SUPPORTING INFORMATION

Translational diffusion of macromolecular assemblies measured using transverse relaxation-optimized PFG-NMR

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Materials

Sample Preparation

The preparation of uniformly [^{15}N,^{2}H]-labeled *E. coli* OmpX reconstituted in Fos-10 mixed micelles has been described previously[1]. The NMR measurements were performed at a protein concentration of 1.2 mM and a Fos-10 concentration of 150 mM in a mixed solvent of 95% H_2O/5% D_2O containing 5 mM sodium phosphate at pH 6.8, 10 mM NaCl and 0.3 % NaN_3. Uniformly [^{15}N,^{2}H]-labeled *Tth* GroEL was prepared by adapting the protocol for production of the unlabeled protein[2,3] along the lines of previous preparations of isotope-labeled *E. coli* GroEL.[4,5] The protein concentration used for the NMR measurements was 0.1 mM (1.4 mM per subunit) in 95% H_2O/5% D_2O containing 100 mM phosphate buffer at pH 6.8, 20 mM KCl and 0.3% NaN_3. As an internal reference for the ^{1}H chemical shifts and for the diffusion constants, 5 µl of a 100 mM 2,2-dimethyl-2-2silapentane-3,3,4,4,5,5-d_6-sulfonate sodium salt (DSS) stock solution was added to the NMR samples.

Methods

Model simulations of the sensitivity of different PFG-NMR schemes

The figure S1 presents simulations for a diffusion delay of Δ = 300 ms (Fig. 1) for protonated and uniformly 80% deuterated α-helices and antiparallel β-sheets. The figure S1A shows that for all these four structures the X-STE experiments has higher sensitivity than ^{15}N-TRO-STE for molecular sizes with rotational correlation times shorter than about 40 ns. Figure 2B shows that the ^{1}H-TRO-STE scheme yields higher sensitivity than ^{15}N-TRO-STE for effective rotational correlation times in the range up to about 10 ns. For larger particles, however, ^{15}N-TRO-STE provides the best sensitivity, specifically for particles with τ_c â 40 ns when compared to X-STE, and for particles with τ_c â 10 ns when compared to ^{1}H-TRO-STE.

NMR Spectroscopy

NMR spectra were acquired on Bruker DRX-700 and AVANCE-800 spectrometers equipped with 5 mm TXI probeheads (Bruker, Billerica, MA), using about 400 µl of protein solution in magnetic susceptibility-matched NMR tubes ('Shigemi tubes'; Shigemi Inc., Allison Park, PA). All experiments were processed and analyzed using the Topspin 1.3 software (Bruker).

PFG-STE measurements of at elevated temperatures

For the translational diffusion measurements with *Tth* GroEL at elevated temperatures, special care was taken to monitor possible convection artifacts.[6] To this end, the plunger position in the Shigemi tube was set at 22 mm from the bottom of the tube, which had empirically been found to minimize convection. The pulsed field gradient strengths in this set-up were calibrated with the residual ^{1}H signal of 99.9% D_2O, using a self-diffusion coefficient of $(1.902 \pm 0.002) \times 10^{-9}$ m^2 s^{-1} for HDO at 25 °C. [7] The translational diffusion constant, D_t, for HDO was then measured at variable temperatures between 25 and 60 °C with Δ = 100 ms (Figure S2A), and at variable Δ values between 100 and 200 ms at 60 °C (Fig S2B) using a PFG-STE-LED experiment[8] (LED: longitudinal encode–decode). A least squares fit of the resulting data shown in Fig. S2A to an Arrhenius relation, $D_t = D_0 e^{-E_a/RT}$, with fit parameters of E_a = 4.10 ± 0.02 kcal/Mol (literature value: 4.5 kcal/Mol over the temperature range 15–45 °C[7]) and D_0 = 1.98 ± 0.01 × 10^{-6}

m^2/s for the activation energy and the diffusion coefficient at infinite temperature, respectively, showed that our experimental set-up yielded D_t values that are not measurably affected by convection. This result is supported by the observation that D_t is independent of Δ if shigemi tubes are used, whereas the measured D_t values are increasing for large Δ values when using conventional tubes, which is indicative for convection artifacts in a homogeneous sample[9,10].

NMR characterization of OmpX/Fos-10 mixed micelles

The following acquisition and processing parameters were used for the 1D ^{1}H-TRO-STE, ^{15}N-TRO-STE and X-STE experiments: t_{max} = 183 ms, ^{1}H sweep width = 16 ppm, 128 transients for each of 16 gradient increments. Before Fourier transformation the data was zero-filled to 2048 complex data points and multiplied with an empirically optimized exponential window function. The X-STE experiment was recorded using the pulse scheme of Sarkar et al..[11]

The effective rotational correlation time of OmpX/Fos-10 micelles, τ_c, was determined from measurements of the signal intensity in the spectral range between 10.0 and 7.0 ppm using the TRACT experiment.[12] A total of 128 relaxation delays ranging from 1 to 128 ms were used, with 128 scans per relaxation delay.

NMR experiments with Tth GroEL

2D [^{15}N,^{1}H]-TROSY and 2D [^{15}N,^{1}H]-CRIPT-TROSY experiments were recorded as described previously.[4,13] In all experiments the acquired data size was 256 × 1024 complex points, t_{1max} = 40.0 ms, t_{2max} = 90.0 ms. 16 and 256 transients per increment were recorded for the 2D [^{15}N,^{1}H]-TROSY and 2D [^{15}N,^{1}H]-CRIPT-TROSY experiments, respectively, which provided the signal-to-noise ratio needed for studies of the temperature dependence of the two spectra. Before Fourier transformation, the data sets were multiplied along the t_1- dimension with a sine bell shifted by 30° [14] and in the t_2 -dimension with an empirically optimized exponential function. The 1D ^{15}N-TRO-STE experiment was acquired with t_{max} = 46 ms, ^{1}H sweep width = 16 ppm, and 1024 transients for each of 10 gradient increments.

Interpretation of the translational diffusion coefficient for Tth GroEL

In the Stokes–Einstein model, the dependence of the translational diffusion constant, D_t, on the solution viscosity, η, the temperature, T, and the hydrodynamic radius, R_h, for a spherical, noninteracting particle is given by

$$D_t = k_B T / (6\pi\eta R_h), \qquad \text{(Eq. S1)}$$

where k_B is the Boltzmann constant. To eliminate the dependence of D_t for GroEL, $D_{t,EL}$, on η and T, we determined the "relative diffusivity", d_{EL},[15,16] for *Tth* GroEL as

$$d_{EL} = D_{t,DSS} / D_{t,EL}, \qquad \text{(Eq. S2)}$$

where $D_{t,DSS}$ is the translational diffusion constant for the reference compound DSS. If $D_{t,EL,}$ and $D_{t,DSS}$ are measured under the same conditions, d_{EL} depends only on the hydrodynamic radii of *Tth* GroEL and DSS, and is independent of η and T.

References

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Table S1. Translational diffusion coefficients for *Tth* GroEL and DSS measured at 25 and 60 ºC.

Temp, °C	$D_{t,EL}$[a] [m^2/s]	$D_{t,DSS}$[b] [m^2/s]	d_{EL}[c]
25.0	$(3.8 \pm 0.2) \times 10^{-11}$	$(48.5 \pm 0.4) \times 10^{-11}$	12.8 ± 0.8
60.0	$(10.1 \pm 0.5) \times 10^{-11}$	$(119.5 \pm 0.9) \times 10^{-11}$	11.8 ± 0.7

[a]The translational diffusion coefficient of *Tth* GroEL, $D_{t,EL}$, was measured using the ^{15}N-TRO-STE experiment (Fig. 1B). The signal intensity over the ^{1}H chemical shift range 8.7 to 9.6 ppm was used for the analysis.
[b]The translational diffusion coefficients of DSS, $D_{t,DSS}$, were measured using the PFG-STE-LED experiment[8], with δ = 1.5 ms and Δ = 100 ms.
[c]The relative diffusivity for *Tth* GroEL, d_{EL}, was calculated using Eq. S2.

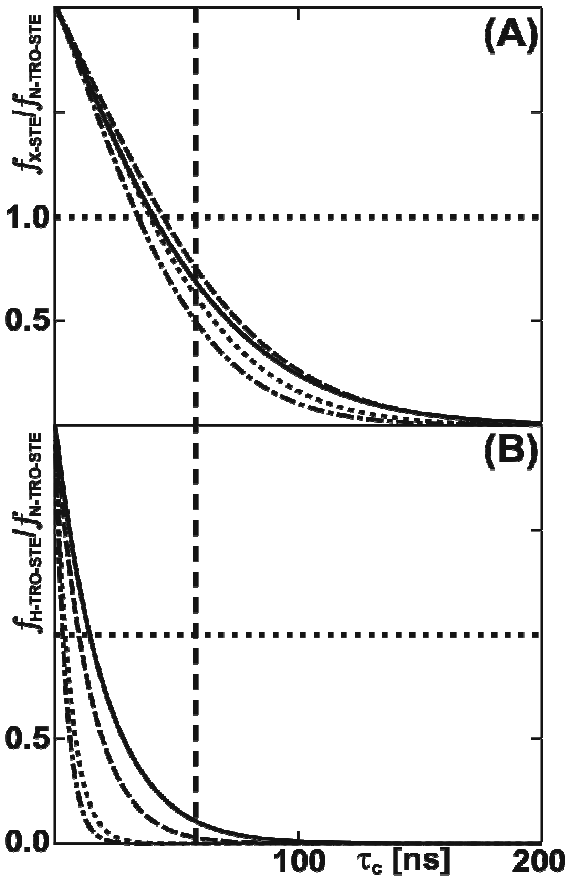

Figure S1. Simulations of sensitivity ratios between different experiments used here for measurements of translational self-diffusion rates. (A) $f_{\text{X-STE}} / f_{\text{N-TRO-STE}}$. (B) $f_{\text{H-TRO-STE}} / f_{\text{N-TRO-STE}}$. The signal attenuation factors $f_{\text{H-TRO-STE}}$ and $f_{\text{N-TRO-STE}}$ were determined using Eqs. (2) and (3), respectively, and the calculation of $f_{\text{X-STE}}$ was based on Eq. (2) in Ferrage et al.[17]. The relaxation rates Γ_{H^+,H^+}, Γ_{N^+,N^+}, $\Gamma^{DD/CSA}_{H^+,H^+N_z}$, $\Gamma^{DD/CSA}_{N^+,H_zN^+}$, Γ_{N_z,N_z} and $\Gamma_{H_zN_z,H_zN_z}$ were evaluated as described previously.[18,19] Data were computed for a ^{1}H frequency of 700 MHz, a diffusion delay Δ of 300 ms (Fig. 1), and for effective rotational correlation times, τ_c, in the range from 0 to 200 ns. For the ^{15}N–^{1}H moiety of residue *i* in different regular protein secondary structures, the following remote protons were considered. α-helix: $^1H^N(i-1)$, $^1H^N(i+1)$, $^1H^N(i-2)$, $^1H^N(i+2)$, $^1H^\alpha(i)$, $^1H^\alpha(i-1)$, $^1H^\alpha(i-2)$, $^1H^\alpha(i-3)$, $^1H^\alpha(i-4)$, $^1H^\beta(i)$ at distances of 2.8, 2.8, 4.2, 4.2, 2.6, 3.5, 4.4, 3.4, 4.2 and 2.5 Å, respectively.[20] Anti-parallel β-sheet: $^1H^N(i-1)$, $^1H^N(i+1)$, $^1H^N(j)$, $^1H^\alpha(i)$, $^1H^\alpha(i-1)$, $^1H^\alpha(j)$, $^1H^\beta(i)$ and $^1H^\beta(i-1)$ at distances of 4.3, 4.3, 3.3, 2.8, 2.2, 3.2, 2.5 and 3.2 Å, respectively, where *j* indicates a long-range contact across the β-sheet. In uniformly 80% ^{2}H-labeled proteins, the $^1H^N$ positions were assumed to be protonated in the extent of 100%, and the H^α and H^β positions were considered to be statistically protonated in the extent of 20%. Sensitivity ratios for ^{15}N–^{1}H moieties in the following environments are shown: 80% deuterated antiparallel β-sheet (solid line), 80% deuterated α-helix (dotted line), protonated antiparallel β-sheet (broken-dotted line) and protonated α-helix (dotted line). The vertical dashed line indicates the experimental τ_c value of 52 ns measured for OmpX/Fos-10 mixed micelles at 4 °C (see also Fig. 2). The horizontal dotted lines are at the value of 1.0 for the sensitivity ratios indicated on the left along the vertical axes.

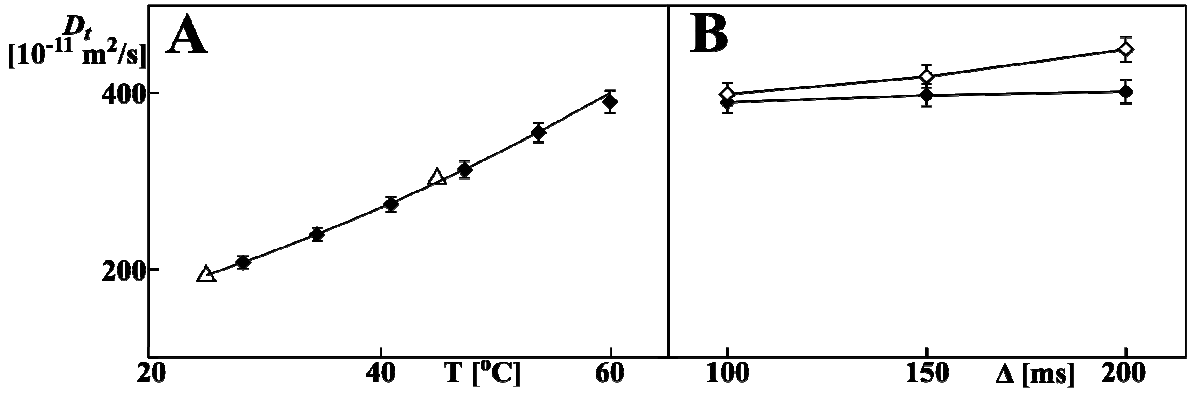

Figure S2: PFG-STE experiments with HDO in 99.9% D_2O. A. Temperature dependence of the self-diffusion of HDO in the temperature range 25–60 °C, measured with a shigemi tube with a plunger position of 22 mm from the bottom of the tube. Filled diamonds and open triangles indicate data points measured using a PFG-STE-LED experiment[8] with δ = 1.5 ms and Δ = 100 ms, and literature values[7], respectively. The line indicates a least square fit using the Arrhenius relationship, $D_t = D_0 e^{-E_a/RT}$. The fit parameters were E_a = 4.10 ± 0.02 Kcal/mol and D_0 = 1.98 ± 0.01 × 10^{-6} m^2/s for the activation energy and the diffusion coefficient at infinite temperature, respectively. B. D_t versus Δ for samples of HDO at 60 °C in a conventional sample tube and in a shigemi tube are plotted as open and filled diamonds, respectively. For measurements of diffusion coefficients, the amplitude of the gradients was incremented linearly in 16 steps. The error bars represent the deviation from the fitted Stejskal–Tanner expression[21].

Listing S1. Bruker AVANCE pulse program for the H-TRO-STE experiment

```
;H-TRO-STE

;Use the Bruker standard macro <dosy> to set-up and start the experiment.

;pl1   : power for 1H
;pl3   : power for 15N
;sp1   : 1H flip up (ph12)
;sp2   : 1H flip down (ph11,ph13)

;spnam1: gauss128_5
;spnam2: gauss128_5

;p1    : 90 degree hard pulse 1H
;p5    : 90 degree hard pulse 15N
;p11   : water flipback pulse [1.1m]

;p21   : spoil gradient pulse [0.5 ms]
;p30   : diffusion gradient pulse (little DELTA)

;d1    : relaxation delay
;d2    : half [H,HNz]-CRINEPT transfer

;d16   : gradient recovery [0.4 ms]
;d20   : diffusion time (big DELTA)

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

#define GRAD1 p21:gp1 d16

#define GRAD_D p30:gp6*diff d16

"DELTA=d20-d2*2-p1*2-p11*2-p21-d16-10u"
"TAU=d2*2-p30-d16-4u"

define list<gradient> diff=<Difframp>

1 10u ze
2 30m
  10u do:f3
  d1
  50u UNBLKGRAD
  10u pl1:f1
  10u pl3:f3
;---------------------------------------[H,HNz]-CRINEPT
  (p11:sp2 ph11:r):f1
```

```
  4u
  6u pl1:f1
  (p1 ph20):f1
  GRAD_D
  TAU 4u
  (p1 ph20 10u p11:sp1 ph12:r):f1 (p5 ph1 2u p5 ph20):f3
;----------------------------------------diffusion delay
  GRAD1
  DELTA
 (p11:sp2 ph13:r):f1
  4u
  6u pl1:f1
  (p1 ph2):f1
;----------------------------------------[HNz,H]-CRINEPT
  GRAD_D
  TAU
  4u BLKGRAD
;----------------------------------------acquisition
  go=2 ph31
  30m mc #0 to 2 F1QF(igrad diff)
exit

ph1 = 0 2 0 2
ph2 = 0 0 1 1

ph11= 2
ph12= 2
ph13= 2 2 3 3

ph31= 0 2 2 0

ph20=0
ph21=1
ph22=2
ph23=3
```

Listing S2. Bruker AVANCE pulse program for the N-TRO-STE experiment

```
;N-TRO-STE

;Use the Bruker standard macro <dosy> to set up and start the experiment.

;pl1   : power for 1H
;pl3   : power for 15N
;sp1   : 1H flip up (ph12)
;sp2   : 1H flip down (ph11, ph13)

;spnam1: gauss128_5
;spnam2: gauss128_5

;p1    : 90 degree hard pulse 1H
;p5    : 90 degree hard pulse 15N
;p11   : water flipback pulse [1.1ms]

;p20   : gradient pulse [1ms]
;p21   : z-Filter gradient pulse [0.5 ms]
;p22   : gradient pulse [0.5 ms]
;p23   : spoil gradient pulse [0.5 ms]
;p24   : z-Filter gradient pulse [0.5 ms]
;p30   : diffusion gradient pulse (little DELTA)

;d1    : relaxation delay
;d2    : half [H,HNz]-CRINEPT transfer
;d3    : half [HzN,N]-CRINEPT transfer

;d16   : gradient recovery [0.4 ms]
;d20   : diffusion time (big DELTA)

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

#define GRAD0  p20:gp0 d16
#define GRAD1  p21:gp1 d16
#define GRAD2  p22:gp2 d16
#define GRAD3  p23:gp3 d16
#define GRAD4  p24:gp4 d16

#define GRAD_D p30:gp6*diff d16

"DELTA=d20-d2*2-d3*4-p1*2-p5*4-p11*2-p21*2-p23-d16*3-20u"
"TAU1=d2*2-p30-d16-4u"
"TAU2=d3*2-p22-d16"

define list<gradient> diff=<Difframp>
```

```
1 10u ze
2 30m
  10u do:f3
  d1
  50u UNBLKGRAD
  10u pl1:f1
  10u pl3:f3
  (p5 ph20):f3
  GRAD0
;----------------------------------------[H,HNz]-CRINEPT
  (p11:sp2 ph11:r):f1
  4u
  6u pl1:f1
  (p1 ph20):f1
  GRAD_D
  TAU1 4u
;----------------------------------------z-filter
  (p1 ph20):f1
  10u
  (p11:sp2 ph12:r):f1
  GRAD1
;----------------------------------------[HzN,N]-CRINEPT
  (p5 ph21):f3
  TAU2
  GRAD2
  (p5 ph1):f3
;----------------------------------------diffusion delay
  GRAD3
  DELTA
  (p5 ph2):f3
;----------------------------------------[N,HzN]-CRINEPT
  GRAD2
  TAU2
  (p5 ph21):f3
  GRAD4
  (p11:sp1 ph13:r):f1
  4u
  6u pl1:f1
  (p1 ph22):f1
;----------------------------------------[HNz,H]-CRINEPT
  GRAD_D
  TAU1
  4u BLKGRAD
;----------------------------------------acquisition
  go=2 ph31
  30m mc #0 to 2 F1QF(igrad diff)
exit
```

ph1 = 1 3 1 3
ph2 = 1 1 3 3

ph11=2
ph12=2
ph13=0

ph31=0 2 2 0

ph20=0
ph21=1
ph22=2
ph23=3