Supporting Information

Reconstitution of Metabolic Reactions within Self-assembled, Multi-compartment Protein Vesicles

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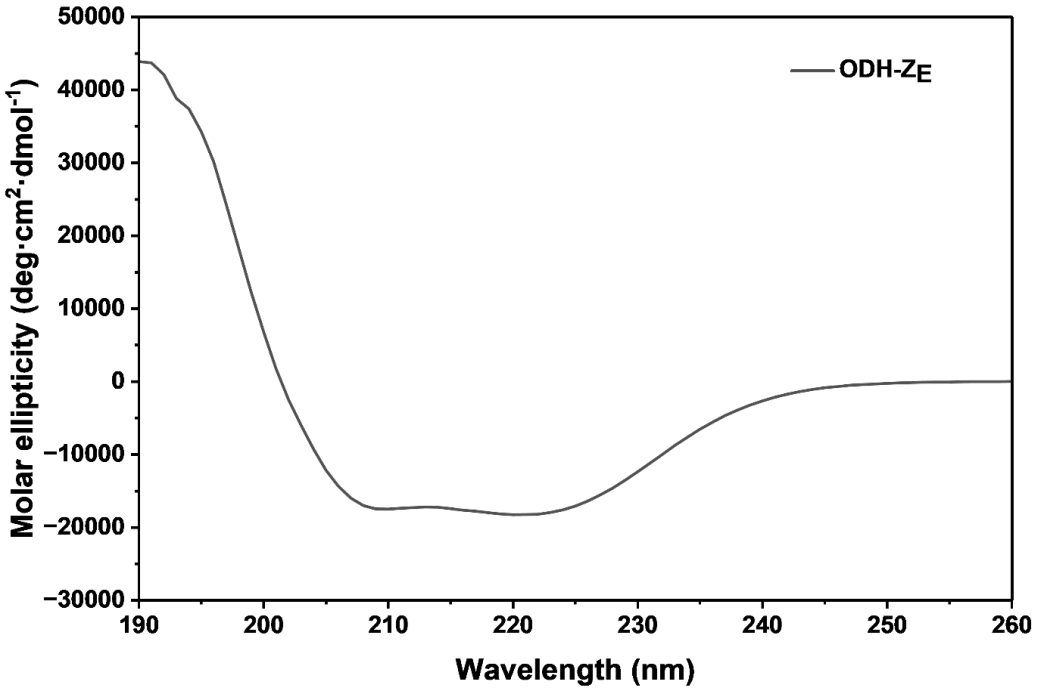

Figure S1. Far-UV CD spectrum of ODH-Z_E fusion proteins (0.2mg/mL) showing negative ellipticity between 205-225 nm.

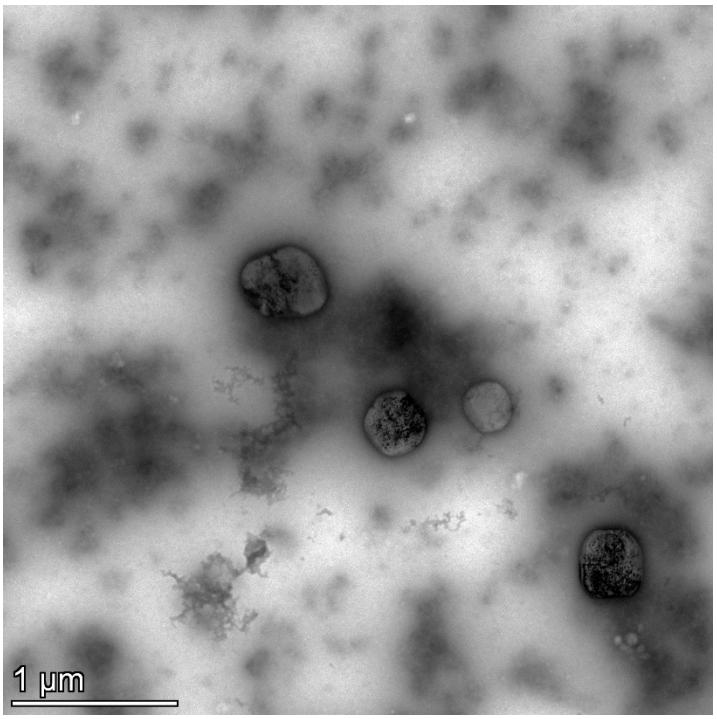

Figure S2. Transmission electron microscope image of ODH-Z_E/Z_R-ELP coacervates formed at 0.137M salt, confirming protein-rich droplets.

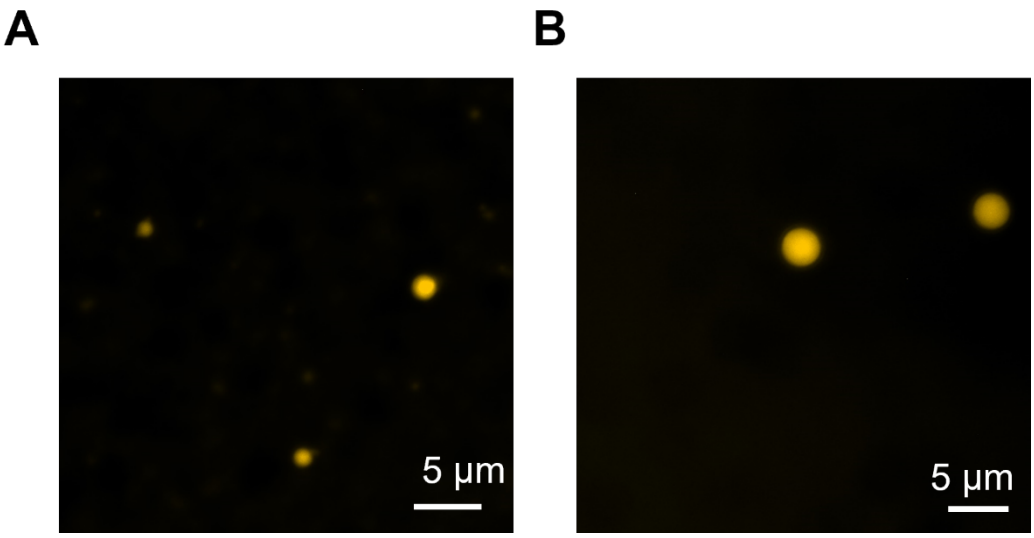

Figure S3. Fluorescence microscope image of ODH-incorporated vesicles encapsulating Rhodamine B (A) at 60μM of Z_R-ELP and 3μM of ODH-Z_E (B) at 120μM of Z_R-ELP and 6μM of ODH-Z_E.

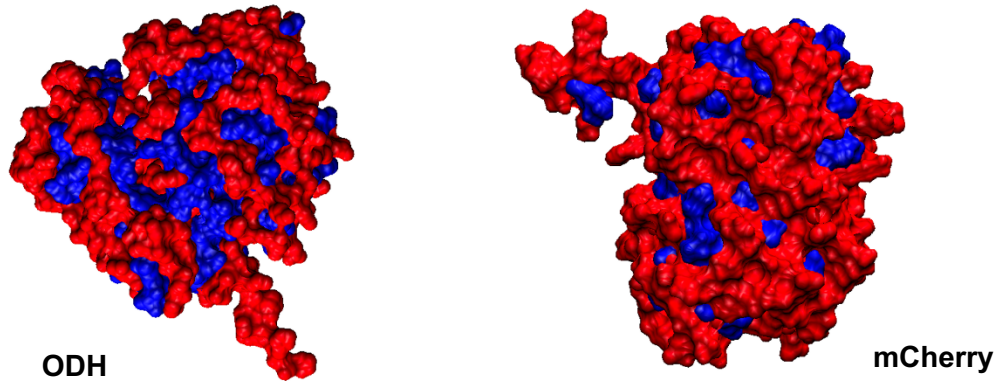

Figure S4. Three-dimensional surface representations of octopine dehydrogenase (ODH, left) and mCherry (right). Hydrophobic amino acid residues are shown in blue, and hydrophilic amino acid residues are shown in red.

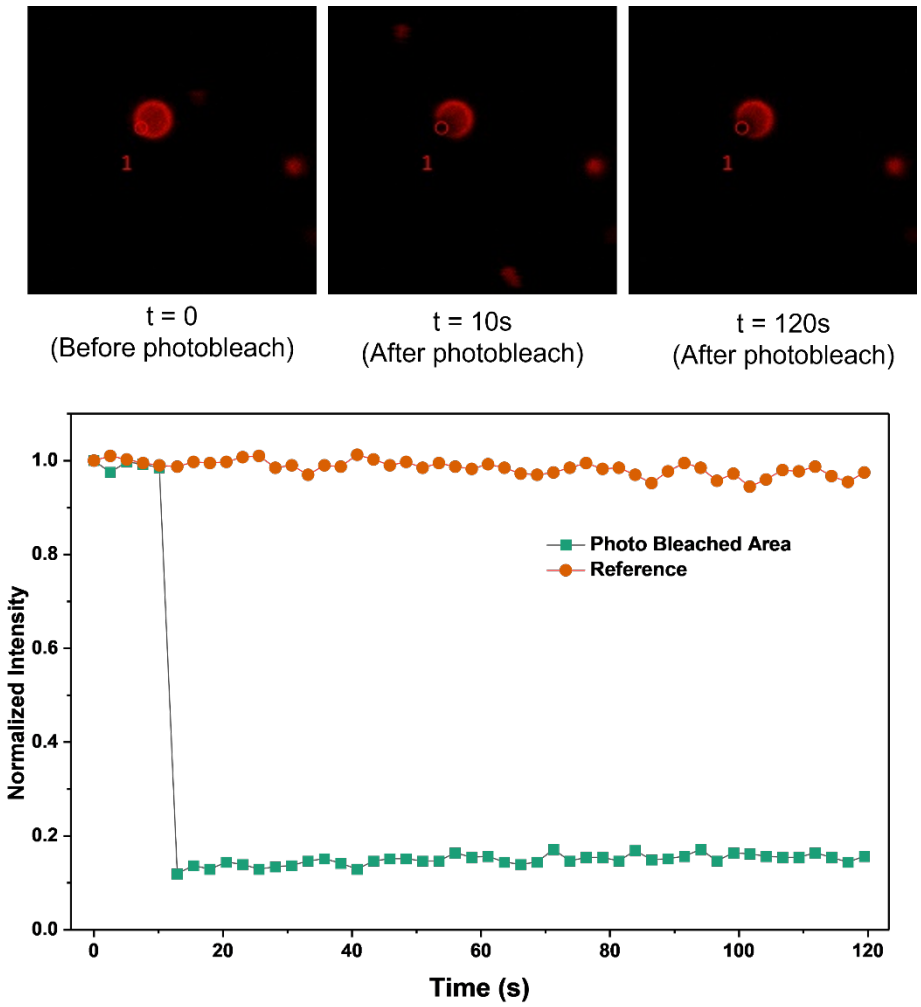

Figure S5. Fluorescence recovery after photobleaching (FRAP) of globular protein vesicle (GPV) membranes. (Top) Representative confocal fluorescence images of mCherry GPVs before and after photobleaching of the region of interest (ROI, indicated by circles). (Bottom) Quantification of fluorescence intensity over time for the photobleached area (green squares) and a non-bleached reference area (orange circles).

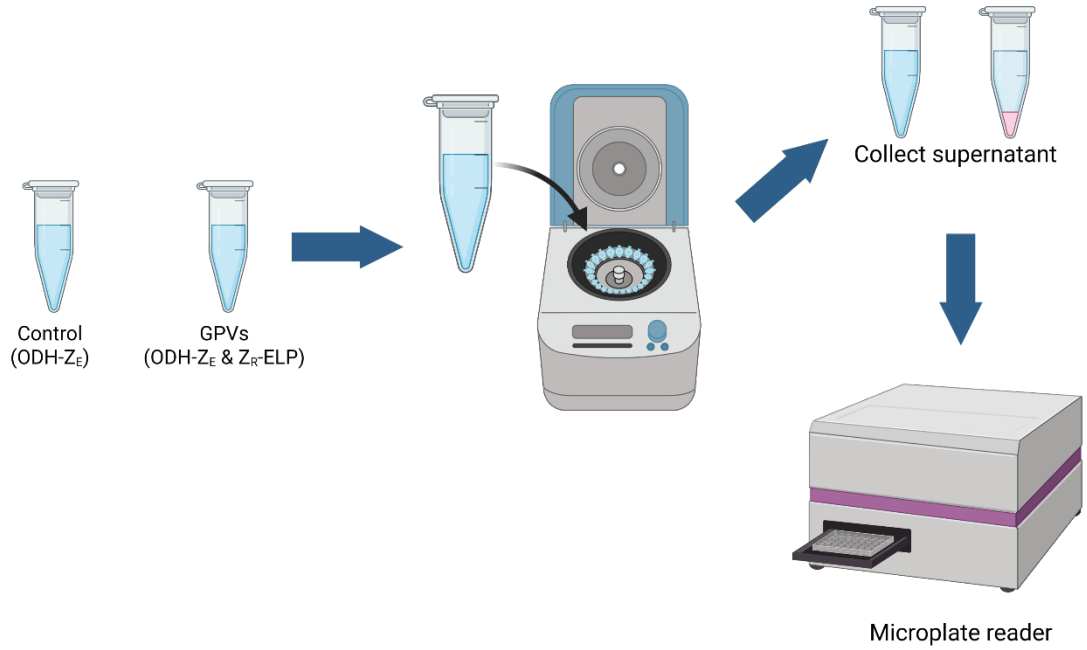

Figure S6. Scheme of the experimental workflow for quantifying enzyme incorporation into globular protein vesicles (GPVs). ODH vesicles were subjected to centrifugation to separate assembled vesicles (pellet) from unincorporated proteins (supernatant). The concentration of unincorporated enzyme in the supernatant was quantified using a fluorescence-based NADH burst assay, allowing estimation of protein incorporation efficiency. Created in BioRender. Shin, J. (2026) https://BioRender.com/2sxszp9

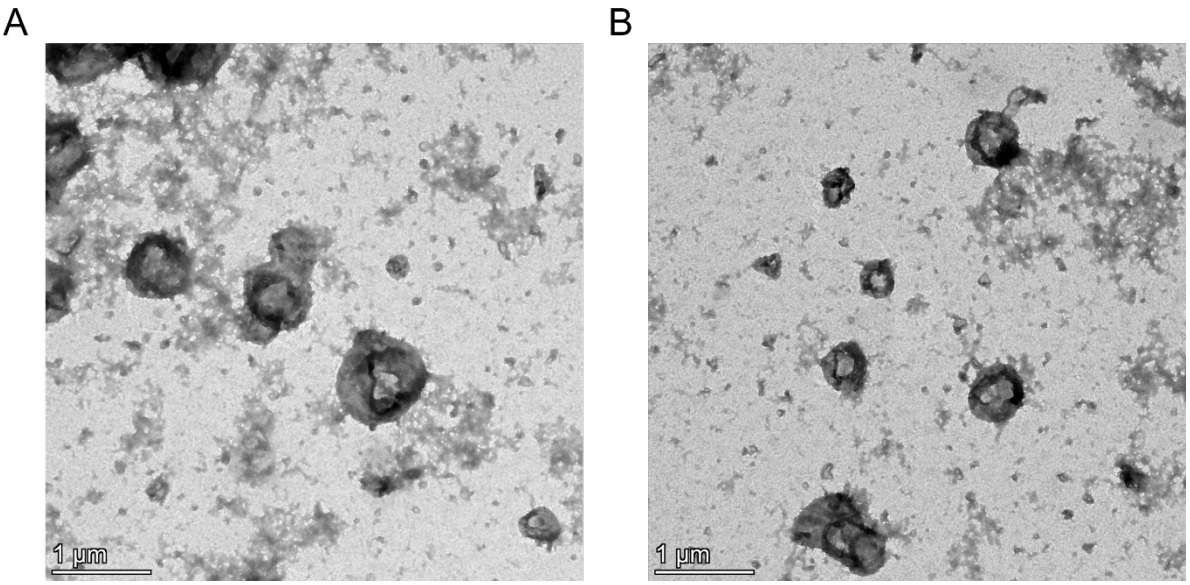

Figure S7. TEM images of ODH-displaying vesicles at high (A) $\chi = 0.1$ and (B) $\chi = 0.2$.