Monomeric α-Synuclein Binds Congo Red Micelles in a Disordered Manner

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SUPPORTING INFORMATION

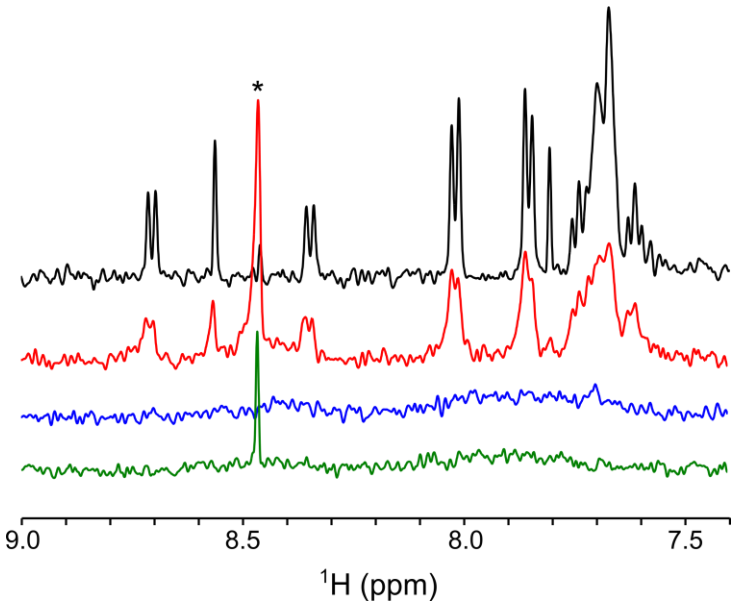

Figure S1. ^{1}H NMR spectra of Congo Red under different conditions: 0.5 µM CR in 1:1 deuterated isopropanol:D2O at pH6 (black); 2 µM CR in 1:1 deuterated isopropanol:D2O at pH 6 (red); 0.5 µM CR in D2O at pH6 (blue), 0.5 µM CR in D2O at pH 8.6 (green). Notice that already at 2 µM in 1:1 isopropanol:D2O there is significant broadening of CR peaks indicative of aggregation. In the absence of isopropanol, CR is strongly aggregated already at 0.5 µM concentration. Buffer pH has a strong effect on macroscopic behavior of CR solutions; in particular, there is higher propensity for precipitate formation at lower pH. However, we observed no narrow peaks in ^{1}H NMR spectra of 0.5 µM CR at pH values as high as 8.6 in the absence of isopropanol.

Experiments were collected on a 500 MHz spectrometer equipped with a cryo-probe at 30 °C. For 0.5 µM samples 16,000 scans were collected, and for the 2 µM sample 1000 scans were collected, so as to match the signal to noise ratios. The spectrum of the 2 µM sample was scaled up 4-fold, to match the signal levels. The asterisk marks a likely impurity.

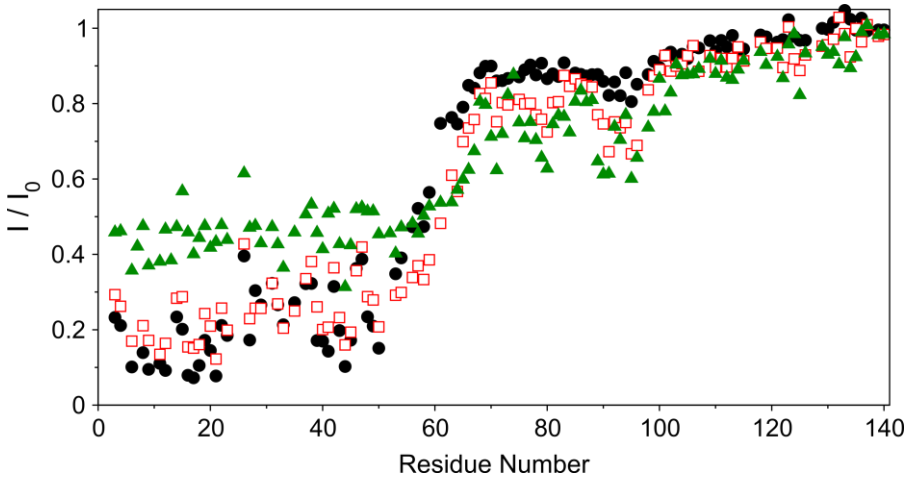

Figure S2. Effect of sample concentration on intensity attenuation (I/I_o) in the presence of Congo Red (15 °C, pH 6.0, $^{2}H/^{15}N$-aS, 500 MHz). A 200 μM sample with equimolar concentrations of aS and CR was prepared, and was subsequently diluted to different concentrations. Shown are relative intensities of HSQC peaks compared to free protein for the following samples: 50 μM aS (black filled circles), 6 μM aS (red open squares), 2 μM aS (green filled triangles).

Acquisition time was 180 ms in the ^{1}H dimension and 165 ms in the ^{15}N dimension. Spectra were apodized with a 72°-shifted sine bell window function in ^{1}H, and with a cosine bell window function in the ^{15}N dimension.

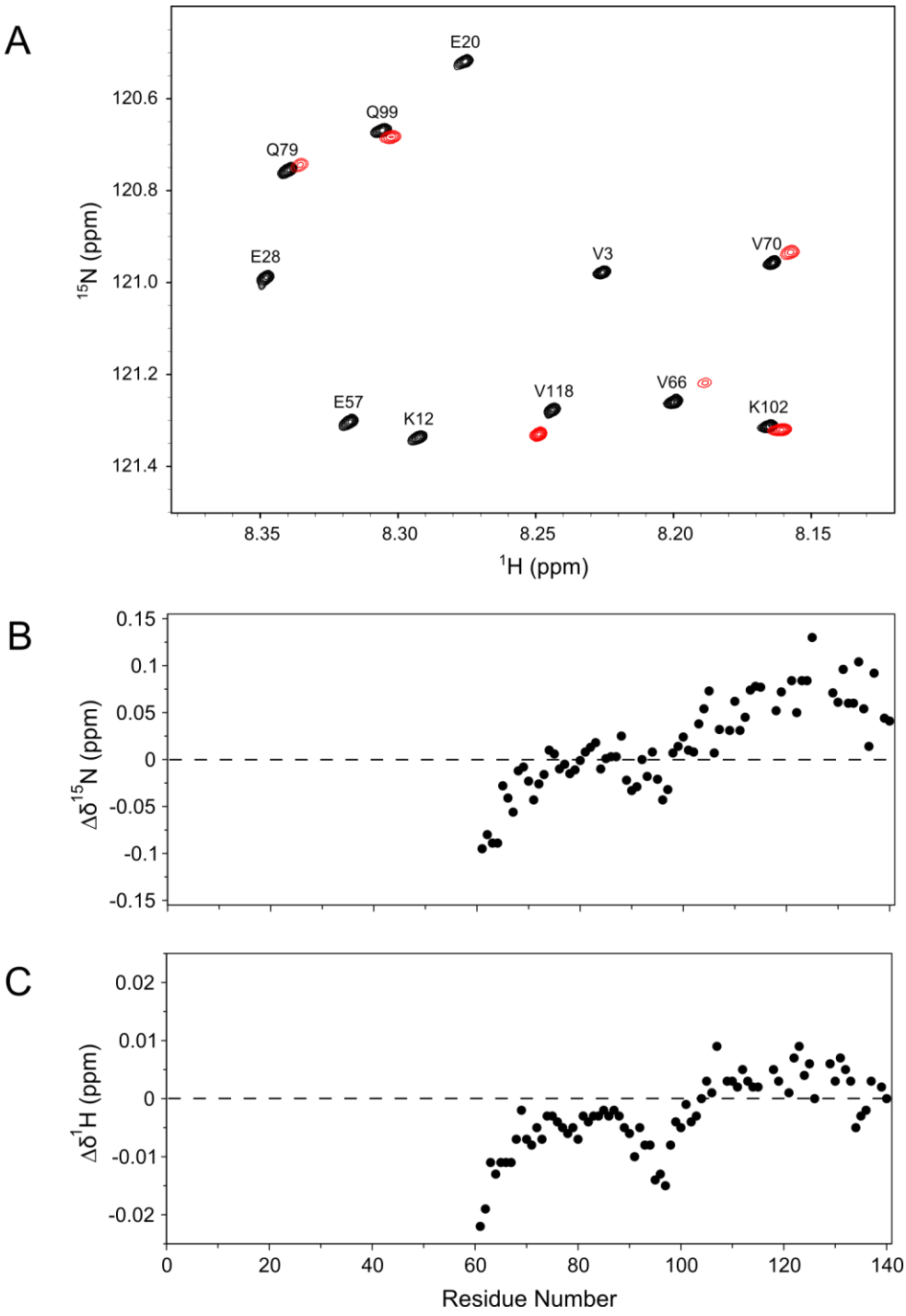

Figure S3. Backbone ^{15}N and $^{1}H^{N}$ chemical shift changes induced by Congo Red. (A) Overlay of expanded small regions of ^{15}N-^{1}H TROSY-HSQC spectra acquired for free aS (black) and at 4:1 CR:aS (red). Spectra were recorded on an 800 MHz spectrometer equipped with a cryo-probe at 15 °C on perdeuterated aS. Acquisition time in each dimension was 750 ms. Spectra were apodized with a 45°-shifted sine bell window function in both dimensions. (B) ^{15}N chemical shift difference and (C) $^{1}H^{N}$ chemical shift difference compared to free aS. The dashed horizontal lines indicate zero change.

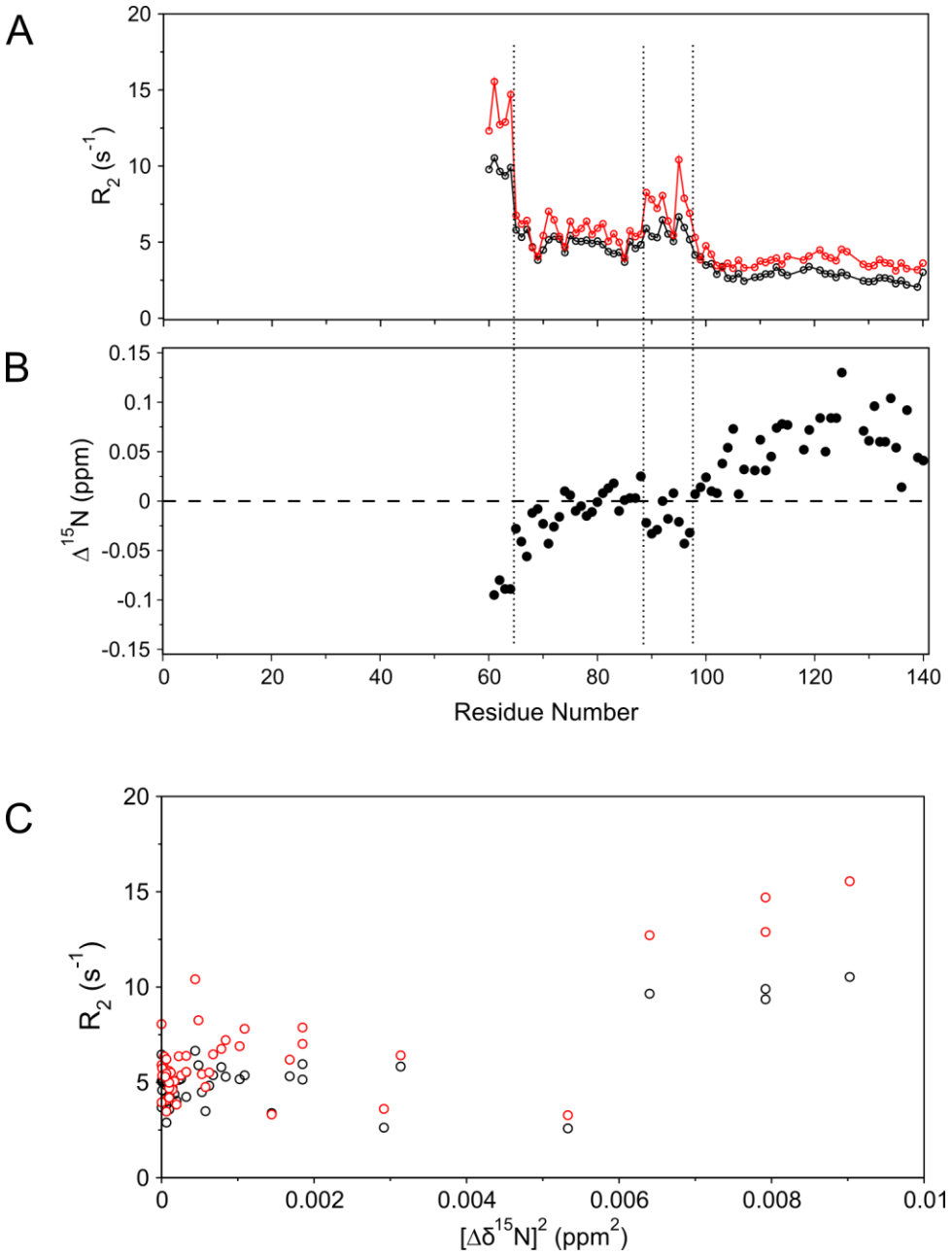

Figure S4. Results of CPMG R_2 relaxation experiments performed on a sample with CR:aS of 4:1. (A) Relaxation rate measured for an effective field of 80 Hz at 500 MHz (black) and 800 MHz (red) as a function of residue number. (B) Change in ^{15}N chemical shift compared to free protein. The dashed horizontal line in B provides the baseline for absence of chemical shift change. The dotted vertical lines serve to delineate regions with distinct behaviors. (C) Correlation of ^{15}N squared chemical shift change with relaxation rate observed at 500 MHz (black) and 800 MHz (red) for residues 61-105.

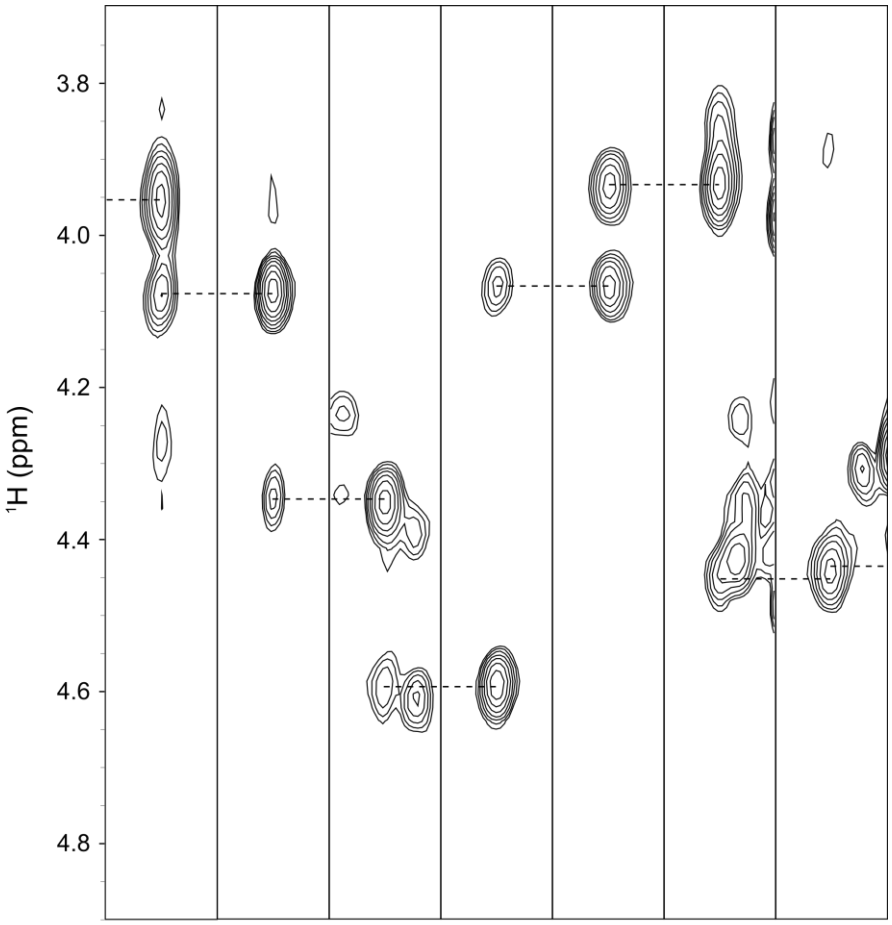

Figure S5. Strip plot of several sequential residues taken from the 600 MHz 3D HHN NOESY-HMQC spectrum of a sample with 1:1 molar ratio of CR:aS. In each strip the stronger peak corresponds to NOE from $^1H^{\alpha}$ of the preceding residue, and the weaker peak is the intraresidue $^1H^{\alpha}$-$^1H^N$ NOE. Notice the absence of $d_{\alpha N}(i, i+3)$ NOE cross peaks, which would be characteristic of α-helix.

Spectra were recorded for fully protonated and ^{15}N-labeled aS, 200 μM, 15°C, pH 6.0. The NOE mixing time was 70 ms. Acquisition times were 54 ms in the ^{15}N dimension and 26 ms in the indirect ^{1}H dimension.

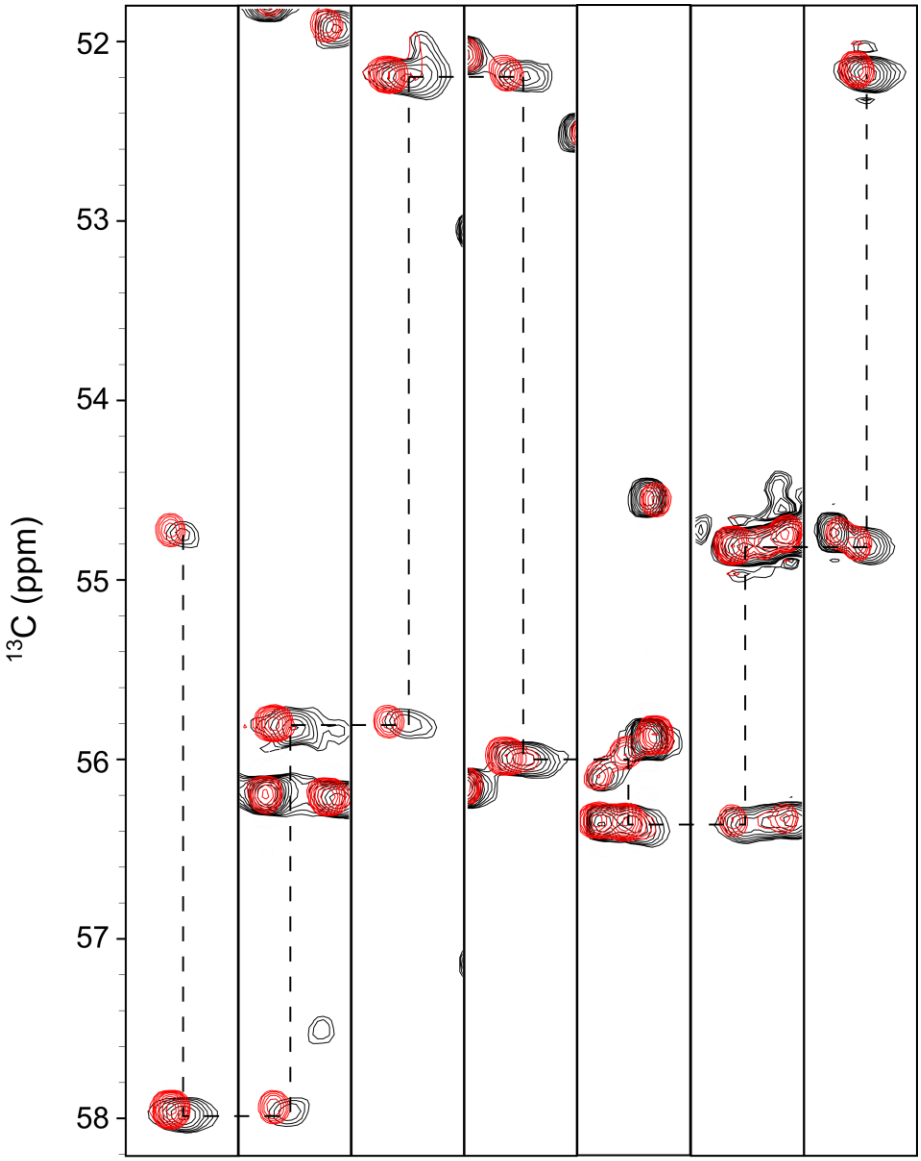

Figure S6. Superimposed strips taken from 3D CT-HNCA spectra of free aS (red), and of aS in the presence of Congo Red at 1:1 molar ratio (black). The spectra were collected on perdeuterated, ^{15}N- and ^{13}C-labeled aS, at 15°C, 200 μM, pH 6.0, on a 500 MHz spectrometer equipped with a cryo-probe. The spectra were folded in the ^{13}C dimension with the carrier at 56 ppm and a spectral width of 10 ppm. Constant-time evolution was used in the ^{13}C dimension with a total acquisition time of 26 ms. Mixed-time evolution (Ying at el, J. Biomol. NMR, 37, 195-204, 2007) was used in the ^{15}N dimension with an acquisition time of 100 ms.

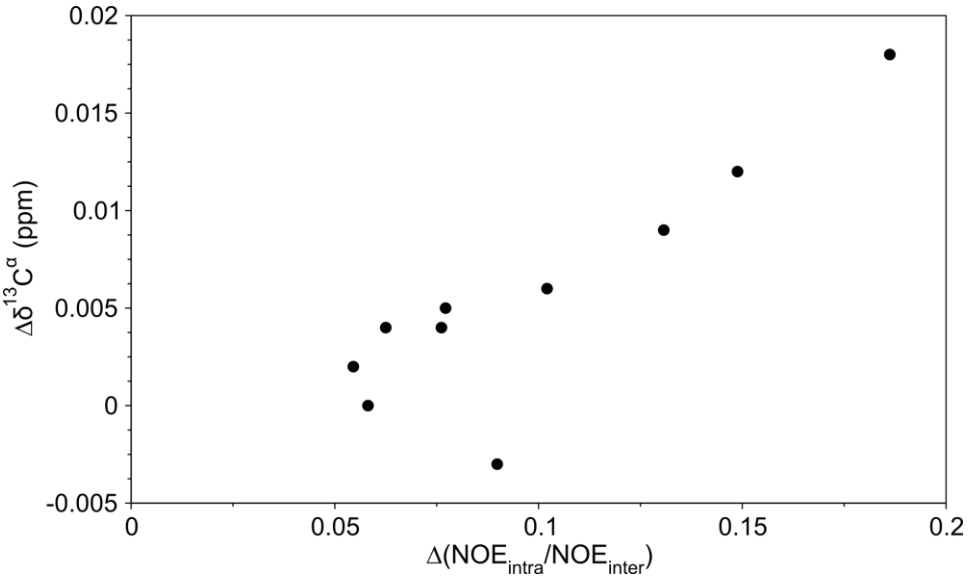

Figure S7. Plot of the change in the $^{13}C^{\alpha}$ chemical shift of residue i versus the change in the intra- to inter-residue H^{α}-H^{N} NOE ratio of residue i+1 $d_{\alpha N}(i+1,i+1)/d_{\alpha N}(i,i+1)$. Data are shown where available for the first 50 residues of aS. Data points that had a Gly as residue i or i+1 are not included. Comparison is between free aS and 200 μM 1:1 CR:aS samples.

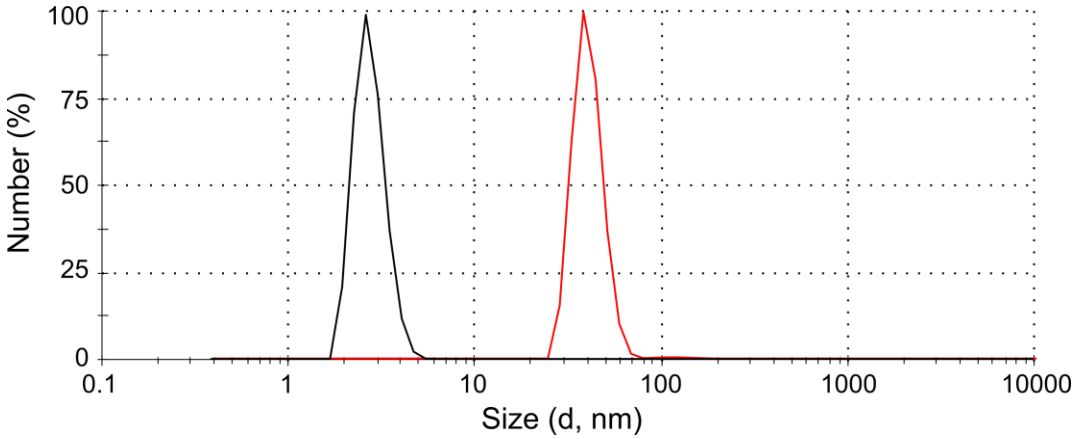

Figure S8. Particle diameter, d, as obtained from dynamic light scattering experiments performed on samples of 200 μM alpha-synuclein + 200 μM Congo Red in 20 mM sodium phospate buffer at pH 6 (red trace), and in 100 mM Tris buffer at pH 7.4 (black trace). The figure shows DLS data weighted by the number of particles. Measurements were performed on a Malvern Zetasizer Nano instrument (using a 532 nm laser) at 15°C.

Table S1. Attenuation of TROSY-HSQC ^{1}H-^{15}N correlation intensities, I/I_o, as a function of CR:aS molar ratio. Four ratios are presented: 1:4, 1:2, 2:1, 8:1. Data used for generating Figure 1B, main text.

Residue	1:4 CR:aS	1:2 CR:aS	2:1 CR:aS	8:1 CR:aS
3	0.705	0.374	—	—
4	0.663	0.335	—	—
6	0.628	0.247	—	—
7	0.633	—	—	—
8	0.667	0.284	—	—
9	0.643	0.241	—	—
11	0.697	0.231	—	—
12	0.720	0.241	—	—
13	0.789	—	—	—
14	0.762	0.393	—	—
15	0.811	0.407	—	—
16	0.805	0.278	—	—
17	0.780	0.246	—	—
18	0.791	0.309	—	—
19	0.830	0.391	—	—
20	0.807	0.377	—	—
21	0.805	0.293	—	—
22	0.806	0.439	—	—
23	0.804	0.403	—	—
26	0.883	0.608	0.130	—
27	0.847	0.423	—	—
28	0.871	0.549	—	—
29	0.848	0.472	0.083	—
31	0.828	0.536	0.103	—
32	0.834	0.520	0.096	—
33	0.819	0.466	0.060	—
35	0.873	0.514	—	—
36	0.856	0.627	—	—
37	0.866	0.551	0.095	—
38	0.835	0.526	0.087	—
39	0.819	0.397	—	—
40	0.834	0.407	—	—
41	0.789	0.347	—	—
42	0.778	0.491	0.107	—
43	0.784	0.421	—	—
44	0.802	0.348	—	—

Table S1 (continued)

Residue	1:4 CR:aS	1:2 CR:aS	2:1 CR:aS	8:1 CR:aS
45	0.806	0.421	—	—
46	0.851	0.582	—	—
47	0.861	0.593	—	—
48	0.875	0.520	0.074	—
49	0.899	0.558	0.115	—
52	0.894	—	—	—
53	0.884	0.615	0.128	—
54	0.879	0.639	0.161	—
56	0.903	0.700	0.234	—
57	0.920	0.736	0.273	—
58	0.903	0.710	0.241	—
59	0.888	0.744	0.314	—
61	0.954	0.911	0.508	—
63	0.960	0.865	0.614	—
64	0.910	0.828	0.551	0.120
65	0.887	0.812	0.592	0.215
66	0.941	0.879	0.704	0.297
67	0.930	0.877	0.692	0.315
68	0.936	0.896	0.761	0.413
69	0.940	0.907	0.812	—
70	0.945	0.908	0.782	0.406
71	0.937	0.895	0.742	0.302
72	0.921	0.878	0.721	0.330
73	0.899	0.862	0.731	0.368
74	0.967	—	—	—
75	0.919	0.873	0.711	0.327
76	0.937	0.892	0.766	0.351
77	0.955	0.911	0.775	0.319
78	0.933	0.896	0.788	0.305
79	0.935	0.902	0.784	0.316
80	0.920	0.878	0.726	0.254
81	0.917	0.875	0.733	0.321
82	0.940	0.890	0.770	—
83	0.960	0.925	0.822	0.420
84	0.930	0.895	0.776	0.365
85	0.939	0.902	0.803	0.410

Table S1 (continued)

Residue	1:4 CR:aS	1:2 CR:aS	2:1 CR:aS	8:1 CR:aS
86	0.918	0.878	0.741	0.382
87	0.904	0.866	0.744	0.380
88	0.952	0.915	0.828	0.366
89	0.952	0.902	0.778	0.252
90	0.942	0.891	0.752	0.235
91	0.926	0.872	0.695	0.163
92	0.931	0.872	0.693	0.195
93	0.917	0.859	0.660	0.206
94	0.961	0.910	0.739	—
95	0.937	0.880	0.659	0.144
96	0.943	0.886	0.737	0.202
98	0.952	0.926	0.832	0.413
99	0.938	0.910	0.819	0.505
100	0.946	0.917	0.844	—
101	0.967	0.944	0.920	0.668
102	0.967	0.952	0.919	0.635
103	0.940	0.920	0.861	0.642
104	0.959	0.944	0.911	0.758
105	0.956	0.946	0.911	0.723
106	0.971	0.952	0.975	0.862
107	0.950	0.943	0.878	0.704
109	0.957	0.940	0.948	0.829
110	0.962	0.958	0.825	0.778
111	0.965	0.952	0.964	0.850
112	0.961	0.946	0.897	0.772
113	0.947	0.924	0.911	0.791
114	0.956	0.934	0.892	0.746
115	0.956	0.936	0.899	0.848
118	0.963	0.937	0.935	0.816
119	0.961	0.951	0.919	0.805
121	0.960	0.939	0.911	0.835
122	0.962	0.970	0.880	0.845
123	0.987	0.995	0.928	—
124	0.965	0.969	0.958	0.914
125	0.978	0.951	0.867	0.865
126	0.974	0.969	0.884	—

Table S1 (continued)

Residue	1:4 CR:aS	1:2 CR:aS	2:1 CR:aS	8:1 CR:aS
129	0.981	0.981	0.929	0.903
130	0.981	0.964	0.967	0.907
131	—	—	0.971	0.949
133	1.001	1.002	0.967	—
134	0.984	0.979	0.980	1.003
135	0.979	0.997	0.993	0.980
136	0.989	0.987	1.003	0.979
137	0.994	0.967	0.959	0.955
139	0.995	0.990	1.000	0.968
140	0.983	0.966	0.966	0.930

Table S2. Chemical shift changes and peak intensity attenuation, I/I_0, for CR:aS of 0.8:1 compared to free aS. Data used for generating Figure 2, main text.

Residue	Delta N	Delta H	I/I_0
3	-0.013	-0.003	0.10
4	-0.016	-0.003	0.10
6	-0.024	-0.005	0.07
7	-0.013	-0.003	0.10
8	-0.007	-0.004	0.10
9	-0.026	-0.004	0.07
11	-0.026	-0.005	0.05
12	-0.023	-0.006	0.06
13	-0.015	-0.001	0.07
14	-0.015	-0.003	0.14
16	-0.027	-0.003	0.07
17	-0.026	-0.002	0.08
18	-0.021	-0.003	0.09
19	-0.016	-0.003	0.13
20	-0.021	-0.003	0.12
21	-0.023	-0.004	0.08
22	-0.012	-0.003	0.18
23	-0.016	-0.003	0.15
24	-0.032	-0.004	0.12
25	-0.016	-0.003	0.18
26	0.001	-0.002	0.32
27	-0.021	-0.003	0.15
28	-0.017	-0.002	0.21
29	-0.018	-0.003	0.17
30	-0.007	-0.002	0.44
31	-0.008	-0.002	0.28
32	-0.007	-0.002	0.25
33	-0.017	-0.002	0.18
35	-0.017	-0.002	0.19
36	-0.006	-0.002	0.30
37	-0.007	-0.002	0.24
38	-0.014	-0.002	0.22
39	-0.017	-0.003	0.13
40	-0.022	-0.002	0.12
41	-0.009	-0.003	0.16
42	-0.006	-0.002	0.32
43	-0.011	-0.003	0.21

Residue	Delta N	Delta H	I/I_0
44	-0.024	-0.003	0.11
45	-0.013	-0.003	0.17
46	-0.010	-0.002	0.27
47	-0.007	-0.002	0.28
48	-0.002	-0.003	0.24
49	-0.013	-0.002	0.21
50	-0.003	-0.005	0.21
51	-0.020	-0.004	0.22
53	-0.013	-0.002	0.28
54	-0.008	-0.002	0.35
55	-0.012	-0.002	0.23
56	-0.008	-0.001	0.41
57	-0.007	-0.001	0.45
58	-0.007	-0.002	0.44
59	-0.005	-0.001	0.53
60	-0.008	-0.001	0.58
61	-0.002	0.000	0.61
62	-0.002	-0.001	0.64
63	-0.002	-0.001	0.67
64	-0.002	-0.001	0.67
65	0.000	0.000	0.79
66	-0.002	-0.001	0.86
67	-0.002	-0.001	0.87
68	0.000	0.000	0.93
69	0.000	0.000	0.92
70	-0.001	0.000	0.91
71	-0.002	0.000	0.77
72	-0.001	0.000	0.75
73	0.000	0.000	0.82
74	0.001	0.000	0.85
75	0.001	0.000	0.90
76	0.000	0.000	0.89
77	0.000	0.000	0.89
78	0.000	0.000	0.77
79	0.000	0.000	0.88
80	0.000	0.000	0.84
81	0.001	0.000	0.86

Table S2 (continued)

Residue	Delta N	Delta H	I/I_0
82	0.000	0.000	0.88
83	0.002	0.000	0.91
84	0.000	0.000	0.91
85	0.000	0.000	0.96
86	0.000	0.000	0.86
87	0.000	0.000	0.89
88	0.001	0.000	0.89
89	0.000	0.000	0.74
90	0.000	0.000	0.86
91	0.000	0.000	0.82
92	0.001	0.000	0.81
93	0.000	0.000	0.86
94	0.001	0.000	0.85
95	0.000	-0.001	0.88
96	-0.001	-0.001	0.82
97	-0.001	0.000	0.91
98	0.002	0.000	0.92
99	0.001	0.000	0.92
100	0.002	0.000	0.89
101	0.001	0.000	0.92
102	0.000	0.000	0.87
103	0.003	0.000	0.96
104	0.004	0.000	0.93
105	0.005	0.000	0.88
106	0.001	0.000	0.99
107	0.002	0.000	0.93
109	0.003	0.000	0.95
110	0.006	0.000	0.90
111	0.002	0.000	0.93
112	0.004	0.000	0.91
113	0.009	0.000	0.96
114	0.010	0.001	1.00
115	0.008	0.000	0.92
118	0.004	0.000	0.92
119	0.009	0.000	0.92
121	0.012	0.000	0.95
122	0.003	0.001	0.88
123	0.011	0.001	0.94
124	0.011	0.000	0.96
125	0.017	0.001	0.90
126	0.020	0.001	0.87
129	0.010	0.001	0.90
130	0.010	0.001	0.88
131	0.013	0.001	0.88
132	0.005	0.001	0.90
133	0.005	0.001	0.90
134	0.010	-0.001	0.87
135	0.010	0.000	1.01
136	-0.005	-0.001	0.87
137	0.012	0.002	0.91
139	0.007	0.001	0.98
140	0.000	0.000	1.02

Table S3A. Attenuation of TROSY-HSQC ^{1}H-^{15}N correlation intensities, I/I_o, as a function of residue number for CR:aS = 0.8:1, for three different ionic strength conditions. Data used for Figure 3A, main text.

Residue	0 mM	100 mM	200 mM
3	0.345	0.593	0.794
4	0.376	0.638	0.716
5	0.278	0.557	0.805
6	0.227	0.512	0.777
8	0.289	0.574	0.857
9	0.248	0.524	0.770
11	0.256	0.492	0.768
12	0.238	0.549	0.815
14	0.414	0.685	0.826
15	0.344	0.756	0.882
16	0.260	0.577	0.820
17	0.234	0.564	0.835
18	0.284	0.620	0.863
19	0.368	0.660	0.837
20	0.311	0.664	0.823
21	0.248	0.587	0.843
22	0.406	0.671	0.855
23	0.391	0.642	0.827
26	0.584	—	—
27	0.366	0.631	0.849
28	0.486	0.713	0.829
29	0.468	0.714	0.860
31	0.530	0.696	0.880
32	0.485	0.717	0.875
33	0.429	0.689	0.893
35	0.451	0.761	0.834
36	0.547	0.784	0.941
37	0.530	0.740	0.922
38	0.487	0.689	0.880
39	0.362	0.652	0.869
40	0.363	0.638	0.875
41	0.344	0.636	0.867
42	0.509	0.750	0.838
43	0.424	0.631	0.813
44	0.301	0.606	0.828
45	0.354	0.540	0.896
46	0.529	0.766	0.897
47	0.647	0.698	0.888
48	0.496	0.737	0.912
49	0.603	—	0.856
50	0.382	0.624	0.824
52	0.521	0.710	0.889
53	0.568	0.757	0.875
54	0.668	0.765	0.900
56	0.659	0.784	0.884
57	0.677	0.811	0.854
58	0.681	0.825	0.900
59	0.694	0.802	0.879
61	0.881	—	—
63	0.800	0.859	0.923
64	0.779	0.843	0.909
65	0.799	0.894	0.917
66	0.853	0.868	0.956
67	0.833	0.856	0.903
68	0.889	0.935	0.958
69	0.878	0.927	0.937
70	0.884	0.921	0.910
71	0.845	0.870	0.912
72	0.850	0.889	0.920
73	0.855	0.888	0.944
74	0.869	0.911	0.926
75	0.844	0.904	0.941
76	0.861	0.856	0.918
77	0.862	0.891	0.905
78	0.892	0.915	0.940
79	0.865	0.950	0.910
80	0.836	0.906	0.920
81	0.851	0.887	0.926
82	0.837	0.929	0.906
83	0.859	0.901	0.887
84	0.857	0.916	0.946
85	0.893	0.934	0.954
86	0.876	0.919	0.948
87	0.895	0.924	0.956

Table S3A (continued)

Residue	0 mM	100 mM	200 mM
88	0.894	0.923	0.944
89	0.848	0.880	0.929
90	0.875	0.921	0.954
91	0.835	0.846	0.942
92	0.859	0.876	0.924
93	0.846	0.916	0.914
94	0.865	0.904	0.930
95	0.853	0.897	0.918
96	0.850	0.879	0.910
98	0.881	0.939	0.925
99	0.885	0.921	0.922
100	0.886	0.895	0.914
101	0.894	0.961	0.928
102	0.924	0.921	0.966
103	0.874	0.925	0.928
104	0.879	0.964	0.919
105	0.908	—	1.027
106	0.921	0.953	0.976
107	0.916	0.971	0.957
109	0.886	0.929	0.952
110	0.893	0.954	0.926
111	0.885	0.963	0.948
112	0.913	0.983	1.020
113	0.919	0.966	0.920
114	0.918	—	1.029
115	0.903	0.925	0.927
116	0.899	0.953	1.004
118	0.923	0.974	0.960
119	0.919	0.970	0.920
121	0.948	0.966	0.980
122	0.988	0.983	0.991
123	0.931	0.983	0.971
124	0.989	0.966	0.989
125	0.923	—	0.929

Residue	0 mM	100 mM	200 mM
126	0.958	0.982	0.959
129	0.932	0.961	0.960
130	0.943	0.988	0.924
132	1.034	—	0.895
133	1.026	1.031	0.984
134	0.975	0.981	1.022
135	0.962	1.000	0.982
136	0.973	—	0.990
137	0.945	1.014	0.986
139	0.943	0.966	0.948
140	0.972	0.986	1.027

Table S3B. Attenuation of TROSY-HSQC ^{1}H-^{15}N correlation intensities, I/I_o, as a function of residue number for CR:aS = 4:1, for three different ionic strength conditions. Data used for Figure 3B, main text.

Residue	0 mM	100 mM	200 mM
59	0.112	—	—
60	0.092	0.044	0.036
61	0.147	0.111	0.115
62	0.111	0.119	0.184
63	0.180	0.145	0.171
64	0.194	0.128	0.151
65	0.349	0.308	0.219
66	0.400	0.355	0.320
67	0.496	0.400	0.354
68	0.603	0.582	0.426
69	0.626	0.579	0.409
70	0.565	0.491	0.391
71	0.462	0.360	0.305
72	0.532	0.408	0.368
73	0.579	0.508	0.397
74	0.612	0.539	0.415
75	0.537	0.376	0.328
76	0.568	0.406	0.367
77	0.526	0.339	0.304
78	0.510	0.394	0.376
79	0.528	0.406	0.325
80	0.483	0.364	0.277
81	0.536	0.386	0.318
82	0.544	0.402	0.292
83	0.591	0.449	0.394
84	0.585	0.477	0.364
85	0.641	0.523	0.423
86	0.594	0.495	0.444
87	0.621	0.492	0.346
88	0.553	0.456	0.347
89	0.442	0.254	0.247
90	0.432	0.245	0.247
91	0.353	0.186	0.199
92	0.391	0.203	0.188
93	0.443	0.295	0.213
94	—	0.373	0.203
95	0.289	0.141	0.144

Residue	0 mM	100 mM	200 mM
96	0.381	0.174	0.171
97	—	0.181	0.141
98	0.607	0.429	0.262
99	0.669	0.528	0.330
100	0.657	0.445	0.398
101	0.746	0.630	0.410
102	0.755	0.611	0.394
103	0.794	0.732	0.431
104	0.799	0.796	0.447
105	0.820	0.726	0.551
106	0.845	0.808	0.582
107	0.790	0.830	0.487
109	0.849	0.901	0.593
110	0.836	0.878	0.596
111	0.847	0.895	0.625
112	0.817	0.877	0.727
113	0.860	0.868	0.631
114	—	—	0.715
115	0.829	0.884	0.666
116	0.812	0.860	0.682
118	0.882	0.919	0.662
119	0.904	0.894	0.688
121	0.931	0.957	0.916
122	0.944	0.952	0.930
123	0.996	0.988	1.022
124	0.999	0.963	0.954
125	0.955	—	—
126	0.999	0.919	0.906
129	0.940	0.922	0.960
130	0.959	0.986	0.957
131	—	—	0.957
134	1.010	0.942	1.004
135	1.038	0.958	0.990
136	0.976	1.098	0.905
137	0.952	0.977	0.937
139	0.944	0.941	0.972
140	0.929	0.946	1.029

Table S4. Relaxation rates measured at 80 Hz CPMG effective field at 500 MHz and 900 MHz as well as squared ^{15}N chemical shift changes relative to free aS. Sample conditions are: 15 °C, pH 6.0, 200 μM aS, 260 μM CR. Data used for generating Figure 4, main text.

Residue	$[\Delta\delta N]^2$, ppm^2	R2, Hz 500 MHz	R2, Hz 900 MHz
3	0.0036	6.5	10.7
4	0.0040	8.6	16.1
6	0.0096	10.9	28.5
8	0.0008	6.3	9.6
9	0.0130	14.4	25.1
11	0.0146	9.9	23.4
12	0.0106	10.6	21.9
14	0.0046	6.6	11.5
15	0.0000	5.8	9.5
16	0.0199	16.6	24.2
17	0.0259	21.0	27.4
19	0.0048	6.4	11.4
20	0.0083	9.7	21.4
21	0.0121	11.7	26.9
22	0.0026	8.2	14.9
23	0.0049	7.8	14.4
24	0.0102	9.7	20.1
26	0.0000	4.3	6.3
27	0.0094	8.8	17.9
28	0.0056	6.2	11.2
29	0.0064	6.2	11.7
31	0.0012	4.8	7.6
32	0.0009	4.2	5.7
33	0.0053	7.9	14.6

Residue	$[\Delta\delta N]^2$, ppm^2	R2, Hz 500 MHz	R2, Hz 900 MHz
35	0.0059	7.4	14.0
36	0.0007	4.9	7.3
37	0.0010	5.0	7.7
38	0.0041	7.7	14.1
39	0.0069	8.8	17.9
40	0.0108	11.1	26.8
41	0.0024	7.8	12.0
42	0.0008	6.5	9.0
43	0.0028	7.2	12.1
44	0.0106	11.0	23.7
45	0.0030	6.0	10.7
46	0.0022	5.9	10.0
47	0.0011	4.8	7.0
48	0.0002	3.8	5.2
49	0.0038	6.7	12.7
53	0.0040	5.1	8.1
54	0.0020	3.9	6.5
56	0.0018	4.1	5.7
57	0.0013	3.2	4.8
58	0.0013	3.5	5.7
59	0.0007	3.4	4.8
60	0.0000	2.7	2.8

Table S5A. Relaxation rates as a function of CPMG effective field measured at 500 MHz. Sample conditions are: 15 °C, pH 6.0, 200 μM aS, 260 μM CR. Data used for determination of the parameters of aS/CR binding, and for generating Figure 5, main text.

Residue	R_2(80 Hz)	R_2(160 Hz)	R_2(240 Hz)	R_2(400 Hz)	R_2(640 Hz)	R_2(960 Hz)
3	6.5	6.1	6.0	5.3	4.5	4.2
4	8.6	8.2	8.1	7.0	6.0	5.5
6	10.9	10.8	9.0	8.9	7.2	6.8
8	6.3	6.2	6.2	5.8	5.2	5.1
9	14.4	12.6	11.9	10.1	8.2	7.5
11	9.9	9.2	8.2	7.1	6.1	4.4
12	10.6	9.4	9.0	7.0	6.5	4.6
14	6.6	5.8	5.6	5.0	4.4	4.0
15	5.8	5.6	5.3	4.7	4.3	4.1
16	16.6	13.1	12.2	9.7	7.5	5.4
17	21.0	19.0	19.2	15.6	11.0	8.9
19	6.4	5.7	5.3	4.8	4.1	3.6
20	9.7	8.7	7.7	6.4	5.3	4.9
21	11.7	9.0	8.8	7.6	6.1	5.2
22	8.2	7.8	7.1	6.5	5.9	5.3
23	7.8	6.9	6.5	5.5	5.1	4.9
24	9.7	9.2	8.2	7.1	5.8	5.2
26	4.3	4.1	3.9	3.6	3.5	3.2
27	8.8	8.0	7.5	6.3	4.9	4.0
28	6.2	5.4	5.1	4.3	3.9	3.5
29	6.2	5.8	5.0	4.2	3.8	3.7
31	4.8	4.2	4.4	3.9	3.7	3.5
32	4.2	4.0	3.8	3.7	3.4	3.4
33	7.9	7.4	6.8	5.9	5.6	4.7
35	7.4	7.0	6.5	5.8	5.0	4.6
36	4.9	4.4	4.3	3.9	3.7	3.5
37	5.0	4.8	4.5	4.2	4.0	3.8
38	7.7	7.4	6.1	5.3	4.7	4.2
39	8.8	8.5	7.8	7.0	6.1	5.4
40	11.1	9.8	9.1	8.0	6.6	5.6

Table S5A (continued)

Residue	R_2(80 Hz)	R_2(160 Hz)	R_2(240 Hz)	R_2(400 Hz)	R_2(640 Hz)	R_2(960 Hz)
41	7.8	6.9	7.0	6.3	5.5	4.9
42	6.5	6.3	6.0	5.7	5.5	5.3
43	7.2	6.8	6.8	6.1	5.8	5.5
44	11.0	10.2	9.7	8.6	7.2	6.1
45	6.0	5.7	5.7	5.1	4.6	4.5
46	5.9	5.6	5.4	4.9	4.4	4.1
47	4.8	4.2	4.0	3.8	3.5	3.7
48	3.8	3.7	3.6	3.4	3.3	3.1
49	6.7	5.9	5.3	4.7	4.1	3.7
53	5.1	4.7	4.6	3.9	3.6	3.3
54	3.9	3.7	3.6	3.4	3.2	3.0
56	4.1	3.7	3.7	3.2	2.9	2.6
57	3.2	3.1	3.1	2.8	2.6	2.5
58	3.5	3.3	3.2	3.1	2.8	2.6
59	3.4	3.1	2.9	2.8	2.6	2.5
60	2.7	2.7	2.6	2.6	2.4	2.3

Table S5B. Relaxation rates as a function of CPMG effective field measured at 900 MHz. Sample conditions are: 15 °C, pH 6.0, 200 μM aS, 260 μM CR. Data used for determination of the parameters of aS/CR binding, and for generating Figure 5, main text.

Residue	R_2(80 Hz)	R_2(160 Hz)	R_2(240 Hz)	R_2(400 Hz)	R_2(640 Hz)	R_2(960 Hz)
3	10.7	10.5	9.5	8.8	7.2	6.1
4	16.1	15.0	14.4	12.2	11.0	9.1
6	28.5	22.6	18.4	16.7	13.5	12.2
8	9.6	9.5	9.4	7.0	7.5	6.6
9	25.1	30.9	22.8	21.9	14.1	9.9
11	23.4	16.8	17.4	14.4	11.0	10.7
12	21.9	23.8	16.9	15.4	13.8	8.9
14	11.5	10.6	9.9	8.1	7.1	5.7
15	9.5	8.3	8.1	7.0	6.3	5.2
16	24.2	23.8	21.0	19.2	15.2	13.3
17	27.4	27.3	29.1	24.2	19.4	17.1
19	11.4	9.5	9.5	7.8	5.1	4.7
20	21.4	18.7	17.7	13.1	10.3	6.2
21	26.9	21.0	16.4	20.2	12.3	8.3
22	14.9	14.6	12.3	11.2	9.3	7.9
23	14.4	11.4	11.0	9.3	7.2	5.7
24	20.1	17.8	18.6	13.6	10.1	9.7
26	6.3	6.1	5.5	5.2	4.3	3.9
27	17.9	17.0	14.3	11.7	10.5	7.1
28	11.2	10.3	8.9	7.3	6.0	5.0
29	11.7	10.4	7.8	6.9	7.0	5.2
31	7.6	6.7	6.8	5.8	4.9	4.6
32	5.7	5.4	4.9	4.7	4.1	3.6
33	14.6	15.0	13.1	12.1	10.1	8.3
35	14.0	13.4	12.2	10.8	8.5	7.3
36	7.3	7.4	6.8	5.7	5.5	5.1
37	7.7	7.1	6.3	6.3	5.0	4.4
38	14.1	13.5	13.5	11.1	9.9	8.6
39	17.9	16.7	15.8	13.9	12.3	10.5
40	26.8	21.9	19.1	15.6	14.2	10.1

Table S5B (continued)

Residue	R_2(80 Hz)	R_2(160 Hz)	R_2(240 Hz)	R_2(400 Hz)	R_2(640 Hz)	R_2(960 Hz)
41	12.0	11.5	10.8	9.2	7.9	6.3
42	9.0	8.6	8.6	7.9	6.9	6.8
43	12.1	11.4	10.9	8.7	7.6	7.5
44	23.7	23.3	19.5	16.7	15.0	8.9
45	10.7	9.5	8.8	7.5	6.7	6.5
46	10.0	9.9	9.5	8.3	7.1	6.6
47	7.0	7.3	6.8	5.7	5.0	4.8
48	5.2	4.7	4.6	4.3	3.9	2.9
49	12.7	12.6	10.9	9.7	8.2	7.2
53	8.1	8.4	7.8	6.7	5.7	4.8
54	6.5	6.0	5.7	5.2	4.5	4.0
56	5.7	6.2	5.8	4.6	4.0	3.3
57	4.8	4.5	4.2	3.6	3.3	2.8
58	5.7	5.0	4.5	4.2	3.3	3.2
59	4.8	4.5	4.4	4.1	3.7	3.3
60	2.8	2.3	2.8	2.4	2.2	2.7

Table S6A. Intraresidue $d_{\alpha N}(i,i)$ NOE build-up rate (s^{-1}) and the ratio $d_{\alpha N}(i,i)/d_{\alpha N}(i-1,i)$ for free aS, as measured in a 600 MHz 3D HNH NOESY-HMQC experiment (200 μM aS). Values for Gly residues only (underscored) were corrected by dividing by 2. Data used for generating Figure 6, main text

Residue	Rate, s^{-1}	Rate error	Ratio	Ratio error
3	0.097	0.003	0.317	0.009
4	0.096	0.003	0.305	0.008
5	0.202	0.005	0.452	0.010
6	0.154	0.003	0.337	0.008
8	0.179	0.004	0.388	0.008
14	0.337	0.003	0.511	0.005
17	0.193	0.003	0.266	0.004
22	0.289	0.004	0.377	0.005
26	0.173	0.002	0.480	0.006
27	0.220	0.003	0.407	0.006
31	0.264	0.004	0.485	0.007
32	0.190	0.003	0.498	0.009
33	0.192	0.004	0.263	0.005
37	0.205	0.003	0.451	0.006
38	0.211	0.003	0.219	0.004
39	0.220	0.003	0.266	0.004
40	0.224	0.004	0.235	0.004
41	0.451	0.005	0.472	0.005
42	0.334	0.008	0.297	0.007
44	0.248	0.004	0.329	0.005
48	0.164	0.002	0.322	0.004
53	0.157	0.003	0.200	0.003
54	0.159	0.003	0.184	0.003
58	0.237	0.003	0.230	0.003
63	0.180	0.003	0.190	0.003
64	0.172	0.003	0.181	0.003
65	0.119	0.004	0.183	0.006
67	0.212	0.003	0.458	0.006
69	0.076	0.002	0.269	0.006
70	0.086	0.002	0.187	0.004
71	0.119	0.003	0.180	0.004
72	0.123	0.003	0.175	0.004
73	0.238	0.003	0.421	0.006
75	0.147	0.003	0.192	0.004
76	0.086	0.003	0.120	0.004
77	0.121	0.003	0.166	0.003
80	0.205	0.004	0.234	0.004
81	0.173	0.003	0.189	0.003
83	0.151	0.002	0.254	0.004
84	0.187	0.003	0.306	0.005
85	0.124	0.002	0.539	0.010
86	0.203	0.003	0.839	0.013
87	0.114	0.003	0.324	0.007
88	0.100	0.002	0.251	0.005
89	0.150	0.002	0.299	0.005
91	0.146	0.003	0.315	0.005
93	0.263	0.004	0.584	0.008
95	0.146	0.003	0.200	0.004
96	0.206	0.003	0.190	0.003
98	0.234	0.003	0.343	0.004
101	0.249	0.003	0.500	0.005
102	0.210	0.002	0.475	0.006
103	0.213	0.003	0.387	0.005
104	0.168	0.002	0.513	0.007
106	0.253	0.002	0.484	0.004
107	0.112	0.002	0.275	0.005
109	0.155	0.002	0.220	0.003
111	0.307	0.002	0.471	0.004
113	0.176	0.003	0.179	0.003
116	0.115	0.003	0.151	0.004
118	0.197	0.003	0.133	0.002
121	0.316	0.004	0.492	0.005
125	0.231	0.003	0.286	0.004
126	0.314	0.005	0.217	0.003
130	0.216	0.003	0.243	0.003
135	0.170	0.003	0.284	0.005
139	0.072	0.002	0.180	0.004
140	0.031	0.002	0.180	0.010

Table S6B. Intraresidue $d_{\alpha N}(i,i)$ NOE build-up rate (s^{-1}) and the ratio $d_{\alpha N}(i,i)/d_{\alpha N}(i-1,i)$ for CR:aS of 1:1, as measured from a 600 MHz 3D HNH NOESY-HMQC spectrum (200 μM aS, 200 μM CR). Values for Gly residues only (underscored) were corrected by dividing by 2. Data used for generating Figure 6, main text.

Residue	Rate, s^{-1}	Rate error	Ratio	Ratio errors
3	0.494	0.023	0.419	0.020
4	0.277	0.015	0.381	0.021
5	0.697	0.034	0.582	0.029
8	0.655	0.039	0.564	0.034
17	0.461	0.031	0.473	0.032
22	0.651	0.018	0.526	0.015
26	0.365	0.009	0.559	0.014
27	0.464	0.015	0.593	0.019
31	0.587	0.016	0.579	0.015
32	0.413	0.015	0.576	0.020
33	0.370	0.015	0.322	0.013
37	0.472	0.012	0.575	0.014
38	0.522	0.018	0.274	0.009
39	0.548	0.023	0.344	0.014
40	0.522	0.022	0.297	0.012
41	0.858	0.032	0.532	0.020
42	0.692	0.019	0.474	0.013
44	0.526	0.022	0.419	0.018
48	0.444	0.013	0.467	0.013
53	0.349	0.009	0.281	0.007
54	0.306	0.009	0.246	0.007
58	0.312	0.008	0.267	0.007
63	0.199	0.005	0.216	0.006
64	0.223	0.006	0.203	0.005
65	0.156	0.005	0.223	0.008
67	0.243	0.005	0.477	0.009
69	0.116	0.003	0.324	0.009
70	0.115	0.004	0.222	0.007
71	0.140	0.005	0.181	0.006
72	0.167	0.004	0.216	0.006
73	0.260	0.005	0.457	0.009
75	0.169	0.005	0.205	0.006
76	0.109	0.005	0.143	0.006
77	0.175	0.005	0.185	0.005
80	0.213	0.005	0.232	0.006
81	0.172	0.005	0.181	0.005
83	0.157	0.004	0.234	0.006
84	0.195	0.005	0.310	0.007
85	0.133	0.004	0.539	0.015
86	0.221	0.005	0.782	0.016
87	0.118	0.004	0.333	0.010
88	0.129	0.004	0.285	0.008
89	0.184	0.004	0.356	0.008
91	0.169	0.004	0.315	0.008
93	0.275	0.005	0.636	0.012
95	0.200	0.005	0.256	0.006
96	0.243	0.006	0.192	0.005
98	0.233	0.004	0.324	0.006
101	0.280	0.004	0.547	0.008
102	0.181	0.004	0.472	0.009
103	0.198	0.004	0.384	0.008
104	0.182	0.004	0.554	0.012
106	0.274	0.004	0.531	0.008
107	0.117	0.004	0.277	0.009
109	0.173	0.004	0.219	0.006
111	0.293	0.004	0.418	0.005
113	0.176	0.005	0.204	0.005
116	0.131	0.005	0.170	0.006
118	0.197	0.005	0.131	0.004
121	0.313	0.005	0.484	0.008
125	0.213	0.005	0.271	0.006
126	0.193	0.005	0.196	0.005
130	0.200	0.004	0.227	0.005
135	0.200	0.005	0.298	0.007
139	0.074	0.003	0.177	0.007
140	0.039	0.003	0.228	0.018

Table S6C. Intraresidue $d_{\alpha N}(i,i)$ NOE build-up rate (s^{-1}) and the ratio $d_{\alpha N}(i,i)/d_{\alpha N}(i-1,i)$ for CR:aS of 4:1, as measured from a 600 MHz 3D HNH NOESY-HMQC spectrum (200 μM aS, 800 μM CR). Values for Gly residues only (underscored) were corrected by dividing by 2. Data used for generating Figure 6, main text.

Residue	Rate, s^{-1}	Rate error	Ratio	Ratio error
64	0.620	0.051	0.287	0.024
67	0.533	0.020	0.518	0.020
69	—	—	0.360	0.015
70	0.279	0.014	0.291	0.014
71	0.375	0.024	0.212	0.014
72	0.344	0.017	0.251	0.012
73	0.510	0.017	0.515	0.017
74	0.298	0.012	0.341	0.014
75	0.358	0.016	0.274	0.012
76	—	—	0.207	0.014
77	0.339	0.021	0.234	0.015
78	0.432	0.019	0.311	0.014
80	0.451	0.026	0.304	0.017
81	0.260	0.019	0.158	0.011
83	0.303	0.013	0.290	0.012
84	0.433	0.017	0.395	0.016
85	0.301	0.011	0.587	0.022
86	0.406	0.015	0.695	0.026
87	0.252	0.012	0.453	0.022
88	0.263	0.014	0.408	0.022
89	0.425	0.023	0.485	0.026
91	0.434	0.031	0.539	0.039
93	0.583	0.032	0.622	0.034
95	0.450	0.042	0.352	0.033
96	0.391	0.037	0.252	0.024
98	0.394	0.015	0.418	0.016
99	0.467	0.015	0.618	0.020
101	0.329	0.011	0.463	0.015
102	0.333	0.010	0.578	0.018
103	0.275	0.010	0.371	0.014
104	0.217	0.008	0.570	0.021
106	0.222	0.007	0.383	0.011
107	0.127	0.007	0.287	0.016
109	0.164	0.007	0.191	0.009

Residue	Rate, s^{-1}	Rate error	Ratio	Ratio error
111	0.268	0.007	0.331	0.009
112	0.118	0.006	0.216	0.012
113	0.174	0.009	0.174	0.009
114	0.220	0.008	0.288	0.010
116	0.115	0.013	0.153	0.017
118	0.166	0.010	0.113	0.007
121	0.277	0.009	0.496	0.017
125	0.147	0.007	0.237	0.011
126	0.123	0.006	0.183	0.009
130	0.123	0.008	0.188	0.012
132	0.238	0.007	0.530	0.016
135	0.145	0.007	0.322	0.015
139	0.103	0.008	0.231	0.017
140	0.034	0.005	0.222	0.032

Table S6D. Change in the ratio $d_{\alpha N}(i,i)/d_{\alpha N}(i-1,i)$ for CR:aS of 1:1 and 4:1 as compared to free aS. Measured from a 600 MHz 3D HNH NOESY-HMQC spectrum. Values for Gly residues are not shown. Data used for generating Figure 6C, main text.

Residue	Δ Ratio CR:aS=1:1	Δ Ratio CR:aS=1:1 error	Δ Ratio CR:aS=4:1	Δ Ratio CR:aS=4:1 error
3	0.102	0.029		
4	0.076	0.029		
5	0.131	0.039		
8	0.175	0.042		
17	0.208	0.036		
22	0.149	0.020		
26	0.080	0.020		
27	0.186	0.025		
32	0.078	0.029		
33	0.058	0.018		
37	0.124	0.020		
38	0.055	0.013		
39	0.077	0.018		
40	0.063	0.017		
42	0.177	0.020		
44	0.090	0.023		
48	0.145	0.018		
53	0.080	0.011		
54	0.063	0.010		
58	0.036	0.010		
63	0.027	0.009		
64	0.022	0.008	0.106	0.027
65	0.040	0.013	—	—
69	0.055	0.015	0.092	0.022
70	0.035	0.011	0.104	0.019
71	0.001	0.010	0.032	0.018
72	0.042	0.009	0.076	0.016
75	0.013	0.010	0.082	0.016
76	0.024	0.010	0.088	0.018
77	0.020	0.009	0.069	0.018
80	-0.001	0.010	0.070	0.022
81	-0.008	0.008	-0.031	0.015
83	-0.019	0.010	0.036	0.016

Table S6D (continued)

Residue	Δ Ratio CR:aS=1:1	Δ Ratio CR:aS=1:1 error	Δ Ratio CR:aS=4:1	Δ Ratio CR:aS=4:1 error
85	0.001	0.025	0.048	0.032
87	0.009	0.018	0.128	0.029
88	0.034	0.014	0.157	0.027
89	0.057	0.013	0.186	0.031
91	0.001	0.013	0.225	0.044
95	0.055	0.010	0.152	0.036
96	0.002	0.008	0.062	0.027
98	-0.019	0.010	0.075	0.020
102	-0.003	0.015	0.103	0.023
103	-0.003	0.013	-0.016	0.019
104	0.041	0.019	0.056	0.028
107	0.002	0.013	0.012	0.021
109	-0.001	0.009	-0.029	0.012
113	0.025	0.008	-0.005	0.012
116	0.019	0.010	0.002	0.021
118	-0.002	0.006	-0.020	0.009
121	-0.008	0.014	0.005	0.022
125	-0.015	0.010	-0.049	0.015
126	-0.021	0.008	-0.033	0.012
130	-0.016	0.008	-0.055	0.015
135	0.014	0.012	0.037	0.019
139	-0.003	0.012	0.051	0.021
140	0.048	0.028	0.042	0.042

Table S7A. Sequential amide-amide NOE cross peak intensities, normalized by the diagonal peak intensity, as measured in a 600 MHz 3D NNH NOESY-HMQC experiment (15 °C, pH 6.0, 200 μM aS; 200 μM CR; 250 ms NOE mixing time). Data used for generating Figure 7B, main text.

Residue	NOE
4	0.143
7	0.057
8	0.062
9	0.147
10	0.091
11	0.107
12	0.102
13	0.142
14	0.111
15	0.110
16	0.112
17	0.100
18	0.093
19	0.123
20	0.116
21	0.121
22	0.107
23	0.103
24	0.069
25	0.061
26	0.079
27	0.089
28	0.082
29	0.090
30	0.063
31	0.063
32	0.090
33	0.073
34	0.044
35	0.054
36	0.084
37	0.092
38	0.087
39	0.103

Residue	NOE
40	0.061
41	0.112
42	0.065
43	0.088
44	0.119
45	0.067
46	0.089
47	0.072
48	0.077
49	0.063
50	0.056
51	0.047
52	0.028
53	0.052
54	0.041
55	0.057
56	0.059
57	0.046
58	0.054
59	0.061
60	0.052
61	0.056
66	0.026
67	0.037
68	0.026
69	0.025
70	0.018
71	0.021
72	0.024
73	0.032
74	0.030
75	0.031
76	0.031
77	0.025

Residue	NOE
78	0.026
79	0.022
80	0.025
81	0.028
82	0.025
83	0.024
84	0.019
85	0.023
86	0.020
87	0.027
88	0.029
89	0.034
90	0.024
92	0.033
93	0.048
94	0.042
95	0.031
96	0.030
98	0.040
99	0.032
100	0.046
101	0.035
104	0.033
106	0.025
107	0.029
110	0.025
111	0.020
112	0.025
113	0.022
114	0.022
115	0.033
119	0.029
123	0.052
124	0.036

Residue	NOE
125	0.033
126	0.030
130	0.024
131	0.026
132	0.021
133	0.024
134	0.020
135	0.021
136	0.018
137	0.015

Table S7B. $d_{NN}(i,i+2)$ amide-amide NOE cross peak intensities, normalized by the diagonal peak intensity, as measured in a 600 MHz 3D NNH NOESY-HMQC experiment (15 °C, pH 6.0, 200 μM aS; 200 μM CR; 250 ms NOE mixing time). Data used for generating Figure 7C, main text.

Residue	NOE
6	0.042
8	0.038
9	0.039
10	0.030
11	0.026
12	0.029
13	0.033
14	0.029
15	0.023
16	0.032
17	0.032
18	0.035
19	0.027
20	0.035
21	0.036
22	0.028
23	0.026
24	0.023
25	0.020
26	0.012
27	0.020
29	0.021
30	0.018
31	0.020
32	0.017
33	0.015
34	0.022
35	0.014
36	0.014
37	0.020
38	0.017
39	0.019
40	0.016
41	0.016
42	0.015
43	0.016

Residue	NOE
46	0.017
47	0.018
48	0.017
49	0.012
50	0.016
51	0.009
52	0.015
53	0.007
54	0.007
55	0.008
56	0.010
57	0.004
58	0.009
59	0.009
60	0.012
61	0.010
62	0.007
67	0.005
68	0.006
69	0.005
74	0.006
83	0.004
87	0.004
88	0.003
89	0.004
91	0.004
92	0.005
93	0.006
94	0.008
95	0.004
96	0.003
99	0.003
101	0.004
103	0.004
106	0.004

Table S7C. $d_{NN}(i,i+3)$ amide-amide NOE cross peak intensities, normalized by the diagonal peak intensity, as measured in a 600 MHz 3D NNH NOESY-HMQC experiment (15 °C, pH 6.0, 200 μM aS; 200 μM CR; 250 ms NOE mixing time). Data used for generating Figure 7C, main text

Residue	NOE
7	0.010
10	0.016
16	0.016
17	0.017
18	0.019
19	0.015
20	0.016
21	0.021
22	0.013
25	0.010
26	0.012
28	0.012
29	0.009
30	0.006
31	0.011
32	0.009
33	0.006
36	0.008
37	0.008
39	0.008
40	0.008
47	0.010
48	0.008
52	0.012
57	0.006
59	0.004
87	0.003
89	0.003
90	0.005
93	0.004
95	0.005

Table S8A. Changes in aS $^{13}C^{\alpha}$ chemical shifts induced by Congo Red, as measured by a 3D CT-HNCA experiment on perdeuterated $^{13}C/^{15}N$-labeled aS (15 °C, pH 6.0, 200 μM aS, 500 MHz) for an CR:aS ratio of 1:1. Data used for generating Fig. 8, main text..

Residue	Delta CA
3	0.004
4	0.009
5	0.011
6	0.014
7	0.020
8	0.018
9	0.028
10	0.023
11	0.015
12	0.014
13	0.007
14	0.009
15	0.026
17	0.019
18	0.012
20	0.023
21	0.012
22	0.019
23	0.013
24	0.020
25	0.020
26	0.018
27	0.016
28	0.022
29	0.011
30	-0.002
31	0.005
32	0.000
33	-0.004
34	0.006
35	0.011
36	0.002
37	0.002
38	0.005

Residue	Delta CA
39	0.004
40	0.003
41	0.002
42	0.011
43	-0.003
44	-0.004
45	0.003
46	0.012
47	0.007
48	0.003
49	0.001
51	0.005
52	0.003
53	0.009
54	-0.006
55	0.003
56	0.003
57	0.013
58	-0.002
59	0.003
60	0.000
62	0.000
63	0.002
64	0.003
65	0.000
66	0.002
67	0.001
68	0.002
69	0.002
70	0.001
71	0.002
72	-0.001
73	0.000
74	0.001

Residue	Delta CA
75	0.001
76	0.000
77	-0.001
78	0.001
79	-0.001
80	-0.001
81	0.000
82	-0.001
84	0.001
85	0.000
86	0.000
87	0.001
88	0.000
89	0.001
90	0.001
91	-0.002
92	0.000
93	0.000
94	0.002
95	0.000
96	0.001
97	-0.001
98	0.010
99	-0.004
100	0.000
101	-0.001
102	-0.002
103	-0.001
104	0.015
105	0.019
106	-0.003
107	-0.002
108	-0.005
109	-0.005

Residue	Delta CA
110	0.012
111	-0.006
112	-0.008
113	-0.011
114	0.021
115	0.020
116	-0.004
117	-0.002
118	-0.010
119	0.040
120	-0.016
121	0.017
122	-0.001
123	0.019
124	-0.025
125	-0.009
126	0.016
127	-0.005
128	-0.017
129	-0.013
130	0.01
131	0.018
132	-0.006
133	-0.004
134	-0.014
135	0.028
136	-0.019
137	0.04
138	-0.002
139	0.037
140	0.011

Table S8B. Changes in aS $^{13}C^{\alpha}$ chemical shifts induced by Congo Red, as measured by a 3D CT-HNCA experiment on perdeuterated $^{13}C/^{15}N$-labeled aS (15 °C, pH 6.0, 200 μM aS, 500 MHz) for an CR:aS ratio of 4:1. Data used for generating Figure 8, main text.

Residue	Delta CA
62	0.112
63	0.107
64	0.073
65	0.036
66	0.026
67	0.017
68	0.025
69	0.027
70	0.027
71	0.028
72	0.002
73	0.022
74	0.028
75	0.032
76	0.036
77	0.049
78	0.027
79	0.039
80	0.015
81	0.018
82	0.002
83	0.016
84	-0.001
85	0.020
86	0.019
87	0.064
88	0.088
89	0.090
91	0.032
92	0.044
93	0.024
94	0.069
95	0.015

Residue	Delta CA
96	0.022
97	0.059
98	0.042
99	-0.018
100	0.025
101	0.010
102	0.001
103	-0.010
104	-0.026
105	-0.006
106	-0.024
107	0.001
108	-0.023
109	-0.017
110	0.007
111	-0.014
112	-0.004
113	-0.021
114	0.008
115	0.024
116	-0.007
117	0.002
118	-0.016
120	-0.046
121	0.004
122	-0.015
123	0.022
124	-0.035
125	0.001
126	0.013
127	0.001
128	-0.025
129	-0.025

Residue	Delta CA
130	0
131	0.023
132	-0.017
133	0
134	-0.034
135	0.039
136	-0.021
137	0.051
138	-0.007
139	0.057
140	0.018

Table S9A. Water-LOGSY effect for 1:1 CR:aS. Experiments were performed on perdeuterated ^{15}N-labeled aS at 5 °C (pH 6.0, 200 μM aS, 500 MHz spectrometer with a room temperature probe). Experiments involved application of a 2 second presaturation pulse (γB_1 =13 Hz vs 0 Hz) followed by a normal HSQC sequence, and were collected in an interleaved manner. Data used for generating Figure 9, main text.

Residue	LOGSY effect	absolute error	Residue	LOGSY effect	absolute error
3	0.639	0.017	41	0.802	0.021
4	0.664	0.017	42	0.831	0.026
6	0.741	0.031	43	0.877	0.029
8	0.691	0.025	44	0.819	0.022
9	0.781	0.034	45	0.796	0.019
11	0.800	0.027	46	0.826	0.015
12	0.745	0.024	47	0.899	0.013
13	0.789	0.019	48	0.802	0.013
14	0.807	0.020	49	0.846	0.018
15	0.779	0.017	50	1.004	0.033
16	0.753	0.017	51	0.973	0.037
17	0.775	0.021	52	0.810	0.012
18	0.804	0.021	53	0.883	0.013
19	0.798	0.018	54	0.865	0.013
20	0.818	0.020	55	0.871	0.017
21	0.815	0.025	56	0.929	0.014
22	0.779	0.019	57	0.904	0.012
23	0.864	0.022	58	0.912	0.014
24	0.864	0.019	59	0.959	0.015
25	0.875	0.022	61	0.960	0.012
26	0.801	0.014	62	0.915	0.011
27	0.845	0.016	63	0.958	0.011
28	0.852	0.015	64	0.951	0.013
29	0.816	0.013	65	1.028	0.015
30	0.868	0.012	66	0.982	0.010
31	0.877	0.017	67	0.989	0.011
32	0.864	0.015	68	1.001	0.010
33	0.825	0.019	69	1.003	0.010
35	0.830	0.015	70	0.955	0.008
36	0.824	0.013	71	0.967	0.009
37	0.759	0.014	72	0.960	0.010
38	0.747	0.014	73	1.012	0.012
39	0.729	0.016	74	0.946	0.009
40	0.746	0.016	75	0.981	0.011

Table S9A (continued)

Residue	LOGSY effect	absolute error
76	0.980	0.011
77	0.978	0.009
78	0.955	0.010
79	1.007	0.011
80	1.005	0.012
81	0.985	0.012
82	0.998	0.011
83	0.997	0.010
84	1.004	0.011
85	1.008	0.011
86	1.011	0.012
87	0.973	0.011
89	0.982	0.011
90	0.990	0.010
91	0.988	0.011
92	0.970	0.011
93	0.977	0.012
94	0.967	0.010
95	0.964	0.010
96	0.984	0.011
98	1.004	0.012
99	1.021	0.012
100	0.991	0.011
101	0.999	0.011
102	0.987	0.010
103	0.998	0.012
104	1.002	0.011
105	1.004	0.011
106	1.001	0.010
107	0.996	0.010
109	1.007	0.010
110	0.986	0.011
111	0.998	0.011
112	0.998	0.010

Residue	LOGSY effect	absolute error
113	0.979	0.010
114	1.007	0.010
115	0.996	0.012
118	0.998	0.012
119	0.992	0.013
121	1.027	0.015
122	0.992	0.015
123	0.975	0.014
124	0.979	0.014
125	0.991	0.013
126	1.003	0.014
129	0.984	0.013
130	0.985	0.012
131	0.985	0.012
132	0.959	0.011
133	1.006	0.011
135	0.990	0.011
136	0.987	0.011
137	0.995	0.010
139	0.981	0.009
140	1.000	0.008

Table S9B. Water-LOGSY effect for 4:1 CR:aS. Experiments were performed on perdeuterated ^{15}N-labeled aS at 5 °C (pH 6.0, 200 μM aS, 500 MHz spectrometer with a room temperature probe). Experiments involved application of a 2 second presaturation pulse (γB_1 =13 Hz vs 0 Hz) followed by a normal HSQC sequence. Data used for generating Figure 9, main text.

Residue	LOGSY effect	absolute error
59	0.341	0.083
60	0.635	0.072
61	0.588	0.033
62	0.643	0.027
63	0.697	0.022
64	0.642	0.025
65	0.833	0.024
66	0.738	0.013
67	0.795	0.015
68	0.786	0.011
69	0.795	0.010
70	0.765	0.009
71	0.759	0.011
72	0.745	0.012
73	0.798	0.013
74	0.775	0.010
75	0.740	0.012
76	0.781	0.012
77	0.772	0.010
78	0.800	0.012
79	0.856	0.013
80	0.846	0.015
81	0.805	0.013
82	0.801	0.011
83	0.825	0.011
84	0.834	0.012
85	0.815	0.012
86	0.808	0.013
87	0.773	0.012
89	0.779	0.013
90	0.773	0.012
91	0.771	0.014
92	0.772	0.016
93	0.776	0.016
95	0.756	0.015
96	0.791	0.015

Residue	LOGSY effect	absolute error
97	0.880	0.018
98	0.851	0.013
99	0.880	0.013
100	0.871	0.012
101	0.876	0.011
102	0.892	0.010
103	0.920	0.012
104	0.946	0.011
105	0.959	0.010
106	0.944	0.009
107	0.973	0.010
109	0.968	0.009
110	0.958	0.010
111	0.978	0.010
112	0.971	0.009
113	0.989	0.010
114	1.003	0.011
115	0.977	0.011
118	1.004	0.011
119	0.978	0.012
121	1.016	0.013
122	0.969	0.013
123	0.955	0.011
124	0.965	0.011
125	0.992	0.011
126	0.979	0.012
129	0.981	0.011
130	0.985	0.010
131	0.978	0.010
132	0.980	0.009
135	0.976	0.009
136	0.951	0.008
137	0.977	0.008
139	0.967	0.007
140	0.989	0.007

Table S10A. HSQC intensity attenuation (I/I_o) of aS caused by an equimolar presence of CR, at 50 μM. Data used for generating Figure S2.

Residue	I/I0
3	0.233
4	0.211
6	0.101
8	0.139
9	0.095
11	0.111
12	0.092
14	0.234
15	0.201
16	0.079
17	0.072
18	0.105
19	0.172
20	0.145
21	0.077
22	0.211
23	0.185
26	0.396
27	0.173
28	0.304
29	0.266
31	0.323
32	0.266
33	0.213
35	0.272
37	0.322
38	0.323
39	0.171
40	0.170
41	0.143
42	0.315
43	0.198
44	0.103
45	0.172

Residue	I/I0
46	0.363
47	0.387
48	0.234
49	0.210
50	0.151
53	0.348
54	0.391
56	0.473
57	0.522
58	0.474
59	0.564
61	0.748
63	0.763
64	0.746
65	0.791
66	0.849
67	0.841
68	0.882
69	0.899
70	0.900
71	0.860
72	0.861
73	0.867
75	0.871
76	0.890
77	0.903
78	0.876
79	0.907
80	0.866
81	0.879
82	0.872
83	0.908
84	0.870
85	0.881

Residue	I/I0
86	0.878
87	0.873
88	0.877
89	0.877
90	0.859
91	0.822
92	0.857
93	0.821
94	0.882
95	0.805
96	0.851
98	0.877
99	0.912
100	0.896
101	0.928
102	0.937
103	0.912
104	0.931
105	0.919
106	0.951
107	0.948
109	0.967
110	0.939
111	0.968
112	0.951
113	0.981
114	0.943
115	0.945
118	0.982
119	0.977
121	0.963
122	0.970
123	1.023
124	0.978

Residue	I/I0
125	0.968
126	0.968
129	1.000
130	0.997
131	1.016
133	1.047
134	1.024
135	0.995
136	1.027
137	0.993
139	0.996
140	0.996

Table S10B. HSQC intensity attenuation (I/I_o) of aS caused by an equimolar presence of CR, at 6 μM. Data used for generating Figure S2.

Residue	I/I0
3	0.293
4	0.262
6	0.170
8	0.211
9	0.172
11	0.135
12	0.164
14	0.284
15	0.288
16	0.154
17	0.151
18	0.161
19	0.243
20	0.210
21	0.122
22	0.257
23	0.199
26	0.428
27	0.229
28	0.257
29	0.257
31	0.324
32	0.268
33	0.204
35	0.250
37	0.335
38	0.381
39	0.261
40	0.200
41	0.207
42	0.364
43	0.232
44	0.160
45	0.194

Residue	I/I0
46	0.357
47	0.420
48	0.288
49	0.279
50	0.208
53	0.292
54	0.299
56	0.339
57	0.370
58	0.333
59	0.386
61	0.482
63	0.610
64	0.567
65	0.699
66	0.735
67	0.758
68	0.827
69	0.814
70	0.855
71	0.752
72	0.803
73	0.797
75	0.811
76	0.799
77	0.801
78	0.770
79	0.759
80	0.725
81	0.803
82	0.805
83	0.874
84	0.846
85	0.866

Residue	I/I0
86	0.852
87	0.848
88	0.844
89	0.770
90	0.746
91	0.673
92	0.752
93	0.736
94	0.750
95	0.667
96	0.689
98	0.836
99	0.874
100	0.890
101	0.928
102	0.886
103	0.899
104	0.906
105	0.927
106	0.954
107	0.886
109	0.927
110	0.896
111	0.920
112	0.897
113	0.920
114	0.950
115	0.913
118	0.963
119	0.947
121	0.947
122	0.896
123	1.004
124	0.919

Residue	I/I0
125	0.888
126	0.929
129	0.953
130	0.956
131	0.971
132	1.029
133	0.986
134	0.924
135	1.000
136	0.964
137	1.009
139	0.979
140	0.983

Table S10C. HSQC intensity attenuation (I/I_o) of aS caused by an equimolar presence of CR, at 2 μM. Data used for generating Figure S2.

Residue	I/I0
3	0.459
4	0.462
6	0.357
7	0.421
8	0.475
9	0.371
11	0.381
12	0.466
13	0.384
14	0.473
15	0.568
16	0.459
17	0.401
18	0.444
19	0.476
20	0.418
21	0.433
22	0.478
23	0.439
26	0.615
27	0.471
28	0.476
29	0.430
31	0.473
32	0.427
33	0.365
35	0.459
37	0.506
38	0.533
39	0.457
40	0.414
41	0.508
42	0.521
43	0.428

Residue	I/I0
44	0.314
45	0.425
46	0.520
47	0.525
48	0.514
49	0.514
50	0.454
52	0.456
53	0.403
54	0.472
56	0.482
57	0.456
58	0.503
59	0.528
61	0.537
63	0.538
64	0.572
65	0.599
66	0.625
67	0.674
68	0.806
69	0.797
70	0.712
71	0.624
72	0.720
73	0.823
74	0.877
75	0.751
76	0.708
77	0.752
78	0.704
79	0.657
80	0.628
81	0.746

Residue	I/I0
82	0.769
83	0.766
84	0.724
85	0.805
86	0.835
87	0.804
88	0.810
89	0.647
90	0.612
91	0.614
92	0.739
93	0.704
94	0.770
95	0.601
96	0.657
98	0.737
99	0.779
100	0.866
101	0.780
102	0.830
103	0.904
104	0.876
105	0.877
106	0.878
107	0.892
109	0.919
110	0.879
111	0.914
112	0.869
113	0.863
114	0.891
115	0.914
118	0.937
119	0.903

Residue	I/I0
121	0.925
122	0.868
123	0.957
124	0.985
125	0.823
126	0.934
129	0.951
130	0.930
131	0.937
132	0.904
133	0.977
134	0.894
135	0.924
136	0.990
137	1.009
139	0.982
140	0.985

Table S11. Backbone ^{15}N and $^{1}H^{N}$ chemical shift changes relative to free aS induced by a 5-fold molar ratio of CR:aS. Data used for generating Figure S3.

Residue	Delta N	Delta H
61	-0.095	-0.022
62	-0.080	-0.019
63	-0.089	-0.011
64	-0.089	-0.013
65	-0.028	-0.011
66	-0.041	-0.011
67	-0.056	-0.011
68	-0.012	-0.007
69	-0.008	-0.002
70	-0.023	-0.007
71	-0.043	-0.008
72	-0.026	-0.005
73	-0.016	-0.007
74	0.010	-0.003
75	0.006	-0.003
76	-0.010	-0.004
77	-0.005	-0.005
78	-0.015	-0.006
79	-0.011	-0.005
80	-0.001	-0.007
81	0.008	-0.003
82	0.013	-0.004
83	0.018	-0.003
84	-0.010	-0.003
85	0.001	-0.002
86	0.003	-0.003
87	0.003	-0.002
88	0.025	-0.003
89	-0.022	-0.005
90	-0.033	-0.006
91	-0.029	-0.010
92	0.000	-0.005
93	-0.018	-0.008
94	0.008	-0.008
95	-0.021	-0.014
96	-0.043	-0.013
97	-0.032	-0.015
98	0.007	-0.008
99	0.014	-0.004
100	0.024	-0.005
101	0.010	-0.001
102	0.008	-0.004
103	0.038	-0.003
104	0.054	0.000
105	0.073	0.003
106	0.007	0.001
107	0.032	0.009
109	0.031	0.003
110	0.062	0.003
111	0.031	0.002
112	0.045	0.005
113	0.074	0.003
114	0.078	0.002
115	0.077	0.002
118	0.052	0.005
119	0.072	0.003
121	0.084	0.001
122	0.050	0.007
123	0.084	0.009
124	0.084	0.004
125	0.130	0.006
129	0.071	0.006
130	0.061	0.003
131	0.096	0.007
132	0.060	0.005
133	0.060	0.003
134	0.104	-0.005
135	0.054	-0.003
136	0.014	-0.002
137	0.092	0.003
139	0.044	0.002
140	0.041	0.000

Table S12. Relaxation rates measured for a sample with CR:aS of 4:1 at 80 Hz CPMG effective field at 500 MHz and 800 MHz, as well as squared ^{15}N chemical shift changes relative to free aS. Data used for generating Figure S4.

Residue	$[\Delta\delta N]^2$, ppm^2	R2, Hz 500 MHz	R2, Hz 800 MHz
61	0.0090	10.5	15.6
62	0.0064	9.6	12.7
63	0.0079	9.4	12.9
64	0.0079	9.9	14.7
65	0.0008	5.8	6.8
66	0.0017	5.3	6.2
67	0.0031	5.8	6.4
68	0.0001	4.7	4.6
69	0.0001	3.8	4.1
70	0.0005	4.5	5.4
71	0.0018	5.1	7.0
72	0.0007	5.4	6.5
73	0.0003	5.2	5.4
74	0.0001	4.3	4.7
75	0.0000	5.4	6.4
76	0.0001	5.1	5.6
77	0.0000	5.0	5.9
78	0.0002	5.1	6.4
79	0.0001	4.9	5.5
80	0.0000	5.1	5.9
81	0.0001	4.8	6.2
82	0.0002	4.4	5.1
83	0.0003	4.2	5.6
84	0.0001	4.3	5.0
85	0.0000	3.7	3.9
86	0.0000	5.0	5.7
87	0.0000	4.6	5.4
88	0.0006	4.8	5.5
89	0.0005	5.9	8.3
90	0.0011	5.4	7.8
91	0.0008	5.3	7.2
92	0.0001	6.5	8.1
93	0.0003	5.5	6.4
94	0.0001	5.0	5.5
95	0.0004	6.7	10.4
96	0.0018	6.0	7.9
97	0.0010	5.2	6.9
98	0.0000	4.1	5.3
99	0.0002	4.0	3.8
100	0.0006	3.5	4.8
101	0.0001	3.6	4.2
102	0.0001	2.9	3.5
103	0.0014	3.4	3.3
104	0.0029	2.6	3.6
105	0.0053	2.6	3.3
106	0.0000	2.9	3.8
107	0.0010	2.4	3.3
109	0.0010	2.7	3.3
110	0.0038	2.7	3.8
111	0.0010	2.9	3.7
112	0.0020	2.9	3.8
113	0.0055	3.4	3.9
114	0.0061	3.0	3.5
115	0.0059	2.8	4.1
118	0.0027	3.2	3.8
119	0.0052	3.4	4.1
121	0.0071	3.2	4.5
122	0.0025	2.9	4.1
123	0.0071	2.9	4.0
124	0.0071	2.7	3.8
125	0.0169	3.0	4.5
129	0.0050	2.5	3.5
130	0.0037	2.4	3.4
131	0.0092	2.4	3.5
132	0.0036	2.7	3.8
133	0.0036	2.6	3.6
134	0.0108	2.6	3.6
135	0.0029	2.3	3.1
136	0.0002	2.5	3.6
137	0.0085	2.2	3.3
139	0.0019	2.1	3.2
140	0.0017	3.0	3.6

Table S13. Correlation of change in $^{13}C^{\alpha}(i)$ chemical shift or residue i with the change in intra- to inter-residue $^{1}H^{\alpha}$-$^{1}H^{N}$ NOE ratio of residue i+1 $d_{\alpha N}(i+1,i+1)/d_{\alpha N}(i,i+1)$. Data are shown where available for the first 50 residues of aS. Data points that had a Gly as residue i or i+1 are not included. Comparison is between free aS and 1:1 CR:aS samples. Data used for generating Figure S7.

Residue	Ratio change	CA CS change
2	0.102	0.006
3	0.076	0.004
4	0.131	0.009
21	0.149	0.012
26	0.186	0.018
32	0.058	0.000
37	0.055	0.002
38	0.077	0.005
39	0.063	0.004
43	0.090	-0.003