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Dynamic Neutron Scattering by Biological Systems

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Abstract

Dynamic neutron scattering directly probes motions in biological systems on femtosecond to microsecond timescales. When combined with molecular dynamics simulation and normal mode analysis, detailed descriptions of the forms and frequencies of motions can be derived. We examine vibrations in proteins, the temperature dependence of protein motions, and concepts describing the rich variety of motions detectable using neutrons in biological systems at physiological temperatures. New techniques for deriving information on collective motions using coherent scattering are also reviewed.

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INTRODUCTION

The first decades of molecular biology witnessed the emergence of the structure-function paradigm, wherein the exquisite three-dimensional architectures of biological macromolecules were used to directly infer functional mechanisms. This approach provides insight into relationships among sequence, structure, and function at the atomic level. For example, the static three-dimensional arrangement of amino acid residues in an enzyme active site was found to form critical interactions needed for catalysis (149).

However, starting around the 1970s, interest in the dynamic aspects of protein structure emerged. Researchers determined proteins exist in different conformations, such as with open or closed domains following hinge-bending motions (88). Spectroscopic experiments and molecular dynamics (MD) simulations indicated proteins explore complex energy landscapes and visit myriad conformational substates (37, 47). Large-amplitude, collective motions in proteins also play important roles in many functional processes, including the entry and exit of molecules in catalytic sites (63) and the allosteric transmission of information through dynamical structural change, leading to the concept of molecular machines (3). Recently, researchers have also explored further dynamical phenomena such as those associated with intrinsic disorder (99) in protein sequences, as well as the concept of conformational selection, wherein proteins explore multiple conformations and the binding configuration is selected by the ligand (84), and also complex mechanisms that involve both induced-fit and conformational selection (46).

MD has been extensively used to investigate biomolecular dynamics. With specialized hardware, these simulations can now extend out as far as the millisecond timescale or for systems of $\sim 10^8$ atoms (132, 133), and they have shown that biomolecular systems exhibit a dazzling variety of diffusive and vibrational dynamics (56, 94). As a result, an apposite quantitative experimental characterization of motional amplitudes, forms, and time dependences is needed. Dynamic neutron scattering, sometimes called inelastic neutron scattering, may be the experimental technique that most directly determines atomic motions. This form of spectroscopy probes both the timescales and length scales of pico- to microsecond atomic motions.

A large body of research exists on the application of dynamic neutron scattering spectroscopy to proteins and other biological systems, and this review does not pretend to be comprehensive (for complementary information, see 24, 39, 69, 71, 136, 158). Here, we emphasize how neutron scattering can be combined with computer simulation to characterize the dynamics of biological systems. We examine vibrations in proteins and some aspects of the temperature dependence of protein dynamics. We discuss the range of motions detectable by neutrons at physiological

temperatures and summarize approaches to nonprotein complex biosystems, using biomembranes and lignocellulosic biomass as examples. New neutron and simulation approaches to determining collective motions are also reviewed.

BASIC NEUTRON PRINCIPLES

Neutron Scattering Functions

Like X-rays, thermal neutron wavelengths (approximately 1 Å) are similar to interatomic distances, and elastic scattering can be used to determine the structures of molecular samples. However, unlike X-rays, which have kiloelectron-volt energies, neutron energies are similar to thermal energies whose measurements lie in the millielectron-volt range (i.e., the energies needed to excite molecular motions). Thus, dynamical excitations change neutron velocities significantly and measurably, as "inelastic" scattering. By determining the energy ($\hbar\omega$, where ω is the frequency) and momentum transfer ($\hbar\boldsymbol{q}$, where $\boldsymbol{q}$ is the scattering vector) dependence of inelastic scattering, one can uniquely and simultaneously obtain both the timescales of motions and their geometries.

A particularly seductive aspect of neutron scattering is the directness of the relationship between particle dynamics and the scattering functions. This directness arises because neutrons scatter from the nuclei of fluctuating atoms and can be appreciated by considering the basic dynamical quantity—in other words, the van Hove function $G(\boldsymbol{r}, t)$ (145),

$$G(\boldsymbol{r},t) = \frac{1}{N}\int \sum_i \sum_j \delta\left[\boldsymbol{r}' + \boldsymbol{r} - \boldsymbol{r}_j(t)\right]\delta\left[\boldsymbol{r}' - \boldsymbol{r}_i(0)\right]\mathrm{d}\boldsymbol{r}', \qquad 1.$$

where $G(\boldsymbol{r}, t)$ is a natural time-dependent generalization of the well-known pair distribution function, $g(r)$, of systems of interacting particles. $G(\boldsymbol{r}, t)$ is a real-space dynamical correlation function describing the spatial and temporal distributions of pairs of particles. It gives the probability of finding a particle at position $\boldsymbol{r}$ at time t given that one of the particles was located at the origin at time $t = 0$. Taking the space Fourier transformation of $G(\boldsymbol{r}, t)$ gives the intermediate scattering function, $I(\boldsymbol{q}, t)$:

$$I(\boldsymbol{q},t) = \int_{-\infty}^{\infty} G(\boldsymbol{r},t)\, e^{-i\boldsymbol{q}.\boldsymbol{r}}\mathrm{d}\boldsymbol{r}. \qquad 2.$$

Coherent and Incoherent Scattering

Neutrons interact with nuclei via the strong nuclear force. The neutron scattering length varies randomly from nucleus to nucleus in a sample because the relative neutron-nucleus spin orientation is random. Therefore, the scattering length at any given site will have an average value and fluctuations from the average. The mean scattering cross section is found from interference terms—the coherent scattering—whereas the fluctuations, which are independent of sites, form incoherent scattering. Incoherent scattering thus detects motions of single atoms, whereas coherent scattering detects pair correlations. $I(q,t)$ as determined by neutrons can thus be separated into incoherent and coherent components:

$$I_{\mathrm{inc}}(q,t) = \sum_i b^2{}_{\mathrm{inc}}\,\langle\exp\left[-i\boldsymbol{q}.\boldsymbol{r}_i(0)\right]\exp\left[i\boldsymbol{q}.\boldsymbol{r}_i(t)\right]\rangle, \qquad 3.$$

$$I_{\mathrm{coh}}(q,t) = \sum_{i.j} b_{i,\mathrm{coh}} b_{j,\mathrm{coh}}\langle\exp\left[-i\boldsymbol{q}.\boldsymbol{r}_i(0)\right]\exp\left[i\boldsymbol{q}.\boldsymbol{r}_j(t)\right]\rangle, \qquad 4.$$

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where $b_{j,\mathrm{inc}}$ ($b_{j,\mathrm{coh}}$) is the incoherent (coherent) scattering length of atom j, $\boldsymbol{r}_j$ is the position vector of that atom, and the brackets denote an ensemble and orientation average. The incoherent neutron scattering cross section, b^2_{inc}, of hydrogen, ^{1}H, is 20 times larger than that of other elements. Thus, incoherent scattering can be used to study hydrogen dynamics. Coherent scattering, in contrast, probes heavy-atom contributions and is well suited for characterizing collective dynamics (55, 82).

The quantity normally measured experimentally is the dynamic structure factor, $S(q,\omega)$, the time Fourier transform of $I(q,t)$:

$$S_{\mathrm{inc}}(q,\omega) = \int_{-\infty}^{\infty} I_{\mathrm{inc}}(q,t)\, e^{-i\omega t}\, \mathrm{d}t, \qquad 5.$$

$$S_{\mathrm{coh}}(q,\omega) = \int_{-\infty}^{\infty} I_{\mathrm{coh}}(q,t)\, e^{-i\omega t}\, \mathrm{d}t. \qquad 6.$$

$S(q,\omega)$ is effectively the amplitude-weighted distribution of the dynamic modes over frequency.

Equations 3 and 4 make clear that all neutron scattering properties can be calculated simply from knowledge of the positions of the atoms as a function of time. This information is the direct result of MD simulation trajectories. Thus, the complementarity of MD to neutron scattering permits, in principle, a complete description of atomic dynamics. Furthermore, there are very few significant approximations embodied in Equations 3 and 4. This contrasts with other techniques for determining dynamical behavior, such as nuclear magnetic resonance (NMR) and optical spectroscopies, in which the computation of measured amplitudes requires hefty assumptions concerning the nature of the dynamics, of probe labels, and/or of electronic variations along the dynamical modes. Efficient software exists to calculate scattering functions from MD atomic trajectories, including nMoldyn (51, 123) and Sassena (80).

Neutron scattering relies on nuclear reactor or spallation sources; the latter is a pulsed production of neutrons obtained by bombarding a target of heavy elements with high-energy particles, typically accelerated protons. New spallation sources include the Spallation Neutron Source in Oak Ridge, Tennessee (USA), and the Japan Proton Accelerator Research Complex in Tokai, Japan. Further, a very powerful European Spallation Source is being constructed in Lund, Sweden.

VIBRATIONS IN PROTEINS

In a classical system at 0 K, there will be no motion and therefore only elastic scattering. As thermal energy is imparted into the system, vibrations will become activated, leading to a reduction of elastic incoherent scattering, which varies as $\sim\exp(-\langle u^2\rangle q^2)$, where $\langle u^2\rangle$ is the average H-weighted atomic mean-square displacement (MSD), and the appearance of inelastic scattering at the vibrational frequencies with intensities $\sim q^2\langle u_i^2\rangle$ from mode i (see **Figure 1**).

Ideally, representing all dynamics in terms of vibrations would be possible, as normal mode analysis (NMA) allows for complete description of the dynamics within the harmonic approximation to the potential function. Moreover, the scattered intensity can be computed directly from knowledge of the frequencies and atomic displacement vectors of an NMA. Accordingly, neutron spectra calculated from atomic-detail protein normal modes were used in the first simulation-based interpretation of protein experiments (134).

The fastest vibrations in molecular systems are bond stretches and bond angle bends, which take place on 1–10^2-fs timescales. These motions are of relatively small amplitude and thus relatively unimportant in biological function. However, because these high-frequency modes are close to harmonic, they can produce sharp lines in an inelastic neutron spectrum. For this reason, neutrons can be useful for testing and parameterizing vibrational and molecular mechanics force fields (43). Nevertheless, it is still possible to misinterpret molecular vibrational neutron spectra, as

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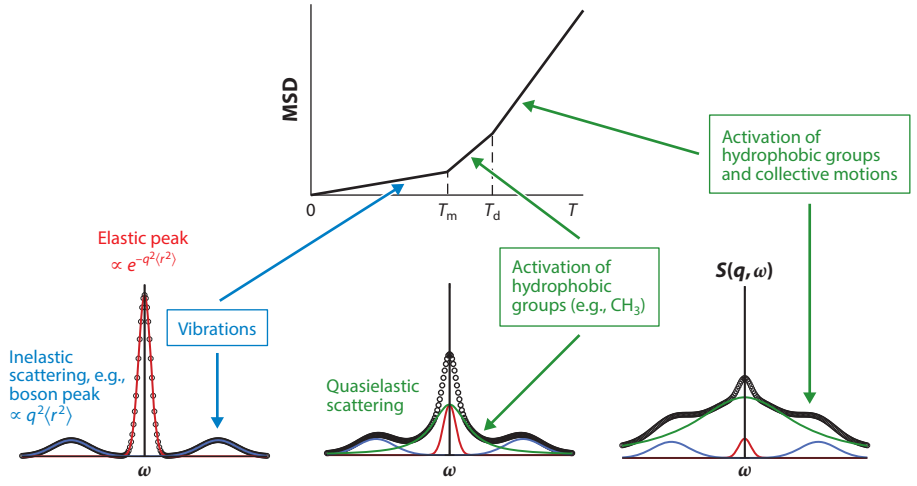


Figure 1

Schematic of the evolution of $S(q,\omega)$ and the MSD with temperature (T) for a globular protein. Elastic (*red*), quasielastic (*green*), and inelastic (*blue*) contributions are shown. Abbreviations: MSD, mean-square displacement; T_d, temperature for dynamical transition; T_m, temperature for activation of methyl motions.

exemplified by a claim that the peptide amino hydrogen is decoupled from the peptide group backbone (65), which may have arisen from an incomplete understanding of neutron recoil effects (66).

Slowing down to the picosecond timescale yields a variety of motions in biological systems. Typically, as the frequency of a vibration decreases, both the amplitude and the number of atoms participating in the vibration tend to increase. Thus, picosecond-period vibrations tend to be delocalized over a macromolecule or, when in a membrane, involve the collective motion of several lipid molecules. These motions can resemble functional conformational change in macromolecules. $S(q,\omega)$ is roughly proportional to the squared amplitude of a harmonic mode; thus, low-frequency motions are of conveniently high scattering amplitudes. Experimentally, neutrons are now complemented by terahertz near-field microscopy, which, in a recent advance, has been used to detect underdamped modes in lysozyme at frequencies >1 meV (1).

Being of large amplitude, low-frequency modes are complicated because they are likely to sample anharmonic regions of the potential surface, making frictional damping from protein and solvent atoms significant. As a result, the spectral signatures of these motions become broadened. Frictional damping can be introduced a posteriori into the harmonic model by assuming that each mode acts as an independent damped Langevin oscillator, leading to good agreement with experimental 300-K scattering (135). Subsequent improvement of the theoretical modeling of overdamped low-frequency modes was performed by Kneller and colleagues, who have worked out much of the detailed theory needed to understand dynamic neutron scattering from sophisticated analytical models of biological system dynamics (15, 52, 73, 74). They derived $S(q,\omega)$ from harmonically vibrating molecules in the presence of friction, describing the essential dynamics by purely diffusive Brownian dynamics in configuration space (72).

A prominent feature observed in low-frequency inelastic neutron and Raman scattering spectra of glass-forming substances is the boson peak, defined as excess in the vibrational density of states over the Debye squared-frequency law. The boson peak may be connected to low-temperature anomalies in the specific heat and thermal conduction of glasses. This peak is the signature of a phase transition in the space of the stationary points of the energy, from a minima-dominated

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phase (with phonons) at low energy to a saddle-point-dominated phase (without phonons) (45). It is visible in proteins and hydration water as a broad band occurring in an energy range of 1.5–3.5 meV, depending on temperature and hydration (see, e.g., 111). MD simulations and NMAs performed on crystalline myoglobin at 150 K indicate that the boson peak originates from $\sim 10^2$ collective, internal harmonic vibrations (78). Accurate description of the environment is essential in reproducing the experimental boson peak form and position. However, internal protein structure is also important: The boson peak position scales systematically with structural motifs, reflecting local rigidity, and disordered proteins appear softer than α-helical proteins, which are softer than β-sheet proteins (115).

The density of states (i.e., the frequency distribution of vibrational modes) is a basic physical quantity obtained from neutron scattering by extrapolating $S(q, \omega)$ to $q = 0$, in a procedure that effectively removes the amplitude weighting. Early studies determined $g(\omega)$ for protein powders and showed that treatment of long-range interactions in NMA is critical for accurate calculations (21). Moreover, within the harmonic approximation, $g(\omega)$ determines the dynamical contribution to the thermodynamics of a condensed-phase system, making it useful in evaluating binding thermodynamics. A change in the vibration spectrum will arise owing to the formation of new intermolecular interactions in the complex.

The change in $g(\omega)$ upon binding a drug (methotrexate) to a cancer target (dihydrofolate reductase) has been determined using inelastic neutron scattering (5). Scattering measurements were performed at 120 K, at which temperature the protein exhibits mostly harmonic vibrational dynamics, thus permitting direct determination of $g(\omega)$. The vibrations of the complex soften significantly relative to those of the unbound protein. The resulting free-energy change calculated from the density-of-states change was 17 ± 4 kJ/mol at 300 K, favoring binding, owing to a large entropic contribution (25 ± 16 kJ/mol) that is partially compensated for by the enthalpy term (8 ± 2 kJ/mol).

The physical origin of the vibrational softening is also of interest. Analytical modeling shows that coupling between protein vibrations and ligand external motion generates entropy-rich low-frequency vibrations in the complex (97). Net entropy gain arising from coupling between the protein and ligand external motion is larger than the loss of vibrational entropy upon complex formation, resulting in the observed vibrational softening upon ligand binding. Experimental vibrational softening has also been fully reproduced using atomic-detail NMA (6). Decomposition of the change in $g(\omega)$ revealed that the largest contributions arise from the structural elements of dihydrofolate reductase critical to stability and function. Normal mode calculations of other protein systems concur with these findings, also indicating an increase in flexibility upon ligand binding and protein-protein association (34, 35, 142). However, at physiological temperatures, a decrease in flexibility upon protein binding to small ligands at physiological temperatures can occur if this process is dominated by changes in anharmonic degrees of freedom (93).

An intriguing and, to our knowledge, still largely unsolved problem in protein dynamics involves the nature of intermolecular and interunit cell motions in protein crystals. Collective motions in protein crystals have been probed using X-ray diffuse scattering and MD (31, 50, 91, 92). But, as stated above, X-rays do not easily yield timescales of the motions of interest. The question therefore remains regarding the extent to which lattice phonons occur in protein crystals. Computer power now enables full atomistic lattice dynamics treatment of a protein crystal (90), but equivalent experimental determination of phonon dispersion relations (if these, as such, exist for protein crystals) has not yet been demonstrated. Dispersion relations have, however, been calculated using MD for protein-water systems and used to demonstrate the presence in the water dynamics of collective modes belonging to the protein (20).

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TEMPERATURE DEPENDENCE OF PROTEIN DYNAMICS

Perhaps the most extensive work using dynamic neutron scattering on protein samples over the past 25 years has concerned the temperature dependence of internal dynamics. Historically, this work dates back at least to studies using Mössbauer spectroscopy, a technique that has considerable commonalities with neutron scattering but examines a much slower timescale than neutron spectrometers do ($\sim 10^{-7}$ s). In 1981, the Lamb-Mössbauer factor f', which is the Mössbauer equivalent of elastic neutron scattering, was used to derive the MSD of the Fe^{2+} ion of metmyoglobin (113, 114). At low temperatures, the displacements are small, originating primarily from vibrational motion. Above ~210 K, a new motional degree of freedom appears in addition to the usual vibrations (113, 114). The authors further stated that such additional motions are specific for biomolecules in their active state and may be described as fluctuations between slightly different conformational substates of the molecule.

As temperature is increased to above ~100 K, diffusive motions may be activated in a protein, corresponding to overdamped harmonic or anharmonic vibrations or transitions between potential wells. These diffusive motions give rise to quasielastic neutron scattering that is centered at $\omega = 0$ (**Figure 1**). Elastic scattering also yields the temperature dependence of the time-dependent MSD, defined as

$$r^2(t) = \frac{I}{N}\sum_{i=1}^{N}[r(t) - r(0)]^2, \qquad 7.$$

where N is the number of atoms; for neutrons, $t(\sim 1/\omega_0)$ is the approximate time limit of the instrument, and ω_0 is the energy resolution. Therefore, the MSD obtained depends on the energy resolution of the instrument used (7, 25); instruments with low energy resolutions will detect only fast motions.

The q-dependence of the incoherent $S(q,0)$ is used to derive the MSD. For normal modes and classical diffusion, single-atom $S(q,0)$ is Gaussian in q. However, for other types of motion or heterogeneity among atoms in the MSDs, non-Gaussian scattering is apparent (for detailed discussions, see 7, 8, 28). Experimental MSD analysis using neutrons has typically involved making the Gaussian assumption, which is valid for $q \to 0$, and assuming all atoms have the same MSD, leading to

$$\langle r^2 \rangle = -6\frac{\mathrm{d}\ln S(q,0)}{\mathrm{d}q^2}. \qquad 8.$$

The temperature dependence of the MSD is then extracted by performing elastic scans (see 26, 27, 39, 85, 86).

An early, eye-opening study on myoglobin demonstrated striking temperature dependence between elastic and quasielastic neutron scattering and the MSD (26, 27). The data were interpreted using an improbable model of jumps of atoms between states at different energy levels, but this study set the stage for much subsequent work. The authors found that the scattering data were well fitted by a mode-coupling approach (26, 27), a nonlinear coupling of density modes inducing structural arrest at a critical temperature. Physically, this approximates the back-flow phenomenon and the cage effect describing concerted motions of particles in liquids.

Since 1989, the temperature dependence of neutron-derived protein MSDs has been studied in detail. Much work has been performed on physical questions concerning the protein dynamical and glass transitions and their solvent dependence (for in-depth discussions of controversies concerning the associated physics, see 18, 24, 126). We now know that there are at least two transitions. MD analysis has demonstrated the activation of motions at low temperature, $T_m \sim 100$ K (49). This hydration-independent transition excites anharmonic motions in hydrophobic and aromatic

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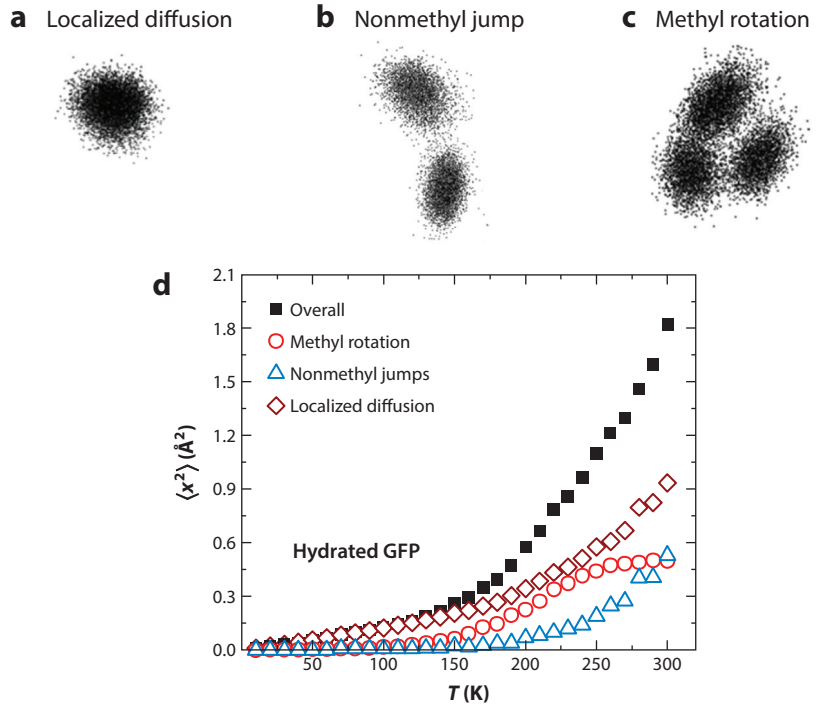


Figure 2

Examples of atomic motions in hydrated green fluorescent protein (GFP) by projection of the molecular dynamics trajectory of individual hydrogen atoms to (*a*) localized diffusion, (*b*) nonmethyl jump (two-site jump in this case), and (*c*) methyl rotation. (*d*) Calculated mean-square displacement of hydrogen atoms, decomposed to three distinct types of atomic motions. Figure adapted from Reference 54.

residues (94), with a strong contribution from methyl group rotations (28, 77, 125, 126, 129). Owing to hydrogen-bonding interactions with the rigid solvent, anharmonic hydrophilic residue motions are not activated at T_m (94).

At higher temperatures, however, hydration plays an activating role. Much experimental and simulation work has suggested that the dynamics of the protein is coupled to that of its surface hydration water (32, 36, 125, 131, 140, 144, 146, 150). At ~180–220 K, another, hydration-dependent, transition occurs (18, 28, 125, 126, 158) during which jumps between minima involving hydrophilic side chains are activated (54, 131, 140, 143). These are strongly coupled to the relaxation rates of the hydrogen bonds they form with hydration water. The higher-temperature transition has been labeled the dynamical transition (26, 27) and occurs at a nominal temperature $\sim T_{d,}$ although the transition in temperature may be quite smooth. Thus, with increasing temperature, first the hydrophobic core of a protein "awakens," followed by the hydrophilic surface (94), a description consistent with elastic scattering studies on homopolymeric amino acids (131).

Neutron scattering experiments have demonstrated that both a protein and its hydration water present the dynamical transition at the same temperature (17, 150). MD has revealed that the thermal activation of translational diffusion of surface water molecules is required for the dynamical transition of folded (140, 144) and disordered proteins (130). The minimum hydration level required for dynamical transition in proteins has been reported to be 20% water (mass ratio) (33, 125, 127, 146).

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A useful neutron quantity is the dynamic susceptibility, $\chi''(q,\omega) = \frac{S(q,\omega)}{n_B(\omega)}$, where $n_B(\omega) = [\exp(\hbar\omega/kT) - 1]^{-1}$ is the Bose occupation number. Although relaxation processes on different timescales can be hard to identify in $S(q,\omega)$, they appear as distinct peaks in $\chi''(q,\omega)$ with associated relaxation times $\tau = 1/\omega$ (125). MD further indicates that $\chi''(q,\omega)$ for incoherent scattering from proteins at 300 K can be decomposed into three distinct classes of motion: localized diffusion, methyl group rotations, and nonmethyl jumps (56). These three classes exhibit qualitatively distinct forms of $\chi''(q,\omega)$ and thus are separable components that can be used to interpret scattering, such as from protein-water interactions and for the temperature dependence of dynamics, and changes such as substrate binding that accompany biological functions.

For protein-water interactions at physiological temperatures, hydration enhances diffusive motions of atoms in protein molecules (125), leading to the intriguing question of whether the hydration effect is mainly local and limited to the protein surface or is global and propagating into the functional core. Neutron χ'' measurements and MD have shown that the major effect of surface hydration visible in neutron spectra is to increase the volume of the localized single-well diffusion of protein atoms and that these diffusive motions are coupled such that the hydration effect propagates from the protein surface into the dry core (53, 96). This finding is supported by neutron scattering experiments on calbindin D9k, the core and surface of which were selectively deuterated, showing that hydration effects on the protein hydrophilic surface and the hydrophobic core are similar (151).

Consistent with the above description, MD simulations of hydrated myoglobin show that the dynamical transition initiated by the solvent-surface interaction propagates to the interior of the protein via collective dynamics distributed over the whole protein in a process that can be described by a very small number of principal component modes (143). The onset of the myoglobin transition at ~180 K is characterized by the appearance of a single double-well principal component mode involving blocks of supersecondary structural elements moving relative to each other. This rigid-body motion qualitatively resembles structural changes seen in proteins in different functional states.

The thermodynamic glass transition occurs at T_g and is observed in proteins using calorimetry and dielectric spectroscopy (for discussion of the relationship between T_d and T_g, see 16, 68). T_d occurs at higher temperatures than does T_g and depends on the energy resolution of the instrument; instruments with low resolution have higher T_d values. There might also be a change in the slope of the MSD at T_g (16), which would show a relationship between short-timescale dynamical changes and long-timescale motions and thermodynamics. However, dynamical changes at T_g may be difficult to separate from T_m. To solve this problem, the relatively short timescales accessible to neutron scattering can be complemented with dielectric spectroscopy. This approach has indicated a specific microsecond backbone relaxation process might be the main structural relaxation of the protein that defines T_g, whereas faster processes present some localized secondary relaxations (70, 71).

Dynamical transitions are also found in nonprotein biological systems (103, 124). For example, experiments and MD on protiated cellulose in D_2O found that cellulose also exhibits a hydration-dependent dynamical transition. However, the increase in the MSD is considerably smaller than that for proteins (117) because the MSD of core nonexchangeable hydrogen atoms (i.e., those not exposed to the solvent) that account for ~70% of the hydrogen in cellulose is nearly independent of temperature and hydration.

DYNAMICS OF BIOLOGICAL SYSTEMS AT PHYSIOLOGICAL TEMPERATURES

We now discuss in more detail motions in proteins and other biophysical systems at physiological temperatures and how they are manifested in neutron scattering spectra. The MSD, as derived by

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incoherent elastic scattering, has been examined in some detail. Hydrogen atoms in proteins have a distribution of MSDs, and early calculations derived from MD on a small protein, simulating an instrument with a very high energy resolution (~0.1 μeV) showed that this motional heterogeneity leads to a breakdown of the Gaussian approximation, indicating the MSD is underestimated by ~30% over the whole temperature range examined (50–300 K) (49). In a study by Vural et al. (148), the origin of the q-dependence of the extracted MSD was investigated and found to arise from neglecting the dynamical heterogeneity.

Incorporating a term proportional to q^4 in the fit, in addition to the Gaussian q^2 term, yields both an improved average MSD and a reliable estimate of the variance of the distribution (8, 157). A further, natural progression is to model a probability distribution of MSDs (102). Several corresponding examples have been reported, including bimodal (28), Weibull (89), and gamma (75, 116) distributions.

The interpretation of incoherent quasielastic scattering in terms of single-particle motions has a rich and long history (9). For small-molecule systems, it is convenient to interpret quasielastic scattering using analytical models of diffusive motion, such as jump models (9, 19), in which the scattering is a sum of q-dependent Lorentzians in ω that can be fitted to the data to yield parameters such as jump times and distances as well as diffusion constants. However, for biological systems, these analytical models oversimplify the dynamics: As discussed above, a variety of motions with heterogeneous geometries and timescales are present. Therefore, it is useful to interpret biological spectra using MD, which can sometimes be a stepping-stone to analytical theory.

A simplified interpretation uses the temperature dependence of elastic incoherent scattering to estimate the resilience of a protein, expressed as an effective force constant derived from the slope of the MSD versus temperature (10, 158). A conceptual problem arises above T_m when barrier-crossing events contribute. In these cases, the force constant is undefined (see 158).

To overcome this problem, dynamics can be understood as decomposed into two components, one conformational involving jumps between potential wells and the other elastic corresponding to fluctuations within the wells. For methyl-bearing side chains, the distribution of barriers between rotamers is heterogeneous (59). These two components can be teased apart by combining quasielastic scattering, light scattering, and MD simulation (54), as the curvature of the potential of mean force for single-well atomic motions is determined by the elastic modulus, which can be determined using light scattering. The conformational component can then be estimated by subtracting the elastic component from the overall MSD, the latter measured using elastic incoherent neutron scattering. The procedure used by Hong et al. (54) could thus be employed to derive the elastic resilience of the protein matrix.

Experiments on systems exploring complex energy landscapes may detect so-called anomalous subdiffusive dynamics, which, for biological macromolecules, has been the subject of considerable experimental and theoretical attention (42, 44, 73, 74, 76, 83, 95, 154). For example, the distance autocorrelation functions probed in single-molecule electron transfer experiments on proteins follow a subdiffusive Mittag-Leffler-like decay with a power-law tail decaying as $\sim t^{-0.51}$ (95) on timescales stretching from microseconds to seconds (76, 154). Neutron scattering experiments (73) and MD simulations (74) have provided evidence for anomalous diffusion existing also in proteins on pico- and nanosecond timescales. Subdiffusion arises from the fractal-like structure of the accessible configuration space (104, 105).

An intrinsic MSD may also be conceptualized, as the long-time converged value, defined as the MSD that appears in the infinite time limit of $I(q,t)$. This corresponds to the value that would be obtained at perfect energy resolution and can be estimated by representing $I(q,t)$ with a stretched exponential function, which is equivalent to a superposition of exponential decay functions representing individual diffusive processes. According to this model, the dynamical

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transition is not an artificial consequence of finite instrumental resolution and a correspondingly limited time window (147). However, the instrumental energy resolution does have a significant effect on the theoretical T_d, shifting it to higher temperature with increasing energy resolution width (22, 62, 101, 131, 152).

The question arises, however, as to whether a single globular protein will ever actually live long enough to see its intrinsic MSD. Studies normally assume that protein dynamics is at thermodynamic equilibrium and ergodic and, therefore, that the ensemble-averaged dynamics reflects the dynamical behavior of one protein molecule averaged over long times. However, MD has shown that the internal dynamics in single globular protein molecules is nonergodic (58). Despite the confinement imposed by the overall average protein structure, the time-averaged MSDs of interatomic fluctuations do not reach stationary values, but instead increase according to a power law over 13 decades. This broken ergodicity is found not only in the global interdomain fluctuations but also in the local dynamics. Thus, ensemble and time averages are no longer the same, and the long-time dynamical behavior of individual protein molecules can no longer be completely represented by ensemble-averaged observations, such as $I(q,t)$. Protein dynamics exhibits aging (i.e., the relaxation function measured at a given observation time window shifts toward a longer time range if the observation time becomes longer). This suggests that functional motions of very similar forms can be observed on many different timescales, ranging from picosecond to second timescales. Thus, picosecond-nanosecond collective motions observed at the dynamical transition may also be present on longer timescales.

The self-similar nonergodic structure of the time dependence of protein dynamics can be represented using a transition network, the nodes of which are metastable conformational substates (58). Transitions between substates are collective diffusive motions, each of which contributes to quasielastic scattering. Markov state modeling (67, 110, 139), which permits MD to be decomposed into segments each representing only individual transitions (109, 112), is used to quantify this dynamics, permitting correlation functions for a variety of experiments to be interpreted using MD in terms of structurally well-defined transitions between specific states (108). This approach has been extended to calculate neutron scattering (81, 156), representing the scattering as a linear combination of Markov processes with exponential decay factors, $\exp(-\kappa_j \tau)$, e.g.,

$$I_{\text{inc}}(q,\tau) = \sum_j \exp\left(-\kappa_j \tau\right) A_j(q), \qquad 9.$$

where $A_j(q)$ is derived from the state-represented individual atomic scattering amplitudes and κ_j are decay rates. Complex biological systems await application of this formalism.

Most dynamic neutron scattering work on proteins has been performed with incoherent scattering using hydrogenated proteins, thus focusing on self-correlations of individual hydrogen atoms. However, coherent scattering, which provides information directly on collective dynamics, promises to be of special biological relevance. $I_{\text{coh}}(q,t)$ can be measured directly using neutron spin echo (NSE) spectroscopy (11, 13, 30, 60), which can measure motions up to microsecond timescales. Functional dynamic modes, such as interdomain motions (11, 60, 64, 137), are particularly susceptible to detection using the NSE technique.

The distance dependence of relative motion can be characterized by the effective diffusion coefficient $D_{\text{eff}}(q)$ (11, 13, 60), which is obtained from the initial decay rate of the NSE-determined $I_{\text{coh}}(q,t)$. Work analyzing NSE and MD from a variety of protein systems has demonstrated that protein interdomain motion follows the principle of de Gennes narrowing (DGN); in other words, $D_{\text{eff}}(q)$ possesses an inverse q-dependence on the interdomain structure factor (55, 57): $D_{\text{eff}}(q) \sim 1/S(q)$ (**Figure 3**). DGN predicts that the rate of interparticle fluctuation is a minimum when the probability of the configuration is a maximum (2, 23). Thus, the rate of interdomain motion

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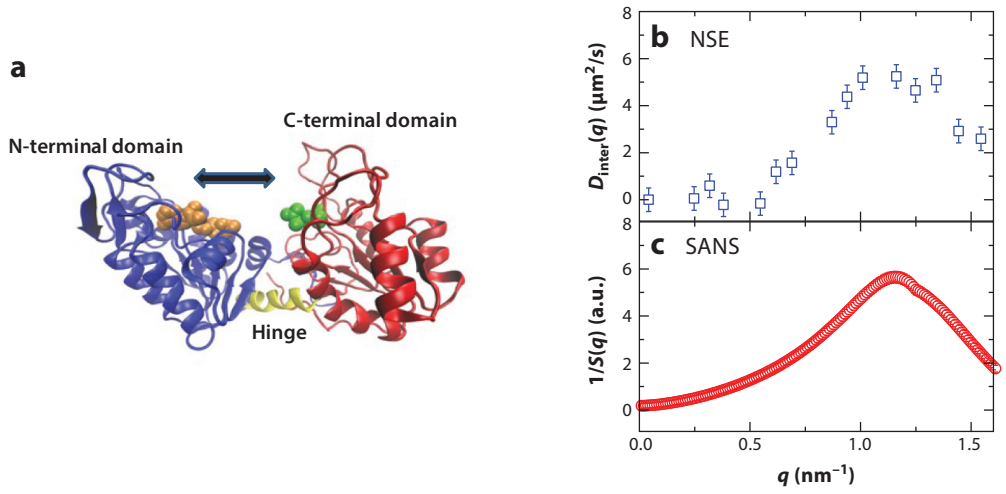


Figure 3

(*a*) Structure of PGK consisting of two domains (*blue* and *red*) of almost equal mass connected by a helix (*yellow*). (*b*) Effective diffusion coefficient for internal motions of PGK in aqueous solution collected using NSE (60). (*c*) Interdomain structure factor, $S(q) = I(q)/[\sum Fi(q)]$, where the form factor of the whole protein molecule, $I(q)$, was measured by SANS and that for each single domain, $Fi(q)$, was calculated based on the crystal structure. Abbreviations: NSE, neutron spin echo; PGK, phosphoglycerate kinase; SANS, small-angle neutron scattering.

is inversely proportional to the probability distribution of the spatial configurations of domains. **Figure 3** demonstrates DGN in the NSE spectrum of an enzyme. However, although DGN may hold for the initial decay, there may well be deviations at longer timescales, and these promise to be of particular interest for future research.

Coherent NSE and backscattering experiments have been interpreted using elastic network models, a simplified coarse-grained NMA that has successfully reproduced the forms of atomistic low-frequency NMA modes in proteins and, in some cases, the distribution of X-ray crystallographic temperature factors (4, 38, 98). Elastic network models have been used to interpret a wide variety of protein mechanisms and experimental data. However, these models do not always reproduce both the forms and the frequencies of the modes concerned. The need for these models to yield a physically reasonable vibrational density of states (i.e., in agreement with neutron scattering results) has been stressed (122).

Although NSE spectroscopy detects the collective internal protein motion in aqueous solution, it is bedeviled by low counting statistics. To counter this problem, higher-flux backscattering experiments can be performed on perdeuterated protein powders to yield a complete description of large-scale collective motions (55). Another recent finding concerns the intriguing temperature dependence of coherent scattering from proteins (82), which reveals the dynamics of heavy atoms (C, N, O) that are masked in incoherent scattering experiments. Dry proteins present an ~200-K dynamical transition that is visible using only coherent scattering, which does not require hydration, although it is significantly amplified when water is present (82).

The value of neutron scattering as a biophysical technique is greatly increased when used for in-depth characterization of protein internal dynamics in combination with other experimental methods for measuring protein dynamics such as NMR spectroscopy: For example, a combination of neutron scattering, NMR, and MD was applied to explore substrate binding to cytochrome P450 (93). All three techniques showed decreased fluctuations in the camphor-bound form. These

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combined experimental and simulation results permitted detailed description of the dynamical change. The observed modifications in the coupling of five specific regions of the enzyme and concomitant closing of the substrate access channel resulted from a network of key interactions comprising side-chain salt bridges and hydrogen bonds connecting the five regions.

Perhaps unsurprisingly, intrinsically disordered proteins have larger atomic MSDs than do folded proteins, exceeding the relatively smaller differences between the MSDs of folded proteins (40). This larger MSD reduces the q-range over which the Gaussian approximation is valid. Furthermore, intrinsically disordered proteins have a higher content of hydrophilic amino acids that interact strongly with water, leading to stronger retardation in the dynamics of the hydration layer (130). Because hydrophilic side chains contain more exchangeable hydrogen atoms, their contribution to overall measured protein dynamics is reduced for these proteins, as polar hydrogen atoms are exchanged for proteins hydrated in D_2O.

Membranes possess local motions and bilayer undulation as well as bending dynamics that occur on nanosecond timescales and 10-nm length scales (14, 41, 100, 107, 119, 120). In pioneering work on collective vibrations in membranes (121), the dispersion relation $\omega(q)$ was derived for the gel and fluid phases of deuterated DMPC bilayers. This relation also confirmed MD predictions of an additional nondispersive (optical) mode (121).

Very recently, a major discovery was made using small-angle neutron scattering: the first direct detection of lipid rafts in biological membranes (106). Complementary dynamic studies combining NSE with neutron scattering length density contrast matching were used to unambiguously determine the mechanical properties of lipid rafts populating unilamellar lipid vesicles (107). Judicious hydrogen/deuterium substitution of the constituent lipids was performed: By also varying the D_2O/H_2O ratio of the solvent, neutron scattering length densities of one phase could be matched to the solvent. This neutron technique enabled isolation of the scattering from only the non-contrast-matched phase. Combining NSE experiments and MD simulations revealed dynamical decoupling of the nanodomains from the surrounding phase, suggesting that mechanical properties connected to the local composition persist even at nanometer length scales.

Variations in relaxation times between different components of a biological system can sometimes be leveraged experimentally to obviate the need for labeling. For example, MD analysis of low-resolution (1–100-ps timescale) elastic scattering from a protein in a cryosolution showed that at lower temperatures ($<$200 K) or on shorter timescales ($<$10 ps) the scattering contributions are proportional to the isotopic nuclear scattering cross sections of each component (48). However, for $T > 200$ K and owing to rapid activation of solvent diffusion, the solvent largely determines the variation with temperature of the total elastic intensity. In contrast, at higher temperatures ($>$240 K) and longer times ($>$100 ps), the protein makes the only significant contribution to scattering because solvent scattering has moved out of the accessible time-space window.

The above properties have been utilized in experiments performed on whole cells. By suitably choosing which region of q and ω to probe, the internal dynamics of a protein population may be observed in vivo without needing specific protein labeling (61). Using this approach, the dynamics in bacterial cells under stress has been examined (87). Softening of the protein dynamics of the stressed cells was reported, consistent with denaturation. Hence, neutron scattering may provide a tool for studying dynamical modifications induced by factors altering protein folds in the proteome of living cells.

Another striking work reported that an extremely halophilic bacterium contains a large population of water molecules with very low mobility, which may originate from water bound to the large amount of K^+ bound within these cells (141). Also remarkable is a distinct effect on cell water dynamics recently reported: As a result of a single point mutation of the photosynthetic system II D1 protein, damping of a collective water vibration decreased upon mutation (128).

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CONCLUSIONS AND FUTURE PERSPECTIVES

Since our previous review in 1991 (136), dynamic neutron scattering of biological systems has changed quite dramatically. Elastic scans have become commonplace and are now used to investigate a wide variety of biological phenomena. MD simulation has been paired with neutrons to provide detailed insight into the motions leading to any given measured scattering profile. Physical concepts from soft matter studies and glass transition phenomena have been applied to protein-water systems. New techniques, including the utilization of coherent scattering methods, have been devised and applied. Dynamic neutron scattering has been applied to complex biological systems, including whole cells.

In the future, ever-more complex biological systems such as plant cell walls, which are composed mostly of crystalline cellulose fibers embedded in a hydrated matrix of hemicellulose and lignin polymers, will continue to be characterized (79, 118). One approach to characterizing such systems is to determine $I(q)$ using small-angle neutron scattering to derive the low-resolution configurational distribution (138). This result is then combined with dynamic neutron scattering experiments and atomic-detail MD (12, 155).

Looking back, we find that isotope labeling, although extremely powerful (153), has perhaps not been applied as frequently as expected, possibly owing to hurdles in producing labeled material. Selective deuteration of components in intact biological systems still serves as the proverbial holy grail of neutron studies (29). This process would facilitate the characterization of individual biopolymers in their complex, heterogeneous native environments. The combination of specific labeling with increased source power and instrumental improvements should enable particularly informative experiments.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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