Electronic Supplementary Material (ESI) for Chemical Communications

Detection of salt bridges to lysines in solution in barnase

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Supplementary material

Table S1. Chemical shift assignments of lysines in barnase

	Cα	Hα	Cβ	Hβ	Cγ	Hγ	Cδ	Hδ	Cε	Hε
K19	53.8	4.54	32.6	2.02/2.26	21.3	1.29/1.34	28.9	1.77	40.9	3.03/3.12
K27	61.0[b]	3.92[b]	31.5[b]	2.04[b]	26.9	1.52	28.8	a	42.0	3.13
K39	55.3	4.53	32.3	1.81/1.97	24.9	1.43	28.3	1.63/1.70	41.1	3.01
K49	52.6[b]	5.15[b]	32.1	1.48/1.99	24.2	1.12/1.15	27.1	1.21/1.45	41.6	2.92
K62	57.3[b]	3.94[b]	32.1[b]	1.68/1.81[b]	25.8	1.46/1.51	28.9	1.88	41.8	3.14
K66	55.5	4.36	34.6	1.43/1.88	23.7	1.37/1.43	29.2	1.55/1.64	41.0	3.01
K98	53.3[b]	5.75[b]	37.5	1.41/1.69	a	a	28.3	0.42/0.85	40.4	2.15/2.27
K108	56.9	3.18	32.5	1.19/1.65	24.4	0.85/1.14	28.7	1.64/1.59	41.3	3.06

Assignments at pH 4.8, 25˚C, 10 mM sodium acetate. [a]Not assigned. All other assignments obtained from CCH-TOCSY except [b]from triple resonance backbone experiments.

Table S2. pK_a values for aspartates in barnase R69K

Residue	pK_a	Δδ (ppm)[a]
D8	3.22 ± 0.06	5.0
D12	3.80 ± 0.05	3.4
D44	3.53 ± 0.06	3.4
D54	3.23 ± 0.09	4.1
D93	2.33 ± 0.05[b]	3.4[b]

[a]Fitted change in ^{13}C' chemical shift between protonated and deprotonated forms. [b]Chemical shift difference fixed at 3.4 ppm.

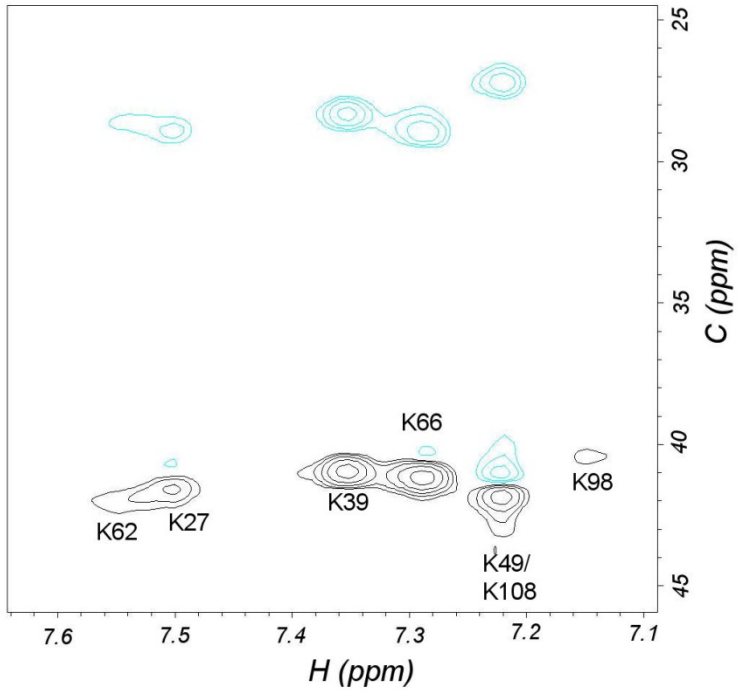

Figure S1. 2D H_3NCECD experiment. Peaks in cyan at the top are from Cδ and are negative.

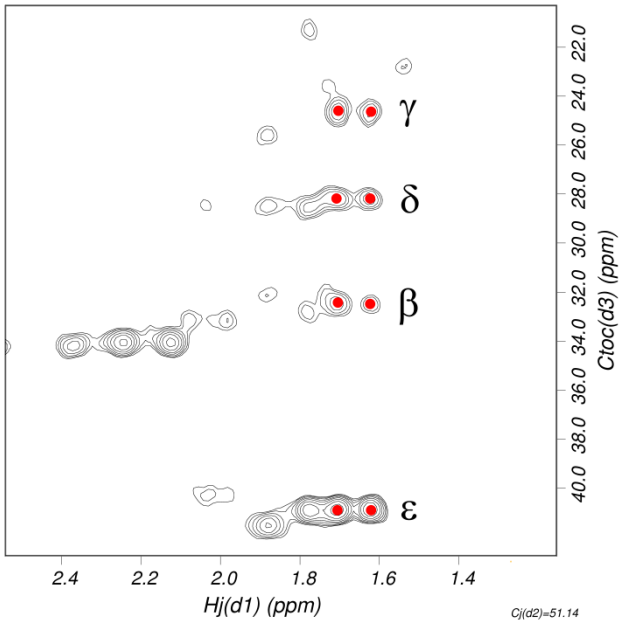

Figure S2. CCH-TOCSY experiment. The figure shows one plane from the 3D experiment through the chemical shift of K39 Cδ (28.3 ppm, folded in to 51.1 ppm).

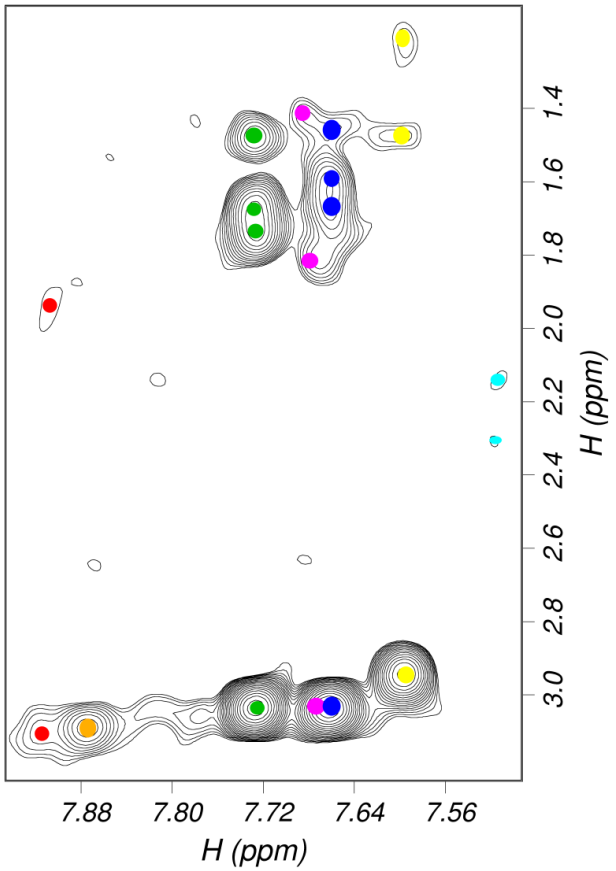

Figure S3. Two-dimensional $^{1}H/^{15}N$ HSQC-TOCSY experiment. Sidechain signals are marked for K62 (red), K27 (orange), K39 (green), K19 (purple), K66 (blue), K49 (yellow) and K98 (cyan).

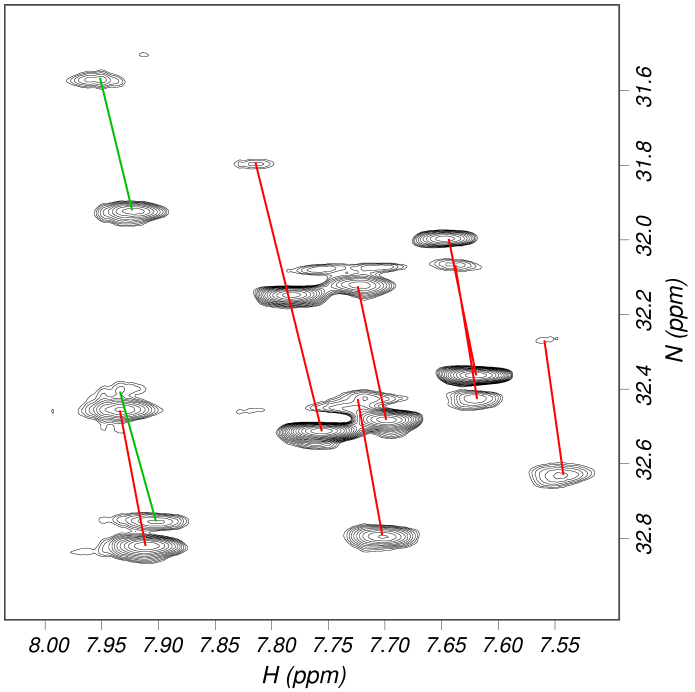

Figure S4. HISQC spectrum of barnase R69K in 20% D_2O, pH 4.8, 3˚C. Connections between NH_3^+ and NH_2D^+ peaks are marked; the two in green are for K27 (top) and K69 (bottom) which are salt-bridged and have different isotope effects.

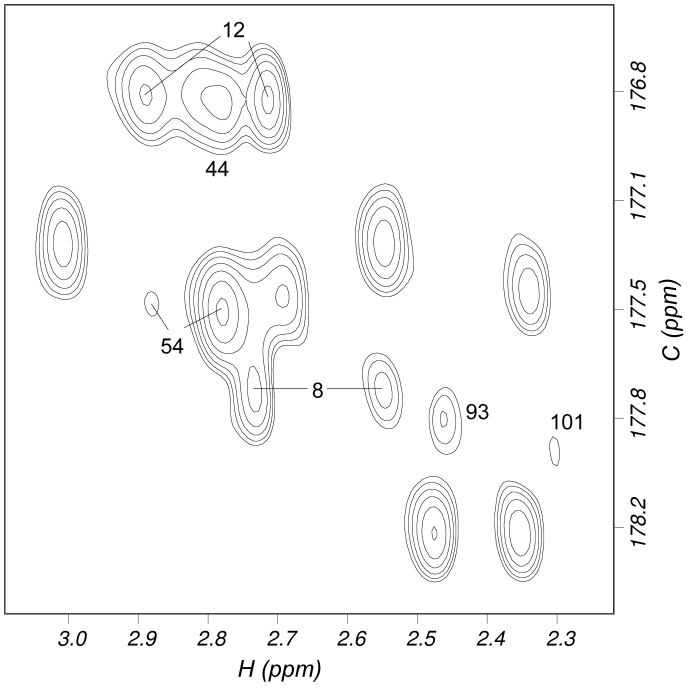

Figure S5. Two-dimensional H(CA)CO spectrum of barnase at pH 5. The residues marked are from aspartates. Unlabelled peaks are glutamate Cγ.

Additional experimental details.

The barnase H102A gene was carried on a pQE-60 plasmid (Qiagen). Barnase was expressed in *Escherichia coli* M15 [pREP4] cells at 37˚C in M9 minimal medium containing 100 μg/ml ampicillin and 100 μg/ml kanamycin. Expression was induced using 1 mM isopropyl-β-D-thiogalacto-pyranoside (Melford), and the culture was incubated for 18 h at 25˚C and harvested by centrifugation (5000*g* for 20 min at 4˚C). Cells were disrupted by sonication in 50 mM Tris-HCl pH 7.4 containing 5 mM $MgCl_2$, EDTA-free complete protease inhibitor (Roche) and DNAse I (Sigma) and centrifuged at 40,000*g* for 40 min at 4˚C. Supernatant was dialysed in 3500 MWCO dialysis tubing (Spectra/Por) overnight against 50 mM Tris-HCl pH 7.4 containing 0.02% sodium azide, and applied to a Q Sepharose Fast Flow column (GE Healthcare) in the same buffer. The run-through was pooled, dialysed overnight against 50 mM sodium acetate pH 5 containing 0.02% sodium azide, applied to a SP Sepharose Fast Flow column (GE Healthcare) equilibrated in the same buffer, and eluted using a linear gradient up to 1 M NaCl over 500 ml. Solutions for NMR were prepared using a Vivaspin 3 kDa MWCO device (Sartorius). For the pH titration, solutions were equilibrated in 33 mM sodium citrate, 33 mM sodium acetate, 33 mM sodium phosphate, 0.02% sodium azide at the appropriate pH and concentrated using a Vivaspin. The pH reported is the mean of the values before and after the NMR measurement, which were typically within 0.05 pH units.

The R69K mutant was prepared by PCR using the primers 5'-CCGGGCAAAAGCGGAAAAACATGGCGTGAAG-3' (forward) and 5'-CTTCACGCCATGTTTTTCCGCTTTTGCCCGG-3' (reverse), using an Agilent Quikchange kit. Mutated plasmids were transformed into XL1-Blue supercompetent cells following the manufacturer's instructions and checked by sequencing.

NMR experiments were carried out on Bruker Avance 600 and 800 instruments (with a cryoprobe at 600 MHz). Temperature was calibrated using d_4-methanol.[1] Standard Bruker experiments were used except for the HISQC and related experiments[2] which were kindly provided by Dr GM Clore (NIH). The H(CA)CO experiment was modified by using a carbon offset of 39 ppm and CH magnetization transfer delays of 1.4 and 1.8 ms. Experiments were carried out in different D_2O:H_2O ratios. For the assignments, 100% H_2O was used to maximise the proportion of signal in the NH_3^+ isotopomer, maintaining a field-frequency lock by using a D_2O capillary.

For estimation of pK_as, chemical shifts were fitted to the standard equation for a single pK_a:

$$\delta = \frac{\delta_{acid} + \delta_{base} 10^{(pH-pK)}}{1 + 10^{(pH-pK)}}$$

Fitting was done using a Levenberg-Marquardt least-squares routine, which also provided an estimate of the error in the fitting. Measured shifts were freely fitted to δ_{acid}, δ_{base} and pK_a. D93 gave an unacceptably large error of 0.3 pH units in fitted pK_a when fitted in this way, because δ_{acid} is poorly defined. It was therefore fitted using a range of total shift changes as seen for other aspartate residues. Fitting with a larger shift range (as seen for D8 and D54) gives an even lower fitted pK_a, implying that the actual pK_a of D93 could in fact be lower than 2.33.

(1) Findeisen, M.; Brand, T.; Berger, S. *Magn. Reson. Chem.* **2007**, *45*, 175-178.
(2) Iwahara, J.; Jung, Y. S.; Clore, G. M. *J. Am. Chem. Soc.* **2007**, *129*, 2971-2980.