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New and Notable

Taking the Pulse of Protein Interactions by EPR Spectroscopy

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ABSTRACT An article by Gaffney et al. in this issue establishes a method using pulse electron paramagnetic resonance spectroscopy to determine the location of protein substrates or binding partners with high precision.

High-resolution structures represent a significant step toward understanding the molecular function of proteins, and x-ray crystallography has been the most important method for generating these structures. However, even when excellent high-resolution structures are available, the molecular mechanisms that promote function may be elusive. Regions of proteins that are important for function may not be resolved, and the structure or position of binding partners or small ligands may not always be revealed, particularly if these ligands do not assume a well-defined configuration. Moreover, the conditions under which x-ray crystallography is carried out may stabilize specific structural substates from a range of conformers that define the functional state of the protein (1). As a result, conformers that represent functional or energetically excited protein conformational states may not be revealed.

A number of exciting new spectroscopic methods based upon magnetic resonance and fluorescence are now being used to probe protein function and they provide a powerful complement to traditional structural biology methods. Among these approaches, site-directed spin labeling is an emerging methodology based upon electron paramagnetic resonance (EPR) spectroscopy that has found a wide-range of applications in defining biomolecular structure and in determining molecular mechanisms. EPR spectroscopy can reveal local dynamics, structure, and structural changes (2–4) and is one of a few methods that can explore membrane protein structure in membranes. Moreover, EPR spectroscopy is particularly well suited to examine dynamic regions of proteins that are difficult to study but play important roles in mediating protein-protein interactions (5). Recent advances in pulse EPR instrumentation combined with the development of sample preparation techniques (see, for example, Zou and McHaourab (6)) have dramatically improved the sensitivity of experiments such as double electron-electron resonance (7). This method measures weak dipolar interactions between spin labels and permits the determination of distances and distance distributions out to 60 Ångstroms or more.

In this issue, an article by Gaffney et al. (8) describes the use of both continuous wave and pulse EPR spectroscopy to determine the location of the substrate-binding site on soybean lipoxygenase. Lipoxygenases are important enzymes in development, which play a role in many lipid-mediated signaling pathways. While there are high-resolution crystal structures for these proteins, the position of the substrate in the active site is not well resolved, particularly at higher pH values where the enzyme is most active. As a result, there is currently debate regarding the likely mode of substrate binding and the dynamics of the substrate in the active site. The authors of this work take a unique approach to map the position of the lipid substrate when bound to lipoxygenase. To minimize the uncertainty that may be associated with rotamers of the spin-labeled side chain, the authors use a modeling approach and identify suitable sites for labeling within the protein interior. In these locations, label conformations are constrained by neighboring residues and the local environment.

The authors measure distances between 10 pairs of labels within the protein, which are found to be consistent with the x-ray structure. These measurements establish grid points for the positions of the spin-labeled side chains within the protein and define likely rotameric configurations for the labels. The authors then measure five distances between protein-associated spin labels and a spin-labeled substrate analog, and use all 15 experimental distances in a distance-geometry approach to define the position of the substrate and its headgroup. Because the positions of the protein-associated labels are well defined, uncertainty in the location of the substrate may be assigned to substrate conformers that were trapped during freezing. This work provides a clear indication of how the substrate must dock to the protein, and conformational heterogeneity that is observed in the headgroup region indicates how twists in the lipid substrate might be accommodated during catalysis. The general method taken in this study establishes an approach that may be used to locate small flexible substrates such as peptides with excellent precision in a macromolecular complex.

This work adds to a number of excellent examples that are documented in recent reviews (see McHaourab et al. (4) and Klare and Steinhoff (9)) in which pulse EPR has been used to measure protein structural changes and examine conformational substates. The G-protein coupled receptor rhodopsin was mapped by a triangulation method generating a matrix of the spin-labeled side chains in dark and light activated states, thereby revealing the extent of the helix movements that

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take place during protein activation (10). Pulse EPR has also been used to characterize the weak self-association of the bacterial chemotaxis CheA histidine kinase (11), and long-range distances obtained by pulse EPR data have been used in combination with small angle x-ray scattering and fluorescence resonance energy transfer spectroscopy to define a structure for the multiprotein ESCRT complex, which is essential in membrane protein recycling and HIV budding (12).

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