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Optical techniques to probe protein dynamics

Knowledge of both protein structure and dynamics is necessary to unravel the molecular mechanisms by which proteins perform their diverse functions. The number of available protein structures has exploded to nearly 60,000 since the first crystallographic analysis of myoglobin by Kendrow in the late 1950s. The study of protein dynamics also has an extensive history, and optical spectroscopy has undoubtedly played a key role. When combined with variables such as temperature or time, the most basic spectroscopic experiment can elucidate protein mechanisms with impressive specificity. The fact that the molecular basis of vision was established by Wald and his predecessors using optical techniques more than 40 years prior to a report of a rhodopsin structure exemplifies the power of optical spectroscopy to elucidate protein function and dynamics in the absence of a static structure.

Spectroscopic study of protein dynamics is, in principle, simple: expose proteins to electromagnetic radiation and characterize changes in the intensity, polarization and frequency of the transmitted, emitted and scattered radiation. Light in the ultraviolet, visible and infrared regions is especially useful because these photons interrogate the electronic and nuclear arrangements in all molecules. Optical techniques provide a wealth of microscopic and macroscopic insight, such as evolution of hydrogen bonds, changes in local polarity and heterogeneity of population. In addition, modern instrumentation enables analysis of protein dynamics on timescales as fast as molecular vibrations and with sensitivities on the single-protein level. Here we present an overview of steady state and time-resolved optical tools used in biophysics with examples from the globular protein myoglobin and the membrane protein rhodopsin.

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KEY RESOURCES

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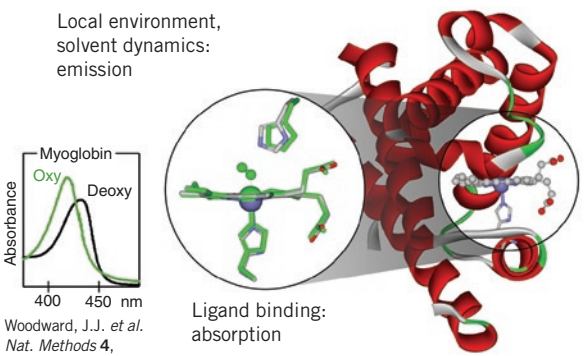
The oxygen-storing heme protein myoglobin (PDB codes 1BZP and 1MBO), highlighting protein changes associated with O_2 binding



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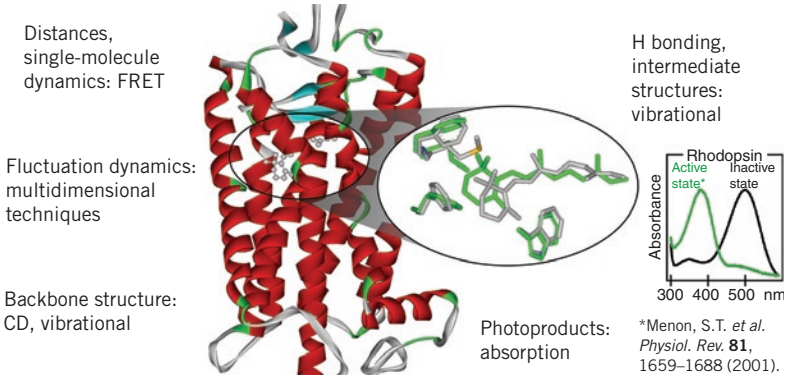
G protein–coupled receptor rhodopsin (PDB codes 1U19 and 2HPY), highlighting protein changes associated with photoactivation



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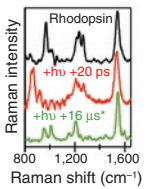
Molecular fingerprints: vibrational

Infrared (IR) absorption and Raman scattering reveal side chain and backbone structures. Site-selective data may be obtained with isotopes and/or resonance Raman (RR) spectroscopy. Structures and reaction/fluctuation dynamics on the timescale of vibrations may be obtained with femtosecond and multidimensional RR and IR.



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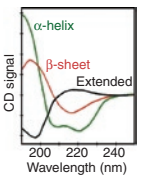
Backbone: circular dichroism

Differential absorption of right- and left-circularly polarized light by a chiral group gives rise to CD signal that reflects structure. Time-resolved CD experiments on the microsecond-to-second timescale revealed global changes in rhodopsin structure.



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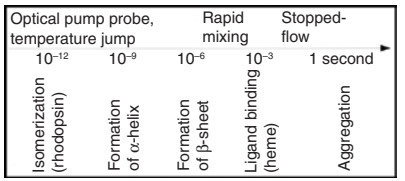
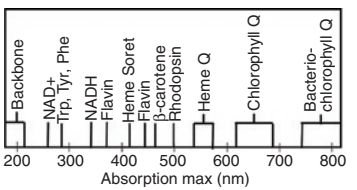
Techniques for time resolution



Dynamics of photo- and temperature-induced reactions are revealed with the highest time resolution, while fast mixing techniques provide insight into substrate-induced reactions.

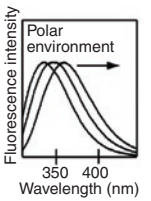
Protein chromophores: absorption



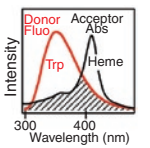
Transient absorption spectroscopy reveals kinetics associated with biological reactions.

Environment and distance: emission

Fluorescence maxima of intrinsic and extrinsic fluorophores with strongly dipolar ground or excited states, such as tryptophan, are sensitive reporters of the local environment. Ultrafast experiments that monitor the temporal evolution of the emission energy, or the dynamic Stokes shift, reveal motional dynamics of local water molecules.



Förster resonance energy transfer (FRET) occurs between donor (D) and acceptor (A) molecules that have spectral overlap. The efficiency for energy transfer scales as r_{DA}^{-6}, where r_{DA} is the D and A separation. FRET pairs may be intrinsic, such as Trp-heme in myoglobin, or exogenous dyes. Single-molecule FRET studies have emerged to provide details of protein dynamics unmasked by the ensemble.



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