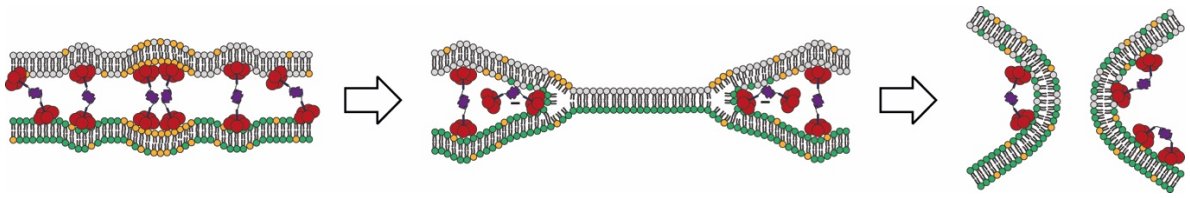
Mechanisms of membrane fusion induced by re-engineered bacterial toxins

W. Bruce TURNBULL [1], Thomas R. BRANSON [1], Wenyue DAI [1], George HEATH [1], Erik KEMPMANN [2], Josef MADL [2], Maria NIKOLOVA [1], Ralf P. RICHTER [1], Winfried RÖMER [2], James F. ROSS [1], Lina SIUKSTAITE [2], Taras SYCH [2],[3], Maren THOMSEN [1], Michael E. WEBB [1], Sarah WEHRUM [2], Gemma C. WILDSMITH [1], Daniel J. WILLIAMSON [1]

[1] University of Leeds, UK, [2] Albert-Ludwigs-University of Freiburg, Germany, [3] Karolinska Institutet, Solna, Sweden

w.b.turnbull@leeds.ac.uk

The cholera toxin B-subunit (CTB) is a pentameric lectin that binds to ganglioside GM1 in lipid bilayers, as a first step to achieve endocytic internalization of its associated toxic A-subunit. The multivalent nature of this interaction enhances the affinity of the toxin protein, but also causes the glycolipids to cluster and induce membrane curvature [1]. This process can be recreated in simple model systems such as Giant Unilamellar Vesicles (GUVs), which shows that CTB is both a sugar-binding and membrane bending protein. However, as CTB has all of its carbohydrate-binding sites on the same face of the pentamer, it does not naturally crosslink membranes unlike other lectins, e.g., LecA which has carbohydrate binding sites that face in opposing directions [2]. Recent we have shown that multimeric assemblies of streptavidin and a site-specifically biotinylated AB_5 complex of CTB can mediate GUV crosslinking and membrane fusion processes [3]. Further investigations of the mechanism of these unprecedented processes will also be presented, including the use of streptavidin mutants with defined valencies to identify the active species in the original study, and the use of parallel and antiparallel coiled-coil motifs to further control the arrangements of the CTB pentamers and their interactions with model membranes. Our results show that protein engineering strategies for controlling the architecture of multivalent lectins can allow repurposing of lectins as nano-scale building blocks and molecular machines for synthetic glycobiology.



Multimeric CTB assemblies crosslink and fuse membranes via a hemifusion diaphragm. Figure adapted from [3] under a Creative Commons CC-BY4.0 licence.

This research has received funding from the European Union's Horizon 2020 research and innovation programme under th Marie Skłodowska-Curie grant agreement number 814029 (synBIOcarb ITN).

Bibliographic references:

[1] H. Ewers, W. Römer, et al. (2009) Nat. Cell Biol. (12) 11-18.
[2] S. Villringer, J. Madl, T. Sych, C. Manner, A. Imberty, W. Römer (2018) Sci. Rep. (8) 1932-1932.
[3] S. Wehrum L. Siukstaite, D. J. Williamson, T. R. Branson, T. Sych, J. Madl, G. W. Wildsmith, W. Dai, E. Kempmann, J. F. Ross, M. Thomsen, M. E. Webb, W. Römer, W. B. Turnbull (2022) ACS Syn. Biol. (11) 3929-3938.