

Supramolecular Disassembly of Facially Amphiphilic Dendrimer Assemblies in Response to Physical, Chemical, and Biological Stimuli

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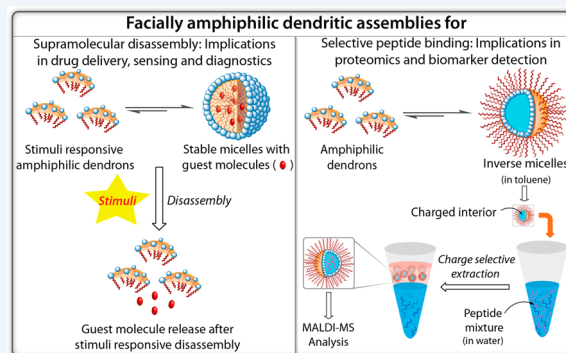
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CONSPECTUS: Supramolecular assemblies formed from spontaneous self-assembly of amphiphilic macromolecules are explored as biomimetic architectures and for applications in areas such as sensing, drug delivery, and diagnostics. Macromolecular assemblies are usually preferred, compared with their simpler small molecule counterparts, due to their low critical aggregate concentrations (CAC) and high thermodynamic stability. This Account focuses on the structural and functional aspects of assemblies formed from dendrimers, specifically facially amphiphilic dendrons that form micelle or inverse micelle type supramolecular assemblies depending on the nature of the solvent medium.

The micelle type assemblies formed from facially amphiphilic dendrons sequester hydrophobic guest molecules in their interiors.

The stability of these assemblies is dependent on the relative compatibility of the hydrophilic and hydrophobic functionalities with water, often referred to as hydrophilic–lipophilic balance (HLB). Disruption of the HLB, using an external stimulus, could lead to disassembly of the aggregates, which can then be utilized to cause an actuation event, such as guest molecule release. Studying these possibilities has led to (i) a robust and general strategy for stimulus-induced disassembly and molecular release and (ii) the introduction of a new approach to protein-responsive supramolecular disassembly. The latter strategy provides a particularly novel avenue for impacting biomedical applications. Most of the stimuli-sensitive supramolecular assemblies have been designed to be responsive to factors such as pH, temperature, and redox conditions. The reason for this interest stems from the fact that certain disease microenvironments have aberrations in these factors. However, these variations are the secondary imbalances in biology. Imbalances in protein activity are the primary reasons for most, if not all, human pathology. There have been no robust strategies in stimulus-responsive assemblies that respond to these variations. The facially amphiphilic dendrimers provide a unique opportunity to explore this possibility.

Similarly, the propensity of these molecules to form inverse micelles in apolar solvents and thus bind polar guest molecules, combined with the fact that these assemblies do not thermodynamically equilibrate in biphasic mixtures, was used to predictably simplify peptide mixtures. The structure–property relationships developed from these studies have led to a selective and highly sensitive detection of peptides in complex mixtures. Selectivity in peptide extraction was achieved using charge complementarity between the peptides and the hydrophilic components present in inverse micellar interiors. These findings will have implications in areas such as proteomics and biomarker detection.



1. INTRODUCTION

Supramolecular assemblies, which are often formed as a result of spontaneous organization of inherently disordered components into stable organized structures, are ubiquitous in nature and have been subjects of investigation in numerous biomimetic architectures.^{1–3} The nature of the assemblies and the fidelity for their formation are usually governed by noncovalent interactions. For example, assemblies such as micelles, liposomes, fibers, and thin films, to name a few, have found profound implications in the field of sensing, drug delivery, and diagnostics.^{4–7} In solution, supramolecular assemblies are often driven by solvophobic forces. For example, the ability of an amphiphilic molecule to form a micelle is determined by its concentration in solution (must be above its critical aggregate concentration) and the hydrophilic–lipophilic balance of the constituent functional groups. In addition, the divergence in whether an amphiphile would form a micelle, a

vesicle, or another assembly is determined by the relative position and volume of its functional groups.^{8–10}

In this Account, we focus on amphiphilic assemblies formed from macromolecular scaffolds, specifically dendrons. Understandably, macromolecular amphiphiles exhibit lower CACs compared with their smaller molecule counterparts. In addition, macromolecular amphiphiles tend to provide more thermodynamically stable aggregates. Therefore, the assemblies achieved from amphiphilic dendrimers and polymers have been of particular interest.

Among the macromolecular assemblies, structures derived from amphiphilic block copolymers have been extensively

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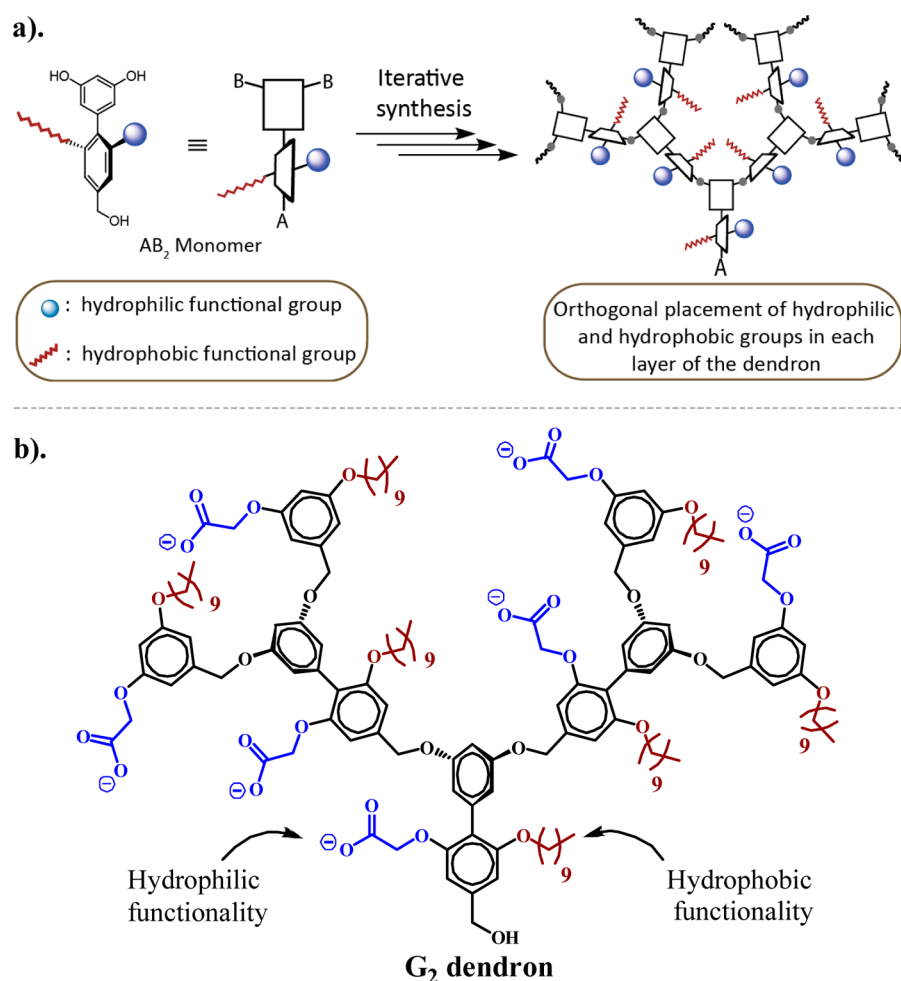


Figure 1. (a) Schematic showing orthogonal placement of amphiphilic units in each layer of the facially amphiphilic dendron.²⁹ (b) Example of a facially amphiphilic G₂ dendron.

studied.^{11–13} Similarly, classical amphiphilic dendrimers are achieved by distinctly differentiating the hydrophilicity of the functional groups that constitute the backbone of the dendrimers, compared to that of the peripheral functional groups. Since these dendrimers have been shown to form a globular shape at higher generations, the surface functional groups at the periphery of the dendrimers form the primary interface with the solvent.^{14–17} Therefore, the variations in the hydrophilicity of the peripheral functional group vs the dendrimer backbone cause the latter to provide unique environment within the dendritic interiors.^{18–20} This feature has been used to demonstrate several unique properties of this supermolecule, including host–guest chemistry and biomimetic encapsulation of electroactive, photoactive, and catalytic functional groups.^{21–25} All these features have been subjects of many reviews over the years and therefore will not be discussed in any detail in this Account.^{14–16,26–28} This Account will primarily focus on the assemblies formed by a unique class of dendrimer-based amphiphiles, called facially amphiphilic dendrons.

2. FACIALLY AMPHIPHILIC DENDRONS

Facially amphiphilic dendrons, first reported by us in 2001,²⁹ are formed from building blocks in which every repeat unit contains both hydrophilic and hydrophobic functional groups. This class of macromolecular amphiphiles was inspired by the

contrafacial amphiphiles, glycosylated cholic acid based amphiphiles, molecular harpoons, and umbrellas studied in the 1990s.^{30–33} Classical dendrimers are synthesized using building blocks that contain an AB_n building block, where A and B are reactively complementary. The most common building block involves an AB₂ monomer; 3,5-dihydroxybenzyl alcohol is an example of a monomer for benzyl ether dendrons.³⁴ Our group envisaged the possibility of introducing a hydrophilic and a hydrophobic functional group at either face of this planar building block by introducing these functional groups in a biaryl moiety. Supported by simple molecular models, we hypothesized that the twist provided by biaryls will place the hydrophilic and the hydrophobic functional groups at a plane orthogonal to that containing the phenolic and the hydroxymethyl functional groups (Figure 1). We hypothesized that the solvophobic forces, combined with macromolecular features of the dendrons, would force these molecules to fold such that the hydrophobic functional groups will be presented within the dendritic interiors, while the hydrophilic functional groups will be presented on the surface. We have found that amphiphilic aggregates formed from these dendrons to be several tens of nanometers in size.³⁵ Although much larger than anticipated, since these molecules are formed in aqueous solutions and since these can act as containers for hydrophobic guest molecules, we called these micelle-like aggregates.

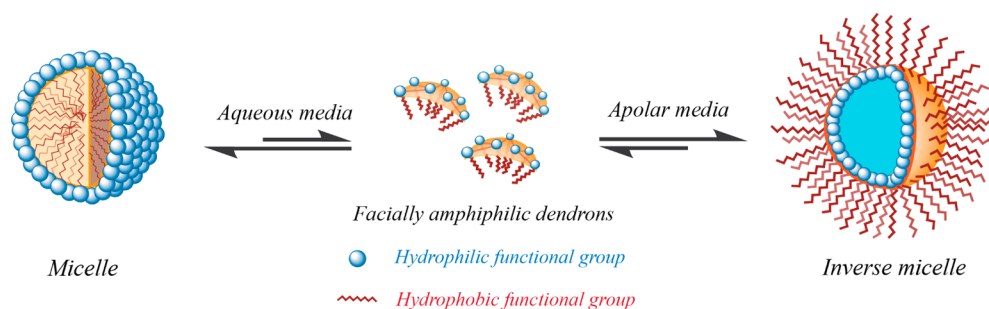
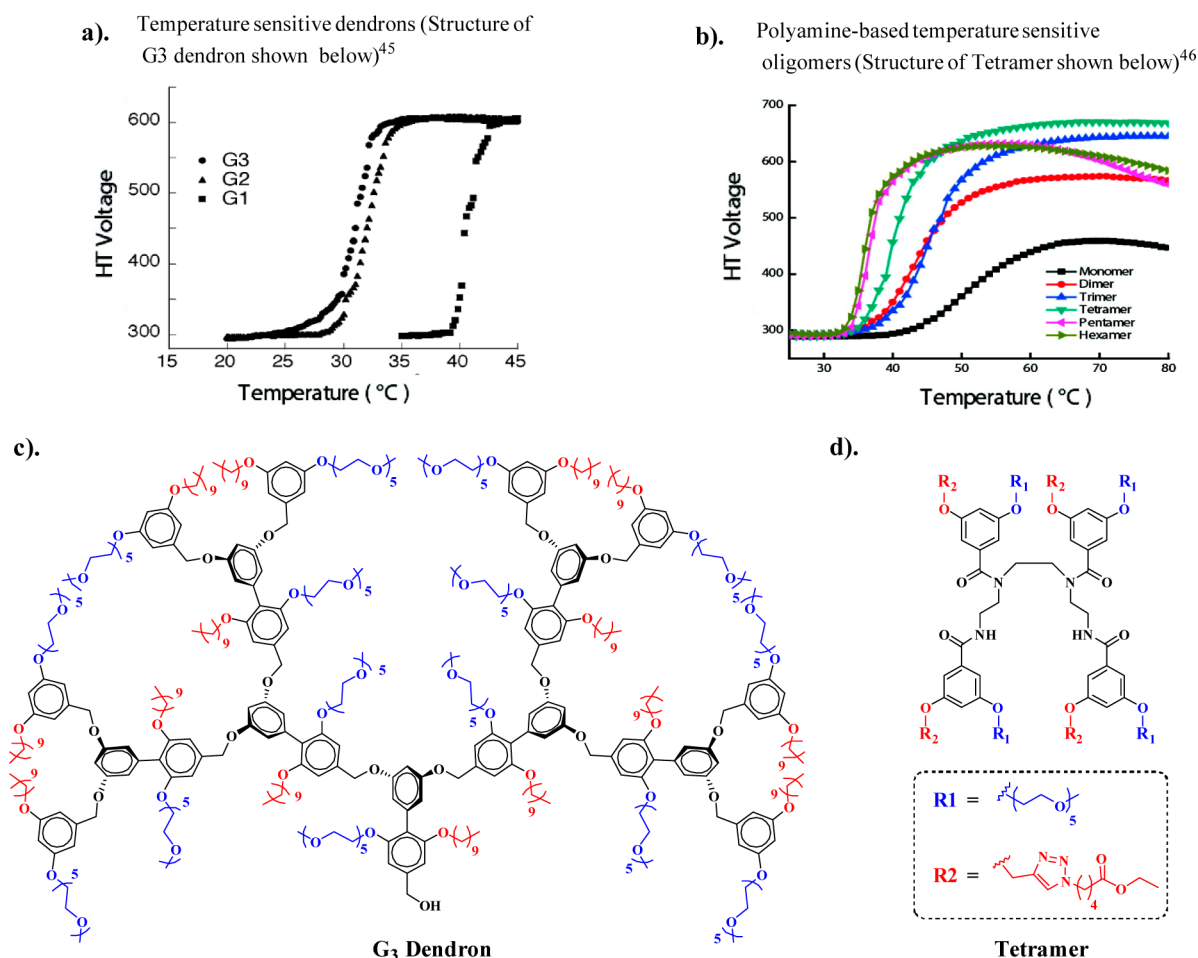


Figure 2. Schematic representation of solvent-dependent supramolecular assemblies formed from facially amphiphilic dendritic structures.

Scheme 1. Temperature Sensitivity of (a) Amphiphilic Dendrons⁴⁵ and (b) Amphiphilic Oligoamine Scaffolds⁴⁶ with SEG Moiety as the Hydrophilic Unit^a and Structures of (c) the G3 Dendron Used in the Study⁴⁵ and (d) the Amphiphilic Oligoamine Scaffold, Represented by the Tetramer⁴⁶



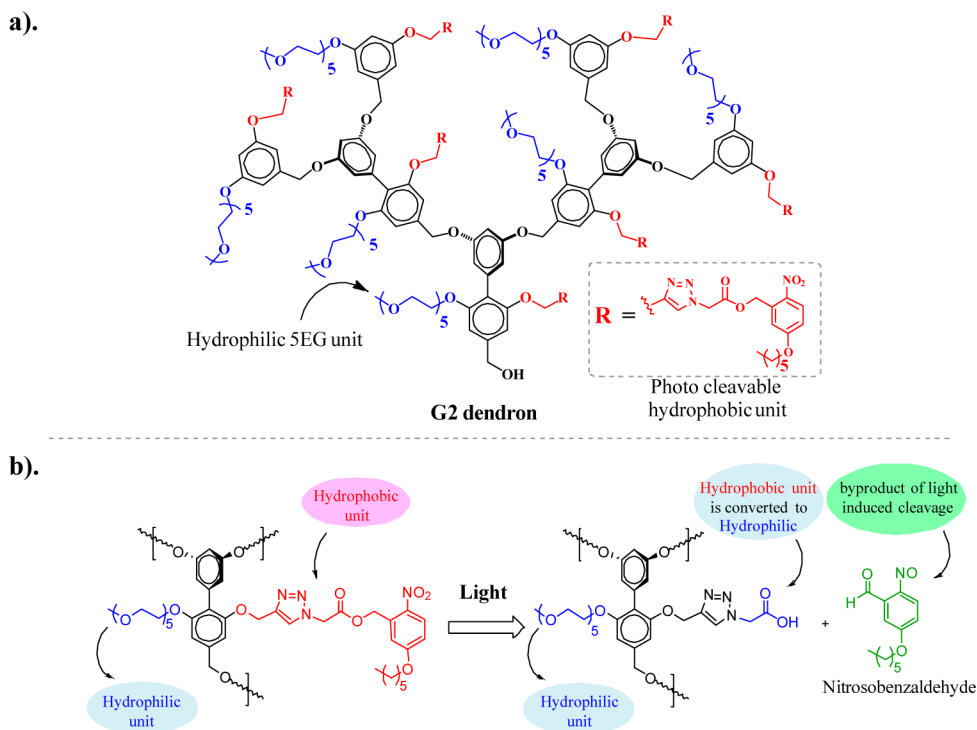
^aIn both plots a and b, the HT voltage indicates the high tension voltage in CD spectrometer (a measure of solution turbidity).

We also hypothesized that these dendrons will not only form hydrophobic nanocontainers in aqueous media but also hydrophilic nanocontainers in apolar media.³⁵ Indeed, these dendrons formed inverse micelle like aggregates that are capable of sequestering hydrophilic guest molecules, when dispersed in toluene (Figure 2). We envisaged that the functional group presentation on the surfaces is dictated by the relative compatibility of the solvent with the functional groups at either face of the dendron. If this hypothesis were correct, then dendrons with oligo(ethylene glycol) (OEG) and decyl moieties as hydrophilic and hydrophobic groups,

respectively, should not form supramolecular assemblies in solvents such as THF or DMF, which was found to be correct.³⁶

Since the assemblies obtained from facially amphiphilic dendrons were found to be quite large, we hypothesized that these molecules simply do not have sufficient backbone flexibility and therefore lack the curvature required to form spherical assemblies. To test this hypothesis, we synthesized a series of structurally similar dendrons using smaller building blocks, where this lack of flexibility is even more amplified.³⁷ Consistent with our model, the resultant assemblies were

Scheme 2. (a) Structure of Second Generation (G2) Photoresponsive Dendron, (b) Light Induced Cleavage of Hydrophobic Unit Causing Simultaneous (i) Release of *o*-Nitrosobenzaldehyde Moiety and (ii) Display of Hydrophilic Carboxylate Functionality on the Dendron⁵¹



indeed larger. Similarly, the availability of all functional groups for recognition, a critical feature for protein-induced disassembly, was ascertained using our studies on interactions of these dendrimers with proteins and with silica surfaces.^{38–40}

3. STIMULI-SENSITIVE AMPHIPHILIC ASSEMBLIES

The fact that these facially amphiphilic dendrons, unlike the classical amphiphilic dendrimers, tend to aggregate in aqueous solutions also presents us with a unique opportunity. First, these molecules exhibit CACs that are comparable to those observed for amphiphilic polymers, while providing the opportunity for developing a well-defined structure–property relationship that is often reserved for small molecules. Thus, these dendrons combine the key advantages offered by both small molecules and polymers. A specific opportunity with these dendron assemblies involves the incorporation of functional groups that make them respond to an environmental change. The structure–property relationships, evolved from these well-defined macromolecular structures, can be used to develop the next generation of stimuli-responsive assemblies that have implications in a variety of areas including drug delivery, sensing, and diagnostics.⁴¹

3.1. Temperature-Responsive Amphiphilic Dendrons

Temperature-responsive systems are widely studied as stimuli-responsive assemblies, where a slight change in temperature results in a rather rapid change in the physical property of the material. Among the various molecules studied, poly(*N*-isopropylacrylamide) (PNIPAAm) and poly(ethylene glycol) (PEG) based polymers are arguably the most studied.^{42–44} Although there are differences in subtle details, the reason for the temperature sensitivity in both of these classes of molecules is attributed to their propensity to extensively hydrogen bond with water. As the temperature of the solution increases, this

hydrogen-bonding network is disrupted, thereby adversely affecting their solubility in the aqueous phase. The temperature at which the solution turns cloudy, the so-called cloud point transition, is often referred to as the lower critical solution temperature (LCST).

Dendrons G1–G3 containing temperature-sensitive penta-(ethylene glycol) (SEG) as the hydrophilic moiety and decyl groups as hydrophobic moiety were synthesized (Scheme 1).⁴⁵ These dendrons self-assemble in aqueous solution to form micelle type assemblies, as determined by dynamic light scattering (DLS) and transmission electron microscopy (TEM) measurements. As observed from turbidity measurements, the LCST transitions for these assemblies were found to be 42, 32, and 31 °C for G1, G2, and G3 respectively. The generation-dependence in the transition temperatures for this class of dendrons is surprising, because all these dendrons form nanoassemblies of more or less the same size. To investigate this further, we synthesized a well-defined series of linear oligomers based on oligoamines (from monomer to hexamer) and found that covalently attaching ethylene glycol moieties provides cooperativity in temperature sensitivity (Scheme 1).⁴⁶ This cooperativity is likely to be the observed generation dependence in temperature sensitivity as well.

Note that the LCST transitions described in the literature, including the ones described above, are based on a phase change in solution, where the soluble molecules fall out of solution in response to an increase in temperature. Recently, we found in the case of assemblies formed by G1 dendrons that there exists another temperature-sensitive transition at lower temperature (17.5 °C) well below the LCST, where the size of assemblies decreased from ~160 nm to ~30 nm, as determined by DLS.⁴⁷ We named this transition a sub-LCST behavior. We investigated the stability of encapsulation of hydrophobic guest

molecules at various temperatures using the recently developed fluorescence resonance energy transfer based (FRET) technique.⁴⁸ Interestingly, we found that the encapsulation was stable at higher temperatures, while the guest molecules exchange dynamically at lower temperatures. This observation seems counterintuitive, because one would expect a dynamic process to be more rapid at higher temperatures. We hypothesized that the SEG units might be even more hydrated due to stronger hydrogen bonding with water at lower temperatures, which decreases the residence time of the dendritic amphiphile in the aggregate. We tested this hypothesis using a pyrene label. Indeed, the dendrons in the micelle-like aggregate rapidly exchange among each other at lower temperatures, while they are “frozen” at higher temperatures. Currently, investigations are underway in our laboratory to investigate the generality of this sub-LCST phenomenon in amphiphilic assemblies.

3.2. Photoresponsive Amphiphilic Dendrons

Light-sensitive materials have several advantages;^{49,50} for example, these materials have the potential of being site-specific and noninvasive in effecting drug delivery. In this design, we envisaged the possibility of achieving a change in hydrophilic–lipophilic balance (HLB) in response to light. For this purpose, we incorporated photosensitive *o*-nitrobenzyl groups containing an alkyl chain as the hydrophobic moiety and SEG unit as the hydrophilic moiety in the dendron;⁵¹ these dendrons formed micelle-like assemblies as anticipated. Exposing the dendron-based assembly to light (360 nm) resulted in the cleavage of the hydrophobic photolabile *o*-nitrobenzyl ester, resulting in the formation of a carboxylic acid moiety on the dendron, while liberating the hydrophobic nitrosobenzaldehyde byproduct (Scheme 2). Since the resultant dendrons are hydrophilic on both faces of the dendron, this molecule loses its assembly-forming capability, resulting in triggered disassembly. This loss of assembly was also confirmed by the light-induced release of noncovalently encapsulated guest molecules (Figure 3). Control dendrons of similar structure without the photosensitive group did not show any response upon photostimulus, confirming the photoresponsive nature of the disassembly.

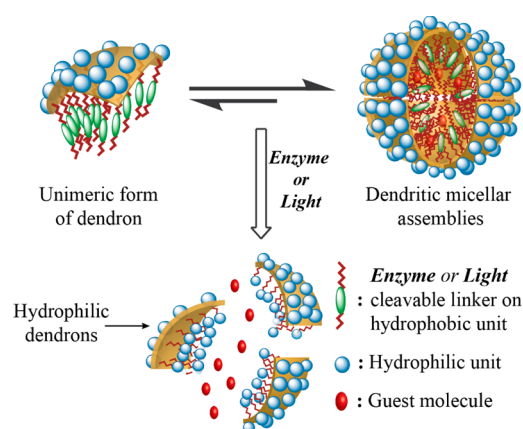


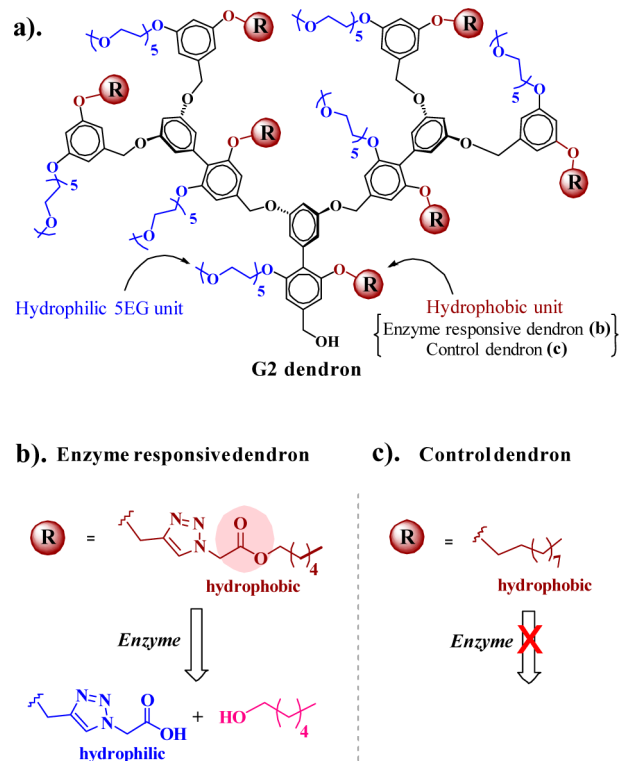
Figure 3. Schematic of disassembly mechanism anticipated, when enzyme or light is used as stimulus. In both these systems, the dendrons lose their amphiphilicity upon exposure to stimulus causing disassembly followed by release of hydrophobic guest molecules.

3.3. Protein-Triggered Disassembly

Many of the stimulus-responsive assemblies are inspired by their implications in biological applications. In this context, while variations in factors such as temperature, pH, and redox changes in a specific biological microenvironment can be considered to be secondary imbalances, most of the pathological imbalances are caused by aberrant protein activity. Therefore, supramolecular systems that respond to these primary imbalances, that is, variations in protein concentrations, are of great significance but are under-explored. The facially amphiphilic dendron-based assemblies provided a unique opportunity to explore this possibility.

3.3.1. Enzymatic Protein-Triggered Disassembly. First, we targeted the dendritic assemblies that are capable of responding to enzymatic activity. For this purpose, dendrons with orthogonally placed hydrophilic SEG and hydrophobic hexyl ester (enzyme substrate) components were prepared (Scheme 3).⁵² Consistent with our prior findings, the G1, G2,

Scheme 3. (a) Structures of Enzyme Responsive G2 Dendron and a Corresponding Control G2 Dendron and Exposure of These Dendrons to Enzyme (PLE) Causing (b) Cleavage of Enzyme-Sensitive Linker, Leading to Loss of Dendron's Amphiphilicity and (c) No Effect on the Control Dendron⁵²



and G3 dendrons exhibited CACs of 4.3, 0.7, and 0.3 μM , respectively, compared with the millimolar CAC observed for the corresponding small molecule amphiphile. Upon exposure of these assemblies to the enzyme, porcine liver esterase (PLE), the reaction-induced disassembly was evident from the fact that micelle-like assembly of ~ 100 nm in size (obtained by DLS) exhibited a time-dependent decrease in size. The assertion is that the observed disassembly is due to the enzyme-induced cleavage of the hydrophobic ester moiety to generate a hydrophilic carboxylate moiety, which should upset the HLB

of the assembly. This assertion is further supported by a control reaction, where PLE did not have any effect on the micelle-like assembly formed from a structurally similar dendron lacking the enzyme-cleavable ester moiety (Scheme 3).

The enzyme-induced HLB change is similar to that of the photosensitive disassembly outlined above (Figure 3). However, note that the photoinduced disassembly does not have the accessibility requirements that are critical in enzyme-induced disassembly. In the latter case, the enzyme cleavable functional groups need to be accessible for the enzyme. Considering that the enzymes are rather large and that the enzyme-responsive units are located in the hydrophobic core of the micelle-like assemblies, we envisaged that equilibrium between the unimeric state and the aggregate state of the dendron must be involved (Figure 3). In this process, the ester moieties are accessible to the enzyme in the unimeric state, while these units are buried in the hydrophobic core in the aggregate state. Even if the equilibrium heavily favors the aggregate state, the dendritic assembly can be ultimately disassembled due to the LeChatelier effect.

The kinetics of the enzyme-induced disassembly process was found to be generation-dependent; the higher generation dendrons were found to disassemble and release the non-covalently encapsulated guest molecules at a much slower rate than the lower generation dendrons. This could be either due to the steric difference in accessibility of the substrate moieties to the enzymes or due to the possibility that higher generation dendrons are likely to have a higher residence time in the aggregate state, that is, the k_{off} in the unimer–aggregate equilibrium is likely to be lower for higher generations (Figure 3). While it might be difficult to distinguish these possibilities, we were interested in identifying whether the latter unimer–aggregate equilibrium would influence the kinetics of disassembly and release. For this purpose, we designed a dendritic molecule containing lipophilic fluorescent precursor functionality, which releases the fluorophore in response to the enzymatic reaction.⁵³ Concurrently monitoring the release of noncovalently sequestered guest molecules and the cleavage of the hydrophobic moiety suggested that there is a clear correlation between covalent bond cleavage and guest molecule release. This then led us to test the possibility of utilizing these dendrons for controlling the release of the guest molecules by limiting the extent of dendron availability in a monomer–aggregate equilibrium. Since the hydrophobic fluorophore in this case was based on coumarin, we utilized the photochemical dimerization of coumarin to test this possibility. Indeed, we observed that the extent of guest molecule release can be precisely controlled by manipulating the extent of cross-linking in these nanoassemblies. These observations further support the notion that the unimer–aggregate equilibrium is involved in the enzyme-induced supramolecular disassembly.

3.3.2. Binding Induced Supramolecular Disassembly Triggered by Proteins. While the enzyme-sensitive disassembly represents a covalent and often irreversible modification of the hydrophilic–lipophilic balance, supramolecular disassembly based on noncovalent interactions is a significant challenge. Noncovalent interaction-based disassembly will have a great impact, because this represents a rather large number of proteins that are critical in biological signal transduction events, but do not have known enzymatic activity. In general, stimuli-sensitive systems based on nonenzymatic proteins are explored to a much lesser extent. We hypothesized that the HLB of a dendron containing a small molecule ligand

(complementary to a specific protein) would be significantly different from that of the protein–dendron complex, because a small molecule is replaced by a rather large hydrophilic surface in the latter system (provided by the bound protein). If we were to design the molecules such that the ligand-bearing dendron will form a stable assembly, then it is possible that disassembly can occur upon protein binding (Figure 4).

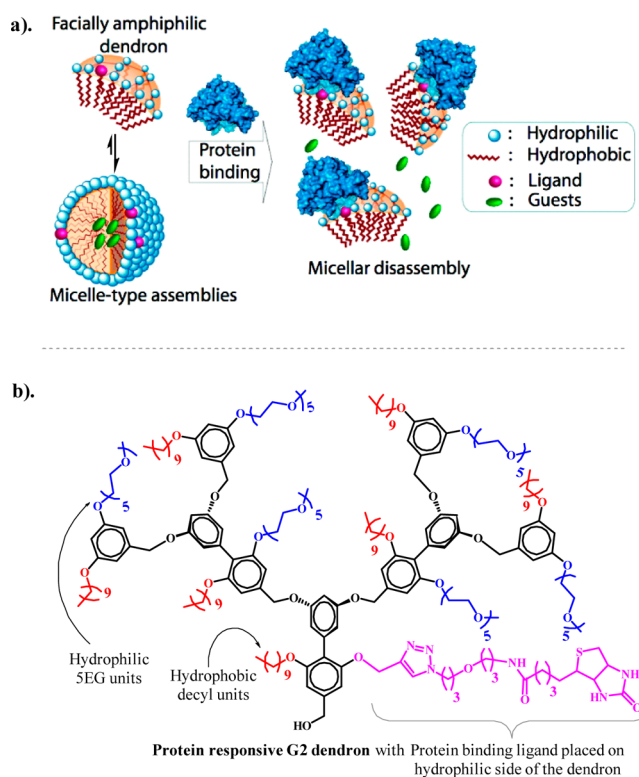


Figure 4. (a) Illustration of protein-induced disassembly; protein binds to the ligand present on the dendron's hydrophilic face, leading to the formation of an overall hydrophilic protein–dendron complex and micelle disassembly. (b) Structure of G2 dendron with biotin (ligand) placed at the core of the dendron as a hydrophilic unit.⁵⁴

We have explored the idea of binding-induced disassembly first with the ligand placed on the hydrophilic side of the amphiphilic dendron (Figure 4).⁵⁴ Dendrons were synthesized by replacing one of their hydrophilic components with a biotin moiety. These dendrons form micelle-like assemblies and thus also encapsulate hydrophobic guest molecules. When these assemblies were exposed to extravidin, a protein that has specific binding complementarity to biotin, the micelle-like aggregates disassembled, as observed with an apparent decrease in size using DLS. However, when these assemblies were exposed to other noncomplementary proteins with diversity in surface charges, no disassembly was observed. These observations support our assertion that the selectivity of these disassembly events is due to a specific ligand–protein interaction. Once the disassembly feature was established through DLS, the guest release feature was then studied by monitoring the release of a hydrophobic fluorescent probe, pyrene. The guest release experiments also supported the specificity of the supramolecular disassembly phenomenon, since exposure to other noncomplementary proteins showed very little release, if any.

Note that, in drug discovery, the most commonly targeted component of the protein is its hydrophobic binding pocket. As a result, most protein-specific small molecule ligands are hydrophobic. Therefore, if we were to ultimately translate these fundamental findings, it is critical that we expand the repertoire of the binding-induced disassembly process to hydrophobic ligand moieties. This is challenging, because these ligands will be buried in the hydrophobic interiors of the micelle-like structures (Figure 5). However, we previously noted the

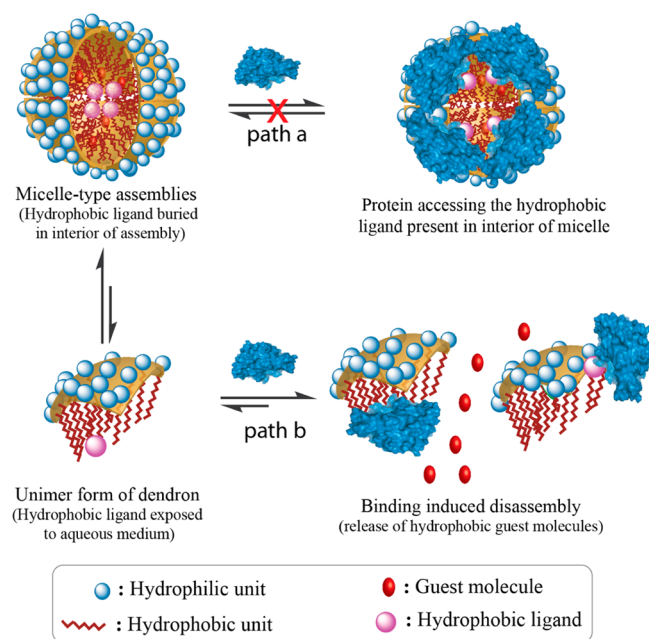


Figure 5. Illustration of protein-induced disassembly pathway available for hydrophobic ligands through the unimer–aggregate equilibrium.⁵⁵

possibility of a unimer–aggregate equilibrium during our investigation of the enzyme-induced disassembly process. In this scenario, a ligand moiety placed on the hydrophobic side of the facially amphiphilic dendron might still be available for protein binding at the unimeric state of the equilibrium. Here too, the LeChatelier type effect can affect the binding-induced supramolecular disassembly. This hypothesis was tested by incorporating the 2,4-dinitrophenyl (2,4-DNP) moiety at the terminus of a decyl chain in the hydrophobic face of the dendron.⁵⁵ It is well-established that anti-DNP immunoglobulin G (aDNP-IgG) exhibits a subnanomolar binding affinity for 2,4-DNP.⁵⁶ The assemblies formed from these 2,4-DNP conjugated dendrons indeed decrease in size and release the encapsulated guest molecules upon exposure to IgG but not to other noncomplementary proteins. This disassembly process was not observed when a control dendron with similar self-assembly characteristics but without the complementary ligand moiety was used. This study greatly expands the scope of the binding-induced supramolecular disassembly, because in principle, this process can be extended to any protein, as long as there is a known ligand binding partner.

So far, we have outlined supramolecular disassembly of facially amphiphilic dendron-based assemblies triggered by a single stimulus. As the next step, we were interested in multiple stimuli-responsive assemblies based on the amphiphilic dendritic systems due to the potential for enhanced selectivity in stimuli responsiveness, which is critical in many targeting applications.⁵⁷ Drawing inspiration from molecular logic

gates,^{58,59} a dual protein stimuli-responsive AND gate design was introduced to the amphiphilic dendron system. By doing so, this system would only respond to the concurrent presence of two different proteins (Figure 6).⁶⁰ To test this possibility, we designed a dendron molecule containing an enzyme-sensitive coumarin ester as the hydrophobic moiety and a protein-specific 2,4-DNP ligand as part of the hydrophilic PEG moiety (Figure 6).⁶⁰ In aqueous solution, the dendron forms micelle-like assemblies (characterized by DLS and TEM) with the hydrophilic moiety exposed on the exterior, and thus the accessibility of the coumarin ester moiety to the PLE enzyme would be minimal. Therefore, there will be no fluorescent product (umbelliferone) formation, when the dendron is exposed to PLE alone. However, we envisaged that the binding interaction between the 2,4-DNP and anti-DNP IgG would cause the equilibrium to be shifted toward the dendron–IgG complex, which is likely to be unimeric in nature. This protein-induced shift in equilibrium favoring the unimer increases the opportunity for PLE to cleave the coumarin ester. When the release of the fluorescent umbelliferone from the coumarin ester cleavage was monitored, the release due to the dual protein triggers was indeed found to be 26 times faster than that due to the enzyme alone. In order to validate whether this difference in release rate is truly due to the specific protein–ligand binding, the system was tested for response to nonspecific binding proteins, and no discernible release was observed. This relay of protein binding and enzymatic cleavage events presents a unique opportunity where molecular release can be achieved only in the presence of both the cleaving enzyme and binding-specific protein. Finally, taking advantage of the reversibly photo-cross-linkable coumarin functionality incorporated in the molecular design, micelles were locked in their aggregate state.⁵³ This cross-linking thus acts as an additional gate. In this case, the de-cross-linking that is necessary to re-establish the unimer–aggregate equilibrium serves as an additional AND gate; note that the dynamic equilibrium is essential for the protein–enzyme AND gate outlined above. When 250 nm light was used as the stimulus, this hypothesis was indeed found to be true. The cross-linked aggregates did not show any response in the presence of enzyme or protein; however, upon de-cross-linking of these assemblies, the dendron–aggregate equilibrium was reestablished, prompting the coumarin release in the presence of enzyme and protein. This combination of light, protein, and enzyme stimulus needed for molecular release significantly enhances the specificity of the system. The molecular design principles developed in this fundamental study will be helpful in downstream drug delivery and sensing applications, which is the present focus in our laboratories.

4. SUPRAMOLECULAR ASSEMBLIES FOR PEPTIDE EXTRACTION

Thus far, we have discussed the design, characterization, and use of micelle-type assemblies formed from facially amphiphilic dendrons, especially as stimuli-responsive assemblies. Herein, we will focus our discussion on the inverse micelle type assemblies formed from facially amphiphilic molecules in apolar medium. The stability and the hydrophilic container properties of these inverse micelles followed by their utility in charged based extraction of hydrophilic molecules will be the subject of discussion in this section. As noted earlier, the facially amphiphilic dendrons can form either micelle or inverse micelle type assemblies depending on the nature of solvent in

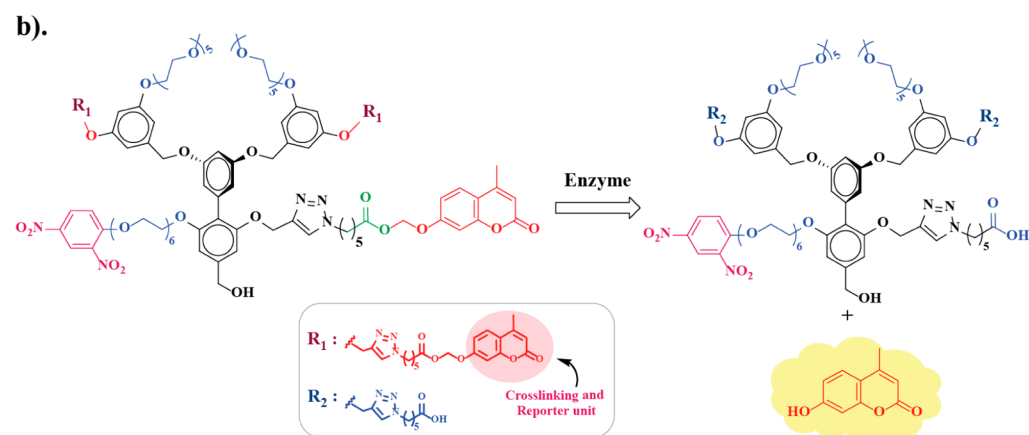
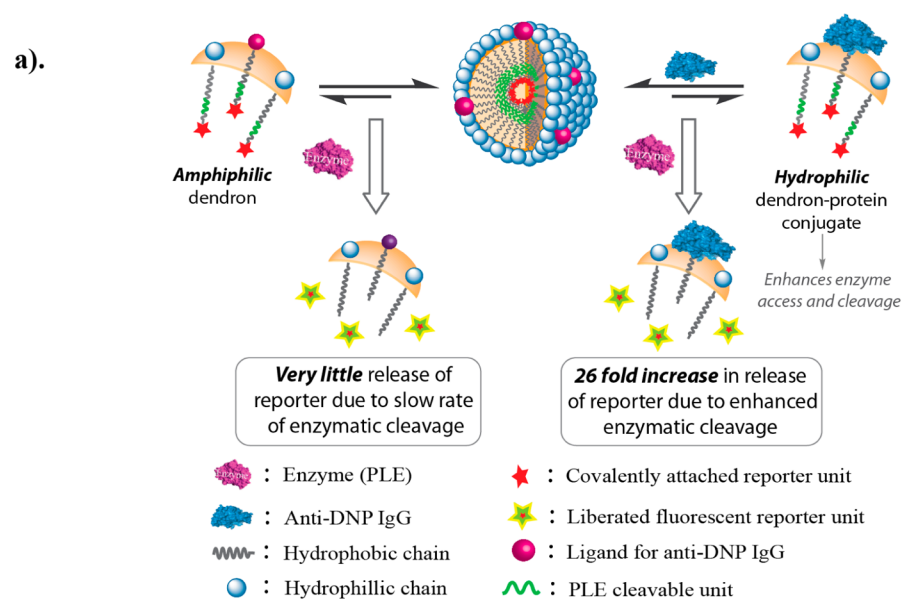
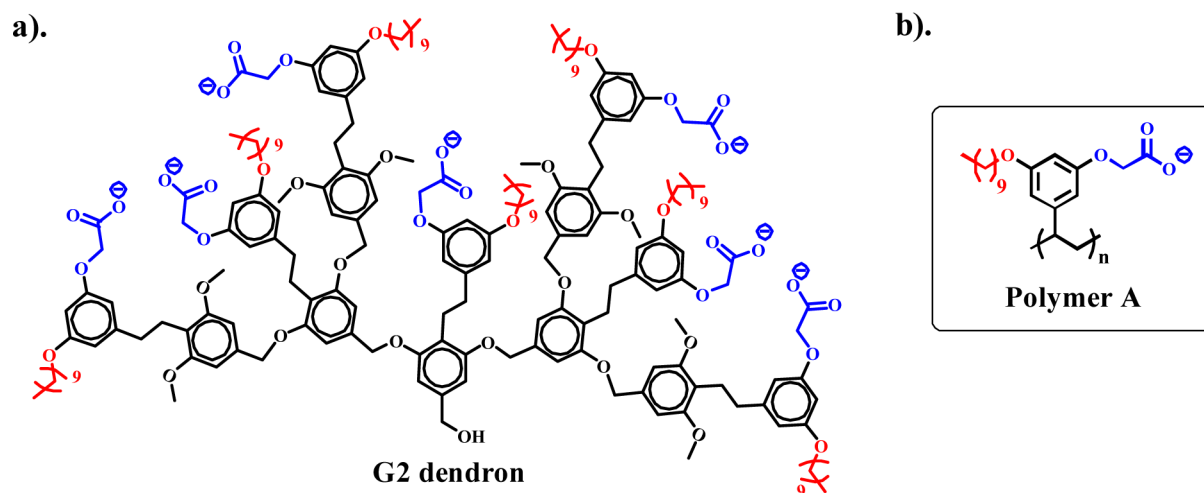


Figure 6. (a) Illustration of dual responsive system. (b) Enzyme induced change in the amphiphilic dendron accompanied by fluorophore release.⁶⁰

Scheme 4. Molecules Used for Charge Selective Peptide Extraction: (a) G2 Dendron⁶² and (b) Homopolymer.⁶¹



which they are dispersed. Considering this, we wanted to test the fate of these assemblies (micelle or inverse micelle type) in a biphasic mixture of polar and apolar solvents. For this purpose, we utilized a facially amphiphilic polymer, polymer A

(Scheme 4),⁶¹ which forms micelle and inverse micelle type assemblies when dispersed in water and toluene medium, respectively. These assemblies, when dispersed in a biphasic mixture of toluene and water, did not equilibrate between the

two solvents. Instead, they retained the initial state of the assembly, that is, these assemblies were micelle-like if the polymer was initially dissolved in water and inverse micelle-like if they were initially assembled in toluene. This suggests that these amphiphilic assemblies are kinetically trapped and do not undergo thermodynamic equilibration in biphasic solvent mixtures. This feature turned out to be the case for facially amphiphilic dendrons as well (Scheme 4).⁶²

Considering the ability of the assemblies to retain their initial structure (micelle or inverse micelle type) in heterogeneous solvent medium, we proposed to test the fate of guest molecules encapsulated in their interior. It was found that though the assemblies remained in initially assembled solvent medium, the guest molecules sequestered in these assemblies partitioned into their thermodynamically favorable solvent. For example, hydrophilic guest molecules encapsulated in the inverse micelle type assemblies partitioned into the aqueous phase when mixed with water, whereas the assemblies themselves remained in the toluene phase. We envisioned the applicability of this property in the context of selective mass transport of hydrophilic guests from aqueous medium to inverse micelle interiors, driven by the complementarity of the functional groups within the inverse micelles with those in the guest molecules. This was first demonstrated using the inverse micelle type assemblies formed by polymer A, which have negatively charged hydrophilic cores. These inverse micelle type assemblies in toluene were extracted with an aqueous solution containing a mixture of positively and negatively charged, Rhodamine 6G and Rose Bengal, respectively.

Selective extraction of positively charged hydrophilic Rhodamine 6G into the inverse micelle interiors was observed (Figure 7). This prompted us to investigate the capability of the inverse micelle type assemblies to extract charged biomolecules. Though these studies were done with dendron and polymer based systems, we will limit our further discussion to only the inverse micelle type assemblies formed by facially amphiphilic dendrons, since the work based on the amphiphilic homopolymers is reviewed elsewhere.⁶³

4.1. Selective Peptide Extraction Using Facially Amphiphilic Dendron Based Inverse Micelles

Analysis of peptides in complex biological mixtures, such as multiprotein digests and cell lysates, is a significant challenge in the field of proteomics.^{64–66} Efficient sample preparation is as essential as accurate sample analysis is in realizing an efficient method that is both selective and sensitive. In this section, we will discuss how this was achieved using a charge selective extraction strategy in combination with sensitive matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) analysis.

Peptides possess a specific isoelectric point (pI), defined by the pH at which the entire peptide is charge neutral. Thus, peptides can be positively or negatively charged in aqueous solutions at a pH below or above their pI, respectively. This charged state of the peptides can electrostatically interact with the complementarily charged interiors of inverse micelle type assemblies. Since the charge on peptides is dictated by the pH of solution, selective variation of solution pH can predictably induce a charge on the peptides of interest, which can be then exclusively extracted using dendrons of opposite charge.

Dendrons of several generations containing a carboxyl moiety as the hydrophilic functionality and a decyl moiety as the hydrophobic functionality were synthesized and used to

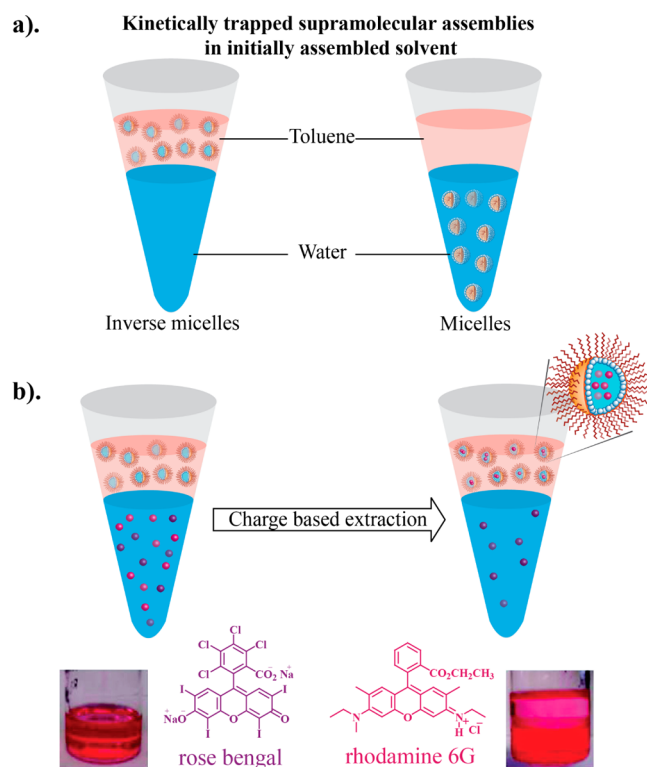


Figure 7. (a) Illustration of assemblies kinetically trapped in initially assembled solvents in heterogeneous solvent mixture. (b) Selective extraction of positively charged rhodamine 6G from water to the toluene phase using inverse micelle assemblies with negatively charged hydrophilic interior.⁶¹

form inverse micelle type assemblies (Scheme 4)⁶² with CACs of 50, 2.5, and 1 μM for G1, G2, and G3 dendrons, respectively.

The selective peptide extraction capability of these inverse micelles was tested by mixing the organic phase (toluene) containing inverse micelles with an aqueous phase (water) containing a mixture of peptides with variable charge (Figure 8). Since the inverse micelles do not disintegrate in heterogeneous solvent mixtures as discussed earlier, only the positively charged peptides should be driven into the water pool of inverse micelles owing to their complementary electrostatic interactions with the negatively charged carboxyl groups. MALDI-MS analysis of these inverse micelles after the extraction indeed suggested selective extraction of positively charged peptides predominantly. This method therefore offers a simple but efficient strategy for selective separation of peptides from complex mixtures, which will likely have considerable implications in the field of protein detection.

5. CONCLUSIONS

Self-assembling supramolecular systems have tremendous implications in various biological applications. We have discussed how structural variations at the molecular level can impart desired stimuli responsive properties in amphiphilic dendron-based systems. We pay particular attention to how disassembly of supramolecular aggregates was achieved using biological stimulus based on enzymatic and nonenzymatic proteins. This was done utilizing covalent modification of the dendrons by the enzymes or through noncovalent interactions between proteins and the assembly forming amphiphilic dendrons. These amphiphilic systems based on facially

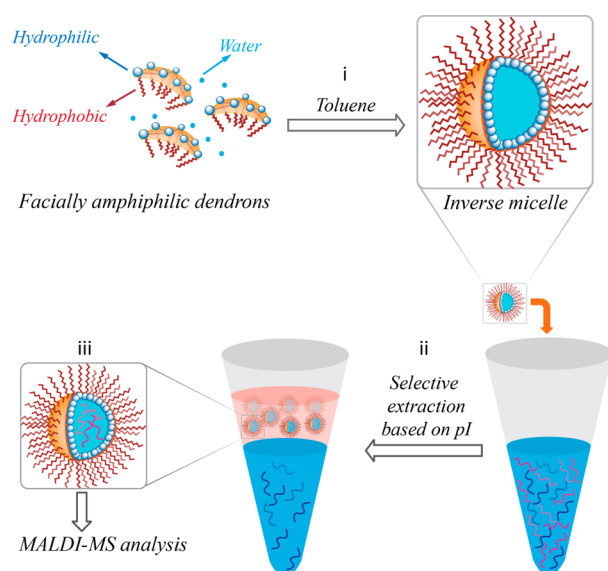


Figure 8. Schematic of selective peptide extraction showing the following stages: (i) formation of inverse micelle assemblies in toluene using amphiphilic dendrons, (ii) extraction of selective peptides present in aqueous phase to the organic phase using inverse micelles, and (iii) analysis of the inverse micelles containing selectively enriched peptides using MALDI-MS.

amphiphilic dendrons provide an excellent model to understand and develop molecular design principles that can be translated to other amphiphilic systems. This is one of our current foci where we are applying these principles to biodegradable polymer systems to realize a more practical platform for stimuli responsive drug delivery.

We have also discussed how an interesting feature of dendron self-assembly (assemblies, kinetically trapped in the initially assembled solvent) was translated into a platform for facile extraction of hydrophilic guest molecules. Selectivity in guest molecule extraction was demonstrated through charge-based interactions between the hydrophilic guest molecules and the amphiphilic dendrons. Utilizing the solution pH-induced charge on peptides, positively charged peptides were selectively extracted from a complex peptide mixture, using negatively charged dendrons; the extracted peptides were then detected using MALDI-MS with good sensitivity. This facile method developed here will have a considerable impact in the field of proteomics and biomarker detection.

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