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

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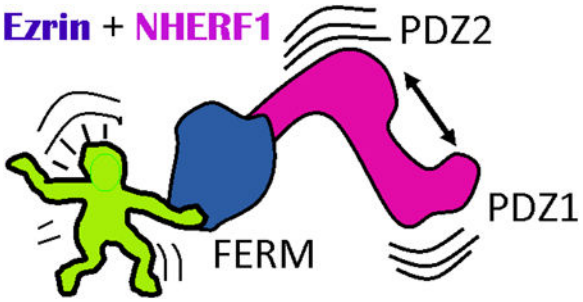
Abstract

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.

Graphical abstract



Keywords

protein dynamics; nanoscale protein motion; nonequilibrium statistical mechanics; NHERF1; ezrin; ERM proteins; PDZ domain; multidomain proteins

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

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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 ~ 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** $D_{\text{eff}}(Q)$ as a function of Q, which is determined by the normalized intermediate scattering function I(Q,t)/I(Q,0):

$$\Gamma(Q)=-\lim_{t\to 0}\frac{\partial}{\partial t}\ln[I(Q,t)/I(Q,0)]$$
$$D_{\text{eff}}(Q)=\frac{\Gamma(Q)}{Q^2} \qquad \text{Eq. 1}$$

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), $D_{\text{eff}}(Q)$ can be accurately estimated by the first cumulant expression (5,6,11,12)*:

$$D_{\text{eff}}(Q)=\frac{k_B T}{Q^2}\frac{\sum_{jl}\left\langle b_j b_l\left(Q\cdot H_{jl}^T\cdot Q+L_j\cdot H_{jl}^R\cdot L_l\right)e^{iQ\cdot(r_j-r_l)}\right\rangle}{\sum_{jl}\left\langle b_j b_l e^{iQ\cdot(r_j-r_l)}\right\rangle} \qquad \text{Eq. 2}$$

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 < > denote an equilibrium average over all protein conformations and scattering lengths, as well as an orientational average over the vector **Q,** so that $\langle Q_a\ Q_b \exp(i\ \mathbf{Qr})\rangle\ Q^{-2} = (1/3)\delta_{ab} j_0(Qr) + [(1/3)\delta_{ab} - (r_a\ r_b\ /r^2)]\ j_2(Qr)$ can be expressed in terms of spherical Bessel functions j.

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 **F**. The **rotational mobility tensor** H^R is defined by the angular velocity response $\boldsymbol{\omega} = H^R\ \boldsymbol{\tau}$ to an applied torque **τ.** 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 translational mobility tensor H^T is a matrix with N^2 **identical** 3×3 rotation elements. This must be so, since H^T yields the velocity response of e.g., subunits B and C to a force applied to subunit A. If the mobility tensor components H_{AB} and H_{AC} are unequal, the velocity response of B and C will be different, B and C will move apart, and the body will no longer remain rigid. Additionally, we showed that for a rigid, uniform (nondeuterated) body, $D_{eff}(Q\rightarrow\infty) = 2\ D_{eff}(Q=0)$(6,12). Large scale numerical simulations are thus not required to demonstrate internal dynamics.

Comparing the calculated $D_{eff}(Q)$ with data allows one to extract the relative degree of dynamic coupling between the various components of the system, for this dynamic coupling is defined by the mobility tensor. For example, a **rigid** two-domain system has a translational mobility tensor

$$H = H_0 \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix} \quad \text{Eq. 3a}$$

with all elements of the tensor equal, and yields [via Eq. 2] the simple result that the *translational* contribution to the effective diffusion constant is given by $D^T_{eff}(Q) = k_BT\ H_0$, independent of Q. By contrast, a two-domain **flexible** protein with internal motion has a translational mobility tensor

$$H=\begin{pmatrix} H_1 & 0 \\ 0 & H_2 \end{pmatrix} \quad \text{Eq. 3b}$$

in principal coordinates. The application of equal forces to the two domains of a flexible protein will then result in their having different velocities, revealing internal motion. For the case where there is one internal translational mode between subunits 1 and 2 with $D_1=k_BTH1$ and $D_2= k_BTH_2$ (5), the *translational* contribution to the effective diffusion constant for this **flexible** system is:

$$D^T{}_{\text{eff}}(Q)=\frac{D_1S_1(Q)+D_2S_2(Q)}{S(Q)} \quad \text{Eq. 3c}$$

Here, $S_1(Q)$ and $S_2(Q)$ are the form factors of the separate individual protein domains, while S(Q) is the form factor of the entire protein. Orientational averages are performed, so that, e.g., $S(Q)=\Sigma_{j1}\, j_0\,(Qr_{j1})$; and $S(Q=0)=N^2$ and $S(Q\rightarrow\infty) = N$, with N the number of beads. Since $D^T{}_{\text{eff}}(Q\rightarrow\infty) = D^R{}_{\text{eff}}(Q\rightarrow\infty)$ ($D^R{}_{\text{eff}}(Q)$ is the rotational contribution to $D_{\text{eff}}(Q)$) while $D^R{}_{\text{eff}}(Q=0) = 0$, $D_{\text{eff}}(Q\rightarrow\infty) = 4\, D_{\text{eff}}(Q=0)$ (6).

If one domain is a fraction × of the whole protein then Eq (2) yields

$$D_{\text{eff}}(Q\rightarrow\infty)/D_{\text{eff}}(Q=0) \sim 2[x^2+(1-x)^2]^{-1} \quad \text{Eq. 4}$$

Similarly the parsing of a polysyndetonic uniform protein into M *equal* domains gives $D_{\text{eff}}(Q\rightarrow\infty) \sim 2M\, D_{\text{eff}}(Q=0)$, related to the transition from coherent to incoherent scattering. The effect can be amplified by selective deuteration (6,16). **The Q dependence of the effective diffusion constant is thus a highly sensitive probe of active internal dynamic modes and correlations** (12,17).

The 3×3 matrix H^R is evaluated here by an inversion of a 3×3 matrix calculated by summing over N bead coordinates n (6) A compact formula arises (6) in terms of a 3×3 matrix inverse, using the protein diffusion constant D_0 and the N structural coordinates of the protein (defined so that $\Sigma_{\,n}\, r_n = 0$):

$$H^R{}_{\alpha\beta}=N(D_0/k_BT)[\textstyle\sum_n(\delta_{\alpha\beta}r^2{}_n - r_{r\alpha}r_{n\beta})]^{-1} \quad \text{Eq. 5}$$

NHERF1: A paradigm of long-range allostery

The multi-PDZ domain scaffolding NHERF1 interacts with a number of physiologically important transmembrane receptors and ion transport proteins in epithelial cells (18,19).

NHERF1 binds to the membrane-cytoskeleton linker protein Ezrin. Previously, we uncovered a prominent long-range allosteric regulatory behavior in NHERF1: Upon Ezrin binding to NHERF1, the binding affinities of the PDZ domains of NHERF1 for target membrane proteins are dramatically increased, even though the PDZ domains are located more than 120 Å away from the Ezrin-binding site (6,20,21*). Thus, Ezrin allosterically modulates NHERF1 to assemble membrane protein complexes and to tether the assembled complexes to the cytoskeletal actin network, which modulates the cell surface targeting and intracellular trafficking of the assembled signaling complexes (22-24).

Long-range allostery is an important consequence of internal protein motion, and can be revealed by NSE

*C*ell signaling ultimately involves cytoskeletal rearrangement via structure sensing and a Rube Goldberg conglomeration of gear selectors, rheostats, and leaky valve faucets. Scaffolding and adapter proteins not only provide a scaffold to dock signaling partners, but also propagate and relay signals allosterically to the site of regulation over a long distance. Our recent study using neutron spin echo spectroscopy reveals that inter-domain motions in NHERF1 on nanometer length-scales and on submicrosecond time scale can propagate allosteric binding signals dynamically. The long-range allostery and nanoscale protein domain dynamics during the interactions of NHERF1 and ezrin provide a paradigm for the mechanisms of how cellular signals can be transmitted in a cellular signaling network (6,12).

NSE reveals the activation of NHERF PDZ domain motion by FERM domain binding

First, we present the results for a rigid body, see Figure 1. We then present the calculation of the model representing the deuterated NHERF1·^{d}FERM and hydrogenated NHERF1·^{h}FERM complexes. Details are given in ref. (6). Figure 2A compares the NSE data with the $D_{eff}(Q)$ from the rigid body model for the hydrogenated and partially deuterated complexes. Figure 2B is the $D_{eff}(Q)$ of the model incorporating internal domain motion between PDZ1 and the rest of the complex. This shows that the long-range allosteric motion is activated by FERM binding.

Deuterium labeling of a domain amplifies the effects of internal motion detected by NSE by masking a portion of the form factor, and, as a result, highlighting the contribution of the terms of internal domain motion (6,15). Because for proteins inertial forces are less important than diffusive, viscous effects, **protein dynamics should be largely independent of the mass of the protein**. The diffusion constant of a deuterated protein is the same as a hydrogenated protein, even though deuterium has twice the mass of hydrogen. Deuterium contrast matching is thus valuable in neutron spin echo spectroscopy experiments.

New horizons: The highly dynamical nature of protein complexes

Protein dynamics presents us with the compelling idea that proteins can communicate *within themselves* to effect long-range allosteric information transfer (6,25). Experimental techniques to observe these essential phenomena in proteins macromolecular complexes are

however in their infancy (26-28). This review discusses an especially promising candidate: neutron spin-echo spectroscopy. We believe that it is impossible not to be excited about the challenges ahead, and the rewards for their successful solution.

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Highlight

- Nanoscale protein dynamics affects cell signaling propagation via long-range allostery in a largely unknown fashion.
- It is difficult at best to probe protein dynamics at these nanoscales since techniques are lacking.
- The novel technique of neutron spin echo spectroscopy (NSE) reveals nanoscale dynamics changes that control the assembly of protein complexes.

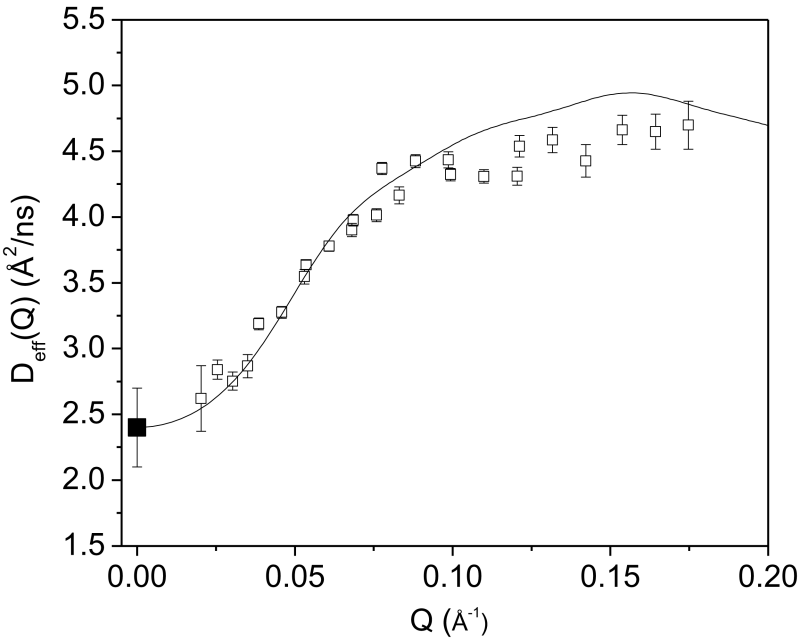

Figure 1. NHERF1 alone can be described by a rigid-body model

Comparing experimental $D_{eff}(Q)$ of NHERF1 (black open squares) with rigid-body calculation (black solid line).

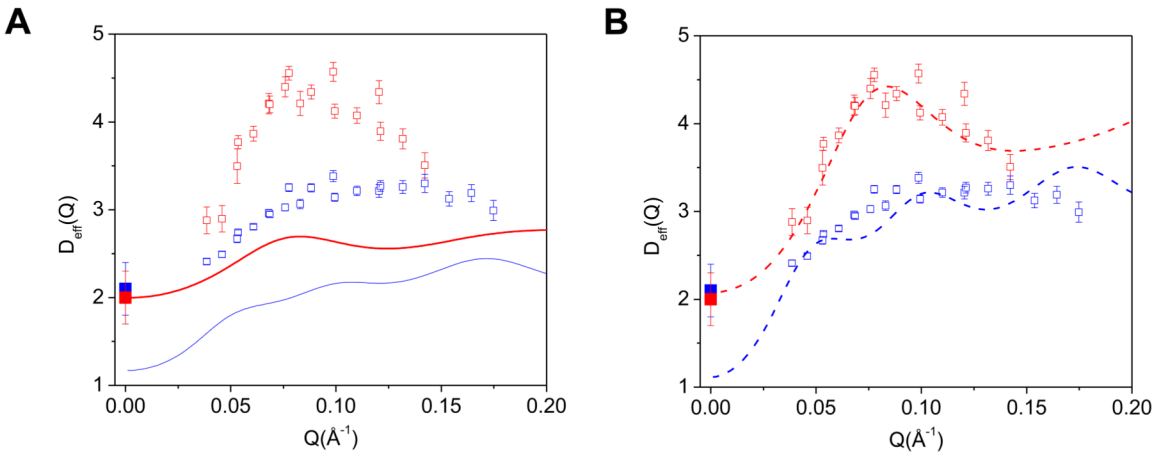

Figure 2. Selective deuteration highlights the activation of domain motion in NHERF1 upon binding to Ezrin FERM domain

(A) Comparing NSE data with rigid model calculations for the NHERF1·^{d}FERM and NHERF1·^{h}FERM complexes using the coordinates of the docked domains. (B) Comparing experimental $D_{eff}(Q)$ with calculations incorporating interdomain motion (via the mobility tensor) between PDZ1 and PDZ2, for NHERF1·^{d}FERM (dashed red line) and NHERF1·^{h}FERM (dashed blue line).