Soft Matter

Supporting Information

Membrane Pumps for the Manipulation and Sorting of Proteins using AC Electric Fields

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Fluorescence Image Analysis

Background images were taken prior to an experiment, and were subtracted from the fluorescence data to allow for the fluorescence and concentration to be directly related. The fluorescence images were analysed to give the relative average charge concentration, $\overline{C}/C_0$, in chosen regions, via the equation

$$\frac{\overline{C}_{sub}(t)}{C_0} = \left(\frac{\int_{sub} I(\mathbf{r},t)d^2r}{\int_{sub} d^2r} - B \right) \left(\left(\int_{ref} I(\mathbf{r},t)d^2r \Big/ \int_{ref} d^2r \right) - B \right)^{-1}, \qquad (1)$$

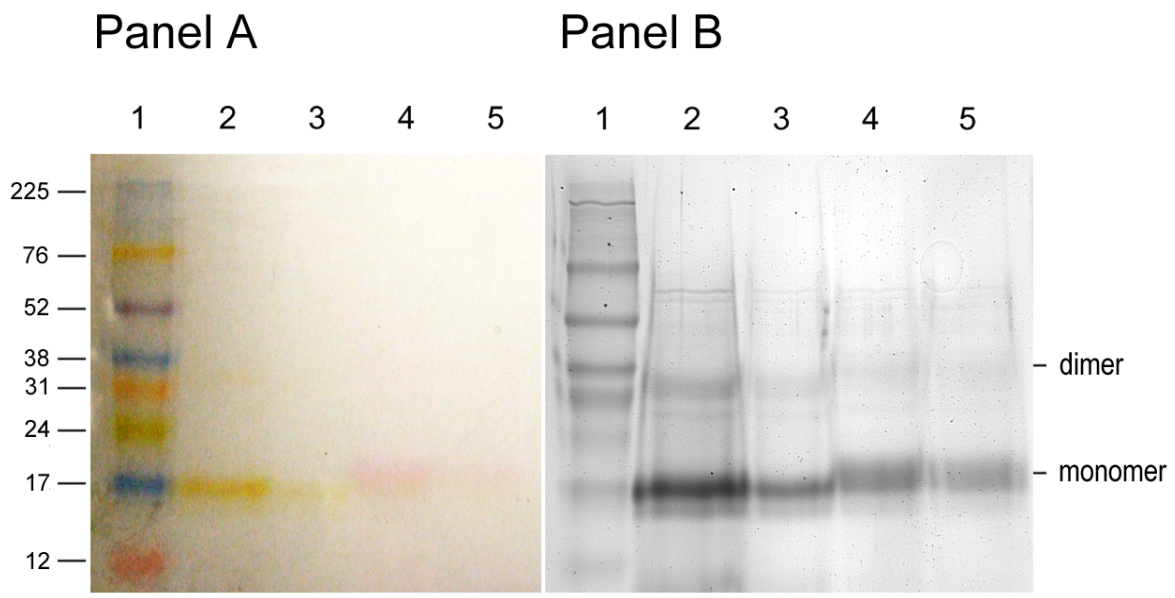
Where $I(\mathbf{r},t)$ represents the fluorescence intensity data at time t, $I(\mathbf{r},0)$ represents the fluorescence intensity data in the image taken at the start of the experiment, and B is the background noise (obtained from an image taken of the same system with no fluorescent components). The suffix *sub* refers to a fluid and connected region in the pattern, whilst the suffix *ref* refers to a reference region. The reference region for the pattern in Figure 2 was the entire enclosed fluid pattern (on the pretence that the total amount of fluorescent material in the whole pattern remains constant). For the pattern in Figures 3 and 4, it was impractical to do this, so the reference region was a patch of Fibronectin that had a small quantity of non-specifically bound fluorescent components (the same components that were present in the sBLM). These fluorescent molecules were immobile, but photobleached at the same rate as the molecules in the sBLM, and as such a patch of the Fibronectin pattern was suitable as a reference region. This accounted for both background noise, and for the photobleaching effect that occurred every time an image was taken, allowing for the increase of charge concentration to be quantified, for direct comparison with theoretical predictions.

Finite Element Model Setup and Analysis

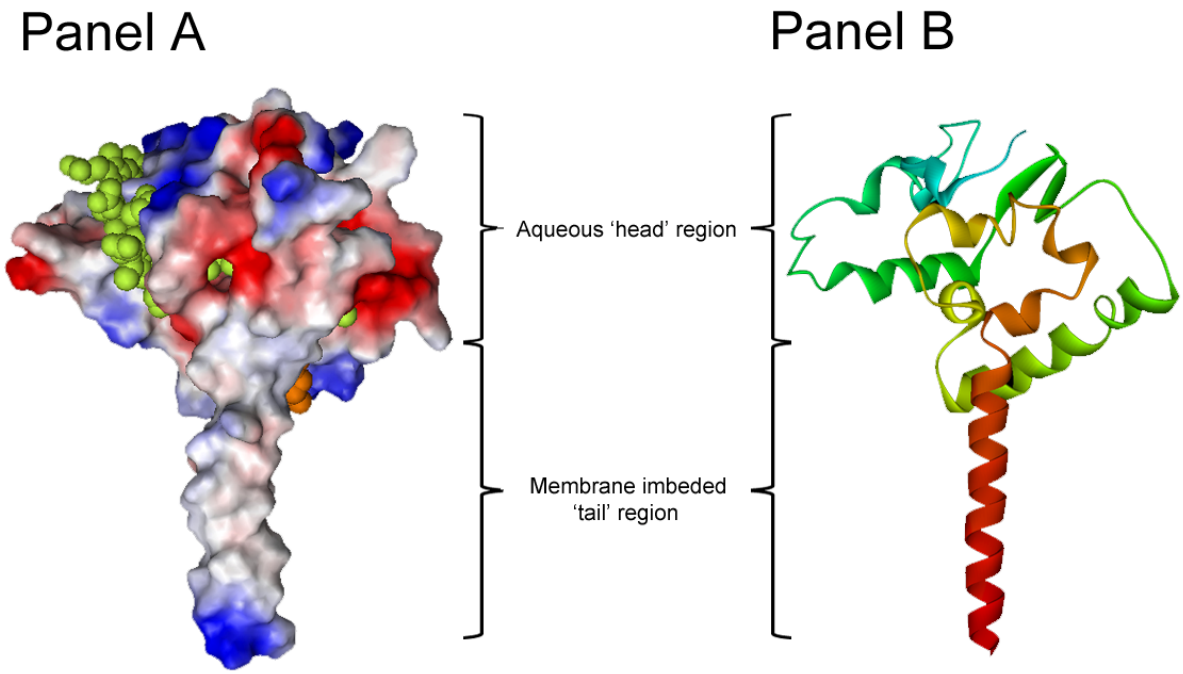
The Nernst-Planck equation was solved in 2D for the patterns shown in Figures 2, 4 and 5, with the electric potential being input as a time-dependent linear gradient across the pattern. The exterior boundary conditions were all set to the symmetry condition $\mathbf{n} \cdot \mathbf{J} = 0$, where $\mathbf{J}$ is the flux of the concentration C. The oscillating sine-wave electric field had an amplitude of 62 V/cm. The solutions to these equations were used to obtain the average charge concentration in regions of interest. The relative average charge concentration $\overline{C}/C_0$ for a given subdomain is defined by the equation

$$\overline{C}_{sub}(t)/C_0 = \int_{sub} C(\mathbf{r},t)d^2r \Big/ C_0 \int_{sub} d^2r, \qquad (2)$$

where C is the concentration of charge and C_0 is the initial concentration of charge. The suffix *sub* refers to any arbitrary region for which there exists values of C throughout.



Supplementary Figure 1: SDS-PAGE analysis of the reconstituted and ATTO565 labeled CymA by SDS-PAGE. Samples were resolved on 15% polyacrylamide gel and photographed under white light (Panel A) before staining with Simply Blue Safe Stain (Panel B). Lane 1, High-Range Rainbow molecular weight ladder (GE); Lane 2, 10 µg of concentrated CymA; Lane 3, 2 µg of CymA proteoliposomes; Lane 4, 2 µg of CymA-ATTO565 Lane 5, 0.5 µg of CymA-ATTO565 proteoliposomes.



Supplementary Figure 2: Electrostatic surface model of CymA (Panel A). The red and blue regions show negative and positive charged areas respectively, whilst the yellow and orange CPK atomic models show the heme prosthetic groups and bound quinone respectively. For clarity, the cartoon model of CymA is shown in the same orientation as the surface model (Panel B). The electrostatic surface model is displayed using Pymol (Delano Scientific) and the cartoon model using Accelrys DS Visualiser 1.7 (Discovery Studio).