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Using NMR spectroscopy to elucidate the role of molecular motions in enzyme function

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ABSTRACT

Conformational motions play an essential role in enzyme function, often facilitating the formation of enzyme-substrate complexes and/or product release. Although considerable debate remains regarding the role of molecular motions in the conversion of enzymatic substrates to products, numerous examples have found motions to be crucial for optimization of enzyme scaffolds, effective substrate binding, and product dissociation. Conformational fluctuations are often rate-limiting to enzyme catalysis, primarily through product release, with the chemical reaction occurring much more quickly. As a result, the direct involvement of motions at various stages along the enzyme reaction coordinate remains largely unknown and untested. In the following review, we describe the use of solution NMR techniques designed to probe various timescales of molecular motions and detail examples in which motions play a role in propagating catalytic effects from the active site and directly participate in essential aspects of enzyme function.

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1. NMR methods and theory

Molecular motions are critical in the function of enzymes. Enzymes change their conformation multiple times during the catalytic cycle and these motions must occur with timescales commensurate with the rate constants that define the reaction mechanism. Characterization of these motions is essential to understanding their role in enzyme chemistry. Solution NMR spectroscopy is the experimental method of choice for analyzing molecular motions over an enormous timescale range. Solution NMR can be performed under a wide range of aqueous conditions including those that approximate physiological pH and [salt].

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Moreover the incorporation of spin-1/2 nuclei into proteins is minimally perturbative and is relatively straightforward. Further, NMR spectroscopy maintains protein integrity, and the experimental library for the study of protein structure and dynamics is constantly improving. Biomolecular NMR has historically been restricted to proteins smaller than 50 kDa, but novel TROSY [1] methods and ^{13}C-methyl labeling strategies [2–5] now enable the study of much larger proteins through enhanced signal-to-noise (S/N) and resolution.

Here, we briefly review the NMR methods designed to characterize macromolecular motions followed by examining several examples from the authors' work that illustrate some of the biological insight that can be obtained through the use of this powerful technique.

1.1. Picosecond-to-nanosecond motions (ps–ns)

NMR techniques are powerful for the study of atomic-resolution protein dynamics over an enormous time scale ranging from picoseconds (ps) to seconds (s) (Fig. 1) [6–9]. Motions on the fast end (ps–ns) of this continuum, which are faster than the overall rotational diffusion of the protein under study, reflect stochastic equilibrium fluctuations in the bond vectors of individual atoms. These stochastic motions modulate the chemical shift anisotropy and dipolar interactions between the nuclei. The identity of nuclei and frequency of the motions determine the rate at which Boltzmann equilibrium is established [10–14]. A comprehensive review of the theoretical aspects of NMR spin-relaxation is given by Palmer et al. [15–17] Here, we present a shortened overview of the spin-relaxation formalism. The mathematical expressions below are useful for describing a heteronuclear spin-1/2 pair system such as the amide proton-nitrogen (^{1}H–^{15}N) located in the protein backbone. The longitudinal, transverse magnetization and cross-relaxation of the ^{15}N heteronucleus relax (R_1, R_2, σ_{IS}) or return to their Boltzmann equilibrium state as described by Abragam [18],

$$R_1 = (d^2/4)[J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I + \omega_S)] + c^2 J(\omega_S) \quad (1)$$

$$R_2 = (d^2/8)[4J(0) + J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I) + 6J(\omega_I + \omega_S)] + (c^2/6)[4J(0) + 3J(\omega_S)] + R_{ex} \quad (2)$$

$$\sigma_{IS} = (d^2/4)[J(\omega_I + \omega_S) - J(\omega_I - \omega_S)] \quad (3)$$

where ω_I and ω_S are the Larmor frequencies of the I (^{1}H) and S (^{15}N) nuclei and c is the chemical shift anisotropy coupling constant = $\Delta\sigma\omega_S/\sqrt{3}$, in which $\Delta\sigma$ is the chemical shift anisotropy value of the S nucleus. R_{ex} is the additional contribution to R_2 that results from conformational exchange motions that occur with μs–ms frequency and is often equal to zero. The dipolar coupling constant d is described by Eq. (4),

$$d = (\mu_0 h \gamma_S \gamma_I / 8\pi^2)\langle r_{IS}^{-3}\rangle. \quad (4)$$

Here, μ_o is the permeability of free space, h is Planck's constant, γ_I and γ_S are the gyromagnetic ratios of nuclei I and S, and $\langle r_{IS}\rangle$ is the average internuclear bond length for the I and S atoms. The spectral density function, $J(\omega)$, is the cosine Fourier transform of the autocorrelation function of the I–S bond vector motion [18].

$$J(\omega) = 2\int_o^\infty C(t)\cos\omega t\ dt \quad (5)$$

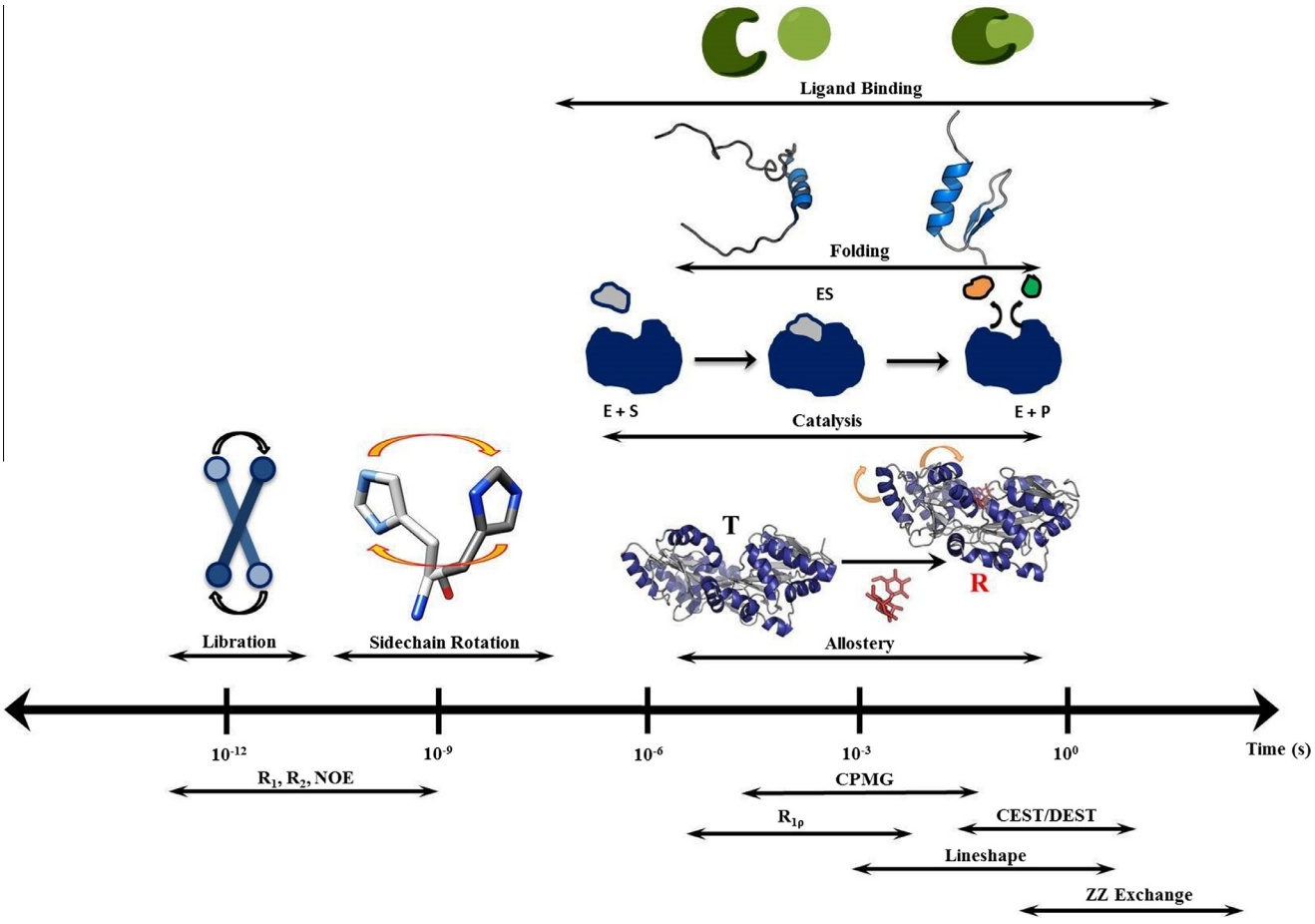

Fig. 1. Timescale depicting enzyme motions. (Top) A cartoon representation of the types and variety of enzyme motions. (Bottom) A list of the types of solution NMR experiments and the timescale of motions to which they are sensitive.