Supporting Information

Measurement of Proton Chemical Shifts in Invisible States of Slowly Exchanging Protein Systems by Chemical Exchange Saturation Transfer

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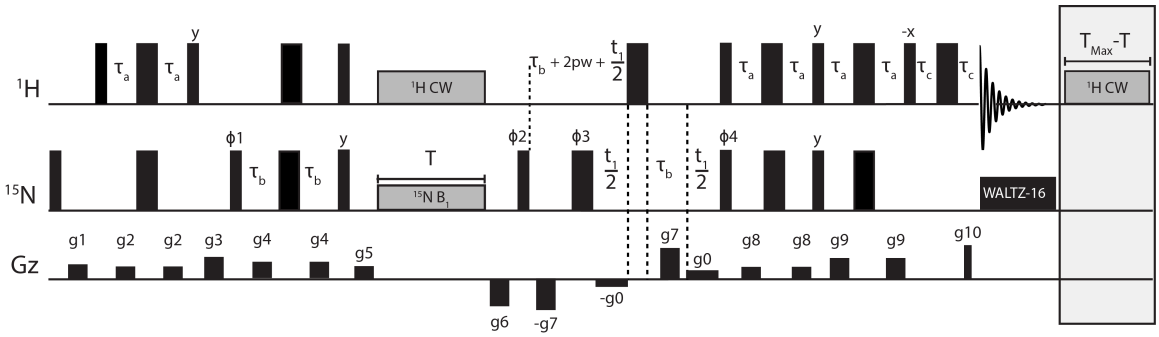

Figure S1 Pulse scheme used to measure $^1H^N$ chemical shifts of invisible states in slowly exchanging protein systems. The sequence is essentially identical to the one previously published to record ^{15}N CEST data[1], except that a weak off-resonance $^1H^N$ CW decoupling field (B_1 strength of about 1kHz) is applied during *T*, instead of composite broadband $^1H^N$ decoupling. Further details regarding the pulse sequence can be found in a previous study.[1]

Table S1 $^{1}H^{N}$ and ^{15}N chemical shift differences between ground and excited states of the A39G FF domain ($\Delta\varpi = \varpi_E - \varpi_G$), along with their uncertainty (σ), as quantified by CEST experiments at 1°C. $^{1}H^{N}$ $\Delta\varpi$ values, assuming that the excited state is a random coil with shifts as predicted by the method of Mulder *et al.*,[2] are also reported for comparison.

Residue	$\Delta\varpi_{H,RC}$ (ppm)	$\Delta\varpi_{H,CEST}$ (ppm)	$\sigma_{H,CEST}$ (ppm)	$\Delta\varpi_{N,CEST}$ (ppm)	$\sigma_{N,CEST}$ (ppm)
Gln 3	-0.20	-	-	0.23	0.02
Ala 5	-0.19	-	-	0.10	0.03
Lys 6	-0.20	-	-	-0.18	0.09
Lys 7	-0.04	-	-	-0.25	0.04
Thr 8	-0.30	-	-	-0.97	0.02
Tyr 9	-0.19	-	-	0.28	0.04
Thr 10	0.25	0.21	0.09	1.70	0.02
Trp 11	-0.04	-	-	-	-
Asn 12	-1.47	-	-	0.55	0.05
Thr 13	0.40	0.35	0.08	4.34	0.02
Lys 14	-0.71	-	-	-0.47	0.05
Glu 15	-0.15	-0.13	0.05	1.94	0.01
Glu 16	0.39	0.16	0.06	1.39	0.02
Ala 17	0.20	0.07	0.10	-1.15	0.02
Lys 18	0.09	0.00	0.07	2.18	0.01
Gln 19	0.39	0.09	0.06	1.00	0.02
Ala 20	0.26	-	-	0.56	0.05
Phe 21	0.32	-	-	0.55	0.05
Lys 22	0.28	-0.06	0.08	3.41	0.01
Glu 23	-0.11	-	-	0.27	0.08
Leu 24	0.46	-	-	-0.46	0.05
Leu 25	0.96	1.01	0.10	2.82	0.01
Lys 26	0.73	0.57	0.08	5.38	0.02
Glu 27	-0.57	-0.73	0.06	-1.95	0.01
Lys 28	0.58	0.66	0.21	6.87	0.03
Arg29	0.44	0.45	0.17	5.96	0.03
Val30	0.74	0.47	0.08	1.64	0.02
Ser32	-0.40	-	-	0.45	0.03
Asn33	0.71	0.65	0.08	4.36	0.01
Ala34	0.74	-	-	0.77	0.02
Ser 35	-0.17	-0.07	0.12	-1.31	0.02
Trp 36	-1.55	-	-	0.48	0.03

Residue	$\Delta\varpi_{H,RC}$ (ppm)	$\Delta\varpi_{H,CEST}$ (ppm)	$\sigma_{H,CEST}$ (ppm)	$\Delta\varpi_{N,CEST}$ (ppm)	$\sigma_{N,CEST}$ (ppm)
Glu 37	-0.93	-0.47	0.06	4.91	0.01
Gln 38	0.92	0.53	0.04	2.46	0.01
Gly 39	-0.35	-0.20	0.10	1.53	0.02
Met 40	-0.53	-	-	-0.97	0.02
Lys 41	1.67	1.41	0.06	6.24	0.01
Met 42	1.05	0.84	0.11	6.31	0.02
Ile 43	1.07	1.06	0.21	12.61	0.04
Ile 44	1.11	0.70	0.16	3.64	0.02
Asn 45	0.73	0.47	0.15	3.94	0.02
Asp 46	0.66	-	-	0.55	0.03
Arg 48	-1.39	-1.06	0.06	2.32	0.01
Tyr 49	-0.08	-	-	-	-
Ser 50	0.85	0.71	0.06	8.27	0.01
Ala 51	0.79	0.61	0.09	1.56	0.02
Leu 52	2.38	2.11	0.07	8.42	0.01
Ala 53	-0.41	-0.41	0.06	-2.79	0.01
Lys 54	0.13	-0.09	0.05	3.33	0.01
Leu 55	-0.82	-1.01	0.06	-6.37	0.01
Ser 56	-0.47	-0.66	0.05	3.65	0.01
Glu 57	1.85	-	-	0.61	0.04
Lys 58	0.62	-	-	0.94	0.02
Lys 59	0.45	0.41	0.08	6.35	0.01
Gln 60	0.74	0.72	0.05	2.44	0.01
Ala 61	0.47	0.56	0.08	2.25	0.01
Phe 62	-0.66	-0.46	0.12	-1.03	0.02
Asn 63	-0.36	-0.47	0.06	2.97	0.01
Ala 64	0.22	0.18	0.06	2.12	0.01
Tyr 65	-0.04	-0.05	0.05	-1.65	0.01
Lys 66	0.28	0.13	0.05	4.25	0.01
Val 67	0.89	0.97	0.05	8.14	0.01
Gln 68	0.87	0.91	0.06	4.45	0.01
Thr 69	0.33	0.35	0.06	1.85	0.01
Glu 70	0.32	-	-	0.62	0.02

Table 2 $^{1}H^{N}$ and ^{15}N chemical shift differences between the ground and the excited states of L99A T4L ($\Delta\varpi = \varpi_E - \varpi_G$), along with their uncertainty (σ), as quantified by CEST experiments at 5°C. Values extracted from CPMG relaxation dispersion experiments at 25°C are also reported for comparison[3]. $\Delta\varpi_{H,CPMG}$ values from CPMG measurements that could not be 'signed' are indicated by '±'.

Residue	$\Delta\varpi_{H,CPMG}$ (ppm)	$\Delta\varpi_{H,CEST}$ (ppm)	$\sigma_{H,CEST}$ (ppm)	$\Delta\varpi_{N,CEST}$ (ppm)	$\sigma_{N,CEST}$ (ppm)
Asn 2	±0.04	-	-	-0.27	0.05
Ile 3	0.23	0.23	0.27	-1.11	0.03
Phe 4	-0.46	-0.85	0.20	-1.44	0.03
Glu 5	-0.09	-	-	-	-
Met 6	±0.24	-	-	0.51	0.10
Leu 7	0.14	0.21	0.17	-2.71	0.03
Arg 8	-0.34	-	-	-	-
Ile 9	0.08	0.44	0.28	1.10	0.05
Asp 10	±0.00	-	-	-	-
Glu 11	-0.16	-0.08	0.24	-3.75	0.04
Gly 12	-	-	-	0.79	0.07
Leu 13	-0.27	-0.30	0.06	-3.08	0.01
Arg 14	0.28	-	-	-0.81	0.03
Leu 15	±0.05	-	-	-0.28	0.09
Lys 16	±0.02	-	-	-0.27	0.09
Ile 17	±0.00	-	-	0.15	0.08
Tyr 18	±0.01	-	-	0.22	0.11
Lys 19	0.02	-	-	0.86	0.02
Asp 20	±0.00	-	-	-	-
Thr 21	±0.04	-	-	-0.64	0.04
Gly 23	±0.01	-	-	-0.36	0.17
Tyr 24	0.06	-	-	-0.25	0.21
Tyr 25	±0.00	-	-	-0.24	0.10
Thr 26	0.07	-	-	-0.49	0.08
Ile 27	0.08	-	-	-0.49	0.06
Gly 28	0.11	-	-	-	-
Ile 29	0.72	0.73	0.16	4.76	0.02
Gly 30	0.21	0.36	0.09	-1.63	0.02
His 31	-0.11	-0.44	0.17	-1.35	0.03
Leu 32	-0.21	-	-	-0.34	0.10
Leu 33	-0.05	-	-	0.59	0.06
Thr 34	±0.01	-	-	-0.42	0.10

Residue	$\Delta\varpi_{H,CPMG}$ (ppm)	$\Delta\varpi_{H,CEST}$ (ppm)	$\sigma_{H,CEST}$ (ppm)	$\Delta\varpi_{N,CEST}$ (ppm)	$\sigma_{N,CEST}$ (ppm)
Lys 35	-0.00	-	-	-0.27	0.13
Ser 36	±0.05	-	-	-	-
Ser 38	±0.00	-	-	-0.08	0.03
Leu 39	±0.00	-	-	-0.36	0.06
Asn 40	±0.00	-	-	0.20	0.20
Ala 41	±0.00	-	-	-0.14	0.23
Ala 42	±0.01	-	-	-	-
Lys 43	±0.00	-	-	0.26	0.14
Ser 44	±0.00	-	-	-	-
Leu 46	±0.00	-	-	0.03	0.82
Asp 47	±0.09	-	-	-0.44	0.10
Lys 48	±0.04	-	-	-0.24	0.08
Ala 49	±0.00	-	-	0.07	0.46
Ile 50	±0.06	-	-	-0.25	0.27
Gly 51	±0.07	-	-	0.27	0.25
Arg 52	0.01	-	-	-	-
Asn 53	0.00	-	-	-	-
Thr 54	±0.01	-	-	0.25	0.17
Asn 55	±0.00	-	-	0.20	0.18
Gly 56	±0.00	-	-	0.31	0.14
Val 57	±0.01	-	-	-0.40	0.12
Ile 58	-0.06	-	-	0.28	0.06
Thr 59	±0.00	-	-	-0.32	0.09
Lys 60	±0.00	-	-	0.29	0.06
Asp 61	±0.00	-	-	0.31	0.12
Glu 62	±0.05	-	-	0.46	0.07
Ala 63	±0.00	-	-	0.10	0.10
Glu 64	0.00	0.02	0.12	-1.60	0.02
Lys 65	0.23	-	-	-	-
Leu 66	±0.04	-	-	-0.29	0.15
Phe 67	-0.17	-	-	0.20	0.20
Asn 68	-0.31	-	-	0.14	0.35
Gln 69	0.19	-	-	-	-
Asp 70	0.52	-	-	-0.42	0.09
Val 71	-0.17	-	-	-0.34	0.16
Asp 72	-0.15	-	-	-0.66	0.08
Ala 73	0.37	-	-	-0.70	0.09
Ala 74	-0.33	-	-	-0.77	0.05
Val 75	-0.56	-0.84	0.16	-2.35	0.03
Arg 76	0.35	-	-	-0.01	0.73
Gly 77	0.21	-	-	0.82	0.04
Ile 78	-0.51	-0.18	0.14	-2.03	0.03
Leu 79	-0.05	-	-	-	-

Residue	$\Delta\varpi_{H,CPMG}$ (ppm)	$\Delta\varpi_{H,CEST}$ (ppm)	$\sigma_{H,CEST}$ (ppm)	$\Delta\varpi_{N,CEST}$ (ppm)	$\sigma_{N,CEST}$ (ppm)
Arg 80	0.32	0.18	0.14	-1.62	0.03
Asn 81	-0.12	-	-	-0.76	0.05
Ala 82	-0.11	0.06	0.15	-1.25	0.01
Lys 83	-0.04	-	-	0.75	0.05
Leu 84	-0.25	-	-	0.27	0.19
Lys 85	-0.33	-0.33	0.12	-1.39	0.02
Val 87	0.06	-	-	0.60	0.03
Tyr 88	-0.06	-	-	-0.29	0.14
Asp 89	±0.02	-	-	-0.76	0.03
Ser 90	0.08	-	-	0.59	0.05
Leu 91	0.20	-0.02	0.17	1.45	0.02
Asp 92	±0.03	-	-	0.66	0.03
Ala 93	-0.03	-	-	-	-
Val 94	0.12	-	-	-0.48	0.09
Arg 95	0.15	-	-	0.64	0.07
Arg 96	-0.08	-	-	0.74	0.04
Ala 97	-0.15	-	-	-0.47	0.09
Ala 98	0.23	-	-	0.33	0.33
Ala 99	0.16	-	-	-	-
Ile 100	-0.46	-0.19	0.09	-2.14	0.02
Asn 101	-0.32	-0.33	0.11	-3.39	0.02
Met 102	0.08	-	-	-	-
Val 103	-0.84	-1.00	0.10	-13.40	0.01
Phe 104	-1.29	-1.22	0.14	2.98	0.02
Gln 105	0.60	0.64	0.11	2.73	0.02
Met 106	0.20	0.05	0.16	4.28	0.03
Gly 107	-0.64	-	-	-	-
Glu 108	-0.29	-0.17	0.09	3.04	0.01
Thr 109	0.22	-	-	-	-
Gly 110	1.09	0.91	0.12	7.13	0.02
Val 111	0.41	0.62	0.14	-1.95	0.02
Ala 112	0.22	0.17	0.08	6.46	0.01
Gly 113	0.90	0.91	0.07	3.51	0.01
Phe 114	0.18	-	-	-0.54	0.06
Thr 115	0.58	-	-	0.30	0.09
Asn 116	-0.69	-	-	-0.46	0.06
Ser 117	-1.05	-1.30	0.12	-4.20	0.02
Leu 118	-1.25	-	-	0.91	0.04
Arg 119	0.89	0.92	0.16	-1.29	0.03
Met 120	-0.20	-0.32	0.20	-1.02	0.03
Leu 121	-0.49	-0.26	0.16	-1.42	0.03
Gln 122	0.47	-	-	-0.61	0.06
Gln 123	±0.07	-	-	0.38	0.11

Residue	$\Delta\varpi_{H,CPMG}$ (ppm)	$\Delta\varpi_{H,CEST}$ (ppm)	$\sigma_{H,CEST}$ (ppm)	$\Delta\varpi_{N,CEST}$ (ppm)	$\sigma_{N,CEST}$ (ppm)
Lys 124	-0.10	-	-	-0.21	0.39
Arg 125	±0.04	-	-	-0.50	0.07
Trp 126	0.13	-	-	0.55	0.04
Asp 127	±0.02	-	-	0.33	0.08
Glu 128	±0.07	-	-	-0.32	0.09
Ala 129	-0.02	-	-	-0.63	0.06
Ala 130	0.11	-	-	0.47	0.07
Val 131	0.09	-	-	-0.01	0.09
Asn 132	±0.14	-	-	0.30	0.17
Leu 133	-0.10	-	-	-0.28	0.16
Ala 134	0.40	0.20	0.16	2.10	0.02
Lys 135	0.11	-	-	-	-
Ser 136	±0.03	0.06	0.06	2.59	0.01
Arg 137	-0.40	0.73	0.33	-1.00	0.04
Trp 138	0.18	-0.03	0.10	-1.49	0.02
Tyr 139	0.23	-	-	-0.07	1.00
Asn 140	-	-	-	0.28	0.13
Gln 141	-0.54	-	-	-0.96	0.05
Thr 142	-0.58	-0.70	0.14	4.31	0.02
Asn 144	0.21	-	-	0.29	0.10
Arg 145	0.07	-0.06	0.13	1.57	0.02
Ala 146	0.44	-	-	0.55	0.09
Lys 147	-0.23	-	-	-	-
Arg 148	0.20	-	-	-	-
Val 149	0.27	-	-	0.20	0.20
Ile 150	-0.27	-	-	0.87	0.06
Thr 151	-0.18	-	-	0.39	0.14
Thr 152	0.08	-	-	-0.84	0.10
Phe 153	0.12	-	-	0.15	0.19
Arg 154	-0.31	-	-	-0.74	0.07
Thr 155	0.07	-	-	0.77	0.07
Gly 156	±0.00	-	-	-	-
Thr 157	±0.04	-	-	-0.06	0.22
Trp 158	-0.09	-	-	-0.25	0.10
Asp 159	±0.02	-	-	-0.35	0.13
Ala 160	±0.06	-	-	-0.46	0.09
Tyr 161	-0.00	-	-	-0.10	0.46
Lys 162	0.09	-	-	-0.01	0.10
Asn 163	0.01	-	-	-0.15	0.13
Leu 164	0.08	-	-	-0.28	0.02

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