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Disassembly of Dendritic Micellar Containers Due to Protein Binding

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A promising approach for the development of drug delivery vehicles is to harness nanoscale materials with stimuli-responsive properties, as these have the potential to enhance drug efficacy and mitigate side effects. Temperature, pH, light, and magnetic fields are among the most frequently used stimuli in these approaches.[1] Utilizing biological stimuli such as proteins for this purpose has recently been geared up,[2] taking advantage of the overexpression of certain proteins at diseased cell sites. While these recent reports are mainly based on enzymatic actions of proteins, molecular systems that respond to nonenzymatic proteins are underexplored and of great significance.[3] For example, responsive assemblies based on receptor−ligand binding interactions are of great interest, because (*i*) these strategies can be applied to a much larger variety of proteins and (*ii*) overexpression of nonenzymatic proteins is highly relevant to a variety of diseases. With this motivation, we report here a dendrimeric system that responds to protein−ligand binding interactions.

The large yet well-controlled architecture makes dendrimers an interesting class of molecules for a variety of applications.[4] In particular, amphiphilic dendrimers have become extremely attractive because (*i*) they can sequester hydrophobic guest molecules in an aqueous milieu[5,6] and (*ii*) they exhibit guest-encapsulation properties at low critical aggregation concentrations (CACs).[5,6] Recently, we reported on a distinct class of amphiphilic dendrimers in which every repeating unit in the dendritic molecule contains both lipophilic (hydrophobic) and hydrophilic functionalities. As a result of the orthogonal placement of these amphiphilic units, these molecules were able to form micelle-type and inverse-micelle-type assemblies in polar and apolar solvents, respectively.[6,2b] Unlike classical amphiphilic dendrimers, our biaryl dendrimers aggregate to form micellar assemblies, and this aggregation is primarily responsible for the hydrophobic guest encapsulation properties of these molecules. Hence, we envisioned that deaggregation of these molecules would cause the micellar assemblies to disassemble, resulting in the release of encapsulated guest molecules. In fact, we have recently demonstrated such a possibility with enzymatic reactions.[2b] To execute the deaggregation through protein−ligand binding interactions, we incorporated a ligand functionality that binds to a specific protein. We hypothesized that dendrimer−protein binding caused by the ligand−receptor interaction would dramatically alter the hydrophilic−lipophilic balance (HLB) of the dendrimer molecule. This is based on the fact that water-soluble globular proteins have hydrophilic surfaces and the binding event replaces a small ligand functionality with a large hydrophilic protein. We conceived that this HLB change could cause the dendrimer to lose its ability to assemble. It then follows that this disassembly would also effect the release of sequestered guest molecules from the micellar interiors in response to protein binding (Figure 1).

To test this hypothesis, we targeted dendrons **G0**−**G2** shown in Chart 1. In these dendrons, the decyl chain acts as the lipophilic unit, and pentaethylene glycol (PEG) was chosen as the hydrophilic unit to reduce nonspecific interactions. Biotin, which is well-known

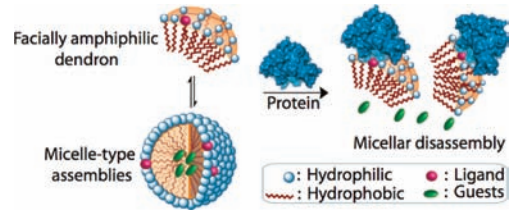
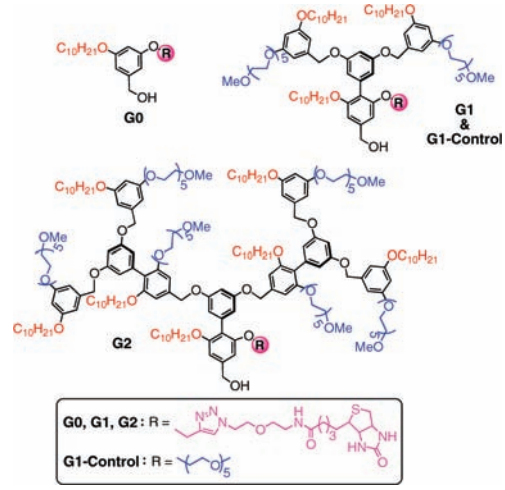


Figure 1. Schematic of protein−ligand binding-induced disassembly of dendritic micellar assemblies and resultant guest release.

Chart 1. Structures of Ligand-Functionalized Self-Assembling Facially Amphiphilic Dendrons



for its strong interaction with avidin, was chosen as the ligand functionality. Biotin was incorporated into the dendron by "click" chemistry, as this allows for easier future ligand variations.[7] We first studied the micellar properties of dendrons **G1** and **G2** using pyrene as the spectroscopic probe.[7] The CACs of **G1** and **G2** were found to be 4.5 and 1.2 μM, respectively, as typically observed for our amphiphilic dendrimers in comparison with their small-molecule counterparts.[2b] The small-molecule dendron **G0** exhibited poor water solubility, presumably as a result of the lack of the PEG unit.

Next, we investigated the protein−ligand binding-induced guest release from the micellar interiors. For this purpose, we used extravidin, a neutral form of avidin with minimal nonspecific interactions. When a 25 μM solution of **G1** was exposed to increasing concentrations of extravidin for 3 h, we observed a decrease in the emission intensity of pyrene (Figure 2a), indicating the release of pyrene from the micellar assemblies. To test whether this decrease in pyrene fluorescence was indeed due to the biotin−extravidin interactions, extravidin was added to a solution of our parent dendron **G1-control**, which lacks the biotin functionality (Chart 1).[6b] With this assembly, we did not observe any significant decrease in the fluorescence of pyrene (Figure 2a), supporting our hypothesis that the guest release in **G1** was in fact

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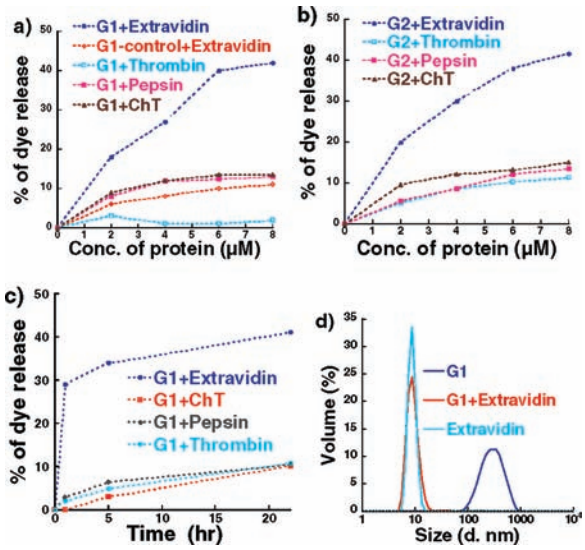

Figure 2. Studies of the disassembly of **G1** (25 μM) and **G2** (5 μM) assemblies. (a, b) Concentration-dependent dye release from (a) **G1** and (b) **G2**. (c) Direct addition of proteins (10 μM) to **G1**; (d) Size variation upon exposure to proteins (10 μM), as determined by DLS.

due to the protein−ligand interaction.[7] Furthermore, to determine whether the guest release was selective to extravidin, we exposed **G1** to proteins with varying pI values, namely, thrombin, pepsin, and chymotrypsin (ChT), and observed only a small percentage of guest release (Figure 2a), probably due to nonspecific interactions. This lack of significant guest release provided further evidence that protein−ligand binding was the most likely reason for the guest release in the extravidin case.[7] Next, we tested whether a similar guest release and disassembly could also be effected in the **G2** dendron. Exposure of 25 μM **G2** to extravidin resulted in only 30% release, as compared with 42% release with **G1**.[7] This is presumably because the higher-generation dendron provides a more tightly packed assembly. In addition, the lower CAC of **G2** allowed us to decrease the starting concentration. Indeed, when 5 μM **G2** was used, a release of ∼45% was obtained. Here too, the guest release was found to be selective to extravidin (Figure 2b).[7]

Three features are noteworthy. (*i*) The guest release was entirely concentration-dependent. For example, in the case of **G1**, addition of 2 μM extravidin caused only 18% release, and no further release was observed. Further release was observed only upon subsequent addition of extravidin (Figure 2a), indicating the concentration-dependent release characteristics. (*ii*) When we exposed **G1** and **G2** solutions straightaway to 10 μM extravidin, we observed immediate releases of ∼30 and 40% in 1 h for **G1** and **G2**, respectively, and an additional 10% release in 5 h (Figure 2c). However, the direct addition of proteins other than extravidin did not result in any significant release of pyrene.[7] These results once again imply that the guest release was controlled by the concentration of protein and eventually afforded almost the same amount of release, regardless of whether protein was added in small portions or all at once. (*iii*) The release observed in these cases was only ∼45%. This could be due to the ability of the hydrophobic chains of our monomeric state of the dendrimer to withhold some amount of pyrene.

Finally, it was important to determine whether the guest release was really due to micellar disassembly. In order to address this, we used dynamic light scattering (DLS) to investigate the transformation in the size of these micellar assemblies upon addition of protein. The initial size of the **G1** assembly was found to be ∼300 nm. Upon addition of 10 μM extravidin, the size decreased to 10 nm, corresponding to size of the protein itself (Figure 2d), while no such decrease in size was observed for other proteins.[7] This dramatic decrease in size supports our hypothesis that the protein binding indeed caused the micellar disassembly, which then resulted in the guest release. A similar size decrease was also observed with **G2**, which further strengthens our disassembly hypothesis.[7]

In summary, we have demonstrated that dendrimer-based amphiphilic nanocontainers can be disassembled in response to an engineered ligand−receptor interaction. Our working hypothesis for the disassembly is based on the alteration in the HLB caused by the binding event. We have shown that such a disassembly event is selective to the protein for which the dendrimer is engineered. We have also demonstrated that these disassembly events can cause a concurrent release of guest molecules. The supramolecular disassembly based on ligand binding outlined here could provide a basis for the design of novel protein-responsive drug delivery and biosensing systems.

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Supporting Information Available: Experimental details and NMR, DLS, and fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) See the Supporting Information for details.

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