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Spin-label EPR Approaches to Protein Interactions

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Citation

Son, M. van. (2014, December 4). *Spin-label EPR Approaches to Protein Interactions*. *Casimir PhD Series*. Retrieved from <https://hdl.handle.net/1887/29986>

Version: Not Applicable (or Unknown)

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Note: To cite this publication please use the final published version (if applicable).

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Issue Date: 2014-12-04

SUMMARY

This thesis describes four studies of the structure and dynamics of biomolecules by electron paramagnetic resonance (EPR). In *Chapter 1*, the principles of this magnetic resonance are introduced.

Membrane fusion, the merging of one membrane vesicle with another, is an essential biomolecular process in eukaryotes. The mechanistic details of this fusion remain to be unravelled. Within this framework, *Chapter 2* presents a study on the interaction of two types of small biomolecules, the peptides E and K. The peptides are designed such that they form a heterodimer when mixed in solution, i.e., one type of peptide forms a dimer with the other type, but not with the same type of peptide. We have investigated the peptides by means of paramagnetic resonance and concluded that heterodimer formation is detectable by this technique. Next, we intend to couple the peptides to membrane vesicles. The model constructs created this way are expected to be capable of membrane fusion. Our ultimate goal is to apply EPR on the model constructs in order to reveal structural information of the constructs during membrane fusion.

Numerous types of protein-protein interactions are involved in cell metabolism, muscle contraction, and signal transduction. These interactions range from static to transient. *Chapter 3* reports on the investigation of the transient interaction between the proteins cytochrome *c* (Cc) and cytochrome *c* peroxidase (CcP). A spin label was placed at the surface of Cc. Conventional EPR (9 GHz) was applied to Cc mixed with different concentrations of CcP. The spectra show that the spin label becomes immobilized upon complex formation. Principal component analysis (PCA) was used to disentangle the EPR spectra. The analysis yielded two pure-component spectra, which

correspond to a slow fraction and a fast fraction of the spin label. The findings are in agreement with previous studies, which shows that the interaction of Cc and CcP involves a static, stereo-specific complex and a more dynamic, loosely bound encounter complex. The PCA analysis proved to be effective and – in combination with EPR – can be considered an excellent tool to study protein-protein interactions.

A useful way to obtain structural information of a biomolecule is by measuring the distance between two points, e.g. between two paramagnetic centres. Distances in the range of 1.5 nm to 6.0 nm are detectable by pulse EPR techniques. Distances shorter than 1.5 nm are more challenging to access. *Chapter 4* explores the possibility of relating the exchange interaction (J coupling) between two spins to short-range distances. We have investigated four peptides that each contain two spin labels, separated by two, three, four, or five amino acids. Previously, a continuous-wave EPR study had shown that in two of these peptides the J coupling is significantly larger than in the other two peptides. In the present study, power-saturation experiments were applied to the peptides to obtain spin-relaxation parameters. We observed that the rate of relaxation increases strongly when the spin labels are closer together. We attribute this to a correspondingly higher J coupling. This makes it possible to discriminate between pairs of spin labels at different positions in the peptides. We posit that power saturation experiments could be used as a tool for short-range distance determination. In contrast to pulse EPR to determine distances, power-saturation experiments can be done in liquid solution and at room temperature, both biologically relevant conditions.

Protein folding is a crucial process in every living cell. Correct protein folding generates a three-dimensional structure that is capable of carrying out a

biological function. Incorrect protein folding is thought to be the cause of certain diseases, such as cystic fibrosis and Alzheimer's disease. *Chapter 5* describes a novel method to obtain experimental data on the folding process. Two spin labels were placed at different positions in the protein flavodoxin. With double electron-electron spin resonance (DEER) we measured the distance between the spin labels on flavodoxin in different concentrations of denaturant. The distance distributions obtained by DEER show that local structure in the unfolding protein can be measured and present evidence for a folding intermediate that is locally more compact than the protein in its native state. We demonstrate that we can follow the unfolding of flavodoxin by DEER and detect changes in local structure upon unfolding.

