Functional Surfaces

Bio-Hybrid Membranes for Biosensing

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Combining natural enzymes with synthetic membranes on solid support enables creation of functional surfaces able to serve for efficient biosensing. Enzymes (laccase and tyrosinase) integrated on soft copolymer mono- and bilayer membranes preserve their activity and specifically detect the presence of phenols. The straightforward approach to create these bio-hybrid membranes allows changing the enzyme type and thus producing functional surfaces for sensitive detection of desired molecules.

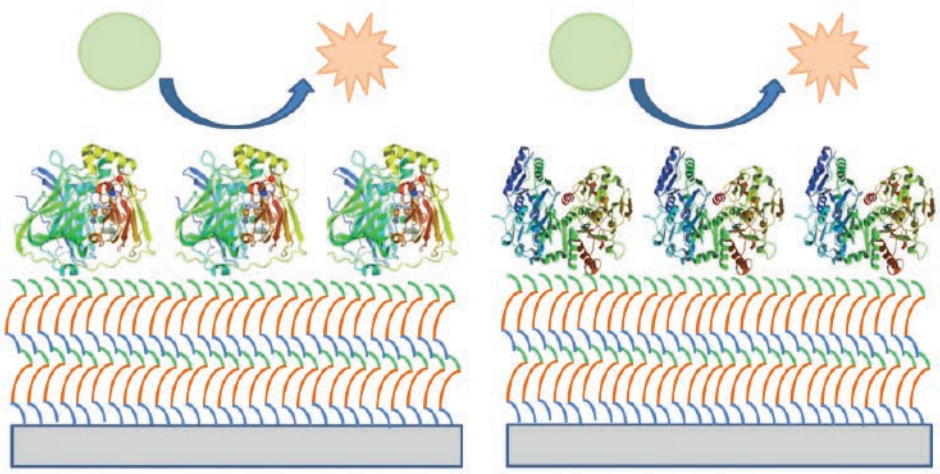

Figure 1. Functional surfaces for phenol biosensing based on attachment of specific enzymes (*a. laccase* and *b. tyrosinase*) on solid supported synthetic membranes. Reproduced by permission of The American Chemical Society [3].

Introduction

Combinations of biomolecules and synthetic assemblies to generate bio-hybrid materials emerge in nanoscience as novel solutions for various domains, such as medicine, catalysis, technology. They benefit from the activity and functionality of the biomolecules (enzymes, proteins, DNA, mimics), while the assemblies represent stable templates or compartments [1]. We created functional surfaces by combining enzymes (laccase and tyrosinase) with soft polymer membranes attached on solid support. These functional surfaces employ simple enzymatic reactions and aim sensitive detection of phenols (green spots in Fig. 1) as proof of concept, because phenols are known as harmful pollutants of drinking and natural waters. The enzymes are attached to synthetic membranes composed of amphiphilic asymmetric block copolymers that are immobilized on solid support in order to form functional surfaces (Fig. 1). Triblock copolymers of poly(ethyleneglycol)-*block*-poly(γ-methyl-ε-caprolactone)-*block*-poly [(2-dimethylamino) ethylmethacrylate], named ABC copolymers, with different hydrophilic and hydrophobic domains are selected to generate solid supported membranes [2]. The advantage of our synthetic membranes is the possibility to use all the options offered by modern polymer chemistry to fine-tune and control the different molecular properties of the copolymers and resulting membranes. Notably, the ability of these amphiphilic copolymers to form uniform and stable films at the air-water interface serves to generate homogeneous monolayers and bilayers upon transfer onto silica solid supports by using the Langmuir–Blodgett (LB) technique. The most promising membranes in terms of their properties (film thickness, wettability, topography and roughness) are used for enzyme attachment and production of functional surfaces. After successful attachment of the enzymes on copolymer membranes by stable noncovalent interactions, both enzymes preserve their activity. As the properties of the synthetic membranes affect the enzymatic activity, they serve to distinguish the most promising bio-hybrid membranes in terms of overall functionality as biosensors for phenols: bilayer films provide the best conditions in terms of overall stability and enzymatic activity [3].

Formation of Membranes

ABC triblock copolymers with poly(ethyleneglycol) as A block, poly(γ-methyl-ε-caprolactone) as B block and poly[(2-dimethylamino) ethylmethacrylate] as C block (PEG_{45}-$PMCL_X$-$PDMAEMA_Y$) were used to self-assemble and form solid-supported polymer membranes. The behavior at the air-water interface of these ABC polymer films was investigated by using Langmuir isotherms (surface pressure-area) and Brewster angle microscopy (BAM). At the optimal surface pressure, ABC films were transferred from the air-water interface to silica plates, resulting in densely packed copolymer monolayers [2]. As differences in the architecture of the copolymer membranes affect the enzymes immobilization and accessibility on the membrane surface, we first evaluated their properties (thickness, hydrophilic/hydrophobic balance, topography and roughness). As A_{45}-B_{101}-C_{27} block copolymer formed the best membranes, it was selected for further combination with the enzymes in order to obtain functional surfaces.

To favor enzyme attachment, copolymer films resulting from different transfer directions (up lifting, arrow up, or down deeping, arrow down, as indicated in Fig. 2A), and sequences (single or two transfers) were prepared [3]. We introduced a new terminology for the polymer membranes formed by Langmuir–Blodgett transfers of ABC block copolymers: the resulting polymer films based on single transfers were labeled as “up” (arrow up) and “down” (arrow down), respectively, while the combination of two transfers were labeled accordingly “down-down”, “up-down”, “down-up” or “up-up”. This terminology is more appropriate to describe the membranes preparation procedure by using amphiphilic block copolymers, compared to the z- and y-type of membranes obtained

from lipid films. The polymer films generated by these transfer directions and sequences were evaluated in terms of thickness, wettability, topography and roughness. Only the "up-up" transfers formed bilayers films (Fig. 2C), while the "up", "up-down" and "down-up" transfers resulted in formation of monolayer films (Fig. 2B).

Enzyme Attachment

The ability of the enzymes to adsorb on the most promising solid supported polymer membranes (monolayer, up-down monolayer and bilayer) was studied by quartz crystal microbalance with dissipation (QCM-D) method and bicinchoninic acid assay (BCA). The enzymes adsorbed on polymer membranes remained stable for at least 18 h at room temperature, according to the QCM-D experiments. Less amounts of each enzyme were absorbed onto the monolayers than onto the bilayer films. Higher amount of both enzymes adsorbed on bilayers is favoured by a higher roughness of these synthetic membranes, with larger surface area available for the enzyme adsorption. Combination of QCM-D and BCA indicated that both enzymes adsorbed on the polymer films in different degrees, depending on the intrinsic architecture of the synthetic membranes. Stable, non-covalent attachment of the enzymes to form bio-hybrid membranes on solid support represents a prerequisite for development of biosensors. Enzymes adsorption,

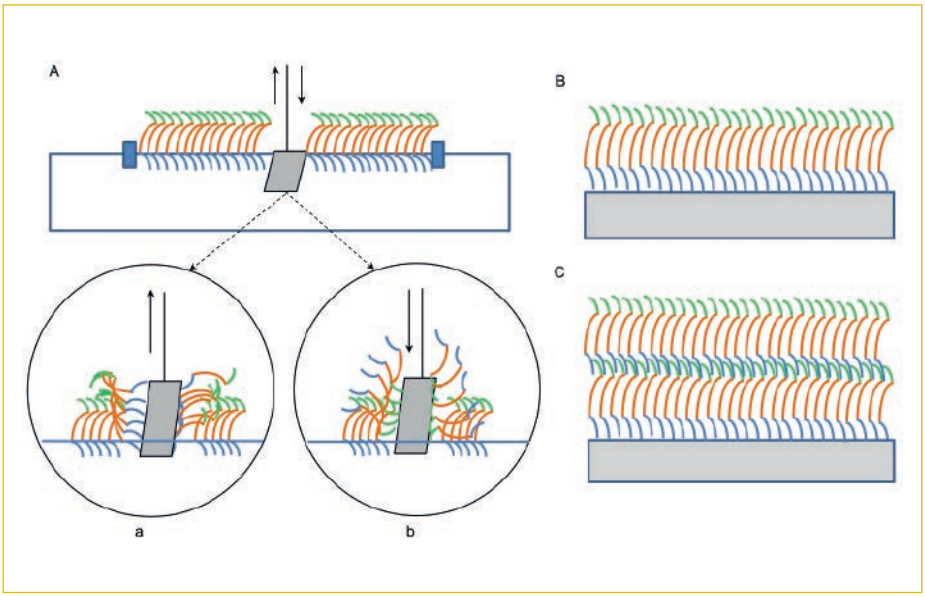

Figure 2. Langmuir–Blodgett transfers to generate the polymer films. A) Polymer transfer onto a hydrophilic solid support (a – up transfer, up lifting; b – down transfer, down dipping), B) Schematic representation of a monolayer film formed by LB transfers and C) Schematic representation of a bilayer film formed by LB transfers.

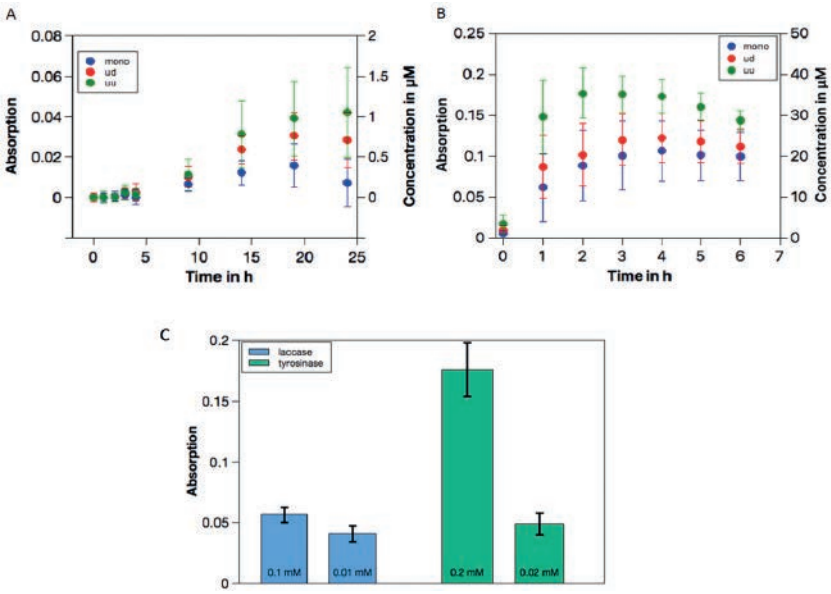

Figure 3. Activity of enzymes immobilized on the A_{45}-B_{101}-C_{27} copolymer films as determined by oxidative product formation: A) laccase with DMP as substrate and B) tyrosinase with 4-MP as substrate. Activity of laccase and tyrosinase attached on the "up-up" bilayer for two different concentrations of the enzyme substrates C): 0.1 mM and 0.01 mM DMP, and 0.2 mM and 0.02 mM 4-MP, respectively. Reproduced by permission of The American Chemical Society [3].

incubation and desorption served to obtain the conditions for production of efficient functional surfaces to sense and convert phenols [3].

Activity of the Attached Enzymes

The activity of biomolecules upon combination with synthetic membranes represents a crucial step in development of functional membranes [4-6]. We studied the activities of the attached enzymes on polymer monolayer and bilayer membranes and compared them with the activity of the free enzymes by using as substrates dimethoxyphenol (DMP) for laccase and 4-methoxyphenol (4-MP) tyrosinase. Both enzymes preserved their activity upon attachment on synthetic membranes with respect to their substrates. The enzymatic activity was tested for different time periods: for laccase 24 h (with maximum activity at 19-24 h) and for tyrosinase 6 h (with maximum activity at 2-3 h). The enzymes activity was different depending on the type of polymer membrane: for monolayer membranes it increased from that corresponding to enzyme attachment on "monolayer up" to that corresponding to enzyme attachment on "monolayer up-down". The highest enzyme activity was obtained when the enzymes were attached on bilayer membranes (Fig. 3A, B).

Both enzymes immobilized on bilayers were tested in terms of their ability to contribute to phenols autooxidation at concentrations 10-fold lower (Fig. 3C). Successful phenols autooxidation demonstrates that lower concentrations of phenol derivatives can be efficiently detected by our functional surfaces. Together, the stability of the solid supported copolymer membranes and the preserved bioactivity of the enzymes indicate that these functional surfaces have high potential upon optimization, for efficient and sensitive phenols biosensing.

Conclusions

In this study we highlighted the potential of functional surfaces produced by taking advantage on the interaction between soft amphiphilic asymmetric copolymer membranes and enzymes (laccase and tyrosinase) to efficiently sense phenols. Triblock copolymer films were prepared by Langmuir–Blodgett transfer technique, resulting in stable, solid-supported membranes for the enzymes absorption. Mono- and bilayers with different properties (film thickness, wettability and roughness) were investigated and the three most promising polymer films (the monolayer, the up-down monolayer and the bilayer) were selected for development of functional surfaces. Both laccase and tyrosinase preserved their enzymatic activity upon adsorption on these polymer films, the best candidate in terms of the overall bio-activity being the bilayer films. Our approach to generate functional surfaces is straightforward and therefore allows extending the sensing of other desired molecules by a simple change of the enzyme types. In the future, these functional surfaces will not only allow to sense one type of molecules (e.g. phenols), but also one day may offer an option for complex detection by using multiple biomolecules simultaneously present in the bio-hybrid membranes.

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