Abstract

Structural biology often employs a combination of experimental and computational approaches to unravel the structure-function paradigm of biological macromolecules. This thesis aims to approach this combination by the application of Pulsed Electron-Electron Double Resonance (PELDOR/DEER) spectroscopy and structural modelling. In this respect, PELDOR spectroscopy in combination with site-directed spin labelling (SDSL) of proteins is frequently used to gain distance restraints in the range from 1.8 to 8 nm. The inter-spin distance and the flexibility of the spin labelled protein domains are encoded in the oscillation and the dampening of the PELDOR signal. The intrinsic flexibility of the commonly used MTSSL (1-Oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) spin label itself can be an obstacle for structural modelling if the flexibility of the label is large compared to the flexibility of the protein domains. In this thesis the investigation of two multi-domain proteins by the 4-pulse PELDOR sequence is presented. At first, the N-terminal polypeptide transport-associated (POTRA) domains of *ana*Omp85, a rigid three domain protein, giving well-defined PELDOR distance restraints, is investigated. The experimental restraints are used for structure refinement of the X-ray structure and reveal a strong impact of the intrinsic flexibility of MTSSL on the accuracy of structural refinement. The second example, K48-linked diubiquitin, is a highly flexible multi-domain protein on which the flexibility of MTSSL is of minor impact on structural modelling. In this case, the distance restraints are utilized to determine conformational ensembles. Due to the high intrinsic flexibility already characterizing diubiquitin the recently developed 7-pulse Carr-Purcell (CP) PELDOR sequence was applied to investigate longer ubiquitin chains. This sequence enables to measure dipolar oscillations with an extended time window, allowing a good separation between inter- and intramolecular contributions even for long distance and broad conformational distributions, thereby providing an increased accuracy of the obtained distance distributions.