Soft Matter

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Journal:	*Soft Matter*
Manuscript ID	SM-COM-06-2019-001243.R1
Article Type:	Communication
Date Submitted by the Author:	23-Jul-2019
Complete List of Authors:	Moon, Jeongbin; Sungkyunkwan University, Chemical Engineering Jo, In-Seong; Sungkyunkwan University, Chemical Engineering Yoon, Jeong Hoon ; Sungkyunkwan University, Chemical Engineering Kim, Yeongha; Sungkyunkwan University, Chemical Engineering Oh, Joon Suk; New York University, Physics; Sungkyunkwan University - Suwon Campus, Polymer Science and Engineering Pine, David; New York University, Department of Physics Yi, Gi-Ra; Sungkyunkwan University, Chemical Engineering

COMMUNICATION

DNA Functionalization of Colloidal Particles via Physisorption of Azide functional Diblock Copolymers

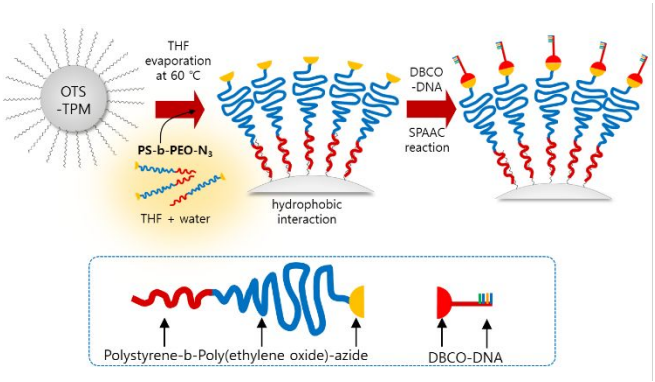
Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Jeongbin Moon,[a] In-Seong Jo,[a] Jeonghoon Yoon,[a] Yeongha Kim,[a] Joon Suk Oh,[b] David J. Pine,[b,c] and Gi-Ra Yi[a*]

DNA-coated inorganic particles can be prepared simply by physical adsorption of azide-functionalized diblock copolymers (polystyrene-*b*-poly(ethylene oxide)-azide, PS-*b*-PEO-N_3) onto hydrophobically-modified inorganic particles, followed by a strain-promoted azide-alkyne cycloaddition (SPAAC, a copper-free click chemistry). This approach is applied to organosilica, silica and titania particles. The DNA-coated colloids are successfully crystallized into colloidal superstructures by a thermal annealing process using DNA-mediated assembly.

Over the past decade, DNA-coated colloids have been intensively investigated as programmable building blocks to construct complex colloidal superstructures via selective and reversible DNA hybridization.[1] It has been demonstrated that DNA-coated nanoparticles could self-assemble into various crystalline lattices by controlling composition, shape and DNA sequences of building blocks.[2] However, it was difficult to obtain crystalline structures using DNA-coated microspheres due to relatively short DNA brushes, which requires uniform and high-density DNA coatings to ensure sub-diffusion at bounded state, otherwise they stick and form kinetically trapped random aggregates.[3] Recently, to address this problem, copper-free click chemistry was introduced to graft DNA strands onto microspheres.[4] The coating density substantially increased to more than 100,000 DNA strands per 1-μm particle and the particles readily self-assembled to colloidal crystals. Another technique was introduced later, in which azide-functionalized diblock copolymers were incorporated onto polymer particles by a swelling and deswelling process followed by grafting of DNA strands onto the end of the block copolymer brushes, which provided flexibility and further increased the coating density to even more than 200,000 DNA strands per 1-μm microspheres.[5] More recently, 100-nm polystyrene (PS) particles with DNA sticky ends were directly synthesized from polymeric micelles.[6] However, those approaches were applied to polymer particles, such as polystyrene and poly(methyl methacrylate), for high-density DNA brushes. DNA-coated inorganic microspheres are relatively very rare. Wang et al. demonstrated that silica and titania can be coated with DNA by introducing an azide group through silane chemistry and a SPAAC reaction.[7]



Scheme 1 Experimental procedures of DNA functionalization on hydrophobically-modified TPM microspheres by physical adsorption of PS-*b*-PEO-N_3 and copper-free click chemistry.

Here, we demonstrate a simple but general alternative approach for coating DNA on inorganic colloids such as organosilica, silica and titania. The surface of inorganic colloids is first hydrophobically modified with octadecyltrimethoxysilane (OTS). The PS end of a diblock copolymer (PS-*b*-PEO-N_3) is then physisorbed onto the OTS through a hydrophobic interaction. Then, dibenzylcyclooctyne-functionalized DNA strands (DBCO-DNA) are coupled to the azide end of the copolymers using SPAAC, which produced similar or higher areal density of DNA brushes (>100,000 strands per 1-μm particle) comparing with previous reports[5,7]. Thereby, the DNA-coated particles readily self-assemble into colloidal crystals under a thermal annealing. As illustrated in Scheme 1, we have prepared monodisperse 1-μm 3-(trimethoxysilyl)propyl methacrylate (TPM) particles by sol-gel reaction and polymerization of TPM as described in a previous report.[8] Then, for the hydrophobic modification, an ethanol dispersion of TPM

[a] *School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea. E-mail: yigira@skku.edu*
[b] *Center for Soft Matter Research, Department of Physics, New York University, New York, New York 10003, USA*
[c] *Department of Chemical & Biomolecular Engineering, Tandon School of Engineering, New York University, Brooklyn, New York 11201, USA*
†Electronic Supplementary Information (ESI) available: Video 1 Repeated association and dissociation of DNA-coated TPM particles. See DOI: 10.1039/x0xx00000x

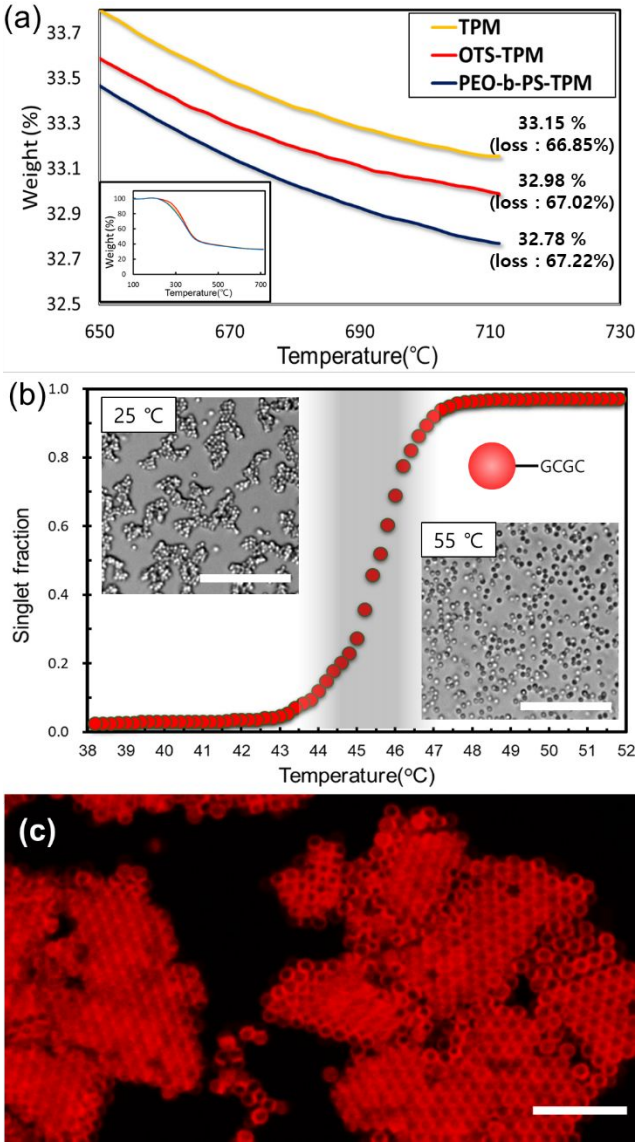


Fig. 1 (a) TGA analysis graph of TPM(yellow), OTS-TPM(red) and PEO-b-PS-TPM(blue). Weight percent of each sample after heat treatment(y-axis) vs temperature(x-axis). (b) Optical microscopy images and melting curve of DNA-coated TPM particles (DNA sequence: Cy3-T10-GCGC). Scale bars are 15-μm (c) Confocal microscopic image of crystallized DNA-TPM particles after thermal annealing at 45 °C for overnight. Scale bar is 5-μm.

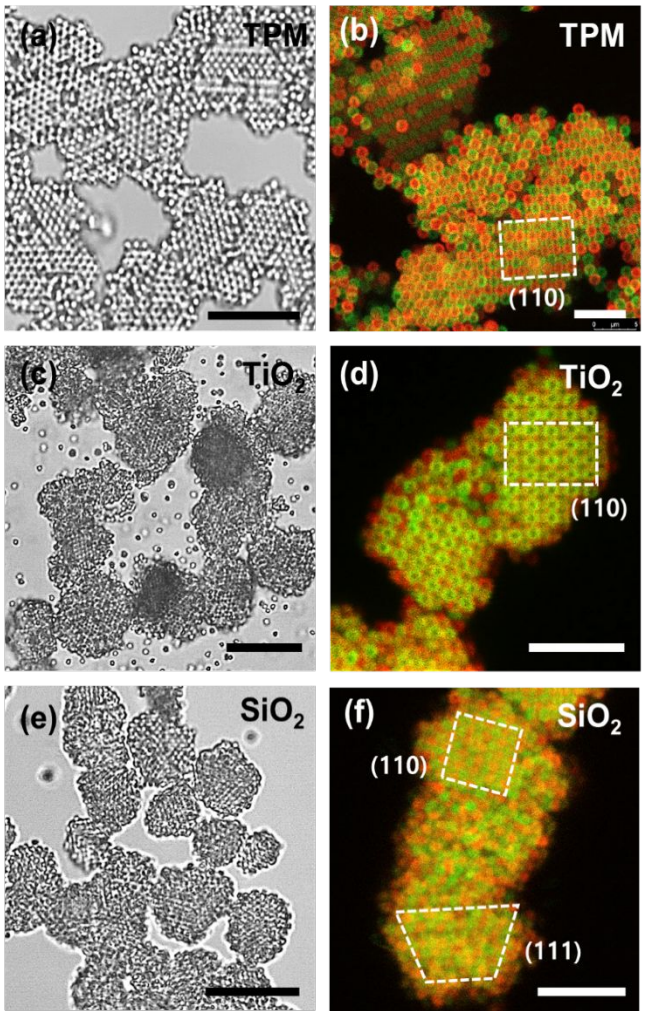


Fig. 2 Confocal microscopy images of DNA-mediated CsCl-like crystalline structures of (a,b) 1-μm TPM (c,d) 750-nm titania (e,f) 600-nm silica particles. The DNA sequence in each sample is same (Cy3-T10-CCTCC(red), Cy5-T10-GGAGG(green)). All of them are annealed under melting temperature (Singlet fraction is from 0.3 to 0.4.) for overnight. Crystal planes of CsCl-like structures are annotated. Scale bars are 10-μm(a,c and e) and 5-μm(b,d and f).

(5 wt.%, 30 ml) was mixed with ammonium hydroxide solution (28 wt.%, 0.3 ml) under gentle stirring. An OTS solution (10 vol.% in chloroform, 0.5 ml) was added and stirred for 2h.[9] The OTS-treated TPM (OTS-TPM) particles are repeatedly washed three times with ethanol and re-dispersed in tetrahydrofuran (THF). The OTS-TPM dispersion in THF (100 μl, 5 wt.%) is mixed with PS-*b*-PEO-N_3 ($M_{w,PS\text{-}b\text{-}PEO}$ = 6,900 g/mol, $M_{w,PS}$ = 1,300 g/mol, and $M_{w,PEO}$ = 5,600 g/mol) solution (100 μl, 1 mM) for 1 hour to coat PS-*b*-PEO-N_3 onto the surface of OTS-TPM. Then, 300 μl of deionized water is added and the sample is annealed at 60°C for 30 minutes for removing THF by evaporation. Azido-PEO-*b*-PS-coated TPM (TPM-N_3) particles are washed with DI water several times and re-dispersed in PBS buffer solution. Finally, the PBS buffer solution (1 wt.%, 200 μl) of TPM-N_3 particles is mixed with DBCO-ssDNA (10 μl, 1 mM, 5'-DBCO-Cy3-T_{10}-GCGC-3') and stirred at 55°C for 24h. The DNA-coated TPM particles are then washed and stored in PBS buffer solution. For TPM, OTS-TPM, and PS-b-PEO-TPM, zeta potential values were -47.1mV, -20.1mV, and -42.6mV, respectively, which confirmed that each surface modification step was done successfully.

In order to estimate the areal densities of surface OTS groups and PS-*b*-PEO-N_3, mass ratios of organic to inorganic compounds in TPM, OTS-TPM, PEO-*b*-PS-TPM were measured by thermal gravimetric analysis (TGA) in which each sample was heated at the rate of 10°C per 1 minute up to 700°C. As shown in Fig. 1a, the weight fraction of organic compound (weight loss in TGA analysis) in TPM and OTS-TPM was 66.85% and 67.02%, respectively. From weight fraction of organic compound in PEO-*b*-PS-TPM (=67.22%), areal density of PS-*b*-PEO brush was estimated as single brush per 20.7 nm^2. This result suggests around 151,800 PS-*b*-PEO-N_3 brushes are attached to 1-μm TPM particles. We grafted fluorophore-labelled (Cy5) DNA strands onto the particles to measure the DNA coating density

in another way. As described in previous report by Oh et al.,[5] fluorescent intensity of single DNA-coated particles was measured using flow cytometry (FACS Aria Fusion, BD biosciences) and then compared with standard calibration curve which was obtained by measuring five different standard fluorescent microspheres (Quantum Cy5MESF, Bangs Laboratories, Inc). Measured coating density is around 148,000 DNA strands per 1-μm TPM particles.

The TPM particles are coated with DNA strands with self-complementary (palindrome) sticky ends (Cy3-T_{10}-GCGC). We confirmed that the particle binding via DNA is thermally reversible as shown in Fig. 1b. When quenched quickly to room temperature, the particles form random aggregates. When the sample is heated to 55 °C, the aggregates quickly dissociate and are completely dispersed, which can be repeated reversibly as shown in Video 1 (ESI†). We measured the singlet fraction of the sample as a function of temperature to locate the melting temperature (T_m) which we define the singlet fraction is 0.5 (Fig. 1b). The aggregates start to rapidly dissociate around 44 °C and the singlet fraction becomes 0.5 around 45.5 °C. All particles are fully dispersed above 47.5 °C. For the crystallization, the sample is annealed slightly below the T_m (45°C), where the particles can stick and roll over each other to find their free energy minimum. The confocal Image in Fig. 1c shows the resultant crystalline structure, a face-centered-cubic (fcc) lattice.

We also prepared two sets of TPM particles coated with complementary DNA strands (not palindromes). The sequences used were Cy5-T_{10}-GGAGG and Cy3-T_{10}-CCTCC. Upon annealing, the particles self-assemble into a CsCl-like structure, as how in the bright field and confocal images in Fig. 2a and 2b.

As mentioned above, we also applied this approach to metal oxide particles such as silica and titania. 750-nm silica and 600-nm titania particles are prepared by sol-gel reaction of tetraethyl orthosilicate (TEOS) and titanium(IV) isopropoxide (TTIP) as described in previous reports.[10] They are then treated with OTS and coated with DNA strands using the same protocol described above. Fig 2c-d and 2e-f show the resultant CsCl-like colloidal crystals composed of the DNA-coated titania and silica microspheres, respectively.

We also applied our approach to coat colloidal clusters with DNA strands. First, we prepared colloidal clusters of TPM particles using an emulsion encapsulation and shrinkage process as described previously[11] and coated the surface with silica (around 10nm) to make the TPM clusters stable.[12] To disperse TPM particles in hexane, we prepared OTS-TPM. 10 wt.% OTS-TPM dispersion (2 ml) was added to 16 ml of Pluronic P123 surfactant solution. Using a homogenizer (IKA T25), this mixture was sheared at 7000 rpm for 30s. The resulting emulsion solution was stored at 100°C to evaporate hexane. To coat bare silica on this OTS-TPM clusters, the solution was diluted to 40 ml. 0.6 ml NH_4OH and 0.2 ml TEOS were added and stirred for 3h. Subsequently, OTS, PS-*b*-PEO-N_3, and DNA strands were coated onto the surface of the TPM clusters. The confocal microscope images in Fig. 3a-c clearly show the DNA-coated clusters. DNA-coated clusters have been attracting considerable attentions recently due to their unique geometry

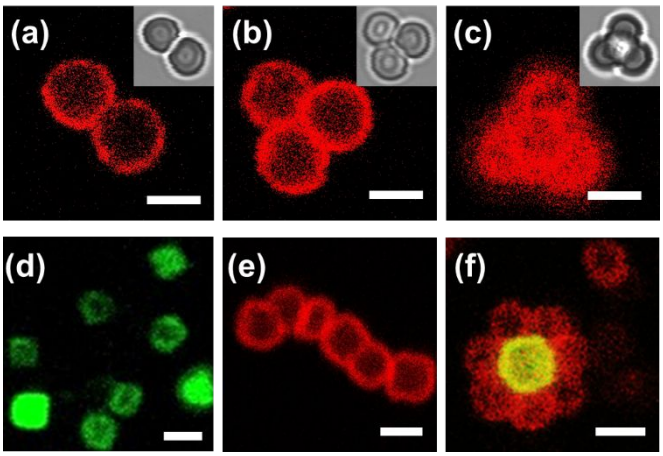


Fig. 3 Fluorescence microscopy images and bright-field images (inset) of DNA-coated TPM clusters (a) dimer (b) trimer (c) tetramer and anisotropic silica hollow cube particles coated with DNA strands (d) Complementary A (Cy5-T_{10}-GGAGG) without A' (e) self-complementary (Cy3-T_{10}-GCGC) and assembled structure. (f) DNA-mediated assembly of hollow silica cube (Cy5-T_{10}-GGAGG, green) and TPM (Cy3-T_{10}-CCTCC, red). Scale bars are 1-μm.

when assembled. Very recently, studies have shown that colloidal $MgCu_2$ laves phase can be realized when tetrahedral clusters and singlets are assembled together, which is a complementary combination of diamond and pyrochlore colloidal structures.[13] Interestingly, both structures are known to have broad photonic bandgaps.[14] To obtain either structure, one of them must be removed from the $MgCu_2$ laves phase. Thus, we believe that the realization of DNA-coated inorganic clusters can present new possibilities.

From a similar point of view, new possibilities can be proposed by coating ssDNA on anisotropic colloidal particles.[15] As an example, we have synthesized hollow silica cubes using previously reported methods.[16] As mentioned above, the physical adsorption of PS-*b*-PEO-N_3 was used to coat ssDNA on hydrophobically-modified anisotropic silica particles and confirmed by confocal microscopy. Fig. 3e-f shows fluorescent images of DNA-coated hollow silica cubes and their assembly. In particular, Fig. 3f shows a cluster assembly of DNA-coated TPM (Cy3-T_{10}-GGAGG, red) and a silica hollow cube (Cy5-T_{10}-CCTCC, green).

In summary, we have developed a simple yet robust method for high-density uniform DNA coating on inorganic colloids via physical adsorption of amphiphilic block copolymers and click chemistry, which resulted in similar or higher areal density of DNA brushes on particles comparing with previous reports.[5,7] These high-density DNA-coated particles can be readily crystallized through a thermal annealing process. This method can be applied to other hydrophobic particles readily as well as anisotropic colloidal particles such as colloidal clusters, cubic or rod-like particles, which would be potentially useful as building blocks to realize laves phase, CaB_6 or even more complex colloidal superstructure. Furthermore, hydrophobic-hydrophobic interaction can be tuned by adjusting solvent property. Therefore, by adding small amount of polar solvent into this system, our DNA coating may become mobile,[17] which is currently under investigation.

Acknowledgement

We acknowledge support from the National Research Foundation of Korea (NRF) (NRF-2019M3A7B8028381). DP acknowledges partial support from the US Army Research Office (W911NF-17-1-0328).

Conflicts of interest

There are no conflicts to declare.

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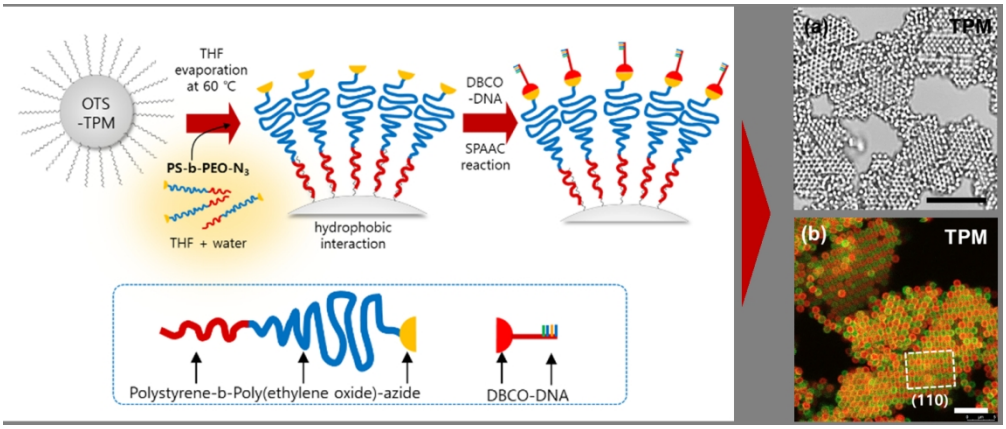
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THF + water
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-DNA
SPAAC
reaction
Polystyrene-b-Poly(ethylene oxide)-azide
DBCO-DNA
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