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Functional dynamics of proteins revealed by solution NMR

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Solution NMR spectroscopy can analyze the dynamics of proteins on a wide range of timescales, from picoseconds to even days, in a site-specific manner, and thus its results are complementary to the detailed but largely static structural information obtained by X-ray crystallography. We review recent progresses in a variety of NMR techniques, including relaxation dispersion and paramagnetic relaxation enhancement (PRE), that permit the observation of the low-populated states, which had been 'invisible' with other techniques. In addition, we review how NMR spectroscopy can be used to elucidate functionally relevant protein dynamics.

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Introduction

Methodological advances in analyzing the dynamics of proteins and protein complexes on a wide range of timescales by NMR spectroscopy (Figure 1) have led to the publication of a large number of applications. For example, relaxation dispersion experiments enable the detection of low-populated functional states of proteins, which had been 'invisible' with other techniques. In addition, formerly uncharacterized encounter complexes have become evident by paramagnetic relaxation enhancement (PRE) analyses. Characterizations of the functional dynamics in large molecular weight systems remain challenging; however, several systems >100 kDa have been analyzed successfully. The importance of dynamics in catalytic activities, signal transduction, protein stability, and protein–protein and protein–ligand interactions has also been discerned by solution NMR techniques. We provide an overview of several specific examples published over the last two years that have had greatly impacted on the field.

Dynamics in proteins revealed by solution state NMR: recent methodological developments and significant findings

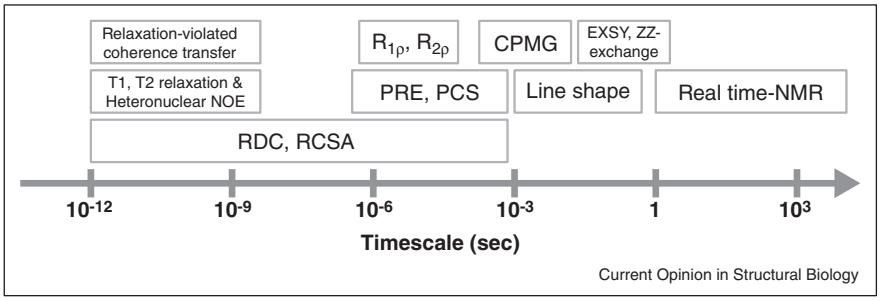
Relaxation dispersion for microsecond–millisecond dynamics

Carr–Purcell–Meiboom–Gill (CPMG) relaxation dispersion is one of the most extensively explored techniques for analyzing microsecond–millisecond timescale dynamics in proteins. Excellent reviews have covered this technique [1–4], and many new experiments, mainly for analyzing side chain dynamics, have been proposed since 2010. For example, new techniques have been introduced that can probe the dynamics of side-chain carbonyl groups [5] and Lys amino groups [6]. In addition, by using a protein expressed in medium containing [^{1}H, ^{13}C]-glucose and D_2O [7,8•], 16 types of side-chain ^{1}H spins can simultaneously be probed in a single relaxation dispersion experiment [7]. Along with the CPMG-type experiments, adiabatic $R_{1\rho}$ and $R_{2\rho}$ relaxation dispersion experiments were also proposed recently, to extend the dynamic range of the analysis without exerting excess stress on the hardware and samples [9]. Nevertheless, the relaxation dispersion method is still limited by a relatively small exchange timescale to be quantified. In practical use, precautions to remove or estimate the systematic errors, and considerations of the intrinsic low sensitivity of the experiments, have to be taken into account for successful application of the strategy.

One of the major strengths of the relaxation dispersion technique is the fact that the chemical shifts of the low-populated (<5%) excited states can be measured. The chemical shifts of the excited states can be incorporated into the structural restraints by using a database approach to obtain the global structures of excited proteins [10••,11] or the local side chain conformations [12,13]. One of these topics will be discussed in detail in a later section. The combined use of aligned media with the relaxation dispersion technique further provides direct structural information through residual dipolar couplings (RDCs) and residual chemical shift anisotropies (RCSAs), leading to the high resolution structures of proteins in their excited states [14,15].

The relaxation dispersion technique is also useful to investigate the energy landscapes of proteins. The relaxation dispersion data for various substrate-bound and cofactor-bound states of dihydrofolate reductase (DHFR) revealed that the interactions between the protein and the ligand(s) change the energy landscape, such that the

Figure 1



Timescales of protein dynamics covered by a variety of NMR methods.

enzyme can efficiently exert its catalytic cycles [16••] (see below for details). Relaxation dispersion was also employed to show that a mutation that abrogates the millisecond timescale fluctuations in DHFR severely impairs the enzymatic function of the protein [17].

The relaxation dispersion technique has also been used to examine rapid protein folding [18], the interactions of intrinsically disordered proteins (IDPs) with their binding partners [19,20], and other phenomena. For example, using NMR titrations and ^{15}N relaxation dispersion, the phosphorylated kinase-inducible domain (pKID) of the transcription factor CREB was found to form an ensemble of transient encounter complexes upon binding to the KIX domain of the CREB binding protein [19]. The encounter complexes evolve via partially folded intermediates to the final bound state, without dissociation from KIX [19]. The structural optimization of the partially folded intermediate into the final bound conformation on the surface of its target protein was nicely reproduced by computing the free-energy landscape, using an enhanced conformational sampling method and all-atom multicanonical molecular dynamics (MD) [21]. It revealed that the free-energy landscape comprised several superclusters separated by free-energy barriers, and when the IDP formed a nonnative complex upon binding, the complex moved on the landscape to fold into the native complex, by increasing the interfacial hydrophobic contacts and the helix content [21]. It is also interesting to note that a millisecond-long MD trajectory of the protein BPTI [22] was employed to predict the outcome of the relaxation dispersion measurements [23]. With further developments in both computational and experimental methods, these two techniques may potentially be used in conjunction for detailed studies of microsecond–millisecond dynamics.

Paramagnetic effects for low-populated long-range structure information

PRE is also sensitive to the existence of low-populated states in proteins and protein complexes. This strategy has been exploited to study encounter complexes [24–27,28•], especially those observed in electron transfer protein interactions [29]. For example, the PRE technique was used to characterize how a bacterial pathogen captures iron in the human body during infections, using iron-regulated surface determinant (Isd) proteins [30]. The PRE-solved IsdA–IsdC hemoprotein complex revealed that transient stereo-specific complex formation is sufficient to mediate heme transfer in the Isd system [30]. Although some of the studies utilized naturally occurring PREs from unpaired electrons within the systems, artificially attached probes were exploited to introduce unpaired electrons in most of the cases. There are two main types of probes that can be introduced site-specifically into a protein. One is stable nitroxide radicals [31,32] and the other is chelated lanthanide ions [33–35]. If the motion of the introduced ions is sufficiently limited, then the chelated lanthanide ions also provide pseudo-contact shift (PCS) and RDC information, which can be used as long-range structure information. Paramagnetic lanthanide tags can also be employed in combination with relaxation dispersion techniques, which potentially enhance the effects of microsecond–millisecond dynamics in the relaxation dispersion NMR spectra and provide structural information on the low-populated states encoded in the PCSs [36]. In this case, the proper selection of an attachment site is critically important to avoid confusing exchange from special fluctuations of the paramagnetic center [36]. In addition, since there is a certain optimal distance for the PRE or PCS effect to be quantified, without apriority knowledge about the structure, the attachment site for the PRE experiment might require optimization in an iterative manner.

The dynamic behavior of IDPs is one of the research fields that has been explored by solution state NMR [20,37,38]. PREs, as well as RDCs, were extensively used in the research to characterize the long-range order in these IDPs and to provide their ensemble structures [39–42]. However, when using RDC information, one should keep in mind that the shallow folding energy landscape of