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Depth profiling in membranes by fluorescence quenching

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Abstract. Membrane penetration depth is an important parameter in relation to membrane structure and organization. A methodology has been developed to analyze the membrane penetration depths of fluorescent molecules or groups utilizing differential fluorescence quenching caused by membrane embedded spin-label probes located at different depths. The method involves determination of the parallax in the apparent location of fluorophores, detected when quenching by phospholipids spin-labelled at two different depths is compared. By use of relatively simple algebraic expressions, the method allows calculation of depth in Å. This method has been used to determine the location of fluorophores in NBD-labelled lipids and anthroyloxy-labelled fatty acids in model membranes and of the membrane embedded tryptophan residues in the reconstituted nicotinic acetylcholine receptor.

Keywords. Fluorescence quenching; spin labelled phospholipids; membrane penetration depth.

Fluorescence quenching is a powerful tool for analysis of membrane structure. Fluorescence quenching by spin-labelled probes has been used to investigate lipid-protein and protein-protein interactions in membranes (London, 1982). Such quenching interactions are short ranged and thus convenient for structure analysis on the scale of molecular dimensions. An important application of quenching in membranes is the determination of the distance (depth) of membrane-bound groups or molecules from the membrane surface (London, 1982; Blatt and Sawyer, 1985). Knowledge of the precise depth of a molecule or group should help define the conformation and topology of membrane probes and proteins.

Recently a method has been developed for measuring the membrane penetration depth utilizing fluorescence quenching by spin-labelled phospholipids and it has been applied to determine penetration depths of the fluorescent groups in a series of NBD-labelled lipids (Chattopadhyay and London, 1986, 1987), anthroyloxy-labelled fatty acids (Chattopadhyay, 1987), and of the membrane embedded tryptophan residues in the reconstituted nicotinic acetylcholine receptor (Chattopadhyay and McNamee, 1989). Analysis of depth in this way is quantitative, yet less complicated than methods utilizing fluorescence energy transfer. In addition, spin labels, unlike other types of quenchers, have the advantage of being quenchers of a wide range of fluorophores including tryptophans.

Two sets of samples are prepared for such depth measurements. The first set contains membranes containing the fluorophore, variable concentrations of a lipid labelled with the quencher at one depth (to be called the shallow quencher), and an unlabelled lipid dioleoyl phosphatidylcholine). The other set contains the same fluorophore, variable concentrations of a different quencher lipid labelled at a different depth (to be called the deep quencher), and the unlabelled lipid. By combining the results of fluorescence intensity measurements from the two sets, the depth of the fluorescent group can be determined by use of the following equation (Chattopadhyay and London, 1987):

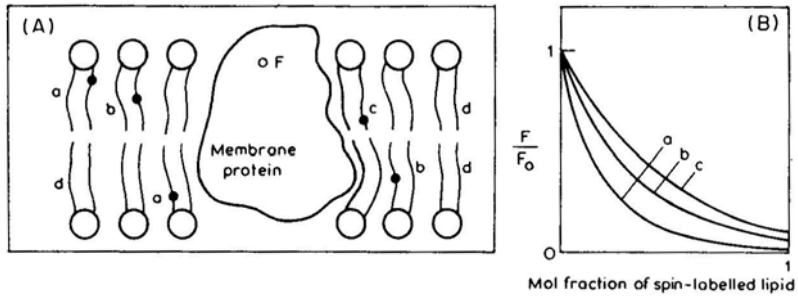

Figure 1. (**A**) A schematic diagram of an integral membrane protein containing a fiuorophore (**F**) in a bilayer containing an ordinary (non quenching) lipid (d) and quenching lipids (a, b, c) in which the quencher group on the lipid is placed at different depths in the membrane. Notice that the vertical distance (depth) of the fiuorophore from the most shallow quencher (a) is the least of the 3 depths. It should be mentioned that in an actual experiment each sample will have only one kind of quenching lipid in addition to an ordinary lipid. (**B**) Differential fluorescence quenching curves for the system described in (**A**). The ordinate is the ratio of fluorescence in the presence (**F**) and absence (Fo) of the quencher. Note that the strongest quenching is obtained with the most- shallow quencher a.

$$Z_{cF} = L_{c1} + \left[\left(-\frac{1}{\pi C} \ln F_1/F_2 - L_{21}^2 \right) \Big/ 2L_{21} \right],$$

where Z_{cF}=depth of the fluorophore from the center of the bilayer, L_{c1} = distance of the center of the bilayer from the shallow quencher 1, L_{21} = difference in depth between the two quenchers, C = two-dimensional quencher concentration in the plane of the membrane, and F_1/F_2 is the ratio of fluorescence intensity from the two sets of samples for the same quencher concentration C. The above equation is derived from an extended version of the Perrin (1924) quenching equation which is applicable to quenching occurring in membranes where fluorophores and quenchers are randomly distributed in the plane of the membrane. It can also be shown that by combining two experiments in this way the effect of restrictions on the minimum allowed lateral approach of fluorophore and quencher is cancelled out. Determination of depth of a fluorophore in a membrane protein by this approach is schematically shown in figure 1. Analysis of depth in this way can also be extended to quenching by probes other than spin labels. Future applications of this method include investigation of the conformation and orientation of hydrophobic peptides and other membrane active molecules in the membrane bilayer.

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