Protein binding monitoring using Surface Plasmon Resonance: kinetics and picomolar detection of proteins in serum samples.

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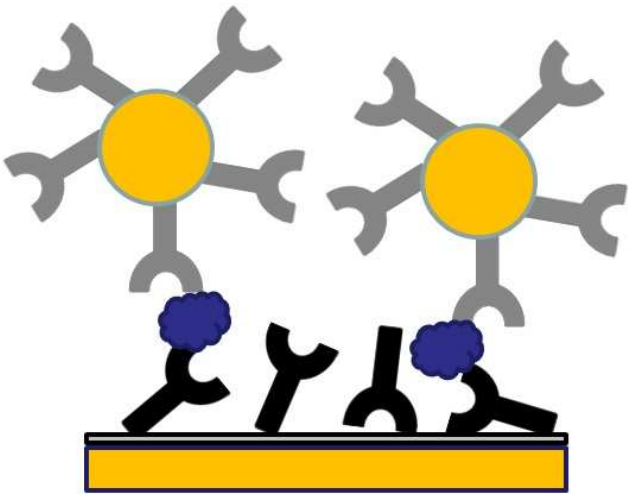

Fig. Immunoassay used for the detection of proteins at low concentration in serum samples.

Surface Plasmon Resonance (SPR) is a widely used tool in bioanalytical laboratories as label-free real-time method for molecular interaction studies.). For detection, one molecule (ligand) is immobilized on the sensor surface (gold) and the binding partner (analyte) is injected in a buffer through the flow cell usually under continuous flow. As the analyte binds to the ligand the protein on the surface gives rise to an increase in the refractive index and this change in refractive index is measured in real time. The fact that the system generates real-time binding data makes it well suited to the analysis of binding kinetics.

First, two unknown protein samples are analyzed using SPR and after experimental optimization binding constants are extracted. It is concluded that the proteins interact in a complex form and a model with two sets of association and dissociation rates is needed to fit with the data. This study is part of the 2011 Global Label-Free Interaction Benchmark Study.

Secondly, the detection of low-concentration proteins in serum samples using functionalized gold-nanoparticle signal-amplification is depicted. In this part, proteins present in a complex medium (human serum) at very low concentration (pg/ml) are detected and quantified using a sandwich immunoassay in which secondary antibodies attached to "bulky" gold nanoparticles cause a larger change in the index of refraction in the vicinity of the surface and, consequently, giving rise to the necessary signal amplification for its detection.