Impact of N-terminal Acetylation of α-Synuclein on its Random Coil and Lipid Binding Properties[†]

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SUPPORTING INFORMATION

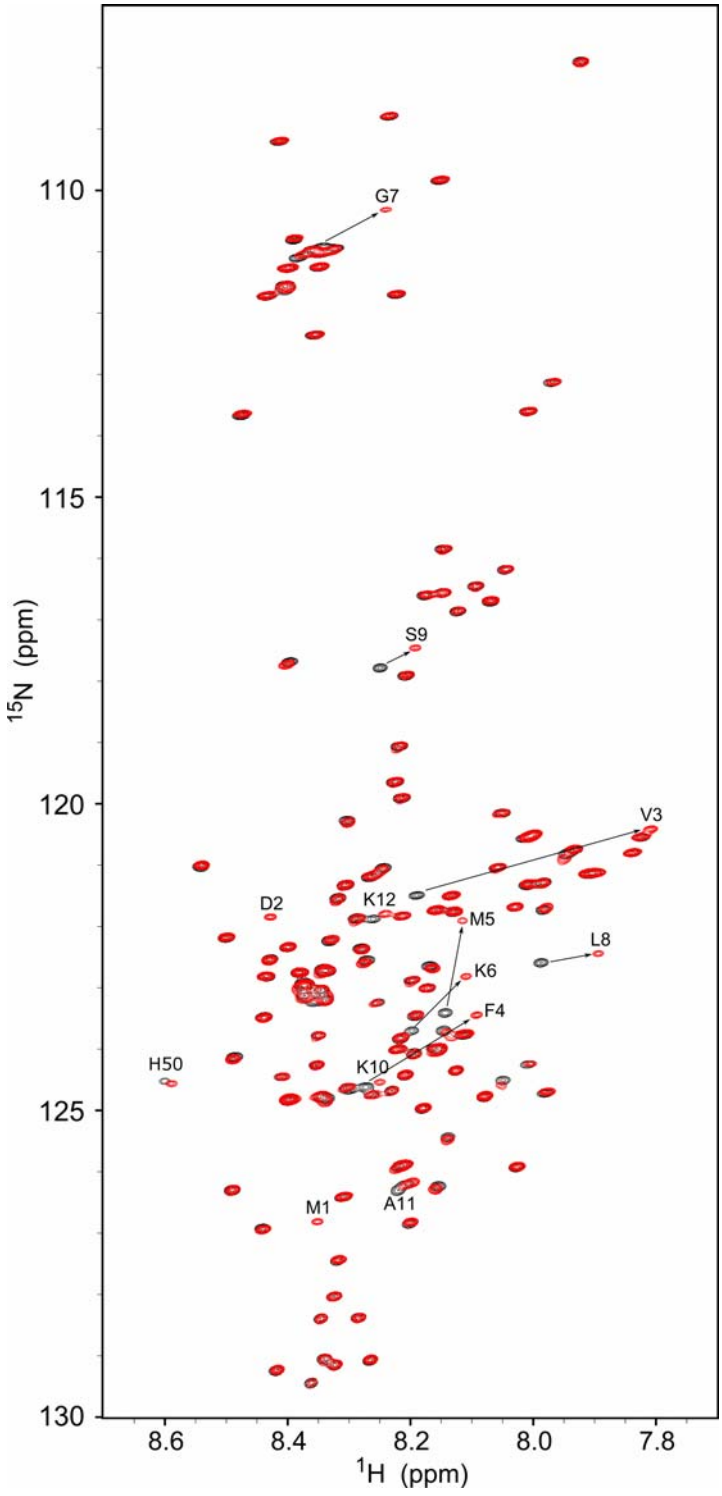

Figure S1. Overlay of 800-MHz TROSY-HSQC spectra of non-acetylated (black) and acetylated (red) aS, measured at 15 °C, pH 6. The prominent changes in peak positions for residues 3-9 are marked with arrows. Correlations for M1 and D2, which become observable in acetylated aS, are labeled, as are peaks for residues 10-12, which undergo small but detectable changes in chemical shifts. The very small difference in position of H50 results from a slightly higher pH (by ~0.1) of the acetylated aS sample. The unmarked A140 correlation (top right corner) is folded in the ^{15}N dimension.

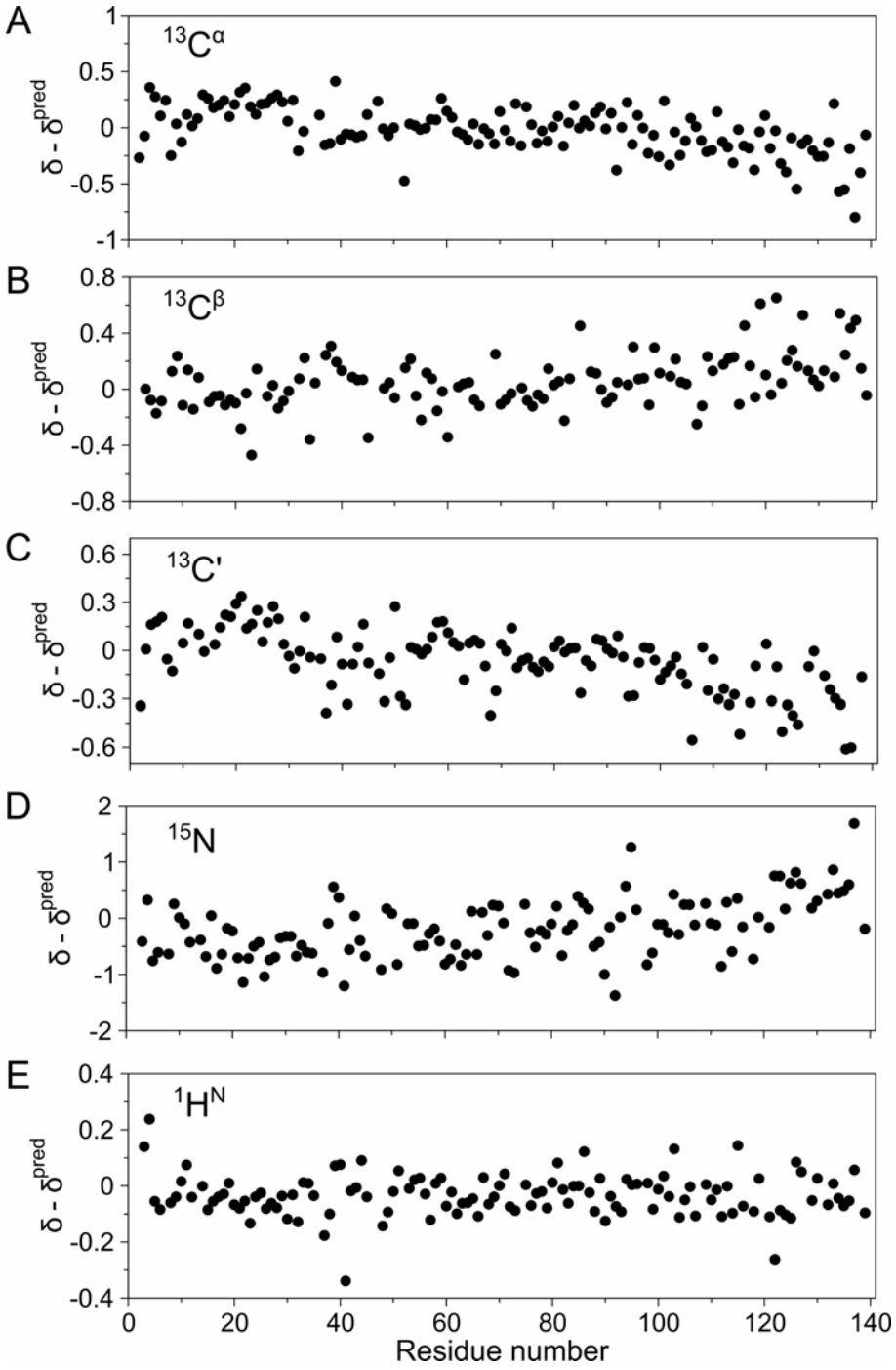

Figure S2. Differences between chemical shifts measured for non-acetylated aS and random coil values predicted based on Kjaergaard *et al.* (*1*) for non-acetylated aS at 15 °C and pH 6. Differences are shown for five nuclei: (A) $^{13}C^{\alpha}$, rmsd=0.21 ppm; (B) $^{13}C^{\beta}$, rmsd=0.20 ppm; (C) $^{13}C'$, rmsd=0.21 ppm; (D) ^{15}N, rmsd=0.56 ppm; (E) $^{1}H^{N}$, rmsd=0.08 ppm.

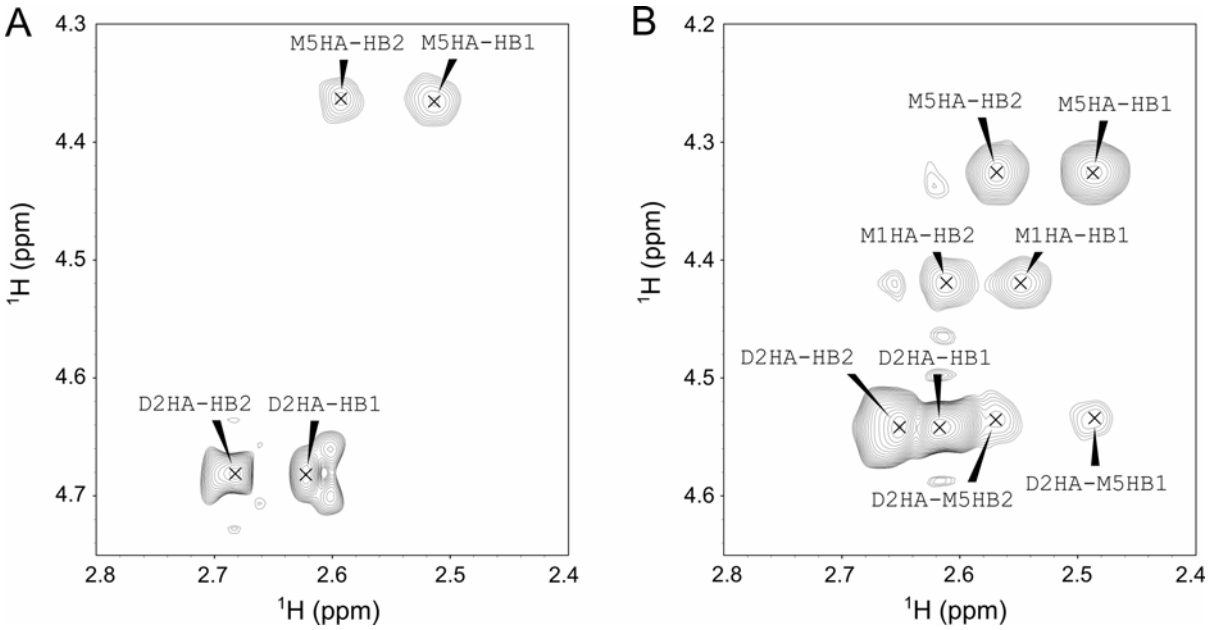

Figure S3. Comparison of small expanded regions of the 900 MHz 2D NOESY spectra of non-acetylated (A) and acetylated (B) 15-residue N-terminal fragment of aS. NOE cross-peaks between H^{α} of Asp2 and H^{β} protons of Met5 are easily observable for the acetylated peptide, but are absent for the non-acetylated peptide.

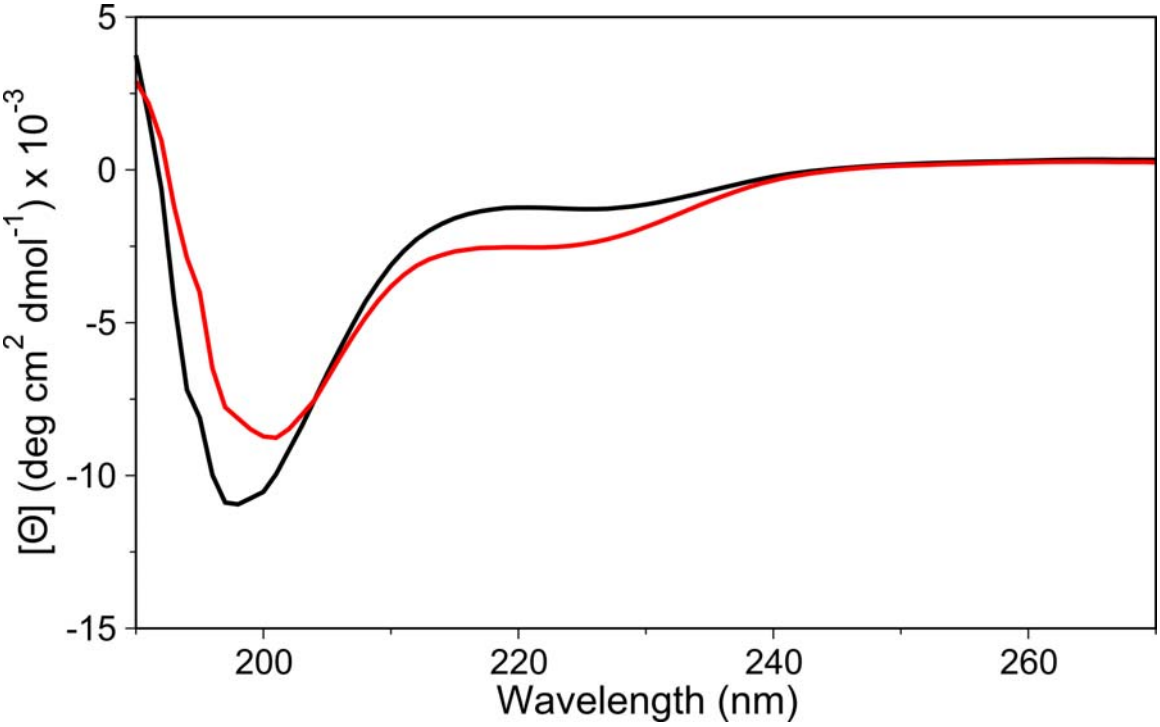

Figure S4. CD spectra of non-acetylated (black) and acetylated (red) 15-residue peptides. The peptide sequences match the first 15 residues of human wild type aS. Both peptides were C-terminally amidated. Spectra were acquired at pH 6, 20 °C, at 100 µM peptide concentration, using a 1 mm path-length cuvette.

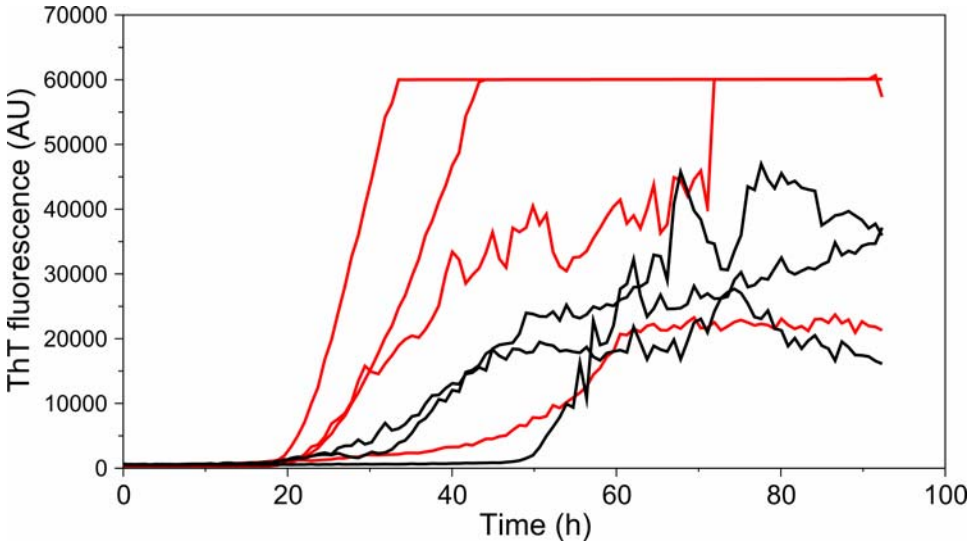

Figure S5. Results of aS fibrillation experiments. Changes in Thioflavin T fluorescence as a function of time are shown for non-acetylated (black) and acetylated (red) WT aS. Each trace represents measurements performed on a single well. Some of the samples reached fluorescence that was higher than the overflow limit of 60,000 AU, and the values of overflowed measurements are truncated to 60,000. Sample conditions were carefully controlled to be identical: 1x PBS pH 7.4, 200 μM protein, 10 μM ThT. Both acetylated and non-acetylated aS were prepared using identical purification protocols, including the high temperature denaturation step. The protein solutions were filtered through 100 kDa Amicon centrifugal filters to remove possible aggregated species prior to the fibrillation experiments.

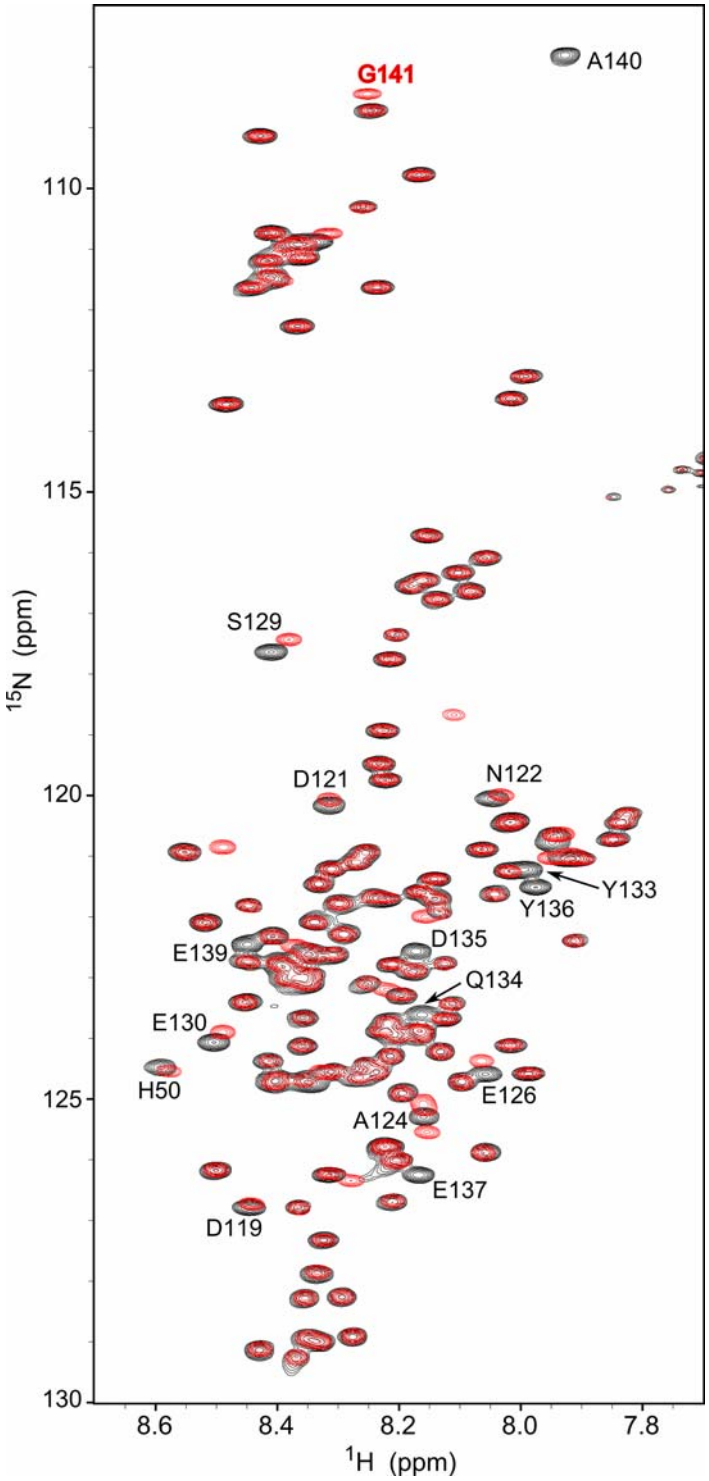

Figure S6. Overlay of 500-MHz TROSY-HSQC spectra of acetylated wild type aS (black) and acetylated His-tagged aS (red). Peaks undergoing significant chemical shift changes are labeled. Note that G141 only exists in the His-tagged aS. Both spectra were recorded in PBS buffer, 5% glycerol, 0.05% BOG, with the pH adjusted to 6. The small difference in position for the H50 correlation results from a very small increase in pH (by ~0.12) over the wild type sample. Note that the A140 resonance is folded in the ^{15}N dimension.

Table S1. Chemical shifts measured for non-acetylated WT aS at 15 °C and pH 6. Grayed out values have lower precision due to partial overlap.

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$
D2	54.217	41.566	175.795	—	—
V3	62.547	32.672	175.890	120.545	8.292
F4	57.936	39.231	175.810	123.651	8.378
M5	55.365	32.702	175.908	122.473	8.244
K6	56.921	32.840	177.128	122.742	8.299
G7	45.313	—	174.144	109.915	8.446
L8	55.155	42.543	177.635	121.620	8.090
S9	58.389	63.779	174.559	116.829	8.352
K10	56.241	33.009	176.442	123.704	8.400
A11	52.661	19.181	177.894	125.390	8.327
K12	56.450	32.959	176.679	120.929	8.368
E13	56.838	30.293	177.021	122.300	8.462
G14	45.319	—	174.014	110.143	8.489
V15	62.571	32.724	176.465	120.152	8.009
V16	62.411	32.694	175.986	125.313	8.314
A17	52.542	19.129	177.653	128.587	8.471
A18	52.701	19.087	177.896	123.773	8.340
A19	52.739	19.091	178.184	123.163	8.305
E20	56.777	30.191	176.937	120.161	8.357
K21	56.732	32.868	177.148	122.341	8.370
T22	62.282	69.810	174.691	115.247	8.151
K23	56.657	32.909	176.672	123.809	8.372
Q24	56.189	29.488	176.589	121.785	8.449
G25	45.359	—	174.242	110.615	8.513
V26	62.590	32.739	176.369	119.772	8.041
A27	52.818	19.011	178.116	127.482	8.453
E28	56.838	30.180	176.665	120.637	8.426
A29	52.710	19.064	177.751	125.000	8.328
A30	52.843	19.061	178.482	123.081	8.260
G31	45.372	—	174.220	107.818	8.341
K32	56.251	33.201	177.017	120.720	8.137
T33	61.925	69.997	174.677	115.619	8.252
K34	56.515	32.925	176.530	123.882	8.502
E35	56.837	30.223	176.948	122.221	8.477
G36	45.317	—	174.016	110.030	8.470
V37	62.341	32.746	175.924	119.591	7.930
L38	54.986	42.505	176.658	125.856	8.304

Table S1 (continued)

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$
Y39	57.881	38.825	175.586	122.511	8.298
V40	62.202	32.830	176.156	123.347	8.104
G41	45.178	—	173.956	112.182	8.072
S42	58.367	63.908	174.794	115.628	8.281
K43	56.446	33.008	176.879	123.496	8.515
T44	61.900	69.914	174.587	115.529	8.198
K45	56.574	32.937	176.494	123.814	8.454
E46	56.823	30.161	176.938	122.013	8.479
G47	45.261	—	173.877	110.030	8.450
V48	62.273	32.792	176.082	119.851	7.944
V49	62.225	32.732	175.924	124.953	8.316
H50	55.392	29.375	174.946	123.667	8.694
G51	45.167	—	173.731	110.652	8.501
V52	62.006	32.972	175.928	119.565	8.122
A53	52.426	19.300	177.822	128.308	8.522
T54	61.835	69.985	174.544	114.919	8.253
V55	62.303	32.856	175.852	123.073	8.268
A56	52.543	19.210	177.778	128.159	8.446
E57	56.627	30.385	176.708	120.957	8.396
K58	56.485	32.995	176.986	122.860	8.459
T59	62.164	69.821	174.648	116.000	8.233
K60	56.664	32.952	176.691	123.694	8.414
E61	56.758	30.144	176.428	122.159	8.458
Q62	55.790	29.512	175.963	121.805	8.446
V63	62.416	32.794	176.349	121.955	8.303
T64	61.784	69.910	174.055	118.161	8.325
N65	53.120	38.906	175.243	121.864	8.542
V66	62.678	32.509	176.863	120.790	8.262
G67	45.355	—	174.666	112.689	8.578
G68	45.043	—	173.733	108.857	8.256
A69	52.311	19.336	177.655	123.811	8.185
V70	62.426	32.713	176.334	120.557	8.236
V71	62.172	32.771	176.289	125.477	8.415
T72	61.847	69.918	174.908	118.724	8.333
G73	45.252	—	174.010	111.393	8.459
V74	62.348	32.849	176.559	119.540	8.105
T75	61.935	69.803	174.079	119.004	8.323

Table S1 (continued)

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$
A76	52.440	19.288	177.560	127.449	8.391
V77	62.225	32.826	176.009	120.129	8.165
A78	52.488	19.152	177.629	128.235	8.431
Q79	55.702	29.622	175.939	120.385	8.414
K80	56.367	33.158	176.678	123.334	8.459
T81	61.903	69.934	174.441	117.009	8.317
V82	62.305	32.820	176.144	123.063	8.327
E83	56.800	30.217	177.026	125.387	8.597
G84	45.288	—	174.141	110.759	8.541
A85	52.871	19.203	178.490	124.002	8.284
G86	45.296	—	174.298	108.226	8.516
S87	58.341	63.894	174.716	115.712	8.178
I88	61.314	38.672	176.263	122.793	8.212
A89	52.604	19.067	177.557	128.132	8.370
A90	52.473	19.167	177.725	123.390	8.231
A91	52.639	19.119	178.132	123.460	8.313
T92	62.004	69.870	175.155	112.642	8.115
G93	45.194	—	173.617	110.721	8.328
F94	57.794	39.590	175.473	120.360	8.113
V95	62.006	33.113	175.393	123.773	8.075
K96	56.370	33.044	176.440	126.496	8.422
K97	56.495	33.196	176.350	123.793	8.503
D98	54.420	41.025	176.209	121.254	8.434
Q99	55.867	29.392	176.013	120.204	8.367
L100	55.378	42.220	177.985	122.852	8.321
G101	45.312	—	174.066	109.833	8.493
K102	56.200	33.150	176.450	120.794	8.235
N103	53.353	38.765	175.314	120.049	8.646
E104	56.681	30.150	176.539	121.373	8.504
E105	56.866	30.099	177.015	121.813	8.486
G106	45.044	—	173.434	110.271	8.453
A107	50.500	18.172	—	124.960	8.131
P108	63.093	32.043	177.048	—	—
Q109	55.713	29.644	175.970	121.207	8.605
E110	56.627	30.399	176.819	122.516	8.543
G111	45.270	—	173.753	110.289	8.505
I112	60.918	38.657	176.237	120.161	8.010

Table S1 (continued)

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$
L113	55.015	42.356	177.138	127.052	8.428
E114	56.398	30.446	175.837	122.240	8.441
D115	54.212	41.036	175.721	121.385	8.383
M116	53.193	32.482	—	122.021	8.270
P117	62.896	32.128	176.698	—	—
V118	61.950	33.054	175.755	120.876	8.317
D119	52.058	41.014	—	125.960	8.541
P120	63.497	32.193	176.920	—	—
D121	54.495	40.787	176.110	119.290	8.405
N122	53.465	39.270	175.370	119.167	8.149
E123	56.747	29.904	176.026	121.643	8.384
A124	52.366	19.131	177.208	124.463	8.246
Y125	57.749	38.976	175.351	119.929	8.048
E126	55.608	30.549	175.415	123.668	8.150
M127	53.302	32.354	—	123.866	8.437
P128	63.059	32.205	176.890	—	—
S129	58.277	63.901	174.807	116.742	8.503
E130	56.467	30.188	176.493	123.177	8.591
E131	56.848	30.155	176.924	121.925	8.486
G132	45.185	—	173.818	110.009	8.429
Y133	58.127	38.788	175.721	120.346	8.088
Q134	55.406	29.840	174.840	122.806	8.247
D135	54.141	41.017	175.442	121.693	8.267
Y136	57.599	39.068	175.074	120.732	8.082
E137	53.509	30.010	—	125.328	8.263
P138	62.914	32.222	176.853	—	—
E139	56.509	30.197	175.365	121.586	8.535
A140	53.797	20.174	—	130.919	8.023

Table S2. Chemical shifts measured for acetylated WT aS at 15 °C and pH 6. Grayed out values have lower precision due to partial overlap.

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$
M1	55.899	32.891	176.594	125.850	8.456
D2	54.554	40.805	176.718	120.888	8.533
V3	63.276	32.325	176.554	119.444	7.913
F4	58.518	39.078	176.352	122.491	8.199
M5	55.774	32.399	176.495	120.956	8.220
K6	57.141	32.744	177.383	121.863	8.215
G7	45.454	—	174.351	109.357	8.346
L8	55.281	42.468	177.727	121.480	8.000
S9	58.521	63.731	174.652	116.494	8.298
K10	56.295	32.958	176.503	123.576	8.354
A11	52.716	19.137	177.954	125.209	8.298
K12	56.480	32.947	176.680	120.825	8.346
E13	56.847	30.220	177.043	122.177	8.463
G14	45.287	—	174.034	110.082	8.486
V15	62.605	32.719	176.464	120.157	8.004
V16	62.461	32.690	176.009	125.254	8.313
A17	52.563	19.121	177.676	128.488	8.466
A18	52.719	19.081	177.910	123.750	8.334
A19	52.747	19.092	178.193	123.140	8.302
E20	56.788	30.185	176.946	120.134	8.354
K21	56.746	32.867	177.156	122.307	8.364
T22	62.290	69.811	174.694	115.220	8.150
K23	56.669	32.904	176.682	123.799	8.369
Q24	56.200	29.489	176.593	121.751	8.449
G25	45.365	—	174.248	110.591	8.511
V26	62.596	32.735	176.368	119.774	8.041
A27	52.821	19.012	178.125	127.455	8.452
E28	56.848	30.179	176.662	120.617	8.425
A29	52.715	19.067	177.748	124.997	8.329
A30	52.833	19.065	178.476	123.080	8.260
G31	45.370	—	174.217	107.817	8.340
K32	56.250	33.200	177.013	120.721	8.135
T33	61.920	70.000	174.673	115.603	8.251
K34	56.515	32.935	176.529	123.882	8.502
E35	56.815	30.209	176.948	122.204	8.478
G36	45.319	—	174.014	110.030	8.470
V37	62.341	32.745	175.924	119.576	7.931
L38	54.989	42.495	176.656	125.856	8.304

Table S2 (continued)

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$
Y39	57.881	38.835	175.583	122.486	8.295
V40	62.201	32.828	176.154	123.347	8.104
G41	45.177	—	173.959	112.164	8.071
S42	58.373	63.909	174.791	115.626	8.278
K43	56.454	33.004	176.877	123.493	8.512
T44	61.900	69.913	174.592	115.501	8.197
K45	56.585	32.941	176.489	123.809	8.452
E46	56.856	30.215	176.938	122.013	8.479
G47	45.263	—	173.876	110.030	8.450
V48	62.274	32.789	176.086	119.829	7.945
V49	62.247	32.746	175.920	124.933	8.314
H50	55.392	29.374	174.938	123.622	8.694
G51	45.167	—	173.726	110.657	8.503
V52	62.013	32.972	175.925	119.565	8.122
A53	52.427	19.300	177.820	128.293	8.522
T54	61.835	69.987	174.542	114.912	8.252
V55	62.308	32.858	175.853	123.070	8.268
A56	52.544	19.212	177.777	128.149	8.444
E57	56.625	30.382	176.707	120.954	8.396
K58	56.476	32.998	176.982	122.860	8.459
T59	62.162	69.823	174.649	115.963	8.232
K60	56.664	32.952	176.692	123.699	8.413
E61	56.745	30.163	176.424	122.123	8.454
Q62	55.789	29.516	175.958	121.864	8.447
V63	62.415	32.791	176.346	121.960	8.306
T64	61.780	69.913	174.051	118.146	8.326
N65	53.122	38.909	175.240	121.862	8.542
V66	62.678	32.508	176.861	120.771	8.264
G67	45.354	—	174.664	112.667	8.579
G68	45.035	—	173.730	108.860	8.256
A69	52.311	19.337	177.653	123.812	8.186
V70	62.424	32.715	176.332	120.541	8.239
V71	62.170	32.780	176.288	125.458	8.415
T72	61.847	69.920	174.906	118.702	8.331
G73	45.252	—	174.010	111.391	8.459
V74	62.341	32.845	176.557	119.540	8.105
T75	61.931	69.807	174.077	118.974	8.322

Table S2 (continued)

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$
A76	52.443	19.290	177.559	127.445	8.391
V77	62.230	32.827	176.008	120.104	8.165
A78	52.487	19.148	177.628	128.207	8.431
Q79	55.703	29.622	175.939	120.377	8.413
K80	56.370	33.158	176.677	123.322	8.459
T81	61.900	69.935	174.438	116.970	8.314
V82	62.303	32.819	176.142	123.070	8.330
E83	56.798	30.219	177.023	125.366	8.597
G84	45.287	—	174.138	110.771	8.541
A85	52.871	19.200	178.491	124.001	8.285
G86	45.297	—	174.298	108.232	8.517
S87	58.351	63.896	174.714	115.718	8.176
I88	61.310	38.674	176.260	122.788	8.216
A89	52.604	19.065	177.554	128.117	8.370
A90	52.472	19.169	177.723	123.394	8.231
A91	52.635	19.125	178.130	123.463	8.314
T92	61.999	69.872	175.153	112.655	8.113
G93	45.193	—	173.613	110.724	8.328
F94	57.798	39.619	175.472	120.338	8.111
V95	62.003	33.114	175.392	123.748	8.081
K96	56.371	33.024	176.444	126.474	8.421
K97	56.488	33.190	176.352	123.793	8.503
D98	54.424	41.025	176.206	121.250	8.433
Q99	55.862	29.394	176.014	120.214	8.368
L100	55.384	42.222	177.985	122.852	8.318
G101	45.310	—	174.070	109.814	8.495
K102	56.203	33.152	176.451	120.793	8.234
N103	53.347	38.768	175.310	120.035	8.645
E104	56.676	30.150	176.539	121.386	8.505
E105	56.862	30.105	177.013	121.813	8.486
G106	45.040	—	173.432	110.272	8.453
A107	50.498	18.174	—	124.964	8.135
P108	63.090	32.046	177.047	—	—
Q109	55.714	29.637	175.972	121.207	8.606
E110	56.625	30.405	176.821	122.524	8.543
G111	45.273	—	173.756	110.295	8.505
I112	60.918	38.658	176.239	120.166	8.013

Table S2 (continued)

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$
L113	55.010	42.353	177.136	127.053	8.428
E114	56.395	30.445	175.840	122.239	8.442
D115	54.214	41.047	175.723	121.394	8.383
M116	53.190	32.477	—	122.040	8.277
P117	62.898	32.125	176.701	—	—
V118	61.937	33.057	175.758	120.865	8.319
D119	52.067	41.019	—	125.971	8.544
P120	63.490	32.200	176.915	—	—
D121	54.491	40.793	176.114	119.298	8.406
N122	53.466	39.273	175.372	119.186	8.154
E123	56.754	29.919	176.029	121.643	8.384
A124	52.364	19.130	177.208	124.486	8.246
Y125	57.743	38.995	175.349	119.934	8.052
E126	55.605	30.563	175.414	123.669	8.151
M127	53.304	32.351	—	123.881	8.440
P128	63.061	32.197	176.888	—	—
S129	58.275	63.903	174.803	116.753	8.503
E130	56.464	30.202	176.493	123.190	8.592
E131	56.840	30.160	176.924	121.925	8.486
G132	45.165	—	173.820	110.009	8.429
Y133	58.128	38.783	175.724	120.331	8.089
Q134	55.407	29.837	174.843	122.778	8.245
D135	54.151	41.034	175.447	121.697	8.267
Y136	57.599	39.064	175.073	120.719	8.080
E137	53.515	30.030	—	125.324	8.261
P138	62.910	32.228	176.852	—	—
E139	56.511	30.207	175.366	121.578	8.535
A140	53.798	20.177	—	130.926	8.023

Table S3. Chemical shifts predicted based on Kjaergaard *et al* for non-acetylated WT aS at 15 °C and pH 6.

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$	$^{1}H^{\alpha}$
M1	55.566	32.807	175.880	122.254	8.541	4.475
D2	54.485	40.819	176.139	122.197	8.429	4.573
V3	62.621	32.670	175.882	120.960	8.152	4.002
F4	57.577	39.309	175.648	123.327	8.140	4.642
M5	55.088	32.874	175.728	123.231	8.299	4.450
K6	56.816	32.925	176.920	123.349	8.383	4.260
G7	45.069	—	174.198	110.570	8.474	3.999
L8	55.404	42.415	177.763	122.256	8.150	4.402
S9	58.354	63.543	174.626	116.575	8.391	4.455
K10	56.369	33.123	176.396	123.692	8.384	4.321
A11	52.543	19.043	177.724	125.488	8.252	4.307
K12	56.434	33.102	176.746	121.356	8.408	4.293
E13	56.756	30.209	176.919	122.621	8.475	4.295
G14	45.025	—	174.021	110.529	8.490	4.013
V15	62.311	32.814	176.426	120.833	8.094	4.135
V16	62.232	32.744	175.948	125.266	8.369	4.102
A17	52.340	19.175	177.509	129.477	8.510	4.304
A18	52.458	19.200	177.674	124.413	8.369	4.283
A19	52.640	19.168	177.973	123.340	8.295	4.282
E20	56.570	30.290	176.646	120.389	8.424	4.259
K21	56.415	33.149	176.810	123.047	8.450	4.383
T22	61.928	69.839	174.553	116.389	8.205	4.342
K23	56.470	33.379	176.507	124.522	8.505	4.333
Q24	56.069	29.344	176.339	122.284	8.488	4.343
G25	45.149	—	174.188	111.043	8.538	4.014
V26	62.372	32.789	176.194	120.810	8.122	4.103
A27	52.554	18.983	177.841	128.223	8.515	4.332
E28	56.545	30.316	176.467	121.328	8.504	4.237
A29	52.481	19.147	177.712	125.346	8.364	4.310
A30	52.785	19.074	178.517	123.402	8.378	4.302
G31	45.126	—	174.331	108.141	8.373	3.970
K32	56.458	33.126	177.022	121.393	8.265	4.397
T33	61.957	69.774	174.468	116.101	8.240	4.362
K34	56.457	33.283	176.572	124.487	8.493	4.342
E35	56.799	30.178	176.924	122.844	8.512	4.285
G36	45.204	—	174.068	110.647	8.515	3.997
V37	62.495	32.503	176.311	120.557	8.107	4.043
L38	55.126	42.197	176.873	125.946	8.404	4.311

Table S3 (continued)

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$	$^{1}H^{\alpha}$
Y39	57.468	38.631	175.502	121.953	8.226	4.654
V40	62.306	32.697	176.241	122.978	8.028	4.083
G41	45.234	—	174.290	113.386	8.411	3.991
S42	58.429	63.821	174.879	116.187	8.299	4.448
K43	56.530	32.942	176.857	123.455	8.521	4.433
T44	61.969	69.846	174.423	115.926	8.107	4.350
K45	56.457	33.283	176.572	124.487	8.493	4.342
E46	56.799	30.178	176.924	122.844	8.512	4.285
G47	45.025	—	174.021	110.529	8.490	4.013
V48	62.282	32.785	176.399	120.764	8.087	4.112
V49	62.295	32.685	175.969	124.782	8.409	4.091
H50	55.392	29.436	174.672	123.584	8.714	4.709
G51	45.025	—	174.017	111.474	8.447	4.016
V52	62.480	32.819	176.265	120.723	8.138	4.087
A53	52.392	19.084	177.802	128.404	8.531	4.428
T54	61.816	70.033	174.537	115.013	8.231	4.363
V55	62.319	33.075	175.875	123.568	8.240	4.123
A56	52.547	19.093	177.770	128.642	8.475	4.305
E57	56.553	30.310	176.624	121.232	8.517	4.249
K58	56.415	33.149	176.810	123.047	8.450	4.383
T59	61.903	69.837	174.467	116.407	8.205	4.352
K60	56.516	33.294	176.580	124.511	8.486	4.328
E61	56.667	30.306	176.378	122.887	8.480	4.253
Q62	55.827	29.495	175.936	122.276	8.545	4.369
V63	62.476	32.755	176.531	122.792	8.365	4.183
T64	61.891	69.861	174.009	118.803	8.385	4.376
N65	53.086	38.981	175.178	121.742	8.587	4.752
V66	62.826	32.628	176.819	121.434	8.370	4.118
G67	45.363	—	174.763	112.585	8.548	4.019
G68	45.096	—	174.135	109.162	8.321	3.974
A69	52.456	19.086	177.907	123.578	8.224	4.363
V70	62.282	32.820	176.295	120.340	8.235	4.126
V71	62.195	32.846	176.293	125.563	8.372	4.219
T72	61.966	69.949	174.768	119.650	8.407	4.380
G73	45.038	—	174.117	112.364	8.547	4.054
V74	62.509	32.840	176.621	120.897	8.152	4.187
T75	61.749	69.883	174.127	118.755	8.319	4.363
A76	52.413	19.410	177.664	127.706	8.461	4.369

Table S3 (continued)

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$	$^{1}H^{\alpha}$
V77	62.364	32.865	176.140	120.642	8.192	4.054
A78	52.517	19.220	177.700	128.455	8.451	4.306
Q79	55.823	29.476	176.039	120.672	8.493	4.307
K80	56.359	33.128	176.655	123.436	8.447	4.392
T81	61.802	69.878	174.381	116.796	8.235	4.402
V82	62.469	33.044	176.154	123.729	8.340	4.132
E83	56.757	30.142	177.013	125.604	8.659	4.304
G84	45.089	—	174.125	110.871	8.542	3.977
A85	52.874	18.751	178.755	123.611	8.284	4.347
G86	45.235	—	174.361	107.955	8.394	4.031
S87	58.324	63.769	174.812	115.548	8.202	4.483
I88	61.182	38.559	176.192	123.293	8.303	4.182
A89	52.418	19.069	177.495	128.559	8.343	4.314
A90	52.483	19.262	177.715	124.390	8.356	4.280
A91	52.510	19.176	178.148	123.616	8.350	4.405
T92	62.382	69.821	175.064	114.018	8.188	4.292
G93	45.191	—	173.657	110.700	8.420	3.953
F94	57.569	39.558	175.759	119.790	8.089	4.685
V95	62.155	32.811	175.675	122.511	8.071	4.049
K96	56.260	32.972	176.515	126.345	8.415	4.271
K97	56.497	33.117	176.331	124.248	8.470	4.286
D98	54.648	41.137	176.195	122.081	8.424	4.556
Q99	55.934	29.095	176.073	120.821	8.450	4.339
L100	55.636	42.105	178.166	122.959	8.333	4.337
G101	45.073	—	174.201	109.943	8.458	3.992
K102	56.532	33.058	176.545	121.051	8.273	4.332
N103	53.391	38.550	175.355	119.624	8.514	4.693
E104	56.927	30.099	176.685	121.661	8.616	4.284
E105	56.983	30.061	177.224	121.571	8.536	4.288
G106	44.959	—	173.990	110.031	8.456	3.984
A107	50.490	18.421	176.166	125.078	8.238	4.614
P108	63.207	32.162	177.028	—	—	4.437
Q109	55.927	29.411	176.219	120.945	8.600	4.318
E110	56.826	30.268	176.874	122.606	8.593	4.296
G111	45.128	—	174.055	110.408	8.519	3.993
I112	61.043	38.480	176.475	121.017	8.119	4.173
L113	55.188	42.137	177.474	126.768	8.429	4.393
E114	56.711	30.217	176.111	122.834	8.538	4.257

Table S3 (continued)

Residue	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C'$	^{15}N	$^{1}H^{N}$	$^{1}H^{\alpha}$
D115	54.229	41.144	176.240	121.033	8.239	4.604
M116	53.355	32.028	174.332	122.174	8.342	4.763
P117	63.078	31.961	177.020	—	—	4.488
V118	62.326	33.110	175.851	121.601	8.408	4.071
D119	52.094	40.404	174.284	125.942	8.515	4.926
P120	63.388	32.091	176.879	—	—	4.422
D121	54.679	40.826	176.423	119.452	8.515	4.558
N122	53.492	38.618	175.471	118.413	8.411	4.672
E123	57.066	29.861	176.529	120.891	8.471	4.182
A124	52.760	18.926	177.547	124.298	8.349	4.252
Y125	57.840	38.697	175.753	119.303	8.163	4.569
E126	56.155	30.386	175.874	122.851	8.065	4.249
M127	53.447	31.826	174.376	123.249	8.387	4.721
P128	63.166	32.073	176.990	—	—	4.522
S129	58.480	63.834	174.811	116.562	8.555	4.428
E130	56.723	30.164	176.617	122.871	8.564	4.337
E131	57.103	30.022	177.081	121.908	8.488	4.228
G132	45.317	—	174.061	109.580	8.496	3.922
Y133	57.913	38.699	176.019	119.483	8.080	4.617
Q134	55.976	29.299	175.175	122.360	8.291	4.234
D135	54.692	40.771	176.054	121.210	8.338	4.478
Y136	57.785	38.632	175.676	120.134	8.135	4.609
E137	54.307	29.518	174.158	123.643	8.206	4.522
P138	63.314	32.073	177.017	—	—	4.379
E139	56.573	30.241	176.629	121.775	8.631	4.232
A140	52.665	19.256	177.827	125.355	8.435	4.305

Table S4. $^{3}J_{HN\text{-}H\alpha}$ couplings for the N-terminal residues of acetylated and non-acetylated aS. Measurements were performed at 900 MHz, 15 °C, pH 6.0 on uniformly ^{15}N-labeled protein. Average $^{3}J_{HN\text{-}H\alpha}$ values and their standard deviations for corresponding residue types, obtained from measurements on non-acetylated aS, are also shown.

Residue	Non-acetylated aS		Acetylated aS		Residue-type average	
	J, Hz	uncertainty	J, Hz	uncertainty	J, Hz	SD, Hz
M1	—	—	6.50	0.02	6.97	0.31
D2	—	—	6.63	0.04	6.89	0.02
V3	7.25	0.04	6.66	0.08	7.60	0.29
F4	7.13	0.03	6.76	0.03	7.02	0.14
M5	7.13	0.06	6.69	0.06	6.97	0.31
K6	6.11	0.07	6.07	0.05	6.66	0.25
L8	7.01	0.03	6.87	0.05	7.00	0.15
S9	6.53	0.07	6.52	0.04	6.62	0.15
K10	—	—	6.85	0.08	6.66	0.25
A11	5.30	0.05	—	—	5.64	0.26
K12	6.64	0.03	6.65	0.02	6.66	0.25
E13	6.18	0.02	—	—	6.52	0.42
V15	7.37	0.07	7.47	0.06	7.60	0.29

Reference

1. Kjaergaard, M., Brander, S., and Poulsen, F. M. Random coil chemical shift for intrinsically disordered proteins: effects of temperature and pH, *J. Biomol. NMR 49*, 139-149.