Supplementary Information for

Validation of X-ray crystal structure ensemble representations of SARS-CoV-2 main protease by solution NMR residual dipolar couplings

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Table S1. Comparison of the agreement between RDCs and X-ray static structures of M^{pro}, when the X-ray structure is either used as a single conformer with the highest occupancy or when the population-weighted average of two available coordinate sets is used.[a]

Structure	Xray				NMR			
	T (K)	Resolution (Å)	N_{alt} [b]	N[a]	$^1D_{NH}$ & $^2D_{C'H}$		$^1D_{NH}$	$^2D_{C'H}$
					$<Da>$	$<Rh>$	Q_{jk}	Q_{jk}
7K3T	100	1.2	25	2	12.727	0.356	0.265	0.265
				1	12.726	0.358	0.274	0.283
7K3T-REDO	-	-	24	2	12.735	0.356	0.265	0.254
				1	12.744	0.358	0.274	0.270
7MHF	100	1.55	17	2	12.641	0.377	0.266	0.253
				1	12.711	0.375	0.267	0.253
7MHG	240	1.53	13	2	12.623	0.384	0.247	0.236
				1	12.725	0.381	0.252	0.236
7MHH	277	2.19	2	2	12.515	0.378	0.299	0.287
				1	12.621	0.375	0.299	0.287
7MHI	298	1.88	3	2	12.521	0.384	0.240	0.247
				1	12.618	0.381	0.241	0.247
7MHJ	298	2.0	2	2	12.514	0.381	0.270	0.283
				1	12.607	0.378	0.270	0.283
7MHK	310	1.96	2	2	12.607	0.378	0.245	0.286
				1	12.609	0.378	0.245	0.286

[a] When only the conformer with the highest occupancy is used for RDC fitting, the number of conformers N is marked as 1. In this work, the RDC fitting of two-conformer presentations (N=2) of the static structure is performed by generating a 100-member ensemble, with each discrete conformer represented *Z* times, where *Z* is the percentage occupancy listed in the PDB entry.

[b] Number of residues (in chain A) for which two alternate conformations were reported and that have experimental RDCs.

Table S2. Weights, <w>, and their standard deviations, σ, for each of the 350 M^{pro} X-ray structures in an ensemble fit to experimental RDCs by the VWFit program. The fit was repeated 10 times, each time omitting a different, randomly selected 10% fraction of residues. The RDC fit of the 10 omitted 10% fractions was used for Q factor calculation (see Table 1). Weight optimization was carried out when considering both $^1D_{NH}$ and $^2D_{C'H}$ (columns 2 and 3), just $^1D_{NH}$ (columns 4 and 5), and just $^2D_{C'H}$ (columns 6 and 7).

PDB id	<w>±σ $^1D_{NH}$ &$^2D_{C'H}$		<w>±σ $^1D_{NH}$		<w>±σ $^2D_{C'H}$		PDB id	<w>±σ $^1D_{NH}$ &$^2D_{C'H}$		<w>±σ $^1D_{NH}$		<w>±σ $^2D_{C'H}$		PDB id	<w>±σ $^1D_{NH}$ &$^2D_{C'H}$		<w>±σ $^1D_{NH}$		<w>±σ $^2D_{C'H}$	
5r7y	0.79	0.95	0.35	0.44	0.42	0.57	6lu7	1.69	1.18	1.64	1.08	0.90	1.00	7maw	0.79	0.75	0.94	0.96	0.59	0.93
5r7z	0.66	1.01	0.27	0.47	0.44	0.64	6lze	2.39	0.55	2.50	0.20	1.40	1.02	7max	1.18	0.91	1.02	1.05	2.00	0.91
5r80	0.17	0.21	0.50	0.40	0.30	0.36	6m03	0.60	0.68	1.27	1.17	0.51	0.59	7maz	1.65	1.21	1.17	1.00	2.27	0.44
5r81	0.27	0.44	0.28	0.32	0.11	0.20	6m0k	2.32	0.76	2.47	0.19	2.18	0.70	7mb0	1.88	1.04	0.67	0.82	1.86	0.68
5r82	0.33	0.30	0.09	0.18	0.25	0.46	6m2n	0.30	0.37	0.86	1.02	0.65	0.84	7mb1	1.06	0.90	1.71	0.77	1.71	0.98
5r83	0.45	0.83	0.58	0.84	0.40	0.65	6m2q	1.79	0.81	1.98	0.89	0.76	0.92	7mb2	1.37	0.96	1.16	0.92	1.39	0.80
5r84	0.26	0.35	0.87	0.92	0.10	0.19	6w63	0.83	0.80	1.13	1.00	0.91	0.91	7mb3	0.57	0.70	1.36	1.09	0.40	0.58
5r8t	0.47	0.50	0.23	0.25	0.33	0.30	6wnp	1.49	0.97	1.04	1.00	1.72	0.83	7mbi	1.43	0.95	1.56	0.92	1.82	0.95
5re4	0.30	0.46	0.46	0.57	0.11	0.21	6wqf	0.75	1.08	1.12	0.93	0.95	0.95	7mhf	0.71	0.83	0.73	0.96	0.63	0.72
5re5	0.24	0.19	0.35	0.69	0.24	0.38	6wtj	1.24	0.95	0.33	0.78	1.06	0.90	7mhg	2.14	0.79	1.64	1.04	1.96	0.98
5re6	0.53	0.80	0.59	0.63	0.60	0.74	6wtk	0.18	0.26	0.77	0.92	0.13	0.15	7mhh	0.54	0.72	0.96	1.00	0.30	0.36
5re7	0.19	0.21	0.91	0.97	0.93	0.87	6wtm	0.71	0.76	1.22	0.91	0.86	0.84	7mhi	1.79	0.89	2.14	0.58	1.57	0.91
5re8	0.25	0.35	0.28	0.42	0.40	0.52	6wtt	1.63	1.02	0.98	0.98	0.99	0.86	7mhj	1.53	1.05	2.19	0.75	1.11	0.87
5re9	0.56	0.82	1.20	1.03	0.74	0.78	6wtt	1.39	0.89	0.91	1.00	2.15	0.76	7mhk	1.91	1.10	2.58	0.10	0.12	0.19
5rea	0.77	0.94	1.34	0.99	0.77	0.68	6xa4	1.64	0.99	0.99	0.87	0.97	0.98	7mhl	0.62	0.73	0.78	0.78	0.84	0.96
5reb	0.96	0.77	1.03	0.89	0.64	0.79	6xb2	1.81	0.92	1.59	0.89	1.19	0.97	7mhm	0.39	0.72	0.15	0.17	0.62	0.92
5rec	0.53	0.62	0.46	0.66	0.31	0.52	6xbh	1.73	1.00	1.46	1.15	2.26	0.38	7mhn	0.14	0.26	0.61	0.78	0.13	0.20
5red	0.64	0.90	0.47	0.47	0.38	0.77	6xbi	0.69	0.82	1.23	0.92	1.11	0.84	7mho	1.41	1.09	2.19	0.49	0.58	0.69
5ree	0.31	0.41	0.40	0.76	0.28	0.73	6xch	0.65	0.84	0.39	0.59	1.05	0.91	7mhp	1.81	1.02	2.11	0.58	2.17	0.49
5ref	0.28	0.66	1.11	1.09	0.41	0.77	6xfn	2.07	0.72	1.07	1.05	1.82	0.77	7mhq	1.10	1.02	1.41	1.07	1.33	0.96
5reg	0.98	0.98	0.44	0.68	0.30	0.38	6xqs	1.19	1.05	0.94	0.93	1.88	1.05	7mlf	1.64	1.00	1.16	0.99	1.64	0.87
5reh	0.53	0.84	0.53	0.94	0.68	0.80	6xqt	1.65	0.85	0.90	0.99	2.57	0.11	7mlg	1.22	1.07	2.04	0.70	1.32	1.10
5rei	0.65	0.80	0.78	1.09	0.26	0.70	6xqu	0.64	0.69	0.92	0.68	1.75	1.10	7mng	0.72	0.89	0.80	0.89	0.47	0.55
5rej	0.21	0.28	0.18	0.29	0.29	0.33	6xr3	2.21	0.33	1.59	0.90	1.28	1.08	7mpb	2.03	0.86	2.33	0.38	1.61	0.79
5rek	0.09	0.19	0.69	0.88	0.42	0.64	6y2e	1.34	0.94	1.77	0.96	0.81	0.67	7mrr	0.52	0.64	0.60	0.88	0.02	0.04
5rel	0.27	0.31	0.12	0.23	0.56	0.90	6y84	0.76	0.85	0.47	0.57	0.50	0.48	7n8c	1.05	1.03	1.59	1.01	0.97	0.95
5rem	0.24	0.63	0.49	0.84	0.28	0.29	6yb7	0.85	0.80	1.25	1.04	0.18	0.22	7n8c	2.21	0.74	1.27	1.01	2.33	0.50
5ren	0.47	0.58	0.17	0.28	0.20	0.24	6ynq	0.24	0.20	0.17	0.31	0.29	0.52	7nev	0.28	0.41	0.11	0.21	0.44	0.64
5reo	0.77	0.97	0.32	0.44	0.22	0.30	6yvf	0.55	0.78	0.16	0.42	0.08	0.16	7nf5	0.61	0.81	1.74	0.88	0.73	0.74
5rep	0.25	0.47	0.35	0.49	0.45	0.79	6zrt	1.20	1.06	0.61	0.84	0.76	0.80	7ng3	0.55	0.65	0.62	0.71	1.69	0.78
5rer	0.54	0.78	0.38	0.45	0.20	0.19	6zru	0.32	0.45	0.66	0.97	0.84	0.67	7ng6	1.28	1.06	0.84	0.87	0.79	0.95
5res	0.45	0.66	0.56	0.82	0.32	0.26	7a1u	0.41	0.84	0.30	0.29	0.18	0.29	7nt1	1.72	0.73	1.63	0.97	1.20	0.70
5ret	0.26	0.30	0.69	0.92	0.20	0.37	7abu	0.67	0.83	0.75	1.00	0.23	0.38	7nt3	1.73	0.90	2.15	0.56	1.39	0.96
5reu	0.43	0.54	0.60	0.61	0.52	0.77	7adw	0.57	0.79	0.64	0.99	0.53	0.81	7ntq	0.56	0.58	1.05	0.96	0.58	0.61
5rev	0.75	0.83	0.85	0.77	0.59	0.50	7aeh	0.61	0.46	1.10	1.12	1.11	0.94	7ntt	1.36	1.13	1.91	0.67	0.89	0.95
5rew	1.07	1.19	0.86	0.98	1.18	1.08	7af0	0.31	0.44	0.51	0.60	1.00	0.95	7ntw	0.75	0.85	0.72	0.90	0.64	0.78
5rex	0.44	0.72	0.51	0.68	0.31	0.57	7aga	0.21	0.30	0.34	0.77	0.57	0.70	7nuk	1.51	1.00	1.72	0.83	0.78	0.91
5rey	0.20	0.56	0.67	0.81	0.18	0.29	7aha	0.29	0.33	0.46	0.52	0.71	0.65	7nwx	0.83	0.87	1.01	0.81	0.91	0.73
5rez	0.83	0.80	0.61	0.75	0.59	0.83	7aku	0.18	0.34	0.24	0.34	0.36	0.57	7nxh	0.22	0.31	0.77	0.74	0.25	0.46
5rf0	0.83	0.98	1.24	1.06	0.48	0.75	7alh	2.28	0.82	1.50	0.94	1.28	0.96	7pfl	1.25	1.04	1.61	0.87	0.90	1.04
5rf1	0.47	0.70	0.38	0.76	0.45	0.84	7ali	1.52	1.11	1.54	0.90	1.06	0.93	7pfm	0.98	0.94	1.05	1.12	1.60	0.70
5rf2	0.70	0.81	0.67	0.92	0.25	0.36	7amj	0.86	0.77	0.56	0.53	0.85	0.74	7phz	1.87	0.89	1.82	0.95	1.65	0.96
5rf3	1.10	1.14	0.63	0.81	0.85	0.77	7ans	1.09	1.01	0.57	0.84	1.04	0.86	7pxz	1.66	0.75	2.11	0.67	1.90	0.75
5rf4	0.42	0.75	0.85	0.84	0.44	0.40	7ap6	0.42	0.81	0.34	0.79	0.32	0.25	7q5e	2.06	0.64	1.83	0.79	0.53	0.80
5rf5	0.13	0.23	1.22	0.94	0.01	0.04	7aqe	0.27	0.35	0.71	0.57	1.17	0.97	7q5f	1.00	1.05	0.89	0.93	1.28	0.94
5rf6	1.05	1.02	0.71	0.70	0.83	0.68	7aqi	0.70	0.75	0.71	0.99	0.47	0.66	7qbb	1.38	0.96	0.95	0.80	1.14	0.88
5rf7	0.75	0.95	0.55	0.82	0.34	0.35	7aqj	0.43	0.57	0.38	0.46	0.79	0.98	7qka	0.69	0.84	0.21	0.47	1.43	0.79
5rf8	0.46	0.80	0.50	0.75	0.86	0.82	7ar6	0.29	0.29	0.43	0.71	0.54	0.72	7qt8	2.13	0.70	2.38	0.37	1.45	0.93
5rf9	0.57	0.77	0.94	0.85	0.53	0.84	7au4	1.18	1.02	1.06	0.84	1.29	0.96	7r7h	0.32	0.46	0.27	0.26	1.61	0.80
5rfb	0.56	0.81	0.25	0.38	0.56	0.80	7avd	1.36	1.05	1.71	0.73	1.50	0.81	7rbz	2.00	0.99	1.70	0.99	1.76	0.93
5rfc	0.18	0.25	0.91	0.84	0.29	0.42	7awr	0.25	0.33	0.86	0.87	0.38	0.48	7rc0	1.93	0.87	2.20	0.48	2.09	0.60
5rfd	1.60	1.18	0.56	0.79	0.94	1.03	7aws	0.21	0.33	0.30	0.40	0.68	0.87	7rfs	0.54	0.82	0.09	0.15	1.32	1.01
5rfe	1.11	1.14	0.82	0.80	1.25	0.83	7aww	0.90	0.95	1.04	0.91	0.51	0.77	7rfw	0.43	0.60	0.66	0.79	0.59	0.84

5rff	0.15	0.27	0.20	0.32	0.63	0.82	7ax6	1.91	0.93	2.09	0.61	1.23	1.11	7rls	1.84	0.97	1.72	0.81	1.46	1.06
5rfg	0.20	0.35	0.16	0.31	0.12	0.19	7axm	0.44	0.65	0.06	0.08	0.98	1.05	7rm2	1.97	0.83	1.64	0.93	1.27	0.65
5rfh	0.42	0.57	0.60	0.88	0.47	0.73	7axo	0.67	0.72	0.09	0.12	0.56	0.91	7rmb	1.49	1.16	1.55	0.93	1.36	0.99
5rfi	0.25	0.30	0.68	0.83	0.20	0.22	7ay7	0.32	0.49	0.73	0.85	0.44	0.78	7rme	1.65	1.00	1.71	0.81	2.09	0.58
5rfj	0.48	0.63	0.96	1.04	0.20	0.29	7b2j	1.87	0.96	1.67	1.10	1.53	0.94	7rmt	1.31	1.05	2.02	0.54	1.73	0.84
5rfk	0.34	0.47	0.35	0.65	0.23	0.34	7b2u	1.81	0.80	0.92	1.01	1.00	0.84	7rmz	1.85	0.69	1.49	0.86	1.73	1.13
5rfl	0.53	0.79	0.57	0.86	0.23	0.28	7b5z	1.15	1.02	2.10	0.71	1.54	1.06	7rn0	1.14	0.86	0.85	1.03	1.37	0.78
5rfm	0.39	0.50	0.96	0.91	0.56	0.89	7b77	1.49	1.15	1.35	0.88	2.31	0.62	7rn1	0.32	0.49	0.38	0.58	0.46	0.71
5rfn	0.23	0.20	0.42	0.49	0.18	0.40	7baj	1.59	1.11	1.98	0.64	0.76	0.88	7rn4	1.89	0.70	1.54	0.83	1.21	0.91
5rfo	0.31	0.84	0.29	0.43	0.12	0.09	7bak	0.82	1.17	0.55	0.59	0.37	0.71	7rnh	1.81	0.86	1.76	0.90	2.42	0.49
5rfp	0.33	0.79	0.60	0.65	0.02	0.04	7be7	2.22	0.83	1.47	0.89	2.36	0.20	7rnk	1.19	1.24	0.92	0.84	2.29	0.40
5rfq	0.46	0.57	0.65	0.78	0.38	0.72	7buy	2.48	0.41	2.29	0.64	1.79	0.95	7rnw	1.60	0.98	1.23	0.91	1.36	1.02
5rfr	0.63	0.62	0.19	0.30	0.36	0.44	7cb7	2.29	0.46	2.10	0.64	2.29	0.45	7s3k	1.15	0.99	0.97	0.94	1.95	0.78
5rfs	0.39	0.78	0.34	0.62	0.16	0.27	7cuu	2.03	0.76	0.92	0.77	1.93	0.78	7s3s	1.24	1.08	1.78	1.00	0.73	0.48
5rft	0.24	0.21	0.70	0.74	0.12	0.14	7cwb	1.57	0.97	2.16	0.77	0.85	0.85	7s4b	1.71	0.95	1.94	0.91	1.49	0.88
5rfu	0.98	0.95	1.00	0.76	0.85	0.94	7d3i	0.29	0.61	0.81	0.78	0.86	0.87	7set	1.56	0.96	0.59	0.90	1.65	0.91
5rfv	0.43	0.46	0.19	0.38	0.22	0.35	7dgb	2.48	0.45	1.91	0.91	1.30	0.86	7sf1	1.02	1.03	0.92	0.84	0.74	0.62
5rfw	1.14	0.99	0.39	0.65	0.76	0.79	7dgf	2.50	0.34	2.05	0.61	2.12	0.56	7sf3	1.25	0.99	1.37	1.00	0.74	0.85
5rfx	0.21	0.32	0.56	0.85	0.24	0.44	7dgh	1.40	1.05	1.58	1.09	0.72	0.95	7sfh	2.09	0.78	1.22	0.97	1.63	0.96
5rfy	0.20	0.41	0.08	0.15	0.33	0.61	7dgi	1.73	0.96	1.66	0.78	1.73	1.01	7sfi	0.74	0.79	1.31	1.06	1.23	0.94
5rfz	0.75	0.76	0.26	0.40	0.69	1.01	7dhj	2.18	0.60	2.39	0.25	1.64	1.04	7sgh	2.25	0.58	2.17	0.61	2.40	0.55
5rg0	0.38	0.48	0.44	0.75	0.31	0.40	7djr	0.51	0.84	0.93	0.88	0.52	0.79	7si9	1.48	0.94	1.91	0.80	1.73	0.93
5rg1	0.48	0.60	0.13	0.23	0.35	0.67	7dpu	2.32	0.79	1.44	1.03	2.37	0.38	7t2t	0.97	0.79	0.75	0.70	0.96	1.14
5rg2	0.40	0.46	0.53	0.74	0.59	0.70	7e18	1.21	1.03	1.37	1.02	1.53	1.12	7tdu	1.82	1.18	2.31	0.47	1.47	1.01
5rg3	0.43	0.69	1.14	0.93	0.50	0.68	7e19	0.39	0.48	0.59	0.67	0.49	0.81	7tdu	1.98	0.69	2.33	0.44	1.87	0.97
5rgg	0.18	0.20	0.20	0.33	0.21	0.45	7e5x	0.80	0.71	0.53	0.66	0.32	0.40	7te0	1.66	0.86	1.91	0.92	0.88	0.87
5rgh	0.65	0.76	0.60	0.51	0.61	0.84	7e6k	2.23	0.89	2.02	0.84	1.79	1.00	7teh	2.08	0.88	2.25	0.42	2.04	0.77
5rgi	0.62	0.84	0.82	0.72	0.50	0.50	7en9	1.13	0.78	1.23	0.99	0.78	0.91	7tek	0.29	0.45	0.32	0.47	0.66	0.54
5rgj	0.90	1.04	0.77	1.03	0.68	1.00	7jfq	2.21	0.88	2.33	0.47	2.11	0.82	7tel	1.24	0.94	0.62	0.87	1.50	0.99
5rgk	0.70	0.66	0.91	1.14	0.74	0.88	7jp1	1.45	1.08	2.12	0.53	0.44	0.57	7tfr	2.01	0.83	1.83	0.98	1.97	0.79
5rgl	0.38	0.47	0.84	0.85	0.14	0.30	7jq3	1.69	1.09	2.44	0.21	2.46	0.44	7tgr	0.65	0.87	0.96	1.05	1.20	0.87
5rgm	0.21	0.41	0.27	0.31	0.46	0.39	7jsu	1.78	0.89	1.66	1.00	0.86	0.92	7tia	1.94	0.91	1.56	0.71	1.66	1.04
5rgn	0.50	0.74	0.70	0.87	0.60	0.70	7jt7	0.61	0.69	0.76	0.71	1.14	1.08	7tiw	0.97	0.95	1.36	1.04	0.72	0.93
5rgo	0.82	0.85	0.93	0.84	0.82	0.91	7ju7	1.97	0.98	2.03	0.97	2.48	0.42	7tix	0.53	0.95	0.20	0.24	1.50	1.08
5rgp	0.17	0.35	0.52	0.76	0.25	0.39	7jun	2.38	0.59	2.33	0.28	1.67	0.99	7tiy	1.15	1.04	1.29	0.99	1.45	0.61
5rgq	0.18	0.34	0.48	0.64	0.06	0.12	7jvz	2.58	0.20	1.95	0.94	2.61	0.08	7tiz	1.37	1.10	1.07	0.74	1.42	1.03
5rgr	1.25	1.05	0.44	0.71	1.30	0.92	7jw8	1.56	1.24	2.20	0.63	2.16	0.73	7tuu	2.53	0.18	2.49	0.12	2.36	0.52
5rgs	0.86	0.87	0.75	1.01	1.10	1.00	7jyc	0.39	0.63	0.21	0.24	1.32	1.07	7ukk	1.48	1.23	1.48	1.16	2.34	0.60
5rgt	0.37	0.74	0.16	0.22	0.54	0.62	7k3t	0.70	0.83	0.52	0.43	0.26	0.23	7vh8	0.63	0.87	0.86	0.77	1.27	1.05
5rgu	0.23	0.42	0.53	0.77	0.10	0.17	7k40	0.60	0.83	1.30	1.08	0.58	0.63	7vjy	1.73	1.04	2.29	0.45	2.16	0.76
5rgv	0.34	0.47	0.82	1.00	0.99	0.93	7k6d	1.57	1.11	0.48	0.66	1.88	0.99	7vjz	1.93	0.90	1.86	0.70	1.85	0.58
5rgw	0.65	0.82	0.68	0.65	1.15	0.97	7k6e	1.01	0.83	0.60	0.79	0.81	0.77	7vk1	0.79	0.71	1.41	0.93	0.72	0.81
5rgx	0.41	0.77	0.39	0.72	1.18	1.04	7khp	1.30	1.17	1.00	0.97	1.08	0.92	7vk2	1.91	0.87	0.90	1.07	1.33	0.84
5rgy	0.13	0.24	0.21	0.34	0.10	0.14	7kph	0.83	0.88	0.69	0.83	0.66	0.65	7wo1	0.67	0.73	0.04	0.07	0.98	1.03
5rgz	0.73	0.59	0.73	0.85	1.29	0.83	7kx5	0.24	0.24	0.11	0.28	0.41	0.77	7wo3	1.78	0.85	2.38	0.36	1.73	0.95
5rh0	0.48	0.80	0.37	0.47	0.76	0.76	7l0d	0.49	0.64	0.43	0.48	0.29	0.38	7wof	2.61	0.31	2.58	0.08	1.82	0.94
5rh1	0.12	0.18	0.12	0.18	0.83	0.86	7l11	1.34	1.12	1.65	0.79	1.27	0.92	7wym	1.38	1.16	1.00	0.81	1.50	0.93
5rh2	0.78	0.99	0.77	0.97	1.11	0.98	7l12	1.25	1.11	1.30	1.06	1.33	0.78	7x6k	2.08	0.81	2.08	0.87	1.88	0.80
5rh3	0.91	0.78	1.55	0.92	1.16	0.79	7l14	1.55	1.08	1.27	0.93	0.82	0.87	7xar	2.28	0.79	1.33	0.93	2.44	0.41
5rh4	0.69	0.75	0.12	0.21	0.85	0.53	7l8i	0.67	0.77	1.80	0.77	1.44	0.99	7z59	1.17	0.96	1.04	0.94	1.30	0.91
5rh5	0.25	0.20	0.19	0.34	1.12	0.88	7l8j	0.10	0.13	0.12	0.14	0.27	0.35	7zqv	1.45	0.98	0.46	0.51	0.95	0.92
5rh6	0.84	1.09	0.83	0.72	1.48	0.81	7lb7	1.92	0.99	1.06	1.02	2.33	0.42	8a4q	2.13	0.88	1.44	0.95	1.68	1.04
5rh7	0.35	0.49	0.21	0.33	1.26	1.15	7lco	0.79	0.62	0.24	0.24	1.73	1.06	8b2t	0.80	1.05	1.67	0.96	1.55	0.83
5rh8	0.14	0.37	0.10	0.20	0.48	0.60	7lcr	2.05	0.68	1.62	0.87	2.23	0.54	8dox	0.29	0.43	0.68	0.89	1.31	1.10
5rh9	0.37	0.80	0.37	0.54	0.88	0.99	7lcs	0.17	0.28	0.27	0.42	0.63	0.66	8dpr	2.14	0.78	1.45	1.08	1.56	1.04
5rha	0.86	0.90	0.61	0.68	0.13	0.16	7lct	0.99	0.93	0.57	0.86	1.25	0.86	8dz0	1.27	1.16	0.49	0.78	1.52	0.99
5rhb	0.63	0.69	0.36	0.34	1.07	0.96	7ldl	0.95	1.10	0.69	0.90	0.10	0.15	8dz2	0.56	0.73	0.72	0.71	0.60	0.88
5rhc	0.78	0.76	0.54	0.75	0.86	1.06	7lkd	0.82	0.81	0.90	0.89	0.84	0.72							
5rhd	0.89	0.83	0.24	0.43	0.16	0.27	7lmd	0.57	0.81	0.44	0.49	0.15	0.20							
5rhe	1.11	1.03	0.94	0.82	0.62	0.55	7lme	1.37	0.90	1.78	0.89	1.61	1.05							
5rhf	0.31	0.44	0.69	0.82	0.56	0.72	7lmf	1.05	0.81	1.41	1.11	1.20	0.90							
5rl0	0.63	0.78	0.34	0.39	0.74	0.99	7ltj	1.76	1.00	2.03	0.89	2.07	0.79							
5rl1	0.40	0.50	0.74	0.86	0.77	0.56	7ltn	1.78	0.79	1.69	0.75	1.89	0.82							

5rl2	0.31	0.60	0.78	0.87	0.90	0.93	7lyh	0.65	0.68	0.34	0.48	0.98	0.96
5rl3	0.86	0.96	0.32	0.68	1.19	1.05	7lyi	0.67	0.98	0.62	0.55	1.92	0.94
5rl4	0.43	0.58	0.41	0.43	0.43	0.54	7mat	1.46	1.05	1.60	0.83	2.08	0.53
5rl5	1.90	1.00	0.42	0.64	1.91	0.98	7mav	1.84	0.79	1.70	0.71	2.11	0.88

Table S3. Distribution of crystallographic space groups for 350 M^{pro} structures in the PDB without missing residues

Space group	Count
C 1 2 1	251
I 1 2 1	30
P 1 2_1 1	27
P 2_1 2_1 2	15
P 2_1 2_1 2_1	14
P 1	9
P 3_2 2 1	3
C 2 2 2_1	1

Table S4. Comparison of RDC agreement of conventionally refined X-ray structures of M^{pro} and their PDB-REDO equivalents.[a]

Structure	X-ray				NMR			
	T (K)	Resolution (Å)	R_{free}	N[b]	$^1D_{NH}$ & $^2D_{C'H}$		$^1D_{NH}$	$^2D_{C'H}$
					$<D_a>$	$<Rh>$	Q_{jk}	Q_{jk}
7K3T	100	1.2	0.187	2	12.727	0.356	0.265	0.265
7K3T-REDO	-	-	0.173	2	12.735	0.356	0.265	0.254
7MHF	100	1.55	0.224	2	12.641	0.377	0.266	0.253
7MHF-REDO	-	-	0.241	2	12.676	0.376	0.274	0.262
7MHG	240	1.53	0.205	2	12.623	0.384	0.247	0.236
7MHG-REDO	-	-	0.251	2	12.676	0.387	0.262	0.274
7MHH	277	2.19	0.253	2	12.515	0.378	0.299	0.287
7MHH-REDO	-	-	0.215	2	12.836	0.388	0.273	0.266
7MHI	298	1.88	0.228	2	12.521	0.384	0.240	0.247
7MHI-REDO	-	-	0.212	2	12.648	0.378	0.259	0.283
7MHJ	298[c]	2.0	0.240	2	12.514	0.381	0.270	0.283
7MHJ-REDO	-	-	0.231	2	12.607	0.381	0.274	0.265
7MHK	310	1.96	0.247	2	12.512	0.381	0.245	0.286
7MHK-REDO	-	-	0.251	2	12.726	0.388	0.271	0.256

[a] All REDO structures are taken from the PDB-REDO Databank (https://pdb-redo.eu/).

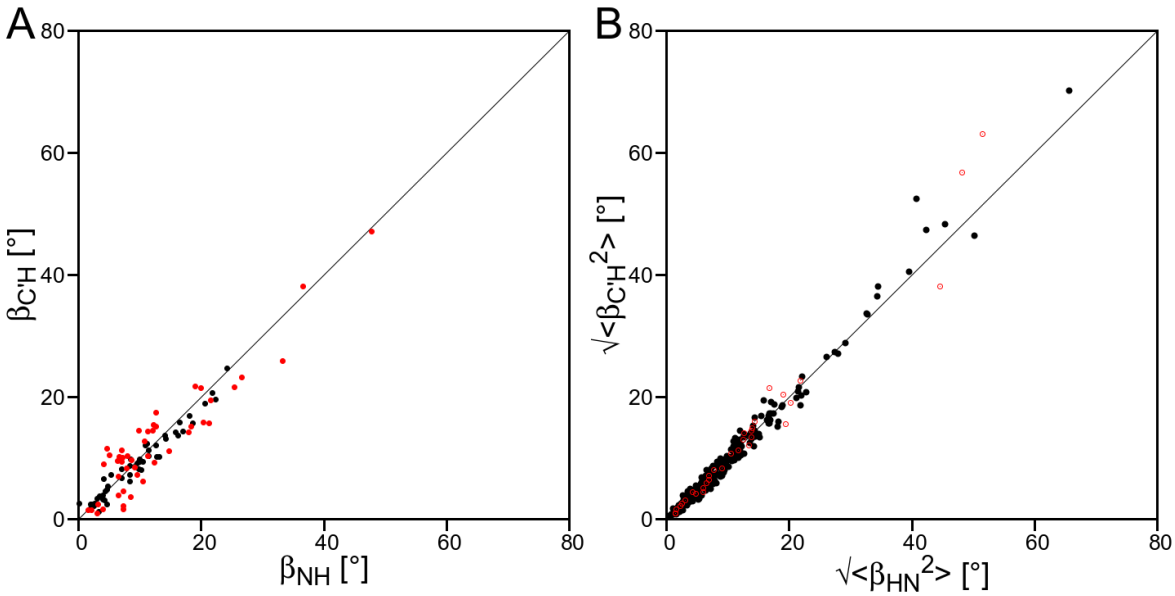

Figure S1. Correlation between angular excursions, β_{NH} and $\beta_{C'H}$, of N-H and C'-H vectors. (A) Correlation between angular excursions, β_{NH} and $\beta_{C'H}$, from their mean orientation for the N-H and C'-H vectors of residues E47 (red) and N53 (black) in the pTLS ensemble of 7K3T. (B) Plot of the RMS amplitude of N-H vector fluctuations, $\sqrt{<\beta_{n,NH}^2>}$ *vs* those of C'-H vectors, $\sqrt{<\beta_{n,C'H}^2>}$ for residues for which both $^1D_{NH}$ and $^2D_{C'H}$ were available. Residues for which one or both RDCs were missing are shown as red open circles.

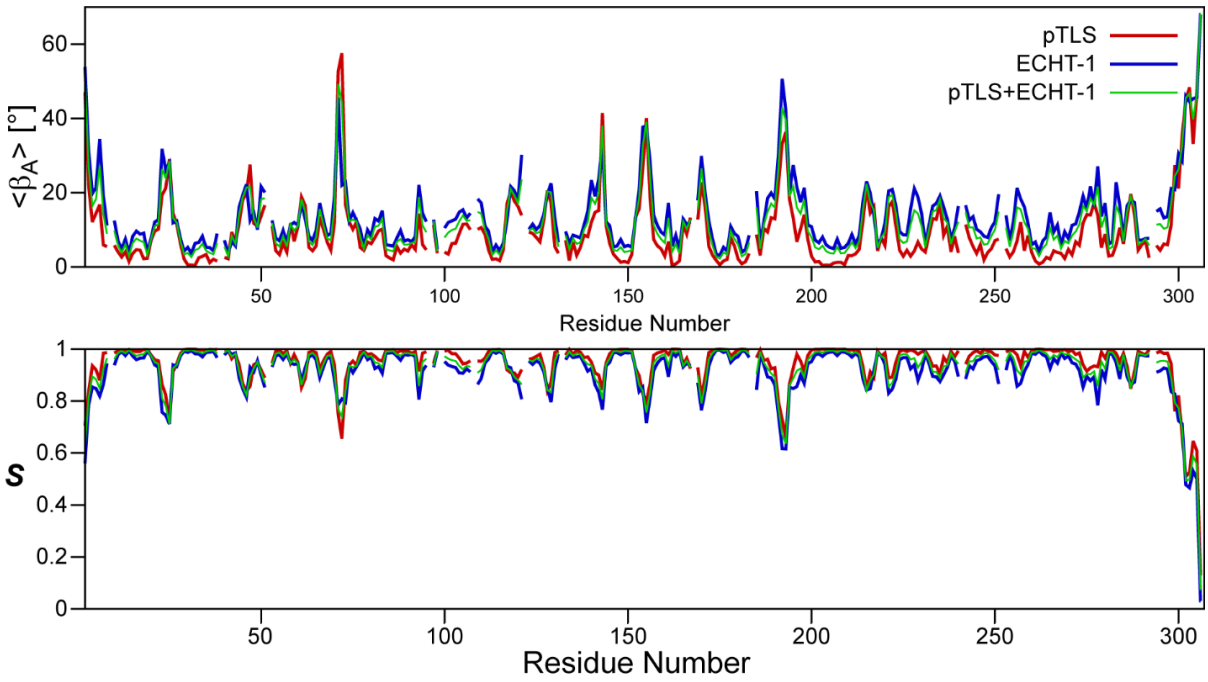

Figure S2. Dynamics distributions in the 7K3T pTLS, ECHT-1, and their combined ensembles. (A) $<\beta_{n,A}>$ as a function of residue number. (B) Corresponding order parameters, ***S***, derived using eq. 3.

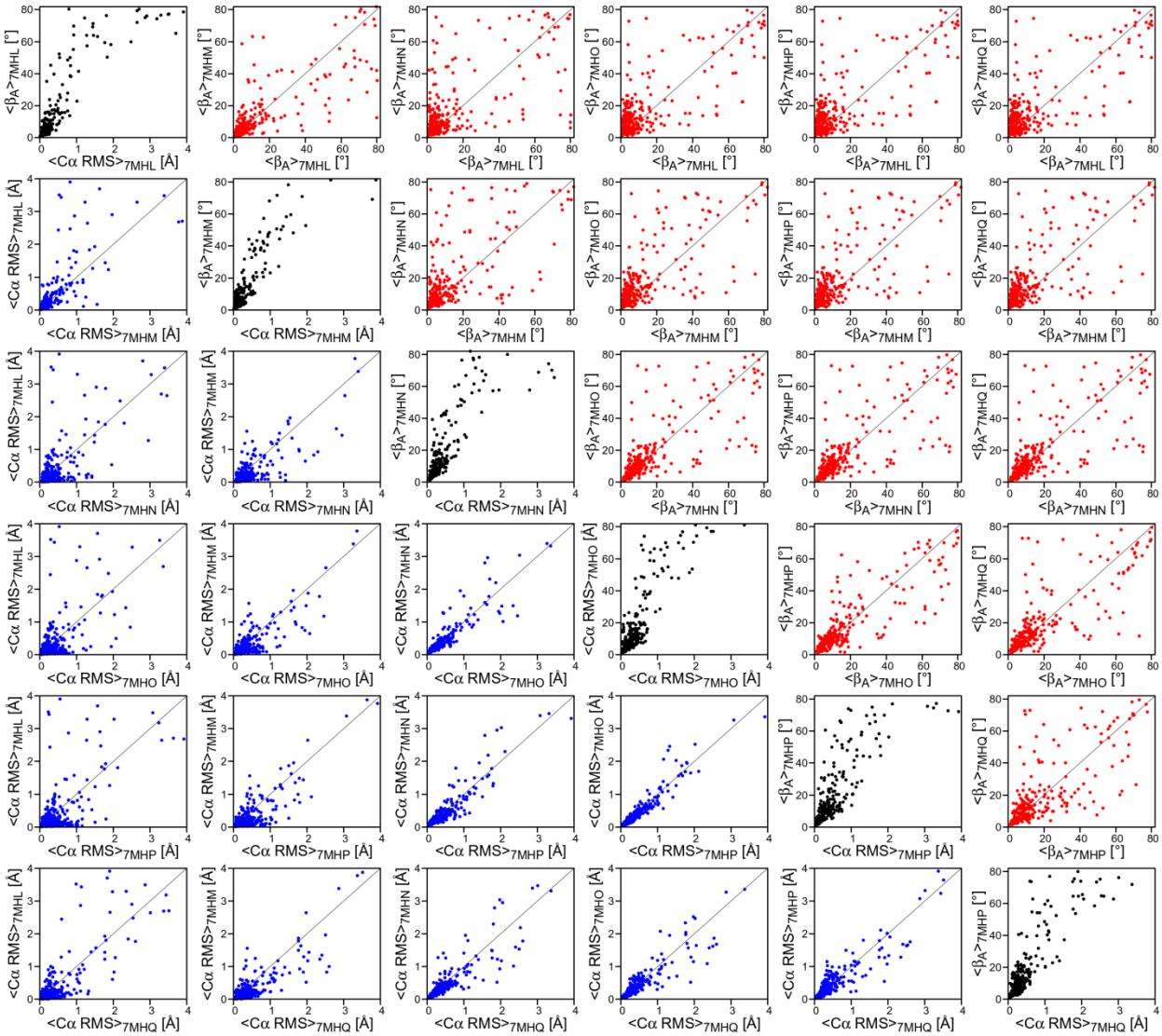

Figure S3. Comparisons of the spread in angular (β_A) excursions and C^α coordinate rmsd for the M^{pro} 7MHL-Q ensembles recorded at different temperatures. Diagonal panels show the correlations between $<\beta_{n,A}>$ excursions and C^α coordinate rmsd (black). The left bottom panels (blue) compare C^α coordinate rmsd for residues in the ensembles The top right 15 panels (red) show correlations between β_A excursions of residues in these six ensembles.

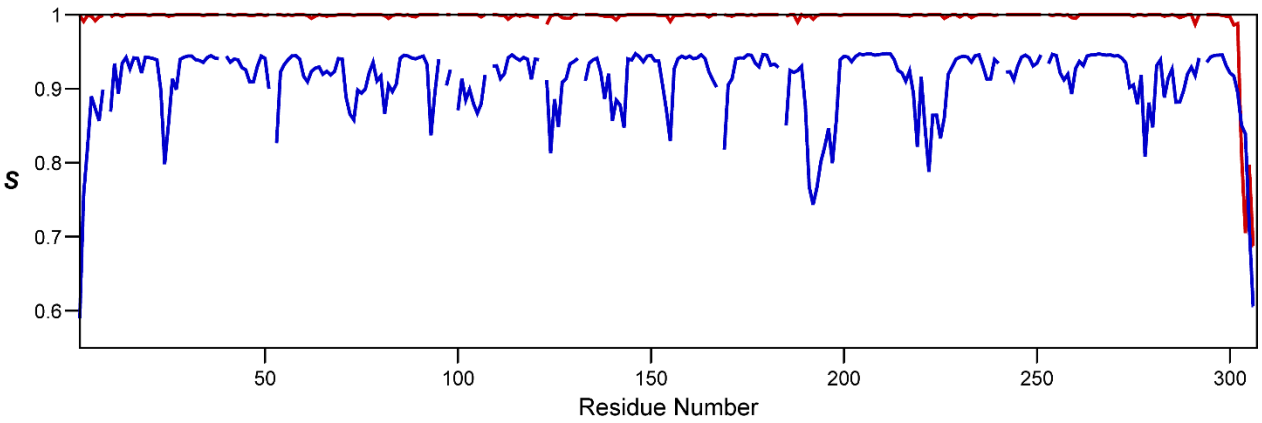

Figure S4. The order parameter ***S*** for the 25-member AF2-M ensemble (red) and predicted $\sqrt{(S^2)}$ values calculated for the top AF2-M model (blue) by using the contact-model based prediction of dynamics (https://spin.ccic.osu.edu/index.php/dynamics/index).[1]

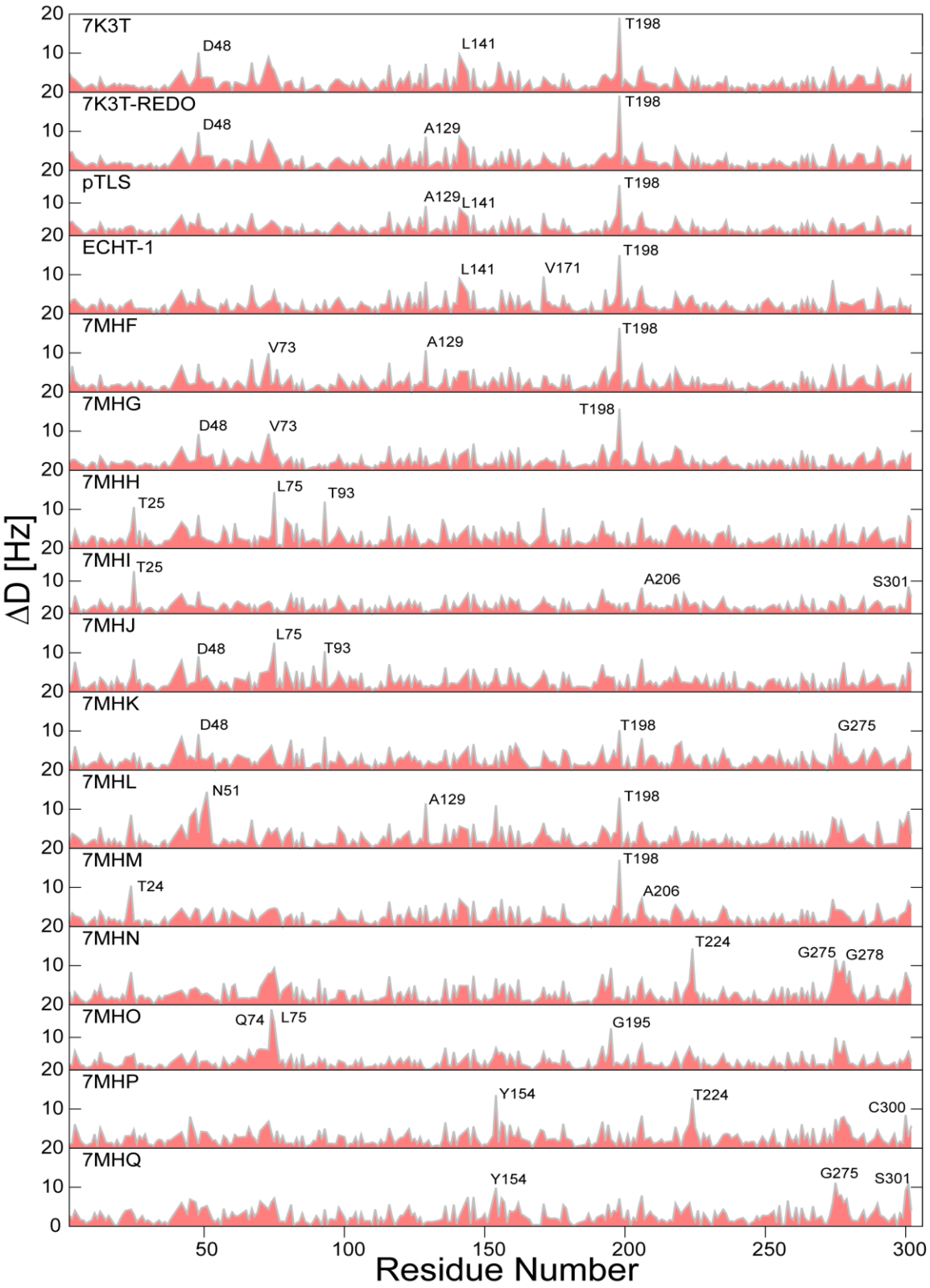

Figure S5. Residuals of the fits of RDCs to the various static and ensemble models of M^{pro}. The plots show the deviations $\Delta D = \sqrt{[({}^1D_{NH}{}^{obs} - {}^1D_{NH}{}^{pred})^2/2 + ({}^1D_{C'H}{}^{obs} - {}^1D_{C'H}{}^{pred})^2/2]}$ as a function of residue number, with the three largest outliers marked.

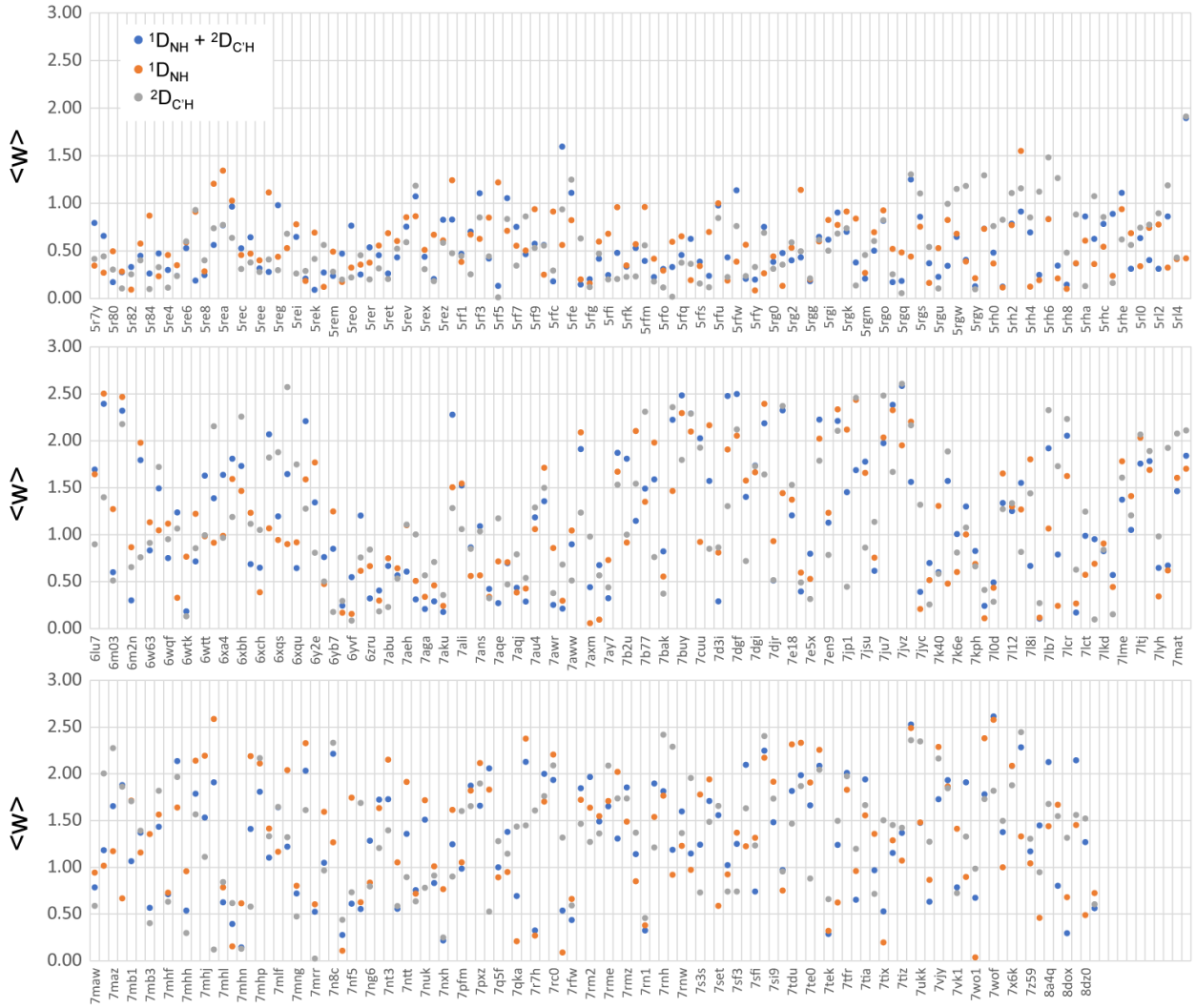

Figure S6. Optimized weights for 350 X-ray structures of M^{pro} (Table S2), obtained from fitting RDCs with the VWfit program. The average weights (<w>) obtained from fitting both $^1D_{NH}$ and $^2D_{C'H}$ (blue) $^1D_{NH}$ (orange), and $^2D_{C'H}$ (grey). The order of the PDB entry codes displayed along the horizontal axis is the same as in Table S2.

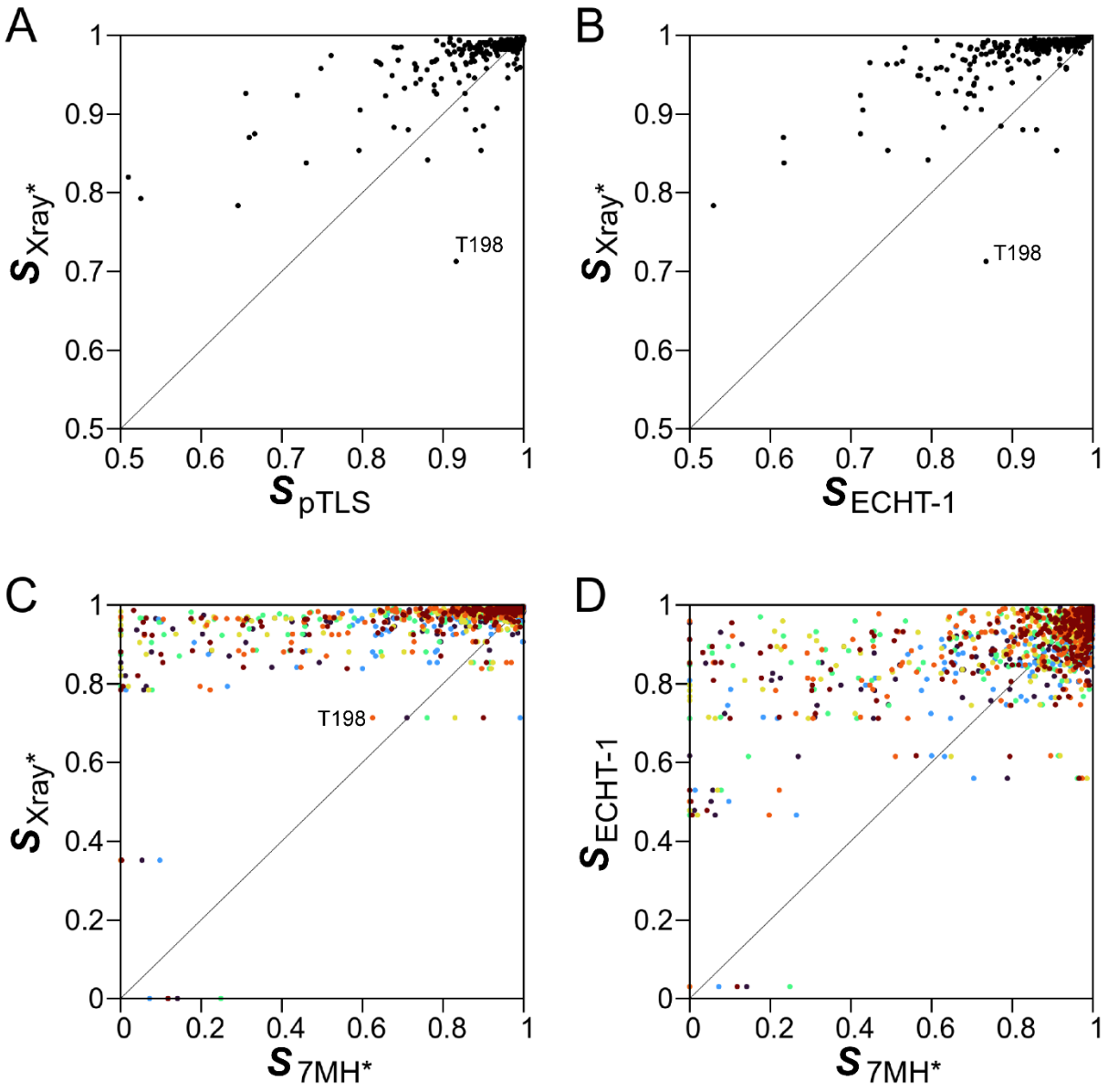

Figure S7. Comparison of backbone amide order parameters, ***S***, derived from different pairs of M^{pro} X-ray ensemble representations. (A) Calculated order parameters for the unweighted ensemble of 350 X-ray structures (Xray*) *versus* corresponding values derived from the 7K3T pTLS ensemble. (B) $\boldsymbol{S}_{Xray*}$ values versus corresponding values in the 7K3T ECHT-1 ensemble. (C) $\boldsymbol{S}_{Xray*}$ values versus corresponding values in the temperature series of ensembles, 7MHL-7MHQ, with colors corresponding to those of Fig.4, main text. (D) $\boldsymbol{S}_{ECHT\text{-}1}$ *versus* $\boldsymbol{S}_{7MH*}$. Values for T198, the only residue showing considerably higher disorder in the Xray* ensemble than in the various ensemble refinements, are marked.

References

1. Li, D. W., Bruschweiler, R. (2009) All-Atom Contact Model for Understanding Protein Dynamics from Crystallographic B-Factors, *Biophys. J.* **96**, 3074-3081.