A general strategy to synthesize chemically and topologically anisotropic Janus particles

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Emulsion polymerization is the most widely used synthetic technique for fabricating polymeric particles. The interfacial tension generated with this technique limits the ability to tune the topology and chemistry of the resultant particles. We demonstrate a general emulsion interfacial polymerization approach that involves introduction of additional anchoring molecules surrounding the microdroplets to synthesize a large variety of Janus particles with controllable topological and chemical anisotropy. This strategy is based on interfacial polymerization mediated by an anchoring effect at the interface of microdroplets. Along the interface of the microdroplets, the diverse topology and surface chemistry features of the Janus particles can be precisely tuned by regulating the monomer type and concentration as well as polymerization time. This method is applicable to a wide variety of monomers, including positively charged, neutrally charged, and negatively charged monomers, thereby enriching the community of Janus particles.

INTRODUCTION
In nature, biological particles with highly anisotropic chemistries and topologies, such as neurons (1), bacteriophages (2), and hemoglobin (3, 4), play important roles in the activities of life (5). Learning from nature, scientists have aimed to synthesize particles that mimic these natural particles because of their great potential for fundamental research and practical applications in cancer diagnostics (6), self-assembled biomaterials (7–9), smart micromachines (10, 11), and energy transporters (12). Emulsion polymerization, as a traditionally leading synthesis technique, has been widely used for the synthesis of polymeric particles (13–15). However, spherical particles are often formed because of interfacial tension (16, 17), which limits flexibility in tuning the topology and chemistry of the particles (18–22). Achieving large-scale synthesis of uniform Janus particles with tunable topology and surface chemistry by emulsion polymerization remains a great challenge. Here, we demonstrate a general emulsion interfacial polymerization approach, which can be scaled up to synthesize a large variety of Janus particles with uniform topology, narrow size distribution, and variability in topology and chemistry.

RESULTS
Rational interfacial design for the large-scale synthesis of Janus particles with tunable topology and surface chemistry
In designing our method, we hypothesized that a dynamic interfacial reaction could be harnessed to break the spherical limitation, such as by making the interfacial reaction start from a specific point rather than from the entire spherical interface of the microdroplets. Here, we attempted to synthesize anisotropic particles by introducing additional anchoring molecules into the interface of the microdroplets. As a proof of concept, we chose to use a typical oil-in-water emulsion system, styrene (St) and divinylbenzene (DVB) in water emulsion, into which, as an example, hydrophilic acrylic acid (AA) was introduced as anchoring molecules. Briefly, when 7 mmol AA anchoring molecules are introduced outside St/DVB-based (13 mmol St, 7 mmol DVB) droplets, they can polymerize to form Janus poly(styrene-co-divinylbenzene) ⊃ poly(acrylic acid) (PSDVB ⊃ PAA) particles with a crescent moon topology and amphiphilicity (the symbol “⊃” represents the orientation of the hydrophobic concave surface and hydrophilic convex surface of the Janus particles). These particles have average diameters of 2.31 ± 0.16 μm and “opening degree” of 104.1 ± 14.5° (the opening degree can be defined as a central angle of sector that corresponds to the concave region of the Janus particle). In contrast, in the absence of AA, the polymerization produced only the spherical particles typical of traditional methods of emulsion polymerization (fig. S1). Energy-dispersive x-ray (EDX) spectroscopy analysis revealed distinct O distribution on the convex/concave surfaces of the particles. For the PSDVB ⊃ PAA particles, the O element only existed in the PAA, implying that the convex surface of the particle was hydrophilic PAA, whereas the concave surface was hydrophobic PSDVB (Fig. 1A). Aberration-corrected scanning transmission electron microscopy (STEM) images of the embedded particles, after microtome sectioning and staining with phosphotungstic acid, revealed two layers. The thickness of the PAA layer in the Janus particles was 53 ± 20 nm (Fig. 1B). When polymerization was scaled up to three times the aforementioned monomer feed, this approach produced uniform Janus particles of approximately 5 g in one batch, providing an effective way to synthesize Janus particles on a large scale. These results demonstrate that the introduction of anchoring molecules can successfully produce Janus particles with hydrophilic convex surfaces and hydrophobic concave surfaces.

We next investigated the influence of concentration of the anchoring molecules and hydrophobic monomers on the topology of the Janus particles. At low AA concentrations of 0.06 M, bread-shaped Janus particles were easily formed. As the concentration of AA increased, the anisotropic topology of the Janus particles gradually changed from bread-shaped to hemispherical-, crescent moon-, and even pistachio-shaped particles at a high AA concentration of 0.3 M (Fig. 1C). When the concentration of AA was further increased, a composite film could be...
obtained, as shown in the ternary phase diagram of AA, St, and DVB (Fig. 1D). Regarding the hydrophobic monomers, when the amount of St or DVB increased, the topology of resultant particles changed from Janus particles (bread, hemisphere, and crescent moon) to porous particles. Bread- and hemispherical-shaped particles were easily produced at molar ratios of $n_{\text{St}}/n_{\text{total}} < 0.625$, $n_{\text{DVB}}/n_{\text{total}} < 0.625$, and $0.1 < n_{\text{AA}}/n_{\text{total}} \leq 0.5$. Crescent moon–shaped particles were formed at molar ratios of $0.25 < n_{\text{St}}/n_{\text{total}} < 0.5$, $0.25 < n_{\text{DVB}}/n_{\text{total}} < 0.5$, and $0.1 < n_{\text{AA}}/n_{\text{total}} \leq 0.25$. Pistachio-shaped particles were easily produced at molar ratios of $0.1 < n_{\text{St}}/n_{\text{total}} < 0.5$, $n_{\text{DVB}}/n_{\text{total}} < 0.25$, and $0.35 < n_{\text{AA}}/n_{\text{total}} < 0.75$ (Fig. 1D). Outside these ranges, spherical porous particles and composite films were produced (fig. S2). Notably, the chemical anisotropy with respect to the amphiphilicity and surface charge of the Janus particles also changed along with their topologies. Therefore, addition of anchoring molecules during emulsion interfacial polymerization can effectively control the topology and surface chemistry features of Janus particles.

The formation mechanism of the Janus particles by emulsion interfacial polymerization

To explore the formation mechanism of Janus particles, we carefully observed the growth process at different polymerization times using optical microscopy and fluorescence microscopy (Fig. 2 and fig. S3). After polymerization proceeded for 15 min, a particle that underwent Brownian motion was observed inside the droplet (Fig. 2, B and C; movie S1; 15 min). This is because the free radicals from water-insoluble 2,2′-azobisobutyronitrile (AIBN) were initially generated inside the droplet and subsequently initiated the polymerization of the hydrophobic St and DVB (Fig. 2A). The particle occasionally collided toward the interface of the droplet, although the precise mechanism is currently unclear. After the particle arrived at the interface of the droplet, the hydrophilic anchoring monomers could interact with the particle and then be initiated to polymerize, rendering the particle anchored at the interface of the droplet and triggering interfacial anchoring polymerization (Fig. 2, B and C; 30 min). Finally, the particle was trapped at the interface of the droplet because of the anchoring effect. Therefore, the subsequent polymerization occurred primarily along the interface of the droplets, resulting in the interfacial preferential growth of the particles (Fig. 2, B and C; 45 and 65 min). The preferential growth of the particle at the interface is based on the equal chemical potential between hydrophobic phases and hydrophilic phases at equilibrium. For the system comprising two immiscible monomer phases at equilibrium, the chemical potential of the hydrophobic phase was equal to that of the chemical potential of hydrophilic phase (23–26). The particle grew at the interface as a result of the increased chemical potential. Until the next new equilibrium appeared, the difference in the chemical potential between hydrophobic phase and hydrophilic phase was again equal to zero. Therefore, to maintain the chemical potential being equal to each other, the polymerization of the monomers at the interface of the droplets was inevitably favored over polymerization at other regions. In this case, the most stable state for the preferential growth of the particles at

Fig. 1. Synthesis and characterization of the Janus particles. (A) High-yield synthesis of typical crescent moon–shaped PSDVB ⊃ PAA Janus particles. The resulting crescent moon–shaped Janus particles exhibited a uniform size. Inset: The opening degree describes the topology features of the Janus particle. The opening degree can be defined as a central angle of sector that corresponds to the concave region of the Janus particle. Scale bar, 10 μm. The EDX spectroscopy analysis indicated different O element distributions on the convex and concave surfaces of the Janus particle. The O element was only observed in PAA, indicating that the convex surface of the particle was hydrophilic PAA, whereas the concave surface was hydrophobic PSDVB. (B) STEM image of embedded Janus particles after microtome cutting and staining with phosphotungstic acid. Scale bar, 500 nm. (C) Scanning electron microscopy (SEM) images of topologically anisotropic Janus particles. The topological geometries of the Janus particles could be well tuned to sequentially achieve bread, hemisphere, crescent moon, and pistachio topologies by regulating the concentrations of the anchoring monomer. Scale bar, 2 μm. (D) Ternary phase-like diagram of AA, St, and DVB for the production of the topologically anisotropic Janus particles. Janus particles were obtained using molar ratios of $n_{\text{AA}}/n_{\text{total}} < 0.625$, $n_{\text{DVB}}/n_{\text{total}} < 0.625$, and $n_{\text{AA}}/n_{\text{total}} < 0.75$. 

the interface may be growth in two directions rather than uniform growth around the interface. Therefore, further preferential growth along the two directions of the interface led to the formation of Janus particles instead of spherical particles.

To better understand this dynamic emulsion interfacial polymerization, we constructed a dissipative dynamics simulation model combined with a stochastic reaction model (see the Supplementary Materials for more details) \(^ 27-35 \). As shown in Fig. 2D, starting from the particle (colored deep yellow), the interfacial anchoring polymerization occurred between the hydrophobic monomers inside microdroplets and external hydrophilic anchoring monomers (figs. S4 and S5). The simulation consistently indicated the preferential growth of the particle along the two directions of the interface (Fig. 2D), in agreement with the experiments. Our simulation also demonstrated that the
The topology of the Janus particle was strongly dependent on the concentration of the hydrophilic anchoring monomers and hydrophobic monomers. As expected, with an increasing concentration of hydrophilic anchoring monomers, hemispherical-, crescent moon-, and pistachio-shaped Janus particles were observed in sequential order (fig. S6), similar to the experimental results. At low concentrations, a small amount of hydrophilic anchoring monomers could not continuously afford polymerization along the interface; thus, bread- or hemispherical-shaped Janus particles were obtained. At high concentrations, sufficient anchoring monomers were continually supplied to polymerize along the interface, enabling the formation of crescent moon-shaped, even pistachio-shaped, Janus particles. Furthermore, the topologies of the particles changed from hemispherical-shaped to crescent moon-shaped and finally to spherical particles with increasing the number of hydrophobic monomers. The polymerization of hydrophobic monomers forms the inner layer of the particle. When the number of hydrophobic monomers is low, the formation of the inner layer along the interface is difficult, thus facilitating the formation of Janus particles. By contrast, when the number of hydrophobic monomers is sufficient, the inner layer can grow synchronously with the interfacial layer, finally forming spherical particles (fig. S7).

The generality of emulsion interfacial polymerization for the synthesis of Janus particles
The emulsion interfacial polymerization strategy was further expanded to the polymerization of other vinyl monomers. We used different...
anchoring monomers, including positively charged, neutrally charged, and negatively charged monomers, to surround the St/DVB-based droplets. A large variety of Janus particles with crescent moon structures were produced, indicating the generality of this strategy (Fig. 3 and figs. S8 to S15). This approach yielded well-defined Janus particles of poly(styrene-co-divinylbenzene) (PSDVB) ⊃ poly(acrylamide) (PAM), poly(styrene-co-divinylbenzene) ⊃ poly(N-isopropylacrylamide) (PSDVB ⊃ PNIPAM), poly(styrene-co-divinylbenzene) ⊃ poly(methacrylamide) (PSDVB ⊃ PMAM) (Fig. 3A), poly(styrene-co-divinylbenzene) ⊃ poly(hydroxyethyl acrylate) (PSDVB ⊃ PHEA), poly(styrene-co-divinylbenzene) ⊃ poly(maleic anhydride) (PSDVB ⊃ PMAH), poly(styrene-co-divinylbenzene) ⊃ poly(hydroxyethyl methacrylate) (PSDVB ⊃ PHEMA)
Janus particles could be modified with Beyond the inherent amphiphilicity of the Janus particles, these well-
the hydrophilic and hydrophobic phases should follow the same polym-
form a stable oil-in-water emulsion interface, (ii) the monomer pairs in
sion systems, a possible principle fort h i se m u l s i o ni n t e r f a c i a lp o l y m e-
PSDVB ⊃ fluorescent fluorescein isothiocyanate (FITC) (Fig.4A). For example,
n o p a r t i c l e sb yc o v a l e to rn o n c o v a l eb o n d i n g( F i g .4 A ) .F o re x a m p l e ,
acids (PSDVB ⊃ PAA, poly(styrene-co-divinylbenzene) ⊃ poly(maleic
m (Fig. S16) and Fe3O4 nanoparticles (fig. S17), onto
convex surface via charge interactions, resulting in multifunctional
Janus particles. On the other hand, the concave surface of the Janus par-
could also be effectively used. For example, Janus particles were
spheric polystyrene (PS) particles to realize shape
complementarity. When the Janus particles with concave diameters of 1.19 ± 0.15 μm were respectively mixed with spherical PS particles with diameters of 0.43 ± 0.04 μm, 1.08 ± 0.05 μm, and 1.42 ± 0.06 μm, many PS particles with diameters less than the concave diameters of Janus particles were trapped in their concaves because of hydrophobic interactions, except for the 1.42-μm-diameter particles. However, when the Janus particles were simultaneously mixed with all three PS particles, the PS particles with diameters of 1.08 ± 0.05 μm were more favorable for capture under current conditions (Fig. 4B and fig. S18, A to D).
In addition, concave surfaces of Janus particles functionalized with aniline were used to selectively recognize live bacteria (Staphylococcus aureus; size, approximately 600 nm), whereas unmodified particles were difficult for this recognition (Fig. 4B and fig. S18, E and F). Functionalizing the concave surfaces of the Janus particles made the concave surfaces positively charged; consequently, they could attract bacteria with negative charges. Moreover, the negatively charged convex surfaces of the Janus particles could repel bacteria. The unique surface charge properties of the Janus particles and their concave sizes enabled them to effectively recognize live bacteria. Therefore, selectively modifying the convex/ concave surfaces of Janus particles may endow Janus particles with more functionality (36, 37).

DISCUSSION
Janus particles with two distinct physical or chemical properties in their surfaces, like the two-faced Roman god Janus, are of importance in funda-
mental research and practical applications, such as self-assembly
superstructures (38, 39), bioimaging and therapeutics (40), solid surfac-
tants (41), panel displays (42), micromotors (43, 44), and optical probes (45). Achieving these applications is largely due to the anisotropic topology and surface chemistry of Janus particles. However, existing
strategies, such as the particle self-assembly technique combined with shading modification (46), microfluidics (41), electrodynamic co-jetting (47), block copolymer self-assembly (48), and swelling emulsion polymerization (23), are still difficult for large-scale synthesis of uniform Janus particles with tunable topology and surface chemistry (49).

Our general emulsion interfacial polymerization method can eff-
effectively synthesize chemically and topologically anisotropic Janus par-
ticles in large scale. This method is in stark contrast to traditional emulsion polymerization methods that produce spherical particles or “snowman-shaped” or “dumbbell-shaped” particles. The emulsion inter-
facial polymerization system has several distinct advantages over traditional emulsion polymerization. (i) Emulsion interfacial polymer-
ization overcomes the limitations of traditional emulsion polymer-
ization. When additional anchoring molecules are introduced outside the microdroplets, polymerization starting from one specific point of the interface can break the uniform growth of particles at the entire interface of the droplets, resulting in the formation of chemi-
cally and topologically anisotropic Janus particles. (ii) Emulsion in-
terfacial polymerization successfully overcomes the current challenges of achieving tunability of the chemical and topological anisotropy of synthetic particles. A wide range of Janus particles (bread, hemisphere, crescent moon, and pistachio) with chemical and topological anisotropy can be readily designed and realized through control of monomer type and concentration as well as polymerization time. (iii) Emulsion interfac-
ial polymerization is general. The emulsion interfacial polymerization strategy can be used for polymerizing various vinyl monomers, includ-
ing positively charged, neutrally charged, and negatively charged ones, greatly enriching the community of Janus particles. With respect to potential applications, we have demonstrated that these Janus particles exhibited remarkable abilities to separate bacteria by using their topological structures and surface chemistry. We strongly believe that our novel method will significantly expand the utility of Janus particles, creating new opportunities in a wide variety of applications, ranging from environment to health, especially in oil-water separation and biological detection.

MATERIALS AND METHODS

Synthesis and characterization of Janus particles
In a typical synthesis, 10 ml of a 1% (v/v) 1-chlorodecane (CD) oil-in-
water emulsion containing 0.25% (w/v) SDS was mixed with 20 ml of an aqueous solution [containing 0.25% (w/v) SDS] of 1% (w/v) non–cross-
linked PS particles at 40°C. The diameter of the PS particles used during polymerization was 1.08 ± 0.05 μm. After 16 hours of magnetic stirring, a 10-ml oil-in-water emulsion [containing 0.25% (w/v) SDS], composed of 13 mmol St, 7 mmol DVB, 0.24 mmol AIBN, and 7 mmol hydrophil-
ic anchoring monomer AA, was prepared by ultrasonic emulsification, and it was subsequently added to the aforementioned solution at 40°C for 6 hours. After 5 ml of 1% (w/v) polyvinyl alcohol was added to the mixture followed by deoxygenation by bubbling with N2 for 15 min at room temperature, the polymerization was performed at 70°C for 14 hours. All polymerizations were carried out in deionized water without using a co-solvent.

Characterization
Janus particles were prepared for SEM (7500, JEOL) by dropping 20 μl of particle aqueous solution onto a silicon wafer (1 cm × 1 cm) at room temperature. After water evaporation, the samples were coated with platinum using a vacuum evaporator for SEM images. The growth

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The synthesis of charged inorganic nanocrystals

**Synthesis of positively charged Ag nanoparticles**

Nearly monodisperse Ag nanoparticles were synthesized in a one-step method (50). In a typical synthesis, 300 mg of AgNO₃ was added to a mixture of 20 ml of liquid paraffin and 6 ml of oleylamine. After deoxygenation by bubbling with N₂ for 20 min and then heated to 160°C for 10 hours. The color of the mixture solution changed from bright orange to brown to black with reaction time. The resulting nanoparticles were washed three times with ethanol. Finally, the resultant Ag nanoparticles were redispersed in ethanol to interact with the anisotropic particles.

**Synthesis of positively charged Fe₃O₄ nanoparticles**

Magnetic Fe₃O₄ nanoparticles were synthesized according to a previously described method (51). In a typical synthesis, 3 mmol Fe(acac)₃ was dissolved in 24 ml of benzyl ether and 6 ml of oleylamine. After deoxygenation by bubbling with N₂ for 20 min, the solution was heated for 1 hour at 110°C for dehydration and then heated to 250°C for 1 hour. After the reaction, the solution was cooled to room temperature. The resultant Fe₃O₄ nanoparticles were washed three times with ethanol. Finally, the resultant Fe₃O₄ nanoparticles were redispersed in ethanol to interact with the anisotropic particles. The inorganic nanoparticles were characterized by a TEM (JEM-2010).

**Selective functionalization of Janus particles**

**FITC-BSA covalent-modified PSDVB ⊃ PAA particles**

Four hundred milligrams of crescent moon-shaped PSDVB ⊃ PAA Janus particles were dispersed in 50 ml of deionized water. Next, 0.2 g of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and 0.4 g of N-hydroxysuccinimide were added to the PSDVB ⊃ PAA Janus particle suspension at 35°C for 10 hours to activate the carboxyl groups of PAA. Finally, 10 mg of activated PSDVB ⊃ PAA nanoparticles was added to 10 ml of a 0.25% (w/v) FITC-BSA aqueous solution at 35°C for 10 hours under magnetic stirring. The resulting particles were centrifuged at 5000 rpm for 5 min and washed three times with deionized water.

**Charge interaction between Ag nanoparticles and PSDVB ⊃ PAA Janus particles**

A positively charged Ag nanoparticle suspension [0.5 ml of 0.5% (w/v)] was added to 0.5 ml of the 1% (w/v) PSDVB ⊃ PAA particle suspension under gentle stirring for 10 hours. The resultant hybrid particles were purified by using a centrifugation-dispersion process. Twenty microliters of ethanol was added to the aforementioned solution, and the resultant solution was centrifuged at 5000 rpm for 5 min. This process was repeated three times to completely remove the unlocked nanoparticles.

**Charge interaction between Fe₃O₄ nanoparticles and PSDVB ⊃ PAA Janus particles**

A positively charged Fe₃O₄ nanoparticle suspension [0.5 ml of 0.5% (w/v)] was added to 1 ml of the 1% (w/v) PSDVB ⊃ PAA particle suspension under gentle stirring for 10 hours. The resulting hybrid particles were purified by using a centrifugation-dispersion process. Twenty microliters of ethanol was added to the aforementioned particle solution, and the resultant solution was centrifuged at 5000 rpm for 5 min.

**Janus particle capture of spherical PS particles**

To capture spherical PS particles with diameters of 0.43 ± 0.04 μm, 10 μl of a PSDVB ⊃ PAA Janus particle suspension (1%, w/v) with 8 μl of a spherical PS particle suspension (0.023%, w/v) was dispersed in 20 μl of deionized water. The solution was then ultrasonicated for 5 min and subsequently used directly for SEM imaging.

To capture spherical PS particles with diameters of 1.08 ± 0.05 μm, 10 μl of a PSDVB ⊃ PAA Janus particle suspension (1%, w/v) with 8 μl of a spherical PS particle suspension (0.364%, w/v) was dispersed in 20 μl of deionized water. Then, the solution was ultrasonicated for 5 min and subsequently used directly for SEM imaging.

To capture spherical PS particles with diameters of 1.42 ± 0.06 μm, 10 μl of a PSDVB ⊃ PAA Janus particle suspension (1%, w/v) with 8 μl of a spherical PS particle suspension (0.43 ± 0.04–μm PS particles (0.023%, w/v), 8 μl of 1.08 ± 0.05–μm PS particles (0.364%, w/v), and 8 μl of 1.42 ± 0.06-μm PS particles (1%, w/v) was dispersed in 20 μl of deionized water. The solution was then ultrasonicated for 5 min and subsequently used directly for SEM imaging.

**Janus particle recognition of S. aureus**

**Bacterial strains and growth conditions**

*S. aureus* were initially grown in sterile LB broth overnight, shaken at 150 rpm at 37°C, and harvested during logarithmic growth.

**Aniline modification of Janus particles**

Twenty milligrams of PSDVB ⊃ PAA particles was dispersed in 1 ml of aniline and shaken overnight at room temperature. The suspension was washed twice with deionized water and finally redispersed in 1 ml of water.

**Recognition of S. aureus by aniline-modified Janus particles**

Two milliliters of *S. aureus* in culture (ca. 1 × 10¹⁰ cells/ml) were mixed with 1 ml of aniline-modified PSDVB ⊃ PAA particles (20 mg/ml) under magnetic stirring for 24 hours at room temperature. In addition, the samples were rinsed with deionized water and filtered to remove salts and planktonic bacteria. The samples were then fixed in a 2.5% glutaraldehyde aqueous solution overnight at 4°C, after which they were rinsed with deionized water. Finally, the samples were dehydrated in a series of ethanol aqueous solutions (30, 50, 70, 85, and 95%), followed by two rinses in 100% ethanol. A control experiment with unmodified Janus particles was also carried out using the same procedures.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/content/full/3/6/e1603203/DC1

fig. S1. Anchoring monomer-mediated interfacial polymerization.

fig. S2. SEM image of topological particles and composite film.

fig. S3. Time-dependent morphology and size evolution of typical crescent moon-shaped PSDVB ⊃ PAA particles.

fig. S4. Schematic illustration of the initial configuration of the simulations.

fig. S5. Illustration of the reaction process controlled by the reaction probability and reaction radius in the copolymerization.

fig. S6. The influence of the concentration of AA on the morphology of Janus particle.

fig. S7. The influence of the number of hydrophobic monomer beads on the morphology of particle.


fig. S15. Characterization of crescent moon-shaped PSDVB ⊃ PMAM particles.

fig. S16. Ag nanoparticle characterization.
REFERENCES AND NOTES


movie S1. Polymerization of 15 min.

table S1. Dissipative particle dynamics simulation interaction parameters between different types of beads.

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