

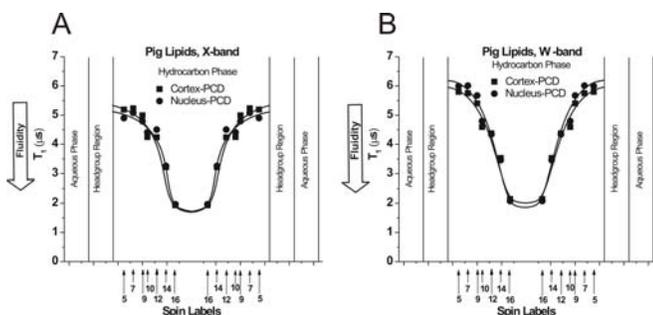
## Research Highlight #141

**Spin-label saturation-recovery EPR at W-band: Applications for eye-lens lipid membranes**Laxman Mainali<sup>a</sup>, Marija Raguz<sup>a,b</sup>, Theodore G. Camenisch<sup>a</sup>, James S. Hyde<sup>a</sup>, Witold K. Subczynski<sup>a</sup><sup>a</sup>Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI 53226, USA<sup>b</sup>Department of Medical Physics and Biophysics, School of Medicine, University of Split, Split, Croatia

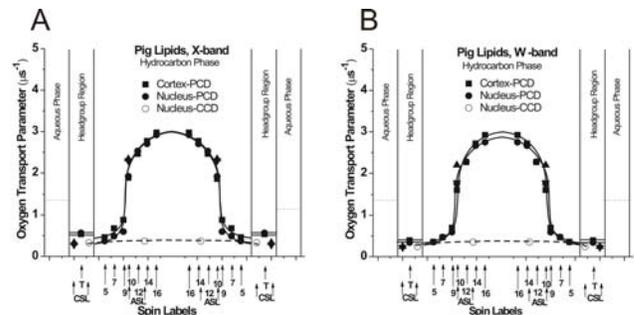
Recently, we used extensive EPR spin-labeling methods (including the saturation recovery [SR] approach) to study the organization and dynamics of lens lipid membranes. These membranes are saturated with cholesterol, which not only saturates the phospholipid bilayer but also leads to the formation of cholesterol bilayer domains (CBDs) within the membrane (Fig. 1). EPR spin-labeling methods provide a unique opportunity to determine the lateral organization of lens lipid membranes (including coexisting membrane domains). Most significantly, EPR spin-labeling methods also make it possible to obtain molecular-level information on the organization and dynamics of cholesterol molecules in the CBD [1]. This information cannot be obtained by differential scanning calorimetry (DSC), X-ray diffraction, or magic-angle-spinning (MAS) NMR, which are methods that have been applied to investigate the lateral organization of lens lipid membranes.

Our previous studies were carried out at X-band using SR EPR spectrometers with a loop-gap resonator (LGR) with a sample volume of 3  $\mu\text{L}$ . To obtain detailed profiles of membrane properties, lipids were extracted from 50 to 100 eye lenses. It is not difficult to obtain these numbers of similar eye lenses (age is the major criterion) from a meat-packing plant. Human lenses are more difficult to obtain in these numbers from eye banks. Additionally, human lenses can be different not only because of age but also because of different health histories among donors. The best solution to this problem is to perform measurements on samples prepared from one or two eyes from a single donor.

Recently, we presented results that demonstrate the feasibility of such measurements [2]. Here, we present profiles of lens lipid membrane properties that were obtained using spin-label EPR at (A) X-band (9.4 GHz) with an LGR with a sample volume of 3  $\mu\text{L}$  and (B) at W-band (94 GHz) with an LGR with a sample volume of 30 nL. Thus, the total amount of sample is 100 times smaller at W-band than at X-band. Results at W-band and X-band include profiles of membrane fluidity (Fig. 2) and the oxygen transport parameter (Fig. 3). In addition, data on discrimination of coexisting membrane domains were obtained. SR EPR at W-band has the potential to be a powerful tool for studying samples of a small volume ( $\sim 30$  nL).



**Figure 2:** Fluidity profiles; profiles of the electron spin-lattice relaxation time,  $T_1$ , for n-PC spin labels at 25°C across cortical and nuclear pig lens lipid membranes recorded at (A) X-band and (B) W-band. PCD: phospholipid cholesterol domain; CBD: cholesterol bilayer domain (see Fig. 1).



**Figure 3:** Profiles of the oxygen transport parameter (oxygen diffusion-concentration product) at 25°C across certain domains in cortical and nuclear pig lens lipid membranes obtained at (A) X-band and (B) W-band. Approximate localizations of nitroxide moieties of spin labels are indicated by arrows.

1. Raguz, M., Mainali, L., Widomska, J., Subczynski, W.K., The immiscible cholesterol bilayer domain exists as an integral part of phospholipid bilayer membranes. *Biochim, Biophys. Acta* DOI: 10.1016/j.bbamem.2010.12.019.
2. Subczynski, W.K., Mainali, L., Camenisch, T. G., Froncisz, W., Hyde, J. S., Spin-label oximetry at Q- and W-band. *J. Magn. Reson.* DOI: 10.1016/j.jmr.2011.01.003.