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EPR distance measurements with Gd(III) - from spin physics to in-cell study of proteins

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Observing proteins structural changes of proteins during function in-side the cell is a challenge yet to be met. The motivation for such studies is the notion the complex cellular environment affects both the conformational equilibrium and the stability of proteins. In this context, distance measurements between two spin labels attached at specific, well defined positions in a protein, by DEER (double electron-paramagnetic resonance) spectroscopy, has been suggested as an attractive method to probe protein's conformations in cells. To realize the potential of such measurements we introduced the use of high spin (S=7/2) Gd(III) spin labels which offer the required stability in the reducing environment of the cell. To establish the utility of Gd(III) spin labels for distance measurements we first carried out Gd(III)-Gd(III) DEER on well-defined synthetic model systems. This was followed by a theoretical analysis of DEER between two high spin centers to evaluate the conditions under which the simple data analysis for extracting the distance distribution for a pair of S=1/2 is valid for Gd(III) as well. In terms of methodology we used a home built, high frequency (95 GHz) EPR spectrometer and implemented chirp pulses to increase sensitivity and found experimental approaches to overcome complications arising from the high spin system. The last step was a large number of applications to proteins in-vitro, using a variety of tags that led confidence to the method. This helped picking up the best Gd(III) tags for in-cell performance in terms of stability, sensitivity and distance resolution, emphasizing that by tuning the chemical structure of the Gd(III) tag all these properties can be optimized. Finally the feasibility of the methodology will be demonstrated on the dimeric BIR1 domain of the X-linked inhibitor of apoptosis protein (XIAP), which shows a reduced stability in the cell as compared to frozen solution and frozen cell extract.