Supporting Information

pmx Webserver: A User Friendly Interface for Alchemistry

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Simulation details

GXG tripeptide simulation setup

The equilibrium simulations of the tripeptides were performed without constructing hybrid structures and topologies for the mutants. Capped tripeptides for every amino acid supported by pmx were placed in a dodecahedron box and solvated with 4049 TIP3P water molecules.[1] For the Charmm22* and Charmm36 force fields the TIP3P water model with the Lennard-Jones parameters on hydrogen atoms was used. Na^+ and Cl^- ions were added to neutralize the system if necessary and to reach a 150 mM salt concentration. For the OPLSAA/L and Charmm force fields the default ion parameters distributed with the Gromacs simulation package were used. For the Amber force fields we used the ions reparameterized by Joung and Cheatham.[2] After the steepest descent energy minimization 20 ns molecular dynamics simulations were performed.

During the course of simulation the system was kept at 300 K temperature by means of the velocity rescaling thermostat[3] with a time constant of 0.1 ps. The pressure was kept at 1 bar using the Parrinello-Rahman barostat[4] with a time constant of 5 ps and compressibility set to 4.6e-5 bar^{-1}. The electrostatic interactions were treated by means of the Particle Mesh Ewald (PME) algorithm[5] where the short range cut-off value was set to 1.2 nm, PME interpolation order was set to 6 and the relative electrostatic interaction strength at the cut-off of 1e-6 was used. The grid spacing for the electrostatic calculations in the reciprocal space was set to 0.1 nm. The van der Waals interactions were switched off between 1 and 1.1 nm. The neighbour list search cut-off of 1.2 nm was used. A dispersion correction for the energy and pressure was applied. All bonds were constrained by means of the LINCS algorithm[6] with the matrix expansion order of 6. The bonded parameters of the water molecules were kept constrained using the SETTLE algorithm.[7] The integration time step of 2 fs was used.

From the equilibrium trajectories, the first 4 ns were discarded, while from the rest 100 snapshots were extracted equidistantly. For every snapshot a hybrid structure of a mutation was introduced. The introduced dummy atoms were energy minimized by keeping the rest of the system frozen. Afterwards, a 20 ps MD simulation was performed to equilibrate velocities. Finally, the alchemical transitions were carried out in 50 ps by enabling the soft-core function with the default set of parameters[8] for the van der Waals and Coulombic interactions.

Gromacs 4.6 version was used for the tripeptide equilibration and transition simulations.

Trp cage alanine scan setup

For both Trp cage investigations the equilibrium simulations were started with the hybrid structures of the mutations already introduced into the system. The calculations were performed used two force fields: Amber99sb*ILDN and Charmm36. For both force fields the TIP3P water model was used. Na^+ and Cl^- ions were added to neutralize the system and reach 150 mM salt concentration.

In the case of alanine scanning study, the "double system in a single box" setup[9] was used. The folded Trp cage protein in the physical state A and a GXG tripeptide in the state B were placed in the same simulation box $\sim$3 nm apart. Position restraints were applied to the C_α atom of the central residue of a tripeptide. Linear removal of the center of mass motion was applied for the folded protein

and the rest of the system during the equilibrium simulations. The equilibrium MD simulations were carried out for 20 ns and the last 8 ns were used to equidistantly extract 100 snapshots for the 100 ps alchemical transitions.

Most of the simulation parameters were retained from the GXG setup described in the previous section, except for the PME order, which was set to 4, the PME grid spacing was set to 0.12 nm, short range electrostatic interaction cut-off of 1.1 nm was used and the relative electrostatic interaction strength at the cut-off of 1e-05 was used. The temperature of the system was kept at 298 K. For the equilibration runs the Verlet cut-off scheme was employed allowing GPU utilization for the short range non-bonded interaction calculations.[10,11] For the alchemical transitions, the Group cut-off scheme was used, thus requiring to adjust the short range electrostatic interaction cut-off to 1.2 nm.

Trp cage stability optimization setup

The simulation parameters for the Trp cage stability optimization analysis were identical to those used in the alanine scanning investigation, with only slight modifications in the simulation setup. Since all the mutations in this case were charge conserving, the "double system in a single simulation box" setup was not applied. The folded Trp cage structures and tripeptides were placed in the simulation boxes separately. The equilibrium simulations were carried out for 10 ns of which the last 8 ns were used to extract 100 snapshots for the 100 ps transition runs.

For the Trp cage alanine scan and stability optimization equilibrium simulations, Gromacs 5.0 was used, while the transitions were carried out with Gromacs 4.6.

Table S1: Amino acid naming conventions used in the mutation libraries for the different force fields.

Amino acid	1 letter code	Amber99sb	Amber99sb*ILDN	OPLSAA/L	Charmm22*	Charmm36
Alanine	A	ALA	ALA	ALA	ALA	ALA
Arginine	R	ARG	ARG	ARG	ARG	ARG
Asparagine	N	ASN	ASN	ASN	ASN	ASN
Aspartate	D	ASP	ASP	ASP	ASP	ASP
Aspartate protonated	B	ASH	ASH	ASPP[a]	ASPP	ASPP
Cysteine	C	CYS	CYS	CYSH	CYS	CYS
Cysteine forming a disulfide bridge[b] with another cysteine	-	CYX	CYX	CYS2	CYS2	CYS2
Glycine	G	GLY	GLY	GLY	GLY	GLY
Glutamate	E	GLU	GLU	GLU	GLU	GLU
Glutamate protonated	J	GLH	GLH	GLUP[c]	GLUP	GLUP
Glutamine	Q	GLN	GLN	GLN	GLN	GLN
Histidine: only Nϵ2 protonated	X	HIE	HIE	HISE	HSE	HSE
Histidine: only Nδ1 protonated	H	HID	HID	HISD	HSD	HSD
Histidine: Nϵ2 and Nδ1 protonated	Z	HIP	HIP	HISH	HSP	HSP
Isoleucine	I	ILE	ILE	ILE	ILE	ILE
Leucine	L	LEU	LEU	LEU	LEU	LEU
Lysine	K	LYS	LYS	LYSH	LYS	LYS
Lysine unprotonated	O	LYN	LYN	LSN[d]	LSN	LSN
Methionine	M	MET	MET	MET	MET	MET
Phenylalanine	F	PHE	PHE	PHE	PHE	PHE
Serine	S	SER	SER	SER	SER	SER
Threonine	T	THR	THR	THR	THR	THR
Tryptophan	W	TRP	TRP	TRP	TRP	TRP
Tyrosine	Y	TYR	TYR	TYR	TYR	TYR
Valine	V	VAL	VAL	VAL	VAL	VAL
Acetyl N-terminal capping group[e]	-	ACE	ACE	ACE	ACE	ACE
N-methylamide C-terminal capping group[e]	-	NME	NME	NAC	CT3	CT3

[a] Protonated aspartate in the mutation library for the OPLSAA/L has been renamed into ASPP, whereas in the native OPLSAA/L it is named ASPH.

[b] Mutations of the sulfur bridge forming cysteine are not supported by pmx.

[c] Protonated glutamate in the mutation library for the OPLSAA/L has been renamed into GLUP, whereas in the native OPLSAA/L it is named GLUH.

[d] Neutral lysine in the mutation library for the OPLSAA/L has been renamed into LSN, whereas in the native OPLSAA/L it is named LYS.

[e] Mutations of the capping groups are not supported by pmx.

Table S2: An example of input for more than 3 mutations to be introduced at once. In this example 4 residues will be mutated. Note that the residues do not need to be entered sequentially. The input may contain two or three values per entry. If three values are entered, the first character will be interpreted as a chain identifier and the second value will mark residue ID. For the entries with two values, the first value will be interpreted as a residue ID and the first residue in the structure matching this ID will be used. The last value in every entry denotes the mutation to be performed.

X	3	A
Y	5	V
20	R	
A	11	D

Table S3: The variants of Trp cage used in the simulations. TC5b and TC10b served as the references to introduce mutations of the other variants. The melting temperature values were experimentally estimated from the CSD (chemical shift deviations) and CD (circular dichroism) measurements.[13]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	T_m, °C (CSD)	T_m, °C (CD)
Reference TC5b	N	L	Y	I	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	42	42
mut TC5a	N	L	**F**	I	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	32	31
mut TC8a	N	L	Y	**A**	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	46	46
mut TC9b	N	**A**	Y	I	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	51	50
mut TC9b_1	N	**A**	Y	I	**A**	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	41	43
mut TC11b1	**G**	**A**	Y	**A**	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	39	41
mut TC11b1_1	**G**	**A**	**F**	**A**	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	29	26
Reference TC10b	D	A	Y	A	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	56	56
mut TC10b_1	D	A	**F**	A	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	47	46
mut TC10b_2	D	A	**L**	A	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	39	-
mut TC10b_3	D	A	**A**	A	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	6	-
mut TC10b_4	D	A	Y	A	Q	W	**A**	K	D	G	G	P	S	S	G	R	P	P	P	S	11	-
mut TC10b_5	D	A	Y	A	Q	W	L	K	D	G	G	P	S	S	G	**K**	P	P	P	S	-	45.8[a]
mut TC8b	D	**L**	Y	A	Q	W	L	K	D	G	G	P	S	S	G	R	P	P	P	S	50	53

[a] T_m value from.[12]

Table S4: Free energy estimates for the capped GXG tripeptides in the Amber99sb force field.

	A	I	V	L	F	Y	W	T	M	C	S	N	Q	D	E	R	K	X	G	Z	H	O	B	J
A	-	12.04 ± 0.29	-71.94 ± 0.23	-70.48 ± 0.36	30.99 ± 0.34	-52.69 ± 0.45	50.87 ± 0.49	-120.53 ± 0.25	6.54 ± 0.32	15.73 ± 0.16	-39.15 ± 0.18	-355.91 ± 0.32	-289.15 ± 0.39	-	-	-	-	-59.00 ± 0.38	-46.09 ± 0.24	-	-52.13 ± 0.30	-46.00 ± 0.47	-211.29 ± 0.24	-181.19 ± 0.33
I	-12.04 ± 0.29	-	-84.88 ± 0.22	-85.54 ± 0.33	16.10 ± 0.38	-68.04 ± 0.47	34.72 ± 0.47	-129.20 ± 0.32	-11.43 ± 0.28	-0.53 ± 0.30	-51.01 ± 0.36	-368.04 ± 0.42	-302.17 ± 0.40	-	-	-	-	-73.64 ± 0.39	-60.92 ± 0.56	-	-68.18 ± 0.45	-58.43 ± 0.40	-228.18 ± 0.42	-199.60 ± 0.38
V	71.94 ± 0.23	84.88 ± 0.22	-	1.18 ± 0.51	100.82 ± 0.38	15.77 ± 0.52	119.15 ± 0.49	-45.27 ± 0.20	76.91 ± 0.37	83.88 ± 0.25	32.95 ± 0.29	-282.46 ± 0.56	-218.78 ± 0.46	-	-	-	-	9.65 ± 0.48	23.08 ± 0.45	-	16.24 ± 0.40	26.16 ± 0.49	-145.77 ± 0.44	-111.91 ± 0.53
L	70.48 ± 0.36	85.54 ± 0.33	-1.18 ± 0.51	-	102.47 ± 0.33	17.55 ± 0.44	120.80 ± 0.42	-50.91 ± 0.55	73.71 ± 0.29	83.57 ± 0.34	30.68 ± 0.39	-282.54 ± 0.28	-217.67 ± 0.40	-	-	-	-	11.99 ± 0.46	24.44 ± 0.52	-	16.66 ± 0.42	23.75 ± 0.54	-142.73 ± 0.28	-113.46 ± 0.34
F	-30.99 ± 0.34	-16.10 ± 0.38	-100.82 ± 0.38	-102.47 ± 0.33	-	-84.44 ± 0.13	18.14 ± 0.35	-151.40 ± 0.46	-25.59 ± 0.33	-15.43 ± 0.29	-72.00 ± 0.38	-387.00 ± 0.41	-320.14 ± 0.40	-	-	-	-	-91.42 ± 0.34	-64.84 ± 0.53	-	-85.11 ± 0.30	-78.79 ± 0.59	-242.20 ± 0.34	-212.52 ± 0.37
Y	52.69 ± 0.45	68.04 ± 0.47	-15.77 ± 0.52	-17.55 ± 0.44	84.44 ± 0.13	-	102.74 ± 0.37	-67.57 ± 0.62	60.49 ± 0.39	67.81 ± 0.36	11.59 ± 0.48	-302.70 ± 0.42	-235.73 ± 0.47	-	-	-	-	-7.17 ± 0.34	19.34 ± 0.55	-	-0.01 ± 0.33	6.58 ± 0.59	-158.68 ± 0.40	-127.61 ± 0.43
W	-50.87 ± 0.49	-34.72 ± 0.47	-119.15 ± 0.49	-120.80 ± 0.42	-18.14 ± 0.35	-102.74 ± 0.37	-	-169.56 ± 0.56	-42.46 ± 0.45	-33.84 ± 0.38	-91.04 ± 0.41	-403.00 ± 0.60	-339.11 ± 0.51	-	-	-	-	-109.86 ± 0.48	-83.84 ± 0.68	-	-102.93 ± 0.40	-97.49 ± 0.73	-258.08 ± 0.49	-231.09 ± 0.43
T	120.53 ± 0.25	129.20 ± 0.32	45.27 ± 0.20	50.91 ± 0.55	151.40 ± 0.46	67.57 ± 0.62	169.56 ± 0.56	-	127.59 ± 0.45	130.82 ± 0.24	80.66 ± 0.22	-239.87 ± 0.77	-167.87 ± 0.51	-	-	-	-	60.38 ± 0.56	73.50 ± 0.43	-	66.23 ± 0.52	73.98 ± 0.58	-104.65 ± 0.50	-61.79 ± 0.54
M	-6.54 ± 0.32	11.43 ± 0.28	-76.91 ± 0.37	-73.71 ± 0.29	25.59 ± 0.33	-60.49 ± 0.39	42.46 ± 0.45	-127.59 ± 0.45	-	8.87 ± 0.26	-46.40 ± 0.30	-356.28 ± 0.38	-293.50 ± 0.29	-	-	-	-	-66.21 ± 0.52	-52.69 ± 0.49	-	-59.49 ± 0.31	-48.98 ± 0.36	-211.92 ± 0.34	-186.42 ± 0.27
C	-15.73 ± 0.16	0.53 ± 0.30	-83.88 ± 0.25	-83.57 ± 0.34	15.43 ± 0.29	-67.81 ± 0.36	33.84 ± 0.38	-130.82 ± 0.24	-8.87 ± 0.26	-	-50.85 ± 0.13	-381.18 ± 0.39	-303.39 ± 0.32	-	-	-	-	-73.91 ± 0.36	-62.38 ± 0.36	-	-68.77 ± 0.32	-61.75 ± 0.49	-246.79 ± 0.34	-196.96 ± 0.36
S	39.15 ± 0.18	51.01 ± 0.36	-32.95 ± 0.29	-30.68 ± 0.39	72.00 ± 0.38	-11.59 ± 0.48	91.04 ± 0.41	-80.66 ± 0.22	46.40 ± 0.30	50.85 ± 0.13	-	-320.39 ± 0.52	-248.98 ± 0.37	-	-	-	-	-19.14 ± 0.45	-6.07 ± 0.31	-	-12.32 ± 0.33	-7.04 ± 0.47	-180.24 ± 0.35	-140.30 ± 0.31
N	355.91 ± 0.32	368.04 ± 0.42	282.46 ± 0.56	282.54 ± 0.28	387.00 ± 0.41	302.70 ± 0.42	403.00 ± 0.60	239.87 ± 0.77	356.28 ± 0.38	381.18 ± 0.39	320.39 ± 0.52	-	63.28 ± 0.64	-	-	-	-	297.35 ± 0.38	309.53 ± 0.52	-	302.69 ± 0.45	307.25 ± 0.73	144.77 ± 0.21	170.66 ± 0.54
Q	289.15 ± 0.39	302.17 ± 0.40	218.78 ± 0.46	217.67 ± 0.40	320.14 ± 0.40	235.73 ± 0.47	339.11 ± 0.51	167.87 ± 0.51	293.50 ± 0.29	303.39 ± 0.32	248.98 ± 0.37	-63.28 ± 0.64	-	-	-	-	-	229.72 ± 0.53	241.52 ± 0.53	-	235.72 ± 0.46	242.38 ± 0.48	79.58 ± 0.50	109.49 ± 0.21
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25.84 ± 0.65	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	-	-	-	-25.84 ± 0.65	-	-	-	-	-	-	-	-	-	-
R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	663.05 ± 0.30	-	-	723.24 ± 0.80	-	-	-	-
K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-663.05 ± 0.30	-	-	-	63.52 ± 0.72	-	-	-	-
X	59.00 ± 0.38	73.64 ± 0.39	-9.65 ± 0.48	-11.99 ± 0.46	91.42 ± 0.34	7.17 ± 0.34	109.86 ± 0.48	-60.38 ± 0.56	66.21 ± 0.52	73.91 ± 0.36	19.14 ± 0.45	-297.35 ± 0.38	-229.72 ± 0.53	-	-	-	-	-	26.01 ± 0.57	-	6.16 ± 0.25	11.20 ± 0.74	-153.19 ± 0.32	-122.24 ± 0.46
G	46.09 ± 0.24	60.92 ± 0.56	-23.08 ± 0.45	-24.44 ± 0.52	64.84 ± 0.53	-19.34 ± 0.55	83.84 ± 0.68	-73.50 ± 0.43	52.69 ± 0.49	62.38 ± 0.36	6.07 ± 0.31	-309.53 ± 0.52	-241.52 ± 0.53	-	-	-	-	-26.01 ± 0.57	-	-	-17.82 ± 0.36	3.84 ± 0.53	-163.62 ± 0.36	-132.62 ± 0.44
Z	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-723.24 ± 0.80	-63.52 ± 0.72	-	-	-	-	-	-	-
H	52.13 ± 0.30	68.18 ± 0.45	-16.24 ± 0.40	-16.66 ± 0.42	85.11 ± 0.30	0.01 ± 0.33	102.93 ± 0.40	-66.23 ± 0.52	59.49 ± 0.31	68.77 ± 0.32	12.32 ± 0.33	-302.69 ± 0.45	-235.72 ± 0.46	-	-	-	-	-6.16 ± 0.25	17.82 ± 0.36	-	-	6.93 ± 0.57	-157.25 ± 0.39	-128.33 ± 0.38
O	46.00 ± 0.47	58.43 ± 0.40	-26.16 ± 0.49	-23.75 ± 0.54	78.79 ± 0.59	-6.58 ± 0.59	97.49 ± 0.73	-73.98 ± 0.58	48.98 ± 0.36	61.75 ± 0.49	7.04 ± 0.47	-307.25 ± 0.73	-242.38 ± 0.48	-	-	-	-	-11.20 ± 0.74	-3.84 ± 0.53	-	-6.93 ± 0.57	-	-163.36 ± 0.70	-135.83 ± 0.50
B	211.29 ± 0.24	228.18 ± 0.42	145.77 ± 0.44	142.73 ± 0.28	242.20 ± 0.34	158.68 ± 0.40	258.08 ± 0.49	104.65 ± 0.50	211.92 ± 0.34	246.79 ± 0.34	180.24 ± 0.35	-144.77 ± 0.21	-79.58 ± 0.50	-	-	-	-	153.19 ± 0.32	163.62 ± 0.36	-	157.25 ± 0.39	163.36 ± 0.70	-	28.81 ± 0.50
J	181.19 ± 0.33	199.60 ± 0.38	111.91 ± 0.53	113.46 ± 0.34	212.52 ± 0.37	127.61 ± 0.43	231.09 ± 0.43	61.79 ± 0.54	186.42 ± 0.27	196.96 ± 0.36	140.30 ± 0.31	-170.66 ± 0.54	-109.49 ± 0.21	-	-	-	-	122.24 ± 0.46	132.62 ± 0.44	-	128.33 ± 0.38	135.83 ± 0.50	-28.81 ± 0.50	-

Table S5: Free energy estimates for the capped GXG tripeptides in the Amber99sb*ILDN force field.

	A	I	V	L	F	Y	W	T	M	C	S	N	Q	D	E	R	K	X	G	Z	H	O	B	J
A	-	9.64 ± 0.33	-70.48 ± 0.26	-70.77 ± 0.40	31.95 ± 0.39	-52.48 ± 0.49	49.20 ± 0.51	-120.98 ± 0.27	6.38 ± 0.34	15.58 ± 0.19	-39.09 ± 0.19	-355.29 ± 0.28	-288.32 ± 0.40	-	-	-	-	-59.86 ± 0.41	-47.32 ± 0.25	-	-51.77 ± 0.30	-46.35 ± 0.47	-211.28 ± 0.25	-179.53 ± 0.36
I	-9.64 ± 0.33	-	-82.02 ± 0.23	-82.14 ± 0.42	13.60 ± 0.37	-71.17 ± 0.46	32.10 ± 0.41	-128.47 ± 0.34	-8.93 ± 0.25	1.88 ± 0.24	-49.15 ± 0.27	-362.43 ± 0.36	-299.21 ± 0.25	-	-	-	-	-75.23 ± 0.43	-63.17 ± 0.48	-	-70.99 ± 0.40	-63.08 ± 0.43	-228.27 ± 0.38	-200.03 ± 0.33
V	70.48 ± 0.26	82.02 ± 0.23	-	0.18 ± 0.52	99.89 ± 0.38	15.44 ± 0.45	117.72 ± 0.45	-45.15 ± 0.21	76.06 ± 0.35	84.42 ± 0.24	32.77 ± 0.29	-280.26 ± 0.52	-217.48 ± 0.37	-	-	-	-	9.78 ± 0.38	21.41 ± 0.41	-	14.50 ± 0.36	22.45 ± 0.53	-146.86 ± 0.46	-112.01 ± 0.45
L	70.77 ± 0.40	82.14 ± 0.42	-0.18 ± 0.52	-	101.65 ± 0.33	15.97 ± 0.43	118.90 ± 0.43	-52.66 ± 0.64	74.05 ± 0.28	83.61 ± 0.36	29.72 ± 0.34	-279.16 ± 0.58	-218.58 ± 0.35	-	-	-	-	8.93 ± 0.52	22.11 ± 0.47	-	16.60 ± 0.36	25.96 ± 0.44	-143.34 ± 0.42	-113.93 ± 0.32
F	-31.95 ± 0.39	-13.60 ± 0.37	-99.89 ± 0.38	-101.65 ± 0.33	-	-84.48 ± 0.14	17.96 ± 0.32	-151.15 ± 0.51	-24.98 ± 0.35	-15.93 ± 0.34	-70.88 ± 0.35	-383.98 ± 0.40	-319.33 ± 0.41	-	-	-	-	-92.16 ± 0.33	-64.25 ± 0.55	-	-85.05 ± 0.30	-78.70 ± 0.54	-243.04 ± 0.36	-211.91 ± 0.35
Y	52.48 ± 0.49	71.17 ± 0.46	-15.44 ± 0.45	-15.97 ± 0.43	84.48 ± 0.14	-	103.68 ± 0.36	-66.83 ± 0.59	60.45 ± 0.43	68.35 ± 0.38	12.93 ± 0.49	-300.44 ± 0.39	-235.60 ± 0.53	-	-	-	-	-7.74 ± 0.33	20.26 ± 0.62	-	0.66 ± 0.35	6.81 ± 0.61	-157.93 ± 0.40	-126.57 ± 0.44
W	-49.20 ± 0.51	-32.10 ± 0.41	-117.72 ± 0.45	-118.90 ± 0.43	-17.96 ± 0.32	-103.68 ± 0.36	-	-169.47 ± 0.60	-41.93 ± 0.44	-33.15 ± 0.37	-88.84 ± 0.43	-402.07 ± 0.46	-337.90 ± 0.65	-	-	-	-	-110.63 ± 0.46	-82.53 ± 0.62	-	-102.50 ± 0.43	-95.49 ± 0.70	-260.39 ± 0.42	-228.53 ± 0.44
T	120.98 ± 0.27	128.47 ± 0.34	45.15 ± 0.21	52.66 ± 0.64	151.15 ± 0.51	66.83 ± 0.59	169.47 ± 0.60	-	127.75 ± 0.49	132.14 ± 0.25	80.73 ± 0.26	-232.51 ± 0.59	-165.87 ± 0.48	-	-	-	-	61.25 ± 0.54	73.61 ± 0.42	-	65.91 ± 0.58	72.87 ± 0.68	-104.90 ± 0.53	-59.30 ± 0.45
M	-6.38 ± 0.34	8.93 ± 0.25	-76.06 ± 0.35	-74.05 ± 0.28	24.98 ± 0.35	-60.45 ± 0.43	41.93 ± 0.44	-127.75 ± 0.49	-	8.87 ± 0.29	-46.69 ± 0.34	-352.57 ± 0.45	-293.85 ± 0.27	-	-	-	-	-66.69 ± 0.56	-54.31 ± 0.46	-	-59.89 ± 0.35	-48.72 ± 0.35	-212.01 ± 0.38	-186.43 ± 0.27
C	-15.58 ± 0.19	-1.88 ± 0.24	-84.42 ± 0.24	-83.61 ± 0.36	15.93 ± 0.34	-68.35 ± 0.38	33.15 ± 0.37	-132.14 ± 0.25	-8.87 ± 0.29	-	-51.05 ± 0.12	-374.81 ± 0.29	-303.45 ± 0.35	-	-	-	-	-74.79 ± 0.36	-62.36 ± 0.31	-	-69.79 ± 0.31	-61.58 ± 0.43	-246.84 ± 0.35	-196.12 ± 0.33
S	39.09 ± 0.19	49.15 ± 0.27	-32.77 ± 0.29	-29.72 ± 0.34	70.88 ± 0.35	-12.93 ± 0.49	88.84 ± 0.43	-80.73 ± 0.26	46.69 ± 0.34	51.05 ± 0.12	-	-314.56 ± 0.36	-248.28 ± 0.35	-	-	-	-	-19.55 ± 0.45	-7.46 ± 0.31	-	-12.51 ± 0.35	-6.18 ± 0.42	-179.63 ± 0.31	-140.12 ± 0.32
N	355.29 ± 0.28	362.43 ± 0.36	280.26 ± 0.52	279.16 ± 0.58	383.98 ± 0.40	300.44 ± 0.39	402.07 ± 0.46	232.51 ± 0.59	352.57 ± 0.45	374.81 ± 0.29	314.56 ± 0.36	-	59.50 ± 0.58	-	-	-	-	293.59 ± 0.39	302.71 ± 0.53	-	298.70 ± 0.39	303.03 ± 0.81	151.67 ± 0.32	167.15 ± 0.44
Q	288.32 ± 0.40	299.21 ± 0.25	217.48 ± 0.37	218.58 ± 0.35	319.33 ± 0.41	235.60 ± 0.53	337.90 ± 0.65	165.87 ± 0.48	293.85 ± 0.27	303.45 ± 0.35	248.28 ± 0.35	-59.50 ± 0.58	-	-	-	-	-	228.49 ± 0.62	241.52 ± 0.54	-	234.66 ± 0.48	241.66 ± 0.47	79.60 ± 0.46	109.00 ± 0.23
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36.69 ± 0.44	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	-	-	-	-36.69 ± 0.44	-	-	-	-	-	-	-	-	-	-
R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	662.45 ± 0.27	-	-	726.49 ± 0.72	-	-	-	-
K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-662.45 ± 0.27	-	-	-	63.45 ± 0.65	-	-	-	-
X	59.86 ± 0.41	75.23 ± 0.43	-9.78 ± 0.38	-8.93 ± 0.52	92.16 ± 0.33	7.74 ± 0.33	110.63 ± 0.46	-61.25 ± 0.54	66.69 ± 0.56	74.79 ± 0.36	19.55 ± 0.45	-293.59 ± 0.39	-228.49 ± 0.62	-	-	-	-	-	26.91 ± 0.62	-	7.28 ± 0.25	11.84 ± 0.69	-152.42 ± 0.38	-120.76 ± 0.48
G	47.32 ± 0.25	63.17 ± 0.48	-21.41 ± 0.41	-22.11 ± 0.47	64.25 ± 0.55	-20.26 ± 0.62	82.53 ± 0.62	-73.61 ± 0.42	54.31 ± 0.46	62.36 ± 0.31	7.46 ± 0.31	-302.71 ± 0.53	-241.52 ± 0.54	-	-	-	-	-26.91 ± 0.62	-	-	-16.50 ± 0.35	4.06 ± 0.49	-162.22 ± 0.37	-129.46 ± 0.45
Z	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-726.49 ± 0.72	-63.45 ± 0.65	-	-	-	-	-	-	-
H	51.77 ± 0.30	70.99 ± 0.40	-14.50 ± 0.36	-16.60 ± 0.36	85.05 ± 0.30	-0.66 ± 0.35	102.50 ± 0.43	-65.91 ± 0.58	59.89 ± 0.35	69.79 ± 0.31	12.51 ± 0.35	-298.70 ± 0.39	-234.66 ± 0.48	-	-	-	-	-7.28 ± 0.25	16.50 ± 0.35	-	-	5.12 ± 0.50	-157.84 ± 0.38	-126.85 ± 0.40
O	46.35 ± 0.47	63.08 ± 0.43	-22.45 ± 0.53	-25.96 ± 0.44	78.70 ± 0.54	-6.81 ± 0.61	95.49 ± 0.70	-72.87 ± 0.68	48.72 ± 0.35	61.58 ± 0.43	6.18 ± 0.42	-303.03 ± 0.81	-241.66 ± 0.47	-	-	-	-	-11.84 ± 0.69	-4.06 ± 0.49	-	-5.12 ± 0.50	-	-163.19 ± 0.73	-134.66 ± 0.47
B	211.28 ± 0.25	228.27 ± 0.38	146.86 ± 0.46	143.34 ± 0.42	243.04 ± 0.36	157.93 ± 0.40	260.39 ± 0.42	104.90 ± 0.53	212.01 ± 0.38	246.84 ± 0.35	179.63 ± 0.31	-151.67 ± 0.32	-79.60 ± 0.46	-	-	-	-	152.42 ± 0.38	162.22 ± 0.37	-	157.84 ± 0.38	163.19 ± 0.73	-	29.27 ± 0.49
J	179.53 ± 0.36	200.03 ± 0.33	112.01 ± 0.45	113.93 ± 0.32	211.91 ± 0.35	126.57 ± 0.44	228.53 ± 0.44	59.30 ± 0.45	186.43 ± 0.27	196.12 ± 0.33	140.12 ± 0.32	-167.15 ± 0.44	-109.00 ± 0.23	-	-	-	-	120.76 ± 0.48	129.46 ± 0.45	-	126.85 ± 0.40	134.66 ± 0.47	-29.27 ± 0.49	-

Table S6: Free energy estimates for the capped GXG tripeptides in the OPLSAA/L force field.

	A	I	V	L	F	Y	W	T	M	C	S	N	Q	D	E	R	K	X	G	Z	H	O	B	J
A	-	83.57 ± 0.30	52.18 ± 0.22	45.73 ± 0.32	59.36 ± 0.48	20.76 ± 0.53	27.48 ± 0.54	16.49 ± 0.30	22.32 ± 0.31	24.21 ± 0.33	20.68 ± 0.21	-143.78 ± 0.28	-135.01 ± 0.45	-	-	-	-	-50.73 ± 0.43	-35.10 ± 0.30	-	46.07 ± 0.50	28.11 ± 0.32	-117.00 ± 0.27	-79.74 ± 0.47
I	-83.57 ± 0.30	-	-35.89 ± 0.22	-36.53 ± 0.27	-4.34 ± 0.41	-43.04 ± 0.49	-34.46 ± 0.60	-67.83 ± 0.55	-65.99 ± 0.40	-84.62 ± 0.47	-63.51 ± 0.43	-231.30 ± 0.52	-233.96 ± 0.30	-	-	-	-	-116.50 ± 0.54	-98.12 ± 0.58	-	-17.82 ± 0.68	-41.38 ± 0.29	-197.78 ± 0.42	-165.80 ± 0.49
V	-52.18 ± 0.22	35.89 ± 0.22	-	-0.59 ± 0.39	9.73 ± 0.50	-29.12 ± 0.51	-23.01 ± 0.58	-39.81 ± 0.22	-29.94 ± 0.51	-48.31 ± 0.18	-35.58 ± 0.24	-195.79 ± 0.39	-194.05 ± 0.47	-	-	-	-	-100.66 ± 0.78	-84.41 ± 0.60	-	-4.73 ± 0.60	-22.82 ± 0.36	-173.77 ± 0.54	-136.46 ± 0.83
L	-45.73 ± 0.32	36.53 ± 0.27	0.59 ± 0.39	-	12.77 ± 0.29	-28.52 ± 0.43	-20.48 ± 0.48	-32.92 ± 0.58	-28.37 ± 0.30	-48.74 ± 0.52	-27.96 ± 0.52	-190.59 ± 0.30	-195.88 ± 0.38	-	-	-	-	-99.78 ± 0.90	-82.18 ± 0.64	-	-2.67 ± 0.34	-19.36 ± 0.39	-170.89 ± 0.32	-143.39 ± 0.42
F	-59.36 ± 0.48	4.34 ± 0.41	-9.73 ± 0.50	-12.77 ± 0.29	-	-38.43 ± 0.14	-32.45 ± 0.37	-24.43 ± 1.83	-37.68 ± 0.37	-39.14 ± 0.32	-33.36 ± 0.53	-195.48 ± 0.89	-192.38 ± 0.41	-	-	-	-	-109.48 ± 0.65	-72.68 ± 0.58	-	-15.91 ± 0.40	-26.49 ± 0.32	-169.37 ± 0.71	-139.88 ± 0.35
Y	-20.76 ± 0.53	43.04 ± 0.49	29.12 ± 0.51	28.52 ± 0.43	38.43 ± 0.14	-	6.04 ± 0.36	11.92 ± 1.58	0.93 ± 0.40	-1.85 ± 0.37	4.92 ± 0.45	-154.98 ± 0.91	-153.28 ± 0.54	-	-	-	-	-69.62 ± 0.90	-34.03 ± 0.66	-	24.53 ± 0.59	11.89 ± 0.42	-132.61 ± 0.69	-101.02 ± 0.45
W	-27.48 ± 0.54	34.46 ± 0.60	23.01 ± 0.58	20.48 ± 0.48	32.45 ± 0.37	-6.04 ± 0.36	-	7.61 ± 1.48	-3.94 ± 0.45	-3.47 ± 0.65	5.48 ± 1.15	-166.71 ± 0.52	-157.24 ± 0.81	-	-	-	-	-80.01 ± 0.44	-38.87 ± 0.72	-	18.36 ± 0.76	8.12 ± 0.62	-141.96 ± 0.54	-103.28 ± 0.49
T	-16.49 ± 0.30	67.83 ± 0.55	39.81 ± 0.22	32.92 ± 0.58	24.43 ± 1.83	-11.92 ± 1.58	-7.61 ± 1.48	-	9.17 ± 0.60	-9.38 ± 0.19	5.07 ± 0.16	-155.70 ± 0.43	-150.10 ± 0.68	-	-	-	-	-85.99 ± 2.89	-69.88 ± 1.26	-	8.32 ± 2.56	12.06 ± 0.57	-147.57 ± 1.59	-97.84 ± 4.70
M	-22.32 ± 0.31	65.99 ± 0.40	29.94 ± 0.51	28.37 ± 0.30	37.68 ± 0.37	-0.93 ± 0.40	3.94 ± 0.45	-9.17 ± 0.60	-	-19.95 ± 0.52	-2.63 ± 0.52	-166.06 ± 0.29	-165.64 ± 0.53	-	-	-	-	-76.81 ± 0.46	-57.88 ± 0.54	-	25.20 ± 0.58	9.65 ± 0.27	-145.96 ± 0.42	-110.92 ± 0.44
C	-24.21 ± 0.33	84.62 ± 0.47	48.31 ± 0.18	48.74 ± 0.52	39.14 ± 0.32	1.85 ± 0.37	3.47 ± 0.65	9.38 ± 0.19	19.95 ± 0.52	-	14.21 ± 0.15	-159.41 ± 0.29	-145.66 ± 0.59	-	-	-	-	-79.11 ± 0.77	-60.54 ± 0.50	-	27.78 ± 0.33	26.16 ± 0.43	-150.50 ± 0.38	-91.34 ± 0.69
S	-20.68 ± 0.21	63.51 ± 0.43	35.58 ± 0.24	27.96 ± 0.52	33.36 ± 0.53	-4.92 ± 0.45	-5.48 ± 1.15	-5.07 ± 0.16	2.63 ± 0.52	-14.21 ± 0.15	-	-161.87 ± 0.40	-154.28 ± 0.60	-	-	-	-	-92.53 ± 0.96	-73.46 ± 0.64	-	23.61 ± 0.52	9.32 ± 0.32	-148.75 ± 0.45	-100.21 ± 0.80
N	143.78 ± 0.28	231.30 ± 0.52	195.79 ± 0.39	190.59 ± 0.30	195.48 ± 0.89	154.98 ± 0.91	166.71 ± 0.52	155.70 ± 0.43	166.06 ± 0.29	159.41 ± 0.29	161.87 ± 0.40	-	-1.57 ± 0.48	-	-	-	-	87.24 ± 0.47	94.74 ± 0.66	-	173.07 ± 1.13	173.73 ± 0.42	37.44 ± 0.25	52.01 ± 0.28
Q	135.01 ± 0.45	233.96 ± 0.30	194.05 ± 0.47	195.88 ± 0.38	192.38 ± 0.41	153.28 ± 0.54	157.24 ± 0.81	150.10 ± 0.68	165.64 ± 0.53	145.66 ± 0.59	154.28 ± 0.60	1.57 ± 0.48	-	-	-	-	-	76.19 ± 1.13	93.98 ± 0.80	-	178.40 ± 0.52	173.52 ± 0.51	19.50 ± 0.47	66.19 ± 0.41
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-7.15 ± 0.39	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	-	-	-	7.15 ± 0.39	-	-	-	-	-	-	-	-	-	-
R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.78 ± 0.48	-	-	101.90 ± 0.51	-	-	-	-
K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-9.78 ± 0.48	-	-	-	84.45 ± 0.60	-	-	-	-
X	50.73 ± 0.43	116.50 ± 0.54	100.66 ± 0.78	99.78 ± 0.90	109.48 ± 0.65	69.62 ± 0.90	80.01 ± 0.44	85.99 ± 2.89	76.81 ± 0.46	79.11 ± 0.77	92.53 ± 0.96	-87.24 ± 0.47	-76.19 ± 1.13	-	-	-	-	-	37.41 ± 0.59	-	94.85 ± 1.26	89.01 ± 0.67	-63.04 ± 0.36	-20.84 ± 0.40
G	35.10 ± 0.30	98.12 ± 0.58	84.41 ± 0.60	82.18 ± 0.64	72.68 ± 0.58	34.03 ± 0.66	38.87 ± 0.72	69.88 ± 1.26	57.88 ± 0.54	60.54 ± 0.50	73.46 ± 0.64	-94.74 ± 0.66	-93.98 ± 0.80	-	-	-	-	-37.41 ± 0.59	-	-	59.65 ± 0.61	69.79 ± 0.63	-67.79 ± 0.49	-37.90 ± 0.62
Z	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-101.90 ± 0.51	-84.45 ± 0.60	-	-	-	-	-	-	-
H	-46.07 ± 0.50	17.82 ± 0.68	4.73 ± 0.60	2.67 ± 0.34	15.91 ± 0.40	-24.53 ± 0.59	-18.36 ± 0.76	-8.32 ± 2.56	-25.20 ± 0.58	-27.78 ± 0.33	-23.61 ± 0.52	-173.07 ± 1.13	-178.40 ± 0.52	-	-	-	-	-94.85 ± 1.26	-59.65 ± 0.61	-	-	-13.02 ± 0.42	-145.90 ± 0.78	-128.35 ± 0.36
O	-28.11 ± 0.32	41.38 ± 0.29	22.82 ± 0.36	19.36 ± 0.39	26.49 ± 0.32	-11.89 ± 0.42	-8.12 ± 0.62	-12.06 ± 0.57	-9.65 ± 0.27	-26.16 ± 0.43	-9.32 ± 0.32	-173.73 ± 0.42	-173.52 ± 0.51	-	-	-	-	-89.01 ± 0.67	-69.79 ± 0.63	-	13.02 ± 0.42	-	-153.60 ± 0.45	-126.08 ± 0.60
B	117.00 ± 0.27	197.78 ± 0.42	173.77 ± 0.54	170.89 ± 0.32	169.37 ± 0.71	132.61 ± 0.69	141.96 ± 0.54	147.57 ± 1.59	145.96 ± 0.42	150.50 ± 0.38	148.75 ± 0.45	-37.44 ± 0.25	-19.50 ± 0.47	-	-	-	-	63.04 ± 0.36	67.79 ± 0.49	-	145.90 ± 0.78	153.60 ± 0.45	-	35.06 ± 0.26
J	79.74 ± 0.47	165.80 ± 0.49	136.46 ± 0.83	143.39 ± 0.42	139.88 ± 0.35	101.02 ± 0.45	103.28 ± 0.49	97.84 ± 4.70	110.92 ± 0.44	91.34 ± 0.69	100.21 ± 0.80	-52.01 ± 0.28	-66.19 ± 0.41	-	-	-	-	20.84 ± 0.40	37.90 ± 0.62	-	128.35 ± 0.36	126.08 ± 0.60	-35.06 ± 0.26	-

Table S7: Free energy estimates for the capped GXG tripeptides in the Charmm22* force field.

	A	I	V	L	F	Y	W	T	M	C	S	N	Q	D	E	R	K	X	G	Z	H	O	B	J
A	-	-17.99 ± 0.34	-50.68 ± 0.22	-55.31 ± 0.49	68.92 ± 0.50	2.89 ± 0.47	74.96 ± 0.57	-97.98 ± 0.25	-13.93 ± 0.35	10.04 ± 0.20	21.90 ± 0.20	-384.46 ± 0.37	-237.49 ± 0.36	-	-	-	-	-131.45 ± 0.39	-	-	-64.98 ± 0.31	-19.29 ± 0.45	-288.94 ± 0.30	-179.47 ± 0.40
I	17.99 ± 0.34	-	3.55 ± 0.18	-75.90 ± 0.38	25.21 ± 0.42	-40.93 ± 0.46	31.98 ± 0.48	-107.44 ± 0.26	12.39 ± 0.27	21.41 ± 0.24	12.55 ± 0.34	-314.59 ± 0.39	-234.29 ± 0.27	-	-	-	-	-175.55 ± 0.49	-	-	-110.69 ± 0.45	-57.78 ± 0.42	-340.22 ± 0.39	-208.44 ± 0.44
V	50.68 ± 0.22	-3.55 ± 0.18	-	-80.55 ± 0.41	53.71 ± 0.38	-12.55 ± 0.37	60.99 ± 0.49	-67.58 ± 0.24	26.35 ± 0.35	42.74 ± 0.18	46.06 ± 0.33	-316.62 ± 0.38	-257.46 ± 0.36	-	-	-	-	-146.45 ± 0.43	-	-	-81.78 ± 0.34	-4.06 ± 0.50	-297.62 ± 0.33	-187.49 ± 0.50
L	55.31 ± 0.49	75.90 ± 0.38	80.55 ± 0.41	-	102.57 ± 0.34	35.57 ± 0.55	109.01 ± 0.51	-60.82 ± 0.45	88.45 ± 0.25	60.20 ± 0.29	58.21 ± 0.37	-271.53 ± 0.43	-158.43 ± 0.32	-	-	-	-	-96.90 ± 0.55	-	-	-33.65 ± 0.48	51.95 ± 0.42	-267.79 ± 0.37	-97.55 ± 0.32
F	-68.92 ± 0.50	-25.21 ± 0.42	-53.71 ± 0.38	-102.57 ± 0.34	-	-66.18 ± 0.12	6.75 ± 0.38	-134.32 ± 0.51	-57.90 ± 0.36	-57.69 ± 0.27	-47.27 ± 0.42	-383.42 ± 0.31	-284.25 ± 0.44	-	-	-	-	-200.22 ± 0.35	-	-	-136.21 ± 0.35	-56.92 ± 0.49	-347.69 ± 0.30	-227.43 ± 0.42
Y	-2.89 ± 0.47	40.93 ± 0.46	12.55 ± 0.37	-35.57 ± 0.55	66.18 ± 0.12	-	71.47 ± 0.37	-68.41 ± 0.55	8.37 ± 0.41	8.88 ± 0.32	18.52 ± 0.43	-317.47 ± 0.37	-218.66 ± 0.45	-	-	-	-	-134.84 ± 0.38	-	-	-69.35 ± 0.41	9.60 ± 0.51	-280.21 ± 0.42	-162.42 ± 0.44
W	-74.96 ± 0.57	-31.98 ± 0.48	-60.99 ± 0.49	-109.01 ± 0.51	-6.75 ± 0.38	-71.47 ± 0.37	-	-140.18 ± 0.57	-63.94 ± 0.46	-64.35 ± 0.38	-53.41 ± 0.45	-391.51 ± 0.45	-294.24 ± 0.45	-	-	-	-	-206.62 ± 0.38	-	-	-141.13 ± 0.43	-65.86 ± 0.54	-355.15 ± 0.41	-236.14 ± 0.55
T	97.98 ± 0.25	107.44 ± 0.26	67.58 ± 0.24	60.82 ± 0.45	134.32 ± 0.51	68.41 ± 0.55	140.18 ± 0.57	-	106.25 ± 0.44	95.69 ± 0.20	113.72 ± 0.20	-263.91 ± 0.40	-118.85 ± 0.41	-	-	-	-	-65.66 ± 0.48	-	-	-1.63 ± 0.43	70.53 ± 0.56	-240.64 ± 0.42	-97.86 ± 0.58
M	13.93 ± 0.35	-12.39 ± 0.27	-26.35 ± 0.35	-88.45 ± 0.25	57.90 ± 0.36	-8.37 ± 0.41	63.94 ± 0.46	-106.25 ± 0.44	-	12.05 ± 0.30	13.17 ± 0.37	-369.40 ± 0.42	-245.69 ± 0.27	-	-	-	-	-140.91 ± 0.48	-	-	-76.71 ± 0.31	-23.87 ± 0.30	-408.08 ± 0.35	-174.66 ± 0.27
C	-10.04 ± 0.20	-21.41 ± 0.24	-42.74 ± 0.18	-60.20 ± 0.29	57.69 ± 0.27	-8.88 ± 0.32	64.35 ± 0.38	-95.69 ± 0.20	-12.05 ± 0.30	-	17.69 ± 0.17	-373.01 ± 0.23	-257.94 ± 0.25	-	-	-	-	-141.75 ± 0.31	-	-	-76.59 ± 0.26	-21.34 ± 0.41	-331.68 ± 0.22	-184.66 ± 0.60
S	-21.90 ± 0.20	-12.55 ± 0.34	-46.06 ± 0.33	-58.21 ± 0.37	47.27 ± 0.42	-18.52 ± 0.43	53.41 ± 0.45	-113.72 ± 0.20	-13.17 ± 0.37	-17.69 ± 0.17	-	-376.27 ± 0.34	-238.85 ± 0.34	-	-	-	-	-152.20 ± 0.36	-	-	-88.24 ± 0.31	-12.26 ± 0.36	-322.51 ± 0.30	-182.98 ± 0.43
N	384.46 ± 0.37	314.59 ± 0.39	316.62 ± 0.38	271.53 ± 0.43	383.42 ± 0.31	317.47 ± 0.37	391.51 ± 0.45	263.91 ± 0.40	369.40 ± 0.42	373.01 ± 0.23	376.27 ± 0.34	-	68.21 ± 0.44	-	-	-	-	182.49 ± 0.48	-	-	246.59 ± 0.40	314.43 ± 0.61	47.99 ± 0.16	149.59 ± 0.53
Q	237.49 ± 0.36	234.29 ± 0.27	257.46 ± 0.36	158.43 ± 0.32	284.25 ± 0.44	218.66 ± 0.45	294.24 ± 0.45	118.85 ± 0.41	245.69 ± 0.27	257.94 ± 0.25	238.85 ± 0.34	-68.21 ± 0.44	-	-	-	-	-	85.10 ± 0.53	-	-	149.71 ± 0.32	207.18 ± 0.33	-61.55 ± 0.34	58.20 ± 0.15
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	77.24 ± 0.55	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	-	-	-	-77.24 ± 0.55	-	-	-	-	-	-	-	-	-	-
R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	544.31 ± 0.30	-	-	633.11 ± 0.54	-	-	-	-
K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-544.31 ± 0.30	-	-	-	87.66 ± 0.57	-	-	-	-
X	131.45 ± 0.39	175.55 ± 0.49	146.45 ± 0.43	96.90 ± 0.55	200.22 ± 0.35	134.84 ± 0.38	206.62 ± 0.38	65.66 ± 0.48	140.91 ± 0.48	141.75 ± 0.31	152.20 ± 0.36	-182.49 ± 0.48	-85.10 ± 0.53	-	-	-	-	-	-	-	64.00 ± 0.22	141.72 ± 0.57	-147.23 ± 0.46	-28.74 ± 0.59
G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-633.11 ± 0.54	-87.66 ± 0.57	-	-	-	-	-	-	-
H	64.98 ± 0.31	110.69 ± 0.45	81.78 ± 0.34	33.65 ± 0.48	136.21 ± 0.35	69.35 ± 0.41	141.13 ± 0.43	1.63 ± 0.43	76.71 ± 0.31	76.59 ± 0.26	88.24 ± 0.31	-246.59 ± 0.40	-149.71 ± 0.32	-	-	-	-	-64.00 ± 0.22	-	-	-	76.63 ± 0.40	-211.55 ± 0.34	-93.32 ± 0.38
O	19.29 ± 0.45	57.78 ± 0.42	4.06 ± 0.50	-51.95 ± 0.42	56.92 ± 0.49	-9.60 ± 0.51	65.86 ± 0.54	-70.53 ± 0.56	23.87 ± 0.30	21.34 ± 0.41	12.26 ± 0.36	-314.43 ± 0.61	-207.18 ± 0.33	-	-	-	-	-141.72 ± 0.57	-	-	-76.63 ± 0.40	-	-309.10 ± 0.45	-192.74 ± 0.28
B	288.94 ± 0.30	340.22 ± 0.39	297.62 ± 0.33	267.79 ± 0.37	347.69 ± 0.30	280.21 ± 0.42	355.15 ± 0.41	240.64 ± 0.42	408.08 ± 0.35	331.68 ± 0.22	322.51 ± 0.30	-47.99 ± 0.16	61.55 ± 0.34	-	-	-	-	147.23 ± 0.46	-	-	211.55 ± 0.34	309.10 ± 0.45	-	144.28 ± 0.41
J	179.47 ± 0.40	208.44 ± 0.44	187.49 ± 0.50	97.55 ± 0.32	227.43 ± 0.42	162.42 ± 0.44	236.14 ± 0.55	97.86 ± 0.58	174.66 ± 0.27	184.66 ± 0.60	182.98 ± 0.43	-149.59 ± 0.53	-58.20 ± 0.15	-	-	-	-	28.74 ± 0.59	-	-	93.32 ± 0.38	192.74 ± 0.28	-144.28 ± 0.41	-

Table S8: Free energy estimates for the capped GXG tripeptides in the Charmm36 force field.

	A	I	V	L	F	Y	W	T	M	C	S	N	Q	D	E	R	K	X	G	Z	H	O	B	J
A	-	-4.04 ± 0.29	-38.71 ± 0.20	-42.49 ± 0.42	73.05 ± 0.49	9.78 ± 0.49	143.79 ± 0.53	-76.33 ± 0.24	7.73 ± 0.46	10.78 ± 0.21	36.56 ± 0.21	-348.82 ± 0.32	-211.59 ± 0.42	-	-	-	-	-128.21 ± 0.33	-	-	-65.23 ± 0.34	4.98 ± 0.47	-289.22 ± 0.33	-157.07 ± 0.40
I	4.04 ± 0.29	-	-2.39 ± 0.19	-79.29 ± 0.32	26.99 ± 0.48	-36.43 ± 0.49	96.04 ± 0.45	-100.09 ± 0.29	20.15 ± 0.28	23.85 ± 0.29	13.73 ± 0.31	-282.59 ± 0.52	-214.70 ± 0.33	-	-	-	-	-173.99 ± 0.39	-	-	-111.86 ± 0.47	-41.71 ± 0.36	-352.48 ± 0.37	-197.62 ± 0.28
V	38.71 ± 0.20	2.39 ± 0.19	-	-78.21 ± 0.33	55.77 ± 0.36	-8.20 ± 0.39	125.42 ± 0.46	-63.01 ± 0.23	37.49 ± 0.36	50.82 ± 0.20	46.57 ± 0.27	-284.53 ± 0.38	-232.42 ± 0.40	-	-	-	-	-145.60 ± 0.33	-	-	-83.77 ± 0.35	12.07 ± 0.41	-303.46 ± 0.33	-171.19 ± 0.41
L	42.49 ± 0.42	79.29 ± 0.32	78.21 ± 0.33	-	104.83 ± 0.36	37.61 ± 0.48	174.24 ± 0.44	-54.77 ± 0.47	98.50 ± 0.62	66.17 ± 0.34	58.71 ± 0.41	-240.17 ± 0.44	-137.49 ± 0.61	-	-	-	-	-95.02 ± 0.37	-	-	-32.85 ± 0.39	62.88 ± 0.53	-280.89 ± 0.30	-89.21 ± 0.37
F	-73.05 ± 0.49	-26.99 ± 0.48	-55.77 ± 0.36	-104.83 ± 0.36	-	-66.12 ± 0.13	62.84 ± 0.35	-134.94 ± 0.62	-57.80 ± 0.42	-59.34 ± 0.31	-49.83 ± 0.42	-381.92 ± 0.33	-281.38 ± 0.35	-	-	-	-	-219.44 ± 0.29	-	-	-156.04 ± 0.31	-56.81 ± 0.50	-348.74 ± 0.37	-228.53 ± 0.43
Y	-9.78 ± 0.49	36.43 ± 0.49	8.20 ± 0.39	-37.61 ± 0.48	66.12 ± 0.13	-	126.87 ± 0.35	-71.27 ± 0.55	7.74 ± 0.43	6.69 ± 0.33	15.49 ± 0.44	-314.48 ± 0.44	-215.72 ± 0.39	-	-	-	-	-153.07 ± 0.34	-	-	-89.63 ± 0.34	8.44 ± 0.52	-280.91 ± 0.40	-163.26 ± 0.43
W	-143.79 ± 0.53	-96.04 ± 0.45	-125.42 ± 0.46	-174.24 ± 0.44	-62.84 ± 0.35	-126.87 ± 0.35	-	-204.32 ± 0.58	-129.00 ± 0.50	-128.20 ± 0.44	-120.48 ± 0.46	-449.31 ± 0.57	-352.77 ± 0.44	-	-	-	-	-282.00 ± 0.52	-	-	-217.44 ± 0.53	-128.33 ± 0.55	-416.29 ± 0.52	-299.45 ± 0.49
T	76.33 ± 0.24	100.09 ± 0.29	63.01 ± 0.23	54.77 ± 0.47	134.94 ± 0.62	71.27 ± 0.55	204.32 ± 0.58	-	107.57 ± 0.50	99.00 ± 0.29	109.56 ± 0.17	-234.68 ± 0.46	-114.24 ± 0.51	-	-	-	-	-66.65 ± 0.58	-	-	-6.13 ± 0.50	79.30 ± 0.60	-250.61 ± 0.48	-90.69 ± 0.56
M	-7.73 ± 0.46	-20.15 ± 0.28	-37.49 ± 0.36	-98.50 ± 0.62	57.80 ± 0.42	-7.74 ± 0.43	129.00 ± 0.50	-107.57 ± 0.50	-	7.08 ± 0.32	5.30 ± 0.49	-350.58 ± 0.36	-234.76 ± 0.28	-	-	-	-	-139.21 ± 0.38	-	-	-75.83 ± 0.31	-22.01 ± 0.28	-432.84 ± 0.27	-177.47 ± 0.34
C	-10.78 ± 0.21	-23.85 ± 0.29	-50.82 ± 0.20	-66.17 ± 0.34	59.34 ± 0.31	-6.69 ± 0.33	128.20 ± 0.44	-99.00 ± 0.29	-7.08 ± 0.32	-	10.18 ± 0.18	-349.38 ± 0.28	-241.63 ± 0.32	-	-	-	-	-139.92 ± 0.27	-	-	-76.68 ± 0.29	-15.73 ± 0.46	-350.47 ± 0.24	-181.80 ± 0.67
S	-36.56 ± 0.21	-13.73 ± 0.31	-46.57 ± 0.27	-58.71 ± 0.41	49.83 ± 0.42	-15.49 ± 0.44	120.48 ± 0.46	-109.56 ± 0.17	-5.30 ± 0.49	-10.18 ± 0.18	-	-345.25 ± 0.40	-227.97 ± 0.41	-	-	-	-	-150.78 ± 0.30	-	-	-87.45 ± 0.27	-4.63 ± 0.55	-335.10 ± 0.36	-174.54 ± 0.49
N	348.82 ± 0.32	282.59 ± 0.52	284.53 ± 0.38	240.17 ± 0.44	381.92 ± 0.33	314.48 ± 0.44	449.31 ± 0.57	234.68 ± 0.46	350.58 ± 0.36	349.38 ± 0.28	345.25 ± 0.40	-	61.21 ± 0.30	-	-	-	-	183.14 ± 0.34	-	-	244.47 ± 0.38	296.35 ± 0.39	-0.46 ± 0.20	124.94 ± 0.57
Q	211.59 ± 0.42	214.70 ± 0.33	232.42 ± 0.40	137.49 ± 0.61	281.38 ± 0.35	215.72 ± 0.39	352.77 ± 0.44	114.24 ± 0.51	234.76 ± 0.28	241.63 ± 0.32	227.97 ± 0.41	-61.21 ± 0.30	-	-	-	-	-	83.14 ± 0.43	-	-	146.55 ± 0.38	197.87 ± 0.39	-96.06 ± 0.25	43.06 ± 0.31
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	76.84 ± 0.41	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	-	-	-	-76.84 ± 0.41	-	-	-	-	-	-	-	-	-	-
R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	895.15 ± 0.33	-	-	981.58 ± 0.61	-	-	-	-
K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-895.15 ± 0.33	-	-	-	91.63 ± 0.65	-	-	-	-
X	128.21 ± 0.33	173.99 ± 0.39	145.60 ± 0.33	95.02 ± 0.37	219.44 ± 0.29	153.07 ± 0.34	282.00 ± 0.52	66.65 ± 0.58	139.21 ± 0.38	139.92 ± 0.27	150.78 ± 0.30	-183.14 ± 0.34	-83.14 ± 0.43	-	-	-	-	-	-	-	66.53 ± 0.20	139.84 ± 0.44	-150.29 ± 0.33	-31.22 ± 0.36
G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-981.58 ± 0.61	-91.63 ± 0.65	-	-	-	-	-	-	-
H	65.23 ± 0.34	111.86 ± 0.47	83.77 ± 0.35	32.85 ± 0.39	156.04 ± 0.31	89.63 ± 0.34	217.44 ± 0.53	6.13 ± 0.50	75.83 ± 0.31	76.68 ± 0.29	87.45 ± 0.27	-244.47 ± 0.38	-146.55 ± 0.38	-	-	-	-	-66.53 ± 0.20	-	-	-	77.07 ± 0.42	-212.68 ± 0.38	-94.08 ± 0.37
O	-4.98 ± 0.47	41.71 ± 0.36	-12.07 ± 0.41	-62.88 ± 0.53	56.81 ± 0.50	-8.44 ± 0.52	128.33 ± 0.55	-79.30 ± 0.60	22.01 ± 0.28	15.73 ± 0.46	4.63 ± 0.55	-296.35 ± 0.39	-197.87 ± 0.39	-	-	-	-	-139.84 ± 0.44	-	-	-77.07 ± 0.42	-	-333.55 ± 0.37	-197.84 ± 0.31
B	289.22 ± 0.33	352.48 ± 0.37	303.46 ± 0.33	280.89 ± 0.30	348.74 ± 0.37	280.91 ± 0.40	416.29 ± 0.52	250.61 ± 0.48	432.84 ± 0.27	350.47 ± 0.24	335.10 ± 0.36	0.46 ± 0.20	96.06 ± 0.25	-	-	-	-	150.29 ± 0.33	-	-	212.68 ± 0.38	333.55 ± 0.37	-	165.24 ± 0.34
J	157.07 ± 0.40	197.62 ± 0.28	171.19 ± 0.41	89.21 ± 0.37	228.53 ± 0.43	163.26 ± 0.43	299.45 ± 0.49	90.69 ± 0.56	177.47 ± 0.34	181.80 ± 0.67	174.54 ± 0.49	-124.94 ± 0.57	-43.06 ± 0.31	-	-	-	-	31.22 ± 0.36	-	-	94.08 ± 0.37	197.84 ± 0.31	-165.24 ± 0.34	-

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