# OPTICAL PROBES FOR ELECTROCHEMICAL EVENTS AND FOR CONFORMATIONAL CHANGES IN BIOLOGICAL MEMBRANES

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## PHOTOSYNTHESIS OF GREEN PLANTS: LIGHT AS SUBSTRATE AND AS PROBE

Green plant photosynthesis is basic to the existence of terrestrial life. The primary processes of photosynthesis occur in the inner membrane system of chloroplasts, named thylakoids. Pigment carrying protein complexes capture quanta of visible light. The energy is channeled by excitation transfer into special chlorophyll complexes where a charge separation is initiated. The photophysical back-reaction is prevented by a series of very rapid electron transfers under partial energy dissipation. The relatively stable product of the electron transport chain is reduced $NADP^+$. Donor for the reducing equivalents is water. As the oxido-reduction reactions are directed across the thylakoid membrane, electrochemical potential differences are created which are used to produce ATP. NADPH and ATP are consumed by the chloroplast to perform useful biological work.
A technically most interesting feature of photosynthesis is the ability to derive reducing equivalents (and/or electrical power) from the ubiquitous *water* at the expense even of *red light*.
In the time domain the above photophysical to electrochemical events cover the range from some pico-sec to some sec. It is advantageous for the student of photosynthesis that most of the partial reactions can be followed by optical spectroscopy, kinetically and sometimes also structurally. Both intrinsic pigments and added indicator dyes have been used for this purpose. Three examples for the application of optical spectroscopy to photosynthesis will be illustrated in this lecture.

*Electrochromic band shifts* of intrinsic pigments have been used as pseudolinear indicators of transient electric fields in the thylakoid membrane. In contrast to artificial voltage probes, changing their conformation or binding, intrinsic and largely immobilized dyes via electrochromism provide a practically unlimited time resolution. The *electrogenic reactions* in the thylakoid membrane are described and also the consumption of electrical energy during the synthesis of ATP (1a,b).
*pH-indicating dyes* have been used to monitor pH-changes not only in the aqueous bulk phase but also in the submicroscopic interior of thylakoids. It may appear difficult to discriminate the supposed pH-response of a dye against its response to other events and also to pH-response from various subcompartments. Appropriate controls are described and the mechanism of *proton injection into thylakoids* is presented. The release of protons during water oxidation will recieve particular attention (2).
A *triplet probe* has been used to monitor *conformational changes of the ATP synthase*. The membrane bound enzyme was covalently and specifically labeled. Two types of conformational changes became apparent upon energization of the thylakoid membrane: an open-

ing and closing (via the triplet lifetime of the label) and a change in the interaction with the membrane (via the rotational diffusion rate of the labeled enzyme in the membrane). The relevance of the conformational changes for activation and activity of the ATP synthase is discussed (3).

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TRANSDUCTION IN VERTEBRATE PHOTORECEPTORS

The molecular basis for the very high quantum sensitivity and for the very large dynamic range of vertebrate photoreceptors is not well understood. The dominant hypothesis that calcium release from the disk interior into the cytosol of retinal rod outer segments transmits, amplifies and regulates between light absorption and the electric response has found only circumstantial evidence.
In this respect it became desirable to record $Ca^{++}$ concentration changes with high sensitivity and time resolution. Flash spectrophotometry with rod outer segments in the presence of the calcium indicating dye arsenazo III has the required properties. The response of the dye to other events than $Ca^{++}$ - concentration changes is clearly to be discriminated and hence allows quantitative measurements. It was observed that light causes the rapid release of $Ca^{++}$ from binding sites in disks at a stoichiometry of 0.5 per excited rhodopsin. However, the released $Ca^{++}$ does not reach the cytosolic space unless a specific ionophore (A23187) is added. The release of calcium from binding sites occurs within 10ms at room temperature and its kinetic parameters closely follow those of the metarhodopsin I/metarhodopsin II transition.

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