SUPPORTING INFORMATION

CONTROL OF PROTON AND ELECTRON TRANSFER IN DE NOVO DESIGNED, BIOMIMETIC BETA HAIRPINS

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Materials and Methods

pK determination. A Hitachi U-3000 spectrophotometer equipped with 1 cm path length cuvettes was employed. The samples were prepared to a concentration of 50 μM and buffered using 10 mM MES-NaOH (pH 5.5), 10 mM HEPES-NaOH (pH 6.0-8.0), 10 mM boric acid-NaOH (pH 8.5-9.5) or 10 mM CAPS-NaOH (pH 10.0-11.5). The solutions were filtered using Acrodisc® 25 mm syringe filters with a 0.45 μm HT Tuffryn membrane. Backgrounds were recorded at each pH using the appropriate buffer. The optical spectrum was recorded between 200 nm and 350 nm. Spectral conditions were as follows: slit width, 2.0 nm; scan speed, 120 nm/min. Deprotonation of tyrosine was monitored by measuring the change in absorbance at 295 nm, at which $TyrO^-$ contributes (*1*). The 295 nm absorption was corrected for baseline instabilities by subtracting out the absorbance at 330 nm. Data were obtained for two to six different samples and were averaged. To calculate tyrosinate concentration, the extinction coefficient was calculated at 295 nm from the pH 11 absorption spectrum. This procedure assumes that tyrosine is completely deprotonated at pH 11, which is one pH unit over the pK reported for tyrosine (*1*). The 295 nm extinction coefficients (L mol^{-1} cm^{-1}) and λmax were: 1664 (292 nm) for tyrosine; 1300 (296 nm) for peptide A; 2146 (294 nm) for peptide C; 1400 (294 nm) for peptide D; 3776 (293 nm) for peptide E; 2911 (293 nm) for peptide F.

Results

Circular dichroism at pH 11. At pH 11, peptide A (Figure S1A), peptide C (His14Cha) (Figure S2B), and peptide D (Arg12Ala; His14Val) (Figure S2C) displayed reversible thermal denaturation between 20ºC and 80ºC. These data support the conclusion that peptides A, C (His14Cha), and D (Arg12Ala; His14Val) are stably folded at pH 11.0. At pH 11.0 and 20ºC,

peptide E (Arg16Ile) displayed a shallow minimum at 199 nm (Figure S1D, red). Increasing the temperature to 80ºC (green) decreased the amplitude of the minimum and shifted the minimum to 201 nm. Cooling peptide E (Arg16Ile) back to 20ºC (post-melt, black) shifted the minimum back to 199 nm. The spectrum collected at 80ºC had an isodichroic point with the 20ºC pre-melt spectrum at 203 nm. This isodichroic point shifts to 208 nm when compared with the 20ºC post-melt spectrum. These data support the conclusion that peptide E (Arg16Ile) forms a stable beta hairpin structure at pH 11.0.

At pH 11 and 20ºC, peptide F (Arg12Ile) displayed a shallow minimum at 198 nm (Figure S1E, red). Increasing the temperature to 80ºC increased the amplitude of the minimum and shifted it to 201 nm (green). Cooling peptide F (Arg12Ile) back to 20ºC (post-melt, black) shifted the minimum back to 198 nm. However the amplitude of the 20ºC post-melt minimum was larger than the amplitude of the 20ºC pre-melt minimum. These data support the interpretation that peptide F can adopt a beta hairpin structure at pH 11.0.

pK determination. Optical titration curves were used to assess the effect of substitutions on the pK of Tyr 5 in peptides A, C (His14Cha), D (Arg12Ala; His14Val), E (Arg16Ile), and F (Arg12Ile) (Figure S2B-F). The pK of tyrosine was determined as a control (Fig. S2A). Tyrosinate and tyrosine are distinguishable in the UV region by the deprotonation-induced red shift of the 270 nm absorption band (see (*2*) and references therein). The deprotonation can be monitored at 295 nm in a titration experiment (*1, 3*) (Figure S2). To determine the pK of tyrosine in solution and in peptides, we performed a least square fit of the optical titration curves to the Henderson-Hasselbach equation (*4*): f(pH) = ((10^(pH-pK))*C)/(((10^(pH-pK))+1)); C is the concentration of the sample**.** The data were fit either from pH 4.0 to pH 10.5 (Figure S2A, B and D) or pH 5.5 to pH 10.5 (Figure S2C and F)). However, the data for peptide E (Figure S2E)

were fit between pH 5.5 and pH 10.0, due to the appearance of a second equivalence point at higher pH in this peptide. The derived pK's were: tyrosine, 9.8 ± 0.1 (Figure S2A); peptide A, 9.3 ± 0.1 (Figure S2B); peptide C, 9.6 ± 0.1 (Figure S2C); peptide D, 9.4 ± 0.3 (Figure S2D); peptide F, 9.6 ± 0.1 (Figure S2CF). In peptide E (Figure S2E), fitting the low pH transition gave a pK of 8.3 ± 0.1 for the first equivalence point.

Assignment of Electrochemical Inflection Points. The electrochemical data from the peptides (Figure S3) were fit with a Nernst equation in which one or more ionizable groups influence the potential, and chi^2 values were used to evaluate the quality of the least square fit. As an example of this procedure, Figure S3 and Table S1 show the results of fitting peptide F (Arg12Ile) data with one (Figure S3A), two (Figure S3B), and three (Figure S3C) ionizable groups. The chi^2 value decreased with the inclusion of more inflection points (Table S1). Additionally, the quality of the fit (Figure S3C, solid line) improved with the inclusion of three ionizable groups, which are present in the peptide. These results support our conclusion that three amino acids (Tyr5, His14, and Arg16) influence the electrochemical behavior of peptide F. A similar procedure was used to evaluate the quality of the fits for all peptide samples.

Electron paramagnetic resonance (EPR) spectroscopy. At pH 5.0, comparison of the tyrosyl radical EPR lineshape in peptide A (Figure S4, red) and in tyrosine solutions (Figure S4A, black), peptide C (Figure S4B, black), peptide D (Figure S4C, black), and peptide F (Figure S4E, black) showed no significant change. However a small change in lineshape was observed when peptide A and peptide E were compared (Figure S4D, red and black).

References

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Table S1. Parameters used to evaluate fits to the electrochemical data, recorded on peptide F (Arg12Ile) (data repeated from Figure 4D.)[†]

# of Ionizable Groups	E^* (V)	S (V/pH)	Tyr^{ox}	pK_1^{ox}	pK_2^{ox}	Tyr^{red}	pK_1^{red}	pK_2^{red}	Chi^2
One[a]	1.4±0.1	0.06±0.01	0.0	-	-	9.6±0.1	-	-	1.35×10^{-2}
Two[b]	1.6±0.1	0.11±0.03	0.0	7.7±0.5		9.6±0.1	5.6±0.3		8.36×10^{-3}
Three[c]	2.1±0.1	0.24±0.01	0.0	6.8±0.6	9.0±0.1	9.6±0.1	4.5±0.3	7.3±0.5	6.36×10^{-3}

[†]Parameters were derived by performing a least squares fit using Igor Pro software. The chi^2 values were used to evaluate the fits. The pK values reported for Tyr^{red} and Tyr^{ox} were determined from the optical titration data (Tyr^{red}) (Figure S2) and from the literature (Tyr^{ox}) (*5*).

[a]The data were fit to the Nernst equation: $E_m = E^* - S \log[\{10^{-pKox} + 10^{-pH}\}/\{10^{-pKred} + 10^{-pH}\}]$, which describes the influence of one ionizable group on the midpoint potential of tyrosine (*2*, *6*).

[b]The data were fit to the modified Nernst equation: $E_m = E^* - S \log[[(\{10^{-pH}\}^2)+(\{10^{-pH}\} * \{10^{-pKox1}\})+(\{10^{-pKox1}\} * \{10^{-pKox2}\})]/[(\{10^{-pH}\}^2)+(\{10^{-pH}\} * \{10^{-pKred1}\})+(\{10^{-pKred1}\} * \{10^{-pKred2}\})]]$, which describes the influence of two ionizable groups on the midpoint potential of tyrosine (*2*, *6*).

[c]The data were fit to the modified Nernst equation: $E_m = E^* - S \log[[(\{10^{-pH}\}^3)+ (\{10^{-pH}\}^2 * \{10^{-pKox1}\}) + (\{10^{-pH}\} * \{10^{-pKox1}\} * \{10^{-pKox2}\}) + (\{10^{-pKox1}\} * \{10^{-pKox2}\} * \{10^{-pKox3}\})]/[(\{10^{-pH}\}^3) + (\{10^{-pH}\}^2 * \{10^{-pKred1}\}) + (\{10^{-pH}\} * \{10^{-pKred1}\} * \{10^{-pKred2}\}) + (\{10^{-pKred1}\} * \{10^{-pKred2}\} * \{10^{-pKred3}\})]]$, which describes the influence of three ionizable groups on the midpoint potential of tyrosine (*2*, *6*).

Figure Legends

Figure S1. Circular dichroism of beta hairpin peptides at pH 11. The panels show data acquired from **(A)** peptide A, **(B)** peptide C (H14Cha), (C) peptide D (H14V; R12A), **(D)** peptide E (R16I), and **(E)** peptide F (R12I). The spectra were collected at 20ºC (red, pre-melt), 80ºC (green), and 20ºC (black, post-melt). See Materials and Methods for spectral conditions.

Figure S2. Optical titration of tyrosine in solution and in beta hairpin peptides, monitoring tyrosinate absorbance at 295 nm. The panels show data acquired from **(A)** tyrosine, **(B)** peptide A, (**C**) peptide C (H14Cha), **(D)** peptide D (H14V; R12A), **(E)** peptide E (R16I), and **(F)** peptide F (R12I). The error bars represent one standard deviation. The superimposed line is a fit of the Henderson-Hasselbach equation. See Materials and Methods for spectral conditions.

Figure S3. Alternative fits for electrochemical titration curves of peptide F (R12I). The data are repeated from Figure 4D. The solid lines show the results of fitting the electrochemical data to Nernst equations involving the influence of (**A)** one, (**B**) two, or **(C)** three ionizable groups. The error bars represent one standard deviation. See Table S1 for fit parameters and Materials and Methods for spectral conditions.

Figure S4. EPR spectra of tyrosyl radicals in beta hairpin peptides at pH 5.0. The radicals were generated by UV photolysis at 108 K. The panels show spectra (black) superimposed on the peptide A spectrum (red), and acquired from (**A)** tyrosine, (**B)** peptide C (H14Cha), **(C)** peptide D (H14Val; R12A), (**D)** peptide E (R16I), and (**E)** peptide F (R12I). To compare spectral

linewidth, the spectra were normalized for any amplitude differences. See Materials and Methods for spectral conditions.

Figure S1

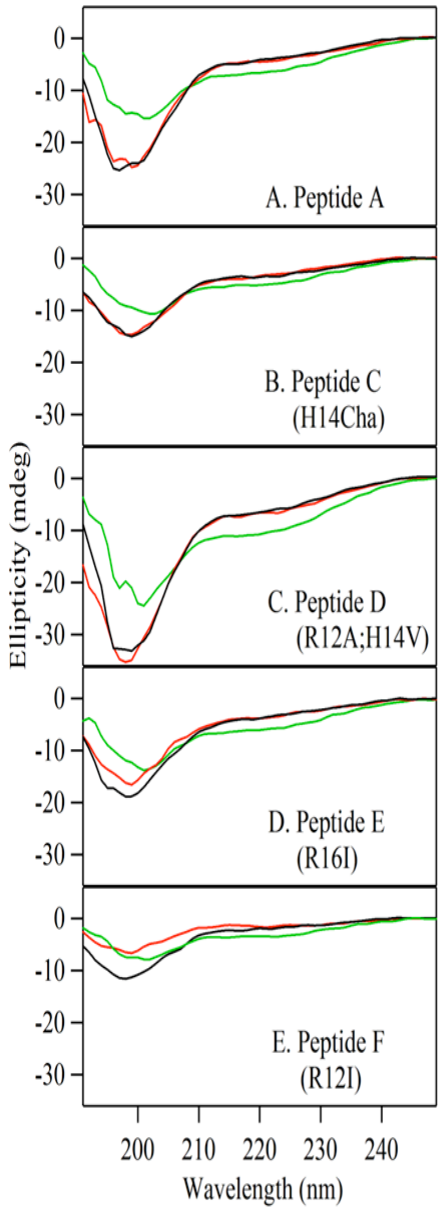

0
-10
-20
-30
A. Peptide A
B. Peptide C
(H14Cha)
C. Peptide D
(R12A;H14V)
D. Peptide E
(R16I)
E. Peptide F
(R12I)
Ellipticity (mdeg)
200
210
220
230
240
Wavelength (nm)

Figure S2

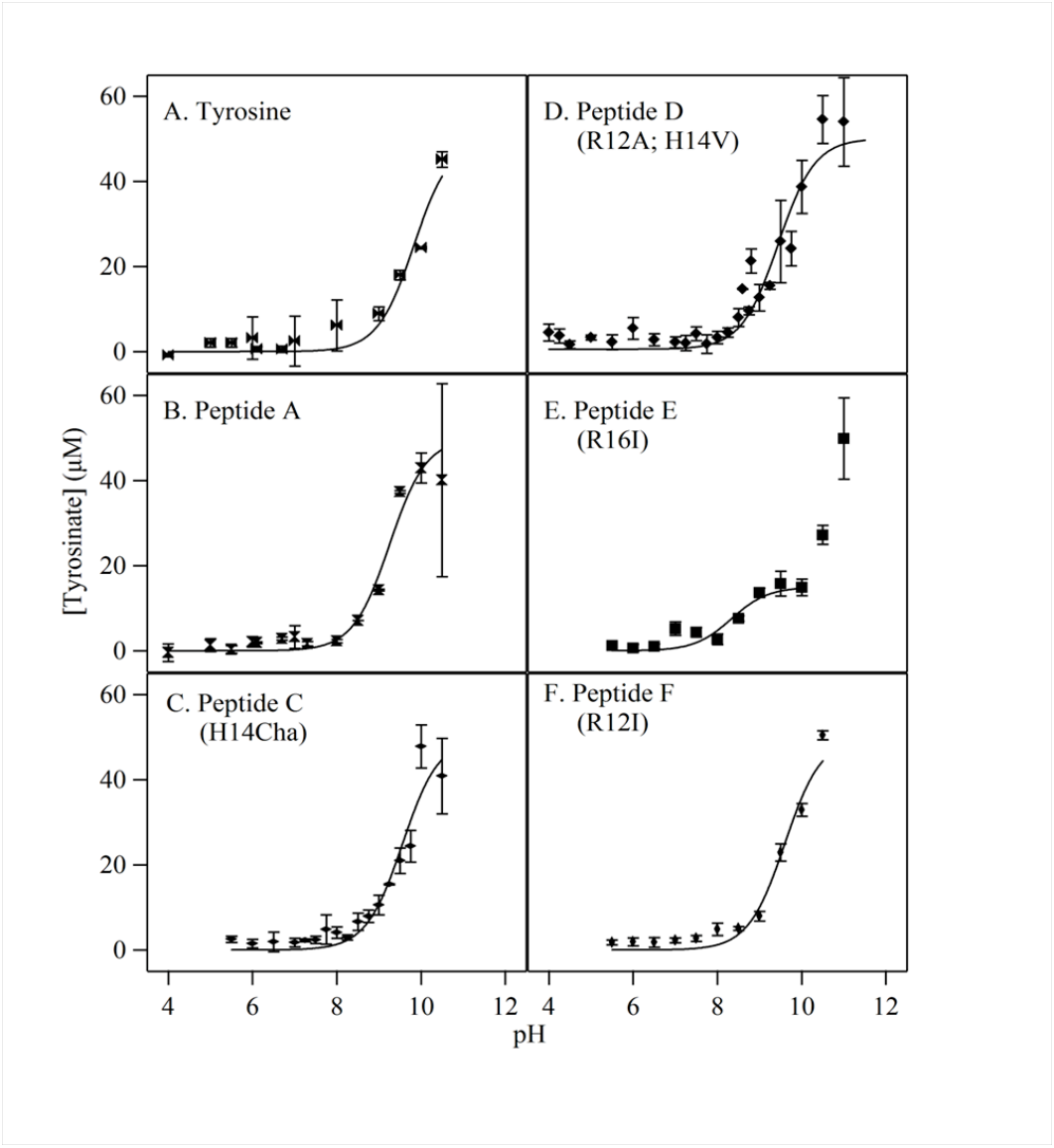

A. Tyrosine
B. Peptide A
C. Peptide C
(H14Cha)
D. Peptide D
(R12A; H14V)
E. Peptide E
(R16I)
F. Peptide F
(R12I)
[Tyrosinate] (μM)
pH
0
20
40
60
4
6
8
10
12

Figure S3

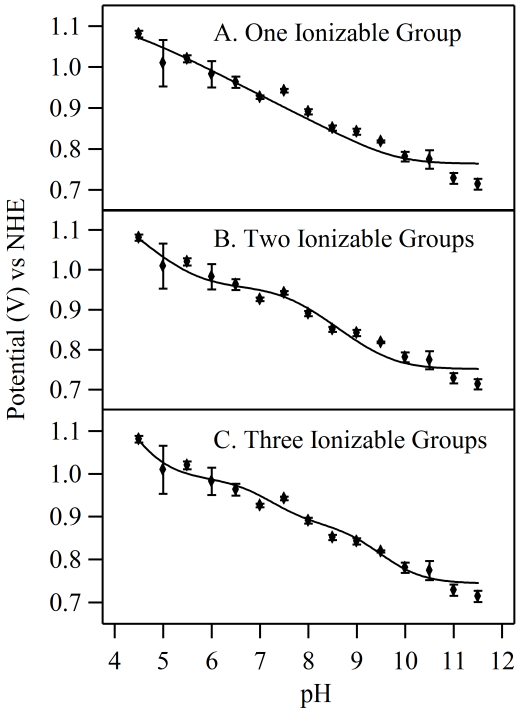
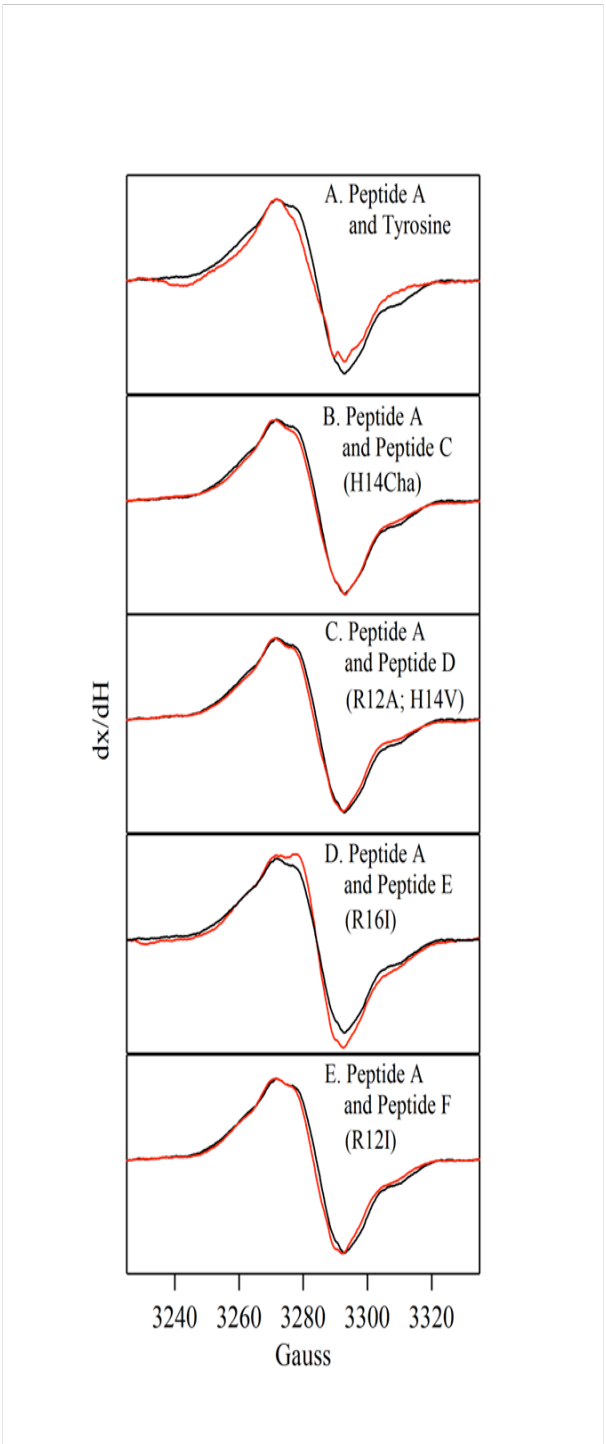

A. One Ionizable Group
B. Two Ionizable Groups
C. Three Ionizable Groups
Potential (V) vs NHE
pH
1.1
1.0
0.9
0.8
0.7
4
5
6
7
8
9
10
11
12

Figure S4


A. Peptide A and Tyrosine
B. Peptide A and Peptide C (H14Cha)
C. Peptide A and Peptide D (R12A; H14V)
D. Peptide A and Peptide E (R16I)
E. Peptide A and Peptide F (R12I)
dx/dH
3240
3260
3280
3300
3320
Gauss