Supporting Material

Phase-Separation and Domain-Formation in Cholesterol-Sphingomyelin Mixture: Pulse EPR Oxygen Probing

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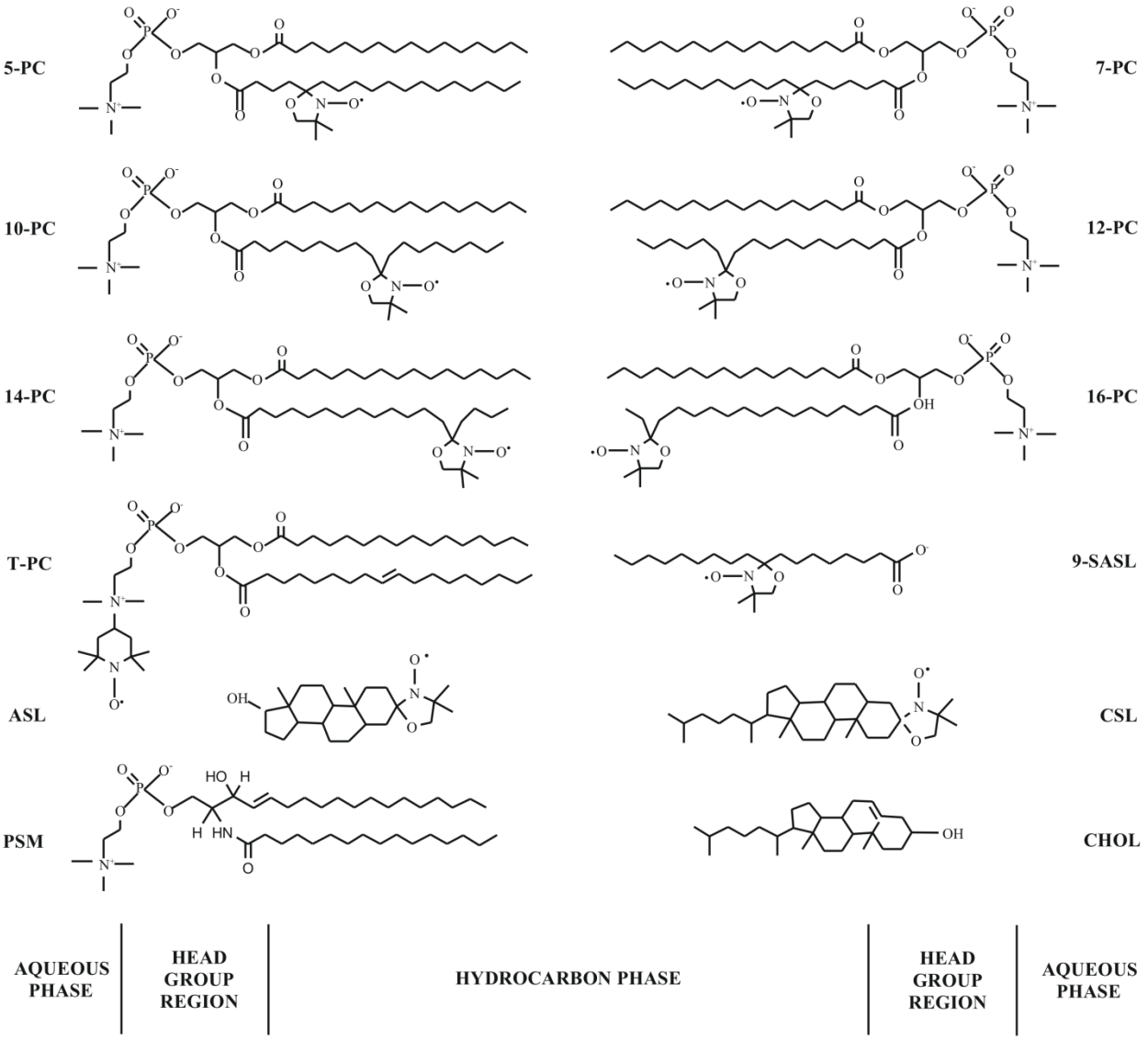

FIGURE S1: Chemical structures of phospholipid-type spin labels (n-PCs, T-PC, 9-SASL) and cholesterol-type spin labels (CSL, ASL). Chemical structures of cholesterol (Chol) and ESM (PSM forms ~86% of ESM) are included to illustrate approximate locations of nitroxide moieties across the membranes.

Outline of theory for evaluating the OTP and NiEDDA accessibility parameter

In dual-probe SR EPR experiments, either molecular oxygen or NiEDDA are introduced in the membrane suspension as a relaxation agent. These relaxation agents induce spin exchange, which leads to faster spin-lattice relaxation of the nitroxide. The rate of bimolecular collisions between either molecular oxygen or NiEDDA and the nitroxide moiety of a spin label placed at a specific location in the membrane is evaluated from the T_1s of the spin label.

The OTP, $W(\mathrm{x})$, was introduced as a convenient quantitative measure of the collision rate between spin label and molecular oxygen (1) :

$$W(\mathrm{x}) = T_1^{-1}(\mathrm{Air},\mathrm{x}) - T_1^{-1}(\mathrm{N}_2,\mathrm{x}) \sim D(\mathrm{x})C(\mathrm{x}) \qquad (1)$$

where $W(\mathrm{x})$ is normalized to the sample equilibrated with air at normal pressure and is proportional to the product of the translational diffusion coefficient, $D(\mathrm{x})$, and the concentration, $C(\mathrm{x})$, of oxygen at a depth "x" in the membrane.

Similarly, the accessibility parameter, $P(\mathrm{x})$, for the water-soluble relaxation agent NiEDDA was defined as:

$$P(\mathrm{x}) = T_1^{-1}(20\ \mathrm{mM\ NiEDDA},\mathrm{x}) - T_1^{-1}(\mathrm{No\ NiEDDA},\mathrm{x}) \qquad (2)$$

Greater $P(\mathrm{x})$ values indicate a greater extent of NiEDDA penetration into the membrane. All accessibility measurements must be performed for deoxygenated samples. The NiEDDA concentration in buffer used for liposome preparation is 20 mM.

When located in two different membrane domains, the spin label alone most often cannot differentiate between the two, giving similar conventional EPR spectra and similar T_1 values. However, even small differences in lipid-packing in these domains can affect partitioning, diffusion, and/or penetration of relaxation agents, which can be easily detected by observing different T_1 values from spin labels in these two locations in the presence of relaxation agents. Collisions with molecular oxygen (Eq. 1), which can be quite different in different domains, form the basis of the discrimination by oxygen transport method developed previously (2). Here, we can also apply a new approach in which collisions with the water-soluble paramagnetic complex NiEDDA (which penetrates differently into different membrane domains) can discriminate these domains.

REFERENCES

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2. Subczynski, W. K., J. Widomska, A. Wisniewska, and A. Kusumi. 2007. Saturation-recovery electron paramagnetic resonance discrimination by oxygen transport (DOT) method for characterizing membrane domains. In Methods in Molecular Biology, Lipid Rafts Humana Press, Totowa. 143-157.