

# Transport and Concentration of Charged Molecules in a Lipid Membrane

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**Abstract:** Lipid membranes are essential for eukariotic cells. They enable them to compartmentalise themselves into different regions, such as the nucleus, mitochondria or the golgi apparatus. The membranes also play an essential part in material transport within the cell. Lipids are also the main components of cell walls, which host membrane proteins. Both are very important in cell replication and are therefore of high interest for drug development. These membranes can be understood as a planar sheet in which the lipid molecules can undergo 2D movement and diffusion, but will not leave their membranous environment. We use solid supported lipid membranes (SLMs) as a model for cell membranes and study their behaviour under the application of external electric fields. We firstly studied different patterns in simulation to optimise their behaviour and then realised them for experimental purposes. We show transport and concentration of membrane components in their native environment, which are vital problems for applications such as drug development and membrane protein investigation.

**Keywords:** Lipid Bilayers, Electric Fields, Electrophoresis, Binding Kinetics, Membrane Proteins

## 1. Introduction

Lipid membranes play several important roles in cell biology. This makes them a relevant research topic for different disciplines. Some very important proteins naturally occur in this membrane, but will denature if taken out of their natural environment. It is therefore of interest to understand the environment the proteins and to develop tools to manipulate and transport membrane proteins without denaturing them.

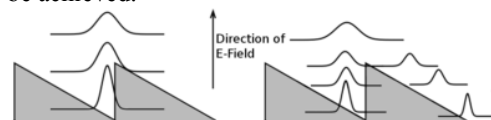
Here solid supported lipid membranes (SLMs) are used as a model for the cell membrane. Since most membrane components, such as proteins or even some lipids are charged, the application of external electric fields can be

used for the manipulation of membrane components. Electrophoretic or electro-osmotic flow will drive membrane components in the desired directions<sup>1,2</sup>.

In order to achieve not only transport in direction of the applied electric field, but also perpendicular to the electric field, we use a patterned lipid bilayer and make use of the idea of Brownian ratchets<sup>3,4</sup>. These ratchets allow us to ‘rectify’ diffusion. The combination of both, free diffusion and ratcheting results in a way to obtain net transport of charged species perpendicular to the applied electric field.

Electrophoresis can also be used in a simpler, DC approach. This results in the disadvantage of the electrodes being further away from the pattern, but the advantage of much shorter experimental time scales is obtained.

The principle of a Brownian ratchet is shown in Figure 1. Starting with the material of interest being concentrated into the ratchet, the electric field is then applied such that the charged material is being driven out of the ratchet. During this process the material will start to freely diffuse. The free diffusion also takes place in a direction perpendicular to the applied field. If the time for free diffusion is chosen such that a certain amount of the charged material moves over the ratchet and into an area above the next ratchet, it will go into the next ratchet, once the electric field is reversed and the charged material is driven back down. Although only small amount of charged material is driven into the next ratchet over one cycle, if the cycle is repeated multiple times, efficient ratcheting can be achieved.



**Figure 1:** Principle of a Brownian ratchet. An initially Gaussian distribution is split into two parts where one part has moved one ratchet further ahead.

## 2. Use of COMSOL Multiphysics

Brownian ratchets and concentrator designs for lipid bilayers were implemented in COMSOL as 2D geometries. As a lipid bilayer is a 2 molecule thick layer, it is a reasonable assumption to consider diffusion in 2D. In both cases COMSOL's chemical reaction engineering module was used to solve the Nernst Planck equation:

$$\frac{\partial c}{\partial t} = \nabla \cdot \left( D \nabla c + \frac{Dze}{k_B T} c \Delta \Phi \right),$$

where  $c$  is the concentration of the species under investigation,  $D$  the diffusion coefficient,  $z$  the charge on the molecule,  $e$  the elementary charge and  $\Phi$  the electric potential. As there was no flow and no magnetic potential, these terms were ignored here.

The diffusion coefficient used here was  $1.5 \mu\text{m}^2/\text{s}$  for Texas Red DHPE<sup>1</sup>. The charge was  $-1$  and the electric potential was  $62 \text{ V/cm}$ , identical to the experiments. For the Brownian ratchets, the electric field was modelled as a piece wise function, which moved charged material into the ratchet for 16 min and out for variable time.

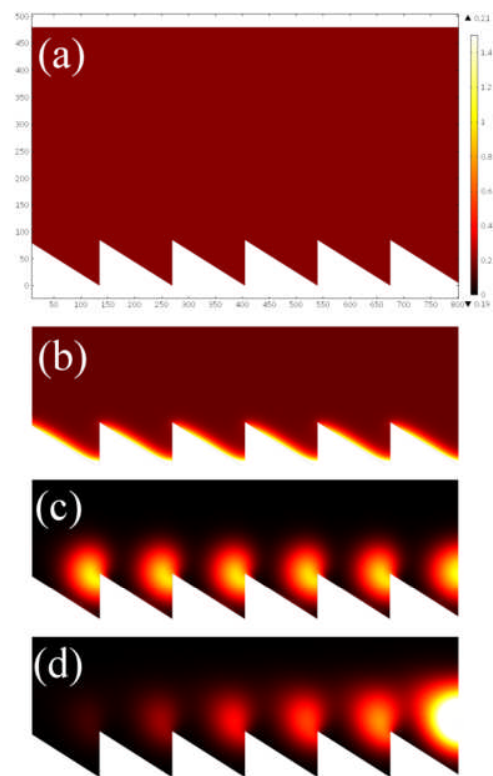
The edges of the pattern were defined as no flux boundaries as the lipid bilayer makes a boundary which can't be penetrated by its components.

## 3. Numerical Model

### 3.1 Brownian Ratchets

Firstly a simple ratchet model was built in COMSOL. It consisted of six teeth combined into one pattern. The electric field used to drive the charged material into the tooth was applied for 16 min, the outward field was applied for 6 min. **Figure 2** shows a time series of the model used. The initial image shows an even distribution of all the material over the pattern.

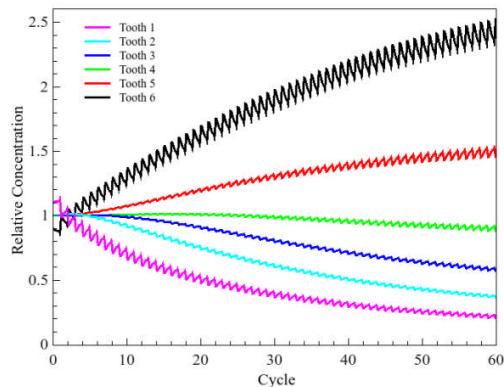
When the electric field is applied, the charged material starts moving into the ratchet, upon reversal of the field the outward movement and also free diffusion are started. When the centre of mass of the charged material reaches the top of the tooth, it can also diffuse over the next tooth.



**Figure 2:** Time series of the Brownian ratchet model. (a) initial configuration: even distribution of the charged material, (b) intermediate state: material driven into the teeth, (c) reversal of the electric field, (d) final state with transport achieved to the right.

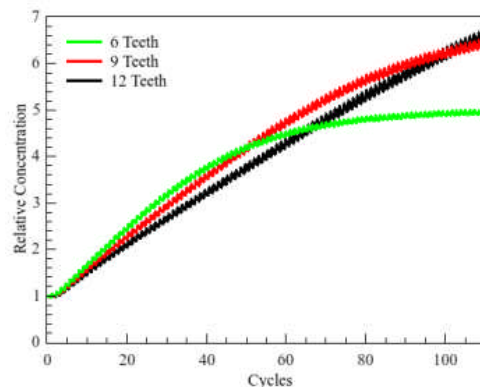
The result after 16 cycles is shown in **Figure 2(d)**, clearly showing the net movement of charged material perpendicular to the applied electric field.

In order to follow the transport of the charged material through the different teeth, we took the surface average of the concentration in the area above each tooth and plotted it against the cycle number. **Figure 3** shows how first ratchets transport their material further to the right where it is accumulated over time. The first teeth are emptied while the last two accumulate more and more of the charged with each cycle. This shows that transport can be achieved perpendicular to the applied electric field using Brownian ratchets.



**Figure 3:** Concentration of charged material over time in the different teeth. The plot shows how the first ratchets pump their material further forwards with each cycle.

The ratchets can also be made much longer than the 6 teeth we have used so far. To show this, we designed ratchets with a length of 6, 9 and 12 teeth. The relative concentration in the final tooth vs. cycle is shown in Figure 4. This shows that the ratchet could theoretically be made to an arbitrary length still achieving efficient transport. The longer the ratchet, the slower is the increase of the relative concentration in the final tooth. This is expected as it takes longer for the charged material to reach the final ratchet, if the ratchet is getting longer. The shorter ratchets level off earlier, than the longer ratchets. This is also as expected, as the total amount of charged material is lower, the shorter the ratchets are. So the maximum amount that can be transported into the final ratchet is less for shorter ratchets. In Figure 4 it can be seen, that for 6 ratchets, the ratcheting process finishes at around 60 cycles, whereas for 9 ratchets this starts happening at around 100 cycles, while the device with 12 ratchets is still transporting at that time and will continue to ratchet for a few more cycles.



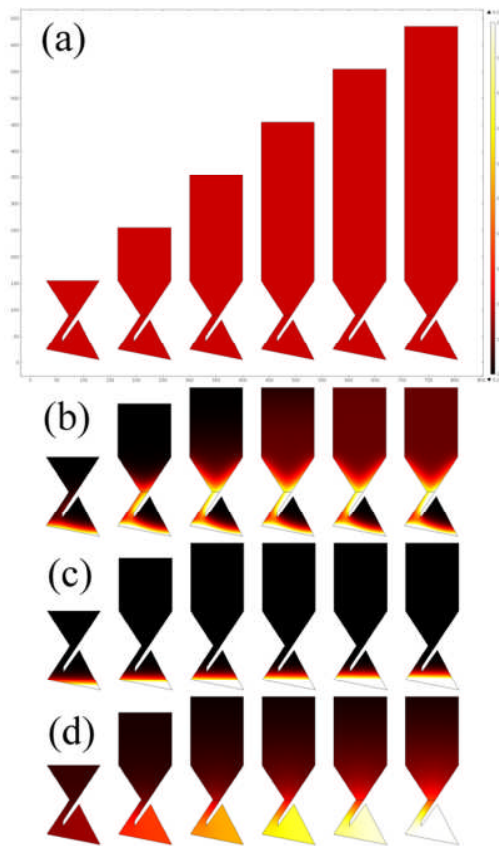
**Figure 4:** Relative concentration of charged material in the final tooth of devices consisting of 6, 9 and 12 ratchets.

#### 4.2 DC Traps

When transport is not the primary goal, but one is aiming to trap material into a given region of interest, other patterns can be more useful than the ratchets shown so far. To achieve trapping of charged material in a region of interest, we have designed traps, which work with a DC principle instead of the AC system shown so far.

The pattern of the trap can be seen in **Figure 5(a)**, it is an array of several traps, which will hold the charged material in the bottom region even after the electric field has been switched off. With an applied electric field the charged material starts moving into the trapping region, as can be seen in **Figure 5(b)** where the smaller traps have already moved all of the material into the trapping region, while the other traps still require more time to fully trap the charged material (see **Figure 5(c)**). To show the trapping behaviour of the patterns, the electric field was then switched off and, as can be seen from **Figure 5(d)**, the charged material remains in the trap, even after 2 h.

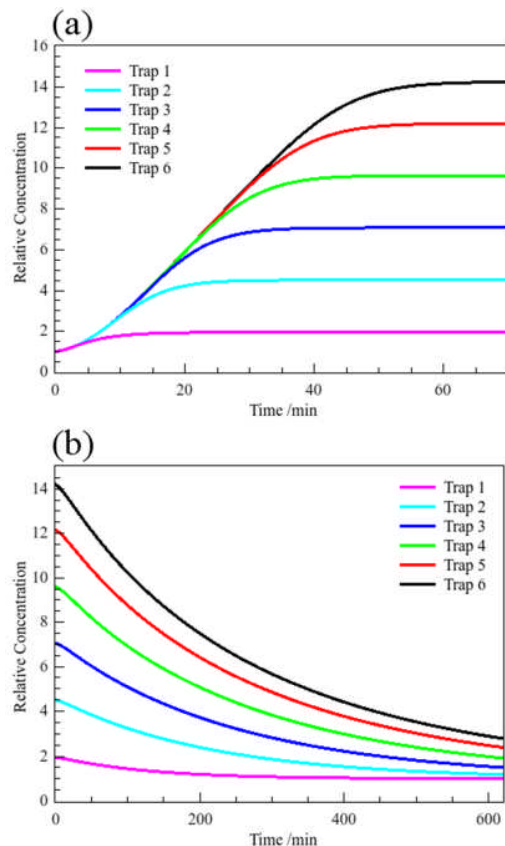
As the traps are in an array with several different sizes of reservoirs for the charged material, the expected behaviour is to get several different concentrations in the trapping regions. As **Figure 6(a)** shows, this is also what the simulations show. With increasing reservoir size the reached final relative concentrations also increase. This shows that all the charged material is moved out of the reservoir regions into the traps. It can also be seen that the traps fill up at



**Figure 5:** A DC trap pattern. The initial distribution is shown in (a), (b) shows the build up in the trapping regions, (c) shows the final state, (d) shows the trapping behaviour, 2 h after the field has been switched off

the same speed initially. As the reservoirs become empty, the relative concentration in the trapping region starts flattening out, finally a steady state is reached. The graph also shows that the spacing between the final relative concentrations is spaced out evenly, as the reservoir sizes are.

When the field is switched off, the charged material in the trap is subject to free diffusion which will drive the material back into equilibrium. The trap design of the lower area of the pattern slows this process down, so that experiments can be carried out on the region of interest, even if the field is switched off. Figure 6(b) shows how the charged material decays with time in the trapping region. The half time of the traps is approximately 250 min, therefore allowing for further experiments, when needed.



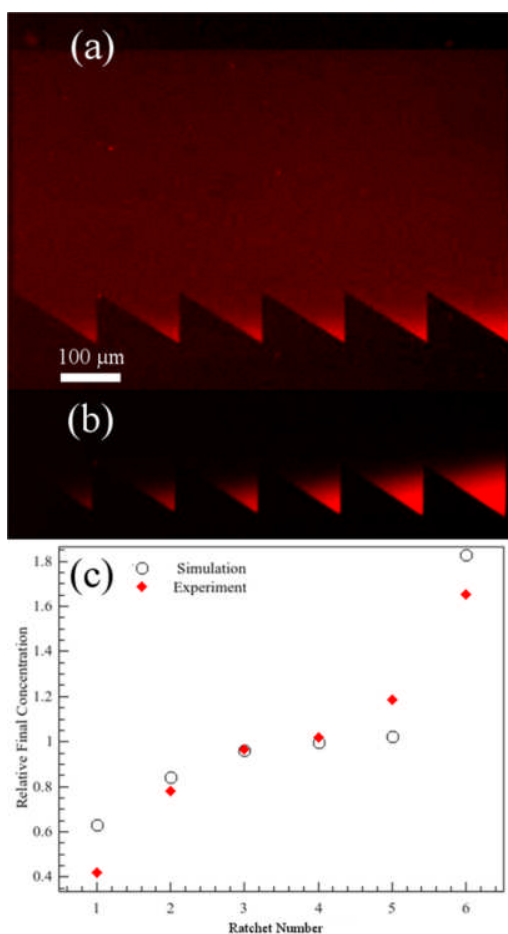
**Figure 6:** Build-up of the charged material in the different trapping regions (a). Traps 1 to 6 refer to the different bottom regions of each pattern seen in **Figure 5** numbered from left to right. After the field has been switched off, the charged material follows an exponential decay (b) with a half time of 250 min.

## 5. Experimental Results

The patterns shown here in simulation first, were realised in a lipid bilayer. The bilayer was formed on a glass cover slip by vesicle fusion<sup>5</sup> of vesicles containing 99.975 mol% POPC and 0.025 mol% Texas Red DHPE as a charged and fluorescent probe. The transport or concentration of the Texas Red DHPE was then followed by fluorescence microscopy. The movement was achieved by the application of an electric field of 62 V/cm in all cases. The patterns were made by pre-patterning the glass surface through micro-contact printing of fibronectin<sup>6,7</sup>, which inhibits bilayer formation where it is printed.

### 5.1 Brownian Ratchets

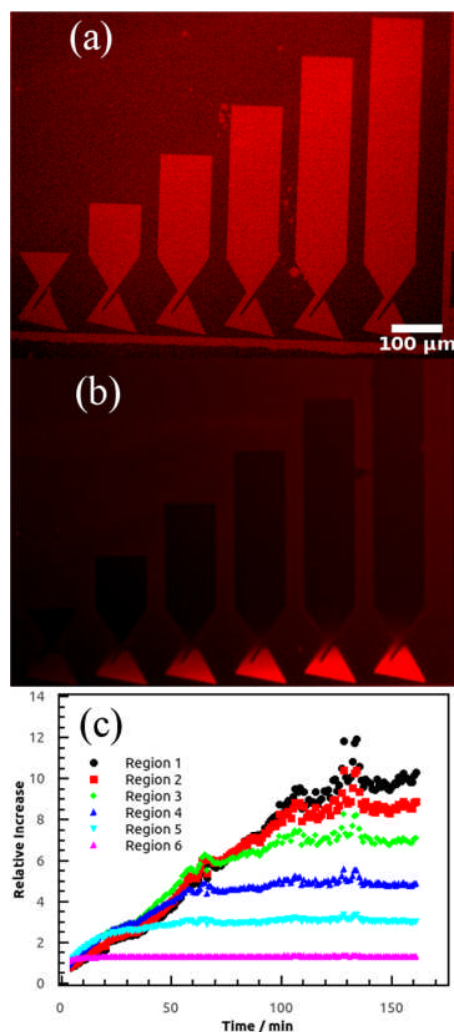
The fabricated patterns with optimised parameters as obtained from the simulation can be seen in Figure 7 where the fluorescence is due to the dye in the lipid bilayer. After 26 cycles of the AC electric field, most of the charged, fluorescent dye has moved to the right (b). We then compared the obtained values for the relative final concentration. Both the general shape of the curve and the exact value agree. This shows that the experiments were accurately predicted from the simulations.



**Figure 7:** (a) Initial fluorescence image of the Brownian ratchet produced in experiment. (b) Ratchet after 26 cycles showing transport to the right. (c) comparison of experimental and simulated final concentrations in the different teeth of the ratchet.

### 5.2 DC Traps

In addition to the ratchets, we also produced an experimental version of the DC traps. The fluorescence image in Figure 8(b) visually already shows an intensity difference in the different trapping regions, showing that the traps generate concentration in different amounts. Figure 8(c) compares well to the simulation results in Figure 6(a). The main difference lies in the biggest trap, where self quenching of the fluorescent dye has the largest impact resulting in an underestimation of the amount of material trapped in the bottom of the pattern.



**Figure 8:** Initial (a) and final (b) fluorescence image of the DC trap pattern. (c) shows the relative concentration in the 6 different traps over time.

## 6. Conclusion

In conclusion we have shown that we can use COMSOL Multiphysics to accurately predict the behaviour of charged components in lipid bilayers. Different patterns were used to achieve different goals, we were able to achieve transport perpendicular to the applied electric field with a Brownian ratchet pattern. This makes use of the combination of a ratchet pattern and free diffusion. Such a design allows for not just moving charged material directly in the direction of the applied electric field. Therefore miniaturisation of the electrodes is possible even with them being very close to the pattern.

The trap design works on a faster DC approach and concentrates and keeps a pre-determined amount of charged membrane component in the trap. With a predicted half time of more than an hour, experiments can still be carried out, even if the electric field is switched off. This could potentially also be used as a biosensor where different concentrations of a certain membrane component are needed in close proximity.

We here used Texas Red DHPE as a charged molecule of interest. The same methods shown here can also be applied to other molecules such as membrane proteins for which concentration and transport is still an important issue. As the ratchets work most efficiently for a given combination of diffusion coefficient and time scale, the ratchets could even provide a mean to sort membrane components on diffusion coefficient.

## 7. References

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## 8. Acknowledgements

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