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Buckled Membranes in Mixed-Valence Ionic Amphiphile Vesicles

Megan A. Greenfield,[†] Liam C. Palmer,[‡] Graziano Vernizzi,[§] Monica Olvera de la Cruz,[‡,§] and Samuel I. Stupp*[,‡,§,¶]

Departments of Chemical & Biological Engineering, Chemistry, Materials Science & Engineering, and Feinberg School of Medicine, Northwestern University, Evanston, Illinois 60208

Received May 1, 2009; E-mail: s-stupp@northwestern.edu

A great challenge in supramolecular chemistry is the design of molecules that can self-assemble into functional aggregates with well-defined 3D structures. To achieve this goal it is important to understand the interplay of attractive and repulsive forces in determining supramolecular shape. It has been hypothesized that when oppositely charged amphiphilic molecules are mixed, they can form vesicles with a periodic 2D ionic (electrostatic) lattice.[1] This tendency opposes the membrane's natural curvature and can result in the buckling of vesicles.[2] Previously, mixtures of monovalent anionic and cationic surfactants have been shown to generate micrometer-sized nonspherical (icosahedral) vesicles when mixed.[3] Since it is well known that the large majority of viruses display icosahedral symmetry,[4] buckled membrane nanostructures are an interesting target for functional self-assembled nanostructures. These systems could also be used to design mineralizations that occur readily on flat molecular assemblies.[5] Here we compute the elastic properties of various mixtures of cationic and anionic molecules and find that the $+3$:-1 stoichiometric ratio of the headgroups maximizes ionic interactions and reinforces the close-packed hexagonal lattice of the hydrophobic tails. Thus, we designed amphiphiles with $+3$ and -1 headgroups to demonstrate the possibility of using electrostatics to generate buckled vesicles (BVs). The amphiphiles were designed to undergo diacetylene topochemical polymerization as an indicator of lateral correlations among molecules in the aggregates. The vesicle surface should then buckle into flat domains, separated by edges, to release the strain of curving an ionic lattice.

The amphiphilic molecules used are **1**, which consists of a $+3$ charged amino acid sequence covalently attached to an alkyl acid containing a diacetylene moiety, and **2**, which is a diacetylenic fatty acid (Figure 1). Diacetylenes can undergo a 1,4-addition polymerization when exposed to UV light. Importantly, this topochemical polymerization requires the positions of the atoms to be at specific distances.[6] Thus higher degrees of polymerization, indicated by a color change to blue,[7] can only occur if the fatty acid tails are locally ordered. This functional group has been previously investigated for the polymerization of 2D polymers[8] and peptide amphiphile micelles[9] and vesicles.[10]

The supramolecular structures of the amphiphiles were characterized by quick-freeze deep-etch (QFDE) transmission electron microscopy (TEM). QFDE is a sample preparation technique that allows high-resolution imaging of hydrated structures while minimizing disruption of the sample due to fixation or processing. Solutions of **1** were composed of micelles with an average diameter of ~10 nm (Figure 2a). In contrast, solutions of a mixture of **1** and **2** contained vesicles with an average diameter of ~200 nm. Some vesicles appeared to buckle on all sides, whereas others have both flat and rounded domains in the same structure.

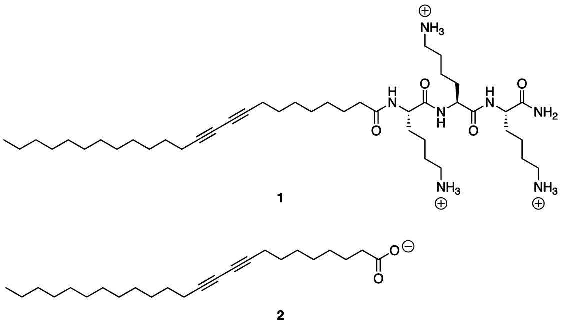

Figure 1. Molecular structure of cation **1** and anion **2**.

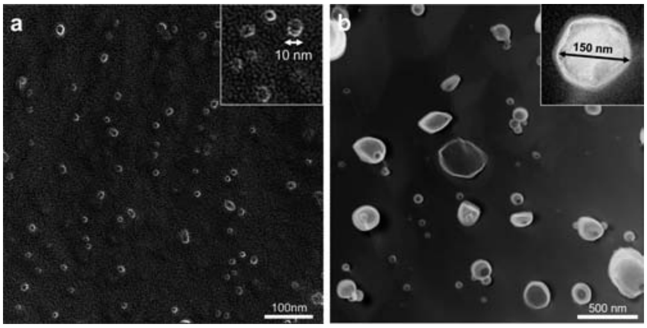

Figure 2. Quick-freeze deep-etch TEM microscopy of (a) micelles formed from **1** and (b) larger buckled vesicles from **1** and **2** in water.

To determine whether these BVs could be polymerized, we irradiated at 254 nm samples of **1** alone, **2** alone, and the catanionic mixture. The samples of **1** alone and **2** alone did not polymerize after UV irradiation, even in solutions of 1 M NaCl. In contrast, the mixed sample turned blue upon irradiation and showed the characteristic trace of polymerized diacetylene (Figure 3). When **1** and **2** were dissolved together in 1 M NaCl no polymerization was observed, indicating that the salt inhibited the formation of the ordered lattice necessary for polymerization. Furthermore, these solutions do not contain BVs. However, we found that adding NaCl *after mixing* **1** and **2** in water does not inhibit their ability to polymerize or to form BVs. The coassembly also polymerized in the presence of KCl and phosphate buffered saline (pH = 7.8), but little or no polymerization was observed in solutions of NaI, $NaClO_4$, or LiCl (Supporting Information (SI)). This is consistent with the Hofmeister series of protein solubilization for anions ($HPO_4^{2-} > Cl^- > I^- > ClO_4^-$) and cations ($K^+ > Na^+ > Li^+$), suggesting that the more highly hydrating ions (I^-, ClO_4^-, and Li^+) cause the ionic lattice to expand and inhibit polymerization. We concluded that only the mixed systems had sufficient internal order to support the topochemical polymerization of the diacetylenes in the hydrophobic core. These data are consistent with the formation of an ionic lattice by the charged headgroups.

[†] Department of Chemical & Biological Engineering.
[‡] Department of Chemistry.
[§] Department of Materials Science & Engineering.
[¶] Feinberg School of Medicine.

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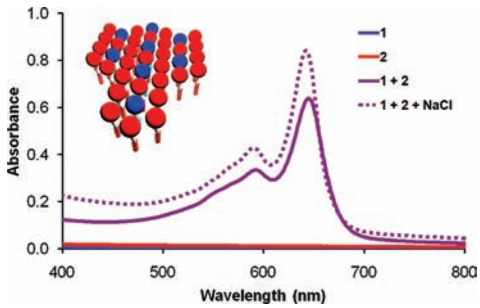

Figure 3. Absorbance spectra show that photopolymerization only occurs with the mixture of **1** and **2**. The inset shows the hexagonal lattice expected to form when **1** and **2** are mixed.

The formation of BVs can be understood as the manifestation of electrostatic interactions among charged headgroups. These headgroups can form a network with strong lateral correlations that can be considered to be a solid membrane.[11] When deformed, these membranes develop internal strains due to the strong intermolecular interactions. A solid membrane can partially relax some of the stretching energy by buckling in a direction orthogonal to the membrane.[12] From a theoretical point of view, solid membranes have been treated as thin, elastic plates. It has been shown that thin elastic spherical shells buckle into faceted shapes when the Föppl−von Kármán number $\gamma = YR^2/\kappa$ is larger than the critical value of $\sim$400, where Y is the Young modulus, κ is the bending rigidity, and R is the linear size of the shell.[13] Elastic theory explains the observed buckling of large viruses or large fullerenes and the lack of facets in self-assembled vesicles with neutral components, where Y is negligible because of the liquid nature of the surface. In the system considered here the strong lateral correlations among charged components generate a cohesive energy and $Y > 0$.

We evaluate the energy E of a regular 2D electroneutral lattice of $+q$ and -1 charges. This energy can be expressed as $E = E_{\mathrm{elect}} + E_{\mathrm{LJ}}$, where $E_{\mathrm{elect}} = l_{\mathrm{B}}/2 \sum_{i,j} q_i q_j/r$ is the electrostatic long-range energy and $E_{\mathrm{LJ}} = 2u \sum_{i,j} ((\sigma/r)^{12} - (\sigma/r)^6)$ is the short-range Lennard−Jones energy. Here $l_{\mathrm{B}} = e^2/\epsilon K_{\mathrm{B}}T$ is the Bjerrum length (where e is the elementary charge, ϵ is the dielectric permittivity, and $K_{\mathrm{B}}T$ is the thermal energy), q_i is the charge at the i-th lattice site, r is the distance among charges, u is the magnitude of the Lennard-Jones potential, and σ is the size of the short-range repulsive potential. The numerical minimization of E confirms that stable hexagonal lattices form for the 2:1 and 3:1 case. However, in the 1:1 case the lattice is square when electrostatics dominate ($l_{\mathrm{B}}/\sigma u > \sim 12.5$) and hexagonal otherwise. We then calculated the Young modulus for the different charges of the cation and find at large $l_{\mathrm{B}}/\sigma u$ that $Y_{3:1} \approx 38.5 l_{\mathrm{B}}/(\sigma u)$, $Y_{3:1}/Y_{1:1} \approx 60$, and $Y_{3:1}/Y_{2:1} \approx 2$. Such values clearly show that Y increases as the charge ratio increases. When salt is added, for screening lengths $\zeta \geq L$, where L is the average distance among trivalent headgroups ($L \approx 2\sigma$), $Y_{3:1}$ remains large (SI). The bending rigidity of the membrane is also affected by electrostatic interactions among charged headgroups. Lau and Pincus have demonstrated that the bending rigidity of a membrane composed of a mixture of anionic and cationic surfactants (overall neutral) in aqueous solution decreases because of electrostatic interactions.[14] We explored the effect of different stoichiometric charge ratios on the membrane's bending rigidity and found that higher stoichiometric ratios yield lower bending rigidities (SI). The decreasing bending rigidity with increasing stoichiometric charge ratio results in an increase of the Föppl−von Kármán number, supporting vesicle buckling at smaller radii, even in the presence of salt.

Interestingly, some of the vesicles were only partially buckled. While mixing the two amphiphiles clearly results in a change of morphology, we cannot exclude the possibility of some local phase separation on the membrane surface. However, the absorbance data indicate local ordering in the mixed system, suggesting coassembly on the molecular scale. As these anionic and cationic amphiphiles mix, they likely form solid-like strongly laterally correlated domains within the fluid vesicle membrane.[15] The partial buckling may originate from the energetic penalty of bending the ionic domains over a curved surface, as described previously by Lipowsky and Dimova.[16] If the amphiphiles were phase segregated rather than mixed, then no change in the vesicle's shape would be expected. Phase-segregated mixtures of neutral amphiphiles over large fluid vesicles have been previously shown to retain their spherical shape.[16,17]

Our results demonstrate that a large charge imbalance between the cationic and anionic head groups of amphiphiles can enable their coassembly into small BVs. In contrast to previous reports of BVs from oppositely charged amphiphiles, the structures described here form without the rigorous exclusion of salt and are even tolerant to physiological salt concentrations. Also, the polymerizable diacetylene groups provide a unique handle to establish the presence of strong intermolecular lateral correlations. These correlations are imposed by the ionic lattice on the vesicle surface and produce the solid-like domains responsible for the observed buckling. Numerical simulations supporting a similar electrostatic-driven buckling mechanism for icosahedral ionic shells have been presented recently.[2] Our work opens a new path for exploring how ionic laterally correlated domains can influence the morphology of self-assembled nanostructures.

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Supporting Information Available: Synthetic procedures, characterization, and additional microscopy, spectroscopy, and theory. This material is available free of charge via the Internet at http://pubs.acs.org.

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