

## **NEWER BIOPHYSICAL METHODS FOR MEMBRANE ANALYSIS IN INTACT RED CELLS**

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Classical biochemistry and conventional energy transfer methods have provided useful knowledge of the structure of the red cell membrane. However, a point of diminishing returns may have been reached. Fortunately, at least two new biophysical approaches offer the likelihood of further extension of our intimate understanding of the membrane. The first of these is Single Photon Radioluminescence. In this technique, an intense tritium label is inserted into a specific membrane lipid, while a suitable fluorophore is used to label a membrane skeletal protein. Analogous to classical fluorescence transfer, energy from the tritium in the lipid is absorbed by the fluorophore on the protein, and the resulting emission can be measured by sensitive single-photon counting. Although requiring specialized counting devices and intense tritium sources, this technique allows distance measurements between the "emitter" and the "receptor" in the 80-200Å range, which is substantially greater than conventional energy transfer. This provides information from sites inaccessible to the former technique, and is particularly suitable for the depths of membrane skeletal arrays.

A second, particularly useful technique, which requires no radioactive source or ultrasensitive counting technology, is Total Internal Reflection. In this microscopic method, special optics are employed to provide very low-angle glancing laser illumination of the surface of the membrane. The resultant light is totally reflected from that surface; however, before leaving the membrane, it penetrates the surface for 100-200Å, as the so-called "evanescent wave". If fluorophores are provided on proteins in this immediately sub-membranous region, they are excited, and can be monitored by micro-spectrographic or micro-photographic methods. Since only fluorophores in the very limited region illuminated by the evanescent wave are excited, the signal-to-noise ratio of such membranes is extraordinarily high, allowing sensitive measurements with minimally disrupting labels. Utilizing green fluorescent protein labels on membrane skeletal elements makes this approach particularly useful for studying the disposition of proteins in the skeletal array of intact living cells. Further ramifications of this method, using photo-bleaching of the labels with ultrafast pulsing laser sources, may provide useful information on the cellular dynamics of those proteins as well.