

# Measuring conformational dynamics and water accessibility of a spin-labeled protein by EPR

Module time 5-6 hours

When 10 a.m.

Where NBCF 04/498

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This module will give an introduction into Electron Paramagnetic Resonance (EPR) spectroscopy and Overhauser Dynamic Nuclear Polarization (ODNP). The experiments will be performed at room temperature at X-band (0.34 T magnetic field, 9.6 GHz irradiating microwave) on a protein spin-labeled with a nitroxide probe (MTSL) and on TEMPOL (4-Hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl) dissolved in water.

We will first perform X-band continuous wave experiments to gain information about the dynamics of the nitroxide probes in the picoseconds to nanoseconds time scale in different environments, and we will learn how to calculate the spin concentration of a given sample.

Additionally, we will detect the NMR spectra of the water molecules surrounding the nitroxide probes, and observe how the intensity of the NMR spectra changes upon irradiation of the EPR transitions. The change in intensity is directly related to the amount of water molecules in contact with the probe, namely its water accessibility. The latter information is complementary to the dynamic data obtained by continuous wave EPR and enables following conformational changes of protein in native environment.

*electron spin 1/2 and hyperfine coupling to  $^{14}\text{N}$  ( $m_I = 1$ )*

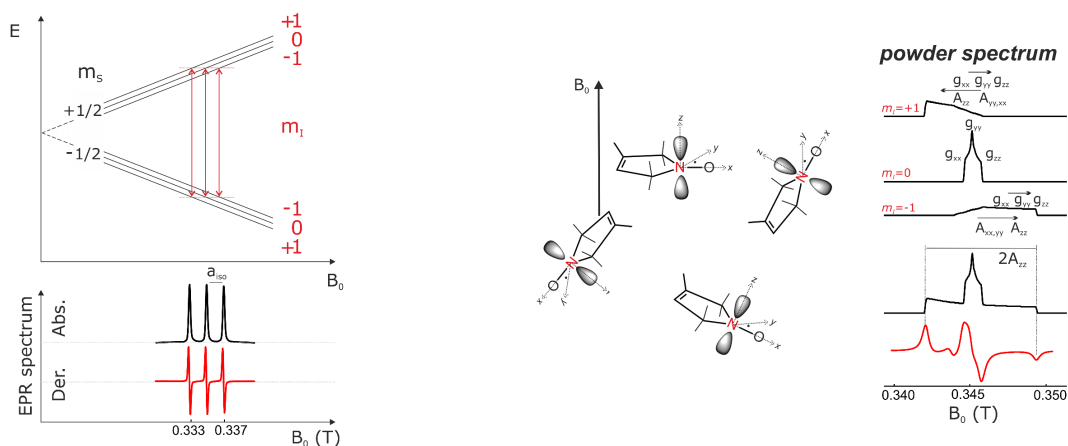


Figure: The spectrum of a nitroxide probe in fast motion (left) and in frozen solution (right).