Supplementary Information

Engineering protein stability with atomic precision in a monomeric miniprotein

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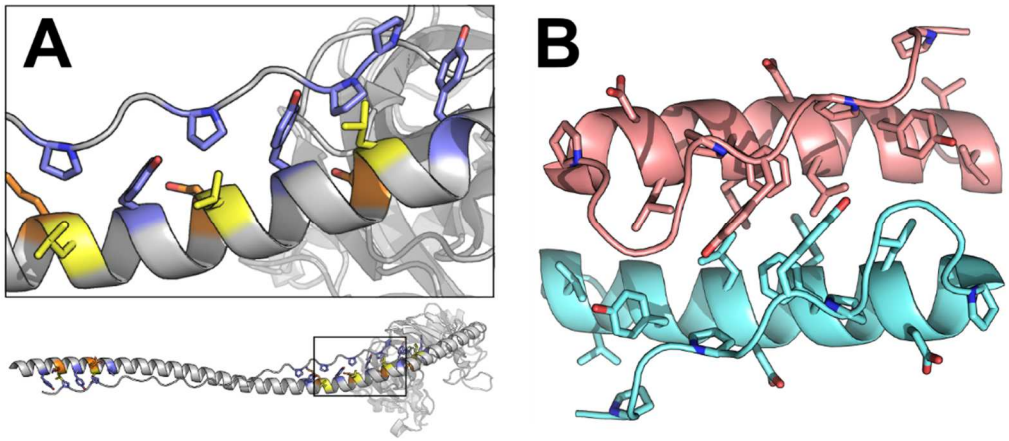
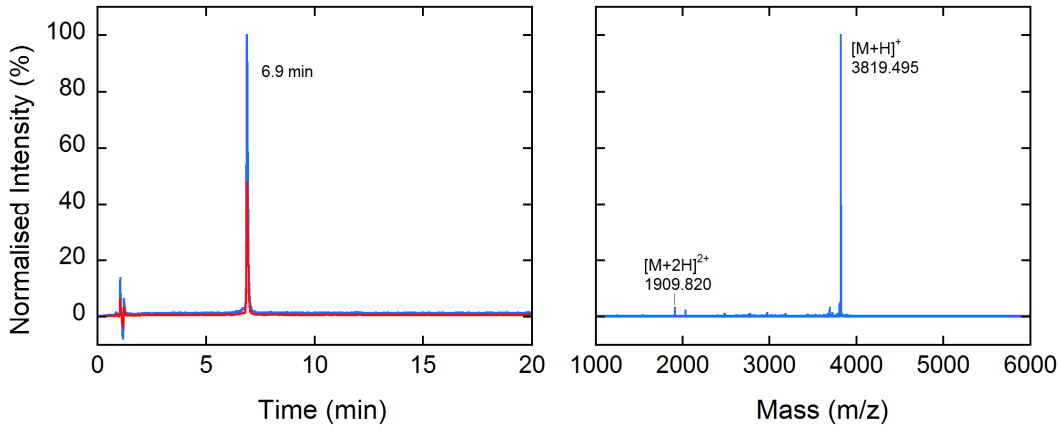
Supplementary Results

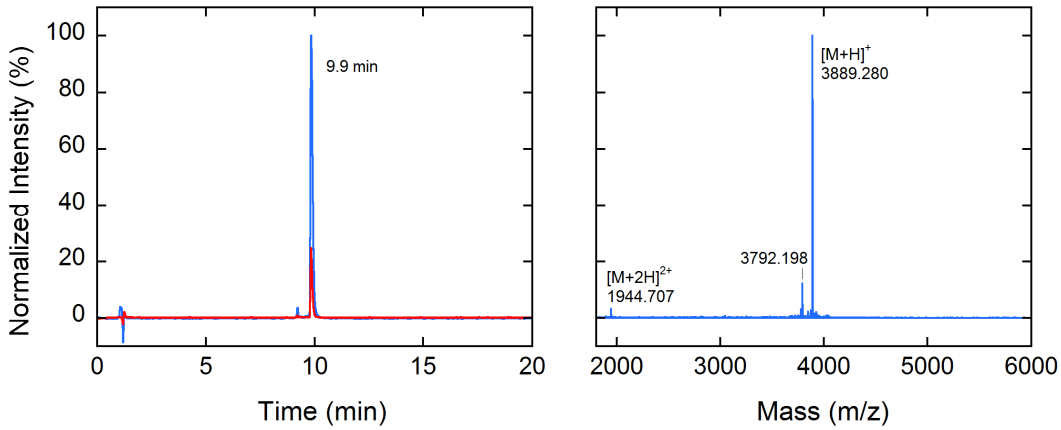


Supplementary Figure 1: Examples of natural proteins with unusual polyproline-II-helix–α-helix conformation. A: A3VP1 of Ag I/II of *Streptococcus mutans* (PDB: 3IOX). **B:** Avian pancreatic polypeptide of *Meleagris gallopavo* (turkey) (PDB: 2BF9).

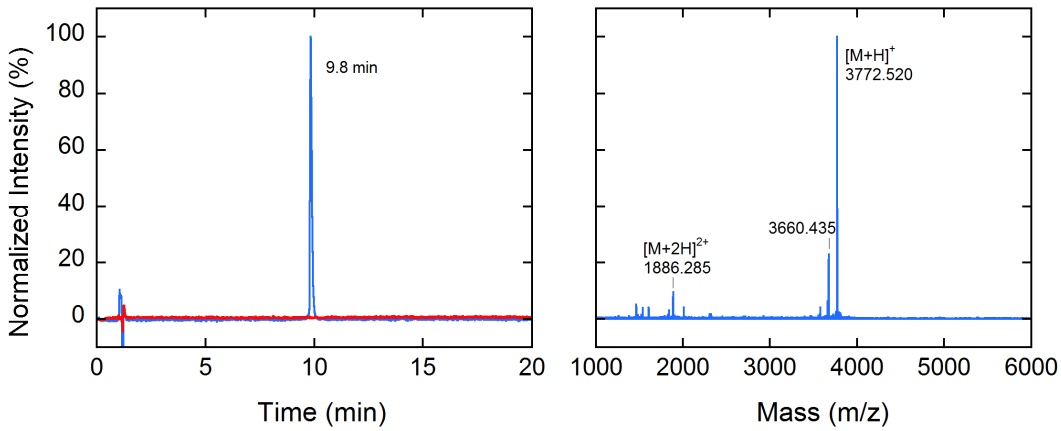
A PPα-Tyr



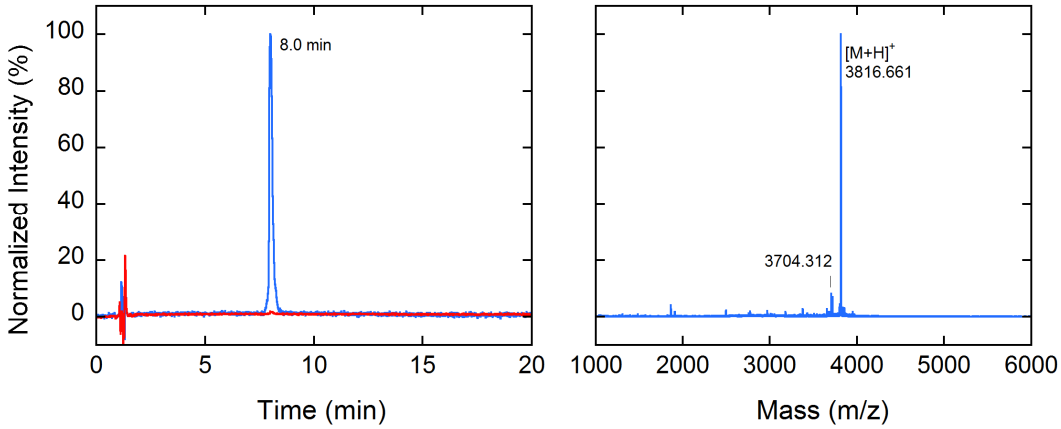
B PPα-Trp



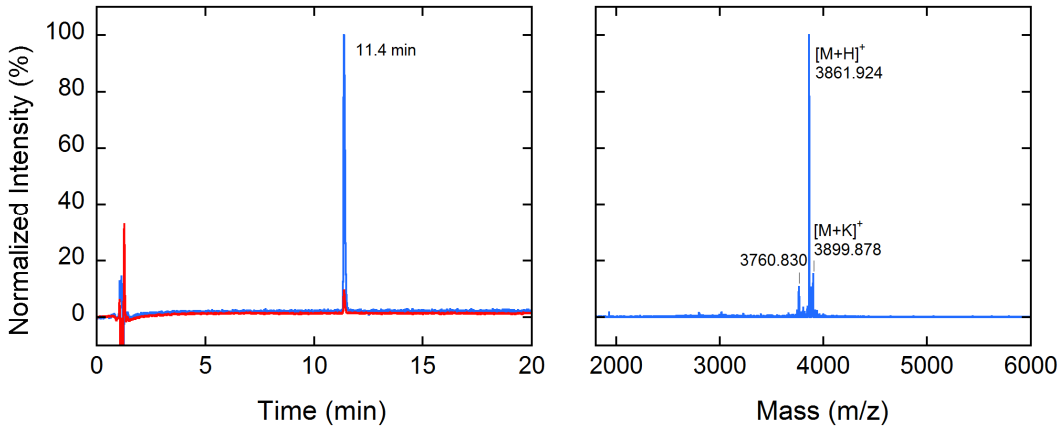
C PPα-Phe



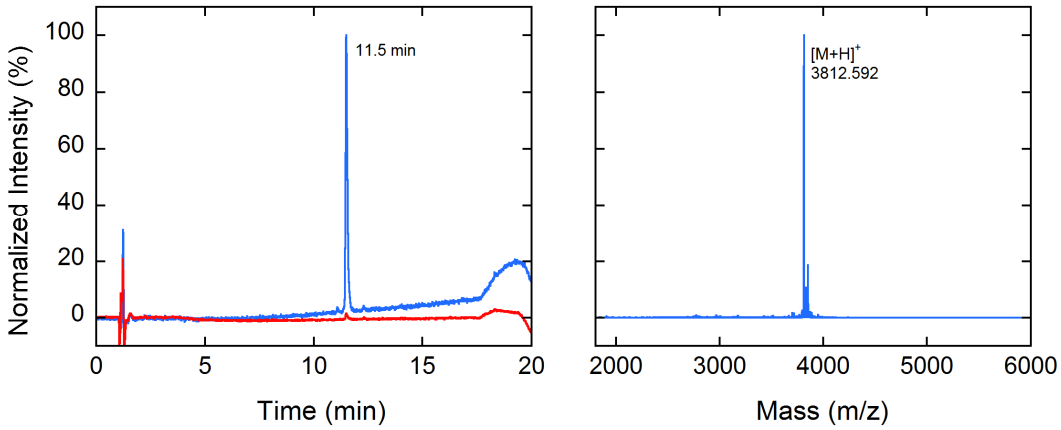
D PPα-ϕNH2



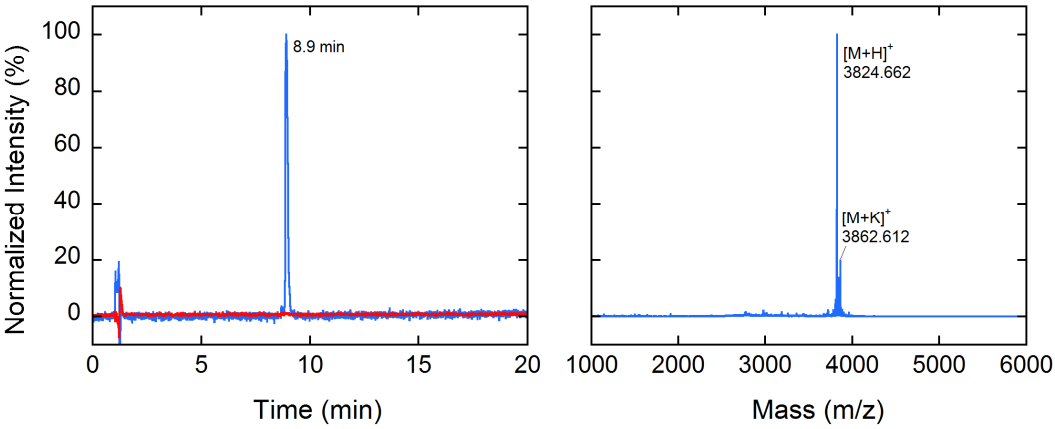
E PPα-ϕOCH3



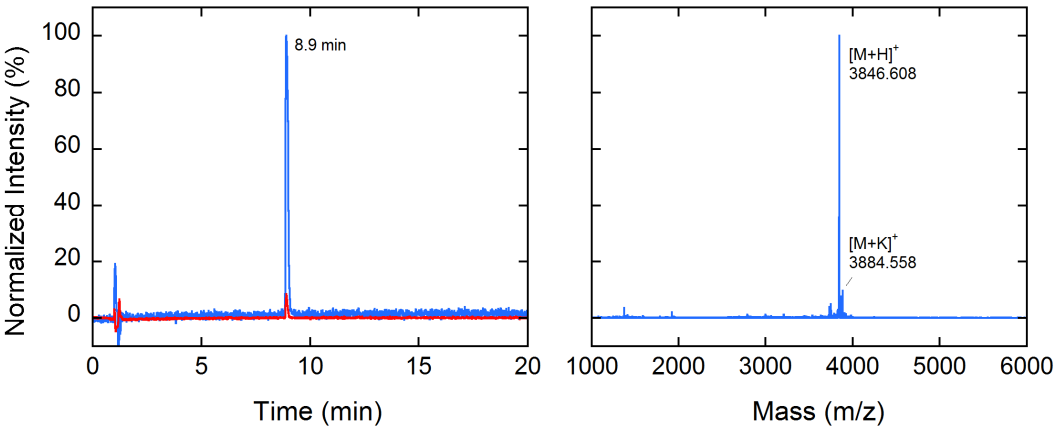
F PPα-ϕCH3



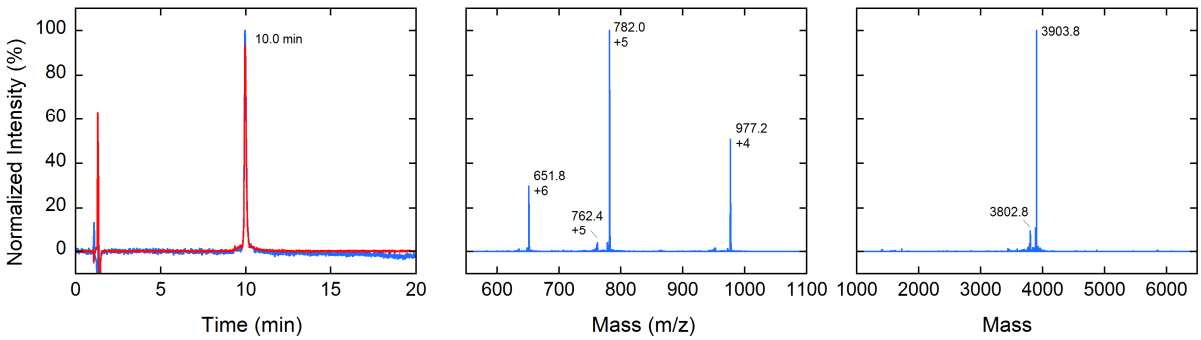
G PPα-ϕF



H PPα-ϕCN

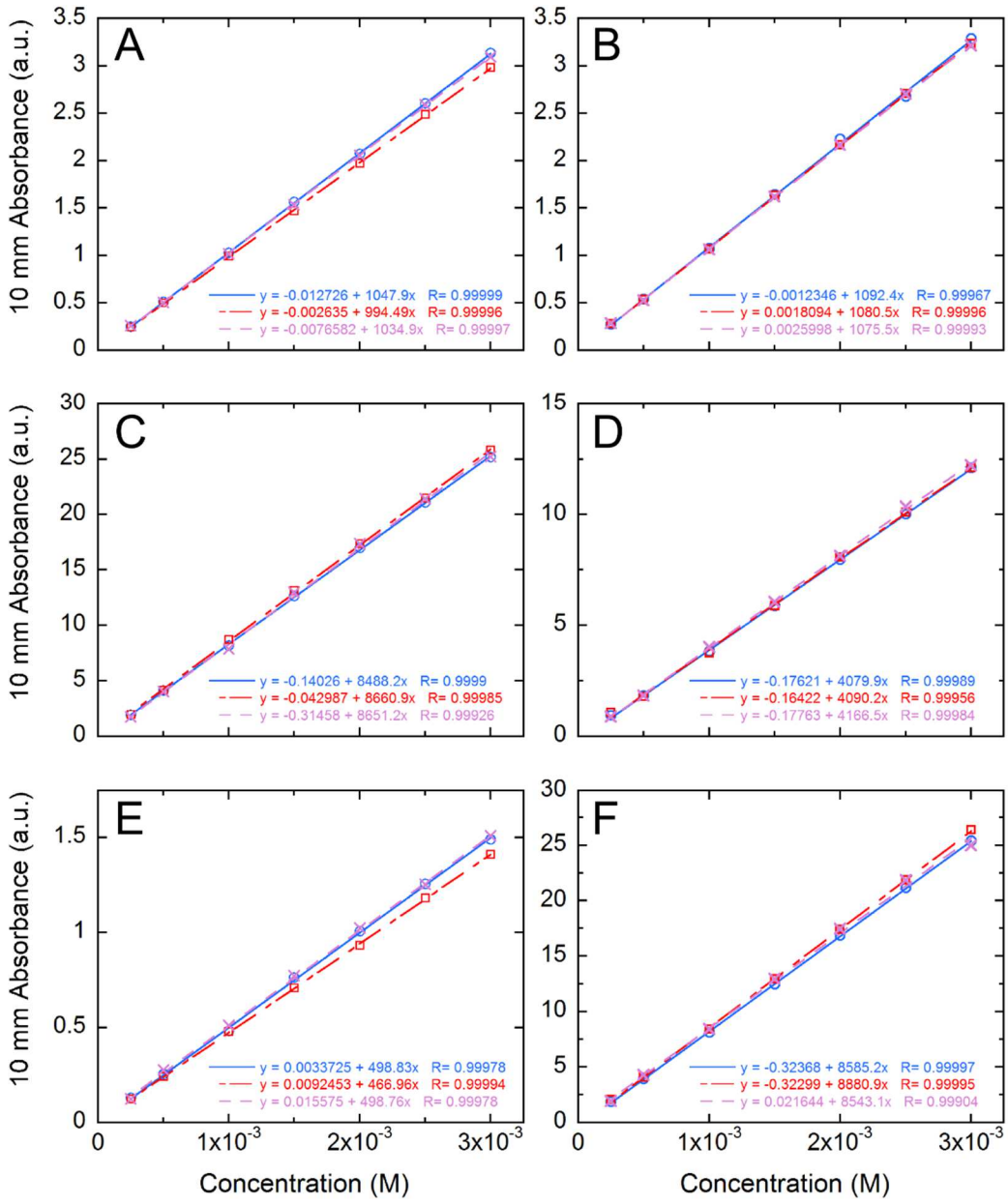


I PPα-ϕNO2



Supplementary Figure 2: Characterizations of the peptides in this study. A-H Analytical HPLC trace (left, monitored at 220 nm, blue and 280 nm, red) and MALDI-TOF mass spectrum (right) of **A:** PPα-Tyr, **B:** PPα-Trp, **C:** PPα-Phe, **D:** PPα-ϕNH_2, **E:** PPα-ϕOCH_3, **F:** PPα-ϕCH_3, **G:** PPα-ϕF, **H:** PPα-ϕCN, and **I:** PPα-ϕNO_2. In **I** the raw mass spectrum is shown centre and the deconvoluted spectrum, right. **Conditions:**

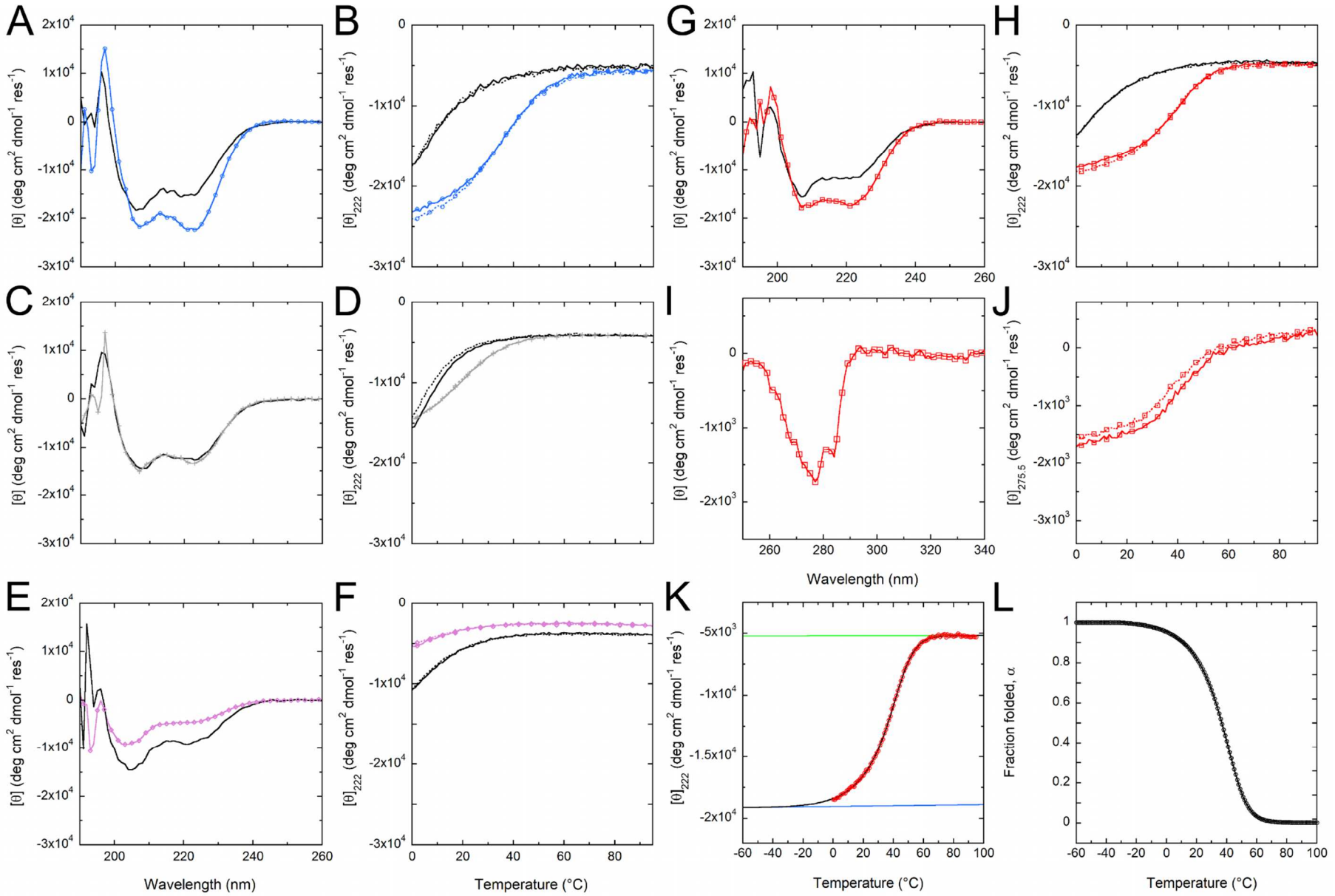
PPα-Tyr:	HPLC gradient = 10-60% buffer B. $[M+H]^+_{calc}$ = 3819.282 Da	PPα-ϕCH_3:	HPLC gradient = 20-60% buffer B. $[M+H]^+_{calc}$ = 3813.365 Da
PPα-Trp:	HPLC gradient = 10-60% buffer B. $[M+H]^+_{calc}$ = 3888.383 Da	PPα-ϕF:	HPLC gradient = 20-60% buffer B. $[M+H]^+_{calc}$ = 3525.254 Da
PPα-Phe:	HPLC gradient = 10-60% buffer B. $[M+H]^+_{calc}$ = 3771.284 Da	PPα-ϕCN:	HPLC gradient = 20-60% buffer B. $[M+H]^+_{calc}$ = 3846.314 Da
PPα-ϕNH_2:	HPLC gradient = 20-60% buffer B. $[M+H]^+_{calc}$ = 3816.329 Da	PPα-ϕNO_2:	HPLC gradient = 10-60% buffer B. $[M]_{calc}$ = 3902.969 Da
PPα-ϕOCH_3:	HPLC gradient = 10-60% buffer B. $[M+H]^+_{calc}$ = 3861.362 Da		



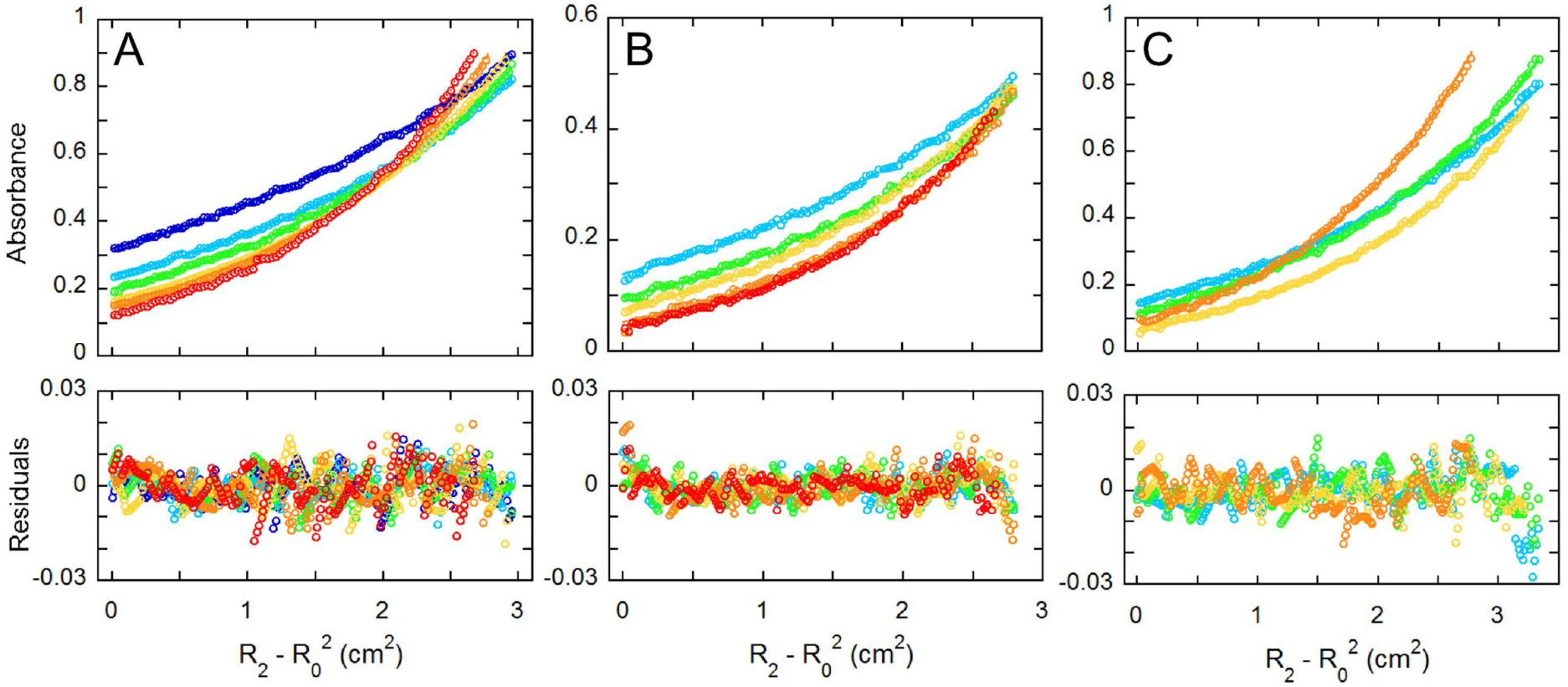
Supplementary Figure 3: Determinations of extinction coefficients for *para*-substituted phenylalanine amino acids. A: *p*-amino-Phe. **B:** *p*-methoxy-Phe. **C:** *p*-methyl-Phe. **D:** *p*-fluoro-Phe. **E:** *p*-cyano-Phe. **F:** *p*-nitro-Phe. **Conditions:**

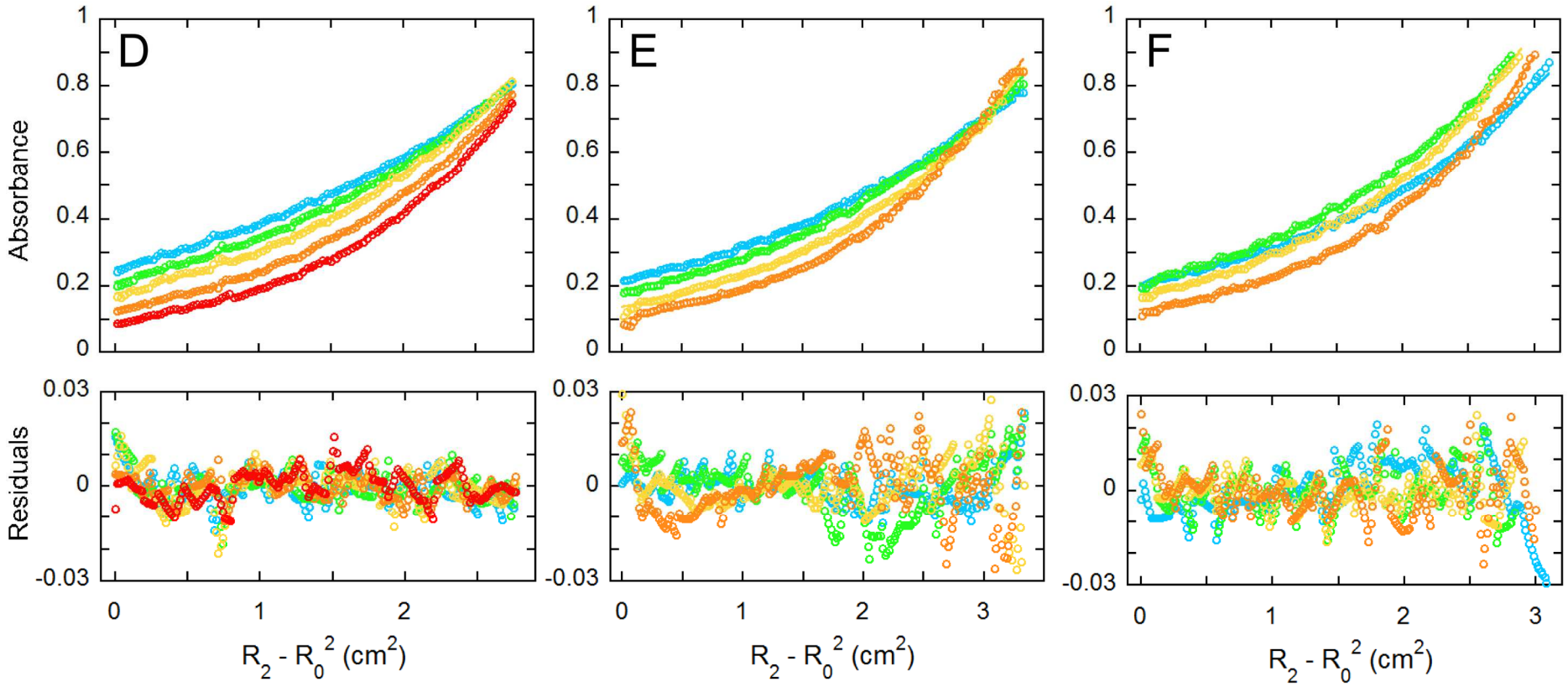
Amino acid	Pathlength (mm)	Wavelength (nm)	Extinction coefficient (M^{-1} cm^{-1})*	Solvent
p-amino-Phe	1	280	1015 ± 29	PBS
p-methoxy-Phe	1	280	1078 ± 9	H_2O
p-methyl-Phe	0.1	214	8573 ± 85	H_2O
p-fluoro-Phe	0.1	214	4151 ± 31	H_2O
p-cyano-Phe	1	280	494 ± 5	H_2O
p-nitro-Phe	0.1	280	8673 ± 235	H_2O:DMSO (1:1 vol%)

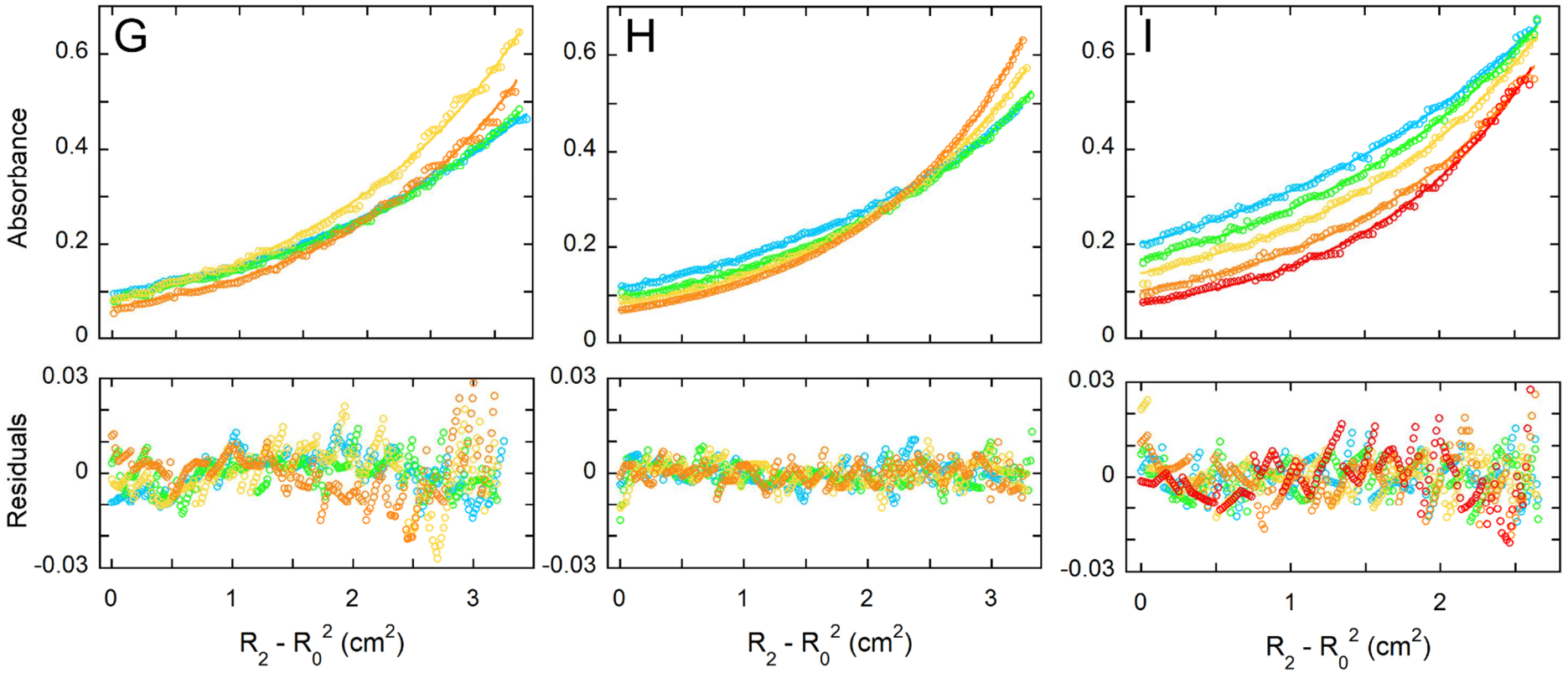
* Mean ± 1 standard deviation of 3 experiments


A
B
C
D
E
F
G
H
I
J
K
L
[θ] (deg cm^2 dmol^{-1} res^{-1})
[θ]$_{222}$ (deg cm^2 dmol^{-1} res^{-1})
[θ]$_{275.5}$ (deg cm^2 dmol^{-1} res^{-1})
Wavelength (nm)
Temperature (°C)
Fraction folded, α

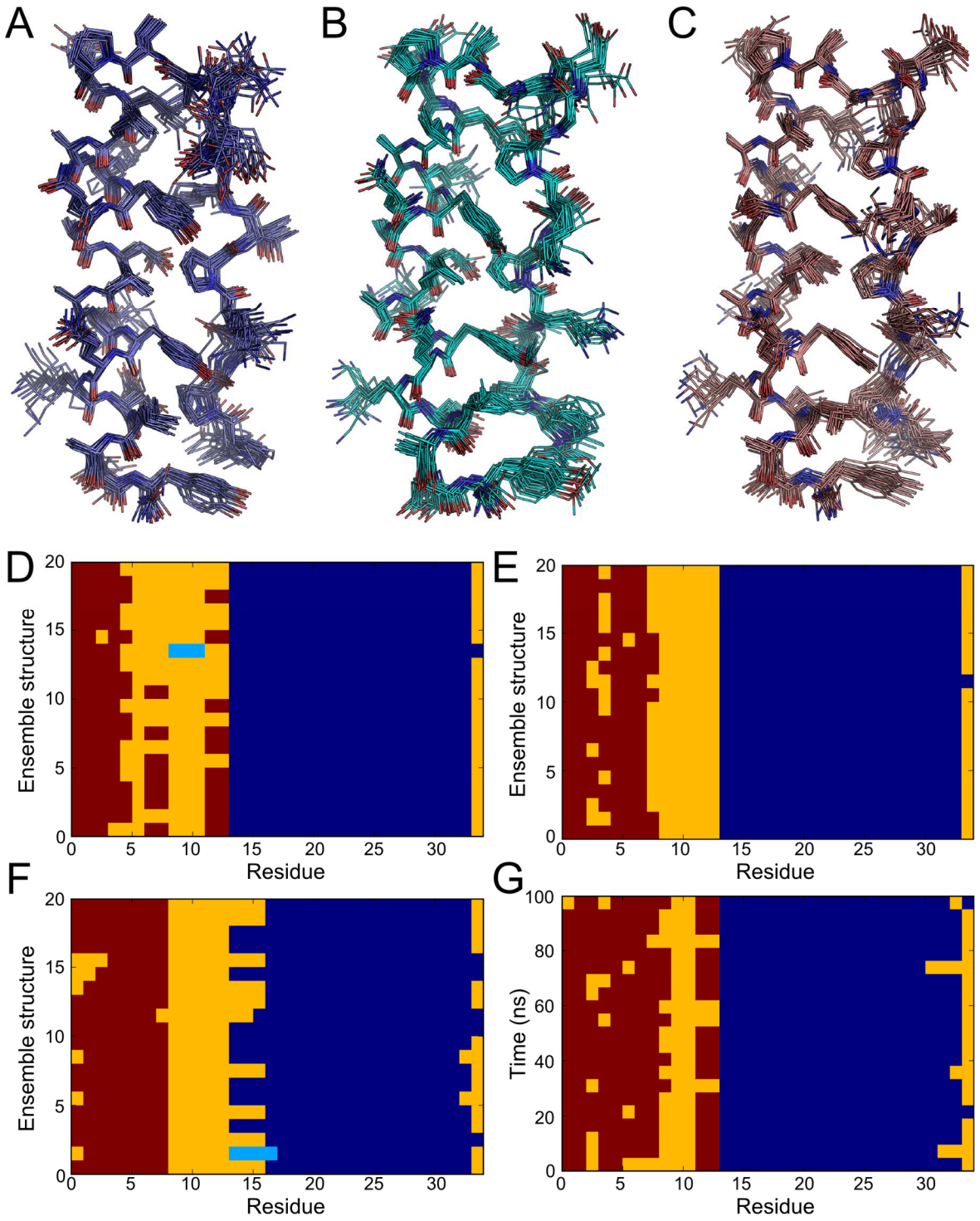
Supplementary Figure 4: Additional CD spectra and thermal denaturation profiles for peptides in this study. CD spectra recorded at 5 °C (**A, C, E, G, I**), and thermal denaturation curves (solid lines) and refolding profiles (dashed lines) monitored at 222 nm (**B, D, F&H**) and at 275.5 nm (**J**) for PPα-Trp (blue circles) and α-Trp (black line), **A&B**; PPα-Phe (gray crosses) and α-Phe (black line), **C&D**; PPα-His (lilac diamonds) and α-His (black line), **E&F**; PPα-Tyr (red squares) and α-Tyr (black line), **G&H** and PPα-Tyr measured in the near UV region, **I&J.** The T_M of PPα-Tyr when measured through the aromatic CD signal at 275.5 nm is 40 °C. **Conditions:** 100 μM peptide in PBS, pH 7.4, 1 mm path length. Near UV spectra were recorded with a 1 cm path length. The identities and purities (>98 %) of α-Tyr, α-Trp, α-Phe and α-His were confirmed by MALDI-TOF mass spectrometry and analytical HPLC respectively (data not shown). Analytical ultracentrifugation sedimentation equilibrium experiments (data not shown) revealed α-Tyr was 1.2 x its calculated monomer mass (99% confidence limits = 2975–3015) and α-Trp was 1.3 x its calculated monomer mass (99% confidence limits = 3297–3402). The fits for α-Phe to a single ideal species were not suitable for the experimental data and indicated aggregation. α-His was not measured. For the Tyr-, Trp- and Phe-containing peptides the full-length PPα miniprotein has significantly more thermal stability than the α-helix alone. Furthermore, the thermal denaturation profiles for the miniproteins are sigmoidal in shape, which suggests cooperative folding between the α-helix and polyproline-II helices. PPα-His and α-His are not well folded, consistent with an electron-poor His ring. **K&L:** An example of the thermal-denaturation curve-fitting procedure. **K:** Thermal-denaturation profile of PPα-Tyr (red circles) and the fitted data (black, solid line), see Materials and Methods for fitting equations. The calculated upper (green) and lower (blue) baselines are also shown. Note, we cannot access the fully folded state of PPα-Tyr experimentally. The lower baseline represents the theoretical CD signal of the fully folded state of the miniprotein and the upper baseline the fully unfolded state. **L:** Fraction folded, α, of PPα-Tyr from curve fitting.


A
B
C
Absorbance
Residuals
$R_2 - R_0^2$ (cm^2)
$R_2 - R_0^2$ (cm^2)
$R_2 - R_0^2$ (cm^2)

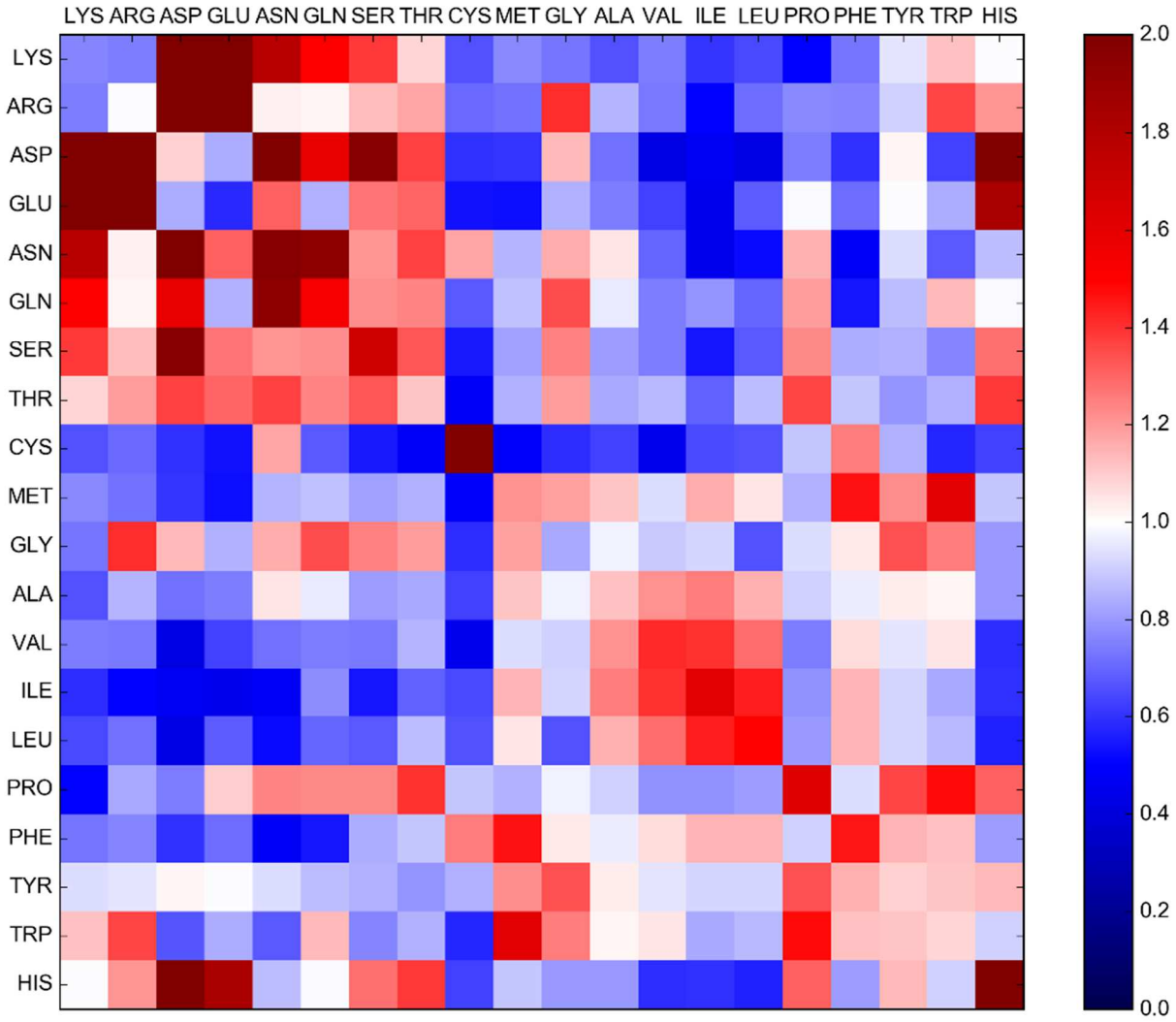

D
E
F
Absorbance
Residuals
$R_2 - R_0^2$ (cm^2)
$R_2 - R_0^2$ (cm^2)
$R_2 - R_0^2$ (cm^2)



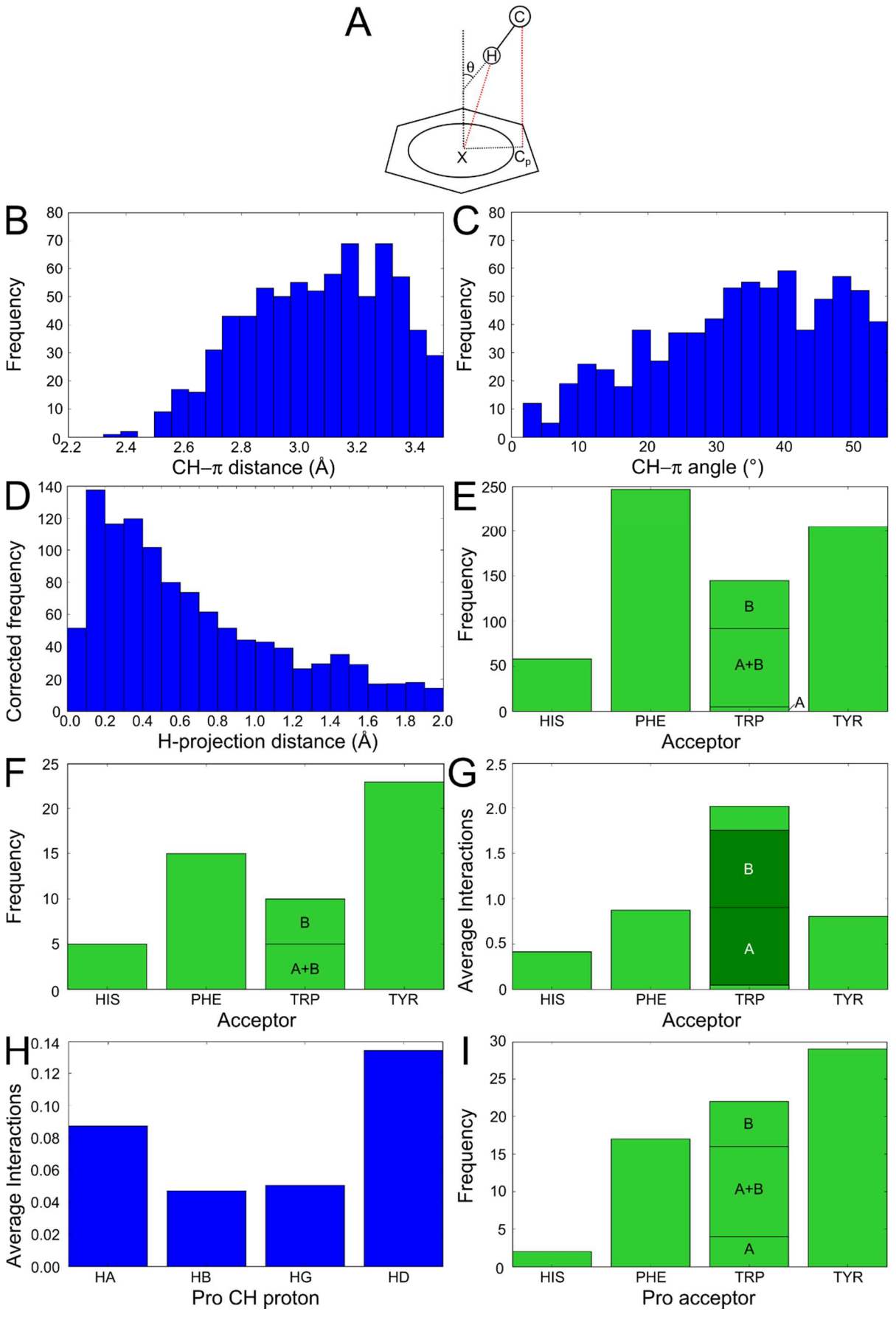
Supplementary Figure 5: Additional AUC data for peptides in this study. Sedimentation equilibrium AUC data (circles, upper panels) and fits to single ideal species (lines, upper panels) with residuals to the fits (lower panels) for PPα peptides. **A:** PPα-Tyr (280 nm). **B:** PPα-Trp (298 nm). **C:** PPα-Phe (235 nm). **D:** PPα-ϕNH_2 (280 nm). **E:** PPα-ϕOCH_3 (280 nm). **F:** PPα-ϕCH_3 (235 nm). **G:** PPα-ϕF (235 nm). **H:** PPα-ϕCN (280 nm). **I:** PPα-ϕNO_2 (325 nm). Conditions: 130 μM peptide in PBS, pH 7.4. **Color key:** 40 krpm (dark blue), 44 krpm (light blue), 48 krpm (green), 52 krpm (yellow), 56 krpm (orange) and 60 krpm (red). The fits returned the following masses: PPα-Tyr, 3613 Da, (0.9 x monomer mass), 99% confidence limits = 3585–3642 Da; PPα-Trp, 3674 (0.9 x monomer mass), 99% confidence limits = 3621–3727 Da; PPα-Phe, 3757 Da (1.0 x monomer mass) 99% confidence limits = 3721–3792 Da; PPα-ϕNH_2, 3588 Da (0.9 x monomer mass), 99% confidence limits = 3561–3615 Da; PPα-ϕOCH_3, 3701 Da (1.0 x monomer mass), 99% confidence limits = 3647–3756 Da; PPα-ϕCH_3, 4183 Da (1.1 x monomer mass), 99% confidence limits = 4024–4975; PPα-ϕF, 3661 (1.0 x monomer mass); PPα-CN, 4134 Da (1.1 x monomer mass); 99% confidence limits = 4099–4168 and PPα-ϕNO_2 (4187, 1.1 x monomer mass), 99% confidence limits = 4107–4267.



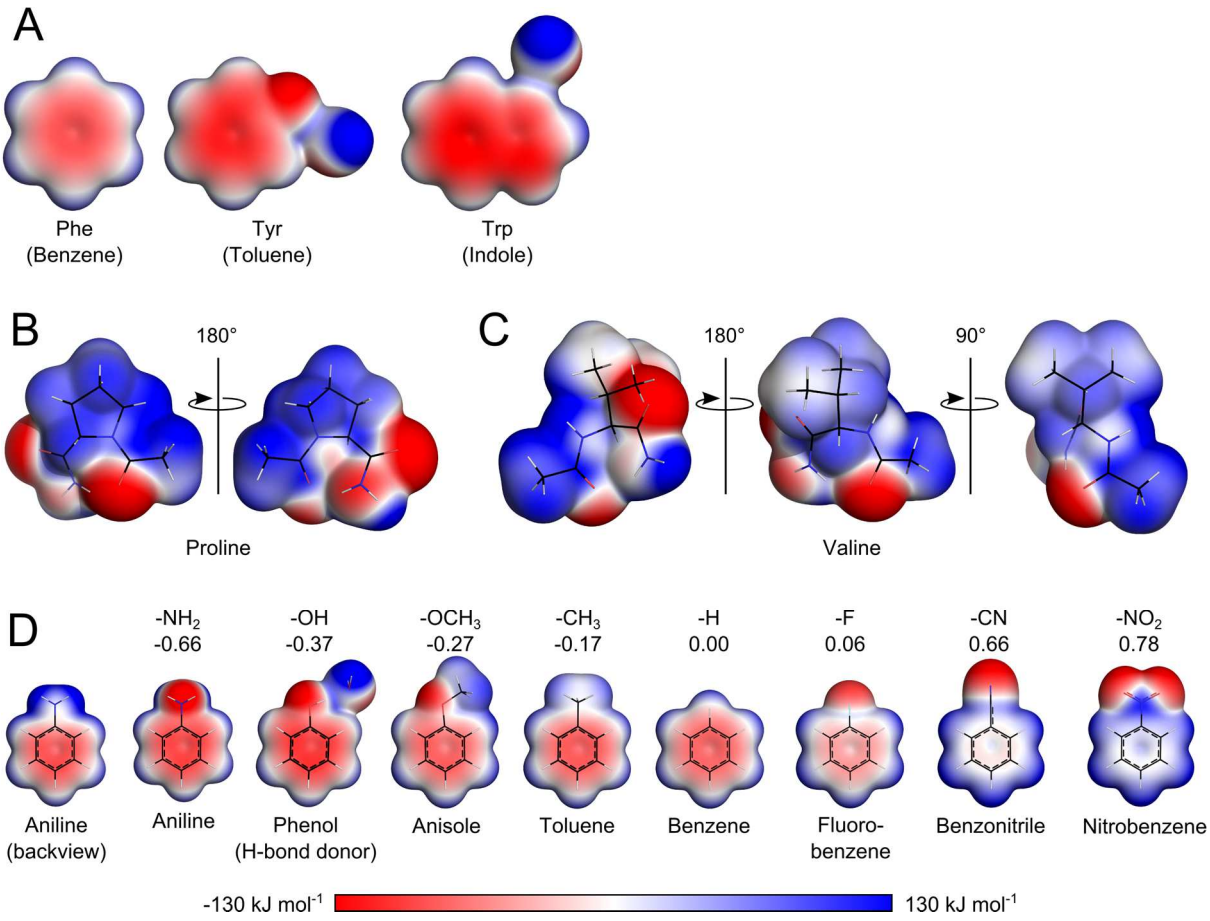
Supplementary Figure 6: NMR ensemble structures and secondary structure assignment of PPα peptides. A-C: NMR ensemble structures of **A:** PPα-Tyr, **B:** PPα-ϕOCH_3, and **C:** PPα-ϕCH_3. Chemical shift assignments, structure calculation and validation statistics are given in Table S1. **D-G:** secondary structure assignment of PPα structures. **D:** PPα-Tyr *in silico* after 100 ns of MD simulation, **E:** PPα-Tyr, **F:** PPα-ϕOCH_3 and **G:** PPα-ϕCH_3. **Key D-G:** Polyproline-II, burgundy; bend, yellow; hydrogen-bonded turn (3, 4 or 5 turn), yellow; 3_{10}-helix (min. length 3 residues), light blue; and α-helix (min. length 4 residues), dark blue. N.B. Residue 0 = *N*-terminal acetyl cap.



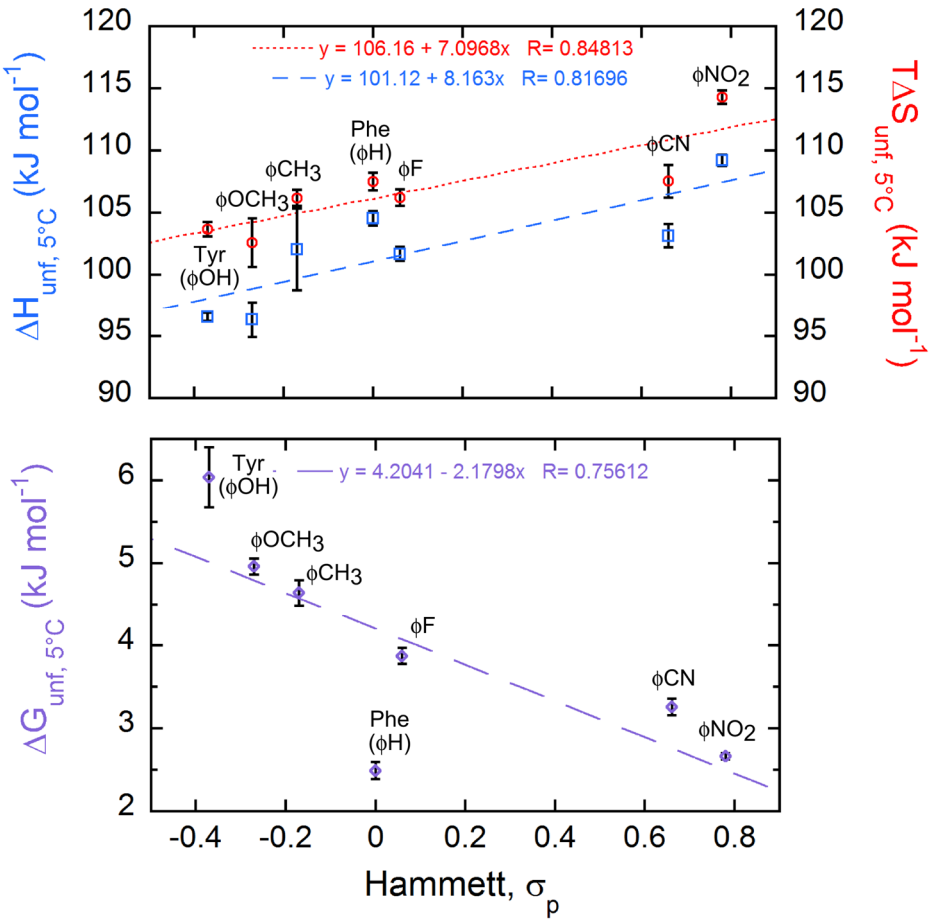
Supplementary Figure 7: Propensities for amino acid side chains to be in close contact. Normalized propensities of side chains to be within 3 Å vs. a random distribution from our high resolution dataset (≤ 1 Å) of X-ray crystal structures from the PDB.


A
C
H
θ
X
C_p
B
Frequency
CH–π distance (Å)
C
Frequency
CH–π angle (°)
D
Corrected frequency
H-projection distance (Å)
E
Frequency
B
A+B
A
HIS
PHE
TRP
TYR
Acceptor
F
Frequency
B
A+B
HIS
PHE
TRP
TYR
Acceptor
G
Average Interactions
B
A
HIS
PHE
TRP
TYR
Acceptor
H
Average Interactions
HA
HB
HG
HD
Pro CH proton
I
Frequency
B
A+B
A
HIS
PHE
TRP
TYR
Pro acceptor

Supplementary Figure 8: Analysis of CH–π interactions in a sub-1 Å dataset and SH3, WW, EVH1 and Profilin binding domains from the PDB. A: Definition of a CH–π interaction parameters. CH–π distance (between the CH proton to center of the aromatic ring, i.e. H–X) ≤ 3.5 Å. CH–π angle (between the vector of the C–H bond and the normal to the plane of the aromatic ring) $\theta \leq 55°$. C-projection distance (between the projection of the CH carbon to the plane of the aromatic ring and the center of the ring) ≤ 1.6 Å for the 5-membered TrpA ring and ≤ 2.0 Å for 6-membered rings (Phe, Tyr and TrpB). **B-D:** Distribution of parameters of CH–π interactions identified in our non-redundant high resolution (≤ 1 Å) dataset of protein X-ray crystal structures from the PDB. **B:** CH–π distance, **C:** CH–π angle, **D:** CH-projection distance (corrected by 1/r). **E-G:** Acceptor identities for all CH–π interactions in the same dataset as B-D. **E:** Frequency of aromatic rings acting as CH–π acceptors, **F:** Frequency of aromatic rings acting as CH–π acceptors for Pro CH–π donors and **G:** Average number of CH–π interactions for aromatic rings as acceptors for all interactions in the dataset. Dark green bars for TrpA and TrpB represent residues where both rings participate in interactions. **H&I:** Residues that participate in CH–π interactions in SH3, WW, EVH1 and Profilin. **H:** Average participation in CH–π interactions as CH donor with binding-domain π-acceptors for Pro CH protons in bound ligands and **I:** Identity of CH–π acceptor residues involved in CH–π interactions of Pro residues in bound ligands.



Supplementary Figure 9: Electrostatic surface potentials (ESPs) of interfacial residues in PPα variants. A: ESPs of Phe, Tyr, and Trp side chains. Tyr and Trp are shown as hydrogen-bond donors to a water molecule to best represent their solvated state. **B:** ESP of Pro and for comparison, **C:** Val. Both are capped at the *N*- and *C*-terminus with acetyl and amide groups respectively. Note the side chain of Pro is more electropositive than Val. **D:** ESPs for *para*-substituted benzene side chains from electron-rich phenol (side chain of Tyr) through electron poor-nitrobenzene (side chain of *para*-nitro-Phe). **Scale:** ≤ -130 kJ mol^{-1} (electropositive, blue) through ≥ 130 kJ mol^{-1} (electronegative, red).



Supplementary Figure 10: Van't Hoff analyses of PPα miniproteins. Enthalpy of unfolding, $\Delta H_{unf,\ 5°C}$ (blue, squares), entropy of unfolding, $\Delta S_{unf,\ 5°C}$ (red, circles) and free energy of unfolding $\Delta G_{unf,\ 5°C}$ (diamonds, purple) at 5°C (where most of the peptides are fully folded) vs. Hammett σ_p constant of *para*-substituents for the PPα peptides. These values were obtained from curve fitting of the thermal denaturation curves obtained by CD spectroscopy. See Materials and Methods for fitting equations. Linear fits of the data are shown by the dotted lines.

A Validation statistics

	PPα-Tyr	PPα-ϕCH$_3$	PPα-ϕOCH$_3$
NMR distance and dihedral constraints			
Distance constraints			
Total NOE	583	561	597
Intra-residue	245	235	224
Inter-residue			
Sequential ($\|i-j\|=1$)	103	98	114
Medium-range ($\|i-j\|<4$)	67	66	81
Long-range ($\|i-j\|>5$)	35	45	44
Ambiguous	133	117	134
Hydrogen bonds	0	0	0
Total dihedral angle restraints			
ϕ	34	36	33
ψ	21	22	22
Structure statistics			
Violations (mean and s.d.)			
Distance constraints (Å)	0.055 ± 0.016	0.057 ± 0.009	0.022 ± 0.001
Dihedral angle constraints (º)	0.03 (± 0.036)	0.44 (± 0.071)	0.34 (± 0.109)
Max. dihedral angle violation (º)	0	0	0
Max. distance constraint violation (> 0.3Å)	5.65 (± 1.52)	6.20 (± 1.57)	0.0 (± 0.00)
Deviations from idealized geometry			
Bond lengths (Å)	0.0027 (± 0.0001)	0.0026 (± 0.0001)	0.0031 (± 0.0001)
Bond angles (º)	0.429 (± 0.010)	0.434 (± - 0.015)	0.462 (± 0.015)
Impropers (º)	0.932 (± 0.084)	1.056 (± 0.089)	1.144 (± 0.144)
Average pairwise r.m.s. deviation** (Å)			
backbone (2º structure)	0.306 (± 0.085)	0.299 (± 0.100)	0.310 (± 0.066)
Heavy atoms (2º structure)	0.673 (± 0.105)	0.693 (± 0.087)	0.700 (± 0.063)
Backbone, all residues	0.514 (± 0.121)	0.520 (± 0.146)	0.474 (± 0.107)
Heavy atoms, all residues	0.825 (± 0.122)	0.828 (± 0.120)	0.821 (± 0.100)

** Pairwise r.m.s. deviation was calculated among 20 refined structures.

100% of each peptide was in allowed regions of Ramachandran space.

B PPα-Tyr

Residue	H	N	HA	CA	CB	Other
Acetyl cap	-	-	-	26.03	-	2.095:H*
Pro1	-	-	3.396	62.42	-	1.708:HB2 1.889:HB3 1.880:HG2 1.935:HG3 3.504:HD2 3.568:HD3 53.21:CD
Pro2	-	-	4.538	64.43	-	2.101:HB2 2.365:HB3 1.638:HG2 1.725:HG3 3.166:HD2 3.216:HD3
Thr3	8.869	116.48	4.279	63.88	71.91	3.926:HB 1.132:HG2* 23.57:CG2
Lys4	8.78	129.22	3.074	56.56	-	1.264:HB2 1.461:HB3 1.215:HG2 1.550:HG3 0.711:HD2 1.269:HD3 2.972:HE2 2.972:HE3 26.45:CD
Pro5	-	-	4.511	64.43	71.91	2.036:HB2 2.400:HB3 1.596:HG2 1.718:HG3 3.072:HD2 3.470:HD3 52.34:CD
Thr6	8.802	118.00	4.126	64.36	-	3.936:HB 1.177:HG2* 23.77:CG2
Lys7	8.727	128.05	3.476	56.99	-	1.520:HB2 1.583:HB3 1.018:HG2 1.340:HG3 1.569:HD2 1.569:HD3 2.810:HE2 2.946:HE3
Pro8	-	-	4.363	64.62	-	1.782:HB2 2.163:HB3 1.692:HG2 1.694:HG3 2.810:HD2 3.050:HD3 52.29:CD
Gly9	8.372	106.63	4.036, 3.867	46.27	-	
Asp10	8.543	117.58	4.337	58.11	-	2.527:HB2 2.603:HB3
Asn11	8.642	114.64	4.677	54.78		2.860:HB2 2.860:HB3 7.027:HD21 7.729:HD22 112.00:ND2
Ala12	7.437	120.78	4.297	54.50	21.95	1.397:HB*
Thr13	7.735	110.03	4.572	-	70.31	4.830:HB 1.454:HG2* 24.58:CG
Pro14	-	-	4.248	67.78	-	1.974:HB2 2.089:HB3 2.260:HG2 2.430:HG3 3.924:HD2 3.984:HD3 52.41:CD
Glu15	9.072	116.53	4.162	62.14	-	1.970:HB2 2.137:HB3 2.330:HG2 2.399:HG3 38.62:CG
Lys16	7.868	120.85	4.175	-	-	1.832:HB2 2.069:HB3
Leu17	8.415	120.51	4.145	-	-	1.647:HB2 1.872:HB3 0.865:HD1* 0.889:HD2*
Ala18	8.421	121.15	4.250	-	-	1.515:HB*
Lys19	8.015	119.80	4.208	-	-	1.967:HB2 2.024:HB3
Tyr20	7.948	117.80	4.563	61.73	-	3.132:HB2 3.221:HB3 7.157:HD* 6.813:HE* 135.30:CD* 120.23:CE*
Gln21	8.105	115.18	3.670	60.00	-	2.167:HB2 2.204:HB3 2.621:HG2 2.621:HG3 7.096:HE21 7.749:HE22 35.72:CG 110.99:NE2
Ala22	7.928	121.99	4.246	-	-	1.563:HB*
Asp23	9.182	121.15	4.418	-	-	2.448:HB2 2.856:HB3
Leu24	9.211	123.85	3.943	59.90	-	1.404:HB2 1.567:HB3 0.829:HD1* 0.974:HD2*
Ala25	7.771	120.51	4.203	-	-	1.518:HB*
Lys26	7.896	118.68	4.114	-	-	1.930:HB2 2.002:HB3
Tyr27	8.260	119.01	4.428	-	-	3.225:HB2 3.276:HB3 7.033:HD* 6.629:HE* 135.50:CD* 119.79:CE*
Gln28	8.407	115.37	3.616	60.87	-	2.044:HB2 2.193:HB3 2.576:HG2 2.701:HG3 7.028:HE21 7.507:HE22 109.30:NE2
Lys29	7.601	120.36	4.106	-	-	1.911:HB2 2.057:HB3
Asp30	8.988	119.70	4.327	-	-	2.510:HB2 2.744:HB3
Leu31	8.986	121.19	3.825	59.77	-	1.379:HB2 1.498:HB3 0.820:HD1* 0.964:HD2*
Ala32	7.584	121.27	4.109	-	-	1.522:HB*
Asp33	8.152	116.23	4.424	-	-	2.707:HB2 2.707:HB3
Tyr34	7.957	120.25	4.164	60.96	-	3.033:HB2 3.221:HB3 7.162:HD* 6.766:HE* 135.30:CD* 120.08:CE*

C PPα-ϕOCH$_3$

Residue	H	N	HA	CA	CB	Other
Acetyl cap	-	-	-	25.99	-	2.087:H*
Pro1	-	-	3.496	62.47	-	1.700:HB2 1.855:HB3 1.861:HG2 1.861:HG3 3.441:HD2 3.564:HD3 53.07:CD
Pro2	-	-	4.515	64.48	-	2.081:HB2 2.442:HB3 1.612:HG2 1.613:HG3 3.077:HD2 3.477:HD3
Thr3	8.676	117.15	4.112	64.28	72.15	3.939:HB 1.194:HG2* 23.79:CG2
Lys4	8.804	129.17	2.949	56.67	-	1.076:HB2 1.140:HB3 1.196:HG2 1.197:HG3 0.603:HD2 1.517:HD3 2.836:HE2 2.836:HE3
Pro5	-	-	4.542	64.54	-	2.116:HB2 2.390:HB3 1.701:HG2 1.739:HG3 3.157:HD2 3.259:HD3
Thr6	8.768	115.78	4.299	63.55		3.941:HB 1.140:HG2*
Lys7	8.780	127.65	3.324	56.97	-	1.552:HB2 0.992:HG2 0.992:HG3 1.307:HD2 1.411:HD3 2.938:HE2 2.939:HE3
Pro8	-	-	4.361	64.66	-	1.778:HB2 2.158:HB3 1.687:HG2 1.688:HG3 2.819:HD2 2.975:HD3
Gly9	8.373	107.12	3.883, 4.017	46.23	-	
Asp10	8.547	118.15	4.335	-	-	2.523:HB2 2.654:HB3
Asn11	8.649	115.11	4.672	54.76	39.57	2.862:HB2 2.862:HB3 7.026:HD21 7.725:HD22 112.47:ND2
Ala12	7.444	121.22	4.291	54.60	21.87	1.394:HB*
Thr13	7.723	110.54	4.575	61.79	70.43	4.830:HB 1.454:HG2*
Pro14	-	-	4.253	67.80	-	1.977:HB2 1.978:HB3 2.091:HG2 2.261:HG3 3.925:HD2 3.983:HD3 52.38:CD
Glu15	9.071	117.01	4.162	62.01	-	1.970:HB2 2.140:HB3 2.339:HG2 2.418:HG3 38.68:CG
Lys16	7.873	121.32	4.180	-	-	1.837:HB2 2.077:HB3
Leu17	8.407	121.05	4.145	-	-	1.658:HB2 1.878:HB3 0.867:HD1* 0.891:HD2*
Ala18	8.426	121.61	4.249	-	-	1.517:HB*
Lys19	8.036	120.28	4.207	-	-	1.970:HB2 2.036:HB3
ϕOCH$_3$20	7.942	118.29	4.591	63.14	-	3.193:HB2 3.264:HB3 7.254:HD* 6.716:HE* 135.19:CD* 118.29:CE* 3.768:HM* 59.82:CM
Gln21	8.092	115.76	3.661	60.75	-	2.171:HB2 2.204:HB3 2.624:HG2 2.624:HG3 7.097:HE21 7.761:HE22 111.41:NE2
Ala22	7.985	122.59	4.236	-	19.95	1.563:HB*
Asp23	9.223	121.50	4.426	-	-	2.465:HB2 2.850:HB3
Leu24	9.145	124.36	3.916	59.79		1.424:HB2 1.597:HB3 0.788:HD1* 0.867:HD2*
Ala25	7.799	121.06	4.202	-	-	1.517:HB*
Lys26	7.933	119.22	4.106	-	-	1.929:HB2 2.014:HB3 1.520:HG2 1.520:HG3
ϕOCH$_3$27	8.234	119.39	4.429	62.80	-	3.256:HB2 3.321:HB3 7.112:HD* 6.808:HE* 135.48:CD* 118.44:CE* 3.815:HM* 59.87:CM
Gln28	8.376	115.97	3.606	60.78	-	2.204:HB2 2.204:HB3 2.575:HG2 2.705:HG3 7.017:HE21 7.531:HE22 109.67:NE2
Lys29	7.658	120.92	4.101	-	-	1.911:HB2 2.052:HB3 1.513:HG2 1.514:HG3
Asp30	9.001	120.00	4.322	-	-	2.519:HB2 2.747:HB3
Leu31	8.846	121.34	3.830	-	-	0.896:HB2 1.509:HB3 0.752:HD1* 0.791:HD2*
Ala32	7.595	121.67	4.110	-	-	1.519:HB*
Asp33	8.136	116.62	4.430	-	-	2.696:HB2 2.696:HB3
ϕOCH$_3$34	7.948	120.60	4.185	-	-	3.085:HB2 3.221:HB3 7.249:HD* 6.912:HE* 135.32:CD* 118.71:CE* 3.801:HM* 59.88:CM

D PPα-φCH$_3$

Residue	H	N	HA	CA	CB	Other
Acetyl cap	-	-	-	26.03	-	2.098:H*
Pro1	-	-	3.664	62.59	-	1.757:HB2 1.896:HB3 1.758:HG2 1.897:HG3 3.496:HD2 3.573:HD3
Pro2	-	-	4.549	64.49	-	2.103:HB2 2.372:HB3 1.708:HG2 1.760:HG3 3.207:HD2 3.302:HD3
Thr3	8.672	114.57	4.33	63.31	72.23	3.937:HB 1.147:HG2* 23.56:CG2
Lys4	8.686	128.41	2.870	56.50	-	1.370:HB2 1.425:HB3 0.647:HG2 1.122:HG3 1.370:HD2 1.370:HD3 2.864:HE2 2.903:HE3
Pro5	-	-	4.495	64.49	-	2.398:HB2 1.561:HG2 1.664:HG3 3.101:HD2 3.421:HD3
Thr6	8.584	115.87	4.126	64.29	72.25	3.946:HB 1.192:HG2* 23.76:CG2
Lys7	8.640	127.25	3.258	56.7	-	1.539:HB* 0.952:HG2 0.952:HG3 1.334:HD2 1.451:HD3 2.929:HE2 2.929:HE3
Pro8	-	-	4.354	64.75	-	1.777:HB2 2.181:HB3 1.696:HG2 1.704:HG3 2.849:HD2 3.006:HD3
Gly9	8.366	106.69	3.866, 4.009	46.37	-	
Asp10	8.536	117.72	4.344	-	-	2.530:HB2 2.654:HB3
Asn11	8.644	114.75	4.670	54.81	39.59	2.862:HB2 2.863:HB3 7.028:HD21 7.726:HD21 111.99:ND2
Ala12	7.489	120.87	4.288	54.71	21.87	1.397:HB*
Thr13	7.700	110.06	4.579	-	70.31	4.827:HB 1.454:HG2* 24.48:CG2
Pro14	-	-	4.257	67.77	-	1.980:HB2 1.982:HB3 2.091:HG2 2.261:HG3 3.925:HD2 3.985:HD3
Glu15	9.074	116.64	4.167	-	-	1.973:HB2 2.141:HB3 2.345:HG2 2.420:HG3
Lys16	7.882	120.75	4.179	-	-	1.830:HB2 2.076:HB3
Leu17	8.398	120.61	4.157	-	-	1.653:HB2 1.893:HB3 0.863:HD1* 0.892:HD2* 25.00:CD1 26.96:CD2
Ala18	8.427	121.39	4.261	-	-	1.521:HB*
Lys19	8.045	119.95	4.210	-	-	1.972:HB2 2.045:HB3 1.523:HG2 1.526:HG3
φCH$_3$20	7.965	118.00	4.588	61.71	-	2.289:HM* 3.174:HB2 3.249:HB3 7.194:HD* 6.974:HE* 25.15:CM 133.98:CD* 134.19:CE*
Gln21	8.019	114.88	3.705	60.68	-	2.180:HB2 2.193:HB3 2.620:HG2 2.688:HG3 7.092:HE21 7.773:HE22 111.27:NE2
Ala22	7.974	122.20	4.235	-	19.96	1.563:HB*
Asp23	9.186	121.25	4.427	-	-	2.475:HB2 2.845:HB3
Leu24	9.086	123.89	3.916	59.71	-	0.931:HB2 1.571:HB3 0.807:HD1* 0.809:HD2*
Ala25	7.811	120.62	4.200	-	-	1.522:HB*
Lys26	7.931	118.77	4.108	-	-	1.928:HB2 2.018:HB3 1.520:HG2 1.521:HG3
φCH$_3$27	8.198	119.12	4.444	-	-	2.338:HM* 3.249:HB2 3.317:HB3 7.066:HD* 7.038:HE* 25.85:CM 133.97:CD* 134.24:CE1
Gln28	8.321	115.08	3.641	60.73	-	2.050:HB2 2.184:HB3 2.561:HG2 2.681:HG3 6.997:HE21 7.490:HE22 109.32:NE2
Lys29	7.708	120.48	4.099	-	-	1.906:HB2 2.046:HB3
Asp30	8.935	119.62	4.325	-	-	2.525:HB2 2.756:HB3
Leu31	8.709	120.71	3.840	59.49	-	1.381:HB2 1.481:HB3 0.762:HD1* 1.013:HD2*
Ala32	7.600	121.11	4.111	-	-	1.509:HB*
Asp33	8.161	116.32	4.433	-	-	2.677:HB2 2.679:HB3
φCH$_3$34	7.976	119.99	4.235	-	-	2.285:HM* 3.092:HB2 3.197:HB3 7.186:HD* 7.168:HE* 25.27:CM 134.08:CD* 134.03:CE1

E PPα-ϕPhe

Residue	H	N	HA	CA	CB	Other
Acetyl cap	-	-	-	26.06	-	2.094:H*
Pro1	-	-	3.693	62.50	-	1.746:HB2 1.939:HB3 1.921:HG2 1.921:HG3 3.545:HD2 3.558:HD3
Pro2	-	-	4.557	64.55	-	2.357:HB* 1.741:HG2 1.830:HG3 3.273:HD2 3.385:HD3
Thr3	8.697	114.94	4.307	63.68	72.20	3.959:HB 1.148:HG2*
Lys4	8.611	128.10	3.067	-	-	1.219:HG2 1.556:HG3 0.705:HD2 1.222:HD3 2.929:HE2 2.929:HE3
Pro5	-	-	4.508	64.75	-	2.070:HB2 2.393:HB3 1.767:HG2 3.140:HD2 3.492:HD3
Thr6	8.618	116.15	4.138	64.21	71.84	3.960:HB 1.181:HG2*
Lys7	8.580	127.18	3.334	-	-	1.458:HB2 1.513:HB3 1.181:HG2 1.181:HG3
Pro8	-	-	4.364	64.88	-	2.202:HB2 2.545:HB3 1.751:HG2 1.817:HG3 2.904:HD2 3.129:HD3
Gly9	8.390	106.74	3.880, 4.012	46.53	-	
Asp10	8.506	117.77	4.361	-	-	2.544:HB2 2.660:HB3
Asn11	8.624	114.91	4.673	-	39.72	2.856:HB2 2.856:HB3 7.024:HD21 7.724:HD22 111.98:ND2
Ala12	7.569	121.04	4.304	-	-	1.398:HB*
Thr13	7.764	110.34	4.585	61.74	-	4.787:HB 1.439:HG2*
Pro14	-	-	4.266	67.55	-	1.976:HB2 2.423:HB3 2.087:HG2 2.250:HG3 3.916:HD2 3.973:HD3
Glu15	9.048	116.77	4.166	61.85	-	1.977:HB2 2.133:HB3 2.343:HG2 2.399:HG3 38.61:CG
Lys16	7.919	120.78	4.183	-	-	1.844:HB2 2.052:HB3
Leu17	8.401	120.60	4.176	-	-	1.667:HB2 1.856:HB3 0.884:HD1* 0.907:HD2*
Ala18	8.418	121.21	4.257	-	-	1.511:HB*
Lys19	8.043	119.64	4.210	-	-	1.963:HB2 2.023:HB3
Phe20	7.998	117.94	4.298	61.36	-	3.146:HB2 3.217:HB3 7.299:HD*
Gln21	8.150	115.71	3.686	60.76	-	2.156:HB2 2.202:HB3 2.617:HG2 2.617:HG3 7.086:HE21 7.746:HE22 110.91:NE2
Ala22	7.983	122.05	4.224	-	-	1.549:HB*
Asp23	9.083	120.80	4.430	-	-	2.483:HB2 2.833:HB3
Leu24	9.021	123.51	3.945	-	-	1.520:HB2 1.521:HB3 0.834:HD1* 0.942:HD2*
Ala25	7.802	120.39	4.191	-	-	1.511:HB*
Lys26	7.923	118.47	4.116	-	-	1.916:HB2 1.989:HB3 1.503:HG2 1.506:HG3
Phe27	8.222	118.87	4.439	-	-	3.302:HB2 3.315:HB3 7.182:HD* 133.93:CD*
Gln28	8.379	115.72	3.675	60.69	-	2.050:HB2 2.184:HB3 2.566:HG2 2.685:HG3 7.011:HE21 7.540:HE22 109.47:NE2
Lys29	7.725	120.39	4.104	-	-	1.900:HB2 2.026:HB3 1.491:HG2 1.494:HG3
Asp30	8.825	119.43	4.339	-	-	2.541:HB2 2.724:HB3
Leu31	8.646	120.68	3.883	59.63	-	1.449:HB2 1.449:HB3 0.813:HD1* 1.023:HD2*
Ala32	7.649	121.18	4.116	-	-	1.499:HB*
Asp33	8.191	116.42	4.437	-	-	2.654:HB2 2.654:HB3
Phe34	7.999	119.83	4.586	-	-	3.214:HB2 3.309:HB3 7.294:HD*

F PPα-ϕF

Residue	H	N	HA	CA	CB	Other
Acetyl cap	-	-	-	26.04	-	2.102:H*
Pro1	-	-	3.554	62.57	-	1.958:HB2 1.958:HB3 1.769:HG2 1.769:HG3 3.548:HD2 3.588:HD3
Pro2	-	-	4.535	64.73	-	2.384:HB* 1.762:HG2 1.762:HG3 3.239:HD2 3.342:HD3
Thr3	8.641	114.79	4.300	63.49	72.20	3.962:HB 1.159:HG2*
Lys4	8.727	127.48	2.983	59.60	-	1.281:HB2 1.470:HB3 0.795:HG2 1.282:HG3 1.592:HD2 1.592:HD3 2.946:HE2 2.946:HE3
Pro5	-	-	4.497	64.64	-	2.015:HB2 2.407:HB3 1.644:HG2 1.740:HG3 3.118:HD2 3.454:HD3
Thr6	8.593	116.44	4.109	61.09	-	3.958:HB 1.198:HG2*
Lys7	8.656	126.78	3.406	56.80	-	1.492:HB2 1.572:HB3
Pro8	-	-	4.374	64.76	-	1.811:HB2 2.188:HB3 1.739:HG2 1.740:HG3 2.933:HD2 3.068:HD3
Gly9	8.377	106.67	3.897, 4.008	46.50	-	
Asp10	8.517	117.72	4.359	-	-	2.543:HB2 2.656:HB3
Asn11	8.643	114.72	4.666	54.84	39.74	2.856:HB2 2.856:HB3 7.729:HD22 7.026:HD21 112.01:ND2
Ala12	7.527	120.91	4.301	54.70	-	1.393:HB*
Thr13	7.744	110.22	4.579	-	-	4.800:HB 1.440:HG2* 24.61:CG2
Pro14	-	-	4.256	67.92	-	1.975:HB2 2.427:HB3 2.090:HG2 2.253:HG3 3.916:HD2 3.978:HD3
Glu15	9.059	116.69	4.164	62.04	-	1.974:HB2 2.134:HB3 2.336:HG2 2.398:HG3 38.71:CG
Lys16	7.900	120.77	4.181	-	-	1.835:HB2 2.065:HB3
Leu17	8.421	120.61	4.155	-	-	1.653:HB2 1.873:HB3 0.876:HD1* 0.893:HD2*
Ala18	8.430	121.39	4.264	-	-	1.509:HB*
Lys19	8.057	119.76	4.211	-	-	1.964:HB2 2.018:HB3
ϕF20	8.000	117.58	4.579	61.91	-	3.208:HB2 3.282:HB3 7.331:HD* 6.962:HE* 135.91:CD* 120.44:CE*
Gln21	8.121	115.82	3.710	60.71	-	2.170:HB2 2.212:HB3 2.606:HG2 2.606:HG3 7.086:HE21 7.767:HE22 35.80:CG 111.02:NE2
Ala22	7.965	122.13	4.233	-	19.95	1.552:HB*
Asp23	9.115	120.76	4.426	-	-	2.472:HB2 2.815:HB3
Leu24	9.018	123.45	3.938	-	-	1.416:HB2 1.574:HB3 0.832:HD1* 1.002:HD2*
Ala25	7.803	120.51	4.203	-	-	1.512:HB*
Lys26	7.932	118.65	4.121	-	-	1.927:HB2 1.994:HB3
ϕF27	8.203	118.50	4.455	-	-	3.281:HB2 3.336:HB3 7.202:HD* 7.036:HE* 135.89:CD* 120.37:CE*
Gln28	8.373	115.92	3.651	60.82	-	2.052:HB2 2.189:HB3 2.564:HG2 2.663:HG3 7.012:HE21 7.562:HE22 109.52:NE2
Lys29	7.677	120.38	4.103	-	-	1.889:HB2 2.036:HB3
Asp30	8.681	120.51	3.860	-	-	2.550:HB2 2.723:HB3
Leu31	8.681	120.51	3.860	59.76	-	1.386:HB2 1.474:HB3 0.792:HD1* 1.046:HD2*
Ala32	7.637	121.22	4.120	-	19.90	1.498:HB*
Asp33	8.218	116.38	4.436	-	42.82	2.676:HB2 2.676:HB3
ϕF34	7.961	119.69	4.238	-	-	3.138:HB2 3.221:HB3 7.318:HD* 7.071:HE* 135.73:CD* 120.18:CE*

G PPα-ϕCN

Residue	H	N	HA	CA	CB	Other
Acetyl cap	-	-	-	26.04	-	2.141:H*
Pro1	-	-	3.648	62.40	-	1.818:HB2 1.818:HB3 1.967:HG2 2.027:HG3 3.623:HD2 3.624:HD3 53.19:CD
Pro2	-	-	4.539	64.49	-	2.018:HB2 2.389:HB3 1.757:HG2 1.757:HG3 3.241:HD2 3.278:HD3
Thr3	8.673	115.05	4.312	63.42	72.02	3.989:HB 1.181:HG2*
Lys4	8.812	126.95	3.001	56.29	-	1.272:HB2 1.544:HB3
Pro5	-	-	4.503	64.41	-	2.001:HB2 2.392:HB3 3.105:HD2 3.408:HD3
Thr6	8.588	116.47	4.113	64.22	71.92	3.983:HB 1.208:HG2* 23.64:CG2
Lys7	8.757	126.14	3.430	56.97	-	1.559:HB2 1.633:HB3
Pro8	-	-	4.373	64.75	-	1.815:HB2 2.167:HB3 1.389:HG2 1.705:HG3 2.928:HD2 3.427:HD3
Gly9	8.389	106.88	3.914, 3.994	46.24	-	
Asp10	8.515	117.84	4.366	-	-	2.560:HB2 2.655:HB3
Asn11	8.651	115.06	4.671	54.66	-	2.852:HB2 2.853:HB3 112.03:Nd 2 7.026:HD21 7.728:HD22
Ala12	7.531	120.98	4.306	54.48	-	1.394:HB*
Thr13	7.741	110.33	4.580	61.72	70.40	4.796:HB 1.445:HG2*
Pro14	-	-	4.260	67.54	-	1.976:HB2 2.428:HB3 2.090:HG2 2.252:HG3 3.917:HD2 3.982:HD3 52.29:CD
Glu15	9.055	116.78	4.166	61.69	-	1.978:HB2 2.137:HB3 2.344:HG2 2.397:HG3 38.50:CG
Lys16	7.905	120.84	4.178	-	-	1.844:HB2 2.068:HB3
Leu17	8.442	120.69	4.133	-	-	1.652:HB2 1.892:HB3 0.868:HD1* 0.895:HD2*
Ala18	8.436	121.57	4.263	-	-	1.521:HB*
Lys19	8.093	119.77	4.221	-	-	1.975:HB2 2.023:HB3
ϕCN20	8.091	117.11	4.633	62.57	-	3.315:HB2 3.374:HB3 7.568:HD* 7.628:HE* 135.34:CD* 137.62:CE*
Gln21	8.159	116.30	3.753	60.62	-	2.193:HB2 2.239:HB3 2.606:HG2 2.606:HG3 7.094:HE21 7.789:HE22 111.11:NE2
Ala22	7.993	122.38	4.233	-	-	1.561:HB*
Asp23	9.092	120.46	4.439	-	-	2.478:HB2 2.769:HB3
Leu24	8.847	123.30	3.927	59.73	-	1.424:HB2 1.609:HB3 0.868:HD1* 0.933:HD2*
Ala25	7.856	120.87	4.212	-	-	1.521:HB*
Lys26	7.973	118.67	4.129	-	-	1.934:HB2 1.990:HB3
ϕCN27	8.209	117.94	4.541	61.88	-	3.380:HB2 3.444:HB3 7.421:HD* 7.716:HE* 134.91:CD* 137.38:CE*
Gln28	8.403	116.43	3.683	60.45	-	2.066:HB2 2.205:HB3 2.550:HG2 2.646:HG3 7.012:HE21 7.596:HE22 35.77:CG 109.59:NE2
Lys29	7.728	120.62	4.102	-	-	1.897:HB2 2.035:HB3
Asp30	8.846	119.17	4.330	-	-	2.527:HB2 2.698:HB3
Leu31	8.473	120.14	3.852	59.41	-	1.388:HB2 1.494:HB3 0.805:HD1* 1.010:HD2*
Ala32	7.651	121.27	4.117	-	-	1.498:HB*
Asp33	8.247	116.40	4.437	-	42.60	2.674:HB2 2.675:HB3
ϕCN34	8.023	119.10	4.350	60.54		3.245:HB2 3.314:HB3 7.505:HD* 7.740:HE* 134.73:CD* 137.40:CE*

Supplementary Table 1: NMR structure calculation and validation statistics, and chemical shift assignments. A: NMR structure calculation and validation statistics for PPα-Tyr, PPα-ϕOCH_3 and PPα-ϕCH_3. **B-G:** NMR chemical shift assignments for **B:** PPα-Tyr, **C:** PPα-ϕOCH_3, **D:** PPα-ϕCH_3, **E:** PPα-ϕPhe, **F:** PPα-ϕF and **G:** PPα-ϕCN. * = equivalent nuclei. CM = methyl C.

A PPα-Tyr

45 CH–π interactions (2.25 ± 0.91 per ensemble structure)

Model	CH donor	CH acceptor
1	Pro1 CA-HA Pro5 CG-HG2	Tyr34 Tyr20
2	Pro1 CA-HA Pro2 CG-HG2 Lys4 CG-HG2	Tyr34 Tyr27 Tyr27
3	Lys4 CA-HA Lys4 CG-HG2 Leu24 CB-HB2 Leu31 CB-HB2	Tyr27 Tyr27 Tyr20 Tyr27
4	Pro1 CA-HA Pro5 CB-HB2 Pro5 CG-HG2	Tyr34 Tyr20 Tyr20
5	Pro1 CA-HA Pro8 CA-HA	Tyr34 Tyr20
6	Pro1 CA-HA	Tyr34
7	Pro2 CG-HG2 Lys4 CA-HA Gly9 CA-HA2	Tyr27 Tyr27 Tyr20
8	Lys4 CA-HA	Tyr27
9	Pro2 CG-HG2 Lys4 CA-HA	Tyr27 Tyr27
10	Pro1 CA-HA Pro2 CG-HG2 Leu24 CB-HB2	Tyr34 Tyr27 Tyr20
11	Pro2 CG-HG2 Lys4 CA-HA	Tyr27 Tyr27
12	Pro1 CA-HA Pro5 CG-HG2	Tyr34 Tyr20
13	Lys4 CA-HA	Tyr27
14	Pro1 CA-HA Lys4 CA-HA Gly9 CA-HA2 Leu31 CB-HB2	Tyr34 Tyr27 Tyr20 Tyr27
15	Pro1 CA-HA Lys4 CA-HA Lys4 CG-HG2	Tyr34 Tyr27 Tyr27
16	Pro1 CA-HA Lys4 CA-HA	Tyr34 Tyr27
17	Pro1 CA-HA Lys4 CA-HA	Tyr34 Tyr27
18	Pro2 CB-HB2	Tyr27
19	Pro1 CA-HA Lys4 CG-HG2	Tyr34 Tyr27
20	Pro1 CA-HA Lys4 CG-HG2	Tyr34 Tyr27

Pro donor total = 24 i.e. 1.2 CH–π interactions per structure.
Leu donor total = 4 i.e. 0.2 CH–π interactions per structure.
Lys donor total = 15 i.e. 0.75 CH–π interactions per structure.
Gly donor total = 2 i.e. 0.1 CH–π interactions per structure.

B PPα-φOCH3

54 CH–π interactions (2.7 ± 0.86 per ensemble structure)

Model	CH donor	CH acceptor
1	Pro1 CA-HA Lys4 CA-HA Leu24 CD2-HD23 Leu31 CB-HB2	4-OCH_3-Phe34 4-OCH_3-Phe27 4-OCH_3-Phe20 4-OCH_3-Phe27
2	Lys4 CA-HA Leu24 CD1-HD12	4-OCH_3-Phe27 4-OCH_3-Phe20
3	Pro1 CA-HA Leu24 CG-HG Leu24 CD1-HD11	4-OCH_3-Phe34 4-OCH_3-Phe20 4-OCH_3-Phe20
4	Pro1 CA-HA Lys4 CA-HA Leu31 CB-HB2 Leu31 CG-HG	4-OCH_3-Phe34 4-OCH_3-Phe27 4-OCH_3-Phe27 4-OCH_3-Phe27
5	Pro1 CA-HA Lys4 CA-HA Leu24 CD1-HD13	4-OCH_3-Phe34 4-OCH_3-Phe27 4-OCH_3-Phe20
6	Pro1 CA-HA Leu24 CD1-HD12	4-OCH_3-Phe34 4-OCH_3-Phe20
7	Pro1 CA-HA Lys4 CA-HA Leu31 CD2-HD22	4-OCH_3-Phe34 4-OCH_3-Phe27 4-OCH_3-Phe27
8	Pro1 CA-HA Lys4 CA-HA Leu24 CD1-HD12 Leu31 CD2-HD22	4-OCH_3-Phe34 4-OCH_3-Phe27 4-OCH_3-Phe20 4-OCH_3-Phe27
9	Pro1 CA-HA Lys4 CA-HA Leu31 CB2-HB2	4-OCH_3-Phe34 4-OCH_3-Phe27 4-OCH_3-Phe27
10	Pro1 CA-HA Lys4 CA-HA Leu24 CD2-HD21	4-OCH_3-Phe34 4-OCH_3-Phe27 4-OCH_3-Phe20
11	Lys4 CA-HA	4-OCH_3-Phe27
12	Lys4 CA-HA Leu31 CB-HB2	4-OCH_3-Phe27 4-OCH_3-Phe27
13	Lys4 CA-HA Leu31 CB-HB2 Leu31 CG-HG	4-OCH_3-Phe27 4-OCH_3-Phe27 4-OCH_3-Phe27
14	Leu24 CD1-HD11	4-OCH_3-Phe20
15	Lys4 CA-HA Leu24 CD1-HD11 Leu31 CB-HB2	4-OCH_3-Phe27 4-OCH_3-Phe20 4-OCH_3-Phe27
16	Pro1 CA-HA Lys4 CA-HA Leu24 CD2-HD22	4-OCH_3-Phe34 4-OCH_3-Phe27 4-OCH_3-Phe20
17	Pro1 CA-HA Leu24 CD2-HD23	4-OCH_3-Phe34 4-OCH_3-Phe20
18	Pro1 CA-HA Lys4 CA-HA Leu31 CB-HB2	4-OCH_3-Phe34 4-OCH_3-Phe27 4-OCH_3-Phe27
19	Pro1 CA-HA Lys4 CA-HA	4-OCH_3-Phe34 4-OCH_3-Phe27
20	Lys4 CA-HA	4-OCH_3-Phe27

	Leu31 CB-HB2 Leu31 CG-HG	4-OCH_3-Phe27 4-OCH_3-Phe27

Pro donor total = 13 i.e. 0.65 CH–π interactions per structure.
Leu donor total = 25 i.e. 1.25 CH–π interactions per structure.
Lys donor total = 16 i.e. 0.8 CH–π interactions per structure.

C $PP\alpha$-ϕCH_3

50 CH–π interactions (2.5 ± 0.76 per ensemble structure)

Model	CH donor	CH acceptor
1	Pro1 CA-HA Leu24 CD2-HD21	4-CH_3-Phe34 4-CH_3-Phe20
2	Lys4 CA-HA Leu24 CD2-HD21	4-CH_3-Phe27 4-CH_3-Phe20
3	Pro1 CA-HA Lys4 CA-HA Leu24 CD2-HD22	4-CH_3-Phe34 4-CH_3-Phe27 4-CH_3-Phe20
4	Lys4 CA-HA Leu24 CD2-HD22	4-CH_3-Phe27 4-CH_3-Phe20
5	Pro1 CA-HA Lys4 CA-HA Leu24 CD2-HD23 Leu31 CB2-HB2	4-CH_3-Phe34 4-CH_3-Phe27 4-CH_3-Phe20 4-CH_3-Phe27
6	Leu24 CD2-HD22	4-CH_3-Phe20
7	Lys4 CA-HA Leu24 CD2-HD23	4-CH_3-Phe27 4-CH_3-Phe20
8	Pro2 CD-HD2 Lys4 CA-HA Leu24 CD2-HD23	4-CH_3-Phe34 4-CH_3-Phe27 4-CH_3-Phe20
9	Pro2 CD-HD2 Lys4 CA-HA Leu24 CD2-HD21	4-CH_3-Phe34 4-CH_3-Phe27 4-CH_3-Phe20
10	Lys4 CA-HA Leu24 CD2-HD21	4-CH_3-Phe27 4-CH_3-Phe20
11	Lys4 CA-HA Leu24 CG-HG	4-CH_3-Phe27 4-CH_3-Phe20
12	Lys4 CA-HA Leu24 CD2-HD23	4-CH_3-Phe27 4-CH_3-Phe20
13	Pro2 CD-HD2 Lys4 CA-HA Leu24 CD2-HD22	4-CH_3-Phe34 4-CH_3-Phe27 4-CH_3-Phe20
14	Lys4 CA-HA Leu24 CD2-HD22	4-CH_3-Phe27 4-CH_3-Phe20
15	Pro2 CD-HD2 Leu24 CD2-HD23	4-CH_3-Phe34 4-CH_3-Phe20
16	Pro1 CA-HA Pro2 CD-HD2 Lys4 CA-HA Leu24 CG-HG	4-CH_3-Phe34 4-CH_3-Phe34 4-CH_3-Phe27 4-CH_3-Phe20
17	Lys4 CA-HA Leu24 CD2-HD22	4-CH_3-Phe27 4-CH_3-Phe20
18	Lys4 CA-HA	4-CH_3-Phe27

	Leu24 CD2-HD22 Leu32 CD2-HD23	4-CH_3-Phe20 4-CH_3-Phe27
19	Pro2 CD-HD2 Lys4 CA-HA Leu24 CD2-HD23	4-CH_3-Phe34 4-CH_3-Phe27 4-CH_3-Phe20
20	Pro1 CA-HA Lys4 CA-HA Leu24 CD2-HD21	4-CH_3-Phe34 4-CH_3-Phe27 4-CH_3-Phe20

Pro donor total = 11 i.e. 0.55 CH–π interactions per structure.
Leu donor total = 22 i.e. 1.1 CH–π interactions per structure.
Lys donor total = 17 i.e. 0.85 CH–π interactions per structure.

Supplementary Table 2: CH–π interactions detected in the NMR structural ensembles of. A: PPα-Tyr, **B:** PPα-ϕOCH_3 and **C:** PPα-ϕCH_3. Potential CH–π interactions were assigned using an operational description adapted from previous studies.[1,2]

A

	Total	No. CH–π as donor	Average CH–π
ALA	664	39	0.06
ARG	310	40	0.13
ASN	346	11	0.03
ASP	432	5	0.01
CYS	162	21	0.13
GLN	299	22	0.07
GLU	355	11	0.03
GLY	682	27	0.04
HIS	140	13	0.09
ILE	371	61	0.16
LEU	575	105	0.18
LYS	378	60	0.16
MET	123	28	0.23
PHE	283	71	0.25
PRO	356	58	0.16
SER	541	17	0.03
THR	547	20	0.04
TRP	102	29	0.28
TYR	254	36	0.14
VAL	500	67	0.10

B

	Contacts with CH–π			
	HIS	PHE	TRP	TYR
ALA	3	12	9	11
ARG	3	10	10	7
ASN	0	4	1	6
ASP	1	4	0	0
CYS	1	6	5	5
GLN	0	5	8	6
GLU	3	2	4	0
GLY	2	8	11	6
HIS	1	4	4	2
ILE	3	20	10	18
LEU	10	35	13	25
LYS	3	15	11	13
MET	2	12	5	5
PHE	7	28	9	16
PRO	5	13	10	19
SER	1	4	6	3
THR	3	9	3	3
TRP	3	9	5	8
TYR	3	14	4	11
VAL	2	17	14	17

C

	Proportion with CH–π			
	HIS	PHE	TRP	TYR
ALA	0.16	0.14	0.24	0.14
ARG	0.14	0.20	0.26	0.13
ASN	0.00	0.18	0.08	0.16
ASP	0.03	0.11	0.00	0.00
CYS	0.20	0.17	0.71	0.24
GLN	0.00	0.19	0.35	0.17
GLU	0.11	0.05	0.21	0.00
GLY	0.17	0.14	0.38	0.10
HIS	0.04	0.17	0.36	0.07
ILE	0.14	0.14	0.22	0.18
LEU	0.33	0.16	0.19	0.16
LYS	0.20	0.38	0.42	0.29
MET	0.22	0.22	0.20	0.13
PHE	0.30	0.19	0.18	0.16
PRO	0.26	0.27	0.30	0.31
SER	0.04	0.07	0.29	0.06
THR	0.09	0.11	0.09	0.05
TRP	0.27	0.18	0.25	0.19
TYR	0.11	0.14	0.10	0.13
VAL	0.09	0.11	0.22	0.15

D

	Proportion x Propensity			
	HIS	PHE	TRP	TYR
ALA	0.13	0.14	0.25	0.15
ARG	0.17	0.15	0.36	0.12
ASN	0.00	0.09	0.05	0.15
ASP	0.06	0.07	0.00	0.00
CYS	0.13	0.21	0.41	0.20
GLN	0.00	0.10	0.40	0.15
GLU	0.20	0.04	0.18	0.00
GLY	0.13	0.15	0.48	0.13
HIS	0.13	0.14	0.33	0.08
ILE	0.09	0.16	0.19	0.16
LEU	0.19	0.18	0.16	0.15
LYS	0.20	0.27	0.48	0.28
MET	0.20	0.33	0.32	0.16
PHE	0.25	0.27	0.21	0.18
PRO	0.34	0.25	0.45	0.42
SER	0.06	0.06	0.22	0.05
THR	0.12	0.10	0.08	0.04
TRP	0.25	0.21	0.27	0.21
TYR	0.12	0.16	0.11	0.14
VAL	0.05	0.12	0.23	0.14

Supplementary Table 3: Distribution of CH–π interactions in our sub-1 Å dataset from the PDB. A: Composition of dataset showing numbers of CH–π donors, and average numbers of CH–π interactions per donor residue. **B:** Numbers of close-contact side-chain – side-chain interactions that involve at least one CH–π interaction (CH donor residues, vertical and aromatic π-acceptor residues, horizontal). **C:** Proportion of close contact side-chain – side-chain interactions that involve CH–π interactions. **D:** Proportion of CH–π interactions in C normalized for propensity (*i.e.*, multiplied by propensity) of the residues to be in close contact.

Peptide	T_M (°C) from first derivative	T_M (°C) from curve fitting	ΔH_{unf} (kJ mol^{-1})	ΔS_{unf} (kJ K^{-1} mol^{-1})	ΔG_{unf} (kJ mol^{-1})	ΔC_p (kJ K^{-1} mol^{-1})
PPα-Tyr	40	36.82	103.4	0.3457	6.136	-0.4598
	39	36.90	103.9	0.3476	6.184	-0.4612
	39	36.92	104.4	0.3484	6.325	-0.3856
	39	35.82	103.0	0.3466	5.509	-0.6224
	39.25 (0.5)	**36.62 (0.5)**	**103.7 (0.59)**	**0.3471 (0.0011)**	**6.039 (0.3617)**	**-0.4822 (0.0999)**
PPα-ϕOCH$_3$	38	33.65	100.9	0.3415	4.938	-0.6221
	37	34.26	104.8	0.3516	5.066	-0.6882
	38	33.49	102.1	0.3458	4.878	-0.5832
	37.67 (0.58)	**33.80 (0.4060)**	**102.6 (1.969)**	**0.3463 (0.0051)**	**4.960 (0.0962)**	**-0.6312 (0.0531)**
PPα-ϕCH$_3$	31	28.57	106.9	0.3622	4.474	-1.010
	31	28.90	105.7	0.3599	4.772	-0.6337
	32	28.81	105.9	0.3602	4.680	-0.6631
	31.33 (0.58)	**28.76 (0.1709)**	**106.2 (0.6509)**	**0.3608 (0.0013)**	**4.642 (0.1525)**	**-0.7688 (0.2091)**
PPα-Phe	20	17.20	107.5	0.3765	2.383	-0.9764
	20	17.73	108.4	0.3781	2.621	-0.8855
	20	17.63	106.6	0.3732	2.514	-0.9329
	20	17.56	107.5	0.3757	2.439	-1.037
	20 (0.0)	**17.53 (0.2298)**	**107.5 (0.7034)**	**0.3759 (0.0021)**	**2.489 (0.1029)**	**-0.9580 (0.0645)**
PPα-F	26	23.85	105.6	0.3638	3.763	-0.6980
	26	23.73	106.1	0.3652	3.935	-0.6536
	26	23.89	106.9	0.3678	3.921	-0.7440
	26 (0.0)	**23.82 (0.0792)**	**106.2 (0.6635)**	**0.3656 (0.0020)**	**3.873 (0.0952)**	**-0.6986 (0.0452)**
PPα-CN	22	21.36	109.0	0.3746	3.371	-0.8708
	22	21.06	107.1	0.3697	3.200	-0.7332
	23	21.47	106.5	0.3681	3.198	-0.7161
	22.33 (0.58)	**21.30 (0.2076)**	**107.5 (1.311)**	**0.3708 (0.0034)**	**3.257 (0.0996)**	**-0.7733 (0.0848)**
PPα-NO$_2$	17	17.12	114.1	0.3922	2.632	-0.8275
	18	17.34	114.9	0.3944	2.709	-0.7264
	17	17.17	113.9	0.3912	2.651	-0.8894
	17.33 (0.58)	**17.21 (0.1169)**	**114.3 (0.5401)**	**0.3926 (0.0016)**	**2.664 (0.0402)**	**-0.8144 (0.0823)**

Supplementary Table 4: Van't Hoff analyses of PPα miniproteins. Thermodynamic parameters of unfolding were obtained by curve fitting of the thermal denaturation profiles (see Materials and Methods). Averages are given in bold typeface and standard deviations in parenthesis. The values for ΔH_{unf}, ΔS_{unf}, ΔG_{unf} are stated for 5°C where all of the peptides are close to fully folded.

A

Domain	PDB ID	Expt.	Resolution (Å)	Domain Chain	Length (residues)	Bound Chain	Length (residues)	No. of CH–π	Length of longest contiguous polyproline II helix (residues)
SH3	1CKA	XRAY	1.50	A	57	B	10	4	5
EVH1	1DDV	XRAY	1.90	A	111	B	6	5	3
WW	1EG4	XRAY	2.00	A	261	P	15	4	5
EVH1	1EVH	XRAY	1.80	A	112	B	7	3	3
SH3	1GBQ	NMR	NA	A	74	B	12	1	3
WW	1K9Q	NMR	NA	A	40	B	6	1	3
SH3	1OV3	XRAY	1.80	A	138	C	18	7	4
WW	1PIN	XRAY	1.35	A	163	B	155	0	3
SH3	1SEM	XRAY	2.00	A	58	C	10	1	3
SH3	1SSH	XRAY	1.40	A	60	B	12	4	8
SH3	1UTI	XRAY	1.50	A	58	D	16	3	6
SH3	1W70	XRAY	1.46	B	60	D	14	2	6
WW	1YWI	NMR	NA	A	41	B	10	6	4
SH3	1YWO	XRAY	1.81	A	64	P	10	3	8
SH3	1ZUK	XRAY	1.90	A	68	C	11	3	8
SH3	2DRM	XRAY	1.35	A	58	E	18	2	4
SH3	2J06	XRAY	1.80	A	65	B	60	0	3
Profilin	2JKG	XRAY	1.89	A	179	P	8	7	6
SH3	2K79	NMR	NA	A	63	B	110	0	3
SH3	2KNB	NMR	NA	B	71	A	81	3	3
WW	2KQ0	NMR	NA	A	49	B	12	1	3
SH3	2LZ6	NMR	NA	B	64	A	76	0	3
WW	2MPT	NMR	NA	A	48	B	14	2	6
Profilin	2PBD	XRAY	1.50	P	139	V	10	8	8
SH3	2ROL	NMR	NA	A	64	B	12	0	3
SH3	2V1R	XRAY	2.10	B	80	P	15	0	4
Profilin	2V8F	XRAY	1.10	A	140	C	21	9	7
SH3	2VKN	XRAY	2.05	A	70	C	12	2	6
SH3	2VWF	XRAY	1.58	A	58	B	15	5	4
SH3	3THK	XRAY	1.70	B	73	D	10	2	3
SH3	3UA7	XRAY	1.50	A	64	E	12	2	6
SH3	4CC7	XRAY	1.97	M	67	N	12	7	5
WW	4E5R	XRAY	1.90	A	56	B	56	1	4
SH3	4HVW	XRAY	0.98	A	61	B	13	3	5
SH3	4J9C	XRAY	1.05	A	63	B	11	9	6
SH3	4LN2	XRAY	1.00	A	68	B	11	4	7
WW	4ROF	XRAY	2.03	A	47	C	14	4	3
SH3	4U5W	XRAY	1.86	B	180	A	149	3	3
							Total	129	177
							Av. per structure	3.39	4.66
							Stdev	2.64	1.72

B

	Total within 4 Å of bound polypeptide	No. CH–π as donor	Average CH–π
ALA	25	4	0.16
ARG	35	8	0.23
ASN	6	0	0.00
ASP	5	0	0.00
CYS	1	0	0.00
GLN	10	0	0.00
GLU	12	0	0.00
GLY	17	0	0.00
HIS	7	0	0.00
ILE	13	3	0.23
LEU	32	13	0.41
LYS	20	8	0.40
MET	4	1	0.25
PHE	9	1	0.11
PRO	149	82	0.55
SER	12	0	0.00
THR	11	0	0.00
TRP	6	0	0.00
TYR	12	0	0.00
VAL	21	1	0.10

C

	Donor			Acceptor		
Component	Residue	C	H	Component	Residue	π–System
1CKA						
PRO	2	CA	HA	PHE	141	PHE
PRO	3	CD	HD3	PHE	141	PHE
LYS	8	CG	HG2	TRP	169	TRPA
LYS	8	CE	HE3	TRP	169	TRPB
1EG4						
PRO	9	CB	HB3	TRP	83	TRPA
PRO	9	CB	HB3	TRP	83	TRPB
PRO	9	CA	HA	TRP	83	TRPB
PRO	10	CD	HD2	TYR	72	TYR
1GBQ						
PRO	2	CA	HA	TYR	7	TYR
1UTI						
PRO	7	CG	HG2	TRP	36	TRPA
LYS	12	CG	HG2	TRP	36	TRPA
LYS	12	CD	HD3	TRP	36	TRPB
1W70						
PRO	363	CD	HD3	PHE	179	PHE
PRO	369	CD	HD3	TRP	207	TRPA
1YWI						
PRO	6	CB	HB3	TYR	30	TYR
LEU	8	CD1	HD12	TYR	28	TYR
LEU	8	CA	HA	TYR	28	TYR
LEU	8	CD1	HD13	TRP	39	TRPB
PRO	9	CG	HG2	TRP	39	TRPA
PRO	9	CD	HD2	TRP	39	TRPB
1YWO						
PRO	2	CA	HA	PHE	11	PHE
PRO	3	CD	HD3	PHE	11	PHE

ARG	8	CD	HD2	TRP	39	TRPB
2K79						
2MPT						
LEU	926	CB	HB3	TRP	505	TRPA
LEU	928	CD2	HD22	TRP	505	TRPA
2LZ6						
2VKN						
LEU	5	CD2	HD23	TRP	40	TRPA
PRO	9	CD	HD2	TYR	11	TYR
4ROF						
PRO	331	CD	HD3	TRP	466	TRPA
PRO	331	CB	HB3	TRP	466	TRPB
PRO	331	CA	HA	TRP	466	TRPB
PRO	332	CD	HD2	TYR	455	TYR
4CC7						
PRO	2	CA	HA	TYR	1522	TYR
PRO	3	CD	HD2	TYR	1570	TYR
PRO	3	CD	HD3	TYR	1522	TYR
PRO	6	CG	HG2	TRP	1554	TRPA
PRO	6	CG	HG2	TRP	1554	TRPB
PRO	10	CB	HB3	TRP	1554	TRPA
PRO	10	CD	HD3	TRP	1554	TRPB
4U5W						
PRO	76	CD	HD2	TYR	136	TYR
PRO	76	CD	HD3	TYR	90	TYR
ARG	81	CD	HD3	TRP	118	TRPA
2V1R						
2PBD						
PRO	203	CB	HB3	TRP	31	TRPA
PRO	204	CD	HD2	TRP	31	TRPB
ALA	206	CB	HB2	TRP	3	TRPA
ALA	206	CB	HB2	TRP	3	TRPB
PRO	207	CD	HD3	TYR	139	TYR
LEU	209	CD2	HD23	TYR	6	TYR
PRO	210	CG	HG3	HIS	133	HIS
PRO	210	CD	HD3	HIS	133	HIS
1ZUK						
PRO	5	CA	HA	PHE	14	PHE
PRO	6	CD	HD2	PHE	63	PHE
ARG	11	CD	HD2	TYR	20	TYR
2JKG						
PRO	1	CB	HB3	TYR	35	TYR
PRO	4	CB	HB3	TRP	7	TRPA
PRO	4	CA	HA	TRP	7	TRPB
PRO	5	CD	HD2	TYR	10	TYR
PRO	5	CG	HG2	TYR	10	TYR
PRO	5	CD	HD3	TRP	7	TRPB
PRO	7	CG	HG3	TYR	5	TYR
3THK						
PRO	74	CA	HA	TYR	12	TYR
PRO	75	CD	HD2	TYR	12	TYR
2KQ0						
PRO	115	CG	HG2	PHE	28	PHE
1K9Q						
PRO	5	CG	HG3	TYR	28	TYR
2J06						
1OV3						
PRO	151	CA	HA	TRP	204	TRPA
PRO	151	CB	HB3	TRP	204	TRPB
PRO	151	CA	HA	TRP	204	TRPB
PRO	152	CD	HD3	TRP	204	TRPA

ARG	158	CD	HD2	TRP	263	TRPA
ARG	158	CD	HD2	TRP	263	TRPB
PRO	160	CG	HG3	PHE	209	PHE
1EVH						
PRO	1002	CG	HG3	TYR	16	TYR
PRO	1002	CB	HB2	TYR	16	TYR
PRO	1005	CD	HD3	PHE	77	PHE
4LN2						
PRO	860	CD	HD2	TYR	922	TYR
PRO	860	CD	HD3	PHE	876	PHE
LEU	865	CD2	HD23	TRP	904	TRPB
PRO	866	CD	HD3	TRP	904	TRPA
1PIN						
1SSH						
PRO	5	CD	HD3	TYR	10	TYR
MET	7	CE	HE2	TYR	56	TYR
ARG	10	CA	HA	TRP	40	TRPA
ARG	10	CG	HG2	TRP	40	TRPB
4J9C						
ALA	1	CA	HA	TRP	110	TRPA
ALA	1	CB	HB3	TRP	110	TRPB
PRO	2	CD	HD2	TRP	99	TRPA
PRO	2	CD	HD2	TRP	99	TRPB
PRO	2	CG	HG2	TRP	99	TRPB
PRO	6	CD	HD3	TRP	99	TRPA
PRO	6	CB	HB3	TRP	99	TRPB
PRO	10	CD	HD2	TYR	70	TYR
PRO	10	CG	HG2	TYR	70	TYR
2ROL						
2V8F						
ILE	1	CG2	HG21	TRP	31	TRPA
ILE	1	CB	HB	TRP	31	TRPB
PRO	2	CD	HD2	TRP	31	TRPB
PRO	4	CD	HD3	TRP	3	TRPA
PRO	4	CB	HB3	TRP	3	TRPB
PRO	5	CD	HD3	PHE	139	PHE
LEU	7	CG	HG	TYR	6	TYR
LEU	7	CD2	HD21	TYR	6	TYR
LEU	7	CD1	HD11	TYR	6	TYR
4E5R						
VAL	329	CB	HB	TYR	335	TYR
2KNB						
ILE	44	CG2	HG21	TYR	299	TYR
PRO	73	CG	HG2	TRP	327	TRPB
ARG	75	CD	HD2	PHE	338	PHE
1SEM						
PRO	2	CA	HA	PHE	163	PHE
4HVW						
LEU	3	CB	HB2	TYR	131	TYR
LEU	11	CD2	HD23	TYR	136	TYR
PRO	12	CD	HD2	TYR	90	TYR
3UA7						
PRO	6	CB	HB3	TYR	91	TYR
PRO	7	CD	HD2	TYR	91	TYR
2DRM						
LYS	3	CA	HA	TYR	11	TYR
PRO	4	CD	HD2	TYR	11	TYR
2VWF						
PRO	3	CA	HA	PHE	7	PHE
PRO	4	CD	HD2	TYR	51	TYR
LYS	11	CA	HA	TRP	35	TRPA

LYS	11	CG	HG2	TRP	35	TRPA
LYS	11	CG	HG2	TRP	35	TRPB
1DDV						
PRO	1002	CB	HB3	PHE	74	PHE
PRO	1003	CD	HD3	TRP	24	TRPA
PRO	1003	CG	HG2	PHE	14	PHE
PRO	1003	CD	HD2	PHE	74	PHE
PHE	1006	CZ	HZ	HIS	12	HIS

Supplementary Table 5: Structural data and CH–π interactions in binding of SH3, WW, Profilin and EVH1 domains from the PDB. A: Identities of binding domain and bound polypeptide in the dataset. **B:** Total number of CH–π interactions, and average number of CH–π interactions per residue within 4 Å of the bound polypeptide chain. **C:** Identities of CH–π donors and acceptors between domain and bound polypeptide.

1 Brandl, M., Weiss, M.S., Jabs, A., Sühnel, J. & Hilgenfeld, R. C-H···π-Interactions in Proteins. *J. Mol. Biol.* **307**, 357-377 (2001).
2 Hudson, K.L. *et al.* Carbohydrate-Aromatic Interactions in Proteins. *J. Am. Chem. Soc.* **137**, 15152-15160 (2015).