molecules

MDPI

Supplementary Tables and Figures

Table S1. Parameters/Input for MD Simulations.

ID Uniprot	PDB	MSE or MET	pH	Temperature (K)	Ionic Strength (M)	Forcefield
P20700	3JTO	MET	7	298	0.100	OPLS
Q12906	3P1X	MET	6.5	298	0.100	OPLS
Q5FJ43	3Q69	MSE	7	293	0.200	OPLS
Q9Y547	1TVG	MET	6.5	298	0.100	OPLS
P65294	3FIF	MSE	5	293	0.100	OPLS
Q24NW5	3LYW	MSE	9	293	0.230	OPLS
Q01826	3NZL	MET	6.5	298	0.100	OPLS
P65294	3FIF	MET	6.5	293	0.100	OPLS
P74795	3C4S	MET	5.5	100	0.135	AMBER
P74795	3C4S	MET	5.5	300	0.135	AMBER
P74795	3C4S	MET	5.5	100	0.135	OPLS
P74795	3C4S	MET	5.5	300	0.135	OPLS
P74795	3C4S	MSE	6.5	100	0.135	OPLS
P74795	3C4S	MSE	6.5	300	0.135	OPLS
Q7VV99	3CPK	MET	6.5	100	0.135	AMBER
Q7VV99	3CPK	MET	6.5	300	0.135	AMBER
Q7VV99	3CPK	MET	6.5	100	0.135	OPLS
Q7VV99	3CPK	MET	6.5	300	0.135	OPLS
Q7VV99	3CPK	MSE	7	100	0.135	OPLS
Q7VV99	3CPK	MSE	7	300	0.135	OPLS
Q8KFZ1	3E0H	MET	6.5	100	0.235	AMBER
Q8KFZ1	3E0H	MET	6.5	300	0.235	AMBER
Q8KFZ1	3E0H	MET	6.5	100	0.235	OPLS
Q8KFZ1	3E0H	MET	6.5	300	0.235	OPLS
Q8KFZ1	3E0H	MSE	4.1	100	0.235	OPLS
Q8KFZ1	3E0H	MSE	4.1	300	0.235	OPLS
Q8ZRJ2	2ES9	MET	6.5	100	0.100	AMBER
Q8ZRJ2	2ES9	MET	6.5	300	0.100	AMBER
Q8ZRJ2	2ES9	MET	6.5	100	0.100	OPLS
Q8ZRJ2	2ES9	MET	6.5	300	0.100	OPLS
Q8ZRJ2	2ES9	MSE	6.5	100	0.100	OPLS
Q8ZRJ2	2ES9	MSE	6.5	300	0.100	OPLS

Table S1 collates Uniprot and PDB IDs of structures used to seed MD (Molecular Dynamics) simulations, whether selenomethionine residues have been replaced with methionine residues (MSE or MET), the forcefield used for MD simulations and the pH, temperature and ionic strength used in each simulation. In general, the pH used in each simulation matched the pH at which the seed structures were obtained. While all simulations were seeded with crystal structures, the ionic strength was that used in NMR-based structure determination. Some "room temperature" simulations were performed at 300 K rather than the exact temperature used in NMR (Nuclear Magnetic Resonance) experiments or crystallization. For some crystal structures calculated using data obtained from cryogenically cooled crystals, simulations were performed at two temperatures, "room temperature" and 100K. The pattern in coordinate variances reported in this paper persists independently of parameters including choice of forcefield, temperature and whether selenomethionines were replaced by methionines.

Table S2. Average Ranks of Backbone Atom B-Factors for Crystallographic Structures.

ID		*Average Ranks*				
Uniprot	**PDB**	**N**	**C'**	**Ca**	**O**	**Std. Error**
O31818	3BHP	2.11	2.70	2.74	2.44	0.172
P74795	3C4S	1.83	2.68	2.89	2.60	0.171
Q7VV99	3CPK	2.23	2.58	2.38	2.80	0.094
Q39VC5	3CWI	1.86	2.93	2.72	2.49	0.153
Q6LYF9	3E0E	2.21	2.49	2.51	2.79	0.127
Q8KFZ1	3E0H	2.34	2.60	2.72	2.34	0.100
E7UZA7	2ES7	2.40	2.52	2.39	2.69	0.104
Q8ZRJ2	2ES9	2.26	2.73	2.62	2.38	0.116
Q9Y3C8	3EVX	2.45	2.53	2.55	2.47	0.100
Q99U58	2FFM	2.50	2.47	2.54	2.49	0.138
Q15811	3FIA	2.23	2.07	2.50	3.20	0.121
P65294	3FIF	2.40	2.40	2.47	2.74	0.166
Q8KNE9	4FPW	2.49	2.48	2.52	2.51	0.090
Q9RZE3	3GGN	2.32	2.61	2.72	2.34	0.101
P71066	2GSV	2.21	2.59	2.62	2.58	0.152
Q7U294	3GW2	2.46	2.40	2.62	2.52	0.125
Q880Y4	3H9X	2.48	2.70	2.36	2.45	0.118
Q8KC80	3IBW	2.45	2.51	2.52	2.52	0.015
Q8U1U6	3IDU	2.42	2.54	2.44	2.60	0.026
P50833	2IM8	2.41	2.51	2.74	2.33	0.115
Q251Q8	3IPF	1.97	2.99	2.40	2.64	0.119
P20700	3JT0	2.39	2.53	2.58	2.50	0.101
B2D8H3	3K63	2.46	2.54	2.43	2.56	0.056
E3YVT8	3LD7	2.34	2.25	2.41	3.00	0.095
Q6N882	3LMO	2.23	2.68	2.67	2.43	0.132
Q24NW5	3LYW	2.21	2.55	2.53	2.70	0.138
Q2S6C5	3MA5	2.48	2.63	2.45	2.45	0.083
P15056	3NY5	2.39	2.57	2.57	2.47	0.049
Q01826	3NZL	1.97	2.12	2.78	3.13	0.136
Q9AAR9	2OOQ	2.13	2.76	2.46	2.65	0.120
Q97RM2	3OBH	2.37	2.71	2.32	2.60	0.136
Q55544	3OSJ	2.39	2.55	2.47	2.58	0.037
Q481E4	2OTA	2.20	2.64	2.62	2.54	0.144
Q12906	3P1X	2.42	2.55	2.47	2.56	0.018
P95883	2QOO	2.33	2.28	2.45	2.94	0.116
Q5FJ43	3Q69	2.18	2.77	2.65	2.41	0.125
Q8EF26	2QTI	2.53	2.14	2.12	3.22	0.142
P03495	2RHK	2.28	2.62	2.58	2.51	0.077
Q8P6W3	1TTZ	2.28	2.74	2.57	2.41	0.146
Q9Y547	1TVG	2.26	2.45	2.53	2.76	0.108

Table S2 reports average ranks of backbone heavy atom B factors. Computation of these averages proceeds by ranking backbone heavy atoms on a per-residue basis than averaging the ranks across all residues. MATLAB's [1] friedman [2] and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results.

Table S3. Average Ranks of Backbone Atom Coordinate Uncertainties for Theseus Superimposed NMR "Ensembles".

ID		*Average Ranks*				
Uniprot	**PDB**	**N**	**C'**	**Ca**	**O**	**Std. Error**
P50833	2HFI	1.96	2.05	2.83	3.16	0.116
P65294	2JN0	1.84	1.92	2.62	3.62	0.183
Q8ZRJ2	JN8	2.21	1.99	2.80	3.00	0.124
P95883	2JPU	1.94	2.08	2.74	3.24	0.114
P71066	2JS1	2.04	2.08	2.68	3.21	0.144
Q8EF26	2JUW	2.11	2.14	2.70	3.05	0.144
O31818	2JVD	1.79	2.21	2.69	3.31	0.186
P74795	2JZ2	1.94	2.17	2.55	3.35	0.159
E7UZA7	2JZT	2.09	2.01	2.68	3.22	0.108
Q9Y3C8	2K07	1.97	2.10	2.68	3.25	0.098
Q6LYF9	2K5V	1.95	1.92	2.56	3.57	0.130
Q8KFZ1	2KCU	2.18	2.13	2.73	2.96	0.100
Q2S6C5	2KCV	2.02	2.18	2.62	3.18	0.130
Q880Y4	2KFP	2.01	2.02	2.66	3.31	0.116
Q15811	2KHN	2.30	1.98	2.80	2.92	0.117
Q7U294	2KKO	2.19	2.27	2.66	2.89	0.124
P03495	2KKZ	1.87	2.13	2.64	3.37	0.112
Q8U1U6	2KL6	1.93	2.08	2.62	3.37	0.124
E3YVT8	2KPP	1.84	2.13	2.46	3.56	0.121
P20700	2KPW	2.03	2.02	2.60	3.35	0.117
P62195	2KRK	2.19	1.94	2.67	3.20	0.139
B2D8H3	2KRT	2.00	2.03	2.59	3.38	0.117
Q6N882	2KW2	1.87	2.23	2.60	3.30	0.129
P15056	2L05	2.09	2.05	2.59	3.27	0.139
Q55544	2L06	2.15	2.16	2.71	2.97	0.104
Q01826	2L1P	2.12	1.89	2.86	3.13	0.142
Q12906	2L33	2.18	2.02	2.54	3.26	0.135
Q97RM2	2L3A	2.22	2.00	2.51	3.27	0.143
Q5FJ43	2LFI	1.99	2.23	2.48	3.30	0.117
Q8KNE9	2LUZ	2.03	2.07	2.61	3.29	0.096
Q99U58	1PQX	1.90	2.26	2.38	3.45	0.135
Q8P6W3	1XPV	2.15	2.14	2.50	3.21	0.146
Q9Y547	1XPW	1.80	2.07	2.61	3.52	0.108
Q9AAR9	2JQN	1.98	1.99	2.60	3.42	0.120
Q481E4	2JR2	2.17	2.04	2.84	2.95	0.148
Q7VV99	2K2E	2.27	1.96	2.62	3.15	0.103
Q39VC5	2K5P	1.56	2.29	2.49	3.65	0.146
Q9RZE3	2KCZ	2.05	2.11	2.58	3.26	0.104
Q8KC80	2KO1	1.92	2.23	2.44	3.41	0.138
Q24NW5	2KPU	1.84	2.13	2.46	3.56	0.121
Q251Q8	2KYI	1.82	2.12	2.54	3.51	0.108

Table S3 reports average ranks of backbone heavy atom coordinate uncertainties calculated from Theseus superimposions. Computation of these averages proceeds by ranking backbone heavy atom coordinate uncertainties on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results.

Table S4. Average Ranks of Backbone Atom Coordinate Uncertainties for FindCore Superimposed NMR "Ensembles".

ID		*Average Ranks*				
Uniprot	**PDB**	**N**	**C'**	**Ca**	**O**	**Std. Error**
P50833	2HFI	1.93	2.05	2.84	3.19	0.116
P65294	2JN0	1.92	1.92	2.63	3.54	0.172
Q8ZRJ2	JN8	2.12	2.09	2.75	3.04	0.120
P95883	2JPU	1.90	2.07	2.74	3.29	0.114
P71066	2JS1	2.03	2.05	2.69	3.24	0.144
Q8EF26	2JUW	2.17	2.03	2.78	3.02	0.143
O31818	2JVD	1.82	2.17	2.76	3.25	0.180
P74795	2JZ2	2.06	2.13	2.61	3.20	0.154
E7UZA7	2JZT	2.13	2.06	2.70	3.12	0.103
Q9Y3C8	2K07	2.07	2.06	2.67	3.19	0.096
Q6LYF9	2K5V	2.31	2.29	2.53	2.88	0.048
Q8KFZ1	2KCU	2.23	2.12	2.73	2.91	0.098
Q2S6C5	2KCV	2.04	2.18	2.62	3.17	0.129
Q880Y4	2KFP	2.01	2.02	2.78	3.19	0.115
Q15811	2KHN	2.09	2.12	2.76	3.03	0.108
Q7U294	2KKO	2.20	2.26	2.68	2.86	0.124
P03495	2KKZ	2.17	2.25	2.61	2.97	0.066
Q8U1U6	2KL6	2.38	2.42	2.53	2.68	0.025
E3YVT8	2KPP	2.05	2.07	2.50	3.38	0.112
P20700	2KPW	2.00	2.03	2.57	3.39	0.115
P62195	2KRK	2.08	1.97	2.66	3.29	0.136
B2D8H3	2KRT	2.01	2.02	2.61	3.36	0.117
Q6N882	2KW2	1.87	2.21	2.62	3.30	0.127
P15056	2L05	2.36	2.33	2.54	2.77	0.052
Q55544	2L06	2.12	2.17	2.71	2.99	0.099
Q01826	2L1P	2.11	1.87	2.86	3.15	0.141
Q12906	2L33	2.08	2.05	2.56	3.31	0.129
Q97RM2	2L3A	2.18	2.01	2.52	3.29	0.142
Q5FJ43	2LFI	2.03	2.20	2.54	3.22	0.112
Q8KNE9	2LUZ	2.02	2.07	2.62	3.29	0.096
Q99U58	1PQX	1.89	2.26	2.43	3.42	0.134
Q8P6W3	1XPV	2.28	2.09	2.51	3.12	0.146
Q9Y547	1XPW	1.80	2.07	2.60	3.52	0.108
Q9AAR9	2JQN	2.01	1.97	2.62	3.40	0.120
Q481E4	2JR2	2.19	2.00	2.81	3.00	0.148
Q7VV99	2K2E	2.23	2.02	2.59	3.16	0.098
Q39VC5	2K5P	1.76	2.19	2.53	3.51	0.137
Q9RZE3	2KCZ	2.09	2.16	2.62	3.13	0.098
Q8KC80	2KO1	1.90	2.23	2.47	3.41	0.138
Q24NW5	2KPU	2.07	1.92	2.68	3.33	0.132
Q251Q8	2KYI	1.94	2.15	2.54	3.37	0.094

Table S4 reports average ranks of backbone heavy atom coordinate uncertainties calculated from FindCore superimposions. Computation of these averages proceeds by ranking backbone heavy atom coordinate uncertainties on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results.

Table S5. MD Simulation Results.

	MSE or		Simulation Length						
Uniprot ID	**MET**	**Forcefield**	**Time (ns)**	**# Frames**	**N**	**C'**	**Ca**	**O**	**Std. Error**
P20700	MET	OPLS	13.6	939	2.17	1.98	2.43	3.41	0.124
Q12906	MET	OPLS	15.8	1097	1.94	2.07	2.49	3.50	0.154
Q5FJ43	MSE	OPLS	16.7	1157	2.19	1.88	2.38	3.55	0.126
Q9Y547	MET	OPLS	18.4	1271	2.10	1.86	2.40	3.65	0.111
P65294	MSE	OPLS	25.8	1787	1.75	2.00	2.58	3.67	0.171
Q24NW5	MSE	OPLS	29.3	2031	1.95	2.09	2.43	3.52	0.139
Q01826	MET	OPLS	30.9	2144	2.04	1.92	2.53	3.51	0.151
P65294	MET	OPLS	36.0	2500	1.88	2.00	2.62	3.50	0.183
P74795	MET	AMBER	36.0	2500	2.53	2.23	2.75	2.49	0.171
P74795	MET	AMBER	36.0	2500	2.46	2.28	2.68	2.58	0.171
P74795	MET	OPLS	36.0	2500	2.18	1.72	2.14	3.96	0.171
P74795	MET	OPLS	36.0	2500	2.23	2.05	2.54	3.18	0.171
P74795	MSE	OPLS	36.0	2500	2.09	2.04	1.93	3.95	0.171
P74795	MSE	OPLS	36.0	2500	2.35	2.12	2.49	3.04	0.171
Q7VV99	MET	AMBER	36.0	2500	2.23	2.11	2.43	3.23	0.095
Q7VV99	MET	AMBER	36.0	2500	2.25	2.06	2.49	3.20	0.095
Q7VV99	MET	OPLS	36.0	2500	2.06	2.05	2.28	3.61	0.095
Q7VV99	MET	OPLS	36.0	2500	2.11	2.13	2.43	3.34	0.095
Q7VV99	MSE	OPLS	36.0	2500	2.11	2.05	2.26	3.58	0.095
Q7VV99	MSE	OPLS	36.0	2500	2.12	2.10	2.41	3.37	0.095
Q8KFZ1	MET	AMBER	36.0	2500	2.10	2.02	2.45	3.43	0.101
Q8KFZ1	MET	AMBER	36.0	2500	2.21	1.96	2.55	3.28	0.101
Q8KFZ1	MET	OPLS	36.0	2500	2.11	1.89	2.24	3.76	0.101
Q8KFZ1	MET	OPLS	36.0	2500	2.07	2.03	2.41	3.48	0.101
Q8KFZ1	MSE	OPLS	36.0	2500	2.17	1.93	2.12	3.78	0.101
Q8KFZ1	MSE	OPLS	36.0	2500	2.03	2.00	2.41	3.57	0.101
Q8ZRJ2	MET	AMBER	36.0	2500	2.06	2.05	2.55	3.34	0.117
Q8ZRJ2	MET	AMBER	36.0	2500	2.12	2.02	2.68	3.18	0.117
Q8ZRJ2	MET	OPLS	36.0	2500	1.93	1.95	2.40	3.72	0.117
Q8ZRJ2	MET	OPLS	36.0	2500	1.84	2.10	2.52	3.55	0.117
Q8ZRJ2	MSE	OPLS	36.0	2500	1.85	2.09	2.21	3.85	0.117
Q8ZRJ2	MSE	OPLS	36.0	2500	1.84	2.10	2.52	3.55	0.117

Table S5 reports average ranks of backbone heavy atom coordinate variance calculated from Theseus superimposions of MD trajectories with the indicated number of snapshops. Computation of these averages proceeds by ranking backbone heavy atom coordinate uncertainties on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results. Simulations are tabulated here in the same order as in Table S1.

Table S6. Average Ranks of N, C', Cα and Cβ B-Factors for Crystallographic Structures.

ID		*Average Ranks*				
Uniprot	**PDB**	**N**	**C'**	**Cα**	**Cβ**	**Std. Error**
O31818	3BHP	2.00	2.56	2.57	2.87	0.172
P74795	3C4S	2.48	2.60	2.55	2.38	0.138
Q7VV99	3CPK	2.19	2.47	2.26	3.07	0.122
Q39VC5	3CWI	2.41	2.55	2.56	2.48	0.166
Q6LYF9	3E0E	2.44	2.45	2.45	2.67	0.090
Q8KFZ1	3E0H	2.24	2.57	2.47	2.72	0.101
E7UZA7	2ES7	2.21	2.62	2.53	2.64	0.152
Q8ZRJ2	2ES9	2.46	2.62	2.41	2.51	0.125
Q9Y3C8	3EVX	2.53	2.37	2.69	2.41	0.118
Q99U58	2FFM	2.45	2.50	2.50	2.55	0.015
Q15811	3FIA	2.42	2.44	2.53	2.61	0.026
P65294	3FIF	1.68	2.63	2.55	3.14	0.171
Q8KNE9	4FPW	2.22	2.51	2.33	2.95	0.115
Q9RZE3	3GGN	1.99	2.37	2.97	2.67	0.119
P71066	2GSV	2.37	2.49	2.53	2.61	0.101
Q7U294	3GW2	2.52	2.50	2.60	2.38	0.056
Q880Y4	3H9X	2.33	2.35	2.37	2.96	0.095
Q8KC80	3IBW	2.13	2.54	2.62	2.71	0.132
Q8U1U6	3IDU	2.08	2.45	2.43	3.03	0.138
P50833	2IM8	2.59	2.61	2.70	2.09	0.083
Q251Q8	3IPF	2.32	2.49	2.49	2.70	0.049
P20700	3JT0	1.84	2.47	2.08	3.60	0.137
B2D8H3	3K63	2.40	2.57	2.74	2.29	0.094
E3YVT8	3LD7	2.17	2.45	2.77	2.61	0.120
Q6N882	3LMO	2.37	2.31	2.71	2.62	0.136
Q24NW5	3LYW	2.41	2.51	2.58	2.50	0.037
Q2S6C5	3MA5	2.12	2.53	2.59	2.77	0.144
P15056	3NY5	2.45	2.48	2.57	2.49	0.018
Q01826	3NZL	2.44	2.60	2.49	2.47	0.116
Q9AAR9	2OOQ	2.05	2.44	2.58	2.93	0.125
Q97RM2	3OBH	2.61	2.30	2.35	2.74	0.142
Q55544	3OSJ	2.22	2.52	2.60	2.66	0.077
Q481E4	2OTA	2.29	2.61	2.78	2.33	0.146
Q12906	3P1X	1.74	2.58	2.67	3.01	0.153
P95883	2QOO	2.21	2.37	2.39	3.03	0.108
Q5FJ43	3Q69	2.15	2.39	2.49	2.97	0.127
Q8EF26	2QTI	2.19	2.45	2.42	2.93	0.100
P03495	2RHK	2.40	2.45	2.66	2.50	0.104
Q8P6W3	1TTZ	2.15	2.52	2.60	2.74	0.116
Q9Y547	1TVG	2.43	2.56	2.57	2.44	0.100

Table S6 reports average ranks of N, C', Cα and Cβ B factors. Computation of these averages proceeds by ranking backbone heavy atoms on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results.

Table S7. Average Ranks of N, C', Cα and Cβ Coordinate Uncertainties for Theseus Superimposed NMR "Ensembles".

ID		*Average Ranks*				
Uniprot	**PDB**	**N**	**C'**	**Cα**	**Cβ**	**Std. Error**
P50833	2HFI	1.76	2.50	2.07	3.67	0.116
P65294	2JN0	1.83	2.46	2.16	3.55	0.098
Q8ZRJ2	JN8	1.98	2.47	2.14	3.41	0.130
P95883	2JPU	2.07	2.47	2.16	3.31	0.100
P71066	2JS1	1.96	2.44	2.34	3.25	0.130
Q8EF26	2JUW	1.86	2.50	2.11	3.53	0.116
O31818	2JVD	2.13	2.49	1.97	3.41	0.117
P74795	2JZ2	2.03	2.39	2.30	3.29	0.124
E7UZA7	2JZT	1.87	2.46	2.29	3.39	0.112
Q9Y3C8	2K07	1.85	2.44	2.12	3.58	0.124
Q6LYF9	2K5V	1.85	2.41	2.37	3.37	0.121
Q8KFZ1	2KCU	1.80	2.50	2.02	3.68	0.183
Q2S6C5	2KCV	1.94	2.43	2.09	3.54	0.117
Q880Y4	2KFP	2.05	2.48	2.06	3.42	0.139
Q15811	2KHN	1.85	2.36	2.08	3.71	0.117
Q7U294	2KKO	1.80	2.48	2.44	3.29	0.129
P03495	2KKZ	2.00	2.33	2.06	3.62	0.139
Q8U1U6	2KL6	2.05	2.50	2.14	3.32	0.104
E3YVT8	2KPP	1.87	2.48	1.87	3.78	0.142
P20700	2KPW	2.11	2.34	2.15	3.40	0.135
P62195	2KRK	2.15	2.28	2.13	3.44	0.143
B2D8H3	2KRT	1.90	2.39	2.36	3.35	0.117
Q6N882	2KW2	1.99	2.52	2.09	3.39	0.124
P15056	2L05	1.97	2.47	2.16	3.41	0.096
Q55544	2L06	1.89	2.30	2.40	3.42	0.135
Q01826	2L1P	2.03	2.42	2.32	3.23	0.146
Q12906	2L33	1.76	2.44	2.17	3.64	0.108
Q97RM2	2L3A	1.95	2.47	2.11	3.47	0.120
Q5FJ43	2LFI	1.95	2.49	2.00	3.57	0.148
Q8KNE9	2LUZ	2.13	2.41	2.03	3.44	0.103
Q99U58	1PQX	1.56	2.47	2.47	3.49	0.146
Q8P6W3	1XPV	1.99	2.46	2.32	3.23	0.104
Q9Y547	1XPW	1.93	2.40	2.41	3.26	0.138
Q9AAR9	2JQN	1.76	2.49	2.15	3.60	0.114
Q481E4	2JR2	1.85	2.41	2.37	3.37	0.121
Q7VV99	2K2E	1.80	2.42	2.24	3.54	0.108
Q39VC5	2K5P	1.93	2.49	2.24	3.35	0.144
Q9RZE3	2KCZ	1.95	2.36	2.09	3.60	0.144
Q8KC80	2KO1	1.63	2.54	2.40	3.44	0.186
Q24NW5	2KPU	1.85	2.36	2.24	3.55	0.159
Q251Q8	2KYI	1.92	2.44	2.10	3.54	0.108

Table S7 reports average ranks of N, C', Cα and Cβ coordinate uncertainties calculated from Theseus superimposions. Computation of these averages proceeds by ranking backbone heavy atom coordinate uncertainties on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results.

Table S8. Average Ranks of N, C', Cα and Cβ Coordinate Uncertainties for FindCore Superimposed NMR "Ensembles".

ID		*Average Ranks*				
Uniprot	**PDB**	**N**	**C'**	**Cα**	**Cβ**	**Std. Error**
P50833	2HFI	1.72	2.51	2.06	3.71	0.116
P65294	2JN0	1.95	2.38	2.17	3.50	0.154
Q8ZRJ2	JN8	1.97	2.46	2.08	3.49	0.103
P95883	2JPU	1.93	2.45	2.07	3.54	0.096
P71066	2JS1	1.75	2.51	2.31	3.43	0.138
Q8EF26	2JUW	2.31	2.49	2.37	2.84	0.048
O31818	2JVD	2.11	2.48	2.13	3.29	0.098
P74795	2JZ2	1.96	2.46	2.31	3.27	0.129
E7UZA7	2JZT	2.04	2.47	2.30	3.19	0.098
Q9Y3C8	2K07	1.84	2.52	2.07	3.57	0.115
Q6LYF9	2K5V	1.95	2.53	2.22	3.30	0.108
Q8KFZ1	2KCU	1.90	2.52	1.95	3.63	0.172
Q2S6C5	2KCV	2.03	2.41	2.27	3.29	0.124
Q880Y4	2KFP	2.16	2.50	2.30	3.04	0.067
Q15811	2KHN	2.37	2.49	2.42	2.72	0.025
Q7U294	2KKO	1.90	2.41	2.39	3.31	0.138
P03495	2KKZ	2.06	2.44	2.19	3.31	0.112
Q8U1U6	2KL6	1.94	2.42	1.96	3.69	0.132
E3YVT8	2KPP	1.93	2.43	2.13	3.51	0.115
P20700	2KPW	1.98	2.46	2.12	3.44	0.136
P62195	2KRK	1.84	2.36	2.09	3.71	0.117
B2D8H3	2KRT	1.78	2.51	2.39	3.32	0.127
Q6N882	2KW2	1.91	2.52	2.17	3.40	0.120
P15056	2L05	1.92	2.44	2.23	3.41	0.094
Q55544	2L06	2.31	2.44	2.33	2.92	0.052
Q01826	2L1P	2.01	2.53	2.15	3.30	0.099
Q12906	2L33	1.84	2.51	1.89	3.77	0.141
Q97RM2	2L3A	2.05	2.42	2.18	3.35	0.130
Q5FJ43	2LFI	2.10	2.31	2.13	3.46	0.142
Q8KNE9	2LUZ	1.98	2.44	2.29	3.29	0.112
Q99U58	1PQX	1.96	2.48	2.15	3.41	0.096
Q8P6W3	1XPV	1.89	2.34	2.37	3.40	0.134
Q9Y547	1XPW	2.15	2.40	2.22	3.23	0.146
Q9AAR9	2JQN	1.72	2.50	2.16	3.62	0.114
Q481E4	2JR2	2.13	2.41	2.07	3.38	0.098
Q7VV99	2K2E	1.78	2.43	2.17	3.62	0.108
Q39VC5	2K5P	1.96	2.47	2.11	3.46	0.120
Q9RZE3	2KCZ	1.97	2.49	1.97	3.57	0.147
Q8KC80	2KO1	1.94	2.49	2.19	3.39	0.144
Q24NW5	2KPU	2.01	2.46	1.99	3.54	0.143
Q251Q8	2KYI	1.70	2.61	2.27	3.42	0.180

Table S8 reports average ranks of N, C', Cα and Cβ coordinate uncertainties calculated from FindCore superimposions. Computation of these averages proceeds by ranking backbone heavy atom coordinate uncertainties on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results.

Table S9. Average Ranks of N, C', Cα and Cβ Coordinate Variances in Theseus Superimposed MD Trajectories.

	MSE or		Simulation Length						
Uniprot ID	**MET**	**Forcefield**	**Time (ns)**	**# Frames**	**N**	**C'**	**Cα**	**Cβ**	**Std. Error**
P20700	MET	OPLS	13.6	939	2.39	2.53	2.21	2.88	0.171
Q12906	MET	OPLS	15.8	1097	2.16	2.30	2.16	3.38	0.095
Q5FJ43	MSE	OPLS	16.7	1157	2.09	2.36	2.27	3.28	0.095
Q9Y547	MET	OPLS	18.4	1271	2.11	2.34	2.22	3.34	0.095
P65294	MSE	OPLS	25.8	1787	2.09	2.35	2.19	3.38	0.101
Q24NW5	MSE	OPLS	29.3	2031	2.19	2.40	2.12	3.30	0.101
Q01826	MET	OPLS	30.9	2144	2.17	2.28	2.05	3.50	0.101
P65294	MET	OPLS	36.0	2500	2.23	2.17	2.06	3.53	0.101
P74795	MET	AMBER	36.0	2500	2.03	2.34	2.17	3.45	0.101
P74795	MET	AMBER	36.0	2500	2.07	2.35	2.20	3.38	0.101
P74795	MET	OPLS	36.0	2500	2.01	2.46	2.15	3.38	0.117
P74795	MET	OPLS	36.0	2500	2.42	2.51	2.23	2.84	0.171
P74795	MSE	OPLS	36.0	2500	2.05	2.50	2.16	3.29	0.117
P74795	MSE	OPLS	36.0	2500	1.97	2.43	2.05	3.55	0.117
Q7VV99	MET	AMBER	36.0	2500	1.94	2.28	2.26	3.52	0.117
Q7VV99	MET	AMBER	36.0	2500	1.83	2.50	2.24	3.44	0.117
Q7VV99	MET	OPLS	36.0	2500	1.83	2.50	2.24	3.44	0.117
Q7VV99	MET	OPLS	36.0	2500	2.26	2.21	1.88	3.65	0.171
Q7VV99	MSE	OPLS	36.0	2500	2.16	2.00	2.23	3.61	0.171
Q7VV99	MSE	OPLS	36.0	2500	2.16	2.42	2.21	3.21	0.171
Q8KFZ1	MET	AMBER	36.0	2500	2.18	2.33	2.28	3.21	0.171
Q8KFZ1	MET	AMBER	36.0	2500	2.20	2.32	2.19	3.29	0.095
Q8KFZ1	MET	OPLS	36.0	2500	2.19	2.36	2.18	3.26	0.095
Q8KFZ1	MET	OPLS	36.0	2500	2.13	2.30	2.17	3.40	0.095
Q8KFZ1	MSE	OPLS	36.0	2500	2.39	2.53	2.21	2.88	0.171
Q8KFZ1	MSE	OPLS	36.0	2500	2.16	2.30	2.16	3.38	0.095
Q8ZRJ2	MET	AMBER	36.0	2500	2.09	2.36	2.27	3.28	0.095
Q8ZRJ2	MET	AMBER	36.0	2500	2.11	2.34	2.22	3.34	0.095
Q8ZRJ2	MET	OPLS	36.0	2500	2.09	2.35	2.19	3.38	0.101
Q8ZRJ2	MET	OPLS	36.0	2500	2.19	2.40	2.12	3.30	0.101
Q8ZRJ2	MSE	OPLS	36.0	2500	2.17	2.28	2.05	3.50	0.101
Q8ZRJ2	MSE	OPLS	36.0	2500	2.23	2.17	2.06	3.53	0.101

Table S9 reports average ranks of N, C', Cα and Cβ coordinate variances calculated from Theseus superimposions of MD trajectories with the indicated number of snapshops. Computation of these averages proceeds by ranking backbone heavy atom coordinate uncertainties on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results. Simulations are tabulated here in the same order as in Table S1.

Table S10. Average Ranks of N, C', Cα and H Coordinate Uncertainties for Theseus Superimposed NMR "Ensembles".

ID		*Average Ranks*				
Uniprot	**PDB**	**N**	**C'**	**Cα**	**H**	**Std. Error**
P50833	2HFI	2.02	3.08	2.43	2.47	0.116
P65294	2JN0	2.07	2.93	2.44	2.55	0.098
Q8ZRJ2	JN8	1.90	2.81	2.20	3.09	0.130
P95883	2JPU	2.17	2.86	2.39	2.57	0.100
P71066	2JS1	2.15	2.95	2.59	2.31	0.130
Q8EF26	2JUW	2.08	3.02	2.46	2.44	0.116
O31818	2JVD	2.20	2.87	2.18	2.75	0.117
P74795	2JZ2	2.16	2.78	2.50	2.56	0.124
E7UZA7	2JZT	1.96	2.93	2.51	2.61	0.112
Q9Y3C8	2K07	1.93	2.72	2.31	3.04	0.124
Q6LYF9	2K5V	1.93	2.76	2.49	2.82	0.121
Q8KFZ1	2KCU	1.86	2.94	2.38	2.82	0.183
Q2S6C5	2KCV	1.89	2.71	2.20	3.20	0.117
Q880Y4	2KFP	2.14	2.86	2.29	2.71	0.139
Q15811	2KHN	2.02	2.77	2.36	2.84	0.117
Q7U294	2KKO	2.08	2.92	2.71	2.29	0.129
P03495	2KKZ	2.14	2.78	2.38	2.70	0.139
Q8U1U6	2KL6	2.17	2.86	2.42	2.54	0.104
E3YVT8	2KPP	2.10	3.01	2.22	2.67	0.142
P20700	2KPW	2.01	2.67	2.29	3.03	0.135
P62195	2KRK	2.15	2.72	2.34	2.79	0.143
B2D8H3	2KRT	2.12	2.79	2.62	2.47	0.117
Q6N882	2KW2	2.17	2.95	2.29	2.60	0.124
P15056	2L05	2.05	2.77	2.32	2.85	0.096
Q55544	2L06	2.02	2.66	2.63	2.69	0.135
Q01826	2L1P	2.13	2.67	2.49	2.72	0.146
Q12906	2L33	1.85	2.85	2.41	2.90	0.108
Q97RM2	2L3A	1.98	2.84	2.36	2.82	0.120
Q5FJ43	2LFI	2.12	2.99	2.32	2.58	0.148
Q8KNE9	2LUZ	2.21	2.79	2.30	2.70	0.103
Q99U58	1PQX	1.79	2.97	2.74	2.49	0.146
Q8P6W3	1XPV	2.19	2.92	2.55	2.34	0.104
Q9Y547	1XPW	1.92	2.61	2.57	2.90	0.138
Q9AAR9	2JQN	2.10	3.06	2.47	2.36	0.114
Q481E4	2JR2	1.93	2.76	2.49	2.82	0.121
Q7VV99	2K2E	1.88	2.82	2.46	2.83	0.108
Q39VC5	2K5P	2.14	2.99	2.46	2.41	0.144
Q9RZE3	2KCZ	2.14	2.91	2.48	2.48	0.144
Q8KC80	2KO1	1.73	3.04	2.69	2.54	0.186
Q24NW5	2KPU	1.89	2.70	2.58	2.83	0.159
Q251Q8	2KYI	2.13	2.89	2.42	2.56	0.108

Table S10 reports average ranks of N, C', Cα and H coordinate uncertainties calculated from Theseus superimposions. Computation of these averages proceeds by ranking backbone heavy atom coordinate uncertainties on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results.

Table S11. Average Ranks of N, C', Cα and H Coordinate Uncertainties for FindCore Superimposed NMR "Ensembles".

ID		*Average Ranks*				
Uniprot	**PDB**	**N**	**C'**	**Cα**	**H**	**Std. Error**
P50833	2HFI	1.96	3.12	2.41	2.50	0.116
P65294	2JN0	1.94	2.67	2.42	2.97	0.154
Q8ZRJ2	JN8	2.14	2.87	2.39	2.60	0.103
P95883	2JPU	2.11	2.88	2.37	2.64	0.096
P71066	2JS1	1.91	2.92	2.53	2.64	0.138
Q8EF26	2JUW	2.28	2.61	2.40	2.70	0.048
O31818	2JVD	2.18	2.83	2.36	2.63	0.098
P74795	2JZ2	2.15	2.93	2.57	2.35	0.129
E7UZA7	2JZT	2.20	2.85	2.53	2.42	0.098
Q9Y3C8	2K07	2.08	3.00	2.40	2.52	0.115
Q6LYF9	2K5V	2.11	2.94	2.44	2.51	0.109
Q8KFZ1	2KCU	1.92	2.93	2.25	2.90	0.172
Q2S6C5	2KCV	2.17	2.77	2.47	2.59	0.124
Q880Y4	2KFP	2.18	2.74	2.44	2.63	0.067
Q15811	2KHN	2.39	2.55	2.46	2.60	0.025
Q7U294	2KKO	1.91	2.64	2.56	2.90	0.138
P03495	2KKZ	2.00	2.66	2.32	3.02	0.113
Q8U1U6	2KL6	2.04	2.73	2.15	3.08	0.132
E3YVT8	2KPP	1.89	2.73	2.25	3.14	0.115
P20700	2KPW	2.10	2.87	2.34	2.69	0.136
P62195	2KRK	2.02	2.76	2.35	2.87	0.117
B2D8H3	2KRT	2.03	2.95	2.69	2.34	0.127
Q6N882	2KW2	2.14	2.96	2.40	2.51	0.120
P15056	2L05	1.98	2.77	2.43	2.83	0.094
Q55544	2L06	2.36	2.60	2.45	2.59	0.052
Q01826	2L1P	2.16	2.91	2.40	2.53	0.099
Q12906	2L33	2.11	3.04	2.20	2.64	0.141
Q97RM2	2L3A	1.97	2.71	2.31	3.01	0.130
Q5FJ43	2LFI	2.10	2.73	2.33	2.84	0.142
Q8KNE9	2LUZ	2.14	2.83	2.53	2.50	0.112
Q99U58	1PQX	2.06	2.77	2.32	2.84	0.096
Q8P6W3	1XPV	1.98	2.68	2.60	2.74	0.134
Q9Y547	1XPW	2.22	2.60	2.35	2.83	0.146
Q9AAR9	2JQN	2.11	3.09	2.50	2.29	0.114
Q481E4	2JR2	2.20	2.76	2.31	2.72	0.099
Q7VV99	2K2E	1.85	2.85	2.43	2.87	0.108
Q39VC5	2K5P	1.99	2.85	2.34	2.81	0.120
Q9RZE3	2KCZ	2.16	2.96	2.30	2.58	0.148
Q8KC80	2KO1	2.14	3.00	2.43	2.44	0.144
Q24NW5	2KPU	2.17	2.96	2.35	2.52	0.143
Q251Q8	2KYI	1.70	3.03	2.51	2.76	0.180

Table S11 reports average ranks of N, C', Cα and H coordinate uncertainties calculated from FindCore superimposions. Computation of these averages proceeds by ranking backbone heavy atom coordinate uncertainties on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results.

Table S12. Average Ranks of N, C', Cα and H Coordinate Variances in Theseus Superimposed MD Trajectories.

	MSE or		Simulation Length						
Uniprot ID	**MET**	**Forcefield**	**Time (ns)**	**# Frames**	**N**	**C'**	**Cα**	**H**	**Std. Error**
P20700	MET	OPLS	13.6	939	2.39	2.61	2.32	2.68	0.171
Q12906	MET	OPLS	15.8	1097	2.12	2.32	2.17	3.39	0.095
Q5FJ43	MSE	OPLS	16.7	1157	2.10	2.57	2.35	2.98	0.095
Q9Y547	MET	OPLS	18.4	1271	2.06	2.50	2.30	3.13	0.095
P65294	MSE	OPLS	25.8	1787	2.03	2.64	2.31	3.02	0.101
Q24NW5	MSE	OPLS	29.3	2031	2.17	2.71	2.27	2.84	0.101
Q01826	MET	OPLS	30.9	2144	2.09	2.28	2.03	3.60	0.101
P65294	MET	OPLS	36.0	2500	2.16	2.16	2.06	3.63	0.101
P74795	MET	AMBER	36.0	2500	2.03	2.56	2.31	3.10	0.101
P74795	MET	AMBER	36.0	2500	2.00	2.59	2.34	3.07	0.101
P74795	MET	OPLS	36.0	2500	2.11	2.80	2.35	2.74	0.117
P74795	MET	OPLS	36.0	2500	2.42	2.68	2.39	2.51	0.171
P74795	MSE	OPLS	36.0	2500	2.19	2.86	2.35	2.60	0.117
P74795	MSE	OPLS	36.0	2500	1.92	2.45	2.12	3.51	0.117
Q7VV99	MET	AMBER	36.0	2500	1.90	2.32	2.25	3.54	0.117
Q7VV99	MET	AMBER	36.0	2500	1.79	2.75	2.41	3.05	0.117
Q7VV99	MET	OPLS	36.0	2500	1.79	2.75	2.41	3.05	0.117
Q7VV99	MET	OPLS	36.0	2500	2.23	2.23	1.82	3.72	0.171
Q7VV99	MSE	OPLS	36.0	2500	2.12	2.02	2.16	3.70	0.171
Q7VV99	MSE	OPLS	36.0	2500	2.19	2.63	2.37	2.81	0.171
Q8KFZ1	MET	AMBER	36.0	2500	2.25	2.67	2.37	2.72	0.171
Q8KFZ1	MET	AMBER	36.0	2500	2.13	2.50	2.34	3.03	0.095
Q8KFZ1	MET	OPLS	36.0	2500	2.15	2.60	2.35	2.90	0.095
Q8KFZ1	MET	OPLS	36.0	2500	2.09	2.36	2.17	3.38	0.095
Q8KFZ1	MSE	OPLS	36.0	2500	2.39	2.61	2.32	2.68	0.171
Q8KFZ1	MSE	OPLS	36.0	2500	2.12	2.32	2.17	3.39	0.095
Q8ZRJ2	MET	AMBER	36.0	2500	2.10	2.57	2.35	2.98	0.095
Q8ZRJ2	MET	AMBER	36.0	2500	2.06	2.50	2.30	3.13	0.095
Q8ZRJ2	MET	OPLS	36.0	2500	2.03	2.64	2.31	3.02	0.101
Q8ZRJ2	MET	OPLS	36.0	2500	2.17	2.71	2.27	2.84	0.101
Q8ZRJ2	MSE	OPLS	36.0	2500	2.09	2.28	2.03	3.60	0.101
Q8ZRJ2	MSE	OPLS	36.0	2500	2.16	2.16	2.06	3.63	0.101

Table S12 reports average ranks of N, C', Cα and H coordinate variances calculated from Theseus superimposions of MD trajectories with the indicated number of snapshops. Computation of these averages proceeds by ranking backbone heavy atom coordinate uncertainties on a per-residue basis than averaging the ranks across all residues. MATLAB's friedman and multcompare functions were used to calculate average ranks as well as standard errors and to assist in tabulating the results. Simulations are tabulated here in the same order as in Table S1.

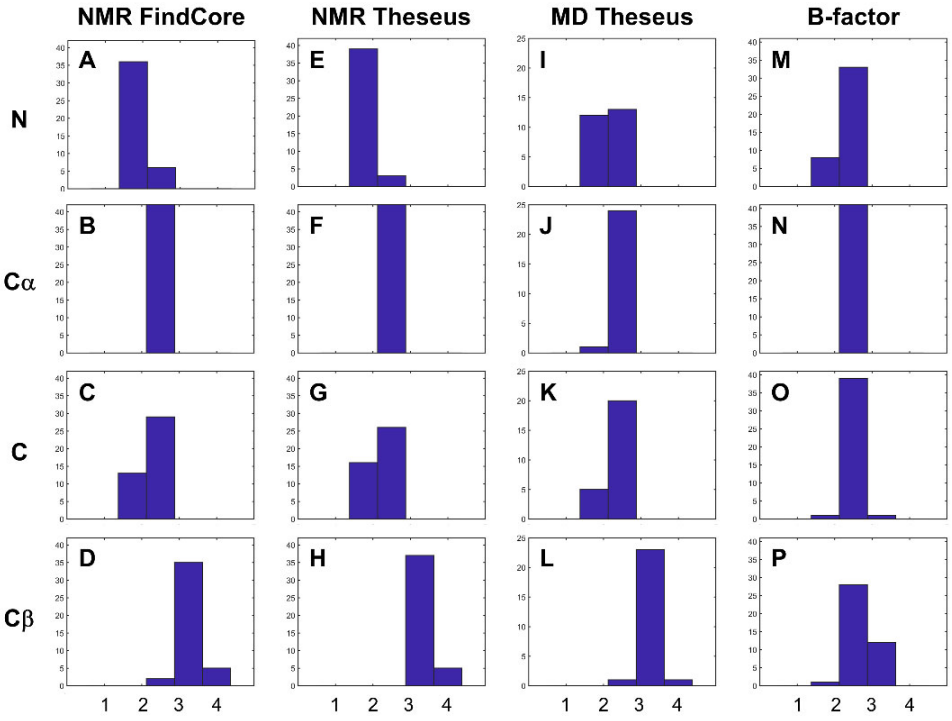

Figure S1. Distribution of average ranks of coordinate uncertainties, variances and B-factors of N, Cα, carbonyl C and Cβ atoms. As described in the main text, atoms in each residue are ranked by (**A**–**D**) coordinate uncertainty of FindCore superimposed NMR ensembles, (**E**–**H**) THESEUS superimposed NMR structures, coordinate variances of (**I**–**L**) THESEUS superimposed MD trajectories and (**M**–**P**) B-factors. For each structure, an average rank is calculated for each backbone heavy atom type: (first row) amide N, (second row) Cα, (third row) carbonyl C and (fourth row) Cβ. For superimposed NMR ensembles (columns one and two) and MD trajectories (column three) a clear pattern is visible: average ranks for amide nitrogen atoms and carbonyl carbon atoms are often lower than average ranks for Cα atoms; average ranks for Cβ atoms are usually higher. When backbone heavy atoms are ranked by B-factor, the average ranks for all backbone heavy atoms typically are between 2-3, although Cβ atoms tend to have higher ranks than N, Cα or carbonyl C atoms.

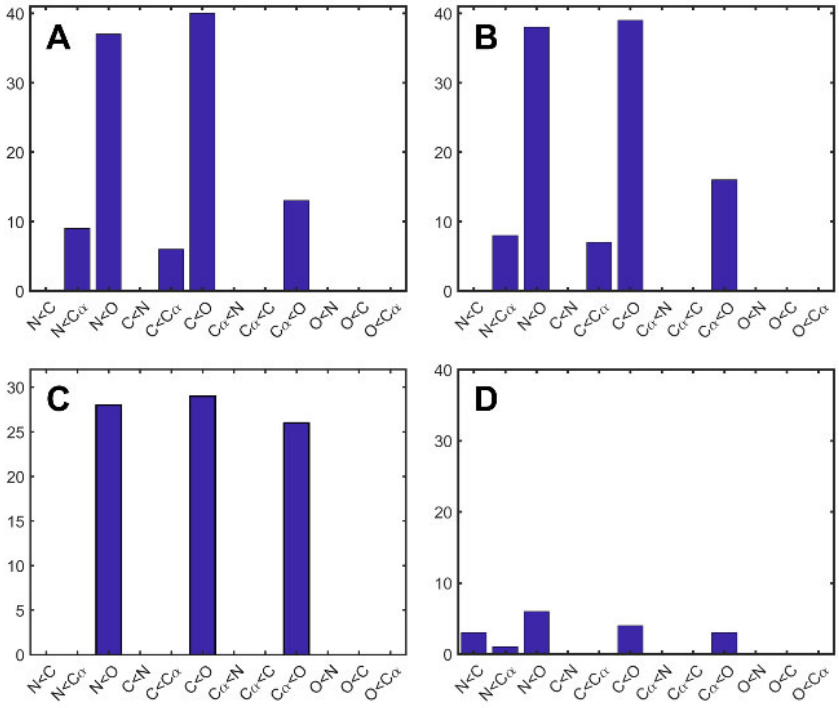

Figure S2. Results of Friedman's Test and subsequent multiple comparisons analysis with Cβ atoms. A bar, associated with a comparison X < Y, that is n units high, indicates that in n structures, the assessed measure of coordinate variability is significantly lower for atom type X than for atom type Y. E.g. in panel A, the bar associated with Cα < Cβ being 39 units high indicates that in 39 NMR ensembles, the coordinate uncertainties (calculated using FindCore superimpositions) for Cα atoms are significantly less (according to Friedman's test) than those for Cβ atoms. Mean ranks are considered significantly different if they differ by more than three standard deviations. Assessed measures of coordinate variability are (**A**) coordinate uncertainties in FindCore superimposed NMR ensembles, (**B**) coordinate uncertainties in THESEUS superimposed NMR ensembles, (**C**) Coordinate uncertainties in THESEUS superimposed MD trajectories and (**D**) crystallographic B-factors. Note that in almost all superimposed NMR ensembles (independent of superimposition method), as well as in almost all THESEUS superimposed MD trajectories, amide nitrogens and carbonyl carbons have significantly lower coordinate uncertainties than Cβ atoms. Only a small number of crystallographic structures have any significant results using the Friedman's test to compare B-factors of different atom types, although these numbers are slightly higher than in the comparisons between backbone heavy atoms shown in Fig. 2.

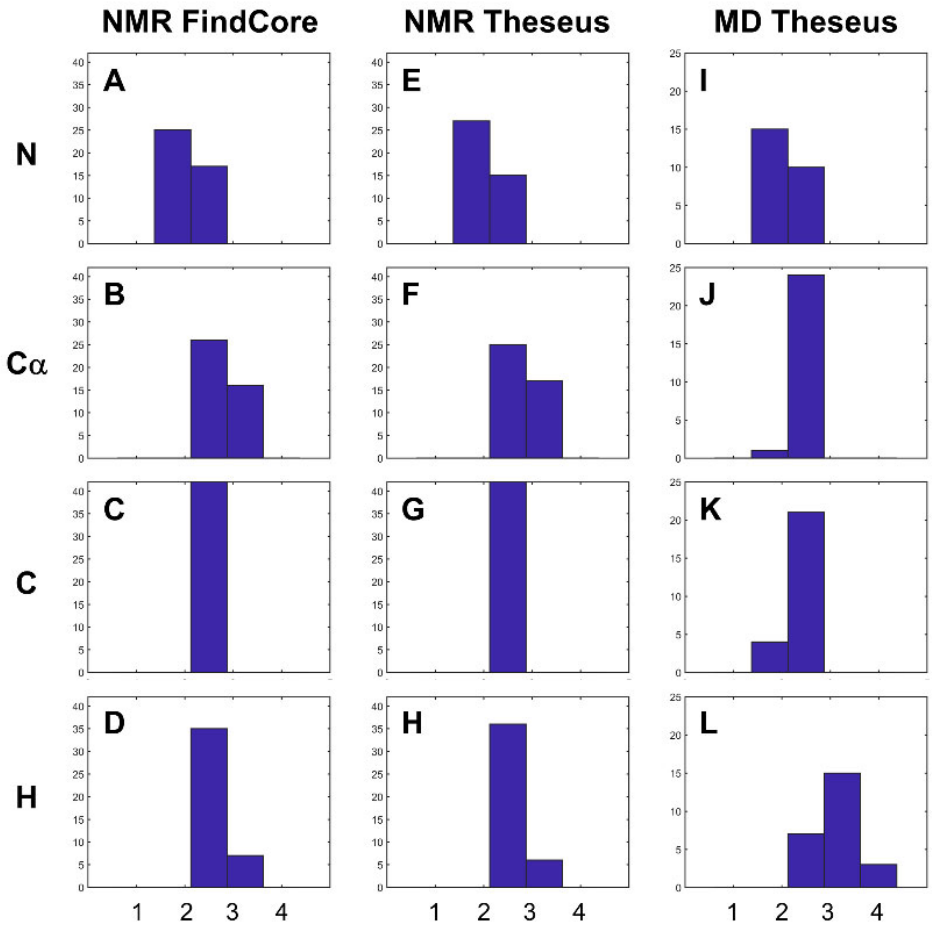

Figure S3. Distribution of average ranks of coordinate uncertainties, variances and B-factors of N, Cα, carbonyl C and amide H atoms. As described in the main text, atoms in each residue are ranked by (**A**–**D**) coordinate uncertainty of FindCore superimposed NMR ensembles, (**E**–**H**) THESEUS superimposed NMR structures and coordinate variances of (**I**–**L**) THESEUS superimposed MD trajectories. For each structure, an average rank is calculated for each backbone heavy atom type: (first row) amide N, (second row) Cα, (third row) carbonyl C and (fourth row) H. For superimposed NMR ensembles (columns one and two) and MD trajectories (column three) a clear pattern is visible: average ranks for amide nitrogen atoms and carbonyl carbon atoms are often lower than average ranks for Cα atoms; average ranks for H atoms are usually higher.

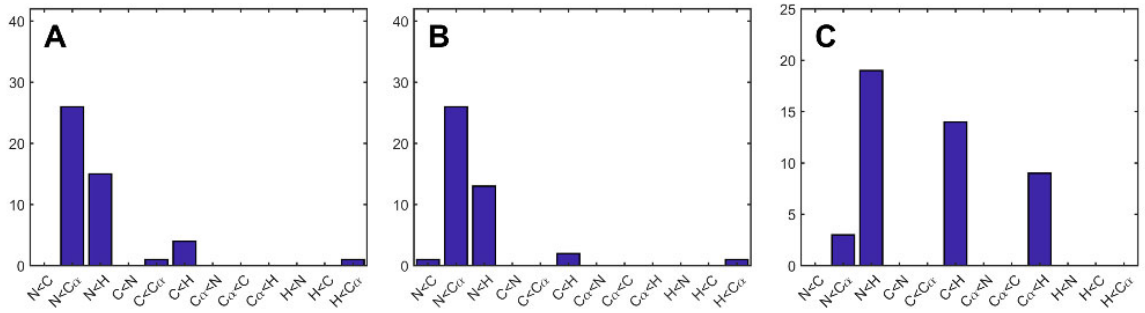

Figure S4. Results of Friedman's Test and subsequent multiple comparisons analysis with amide H atoms. A bar, associated with a comparison X < Y, that is n units high, indicates that in n structures, the assessed measure of coordinate variability is significantly lower for atom type X than for atom type Y. E.g. in panel A, the bar associated with Cα < H being 39 units high indicates that in 39 NMR ensembles, the coordinate uncertainties (calculated using FindCore superimpositions) for Cα atoms are significantly less (according to Friedman's test) than those for amide H atoms. Mean ranks are considered significantly different if they differ by more than three standard deviations. Assessed measures of coordinate variability are (**A**) coordinate uncertainties in FindCore superimposed NMR ensembles, (**B**) coordinate uncertainties in THESEUS superimposed NMR ensembles and (**C**) Coordinate uncertainties in THESEUS superimposed MD trajectories. Note that amide H coordinate uncertainties and variances are significantly higher than corresponding backbone heavy atom uncertainties and variances in fewer cases than seen for Cβ and carbonyl O atoms.

References

1. MATLAB. (2017). version 9.2.0.538062 (R2017a). Natick, Massachusetts: The MathWorks Inc.
2. M Hollander, DA Wolfe, E Chicken, Nonparametric Statistical Methods, 3rd ed., 2015, pp. 1-10.