**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 Kendrew 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 techniques with examples from the globular oxygen-storing heme protein myoglobin (PDB codes 1BZP and 1MBO), highlighting protein changes associated with O₂ binding and the gas-protein–coupled receptor rhodopsin (PDB codes 1U19 and 2HPY), highlighting protein changes associated with photoactivation.

### 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.

### 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.

### 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.

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