

SUPRAMOLECULAR INTERFACIAL ARCHITECTURES FOR OPTICAL BIOSENSING

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INTRODUCTION: Any optical and/or electrical biosensor (transducer) surface requires a functional coating that allows for a highly specific and selective coupling via recognition and binding of the analyte molecule of interest from solution while simultaneously minimizing the response caused by non-specific binding. To this end we design, synthesize, assemble, and characterize (both structurally and functionally) supramolecular interfacial architectures with chemical features that vary for different types of bio-analytes. For the specific case of biosensor applications based on surface plasmon optical detection principles we will discuss in this contribution the different strategies optimized for DNA hybridization and protein binding assays. This will be complemented by examples for the build-up of tethered lipid bilayers for the construction of membrane chips.

METHODS: All optical detection studies were carried out with the recently introduced surface-plasmon fluorescence spectroscopy that combines the optical field-enhancements obtainable at resonant excitation of a surface plasmon mode with fluorescence detection principles. This way extreme limits of detection of binding events can be achieved provided the energy transfer quenching mechanisms operating for chromophores located near metal substrates are taken into account. For the tethered lipid bilayer membranes the metal substrate that carries the surface-plasmon mode simultaneously acts as the working electrode in a 3-electrode-electrochemical cell allowing for the simultaneous assessment of membrane properties by electrochemical and impedance spectroscopic techniques.

RESULTS: For surface hybridization studies we use an architecture that is based on a generic binding matrix built from biotinylated mixed self-assembled thiol monolayers and a streptavidin layer. Biotinylated oligonucleotide catcher probes or their uncharged peptide nucleic acid (PNA) analogues are immobilized via this binding matrix at the sensor surface in contact with a flow cell. The injection of target oligonucleotides carrying a chromophore then allows for the on-line observation of the association (hybridization) and dissociation processes, as well as

for the evaluation of affinity constants (by titration experiments) as a function of the length of the oligos, matching and mismatching base pairs (for SNPs detection), the temperature, pressure, ionic strength, etc. For protein binding studies we demonstrate that the use of hydrogels or polymer brushes offer a significant sensitivity advantage in terms of a high binding site density but also for the optimization of the distance dependent efficiency for chromophore excitation and fluorescence emission. We demonstrate that detection limits in the attomolar concentration range are possible. Finally, we introduce a novel thiolipid system that allows for the construction of electrically tight bilayers with a capacity in the range of $C = 0.5 \mu\text{F}/\text{cm}^2$ and an electrical resistivity in excess of $R = 10 \text{M}\Omega\text{cm}^2$. The incorporation of the ion-carrier valinomycin results in a reversible increase of the K^+ -selective membrane conductivity by more than 4 orders of magnitude. The consequences of this breakthrough will be discussed. Moreover, the use of polymerizable lipids for the preparation of patterned tethered bilayers to be used in membrane chips with multiple corrals will be discussed.

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ACKNOWLEDGEMENTS: This work was funded by the EU (QLK1-2000-01658, DNA-Track).