THE JOURNAL OF PHYSICAL CHEMISTRY B

ARTICLE

pubs.acs.org/JPCB

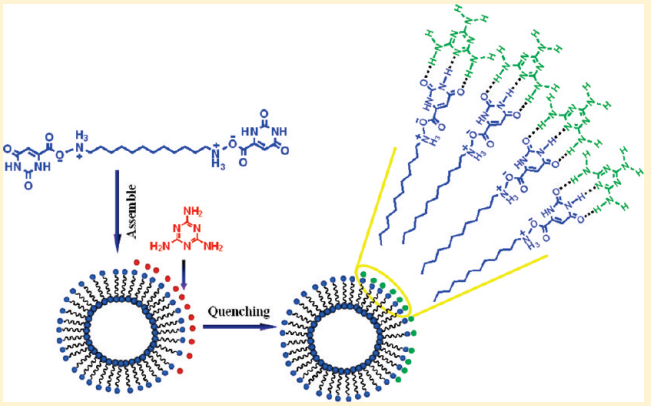
Molecular Recognition of Melamine by Vesicles Spontaneously Formed from Orotic Acid Derived Bolaamphiphiles

Zhong-Xiu Chen,*[,†] Xiao-Xia Su,[‡] and Shao-Ping Deng[‡]

[†]Department of Applied Chemistry, College of Food & Biology Engineering, and [‡]Food Sensory Lab, Zhejiang Gongshang University, Hangzhou, Zhejiang 310035, P. R. China

Supporting Information

ABSTRACT: Molecular recognition by means of multiple hydrogen bonds is of great importance in biological functions. In this paper, an orotic acid derived bolaamphiphile 1,12-diaminododecane diorotate (DDO) with molecular recognition function moieties was designed. Both self-aggregation behavior and molecular recognition with melamine were extensively examined. This bolaamphiphile itself can form vesicles easily in aqueous solutions at 25 °C. Steady-state fluorescence was used to characterize the detailed molecular recognition process. The fluorescence of melamine was quenched more effectively by the spontaneously formed vesicles than by the monomers of the surfactant. Two mechanisms were involved in the fluorescence quench process. At lower concentration, the fluorescence of melamine was found to be quenched by static complex formation. While at higher concentration, both static and dynamic quenching mechanisms coexisted in interaction process. Thermodynamic parameters measured by isothermal titration calorimetry showed that the free energy (ΔG) is negative, indicating that binding of DDO molecules with melamine is favorable energetically. Hydrogen-bonded interactions contribute comparatively a lot for the DDO monomer binding with melamine; at the higher concentration above its critical aggregation concentration, the dissociation of the aggregates take place and lead to an entropically driven molecular recognition process. As complicated binding sites can be constructed through self-assembly at the vesicle interface rather than simple molecular modules, this bolaamphiphile with the molecular recognition functional group may make it possible to generate well-defined recognition sites to mimic biomolecular receptors. Moreover, the present research will give a guide to design chemosensors for melamine detection based on molecular recognition.



1. INTRODUCTION

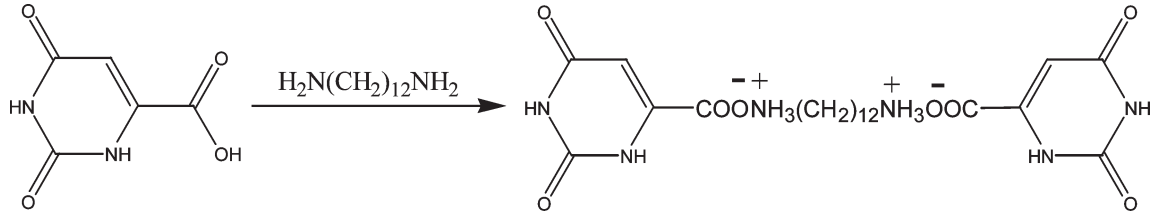
Molecular recognition by means of multiple hydrogen bonds is of great importance in biological functions, because the mutual recognition of complementary bases in nucleic acids is the basis of their stable double-helix structure and the most efficient mechanism of accumulating, storing, and evolving genetic information.[1] Analysis and mimicry of the molecular recognition processes not only will lead to a better understanding of biomembrane functions and processes but also can be helpful in the development of novel medical and biological sensors. Complementary binding sites can be used to program the self-assembly of donor and acceptor components into molecular heterojunctions, leading to the deliberate formation of functional architectures.

Melamine is often involved in the designed molecular recognition system due to its special structure which makes it a promising model compound for molecular recognition purposes.[2] Molecular recognition of barbituric acid by amphiphilic melamine-type monolayers has been reported.[3] Mixtures of alkoxyphenyl-substituted melamine with the complementary benzoic acids form discrete hydrogen-bonded heterodimers with elongated central core.[4] Chiral melamine derivatives have been designed for their potential application to chiral discrimination as chiral selectors.[5] Codeposition from a solution of symmetric melamine-terminated electron-donor oligomers with a complementary barbiturate-labeled electron-acceptor fullerene resulted in homogeneous films, and the photovoltaic response was greatly enhanced in the presence of hydrogen-bonded organic devices.[6] Recently, Lu's group[7] reported using melamine to control the assembly status of cyanuric acid derivative stabilized gold nanoparticles through hydrogen-bonding recognition, thus allowing the tuning in optical properties of gold nanoparticles. Obviously, all of the molecular recognition system was designed based on proton acceptors and donors

Received: July 10, 2010
Revised: September 23, 2010
Published: December 29, 2010

ACS Publications © 2010 American Chemical Society

Scheme 1. Synthesis for Bolaamphiphiles DDO



character in melamine structure, which makes it easy to form complementary hydrogen bonding with other molecules.

Realization of effective hydrogen bonding in water is especially significant due to its relevance to biological recognition.[8] However, it is reported that hydrogen bonding is usually suppressed or less effective for specific recognition in aqueous solution, since the molecules are surrounded by bulk water.[9] Accordingly, biological systems prefer to adopt interfacial environments for molecular recognition. The pioneering work of Kunitake and co-workers[10] have demonstrated that binding constants of adenosine monophosphate to guanidinium functionality in aqueous aggregates, such as micelles or bilayer vesicles, are significantly larger than those between molecularly dispersed guanidinium and phosphate in water. Therefore, many efforts were devoted to design kinds of amphiphiles to provide an appropriate interface for effective molecular recognition.[11−17] For example, control of enantioselectivity of amino acid recognition can be realized by dynamic motion of a polycholesteryl-substituted cyclen complex host molecule at the air−water interface.[14] An LB film composed of 4-*n*-dodecyl-6-(2-thiazolylazo) resorcinol was reported for naked-eye detection of cadmium ions[15] and a peptide-amphiphile for selective recognition of paraoxon.[16] Hierarchically self-assembled structure of the amphiphilic and plate-like codendrimer was designed which can provide multiple H-bonding between molecules.[17] Compared with conventional amphiphiles, bolaamphiphiles in which two hydrophilic head groups are covalently linked by a hydrophobic alkyl chain show abundant interfacial conformations and the potential to form nanostructures in organized assemblies with many interesting properties.[18−20] Among various organic supramolecular structures self-assembled by bolaamphiphiles,[21] vesicles have drawn much attention because it can be used as models of biological membranes or drug transport systems.[22,23] The slower rate of fusion of monolamellar membranes makes bolaamphiphiles useful in pharmaceutical preparation of liposomes.[24] Introducing some molecular recognition groups to the nanoscale molecular assemblies based on bolaamphiphiles was considered to be a good way for constructing biological models to understand the unique specificity of the functions performed by biological structures. Bolaamphiphiles connected by two crowns may form stable vesicles[25] and this crown ethers-based bolaamphiphiles can be used for sensors for ions and molecular scaffolds.[26] Certain crown bolaamphiphiles function as "membrane disruptors".[27] Porphyrin is another functional group connected with self-assembly of bolaamphiphile and was designated as hydrophobic cavities in molecular monolayers on aminated silica particles which are useful as model systems for the binding of small molecules to proteins in water.[28] Till now, molecular recognition group attached on the bolaamphiphiles vesicles mainly focus on crown ether and porphyrin moiety, there are few reports related to the vitamin-like group. Actually, vitamin based amphiphiles such as ascorbyl derivatives were reported to form coagels, micelles, or gel-like dispersions.[29] The only bolaform surfactant in which the ascorbic acid units act as the polar headgroups, was reported to form hollow nanotubes.[30]

In this paper, a novel bolaamphiphile 1,12-diaminododecane diorotate (DDO) with a vitamin-like moiety as the recognition group was synthesized and was found to form vesicles spontaneously. Its potential hydrogen donor and acceptor on the hydrophilic moiety prompt us to study its interaction with melamine. It was found that the bolaamphiphiles can recognize melamine though complementary hydrogen bonds and the interaction between the two molecules lead to fluorescence quench of melamine. Detailed examination revealed that the mechanism of the quench process differed with the concentration of melamine. As most of the reported bolaamphiphiles can form vesicles with the need of other surfactant[31,32] or in extreme pH conditions,[33] these easily formed vesicles with the molecular recognition functional group in aqueous medium can be used as models of biological membranes or artificial cells. The study of these bolaamphiphiles has been driven by fundamental research with the objective of preparing "designer assemblies" to manipulate the molecular recognition processes in artificial systems. Meanwhile, few reports focus on the fluorescence of melamine; the present molecular recognition at interfaces not only has relevance to biological systems but also is important for modern applications such as high sensitivity sensors for potential detection of melamine by fluorescence quenching.

2. EXPERIMENTAL SECTION

Materials. Melamine (99%) and pyrene (97%) were purchased from Sigma-Aldrich and were used without further purification. L-Orotic acid (95%) and 1,12-diaminododecane (98%) were from Shanghai Chemical Reagent Company. Ultrapure Millipore (B0500891) water (18.2 MΩ) was used for the preparation of all solutions.

Synthesis of Orotic Acid Derived Bolaamphiphiles. L-Orotic acid (1.5610 g, 10 mmol) and a suspension of 1,12-diaminododecane (1.0018 g, 5 mmol) in 50 mL of water were introduced into a flask equipped with a magnetic stir bar. After reaction for 48 h, excess reactants were removed via centrifugation. The crude product was further dissolved in ethanol and filtered. The filtrate was collected and dried in vacum to give the final product 1,12-diaminododecane diorotate (DDO). The structure was confirmed by ^{1}H NMR spectra. ^{1}H NMR (400 MHz, D_2O): δ 6.07 (2 H, s), 2.89 (4 H, t, J = 6 Hz), 1.59−1.53 (4 H, m), 1.30−1.21 (16 H, m). FT-IR (KBr) ν_{max} (cm^{-1}): 3417, 3325, 3088, 2851, 1701, 1617, 1571, 1492, 1384, 1320, 923, 890, 774, 546, 443.

Steady-State Fluorescence Spectral Measurements. Steady-state fluorescence spectra experiments were monitored on the

dx.doi.org/10.1021/jp106385x |*J. Phys. Chem. B* 2011, 115, 1798–1806

spectrometer Hitachi F7000 at 25 °C. Fluorescence measurements of melamine were performed in aqueous solutions which were degassed before measured. The excitation wavelength was 250 nm with both the exciting and emitting slit of 5.0 nm.

Determination of the Critical Aggregation Concentration (CAC) of DDO. The CAC values for DDO were obtained by monitoring the pyrene I_1/I_3 by using the steady-state fluorescence measurements on a Hitachi F-7000 fluorescence spectrophotometer at 25 °C. The excitation wavelength was 334 nm with the exciting and emitting slit of 2.5 and 5.0 nm, respectively. Fluorescence measurements were performed in aqueous solutions which were degassed before measured. The fluorescence probe was kept constant ([pyrene] = 0.5 μmol L^{-1}). Fluorescence emission spectra of these solutions were recorded employing an excitation wavelength of 334 nm, and the intensities I_1 and I_3 were measured at the wavelengths corresponding to the first and third vibronic peaks located at ca. 373 and 384 nm respectively. The ratio of I_1/I_3 was plotted as a function of the total surfactant concentration. The CAC was taken as a point of intersection, by fitting the data into linear equations.

Dynamic Light Scattering (DLS) and Conductivity Measurement. The dynamic light scattering (DLS) measurements were determined by a Zetasizer Nano-ZS (Malvern Instruments Ltd., U.K.). The size and conductivity measurements were performed using a laser−Doppler velocimetry technique. The instrument uses a laser at a wavelength of 632.8 nm and detects the scattered light at an angle of 173°. All measurements were performed in a temperature-controlled chamber at (25 ± 0.01) °C. All the samples were filtered through a 0.8 μm cellulose acetate filter before measurement. Equilibration time was 2 min, and each experiment was repeated two or more times.

Transmission Electron Microscopy (TEM). TEM micrographs were obtained with a Hitachi 7650 transmission electron microscope (working voltage of 120 kV) by phosphortungstic acid (1% (w/v)) as the staining agent. One drop of the sample solution (10 μL) was placed onto a carbon Formvar-coated copper grid (200 mesh). Filter paper was employed to suck away the excess liquid. Then one drop of the staining agent was placed onto the copper grid. The sample was allowed to stand for 30 s, and then any excess solution was removed by filter paper. The samples were last visualized under the transmission electron microscope and each experiment was repeated two or more times.

Isothermal Titration Calorimetry. An isothermal titration calorimeter (VP-ITC, Microcal Inc., Northampton, MA) was used to measure enthalpies associated with bolaamphiphiles DDO−melamine interactions at 298 K. Bolaamphiphiles in the syringe was of 4 mM. The concentration of melamine has been screened until a reasonable ITC profile was achieved, along with the smallest chi-square (chi^2) value of the final data fitting. In a typical experiment, melamine solution (30 mM) was placed in the 1430 μL sample cell of the calorimeter and bolaamphiphiles solution (4 mM) was loaded into the injection syringe. Both solutions were prepared in deionized water and were degassed before use. The reference cell was filled with deionized water. The first drop was set to 2 μL, and then DDO solution was titrated into the sample cell as a sequence of 27 injections of 10 μL aliquots. The duration of each injection was 60 s, and there was an interval of 360 s between successive injections to achieve complete equilibration. The solution in the titration cell was stirred at a speed of 307 revolutions min^{-1} throughout the experiment. Control experiments included the titration of bolaamphiphiles into water, water into melamine, and water into water. The last two controls resulted in small and equal enthalpy changes for each successive injection of water and, therefore, were not further considered in the data analysis. Raw data were obtained as a plot of heating rate (μcal s^{-1}) against time (min). These raw data were then integrated to obtain a plot of observed enthalpy change per mole of injected melamine (ΔH, kcal mol^{-1}) against molar ratio (bolaamphiphile/melamine). Binding stoichiometries, enthalpies, and equilibrium association constants were determined by fitting the data to a two-sites binding model with MicroCal Origin 7 using a nonlinear least-squares approach (Levenberg−Marquardt algorithm). All data show the average and standard deviation of three independent titrations.

3. RESULTS AND DISCUSSION

Aggregation Behavior of Bolaamphiphiles DDO. Figure 1A shows the electrical conductivity plot against DDO concentration. With the increase of DDO concentration, the electrical conductivity of the surfactant solution increase but show a breakpoint in the plot at 2.3 × 10^{-4} M which corresponds to the critical aggregation concentration (CAC) values of DDO. The polarity of the hydrophobic domains of the aggregates can be estimated by a fluorescence probe technique. Pyrene is a well-known fluorescence probe for micropolarity studies because of its solubilization site in micellar interiors.[34] The intensity ratio I_1/I_3 of the third and the first vibronic peaks of the pyrene fluorescence spectrum is very sensitive to solvent polarity[35] and therefore has been widely used as a measure of the polarity of the microenvironment of the probe. Because low values of I_1/I_3 indicate a nonpolar environment whereas high values indicate a polar environment, the apparent micropolarity of the aggregates was estimated by measuring the I_1/I_3 ratio at various surfactant concentrations. The data plotted in Figure 1B shows a decrease of the I_1/I_3 ratio with the increase of DDO concentration. The concentration (2.8 × 10^{-4} M) corresponding to the inflection point can be taken as the CAC. This value is very close to that obtained from electrical conductivity studies but is larger than that determined by ITC method (1.4 × 10^{-4} M, see the Supporting Information). Guo's group[31] has reported a histidine-derived bola surfactant, but micellization of this surfactant took place at a lower concentration.

It is noteworthy that two break points existed in fluorescence spectra plot as shown in Figure 1B. It was also observed in the surface tension isotherms of an amphiphilic pyrimidinic macrocycles derivatives -polymer mixture and some conventional surfactant/polymer systems.[25,26,36] The two transition points in surface tension curve of the fluorocarbon and hydrocarbon mixed system is due to the mutual hydrophobicity of the hydrocarbon and fluorocarbon chains.[37,38] In the case of polymer and surfactant system, the two break points may be the evidence of the surfactant adsorption on the polymer and the surfactant micelle formation respectively.[39] In the mixed system of a cationic bolaamphiphile (biphenyl-4,4-bis(oxyhexamethylenetrimethylammonium bromide), BPHTAB) and sodium dodecyl sulfate or $C_9H_{19}COONa$, vesicles and elongated aggregates exist while the total surfactant concentration is between the two break point.[36] In our DDO solutions, only one kind of hydrophobic chains exist and whether the two transition points came from the formation of different surfactant aggregates needs to be further studied.

The size distributions of DDO aggregates determined by DLS are shown in Figure 2. At 1.0 × 10^{-3} M DDO (above its CAC), one broad hydrodynamic radius distribution was observed. The distribution centering at 295 nm corresponds to DDO aggregates. The sigmoidal part for the second transition in Figure 1B usually

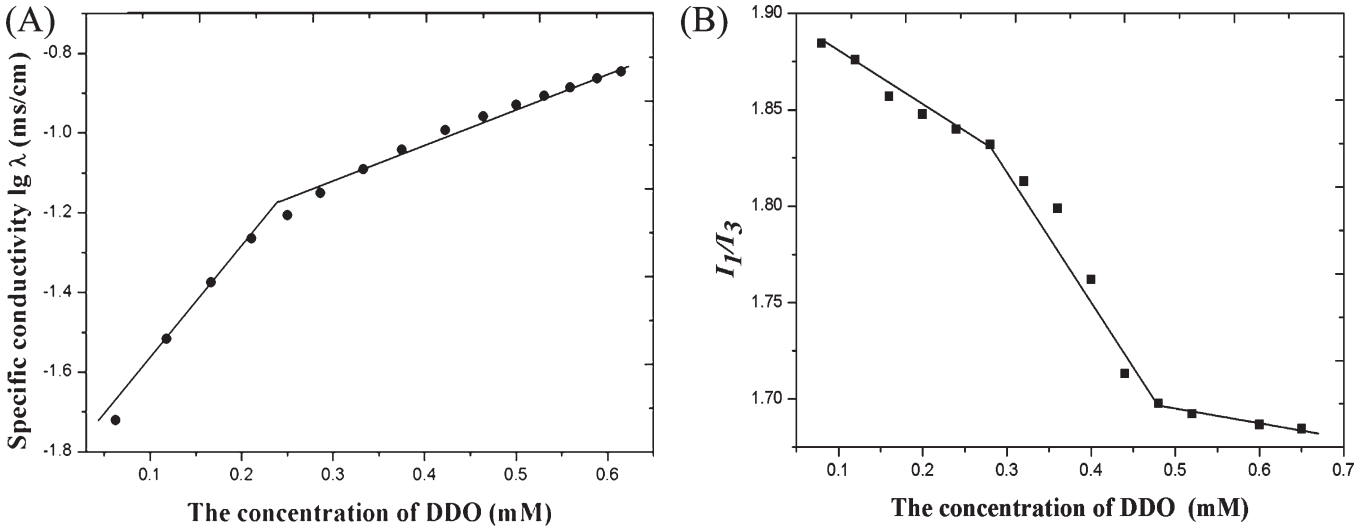


Figure 1. (A) Specific conductivity versus DDO concentration. (B) The I_1/I_3 band ratio in the fluorescence spectra of pyrene dissolved in various aggregates as a function of DDO concentration.

evidenced vesicle formation.[40] Transmission electron micrographs shown in Figure 3 confirmed the formation of vesicles. It was reported that the length of chain connected to the polar group of the bolaamphiphiles is critical for the vesicle formation. The presence of a long spacer leads to a micelle because of easier folding, whereas short spacers lead to vesicle formation.[41] Monolayer vesicles are formed from double short-chain C_{12} bolaamphiphiles in aqueous medium, and single-chained bolaamphiphiles possessing a short spacer (C_8 or C_{12}) hydrophobic have only been shown to form vesicles when possessing a planar (urocanic acid- comprising an imidazole unit) headgroup.[42] In crowns-based bolaamphiphiles, when the chain connecting the two aza-15-crown-5 residues is $(CH_2)_{16}$ rather than $(CH_2)_{12}$, micelles are formed instead of liposomes.[43] Based on the current research, we assume that the present bolaamphiphiles which bear a C_{12} as the hydrophobic chain, and a planar pyrimidine ring as the polar group form a monolayer vesicle as shown in Figure 4.

Among a great variety of self-assembled organic supermolecular structures formed from bolaamphiphiles,[21] only few reports focus on the spontaneous formation of vesicles. The known stable vesicular aggregates spontaneously formed from cryptands-derivatized bolaamphiphiles.[44] And, the aggregates formed from symmetrical bolaamphiphiles which was derived from (*E*) urocanic acid (3-[1H-imidazolyl-(4)-yl]-propenoic acid) was strongly dependent on pH, structure, and position of the connecting links.[33] Only for the N-alkylated derivatives, vesicles were obtained and pH variation did not alter the aggregates formed. An unsymmetrical bolaamphiphiles with urocanic acid as one polar head and a carboxylic acid as the other polar group were reported to form vesicles at pH 9.9. Many bolaamphiphiles-based vesicles can only prepared in extreme conditions and tended to form vesicles only in the presence of other surfactants.[31,32] As vesicles represent good models for studying biological membranes, the DDO molecule presented here which form vesicles spontaneously in neutral conditions seems to be a great potential candidate to mimic the biological cell membranes.

3.2. Molecular Recognition of Melamine by Bolaamphiphiles DDO. Interfacial molecular recognition and construction of supramolecular assemblies have received considerable attention in the past decades.[45] As is well-known, biological receptors are

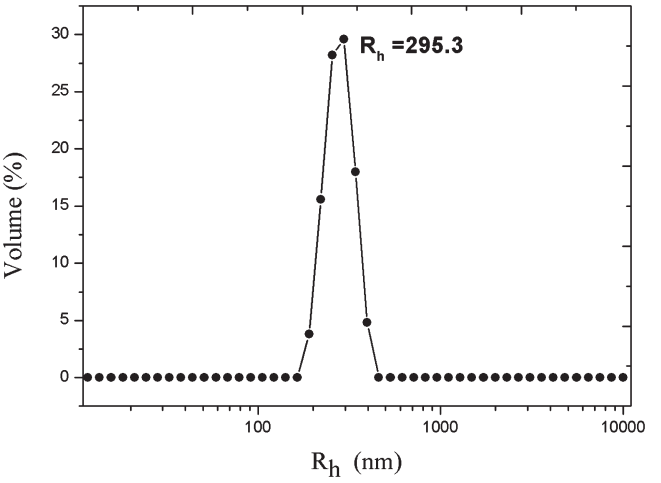


Figure 2. Size distributions of DDO solution (C_{DDO} = 1.0 mM).

situated at interfaces of biological systems at which specific molecular recognition processes under decisive participation of hydrogen bonds occur. As the bolaamphiphiles DDO can form monolayer vesicles, it can be a model system for molecular recognition between an amphiphilic host-bilayer and a nonsurface-active molecule dissolved in the aqueous subphase to study essential features of the supramolecular structures which can help to understand molecular recognition processes at biological interfaces. Hydrogen bond based biomimetic recognition systems have been found effective at the air−water interface. As the DDO molecule bears a heterocyclic moiety which can act as the hydrogen acceptor, it can be expected to be the host molecule to recognize some molecules with potential hydrogen donors. Melamine and cyanurate derivatives are some of the famous and widely studied hydrogen bond-directed assemblies in supermolecular chemistry,[2] and molecular recognition between synthetic hydrogen-bonding phospholipids and melamine has been reported,[46] but rare are reports about melamine fluorescence properties during the molecular recognition. To assess this possibility, we have performed an analysis of the interaction of DDO with melamine using the fluorescence approach.

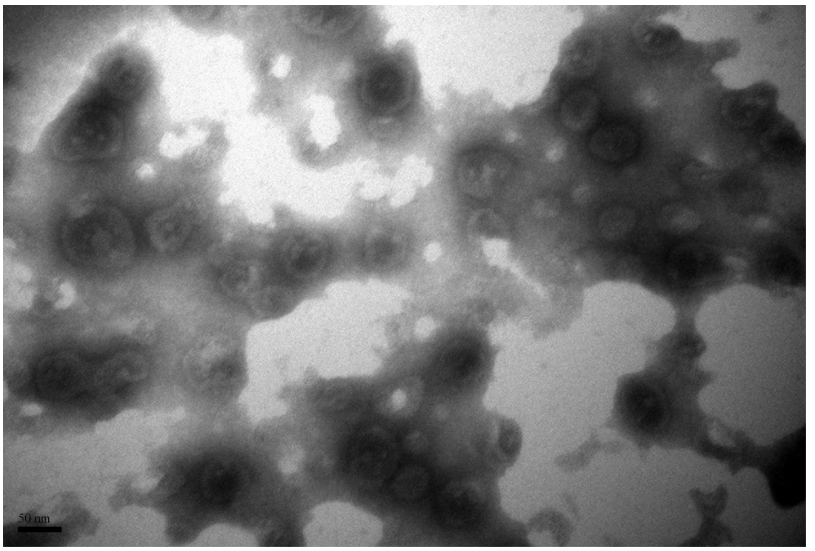


Figure 3. Transmission electron micrograph of DDO vesicles (C_{DDO} = 1.0 mM).

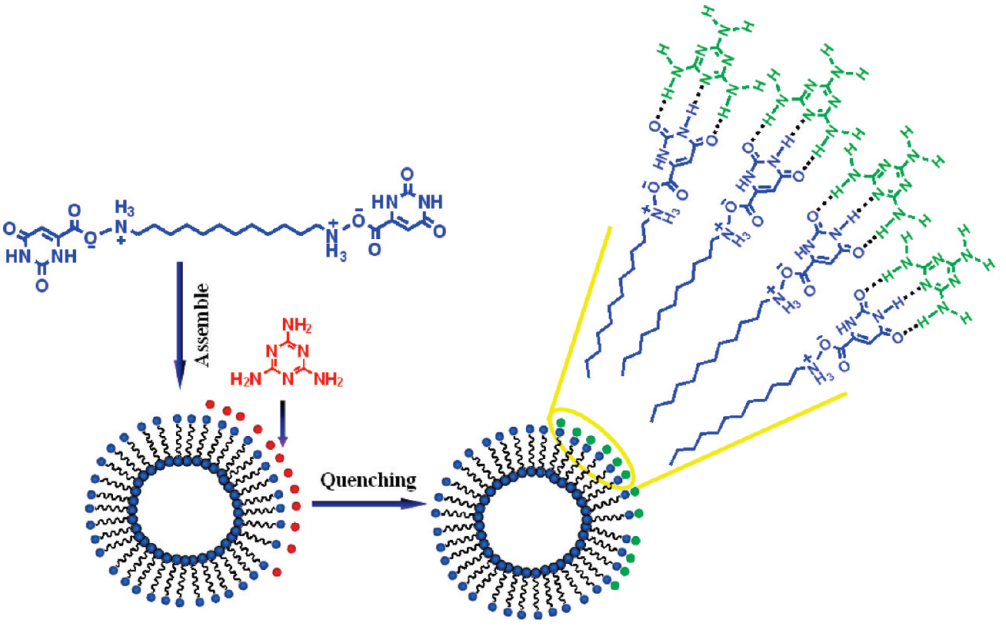


Figure 4. Schematic depiction of molecular recognition of melamine by DDO vesicles.

Figure 5 shows the fluorescence intensity of melamine (1 mM) in the presence of different molecules. Obviously, the fluorescence of melamine was quenched by DDO. Some amino acids and sodium dodecyl sulfate (SDS) surfactant (below or above its critical micelle concentration) was selected in the control experiment. SDS shows no quenching results whether in monomer state or in micelles, which excludes the possibility that amphiphilic surfactant or the micelles cause the disappearance of melamine fluorescence. Amino acids such as threonine, proline, histidine and cysteine also have poor effects on the fluorescence of melamine, which means the quenching melamine fluorescence was not due to the dielectric property change caused by the polar amino acid dissolved in the water. Orotic acid, whose structure is the same as the headgroup of DDO shows greater quenching effect than other amino acids. However, orotic acid molecules can not form organized membranes or vesicles which limit its further use in the biomimetic research. Accordingly, DDO molecule which forms vesicles spontaneously has great advantages to mimic the biological cell membranes.

All of these facts are indicative of a strong molecular recognition taking place between amphiphiles DDO and melamine.

The fluorescence data were analyzed by the well-known Stern−Volmer equation

$$F_0/F = 1 + K_{SV}[Q], \qquad K_{SV} = 11037.56\ (M^{-1})$$

In this equation F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively; K_{SV} is the Stern−Volmer quenching constant, and $[Q]$ is the concentration of DDO. A plot of F_0/F versus $[Q]$ yields an intercept of one on the y axis and a slope equal to K_{SV} as shown in Figure 6. A linear Stern−Volmer plot is generally indicative of a single class of fluorophores all equally accessible to quencher.

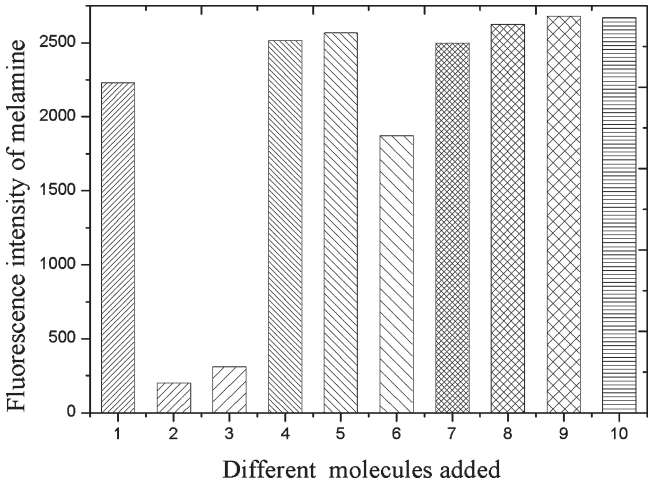


Figure 5. Fluorescence intensity of melamine (1 mM) in the presence of different molecules: (1) none, (2) amphiphiles DDO (0.5 mM), (3) orotic acid (0.5 mM), (4) threonine (0.5 mM), (5) proline (0.5 mM), (6) histidine (0.5 mM), (7) cysteine (0.5 mM), (8) SDS (0.5 mM), (9) SDS (5.0 mM), and (10) SDS (10.0 mM).

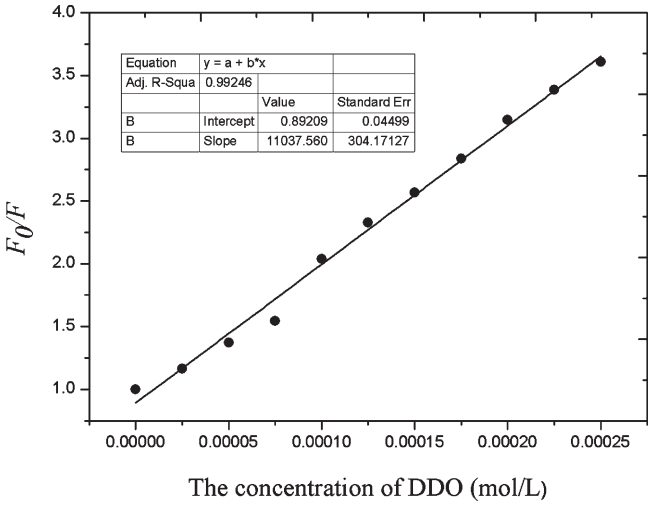


Figure 6. Stern−Volmer plots of fluorescence quenching by DDO. (The concentration of melamine is 5×10^{-5} M.)

As both static and dynamic quenching can result in linear Stern−Volmer plot, lifetime measurement is a good way to distinguish static or dynamic quenching involved. But in our fluorescence decay measurement, we found that there's too much deviation due to fast decay of melamine fluorescence. It is reported that fluorophore and quencher complexes typically exhibit different absorption behavior in comparison with the free fluorophore due to intermolecular interaction in the ground state. Thus, a change in the absorption profile in the presence of quencher can be indicative of a static quenching mechanism. However, it is important to note that both dynamic and static quenching processes operate concurrently in many systems.[47]

Careful examination of the absorption spectra declared that both static and dynamic quenching mechanisms are involved in the interaction of melamine at different concentration with DDO amphiphiles. As was shown in Figure 7, when melamine is at a lower concentration, the emission spectrum of melamine in the presence of quenchers showed a red shift from 360 to 364 nm,

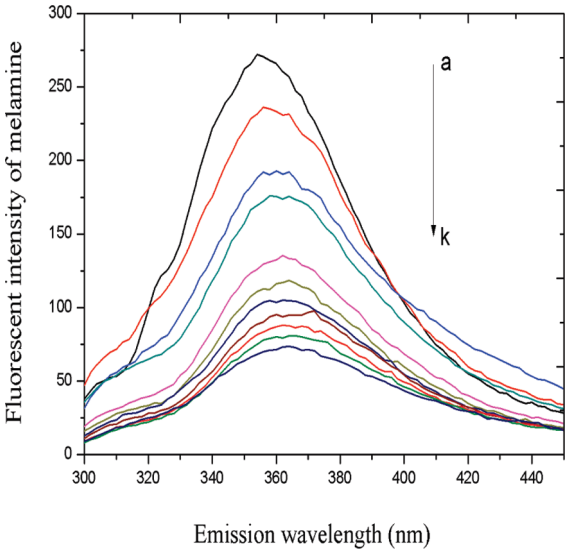


Figure 7. Fluorescence emission spectra of melamine (5×10^{-5} M) in the presence of different concentrations of quencher at 25 °C. The concentration of quencher were (a) 0, (b) 2.5, (c) 5.0, (d) 7.5, (e) 10.0, (f) 12.5, (g) 15.0, (h) 17.5, (i) 20.0, (j) 22.5, and (k) 25.0×10^{-5} M. The excitation wavelength was 250 nm.

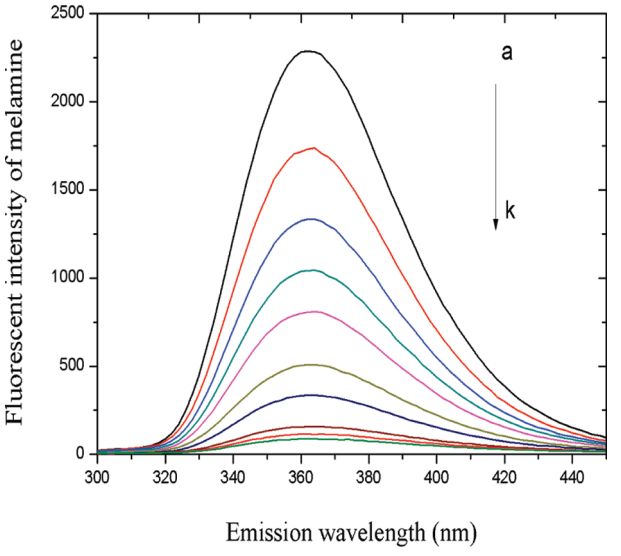


Figure 8. Fluorescence emission spectra of melamine (1×10^{-3} M) in the presence of different concentrations of quencher at 25 °C. The concentration of quencher were (a) 0, (b) 5.0, (c) 1.0, (d) 1.5, (e) 20.0, (f) 30.0, (g) 40.0, (h) 50.0, (i) 60.0, (j) 70.0 and (k) 80.0×10^{-5} M respectively. The excitation wavelength was 250 nm.

which was due to ground-state complex formation. The red shift was also observed in other molecular recognition systems. Intermolecular binding of pyrenyl static excimer of monopyrenylalkylamine derivative with Cu^{2+} ion was observed to shift red 15 nm in the excitation spectra.[48] Brun et al[49] reported that the interaction of some isomeric palladium tetrakis (*N*-methyl-*x*-pyridinium) porphyrins with DNA exhibited a red shift both in the Soret band and the Q bands maximum due to either intercalate between base pairs or binding to the exterior of the duplex. However, formation of complexes may also result in blue shift in the spectra. During the interaction of bovine serum

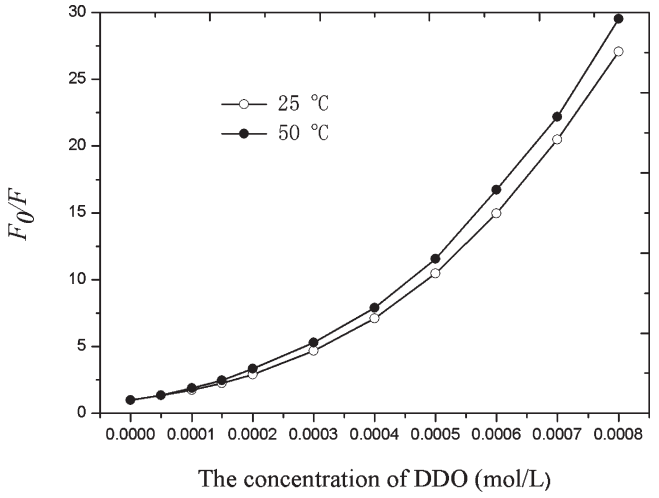


Figure 9. Stern−Volmer plots of fluorescence quenching by DDO quencher at different temperature (The concentration of melamine is 1×10^{-3} mol/L.)

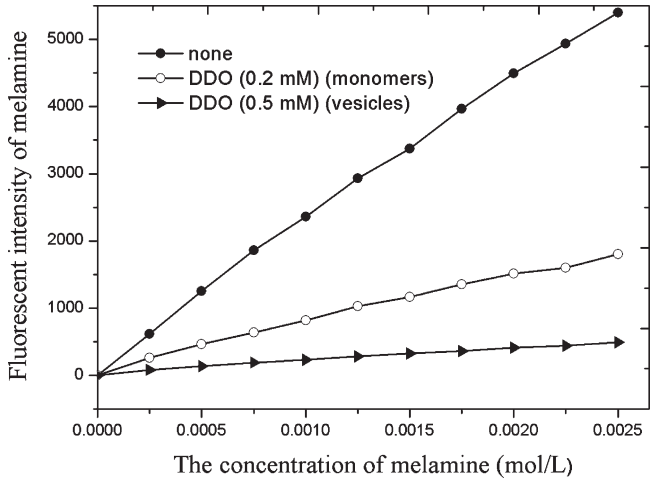


Figure 10. Fluorescent intensity of melamine quenched by DDO molecules in monomer state and in vesicles, respectively.

albumin (BSA) with p-aminoazobenzene (PAAB), the fluorescence intensity of BSA decreased regularly with the increasing concentration of PAAB and the maximum emission wavelength underwent an obvious blue shift of up to 7 nm, which was attributed to the conformation change of BSA.[50] Quenchers such as acrylamide and I^- generally are able to quench the more exposed, red tryptophanyl residues, and there will be a tendency for the fluorescence to be blue-shifted with the addition of quencher. Proteins having only a single tryptophanyl residue also show a slight blue shift on quenching in some cases.[51] Thus, difference spectra obtained by comparing the fluorescence in the absence and presence of quencher can aid in the characterization of the various emitting components. By the excitation spectrum which is a useful method to differentiate a dynamic excimer from a static one, the static quenching mechanism can be derived from the perturbation of the absorption spectrum of melamine fluorophores at a low concentration.

In contrast, when melamine is in a higher concentration, the maximum emission spectrum of melamine did not change with the increase of quencher concentration, which means the quencher

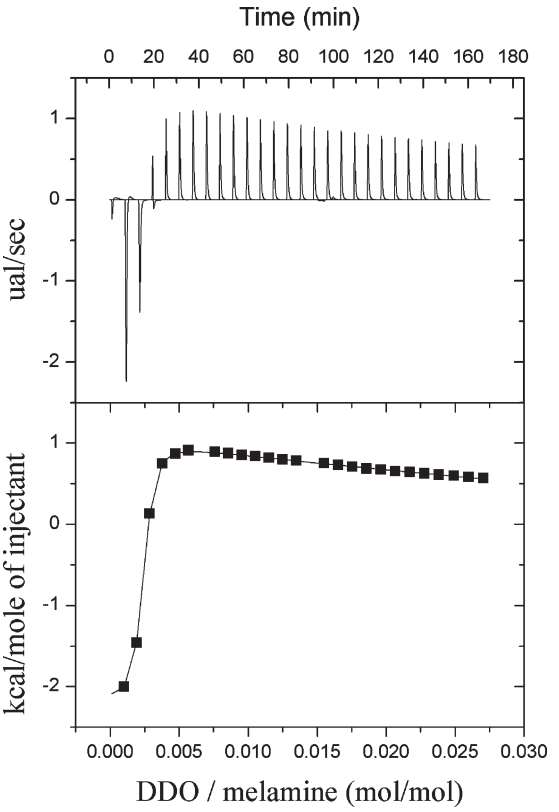


Figure 11. Isothermal titration calorimetry for amphiphilic molecules binding and interaction to melamine at 298 K. The upper panel shows heat flow for each injection (microcalories per second) as a function of time (minutes); the lower panel shows the integration of each peak (kilocalories per mole injectant) as a function of quencher to melamine molar ratio by subtracting the heat of dilution. The quencher in the concentration of 4 mM was put in the syringe and melamine (30 mM) was put in the cell.

only affects the excited states of the fluorophores, and, collisional quenching was expected. Figure 9 shows the Stern−Volmer plots at the concentration of 1 mmol/L. Higher quenching efficiency was observed at 50 °C compared to that of 25 °C, this was due to higher temperatures resulting in faster diffusion and hence larger amounts of collisional quenching. This further proved that the dynamic quenching was involved at a higher melamine concentration. However, in many instances the fluorophore can be quenched both by collisions and by complex formation with the same quencher. The characteristic feature of the Stern−Volmer plots in such circumstances is an upward curvature, concave toward the *y* axis. To account for this curvature an additional quenching mechanism, usually referred to as static quenching, has been considered by many workers.[52−55] The upward bending of the curves in Stern−Volmer plot has been reported in some literature for cationic quenchers with the existence of labeled anionic polyelectrolyte due to the increase in the static quenching,[52−54] and in the interaction of cetylpyridinium chloride with bovine serum albumin.[55] The noticeable upward curvature in the Stern−Volmer plot of the data can be due to a strong cooperativity in the adsorption and/or to static quenching by the bound surfactant. The observed upward Stern−Volmer plot curvature in Figure 9 implies a combined quenching mechanism in this molecular recognition interaction. Furthermore, the upward curvature also is evidence that the fluorescence of melamine was quenched

Table 1. Thermodynamic Parameters Derived from ITC Profiles for Association of DDO Quencher with Melamine at 298 K[a]

	n (binding ratio)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (cal K^{-1} mol^{-1})	K_a (M^{-1})
1	$0.00204 \pm 7.78 \times 10^{-6}$	−8.40	-2.18 ± 0.016	20.9	$1.51 \times 10^6 \pm 3.11 \times 10^5$
2	$0.0369 \pm 9.89 \times 10^{-4}$	−4.34	1.59 ± 0.019	19.9	$1.49 \times 10^3 \pm 458$

[a] ΔH and K were evaluated directly from the titration data using the Origin software. Association constant K = [binding complex]/[quencher]-[melamine]. ΔG and $T\Delta S$ were calculated using $\Delta G = -RT \ln K$ and $\Delta G = \Delta H - T\Delta S$. Mean and standard deviation values from the fitted data of three independent experiments are shown.

more effectively by the spontaneously formed vesicles (concentration above the CAC) than by the monomers of the surfactant. Figure 10 shows the fluorescent intensity of melamine in the presence of DDO at 0.2 mM (mono state) and 0.5 mM (above CAC, vesicles state) respectively. It can be seen that the quenching efficiency was greatly increased when DDO molecules aggregate into vesicles (see the Supporting Information).

It has been well documented that sensitive isothermal titration calorimetry (ITC) is the direct and usually preferred method to measure the heat released in the course of binding and interaction.[56] The thermodynamic parameters obtained by ITC can provide important insights into the underlying physical basis of donor-receptor interactions, which are essential for a better understanding of the kinetics and thermodynamics during molecular recognition processes.[57] Figure 11 shows ITC mesurement for DDO amphiphilic molecules binding and interaction to melamine at 298 K. The inflection point at the ratio of 0.005 (quencher/melamine, mol/mol) was observed. It can be seen that the enthalpy change associated with each injection was exothermic for the first few injections, decreased appreciably after a certain number of injections, and then reached endothermic value for the following injections. An upward pattern of the ITC titration peaks was also observed. The heat flow of the first few drops is exothermic after which it tends to be endothermic. To further investigate the contribution of enthalpy or entropy to the association between the DDO and melamine, the ITC profiles were fit with two sets of binding sites models to yield reasonable thermodynamic parameters of the experimental data. These fitting were derived using ΔH, the association constant (K), and the binding stoichiometry as free-floating parameters. The binding thermodynamic parameters that emerged from these fittings are summarized in Table 1.

It can be seen that both of the free energy (ΔG) is negative indicating that binding of DDO molecules with melamine is favorable energetically. Two kinds of equilibrium association constants existed in the binding process. Enthalpy changes contribute to the association process of DDO with melamine in the first step because of negative ΔH_1. The thermodynamic data herein clearly imply that H-bonded interactions contribute comparatively a lot for the DDO monomer binding with melamine. With the increasing of DDO concentration injection, positive enthalpy changes were observed which means other forces driving the binding reaction and contributing the bulk of the favorable binding free energy. We assume that when DDO which is above its critical aggregation concentration were added into melamine solution, dissociation of the aggregates take place and this process needs more heat which results in the endothermic reaction; meanwhile, the dissociation creates disorder in the system and thus leads to a favorable entropic gain to various degrees, arising from the loss of conformational freedom upon association. The resultant positively integrated heat value demonstrates that the association between DDO and melamine is an entropically driven process.

4. CONCLUSIONS

An orotic acid derived bolaamphiphile (DDO) with molecular recognition function moiety was found to form vesicles spontaneously in aqueous solutions at 25 °C and show molecular recognition to melamine. The direct evidence for molecular recognition of DDO-melamine is observed through steady-state fluorescence spectroscopy. The fluorescence of melamine was quenched more effectively by the spontaneously formed vesicles than by the monomer state of the DDO. Fluorescence of melamine at lower concentration was found to be quenched by static complex formation. While at higher concentration, both static and dynamic quenching mechanisms were involved in the interaction process. Thermodynamic parameters measured by ITC showed that the free energy (ΔG) is negative, indicating that binding of DDO molecules with melamine is favorable energetically. Two kinds of equilibrium association constants existed in binding process. Hydrogen-bonded interactions contribute comparatively a lot for the DDO monomer binding with melamine; at the higher concentration above its critical aggregation concentration, the dissociation of the aggregates take place and lead to an entropically driven molecular recognition process. As complicated binding sites can be constructed through self-assembly at the vesicle interface rather than simple molecular modules, these boalamphiphiles with the molecular recognition functional group may make it possible to generate well-defined recognition sites to mimic biomolecular receptors. Moreover, as the determination of melamine is of biological, clinical, and food industry importance, the present research will give a guide to design chemosensors for melamine detection based on molecular recognition.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details of the synthesis and CAC determined by ITC along with fluorescence quenching efficiency by different concentration of DDO. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 011-86-13735802398. Fax: 011-86-571-88071024. E-mail: zhxchen@ustc.edu.

■ ACKNOWLEDGMENT

This work was financially supported by the National Natural Science Foundation of China (NSFC, No. 20973155). The authors are grateful to the National Nature Science Foundation of China (No. 30770536) and the project sponsored by the scientific research foundation from the education department of Zhejiang Province for the Chinese scholars who studied abroad. We thank Diane M. Reader and Douglas W. Reader at Twin City

of Minnesota for their valuable advice and English grammar checking.

REFERENCES

(1) Lehn, J. M. *Supramolecular Chemistry*; VCH: Weinheim, Germany, 1995.
(2) (a) Zhang, H.-M.; Xie, Z.-X.; Long, L.-S.; Zhong, H.-P.; Zhao, W.; Mao, B.-W.; Xu, X.; Zheng, L.-S. *J. Phys. Chem. C* **2008,** *112*, 4209–4218. (b) Vollhardt, D.; Liu, F.; Rudert, R.; He, W. *J. Phys. Chem. B* **2005,** *109*, 10849–10857. (c) Vollhardt, D.; Liu, F.; Rudert, R. *ChemPhysChem* **2005,** *6*, 1246–1250.
(3) Kovalchuk, N. M.; Vollhardt, D.; Fainerman, V. B.; Aksenenko, E. V. *J. Phys. Chem. B* **2007,** *111*, 8283–8289.
(4) Kohlmeier, A.; Nordsieck, A.; Janietz, D. *Chem. Mater.* **2009,** *21*, 491–498.
(5) Liu, Q.; Zhang, S.; Wu, B.; Guo, J.; Xie, J.; Gu, M.; Zhao, Y.; Yun, L.; Liu, K. *Anal. Chem.* **2005,** *77*, 5302–5310.
(6) Huang, C.-H.; McClenaghan, N. D.; Kuhn, A.; Hofstraat, J. W.; Bassani, D. M. *Org. Lett.* **2005,** *7*, 3409–3412.
(7) Ai, K.; Liu, Y.; Lu, L. *J. Am. Chem. Soc.* **2009,** *131*, 9496–9497.
(8) Ariga, K.; Kunitake, T. *Acc. Chem. Res.* **1998,** *31*, 371–378.
(9) Fersht, A. R. *Trends Biochem. Sci.* **1987,** *12*, 301–304.
(10) Onda, M.; Yoshihara, K.; Koyano, H.; Ariga, K.; Kunitake, T. *J. Am. Chem. Soc.* **1996,** *118*, 8524–8530.
(11) Miao, W.; Du, X.; Liang, Y. *J. Phys. Chem. B* **2003,** *107*, 13636–13642.
(12) Wang, Y.; Du, X.; Miao, W.; Liang, Y. *J. Phys. Chem. B* **2006,** *110*, 4914–4923.
(13) Corvis, Y.; Korchowiec, B.; Korchowiec, J.; Badis, M.; Mironiuk-Puchalska, E.; Fokt, I.; Priebe, W.; Rogalska, E. *J. Phys. Chem. B* **2008,** *112*, 10953–10963.
(14) Michinobu, T.; Shinoda, S.; Nakanishi, T.; Hill, J. P.; Fujii, K.; Player, T. N.; Tsukube, H.; Ariga, K. *J. Am. Chem. Soc.* **2006,** *128*, 14478–14479.
(15) Prabhakaran, D.; Yuehong, M.; Nanjo, H.; Matsunaga, H. *Anal. Chem.* **2007,** *79*, 4056–4065.
(16) Wang, C.; Li, C.; Ji, X.; Orbulescu, J.; Xu, J.; Leblanc, R. M. *Langmuir* **2006,** *22*, 2200–2204.
(17) Yang, M.; Wang, W.; Lieberwirth, I.; Wegner., G. *J. Am. Chem. Soc.* **2009,** *131*, 6283–6292.
(18) Sistach, S.; Rahme, K.; Pérignon, N.; Marty, J. D.; Viguerie, N. L.; Gauffre, F.; Mingotaud, C. *Chem. Mater.* **2008,** *20*, 1221–1223.
(19) Li, Q.; Mittal, R.; Huang, L.; Travis, B.; Sanders., C. R. *Biochemistry* **2009,** *48*, 11606–11608.
(20) Vemula, P. K.; Cruikshank, G. A.; Karp, J. M.; John, G. *Biomaterials* **2009,** *30*, 383–393.
(21) Yan, Y.; Lu, T.; Huang, J. *J. Colloid Interface Sci.* **2009,** *337*, 1–10 and references therein.
(22) Mizoshita, N.; Seki, T. *Langmuir* **2005,** *21*, 10324–10327.
(23) Sun, X.-L.; Biswas, N.; Kai, T.; Dai, Z.; Dluhy, R. A.; Chaikof, E. L. *Langmuir* **2006,** *22*, 1201–1208.
(24) Elferink, M. G. L.; van Breemen, J.; Konings, W. N.; Driessen, A. J. M.; Wilschut, J. *Chem. Phys. Lipids* **1997,** *88*, 37–43.
(25) Goddard, E. D. *J. Colloid Interface Sci.* **2002,** *256*, 228–235.
(26) Gokel, G. W.; Leevy, W. M.; Weber, M. E. *Chem. Rev.* **2004,** *104*, 2723–2750.
(27) Fyles, T. M.; Zeng, B. *J. Org. Chem.* **1998,** *63*, 8337–8345.
(28) Bhosale, S.; Bhosale, S.; Wang, T.; Kopaczynska, M.; Fuhrhop, J.-H. *J. Am. Chem. Soc.* **2006,** *128*, 2156–2157.
(29) Lo Nostro, P.; Ninham, B. W.; Ambrosi, M.; Fratoni, L.; Palma, S.; Allemandi, D.; Baglioni, P. *Langmuir* **2003,** *19*, 9583–9591.
(30) Ambrosi, M.; Fratini, E.; Alfredsson, V.; Ninham, B. W.; Giorgi, R.; Lo Nostro, P.; Baglioni, P. *J. Am. Chem. Soc.* **2006,** *128*, 7209–7214.
(31) Chen, Y.; Liu, Y.; Guo., R. *J. Colloid Interface Sci.* **2009,** *336*, 766–772.
(32) Bhattacharya, S.; Biswas, J. *Langmuir* **2010,** *26*, 4642–4654.
(33) Sirieix, J.; de Viguerie, N. L.; Rivière, M.; Lattes, A. *New J. Chem.* **2000,** *24*, 1043–1048. (b) Sirieix, J.; de Viguerie, N. L.; Rivière, M.; Lattes, A. *Langmuir* **2000,** *16*, 9221–9224.
(34) Thomas, J. K. The Chemistry of Excitation at Interfaces. In *ACS Monographs*; American Chemical Society: Washington, DC, 1984; Vol. 181.
(35) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977,** *99*, 2039–2044.
(36) Han, F.; He, X.; Huang, J.; Li, Z.; Wang, Y.; Fu, H. *J. Phys. Chem. B* **2004,** *108*, 5256–5262.
(37) Mukerjee, P.; Mysels, K. J. *Colloidal Dispersion and Micellar Behavior*; American Chemical Society: Washington, DC, 1975; p 239.
(38) Ueno, M.; Shioya, K.; Nakamura, T.; Meguro, K. *Colloid and Interface Science*; Academic Press: New York, 1977; p 411.
(39) Jones, M. N. *J. Colloid Interface Sci.* **1967,** *23*, 36–42.
(40) Maiti, K.; Mitra, D.; Mitra, R. N.; Panda, A. K.; Das, P. K.; Rakshit, A. K.; Moulik, S. P. *J. Phys. Chem. B* **2010,** *114*, 7499–7508.
(41) Visscher, I.; Engberts, J. B. F. N. *Langmuir* **2000,** *16*, 52–58.
(42) Franceschi, S.; Andreu, V.; Viguerie, N.d.; Riviere, M.; Lattes, A.; Moisand, A. *New J. Chem.* **1998,** *22*, 225–231.
(43) Munoz, S.; Mallen, J.; Nakao, A.; Chen, Z.; Gay, I.; Echegoyen, L.; Gokel, G. W. *J. Am. Chem. Soc.* **1993,** *115*, 1705–1711.
(44) Bandyopadhyay, P.; Bharadwaj, P. K. *Langmuir* **1998,** *14*, 7537–7538.
(45) Vollhardt, D.; Liu, F.; Rudert, R.; He, W. *J. Phys. Chem. B* **2005,** *109*, 10849–10857.
(46) Ma, M.; Paredes, A.; Bong, D. *J. Am. Chem. Soc.* **2008,** *130*, 14456–14458.
(47) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Kluwer Academic/Plenum Publishers: New York, 1999.
(48) Kim, H. J.; Hong, J.; Hong, A.; Ham, S.; Lee, J. H.; Kim, J. S. *Org. Lett.* **2008,** *10*, 1963–1966.
(49) Brun, A. M.; Harriman, A. *J. Am. Chem. Soc.* **1994,** *116*, 10383–10393.
(50) Zhang, Y.-Z.; Zhou, B.; Liu, Y.-X.; Zhou, C.-X.; Ding, X.-L.; Liu, Y. *J. Fluoresc.* **2008,** *18*, 109–118.
(51) Eftink, M. R.; Ghiron, C. A. *Anal. Biochem.* **1981,** *114*, 199–227.
(52) Delaire, J. A.; Rodgers, M. A. J.; Webber, S. E. *J. Phys. Chem.* **1984,** *88*, 6219–6227.
(53) Yamamoto, H.; Mizusaki, M.; Yoda, K.; Morishima, Y. *Macromolecules* **1998,** *31*, 3588–3594.
(54) Morishima, Y.; Ohgi, H.; Kamachi, M. *Macromolecules* **1993,** *26*, 4293–4294.
(55) Díaz, X.; Abuin, E.; Lissi, E. *J. Photochem. Photobiol. A: Chem.* **2003,** *155*, 157–162.
(56) Jelesarov, I.; Bosshard, H. R. *J. Mol. Recognit.* **1999,** *12*, 3–18.
(57) Chen, Z.-X.; Guo, G.-M.; Deng, S.-P. *J. Agric. Food Chem.* **2009,** *57*, 2945–2954.