Molecularly controlled functional architectures

This paper summarizes some of our efforts in designing and synthesizing bio-functional layers at solid/solution interfaces, characterizing their structure and dynamics, and optimizing their functional properties. We explore different materials and architectures, focusing here on hydrogels and lipid bilayer membranes.

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The design and synthesis of molecularly or supra-molecularly defined interfacial architectures have seen in recent years a remarkable growth of interest and scientific research activities for various reasons. On the one hand it is generally believed that the construction of an interactive interface between the living world of cells, tissue or whole organisms and the (inorganic or organic) materials world of technical devices, like implants or medical parts requires the proper construction and structural (and functional) control of this organism-machine interface[1]. We are still at the very beginning of generating a better understanding of what is needed to make an organism tolerating implants, to guarantee the bidirectional communication between microelectronic devices and living tissue or to simply construct an interactive biocompatibility of surfaces in general[2].

On the other hand, the relatively simple interface between a technical transducer used in a biosensor format and the analyte solution of interest constitutes a challenge for the supra-molecularly controlled assembly of the interfacial architecture. In addition to the need to optimize the specific interaction between the binding sites at the sensor surface and the analyte from solution, one of the major tasks for the proper design of the interfacial bio-functional architecture, actually, is the minimization of the non-specific binding of biomolecules from a physiological solution[3,4]. Typically, these species are by far in excess and at concentrations for which already rather weakly affine binding sites generate a significant interfacial signal interfering with that originating from the specifically bound biomolecules of interest.

The gene chip is a well-established sensor platform for the detection of oligonucleotides, PCR amplicons, generic DNA, etc., for a variety of biological and medical applications. Many questions remain to be solved associated, for example, with the fact that DNA intrinsically is a highly charged polyelectrolyte system. In a dilute bulk solution, at an interface this feature can cause all kinds of problems related, for example, to the Coulombic interactions of the surface-attached capture probes with the analyte target strands binding from solution, or the possible cross-talk between neighboring hybridization sites, to mention but a few.

Not quite the same level of matureness, however, already beyond a purely experimental stage are arrays that detect various kinds of proteins, with applications ranging from monitoring expression levels

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to helping in cancer diagnostics and other disease detection. Here, the practical problems are still much more serious than in the case of the gene chip but first commercial products are appearing on the market.

And finally, a membrane chip does not exist at all; for many years it has been speculated that there has been significant activity in Australia – headed by Bruce Cornell. However, despite some interesting general scientific papers[5,6] no report about the successful introduction of a real product has appeared so far. Moreover, the Australian group focused on the use of gramicidin, a well-known pore-forming peptide that upon dimerization opens an ion channel through the membrane. Our approach is much more general (and allows for a broad range of potential application from binding studies in drug development addressing membrane-integral receptors, to the elucidation of membrane associated pathogenic processes like the amyloid plaque formation in the development of Alzheimer's disease[7]), it allows for the use of the developed platform as a 'phantom cell' for a detailed evaluation of the essential processes underlying cell-cell contact[8], i.e., in cancer development or for general tissue engineering purposes, or for the development of strategies to overcome current concerns for classical antibiotics which find more and more bacterial strains resistant to the traditionally applied antibiotic drugs[9].

In this paper, we will concentrate on just the biosensor aspect and focus on two architectures, i.e., the fabrication and functionalization of a hydrogel matrix used in optical bio-affinity studies of a classical target, the prostate specific antigen (PSA) and the implementation of a novel model membrane platform, the tethered lipid bilayer for the cell-free expression of membrane proteins, in particular, G-protein-coupled receptors (GPCR). The development of a variety of surface analytical tools that offer a detailed picture of the interface, and any molecular architecture that is assembled to it, has opened the window of opportunities to a much better and deeper understanding of the organization and structural characteristics of any supramolecular coating that is prepared at the interface with the aim at controlling its functional performance. Hence, in the examples for supramolecular interfacial architectures that we will discuss below we will put particular emphasis on the correlation between the requirements of the transduction principles and how one can design the surface architecture to meet these needs.

Supramolecular interfacial architecture for bio-affinity studies

Grafted hydrogel layers for biosensor functionalization

Our strategy for the preparation of highly swollen hydrogels is based on the synthesis of ter- (or even quarter-) polymers incorporating monomers that control the base properties of the resulting hydrogel. This includes the manipulation of its hydrophilic/hydrophobic character which influences parameters like the collapse temperature (lower critical solution temperature, LCST). Other monomers can be used for the covalent coupling of the binding partners, e.g., antigens

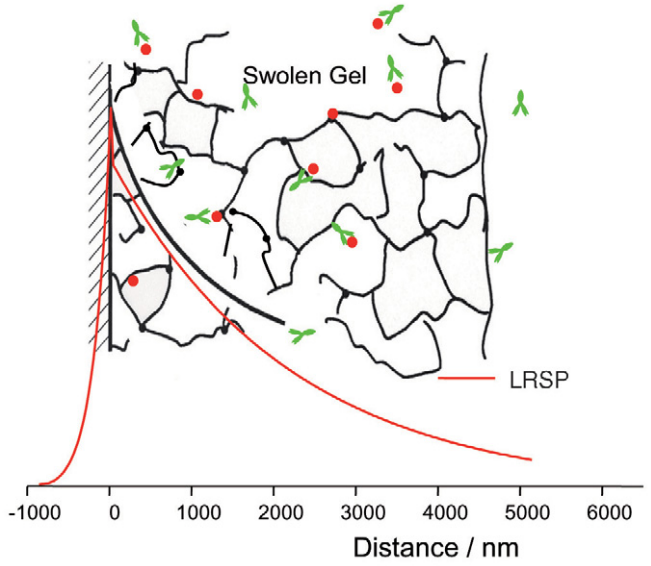

Fig. 1 The interfacial architecture of a grafted, highly swollen hydrogel, functionalized by binding sites (red dots) to which analyte molecules from solution (green objects) can bind. For comparison, the extended electromagnetic field of a long range surface plasmon (LRSP) mode propagating along the solid/solution interface is plotted (red curve). Note that the gel as well as the evanescent field extends some micrometers into the analyte solution.

or antibodies, organic dyes, (semiconducting or even magnetic) nanoparticles, etc. And finally, this concept includes cross-linking units which in our case function by the hydrogen abstraction properties of benzophenon units[10]. After spin-coating this copolymer onto a substrate, which is pre-conditioned by a self-assembled monolayer of benzophenon derivatives of long alkyl chain molecules, the benzophenon moieties are photo-activated by UV light resulting in a simultaneous cross-linking and grafting of the polymer layer to the substrate. Immersion into a buffer solution then leads to the formation of the hydrogel that can swell to a thickness that exceeds its dry value by up to a factor of 40[11]. This means that these matrices are very open structures allowing for an easy access of the analytes by diffusion from the bulk solution to their stationary partners inside the gel resulting in a nearly unrestricted affinity binding reaction[12]. This is schematically sketched in Fig. 1. Here, the swollen thickness is chosen so as to match to the evanescent tail of the optical field of a long-range surface plasmon mode (cf. below) extending also some few μm into the buffer medium.

Long range surface plasmon fluorescence spectroscopy

Among the many experimental techniques well-suited for the structural and functional characterization of such interfacial architectures in contact with an aqueous phase surface plasmon resonance (SPR) spectroscopy[13,14] has gained an enormous popularity as an ultra sensitive optical technique[15]. In particular, the introduction of a