Supporting Information

Multiphase water-in-oil emulsion droplets for cell-free transcription-translation

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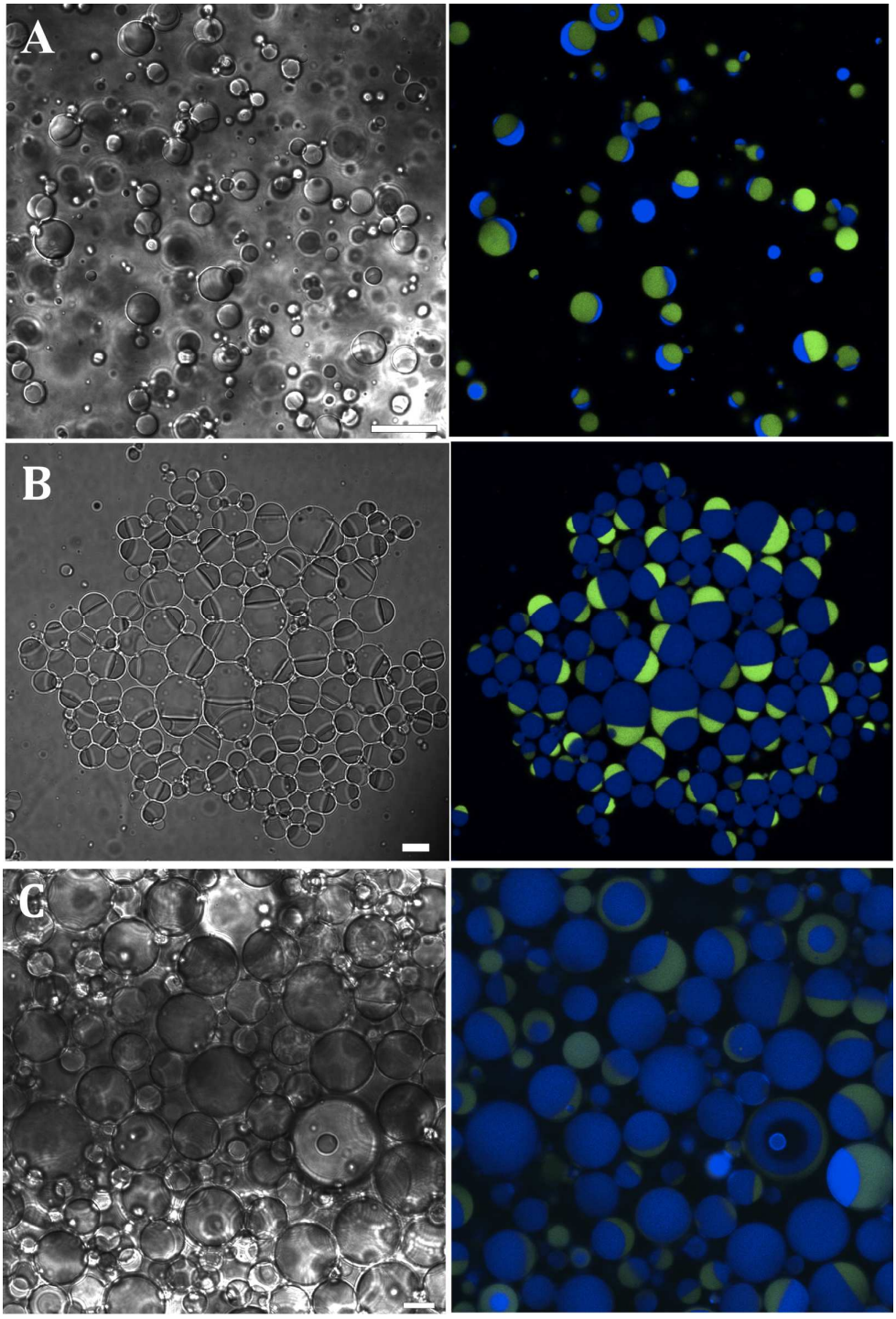

Figure S1. Direct encapsulation of ATPS in w/o emulsion droplets with wider field of view to show droplet populations. Transmitted (DIC) and confocal fluorescence images of w/o emulsion droplets containing different ATPS compositions are shown on the left and right, respectively. (A) 10% dextran, 7% PEG, (B) 19.8% dextran, 2.8% PEG, and (C) 18.5% dextran, 5.5% PEG. For visualization, each ATPS additionally contained 7.2 μg Alexa 488 labeled PEG (green) and 4 μg Alexa 647 labeled dextran (blue). The scale bar is 25 μm (A), 10 μm (B), 10 μm (C).

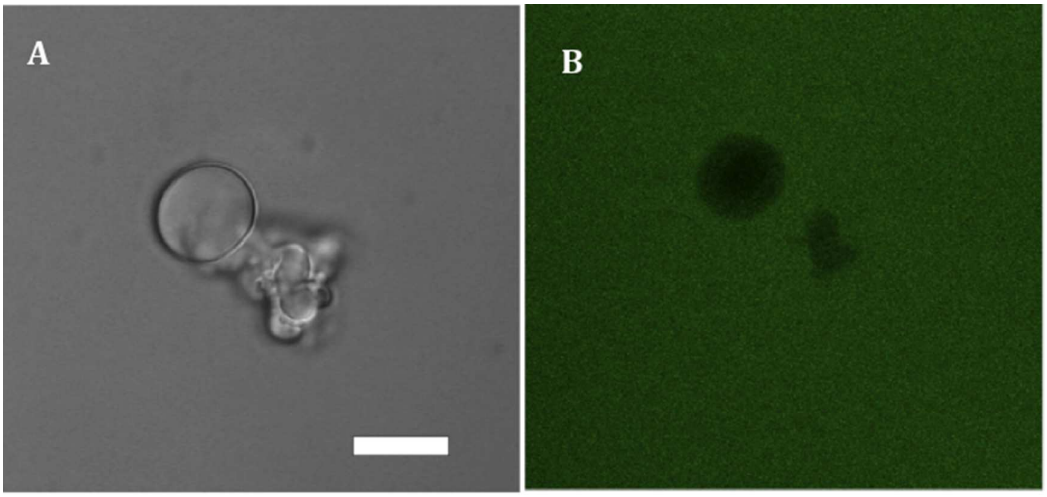

Figure S2. Attempted direct encapsulation of ATPS in POPC lipid vesicles performed at a constant temperature where ATPS remains phase-separated. Note that the yield of giant vesicles or other large vesicle-like material was quite low in these experiments due to the mechanical mixing. Vortexing lipid with a phase-separated ATPS is not a viable means of encapsulation. Transmitted light (A) and fluorescence (B) microscopy images are shown. Vesicles were made as described in the methods and resuspended in an ATPS composition of 10% dextran 10 kDa, 8% PEG 8 kDa and vortexed. The PEG phase was labeled with PEG 5 kDa conjugated with Alexa488. The scale bar represents 10 μm.

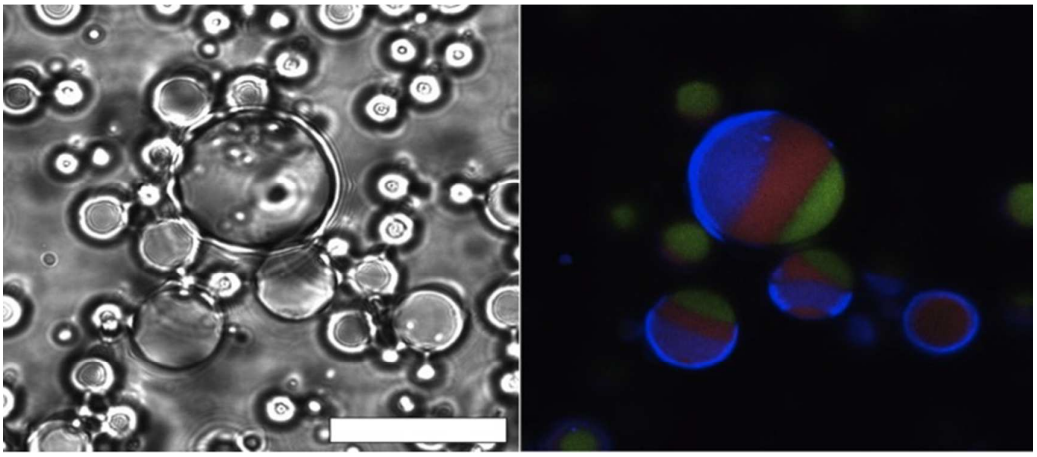

Figure S3. A3PS in w/o emulsion droplets. A3PS consisted of 19.8% dextran, 2.8% PEG, 5% Ficoll. Transmitted light (left) and fluorescence (right) microscopy images are shown. Aqueous phases were visualized with 3.6 μg Alexa 488 labeled PEG (green), 0.8 μg Alexa 647 labeled dextran 40 kDa (blue), and 12 μg tetramethylrhodamine isothiocyanate labeled Ficoll (red). The scale bar represents 25 μm.

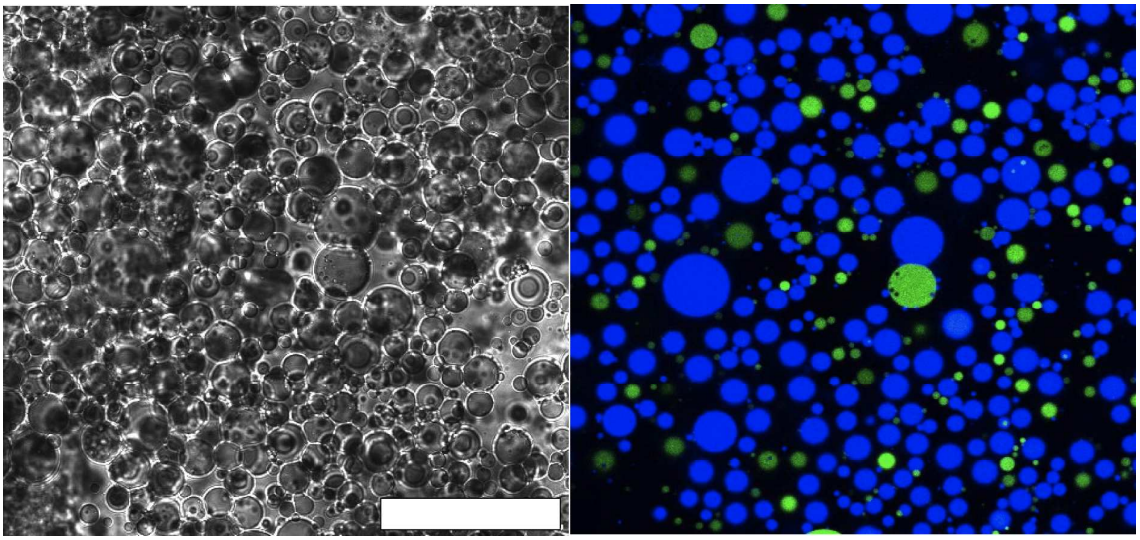

Figure S4. A modified emulsion composition does not directly encapsulate ATPS efficiently. The emulsion was stabilized with 2% Span 80, 3% Tween 80 in mineral oil, as opposed to the more standard composition of 4.5% Span 80, 3% Tween 80. The ATPS consisted of 19.8% dextran 10 kDa, 2.8% PEG 8 kDa. (A) Transmitted light and (B) fluorescence confocal microscopy images are shown. Alexa 647 labeled dextran 20kDa (shown here in red) and Alexa 488 labeled PEG 5 kDa (shown in green) were used for visualization. The scale bar represents 75 μm.

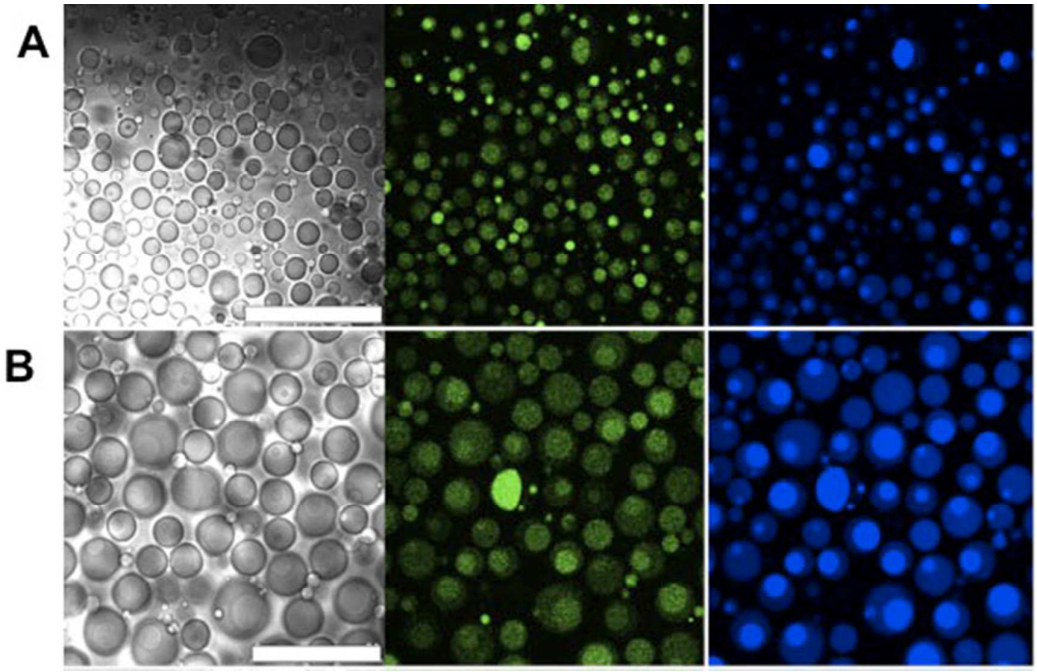

Figure S5. Expression at 2 h and 4 h in w/o emulsion droplets containing an ATPS composition of 10% dextran 10%, 7% PEG. Left-hand panels are transmitted light (DIC), central panels are fluorescence from expressed protein (mYPet), and right-hand images correspond to emission from Alexa647-labeled dextran 40 kDa to show the location of the dextran-rich phase. Images were acquired after incubation at 37 °C for (A) 2 h, and (B) 4 h. Scale bars represent 50 μm. Note that although the droplets in panel A are on average smaller than those in panel B, this is not representative of a change in droplet size with time. A range of droplet sizes was observed at all timepoints.

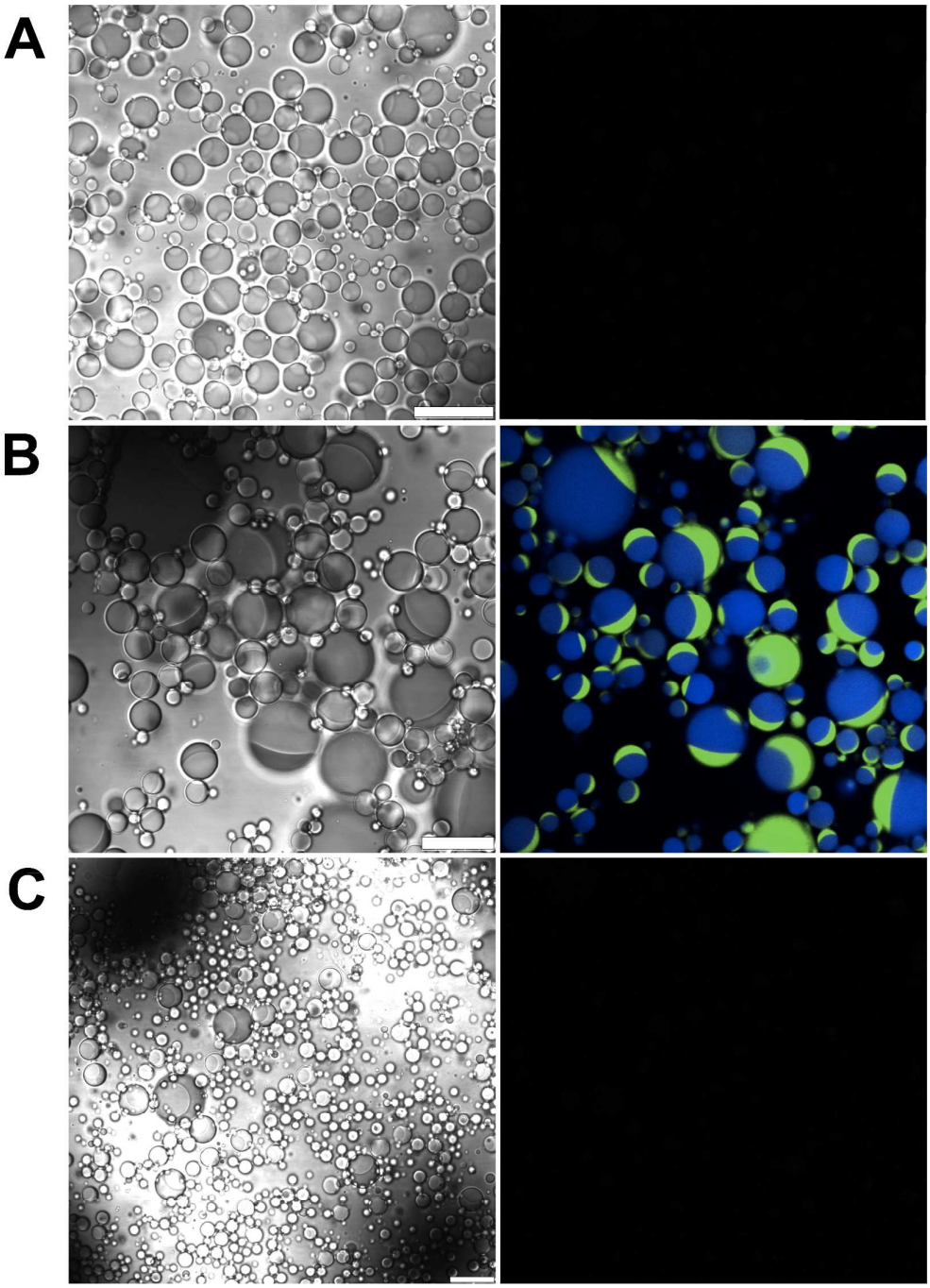

Figure S6. Controls for protein expression experiments in droplets containing an ATPS composition of 10% dextran, 7% PEG. Transmitted light (left) and fluorescence (right) microscopy images are shown. (A) W/o emulsion droplets containing ATPS, the PURE system, but not DNA coding for mYPet. (B) Same as panel A except that the polymers are labeled with Alexa 647-dextran (blue) and Alexa 488-PEG (green). (C) W/o emulsion droplets containing ATPS, the PURE system, and DNA coding for mYPet at time zero. That is, the needed components are present for expression as seen in Figure 3 but the image was taken immediately after the components were assembled at room temperature. Scale bars for all the images represent 25 μm.

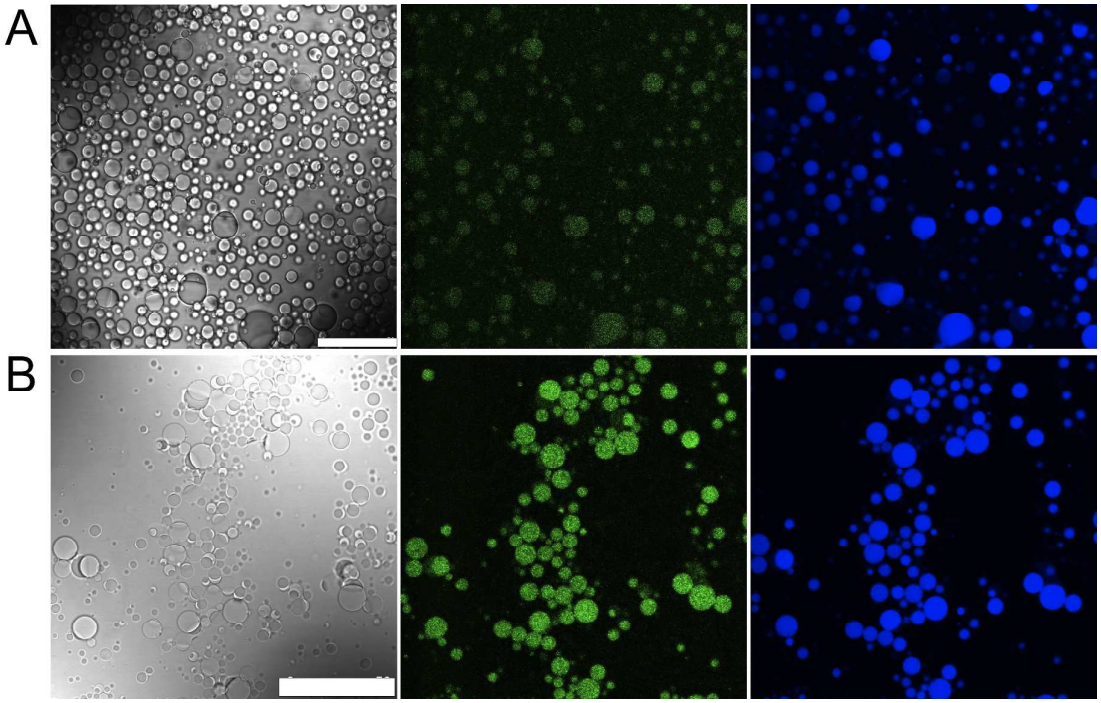

Figure S7. Expression at 2 h and 4 h for droplets containing an ATPS composition of 19.8% dextran, 2.8% PEG. Left-hand panels are transmitted light (DIC), central panels are fluorescence from expressed protein (mYPet), and right-hand images correspond to emission from Alexa647-labeled dextran 10 kDa to show the location of the dextran-rich phase. Images were acquired after incubation at 37 °C for (A) 2 h, and (B) 4 h. Scale bars for all the panels represent 50 μm.

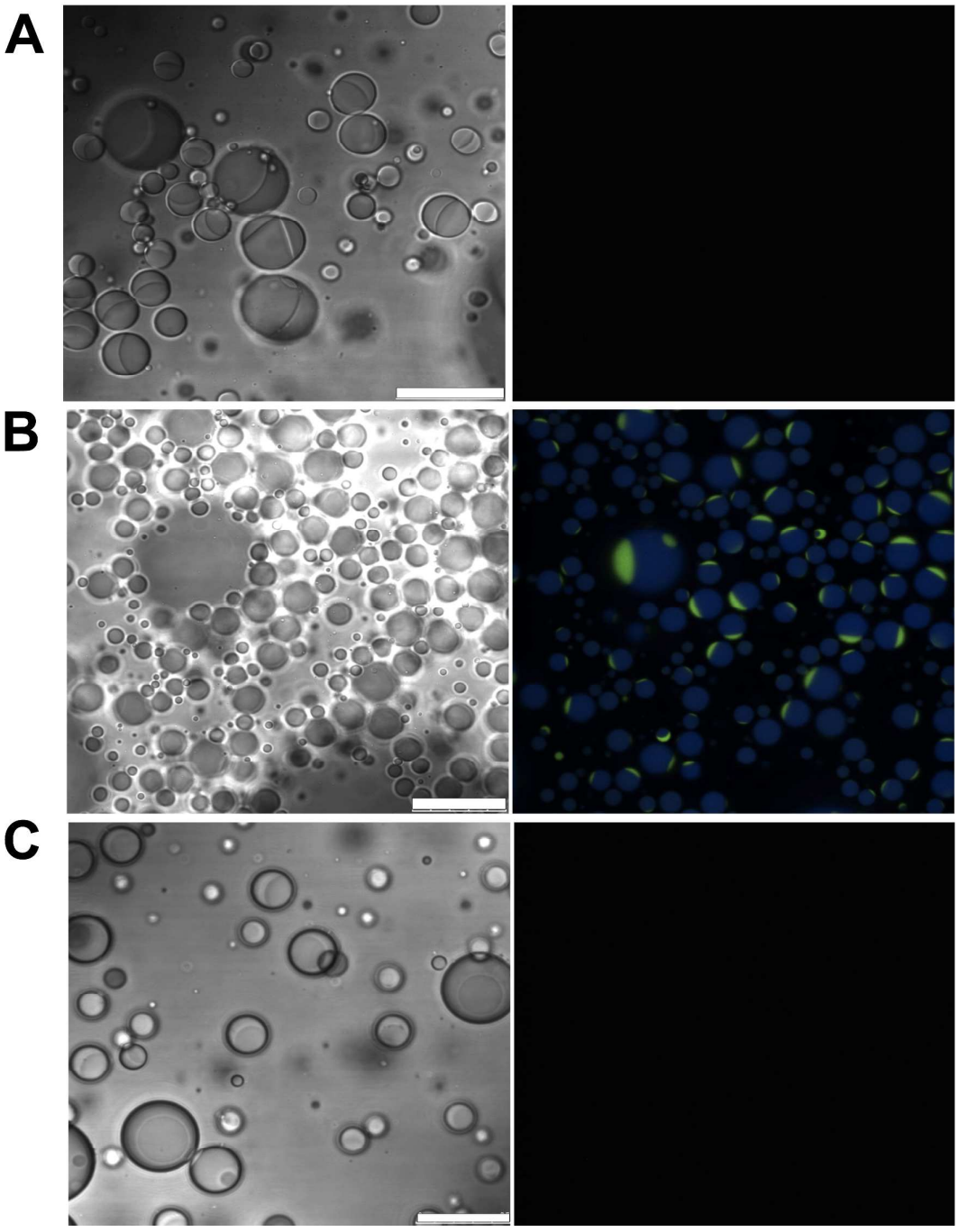

Figure S8. Controls for protein expression in w/o droplets containing an ATPS composition of 19.8% dextran, 2.8% PEG. Transmitted light (left) and fluorescence (right) microscopy images are shown. (A) W/o emulsion droplets containing ATPS, the PURE system, but not DNA coding for mYPet. (B) Same as panel A except that the polymers are labeled with Alexa 647-dextran (blue) and Alexa 488-PEG (green). (C) W/o emulsion droplets containing ATPS, the PURE system, and DNA at time zero. That is, the needed components are present for expression as seen in Figure 3 but the image was taken immediately after the components were assembled at room temperature. Scale bars represents 25 μm.

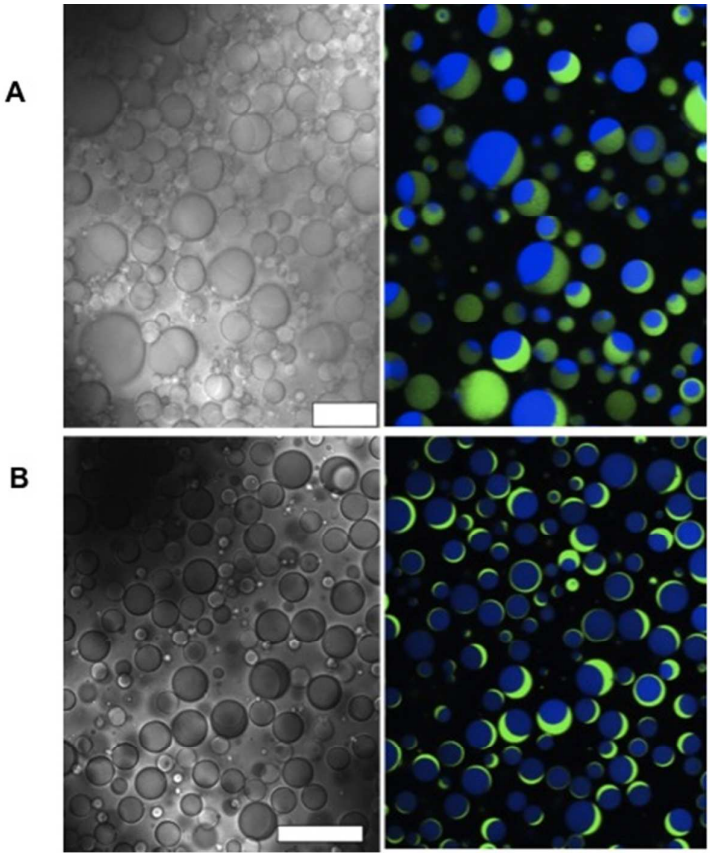

Figure S9. Incubation at 37°C for 2 h does not prevent ATPS encapsulation in w/o emulsion droplets. (A) ATPS composition of 10% dextran, 7% PEG. Scale bar represents 25 μm. (B) ATPS composition of 19.8% dextran, 2.8% PEG labeled in the same way. Scale bar represents 50 μm. Polymers are labeled with Alexa 647-dextran (blue) and Alexa 488-PEG (green).

Methods

Materials. Polyethylene glycol (PEG) 8,000 Da, PEG 5,000 Da, Ficoll 400,000 Da, PEG 5,000 Da (o-(2-aminoethyl)-o-methylpolyethylene), tetramethylrhodamine isothiocyanate conjugated Ficoll 40,000 Da, mineral oil, Span 80, and Tween 80 were from Sigma-Aldrich. PEG 20,000 Da was from Nektar. Alexa 647 conjugated dextran 10,000 Da and Alexa 488-carboxylic acid were from Life technologies. Distilled water was purified to a resistivity of ≥ 18.2 MΩ with a Barnstead NANOPure Diamond system.

A2PS and A3PS compositions. Three different ATPS compositions were encapsulated in w/o emulsions, including 19.8% (w/w) dextran 10 kDa, 2.8% (w/w) PEG 8 kDa; 10% (w/w) dextran 10 kDa, 7% (w/w) PEG 8 kDa; 18.5%(w/w) dextran 10 kDa, 5.5% (w/w) PEG 8 kDa. The ATPS compositions used for the expression of mYPet in w/o emulsion droplets were 20% (w/w) dextran, 14% (w/w) PEG and 39.6% (w/w) dextran and 5.6% (w/w) PEG before two-fold dilution with T/T components. The PEG was labeled using Amicon Ultra 0.5 mL centrifugal filters with a cut off of 10 kDa. Each solution was made by directly dissolving the components in 5 mL deionized H_2O with stirring with a magnetic stir plate. The A3PS composition was 19.8% (w/w) dextran 10 kDa, 2.8% (w/w) PEG 8 kDa, 5%(w/w) Ficoll 400 kDa. To produce the A3PS, 1 mL of 30% (w/w) Ficoll 400 kDa was first prepared and then added to a previously made ATPS of 19.8% (w/w) dextran 10 kDa, 2.8% (w/w) PEG 8 kDa with a positive displacement pipet (Gilson).

Encapsulation of ATPS in vesicle. Vesicles were made with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). Lipid stocks were made by dissolving 1 g POPC in 25 mL chloroform to obtain a 40 mg/mL solution. With a glass pipette an aliquot of the lipid stock was transferred to a round bottom flask. The organic solvent was then evaporated with a Buchi Rotovapor R-210 for 30 min. This procedure resulted in the formation of a thin lipid film. Next, 1 mL of ATPS was added to hydrate the lipid, giving a final concentration of 70 mM POPC. Vesicles were then formed by dispersing the lipid by vortexing at 3200 rpm.

Encapsulation of ATPS and A3PS in w/o emulsion. The oil phase was freshly prepared by mixing 4.5% (vol/vol) Span 80, 0.5% (vol/vol) Tween 80 with 0.95 mL mineral oil. This mixture was vortexed at 3,200 rpm until the Span 80 was completely dissolved in the mineral oil. 0.05 mL of bulk ATPS or bulk A3PS was added to 0.95 mL of the oil phase and vortexed at 3,200 rpm until the dispersion became cloudy.

Transcription-translation inside of w/o emulsions containing aqueous multiphase systems. 25 μL of the PURExpress[1] *in vitro* protein synthesis kit (New England BioLabs) containing 250 ng plasmid template encoding mYPet (RL001A[2]) was added to 25 μL of previously made ATPS with gentle mixing in a microcentrifuge tube. The resulting final volume of 50 μL was added to 0.95 mL of the oil phase plus surfactants. The sample was incubated at 37 °C for at least 6 h. Images were acquired every 2 h by confocal microscopy.

Confocal microscopy. Aqueous phases were visualized by including fluorescently labeled polymers during ATPS and A3PS formation. For the dextran enriched phase, either Alexa 647 conjugated with dextran 10 kDa or dextran 40 kDa was used at a final concentration of 4 μg/μL for ATPS and 0.8 μg/μL for A3PS. The PEG enriched phase was labeled with Alexa 488 conjugated with PEG 20 kDa or PEG 5 kDa was used at a final concentration of 7.2 μg/μL for ATPS and 3.6 μg/μL for A3PS. 12 μg/μL Ficoll labeled with tethramethylrhodamine isothiocyanate enriched was used to label the Ficoll enriched phase.

All samples were observed in chambers constructed by placing a 20 x 5-mm silicon spacer (Molecular Probes) onto a microscope slide. Images were obtained with a LSM-5 Pascal Laser confocal microscope (Carl Zeiss, Inc.) equipped with a plan-apochromat 63X oil immersion objective (1.4 NA) and Pascal software. The images were analyzed with ImageJ.[3]

Supplemental References

(1) Shimizu Y.; Inoue A.; Tomari Y.; Suzuki, T.; Yokogawa, K.; Nishikawa K., Ueda T. Cell-free translation reconstituted with purified components. *Nat. Biotechnol.* **2001**, *19*, 751-5.

(2) Lentini R.; Forlin. M.; Martini L.; Del Bianco C.; Spencer A.C.; Torino D.; Mansy S.S. **2013.** *ACS Synth Bio. 2*, 482–489.

(3) Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods*, **2012**, *9*, 671-675.