Electronic Supplementary Material (ESI) for Chemical Science.

The rippled β-sheet layer configuration—a novel supramolecular architecture based on predictions by Pauling and Corey

SUPPORTING INFORMATION

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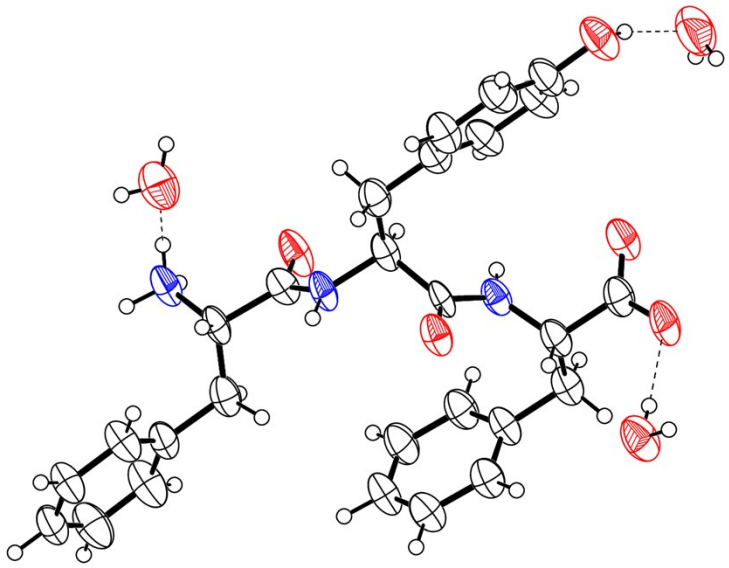

Figure S1. Thermal ellipsoid plot (50% probability level) of FYF $\cdot 3H_2O$. Color code: O red, N blue, C grey, H white spheres of arbitrary radius.

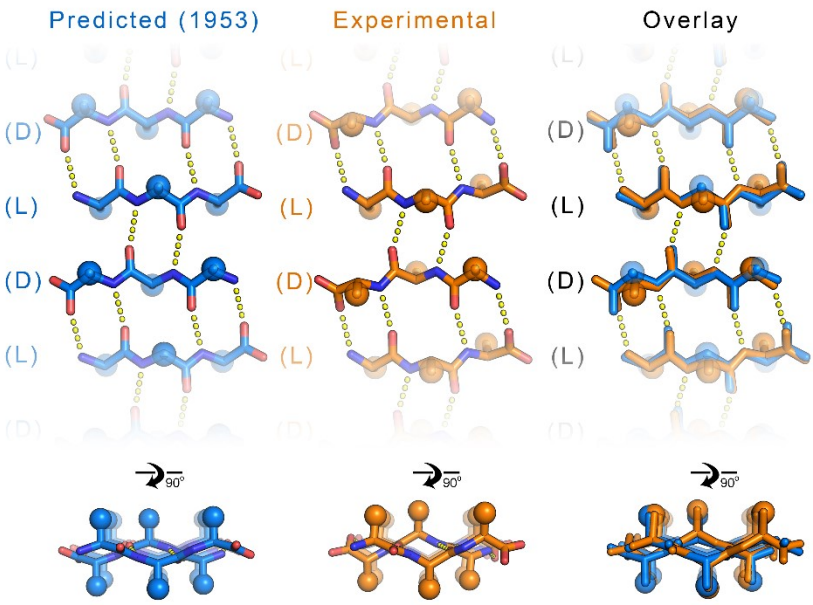

Figure S2. The hypothetical periodic rippled antiparallel β-sheet as predicted by Pauling and Corey (left), our experimentally determined X-ray structure of the periodic antiparallel rippled β-sheet $[FYF:fyf]_n$ layer (middle), as well as their superposition (right). C_β carbons are shown as spheres; full sidechains omitted to enhance clarity. Hydrogen bonds are shown as dotted lines.

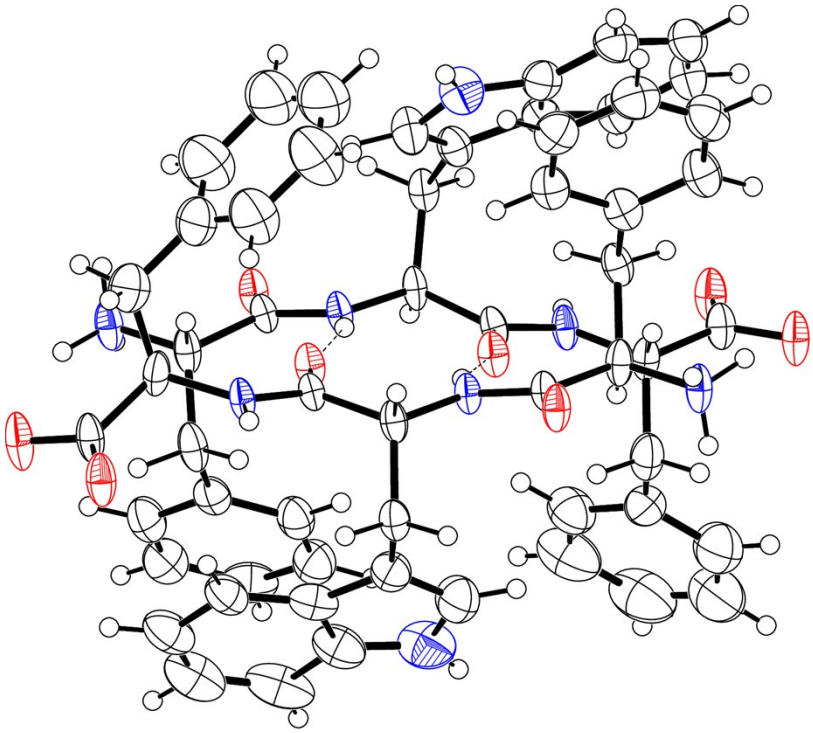

Figure S3. Thermal ellipsoid plot (50% probability level) of FWF:fwf. Color code: O red, N blue, C grey, H white spheres of arbitrary radius.

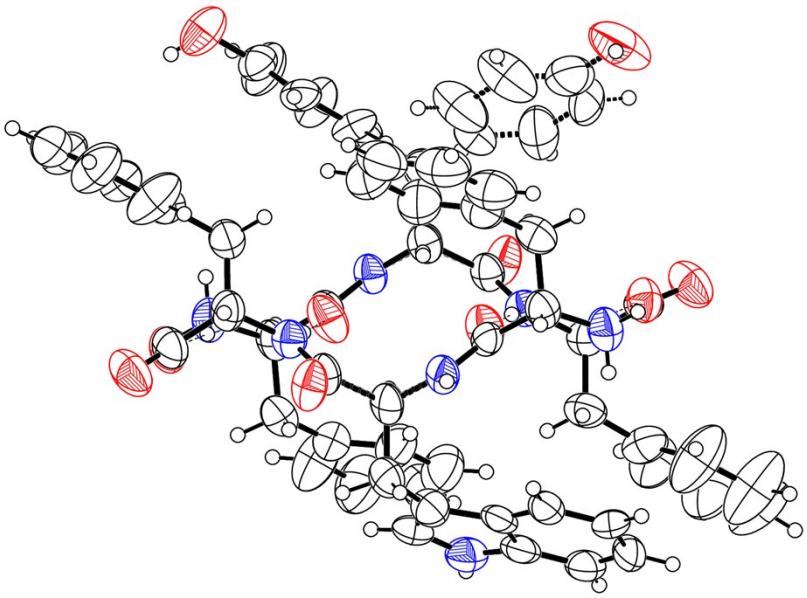

Figure S4. Thermal ellipsoid plot (30% probability level) of FWF:fyf. Color code: O red, N blue, C grey, H white spheres of arbitrary radius. Solvent and disordered side chain omitted for clarity.

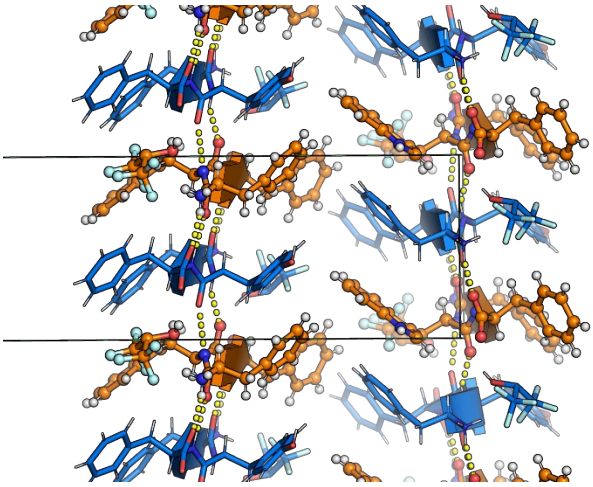

Figure S5. Two [FWF:fyf]n columns are mated *via* a steric zipper interface into a rippled β-sheet fibril. The (*L*,*L*,*L*) tripeptides shown in brown and the (*D*,*D*,*D*) tripeptides shown in blue.

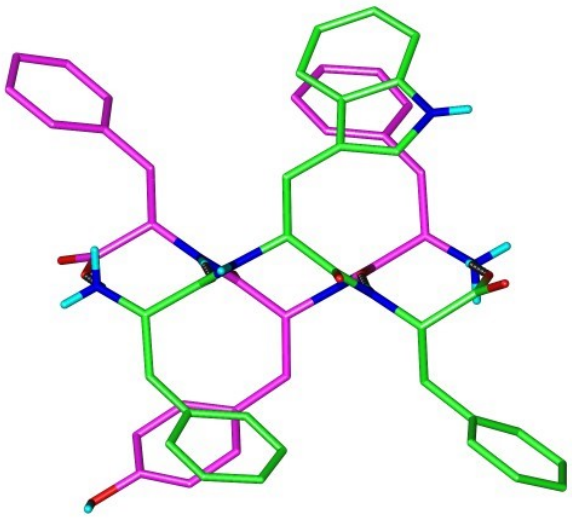

Figure S6. The alternative set of rotamers for [FWF:fyf]n, shown for the antiparallel rippled β-sheet dimer.

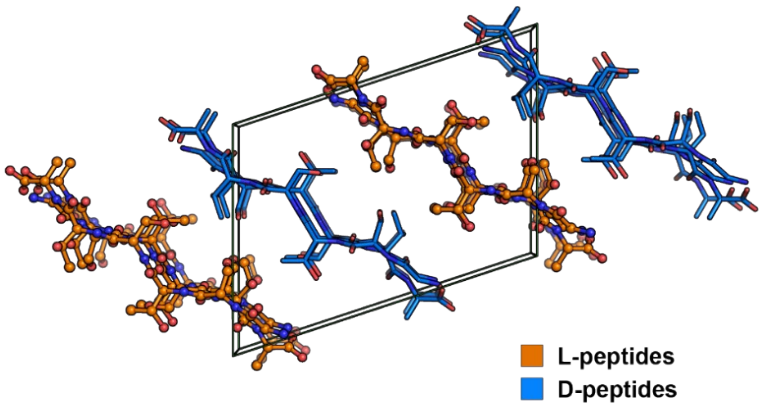

Figure S7. The L- and D-peptides self-sort into homochiral pleated sheets in the GSTSTA:gststa crystal (PDB ID code 6m7m). The crystal is viewed down the H-bonding axis.

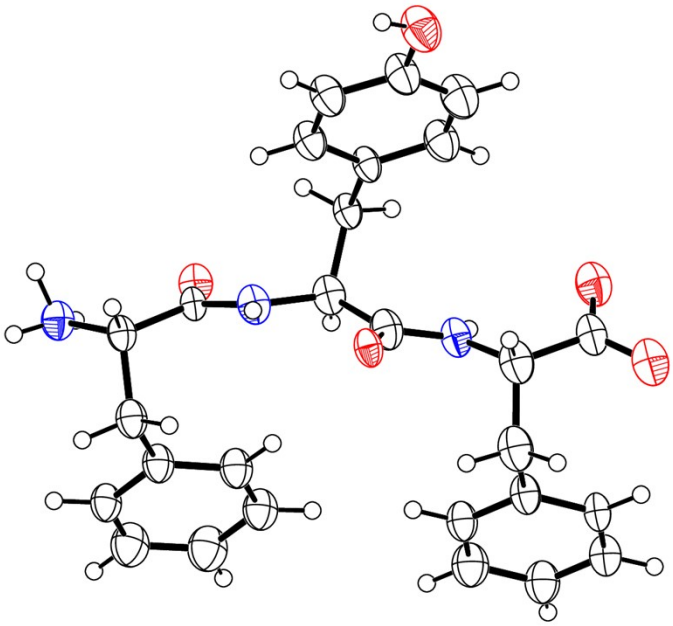

Figure S8. Thermal ellipsoid plot (50% probability level) of FYF. Color code: O red, N blue, C grey, H white spheres of arbitrary radius.

Materials and methods

Peptide synthesis and crystallization

All peptides were synthesized on pre-loaded Wang resins by standard Fmoc based, solid-phase peptide chemistry. All syntheses were performed manually at 0.2 mM scale relative to resin loading. The peptides were cleaved and deprotected with a mixture consisting of trifluoroacetic acid (10 mL), tri-isopropylsilane (1 mL), and liquefied phenol (0.5 mL). The cleavage solution was added to the resins and agitated for 2 h. The solution was then evaporated to 2 mL under nitrogen gas, and the peptides precipitated with cold diethyl ether and centrifuged at 6000 rpm. The peptide pellet was washed with cold diethyl ether, dried, dissolved in 1:1 acetonitrile:water, flash frozen in liquid nitrogen, and lyophilized. No further purification was performed prior to crystallization.

Crystallization and crystal structural determination

[FYF:fyf]n

Solutions of the L-FYF and D-fyf peptides were prepared separately by dissolving 6 mg of each individual peptide in 300 μL of hexafluoroisopropanol. The resulting solutions were combined. Nanopure water (3 mL) was subsequently added. Colorless needles formed upon leaving the solution standing overnight. The needles were approximately 5 microns thick, making them suitable for X-ray diffraction at microfocal beamline 24-ID-E of the Advanced Photon Source located at Argonne National Laboratory. Crystals were cooled to a temperature of 100 K. Diffraction data from three crystals were indexed, integrated, scaled, and merged using the programs XDS and XSCALE.[1] Data collection statistics are reported in Table S1. An atomic model was obtained by direct methods using the program ShelxD.[2] The model was refined using the program SHELXL,[3] and manually edited using the graphics program Coot.[4] Some of the structure illustrations were created using PyMOL.[5]

Table S1. Data collection and refinement statistics for the structure of racemic FYF:fyf

Peptide Sequence	FYF:fyf
X-ray Source	APS 24-ID-E
Space group	*C*2/*c*
Resolution (Å)	1.10 (1.13-1.10)*
Unit cell lengths: a,b,c (Å)	22.02, 9.57, 25.84
Unit cell angles: α,β,γ (°)	90.0, 102.35, 90.0
Measured reflections	30513 (670)
Unique reflections	1905 (81)
Overall completeness (%)	91.8 (57.0)
Overall redundancy	16.0 (8.2)

Overall R_{merge}	0.082 (0.155)
$CC_{1/2}$	99.9 (98.6)
Overall I/δ	25.5 (10.7)
Refinement R_{work} / R_{free}	 0.055 / 0.066
RMSD bond length (Å)	0.015
RMSD angle (°)	1.4
Number of peptide atoms (incl. H)	64
Number of water molecules	3
Number of other solvent atoms	0
Average B-factor of peptide ($Å^2$)	5.1
Average B-factor of water ($Å^2$)	16.3
Average B-factor other solvent ($Å^2$)	N/A
PDB ID code	8DDH

*Values in parentheses correspond to highest resolution shell.

[FWF:fwf]n

Solutions of the L-FWF and D-fwf peptides were prepared separately by dissolving 1 mg of each individual peptide in a solution of trifluoroethanol in Nanopure water (1:10, 1.1 mL). The resulting solutions were combined. Colorless needles formed upon leaving the solution standing for 3 weeks. A suitable colorless needle with dimensions of 0.26 × 0.04 × 0.03 mm^3 was used for single-crystal X-ray diffraction data collection at 100 K on a Rigaku XtaLAB Synergy-S diffractometer using Cu Kα radiation (λ = 1.54 Å). Data collection, processing and reduction were performed with CrysAlisPro. After face indexing, a numerical absorption correction was applied using gaussian integration. An empirical absorption correction using spherical harmonics was applied with the SCALE3 ABSPACK scaling algorithm. The structure was solved by intrinsic phasing using ShelXT and refined with ShelXL using Olex2.[6] All non-hydrogen atoms were refined anisotropically using standard procedures.[7] Atomic displacement parameters for hydrogen atoms were fixed to 1.2 × U_{iso} of the atoms to which they are attached. All hydrogen atoms were placed at geometrically calculated positions and refined using a riding model. For the ammonium groups, a modified riding model was used in which H atoms are allowed to rotate about the C–N bond. The N-H distances in the ammonium and amide/indole groups were constrained to 0.91 Å and 0.88 Å, respectively. The O–H distance of the carboxylic acid was constrained to 0.84 Å. All H-atom positions were consistent with the hydrogen-bonding patterns dictated by the heavier atoms. A pocket of disordered solvent molecules (66 e^- in 176 $Å^3$; consistent with 1 trifluoroethanol and 1 water) could not be satisfactorily refined. The contribution to this disordered solvent to the observed structure factors was masked using Olex2.

Table S2. Data collection and Refinement statistics for racemic FWF:fwf.

T (K)	100(2)
λ (Å)	1.54184
Crystal System	Monoclinic
Space group	$P2_1/c$
a (Å)	9.5344(5)

b (Å)	23.7071(16)
c (Å)	24.8206(12)
β (°)	99.040(5)
Volume (Å^3)	5540.6(5)
Z	8
ρ_{calc} (Mg/m^3)	1.197
Size (mm^3)	0.26 × 0.04 × 0.03
θ range (°)	2.593 – 67.078
Total data	42092
Unique data	9787
Parameters	670
Completeness	98.7%
R_{int}	8.73%
R_1 (I > 2σ)	8.17%
R_1 (all data)	14.11%
wR_2 (I > 2σ)	22.23%
wR_2 (all data)	26.16%
S	1.049
Min, max (e Å^{-3})	–0.466, 0.568
Deposition number	CCDC 2168013

[FWF:fyf]n

Solutions of the L-FWF and D-fyf peptides were prepared separately by dissolving 1.5 mg of each individual peptide in 50 µl and 100 µl of hexafluoroisopropanol, respectively. The resulting solutions were combined. Nanopure water (1 mL) was subsequently added. Colorless needles formed upon leaving the solution standing for several days.

Table S3. Data collection and Refinement statistics for FWF:fyf.

Peptide Sequence	FWF:fyf
X-ray Source	APS 24-ID-E
Space group	$P2_1$
Resolution (Å)	1.10 (1.13-1.10)*
Unit cell lengths: a,b,c (Å)	9.66, 26.26, 11.63
Unit cell angles: α,β,γ (°)	90.0, 95.12, 90.0
Measured reflections	13143 (308)
Unique reflections	2242 (113)
Overall completeness (%)	93.8 (66.1)
Overall redundancy	5.9 (2.7)
Overall R_{merge}	0.076 (0.255)
$CC_{1/2}$	99.8 (94.3)
Overall I/δ	13.0 (3.6)
Refinement R_{work} / R_{free}	

	0.092 / 0.127
RMSD bond length (Å)	0.017
RMSD angle (°)	2.7
Number of peptide atoms (incl. H)	168
Number of water molecules	1
Number of other solvent atoms	22
Average B-factor of peptide (Å^2)	12.9
Average B-factor of water (Å^2)	14.8
Average B-factor other solvent (Å^2)	14.9
PDB ID code	8DDF

[FYF:FYF]n

A solution of the L-FYF peptide was prepared by dissolving 5 mg of the peptide in 3 mL of nanopure water (3 mL). Colorless needles, suitable for micro-electron diffraction formed after several hours. 3 µL was deposited directly from the batch suspension on to an ultrathin carbon/lacey TEM grid using the dropcast technique. The sample was inserted at room temperature in a specialized sample holder designed for cryo – electron microscopy, and subsequently cooled to -177 °C. Electron diffraction data was collected at a Tecnai F30 TEM operating at 300 kV with a flux density of approximately 0.0192 electrons per square Angstrom per frame, such that the total accumulated dose by a crystal during a typical tilt series was approximately 8.04 MGy. Single crystals were located on the grid and centered in the microscope's selected area aperture, and continuous rotation diffraction tilt series were collected for each. Data was processed in XDS with a high-resolution limit of 0.9 Angstroms, and intensities from two crystals were merged in space group *C*2 to give an overall completeness of 71%, which was sufficient to resolve a structure solution by direct methods using SHELXD. Merging data from up to three additional crystals did not enhance the completeness of the data beyond 75%, at the expense of higher R_{merge} values.

Structure refinement was performed in PHENIX, where a final R_{work}/R_{free} of 16.85% and 17.08% respectively were reached after the addition of hydrogen atoms and anisotropic treatment of non-H atoms using PHENIX's TLS (Translation/Libration/Screw) routine. Individual anisotropic refinement of B-factors was also attempted in PHENIX, but this yielded a greater gap between R_{work} and R_{free}, without any improvement to R_{work} over the TLS result. As such, the refined structure reported is the iteration refined using TLS.

Table S4: Data collection and Refinement statistics for the structure of homochiral FYF

Peptide Sequence	FYF Crystal 1	FYF Crystal 2	FYF merge
Electron Source	Tecnai F30 TEM	Tecnai F30 TEM	
Space group	*C*2	*C*2	*C*2
Resolution (Å)	0.90 (1.00-0.90)*	0.90 (1.00-0.90)	0.90 (1.00-0.90)
Unit cell lengths: a,b,c (Å)	23.14, 4.84, 19.79	23.08, 4.84, 19.55	23.14, 4.84, 19.79
Unit cell angles: α,β,γ (°)	90.00, 107.05, 90.00	90.00, 106.62, 90.00	90.00, 107.05, 90.00
Measured reflections	3015 (801)	2956 (835)	5980 (1639)

Unique reflections	1097 (288)	987 (275)	1237 (332)
Overall completeness (%)	63.0 (62.6)	57.3 (60.2)	71.0 (72.2)
Overall redundancy	2.7 (2.8)	3.0 (3.0)	4.8 (2.7)
Overall R_{merge}	0.106 (0.320)	0.092 (0.206)	0.113 (0.323)
$CC_{1/2}$	99.1 (60.6)	99.1 (92.3)	99.1 (64.3)
Overall I/δ	6.7 (3.5)	8.1 (4.9)	8.6 (5.0)
Refinement R_{work} / R_{free}			0.169 / 0.185
RMSD bond length (Å)			0.020
RMSD angle (°)			1.25
Number of peptide atoms (incl. H)			64
Number of water molecules			0
Number of other solvent atoms			0
Average B-factor of peptide ($Å^2$)			3.3
Average B-factor of water ($Å^2$)			N/A
Average B-factor other solvent ($Å^2$)			N/A
PDB ID code			8DDG

*Values in parentheses correspond to highest resolution shell.

References

[1] Kabsch, W. XDS. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2010**, *66* (2), 125–132.

[2] Sheldrick, G. M. Experimental Phasing with SHELXC/D/E: Combining Chain Tracing with Density Modification. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2010**, *66* (4), 479–485.

[3] Sheldrick GM. A short history of SHELX. Acta Crystallographica Section A: Foundations of Crystallography. 2008;64(1):112–22.

[4] Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. Features and Development of Coot. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2010**, *66* (4), 486–501.

[5] The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.

[6] (a) G. M. Sheldrick, SHELXT - Integrated space-group and crystal-structure determination, *Acta Crystallogr. Sect. A Found. Crystallogr.*, 2015, **71**, 3–8; (b) G. M. Sheldrick, Crystal structure refinement with SHELXL, *Acta Crystallogr. Sect. C*, 2015, **71**, 3–8; (c) O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, OLEX2: A complete structure solution, refinement and analysis program, *J. Appl. Crystallogr.*, 2009, **42**, 339–341.

[7] P. Muller, Practical suggestions for better crystal structures, *Crystallogr. Rev.*, 2009, **15**, 57–83.