Supporting Information

High resolution structure of a β-peptide bundle

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General Methods. Fmoc-protected ⟨-amino acids, *O*-Benzotriazole-*N*,*N*,*N'*,*N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU), *N*-Hydroxybenzotriazole (HOBt), and Wang resin were purchased from Novabiochem (San Diego, CA). Dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), *N*-methyl morpholine (NMM), trifluoroacetic acid (TFA), and piperidine (Pip) were purchased from American Bioanalytical (Natick, MA). *N*-Fmoc-®^{3}-*p*-iodohomophenylalanine was purchased from Peptech (Burlington, MA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO). Mass spectra were acquired with an Applied Biosystems Voyager-DE Pro MALDI-TOF mass spectrometer (Foster City, CA). Reverse-phase HPLC was performed using a Varian Prostar HPLC and Vydac analytical (C8, 300 Å, 5 ⌈m, 4.6 mm x 150 mm) or preparative (C8, 300 Å, 5 ⌈m, 25 mm x 250 mm) columns, using water/acetonitrile gradients containing 0.1% TFA. ®^{3}-peptides were synthesized using a Symphony/Multiplex peptide synthesizer (Protein Technologies, Tuscon, AZ). CD spectra were acquired with a Jasco J-810 Spectropolarimeter (Jasco, Tokyo, Japan) equipped with a Peltier

temperature control. Analytical ultracentrifugation was performed using a Beckman XL-I instrument (Beckman, Fullerton, CA).

®-peptide synthesis and purification. ®-peptides were synthesized on a 25 μmole scale using standard Fmoc chemistry and Wang resin loaded with ®^{3}-homoaspartic acid as described.[1] One cycle of peptide elongation consisted of the following steps: First, the loaded resin was washed with DMF (6 X 30 sec) and the terminal Fmoc protecting group removed with 20% Pip in DMF (1 X 2 min, 2 X 8 min). This deprotection step was supplemented with a second deprotection step using diazabicyclo[5.4.0]undec-7-ene (DBU) after five residues had been attached to the resin (1 X 2 min 20% Pip/DMF, 2 X 8 min 20% Pip/DMF, 1 X 8 min 2:2:96 Pip/DBU/DMF, and 1 X 8 min 20% Pip/DMF). After deprotection, the resin was washed with DMF (6 X 30 sec) and the next ®^{3}-amino acid coupling reaction was carried out for 30 min using 2 equiv. of the appropriate ®^{3}-amino-acid, 1.8 equiv. HBTU, 2 equiv. HOBt, and 5 equiv. diisopropylethylamine (DIEA). This coupling was then repeated with fresh reagents for another 30 min. The resin was then washed with DMF (6 X 30 sec) before the next cycle of elongation. This elongation cycle was repeated until the ®-peptide sequence was complete. Once the final Fmoc protecting group had been removed, the resin was washed with DMF (8 X 30 sec) and methylene chloride (8 X 30 sec), dried for 20 min under nitrogen, and treated with a cleavage cocktail composed of 1% v/v each of water, triisopropylsilane and phenol in TFA (2 X 60 min). The cleaved peptides were precipitated with 50% v/v pentane/ether. After decantation and drying with nitrogen, the precipitated peptides were dissolved in 30% v/v acetonitrile in water and purified by HPLC.

The success of each synthesis was assessed first by HPLC and MALDI-TOF analysis of the crude reaction mixture. ®-peptides were then purified to homogeneity by reverse-phase HPLC.

The identities and purities of purified ®-peptides were assessed by analytical HPLC and mass spectrometry. MALDI mass spectra were obtained using peptide samples in ⟨-cyano-4-hydroxycinnaminic acid (CHCA) matrix. The masses found were: Zwit-1F (m/z observed, calculated): 1636, 1636; Zwit-1F* (m/z observed, calculated): 1762, 1762. Following purification, ®-peptides were lyophilized and reconstituted in phosphate buffer (10 mM NaH_2PO_4, 200 mM NaCl, adjusted to pH 7.1 with NaOH) or PBC buffer (1 mM phosphoric, boric, and citric acids, adjusted to pH 7.0 with NaOH) for subsequent characterization.

Circular Dichroism (CD). Wavelength-dependent CD spectra were obtained in PBC buffer at 25 ºC in continuous scan mode with a 1 nm data pitch, 50 nm/min scanning speed, 4 sec response, 1 nm band width, and 3 accumulations. Wavelength-dependent scans of various concentrations of Zwit-1F are shown in Figure 1 in the main text. Zwit-1F* was observed to exhibit similar concentration-dependent increases in structure as monitored by changes in molar residue ellipticity (Figure SI-1). Temperature-dependent CD spectra were obtained at 205 nm, between 5 and 95 ^{o}C, using the variable temperature module provided with the instrument. Data were collected with a 1 °C data pitch, 5 s. delay time, 1 °C/min. temperature slope, 4 s. response time, and 1 nm band width. Figure 1 in the main text illustrates the change in the MRE at 205 nm for a 100 µM sample of Zwit-1F.

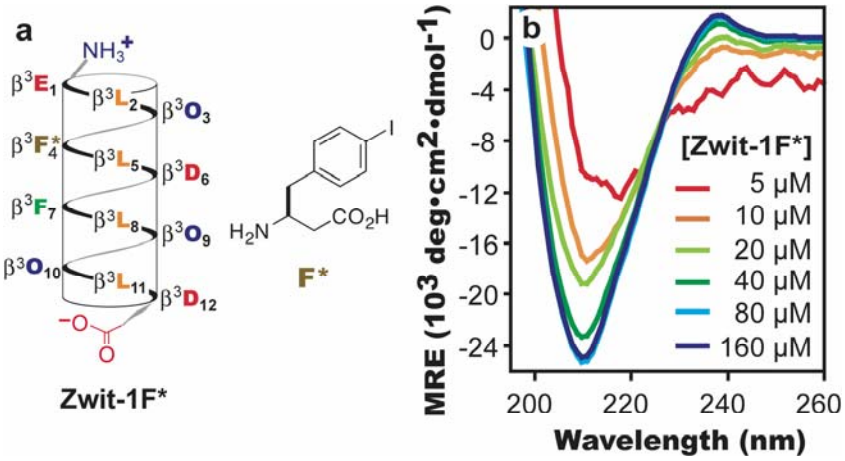

Figure SI-1. Zwit-1F* and its self-association monitored by circular dichroism spectroscopy (CD). (**a**) Helical net representation of Zwit-1F* and the structure of β^3-4-iodohomophenylalanine (F*). (**b**) Wavelength-dependent CD spectra of Zwit-1F* at the concentration shown at 25 °C in PBC buffer, pH 7.1.

Sedimentation Equilibrium. Samples analyzed using analytical ultracentrifugation were prepared by dissolving HPLC-purified and lyophilized ®-peptides in PBC buffer at the desired concentrations (100 μM, and 250 μM). The samples were then centrifuged to equilibrium at 25 °C at three different speeds (42,000, 50,000, and 60,000 rpm) in an AN 60-Ti 4-hole rotor equipped with six-channel, carbon-epoxy composite centerpieces supplied by Beckman. Absorbance was monitored at 230 nm. Data were collected with a 0.001 cm step size, and successive scans were initiated at 3-hour intervals. Samples were judged to have reached equilibrium when no significant change in radial concentration was observed in 3 successive scans using the program Match within the Heteroanalysis software suite (available from the National Analytical Ultracentrifugation Facility website, http://vm.uconn.edu/~wwwbiotc/uaf.html). The partial specific volume of each β-peptide was calculated from the amino acid composition according to Durchschlag and Zipper.[2] The data were fit to a monomer-Nmer equilibrium model using Heteroanalysis software. The results of a representative global fitting are: Fixed parameters: monomer MW = 1636, V-bar = 0.793, d = 1.000, ε_{230} = 950 M^{-1}, and n = 6 or 8. A stoichiometry of an even number of subunits was assumed given the observed 1:1 stoichiometry of Acid-1F/Base-1F. Data fit with these parameters and the resulting residuals are shown in Figure SI-2.

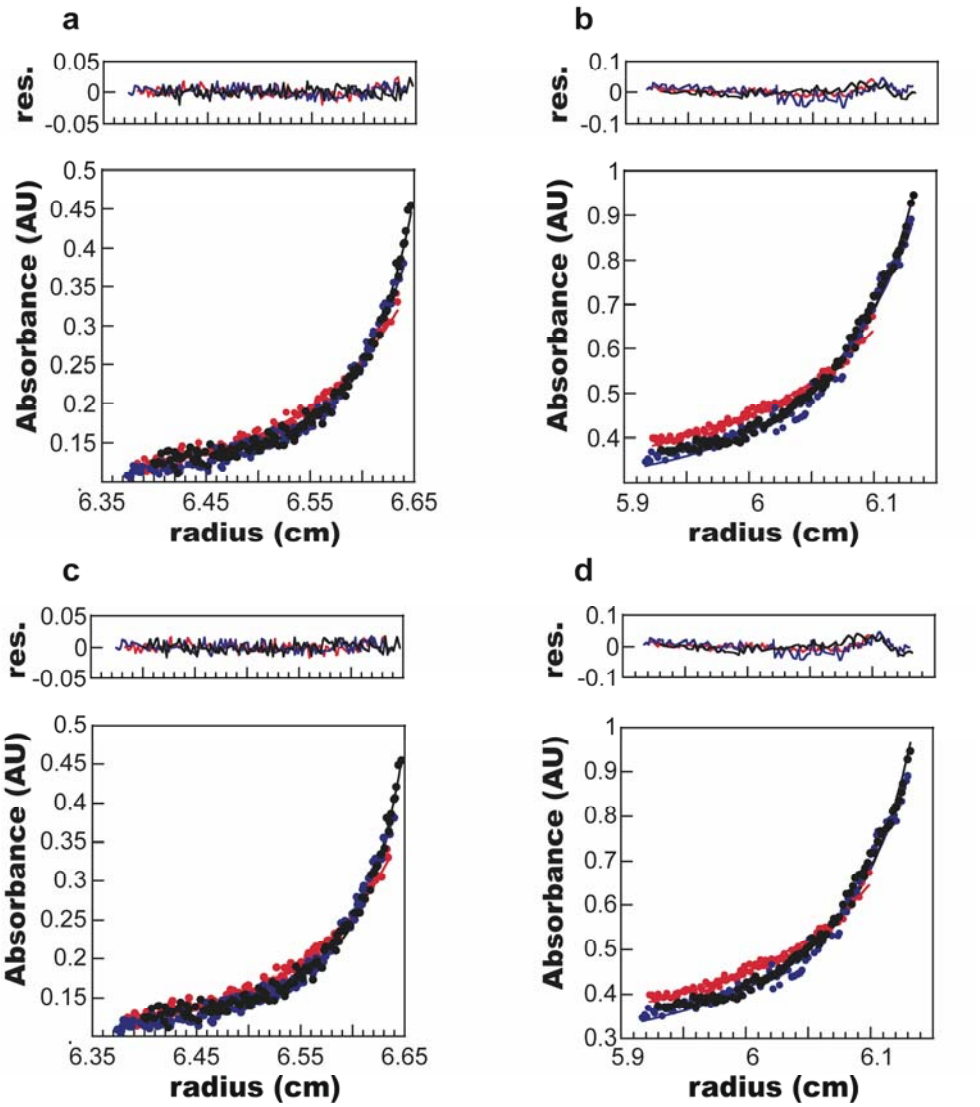

Figure SI-2. Sedimentation equilibrium analysis of Zwit-1F oligomerization. Samples containing (**a** and **c**) 100 µM Zwit-1F monomer or (**b** and **d**) 250 µM Zwit-1F monomer were prepared in PBC buffer (pH 7.1) and centrifuged to equilibrium at speeds of 42 (red), 50 (blue), or 60 krpm (black) and 25 ^{o}C. Experimental data are shown as points; lines indicate fits to (**a** and **b**) monomer-hexamer or (**c** and **d**) monomer-octamer models.

Structure Determination. Zwit-1F and Zwit-1F* (1mM) crystallized by hanging drop vapour diffusion over 1.5M and 0.4M Na,K Tartrate, respectively. Crystals were soaked in a cryoprotectant of mother liquor containing 15% glycerol and X-ray diffraction data collected at

100K at the Cornell High Energy Synchrotron Source beamline A1 (0.946 Å) and the Yale Center for Structural Biology (1.54 Å) for Zwit-1F and Zwit-1F*, respectively (See Table SI-1). Data processing and reduction were performed using HKL2000.[3] Heavy atom location, single-wavelength anomalous phasing and density modification were conducted using CNS.[4] Initial phases were calculated to 2.6 Å for Zwit-1F* using single-wavelength anomalous diffraction techniques, giving a phasing power of 2.7 and figure of merit of 0.35, which improved to 0.83 following density modification. Electron density maps showed four unambiguous 3_{14}-helices, which were built and refined in XFIT.[5] Following several rounds of refinement, the Zwit-1F* model was used as a starting model for the refinement of Zwit-1F. After initial refinement of Zwit-1F in CNS was complete, the model was further refined with SHELX-97,[6] retaining an identical R_{free} set representing 10% of the data. Data were of sufficient resolution to apply anisotropic displacement parameters, as indicated by a 4% decrease in R_{work} and a 3.2% decrease in R_{free}. Due to the relatively high resolution of the structure, dual conformers for several residues could be resolved. The side chains of ®^{3}-Orn3 in three monomers, ®^{3}-Orn10 in one monomer and the "mainchain" (defined by conformation) of ®^{3}-Asp12 in one monomer are modelled in two conformations. Figures were prepared using PyMol.[7]

Table SI-1: Data collection, phasing and refinement statistics (**SAS**)

	Zwit-1F	Zwit-1F*
Data collection		
Space group	$P4_12_12$	$P4_12_12$
Cell dimensions		
a, *b*, *c* (Å)	44.5, 44.5, 73.4	43.9, 43.9, 73.7
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	50-1.45 (1.5-1.45)[a]	100-2.45 (2.54-2.45)
R_{sym} or R_{merge}	4.4 (46.0)	10.7 (37.2)
I/σI	43.5 (4.0)	29.5 (6.0)
Completeness (%)	98.8 (92.7)	99.9 (99.2)
Redundancy	10.3 (8.9)	13.8 (9.3)
Refinement		
Resolution (Å)	50-1.45	
No. reflections	13,619	
$R_{work/}$ R_{free} (%)	14.2 / 19.2	
No. atoms		
Protein	487	
Water	94	
B-factors		
Protein	20.70	
Water	43.48	
r.m.s deviations		
Bond lengths (Å)	0.011	
Bond angles (°)	2.0	

[a]Highest resolution shell is shown in parenthesis

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