

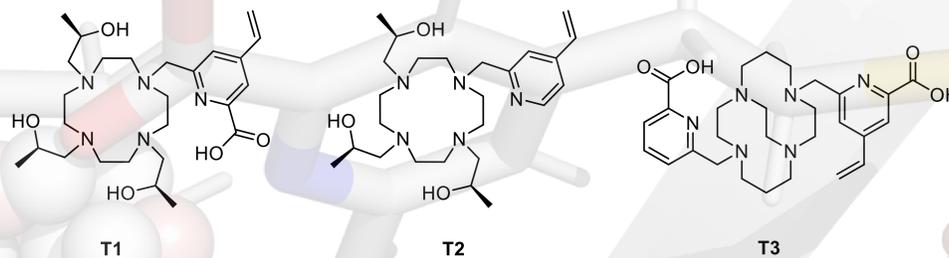
# Design of new paramagnetic tags for NMR Spectroscopy: New strategies to yield fast, selective and irreversible tagging of protein

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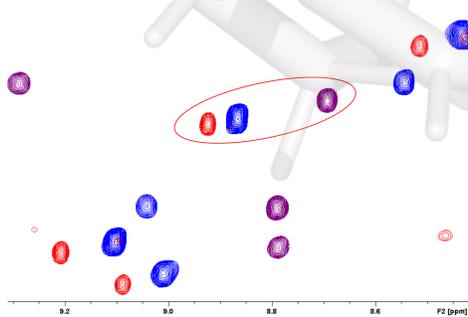
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## INTRODUCTION

Paramagnetic restraints have been used in Biomolecular NMR for the last three decades in order to elucidate and refine structures but also to characterize protein/ligand interactions. Pseudo Contact Shifts (PCS) are the most commonly used restraints, consisting in measuring a shift between a paramagnetic species and a diamagnetic reference, whose magnitude depends on the distance from the paramagnetic centre<sup>1</sup>. A common technique to generate such restraints consists in the attachment of Lanthanides ions to the protein via a Lanthanide-Binding-Tag (LBT). Here, we present 3 novel LBTs. They all feature a vinyl-pyridine moiety for a conjugation through irreversible thiol-ene reaction. Their paramagnetic performances as well as conjugation conditions are discussed.



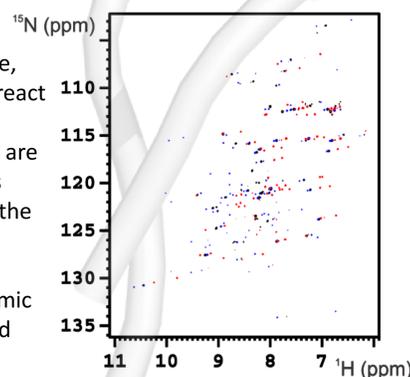
## PHOTO-CATALYZED THIOLENE REACTION



2D <sup>1</sup>H-<sup>15</sup>N HSQC of GB1 T53C tagged with T1  
Lanthanum (dia)  
Ytterbium (para)  
Dysprosium (para)

- Cysteine-selective reaction, only one set of peaks is observed
- Fast and irreversible paramagnetic tagging<sup>2</sup>
- Easy-to-synthesise T1 and T2

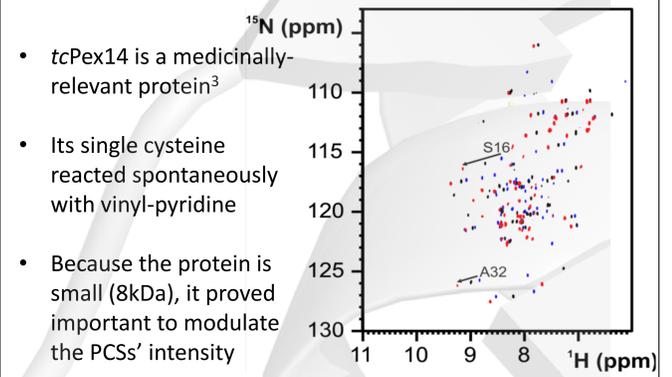
## THE CHEMICAL INERTNESS OF T3



2D <sup>1</sup>H-<sup>15</sup>N HSQC of GB1 T53C tagged with T3  
Lanthanum (dia)  
Ytterbium (para)  
Dysprosium (para)

- At room temperature, GB1's T53C doesn't react
- Because GB1 and T3 are thermostable, it was possible to perform the reaction at 70°C
- Because T3 is a racemic mixture, we obtained two set of peaks
- It was possible to isolate and analyse separately both sets

## TOWARDS FRAGMENT PLACEMENT ON tcPex14



2D <sup>1</sup>H-<sup>15</sup>N HSQC of tcPex14 tagged with vdp  
Diamagnetic (Lu)  
Paramagnetic (Yb)  
Paramagnetic (Tm)

- tcPex14 is a medically-relevant protein<sup>3</sup>
- Its single cysteine reacted spontaneously with vinyl-pyridine
- Because the protein is small (8kDa), it proved important to modulate the PCSs' intensity
- We do this in an effort to characterize the binding mode of small ligands using PCS (Poster #481)

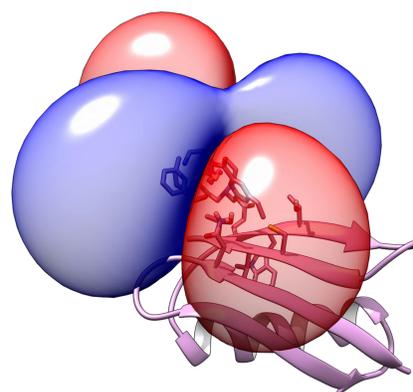
## CONCLUSION

In this work, we presented different vinyl-pyridine based LBTs. We show that they offer a versatile way of introducing paramagnetic restraints on different kind of proteins. Those ligands offer the possibility to easily generate NMR-restraints. While T3 offers an outstanding kinetic inertness, T1 and T2 present different coordinating environment which is expected to influence the paramagnetic properties of the Lanthanide and the interaction between the tag and the protein's surface.

This is particularly attractive in the field of structure-based drug discovery where it has been shown that paramagnetic restraints can help determine the binding-pose of small ligands even in the absence of X-ray data. We are working in this direction, with a focus on tcPex14.

	Metal	Q	$\Delta\chi_{ax}$ (ppm)	$\Delta\chi_{rh}$ (ppm)
T1	Yb	0.099	1.54	-0.531
	Dy	0.071	-5.82	2.03
T3.A	Yb	0.088	3.31	-1.82
	Dy	0.138	-17.33	9.17
T3.B	Yb	0.063	-16.07	7.77
	Dy	0.041	57.94	-28.22

PCS-based tensor values for GB1 T53C tagged with T1 and T3.  
The PCS were analyzed with FANTEN



PCS-Isosurface (1ppm) for GB1 T53C tagged with T3.B

References: 1. Bertini, I., Luchinat, C. & Parigi, G. *PNMRS*, 40, 249–273 (2002). 2. Li, Q.-F., Yang, Y., Maleckis, A., Otting, G. & Su, X.-C. *Chem. Commun.* 48, 2704 (2012). 3. Dawidowski, M. et al. *Science* (80-.). 355, 1416–1420 (2017).

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