UNTANGLING Tn ANTIGEN STRUCTURE AND ITS FIRST HYDRATION SHELL

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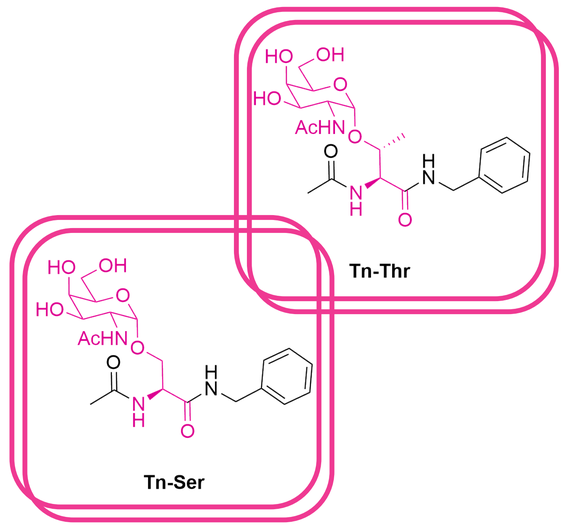
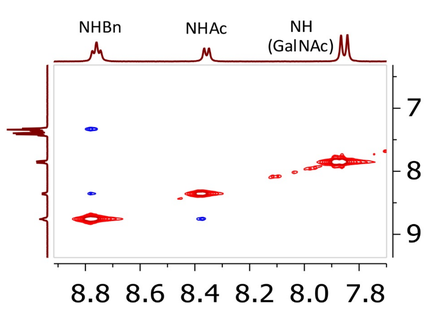
INTRODUCTION

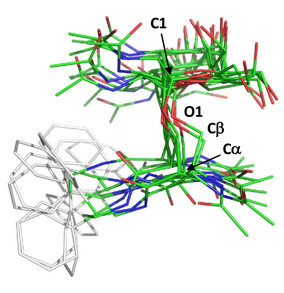
The Tn antigen is one of the most widely studied antigens related to cancer. It comprises an *N*-acetyl-D-galactosamine (GalNAc) linked to either serine (Ser) or threonine (Thr) residues *via* an α-*O*-glycosidic linkage (α-*O*-GalNAc-Ser/Thr) [1]. Even though both have been reported to be identical from a conformational viewpoint, herein we experimentally demonstrate their distinction. As we have postulated before, [2] there are key differences concerning the geometry of the glycosidic linkage and the Ψs torsion angle, which actually affects their interaction with biological targets, such as antibodies,[3] or when acting as antifreeze proteins. To shed some light on the distinct conformational behavior of these entities, derivatives **Tn-Thr** and **Tn-Ser** have been synthesized, carrying a benzylamide group as a chromophore, required for the gas phase experiments (see below) .



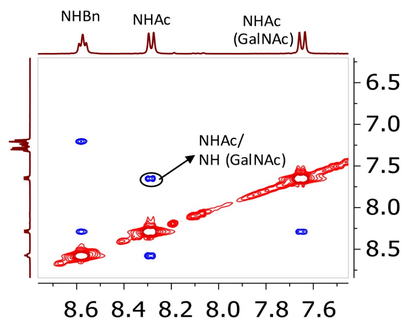
CONFORMATIONAL ANALYSIS IN SOLUTION

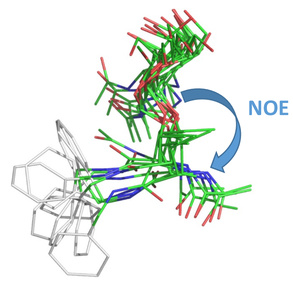
This study combines NMR data and molecular dynamics (MD) simulations. While in **Tn-Thr** derivative the carbohydrate moiety is almost perpendicular to the peptide backbone, in the **Tn-Ser** variant, the sugar adopts a parallel disposition. This characteristic 3D orientation of the carbohydrate in both compounds leads to different first hydration shell. Our studies suggest the existence of bridging water molecules in both antigens between the carbohydrate and the peptide moieties.



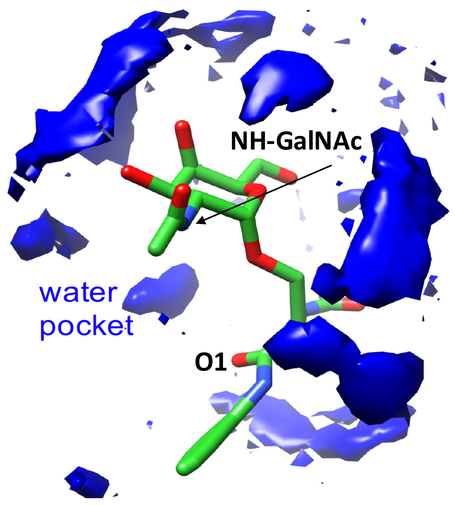


Tn-Ser
Ψ (C1-O1-Cβ-Cα) = 180º
flexible structure
alternate conformation
parallel arrangement

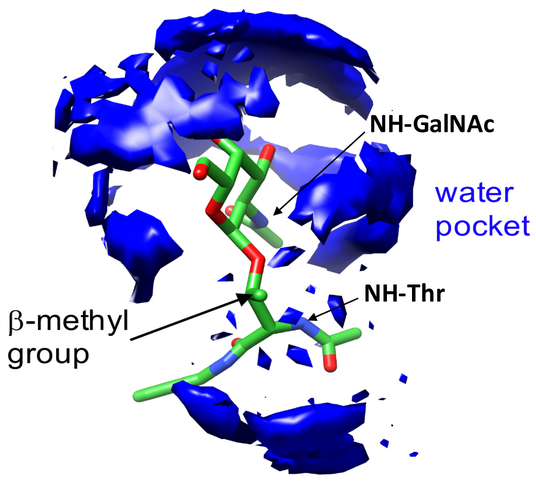




Tn-Thr
Ψ (C1-O1-Cβ-Cα) = 120º
rigid structure
eclipsed conformation
perpendicular arrangement

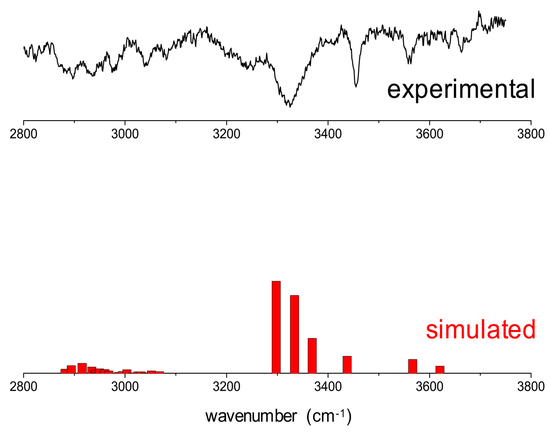


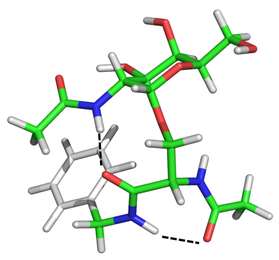
Interestingly, in the **Tn-Ser** derivative a water pocket it is found between O1 and NH-GalNAc amide, whereas in **Tn-Thr** it accommodates between NH-GalNAc and NH-Thr.
Moreover, our calculations point out that glycosidic linkage of **Tn-Thr** may adopt an eclipsed conformation to circumvent the disruption of the first hydration shell around the GalNAc moiety that the β-methyl group could cause in the staggered structure exhibited by **Tn-Ser**.



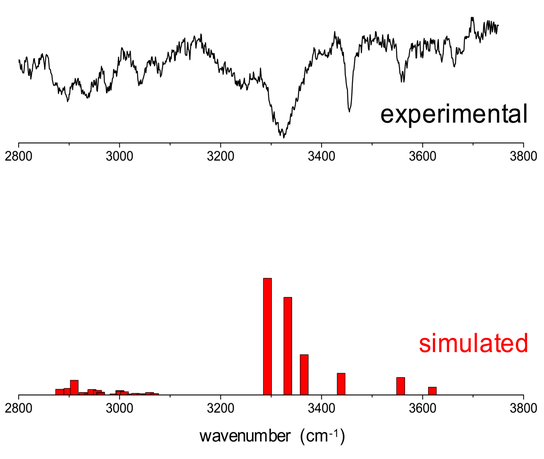
CONFORMATIONAL ANALYSIS IN GAS PHASE

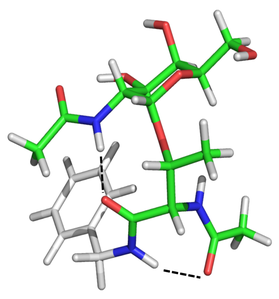
The conformational analysis of both derivatives was then studied in the gas phase through a combination of infrared-ultraviolet double resonance ion-dip (IRID) experiments [4] and DFT calculations. It is worth noting that the infrared spectra of these compounds are virtually identical, showing an exo-anomeric/*syn* conformation for the glycosidic linkage. Besides, the lowest energy structures that reproduce the experimental spectra are identical to each other, supporting the IRID results. Therefore, ***these data demonstrate the key role that the aforementioned water molecules play in the 3D-disposition of these antigens***.





Tn-Ser
glycosidic linkage
(ϕ=88.0,ψ=164.3,χ^1=58.8)





Tn-Thr
glycosidic linkage
(ϕ=90.8,ψ=163.9,χ^1=55.4)

ACKNOWLEDGEMENTS

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