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).

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. Extrapolating the ODNP data at infinite microwave power will allow to extract the translation diffusion dynamics of the water surrounding the spin label.
The latter information is complementary to the dynamic data obtained by continuous wave EPR and enables following conformational changes of proteins in native environment (Figure 1).

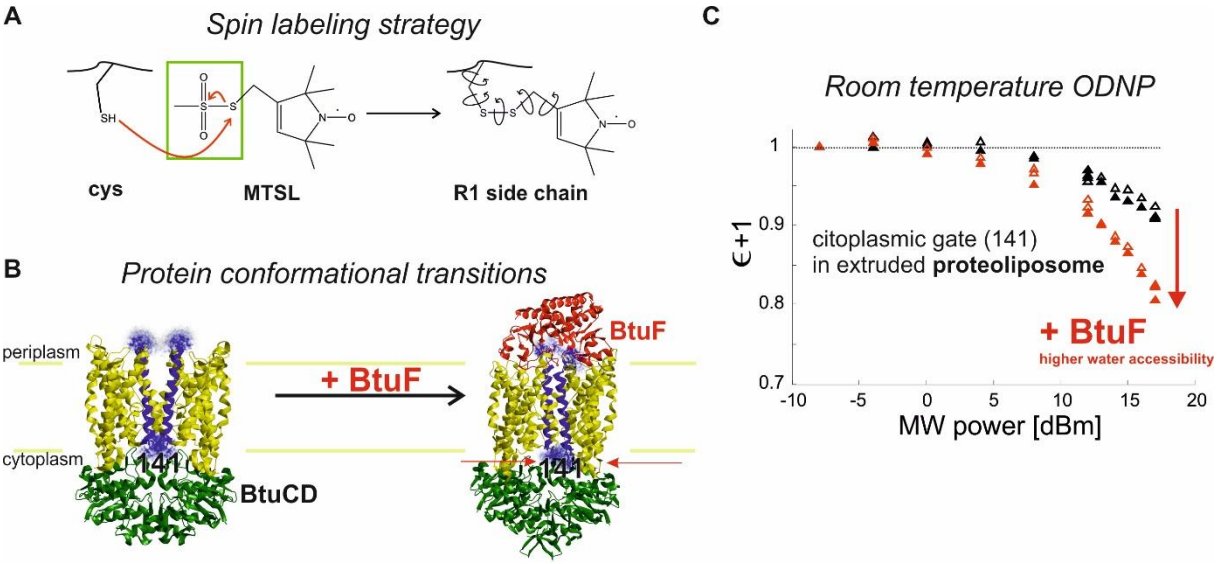

Fig. 1. Monitoring water accessibility via ODNP. A) Spin labels are attached to engineered cysteines in a protein. B) Changes in the overall conformation of a membrane protein are tightly coupled to changes in water accessibility at specific regions. This example shows the binding of a periplasmic BtuF protein to the ABC transporter BtuCD. The cytoplasmic gate (cysteine at position 141) becomes less water accessible upon binding. C) ODNP measurements reveal the decreased water accessibility at the cytoplasmic gate upon BtuF binding at the periplasmic gate.