FT-EMR STRUCTURAL STUDY OF BIOMOLECULES WITH DOUBLE ELECTRON- ELECTRON RESONANCE EXPERIMENTS AT 94 GHZ

M. Bonora, L.-C. Brunel, J. van Tol (NHMFL); A. Beth, E. Hustedt (Vanderbilt University, Nashville)

Introduction

Double Electron-Electron Resonance (DEER) is a recently introduced FT-EMR experiment that allows to quantify the dipolar interaction between two paramagnetic centers, and to extract the distance between them in the range 2-8 nanometers [1]. This experiment has been used to solve a number of biophysical problems, including the determination of distance constraints in proteins [2], [3]. We are looking forward to combine the ability of DEER in measuring interspin separations on molecular scale, with the improved g-resolution obtained at high frequencies in EMR spectra of proteins bi-labeled with paramagnetic nitroxide probes. If successful, this approach will lead to a better comprehension of the consistency of the structural models of proteins and the reliability of the distribution of distances achieved with DEER.

Experimental

Solutions of mutants of the bacteriophage T4 lysozyme bi-labeled with nitroxides ([300 µM]) have been prepared in Vanderbilt University. The samples have been studied in NHMFL with 94 GHz DEER FT-EMR at T = 60 K.

Results and Discussion

Some 94 GHz DEER results are shown in Fig. 1. We were able to measure interspin separations of about 28, 33 and 46 Å in the T4 lysozyme and to estimate the distribution of distances for the different mutants.

![Fig.1](image)

**Fig.1.** Experimental 94 GHz DEER profiles (black) and their simulations (red) for three nitroxide bi-labeled T4 mutants. The insets show a cartoon of the protein with the positions of the labels.

Conclusions

We demonstrated the feasibility of high-frequency DEER on a bi-labeled protein. Work to take advantage of the enhanced spectral EMR resolution at 94 GHz is in progress.

Acknowledgements

This work is supported by an FSU Research Foundation PEG grant.

References