

## Probing DNP Enhancements for Biological Applications

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Dynamic Nuclear Polarisation (DNP) is used to transfer the high spin polarization of unpaired electrons to coupled nuclear spins. Stable radicals are added to a solution of the analyte and irradiating with microwaves is applied at the EPR lines of the radical. At temperatures of 1.5K polarisation times of 1-3h are required for optimal polarisation of  $^{13}\text{C}$  and  $^{15}\text{N}$ . In such experiments enhancements of  $>10,000$  were achieved [1] by rapidly warming up polarized samples to approx. 300K where spectra are recorded after transfer to a high field magnet. Various experiments were designed to polarize at low temperature and observe the signal at higher temperatures [1,2]. This implementation of DNP requires efficient transfer of polarisation from stable radicals to the analytes which is facilitated by an optimal contact between the radical and the analyte in a glass state formed at low temperature. We have studied enhancements for different radicals to optimise polarisation for different substances, including typical metabolites found in body fluids. The life time of the polarisation after the transfer depends primarily on the longitudinal relaxation time of the polarised molecules which is usually long for quaternary carbons. We have therefore studied the possibility of polarisation transfer from long-lived  $^{13}\text{C}$  labelled carbonyl groups to carbon atoms with a shorter  $T_1$  relaxation time via a  $^{13}\text{C}$ -nuclear Overhauser effect. This requires that a  $^{13}\text{C}$  labelled carbon atom is introduced into the analyte, for example by acetylation with an isotopically labelled acetyl group. This principle can be used to enhance the life-time of polarization and to further enhance the observed polarisation at room temperature. It can also be exploited for two-fast dimensional HMQC spectra which can be obtained during the life time of labelled carbonyl atom. Moreover, polarisation transfer in the glass state from isotopically labelled carbonyl groups allows not only an intramolecular transfer but also an intermolecular transfer of polarisation when labelled compounds termed co-polarisation agents are mixed into the glass state. This has substantially broadened the applicability of DNP to substances which are otherwise difficult to polarise, including citrate and glucose. This new principle can be employed in many ways for NMR based metabolomics.

## References

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