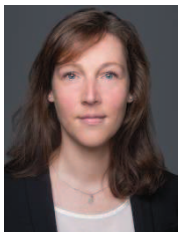
Grafting PNIPAAm from β-barrel shaped transmembrane nanopores

Charan H., Kinzel J., Glebe U., Anand D., Mirzaei Garakani T., Zhu L., Bocola M., Schwaneberg U., Böker A. (2016). Grafting PNIPAAm from β-barrel shaped transmembrane nanopores, *Biomaterials* 107, 115-123.

Development of functional building blocks by polymer chain growth from the surface of transmembrane protein FhuA for assembly in membranes



Combining unique properties of transmembrane proteins e.g. generating membrane potential gradients or translocation of compounds with specific functions of polymers like temperature and pH stimuli formed conjugates enable new applications. For generation of hybrid protein-polymer membranes assembly of conjugates is a well-known strategy. The two main methods are 1) grafting polymer chains to protein surfaces and 2) grafting polymer chains from protein surfaces. Using globular proteins is well established but investigation of membrane proteins for conjugate formation was not performed before. We established conjugate formations of a transmembrane protein for the first time using the β-barrel protein ferric hydroxamate uptake protein component A (FhuA), an outer membrane protein of *Escherichia coli*. FhuA was reengineered to redistribute lysines from positions over the whole protein to well-defined positions symmetrically in a rim above the hydrophobic region. So up to 11 lysines are only orientated towards outside of the protein channel ensuring a growth of polymer chains exclusively outside of the transmembrane protein and prevent a channel blocking upon polymerization. Prior polymerizing N-isopropylacrylamide (NIPAAm) from FhuA, a water-soluble initiator for controlled radical polymerization (CRP) was covalently attached to lysines (**Fig. 1**). Characterization of conjugates was performed by circular dichroism spectroscopy, gradient SDS-PAGE, MALDI-ToF mass spectrometry, dynamic light scattering, transmission electron microscopy, size exclusion chromatography and analytical ultracentrifugation (**Fig. 2**). Membrane synthesis of functional nanosized building blocks of FhuA-PNIPAAm is currently investigated. Functionalization of hybrid membranes for chiral separations is envisioned with applications in productions of sweetener, insecticides and pharmaceuticals.

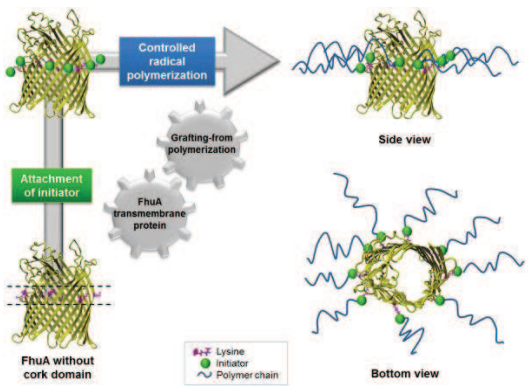


Fig. 1. Overview figure of conjugate formation based on re-engineered transmembrane protein FhuA with up to 11 lysines (magenta) via attaching a water-soluble CRP initiator (green).

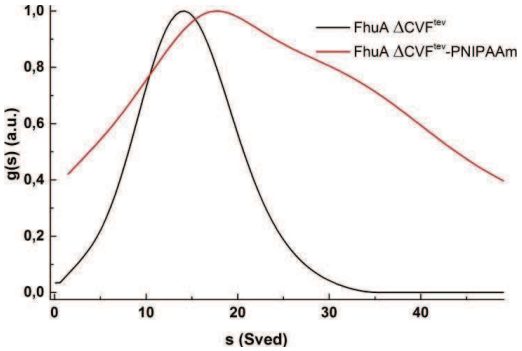


Fig. 2. Sedimentation coefficients determined by analytical ultracentrifugation. Increased coefficient and broader distribution lead to the conclusion of an increase of molecular weight due to attached polymer chains (red) in comparison to unmodified FhuA (black).