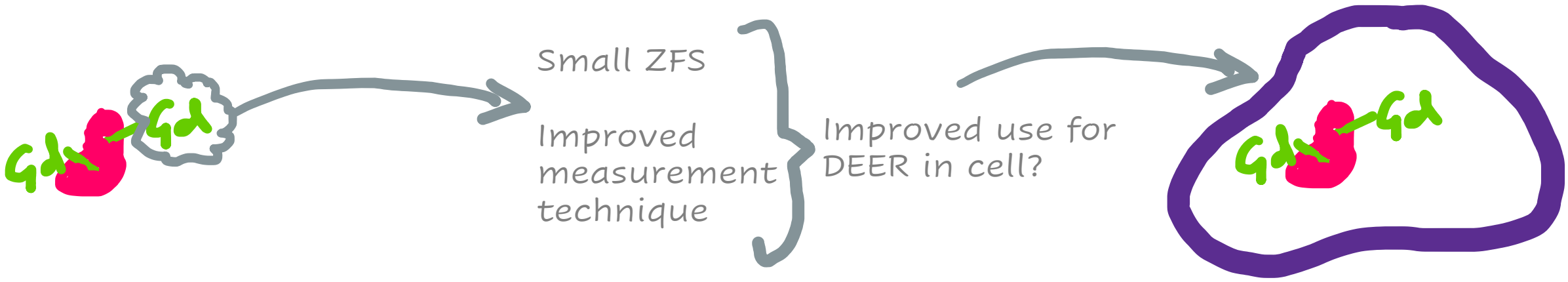
The use of Gd(III) with narrow ZFS for DEER

Janet E. Lovett[a*], Anokhi Shah[a], Hassane EL Mkami[a], Robert I. Hunter[a], Paul A. S. Cruickshank[a], Michael J. Taylor[a], Graham M. Smith[a], Amandine Roux[b], Matthieu Stark[b], Jackie A. Mosely[b], David Parker[b], Michael Stevens[c], David G. Norman[c], Akiva Feintuch[d], Mian Qi[e], Adelheid Godt[e]

[a] *University of St Andrews, School of Physics and Astronomy and the BSRC, UK,* [b] *University of Durham, Department of Chemistry, UK,* [c] *College of Life Sciences, University of Dundee, UK,* [d] *Department of Chemical Phyiscs, Weizmann Institute of Science, Israel,* [e] *Faculty of Chemistry and Center of Molecular Materials, Bielefeld University, Germany.*

* jel20@st-andrews.ac.uk

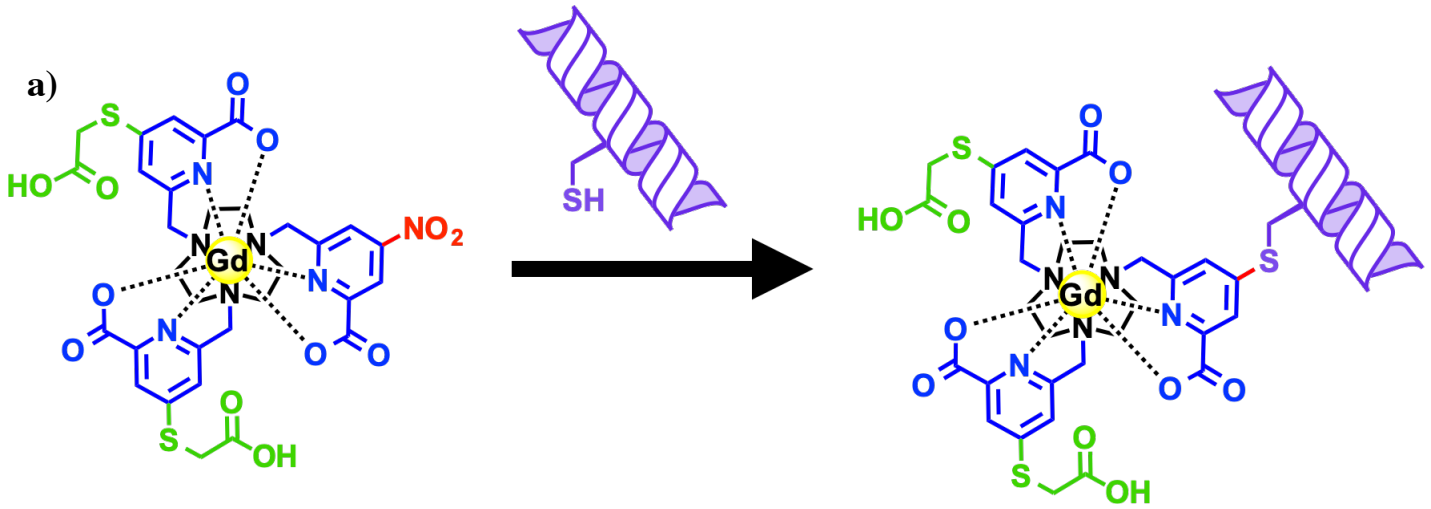


Why bother?

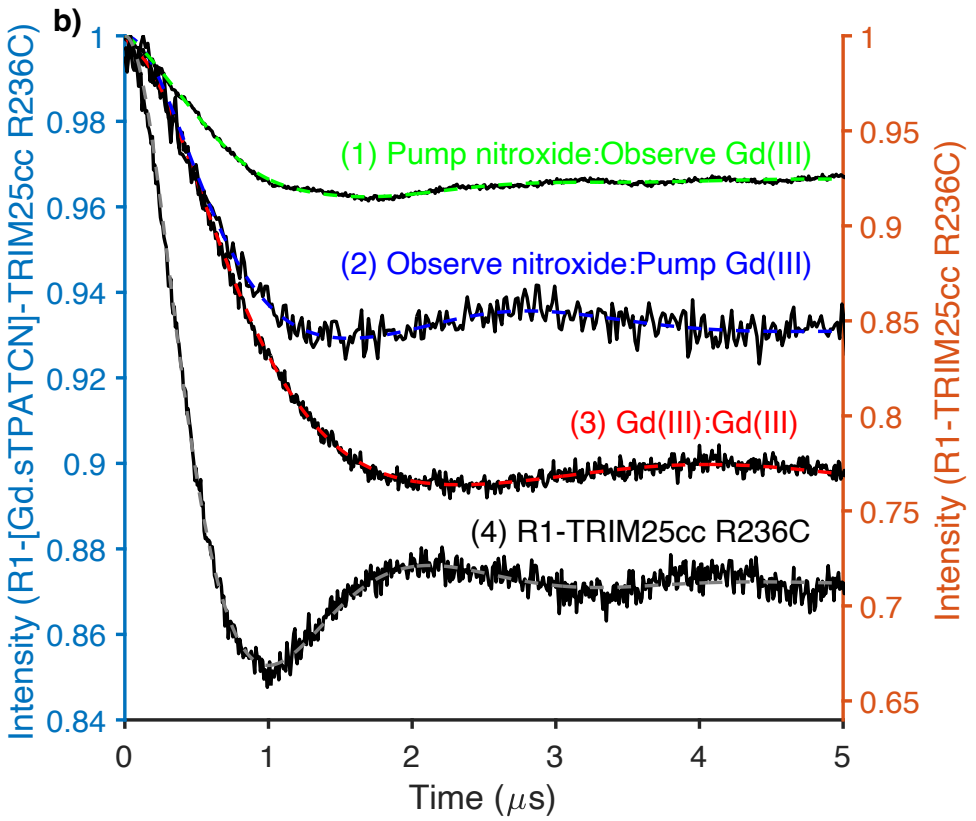
- Gd(III) labels have been proven to be useful for in cell measurements:
  - the paramagnetic centre remains within the cell;
  - Gd(III) has a signal unique from the background of the cell's.
- Ideally the Gd(III) DEER signal needs to be measurable at low concentrations.
  - An additional challenge to this is the low modulation depth of the DEER for Gd(III-Gd(III).
- Gd(III) labels often lead to broad DEER distributions:
  - this has root-cause not only in the size of the complex/linker system
  - but also in the underlying physics of measuring the Gd(III) with pulsed EPR.

A Gd(III) spin label with a narrow ZFS and short linker: [Gd.sTPATCN]-SL

Shah et al., *Inorg Chem.*, **2019**, 58, 3015



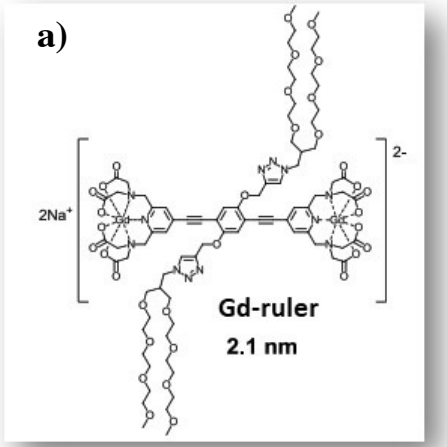
- New Gd(III) spin label for non-reducible linkage to cysteines with a short tether, see figure a.
- FWHH of the central transition comparable to the shortest measured at c.a. 2.5 mT at Q-band and 1.1 mT at W-band (both at 10 K).
- DEER EPR measurements of the dipolar coupling between spin label pairs or spin label to nitroxide (R1) labels on a protein (TRIM25cc, figure b) show that the Gd label occupies a different conformational space to R1 but that the overall signal is promising.

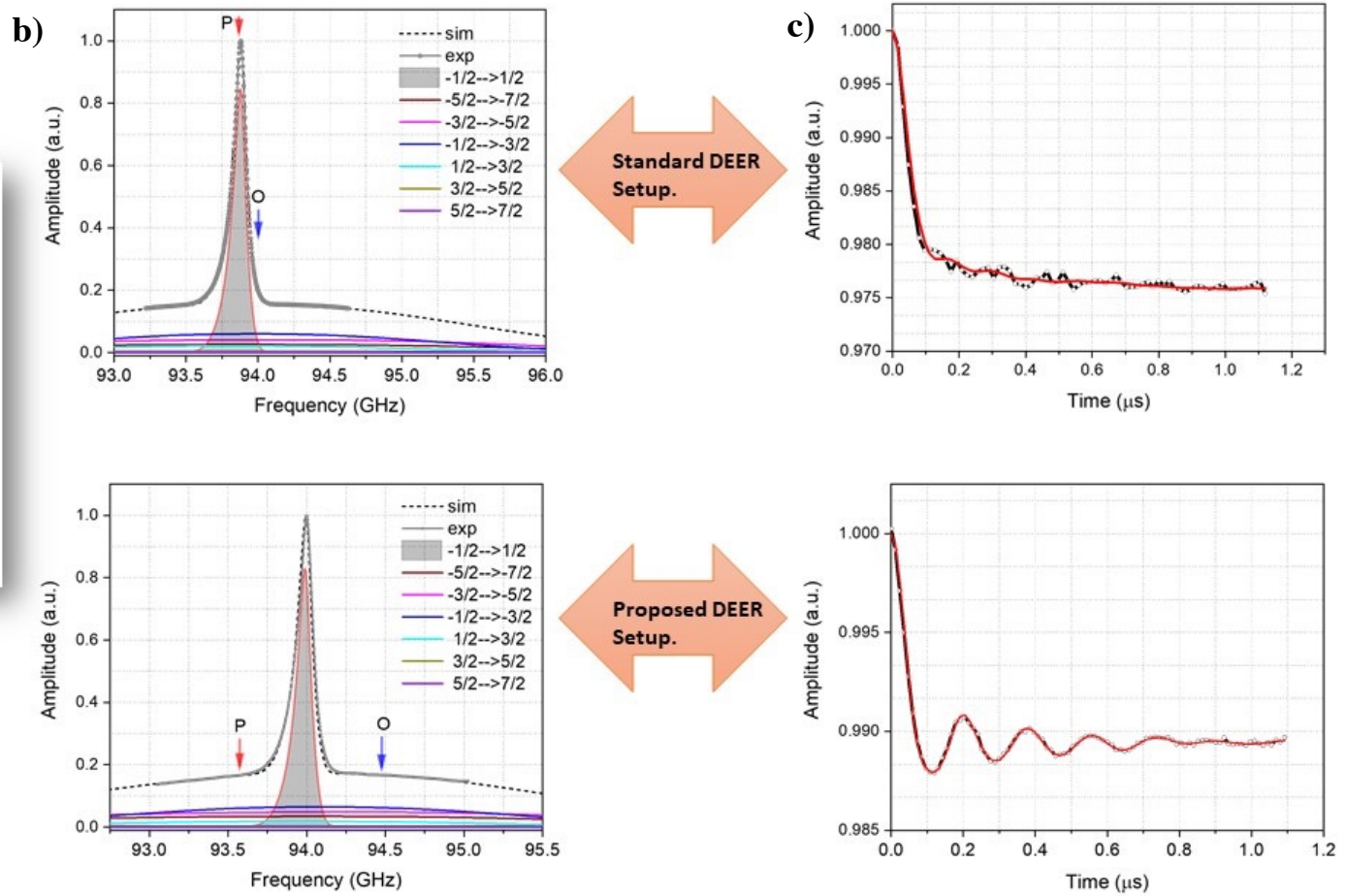


Improving the DEER measurement of Gd(III)-Gd(III) distances

EL Mkami et al., *Magn Reson.*, **2020**, 1, 301

- HiPER (W-band) DEER EPR measurements on a PyMTA Gd ruler (figure a) show that significant improvements in measurement quality can be given by modifying the pump and probe positions from the traditional set up of pumping the Gd(III) central transition (figures b and c).





Where next?

- Further optimization of labelling conditions and in cell testing of [Gd.sTPATCN]-SL.
- Further optimization and testing of HiPER and at lower frequencies (using commercial spectrometers) for measuring Gd(III) complexes of varying ZFS at lower concentrations.
- In cell work combining our findings!