Table 1. pK_a^N values detremined experimentally and calculated with the program PROPKA, pK_a^D values reported by Nozaky and Tanford, percent accesible surface area and interactions of the ionzable groups of α-sarcin used in the calculations.

Res. Nº	Exper[a].	ASA %	**H-bond**/Electros. interaction	pK_a^D [b]	PROPKA[c]
His 35	6.3 ± 0.1	34	E31,K129	6.3	6.72 (surface)
His 36	6.8 ± 0.1	43	D105	6.3	6.50 (surface)
His 50	7.7 ± 0.1	18	Y48,E96,R121	6.3	2.49 (surface)
His 82	7.3 ± 0.1	11	**D41,** D91	6.3	7.41 (surface)
His 92	6.9 ± 0.1	18	R78, K81	6.3	6.83 (surface)
His104	6.5 ± 0.1	27	D102, E115	6.3	6.40 (surface)
His137	5.8 ± 0.1	6	**G143**,R121	6.3	2.03 (buried)
His 150	7.6 ± 0.1	46	C-term	6.3	7.01 (surface)
Glu 19	4.6 ± 0.1	22	K21	4.4	4.59 (surface)
Glu 31	4.6 ± 0.1	17	H35	4.4	4.78 (surface)
Glu 96	5.2 ± 0.1	1	H50,R121	4.4	5.03 (buried)
Glu 115	4.9± 0.1	36	H104	4.4	4.50 (surface)
Glu 140	4.2 ± 0.1	26	Y18	4.4	3.90 (surface)
Glu 144	4.3 ± 0.1	44		4.4	4.24 (surface)
Asp 9	3.9 ± 0.1	10	K139	4.0	4.29 (surface)
Asp 41	<3.0	5	**H82**,**W51**	4.0	2.90 (buried)
Asp 57	4.2 ± 0.1	33		4.0	4.01 (surface)
Asp 59	4.0 ± 0.1	35		4.0	3.21 (surface)
Asp 75	3.8± 0.1	22	R78	4.0	3.66 (surface)
Asp 77	3.0 ± 0.1	19	Y56	4.0	3.43 (surface)
Asp 85	3.8 ± 0.1	26	K43	4.0	4.01 (surface)
Asp 91	<3.0	3	H82,Y93,Y126	4.0	3.67 (surface)
Asp 102	<3.0	46	H104	4.0	3.74 (surface)
Asp 105	<3.0	30	H36	4.0	3.14 (surface)
Asp 109	3.7 ± 0.1	37		4.0	3.87 (surface)
N-term[d]	n.d.	88		7.7	7.79 (surface)
C-term	3.5 ± 0.1	99		3.8	2.76 (surface)

[a] Experimental pK_a^N values from Pérez-Cañadillas et al. (1998) *Biochemistry 37*, 15865-76.
[b] pK_a^D values from Nozaki, and Tanford. (1967) *Methods Enzymol 11*, 715-734.
[c] Calculated pK_a^N values and residue location according to PROPKA program (Li et al. 2005, *Proteins 61*, 704-721) with α-sarcin 1DE3.pdb structure.
[d] N-term pK_a^N value not determined (n.d.) and not included in electrostatic calculations.

Table 2. Experimental pK_a^N values of the histidines and glutamic acid 96 of E96Q, H50Q, H137Q, H50/137Q α-sarcin mutants used in the calculations.

Protein	H35	H36	H50	H82	H92	E96	H104	H137	H150
E96Q	6.3±0.2	7.3±0.2	6.4±0.2	n.d.	7.2±0.1	-	n.d.	5.9±0.1	7.9±0.1
H50Q	6.2±0.2	n.d.	-	7.4±0.1	7.3±0.1	5.0±0.1	n.d.	6.1±0.1	7.3±0.1
H137Q	6.3±0.2	n.d.	>8	7.4±0.1	7.1±0.1	5.7±0.1	6.6±0.2	-	7.7±0.1
H50/137Q	6.3±0.2	7.3±0.2	-	7.0±0.1	7.4±0.1	5.4±0.1	7.3±0.2	-	7.6±0.1

n.d. not determined

All reported standard errors in Table 1 and Table 2 reflect the precision of the experimental data fitting and do not include the uncertainty in the pH determination of the samples, which is estimated to be ± 0.1 unit.

Table 3. $^1H/^2H$ exchange rate constants of α-sarcin amide protons at pH* 5.2 and 35 ºC.

k_{obs} (min^{-1})	Nº residues	Residues
>600	2	V2, S74
$9.4\cdot10^{-2}$-600	61	T3, L7, K11, K14, T15, N16, Y18, T20, R22, Q27, N28, L39, D41, G42, K43, T44, G45, S46, S47, Y48, W51, T53, G55, G58, D59, G60, K61, L62, K64, G65, T67, K70, G72, D75, C76, H82, S83, K84, D85, G86, N87, G88, K89, T90, D102, D105, D109, S110, K111, K112, K114, E115, N128, T138, K139, E140, N141, Q142, G143, E144, L145
$4.0\cdot10^{-2}$-$9.4\cdot10^{-2}$	6	N26, S40, R66, D91, K107, F108
10^{-2}-$4.0\cdot10^{-2}$	18	N12, K17, K29, S32, H35, A37, N54, D57, K73, D77, R78, H104, N116, A120, K129, F131, L147, S149
10^{-3}-10^{-2}	10	N8, D9, Q10, E19, K21, S34, L94, G103, G118, H137
10^{-4}-10^{-3}	9	L24, N33, Y56, I69, K81, H92, Y93, F100, Y106
$5.7\cdot10^{-6}$-10^{-4}	8	W4, T5, C6, L23, A30, E31, H50, K146
$<5.7\cdot10^{-6}$	19	Y25, F52, L95, E96, F97, T99, R121, V122, I123, Y124, T125, Y126, V130, C132, G133, I134, I135, A136, C148

Table 4. Values of T_m, ΔH_m, and ΔG_{D-N} at 35 ^{0}C for α-sarcin and H50Q, E96Q, H137Q, H50/137Q variants.

α-sarcinn			
pH	T_m (^{0}C)	ΔH_m (kcal/mol)	ΔG (kcal/mol)
2.72	38.7±0.1	89±3	1.1±0.1
3.03	44.7±0.1	92±4	2.6±0.2
3.11	46.0±0.1	89±5	2.8±0.2
3.47	52.3±0.2	79±4	3.5±0.3
3.7	52.9±0.2	104±7	5.0±0.3
4.12	60.6±0.2	89±4	5.5±0.4
4.5	63.0±0.1	105±4	7.1±0.5
4.93	64.3±0.2	116±7	8.2±0.6
5.31	63.8±0.1	136±8	9.8±0.7
5.6	63.0±0.1	137±7	9.8±0.7
5.79	61.9±0.1	120±6	8.1±0.6
5.83	61.6±0.1	139±8	9.5±0.7
5.94	61.1±0.1	127±6	8.5±0.6
6.15	59.0±0.1	119±6	7.4±0.5
6.4	56.47±0.07	141±6	8.2±0.6
6.92	51.70±0.07	145±6	7.0±0.5
7.32	47.47±0.08	137±6	5.0±0.3
7.53	45.44±0.08	121±5	3.7±0.3

H50Q				E96Q			
pH	T_m (^{0}C)	ΔH_m (kcal/mol)	ΔG (kcal/mol)	pH	T_m (^{0}C)	ΔH_m (kcal/mol)	ΔG (kcal/mol)
3.25	45.03±0.09	80±2	2.3±0.2	3.49	49.72±0.07	126±4	5.3±0.4
4.45	58.9±0.1	107±5	6.5±0.5	4.74	60.57±0.06	139±4	9.3±0.7
5.02	61.0±0.1	133±6	8.9±0.7	5.78	60.39±0.06	147±5	9.8±0.7
5.76	59.0±0.1	126±6	7.9±0.6	6.37	56.91±0.05	156±5	9.3±0.7
5.59	70.3±0.7	114±3	9.1±0.7	6.96	52.54±0.06	156±6	7.7±0.6
6.21	56.70±0.09	130±6	7.5±0.6	7.26	50.01±0.06	159±6	6.9±0.5
6.96	51.85±0.06	138±5	6.6±0.5	7.5	48.51±0.05	149±5	5.9±0.4
7.53	47.74±0.08	130±6	4.8±0.4				

H137Q				H50/137Q			
pH	T_m (^{0}C)	ΔH_m (kcal/mol)	ΔG (kcal/mol)	pH	T_m (^{0}C)	ΔH_m (kcal/mol)	ΔG (kcal/mol)
2.9	38.43±0.06	96±2	1.0±0.1	3.63	48.0±0.1	111±6	4.2±0.3
3.92	55.04±0.07	129±4	7.1±0.5	4.61	55.2±0.1	114±6	6.1±0.5
4.15	56.9±0.2	141±17	8.3±0.6	5.54	55.6±0.1	117±6	6.4±0.5
4.72	59.36±0.08	136±5	8.7±0.7	5.73	55.1±0.1	121±7	6.5±0.5
4.75	58.79±0.07	139±5	8.8±0.7	6.14	53.2±0.1	109±5	5.4±0.4
5.23	59.27±0.06	143±5	9.2±0.7	6.43	51.6±0.1	130±7	6.1±0.5
5.37	59.5±0.2	131±15	8.4±0.6	6.49	51.2±0.1	127±7	5.8±0.4
5.99	56.7±0.2	153±16	9.1±0.7	6.95	47.9±0.1	113±5	4.2±0.3
6.96	49.21±0.06	150±5	6.2±0.5	7.62	43.1±0.1	118±6	2.8±0.2
7.48	43.80±0.05	144±4	3.8±0.3				

Figure 1. Comparison of the $\partial \Delta G/\partial$ pH profiles for α-sarcin calculated with two different sets of pK_a^D values.