FUNCTIONAL BIO-NETWORKS FROM NANOLITER WATER DROPLETS

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Supporting Information

Experimental Section

Reagents and Proteins

1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC, Avanti), hexadecane (Sigma), β-cyclodextrin (Sigma), γ-cyclodextrin (Sigma), heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (Cyclolab) were used as received. Wild-type (WT), M123R[1] and M113F/K147N[2] αHL heptamers were prepared by *in vitro* transcription and translation (IVTT), followed by oligomerization on red blood cell membranes. After separation by SDS-PAGE, the heptamer band was cut from the gel and the protein was extracted[3]. Typically, αHL samples were diluted between 100 to 10,000 times in the buffer that was used to form the protein droplets. After dilution, any detergent remaining from the gel purification did not affect the stability of the DIB. Bacteriorhodopsin (as purple membrane) from *Halobacterium salinarum* was purchased from Sigma. BR (1 mg) was solubilized by sonication for 30 minutes in 40 μl of a one to one mixture of buffer (10 mM HEPES, 100 mM NaCl, pH 7.5) and 0.01% dodecylmaltoside (DDM) in water, which yielded a dark purple suspension. When preparing BR droplets, the stock suspension of BR was diluted 10-fold in 10 mM HEPES, 100 mM NaCl, pH 7.5.

Network Platform

A 10 x 10 x 4 mm Perspex well was micromachined with an array of divots on the bottom surface, with a center-to-center spacing of 700 μm. Each divot had a diameter of 1 mm. The divots served as an “egg crate” to keep droplets from rolling away during puncture with an electrode. Electrodes were threaded through 200 μm diameter holes that were drilled through the bottom of the divots. The underside of the cell was sealed with UV curable glue to ensure that the oil did not leak around the electrodes. All electrodes were soldered to a common wire which was connected to the working (as opposed to grounded) end of a patch clamp headstage. To remove a droplet from a network, an agarose-tipped electrode was plunged into the desired droplet using a micromanipulator and lifted slowly. Droplets could also be separated by driving a plastic pipette tip between a DIB interface. This technique was used to removed the red droplets in Figure 3c.

Rapid Screening Platform

Electrical recordings were performed in a 1 mL Perspex bath filled with 10 mM DPhPC in hexadecane. The end of a 10 μL disposable plastic pipette tip was fixed vertically to the bottom of the chamber with the narrow end facing up. A 100 μm-diameter Ag/AgCl electrode protruded 0.5 mm through the top of the pipette tip. This assembly formed a "tee" onto which droplets were positioned. The second electrode was a section of Ag wire that had been melted at the end to form a 200 μm-diameter ball. This was treated with NaClO to create an Ag/AgCl electrode and then coated with an ~200 μm-thick layer of 5% (w/v) agarose in water. The agarose served as a hydrophilic anchor from which droplets could be hung. The agarose-coated Ag/AgCl electrode was

mounted vertically (ball-end down) onto a micromanipulator (NMN-21, Narishige) which allowed suspended droplets to be accurately positioned. Typically, 200 nL droplets (diameter ~720 μm) were deposited onto the "tee" and "ball" electrodes by placing the tip of a plastic pipette under the oil very close to the electrode and carefully extruding the droplet out of the pipette tip onto the electrode. To form an array of droplets (200 nL), "tees" were added to the recording bath and their Ag/AgCl electrodes were connected to create a common working electrode, while the grounded electrode was connected to the micromanipulator. When recording through a three-droplet chain, two tees were used, with an electrode in each. A "ball" electrode was used to hold the middle droplet, but was not connected to the amplifier. Droplets (200 nL) were placed on their respective "ball" and "tees" and stabilized for thirty minutes before the center droplet was moved into position between the two terminal droplets.

Electrical Recording

The electrodes were connected to a patch-clamp amplifier (Axopatch 200B; Axon Instruments). The currents were filtered with a low-pass Bessel filter (80 dB/decade) with a corner frequency of 2 kHz and then digitized with a DigiData 1320 A/D converter (Axon Instruments) at a sampling frequency of 5 kHz. The Perspex recording-cell platform and amplifying headstage were enclosed in a metal box, which served as a Faraday cage.

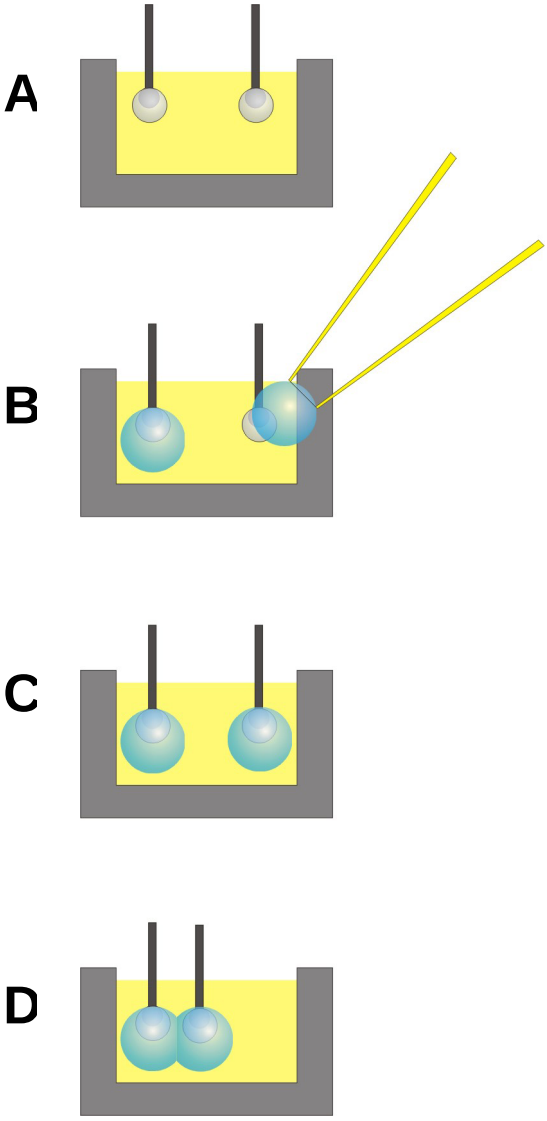

Figure S1. Formation of a DIB. a) Two 100 µm thick Ag/AgCl electrodes were coated with a layer of 5% (in water) agarose gel at the tip and then submerged under an oil/lipid bath. b) A 200 nL aqueous droplet was hung from each electrode by using a hand-held pipette. c) The droplets were stabilized in the oil for thirty minutes. d) The two droplets were brought together using a micromanipulator and a bilayer spontaneously formed.

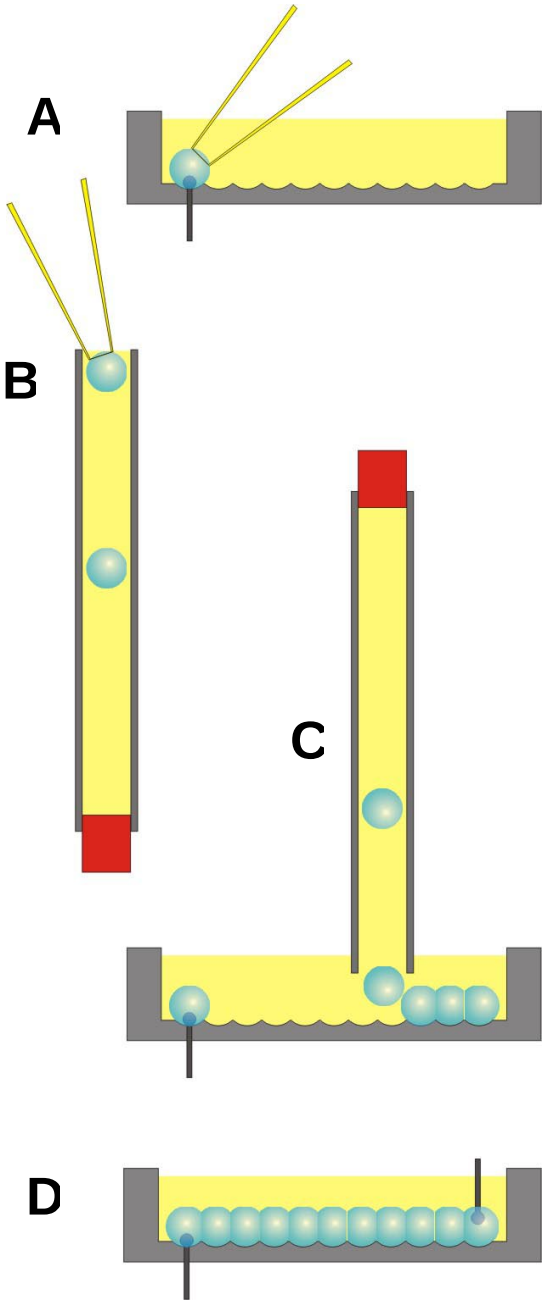

Figure S2. Formation of a DIB network. a) A drop was deposited on an Ag/AgCl electrode protruding through the bottom of a dimpled Perspex surface using a pipette. b) A 20 cm long tube was capped at the bottom and filled with the oil/lipid mixture. Droplets were added into the top using a pipette. c) After the droplets fell nearly to the bottom, the tube was inverted and the tip placed into the oil bath to allow the droplets to fall into the dimples below. d) The last droplet connected the rest of the network to the droplet containing the fixed electrode.

Video Captions

Supporting Video 1 – An agarose-coated electrode was plunged into pink droplet, lifted from the network and then moved out of the field of view. This process did not damage the DIB network. An image was taken once every 5 seconds and the video is played back at 30 frames per second.

Supporting Video 2 – A stabilized yellow droplet was introduced into the free space in the network just before the start of the video. After the droplet settled to the bottom, bilayers formed at all four adjacent interfaces. An image was taken once every 5 seconds and the video is played back at 30 frames per second.

References

(1) Madathil, R. et al, in preparation.
(2) Kang, X. F.; Gu, L. Q.; Cheley, S.; Bayley, H. *Angew. Chem.-Int. Edit.* **2005**, *44*, 1495-1499.
(3) Cheley, S.; Malghani, M. S.; Song, L. Z.; Hobaugh, M.; Gouaux, J. E.; Yang, J.; Bayley, H. *Protein Eng.* **1997**, *10*, 1433-1443.