Self-limiting self-assembly of supraparticles for potential biological applications†

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Nanotechnology has largely spurred the development of biological systems by taking advantage of the unique chemical, physical, optical, magnetic, and electrical properties of nanostructures. Self-limiting self-assembly of supraparticles produce new nanostructures and display great potential to create biomimicking nanostructures with desired functionalities. In this minireview, we summarize the recent developments and outstanding achievements of colloidal supraparticles, such as the driving forces for self-limiting self-assembly of supraparticles and properties of constructed supraparticles. Their application values in biological systems have also been illustrated.

Introduction

Terminal supraparticles (SPs) assembled with individual building blocks are widespread in living systems,1 and exemplified by lipid bilayer membranes,2 viruses,3 carboxysomes,4 exosomes,5 and endosomes.6 These natural nanoscale assemblies play significant roles to sustain the normal operation of biological systems, taking advantage of their specific morphology and components, such as signal transduction,7 biological catalysis,3,8 controllable synthesis,9 and cargo protection.10 These nanostructures exhibit specific morphologies with an assembly based on characteristic dimensions from the nano- to micro-scale.

With regard to the construction mechanism of natural organelles, the method of self-limiting self-assembly plays an important role in the construction of intricate and functional nanostructures.3,7,11 Unlike DNA base pairing,12,13 protein templates,14 and fiber15 driven nanostructures, SPs are constructed based on the balanced non-covalent interaction between repulsive and attractive forces including hydrogen bonding, electrostatic interaction, and hydrophobic/hydrophilic anisotropy16 between the ions, nanoparticles (NPs), solvents, and stabilizers in synthetic colloids.17,18 The versatility and cost-effectiveness properties of constructed SPs are important evaluation criteria when they are considered for future applications. Complexity and uniformity are also remarkable characteristics of terminal SPs,17,19,20 effecting their biological properties and units.21 They are also a potent tool for addressing the needs of replicating biological functions of nanoassemblies composed of robust inorganic components with special physical, chemical, optical, and magnetic properties (Scheme 1).

Moreover, inspired by the superiority of the “bottom-up” assembly method existing mainly in living systems, self-limiting self-assembly has overcome the limitations of biological systems and become more and more popular in recent years to create artificial nanoassemblies.22–30 Compared with natural organelles, the constituents of artificial SPs have been largely expanded, including proteins, polymers, and lipids, or semi-
conductor NPs,17,28,31,32 metal NPs,33–36 and magnetic NPs,37 or even both above-mentioned types of constituents.16,25,26,38 Due to the flexibility of building blocks,21,39–45 SPs can be constructed with complex compositions and various morphologies (Fig. 1),46–51 such as spherical NPs (Fig. 1A–1C, 1N),17,52,53 virus-like nanoshells (Fig. 1D–1I),54 and extended architectures including layer-by-layer films,55 superlattices,56–58 sheets (Fig. 1J–1L),59,60 metal nanostars19,61 (Fig. 1M) and helical nanoribbons (Fig. 1O and 1P).62–64 The realization of terminal biomimicking superstructures from inorganic NPs38 makes it possible to replicate these biological superstructures and some of their functions.

Artificial nanoassemblies can bridge the gap between natural and artificial structures, and impart collective and novel properties that do not exist in nature,3,65 for instance plasmonic, excitonic states, and magnetic properties.24,66,67 These specific properties could also benefit the development of other fields, such as optoelectronics,68 construction technology, biomedical applications,53,55,64 environmental protection,56,71,72 and food safety.43 Reviewing the research on SPs and understanding the relationship between artificial materials and natural biological molecules are necessary to further identify the assembly mechanism, construct unique nanostructures with specific functions, and explore their potential for biological applications. In this minireview, we investigate the assembly mechanism of self-limiting self-assembly, classify their multiple properties, and discuss the existing biological studies on SPs. The underlying value and the potential applications of SPs in living systems are also discussed.

Driving forces of the self-limiting self-assembly approach

The spontaneous formation of uniform superstructures observed in biological, inorganic, or colloidal systems suggests that there must be a generic mechanism behind those distinct systems. Understanding how self-limited assemblies are formed could provide a versatile approach to control the dimensions and shape of synthetic nanoassemblies (Fig. 2).16,18,25,71,74 As demonstrated previously, self-limitation of the assembly process is achieved through the renormalized balance of the repulsive and attractive interactions between the subunits (Fig. 2A). Understanding the driving forces during the construction of self-limited SPs is important (Fig. 2B–E).17,54,75 Herein, the driving forces between building blocks are classified and discussed in detail to facilitate the understanding of spontaneous assembly.

van der Waals forces, as one type of long range attractive interaction, can be classified into Keesom forces (permanent–permanent dipole interactions), Debye forces (permanent–induced dipole interactions), and London dispersion forces (fluctuating dipole-induced dipole interactions).76,77 Keesom forces, as one type of short range attractive interaction, can be classified into London forces (permanent–permanent dipole interactions), Debye forces (permanent–induced dipole interactions), and medium-range dipole–dipole interactions (permanent–induced dipole interactions). However, for short range attractive interactions, it is difficult to determine the driving forces due to the complexity of the interactions. Therefore, the classification of driving forces for short range attractive interactions is not discussed.
interactions are an attractive interaction over various rotational orientations of dipoles. Debye forces are present between the polarized atoms or molecules and permanent rotating dipoles. Due to the chemical environment of the crystal structure and the surface ligands of SPs, both Keesom and Debye forces include at least one permanent dipole. This also indicates the
existence of electric dipole moments in building blocks. Dispersion forces are generated between two instantaneous dipoles, which are an attractive force component of van der Waals forces caused by fluctuating polarizations in the electron distribution of atoms. The order of the relative interaction strength from strong to weak is as follows: Keesom forces, Debye forces, and London dispersion forces. van der Waals interactions are commonly used to eliminate electrostatic and steric repulsive forces during the self-limited self-assembly process.

Electrostatic interactions include repulsive and attractive forces.25,78–80 When colloidal building blocks are identically
charged, electrostatic repulsion will occur between them and result in the overlapping of an electrical double layer surrounding NPs, which can prevent the coagulation or aggregation of the colloids and maintain their stability. Similar to van der Waals force, electrostatic force also plays an important role in the assembly of SPs. Electrostatic interactions between NPs not only drive the assembly of the superstructures, but also maintain the structural stability of terminal nanassemblies. The charge on building blocks is usually identical for the construction of SPs, which can eliminate the attractive interaction of van der Waals forces between NPs and drive the assembly of SPs. The strength of electrostatic forces between building blocks can regulate the pH and electrolyte concentration of the assembled colloid.

As a special kind of electrostatic dipole–dipole interaction between hydrogen acceptor and donor, hydrogen bonds with a directional, linear, and straight structure, are a kind of significant molecular interaction with wide existence in nature, and they play crucial roles to keep the stability of SPs. Hydrogen bonding can induce self-assembly, which was exemplified by two or three dimensional nanostructures-assembled building blocks through hydrogen bonding, such as yttrium fluoride (YF₃) SPs. The strength of hydrogen bonding could be regulated by controlling the pH value since deprotonated acid cannot form hydrogen bonds at a high pH value.

Understanding these interactions is essential for the technological implementation of terminal SPs synthesized with different dimensions, collective properties, and predictive biological responses. Nanostructures with desired functionalities can be better predicted and applied by understanding the effects of ionic strength, size, material, and ligand coatings on the interactions between NPs.

### New properties of constructed supraparticles

Micelle-like inorganic supraparticles (SPs) display excellent flexibility in the regulation of their sizes, components, morphologies, and optical properties based on the self-limiting self-assembly method. The ordered arrangement of building blocks and spatial confinement of subunits endowed SPs with novel and collective properties (Fig. 3), which make SPs hold great potential for fundamental or biological applications.

![Fig. 3](image-url)
Morphology is one of the most significant characteristics of SPs. By changing the colloidal environment, including temperature, ionic strength, pH value, stabilized ligands, and compositions, the morphology of SPs can be flexibly regulated,\textsuperscript{17,34,54} such as vesicle-like nanoshells and capsule-like spherical metal–semiconductor nanostructures.\textsuperscript{17,20} It is known that nanoshells are extensively found in living systems and play significant roles in maintaining the pH, ionic strength, and pressure of biological environments, for example, carboxysomes, vacuoles, and vesicles. Yang et al. reported a kind of artificial capsule-like nanoshells assembled with cadmium sulfide (CdS) NPs based on a self-limiting method (Fig. 3A and 3B), which bridged the gap between inorganic and natural organic self-assembled nanosystems.\textsuperscript{54}

These artificial nanoshells indicate great potential for substance protection and transportation, reaction control, and homogeneous catalysis in biological systems. Spherical zinc sulfide (ZnS) SPs and gold (Au)-ZnS SPs with interior pores showed higher catalytic and enantioselective ability compared with individual building blocks of ZnS NPs and Au NPs (Fig. 3C and 3D), which clearly illustrated the important roles of the three dimensional chiral space in terminal SPs.\textsuperscript{34}

Compared with individual NPs and organic subunits, assembled SPs have novel optical properties, such as core-satellite nanostructures assembled with Au NPs and Au nanorods (NRs),\textsuperscript{86} and side by side assemblies constructed with Au NPs,\textsuperscript{87} Au-Ferroferric oxide (Fe\textsubscript{3}O\textsubscript{4}) SPs, which effectively integrate plasmonic and magnetic materials, showed excellent comprehensive performance in photothermal therapy and magnetic resonance imaging.\textsuperscript{19,61} The ordered arrangement of building blocks can also create new optical properties, such as the strong surface enhanced Raman scattering (SERS) enhancement ability, and red/blue shift or split of the excitation peak (Fig. 3E). With the self-limiting self-assembly method, SPs can also be constructed into unique morphologies with high circular dichroism (CD) optical activities.\textsuperscript{88–96} When achiral Au NRS and chiral cadmium telluride (CdTe) NPs were assembled, chiral core–shell SPs could be constructed by modulating the molar ratio of the NRS and NPs. In addition, with increasing NP content, nanoassemblies with single NR in a shell of NPs could be formed.\textsuperscript{91} Besides the chiral optical properties, the photon luminescence properties of quantum dots (QDs) can also be quenched by their neighboring metal NPs due to the Förster-resonance-energy transfer or enhanced by plasmon resonance excitation of metal NPs.\textsuperscript{92,93} Chiral nanostructures of scissor-like SPs can also be formed with strong chiral optical rotatory activity based on NP dimers attributed to the chiral interactions between CdTe NPs in the shell. According to numerical simulation, the chiral optical band of plasmon–exciton assemblies is attributed to the coupling effects between longitudinal/transverse plasmon modes and NP excitonic state.\textsuperscript{94} The optical properties of chiral NR-NP assemblies can combine exciton and plasmon properties, which has great potential for chiral catalysis and chiral sensing.\textsuperscript{91} Wurtzite zinc selenium (ZnSe) NR couples synthesized with twining structures showed low photoluminescence polarization anisotropy compared with individual NR as confirmed by single-particle fluorescence, and the composition of NR couples was altered by the cation exchange approach. Using this method, a family of NR-couple structures with diverse compositions and controlled properties can be constructed to study the electronic coupling effects between individual NR, and can also be further used in photocatalysis, optics, and optoelectronic devices.\textsuperscript{95} Colloidal SPs can also be constructed with stable, broad, and tunable emissions for promising biological applications by collecting QDs together, such as the Cd(Se, ZnS) core/(Cd, Zn)S shell nanocrystals that emit three kinds of colors including red, green, and blue.\textsuperscript{52}

Magnetic properties, which play important roles in biological systems for bioimaging and therapy of controllable diseases, are another significant factor of SPs. Due to their collective effects and spatial confinement, the magnetization of SPs is usually stronger than that of individual building blocks (Fig. 3F–3H). From the viewpoint of biomedical applications, magnetic SPs are amenable to secondary structures, which can promote magnetic responsiveness and easy function design.\textsuperscript{66} Xia et al. developed Fe\textsubscript{3}O\textsubscript{4} SPs that acted as an off-on magnetic resonance imaging (MRI) switch in the tumor microenvironment; besides that they could be excreted from living bodies due to their quasi-amorphous structure and hierarchical topology design.\textsuperscript{67} The preference of novel Fe\textsubscript{3}O\textsubscript{4} SPs can be justified based on three aspects: first, the surface to volume ratio of SPs is smaller than that of Fe\textsubscript{3}O\textsubscript{4} NPs, which obviously can lead to a rather low $r$\textsubscript{1} relaxivity. Second, Fe\textsubscript{3}O\textsubscript{4} SPs showed a high signal-to-noise ratio for tumor imaging due to their dramatic disassembly/degradation-induced active $T_2$-weighted signal readout. Third, Fe\textsubscript{3}O\textsubscript{4} SPs could be disassembled/decomposed to facilitate the clearance/excretion from living systems without obvious kidney damage at an appropriate dosage.\textsuperscript{22} Above examples illustrated the excellent magnetic properties and multiple functions of SPs, which have great potential for biological applications. In addition, magnetic NPs can be assembled with quantum dots to form magnetofluorescent SPs (Fig. 3I and 3J), which are constructed with a close-packed magnetic NP “core” and surrounded by a fluorescent quantum dots “shell”. These specific SPs can be further coated with silica to serve as a magnetic resonance and in vivo multi-photon imaging probe.\textsuperscript{19} Magnetic materials can also be assembled with metal NPs to form magnetoplasmonic particles for biological imaging and photon thermal therapy. Due to the excellent biocompatibility and strong magnetic responsiveness, magnetic SPs are also excellent candidates for biomedical applications. The excitation difference of a gold monomer, a hexamer, and a heptamer could also illustrate the collective and creative properties of assembled nanostructures very well (Fig. 3E).

SPs can be as bio-mimicking enzymes for biological catalysis and photocatalysis, like polymerization of amino acids and degradation of contaminants.\textsuperscript{30,34} Chiral ZnS SPs and Au-ZnS SPs were successfully synthesized with multi-pores, which exhibited excellent enantioselective catalysis for tyrosine.
dimerization due to the confined space. Compared with individual NPs, the catalytic ability of SPs could be largely enhanced and modulated by regulating the morphology and composition of building blocks. One typical example is the SPs assembled with CdTe NPs and cytochrome C. The combination of inorganic NPs (CdTe) and biological molecules (enzyme) into tightly packed components could construct SPs with uniform size and morphology, which can enhance the catalytic efficiency due to the effective charge and exciton transportation in SPs. The combination of organic molecules into superstructures can be achieved by self-limiting self-assembly to improve the desired properties, for example, protein nanowires assembled with supermolecules are able to incorporate artificial antioxidative glutathione peroxidase, leading to enhanced antioxidative activity, compared with the catalytic abilities of the monomer. These examples illustrated the potential of SPs for biological applications, and meanwhile, inspired us to create new SPs with superior catalytic performance for specific biological application.

Biological application of supraparticles

As described previously, SPs can be constructed with biomimicking morphologies, various compositions, and excellent stability and biocompatibility. Perfluorooctanoic acid-functio-

Fig. 4 (A) Schematic of Fe₃O₄ SPs assembly process. Computed tomography (CT), photoacoustic (PA), and magnetic resonance (MR) in vivo multimodal tumor imaging. This figure has been adapted from ref. 61 with permission from Wiley-VCH, copyright 2018. (B) Active T1-weighted MRI of tumor cells and disassembly for renal clearance based on Fe₃O₄ SPs. This figure has been adapted from ref. 67 with permission from American Chemical Society, copyright 2020. (C) TEM image and 3D tomography images of CuₓCoₙS SPs. (D) Confocal images of human breast cancer cell line (MCF-7) stained with hydroxyphenyl fluorescein (HPF) and 2',7'-di-chlorofluorescein (DCF) for hydroxyl radical and ROS detection after various treatments with CuₓCoₙS SPs, respectively. Scale bar = 100 mm. (E) Live and dead cell imaging after various treatments with CuₓCoₙS SPs. Scale bar = 100 mm. (F) TEM and CD spectra of Co₃O₄ SPs. These figures have been adapted from ref. 99 with permission from Wiley-VCH, copyright 2019. (G) Chiral-specific stability of SPs in vivo and in vitro. In vivo imaging system images of four groups of mice before and after intravenous injection of phosphate-buffered saline (PBS), L-, D-, and DL-SPs. This figure has been adapted from ref. 53 with permission from Wiley-VCH, copyright 2020.
nalized nanodiamond SPs exhibited high penetration of the cell membrane, and could be used as drug carriers for chemotherapy. SPs constructed with metal, semiconductor, or magnetic materials exhibited excellent photothermal stability, low specific interactions, and good biocompatibility due to plasmonic coupling between the core and shell materials, providing a favorable tool in biological imaging and photodynamic therapy of tumors, such as Au@Cu2−xSeSPs and Fe3O4@Au SPs (Fig. 4A). Based on monodisperse Fe2O3 SPs, a distinct off-on MRI switch was constructed for tumor imaging with high resolution and a low signal to noise ratio (Fig. 4B). UFO-shaped plasmonic SPs constituted with Au NPs and manganese dioxide (MnO2) nanosheets were successfully used to monitor the vesiculation of cell membrane through the change of localized surface plasmon resonance (LSPR) that was caused by the morphology variation of MnO2 nanosheets. By observing the change in LSPR signal, dynamic interactions between SPs and cell membranes could be monitored. Moreover, heterogeneous copper cobalt sulfide (CuCoS) SPs constructed with a self-limited self-assembly strategy exhibited excellent catalytic ability for the production of ROS in cancer cells. Compared with homogeneous copper sulfide (CuS) SPs, cobalt sulfide (CoS) SPs, and even a commercial reactive oxygen species (ROS) production agent, artificial CuCoS SPs not only displayed good biocompatibility but also exhibited excellent catalytic ability due to their specific morphology and the combination of different compositions (Fig. 4C), which is successfully applied to induce the apoptosis of cancer cells in medical research. Taking advantage of their porous structures, CuCoS SPs can also be loaded with cyanine 5.5 and designed as a target-responsive switch for cancer cell imaging (Fig. 4D and 4E). Interestingly, engineering SPs with chiral optical properties showed different interactive abilities with living cells and proteins. SPs with d-chirality exhibited three-fold higher cell membrane penetration in breast, cervical, and multiple myeloma cancer cells and more stable adhesion to lipid layers than l-SPs (Fig. 4F and 4G), and displayed a longer biological half-life likely due to the opposite chirality, which protected them from endogenous proteins. These chiral SPs provide a new method for controlling drug delivery systems, which can be used for tumor marker detection and disease therapy. Based on the self-limiting self-assembly approach, a kind of SP with red-to-near-infrared luminescence signals were constructed based on biocompatible silicon quantum dots for fluorescence bioimaging. Au@CdS hybrid core–shell SPs were successfully used for quantitative detection of dopamine in real time if modified on the electrode. Based on the advantages of stability, sensitivity, and selectivity, Au@CdS SPs have exhibited considerable advantages in the field of medical diagnosis. Overall, self-limiting self-assembly provides a promising technique for constructing multiple functional SPs, which have great potential for solving difficulties in living systems. Summarizing and classifying the specific properties of SPs could provide guidance for their potential functions in biological systems and solve the serious problems based on nanotools.

Summary and outlook for supraparticles

Self-limiting self-assembly provides a useful toolbox for creating exciting and novel nanomaterials. Their versatile, convenient, economic, and flexible properties provide an assembly strategy with obvious superiority and powerful competition for further applications compared with other assembly methods. Giving some examples like its amplified effects of collective motion, subtle nanoscale anisotropies (such as chirality), and non-additivity of their interactions, these are all meant to further facilitate amazingly complex biomimetic structures, such as nanowires, nano-ribbons, multilayers, and helix. The organization of these assemblies rivals in complexity to size-limited assemblies found in biological systems, such as viruses and micelles. These artificially designed assemblies have become a rival in complexity to size-limited biological substances in living systems. In addition, the difference of NPs in chemistry and assembling conditions can lead to a wide variety of self-organization morphology. Composites constructed by nanoscale assemblies made from different compositions have a unique combination of properties, which is very meaningful to provide potential functions for the future development of biological systems.

Summarizing the existing colloidal SPs, understanding the underlying mechanism of the assembly process, and exploring their application potential in living systems not only help us to recognize the generality of self-limiting self-assembly, but also inspire us to construct goal-guided nanostructures with superior biological functionalities. The specific morphology and excellent optical, electrical, and catalytic properties of SPs result in them having considerable potential to solve difficult biological problems, for example, biological imaging, disease diagnosis, cancer therapy, and drug delivery. This review detailed the development of terminal SPs and offered new perspectives for supporting living systems based on the self-limiting self-assembly strategy.

Competing interests

The authors declare no competing financial interests.

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