

Droplet-hydrogel lipid bilayers as an alternative model for studying mechanosensation

K.R. Rosholm,¹ M.A.B. Baker,² P. Ridone,¹ Y. Nakayama,¹ N. Bavi,¹ P.R. Rohde,¹ L.G. Cuello,¹ L.K. Lee² and B. Martinac,¹ ¹Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia and ²EMBL Australia Node for Single Molecule Science, UNSW, NSW 2052, Australia.

The droplet on hydrogel bilayer (DHB) is a novel platform for investigating the function of ion channels (Leptihn *et al.*, 2013). This setup allows tight control of all bilayer components and the option to perform parallel recordings of channel current and bilayer fluorescence. Here we present the reconstitution and activation of the prototypical MS channel MscL, the large conductance MS channel of *E. coli*, in DHBs. By selectively stretching the droplet monolayer with nanolitre injections of buffer we activated the channel and the tension was scaled with the injected volume (Figure), allowing us to control and quantify the DHB tension and relate it to the observed channel activity. Finally, we used MscL as tension sensor, revealing that the DHB equilibrates the induced tension over time, likely by lipid insertion into the droplet monolayer. Our study establishes a method to controllably activate MS channels in DHBs and enables the future measurement of protein conformational change *via* fluorescence while recording single channel activity of of MS channels.

