From Ensemble Binding to Single-Molecule Statistics in large area biosensing devices

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Plasmonic and potentiometric platforms are well-established label-free approaches for immuno and molecular detections. However, when operated without amplification strategies, their analytical sensitivity has traditionally been limited to the nanomolar concentration range. In this lecture, I will discuss a comprehensive physical and statistical framework showing that biosensing responses over an exceptionally broad dynamic range, from nicromolar down to 10-zeptomolar concentrations, are governed by two distinct sensing regimes.

The study focuses on centimeter-scale sensing interfaces densely functionalized with pH-conditioned recognition layers, where electrostatic and ion-mediated effects play a central role in transducing molecular recognition events. This is called the Single-Molecule with a Large Surface (SiMoLS) systems. At ultralow analyte concentrations, the sensor response is no longer described by ensemble-averaged affinity interactions. Instead, it is dictated by Poisson statistics associated with stochastic single- and few-molecule capture events occurring on large-area interfaces. In this regime, each binding event can trigger a local pH-conditioned mediated dielectric and electrostatic reorganization of the interfacial layer, producing a measurable plasmonic or potentiometric signal.

This mechanism enables direct, label-free detection at concentrations as low as 10–100 zM with 99% statistical confidence, while keeping false-positive and false-negative rates below 1%. As the analyte concentration increases, the sensing behavior progressively evolves toward a classical Maxwell–Boltzmann regime, characterized by conventional binding isotherms and ensemble-averaged equilibrium affinity.

By explicitly accounting for noise, uncertainty propagation, and statistically robust decision criteria, the full binding curves can be modeled across both regimes, allowing the extraction of equilibrium parameters, including an apparent equilibrium constant specific to the ultra-dilute limit. These results clarify some key aspects of the physical origin of extreme sensitivity in large-area biosensing platforms and highlight the role of electrostatic, ion-mediated interfacial phenomena in the design of next-generation single-molecule sensors operating without labels.

Mechanistically, the two regimes arise from the responsive nature of the pH-conditioned capturing layer. At ultralow antigen concentrations, a single antigen–antibody binding event is proposed to nucleate a localized domain where neighboring antibodies undergo collective dielectric and electrostatic rearrangement, likely involving the cooperative hydrogen-bond networks. This converts a discrete molecular event into a mesoscopic dielectric perturbation detectable by plasmonic or potentiometric transducers. At higher concentrations the formation of a continuous layer of target molecules that are affinity bound to the capturing layer is seen and the response is governed by ensemble-averaged affinity-bindings.

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