Electronic Supplementary Information for

"Comparison of C-H···π and Hydrophobic Interactions in a ββα Hairpin Peptide: Impact on Stability and Specificity"

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Table S1: Comparison of the fraction folded from glycine chemical shift difference and composite Hα.
Tables S2, S3: Thermal analysis of fraction folded from glycine chemical shift difference and composite Hα.
Tables S4, S5: Thermodynamic parameters from glycine chemical shift difference and composite Hα.
Tables S6-S22: Assignment of protons in peptides at 298K.

Error Analysis:

The error intrinsic in the determination of the fraction folded originates from the uncertainty in the glycine chemical shift difference and in the temperature. The error in chemical shift is ± 0.005 ppm and the error in temperature is ± 0.5 K, which translates to ±1% fraction folded uncertainty. The propagation of error results in an error of ±0.05 kcal mol^{-1} for the investigated peptides. Due to the lower fraction folded of the control peptides the error can become as large as 0.1 kcal mol^{-1}.

The error intrinsic in double mutant cycles is determined as the propagation of error from the fraction folded of each of the individual peptides. As the error is a factor of the fraction folded the relatively low fraction folded of the double mutants will propagate the largest error. However, all double mutant cycles share the VS peptide as the double mutant. Consequently, this peptide will not have independent and random error and is not used to determine the error in the double mutant cycles. The error that results from three members of double mutant cycles is ±0.1 kcal mol^{-1}.

Comparison of Fraction Folded as determined from Gly and Hα.

Using the Hα chemical shift yields similar results for the fraction folded as the glycine residue at every temperature. However, not every Hα resonance can be determined at every temperature due to saturation of the water resonance. Consequently, the composite Hα fraction folded is more irregular than the Glycine chemical shift difference. Nonetheless, as can be seen from Tables S1 through S3, they yield the same fraction folded trends.

Table S1. Comparison of fraction folded from the Glycine chemical shift and Hα of positions 2,4,6,9,10 and 11 at 298K.

	Fraction Folded	
Peptide	Gly	Hα
ChaK	53	52
ChaNle	79	77
FK	51	49
FNle	65	68
WK	77	74
WNle	88	87

Table S2. The fraction folded of **WNle** from the Glycine chemical shift difference and H chemical shifts (residues 2,4,6,9,10 and 11).

Temp	Fraction Folded	
(K)	Gly	H
276	0.84	0.81
280	0.84	0.81
284	0.85	0.83
289	0.86	0.84
293	0.86	0.84
298	0.85	0.85
302	0.85	0.84
306	0.84	0.82
311	0.82	0.8
315	0.80	0.77
320	0.76	0.73

Table S3. The fraction folded of **ChaK** as determined by Glycine chemical shift differences and H chemical shifts (residues 2,4,6,9,10 and 11).

	Fraction Folded	
Temperature (K)	Gly	H
275	0.54	0.51
280	0.55	0.52
284	0.55	0.53
288	0.55	0.54
293	0.54	0.54
297	0.53	0.53
302	0.52	0.52
306	0.50	0.50
310	0.49	0.49
315	0.47	0.47
319	0.45	0.45
324	0.42	0.45
328	0.40	0.44

Comparison of thermal parameters derived from Gly and Ha data.
The DH, DS, and DCp values derived from Gly splitting and Ha chemical shifts are quite similar, such that for WNle, they are within the fitting error, whereas for ChaNle, the differences are outside of the error as determined from the fitting. The comparison of these two sets of data provides an estimate for the actual error in these values.

Table S4. The thermodynamic parameters of folding of **WNle** from glycine chemical shift difference and H fraction folded.[a]

	ΔH°	ΔS°	ΔC_p°
WNle Gly	-1410 (90)	-1.2 (0.3)	-170 (10)
WNle H	-1450 (140)	-1.5 (0.5)	-200 (20)

a. Units are cal mol^{-1} for $\Delta H°$ and cal $mol^{-1}K^{-1}$ for $\Delta S°$ and $\Delta Cp°$. Values in parentheses are the error as determined from the fitting.

Table S5. The thermodynamic parameters of folding of **ChaK** from glycine chemical shift difference and H fraction folded.[a]

	H	S	C_p
ChaK Gly	-1750 (30)	-5.6 (0.1)	-115 (4)
ChaK H	-1020 (130)	-3.3 (0.4)	-100 (16)

a. Units are cal mol^{-1} for H° and cal $mol^{-1}K^{-1}$ for S° and Cp°. Values in parentheses are the error as determined from the fitting.

Assignment of Protons in Investigated and Control Peptides (ppm):

Table S6. Peptide FK Ac-Arg-**Phe**-Val-Glu-Val-Asn-Gly-Orn-**Lys**-Ile-Leu-Gln-NH_2

						Amide	Amine
R	4.28	1.69	1.49	3.14		8.13	7.15,6.65
F	4.98	2.95, 2.91	H2,6 7.11	H3,4,5 7.31		8.31	
V	4.17	1.97	0.91			8.64	
E	4.72	2.00, 1.92	2.3			8.48	
V	4.27	2.01	0.87			8.48	
N	4.54	2.98, 2.76				9.13	
G	4.03,3.80					8.55	
O	4.51	1.82	1.68	3.02		7.95	7.66
K	4.49	1.65	1.21	1.46, 1.37	2.73	8.43	7.46
I	4.35	1.88	1.43, 1.20, 0.89	0.89		8.7	
L	4.30	1.57	1.62	0.76, 0.73		8.47	
Q	4.31	2.08, 1.89	2.3			8.48	

Table S7. Peptide WK: Ac-Arg-**Trp**-Val-Glu-Val-Asn-Gly-Orn-**Lys**-Ile-Leu-Gln-NH_2

						Amide	Amine
R	4.31	1.66	1.52	3.15		8.07	7.14,6.65
W	5.06	3.13, 3.06	7.22, 7.48	7.07, 7.22	7.24	8.32	
V	4.2	1.97	0.93			8.84	
E	4.89	2.04, 1.95	2.29			8.52	
V	4.42	1.9	0.88			8.93	
N	4.46	3.09, 2.77				9.37	
G	4.08,3.74					8.63	
O	4.6	1.83	1.74	3.06		7.86	7.65
K	4.73	1.71	1.27	1.39	2.61	8.51	7.32
I	4.54	1.91	1.42, 1.22; 0.90	0.9		9.06	
L	4.07	1.37	1.11	0.56		8.33	
Q	4.36	2.04, 1.89	2.39			8.64	

Table S8. Peptide **FNle**: Ac-Arg-**Phe**-Val-Glu-Val-Asn-Gly-Orn-**Nle**-Ile-Leu-Gln-NH_2

	□	□	□	□	□
R	4.31	1.65	1.52	3.15	
F	5.01	2.92	H2,6 7.11	H3,4,5 7.32	
V	4.17	1.96	0.92		
E	4.75	1.96	2.22		
V	4.31	1.96	0.87		
N	4.50	3.01, 2.79			
G	4.06,3.76				
O	4.57	1.79	1.72	3.02	
Nle	4.54	1.65	1.18	1.12	0.83
I	4.42	1.89	1.45, 1.23, 0.88	0.88	
L	4.54	1.65	1.15	0.73	
Q	4.31	2.07, 1.96	2.32		

Table S9. Peptide **WNle**: Ac-Arg-**Trp**-Val-Glu-Val-Asn-Gly-Orn-**Nle**-Ile-Leu-Gln-NH_2

	□	□	□	□	□
R	4.43	1.73	1,65, 1.54	3.16	
W	5.07	3.05	7.04, 7.20,7.21	7.22, 7.44	
V	4.23	1.94	0.92		
E	4.88	2.06, 1.90	2.25		
V	4.48	2.01	0.87		
N	4.43	3.08, 2.77			
G	3.96,3.57				
O	4.68	1.9	1.81, 1.74	3.04	
Nle	4.72	1.88	1.22	1.07	0.79
I	4.62	1.89	1.43, 1.20, 0.88	0.88	
L	3.94	1.26	0.4	0.12	
Q	4.33	2.03, 1.86	2.25		

Table S10. Peptide **ChaK**: Ac-Arg-**Cha**-Val-Glu-Val-Asn-Gly-Orn-**Lys**-Ile-Leu-Gln-NH_2

	□	□	□	□	□	□
R	4.33	1.73	1.6	3.19		
Cy	4.66	1.64	1.41	1.27	1.1	0.75
V	4.15	1.99	0.9			
E	4.64	1.93	2.23			
V	4.24	1.99	0.87			
N	4.54	2.97, 2.77				
G	4.03,3.81					
O	4.52	1.77	1.7	3		
K	4.55	1.73	1.43	1.65	2.95	
I	4.32	1.86	1.40, 1.12, 0.87	0.87		
L	4.44	1.58	1.55	0.87		
Q	4.32	2.08, 1.87	2.33			

Table S11. Peptide **ChaNle**: Ac-Arg-**Cha**-Val-Glu-Val-Asn-Gly-Orn-**Nle**-Ile-Leu-Gln-NH_2

	□	□	□	□	□	□
R	4.38	1.72	1.62	3.18		
Cy	4.77	1.63	1.32	1.13	1.1	0.69
V	4.16	1.93	0.91			
E	4.69	2.01, 1.84	2.18			
V	4.3	1.98	0.87			
N	4.46	3.02, 2.77				
G	4.08,3.75					
O	4.62	1.72	1.72	3.03		
Nle	4.63	1.72	1.27	1.2	0.78	
I	4.42	1.84	1.41, 1.150.87	0.87		
L	4.47	1.61	1.53	0.86		
Q	4.35	2.14, 1.98	2.36			

Table S12. Peptide **ChaS**: Ac-Arg-**Cha**-Val-Glu-Val-Asn-Gly-Orn-**Ser**-Ile-Leu-Gln-NH_2

	□	□	□	□	□	□
R	4.31	1.85	1.79,1.73	3.18		
Cy	4.59	1.62	1.49	1.27	1.12	0.94
V	4.14	1.99	0.91			
E	4.65	2.01, 1.92	2.33			
V	4.19	2	0.9			
N	4.59	2.98, 2.77				
G	4.02,3.84					
O	4.53	1.79	1.71	3.01		
S	4.61	3.8				
I	4.3	1.87	1.40, 1.17, 0.89	0.89		
L	4.41	1.62	1.51	0.87		
Q	4.31	2.11, 1.95	2.34			

Table S13. Peptide **FS**: Ac-Arg-**Phe**-Val-Glu-Val-Asn-Gly-Orn-**Ser**-Ile-Leu-Gln-NH_2

	□	□	□	□	□	Amide
R	4.26	1.64	1.51	3.13		8.25
F	4.84	3.05, 2.92	H2,6 7.15	H4 7.29 H3,5 7.31		8.32
V	4.14	2	0.91			8.59
E	4.6	1.98	2.37			8.43
V	4.18	1.99	0.88			8.38
N	4.6	2.96, 2.78				
G	4.01,3.85					8.51
O	4.52	1.85	1.75	3.02		7.99
S	4.6	3.76				
I	4.33	1.9	1.42, 1.21; 0.90	0.9		8.6
L	4.26	1.54	1.48	0.78		8.13
Q	4.3	2.10, 1.94	2.33			8.42

Table S14. Peptide **WS**: Ac-Arg-**Trp**-Val-Glu-Val-Asn-Gly-Orn-**Ser**-Ile-Leu-Gln-NH_2

	□	□	□	□	□
R	4.33	1.64	1.49	3.11	
W	4.9	3.09	7.04, 7.20,7.21	7.41, 7.46	
V	4.16	1.98	0.91		
E	4.76	1.96	2.37		
V	4.3	1.98	0.86		
N	4.52	3.01, 2.76			
G	4.05,3.79				
O	4.59	1.74	1.74	3.03	
S	4.82	3.78			
I	4.45	1.86	1.41, 1.19; 0.89	0.89	
L	4.04	1.34, 1.09	0.78	0.56, 0.37	
Q	4.3	2.06, 1.88	2.29		

Table S15. Peptide **VK**: Ac-Arg-**Val**-Val-Glu-Val-Asn-Gly-Orn-**Lys**-Ile-Leu-Gln-NH_2

	□	□	□	□	□
R	4.33	1.75	1.61	3.18	
V	4.22	2.02	0.89		
V	4.15	1.99	0.91		
E	4.66	1.96, 1.90	2.27		
V	4.27	1.99	0.91		
N	4.59	2.95, 2.77			
G	4.01,3.86				
O	4.47	1.77	1.61	3.01	
K	4.41	1.71	1.47, 1.31	1.65	2.96
I	4.26	1.85	1.44,1.170.87	0.87	
L	4.47	1.85	1.61	0.89	
Q	4.33	2.10, 1.95	2.34		

Table S16. Peptide **VS**: Ac-Arg-**Val**-Val-Glu-Val-Asn-Gly-Orn-**Ser**-Ile-Leu-Gln-NH_2

	□	□	□	□	□
R	4.32	1.59	1.75	3.18	
V	4.18	2.01	0.89		
V	4.13	2.00	0.91		
E	4.60	1.91	2.31		
V	4.24	1.98	0.90		
N	4.62	2.93, 2.78			
G	3.99,3.87				
O	4.5	1.89	1.75	3.01	
S	4.56	3.81			
I	4.26	1.88	1.40,1.18,0.89	0.89	
L	4.41	1.60	1.60	0.89	
Q	4.32	2.11, 1.96	2.34		

Table S17. Peptide **CWKC**: Ac-Cys-Arg-**Trp**-Val-Glu-Val-Asn-Gly-Orn-**Lys**-Ile-Leu-Gln-Cys-NH_2

	α	β	γ	δ	ε	Amide	Amine
C	5.23	3.01, 2.41				8.36	
R	4.63	1.8	1.67, 1.50	3.17		8.72	7.11, 6.67
W	5.15	3.10, 2.94	7.00,7.23,7.24	7.28, 7.51		8.65	
V	4.25	1.93	0.91			9.07	
E	5.06	2.00, 1.89	2.19			8.55	
V	4.59	2.06	0.87			9.54	
N	4.41	3.11, 2.76				9.6	
G	4.13,3.69					8.68	
O	4.7	1.85	1.73	3.04		7.77	7.66
K	4.97	1.72	1.41, 1.31	1.72	2.57	8.54	7.21
I	4.73	1.85	1.42,1.180.87	0.87		9.41	
L	3.92	1.31, 0.78	0.37	0.09, -0.33		8.32	
Q	4.57	2.10, 1.82	2.23			9.05	
C	5.05	2.94				8.91	

Table S18. Peptide **CWNleC**: Ac-Cys-Arg-**Val**-Val-Glu-Val-Asn-Gly-Orn-**Ser**-Ile-Leu-Gln-Cys-NH_2

	α	β	γ	δ	ε
C	5.21	2.97, 2.39			
R	4.6	1.78	1.64, 1.49	3.15	
W	5.1	3.01			
V	4.22	1.92	0.9		
E	4.92	2.03, 1.83	2.19		
V	4.53	2	0.86		
N	4.39	2.74, 3.08			
G	4.12,3.69				
O	4.71	1.85	1.73	3.03	
Nle	4.92	1.69	1.27	1.09	0.68
I	4.71	1.84	1.40,1.19,0.87	0.87	
L	3.86	1.25, 0.71	0.37	-0.06, -0.35	
Q	4.55	2.07, 1.80	2.21		
C	5.03	2.9			

Table S19. Peptide **VNle**: Ac-Arg-**Val**-Val-Glu-Val-Asn-Gly-Orn-**Nle**-Ile-Leu-Gln-NH_2

	α	β	γ	δ	ε
R	4.35	1.75	1.60	3.17	
V	4.25	1.99	0.86		
V	4.16	1.95	0.89		
E	4.74	1.97, 1.86	2.24		
V	4.35	1.94	0.88		
N	4.54	2.99, 2.77			
G	4.06,3.83				
O	4.54	1.80	1.71	3.00	
Nle	4.44	1.66	1.25, 1.21	1.23	0.82
I	4.28	1.84	1.40,1.17,0.86	0.86	
L	4.50	1.59	1.59	0.86	
Q	4.34	2.10, 1.93	2.31		

Table S20. Ac-Arg-Ser-Val-Glu-Val-Asn-Gly-NH_2

	α	β	γ	δ	ε	Amide	Amine
R	4.33	1.82, 1.73	1.64	3.19		8.28	7.19,6.66
S	4.49	3.85				8.43	
V	4.10	2.06	0.92			8.26	
E	4.38	2.04, 1.94	2.37			8.39	
V	4.15	2.09	0.92			8.16	
N	4.69	2.87, 2.78				8.63	
G	3.94					8.41	

Table S21. Ac-Asn-Gly-Orn-Lys-Ile-Leu-Gln-NH_2

	α	β	γ	δ	ε	Amide	Amine
N	4.67	2.82				8.44	
G	3.94					8.55	
O	4.35	1.88,1.78	1.7	3.01		8.13	7.64
K	4.31	1.77	1.42	1.69	2.99	8.33	7.56
I	4.14	1.85	1.48,1.20,0.89	0.89		8.25	
L	4.38	1.65	1.61	0.93,0.88		8.37	
Q	4.30	2.12,1.99	2.37			8.33	

Table S22. Ac-Asn-Gly-Orn-Ser-Ile-Leu-Gln-NH_2

	α	β	γ	δ	ε	Amide	Amine
N	4.69	2.82				8.42	
G	3.94					8.56	
O	4.42	1.91, 1.80	1.70	3.01		8.18	7.64
S	4.47	3.85				8.35	
I	4.19	1.88	1.44, 1.20, 0.90	0.90		8.23	
L	4.36	1.61	1.61	0.92, 0.86		8.25	
Q	4.30	2.12, 1.98	2.36			8.27	