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## Depletion-interaction-driven assembly of golf ball-like particles for development of colloidal macromolecules

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### Abstract

Colloidal molecules created by clustering monodisperse particles are a significant model system of atoms and molecules in colloidal scale. To attain modelling of complex or high-molecular-weight molecules such as hyper-branched polymers and proteins, it is required to develop a new assembling system which can prepare three-dimensionally designed colloidal molecules. In this study, we proposed an approach for complex colloidal molecules created by association between golf ball-like particles and spherical particles via depletion interaction. Several golf ball-like particles joined together, which formed joined particle assemblies at the spherical particles acting as their joints in a depletant solution. The number of golf ball-like particles in a particle assembly was increased by increasing the concentration of the depletant, leading to the formation of branched colloidal chains of the golf ball-like particles. Because the golf ball-like particles in the colloidal chain flexibly rotated at their joints, the conformation of the chains varied in the depletant solution. The present results demonstrated that the golf-ball like particle that can be joined to form colloidal macromolecules with high association number and high flexibility will be a promising building block for complex colloidal model systems.

**Keywords :** Depletion interaction; Golf ball-like particles; Lock and key; Particle assembly

**Abbreviations:** GB particles, golf ball-like particles; MPTMS, 3-Methacryloxypropyltrimethoxysilane; NH<sub>3</sub>, ammonia aqueous solution; MMA, methylmethacrylate; PMMA, poly(methylmethacrylate); NaOH, aqueous sodium hydroxide solution; VA-057, 2,2'-azobis[*N*-(2-carboxyethyl)-2-methylpropionamide]; HCl, aqueous hydrochloric acid; KPS, potassium persulfate; PEG, poly(ethylene glycol) methyl ether; PSt, polystyrene; SiO<sub>2</sub>, silica;  $r_s$ , radius of spherical particles;  $r_d$ , radius of depletants;  $\Delta V$ , overlapping volume of depletion layers

### 1. Introduction

Assemblies of monodisperse particles used as building blocks play an important role of colloidal model systems resembling atomic or molecular systems. In 2000s, assemblies of oppositely charged monodisperse particles were reported to reproduce crystalline structures composed of atoms and molecules in colloidal scale [1–3], leading to microscopic visualization of condensed matter physics including phase transitions and nucleation [4–6]. Clustered particles having geometric shapes similar to molecules, which are known as “colloidal molecules,” were diversely created by clustering monodisperse particles with different sizes, clustered angles and number ratios of element particles [7–9]. The clusters of monodisperse particles was attained by various approaches, such as formation of chemical bounds between surface-modified particles [10,11], adsorption of particles in oil-in-water emulsion systems [12,13], and phase separation of immiscible materials in a particle [14,15]. Janus particles which feature two different surface moieties in a single colloid are well employed for preparation of colloidal molecules [16–18]. Template-assisted self-assembly of colloids is one of the useful pathways to precisely fabricate the molecules on a substrate having periodic patterns [19,20].

Depletion interaction between different particles can also be a feasible approach to diverse shapes of the colloidal molecules because neither surface-modification of particles nor application of external fields to elemental particles is required for the creation of colloidal molecules. The depletion interaction acting upon particles suspended in a solution of macromolecules that were not adsorbed on the

particles was proposed by Asakura and Oosawa [21,22]. Such macromolecules to induce the depletion interaction are called depletants. The particles in the solution of depletants are surrounded by a depletion layer within which the center of depletants is not accessible. Overlapping of the depletion layers induces attractive interparticle forces due to repelling medium between the particles by osmotic pressure. The interparticle depletion force ( $F_{dep}$ ) can be expressed with the following Eq. (1) [23].

$$F_{dep}(r) = -n_b kT \times \Delta V_{OV}(r)$$

Where  $r$ : the distance between centers of the particles,  $n_b$ : bulk number density of depletants,  $k$ : Boltzmann's constant,  $T$ : temperature, and  $\Delta V_{OV}(r)$ : the overlapping volume of the depletion layer.

Sacanna et al. reported that the depletion interaction is a practical approach to assemble differently shaped particles that are various combinations of dented colloids (lock particles) and spherical particles (key particles). A variety of colloidal molecules were created by mixing key particles with lock particles having different numbers of dents at different number ratios of locks to key particles [24,25]. Since the particles are not completely fixed together, the lock particles exhibited flexible motions at their joints in a depletant solution.

Assembly of particles in a depletant solution is reminiscent of increasing molecular weight of polymers (i.e. colloidal polymerization). Patchy particles with a specific surface being able to bind to other particle surfaces are one of promising building blocks to create colloidal macromolecules via polymerization of colloidal molecules [26–29].

Application of an external field has also been employed for colloidal polymerization [30–32]. For instance, dumbbell-like composite particles responsive to external fields could behave as monomer molecules in a colloidal chain. Two-dimensionally extended or shortened chain structures were controlled by switching application of electric and/or magnetic fields, exhibiting reversible variations in dumbbell conformation in the colloidal chain [33]. These colloidal behaviors suggest that assemblies of nonspherical particles can be a model of elastic polymer chains showing different conformation depending on circumstance factors (e.g. temperature and pH values).

In the present work, a new assembling system to provide three-dimensional colloidal polymerization via depletion interaction is proposed for a nonspherical colloid having multiple dents (i.e. golf ball-like particles), which are previously reported by our group [34]. The golf ball-like particles, hereafter referred to as GB particles, were prepared by a combined method of heterocoagulation between soft silicone oil droplets and hard spherical template particles, polymerization of the oil droplets, and removal of the template particles. The multiple dents formed on a single particle by the template method suggests 3D network formation by joining the GB particles (lock particles) and spherical particles (key particles) in the presence of depletants. The GB particles that can be joined to form 3D colloidal molecules with high association number and high flexibility can be promising model colloids for hyper-branched molecules and proteins.

## 2. Materials and methods

### 2.1 Materials

3-Methacryloxypropyltrimethoxysilane (MPTMS, 95%) was obtained from JNC Corporation (Tokyo, Japan). Methylmethacrylate (MMA, 98%), ammonia aqueous solution ( $\text{NH}_3$ , 25 wt%), aqueous sodium hydroxide solution (NaOH, 0.1 M), aqueous hydrochloric acid (HCl, 0.1 M), potassium persulfate (KPS, 95%), and 2,2'-azobis[*N*-(2-carboxyethyl)-2-methylpropionamide] (VA-057, 95%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Poly(ethylene glycol) methyl ether (PEG, number average molecular weight: 5000) was purchased from Sigma-Aldrich Japan (Tokyo, Japan). The inhibitor of MMA was removed by an inhibitor removal column, and water was deionized in advance ( $>18.2 \text{ M}\Omega \text{ cm}$ ).

### 2.2 Synthesis of GB particles

The GB particles were prepared by a heterocoagulation between negatively charged oil droplets and PMMA particles [34]. Oil-in-water emulsions were prepared by hydrolyzing MPTMS in an aqueous ammonia solution [35–37]. After stirring for 4 min at 35 °C, the mixed aqueous solution was kept for 24 h without stirring. The concentration of MPTMS and  $\text{NH}_3$  were 2 vol% and 200 mM, respectively.

The PMMA particles used as templates for preparing GB particles were prepared by soap-free emulsion polymerization with an amphoteric initiator, VA-057 [38]. In the polymerization, the monomer of MMA was added into an aqueous NaOH solution which was bubbled with nitrogen for 30 min. After stirring for 30 min at 70 °C, an aqueous solution of VA-057 was added to the solution as an initiator. The polymerization of MMA was conducted at  $[\text{MMA}] = 350 \text{ mM}$ ,  $[\text{NaOH}] = 2.5 \text{ mM}$  and  $[\text{VA-057}] = 10 \text{ mM}$ .

To prepare the GB particles, PMMA particles were mixed with the suspension of MPTMS droplets at the HCl concentration of 40

mM under stirring at 35 °C. PMMA particles negatively charged in water were mixed with the oil droplets of MPTMS in the aqueous suspension of HCl to lower the surface charge density of PMMA particles for heterocoagulation with the oil droplets. After 15 min stirring, the suspension was heated to 70 °C and then an aqueous solution of KPS was added as an initiator to polymerize the oil droplets having a polymerizable functional group in MPTMS molecules. The preparation of GB particles was conducted at [KPS] = 2 mM and an oil droplet concentration of 0.4 vol%. The number ratio of GB particles and PMMA particles was 1:120.

### 2.3 Creation of particle assemblies in a depletant solution

The GB particles and spherical particles were mixed in PEG solution and left for 30 min without stirring in the solution. The number ratio of GB particles and spherical particles was 1:20, and the total volume of particles was 0.05 vol%. The formed particle assemblies in the solution were observed with an optical microscope (Nikon, C2Si) under 150× magnification.

### 2.4 Characterization

The synthesized particles were observed with FE-TEM (Hitachi, HD-2700). Zeta potentials of the particles were measured with an electrophoretic light scattering (ELS) photometer (Otsuka Electronics Co., ELSZ-2).

## 3. Results and discussion

### 3.1 Particle characterization

Fig. 1(a)–(c) present SEM and TEM images of the GB particles prepared by our method [34], showing 300 nm-sized dimples uniformly distributed on the particle surface. The average sizes of particles and the coefficient of variation of the sizes in the TEM image (b) were 968 nm and 13%, respectively. The GB particles exhibited a negative zeta potential (−59 mV) because of the anionic initiator KPS used in the synthesis of GB particles. The low concentration of MPTMS polymerized in the synthesis of GB particles is expected to be a high surface charge density due to a high amount of anionic radicals incorporated into a single GB particle.

Fig. 1(d) shows TEM image of PMMA particles used as a template for imprinting the dimples on soft oil droplets which were polymerized after the imprinting. Although the particles in the TEM image were non-spherical and deformed, the deformation was caused by the electron beam in TEM observation [39]. The PMMA particles were monodisperse with an average diameter of 330 nm and a coefficient of variation of 2.4%. The diameter and depth of the GB particles estimated in TEM image were approximately 300 nm and 97 nm, respectively (see Fig. S1).

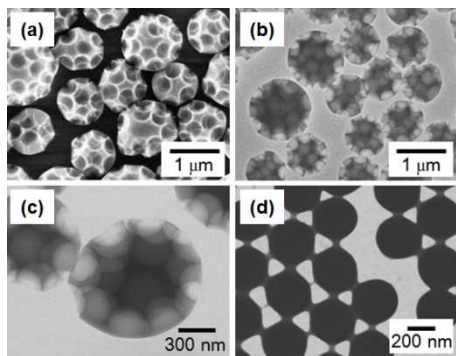


Fig. 1 SEM and TEM images of the golf ball-like particles (a–c) and TEM image of 330-nm PMMA particles (d).

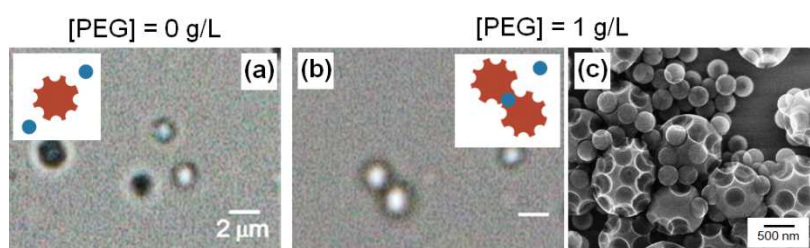
### 3.2 GB particle assemblies in a depletant solution

Colloidal stabilities in the coexistence of GB particles and PMMA particles were examined in water with an optical microscope. Fig. 2(a) is a typical snapshot of Movie 1 showing the GB particles suspended in water. Most GB particles, which are illustrated in the inset of Fig. 2(a), were independently dispersed in water as shown in Movie 1. The submicron-sized PMMA particles were, however, not clearly captured in the snapshot due to their strong Brownian motion, which were supposedly illustrated with blue dots in the same inset. Due to the negative zeta potentials of −59 mV for GB particles and −23 mV for PMMA particles, the two negatively charged particles could be scarcely heterocoagulated in the depletant solution.

Fig. 2(b) shows a typical snapshot of Movie 2 for the coexistence of the particles at PEG concentration of 1 g/L. As shown in Fig. 2(b) and Movie 2, some nonspherical particles which were larger than a single GB particle were observed, and they exhibited Brownian

motion differently from a single GB particle. The results suggested that some GB particles were joined to other particles in the depletant solution. Another interesting feature is each part of the joined particles was freely rotated at its joint. Fig. 2(c) shows SEM image of the GB particles whose samples were prepared by drying the depletant solution in the coexistence of PMMA particles. In the SEM image, some of the GB particles incorporated PMMA particles into their dimples, and were very closely located at the joint PMMA particles. The combined observations with an optical microscope and SEM can experimentally support that GB particles were associated with each other with the assistance of PMMA particles in the depletant solution.

The specific association of GB particles in Fig. 2(b) was compared with that in the depletant solution without PMMA particles. As shown in Movie 3, no association of GB particles was observed in the absence of PMMA particles, meaning that the added PEG molecules were not effective as flocculants for the GB particles. The specificity in association was checked using negatively charged, spherical polystyrene (PSt) particles (without any dimples) with an average size almost the same as the GB particles. No association between the PSt particles was observed in the presence of PMMA particles in the depletant solution (see Fig. S2). These comparative experiments strongly supported that overlapping volumes of the deplete region surrounding the particles could be a dominant factor for the association behavior in the coexistence of differently shaped particles electrostatically stabilized in the depletant solution.

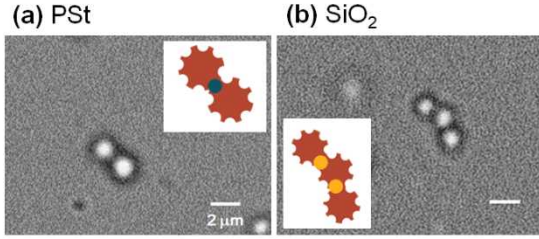


**Fig. 2** Optical microscope images of particle suspensions of golf ball-like particles and spherical particles observed in water (a) and in PEG solution. (b). SEM image of the particle assemblies obtained in PEG solution (c) ([PEG] = 1 g/L). In the insets of (a) and (b), spherical PMMA particles, which are not shown in the microscope image, are illustrated with blue dots.

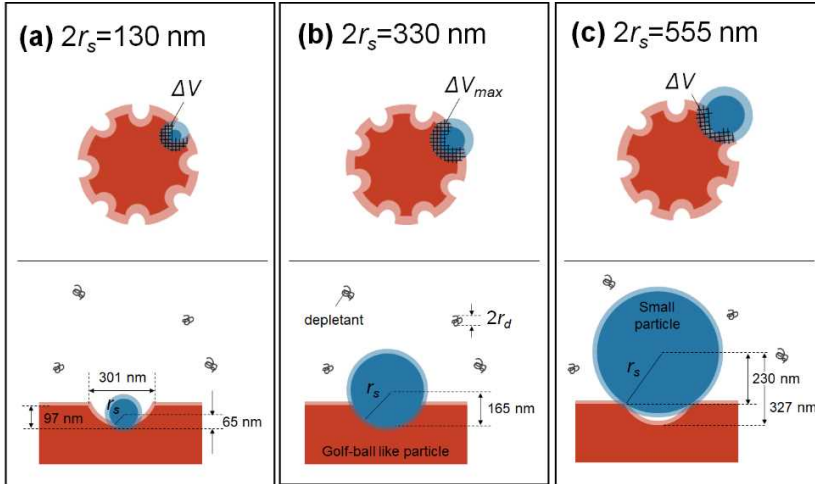
### 3.3 Formation of particle assemblies with different key particles

The strong dependence on particle shapes determining overlapping volumes between particles could expect weak dependence of particle association on surface chemical compositions of particles. Fig. 3 indicates optical microscope images of GB particles associated in the coexistence of PSt or silica ( $\text{SiO}_2$ ) particles with average sizes similar to PMMA particles shown in Fig. 2(d). As illustrated in the insets of Fig. 3(a) and (b), the existence of spherical particles in the depletant solution facilitated the association of GB particles although PSt and  $\text{SiO}_2$  had zeta potentials higher than PMMA particles (PSt:  $-39$  mV and  $\text{SiO}_2$ :  $-49$  mV). These results experimentally support the strong dependence on particle shapes instead of chemical compositions of the particle surfaces in the depletion interaction acting upon particles electrostatically stabilized.

The association of GB particles in the depletant solution should be governed by the size of joint particles [40,41]. The roles of joint particles were examined by experiments in which GB particles were observed in the presence of particles smaller or larger than the PMMA particles in Figs. S3(a) and (b). As seen in Movie 4 for 130-nm PSt particles and Movie 5 for 555-nm PSt particles, GB particles in the presence of the smaller or larger PSt particles were dispersed in the depletant solution without any association. The difference in overlapping volumes between GB particles and the PSt particles is schematically depicted in Fig. 4 where the overlapping layers are indicated with hatching in upper column. As shown in the cross sectional images indicated lower columns in Fig. 4, the overlapping volume  $\Delta V_{OV}$  can be maximized by using 330-nm joint particles geometrically well matched with dimples on GB particles. The depletant diameter ( $2r_d$ ) estimated according to the previous report [42] was 3 nm in water. Although the percentage of GB particles incorporating PSt particles into their dimples could not be null, the percentage must have been too low to form the assembly of GB particles with high association numbers in the depletant solution.



**Fig. 3** Optical microscope images of the particle assemblies observed in PEG solution (1 g/L) in the presence of 326-nm PSt particles (a) and 329-nm SiO<sub>2</sub> particles (b). In the insets, PSt and SiO<sub>2</sub> particles are illustrated with green and yellow dots, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4** Schematic diagrams of the overlapping excluded volume ( $\Delta V$ ) between a golf ball-like particle and a small particle with diameter ( $2r_s$ ): 130 nm (a), 330 nm (b) and 555 nm (c).

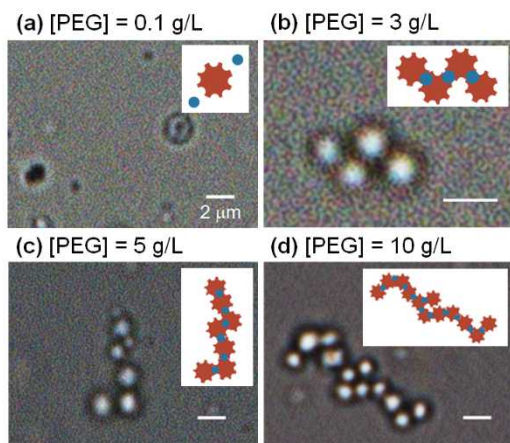
### 3.4 Formation of particle assemblies with different concentration of PEG

The effect of depletant concentration on the association behavior of GB particles was examined in concentration range of 0.1–10 g/L. Fig. 5 shows snapshots of GB particles observed in the coexistence of 330-nm PMMA particles in a different PEG concentration range 3–10 g/L. The chain-like structures consisting of GB particles were observed rather than cluster structures in the solution. The GB particles and PMMA particles can be assembled slowly via depletion interaction. As shown in Fig. S4, the second GB particle should be joined to the opposite site of the first joint between a GB particle and a PMMA particle due to the steric hindrance of GB particles, resulting in the formation of the chain-like structures rather than clusters of the particles. In contrast, the disordered clusters of GB particles were observed when the GB particles were rapidly aggregated in the presence of PMMA particles in electrolyte aqueous solutions (see Fig. S5).

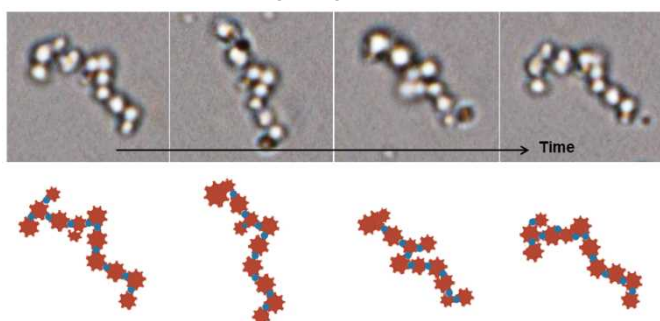
The number of GB particles forming particle assemblies was increased by the PEG concentration at which GB particles formed chain-like structures reminiscent of polymer chains. The increase in the number of GB particles in the chains can be explained by Eq. (1) in which interparticle depletion force is proportional to the number density of depletants in solution. The PEG molecules may be adsorbed on the particle surfaces both GB particles and PMMA particles in the solution. However, little random clustering of GB particles were observed at high PEG concentrations, indicating that the GB particles were predominantly assembled by the depletion interaction.

Fig. 6 presents snapshots of GB particles in the branched chain for approximately 28 s in Movie 6. The conformation was varied at the depletant concentration of 10 g/L where the chain was branched. According to the movie, some conformation of GB particles in Fig. 6 were apparently caused by the rotation of each particle at the joints. This result newly proposes multipoint lock-and-key system to realize the colloidal polymerization for 3D network of particles. The results demonstrated that the depletion-interaction-driven assembling system can be employed to create flexible colloidal chains without any application of external fields such as electric and/or magnetic field [30–33].

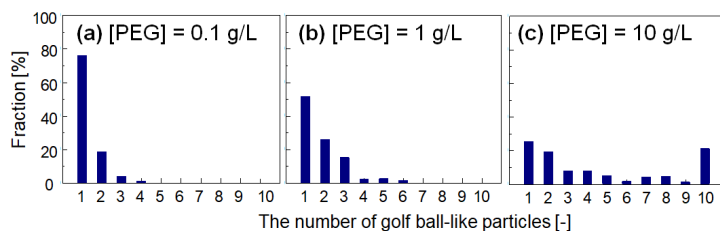
The numbers of GB particles in an assembly were manually counted for 200 GB particles or more in snapshots of the optical microscope images. Fig. 7 shows the number fractions of GB particles assembled in a corresponding assembly from monomer to decamer. Assemblies larger than decamer is included in the column of decamer in Fig. 7. At the depletant concentration of 0.1 g/L or lower, approximately 80% of GB particles were well dispersed and belonged to the state of a single particle. An increase in the depletant concentration to 1 g/L raised the number fractions of dimers and trimers. The fraction numbers in details are summarized in Table S1. Further increase to 10 g/L lowered the number fraction of monomer to 30% or lower. The number fraction of decamer and larger was dramatically increased by the increase to 10 g/L.



**Fig. 5** Optical microscope images of particle assemblies observed in PEG solution at different concentration of PEG: 0.1 g/L (a), 3 g/L (b), 5 g/L (c) and 10 g/L (d). In the insets, spherical PMMA particles, which are not shown in the microscope image, are illustrated with blue dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



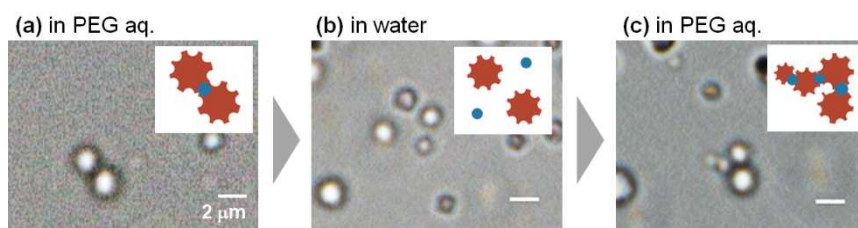
**Fig. 6** Snapshots of [Movie 6](#) observed with an optical microscope and schematic diagrams of particle assemblies observed in PEG (10 g/L) solution.



**Fig. 7** Fraction of the number of the golf ball-like particles in a corresponding observed in PEG solution with different concentration of PEG: 0.1 g/L (a), 1 g/L (b) and 10 g/L (c).

### 3.5 Reversibility between disperse and assembled states of GB particles

The reversibility between dispersed and assembled states of GB particles was examined in an additional experiment where the assembly of GB particles (Fig. 8(a)) settled in a vessel was put into water without any depletants. As shown in Fig. 8(b), the particles collected by the settlement could be redispersed in water without any association of GB particles. Then the dispersed particles were associated again in the depletant solution of 1 g/L, suggesting that GB particles in the solution were entropically jointed at the joints of PMMA particles by the depletion interaction.



**Fig. 8** Optical microscope images of the particle assemblies observed in PEG solution (a), after solvent displacement to water (b) and after redispersing again to PEG solution (c) ([PEG] = 1 g/L). In the insets, spherical PMMA particles, which are not shown in the microscope image, are illustrated with blue dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

#### 4. Conclusion

In the present work, we demonstrated a new approach for depletion-interaction-driven colloidal assembling system to form multiple joints between spherical polymer particles and golf ball-like particles having dents on their surfaces in a depletant solution. The depletion interaction between the golf ball-like particles (lock) and the spherical particles (key), which were electrostatically stabilized, induced a slow assembly of the particles and facilitated the formation of colloidal chains whereas previous colloidal chains were generally formed by application of external fields [30–33]. The number of golf ball-like particles in a single chain was increased by increasing the concentration of the depletant. Branched structures of the colloidal chains were also formed by the depletion interaction at a high depletant concentration. The depletion interaction between lock and key particles was allowed to vary the conformation of the colloidal chains by rotating the lock particles at the key particles which acted as their joints. The introduction of golf ball-like particles into the depletion-interaction-driven assembling system of colloid which were previously reported [7,24,25,43] was found to increase the numbers of particles assembled by the depletion interaction and create colloidal macromolecules with high association numbers and flexibility.

The present work proposed a spherical shape for multiple recognition of counter-particles in the deplete solution. Other shapes such as cylinders and plates can also be used to assemble element particles as if a variety of stereo-selective antigen-antibody interaction act on biomolecules. Such different interactions to assemble colloids can diversify colloidal assemblies with different colloidal angles and flexibility in the assembly. Colloidal polymerization in which a large number of colloid are stereo-selectively assembled expects more complex colloids such as colloidal block copolymers and proteins.

#### Acknowledgements

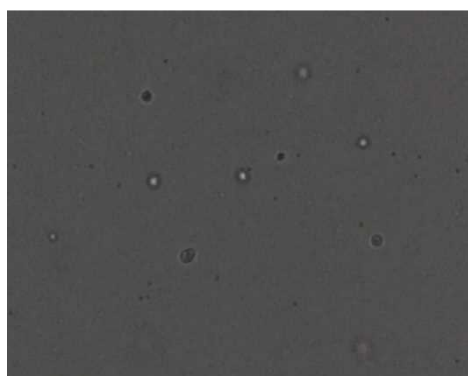
The authors thank A. Arakawa for the measurements of STEM images and technical support staff in the department of Engineering, Tohoku University. This research was mainly supported by the Ministry of Education, Culture, Sports, Science and Technology (JSPS KAKENHI Grant Numbers 16J03375, 15K0222, 17H02744, 17K19020).

#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jcis.2018.08.117>

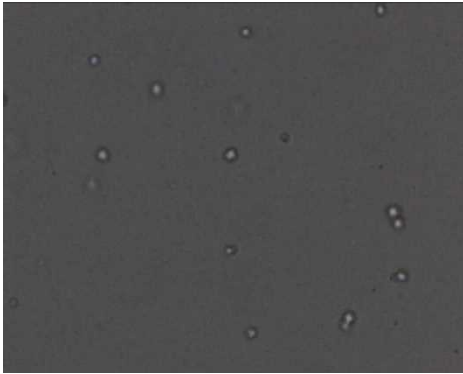
#### Extras

Supplementary video 1

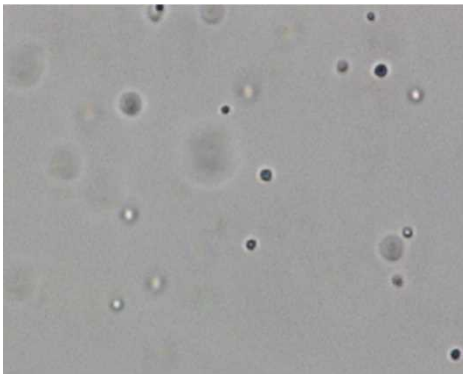




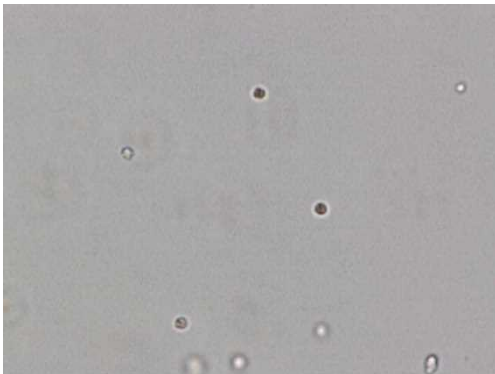
Supplementary video 2



Supplementary video 3



Supplementary video 4

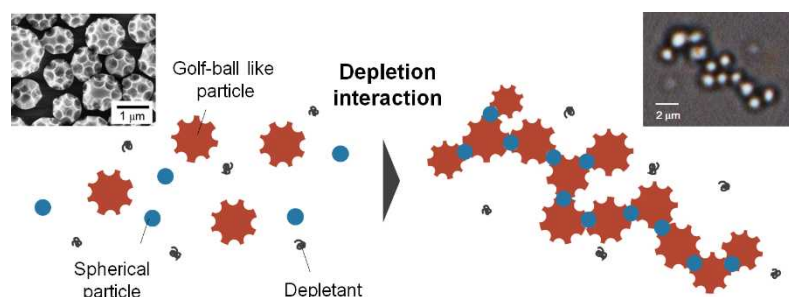


Supplementary video 5





### Graphical abstract



### Highlights

- Golf ball-like particles to form assemblies via depletion interaction.
- Formation of branched colloidal chains in the depletant solution.
- The particles moving flexibly at spherical particles acting as their joints.
- Various conformation of the colloidal chains observed in the solution.

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