

Membrane-mediated protein interactions drive membrane protein organization

Description of Supplementary Movies

Supplementary Movie 1. High-Speed Atomic Force Microscopy movie of the experimental design to study membrane-mediated interactions (see Fig. 1g). Following the physisorption of the reconstituted AqpZ membranes to the mica (see Methods), AqpZ arrays were observed on the bare mica (~0s). The addition of lipids to the HS-AFM fluid chamber (~47s) initiated bilayer spreading and membrane fusion (~230s). During this process AqpZ molecules started to diffuse and explored the entire membrane (~290s). 100% membrane coverage was achieved after ~365s, and 100% coverage of the membrane by diffusing molecules was observed after ~415s. Imaging parameters: 1 frame/s, 1 nm/pixel.

Supplementary Movie 2. Overview High-Speed Atomic Force Microscopy movie of AqpZ diffusing in the membrane and associating/dissociating to and from arrays (see Fig. 1i). HS-AFM imaging reveals membrane diffusing AqpZ as transient streaks in scan lines. Imaging parameters: 1 frame/s, 1 nm/pixel.

Supplementary Movie 3. Overview High-Speed Atomic Force Microscopy movie of large AqpZ 2D-arrays, ~40 minutes after continuous bilayer formation (see Fig. 2a). The AqpZ 2D-arrays changed shape with local growth and contraction but without global changes in array size. Imaging parameters: 1 frame/s, 1 nm/pixel.

Supplementary Movie 4. High-Speed Atomic Force Microscopy movie of AqpZ 2D-array dynamics, ~120 minutes after continuous bilayer formation (see Fig. 2b). Single-molecule membrane-mediated association/dissociation dynamics to and from the AqpZ array edges were observed. Imaging parameters: 1 frame/s, 0.5 nm/pixel.

Supplementary Movie 5. High-Speed Atomic Force Microscopy movie (intermediate magnification) view of AqpZ 2D-array dynamics, ~40 minutes after continuous bilayer formation (Fig. 2c). Imaging parameters: 1 frame/s, 0.33 nm/pixel.

Supplementary Movie 6. High-Speed Atomic Force Microscopy movie (high magnification) of AqpZ 2D-array dynamics, ~15 minutes after continuous bilayer formation (see Fig. 2d). Imaging parameters: 1 frame/s, 0.17 nm/pixel.

Supplementary Movie 7. High-Speed Atomic Force Microscopy movie of AqpZ 2D-array dynamics at a faster imaging rate. The imaging rate was set to be faster (250ms) than the fast AqpZ dissociation time constant. Imaging parameters: 4 frame/s, 0.5 nm/pixel.

Supplementary Movie 8. Membrane protein automata (see Extended Data Fig. 10). Top row: simulations of AqpZ array dynamics with different hydrophobic mismatch values. Bottom row: simulations favoring micro-geometries 2-4, respectively. See Supplementary Note 2 for a detailed description of the membrane protein automata.

Supplementary Movie 9. High-Speed Atomic Force Microscopy movie of non-tetrameric AqpZ-W14A oligomers along the 2D-array edges in C20 lipids (see region 1, Fig. 4a). Imaging parameters: 1 frame/s, 0.5 nm/pixel.

38 **Supplementary Movie 10. High-Speed Atomic Force Microscopy movie of non-tetrameric AqpZ-**
39 **W14A oligomers along the 2D array edges in C20 lipids (region 2, Fig. 4b).** Imaging parameters: 1
40 frame/s, 0.5 nm/pixel.