

Local interactions of the asparagine and histidine aminoacids in a protein chain: gas phase conformer-selective IR spectroscopy of model molecules

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Polar residues of proteins, having both donor and acceptor sites, are known by biochemists as promoters of secondary structures such as gamma- and beta-turns. The possibility to locally anchor their side chain (SC) to the main chain (MC) through H-bonding involving side chain polar sites is expected to be at the heart of this behavior.

We will document this H-bond-based anchoring through a gas phase approach that consists i) first, in determining the UV spectroscopic features of the conformations adopted by model peptides (Scheme 1) having a polar neutral residue, such as asparagine (Asn^[1]) and histidine (His^[2]), and ii) second, in determining the intramolecular H-bonds interactions that stabilize them, using a double resonance IR/UV laser spectroscopic approach based on laser-desorbed jet-cooled molecules coupled to quantum chemistry calculations.^[1,2] It will be shown that the local anchoring of the Asn and His side chains (respectively bearing a carboxamide and an imidazole ring) consists of SC-MC and MC-SC H-bonds, which are consistent with the γ - and β -turn backbone secondary structures observed in proteins and contribute to stabilize them.

These gas phase structural data, relevant to a hydrophobic environment, have been used as a reference^[1,2] to assess the anchoring taking place in high resolution crystallized proteins of the Protein Data Bank.^[3] This approach reveals that the stronger MC-SC bonds are preserved in many cases whereas the SC-MC bonds are always disrupted, in qualitative agreement with the gas phase ranking of their strength.

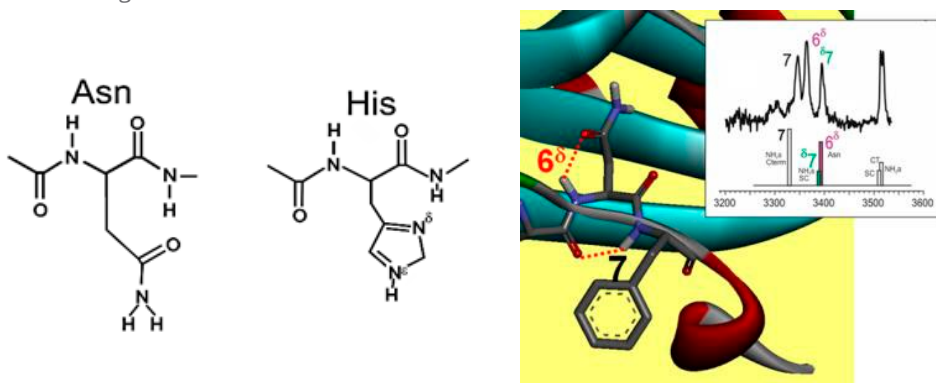


Figure: Example of a conformer-selective IR spectrum (insert) of a model peptide containing an asparagine residue ($-\text{CH}_2-\text{CONH}_2$ side chain) in the NH stretch region, recorded by IR/UV double resonance spectroscopy, illustrating the presence of local main chain/side chain H-bonds, together with an example of the same H-bonding pattern (coloured picture) as found in a protein of the Protein Data Bank.^[3]

[1] Habka et al. *Phys.Chem.Chem.Phys.*, 2018, **20**, 3411

[2] Sohn et al. *Phys.Chem.Chem.Phys.*, 2017, **19**, 17128

[3] Berman et al. *Nucleic Acids Res.*, 2000, **28**, 235

Water Sculpts the Distinctive Shapes and Dynamics of the Tn Antigens: Implications for their Molecular Recognition

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The Tn antigen is one of the most widely studied antigens related to cancer. It includes two variants, GalNAc-O-Thr and GalNAc-O-Ser which differ only in a methyl group. However, despite their structural simplicity and similarity, they display different conformations in solution, which can have significant biological repercussions.[1]

The NMR data and molecular dynamics (MD) simulations show that in GalNAc-O-Thr derivative the carbohydrate moiety is almost perpendicular to the peptide backbone and in the glycosylated Ser variant, the sugar adopts a parallel disposition.[2] This characteristic 3D orientation of the carbohydrate in both compounds leads to different first hydration shells and postulates the existence of water molecules in both antigens that bridge the carbohydrate and the peptide moieties.

A multidisciplinary approach that includes the synthesis, experimental and theoretical study of the Tn antigens in the gas, solution and solid phases has been applied to deduce the key role of water in the modulation of the conformational preferences of these molecules and therefore in their presentations for interacting with protein receptors. Both modified Tn antigens were evaluated in the gas phase, using mass- and conformer-selected infrared laser spectroscopy and coupled with quantum chemical computations.[3] The results obtained were supported by crystal structures of two designed glycopeptides bound to an antibody. [4]

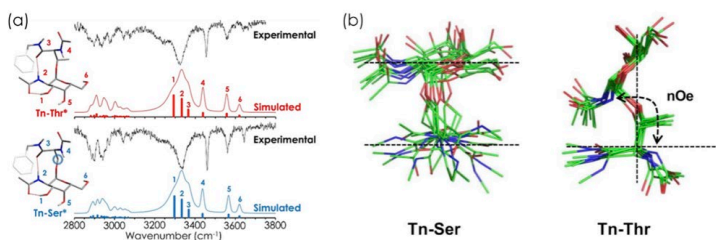


Figure 1. (a) IRID spectra of Tn-Thr* and Tn-Ser* Antigens. (b) Conformation in solution of Tn-Thr and Tn-Ser.

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- [2] Corzana, F.; Busto, J.H.; Jiménez-Osés, G.; García de Luis, M.; Asensio, J.L.; Jiménez-Barbero, J.; Peregrina, J.M.; Avenoza, A. J. *Am. Chem. Soc.* **2007**, *129*, 9458.
- [3] Cocinero, E. J.; Çarçabal, P.; Vaden, T. D.; Simons, J. P.; Davis, B. G. *Nature* **2011**, *469*, 76.
- [4] Bermejo, I. A.; Usabiaga, I.; Compañón, I.; Castro-Lopez, J.; Insausti, A.; Fernández, J. A.; Avenoza, A.; Busto, J. H.; Jiménez-Barbero, J.; Asensio, J. L.; Peregrina, J. M.; Jiménez-Oses G. Hurtado-Guerrero, R.; Cocinero, E. J.; Corzana F. *J. Am. Chem. Soc.* [in press 2018; DOI: 10.1021/jacs.8b04801]

Testing the parity symmetry in cold chiral molecules using vibrational spectroscopy

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Parity violation (PV) has never been observed in chiral molecules. Caused by the weak nuclear force, PV should lead to a tiny energy difference between the enantiomers of a chiral molecule, and in turn to frequency differences in the rovibrational spectra of the two enantiomers of a chiral molecule. A successful PV measurement will shed some light on the origins of biomolecular homochirality. It can also constitute a test of the standard model in the low-energy regime and a probe of physics beyond it, and serve as a stringent benchmark in relativistic quantum chemistry calculations. We present our ongoing work towards developing the technologies needed for measuring PV in chiral molecules via Ramsey interferometry in the mid-infrared. This includes amongst other things developing frequency stabilised quantum cascade lasers calibrated against primary standards and a buffer-gas source of organo-metallic species of interest for a PV measurement formed using laser ablation of solid-state molecules in a cryogenic cell containing gaseous helium at 4 K. We also present the results of preliminary spectroscopic investigations conducted on various species, in particular methyltrioxorhenium (MTO), an achiral test molecule from which promising chiral derivatives have recently been synthesized. We report on the high-resolution spectroscopy of MTO, both in cells and in molecular beams, at various temperatures and resolutions.

Hydrated Lewis antigens in the gas phase: building up the environment or peeling off the solvent?

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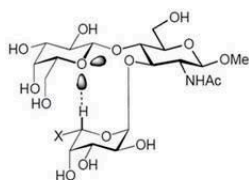
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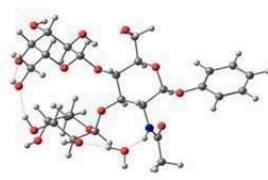
C3.A

Sialyl LewisX and the structurally related trisaccharide Lewis X antigen (LeX) are important carbohydrate as they are recognized by several selectins involved in fundamental pathogenic biological processes. As a consequence, they have been the target of many studies trying to design drugs mimicking them. Most molecular recognition processes are initially driven by "shape recognition" between the receptor and the ligand. A good understanding of the conformational preferences of a molecule is then a requisite starting point for the design of mimetic drugs. In that view, there has been tremendous efforts in resolving the structural properties (conformation, flexibility, specificity) of LeX bound to selectins or in a solvent. These have shown that the bioactive conformation of LeX adopts a stacked arrangement of its Fucose and Galactose components, stabilized by an unconventional CH → O "hydrogen bonding".^[1] In contrast, previous gas phase studies showed that the intrinsic preferred conformation of Lewis X, free of any perturbation from the environment, was stabilized by cooperative OH → O interactions where the Fucose and Galactose units are hydrogen bonded instead of stacked.^[2] This suggested an important role of the solvent in the bio-activity of LeX. In this study, we have then studied the conformational preferences of the hydrated clusters of LeX by a combination double resonance IR-UV laser spectroscopy experiments and computational quantum chemistry conformational landscape exploration. Thanks to the stabilization of large hydrated clusters of LeX we could observe, in the gas phase, its solvent phase, bioactive conformation.

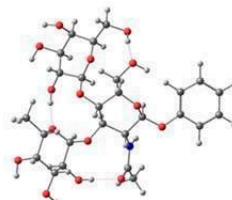
Our results show how important structural water molecules are in the shaping and fine-tuning of carbohydrate bio-active conformations. Their effect must be well taken into account to better scrutinize their functioning and progress towards a rationalization of the design of new drug-like mimetics.



Bioactive Lewis X



Stacking conformation
Of Lewis X - H₂O



Cooperative conformation
of isolated Lewis X

[1] Zierke *et al.*, *J. Am. Chem. Soc.* (2013) **135**, p. 13464

[2] Su *et al.*, *Chem. Phys. Lett.* (2009), **477**, p. 365

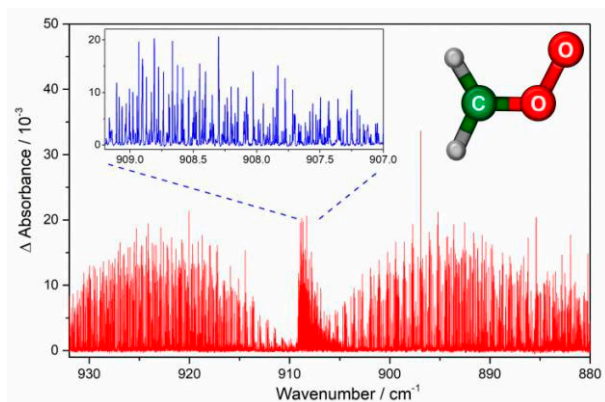
High-resolution ro-vibrational spectrum of the simplest Criegee intermediate CH₂OO between 880 and 932 cm⁻¹

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The Criegee intermediates, carbonyl oxides produced in the ozonolysis of unsaturated hydrocarbons, play important roles in atmospheric chemistry. CH₂OO is the simplest Criegee intermediate and its characterization is important for investigations of its reaction mechanisms and molecular structure¹⁻⁵. In this work, we report the high-resolution ro-vibrational absorption spectrum of CH₂OO recorded with a tunable cw external-cavity quantum cascade laser (EC-QCL) coupled with a Herriott multi-pass absorption cell; the system covers a wide spectral range (880–932 cm⁻¹) with optical resolution 0.0015 cm⁻¹. Over one thousand lines are assigned to determine the spectral constants of the OO-stretching (ν_6) mode of CH₂OO. Perturbations in some rotational levels of this mode are also identified and analyzed. Furthermore, a group of lines near 899.5 cm⁻¹ are identified to be associated with the hot band because their temporal profiles are distinct from those of the ν_6 band.



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- [2] J. M. Beames, F. Liu, L. Lu, and M. I. Lester, *J. Am. Chem. Soc.*, 2012, 134, 20045.
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- [4] M. Nakajima and Y. Endo, *J. Chem. Phys.*, 2013, 139, 101103.
- [5] Y.-P. Lee, *J. Chem. Phys.*, 2015, 143, 020901.