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Visualizing the nanoscale: protein internal dynamics and neutron spin echo spectroscopy

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The most complex molecular machines are proteins found within cells. Protein dynamics, in particular dynamics on nanoscales, presents us with a novel paradigm for cell signaling: the idea that proteins and protein complexes can communicate directly *within themselves* to effect long-range information transfer, via coupled domains and correlated residue clusters. This idea has been little explored, in large part because of a paucity of experimental techniques that can address the necessary questions. Here we review recent progress in developing a promising new approach, neutron spin echo spectroscopy.

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Proteins MOVE! Far from the static objects seen in textbooks, proteins are dynamic actors whose internal dynamics stimulates and controls a medley of essential biological processes. The natural scale for these internal motions is that of nanoseconds to microseconds and nanometers, so we therefore refer to them as *nanoscale motions*. Paradoxically, the visualization of this nanoscale activity requires using large-scale sophisticated neutron scattering techniques at spallation neutron sources and nuclear reactors. Our course of action is thus very much an adventure in *Big Science*, and requires traveling through an interdisciplinary conurbation between nuclear physics, nonequilibrium statistical mechanics, molecular biology and protein chemistry in order to reach its goals. We therefore give an outline of the field in hopes that the reader will share our enthusiasm for this burgeoning endeavor. We begin by summarizing basic properties of nanoscale protein motions.

Protein motions are overdamped, creeping movements rather than underdamped oscillations. The environment in which proteins act is one of low Reynolds number (usually abbreviated *Re*), which is the ratio of the magnitude of inertial forces to that of the forces that arise from the viscous drag that opposes motion. If inertial forces are more important, Reynolds number is large, and forces are proportional to mass times acceleration. If viscous drag is more important, the Reynolds number is small (<1000), and mechanical forces are proportional to the velocity of the protein, incorporating a concept known as the mobility tensor. Reynolds number can be *estimated* by the simple formula $Re = LV/\nu$, where L is a characteristic length scale of the protein, V is a characteristic velocity, and ν is the kinematic viscosity of the solvent (for water, $\nu = 10^5$ Å^2/ns). Simple calculations show that even a complex as large as a ribosome is still in the overdamped regime [1••]. The environment of a protein thus has more in common with playing badminton at the bottom of a swimming pool full of molasses (low *Re*) than in crossing the Atlantic in the Titanic (high *Re*).

Proteins obey Brownian dynamics. Protein dynamics arises as a result of an interplay between the mechanical forces mentioned above, and the thermal forces that arise from the collision of the protein with solvent molecules. These thermal forces are random in magnitude and direction, and lead to the protein undergoing *diffusion*. A freely diffusing object displays what is called *Brownian motion*, with frequent changes in the direction and speed of its movement. The world in which proteins operate is therefore characterized by the presence of a significant amount of noise and the resultant diffusion of protein subunits arising from thermal motion. This thermal motion is essential for the protein to reach its equilibrium state [2••].

The relaxation timescales of these overdamped internal diffusive motions are of order L^2/D, where D is the overall diffusion constant of the protein and L is the characteristic length scale of the motion. This follows from a Brownian analysis of simple harmonic motion [3•]. According to the domain concept of structural biology [4] and the references therein, proteins can be considered as being comprised of somewhat rigid domains connected by soft spring linkers. Since protein diffusion constants are of the order of a few Å^2/ns, we see that for domains connected by linkers of the order of $L \sim 10$ Å or so, the relaxation timescales of nanometer internal modes will be of the order of nanoseconds, with rotational relaxation times that are a factor

of 4 or so longer. This justifies our use of the word nanoscales for these motions. The scale of such motions is optimal for neutron spin echo spectroscopy [5•,6••,7•].

Neutron spin-echo spectroscopy (NSE) is unique in its capacity to determine nanoscale motions. NSE is a quasielastic neutron scattering technique that employs the Larmor precession of neutron spins in a magnetic guide field as a clock to measure extremely small changes in velocities of scattering neutrons [8,9], and thus enables the detection of very small energy changes corresponding to nanosecond-to-microsecond dynamics. This is a dynamic regime that is difficult or impossible to observe with other methods [10].

Neutron spin echo spectroscopy measures the intermediate scattering function I(Q,t), which is the spatial Fourier transformation of the space-time van Hove correlation function $G(r,t)(2)$, $I(Q,t) = \int_V G(r,t)\exp(-iQ\cdot r)dr$, where Q is the scattering vector. (The designation 'intermediate' arises precisely because only one of the variables of $G(r,t)$ is Fourier transformed.)

Nanoscale motions determine I(Q,t). For a given Q, $I(Q,t)$ typically can be fit to a single exponential in time (and is difficult to fit to more exponentials, because of the problem of separating rotational diffusion from internal mode relaxation). A natural way to interpret neutron scattering data is therefore to examine the effective diffusion constant $D_{eff}(Q)$ as a function of Q, which is determined by the normalized intermediate scattering function $I(Q,t)/I(Q,0)$:

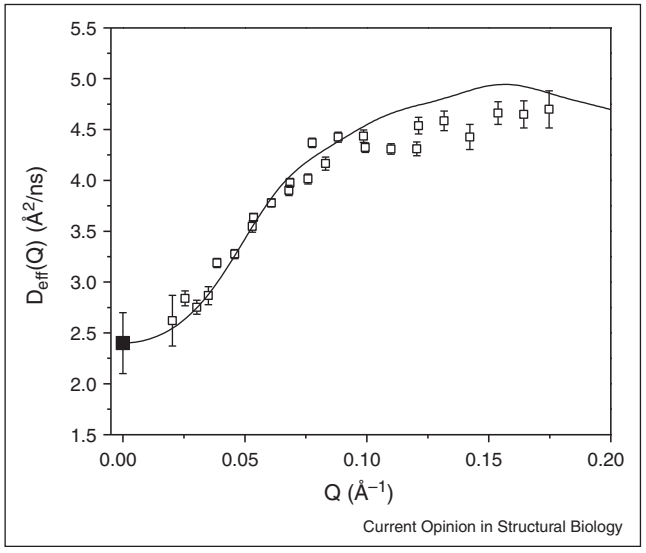
$$\Gamma(Q) = -\lim_{t\to 0}\frac{\partial}{\partial t}\ln\left[\frac{I(Q,t)}{I(Q,0)}\right]$$
$$D_{eff}(Q) = \frac{\Gamma(Q)}{Q^2} \quad (1)$$

where $I(Q,0)$ is the static form factor. As $I(Q,t)/I(Q,0)$ is generally amenable to a single-exponential fit in time (see Figure 1), $D_{eff}(Q)$ can be accurately estimated by the first cumulant expression [5•,6••,11,12]:

$$D_{eff}(Q) = \frac{k_BT}{Q^2} \times \frac{\sum_{jl}\langle b_jb_l(Q\cdot H^t_{jl}\cdot Q + L_j\cdot H^R_{jl}\cdot L_l)e^{iQ\cdot(r_j-r_l)}\rangle}{\sum_{jl}\langle b_jb_le^{iQ\cdot(r_j-r_l)}\rangle} \quad (2)$$

which is a generalization of the remarkable Akcasu–Gurol (AG) formula [2••,13•,14•] to rotational motion [5•]. (This formula is an elementary sum-rule result that results from the fact that the associated Smoluchowski equation forms a Sturm–Liouville system.) Here, b_j is the scattering length of a subunit j, H^T is the translational mobility tensor, and H^R is the rotational mobility tensor. The coordinates of the various subunits ('subunits' are scattering centers that can be atoms, beads, or domains, generically called beads here) are taken relative to the center of *friction* of the protein, and are given by $\mathbf{r}_j$ (note that $\Sigma\mathbf{r}_j = 0$); k_BT is the usual temperature factor; and $\mathbf{L}_j = \mathbf{r}_j \times \mathbf{Q}$ is the torque vector for each coordinate. The brackets $\langle\ \rangle$ denote an equilibrium average over all protein conformations and scattering lengths, as well as an orientational average over the vector $\mathbf{Q}$, so that $\langle Q_aQ_b\exp(i\mathbf{Qr})\rangle \quad Q^{-2} = (1/3)\delta_{ab}j_0(Qr) + [(1/3)\delta_{ab} - (r_ar_b/r^2)]j_2(Qr)$ can be expressed in terms of spherical Bessel functions j.

Figure 1



NHERF1 alone can be described by a rigid-body model. Comparing experimental $D_{eff}(Q)$ of NHERF1 (black open squares) with rigid-body calculation (black solid line).

The AG approach described in Eq. (2) is valid for either rigid bodies or rigid-body subunits connected by soft spring linkers [5•,11]. The translational mobility tensor H^T is defined by the velocity response $\mathbf{v} = H^T\mathbf{F}$ to an applied force $\mathbf{F}$. The rotational mobility tensor H^R is defined by the angular velocity response $\boldsymbol{\omega} = H^R\boldsymbol{\tau}$ to an applied torque $\boldsymbol{\tau}$. In practice, the structural coordinates of a protein may be obtained from high-resolution crystallography or NMR or from low-resolution EM, SAXS or SANS. Comparison of the calculations Eq. (2) to experimental $D_{eff}(Q)$ thus allows one to test models of the mobility tensors. The rotational mobility tensor H^R can be determined from the translational mobility tensor H^T [6••,15]. We point out that the first cumulant expression is explicitly independent of the spring constant of a linker connecting the domains. It is thus not necessary to fit multiple time exponentials to separate translational, rotational, and internal modes in order to reveal internal dynamics.

The mobility tensor directly reveals internal degrees of freedom. For a rigid body composed of N identical subunits, the