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A Biocompatible Surfactant with Folded Hydrophilic Head Group: Enhancing the Stability of Self-Inclusion Complexes of Ferrocenyl in a β-Cyclodextrin Unit by Bond Rigidity

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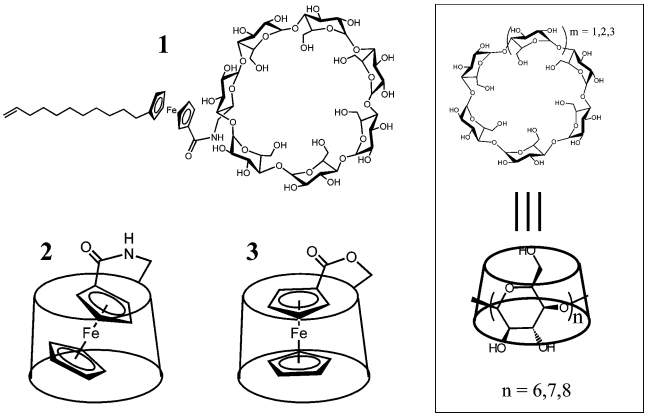
Abstract: This work reports a new biocompatible surfactant structure, of which the hydrophilic head group is composed of a folded, stable self-inclusion complex of a ferrocenyl substituted β-cyclodextrin (βCD). While multiple intra- or intermolecular complexes can exist for this amphiphile, the molecule folds into a unique intramolecular complex with well-defined conformation, in which part of the aliphatic chain and the ferrocene group are both included in the annular cavity of βCD. Study of different isosteric covalent linkages indicates that this folded structure is stable against displacement by the presence of other small guest molecules. Furthermore, in contrast to ferrocene-CD conjugates that are without the aliphatic chain, the presence of small guest molecules in solution does not influence at all the induced circular dichroism signal of this amphiphile, indicating a sterically congested, but stable, folded conformation of the inclusion complex. This new amphiphile is surface active and, more importantly, does not denature the membrane protein bacteriorhodopsin. Finally, because this surfactant forms self-assembled aggregates, this work introduces a folded structure into soft matters formed by amphiphiles in water.

Introduction

Amphiphilic molecules and liquid crystals are two classes of organic molecules, for which the details of the molecular structure determine the properties of the assembly or the phases at the meso- or macroscopic level. The phase behaviors and the anisotropic properties of these assemblies are fundamentally interesting[1] and can be tremendously useful for biological studies. For instance, the cubic phase formed by lipid bilayers has been demonstrated to successfully recrystallize membrane proteins.[2] Liquid crystal materials have been used to amplify and transduce protein binding events into optical signals without any chemical or radioactive labels.[3,4] Recent progress in the study of amphiphilic molecules has also shown significant advances in making new molecular and self-assembled structures[5] as well as in exploring new functions and applications. Particularly, gene delivery is accomplished by controlling the oxidation state of a novel redox-active surfactant.[6] Templated growth of biomimetic bond materials has been demonstrated by carefully engineering the molecular details of a peptide surfactant that self-assembles into a well-defined structure that exhibits targeted functions.[7]

Scheme 1



In this work, we aim to build a new amphiphilic structure that incorporates two distinct properties into its molecular structure. First, this surfactant molecule should consist of a switchable moiety that can be reversibly toggled between two states by external stimuli. Second, the assemblies formed by this surfactant should be biocompatible for working with proteins and cells. Specifically, unlike traditional ionic surfactants, this molecule should retain the native structure of a folded protein.

Our design of the switchable surfactant involves covalently linking an alkenyl ferrocene with a β-cyclodextrin (βCD) (Scheme 1). Cyclodextrin (CD) is known to form a wide variety of inclusion complexes both intra- and intermolecularly. When covalently derivatized at the 5′ position (small annular face) or

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2′/3′ position (large annular face) with a substituent of proper size, the cyclodextrin can form either intramolecular (self-inclusion)[8] or intermolecular complexes with the substituent, where the substituent is inserted in the annular cavity of the CD.[9] For these studies of inclusion complexation of small molecules, the size of the ensemble of different possible conformations is often small and negligible.[10]

Inspired by the ubiquitous folding of biomolecules and artificially designed foldamers in water,[11] we set out to explore if molecular folding can be designed into the structure of an amphiphile based on inclusion complexes of cyclodextrin. Whereas the folding of a protein or a foldamer involves a backbone of amide bonds, we study the multiple folding possibility of a ferrocene group with β-cyclodextrin. By introducing a ferrocene group tethered with an aliphatic chain at the 5′ position of βCD (Scheme 1), amphiphilic structure **1** can exist in multiple modes of complexation and folding to arrive at different conformations (Figure 1). The complexation can be either intramolecular or intermolecular. Two possible intramolecular complexations exist. One proceeds with direct complexation leaving the aliphatic chain trailing out of the 5 smaller annular face (structure **A** in Scheme 1). The other one involves threading the aliphatic chain into the annular cavity first to give an intermediate structure **B**, and then further binds the ferrocene in the βCD cavity to afford the final structure **C**. For intermolecular complexation, three possible pathways exist. In these pathways, the aliphatic chain and the ferrocene can either thread through the 5′ small annular face or thread through the 3′ large annular face to afford dimeric structure **D** or oligomeric structures **E** and **F**.

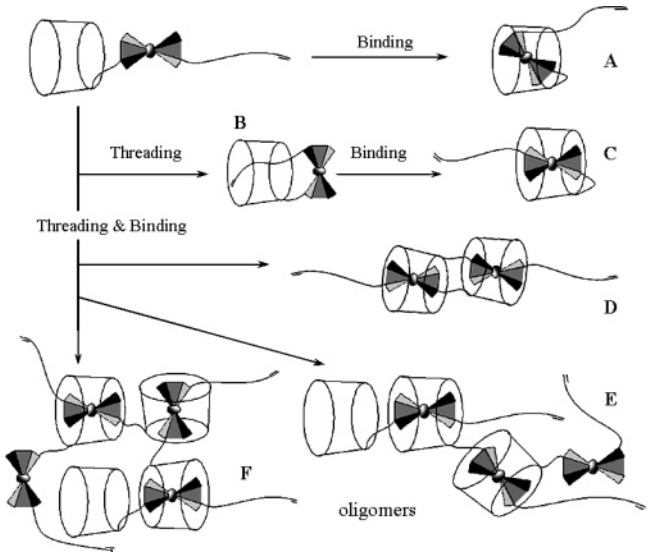

Figure 1. Possible modes of inclusion complexes and different folded conformations of amphiphile **1**. Structure A shows a folded conformation in which both the aliphatic chain and ferrocene are included in the βCD. Structure **B** shows an intermediate, in which the aliphatic chain threads through the βCD. Structure **C** shows a self-threading complex. Structure **D** shows a dimer formed by intermolecular threading. Structures **E** and **F** show different oligomers formed by intermolecular complexation.

Amphiphile **1** offers two new properties that are fundamentally interesting and potentially useful. First, certain molecular assemblies in the form of both liquid crystals (cubic phase) and micellar aggregate do not denature protein folding structure.[12] In particular, it is well-known that some non-ionic surfactants such as Brij and Triton are used to stabilize protein structure and reduce physical adsorption of proteins on surfaces.[13] Because cyclodextrin is non-ionic and mildly hydrophilic, amphiphilic molecules using βCD as part of the hydrophilic head group have the potential to retain the native folding of a protein. Second, this inclusion complex has the potential to exhibit reversible switching in the conformation triggered by the control of the oxidation states of the ferrocene group. Kaifer and co-workers demonstrated that by introducing a viologen group onto the CD, the complexation can be controlled electrochemically.[9c] In this work, we derivatize a redox-active group, ferrocenyl alkene, on the 5′ face of the CD. The control of the oxidation state of ferrocene can trigger a change in the conformation of the inclusion complex formed between the ferrocene and the CD. This oxidation-triggered conformational change can in turn cause changes at the level of supramolecular assembly.

For the formation of intramolecular complexation, the nature and the geometry of the covalent linkage between the ferrocene groups and βCD plays a critical role for its stability. While the ester bond has been previously used to link ferrocene groups with the βCD covalently,[14] we choose the amide bond for amphiphile **1** because the amide bond is generally more robust than the ester bond toward hydrolysis in aqueous solutions. Because the amide and ester bonds are isosteric, as they share

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the same number of atoms in the skeleton of the bonding geometry, the amide linkage should also have a propensity to allow the formation of a self-inclusion complex. Isosteric molecules have often been used to study the effect of conservative changes in bond geometry on biological structures[15] and drug activities.[16] In this work, we use this pair of isosteres to study the structure and stability of the formed self-inclusion complexes.

Few examples have demonstrated inclusion complexes that have the same level of complexity as **1**. Below we characterize the preferred conformation and stability of the inclusion complexes of amphiphile **1**, with comparison to molecules **2** and **3** (Scheme 1). We also examined the hydrodynamic size of the aggregates formed by **1**. Finally, we studied the influence of the assembly formed by amphiphilic molecule **1** on the folded structure of the membrane protein bacteriorhodopsin (BR).

Experimental Section

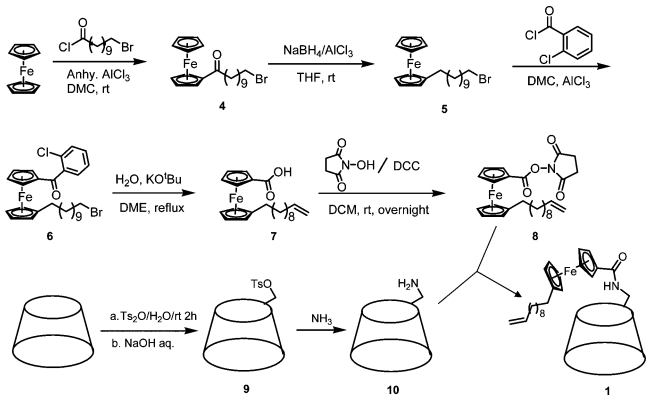
Materials and General Methods. Chemicals and solvents were purchased from Aldrich Chemical Co., Acros Organics, VWR International Inc., Pfaltz & Bauer, and Pharmca. THF was distilled from sodium metal and benzophenone, and CH_2Cl_2 was distilled from calcium hydride. All aqueous solutions were prepared with deionized water having a resistivity greater than 18.2 MΩ-cm (Milli-Q Biocel A10, Millipore, Bedford, MA).

Instrumentation. Circular dichroism spectra were recorded on an Aviv model 202 circular dichroism spectropolarimeter. Samples with guest molecules were recorded at 25 °C in a mixed solvent of 80% water and 20% ethylene glycol (EG). UV−vis absorbance was measured in water on a Beckman-DU640 spectrophotometer. NMR spectra were acquired on a Bruker 300 MHz spectrometer. Nuclear Overhause effect spectroscopy (NOESY) spectra of **1** (∼0.7 mg in 0.5 mL D_2O) were recorded on a Bruker 500 MHz spectrometer with a mixing time of 1.8 s. LCMS and HPLC were recorded on a Shimadzu LCMS-2010A spectrometer with an Alltima C18 5u, 150 mm × 4.6 mm (solvent: 10% MeCN to 20%MeCN in water).

Synthesis of Compounds 1, 2, and 3. Compound **3** was prepared according to the previously reported literature procedure.[14d] Compound **2** was prepared by coupling mono-6-deoxy-6-amino-β-cyclodextrin[17] with ferrocenecarboxylic acid. Compound **1** was prepared by a converging synthetic route where a monosubstituted amino β-cyclodextrin[17] was coupled with alkenyl ferrocenecarboxylic acid (Scheme 2).[18,19] Experimental details of the synthesis are shown in Supporting Information.

Denaturation of Bacteriorhodopsin (BR) in Detergent. [21] A suspension of 1 mg of BR in 0.8 mL of deionized water was sonicated in a centrifuge tube for 15 s, and then placed on ice for 30 s. This cycle was repeated five times, and the solution was centrifuged. The clear purple solution was retrieved from the precipitate. A total of 0.2 mL of 0.125% NaN_3 was added to the purple solution. Sodium dodecyl sulfate (SDS) or surfactant **1** was added to the BR solution to afford samples containing 0.21 mg/mL BR with (A) 0.24 mM (0.035 wt %) of **1**, (B) 0.15 mM (0.020 wt %) of **1**, or (C) 8.7 mM (0.25 wt %) of SDS. The addition of SDS caused the BR solution to become yellow, but samples with **1** retained the reddish color in the BR solution for at least two weeks in the refrigerator (4 °C).

Scheme 2



Results and Discussion

In order to elucidate the structural details and the potentially preferred conformation of the inclusion complex of **1**, we prepared isosteric molecules **2** and **3** for structural characterization comparison. Molecule **2** has an amide and molecule **3** has an ester linkage between the ferrocene and βCD. For molecule **3**, past studies have demonstrated that the presence of other small guest molecules in solution can change the orientation[8j,22] or completely displace[23] the included ferrocene from the annular ring of β-cyclodextrin.

Effect of Bond Rigidity on the Stability of the Self-Inclusion Complex. Amide and ester bonds differ in many aspects of molecular detail.[16] Perhaps the most significant difference is that amide bonds have preferred flat conformations with either trans or cis conformation, whereas ester bonds are more flexible.[16] Thus, study of these two molecules provides an opportunity to decipher the effect of bond rigidity on the structure and stability of the inclusion complexes.

Although cyclodextrins contain chiral sugars, they lack UV-active chromophores, and thus have no observable signal from circular dichroism. However, when an achiral chromophore (such as ferrocene) is inserted in the annular core of cyclodextrin, a large circular dichroism signal is observed.[24] This so-called induced circular dichroism is instrumental for deciphering the structure of CD inclusion complexes that involve small molecules with chromophores.[24] The intensity of circular dichroism is dictated by the electronic transition of the chromophore in a chiral environment and is characterized by the rotation strength. Their relationship is described by the sinusoidal

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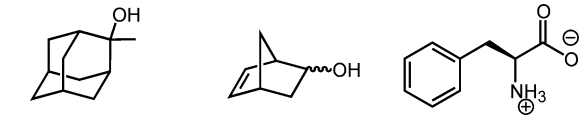
Scheme 3



2-methyl-2-adamantanol 5-norbornen-2-ol phenylalanine

function called the Kirkwood−Tinoco coupled oscillator.[25] When applied to the cases of induced circular dichroism, the same sinusoidal relationship holds.[14b,26] Specifically, inclusion of ferrocene in βCD or γCD produces a positive or negative signal, respectively, of induced circular dichroism at about 470 nm. This result corresponds to the long axis of ferrocene being aligned vertically (positive signal at 470 nm) in the annular cavity of βCD, but horizontally (negative signal at 470 nm) in γCD. Using the Kirkwood−Tinoco coupled oscillator, the tilt angle of the ferrocene with respect to the *z*-axis through the center of the cyclodextrin can be readily estimated by calculating the attenuation of the rotational strength of the complex—using the intensity of the induced circular dichroism at 470 nm (See Supporting Information for details).

Induced Circular Dichroism of 3 with and without Guest Molecules. Because the self-inclusion of ferrocene-appended βCD via ester linkage (molecule **3**) has been reported previously,[14b,c,d] we choose a new set of small guest molecules—2-methyl-2-adamantanol, 5-norbornen-2-ol, and phenylalanine (Scheme 3)—to investigate the effect of the guest molecules' properties on the self-inclusion complexes **3**, **2**, and **1**. 2-Methyl-2-adamantanol and 5-norbornen-2-ol bear a similar chemical functional group, but 2-methyl-2-adamantanol is larger in size than 5-norbornen-2-ol. Phenylalanine, an amino acid, exists as a Zwitterion in aqueous solution, and thus has the highest polarity among the three guest molecules. Because molecules with structures similar to 2-methyl-2-adamantanol and 5-norbornen-2-ol have been shown to influence the structure of self-inclusion complexes,[14b,c,d] we chose a new set of molecular structures to investigate the generality of the effects imposed by the small molecules.[27] Furthermore, there is a lack of extensive studies on the effect of nonpolar amino acids on the stability of self-inclusion complexes. We reason that the nonpolar amino acids can also influence the self-inclusion complexes by inserting the nonpolar group on the amino acid residue into the cavity of the CD, while leaving the Zwitterion portion in the aqueous solvent. Phenyl groups are known to form inclusion complexes with cyclodextrins.[8j] Thus, phenylalanine, having a benzyl group, provides an opportunity for investigating the effect of amino acids on the self-inclusion complexes.

For molecule **3**, the induced circular dichroism shows two positive bands at 470 and 350 nm, which is consistent with the ferrocene being oriented vertically along the *z*-axis inside the annular cavity of the CD (Figure 2).[14b] Upon addition of the guest molecule 2-methyl-2-adamantanol, both bands of induced circular dichroism at 470 and 320 nm drastically decreased from positive peaks to negative troughs with significantly lower

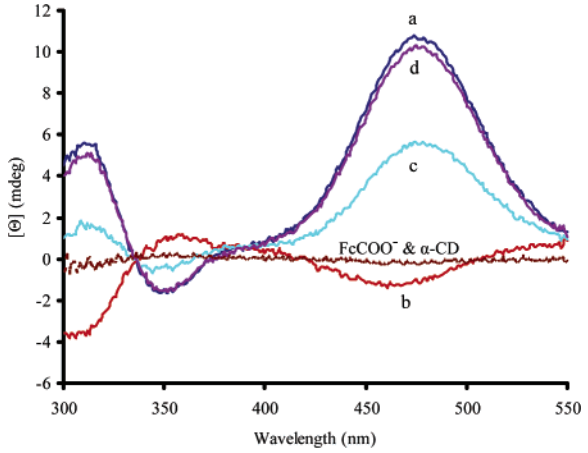


Figure 2. Circular dichroism spectra of **3** alone (a, 0.50 mM) and in the presence of 20 eq. of guest (b, 2-methyl-2-adamantanol; c, 5-norbornen-2-ol; d, phenylalanine) and $FcCOO^-$ (0.85 mM) with α-cyclodextrin (0.85 mM) (dot) at 25 °C in an aqueous solution containing 20% ethylene glycol.

intensity. However, for the guest molecule 5-norbornen-2-ol, the intensity of bands at 470 and 320 nm only decrease moderately and remain positive. For phenylalanine, there is essentially no change in the induced circular dichroism of **3**. These results suggest that the ferrocene is completely displaced from the cavity of βCD by 2-methyl-2-adamantanol (Figure 4), but only partially displaced by 5-norbornen-2-ol and not disrupted at all by phenylalanine.[14e,23e,28] Inspecting the structures of all three guest molecules, we believe that the large displacement of ferrocene by 2-methyl-2-adamantanol is primarily due to its large size in comparison to 5-norbornen-2-ol because the large hydrophobic group of 2-methyl-2-adamantanol provides a better molecular fit in the cavity of βCD. The inability of phenylalanine to displace the ferrocene can be attributed to a wide range of possibilities, including close proximity between the benzyl group and the Zwitterion, and the strong solvation in the aqueous solvent.

Induced Circular Dichroism of 2 with and without Guest Molecules. The induced circular dichroism of molecule **2** (Figure 3) differs substantially from **3** in both the absence and the presence of small guest molecules. Under the same conditions, **2** exhibits the same positive band at 470 and 320 nm as that of **3**, except that the intensity of the band at 470 nm is only half of that for **3**. Using the Kirkwood−Tinoco coupled oscillator expression,[25] this reduction of the intensity corresponds to ferrocene group tilt angle of 33° from the *z*-axis in the CD cavity (Figure 4). The similar intensity of the positive bands at 320 nm of **2** and **3** are consistent with multiple transitions with polarizations along the ferrocene moiety *z*-axis and other polarization, in the $X-Y$ plane.[29,26b] We note that all the changes in the induced circular dichroism upon addition of small guest molecules appear to be instantaneous and stable over time. The spectra of induced circular dichroism are indistinguishable whether the samples are prepared immediately or aged overnight.

Upon addition of 2-methyl-2-adamantanol to the solution of **2**, the band of induced circular dichroism at 470 nm did not exhibit any noticeable changes. But, the positive band at around 320 nm decreased drastically to a negative trough, and the peak

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(27) We have also studied the effect of guest molecule *l*-borneol, which has been published previously. Our result show that *l*-borneol exhibits the same effect as guest molecules 2-methyl-2-adamantanol (please see Supporting Information).
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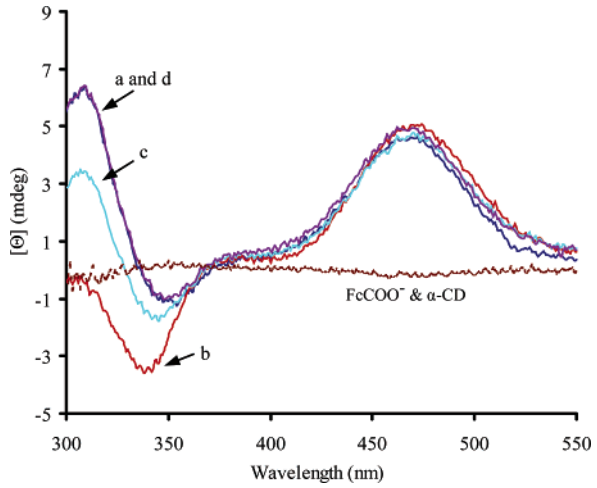

Figure 3. Circular dichroism spectra of **2** alone (a, 0.50 mM) and in the presence of 20 equiv guests (b, 2-methyl-2-adamantanol; c, 5-norbornen-2-ol; d, phenylalanine) and control: $FcCOO^-$ (0.85 mM) with α-cyclodextrin (0.85 mM) (dot) at 25 °C in an aqueous solution containing 20% ethylene glycol.

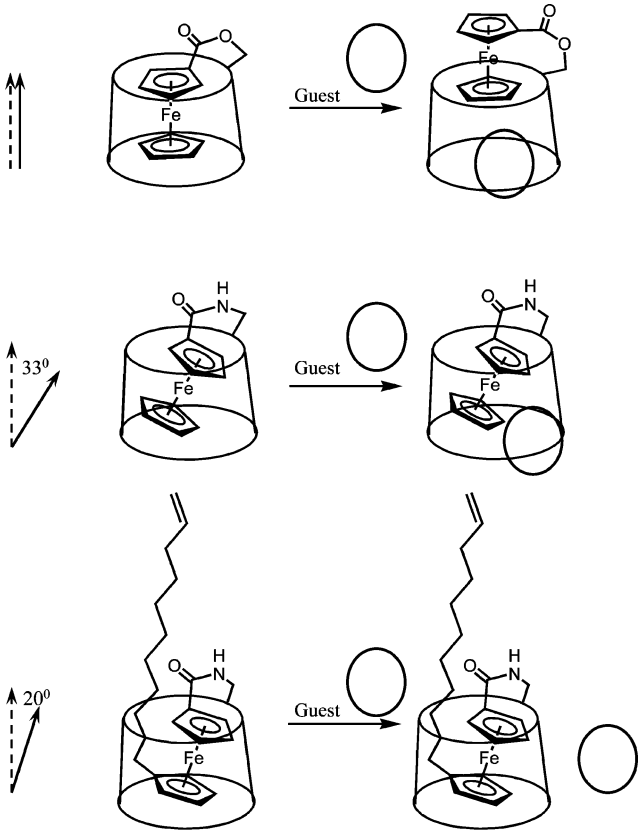

Figure 4. Schematic illustration of conformation of **1**, **2**, and **3** in the absence or presence of guests 2-methyl-2-adamantanol. The dotted arrow indicates the *z*-axis of the annular cavity of the cyclodextrin ring; the solid arrow indicates the long axis of the ferrocene moiety on the complex.

maximum shifted from 320 to 340 nm. Because the band at 470 nm is considered to be due to d−d transition of Fe(II),[29a,b,c] the magnitude of the band is not expected be affected by translating the ferrocene in the *X*−*Y* plane of the cavity of βCD.[26b] As a result, the invariance of the band at 470 nm suggests that the addition of the guest molecule 2-methyl-2-adamantanol does not cause the ferrocene to be either rotated relative to the *z*-axis nor displaced from βCD cavity. The band at 320 nm is involved with transitions of multiple polarizations along the *x*-, *y*-, and *z*-axes, and thus it is difficult to pinpoint a single transition.[26b] Together with the invariance of the band at 470 nm, this result suggests a translocation of the ferrocene in the *X*−*Y* plane to accommodate the partial insertion of the guest molecule 2-methyl-2-adamantanol into the cavity from the large annular face (Figure 4).[14b,30] When inspecting the effect from other small guest molecules—5-norbornen-2-ol and phenylalanine—on the self-inclusion structures of **2**, we observe a consistent effect on the induced circular dichroism of molecule **2**: The band at 470 nm remains unchanged upon addition of all guest molecules tested, but the band at 320 nm decreases to different extents depending on the size of the guest molecules. For 2-methyl-2-adamantanol, the band at 320 nm reduces to the greatest extent. But for 5-norbornen-2-ol, the band at 320 nm reduces to only half of its original intensity (i.e., without the presence of guest molecules). Phenylalanine has no observable influence on the induced circular dichroism of **2**. Overall, our results suggest that the ferrocene in molecule **2** is not displaced or excluded from the annular cavity of the βCD ring, and some guest molecules can partially insert into the βCD ring to displace the ferrocene from the center of βCD.

It is important to note that although the influence of each small guest molecule on **2** is different from that on **3**, the trend of the influence on both complexes is the same: 2-methyl-2-adamantanol imposes a significant impact on the structure of the self-inclusion complex of **3** and **2**, while 5-norbornen-2-ol has a moderate influence and phenylalanine has no impact on the self-inclusion complexes.

For a control experiment, we examined the circular dichroism of a mixture of αCD and ferrocene carboxylate in water (0.85 mM, pH = 8.8). Because αCD consists of only six glucopyranose monomers (whereas βCD consists of seven glucopyranose monomers) and thus has a smaller annular diameter, αCD cannot form a one-to-one inclusion complex with ferrocene.[31] Circular dichroism of this system shows two bands with intensities close to zero: a negative band at 470 nm and a positive band at 340 nm. This result supports the general interpretation that the ferrocene group is not inserted in αCD, but is inserted in the cavity of βCD for all three molecules **3**, **2**, and **1** (please see later).

When comparing the covalent linkage from an ester bond to an amide bond, the induced circular dichroism responds differently upon addition of small guest molecules. For amide linkage of **2**, the positive band at 470 nm is not affected upon addition of any of the guest molecules investigated, indicating that ferrocene is not excluded from the annular cavity. For ester linkage of **3**, the band at 470 nm is reduced to a small negative band consistent with ferrocene being displaced by the small molecules. This result suggests that the self-inclusion complex with an amide linkage is more stable against the displacement

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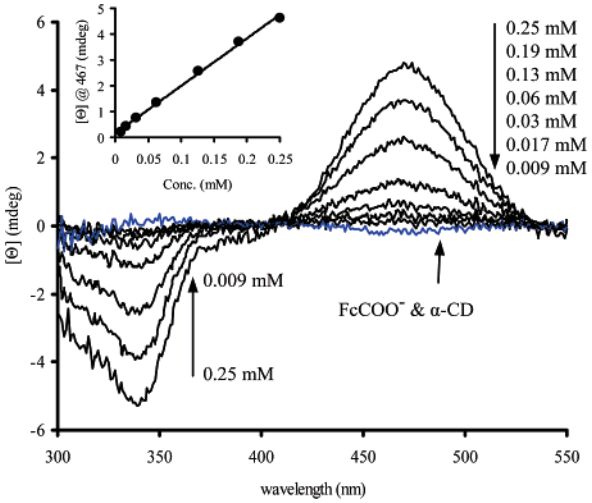

Figure 5. Circular dichroism spectra of **1** (black) at different concentrations and $FcCOO^-$ (0.85 mM) with α-cyclodextrin (0.85 mM) (blue) at 25 °C in 20% EG aqueous. The insert shows the plot of peak intensity of the band at 467 nm versus the concentration of **1**.

by guest molecules than that with an ester linkage. This result is also consistent with the amide bond being more rigid than the ester bond and thus hindering the molecular motion of ferrocene being displaced from the *β*CD.

Induced Circular Dichroism of 1 with and without Guest Molecules. When an aliphatic chain is attached to the ferrocene, amphiphile **1** can in principle form either inter- or intramolecular complexes (Scheme 1). Circular dichroism of amphiphilic molecule **1** shows a band at 470 nm that is similar to that observed in molecule **2** (Figure 5). This result strongly suggests that the ferrocene is included in the annular cavity of *β*CD. Under the same conditions as that for molecule **3**, we identify a retention of 83% in the peak intensity in the 470 nm band. On the basis of the Kirkwood-Tinoco expression, this reduction corresponds to the ferrocene being oriented approximately 20° from the *z*-axis of *β*CD in **1** (Figure 4). Further investigation of the effect of concentration on the induced circular dichroism of **1** indicates that the intensity of the band at 470 nm decreases linearly over 30-fold dilutions (from 0.25 to 0.009 mM) of **1** in water (inset of Figure 5). This result is consistent with an intramolecular rather than intermolecular complexation of **1** in solution. We note that in a previous work that characterizes an intermolecular complexation between a *β*CD and a pyrene moiety,[9d] the signal intensity shows the characteristic curvature for intermolecular binding (plateau at high concentration) in the same concentration range as shown in Figure 5. Furthermore, we used NMR spectroscopy to characterize the key proton−proton interaction (nuclear Overhauser effect) that identifies the specific mode of the self-inclusion complex (see later).

In contrast to both **2** and **3**, the *addition of each of the small guest molecules* causes no observable change in the entire spectrum of circular dichroism of **1** (see Supporting Information). Comparing the circular dichroism spectra of **1** and **2**, the induced circular dichroism of **1** (with or without addition of small guest molecules) appears to be identical to the induced circular dichroism of complex **2** with the addition of 2-methyl-2-adamantanol. This result leads us to suggest a unique conformation of **1** in the form of **A** (Scheme 1). In this conformation of structure **A**, the aliphatic chain is partially embedded in the annular cavity along with the ferrocene group. Because the aliphatic chain occupies some of the volume of the cyclodextrin cavity, this congestion shifts the ferrocene moiety from the center of the *β*CD. Furthermore, because of this congestion, the aliphatic chain functions as a small guest molecule inside the cavity of cyclodextrin that prevents any further inclusion of other small guest molecules.

This result also suggests that other conformations (**D**, **E**, and **F**) are not favored for two reasons. First, the intermolecular complexations (**D**, **E**, and **F**) (Figure 1) do not seem to have a structural constraint for the ferrocene to be tilted from the *z*-axis inside the cavity of *β*CD, and thus the signal at 470 nm should not be attenuated. Second, for the ferrocene to be displaced from the cavity of *β*CD in the intermolecular complexes, the amide linkage does not have to go through a conformational change that is required for the intramolecular complex, which includes a cis to trans transformation. Thus, it should be possible to displace the ferrocene should the complexation be intermolecular.

Figure 4 summarizes the effectiveness of the guest molecule, 2-methyl-2-adamantanol, at displacing the ferrocene in **1**, **2**, and **3**. For molecule **3**, the included ferrocene adopts a conformation where the long axis of the ferrocene is parallel to the *z*-axis of the cavity of the CD and is displaced (or excluded) to the outside of the cavity in the presence of 2-methyl-2-adamantanol. For molecule **2**, the included ferrocene has an orientation with the long axis of the ferrocene tilted about 33° from the *z*-axis of the *β*CD and is only shifted from the center of the cavity but still included in the *β*CD. For molecule **1**, the included ferrocene is tilted 20° from the *z*-axis of the *β*CD and is not affected by the presence of guest molecules.

Structural Study of 1 by Proton NMR Spectroscopy. Although induced circular dichroism of amphiphile **1** suggests an intramolecular complexation of ferrocene group in the annular cavity of *β*CD, the results do not rigorously eliminate the threaded conformation **C**. To further reveal detailed structural features of **1**, nuclear Overhauser enhancement spectroscopy (^{1}H NOESY) was employed to examine the interproton distance within the molecules (Figure 6). NOESY is particularly useful for determining the conformation of a molecule because the NOE signal is a through-space effect that arises when two protons are close (within 5 Å) in space.[32] When the NOE signal arises between two protons that are far apart in bonding connectivity in a molecule (so-called long-range NOE), the result suggests that the molecule is folded in a certain way such that the two protons are close in space. Because amphiphile **1** can, in principle, form either intra- or intermolecular complexes, and because the complexes can adopt different conformations (Scheme 1), the existence of the long-range NOE becomes invaluable at eliminating conformations that cannot be distinguished by the circular dichroism alone.

Inspecting the ^{1}H NMR spectroscopy of **1**, we first note that the chemical shifts of the protons on the ferrocene have been split into a multiplet consisting of twelve peaks in the region of 4.1 to 4.6 ppm. For a disubstituted ferrocene such as molecule **6**, the eight protons usually exhibit four peaks with equal intensity because of the symmetry of the molecule (see Supporting Information for the proton NMR of **6**). One possible interpretation for the splitting of these ferrocene protons in **1** can be attributed to the possibility that the ferrocene group is

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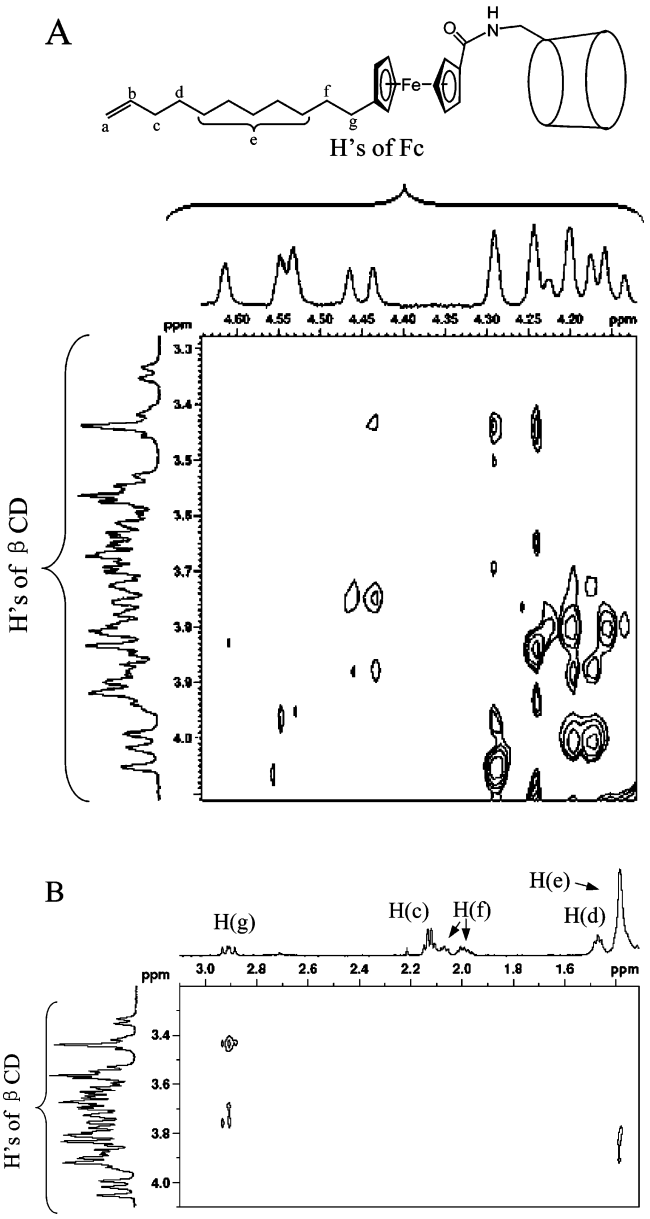

Figure 6. Selected region of 2-dimensional NOESY ^{1}H NMR spectrum of **1** in D_2O at ambient temperature. Long-range NOE between (A) protons of ferrocene group and protons of βCD and (B) protons of aliphatic chain and protons of βCD.

located in a highly chiral microenvironment, and each of the protons experiences a different chemical environment.

Inspecting the ^{1}H NOESY of **1**, we observed two sets of long-range NOE that are important for deciphering the structure of the conformation of surfactant **1**. First, we observed NOE between the protons on the ferrocene and the protons on the cyclodextrin (Figure 6A). This result is consistent with an inclusion complex where the ferrocene is inserted in the annular cavity of the βCD. Second, and more importantly, we observed considerably strong NOE between the protons on the aliphatic chain (H_e) and on the cyclodextrin (Figure 6B). This second set of long-range NOE signals helps eliminate conformations that are not present in the solution. Because the group of proton H_e belongs to the central portion of the aliphatic chain, they would be too far away from the protons on the βCD in the conformation **C**, **D**, **E**, or **F** (Figure 1). Conformation **B** is also eliminated because the induced circular dichroism and NOE

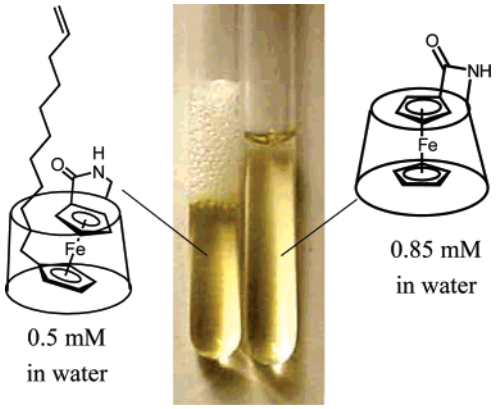

Figure 7. Amphiphilic molecule **1** (0.075 mM) and model molecule **2** (0.85 mM) in water after pipetting air into the solution.

indicate that the ferrocene is inserted in the annular cavity of βCD. Together, the results from NOESY and circular dichroism indicate that the most likely conformation of **1** is in the form of folded structure **A**. Furthermore, because of the alkenyl substitution on the cyclopentadienyl ring of ferrocene that are immersed in the βCD cavity, the ferrocene group is likely to be shifted from the center of the annular cavity because of the crowded congestion. This structure (conformation **A**) also supports the interpretation that each of the eight protons on the ferrocene is located in a different position in a chiral microenvironment.

Surface Activity of 1. Because molecule **1** is amphiphilic, we conducted a simple experiment to test if **1** is surface active. Figure 7 shows the solution of molecule **1** (0.075 mM) and molecule **2** (0.85 mM) in water. Upon pipetting air into each of the solution, an intense foaming was observed in the solution containing molecule **1**, whereas the solution containing molecule **2** did not show any noticeable foaming. This result suggests that molecule **1** is surface active in its reduced state. βCD is known to have an anomalously low solubility in water compared to αCD or γCD.[33] For molecule **1**, the burying of part of the aliphatic chain inside the annular core of CD may have reduced the overall hydrophobicity of the molecule and rendered it water-soluble for the observed foaming. The quantitative measurement of the surface activity is the subject of another study.

Self-Assembly of Surfactant Molecule 1. Amphiphilic molecules that show surface activities do not necessarily self-assemble to form aggregates in aqueous solution, although they often do. For example, cationic bola surfactants containing ferrocenium and ammonium groups are highly surface active, but do not form aggregates in solution.[34] Because our primary interest is to investigate whether this surfactant can be biocompatible in retaining the native folding of membrane proteins, it is important to characterize the aggregate formation in aqueous solution. Dynamic light scattering of surfactant **1** shows a small critical aggregate concentration of **1** around 7 μM. This low critical concentration indicates that surfactant **1** has a high propensity to form aggregates in water and is consistent with other assembled structures that involve cyclodextrin.[35]

Biocompatibility with Membrane Proteins. Membrane proteins are critical for a wide range of vital biological activities

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including transportation of ions and metabolites (pumps and channels), as well as molecular recognition of hormones and neutransmitters (receptors). Because membrane proteins are amphiphilic in nature, the structural elucidation of membrane proteins is the most difficult among all the biomolecules. To date, recrystallization of membrane protein assisted by non-denaturing surfactants seems to be the most promising means. The type and the number of the surfactants suitable for recrystallization are, in spite of strong efforts, rather limited. So far, only certain sugar-based surfactants and the liquid crystalline phase of lipids (cubic phase) are known to retain the native folding structure of a membrane protein and sometimes produce workable crystals of membrane proteins.[2,36] Furthermore, the conditions suitable for membrane protein recrystallization are highly empirical and elusive.[37]

In this work, we examined the ability of the assemblies formed by **1** to retain (or to denature) the native folded structure of a membrane protein, bacteriorhodopsin.[38] Bacteriorhodopsin (BR) is an integral membrane protein that catalyzes the light-induced transfer of protons across the membrane. This membrane protein contains a single polypeptide chain of 248 amino acids that form seven α-helices, which are largely embedded in the lipid bilayer. In the hydrophobic core of the seven α-helices, an all-trans retinal is bound covalently to a specific lysine residue (Lys 216) via a protonated Schiff base. This unique binding of retinal provides a convenient window for monitoring folded and unfolded states of BR. When bound with retinal, the whole folded BR affords a unique transition in a UV−vis spectrum with λ_{max} at ∼560 to 570 nm, which also accounts for the purple color of BR. When treated with denaturant such as sodium dodecyl sulfate (SDS), bacteriorhodopsin denatures through an intermediate state ($t_{1/2}$ < 1 min), in which the retinal is still attached, but its structural interaction with the rest of the protein is lost. The denaturation of BR further proceeds to the state where the retinal binding is lost and the secondary structure of BR is partially denatured, which affords samples with a yellowish color. The free unbound retinal has a UV transition with λ_{max} at ∼380 nm.

Figure 8 shows the UV−vis absorption of bacteriorhodopsin in the presence of molecule **1** (0.24 and 0.15 mM) and SDS (8.7 mM). In presence of SDS, the sample turned yellow (inset of Figure 8), and the transition at 570 nm diminished to the baseline, and a new transition at 395 nm emerged. This result is consistent with the denaturation of BR, which causes the loss of the bound retinal from BR and the increase of the concentration of free retinal in the sample solution. We did not attempt to capture the intermediate state of the denaturation of BR. In contrast, in the presence of surfactant **1** above the critical concentrations of aggregation formation, the BR sample remains purple in color (inset of Figure 8), and the peak for the transition at 570 nm is retained at concentrations of 0.24 and 0.15 mM of **1** without noticeable changes. Together, these two results indicate that aggregation of **1** does not denature bacteriorhodopsin. The current state of the art membrane recrystallization often includes the use of biocompatible surfactants, and the screening of thousands of different conditions with small variations. Molecule **1** provides an additional surfactant structure and aggregration morphology that has the potential for recrystallizing and studying the structure of membrane proteins.

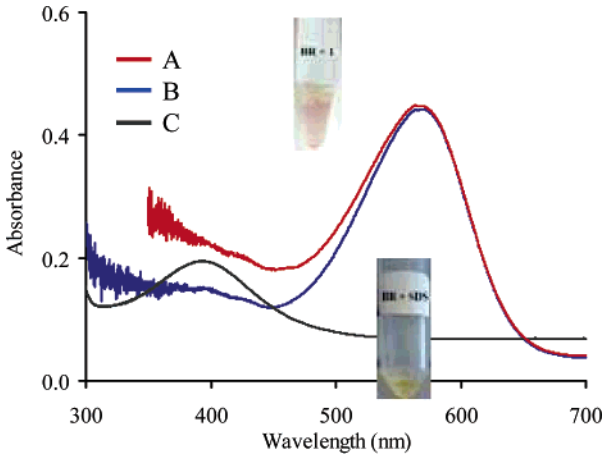

Figure 8. UV−vis spectrum of bacteriorhodopsin with 0.24 mM of **1** (A), 0.15 mM of **1** (B), and 8.7 mM of SDS (C).

Conclusion

A new amphiphilic molecule, **1**, composed of a switchable (redox-active) group in an inclusion complex is described. Most notably, this molecule folds into a specific self-inclusion complex via a unique path of intramolecular interactions, among several different possible pathways that could lead to other structures. By using a more rigid covalent bond (amide rather than ester bond) that connects the key components of this molecular structure, the stability of the self-inclusion complex against displacement by small guest molecules is enhanced. This new amphiphilic molecule is surface active and forms aggregates. Furthermore, the self-assembled aggregates of **1** retain the native folding of the membrane protein bacteriorhodopsin. Because of the unique structure of this amphiphile, molecule **1** forms the basis for a new class of surfactants, of which the chain length and structure of the aliphatic chain can be systematically varied and studied. The control of the oxidation states, molecular conformation, changes in the supramolecular assembly, and the potential for drug loading and release are the subject of our ongoing research.

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Supporting Information Available: Synthsis of 2 and 3, NMR spectra of all compounds; induced circular dichroism of **1**, **2**, and **3** with and without *l*-borneol; NOESY, UV−vis, LCMS, and HPLC trace of surfactant **1**; the dependence of rotational strength of a chromophore on angle tilted from the *z*-axis. This material is available free of charge via the Internet at http://pubs.acs.org.

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