Cell-free expression and electrophysiological measurements of the KcsA channel in interdroplet bilayers

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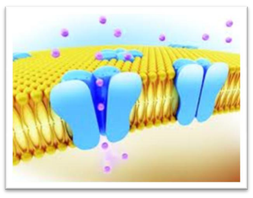
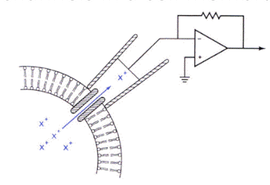
Introduction

Ion channels constitute important targets for pharmacological drugs [1] and can be characterised at a single-channel level with a number of electrophysiology techniques. These include patch clamping, where a glass pipette makes a seal with a cell membrane, or bilayer lipid membranes, where purified ion channels are introduced into an aperture-suspended bilayer of synthetic lipids. The first method requires precise positioning and clamping of the glass pipette over a small membrane patch, while the second method requires protein purification, reconstitution into proteoliposomes and incorporation into the lipid bilayer. Both methods require overexpression to generate sufficient amounts of the channel of interest, which may be toxic to the cell [2]. Membrane proteins can also be obtained by cell-free protein expression, an *in-vitro* method which uses a stabilised cell lysate to express proteins from a supplied DNA template, with or without the addition of detergents or liposomes [3]. This method does not require significant molecular biology infrastructure but is not suitable for large amounts of protein because of the high cost of commercial cell lysates. We have explored the potential of cell-free expression of a small potassium channel, KcsA from *Streptomyces lividans*, for electrophysiological characterisation in microdroplets with the motivation that microvolume cell-free expression is economical and that microdroplet technology has the potential to be scaled up to array format for high-throughput studies, including drug screening.



ion channels in a cell membrane



patch-clamp electrophysiology

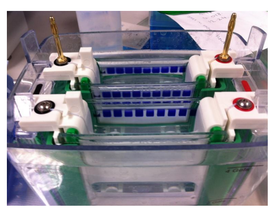
Ion channel expression and characterisation: cell-based *vs* cell-free

Start: → (~2-3 days) → **Overexpression of milligrams of ion channel**

Litre-scale *E. coli* culture for IPTG-induced overexpression from channel-encoding plasmid.

→ (~2-5 days) → **Purification & verification**

SDS-PAGE analysis of detergent-solubilised affinity-column purified channels.



→ (~2-3 hours) → **Channel reconstitution into lipid vesicles**

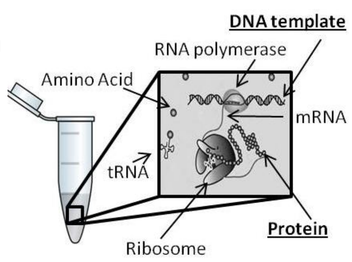
Proteoliposome formation by detergent-depletion.

→ **Proteoliposome fusion with pure-lipid bilayer for electrical characterisation**

Warner Cups for formation of aperture-suspended lipid bilayers.

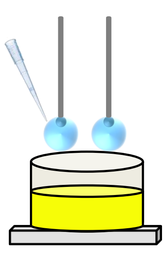
Start: → (~2 hours) → **Ion channel expression in a commercial cell lysate from custom DNA template**

50 µl cell-free expression mixture containing an *E. coli* S30 lysate supplemented with RNA polymerase, an ATP regeneration system and liposomes.



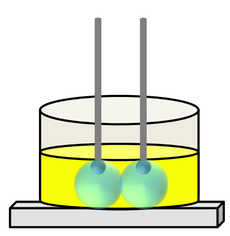
→ (~1-2 minutes) → **The cell-free expression product is introduced as microdroplets in an oil phase**

SU-8 coated support with top reservoir of 20 mg/ml asolectin in decane.



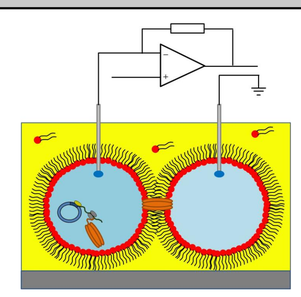
→ (~1-2 minutes) → **Two droplets are brought into contact to form an interdroplet bilayer [4]**

The agar-coated Ag/AgCl electrodes in each droplet enable droplet manipulation.



→ (days) → **Electrical measurements of the interdroplet bilayer**

Electrodes are attached to headstage and amplifier for bilayer current measurements.



Results

The interdroplet bilayers became unstable and fused after 8 minutes on average (n=8) when one droplet contained the undiluted cell-free mixture.

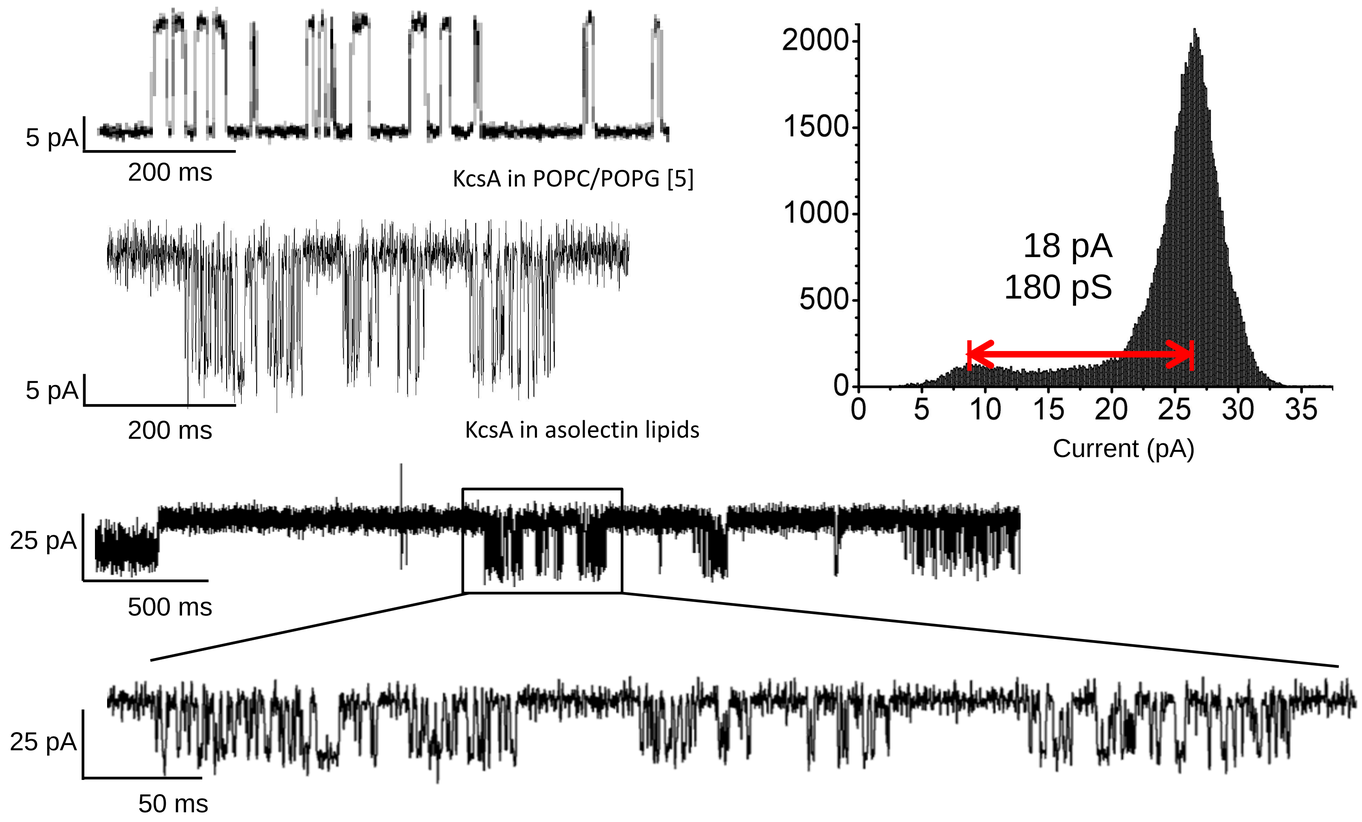
The average lifetime was improved to 21 minutes by diluting the mixture (n=6) and to >30 minutes by also adding lipid vesicles (n=3).

Channel activity was observed at pH 4, suggesting that the cell-free expressed KcsA becomes inserted into the interdroplet bilayer.

The mean conductance of the channel was 180 pS and the lifetime of the individual gating events is 2-20 ms in bursts of activity lasting 50-500 ms.

The mean conductance, inactivity-separated current bursts, and pH-induced gating are consistent with KcsA literature data, for example 16 pA current steps (conductance = 160 pS) which last from 20-40 ms in POPC/POPG [5].

To verify our current measurements we purified cell-free expressed His-tagged KcsA; a 18 kDa band (~KcsA monomer) was observed on SDS PAGE gels.



Conclusion

Confirming previous observations [6], we found that the cell-free mixture destabilises interdroplet bilayers, possibly because of the high protein content of the S30 lysate, but bilayer lifetime can be improved by the addition of liposomes to the cell-free mixture and by dilution of the mixture.

Significantly, our results show that we can express KcsA using a commercial cell-free system and can obtain electrophysiological data without any purification or reconstitution of the KcsA channel. Channel insertion into the interdroplet bilayer could be facilitated by KcsA association with liposomes in the cell-free mixture, as observed for MscL [7].

Cell-free expression of ion channels in microdroplets in combination with spontaneous bilayer insertion has the potential to substantially accelerate ion channel electrophysiology studies while avoiding the need for cell cultures and significant molecular biology infrastructure. Moreover, the microdroplet format is amenable to parallelization, for example for high-throughput drug screening applications.

References

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