Observation of α-Helical Hydrogen Bond Cooperativity in an Intact Protein

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Supporting Information

Methods and Materials

Protein expression and purification. Details regarding the procedure of GB3 expression and purification are as described previously.[1] Final samples were prepared in buffer consisting of 10 mM sodium acetate, pH 4.3, or 10 mM sodium phosphate at pH 7.0 in a 15%/85% (v/v) H_2O/D_2O mixture. The pH was read directly from a glass electrode pH meter without correction for the isotope effect.

NMR spectroscopy and exchange rate fitting. All NMR experiments were carried out on a Bruker Avance 600-MHz spectrometer, equipped with a z-axis gradient, triple resonance, cryogenic probe. For each sample, 3–4 mg of ^{15}N-labeled protonated GB3 powder was added to a 500 μl 15%/85% H_2O/D_2O mixed buffer and transferred immediately to a NMR tube which was then inserted into the NMR spectrometer. Recordings of gradient-enhanced 2D ^{15}N-^{1}H HSQC spectra with the Rance-Kay readout scheme[2] were started 45 minutes after the mixing. The first five spectra each had an acquisition time of 15 minutes and then the acquisition time was increased to 30 minutes by doubling the number of scans. A total of 25 spectra were recorded over a period of 12 h. The acquisition times were 70.5 ms (^{15}N) and 83.0 ms (^{1}H) with the data matrices consisting of 94*×1024* data points, where N* indicates N complex points. The same experiments were performed for the WT (which includes a E27Q mutation to the original GB3 sequence), V21A, V21L, E24Q, T25A, T25S, D36N, D36E, N37A, and N37Q, at low pH (4.3) and temperatures (275 K and 280 K), as well as WT, K28L, A29S, K31L, and Q32N, at high pH (7.0) and temperatures (298 K and 303 K for WT, K28L, and K31L, and 293 K and 298 K for A29S and Q32N). The assignment of the WT spectrum was obtained from a HNCACB spectrum (48*(^{13}C)×20*(^{15}N)×1024*(^{1}H) data points, and acquisition times of 4.3 ms (^{13}C), 10.0 ms (^{15}N), and 121 ms (^{1}H)).[3] The assignments of the mutants were made by comparing their ^{15}N-^{1}H HSQC and ^{15}N-edited NOESY spectra (40*(^{1}H, indirect)×20*(^{15}N)×1024*(^{1}H, direct) data points with corresponding acquisition times of 4.8 ms, 10.0 ms, and 121 ms, respectively) with those of the WT.

For the first order H/D exchange reaction, the concentration of a N–H changes as follows

$$[NH](t) = [NH](0)\left(\exp(-kt) + c\right) \quad \text{(S1)}$$

where [NH] is the concentration of the amide N–H, and k is the exchange rate. The constant $c = 0.15/0.85 = 0.18$ is derived from the fact that the solvent contains 15% of H_2O so that at the end of the exchange the concentration should be 15% of the initial value. The N–H concentration is proportional to the peak volume in the ^{15}N-^{1}H HSQC spectrum, and the rate k therefore can be determined from fitting of the time dependence of the peak volume:

$$I(t) = I(0)\left(\exp(-kt) + c\right) \quad \text{(S2)}$$

where I(t) is the peak volume at time t. One slight problem with the peak volume measurement is that the peak position changes slightly as the H/D exchange proceeds so that the region defining the peak has to be large enough for its volume measurement to remain accurate. For a peak that has other peaks nearby, this becomes difficult and only the peak height can be determined with good accuracy (about 10 amides are in such a scenario for GB3). Unlike the peak volume, the peak height is affected by the exchange rate caused by the variable isotope shift dispersion, which has the effect of slightly broadening the linewidth, primarily in the ^{15}N dimension. As a result, using peak height in the fitting of eq. S2 tends to slightly overestimate the exchange rate k. Figure S1 shows that the exchange rate fitted using the peak height is about 7.4% larger than that obtained from the peak volume fitting of well separated peaks of WT GB3 at 298 K and 303 K, with the peak volume and height extracted using the NMRView[4] and NMRPipe[5] programs, respectively. In the exchange rate fitting for all the measurements, the peak height was used and then the rate was corrected using the linear equation k_volume = k_height/1.074 in Figure S1.

Relationship between Δδ and *k*. It is observed from the experiments that as the backbone amide H/D exchange proceeds, the ^{1}H, ^{15}N chemical shifts in the ^{15}N-^{1}H HSQC spectra also change. A simple model can be used to help understand these changes. Assuming there are only two amides (amide 1 and amide 2) in the protein,

the H/D isotope state in amide 2 affects the chemical shift of amide 1 and vice versa. Taking the ^{15}N of amide 1 as an example, its value δ equals to δ_D if amide 2 has N–D or δ_H if amide 2 has N–H. If amide 2 has a mixture of H/D, a linear approximation is introduced, $\delta = p_H \times \delta_H + p_D \times \delta_D$, where p_H and p_D are the population of amide 2 having N–H and N–D, respectively. In principle, δ_1 is also affected by the H/D state of amide 1. But since the ^{15}N-^{1}H HSQC peak of amide 1 appears only when it has N–H, the D substitution at amide 1 has no effect on its peak position and simply decreases its signal intensity. Following eq S2, $p_H = I(t)/I(0)$, $p_D = 1 - p_H$, δ of amide 1 can be written as

$$\delta(t) = \delta_H\left(\exp(-kt) + c\right)/(1 + c) + \delta_D\left(1 - \exp(-kt)\right)/(1 + c) \quad \text{(S3)}$$

or

$$\delta(t) = \delta_H + (\delta_H - \delta_D)\,(\exp(-kt) - 1)/(1 + c) \quad \text{(S4)}$$

Similarly if there are two amides (amides 2 and 3) with isotope effects that contribute to the chemical shift δ of amide 1, this δ can be written as

$$\delta = p_{2H,3H} \times \delta_{2H,3H} + p_{2H,3D} \times \delta_{2H,3D} + p_{2D,3H} \times \delta_{2D,3H} + p_{2D,3D} \times \delta_{2D,3D} \quad \text{(S5)}$$

where

$$p_{2H,3H} = (\exp(-k_1 t) + c)\,(\exp(-k_2 t) + c)/(1 + c)^2 \quad \text{(S6)}$$

$$p_{2H,3D} = (\exp(-k_1 t) + c)\,(1 - \exp(-k_2 t))/(1 + c)^2 \quad \text{(S7)}$$

$$p_{2D,3H} = (1 - \exp(-k_1 t))\,(\exp(-k_2 t) + c)/(1 + c)^2 \quad \text{(S8)}$$

$$p_{2D,3D} = (1 - \exp(-k_1 t))\,(1 - \exp(-k_2 t))/(1 + c)^2 \quad \text{(S9)}$$

and $\delta_{2H,3H}$, $\delta_{2H,3D}$, $\delta_{2D,3H}$, and $\delta_{2D,3D}$ are the chemical shifts when the isotope states of amides 2 and 3 are HH, HD, DH, and DD, respectively. Incorporating eq. S6– eq. S9 to eq. S5 gives

$$\delta(t) = \delta_{2H,3H} + \lambda_1(\exp(-k_1 t) - 1)/(1 + c) + \lambda_2(\exp(-k_2 t) - 1)/(1 + c) \quad \text{(S10)}$$

with the assumption that $\delta_{2H,3H} - \delta_{2D,3H} = \delta_{2H,3D} - \delta_{2D,3D} = \lambda_1$ and $\delta_{2H,3H} - \delta_{2H,3D} = \delta_{2D,3H} - \delta_{2D,3D} = \lambda_2$. Eq. S10 can be easily generalized for a protein with N amides:

$$\delta_i(t) = \delta_{i0} + \sum_{j=1}^{n} \lambda_{ij}(\exp(-k_j t) - 1) / (1 + c) \quad \text{(S11)}$$

where δ_{i0} corresponds to the ^{15}N chemical shift of the measured amide *i* with all other amides having N–H. The same equation can be derived for the amide ^{1}H chemical shift. The fitting of peak intensities and $\Delta\delta$ were performed using Matlab programs developed in-house.

Quantum mechanical calculations. A tri-N-methylactamide ($(NMA)_3$) H-bonding complex was built with peptide bond coordinates that match those of the peptide groups that include the amides of α-helical residues F30, Y33 and D36 in the structure of GB3. The quantum mechanical (QM) calculations were performed using the MP2 method which produces a geometry and interaction energy for H-bonded systems that agree well with higher level QM methods.[6,7] The complex was geometry-optimized at the MP2/6-311+g* level with all the H-bond geometric parameters fixed except the distance d_{NO} between NMA_2 and NMA_1. After the geometry optimization, d_{NO} was increased with a step of 0.005 Å while all other geometric parameters were fixed by translating all NMA_1 atoms in the direction of the NMA_2-N$^{\cdots}$O-NMA_1 H-bond (Figure 3A). Chemical shifts were calculated using the GIAO method[8,9] at the MP2/6-311+g* level. Single point MP2/6-311+g* calculations were also performed for each structure and then the natural bond orbital (NBO) charge was extracted for each atom. The electric dipole moment of each NMA was calculated manually using the NBO charges with the origin set at the midpoint of the (H)N–C(O) bond, yielding values of 5.7, 5.6, and 6.0 Debye for NMA_1, NMA_2 and NMA_3 respectively. The H-bond energy was calculated using the equation E(H-bond) = E(NMA_1-NMA_2-NMA_3) – E(NMA_1) – E(NMA_2-NMA_3) for the NMA_1-NMA_2 H-bond, and E(H-bond) = E(NMA_1-NMA_2-NMA_3) – E(NMA_3) – E(NMA_1-NMA_2) for the NMA_2-NMA_3 H-bond, yielding an energy of 8.0 kcal/mol for the former and 8.1 kcal/mol for the latter after the correction of the Basis set Superposition error (BSSE).[10] Gaussian 09 was used for all quantum mechanical calculations.[11]

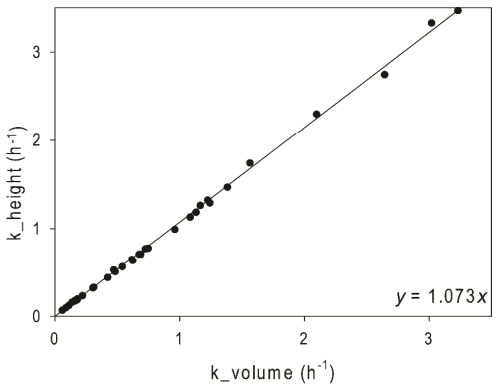

Figure S1. Correlation between the H/D exchange rate (*k*_volume) obtained by fitting the peak volume and that (*k*_height) obtained by fitting the peak height for well separated peaks in the ^{15}N-^{1}H HSQC spectra of WT GB3 at 298 K and 303 K. The best fitted line is $y = 1.073x$, with the correlation coefficient R^2 of 0.99.

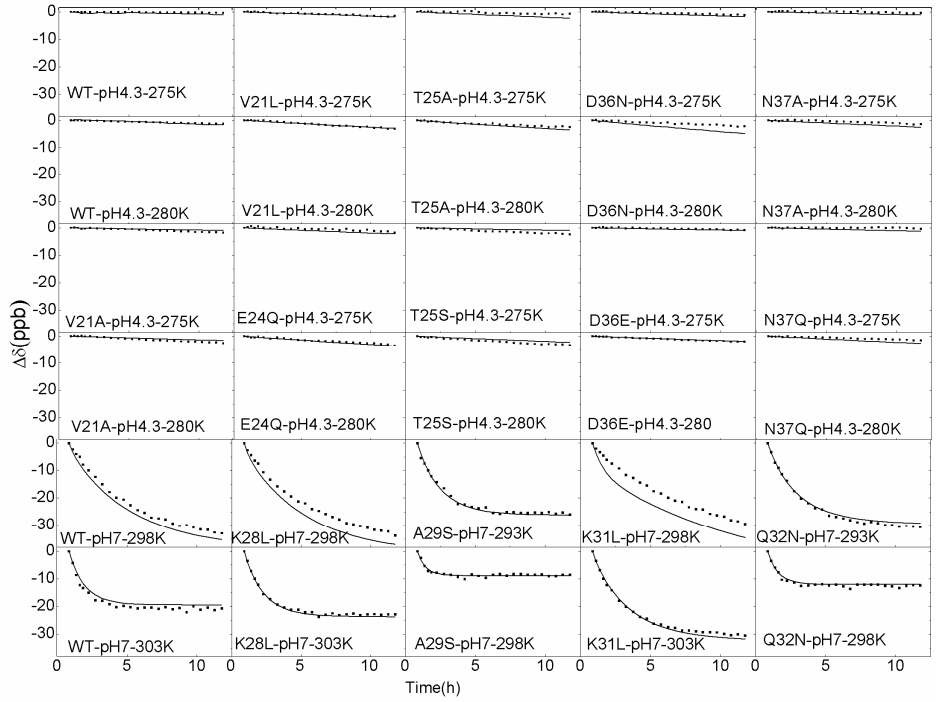

Figure S2. Change of the backbone amide ^{15}N chemical shift, Δδ, of A29 as a function of time after the protonated sample was dissolved in 85%D_2O/15%H_2O. The dots are the experimental numbers whereas the solid lines represent the global fit of Δδ to eq. 1. The deviation between the experimental and predicted Δδ values at 298 K for K28L and K31L is likely due to the $\lambda_{ij} = \lambda_l$ assumption. The deviation of Δδ values in principle affects the fitted λ_l values. However, there are a total of 210 fitted curves, so the effect of small deviations of a few curves on λ_l values is small.

Table S1. Residue specific exchange rates k (h^{-1}) for the "WT" and mutants at various temperature and pH values

Res.	WT		V21A		V21L		E24Q		T25A	
	275 K	280 K	275 K	280 K	275 K	280 K	275 K	280 K	275 K	280 K
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.000	0.000	0.001	0.000	0.000	0.001	0.003	0.003	0.003	0.003
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.002	0.002	0.002
7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
8	0.020	0.043	0.024	0.047	0.024	0.048	0.024	0.048	0.024	0.050
9	0.419	0.764	0.500	0.993	0.490	0.852	0.434	0.750	0.477	0.855
10	f[a]	f	f	f	f	f	f	f	f	f
11	f	f	f	f	f	f	f	f	f	f
12	f	f	f	f	f	f	f	f	f	f
13	0.228	0.451	0.274	0.543	0.267	0.512	0.234	0.461	0.252	0.482
14	0.336	0.693	0.390	0.837	0.389	0.774	0.352	0.638	0.358	0.678
15	2.547	f	f	f	f	f	2.109	f	2.328	f
16	0.068	0.142	0.069	0.136	0.080	0.165	0.070	0.142	0.068	0.132
17	0.755	1.525	1.039	2.363	1.000	1.774	0.629	1.237	0.715	1.661
18	0.071	0.155	0.061	0.124	0.094	0.200	0.072	0.155	0.111	0.212
19	4.115	f	f	f	f	f	f	f	f	f
20	0.080	0.183	0.161	0.322	0.142	0.293	0.088	0.189	0.069	0.156
21	f	f	f	f	f	f	f	f	f	f
22	0.042	0.107	0.049	0.115	0.049	0.114	0.053	0.129	0.027	0.066
23	1.177	2.181	2.390	f	2.094	f	1.441	2.868	2.513	f
24	0.165	0.354	0.249	0.533	0.238	0.506	0.333	0.666	0.209	0.432
25	0.006	0.023	0.019	0.056	0.021	0.061	0.016	0.048	0.000	0.008
26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
27	0.000	0.000	0.000	0.000	0.001	0.002	0.002	0.003	0.002	0.002
28	0.000	0.001	0.001	0.002	0.003	0.004	0.003	0.006	0.004	0.005
29	0.001	0.003	0.002	0.004	0.003	0.005	0.004	0.007	0.003	0.005
30	0.000	0.000	0.000	0.000	0.001	0.001	0.002	0.002	0.002	0.002
31	0.000	0.000	0.000	0.000	0.001	0.002	0.002	0.003	0.002	0.002
32	0.001	0.004	0.003	0.005	0.004	0.007	0.004	0.007	0.004	0.007
33	0.003	0.006	0.004	0.007	0.005	0.007	0.006	0.008	0.007	0.010
34	0.000	0.000	0.000	0.000	0.001	0.002	0.002	0.003	0.002	0.002
35	0.000	0.004	0.002	0.004	0.003	0.007	0.003	0.008	0.003	0.007
36	0.033	0.069	0.029	0.059	0.034	0.070	0.039	0.080	0.040	0.078
37	0.000	0.011	0.000	0.009	0.000	0.012	0.000	0.014	0.000	0.011
38	0.107	0.204	0.129	0.230	0.122	0.224	0.113	0.209	0.135	0.250
39	0.002	0.001	0.002	0.000	0.003	0.001	0.003	0.002	0.006	0.000
40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
41	1.877	f	1.757	f	1.743	f	1.677	f	1.582	f
42	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
43	0.042	0.096	0.061	0.129	0.055	0.114	0.054	0.118	0.048	0.110
44	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.002	0.001	0.002

45	2.031	f	2.410	f	f	f	2.038	f	1.697	f
46	0.000	0.000	0.000	0.000	0.002	0.002	0.003	0.004	0.003	0.003
47	f	f	f	f	f	f	f	f	f	f
48	0.657	1.234	0.913	1.926	0.849	1.445	0.668	1.221	0.628	1.319
49	0.040	0.093	0.056	0.120	0.051	0.106	0.044	0.097	0.045	0.103
50	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
51	0.000	0.000	0.001	0.000	0.001	0.002	0.002	0.003	0.003	0.002
52	0.000	0.000	0.000	0.000	0.001	0.001	0.002	0.002	0.003	0.002
53	0.000	0.000	0.000	0.000	0.001	0.001	0.002	0.002	0.002	0.002
54	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000
55	0.001	0.001	0.002	0.002	0.002	0.003	0.003	0.003	0.003	0.004
56	0.029	0.060	0.025	0.050	0.028	0.058	0.031	0.065	0.031	0.060

Res.	T25S		D36N		D36E		N37A		N37Q	
	275 K	280 K	275 K	280 K	275 K	280 K	275 K	280 K	275 K	280 K
3	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.000
4	0.000	0.000	0.001	0.002	0.000	0.000	0.001	0.001	0.001	0.001
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.001
7	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
8	0.023	0.045	0.025	0.051	0.025	0.050	0.009	0.019	0.007	0.015
9	0.483	0.828	0.412	0.722	0.500	0.882	0.130	0.257	0.183	0.361
10	f	f	f	f	f	f	f	f	f	f
11	f	f	f	f	f	f	f	f	f	f
12	f	f	f	f	f	f	f	f	1.926	f
13	0.251	0.476	0.237	0.472	0.263	0.521	0.000	0.000	0.000	0.000
14	0.368	0.709	0.345	0.679	0.391	0.784	0.158	0.328	0.239	0.458
15	2.652	f	f	f	3.134	f	1.772	2.980	3.305	f
16	0.076	0.153	0.071	0.149	0.080	0.162	0.023	0.051	0.034	0.074
17	0.912	1.650	1.037	2.005	1.041	1.954	0.462	0.871	0.929	1.691
18	0.115	0.235	0.059	0.128	0.077	0.164	0.031	0.066	0.037	0.083
19	f	f	f	f	f	f	1.966	f	4.292	f
20	0.080	0.161	0.087	0.199	0.104	0.219	0.059	0.112	0.073	0.159
21	f	f	f	f	f	f	3.636	f	f	f
22	0.034	0.078	0.046	0.116	N/A[b]	N/A[b]	0.038	0.087	0.048	0.116
23	2.006	3.850	1.073	2.099	1.332	2.549	0.625	1.317	0.985	1.926
24	0.261	0.534	0.176	0.376	0.195	0.396	0.134	0.284	0.164	0.344
25	0.037	0.087	0.007	0.025	0.007	0.026	0.004	0.020	0.007	0.024
26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
27	0.000	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.001
28	0.002	0.005	0.002	0.004	0.001	0.002	0.001	0.002	0.002	0.003
29	0.002	0.004	0.003	0.007	0.002	0.004	0.001	0.002	0.003	0.005
30	0.000	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.001
31	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.001	0.001	0.001
32	0.003	0.007	0.004	0.011	0.003	0.006	0.003	0.007	0.003	0.007
33	0.005	0.008	0.001	0.005	0.001	0.002	0.004	0.007	0.020	0.034
34	0.000	0.001	0.002	0.006	0.000	0.001	0.001	0.001	0.000	0.001

35	0.003	0.007	0.008	0.019	0.003	0.007	0.000	0.003	0.000	0.002
36	0.038	0.079	0.056	0.119	0.015	0.032	0.084	0.172	0.046	0.090
37	0.000	0.014	0.032	0.087	N/A[b]	N/A[b]	0.001	0.007	0.002	0.005
38	0.129	0.229	0.312	0.610	0.208	0.375	0.013	0.027	0.017	0.034
39	0.002	0.000	0.000	0.003	0.001	0.000	0.001	0.002	0.020	0.003
40	0.000	0.000	0.000	0.015	0.000	0.000	0.001	0.000	0.003	0.000
41	1.724	f	1.884	f	1.903	f	1.107	1.923	1.200	2.020
42	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.007	0.003	0.003
43	0.050	0.100	0.052	0.002	0.059	0.123	0.026	0.056	0.045	0.097
44	0.000	0.000	0.001	0.006	0.000	0.000	0.000	0.000	0.000	0.001
45	1.906	f	2.036	f	2.349	f	1.009	1.901	1.709	f
46	0.001	0.000	0.002	0.004	0.001	0.001	0.001	0.001	0.002	0.002
47	f	f	f	1.299	f	f	f	f	f	f
48	0.729	1.259	0.679	0.103	0.861	1.525	0.414	0.771	0.663	1.226
49	0.047	0.097	0.046	0.000	0.052	0.111	0.031	0.070	0.047	0.103
50	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
51	0.000	0.000	0.001	0.002	0.000	0.000	0.000	0.001	0.001	0.001
52	0.000	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.001	0.001
53	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.001	0.001
54	0.000	0.000	0.000	0.004	0.000	0.000	0.004	0.023	0.166	0.345
55	0.001	0.002	0.002	0.059	0.001	0.002	0.001	0.002	0.001	0.002
56	0.027	0.059	0.029	0.060	0.026	0.055	0.022	0.048	0.020	0.044

Res.	WT		K28L		A29S		K31L		Q32N	
	298 K	303 K	298 K	303 K	293 K	298 K	298 K	303 K	293 K	298 K
3	0.918	f	1.056	f	N/A[c]	f	0.875	f	1.222	f
4	0.395	1.526	0.534	1.629	0.505	1.596	0.411	1.527	0.681	2.181
5	0.144	0.656	0.205	0.672	0.195	0.666	0.140	0.616	0.266	0.895
6	0.060	0.303	0.084	0.304	0.078	0.297	0.060	0.275	0.108	0.434
7	0.156	0.586	0.205	0.577	0.189	0.567	0.146	0.539	0.236	0.722
8	f	f	f	f	f	f	f	f	f	f
9	f	f	f	f	f	f	f	f	f	f
10	f	f	f	f	f	f	f	f	f	f
11	f	f	f	f	f	f	f	f	f	f
12	f	f	f	f	f	f	f	f	f	f
13	f	f	f	f	f	f	f	f	f	f
14	f	f	f	f	f	f	f	f	f	f
15	f	f	f	f	f	f	f	f	f	f
16	f	f	f	f	f	f	f	f	f	f
17	f	f	f	f	f	f	f	f	f	f
18	f	f	f	f	f	f	f	f	f	f
19	f	f	f	f	f	f	f	f	f	f
20	f	f	f	f	N/A[c]	f	f	f	f	f
21	f	f	f	f	f	f	f	f	f	f
22	f	f	f	f	f	f	f	f	f	f
23	f	f	f	f	f	f	f	f	f	f
24	f	f	f	f	f	f	f	f	f	f
25	f	f	f	f	f	f	f	f	f	f

26	0.486	1.616	1.012	2.481	0.439	1.326	0.858	2.561	0.613	1.750
27	0.296	1.171	0.490	1.282	0.383	1.231	0.365	1.294	0.438	1.417
28	1.144	3.222	0.378	0.912	1.847	4.190	0.695	2.002	1.277	3.084
29	3.263	f	1.481	3.014	f	f	1.618	4.274	2.019	4.427
30	0.168	0.721	0.175	0.574	0.720	2.153	0.114	0.495	0.304	1.042
31	0.238	1.055	0.239	0.840	0.460	1.421	0.063	0.276	0.612	1.874
32	3.095	f	2.533	f	f	f	1.330	3.463	f	f
33	0.520	1.363	0.534	1.169	0.593	1.332	0.333	0.919	2.453	f
34	0.327	1.169	0.411	1.148	0.408	1.237	0.188	0.721	0.680	1.712
35	1.100	3.224	1.238	3.196	1.323	3.175	0.696	2.100	2.284	5.125
36	2.138	f	2.318	4.835	2.218	4.205	1.341	3.258	3.980	f
37	1.048	2.554	1.194	2.631	1.074	2.247	0.689	1.867	2.054	4.038
38	f	f	f	f	f	f	f	f	f	f
39	0.213	0.587	0.278	0.599	0.196	0.477	0.154	0.448	0.340	0.755
40	f	f	f	f	f	f	f	f	f	f
41	f	f	f	f	f	f	f	f	f	f
42	0.182	0.568	0.187	0.455	0.175	0.437	0.092	0.305	0.168	0.471
43	f	f	f	f	f	f	f	f	f	f
44	0.084	0.406	0.115	0.412	0.095	0.377	0.085	0.374	0.135	0.531
45	f	f	f	f	f	f	f	f	f	f
46	0.116	0.483	0.153	0.490	0.130	0.459	0.121	0.480	0.177	0.601
47	f	f	f	f	f	f	f	f	f	f
48	f	f	f	f	f	f	f	f	f	f
49	f	f	f	f	f	f	f	f	f	f
50	1.197	f	1.163	2.468	1.214	2.393	1.029	2.682	1.095	2.407
51	0.178	0.757	0.257	0.815	0.212	0.750	0.197	0.824	0.301	1.003
52	0.161	0.656	0.234	0.719	0.192	0.660	0.181	0.720	0.276	0.873
53	0.180	0.714	0.266	0.788	0.220	0.740	0.205	0.787	0.311	0.977
54	0.067	0.307	0.094	0.337	0.078	0.288	0.074	0.326	0.110	0.424
55	0.109	0.466	0.154	0.493	0.118	0.418	0.114	0.478	0.155	0.561
56	0.601	1.227	0.595	1.043	0.526	0.908	0.493	0.965	0.470	0.852

[a.] Hydrogen exchange is faster than 6 h^{-1}, and the rate cannot be determined quantitatively from the recorded data.

[b.] The exchange rates are not available due to overlap between D22 and N37.

[c.] The exchange rates are not available due to overlap between Y3 and A20.

Table S2. F-test analysis of the fitting models

	m = 1		m = 2		m = 3		m = 4	
	χ^2 (ppb^2)	p-value	χ^2 (ppb^2)	p-value[a]	χ^2 (ppb^2)	p-value[b]	χ^2 (ppb^2)	p-value
^{15}N	25.6	N/A	21.3	2×10^{-8}	5.038	$<10^{-8}$	5.035	0.94
^{1}H	0.288	N/A	0.267	4.3×10^{-4}	0.134	$<10^{-8}$	0.130	0.052

[a]. P-value was calculated for the F-test using an online calculator at: http://www.danielsoper.com/statcalc

[b.] The p-value for the F-test from model 2 (m = 2) to model 3 (m = 3) is < 10^{-8}, the smallest number available from the calculator.

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