The goal of the proposed project is to develop and use solid-state nuclear magnetic resonance (SSNMR) techniques to probe the structure and dynamics of the intermediate conformations of an ATP Binding Cassette (ABC) transporter. Experiments designed for proton detection of triply labeled (^{2}H, ^{13}C, ^{15}N) protein with ^{1}H at exchangeable sites will be performed and optimized. Experiments designed with optimal control protocols will be used to improve the polarization transfer efficiency by utilizing the dipolar and scalar couplings simultaneously. Finally, dynamic nuclear polarization (DNP) will be used to test the feasibility of using the technique on delicate membrane proteins as a final means to enhance sensitivity.

The ATP-binding cassette (ABC) transporters are ubiquitous membrane proteins that couple the hydrolysis of ATP to transport a diverse array of substrates across biological membranes. They have been detected in all genera of the three kingdoms of life. ABC transporters contain two highly conserved nucleotide binding domains that hydrolyse ATP and two transmembrane domains that provide a translocation pathway for the substrate. ABC transporters are involved in several human inherited diseases. For example there are ABC transporters (e.g. MDR1) that export drug molecules from cancer cells. That makes them ideal drug targets[1]. Relatively little is known about the structure-function relationship of these proteins at an atomic level of detail. Therefore, it is proposed to perform SSNMR experiments on these very interesting membrane proteins. Recent work from the Oschkinat lab has demonstrated the ability to produce trapped intermediate samples of the arginine transporter ArtMP-J from *Geobacillus stearothermophilus* in sufficient quantity for SSNMR. The production of more samples will be done in collaboration with Vivien Lange and Britta Kunert.

Multidimensional SSNMR on fully or extensively labeled proteins is rapidly progressing as a means to probe systems that are exceedingly difficult or impossible to study by the established methods of x-ray crystallography or solution NMR such as membrane proteins. Structural work has been completed on small globular proteins such as SH3[2] in the Oschkinat lab, and my previous work on GB1[3] and several others. Examples of membrane proteins probed by SSNMR such as cytochrome bo_3 oxidase[4], and OMP-G[5]. The primary limitation thus far has been the sensitivity of the experiments. The inclusion of lipids reduces the space for protein molecules in the preparation of SSNMR samples which only exaggerates the problem. We intend to investigate multiple means of improving the sensitivity spectroscopically, as described below.

Most previous SSNMR methods borrow heavily from established solution NMR techniques except for the use of proton detection. The use of carbon detection was necessary due to the vast dipole-dipole network that is responsible for the broadening of the ^{1}H lineshape. It has been shown that triply labeled (^{2}H, ^{13}C, ^{15}N) and ^{1}H-exchanged samples give narrow ^{1}H linewidths[6]. Further dilution of the exchangeable protons[7] or faster spinning[8] have been shown to further reduce the ^{1}H linewidth. The impetus for proton detection is 2 fold. First, the higher gyromagnetic ratio results in a more sensitive experiment. Secondly, even if proton dilution were to negate the sensitivity gain, the spectral resolution gained from another independent and generally well dispersed resonance more than makes up for the loss. The optimal implementation for model systems, not to mention the more complicated and delicate case of membrane proteins, remains unsolved. This proton detection work will be done in collaboration with Ümit Ackbey, Sascha Lange, and members of the Bernd Reif group, also at the FMP.

Signal enhancement and transfer in SSNMR has most typically been achieved through the use of dipole couplings and cross polarization. This is convenient as the experiments are optimal at short mixing times (1-2 ms for ^{1}H-X CP, and <10 ms for low gamma CP) thereby minimizing the signal lost to the numerous relaxation mechanisms active in SSNMR. Recent improvements in hardware design have allowed the use of scalar coupling modulated pulse sequences. In rigid systems, it is expected that CP will be more efficient, however in dynamic systems, the solution type experiments are expected to be superior. In complicated systems such as proteins, it is possible to have rigid regions, highly dynamic regions, and regions with intermediate dynamics. Therefore, it is an open question, especially in the largely unexplored realm of membrane proteins, which of the two transfer schemes produces the greatest intensity from the largest number of spins. We will apply optimal control algorithms[9] to find a solution which allows both the dipolar transfer and the scalar transfer mechanism to be active. It is hoped that this will improve the transfer efficiency, and therefore increase sensitivity.

The advent of the cold metal probes in solution NMR increased sensitivity by a factor of 3-5 times. This "modest" gain in sensitivity has spurned the development of faster acquisition schemes, novel experiments, and the ability to study of larger systems with less sample. DNP holds even more promise with theoretical gains of >300, and practical gains of ~30 times[10]. While this method is in it's infancy in regards to protein NMR, the FMP is fortunate enough to be the first customer of a commercial SSNMR MAS DNP instrument. The prospects for such huge gains in sensitivity are very exciting and warrant close examination.

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