Quantifying the Self-Assembly Behavior of Anisotropic Nanoparticles Using Liquid-Phase Transmission Electron Microscopy

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CONSPECTUS: For decades, one of the overarching objectives of self-assembly science has been to define the rules necessary to build functional, artificial materials with rich and adaptive phase behavior from the bottom-up. To this end, the computational and experimental efforts of chemists, physicists, materials scientists, and biologists alike have built a body of knowledge that spans both disciplines and length scales. Indeed, today control of self-assembly is extending even to supramolecular and molecular levels, where crystal engineering and design of porous materials are becoming exciting areas of exploration. Nevertheless, at least at the nanoscale, there are many stones yet to be turned. While recent breakthroughs in nanoparticle (NP) synthesis have amassed a vast library of nanoscale building blocks, NP–NP interactions in situ remain poorly quantified, in large part due to technical and theoretical impediments. While increasingly many applications for self-assembled architectures are being demonstrated, it remains difficult to predict-and therefore engineer-the pathways by which these structures form. Here, we describe how investigations using liquid-phase transmission electron microscopy (TEM) have begun to play a role in pursuing some of these long-standing questions of fundamental and far-reaching interest.

Liquid-phase TEM is unique in its ability to resolve the motions and trajectories of single NPs in solution, making it a powerful tool for studying the dynamics of NP self-assembly. Since 2012, liquid-phase TEM has been used to investigate the self-assembly behavior of a variety of simple, metallic NPs. In this Account, however, we focus on our work with anisotropic NPs, which we show to have very different self-assembly behavior, and especially on how analysis methods we and others in the field are developing can be used to convert their motions and trajectories revealed by liquid-phase TEM into quantitative understanding of underlying interactions and dynamics.

In general, liquid-phase TEM studies may help bridge enduring gaps in the understanding and control of self-assembly at the nanoscale. For one, quantification of NP–NP interactions and self-assembly dynamics will inform both computational and statistical mechanical models used to describe nanoscale phenomena. Such understanding will also lay the groundwork for establishing new and generalizable thermodynamic and kinetic design rules for NP self-assembly. Synergies with NP synthesis will enable investigations of building blocks with novel, perhaps even evolving or active behavior. Moreover, in the long run, we foresee the possibility of applying the guidelines and models of fundamental nanoscale interactions which are uncovered under liquid-phase TEM to biological and biomimetic systems at similar dimensions.

1. INTRODUCTION

Liquid-phase transmission electron microscopy (TEM) has the unique ability to resolve the motions and trajectories of single nanoparticles (NPs) in solution. This capability has made it a powerful addition to the arsenal of methods used to probe nanoscale self-assembly behavior. For example, while small-angle X-ray scattering (SAXS) can be used to probe nanoscale structural evolution, it can only do so on the ensemble level. Conventional TEM and cryogenic EM have single NP resolution, but are limited to imaging dried or flash-frozen samples; continuous self-assembly dynamics cannot be elucidated directly. Even simulations can find challenges: it is computationally taxing to incorporate the large number of particles necessary to probe assembly processes, and interaction potentials used in simulations are often derived from micrometer-scale colloidal behavior, which does not merely rescale to the nanoregime. The paucity of experimental data on nanoscale dynamics complicates the validation of these models. With these and other motivations, liquid-phase TEM

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has already been used to investigate self-assembly dynamics in a variety of systems, chiefly of spherical metallic NPs.\textsuperscript{7–12}

In this Account, we focus on our work studying the self-assembly of anisotropic NPs (section 2),\textsuperscript{13} and particularly on quantifying their self-assembly behavior (section 3).\textsuperscript{14,15} Anisotropic NPs have intrinsically different self-assembly properties, largely because their interactions are highly directional.\textsuperscript{16,17} A quantitative approach to describing their dynamics, meanwhile, offers deeper insight into the nanoscale interactions governing assembly, and into how the kinetic pathways taken to a final structure can be manipulated. For example, can we control a kinetic pathway to bias one product among many possibilities? Can we map out the complete self-assembly free energy landscape, much like extensive efforts in protein folding? Understanding these dynamics “rules” will be a critical prerequisite to manipulating self-assembly of active soft matter, where building blocks are not static, but self-propelled and evolving.\textsuperscript{18–22}

In subsequent sections, we present a few major areas in which liquid-phase TEM investigations of self-assembly are still coming into their own (section 4). Understanding of how the electron beam affects building blocks, their surface ligands, their environment, and their interactions, as well as how NPs move and interact with and within the confined liquid-phase TEM chamber, are imperative for meaningful interpretation of liquid-phase TEM movies and for connecting in situ observations to self-assembly studies outside TEM. These factors are still being fully characterized.\textsuperscript{8,23} At the same time, we provide evidence that, when properly considered, these features can potentially serve as unique tools for the manipulation of NP self-assembly in a variety of configurations.\textsuperscript{15} We conclude by outlining the future directions to be taken by in situ self-assembly investigations, toward new building blocks, new crosstalk between modalities, and new paradigms in soft matter.

### 2. THE ROLE OF ANISOTROPY IN LIQUID-PHASE NP SELF-ASSEMBLY

Metallic NPs (Pt-Fe,\textsuperscript{10} Pt,\textsuperscript{7,24} Au,\textsuperscript{8,9,11,12,14,15} etc.) have been a practical starting point for liquid-phase TEM studies of self-assembly. For one, they exhibit high contrast against a liquid background, which makes it possible to achieve nanometer to atomic resolution.\textsuperscript{25} Most metallic NPs are also highly stable under prolonged electron beam exposure, which is required to capture continuous assembly dynamics. In particular, although NPs have diffusion coefficients in bulk water on the order of $10^{-6}$–$10^{-8}$ nm$^2$/s (per the Stokes–Einstein relation, for NPs between 100 and a few nanometers in size), NP motions in liquid-phase TEM chambers can be three to six orders of magnitude slower, likely because of an increase in solvent viscosity during imaging or the involvement of NP–substrate attractions.\textsuperscript{26,27} While these effects bring the time scale of NP motions up to the temporal resolution of conventional liquid-phase TEM instrumentation, when coupled with the fact that self-assembly requires millions of collisions to occur within a

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<th>Assembled structure and schematic</th>
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Figure 1. Overview of building blocks and structures observed in liquid-phase TEM self-assembly studies. Most work has considered spherical metallic NPs (Au, Pt, Pt-Fe), while anisotropic particles have only recently been studied. EM images adapted from refs 7, 8, 10–15. Copyright 2013, 2012, 2017, 2016, and 2015 American Chemical Society. Copyright 2016 Nature Publishing Group. Copyright 2015 and 2016 American Chemical Society.
concentrated NP suspension, the consequence is that self-assembly time scales range from minutes to hours. During time spans of this length, NPs otherwise susceptible to sustained electron beam exposure can restructure or decompose, depending on the beam dose.13

Most of the aforementioned studies have investigated the self-assembly of spherical metallic NPs, since high quality, monodisperse samples are typically easy to make. Anisotropic NPs, on the other hand, require more sophisticated synthesis methods28,29 and their self-assembly has only recently been investigated under liquid-phase TEM. These NPs are distinguished from spheres by their intrinsically directional interactions, either because of their shape and symmetry,16,17,30 or because of their heterogeneous surface ligand coverage31 arising from the different binding energies32 and curvatures33 across a NP’s surface. These directional interactions have the potential to mimic the surface heterogeneity of biomolecules, a feature which, in nature, leads to assemblies with novel symmetries, hierarchies, and multiplex functions.34

The representative NP building blocks and structures observed in liquid-phase TEM self-assembly are summarized in Figure 1. Closely packed structures of spherical NPs typically form when the electron beam agitates and gradually evaporates the solvent, whereupon convection sweeps NPs together.24 Loosely packed, one-dimensional (1D) chains,8,11,12 which rarely result from solvent-drying, are formed when the solvent is preserved throughout self-assembly and when, consequently, liquid-mediated NP–NP interactions serve as the major driving force for assembly.

2.1. Shape Effects: Directional Interactions in a Liquid Environment

The directionality of liquid-mediated NP–NP interactions is highly dependent on NP shape. For example, when Sutter et al. used liquid-phase TEM to study the assembly of CdSe/CdS octapods (Figure 1), they observed that these particles assembled into chains, much like assemblies of spherical NPs in liquid.14 However, the anisotropy of the octapods restricts the number and orientation of attachments which are involved in chain formation; the branching of octapods leads to a "pod-to-pod" particle connection, which can only occur in specific orientations.

In our work, we have investigated the liquid-phase self-assembly of NPs which, unlike both spheres and octapods, lack radial symmetry and have interactions of more pronounced directionality. For example, Au nanoprisms experience a van der Waals attraction in the face-to-face orientation which is hundreds of times larger than in the side-by-side configuration.15 This directionality promotes assembly into 1D lamellar superlattices in a liquid environment (Figure 2A, B), with a characteristic lattice constant (i.e., the center-to-center spacing between adjacent prisms) determined by a balance between van der Waals attraction between the prisms and electrostatic repulsion between charged ligand coatings (Figure 2C). We monitored the response of this lattice constant to liquid condition changes under liquid-phase TEM at the single NP level. We expect this capability to track nanoscale particles directly, are less expected.14 In solvent evaporation-driven self-assembly, Au nanorods often assemble side-by-side into bundles when concentrated by drying.30 In liquid, however, Au nanorods diffuse freely throughout the assembly process, whereby they sample the assembly free energy landscape with statistics governed by rod–rod interactions.14,36 Nanorods coated with charged ligands eventually assemble tip-to-tip (Figure 2E, F), a configuration relevant for plasmonic coupling of the electromagnetic fields concentrated at individual rod tips. We attributed tip-selective attachment to the nonuniform rod shape and, consequently, different interaction microenvironments associated with different modes of approach. In particular, given the appropriate liquid conditions, two nanorods approaching side-by-side experience a stronger electrostatic repulsion than those nearing tip-to-tip, since the highly curved tip surface has a lower density of charges (Figure 2D). Rods orient to minimize the repulsions encountered as they approach one another, whereupon short-range van der Waals attraction "locks" them into 1D chains.

In general, shape anisotropy strongly impacts the directionality of NP–NP interactions in solution and, as such, is a powerful tool for engineering new architectures and new self-assembly dynamics. Moreover, we expect systematic studies on diverse anisotropic shapes will provide insight not just into NP self-assembly, but also into addressing fundamental questions in other fields, such as historic geometric puzzles concerning 3D anisotropy and space tessellation.15 For example, tetrahedral particles have been shown in computer simulations to pack into exotic 3D structures, including chiral chains and quasi-crystals.36 It will be intriguing to image such symmetry-breaking processes from the bottom-up in real-time.

2.2. Shape- and Solvent-Mediated Ligand Organization in NP Self-Assembly

Ligands are often used to passivate high-energy NP surfaces during synthesis. They are also involved in the majority of NP–NP interactions; while van der Waals and dipole–dipole interactions chiefly depend on the NP core composition, electrostatic, hydrophobic, steric, and depletion interactions are...
predominantly associated with ligand properties (e.g., their conformation, charge, thickness, spatial distribution, etc.). Functional moieties on ligand molecules can also elicit specific binding interactions, such as hydrogen bonding,\textsuperscript{12,36,39} DNA hybridization,\textsuperscript{11,40} or dithiol bonding.\textsuperscript{41}

Ligand organization on a NP surface is highly sensitive both to facet binding energies and to curvature. For example, Choi et al. calculated that oleic acid ligands bind more strongly to the \{111\} facets of PbS NPs, so \{100\} facets are more susceptible to reductions in coating density during drying (Figure 3A).\textsuperscript{32} As such, reducing ligand density effectively leads to asymmetric repulsion and the formation of body-centered cubic (bcc) assemblies, rather than face-centered cubic (fcc) structures observed for uniformly coated particles (Figure 3B). Walker et al., meanwhile, demonstrated that the combination of positive and negative curvature on nanoscopic “dumbbells” leads to heterogeneous packing of ligands across a NP surface (Figure 3C). This heterogeneity leads to uniform electrostatic repulsion at low pH but patchy repulsion at high pH (Figure 3D).\textsuperscript{33}

Ligand mediation of NP assembly is also intimately coupled with solvent properties. For example, pH dictates the protonation state (and therefore charge) of basic or acidic ligands.\textsuperscript{15} Likewise, hydrophobic, depletion, and steric interactions are also closely associated with solvent properties; they are entropic effects where assembled states are favored due to an increase in the entropy of ligand or solvent molecules.\textsuperscript{42} One example involves polymer coatings on spherical NPs, which can produce isotropic interactions in a good solvent, where chains are extended, or directional interactions in a poor solvent, where chains are collapsed at the NP surface (Figure 3E).\textsuperscript{31} In this regard, two especially relevant capabilities afforded by liquid-phase TEM are (i) in situ modulation of solvent conditions, either based on quantitative knowledge of electron beam-solvent interactions (more in section 4), or simply through solvent exchange using a liquid flow holder;\textsuperscript{43} and (ii) real-time observation of the concomitant dynamics.

3. MAPPING NANOSCALE INTERACTIONS BY SAMPLING NP TRAJECTORIES UNDER LIQUID-PHASE TEM

NP–NP interactions determine the overall self-assembly free energy landscape and the possible kinetic pathways to a final structure. Existing force field models used for simulations would benefit immensely from advanced experimental measurements. Our previous work demonstrated a potentially general workflow which can be used to quantify NP–NP interactions based on automated trajectory tracking under liquid-phase TEM.\textsuperscript{14} Namely, by analyzing the relative distances and orientations of a statistically significant number of interacting NP pairs, effective potentials can be extracted either by Boltzmann inversion, for simple pairwise systems, or by
iterative Boltzmann inversion, for highly concentrated suspensions or systems with multiple degrees of freedom.

As a proof-of-concept, we used liquid-phase TEM to image an aqueous suspension of positively charged Au nanorods and quantify their orientation- and separation-dependent interactions.14 Currently, the relevant features to be tracked are determined in an ad hoc manner. However, for rods, tracking tip positions is sufficient to characterize rod configurations. A mapping of ~10 000 rod pairs around a central reference rod was obtained by automatically tracking rods in TEM movies (Figure 4A). An otherwise stable dispersion of nanorods was triggered by the electron beam to self-assemble. However, as indicated in the configuration map, rods have a circular “exclusion zone” tangent with their tips, which is consistent with exclusively tip-to-tip assembly. Also from the raw configuration plot, we computed a radial distribution function, $g(r)$, which describes the particle density distribution (C). (D) The pairwise particle interaction energy $u(r)$ can be calculated from $g(r)$ directly. In this case, the form of $u(r)$ is consistent with the electrostatic repulsion between nanorods. (E) At lower ionic strength, when electrostatic repulsion is stronger, this exclusion zone expands. Adapted from ref 14. Copyright 2015 American Chemical Society.

Figure 4. Quantification of solution-phase NP–NP interactions. (A) Tracking the relative positions of ~10 000 nanorods permits a mapping of their spatial interactions, which exhibits a circular zone of exclusion. (B) Mapping of NP spatial distributions can be converted into a radial distribution function, $g(r)$, which describes the particle density distribution (C). (D) The pairwise particle interaction energy $u(r)$ can be calculated from $g(r)$ directly. In this case, the form of $u(r)$ is consistent with the electrostatic repulsion between nanorods. (E) At lower ionic strength, when electrostatic repulsion is stronger, this exclusion zone expands. Adapted from ref 14. Copyright 2015 American Chemical Society.

4. CONNECTING “IN SITU” OBSERVATIONS TO “OUTSIDE TEM” SELF-ASSEMBLY STUDIES

Inevitably, imaging with a high-energy electron beam impacts the sample, especially when imaging in liquids. At high dose rates (300–10 000 e$^-$/Å$^2$ s), we and others have shown that the beam can restructure even metallic NPs, likely due to a combined effect of reactive radical formation and the decomposition of stabilizing ligands.15,65 Consequently, to keep conditions maximally native, most NP self-assembly studies are performed in a low dose rate range (0.2–100 e$^-$/Å$^2$ s).15,65 However, even at lower dose rates, the beam can impact self-assembly processes, by altering NP motions and NP–NP–substrate interactions.

4.1. The Role of Radiolysis in NP–NP Interactions at Low Electron Beam Doses

Even during low-dose liquid-phase TEM imaging, energy transfer from the electron beam to the solvent can induce complex radiolysis reactions that produce reactive radicals, ions, and gases in solution. Recent work in characterizing radiolysis products in diverse solvents has been reviewed by Woehl and Abellan,46,47 but here we focus on water, since this is the solvent most commonly used in metallic NP self-assembly studies. Pioneering work has revealed that radiolysis of water is highly localized to areas illuminated by the beam and reaches equilibrium within seconds.49,50 but OH$^-$, H$^+$, and other species generated can alter liquid conditions, such as pH and ionic strength (Figure 5A).

We recently conducted parallel and correlative liquid-phase TEM and SAXS studies using 1D lamellar superlattices of Au nanoprism as the “ionic strength meters” to quantify electron beam induced solution changes inside liquid-phase TEM (Figure 5).15 In particular, since the lattice constant of superlattices responds selectively to a solution’s ionic strength (not to temperature, for example), by comparing the lattice constant changes measured in liquid-phase TEM (Figure 5B) upon irradiation to those measured by SAXS (Figure 5C) and those predicted by theoretical modeling (Figure 5D) in response to ionic strength increases, we formulated a relationship between dose rate and beam-induced ionic strength elevation. We showed that the ionic strength inside the liquid-phase TEM chamber can increase up to 6 times at dose rates between 0.2 and 5.5 e$^-$/Å$^2$ s) and impact the self-assembly process (Figure 5E). This work also revealed important details on ligand integrity. For example, when higher dose rates (6–300 e$^-$/Å$^2$ s) were applied, we observed that the distance between nanoprisms could not fall below twice the ligand length, indicating that these ligands were still intact (Figure 5E, regime 2). However, with beam dose rates higher than 300 e$^-$/Å$^2$ s), irreversible damage occurred; superlattices...
collapsed over the ligand steric hindrance into a fused gold piece.

We expect that similar strategies can be used to quantify other beam-induced effects, which can then be utilized to control self-assembly processes while minimizing deleterious repercussions. Park and co-workers have implemented beam-induced local solvent evaporation to trigger NP close-packing.\textsuperscript{7,24} Other groups have reported approaches like ours, where beam illumination induces assembly by increasing the ionic strength.\textsuperscript{8} Such in situ triggering allows one to track the full course of self-assembly, beginning with early stage kinetics. In the cases where the radiolysis effects have not been quantified, radical scavengers or radiation-resistant solvents can be used to mitigate potentially detrimental radiation effects.\textsuperscript{48,51,52}

4.2. The Electron Beam and Liquid-Phase TEM Substrates

Several groups have observed that liquid-phase TEM substrates—especially the SiN\textsubscript{x} substrates which are so far the most commonly used in self-assembly studies—can be prone to the electron beam in ways beyond the ionic strength effects discussed above, and with a direct impact on NP–substrate interactions. The nature of these interactions, however, is not yet clear. Liu et al., for example, observed that positively charged Au NPs initially adsorbed to a SiN\textsubscript{x} substrate started to diffuse upon beam irradiation, while negatively charged Au NPs remained immobilized.\textsuperscript{8} They speculated that beam exposure generates a positive substrate surface potential (repulsive for positively charged NPs and attractive for negatively charged NPs), based on a balance between input and output charges on the exposed area (Figure 6A, C). Meanwhile, Chee et al. proposed that the SiN\textsubscript{x} surface exposes a surface charge that depends on pH (SiNH\textsubscript{3}\textsuperscript{+} at low pH, SiO\textsuperscript{−} at high pH), which can be mediated through beam dose rates (Figure 6B), and makes the motions of charged nanocubes Lévy flight-like because of heterogeneous electrostatic NP–substrate interactions (Figure 6D).\textsuperscript{23}

While other substrates, such as silicon or graphene, can be less susceptible to the charging or roughness effects associated with SiN\textsubscript{x},\textsuperscript{24,27,53} NP–substrate interactions are always a vital consideration. Liquid-phase TEM uses “sandwiching” configurations, where samples lie between substrates only 50–500

\begin{figure}[h]
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\caption{Electron beam and NP–NP interactions. (A) Beam-induced radiolysis produces reactive species which impact self-assembly behavior. Blue dots represent electrons (e\textsuperscript{−}), red dots represent ionic species (H\textsuperscript{+}, OH\textsuperscript{−}, etc.) and green dots represent radicals (H, OH, etc.). (B) Radiolysis of water produces H\textsuperscript{+} and OH\textsuperscript{−}, which screen electrostatic repulsion between nanoprism. This reduces the lattice constant in a dose-dependent manner. Parallel SAXS (C), TEM, and theoretical calculations (D) were used to determine this lattice spacing as a function of ionic strength. This dependence can be converted into a relationship between dose rate and an effective ionic strength (E). Adapted from ref 15. Copyright 2016 American Chemical Society. Scale bar: 50 nm.}
\end{figure}
First studies and statistical mechanics will mutually benefit both fields. Self-assembly studies will require statistical mechanical frameworks to translate new knowledge of fundamental interactions, fluctuations, and motions at the nanoscale into free energy landscapes, phase behavior, and the self-assembly design rules needed to optimize architecture-dependent properties and applications. Liquid-phase TEM studies will reciprocate by providing quantitative measurements of nanoscale interactions and motions that have, until recently, been an experimental and theoretical challenge.

Third, we see great potential in developing multimodal imaging techniques for studying nanoscale self-assembly dynamics, by interfacing liquid-phase TEM with methods such as element/oxidative states mapping (electron energy loss spectroscopy, energy-dispersive X-ray spectroscopy), 3D structural elucidation (tomography, diffraction), and optical microscopy. In this way, the understanding obtained will span length scales seamlessly, from atomic and single-NP levels all the way to the micrometer-scale.

Fourth, recent breakthroughs in active micrometer-scale colloids driven by external energy have demonstrated fundamentally new self-organization and phase behaviors depending on steady-state, not thermodynamic equilibrium;18−22 we see great promise in designing and imaging nanoscale active matter, where NPs fluctuate spatiotemporally under chemical or electrical cues and stabilize in time-varying patterns. This strategy to arrange NPs may lead to structures much more closely resembling living systems, which are often out of equilibrium.

Last but not least, we expect more experiments will eventually develop a quantitative understanding of general electron beam effects (e.g., radiolysis, ligand chemistry, NP diffusion, NP−substrate interactions), enabling direct knowledge transfer from liquid-phase TEM to conditions outside, for practical applications. In sum, new NP systems, advanced particle tracking algorithms, ultrafast and ultrasensitive detectors, new understanding of beam effects, and new theories for nanoscale interactions can all iteratively benefit studies on NP self-assembly.

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**Notes**

The authors declare no competing financial interest.

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