

Supplementary Information for:

Remodeling a β -peptide bundle

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Methods

General Methods	2
β -Peptide Bundle Design using Rosetta	2
β -Peptide Synthesis and Purification	4
Circular Dichroism Spectroscopy (CD)	6
Sedimentation Equilibrium Analytical Ultracentrifugation (SE-AU)	8
1-Anilino-8-naphthalenesulfonate (ANS) Binding	11
NMR analysis of hydrogen/deuterium exchange	12

Tables

Table S1. Redesigned sequences from Rosetta fixed-backbone designs.	3
Table S2. Rosetta terms for non-polar atom-atom packing in the core sequence redesign of the Acid-1Y octameric β -peptide bundle	4
Table S3. Fits of isothermal circular dichroism data to monomer-octamer (1-8), monomer-dimer-octamer (1-2-8) and monomer-tetramer-octamer (1-4-8) models	7
Table S4. Fits of sedimentation equilibrium analytical ultracentrifugation data for Acid-1Y ^{FF} and Acid-1Y ^{FF*} to monomer- <i>n</i> -mer models	8

Figures

Fig. S1. Self-association of designed β -peptide bundles showing data fits	6
Fig. S2. Sedimentation equilibrium analytical ultracentrifugation analysis of Acid-1Y ^{FF} bundle stoichiometry and association constant	9
Fig. S3. Sedimentation equilibrium analytical ultracentrifugation analysis of Acid-1Y ^{FF*} bundle stoichiometry and association constant	10
Fig. S4. Fluorescence emission of 1-anilino-8-naphthalenesulfonate (ANS) in the presence of Acid-1Y ^{FF} and Acid1Y ^{FF*}	12

References	14
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General Methods. Fmoc-protected amino acids, *O*-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU), and Wang resin were purchased from Novabiochem (San Diego, CA). (7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP) was purchased from Oakwood Products, Inc. (West Columbia, SC). 1-Hydroxy-7-azabenzotriazole (HOAt) was purchased from Chempep, Inc. (Miami, FL). Fmoc-L-pentafluorophenylalanine was purchased from Anaspec (Fremont, CA). Dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), *N*-methylmorpholine (NMM), trifluoroacetic acid (TFA) and piperidine (Pip) were purchased from American Bioanalytical (Natick, 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 µm, 4.6 mm X 150 mm), semi-preparative (C8, 300 Å, 10 µm, 10 mm X 250 mm), or preparative (C8, 300 Å, 5 µm, 25 mm X 250 mm) columns, using water/acetonitrile gradients containing 0.1% TFA. Circular dichroism (CD) spectra were acquired with a Jasco J-810 Spectropolarimeter (Jasco, Tokyo, Japan) equipped with a Peltier temperature control module. Analytical ultracentrifugation (AU) was performed using a Beckman XL-I instrument. ¹H NMR experiments were performed on Bruker 500 MHz and Varian Inova 800 MHz instruments. Sedimentation Equilibrium (SE)-AU, CD, fluorescence, and NMR experiments were performed in phosphate buffer (10 mM NaH₂PO₄, 200 mM NaCl, pH adjusted to 7.1 with NaOH).

β-Peptide Bundle Design using Rosetta. The Rosetta macromolecule modeling software¹⁻⁴ was used to identify variants of the β³-peptide Acid-1Y bundles that would potentially assemble into well-folded, octameric β-peptide bundles. The only Rosetta extension required for modeling β³-peptides was the specification of initial bond lengths and angles, which were derived from the Acid-1Y crystallographic model (for the backbone) and from natural proteins (for the side chains). All eight non-equivalent homoleucine core residues in the crystallographic model (positions 2, 5, 8 and 11 in two non-equivalent monomers) were remodeled. These side chains were allowed to sample β-peptide

variants of the twenty proteinaceous amino acids with Rosetta's fixed backbone design algorithm *fixbb*.⁵ Symmetry was enforced across all 32 side chains using Rosetta's *FixbbLinkingRotamerSimAnnealer*.⁶ All calculations were carried out in the recently developed Rosetta 3 framework.⁷ Example command-lines are given below; and all calculations are being made available in the β -peptide_modeling application contained in Rosetta 3.4.2 (freely available to academics at <http://www.rosettacommons.org>).

Table S1. Redesigned sequences from Rosetta fixed-backbone designs.

Score terms ^a	Symmetry Enforced?	Sequence
Old score	Yes	(LLLL LLLL) × 4
Current	Yes	(LLLL LLLL) × 4
MM	Yes	(LLLL LLLL) × 4
Old score + softrep	Yes	(MILI FLLL) × 4
Current + softrep	Yes	(MIAI FLFL) × 4
MM + softrep	Yes	(MILI FLLL) × 4
Old score	No	LIFI LLLL LLLL LLLL RLLI FITI LILI LMML
Current	No	LLLI LLLL LLLI LLLL RILI FLLL LILI LLLL
MM	No	LLLI LLLL LLLL LLLL LLLI LLML LILL LLAL
Old score + softrep	No	MILI FLVI LPFL LFLL MIFI FLLL MILL FLVI
Current + softrep	No	MILI FMLL MPAI FFFI MPLI FMML MILI FLVI
MM + softrep	No	MILI FLLL LLAI LLFL MILI FMML MILL FLVM

^a 'Old score' refers to Rosetta all-atom energy function available in pre-release versions of Rosetta 3.0; 'Current' refers to Rosetta 3.4 all-atom energy function. 'softrep' refers to smoothed potential with lower penalties for clashes, used in early stages of modeling or design⁸. 'MM' refers to calculations with additional terms *mm_twist*, *mm_lj_intra_rep*, and *mm_lj_intra_atr* introduced in reference.⁹

The Rosetta all-atom energy function for protein design was used for the optimization; it accounts for van der Waals interactions between side chains (attractive and repulsive forces), the hydrophobic effect and polar burial penalties (solvation) and hydrogen bonding (here, primarily fixed backbone-backbone interactions). To avoid incorrect biases from natural protein crystals, the backbone-independent version of Dunbrack's rotamer library⁷ was used for the side chain search and a knowledge-based term favoring frequently observed rotamers in natural proteins was omitted. These approximations will necessarily lead to inaccuracies in energy estimates. Nevertheless, by modeling the non-polar interactions of the molecule's side chains at an all-atom level, the calculations were still useful in suggesting novel variants of well-packed cores. For example, as a basic consistency check, the original sequence (native core: L₂, L₅, L₈, L₁₁ in both non-equivalent monomers) was returned as the optimal core sequence in a symmetric complete re-design of the Acid-1Y core with the standard Rosetta protein energy function.¹⁰

The Rosetta energy function has been improved⁷ since our original calculations (which made use of an early version of Rosetta 3.0, internal revision number 17858). These improvements were also systematically explored in this symmetric sequence re-design test. First, the van der Waals potential has been smoothed to have continuous derivatives. Second, additional terms *mm_twist*, *mm_lj_intra_rep*, and *mm_lj_intra_atr* (called herein the MM potential) have been introduced and calibrated to better model non-canonical amino acids.⁹ Redesigning the eight Acid-1Y core positions returned the all-homoleucine core. In each of these calculations, symmetry constraints required that the substitution of side-chain identity or rotameric conformation at one of the eight core positions be copied to three other positions related by symmetry. Lifting this symmetry constraint led to sequences that were not all-leucine cores (see Table S1). These sequences have not yet been explored due to the complications of preparing and mixing multiple different β -peptide components in exact stoichiometries.

For calculations exploring core substitutions with backbone flexibility, side chains were repacked with the *fixbb* algorithm and the *softrep* energy function,⁸ followed by continuous minimization of all backbone and side chain torsions and rigid-body degrees of freedom positioning the eight monomers. For the minimization, the standard Rosetta energy function rather than the *softrep* variant was employed to better discriminate precise hydrophobic packing; weak harmonic constraint potentials with spring constants of $K = 0.08 \text{ kT/rad}^2$ were applied to all backbone torsional degrees of freedom (four per β -peptide residue) to prevent large conformational excursions (given as *dihedral* in Table S2). The total Rosetta energy of the homophenylalanine-containing variant Acid-1Y^{FF} was not as low as the starting conformations, mainly due to increases in *dihedral*; however the lower energies for summed Lennard-Jones terms *fa_atr* and *fa_rep* and solvation *fa_sol* suggested that the packing of non-polar residues was better than the starting homoleucine-containing bundle Acid-1Y. As above, these calculations have been repeated in the more recent Rosetta 4.3 framework, including use of terms *mm_twist*, *mm_lj_intra_rep*, and *mm_lj_intra_atr* to replace the *dihedral* constraints. In these repeated calculations, the summed non-polar packing terms were comparable in Acid-1Y and Acid-1Y^{FF} (within 2 Rosetta units; approximately 1 k_BT).

Table S2. Rosetta terms for non-polar atom-atom packing in the core sequence redesign of the Acid-1Y octameric β -peptide bundle. The van der Waals forces are accounted for in the attractive (atr) and repulsive (rep) terms. The hydrophobic effect is represented by the solvation term (sol). Energies are given in Rosetta units, which approximately corresponds to 1 k_BT.¹¹

Variant	Core Sequence ^a	<i>fa_atr</i>	<i>fa_rep</i>	<i>fa_sol</i>	nonpolar sum ^b	<i>hbond</i>	<i>dihedral</i> / MM ^c	sum
Acid-1Y (old score)	LLLL LLLL	-409	29	181	-200	-89	0	-298
Acid-1Y ^{FF} (old score)	LFFL LFFL	-436	25	187	-224	-102	134	-192
Acid-1Y (current)	LLLL LLLL	-440	53	185	-202	-90	3	-288
Acid-1Y ^{FF} (current)	LFFL LFFL	-442	58	186	-198	-89	109	-178

Acid-1Y (MM)	LLLL LLLL	-499	65	197	-237	-86	106	-218
Acid-1Y ^{FF} (MM)	LFFL LFFL	-528	87	206	-235	-84	193	-126

^a The sequences are positions 2,5,8, and 11 of the non-equivalent “outer” and “inner” monomers, respectively.

^b Sum of *fa_atr*, *fa_rep*, and *fa_sol*.

^c For the first four entries, the dihedral constraint score is shown. For the last two entries, we show the sum of the molecular mechanics scores (*mm_twist*, *mm_lj_intra_rep*, and *mm_lj_intra_atr*) since dihedral constraints are not used in those calculations.

After our work, a study from Shandler and colleagues¹² proposed a molecular-mechanics-based potential for β -peptide side chains. Interestingly, our re-designed core β^3 -phenylalanine rotamer conformations turn out to be similar to conformations proposed in that study to be energetic minima in the 3_{14} helix context. These rotamers are also similar to those observed in the previous Zwit-1F crystal structure, but at non-core positions. The four (χ_1 , χ_2) values in our new designed core phenylalanine side chains are (-53° , 112°), (-57° , 138°), (-59° , 139°), and (-76° , 176°), with other positions related by symmetry. The first 3 conformers fall within the dominant energy well in the molecular mechanics-based prediction (-80° to -40° , 90° to 135°), and the fourth conformer is close by.

Rosetta command lines. The Rosetta command lines used in this work are listed below for reference.

1. β^3 -peptide peptide redesign

```
beta_peptide_modeling.<exe> -database <database> -force_field  
beta_peptide -native acdy_LLLL_LLLL.pdb -algorithm redesign -ex1 -ex2 -  
packing::pack_missing_sidechains false -packing::extrachi_cutoff 0 -  
repack_res 2 5 8 11 14 17 20 23 -n_repeat 4 -repeat_size 24
```

2. Repacking and minimization for mutated sequence

Repack:

```
beta_peptide_modeling.<exe> -database <database> -force_field  
beta_peptide_soft_rep_design -native acdy_LFFL_LFFL.pdb -algorithm
```

```
repack -ex1 -ex2 -packing::pack_missing_sidechains false -  
packing::extrachi_cutoff 0 -repack_res 2 5 8 11 14 17 20 23 -n_repeat 4  
-repeat_size 24
```

Minimize:

```
beta_peptide_modeling.<exe> -database <database> -force_field  
beta_peptide -native acdy_LFFL_LFFL_repack.pdb -algorithm minimize -ex1  
-ex2 -packing::pack_missing_sidechains false -packing::extrachi_cutoff 0
```

The two commands above are run sequentially to obtain the final model for the redesigned sequence.

All the commands listed above used the standard Rosetta protein scoring function with symmetry enforced. Extra flags/modifications that are used for old scoring (no smoothing), non-symmetric redesign, and MM potential are given below.

`-score::no_smooth_etables true` Use the old Rosetta attractive/repulsive score without smoothing.

`-no_symmetry true` Disable the symmetry-based redesigning.

`-force_field beta_peptide_mm / -force_field beta_peptide_soft_rep_mm` Use the molecular mechanics-based Rosetta scoring files. Replace the corresponding “`-force_field`” flags with the MM ones in the above command lines. In addition, the extra flags “`-apply_dihedral_cst false`” should be used to turn off dihedral constraint when the MM potential is used.

β -Peptide synthesis and purification. The Arndt-Eistert homologation method¹³ was used to synthesize *N*-Fmoc-(*S*)- β^3 -amino acids from the corresponding α -amino acid precursor as described previously.¹⁴ β -peptides were synthesized in a CEM MARS microwave reactor on pre-loaded Fmoc- β^3 -Asp(*t*Bu)-OH Wang resin¹⁵ (25 μ mol scale) in a glass reaction vessel (Ace Glass, Vineland, NJ) that was pretreated with SigmaCote (Sigma Aldrich). The resin (46.6 mg of 0.537 mmol/g) was allowed to swell for 1 h in 8 mL DMF on a rotary shaker. After swelling, the resin was washed 6 times

with 4 mL DMF, including 3 times of swirling the reaction vessel (RV) to break up any resin clumps. Subsequently, the Fmoc protecting group was removed using 4 mL of 20% piperidine/DMF and microwave irradiation (microwave deprotection program: 50% power at 400 W maximum; ramp to 70 °C for 2 min.; hold at 70 °C for 4 min.; cool for 5 min) with magnetic stirring. Following deprotection, the resin was washed using the same wash procedure as above (6 x 4 mL DMF, including 3 x with vigorous shaking of the RV). Coupling solution was prepared in a separate vial. This cocktail consisted of the appropriate Fmoc- β^3 -amino acid (75 μ mol), PyAOP (75 μ mol), and HOAT (75 μ mol) resuspended in 2 mL DMF. To this was added 35 μ L diisopropylethylamine (DIPEA), and the 2 mL solution was added to the resin in the RV. The reaction mixture was then exposed to microwave irradiation with magnetic stirring (coupling program: 50% power at 400 W maximum; ramp to 60 °C for 2 min.; hold at 60 °C for 6 min.; cool for 5 min). After coupling, the resin in the RV was extensively washed with DMF. To prepare for another coupling, the resin was deprotected as previously described. The cycle of deprotection-wash-coupling-wash was performed until the peptide was synthesized in full. The solution used for deprotection was always prepared immediately before use.

At the end of the synthesis, the last Fmoc protecting group was removed using the deprotection protocol. The resin was washed alternately with DMF and methanol for a total of 16 washes, followed by an additional 8 consecutive methanol washes, and dried at least 30 min under N₂. The dry resin was treated with three 4 mL portions of cleavage solution (TFA with 1% v/v each of H₂O, phenol, and triisopropyl silane) for 1 hr, 1 hr, and 30 min. The cleavage solution was collected in a 50 mL round bottom flask and was rotovaped to remove the TFA. The remaining 1 mL of solution was resuspended in 50:50 CH₃CN/H₂O for HPLC purification.

The success of each synthesis was assessed by both HPLC and MALDI-TOF analysis of the crude reaction mixture. β -peptides were then purified to homogeneity by reverse-phase HPLC. The identities and purities of the purified β -peptides were assessed by analytical HPLC and mass spectrometry. MALDI mass spectra were obtained using peptide samples in α -cyano-4-hydroxycinnamic acid (CHCA) matrix. The masses found were: Acid-1Y^{FF} (m/z observed,

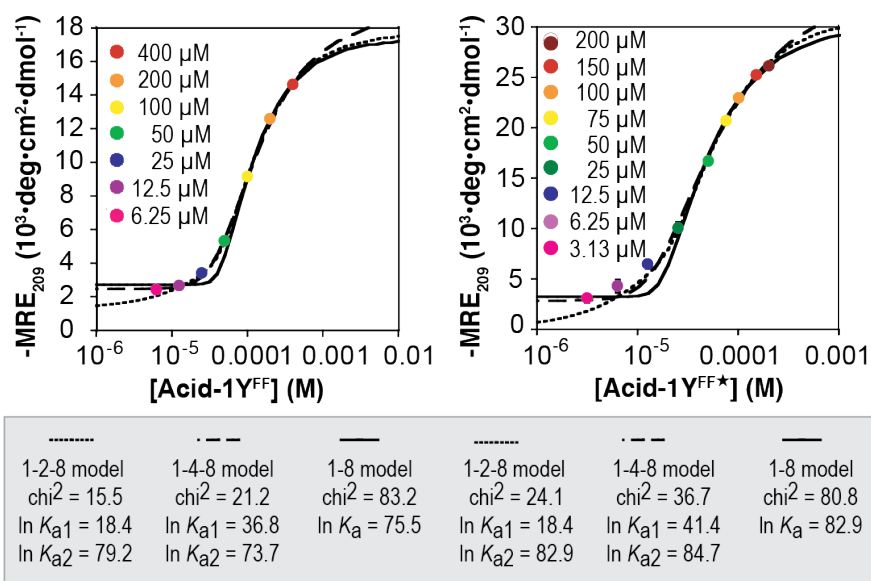


Fig. S1. Self-association of the Acid-1Y^{FF} and Acid-1Y^{FF*} bundles showing data fits. Circular dichroism spectra of Acid-1Y^{FF} and Acid-1Y^{FF*} as a function of concentration. Plots of MRE₂₀₉ as a function of [β-peptide] were fit to monomer-dimer-octamer (1-2-8), monomer-tetramer-octamer (1-4-8) or monomer-octamer (1-8) equilibria; error analysis and calculated values are shown.

[ot-MW-Abs.htm](http://encorbio.com/protocols/Prot-MW-Abs.htm))

Circular Dichroism (CD). The wavelength dependent CD spectra of Acid-1Y^{FF} and Acid-1Y^{FF*} at concentrations between 3.13 and 400 μM were acquired at 25 °C using a Jasco J-810 Spectropolarimeter (Jasco, Tokyo, Japan). Relevant machine settings include: continuous scan mode, 1.0 nm data pitch, 50 nm/min scanning speed, 4 sec response, 0.5 nm band width and 3 accumulations. The concentration dependence of the molar residue ellipticity (MRE) minimum (at 209 nm for Acid-1Y^{FF} and 212 nm for Acid-1Y^{FF*}) was determined by fitting of the total peptide monomer concentration ([Peptide]_{Total}) as a function of the experimentally determined MRE using the following equation in Kaleidagraph, Version 3.6 (Synergy Software; Reading, PA).¹⁶ MRE_{Mon} represents the MRE of the β-peptide monomer; MRE_{nmer} represents the MRE of the β-peptide oligomer, where n = the oligomerization state.

calculated): 1748, 1748; and
 Acid-1Y^{FF*} (m/z observed,
 calculated): 1840, 1839.
 Following purification, β-peptides
 were lyophilized and
 reconstituted in buffer (described
 above) for characterization.
 Concentrations were determined
 by UV absorbance at 280 nm,
 where 1490 M⁻¹ cm⁻¹ was used
 as the molar extinction
 coefficient for tyrosine.

<http://encorbio.com/protocols/Pr>

$$[Peptide]_{Total} = \left\{ \frac{(MRE_{Exp} - MRE_{Mon})(1/K_a)}{n(MRE_{Nmer} - MRE_{Mon}) \left[1 - \left(\frac{MRE_{Exp} - MRE_{Mon}}{MRE_{Nmer} - MRE_{Mon}} \right) \right]^n} \right\}^{1/(n-1)}$$

Using this equation and the fixed parameters $MRE_{Mon} = 2991 \pm 300 \text{ deg}\cdot\text{cm}^2\cdot\text{dmol}^{-1}$ and $MRE_{Nmer} = 17,052 \pm 150 \text{ deg}\cdot\text{cm}^2\cdot\text{dmol}^{-1}$, we calculate a $\ln K_a = 75.5 \pm 0.5$ for the Acid-1Y^{FF} bundle. For the Acid-1Y^{FF*} bundle, the $\ln K_a$ calculated was 73.0 ± 0.5 ($MRE_{Mon} = 3000 \text{ deg}\cdot\text{cm}^2\cdot\text{dmol}^{-1}$; $MRE_{Nmer} = 30,014 \pm 329 \text{ deg}\cdot\text{cm}^2\cdot\text{dmol}^{-1}$).

Fits of peptide circular dichroism data were also carried out to a more general equilibrium involving multiple species e.g., a monomer, tetramer, and an octamer. If the fraction of peptide within each n -mer species is f_n , these values satisfy the equations:

$$f_n = n f_1 (p f_1 / \kappa_n)^{n-1} \quad (1)$$

where p is the peptide concentration and the constants κ_n are related to standard association constants by:

$$\ln K_a = -n \ln(\kappa_n / 1 \text{ M}) \quad (2)$$

The fractions in each species sum to 1:

$$f_1 = 1 - \sum_{n>1} f_n \quad (3)$$

For given κ_n , the equations were solved numerically in MATLAB by iteratively computing updates to the f_n (for $n > 1$) with eq. 1 and updating f_1 with eq. 3. To optimize over κ_n , grid searches were carried out over values from 10^{-6} M to 10 M (in $10^{0.25}$ M increments). Errors were estimated by bootstrapping the data, i.e., by repeating the entire fitting analysis with replicates generated by random selection of available data with replacement.¹⁷ Association constants calculated were as follows: Acid-1Y^{FF} 1mer-2mer-8mer model, $\ln K_1 = 18.4 \pm 4.1$ and $\ln K_2 = 79.2 \pm 9.8$; Acid-1Y^{FF} 1mer-4mer-8mer model, $\ln K_1 = 36.8 \pm 3.2$ and $\ln K_2 = 73.7 \pm 11.2$; Acid-1Y^{FF*} 1mer-2mer-8mer model, $\ln K_1 = 18.4 \pm 4.0$ and $\ln K_2 = 82.9 \pm 2.3$; Acid-1Y^{FF*} 1mer-4mer-8mer model, $\ln K_1 = 41.4 \pm 4.6$ and $\ln K_2 = 84.7 \pm 3.0$.

Table S3. Fits of isothermal circular dichroism data to monomer-octamer (1-8), monomer-dimer-octamer (1-2-8) and monomer-tetramer-octamer (1-4-8) models. P values below 1×10^{-3} for the simplest monomer-octamer (1-8) model strongly disfavor those fits, while P values above 0.1 for monomer-dimer-octamer (1-2-8) and monomer-tetramer-octamer (1-4-8) models show that either model is consistent with the data. # = number of parameters

Construct	Fit	$\ln K_1$	$\ln K_2$	c^2	N	#	P
Acid-1Y ^{FF}	1-8	–	75.5	83.2	21	3	2.3×10^{-10}
Acid-1Y ^{FF}	1-2-8	18.4	79.2	15.5	21	5	0.48
Acid-1Y ^{FF}	1-4-8	36.8	73.7	21.2	21	5	0.17
Acid-1Y ^{FF*}	1-8	–	82.9	80.8	36	3	6.9×10^{-6}
Acid-1Y ^{FF*}	1-2-8	18.4	82.9	24.1	36	5	0.81

Table S4. Fits of sedimentation equilibrium analytical ultracentrifugation data for Acid-iY^{FF} and Acid-1Y^{FF*} to monomer- n -mer models

Acid-1Y ^{FF}			Acid-1Y ^{FF*}		
n	$\ln K$	RMSD	n	$\ln K$	RMSD
2	15.496	0.04950	2	15.872	0.04926
3	25.883	0.03906	3	27.349	0.03880
4	51.271	0.02872	4	40.714	0.02894
5	110.564	0.01973	5	53.217	0.02039
6	54.738	0.01294	6	54.896	0.01320

7	58.615	0.00821	7	62.379	0.00766
8	64.763	0.00778	8	70.662	0.000702
9	69.020	0.01026	9	79.094	0.01122
10	74.962	0.01246	10	84.711	0.01650
Float n	62.091	0.00731	Float n	67.223	0.00646
	n = 7.57			n = 7.59	

Temperature-dependent CD spectra were obtained at 209 nm and 212 nm (for Acid-1Y^{FF} and Acid-1Y^{FF*}, respectively) between 5 and 95 °C, using the Peltier temperature control module provided with the instrument. Relevant settings include: 1 °C data pitch, 5 sec delay time, 1 °C/min temperature slope, 4 sec response time, and 1 nm band width. In the case of both Acid-1Y^{FF} and Acid-1Y^{FF*}, the first derivative of the MRE was calculated at each of four β -peptide (monomer) concentrations. The T_m values reported represent the maximum of the $\delta\text{MRE}_{\text{min}}/\delta T$ plots. Fig. 2b,d in the main text show plots of $\delta\text{MRE}_{\text{min}}/\delta T$ as a function of temperature for Acid-1Y^{FF} and Acid-1Y^{FF*}.

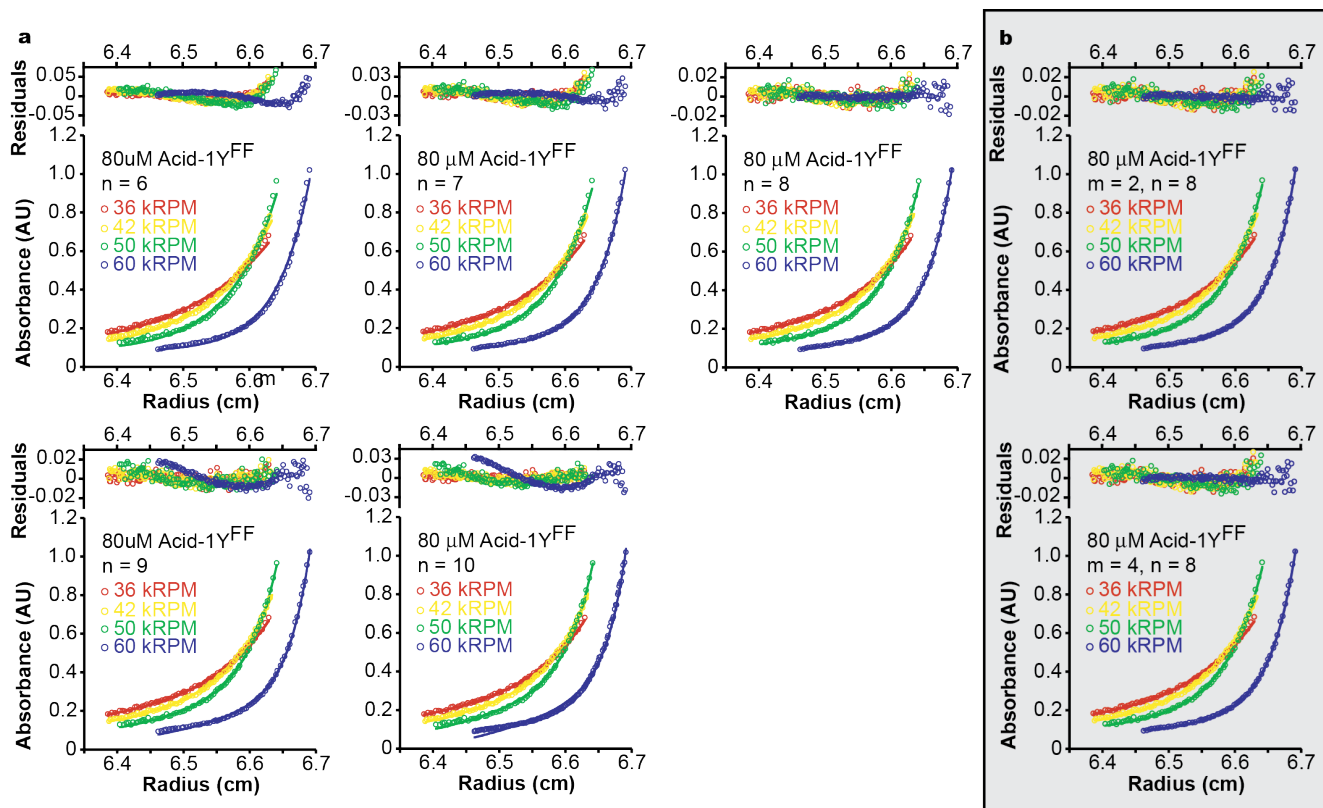


Fig. S2. Sedimentation equilibrium analytical ultracentrifugation analysis of bundle stoichiometry and association constant: Acid-1Y^{FF}. Plots illustrating monomer-*n*-mer equilibrium fits to experimental data at 80 μ M concentrations (monomer) of Acid-1Y^{FF}. **(A)** Representative data fits and residuals of Acid-1Y^{FF} monomer-*n*-mer and **(B)** monomer-*m*-mer-*n*-mer equilibrium models. See also Table S4.

Sedimentation Equilibrium Analytical Ultracentrifugation. HPLC-purified, lyophilized samples of Acid-1Y^{FF} and Acid-1Y^{FF*} were resuspended in phosphate buffer (10 mM NaH₂PO₄, 200 mM NaCl, pH adjusted to 7.1 with NaOH) at concentrations of 20, 80 and 200 μ M, and centrifuged at 25 °C to equilibrium at four speeds (36,000, 42,000, 50,000 and 60,000 rpm). Centrifugation was performed using an AN 60-Ti 4-hole rotor equipped with six-channel, carbon-epoxy composite centerpieces (Beckman). Absorbance was monitored at both 230 and 280 nm. Data were collected with a step size of 0.001 cm with scans occurring at 1 h intervals. Samples were determined to have reached equilibrium when no significant changes in radial concentration were observed in three successive scans, as determined using the Match sub-routine within the Heteroanalysis software suite (available

from the National Analytical Ultracentrifugation Facility website,

<http://vm.uconn.edu/~wwwbiotc/uaf.html>).

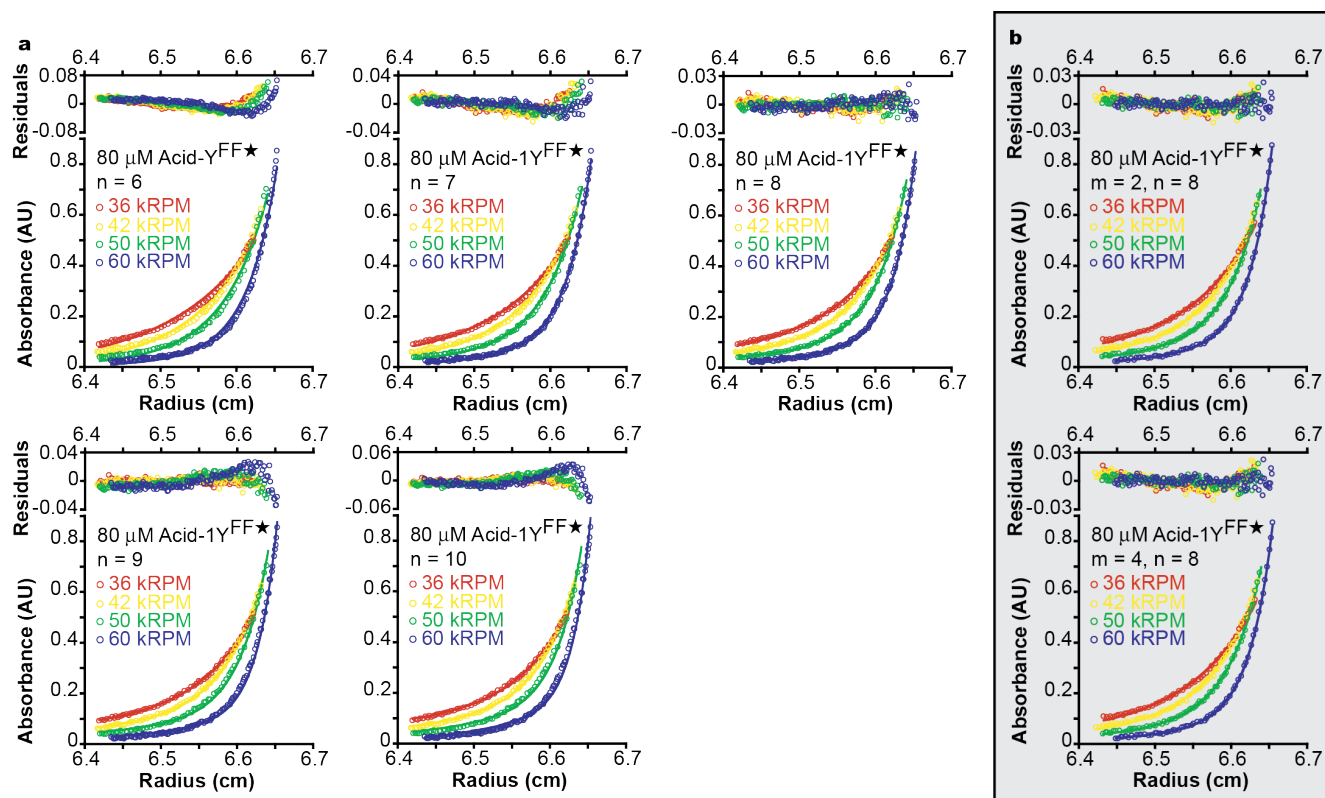


Fig. S3. Sedimentation equilibrium analytical ultracentrifugation analysis of bundle stoichiometry and association constant: Acid-1Y^{FF*}. Plots illustrating monomer-*n*-mer equilibrium fits to experimental data at 80 μ M concentrations (monomer) of Acid-1Y^{FF*}. **(A)** Representative data fits and residuals of Acid-1Y^{FF*} monomer-*n*-mer and **(B)** monomer-*m*-mer-*n*-mer equilibrium models. See also Table S4.

The partial specific volume of each β -peptide was calculated on the basis of functional group composition according to Durchschlag and Zipper.¹⁸ The data were initially fit to a monomer-*n*-mer equilibrium model using Heteroanalysis software, assuming fast monomer-*n*-mer equilibration compared to diffusion within the ultracentrifuge profile. Fixed parameters for Acid-1Y^{FF}: monomer MW = 1748 Da; \bar{v} = 0.83070 cm³/g; d = 1.00674 g/mL; ϵ_{280} = 2906 (accounting for the 1.2 cm path length) M⁻¹•cm⁻¹; and n = 8. Fitted parameters for Acid-1Y^{FF}: $\ln K_a$ = 64.8 \pm 0.1; root mean square deviation (RMSD) = 0.00778; and baseline deviation < 0.02. Fixed parameters for Acid-1Y^{FF*}: monomer MW = 1839 Da; \bar{v} = 0.77591 cm³/g; d = 1.00674 g/mL; ϵ_{280} = 2906 (accounting for the

1.2 cm path length) $M^{-1}\cdot cm^{-1}$; and $n = 8$. Fitted parameters for Acid-1Y^{FF*}: $\ln K_a = 70.7 \pm 0.1$; RMSD = 0.00702; and baseline deviation < 0.03. For both Acid-1Y^{FF} and Acid-1Y^{FF*}, fitting to all other values of n between 2 and 10 resulted in larger RMSD values, and greater and more systematic residuals (Fig. S2a and S3a). When n was allowed to float, it fit best to 7.57 for Acid-1Y^{FF} and 7.59 for Acid-1Y^{FF*}.

The data were also fit to a monomer- m -mer- n -mer equilibrium model using Heteroanalysis (Fig. S2b and S3b). Fixed parameters for Acid-1Y^{FF} were the same as above, with the additional parameter m set to either 2 or 4 (Fig. S2b). For $m = 2$: $\ln K_1 = 8.143 \pm 0.4$; $\ln K_2 = 68.0663 \pm 0.7$; RMSD = 0.00735; and baseline deviation < 0.02. For $m = 4$: $\ln K_1 = 27.600 \pm 0.2$; $\ln K_2 = 68.150 \pm 0.3$; RMSD = 0.00714; and baseline deviation < 0.02. Fixed parameters for Acid-1Y^{FF*} were also the same as above, and m was set to either 2 or 4 (Fig. S3b). For $m = 2$: $\ln K_1 = 11.560 \pm 0.4$; $\ln K_2 = 79.707 \pm 1.3$; RMSD = 0.00640; and baseline deviation < 0.02. For $m = 4$: $\ln K_1 = 29.742 \pm 0.2$; $\ln K_2 = 72.644 \pm 0.2$; RMSD = 0.00634; and baseline deviation < 0.02. When m was allowed to float, it fit best to 4.14 for Acid-1Y^{FF} and 4.02 for Acid-1Y^{FF*}. The F-test was used to determine whether the lower RMSD values observed for the monomer-tetramer-octamer fits were significant relative to the monomer-octamer fits, or whether they represented an artifact caused by sampling more parameters. The F-test was conducted using the GraphPad software (available online at <http://www.graphpad.com/quickcalcs/AIC1.cfm>). The following equation was used to calculate the sum-of-squares (SS), where n is the number of data points: $SS = (RMSD^2)(n)$. For both Acid-1Y^{FF} and for Acid-1Y^{FF*}, the probability that the monomer-tetramer-octamer equilibrium was the correct model was greater than 1,000,000:1, corresponding to $P < 10^{-6}$.

1-Anilino-8-naphthalenesulfonate (ANS) Binding. Stock solutions of 800 μM Acid-1Y^{FF}, 1000 μM Acid1Y^{FF*} and 20 μM ANS were prepared in phosphate buffer (10 mM phosphate, 200 mM NaCl, pH 7.1). Binding reactions were performed by mixing 80 μL of the ANS stock solution with an appropriate volume of the Acid-1Y^{FF} or Acid1Y^{FF*} stocks and diluting with phosphate buffer to a final volume of

160 μL to produce 0, 12.5, 25, 50, 100, 150, and 200 μM solutions of Acid-1Y^{FF} and 0, 1.5625, 3.125, 6.25, 12.5, 25, 50, 100, 150, and 200 μM solutions of Acid1Y^{FF*}. The fluorescence intensity of each solution (counts/s) was measured at 25 °C using a Photon Technology International (Lawrenceville, NJ) Quantamaster C-60 spectrofluorimeter and a 1 cm path length Hellma (Mullheim, Germany) cuvette. Each sample was excited at 350 nm (4 nm slit width) and the emission measured at 1 nm intervals between 400 and 600 nm (Fig. S4).

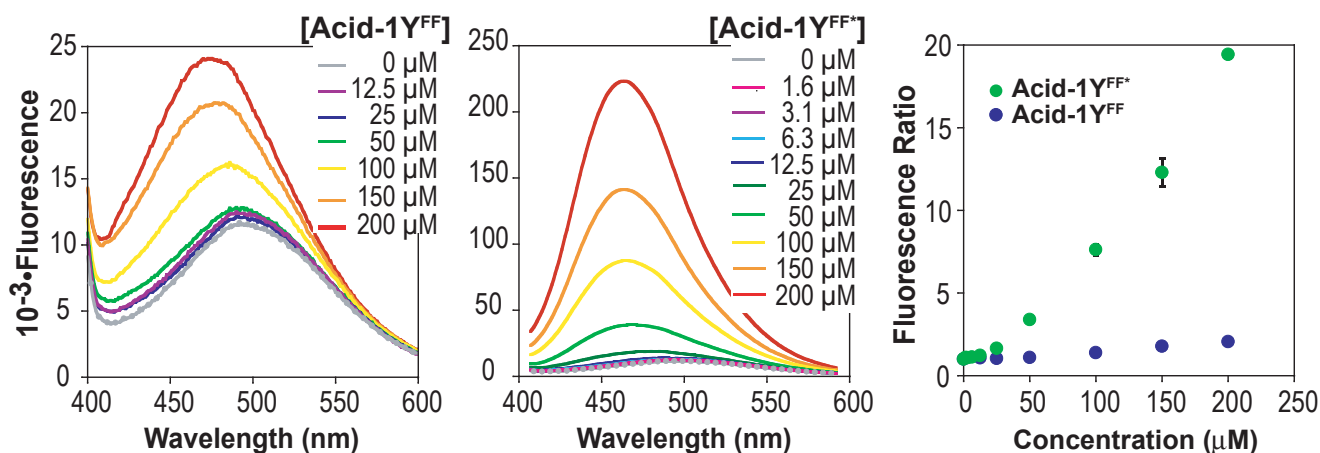


Fig. S4. Fluorescence emission of 1-anilino-8-naphthalenesulfonate (ANS) in the presence of Acid-1Y^{FF} and Acid1Y^{FF*}. Fluorescence is in counts $\cdot\text{s}^{-1}$. The fluorescence ratio is defined as the maximal ANS fluorescence at each β -peptide concentration divided by the maximal ANS fluorescence in the absence of added β -peptide. The final [ANS] = 20 μM .

NMR analysis of hydrogen/deuterium exchange. The rate of exchange of amide N-H protons within the Acid-1Y^{FF} and Acid1Y^{FF*} bundles were determined at 25 °C by NMR using the WATERGATE solvent suppression pulse sequence ZZPWG (see www.bruker.com for pulse sequence information) and procedures described previously.^{5, 19} In brief, we first acquired ¹H spectra of Acid-1Y^{FF} and Acid1Y^{FF*} at concentrations of 750 and 549 μM , respectively, in a 9:1 mixture of H₂O and D₂O, and the location of the H₂O resonance was identified.¹⁹ The transmitter offset (⁰¹P) was set to this frequency for ZZPWG acquisitions. In order to acquire deuterium exchange data, the Acid-1Y^{FF} sample was dissolved in phosphate buffer (10 mM NaH₂PO₄, 200 mM NaCl, pH adjusted to 7.1 with NaOH) spiked with 1 mM TMS⁺ and lyophilized. The lyophilized sample was resuspended in D₂O at

a concentration of 750 μM just prior to insertion into the instrument. Any peptide that could not be resolubilized was removed from the solution by centrifugation at 5000 rpm for 1 minute before addition to the NMR tube. Prior to loading the Acid-1Y^{FF} sample, the spectrometer was locked and shimmed on a sample of Zwit-1F in 9:1 H₂O/D₂O in phosphate buffer. Due to the lack of amide protons, the data for Acid-1Y^{FF} was not further processed. For Acid1Y^{FF*}, a standard spectrum was acquired using a 500 μM sample of Acid1Y^{FF*} in the aqueous buffer. This standard was also used to lock and shim the instrument prior to running the H/D NMR acquisition. A separate sample was prepared in D₂O as described above, resulting in a concentration of 549 μM . Spectra were measured every 10 minutes, for 120 total spectra, with 16 scans per spectrum. Mestrelab Mnova (Mestrelab Research, Escondido, CA) was used for data processing, with 30 Hz line broadening and Bernstein polynomial baseline correction. Integrated areas were extracted from the same regions in each spectrum of the time series. We measured the pD of the sample after exchange was complete using a pH meter (Thermo Orion Model 410, Beverly, MA). The sample was found to be at pH 5.77, corresponding to a pD of 6.17.²⁰ Protection factors were calculated as previously described.¹⁹

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