Study on self-assembly of colloidal particles at high ionic strength with stimulated emission depletion microscopy

Bhanu B. Neupane1,2 | Yaning Zhong1 | Gufeng Wang1

Abstract
Understanding the spatial organization of nano- and micro-sized particle is very important in the fabrication of complex structures having unprecedented properties. Study on self-assembly of submicroscopic colloidal particles at high ionic strength solution at single particle resolution can provide new insight into the nanoscale interactions. In this study, we studied the self assembly behavior of negatively charged 0.2 and 1 μm colloidal particles at high ionic strength on glass-solution interface that is, in situ environment. The self-assembled 0.2 μm particles could not be resolved with conventional confocal and epi-fluorescent microscopy, so a home-built continuous wave stimulated emission depletion (STED) microscope was used for the study. We found that particles self-assemble into ordered and disordered structures at higher and lower ionic strength solution, respectively. The optical imaging methods allowed us to measure inter-particle gap at second energy minimum directly. Interestingly, we found that the inter-particle gap in the wet self-assembly higher than the classical Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory predicted. The in situ investigation of particle self-assembly at high ionic strength will provide more insight for the understanding nanoscale interactions.

KEYWORDS
DLVO theory, ionic strength, negatively charged, resolution, self-assembly, stimulated emission depletion

1 | INTRODUCTION

In recent years, nano and micro particle self-assembly (SA) gains much attention because it offers an effective “bottom up” approach to fabricate macroscopic materials having unique optical, electronic, magnetic, or catalytic properties.1-7 The construction units used in self-assembling can be inorganic, polymeric, metallic or semiconductor nanoparticles. The self-assembled structures can be formed in both dried and wet conditions using different methods.8,9 In simple drop-casting and drying methods, a drop of colloidal suspension is allowed to evaporate slowly on a substrate and the particles self-assemble into a tightly packed 2D or 3D crystalline structure due to capillary force.10 Indeed, many of the
self-assemblies have to be dried before they can be observed or used. However, drying usually results in the formation of various types of defects such as cracks because of limited mobility of the particles during the drying process. To improve the quality of the self-assembled structure, various methods have been used to control the feeding speed of the particles or the solvent evaporation speed, for example, convective assembling spin coating and utilizing patterned surfaces as templates to enrich particles before drying. Also, methods that use SA in liquid environments are also available such as sedimentation in gravity, flow induced, and external fields like magnetic fields, electric fields etc.

Different techniques are in practice to study the SA of particles such as microscopic, and light, X-ray, and neutron scattering techniques. Choice of method depends on the size and nature of particle and the environment (dry and wet). Conventional optical microscopies have diffraction limited resolution and being used to observe the assembly and disassembly of micro particles in solution phase and in liquid-glass interface. However, due to the small size of the particles, it becomes challenging to monitor and characterize self-assemblies of sub-microscopic particles in situ. For small particles, current dominant methods to characterize their self-assemblies formed under wet conditions are still to transfer or deposit them on a solid substrate and dry them before observation using electron microscopy or scanning probe microscopy. It is unclear whether dried nanoparticle assembly will keep the same structure as the wet ones.

In recent years, with the carefully designed, ultrathin microfluidic cells, in situ transmission electron microscopy observation of nanoparticle SA at wet environment becomes feasible. This method provides excellent spatial resolution of few nanometers for metallic or semiconductor particles and modest improvement in resolution for soft materials, for example, biological cells and polymers as compared to optical microscopy. However, the observation chamber is usually very thin, which may interfere with the inter-particle interactions as reported in the literature.

The SA of particles having diverse shape, size, and composition at different conditions are being explored in literature. Among them, polymeric colloidal particles are being explored since long time as a model system to understand the nature of interactions between charged surfaces. Literature studies have reported the dependence of SA of polymeric colloidal particles (size bigger than diffraction limit) on concentration, surface charge, pH, and low to high salt concentration. It would be interesting to explore how smaller sized colloidal particles (smaller than diffraction limit) self-assemble in high ionic strength solution. This is because some interactions, for example, the electrostatic force between the particles, do not scale with the particle size. As the particle dimension drops by orders of magnitude, these forces remain similar but functioning on a much smaller scale. In addition, submicroscopic particles are more susceptible to thermal activities due to their small size. Thus, it is reasonable to question whether sub-microscopic particles will behave similarly as micro-particles in self-assembling especially in presence of high ionic strength.

The advent of sub-diffraction limit (or popularly named super-resolution) optical imaging techniques provided a new opportunity to study in situ SA of particles smaller than diffraction limit. Among these techniques, stimulated emission depletion (STED) microscopy is particularly promising because it truly reduces the illumination point spread function thus can be used in systems with high dye density. In addition, it has a temporal resolution in milliseconds to seconds, similar to confocal microscopy. Thus, dynamic events can be monitored. Since its proposal and experimental demonstration in recent years, STED microscopy has achieved spatial resolution improvement more than one order of magnitude over the diffraction limited resolution and gained broad interests in scientific and engineering communities.

In this work, using a home built continuous wave STED microscope having lateral resolution of around 70 nm, we studied SA of 200 nm particles on solution-glass interface in the presence of medium to high ionic strength. The inter-particle gap was calculated using classical DLVO theory and compared with the experimental result. A comparative study on the SA formed at dry and wet conditions was made. For comparison, we also studied the SA of 1000 nm particles at similar conditions using conventional epi-fluorescent microscopic technique.

2 MATERIALS AND METHODS

2.1 Sample preparation

Carboxylated polystyrene colloidal particles with a nominal size of 200 and 1000 nm diameters were acquired from Invitrogen. Their actual sizes were $190 \pm 8$ and $980 \pm 20$ nm, respectively, as measured with scanning electron microscopy (SEM) from dried, and tightly packed particle assemblies. The zeta potentials measured in $8.0 \text{mM}$ phosphate buffer at pH 9.5 was $-100 \pm 6$ and $-80 \pm 5$ mV, respectively, each from three repeated experiments. The flocculation concentration
of NaCl for the particles was approximately 600 mM judged by visual inspection. The absorption and fluorescence emission maxima of aqueous suspension of these particles were 505 and 515 nm, respectively.

The particle SA under drying conditions was prepared by drying the aqueous suspension at ambient temperature. For fluorescence measurements, the dried sample was re-submerged in de-ionized water before microscopic measurements. The SA was prepared on a glass coverslip (22 × 22 mm, No. 1.5, Corning, New York) having root-mean-square surface roughness of 0.4 ± 0.2 nm. To remove the surface impurities, substrate was sonicated in soap water once, then in DI water three times, and ethanol once, each for 15 minutes and finally dried with high purity N₂ gas. The self-assemblies of nanoparticles under wet conditions were prepared by mixing colloidal suspensions with NaCl solution at different concentration. In the suspension, a fixed particle concentration of 0.02% (w/v) was maintained. The suspensions were then dropped on the top of pre-cleaned cover glasses and placed in a wet chamber at 4°C over night (12 hours) for self-assembling to occur. No solvent evaporation was observed in the self-assembly process.

2.2 Confocal and STED microscopy

The schematic and detailed description of our homebuilt inverted and continuous wave STED microscope was reported previously. Briefly, the excitation was provided by an Ar⁺ laser at 488 nm and the depletion was at 592 nm using a continuous wave fiber lasers. The depletion beam was shaped into a donut profile using a 0-2 π vortex phase plate (VPP-1a, RPC photonics, New York). The fluorescence photons were collected through a 50 μm, multimode fiber. The excitation and depletion laser power at the objective (Plan Apo, 100 ×, NA 1.40, Oil) back aperture were approximately 15 μW and approximately 600 mW, respectively. When operated in the confocal mode, the depletion beam was turned off. The integration time was 1.0 ms unless specified. The fluorescence signals were collected by an avalanche photodiode (Perkin-Elmer, SPCM-AQRH-15-FC) and counted by a counter board. The acquired data were converted to images using a home-written NIH ImageJ program. MATLAB and NIH ImageJ were used to analyze the collected images. The spatial resolution of the confocal and the STED microscope was characterized each time before experiments by measuring 45 nm fluorescent nanoparticles (Invitrogen) immobilized on pre-cleaned cover glasses. To prevent the sample drying during imaging, a simple wet chamber was designed in which the coverslip was glued to a glass slide with a transparent nail polish. With this arrangement, the solvent drying was not observed during imaging.

2.3 Epi-fluorescence microscopy

Epi-fluorescence images were acquired on an upright Nikon Eclipse 80i microscope. The signal was collected by either a NA 1.40 (Plan Apo, 100× /1.40, oil) or a NA 1.49 objective. An Andor iXon 897 camera (512 × 512 imaging array, 16 μm pixel size) or a Hamamatsu ORCA-Flash 2.8 CMOS camera (1920 × 1440, imaging array, 3.63 μm pixel size) was used to image the samples.

2.4 SEM imaging

Ten microliters of concentrated aqueous suspension of the nanoparticles was dispensed on the coverslip and air dried at ambient temperature. Before SEM measurement, all samples were vacuum dried and then a thin layer of Pd-Au (<5 nm) was coated to make the sample conductive. Sample coverslips were directly mounted to the sample holder and imaged with JEOL JSM-6400F field emission scanning electron microscope.

2.5 Pairwise particle potential energy predicted by DLVO theory

The quantitative analysis of the interactions between particle-particle was first provided by Derjaguin and Landau in 1941 and by Verwey and Overbeek in 1948 independently, hence the theory is named DLVO theory. In DLVO theory, the interactions between a pair of colloidal particles include an attractive London-van der Waals force and a repulsive electrostatic force. When the gap between two particles is comparable to their size \((H > a/100)\), the van der Waals potential energy between the particle pair \((V_{A,PP})\) is⁴⁸:
\[ V_{A,PP} = -\frac{A_{131}}{6} \left\{ \frac{2a^2}{H^2 + 4aH} + \frac{2a^2}{(H + 2a)^2} + \ln \left[ \frac{H^2 + 4aH}{(H + 2a)^2} \right] \right\} \]  

(1)

where \( a \) is the particle radius; \( H \) is the gap between particle-particle; \( A_{131} \) is Hamaker constant. When the gap is much smaller than the particle size, the pairwise potential energy reduces to \(^{48}\):

\[ V_{A,PP} = \frac{A_{131} a}{12H} \]  

(2)

In vacuum, Hamaker constant of most condensed phases are on the order of magnitude \( 0.4 \sim 4 \times 10^{-19} \) J.\(^{48}\) In aqueous solutions, based on Lifshitz Theory, Hamaker constant of polystyrene-water-polystyrene system could be estimated to be between \( 0.95 \times 10^{-20} \) and \( 1.3 \times 10^{-20} \) J.\(^{48}\) Literature reported values can range between \( 0.4 \times 10^{-20} \) and \( 1.3 \times 10^{-20} \) J.\(^{48,49}\) In the estimation below, we used the relatively larger reported value (\( 1.3 \times 10^{-20} \) J) to calculate the particle-particle attraction potentials.

To complete DLVO theory, the repulsion between the same charges on the particles also should be considered. Under the thin electrical double layer (EDL) assumption (\( \kappa a > 1 \)), that is, Smoluchowski limit, the potential energy caused by electrostatic repulsion between the particle-particle (\( V_{R,PP} \)) is\(^{31,47,50}\):

\[ V_{R,PP} = 32\pi\varepsilon\varepsilon_0 a \left( \frac{k_BT\Psi_P}{Ze_0} \right)^2 \ln[1 + e^{-\kappa H}] \]  

(3)

where

\[ \Psi_P = \tanh \left[ \frac{Ze_0\phi_P}{4k_BT} \right] \]  

(4)

\[ \kappa^2 = \frac{2e^2N_AI}{\varepsilon\varepsilon_0 k_BT} \]  

(5)

\( \varepsilon \) is the dielectric constant; \( \varepsilon_0 \) is the vacuum permittivity; \( k_B \) is Boltzmann constant; \( T \) is the room temperature; \( Z \) is the valence of the ion; \( e_0 \) is the charge of an electron; \( \phi \) is the surface potential; \( N_A \) is Avogadro’s constant; \( I \) is the ionic strength; \( 1/\kappa \) is the characteristic Debye length of the electrolyte, or the thickness of the double layer. The total potential energy \( V_{T,PP} \):

\[ V_{T,PP} = V_{A,PP} + V_{R,PP} \]  

(6)

Using Equations (1)-(6), the pairwise particle potential energy between a pair of particles was calculated.

3 | RESULTS

3.1 | Self-assembly at high concentration of salt

In this study, we used our home built STED microscope (resolution of approximately 70 nm) to study self-assemblies of 200 nm, carboxylated polystyrene colloidal particles under wet conditions on solution-glass interface. These particles had nominal diameter of 200 nm and actual size of 190 ± 8 nm as characterized by SEM imaging (Figure 1). They were stable in solutions when the salt concentration was below the flocculation concentration, which is approximately 600 mM at pH 7.0. In the absence of salt, they did not readily adsorb onto glass surface. However, when NaCl salt was added in hundreds of mM, these particles adsorbed onto the surface quickly. Overtime, they formed a particle layer or multiple layers, which was usually thought to be random aggregates.

Conventional optical microscopy does not have the spatial resolution to resolve the structure of SA pattern. For example, under an epi-fluorescence microscopy with a numerical aperture as high as 1.49, the particle assembly formed in the presence of 500 mM NaCl just shows as a smeared image (Figure S1).
FIGURE 1 SEM image of self-assembly of 200 nm particles on glass surface under drying condition. Scale bar is 1 μm. The measured particle size was 190 ± 8 nm. The particles were re-wetted before observation.

To study the SA 200 nm particles formed at different salt concentration, we used both confocal and STED microscope. The lateral resolution of confocal and STED microscope was 250 nm and 70 nm, respectively. In comparison to epi-fluorescence microscopic image shown in Figure S1, image quality is improved in confocal but neither the particles nor their assembling pattern is resolved (Figure S2A-F). Thus, conventional diffraction limited optical microscopy is incapable of resolving the particle assembling patterns of 200 nm particles formed under wet conditions. It is interesting to note that, the different SA patterns formed at different ionic strength are nicely resolved in the STED microscopic images (Figure 2A-F). In comparison to dry SA shown in Figure 1, wet SA show noticeable inter-particle gap. Note that the STED images were taken in aqueous phase and SEM cannot image the particles in such environment.

We also studied the SA behavior of 1000 nm (actual size 980 ± 20 nm) particles at different ionic strength. Because of larger size of the particles the SA pattern can be resolved even with an epi-fluorescent microscope. The images collected at different ionic strength are shown in Figure 3A-E. It is interesting to note the different SA pattern with noticeable inter-particle gap observed in the images at different ionic strength.

3.2 Calculation of particle-particle interactions

Using the classical DLVO theory (Equations (1)-(5)), we calculated the attractive $V_{A,PP}$, repulsive $V_{R,PP}$, and the total potential $V_{T,PP}$ at different distance of separation ($H$) between paired 190 and 980 nm particles in the presence of 250 mM NaCl (Debye length approximately 0.61 nm). The data for 190 and 980 nm particles are plotted in Figure 4A, B, respectively. It is to be noted that the for both particles combined interactions ($V_{T,PP}$) show a minimum when two particles are approximately 4 nm apart. The depth of potential energy minimum is much higher than thermal energy ($3/2k_BT$) for 980 nm particles.

The input parameters used in the calculation are provided in Table 1.
**FIGURE 3** The epi-fluorescent microscopic images of 1000 nm particles (actual size 980 ± 20 nm) self-assembled on glass surface in the presence of different salt concentrations. A, NaCl concentration 500 mM; B, 350 mM; C, 250 mM; D, 125 mM; and E, 25 mM. A scale bar of 10 μm is shown in A, and applies to all images.

**FIGURE 4** Inter-particle spacing predicted by DLVO theory for 190 nm A, and 980 nm B, particles.

| Particles | Polystyrene (PS) carboxylated |
|-----------|------------------------------|
| $T$ (K) | 298.3 |
| $A_{PS-water-PS}$ (J) | $1.3 \times 10^{-20}$ |
| $\phi_P$ for 190 and 980 nm particles | $-100$ and $-80$ mV, respectively |
| $\varepsilon$ (water) | 78.3 |

**TABLE 1** Parameters used in the estimation of particle-particle interactions

**4 | DISCUSSIONS**

**4.1 | Comparison of self-assembly at dry and wet conditions**

In dry condition the 200 nm colloidal particles form hexagonal packing (Figure 1). The particles are in close contact with each other due to the strong capillary force during drying. The self-assemblies formed at wet conditions (Figure 2A-F) are completely different than those formed at drying conditions. Two features can be identified immediately: (a) The particles self-assemble in orders and the self-assembled structure is dependent on the salt concentration and (b) Particles assemble loosely with significant inter-particle gaps in all of these patterns. In the presence of 500 mM NaCl, the particles are arranged into quasi-square packings (Figure 2A). Especially, the particles are not in contact with each other but show a significant gap between them. The average particle center-to-center distance is $240 \pm 20$ nm (average over 100 inter-particle pairs), giving a gap of 50 nm between particles. The inter-particle gap across the particles is more obvious in Figure 5A, B that show the enlarged portion of Region 1 and a typical line intensity profile, respectively.
The SA pattern at 350 mM concentration is slightly different than 500 mM and consists of quasi-square packing and parallel chains with average inter-particle gap of approximately 55 nm (Figure 2B). When the salt concentration is decreased to 250 mM, the self-assembled structure becomes more heterogeneous, containing quasi-squares, quasi-hexagons, and parallel chains (Figure 2C). The inter-particle distance also becomes more heterogeneous. Gradual transitions between square-packing and parallel chains can be identified at the connecting areas. In quasi-squarely packed regions (e.g., Region 2), the inter-particle distance was 260 ± 20 nm giving a gap of 70 nm between the particles. To depict the inter-particle gap, an enlarged image and a typical line profile of region 2 is plotted in Figure 5C, D, respectively. When all patterns (quasi squares and hexagons and linear) are included, we found average center-to-center distance over 100 particle pairs of 255 ± 25 nm and gap of approximately 65 nm.

As the salt concentration is further decreased to 125 mM or below, the particle pattern becomes random (Figure 2D-F). Short and bent chains consisting of 3-4 particles can be identified everywhere. For 125 mM concentration, the with particle center-to-center distance of 295 ± 35 nm (average over 100 particle pairs) was observed within the short chains giving gap of around 100 nm. These chains are bent and not aligned parallel. The whole images for 10, 25, and 125 mM show a typical gel-like pattern with inter-connected loops composed of short chains and randomly immobilized particles (Figure 2D-F).

All the particle self-assemblies started from the same particle concentration (at neutral pH and a fixed particle concentration of 0.02% w/v). In the images, they show different surface densities because of heterogeneous distribution. The particles in low salt solution are most evenly distributed across the whole glass slide surface, while the particles at high salt concentrations tend to gather locally.

The particle assembly is a compromise among particle-particle, and particle-surface interactions. At high salt concentration range, the particles assemble into an order structure, indicating that the particle-particle interactions among particles dominate the assembly. At low salt concentration, the interactions among particles that drive the particles to assemble are disrupted by the particle surface interaction. So, ordered assembly is not formed at low salt concentration. Overall, Figure 2A-F gives a typical set of self-assembling patterns of 200 nm particles as a function of salt concentration:

**FIGURE 5** STED image of self-assembly of 200 nm particles and the typical line profiles across the particles. The images correspond to the highlighted regions in Figure 2D-F, respectively. A and B, In the presence of 500 mM NaCl on glass surface; C and D, 250 mM NaCl; E and F, 125 mM NaCl
from ordered quasi-square packing at high salt concentration, to mixed quasi-squares and chains at medium salt concentration, to finally gel-like random patterns with short chains at low salt concentrations. Such a packing trend as a function of salt concentration can be repeatedly produced.

As a comparison, the SA of 1000 nm particle, in consistent with 200 nm particles show gel like patterns at low salt concentration and more organized patterns at high concentration. The images of 1000 nm particles were collected at lower resolution mode and at larger image pixel size, the inter-particle distance measurement can have higher uncertainties. Nonetheless, we measured the inter-particle distance to get rough idea on the particle packing. The measured center-to-center particle distance at 500, 350, 250, 125, and 25 mM is $1170 \pm 44$, $1160 \pm 48$, $1140 \pm 38$, $1145 \pm 47$, and $1180 \pm 44$ nm respectively. This shows that particle packing is not linear as in case of 200 nm particles. More clearly, sparse packing is observed at high salt concentration (Figure 3A) followed by more dense packing (Figure 3B-D) then a sparser packing again at lower concentration (Figure 3E). The observed differences in the SA 200 and 1000 nm particles might be due to difference in size. As the particle size decreases, the forces remain similar but functioning on a much smaller particle and this could influence the assemblies.

### 4.2 Prediction on the inter-particle gap

Under most of our experimental conditions, the Debye distances are close to or smaller than 1.0 nm, much smaller than the particle sizes (e.g., 190 nm in diameters). Thus, thin EDL assumption applies. The van der Waals interaction is short-ranged, which decreases to the level of thermal energy ($1.5k_BT$, or $6.2 \times 10^{-21}$ J) at a gap of approximately 4% of the particle diameter (i.e., approximately 8 nm for 190 nm particle and 40 nm for 980 nm particle, respectively, Figure 4). The electric repulsion is also short-ranged under our experimental conditions. The combined interactions show a second minimum of the total potential energy of approximately 4 nm away from the contact point ($H = 0$ nm) for 190 nm particles. The first minimum is at the contact point, which is not show on the Figure 4. Both the van der Waals interaction working ranges and the second potential energy minimum (several nanometers) are one order of magnitude smaller than the observed particle-particle spacing in the wet self-assemblies (50-70 nm).

Why the particles come together and form ordered structure at medium to high salt concentrations? When these particles are aligned in order, the distance between the particles must represent a balanced position laterally, or a potential energy minimum, under the influence of both attractive and repulsive forces. Under current dominant theory in colloidal and interfacial science, the DLVO theory, the interactions between colloids include an attractive van der Waals force and a repulsive electrostatic force. The two forces combined generate a second potential energy minimum at several nm apart for paired particles at equilibrium, with the first energy minimum at their contact. The energy barrier between the two energy minima is larger than the thermal energy $3/2k_BT$, where $k_B$ is the Boltzmann constant, and $T$ is the temperature. This theory successfully explains the stability of colloidal dispersions and its dependence on the ionic strength of the electrolyte.

However, this theory does not explain the observed gaps between the small nanoparticle assemblies. The main difficulty comes from the short working distance of both electrostatic interaction and the van der Waals interaction in the presence of high concentration of salt. For example, the Debye length at 250 mM NaCl for the particle is only approximately 0.6 nm; the potential energy caused by van der Waals attraction between a pair of 190 nm polystyrene particles ($V_{\text{A,PP}}$) drops quickly in approximately 8 nm to be below the thermal energy ($1.5k_BT$) (Figure 4).

The working distances of both interactions are approximately one order of magnitude smaller than the measured gaps in their self-assemblies (50-70 nm). Here, the full version of the attractive van der Waals interaction equation (Equation (1)) was used:

$$V_{\text{A,PP}} = -\frac{A_{131}}{6} \left\{ \frac{2a^2}{H^2 + 4aH} + \frac{2a^2}{(H + 2a)^2} + \ln \left[ \frac{H^2 + 4aH}{(H + 2a)^2} \right] \right\}$$

where $H$ is the gap between particles; $a$ is the radius of the particles; $A_{131}$ is Hamaker constant; the subscript 1 denotes the materials of the particles, which is polystyrene, and 3 denotes the medium, which is aqueous solution. For polystyrene particle pairs in aqueous solution, Hamaker constant measured from micrometer-sized polystyrene particles is usually reported to be in the range of $0.4 \sim 1.3 \times 10^{-20}$ J. In the estimation, the relatively larger reported value of $1.3 \times 10^{-20}$ J was used. Our estimation is consistent with literature reports where van der Waals interaction becomes negligible when the gap-to-radius ratio decreases to $1/20-1/10$.25,52
Under our experimental conditions, there was no flow or external electric field. The ordered structure is not a mere reflection of the pattern of the surface adsorptive sites since particles self-assemble into different patterns at different salt concentrations. The large inter-particle gap observed in this study cannot be explained by the conventional DLVO theory. The failure of DLVO theory at high ionic strength for high surface change particles is due to multiple over-simplification made in the theory that cannot account the effect of hydration forces, ion-ion correlation, steric effects. We also like to mention that long range interaction was also reported previously. For example, long-range attractive force between plate-like gold particles (2 μm diameter and 10-20 nm thickness) at medium salt concentration was reported by Okomoto and Hachisu. Several attempts have been made to account the limitations of DLVO theory. Long-range attractive interaction between objects carrying like charges has been reported and different theories have been proposed to explain this phenomenon. Sogami and Ise proposed that like-charged macroions with high surface charges show long-range attraction and short-range repulsion. Grier's and other groups confirmed this long-range attraction force between the samely charged particle pairs, but only when the particle pairs are near one, or a pair of, samely charged walls. The attractive interaction was explained in terms of the counter ions gathered between the particle pairs. Li et al used multiscale coarse-grained molecular dynamics approach to account the ionic correlations. It was found that nanoparticles, depending on the surface charge density and ionic strength, show remarkably strong long-range repulsions or attractions. The molecular dynamics simulation may help to explain the large inter-particle gap observed in our case. Although our study was focused on the SA behavior at high ionic strength on negatively charged substrate, it would be interesting to explore the stability and degree of order of the aggregates formed under this condition.

5 | CONCLUSION

To summarize, we demonstrate that using a STED microscope, we are able to in situ resolve the self-assembled structures of particles smaller than the diffraction limit on water-glass surface. We found that highly charged polystyrene particles show completely different self-assembling behavior under wet conditions than being dried on substrate surface. Unlike drying caused self-assemblies where particles are closely packed due to strong capillary force, under wet conditions and without solvent evaporation, the particles self-organize into ordered patterns that are dependent on the salt concentration in the solution. Most interestingly, the self-assembled structures, even in the presence of high concentration of salt, are loosely packed and show significant inter-particle gaps. The gaps are one order of magnitude large than van der Waals interaction distance or the characteristic Debye length. In summary, this study shows that in situ investigation of particle self-assembling is important and will provide more insight for the understanding nanoscale interactions.

ACKNOWLEDGMENTS
This work was supported by the North Carolina State University start-up fund and FRPD award to G.W. We acknowledge Analytical Instrumentation Facility (AIF) center, North Carolina State University for providing SEM measurements.

PEER REVIEW INFORMATION
Engineering Reports thanks the anonymous reviewers for their contribution to the peer review of this work.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/eng2.12233.

AUTHOR'S NOTE
Bhanu Bhakta Neupane was with Chemistry Department, North Carolina State University, Raleigh, North Carolina, USA when the work was carried out.

CONFLICT OF INTEREST
Authors have no conflict of interest relevant to this article.

AUTHOR CONTRIBUTIONS
Bhanu Neupane: Conceptualization-Equal, Data curation-Lead, Writing-original draft-Equal. Yaning Zhong: Data curation-Equal, Methodology-Equal. Gufeng Wang: Conceptualization-Equal, Funding acquisition-Equal, Writing-review and editing-Equal.
REFERENCES

1. Takeda S, Wiltzius P. Growth of highly ordered colloidal crystals using self-assembly at liquid-liquid interfaces. Chem Mater. 2006;18:5643-5645.
2. Lin Y, Skaff H, Emrick T, Dinsmore AD, Russell TP. Nanoparticle assembly and transport at liquid-liquid interfaces. Science. 2003;299:226-229.
3. Binder WH. Supramolecular assembly of nanoparticle melts at liquid-liquid interfaces. Angew Chem Int Ed. 2005;44:5172-5175.
4. Ariga K, Hill JP, Lee MV, Vinu A, Charvet R, Acharya S. Challenges and breakthroughs in recent research on self-assembly. Sci Technol Adv Mater. 2008;9:014109.
5. Tang Z, Zhang Z, Wang Y, Glotzer SC, Kotov NA. Self-assembly of CdTe nanocrystals into free-floating sheets. Science. 2006;314:274-278.
6. Dahal B, Mukhiya T, Ojha GP, et al. In-built fabrication of MOF assimilated B/N co-doped 3D porous carbon nanofiber network as a binder-free electrode for supercapacitors. Electrochim Acta. 2019;301:209-219.
7. Katayama Y, Kalaj M, Barcus KS, Cohen SM. Self-assembly of metal–organic framework (MOF) nanoparticle monolayers and free-standing multilayers. J Am Chem Soc. 2019;141:20000-20003.
8. Zhang J, Sun Z, Yang B. Self-assembly of photonic crystals from polymer colloids. Cur Opin Colloid Interface Sci. 2009;14:103-114.
9. Pusey PN, Van Megen W. Phase behaviour of concentrated suspensions of nearly hard colloidal spheres. Nature. 1986;320:340-342.
10. Verwey EJW, Overbeek JHG, Van Nes K. Theory of the Stability of Lyophobic Colloids. 1955;10(2):224-225.
11. Overbeek JTG. On the electrostatic interaction in macroionic solutions and suspensions. J Chem Phys. 1987;87:4406-4408.
12. Pusey PN, Van Megen W. Phase behaviour of concentrated suspensions of nearly hard colloidal spheres. Nature. 1986;320:340-342.
13. Verwey EJW, Overbeek JHG, Van Nes K. Theory of the Stability of Lyophobic Colloids. 1955;10(2):224-225.
35. Betzig E, Patterson GH, Sougrat R, et al. Imaging intracellular fluorescent proteins at nanometer resolution. *Science*. 2006;316:1642-1645.
36. Schermelleh L, Carlton PM, Haase S, et al. Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy. *Science*. 2008;320:1332-1336.
37. Hell SW, Wichmann J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. *Opt Lett*. 1994;19:780-782.
38. Willig KI, Keller J, Bossi M, Hell SW. STED microscopy resolves nanoparticle assemblies. *N J Phys*. 2006;8:106.
39. Iketaki Y, Watanabe T, Bokor N, Fujii M. Construction of super-resolution microscope based on cw laser light source. *Rev Sci Instrum*. 2006;77:063112.
40. Li Q, Wu SS, Chou KC. Subdiffraction-limit two-photon fluorescence microscopy for GFP-tagged cell imaging. *Biophys J*. 2009;97:3224-3228.
41. Gould TJ, Myers JR, Bewersdorf J. Total internal reflection STED microscopy. *Opt Express*. 2011;19:13351-13357.
42. Galiani S, Harke B, Vicidomini G, et al. Strategies to maximize the performance of a STED microscope. *Opt Express*. 2012;20:7362-7374.
43. Kuang C, Zhao W, Wang G. Far-field optical nanoscopy based on continuous wave laser stimulated emission depletion. *Rev Sci Instrum*. 2010;81:053709.
44. Puthukodan S, Murtezi E, Jacak J, Klar TA. Localization STED (LocSTED) microscopy with 15 nm resolution. *Nanophotonics*. 2020;9(4):783-792.
45. Chen F, García-López V, Jin T, et al. Moving kinetics of nanocars with hydrophobic wheels on solid surfaces at ambient conditions. *J Phys Chem C*. 2016;120:10887-10894.
46. Neupane B, Chen F, Sun W, Chiu DT, Wang G. Tuning donut profile for spatial resolution in stimulated emission depletion microscopy. *Rev Sci Instrum*. 2013;84:043701.
47. Derjaguin BV, Landau L. Actaphysicochim. URSS. 1941;14:633.
48. Israelachvili JN. *Intermolecular and Surface Forces: Revised Third Edition*. Massachusetts: Academic Press; 2011.
49. Bevan MA, Prieve DC. Direct measurement of retarded van der Waals attraction. *Langmuir*. 1999;15:7925-7936.
50. Mori Y, Kimura K, Tanigaki M. Influence of particle-wall and particle-particle interactions on retention behavior in sedimentation field-flow fractionation. *Anal Chem*. 1990;62:2668-2672.
51. Israelachvili JN. *Intermolecular and Surface Forces*. Massachusetts: Academic Press; 2015.
52. Pailthorpe BA, Russel WB. The retarded van der Waals interaction between spheres. *J Colloid Interface Sci*. 1982;89:563-566.
53. Boström M, Williams DRM, Ninham BW. Specific ion effects: why DLVO theory fails for biology and colloid systems. *Phys Rev Lett*. 2001;87:168103.
54. Pashley RM. DLVO and hydration forces between mica surfaces in Li+, Na+, K+, and Cs+ electrolyte solutions: a correlation of double-layer and hydration forces with surface cation exchange properties. *J Colloid Interface Sci*. 1981;83:531-546.
55. Zwanikken JW, de la Cruz MO. Tunable soft structure in charged fluids confined by dielectric interfaces. *Proc Natl Acad Sci*. 2013;110:5301-5308.
56. González-Mozuelos P, Yeom MS, de la Cruz MO. Molecular multivalent electrolytes: microstructure and screening lengths. *Eur Phys J E*. 2005;16:167-178.
57. Okamoto S, Hachisu S. On the long-range attractive force between plate-like gold particles: I. fibrous aggregation. *J Colloid Interface Sci*. 1973;43:30-35.
58. Sogami I, Ise N. On the electrostatic interaction in macroionic solutions. *J Chem Phys*. 1984;81:6320-6332.
59. Schmitz KS, Mukherjee AK, Bhuiyan LB. Screened coulomb pair potential in colloidal interactions in suspensions revisited. *J Phys Chem B*. 2003;107:10040-10047.
60. Belloni L. Ionic condensation and charge renormalization in colloidal suspensions. *Colloids Surf A Physicochem Eng Asp*. 1998;140:227-243.
61. Wu J, Bratko D, Prausnitz JM. Interaction between like-charged colloidal spheres in electrolyte solutions. *Proc Natl Acad Sci*. 1998;95:15169-15172.
62. Apostol M, Cune LC. On the dynamics of the lyophobic colloids. *Chem Phys*. 2020;531:110660.
63. Sogami I. Effective potential between charged spherical particles in dilute suspension. *Phys Lett A*. 1983;96:199-203.
64. Kepler GM, Fraden S. Attractive potential between confined colloids at low ionic strength. *Phys Rev Lett*. 1994;73:356-359.
65. Carbajal-Tinoco MD, Castro-Román F, Arauz-Lara JL. Static properties of confined colloidal suspensions. *Phys Rev E*. 1996;53:3745-3749.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Neupane BB, Zhong Y, Wang G. Study on self-assembly of colloidal particles at high ionic strength with stimulated emission depletion microscopy. *Engineering Reports*. 2020;2:e12233. [https://doi.org/10.1002/eng2.12233]