Supporting Information for

Quinary structure modulates protein stability in cells

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Table S1. Backbone amide proton exchange rates (k_{obs}, s^{-1}) and corresponding $\Delta G^{\circ'}_{op}$ (kcal/mol) values for wild type (T2Q) GB1 in buffer (20 mM citrate, 150 mM NaCl, pH 5.8, 37 °C). The in-cell data have been published (*1*). [a]SDM: standard deviation of the mean from three trials. pH is corrected for the isotope effect. For glass electrodes in D_2O, pH = pH_{read} + 0.4 (*2*).

residue	$k_{obs,buff}$	SDM[a]	$\Delta G^{\circ'}_{op,buff}$	SDM[a]
Y3	3.88E-05	4.92E-06	6.31	0.10
K4	4.87E-05	9.70E-06	6.46	0.02
V26	2.18E-05	2.22E-06	7.04	0.12
K28	4.97E-05	6.37E-06	5.97	0.04
V29	1.84E-05	3.78E-06	6.00	0.04
K31	1.32E-05	3.10E-06	7.17	0.09
A34	2.23E-05	2.55E-06	6.77	0.05
T44	1.34E-05	1.02E-06	6.89	0.06
D46	1.37E-05	2.74E-06	6.74	0.02
T51	2.79E-05	1.99E-06	6.64	0.10
F52	1.80E-05	5.34E-06	6.72	0.13
T53	2.54E-05	4.99E-06	6.73	0.14

Table S2. Backbone amide proton exchange rates (k_{obs}, s^{-1}) and corresponding $\Delta G^{\circ'}_{op}$ (kcal/mol) values for D40K GB1 in cells and in buffer (20 mM citrate, 150 mM NaCl pH 5.8, 37 °C). pH is corrected for the isotope effect. For glass electrodes in D_2O, pH = pH_{read} + 0.4 (*2*). [a]SDM: standard deviation of the mean from three trials. [b]$\Delta\Delta G^{\circ'}_{op,cell,D40K}$ = $\Delta G^{\circ'}_{op,cell,D40K} - \Delta G^{\circ'}_{op,buff,D40K}$. [c]From propagation of error, $\sigma = \sqrt{(\delta_{\Delta G^{\circ'}_{op,cell}})^2 + (\delta_{\Delta G^{\circ'}_{op,buff}})^2}$.

residue	$k_{obs,cells}$	SDM[a]	$k_{obs,buff}$	SDM[a]
Y3	9.73E-04	±4.46E-05	1.80E-05	±1.52E-06
A26	9.45E-04	±5.48E-05	9.43E-06	±2.27E-06
K28	9.73E-04	±4.74E-05	3.41E-05	±1.48E-06
V29	6.30E-04	±4.87E-05	1.61E-05	±1.62E-06
K31	9.28E-04	±1.92E-05	1.16E-05	±1.95E-06
A34	7.17E-04	±8.10E-05	1.06E-05	±1.88E-06
T44	6.77E-04	±7.96E-06	9.54E-06	±3.14E-06
D46	6.84E-04	±5.68E-05	1.07E-05	±2.45E-06
T51	9.59E-04	±1.79E-05	1.35E-05	±2.79E-06
F52	8.60E-04	±1.09E-04	1.02E-05	±2.59E-06
T53	9.42E-04	±3.27E-05	1.08E-05	±2.24E-06

residue	$\Delta G^{\circ'}_{op,cell,D40K}$	SDM[a]	$\Delta G^{\circ'}_{op,buff,D40K}$	SDM[a]	$\Delta\Delta G^{\circ'}_{op,cell,D40K}$[b]	uncertainty[c]
Y3	4.20	±0.03	6.59	±0.02	-2.39	±0.03
A26	4.60	±0.04	7.43	±0.11	-2.82	±0.12
K28	4.07	±0.03	6.07	±0.02	-2.00	±0.03
V29	3.75	±0.05	5.94	±0.02	-2.19	±0.05
K31	4.36	±0.01	7.02	±0.07	-2.65	±0.07
A34	4.57	±0.07	7.11	±0.07	-2.55	±0.10
T44	4.27	±0.01	6.94	±0.18	-2.67	±0.18
D46	4.23	±0.05	6.78	±0.11	-2.55	±0.12
T51	4.38	±0.01	6.97	±0.08	-2.59	±0.08
F52	4.33	±0.08	7.04	±0.12	-2.71	±0.14
T53	4.31	±0.02	7.03	±0.09	-2.72	±0.09

Table S3. $\Delta\Delta G^{\circ'}_{op,mut}$ ($\Delta G^{\circ'}_{op,D40K} - \Delta G^{\circ'}_{op,wt}$, kcal/mol) caused by the D40K mutation in cells and in buffer (20 mM citrate, pH 5.8, 37 °C). pH is corrected for the isotope effect. For glass electrodes in D_2O, pH = pH_{read} + 0.4 (*2*). [a]From propagation of error.

residue	$\Delta\Delta G^{\circ'}_{op,mut}$	uncertainty[a]	$\Delta\Delta G^{\circ'}_{op,mut}$	uncertainty[a]
Y3	-1.18	±0.04	0.34	±0.10
A26	-1.41	±0.06	0.44	±0.16
K28	-1.08	±0.05	0.09	±0.04
V29	-1.35	±0.05	-0.09	±0.04
K31	-1.37	±0.07	-0.06	±0.11
A34	-1.29	±0.08	0.34	±0.09
T44	-1.63	±0.07	-0.03	±0.19
D46	-1.49	±0.12	0.04	±0.11
T51	-1.23	±0.06	0.35	±0.13
F52	-1.50	±0.11	0.20	±0.17
T53	-1.22	±0.07	0.42	±0.16

Table S4. Backbone amide proton exchange rates (k_{obs}, s^{-1}) and corresponding $\Delta G^{\circ'}_{op}$ *(kcal/mol)* values for I6L GB1 in buffer (20 mM citrate, 150 mM NaCl pH 5.8, 37 °C). The in-cell data have been published (*1*). pH is corrected for the isotope effect. For glass electrodes in D_2O, pH = pH_{read} + 0.4 (*2*). [a]The k_{obs} values are the average of two trials. [b]The uncertainty is the range. [c]From propagation of error.

residue	k_{obs}	uncertainty[b]	$\Delta G^{\circ'}_{op}$ [c]	uncertainty[c]
Y3	9.13E-05	±1.80E-06	5.59	±0.06
K4	6.39E-05	±3.15E-06	5.93	±0.10
A26	3.85E-05	±4.50E-06	6.51	±0.14
K28	7.42E-05	±4.30E-06	5.59	±0.10
V29	4.85E-05	±6.50E-06	5.26	±0.15
K31	4.50E-05	±9.00E-06	6.17	±0.20
A34	6.97E-05	±7.00E-07	5.93	±0.06
T44	6.75E-05	±1.15E-05	5.63	±0.18
D46	6.50E-05	±1.50E-05	5.64	±0.21
T51	6.65E-05	±1.65E-05	5.97	±0.23
F52	6.00E-05	±1.60E-05	5.91	±0.24
T53	6.90E-05	±1.21E-05	5.86	±0.18

Table S5. $\delta\Delta\Delta G^{\circ'}_{op,int}$ (=$\Delta\Delta G^{\circ'}_{op,mut,cell} - \Delta\Delta G^{\circ'}_{op,mut,buff}$, kcal/mol) values for I6L and D40K GB1. [a]From propagation of error.

residue	$\delta\Delta\Delta G^{\circ'}_{op,int,I6L}$	uncertainty[a]	$\delta\Delta\Delta G^{\circ'}_{op,int,D40K}$	uncertainty[a]
Y3	-1.00	±0.13	-1.46	±0.11
K4	-0.67	±0.15	-	-
A26	-0.84	±0.19	-1.79	±0.17
K28	-0.62	±0.12	-1.17	±0.06
V29	-0.64	±0.16	-1.29	±0.07
K31	-0.53	±0.23	-1.22	±0.13
A34	-0.83	±0.10	-1.63	±0.12
T44	-2.71	±0.21	-3.98	±0.20
D46	-0.63	±0.24	-1.54	±0.16
T51	-0.75	±0.26	-1.56	±0.14
F52	-1.05	±0.29	-1.82	±0.21
T53	-0.56	±0.25	-1.52	±0.18

Table S6. Backbone amide proton exchange rates (k_{obs}, s^{-1}) and corresponding $\Delta G^{\circ'}_{op}$ (kcal/mol) values for D40N GB1 in cells and in buffer (20 mM citrate, 150 mM NaCl pH 5.8, 37 °C). pH is corrected for the isotope effect. For glass electrodes in D_2O, pH = pH_{read} + 0.4 (*2*). [a]SDM: standard deviation of the mean from three trials. [b]$\Delta\Delta G^{\circ'}_{op,cell,D40N}$ = $\Delta G^{\circ'}_{op,cell,D40N} - \Delta G^{\circ'}_{op,buff,D40N}$. [c]From propagation of error.

residue	$k_{obs,cells}$	SDM[a]	$k_{obs,buff}$	SDM[a]
Y3	5.23E-04	±4.12E-05	1.64E-05	±1.60E-07
K4	1.17E-03	±3.00E-04	2.58E-05	±2.10E-07
A26	4.76E-04	±5.64E-05	6.88E-06	±4.25E-07
K28	5.25E-04	±4.34E-05	3.53E-05	±2.05E-07
V29	4.31E-04	±7.97E-05	1.52E-05	±8.00E-08
K31	4.87E-04	±5.33E-05	7.95E-06	±4.50E-07
A34	4.91E-04	±5.11E-05	1.11E-05	±2.40E-07
T44	4.05E-04	±9.15E-05	7.65E-06	±1.50E-07
D46	4.27E-04	±7.99E-05	7.77E-06	±1.30E-07
T51	5.30E-04	±4.36E-05	1.14E-05	±1.50E-07
F52	5.35E-04	±2.87E-05	7.14E-06	±5.05E-07
T53	4.84E-04	±5.67E-05	8.94E-06	±3.15E-07

residue	$\Delta G^{\circ'}_{op,cell,D40N}$	SDM[a]	$\Delta G^{\circ'}_{op,buff,D40N}$	SDM[a]	$\Delta\Delta G^{\circ'}_{op,cell,D40N}$[b]	uncertainty[c]
Y3	4.59	±0.05	6.72	±0.006	-2.13	±0.05
K4	4.25	±0.19	6.55	±0.005	-2.30	±0.19
A26	5.03	±0.07	7.64	±0.04	-2.61	±0.08
K28	4.45	±0.05	6.11	±0.004	-1.66	±0.05
V29	4.00	±0.11	6.05	±0.003	-2.04	±0.11
K31	4.77	±0.07	7.30	±0.03	-2.53	±0.08
A34	4.80	±0.06	7.13	±0.011	-2.33	±0.06
T44	4.62	±0.13	7.04	±0.01	-2.42	±0.13
D46	4.54	±0.11	6.99	±0.01	-2.45	±0.11
T51	4.75	±0.05	7.12	±0.01	-2.37	±0.05
F52	4.62	±0.03	7.28	±0.04	-2.66	±0.05
T53	4.72	±0.07	7.18	±0.02	-2.45	±0.08

Table S7. $\Delta\Delta G^{\circ'}_{op,mut}$ ($\Delta G^{\circ'}_{op,D40N} - \Delta G^{\circ'}_{op,wt}$, kcal/mol) caused by the D40N mutation in cells and in buffer (20 mM citrate, pH 5.8, 37 °C). pH is corrected for the isotope effect. For glass electrodes in D_2O, pH = pH_{read} + 0.4 (*2*). [a]From propagation of error.

residue	$\Delta\Delta G^{\circ'}_{op,mut,cell}$	uncertainty[a]	$\Delta\Delta G^{\circ'}_{op,mut,buff}$	uncertainty[a]
Y3	-0.79	±0.06	0.41	±0.10
K4	-1.01	±0.19	0.10	±0.02
A26	-0.99	±0.09	0.60	±0.12
K28	-0.74	±0.06	0.14	±0.04
V29	-1.14	±0.11	0.05	±0.04
K31	-1.02	±0.10	0.13	±0.09
A34	-1.11	±0.08	0.36	±0.06
T44	-1.32	±0.15	2.44	±0.06
D46	-1.23	±0.15	0.26	±0.02
T51	-0.91	±0.08	0.47	±0.10
F52	-1.05	±0.08	0.55	±0.13
T53	-0.85	±0.10	0.45	±0.14

Table S8. $\delta\Delta\Delta G^{\circ'}_{op,int}$ ($\Delta\Delta G^{\circ'}_{op,mut,cell} - \Delta\Delta G^{\circ'}_{op,mut,buff}$, kcal/mol) values for D40N GB1.

[a]From propagation of error.

residue	$\delta\Delta\Delta G^{\circ'}_{op,int,D40N}$	**uncertainty**[a]
Y3	-1.19	±0.11
K4	-1.11	±0.19
A26	-1.59	±0.15
K28	-0.88	±0.08
V29	-1.19	±0.12
K31	-1.15	±0.14
A34	-1.47	±0.10
T44	-3.76	±0.16
D46	-1.49	±0.15
T51	-1.38	±0.13
F52	-1.60	±0.16
T53	-1.30	±0.17

Table S9. Backbone amide proton exchange rates (k_{obs}, s^{-1}) and corresponding $\Delta G^{\circ'}_{op}$ (kcal/mol) values for D40A GB1 in cells and in buffer (20 mM citrate, 150 mM NaCl pH 5.8, 37 °C). pH is corrected for the isotope effect. For glass electrodes in D_2O, pH = pH_{read} + 0.4 (*2*). [a]SDM: standard deviation of the mean from three trials. [b]$\Delta\Delta G^{\circ'}_{op,cell,D40A}$ = $\Delta G^{\circ'}_{op,cell,D40A} - \Delta G^{\circ'}_{op,buff,D40A}$. [c]From propagation of error.

residue	$k_{obs,cells}$	SDM[a]	$k_{obs,buff}$	SDM[a]
Y3	6.04E-04	±1.15E-04	3.71E-05	±6.04E-06
K4	9.65E-04	±2.63E-04	4.10E-05	±4.75E-06
A26	4.67E-04	±6.70E-05	1.48E-05	±2.37E-06
K28	6.09E-04	±1.03E-04	5.84E-05	±9.67E-06
V29	2.81E-04	±4.91E-05	2.64E-05	±4.80E-06
K31	5.22E-04	±1.96E-04	1.74E-05	±3.24E-06
A34	4.89E-04	±1.21E-04	1.95E-05	±1.38E-06
T44	3.23E-04	±4.81E-05	1.15E-05	±1.20E-06
D46	3.30E-04	±4.03E-05	1.36E-05	±1.82E-06
T51	5.72E-04	±1.08E-04	1.97E-05	±2.62E-06
F52	5.14E-04	±9.10E-05	1.61E-05	±2.44E-06
T53	5.37E-04	±1.03E-04	1.91E-05	±2.84E-06

residue	$\Delta G^{\circ'}_{op,cell,D40A}$	SDM[a]	$\Delta G^{\circ'}_{op,buff,D40A}$	SDM[a]	$\Delta\Delta G^{\circ'}_{op,cell,D40A}$[b]	uncertainty[c]
Y3	4.52	±0.11	6.45	±0.02	-1.94	±0.12
K4	4.36	±0.15	6.49	±0.05	-2.13	±0.16
A26	5.05	±0.09	7.40	±0.03	-2.35	±0.09
K28	4.37	±0.10	6.03	±0.02	-1.66	±0.10
V29	4.26	±0.10	5.95	±0.02	-1.68	±0.10
K31	4.80	±0.22	7.06	±0.01	-2.26	±0.22
A34	4.83	±0.14	7.00	±0.08	-2.17	±0.16
T44	4.74	±0.09	7.00	±0.07	-2.27	±0.11
D46	4.69	±0.07	6.86	±0.03	-2.17	±0.08
T51	4.72	±0.11	7.01	±0.04	-2.29	±0.12
F52	4.66	±0.11	7.01	±0.03	-2.36	±0.11
T53	4.67	±0.11	6.94	±0.03	-2.27	±0.12

Table S10. $\Delta\Delta G^{\circ'}_{op,mut}$ ($\Delta G^{\circ'}_{op,D40A} - \Delta G^{\circ'}_{op,wt}$, kcal/mol) caused by the D40A mutation in cells and in buffer (20 mM citrate, 150 mM NaCl pH 5.8, 37 °C). pH is corrected for the isotope effect. For glass electrodes in D_2O, $pH = pH_{read} + 0.4$ (*2*). [a]From propagation of error.

residue	$\Delta\Delta G^{\circ'}_{op,mut,cell}$	uncertainty[a]	$\Delta\Delta G^{\circ'}_{op,mut,buff}$	uncertainty[a]
Y3	-0.86	±0.12	0.14	±0.10
K4	-0.90	±0.16	0.04	±0.06
A26	-0.98	±0.17	0.36	±0.12
K28	-0.82	±0.10	0.06	±0.04
V29	-0.88	±0.11	-0.05	±0.04
K31	-0.98	±0.11	-0.11	±0.09
A34	-1.08	±0.23	0.23	±0.10
T44	-1.20	±0.15	2.41	±0.09
D46	-1.08	±0.11	0.12	±0.04
T51	-0.94	±0.13	0.36	±0.10
F52	-1.01	±0.13	0.29	±0.13
T53	-0.90	±0.13	0.22	±0.14

Table S11. $\delta\Delta\Delta G^{\circ'}_{op,int}$ ($\Delta\Delta G^{\circ'}_{op,mut,cell} - \Delta\Delta G^{\circ'}_{op,mut,buff}$, kcal/mol) values for D40A GB1.

[a]From propagation of error.

residue	$\delta\Delta\Delta G^{\circ'}_{op,int,D40}$	uncertainty[a]
Y3	-1.00	±0.16
K4	-0.94	±0.17
A26	-1.34	±0.16
K28	-0.88	±0.11
V29	-0.83	±0.11
K31	-0.88	±0.24
A34	-1.31	±0.18
T44	-3.61	±0.15
D46	-1.20	±0.14
T51	-1.30	±0.16
F52	-1.30	±0.18
T53	-1.12	±0.19

Fig. S1. Expanded thermodynamic cycle from Fig. 1, depicting equilibria between native (N) and denatured (D) states of wt GB1 and its variants. Intracellular interactions (red) cause deviations in values of $\Delta\Delta G'^{\circ}_{mut,cell}$ and $\Delta\Delta G'^{\circ}_{mut,buff}$.

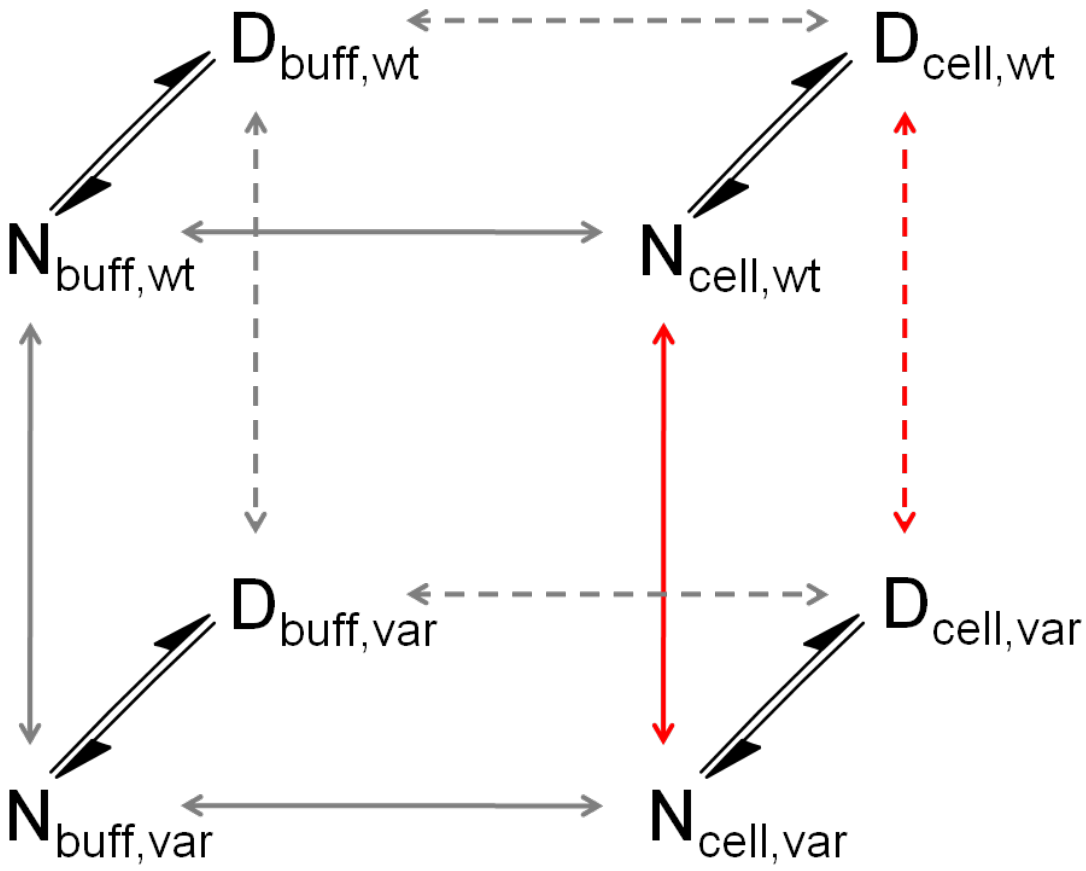
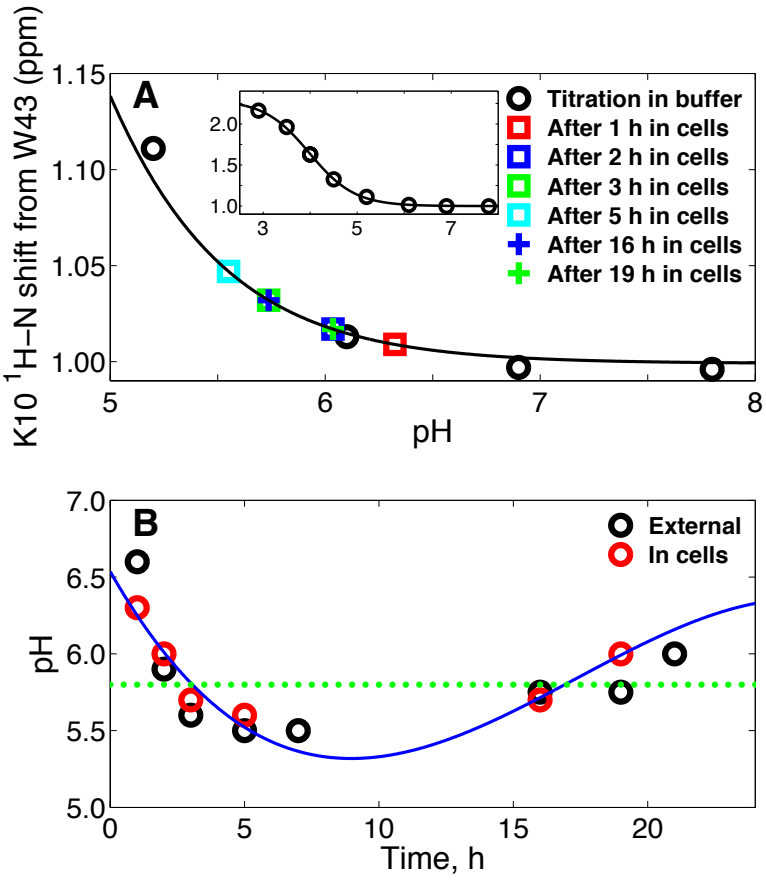


Fig. S2 Cytoplasmic pH. A) pH dependence of the K10 amide proton shift relative to that of the pH-insensitive amide proton of W43 (10.507 ppm). pH is corrected for the isotope effect. For glass electrodes in D_2O, pH = pH_{read} + 0.4 (*2*). The inset shows the entire titration curve constructed from GB1-expressing lysates in 50 mM citrate, 50 mM bis-tris propane, 50 mM HEPES, 50 mM borate, 5% D_2O, 0.1% DSS, 298 K. The curve is from a fit to the Henderson-Hasselbach equation, $\delta = \delta_{low} - \frac{\delta_{low} - \delta_{high}}{1+10^{n(pK_a - pH)}}$, where δ_{low} and δ_{high} are the low and high pH shift plateaus and n is the number of protons. The derived pK_a, 4.0, which probably reflects that of E56 to which the backbone amide forms a hydrogen bond, is consistent with literature values (*3*). In-cell spectra were acquired as a function of time and shift changes overlaid onto the curve. **B)** Time dependence of the external (glass electrode) and internal (from data in A) pH. The dashed horizontal line represents the pH used to derive the intrinsic rates. The curve is of no theoretical significance.


A
B
K10 ^{1}H–N shift from W43 (ppm)
pH
Time, h
Titration in buffer
After 1 h in cells
After 2 h in cells
After 3 h in cells
After 5 h in cells
After 16 h in cells
After 19 h in cells
External
In cells

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