Accurate orientation PELDOR measurements and analysis using rigid spin labels at high fields

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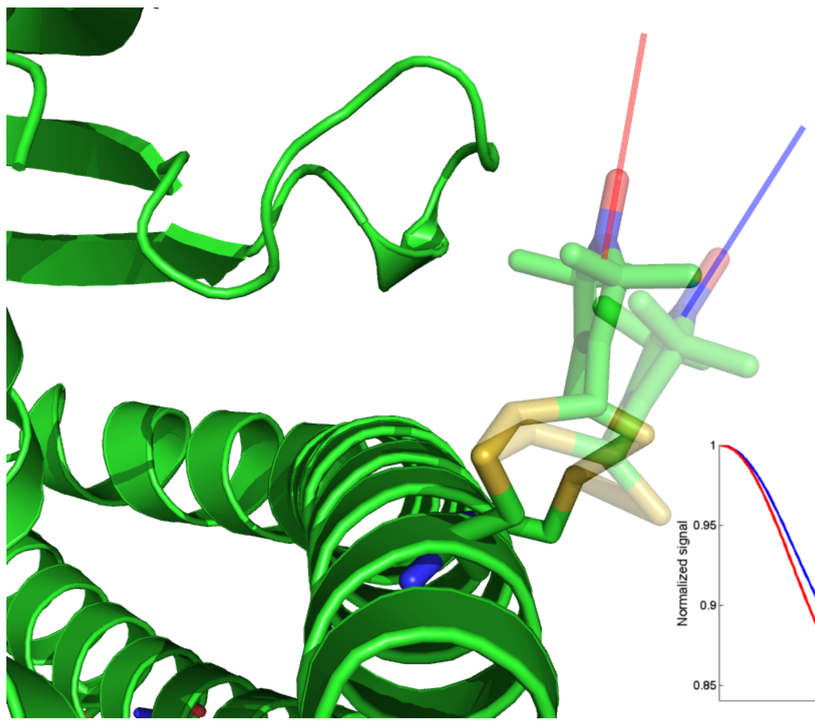
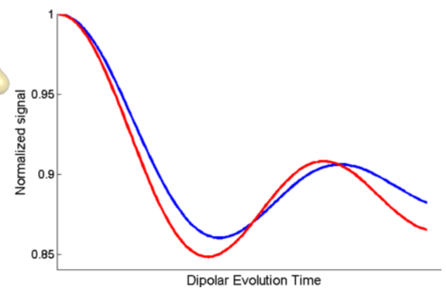
Methods for extracting orientation information



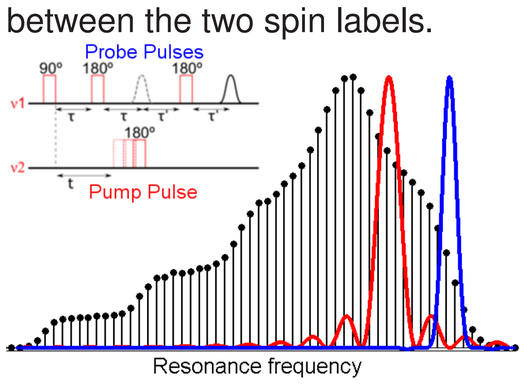
A change in the spin label's orientation relative to the other spin label results in a change in the observed PELDOR signal

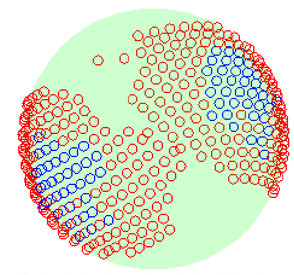


Orientation selective pulsed electron-electron double resonance (PELDOR) spectroscopy at high field has a strong dependence on spin label orientation [1]. **Mutual orientation of the spin labels can be resolved to a better than 5 degree accuracy by doing several optimized PELDOR experiments at W-band.**

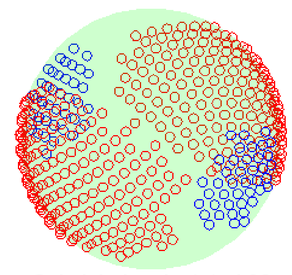
Optimized orientation selective PELDOR

PELDOR experiments use two pulsed microwave sources at different frequencies; the pump frequency and the probe frequency. The pump pulse inverts one of a pair of spins, causing a local magnetic field variation around the other spin. This is detected with a refocused echo using the probe pulse. Due to anisotropic effects at high fields this experiment can resolve differences in orientation between the two spin labels.





Spin labels orientated in the same direction

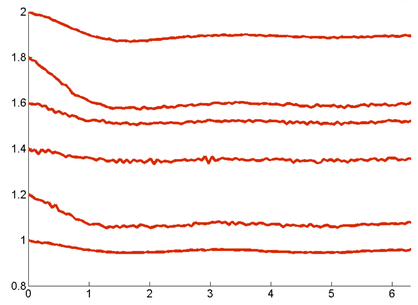


Spin labels orientated 90 degrees apart around Z

Shown above is the excitation of an orientation selective PELDOR experiment, left as a plot of resonance frequency and right geometrically. It can be shown empirically by examining these plots that there exists optimal choices of pump and probe combinations. This can be automated using statistical methods. **It is possible to optimize this experiment such that it provides maximal differences in modulation depth for varying orientations with minimal ambiguity from the fewest possible different excitations.**

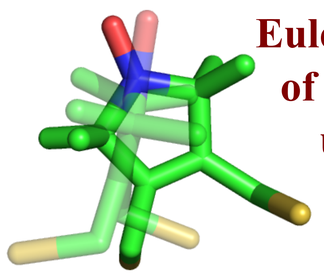
A method for interpretation of orientation selective PELDOR

Set of PELDOR traces

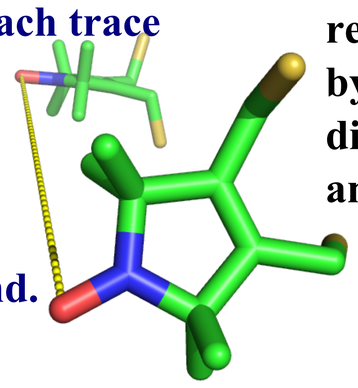


PELDOR traces of VPS75 1619 sample

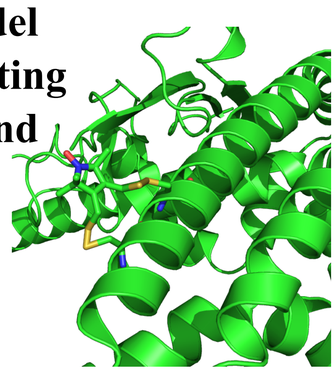
Extract the modulation depths of each trace to calculate relative orientation and distribution Euler angles (αβγ) of spin label pair using a library approach.



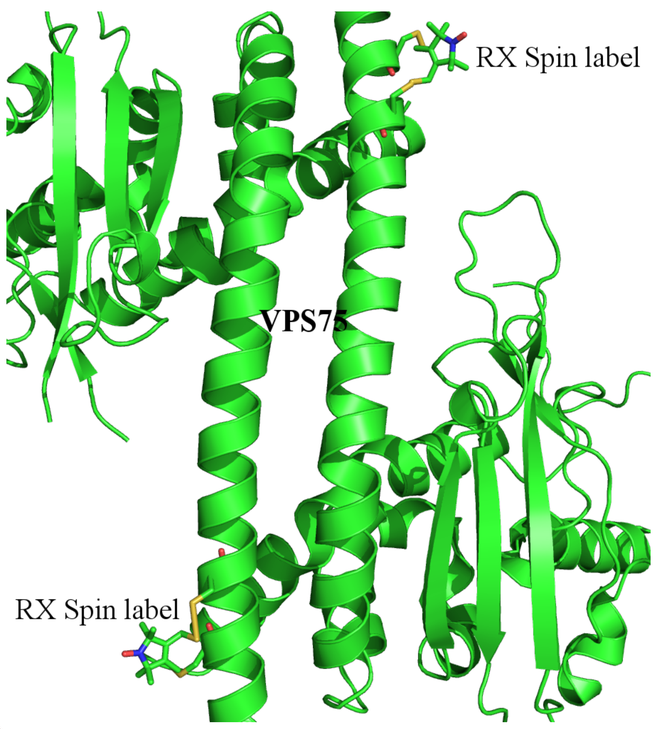
Extract the dipolar frequency components of each trace to find relative position and distance (rθϕ). Initial distances derived at X band.



Fitting methods to refine model by correlating distance and angles.

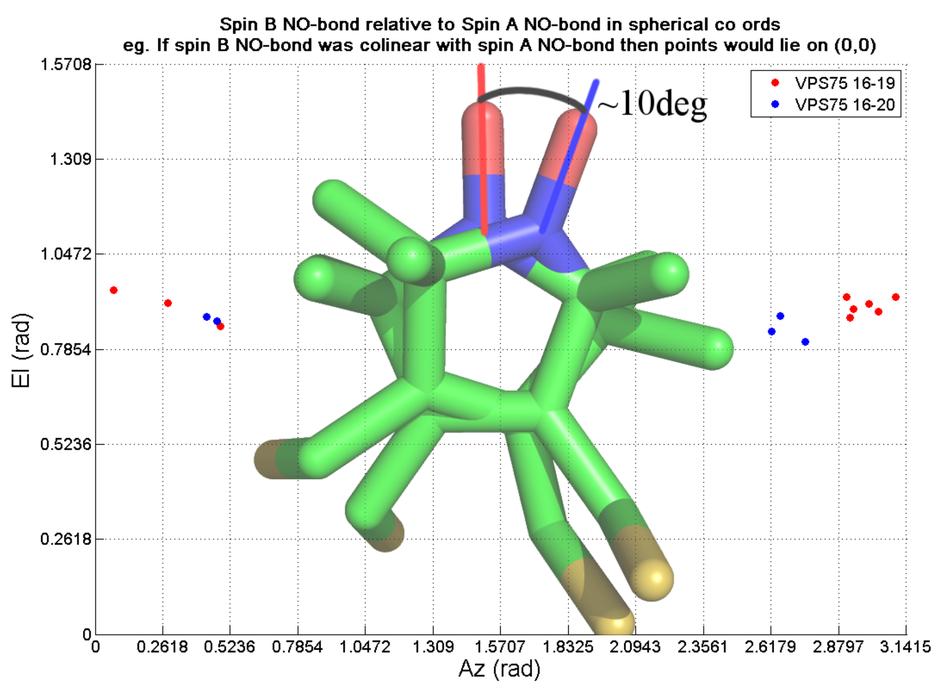


Key results for RX spin labeled VPS75



Vacuolar protein sorting-associated protein 75 (VPS75) has been prepared into two samples, one labeled with RX spin labels [2] at the residue sites 16 and 20 and the other at residue sites 16 and 19. This difference in attachment leads to a subtle change in relative orientation and distance between the spin labels. At X band the distance distributions show a change of approximately 3Å.

At high field, using the HiPER spectrometer, it has been possible to resolve this change in orientation, with the nitroxide bond pointing direction having a difference of approximately 10°.



Ongoing work

- **Derive a generic automated method to optimize PELDOR experiments at W band.**
- **Use of highly efficient simulations to find optimal experimental parameters dynamically during the experiments.**
- **A metric for disorder - By examining the solution space for a rigid system and then comparing this to cases of increased disorder it is possible to conclude a metric of disorder.**

References

1 Reginsson, G. W., Hunter, R. I., Cruickshank, P. A., Bolton, D. R., Sigurdsson, S. T., Smith, G. M., & Schiemann, O. (2012). W-band PELDOR with 1kW microwave power: Molecular geometry, flexibility and exchange coupling. Journal of Magnetic Resonance, 216, 175-182.

2 Fleissner, M. R., Bridges, M. D., Brooks, E. K., Cascio, D., Kálai, T., Hideg, K., & Hubbell, W. L. (2011). Structure and dynamics of a conformationally constrained nitroxide side chain and applications in EPR spectroscopy. Proceedings of the National Academy of Sciences, 108(39), 16241-16246.