

MEMBRANES AND VESICLES

Aqueous Phase Separation in Vesicles: Wetting Phenomena and Nanotube Formation



Aqueous solutions containing two species of water-soluble polymers such as dextran and polyethylen glycol (PEG) undergo phase separation as soon as the polymer concentrations exceed a few weight percent, see **Fig. 1**.

Membranes and vesicles suspended in such a solution are then exposed to two different aqueous phases. Such lipid/polymer systems undergo complete-to-partial wetting transitions [1],

exhibit effective and intrinsic contact angles [2], and lead to the formation of membrane nanotubes [3].

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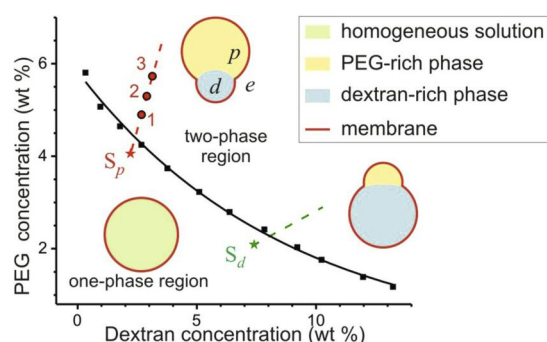


Figure 1: Phase diagram of aqueous solution of PEG and dextran. The solution undergoes phase separation into a PEG-rich phase p and a dextran-rich phase d as soon as the concentration of one of the polymers exceeds a few percent. The black line represents the binodal, i.e. the boundary between the one-phase and the two-phase region. The red and the green lines indicate two different deflation trajectories starting from two initial states S_p and S_d in the one phase region.

A convenient method to induce phase separation within the vesicles is by osmotic deflation. Osmotically active particles such as sugar molecules are added to the exterior solution, and the resulting osmotic unbalance leads to the permeation of water through the vesicle membranes and to a reduced volume of the vesicles. Since the dissolved polymers cannot permeate the membrane, the polymer concentration is increased, and the aqueous solution within the vesicle forms two separate phases, a PEG-rich and a dextran-rich phase, see **Figs. 2** and **3**.

In **Fig. 2**, the aqueous solution within the vesicle is initially homogeneous, see **Fig. 2(a)**, and then forms, during successive deflation steps, a dextran-rich droplet (light green) and a PEG-rich droplet (dark green), see **Fig. 2(b) - (f)**.

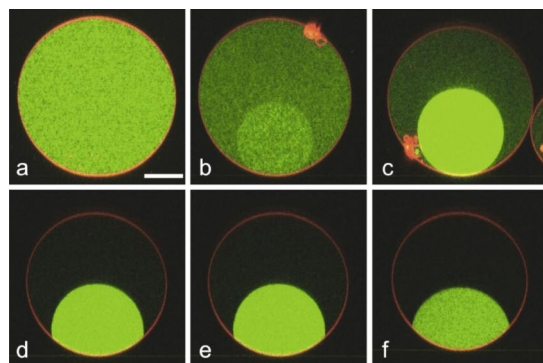


Figure 2: Confocal micrographs of a lipid vesicle (red line) which encloses a PEG-rich droplet (dark green) and a dextran-rich droplet (light green). As the vesicle is deflated from (c) to (d), the membrane undergoes a transition from complete to partial wetting by the PEG-rich phase. Further deflation from (d) to (f) leads to an increasing value of the contact angle between the membrane and the PEG-rich phase. Scale bar: 20 μm . [1]

Inspection of **Fig. 2** shows that the contact angle between the PEG-rich droplet and the membrane is close to zero for small deflation as in **Fig. 2(b)** and **(c)** but starts to increase for larger deflation as in **Fig. 2(d) - (f)**. Therefore, the system undergoes a complete-to-partial wetting transition as the vesicle is deflated from **Fig. 2(c)** to **(d)**.

The vesicle shapes shown in **Fig. 2(d) - (f)** are somewhat special since they stay essentially spherical even though the membrane is partially wet by both phases. In general, partial wetting of the membrane leads to a kink along the contact line, at which the membrane is pulled by the interface between the PEG-rich and dextran-rich phase, see **Fig. 3(a)**. In all cases, this contact line divides the membrane into two distinct segments, separating the two aqueous phases, α and β , within the vesicle interior from the exterior solution, γ . In general, these two membrane segments experience two

distinct mechanical tensions, $\Sigma_{\alpha\gamma}$ and $\Sigma_{\beta\gamma}$. In mechanical equilibrium, these two tensions must be balanced, along the contact line, by the interfacial tension $\Sigma_{\alpha\beta}$ between the two liquid phases, see **Fig. 3(a)** and **(b)**.

The kink shown in **Fig. 3(a)** 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. 3(b)**, which implies the existence of an intrinsic contact angle Θ_{in} . In contrast to the three contact angles shown in **Fig. 3(a)**, the intrinsic contact angle represents a material parameter that is independent of the vesicle geometry. **[2]**

Another unexpected aspect of the aqueous phase separation within the vesicles is the formation of membrane nanotubes, see **Fig. 4**. The tubes have a diameter below optical re-

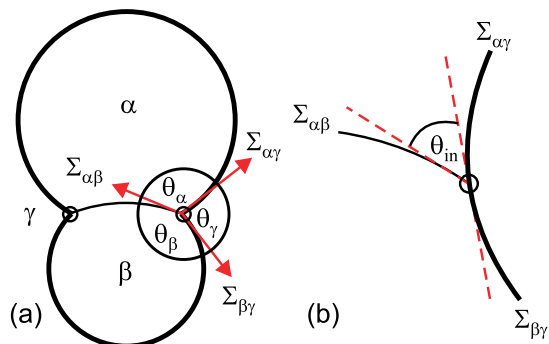


Figure 3: (a) Cross-section of a vesicle enclosing one α (top) and one β (bottom) droplet suspended in the exterior solution γ . When viewed with optical resolution, the vesicle shape exhibits a sharp kink along the contact line (\odot) and can be characterized by three effective contact angles θ_α , θ_β , and θ_γ . These contact angles are related to the three tensions $\Sigma_{\alpha\beta}$, $\Sigma_{\alpha\gamma}$, and $\Sigma_{\beta\gamma}$ via the force balance along the contact line; and (b) Enlarged view close to the contact line: Intrinsic contact angle Θ_{in} between the two planes that are tangential to the α_β interface and to the smoothly curved vesicle membrane, respectively, at a certain point of the contact line. **[2]**

solution and become only visible when fluorescently labeled. The tubes form during the phase separation process and are stable after this process has been completed **[3]**. A theoretical analysis of the deflated vesicles reveals that these membrane tubes are stabilized by negative spontaneous curvature. 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 $-1/(240 \text{ nm})$ 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.

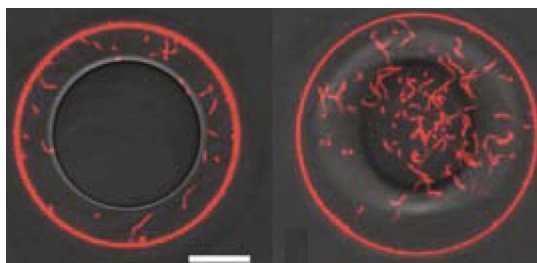


Figure 4: Membrane nanotubes (short red segments) extending from the vesicle membrane (red circle) into the vesicle interior. The nanotubes are below optical resolution and only visible when fluorescently labeled. These tubes are induced by the aqueous phase separation and stabilized by the spontaneous curvature of the membranes. The latter curvature is negative and about $-1/(240 \text{ nm})$. The tube may then have a cylindrical shape with radius 120 nm, a necklace-like morphology consisting of small spheres with a radius of 240 nm, or some intermediate morphology. **[3]**

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References:

- [1]** Y.H. Li, R. Lipowsky, and R. Dimova. Transition from complete to partial wetting within membrane compartments. *JACS*, **130**:12252–12253, 2008.
- [2]** H. Kusumaatmaja, Y.-H. Li, R. Dimova, and R. Lipowsky. Intrinsic contact angle of aqueous phases at membranes and vesicles. *Phys. Rev. Lett.*, **103**: 238103, 2009.
- [3]** Y.-H. Li, R. Lipowsky, and R. Dimova. Membrane nanotubes induced by aqueous phase separation and stabilized by spontaneous curvature. *Proc. Nat. Acad. Sci. USA*, **108**: 4731-4736, 2011.