

Supporting Information for:

Protein-Crowder Charge and Protein Stability

Mohona Sarkar, Joe Lu and Gary J. Pielak*

* Correspondence: gary_pielak@unc.edu

Table S1: Proteomic analysis of proteins that bound the diethylaminoethyl (DEAE) cellulose column. The list comprises proteins with two or more identified peptides, their percent sequence coverage and calculated isoelectric point (pI). Of the 200 proteins, 194 had pI values of less than 7.0. Proteins that are also identified in the unbound fraction are highlighted in yellow.

Peptides	%Coverage	Names	pI
32	56.02	60 kDa chaperonin	4.9
25	64.21	Elongation factor Tu 1	5.5
24	40.91	Elongation factor G	5.4
23	60.51	Tryptophanase	6.2
21	58.99	Isocitrate lyase	5.3
19	35.74	Chaperone protein DnaK	5.0
18	57.88	Phosphoglycerate kinase	5.2
18	58.51	Succinyl-CoA ligase [ADP-forming] subunit beta	5.5
18	40.95	Acetyl-coenzyme A synthetase	5.8
17	26.49	Aconitate hydratase 1	5.9
15	22.20	Aconitate hydratase 2	5.4
15	45.90	Citrate synthase	6.7
14	45.91	Isocitrate dehydrogenase [NADP]	5.3
13	50.21	Aspartate ammonia-lyase	5.3
13	58.01	Glyceraldehyde-3-phosphate dehydrogenase A	7.1
12	39.12	Enolase	5.5
11	36.11	Phosphoenolpyruvate carboxykinase [ATP]	5.7
11	30.64	Galactitol-1-phosphate 5-dehydrogenase	6.4
10	52.68	Transaldolase B	5.2
10	17.97	Chaperone protein ClpB	5.5
10	31.22	Transketolase 1	5.7
10	15.67	Glycine dehydrogenase [decarboxylating]	6.0
10	23.19	Pyruvate kinase I	6.1
9	15.75	Carbamoyl-phosphate synthase large chain	5.3
9	28.29	Phosphate acetyltransferase	5.4
9	22.32	Pyruvate dehydrogenase E1 component	5.7
9	37.14	D-tagatose-1,6-bisphosphate aldolase subunit GatZ	5.8
9	35.61	Aspartate aminotransferase	5.8
9	36.49	Fructose-bisphosphate aldolase class 2	5.9
9	65.15	Flavoprotein WrbA	5.9
9	33.14	Fructose-bisphosphate aldolase class 1	6.7
8	30.22	Phosphopentomutase	5.3
8	36.69	Argininosuccinate synthase	5.4
8	27.85	Dihydrolipoyl dehydrogenase	6.2
8	30.99	4-aminobutyrate aminotransferase GabT	6.2
8	35.96	Succinylornithine transaminase	6.4
8	28.78	Serine hydroxymethyltransferase	6.5
8	25.00	Fumarate hydratase class I, aerobic	6.6
8	39.79	Succinyl-CoA ligase [ADP-forming] subunit alpha	6.8
7	25.82	Glutamate-1-semialdehyde 2,1-aminomutase	4.8
7	12.12	Phosphoenolpyruvate synthase	5.1
7	45.99	Alkyl hydroperoxide reductase subunit C	5.2
7	41.22	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI	5.9
7	26.18	Soluble pyridine nucleotide transhydrogenase	6.6
7	45.49	Glucosamine-6-phosphate deaminase	6.9

6	43.79	Glucose-specific phosphotransferase enzyme IIA component	4.8
6	21.28	DNA-directed RNA polymerase subunit alpha	5.1
6	21.09	Lactaldehyde dehydrogenase	5.2
6	49.48	10 kDa chaperonin	5.2
6	27.92	Elongation factor Ts	5.3
6	15.88	Asparagine--tRNA ligase	5.3
6	13.31	NADP-dependent malic enzyme	5.5
6	20.44	3-oxoacyl-[acyl-carrier-protein] synthase 1	5.5
6	30.77	Aminomethyltransferase	5.6
6	16.33	CTP synthase	5.9
6	34.67	Cysteine synthase A	6.1
6	45.60	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	6.2
6	24.26	Cysteine desulfurase	6.4
6	10.33	Aldehyde-alcohol dehydrogenase	6.8
5	18.98	Trigger factor	4.9
5	22.26	ADP-L-glycero-D-manno-heptose-6-epimerase	4.9
5	4.47	DNA-directed RNA polymerase subunit beta	5.3
5	20.39	6-phosphofructokinase isozyme 2	5.4
5	8.57	Glycerol kinase	5.5
5	19.07	Aspartate-semialdehyde dehydrogenase	5.6
5	20.31	Aldehyde dehydrogenase B	5.6
5	48.95	Nucleoside diphosphate kinase	5.8
5	19.44	Peptidase B	5.9
5	19.10	2-amino-3-ketobutyrate coenzyme A ligase	6.0
5	16.46	Gamma-aminobutyraldehyde dehydrogenase	6.0
5	10.26	Formate acetyltransferase 1	6.0
5	14.12	Succinate dehydrogenase flavoprotein subunit	6.3
5	20.00	2,5-diketo-D-gluconic acid reductase A	6.5
5	21.80	Arginine N-succinyltransferase	6.5
5	49.11	UPF0438 protein YifE	6.6
5	16.67	Pyruvate kinase II	6.7
5	5.33	DNA-directed RNA polymerase subunit beta'	7.1
4	36.52	Glutaredoxin-4	4.8
4	43.67	Bacterioferritin	4.8
4	15.22	ATP synthase subunit beta	5.0
4	9.19	6-phosphogluconate dehydrogenase, decarboxylating	5.2
4	8.74	Proline--tRNA ligase	5.2
4	31.58	S-ribosylhomocysteine lyase	5.4
4	15.77	Succinate-semialdehyde dehydrogenase [NADP(+)] GabD	5.6
4	10.99	Uncharacterized ABC transporter ATP-binding protein YjjK	5.6
4	21.76	Purine nucleoside phosphorylase DeoD-type	5.7
4	31.76	Triosephosphate isomerase	6.0
4	4.90	Isoleucine--tRNA ligase	6.0
4	6.96	Ribonucleoside-diphosphate reductase 1 subunit alpha	6.2
4	19.48	Uncharacterized zinc-type alcohol dehydrogenase-like protein YahK	6.2
4	10.14	Pyruvate dehydrogenase	6.3
4	13.49	Transketolase 2	6.3
4	14.66	L-threonine 3-dehydrogenase	6.4

3	39.29	Thiol peroxidase	4.9
3	9.03	tRNA modification GTPase MnME	5.0
3	32.05	Putative peroxiredoxin bcp	5.2
3	9.41	Oligopeptidase A	5.3
3	5.63	Aminopeptidase N]	5.3
3	23.14	7-alpha-hydroxysteroid dehydrogenase	5.4
3	18.27	Uracil phosphoribosyltransferase	5.5
3	13.81	Phosphoserine aminotransferase	5.6
3	8.44	Protein pmbA	5.6
3	13.55	Phosphoglucomutase	5.7
3	17.81	6-phosphofructokinase isozyme 1	5.7
3	15.69	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	5.7
3	9.43	Tyrosine--tRNA ligase	5.8
3	18.26	NADPH-dependent curcumin reductase	5.8
3	9.03	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]	5.9
3	6.51	Catalase HP11	5.9
3	10.81	Putative glyceraldehyde-3-phosphate dehydrogenase C	6.1
3	16.46	3-oxoacyl-[acyl-carrier-protein] synthase 2	6.1
3	30.41	Ferric uptake regulation protein	6.1
3	8.50	Fatty acid oxidation complex subunit alpha	6.2
3	11.19	Glutamine--tRNA ligase	6.3
3	12.57	Carbamoyl-phosphate synthase small chain	6.4
3	24.43	Putative UPF0012 hydrolase YbeM	6.4
3	16.60	Inosine-5'-monophosphate dehydrogenase	6.4
3	5.36	2-oxoglutarate dehydrogenase E1 component	6.5
3	16.92	Fumarate hydratase class II	6.6
3	8.33	Bifunctional polymyxin resistance protein ArnA	6.9
3	21.31	3-oxoacyl-[acyl-carrier-protein] reductase FabG	7.4
3	26.06	50S ribosomal protein L10 unit	9.0
2	18.71	Protein-export protein SecB	4.4
2	7.42	USG-1 protein	4.5
2	11.11	Transcription elongation protein NusA	4.6
2	21.49	50S ribosomal protein L7/L12 unit	4.7
2	16.37	3-mercaptopyruvate sulfurtransferase	4.7
2	4.87	Phosphoenolpyruvate-protein phosphotransferase	4.9
2	18.58	Vitamin B12 transport periplasmic protein BtuE	4.9
2	25.78	NifU-like protein	4.9
2	19.68	Glutaminase 1	5.0
2	10.96	PTS-dependent dihydroxyacetone kinase, dihydroxyacetone-binding subunit dhaK	5.0
2	13.20	Glycine--tRNA ligase alpha subunit	5.0
2	13.92	2-dehydro-3-deoxygluconokinase	5.0
2	24.19	UPF0502 protein YceH	5.1
2	11.44	Molybdopterin molybdenumtransferase	5.1
2	4.29	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	5.2
2	8.79	D-alanine--D-alanine ligase A	5.2
2	14.17	UPF0135 protein Ybgl	5.2
2	9.94	Chaperone protein HtpG	5.2

2	6.14	Lysine--tRNA ligase, heat inducible	5.2
2	31.41	6,7-dimethyl-8-ribityllumazine synthase	5.3
2	7.29	S-adenosylmethionine synthase	5.3
2	4.60	Argininosuccinate lyase	5.3
2	25.00	Universal stress protein A	5.3
2	4.53	Phenylalanine--tRNA ligase beta subunit	5.3
2	5.00	Leucine--tRNA ligase	5.3
2	6.55	NAD-dependent malic enzyme	5.3
2	7.33	Ketol-acid reductoisomerase	5.3
2	13.45	Aerobic respiration control protein ArcA	5.3
2	19.34	Stringent starvation protein A	5.3
2	4.31	Valine--tRNA ligase	5.3
2	8.35	ATP-dependent protease ATPase subunit HslU	5.4
2	7.42	Cytosol non-specific dipeptidase	5.4
2	16.89	Ribose-5-phosphate isomerase A	5.4
2	5.87	Bifunctional protein HldE	5.4
2	7.26	Glycine--tRNA ligase beta subunit	5.4
2	10.95	Protein yeeZ	5.5
2	8.96	Glutamine synthetase	5.5
2	5.37	Arginine--tRNA ligase	5.5
2	5.58	Serine--tRNA ligase	5.5
2	10.90	Thioredoxin reductase	5.5
2	9.03	ATP phosphoribosyltransferase	5.6
2	10.78	Ornithine carbamoyltransferase chain I	5.7
2	6.72	Alkyl hydroperoxide reductase subunit F	5.7
2	4.70	Peptidyl-dipeptidase dcp	5.7
2	8.33	Malate dehydrogenase	5.8
2	14.81	Glyoxylate/hydroxypyruvate reductase B	5.8
2	6.87	Alpha-galactosidase	5.8
2	3.25	Methionine--tRNA ligase	5.9
2	7.04	Asparagine synthetase B [glutamine-hydrolyzing]	5.9
2	6.78	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase	5.9
2	5.03	Dihydroxy-acid dehydratase	5.9
2	7.43	Periplasmic trehalase	5.9
2	6.37	Histidine--tRNA ligase	5.9
2	4.65	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	5.9
2	2.05	Bifunctional protein putA	6.0
2	9.48	Xaa-Pro dipeptidase	6.0
2	7.53	Fructose-1,6-bisphosphatase class 1	6.0
2	13.31	NADP-dependent 3-hydroxy acid dehydrogenase YdfG	6.0
2	9.89	Molecular chaperone Hsp31 and glyoxalase 3	6.0
2	8.92	Protein CsiD	6.2
2	16.21	Uridine phosphorylase	6.2
2	9.85	Acetylornithine/succinyl-diaminopimelate aminotransferase	6.2
2	12.90	Oxygen-insensitive NAD(P)H nitroreductase	6.2
2	46.81	Galactitol-specific phosphotransferase enzyme IIB component	6.3
2	12.66	Transaldolase A	6.3
2	9.00	Acetate kinase	6.3

2	11.65	Uncharacterized protein YciO	6.4
2	16.59	Thymidine kinase	6.4
2	6.94	NADP-specific glutamate dehydrogenase	6.4
2	16.67	Phosphoheptose isomerase	6.4
2	26.85	50S ribosomal protein L9 unit	6.6
2	3.72	2,4-dienoyl-CoA reductase [NADPH]	6.6
2	15.93	Ribosome-associated inhibitor A	6.7
2	11.56	Protein tas	6.8
2	21.31	Putative NAD(P)H nitroreductase ydjA	6.8
2	13.70	3-ketoacyl-CoA thiolase	6.8
2	14.44	2-dehydro-3-deoxyphosphooctonate aldolase	6.8
2	20.40	Osmotically-inducible protein Y	6.8
2	28.17	Universal stress protein D	6.9
2	16.22	GTP cyclohydrolase 1	7.3
2	9.96	Uridylate kinase	7.9

Table S2: Proteomic analysis of proteins that did not bind to the DEAE cellulose column. The list comprises proteins with two or more identified peptides, their percent sequence coverage and calculated isoelectric point (pI). Of the 193 proteins identified, only 71 had pI greater than 7.0. Proteins that are also identified in the bound fractions are highlighted in yellow.

Peptides	%Coverage	Names	pI
37	94.23	Malate dehydrogenase	5.8
34	79.15	Glyceraldehyde-3-phosphate dehydrogenase A	7.1
22	57.08	sn-glycerol-3-phosphate-binding periplasmic protein	6.7
21	71.92	Lysine-arginine-ornithine-binding periplasmic protein	5.9
21	71.2	Transaldolase A	6.3
21	71.19	Glutamate/aspartate periplasmic-binding protein	8.5
20	57.27	Periplasmic oligopeptide-binding protein	6.5
20	59.63	Periplasmic dipeptide transport protein	6.7
19	78.11	Osmotically-inducible protein Y	6.8
17	68.85	Histidine-binding periplasmic protein	5.6
16	33.08	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	5.7
15	70.78	Putative ABC transporter arginine-binding protein 2	6.1
15	62.79	Glutaredoxin-2	8.0
14	58.14	Protein yhbO	5.5
14	32.64	Malate synthase G	6.2
14	59.12	ABC transporter periplasmic-binding protein YtfQ	7.1
14	55.91	Putative ABC transporter periplasmic-binding protein YdcS	7.4
13	63.55	Adenylate kinase	5.8
13	30.29	Biotin carboxylase	7.1
13	59.32	50S ribosomal protein L6	9.7
12	60	Ribosome-recycling factor	6.9
11	95.65	UPF0337 protein YjbJ	5.6
11	58.04	Peroxiredoxin OsmC	5.9
11	50.34	D-ribose-binding periplasmic protein	7.5
11	45.56	Glutamine-binding periplasmic protein	8.5
10	91.19	Superoxide dismutase [Fe]	5.9
10	69.23	Uncharacterized protein YahO	6.1
10	28.9	Outer membrane protein A	6.4
10	52.73	2,5-diketo-D-gluconic acid reductase A	6.5
10	35.19	Erythronate-4-phosphate dehydrogenase	6.7
10	48.32	Quinone oxidoreductase 1	8.3
10	47.41	FKBP-type peptidyl-prolyl cis-trans isomerase FkpA	8.5
9	61.35	UPF0234 protein YajQ	6.2
9	76.21	Superoxide dismutase [Mn]	7.0
8	78.91	Enamine/imine deaminase	5.5
8	51.64	KHG/KDPG aldolase	5.7
8	41.46	3'(2'),5'-bisphosphate nucleotidase CysQ	6.0
8	25	Uncharacterized sulfatase YdeN	6.0
8	15.56	Periplasmic beta-glucosidase	6.2
8	48.98	Uncharacterized oxidoreductase YghA	6.8
8	41.98	ABC transporter arginine-binding protein 1	7.4
8	29.77	Protein TolB	7.6
7	61.47	Thioredoxin-1	4.9

7	40.15	Deoxyribose-phosphate aldolase	5.7
7	46.95	Peptidyl-prolyl cis-trans isomerase B	5.8
7	40.53	Methionine aminopeptidase	6.0
7	24	Uncharacterized protein YjgR	6.3
7	21.9	Probable D,D-dipeptide-binding periplasmic protein DdpA	6.7
7	59.12	Transcription antitermination protein NusG	6.8
7	41.98	Ecotin	7.1
7	39.49	Protein YdgH	9.3
7	58.45	50S ribosomal protein L11	9.6
6	79.52	Protein YciN	5.7
6	85.88	Phosphocarrier protein HPr	5.8
6	34.32	Putative carboxymethylenebutenolide	6.0
6	45.37	Nitrate/nitrite response regulator protein NarL	6.1
6	15.25	Glucans biosynthesis protein D	6.3
6	64.74	Superoxide dismutase [Cu-Zn]	6.4
6	64.63	Uncharacterized SufE-like protein YgdK	6.6
6	25.53	L-arabinose-binding periplasmic protein	6.6
6	28.53	Glyoxylate/hydroxypyruvate reductase A	6.8
6	15.7	Esterase FrsA	6.9
6	18.46	Chaperone SurA	7.0
6	19.37	Glucans biosynthesis protein G	7.2
6	78.26	Cold shock-like protein CspC	7.2
6	73.72	Uncharacterized protein YccU	7.3
6	34.51	N-acetylneuraminate epimerase	7.7
6	20.25	Periplasmic serine endoprotease DegP	8.6
5	19.18	Mannose-6-phosphate isomerase	5.4
5	19.8	Elongation factor Tu 1	5.5
5	27.4	Inner membrane protein yebE	5.5
5	44.44	Sugar phosphatase YfbT	6.1
5	48.87	Ribosome-binding factor A	6.2
5	35.16	Uncharacterized protein YcgM	6.3
5	26.19	Putative glucose-6-phosphate 1-epimerase	6.3
5	33.03	Glycine betaine-binding periplasmic protein	6.4
5	42.14	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	6.5
5	18.9	Arabinose 5-phosphate isomerase KdsD	6.7
5	19.19	Blue copper oxidase CueO	6.8
5	28.87	Universal stress protein D	6.9
5	21.01	Thiosulfate-binding protein	8.1
5	38.69	G/U mismatch-specific DNA glycosylase	9.1
5	18.26	Probable L,D-transpeptidase YnhG	9.4
5	48.94	50S ribosomal protein L25	9.6
5	36.02	Chaperone protein skp	9.7
4	12.04	60 kDa chaperonin	4.9
4	42.52	Autonomous glycyl radical cofactor	5.2
4	21.85	Sugar phosphatase YidA	5.3
4	22.39	Tryptophan synthase alpha chain	5.5
4	14.18	Succinyl-CoA ligase [ADP-forming] subunit beta	5.5
4	24.24	D-xylose-binding periplasmic protein	5.6
4	22.59	Transcriptional regulatory protein RstA	5.7
4	18.69	Cytoskeleton protein RodZ	5.8
4	32.69	Probable quinol monooxygenase YgiN	6.2

4	30.63	Sugar phosphatase YbiV	6.4
4	28.45	Transcriptional regulatory protein OmpR	6.4
4	23.53	Probable L,D-transpeptidase YbiS	6.4
4	24.42	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase	6.6
4	20.73	Uncharacterized protein YggE	6.6
4	15.05	Periplasmic AppA protein	6.7
4	33.5	Outer-membrane lipoprotein carrier protein	6.8
4	26.92	Acyl-CoA thioesterase I	7.6
4	10.35	Sulfite reductase [NADPH] hemoprotein beta-component	7.6
4	14.58	TPR repeat-containing protein YfgC	7.7
4	11.92	Periplasmic murein peptide-binding protein	8.4
4	47.83	Acylphosphatase	8.5
4	17.56	Uncharacterized protein YncE	9.2
4	19.62	50S ribosomal protein L3	9.9
4	35.42	50S ribosomal protein L15	11.2
3	11.11	Septum site-determining protein MinD	5.4
3	10.65	Enolase	5.5
3	14.24	Outer membrane protein assembly factor BamC	5.6
3	16.08	Quinone oxidoreductase 2	5.6
3	26.38	Nitrogen regulatory protein	5.9
3	15.43	Putative quinone oxidoreductase YhdH	5.9
3	12.09	Periplasmic pH-dependent serine endoprotease DegQ	5.9
3	45.71	Cold shock protein CspA	5.9
3	10.04	Protein yhjJ	6.0
3	17.7	Putative osmoprotectant uptake system substrate-binding protein osmF	6.1
3	11.38	Anhydro-N-acetylmuramic acid kinase	6.1
3	38.3	Galactitol-specific phosphotransferase enzyme IIB component	6.3
3	4.4	Protein yhgF	6.3
3	26.37	Probable Fe(2+)-trafficking protein	6.3
3	18.22	Glycerophosphoryl diester phosphodiesterase	6.5
3	6.45	Tail-specific protease	6.6
3	22.92	3-demethylubiquinone-9 3-methyltransferase	6.6
3	14.29	Cystine-binding periplasmic protein	6.6
3	18.4	Uncharacterized GST-like protein YghU	6.7
3	14.16	D-erythrose-4-phosphate dehydrogenase	6.8
3	19.03	Succinyl-CoA ligase [ADP-forming] subunit alpha	6.8
3	13.77	Cell division protein ZapD	6.8
3	9.97	D-allose-binding periplasmic protein	6.9
3	25.3	Periplasmic protein CpxP	6.9
3	25.69	Transcriptional regulator SlyA	7.1
3	22.05	Uncharacterized protein YhhK	7.1
3	50.6	Glutaredoxin-3	7.2
3	13.08	Riboflavin biosynthesis protein RibD	7.6
3	31.52	Chorismate--pyruvate lyase	8.0
3	11.91	Glutathione-binding protein GsiB	8.3
3	28.81	Protein YebF	8.6
3	22.63	Peptidyl-prolyl cis-trans isomerase A	8.9
3	40.13	Protein CreA	9.0
3	52.22	DNA-binding protein HU-alpha	9.6

3	22.33	30S ribosomal protein S10	9.7
3	39.73	Uncharacterized protein YhhA	10.9
2	36.51	Uncharacterized protein YbgS	4.9
2	45.61	RutC family protein YoaB	5.2
2	17.46	Uncharacterized metalloprotease YggG	6.1
2	21.09	DNA polymerase III subunit chi	6.2
2	4.46	Tryptophanase	6.2
2	12.5	Uncharacterized protein YeiR	6.5
2	13.6	Ribosomal RNA small subunit methyltransferase J	7.1
2	9.69	Right origin-binding protein	7.2
2	14.34	3-oxoacyl-[acyl-carrier-protein] reductase FabG	7.4
2	10.57	Uncharacterized protein YcbX	7.6
2	23.89	Uncharacterized protein YebY	8.0
2	5.78	Protein HemY	8.5
2	11.95	Sigma-E factor regulatory protein RseB	8.6
2	12.89	Peptidyl-tRNA hydrolase	9.0
2	40.37	UPF0265 protein YeeX	9.3
2	21.82	UPF0339 protein YegP	9.4
2	13.25	50S ribosomal protein L1	9.6
2	16.26	50S ribosomal protein L14	10.4

Table S3: ΔG_{op}° (kcal/mol) for the I29A;I37H variant in buffer (20 °C, pH 7.0, 50 mM sodium phosphate). Protons that exchange upon global unfolding¹ are bolded (SEM, standard error of the mean).

Residue	Trial 1	Trial 2	Trial 3	AVERAGE \pm SEM
Trp5	6.56	6.79	6.72	6.69 \pm 0.07
Glu7	—	—	6.94	6.94
Leu8	6.37	6.58	6.57	6.51 \pm 0.07
Val9	5.88	6.11	6.03	6.01 \pm 0.07
Gly10	5.88	6.04	6.04	5.99 \pm 0.05
Lys11	7.13	7.33	7.26	7.24 \pm0.06
Val13	6.11	6.30	6.27	6.23 \pm 0.06
Ala16	6.94	7.15	7.12	7.07 \pm 0.07
Lys17	—	—	5.26	5.26
Lys18	5.89	6.17	6.05	6.04 \pm 0.08
Val19	6.67	6.70	6.78	6.72 \pm 0.03
Ile20	6.89	6.66	7.52	7.2 \pm0.25
Leu21	6.38	6.30	6.72	6.47 \pm0.13
Gln22	6.63	6.84	6.79	6.75 \pm 0.06
Asp23	6.82	7.02	6.95	6.93 \pm 0.06
Lys24	6.51	6.69	6.67	6.62 \pm 0.06
Ala27	5.75	5.96	5.91	5.87 \pm 0.06
Ile30	6.84	7.75	6.94	7.1 \pm0.3
Leu32	6.71	6.91	6.90	6.84 \pm 0.06
Val34	4.10	4.23	4.09	4.14 \pm 0.04
Arg46	6.72	6.94	6.89	6.85 \pm 0.07
Val47	6.85	7.02	7.01	6.96 \pm0.06
Arg48	6.86	7.04	7.04	6.98 \pm 0.06
Leu49	6.79	7.01	6.94	6.91 \pm0.07
Phe50	6.75	6.79	6.84	6.79 \pm0.03
Val51	6.58	6.81	6.66	6.68 \pm0.07
Asp52	6.57	6.83	6.72	6.71 \pm 0.08
Asp55	6.28	6.50	5.36	6.1 \pm 0.3
Asn56	7.38	7.60	7.54	7.51 \pm 0.07
Ala57	7.20	7.22	7.13	7.18 \pm 0.03
Ala58	6.77	7.01	6.87	6.88 \pm 0.07
Glu59	7.62	7.80	7.76	7.73 \pm 0.05
Arg62	6.71	6.90	6.88	6.83 \pm 0.06
Val63	6.80	6.93	6.96	6.90 \pm 0.05
Gly64	5.62	6.49	5.76	5.9 \pm 0.3

Table S4: ΔG_{op}^0 (kcal/mol) for the I29A;I37H variant in 100.0 g/L total-protein lysate (20 °C, pH 7.0, 50 mM sodium phosphate). Protons that exchange upon global unfolding¹ are bolded (SEM, standard error of the mean).

Residue	Trial 1	Trial 2	Trial 3	AVERAGE \pm SEM
Trp5	6.07	5.98	5.94	6.00 \pm 0.04
Leu8	6.05	5.97	5.76	5.93 \pm 0.09
Val9	5.51	5.46	5.36	5.44 \pm 0.04
Lys11	6.82	6.82	6.85	6.83 \pm0.01
Val13	5.72	5.75	5.73	5.73 \pm 0.01
Ala16	6.50	6.50	6.38	6.46 \pm 0.04
Lys18	5.30	5.65	5.46	5.5 \pm 0.1
Val19	6.14	6.10	5.94	6.06 \pm 0.06
Ile20	6.32	6.19	5.84	6.1 \pm0.14
Leu21	5.99	5.92	5.64	5.9 \pm0.11
Gln22	6.30	6.24	6.19	6.24 \pm 0.03
Asp23	6.51	6.52	6.54	6.52 \pm 0.01
Lys24	6.14	6.13	6.06	6.11 \pm 0.03
Ala27	5.44	5.37	5.41	5.41 \pm 0.02
Ile30	6.25	6.21	5.97	6.14 \pm0.09
Leu32	6.32	6.27	6.07	6.22 \pm 0.08
Val34	—	4.53	5.08	4.8 \pm 0.6
Arg46	6.44	6.39	6.24	6.36 \pm 0.06
Val47	6.46	6.36	6.23	6.35 \pm0.07
Arg48	6.61	6.50	6.47	6.53 \pm 0.04
Leu49	6.49	6.47	6.34	6.43 \pm0.05
Phe50	6.34	6.28	6.13	6.25 \pm0.06
Val51	6.30	6.17	5.87	6.11 \pm0.13
Asp52	6.19	6.15	6.01	6.12 \pm 0.05
Ala57	6.54	6.50	6.33	6.46 \pm 0.06
Ala58	6.35	6.40	6.20	6.32 \pm 0.06
Glu59	7.10	7.06	6.92	7.03 \pm 0.05
Arg62	6.33	6.29	6.25	6.29 \pm 0.02
Val63	6.23	6.25	6.06	6.18 \pm 0.06
Gly64	5.16	5.10	4.76	5.0 \pm 0.12

Table S5: ΔG_{op}° (kcal/mol) for the I29A;I37H variant in 100.0 g/L anionic-protein lysate (20 °C, pH 7.0, 50 mM sodium phosphate). Protons that exchange upon global unfolding¹ are bolded (SEM, standard error of the mean).

Residue	Trial 1	Trial 2	Trial 3	AVERAGE \pm SEM
Trp5	5.88	5.92	6.25	6.0 \pm 0.12
Leu8	5.93	5.68	6.20	5.9 \pm 0.15
Val9	5.47	5.37	5.74	5.5 \pm 0.1
Lys11	6.87	6.72	7.05	6.9 \pm0.1
Val13	5.83	5.85	6.04	5.91 \pm 0.07
Ala16	6.72	6.44	6.73	6.63 \pm 0.1
Lys18	5.80	5.90	5.80	5.83 \pm 0.03
Val19	6.22	5.95	6.45	6.2 \pm 0.1
Ile20	6.30	5.68	6.63	6.2 \pm0.3
Leu21	5.87	5.65	6.23	5.9 \pm0.17
Gln22	6.23	6.29	6.59	6.4 \pm 0.11
Asp23	6.60	6.38	10.54	6.5 \pm 0.22
Lys24	6.28	6.22	6.43	6.31 \pm 0.06
Ala27	5.84	5.69	5.79	5.77 \pm 0.04
Ile30	6.34	5.66	6.47	6.2 \pm0.25
Leu32	6.27	5.87	6.52	6.2 \pm 0.19
Val34	5.51	5.05	4.80	5.1 \pm 0.21
Arg46	6.49	6.36	6.69	6.5 \pm 0.1
Val47	6.38	5.97	6.69	6.4 \pm0.21
Arg48	6.78	6.61	6.87	6.75 \pm 0.08
Leu49	6.53	6.25	6.63	6.5 \pm0.11
Phe50	6.48	6.25	6.61	6.4 \pm0.11
Val51	6.19	5.86	6.45	6.2 \pm0.17
Asp52	6.29	6.09	6.41	6.26 \pm 0.09
Ala57	6.38	6.27	6.66	6.4 \pm 0.12
Ala58	6.35	6.14	6.65	6.4 \pm 0.15
Glu59	7.16	6.83	7.42	7.1 \pm 0.17
Arg62	6.47	6.29	6.57	6.44 \pm 0.08
Val63	6.17	6.11	6.48	6.2 \pm 0.11
Gly64	4.89	4.48	5.17	4.8 \pm 0.2

Table S6: Conductivity (mS/cm) of NaCl solutions and 100 g/L total-protein lysate in buffer.

NaCl (mM)	Conductivity (mS/cm)
0	0.04
20	2.02
40	3.99
60	6.02
80	7.94
100	9.6
120	11.4
140	13.4
160	15.4
180	17.2
200	19.1
<hr/>	
50 mM phosphate, pH 7.0	9.37
lysate (100 g/L) in 50 mM phosphate, pH 7.0	17.7

References:

- (1) Neira, J. L., Itzhaki, L. S., Otzen, D. E., Davis, B., and Fersht, A. R. (1997) Hydrogen exchange in chymotrypsin inhibitor 2 probed by mutagenesis. *J. Mol. Biol.* 270, 99-110.