NMR Methods to Study Dynamic Allostery

Sarina Grutsch¹, Sven Brüschweiler², Martin Tollinger¹ *

¹ Institute of Organic Chemistry, Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innsbruck, Austria, ² Department of Computational & Structural Biology, Max F. Perutz Laboratories, Campus Vienna Biocenter 5, Vienna, Austria

* martin.tollinger@uibk.ac.at

Abstract

Nuclear magnetic resonance (NMR) spectroscopy provides a unique toolbox of experimental probes for studying dynamic processes on a wide range of timescales, ranging from picoseconds to milliseconds and beyond. Along with NMR hardware developments, recent methodological advancements have enabled the characterization of allosteric proteins at unprecedented detail, revealing intriguing aspects of allosteric mechanisms and increasing the proportion of the conformational ensemble that can be observed by experiment. Here, we present an overview of NMR spectroscopic methods for characterizing equilibrium fluctuations in free and bound states of allosteric proteins that have been most influential in the field. By combining NMR experimental approaches with molecular simulations, atomistic-level descriptions of the mechanisms by which allosteric phenomena take place are now within reach.

Introduction

In allosteric proteins, information about binding events is communicated between remote sites that are linked by a network of interactions. This allosteric communication process typically involves a specific redistribution of the accessible conformational states and can, but does not have to, produce an experimentally observable structural rearrangement [1–3]. In either case, allosteric communication is fundamentally dynamic in nature [4–8] and can be experimentally studied in a quantitative manner by nuclear magnetic resonance (NMR) spectroscopy. It is of particular interest to identify and characterize equilibrium dynamics of allosteric proteins, i.e., conformational fluctuations that are permanently present under equilibrium conditions. Long-range fluctuations can involve networks of protein sites spanning distances up to 20 Å and more, and their modulation in response to specific binding of a ligand molecule can be essential for allosteric communication between remote binding sites.

The last decade has witnessed the development and optimization of exciting new NMR spectroscopic methods to study such equilibrium dynamics in the free and bound states of biomolecules in atomistic detail. The experimental characterization of such processes in proteins and (ribo)nucleic acids is now feasible for timescales ranging from picoseconds to seconds (and slower), at atomic resolution and in a quantitative manner [9,10]. While pico- to nanosecond timescale equilibrium dynamics are commonly studied by combined measurement of diverse spin relaxation parameters pertaining to NMR-active nuclei, processes occurring in microseconds and milliseconds are accessible through experiments that are sensitive to the presence of exchange line broadening in NMR spectra. Among these, relaxation dispersion
techniques are particularly useful to characterize conformational heterogeneity and transitions between different conformational states, including high-energy states that are populated to very low degrees. Dynamic processes can also be studied by residual dipolar couplings, magnetization transfer techniques, or real-time observation.

With respect to allosteric proteins, application of these NMR techniques has revealed subtle details of dynamic coupling mechanisms. Most strikingly, the focus of interest has steadily been shifting from very fast (pico- to nanosecond) processes toward the micro- to millisecond time regime and beyond. This has been afforded, on the one hand, by methodological advancements, which now facilitate rapid and efficient acquisition of relaxation dispersion data and the implementation of this technique as a standard experimental tool in most NMR laboratories. On the other hand, the development of new procedures for site-specific isotope labeling has prompted the application of NMR spectroscopy to proteins and protein complexes of high-molecular weight that had not been amenable to investigation before. Because NMR experiments by nature yield quantitative information, these data provide extremely valuable parameters for computational chemists. It is, thus, not surprising that computer simulation techniques have been applied to slower timescale allosteric processes in the recent past using enhanced sampling protocols. Together, simulation and experiment have provided exciting insights into intricate molecular mechanisms of allosteric proteins.

In this review, we provide an overview of the most commonly used NMR spectroscopic techniques to study dynamic allosteric phenomena, along with recent methodological developments. These experimental approaches are illustrated using five representative proteins and protein complexes [11–15], for which the allosteric mechanisms have been characterized in detail (Fig 1).

**Relaxation Dispersion Experiments**

Dynamic processes occurring on a timescale of micro- to milliseconds can contribute to the line-widths of resonances in NMR spectra through exchange line broadening, provided that the chemical shifts of the involved states are different (Fig 2). NMR relaxation dispersion (RD) techniques are excellent experimental tools to probe such processes quantitatively and at atomic resolution [10]. In these experiments, relaxation rate constants are measured under the effect of an adjustable radio frequency field, which is typically achieved by application of a series of refocusing pulses with variable pulse spacing (Carr-Purcell-Meiboom-Gill [CPMG] experiments) or using a continuous radio frequency field [16]. In either case, relaxation dispersion profiles are obtained, which are characteristic for the kinetic, thermodynamic, and structural features of the underlying dynamic process. RD experiments provide information about micro- to millisecond dynamic processes so long as the involved states are populated to more than ~0.5%, corresponding ΔG more than ~3 kcal/mol at 25°C.

From an experimental perspective, interference due to scalar couplings between adjacent NMR-active nuclei can result in contributions to RD profiles that are not related to dynamics [17]. Standard RD experiments thus pertain to nuclei that (i) are effectively "isolated" (scalar couplings less than ~1 Hz) from each other, such as backbone amide 15N, and (ii) can be isotope-labeled by bacterial expression without requiring specific labeling schemes. CPMG-based RD experiments have also been devised for 1H and 13C nuclei in the protein backbone, in protein side chains, along with relaxation-optimized experiments for probing high-molecular weight proteins [10]. In many of these experiments, scalar couplings are either suppressed experimentally or eliminated by use of specifically isotope-labeled protein samples [18]. With the exception of deuteration, which modulates the strengths of van der Waal's interactions and can, thus, have an effect on structural dynamics, isotope labeling is generally considered...
**Fig 1. Three-dimensional structures of allosteric proteins.** (A) The homodimeric catabolite activator protein (CAP) bound to two molecules of cAMP (green spheres; Protein Data Bank [PDB] identifier 1G6N) [11]. (B) The KIX domain of CREB-binding protein (CBP; blue) in complex with the peptides mixed-lineage leukemia (MLL; top, dark green, residues 2,840–2,858) and phosphorylated kinase-inducible domain (pKID; light green, residues 116–149; PDB identifier 2LXT) [12]. (C) The PBX1 homeodomain (PBX-HD, blue) bound to DNA (green) and the HoxB1 homeodomain peptide (light blue, residues 177 –185; PDB identifier 1B72) [13]. (D) The 20S core particle proteasome (20S CP); α- and β-subunits are shown in light and dark blue, respectively (PDB identifier 3C91) [14]. (E) The heterodimeric enzyme imidazole glycerol phosphate synthase (IGPS), subunits HisH (light blue) and HisF (dark blue). The allosteric effector PRFAR (dark green spheres) and the substrate glutamine (light green spheres) are shown (PDB identifier 1OX5) [15]. Prepared using PyMOL (The PyMOL Molecular Graphics System, Version 1.41, Schrödinger LLC).

doi:10.1371/journal.pcbi.1004620.g001
Fig 2. Relaxation dispersion experiments. (A) Transitions (exchange) between two states, A and B, causes line broadening of resonances in NMR spectra if the chemical shifts of the two states are different ($\Delta \omega \neq 0$) and the exchange rate constant, $k_{ex}$, is in the micro- to millisecond time range. (B) In the typical experimental setup for CPMG relaxation dispersion measurements, resonance intensities at multiple protein sites (e.g., all backbone amide NH groups) are measured at variable CPMG frequencies (bottom). Relaxation dispersion profiles are obtained by converting these intensities to transverse relaxation rates (top). (C) Analysis of RD profiles yields information on kinetic ($k_{ex}$), thermodynamic (fractional populations $p_A, p_B$), and structural ($\Delta \omega$) parameters of the underlying dynamic exchange process(es). RD experiments provide this information only for protein sites with different local structures in states A and B ($\Delta \omega \neq 0$).

doi:10.1371/journal.pcbi.1004620.g002
noninvasive. For (ribo)nucleic acids, due to significant resonance overlap in NMR spectra and the fairly complex scalar coupling network, site-specific isotope labeling plays a major role in developing CPMG-based RD experimental schemes [19].

Experimental RD data are analyzed by fitting exact [20] or approximative analytical equations that are available for certain timescales [10] or by use of numerical solutions of the Bloch equations, including magnetization exchange effects [21]. For a simple two-state exchanging system \( A < \rightarrow B \), analysis of RD data yields information on the kinetics of the underlying dynamic process in terms of the exchange rate constant, \( k_{ex} \), the chemical shift difference between the states, and their fractional populations. Temperature-dependent experiments enable the quantification of thermodynamic parameters \( \Delta H \), \( \Delta S \), and activation-free energies \( E_a \) by standard van’t Hoff and Arrhenius analysis. In practice, RD profiles of multiple "reporters" (i.e., nuclei) recorded at two or more static magnetic field strengths are fit together to obtain an accurate description of the underlying dynamic process(es) [22]. Data analysis typically involves a collective motional model, assuming a common exchange rate constant and fractional populations but nucleus-specific chemical shift differences between states. By this approach, collective motions (in which all nuclei sense the same kinetics) can be identified while outliers become evident [23].

Relaxation dispersion NMR techniques have been employed to examine the allosteric mechanisms of various proteins and protein complexes with molecular weights up to 230 kDa [24–29]. As an example, for the catabolite activator protein (CAP, Fig 1A), RD studies revealed the dynamic process through which two ligand binding sites that are more than 24 Å apart mediate allostery [30]. CAP is a homodimeric protein in which each subunit contains a ligand (cAMP) binding site at its N-terminus and a DNA-binding domain at its C-terminus. While the two cAMP molecules bind to CAP at distinct sites, binding of the first cAMP ligand significantly enhances micro- to millisecond dynamics of CAP of almost all amino acids in both subunits, thereby linking the two binding sites. Subsequent binding of the second cAMP molecule is accompanied by substantial rigidification of the entire protein backbone and a near complete loss of dynamics, further highlighting the role of conformational entropy for allosteric regulation in CAP. In addition, RD data of different variants of CAP were used to characterize the regulation of the DNA-binding domain, which is stimulated by cAMP [31], and for analyzing the role of sparsely populated ("excited") states in allosteric inhibition [32]. In a recent computational study using coarse-grained elastic network models (ENMs) of CAP, low-frequency correlated motions throughout the protein were found to be affected by binding both the first and the second molecules of cAMP, suggesting that allostery is indeed mediated by equilibrium fluctuations [33]. All-atom molecular dynamics (MD) simulations of micro- to millisecond dynamics in CAP are not available to date.

Allosteric coupling is notably different for the KIX domain of CREB-binding protein (CBP), a three-helix bundle protein that physically interlinks transcription factors via the formation of a ternary complex (Fig 1B). In KIX, ligand peptides are bound to remote binding sites, yet binding of either ligand mutually enhances the affinity for the second ligand. NMR relaxation dispersion experiments showed that allosteric communication proceeds through a defined redistribution of accessible conformational states [34]. Using backbone and side-chain RD experiments, it was shown that KIX bound by only one ligand peptide (mixed-lineage leukemia [MLL]) is conformationally heterogeneous in solution. Indeed, in this binary complex, an "excited" state is populated (to 7% ± 0.3% at 25°C) in which KIX structurally resembles the ternary complex. Thus, even in the absence of an interaction partner for the second binding site,
the remote binding surface appears to be partially preformed. NMR titration experiments further imply that binding of the second ligand is accomplished through a redistribution of states to form the ternary complex. Moreover, the RD data showed that allostery coupling proceeds through a network of residues that bridge the two binding sites in KIX at a rate $k_{ex}$ of $330 \pm 40s^{-1}$, including part of the hydrophobic core. The allosteric communication pathway in KIX was recently examined in atomistic detail by combining all-atom molecular dynamics with enhanced sampling techniques [35]. Using well-tempered ensemble metadynamics to probe conformational states with millisecond lifetimes, the presence of an excited state in the binary complex of KIX with MLL was verified, and its structural similarity to the ternary complex (rather than the binary complex) was confirmed. Of note, this molecular simulation study pointed out the critical role of side-chain dihedral angle variations of amino acid residues in the KIX hydrophobic core as a means for transmitting allostery information between binding sites.

Relaxation dispersion experiments are not restricted to simple two-state processes. However, the analysis of RD data becomes increasingly complex with increasing numbers of exchanging states and, hence, the number of adjustable parameters in the fitting procedure. Characterization of processes that involve three interconverting states requires RD measurement of different single-quantum and multiple-quantum (MQ) coherences [36], controlled perturbation of the equilibrium through temperature variation or addition of ligand [37], or orthogonal NMR experiments to constrain adjustable parameters (e.g., chemical shifts measurement in heteronuclear single quantum coherence [HSQC] spectra or relaxation rates) [23,38]. An interesting case involving (at least) three relevant conformational states is the allosteric PBX1 homeodomain (PBX-HD) [39,40]. This small DNA-binding protein comprises three $\alpha$-helices that are packed against each other and an unstructured 15-residue extension at the C-terminus. DNA-binding is accompanied by folding of the C-terminal segment to a fourth helix, which enhances the binding of transcription factors to a remote site on the protein surface (Fig 1C). Elaborate RD NMR analysis showed that the disorder-to-helix transition of the C-terminal segment in PBX-HD already occurs, to a low extent ($4.5 \pm 0.8\%$ at $25^\circ$C), in the absence of DNA at a rate $k_{ex}$ of $2300 \pm 200s^{-1}$. Folding of the C-terminal segment is accompanied by a concerted restructuring of the remainder of the protein, including residues that are involved in the recognition of transcription factors, which implies a functional role of these dynamics in the allosteric communication mechanism of PBX-HD. Valuable insights into the energetics of this process were derived from temperature-dependent RD measurements [39]. Moreover, combination of RD measurements with orthogonal NMR relaxation techniques revealed the presence of an additional high-energy state in the PBX-HD conformational ensemble, with an exchange rate, $k_{ex}$, of $9,500 \pm 2,300s^{-1}$, in which the C-terminal extension is locally misfolded [40]. The characterization of this state, which represents an off-pathway folding intermediate that corresponds to a kinetic dead end, highlights the complexity of the conformational ensemble that is present in solution.

**Magnetization Exchange Experiments**

Conformational transitions can involve motions on even slower timescales, in which case NMR relaxation techniques that monitor magnetization exchange are suitable [41]. In these experiments, chemical shifts are recorded after a variable delay period, during which exchange between states occurs. During this delay, which is limited by the relaxation properties of the system, conformational states have a finite probability of converting to each other. The resultant spectrum thus contains cross-peaks that reflect the interconversion between states, along with diagonal peaks deriving from each conformer (Fig 3). By monitoring both the emergence
of cross-peaks rate and the decay of diagonal peaks, the exchange kinetics between conforma-
tional states and, hence, their mean lifetimes, can be determined [10].

Methyl-TROSY-based magnetization exchange experiments have provided exciting insights
into slow allosteric transitions of a number of high-molecular weight proteins and protein
complexes [42,43]. For the archaean 20S core-particle (CP) proteasome, analysis of methyl-
TROSY magnetization exchange spectra revealed a delicate balance of different conformational
states in solution that are critical for its function [44]. The 20S CP, a large protein complex that
forms a barrel-like structure around a central proteolytic chamber (Fig 1D), maintains cellular
homeostasis by selectively degrading misfolded or damaged proteins. Substrate entry is restricted by 13 Å gated pores at either side of the proteasome barrel that are regulated through binding of activators (regulatory particles). The magnetization exchange data showed that the gate, which is formed by N-terminal residues of the \( \alpha \)-subunits in the 20S CP, exists in dynamic equilibrium between different states, one higher-populated ground state and two lower-populated states. It could further be shown that, in these less-populated conformers, the entrance to the proteolytic chamber is blocked, while in the ground state this is not the case. The NMR experimental data thus directly relate to the conversion of inactive states of 20S CP to its activated state, and vice versa. Of note, in X-ray crystallographic studies of the 20S CP, electron density has not been observed for the proteasome gating residues [45].

**NMR Order Parameters**

In addition to slow timescale motions, which frequently report on redistributions of states in conformational ensembles, allosteric proteins in many cases display functionally relevant pico- to nanosecond dynamics. Motions in this time regime typically reflect stochastic fluctuations of bond vectors. Experimentally, such fast dynamic processes can be probed by NMR spin relaxation measurements that monitor different relaxation modes (Fig 4) [46,47]. The experimental data are typically interpreted within the “model-free” analytical strategy in terms of generalized squared order parameters \( S^2 \) [48], which provide a generic measure of the amplitudes of bond vector fluctuations, along with the timescale (\( \tau_e \)) of these motions. As such, \( S^2 \) reports on the degree of spatial restriction of internal motion of a particular bond vector. Values of \( S^2 \) can vary from zero to one, with one corresponding to a rigid bond vector and zero corresponding to complete flexibility.

Notably, the experimental relaxation data may require additional fitting parameters besides the standard \( S^2 \) and \( \tau_e \) pair, indicative of additional motions and/or micro- to millisecond dynamics [49]. While the “model-free” strategy has been dominating the analysis of pico- to nanosecond timescale dynamics, the use of specific motional models, such as the Gaussian axial fluctuation model [50], can be a practicable alternative for data interpretation, providing valuable information regarding the details of internal motions [51]. Moreover, the spectral density function that underlies NMR relaxation phenomena can be mapped at several frequencies [52].

Standard experimental setups typically involve the measurement of backbone amide NH bond vectors [53], but experiments for determining order parameters are also available for side-chain positions in amino acids, such as \(^2\)H nuclei in methyl groups of partly deuterated proteins [54]. Because \(^{13}\)C-\(^{13}\)C scalar interactions in proteins or nucleic acids represent a major complication for the measurement of \(^{13}\)C relaxation in protein side-chains, site-specific labeling (reviewed in [46]) and/or experimental schemes for the suppression of \(^{13}\)C-\(^{13}\)C scalar couplings are required [55]. Moreover, to provide an improved physical description of bond motions, it is possible to probe the relaxation parameters of multiple bond vectors of overlapping functional groups (such as backbone amide NH and \(^{13}\)C\(\alpha\)-\(^{13}\)CO bond vectors) [47].

NMR order parameters have been used as surrogates for conformational entropy, as they provide a direct measure of a local increase or decrease of the flexibility of bond vectors, for example, upon binding of a ligand molecule [56,57]. In addition, because order parameters are sensitive to pico- to nanosecond timescale dynamics, such data presents an ideal interface between experimental techniques and molecular-dynamics-based approaches. Several studies are available in the literature that reproduce and, thereby, validate protein NMR order parameters from (sub-)microsecond MD trajectories [58]. Since computer simulations provide dynamic information of all bond vectors in a protein, not only those that are easily observable by NMR, the amount of information that is available for identifying correlated motions in proteins is significantly extended.
Most importantly, MD simulations complement NMR order parameters by providing collections of atomic coordinates that correctly represent the underlying dynamic processes. Given the nonlinear relationship between NMR relaxation and protein structure(s), however, a comprehensive representation of pico- to nanosecond dynamics data is not a trivial task. Not only are accurate, experimentally validated force fields essential and critical, but so is sufficient conformational sampling of the system. Sampling efficiency can be improved by use of enhanced sampling approaches such as replica exchange MD (REMD) [59] or accelerated MD (AMD) [60]. Quite generally, molecular dynamics studies have shown that structural

**Fig 4. Determination of NMR order parameters.** (A) Processes in the pico- to nanosecond time regime can be probed by experiments that monitor the relaxation rates of different spin modes. Relaxation rates at multiple sites in a protein are determined from exponential fits of resonance intensities in a time series. (B) Analysis of the experimental data within the model-free approach separates nanosecond timescale contributions arising from rotational diffusion of the protein as a whole ($\tau_c$) from (typically) picosecond contributions due to internal bond vector fluctuations, for which amplitudes ($S^2$), timescale ($\tau_e$), and, if applicable, information on additional motions are obtained.

doi:10.1371/journal.pcbi.1004620.g004
fluctuations that are consistent with the experimental order parameters can indeed be reproduced by simulation [61]. The experimental data match the values that can be calculated from MD trajectories, demonstrating the synergistic potential of molecular dynamics and NMR.

NMR experimental data and molecular simulation have been integrated by using order parameters (along with structural information) as restraints in MD approaches to generate a structural ensemble of ubiquitin that adequately represents pico- to nanosecond timescale dynamics [62]. The obtained structures were cross-validated by back-calculating NMR observables that were not used for structure generation, such as scalar and residual dipolar couplings, showing high agreement. For ubiquitin, the dynamics-refined structural ensemble displays a significantly higher level of heterogeneity than ensembles obtained by standard NMR structure determination protocols. Most strikingly, integration of NMR and molecular simulation revealed a high degree of side-chain rotameric heterogeneity of hydrophobic residues that is not traceable by using standard NMR and X-ray structure determination protocols. It was hypothesized that the liquid-like mobility of the hydrophobic core in ubiquitin may well be a general feature of proteins that is essential for biological function [62].

With respect to allosteric proteins, NMR order parameters have revealed the dynamic nature of a number of different systems [63–65]. In CAP (Fig 1A), for example, differences of backbone amide NMR order parameters of different ligand complexes were used to estimate changes in conformational entropy upon cAMP binding [30]. Comparison with the calorimetrically measured difference in entropy between the two sequential cAMP binding steps confirmed that the observed negative cooperativity in this protein is indeed due to alterations in flexibility. Because ligand binding to CAP occurs without measurably changing the three-dimensional structure of this protein, CAP is considered a model system for purely dynamics-driven allostery. NMR order parameters have also revealed the dominating effect of conformational entropy in the allosteric activation of the DNA-binding domain by cAMP binding [66]. The CAP experimental data have been verified by extensive molecular dynamics simulations, further highlighting the entropic nature of allosteric coupling [67]. In a recent MD study, several side chain interactions that change the protein’s internal force network were identified, providing further insight into mechanistic details of the CAP allosteric mechanism [68]. Moreover, using force distribution analysis, a subset of protein sites in CAP could be identified that act as an allosteric communication pathway between the two cAMP and the DNA binding sites.

For the allosteric KIX domain of CBP (Fig 1B), backbone amide order parameters $S^2$ indicate that binding of MLL peptide leads to an overall loss of pico- to nanosecond timescale dynamics in the KIX backbone [12]. Upon complex formation, MLL directly packs against the L12-G2 loop connecting helices α1 and α2, which is accompanied by a substantial rigidification of this particular region of KIX. In a molecular dynamics study, the mechanistic significance of the L12-G2 loop for allosteric communication KIX was first recognized [69]. It could be shown that rigidification of the L12-G2 loop acts as a dynamical switch in KIX that triggers allosteric communication between binding sites. In addition, MD simulations using a topology-based Go-like model showed that stabilization of the L12-G2 loop upon MLL binding to KIX reduces structural dynamics and lowers the entropic cost for binding the second ligand peptide at the remote allosteric site [70]. These data thus provide a causal link between changes in structural dynamics upon ligand binding and its effects on affinities in the KIX domain.

Experimentally, pico- to nanosecond timescale dynamic processes can also be probed by measuring NMR cross-correlated relaxation rates [71]. For high-molecular weight proteins, dipolar $^1H-^1H$ cross-correlated relaxation rates are directly related to order parameters and can thus be employed to characterize the amplitude of motion of a particular moiety. In case of methyl groups, $S^2_{axis}$ is obtained, i.e., the generalized order parameter that describes the
amplitude of motion of the methyl 3-fold axis [72]. Intramethyl $^1$H-$^1$H cross-correlated relaxation rates can be measured using highly deuterated, methyl-labeled samples, employing experiments that monitor the buildup of methyl $^1$H double- or triple-quantum magnetization [73,74].

For the allosteric enzyme imidazole glycerol phosphate synthase (IGPS), dipolar $^1$H-$^1$H cross-correlated relaxation rate constants, $\eta$, were determined for the apo-form of the enzyme, along with different binary and ternary complexes [75]. IGPS is a heterodimeric protein (Fig 1E) in which each monomer subunit catalyzes a different reaction. Effector binding to one subunit (HisF) accelerates the hydrolysis of glutamine by the other subunit (HisH) across a distance of $>25$ Å. $^1$H-$^1$H cross-correlated relaxation rate constants for the different complexes indicated a site-specific response of pico- to nanosecond timescale motions upon binding of effector. In agreement with these data, a 100-nanosecond all-atom MD simulation revealed a variety of correlated motions on the nanosecond timescale, bridging the effector binding and active sites [76]. Detailed analysis of the MD trajectories using generalized correlation coefficients further showed that the entropically driven effector binding partly promotes structural changes in HisF and HisH that are responsible for the inactive-to-active allosteric transition of this enzyme. Because motions occurring on the millisecond timescale, detected by RD measurements, are required for optimal HisH activity, the authors hypothesized that these nanosecond dynamics might represent the initial fluctuations that ultimately lead to the formation of the fully active enzyme [75].

**Residual Dipolar Couplings and Chemical Shifts**

The experiments described above directly probe dynamic processes, such as conformational exchange or bond vector fluctuations, through spin relaxation measurements. These methods are ideally complemented by NMR parameters that are dynamically averaged in conformational ensembles, such as chemical shifts or dipolar couplings. While these observables do not yield quantitative information on the timescales of dynamic processes per se, they can provide invaluable information about the dynamic nature of allosteric proteins and protein complexes.

As an example, residual dipolar couplings (RDCs) represent a rich source of structural information and are routinely used for NMR structure determination, as they report on the relative orientation between vectors connecting pairs of interacting nuclei (e.g., the backbone NH bond vector) and the magnetic field [77]. In addition, in cases in which dynamics cause the dipolar vector to reorient, the magnitude of a given RDC value corresponds to the population-weighted averaged value over all orientations (Fig 5) [78]. This particular feature of RDCs provides the basis for their use as sensitive reporters on dynamic processes, spanning a broad time scale window that ranges from picoseconds all the way to milliseconds. In order to extract this information from the experimental data, however, both dynamical and structural parameters must be taken into account. Typically, RDCs are measured using multiple differently aligned samples and subsequently fit by use of explicit motional models, some of which yield per-residue generalized order parameters, or by generating ensembles of structures that adequately represent the experimental data [79]. Because dipolar coupling data by themselves do not report on timescales, this information is only indirectly available by comparison of RDC-derived order parameters with values obtained from spin relaxation data [79].

From an extensive set of RDC data that were recorded under variable experimental conditions, correlated dynamics in the immunoglobulin-binding B1 domain of streptococcal protein G were characterized [80]. A total of up to 27 RDCs were collected for the peptide planes of each amino acid and analyzed employing a three-dimensional Gaussian axial fluctuation model. The results indicate the presence of correlated motions amino acids across the central
Based on these observations, it was concluded that long-range information transfer between remote parts of the structure is transmitted collectively through a network of residues that are connected via interstrand hydrogen bonds. Notably, RDC-derived order parameters were found to be consistently lower than the $S^2$ values that were extracted from $^{15}\text{N}$ relaxation experiments, implying that the correlated dynamics involve motions that are slower than picoseconds.

As with NMR order parameters, molecular simulations have been employed to generate physically plausible structural ensembles that are consistent with the experimental RDC data. For ubiquitin, a 116-member structural ensemble was generated using a large RDC dataset as input restraints that covers solution dynamics up to the microsecond timescale [81]. The RDC ensemble embraces all structural heterogeneity that is observed in crystal structures of ubiquitin and ubiquitin complexes, indicating that microsecond and submicrosecond dynamics account for a large proportion of the structural variability that is required for molecular...
recognition. While RDC ensembles of allosteric proteins are not available to date, it is clear that this approach has the potential to substantially improve our understanding of allosteric mechanisms.

NMR chemical shifts, like residual dipolar couplings, are exquisitely sensitive to even small changes in structure. Because NMR chemical shifts observed for each peak correspond to an average over the chemical shifts in each conformer in the ensemble, analysis of peak positions in NMR spectra yields quantitative information on the populations of different conformational states. Moreover, chemical shifts contain information about dynamics, since conformational averaging affects the experimentally observed values. Significant deviation of NMR chemical shifts from random-coil values thus indicate a relatively rigid and well-defined structure, while proximity of chemical shifts to random coil values is a manifestation of conformational averaging. By use of empirically derived relationships between chemical shifts and flexibility, this feature can be employed to predict dynamic properties at protein backbone and side-chain sites using chemical shift data [82,83]. The timescale of dynamic processes that is covered by this method ranges from picoseconds to milliseconds [84].

With respect to dynamic allostery, NMR chemical shift measurements have predominantly been employed for analyzing the population shifts of conformational states [85–87]. A particularly powerful experimental probe is methyl carbon $^{13}$C chemical shifts of isoleucine, leucine, and valine, which provide a very sensitive measure of the side-chain conformations of these residues [88]. Assuming that conformational sampling of side-chain dihedral angles ($\chi_2$ in leucine and isoleucine, $\chi_1$ in valine) can be described in terms of jumps between a limited number of rotameric states, experimentally determined methyl $^{13}$C chemical shifts of these residues can be used to determine the populations of these rotameric states [89–91]. This particular feature was used to characterize allosteric coupling in the KIX domain [12]. Methyl $^{13}$C chemical shifts were used to analyze the isoleucine, leucine, and valine side chain rotameric states in different complexes of KIX. The data revealed that the transition from the binary complex of KIX (with the MLL peptide bound) to the ternary complex (with both MLL and pKID peptides bound) (Fig 1B) is accompanied by a collective change of KIX side-chain rotameric states in the hydrophobic core. The redistribution of rotameric states involves residues that had previously been recognized as being part of the allosteric network [34]. Notably, this effect is not captured by the NMR structural bundles of KIX complexes, which represent the highest-populated rotamers of these residues in solution. Using metadynamics, the allosteric communication pathways of KIX were probed computationally, revealing tight coupling of the dynamics of the L12-G2 loop connecting helices $\alpha_1$ and $\alpha_2$ and the restructuring of the hydrophobic core [35]. It was concluded that binding of the MLL to KIX moves the L12 loop close to the ligand peptide, which results in a less compressed hydrophobic core that is susceptible to a redistribution of rotameric states of allosteric network residues. Ultimately, this process leads to an increase of the binding affinity for the second ligand.

Of specific interest are side-chain methyl-TROSY experiments [92], which have been applied to monitor population shifts for very high-molecular weight allosteric proteins up to 670 kDa [93–95]. For example, it could be demonstrated that activator binding to 20S CP (Fig 1D) modulates the relative populations of conformational states that are present in solution [96]. Moreover, the methyl-TROSY $^{13}$C-$^1$H chemical shift data revealed that the activator binding site is linked to the active site of the 20S CP through an allosteric network spanning a distance of 80 Å. It was further shown that the function of the 20S CP is perturbed by changing the relative distribution of conformers. Binding of an allosteric inhibitor, which shifts the position of the equilibrium to the inhibited state, was monitored by methyl $^1$H and $^{13}$C chemical shifts and resulted in a loss of function. In addition, population shifts were induced by
mutation of amino acid residues that make key contacts with allosteric activators, resulting in an approximately linear dependence of chemical shifts observed in methyl-TROSY spectra [96].

While these examples do not exploit NMR chemical shifts as reporters for dynamics, they clearly demonstrate the dynamic nature of conformational ensembles of proteins in solution. Indeed, chemical shift responses to perturbations are very commonly employed as diagnostic tools for identifying coupled networks within allosteric proteins or protein complexes. This task becomes increasingly challenging, however, in cases in which allosteric proteins lack significant long-range structural responses to perturbations, indicative of fundamentally dynamics-driven allostery [97]. In such cases, covariance analysis of chemical shift changes caused by binding different effector molecules can be employed [98]. Using this approach, protein sites with large chemical shift variations upon perturbation as well as those that display only subtle variations are taken into account, so long as their chemical shift responses are correlated. Covariance analysis of chemical shifts is, thus, capable of identifying networks of coupled residues that comprise both structural and dynamic components. As a matter of fact, this approach is particularly effective in identifying allosteric coupling networks within partially unstructured and highly dynamic regions, which are common in proteins that are involved in signaling and often remain elusive to structure-based techniques [99]. Chemical shift-based perturbation analysis of allosteric networks is complemented by computational approaches using elastic network models. Scanning for protein sites that dynamically respond to perturbations of ENMs, such as ligand binding, can identify coupled networks within a protein that are related to allosteric communication [100].

Outlook

The above examples show that allosteric communication in many cases includes structural transitions, accomplished through a defined and dynamic redistribution of the conformational ensemble. As proposed by Cooper [101], allostery can in principle be completely dynamic in nature without requiring any structural changes at all. The question thus remains: how small a structural change shall be considered significant? From a practical perspective, this represents a challenge for experimental techniques that are exquisitely sensitive to structural changes. Crystallographic studies of proteins have proven the exclusive ability of high-resolution X-ray diffraction techniques to detect even small differences between three-dimensional structures. Likewise, methodological advancements in NMR spectroscopy have enabled the observation of conformational states in solution that are only sparsely populated as well as transitions between them.

Future developments in this direction, along with the enhanced spectrometer performance that is afforded by cryogenically cooled probes and higher magnetic field strength spectrometers, will further boost the sensitivity of NMR experiments. This will enable NMR studies of even higher-molecular-weight allosteric proteins and protein complexes. Slow conformational transitions in such systems are likely outside the time regime that can be monitored by current magnetization transfer techniques, requiring the development of novel experimental approaches such as the use of long-lived spin states for detecting very slow exchange processes [102]. Of note, data acquisition of two-dimensional methyl-TROSY spectra in less than 5 seconds has been reported [103], providing an experimental tool for real-time observation of conformational transitions in higher-molecular-weight assemblies. NMR real-time approaches to characterize slow conformational transitions have been particularly useful for medium-sized ribonucleic acids in the past [104]. A number of allosteric ribozymes and riboswitch aptamers have been described, in which remote parts of the RNA are likely dynamically coupled [105,106].
Moreover, it is foreseeable that even lower-populated states will become amenable to experimental characterization, further increasing the proportion of the conformational ensemble that can be observed by experiment. Recent crystallographic studies have highlighted the capability of room temperature X-ray data collection to model multiple conformers in crystals [107]. These techniques thus offer intriguing synergistic potential for NMR spectroscopy and X-ray crystallography to characterize conformational heterogeneity at great detail by use of orthogonal approaches. Distinctive structural features that are difficult to grasp with standard structure determination protocols, such as side-chain rotameric distributions, are likely to attract considerable attention in the future. It is tempting to speculate that ligand-induced modulation of side-chain heterogeneity, as observed for the KIX domain, may represent a common aspect of allosteric communication. Taken together, experimental advances will present new challenges for computational techniques with respect to both the timescale that is accessible by computer simulations as well as the size of the biomolecules that are investigated. All-atom metadynamics [108] and AMD [109] represent but two approaches that are available to date for sampling millisecond timescale events, setting the stage for future developments toward even slower timescale motions. Only a combination of experimental and computational techniques will succeed in providing a comprehensive and authentic view of dynamic allostery in biomolecules.

References

1. Gunasekaran K, Ma B, Nussinov R. Is allosteroy an intrinsic property of all dynamic proteins? Proteins. 2004; 57(3):433–43. PMID: 15382234
2. Tsai CJ, del Sol A, Nussinov R. Allostery: absence of a change in shape does not imply that allosteroy is not at play. J Mol Biol. 2008; 378(1):1–11. doi: 10.1016/j.jmb.2008.02.034 PMID: 18353365
3. Motlagh HN, Wrabl JO, Li J, Hilser VJ. The ensemble nature of allosteroy. Nature. 2014; 508 (7496):331–9. doi: 10.1038/nature13001 PMID: 24740064
4. Kern D, Zuiderweg ER. The role of dynamics in allosteric regulation. Curr Opin Struct Biol. 2003; 13 (6):748–57. PMID: 14675554
5. Swain JF, Gierasch LM. The changing landscape of protein allosteroy. Curr Opin Struct Biol. 2006; 16 (1):102–8. PMID: 16423525
6. Smock RG, Gierasch LM. Sending signals dynamically. Science. 2009; 324(5924):198–203. doi: 10. 1126/science.1169377 PMID: 19359576
7. Tsai CJ, Del Sol A, Nussinov R. Protein allosteroy, signal transmission and dynamics: a classification scheme of allosteric mechanisms. Mol Biosyst. 2009; 5(3):207–16. doi: 10.1039/b819720b PMID: 19225609
8. Nussinov R, Tsai CJ. Allostery without a conformational change? Revisiting the paradigm. Curr Opin Struc Biol. 2015; 30:17–24.
9. Mittermaier A, Kay LE. New tools provide new insights in NMR studies of protein dynamics. Science. 2006; 312(5771):224–8. PMID: 16614210
10. Palmer AG 3rd. Chemical exchange in biomacromolecules: past, present, and future. J Magn Reson. 2014; 241:3–17. doi: 10.1016/j.jmr.2014.01.008 PMID: 24698076
11. Passner JM, Schultz SC, Steitz TA. Modeling the cAMP-induced allosteric transition using the crystal structure of CAP-cAMP at 2.1 A resolution. J Mol Biol. 2000; 304(5):847–59. PMID: 11124031
12. Bruschweiler S, Konrat R, Tollinger M. Allosteric communication in the KIX domain proceeds through dynamic repacking of the hydrophobic core. ACS Chem Biol. 2013; 8(7):1600–10. doi: 10.1021/cb4002188 PMID: 23851431
13. Piper DE, Batchelor AH, Chang CP, Cleary ML, Wolberger C. Structure of a HoxB1-Pbx1 heterodimer bound to DNA: role of the hexapeptide and a fourth homeodomain helix in complex formation. Cell. 1999; 96(6):587–97. PMID: 10052469
14. Rabl J, Smith DM, Yu Y, Chang SC, Goldberg AL, Cheng Y. Mechanism of gate opening in the 20S proteasome by the proteasomal ATPases. Mol Cell. 2008; 30(3):360–8. doi: 10.1016/j.molcel.2008.03.004 PMID: 18471981
15. Chaudhuri BN, Lange SC, Myers RS, Davisson VJ, Smith JL. Toward understanding the mechanism of the complex cyclization reaction catalyzed by imidazole glycerolphosphate synthase: crystal structures of a ternary complex and the free enzyme. Biochem. 2003; 42(23):7003–12.
16. Palmer AG 3rd, Massi F. Characterization of the dynamics of biomacromolecules using rotating-frame spin relaxation NMR spectroscopy. Chem Rev. 2006; 106(5):1700–19. PMID: 16683750
17. Mulder FA, Hon B, Mittermaier A, Dahlquist FW, Kay LE. Slow internal dynamics in proteins: application of NMR relaxation dispersion spectroscopy to methyl groups in a cavity mutant of T4 lysozyme. J Am Chem Soc. 2002; 124(7):1443–51. PMID: 11841314
18. Lundstrom P, Ahnér A, Blissing AT. Isotope labeling methods for relaxation measurements. Adv Experim Med Biol. 2012; 992:63–82.
19. Alvarado LJ, Longhini AP, LeBlanc RM, Chen B, Kreutz C, Dayie TK. Chemo-enzymatic synthesis of selectively 13C/15N-labeled RNA for NMR structural and dynamics studies. Meth Enzymol. 2014; 549:133–62. doi: 10.1016/B978-0-12-801122-5.00007-6 PMID: 25432748
20. Baldwin AJ. An exact solution for R2,eff in CPMG experiments in the case of two site chemical exchange. J Magn Reson. 2014; 244:114–24. doi: 10.1016/j.jmr.2014.02.023 PMID: 24852115
21. McConnell HM. Reaction rates by nuclear magnetic resonance. J Chem Phys. 1958; 28:430–1.
22. Kovrigin EL, Kempf JG, Grey MJ, Loria JP. Faithful estimation of dynamics parameters from CPMG relaxation dispersion measurements. J Magn Reson. 2006; 180(1):93–104. PMID: 16458551
23. Farber PJ, Mittermaier A. Relaxation dispersion NMR spectroscopy for the study of protein allostery. Biophys Rev. 2015; 7(2):191–200.
24. Lipchock JM, Loria JP. Nanometer propagation of millisecond motions in V-type allostery. Structure. 2010; 18(12):1596–607. doi: 10.1016/j.str.2010.09.020 PMID: 21134639
25. Oyen D, Fenwick RB, Stanfield RL, Dyson HJ, Wright PE. Cofactor-Mediated Conformational Dynamics Promote Product Release From Escherichia coli Dihydrofolate Reductase via an Allosteric Pathway. J Am Chem Soc. 2015; 137(29):9459–68. doi: 10.1021/jacs.5b05707 PMID: 26147643
26. Villali J, Pontiggia F, Clarkson MW, Hagan MF, Kern D. Evidence against the “Y-T coupling” mechanism of activation in the response regulator NtrC. J Mol Biol. 2014; 426(7):1554–67. doi: 10.1016/j.jmb.2013.12.027 PMID: 24406745
27. Shi L, Kay LE. Tracing an allosteric pathway regulating the activity of the HslV protease. Proc Natl Acad Sci USA. 2014; 111(6):2140–5. doi: 10.1073/pnas.1318476111 PMID: 24469799
28. Neudecker P, Korzhnev DM, Kay LE. Assessment of the effects of increased relaxation dispersion data on the extraction of 3-site exchange parameters characterizing the unfolding of an SH3 domain. J Biomol NMR. 2006; 34(3):129–35. PMID: 16604422
29. Townsend PD, Rodgers TL, Pohl E, Wilson MR, McLeish TC, Cann MJ. Global low-frequency motions in protein allostery: CAP as a model system. Biophys Rev. 2015; 7(2):175–82. PMID: 26000062
30. Palazzesi F, Barducci A, Tollinger M, Parrinello M. The allosteric communication pathways in KIX domain of CBP. Proc Natl Acad Sci USA. 2013; 110(35):14237–42. doi: 10.1073/pnas.1313548110 PMID: 23940332
31. Neudecker P, Korzhnev DM, Kay LE. Assessment of the effects of increased relaxation dispersion data on the extraction of 3-site exchange parameters characterizing the unfolding of an SH3 domain. J Biomol NMR. 2006; 34(3):129–35. PMID: 16604422
32. Grey MJ, Wang CY, Palmer AG. Disulfide bond isomerization in basic pancreatic trypsin inhibitor: Multisite chemical exchange quantified by CPMG relaxation dispersion and chemical shift modeling. J Am Chem Soc. 2003; 125(47):14324–35. PMID: 14624581
38. Li P, Martins IR, Amarasinghe GK, Rosen MK. Internal dynamics control activation and activity of the autoinhibited Vav DH domain. Nat Struct Mol Biol. 2008; 15(6):613–8. doi:10.1038/nsmb.1428 PMID: 18488041

39. Farber PJ, Mittermaier A. Concerted dynamics link allosteric sites in the PBX homeodomain. J Mol Biol. 2011; 405(3):819–30. doi:10.1016/j.jmb.2010.11.016 PMID: 21087615

40. Farber PJ, Slager J, Mittermaier AK. Local folding and misfolding in the PBX homeodomain from a three-state analysis of CPMG relaxation dispersion NMR data. J Phys Chem B. 2012; 116(34):10317–29. doi:10.1021/jp306127m PMID: 22845760

41. Montelione GT, Wagner G. 2D Chemical exchange NMR spectroscopy by proton-detected heteronuclear correlation. J Am Chem Soc. 1989; 111(8):3096–8.

42. Imai S, Osawa M, Takeuchi K, Shimada I. Structural basis underlying the dual gate properties of KcsA. Proc Natl Acad Sci USA. 2010; 107(14):6216–21. doi:10.1073/pnas.0911270107 PMID: 20212150

43. Audin MJ, Dom G, Fromm SA, Reiss K, Schutz S, Vorlander MK, et al. The archaeal exosome: identification and quantification of site-specific motions that correlate with cap and RNA binding. Angew Chem. 2013; 52(32):8312–6.

44. Religa TL, Sprangers R, Kay LE. Dynamic regulation of archaeal proteasome gate opening as studied by TROSY NMR. Science. 2010; 328(5974):98–102. doi:10.1126/science.1184991 PMID: 20360109

45. Förster A, Masters EI, Whitby FG, Robinson H, Hill CP. The 1.9 Å structure of a proteasome-11S activator complex and implications for proteasome-PAN/PA700 interactions. Mol Cell. 2005; 18(5):589–99. PMID: 15916965

46. Igumenova TI, Brath U, Akke M, Palmer AG, 3rd. Characterization of chemical exchange using residual dipolar coupling. J Am Chem Soc. 2007; 129(44):13396–7. PMID: 17929930

47. Jarymowycz VA, Stone MJ. Fast time scale dynamics of protein backbones: NMR relaxation methods, applications, and functional consequences. Chem Rev. 2006; 106(5):1624–71. PMID: 16683748

48. Lipari G, Szabo A. Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 1. Theory and range of validity. J Am Chem Soc. 1982; 104(4546–4559).

49. Clore GM, Szabo A, Bax A, Kay LE, Driscoll PC, Gronenborn AM. Deviations from the Simple 2-Parameter Model-Free Approach to the Interpretation of N-15 Nuclear Magnetic-Relaxation of Proteins. J Am Chem Soc. 1990; 112(12):4989–91.

50. Bruschweiler R, Wright PE. Nmr Order Parameters of Biomolecules—a New Analytical Representation and Application to the Gaussian Axial Fluctuation Model. J Am Chem Soc. 1994; 116(18):8426–7.

51. Daragan VA, Mayo KH. Motional Model Analyses of Protein and Peptide Dynamics using 13C and 15N NMR relaxation. J Magn Reson Spectrosc. 1997; 31:63–105.

52. Peng JW, Wagner G. Mapping of Spectral Density-Functions Using Heteronuclear Nmr Relaxation Measurements. J Magn Reson. 1992; 98(2):308–32.

53. Kay LE, Torchia DA, Bax A. Backbone dynamics of proteins as studied by 15N inverse detected heteronuclear NMR Relaxation Measurements. J Am Chem Soc. 1990; 112(12):4989–91.

54. Muhandiram DR, Yamazaki T, Sykes BD, Kay LE. Measurement of 2H T1 and T1.rho. relaxation times in uniformly 13C-labeled and fractionally 2H-labeled proteins in solution. J Am Chem Soc. 1995; 117:11536–44.

55. Stone MJ. NMR relaxation studies of the role of conformational entropy in protein stability and ligand binding. Acc Chem Res. 2001; 34(5):379–88. PMID: 11352716

56. Frederick KK, Marlow MS, Valentine KG, Wand AJ. Conformational entropy in molecular recognition by proteins. Nature. 2007; 448(7151):325–9. PMID: 17637663

57. Allison JR. Assessing and refining molecular dynamics simulations of proteins with nuclear magnetic resonance data. Biophys Rev. 2012; 4(3):189–203.

58. Sugita Y, Okamoto Y. Replica-exchange molecular dynamics method for protein folding. Chem Phys Lett. 1999; 314:141–51.

59. Hamelberg D, Morgan J, McCammon JA. Accelerated molecular dynamics: a promising and efficient simulation method for biomolecules. J Chem Phys. 2004; 120(24):11191–29. PMID: 15268227

60. Pastor N, Amero C. Information flow and protein dynamics: the interplay between nuclear magnetic resonance spectroscopy and molecular dynamics simulations. Front Plant Sc. 2015; 6:306.
Petit CM, Zhang J, Sapienza PJ, Fuentes EJ, Lee AL. Hidden dynamic allostery in a PDZ domain. Proc Natl Acad Sci USA. 2009; 106(43):18249–54. doi: 10.1073/pnas.0904491106 PMID: 19828436

Volkmann BF, Lipson D, Wemmer DE, Kern D. Two-state allostERIC behavior in a single-domain signaling protein. Science. 2001; 291(5512):2429–33. PMID: 11264542

Leung HT, Kukic P, Camilloni C, Bemporad F, De Simone A, Aprile FA, et al. NMR characterization of the conformational fluctuations of the human lymphocyte function-associated antigen-1 I-domain. Prot Sci. 2014; 23(11):1596–606.

Tzeng SR, Kalodimos CG. Dynamic activation of an allosteric regulatory protein. Nature. 2009; 462(7271):368–72. doi: 10.1038/nature08560 PMID: 19924217

Li L, Uversky VN, Dunker AK, Meroueh SO. A computational investigation of allostery in the catabolite activator protein. J Am Chem Soc. 2007; 129(50):15668–76. PMID: 19041839

Louet M, Seifert C, Hensen U, Grater F. Dynamic Allostery of the Catabolite Activator Protein. Proc Natl Acad Sci USA. 2009; 106(43):18249–54. doi: 10.1073/pnas.0904491106 PMID: 19828436

Tjandra N, Bax A. Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium. Science. 1997; 278(5340):1111–4. PMID: 9353189

Korkmaz EN, Nussinov R, Haliloglu T. Conformational control of the binding of the transactivation domain of the MLL protein and c-Myb to the KIX domain of CREB. PLoS Comput Biol. 2012; 8(3):e1002420. doi: 10.1371/journal.pcbi.1002420 PMID: 22438798

Law SM, Gagnon JK, Mapp AK, Brooks CL. Prepaying the entropic cost for allosteric regulation in proteins. J Am Chem Soc. 2005; 127(43):14970–1. PMID: 16172390

Berjanskii MV, Wishart DS. A simple method to predict protein flexibility using secondary chemical shifts. J Am Chem Soc. 2005; 127(43):14970–1. PMID: 16248604

Berjanskii MV, Wishart DS. A simple method to measure protein side-chain mobility using NMR chemical shifts. J Am Chem Soc. 2013; 135(39):14536–9. doi: 10.1021/ja407509z PMID: 24032347

Berjanskii MV, Wishart DS. Application of the random coil index to studying protein flexibility. J Biomol NMR. 2008; 40(1):31–48. PMID: 17985196

Masterson LR, Mascioni A, Traaseth NJ, Taylor SS, Veglia G. Allosteric cooperativity in protein kinase A. Proc Natl Acad Sci USA. 2008; 105(2):506–11. doi: 10.1073/pnas.0709214104 PMID: 18178622
86. Zhuravleva A, Gierasch LM. Allosteric signal transmission in the nucleotide-binding domain of 70-kDa heat shock protein (Hsp70) molecular chaperones. Proc Natl Acad Sci USA. 2011; 108(17):6987–92. doi: 10.1073/pnas.1014448108 PMID: 21482798

87. Vashisth H, Storaska AJ, Neubig RR, Brooks CL 3rd. Conformational dynamics of a regulator of G-protein signaling protein reveals a mechanism of allosteric inhibition by a small molecule. ACS Chem Biol. 2013; 8(12):2778–84. doi: 10.1021/cb400568g PMID: 24093330

88. London RE, Wingad BD, Mueller GA. Dependence of amino acid side chain 13C shifts on dihedral angle: application to conformational analysis. J Am Chem Soc. 2008; 130(33):11097–105. doi: 10.1021/ja802729q PMID: 18652454

89. Mulder FA. Leucine side-chain conformation and dynamics in proteins from 13C NMR chemical shifts. Chembiochem. 2009; 10(9):1477–84. doi:10.1002/cbic.200900086 PMID: 19466705

90. Hansen DF, Neudecker P, Kay LE. Determination of valine side-chain rotamer conformations in proteins from methyl 13C chemical shifts: application to the 360 kDa half-proteasome. J Am Chem Soc. 2011; 133(21):8272–81. doi:10.1021/ja2014532 PMID: 21545099

91. Kalodimos CG. NMR reveals novel mechanisms of protein activity regulation. Prot Sci. 2011; 20(5):773–82.

92. Selvaratnam R, Chowdhury S, VanSchouwen B, Melacini G. Mapping allostery through the covariance analysis of NMR chemical shifts. Proc Natl Acad Sci USA. 2011; 108(15):6133–8. doi:10.1073/pnas.1017311108 PMID: 21447788

93. Ameo C, Schanda P, Dura MA, Ayala I, Marion D, Franzetti B, et al. Fast two-dimensional NMR spectroscopy of high molecular weight protein assemblies. J Am Chem Soc. 2009; 131(10):328–34. PMID: 17212412

94. Breaker RR, Joyce GF. The expanding view of RNA and DNA function. Chem & Biol. 2014; 21(9):1059–65.

95. Woldeyes RA, Sivak DA, Fraser JS. E pluribus unum, no more: from one crystal, many conformations. Curr Opin Struct Biol. 2014; 28:56–62. doi: 10.1016/j.sbi.2014.07.006 PMID: 25113271
108. Tiwary P, Parrinello M. From metadynamics to dynamics. Phys Rev Lett. 2013; 111(23):230602. PMID: 24476246

109. Pierce LC, Salomon-Ferrer R, Augusto FdOC, McCammon JA, Walker RC. Routine Access to Millisecond Time Scale Events with Accelerated Molecular Dynamics. J Chemical Theory Comput. 2012; 8(9):2997–3002.