FRET and TIRF microscopy for single molecule characterisation of synergistic antimicrobial peptides in artificial bilayers

Miss Hannah Baird[1], Dr William David Jamieson[1], Dr Oliver Castell[1]
[1]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom

Background incl. aims

Droplet Interface Bilayers (DIBs) function as artificial membrane models for electrical and optical measurements [1]. This project uses total internal reflection fluorescence (TIRF) microscopy and Förster Resonance Energy Transfer (FRET) in DIBs to investigate the dynamics of peptides and proteins interacting with membranes. Membrane pores have vital roles in neuronal and cardiac signalling, as well as in the mechanism of certain antibiotics.
The lytic antimicrobial peptides Magainin 2 (Mag2) and PGLa, part of the immune system of the African clawed frog (Xenopus laevis), have a synergistic antimicrobial effect when applied to Gram-negative bacteria in a 1:1 ratio. The aim of this project is to uncover the mechanism behind this synergy at the membrane level.

Methods

When an agarose surface and a droplet are submerged in a lipid/oil environment, a monolayer spontaneously self-assembles at the aqueous-oil interface. DIBs form when the lipid monolayers make contact. The protein or peptide of interest, contained within the droplet, can then form pores in this bilayer. Ions flux through these pores, and this pico-ampere current can be measured by electrophysiology. Simultaneously, TIRF microscopy is used to observe ion flux by illuminating the fluorescent calcium binding dye, Fluo-8, with totally internally reflected laser light.

Alternatively, the single-molecule dynamics of fluorescently tagged peptides on the membrane surface can be investigated. By tagging PGLa with a donor fluorophore and Mag2 with an acceptor fluorophore, single molecule FRET with TIRF microscopy can act as a so called "molecular ruler"[3]. When the donor fluorophore is being excited and is less than 10nm from the acceptor fluorophore, a non-radiative energy transfer can occur from the donor to the acceptor, causing the acceptor to fluoresce. This offers distance-dependent insights into the interaction and dynamics of Mag2-PGLa. The formation of heterodimers and higher order structures is of particular interest in these experiments.

Results

Spatiotemporal data from electrophysiology and TIRF experiments has revealed that the Mag2-PGLa pore is a highly dynamic pore that visits multiple conductance states. Single molecule FRET data of Mag2 and PGLa has been obtained for the first time, confirming the existence of Mag2-PGLa heterodimers. Data suggests that very few Mag2-PGLa heterodimers exist at any time. These experiments have allowed better understanding of the interaction of the peptides with one another and with the membrane, thus shining a light on the mechanism behind the Mag2-PGLa synergy.

Conclusion

DIBs, TIRF microscopy, and single-molecule FRET have been employed to investigate the dynamics of Mag2 and PGLa interacting with one another and the membrane. Experimental results reveal the highly dynamic nature of the Mag2-PGLa pores and confirm the existence of Mag2-PGLa heterodimers. These findings provide valuable insight into the molecular mechanism driving the Mag2-PGLa synergy. Future work will focus on achieving pseudo-simultaneous pore-imaging, electrophysiology, and FRET of Mag2 and PGLa.

https://doi.org/10.1051/bioconf/202412915010

Keywords:

TIRF FRET AMPs DIBs

Reference:

[1] S. Leptihn, O.K. Castell, B. Cronin, E. Lee, L.C.M. Gross, D.P. Marshall, J.R. Thompson, M. Holden, M.I. Wallace, Nat. Protoc. 8, 6 (2013).
[2] J. Zerweck, E. Strandberg, O. Kukharenko, J. Reichert, J. Bürck, P. Wadhwani, A.S. Ulrich, Sci. Rep. 7, 13153 (2017).
[3] S.J. Holden, S. Uphoff, J. Hohlbein, D. Yadin, L. Le Reste, O.J. Britton, A.N. Kapanidis, Biophys. J. 99, 9 (2010).