SPRi Imaging of Oligosaccharides/Proteins Interactions

MERCEY Emilie[1], SADIR Rabia[2], MAILLART Emmanuel[3], ROGET André[1], LORTAT-JACOB Hugues[2], LIVACHE Thierry[1]

1 *CREAB, UMR 5819 (CEA-CNRS-UJF), DRFMC. CEA Grenoble ; 17, rue des Martyrs 38054 GRENOBLE Cedex 9, France.*

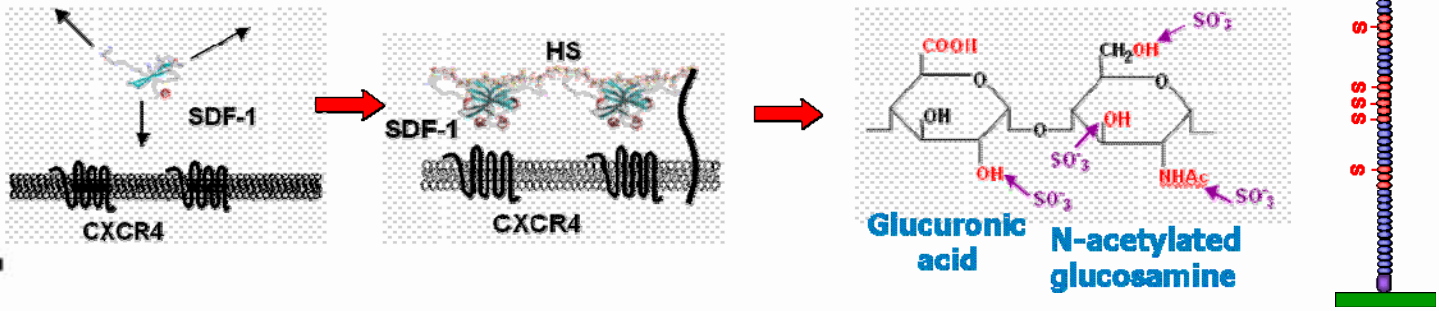
2 *Laboratoire d'Enzymologie Moléculaire, Institut de Biologie Structurale UMR 5075 (CEA-CNRS-UJF), 41 rue Jules Horowitz, 38027 Grenoble Cedex 1, France.*

3 *GenOptics SA, Orsay, France*

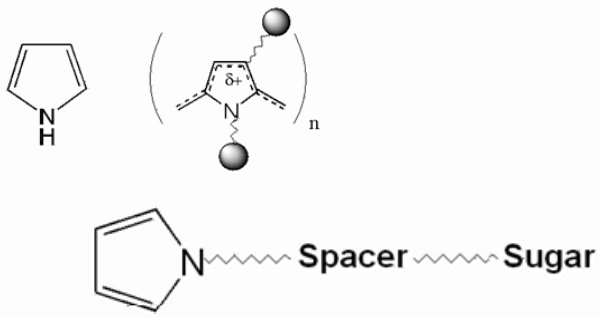
Introduction

The majority of proteins are glycosylated: they possess oligosaccharide chains and are hence termed glycoproteins. Oligosaccharides, also called carbohydrate or sugar, are often quite large (as large as some protein domains for example) and they have many functions in molecular interactions.

In mammals, sulfated glycosaminoglycans (GAGs) are among the carbohydrates which play an essential physiological role. For example, heparin (also known as an anti-coagulant) and heparin sulfate (HS) are linear polysaccharides expressing a high chemical heterogeneity that enables a wide range of very diverse functions, essentially through direct interactions with a number of proteins.

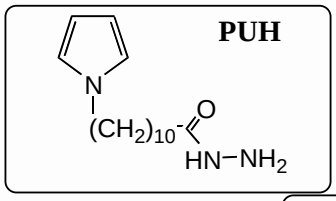


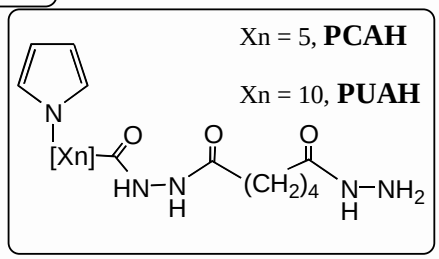
This application note highlights the development of an oligosaccharide chip (surface functionalization, density of immobilization, spacer arm length) on the same SPRi platform using conductive polymers. These results are highlighted through the example of interactions between HS and SDF-1 α chemokine.



Experiment

The oligosaccharide chip has been optimized using HP6 and Chondroitine Sulfate (CS) conjugated to a pyrrole via different spacer arms.





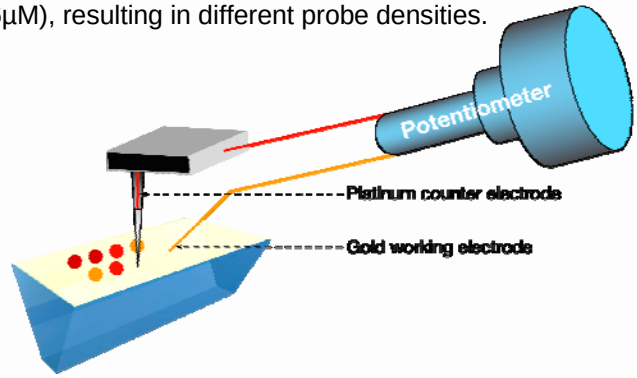
Surface Chemistry

Two strategies has been compared to conjugate different pyrrole-linker to oligosaccharide by direct coupling or sequential coupling.
Three different "pyrrole-spacer arms–oligosaccharides" have been obtained with various string lengths:

1. PUH: Pyrrole Undecanoyl Hydrazide
2. PCAH: Pyrrole Caproyl Adipate Hydrazide
3. PUAH: Pyrrole Undecanoyl Adipate Hydrazide

Spotting of Oligosaccharide on the SPRi-Biochip™

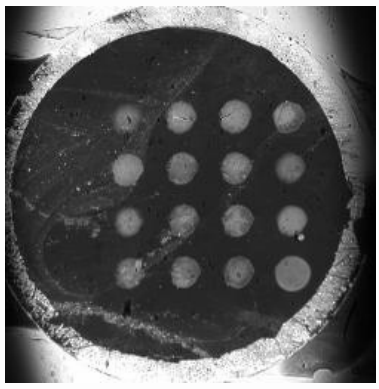
Oligosaccharides were electrodeposited on the SPRi-biochip™ at different concentrations (100, 25, 6.25 and 1.56µM), resulting in different probe densities.



Pattern and image of SPRi-biochip™ functionalized

- *Each sequence is adressed separately*
- *A mixture, diluted in a phosphate buffer, of pyrrole and pyrroles coupled with oligosaccharide is copolymerized*
- *Spot thickness is controlled by the injected charge via a voltage pulse. Spot diameter: 100 µm < ϕ <500 µm*
- *Up to 400 spots*

PCAH-HP6 100 µM	PUAH-HP6 100 µM	PUH-HP6 100 µM	HP6 50 µM
PCAH-HP6 25 µM	PUAH-HP6 25 µM	PUH-HP6 25 µM	PCAH-CS 50 µM
PCAH-HP6 6.25 µM	PUAH-HP6 6.25 µM	PUH-HP6 6.25 µM	ppy
PCAH-HP6 1.56 µM	PUAH-HP6 1.56 µM	PUH-HP6 1.56 µM	ppy



SPRi interactions monitoring

The SPR imaging technology developed by GenOptics is a label-free highly sensitive method of visualization. Thanks to a video CCD camera the whole area of the biochip can be visualized. This enables the functionalization of biochips in array format and the collection of data from all the different spots.

A broad monochromatic polarized light (at a specific wavelength) illuminates the whole functionalized area of the SPRi-biochip™ which is connected with a detection chamber. A CCD video camera gives access to array format (up to 400 spots) by image capture of all local changes at the surface of the SPRi-biochip™.

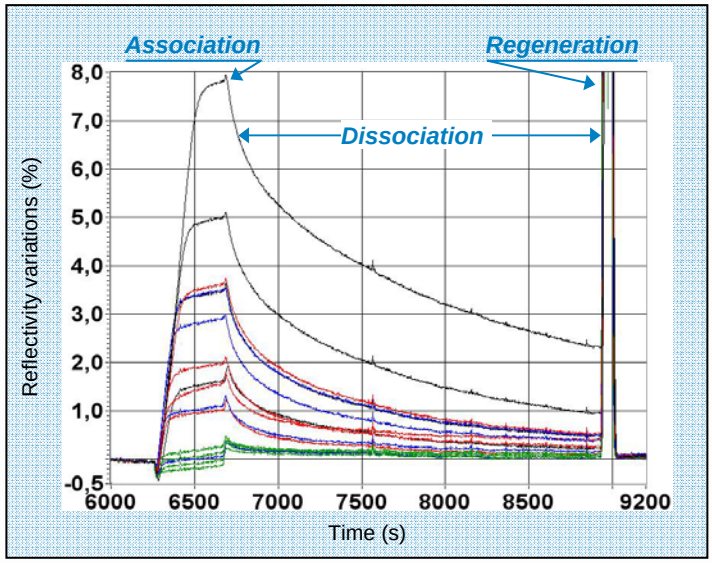
Results

After introduction of the oligosaccharide chip in GenOptics SPRi instrument, different concentrations of SDF1α were injected in the flow cell. The chip was regenerated with saline buffer between each injection of chemokine.

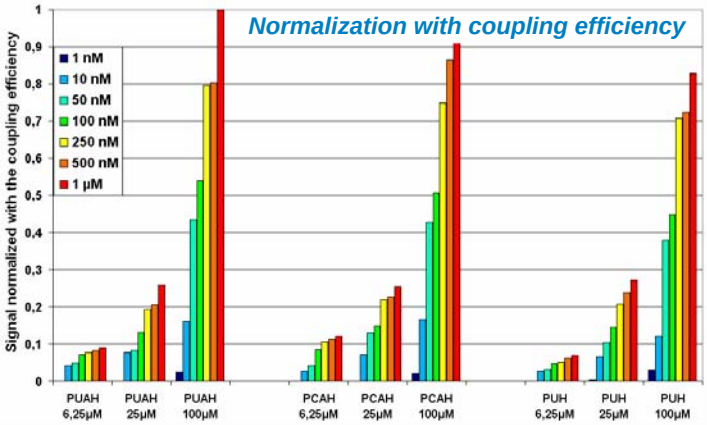
When SDF1α was injected at 250nM, we observe interaction curves between SDF1α and HP6 which vary with spacer arm length and HP6 probe density. The signal intensity increases with HP6 probe density and PUH spacer arm is better than PCAH and PUAH

The four green curves correspond to negative probes and reveal no interaction. The quick and easy regeneration allows the sensor to be used more than 10 times without loss of signal.

The 16 kinetic curves allow the relationship between oligosaccharides and proteins (chemokine) to be determined. Genoptics technology makes it possible to analyze the influence of the spacer on the molecule interactions and to optimize the density of the oligosaccharide probes which are linked at the surface.



Influence of spacer arm length and HP6 probe density on the interaction kinetics resulting from the injection of 250nM SDF-1α.



- ***Kinetic results***

  kass(HP6/SDF) = 7.1 10^4 ± 2 10^4 $M^{-1}.s^{-1}$
  kdiss(HP6/SDF) = 1.4 10^{-2} ± 0.1 $10^{-2}.s^{-1}$
  KD(HP6/SDF) = 195 ± 50 nM

- ***These results indicate that SDF1α reacts***

Conclusion

Combining Polypyrrole with SPRi provides a unique method for fast, easy and reliable studies of oligosaccharide interactions on a biochip. The GenOptics platform, based on SPRi technology, is a powerful device for glycobiology, a rapidly expending area of research.

- This study demonstrates the process of grafting natural oligosaccharides on SPRi-Biochip™.
- SPR imaging is ideal to study protein-oligosaccharide interactions without labeling neither the probes nor the targets and to monitor multiple (at least 25) kinetic interactions in real time.
- This approach enable direct studies of synthetic or natural oligosaccharides properties to be developed even further.

HORIBA JOBIN YVON
GENOPTICS BIO INTERACTIONS

Find us at **www.jobinyvon.com/SPRi** or telephone:

France: +33 (0)1 64 54 13 00
Germany: +49 (0)89 46 23 17-0
China: +86 (0)10 8567 9966
USA: +1 732 494 86 60
UK: +44 (0)20 8204 8142
Other Countries: +33 (0)1 64 54 13 00
Japan: +81 (0)3 3861 8231
Italy: +39 0 2 5760 3050