Lipid Membranes in Contact with Aqueous Phases of Polymer Solutions

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The interior of living cells is crowded with macromolecules and organelles. The weight fraction of proteins, RNAs and polysaccharides is on the order of 20–30 %. Interactions between macromolecules in water can lead to the formation of coexisting aqueous phases. Thus, in the concentrated interior of the cell, local phase separation may occur, involving microcompartmentation, which in turn can affect, e.g., cell functioning and the performance of cytoplasmic proteins.

The phenomenon of phase separation is often observed in solutions of two polymer species. The most well studied aqueous two-phase system (ATPS) formed by macromolecules is the one of poly(ethylene glycol) (PEG) and dextran. We studied the phase separation of this system in the closed compartment of lipid vesicles as a model for biological microcompartmentation. Giant lipid vesicles loaded with polymer solutions typically contain two droplets with different polymer compositions, formed by phase separation within the vesicle interior **[1]**. We employed these cell-sized biomimetic systems to study the wetting behavior of the polymer phases on the membrane **[2, 3]**, the reorganization of the lipid bilayer arising from molecular crowding **[4]** and the resulting morphological shapes adopted by vesicles loaded with ATPS **[5]**.

Aqueous Two-Phase Polymer Solutions of Dextran and PEG

Above a total polymer weight fraction of a few weight percent, aqueous solutions of PEG and dextran demix. The corresponding two-phase region is bounded by the binodal line within the phase diagram of the system (**Fig. 1**). At concentrations below the binodal the polymer solution is homogeneous. Above the binodal, the solution undergoes phase separation and the compositions of the coexisting phases are given by the tie lines in the phase diagram. A variety of methods for tie-line determination has been explored in the literature based on the use of different experimental techniques. Recently, we proposed a relatively simple approach based on density measurements of the phases **[6]**.

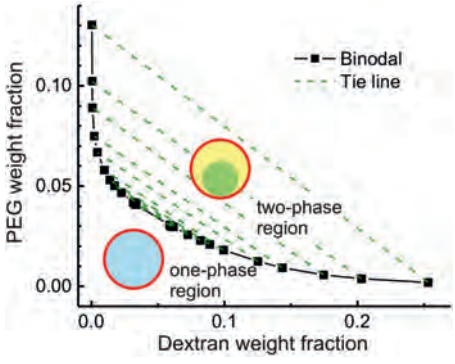

*Fig. 1: Binodal and tie lines of the aqueous solution of dextran (molecular weight 400-500 kg/mol) and PEG (molecular weight 8 kg/mol) measured at 24 °C **[6]**. Below the binodal the polymer solution is homogeneous; above the binodal it undergoes phase separation. The insets schematically illustrate the vesicle membrane (red) enclosing the homogeneous solution (blue) or the two liquid droplets consisting of dextran-rich (green) and PEG-rich (yellow) phases.*

Membrane Wetting and Wetting Transition

Liquid droplets at interfaces may exhibit zero or nonzero contact angles corresponding to complete or partial wetting, respectively. As one varies the liquid composition, the system may undergo a transition from complete to partial wetting. Such a transition can also occur for an aqueous solution enclosed within a vesicle **[2]**. In this case, the substrate is the lipid membrane.

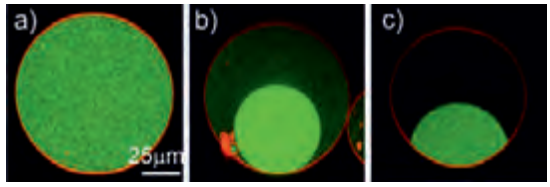

Fig. 2: Phase separation inside a vesicle (confocal vertical cross-sections). The membrane is labelled in red; a small fraction of dextran is labelled in green. As the external osmolarity is increased in a stepwise manner, phase separation occurs (a, b) and the dextran-rich drop (green) undergoes a wetting transition (b, c).

We used giant vesicles encapsulating PEG-dextran solutions in the one-phase state (**Fig. 2a**). In order to obtain vesicles containing two phases, we raise the interior polymer concentration above the binodal by exposing the vesicles to a hypertonic medium, i.e., by osmotic deflation. The polymer concentration inside increases, leading to phase separation (**Fig. 2a, b**). The dextran-rich phase is heavier and thus, the dextran-rich droplet is always located at the bottom of the vesicle. When the external osmolarity is further increased, the dextran-rich phase starts to wet the membrane (**Fig. 2c**). The morphology change of the dextran-rich droplet indicates a wetting transition from complete wetting of the PEG-rich phase in **Fig. 2b** to partial wetting in **Fig. 2c**.

Wetting-Induced Budding

When both phases wet the membrane, the smaller one may bud out of the vesicle upon further deflation (**Fig. 3a-c**). Initially, for weak deflation, the vesicle is approximately spherical (**Figs. 2c and 3b**). Upon further deflation, the dextran-rich phase starts to form a bud away from the PEG-rich phase (**Fig. 3c**). The excess area arising from deflation is utilized by the vesicle to undergo morphological changes and a budding transition **[5]**. The direction of budding can be reversed if the phase separation occurs in the vesicle exterior **[5]**.

In mechanical equilibrium, the two membrane tensions $\hat{\Sigma}_{pe}$ and $\hat{\Sigma}_{de}$ must be balanced along the contact line (where the external medium, the PEG-rich phase and the dextran-rich phase are in close proximity) by the interfacial tension Σ_{pd} between the two liquid phases (**Fig. 3d**). The interfacial tension Σ_{pd} pulls on the membrane towards the vesicle interior. When Σ_{pd} is small, the membrane tensions can easily balance this pulling force in the normal direction and the contact angle θ_e remains close to 180 degrees. As the interfacial tension Σ_{pd} increases and the vesicle is deflated further, the membrane tension can no longer sustain the quasi-spherical vesicle shape, the membrane bends along the contact line and the dextran-rich phase buds out. The budding event sig-

nificantly reduces the interfacial energy by decreasing the interfacial area between the two liquid phases.

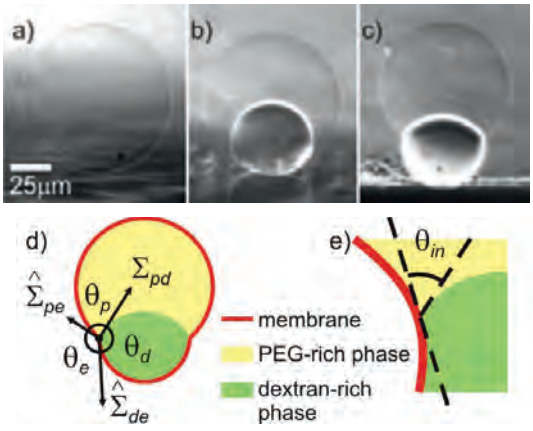

Fig. 3: (a-c) Side-view phase contrast images of a budding vesicle. After phase separation (a, b), the interior solution consists of PEG-rich (lighter) and dextran-rich (heavier) droplets. Further deflation of the vesicle causes the dextran-rich droplet to bud out as shown in (c). In the sketch in (d), the three effective contact angles as observed with optical microscopy are indicated as well as the two membrane tensions and the interfacial tension Σ_{pd}. The intrinsic contact angle θ_{in}, which characterizes the wetting properties of the membrane by the PEG-rich phase at the nanometer scale is sketched in (e).

The kink in the vesicle membrane shown in **Fig. 3c, d** is observed by optical microscopy but cannot persist to small length scales, since such a kink would imply an infinite bending energy of the membrane. Therefore, when viewed with suboptical resolution, the membrane must be smoothly curved as in **Fig. 3e**, which implies the existence of an intrinsic contact angle θ_{in}. In contrast to the three contact angles shown in **Fig. 3d**, the intrinsic contact angle represents a material parameter that is independent of the vesicle geometry **[3]**.

Formation of Membrane Nanotubes

Upon vesicle deflation, excess area is created. Depending on the membrane tension and spontaneous curvature, the area created during deflation may lead to vesicle budding as shown above, and/or may be involved in creating membrane nanotubes **[4]**, which have a diameter below optical resolution and become visible when fluorescently labelled (**Fig. 4**). The tubes form during the phase separation process and are stable after this process has been completed. They are always in contact with the PEG-rich phase and adsorb onto the two-phase interface forming a layer or meshwork of tubes (**Fig. 4b**). When the interface becomes overcrowded, hundreds of tubes protrude into the PEG-rich phase (**Fig. 4c**).

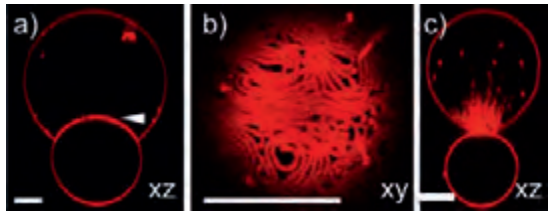

Fig. 4: Tube formation in vesicles with internal phase separation. (a) Vertical xz-section showing adsorption of tubes onto the two-phase interface (arrowhead). (b) Horizontal xy-section at the z-position of the arrowhead in (a) showing tubes at the two-phase interface. (c) Vertical xz-section of a vesicle with overcrowded two-phase interface; the tubes protrude into the upper PEG-rich phase. The scale bars correspond to 15 µm.

A theoretical analysis of the deflated vesicles reveals that these membrane tubes are stabilized by negative spontaneous curvature **[4, 7]**. Using the large separation of length scales between the tube diameter and the overall size of the vesicles, the spontaneous curvature can be calculated and is found to be about –1/(240 nm) for a certain range of polymer concentrations. The nanotubes can also be retracted back into the mother vesicle by increasing the membrane tension via micropipette aspiration of the vesicle.

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