Highly Perturbed pKa Values in the Unfolded State of Hen Egg White Lysozyme

John Bradley, Fergal O'Meara, Damien Farrell, and Jens Erik Nielsen

School of Biomolecular and Biomedical Science, Centre for Synthesis and Chemical Biology, University College Dublin Conway Institute, University College Dublin, Dublin, Ireland
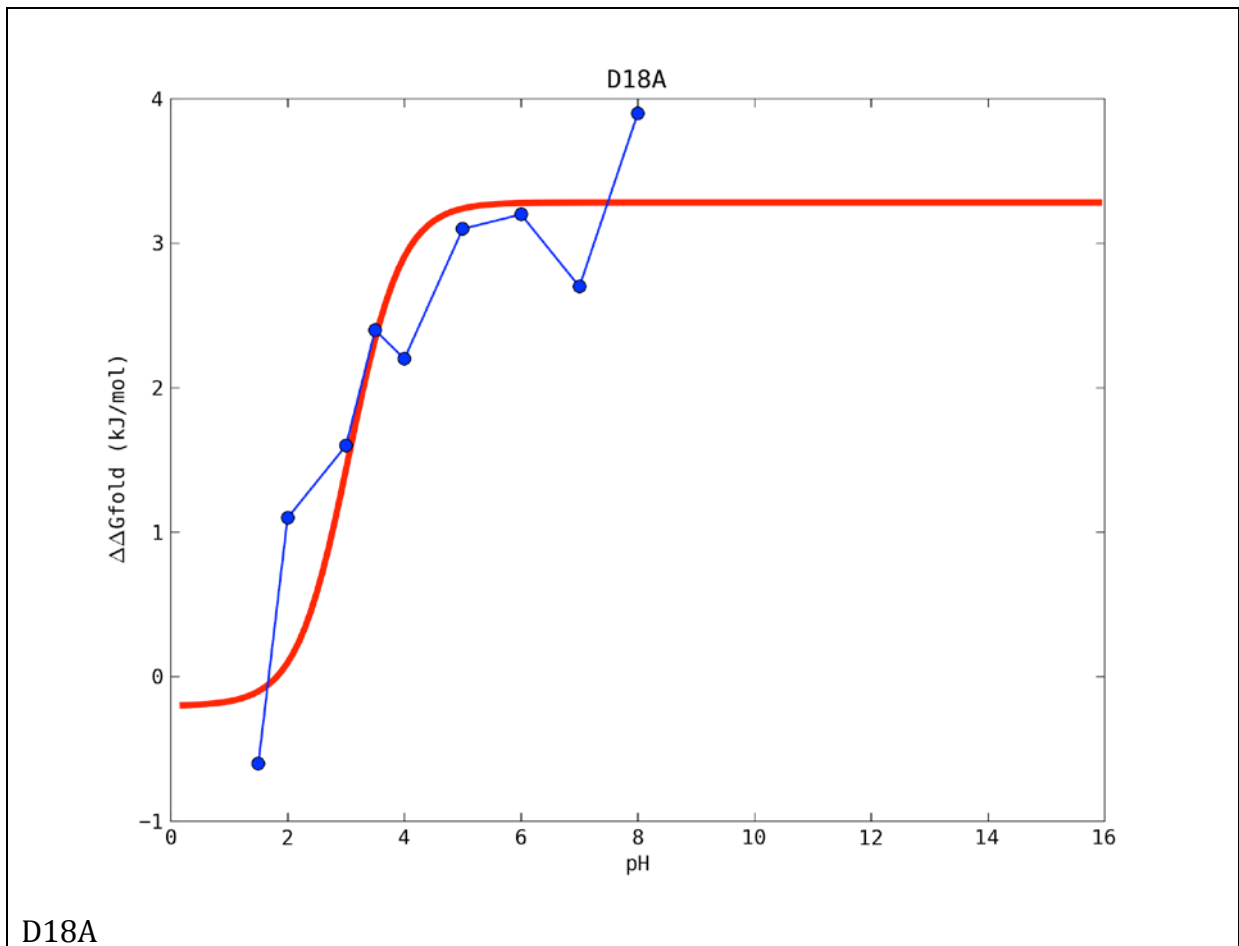
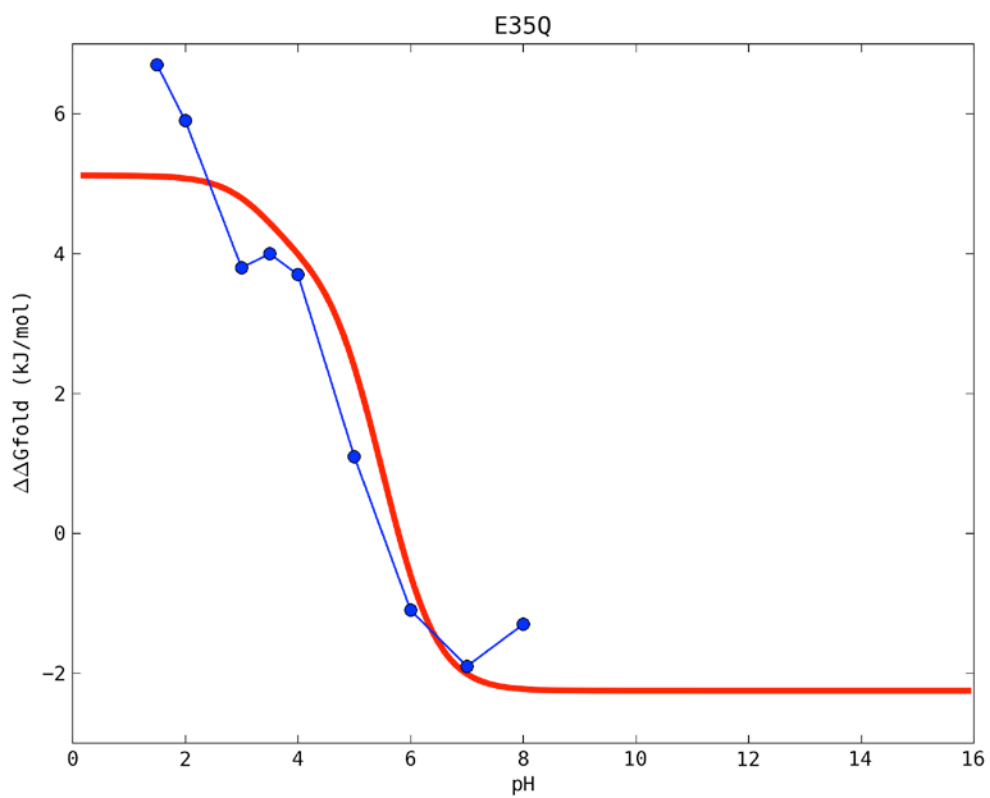**Supplementary material**

Figure 1

CD-wavelength scans of wild type Hen Egg White Lysozyme before and after two thermal denaturation runs (10-95°C) in a 50mM Britton-Robinson buffer as described in Materials and Methods.
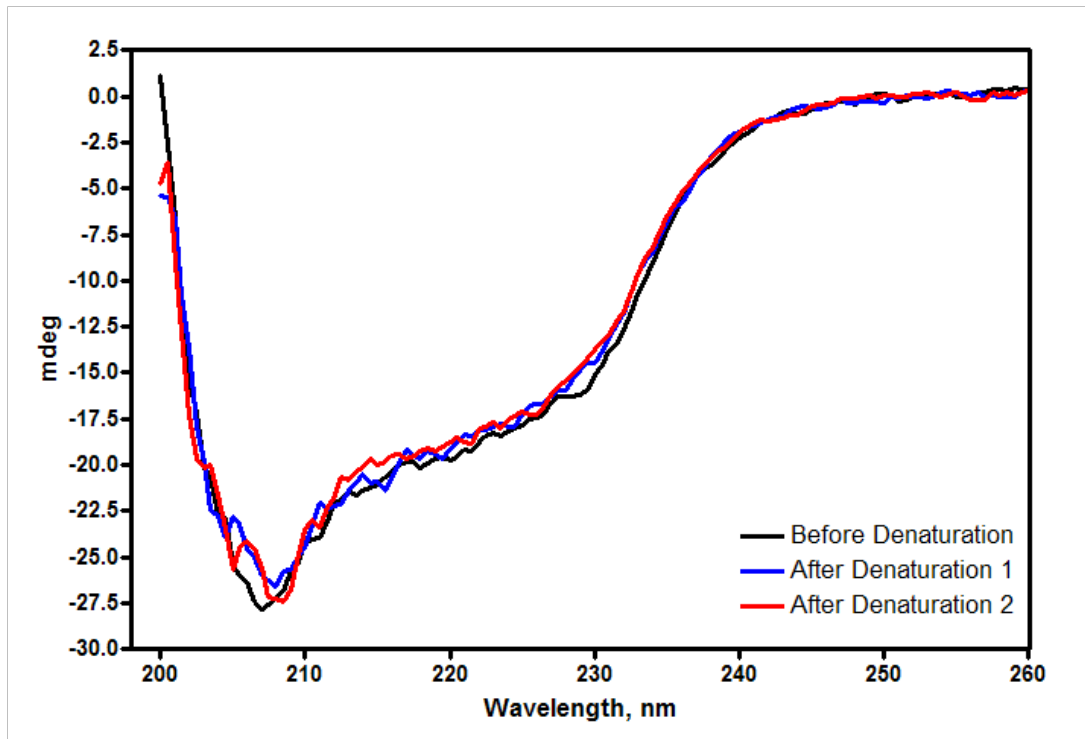
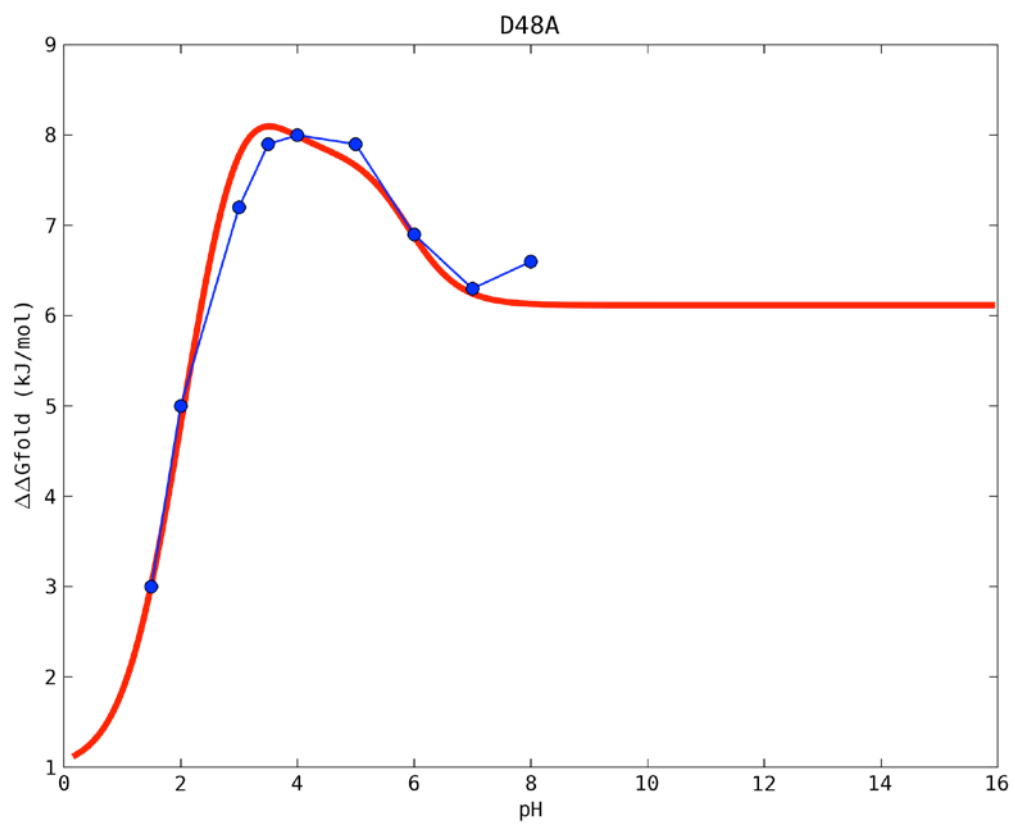## Figure 2

Fits of the differential pH-stability profiles for the eight mutants of HEWL.

D18A

E35Q

D48A

D52A

D66A

D101A
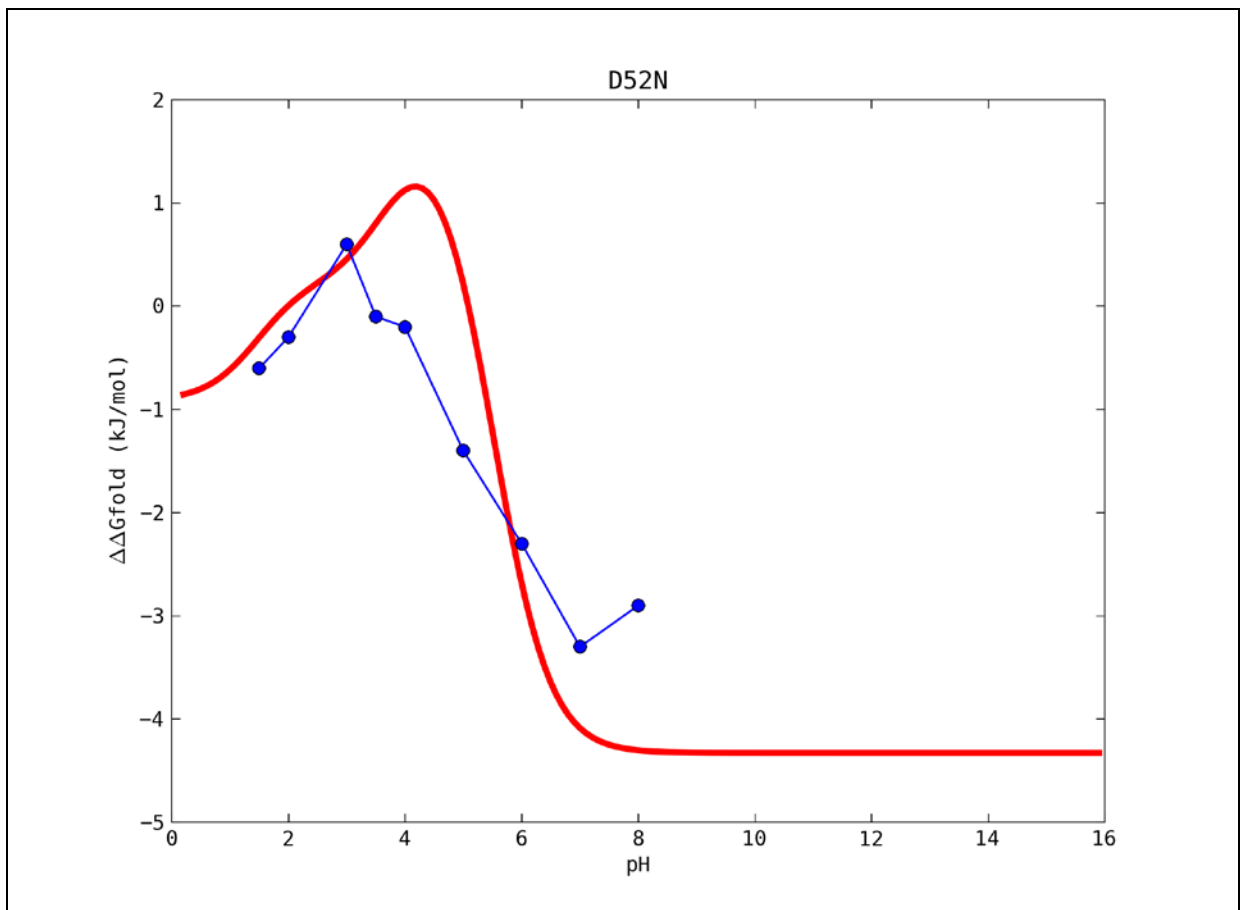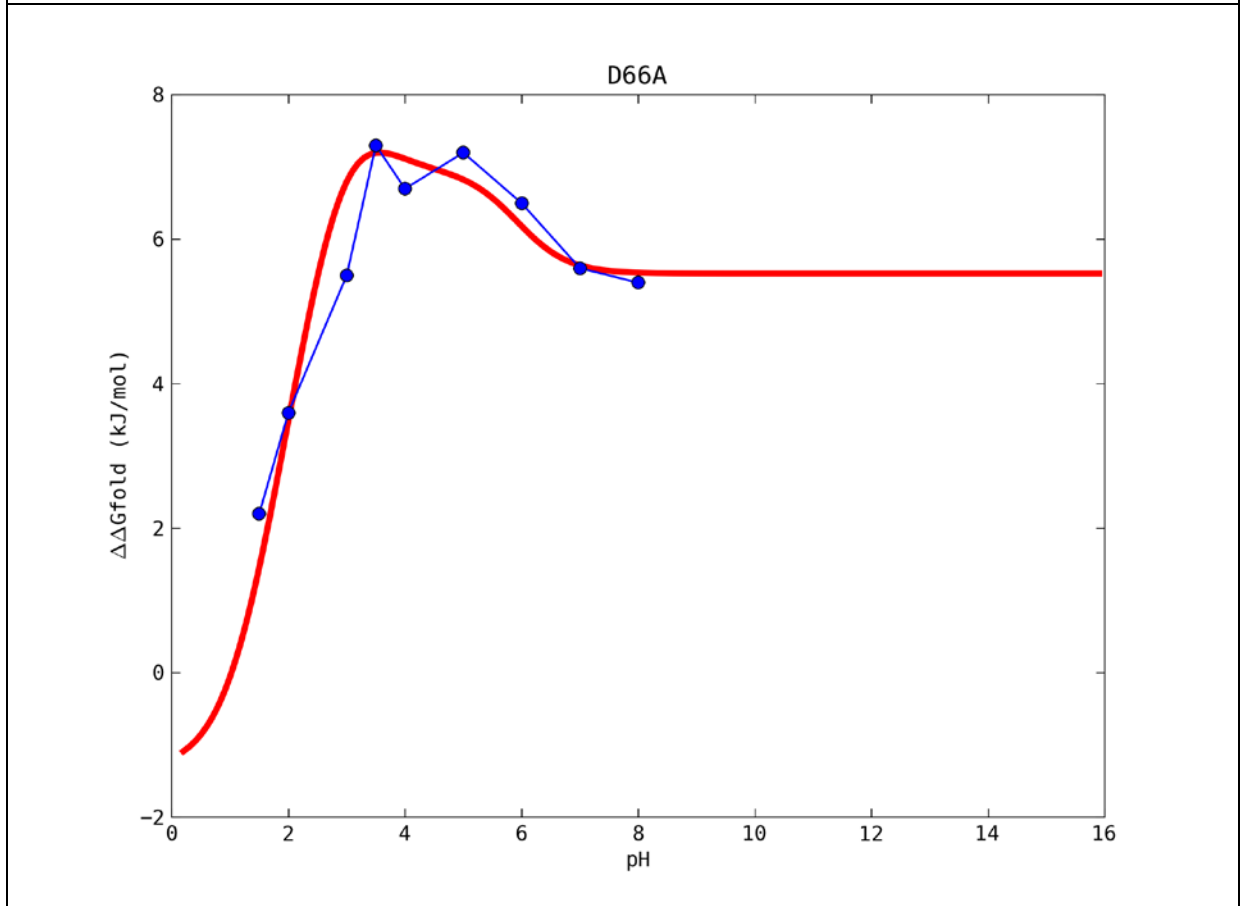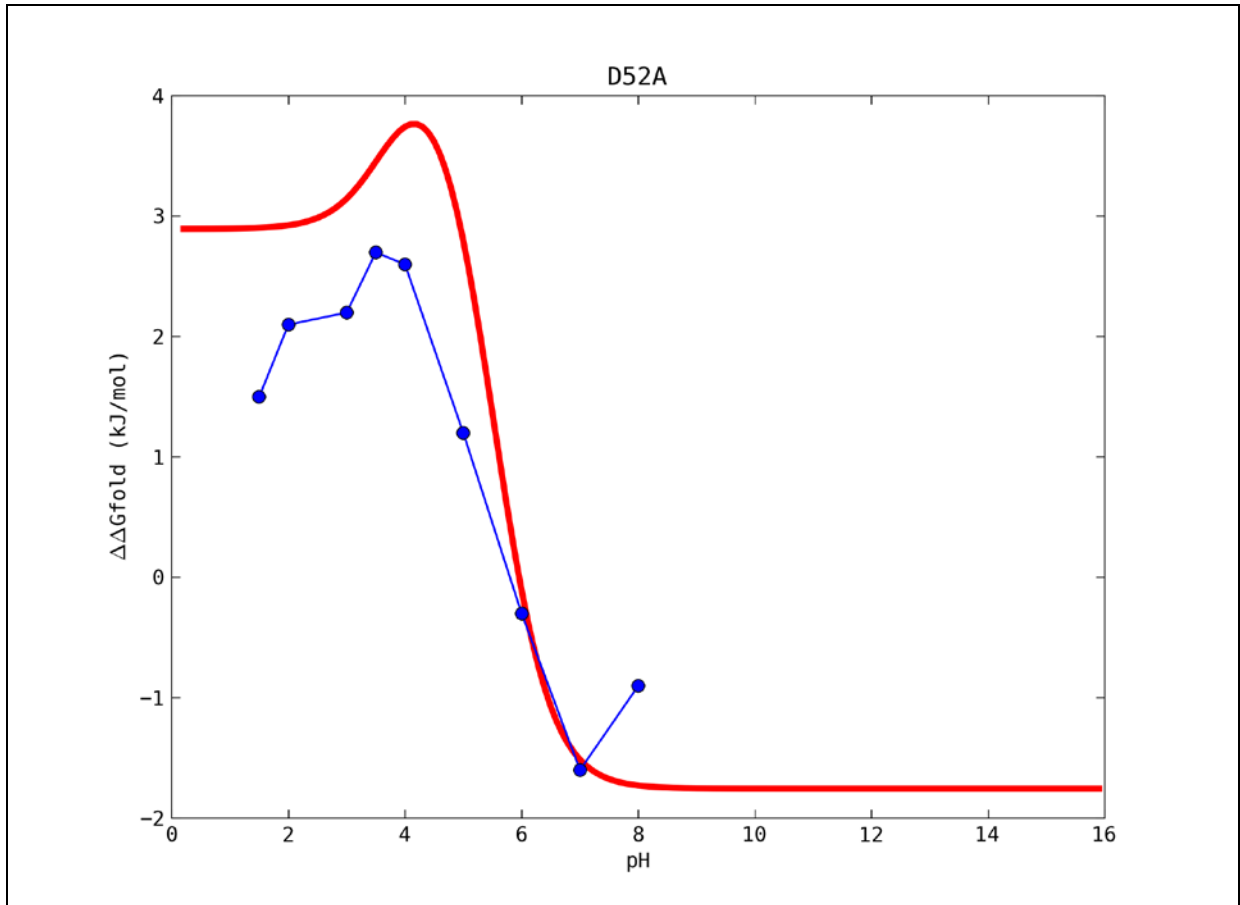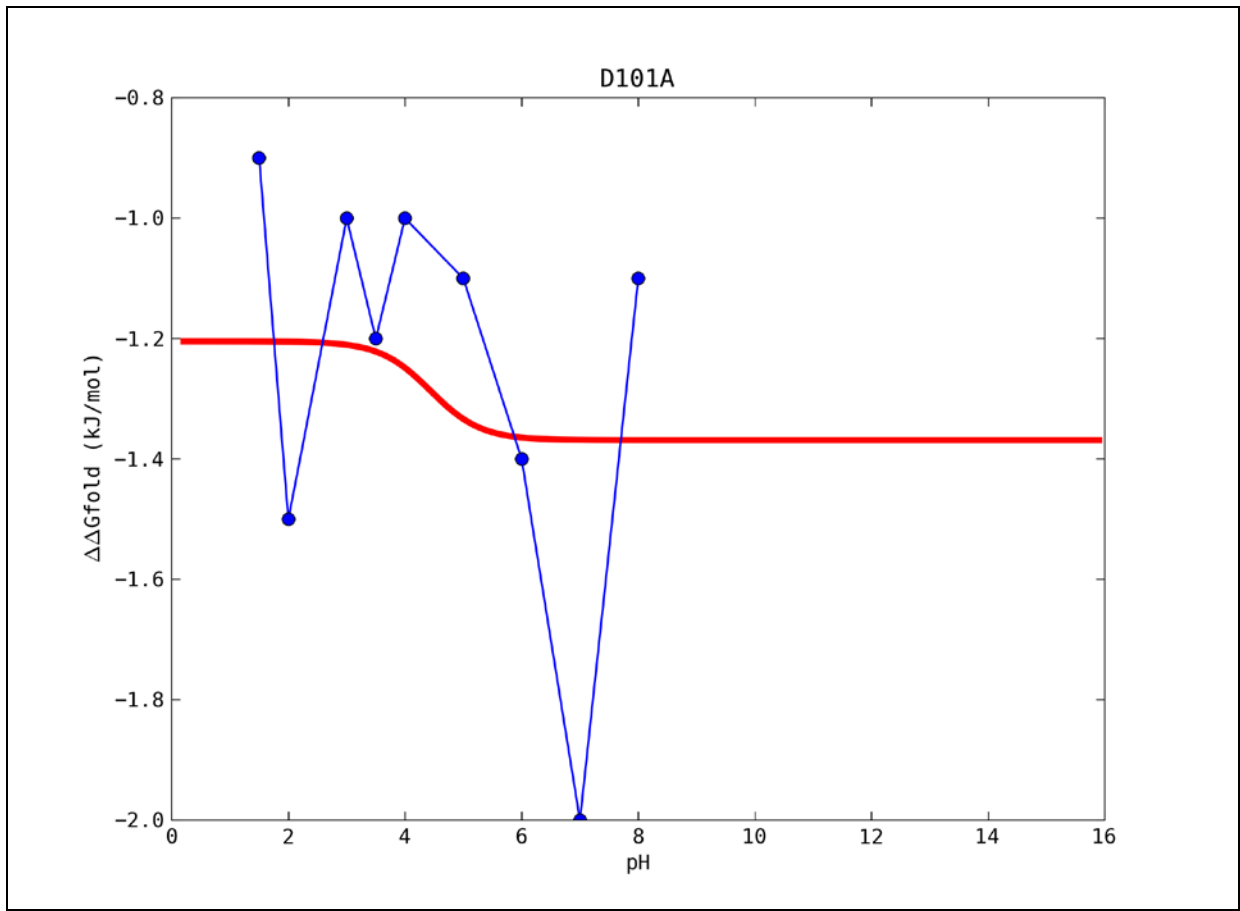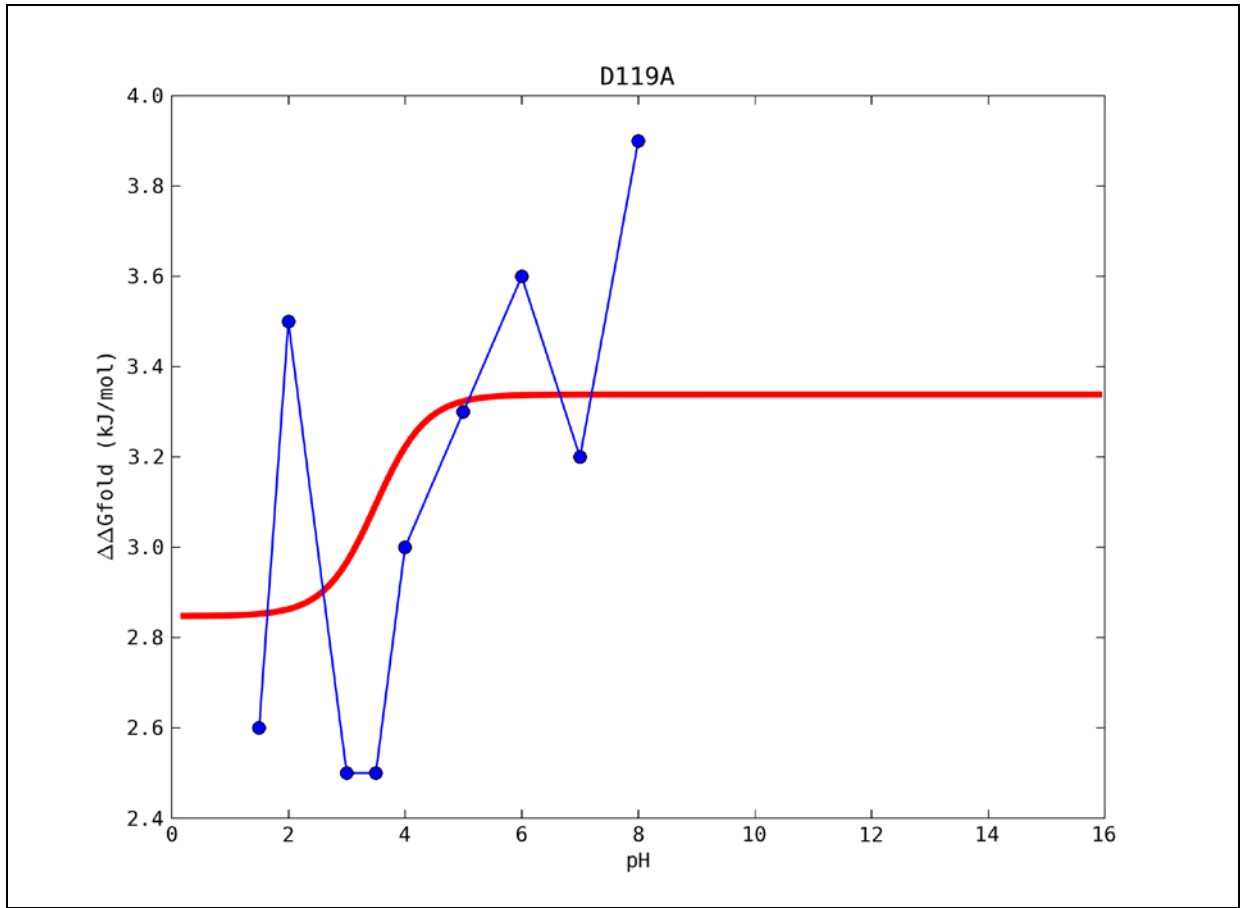
Figure SI3

Fit of the global HEWL pH-stability profile using the $pK_{a,U}$ values determined using mutant data, and pK_a values for Glu7, Asp87, C-terminus, N-terminus and a lysine determined using the progressive fitting strategy.

Figure SI4

Hen Egg White Lysozyme (PDB ID 2LZT) with all acidic groups and His 15 indicated. Figure produced with Yasara (http://www.yasara.org).
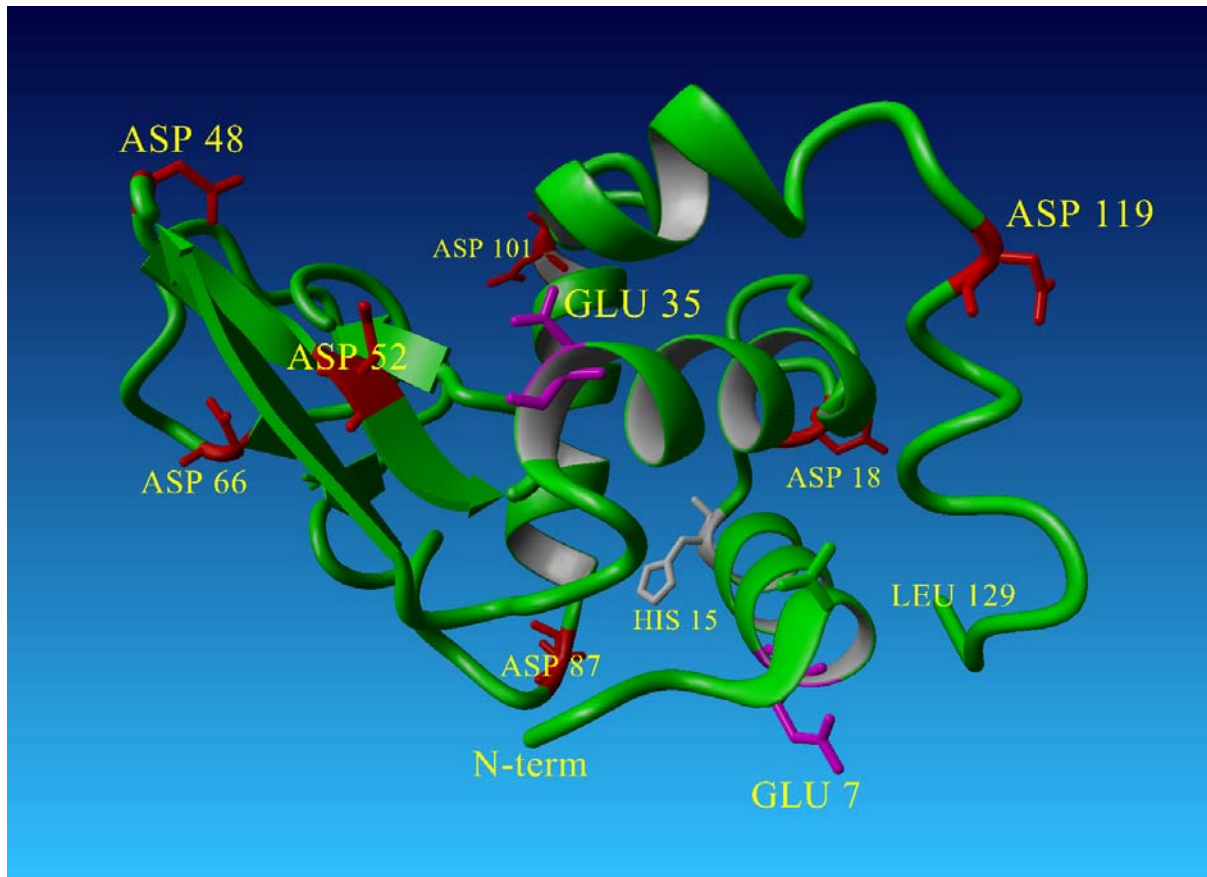
Table SI1

$\Delta S°_m$ values (in J/mol/K) found by Van't Hoff analysis.

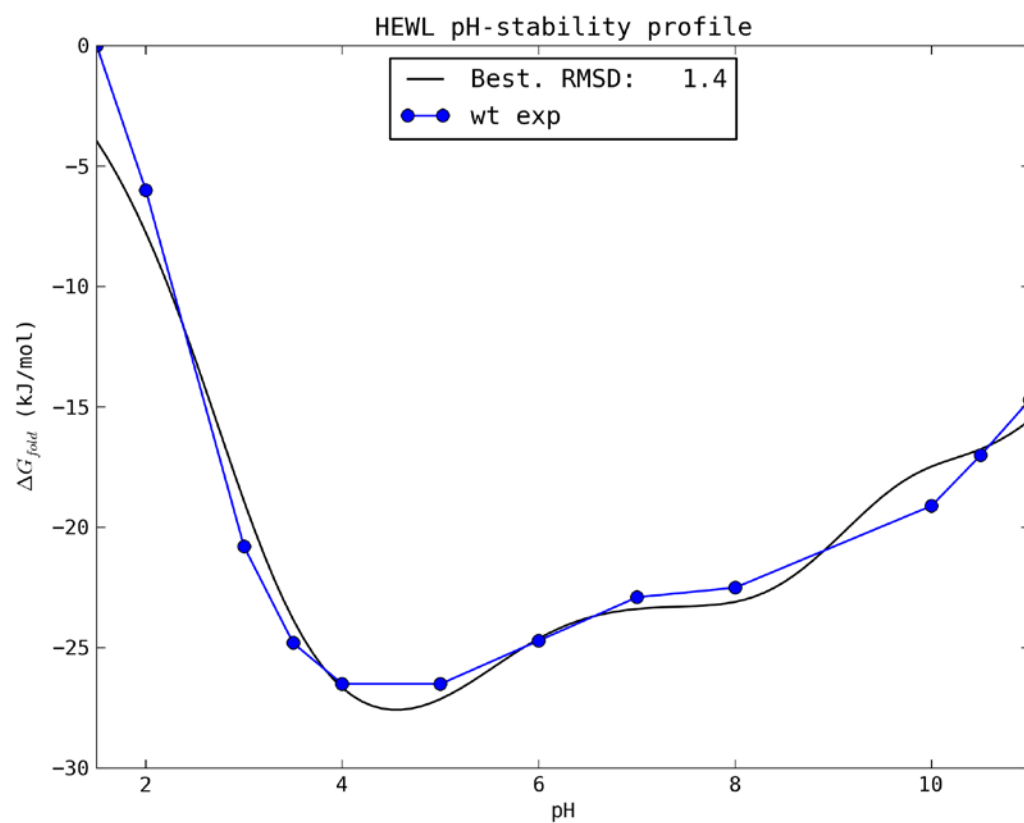| pH	WT	D18A	E35Q	D48A	D52A	D52N	D66A	D101A	D119A
1.50	0.84	0.76	0.79	0.67	0.79	0.90	0.82	0.88	0.73
2.00	0.94	0.77	1.51	0.73	0.94	1.08	1.00	1.05	0.85
3.00	1.15	0.97	1.62	0.79	1.09	1.07	1.15	1.12	1.03
3.50	1.25	1.05	1.79	0.85	1.24	0.98	1.19	1.16	1.01
4.00	1.12	0.97	0.89	0.93	1.18	1.03	1.23	1.13	1.07
5.00	1.17	0.97	1.18	0.92	1.20	1.04	1.15	1.34	1.09
6.00	1.26	1.05	1.18	0.90	1.26	1.34	1.08	1.28	1.20
7.00	1.25	1.07	1.17	0.89	1.23	1.24	1.05	1.25	1.18
8.00	1.21	1.13	1.32	1.01	1.35	1.26	1.23	1.29	1.23
9.00	0.68	0.63	1.25	1.00	0.84	0.91	0.69	0.67	0.64
10.00	1.43	1.32	1.41	1.07	1.66	1.38	1.31	1.61	1.20
10.50	1.52	1.58	1.38	1.13	1.71	1.75	1.27	1.76	1.33
11.00	1.52	1.64	0.63	1.09	1.82	1.65	1.18	2.03	1.54

Table SI2

ΔH° values (in kJ/mol) found by Van't Hoff analysis.

pH	WT	D18A	E35Q	D48A	D52A	D52N	D66A	D101A	D119A
1.5	266.4	242.3	246.1	210.3	251.1	285.9	257.3	319	314.6
2	3.4.5	249	517.8	233.1	303.3	350.4	320.8	326.7	321.4
3	395.3	331.3	549.3	264.4	369.4	364.6	385.5	343.8	339.6
3.5	433.2	362.7	602.3	286.6	426.5	339.3	403.8	402.9	344.3
4	391.4	337.8	284.1	317.4	407.7	359.5	418.1	396.5	345.8
5	407.8	334.5	397.8	312.2	419	365.4	391.6	468.5	345.4
6	437.2	360.5	400.9	304.1	437	467.3	367.5	446.9	343
7	431.2	365.6	403.9	298.8	425.4	431.8	354	435.2	341.2
8	416.6	384	460.1	340.3	467.7	439.4	416	444.7	340
9	237	216	433.9	333.7	293	316.5	234.2	235.2	343.3
10	487.5	447.3	489.3	358.5	567.6	471.5	438.9	552.9	334.6
10.5	513.5	531.8	478.4	379	577.9	597.4	422.7	600.7	332.3
11	508.3	547.6	218.9	363.9	614.2	555	389.6	686.4	328.7

Table SI3

T$_m$ values in Kelvin obtained from the Van't Hoff analysis.

pH	T$_m$ WT	T$_m$ D18A	T$_m$ E35Q	T$_m$ D48A	T$_m$ D52A	T$_m$ D52N	T$_m$ D66A	T$_m$ D101A	T$_m$ D119A
1.50	317.8	318.50	309.80	314.20	316.20	318.60	315.20	319.00	314.60
2.00	324.9	323.60	317.90	319.10	322.50	325.30	320.70	326.70	321.40
3.00	342.5	340.60	338.00	334.10	339.90	341.80	335.90	343.80	339.60
3.50	347.3	344.50	342.60	338.00	344.10	347.40	338.50	348.60	344.30
4.00	349.3	346.70	345.00	339.80	346.30	349.60	341.40	350.50	345.80
5.00	349.2	345.60	348.00	340.00	347.90	351.00	340.80	350.70	345.40
6.00	347.2	343.30	348.50	339.00	347.60	350.20	339.60	348.90	343.00
7.00	345.1	341.90	347.30	337.60	347.00	349.10	338.40	347.50	341.20
8.00	344.6	340.10	346.10	336.70	345.70	347.80	338.10	345.90	340.00
9.00	346.7	344.50	349.60	334.80	348.40	348.00	341.80	350.30	343.30
10.00	340.5	338.10	341.70	336.30	341.00	342.70	334.50	343.20	334.60
10.50	338.0	335.90	339.30	334.20	338.60	341.10	332.40	340.70	332.30
11.00	335.3	333.40	337.30	332.50	336.80	336.90	330.60	338.80	328.70

Table SI4

Mutant/ΔS°_m (J/mol/K)	pKa, U				
	0.84	1.15	1.15±10%	1.15±20%	1.15±30%
D18A	3.4 (0.2)	3.4 (0.2)	3.4 (0.2)	3.3 (0.1)	3.1 (0.1)
E35Q	5.0 (0.2)	4.4 (0.2)	4.4 (0.3)	4.5 (0.2)	4.6 (0.4)
D48A	2.9 (0.3)	2.0 (1.9)	2.2 (0.3)	2.2 (0.3)	1.5 (3.1)
D52A	4.0 (0.2)	3.0 (0.3)	3.0 (0.2)	3.0 (0.2)	3.2 (0.2)
D52N	4.1 (0.2)	3.1 (0.2)	3.1 (0.2)	3.2 (0.3)	3.2 (0.4)
D66A	3.1 (0.1)	2.4 (0.3)	2.5 (0.4)	2.5 (0.3)	2.2 (0.4)
D101A	4.4 (0.2)	4.3 (0.3)	4.3 (0.2)	4.3 (0.2)	4.3 (0.2)
D119A	3.6 (0.2)	3.9 (0.3)	3.9 (0.2)	3.7 (0.3)	3.8 (0.3)

The effect of using a different ΔS°_m for calculating $\Delta\Delta G^{\circ}$ values from ΔT_m on the fitted pKa,U values. The value of ΔS°_m used for the main results is that of the wild type at pH 1.5 (0.84 J/mol/K), and is showed in the first column. In the following columns the average value of ΔS°_m across all mutants and pH values (1.15 J/mol/K) is used with an increasing amount of random Gaussian noise added. It is seen that although the use of a different ΔS°_m value does produce different pKa, U values, the effect is moderate for D18, E35, D101 and D119, whereas the effect for D48, D52 and D66 is to predict even more highly shifted pKa,U values.