New and Notable

Generating Accurate Contact Maps of Transient Long-Range Interactions in Intrinsically Disordered Proteins by Paramagnetic Relaxation Enhancement

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The theory of paramagnetic relaxation for a rigid paramagnetic center was first set forth by Solomon (1) and Bloembergen (2) in the mid-1950s and early 1960s. The PRE is caused by magnetic dipolar interactions between a nucleus and the unpaired electron of a paramagnetic center and results in an increase in the relaxation rate of nuclear magnetization. Paramagnetic systems with an isotropic *g*-tensor only give rise to PRE effects, while systems with an anisotropic *g* tensor also result in pseudo-contact shifts. The observed PRE is proportional to the r^{6} separation between the observed nucleus (e.g., a proton) and the paramagnetic center, and because the magnetic moment of an unpaired electron is large, the effects can extend to separations as long as ~35 Å (e.g., in the case of Mn^{2}). The most reliable approach for making use of the PRE is to measure the transverse PRE rate (G_2) which is obtained by taking the difference in transverse relaxation rates between the paramagnetic (e.g., Mn^{2} or MTSL) and diamagnetic (e.g., Ca^{2} or MTS) states of the system (3,4).

The potential of the PRE for protein studies was first demonstrated in the mid-1980s (5–7) but only gained widespread use in the last decade (8–10) when methods for introducing surface-exposed cysteines became routine, thereby allowing relatively facile introduction of paramagnetic labels at any position of the surface of the protein. In these early applications, PREs were qualitatively interpreted and converted into approximate distance ranges with large error bounds owing to the fact that the paramagnetic labels are generally attached to the protein via several rotatable bonds (7) and therefore sample a relatively wide region of conformational space. As a result, the utility of the PRE in structure determination was limited. This problem was resolved when Iwahara et al. (3) developed the relevant theoretical framework for dealing with mobile spin labels and implemented methods for direct structure refinement against the PRE data using an ensemble representation for the paramagnetic labels.

In 2006, it was realized that the r^{6} $^{1/6}$ dependence of the PRE, coupled with the fact that the magnitude of the PRE is large, should permit one to observe the footprint of transient invisible states on the spectrum of the major visible species, providing there are distances between the paramagnetic label and the protons of interest that are shorter in the sparsely-populated species than the major one (11). In the fast exchange limit, when the exchange rate is significantly larger than the difference in PRE rates between the two species, the PREs observed on the proton resonances of the major species will simply be population-weighted averages of the PREs in the two states. This insight led to the first direct demonstration of proteins sliding along the DNA as well as translocating from one DNA to another without dissociating into free solution (11,12). The PRE profile observed for the specific complex of the transcription factor HoxD9 with DNA at low salt (20 mM NaCl) was in complete agreement with the structure of the complex. At moderately higher salt concentrations (100 and 150 mM NaCl), where binding is still very tight (K_D ~ 1.5 nM), dissociation from the DNA is slow (<0.01 s ^{1}) and the ^{1}H-^{15}N correlation spectrum of the complex remains unchanged, the observed PRE profiles are typical of a nonspecific complex (11). These PRE profiles, which were completely unexpected, reflect the search process whereby the protein transiently samples nonspecific sites on the DNA at very low occupancy (<1%), some of which come into close proximity to the paramagnetic labels. Building on this work, PREs were then used to directly demonstrate the existence of encounter complexes in protein-protein association (13–15), the presence of rapid transitions between major open and minor (~5%) partially closed states of maltose binding protein (16), and the transient formation of active dimers of the HIV-1 protease precursor that lead to the initial intramolecular cleavage events that generate the active mature protease (17).

A substantial amount of work has been published on denatured and disordered proteins using PREs with the aim of detecting transient compact species, starting in 1997 when Gillespie and Shortle (18,19) made a first attempt to characterize the denatured state of a mutant of Staphylococcal nuclease based on PRE data. A limitation of that work, as well as much of the subsequent work along these lines (20–23), was the inability to quantitatively characterize transient long-range interactions in terms of a unique and easily understandable representation. In a pioneering study in the *Biophysical Journal*, Silvestre-Ryan et al. (24) demonstrate that it is indeed possible to obtain very reliable high-resolution, long-range contact maps for disordered proteins from PRE data obtained with labels separated by ~15 residues (one label per sample) even when these transient species have occupancies as low as 1%. The key insight in this work is that a single structure

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satisfying all the PRE data can be readily obtained by appropriate refinement methods (in this instance, Monte Carlo-based simulated annealing). This structure does not represent an actual physical structure nor does it reflect the conformational space sampled by disordered/denatured proteins. The contact map, however, derived from this single structure provides a highly accurate picture of all the transient long-range interactions sampled by the system. Indeed, the contact maps generated from a single structure are almost identical to those obtained from ensemble calculations comprising 5–10 members. Moreover, the use of larger ensembles (>20) is in fact detrimental, as it results in contact dilution reflecting the ambiguity of PREs when one attempts to recover both distance and populations simultaneously.

With a series of theoretical calculations in hand to firmly establish the validity of this approach, Silvestre-Ryan et al. (24) then proceeded to generate a long-range contact map for the acid-denatured state of apomyoglobin from previously published PRE data (22). The contact map reveals local collapse at the N- and C-terminal regions that include both native and nonnative interactions between helices formed in the folded state.

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