ChemComm

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Journal:	*ChemComm*
Manuscript ID	CC-COM-05-2018-003637.R1
Article Type:	Communication

Journal Name

COMMUNICATION

www.rsc.org/

Compressible Colloidal Clusters from Pickering Emulsions and Their DNA Functionalization

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

In-Seong Jo[a], Joon Suk Oh[b], Shin-Hyun Kim[c], David J. Pine[b, d], and Gi-Ra Yi*[a]

Colloidal clusters were prepared by assembling azide-functionalized non-crosslinked polymer particles using fluorinated oil-in-water emulsion droplets. The particles were adsorbed on the droplet interface, which were packed to form clusters during slow evaporation of the oil. Then, clusters were coated by DNA using an alkyne-azide cycloaddition (SPAAC) reaction. As the particles are not crosslinked, the shape of the DNA-coated clusters can be further modified to control compression ratio through plasticization.

DNA-coated nanoparticles have been extensively investigated as building blocks for constructing nanostructures for nanoplasmonics or nanoelectronics applications since they can readily self-assemble into superstructures as programmed.[1-4] Recently, DNA-coated microspheres (>100 nm) have also been explored for photonic crystals or glasses which could manipulate light as designed in highly efficient manners.[5-7] If their assembled structures are periodic or aperiodic at acoustic wavelength scales, those colloidal systems are potentially also useful for manipulating sounds.[8] Unlike nanoparticles whose diameters are comparable to DNA coating layers, it is of great importance to have high-density DNA coating on microparticles to facilitate effective reconfiguration of kinetically trapped structures to equilibrium structures.[9] Previous studies have shown that the minimum DNA density for reconfiguration to occur is 30,000 strands per 1-μm particles (or 25% of areal coverage).[10] Wang and coworkers demonstrated that 1-μm particles coated with 100,000 DNA strands could readily self-assemble into fcc crystal structures or CsCl-like crystal structures through DNA-mediated assembly.[11] More recently, Oh and coworkers reported an efficient method to coat 1-μm polymeric particles with more than 200,000 DNA strands using modified swelling-deswelling method and click chemistry and also demonstrated the DNA-coated particles could successfully self-assemble into colloidal crystals.[12]

Generally, spheres can self-assemble into relatively simple colloidal superstructures. To achieve more complex structures, one can utilize pre-assembled clusters as a building block.[13-15] Recently, Ducrot and coworkers reported colloidal Laves phases ($MgCu_2$) using DNA-mediated assembly of colloidal tetrahedra and spheres, which could be further developed for diamond- or pyrochlore-like colloidal superstructures, known to possess largest photonic band gaps.[16] In addition, various kinds of colloidal clusters can be exploited to generate new colloidal phases that cannot be achieved with simple spherical systems.[17] For instance, octahedral colloidal clusters and spheres could self-assemble into $AuCu_3$ and CaB_6 superlattices.

In this new scheme for DNA-mediated colloidal assembly with pre-assembled clusters, DNA sequences, areal density of DNA, particle number density, and size ratio are key parameters for determining equilibrium structures. The compression ratio, the ratio of center-to-center distance to particle diameter ($r_{cc}/2a$) of clusters is another important parameter for structure formation.[16] However, it has been difficult to control the compression ratio of colloidal clusters with current methods, as crosslinked polymeric microspheres need to be used to form clusters within oil-in-water emulsion.[18-21] Here, we use non-crosslinked polystyrene particles for colloidal clusters instead, which can be merged partially through solvent annealing. In this way, the compression ratio can be precisely controlled. By introducing azide (N_3) functional groups onto polymer particles, particles can be coupled with dibenzocyclooctyl (DBCO)-functionalized DNA strands through strain-promoted alkyne-azide cycloaddition (SPAAC) reaction (also known as copper-free click chemistry).[10, 11, 22]

The polystyrene particles were synthesized by surfactant-free emulsion polymerization as described in the supplementary materials. Their mean diameter and standard deviation were 1.05 μm and 0.05 μm, respectively, which were measured by analyzing in-line holographic patterns of particles in microfluidic channels

[a] School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea
[b] Center for Soft Matter Research, Department of Physics, New York University, New York, New York 10003, United States
[c] Department of Chemical & Biomolecular Engineering, KAIST, Daejeon 34141, Republic of Korea
[d] Department of Chemical & Biomolecular Engineering, Tandon School of Engineering, New York University, Brooklyn, New York 11201, United States
† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

(xSight, Spheryx Inc.). Amphiphilic polystyrene-*b*-polyethylene oxide diblock copolymers with an azide group at the end (PS-*b*-PEO-N_3) were the coupled to the particle surfaces using a swelling and deswelling process.[12] **Figure 1a** illustrates the formation of colloidal clusters and

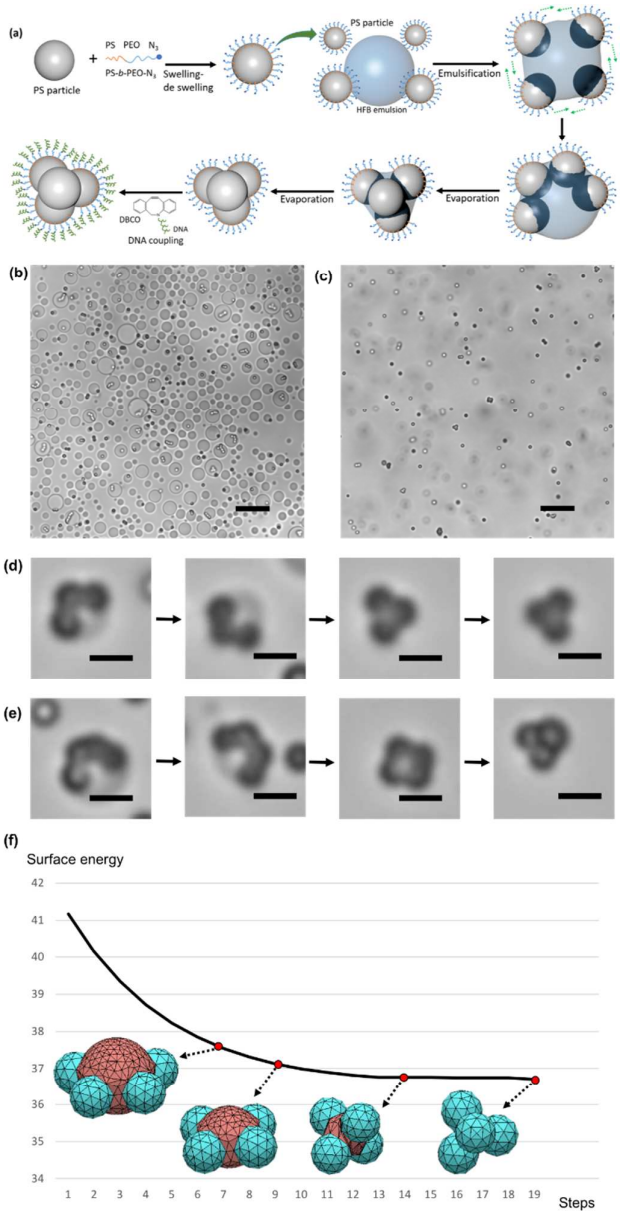

Figure 1. (a) Schematic for incorporation of PS-b-PEO-N_3 into PS particles and fabrication of colloidal clusters using oil-in-water emulsions. Hexafluorobenzene (HFB) was emulsified into an aqueous suspension of the PS particles by sonication. The particles spontaneously adsorbed on droplet interfaces and formed aggregates. The evaporation of HFB leads to particle packing into clusters. Afterward, DNA was coated on the surface of the clusters through a coupling reaction between PS-b-PEO-N_3 and DBCO-DNA. **(b, c)** Optical microscope images of HFB-in-water droplets containing the PS particles at the interface and resulting clusters. The scale bar is 10 μm. Timeline optical microscope images of HFB droplets that contain **(d)** three and **(e)** four PS particles under 50 mbar in 10 minutes. Linear chains of three and four particles in droplets turn to triangle and tetrahedron, respectively. Scale bar is 2 μm. **(f)** Surface evolver simulation for the formation of tetrahedron cluster from four particles confined at the interface of a droplet. Graph shows that surface energy of emulsion interface (red colored) decreases gradually as the volume of emulsion drop is reduced. Four particles on the interface closely packed with each other when the droplet volume goes to 0.

functionalization of DNA strands. We emulsified hexafluorobenzene (HFB) in water using sonication in the presence of azide-functionalized PS particles. The particles adsorbed to the interface between water and oil phases to minimize interfacial energy, forming particle-stabilized emulsions, known as 'Pickering' emulsions. **Figure 1b** shows the optical images of Pickering emulsions, where polydisperse HFB-in-water emulsion droplets (5-50μm), containing various number of PS particles ranging from 1 to 10, were produced. Typically, 100-μl solution of 1-wt% PS particle was diluted with 3 ml of deionized water into which hexafluorobenzene (HFB) oil (300 μl) was then emulsified with probe-type ultrasonic homogenizer for 10 seconds. Average diameter of emulsion was 5.76 μm. We gradually removed the HFB oil droplet by evaporation under low pressure (50 mbar) at room temperature for 15 minutes. As a result, the PS particles were assembled into uniform clusters with given number of constituent particles as shown in **Fig. 1c**. Their distribution of the number of constituent particles are plotted in **Fig. S1e**, in which trimer and tetramer are major clusters. Finally, the colloidal clusters are functionalized with dibenzocyclooctyl (DBCO)-DNA through strain-promoted alkyne-azide cycloaddition (SPAAC) reaction. By adjusting sonication time for emulsification, we could control the size distribution of emulsion and then the number of constituent particles (N) as shown in **Fig. S1**. In case of shorter sonication time (3 s), we obtained larger emulsion with average diameter of 6.99 μm (**Fig. S1a**) and larger colloidal clusters which have more than six particles (N>6) in clusters (**Fig. S1d**). By contrast, with longer sonication time (30 s), smaller emulsion with average diameter of 4.78 μm (**Fig. S1c**) and smaller colloidal clusters were obtained, of which majority was dimer (N=2) (**Fig. S1f**).

Interestingly, in Pickering emulsion, the PS particles are not homogeneously distributed on the surface of HFB droplet, but aggregated in linear or branched chains as shown in **Fig. 1b or S1b**. Then, as HFB oil evaporates, they gradually assemble into well-defined configurations of 'minimal second moment'[19] as shown in **Fig. 1d** and **1e**, implying that particles are loosely aggregated so that they can rearrange into symmetric structures. HFB can be wetted preferentially more on polymer particles at interface which caused attractive forces between particles.[13] With a surface evolver simulation,[23] we confirmed that the chains of particles reform into well-defined clusters as shown in **Fig. 1f**, as they are aggregated but flexible.

Since our clusters are prepared from polydisperse emulsions, there is a distribution of cluster sizes. However, clusters with the same number of particles have the same configuration in each cluster. To fractionate of clusters, density gradient centrifugation technique is used. To this end, we made 5-60% glycerol gradient solution in 15-ml glass test tube using a density gradient maker, on which cluster

solution (~0.4 ml) was loaded very carefully along the side wall of test tube. Then, the sample was centrifuged for 30 minutes at 1300 RCF, forming discrete bands as shown in **Fig. 2a**.

We carefully collected the clusters from each band using a syringe. SEM images in **Figure 2(b-e)** confirm that most of clusters in same band have the same configuration. As shown in histogram of Fig. 2j, 76.5%, 72.5%, 67.5% of dimer, trimer, tetramers are collected from corresponding band after density gradient separation. Since the particles have azide group on the surface, DBCO-functionalized DNA strands can be coupled onto the surface of PS clusters through SPAAC reaction. Because a fluorophore (Cy5) was inserted in the DNA strands, we were able to confirm that the clusters were coated with DNA strands in the confocal images of the clusters (**Fig. 2f-I**). We used flow cytometry to measure the DNA density on the PS particle. The areal density of DNA is 286,000 per 1-μm-diameter particle. We also verified that the DNA-coated particles were stable for more than 3 months, exhibiting similar thermal responses over 3 months.

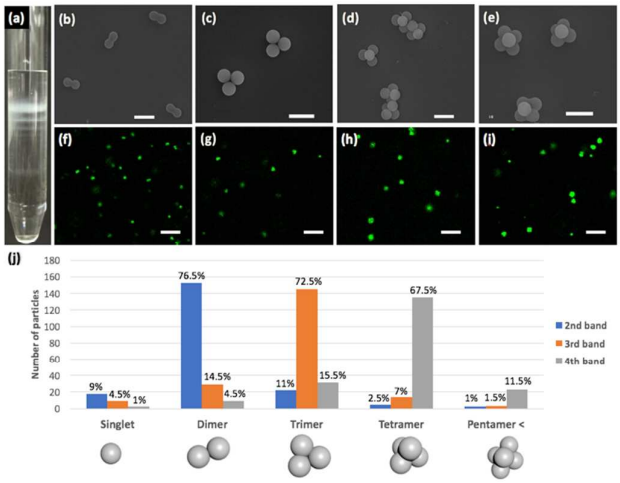

Figure 2. (a) Test tube containing a cluster suspension of 1-μm PS microspheres separated by density gradient centrifugation. **(b-e)** Scanning electron micrograph images of colloidal clusters extracted from each band. Isolation of the fractions and subsequent FESEM analysis showed that these bands can be assigned to **(b)** doublets, **(c)** triplets, **(d)** tetramers (tetrahedra), and **(e)** pentamers. Scale bar is 2 μm. **(f-i)** Confocal microscope analysis of the clusters after clicking DNA onto azide functional group their surface: **(f)** doublets, **(g)** triplets, **(h)** tetrahedrons, **(i)** pentamers. Each cluster shows bright fluorescent signal. Scale bar is 5 μm. **(j)** Histograms showing the number ratio of clusters from three distinct bands after density gradient centrifugation, where 200 clusters were analyzed per each band. Second band (blue) from test tube contains dimers over 70%, and third band (orange) contains trimers over 70%. Fourth band (gray) contains tetramers 67.5% of all.

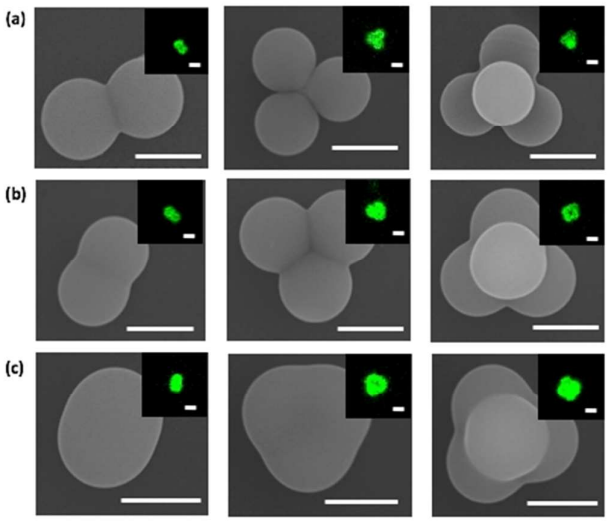

Figure 3. SEM images of DNA-coated colloidal clusters with different compression ratios by solvent annealing in a mixture of water and THF. The fraction of THF in the mixture was set to **(a)** 10 v/v%, **(b)** 15 v/v%, and **(c)** 20 v/v%. Confocal images (inset) confirm that Cy5-DNA strands remain on the surfaces after the treatment. Scale bar is 1μm.

Unlike the colloidal clusters used in a previous report[19], the particles in our clusters are not crosslinked so that they can be deformed as much as desired by a simple swelling process. As already mentioned, the compression ratio of clusters is one of the key parameters for building up complex colloidal superstructures such as $MgCu_2$ crystals and colloidal diamond structure.[16] We have treated DNA-coated clusters with the mixture of THF and water. As THF is a good solvent for PS, it can plasticize the clusters and cause them to deform. Importantly, the deformation can be tuned by the amount of THF added. Moreover, the deformation can be readily halted by adding an excess of water. Since THF is miscible with water, we can rapidly reduce the fraction of THF. As shown in the SEM images of **Fig. 3**, the DNA-coated clusters of two, three and four PS particles are successfully deformed after the treatment and the compression ratio ($r_{cc}/2a$) or degree of deformation increases from 0.36 to 0.90 as the mixing ratio of THF in water increases. Importantly, we also confirmed that DNA-coating was stable after the solvent treatment in the confocal images of insets of **Fig 3**.

In summary, we developed a simple method to produce compressible colloidal clusters by stabilizing hexafluorobenzene droplet in water with non-crosslinked polystyrene microspheres containing azide functional groups on the surface. Then, the clusters were coated with DNA strands through SPAAC reaction. Next, we demonstrated a partial deformation of the DNA-coated clusters by swelling-induced plasticization with THF. We could precisely adjust the compression ratio of the clusters from 0.9 to 0.36. We also confirmed that the DNA-coating was stable upon solvent treatment. These precisely-tuned DNA-coated clusters with various compression ratios can serve as new building blocks for complex colloidal superstructures such as pyrochlore or diamond structures.

We acknowledge support from the by the National Research Foundation of Korea (NRF) (NRF-2010-0029409, and NRF-2014M3A9B8023471). DP acknowledges partial support from the US National Science Foundation under Award Number DMR-1610788.

Conflicts of interest

There are no conflicts to declare.

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