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Aqueous Phase Separation in Giant Vesicles

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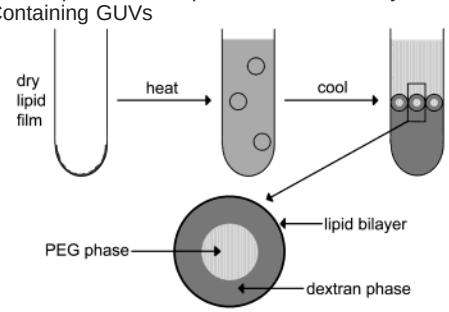
We report the encapsulation of an aqueous two-phase system (ATPS)[1] within giant unilamellar vesicles (GUV).[2] Phospholipid vesicles have long been recognized as models for the cell membrane.[3,4] Recently, they have been investigated as reaction vessels[5] and in the development of primitive functional mimics of living cells, in which biochemical reactions are carried out within the liposome.[6] The cytoplasm of biological cells contains high concentrations of macromolecules (e.g., an *E. coli* cell contains ~340 mg/mL of proteins and nucleic acids).[7] This leads to macromolecular crowding[8] and has been postulated to result in phase separation of the cytoplasm.[9] Bulk aqueous solutions of two chemically dissimilar polymers will phase separate at concentrations above a few weight percent; the resulting ATPS have applications in biomolecule separations and cell fractionation.[10] If phase separation does occur within living cells, it would lead to microcompartmentation and might explain some observations of cell structure and function.[11]

We have encapsulated an ATPS composed of poly(ethylene glycol) (PEG), dextran, and water within GUVs using the strategy shown in Scheme 1. Vesicles were prepared above the phase transition temperature of the polymer solution. Upon cooling to room temperature, the polymer solution phase separated both within the vesicles and in the bulk solution. ATPS-containing vesicles were collected from the bulk interface for study.

Scheme 1. Preparation of Aqueous Two-Phase System (ATPS)-Containing GUVs



Scheme 1 requires an ATPS that can be converted from one to two phases via a modest temperature change. We have identified a PEG/dextran composition (3.75% PEG 8000 g/mol, with 4.5% dextran 482 000 g/mol in H_2O) that is biphasic at 25 °C and can be converted to a single phase at the elevated temperatures used during vesicle preparation (50 °C). Vesicles composed of egg phosphatidylcholine (PC):DOPG (in a 9:1 ratio) with <0.05 mol % rhodamine-tagged DOPE were prepared by a modification to published protocols using this ATPS as the aqueous phase (a detailed protocol is available as Supporting Information).[12,13] To characterize the polymer phases following separation, fluorescently tagged polymers (i.e., FITC-dextran or FITC-PEG) were incorporated during preparation. This protocol yielded not only the desired ATPS-GUVs but also significant numbers of multilamellar vesicles and smaller uni- and oligolamellar vesicles, as is typical for GUV preparations.[12,14] The yield of ATPS-GUVs was quite high, however, with many of the unilamellar vesicles observed showing phase separation (Supporting Information).

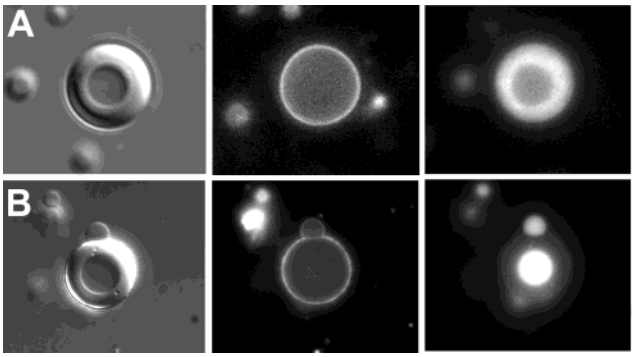


Figure 1. Optical microscopy of representative ATPS-containing liposomes. (A) 6.5 μm ATPS-GUV labeled with 0.001 wt % FITC-dextran 500 000 g/mol. (B) 12 μm ATPS-GUV labeled with 0.001 wt % FITC-PEG 5000 g/mol. (left panels) Transmitted light images taken with differential interference contrast (DIC) optics, showing ATPS-containing vesicles. (center panels) Fluorescence images showing location of rhodamine labeled lipid. (right panels) Fluorescence images of FITC-tagged polymers. T = 25 °C.

Figure 1 shows representative ATPS-containing liposomes from preparations labeled with a small mole fraction of FITC-dextran (1A) or FITC-PEG (1B). Phase separation is visible even in the transmitted light (DIC) images of these vesicles (left panels). The ATPS-GUVs appear as two concentric circles, with different degrees of contrast to the inner and outer shell. The inner circle is the result of the interface formed between the encapsulated polymers upon cooling. The lack of a lipid membrane at this interface is evident in Figure 1 (center panels), which displays fluorescence from rhodamine-tagged DOPE lipids incorporated in the bilayer. The identity of the inner and outer phases was determined by imaging FITC-tagged polymers (Figure 1, right panels).[15] In every case, the PEG-rich phase was surrounded by the dextran-rich phase, which bordered the GUV membrane. In bulk preparations, the more dense dextran-rich phase forms on the bottom. In microvolumes, the effect of gravity is less important, and surface interactions can dominate. Thus, the phase with higher affinity for the lipid bilayer surface is expected to wet the bilayer preferentially.[16] However, incorporation of 0.05 mole fraction of either PEG 1000 or PEG 5000-modified DOPE lipid within the bilayer did not reverse the relative positions of the two phases (Supporting Information). Experiments are currently underway to determine the impact of phase density relative to that of surface chemistry in these microenvironments.

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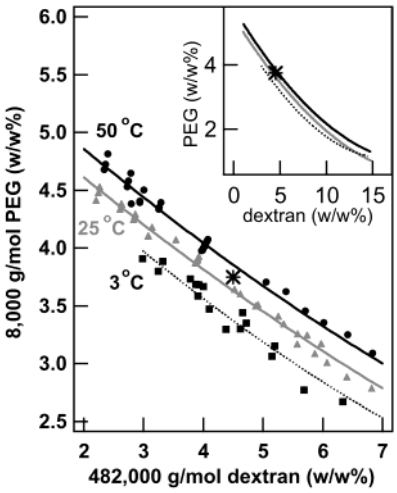

Figure 2. Experimentally determined phase diagram for PEG 8000 g/mol-dextran 482 000 g/mol ATPS at 3, 25, and 50 °C. Solutions exist as two phases at compositions above the binodal curve for a given temperature. An asterisk marks the composition used in this work. (inset) A larger portion of the phase diagram for this system. Lines shown are polynomial best fits to the data and are included to guide the eye.

In Figure 1B, a portion of the PEG phase has been expelled from the vesicle, but remains attached within a contiguous lipid membrane. We observe some of these structures in ATPS-GUV samples at room temperature and can induce PEG-phase expulsion by heating the vesicles. Bulk ATPS of this composition converts to a single phase above 50 °C. In contrast, heating encapsulated ATPS to 50 °C did not promote the formation of a single phase but instead resulted either in no change or in the expulsion of the inner PEG phase; each outcome occurred with approximately equal frequency. Expulsion reduces the contact area between PEG- and dextran-rich phases, but requires an expansion in lipid bilayer surface area. Thus, one explanation for these results is that the elevated temperature facilitates membrane area expansion,[17] which may promote the escape of the PEG-rich phase before conversion to a single phase can occur.[18] The ejected PEG phase remains in close contact with the exterior surface of the parent vesicle and is encapsulated by the lipid bilayer. Interestingly, while we often observe partial expulsion (Figure 1B) in as-prepared samples, heating-induced expulsion leads to complete removal of the PEG phase from the interior into an attached "daughter vesicle".[19] Further experiments are underway to understand the differences in phase behavior for encapsulated ATPS as compared to bulk.

Figure 2 shows the phase diagram for the PEG 8000/dextran 482 000 ATPS at 3, 25, and 50 °C as determined using the "cloud point determination" method.[20] The curves shown are binodals, which delineate the two-phase region (above) from the single-phase region (below). As the temperature is increased, the phase diagram for ATPS-forming polymer/polymer/water mixtures shifts to favor a single phase over two phases. An asterisk marks the composition of the ATPS used in these GUV encapsulation experiments, which lies between the 50 and 25 °C binodals. Although the separation between these binodals is quite small, the 3.75% PEG 8000 g/mol, 4.5% dextran 482 000 g/mol system can be reliably controlled by converting between 50 and 25 °C. Note that polymer compositions corresponding to any other point between these binodals also meet the temperature conditions necessary for ATPS encapsulation. Choice of different polymer compositions within this range will control the volumes of the two phases.[20] The shift in binodal curve position is as significant between 25 and 3 °C as it is between 50 and 25 °C; thus, by appropriate choice of polymer concentrations, it should be possible to encapsulate ATPS within GUVs at 25 °C. Lower temperature synthesis will facilitate incorporation of biomacromolecules and may inhibit PEG-expulsion in ATPS-GUVs.

Herein we have reported the spatial organization of distinct aqueous phases within cell-sized containers. Such structures are interesting not only from the standpoint of materials assembly, but also as primitive models of microcompartmentation in living cells.

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Supporting Information Available: Detailed synthetic protocol and additional images of ATPS-GUVs, including ATPS-GUVs prepared with 0.05 mol % PEG 1000-DOPE lipid, and a larger plot of data from the inset of Figure 2 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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