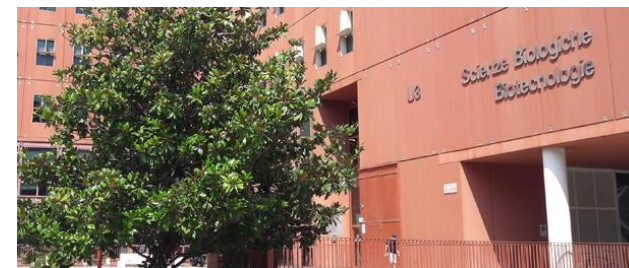


# MOLECULAR RECOGNITION OF HISTO-BLOOD GROUP ANTIGENS BY HUMAN GALECTIN-1: AN NMR VIEW



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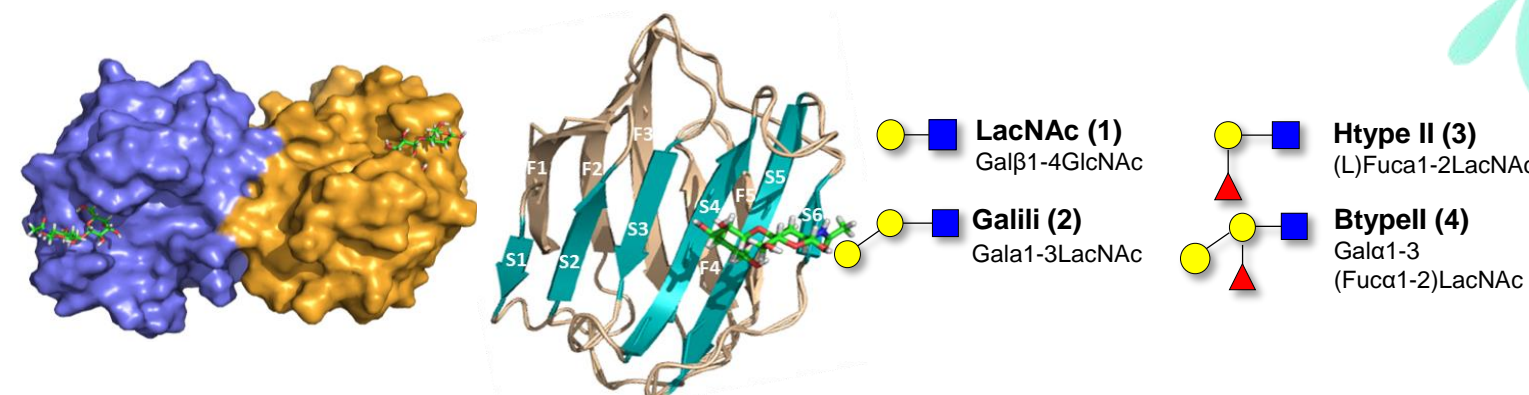
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## INTRODUCTION

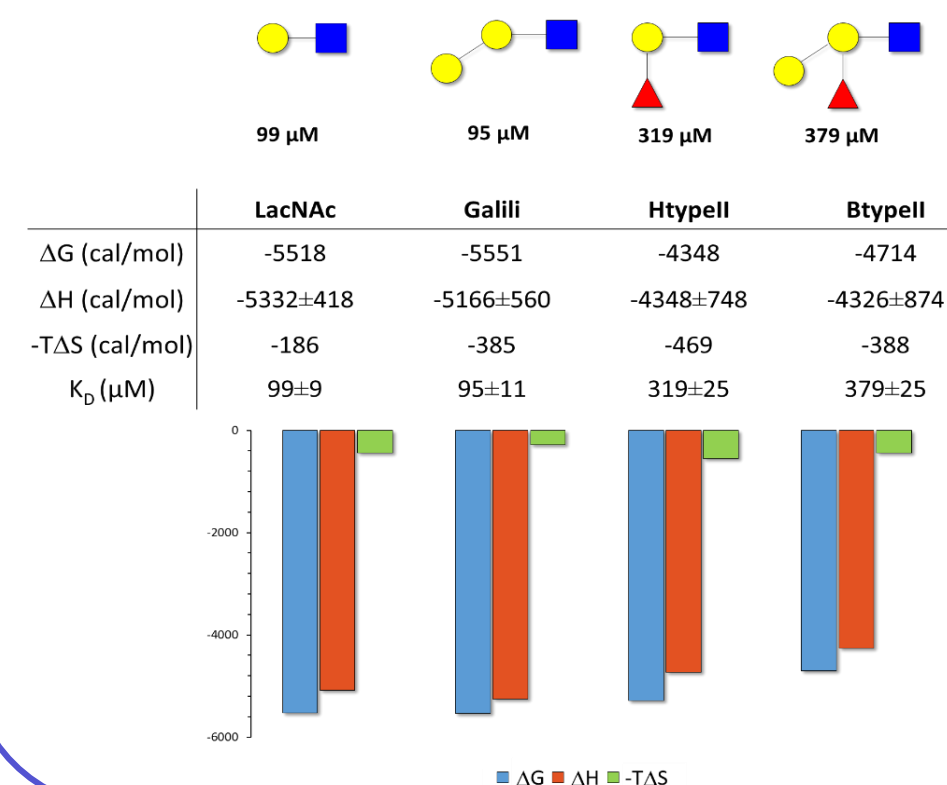
Protein-glycan interactions are linked with several diseases, including infection, cancer or autoimmune disorders. In this context, human galectin-1 (*hGal*-1), an homodimeric  $\beta$ -galactoside binding lectin (**Figure 1**), is involved in a large variety of relevant biological processes such as inflammatory responses, differentiation trafficking, survival of immune cells and establishment and maintenance of T-cell tolerance and homeostasis *in vivo*<sup>[1]</sup>.



**Figure 1.** On the left: *hGal*-1 complexed with LacNAc. In the middle: a monomer of *hGal*-1. On the right: ligands used in the study.

Considering its biological relevance and its growing importance as biomedical target, we have investigated the binding of *hGal*-1 with naturally occurring oligosaccharides containing LacNAc structures. In particular, we studied the molecular recognition of blood group antigens H type II and B type II and their constituent fragments: LacNAc and its 3'-O- $\alpha$ -Gal derivative.

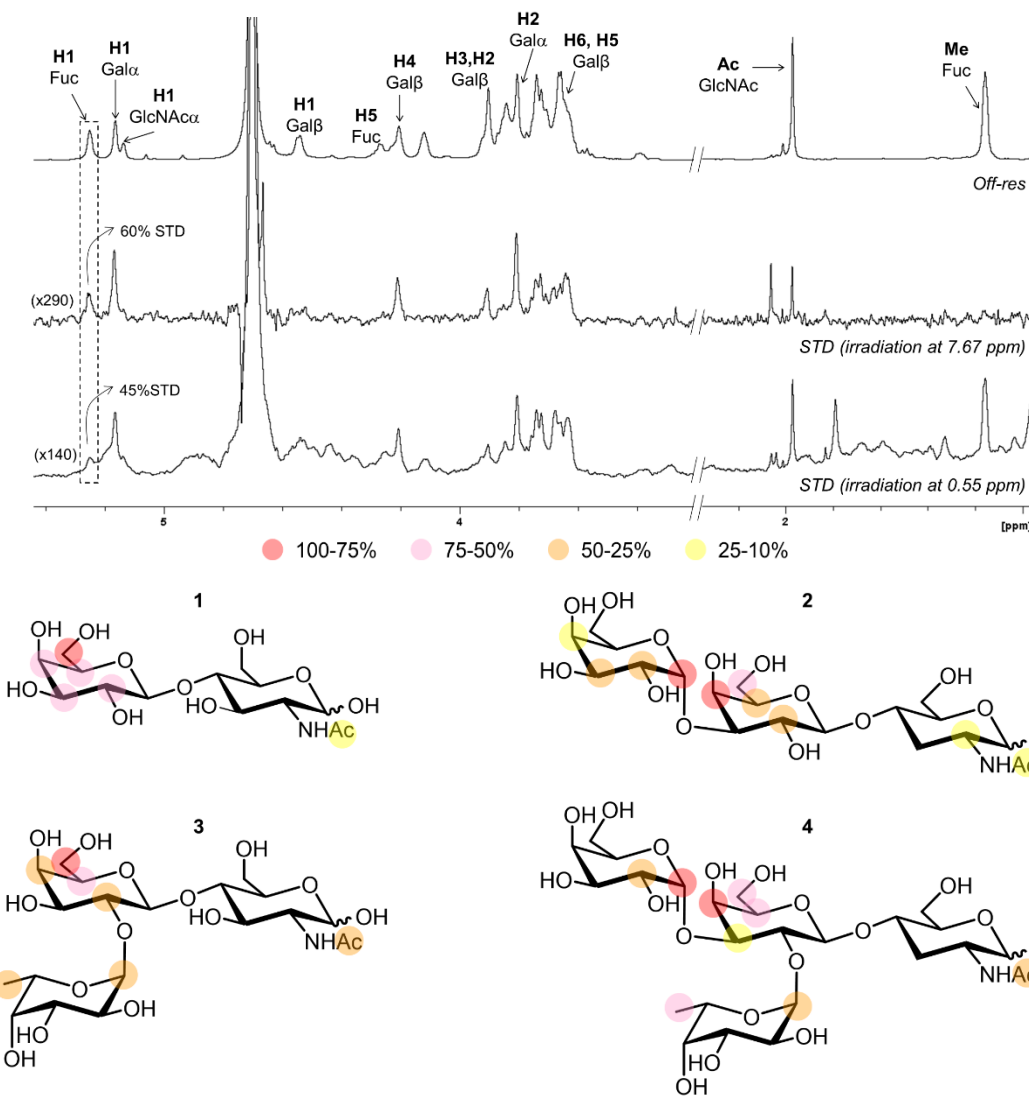
## 1.THERMODYNAMIC PROFILE



The thermodynamic profile was deduced by using Isothermal Calorimetry Titration (ITC). The presence of the Fucose unit and the growing complexity and rigidity of the ligand reduces the binding affinity. For all the ligands the binding entropy is positive, a fact which is rather unusual for lectin-sugar interactions. Differential motion upon binding is probably at the heart of this feature.

## 2.STD-NMR STUDIES

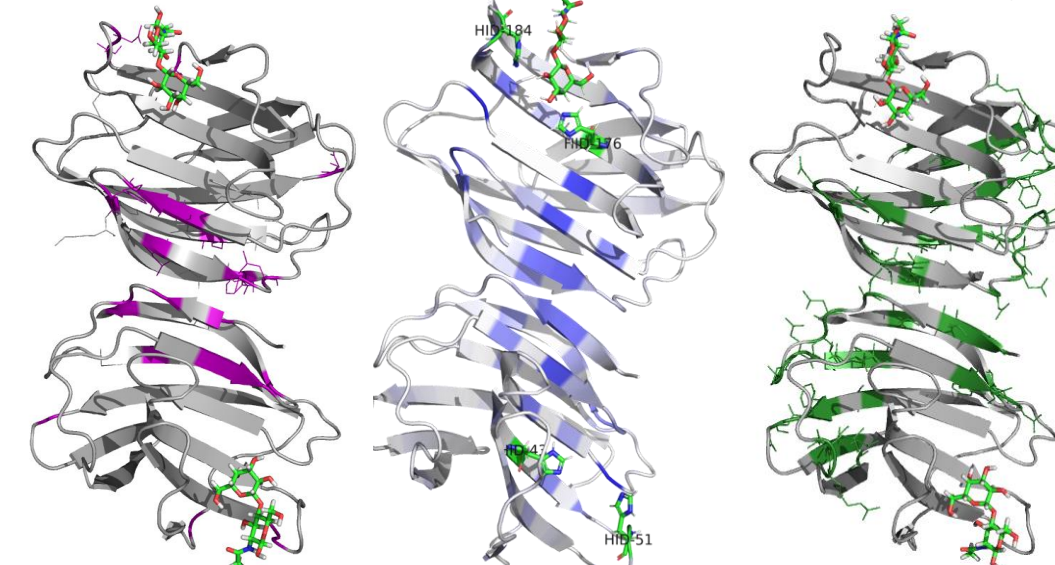
The binding features from the ligand perspective were determined with STD-NMR experiments. The sugars share a common binding mode: significant STD signals for the protons of the central  $\beta$ -Gal were observed (key binding epitope). For the fucosylated ligands, STD signals were observed for the anomeric proton of the Fuc unit, which increased upon irradiation of the aromatic region of the lectin, as shown in **Figure 2**.



**Figure 2.** Comparison between  $^1\text{H}$ -STD-NMR spectra of B type II and *hGal*-1 off-resonance spectrum, STD with irradiation in aromatics and aliphatics. Spectra recorded at 600 MHz. 1, 2, 3, 4: STD epitope map for the interaction of LacNAc, Galili and B type II with *hGal*-1.

## 4. DYNAMIC ASPECTS

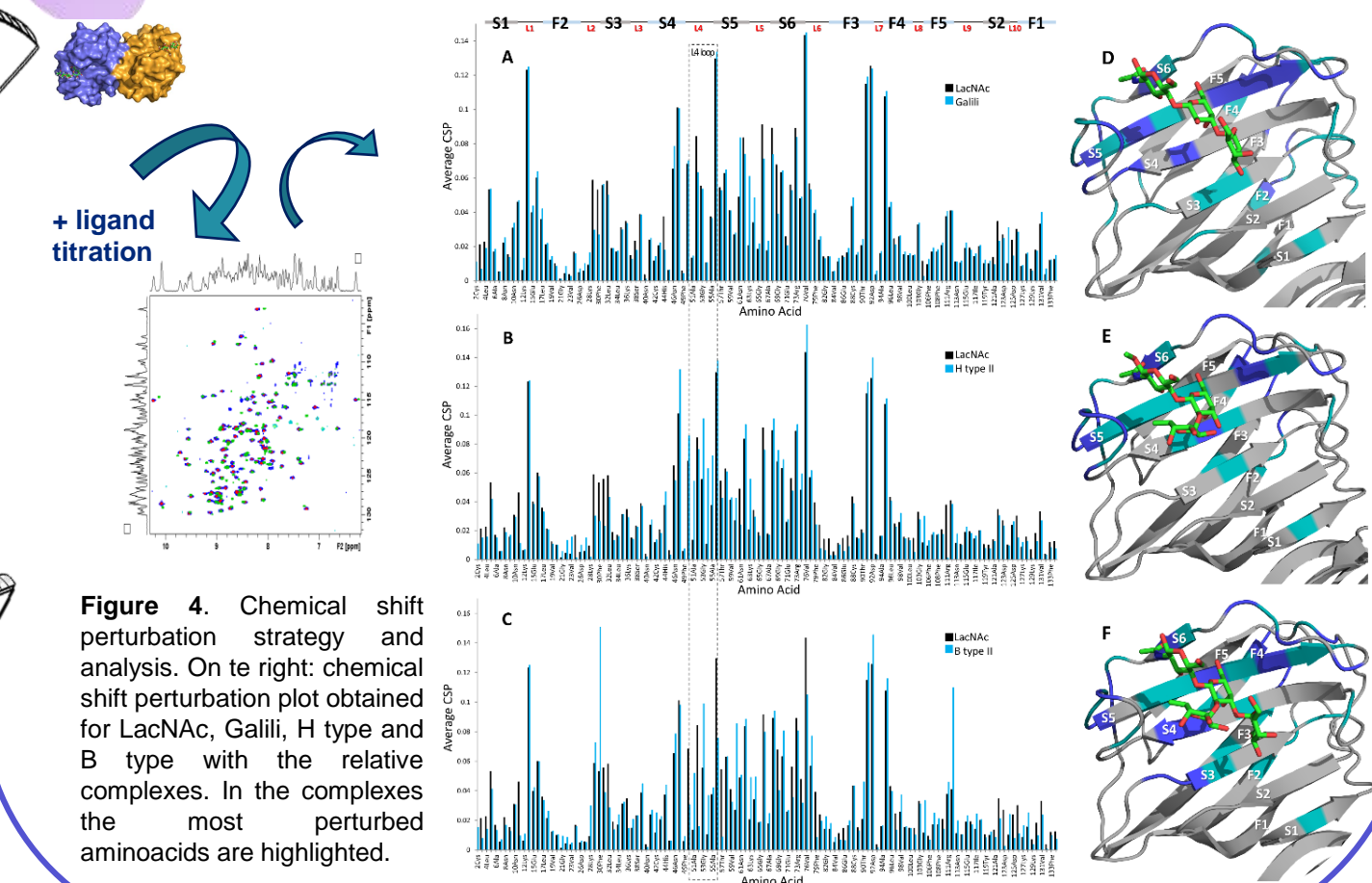
In order to understand the protein dynamic upon binding and explain the ITC data, CPMG relaxation experiments were performed. The results revealed that several amino acids (located at the interface of the two monomers) suffer relevant motions in the milliseconds timescale only upon binding.



**Figure 6.** A) *hGal*1/LacNAc results of relaxation dispersion analysis. B) *hGal*1/LacNAc results of allosteric channels analysis. C) *hGal*1/LacNAc results of CSP experiments

## 3.CHEMICAL SHIFT PERTURBATION ANALYSIS

Protein-based NMR experiments were used to extend the understanding of the binding features. Heteronuclear  $^{15}\text{N}$ - $^1\text{H}$  Transverse Relaxation Optimized Spectroscopy (TROSY) experiments were acquired in the presence of increasing ligand/protein ratios. The glycans display a common binding mode that resembles that described for LacNAc: the main chemical shift changes are in residues located in the strands S3 S4 S5 and S6 of the Carbohydrate Recognition Domain (CRD). However also residues that lay far away from the canonical binding site result perturbed, proposing a conformational change of the whole structure of the protein upon binding. Fucosylated ligands displayed an higher perturbation for the amino acids of the flexible loop in comparison with the other ligands.



**Figure 4.** Chemical shift perturbation strategy and analysis. On the right: chemical shift perturbation plot obtained for LacNAc, Galili, H type and B type with the relative complexes. In the complexes the most perturbed aminoacids are highlighted.

## CONCLUSIONS

Entropy favours the molecular recognition process. The binding epitope of the histo-blood group antigens (STD) has been elucidated. The Fuc unit participates in the binding but without increasing the affinity. Not only residues of the CRD of the protein are affected upon binding, but also some residues far away from the CRD display certain conformational rearrangement (CSP) and undergo critical motions in the milliseconds timescale. Overall, these results provide an analysis, from a molecular point of view, of the role of rigidity of the ligand and its presentation for the binding process, necessary to guide the design and development of galectin-based therapeutic tools.

### Acknowledgements

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### References

[1] J. Camby et al., *Glycobiology*, **2006**, Vol. 16, No. 11, pp 137R-157R.

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