43: Study of biomolecular interactions by surface plasmon resonance biosensor analysis (BIAcore technology)

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The surface plasmon resonance (SPR) -based biosensor technology allows realtime detection and monitoring of biomolecular binding events. In a BIAcore experiment, one of the interacting molecules (the ligand) is immobilized on the biosensor surface (sensor chip), while the other one (the analyte) is delivered to the surface in a continuous buffer flow. Formation and dissociation of complexes between the two interactants are followed by SPR, which detects mass changes at the surface of the sensor chip. The resonance signal is recorded as a sensorgram (Figure 1). This technology requires no labelling of the molecules and provides quantitative information on kinetic parameters (association and dissociation rate constants, k_a and k_d) and affinity constants (K_D). It allows characterization of molecular interactions involving proteins, small molecules, carbohydrates, lipids, nucleic acids, or even whole cells (bacteria, viruses…). Applications include epitope mapping, molecular assembly, ligand fishing and small molecule screening.

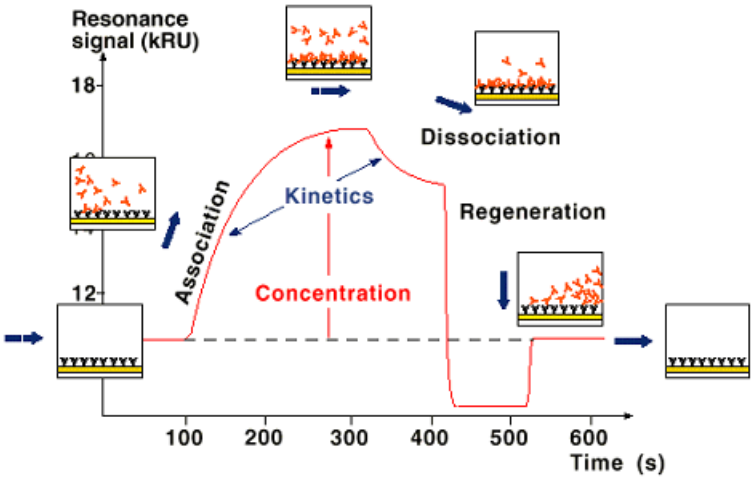

Figure 1: A sensorgram (http://www.biacore.com)

The principles and fields of application of the BIAcore technology in life sciences will be briefly reviewed at the beginning of the practical course, which will focus on an example of a standard kinetic study of biomolecular interactions between an antibody and its antigen, performed on one of the latest generation SPR instrument (Biacore T200). The practicals will consist of the setup of the experiment, pre-concentration and pH scouting assays, immobilization of the ligand on a sensor chip, control of the binding capacity of the immobilized ligands and of a kinetic experiment (choice of experimental conditions: flow rate, duration of injection, regeneration solution and sample preparation). The principles of evaluation of kinetic data obtained with the BIAcore technology will be introduced, followed by analysis of kinetic data of antibody-antigen interactions (data preparation for the evaluation, choice of an interaction model, validation of the selected model and calculation of kinetic and affinity constants).

Student profile: basic knowledge in protein biology is required.

Reference: Rich R. L. and Myszka D. G. (2000) Advances in surface plasmon resonance biosensor analysis. *Curr. Opin. Biotechnol.* 11:54-61