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Supporting Material

Title: Activation of nanoscale allosteric protein domain motion revealed by

Authors: Bela Farago, Jianquan Li, Gabriel Cornilescu, David J.E. Callaway, and Zimei Bu

Appendix

In this appendix, we present a simple proof of the statement that, **for a uniform rigid body**, the effective diffusion constant $D_{eff}(Q)$ at infinite Q is twice that at zero Q, i.e., $D_{eff}(Q\rightarrow\infty) = 2\ D_{eff}(Q=0)$. This result is independent of the shape or size of the body. (We explicitly point out however that the body must have a uniform scattering length density, thus it cannot be partially deuterated). This section also provides an elementary example of the algebraic manipulations needed to perform calculations utilized in the text.

We take the rigid body as consisting of a collection of N identical subunits (which can be atoms, dummy atoms, domains, etc). The case of a continuous solid can be reached by an appropriate limit of large N if desired. First, we note that for a rigid body, the rotational and translational mobility tensors are simply 3x3 matrices, identical for each subunit. This must be so, since otherwise a force applied to a given subunit would result in different resultant velocities for other subunits, and the body would not remain rigid. Thus we see that **the mobility tensor defines and characterizes internal motion for a body**. We also make the simple observation that $\exp(i\mathbf{Qr})$ is one for all values of the vector **r** at zero **Q**, and that, as Q increases without bound, this quantity is one when r is zero and equals zero otherwise. The remainder of our analysis begins with the Akcasu-Gurol (**AG**) formula Eq. (2). We shall adopt the convention that the indices that identify a given subunit will be labeled with Latin subscripts (m,n,…) while spatial indices (x,y,z) will be indicated with Greek symbols (α, β, γ, …), and are typically omitted for clarity, with vector quantities indicated by bold script. We note that at Q=0, the contribution to $D_{eff}(Q)$ from translational diffusion is $D^T_{eff}(Q=0) = k_BT\ (\Sigma_{mn}\ H^T_{\ mn})/N^2$, while at infinite Q this contribution becomes $D^T_{eff}(Q\rightarrow\infty) = k_BT\ (\Sigma_{nn}\ H^T_{\ nn})/N = k_BT\ \ Tr(H^T)/N$. We point out for later use that for a rigid body, $D^T_{eff}(Q=0) = D^T_{eff}(Q\rightarrow\infty)$ since H^T is independent of n. By contrast, $D^R_{eff}(Q)$, the contribution to $D_{eff}(Q)$ from rotational diffusion, is zero at Q=0, since $\Sigma_n\ \mathbf{r}_n = 0$ by definition of our coordinate system. Of course, $D_{eff}(Q) = D^T_{eff}(Q) + D^R_{eff}(Q)$. The calculation of the contribution of rotational diffusion at large Q is slightly more involved, and will now be considered in detail.

The contributions to rotational diffusion are normally evaluated using the usual methods for systems involving rigid constraints, such as Lagrange multipliers or generalized coordinates (see Doi and Edwards (42), sec 3.8). The following course is simpler and suffices here. The angular velocity vector of the rigid object is given by $\boldsymbol{\omega} = H^R\ \boldsymbol{\tau}$, with the torque $\boldsymbol{\tau} = \Sigma_n\ \mathbf{r}_n \times \mathbf{F}_n$. The vector force $\mathbf{F}_n$ on subunit n is given in terms of the velocity $\mathbf{v}_n$ and overall angular velocity $\boldsymbol{\omega}$ by **Eq. (3)**. Thus for an arbitrary 3-component vector $\boldsymbol{\omega}$

$$\boldsymbol{\omega} = H^R{}_{\Sigma mn}\ \mathbf{r}_m \times (N^2H^T)^{-1}{}_{mn}\ (\boldsymbol{\omega} \times \mathbf{r}_n) \qquad \textbf{(A.1)}$$

We note that Eq. (A.1) is of the form $\boldsymbol{\omega} = M\boldsymbol{\omega}$ for an arbitrary vector $\boldsymbol{\omega}$, implying that M is the identity matrix. It immediately follows that the 3x3 matrix H^R can be evaluated by an inversion of a 3x3 matrix calculated by summing over subunit coordinates n. *The rotational mobility tensor is thus determined by the translational mobility tensor.* In the simplified case (adopted in this work) where the x, y, and z principal components of the translational mobility tensor H^T are all set equal to a friction constant $1/\zeta$ (see discussion surrounding eq. 3), a compact formula arises in terms of a 3x3 matrix inverse, arising from the protein diffusion constant D_0 (measured by NMR) and the N structural coordinates of the protein (defined so that $\Sigma_n\ r_n = 0$):

$$H^R_{\alpha\beta} = N (D_0/k_BT) \; [\Sigma_n (\delta_{\alpha\beta} r^2_n - r_{r\alpha} r_{n\beta})]^{-1} \qquad (A.2)$$

To complete the proof that $D_{eff}(Q\rightarrow\infty) = 2\, D_{eff}(Q=0)$, we now use the fact that $\boldsymbol{\omega}$ is arbitrary, set $\boldsymbol{\omega} = H^T \mathbf{Q}$ in equation (A.1), contract the remaining vector index on the left hand side with $\mathbf{Q}$, and perform an average $< \ldots >$ over the orientation of $\mathbf{Q}$:

$$\begin{aligned} D^T_{eff}(Q\rightarrow\infty) &= k_BT \, \Sigma_n <\mathbf{Q}\, H^T \mathbf{Q}>/(NQ^2) \\ &= k_BT \, \Sigma_n < (\mathbf{Q} \times \mathbf{r}_n)\, H^R \, (\mathbf{Q} \times \mathbf{r}_n) >/(NQ^2) \\ &= D^R_{eff}(Q\rightarrow\infty) \end{aligned} \qquad (A.3)$$

from which we see that at large Q the translational and rotational contributions to the AG formula are identical, so $D_{eff}(Q=0) = D^T_{eff}(Q=0) = D^T_{eff}(Q\rightarrow\infty) = D^R_{eff}(Q\rightarrow\infty)$. Since $D_{eff}(Q) = D^T_{eff}(Q) + D^R_{eff}(Q)$ this completes the proof.

We will also demonstrate that in general for a flexible system one **only** has the bound $D_{eff}(Q\rightarrow\infty) \geq D_{eff}(Q=0)$. We consider a system with no rigid constraints, so there is only one mobility tensor H. We also omit spatial indices in the interests of clarity.

Note that the second law of thermodynamics assures us that the power dissipated by a system is generally non-negative (Doi and Edwards (1), Eq.3.18), therefore

$$\Sigma_n \mathbf{v}_n \mathbf{F}_n = \Sigma_{mn} \mathbf{F}_m \, H_{mn} \, \mathbf{F}_n \geq 0 \qquad (A.4)$$

for any set of applied forces **F,** and therefore the mobility tensor is positive semidefinite (has no negative eigenvalues). If we choose **F** to have only two nonzero components, $F_m = 1$ and $F_n = -1$, we see that (A.4) implies that H is dominated by its diagonal elements:

$$H_{mm} + H_{nn} \geq H_{mn} + H_{nm} \qquad (A.5)$$

We now sum over all indices m and n, and note that (A.5) implies

$$D_{eff}(Q\rightarrow\infty) = k_BT \, Tr\,(H)/N \geq k_BT \, \Sigma_{mn} H_{mn} /N^2 = D_{eff}(Q=0) \qquad (A.6)$$

The inequality approaches the equality $D_{eff}(Q\rightarrow\infty) = D_{eff}(Q=0)$ when all elements of H are equal (the delicate singular limit of a stiff but still flexible body, discussed in (2)). In the other extreme limit, when the mobility tensor is entirely diagonal (the limit of non-interacting subunits in a flexible system) we have

$$D_{eff}(Q\rightarrow\infty) = N \, D_{eff}(Q=0) \qquad (A.7)$$

For a system with an infinite number N of subunits, $D_{eff}(Q\rightarrow\infty)$ thus increases without bound in the case of a diagonal mobility tensor (cf. the Rouse model of polymers). Thus we see that the uniform rigid body result $D_{eff}(Q\rightarrow\infty) = 2\ D_{eff}(Q=0)$ is quite unusual.

1. Doi, M., and S. F. Edwards. 1986. The theory of polymer dynamics. Oxford University Press, Oxford.
2. Bu, Z., R. Biehl, M. Monkenbusch, D. Richter, and D. J. Callaway. 2005. Coupled protein domain motion in Taq polymerase revealed by neutron spin-echo spectroscopy. Proc Natl Acad Sci U S A 102:17646-17651.

	Concentration (mg/ml)	D_0 (Å^2/ns)
NHERF1	11.9	2.4±0.3
NHERF1·^{h}FERM	6.5	2.1±0.3
NHERF1·^{d}FERM	3.4	2.0±0.3

Table SI. Translational diffusion constants D_0 obtained from pulsed field gradient NMR.

Supporting Figures

Figure S1. (A) I(Q,t)/I(Q,0) vs. t plot for NHERF1·^{h}FERM and (B) I(Q,t)/I(Q,0) vs. t plot for NHERF1·^{d}FERM obtained from NSE experiements. The lines in the plots are single exponential fit to the NSE data

Figure S2. (A) Size-exclusion chromatograph of NHERF1·FERM complex, NHERF1, and FERM. **(B)** SDS PAGE analysis of NHERF1·^{d}FERM (lane 2) and NHERF1·^{h}FERM (lane 3). The electrophoresis experiments were performed on samples used for NSE experiments after the neutron scattering experiments.

Figure S3. For the hydrogenated NHERF1·^{h}FERM complex, the difference in $D_{eff}(Q)$ between the rigid-body model and domain-motion models is very small, but is significantly increased in the deuterated complex. **(A)** Comparing the rigid-body calculation with the domain-motion calculation in the four-point model in the hydrogenated NHERF1·^{h}FERM complex. NSE data from the NHERF1·^{h}FERM (blue open squares), the four-point rigid-body model (black line), four-point model incorporating domain motion between PDZ1 and PDZ2 (red line), four point model incorporating domain motion between PDZ1 and PDZ2 and finite size form factor of 20 Å radius for the FERM domain, PDZ1 and PDZ2 (blue line). D_0 at Q=0 Å^{-1} as measured from PFG NMR is shown in blue solid square. **(B)** Comparing the rigid-body calculation with the domain-motion calculation in the four-point model in the deuterated NHERF1·^{d}FERM complex. NSE data from the NHERF1·^{d}FERM (red open squares), the four-point rigid-body model (black line), four-point model incorporating domain motion between PDZ1 and PDZ2 (red line), four point model incorporating domain motion between PDZ1 and PDZ2 and finite size form factor of 20 Å radius for the FERM domain, PDZ1 and PDZ2 (blue line). D_0 at Q=0 Å^{-1} as measured from PFG NMR is shown in red solid square.

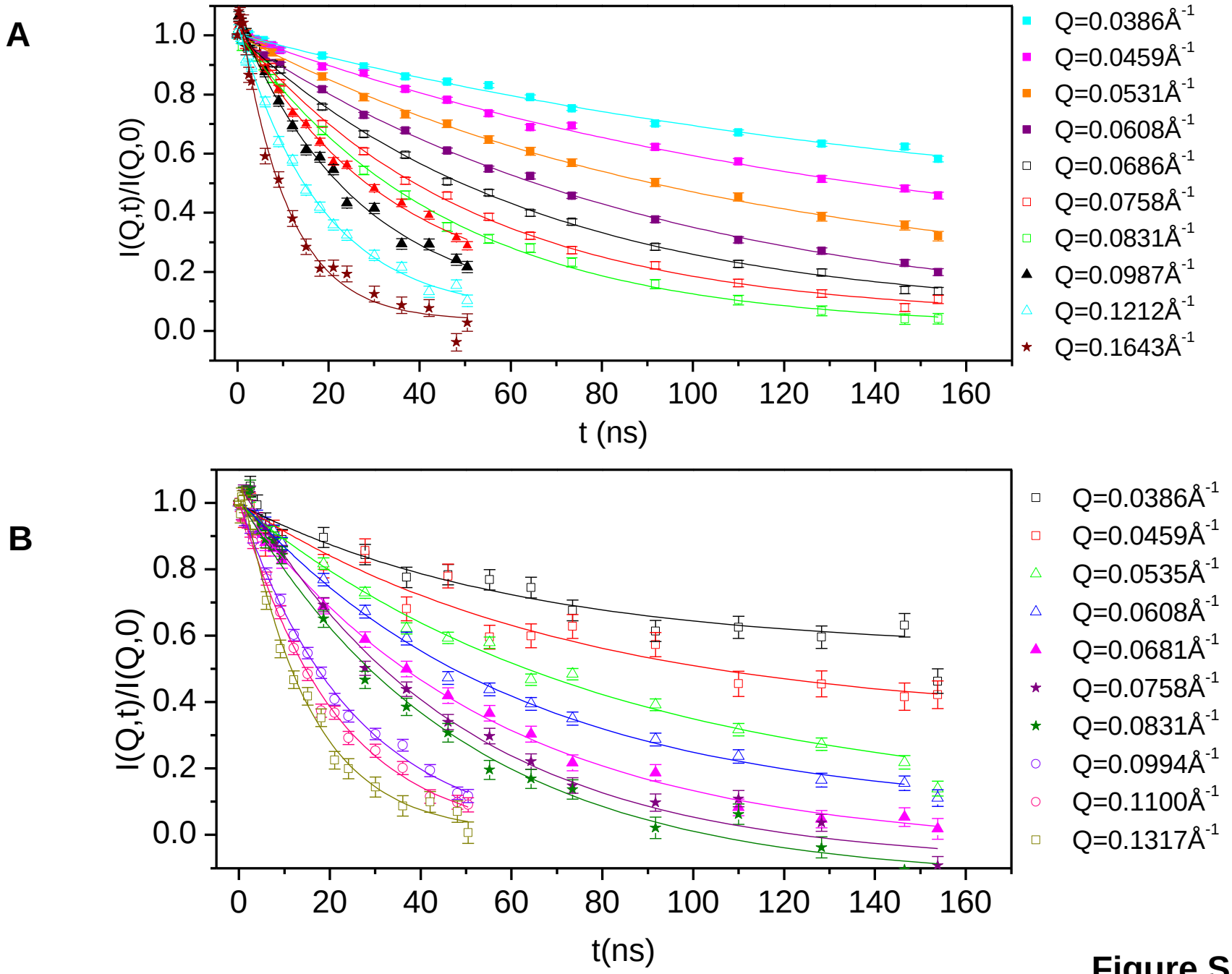
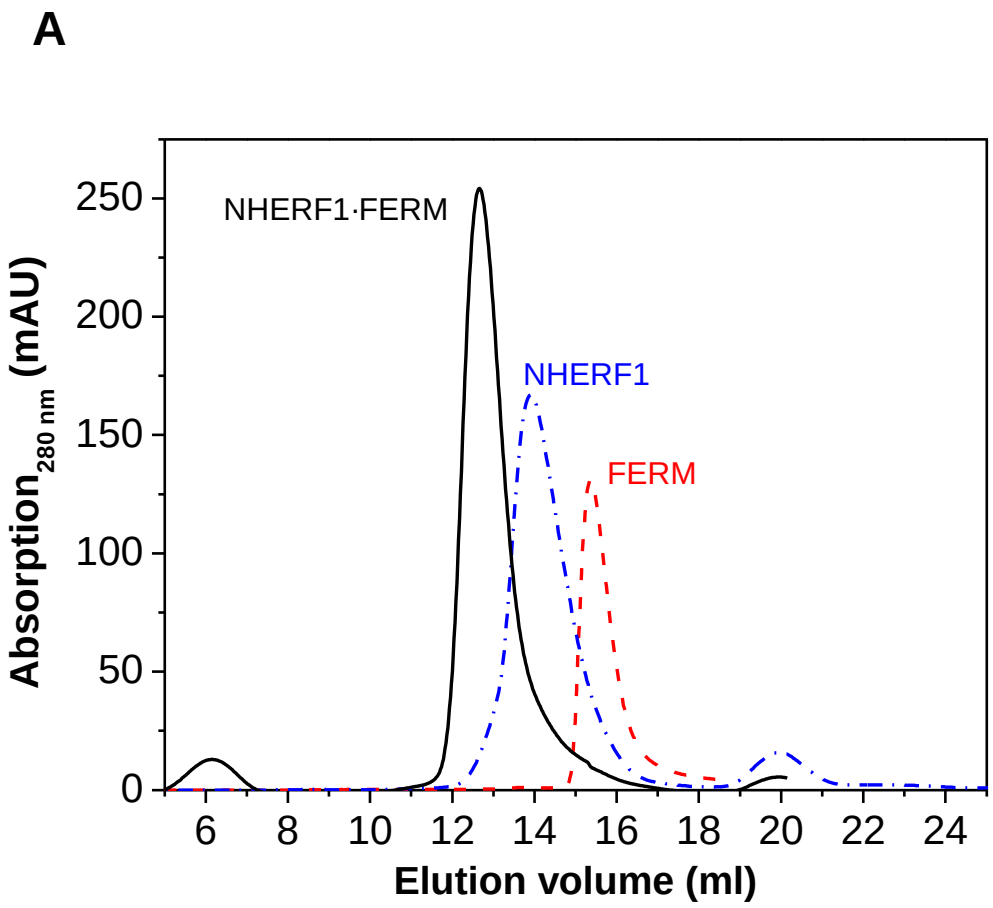

A
I(Q,t)/I(Q,0)
t (ns)
Q=0.0386Å-1
Q=0.0459Å-1
Q=0.0531Å-1
Q=0.0608Å-1
Q=0.0686Å-1
Q=0.0758Å-1
Q=0.0831Å-1
Q=0.0987Å-1
Q=0.1212Å-1
Q=0.1643Å-1
B
I(Q,t)/I(Q,0)
t(ns)
Q=0.0386Å-1
Q=0.0459Å-1
Q=0.0535Å-1
Q=0.0608Å-1
Q=0.0681Å-1
Q=0.0758Å-1
Q=0.0831Å-1
Q=0.0994Å-1
Q=0.1100Å-1
Q=0.1317Å-1

Figure S1


A
NHERF1·FERM
NHERF1
FERM
Absorption280 nm (mAU)
Elution volume (ml)
0
50
100
150
200
250
6
8
10
12
14
16
18
20
22
24

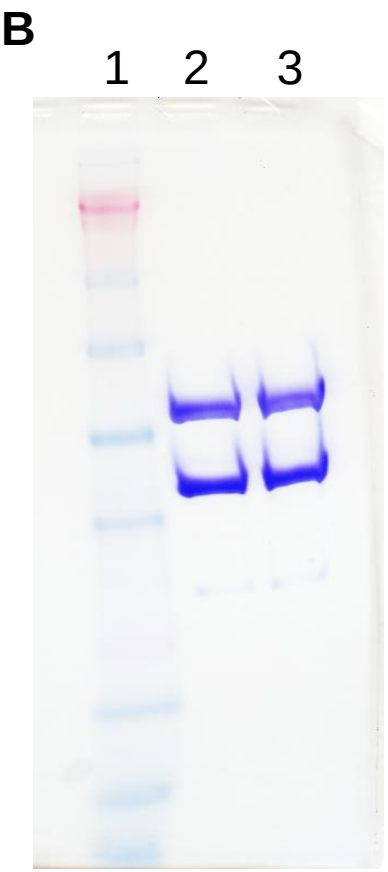

B
1
2
3

Figure S2

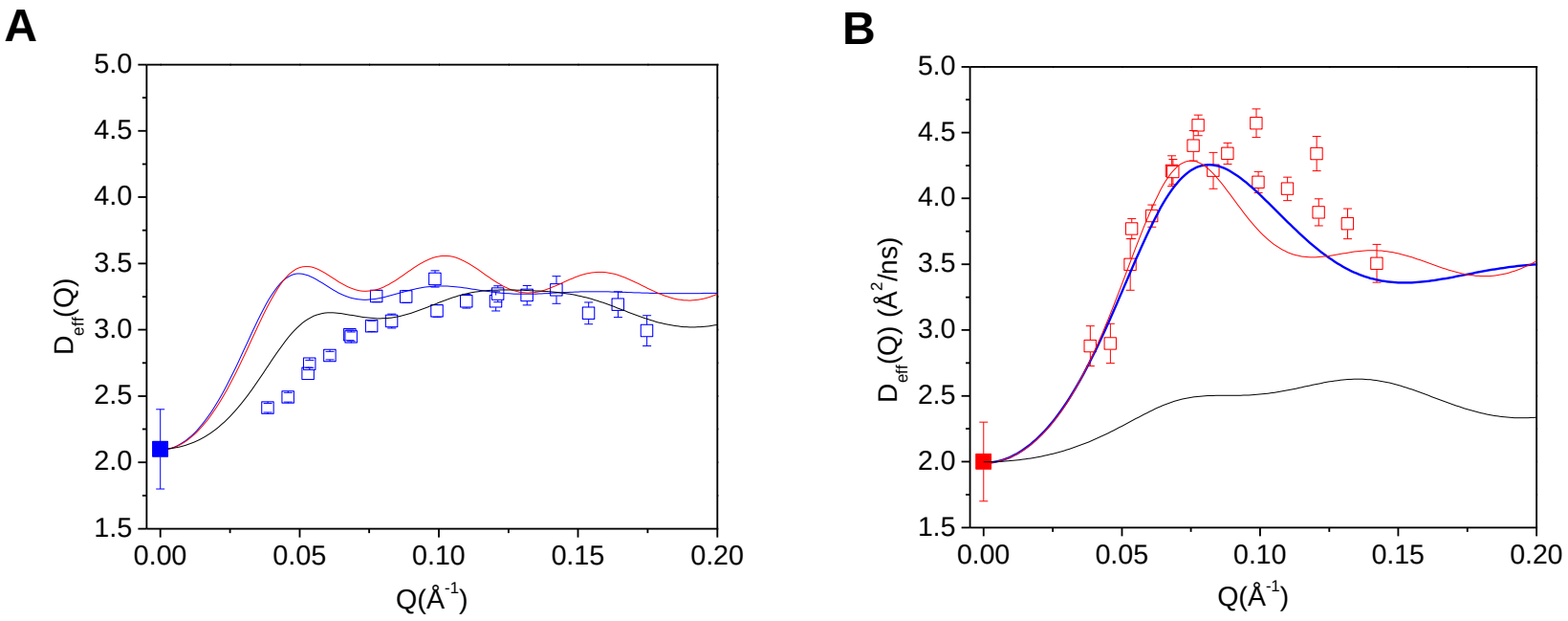

A
B
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0.00
0.05
0.10
0.15
0.20
D_eff(Q)
D_eff(Q) (Å²/ns)
Q(Å⁻¹)

Figure S3