Biomimetic membrane systems on graphene based sensor-devices for biomedical diagnoses application

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Approximately 20% of the open DNA reading frames in complex organisms encode membrane-associated proteins [1]. Despite their abundance and key roles in cell adhesion, recognition, motility, energy production, transport of nutrients and cholesterol, our knowledge of the structure-function relationship for membrane proteins is rather limited, and lags far behind that of soluble proteins [2-4]. In part, this is due to limited biophysical tools that are available to adequately probe the physical-chemical principles underlying membrane protein function [5].
Similarly unsatisfying is the situation with respect to the implementation of platforms for bio-sensors that are able to address the many important interactions between ligands and membrane integral receptors, despite the fact that

- 50% of the interesting drug targets currently investigated for the screening of new medications are transmembrane proteins,
- membrane disrupting peptides are important toxins and promising antibiotics, and that
- current sensing platforms for membrane proteins/processes (e.g., approaches based on whole cell recordings with patch-clamp techniques) are lacking in versatility and flexibility, and are not suitable for “layman” use.

Many questions pertaining to membrane processes in general and ligand/membrane receptor interactions, in particular, could be addressed by solid-supported or tethered lipid bilayers. These are novel model membrane platforms that allow for a simultaneous characterization of the structural and the functional aspects of membrane processes and the evaluation of the correlation between both. The basic structural unit that we propose for this novel sensor platform is the tethered lipid bilayer membrane displayed in Figure 1. The experimental realization of this architecture has been demonstrated in our group already several years ago [6,7]. By now, we have developed a molecular tool kit for the (self-) assembly and covalent attachment of tethered monolayers onto supports of different materials classes, including
- noble metals (Au, Ag, etc) via thiols,
- oxide surfaces, e.g., SiO_2, TiO_2, Ta_2O_5.

The essential properties of these solid-tethered membranes include:

- membrane fluidity with lateral diffusion coefficients of the lipid constituents of D ~ 1µm2/sec.
- membrane capacities of C = 0.5 µF/cm^2
- resistivities in excess of R = 10 MΩcm^2.

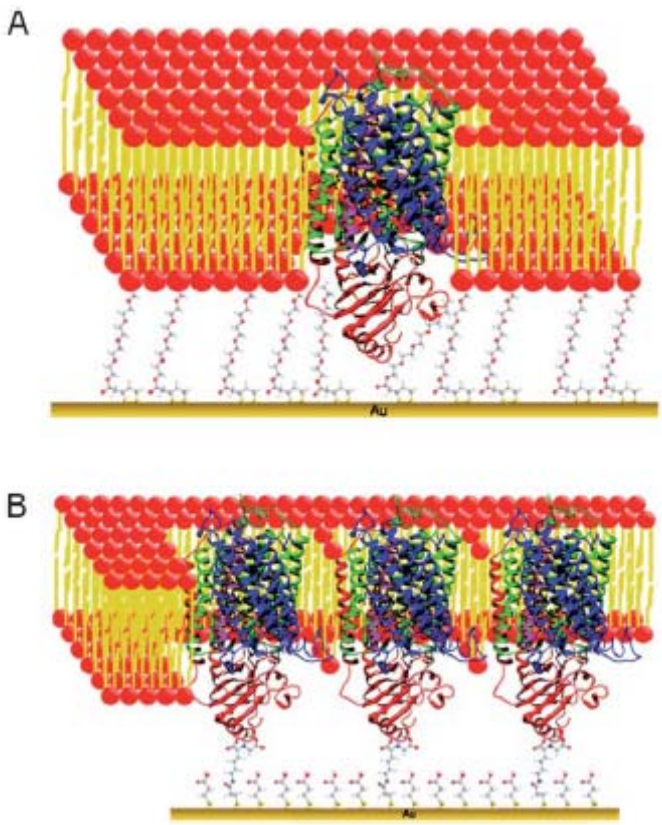

Figure 1: *Schematics of the conventional tethered bilayer lipid membrane (tBLM) (A) as compared to the protein-tethered bilayer lipid membrane (ptBLM) (B), both with an incorporated membrane protein, cytochrome c oxidase from R. sphaeroides used as an example.*

We have already demonstrated that a multitude of different membrane-proteins like Cytochrome c Oxidase (CcO), the bc1-complex, photosynthetic reaction centre from bacteria as well as the ion channel $ICln_{159}$ can be incorporated in our membrane system by retaining their natural functions [7-10]. Conformational changes of such proteins incorporated in a lipid bilayer system, as a function of perturbation could be monitored with Surface Enhanced Infrared Absorption Spectroscopy (SEIRAS) [7-10]. Besides the detection of different conformational states of a protein, we were also able to monitor structural changes at the inorganic redoxcentres located in redox-proteins like CcO or bc1-complex. Such changes of the inorganic compounds like heme-structures or Fe-S clusters could be monitored by the use of Surface Enhanced Raman Spectroscopy (SERS) [11,12]. Using both complementary spectroscopic techniques allows us to get a detailed overview of which parts of a protein that takes part in electron transfer, which redox-centres were addressed and how fast the electron transfer took place.

Such an example shows, which potential such biomimetic membrane systems have. Monitoring such protein dynamics could potentially help to understand the functionality of such membrane proteins.

The next step to build up a biomimetic electrochemical sensor platform is the incorporation of membrane proteins that act as the functional units in the final device. Examples of such functional units are membrane based ligand-receptors. Those receptors are crucial for cell-cell communication or detection of specific ligands. Such biomimetic membrane architectures functionalized with such receptor proteins could also be developed as drug screening platforms. One example for a membrane based receptor with pharmaceutical relevance is the Somatostatin-receptor (SSTR). Totally, five somatostatin receptors are known: SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5. Somatostatin acts at many sites to inhibit the release of many hormones and other secretory proteins. From the pharmaceutical point of view it is from great interest to investigate the binding kinetics of different pharmaceutical relevant ligands, such as Somatostatin 14 (SST14).

With the help of Infrared-spectroscopy it could be monitored if the binding event of such a ligand initiates a conformational change in the structure of the protein backbone of the used somatostatin receptors. If a conformational change takes place during the binding event the next question will be, if there are significant differences between the conformational changes of the used receptor, as a function of different added ligands to the system.
If there is no conformational change of the used recptor during the ligand binding, Infrared-spectroscopy can not be used to monitor the binding event of the ligands.

Besides the spectroscopic investigation of such binding events, field effect-transistors (FET) could be used to monitor such binding events electrochemically. Such transistor systems are independent of conformational changes. Such systems monitor very sensitive the change of the electric field during binding processes.

Recently we have developed an improved graphene synthesis method, resulting in large graphene oxide flakes, which can be reduced by hydrazine vapour more effectively compared with the existing methods for fabricating graphene [13-16]. With this approach, a high-performance and low-voltage operating graphene field-effect transistor (FET) was fabricated (figure 2) [17].

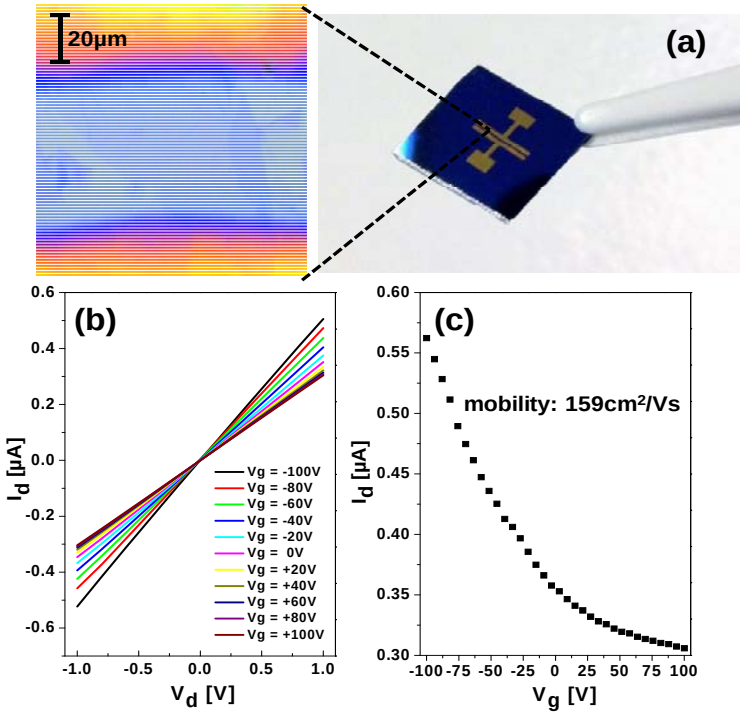

Figure 2: *(a) Optical micrograph of the fabricated graphene device. (b) Output characteristics of the transistor device under different applied gate voltages. (c) Current – voltage transfer curve of bottom-gated graphene FET at a drain-source bias of Vd = 0.1V.*

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(1-pyrenesulfonyl), see figure 3, will be immobilized by π-π-interaction, on top of the reduced graphene surface. First measurements have indicate that the assembly of the pyrene group and the grapheme layer are robust enough for a continuous flow of analyte over the grapheme based sensor.

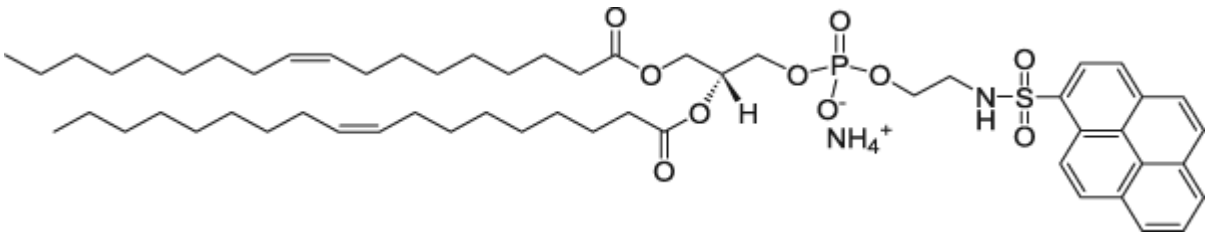

Figure 3: *Chemical structure of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(1-pyrenesulfonyl).*

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(1-pyrenesulfonyl), immobilized on top of the used graphene surface, will act as an anchor for a supported membrane. This membrane-architecture will be very similar to the membrane-architecture of the tethered bilayer lipid membrane (tBLM) system, developed in our group.
Additionally to the idea of forming a tBLM system, it could be also tested, if a ptBLM (protein tethered bilayer lipid membrane) system could be formed on top of the graphene based sensor as well. Therefore a Ni-NTA-linker, functionalized with Pyrene will be used to immobilize a his-taged protein, like Somatostation-receptors Type 3 and 5, which are available in our group. This his-taged protein will then be used to tether the membrane between the proteins immobilized on the sensor surface [7,9,10].

The membrane formation will be monitored with the help of SPR spectroscopy as well as with QCM.
The quartz crystal microbalance with dissipation monitoring (QCM-D) has emerged as one of the leading techniques to study the kinetics of lipid membrane assembly at interfaces thanks to its ability to distinguish interfacial conformations such as adsorbed vesicles from planar lipid membranes. We will therefore use QCM-D to study the formation of this new type of tethered membranes, where the assembly of lipid bilayers onto graphene in itself is of considerable general interest and importance. The characterization will be an important step to optimize formation of membranes with sufficient quality for probing the Somatostatin receptor functionality by FET.

Combining FETs with a biomimetic membrane-system appears to have potential for extension into a fully integrated system, providing an inexpensive, fast, and selective sensor platform for a wide range of applications in biomedical use and microarrays as well as screening for the affinity constant of specific antibodies.

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