Supporting information

Automatic structure-based methyl NMR resonance assignment in large proteins

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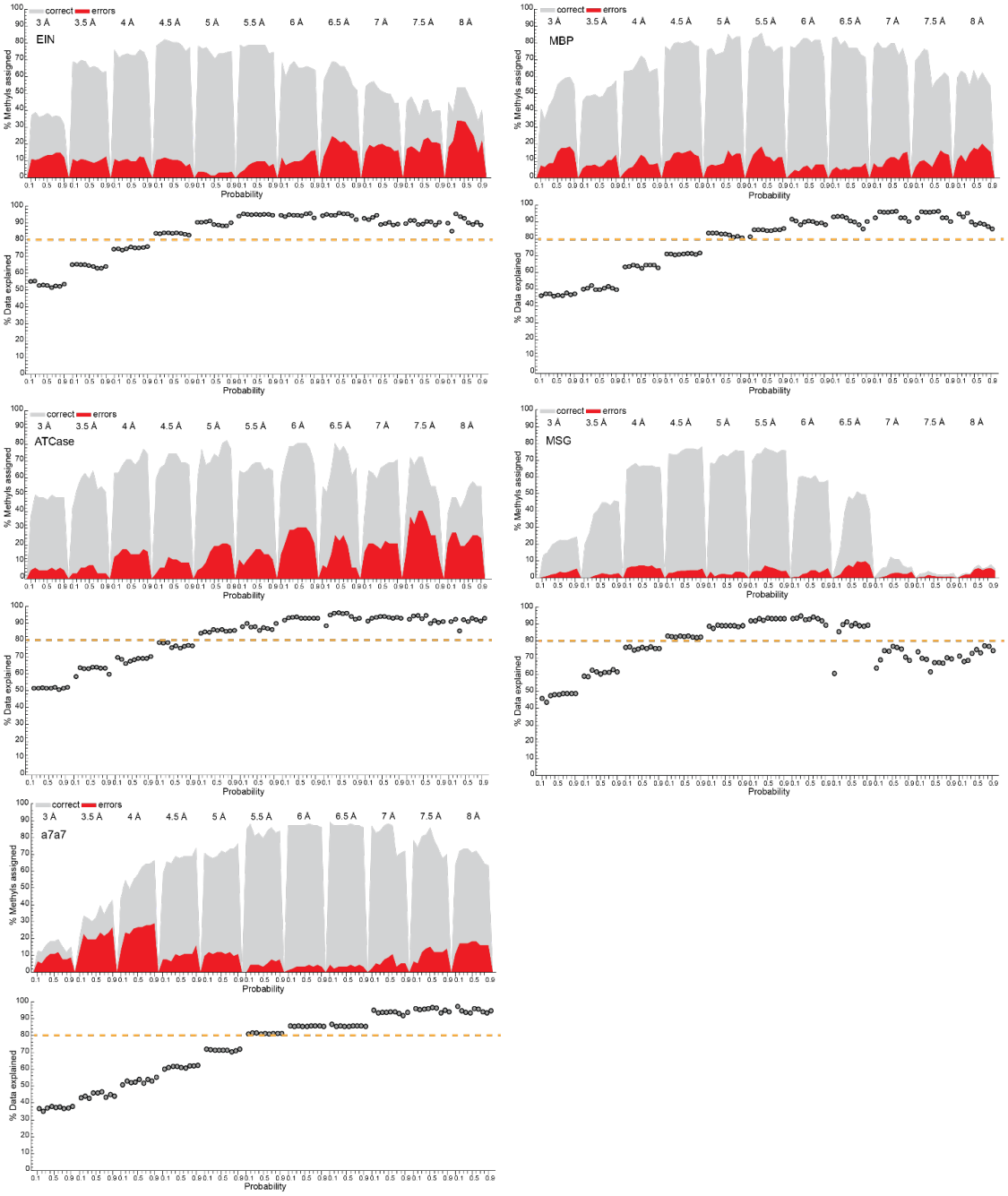

Figure S1. Parametrization of the distance cutoff d_{cut} and the probability of expected methyl-methyl NOE contacts, p_{NOE}. For each protein, the upper panel shows the percentage of correct (*grey*) and erroneous (*red*) strong (i.e. confident) assignments for given d_{cut} and p_{NOE} values. Assignment percentages are relative to the number of reference assignments. For each protein, the lower panels show for each protein the percentage of explained experimental NOESY peaks. The distances at which ~80–85% of the data are explained generally led to the most reliable assignments. For the largest dataset (MSG) with >250 methyls, the algorithmic performance significantly deteriorates for d_{cut} > 6.5 Å (equivalent to >11.5 Å C–C distance), resulting in an

unreliable assignment. Note that such large distance cutoffs are unrealistic in practice and unlikely to be generally required.

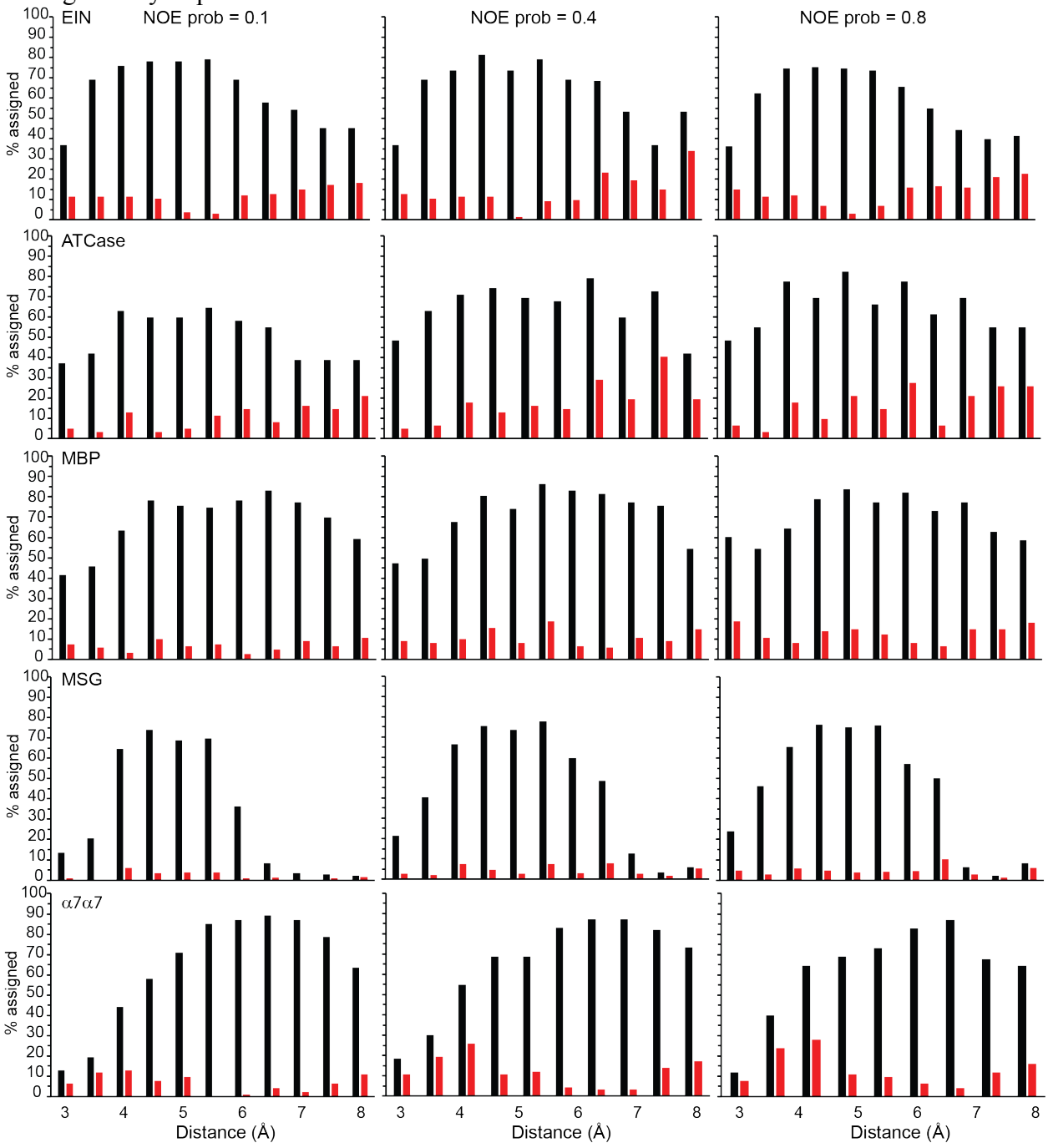

Figure S2. Accuracy of the methyl assignments obtained for the different values of NOE probabilities over a range of NOE distance cutoffs, d_{cut}. The percentage of accurately (*black*) and erroneously (*red*) assigned methyl groups is shown for the NOE probability values of 0.1, 0.4, and 0.8 in the first, second, and third column, respectively.

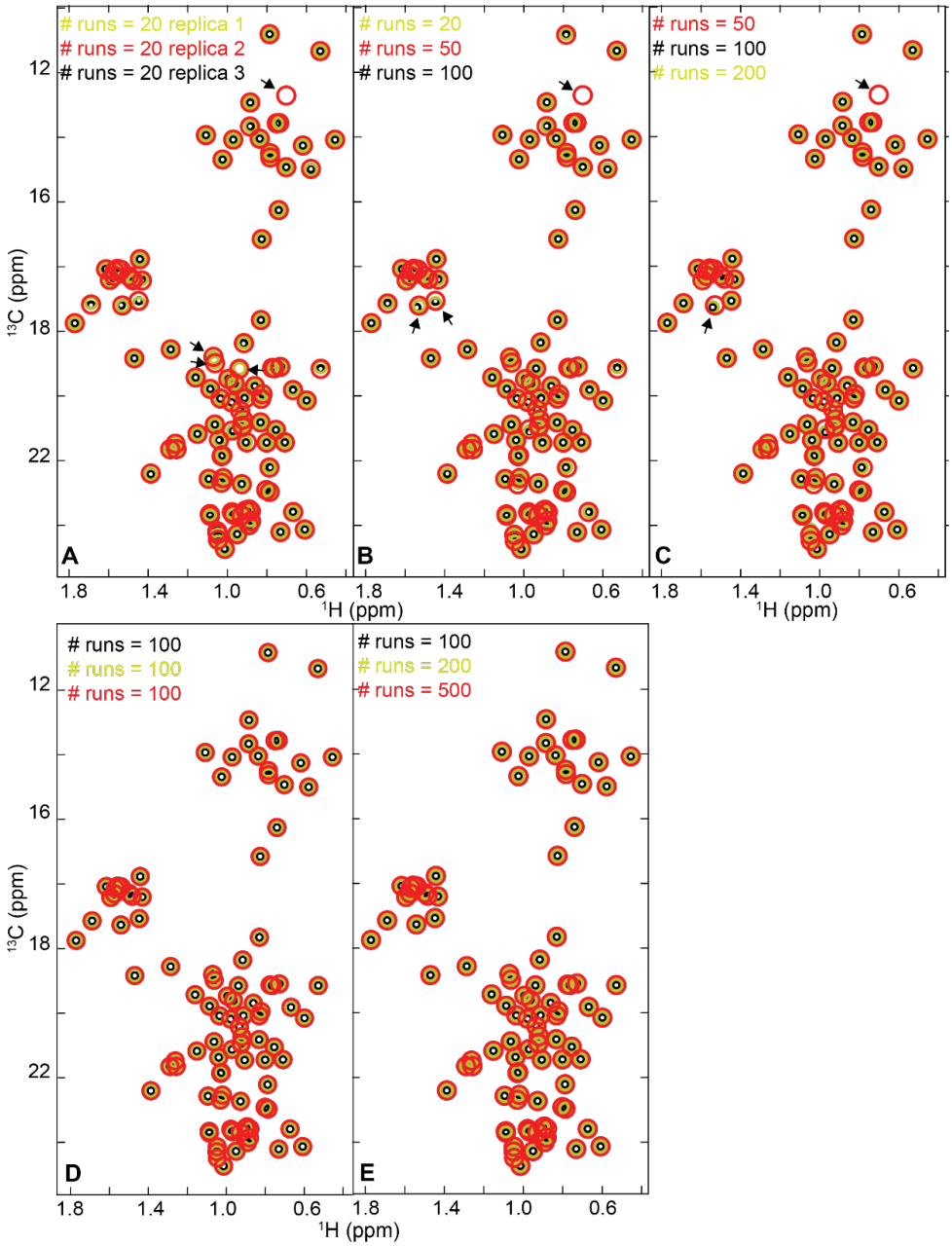

Figure S3. Determination of the optimal number of individual assignment runs for automatic methyl resonance assignment with MethylFLYA. Positions of the "strong" (i.e. confident) MethylFLYA-derived methyl assignments for EIN are shown in ^{1}H-^{13}C correlation plots as circles with different colors and increasing diameters. **(A)** Running 20 parallel assignment calculations in three replicates, each from a different random starting point, shows some differences in the derived strong assignments between the replicates (arrows). **(B-C)** Increasing the number of calculations to 50 shows that the differences persist when compared to the higher number of parallel calculations (100 or 200). **(D)** Running 100 parallel MethylFLYA calculations is sufficient for the reproducibility of strong assignments. **(E)** A further increase in the number of parallel calculations (e.g. 200, 500) results in sets of strong assignments that are consistent with the set of 100 parallel calculations.

Table S1. Methyl resonance assignments by MethylFLYA, MAGMA, MAP-XSII, and FLAMEnGO2.0. The methyl groups with reference assignment are listed, with the ^{13}C and ^{1}H chemical shift values in ppm in columns '13C' and '1H'. Agreement or disagreement of the confident assignments by the four algorithms with the reference assignment is indicated by '=' (agreement) or '!' (disagreement) signs. The first sign is for ^{13}C, the second for ^{1}H. Missing, non-confident, or ambiguous assignments are left blank, except in the case of MethylFLYA, where also the weak (non-confident) assignments are indicated in parentheses. Filtered peak lists were used.

ATCase

	Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO		Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO
7	LEU CD1	24.700	0.825	==				74	LEU CD1	25.859	0.789	(==)		!!	!!
7	LEU CD2	23.351	0.779	==				76	LEU CD1	25.036	0.788	==		==	
9	VAL CG1	20.219	0.870	==				76	LEU CD2	24.234	0.659	==		==	
9	VAL CG2	21.153	0.879	==				83	VAL CG1	21.482	0.783	==			
12	ILE CD1	13.256	0.486	(==)		!!	==	83	VAL CG2	21.844	0.779	==			
17	VAL CG1	20.691	0.894	(==)				86	ILE CD1	11.600	0.602	(==)			==
18	ILE CD1	13.418	0.734	==		==		91	VAL CG1	21.777	0.944	==		==	
21	ILE CD1	14.203	0.678	(==)		==		91	VAL CG2	21.184	0.954	==		==	
30	LEU CD1	25.344	0.851	==	==			92	VAL CG1	21.705	1.032	==			
30	LEU CD2	23.065	0.650	==	==			92	VAL CG2	18.600	0.761	==		==	
32	LEU CD1	23.190	0.364	==			==	99	LEU CD1	25.443	0.739	(==)	==	==	
32	LEU CD2	24.650	0.286	==			==	99	LEU CD2	24.463	0.751	(==)	==	==	
35	LEU CD1	24.537	0.727	==	==			103	ILE CD1	13.711	0.794	==	==	==	==
35	LEU CD2	21.752	0.733	==	==			106	VAL CG2	19.857	0.939	==		==	
42	ILE CD1	14.481	0.676	!!	==	==		107	LEU CD1	25.746	0.448	==			
44	ILE CD1	16.259	0.769	!!	==	==		107	LEU CD2	21.905	0.533	==			
46	LEU CD2	22.309	0.823	==	==		!!	127	VAL CG1	21.790	0.696	==		==	!!
48	LEU CD1	22.510	0.844	(!!)				127	VAL CG2	20.699	0.620	==		==	==
48	LEU CD2	27.064	0.917	(!!)				134	ILE CD1	13.073	0.492	==	==	==	==
58	LEU CD1	24.286	0.781	(==)		==		136	LEU CD1	25.551	0.682	(==)		==	
58	LEU CD2	26.475	0.732	(==)				136	LEU CD2	25.695	0.588	(==)		==	
59	ILE CD1	14.045	0.754	=!		==		149	VAL CG1	21.889	1.161	==			
61	ILE CD1	14.380	0.679	=!		==		149	VAL CG2	20.361	0.954	==		==	
66	LEU CD1	24.699	0.617	(==)		!!		150	VAL CG1	22.056	0.591	==		==	!!
66	LEU CD2	21.671	0.481	(==)				150	VAL CG2	22.442	0.733	==		==	==
71	VAL CG1	21.088	0.790	==				151	LEU CD1	23.053	0.512	==			
71	VAL CG2	23.701	0.902	==				151	LEU CD2	24.279	0.458	==			

EIN

	Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO		Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO
6	LEU CD1	23.060	0.755	==	==			137	LEU CD1	22.080	1.036	==		==	
6	LEU CD2	25.480	0.891	==	==			137	LEU CD2	25.720	1.088	==			
11	ILE CD1	14.030	0.834	==	==	==		138	LEU CD1	25.620	1.089	==	==	==	
12	ALA CB	23.660	1.258	==	==	==		138	LEU CD2	24.580	1.095	==	==	==	
16	ALA CB	20.550	1.287	==	==	==		141	ILE CD1	14.690	1.022	==	==	==	
17	LEU CD1	25.850	0.884	==		!!		142	LEU CD1	26.290	0.949	==	==	==	
17	LEU CD2	23.840	1.025	==		!!		142	LEU CD2	22.890	1.063	==	==		
18	LEU CD1	25.640	0.978	==	==	!!		147	ILE CD1	12.910	0.884	(==)	==	==	
18	LEU CD2	23.450	0.800	==	==			149	LEU CD2	23.210	0.850	==		!!	
19	LEU CD1	25.500	0.963	(==)		!!		151	ALA CB	19.030	1.427	(==)	==	==	
19	LEU CD2	23.980	0.890	(==)		!!		152	ILE CD1	13.680	0.883	==	==	==	
24	ILE CD1	14.070	0.970	==	==	==		156	VAL CG1	22.100	0.832	==		!!	
26	ILE CD1	11.330	0.531	==	==	==		156	VAL CG2	17.160	0.826	==		!!	
31	ILE CD1	14.940	0.697	==	==	==		157	ILE CD1	14.070	0.457	==	==	==	
33	ALA CB	18.340	1.573	==	==			158	LEU CD1	25.840	0.976	==		==	
36	VAL CG1	21.790	1.087	==				159	VAL CG1	23.450	0.710	==		==	
36	VAL CG2	23.440	1.255	==		==		159	VAL CG2	21.190	0.761	==		==	
40	VAL CG1	21.440	1.161	==		==		160	ALA CB	24.410	1.387	==	==	==	
40	VAL CG2	23.690	1.294	==		==		161	ALA CB	18.120	1.617	==		==	
44	LEU CD1	21.810	0.669	==	==			163	LEU CD1	25.570	0.670	(==)			
44	LEU CD2	25.730	0.927	==	==			163	LEU CD2	24.230	0.782	(==)			
50	ALA CB	18.380	1.482	==				169	ALA CB	19.220	1.459	(==)		!!	
54	LEU CD1	26.170	1.046	==	==			176	VAL CG1	22.150	0.600	==		!!	
54	LEU CD2	23.180	1.151	(==)	==			176	VAL CG2	22.060	0.914	==		!!	
57	ILE CD1	13.640	0.742	(=!)		!!		177	LEU CD1	26.030	0.889	==	==	==	
61	ALA CB	18.520	1.593	(==)		!!		177	LEU CD2	21.700	0.860	==	==		
71	ALA CB	18.220	1.530	(!!)		==		180	ILE CD1	16.240	0.740	==	==	==	
72	ILE CD1	12.740	0.697	==		==		183	ALA CB	20.740	1.604	(!!)		==	
77	ILE CD1	13.390	0.888	(==)				194	ALA CB	18.450	1.470	(==)			
79	LEU CD1	23.460	0.903	==				197	LEU CD1	26.601	1.011	==			
79	LEU CD2	25.500	0.916	==				199	LEU CD1	26.133	0.840	(=!)			
80	LEU CD1	26.580	1.046	==				201	ALA CB	22.220	1.031	(!!)			
80	LEU CD2	24.410	1.011	==				202	ILE CD1	13.550	0.731	==	==	==	

85	LEU CD1	24.750	1.031	==		==			203	VAL CG1	19.700	0.729	(==)				
85	LEU CD2	24.730	0.927	==		==			208	VAL CG1	22.860	0.922	==		==		
89	ILE CD1	14.270	0.619	==	==	==			208	VAL CG2	19.660	0.830	==		==		
90	ILE CD1	13.940	1.107	==	==	==			212	VAL CG1	23.000	0.925	==		==		
91	ALA CB	18.060	1.544	==	==				212	VAL CG2	22.820	0.836	==		==		
92	LEU CD1	26.780	1.011	==	==				218	LEU CD1	26.130	0.609	==	==	==		
92	LEU CD2	22.750	0.915	==	==				218	LEU CD2	24.900	0.806	==	==	==		
93	ILE CD1	14.460	0.786	==	==	==			219	ILE CD1	14.980	0.580	==		==		
100	ALA CB	19.750	1.771	==		==			220	LEU CD1	24.990	0.781	==	==	==		
102	ALA CB	18.270	1.496	==		==			220	LEU CD2	26.220	0.731	==	==	==		
103	ALA CB	20.840	1.470	==					222	ALA CB	18.140	1.568	==	==	==		
104	ALA CB	17.780	1.442	==		==			223	VAL CG1	20.380	0.918	(==)				
107	VAL CG1	21.610	0.961	==		==			223	VAL CG2	21.200	0.936	(==)		==		
107	VAL CG2	23.870	1.031	==		==			227	VAL CG1	21.070	0.718	==		==		
108	ILE CD1	10.820	0.787	==	==	==			227	VAL CG2	21.940	0.818	==		==		
112	ALA CB	18.440	1.429	==		==			229	VAL CG1	21.470	0.996	==		==		
114	ALA CB	18.000	1.540	==					229	VAL CG2	21.080	0.786	==		==		
115	LEU CD1	25.590	0.866	==	==	==			235	VAL CG1	21.160	0.527	==		==		
115	LEU CD2	23.090	0.976	==	==				236	ILE CD1	13.530	0.754	==		==		
118	LEU CD2	22.450	0.945	(!=)					241	ALA CB	17.870	1.594	(==)				
123	LEU CD1	26.150	1.062	==					242	VAL CG1	21.020	0.981	(=!)		!!		
127	ALA CB	19.170	1.688	==		==			242	VAL CG2	22.040	1.167	(!)				
130	VAL CG1	22.207	0.976	==		==			246	VAL CG1	21.100	1.041	(==)				
130	VAL CG2	23.390	1.038	==		==			246	VAL CG2	20.840	1.075	(==)				
133	ILE CD1	14.630	0.784	==	==	==			247	ALA CB	19.240	1.534	(==)				

MBP

	Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO		Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO
2	ILE CD1	13.054	0.402	(=!)	==	!!		183	VAL CG1	22.416	0.882	==			==
7	LEU CD1	25.203	0.267	==	==	!!	==	183	VAL CG2	22.420	0.841	==		==	==
7	LEU CD2	24.064	0.615	==	==		==	192	LEU CD1	24.249	1.085	==	==	==	==
8	VAL CG1	20.962	0.876	(!)				192	LEU CD2	25.330	0.845	==	==	!!	==
8	VAL CG2	21.211	0.923	(=!)				195	LEU CD1	23.706	0.893	==	==		
9	ILE CD1	14.523	0.346	==	==	!!	==	195	LEU CD2	25.191	0.845	==	==		
11	ILE CD1	13.105	0.107	==	==	!!		196	VAL CG1	21.649	0.969	==			
20	LEU CD1	24.725	0.876	==				196	VAL CG2	23.319	0.856	==			
20	LEU CD2	26.879	0.638	==		!!		198	LEU CD1	25.598	1.018	(!!)			
23	VAL CG1	22.314	0.823	==				198	LEU CD2	23.461	0.813	(!!)			
23	VAL CG2	23.403	1.252	==				199	ILE CD1	14.222	0.743	==	==		==
33	ILE CD1	10.138	0.574	==	==		==	212	ILE CD1	13.110	0.867	(==)			==
35	VAL CG1	20.685	0.331	==				226	ILE CD1	11.775	0.060	==			==
35	VAL CG2	22.360	0.760	==				240	VAL CG1	20.890	0.585	(=!)			
37	VAL CG1	22.840	0.951	==				240	VAL CG2	21.830	0.788	(!!)			==
37	VAL CG2	20.673	0.766	==				244	VAL CG1	22.083	1.072	(!!)			
43	LEU CD1	26.677	1.101	==	==			244	VAL CG2	21.606	0.636	(=!)			
43	LEU CD2	26.431	1.133	==	==	!!		246	VAL CG1	20.441	0.969	==			
50	VAL CG1	21.260	0.967	(!!)				246	VAL CG2	21.596	0.983	==		!!	
50	VAL CG2	19.894	0.906	(!!)				247	LEU CD1	25.833	0.663	(!=)			==
59	ILE CD1	13.790	0.735	==	==			247	LEU CD2	23.824	0.645	(!!)			
60	ILE CD1	14.568	0.452	==	==	==		259	VAL CG1	21.509	0.925	==			==
75	LEU CD1	25.725	0.638	(=!)	==			259	VAL CG2	22.697	0.980	==			==
75	LEU CD2	20.664	0.538	(!!)	==			261	VAL CG1	20.308	0.679	==			==
76	LEU CD1	27.430	0.897	==	==			261	VAL CG2	20.936	0.565	==			
76	LEU CD2	21.891	0.538	==	==	==		262	LEU CD1	22.681	0.806	==	==		
79	ILE CD1	12.313	0.161	==	==	==		262	LEU CD2	25.314	0.885	==	==		
89	LEU CD1	26.152	0.526	==	==			266	ILE CD1	13.882	0.915	==	==		
89	LEU CD2	25.298	0.484	==	==			275	LEU CD1	25.202	0.933	==	==		==
97	VAL CG1	22.085	0.578	==			==	275	LEU CD2	22.493	0.947	==	==		==
97	VAL CG2	19.363	1.347	==			==	280	LEU CD1	25.779	0.672	==	==		==
103	LEU CD1	24.748	0.490	==	==			280	LEU CD2	23.405	0.710	==	==		
103	LEU CD2	24.100	0.972	==	==			284	LEU CD1	23.945	0.843	==			
104	ILE CD1	14.779	0.845	==	==			284	LEU CD2	27.398	1.039	==			
108	ILE CD1	9.981	0.637	==	==	==		285	LEU CD1	25.695	1.052	==	==		
110	VAL CG1	20.954	0.767	==				285	LEU CD2	24.003	1.119	==	==		
110	VAL CG2	20.424	0.476	==				290	LEU CD1	27.886	1.074	==	==		
115	LEU CD1	22.828	0.684	(==)				290	LEU CD2	21.864	0.792	==	==		
115	LEU CD2	24.597	0.587	(!)				293	VAL CG1	21.422	1.004	==			
116	ILE CD1	13.706	0.459	(!!)				293	VAL CG2	22.246	0.901	==			
121	LEU CD1	24.729	0.665	==				302	VAL CG1	22.153	1.059	==			
121	LEU CD2	22.791	0.779	==				302	VAL CG2	19.070	1.262	==			
122	LEU CD1	25.548	0.930	(!!)				304	LEU CD1	24.263	0.848	(==)	==		
122	LEU CD2	26.848	0.937	(!!)				304	LEU CD2	26.586	0.702	(==)	==		
132	ILE CD1	12.360	0.560	==				311	LEU CD1	26.326	0.870	==			==
135	LEU CD1	24.040	0.888	(!!)				311	LEU CD2	22.652	0.797	==			==
135	LEU CD2	24.317	0.882	(!=)				317	ILE CD1	12.483	1.118	(==)	==		==
139	LEU CD1	25.914	0.787	=!				329	ILE CD1	12.362	0.821	==	==	==	==
139	LEU CD2	22.927	0.852	!!				333	ILE CD1	12.253	-0.138	(==)		==	==
147	LEU CD1	22.500	0.683	(==)				343	VAL CG1	21.297	0.863	==		==	==

147	LEU CD2	27.555	0.936	(==)					343	VAL CG2	23.236	0.168	==		==	==	
151	LEU CD1	26.232	0.744	==					347	VAL CG1	20.468	0.918	==				
151	LEU CD2	23.579	0.135	==		!!			347	VAL CG2	22.282	1.043	==				
160	LEU CD1	19.602	-1.003	(==)	==	==			348	ILE CD1	12.965	0.869	(==)	==		==	
160	LEU CD2	25.084	-0.302	(==)	==	!!			357	VAL CG1	21.315	0.991	==				
161	ILE CD1	13.081	0.760	==	==		==		357	VAL CG2	23.401	1.045	==		!!		
178	ILE CD1	13.905	0.853	(==)		==	==		361	LEU CD1	25.264	0.892	==	==		==	
181	VAL CG1	21.539	0.823	==		==	==		361	LEU CD2	19.605	0.722	==	==	==	==	
181	VAL CG2	22.538	0.711	==					368	ILE CD1	15.285	0.693	==	==	==	==	

MSG

	Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO		Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO
5	ILE CD1	12.833	0.820	(==)				348	VAL CG1	20.967	0.163	==			
10	LEU CD1	24.486	0.915	==				348	VAL CG2	21.448	0.402	==			
10	LEU CD2	22.364	0.868	==				349	ILE CD1	14.919	0.778	==			
12	ILE CD1	15.537	0.008	==				361	ILE CD1	12.815	0.999	==		!!	
20	VAL CG1	21.566	0.463	==				362	LEU CD1	24.066	1.105	==			
20	VAL CG2	23.955	0.739	==				362	LEU CD2	27.381	0.769	==			
24	VAL CG1	20.604	0.541	==				365	VAL CG1	20.364	-0.085	==			
24	VAL CG2	22.556	0.137	==				365	VAL CG2	21.867	0.566	==			
25	LEU CD1	24.621	0.295	==	==			370	ILE CD1	13.820	0.871	==	==		
25	LEU CD2	20.071	-0.285	==	==			372	LEU CD1	23.064	0.863	==	==		
30	LEU CD1	25.838	0.755	==	==			372	LEU CD2	25.825	1.033	==	==	!!	
30	LEU CD2	22.144	0.817	==	==			375	LEU CD1	25.628	0.885	==	==		
42	ILE CD1	14.704	0.680	==	==			375	LEU CD2	22.069	0.721	==	==		
43	VAL CG1	20.943	0.678	==				386	VAL CG1	19.406	0.670	==			
43	VAL CG2	24.763	1.051	==				386	VAL CG2	21.071	0.726	==			
46	LEU CD1	26.301	0.806	(==)				388	ILE CD1	12.747	0.290	==	==		
46	LEU CD2	22.903	0.827	(==)				389	VAL CG1	22.383	0.720	(==)			
53	LEU CD1	25.988	0.305	(==)				389	VAL CG2	20.605	0.437	(==)			
54	LEU CD1	24.942	1.101	==				399	VAL CG1	21.973	0.912	==			
54	LEU CD2	19.976	0.673	==				399	VAL CG2	20.715	0.989	==			
60	ILE CD1	12.120	0.769	==	==			409	ILE CD1	13.073	0.548	==	==		
64	LEU CD1	25.428	0.904	==	==			413	LEU CD1	26.081	0.718	==	==		
64	LEU CD2	24.554	0.751	==	==			413	LEU CD2	22.095	0.807	==	==		
75	VAL CG2	20.637	0.815	(==)				420	LEU CD1	24.802	0.469	==	==		
85	LEU CD1	24.801	-0.085	==	==			420	LEU CD2	22.504	0.373	==	==		
85	LEU CD2	20.081	-0.210	==	==			424	ILE CD1	14.922	0.745	==	==	==	
88	LEU CD1	23.547	0.823	==	==			433	LEU CD1	27.191	0.971	==			
88	LEU CD2	25.333	0.674	==	==			433	LEU CD2	25.452	0.888	==			
91	LEU CD1	25.353	0.901	(!!)				435	LEU CD1	27.524	1.194	==	==		
91	LEU CD2	24.612	0.903	(!!)				435	LEU CD2	21.476	0.801	==	==	==	
98	VAL CG1	20.318	0.852	(==)				439	ILE CD1	13.782	0.790	==		!!	
100	VAL CG1	21.311	0.958	==				446	VAL CG1	21.292	0.980	==			
100	VAL CG2	22.218	0.857	==				446	VAL CG2	22.836	1.134	==			
105	ILE CD1	10.255	0.810	(!=)	!!			449	ILE CD1	14.145	0.642	==		!!	
109	ILE CD1	14.490	0.724	(==)	!!			464	VAL CG1	20.503	0.474	==			
117	LEU CD1	25.348	0.578	==				464	VAL CG2	17.943	0.670	==			
117	LEU CD2	26.534	0.593	==				471	LEU CD2	22.194	1.075	(==)	==		
118	VAL CG1	19.382	0.230					490	VAL CG1	19.622	0.904	==			
118	VAL CG2	17.697	-0.834	(!)		!!		490	VAL CG2	20.949	0.854	==			
119	VAL CG1	21.619	0.737	==				491	LEU CD1	25.082	0.561	==	==		
119	VAL CG2	18.891	0.687	==				491	LEU CD2	20.686	0.282	==	==		
128	LEU CD1	24.941	0.572	(==)				494	LEU CD1	25.945	0.627	==	==		
128	LEU CD2	24.322	0.607	(==)				494	LEU CD2	22.337	0.670	==	==		
138	LEU CD1	23.192	0.768	(==)				498	LEU CD1	25.082	0.761	==	==	==	
138	LEU CD2	26.304	0.748	(==)				498	LEU CD2	23.324	0.619	==	==	==	
142	LEU CD1	27.936	0.456	==	==			504	ILE CD1	13.295	0.722	==			
142	LEU CD2	21.862	-0.448	==	==			514	LEU CD1	25.980	1.066	==			
147	ILE CD1	13.610	0.262	==	==			514	LEU CD2	22.371	0.937	==			
148	ILE CD1	11.341	0.390	==	==			526	LEU CD1	23.498	0.115	(==)			
166	VAL CG1	21.207	0.848	==				526	LEU CD2	22.406	0.100	(==)		==	
166	VAL CG2	22.805	0.924	==				535	VAL CG2	17.974	0.916	==			
167	ILE CD1	14.215	0.831	(!!)				543	LEU CD1	25.180	0.572	==	==		
170	VAL CG1	22.279	1.178	==		==		543	LEU CD2	21.491	0.250	==	==		
170	VAL CG2	25.567	1.376	==				546	LEU CD1	25.237	0.781	(==)			
178	LEU CD1	26.784	0.611	==		==		546	LEU CD2	20.492	0.261	(==)			
178	LEU CD2	26.758	0.726	==		==		553	VAL CG1	22.981	1.087	(==)			
180	LEU CD1	24.390	-0.082	==	==			553	VAL CG2	20.128	1.115	(==)			
180	LEU CD2	22.737	0.359	==	==			556	VAL CG1	21.888	0.894	(==)			
188	VAL CG1	21.750	0.407	==				556	VAL CG2	20.935	0.865	(!=)			
188	VAL CG2	23.700	0.743	==				560	ILE CD1	13.815	0.603	==	==		
189	VAL CG2	18.759	0.541	==				572	LEU CD1	26.490	0.721	==	==		
193	VAL CG1	19.420	0.703	==				572	LEU CD2	23.384	1.055	==	==		
193	VAL CG2	19.366	0.205	==				573	LEU CD1	24.348	0.989	==			
194	VAL CG2	20.321	0.773	(=!)				573	LEU CD2	25.639	0.950	==			
198	LEU CD1	22.639	0.663	==	==			576	LEU CD1	24.877	0.826	==	==		
198	LEU CD2	26.102	0.813	==	==			576	LEU CD2	24.663	1.214	==	==		

200	ILE CD1	16.119	0.746	==	==	==		577	LEU CD1	26.739	0.781	(!!)			
202	LEU CD1	24.801	0.768	==	==			577	LEU CD2	22.528	0.738	(!)			
202	LEU CD2	22.680	0.501	==	==			579	ILE CD1	14.656	0.757	==	==	==	
210	LEU CD1	28.067	1.090	==		==		581	VAL CG1	20.609	0.530	==			
210	LEU CD2	22.536	0.904	==		==		581	VAL CG2	20.334	0.527	==			
217	VAL CG1	21.989	0.800	==				592	ILE CD1	13.646	0.825	==		==	
217	VAL CG2	21.929	0.880	==				596	LEU CD1	26.922	1.050	==		==	
229	ILE CD1	10.616	0.562	==	==	==		596	LEU CD2	24.904	1.053	==			
230	LEU CD1	21.754	0.680	==				600	VAL CG1	22.462	0.752	==		==	
230	LEU CD2	24.072	-0.499	(==)				600	VAL CG2	25.068	0.974	==			
231	LEU CD1	25.413	0.876	==				603	ILE CD1	14.352	0.502	(==)		==	
231	LEU CD2	24.834	1.069	==				604	LEU CD1	27.761	0.912	==	==		
236	LEU CD1	25.156	0.804	(==)	==			604	LEU CD2	23.071	0.754	==	==		
236	LEU CD2	22.500	0.699	(==)	==			607	VAL CG1	23.104	1.033	==		==	
238	ILE CD1	13.747	0.503	==	==	==		607	VAL CG2	26.709	1.120	==		==	
240	LEU CD1	24.913	0.632	==	==	==		608	VAL CG1	20.748	1.329	(==)			
240	LEU CD2	24.407	0.715	==	==			608	VAL CG2	21.975	1.155	(==)			
242	ILE CD1	12.365	0.664	==		==		611	VAL CG1	21.774	0.864	==			
248	ILE CD1	12.276	0.756	(!!)				611	VAL CG2	25.146	1.349	==		==	
256	ILE CD1	11.691	0.901	==		==		623	ILE CD1	14.613	0.784	(==)			
259	VAL CG1	19.992	0.651	==				626	VAL CG1	20.784	0.805	==			
259	VAL CG2	21.191	0.648	==		==		626	VAL CG2	21.600	0.882	==			
260	ILE CD1	11.138	0.651	(==)		!!		628	LEU CD1	25.544	0.894	==			
261	VAL CG1	22.279	0.898	==				628	LEU CD2	23.460	0.678	==			
261	VAL CG2	21.020	0.924	==				635	LEU CD1	27.517	0.867	==			
265	ILE CD1	9.490	0.378	(==)	==			635	LEU CD2	25.306	1.020	==			
268	ILE CD1	13.602	0.655	(==)		!!		642	ILE CD1	15.941	0.647	==		==	
269	LEU CD1	26.885	0.876	==				646	LEU CD1	23.659	0.863	==			
269	LEU CD2	22.419	0.655	==				646	LEU CD2	25.235	0.829	==			
275	VAL CG1	23.138	0.833	(==)				650	ILE CD1	8.057	0.581	==		==	
275	VAL CG2	21.430	0.785	(==)				651	LEU CD1	28.466	1.193	==		==	
278	VAL CG1	21.032	0.423	(==)				651	LEU CD2	25.318	1.057	==		==	
278	VAL CG2	18.142	0.642	(==)				656	VAL CG1	22.427	0.763	==		==	
284	ILE CD1	13.198	0.745	(==)				656	VAL CG2	24.262	1.119	==		==	
286	LEU CD1	25.696	0.881	(==)				660	LEU CD1	25.900	0.717	==	==		
286	LEU CD2	26.157	0.768	(==)				660	LEU CD2	23.362	0.479	==	==		
291	LEU CD1	25.289	0.601	==	==			666	VAL CG1	19.705	0.899	==			
291	LEU CD2	26.260	0.771	==	==			666	VAL CG2	21.958	1.127	(==)			
293	LEU CD1	25.478	0.930	(=!)				667	VAL CG1	22.628	1.058	==			
293	LEU CD2	24.044	0.778	(!=)				667	VAL CG2	23.404	1.131	==			
298	LEU CD1	23.575	1.052	(=!)				696	LEU CD1	26.693	0.738	==			
298	LEU CD2	25.239	0.821	(==)				696	LEU CD2	24.144	0.903	==			
313	LEU CD1	25.130	0.938	(=!)				697	ILE CD1	14.757	0.574	(==)		==	
313	LEU CD2	24.015	0.807	(==)				699	LEU CD1	25.034	0.907	==			
327	ILE CD1	13.073	0.572	==				699	LEU CD2	22.156	0.881	==			
329	LEU CD1	25.272	0.204	(==)				711	LEU CD1	25.811	0.806	==			
329	LEU CD2	22.249	0.625	==				711	LEU CD2	22.836	1.022	==			
334	LEU CD2	27.669	0.780	(==)	==			712	LEU CD1	24.727	0.182	==			
335	LEU CD1	25.213	0.818	(==)				712	LEU CD2	22.944	0.057	==		!!	
335	LEU CD2	22.086	0.633	(!!)				717	LEU CD1	25.176	0.980	(==)			
337	ILE CD1	14.606	0.875	==	==			717	LEU CD2	23.695	0.956	(==)			
343	LEU CD1	22.988	0.988	==											
343	LEU CD2	25.785	0.745	==											

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	Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO		Methyl	13C	1H	FLYA	MAGMA	MAPXS	FLAMENGO
21	LEU CD1	25.996	0.607	(=!)	==	==	==	113	VAL CG1	20.996	0.301	==		==	==
24	VAL CG1	24.215	0.930	==			==	113	VAL CG2	21.787	-0.033	==		==	==
24	VAL CG2	21.800	0.873	==		==	==	116	VAL CG1	22.011	0.950	==		==	!!
31	VAL CG1	21.101	0.975	==		==	==	116	VAL CG2	21.613	0.940	==		==	!!
38	LEU CD1	26.082	0.639	==	==		==	134	VAL CG1	22.670	0.742	==		==	==
38	LEU CD2	28.507	0.701	==	==	==	==	134	VAL CG2	23.838	0.872	==		==	==
46	VAL CG1	19.945	0.821	==		==	==	136	LEU CD1	27.678	0.667	==	==	==	!!
46	VAL CG2	22.010	0.938	==		==	==	136	LEU CD2	26.611	0.410	==	==	==	!!
47	LEU CD1	25.259	0.714	==	==	==	==	137	ILE CD1	14.071	0.374	==	==	==	==
47	LEU CD2	28.341	0.572	==	==	==	!!	141	ILE CD1	12.458	0.616	==	==	==	==
48	LEU CD1	26.717	0.608	==	==	==	==	144	ILE CD1	11.757	0.762	==	==		==
48	LEU CD2	23.970	0.748	==	==	==	!!	148	LEU CD1	25.245	0.731	==	==	==	==
49	ILE CD1	14.168	0.594	==	==	==	==	148	LEU CD2	26.003	0.454	==	==	==	==
54	VAL CG1	20.875	0.923	==		==	==	157	ILE CD1	13.663	0.638	(==)	==	==	==
54	VAL CG2	21.440	0.918	==		==	==	165	ILE CD1	13.629	0.425	==	==	==	==
58	LEU CD1	21.343	0.440	(!!)	==		==	172	VAL CG1	23.655	0.696	==		==	==
58	LEU CD2	26.121	0.784	(=!)	==	==	==	172	VAL CG2	22.860	0.799	==		==	==
59	ILE CD1	13.464	0.791	(==)	==	==	==	173	VAL CG1	21.461	0.946	==		==	==
64	ILE CD1	12.983	0.799	==	==	==	==	173	VAL CG2	23.532	0.982	==		==	==
67	ILE CD1	13.905	0.542	==	==	==	==	176	LEU CD1	25.759	0.638	(==)	==	==	==
69	LEU CD1	24.774	0.755	==	==	==	==	176	LEU CD2	22.386	0.901	(==)	==	==	==
69	LEU CD2	22.438	0.425	==	==	==	==	184	LEU CD1	25.965	0.733	==	==	==	==

70	ILE CD1	11.698	0.660	==	==	==	==	184	LEU CD2	22.537	0.806	==	==	==	==
74	VAL CG1	20.747	0.770	==		==	!!	190	VAL CG1	21.380	-0.176	==		==	==
74	VAL CG2	22.071	0.947	==		==	!!	190	VAL CG2	23.016	0.630	==		==	==
77	VAL CG1	22.491	0.733	==		==	==	192	LEU CD1	24.522	1.077	==	==	==	==
77	VAL CG2	21.784	0.755	==			==	192	LEU CD2	26.319	0.975	==	==	==	==
81	LEU CD1	22.548	1.077	==	==	==	==	194	ILE CD1	10.361	0.506	==	==	==	==
81	LEU CD2	26.063	1.194	==	==	==	==	197	LEU CD1	23.729	0.645	==	==	==	==
82	VAL CG1	21.933	1.173	==		==	==	197	LEU CD2	25.212	0.652	==	==	==	==
82	VAL CG2	21.877	1.099	==		==	==	201	LEU CD1	25.766	0.784	==	==	==	==
87	VAL CG1	20.901	1.033	(==)			!!	201	LEU CD2	22.016	0.660	==	==	==	==
87	VAL CG2	20.479	1.151	(==)		!!	!!	207	LEU CD1	24.814	0.835	==	==	==	==
88	LEU CD1	25.077	0.242	==	==	==	!!	207	LEU CD2	26.319	0.762	==	==	==	==
88	LEU CD2	20.700	-0.095	==	==	==	!!	212	ILE CD1	14.420	0.403	==	==	==	==
89	VAL CG1	21.464	0.784	==			!!	215	ILE CD1	16.367	1.051	==	==	==	==
89	VAL CG2	23.461	0.711	==		==	!!	217	VAL CG1	20.710	0.916	==			!!
94	ILE CD1	13.229	0.793	(==)	==	==	==	217	VAL CG2	21.220	0.945	==		==	!!
106	LEU CD1	24.657	0.440	==	==	==	==	223	ILE CD1	11.794	0.782	==	==	==	==
106	LEU CD2	23.969	0.227	==	==	==	==	229	VAL CG1	21.420	1.023	==		==	==
107	VAL CG1	22.135	0.828	==		==	!!	229	VAL CG2	23.133	1.180	==		==	==
107	VAL CG2	21.775	0.745	==			!!	233	LEU CD1	25.610	0.872	==	==	==	==
109	ILE CD1	13.179	0.726	==	==	==	==	233	LEU CD2	22.819	0.696	==	==	==	==
112	LEU CD1	23.297	0.652	==	==	==	==								
112	LEU CD2	26.192	0.634	==	==	==	==								

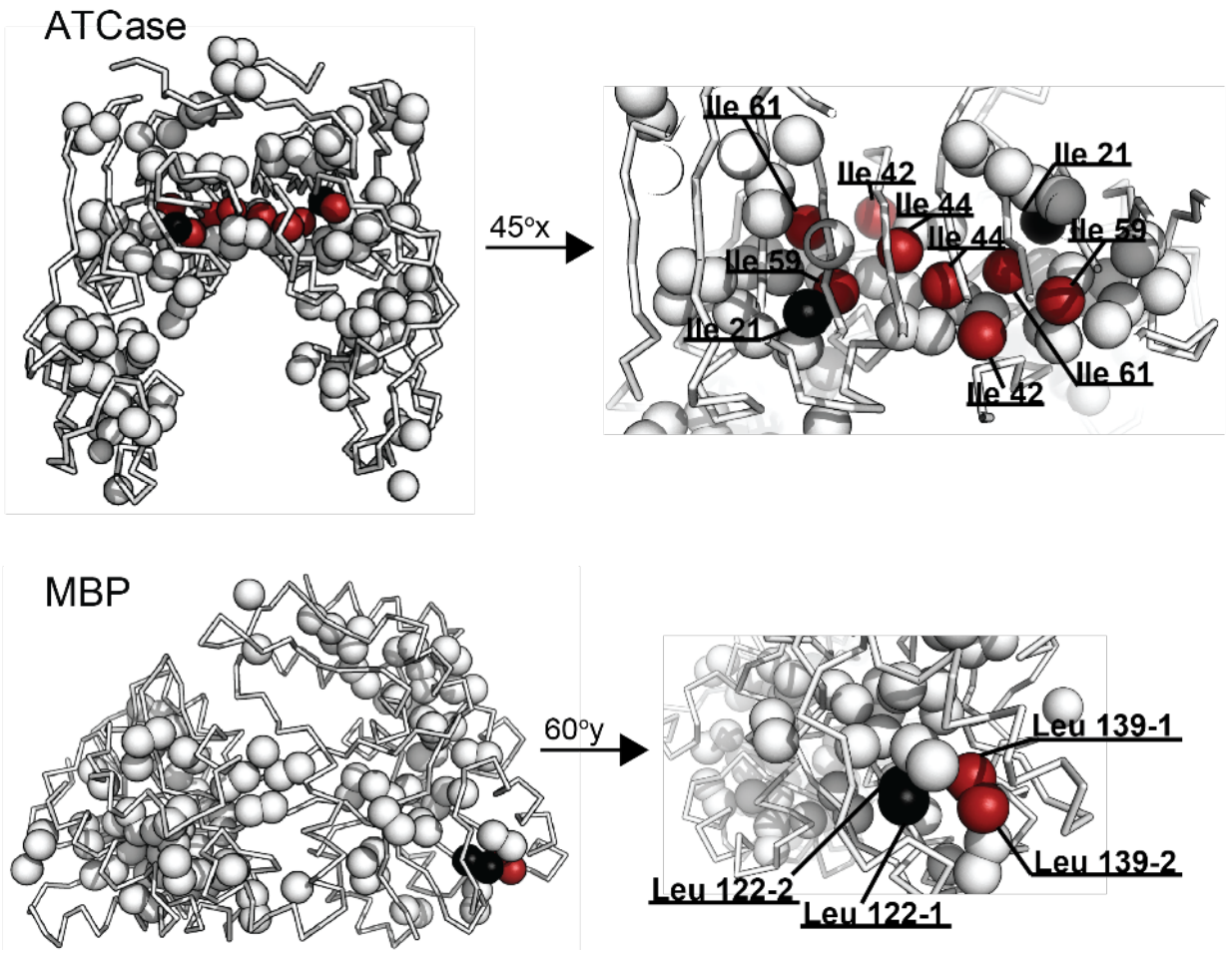


Figure S4. Sources of errors in the automatic methyl resonance assignments generated by MethylFLYA. Carbon atoms of the erroneously assigned methyls are shown as red spheres, whereas their correct assignment positions are given in black spheres, or exceptionally in red when the assignment at those positions is also incorrect (i.e. for assignment swaps such as Ile42↔Ile44). The mis-assigned resonances belong to nearby methyl groups. For ATCase, the assignment errors cluster at the interface of the two subunits of the homodimer.

Table S2. Summary of errors in the automatic methyl resonance assignments generated by MethylFLYA. The mis-assigned methyls are assigned to spatially proximal residues.

Protein	Methyl group	Erroneously assigned to	C–C distance (Å) between correct and erroneous assignment
ATCase	Ile 42 δ_1	Ile 44 δ_1	5.5
	Ile 44 δ_1	Ile 42 δ_1	5.5
	Ile 59 δ_1	Ile 21 δ_1	4.0
	Ile 61 δ_1	Ile 59 δ_1	5.9
MBP	Leu 139 δ_1	Leu 122 δ_1	5.2
	Leu 139 δ_2	Leu 122 δ_2	6.9

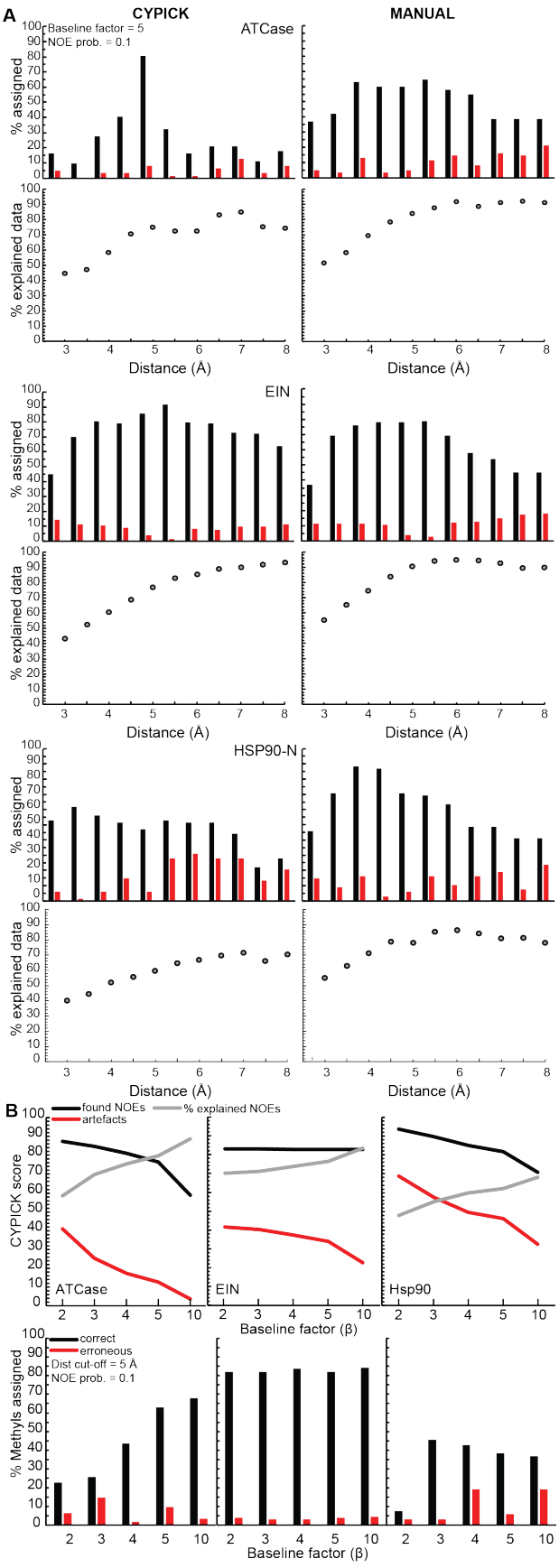


Figure S5. Parameter optimization for automatic NOESY peak picking with CYPICK. **(A)** Percentage of accurately (black) and erroneously (red) assigned methyls as a function of methyl ^{1}H–^{1}H NOE distance cutoffs, d_{cut}, at fixed NOE probability value p_{NOE} = 0.1. Results using the automatically generated CYPICK NOESY lists are in the left columns, and results obtained using the manually prepared NOESY lists are in the right column. **(B)** Varying baseline factors for automatic peak picking of methyl-methyl NOESY spectra using CYPICK at fixed distance cutoff d_{cut} = 5 Å and NOE probability value p_{NOE} = 0.1. Variation of the CYPICK find score (*black*), artifact score (red), and the percentage of explained inter-methyl NOEs (*grey*) as a function of the baseline factor. For all three proteins, a fixed distance cutoff d_{cut} = 5 Å and an NOE observation probability p_{NOE} = 0.1 were used to generate the expected methyl-methyl NOEs. Assignment percentages are relative to the number of reference assignments.

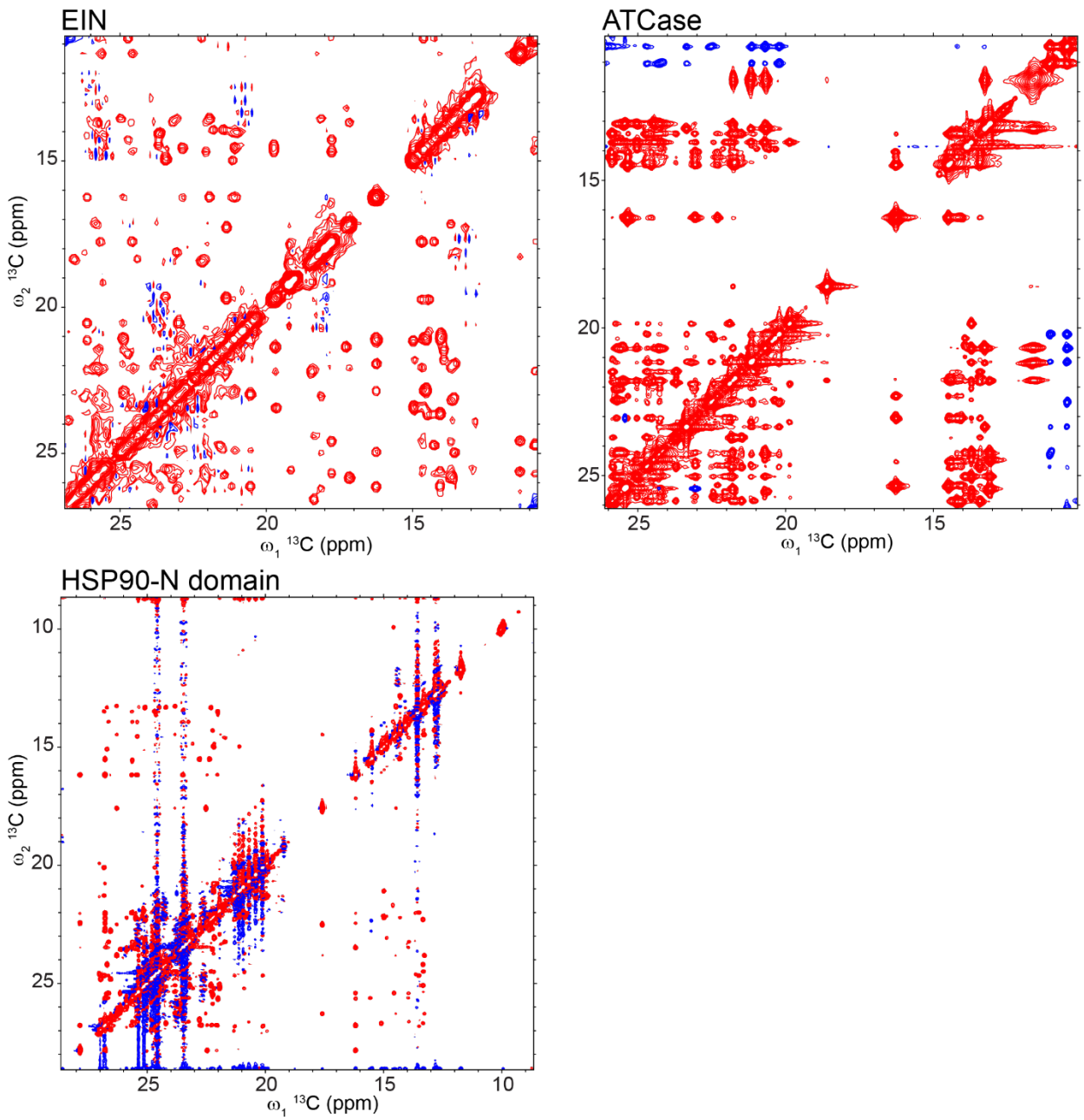


Figure S6. 2D $^{13}C(\omega_1)$-$^{13}C(\omega_2)$ projections of 3D CCH NOESY (ATCase, HSP90) or 4D HCCH NOESY spectra (EIN). All spectra are plotted with contour levels starting at a signal-to-noise ratio of three, as determined by the software Sparky (T. D. Goddard and D. G. Kneller, University of California, San Francisco). Positive and negative contours are colored in red and blue, respectively. The spectra were acquired previously by Venditti *et al.*[27] (EIN), Velyvis *et al.*[24] (ATCase), and Shah *et al.*[41] (HSP90).

Table S3. MethylFLYA computation times (h) for different combinations of input NMR data, as in Figure 3.

Protein	Filtered NOEs	Unfiltered NOEs	L, V=LV	2L, 2V	L, V=LV, 2LV
EIN	0.52	0.50	0.54	0.48	0.54
ATCase	0.38	0.40	0.40	0.36	0.41
MBP	0.46	0.45	0.53	0.52	0.59
MSG	1.23	1.23	1.53	1.20	1.24
$\alpha_7\alpha_7$	0.50	0.51	0.60	0.69	0.79

Calculations were performed using 100 Intel Xeon E5-2690 processor cores in parallel.

Table S4. Results of the CYPICK application to the 3D CCH NOESY (ATCase, HSP90) and 4D HCCH NOESY (EIN) spectra. The results are given for the baseline factor $\beta = 5$. A find score indicates which percentage of NOE peaks from the reference were also found by CYPICK. Overall, find, and artifact scores are defined by Würz *et al.*[37]

Protein	Peaks (reference)	Peaks (CYPICK)	Overall score (%)	Find score (%)	Artifact score (%)
EIN	618	775	74	83	34
ATCase	563	495	74	77	13
HSP90	409	624	68	82	46

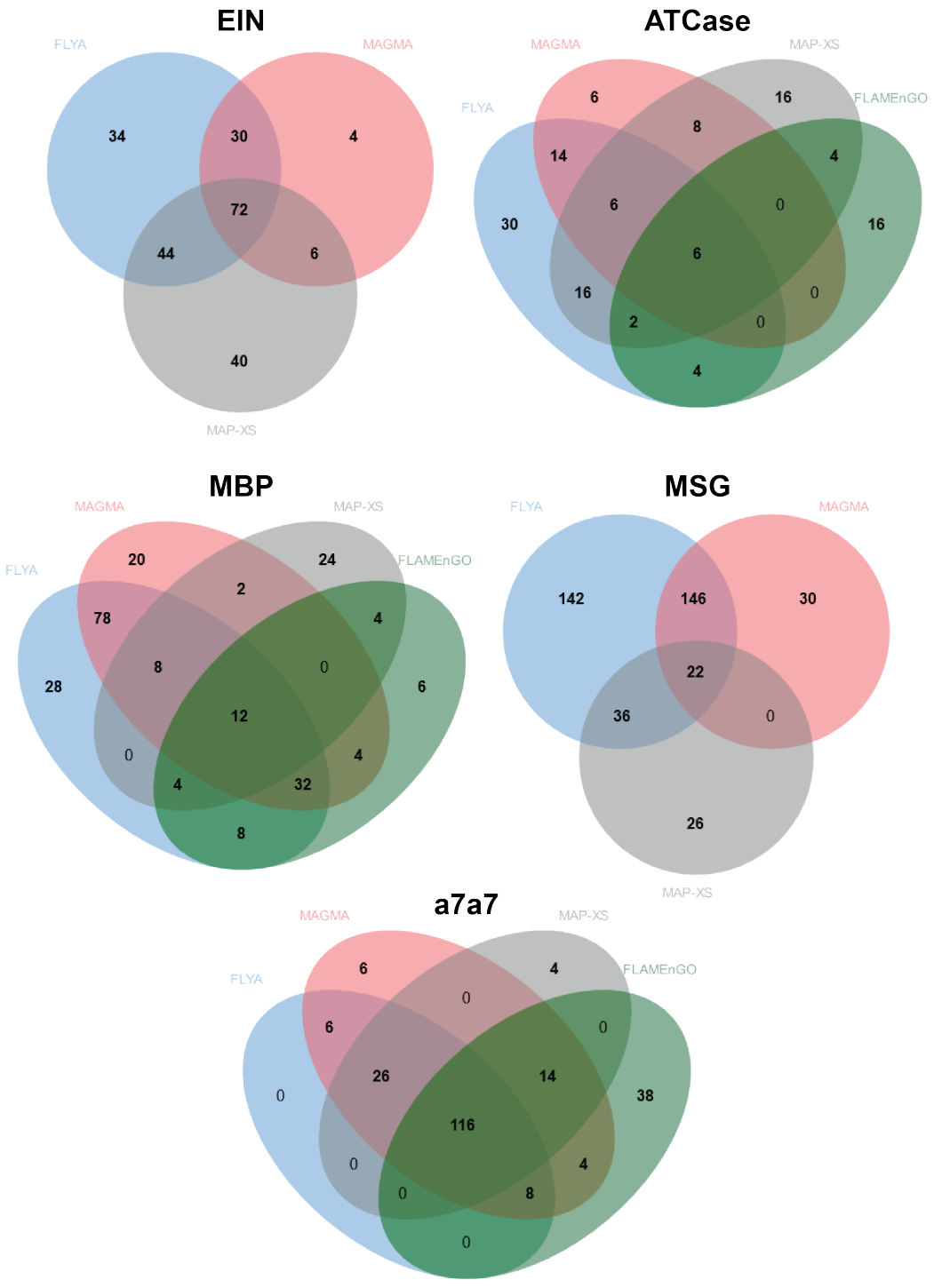
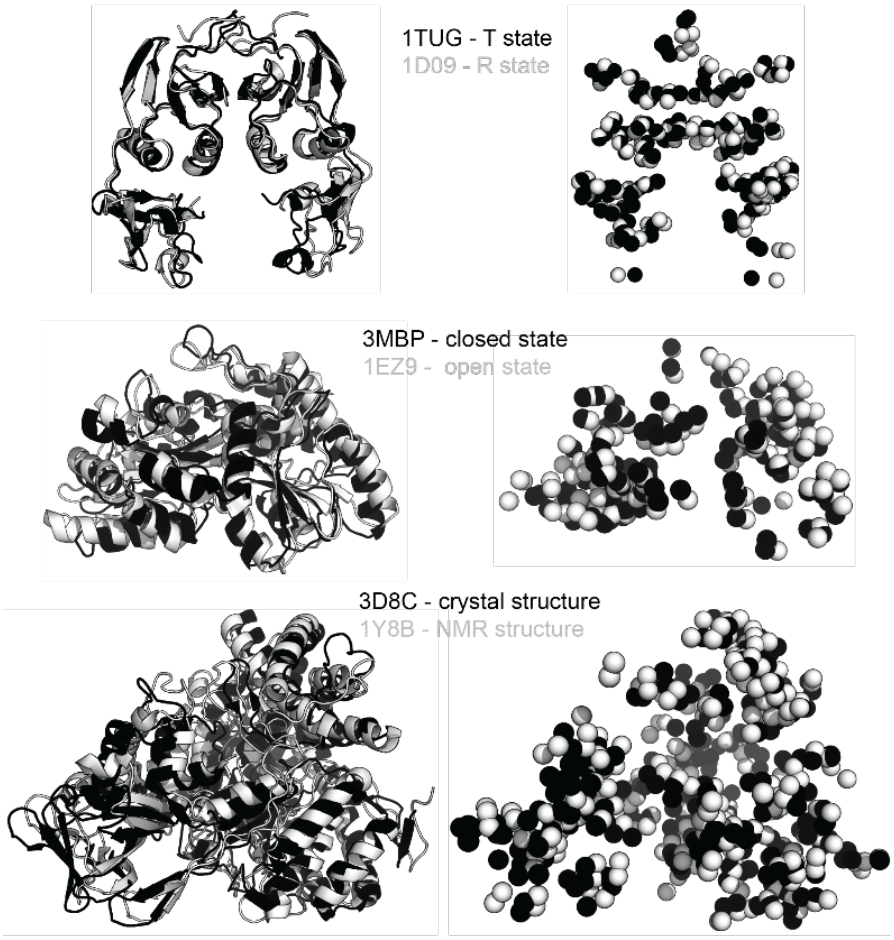


Figure S7. Intersection of assignments generated with different automatic methyl assignment protocols. The illustration of the intersections of assignment solutions from MethylFLYA, MAGMA, FLAMEnGO2.0, and MAP-XSII are shown for the indicated benchmark cases. In the case of FLAMEnGO2.0, no confident (100%) assignments were found for EIN and MSG.



Protein	PDB ID	correct	error
atcase	1d09 (Rstate)	36	3
	1tug (Tstate)	36	1
mbp	1ez9 (open)	83	2
	3mbp (closed)	75	2
msg	1y8b (NMR)	129	0
	1d8c (X-ray)	173	0

Figure S8. MethylFLYA performance on different input structures for three enzymes in the benchmark. Differences in backbone conformations between the different protein states are shown with protein structures in cartoon representation (*right* column). In the *left* column, the positions of the methyl carbons are indicated with spheres for each of the conformers, with colors matching those assigned to the backbone (*right*). The total number of accurate and erroneous "strong" methyl assignments generated with MethylFLYA using different conformers is summarized in the table (bottom row).

Supplementary methods

An example MethylFLYA automated methyl assignment calculation for the N-terminal domain of E. coli Enzyme I (EIN) can be downloaded from http://www.cyana.org/methylflya.tgz.

The complete assignment calculation is performed by first running the *RUN.cya* macro that calls *PREP.cya* to make expected peaks using three different NOE distance cutoffs, and starts parallel FLYA automated assignment runs with *CALC.cya* for the different NOE distance cutoffs. After completing the FLYA runs, consensus chemical shifts are obtained with *CONSOL.cya*, which must be run separately.

Input files:

demo.seq	amino acid sequence
demo.pdb	3D structure
C13HSQC.peaks	2D [^{1}H,^{13}C]-HMQC peak list with amino acid type assignments (peaks are assigned to methyl groups of the correct amino acid type but arbitrary residue number)
HCcCH.peaks	short mixing-time 4D CCNOESY for intraresidual Leu/Val connections
CCNOESY.peaks	4D CCNOESY peak list, unassigned
ref.prot	reference chemical shifts (for comparison only)
init.cya	initialization macro
RUN.cya	automated assignment calculation (calls *PREP.cya*, *CALC.cya*)
PREP.cya	prepare peak lists for FLYA
CALC.cya	run FLYA assignment calculation
CONSOL.cya	determine consensus chemical shifts

The preparation macro, *PREP.cya*, performs the following tasks:

1. The [^{1}H,^{13}C]-HMQC peak list, *C13HSQC.peaks*, which is assigned to methyls of the correct amino acid type (and arbitrary residue numbers that are not used), is split into four amino acid type-specific peak lists, C13HSQC_*X*.peaks, with *X* = A, I, L, V.
2. The peaks in the unassigned short mixing-time 4D CCNOESY peak list, *HCcCH.peaks* (formally treated as an HCCH TOCSY-type experiment), which contains intraresidual connections between the two methyl groups of Leu or Val, are assigned to amino acid types (and irrelevant, arbitrary residue numbers) according to the closest [^{1}H,^{13}C]-HMQC peaks, and the peak list is split into two amino acid type-specific peak lists, *HCcCH_L.peaks* and *HCcCH_V.peaks*.
3. The unassigned 4D NOESY peak list, *CCNOESY.peaks*, is treated similarly, and split into 16 amino acid pair type-specific peak lists, *CCNOESY_XY*.peaks, with *X*, *Y* = A, I, L, V.
4. The macro *peaklists.cya* that specifies the generation of expected peaks during the FLYA calculations in *CALC.cya* is written.

The *RUN.cya* macro creates three subdirectories *demo_d4.5*, *demo_d5.0*, and *demo_d5.5* for the three FLYA calculations with different NOE distance cutoffs, d_{cut} = 4.5, 5.0, 5.5 Å. In each of these three directories, the input files are copied, the *PREP.cya* macro is executed, and three jobs of 100 individual FLYA assignment runs each are started with *CALC.cya*. The individual assignment runs are executed in parallel on different processors, if available.

Subsequently, consensus assignments are generated with the *CONSOL.cya* macro that produces the following main output files:

consol.prot	consensus chemical shift lists (in XEASY format)
consol-strong.prot	strong (confident) consensus chemical shifts (in XEASY format)
consol.tab	table of consensus methyl assignments
consol.pdf	plot of consensus methyl assignments

Optionally (not used in this paper), already known, partial methyl assignments can be included in the calculation by specifying their shifts in a chemical shift list file, e.g. *fix.prot*, and adding the line 'shiftassign_fix := fix.prot' to the *CALC.cya* macro.

For more details on specifying the partial assignments, comparing results to the known reference, or other generic FLYA input file requirements, macros, and output files please see: http://www.cyana.org/wiki/index.php/Tutorials.

Experiment definitions in the CYANA library

The CYANA library (*cyana.lib*) contains definitions of the experiments necessary for automatic methyl resonance assignment with MethylFLYA:

```
SPECTRUM C13HSQC C H
 0.980 C:C_A* H:H_A*

SPECTRUM CCNOESY3D C1 C2 H1
 0.900 C1:C_A* H1:H_A* ~4.0 H_A* C2:C_A*
 0.800 C1:C_A* H1:H_A* ~4.5 H_A* C2:C_A*
 0.700 C1:C_A* H1:H_A* ~5.0 H_A* C2:C_A*
 0.600 C1:C_A* H1:H_A* ~5.5 H_A* C2:C_A*
 0.500 C1:C_A* H1:H_A* ~6.0 H_A* C2:C_A*

SPECTRUM CCNOESY H1 H2 C2 C1
 0.900 C1:C_A* H1:H_A* ~4.0 H2:H_A* C2:C_A*
 0.800 C1:C_A* H1:H_A* ~4.5 H2:H_A* C2:C_A*
 0.700 C1:C_A* H1:H_A* ~5.0 H2:H_A* C2:C_A*
 0.600 C1:C_A* H1:H_A* ~5.5 H2:H_A* C2:C_A*
 0.500 C1:C_A* H1:H_A* ~6.0 H2:H_A* C2:C_A*

SPECTRUM HCcCH H1 H2 C2 C1
 1.000 H1:H_ALI C1:C_ALI C_ALI C2:C_ALI H2:H_ALI
```

The header line of an experiment definition starts with the word SPECTRUM and gives the name of the spectrum type and a list of labels that correspond to the nuclei that constitute the direct and indirect dimensions, one for each spectral dimension.

Subsequent rows specify the magnetization transfer pathways for the experiment. The first number denotes the peak observation probability. It is followed by a linear list of atom types that defines a molecular fragment, in which atoms must be of the given types, e.g. H_ALI for aliphatic hydrogens, H_A* for aliphatic or (for MethylFLYA irrelevant) aromatic hydrogens, C_ALI for aliphatic carbons, etc., as defined in the ATOMTYPES section at the beginning of the CYANA residue library. Atoms must be connected to the next atom in the list either by a covalent bond or, in the instances where a tilde followed by a number is given, by an NOE, i.e. a distance shorter than the given cutoff (in Å) in the 3D structure. An expected peak is generated whenever a

molecular fragment matches the covalent structure and, in case of NOEs, the 3D protein structure. The nuclei for which the frequency is measured in the experiment are identified by labels, followed by a colon. There must be as many labels as in the header (SPECTRUM) line, corresponding to the dimensionality of the spectrum.

See http://www.cyana.org/wiki/index.php/Residue_library_file for more details about the CYANA library.

Input peak list format

Input peak lists must contain a header line starting with #SPECTRUM that specifies the spectrum type and the labels, which must match the corresponding entry in the CYANA library, but may be permuted to indicate the order in which data for the spectral dimensions is given in peak list columns. For instance, a 4D NOESY peak list in XEASY format may start as follows:

```
# Number of dimensions 4
#SPECTRUM CCNOESY H1 C1 H2 C2
 872   0.230  19.382   0.655  13.602 1 U   1.000E+02  0.000E+00 e 0 -  -  -  -
 874  -0.834  17.697   0.655  13.602 1 U   1.000E+02  0.000E+00 e 0 -  -  -  -
 883   0.848  21.207   0.390  11.341 1 U   1.000E+02  0.000E+00 e 0 -  -  -  -
 887   0.924  22.805   0.390  11.341 1 U   1.000E+02  0.000E+00 e 0 -  -  -  -
 894   1.376  25.567   0.390  11.341 1 U   1.000E+02  0.000E+00 e 0 -  -  -  -
 901   0.407  21.750   0.746  16.119 1 U   1.000E+02  0.000E+00 e 0 -  -  -  -
```

Following the header, data for each peak is given on one line: peak number, peak position (ppm; 4 real numbers), "1 U", peak volume, volume error (if known), "e 0", and assignment ('–' if unassigned). Assignments, if present, are given in the form *A.r*, where *A* denotes an atom name and *r* a residue number.

Table of consensus chemical shifts

The consolidation of the chemical shift assignments from individual assignment runs into consensus chemical shifts is documented in the MethylFLYA output file *consol.tab*, which contains the final methyl assignment results. The beginning of an example *consol.tab* file is given below.

```
Atom  Residue      Ref    Shift     Dev  Extent  inside   inref
QD1   ILE    2            0.739            300.0   100.0     0.0  strong
CD1   ILE    2           16.246            300.0    99.9     0.0  strong
QD1   ILE    5            0.843            300.0    37.1     0.0
CD1   ILE    5           12.874            300.0    62.7     0.0
QD1   LEU    6   0.755    0.756  -0.001    300.0    99.5   100.0  strong=
QD2   LEU    6   0.891    0.891   0.000    300.0    96.4    97.0  strong=
CD1   LEU    6  23.060   23.065  -0.005    300.0    98.3    98.3  strong=
CD2   LEU    6  25.480   25.557  -0.077    300.0    96.8    97.0  strong=
```

The first three columns in the file list the atom type, residue type, and residue number for each assigned atom. When a reference assignment is known, the value of the known reference chemical shift is listed in the fourth column. The consensus chemical shift (i.e. the MethylFLYA result) is given in the fifth ("Shift") column. When applicable, its deviation from the reference assignment is given in the 'Dev' column in ppm. The 'Extent' column refers to the number of individual assignments runs, in which an assignment for the given atom was obtained. Given that a hundred

calculations are run at each of three distance cutoffs, the consolidation runs over 300 individual calculations. The next two columns ‘inside’ and ‘inref’ respectively specify the percentage of assignments for this atom in the (300) individual runs that agree (within the chemical shift tolerance specified in the init.cya file) with the consensus or reference assignment, respectively. The final column indicates whether an assignment is ‘strong’ (i.e. confident). For the calculations in this paper, assignments are classified as strong if and only if the percentage in the ‘inside’ column is 80% or more. When a reference assignment is known, a ‘=’ is appended to the last column if the MethylFLYA assignment match the reference assignment (within the chemical shift tolerance), or ‘!’ if the two assignments differ by more than the chemical shift tolerance.