Supporting Information

Aqueous Phase Separation in Giant Vesicles

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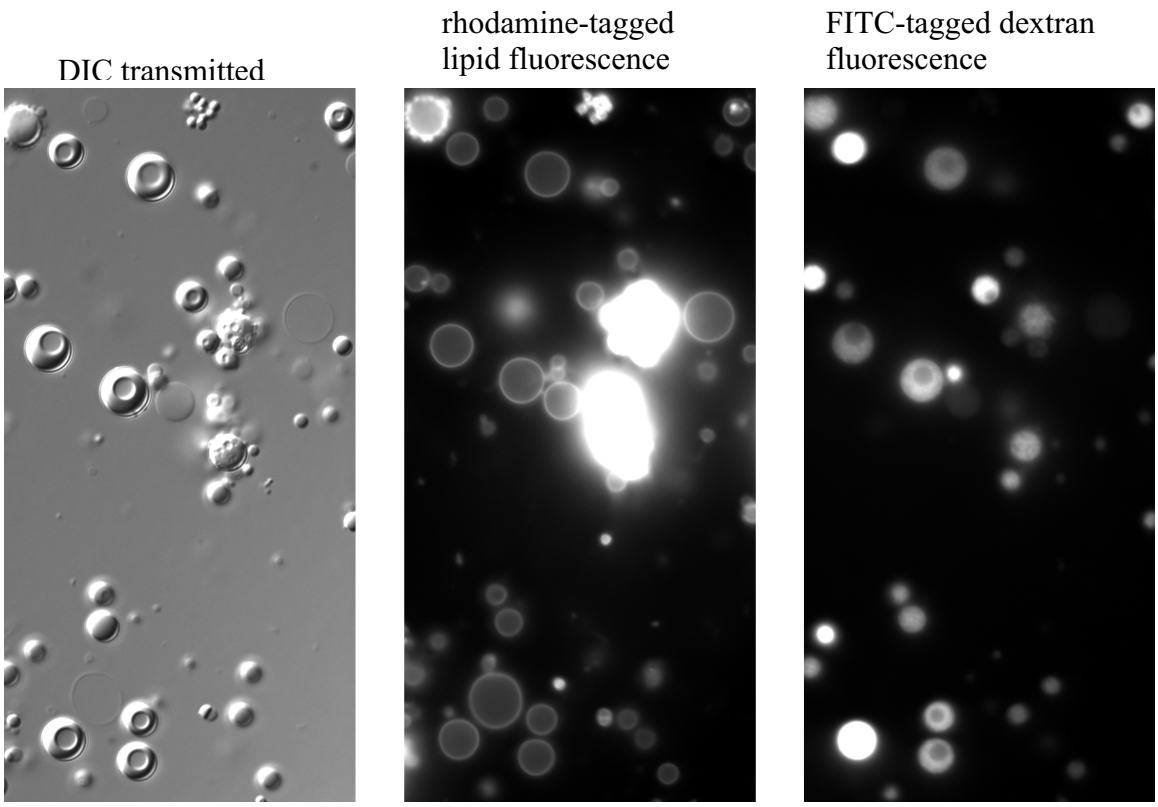
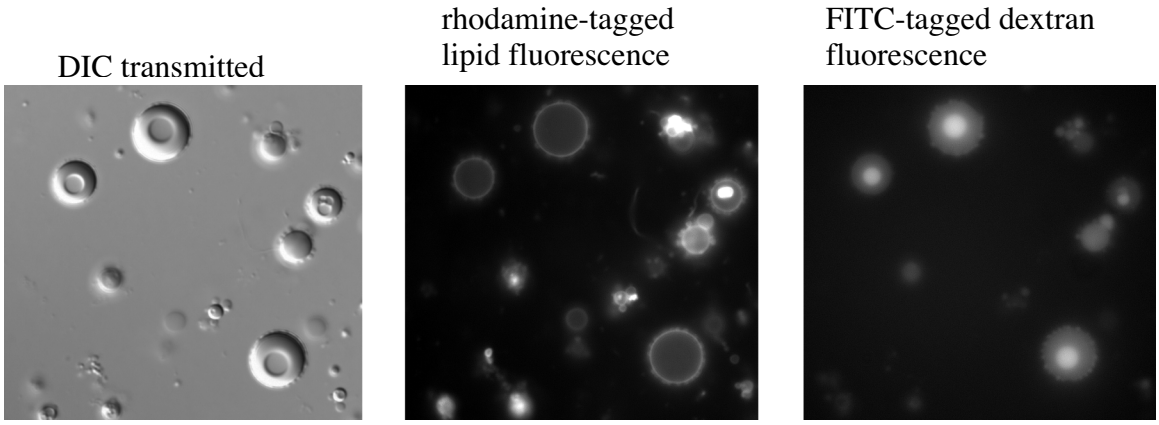
ATPS-GUV preparation protocol:

To prepare vesicles, 90 μl of egg phosphatidylcholine (Avanti, 10 mg/ml in $CHCl_3$), 10 μl of DOPG (Avanti, 10 mg/ml in $CHCl_3$), and 2 μl of DOPE-rhodamine (Avanti, 0.33 mg/ml in $CHCl_3$) were dried under argon to form a thin lipid film inside a 4 dram glass vial. The film was then dessicated for six hours under vacuum. Prior to ATPS addition, lipid films were pre-hydrated by blowing argon saturated with water gently over the film surface for one minute. The polymer solution used to hydrate the films was added at 50°C (as a single phase), and the lipids were hydrated overnight at this temperature. Following hydration, the solution was cooled to room temperature, whereupon phase separation occurred. The liposomes were collected from the bulk ATPS interface with a micropipet and transferred to a centrifuge tube. Samples were prepared by diluting 2 μl of this ATPS-vesicle solution with 70 μl of 0.5 M sucrose.

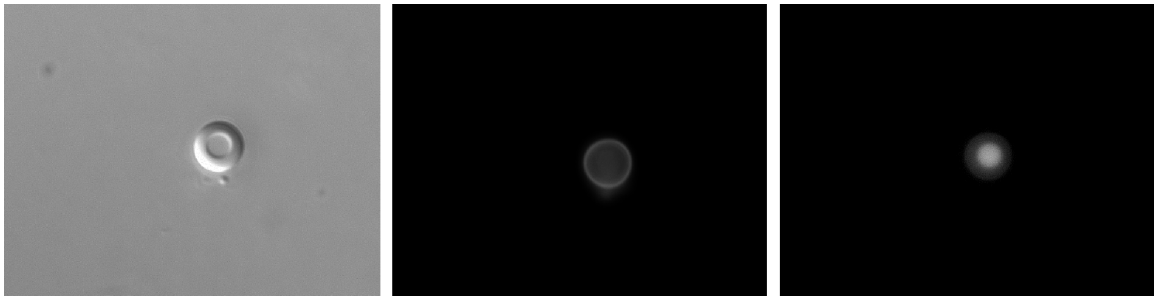
We notice slight differences in the temperature-dependent phase behavior for different lots of polymers from the same manufacturer, due to batch-to-batch differences in average molecular weight. The experiments described in this manuscript were conducted using 8,000 g/mol PEG [Sigma lot# 20K0211] and 482,000 g/mol dextran [Sigma lot # 81K1082, sold as "dextran 500,000"]. We routinely determine new phase diagrams when new lots of polymers are used, even if the polymer molecular weight is reported to be the same for the new lot as for the old lot.



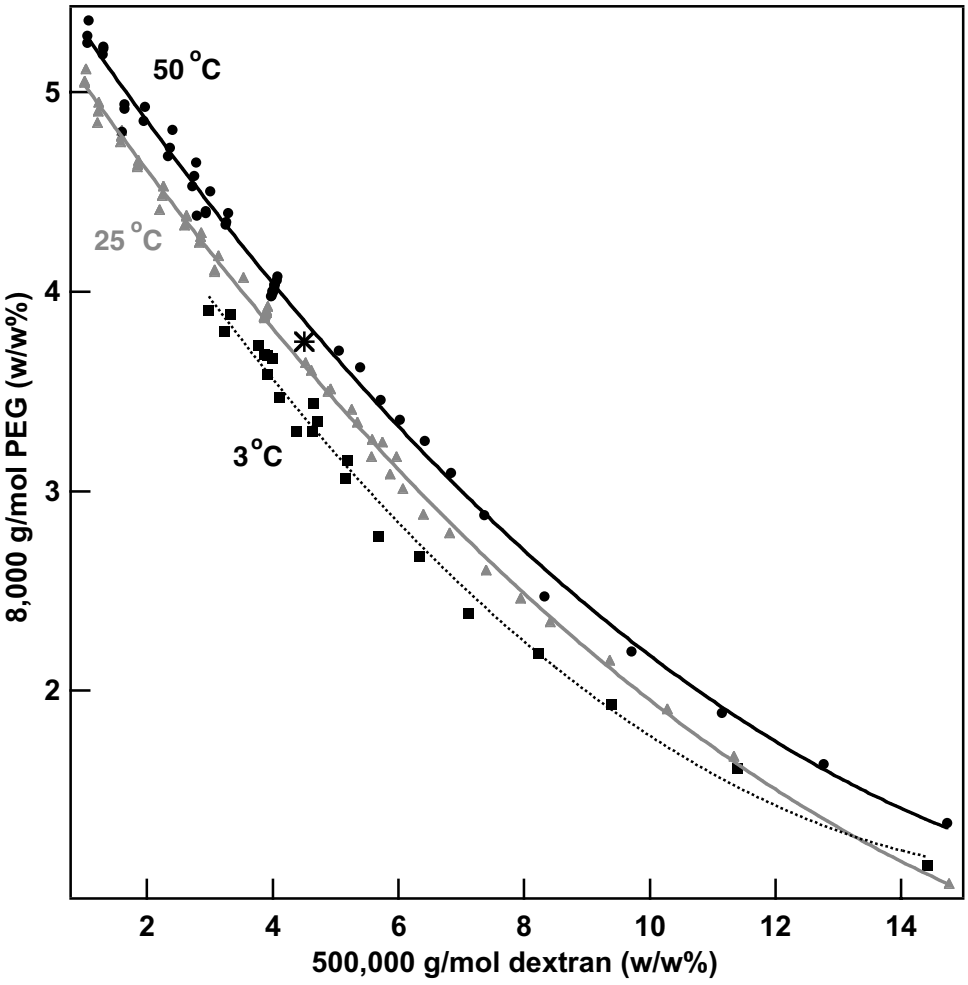
Supporting Information Figure 1. Optical microscopy and fluorescence images from a typical preparation of ATPS-liposomes (prepared as described in the text), that have been labeled using <0.05 mole % rhodamine-DOPE, and with 0.001 wt % FITC-dextran 500,000 g/mol in the aqueous phase.



Supporting Information Figure 2. Optical microscopy and fluorescence images from a typical preparation of ATPS-liposomes (prepared as described in the text), that have been labeled using <0.05 mole % rhodamine-DOPE, and with 0.001 wt % FITC-PEG 5,000 g/mol in the aqueous phase.



Supporting Information Figure 3. Representative image of an ATPS-liposome that has been doped with 0.05 mole % of DOPE-PEG 1000 lipid. The DIC (left) and rhodamine (center) images from this sample indicate that the ATPS is encapsulated within the vesicle. However, the fluorescence from the FITC-labeled PEG (0.001 % by wt) shows that the incorporation of the PEGylated lipid does not reverse the position of the encapsulated polymer phases.



Supporting Information Figure 4. Larger view of experimentally determined phase diagram for PEG 8000 g/mol – dextran 500,000 g/mol ATPS as a function of temperature. The asterisk marks the composition used in this work. Lines are polynomial best fits to the data, and are included to guide the eye.