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Functional surface engineering by nucleotide-modulated potassium channel insertion into polymer membranes attached to solid supports

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ABSTRACT

Planar solid-supported membranes based on amphiphilic block copolymers represent promising systems for the artificial creation of structural surfaces. Here we introduce a method for engineering functional planar solid-supported membranes through insertion of active biomolecules. We show that membranes based on poly(dimethylsiloxane)-*block*-poly(2-methyl-2-oxazoline) (PDMS-*b*-PMOXA) amphiphilic diblock copolymers, which mimic natural membranes, are suitable for hosting biomolecules. Our strategy allows preparation of large-area, well-ordered polymer bilayers *via* Langmuir–Blodgett and Langmuir–Schaefer transfers, and insertion of biomolecules by using Bio-Beads. We demonstrate that a model membrane protein, the potassium channel from the bacterium *Mesorhizobium loti*, remains functional after insertion into the planar solid-supported polymer membrane. This approach can be easily extended to generate a platform of functional solid-supported membranes by insertion of different hydrophobic biomolecules, and employing different types of solid substrates for desired applications.

1. Introduction

Biological membranes are complex structures, consisting of phospholipids, proteins, and oligosaccharides, where a variety of processes, such as active and passive transport across the membrane or molecular recognition interactions, occur simultaneously. Because of its complexity, it has not yet been possible to reconstruct a cell membrane, and simplified membrane models, mainly based on phospholipids, have been developed to investigate selected processes that take place in membranes. Phospholipids have been preferred because they are components of the natural membranes and can be used for *in vivo* applications [1]. However, as they are not sufficiently stable for long-term experiments, other models based on amphiphilic block copolymers have been introduced [2]. These are an improved alternative to phospholipids, because they self-assemble in specific aqueous conditions, and have increased mechanical stability [3]. In addition, their structure and properties can be designed through copolymer chemical engineering [4].

Vesicles, which find applications, e.g. in drug delivery [5], or as nanoreactors [6], are frequently studied membrane models. Because of their hollow structure, the bilayer is hydrated on both sides, which make them similar to natural membranes. Appropriate insertion of membrane proteins such as Complex I in a desired position has allowed the development of nanodevices to conduct electron transfer from the environment to a specific location inside the membrane [7]. Additionally, successful insertion of channel proteins in the membrane of polymer vesicles loaded with catalysts (enzymes, proteins, mimics) has allowed the exchange of molecules with the environment, and supported *in situ* functionality of these nanoreactors.

Another membrane model is a freestanding membrane, which has been used for investigations of protein insertion mechanisms [8]. Such planar membranes are accessible from both sides, and act as perfect insulators; hence insertion of a protein can be detected by a change in electrical properties of the system [9]. While there are examples of freestanding polymer membranes, which have successfully hosted membrane proteins, the disadvantages of this system, i.e. a low stability and difficult to handle, make it of low technological interest.

Planar solid-supported membranes represent a step further as membrane models in terms of increased stability and preserved fluidity [10]. Therefore, they are more appropriate for insertion of membrane proteins and the study of protein functions or biomolecule–surface interactions. These membranes can be used for

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investigating multivalent ligand-receptor binding, biomimetic sensing, or drug screening [11,12]. Lipid solid-supported membranes have been broadly investigated, and numerous examples of adsorption of biomolecules into these membranes have been reported, e.g. incorporation of ATPase [13], cytochrome c oxidase [14], α-hemolysin (α-He) [15], or outer membrane proteins (OmpF and OmpA) [16]. However polymer membranes are more appropriate for applications, because they surpass lipid membranes in the respect of stability, i.e. their structures can persist for a few hours even after drying [17]. Lipids form rigid monolayers in a liquid condensed state (Fig. S1, Supplementary Information) while the polymer films are in a liquid expanded state at the air–water interface [18,19]. This indicates that an appropriate selection of the polymer blocks leads to a resulting membrane more flexible than lipid membranes and thus better suited for biomolecule insertion, as previously reported by insertion of Complex I in the polymer membrane of vesicles [7].

The drawback of solid-supported membranes is the risk of protein denaturation, as the result of direct contact between transmembrane protein and substrate, but this problem can be overcome by separating the solid substrate from the membrane with a spacer [20].

Dorn et al. were the first to investigate interactions between a planar solid-supported polymer membrane, formed by poly(-butadiene)-*block*-poly(ethylene oxide) (PB-*b*-PEO) block copolymer, and a polypeptide polymyxin B [21]. However the peptide was adsorbed into the polymer membrane only temporarily, and slowly diffused back into the solution. A further step was realized by incorporating water soluble α-He into solid-supported PB-*b*-PEO membrane when the membrane was destabilized by an electrical current [22]. This method allowed a permanent and functional insertion of the protein, as proved by a flow of ions through the membrane until Donnan equilibrium was reached.

However, except for this example of α-He insertion supported by membrane destabilization through application of electric current, there have been no other methods reported for insertion of membrane proteins in solid-supported polymer membranes. This is explained by the complex scenario and requirements that are necessary for functional insertion of a membrane protein: i. a homogeneous and stable membrane, ii. a membrane with sufficient fluidity to host a protein, and iii. the presence of a spacer between the substrate and membrane (e.g. polymer layer or a water reservoir) to prevent protein denaturation, which can occur as the result of interactions with the hard support.

Here we present a new approach for incorporating membrane proteins into large area, solid-supported polymer membranes without using an electrical current to destabilize the membrane (Fig. 1). In this approach, well-organized polymer bilayers have been prepared by film transfer techniques. We selected poly(-dimethylsiloxane)-*block*-poly(2-methyl-2-oxazoline) (PDMS-*b*-PMOXA) diblock copolymer as a good candidate for forming an artificial membrane, as its hydrophobic PDMS block provides the necessary flexibility for the membrane [23], while the water soluble PMOXA block acts as a buffer to prevent protein interactions with the solid substrate. In order to characterize the polymer membrane, a combination of surface analysis techniques were used, i.e. atomic force microscopy (AFM), ellipsometry, contact angle measurements, and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). To perform a functional insertion of the membrane protein into the polymer membrane, both the protein and polymer membrane have to be destabilized. For this purpose, we used Bio-Beads which are non-polar, polystyrene beads capable of adsorbing organic materials from aqueous solutions, and thus providing a reproducible and simple way to obtain polymer vesicles [7] and liposomes with inserted proteins [24]. However Bio-Beads have never been used previously for protein incorporation into planar solid-supported polymer membranes.

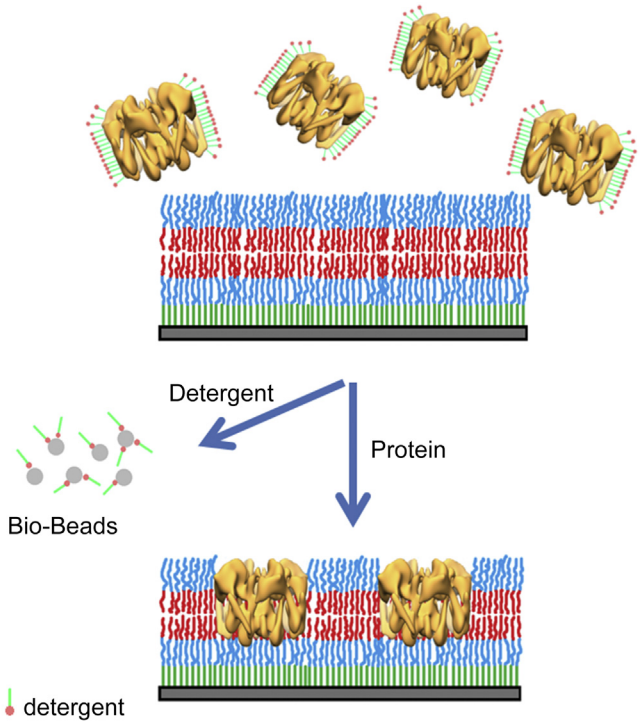

Fig. 1. Schematic representation of membrane protein insertion into solid-supported polymer membrane with usage of Bio-Beads.

The critical point of the whole procedure is the preparation of defect-free homogeneous membranes, because each defect influences the conductance measurement. It is also of high importance that protein destabilization is gentle, because too rapid detergent removal can induce protein precipitation. Our approach is an easy and straightforward way to engineer functional surfaces by protein insertion into solid-supported polymer membranes. Besides its simplicity, the advantage of this method is the possibility of employing different solid substrates of unrestricted sizes, which represents a key point for development of large functional surfaces for technological, medical or environmental applications. In addition, this approach can be easily extended in terms of functionality by insertion of other hydrophobic proteins and biomolecules.

2. Materials and methods

2.1. Polymer synthesis and characterization

We used PDMS-*b*-PMOXA diblock copolymers with an aldehyde end-group. The polymer with a molar mass of 5735 g mol^{-1} was composed of 65 PDMS units and 12 PMOXA units. It was synthesized according to the procedure described by Egli et al. [25]. The molecular mass of the polymer and length of the blocks were calculated from the ^{1}H NMR spectra. GPC data showed PDI of the PDMS-*b*-PMOXA diblock copolymer to be 1.67.

Oxidation of the hydroxyl end-group was performed using Dess-Martin periodinane (DMP, Aldrich) [26]. PDMS-*b*-PMOXA-OH (200 mg) and DMP (17 mg, 40 μmol) were added to a two-neck round bottom flask, closed, and degassed. Then anhydrous dichloromethane (10 ml) was introduced under a stream of argon, and the reaction mixture was stirred for 24 h at room temperature. The modified polymer was purified by dialysis (Spectrapor® with MWCO 3500 Da) in ethanol for 18 h.

2.2. MloK1 expression and purification

Full-length, cyclic nucleotide-modulated potassium channel MloK1 was expressed and purified to homogeneity as described in Ref. [27]. Briefly, *Escherichia coli* cells containing His-tagged MloK1 construct were grown in LB medium at 37 °C.

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