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Commentary

Importance Aspects of Anti-fouling Materials for Label Free Kinetics

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DESCRIPTION

Label free binding kinetics is essential to describe the interaction mechanisms of proteins, antibodies, oligonucleotides, and more. Differently from labelled or end-point methods, label-free kinetics allows for real time investigation of binding reactions, removing the constraint of having a third interacting element. The possibility of studying the behavior of a wide range of bio-agents in their native conformation is attractive for a number of reasons. Label free kinetic characterization of a drug receptor interaction is the least artificial way to measure the concentration dependent response of the ligands to the compound The sensors currently available on the market enable the investigation of hundreds of probes at a time against the single antigen as well as a single probe against multiple targets. One of the limitations of label free kinetic measurements is the requirement to immobilize one of the two interacting agents on the sensor surface in order to measure the amount of target binding to the ligands.

The surface of the sensor needs to be chemically activated and functionalized by coating it with a specifically designed material containing reactive groups that can stably anchor molecules desaturating their structure. More over such material needs to attach the probe molecules to form high density layer, otherwise being repulsive to other non-specific interactions such as anti-fouling. Achieving stable high density immobilization of biomolecules is the main goal of the field of surface chemistry for biosensors has seen significant development in the past few years due to the dedicated materials with very specific features.

Ideal surface needs to have all the characteristics enumerated in order to adapt different sensor materials. While most sensors are made of glass or hard plastics are some of the feature metallic component. For example Surface Plasmon Resonance (SPR) sensors, the current standard in label free kinetics base their working principle on a thin layer of the gold which coats on glass prism.

Hence, SPR dedicated surface chemistry needs to adapt to an unusual impractical chip materials. In some cases multilayer probe immobilization is also provided, to increase probe density without causing steric hindrance.

CM-dextran matrices for SPR biosensors

SPR was a label free optical sensing tool relies on resonant coupling of the evanescent field with the plasmonic excitation of electrons in the gold layer to measure refractive index variations. SPR biosensors provide real time monitoring interactions of several analytes enabling fast biomolecular kinetics measurements with the high specificity and sensitivity without labelling. SPR platforms have been utilized for the detection of viruses, biosimilar molecules and proteins. Studies shows accurate and early recognition of the infectious diseases biomarkers can be performed in real time. Today, these biosensors are utilized in many areas including biochemical studies such as investigation of DNA–DNA interactions and also in agricultural settings.

Epoxy silanization and SAM monolayers

Label free sensors are not based on metal surfaces. The simplest and cheapest way to anchor molecules in glass surface is to salinize the surface by utilizing bi dimensional epoxysilane coating. Epoxysilane polymers such as trimethoxysilane have two functional ends which perform two different tasks such as silane groups bind covalently to plasma activated silica surfaces by reacting with the OH groups. Epoxy end reacts with amine groups in proteins or other amine modified biomolecules linking them to the surfaces. The process of coating with epoxysilane polymers is both cost effective and relatively fast. It can be safely performed in any laboratory and does not require any particular expertise. Hence, it may remain one of the most functionalization methods in biosensing applications which despite to having some pretty significant disadvantages. One of the main issues is when dealing with epoxide coatings is surface inhomogeneity as well as spot to spot variation. Coffee ring effect has also been observed in some cases by causing uneven probe distribution across the spot. The majority of these types of problems can be correlated to the nature of the immobilization

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reaction on the epoxide surfaces. Coating a the glass surface with GLYMO will form a Self-Assembled Monolayer (SAM) of around 2 nm in thickness that will react with the molecules, primary with the amine groups to anchor them to the surface. The high reactivity of the SAM implies that the probes will be strongly attracted towards surface is to the point that their structure might be distorted and epitopes could be inaccessible for the binding. This type of issue is particularly relevant with protein probes. Proteins can be classified as soft proteins and hard proteins which refer to their inclination to denaturate. If the protein contains a large number of disulfide bonds it may be more resistant to denaturation and it will be classified into hard protein. One of the examples for the hard protein is Bovine Serum Albumin (BSA) commonly used as a model for protein-binding assays which contains 17 disulfide bonds. On the other hand α-lactalbumin is identified as a soft protein which containing only 4 disulfide bonds. Immunoglobulin (IgG) is also a soft protein in which most antibodies belong to this type of category that highlights the importance of being able to efficiently handle this probe. Immobilizing soft proteins is more complicated due to their delicate structure which requires particular care when strongly attaching them to the surface. Epoxide coatings are usually not ideal in this due to their tendency to strongly pull the molecule towards the surface from multiple sides and flatten.