Membrane transport kinetics as measured by stopped-flow

I. Introduction

Stopped-flow spectrometers can be used to rapidly mix two or more solutions to induce a reaction in a cuvette and to follow their kinetics through the change of the mixture's optical properties. This allows the measurement of rapid kinetics in the time range of 1 ms to several minutes.

A particularly smart application is the fast tracking of transient kinetics of water and solute transport across the membrane of small vesicles. Such a reaction can be generated by mixing purified membrane vesicles with a hyper-osmotic solution. The imposed concentration gradient induces a fast flow of water out of the vesicles.

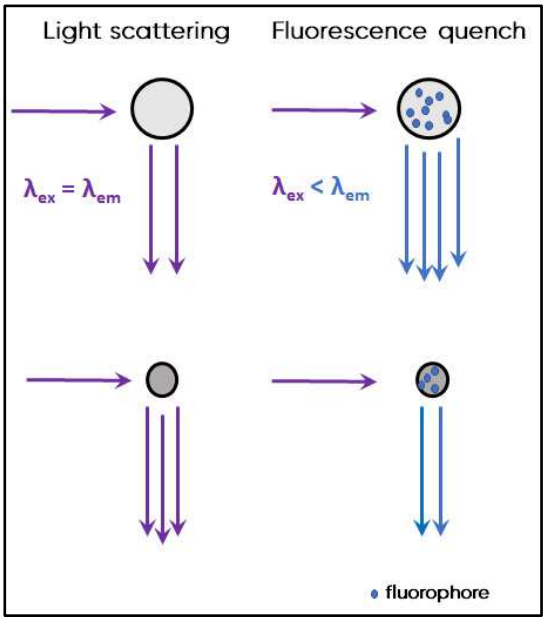

Figure 1 **Principle of light scattering and fluorescence quench techniques used to measure shrinkage of membrane vesicles.**

As a consequence, the volume of the vesicles is reduced and the concentration of the inner medium increases. This shrinkage kinetics will stop when the concentration of the inner medium has reached that of the surrounding one. The rapid change of size of the vesicles can be detected by means of the scattered light intensity at 90° (Figure 1 left side). The intensity will increase when the size of the vesicles decreases. Alternatively, vesicles can be loaded with a fluorescent dye with concentration dependent quenching properties. In short, the emitted light will be quenched when the concentration of the dye inside the vesicles increases, thus a decrease in light intensity with decreasing size will be detected (fluorescence quench technique; Figure 1 right side).

II. Aquaporins, an important water channel

Aquaporins are important and well-studied water channel membrane proteins. They form pores in the membrane of biological cells, mainly facilitating the transport of water between cells.[1] The cell membranes of a variety of different bacteria, animal and plant cells contain aquaporins through which water can flow more rapidly into and out of the cell than by diffusing through the phospholipid bilayer (Figure 2).

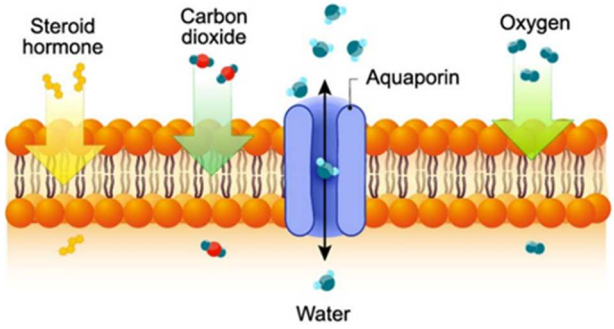

Figure 2 **Schematics of aquaporin water channel embedded in a phospholipid layer.**

After extensive study in their natural environment[2],[3],[4], it has been possible to isolate and incorporate aquaporins into membranes using proteoliposomes, for

example, to optimize water purification processes.[5] These so-called biomimetic membranes represent a highly efficient alternative to traditional membranes, due to improved water flux, without compromising on selectivity.

By following the swelling or shrinking of vesicles using a stopped-flow it is possible to obtain information about the water permeability of the cell. The osmotic shock induces a change of vesicle size and generates a change in light scattering. The osmotic water permeability (P_f) is directly proportional to the observed rate constant using the equation:

$P_f = K.V_0/A_v.V_w.C_{out}$

Where V_0 is the initial mean vesicle volume, A_v the mean vesicle surface, V_w the molecule volume of water, C_{out} the external osmolality and K the observed rate constant as measured by fitting the stopped-flow data with a single exponential. V_0/A_v is determined using dynamic light scattering prior to the stopped-flow measurement so P_f varies linearly with the observed rate constant.

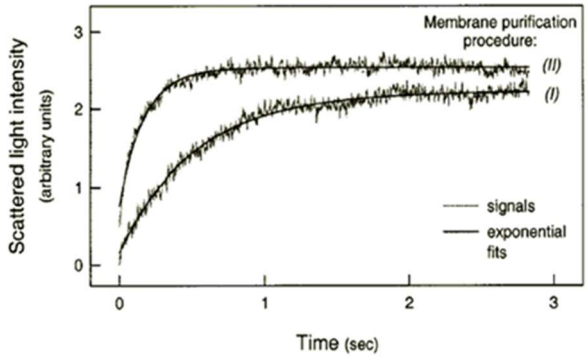

Figure 3 Example of shrinking kinetics.[6]

The shrinking kinetics shown in Figure 3 were obtained using a SFM-300 by carrying out a 1:1 mixing ratio leading to a 253 mosmol.kg^{-1} H_2O inward osmotic gradient. It shows the influence of a different membrane treatment on the water permeability of the vesicles. Single exponential fits are then used to measure K.

III. Experimental considerations

1. Stopped-flow considerations

All BioLogic stopped-flow models can be used for transport membrane kinetics. A two syringe stopped-flow system (SFM-2000 or µSFM) is the perfect mixer for single mixing kinetics or if the sample size is highly limited. A 3 or 4 syringe stopped-flow (SFM-3000/4000) is preferred when users want to automatically vary the amplitude of the osmotic shock at each stopped-flow injection, or when he/she needs more modularity. For example when thereis a need to study the influence of different inhibitors on water permeability.

2. Detector considerations

MOS-200 is the ideal rapid kinetics spectrometer for membrane transport studies. The coupling to the stopped-flow is easy and can be carried out in a few minutes, it offers oustanding sensitivity and data can be collected every 10µs to match the most rapid experiments. A fiber optics bundle brings monochromatic light to the observation cuvette. A high sensitivity photomultiplier tube (PMT) is directly attached to the observation head at 90° to excitation to exclude excitation wavelength in fluorescence mode or to extract scattered signal (Figure 4). In fluorescence mode a cut-off filter is placed inside the PMT to descriminate excitation wavelength.

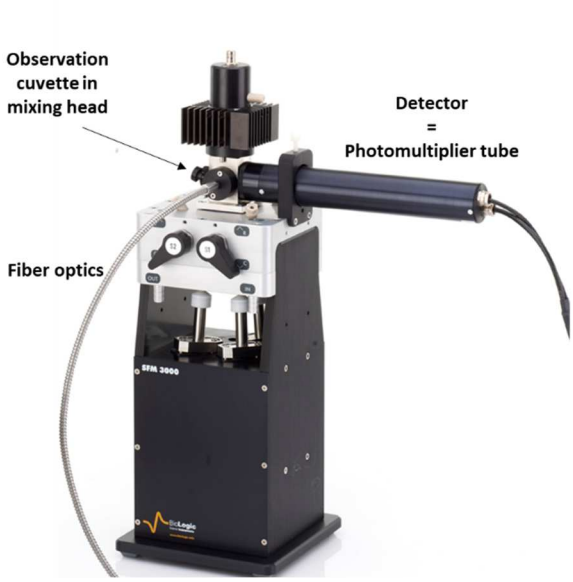

Figure 4 Example of stopped-flow set-up for fluorescence or 90° light scattering.

3. Which wavelength to use?

When using fluorescence detection the choice of excitation wavelength and cut-off wavelength is obvious as it matches the physical properties of the fluorophore.

When carrying out 90° light scattering experiments, the only consideration is to select

an excitation wavelength that is larger than mean diameter of the vesicles studied. The size of vesicles in stopped-flow experiments is often between tens of nanometers and 300 nm so an excitation wavelength higher than 300 nm is required. In such scenarios it is recommended to select a wavelength corresponding to a mercury line of the XeHg lamp (365nm, 410nm, 435 nm ,546nm or 577 nm in order to obtain the best signal to noise ratio). The user can expect a 10 times better signal to noise ratio using one of the above-mentioned mercury lines.

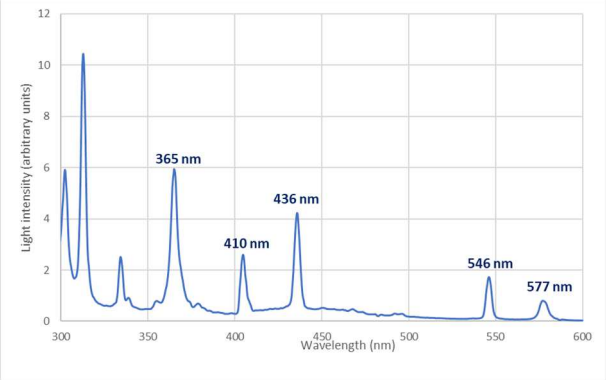

Figure 5 Xe/Hg lamp spectrum

IV. Unique advantages of BioLogic SFM for membrane transport kinetics.

BioLogic stopped-flow mixers (SFM series or µSFM) are equipped with independent stepping motors. This syringe drive technology offers precision and modularity. Combined with the performance of the stop mechanism (hard-stop electrovalve) this system offers unique advantages to work with membrane vesicles or other pressure sensitive samples:

• Sample volume per experiment can be reduced to 10 µl. It saves precious vesicle preparation time. The handling of such small volumes is made possible thanks to the ten nanoliter precision stepping motor steps and because of the internal design of the mixer.

• Exact control of total flow rate, thus minimizing sheer forces during the flow that might affect integrity of membrane vesicles. The SFM is ideal to handle pressure sensitive samples.

• Continuously variable mixing ratios from 1:1 to 1:100. The user can easily program in Biokine software a dilution series to study concentration effects e.g. of inhibitors (refer to Figure 6).

• Stopping of flow *via* a precisely synchronised hard stop to avoid any over-pressure artefacts as induced by simple stop syringes.

• Berger Ball Mixer technology: homogeneous and efficient mixing in the most stringent conditions.

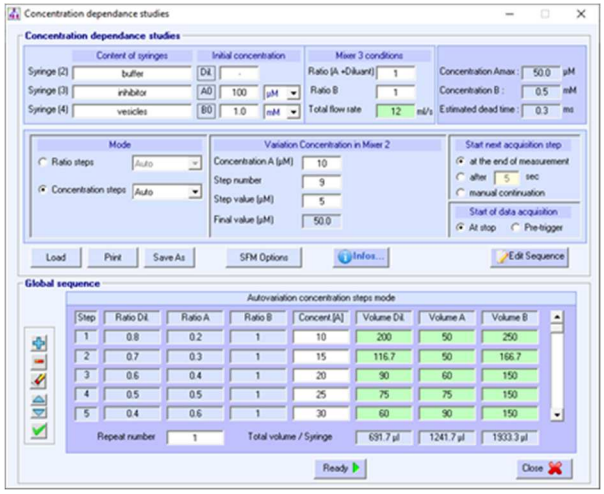

Figure 6 Example of driving sequence to vary automatically the concentration of inhibitor.

V. Conclusion

BioLogic SFM stopped-flow are the ideal mixers for membrane transport kinetics. Stopped-flow are the only instruments able to quantify the efficiency of aquaporin channels by measuring the water permeability following an osmotic shock. Thanks to the independent stepping motor drive, user can fully control injection volumes, mixing ratios and pressure to ensure the integrity of the vesicles or membranes during the experiment.

Do not hesitate to contact our team if you have questions, need more information or would like to obtain other references or links to laboratories where you can see our SFM-2000/3000/4000 or µSFM in action.

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