# Soutenance ibs

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## Soutenance de thèse

Vendredi 16 Mars 2012

**A 13h - Salle des séminaires de l'IBS**

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Groupe Structure et Activité des Glycosaminoglycanes

# Development of a binding assay between the HIV-1 envelope protein (gp120) and coreceptors CCR5/CXCR4 by Surface Plasmon Resonance: Screening and optimization of viral entry inhibitors

**Thèse de Doctorat de l'Université Joseph Fourier**

Cell-associated Heparan Sulphate (HS) binds the V3 loop of HIV-1 gp120 aiding in viral infectivity. However, a soluble HS polyanion has antiviral properties once conjugated to mCD4 (mCD4-$HS_{12}$) showing nM activity against HIV-1 *in vitro*. Due to the structural complexity of HS, screening differently sulphated oligosaccharides to improve the molecule's activity would be cumbersome, thus to obtain a more specific, higher affinity and easier to produce moiety, collaborators synthesized HS mimetic peptides. We aimed to screen these peptides for their capacity to inhibit HIV-1 entry. Thus we set-up a platform whereby solubilised CCR5 and CXCR4 were immobilized on biosensors and used to screen for molecules that inhibited gp120-CD4 binding to the coreceptors. To control the solubilization process, CXCL12 (CXCR4 natural ligand), was injected over the immobilized CXCR4. The affinities of CXCL12 isoforms ($\alpha$ and $\gamma$) for CXCR4 were in range of values from different techniques, proving the functionality of our system and enabling an investigation of the binding mechanisms of the two isoforms with CXCR4 and their regulation by HS. The system was subsequently used to screen the inhibitory capacity of the HS mimetic peptides. Each peptide, $[S(XDXS)_n]$, contained amino acids that mimic the hydroxyl, carboxyl and sulphate groups on HS chains. The peptide containing sulphotyrosine residues conjugated to mCD4 (mCD4-$P3YSO_3$) displayed nM $IC_{50}$ for simultaneously inhibiting gp120 binding to HS, CD4, antibody, coreceptors and HIV-1 infection *in vitro*. This is the first bivalent entry inhibitor that targets both R5 and X4 viruses and the concept of a HS mimetic peptide lends itself to structural-functional analysis of HS chains binding to proteins, a novel technique in this field.