Membrane bilayer-mediated clustering and functional interaction of MscL, the mechanosensitive channels of large conductance from *E. coli*

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Mechanosensitive channels allow bacteria to respond to osmotic stress by opening a nanometer-sized pore in the cellular membrane. Most studies have focused on individual proteins and assumed no interactions with neighboring proteins. In this study we systematically observed the formation of MscL clusters over a wide range of protein/lipid ratios in lipid bilayers of varying lipid composition. We evaluated the spatial distribution of channels in a lipid bilayer using patch-clamp electrophysiology, fluorescence and atomic force microscopy, and neutron scattering and reflection techniques, coupled with mathematical modelling of the mechanics of a membrane crowded with proteins. MscL is closely packed within each cluster but is still active and mechanosensitive. However, the channel activity is modulated by the presence of neighbouring proteins, indicating membrane-mediated protein-protein interactions. The underlying principles of self-assembly may also play a role in the *in vivo* behaviour of MscL since native expression levels of MscL increase from approximately five copies per bacterial cell in various stages of cell growth. Furthermore, fluorescence images from *Escherichia coli* expressing MscL-GFP fusion proteins suggest that the channel distribution is non-uniform Collectively, these results suggest that MscL self-assembly into channel clusters plays an osmoregulatory functional role in the bacterial cell membrane.