Proceedings Paper:
Booth, A, Marklew, C, Ciani, B et al. (1 more author) (2017) Developing ESCRT-III as a toolkit for bottom-up construction of eukaryote-like artificial cells. In: European Biophysics Journal. 19th IUPAB Congress and 11th EBSA Congress, 16-20 Jul 2017, Edinburgh. Springer Nature , S46-S46.

https://doi.org/10.1007/s00249-017-1222-x

Developing ESCRT-III as a toolkit for bottomup construction of eukaryote-like artificial cells

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The Endosomal Complex Required for Transport (ESCRT) is a ubiquitous class of proteins involved in most membrane remodeling processes in eukaryotic cells. Previous studies have demonstrated that ESCRTs can be reconstituted in artificial lipid vesicle systems, notably giant unilamellar vesicles (GUVs), where they are able to induce the formation of intraluminal vesicles (ILVs), encapsulating material from the extravesicular solution.[1] Our aim is to develop the ESCRT proteins as a toolkit to generate GUV-derived structures, with multiple compartments containing different chemical environments, enzymes or molecular probes. The resulting cell-like structures may find applications in synthetic biology, drug delivery, diagnostics and the development of nano-reactor technology.[2] Using confocal microscopy and flow cytometry, we have gained insights into how protein stoichiometry and membrane mechanics influence the size and number of the ILV compartments obtained. We will further develop the ESCRT toolkit using the ATP-ase Vps4 to induce multiple encapsulation events and introduce rationally designed ESCRT chimera proteins to further simplify the system.

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