Supplementary information

TOWARD ELECTROPHORETIC SEPARATION of MEMBRANE PROTEINS in SUPPORTED $n$-BILAYERS

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Supporting Videos

An inverted fluorescence microscope (Leica) and a camera (Hamamatsu C2400-77: 768 x 494 pixels, 30 MHz) equipped with a fluorescence intensifier suitable for fluorescence imaging (magnification x 400) were used to carry out protein velocity measurements from recorded videos (1 to 5). As illustrations, series of snapshots extracted from videos at different times are shown in the Results and Discussion section (figures 2-3).

Drift velocity measurements were performed as described in Harb et al.\textsuperscript{15}. Briefly, the drift velocity of at least 10 spots measured on three different locations from each sample was obtained by dividing distance traveled by the particles by time. Reproducibility was within 10 \%. The primary source of error was the measurement of the displacement, due to the resolution of the microscope (± 1µm).

All videos are in real time. The camera is black and white and SQR and αHL are not easy to distinguish through the spot intensity. They have only different mobilities. It is why spots in videos were manually colored (SQR in red, αHL in blue), excepted video 5. In video 5, which shows the migration of three objects, the “natural” level of gray and/or the difference in mobilities enable to differentiate the objects.

Video S1: Electrophoretic migration of SQR (red) and αHL (blue) inserted in EggPC supported multibilayer under 3V/cm at pH 7.2. SQR migrates toward the positive electrode and αHL migrates toward the negative electrode. Yellow ellipses highlight situations where proteins do not stick together and manage to find a way to glide past each other.

Video S2: Electrophoretic migration of SQR (red) and αHL (blue) inserted in EggPC supported multibilayer under 3V/cm at pH 9.2. SQR and αHL race each other toward the positive electrode. The yellow ellipse highlights a situation where SQR catches up αHL, does not stick with and manage to find a way to overtake it.

Video S3: Electrophoretic migration of SQR (red) and αHL (blue) inserted in EggPC supported multibilayer under 3V/cm at pH 6.8. This pH equals the pI of SQR. Then, SQR is immobile and αHL migrates toward the negative electrode.
**Video S4:** Electrophoretic migration of SQR (red) and αHL (blue) inserted in EggPC supported multibilayer under 3V/cm at pH 8.3. This pH equals the pI of αHL. Then, SQR migrates toward the positive electrode and αHL is immobile.

**Video S5:** Electrophoretic migration of a mixture of three objects: trimeric monotopic SQR, heptameric α-HL and dimers of heptameric α-HL at pH 7.2 under 3V/cm in a DMPC supported double bilayer. The different objects have their original gray (trimeric SQR: and heptameric α-HL: light gray; the dimer of heptameric α-HL: brilliant gray). All objects SQR and αHL race each other toward the positive electrode: the fastest are SQR, the slowest are dimer of heptameric α-HL.

**Scheme S1**

Scheme S1: Schematic diagram of the cell used to carry out protein velocity measurements in double SLB: (a) video setup for electrophoretic mobility measurements: cell positioned on an inverted microscope equipped with a camera; (b) zoom on the system: SQR (red ellipses) and α-HL (blue mushrooms) inserted on top of double bilayer.

The sample (supported bilayer + inserted proteins) was transferred underwater in a thermostated electrophoretic cell. The cell setup was home-made (Figure 1): a parallelepipedic tank (30 mm
x 25 mm x 5 mm) prepared using two glass plates, giving an interior liquid volume of 3 ml. Near one plate, a Peltier element and a heat sink were used to either cool down or heat the cell. Water in the cell was replaced by gently rinsing and overflowing several times with the buffer (PB 10 mM, NaCl 100 mM and NaN$_3$ 0.2 mM) at the desired pH. The cell was then positioned under microscope to take pictures or to record videos.

Platinum electrodes (diameter = 0.25 mm) were positioned in the cell 30 mm apart. The tension was applied between the Pt electrodes, with an electric field of 3 V/cm applied for 20 s. The solvent was replaced after each velocity measurement to remove electrolysis products. This also helped to eliminate any possibility of temperature increase due to Joule heating or pH change; tracking by thermocouple when electric field was on showed no changes.