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Synthetic and bio-hybrid nanoscale layers with tailored surface functionalities

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Abstract

We examine the prospective routes for the design of synthetic/biomacromolecular/inorganic film assemblies for photothermal cell based on biomimetic approach. We demonstrate examples of channel proteins immobilized onto surfaces of silicon single crystals modified with Langmuir–Blodgett and self-assembled monolayers. These proteins can be immobilized in intact, closed-pore conformation. Their state within photosensitive monolayers can be controlled by the photoisomerization reaction triggered by UV light.

Keywords: Photosensitive monolayers; Nanoscale layer; Protein adsorption; Membrane channel proteins

1. Introduction

Biomimetics allows researchers to tap into the ingenious designs of nature to inspire improvements on current sensing technology. Often, a unique biomaterial microstructure and specific properties are targeted in such an approach. Recent developments in the advanced materials sciences have given unparalleled access to biological systems at all levels of structural organization, including nearly in vivo conditions. This has provided an opportunity to uncover the rules governing in situ functioning of biomolecular structures and the routes to designs of microsensors based on these rules. Recent studies have extensively discussed mechanisms of heat detection in the physiological response of such biological species as snakes and beetles [1–6]. In both species, IR-sensitive elements are represented by a compliant thin membrane film deflecting in respond to a change in heat flux. The structure of these biological IR receptors is remarkable for their ability to monitor thermal flux with high sensitivity. Suspended membrane structure recalls the design of a photothermal Golay cell introduced many years ago. The working principle of this cell is based on the detection of sub-micrometer-scale deviations of a flexible metal membrane caused by gas expanding in a sealed rigid cell (Fig. 1) [7,8], limits dramatically thermal sensitivity of the Golay cell and its spatial resolution. In a micromachined version of the Golay cell proposed very recently, a sensitive element is composed of a solid silicon membrane of a micrometer thickness that improves sensitivity and resolution to some extent [9].

Considering available findings on biological receptors, several additional steps could be suggested to increase the thermal sensitivity of existing thermal sensors. The design of the sensing elements from compliant membrane should provide sensibility to the structure far greater than the rigid membrane in current Golay cell design (Fig. 2). A further improvement may be seen in the multilayered structure of these membranes unlike a silicon membrane of the Golay cell. As previously noticed in biological IR receptors, not only does this structure provide a considerably faster response, but it also allows a much higher probability of detection of local temperature fluctuations. Free-suspended compliant membranes can be more sensitive under stress conditions, and thus are susceptible to consistent external disturbances.

A compliant layer with imbedded ion channel proteins can be designed as a compliant stress-sensitive element (Fig. 3). Indeed, on a molecular level, calcium ion channels act as ion current modulators in response to change in membrane tension. Similar ion channels are ubiquitous in thermal and mechanical sensors in different live species from bacteria to humans.

2. Results and discussion

A critical step in designing of prospective sensors based on silicon micromachined devices and sensitive biomacromolecule containing layers is immobilization of very compliant membrane proteins on a solid surface with

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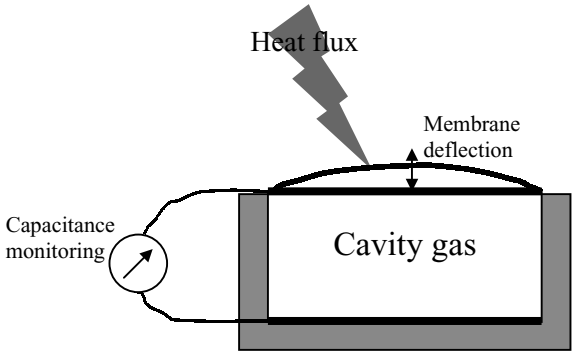

Fig. 1. Schematics of the photothermal Golay cell based on inorganic material and operational principles discussed in Refs. [8,9].

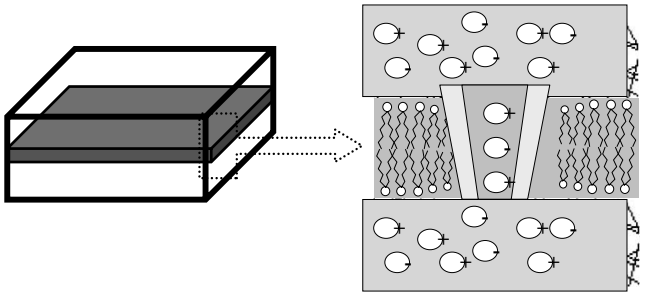

Fig. 3. The compliant interlayer with protein/lipid/hydrogel complex.

preservation of their conformation [10]. Here, we discuss our recent studies on immobilization of channel protein on solid surfaces of silicon single crystals, which are widely used in manufacturing of microsensors. The fabrication and characterization procedures are discussed elsewhere (e.g., see [11]).

Briefly, the solid substrates were freshly cleaned atomically smooth silicon wafers (Semiconductor Processing Co.). Silicon wafers were treated for 10 min in an ultrasonic bath at room temperature and cleaned with a "piranha solution" (30% concentrated hydrogen peroxide, 70% concentrated sulfuric acid) for 1 h. The substrates were rinsed with Nanopure water and dried with dry nitrogen. Adsorbed monolayers of the protein were prepared by placing a 20 μl droplet of a protein solution on a bare or a modified silicon wafer. Substrates were rinsed by water to remove excess protein after 1 min exposure. The Langmuir–Blodgett (LB) lipid monolayers at the air–water interface were compressed to a condensed state (30 mN/m) and deposited onto the silicon substrate on an automated LB system (Riegel and Kirstein, GmbH) located within a laminar clean air hood. In our research, we have studied α-toxin from *Staphylococcus aureus* as a model ion channel. Staphylococcal α-hemolysin forms a large central pore in the open state, which can be used for the modulation of the ionic conductivity through membrane [12–14]. Atomic force microscopy (AFM) measurements were performed using Nanoscope IIIa, Dimension 3000 and Multimode microscopes (Digital Instruments) according to a usual procedure adapted in our lab [15]. Tapping mode topographic and phase images were recoded in the air using silicon cantilevers.

As we observed with high resolution AFM imaging, a direct deposition of α-hemolysin on a highly hydrophilic, bare silicon surface under ambient conditions results in the fabrication of a dense monolayer or globular individual molecules depending upon initial concentration of solution (Fig. 4). In this state, AFM did not confirm presence of open/close-pore structures of individual protein molecules. Contrary, protein macromolecules on a bare silicon are in collapsed, compressed shape and structural characteristics are different from intact conformation. On the other hand, immobilization

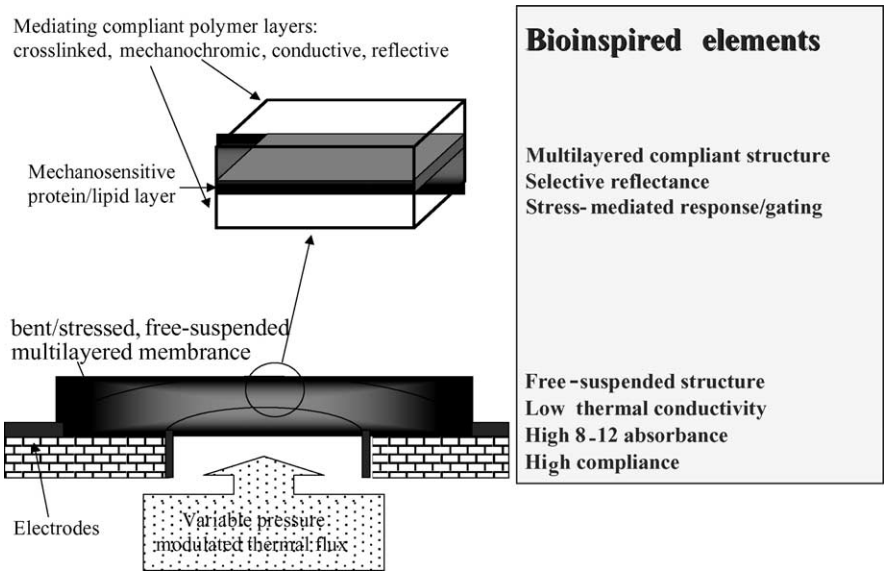

Fig. 2. Schematics of a free-suspended, complaint multilayered membrane with incorporated mechanosensitive interlayer and a list of bio-inspired elements.

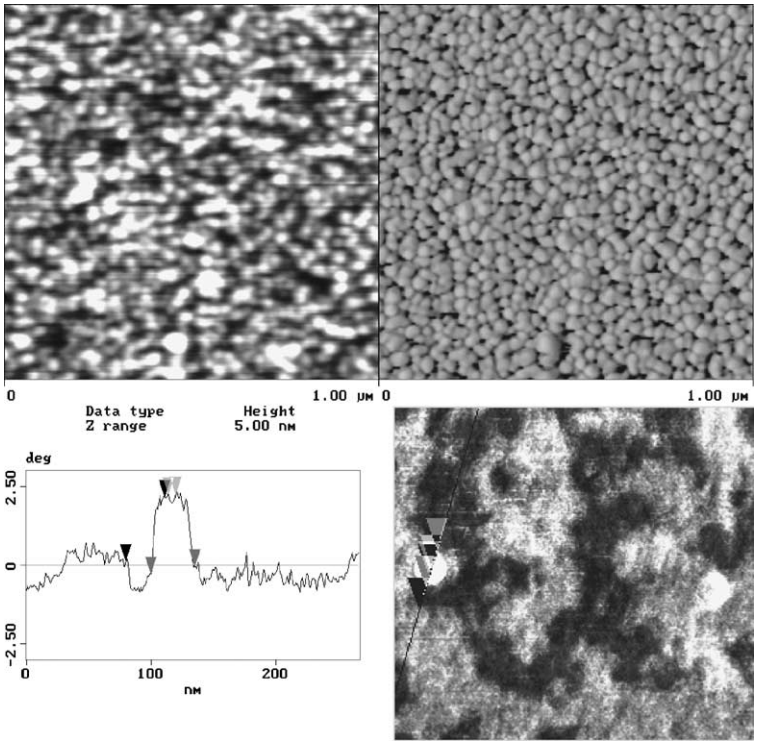

Fig. 4. AFM images of immobilized ion channel protein, topography (left) and phase (right). Top: Collapsed α-hemolysin adsorbed on a bare silicon from concentrated solution, 1 μm × 1 μm. Bottom: LB lipid monolayer with hemolysin proteins in close-pore conformation (a white round bump), 400 nm × 400 nm, and corresponding cross-section (left).

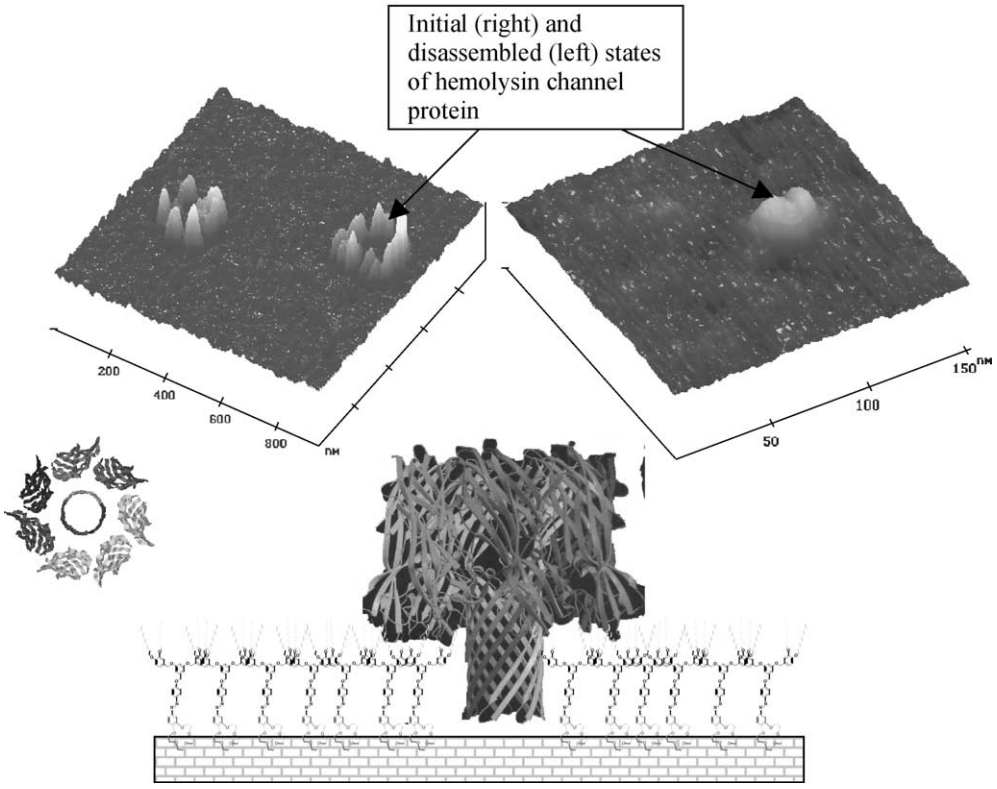

Fig. 5. Bottom: A scheme of α-hemolysin immobilization in a LB monolayer from photosensitive monodendron molecules; top: 3D views of intact protein (right) and disassembled protein after photochromic isomerization of dendritic monolayer (amphiphilic monodendron containing a benzyl-15-crown-5 polar focal point and four dodecyl tails as peripheral groups [17]); model of seven subunit of α-hemolysin as obtained by structure visualization using Raswin Molecular Graphics v2.6 software (http://www.openrasmol.org) (left).

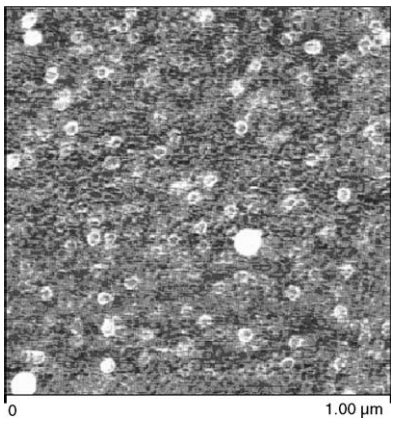

Fig. 6. AFM topographical image of open-pore α-hemolysin protein immobilized in a loosely packed alkylsilane self-assembled monolayer.

of these proteins onto the silicon surface mediated by a monolayer of lipid molecules supported by a polymer gel water-containing sub-layer resulted in their positioning predominantly in-between lipid domains (see white dots in Fig. 4). The presence of a light depletion on top of the molecules as labeled by markers in a cross-section in Fig. 4, indicates their closed-pore structure.

Similarly, the preservation of a closed-pore conformation can be achieved by immobilization of α-hemolysin in LB monolayer of photochromic dendrimer molecules by adsorption from a water subphase directly in LB trough (Fig. 5). In this monolayer deposited on a solid substrate, protein preserves its initial conformational state. Photoisomerization of the supporting photosensitive dendrimer molecules within the monolayer initiated by UV illumination at 365 nm [16] causes photochromic conversion within monolayers that, in turn, changes the intralayer pressure and triggers "disassembling" of the protein (Fig. 5). Clearly seen are seven subunits grouped similarly to their initial arrangement in an assembled protein as can be seen from comparison with corresponding molecular model (Fig. 5). Finally, this protein immobilized on a loosely packed alkylsilane self-assembled monolayer, possess a clearly visible open-pore microstructure with a wide-open central pore (Fig. 6).

In conclusion, we demonstrated that channel protein with large pore can be immobilized into organic monolayer on a surface of a silicon wafer via self-assembly or LB deposition. Conformational state (close–open pore state) of the protein could be controlled by the nature of the supporting monolayer and its structural organization. The incorporation of the channel proteins in thin free-standing membranes is another challenging step.

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