Reconstitution of clathrin/ AP-2-mediated endocytosis on membrane sheets to study effects of multivalent inhibitors

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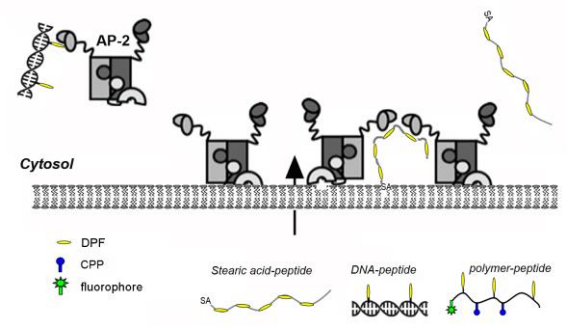

Abstract:

Clathrin-mediated endocytosis (CME), an important cellular mechanism for the uptake of nutrients or growth factors, and for cell entry of pathogens, is based on multivalent protein networks centered around clathrin and the heterotetrameric AP-2 complex. Within the current funding period peptide-based tools have been developed in projects B01 (DNA-peptide conjugates) and B05 (peptide-lipid conjugates) that target key interaction sites within the ear domains of AP-2. The proposed project builds on the recent development of plasma membrane sheets from a variety of cell types that allow access to the cytoplasmic face of the membrane. Addition of nucleotides and cytosol containing labeled endocytic proteins (i.e. fluorophore-labeled clathrin, AP-2, or dynamin, recombinant eGFP-tagged BAR domain proteins etc.) results in endocytic vesicle formation (Wu et al., *Nature Cell Biol.* 2010), a process that can be studied by live cell confocal and super-resolution microscopy. The establishment of this assay will allow us to assess the effects of multivalent peptide conjugates (made in B01 and B05) harboring binding sites for AP-2 or ear domains at different spacings or with spacers of different rigidity with respect to endocytic protein recruitment and the formation of endocytic intermediates or clathrin-coated vesicles.

Publication/s:

Stahlschmidt W, Robertson MJ, Robinson PJ, McCluskey A, Haucke V. (2014): J Biol Chem. 2014 Jan 9. [Epub ahead of print]

von Kleist L, Stahlschmidt W, et al (2011): Cell 146:471-84

Wu M, Huang B, Graham M, Raimondi A, Heuser JE, Zhuang X, De Camilli P. (2010): Nat Cell Biol. 12:902-8