Placing fragments with lanthanide tags using paramagnetic NMR

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Paramagnetic NMR using lanthanide binding tags has proven to be very useful for structural characterization of proteins and strongly bound ligands in slow exchange. This is based on the distance and orientation dependence of pseudocontact shifts (PCS) induced by the paramagnetic center. A combination of NMR titrations and binding kinetics from other techniques such as AlphaScreen™ can be used to tackle the challenge of using this approach with molecules binding in fast exchange, for example fragment molecules in drug discovery programs, to characterise the binding pose. This could aid in fragment placement for key drug development projects where crystallisation or soaking fragments is difficult, as is often the case in early stage drug discovery. We use DOTA-M8[a] and vinyl-dipicolinic acid[b] to determine the binding site of a small fluorinated fragment that binds in fast exchange to *Trypanosoma cruzi* PEX14 N-terminal domain using Lutetium, Ytterbium and Thulium. This is a medically relevant target in the treatment of Chagas Disease, prevalent in South America: interruption of the Protein-Protein Interface (PPI) between this and PEX5 has been shown to lead to death of the parasite[c] but soaking and co-crystallisation with fragments has proved challenging.

[a]DOTA-M8: An Extremely Rigid, High-Affinity Lanthanide Chelating Tag for PCS NMR Spectroscopy (Häussinger et al, JACS 2009)
[b]A Dipicolinic Acid Tag for Rigid Lanthanide Tagging of Proteins and Paramagnetic NMR Spectroscopy (Su et al, JACS 2008)
[c]Inhibitors of PEX14 disrupt protein import into glycosomes and kill Trypanosoma parasites (Dawidowski et al, Science 2017)