Direct Investigation of Slow Correlated Dynamics in Proteins via Dipolar Interactions

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NMR spectroscopy

$\Gamma_{HNiNi/HNi+1Ni+1}$ + $\Gamma_{HNiNi+1/HNi+1Ni}$ measurements. 'reference' and 'trans' spectra of 3D ct-^{13}C'-HN(CA)CON [ct-$^{13}C^{\alpha}$-HNCA(CO)N] experiments (derived from a 2D version presented in reference 1 [1] were recorded with 36(N, t_1) x 40(C', t_2)[20(C^{α}, t_2)] x 512(H^N, t_3) complex points, t_{1max} = 18.0 ms, t_{2max} = 26.4[6.6] ms, t_{3max} = 51.2 ms, an interscan delay of 1.0 s, τ_{MQ} = 43 ms, and typically 16[32] scans per increment resulting in a measurement time of 1[2][2] day. Because the 'trans' spectra are considerably less sensitive they were typically recorded twice and added up thereby effectively doubling the number of scans. The time domain data were multiplied with a square cosine function in the direct dimension and cosine functions in the indirect dimensions and zero-filled to 256 x 128 x 2048 complex points. Spectra with ^{13}C' and with $^{13}C^{\alpha}$ evolution were recorded twice and three times, respectively, such that averaged CCR rates with associated random errors could be obtained.

$\Gamma_{d(HAiCAi)/d(HAi-1CAi-1)}$ + $\Gamma_{d(HAiCAi-1)/d(HAi-1CAi)}$ measurements. 'reference' and 'trans' spectra of the 2D HNCA(CA) experiment put forward in Charpin et al. [3] and a 3D $^{13}C^{\alpha}$-HNCA(CO)CA experiment were recorded with 64(N, t_1) x 512(H^N, t_2) and 36(N, t_1) x 24(C^{α}, t_2) x 512(H^N, t_3) complex points, t_{1max} = 32.0 ms, t_{2max} = 51.2 ms and t_{1max} = 18.0 ms, t_{2max} = 7.92 ms, t_{3max} = 51.2 ms, an interscan delay of 1.0 s, τ_{MQ} = 28 ms, and typically 256 and 32 per increment resulting in a measurement time of 0.5 and 2 days, respectively. Because the 'trans' spectra are considerably less sensitive than the 'reference' spectra they were typically recorded twice and added up thereby effectively doubling the number of scans. The time domain data were multiplied with a square cosine function in the direct dimension and cosine functions in the indirect dimensions and zero-filled to 1024 x 4096 and 256 x 128 x 2048 complex points. All spectra were recorded twice so that average CCR rates and associated random errors could be obtained.

$\Gamma_{HNiNi/HAiCAi} + \Gamma_{HAiNi/HNiCAi}$ measurements. The two experiments used previously to determine the intraresidual $\Gamma_{d(HN)/d(H\alpha C\alpha)} + \Gamma_{d(H\alpha N)/d(HC\alpha)}$ CCR rate were repeated.[4] The first approach relies on the ACE method realized in a 3D ct-HNCA experiment.[4]

In the second approach, the DIAI method is realized with a pair of 3D HNCA pulse sequences ('reference' and 'trans') was used as presented in Pelupessy et al. in 2 days.[5]

In addition, a 3D ct-HNCA MMQ experiment was used. As proposed in Yang and Kay,[6] the ZQ and DQ coherences are superimposed resulting in 4 components to be evaluated. The spectrum was recorded twice with τ_{MQ} = 31.0 ms or τ_{MQ} = 33.5 ms, 50(MQ[N,C^{α}], t_1) or 55(MQ[N,C^{α}], t_1) x 36(N, t_2) x 512(H^N, t_3) complex points, t_{1max} = 25.0 ms or t_{1max} = 27.5 ms, t_{2max} = 18.0 ms, t_{3max} = 63.28 ms, an interscan delay of 1 s or 0.92 s and 16 or 24 scans per increment resulting in a measurement time of 1.5 or 2 days. The time domain data were multiplied with a square cosine function in the direct dimension and cosine functions in the indirect dimensions and zero-filled to 256 x 128 x 2048 complex points.

$\Gamma_{HNiNi/HAi-1CAi-1} + \Gamma_{HAi-1Ni/HNi-1CAi-1}$ measurements. Three experiments used previously were repeated .[4] The first approach relies on ACE realized in a 3D ct-HN(CO)CA experiment. In the second and third approach, the DIAI method is realized with two types of 2D HN(CO)CA experiments.[7,8] In the third approach, the standard formula to extract the CCR rate is modified to account for the three following effects: First, the temporary interconversion of the ZQ and DQ coherences reverses the relevant CCR. Thus, the effective time during which the relevant CCR occurs is actually $\tau_{MQ,effective} = \tau_{MQ} - 1/(2J_{HN}) + 1/(2J_{HC})$. Second, for the same reason, the ratio of the intensities is modified by a term proportional to $(1/(2J_{HN}) - 1/(2J_{HC}))$ and the CCR rate itself , see equation 2b in Pelupessyet al.[8] Here, the CCR rates obtained from the DIAI 2D experiment mentioned above are used as an estimate for this correction term. Third, the MQ[$^{15}N_i$,$^{13}C^{\alpha}_{i-1}$] coherences scalar couple to $^{15}N_{i-1}$ and $^{13}C^{\alpha}_i$ via $J_{Ni-1C\alpha i-1}$ and $J_{NiC\alpha i}$. Because the inversion pulses

on the MQ coherences are non-uniformly shifted in the 'trans' experiment, the intensities of the 'ref' and 'trans' spectra are affected in different ways. Here, it is assumed that both couplings have uniform values of 9 Hz. This results in rescaling of the 'ref' and 'trans' intensities of 1/0.49 and 1/0.55, respectively.

All spectra were processed and analyzed using the software packages NMRPipe.[9] Peak heights were determined by parabolic interpolation. Peak positions were determined in the reference spectrum with typically much higher peak intensity and fitted at the according position in the transfer spectra.

Corrections from full relaxation-matrix analysis. Corrections to the apparent CCR rates were calculated as outlined in Vogeli and Yao.[4] The most important coefficients in the relaxation matrix were looked up or estimated individually for each CCR rate. For the H^N-N/H^N-N CCR rates, the dipolar cross-relaxation rates W_0 were taken from Vogeli et al. if available and calculated otherwise.[10] For the H^α-C^α/H^α-C^α CCR rates, all cases yield errors in the per-mil range and no detailed analysis was carried out.[11] For the intraresidual and sequential H^N-N/H^α-C^α CCR rates, W_0 rates and proton autorelaxation rates were taken from Vogeli et al. if available.[10] Otherwise, the W_0 rates were estimated from the distances and averages were used for the autorelaxation rates.

Experimental errors of the CCR rates. The standard deviations of the averages are 0.19, 1.37, 0.20 and 0.26 s^{-1} for the H^N-N/H^N-N, H^α-C^α/H^α-C^α, intraresidual H^N-N/H^α-C^α and sequential H^N-N/H^α-C^α CCR rates, respectively. Considering ranges of measured values of 6.73, 43,9, 12.4 and 12.7 s^{-1}, the errors correspond to 2.8, 3.1, 1.6 and 2.1 %.

Prediction of CCR rates in model-free analysis and structure validation. The anisotropic diffusion tensor published in Hall and Fushman was used after uniform rescaling to match the effective overall tumbling times of 3.41 ns and 4.03 ns of the deuterated and protonated samples, respectively.[12] The errors are on the order of 1 %. For the prediction of the CCR rates expected for uncorrelated motion of the two vectors the RDC order parameters published in references Yao et al. and Sabo et al. [13,14] The CCR rates predicted for the dynamic model are shown in Tables S5 to S8.

Errors of the correlation factor F_{corr}. The errors of F_{corr} are propagated errors based on the experimental errors of the CCR rates and of the RDC order parameters S^{RDC}. If one of the errors of S^{RDC} is not available a typical error is assumed. If one of the measuring methods does not yield a value for a CCR rate the errors of the remaining methods are used. If one of the S^{RDC} values is not available the average of the preceding and succeeding residues is used (or the average over the whole sequence in the case of H^{α}–C^{α}/H^{α}–C^{α}). If at least one of these modifications is applied it is indicated in the SI tables. In principle, there is an additional error contribution from the uncertainty in the predicted CCR rate. A reasonable error for the H^{N}-N and H^{α}–C^{α} vector orientations is on the order of 2°. Numerical simulations suggest that this additional error is small in most cases and that the conclusions will be the same.

Ensemble Structure Calculations. The ensemble restrained simulation protocol was adapted from that used by Clore and Schwieters, with a few changes.[15] The simulations the Amber 99SB force field as implemented by Case et al. and the final structures were minimized including energy terms for generalized Born implicit solvent as implemented in NIH-Xplor.[16] The backbone and sidechain ^{3}J couplings were removed as restraints, as were the B-factor and order

parameter restraints. A relative atomic position (RAP) restraint with a 0.5 Å threshold was applied to the backbone heavy atoms of residues in regular secondary structure elements to ensure the single tensor approxiamation by maintaining the alignment in the laboratory frame.[17] The rest of the heavy atoms were kept in approximate position using a second loose RAP restraint with a 2.5 Å threshold. The simulations were restrained using flat bottomed potentials with six sets of H^N–N and H^α–C^α RDCs reported by Yao et al.[13] and by the four sets of isotropic CCRs described above. The experimental error for the H^N–N RDCs was 0.1 Hz and for H^α–C^α RDCs 0.2 Hz, experimental errors used to restraint the CCRs are given in tables S1-S4. Ensembles of size 1, 2, 4, 8 and 16 replicas were generated using NIH-XPLOR modified to contain an ensemble averaged isotropic CCR restraint. Control simulations were run with the w_{CCR} set to zero. The dihedral correlations were calculated as previously described.

In principle, the CCR rate correction for anisotropic tumbling works best for overall tumbling that is not highly anisotropic and for smaller fluctuations. On the other hand, if the fluctuations are large, many directions are sampled and the relative impact of overall anisotropic tumbling on the CCR rates will become small and the impact of correlated motion on the CCR rates cannot be masked by anisotropic tumbling. In our case, the corrections are relatively small. The largest corrections are ca. 5%, but the majority are much smaller. Even in the extreme case of 5%, the introduced error of F_{corr} would be ca. 2-3%.

Quantification of correlated motion in the work of Clore and Schwieters.[17] The correlation between two bond vector orientations was quantified by using the correlation function C_{kl} between residues *k* and *l* as introduced by Clore and Schwieters.[17] It is essentially a measure of how closely the bond vectors of members of each ensemble line up with those from all other calculated ensembles. C_{kl} was calculated as follows: v_{ikm} is the unit vector along a bond vector of

residue k of member m (0 or 1) in the ensemble i. It is assumed that each vector can be binned into one of two orientations. For two residues k and l and two ensembles i and j, the ensembles are said to coincide if $f(i,j; k,l) = 1$, which is the case for either $v_{ik0} \cdot v_{jk0} > v_{ik0} \cdot v_{jk1}$ and $v_{il0} \cdot v_{jl0} > v_{il0} \cdot v_{jl1}$, or for $v_{ik0} \cdot v_{jk0} < v_{ik0} \cdot v_{jk1}$ and $v_{il0} \cdot v_{jl0} < v_{il0} \cdot v_{jl1}$. Otherwise, $f(i,j; k,l)$ is set to 0. Averaging over all ensembles, the correlation, C_{kl}, between residues k and l is defined as $C_{kl} = -1 + 2/N^2 \Sigma_{ij} f(i, j; k, l)$, where i and j are summed over all ensembles and N is the number of these ensembles. C_{kl} scales between 0 and 1, which indicate no correlation and perfect correlation, respectively.

Calculation of sheet bending and twisting. For each member of each ensemble, the backbone atoms excluding C^{α} were fitted to the catenoid shape using the generalized cylinder fitting method using two parameters,[18] the bending parameter and the inclined angle of β-strands that describe the shape of the β-sheet.[19]

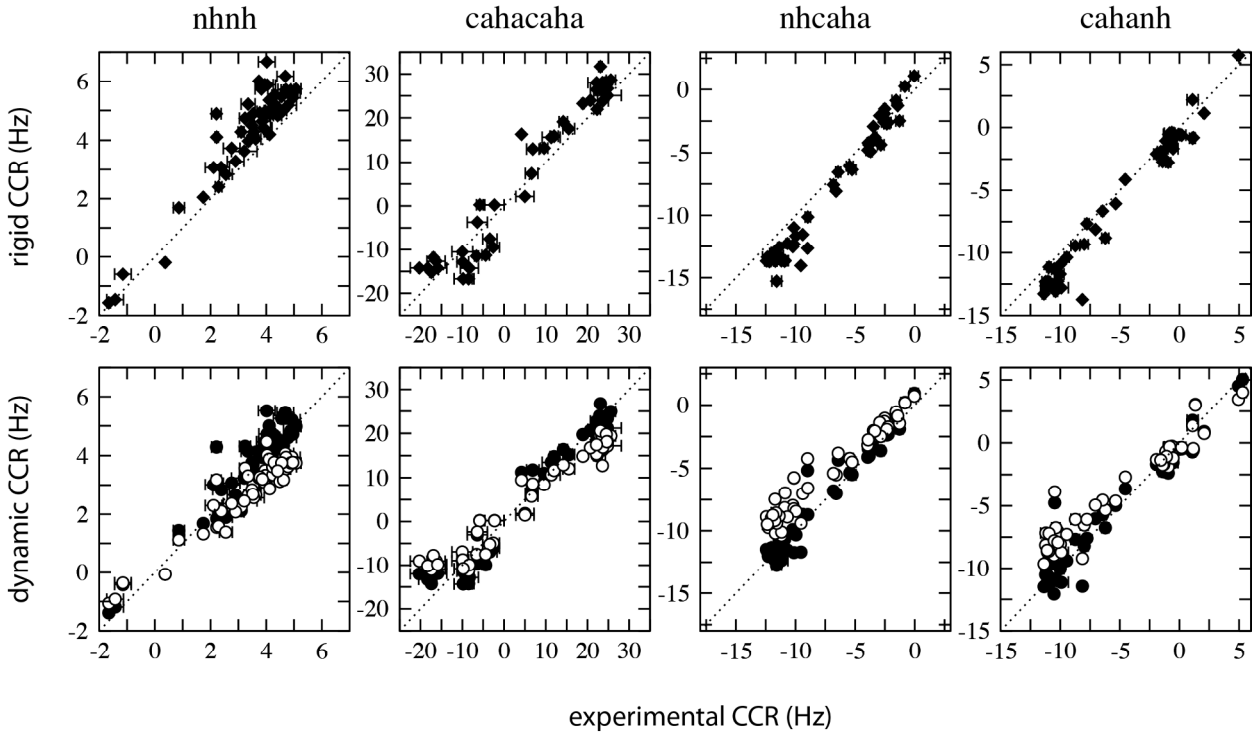

Figure S1. Correlation of the calculated and experimental CCR rates. In the top panel, a rigid molecule was assumed, while a dynamic model using the experimental RDC order parameters was applied in the bottom panel. The RDC order parameters were extracted using either the iterative DIDC (filled circles) or ORIUM (empty circles) approaches. Experimental errors on the CCR rates are indicated with error bars.

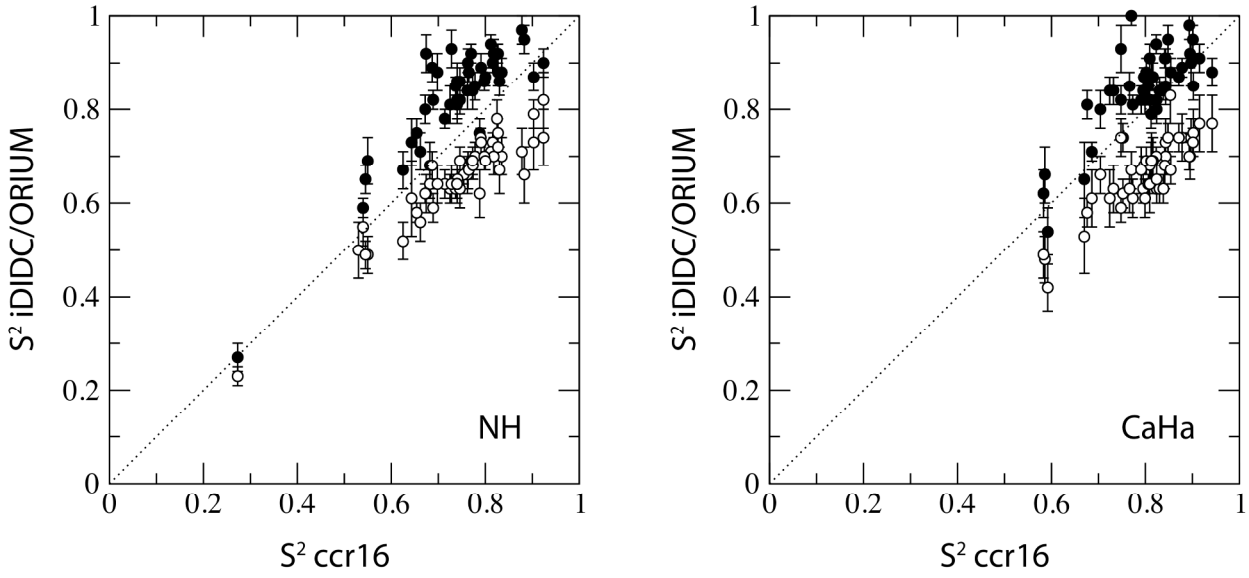

Figure S2. Correlation of the H^N-N and H^α–C^α order parameters of the calculated ensemble with the ORIUM (open circles) and iDIDC (filled circles) order parameters for a representative ensemble of Ccr16. Note that some values are close to 1 (perfect rigidity), but given the error they are compatible with physically meaningful values.

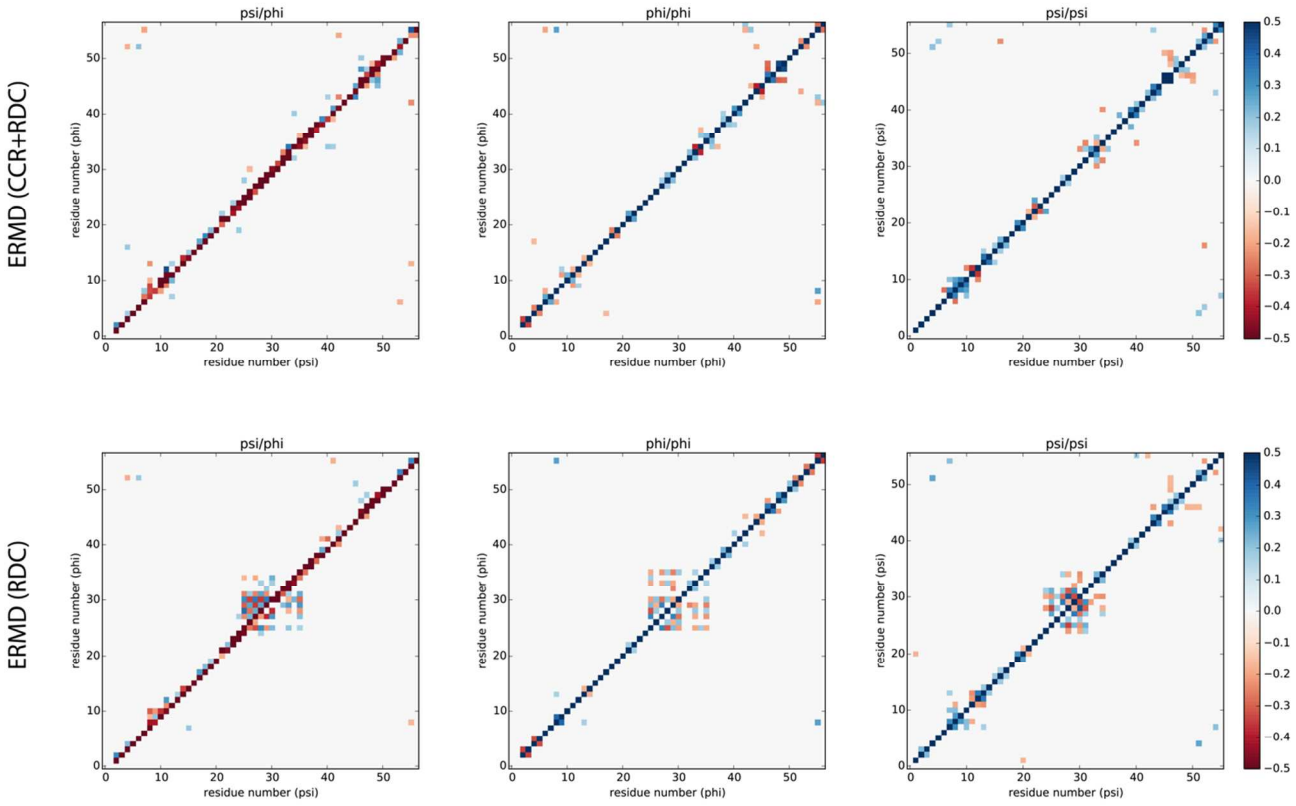

Figure S3. Dihedral correlation plots for ϕ and ψ, calculated for 800 structures from 50 ensembles of 16 members.

Table S1. Experimental $\Gamma = \Gamma_{HNiNi/HNi+1Ni+1} + \Gamma_{HNiNi+1/HNi+1Ni}$ CCR rates obtained from GB3 at T = 298 K and $\tau_{c,eff}$ = 3.41 ns.

res # [a]	aa type	Γ (C'+ C·), corrected [s^{-1}] [bc]	ΔΓ(C'+ C$^{\alpha}$) [s^{-1}] [d]	ΔΓ(C'+ C$^{\alpha}$) [s^{-1}] [e]	correction to Γ [s^{-1}] [c]	isotropic Γ	error for isotropic Γ
3	TYR	3.24	0.20	0.05	-0.02	3.35	0.20
4	LYS+	3.53	0.02	0.18	-0.02	3.56	0.02
5	LEU	3.97	0.14	0.10	-0.03	4.28	0.14
6	VAL	3.91			-0.02	4.22	0.30
7	ILE	3.36	0.06	0.11	-0.02	3.77	0.06
8	ASN	0.86	0.20	0.17	-0.05	1.29	0.20
9	GLY	-1.15			-0.01	-1.61	0.30
10	LYS+	3.10	0.11	0.12	-0.16	3.33	0.11
11	THR	0.37	0.04	0.06	-0.38	0.53	0.04
12	LEU	2.22	0.06	0.04	-0.01	2.47	0.06
13	LYS+	3.83	0.23	0.10	-0.03	4.09	0.23
14	GLY	3.74	0.01	0.20	-0.04	4.02	0.01
15	GLU	3.95			-0.03	4.17	0.30
16	THR	3.35	0.25	0.16	-0.03	3.52	0.25
17	THR	3.97			-0.02	4.10	0.30
18	THR	4.04	0.30	0.18	-0.03	3.96	0.30
19	LYS+	3.62	0.19	0.05	-0.01	4.11	0.19
20	ALA	4.71	0.17	0.13	-0.03	5.23	0.17
21	VAL	1.74	0.03	0.19	-0.13	2.04	0.03
22	ASP	4.69			-0.04	4.59	0.30
23	ALA	3.49	0.08	0.05	-0.13	3.27	0.08
24	GLU	4.24			-0.22	3.75	0.30
25	THR	4.23			-0.26	4.07	0.30
26	ALA	4.92			-0.25	4.67	0.30
27	GLU	4.79			-0.16	4.35	0.30
28	LYS+	4.37			-0.22	3.94	0.30
29	ALA	4.71	0.29	0.12	-0.22	4.39	0.29
30	PHE	4.49	0.03	0.13	-0.20	4.04	0.03
31	LYS+	4.62	0.12	0.05	-0.19	3.96	0.12
32	GLN	4.88	0.26	0.12	-0.20	4.39	0.26
33	TYR	5.07	0.18	0.12	-0.19	4.61	0.18
34	ALA	4.96	0.03	0.06	-0.14	4.42	0.03
35	ASN						
36	ASP	2.90			-0.18	2.66	0.30

37	ASN	-1.66	0.06	0.10	-0.23	-1.78	0.06
38	GLY	2.38			-0.18	2.02	0.30
39	VAL	2.76			-0.01	2.30	0.30
40	ASP	4.02			-0.02	3.46	0.30
41	GLY	2.21	0.17		-0.04	2.00	0.17
42	VAL	2.29	0.12	0.25	-0.01	2.66	0.12
43	TRP	2.11			-0.02	2.05	0.30
44	THR	4.11	0.00	0.05	-0.03	4.40	0.01
45	TYR	4.29	0.09	0.13	-0.02	4.49	0.09
46	ASP	3.55	0.38	0.07	-0.03	3.79	0.38
47	ASP	2.55	0.23	0.06	-0.14	2.81	0.23
48	ALA	3.52	0.03	0.10	-0.19	3.34	0.03
49	THR	-1.43			-0.30	-1.28	0.30
50	LYS+	3.77	0.22	0.05	-0.20	3.88	0.22
51	THR	3.86			-0.02	3.95	0.30
52	PHE	3.21	0.46	0.31	-0.01	3.42	0.46
53	THR	4.56	0.30	0.25	-0.02	4.87	0.30
54	VAL	4.41	0.15	0.06	-0.03	4.76	0.15
55	THR	3.24	0.20	0.05	-0.02	3.35	0.20

[a] Residue number refers to position i in the amino acid sequence.

[b] Averaged values from the 3D C'- and C$^{\alpha}$-resolved experiments.

[c] Correction to the apparent CCR rate calculated with the full relaxation-matrix approach (Vögeli 2013).

[d] Random error obtained from half the pairwise r.m.s. deviation between the values from 3D C'- and C$^{\alpha}$-resolved experiments.

[e] Error obtained from the propagated errors of the individual 3D C'- and C$^{\alpha}$-resolved experiments.

Table S2. Experimental $\Gamma = \Gamma_{HAiCAi/HAi\text{-}1CAi\text{-}1} + \Gamma_{HAiCAi\text{-}1/HAi\text{-}1CAi}$ CCR rates[a] obtained from GB3 at T = 298 K and $\tau_{c,eff}$ = 4.03 ns.

res # [b]	aa type	Γ(2D+ 3D) [s^{-1}] [c]	ΔΓ(2D+ 3D) [s^{-1}] [d]	ΔΓ(2D+ 3D) [s^{-1}] [e]	isotropic Γ	error for isotropic Γ
3	TYR	22.9	0.3	0.4	24.85	0.30
4	LYS+	15.5	1.4	0.7	15.57	1.40
5	LEU	22.3	0.8	0.2	22.98	0.80
6	VAL	23.4	0.5	0.6	24.57	0.50
7	ILE	24.8	3.3	0.4	26.02	3.30
8	ASN	22.2	2.1	0.8	24.01	2.10
9	GLY	1.5	1.5	0.1		
10	LYS+	-0.9	3.5	0.5		
11	THR	4.2	0.0	0.6	4.23	0.10
12	LEU	-2.6	1.5	0.2	-2.49	1.50
13	LYS+	23.6	0.2	0.2	24.91	0.20
14	GLY	1.4	0.5	0.4		
15	GLU	5.2				
16	THR	24.9	0.6	0.7	26.81	0.60
17	THR	11.3			11.04	2.17
18	THR	25.6	1.1	0.8	27.02	1.10
19	LYS+	6.6	1.5	0.9	4.66	1.50
20	ALA					
21	VAL					
22	ASP	9.6	0.9	0.1	10.08	0.90
23	ALA	-6.6	0.5		-9.18	0.50
24	GLU	-8.5	1.3	0.2	-6.56	1.30
25	THR	-16.3			-16.42	2.17
26	ALA	-10.0	2.5	0.4	-13.78	2.50
27	GLU					
28	LYS+	-20.3			-19.92	4.79
29	ALA	-9.9	1.4	0.9	-10.82	1.40
30	PHE	-3.4	1.7	0.3	-7.35	1.70
31	LYS+	-17.4	0.4	0.5	-16.72	0.40
32	GLN	-18.3			-18.45	3.32
33	TYR	-16.9	0.3	0.6	-19.05	2.05
34	ALA	-4.4	0.9	0.7	-6.48	0.90
35	ASN	-8.3			-7.88	2.17
36	ASP	-15.9			-16.35	2.17
37	ASN	-5.8	0.8	0.1	-8.35	0.80

38	GLY	1.3	1.2	0.2		
39	VAL	2.6	0.4	0.1		
40	ASP	23.1	0.4	1.0	19.84	0.40
41	GLY	1.2	0.7	0.1		
42	VAL	1.5	1.0	0.2		
43	TRP	12.0	1.0	0.5	12.24	1.00
44	THR	24.5	0.4	0.5	26.01	0.40
45	TYR	20.7	0.2	0.9	21.35	0.20
46	ASP	23.7	1.3	1.4	25.07	1.30
47	ASP	6.9	1.5	0.5	6.04	1.50
48	ALA	-9.8			-7.36	2.17
49	THR	5.0			4.42	2.17
50	LYS+	-2.3	2.3	0.3	-0.01	2.30
51	THR	-6.4	2.4	0.3	-9.36	2.40
52	PHE	18.9	0.3		19.70	0.30
53	THR	24.8	0.1	0.7	25.92	0.10
54	VAL	24.6	0.3	0.3	26.03	0.30
55	THR	22.2	1.5	0.3	23.51	1.50
56	GLU	14.2	1.0	0.2	15.16	1.00

[a] If a glycine residue is involved the CCR rate is the sum of the individual rates corresponding to $H^{\alpha 1}$ and $H^{\alpha 2}$.

[b] Residue number refers to position i in the amino acid sequence.

[c] Averaged values from the 2D and 3D measurements; values from 2D scaled to match 3D.

[d] Random error obtained from half the pairwise r.m.s. deviation between the 2D and 3D measurements; values from 2D scaled to match 3D.

[e] Error obtained from the propagated errors of the individual 2D and 3D measurements.

Table S3. Experimental $\Gamma = \Gamma_{HNiNi/HAiCAi} + \Gamma_{HAiNi/HNiCAi}$ CCR rates[a] obtained from GB3 at T = 298 K and $\tau_{c,eff}$ = 4.03 ns.

res # [b]	aa type	Γ(ACE+DIAI+MMQ), corrected [s^{-1}] [c]	ΔΓ(ACE+DIAI+MMQ) [s^{-1}] [d]	ΔΓ(ACE+DIAI+MMQ) [s^{-1}] [e]	correction to Γ(DIAI) [s^{-1}] [f]	correction to Γ(ACE) [s^{-1}] [f]	correction to Γ(MMQ) [s^{-1}] [f]	isotropic Γ	error for isotropic Γ
3	TYR	-11.19	0.55	0.09	-0.12	-0.15	0.03	-11.84	0.55
4	LYS+	-12.19	0.13	0.06	-0.19	-0.16	-0.04	-12.68	0.13
5	LEU	-12.27	0.03	0.26	-0.12	-0.17	0.00	-12.65	0.03
6	VAL	-11.69	0.14	0.17	-0.08	-0.17	0.03	-12.18	0.14
7	ILE	-11.36		0.02	-0.15	-0.15	0.02	-12.10	0.34
8	ASN	-11.72	0.16	0.13	-0.17	-0.13	0.02	-12.23	0.16
9	GLY								
10	LYS+	-3.70	0.13	0.13	-0.04	-0.06	-0.02	-3.79	0.13
11	THR	-10.14	0.05	0.09	0.16	-0.17	0.12	-9.91	0.05
12	LEU	-8.99	0.08	0.06	-0.18	-0.13	-0.01	-9.48	0.08
13	LYS+	-10.87	0.27	0.01	-0.19	-0.12	-0.05	-11.04	0.27
14	GLY								
15	GLU	-9.40	0.05	0.60	-0.16	-0.12	-0.02	-9.84	0.05
16	THR	-6.60	0.07	0.08	-0.17	-0.11	-0.01	-6.41	0.07
17	THR	-10.22	0.06		-0.26	-0.13	-0.08	-10.53	0.06
18	THR	-6.82	0.23	0.02	-0.09	-0.08	-0.03	-6.35	0.23
19	LYS+	-11.17	0.11	0.09	0.12	-0.21	0.13	-11.52	0.11
20	ALA	-6.42	0.22	0.04	-0.11	-0.07	-0.06	-6.98	0.22
21	VAL	-1.28	0.30	0.11	0.06	-0.06	-0.02	-0.85	0.30
22	ASP	-5.45	0.29	0.12	-0.16	-0.06	-0.09	-5.53	0.29
23	ALA	-1.57	0.35	0.05	-0.03	-0.04	-0.08	-0.52	0.35
24	GLU	-2.52	0.10	0.04	-0.04	-0.03	-0.09	-1.92	0.10
25	THR	-5.26	0.33	0.21	-0.09	-0.08	-0.02	-5.50	0.33
26	ALA	-2.59	0.19	0.02	-0.05	-0.06	-0.06	-2.79	0.19
27	GLU	-0.04		0.23	-0.04	-0.04	-0.08	0.77	0.34
28	LYS+	-2.55	0.13		-0.03	-0.03	-0.08	-1.99	0.13
29	ALA	-3.47	0.00	0.02	-0.06	-0.04	-0.09	-4.22	0.01
30	PHE	-3.83	0.18	0.02	-0.08	-0.05	-0.07	-3.79	0.18
31	LYS+	-2.94	0.03	0.03	-0.04	-0.04	-0.08	-2.19	0.03
32	GLN	-3.94	0.15	0.05	-0.08	-0.05	-0.06	-3.56	0.15
33	TYR	-0.83		0.23	-0.01	-0.01	-0.09	-1.60	0.34
34	ALA	-2.23	0.24	0.04	-0.05	-0.04	-0.08	-1.35	0.24
35	ASN	-2.34	0.25		-0.04	-0.05	-0.06	-1.88	0.25

36	ASP	-1.43	0.03		-0.04	-0.03	-0.09	-1.65	0.03
37	ASN	-10.91	0.08	0.10	-0.23	-0.12	0.04	-10.69	0.08
38	GLY								
39	VAL	-11.58	0.40	0.07	-0.42	-0.12	-0.05	-9.93	0.40
40	ASP	-9.55	0.01	0.05	-0.33	-0.11	-0.03	-7.74	0.01
41	GLY								
42	VAL	-10.14	0.09	0.22	-0.19	-0.12	-0.03	-10.53	0.09
43	TRP	-9.97	0.01	0.15	-0.12	-0.13	0.00	-10.38	0.01
44	THR	-10.68	0.13	0.14	-0.20	-0.11	-0.02	-10.94	0.13
45	TYR	-11.37	0.13	0.04	-0.09	-0.17	-0.02	-11.82	0.13
46	ASP	-12.19	0.00	0.16	-0.24	-0.13	-0.01	-12.65	0.01
47	ASP	-2.85	0.28	0.06	0.03	-0.09	-0.01	-1.61	0.28
48	ALA	-3.34	0.16	0.00	-0.08	-0.04	-0.08	-3.05	0.16
49	THR	-11.63	0.41	0.32	-0.23	-0.11	0.05	-11.48	0.41
50	LYS+	-3.90	0.09		-0.20	-0.10	-0.03	-3.22	0.09
51	THR	-12.44	0.15		-0.32	-0.14	-0.08	-12.96	0.15
52	PHE	-12.02	0.03	0.15	-0.03	-0.18	0.03	-12.51	0.03
53	THR	-11.74	0.18	0.16	-0.23	-0.15	-0.05	-12.17	0.18
54	VAL	-11.91	0.27	0.20	-0.14	-0.15	-0.03	-12.31	0.27
55	THR	-12.36	0.41		-0.11	-0.17	0.03	-12.86	0.41
56	GLU	-8.97	0.22	0.06	-0.13	-0.10	-0.01	-9.78	0.22

[a] If a glycine residue is involved the CCR rate is the sum of the individual rates corresponding to $H^{\alpha 1}$ and $H^{\alpha 2}$.

[b] Residue number refers to position i in the amino acid sequence.

[c] Averaged values from the DIAI experiment and the average of the ACE and MMQ experiments (relative weights 2:1:1 for DIAI:ACE:MMQ). Corrected by a uniform scaling of 1.014.

[d] Random error obtained from half the pairwise r.m.s. deviation between the DIAI and the average of the ACE and MMQ measurements; values from DIAI and MMQ scaled to match ACE.

[e] Error obtained from the propagated errors of the individual DIAI, ACE and MMQ measurements.

[f] Correction to the apparent CCR rate calculated with the full relaxation-matrix approach (Vögeli 2013).

Table S4. Experimental $\Gamma = \Gamma_{HNiNi/HAi-1CAi-1} + \Gamma_{HAi-1Ni/HNiCAi-1}$ CCR rates[a] obtained from GB3 at T = 298 K and $\tau_{c,eff}$ = 4.03 ns.

res # [b]	aa type	Γ(ACE+ DIAI), corrected [s^{-1}] [c]	ΔΓ(ACE+ DIAI) [s^{-1}] [d]	ΔΓ(ACE+ DIAI) [s^{-1}] [e]	correction to Γ(1. DIAI) [s^{-1}] [f]	correction to Γ(2. DIAI) [s^{-1}] [f]	correction to Γ(ACE) [s^{-1}] [f]	isotropic Γ	error for isotropic Γ
3	TYR	-10.01	0.33	0.45	-0.62	-0.53	-0.24	-10.77	0.33
4	LYS+	-7.98	0.33	0.19	-0.50	-0.41	-0.18	-7.94	0.33
5	LEU	-9.99	0.08	0.18	-0.60	-0.55	-0.22	-10.04	0.08
6	VAL	-11.37	0.15	0.16	-0.69	-0.65	-0.27	-11.86	0.15
7	ILE	-11.07	0.16	0.22	-0.68	-0.64	-0.23	-11.80	0.16
8	ASN	-11.20	0.44	0.15	-0.70	-0.66	-0.22	-11.96	0.44
9	GLY	-6.22	0.30	0.23	-0.39	-0.32	-0.12	-7.25	0.30
10	LYS+								
11	THR	-0.58	0.29	0.17	-0.05	0.06	-0.02	-0.28	0.29
12	LEU	1.32	0.13	0.08	0.04	0.20	0.01	1.15	0.13
13	LYS+	-10.45	0.24	0.18	-0.69	-0.62	-0.19	-10.88	0.24
14	GLY	-10.20	0.29	0.39	-0.66	-0.63	-0.17	-10.76	0.29
15	GLU								
16	THR	-8.73	0.37	0.12	-0.57	-0.48	-0.17	-8.92	0.37
17	THR	-1.92	0.23	0.11	-0.15	0.16	-0.17	-1.03	0.23
18	THR	-6.45	0.12	0.24	-0.40	-0.32	-0.14	-6.16	0.12
19	LYS+	-5.35	0.07	0.24	-0.28	-0.22	-0.15	-3.57	0.07
20	ALA	-10.37	0.35	0.16	-0.67	-0.62	-0.20	-11.30	0.35
21	VAL	-7.04	0.05		-0.28	-0.30	-0.21	-8.00	0.05
22	ASP	2.07	0.09	0.20	0.10	0.28	0.01	2.63	0.09
23	ALA	-4.54	0.09	0.22	-0.28	-0.17	-0.12	-4.26	0.09
24	GLU	0.04	0.12		-0.02	0.12	0.00	0.10	0.12
25	THR	-0.90			-0.07	0.04	-0.02	-0.43	0.45
26	ALA	-0.94	0.21	0.25	-0.07	0.04	-0.01	-0.68	0.21
27	GLU	-0.68			-0.06	0.06	-0.02	-1.67	0.45
28	LYS+	-0.68	0.13	0.14	-0.06	0.06	-0.01	0.05	0.13
29	ALA	-0.64	0.19	0.08	-0.06	0.06	-0.01	-0.29	0.19
30	PHE	-0.53	0.42		-0.05	0.07	-0.01	-0.98	0.42
31	LYS+	-1.31	0.12	0.12	-0.09	0.00	-0.02	-2.19	0.12
32	GLN	-1.15	0.07	0.21	-0.08	0.02	-0.01	-0.51	0.07
33	TYR	-1.41	0.31	0.12	-0.10	0.00	-0.02	-1.09	0.31
34	ALA	-0.82	0.45	0.30	-0.06	0.05	-0.01	-1.75	0.45
35	ASN	-1.09	0.05		-0.08	0.02	-0.02	-0.68	0.05
36	ASP	-1.56	0.30	0.29	-0.10	-0.01	-0.01	-1.16	0.30

37	ASN	1.09	0.24	0.13	0.03	0.19	0.00	1.70	0.24
38	GLY	4.96	0.15	0.28	0.25	0.51	0.02	5.08	0.15
39	VAL								
40	ASP	-10.38	0.16	0.13	-0.68	-0.64	-0.17	-8.87	0.16
41	GLY	-8.14	0.07	0.11	-0.55	-0.50	-0.11	-6.63	0.07
42	VAL								
43	TRP	-9.45	0.21	0.54	-0.59	-0.47	-0.23	-10.49	0.21
44	THR	-11.23	0.31	0.27	-0.72	-0.69	-0.20	-11.73	0.31
45	TYR	-7.76	0.19	0.21	-0.45	-0.41	-0.16	-8.14	0.19
46	ASP	-10.94	0.18	0.18	-0.71	-0.67	-0.22	-11.32	0.18
47	ASP	-10.51	0.15	0.17	-0.53	-0.55	-0.27	-11.12	0.15
48	ALA	1.17	0.12	0.14	0.04	0.20	0.01	1.24	0.12
49	THR	0.20	0.35		-0.02	0.13	-0.01	0.21	0.35
50	LYS+	5.30	0.31	0.43	0.24	0.51	0.03	5.57	0.31
51	THR	1.10	0.47	0.21	0.03	0.23	-0.02	2.80	0.47
52	PHE	-9.91	0.59		-0.57	-0.49	-0.26	-10.19	0.59
53	THR	-10.50	0.49	0.38	-0.67	-0.62	-0.21	-10.78	0.49
54	VAL	-10.20	0.11	0.20	-0.62	-0.57	-0.22	-10.67	0.11
55	THR	-10.96	0.14	0.24	-0.67	-0.60	-0.26	-11.46	0.14
56	GLU	-11.08	0.16	0.16	-0.71	-0.66	-0.23	-11.93	0.16

[a] If a glycine residue is involved the CCR rate is the sum of the individual rates corresponding to $H^{\alpha 1}$ and $H^{\alpha 2}$.

[b] Residue number refers to position i in the amino acid sequence.

[c] Averaged values from the ACE and two DIAI measurements; values from DIAI scaled to match ACE.

[d] Random error obtained from standard deviation of the average of the individual data sets; values from DIAI scaled to match ACE.

[e] Error obtained from the propagated errors of the individual ACE and DIAI measurements.

[f] Correction to the apparent CCR rate calculated with the full relaxation-matrix approach (Vögeli 2013).

Table S5. Predicted F_{corr} for $\Gamma = \Gamma_{HNiNi/HNi+1Ni+1} + \Gamma_{HNiNi+1/HNi+1Ni}$ for GB3.

res # [a]	aa type	Γ (predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}
		iterative DIDC			*ORIUM*			*Current Work*		
3	TYR	4.31	0.75	0.05	3.57	0.91	0.06	3.432	0.97	0.01
4	LYS+	3.72	0.95	0.02	2.99	1.18	0.04	3.392	1.05	0.01
5	LEU	4.31	0.92	0.04	3.49	1.14	0.07	4.492	0.9	0.02
6	VAL	3.98	0.98	0.08	3.11	1.26	0.12	3.772	1.04	0.02
7	ILE	3.49	0.96	0.03	2.66	1.26	0.06	3.002	1.14	0.02
8	ASN	1.44	0.6	0.14	1.12	0.77	0.18	1.312	0.81	0.05
9	GLY	-0.42	2.74	0.72	-0.36	3.2	0.85	-0.892	1.47	0.32
10	LYS+	2.12*	1.46	0.11	2.24	1.38	0.11	2.142	1.47	0.1
11	THR	-0.07*	-5.64	0.78	-0.07	-5.57	0.73	-0.382	-0.94	0.06
12	LEU	1.85	1.2	0.08	1.55	1.43	0.09	1.722	1.4	0.09
13	LYS+	4.06	0.94	0.07	3.25	1.18	0.1	3.722	1.06	0.02
14	GLY	4.14	0.9	0.04	3.24	1.15	0.06	3.812	1.03	0.02
15	GLU	4.46	0.89	0.07	3.49	1.13	0.1	3.982	1.03	0.02
16	THR	4.15	0.81	0.06	3.31	1.01	0.08	3.712	0.95	0.02
17	THR	4.05	0.98	0.08	3.33	1.19	0.1	3.472	1.16	0.02
18	THR	4.73*	0.85	0.08	4.02*	1	0.09	3.922	0.98	0.04
19	LYS+	2.91*	1.24	0.1	2.65*	1.37	0.1	3.152	1.22	0.04
20	ALA	4.01	1.18	0.07	3.55	1.33	0.1	3.732	1.3	0.02
21	VAL	1.68	1.04	0.04	1.33	1.31	0.09	1.652	1.13	0.04
22	ASP	5.46	0.86	0.06	4.07	1.15	0.08	4.292	1.07	0.02
23	ALA	3.76	0.93	0.03	2.83	1.23	0.05	3.022	1.1	0.03
24	GLU	4.16*	1.02	0.08	3.34	1.27	0.1	3.702	1.1	0.05
25	THR	4.54*	0.93	0.07	3.62	1.17	0.09	3.912	1.04	0.02
26	ALA	5.2	0.95	0.06	3.95	1.25	0.09	4.182	1.13	0.02
27	GLU	4.61	1.04	0.07	3.63	1.32	0.1	4.102	1.1	0.02
28	LYS+	4.58	0.96	0.07	3.65	1.2	0.09	4.012	1.06	0.02
29	ALA	5.26*	0.9	0.06	3.94	1.2	0.08	4.182	1.08	0.02
30	PHE	4.49*	1	0.04	3.12	1.44	0.05	3.542	1.2	0.03
31	LYS+	4.35	1.06	0.04	3.18	1.45	0.06	3.962	1.11	0.02
32	GLN	4.83	1.01	0.06	3.75	1.3	0.07	4.272	1.1	0.02
33	TYR	4.98	1.02	0.04	3.75	1.35	0.07	4.182	1.16	0.02
34	ALA	4.73	1.05	0.03	3.76	1.32	0.05	4.382	1.08	0.01
35	ASN	na	na	na	na	na	na	na	na	na
36	ASP	2.65	1.09	0.12	2.08	1.4	0.15	2.312	1.18	0.06
37	ASN	-1.38	1.2	0.05	-1.06	1.56	0.07	-1.212	1.38	0.09

38	GLY	2.84	0.84	0.11	2.1	1.13	0.15	2.262	1.02	0.1
39	VAL	3.07^	0.9	0.1	2.36	1.17	0.13	2.452	1.13	0.26
40	ASP	5.52^^	0.73	0.07	4.47*	0.9	0.08	2.6618	1.49	0.42
41	GLY	4.29^	0.52	0.05	3.18*	0.7	0.07	1.4015	1.6	0.48
42	VAL	2.18	1.05	0.06	1.58	1.45	0.09	1.502	1.54	0.13
43	TRP	3.01	0.7	0.1	2.29	0.92	0.14	2.042	0.99	0.02
44	THR	5.01	0.82	0.03	3.88	1.06	0.05	4.472	0.94	0.01
45	TYR	4.68	0.92	0.03	3.77	1.14	0.05	4.242	1.02	0.02
46	ASP	3.36	1.06	0.12	2.56	1.39	0.16	2.822	1.24	0.06
47	ASP	1.89	1.35	0.13	1.39	1.84	0.19	1.692	1.55	0.11
48	ALA	3.51	1	0.03	2.69	1.31	0.05	2.962	1.18	0.03
49	THR	-1.18	1.21	0.26	-0.92	1.55	0.33	-1.192	1.15	0.05
50	LYS+	4.13	0.91	0.06	3.27	1.15	0.08	3.842	1.01	0.01
51	THR	3.94	0.98	0.08	3.2	1.21	0.1	3.522	1.08	0.01
52	PHE	3.24	0.99	0.14	2.45	1.31	0.2	2.922	1.08	0.03
53	THR	5.26	0.87	0.06	3.75	1.22	0.11	4.712	0.98	0.01
54	VAL	4.44	0.99	0.04	3.48	1.27	0.08	4.352	1.03	0.01
55	THR	3.67	1.12	0.03	2.87	1.44	0.07	3.372	1.25	0.02

[a] Residue number refers to position i in the amino acid sequence

* Individual S^2 value was missing and has been substituted for the average of neighboring residues

^ Individual S^2 value was missing and has been substituted for the average for the whole protein.

Table S6. Predicted F_{corr} for $\Gamma = \Gamma_{HAiCAi/HAi+1CAi+1} + \Gamma_{HAiCAi+1/HAi+1CAi}$ for GB3. [a]

res # [b]	aa type	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}
		iterative DIDC			*ORIUM*			*Current Work*		
3	TYR	15.23	1.02	0.09	12.03	1.29	0.13	13.732	1.1	0.03
4	LYS+	19.09	1.17	0.06	14.99	1.49	0.08	19.222	1.14	0.01
5	LEU	23.02	1.02	0.04	19.61	1.19	0.06	22.872	1.04	0.01
6	VAL	21.16	1.17	0.16	17.22	1.44	0.21	19.972	1.13	0.02
7	ILE	20.85	1.06	0.11	15.4	1.44	0.17	18.712	1.22	0.02
8	ASN	na	na	na	na	na	na	na	na	na
9	GLY	na	na	na	na	na	na	na	na	na
10	LYS+	11.22*	0.37	0.02	9.45*	0.44	0.05	3.5418	1.12	0.22
11	THR	-6.08*	0.43	0.25	-4.91*	0.53	0.31	-1.4015	1.62	0.55
12	LEU	16.23	1.45	0.09	12.72	1.85	0.15	15.372	1.54	0.02
13	LYS+	na	na	na	na	na	na	na	na	na
14	GLY	na	na	na	na	na	na	na	na	na
15	GLU	24.12	1.03	0.05	19.55	1.27	0.06	20.722	1.23	0.01
16	THR	13.49	0.84	0.16	10.68	1.06	0.21	11.432	1.1	0.02
17	THR	24.96	1.03	0.05	19.42	1.32	0.07	23.522	1.1	0.01
18	THR	6.43*	1.03	0.25	5.77	1.14	0.26	5.142	1.14	0.05
19	LYS+	na	na	na	na	na	na	na	na	na
20	ALA	na	na	na	na	na	na	na	na	na
21	VAL	10.97	0.88	0.09	8.42	1.14	0.12	10.352	0.91	0.04
22	ASP	-9.73	0.68	0.06	-7.52	0.88	0.08	-8.092	0.9	0.02
23	ALA	-14.27	0.6	0.09	-11.22	0.76	0.12	-12.582	0.75	0.03
24	GLU	-10.93*	1.49	0.2	-9.02*	1.81	0.26	-10.002	1.17	0.03
25	THR	-8.93*	1.12	0.28	-6.92*	1.45	0.37	-9.192	1.14	0.04
26	ALA	na	na	na	na	na	na	na	na	na
27	GLU	-12	1.69	0.18	-9	2.26	0.25	-10.792	1.16	0.04
28	LYS+	-11.44	0.87	0.12	-8.74	1.13	0.17	-10.362	1.06	0.02
29	ALA	-6.92*	0.49	0.25	-5.23*	0.65	0.33	-4.662	1.08	0.1
30	PHE	-14.30*	1.22	0.05	-11.03*	1.58	0.1	-13.532	1.17	0.02
31	LYS+	-13.27	1.38	0.17	-10.13	1.81	0.22	-11.652	1.14	0.02
32	GLN	-10.36	1.63	0.04	-7.75	2.18	0.07	-9.602	1.26	0.02
33	TYR	-9.94	0.44	0.09	-7.51	0.59	0.12	-8.932	0.63	0.03
34	ALA	-12.83	0.65	0.17	-9.91	0.84	0.22	-11.122	0.9	0.03
35	ASN	-11.87	1.34	0.19	-9.83	1.62	0.23	-10.552	1.21	0.03
36	ASP	0.2	-28.43	3.97	0.17	-35.06	5.07	-1.943	2.89	0.12
37	ASN	na	na	na	na	na	na	na	na	na
38	GLY	na	na	na	na	na	na	na	na	na
39	VAL	26.76^	0.86	0.04	20.58^	1.12	0.08	18.152	1.28	0.31

40	ASP	na	na	na	na	na	na	na	na	Na
41	GLY	na	na	na	na	na	na	na	na	na
42	VAL	14.77	0.81	0.07	11.68	1.03	0.1	12.352	1.08	0.02
43	TRP	23.92	1.02	0.03	19.76	1.24	0.08	23.102	1.07	0.01
44	THR	20.67	1	0.03	16.8	1.23	0.08	20.372	1.02	0.01
45	TYR	20.59	1.15	0.07	16.87	1.41	0.1	21.862	1.06	0.01
46	ASP	11.77	0.59	0.13	8.58	0.8	0.18	8.082	1.02	0.06
47	ASP	-14.37	0.68	0.15	-10.76	0.91	0.2	-12.882	0.84	0.02
48	ALA	1.79	2.79	1.21	1.42	3.53	1.54	4.492	0.9	0.09
49	THR	0.21	-10.92	10.92	0.16	-13.99	14	-1.832	1.07	0.11
50	LYS+	-3.05	2.1	0.79	-2.37	2.69	1.02	-3.042	1.38	0.14
51	THR	19.76	0.96	0.03	14.87	1.27	0.05	18.572	1.02	0.01
52	PHE	23.33	1.06	0.03	18.13	1.37	0.06	22.792	1.1	0.01
53	THR	23.64	1.04	0.03	18.12	1.36	0.07	22.052	1.13	0.01
54	VAL	22.73	0.98	0.07	17.43	1.27	0.11	21.022	1.08	0.02
55	THR	16.34^	0.87	0.07	12.90^	1.1	0.12	15.692	0.95	0.05

[a] If a glycine residue is involved the CCR rate is the sum of the individual rates corresponding to $H^{\alpha 1}$ and $H^{\alpha 2}$

[b] Residue number refers to position i in the amino acid sequence

* Individual S^2 value was missing and has been substituted for the average of neighboring residues

^ Individual S^2 value was missing and has been substituted for the average for the whole protein

Table S7. Predicted F_{corr} for $\Gamma = \Gamma_{HNiNi/HAiCAi} + \Gamma_{HAiNi/HNiCAi}$ for GB3.[a]

res #[b]	aa type	Γ (predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}
		iterative DIDC			*ORIUM*			*Current Work*		
3	TYR	-12.36	0.91	0.05	-10.38	1.08	0.07	-11.382	1.02	0.01
4	LYS+	-11.93	1.02	0.02	-9.3	1.31	0.04	-11.072	1.12	0.01
5	LEU	-12.03	1.02	0.03	-9.73	1.26	0.05	-11.802	1.05	0.01
6	VAL	-11.97	0.98	0.03	-10.2	1.15	0.06	-12.112	0.98	0.01
7	ILE	-11.52	0.99	0.04	-8.61	1.32	0.08	-10.192	1.14	0.02
8	ASN	-9.92	1.18	0.06	-7.47	1.57	0.1	-9.032	1.32	0.02
9	GLY	na	na	na	na	na	na	na	na	na
10	LYS+	-3.16	1.17	0.05	-2.83	1.31	0.09	-5.262	0.78	0.07
11	THR	-5.77**	1.76	0.15	-5.75*	1.76	0.21	-7.562	1.32	0.08
12	LEU	-5.17	1.74	0.12	-4.24	2.12	0.14	-5.072	1.85	0.03
13	LYS+	-8.68	1.25	0.07	-6.96	1.56	0.12	-8.822	1.23	0.02
14	GLY	na	na	na	na	na	na	na	na	na
15	GLU	-8.69	1.08	0.04	-7.01	1.34	0.06	-8.632	1.14	0.02
16	THR	-7.01	0.94	0.04	-5.52	1.2	0.04	-6.042	1.12	0.02
17	THR	-9.89	1.03	0.02	-7.95	1.29	0.04	-9.292	1.13	0.02
18	THR	-6.8	1	0.04	-5.41	1.26	0.06	-6.102	1.1	0.02
19	LYS+	-10.54**	1.06	0.1	-10.10*	1.11	0.06	-10.172	1.09	0.04
20	ALA	-4.36	1.47	0.12	-3.82	1.68	0.14	-4.932	1.34	0.04
21	VAL	-1.9	0.67	0.16	-1.47	0.87	0.21	-1.502	0.73	0.09
22	ASP	-5.37	1.01	0.06	-4.22	1.29	0.08	-4.932	1.14	0.03
23	ALA	-0.74	2.12	0.48	-0.54	2.89	0.66	-1.252	1.09	0.24
24	GLU	-1.3	1.94	0.09	-1.05	2.4	0.13	-1.942	1.25	0.09
25	THR	-5.51**	0.96	0.07	-4.52*	1.16	0.09	-4.182	1.25	0.12
26	ALA	-2.28	1.14	0.09	-1.72	1.51	0.12	-2.162	1.21	0.05
27	GLU	0.96	-0.04	0.36	0.71	-0.06	0.48	0.672	0.37	0.11
28	LYS+	-1.66	1.54	0.08	-1.32	1.93	0.11	-2.162	1.15	0.06
29	ALA	-2.68	1.3	0.02	-2.05	1.7	0.07	-2.622	1.38	0.06
30	PHE	-4.04**	0.95	0.06	-2.99*	1.28	0.1	-4.252	0.92	0.05
31	LYS+	-1.93	1.52	0.04	-1.4	2.1	0.08	-2.542	1.11	0.09
32	GLN	-4.13	0.95	0.04	-3.18	1.24	0.05	-4.282	0.92	0.03
33	TYR	0.22	-3.82	1.56	0.16	-5.05	2.07	-0.473	1.96	0.22
34	ALA	-2.32	0.96	0.11	-1.75	1.28	0.15	-1.912	1.04	0.1
35	ASN	-2.37	0.99	0.11	-1.94	1.21	0.13	-2.022	1.19	0.09
36	ASP	-1.06	1.35	0.04	-0.88	1.62	0.06	-1.732	0.89	0.06
37	ASN	-10.67	1.02	0.03	-8.15	1.34	0.05	-9.142	1.17	0.03

38	GLY	na	na	na	na	na	na	na	na	Na
39	VAL	-12.76	0.91	0.03	-9.39	1.23	0.06	-11.222	1.01	0.01
40	ASP	-11.70^^	0.82	0.04	-9.40^	1.02	0.07	-6.992	1.35	0.43
41	GLY	na	na	na	na	na	na	na	na	na
42	VAL	-11.74	0.86	0.02	-8.23	1.23	0.05	-9.602	1.07	0.02
43	TRP	-10.31	0.97	0.02	-8.43	1.18	0.05	-8.152	1.25	0.02
44	THR	-11.53	0.93	0.03	-8.88	1.2	0.07	-10.882	0.99	0.01
45	TYR	-10.78	1.05	0.03	-8.82	1.29	0.07	-10.982	1.06	0.01
46	ASP	-11.71	1.04	0.03	-9.44	1.29	0.04	-11.162	1.11	0.01
47	ASP	-3.62	0.79	0.08	-2.49	1.14	0.13	-2.412	1.05	0.1
48	ALA	-2.62	1.28	0.07	-2.08	1.6	0.1	-2.792	1.34	0.24
49	THR	-11.27	1.03	0.04	-8.56	1.36	0.06	-10.362	1.14	0.02
50	LYS+	-3.45	1.13	0.04	-2.76	1.41	0.07	-2.922	1.3	0.05
51	THR	-11.48	1.08	0.03	-8.87	1.4	0.06	-10.232	1.24	0.01
52	PHE	-11.34	1.06	0.03	-8.97	1.34	0.04	-11.042	1.11	0.01
53	THR	-12.36	0.95	0.03	-9.17	1.28	0.08	-11.042	1.08	0.01
54	VAL	-11.87	1	0.03	-8.74	1.36	0.08	-10.832	1.11	0.01
55	THR	-11.62	1.06	0.04	-9.48	1.3	0.09	-11.022	1.13	0.01
56	GLU	-8.70^	1.03	0.05	-6.58^	1.36	0.1	-8.522	1.12	0.04

[a] If a glycine residue is involved the CCR rate is the sum of the individual rates corresponding to $H^{\alpha 1}$ and $H^{\alpha 2}$

[b] Residue number refers to position i in the amino acid sequence

* Individual S^2 value was missing and has been substituted for the average of neighbouring residues

^ Individual S^2 value was missing and has been substituted for the average for the whole protein

Table S8. Predicted F_{corr} for $\Gamma = \Gamma_{HNiNi/HAi\text{-}1CAi\text{-}1} + \Gamma_{HAi\text{-}1Ni/HNiCAi\text{-}1}$ for GB3.[a]

res #[b]	aa type	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}	Γ(predicted, S^2) [s^{-1}]	F_{corr}	ΔF_{corr}
		iterative DIDC			*ORIUM*			*Current Work*		
3	TYR	-10.06^	1	0.05	-8.11^	1.23	0.09	-8.502	1.21	0.1
4	LYS+	-8.2	0.97	0.05	-6.54	1.22	0.07	-6.992	1.14	0.02
5	LEU	-9.84	1.01	0.03	-8.04	1.24	0.04	-8.702	1.14	0.01
6	VAL	-11.49	0.99	0.03	-9.63	1.18	0.05	-10.832	1.06	0.01
7	ILE	-11.05	1	0.03	-8.68	1.28	0.07	-10.342	1.09	0.01
8	ASN	-9.76	1.15	0.07	-7.18	1.56	0.11	-8.582	1.33	0.02
9	GLY	-6.76*	0.92	0.07	-5.31*	1.17	0.14	-6.562	1.02	0.02
10	LYS+	na	na	na	na	na	na	na	na	na
11	THR	-0.37*	1.56	0.78	-0.33*	1.77	0.91	-0.5818	0.64	0.4
12	LEU	3.07*	0.43	0.05	3	0.44	0.06	2.302	0.65	0.1
13	LYS+	-4.76	2.19	0.17	-3.88	2.7	0.21	-4.862	2.16	0.03
14	GLY	-8.66*	1.18	0.09	-7.05*	1.45	0.14	-8.632	1.21	0.02
15	GLU	na	na	na	na	na	na	na	na	na
16	THR	-7.68	1.14	0.07	-6.08	1.43	0.09	-7.222	1.21	0.02
17	THR	-1.68	1.15	0.14	-1.31	1.46	0.18	-1.592	1.23	0.16
18	THR	-5.73	1.12	0.03	-4.55	1.42	0.05	-5.122	1.23	0.02
19	LYS+	-4.98*	1.07	0.08	-4.63	1.16	0.05	-2.992	1.39	0.14
20	ALA	-7.99*	1.3	0.11	-6.76*	1.53	0.14	-7.772	1.34	0.04
21	VAL	-6.03	1.17	0.05	-4.95	1.42	0.05	-6.072	1.22	0.02
22	ASP	0.88	2.34	0.14	0.73	2.83	0.24	na	na	na
23	ALA	-3.65	1.25	0.04	-2.69	1.69	0.09	-1.952	2.33	0.25
24	GLU	-0.48	-0.08	0.25	-0.38	-0.1	0.31	-0.4214	-0.49	0.14
25	THR	-0.44*	2.03	1.02	-0.35*	2.59	1.3	-1.2219	0.61	0.08
26	ALA	-2.38*	0.39	0.09	-1.89	0.5	0.11	-2.022	0.52	0.06
27	GLU	-0.93	0.73	0.48	-0.69	0.99	0.66	-1.202	0.81	0.09
28	LYS+	-1.02	0.67	0.13	-0.79	0.86	0.17	-1.102	0.42	0.05
29	ALA	-0.35	1.82	0.54	-0.27	2.33	0.7	-1.172	0.51	0.06
30	PHE	-1.55*	0.34	0.27	-1.19*	0.44	0.35	-0.742	1.03	0.18
31	LYS+	-2.26*	0.58	0.06	-1.69	0.77	0.07	-0.888	2.01	0.42
32	GLN	-0.96	1.19	0.08	-0.68	1.68	0.12	-0.702	1.53	0.18
33	TYR	-2.25	0.63	0.14	-1.73	0.81	0.18	-2.742	0.49	0.03
34	ALA	-0.41	2.01	1.1	-0.31	2.64	1.45	-0.812	1.27	0.16
35	ASN	-1.4	0.78	0.04	-1.07	1.02	0.06	-1.992	0.47	0.02
36	ASP	-1.58	0.99	0.19	-1.36	1.15	0.22	-1.972	0.77	0.06
37	ASN	-0.7	-1.55	0.34	-0.54	-2.01	0.45	-1.401	-0.86	0
38	GLY	4.53*	1.09	0.05	3.46*	1.43	0.09	4.032	1.23	0.02

39	VAL	na	na	na	na	na	na	na	na	Na
40	ASP	-10.84^	0.96	0.04	-8.15^	1.27	0.09	-7.562	1.33	0.16
41	GLY	-11.46^^	0.71	0.04	-9.20^	0.88	0.06	-8.342	0.93	0.24
42	VAL	na	na	na	na	na	na	na	na	na
43	TRP	-9.38	1.01	0.04	-7.28	1.3	0.07	-8.082	1.19	0.02
44	THR	-10.52	1.07	0.04	-8.13	1.38	0.08	-9.502	1.19	0.01
45	TYR	-7.6	1.02	0.04	-6.09	1.27	0.07	-7.982	0.99	0.02
46	ASP	-9.1	1.2	0.05	-7.2	1.52	0.06	-9.092	1.2	0.02
47	ASP	-12.07	0.87	0.02	-8.95	1.17	0.05	-9.812	1.09	0.02
48	ALA	-0.57	-2.04	0.23	-0.44	-2.64	0.3	0.6311	2.06	0.4
49	THR	-0.47	-0.43	0.75	-0.35	-0.57	1	-1.452	-0.02	0.07
50	LYS+	5.06	1.05	0.07	4.02	1.32	0.09	4.612	1.15	0.02
51	THR	1.78	0.62	0.26	1.36	0.81	0.35	1.262	1.21	0.11
52	PHE	-11.14	0.89	0.06	-8.71	1.14	0.08	-9.872	1	0.01
53	THR	-9.65	1.09	0.06	-7.79	1.35	0.08	-8.622	1.21	0.02
54	VAL	-11.03	0.92	0.02	-7.92	1.29	0.08	-9.662	1.07	0.01
55	THR	-11.32	0.97	0.03	-8.63	1.27	0.07	-9.942	1.11	0.02
56	GLU	-10.52^	1.05	0.05	-8.54^	1.3	0.1	-8.962	1.27	0.06

[a] If a glycine residue is involved the CCR rate is the sum of the individual rates corresponding to $H^{\alpha 1}$ and $H^{\alpha 2}$

[b] Residue number refers to position i in the amino acid sequence

* Individual S^2 value was missing and has been substituted for the average of neighboring residues

^ Individual S^2 value was missing and has been substituted for the average for the whole protein

Table S9. R.m.s. deviation, slope *s* and Pearson's correlation coefficient *r* between experimental and predicted CCR rates obtained from a rigid GB3 model.

CCR rate	coordinates	r.m.s.d. $[s^{-1}]^{*}$	s^{*}	r^{*}
$R_{HNiNi/HNi+1Ni+1} + R_{HNiNi+1/HNi+1Ni}$	2OED-DIDC†,‡, pairs *i* = 40, 41 excluded	1.51	0.72	0.96
	2OED-DIDC$^{\#,\ddagger}$, pairs *i* = 40, 41 excluded	0.97	0.81	0.96
$R_{HAiCAi/HAi-1CAi-1} + R_{HAiCAi-1/HAi-1CAi}$	2OED-DIDC†,‡, pairs *i* = 11, 40 excluded	6.27	0.76	0.97
	2OED-DIDC$^{\#,\ddagger}$, pairs *i* = 11, 40 excluded	4.16	0.88	0.97
$R_{HNiNi/HAiCAi} + R_{HAiNi/HNiCAi}$	2OED-DIDC†,‡	2.89	0.75	0.98
	2OED-DIDC$^{\#,\ddagger}$	1.57	0.86	0.98
$R_{HNiNi/HAi-1CAi-1} + R_{HAi-1Ni/HNiCAi-1}$	2OED-DIDC†,‡	2.63	0.74	0.98
	2OED-DIDC$^{\#,\ddagger}$	1.61	0.85	0.98

* x axis is the predicted and y axis the experimental rate.

† r_{HN} = 1.02 Å and $r_{H\alpha C\alpha}$ = 1.09 Å are assumed.

‡ Coordinates from RDC-refined X-ray structure (14) whose H^{N} and H^{α} proton positions were subsequently optimized with RDCs (12, 13).

$^{\#}$ r_{HN} = 1.041 Å and $r_{H\alpha C\alpha}$ = 1.117 Å are assumed.

Table S10. Cross validation of ensembles with CCR and ^{3}J coupling data

	CCR rmsd (Hz) [a]				^{3}J coupling rmsd (Hz) [b]			
	H^NN/H^NN	$C^\alpha H^\alpha/C^\alpha H^\alpha$	$H^NN/C^\alpha H^\alpha$	$C^\alpha H^\alpha/H^NN$	H^NH^α	C'C'	H^NC'	H^NC^β
Expt. Err	0.19	1.37	0.20	0.26	0.14	0.03	0.10	0.07
	Original Ensemble							
Ccr16 [c]	0.15	2.02	0.25	0.29	0.55	0.25	0.39	0.72
	Cross Validation (Bold excluded dataset)							
test1 [d]	**0.54**	2.05	0.25	0.29	0.54	0.26	0.40	0.71
test2 [d]	0.15	**3.54**	0.26	0.30	0.54	0.23	0.39	0.73
test3 [d]	0.15	2.04	**1.07**	0.29	0.67	0.24	0.41	0.69
test4 [d]	0.15	1.95	0.25	**1.13**	0.54	0.26	0.41	0.71

[a] bond lengths of 1.041 and 1.117 Å were used to calculate CCRs from structures to account for libration motions that are not present in static structures

[b] Karplus parameters used were those for the fits to Ace-Ala-NMe [20], experimental data [21,22]

[c] Average values calculated from 20 ensembles

[d] Average values calculated from 5 ensembles, tests 1-4 are the same calculation as used for ccr16, but with a single CCR dataset omitted in each case

Table S11. H^N-N and H^α-C^α order parameters S used to calculate F_{corr}.

res #	aa type	S (H^N-N)[a]	ΔS (H^N-N)[a]	S (H^α-C^α)[b]	ΔS (H^α-C^α)[b]	S (H^N-N)[c]	ΔS (H^N-N)[c]	S (H^α-C^α)[c]	ΔS (H^α-C^α)[c]
2	GLN			0.95	0.02			0.85	0.03
3	TYR	0.90	0.02	0.94	0.02	0.88	0.02	0.86	0.03
4	LYS+	0.94	0.02	0.91	0.03	0.85	0.01	0.79	0.02
5	LEU	0.93	0.03	0.95	0.05	0.84	0.02	0.86	0.02
6	VAL	0.93	0.03	0.92	0.03	0.86	0.03	0.87	0.03
7	ILE	0.92	0.03	0.92	0.03	0.82	0.02	0.79	0.03
8	ASN	0.93	0.03	0.81	0.07	0.82	0.03	0.69	0.04
9	GLY	0.91	0.04			0.81	0.02		
10	LYS+	0.80	0.03	0.84	0.02	0.74	0.03	0.78	0.04
11	THR	0.76				0.71	0.04		
12	LEU	0.51	0.06	0.79	0.07	0.48	0.02	0.70	0.04
13	LYS+	0.83	0.03	0.73	0.07	0.79	0.03	0.65	0.04
14	GLY	0.84	0.05			0.72	0.03		
15	GLU	0.81	0.05	0.90	0.05	0.75	0.03	0.81	0.02
16	THR	0.89	0.04	0.96	0.05	0.79	0.02	0.86	0.02
17	THR	0.87	0.02	0.89	0.03	0.80	0.02	0.79	0.02
18	THR	0.91	0.03	0.97	0.03	0.84	0.02	0.85	0.02
19	LYS+	0.89						0.91	0.03
20	ALA	0.83	0.06	0.81	0.10	0.80	0.02	0.73	0.05
21	VAL	0.91	0.06	0.90	0.03	0.78	0.05	0.76	0.02
22	ASP	0.88	0.02	0.92	0.04	0.83	0.01	0.84	0.02
23	ALA	0.91	0.02	0.91	0.03	0.79	0.01	0.78	0.04
24	GLU	0.87	0.03	0.93	0.02	0.79	0.03	0.86	0.02
25	THR	0.90				0.86	0.01		
26	ALA	0.92	0.02	0.91	0.03	0.84	0.01	0.79	0.02
27	GLU	0.94	0.03	0.90	0.03	0.84	0.02	0.77	0.02
28	LYS+	0.91	0.02	0.94	0.02	0.84	0.02	0.82	0.01
29	ALA	0.90	0.02	0.95	0.02	0.85	0.01	0.82	0.03
30	PHE	0.91				0.81	0.01		
31	LYS+	0.95	0.04	0.97	0.03	0.79	0.03	0.86	0.02
32	GLN	0.89	0.02	0.95	0.03	0.81	0.01	0.82	0.01
33	TYR	0.91	0.02	0.91	0.02	0.82	0.01	0.79	0.02
34	ALA	0.91	0.04	0.96	0.03	0.80	0.03	0.84	0.03
35	ASN	0.87	0.03	0.94	0.03	0.87	0.02	0.83	0.02
36	ASP	0.89	0.02	0.89	0.03	0.83	0.01	0.83	0.02
37	ASN	0.88	0.03	0.90	0.02	0.76	0.01	0.78	0.03
38	GLY	0.94	0.04			0.89	0.02		
39	VAL	0.86	0.02	0.92	0.02	0.77	0.02	0.80	0.01

40	ASP	0.90				0.82	0.02		
41	GLY	0.77							
42	VAL	0.95	0.04	0.99	0.03	0.80	0.03	0.84	0.02
43	TRP	0.93	0.03	0.94	0.03	0.82	0.02	0.88	0.03
44	THR	0.91	0.06	0.91	0.03	0.91	0.03	0.80	0.04
45	TYR	0.92	0.04	0.96	0.03	0.80	0.03	0.88	0.03
46	ASP	0.94	0.03	0.91	0.04	0.85	0.02	0.81	0.02
47	ASP	0.87	0.06	1.00	0.02	0.70	0.03	0.82	0.02
48	ALA	0.83	0.04	0.86	0.03	0.70	0.02	0.79	0.02
49	THR	0.91	0.03	0.97	0.02	0.79	0.01	0.84	0.01
50	LYS+	0.94	0.04	0.91	0.03	0.80	0.02	0.82	0.03
51	THR	0.95	0.03	0.91	0.03	0.83	0.02	0.78	0.03
52	PHE	0.95	0.04	0.94	0.03	0.83	0.02	0.82	0.02
53	THR	0.95	0.03	0.93	0.03	0.81	0.04	0.83	0.03
54	VAL	0.94	0.03	0.92	0.03	0.84	0.03	0.79	0.03
55	THR	0.96	0.03	0.94	0.03	0.85	0.04	0.83	0.04
56	GLU	0.95	0.04			0.80	0.03		

[a] Taken from reference 13. The samples used in that study were deuterated, while the mutants were the same as in the original study with protonated samples. [23]

[b] Taken from reference 23.

[c] Taken from reference 14.

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