Differential phospholipid-binding of α-synuclein variants implicated in Parkinson's disease revealed by solution NMR spectroscopy[†]

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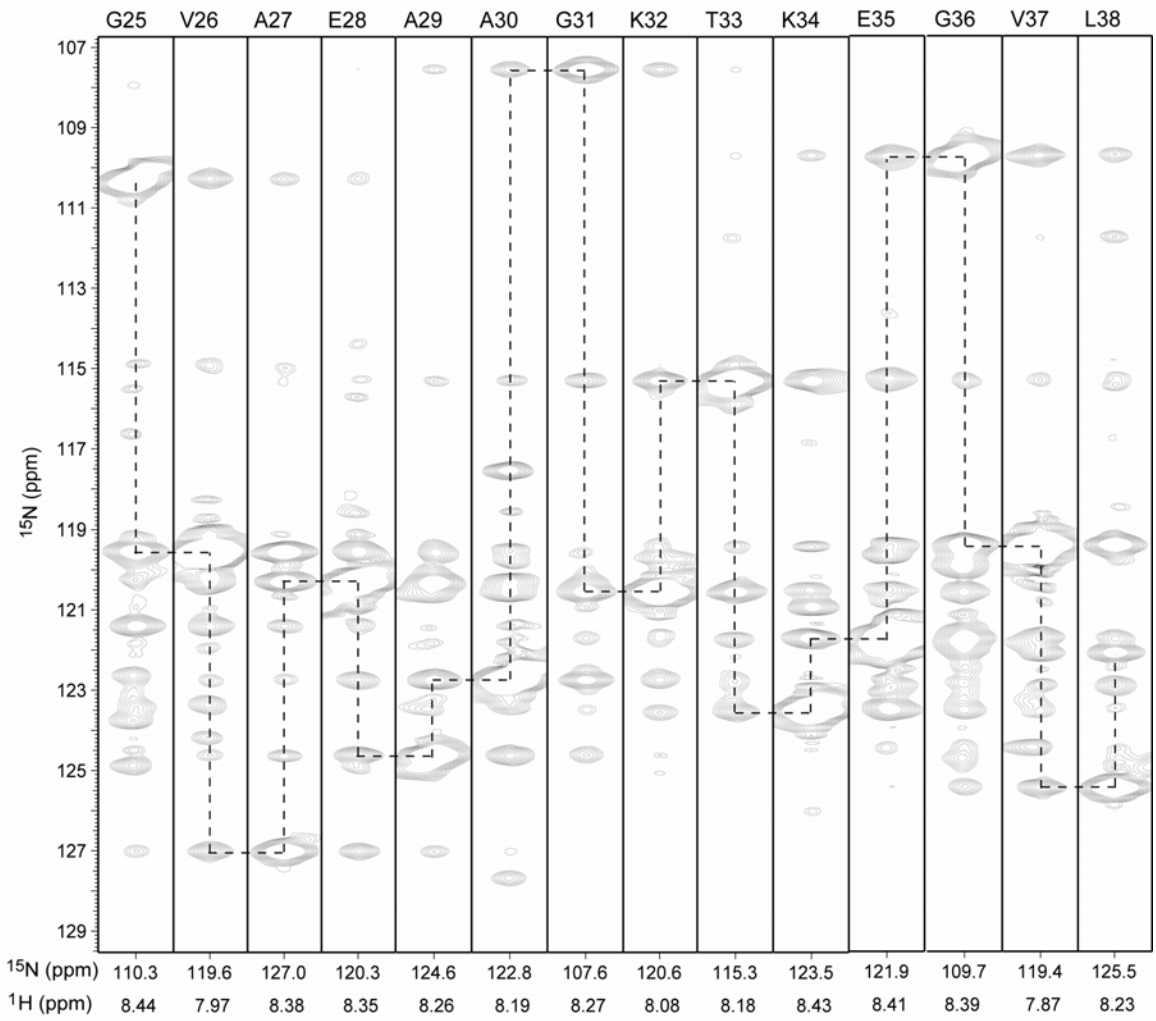
SUPPORTING INFORMATION

Supporting Information Table 1. ^{1}H and ^{15}N resonance assignments of WT αS and its three disease variants (20 ºC, 20 mM Na_2HPO_4, pH 6.0, 94%/6% H_2O/D_2O, 0.02% NaN_3).

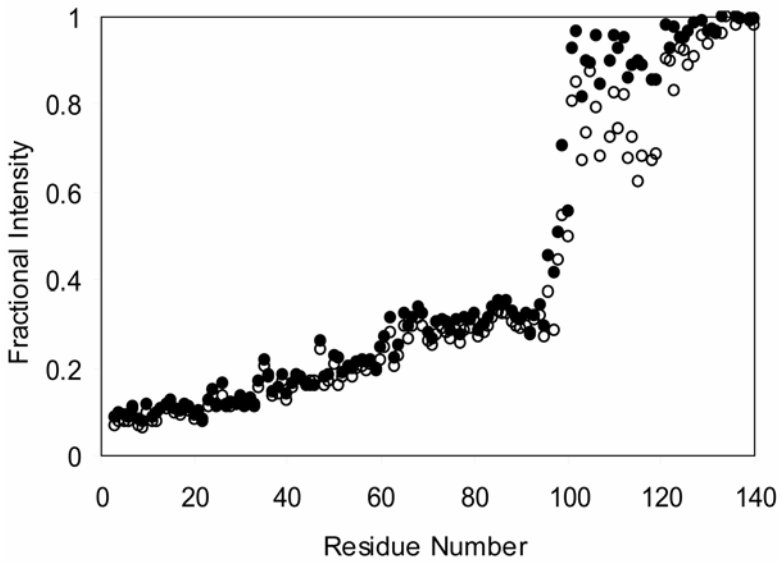
Residue Number	Residue Type	WT αS ^{1}H (ppm)	WT αS ^{15}N (ppm)	A30P αS ^{1}H (ppm)	A30P αS ^{15}N (ppm)	E46K αS ^{1}H (ppm)	E46K αS ^{15}N (ppm)	A53T αS ^{1}H (ppm)	A53T αS ^{15}N (ppm)
3	V	8.217	120.284	8.215	120.284	8.216	120.333	8.213	120.284
4	F	8.297	123.272	8.297	123.286	8.299	123.329	8.295	123.281
5	M	8.177	122.154	8.177	122.162	8.179	122.207	8.176	122.156
6	K	8.22	122.46	8.221	122.474	8.224	122.524	8.22	122.469
7	G	8.364	109.671	8.364	109.685	8.369	109.725	8.365	109.673
8	L	8.021	121.51	8.021	121.521	8.024	121.57	8.02	121.52
9	S	8.279	116.53	8.28	116.544	8.283	116.59	8.279	116.538
10	K	8.322	123.464	8.323	123.472	8.325	123.524	8.322	123.472
11	A	8.248	125.017	8.249	125.034	8.251	125.076	8.248	125.028
12	K	8.284	120.661	8.289	120.554	8.285	120.726	8.283	120.68
13	E	8.384	121.97	8.384	121.995	8.385	122.034	8.383	121.987
14	G	8.407	109.874	8.406	109.887	8.411	109.934	8.408	109.878
15	V	7.933	120.011	7.933	120.011	7.933	120.057	7.932	120.02
16	V	8.224	124.803	8.226	124.835	8.226	124.854	8.223	124.816
17	A	8.38	128.064	8.381	128.124	8.382	128.119	8.379	128.071
18	A	8.252	123.421	8.252	123.464	8.254	123.479	8.251	123.434
19	A	8.221	122.834	8.222	122.875	8.223	122.881	8.22	122.84
20	E	8.278	119.874	8.282	119.905	8.279	119.915	8.278	119.886
21	K	8.29	122.034	8.295	122.093	8.29	122.086	8.288	122.044
22	T	8.078	114.947	8.078	114.968	8.082	115.004	8.078	114.956
23	K	8.291	123.517	8.292	123.607	8.294	123.566	8.29	123.529
24	Q	8.371	121.479	8.372	121.551	8.373	121.52	8.371	121.485
25	G	8.434	110.351	8.431	110.452	8.438	110.4	8.435	110.352
26	V	7.967	119.62	7.958	119.366	7.969	119.646	7.966	119.629
27	A	8.375	127.082	8.363	126.684	8.378	127.119	8.375	127.092
28	E	8.345	120.367	8.29	120.558	8.345	120.401	8.344	120.382
29	A	8.255	124.693	8.378	127.615	8.25	124.713	8.253	124.696
30	A	8.188	122.811			8.189	122.861	8.187	122.818
31	G	8.267	107.622	8.436	108.905	8.269	107.682	8.267	107.623
32	K	8.073	120.624	8.128	120.857	8.078	120.687	8.072	120.635
33	T	8.177	115.374	8.201	115.642	8.182	115.424	8.176	115.386
34	K	8.417	123.594	8.446	123.713	8.419	123.653	8.418	123.591
35	E	8.403	121.907	8.402	121.916	8.408	121.837	8.403	121.931
36	G	8.386	109.772	8.398	109.842	8.39	109.813	8.385	109.784
37	V	7.862	119.462	7.864	119.486	7.858	119.502	7.86	119.473
38	L	8.222	125.509	8.227	125.568	8.224	125.551	8.222	125.528
39	Y	8.217	122.151	8.22	122.189	8.22	122.172	8.216	122.168
40	V	8.037	122.981	8.039	122.996	8.047	122.898	8.036	122.99
41	G	7.996	111.8	7.995	111.819	7.993	111.769	7.995	111.8
42	S	8.206	115.509	8.207	115.52	8.221	115.535	8.206	115.518
43	K	8.436	123.269	8.438	123.285	8.464	123.233	8.436	123.284
44	T	8.127	115.268	8.128	115.28	8.082	115.004	8.128	115.28
45	K	8.371	123.531	8.372	123.544	8.339	124.175	8.37	123.55
46	E	8.404	121.766	8.415	121.895	8.368	123.037	8.406	121.791

47	G	8.376	109.814	8.377	109.835	8.407	110.328	8.38	109.816
48	V	7.875	119.715	7.875	119.739	7.961	119.9	7.874	119.742
49	V	8.226	124.5	8.227	124.53	8.25	124.713	8.228	124.536
50	H	8.6	123.202	8.59	123.289	8.608	123.415	8.582	123.332
51	G	8.428	110.402	8.429	110.468	8.434	110.472	8.422	110.462
52	V	8.038	119.491	8.034	119.483	8.036	119.507	8.101	119.536
53	A	8.438	127.813	8.437	127.828	8.442	127.831	8.351	118.617
54	T	8.17	114.601	8.171	114.615	8.169	114.608	8.229	117.611
55	V	8.179	122.679	8.179	122.695	8.177	122.678	8.214	122.845
56	A	8.359	127.645	8.359	127.663	8.358	127.636	8.364	127.76
57	E	8.312	120.639	8.313	120.658	8.311	120.645	8.315	120.658
58	K	8.375	122.53	8.375	122.548	8.37	122.531	8.371	122.572
59	T	8.154	115.625	8.154	115.636	8.151	115.588	8.154	115.631
60	K	8.327	123.382	8.327	123.402	8.322	123.409	8.326	123.401
61	E	8.377	121.818	8.377	121.838	8.374	121.815	8.377	121.844
62	Q	8.372	121.522	8.371	121.556	8.371	121.54	8.372	121.523
63	V	8.225	121.658	8.226	121.678	8.224	121.673	8.224	121.677
64	T	8.246	117.77	8.247	117.788	8.246	117.775	8.246	117.785
65	N	8.465	121.562	8.465	121.581	8.464	121.605	8.464	121.575
66	V	8.188	120.557	8.19	120.577	8.19	120.612	8.188	120.574
67	G	8.49	112.32	8.491	112.342	8.496	112.389	8.491	112.329
68	G	8.185	108.75	8.185	108.764	8.188	108.802	8.185	108.752
69	A	8.115	123.648	8.115	123.661	8.114	123.7	8.114	123.656
70	V	8.15	120.226	8.151	120.248	8.151	120.275	8.149	120.241
71	V	8.318	124.961	8.319	124.987	8.32	125.009	8.318	124.983
72	T	8.246	118.26	8.247	118.283	8.247	118.291	8.246	118.275
73	G	8.376	111.1	8.376	111.121	8.381	111.151	8.376	111.107
74	V	8.032	119.42	8.032	119.454	8.033	119.476	8.03	119.432
75	T	8.236	118.509	8.237	118.531	8.235	118.509	8.236	118.525
76	A	8.304	127.017	8.305	127.036	8.304	127.044	8.304	127.03
77	V	8.075	119.761	8.076	119.78	8.073	119.778	8.074	119.777
78	A	8.338	127.703	8.339	127.725	8.338	127.722	8.338	127.717
79	Q	8.325	120.065	8.326	120.086	8.323	120.098	8.324	120.082
80	K	8.376	122.995	8.376	123.019	8.372	123.027	8.375	123.014
81	T	8.234	116.637	8.235	116.66	8.234	116.655	8.234	116.655
82	V	8.24	122.695	8.246	122.703	8.248	122.713	8.247	122.683
83	E	8.511	124.939	8.512	124.964	8.513	124.971	8.511	124.961
84	G	8.454	110.468	8.455	110.489	8.456	110.523	8.454	110.477
85	A	8.213	123.829	8.213	123.842	8.214	123.888	8.211	123.836
86	G	8.433	107.994	8.434	108.013	8.438	108.047	8.434	108
87	S	8.11	115.59	8.111	115.602	8.111	115.648	8.109	115.599
88	I	8.141	122.595	8.142	122.613	8.143	122.647	8.141	122.608
89	A	8.289	127.674	8.29	127.696	8.29	127.699	8.289	127.686
90	A	8.147	123.067	8.148	123.088	8.147	123.104	8.147	123.08
91	A	8.231	123.126	8.232	123.147	8.231	123.162	8.231	123.142
92	T	8.037	112.426	8.038	112.442	8.035	112.444	8.036	112.439
93	G	8.249	110.464	8.249	110.483	8.251	110.517	8.249	110.468
94	F	8.045	120.215	8.046	120.23	8.047	120.268	8.044	120.227
95	V	8.008	123.367	8.009	123.389	8.011	123.399	8.007	123.383
96	K	8.337	126.079	8.338	126.104	8.341	126.143	8.337	126.095

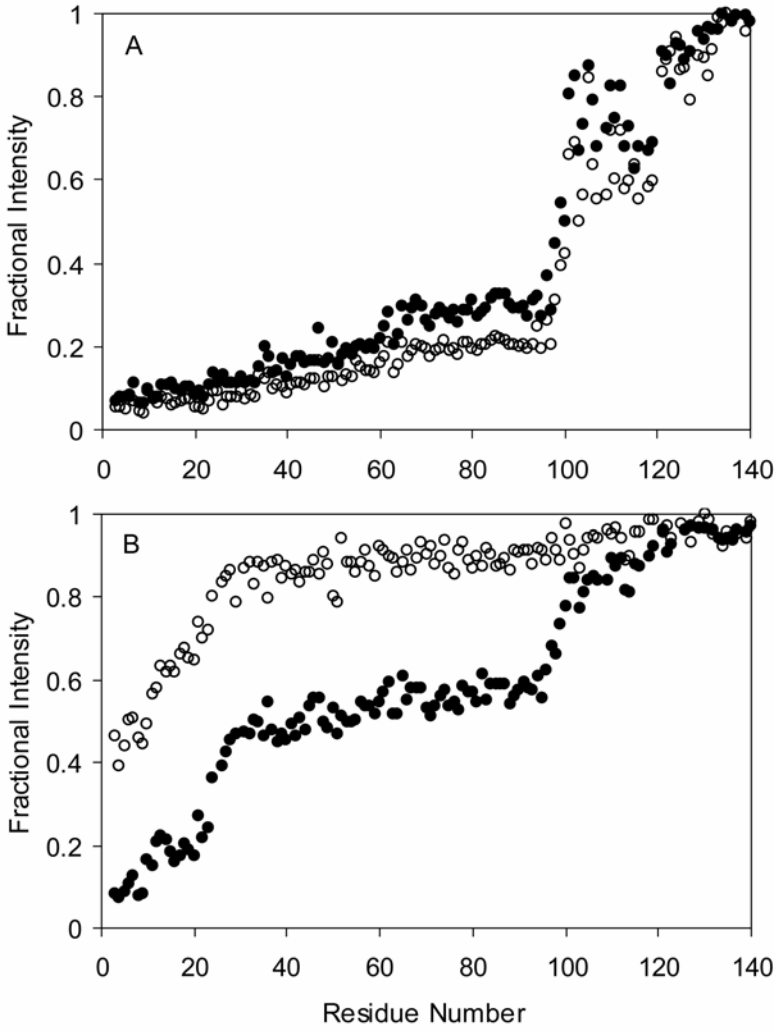
97	K	8.418	123.555	8.419	123.576	8.422	123.629	8.418	123.591
98	D	8.361	120.97	8.362	121	8.365	121.026	8.361	120.993
99	Q	8.299	120.016	8.299	120.034	8.299	120.071	8.297	120.033
100	L	8.244	122.673	8.244	122.7	8.244	122.722	8.243	122.696
101	G	8.417	109.574	8.417	109.596	8.421	109.635	8.417	109.582
102	K	8.161	120.687	8.161	120.7	8.163	120.75	8.16	120.7
103	N	8.566	119.809	8.567	119.837	8.571	119.883	8.567	119.832
104	E	8.432	121.205	8.434	121.228	8.437	121.28	8.433	121.229
105	E	8.417	121.611	8.418	121.631	8.413	121.709	8.417	121.635
106	G	8.375	110.026	8.376	110.051	8.38	110.085	8.376	110.035
107	A	8.068	124.793	8.067	124.804	8.069	124.843	8.067	124.799
108	P								
109	Q	8.522	120.914	8.523	120.938	8.525	120.983	8.522	120.941
110	E	8.465	122.251	8.466	122.285	8.468	122.319	8.465	122.286
111	G	8.426	110.047	8.426	110.066	8.428	110.089	8.426	110.054
112	I	7.941	120.098	7.943	120.116	7.943	120.143	7.941	120.117
113	L	8.344	126.708	8.346	126.744	8.346	126.747	8.345	126.75
114	E	8.365	121.992	8.368	122.032	8.372	122.055	8.367	122.042
115	D	8.311	121.164	8.312	121.202	8.313	121.235	8.311	121.205
116	M	8.207	121.831	8.209	121.851	8.204	121.857	8.208	121.85
117	P								
118	V	8.228	120.562	8.227	120.574	8.223	120.557	8.226	120.577
119	D	8.453	125.563	8.454	125.597	8.45	125.572	8.453	125.601
120	P								
121	D	8.34	119.105	8.341	119.15	8.337	119.105	8.339	119.16
122	N	8.099	118.949	8.1	118.967	8.083	118.937	8.098	118.967
123	E	8.313	121.4	8.315	121.43	8.312	121.428	8.314	121.443
124	A	8.169	124.234	8.169	124.267	8.165	124.173	8.168	124.275
125	Y	7.981	119.69	7.984	119.723	7.973	119.612	7.983	119.74
126	E	8.095	123.349	8.101	123.379	8.111	123.238	8.101	123.405
127	M	8.364	123.604	8.368	123.63	8.368	123.56	8.368	123.634
128	P								
129	S	8.42	116.453	8.422	116.478	8.41	116.423	8.421	116.487
130	E	8.513	122.963	8.516	123.001	8.518	122.975	8.516	123.01
131	E	8.415	121.768	8.418	121.811	8.418	121.696	8.415	121.815
132	G	8.349	109.73	8.35	109.74	8.344	109.696	8.35	109.732
133	Y	8.022	120.213	8.024	120.224	8.017	120.19	8.022	120.229
134	Q	8.179	122.509	8.181	122.519	8.193	122.419	8.178	122.533
135	D	8.199	121.466	8.2	121.508	8.203	121.505	8.198	121.518
136	Y	8.014	120.493	8.015	120.486	8.011	120.41	8.012	120.476
137	E	8.195	125.08	8.201	125.099	8.207	125.058	8.201	125.115
138	P								
139	E	8.453	121.304	8.455	121.333	8.458	121.357	8.454	121.339
140	A	7.942	130.629	7.942	130.566	7.939	130.61	7.94	130.608



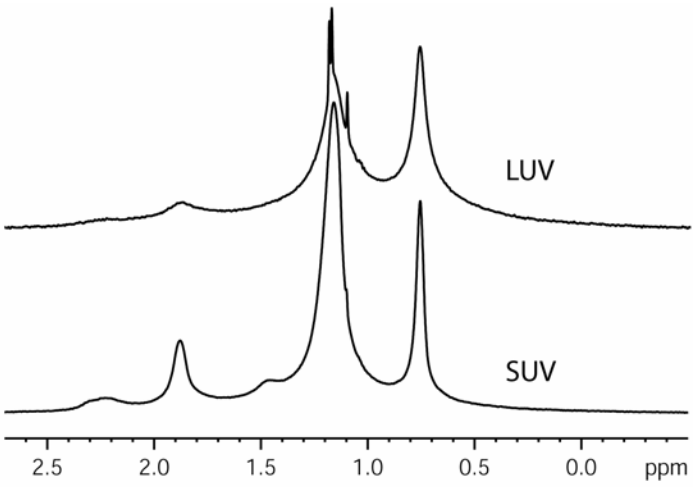
Supporting Information Figure 1. Series of strip plots showing ^{1}H-^{1}H amide NOEs for residues 25-38 of 600 μM A53T αS in the presence of 0.06% SUV. Strips are taken orthogonal to the ^{1}H frequency axis of a 600-MHz 3D HMQC-NOESY-HMQC spectrum. The NOE mixing time was 100 ms.



Supporting Information Figure 2. Signal attenuation in the ^{1}H-^{15}N HSQC spectra of 500 μM WT αS in the presence of 0.1% SUV (filled circle) or 0.1% LUV (open circle), plotted as a function of residue number.



Supporting Information Figure 3. ^{1}H-^{15}N HSQC signal attenuation profiles observed upon addition of LUV lipid mixtures to solutions containing both labeled and unlabeled WT and A30P αS. The attenuation patterns show competition for lipid between the tighter binding WT and weaker binding A30P mutant forms of αS. (A) 500 μM WT αS in the presence of 0.1% LUV (filled circle) and 300 μM WT αS in the presence of 0.1% LUV plus 300 μM unlabeled A30P αS (open circle). (B) 500 μM A30P αS in the presence of 0.1% lipid (filled circle) and 300 μM A30P αS in the presence of 0.1% lipid plus 300 μM unlabeled WT αS (open circle). Note that only the signal of the labeled form of αS is observed (and reflected in the intensity patterns) whereas the unlabeled fraction is NMR-invisible and does not significantly contribute to spectral intensity due to the low natural isotopic abundance (~0.3%) of ^{15}N.



Supporting Information Figure 4. 600 MHz ^{1}H NMR spectra of the aliphatic region of solutions of 0.1% w/v SUV and LUV lipid mixtures, recorded in H_2O solution using a "1-1-echo scheme" and a total echo delay of 600 μs during which pulsed field gradients were used for water suppression.