TOWARD ELECTROPHORETIC SEPARATION of MEMBRANE PROTEINS in SUPPORTED n-BILAYERS

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Toward electrophoretic separation of membrane proteins in supported n-bilayers

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ABSTRACT: Membrane proteins are key constituents of the proteome of cells but are poorly characterized, mainly because they are difficult to solubilize. Proteome analysis involves separating proteins as a preliminary step towards their characterization. Currently, the most common method is “solubilizing” them with sophisticated detergent and lipid mixtures for later separation via, for instance, sodium dodecyl sulfate polyacrylamide gel electrophoresis. However, this later step induces loss of 3D structure (denaturation). Migration in a medium that mimics the cell membrane should therefore be more appropriate. Here, we present a successful electrophoretic separation of a mixture first of two and then of three different membrane objects in supported n-bilayers. These “objects” are composed of membrane proteins Sulfide Quinone Reductase and α-Hemolysin. Sulfide Quinone Reductase forms an object from three monomers together and self-inserts into the upper leaflet. α-Hemolysin inserts as a spanning heptamer into a bilayer or can build stable dimers of α-Hemolysin heptamers under certain conditions. By appropriately adjusting pH it proved possible to move them in different ways. This work holds promise for separating membrane proteins without losing their 3D structure, thus their bioactivity, within a lipidic environment that is closer to physiological conditions and for building drug/diagnostic platforms.

1 Introduction

Membrane proteins play an important role in biological processes. Located at the boundary surface between the inside and the outside of the cell, they play many roles, acting as a gate in active/passive transport of ions/molecules through membranes and thereby ensuring reception/transmission of signals, modeling and adhesion, intercellular recognition, extracellular matrix binding, enzymatic activity, etc. Although they constitute roughly 30% of the proteome of a mammalian cell, i.e. close to 30 000 proteins, only 999 (≈ 3.3 %) 3D structures are known [1]. Yet membrane proteins represent 50% of pharmaceutical targets, knowledge of 3D structure being crucial to researchers seeking to design better-targeted drugs.

This lack of characterization stems from issues with purifying, crystallizing and functionally reconstituting membrane proteins. The main reason is that they are extremely difficult to handle in a water environment without denaturing them. In practice, a mixture of detergents and lipids must be used to stabilize the 3D structure. In addition, the environment is very difficult to reproduce in a fully biomimetic manner, and can wind up being completely different, for example with a separation method like SDS-PAGE. In that case, because of denaturing conditions, the 3D structure is completely lost. As a successful alternative, methods like Blue Native and Clear Native PAGE [2-6] involve one-step isolation of membrane proteins using nonionic detergents such as dodecyl maltoside, Triton X-100 or digitonin under conditions enabling to solubilize biological membranes. By carefully selecting and adjusting the detergent and its concentration, it is possible to create conditions that are not harmful to the membrane proteins or the complexes, owing to the presence of some of the lipids from the solubilized membranes. Then, separated bands can be excised and run in another dimension for final separation with various denaturing techniques (SDS-PAGE, doubled SDS-PAGE and IEF/SDS 3D PAGE.

Some years ago another strategy was used in [7]. In this work, the authors were able to report progress in the knowledge of membrane proteins, by identifying complexes playing vital biological roles in Saccharomyces cerevisiae. They identified complexes with 1590 putative integral, extrinsic and lipid-anchored membrane proteins using tandem affinity purification and mass spectrometry.

More recently Okamoto et al. separated for the first time two membrane pore-forming proteins in multilayer lipid membranes at one pH [8]. Embedding the membrane proteins in a medium which partially mimics their natural environment meant that they could be separated without denaturation. Bao et al have reported a 25-fold increase concentration of trans-membrane protein–proteorhodopsin incorporated into supported lipid bilayers using electric fields [9].

We propose here to carry out electrophoretic separation of a mixture of two objects at differing pHs in differing lipidic systems (different chemical nature and number of bilayers) and then to

separate a mixture of three objects: the two previous objects plus a dimer of one of them. These objects are composed of membrane proteins Sulfide Quinone Reductase (SQR) or α-Hemolysin (-HL), model proteins easily handled in aqueous solution before insertion. Sulfide Quinone Reductase forms an object from three monomers together with some detergent and self-inserts into the upper leaflet of the bilayer. α-Hemolysin inserts as a spanning heptamer into the bilayer or can build stable dimers of α-Hemolysin heptamers under certain conditions. In previous papers [10-13] we measured some physico-chemical properties (diffusion coefficient, electrophoretic mobility, and charge) of these two different objects alone, taken as models in such an environment. They were chosen because they are very different: one is monotopic (inserts in one leaflet); the other is transmembrane (spans the two leaflets).

We use supported phospholipidic n-bilayers of two different lipids with n which can be set to 1, 2 or ≅ 35. One single bilayer (n=1) constitutes the basic system. A double bilayer (n=2) provides another test system. A multibilayer (n ≅ 35), which has the advantage of being very easy to build, provides a third test system and may improve membrane protein loading capacities for what we hope will become routine separation.

This study confirms this method as one that can be routinely used to carry out real electrophoretic separation of membrane proteins, starting from a cell or a cell lysate. Furthermore, it can be used as a drug or diagnostic platform, once proteins have been separated.

Starting with a mixture of known objects, conditions can be chosen so as to immobilize either one or the other object, move them in opposite directions, etc. Later, starting with an unknown mixture, after a screening of the pHs, separating conditions can be chosen in accordance with each situation.

2 Materials and Methods

2.1 Sample

2.1.1. Supports

The slides used as supports were 2.5x 2.5 cm2, either mica cleaved on both sides just before use (JBG-Metafix, France) to support the double bilayer or glass (Marienfield, cut edges, France) to support the single bilayer or the multibilayer. They were cleaned by immersion for 10 min in freshly prepared alcoholic NaOH, then thoroughly rinsed with ultra-pure water and sonicated 3 times for 5 min in ultra-pure water.

2.1.2. Lipids

All lipids were purchased from Avanti Polar Lipids (Coger, France) and used without further purification. Supported single and double bilayers were prepared using 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, Mw 734 g·mol −1). Supported multibilayers were prepared

using either DMPC or L-α-phosphatidylcholine (EggPC, Mw 770 g·mol −1).
The single bilayer and double bilayer of DMPC were built using the Langmuir-Blodgett transfer technique (for details, see [14]). Lipid molecules solubilized in chloroform (∼1 mg/ml) were deposited on the water subphase (18 MΩcm, MilliQ, 15 °C) of a Langmuir trough (KSV Minitrough 361 mm × 74 mm, Finland), equilibrated for 10 minutes to allow complete solvent evaporation and then compressed to 30 mN/m. Transfer ratios were close to 1.
For the double bilayer on mica, the fourth monolayer, closing the second bilayer, was transferred using the Langmuir-Schaefer (LS) technique. Transfer ratios were close to 1 for the first three monolayers and 0.8 for the fourth monolayer.
The multibilayer was formed via the lipid film hydration method. 20 µL of lipids (either DMPC or EggPC) in chloroform (1mg/mL) were first desiccated under vacuum for 1 h on a glass slide. Then the slide was positioned in our incubation cell (a TeflonTM cylinder embedded in steel, 1 cm diameter) and the film was rehydrated at room temperature with ultrapure water (18 MΩ·cm, MilliQ) for 30 min. Obtaining a multibilayer can be difficult. Several articles detail how Multilamellar Vesicles (MLV) can be produced via rehydration of dry lipidic film. We noticed that it usually involves supplying energy to the system, for instance through strong agitation or electric fields. Failing this, the outermost bilayer of a rehydrated DOPC film will spontaneously peel off (possibly bilayer by bilayer). However, it is also reported that peeling does not occur when starting from DPPC in gel phase [15]. More recently, Garcia-Meynes et al [16] carried out AFM experiments on supported rehydrated multibilayer stacks in buffer solution. Keeping the same buffer without rinsing, they avoided turbulences and thus detachment of the lipid stacks.
In our system, we obtain a supported multibilayer and this is ensured by the following:
- Removal, washing and addition to the Teflon cell were performed very gently and along the wall of the cell, to minimize turbulence.
- Working in pure water on glass increases electrostatic interactions because of the low ionic strength. This helps stabilize the rehydrated multibilayer at the glass surface.
- Qualitatively, under the microscope, the sample appeared flat: there was no need to adjust the z direction to remain in focus, and no sign of the existence of slowly “swimming” MLVs.
Additionally, the electrophoretic mobilities of our objects were analogous when measured in single or double bilayers. Experiments were performed in replicate reproducibly in all systems (at least twice, sometimes more).
Assuming a lipid head molecular area of 0.63 nm2 [17], given the amount of dried lipids (20 µL from a solution 1mg/mL) and the area exposed to rehydration (a circle 1 cm in diameter), we estimated a number of bilayers close to ≈ 35 in the multibilayer.

Supported Lipidic Bilayers (SLB) were kept under water throughout the experiment and used directly after preparation.

2.1.3. Proteins

α-Hemolysin (α-HL), extracted from Staphylococcus aureus, was purchased from Sigma Aldrich Inc and used without further purification.

Sulfide Quinone Reductase (SQR) was extracted from A. aeolicus membrane with dodecyl-β-D-Maltoside (DDM) and purified as previously described [18]. Following purification, SQR was dialyzed against 50 mM HEPES buffer pH 7.2, 0.1% DDM using Vivaspin ultrafiltration spin columns (Sartorius stedim), concentrated at about 2 mg/ml and stored at -20 °C.

2.1.3.1 Protein Labeling

We labelled the proteins using a kit from Molecular Probes (Invitrogen) to covalently link AlexafluorTM 488 fluorescent dye to the protein, as described in [19]. The initial concentration of the protein was 0.4 mg/ml in PBS 1x (with DDM in the case of SQR), the reaction time was 15 min for α-HL and 40 min for SQR. This yields an average labelling of 0.2 mole Alexa 488/mole protein.

After purification, the solution containing the labelled protein was mixed with the proper amount of a solution of the same protein unlabelled, to reach a final solution of α-HL with 1/7 labelled monomer and a solution of SQR with 1/3 labelled monomer. This rather low-labelled monomer content rules out any disturbance of the insertion mechanism of the protein and of the dynamics of the final object. Final aliquots of protein (0.2 µM) in Phosphate Buffer (PB) 20mM and NaCl 100 mM pH 7 were stored at -18 °C before use (with DDM in the case of SQR).

2.1.3.2 Protein Insertion

SQR inserts as a trimeric integral monotopic (lipid-anchored in only one leaflet) membrane protein at the end of the process [20-21] and we define it as the first object.

Incubated over a fluid bilayer, α-HL monomers in solution assemble as heptameric pre-pores at the surface of the bilayer, turning into transmembrane (spanning) heptameric pores [22-23]. We define it as the second object.

Incubating α-HL over a lipid bilayer consisting of a mixture of fluid/gel regions (half transition) promotes the formation of dimers of α-HL heptameric pores [10], we define it as the third object of our protein mixture.

The composition of the aliquots leads on average to one fluorescent label per object (the heptameric pore for α-HL and the SQR trimer for SQR) and to two fluorescent labels per dimer of

heptameric -HL.

2.1.3.3 Preparing a system containing a mixture of 2 objects: trimeric SQR and single heptameric -HL

The volume over a slide with the bilayer (or multibilayer) positioned in our incubation cell was 1 ml. First, 700 µl of water were removed, leaving the bilayer under the remaining 300 µl, and 300 µl of an SQR aliquot were added. This final volume of 600 µl of protein (SQR 0.1 µM in PB 10mM NaCl 100 mM, pH 7.2) was incubated for 12 hours at 33 °C (bilayer in the fluid phase), after which 300 µl of the supernatant were discarded and the excess of SQR was gently removed by adding and pipetting 500 µl of distilled water 10 times.

The sample was returned to the incubation cell and again immersed under 1 ml of distilled water, 700 µl of which were removed again. Then 300 µl of an aliquot of -HL were added to the incubation cell following the same protocol (12 hours at 33 °C).

2.1.3.4 Preparing a system containing a mixture of 3 objects: SQR, single heptameric -HL, and dimer of heptameric -HL

The incubation was carried out over a DMPC double bilayer. The first two steps were as described above for the mixture of two objects. Formation of dimers of heptameric -HL depends on temperature and time. To obtain inserted dimers of heptameric -HL, the solution was brought back to 22 °C, a temperature where gel and fluid lipid regions coexist and which is thus conducive to the formation of dimers of heptameric -HL. An extra 12 hours of incubation was carried out, which added to the initial 12 h incubation of -HL gave a total of 24 hours, found to be optimal to obtain a large number of dimers [10].

2.1.4 Systems

The different systems of our study are summarized in Table 1.

Table 1: Systems, pH and incubation temperature in different supported lipidic bilayer systems

Mixture	Conditions	DMPC single bilayer	DMPC double bilayer	DMPC Multibilayer	EggPC Multibilayer
of 2 Objects	pH	6.8; 8.3; 9.2	-------	6.8; 8.3; 9.2	6.8; 7.2; 8.3; 9.2
	Incubation T(°C)	33	-------	33	33
of 3 Objects	pH	-------	9.2	-------	-------
	Incubation	-------	33 °C (SQR &	-------	-------

	T(°C)		α-HL) then 22 °C to obtain α-HL dimers		

The temperature used to measure electrophoretic mobilities was 35 °C for all systems, a temperature where bilayers are fluid. For a given species, for a given pH, all mobilities were identical whatever the system (single bilayer, double bilayer, and multibilayer, composed of EggPC or DMPC) and also identical to the value measured on the object when alone and not in a mixture.

2.2 Videomicroscopy

2.2.1 Cell

The sample (supported bilayer + inserted proteins) was transferred underwater in a thermostated electrophoretic cell (see [11] and Supplementary information S1).

2.2.2 Video

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. Videos of migration of proteins under electric field (3V/cm) at different pHs are available in the Supplementary information files S2-S6 (video 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).

2.3 Electrophoretic mobility

The resulting effective mobility of an object (μ_{eff}) is the sum of all contributions [11]:

$$\mu_{eff} = \mu_E + \mu_{lipid} + \mu_{EOF} \quad \text{(eq. \#1)}$$

We showed that μ_{lipid} and μ_{EOF} were negligible in our conditions. We thus get:

$$\mu_{eff} = \mu_E \quad \text{(eq. \#2)}$$

We obtain the mobility of the object directly by simply dividing its velocity by the field value. Drift velocity measurements were performed as described in Harb et al. [11]. Briefly, the velocity of at least 10 spots measured on three different locations in the bilayer from each sample was obtained by dividing distance traveled by the objects 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).

3. Results and Discussion

3.1 Characterization of the different systems

3.1.1 Mixture of 2 objects

After incubation of SQR, the support was placed under a fluorescence microscope and a density of 426 spots/mm2 was observed. After incubation of -HL and washing, the spot density observed for -HL was 177 spots/mm2.

3.1.2 Mixture of 3 objects

We observed 392/mm 2 spots versus 426 spots/mm 2 (mixture of 2) for SQR, a reproducibility of insertion to that of the protein alone, which saturated at around 400 spots/mm 2 in [12]. There was the same trend for α-HL with 182 spots/mm 2 versus 177 spots/mm 2 (mixture of 2) and ≈180 spots/mm2 for the protein alone [10]. For the dimer of heptameric α-HL, we found 130 spots/mm2, which is in good agreement with the 139 spots/mm2 obtained after 24 h incubation in [10]. Number of spots varied from one experiment to another by around 5 %.

In addition, these reproducible densities shed light upon the mechanism of dimer formation: the dimers are built independently, their formation does not consist in dimerization of already-inserted heptameric α-HL.

3.1.3 Identification of objects

Fluorescence intensity in arbitrary unit (a.u)

In previous work [10], we observed an average intensity close to 38 a.u unit. for trimeric SQR and heptameric α-HL. The same intensity was observed here, corresponding to the label content of each object. For the dimer of heptameric α-HL alone, the average intensity was ≈ 70 a.u, roughly twice the previous intensity, as expected.

In the mixture of two objects, we observed spots having an average intensity of 38 a.u. In the mixture of three objects we observed spots having the same intensity and spots having an average intensity of 67-70 a.u, indicating the presence of the three objects.

Differentiation between SQR and α-HL

For both mixtures (2 or 3 objects):

 SQR was incubated first, then videoed and analyzed. Recording free particle trajectories with our video setup, and treating them with Image J developed by the MOSAIC GROUP at ETH Zurich [25], we determined diffusion coefficients D of SQR at zero electric field (with the plugin), finding values of D in good agreement with previous reports [12].

 Next, α-HL was incubated. We observed two populations, each with its own mobility, in agreement with those measured on protein alone [11]. This enabled us to distinguish SQR

from α-HL.

 No video measurements of diffusion coefficients were performed for -HL but total spot density was observed to have increased, indicating that SQR and α-HL were now inserted in the same bilayer.

 Each determination (fluorescence intensity and electrophoretic mobility) was carried out on 10 spots at least. Using these determinations, we obtained a position pattern for a given object that was then used, in a sort of feedback loop, to selectively color the spots on the figures of the article (SQR in red, α-HL in blue) for easier discrimination.

3.2 Relevant pHs for protein separation

We wished to visualize, and thus to predict, which pH(s) could be used to separate the objects. Our previous studies [11, 13] provided data on how electrophoretic mobility of each protein depends on pH, enabling us to draw Figure 1 to compare them.

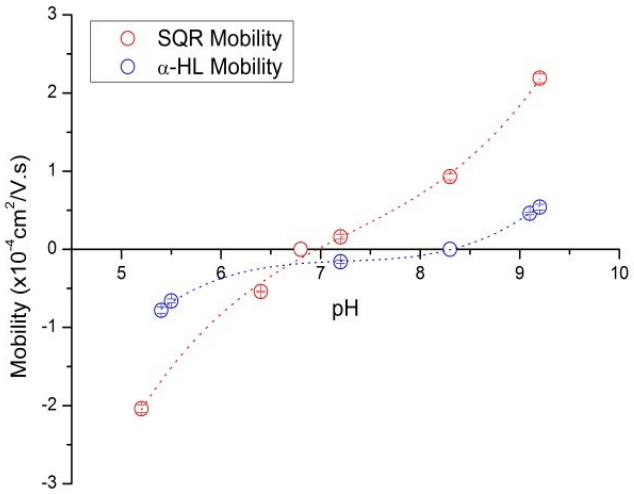

Figure 1: Electrophoretic mobility of trimeric SQR alone and heptameric α-HL alone as a function of pH.

3.3 Separation of 2 objects (trimeric SQR + heptameric α-HL)

At pH 7.2, both proteins have mobility, but in opposite directions since they have opposite charges (see fig.1). The complete sequence is shown in video 1 (S2). They frequently appear to simply go their own way, ignoring the existence of other species. However, there are some situations where two different objects approach each other closely enough to collide, with potentially varying results. The question is: will they join together and stop migrating? or will they migrate as one object with a mobility that is derived from their opposite mobilities?

We were able to capture these particular situations (bottom/left and top/right, yellow ellipses in video 1). We focus on one of these events from the movie, detailed in figure 2: we extracted some snapshots, time interval 2.3 s to 6.8 s, from video 1 (S2).

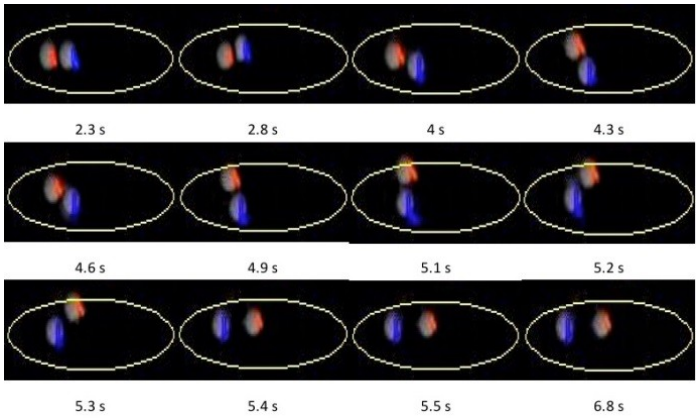

Figure 2: Zoom on SQR (red) bumping into -HL(blue) inserted in EggPC supported multibilayer at pH = 7.2 under a 3 V/cm electric field with the time interval 2.3 s to 6.8 s extracted from video 1 (S2). Scale bar: 50 µm.

In the movie, the objects start to bump into each other at first, bounce, and finally manage to find a way to glide past each other before following separate paths. The striking feature is that, even though they have opposite charges, they do not stick to each other. We suggest that they do not stick to each other because each object is surrounded by a belt of bound lipids. This is a very interesting result since it proves that, even with opposite charges, separation is still possible.
The complete sequence at pH 9.2 is shown in video 2 (Supplementary information S3). As figure 1 shows, the difference in mobility is maximal, meaning that this is the pH at which separation is fastest and most efficient for this system. At this pH, all objects have a negative charge and race toward the positive electrode as shown by the red (for SQR) and blue (for -HL) arrows. SQR migrates faster (µ SQR = 2.2x10 -4 cm 2 /V.s) than -HL (µ -HL = 6x10 -5 cm 2 /V.s), the mobilities being the same as when the proteins were alone. SQR either overtakes -HL easily while migrating whenthey are in distinct lanes or may overtake by changing lanes when SQR catches up with -HL on the same trajectory (see yellow ellipse on video 2 around t=13 s). Again, we observe no sticking to each other or overcrowding that would prevent separation.
At pH 6.8, the SQR is motionless, while the α-HL moves toward the negative electrode (see video 3- Supplementary information S4). At this pH, -HL (blue spots) has a positive charge whereas SQR (red spots) has reached its pI (isoelectric point) [11, 13]. We measured the mobility of -HL, and found µ = -1.9 x10 -5 cm 2 /V.s, which is in good agreement with previous results [11].
The next experiment is intended to reverse roles for SQR and -HL; at pH = 8.3, -HL reached

its pI and SQR had a net negative charge. The SQR moves toward the positive electrode (see video 4- Supplementary information S5) but the -HL is immobile. We measured the mobility of SQR, finding a value of 1.0 x10 -4 cm 2 /V. s, which is in good agreement with our previous results [13]. All these scenarios at differing pHs will lead to the separation of the proteins.

3.4 Separation of 3 objects (trimeric SQR + heptameric α-HL+ di-heptameric α-HL)

As a final experiment, we prepared a mixture of three objects: trimeric SQR, heptameric -HL and dimer of heptameric -HL. We constructed a system composed of a double bilayer into which we inserted these three objects, as described in the Materials and Methods section. The experiment was run at pH 9.2, where all objects are negatively charged. They race each other toward the positive electrode (video 5- Supplementary information S6). In this video, the different objects are shown in different shades of gray (trimeric SQR: the lightest, heptameric -HL: medium gray and the dimer of heptameric -HL: the darkest). In figure 3, as illustration of video 5, trimeric SQR and heptameric -HL are shown in their usual colors, red and blue respectively, and the third object, the dimer, is in yellow (same color code for the arrows). Circles/ellipses illustrate situations where the three objects (red, blue and yellow) are close at the beginning and where distances between them increase with time.

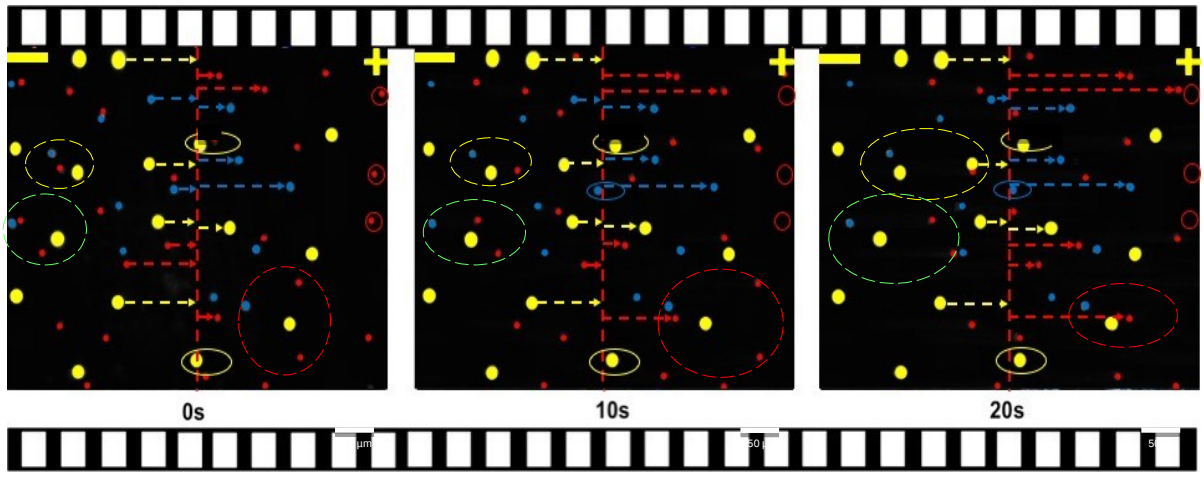

Figure 3: Images of fluorescent SQR (red), -HL(blue) and dimeric -HL(yellow) electrophoretic migration recorded at 0 s, 10 s and 20 s extracted from video 5 (S6). The vertical red dotted line is an arbitrary reference line to highlight the position change of objects. Scale bar:50 µm.

The fastest is SQR and the slowest is the dimer. Each object has its mobility value, determined during previous work on protein alone. This experiment shows again that it is possible to electrophoretically separate a mixture of membrane proteins in supported lipid bilayers in a short time and with a fairly low electric field.

All the mobilities recorded during these experiments are presented in table 2.

Table 2: Mobilities μ of the different objects as a function of pH and of x, the number of species present in the mixture. Values of μ: ±10%.

Mobility (10^{-5}cm²/V.s) of	**SQR**			**αHL**			**di- αHl**	
System with *x* objects **pH**	***x*= 1**	***x*=2**	***x*=3**	***x*= 1**	***x*=2**	***x*=3**	***x*=1**	***x*=3**
5.2-5.5	-20.6	-	-	-8.2	-	-	-	-
6.4	- 6.0	-	-	-	-	-	-	-
6.8 (pI of SQR)	0	0	-		-1.9	-	-	-
7.2	1.6	1.6	-	-1.9	-2.1	-	- 1,0	-
8.3 (pI of αHL)	9.9	10.0	-	0	0	-	-	-
9.2	22.0	22.2	21.8	5.8	6.0	6.1	-	1.9

4. Conclusion

This work provides new insights into the feasibility of an electrophoretic separation of membrane proteins in single, double and multi supported bilayers. Beyond separation to establish 3D structure, thus keeping their bioactivity, of individual membrane proteins, this protocol can be use as test platform for drug or diagnostic. It reveals that SQR remains immobile (when at its pI) while -HL can migrate alone or vice versa, which is achieved by suitably adjusting the pH of the buffer. We also demonstrated that when proteins are charged (mixture of 2 or 3 objects), separation is feasible due to the differing mobility that results from their differing structures and charges. If the proteins interact while migrating, they do not stick together, probably due to the existence of bound lipids which act as a bumper, avoid each other and continue migrating. This is a very interesting result since even in these situations, separation occurs. Compared to standard electrophoretic methods, we observed clear separation of the three objects in substantially shorter distances (microns) and times (seconds), and with a weak field (3V/cm). In addition, this system is easy to build using rehydration of dried lipid film, and the resulting multibilayer will certainly increase membrane protein loading capacities. We hope to generalize to other membrane proteins after solving the next challenge which is to insert an unknown membrane protein mixture from living systems into the supported multibilayer. We suggest two methods as potentially feasible: direct fusion of cells on an already-present supported bilayer or healing an intentionally-created scratch in a multibilayer with a low-detergent solubilized biological membrane.

Supporting Information.

S1: Schematic diagram of the video/electrophoretic cell and its description. Protocol to make videos (text and diagram, pdf)

S2 to S6 : Video 1 to 5 (.avi file)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; EggPC, L-α-phosphatidylcholine (Egg, Chicken); SLB, Supported Lipidic Bilayer; SQR, Sulfide Quinone Reductase, -HL, Alpha Hemolysin.

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