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Combining Neutron Scattering, Deuteration Technique, and Molecular Dynamics Simulations to Study Dynamics of Protein and Its Surface Water Molecules

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Abstract Protein internal dynamics is essential for its function. Exploring the internal dynamics of protein molecules as well as its connection to protein structure and function is a central topic in biophysics. However, the atomic motions in protein molecules exhibit a great degree of complexities. These complexities arise from the complex chemical composition and superposition of different types of atomic motions on the similar time scales, and render it challenging to explicitly understand the microscopic mechanism governing protein motions, functions, and their connections. Here, we demonstrate that, by using neutron scattering, molecular dynamics simulation, and deuteration technique, one can address this challenge to a large extent.

Keywords Neutron scattering; Deuteration techniques; Molecular dynamics simulations; Protein; Hydration water; Dynamics

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INTRODUCTION

Protein is the life engine, and conducts most bio-functions in living matters. Protein internal motions, especially the large-amplitude, collective atomic motions, are essential for many functional processes, including the entry of substrates into catalytic sites,^[1] allosteric conformational change,^[2] and enzymatic reactions.^[3] Moreover, water is the solvent of life, playing a crucial role in determining the native structure, dynamics, and function of biological macromolecules.^[4–8] The diffusive motions of water molecules not only aid the transportation of function-required essential ingredients, *e.g.*, protons, ions, and substrates across membranes and into the catalytic site of enzymes,^[4,9,10] but also render internal flexibility to the biomacromolecules. Hence, studying dynamics of protein and its surface water molecules is of great importance towards the understanding of the microscopic mechanism governing the protein function.

Various spectroscopic methods, including dielectric spectroscopy, nuclear magnetic resonance, terahertz spectroscopy, and ultrafast optical spectroscopy, *etc.*, have been applied to characterize the dynamics of biomacromolecules and their surface water molecules.^[11–14] However, these spectroscopic methods furnish only the temporal information on the dynamical process probed due to the lack of spatial resolution.

Fluorescence resonant energy transfer (FRET) can convey both spatial and temporal information of biomacromolecules by measuring the fluctuation of two optical labels in the system,^[15] which, however, lacks atomic resolution.

Neutron scattering is particularly informative for investigating the dynamics of biological systems. It provides both spatial and temporal information, and the probed tempo-spatial range spans from tens of femtosecond to hundreds of nanoseconds, and from sub angstrom to tens of nanometers, which are highly relevant for the bio-functions.^[16–26] Moreover, neutron signals contain both incoherent and coherent components: the former represents the self-motion of atoms while the latter characterizes the inter-atom motions. As the incoherent neutron scattering cross-section of hydrogen atoms is extremely large, one can make use of this unique feature to study a designed dynamic component or dynamics of a chosen region in the biological system by proper deuteration.^[18,22] Additionally, the all-atom molecular dynamics (MD) simulations can easily reach 100 nanoseconds or microseconds for a 100000 atom systems at the present time, which complements the neutron scattering experiments to provide microscopic details.

The dynamics in the protein/water system involves a great degree of complexity. The system is composed of both water and protein while the later contains twenty different types of amino acid “monomers”. Moreover, atoms in a protein molecule present different kinds of vibrational, diffusive, jump-like, and even large-scale collective motions, and they might overlap in time.^[18,25] These complexities cloud the un-

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derstanding of dynamics in the protein-water system as well as its connection to the bio-functions. A combination of neutron scattering, MD simulation, and deuteration technique can be used to address this challenge to a large degree. The present article mainly summarizes the work conducted in our group and is organized in three sections: I. Illustration on decomposition of different dynamical components in the protein/water system by combining neutron scattering, MD simulation, and deuteration technique; II. Using the decomposition method to explore the microscopic origin of protein dynamical transition, where the protein molecules transit from the rigid nonfunctional form to a flexible functional state upon heating beyond ~200 K. III. Perspectives of the future application of this combined technology to further the study of dynamics in biological and polymeric systems, and of the emerging opportunities to apply the Chinese neutron sources on soft-matter sciences.

ILLUSTRATION ON DECOMPOSITION OF DIFFERENT DYNAMICAL COMPONENTS IN THE PROTEIN-WATER SYSTEM BY COMBINING NEUTRON SCATTERING, MD SIMULATION, AND DEUTERATION TECHNIQUE

Self-motions of Protein Atoms

Neutron is highly sensitive to hydrogen atoms. The incoherent cross section of hydrogen atoms, which represents self-motions of hydrogen atoms, is one order of magnitude larger than the coherent and incoherent cross sections of other elements. Hence, the neutron signals collected on regular protein samples, which are full of hydrogens, mainly reflect the self-atomic motions of hydrogen atoms. However, protein hydrogen atoms can present complex dynamical behaviors, conducting various vibrational and diffusive motions in the same time window, which renders it difficult to determine the connection between a specific motion and the protein function. Here, by combining neutron scattering and molecular dynamics simulation, we decomposed the dynamics of protein hydrogen atoms in a hydrated powder around room temperature on the pico-to-nanosecond time scales into three different classes: localized diffusion confined in a single energy basin, non-methyl jumps among multi-sites, and three-fold rotations of methyl hydrogens (Fig. 1a).^[18] These three different atomic motions contribute vast differently to the neutron susceptibility spectra (Fig. 1b). $\chi''(q, \nu)$. $\chi''(q, \nu) = S(q, \nu)/n_B(\nu)$, where $n_B(\nu)$ is the Bose factor $n_B(\nu) = \left[\exp\left(\frac{h\nu}{kT}\right) - 1 \right]^{-1}$, and $S(q, \nu)$ is the dynamical structure factor, *i.e.*, the distribution of scattered neutron as a function of scattering wave vector q and energy transfer $h\nu$ (h is the Planck constant). As seen in Fig. 1, the methyl rotation presents a pronounced peak around 10 GHz, yielding a relaxation time about 22 pico seconds. It corresponds to an energy barrier of 2.8 kcal/mol by assuming an Arrhenius temperature dependence, $\tau = \tau_0 \exp(\Delta E/kT)$, with $\tau_0 \sim 0.18$ ps. In contrast, the localized diffusion of hydrogen atoms in single energy basins presents a rather flat background while the multi-site jumps contribute mostly to the low frequency be-

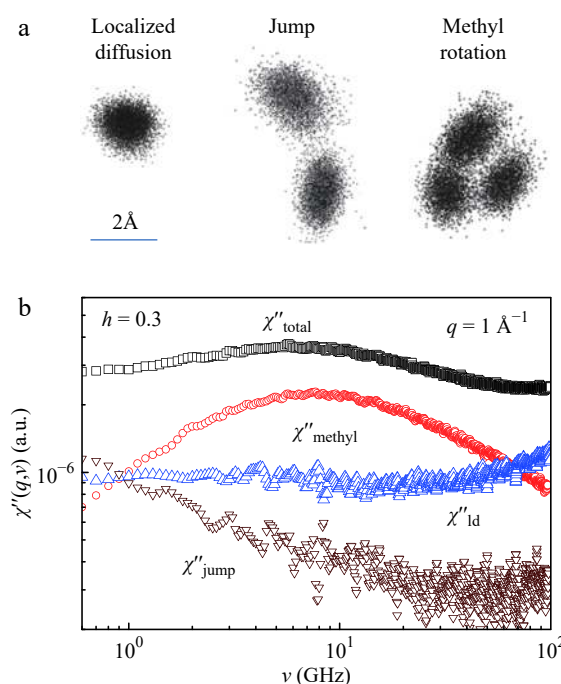


Fig. 1 Typical examples of scatter plot projections of 10 ns hydrogen-atom trajectories from MD on lysozyme: (a) Diffusion in a single energy basin (the δ hydrogen atom of TRP63), and diffusion in clusters plus jumping between two clusters (a γ hydrogen atom of ARG150) and three clusters (a methyl hydrogen atom of VAL2); (b) MD neutron susceptibility spectra of the whole protein (χ''), and the contributions from three different types of atomic motions. Here, the studied protein system is a hydrated powder sample, where the hydration level is 0.3 gD₂O/gprotein. The MD simulation system mimics the experimental condition for hydrated powders, containing two randomly oriented protein molecules at the proper hydration level. It is conducted at room temperature and ambient pressure. The total simulation time is 200 ns long, with the second 100 ns used for analysis. (Reprinted with permission from Ref. [18]; Copyright (2011) American Physical Society)

low 1 GHz, indicating relaxation processes longer than 1 ns.

Using the above decomposition method, we studied the hydration effect on these three different atomic motions in proteins and found that rotation of methyl group is essentially hydration independent while the amplitude of localized diffusion is greatly enhanced upon hydration with the non-methyl multi-site jumps being just slightly affected by the water content (Fig. 2).^[23]

Large-scale Collective Atomic Motions in Protein

In addition to motions of individual atoms and side groups, a protein molecule also conducts nanometer-scale collective dynamical modes involving its backbone, and the latter is of direct relevance to various enzymatic function of the biomacromolecules. However, as described before, the neutron signals measured in a regular protein system is dominated by self-motions of the hydrogen atoms. To highlight the dynamics of protein heavy atoms, especially the protein backbone, one can use fully deuterated proteins. In a deuterated protein, all hydrogen atoms including the $-\text{CH}$ are replaced by deuterium. The deuterated proteins are extracted from *E. coli* cells, which were raised in D₂O medium and fed by deuterated sugar sources.^[16,22] One example is presented in

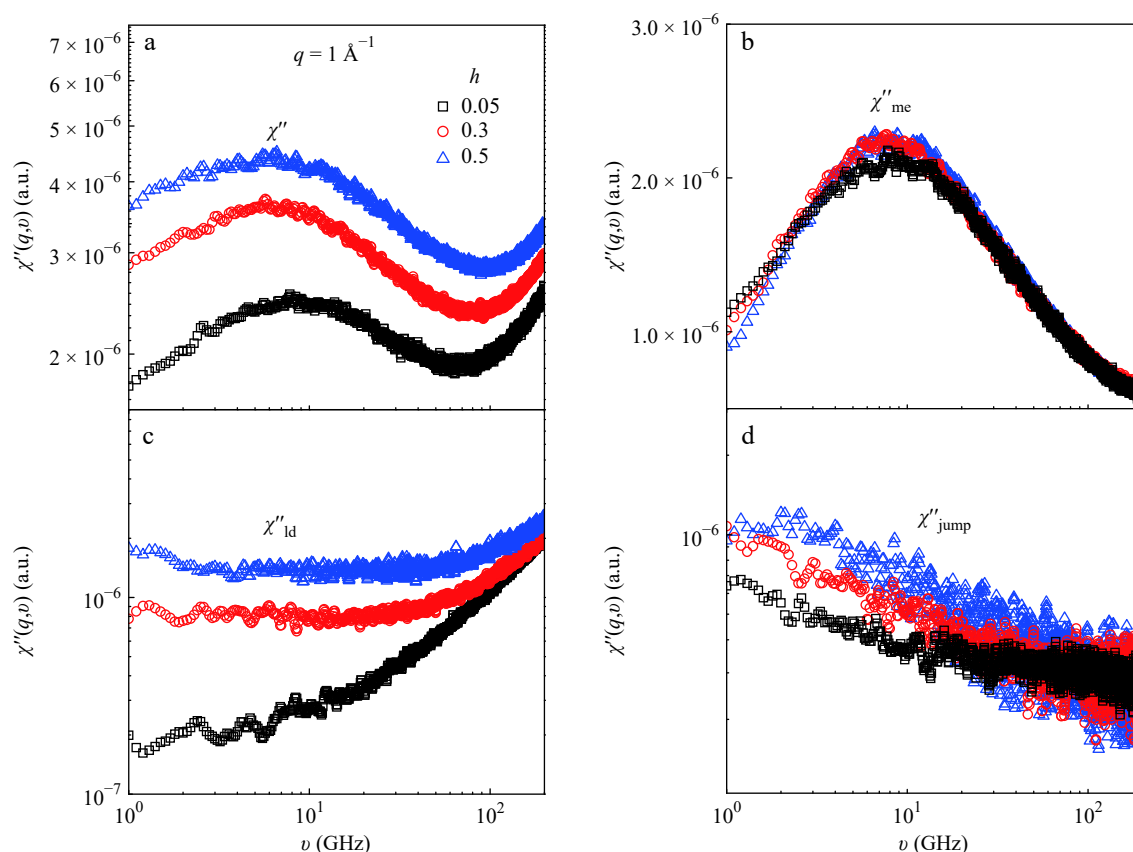


Fig. 2 Susceptibility spectra of lysozyme at different hydration levels (h): (a) total protein dynamics (χ''), (b) methyl group rotation (χ''_{me}), (c) localized diffusion (χ''_{ld}), and (d) jumps (χ''_{jump}) at $q = 1 \text{ \AA}^{-1}$. The simulation setup is similar to that as described in the caption of Fig. 1. (Reprinted with permission from Ref. [23]; Copyright (2012) American Physical Society)

Fig. 3, where the neutron spectra of dynamical structure factor, $S(q, \Delta E)$ ($\Delta E = \hbar\nu$) measured on perdeuterated Cytochrome P450 and the hydrogenated one are displayed in Figs. 3(a) and 3(b), respectively. $S(q, \Delta E)$ measured on the perdeuterated sample is dominated by coherent neutron signals, while the one collected on the hydrogenated protein weights more heavily on incoherent ones. The ratio between these two is denoted as $\gamma(q)$. As shown in Refs. [27–29], a system undergoes in-phase collective motions, *i.e.*, constituting units moving synchronously in time with similar amplitudes, will exhibit $\gamma(q) \propto I(q)$ while $\gamma(q)$ is a constant if the motion is completely random and uncorrelated. As shown in Figs. 3(c) and 3(d), the q dependence of $\gamma(q)$ derived experimentally and from simulation resembles that of $I(q)$. The neutron spectra collected are in the energy window from 1 μeV to 15 μeV , and $\gamma(q)$ resembles $I(q)$ in the q range from $0.3 \text{ \AA}^{-1}$ to $1.7 \text{ \AA}^{-1}$, indicating collective atomic motions on the length scale of a few nanometers over the time window from 100 ps to 1 ns.

To determine the forms of large-scale collective motions in CYP101 probed experimentally, we performed a normal mode analysis using a commonly-used elastic network model,^[30] which assumes that all protein residues move synchronously along the eigenvectors of the normal modes. The q dependence of $D_{\text{inter}}(q)$ resulting from different normal modes was calculated and typical examples are presented in Fig. 4(a). Here, $D_{\text{inter}}(q)$ is the interatomic mobility derived

from the neutron data, and more details can be found in Ref. [16]. By comparing to the experimental data, we found that $D_{\text{inter}}(q)$ of the first normal mode matches the experimental values the best (Fig. 4b).^[16]

By minimizing the difference in $D_{\text{inter}}(q)$ between the experimental data and the first normal mode, we found that the amplitude of the normal mode is $\langle x^2(\Delta t) \rangle_{\text{NM}} = 0.41 \text{ \AA}^2$. The catalytic site of CYP101 is deeply buried, and the substrate enters the site *via* a channel (Fig. 4c), the entrance of which is formed by the B' helix and the F/G loop. Setting the amplitude of Mode 1 as $\langle x^2 \rangle_{\text{NM}} = 0.41 \text{ \AA}^2$, the gate of the substrate-access channel, approximated here as the distance between GLY189 in the F/G loop and ARG89 in the B' helix, varies by $\sim 0.5 \text{ \AA}$ on the experimental time scale of $\sim 1 \text{ ns}$.^[16]

In Situ Study of the Dynamics of Protein-surface Water Molecules

Water is the solvent of life, playing a crucial role in determining the native structure, dynamics, and function of biological macromolecules.^[4,5,7,8] Spectroscopic methods, including dielectric spectroscopy, nuclear magnetic resonance, terahertz spectroscopy, and ultrafast optical spectroscopy, *etc.*, have been applied to explore water dynamics on protein surface,^[11–14,31–34] but the measured signals are always clouded by the contribution from protein molecules. This difficulty can be overcome by performing neutron scattering using

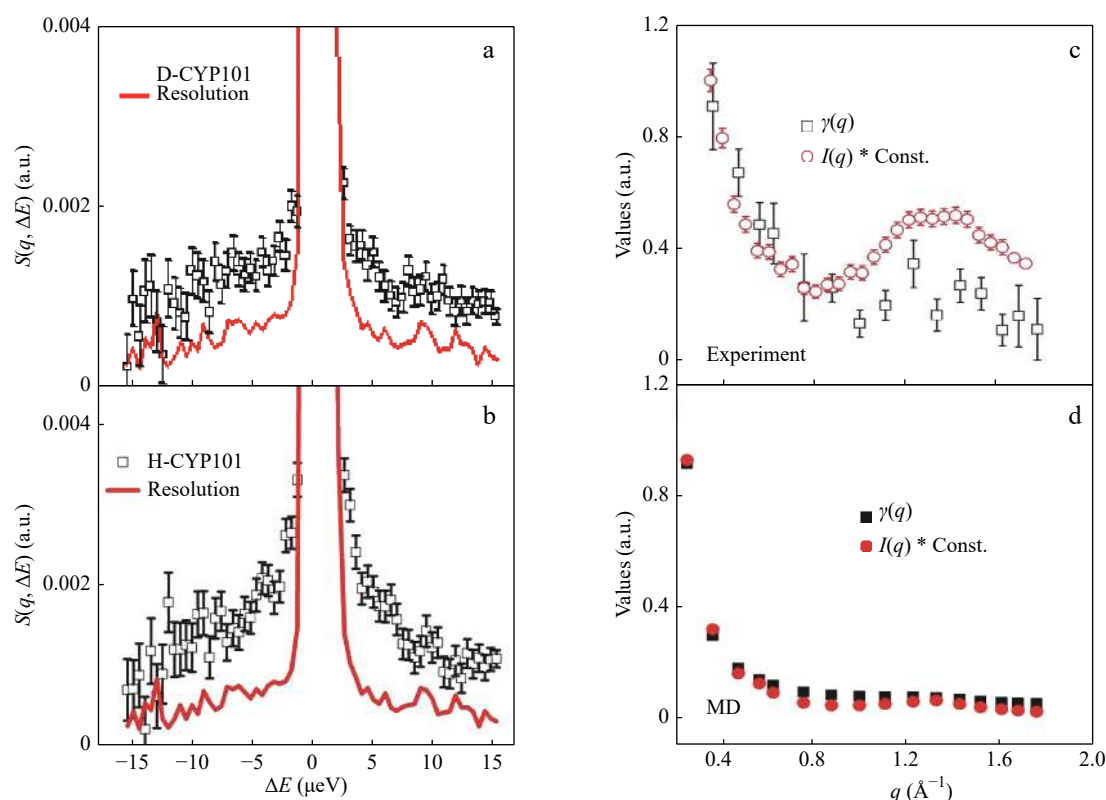


Fig. 3 Quasi-elastic neutron spectra of CYP101, $S(q, \Delta E)$, averaged over the q windows from $0.35 \text{ \AA}^{-1}$ to $1.75 \text{ \AA}^{-1}$, measured on (a) D-CYP101 and (b) H-CYP101 at 285 K. The red curves are the resolution function measured on vanadium. The raw data have been smoothed by averaging 5 neighboring data points. Comparison of the q dependence of $\gamma(q)$ with that of $I(q)$ obtained (c) experimentally and (d) from simulation. $\gamma(q)$ is the ratio of the coherent to incoherent $S(q, \Delta E)$ intensity both integrated over the energy window from $5 \text{ \mu eV}$ to $10 \text{ \mu eV}$ at a given q at 285 K. Error bars throughout the text represent one standard deviation. (Reprinted with permission from Ref. [16]; Copyright (2016) Science Advances) (The online version is colorful.)

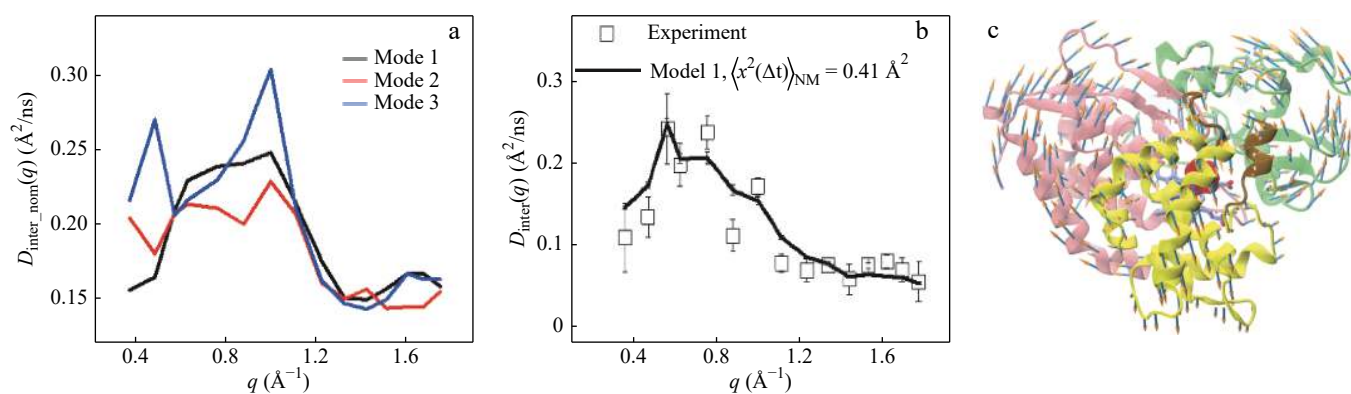


Fig. 4 (a) $D_{\text{inter_norm}}(q)$ derived from the first three lowest-frequency normal modes; (b) Comparison of $D_{\text{inter}}(q)$ measured experimentally and that derived from the first normal mode, the latter being corrected by the inter-protein structure factor and multiplied by the amplitude ($\langle x^2 \rangle_{\text{NM}} = 0.41 \text{ \AA}^2$) required to minimize the difference between experimental $D_{\text{inter}}(q)$ and that derived from the first normal mode; (c) Three parts (marked as green, yellow, and pink) in the protein rotate against each other. The region marked red is the catalytic site. The entrance of the substrate access channel formed by the B' helices and F/G loops is marked brown. (Reprinted with permission from Ref. [16]; Copyright (2016) AAAS)

perdeuterated proteins. Fig. 5(a) shows the experimental and MD-derived neutron susceptibility spectra of perdeuterated protein hydrated in H_2O at the hydration level of $0.4 \text{ g}_{\text{water}}/\text{g}_{\text{protein}}$, *i.e.*, about one single layer of water molecules surrounding the protein surface. The MD-derived neutron spectra are in quantitative agreement with the experimental ones, justifying our simulation models. The q dependence of

the relaxation time, τ , *i.e.*, approximately the time required for water molecules to diffuse a distance about $2\pi/q$, derived from the experiment and from MD simulation is presented in Fig. 5(b). τ shows a power law dependence with q , $\tau \propto q^{-n}$. The exponent n is found to be 2 for bulk water but 2.5 for protein-surface water molecules. Using a simple dimensional argument, one can derive that the mean-squared atomic dis-

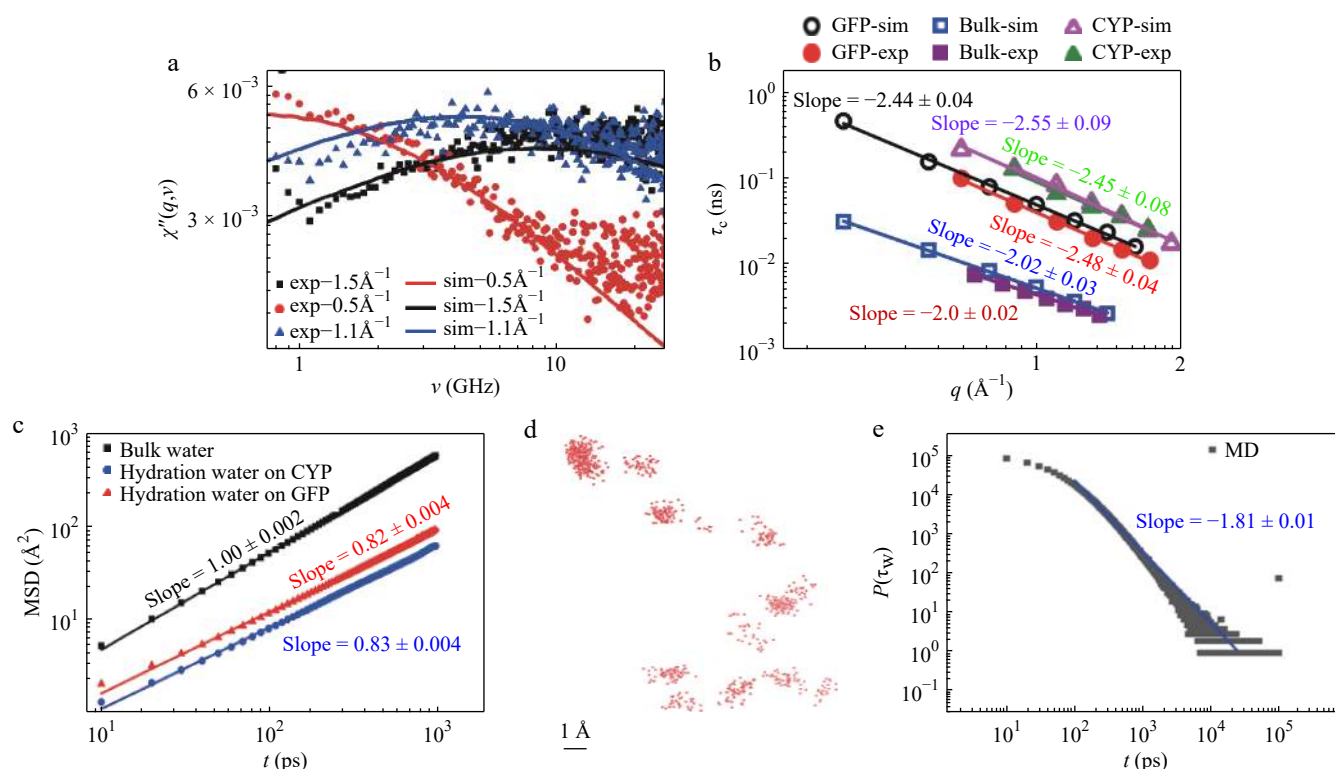


Fig. 5 (a) Neutron susceptibility spectra, $\chi''(q, \nu)$, for hydration water derived from experiment and from MD simulation on perdetrated GFP at various q ; (b) q dependence of relaxation time, τ , derived experimentally and from MD simulation for bulk water and protein-surface hydration water; (c) MD-derived MSD for both hydration and bulk water in the time window from 10 ps to 1 ns (Black for bulk water, red for water on GFP, and blue for water on CYP. Solid lines are power-law fits, with the exponents displayed accordingly.); (d) The scatter plot obtained by projection of the MD trajectory of a selected water molecule on CYP (The position of the oxygen atom of water molecule is projected at every 100 ps for a continuous trajectory of 100 ns.); (e) MD-derived distribution of trapping time, $P(\tau_w)$. (Reprinted with permission from Ref. [22]; Copyright (2018) American Physical Society)

placement (MSD) is proportional to time in the power of $\beta = 2/n$. Hence, the bulk water molecules conduct normal diffusion while the protein surface water molecules exhibit sub-diffusive motions with the exponent β equal to 0.8. These are further confirmed by the analysis of time dependence of MSD derived from MD (Fig. 5c).^[22] Further analysis of MD trajectories shows that this sub-diffusive motion results from

the temporal disorder as water molecules perform stochastic jumps among trapping sites formed on the protein surface (Fig. 5d), and the associated trapping time obeys a broad distribution (Fig. 5e). However, once the time scale explored exceeds nanoseconds, the motion of water molecules transit to normal diffusion (Fig. 6a) due to a many-body volume-exclusion effect (Fig. 6b, see more details in Ref. [22]).

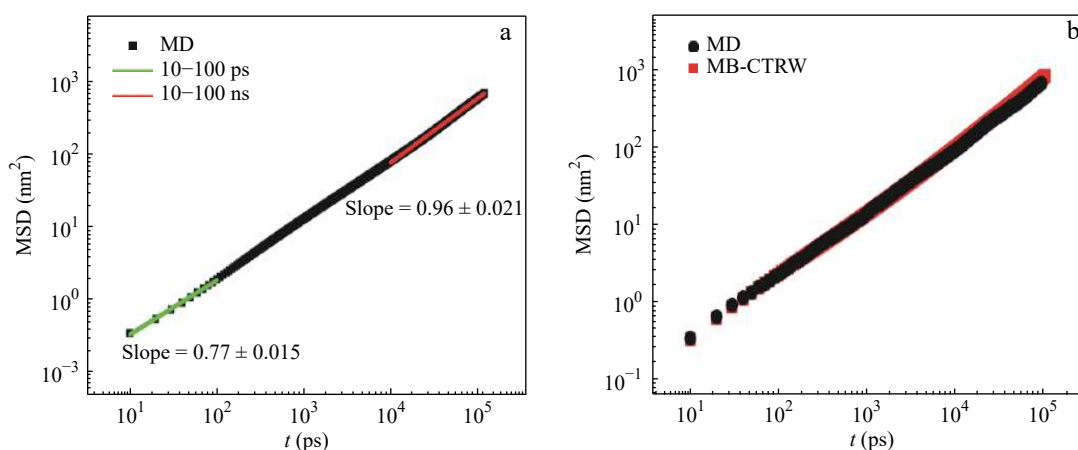


Fig. 6 Statistical results derived for hydration water on CYP: (a) Mean square atomic displacement (MSD), $\langle X^2(\Delta t) \rangle$, derived from MD (black solid square) (Green and red lines represent power-law fits in a time window from 10 ps to 100 ps and from 10 ns to 100 ns, respectively.); (b) MSD calculated directly from MD (black dots) and from Monte Carlo simulation based on Model MB-CTRW (red dots, many-body continuous time random walk) (Reprinted with permission from Ref. [22]; Copyright (2018) American Physical Society)

COMBINING NEUTRON SCATTERING, MD SIMULATION, AND DEUTERATION TECHNIQUE TO EXPLORE THE MICROSCOPIC ORIGIN OF THE PROTEIN DYNAMICAL TRANSITION

Contribution from Different Types of Self-motions of Protein Hydrogen Atoms

In Section I, we have shown that, by combining neutron scattering, MD simulation, and deuteration technique, one can decompose different types of atomic motions or dynamics of different components in the protein-water system. In this section, we will illustrate how this methodology can be used to illustrate the microscopic origin of protein dynamical transition. Protein molecules will present a transition around 200 K, at which internal protein motions change from non-functional, glass-like, rigid, harmonic vibrations to incorporating liquid-like, flexible, anharmonic dynamics required for function.^[4,35–39] It has been shown that this dynamical transition will be significantly enhanced with the presence of

water. However, it is unclear how different types of atomic motions contribute to it. We decomposed self-motions of the protein hydrogen atoms and studied its temperature dependences. As shown in Fig. 7, the rotation of methyl groups is essentially hydration independent while the localized diffusion of protein hydrogen atoms is greatly amplified upon hydration, starting around the dynamical transition temperature. It indicates that localized diffusion of protein hydrogen atoms is the major dynamical species contributing to the dynamical transitions.

Contributions from Protein Backbone

Although the underlying mechanism for protein dynamical transition remains controversial, its presence is generally believed to require a minimum amount of water ~ 0.2 g_{water}/g_{protein}. However, the energy landscape of a globular protein is intrinsically complex and highly anharmonic, leading to the question as to whether the protein dynamical transition may be an intrinsic property of dry proteins.

By performing neutron scattering on lyophilized (hydration level, $h \sim 0.02$, Fig. 8a) perdeuterated cytochrome P450

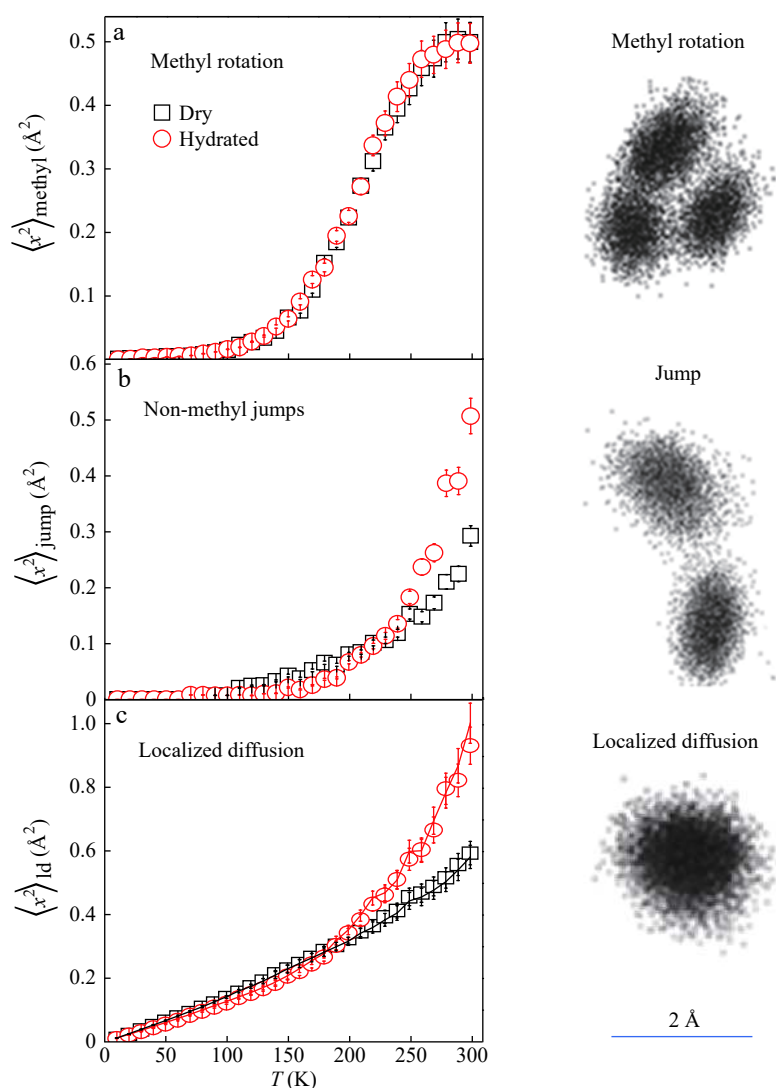


Fig. 7 Contribution to the overall MSD derived from MD by (a) methyl rotation, (b) non-methyl multi-site jumps, and (c) localized diffusion of protein hydrogen atoms in dry ($h = 0.05$ g_{water}/g_{protein}) and hydrated form (0.4 g_{water}/g_{protein}) (The simulation setup is similar to that described in the caption of Fig. 1) (Reprinted with permission from Ref. [24]; Copyright (2013) American Physical Society)

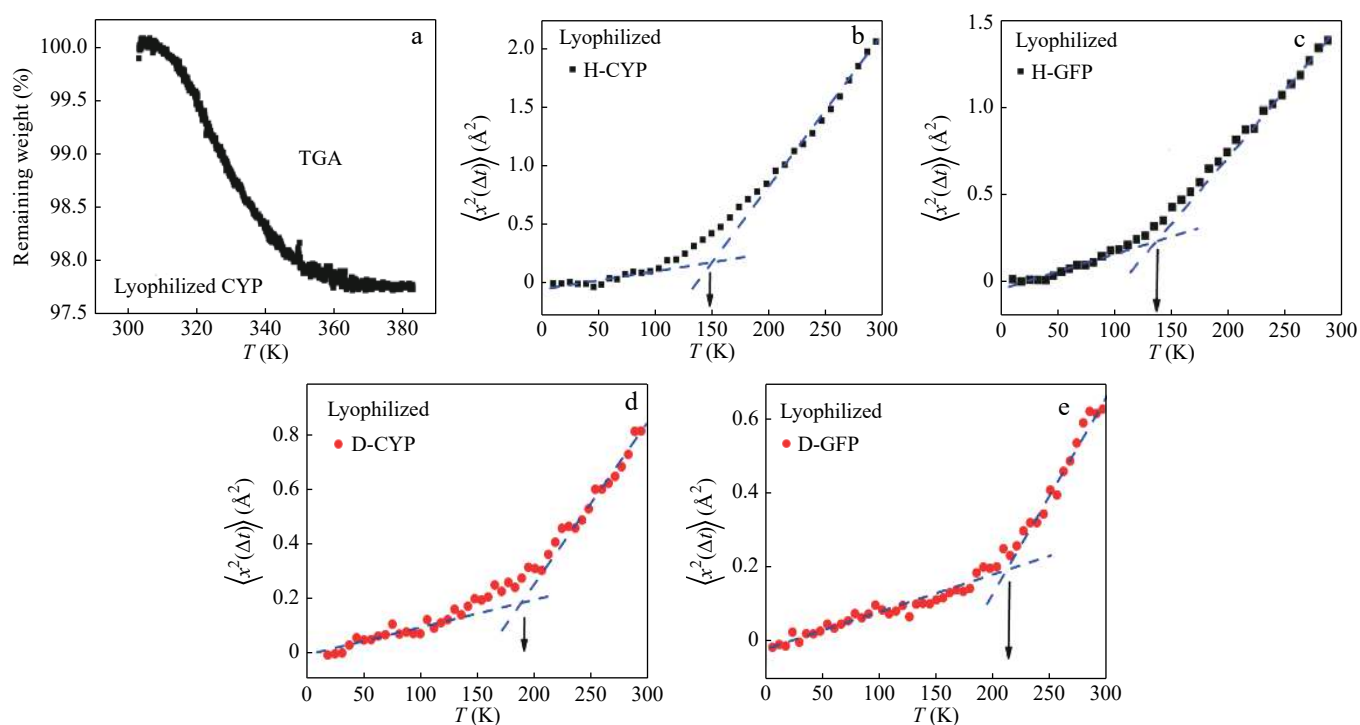


Fig. 8 (a) Temperature-induced weight loss of lyophilized CYP measured by a thermogravimetric analyzer; Experimental MSD $\langle x^2(\Delta t) \rangle$ derived from dry ($h = 0.02$) (b) H-CYP, (c) H-GFP, (d) D-CYP, and (e) D-GFP (Here, H- and D- denote hydrogenated and deuterated, respectively. The notations are the same as in Fig. 9.) (Reprinted with permission from Ref. [20]; Copyright (2017) American Physical Society)

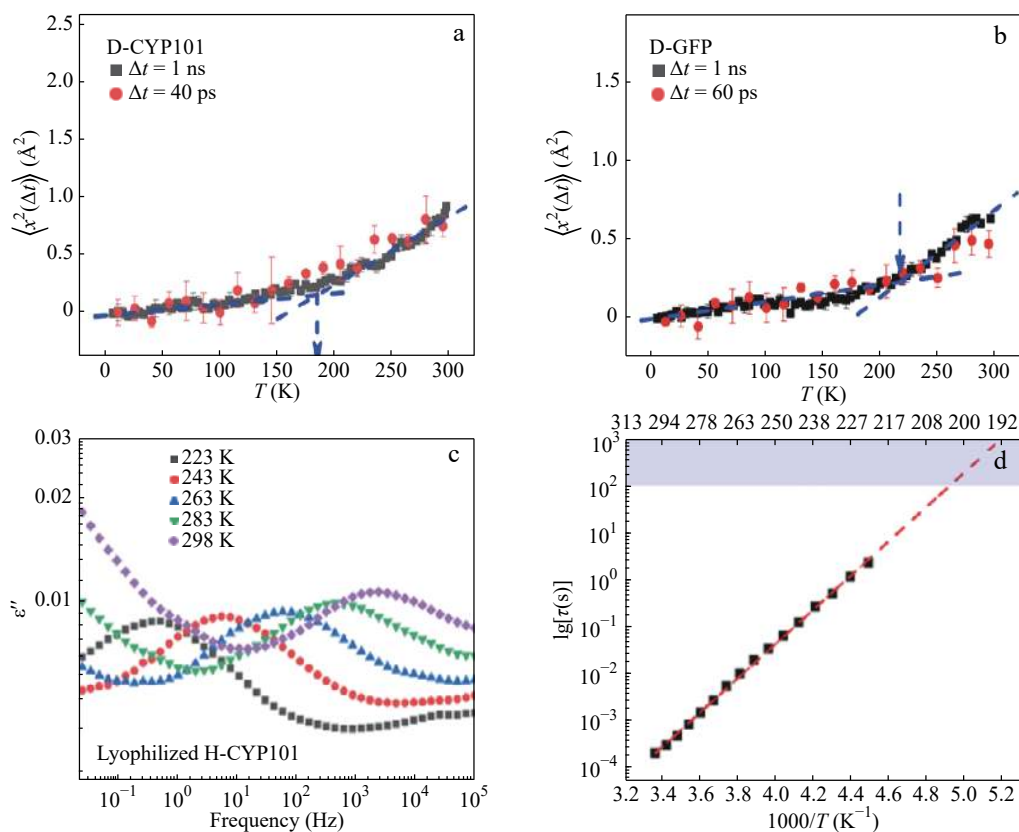


Fig. 9 Experimental MSD $\langle x^2(\Delta t) \rangle$ derived from lyophilized (a) D-CYP101 and (b) D-GFP; (c) Dielectric loss spectra of lyophilized H-CYP101 measured at selective temperatures; (d) Relaxation times of H-CYP101 derived from (c) as a function of reciprocal temperature (The red dashed line is a linear extrapolation towards the low temperatures, and the blue shaded area indicates the time window from 100 s to 1000 s.) (Reprinted with permission from Ref. [25]; Copyright (2018) American Chemical Society)

(CYP) (Fig. 8d) and green fluorescent protein (GFP) (Fig. 8e), we showed that the motions of heavy atoms in an essentially dry protein present a dynamical transition at ~200 K, calling into question the requirement for water.^[20] Here, perdeuterated sample is used to highlight the neutron signals resulting from the collective inter-atomic motions of heavy atoms, especially those in protein backbone.^[16]

The intriguing question is what causes the 200 K dynamical transition of protein heavy atoms in the dry form. We performed elastic scan neutron measurement on both lyophilized perdeuterated CYP and GFP using instruments with different resolutions (Figs. 9a and 9b), and found this transition in dynamics of protein heavy atoms is resolution independent, indicating it behaves like a critical phenomenon rather than a relaxation process entering the experimental time window upon heating.^[25] Further dielectric measurement demonstrates that it results from unfreezing of the relaxation of protein structures within the experimental equilibrium time (100–1000 s) upon heating, which renders global flexibility to the biomacromolecules (Figs. 9c and 9d).

CONCLUSIONS AND OUTLOOK

The present article highlights the methodology by combining neutron scattering, MD simulation, and deuteration technique to decompose different dynamical processes or dynamics of different components in the protein-water system, and demonstrates its power in exploring the microscopic origin of protein dynamical transition as viewed from different aspects. This methodology can also be applied to study other scientific questions in biology and polymers. Just to name a few, to understand how different types of atomic motions, collective backbone motions, and water dynamics contribute to protein folding/unfolding, protein-ligand binding, protein-protein docking, glass transition of polymer with plasticizer or anti-plasticizers, etc. It is of importance to note that three new neutron facilities were just built up in China: China Spallation Neutron Source at Dongguan, China Advanced Research Reactor at Beijing, and Mianyang Research Reactor in Sichuan. Tens of advanced neutron instruments are either in operation or under construction, and they will ensure the bright future for dynamical and structural studies in a broad category of biological and polymeric materials in this country.

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