LIGAND-BASED NMR TECHNIQUES TO STUDY BIOMOLECULAR INTERACTIONS: A FOCUS ON SATURATION TRANSFER DIFFERENCE (STD) NMR

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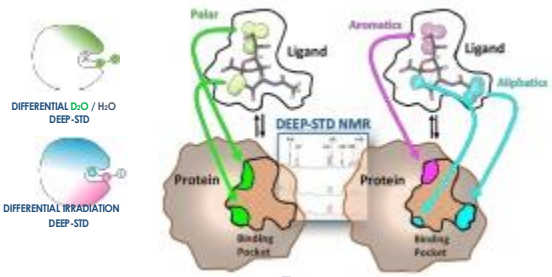

This course is intended for undergraduate and graduate students, as well as for more experienced researchers that want to deepen their knowledge about ligand-based NMR techniques for the study of biomolecular interactions, particularly focusing on the applications of the *Saturation Transfer Difference (STD) NMR* technique. The students should have a background in the basic principles of 1D NMR spectroscopy (2D NMR desirable), as well as in basic structural biochemistry (biomolecular structure).

Dates: 12th, 19th, 26th May, 1pm-3pm (Sao Paulo time zone)

Limit of attendees: up to 100. Overbook will be evaluated.

CONTENT

1.- Binding equilibria and chemical exchange

2.- Ligand-based NMR methods (I). Methods based on relaxation

- 2.1. Transverse relaxation
- 2.2. Longitudinal relaxation
- 2.3. Paramagnetic relaxation
- 2.4. ^{19}F NMR relaxation

3.- Ligand-based NMR methods (II). Methods based on the nuclear Overhauser effect (nOe).

- 3.1. Transfer of *intra-molecular* NOE: exchange-transferred NOE.
- 3.2. Transfer of *inter-molecular* NOE:
  - 3.2.1.- ILOEs and INPHARMA
  - 3.2.2.- WaterLOGSY
  - 3.2.3.- Brief introduction to STD NMR

4.- Ligand-based NMR methods (III). Methods based on translational diffusion.

5.- Coming into the details of *Saturation Transfer Difference (STD) NMR*

- 5.1. Introduction to Saturation Transfer. Chemical exchange
- 5.2. Spin diffusion and STD NMR outcomes

5.3. Ligand screening
5.4. Optimisation of experimental conditions
5.5. Getting structural information from the ligand: Group Epitope Mapping
5.6. Kinetics of STD NMR. T_1-bias
5.7. STD NMR build-up curves. Analysis of initial growth rates

6. Applications of *Saturation Transfer Difference (STD) NMR* (I)

6.1. Saturation Transfer Double Difference (STDD) NMR. On-cell STD NMR
6.2. Affinity measurements. Binding isotherms. Concept of "fast rebinding". Initial growth rates approach.

7. Applications of *Saturation Transfer Difference (STD) NMR* (II)

7.1. Validation of 3D structures of protein-ligand complexes. Docking. MD.
7.2. Complete Relaxation and Conformational Exchange Matrix Approach (CORCEMA-ST)
7.3. Multiple ligand binding modes. Concept of "fast cross-rebinding"

8. Applications of *Saturation Transfer Difference (STD) NMR* (III). Novel Approaches.

8.1. Can we get information about the protein? DEEP-STD NMR
8.2. Spatially resolved STD NMR
8.3. Application of STD NMR to soft-matter (organo- and hydro-gels)
8.4. *spin*-STD (SSTD) NMR. Kinetics of chemical exchange

COURSE MATERIAL

We will not follow one piece of material. There are, however, different materials that can be used to prepare, follow, and deepen into knowledge even beyond the course (see REFERENCES). Among them, excellent discussions about the basics of NMR for ligand-receptor interactions can be found in:

(Book) T. Claridge, HIGH-RESOLUTION NMR TECHNIQUES IN ORGANIC CHEMISTRY, Elsevier, 2016. (Chapters 11; also Chapter 9 for NOE).

(Review) S. Walpole, S. Monaco, R. Nepravishta, J. Angulo, STD NMR as a Technique for Ligand Screening and Structural Studies, *Methods in Enzymology*, Elsevier 2018.

(Review) A.D. Gossert, W. Jahnke, NMR in drug discovery: A practical guide to identification and validation of ligands interacting with biological macromolecules, *Progress in Nuclear Magnetic Resonance Spectroscopy*, 97 (2016) 82-125.

(Review) J.W. Peng, J. Moore, N. Abdul-Manan, NMR experiments for lead generation in drug discovery, *Progress in Nuclear Magnetic Resonance Spectroscopy*, 44 (2004) 225-256.

The Lecturer: Dr Jesús Angulo

Jesús Angulo obtained his MSc and PhD in Chemistry from the University of Seville (US). He was a postdoc in the group of Prof. Thomas Peters at the University of Lübeck. He then was a "Juan de la Cierva" and "Ramón y Cajal" fellow at the CSIC in Seville (IIQ, 2006-2013). In 2013 he joined the School of Pharmacy at the University of East Anglia, becoming Associate Professor. Since 2020 he is a *Senior Distinguished Researcher* at the US leading the Biomolecular Interactions & Structural Glycobiology group, focusing on developing advanced NMR ligand-observed techniques for protein-ligand interactions.

REFERENCES

Articles

M. Mayer, B. Meyer, Characterization of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy, *Angewandte Chemie International Edition*, 38 (1999) 1784-1788.

M. Mayer, B. Meyer, Group Epitope Mapping by Saturation Transfer Difference NMR To Identify Segments of a Ligand in Direct Contact with a Protein Receptor, *Journal of the American Chemical Society*, 123 (2001) 6108-6117.

J. Angulo, I. Diaz, J.J. Reina, G. Tabarani, F. Fieschi, J. Rojo, P.M. Nieto, Saturation Transfer Difference (STD) NMR Spectroscopy Characterization of Dual Binding Mode of a Mannose Disaccharide to DC-SIGN, *Chembiochem*, 9 (2008) 2225-2227.

J. Angulo, P.M. Enriquez-Navas, P.M. Nieto, Ligand-Receptor Binding Affinities from Saturation Transfer Difference (STD) NMR Spectroscopy: The Binding Isotherm of STD Initial Growth Rates, *Chemistry-a European Journa*l, 16 (2010) 7803-7812.

J. Angulo, P.M. Nieto, STD-NMR: application to transient interactions between biomolecules-a quantitative approach, *European Biophysics Journal with Biophysics Letters*, 40 (2011) 1357-1369.

S. Monaco, L.E. Tailford, N. Juge, J. Angulo, Differential Epitope Mapping by STD NMR Spectroscopy To Reveal the Nature of Protein–Ligand Contacts, *Angewandte Chemie International Edition*, 56 (2017) 15289-15293.

R. Nepravishta, S. Walpole, L. Tailford, N. Juge, J. Angulo, Deriving Ligand Orientation in Weak Protein–Ligand Complexes by DEEP-STD NMR Spectroscopy in the Absence of Protein Chemical-Shift Assignment, *ChemBioChem*, 20 (2019) 340-344.

R. Nepravishta, S. Monaco, J. Munoz Garcia, Y. Khimyak, J. Angulo, Spatially Resolved STD-NMR Applied to the Study of Solute Transport in Biphasic Systems. Application to Protein-Ligand Interactions, *Natural Product Communications*, (2019).

Books

D. Neuhaus, M.P. Williamson, THE NUCLEAR OVERHAUSER EFFECT IN STRUCTURAL AND CONFORMATIONAL ANALYSIS, John Wiley & Sons, April 2000
T. Claridge, HIGH-RESOLUTION NMR TECHNIQUES IN ORGANIC CHEMISTRY, Elsevier, 2016.