

**Lipid Domains: EPR Discrimination by Oxygen Transport.**

Anna Wiśniewska, Witold K. Subczyński

Discrimination by oxygen transport (DOT) method is a dual-probe saturation recovery EPR approach, in which the observable parameter is a spin-lattice relaxation time ( $T_1$ ) of spin labels, and the measured value is the bimolecular collision rate between molecular oxygen and the nitroxide moiety of a spin label. The method is based on the variation of the local diffusion-concentration product of oxygen in different membrane domains. Membrane domains can be characterized by profiles of the oxygen diffusion-concentration product in situ without the need for physical separation, which provides useful information about internal dynamics in each domain. The DOT method is especially suitable for obtaining time-space characteristics of small transient domains in model and biological membranes. The time scale of the DOT method is approximately 0.1 to 100  $\mu\text{s}$ , and we are able to obtain reliable lipid exchange rates between domains when these rates fit this time window. The sensitivity of the method can be improved by using higher microwave frequencies, such as Q or W-band. Using the DOT method, we are able to discriminate and characterize solid-ordered, liquid-disordered and liquid-ordered domains in membranes made of binary mixtures of phosphatidylcholine and cholesterol or sphingomyelin and cholesterol, and of ternary raft-forming mixtures.