Executive Summary

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Project "Functional surfaces through immobilization of nanoreactors and biomimetic block copolymer membranes"

The major goal of this project was to develop and optimize functional surfaces for biomaterials and biosensors by the immobilization of protein-polymer based nanoreactors and protein-decorated copolymer membranes. In this respect, we developed novel types of smart surfaces with two distinct approaches: (i) immobilization of functional nanoreactors on solid supports, and (ii) preparation of solid-supported block copolymer membranes in which proteins are inserted. To achieve the goals of the project various milestones were planned and achieved successfully:

i) In order to introduce permeability into impermeable polymer membranes an elegant approach is to insert channel membrane proteins to act as pores. By genetic engineering, a membrane protein (OmpF) was modified with a polyhistidine tag, which can bind antibodies, and thus serve to change the channel permeability by steric interactions. The functionality of the genetically modified porins was proven by their reconstitution in the membrane of vesicular assemblies, which serve as compartments for the design of nanoreactors. Such His-tagged protein could also be a hub for other enzymes to generate a smart surface, e.g. for biosensors.

ii) In order to obtain active surfaces able to produce antibiotics with required time and space precision, nanoreactors were immobilised on a solid support. Nanoreactors that produce antibiotics were prepared by reconstitution of wild-type OmpF in polymer vesicles (polymersomes), and encapsulation of an enzyme, penicillin acylase, in their cavities. A simple and efficient method suitable for medical and pharmaceutical applications was developed to immobilise the nanoreactors on surfaces. The attachment approach led to covalent immobilization, preserved the vesicular structure of the nanoreactors, and did not influence the enzymatic activity of the encapsulated catalyst. Such functional surfaces efficiently synthesized antibiotics, and induced a strong inhibitory effect against E. coli bacterial growth for long periods of time (up to 7 days) under physiological conditions. The covalent immobilization approach can be applied for different, functional nanoreactor surfaces, including those of implants. Such nanoreactors serve to produce and release antibiotics "on demand", and represent a new example of how nanoscience can contribute in a responsive manner to applications in the medical domain. The system with the best functionality and stability will now be considered for scaling-up and technology transfer. In this respect we participate in a COST Action in which the main goal is the development of new nanoscience-approaches against infections resulting from implant therapy.

iii) Biomimetic block copolymer membranes immobilized on planar solid supports were prepared and decorated with membrane proteins for biosensing applications. One approach was to use the grafting-from approach, which offers a great structural variety in selecting polymers, polymer topology and composition. A two-step synthesis was developed in our lab to synthesize the initiator, which can be anchored to a solid surface and allows an extended graft-from polymerization. The first immobilized amphiphilic polymers were prepared by the surface-initiated atom transfer radical polymerization (ATRP) approach, which is a homogenous and reproducible synthesis method, resulting in controllable density and chain length of the copolymer. To optimize the membrane conformation, and mobility (fluidity) for lateral protein reconstitution, the individual polymer block lengths, the hydrophilic/hydrophobic ratio, and the grafting density were fine-tuned by adjusting: (i) the reaction time, (ii) the

quantity of monomer, (iii) the quantity or nature of the catalyst and (iv) the surface coverage with initiator. Protein insertion onto solid-supported block copolymer membranes was achieved with two approaches: application of electrical field and the use of Bio-Beads. They allowed successful insertion of alpha-haemolysin (aHL) insertion and the water insoluble membrane protein MloK. These methods are in no way limiting, and intuitively lead to broader applications by substituting one membrane protein with another.

iv) In order to select the most appropriate system for further applications, we compared the two active surfaces - immobilised nanoreactors and enzymes inserted in the solid supported block copolymer membranes - with respect to: (i) functionality, (ii) robustness and (iii) sensitivity. We investigated the “active surfaces” designed by immobilisation of polymersomes with inserted OmpF and “active surfaces” obtained by insertion of OmpF in solid-supported polymer membranes: both showed the function of the channel membrane protein for substrate molecules or ion transport and have long term stability.