

23.1 Introduction to Potentiometric Probes

Potentiometric optical probes enable researchers to perform membrane potential measurements in organelles and in cells that are too small to allow the use of microelectrodes. Moreover, in conjunction with imaging techniques, these probes can be employed to map variations in membrane potential across excitable cells and perfused organs,¹ with spatial resolution and sampling frequency that are difficult to achieve using microelectrodes.

Overview of Applications for Potentiometric Probes

The plasma membrane of a cell typically has a transmembrane potential of approximately -70 mV (negative inside) as a consequence of K^+ , Na^+ and Cl^- concentration gradients that are maintained by active transport processes. Potentiometric probes offer an indirect method of detecting the translocation of these ions, whereas the fluorescent ion indicators discussed in Chapter 22 can be used to directly measure changes in specific ion concentrations. Increases and decreases in membrane potential — referred to as membrane hyperpolarization and depolarization, respectively — play a central role in many physiological processes, including nerve-impulse propagation, muscle contraction, cell signaling and ion-channel gating. Potentiometric probes are important tools for studying these processes, as well as for visualizing mitochondria (which exhibit transmembrane potentials of approximately -150 mV, negative inside matrix) (Section 12.2), for cell-viability assessment (Section 15.2) and for high-throughput screening for new drug candidates. Potentiometric probes include the cationic or zwitterionic styryl dyes, the cationic carbocyanines and rhodamines, the anionic oxonols and hybrid oxonols and merocyanine 540. The class of dye determines factors such as accumulation in cells, response mechanism and toxicity. Surveys of techniques and applications using membrane potential probes can be found in several reviews.^{2–9}

Selecting a Potentiometric Probe

Selecting the best potentiometric probe for a particular application can be complicated by the substantial variations in their optical responses, phototoxicity and interactions with other molecules. Probes can be divided into two categories based on their response mechanism:

- Fast-response probes (usually styrylpyridinium dyes; Section 23.2) operate by means of a change in their electronic structure, and consequently their fluorescence properties, in response to a change in the surrounding electric field (Figure 23.1). Their optical response is sufficiently fast to detect transient (millisecond) potential changes in excitable cells, including single neurons, cardiac cells and intact brains. However, the magnitude of their potential-dependent fluorescence change is often small; fast-response probes typically show a 2–10% fluorescence change per 100 mV.
- Slow-response probes (Section 23.3) exhibit potential-dependent changes in their transmembrane distribution that are accompanied by a fluorescence change (Figure 23.1). The magnitude of their optical responses is much larger than that of fast-response probes (typically a 1% fluorescence change per mV). Slow-response probes, which include cationic carbocyanines and rhodamines and anionic oxonols, are suitable for detecting changes in average membrane potentials of nonexcitable cells caused by respiratory activity, ion-channel permeability, drug binding and other factors.

Calibration of potentiometric probes can be accomplished by imposing a transmembrane potential using valinomycin (V-1644, Section 22.1) in conjunction with externally applied K^+ solutions.^{10,11} The descriptions in this chapter focus on the response characteristics of the various types of potential-sensitive dyes offered by Molecular Probes.

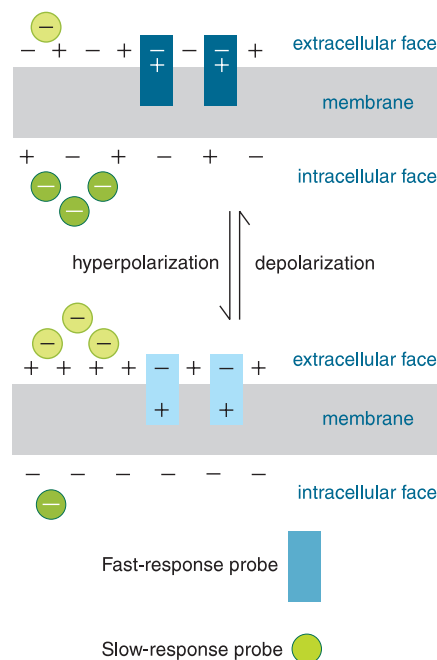


Figure 23.1 Response mechanisms of membrane potential-sensitive probes. Fast-response probes undergo electric field-driven changes of intramolecular charge distribution that produce corresponding changes in the spectral profile or intensity of their fluorescence (represented by color changes in the illustration). Slow-response probes are lipophilic anions (in this illustration) or cations that are translocated across membranes by an electrophoretic mechanism. Fluorescence changes associated with transmembrane redistribution (represented by color changes in the illustration) result from sensitivity of the probe to intracellular and extracellular environments. Thus, potentiometric response speeds directly reflect the time constants of the underlying processes — fast intramolecular redistribution of electrons versus relatively slow transmembrane movement of entire molecules.

References

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