Modern methods in experimental physics:
site-directed spin labeling EPR

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Problem Set 2

Questions - to be discussed in the next lecture, and experimentally verfied in the lab

a. Which limitations exist in choosing the modulation amplitude in a cw EPR experiment? Is the optimal modulation amplitude dependent on the temperature of the sample?

b. Describe the spectral effects in the derivative line of an EPR spectrum created by decreasing the conversion time from 40 to 5 ms when the time constant of the lock-in amplifier is 10 ms.

Problem 1: Lineshape analysis: continuous wave power saturation measured using EPR derivative spectra

Radicals, transition metals, triplet states are characterized by extremely different relaxation times, which are also strongly temperature dependent. When you want to measure the cw EPR spectrum of a particular radical at a certain temperature, you must optimize the experimental conditions to have the maximum signal intensity with a reliable and reproducible lineshape. One key point is the applied microwave power (related to the B_1 intensity) which should be optimized to avoid saturation of the EPR signal, which is related to signal distortion (line broadening).

a. Write down the equation describing the EPR signal absorption and its derivative (why the derivative? EPR spectra are measured vith a B_0 field modulation and lock-in detection which give experimentally the first derivative of the absorption lines). (hint: use the steady state solution of the Bloch equations M_y and calculate the first derivative with respect to the resonance offset). Write the expression for the amplitude of the peak to peak intensity of the first derivative signal I'_{pp} (hint: use the second derivative of M_y). This expression holds true for homogeneous EPR lines.

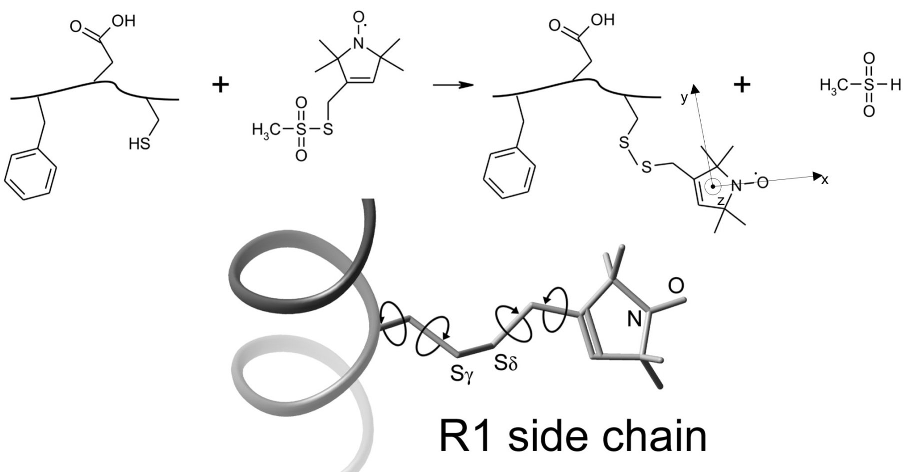

Figure 1: In the site directed spin labeling technique a nitroxide radical is attached via a disulfide bond to an engineered cysteine, leading to a non natural side chain usually called R1. The radical used here is the methanethiosulfonate spin label MTSSL. The g tensor coordinate system shown. The EPR signal arises only from the attached label, independently of the size of the protein under investigation.

b. Plot I'_{pp} *versus* $\omega_1 = \gamma B_1$. Use the following parameters describing the X-band spectra of MTSSL attached to a specific position in a membrane protein at room temperature, with $T_1 = 1$ μs and $T_2 = 500$ ns. How does the saturation curve change by decreasing both relaxation times by one order of magnitude? Consider $M_0 = 1$ and $\omega_1/2\pi$=[0-600 kHz]. Which region of the plot enables to detect a signal lineshape without saturation distortion? The field strength in the resonant cavity B_1 or $\omega_1 = \gamma B_1$ is proportional to the square root of the incident microwave power. The plot I'_{pp} *versus* $\sqrt{P_{mw}}$ is called saturation curve.