Soutenance ibs

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Soutenance de thèse

Mardi 15 Mai 2012

A 14h - Salle des séminaires de l'IBS

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Exploring new approaches for GPCR studies at the molecular level: application to chemokine receptors

Thèse de Doctorat de l'Université Joseph Fourier

Chemokine receptors are critical regulators of cell migration in the context of immune surveillance, inflammation and development. The GPCRs (G protein-coupled receptors) CCR5 and CXCR4 are specifically implicated in cancer metastasis and HIV-1 infection. An expression system to over-express these two GPCRs was developed. To overcome the toxicity problem of membrane protein expression in bacterial system, the production approach consists in targeting the proteins towards *E. coli* inclusion bodies thanks to a N-terminal fusion allowing a high yield expression. After purification under denaturing conditions, these GPCRs were then folded using original polymeric surfactants: the amphipols. The validation of this new approach for the chemokine receptor production is one of the goals of this work. In order to assess the functionality of the folded proteins, series of tools have been developed: engineered chemokine ligands (RANTES for CCR5 and SDF1a for CXCR4) were produced.

The functionality of chemokines was evaluated at cellular and molecular levels. Interaction between the receptor folded in amphipols and its ligand was evaluated using Surface Plasmon Resonance (SPR) technique. Several types of surfaces, functionalized with the chemokine receptor/amphipol complex have been explored in this work. At the end of this project the productions of chemokines and their receptors has been set up. These established tools open the way to future studies, at the molecular level, in order to, for instance, investigate receptor dimerization and complex stoichiometry.