Mini-workshop

Surface Plasmon Resonance and CD18 Integrin ligand specificity

Thursday April 20, 2017 - 9h30-12h

Rhône Meeting room (bât. Irstea, ground floor)

Program

9h30 – Opening and welcome of the participants (Jean-Marc Lancelin)

9h45-10h45 : Thomas Vorup-Jensen, MSc PhD DMSc, Dept of Biomedicine, Aarhus University, Aarhus, Denmark

CD18 Integrin Ligand Specificity in Immunity and Immunopharmacology

11h - 11h 20 : Alexey Ferapontov, BSc, Dept of Biomedicine, Aarhus University, Aarhus, Denmark

Principles and Immunological Applications of Surface Plasmon Resonance (SPR)

11h20 – 11h40 : Kristian Juul-Madsen, MSc, Dept of Biomedicine, Aarhus University, Aarhus, Denmark

Analyzing heterogeneous binding in SPR-experiments

11h40 - 12h00 – Discussion and questions.

please notice of your participation to jean-marc.lancelin@isa-lyon.fr, tel 04 37 42 35 46

CD18 Integrin Ligand Specificity in Immunity and Immunopharmacology

Thomas Vorup-Jensen, MSc PhD DMSc

Professor, Dept of Biomedicine, Aarhus University, Aarhus, Denmark

e-mail: vorup-jensen@biomed.au.dk

The family of beta-2 (CD18) integrins includes four members, namely CD11a/CD18 (also known as lymphocyte function-associated antigen [LFA]-1, or integrin $\alpha_L\beta_2$) CD11b/CD18 (Mac-1, complement receptor 3, or $\alpha_M\beta_2$), CD11c/CD18 (p150,95, complement receptor 4, or $\alpha_X\beta_2$), and CD11d/CD18 ($\alpha_D\beta_2$). These receptors serve a wide range of functions in the immune system, including extravasation of leukocytes to zones of inflammation, support of the immunological synapse between T lymphocytes and antigen presenting cells, and phagocytosis by macrophages and neutrophil granulocytes. Although structurally similar, the CD18 integrins are nevertheless binding ligands in fundamentally different ways. In this talk, I will focus on the ligand recognition by the CD11b/CD18 and CD11c/CD18 receptors. Both of these receptors bind complement as a part of phagocytosis. However, in addition, a large multitude of structurally and functionally unrelated ligands have also been reported. Recent progress have identified certain patterns distinguishing ligands for CD11b/CD18 and CD11c/CD18. In particular surface plasmon resonance-based assays have been helpful in quantifying the interaction with ligands. Structural analysis of the ligand recognition by CD11b/CD18 using X-ray crystallography have considerably strengthened our knowledge of these interactions at the atomic level. The ligand binding by these receptors contributes a fascinating picture of the diversity in protein-ligand binding. Taken together with the recent characterization of soluble, ligand-binding forms of the CD18 integrins, new perspectives are added on the immunopharmacology targeting these receptors.

Principles and Immunological Applications of Surface Plasmon Resonance (SPR)

Alexey Ferapontov, BSc

Dept of Biomedicine, Aarhus University, Aarhus, Denmark

e-mail: alexf@biomed.au.dk

An understanding of how the immune system works on the molecular level is a vital part of immunological research. In immunochemistry, knowledge of the binding interactions between the biomolecules can provide important information about the mechanism of immune responses. While different approaches can be used for their studies, a tried and tested option is assays based on surface plasmon resonance (SPR). In this presentation, the basic principles of SPR will be reviewed, including the SPR setup, the theoretical physical background behind this technique, and mechanistic views on surface plasmons: what are they, how they are generated and what role do they play in an SPR experiment. It will be addressed how the adsorption or covalent immobilization of a material on the studied surface affects the surface plasmons, and why this lead to a change in the SPR signal. It will be discussed what is necessary to perform an SPR experiment, how the SPR data can be interpreted and evaluated, and what are the possible advantages and limitations of this technique. Several examples of SPR applications in immunological studies will be discussed.

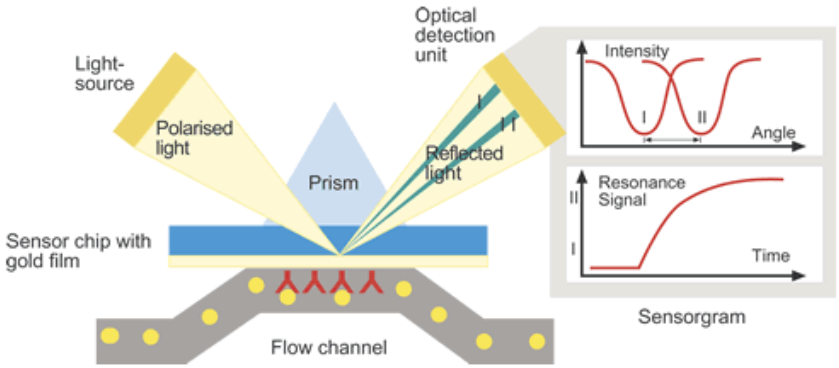

Figure 1. Schematic of the SPR experimental setup and representative data (sensogram) collected during SPR measurements. (http://www.rci.rutgers.edu/~longhu/Biacore/)

Analyzing heterogeneous binding in SPR-experiments

Kristian Juul-Madsen, MSc

PhD student, Dept of Biomedicine, Aarhus University, Aarhus, Denmark

e-mail: juul-madsen@biomed.au.dk

In many SPR experiments, the goal is to estimate the affinity between a ligand and an analyte. The simplest interaction between ligand and analyte is a 1:1 interaction. The Langmuir model, with the analyte A and the ligand L forming an AL complex, efficiently describes this interaction. Unfortunately, analyte ligand binding are rarely a simple 1:1 interaction. The Langmuir model assumes that interactions are homogenous. However, the homogeneous immobilization of ligand onto a surface is not trivial. The coating often results in a continuum of slightly different kinetic and thermodynamic properties due to different distributions of orientations on the surface. This gives a non-uniform surface, and classes of binding interaction need evaluation. Furthermore, the binding of some ligands is known to involve multiple binding sites. Hence, this requires that multiple binding sites can be distinguished in the SPR data. Some degree of approximation is required to enable such distinction, but it gives a more comprehensive image of the binding interaction than the simpler 1:1 reaction model. A model has developed that takes into account the different classes of binding in a SPR experiment. The model is based on an integral equation that describes the surface as a continuous two-dimensional distribution of affinity and kinetic constants. In this presentation, the model will be reviewed for the data analysis of SPR experiments.

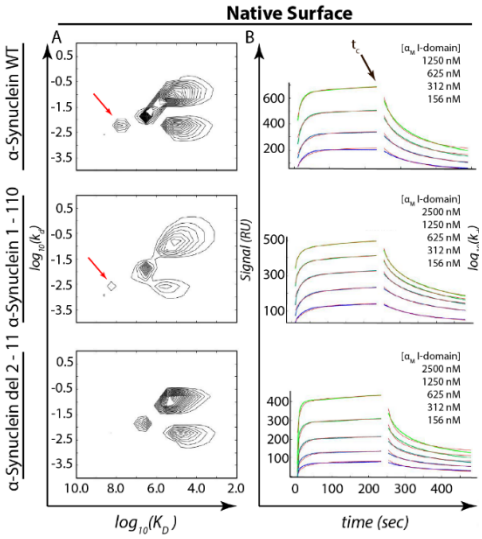

Figure 1. SPR-data analyzed with EVILFIT. *A*, The 2D-distribution of binding sites. *B*, The Sensograms and fit used for analysis.