Partitioning of Polarization Agents for Dynamic Nuclear Polarization in Living Biomass

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Insights from structural biology are sometimes limited because the manipulations of the samples required for biophysical examination of a biomolecule may alter its molecular structure. This is a particular concern for biomolecules with environmentally-sensitive conformations, such as proteins that can adopt more than one stable conformation or those that have regions of intrinsic disorder. In such cases, it may be difficult, if not impossible to fully reconstitute the biologically relevant conformation with a purified sample. Yet, there is increasing evidence of the importance of these regions for biological activity. Nuclear Magnetic Resonance (NMR) spectroscopy has exquisite specificity for NMR active nuclei, which are non-perturbative probes that offer the possibility of atomic-level information. However, a limitation is experimental sensitivity. The addition of dynamic nuclear polarization (DNP) to MAS NMR can increase the sensitivity of experiments to the point that observation of a protein at its endogenous concentration can potentially be accomplished in reasonable experimental times.

However, the DNP MAS NMR, like all techniques imposes constraints on the sample. Sample preparation can significantly impact how effective the technique is and thus determining if and how optimizing sample preparation for living samples is a critical step towards the goal of in vivo structural biology. Here, we investigated the effects of AMUPol when incubated with live cells by analyzing the representative NMR signals for the major biomass components: protein, lipid, RNA, and cell wall. We determined the DNP enhancement and the degree of partitioning of the polarization agent AMUPol of all the biomass components. We describe the sample preparation methods that maximize enhancements and maintaining cell viability at low experimental temperature for both bacteria and yeast.