Conformationally Switchable Water-Soluble Fluorescent Bischolate Foldamers as Membrane-Curvature Sensors

Roshan W. Gunasekara
Iowa State University, roshan@iastate.edu

Yan Zhao
Iowa State University, zhaoy@iastate.edu

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Conformationally Switchable Water-Soluble Fluorescent Bischolate Foldamers as Membrane-Curvature Sensors

Roshan W. Gunasekara and Yan Zhao*

Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111, United States

Supporting Information

ABSTRACT: Membrane curvature is an important parameter in biological processes such as cellular movement, division, and vesicle fusion and budding. Traditionally, only proteins and protein-derived peptides have been used as sensors for membrane curvature. Three water-soluble bischolate foldamers were synthesized, all labeled with an environmentally sensitive fluorophore to report their binding with lipid membranes. The orientation and ionic nature of the fluorescent label were found to be particularly important in their performance as membrane-curvature sensors. The bischolate with an NBD group in the hydrophilic \( \alpha \)-face of the cholate outperformed the other two analogues as a membrane-curvature sensor and responded additionally to the lipid composition including the amounts of cholesterol and anionic lipids in the membranes.

INTRODUCTION

Lipids are the main ingredients of biological membranes, whether plasma membranes that define the boundary of a cell or membranes of intracellular organelles that isolate them from the cytoplasm.\(^1\) In comparison to proteins, nucleic acids, and polysaccharides, these small-molecule amphiphiles seem to lack the usual biological sophistication. However, continued studies in recent decades revealed that lipids have their unique way of complexity that in some ways rival or even surpass what is found in the other more complex biomacromolecules.\(^2\) For example, biological membranes are made of complex mixtures of lipids, whose composition is subject to change at different stages of the cell life and even in response to biological conditions of the cell.\(^3\) Membranes are not homogeneous mixtures of lipid molecules either. Heterogeneity is found both horizontally within the same leaflet of a bilayer membrane and vertically across the two leaflets.\(^4,5\) Phase-separated lipid domains play important roles in biological processes such as membrane protein assembly and signal transduction. Thus, even without secondary and tertiary structures, lipid molecules \textit{communally} could change their chemical composition and dynamic structures, similar to what proteins do at the primary and secondary structural levels. Because these changes and the dynamics of lipids occur in a self-assembled ensemble, the complexity is no smaller than the individual complexity displayed by the other seemingly more sophisticated biomacromolecules.

Another level of complexity in lipid assemblies comes from membrane curvature.\(^6\) Larger plasma membranes are, as a result of simple geometry, flatter or have smaller curvature than membranes of intracellular organelles. Certain organelles have notably highly curved membranes including endoplasmic reticulum and golgi apparatus. However, membrane curvature is a not a static property of biological membranes. In processes such as cellular movement, division, and vesicle fusion and budding, membrane curvature is actively modulated by proteins to enable these processes. Curvature modulation is an extremely important and intriguing process, as it translates molecular interactions between lipids and proteins into mechanical movement/rearrangement of cells and vesicles.\(^7\)

For these reasons, there is strong interest in developing tools that can sense membrane curvature, particularly under dynamic conditions.\(^8,9\) In nature, certain proteins are known to be specific membrane-curvature sensors. BAR domains are coiled-coil bundles of proteins; these arc-shaped structures can associate with membranes with matching curvatures.\(^10,11\) Other membrane sensors such as ALPS do not have specific secondary/tertiary structures on their own but recognize the hydrophobic defects in highly curved membranes. The most interesting feature of this class of membrane sensors is that they stay soluble in water in the presence of low-curvature membranes (e.g., liposomes with \( R > 100 \) nm) but selectively bind high-curvature membranes (e.g., liposomes with \( R < 50 \) nm), meanwhile switching from a random conformation to an \( \alpha \)-helix on the surface of the membrane.\(^12,13\)

Because of the complexity of the natural protein-based curvature sensors, scientists in recent years became interested in developing small-molecule-based sensors, for their ease of synthesis and more straightforward structure–activity correlation. A cyclic peptide derived from Synaptotagmin-I (a protein
possibly involved in calcium-dependent membrane trafficking and fusion)\textsuperscript{14,15} and a 25-mer peptide (MARCKS-ED) were both shown recently to be effective curvature sensors.\textsuperscript{16}

In this work, we report that simple bischolate foldamers could be used as effective membrane-curvature sensors. The location and the nature of the fluorescent probe on the foldamer turned out particularly important in their ability to act as membrane-curvature sensors. Our most effective foldamer sensor responded not only to membrane curvature but also to the cholesterol content and the amount of negatively charged lipids in predictable fashions.

**EXPERIMENTAL SECTION**

The general methods and the syntheses of the compounds are reported in the Supporting Information.

**Liposome Preparation.** A chloroform solution of POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and POPG (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-1'-rac-glycerol sodium salt) was placed in a 10 mL test tube and dried under a stream of nitrogen at room temperature using an Avanti Mini-Extruder to produce the lipid film. A solution of PBS buffer (1.0 mL, pH = 7.4) was added to the test tube containing thin lipid film. Rehydration of the lipid was allowed to continue for 90 min with frequent vortexing at 4 °C. The lipid suspensions of the resulting multilamellar vesicles were subjected to 10 freeze-thaw cycles. The resulting mixture was then extruded 29 times through a polycarbonate filter (diameter = 19 mm, pore diameters of 30, 100, and 400 nm) at room temperature using an Avanti Mini-Extruder to produce the desired LUVs. Each LUV was diluted in PBS to a lipid concentration of 15–25 μM, and their size was analyzed by DLS. Intensity data from each sample were collected in five replicates and analyzed by the Precision Deconvolve software.

**Fluorescence Enhancement Assay.** Different bischolates were mixed with the above prepared LUV solutions to afford a solution with [bischolate] = 0.10 μM and [total lipids] = 300 μM in PBS buffer. The samples were transferred to 10 mm cuvettes, and fluorescence spectra were recorded on a Varian Cary Eclipse spectrophotometer. Fluorescence spectra were also obtained without liposomes in the solution. The excitation wavelength (λ\textsubscript{ex}) was 340, 470, and 470 nm for bischolate 1, 3, and 4, respectively. The excitation slit width was 10 nm, and the emission slit width was 20 nm. The fluorescence enhancement was obtained by averaging two scans and normalizing the emission intensity of the probe in the presence of the LUVs to the intensity in the absence of the LUVs.

**RESULTS AND DISCUSSION**

**Design and Synthesis of Bischolate Membrane-Curvature Sensors.** Foldamers are synthetic mimics of biomacromolecules with controlled conformations.\textsuperscript{17–19} Our group has been interested in foldamers made of cholic acid, a natural facial amphiphile.\textsuperscript{20–22} The amphiphilicity of the building block and the resultant foldamers allows these compounds to interact with lipid membranes in tunable fashions.\textsuperscript{23–25} Since amphipathic peptides act as membrane-curvature sensors,\textsuperscript{8,12–16,34,25} and facially amphiphilic cholate derivatives have been used by researchers as amphiphilic peptide mimics,\textsuperscript{28,29} we reasoned that appropriate cholate foldamers might be able to bind membranes in a curvature-dependent fashion.

Bischolate 1 was recently synthesized in our group.\textsuperscript{30} The molecule has two cholates connected head-to-tail with a glutamic acid tether in between. The glutamic acid was introduced to facilitate the cholate–cholate interactions. Our previous work suggests that a C4 tether allows the facial amphiphiles to interact with each other readily.\textsuperscript{31} The molecule contains two carboxylates: one from one of the cholates and the other from the glutamate tether. These ionic groups (including the sulfonate on the fluorophore) make the molecule soluble in water, a feature important to aqueous-based applications such as membrane-curvature sensing. Natural cholic acid has a hydrophilic α-face with three hydroxyl groups and a hydrophobic β-face made of hydrocarbon. Bischolate 1 has the terminal hydroxyl inverted and replaced with an amido and a triazolyl group, respectively. The triazolyl was used to introduce the aminophathalamenesulfonate group, which is analogous to the more common environmentally sensitive fluorophore dansyl.\textsuperscript{32} Its environment-dependent emission allowed us to characterize its conformation in different solvents (Scheme 1).

Our previous work shows that, in polar solvents (i.e., >50% water in methanol), bischolate 1 folds hydrophobically, with the polar groups exposed to the solvents. In nonpolar solvents (i.e., >30% THF in methanol), the molecule adopts a conformation with the polar groups point inward, solvated by polar solvents concentrated in the center of the molecule.\textsuperscript{30} At intermediate polarity (namely, in between 30% THF/methanol and 50% methanol/water), the molecule adopts an unfolded, random
conformation, with both the hydrophilic and hydrophobic faces exposed. The solvent-induced conformational change between a micelle-like conformation with exposed hydrophilic groups and a reverse-micelle-like conformation with buried hydrophilic groups has been observed multiple times for both cholate foldamers and nonfoldamers under similar conditions.

The above conformational change was established through the solvent-sensitive emission and its comparison with the control compound 2 (Scheme 1). The environmentally dependent switchable conformations seem to be perfect for a membrane-curvature sensor and resemble ALPS in its transition from a random conformation in water to the amphipathic α-helix upon binding with a highly curved membrane.

In this work, we synthesized two additional bischolates, 3 and 4. Molecule 3 is identical to 1 in every aspect except that the ionic aminonaphthalenesulfonate was replaced by another environmentally sensitive NBD fluorophore. NBD absorbs and emits at a longer wavelength than aminonaphthalenesulfonate derivatives. As will be shown in our discussion below, the (ionic or nonionic) nature of the fluorophore turns out to be a key parameter to the compound’s performance as a membrane curvature-sensor. Molecule 4 is similar to 3, having the nonionic NBD fluorescent label, except that the label is located on the α-face of the attached cholate.

Evaluation of Bischolate Membrane-Curvature Sensors. The performance of these bischolates as membrane-curvature sensors was evaluated by a fluorescence enhancement assay used by many researchers. Briefly, a given concentration of liposomes of a certain size was added to an aqueous solution of the membrane-curvature sensor. Association of the sensor with the hydrophobic membranes enhances its migration from an aqueous phase to a more environmentally sensitive aminonaphthalenesulfonate is also helix upon binding with a highly curved membrane. The concentration of liposomes of a certain size was added to an aqueous solution of the membrane-curvature sensor. Association of the sensor with the hydrophobic membranes enhances its migration from an aqueous phase to a more environmentally sensitive aminonaphthalenesulfonate is also helix upon binding with a highly curved membrane.12,13 The from a random conformation in water to the amphipathic membrane-curvature sensor and resemble ALPS in its transition dependent switachable conformations seem to be perfect for a membrane-curvature sensor either, as its emission intensity displayed a very small response to the membrane curvature.

The largest emission enhancement was observed for compound 4, which has the same fluorophore as 3 but the opposite stereochemistry for the NBD group. This bischolate was clearly the best curvature sensor among the three, not only because of its strongest emission enhancement but, more importantly, a clear increase of emission intensity with increasing membrane curvature or decreasing liposome size (Figure 1, ). The NBD-functionalized bischolate 3 experienced a stronger enhancement of emission in the presence of the liposomes (Figure 1, ), possibly due to the nonionic nature of its fluorophore. Nonetheless, despite its stronger emission enhancement, this compound is not a good membrane-curvature sensor, as its emission enhancement did not display a monotonous trend as a function of the liposome size (Figure 1, ).
the largest and most hydrophobic surfaces are the cholate $\beta$-faces.

The emission wavelength of the NBD group of 3 and 4 in aqueous buffer was quite similar (545–550 nm). There was no significant difference between the two over half of dozen measurements in our hands. The results suggest that the water exposure of the NBD groups in 3 and 4 was similar when the two compounds were dissolved in water. The conclusion is reasonable, as the two compounds are expected to fold in water through the hydrophobic association of the $\beta$-faces. In compound 4, the NBD group was on the water-exposed $\alpha$-face; folding is thus not expected to bury the fluorophore. In compound 3, although the NBD group was on the hydrophobic $\beta$-face of the cholate, its large size suggests that it is unlikely to be shielded from water contact either, at least not completely.

As the molecules (3 and 4) enter the membrane, totally different situations occurred. The maximum emission wavelength ($\lambda_{em}$) of the NBD of 3 occurred at 521 nm, regardless of the liposome sizes. In contrast, probe 4 emitted at 538 nm, also independent of the liposome sizes. Since these wavelengths are both blue-shifted in comparison to their emission wavelength in water (545–550 nm), the probes were undoubtedly interacting with the hydrophobic membranes after the addition of the liposomes. This conclusion is also consistent with the large increase of their emission intensity caused by the addition of the liposomes (Figure 1). The fact that the NBD of 3 emitted at a significantly lower wavelength that the NBD of 4 suggests that the former was located in a more hydrophobic microenvironment than the latter.37 Given the location of the NBD on the hydrophobic $\beta$-face of the cholate in 4, this is an extremely likely situation: as 3 enters the membrane, it has to hide its hydrophilic groups from the lipid hydrocarbon (Scheme 1, left), similar to the folded 1 in nonpolar solvents (Scheme 1). Since the NBD group is on the opposite site of the hydrophobic $\alpha$-face, it should be in a fairly hydrophobic microenvironment. For compound 4, the small change in $\lambda_{em}$ upon binding with the membranes suggests that its NBD group was in a fairly polar environment, in agreement with the schematic representation of the folded bischolate in the membrane (Scheme 2, right).

The emission wavelengths ($\lambda_{em}$) of 3 and 4 were reasonable from the location of the NBD group and the presumed reverse-micelle-like conformation of these molecules in a nonpolar membrane environment. What remains puzzling was the fact that bischolate 4 enjoyed a stronger emission enhancement than 3 under similar conditions (Figure 1). Normally, one expects a fluorophore entering a more hydrophobic environment should emit more strongly. However, it has been reported that for primary amine-derived NBD derivatives such as 3 and 4, the quantum yields of the compounds were also influenced by other factors.37 Although the general trend is that such compounds emit more strongly in less polar solvents, n-propylamino-NBD was found to emit very weakly in highly nonpolar solvents such as hexane and cyclohexane but much more strongly in solvents of intermediate polarity including ethyl acetate, THF, dioxane, acetone, and chloroform. Since the NBD group of 3 was on the $\beta$-face of the cholate, it would be expected to be in a more hydrocarbon-like environment than that of 4. Thus, the stronger emission of 4 than 3 in the membrane-bound state is consistent with the behavior of n-propylamino-NBD in different solvents.

Our study so far indicates that bischolate 4 was the best membrane-curvature sensor for its high sensitivity and direct correlation between the emission enhancement and membrane curvature. We wondered whether the sensitivity was a result of a stronger binding with higher curvature membranes or some other factors. The binding affinity of a membrane-curvature sensor to the membrane can be determined by fluorescence titration, in which the concentration effect of the liposomes on the emission intensity of the sensor is measured:27,39 The affinity is defined as the apparent molar partition coefficient ($K_p$) of the probe between lipid membranes and the aqueous solution and is described by the following equations:

$$\% \text{ bound} = \left( \frac{F - F_0}{F_{100} - F_0} \right) \times 100\% \quad (1)$$

$$\% \text{ bound} = \left( \frac{C_p K_p}{1 + C_p K_p} \right) \times 100\% \quad (2)$$

in which $F$ is the maximum NBD emission intensity at a given lipid concentration, $F_0$ is the NBD emission intensity in the buffer in the absence of lipids, $F_{100}$ is the emission intensity at lipid saturation, and $C_p$ is the concentration of the accessible lipids in the sample. For large unilamellar vesicles (LUVs) as we have, the accessible lipids amount to 50% of the total lipid concentration.27

Figure 2 shows the fluorescence titration of probe 4, our best membrane-curvature sensor, by liposomes of different sizes. The data fit quite nicely to eq 2 and allowed us to understand the binding affinities of the probe to the membranes. The binding data are summarized in Table 1, which also contains the data for probes 1 and 3.

For biologically derived membrane-curvature sensors such as amphipathic $\alpha$-helices, it has been found that their preferential binding to highly curvature membranes is mainly caused by a higher number of hydrophobic binding sites (i.e., packing defects) rather than a stronger binding affinity.26 For liposomes ranging from 75 to 500 nm in size, for example, the binding affinity displayed a mere 2–2.5-fold increase with decreasing liposome sizes. Our binding data in general showed the same trend. For all three probes, the apparent molar partition coefficient ($K_p$) increased with decreasing liposome sizes. The

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Scheme 2. Schematic Representation of Possible Conformations of the Folded 3 and 4 in the Lipid Bilayer

“The carboxylic acid in the hydrophobic membrane is shown to be protonated whereas that in the aqueous environment to be deprotonated.”
largest increase was almost 4-fold. Thus, the binding affinity of these bischolate probes was somewhat more sensitive to the membrane curvature than that of the naturally occurring amphipathic \( \alpha \)-helices. One possibility for the more sensitive binding affinity could originate from the size of the hydrophobic groups in our probes. Amphipathic \( \alpha \)-helices rely on their hydrophobic amino acid side chains to bind the hydrophobic packing defects in the membrane. Since these groups are much smaller than the cholate groups in our probes, they might be able to fit into many smaller binding sites that our probes simply cannot bind. If this is indeed the case, our probe could be sensing preferentially the larger hydrophobic packing defects in comparison to natural \( \alpha \)-helical membrane-curvature sensors. Such a feature makes our probe complementary to the natural sensors and may be useful in certain settings.

There are two other important conclusions we can draw from the binding data. First, although the binding affinity of all our probes displayed a monotonous increase with decreasing liposome sizes and thus increasing membrane curvatures (Table 1), only probe 4 showed monotonous changes in emission enhancement as a function of the liposome size (Figure 1).

Clearly, in addition to binding affinity, other factors such as the location of the fluorophore in the probe (and in turn the location in the membrane) and the ionic nature of the fluorophore are all important to the emission intensity. Since measuring the binding affinity involves a substantially larger amount of work, probe 4 remains the sensor of choice in our study. Second, the binding affinity of the probe to lipid membranes overall correlates with the fluorescence enhancement for the three probes, particularly for the smaller liposomes. The binding affinity, for example, followed the order of 4 > 3 > 1 for liposomes 58 and 83 nm in size. The binding affinity seemed to make sense from the viewpoint of hydrophobic driving force in the binding. Probe 1 has a total of three ionic groups (two carboxylates and one sulfonate); its strong hydrophilicity is expected to lower the hydrophobic driving force for the binding. In addition, our membranes were overall negatively charged, with a ratio of [POPG]/[POPC] = 10/1. A larger number of anionic groups in 1 also translates to a stronger repulsion by the liposomes. The stronger binding of 4 over 3 by the liposomes also seems to be reasonable. The NBD group of 4 is on the hydrophilic \( \alpha \)-face of the cholate and thus is completely exposed to water in the water-soluble state. The unfavorable water contact for the NBD probably provided a stronger hydrophobic driving force for the probe to enter the membrane. For probe 3, although the NBD group must also have significant water exposure (as discussed above in terms of its emission wavelength), its location on the hydrophobic \( \beta \)-face of the cholate suggests that it should be in partial contact with the other folded hydrophobic cholate. Undoubtedly, more hydrophobic contact prior to binding to the membranes means that part of the hydrophobic surface is already buried and thus is equivalent to a lower hydrophobic driving force for the binding.

All the data so far suggest that probe 4 is the best membrane-curvature sensor among the three, displaying the strongest emission enhancement (Figure 1), a monotonous response to lipid curvatures (Figure 1), and the strongest binding for lipid membranes (Table 1). It is known that the composition of lipids also has an impact on the number of hydrophobic binding sites in the membranes. Cholesterol, for example, is able to reduce the number of hydrophobic defects in a membrane. All three probes as a function of liposome sizes for POPC/POPG and

\[ \text{Table 1. Apparent Partition Coefficients (} K_p \text{) for the Oligocholate Foldamers in PBS Buffer (pH 7.4)} \]

<table>
<thead>
<tr>
<th>entry</th>
<th>liposome size (nm)</th>
<th>probe</th>
<th>( K_p (10^3 \text{M}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>1</td>
<td>41 ± 7</td>
</tr>
<tr>
<td>2</td>
<td>83</td>
<td>1</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>3</td>
<td>141</td>
<td>1</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>3</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>5</td>
<td>83</td>
<td>3</td>
<td>39 ± 7</td>
</tr>
<tr>
<td>6</td>
<td>141</td>
<td>3</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>4</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>8</td>
<td>83</td>
<td>4</td>
<td>64 ± 10</td>
</tr>
<tr>
<td>9</td>
<td>141</td>
<td>4</td>
<td>22 ± 4</td>
</tr>
</tbody>
</table>

\[ [\text{Bischolate}] = 0.10 \mu\text{M, } [\text{POPC}]/[\text{POPG}] = 10/1. \text{ The binding data were obtained by nonlinear least-squares curving fitting of the binding data to eq 2.} \]

Figure 2. Binding of bischolate 4 in PBS buffer (pH 7.4) by LUVs with the average diameter of (a) 58, (b) 83, and (c) 141 nm. The smooth curves are nonlinear least-squares curving fitting of the binding data to eq 2. [Bischolate] = 0.10 \pM. \( C_s \) is the concentration of the accessible lipid and is 50% of the total lipid in the sample.

Figure 3. (a) Enhancement in the fluorescence emission of bischolate foldamer 1 (●), 3 (○), and 4 (□) induced by LUVs of different sizes made of 10:1 POPC/POPG consisting of 30 mol % cholesterol. (b) Enhancement in the fluorescence emission of oligocholate foldamer 4 induced by LUVs made of 10:1 POPC/POPG consisting of 0 (▪), 10 (◇), and 30 mol % (▲) cholesterol. \( I_0 \) and \( I_e \) represent the maximum emission intensity of the probe in the presence of LUVs and in PBS buffer (pH 7.4), respectively. [Bischolate] = 0.10 \pM. [Lipids] = 300 \pM.
membranes consisting of 30 mol % cholesterol. Probe 4 clearly remained as the most sensitive membrane-curvature sensor in the high cholesterol membranes. Overall, the inclusion of cholesterol in the lipids lowered the fluorescence enhancement, especially for probes 3 (◇) and 4 (□). The trend could be seen more clearly in Figure 3b, showing the $I/I_0$ curve with 30 mol % (▲) cholesterol significantly lower than those with 0 (■) and 10 mol % (●) cholesterol.

Our membrane-curvature sensor (i.e., 4) is an anionic foldamer. Since its binding with the membrane is driven by hydrophobic interactions and electrostatic repulsion exists between the anionic foldamer and the negatively charged POPC/POPG membranes, we wanted to see whether the electric potential of the liposomes will influence the performance of the sensor. Figure 4a shows the fluorescence enhancement of 4 in the presence of the same concentrations of 50:1 (◇), 10:1 (◇), and 0:1 (▲) POPC/POPG liposomes. Overall, the membranes consisting the least amount of anionic lipids (i.e., POPG) showed the largest enhancement, while those with the highest amount of POPG showed the smallest enhancement. Thus, electrostatic repulsion between the membranes and the sensor indeed impacted the binding negatively. As shown in Figure 4b, increasing POPG in the lipid formulation consistently lowers the emission of the probe, most likely due to weaker binding between the more negatively charged membranes and the anionic sensor.

**CONCLUSIONS**

In comparison to the membrane curvature-sensors found in nature (mainly proteins and amphipathic α-helices), the bischolates reported in this work is much easier to synthesize. Importantly, their ability to act as curvature sensors can be tuned rationally as shown by this study. Their design is highly modular, and each component in the structure has specific functions: the amphiphilic β-cholates interacting with water and membranes in a predictable fashion; the glutamic acid tether provides the flexibility for the two cholates to interact intramolecularly; the anionic carboxylates on the cholate and the glutamic acid make the probe water-soluble; the environmentally sensitive fluorophore reports the transition from the water-soluble state to the membrane-bound state in a curvature-dependent fashion. It is significant that the ionic nature and the orientation of the fluorescent label are critical to the performance of the bischolates as the sensor. Their simple synthesis as compared to the natural membrane-curvature sensors makes them potentially useful tools in biophysics and biochemistry.

**ASSOCIATED CONTENT**

Supporting Information

Synthesis and spectroscopic characterization of the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

*Phone 515-294-5845; Fax 515-294-0105; e-mail zhaoy@iastate.edu (Y.Z.).

**Notes**

The authors declare no competing financial interest.

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Medium: Involvement of a TICT State?


(40) The binding was weak in all three cases for the largest liposomes.


(38) Olson, F.; Hunt, C. A.; Szoka, F. C.; Vail, W. J.; Papahadjopoulos, D. Preparation of Liposomes of Defined Size


(40) The binding was weak in all three cases for the largest liposomes.


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