Localized DNP Enhancement in Biomolecules

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With increasing complexity of biomolecular assembly, sensitivity as well as specificity play a major role in NMR-based structural biology. Dynamic nuclear polarization (DNP) has shown tremendous potential to increase sensitivity in numerous applications.[1] Even though in conventional DNP experiments uniform signal enhancements are typically obtained, DNP itself can act as a source of specificity as well.[2] In this presentation, two methods allowing to introduce a large degree of specificity to DNP-enhanced MAS NMR spectra will be presented.

The first method utilizes the distance dependence of the dipolar hyperfine interaction between the electron spin (source) and nuclear spin (target). The hyperfine interaction is mediating the initial step of the complex mechanism of the overall DNP transfer. By microwave irradiation, electron-nuclear coherences are generated which finally result in nuclear hyperpolarization. If subsequent spin diffusion is restricted, this transfer dynamic can act as a measure for hyperfine interaction. However, the competing paramagnetically enhanced spin-lattice relaxation counteracts the creation of a polarization gradient, theoretically leading to uniform, distance-independent DNP enhancement. Nevertheless, the direct-DNP build-up rate can act as a direct measure of this interaction and can thus yield distance information in biomolecules. We will present experimental evidence for this elusive DNP distance dependence on a ubiquitin model protein spin-labeled with a Gd^{3+} chelate tag.[3] By correlating the ^{15}N build-up rates with computationally assisted structural modeling of the paramagnetically labeled protein, the theoretically r^{-6} dependence is observed. However, the paramagnetic Gd^{3+} generates a bleaching volume with a radius of 12 Å within which no nuclear spin contributes to the measured rate. This opens up the possibility to extract distance restraints directly from direct DNP which can be further used in structural modeling.

The second method employs efficient indirect DNP (via ^{1}H), but introduces methyl groups as localized polarization transfer pathways between ^{1}H and ^{13}C. The introduction of specifically ^{13}C-labeled methyl groups via different biomolecular methods enables the discrete placement of antennas for hyperpolarization within the carbon network. Based on heteronuclear cross-relaxation, SCREAM-DNP (specific cross-relaxation enhancement by active motions under DNP) can thus probe the local environment around these highly dynamic functions.[4] This is especially interesting in the context of biomolecular complexes and specific contacts between binding partners.[5] Examples and applications on proteins, RNA, and ribonucleoproteins (RNPs) will be presented and future implications discussed.

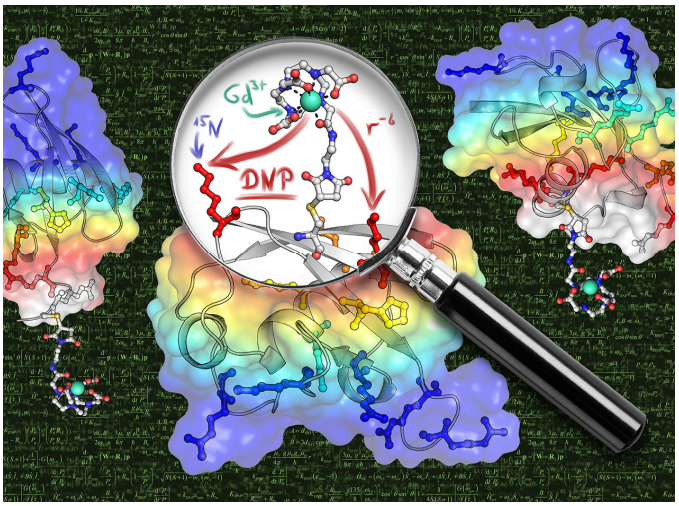

Figure 1. Artist's sketch of site-specific DNP from a Gd^{3+}-tag to ^{15}N nuclei in the local environment.

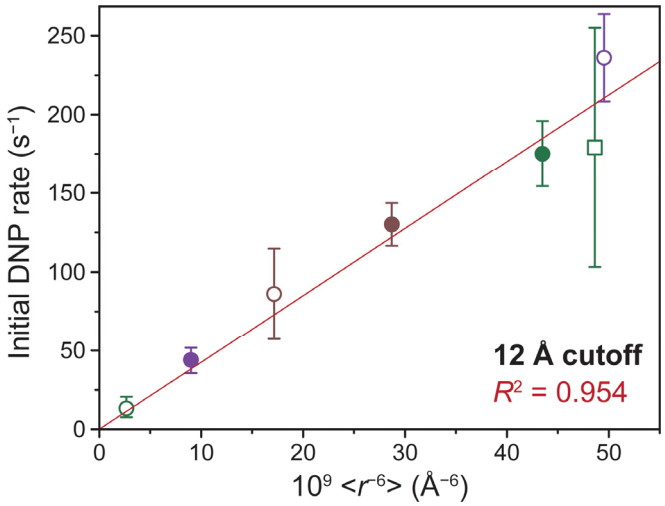

Figure 2. Distance dependence of the experimentally measured DNP build-up rate as a function of the mean distance between Gd^{3+} label and specific amino acid residues in ubiquitin.

References

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2. R. Rogawski and A. E. McDermott, *Arch. Biochem. Biophys.*, 2017, **628**, 102-113.
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4. V. Aladin and B. Corzilius, *eMagRes*, 2020, **9**, 1-9.
5. V. Aladin et al., *Angew. Chem. Int. Ed.*, 2019, **58**, 4863-4868.