing and capping agent,\textsuperscript{34} and has also enabled the formation of highly-ordered nanoparticle assemblies.\textsuperscript{35} In general, the assembly of these elementary nanoparticles into well-defined, functional networks is hampered by the complexities associated with nanoparticle bonding interactions. The formation and stabilization of superlattices involves a complex balance between several forces. Forces between nanoparticle cores include van der Waals attractions and electrostatic interactions described by DLVO theory\textsuperscript{36,37} (named for Derjaguin and Landau, Verwey and Overbeek). Additional forces between polymer ligands include van der Waals, steric, electrostatic, hydrophobic/hydrophilic, solvation/depletion, friction/lubrication, and capillary forces.\textsuperscript{17}

At very small separations, adhesive London and van der Waals forces dominate the interparticle potential, as first described by Hamaker in 1937.\textsuperscript{38} For two particles of the same size it is expressed as

\[
V_{\text{vdW}} = -\frac{A}{12} \left[ \frac{4R^2}{C^2 - 4R^2} + \frac{4R^2}{C^2} + 2\ln \frac{C^2 - 2R^2}{C^2} \right],
\]

where \( A \) is the Hamaker constant (typically 1.95 eV for a gold particle pair), and \( C \) is the center-to-center distance. It can be shown that this potential scales with the separation, \( S \), as \( S^{-1} \) at very short separations, but scales as the familiar \( S^{-1} \) van der Waals potential at larger separations.\textsuperscript{9} While the Hamaker potential by itself can adequately model systems comprising short-ligand-capped particles in nonpolar solvents,\textsuperscript{39} the repulsive forces from the corona can also contribute to the interparticle potential. The total potential energy of a two-particle system is
\begin{equation}
V = V_{\text{vdW}} + V_{\text{steric}}.
\end{equation}

The steric term can be modeled as

\begin{equation}
V_{\text{steric}}[\text{eV}] \approx \frac{100Rh_0^2}{(C - 2R)\pi \rho^2} k_B T e^{-\frac{(C - 2R)}{h_0}},
\end{equation}

where \( h_0 \) is the corona height, \( \rho \) is the diameter of a single polymer footprint on the surface, and \( k_B T \) is the thermal energy.\(^{40}\) The corona height is the average height that the capping polymers extend above the nanoparticle surface, and is described by polymer brush theory. Considering only steric repulsion, the average height is a function of the number of subunits within the chain, the density of polymers affixed to the surface, and the curvature of the surface which effectively reduces the ligand density as the chains extend radially away from the surface. When comparable to the nanoparticle diameter, the corona height, \( h_0 \), scales as

\begin{equation}
h_0 \sim N^{3/5} \sigma^{1/5} R^{2/5},
\end{equation}

where \( N \) is the number of monomer subunits, and \( \sigma \) is the surface density of ligands.\(^{41}\) The steric forces produced by the corona cause the nanoparticles to behave as soft spheres.

Figure 1.1A illustrates the contributions from van der Waals attraction and steric repulsion. Aggregation due to van der Waals attractive forces can be induced when the steric repulsion is small due to a short corona, resulting in an equilibrium distance between the two particles. As the corona height increases, the stable equilibrium diminishes until van der Waals attraction is negligible (Figure 1.1B). At this point, additional attractive forces between the polymer chains within the adjacent corona are required to stabilize the nanoparticle assembly.\(^{42,43}\) These forces may include short-range nonspecific interactions, such as hydrophobic forces between
interdigitated ligands, interchain van der Waals attraction, and electrostatic interactions.

Specific interactions afforded by biomolecules can also provide a stable and tunable method for mitigating interparticle interactions. In the next section, the DNA molecule, its properties, and the advantages it has over traditional synthetic ligands in regulating nanoparticle self-assembly will be discussed.
**Figure 1.1.** The interplay between attractive and repulsive forces. (A) Steric repulsion competes with van der Waals attraction, resulting in a stable equilibrium separation. (B) The shape of the potential varies as the ratio, $\chi = \frac{h_0}{R}$, increases. The parameters used here are $R = 3 \text{ nm}$, $\rho = 0.43 \text{ nm}$, and $k_B T = 25.9 \text{ meV}$. 
1.3 USING DNA TO PROGRAM INTERACTIONS

1.3.1 Rationale for Using DNA

The same Watson-Crick base-pairing inherent to genome structure, replication, and transcription within a cell can be exploited to guide the assembly of nanomaterials by enabling “programmable” interactions. This capability gives nucleic acids a unique advantage over other polymers in that it provides a means by which novel nanostructures can be rationally designed, facilitating well-defined connectivity between various functional moieties. From a structural viewpoint, DNA can come in single-stranded (ssDNA) or double-stranded (dsDNA) form: the former being relatively flexible with a persistence length of less than 1 nm while the latter being far more rigid with a persistence length of 50 nm. In principle, the rigidity can be tailored by combining ssDNA and dsDNA in unique ways to produce distinctive structures of varying topologies.44 The double helix also has a well-defined, predictable nanoscale structure with a helical periodicity of about 10.5 base-pairs and an average separation of 0.34 nm between each base-pair. From a chemical viewpoint, numerous biochemical techniques, including a vast assortment of well-characterized enzymes, can be utilized to process DNA strands with angstrom-level precision.45

Two representative strategies for directing the assembly of nanoparticles are template engineering, which relies on a scaffold that has predefined positions for which nanoparticles can attach, and corona engineering, in which the corona surrounding the particle provides the mechanism for self-assembly.8 These two strategies together, combined with advances in nanoparticle synthesis and functionalization, have provided a means for developing the “plasmonic molecular world” by enabling the rational design of a nearly limitless array of nanoparticle systems (Figure 1.2).8 Each strategy and their associated advantages and limitations will be discussed in the subsequent sections.
Figure 1.2. Topologies and structures possible through rational design. Reprinted from Reference 8.
1.3.2 Template Engineering

Template engineering involves the use of well-defined DNA subunits that can self-assemble into periodic scaffolds for which functional materials can be attached. These branched subunits, originally pioneered by Seeman, are composed of multi-stranded double- and triple-crossover motifs that enhance the rigidity of the overall structure, enabling the formation of well-defined tiling patterns. In addition, each tile can be engineered to have overhangs for which nanoscale objects, such as metallic nanoparticles, quantum dots, and proteins, can be attached. Such methods afford control over not only the lattice spacing, but also control over the heterogeneity of the lattice. For example, periodic arrays of different sized nanoparticles have been constructed.

Another competing strategy, known as DNA origami, provides a means for generating well-defined, arbitrary patterns that, unlike tile-based strategies, are bounded in overall size. DNA origami utilizes a “one-pot” process in which a large single strand is folded into a desired shape through hybridization with many smaller strands. Like tile-based templates, DNA origami can organize the arrangement of nanomaterials in a well-defined manner. Furthermore, DNA origami scaffolds have been incorporated into structures that have been lithographically patterned, illustrating a potential route towards the integration of “bottom-up” nanoscale materials into “top-down” solid-state devices. While advantageous in their ability to provide a rigid frame for spatially controlling the locations of nanoparticles, scaffolding strategies are generally limited in scale and overall yield and must be prepared and maintained under optimal conditions, as complex nanoscale forces can easily cause these structures to deviate from their desired shapes.
1.3.3. Corona Engineering

Corona engineering involves modifying the surfaces of metallic nanoparticles with polymer ligands, for which the assembly process is governed mainly by the interactions that occur between the ligands of neighboring particles. DNA, in particular, has received much attention as a corona material due to its molecular recognition capability and structural versatility. Through sequence-specific hydrogen bonding, the adhesive forces between nanoparticles can be engineered to mimic chemical bonds between atoms. In general, the forces within DNA-nanoparticle systems include, but are not limited to, attractive van der Waals forces between both metallic cores and ligands, repulsive steric forces between ligands, electrostatic repulsion between DNA within the corona and between neighboring coronae, attractive Watson-Crick base pairing, nonspecific hydrogen bonding, and hydrophobic interactions between bases.\textsuperscript{16} Though complex, DNA molecules have the ability to mitigate these forces. For example, van der Waals forces between nanoparticle cores can be sterically balanced by tuning the DNA length as well as controlling the density of DNA on the nanoparticle surface, and electrostatic interactions can be tuned through pH and ionic strength. The sequence can not only be engineered in terms of length to control interparticle spacing, but can also impart bonding strength and specificity. Control over these parameters can overcome relatively chaotic thermodynamic pathways to yield unique molecule-like architectures, from simple dimers to large-scale 3D crystals.\textsuperscript{58-62}

Molecule-like nanoparticle constructs, or “nanocrystal molecules”, were first demonstrated by Alivisatos and co-workers using the concept of monofunctionalization – a method for attaching single DNA strands to nanoparticles in a one-to-one stoichiometric ratio.\textsuperscript{63} In this seminal work, 1.4 nm particles were used to ensure that a minimum of one single DNA strand could be attached, resulting in
discrete homodimeric and homotrimeric nanoparticle assemblies. While one-to-one conjugation of small nanoparticles is facilitated by steric competition of DNA strands for the limited surface area, the lack of this surface-area restriction for larger particles makes it nontrivial to scale up these assemblies. Consequently, more complex systems, such as multi-particle systems of varying symmetries and chiralities, often require more elaborate modification techniques and purification steps to isolate the desired particles from stoichiometric mixtures.\textsuperscript{63-67} Recent examples include utilizing both capture and blocking sequences in varying stoichiometric ratios,\textsuperscript{68} and anisotropic nanoparticle functionalization strategies based on geometric restrictions.\textsuperscript{60,69}

In parallel to the development of nanocrystal molecules, 3D extended nanoparticle arrays, or \textit{supercrystals}, have been produced using corona engineering strategies. The use of DNA to direct the assembly of large-scale nanoparticle assemblies was pioneered by Mirkin and co-workers in 1996.\textsuperscript{70} DNA conjugated to gold nanoparticles by means of thiol-gold covalent bonds (Figure 1.3) were shown to link the particles together into large aggregates through DNA hybridization, as evidenced by a distinct absorbance shift owing to plasmon coupling between nanoparticles. While the resulting aggregates were amorphous in structure, the approach of Mirkin and co-workers provided a framework for the development of nanoparticle supercrystals, which were realized a decade later.

In 2008, both Gang and Mirkin independently reported highly-ordered arrays of gold nanoparticles organized through DNA-based interactions. In the approach of Gang and co-workers,\textsuperscript{58} a binary system comprising two types of ssDNA-capped nanoparticles with complementary sequences was produced by mixing the particles together. Each of the two types of particles possessed flexible spacer regions of varying length followed by terminal base-pairing regions that could bridge the particles together, resulting in an ordered, three-dimensional nanoparticle array with a
body-centered cubic (BCC) crystal structure. In Mirkin’s approach, a linker stand was used to form a bridge between two types of nanoparticles to induce crystallization, which was reversible over multiple heating and cooling cycles. When the linker contained two identical, self-complementary regions, the resulting crystal structure was face-centered cubic (FCC). However, the crystal structure was BCC when two different, non-self-complementary sequences were used. In both of these binary systems, DNA acted as a bridge while the nanoparticles accounted for the majority of the crystallites’ masses and were also responsible for their optical properties. A general design consideration is that the ratio of hydrodynamic radii and the linker ratio between types of particles govern the crystal structure phase diagram, leading to a variety of potential crystalline assemblies.
Figure 1.3. Schematic of a DNA-capped nanoparticle.
1.4 SIGNIFICANCE OF THIS DISSERTATION

Considerable research effort has been devoted to the investigation of DNA-driven control over the self-assembly of inorganic nanoparticles. While certain success can be attributed to the unique base-pairing nature of DNA, a fundamental aspect has often been ignored. Namely, what is the effect of DNA on nanoparticle self-assembly if Watson-Crick base-pairing potential is removed entirely and the DNA molecule is treated as a pure polyelectrolyte? This would still leave several other parameters to consider, such as DNA length, surface density, pH, and ionic strength to name a few.

In this dissertation, I will discuss a strategy in which DNA is treated as a generic polymer corona that can be engineered from a more structural, rather than biological, perspective. Control over the corona thickness and mechanical strength can influence how it deforms when it is in close proximity to other coronae, leading to entanglement and nonspecific stabilization forces. Entropic interactions, rather than enthalpic, will therefore be shown to provide the driving force behind self-assembly. Although this entropy-driven strategy may lack the advantage associated with base-pairing, careful control over the environmental conditions and geometric constraints can achieve comparable results. Furthermore, stable structures can be produced in environments that are unfavorable for base-pairing, resulting in large-scale two- and three-dimensional crystalline formations.
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CHAPTER TWO:
MECHANICAL ANALYSIS OF FREE-STANDING NANOPARTICLE
SUPERLATTICE MEMBRANES


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2.1 BACKGROUND

There has been an increasing demand for novel strategies to design flexible substrates and components for electronic devices. One such strategy has been the development of free-standing, ultrathin nanomembranes comprised of both organic and inorganic materials.\(^1\) These represent a relatively new class of materials that possess nanoscale thickness across macroscopic dimensions, giving rise to unique mechanical properties for which a broad spectrum of applications in nanoscale separations, sensing, and energy harvesting may be realized. For example, free-standing nanomembranes are being investigated as prospective sensing elements for thermal and acoustic microsensors.\(^2\) The feasibility of nanocomposite-based membranes has also been demonstrated in the design of energy storage devices, potentially leading to flexible supercapacitors.\(^3,4\)

Due to the unique properties of inorganic nanoparticles outlined in the previous chapter, intense efforts have been devoted toward the incorporation of nanoparticles into free-standing nanomembranes in order to tailor their properties to specific applications. However, despite success in developing a broad genus of nanoparticles within the last several years, it still remains a challenge to organize these particles into well-defined architectures.\(^5\) While inorganic nanoparticles have been incorporated into free-standing nanomembranes through a variety of techniques, including spin-assisted assembly,\(^6,7\) chemical crosslinking,\(^8\) and surfactant templating strategies,\(^9\) achieving highly-ordered arrays in the free-standing format is less straightforward.

Two representative techniques were independently proposed by Jaeger’s group\(^10\) and our group.\(^11\) Jaeger’s strategy involved the use of hydrophobic, dodecanethiol-capped gold nanoparticles.\(^10\) The particles were suspended in toluene and then a small volume was placed on a droplet of water. As the toluene evaporated, the particles packed densely into an ordered layer on top of the water droplet, and were
subsequently transferred to a mesh grid. This produced single-particle thick, free-standing membranes of highly-ordered particles that spanned several micrometer-scale holes. Mechanical analysis revealed that the Young’s moduli of these membranes were surprisingly large (on the order of 5–40 GPa), and their stabilizing forces were attributed to the dense-packing of alkyl ligands in the interstices between the particles, with little contribution from the van der Waals attractions between the gold nanoparticle cores. Although this approach successfully produced highly-ordered, free-standing nanomembranes, the interparticle spacing was constrained to a small range (1–3 nm) owing to synthetic limitations on the length of alkyl chains. Furthermore, because the resulting free-standing regions formed a continuous film that spanned the entire substrate, this strategy was not capable of forming discrete membranes – a feature that would be useful for the integration of superlattices into well-defined solid-state devices.

Our group first reported planar nanoparticle superlattices (long-range-ordered nanoparticle arrays) self-assembled from ssDNA-capped nanoparticles. The nanoparticles were originally dispersed in water, but formed ordered arrays due to controlled evaporation.\textsuperscript{12} This evaporation-driven approach was later applied to a microfabricated porous substrate, resulting in the formation of free-standing superlattice membranes.\textsuperscript{11} Furthermore, it overcame the limitations of Jaeger’s approach by enabling the formation of discrete membranes confined to an arbitrarily shaped microhole. DNA allowed for a wider and more tunable range of up to 20 nm separation for which the plasmon coupling between nanoparticles could be regulated. Interestingly, no base-pairing was required as stable membranes could be produced from both base-pairing and non-base-pairing sequences.

This chapter describes the fabrication of free-standing nanoparticle superlattices assembled from DNA-capped nanoparticles, as well as the methodologies
used to investigate their mechanical and morphological properties. A discussion of the results will provide insight into the mechanisms behind the assembly process, design considerations, and implications on the scaling laws of polymer brush theory. It also raises important questions that will serve as a basis for the subsequent chapters.

2.2 MATERIALS AND METHODS

2.2.1 Synthesis of DNA-Capped Gold Nanoparticles

The gold nanoparticles (AuNPs) used in these experiments had been synthesized previously based on protocols from the literature,\textsuperscript{13,14} and had diameters of 12.8 ± 2.0 nm. Single-stranded DNA sequences were ordered from Integrated DNA Technologies with thiol groups conjugated to their 5’ ends. AuNPs were capped with DNA by first deprotecting the DNA with dithiothreitol (DTT), purifying out the DTT using NAP-5 columns (GE Healthcare), and then incubating the DNA solution with the AuNP solution in a ratio of 1000:1 (DNA:AuNPs) to ensure maximum DNA surface density. The mixture was incubated at room temperature for approximately 12 hours, after which sodium chloride was added to a concentration of 1 M to reduce electrostatic repulsion and further increase DNA surface density. The mixtures were incubated for another 10–12 hours, and then centrifuged and exchanged with ultrapure water to remove the free ssDNA. Three different batches were prepared for mechanical studies in which each sequence comprised 15, 30, or 50 thymine bases, hereafter referred to as batches T15, T30, and T50, respectively.

2.2.2 Preparation of Membranes

Silicon substrates supporting free-standing silicon nitride films were purchased from SPI Supplies. The films were 200 nm thick and consisted of circular perforations of 2 µm in diameter with a 4 µm pitch. Copper mesh grids with 7 µm square holes
(Ted Pella, Inc.) were also used but only for imaging analysis purposes. In order to produce free-standing nanoparticle superlattices, an approximately 40 nM solution of DNA-capped AuNPs was deposited onto a silicon nitride substrate and allowed to dry under ambient conditions.

2.2.3 Nanoindentation Experiments

Nanoindentation, also known as force spectroscopy, is a technique for measuring the mechanical properties of nanoscale materials (Figure 2.1A). It can be performed using an atomic force microscope (AFM), which is capable of both surface imaging and mechanical analysis depending on the type of probe. For example, a microsphere probe is ideal for measuring the elasticity of free-standing films because it can apply a greater amount of force distributed over a wider area, thus reducing the probability of membrane rupture and local variations. However, microsphere probes are too large for imaging. Nanoprobes, on the other hand, are sharp and can be used for imaging, yet they may require blunting in order to prevent membrane rupture, which can potentially compromise image quality.

A single nanoindentation experiment consisted of changing the height of the cantilever above the membrane by means of a piezoelectric crystal \((Z_{\text{piezo}})\), and then measuring the deflection of a laser off of the tip of the cantilever \((d_{\text{deflection}})\). A schematic of a typical deflection measurement is shown in Figure 2.1B. This raw data was converted into a force-displacement curve by multiplying the measured deflection by the spring constant of the cantilever and plotting it against the indentation depth, which was computed from \(d = Z_{\text{piezo}} - d_{\text{deflection}}\).

Force-displacement curves of clamped, circular, free-standing membranes have two regimes: 15,16 a linear regime for small displacements in which the response follows Hooke’s law, and a nonlinear regime for large displacements that is
independent of pre-strain within the membrane and is governed instead by the intrinsic elastic response of the membrane (Fig 2.1C). The force as a function of the ratio of membrane displacement to membrane radius, $\delta = d / R$, can be expressed as

$$F(\delta) = \frac{\pi R l}{3} (3\varepsilon \delta + E \delta^3),$$

(2.1)

where $l$ is the membrane thickness, $\varepsilon$ is the membrane pre-strain, and $E$ is Young’s modulus.\textsuperscript{1,10,17} It is assumed that Poisson’s ratio for this material is 1/3. This model is presumed valid when the probe diameter is small compared to the membrane radius. For small displacements, the term linear in $\delta$ will dominate, which is due to the in-plane stress, or pre-strain, that occurs by fixing the boundary of the membrane to a solid support. Note, however, that greater indentation depths result in a cubic dependence on $\delta$, which occurs when the pre-strain pressure is greatly exceeded by the probe. Although the elastic modulus can be determined in this regime, such high forces can rupture the membrane prior to the cubic transition. This problem will be discussed again later in this chapter.

Force-displacement curves and surface topography measurements were acquired using the tapping mode operation of a Dimension 3100 AFM (Digital Instruments) at the Cornell Nanobiotechnology Center. PointProbe-Plus ZEILR silicon probes (Nanosensors) were blunted prior to use in nanoindentation experiments by scanning a steel surface in contact mode for several minutes. High resolution images of membranes were obtained using probes as received. Although blunted tips resulted in poor image quality, the images obtained were adequate for locating the membranes prior to indentation. Only one probe was used in all experiments to reduce experimental uncertainty. The cantilever spring constant was taken to be 1.7 N/m, as reported by the manufacturer.
Figure 2.1. Force spectroscopy on flexible membranes. (A) An illustration of an AFM probe indenting a free-standing superlattice membrane in the center. Reprinted with permission from Reference 1. Copyright © 2009 Elsevier Ltd. (B) A single measurement consists of an approach and retract cycle (hysteresis is exaggerated to show detail). On the approach phase, no probe deflection occurs until the tip encounters the surface, which happens abruptly due to attractive forces between the tip and surface. Greater deflection occurs as the tip is brought closer to the surface by means of the piezoelectric crystal. As the tip retracts, the abrupt jump out of contact is typically greater due to a larger number of adhesive forces that resulted from driving the tip into the surface. (C) For the same type of membrane under various pre-strains, a cubic transition occurs when the indentation depth becomes comparable to the membrane radius.
2.2.4 Geometric Analysis

Thin, paper-like materials can display high degrees of curvature in response to large deformation forces when there is little structural support. An interesting property of such materials is that the response is scale invariant, and can be described by the scaling laws of crumpled elastic sheets.\textsuperscript{18} Similar to folded paper, nanoscale materials can display buckled, crumpled, and folded morphologies that are governed by their intrinsic elastic properties. The relationship between the folding ridge dimensions and the elastic parameters is given by

\[
\frac{1}{C_0 L^0} = \frac{B}{G} \approx l^2,
\]

(2.2)

where \(C_0\) is the curvature and \(L\) is the length of a folded ridge, respectively, \(B\) is the bending modulus, and \(G\) is the stretching modulus. For isotropic materials, the ratio of the two moduli approximates to the square of the membrane thickness, \(l\). Consequently, this analysis can be useful for estimating the thickness of a membrane simply by measuring its folding ridges. For example, Russell and co-workers used confocal microscopy to observe folding ridges within crosslinked CdSe nanoparticle membranes.\textsuperscript{8} \(C_0\) and \(L\) pairs were measured from several ridges in the microscope images, yielding a range of 2–7 nm that was in good agreement with the estimated membrane thickness of 5 nm.

2.3 RESULTS

2.3.1 Formation of Membranes

After drying a droplet containing DNA-capped nanoparticles on a microperforated substrate, superlattice membranes spanned only the microholes with very few stray nanoparticles found on the surrounding silicon nitride support (Figure 2.2). The most consistent results were achieved using nanoparticle concentrations ranging
from 30–60 nM. In general, the nanoparticles within the membranes were hexagonally-packed, with the exception of defects that occurred at the circular perimeter, suggesting that the nanoparticle interactions governed the ordering rather than the geometry of the substrate. The surfaces of these membranes were flat and relatively smooth, and were recessed into the 200 nm-thick substrate to a depth of 100–180 nm. Furthermore, the membranes were robust enough to withstand high vacuum, repeated atomic force imaging, and electron beam irradiation. They also lasted over 1 year under ambient conditions with no observable change in quality.

When microholes larger than 2 µm in diameter were used, membranes were not able to form completely. Figure 2.3A shows a free-standing membrane that was partially detached from the edge of its hole. It is likely that the membrane originally spanned the hole, but detached from the substrate and folded in on itself, producing a rippled appearance. Another characteristic of these detached membranes was that the interparticle spacing was generally smaller along the direction perpendicular to the boundary of the detached region. This suggested that the membranes were pre-strained during their formation, but relaxed along one direction after detachment from the substrate (Figure 2.3B).

The proposed mechanism through which the membranes form is shown in Figure 2.4. After a drop is deposited via pipette and begins to recede, its contact line becomes pinned as it passes over the microholes leaving behind satellite microdroplets in its wake. As water begins to evaporate from both sides of the microdroplets, the motions of the nanoparticles become spatially restricted. Consequently, over an optimal range of nanoparticle concentrations, nanoparticles will be packed together into a single layer forming a two-dimensional membrane stabilized by the interactions between the nanoparticles and the substrate perimeter.
**Figure 2.2.** Various visualizations of free-standing superlattice membranes. Left: Transmission electron microscopy (TEM) images reveal the hexagonal packing arrangement. An inset shows the fast Fourier-transform (FFT). Middle: 3D TEM tomographic reconstructions show that the membranes are of single-particle thickness. Right: AFM imaging shows a series of perforations in which some contain membranes and others do not. Reprinted from Reference 11.
Figure 2.3. Compression within detached membranes. (A) Detached membranes were typically observed when the hole dimensions exceeded 2 µm, and exhibited a wrinkled, paper-like appearance. (B) The unsupported side of a detached membrane had less pre-strain along the direction parallel to the detachment, resulting in a shorter interparticle spacing in that direction. Reprinted from Reference 11.
Figure 2.4. The superlattice membrane assembly process. (A) After a droplet is placed on the grid, it evaporates and leaves behind satellite microdroplets in the perforations. (B) As the microdroplets dry, volume restrictions force the particles to pack close together.
2.3.2 Corona Height Scaling

In the previous chapter, Eq. 1.5 described how the polymer corona height scales for nanoparticles. A more general model that accounts for surface curvature is given by

\[ h_0 \sim N^{3/(3+D)} \sigma^{1/(3+D)} R^{D/(3+D)}, \]  

(2.3)

where \( D \) represents the dimensionality of the system in which \( D = 0 \) corresponds to a planar surface, \( D = 1 \) corresponds to a cylindrical surface, and \( D = 2 \) corresponds to a spherical surface.\(^{19}\) Based on Eq. 2.3, we can express the brush height more compactly as

\[ h_0 = \alpha N^\nu, \]  

(2.4)

where \( \alpha \) is a proportionality constant that absorbed surface density and nanoparticle radius, and also accounts for excluded volume effects. The scaling factor, \( \nu \), corresponds to the scaling law that arises from the dimensionality of the system.

The scaling factor can be extracted from a best fit of the experimental data, with the constraint that the ssDNA brush densities and nanoparticle radii are the same for all \( N \). While this can be easily confirmed for nanoparticle radius, assuming that brush density is independent of \( N \) is also a reasonable assumption.\(^{20}\) Figure 2.5 shows a best fit of Eq. 2.4 to experimentally measured brush heights for un-deformed membranes and compressed membranes, resulting in \( \nu = 0.55 \) for un-deformed membranes and \( \nu = 1.06 \) for compressed membranes.
Figure 2.5. Brush height scaling behavior in superlattice membranes. (A) Interparticle spacings measured within fully attached, un-deformed membranes. (B) Interparticle spacings measured along the direction of relaxation. The scaling law for un-deformed membranes is in agreement with the free particle dimensionality. The deformed membranes behave more like polymer brushes on a flat surface, most likely due to the high level of compression and interdigitation of the polymer layers.
2.3.3 Geometric and Mechanical Analysis

Using scanning TEM tomography of partially-attached membranes, the curvature and sizes of ridges in the membranes were measured (Figure 2.6). The measured thicknesses were estimated from Eq. 2.2 to be between 37 nm and 66 nm. More dramatically deformed membranes provided a lower limit on membrane thickness, for which the smallest value of 37 nm was taken to be the membrane thickness in subsequent calculations. This was also in agreement with the membrane thickness estimated from dynamic light scattering data.\(^\text{11}\)

Superlattice membranes of different DNA lengths (T15, T30, and T50) were indented with a worn silicon probe. Each membrane was indented at the center multiple times to demonstrate reproducibility of the measurement, and the results were shown to be fairly consistent for multiple membranes of the same DNA length. Even after multiple indentations, no damage was ever observed unless the membrane was punctured. Individual ruptures did not nucleate after repeated puncturing (Figure 2.7).

The probe was blunted prior to force spectroscopy experiments (Figure 2.8). Force-displacement curves for the three different types of membranes are shown in Figure 2.9, and their corresponding spring constants are shown in the inset. The resulting curves were fairly linear, but would typically break at higher indentation depths when \(d/l > 3\). The fact that linear behavior dominated the force spectra suggested that pre-strain was significant within the membranes, and is likely due to the firm attachment of the membranes to their respective silicon nitride substrates. Consequently, this made it relatively difficult to estimate Young’s moduli since the nonlinear regime was unreachable prior to puncturing the membranes. Reliable fitting could only be performed with T30 membrane data, yielding \(E = 6.5 \pm 1.6\) GPa. This data was comparable to the values reported by Jaeger and co-workers for alkyl-based membranes.\(^\text{10}\)
Figure 2.6. Geometric analysis of ridges. Each measurement of a ridge yielded a corresponding curvature and ridge length. Only the sharpest ridges could provide a lower limit on the membrane thickness. Reprinted from Reference 11.
Figure 2.7. Membrane puncturing experiments. (A-F) A single membrane was punctured in one location and imaged. Note that no nucleation occurred with each subsequent puncture step. Reprinted from Reference 11.
**Figure 2.8.** Mechanical wearing of an AFM probe. The radii of curvature before and after wearing were estimated to be 7 nm and 100 nm, respectively. This probe was used exclusively in all of the force spectroscopy measurements to reduce experimental uncertainty. Reprinted from Reference 11.
Figure 2.9. Force displacement curves for different types of membranes. The spring constants were taken to be the slopes, and are shown in the inset. Reprinted from Reference 11.
2.4 DISCUSSION

The entropy-driven strategy for fabricating free-standing nanoparticle superlattices represents an unconventional use of DNA in that its base-pairing nature is not utilized to achieve the end result. Instead, DNA plays a more generic structural role by permitting a wider range of interparticle spacings within the superlattice membranes than has been achieved with other types of polymer ligands. In addition, this strategy significantly improved control over both internal order and overall shape of superlattices by utilizing microhole-confined self-assembly, in contrast to non-uniform evaporation kinetics and solvent fluctuation effects that may arise from dewetting on a solid substrate. This strategy further overcomes stability issues associated with transferring biomolecule-based assemblies to solvent-free environments, potentially expanding the applicability of such materials in solid-state devices. Overall, these characteristics will facilitate the systematic investigation of substrate-free self-assembly phenomena and plasmonic coupling within large-scale nanoparticle systems in the absence of substrate-induced interference.

Dense DNA packing and negligible ionic strength likely inhibits Watson-Crick base-pairing, and therefore interactions between DNA-capped nanoparticles must be dominated by a balance between electrostatic repulsion and van der Waals attractions: a common mechanism of colloidal hard-sphere assembly. Unlike hard-sphere systems, however, ordered structures are maintained by the interactions between the soft polymer coronae. The nanoparticle cores, per se, are not responsible for this stabilization as it can be assumed that the interparticle van der Waals potential falls off rapidly as the center-to-center spacing is increased. During the assembly process, particularly in the late stages of drying, the soft DNA coronae likely interdigitate or deform through nonspecific interactions, as has been observed with alkyl-based superlattices. In order to investigate the effects of base-pairing, a comparison was
made between membranes fabricated using poly-thymine DNA and membranes containing DNA ligands with self-complementary regions.\textsuperscript{11} It was found that the self-complementary ligands did not improve the degree of internal order compared with poly-thymine ligands, suggesting that a dense coating of DNA was the only requirement.

The interparticle spacing for both the fully and partially-attached membranes can be adjusted over a wide range by setting the DNA sequence length.\textsuperscript{11} In particular, the edge-to-edge interparticle spacing could range between $0.9 \pm 0.2$ nm to $19.6 \pm 1.7$ nm, which was a much wider range than has been achieved for alkyl ligands.\textsuperscript{23} Interestingly, the membrane stiffness increased with decreasing ligand length. Since the ligands, rather than the metallic cores, are the dominant factors governing the membrane stabilization, it should follow that the ligand-ligand interactions must affect the elasticity and mechanical strength. Consequently, shorter ligands will likely produce a higher ligand density (and therefore, greater crosslinking density) in the interstices between the nanoparticles, while longer ligands will tend to be less dense as radial extension from their nanoparticle cores will cause them to spread out.

Although a densely-packed DNA corona was necessary to preserve internal order in both fully- and partially-attached membranes, fully-attached membranes were unable to form in microholes that exceeded 2 µm in diameter. In order to explain this phenomena, a failure model was developed by Hui and Long in collaboration with our group.\textsuperscript{24} Two mechanisms of failure were considered: adhesive and cohesive failure. In adhesive failure, a crack nucleates along the perimeter of the membrane. In cohesive failure, on the other hand, crack nucleation occurs within a region of the membrane that is adjacent to the supporting interface. Both modes of failure can be characterized by a factor, $W$, which is defined as the energy required to detach the membrane per unit area of the membrane at the supporting interface. A simple scaling
argument was derived to show why membranes with larger radii are likely to fail, given by

\[ \frac{E\varepsilon^2 R}{2(1-\nu)W} > 1, \]

where \( \nu \) is Poisson’s ratio. Although this argument is simplistic in that it assumes total detachment from the entire support, it predicts a bonding energy that is on the scale of 15–50 mJ/m² based on our previously reported experimental results.\(^{11}\) This range is comparable to the energy of van der Waals interactions,\(^{25}\) further suggesting that these are the dominant forces responsible for adhesion of the membranes to their silicon nitride supports. In reality, however, a more likely scenario is that a small defect present at the interface begins to nucleate. This occurs when the energy release rate of a crack exceeds the adhesion per unit area, \( W \). Finite element modeling revealed a critical membrane radius beyond which crack nucleation will occur.\(^{24}\) In addition, stiffer membranes were also predicted to detach more easily than softer membranes, consistent with preliminary experimental results. Overall, these insights may prove to be a useful guide in the design of other types of free-standing membranes.

An interesting observation in the corona height behavior suggests that the effective corona height in un-deformed membranes is similar to that of spherical particles (\( \nu = 0.6 \)), while deformed membranes approximate the scaling behavior of a planar surface (\( \nu = 1 \)). This suggests that, while the scaling laws shown in Eq. 2.3 hold for the coronae of free particles or particles that are loosely packed together, the scaling laws can deviate depending on the local environment. While few works have addressed the effect of nearby corona interactions in terms of shear forces and the effects of longitudinal compressive forces on free energy,\(^{26-28}\) none have addressed the scaling properties of the polymer brush height.
While the proposed mechanism of Figure 2.4 may be accurate during the dewetting stage of membrane fabrication, additional experiments were needed to understand the formation of the nanoparticle crystals at the final stages of drying. The following chapters will discuss both real-time and spatial characterization techniques that were implemented to shed light on the self-assembly process.
REFERENCES


CHAPTER THREE:
CRystallization of DNA-Capped Nanoparticles
Measured in Real-Time


1 Biological & Environmental Engineering, Cornell University, Ithaca, NY, USA
2 Cornell High Energy Synchrotron Source, Cornell University, Ithaca, NY, USA
3 Theoretical & Applied Mechanics, Cornell University, Ithaca, NY, USA
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3.1 BACKGROUND

The assembly of organically-capped nanoparticles into well-defined supercrystalline structures, such as free-standing superlattice membranes, involves the elastic deformation of soft, spring-like polymer layers. Such systems differ from conventional colloidal hard-sphere assembly in that the forces of interaction are distributed throughout the corona rather than confined to the surface, though the same types of stabilizing forces are present. Despite numerous studies of soft-corona nanoparticle assemblies,1,2 the focus has been on equilibrium structures rather than on how the soft coronae deform dynamically during the crystallization process.

DNA-capped nanoparticles can serve as a unique model system for investigating nanoscale self-assembly, especially since the effective size of the soft corona can be precisely tuned over a wide range. In an effort to better understand the mechanism of crystal formation, recent research efforts by several groups, including ours,3,4 have employed synchrotron radiation, in particular small angle X-ray scattering (SAXS), to reveal the nature of such interactions. Gang and Mirkin, for example, have studied the sequence- and temperature-dependent nanoparticle crystallization in bulk buffered solutions.5-7 This chapter, however, will focus on the effects of sequence length and specific versus nonspecific interactions and their effects on the real-time crystallization dynamics within a drying-mediated self-assembly process.

3.2 MATERIALS AND METHODS

3.2.1 Synthesis of DNA-Capped Gold Nanoparticles

Gold nanoparticles were both synthesized and chemically conjugated to ssDNA as described in Chapter Two. The sequences used were either non-base-
pairing with the sequence SH-5’-poly(dT)$_N$, in which $N = 5, 15, 30, 70, \text{ or } 90$, or base-pairing with the sequence SH-5’-TGTAC.

### 3.2.2 Experimental Setup

SAXS experiments were performed at the D1 station of the Cornell High-Energy Synchrotron Source (CHESS) in order to probe the \textit{in situ} growth of DNA-capped nanoparticle supercrystals during water evaporation. X-rays from a hardbent dipole magnet were monochromatized with a pair of Mo$_2$B$_4$C multilayers yielding an X-ray beam with a wavelength of 1.25 Å and a bandwidth of 1.5%. The beam was collimated to form a circular spot 0.5 mm in diameter, producing an average flux of approximately $10^{11}$ photons/s. Scattered X-rays were detected with a CCD-type area detector (MedOptics) at sample-to-detector distances of 1041 mm and 1569 mm, as calibrated using a silver behenate standard. A photodiode integrated into the beamstop monitored the transmitted intensity.

For a single experiment, a 1.6 $\mu$L droplet of 800 nM DNA-capped nanoparticle solution was placed on a 25 $\mu$m thin Kapton® polyimide film (Dupont) oriented vertically (Figure 3.1). The film was mounted to a metal stand with a hole in the center for the X-ray beam to pass through. The droplet volume could be dynamically controlled by adjusting the chamber humidity with a helium gas flow. A thermohygrometer was also present to monitor the temperature and relative humidity within the chamber. The chamber rested on a motorized stage that could adjust the relative beam-to-sample position in three dimensions. Time series were collected for each sample with typical exposure times between 0.5 and 3 s and at intervals between 0.5 and 3 min, depending on the conditions of the experiment. A complete time series typically lasted between 30 and 60 min.
Figure 3.1. Schematic of the experimental setup for transmission SAXS. The thin polyimide film is not shown, but is affixed to the vertical sample holder. Reprinted with permission from Reference 4. Copyright © 2011 American Chemical Society.
3.2.3 Data Processing and Analysis

For transmission SAXS through a fluid, the X-ray scattering intensity can be expressed as

\[ I(q) = CVF(q)S(q), \]

where \( C \) is the concentration of the nanoparticles, \( V \) is the total volume irradiated by the finite sized X-ray beam, \( F(q) \) is the form factor, and \( S(q) \) is the structure factor. The radial scattering vector in crystallographic units is given by

\[ q = \frac{4\pi}{\lambda \sin(\theta)}, \]

where \( \theta \) is the scattering angle, and \( \lambda \) is the wavelength. One-dimensional SAXS spectra were obtained by integrating the two-dimensional detector images azimuthally over a quadrant using FIT2D software.\(^8\,9\) The structure factor, \( S(q) \), from each SAXS pattern was obtained by dividing out the experimental form factor, \( F(q) \), obtained from a scan of non-interacting, free nanoparticles at low concentration at the beginning of the experiment.

3.3 RESULTS

3.3.1 Observation of Dynamic Crystallization

The crystallization of DNA-capped nanoparticles was mapped temporally over the lifetime of droplet evaporation by obtaining a time series of SAXS scans, which was taken near the contact line of the droplet. SAXS spectra and their corresponding one-dimensional structure factors are shown in Figure 3.2 for base-pairing and non-base-pairing sequences at various time points. It was found that the onset of crystallization occurred immediately after the droplet was placed in the sample chamber for the base-pairing sequences (Figure 3.2A). This rapid onset was likely
triggered by specific Watson-Crick base-pairing, which is consistent with the mechanism reported by both Gang and Mirkin.\textsuperscript{5,6} For non-base-pairing sequences, the SAXS patterns exhibited diffuse scattering that was characteristic of the form factors for randomly distributed nanoparticles (Figure 3.2B). As the droplet began to evaporate, the structure factor began to emerge indicating the onset of crystallization. In this situation, evaporation-driven volume restriction was the likely mechanism of crystallization. Despite these two mechanisms, however, crystallites of each could both be indexed to face-centered cubic (FCC) lattices.\textsuperscript{3}

The evolution of the SAXS spectra for non-base-pairing DNA after the onset of crystallization is shown in Figure 3.3. The emergence of the first-order Bragg peak occurred first, followed by subsequent higher order peaks. As time progressed, all of the peaks shifted continuously in the positive $q$ direction, which corresponded to a decrease in interparticle spacing. Interestingly, the Bragg peaks in all of these 1D plots can be indexed to FCC lattices, revealing uniform, continuous shrinking of the lattice over time. Note that at $\sim$4300 s, a rapid shift in peak positions occurs while higher order scattering peaks vanished, indicating the formation of aggregates with short-range order. At $\sim$4700 s, the droplet was completely dried.
Figure 3.2. Time progression of nanoparticle crystallite formation. Results for (A) a base-pairing DNA corona, and (B) a non-base-pairing DNA corona. Reprinted with permission from Reference 3. Copyright © 2010 Wiley-VCH Verlag GmbH & Co.
Figure 3.3. Evaporation-driven crystallization without base-pairing. The droplet contained poly(dT)15-capped nanoparticles. Reprinted with permission from Reference 3. Copyright © 2010 Wiley-VCH Verlag GmbH & Co.
3.3.2 *Humidity Cycling*

The dynamic, reversible behavior of the nanoparticle crystallites was demonstrated by placing the droplet into a sealed chamber in which the humidity was varied. This was done by placing a water reservoir within the chamber and allowing the chamber to initially equilibrate to 100% relative humidity (RH). The humidity could be regulated by adjusting the flow of helium gas through the chamber. Under 50% RH, water evaporated from the droplets, while at 100% RH, the droplets would be restored to their original volumes. This was repeated over numerous cycles with only 1–2 minutes in between each cycle (Figure 3.4). The interparticle spacing decreased upon evaporation, and increased upon swelling. The decrease in interparticle spacing was likely due to two factors: (1) increased crowding of nanoparticles, ultimately leading to corona compression, and (2) increased ionic strength leading to greater charge screening and, hence, less electrostatic repulsion between DNA coronae.
Figure 3.4. Humidity cycling experiments. The humidity was adjusted between 100% and 50% RH for several cycles with approximately 1-2 minutes needed between each cycle to equilibrate. Reprinted with permission from Reference 3. Copyright © 2010 Wiley-VCH Verlag GmbH & Co.
3.3.3 Modeling of Corona Deformation

As proposed in the previous chapter, densely-packed DNA molecules anchored to the nanoparticle surface behave as polymer brushes with equilibrium height, $h_0$. In addition, it was assumed that the DNA corona follows a $\nu = 1$ scaling law when highly compressed. The undeformed, equilibrium brush height can be expressed as

$$h_0 = N(\eta \sigma b^2)^{1/3}, \quad (3.3)$$

where $\eta$ is a scaling constant that depends on excluded volume effects, and $\sigma$ is the number of DNA strands per unit area of nanoparticle.\textsuperscript{10}

It was assumed that DNA coronae experience isotropic deformation during the late stages of drying and after complete drying. Based on the model depicted in Figure 3.5A, the deformation from equilibrium of the DNA corona can be expressed as

$$d = h_0 + R - \frac{D_{NN}}{2}, \quad (3.4)$$

where $D_{NN}$ is the center-to-center nearest neighbor spacing. The compression ratio can be written as

$$\lambda = \frac{d}{h_0 + R}, \quad (3.5)$$

which accounts for the compression of the entire particle and not just the soft corona. Note that the compression ratio can never approach 100% since this would require deformation of the solid particle core. It is also helpful to define a dimensionless softness parameter

$$\chi = \frac{h_0}{R}. \quad (3.6)$$
The softness parameter was taken as the ratio of the equilibrium corona height (the hydrodynamic radius as measured with dynamic light scattering) to the nanoparticle radius (taken as 13.4 nm).

Each ssDNA strand was assumed to respond linearly like a spring under a force, $F$, according to

$$F = k_E d,$$  \hspace{1cm} (3.7)

where $k_E$ is the entropic spring constant. Applying the entropic spring argument, the spring constant of an ideal polymer chain in three dimensions is given by

$$k_E = \frac{3k_B T}{Nb^2},$$  \hspace{1cm} (3.8)

where $k_B T$ is the thermal energy, $N$ is the number of Kuhn segments, and $b$ is the Kuhn length. Combining Eqs. 3.3 and 3.8 yields

$$k_E = \frac{3k_B T (\eta \sigma)^{1/3}}{h_b b^{4/3}}.$$  \hspace{1cm} (3.9)

Further combining Eqs. (3.7) and (3.9), the deformation can now be expressed in terms of the equilibrium brush height as

$$d = \frac{F b^{4/3}}{3k_B T (\eta \sigma)^{1/3} h_b}.$$  \hspace{1cm} (3.10)

By combining Eqs. 3.5, 3.6, and 3.10, the compression ratio can now be written in terms of the nanoparticle softness as

$$\lambda = \frac{\chi}{1 + \chi} \left[ \frac{F b^{4/3}}{3k_B T (\eta \sigma)^{1/3}} \right].$$  \hspace{1cm} (3.11)

In order to account for the stiffness of very short polymer brushes that resist elastic deformation, Eq. 3.11 was modified with an empirical correction factor, yielding
where $\beta = \frac{F b^{4/3}}{(3k_B T (\eta \sigma)^{1/3})}$.

The compression ratios of fully-dried DNA-capped nanoparticles of varying softness and a corresponding fit of Eq. 3.12 are shown in Figure 3.5B. The prefactor, $\beta$, and $\lambda_0$ were used as the fitting parameters. Note that this fit assumes that all fully-dried nanoparticle crystals experience the same compressive force regardless of their equilibrium brush height, and that the compression ratio is only a function of the particle softness. A comparison was also made to free-standing nanoparticle superlattice data, but overall compression was less than that of the 3D crystals. This could possibly be attributed to a smaller amount of force exerted on the nanoparticle corona due to opposing pre-strain forces. The entropic spring model of the DNA corona was additionally applied to conventional alkyl-corona nanoparticles, and agrees reasonably well with the data reported by Martin and co-workers. The softness range, however, was limited due to ligand length restrictions.
Figure 3.5. Modeling of the soft corona. (A) Relevant dimensions of the nanoparticle system. It is assumed that the coronae are in tangential contact before deformation, but become compressed and interdigitated due to drying effects. (B) A fit of the model to dried 3D crystals, free-standing membranes, and alkyl corona data from Ref. 12. Reprinted with permission from Reference 3. Copyright © 2010 Wiley-VCH Verlag GmbH & Co.
3.4 DISCUSSION

Using synchrotron-based SAXS, the crystallization of a colloidal droplet of DNA-capped nanoparticles was observed in real-time and in situ. These experiments demonstrated the gradual progression of the crystallization process, revealing the mechanism by which ordered crystallites form and how the lattice constant continuously changes owing to the spring-like nature of the DNA corona. Self-hybridizing sequences could trigger the onset of crystallization, which was later aided by the evaporation kinetics, whereas non-base-pairing sequences, poly(dT)\textsubscript{N}, resulted in crystallization much later in the drying process. By controlling the lengths of DNA sequences, it was possible to relate the nanoparticle softness to the total compression at the late stages of evaporation. This behavior could be described by a simple entropic spring argument, and could also be extended to alkyl coronae. These results suggest that the crystallization process can be tailored simply by controlling the corona properties. For example, using double-stranded DNA or branched DNA motifs could affect the stiffness of the corona, and potentially allow for greater control over the process. Such corona engineering strategies may emerge as alternative routes towards the rational design of nanoparticle assemblies.

Despite the base-pairing-driven pathway of recently reported DNA nanoparticle crystals, the drying-mediated pathway is an entropic process. In the absence of base-pairing interactions, poly-thymine coronae are electrostatically repulsive, and therefore crystallization can only occur when volume restriction causes the nanoparticle concentration to exceed a critical threshold, much like colloidal hard-spheres. Beyond this threshold, van der Waals forces between the ligands and possibly nonspecific hydrogen bonding between DNA bases help stabilize the crystals.

While the experiments described in this chapter were able to probe the crystallization in real-time, crystallites were often found near the droplet edges with
very little scattering signal produced at the droplet centers. It would be plausible to suggest that drying-mediated crystallization plays a greater role at the droplet edge due to competition between the inward movement of the air-liquid interface and the crystallite diffusion. In the next chapter, we will supplement the real-time data with detailed spatial information in order to better understand how these crystallites nucleate.
REFERENCES


CHAPTER FOUR:
INVESTIGATING NANOPARTICLE SELF-ASSEMBLY AT THE
AIR-LIQUID INTERFACE

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4.1 BACKGROUND

The previous chapters have discussed a drying-mediated strategy to produce nanoparticle supercrystals. In general, the methods of generating supercrystals can be summarized in two distinct strategies: drying-mediated nanoparticle crystallization and DNA-programmable crystallization.1 The drying-mediated strategies are entropic processes driven by evaporation of a solvent in which the nanoparticles are dispersed, forcing them to pack together at a solid or liquid interface with the aid of optimized nonspecific forces.2-15 DNA-programmable strategies, which are a relatively recent addition, are enthalpic processes that exploit Watson-Crick base-pairing between DNA strands on adjacent particles to drive the assembly in bulk solution.16-19

Both of these strategies have strengths and weaknesses. For example, while DNA-programmable crystallization allows for wider ranges of the interparticle spacing and a great measure of control over the crystal structure, the resulting crystals are typically limited to the solution phase posing a potential problem for solid-state device integration. On the other hand, while drying-mediated crystallization can occur at a variety of different interfaces, the interparticle spacing of the resulting superlattices is constrained by the synthetic limitations on capping ligand length. Another shortcoming of drying-mediated strategies is that superlattices often show cracks due to kinetic trapping effects during the drying process, which may have a tendency to nucleate according to our previous modeling studies.20 In addition, far-from-equilibrium drying effects may dominate and obscure the more subtle effects of nanoparticle corona interactions. While truly equilibrated systems are usually difficult to study, it is important to understand the parameters at work so that deposition and transfer techniques can be refined to avoid kinetic trapping or cracking.

Layers on a liquid surface, however, are not constrained in their self-assembly kinetics by a diminishing amount of solvent, and thus true equilibrium structures can
be prepared and studied. Although hydrophobically- and amphiphilically-capped nanoparticles assembled into Langmuir films have been extensively studied,\textsuperscript{2,21-23} few studies have addressed the assembly of hydrophilically-capped nanoparticles into Gibbs layers. Gibbs layers, in contrast to Langmuir layers, are equilibrium systems that form when the solute is capable of lowering the surface tension of the solvent, in which their surface coverage is a function of solute concentration in the bulk subphase.\textsuperscript{24,25} Nanoparticles coated with DNA can introduce additional parameters into the Gibbs system, and can provide a unique model for their investigation.

In this chapter, we will discuss how a variant of grazing-incidence SAXS (GISAXS), called parallel SAXS (parSAXS), was used to directly investigate the interfacial phenomena of droplets containing DNA-capped nanoparticles.\textsuperscript{26} This chapter will pick up where the previous chapter left off by focusing on the spatial aspect of nanoparticle crystallization, thereby fortifying our picture of the crystallization process.

4.2 MATERIALS AND METHODS

4.2.1 Synthesis of DNA-Capped Gold Nanoparticles

Gold nanoparticles were both synthesized and chemically conjugated to ssDNA as described in Chapter Two. The sequences used consisted of polythymine spacers that ended with a self-complementary base-pairing region: 5'-SH-C\textsubscript{6}-\texttt{(dT)}\textsubscript{N}-CTCATGAG, where N = 7, 15, or 30. The base-pairing region was used to promote rapid crystallization of DNA-capped nanoparticles, as discussed in the previous chapter.
4.2.2 Experimental Setup

SAXS experiments were performed at the D1 station at CHESS. The incident X-ray beam had a flux on the order of $10^{12}$ photons s$^{-1}$ mm$^{-2}$ with a bandwidth of 1.5% using a multilayer monochromator. The beam had a total energy of 10 keV with a wavelength of 1.24 Å, and the dimensions were 0.3 mm (horizontal) by 0.2 mm (vertical). A MedOptics CCD detector located 993 mm from the sample was used to capture the scattered images, with integration times between 0.1 and 1 s. Some images, however, were obtained at the SAXS/WAXS beamline of the Australian Synchrotron for qualitative purposes.

A single experiment consisted of placing a 1.5 µL droplet of DNA-capped nanoparticle solution on a clean silicon wafer shard. The silicon substrate was placed in an environmental chamber similar to the one described in Chapter Three, except that the sample was oriented such that the plane of the substrate was parallel to the incident X-ray beam (Figure 4.1). The droplet volume could be stabilized for spatial scanning by controlling the vapor pressure in the chamber with a reservoir of the same salt concentration as the droplet.
Figure 4.1. Schematic of the experimental setup for parSAXS. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
4.2.3 Data Processing and Analysis

The intensity of the two-dimensional spectrum produced by a GISAXS measurement of nanoparticles on a solid support can be modeled as

\[ I(q) = V(q_z)S(q_x)F(q_x^*, q_z^*) , \quad (4.1) \]

where \( V(q_z) \) is the Vineyard factor that accounts for scattering from the substrate and depends on the incident beam angle, \( S(q_x) \) is the structure factor of the nanoparticle monolayer, and \( F(q_x^*, q_z^*) \) is the form factor that arises due to the shape-induced scattering from individual, non-interacting nanoparticles.\(^{27}\) For a parSAXS measurement, the incident angle of the X-ray beam is 0º with respect to the substrate. Consequently, we can assume that the Vineyard factor will be unity since there will be no substrate scattering effects. The form factor for spherical particles can be expressed as

\[ F(q) = \int_0^\infty N(R)P(q, R)R^6dR , \quad (4.2) \]

where

\[ P(q, R) = (3[\sin(qr) - qR \cos(qR)]/(qR)^3)^2 \quad (4.3) \]

and

\[ N(R) = \frac{\exp[-(R - R_0)^2/(2\sigma_R^2)]}{(2\pi\sigma_R^2)^{1/2}} , \quad (4.4) \]

It is assumed that the particle sizes follow a Gaussian distribution with a mean radius \( R_0 \) and standard deviation \( \sigma_R \). The structure factor can be expressed as

\[ S(q_s) = S_0(q_s)D(q_s) + 1 - D(q_s) \quad (4.5) \]

in most cases,\(^{28}\) although it can be approximated as

\[ S(q_s) \approx S_0(q_s)D(q_s) \quad (4.6) \]
when dilute scattering effects at greater scattering angles are minimal.\textsuperscript{29} \( S_0(q_x) \) contains information about the Bragg peak locations and is given by

\[
S_0(q_x) = \sum_{\{hk\}} m_{hk} L(q_x), \ m_{h0} = m_{hh} = 6, m_{hk} = 12.
\] (4.7)

The \( m_{hk} \) factors are multiplicities of the \( hk \) reflections for a simple, two-dimensional hexagonal packing arrangement.\textsuperscript{27} The static Debye-Waller factor, \( D(q_x) \), which accounts for local disorder within the unit cell, is given by

\[
D(q_x) = \exp(-\sigma_{DW}^2 q_x^2).
\] (4.8)

Higher order Bragg peaks are more sensitive to the displacement from their optimal lattice positions, \( \sigma_{DW} \), and decay exponentially at higher scattering angles. The shapes of Bragg reflections are described by the Lorentzian function

\[
L(q_x) = \frac{1}{1 + \frac{D_g^2 (q_x - q_{hk})^2}{4\pi^2}},
\] (4.9)

where \( D_g \) is the average grain size of the superlattice, and \( q_{hk} \) corresponds to a particular Bragg reflection. The relationship between grain size and the full width at half-maximum of a particular diffraction peak, \( B_{hkl} \), is given by the Scherrer equation,

\[
D_g = \frac{K\lambda}{B_{hkl} \cos \theta_{hkl}}.
\] (4.10)

Taking into account resolution effects due to the finite footprint of the beam and sample, the diffraction peak width in Eq. 4.10 can be written as

\[
B_{hkl} = (B_{exp}^2 - B_{res}^2)^{1/2},
\] (4.11)

where \( B_{exp} \) is the experimentally measured width.\textsuperscript{30} \( B_{res} \) is based on the resolution of the SAXS setup, according to
\[ B_{\text{res}} = \left( B_{\text{div}}^2 + B_{\text{BW}}^2 + B_{\text{geo}}^2 \right)^{1/2}. \]  

(4.12)

The beam divergence, \( B_{\text{div}} \), is the ratio of the horizontal source size to source-to-sample distance given by

\[ B_{\text{div}} = \frac{\sigma_H}{L_{\text{source}}}, \]  

(4.13)

which is 0.16 mrad for the D1 station at CHESS. The radial divergence is

\[ B_{\text{BW}} = 2 \frac{\Delta E}{E} \tan \theta_{hkl}, \]  

(4.14)

where \( \Delta E/E = 1.5\% \) for the beam used in this work. The geometric smearing is

\[ B_{\text{geo}} = \frac{w}{L} \tan 2\theta_{hkl}, \]  

(4.15)

where \( w \) is the width of the footprint, and \( L \) is the sample-to-detector distance. A worst-case scenario of \( w = 2 \) mm was assumed, in which the beam completely overlaps with the sample.

4.3 RESULTS

4.3.1 Raster Scanning of Droplet Profiles

Sessile droplets were spatially-mapped in the vertical and horizontal directions using parSAXS in a raster scanning mode. A representative scan profile is shown in Figure 4.2. Scans near the apex of a droplet revealed information about the air-liquid interface due to the comparable scattering between the surface area and small bulk volume contained within the scattering cross-section. However, as the relative beam position was decreased and the beam passed directly through the bulk of the droplet, the bulk volume dominated and no interfacial information could be revealed. Such scans not only revealed scattering events occurring at the interface, but also showed the locations of precipitates within the sample.
Figure 4.2. Representative parSAXS raster scan. Scattering streaks indicative of crystallization were often observed along the edge of the droplets at the air-liquid-solid interface due to evaporative effects that occur during sample loading and equilibration of the vapor pressure. Therefore, parSAXS data was extracted from scans near droplet apex to ensure that the data corresponded to Gibbs monolayers and not dry deposits. Dilute scattering dominated the spectra from the bulk interior of the droplet, which arose from randomly dispersed particles and not ordered particles. The form factor scattering of the bulk solution dominated the scattering and drowned out any scattering signal from the surfaces that the beam passed through. Monolayer scattering was often observed 2–3 steps into the droplet interior, which was due to the finite beam footprint. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
4.3.2 Representative Spectra

Figure 4.3 gives a closer look at a single parSAXS measurement at the air-liquid interface. Spectra obtained near or at the air-liquid interface contain a specular reflection that extends from the beam position in a direction that is perpendicular to the surface at which the beam passes through. Each contained the diffuse background due to dilute scattering of free nanoparticles in solution. Under certain conditions, parallel streaks (Bragg reflections) were observed along with the specular streak, which were indicative of nanoparticle monolayers. Sometimes only a single, first-order Bragg reflection was observed, indicating the existence of a two-dimensional monolayer with short-range order. A single ring, on the other hand, suggested the presence of three-dimensional aggregates with short-range order. Multiple, concentric rings would correspond to a three-dimensional polycrystalline aggregate, however this was never observed. Composite spectra often resulted from simultaneous scattering events from both monolayers and aggregates due to the footprint of the beam. The representative spectra obtained from parSAXS measurements at the air-liquid interface are illustrated in Figure 4.4. While Bragg peaks were observed near the interface under a variety of salt conditions, highly-ordered monolayers were only observed over a limited range. For example, Figure 4.5 shows a parSAXS spectrum of a highly-ordered monolayer near the droplet apex in which fifth order Bragg peaks were observed.
Figure 4.3. Schematic of a parSAXS experiment. The sample stage is positioned such that the X-ray beam is passed through the droplet parallel to the plane of the substrate. The coordinate system of the scattering vector is defined such that $q_z$ is parallel to the specular reflection of the incident beam off of the droplet surface, and $q_x$ is perpendicular to $q_z$ and thus parallel to the local surface segment. The specular reflection is an artifact inherent to this type of experiment, and is ignored in the subsequent data analysis. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
Figure 4.4. Representative spectra observed in parSAXS experiments. The angle of the scattered streaks is arbitrary in order to show all of the features. The form factor background is not shown. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
Figure 4.5. An example of a highly ordered monolayer. (A) This image was acquired at the SAXS/WAXS beamline of the Australian Synchrotron using nanoparticles capped with $N = 7$ ligands at an NaCl concentration of 500 mM. (B) The relative positions of the Bragg peaks with respect to the first-order peak correspond to a simple hexagonal lattice. Note that the experimental form factor acquired from scans of a droplet interior was divided out to obtain the structure factor. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
4.3.3 Effects of Ionic Strength on Crystallinity

Measurements were taken at the droplet apex over a range of sodium chloride concentrations, as shown in Figure 4.6. At low concentrations (100 mM and below), only the form factor was visible with no scattering from the interface. This is likely because the salt is unable to adequately screen the negative ssDNA backbone, resulting in strong electrostatic repulsion between DNA coronae. At high concentrations (750 mM NaCl and above), only a ring-like scattering pattern was observed, indicating that the particles precipitated out of solution. Within this upper and lower limit, scattering due to Gibbs monolayers was observable. Furthermore, at a concentration of 500 mM the crystalline order was optimal in terms of narrow peak width and the presence of higher order scattering peaks.

Figure 4.7A shows a fit of Eq. 4.6 to the data for 500 mM NaCl using \( D_g \) and \( \sigma_{DW} \) as fitting parameters, after correcting for resolution effects. A plot of the average grain size versus sodium chloride concentration is shown in Figure 4.7B. The optimal grain size occurred at 500 mM, corresponding to an average grain diameter of 365 nm and a 2.87 nm deviation from perfect order. These results suggest that crystalline order is highly sensitive to the ionic strength, although disordered Gibbs monolayers can be obtained over a wider range of salt concentrations.
Figure 4.6. Effects of ionic strength on monolayer formation. Monolayers formed from particles with $N = 15$ spacers were observed at the droplet apex for NaCl concentrations ranging from 250 to 750 mM, and are crystalline at 500 mM. Only form factor scattering was observed at 100 mM. At 1000 mM, only an aggregate with short range order was observed with no scattering occurring from the interface. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
Figure 4.7. Fit of the theoretical structure factor. (A) A fit for when [NaCl] = 500 mM. (B) Average grain size versus salt concentration for nanoparticles capped with $N = 15$ spacers. The optimal grain size occurred at [NaCl] = 500 mM. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
4.3.4 Brush Height Modeling in a Gibbs System

The DNA corona is computed from the nearest neighbor spacing, as determined from parSAXS. For a two-dimensional hexagonal lattice, the nearest neighbor spacing for a given set of Miller indices \((hk)\) can be determined from

\[
D_{\text{NN}} = \frac{4\pi}{q_{hk}} \left(\frac{h^2 + h k + k^2}{3}\right)^{1/2},
\]  

(4.16)

or more simply

\[
D_{\text{NN}} = \frac{4\pi}{q_{10}^3} \frac{1}{2},
\]  

(4.17)

when the \(q_{10}\) peak position is used. Based on the schematic in Figure 4.8, the brush height, \(h_0\), was modeled as

\[
h_0 = \left( D_{\text{NN}} - 2R - L_{\text{BP}} - 2L_{\text{6C}} \right)/2,
\]

(4.18)

where \(L_{\text{BP}} = 2.72\) nm (for 8 bases at 0.34 nm/base), it was assumed that \(L_{\text{6C}} = 0.5\) nm for the alkyl spacers, and \(R = 6.7 \pm 0.7\) nm based on a fit of Eq. 4.2 to the experimental form factor.

In a Langmuir layer, the monolayer will be compressed with increasing nanoparticle monolayer concentration, while in a Gibbs layer the surface density is controlled by the bulk concentration. The surface coverage of a Gibbs layer, \(\Gamma\), is described by the well-known Gibbs adsorption equation

\[
\Gamma = -\frac{C}{RT} \frac{\partial \gamma}{\partial C}_{T,P},
\]

(4.19)

where \(C\) is the concentration of nanoparticles in the subphase, \(\gamma\) is the surface tension, \(R\) is the gas constant, \(T\) is the temperature, and \(P\) is the pressure. Since surface coverage is driven largely by nanoparticle concentration, it can be envisioned that compressive forces directed laterally along the interface would force particles back
into the subphase rather than compress their coronae. It was found that varying the
bulk concentration while holding the ionic strength constant did not result in any
significant compression in terms of interparticle spacing and corona height, consistent
with the expectation of a Gibbs layer (Figure 4.9A).

Following an analysis similar to that of Chapter Three, the entropic spring
model can be used to model a Gibbs system using the brush height scaling relation for
spherical, uncompressed particles. Recall that the brush height scales as
\[ h_0 \sim N^{3/5} \sigma^{1/5} R^{2/5}, \]
according to Eq. 1.5. If there is any compression of the DNA
corona, the compressed corona can be expressed as
\[ h'_0 = h_0 (1 - \lambda), \] (4.20)
where \( \lambda = d / h_0 \) is the compression ratio and \( d \) is the compression distance. Recall
from Chapter Three that the DNA chains on the nanoparticle surface can be modeled
as a collection of parallel springs with spring constant \( k_E \), where \( F = k_E d \) is the
isotropically distributed equilibrium force acting on the corona, and \( k_E = 3 k_B T / N b^2 \)
according to Eq. 3.8. After making the appropriate substitutions into Eq. 4.20, the
compressed corona height becomes
\[ h'_0 = a N^{3/5} \sigma^{1/5} R^{2/5} - \frac{N b^2 F}{3 k_B T}, \] (4.21)
where \( a \) is a proportionality constant.

Figure 4.9B shows how the corona height varies with DNA spacer length.
Assuming constant ligand density and using \( a \) and \( F \) as fitting parameters, the best fit
was achieved using \( F = 0 \), consistent with the uncompressed corona of Eq. 1.5.
Furthermore, because the DNA brush height scales only with \( N \), it can be assumed in
the subsequent analysis that the ligand density is independent of DNA length.
Figure 4.8. Modeling the brush height in a Gibbs monolayer. The nearest neighbor spacing, $D_{\text{NN}}$, is experimentally determined from parSAXS experiments. The particle radius, $R$, is determined from fitting Eq. 4.2 to the experimental form factor. The base-pairing region is modeled as a rigid spacer that separates adjacent nanoparticles. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
Figure 4.9. Parameters affecting brush height. Each data point was based on three different measurements along the air-liquid interface. As indicated by the error bars, the measurements were fairly uniform along the droplet profile and the effect of the air-liquid-substrate meniscus was negligible. (A) Nearest neighbor spacing was not significantly affected by changes in nanoparticle concentration. (B) The corona height at constant density is dependent only on spacer length and is consistent with that of an uncompressed, free-particle corona. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
4.3.5 Modeling the Effects of Ionic Strength on Corona Height

The brush height within monolayers was measured for the three different spacers at various sodium chloride concentrations. It was expected that higher concentrations of monovalent salt ions would decrease the interparticle spacing by electrostatic screening and thus reduce intrastrand repulsive charges, while reducing the salt concentration would increase the intrastrand repulsion and, consequently, the interparticle spacing (Figure 4.10A). As reported by Gang and co-workers, reversible shrinking and swelling driven by changes in ionic strength occurred in three-dimensional DNA-capped nanoparticle crystals, however, the trend was never fit to a theoretical model.

Recall from Chapter Three that the corona softness parameter was defined as $\chi \equiv h_0 / R$, according to Eq. 3.6, and was used to model the corona compression after solvent evaporation. Equating this to a model first proposed by Daoud and Cotton, but later modified to account for electrostatic interactions, yields

$$\chi = h_0 / R = (1 + kNbR^{-1}(\eta\sigma / L_K)^{1/3})^{3/5} - 1,$$

(4.22)

where $k$ is a proportionality constant typically taken as unity, $N$ is number of bases, $b$ is the length per base (0.65 nm/base for ssDNA), $\sigma$ is the surface density of polyelectrolyte chains, $\eta$ is the excluded volume parameter, and $L_K$ is the Kuhn length.

Guo and Ballauff proposed that modifications may be needed to achieve greater quantitative agreement with experimental data. Hence, two arguments were applied: (1) the theory of Argillier and Tirrell, which approximates the excluded volume as $\eta \sim L_K^3$, and (2) the theory of Barrat and Joanny, which predicted that the persistence length varies linearly with the Debye screening length, $L_K \sim \kappa^{-1}$, for flexible polyelectrolytes. The Debye screening length for water at 25ºC is given by
\[ \kappa^{-1} = (8\pi N_A L_b I)^{-1/2}, \]  
(4.23)

where \( N_A \) is Avogadro’s number, \( I \) is the ionic strength (in \( \text{mol/m}^3 \)), and \( L_b \) is the Bjerrum length (0.714 nm). Eq. 4.23 simplifies to

\[ \kappa^{-1} (\text{nm}) = 0.304 C^{-1/2} \]  
(4.24)

for added concentrations, \( C \) (in \( \text{mol/L} \)), of NaCl. After making the appropriate substitutions, Eq. 4.22 can now be expressed as

\[ h_0 / R = (1 + k N_{\text{eff}} b R^{-1} (\sigma / \kappa^2)^{1/3})^{3/5} - 1, \]  
(4.25)

where \( k \) was allowed to absorb any additional multiplicative proportionality constants. The expression has also been modified with \( N_{\text{eff}} = N + N_0 \), where \( N_0 \) is an empirical additive term to account for an effective increase in the spacer segment. This increase can be attributed to limited conformational freedom of the single-stranded spacer in the vicinity of the more densely-charged hybridization region,\(^{37}\) as well as to the contribution from single-stranded regions that are only partially hybridized.

An estimate of \( k \approx 2.7 \) can be made from the parameters reported by Gang and co-workers:\(^{38}\) \( h_0 = 8 \text{ nm}, \ R = 6 \text{ nm}, \ N = 30, \ b = 0.65 \text{ nm/base}, \ C = 0.3 \text{ M}, \) and \( \sigma = 0.145 \text{ chains/nm}^2 \). Using \( \sigma \) and \( N_0 \) as the only fitting parameters (assuming a constant oligonucleotide density, as discussed previously), an excellent fit of the model to the experimental data was achieved, yielding \( N_0 = 2.45 \) and \( \sigma = 0.17 \text{ chains/nm}^2 \) for all three nanoparticle systems (Figure 4.10B). The oligonucleotide footprint was \( \sigma^{-1} = 5.9 \text{ nm}^2 \) for when \( R = 6.8 \text{ nm} \), which compared reasonably well with the experimentally measured footprint of \( 6.0 \pm 1.0 \text{ nm}^2 \) for \( R = 7.5 \text{ nm} \), as reported by Mirkin and co-workers.\(^{39}\)
Figure 4.10. Electrostatic screening model of brush height. (A) Repulsive charges between ssDNA strands increase the interparticle spacing unless screened. Only one strand per particle is shown for clarity. (B) A fit of the modified Daoud-Cotton model to the brush height versus sodium chloride concentration for droplets containing nanoparticles with different ssDNA spacer lengths. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
4.3.6 Dynamic Shrinking/Swelling Experiments

The ionic strength could be varied dynamically by controlling the vapor pressure within the experimental chamber. Pure water was placed in the sample cell reservoir, causing diffusive transport of water through the vapor phase to the droplet due to its greater salt content. In order to evaporate the droplet, helium gas was flowed through the cell to dilute the water vapor in the cell. The droplet swelled again when the helium flow was stopped. Since the particle and salt content within the droplet were fixed, water vapor uptake and evaporation caused both particle and salt concentrations to vary according to the salt concentration in the reservoir.

Gibbs monolayers, which were already present in the initial droplet, were still present even after evaporating the droplet down to 25% of the original volume, as shown in Figure 4.11A–B. This resulted in a four-fold increase in salt and nanoparticle concentration. The monolayers were maintained even after rehydration of the droplet (Figure 4.11C). While the nanoparticle concentration also increased upon evaporation, it did not seem to play a significant role in monolayer formation. However, if the droplet was evaporated such that the salt concentration exceeded the crystallization threshold, the nanoparticles precipitated out and thus no monolayers were observed when the droplet recovered to its original volume. These results were in agreement with cycling phenomena of Chapter Three, and further support the hypothesis that the sharp shift in the first order peak in Figure 3.3 corresponds to a sudden, irreversible aggregation of nanoparticles.
Figure 4.11. Raster scans of a droplet undergoing dynamic shrinking/swelling. The droplet contained nanoparticles capped with $N = 7$ spacers at an NaCl concentration of 1000 mM. (A) At 100% relative humidity (RH), the droplet was initially in equilibrium with the chamber. (B) After being at 68% RH for 2 hours, the droplet shrank significantly. (C) When the humidity returned to 100% RH for an additional 80 minutes, the droplet regained its original shape. Reprinted with permission from Reference 26. Copyright © 2011 American Chemical Society.
4.4 DISCUSSION

The parSAXS technique is a special case of GISAXS in which the angle of incidence is 0º, making it uniquely capable of observing interfacial phenomena while avoiding scattering effects that result from X-ray reflection off of the supporting substrate. This technique complements our previous strategy of real-time measurements by providing spatial information regarding DNA-capped nanoparticle crystallization. In addition, measurements could be performed on a microliter-scale droplet of solution, which is particularly useful for when sample quantity is limited.

The analysis in this chapter revealed that DNA-programmable crystals are not limited to bulk solution and can also be produced at the air-liquid interface, which has important implications in drying-mediated assembly strategies. By varying parameters, such as spacer length, concentration, and ionic strength, parSAXS measurements could resolve the effects on the resulting monolayers. For example, no significant changes in interparticle spacing were observed when the nanoparticle concentration was varied, and changes in spacer length were consistent with polymer brush theory for uncompressed brushes anchored to a spherical surface. Both of these results were in agreement with our hypothesis that DNA-capped nanoparticles form Gibbs monolayers. We can attribute all of the interactions between nanoparticles to DNA corona interactions, rather than to core-core interactions. Using Eq. 1.2, the van der Waals attractive force between cores at the smallest observed interparticle spacing (22.1 nm) is $V_{vdW} = 5.7 \text{ meV}$. This is five times less than the thermal energy, which is $k_B T = 25.6 \text{ meV}$ at 25°C.

Amorphous and crystalline monolayers of nanoparticles were obtained depending on the ionic strength of the solution, though crystalline monolayers occurred over a narrower range of salt concentrations. The change in interparticle spacing for different salt concentrations could be described by a modified form of the
Daoud-Cotton model. This model highlights the important parameters that regulate interparticle spacing, and could easily be extended to 3D nanoparticle crystals in solution. One of the implications of our modifications to the model is that increasing the ionic strength effectively decreases the persistence length of the ssDNA corona. Consequently, this is an important design consideration for DNA nanostructures, particularly those that exploit the flexibility of ssDNA components under various buffer conditions and local environments.

Due to the dynamic nature of Gibbs layers in which particles freely exchange between bulk and surface reservoirs, the air-liquid interface provides a unique platform for the investigation and preparation of equilibrium nanostructures. The ability to control spatial crystallization of bio-functionalized nanoparticles, particularly at 2D interfaces, may open up exciting routes toward programmable 2D plasmonic materials. Knowledge of the interactions at the air-liquid interface may help facilitate the integration of nanostructures of aqueous origin into solid-state devices.
REFERENCES


CHAPTER FIVE:
CONCLUSIONS AND FUTURE DIRECTIONS
5.1 IMPLICATIONS AND FUTURE WORK

Superlattices and crystals comprised of DNA-capped nanoparticles were produced through an entropy-driven process without the requirement of specific Watson-Crick base-pairing. Nanoparticle superlattices were produced by dewetting a colloidal droplet over a microfabricated substrate. The boundaries of the superlattice membranes were fixed due to volume confinement within the substrate, and the interparticle spacing could be set by adjusting the ssDNA length. The crystallization process was also investigated in real-time using SAXS, revealing the dynamics of soft-corona nanoparticle crystallization. These experiments were performed during droplet evaporation, but without substrate-imposed restrictions. It was found that the base-pairing sequences resulted in faster crystallization, while volume restrictions were necessary when no base-pairing was possible. Once again the DNA length was a key parameter governing the interparticle spacing, which additionally provided insight into the spring-like nature of the corona.

While the real-time studies provided temporal information about the crystallization process, additional experiments were needed to observe the spatial behavior. The air-liquid interface was directly probed using parSAXS, revealing the formation of Gibbs monolayers. Ionic strength was found to be another key parameter since monolayers formed hexagonal packing arrangements over a narrow range of salt concentration, and the crystalline order was highly sensitive to the ionic strength. Through thermodynamic models, it is possible to tune the biomolecular interactions at this interface and achieve a level of control. It is likely that crystalline structures that form at the interface serve as catalysts for the nucleation of larger crystals when a concentration threshold is exceeded. The mechanism would be similar to that reported by Jaeger and co-workers, in which the evaporation kinetics drove the nucleation of monolayers at the air-liquid interface.1
The following sections describe possible avenues of exploration to supplement the work discussed herein.

5.1.1 Additional Ionic Strength Studies

In order to transfer the Gibbs monolayer superlattices to a solid support, it is important to eliminate or minimize the effects of salt crystals that form after complete evaporation. One approach may be to use multivalent salts, which could provide the same ionic strength conditions but with less salt overall. Preliminary experiments comparing magnesium chloride to sodium chloride resulted in precipitation of nanoparticles at much lower concentrations of MgCl₂ than NaCl. Furthermore, MgCl₂ produced a very sharp transition from Gibbs monolayers to complete precipitation with no observable form factor, which never occurred with NaCl. Future experiments will investigate the effects of divalent cations and how their relative sizes affect DNA screening, and additional modeling may be needed in order to predict the interparticle spacing.

5.1.2 Dynamic Formation of Binary Systems

The volume and ionic strength in a droplet can be controlled by preparing a reservoir in the sealed chamber with a fixed salt concentration that is different from that of the droplet. For the vapor pressures to equilibrate, the droplet will either shrink or expand to match the salt concentration of the reservoir. Thus, if the original volume is known, the final volume can also be determined. As a result, a single droplet of nanoparticles can be “stepped down” in volume to find ideal salt conditions for producing phase transitions.

As proposed by Mirkin and co-workers,² the size ratio of two types of nanoparticles is a factor that governs which type of crystal structure is the most
thermodynamically favorable. With this in mind, consider a binary system comprising two types of particles: A and B. At a given ionic strength, both particles A and B have the same hydrodynamic diameter. Suppose, however, that particle A is softer than B, that is, the core of A is smaller than that of B, but the corona height of A is greater than that of B. At different ionic strengths, the coronae of each type of particle will be affected differently, resulting in different diameter ratios. Using controlled evaporation, the ionic strength can be gradually changed, which, in turn, will scale the diameter ratio between A and B. This strategy could enable a sampling of various ratios until a thermodynamically optimal ratio for binary crystal formation is found.

5.1.3 Surface Tension Analysis and Simulation of Gibbs Monolayers

Recall that the Gibbs adsorption equation from Eq. 4.19 describes the relationship between surface tension and solute concentration. The surface coverage of a Gibbs monolayer at a particular equilibrium solute concentration, \( C_{eq} \), will be

\[
\Gamma(C_{eq}) = \Gamma_x \frac{C_{eq}}{a_L + C_{eq}}, \quad (5.1)
\]

where \( \Gamma_x \) is the maximum possible surface coverage, and \( a_L \) is the Langmuir parameter which corresponds to the solute concentration at half-maximum surface coverage.\(^3\) Inserting Eq. 5.1 into Eq. 4.19 and integrating yields the Langmuir-Szyszkowski equation,

\[
\gamma(C_{eq}) = \gamma_0 - \Gamma_x RT \ln(1 + C_{eq} / a_L), \quad (5.2)
\]

which predicts the surface tension of the solution based on the known surface tension of the solvent alone, \( \gamma_0 \). In practice, three regimes of adsorption are generally observed: (1) at low concentrations, particles are merely diffusing to the interface (no change in surface tension), (2) at medium concentrations, particles start to accumulate
at the interface (decrease in surface tension), and (3) at high concentrations, surface tension saturates as particles reach an equilibrium between the surface and bulk. Note that Eq. 5.2 cannot account for the third regime and breaks down since it predicts that surface tension will continually decrease at higher solute concentrations.

While the parSAXS data in the previous chapter showed that the interparticle spacing was independent of nanoparticle concentration and suggested the formation of monolayer islands, these experiments were unable to provide information about surface coverage – a vital piece of information. In theory, it should be possible to obtain this information from surface tension measurements of DNA-capped nanoparticle solutions. Preliminary surface tension measurements were performed in air using a Ramé-Hart goniometer with a pendant-drop setup under controlled humidity. Complications arose, however, in which nanoparticle residues adhered strongly to the pipette tip regardless of the material it was made of. This was typically observed any time salt was added to the solution. In addition, it has been reported that monolayers may take hours to equilibrate, and hence the volume of the droplet needs to remain constant during this time. 4 Both of these problems can be solved if future experiments are performed within a liquid cell. This will eliminate evaporative effects, yet still provide a value for surface tension that is independent of the surrounding medium.

Monte Carlo simulations may be helpful in understanding the effects of salt concentration on surface coverage, and help corroborate some of the data obtained through SAXS experiments. Stochastic lattice models have been used to simulate drying-mediated assembly of nanoparticles, 5 in which the change of a single lattice element is accepted based on the Metropolis algorithm. 6 The lattice contains three phases: liquid, gas, and solid. The solid nanoparticle phase is conserved and a single particle can only move if all cells in the immediate direction of movement are liquid.
Consequently, nanoparticle diffusion is governed by a random walk biased by interactions with surrounding liquid, gas, and neighboring nanoparticles. Ionic strength can be modeled by adjusting the interaction potential between two nanoparticles, and a stable air-liquid interface can be simulated by adjusting the chemical potential and temperature.

5.2 OUTLOOK

The fundamental studies described in this dissertation are not only important to understanding some underlying details of entropy-driven self-assembly, but have broader implications in bio-mediated self-assembly strategies that seek to integrate biomolecule-based components with solid-state devices. This is becoming a new direction in the field of DNA nanotechnology as some recent efforts, in addition to those described herein, have combined DNA-based self-assembly with lithographically-patterned surfaces.\textsuperscript{7-9} With the development of more novel strategies to direct the synthesis of highly-ordered nanoscale materials, it is expected that biomolecule-based assembly will play a crucial role in the future of optical and electronic devices.
REFERENCES


