Porated liposomes: towards new nanomotors model

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Cells and microorganisms like bacteria use chemotaxis to move directly in response to concentration gradients of nutrients and toxins [1]. Likewise, synthetic delivery systems could take inspiration from nature to mimic this kind of transport. Ultimately, it could guide nanomotors in the body in a specific way, according to concentration gradients occurring physiologically [2].

Janus colloids/particles are the most studied chemotactic synthetic systems. They consist of spheres with one hemisphere covered with a catalyst, which results in a chemical gradient across its surface. This asymmetry is necessary to induce phoretic motion [3].

Envisioning therapeutical applications, proteins could be used as a catalyst in developing biocompatible motors, as their substrate and metabolites are also biocompatible and very versatile. Most of these nanomotors use proteins asymmetrically distributed on the surface of nanoparticles or nanovesicles [4]. However, these objects are unknown to the immune system. If injected intravenously, they tend to be covered with protein corona once in the blood, which can change their surface chemistry [5].

Therefore, we present a system with the basic characteristics to achieve a minimal chemotactic cell in which the enzyme is encapsulated inside a vesicle (Figure 1). It consists of an asymmetric liposome of 100 nm (made of soy phosphatidylcholine) with encapsulated glucose oxidase. The asymmetry is given by the presence of pores in the membrane, inserted by the protein alpha-hemolysin. Mass ratios of 0.075 and 0.1 Hly/lipid were used, resulting in ~2 and ~3 pores per liposome (Figure 2).

When the liposome is placed in an environment with glucose, the catalysed reaction occurs in its lumen. The products diffuse outwards through the pores, creating a local concentration gradient. The asymmetric distribution of products along its surface generates a slip velocity that moves the vesicle in response to the glucose concentration gradient (Figure 3).

The motion of the liposomes labelled with rhodamine octadecyl ester perchlorate (1%) was investigated in an Ibidi microfluidic (Figure 4). A concentration gradient of 0.05 M of glucose was established in the channel. For this particular channel, a higher concentration gradient of glucose would induce advective flow, which would hide the movement caused by chemotaxis [6].

In this experimental setup, several phenomena can be observed. The presence of the concentration gradient itself results in a difference in the interaction potential between the solute and the channel walls along the x direction. In the same way, the interaction between the solute and the vesicle's external surface will be different in the x direction, which will give rise to a slip velocity. This passive movement, called diffusioosmophoresis, happens even in inactive particles (pristine liposomes, for example).

On the other hand, active movement is related to locomotion in response to the gradient of the products generated by the enzyme-catalysed reaction. The concentration of products that is asymmetrically distributed on the surface of the liposome will result in a slip velocity that will be aligned with the concentration gradient of glucose: chemotaxis.

The movement of pristine liposomes, liposomes with encapsulated GOX with no pores (GOX-L) and with ~2/3 pores (GOX-L-Hly), was imaged with a confocal microscope and analysed with the program Trackpy.

While the pristine liposome and the one with encapsulated glucose oxidase (GOX-L) presented a velocity towards low glucose concentration, the displacement of liposomes with pores (~3) was reverted to the opposite direction. The movement of the pristine liposome and the GOX-L is due to diffusioosmophoresis. In the absence of a glucose concentration gradient, vesicles present only Brownian motion (Figure 5).

For active vesicles, the drift velocity combines diffusioosmophoresis and chemotaxis. With ~2 pores, the chemotactic rate is on the same magnitude as the diffusioosmophoresis, cancelling any drift movement. With ~3 pores, the chemotactic velocity suppresses the diffusioosmophoresis, resulting in a net drift with a speed of ~-0.7 μm/s towards high glucose concentrations (Figure 6).

In conclusion, the experiment in the microfluidic device highlights the contribution of diffusioosmophoresis and chemotaxis to the vesicle's movement. It was observed that the porated liposomes with encapsulated glucose oxidase could move in response to a glucose concentration

gradient. Its direction could be controlled according to the presence/absence of pores in its membrane.

References

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Figures

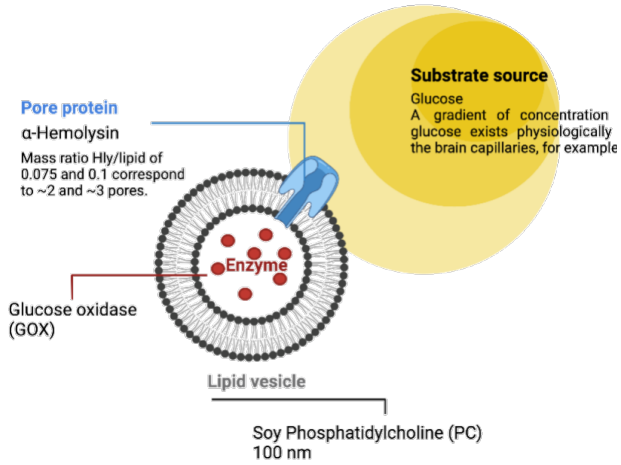

Figure 1. Scheme of a porated liposome with encapsulated glucose oxidase placed in an environment with a concentration gradient of glucose.

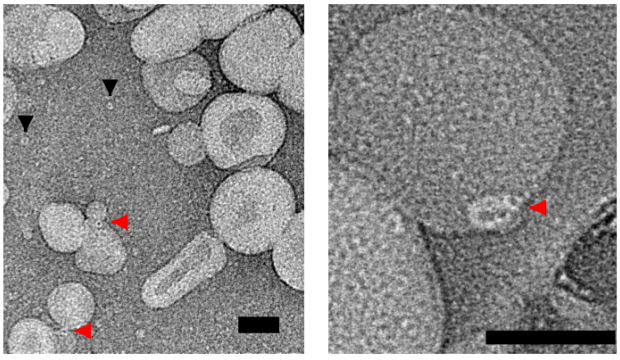

Figure 3. TEM micrographs of liposomes incubated with α - Hemolysin. Black and red arrows indicate Hly unbound (black) and inserted into liposomes (red), respectively. Scale bar, 50 nm.

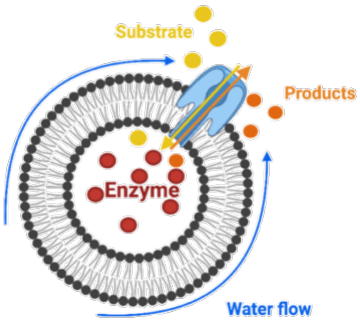

Figure 3. Illustration of the reaction occurring inside the porated lipid vesicle indicating the substrate and product diffusion and the generated water flow.

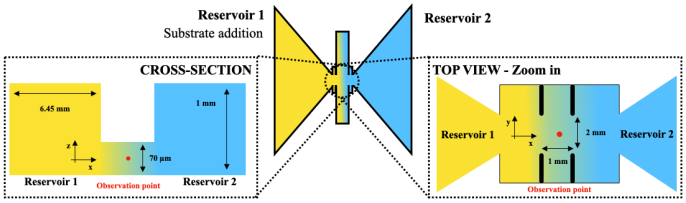

Figure 4. Top view of the Ibidi microfluidic device and an insert of the cross-section view.

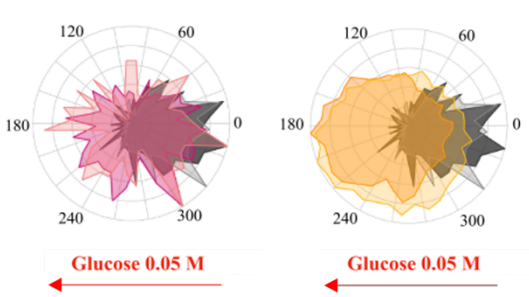

Figure 5. Polar histogram with displacements of pristine liposome (grey) and liposomes with 2 pores (pink) and 3 pores (orange).

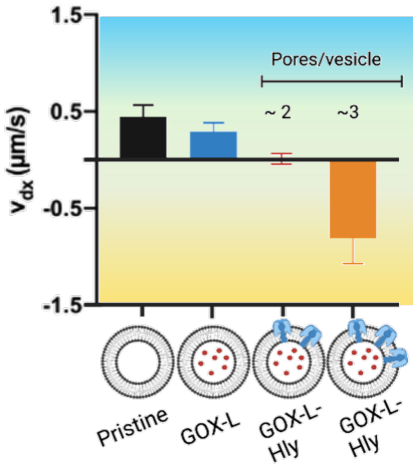

Figure 6. Drift velocities of pristine liposomes, GOX encapsulated liposomes without and with pores.