Non-equilibrium hydrogen exchange for determination of H-bond strength and water accessibility in solid proteins.

Supporting Information

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Site-resolved hydrogen non-equilibrium exchange rates

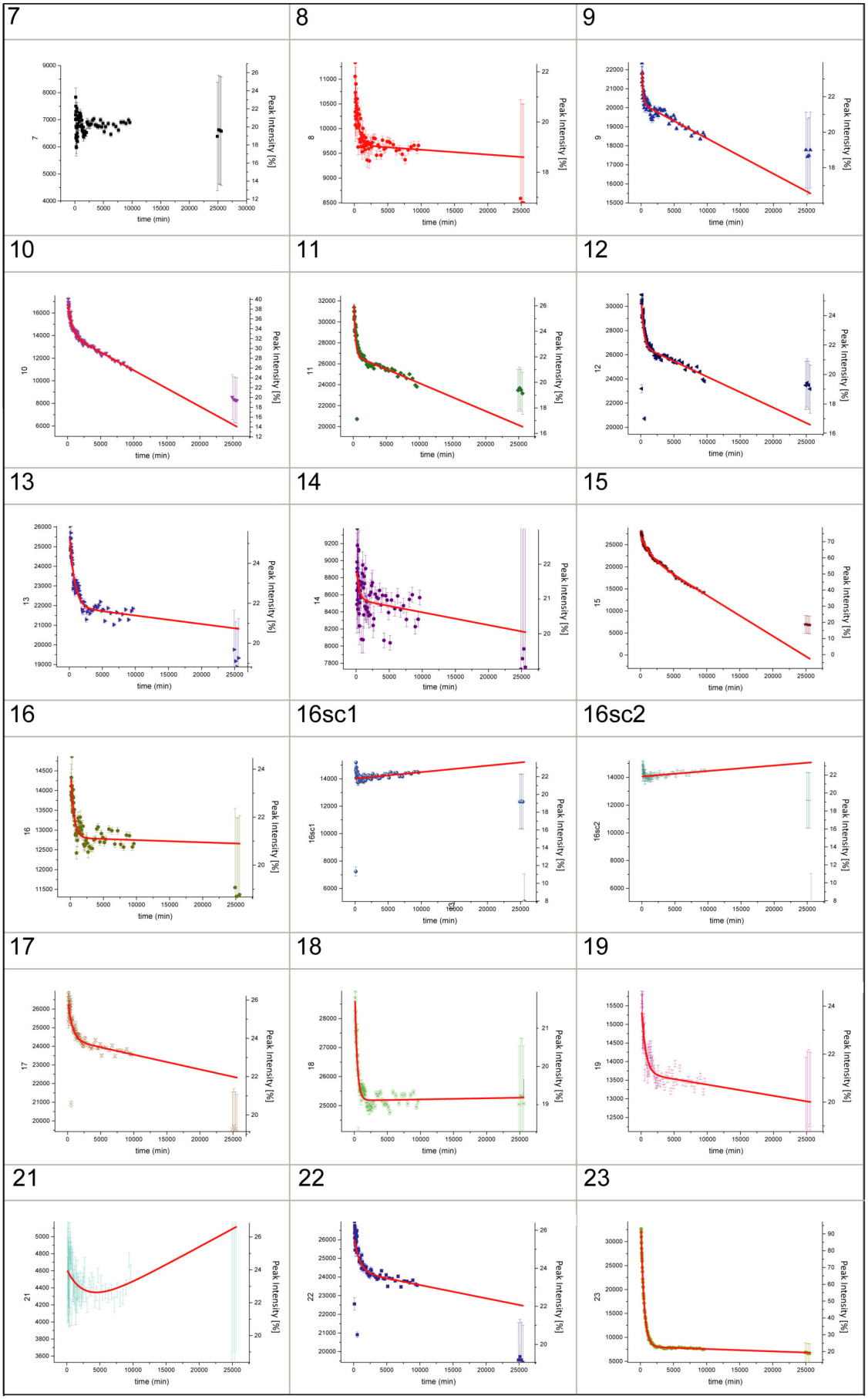
residue	k (Eq. 2) [10^{-3} ·min^{-1}]	Error [10^{-3} · min^{-1}]	Adj. R^2	k_1 (Eq. 1) [10^{-3} ·min^{-1}]	Error [10^{-3} · min^{-1}]	Adj. R^2
7	eq.*			eq.*		
8	1.92#	0.424	0.66	1.92#	0.427	0.65
9	5.05	1.39	0.87	5.20	1.57	0.89
10	1.63	0.162	0.98	2.03	0.299	0.98
11	4.26	1.23	0.71	4.39	1.45	0.70
12	overlap			overlap		
13	1.35	0.185	0.87	1.40	0.336	0.87
14	eq.*			eq.*		
15	0.671	0.116	0.99	3.69	0.791	0.99
16	2.07#	0.447	0.63	2.07#	0.451	0.63
17	overlap			overlap		
18	overlap			overlap		

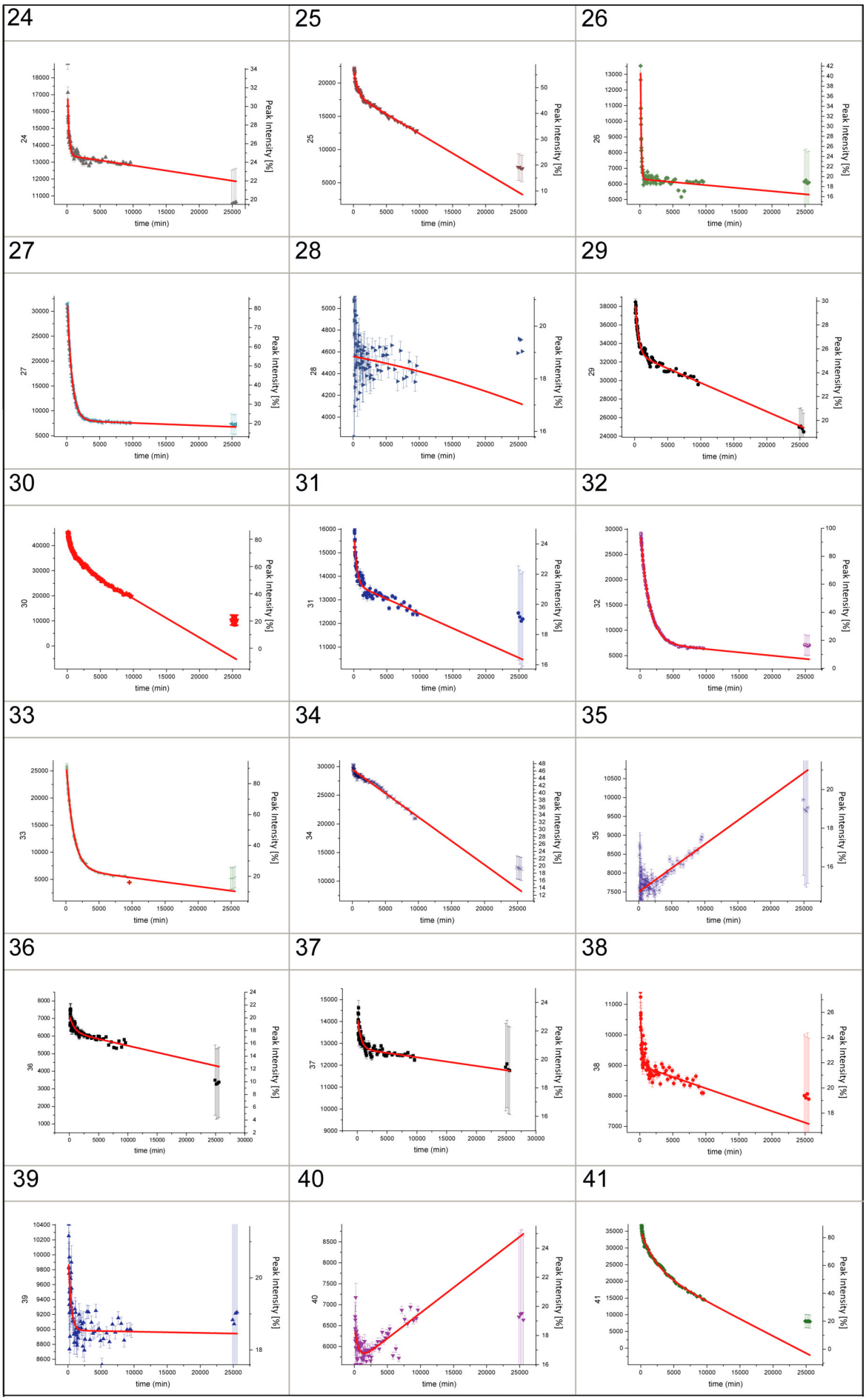
19	1.63	0.349	0.72	1.63	0.351	0.72
21	eq.*			eq.*		
22	overlap			overlap		
23	2.19	0.0231	1.0	2.20	0.0338	1.0
24	4.73	0.600	0.84	5.48	0.805	0.85
25	1.77	0.198	0.99	2.86	0.464	0.99
26	9.61	0.992	0.85	9.87	1.10	0.85
27	1.45	0.0153	1.0	1.46	0.0219	1.0
28	eq.*			eq.*		
29	2.05	0.191	0.93	2.49	0.343	0.96
30	0.780	0.0860	0.99	1.57	0.244	1.0
31	2.51	0.416	0.90	3.12	0.688	0.90
32	0.748	0.0150	1.0	0.790	0.0422	1.0
33	0.935	0.0193	1.0	0.963	0.0381	1.0
34	eq.*			eq.*		
35	eq.*			eq.*		
36	overlap			overlap		
37	1.79	0.340	0.84	1.79	0.342	0.80
38	3.63	0.892	0.73	3.63	0.898	0.73
39	2.54#	0.838	0.39	2.47#	0.692	0.38
40	eq.*			eq.*		
41	0.421	0.0625	0.99	0.719	0.240	0.99
42	2.95	0.397	0.83	3.00	0.488	0.83
43	4.37#	1.20	0.65	3.70#	1.14	0.65
44	1.69	0.276	0.90	1.85	0.438	0.90
45	eq.*			eq.*		
46	7.77#	1.68	0.61	7.81#	1.80	0.61

49	1.62#	0.470	0.58	1.62#	0.473	0.57
50	2.34	0.493	0.83	2.34	0.496	0.83
51	11.5	0.955	0.89	11.5	0.937	0.88
52	0.444	0.0321	1.0	0.700	0.0997	1.0
53	3.57	0.461	0.86	3.57	0.465	0.86
55	1.46	0.149	0.94	1.63	0.283	0.94
56	eq.*			eq.*		
57	1.40	0.322	0.85	1.72	6.94	0.85
58	1.52	0.227	0.83	1.59	0.395	0.83
59	overlap			overlap		
60	2.03#	0.524	0.44	3.45#	1.01	0.35
61	1.70#	0.329	0.70	1.76#	0.544	0.70
62	1.42#	0.194	0.58	1.47	0.343	0.84

Table S1: Site-resolved non-equilibrium hydrogen exchange rates expressed in 10^{-3} min^{-1}. The table contains all the exchange rates from unambiguous peaks with an exchange slow enough for non-equilibrium measurements but faster than the rate originating from water accessibility hindrance. *k* (Eq. 1) and *k* (Eq. 2) are fitted using equation 1 and 2 (main text), respectively. Data colored in red and marked with a hash are fitted with an adjusted R^2 value lower than 0.70 and therefore need to be excluded from consideration. These can be considered to represent fast exchange, where equilibrium protonation levels have almost been reached at the first time point. Residues marked with eq.* were already at their equilibrium intensity before the first measured time point and were counted as exchanging too fast for recording. These are depicted as ruby-colored columns in Fig. 3 (main text). Residues marked as “overlap” are ambiguous.

Peak intensities from 76 consecutive 2D HN-spectra

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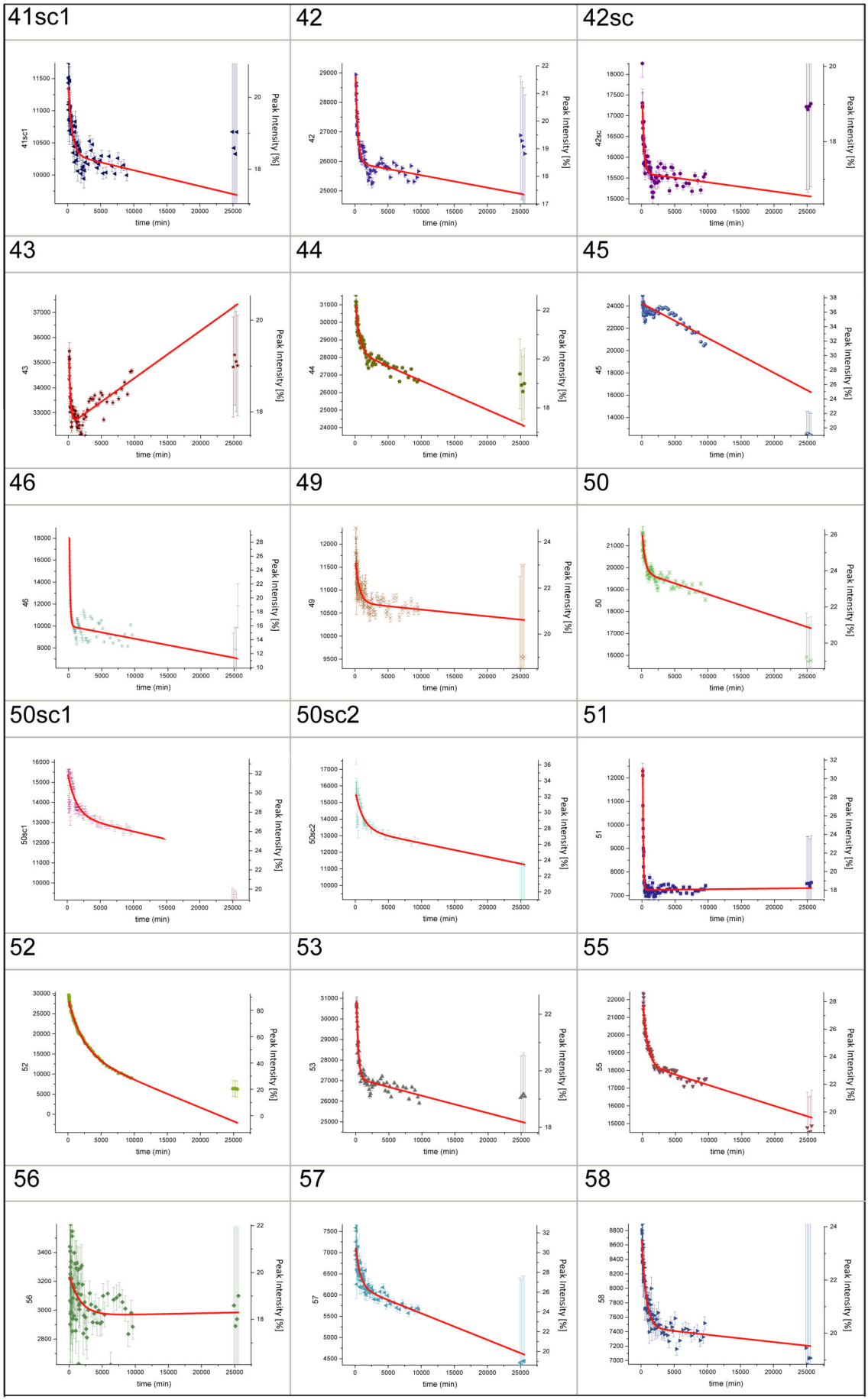
time (min)
Peak Intensity [%]


41sc1
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42sc
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50sc1
50sc2
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time (min)
Peak Intensity [%]

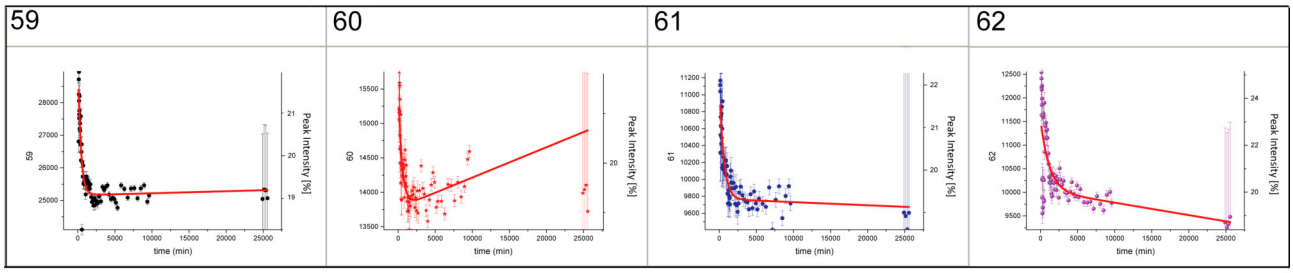


Fig S1: Peak intensities from 76 consecutive 2D H/N spectra measured over two weeks with increasing numbers of scans as described in the main text. Numbers 7 till 62 correspond to residue E7 till D62 of chicken α-spectrin. 16Sc1, 16sc2, 50sc1, 50sc2, 41sc and 42sc are corresponding to side chain amides. All graphs are fitted using equation 2 (main text). Protonation percentage scales on the right are approximate due to unknowns in the conversion from the individual intensities.

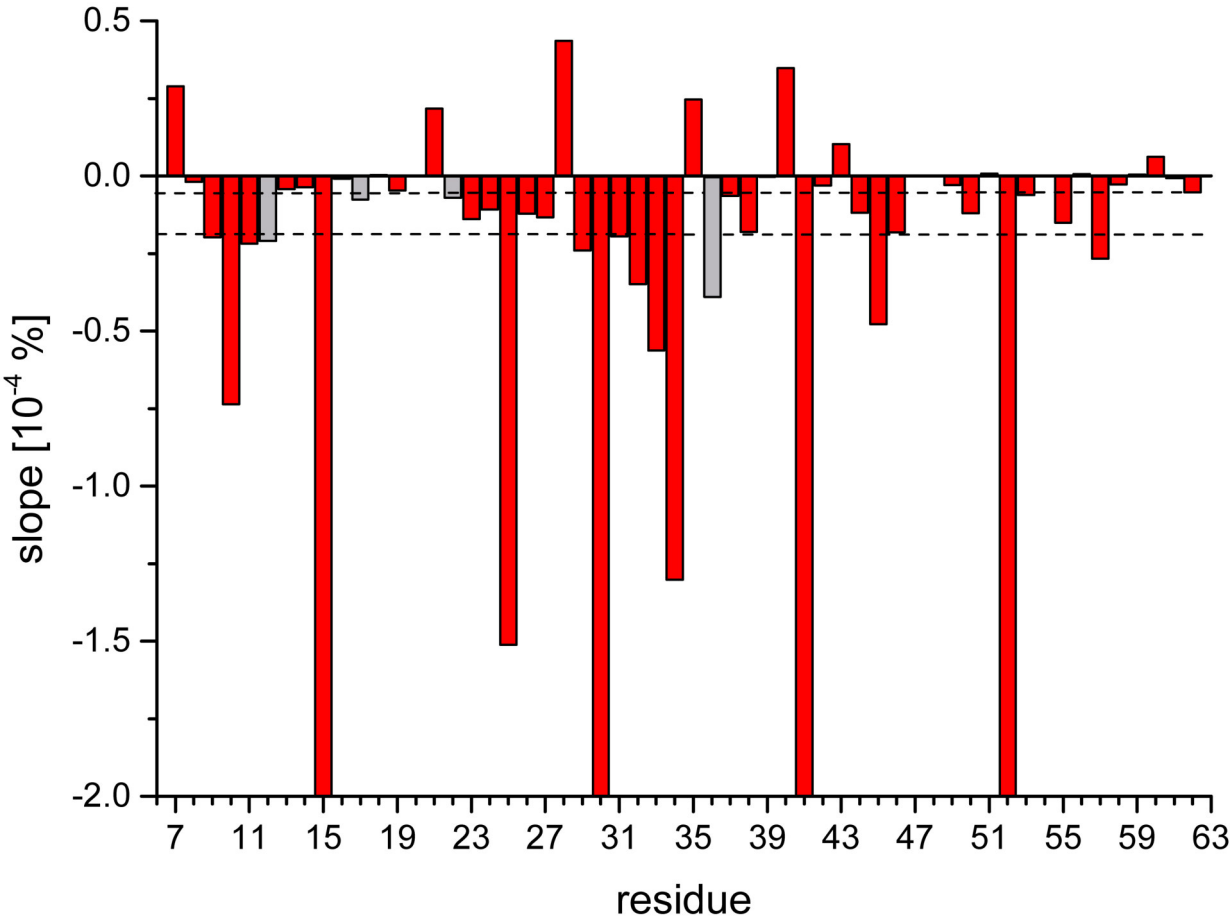
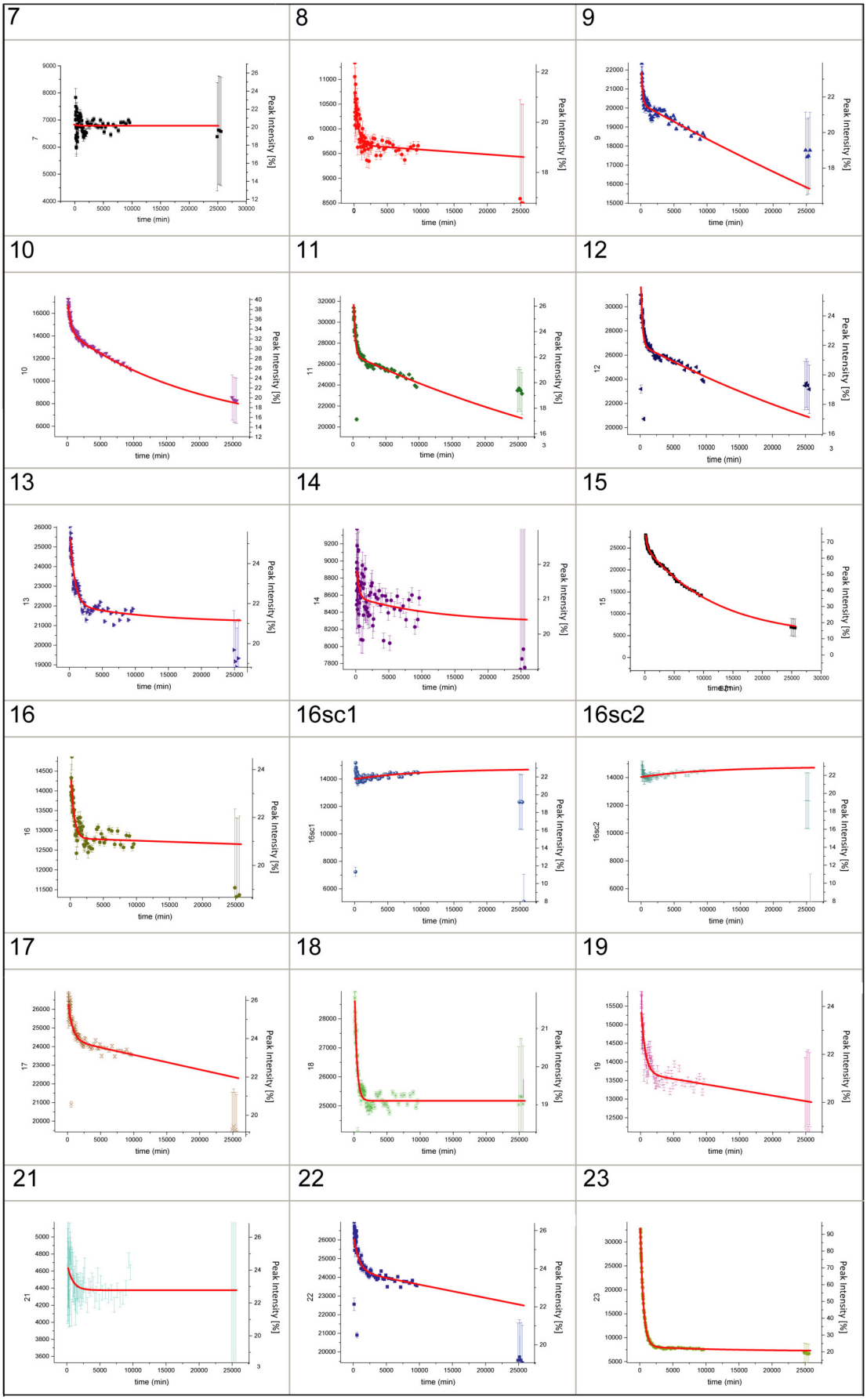


Fig. S2: Slope of the decay curves shown in figure S1, fitted using equation 2 (main text). In order to classify the residues qualitatively as accessibility-hindered or non-accessibility-hindered, two borders of slope D were defined. An upper border of $1.9 \cdot 10^{-5}$ % · min^{-1} in magnitude identifies all decay curves with a slow exchange component, as belonging to diffusion-hindered residues. A lower border of $5.2 \cdot 10^{-6}$ % · min^{-1} in magnitude categorizes decay curves bearing no such slow-decay component. These curves belong to residues that are water-accessible. Some decay curves show a positive slope, this originates mostly from low signal to noise and therefore inaccurate fitting. Another explanation for the existence of positive slopes is the slight increase of the proton concentration in the buffer due to the release of amide protons (as described in the main text).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16sc1
16sc2
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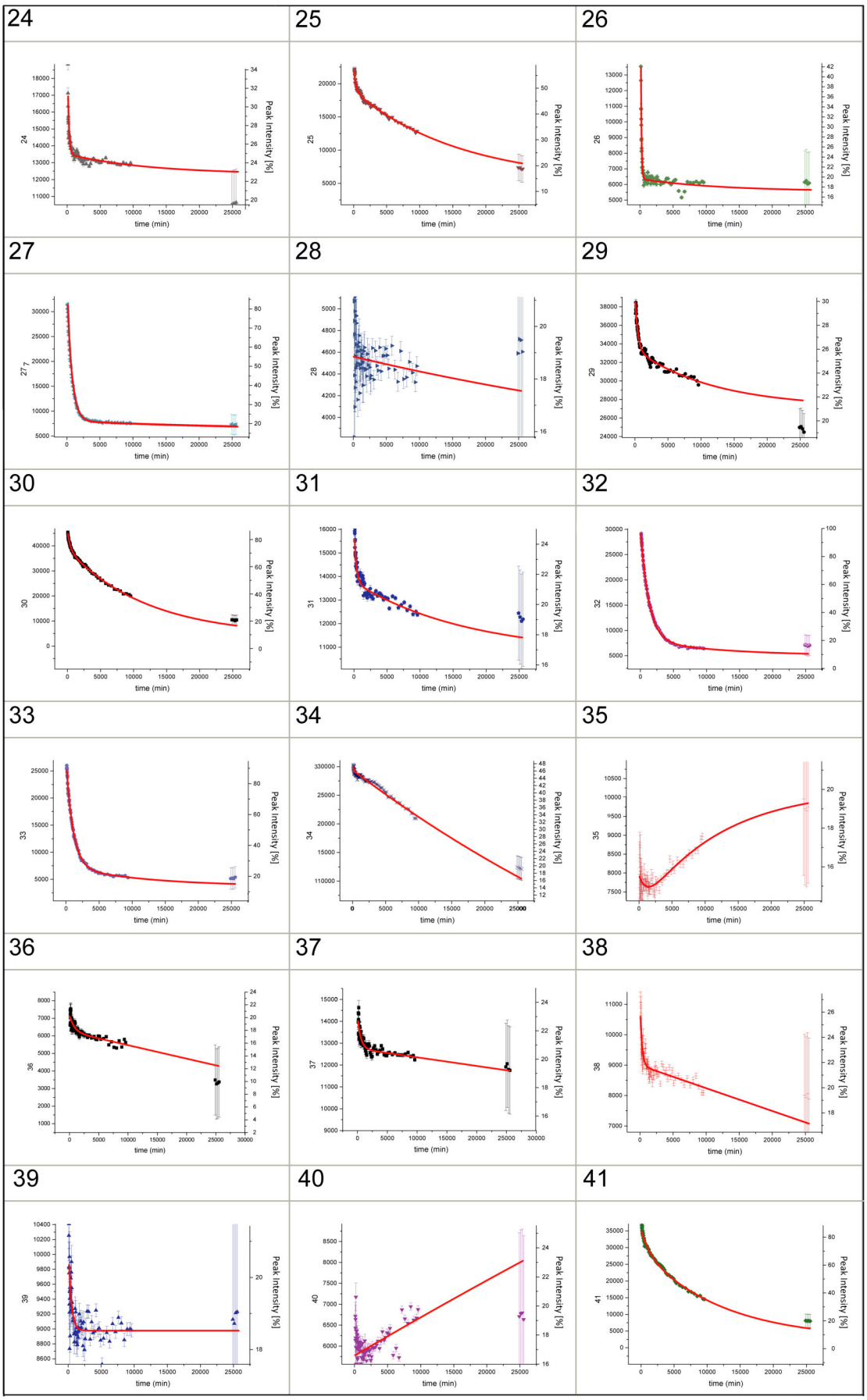
time (min)
Peak Intensity [%]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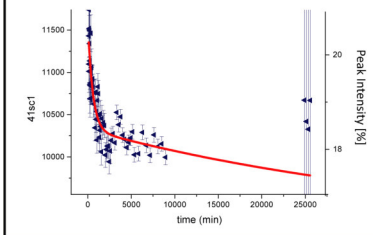
time (min)
Peak Intensity [%]

41sc1



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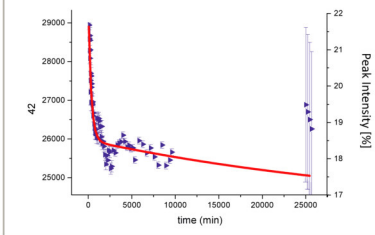
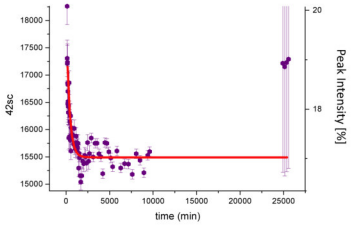


42sc



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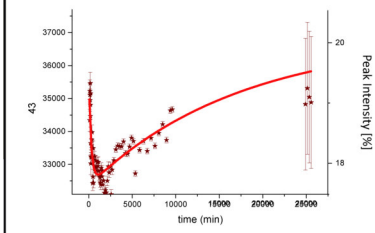

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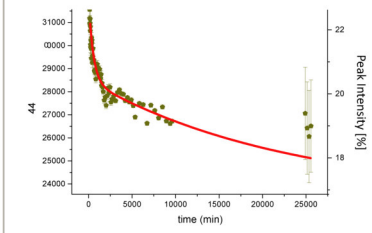

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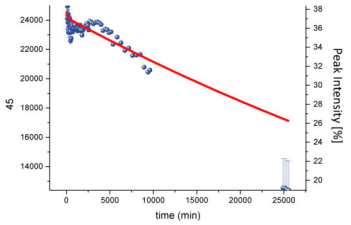

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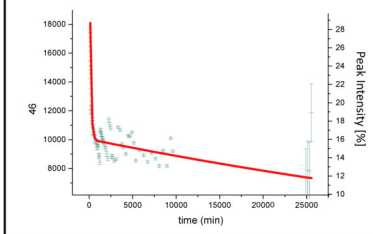

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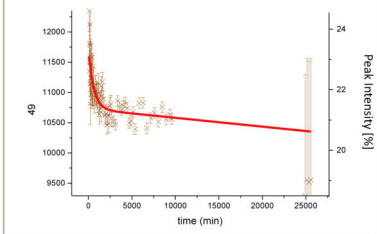

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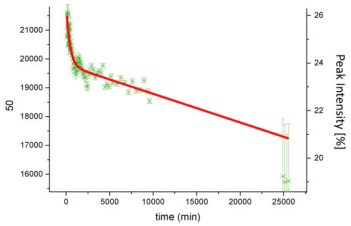
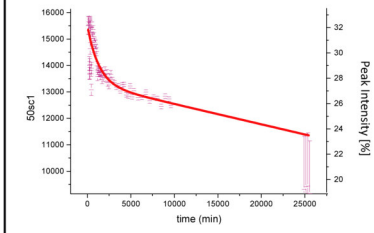
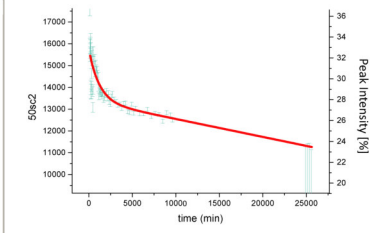


50sc1



50sc2



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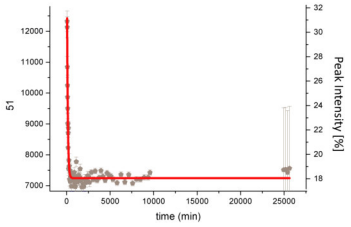

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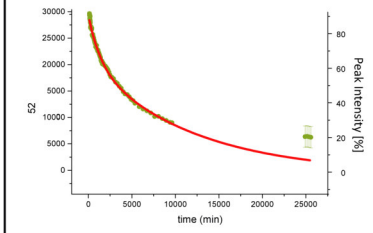

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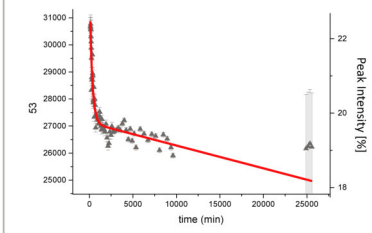

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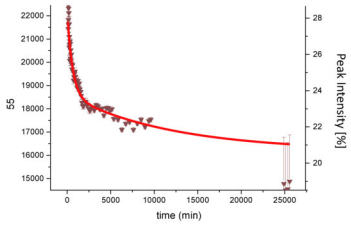

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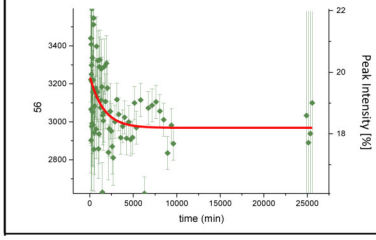

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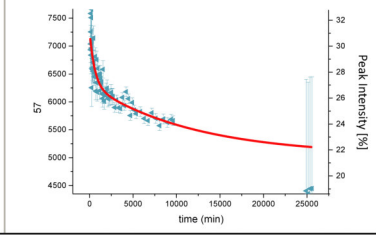

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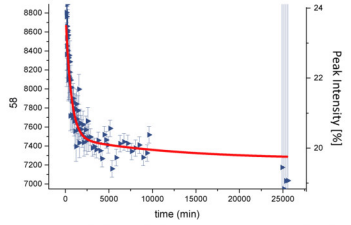

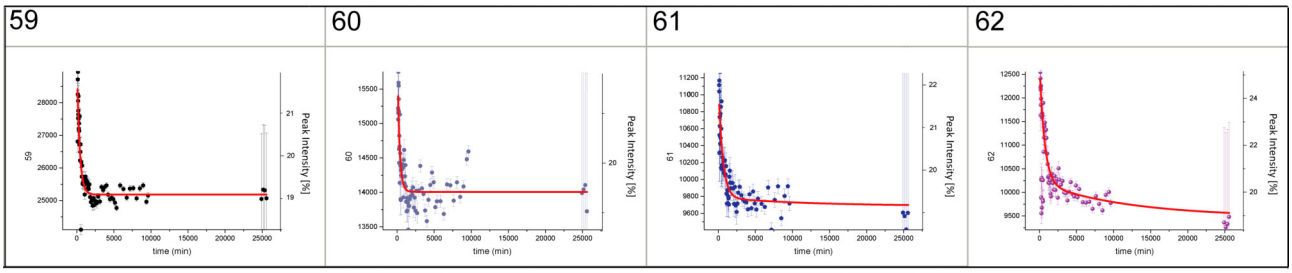

Fig S3: Peak intensities from 76 consecutive 2D H/N spectra measured over two weeks with increasing numbers of scans as described in the main text. Numbers 7 till 62 correspond to residue E7 till D62 of chicken α-spectrin. 16Sc1, 16sc2, 50sc1, 50sc2, 41sc and 42sc correspond to side chain amides. All graphs are fitted using equation 1 (main text). Protonation percentage scales on the right are approximate due to unknowns in the conversion from the individual intensities.