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Supramolecular dendrimer capsules by cooperative binding^{†,‡}

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Abstract

We exploit the unique features of dendrimers as molecular nanospheres with many peripheral binding sites to show that maximizing cooperative binding enables a novel modular self-assembly approach to construct supramolecular capsules.

Cooperativity is a general principle that governs multivalent binding between two entities, *e.g.* multivalent ligand-receptor interactions, as well as subunit binding in self-assembly processes, *e.g.* collective base pairing in DNA double-helix formation and assembling of protein subunits into viral capsid or cytoskeleton superstructures.¹ Cooperative multivalent binding in molecular recognition has been well studied.¹ However, limited effort has been made to combine and maximize multivalent and subunit cooperative binding towards constructing functional supramolecular self-assemblies from large numbers of subunits. Here, we explore dendrimers as molecular nanospheres with highly-branched peripheral binding sites to show a new concept that maximizing cooperative binding enables a novel modular self-assembly approach to construct functional supramolecular capsules, Scheme 1.

Dendrimers have a highly branched architecture emanating from the core, thus can provide many binding sites at the periphery. They are globular in topology, monodispersed in size, and nanometers in dimension (1–10 nm).² Dendrimers and their conjugates are not only of great interest to molecular recognition studies, but have also been vigorously pursued in the fabrication of supramolecular self-assemblies, ranging from dimers, oligomers, megamer clusters, rotaxane complexes, to large assemblies such as vesicles, fibers, gels and liquid crystals.³ Despite tremendous achievements, such dendrimer self-assemblies, however, were largely based on traditional amphiphile hydrophobic effect, guest–host complexation, or dimeric/oligomeric linkages *via* H-bonding or metal coordination. The unique features of dendrimers as molecular nanospheres with highly-branched binding sites are yet to be fully exploited.

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To demonstrate the generality of the cooperative binding concept, here we studied two types of dendrimers that differ significantly in composition, branching motif and rigidity—Fréchet-type dendrimers with $1 \rightarrow 2$ aryl branching motif and ether connectivity, and Newkome-type dendrimers with $1 \rightarrow 3$ C-branching motif and amide connectivity, Fig. 1A and 2A. Each dendrimer was functionalized with carboxyl peripheral groups to allow for potential electrostatic interactions in water. The benzyl ether interior of the Fréchet dendrimers is rather rigid and highly hydrophobic, whereas the Newkome dendrimers have much more conformational freedom around sp^3 -C branching points, and their amide rich interior is much more hydrophilic. Consistently, our molecular Dynamics (MD) simulations on the Newkome dendrimers demonstrated a large degree of conformational flexibility and revealed water penetration into their amide-rich interior, Fig. 2A(II), ESI. The distinct difference between these two types of dendrimers was also confirmed by solubilization of hydrophobic fluorescent dyes inside the Fréchet dendrimers, but not by the Newkome dendrimers.

The carboxyl peripheral groups can completely dissociate into carboxylate anions ($-\text{COO}^-$) at neutral pH in aqueous solution, and both types of dendrimers dispersed as individual molecules upon dissolution in water, Fig. 1A(II) and 2A(III). Although the Fréchet dendrimers have highly hydrophobic benzyl ether interiors, their hydrophobic associations into bilayer vesicles are unlikely due to their conformational rigidity. In fact, such Fréchet dendrimers are well known as unimolecular micelles in water, where the hydrophobic benzyl ether interior collapses into a rigid core surrounded by closely packed polar groups at the surface.⁴ Direct Fluorescent Microscopy (FM) imaging of dye-labeled Fréchet- COO^- dendrimers revealed their Brownian motion in water as dispersed individual molecules, Fig. 1A(II). Cryogenic-Transmission Electron Microscopy (Cryo-TEM) with resolution in nanometers showed similar results to those obtained by FM, Fig. 1A (II, inset), where the diameters of the fourth-generation-(G4)-Fréchet- COO^- dendrimers (with 32 peripheral groups) and second-generation-(G2)-Fréchet- COO^- dendrimers (with 8 peripheral groups) were measured as 5 nm and 3 nm respectively. Cryo-TEM studies on Newkome- COO^- dendrimers showed that they are also dispersed molecular nanospheres with average diameters of 4 nm and 2.5 nm for G2 (with 36 peripheral groups) and G1 (with 12 peripheral groups) respectively.

Regardless of core composition and hydrophobicity, branching motif or generations, upon the addition of divalent Ca^{2+} , the dendrimer- COO^- nanospheres spontaneously assembled into submicron hollow capsules, Fig. 1B(I) and 2(B). Both FM and TEM imaging of these capsules revealed the projection of hollow cavities and dense membranes. Negative staining in TEM, Fig. 1B(II), further resolved the enclosed, ragged surface topology of these capsules, which appear to be composed of large numbers of tightly bound dendrimer nanospheres. The enclosed capsule structure was further confirmed by their ability to encapsulate guest materials into their cavity, as demonstrated in Fig. 1B(III), using red-fluorescent anticancer drug doxorubicin. Based on the membrane volumes of the capsules and the sizes of the dendrimer nanosphere molecules, we estimate these dendrimer capsules to contain hundreds to more than tens of thousands of dendrimer nanosphere units. These capsules were also observed to be robust and stable against shear and rupture stress.

Although both types of dendrimers formed capsules with Ca^{2+} , they differed distinctively in their response to Ca^{2+} . Fréchet- COO^- dendrimers required excess Ca^{2+} than total carboxylates to assemble, *i.e.* $\text{Ca}^{2+}:\text{COO}^- \sim 4$, whereas Newkome- COO^- dendrimers, regardless of generations, began to form monolayer capsules at $\text{Ca}^{2+}:\text{dendrimer} \sim 3:1$, where the Ca^{2+} stoichiometry is far below carboxylates, *i.e.* $\text{Ca}^{2+}:\text{COO}^- = 1:8$ for G2, Fig. 2B. Newkome dendrimers also tend to form smaller and thinner capsules than Fréchet- COO^- dendrimers. Capsules from G4-Fréchet- COO^- dendrimers have average cavity sizes around 100 nm and their membrane thickness can grow up to 100 nm at high Ca^{2+} concentrations, whereas G2-Newkome dendrimers with comparable number of peripheral groups formed capsules with average cavity sizes around 50 nm and their thickness can grow from monolayer to a maximum of 20 nm with increasing Ca^{2+} concentrations, Fig. 2B. The size and thickness of the capsules also depended on the generation of the dendrimers. For both types of dendrimers, the smaller lower-generation dendrimers formed smaller cavities with thinner membranes, Fig. S1, ESI.

Such Ca^{2+} induced capsule formation is distinctively different from the classical amphiphile hydrophobic-effect driven bilayer vesicles, as this process is independent of hydrophobicity and the membrane thickness can grow from monolayer to far beyond bilayers. Although Ca^{2+} is commonly used for inducing coil and aggregation of carboxylate based polyelectrolytes,⁵ the formation of the ordered hollow capsules here is surprising. Hollow capsule formation, such as in viral capsids, generally proceeds by a nucleation and growth pathway, where an oligomeric patch forms first followed by bending and expansion into a shell to minimize the surface energy.⁶ The dendrimers here share some common features with proteins, as they are nanometers in size and spherical in shape with many binding sites at periphery. Divalent Ca^{2+} can not only bind with COO^- as counterion, but can also form $\text{COO}^- \text{--} \text{Ca}^{2+} \text{--} \text{COO}^-$ salt-bridges linking between dendrimer nanospheres. The highly branched feature of dendrimers would also allow for multiple Ca^{2+} salt-bridges between dendrimers, as well as three-dimensional binding into multiple layers with sufficient Ca^{2+} , consistent with the observed capsule thickening with the increasing Ca^{2+} .

To better understand the $\text{Ca}^{2+}/\text{COO}^-$ binding that underlies the capsule formation, we used isothermal titration calorimetry (ITC) to study the energetics of the binding process. Ca^{2+} was added incrementally into the aqueous solution of the more sensitive Newkome- COO^- dendrimers. The heat changes of the titration and the integrated heat per mole of added Ca^{2+} *versus* the molar ratio of $\text{Ca}^{2+}:\text{Dendrimer}$ were plotted in Fig. 3. Interestingly, the titration was multiphasic and was best fitted with the two independent binding site model. The most obvious distinction among sites possible in this system is between individual $\text{Ca}^{2+}\text{--COO}^-$ counterion binding and $\text{COO}^- \text{--} \text{Ca}^{2+} \text{--} \text{COO}^-$ salt-bridge forming to join two dendrimers. The parameters reported by ITC for two processes were $K_1 = 3.1 \times 10^3 \text{ M}^{-1}$, $H_1 = 7.5 \text{ kcal/mol}$, $T \Delta S_1 = 12.2 \text{ kcal/mol}$, $G_1 = -4.7 \text{ kcal/mol}$; and $K_2 = 4.8 \times 10^4 \text{ M}^{-1}$, $H_2 = -3.4 \text{ kcal/mol}$, $T \Delta S_2 = 1.7 \text{ kcal/mol}$, $G_2 = -5.1 \text{ kcal/mol}$. Both binding events are spontaneous processes with release of free energy. The first process is endothermic and entropically driven, consistent with disruption of the ordered water solvation shell upon individual counterion binding.⁷ Average binding constant K_1 is also similar to the previously reported $\text{Ca}^{2+}\text{--COO}^-$ counterion binding constant.⁸ In contrast, the second Ca^{2+} binding process is exothermic and

enthalpically driven with the average K_2 ten times greater than K_1 . The isotherm is also sigmoidal, indicating a positive cooperative effect. The second process is likely the $\text{COO}^- - \text{Ca}^{2+} - \text{COO}^-$ salt-bridge binding that assembles dendrimers into capsules, where the formation of the highly ordered dendrimer nanosphere lattices would yield exothermic lattice energy and entropy loss. In addition, as two dendrimers are brought together by the first Ca^{2+} salt-bridge formation, their proximity would facilitate the subsequent formation of Ca^{2+} salt bridges between them and maximize the cooperativity. Multiple bonding in turn can enhance the binding strength between dendrimers, and stabilize the self-assembly structure and account for the robustness of these capsules.

The Ca^{2+} induced cooperative binding of dendrimers also explains for the observed differences between Fréchet- and Newkome- COO^- dendrimers. As rigid spheres with tightly packed carboxylate groups at the surface, Fréchet dendrimers require addition of more Ca^{2+} to bind and screen the densely negative surface charges first, before they can be brought to proximity to form Ca^{2+} salt-bridges.⁹ In contrast, the carboxylate groups in the highly flexible Newkome dendrimers are more like independent point charges, which can extend to form Ca^{2+} salt-bridges much more freely. The flexibility of Newkome dendrimers would also enable bending into much smaller shells as observed. Quantitatively, since at least one Ca^{2+} salt-bridge is needed to link between every two Newkome dendrimers and each dendrimer sphere would be surrounded by six neighbors in close-packing, a minimum ratio of $\text{Ca}^{2+} : \text{dendrimer} = 6/2$ (3 : 1) would be required to induce the capsule formation for Newkome dendrimers, which is consistent with our experimental finding.

Cooperative binding not only allows for flexible supramolecular dendrimer capsule fabrication, but also possesses the advantage of ease in controlling disassembly towards release. Since the capsules are bound by Ca^{2+} salt-bridges, removal of Ca^{2+} would cause the capsules to disassemble into dendrimer monomers. Fig. 4 shows that as EDTA, an effective Ca^{2+} chelator that can withdraw Ca^{2+} from the capsules, was added to Fréchet- $\text{COO}^-/\text{Ca}^{2+}$ capsule solutions, the capsules quickly disassembled with time, which was directly observed by FM.

In summary, we have exploited the unique features of dendrimers as molecular nanospheres with highly-branched binding sites and demonstrated their ability to maximize cooperative binding, which enables a novel modular self-assembly approach to construct supramolecular capsules. Such capsules by cooperative binding are tunable in size and thickness, controllable in disassembly, and can be potentially used for a wide variety of encapsulation applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Ca²⁺ induced self-assembly of Fréchet-COO⁻ dendrimers into supramolecular capsules in water. (A) I. Molecular structure; II. FM and cryo-TEM (inset) imaging on dispersed Fréchet-COO⁻ dendrimers. (B) I. Cross-section projection of the Ca²⁺ induced capsules imaged by FM and TEM (inset); II. Surface topology of the capsules imaged by negatively-stained TEM; III. Encapsulation of red-fluorescent Dox by the capsules.

Fig. 2.

Ca^{2+} induced self-assembly of Newkome- COO^- dendrimers into supramolecular capsules in water. (A) I. Molecular structure; II. MD simulation. Grey/red contour—deviation in conformations. III. Cryo-TEM imaging on dispersed Newkome- COO^- dendrimers in water. (B) Scaling and illustration of the capsule thickness with the Ca^{2+} /carboxylate molar ratio.

Fig. 3.

ITC analysis on Ca^{2+} binding to Newkome- COO^- dendrimers. Left panel shows the calorimetric titrations; Right panel displays the integrated heat values as a function of molar ratio. The solid line represents the curve fit to a two independent binding site model.

Fig. 4.

FM imaging on the disassembly of the Fréchet-COO⁻/Ca²⁺ capsule by the addition of EDTA. Scale bar: 1 μ m.

Scheme 1.

Schematic illustration of the cooperative binding of dendrimers into supramolecular capsules.