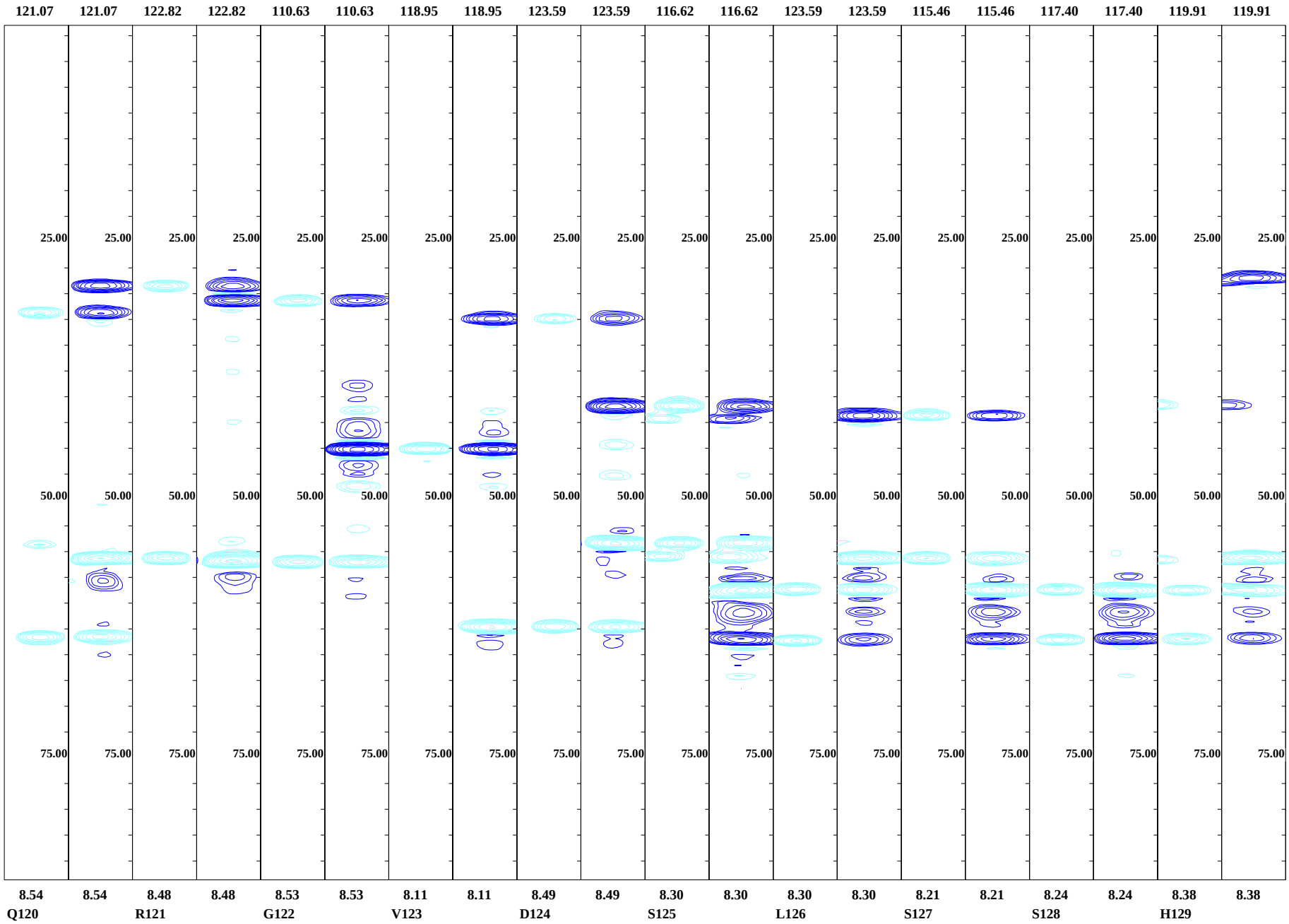
APPENDIX B

Supplementary NMR data

Sequential assignment by triple resonance experiments

The sequential assignment of the Zα domain (residues 119 – 200) was verified by using triple resonance NMR experiments [18]. 2D strips were extracted from 3D CBCA(CO)NH and CBCANH spectra using the NMR analysis program Aurelia (Bruker). For each residue of Zα (except for the four prolines and the first two N-terminal residues) a pair of strips is shown aligned in sequential order (fig. 60).

fig. 60 **Sequential backbone assignment of Zα by using 3D CBCA(CO)NH and CBCANH triple resonance spectra (see following 9 pages).** The backbone ^{15}N chemical shift is listed above and the H_N chemical shift is listed below each strip. A ^{13}C chemical shift axis is represented at the long axis of a strip. The left strip of each pair originates from the CBCA(CO)NH spectrum showing the Cα and Cβ shifts of the residue one residue ahead in the primary sequence (i-1) in light gray color. The right strip of a pair originating from the CBCANH spectrum, shows the Cα and Cβ shifts of that very residue (i) in light gray and black color, respectively. In addition, it shows the Cα and Cβ shifts of the residue one residue ahead in sequence (i-1) with less intensity in light gray and black color, respectively. In conjunction with the information from the ^{15}N NOESY-HSQC sequential assignment approach, this data allowed unambiguous sequential assignment even for the poorly dispersed N-terminal residues of Zα.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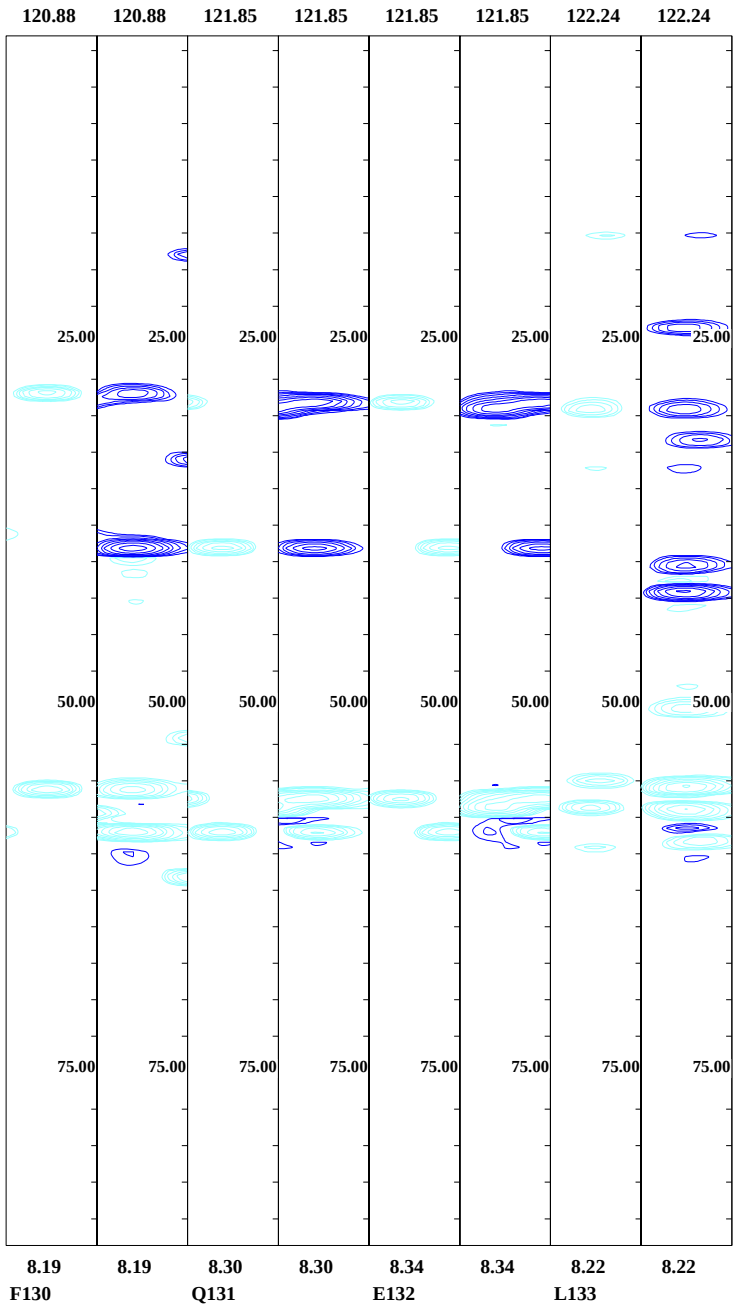
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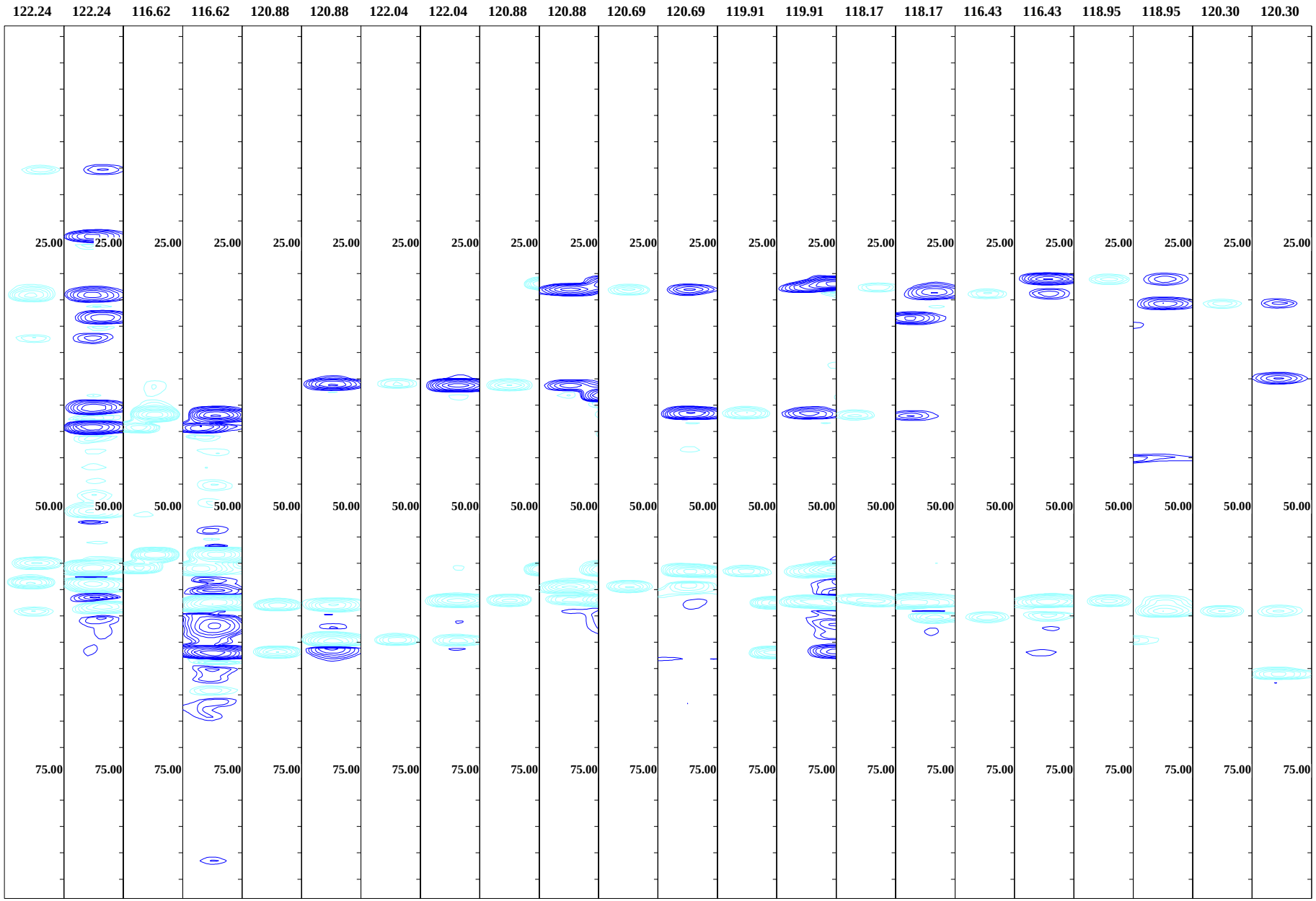
E132
L133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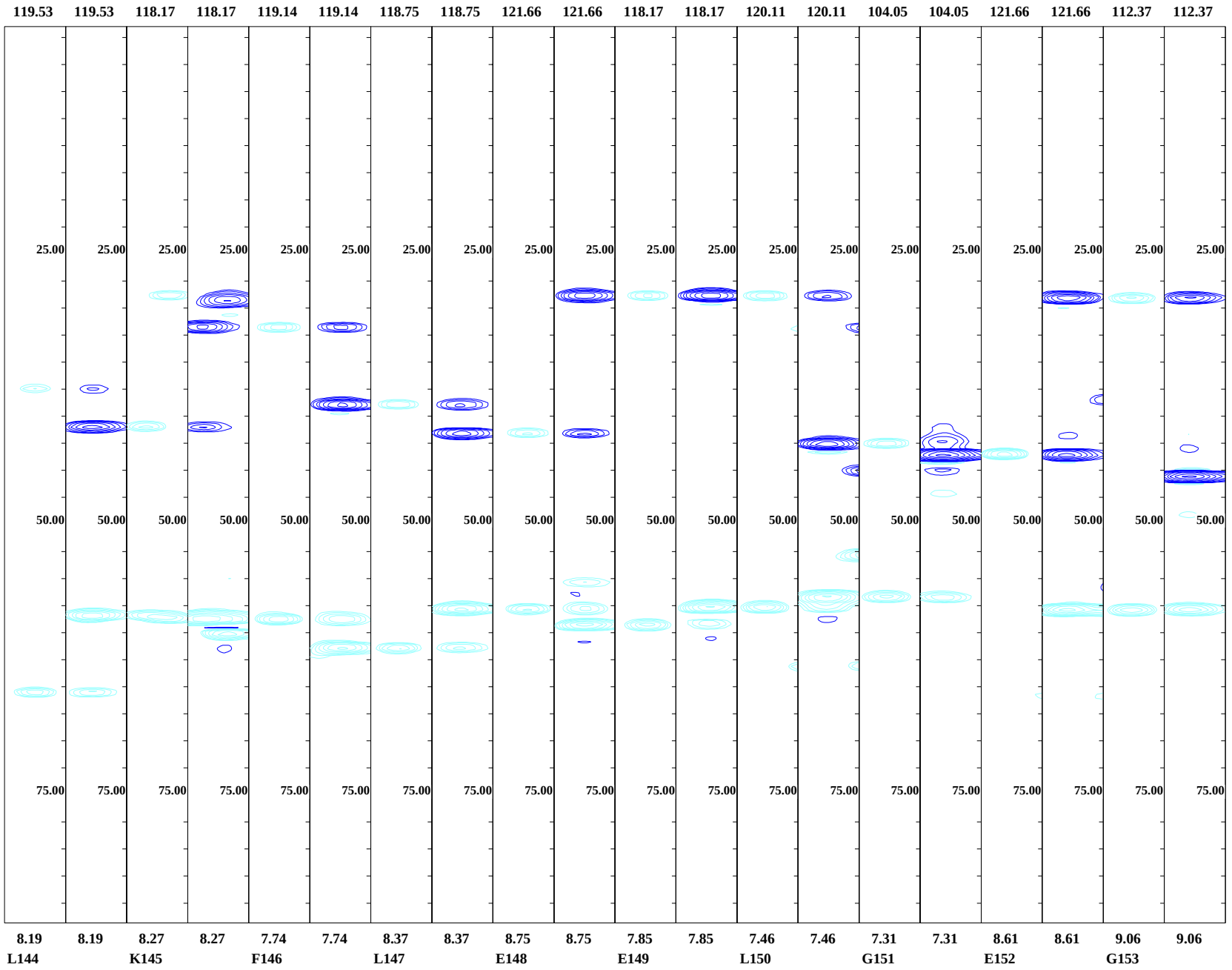
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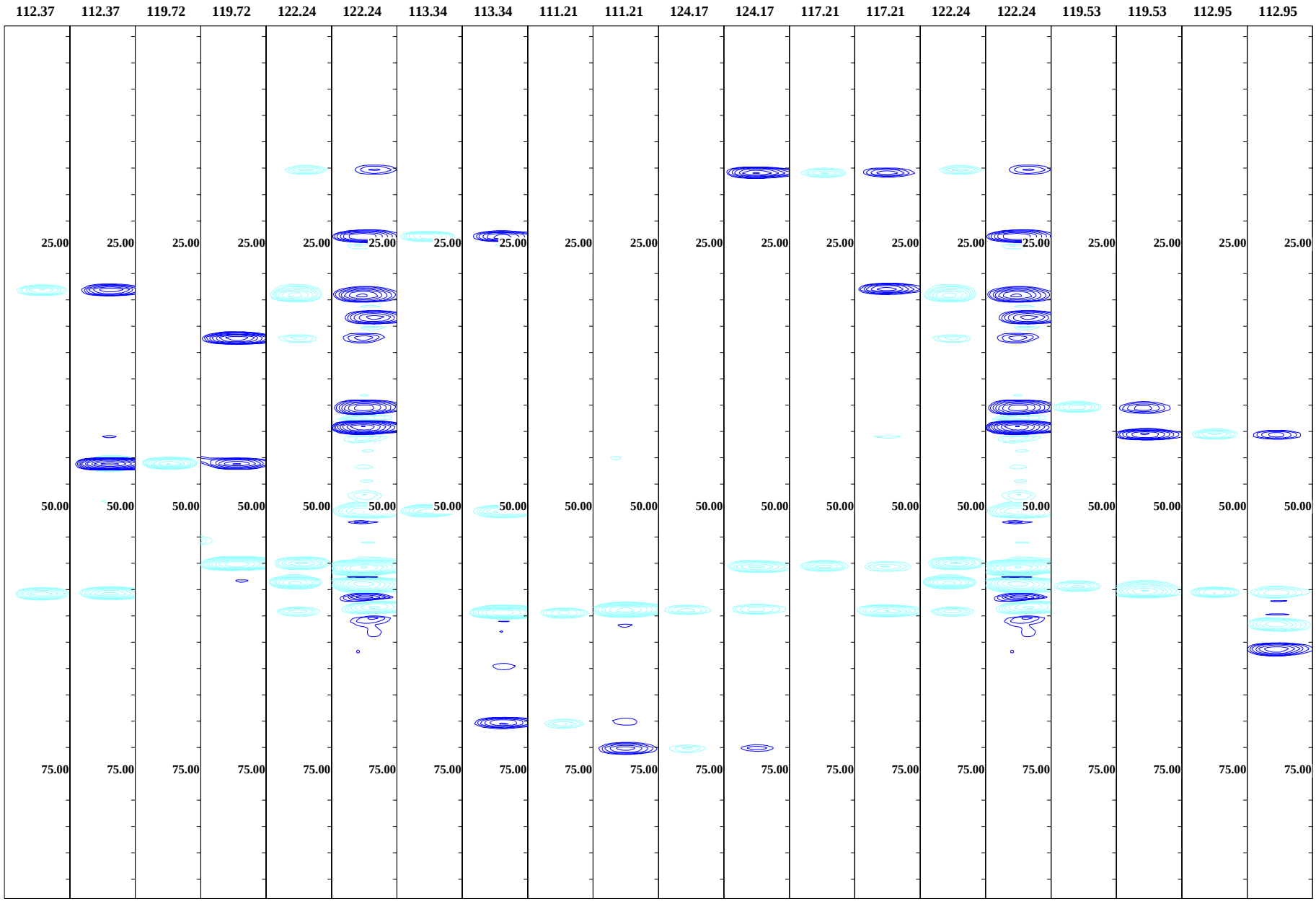
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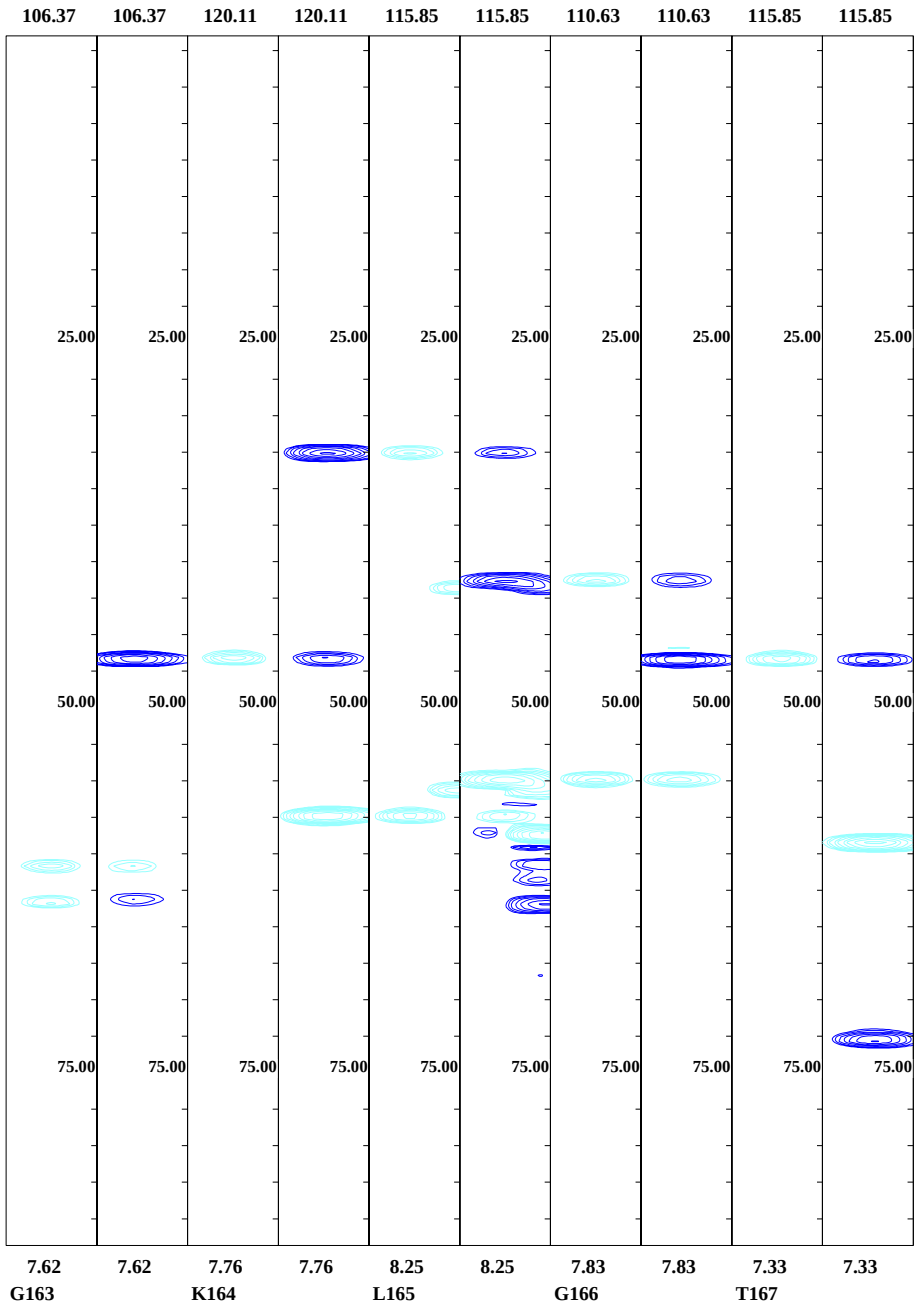
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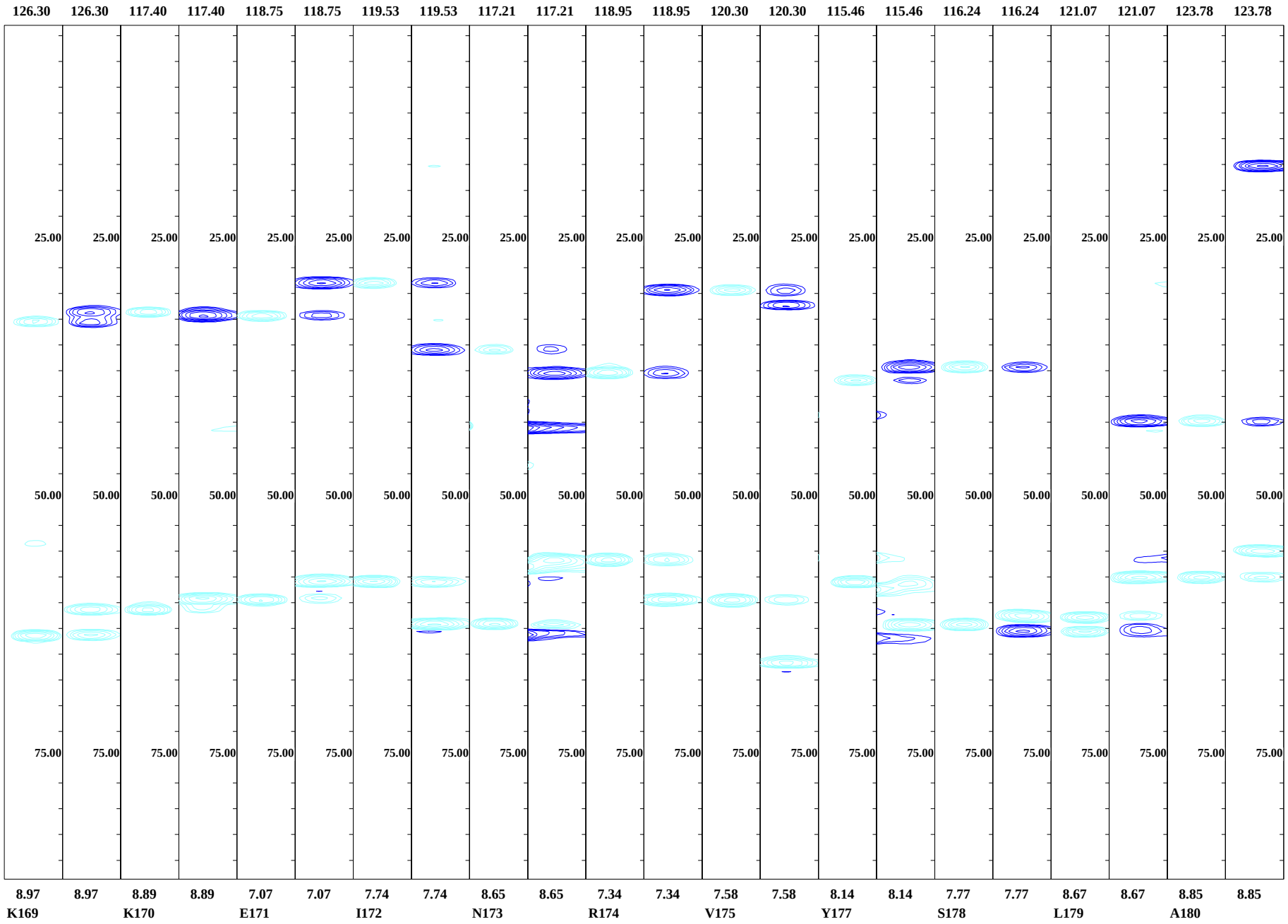
L165
G166
T167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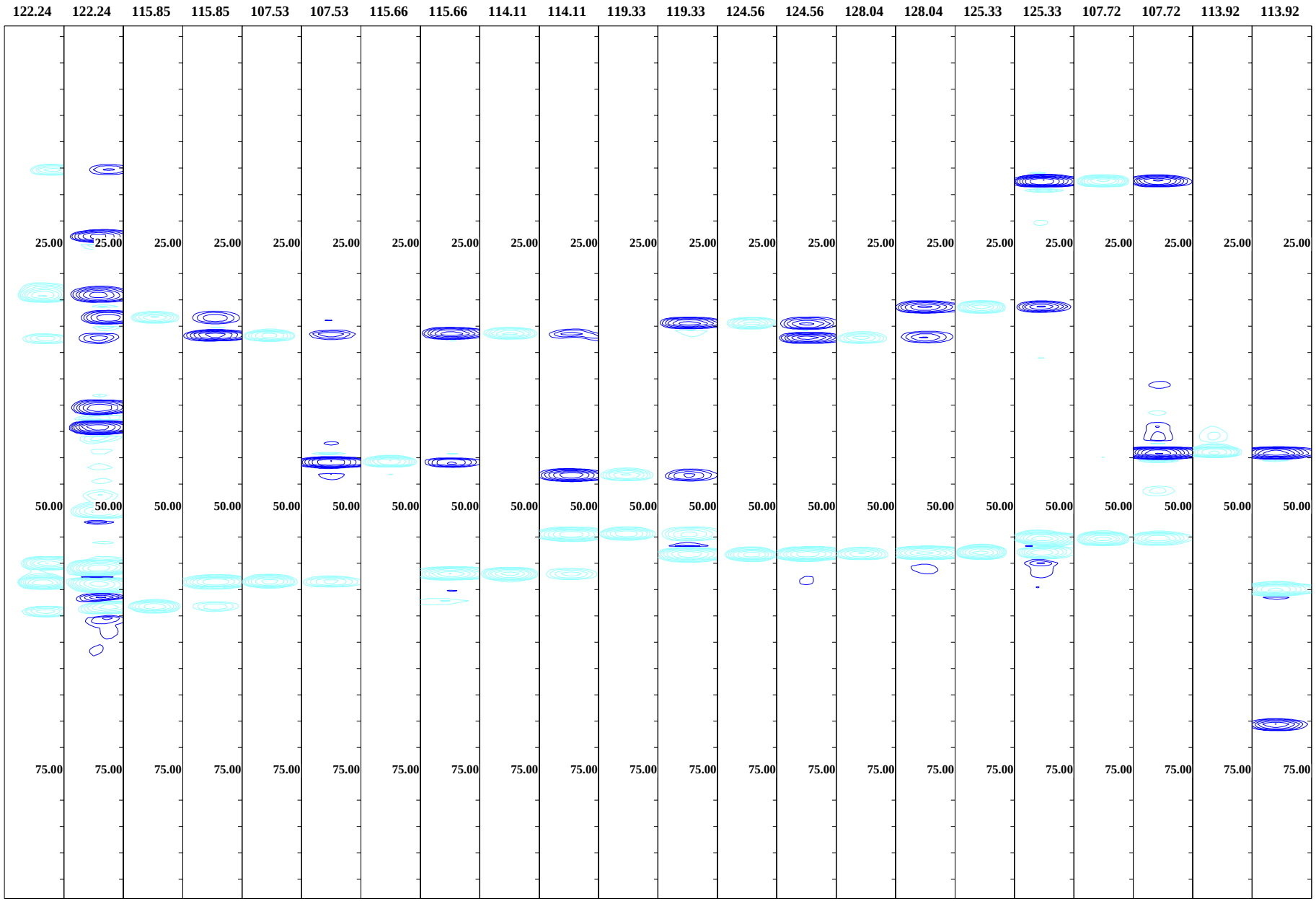
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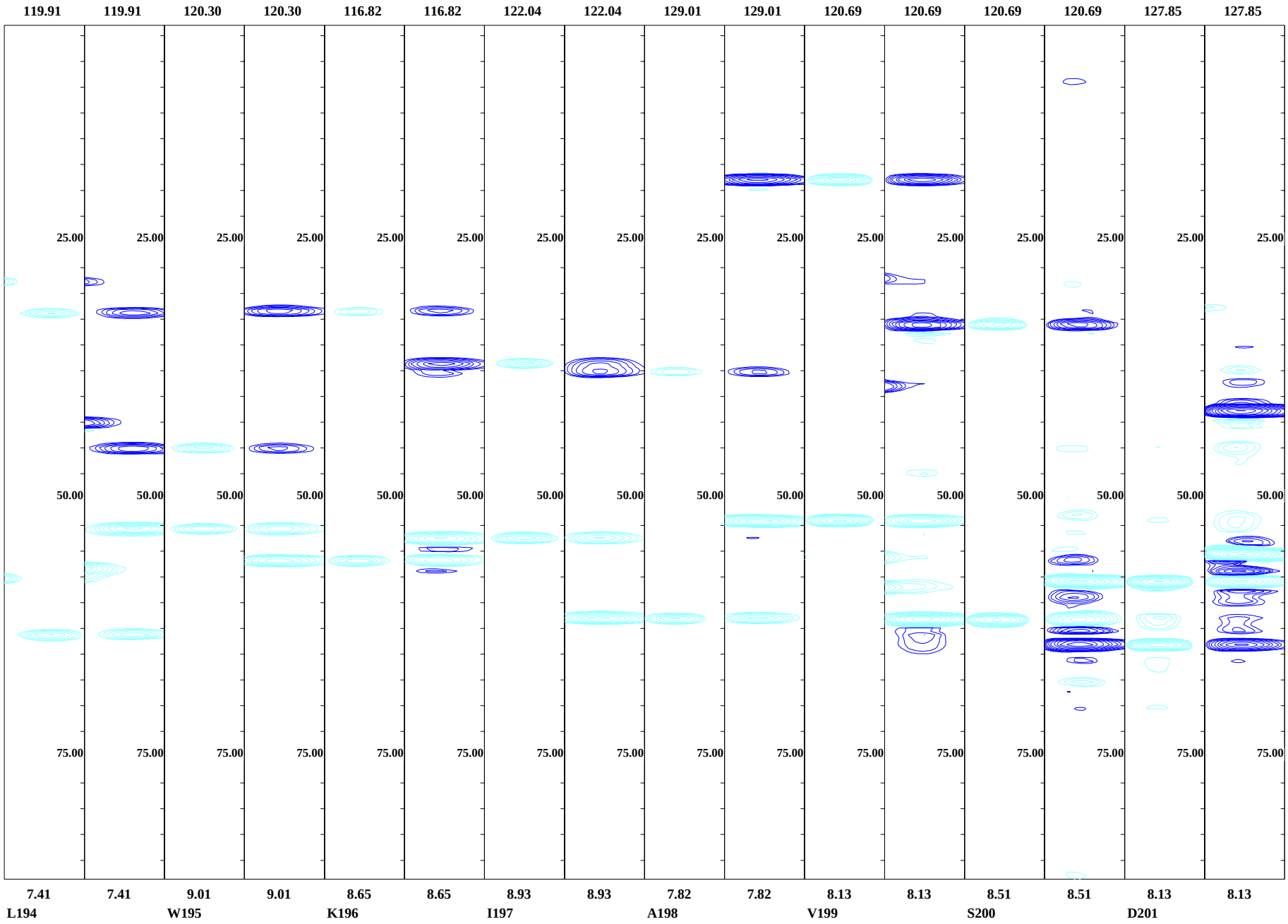
K181 K182 G183 K184 L185 Q186 K187 E188 A189 G190 T191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L194 W195 K196 I197 A198 V199 S200 D201

Chemical shift assignment list of Zα

The chemical shift table of the Zα domain (residues 119 - 200) from human ADAR1 contains the complete ^{1}H, ^{15}N and ^{13}C resonance assignment (table 13 and table 14). Residues are numbered according to the sequence of human ADAR1. The serine at the N-terminus and the aspartic acid at the C-terminus are vector-encoded. All chemical shifts are in ppm which were referenced to external DSS. ^{15}N and ^{13}C shifts were indirectly calibrated to the ^{1}H chemical shift of DSS by using magnetogyric ratios [22]. Atoms are named following the recommendations given in [22] except for methylene ^{1}H atoms which are numbered '1' and '2' rather than '2' and '3'.

The ^{1}H and backbone ^{15}N chemical shifts are shown in table 13, and the ^{13}C and side chain ^{15}N chemical shifts (Nε, Nδ) are shown in table 14. The stereo-specific assignment is arbitrary because the solution of Zα was calculated by floating stereo-specific assignment. If geminal ^{1}H resonances are degenerate the common chemical shift is given in the first column (β1, γ1, δ1 or ε1). The Cγ2 and Cδ2 resonances of valine and leucine residues are presented in the Cδ and Cε column, respectively.

table 13 **The ^{1}H chemical shifts of Zα.**

residue		H_N	N	Hα1	Hβ1	Hβ2	Hγ1	Hγ2	Hδ1	Hδ2	Hε1	Hε2
	S	8.714	117.3	4.846	3.881							
119	P			4.485	1.932	2.333	2.057		3.747	3.880		
120	Q	8.524	121.1	4.331	1.999	2.111	2.407				7.558	6.909
121	R	8.460	122.8	4.379	1.799	1.902	1.669		3.225		7.255	
122	G	8.517	110.6	4.031	Hα2: 3.983							
123	V	8.091	119.0	4.147	2.125		0.941	0.964				
124	D	8.479	123.6	4.670	2.705	2.759						
125	S	8.290	116.5	4.398	3.880	3.936						
126	L	8.302	123.6	4.363	1.637	1.743	1.672		0.882	0.942		
127	S	8.213	115.5	4.409	3.909							
128	S	8.233	117.5	4.400	3.855							
129	H	8.378	120.0	4.694	3.135	3.250				7.152	8.259	
130	F	8.191	120.9	4.546	3.095	3.152	Hζ: 7.345		7.277		7.383	
131	Q	8.305	121.8	4.243	1.994	2.081	2.319				7.527	6.891
132	E	8.346	121.8	4.217	2.093	2.018	2.326					
133	L	8.211	122.3	4.360	1.613	1.696	1.67		0.88	0.944		
134	S	8.311	116.5	4.385	3.877	3.930						
135	I	7.860	120.9	4.084	1.805		1.061	1.181	0.796	Hγ2: 0.786		
136	Y	8.002	121.9	4.553	2.941	3.171			7.115		6.806	
137	Q	8.250	120.8	4.191	2.108		2.426				6.865	7.552
138	D	8.373	120.6	4.496	2.792							
139	Q	8.419	120.0	4.054	2.035	2.141	2.405	2.453			6.806	7.385

residue		H_N	N	Hα1	Hβ1	Hβ2	Hγ1	Hγ2	Hδ1	Hδ2	Hε1	Hε2
140	E	8.265	118.2	3.712	1.973	2.266	2.080	2.407				
141	Q	8.034	116.5	3.987	2.174		2.544	2.474			6.891	7.578
142	R	8.005	118.9	3.994	1.874		1.588		3.189		7.377	
143	I	7.979	120.3	3.568	1.828		1.695	0.855	0.699	Hγ2: 0.656		
144	L	8.192	119.6	3.940	1.775	1.598	1.788		0.848			
145	K	8.286	118.3	4.069	1.912		1.522	1.437	1.706		2.98	
146	F	7.748	119.2	4.158	3.145	3.226	Hζ: 7.282		7.118		7.283	
147	L	8.382	118.7	4.078	2.031	1.552	2.031	1.931		1.025	0.822	
148	E	8.742	121.5	3.949	2.132	2.291	2.293	2.552				
149	E	7.847	118.1	4.026	2.022		2.285	2.542				
150	L	7.473	120.1	3.998	1.563	1.782	1.721		0.777	0.886		
151	G	7.330	104.1	4.381	Hα2: 3.751							
152	E	8.609	121.5	4.108	2.033		2.316	2.371				
153	G	9.053	112.3	4.090	Hα2: 3.849							
154	K	7.341	119.8	4.464	1.78		1.245	1.412	1.689		3.044	
155	A	8.211	122.3	5.218	1.328							
156	T	8.845	113.4	5.081	4.100		1.238					
157	T	8.540	111.2	5.223	4.902		1.408					
158	A	9.836	124.2	3.940	1.479							
159	H	9.017	117.2	4.174	3.494	3.225				7.367	8.528	
160	D	8.211	122.3	4.328	2.758	2.910						
161	L	7.912	119.5	3.554	1.104	1.901	0.605		0.53	1.688		
162	S	8.364	113.0	4.134	3.878	3.923						
163	G	7.633	106.3	3.961	Hα2: 3.839							
164	K	7.758	120.1	4.180	1.624	1.637	1.477		1.709		3.056	
165	L	8.246	115.9	4.326	1.570		1.429		0.558	0.644		
166	G	7.847	110.5	3.931								
167	T	7.326	115.9	4.761	3.494		1.226					
168	P			4.365	1.896	2.575	2.150	2.045	3.641	4.002		
169	K	8.959	126.4	3.692	1.906		1.411	1.464	1.783		3.048	3.107
170	K	8.886	117.5	4.180	1.776	1.942	1.416	1.544	1.753		3.040	
171	E	7.085	118.8	4.260	2.004		2.234					
172	I	7.750	119.5	3.517	2.084		1.334	1.042	0.549	Hγ2:0.767		
173	N	8.654	117.2	4.336	2.709				7.442	6.831		
174	R	7.352	118.8	4.051	1.981	2.040	1.615	1.802	3.200	3.277	7.544	
175	V	7.587	120.2	3.725	1.999		0.854	0.966				
176	L	8.559	121.4	3.403	0.972	-0.939	1.313		-0.025	-0.385		
177	Y	8.128	115.6	4.236	3.160	3.091			7.449		6.969	
178	S	7.776	116.2	4.340	4.196	4.106						
179	L	8.660	121.1	4.053	1.2	2.152	1.868		0.795	0.875		
180	A	8.844	123.7	4.630	1.765							
181	K	8.211	122.3	4.199	2.116		1.646	1.565	1.788		3.053	

residue		H_N	N	Hα1	Hβ1	Hβ2	Hγ1	Hγ2	Hδ1	Hδ2	Hε1	Hε2
182	K	7.599	115.8	4.398	1.883	2.153	1.780	1.590	1.619	1.771	2.963	
183	G	8.027	107.5	4.242	Hα2: 3.964							
184	K	8.023	115.7	4.490	1.784	1.951	1.478		1.7		3.033	
185	L	6.940	114.2	5.227	1.467	1.797	0.841		0.859	0.848		
186	Q	9.313	119.4	4.641	1.881		2.223	2.169			7.627	6.937
187	K	8.355	124.7	4.199	1.092	0.658	0.448	-0.079	1.236	1.355	2.766	2.71
188	E	9.123	128.1	4.447	1.709	1.946	2.071					
189	A	8.325	125.3	4.085	1.355							
190	G	7.789	107.7	4.063	Hα2: 3.842							
191	T	8.293	113.9	4.621	4.1		1.169					
192	P			5.216	2.358	2.598	1.933	2.165	3.553	3.629		
193	P			4.150	1.473	2.029	2.248	2.182	3.808	3.950		
194	L	7.401	120.0	4.850	1.273	1.441	1.566		0.763	0.823		
195	W	9.014	120.3	5.225	2.787	2.928	Hδ1: 7.14 Hε1: 9.895 Hε3: 7.176 Hζ2: 7.152 Hζ3: 6.631 Hη2: 6.253 Nε1: 128.3					
196	K	8.662	116.8	5.086	1.823		1.402	1.207	1.545		2.826	2.764
197	I	8.936	122.1	4.166	1.857		1.614	1.296	0.965	Hγ2: 1.041		
198	A	7.839	129.1	4.329	1.176							
199	V	8.115	120.6	4.238	2.117		0.978	0.992				
200	S	8.502	120.5	4.544	3.894							
	D	8.127	127.8	4.451	2.709							

table 14 **The ^{13}C chemical shifts of Zα.**

residue		Cα	Cβ	Cγ	Cδ	Cε	Nε, Nδ2, Cγ2
	S	56.66	63.23				
119	P	63.39	32.06	27.37	50.78		
120	Q	55.78	29.48	33.79			Nε2 112.8
121	R	56.12	30.88	27.01	43.18		Nε 84.9
122	G	45.28					
123	V	62.46	32.69	20.24	21.07		
124	D	54.38	41.14				
125	S	58.93	63.58				
126	L	55.79	41.97	26.97	23.12	24.74	
127	S	58.78	63.58				
128	S	58.88	63.56				
129	H	55.75	28.66		119.8	137.8	
130	F	58.63	39.26		131.6	131.6	Cζ 131.6
131	Q	56.38	29.29	33.60			Nε2 112.3
132	E	56.97	29.81	36.11			
133	L	55.57	42.28	27.07	23.12	24.74	
134	S	59.08	63.51				
135	I	62.48	38.22	27.14	13.18		Cγ2 17.27
136	Y	58.66	38.31		133.2	118.2	
137	Q	57.37	29.24	34.04			Nε2 112.0
138	D	55.86	40.95				
139	Q	58.67	29.01	33.74			Nε2 110.8
140	E	60.25	29.64	37.42			
141	Q	58.73	28.25	34.08			Nε2 112.2
142	R	59.65	30.56	27.89	43.50		Nε 85.0
143	I	65.57	37.65	30.61	13.81		Cγ2 17.28
144	L	58.59	41.17	27.02	23.62	24.94	
145	K	58.89	31.93	24.87	28.67	41.89	
146	F	61.55	39.17		132.0	131.0	Cζ 129.7
147	L	57.95	41.78	28.15	24.24	27.11	
148	E	59.38	29.14	35.6			
149	E	57.75	29.10	36.4			
150	L	56.81	42.75	26.16	23.48	24.96	
151	G	43.76					
152	E	57.99	29.29	35.88			
153	G	45.73					
154	K	55.18	33.84	24.83	28.19	42.49	
155	A	50.25	24.17				
156	T	59.75	70.25	19.09			
157	T	59.58	72.62	21.93			
158	A	55.46	18.19				
159	H	59.63	29.18		120.1	136.3	

residue		Cα	Cβ	Cγ	Cδ	Cε	Nε, Nδ2, Cγ2
160	D	57.34	40.36				
161	L	57.74	42.93	26.69	24.53	26.69	
162	S	60.93	63.23				
163	G	46.80					
164	K	57.50	32.77	24.89	28.32	41.89	
165	L	55.09	41.52	27.82	25.75	23.14	
166	G	46.85					
167	T	59.36	72.82	20.82			
168	P	63.29	32.98	27.82	51.76		
169	K	60.84	32.11	25.57	28.95	41.50	
170	K	59.84	32.40	24.88	29.31	41.61	
171	E	58.05	29.21	35.67			
172	I	62.18	35.69	27.23	10.21		Cγ2 17.97
173	N	56.05	37.95				Nδ2 109.8
174	R	59.87	29.89	27.09	43.40		Nε 83.9
175	V	65.88	31.38	23.14	22.61		
176	L	58.11	38.64	25.0	21.79	24.15	
177	Y	62.24	37.35		133.4	117.7	
178	S	61.34	62.86				
179	L	57.67	42.58	27.18	26.13	23.89	
180	A	55.12	17.90				
181	K	59.24	31.88	25.04	29.03	41.84	
182	K	56.84	33.54	26.05	29.80	41.69	
183	G	45.56					
184	K	56.13	33.41	24.95	28.15	42.16	
185	L	52.44	46.78	28.61	23.90	24.84	
186	Q	54.23	32.43	33.90			Nε2 112.5
187	K	54.24	33.81	23.85	29.13	41.50	
188	E	54.12	30.89	34.80			
189	A	52.81	18.99				
190	G	44.69					
191	T	57.62	70.38	20.56			
192	P	62.82	33.63	24.80	49.90		
193	P	63.22	32.14	27.52	50.61		
194	L	52.93	45.18	27.24	24.13	25.31	
195	W	Cα: 56.05 Cβ: 31.91 Cδ1: 126.4 Cε3: 119.0 Cζ2: 114.3 Cζ3: 121.5 Cη2: 123.5					
196	K	53.87	37.04	23.13	29.56	41.79	
197	I	61.60	37.67	28.42	12.41		Cγ2 17.58
198	A	52.22	19.24				
199	V	61.69	33.22	20.31	21.32		
200	S	58.05	64.14				
	D	55.43	41.57				

HNHα coupling constants of Zα

The HNHα coupling constants (table 15), J(HNHA), of human Zα (residues 119-200) were determined from a 3D ^{15}N-edited HNHA spectrum (ξ=14ms) by the method of Vuister and Bax using integrated diagonal and cross peaks [19]. Apparent coupling constants were multiplied by a factor of 1.11 to correct for Hα spin flips [19]. J(HNHA) values smaller than 6 Hz are diagnostic of α-helical secondary structure, while J(HNHA) values larger than 8 Hz are diagnostic of extended chain conformation (β-strand) [22]. The secondary structure information derived from these coupling constants is in good agreement with the high resolution NMR structure of Zα (column „Secondary Structure derived from high resolution NMR structure"). The coupling constants were converted into dihedral phi angles applying the Karplus equation parameterized for proteins [194]. Allowing a tolerance of 30°, no dihedral angle restraint showed violations greater than 2° during structure calculation by dynamic simulated annealing. The coupling constants measured for glycines were not used for structure calculation because no stereo-specific assignment was available for the pair of Hα atoms. Empty boxes indicate that the peak volumes could not be determined due to spectral overlap.

table 15 **HNHα coupling constants in human Zα**

#	residue	J(HNHA) in Hz (±1)	Secondary structure derived from high resolution NMR structure
119	**P**	-	
120	**Q**		
121	**R**		
122	**G**		
123	**V**	6.6	
124	**D**		
125	**S**		
126	**L**	7.5	
127	**S**	6.4	
128	**S**	5.9	
129	**H**	5.0	
130	**F**	4.9	
131	**Q**	6.1	
132	**E**	6.6	
133	**L**		
134	**S**		
135	**I**	6.0	
136	**Y**	6.9	α1
137	**Q**	4.7	α1
138	**D**		α1
139	**Q**		α1
140	**E**		α1

141	**Q**	3.4	α1
142	**R**	4.0	α1
143	**I**	3.8	α1
144	**L**	4.4	α1
145	**K**		α1
146	**F**		α1
147	**L**	3.3	α1
148	**E**	3.0	α1
149	**E**	5.5	α1
150	**L**	4.8	α1
151	**G**	8.5; 3.4	
152	**E**	3.4	
153	**G**	5.6; 5.6	
154	**K**	6.7	
155	**A**		β1
156	**T**	9.2	β1
157	**T**		β1
158	**A**		α2
159	**H**	< 3	α2
160	**D**		α2
161	**L**	3.2	α2
162	**S**	3.1	α2
163	**G**	5.4; 5.4	α2
164	**K**		α2
165	**L**	6.9	α2
166	**G**		
167	**T**	8.1	
168	**P**	-	
169	**K**	< 3	α3
170	**K**	< 3	α3
171	**E**	7.5	α3
172	**I**		α3
173	**N**	2.5	α3
174	**R**	3.1	α3
175	**V**	5.3	α3
176	**L**		α3
177	**Y**	< 3	α3
178	**S**	4.5	α3
179	**L**	3.6	α3
180	**A**	3.8	α3
181	**K**		α3
182	**K**	7.1	α3
183	**G**	7.3; 5.7	

184	**K**		
185	**L**		β2
186	**Q**	10.0	β2
187	**K**		β2
188	**E**	10.0	β2
189	**A**		
190	**G**		
191	**T**	9.7	
192	**P**	-	
193	**P**	-	
194	**L**	8.0	β3
195	**W**	9.2	β3
196	**K**		β3
197	**I**	4.4	β3
198	**A**	6.3	
199	**V**	8.5	
200	**S**	9.7	