ADVANCED MATERIALS

Supporting Information

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Bioinspired Flexible and Tough Layered Peptide Crystals

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Supplementary Information

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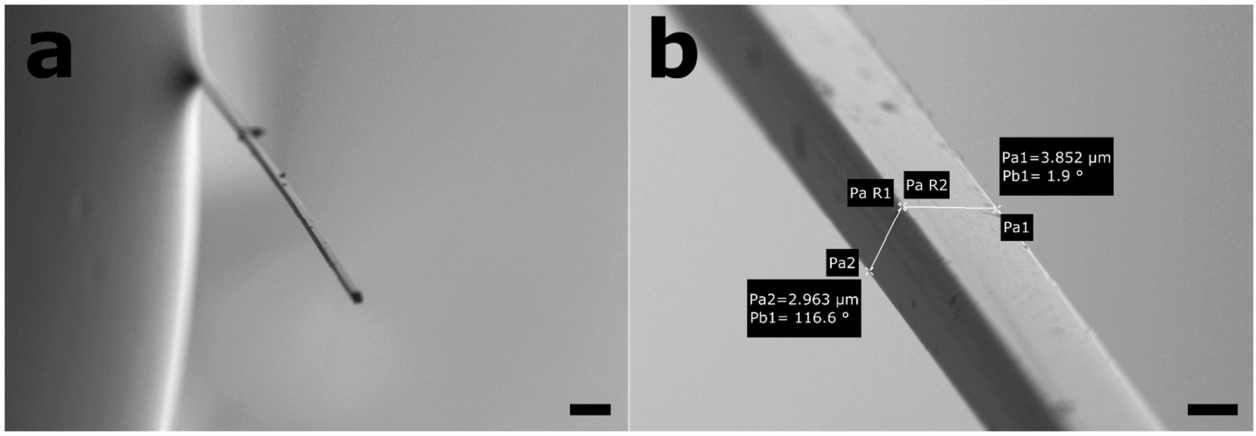

Figure S1. High resolution scanning electron microscopy images. Scale bar is (a) 20 µm and (b) 2 µm.

complex	Boc-FF
CCDC Deposition #	**1524591**
Formula	**C23 H28 N2 O5**
Crystal description	**Colourless needle**
Crystal size, [mm^3]	**0.300 x 0.010 x 0.010**
FW, [$g.mol^{-1}$]	**412.47**
Crystal system	**Monoclinic**
Space group	***C*2**
Unit cell **a, [Å]** **b, [Å]** **c, [Å]** **β, [°]**	 **47.744(12)** **6.1923(12)** **15.007(4)** **102.327**
Cell volume, [$Å^3$]	**4334.5(18)**
Z	**8**
ρ_{cacld}, [$g.cm^{-3}$]	**1.264**
µ, [mm^{-1}]	**0.089**
No. of reflections	**19710**
No. of unique reflections	**8110**
R_{int}	**0.074**

No. of parameters (restraints)	**562/1**
Final R[a]	**0.0706**
Final wR2[b]	**0.1397**
GooF	**1.139**

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Table S1. Crystallographic data of Boc-Phe-Phe

[a] for data with $I > 2\sigma(I)$. [b] for all data.

Sample	L (μm)	W* (μm)	T* (μm)	I_a (m^-24)	I_c (m^-24)	I_1 (μm)	E_x (GPa)	$E_{c/z}$ (GPa)
1	464.0	6.0	21.2	4764.1	381.6	453.4		18.94
						427.0		18.72
						453.1	4.49	
						417.5	5.02	
2	809.0	15.3	5.8	1731.1	248.8	801.1	6.65	
						668.4	5.53	
						790.2	-	-
3	756.0	24.0	8.5	9792.0	1228.3	746.1	5.01	
						721.3	6.79	
						732.2		16.32
						679.7		20.27
						683.7		25.02
6	892.0	29.7	15.0	32747.6	8353.1	821.7	5.31	
						855.4		11.48
						793.0		13.01
7	503.0	15.9	7.8	2612.8	628.8	443.6	3.51	
						435.2	4.11	
						407.9		15.14
8	626.0	19.5	8.1	5024.8	873.9	619.9	6.35	
						573.3	7.25	
						597.2		13.24
						552.2		13.73
						366.1		12.62
						368.1		12.15

Table S2. Summary of the bending test results.
* The terms thickness and width refer to the rod dimension perpendicular and parallel to the silicon wafer, respectively. The plane direction was determined during the SEM measurement as well. The moment of inertia was then calculated in each direction.

Sample	Cross-section area (μm^2)	Length (μm)	Force (mN)	Strength (MPa)	Strain (%)	Modulus (GPa)
1	26.94	372	4.72	175.0	5.1	3.2
2	11.67	388	2.52	216.0	3.8	6.7
4	37.02	328	2.75	74.0	3.8	2.3
5	193.77	378	8.27	43.0	3.6	1.3
6	38.78	328	2.99	77.0	5.2	1.9
9	54.95	437	4.32	78.7	5.3	1.3
10	61.21	401	3.88	63.5	4.6	1.8
11	50.29	433	4.93	98.1	10.6	2.1
12	21.45	463	2.32	107.7	3.5	3.1
14	17.1	404	2.64	155.0	4. 5	6.5
15	18.29	342	1.67	91.1	4.6	3.7
Avg	**45.4**	**400**	**3.6**	**104.3**	**5.0**	**3.1**
StDev	**41.2**	**44**	**1.6**	**45.5**	**2.0**	**1.9**

Table S3. Summary of the tensile test results.

	a [Å]	b [Å]	c [Å]	β (Degree)
Crystallographic	47.74	6.19	15.01	102.33
DFT (PBE+TS-vdW)	47.1 (-1.3%)	6.1 (-1.4%)	14.84 (-1.1%)	101.4

Table S4. Crystallographic and DFT-computed lattice parameters. The relative error of the theory with respect to crystallographic parameters is given in the parentheses.

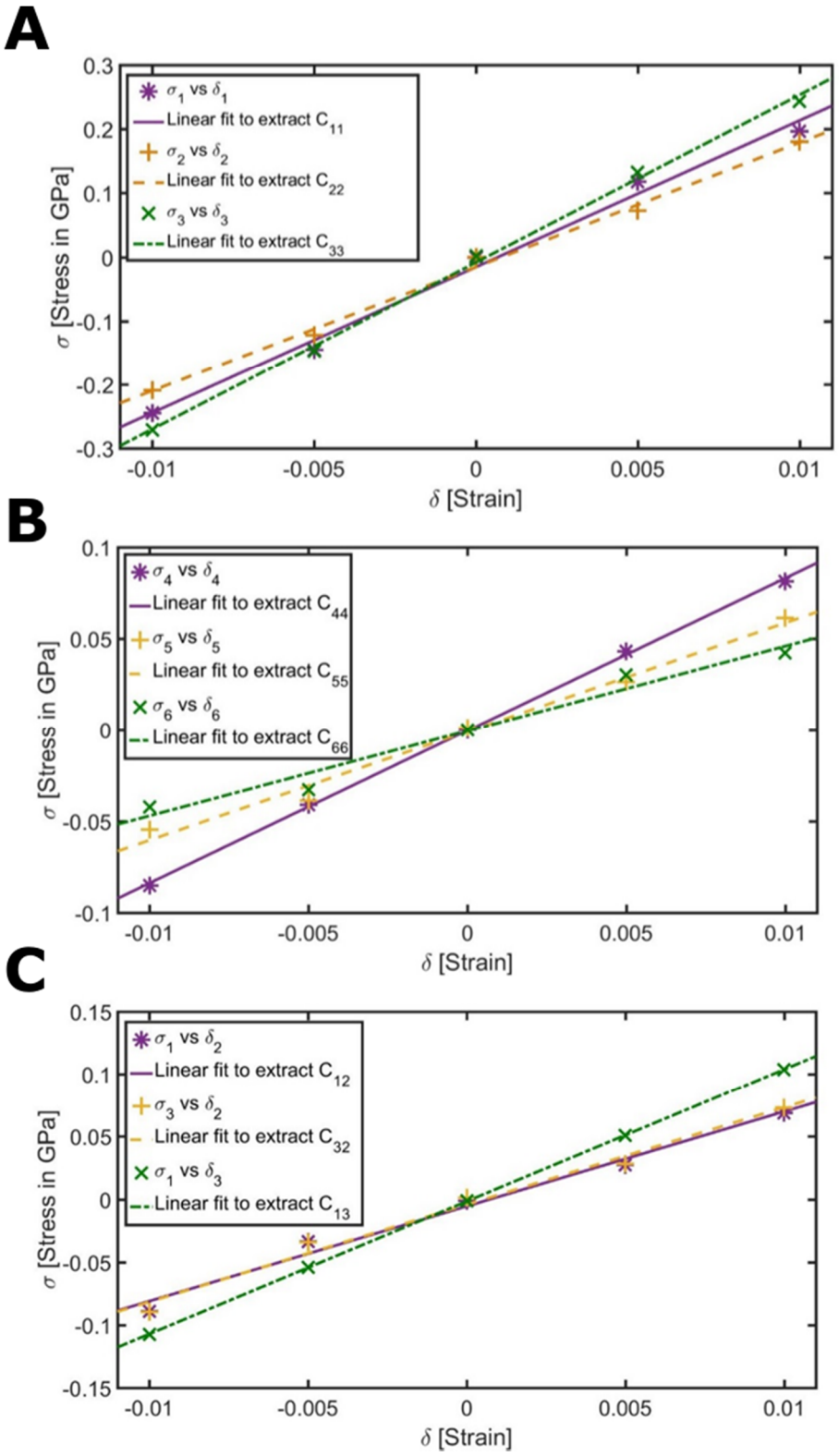

Figure S2. Stress-strain curves of the pristine Boc-FF crystal obtained using dispersion-corrected DFT, from which elastic constants were extracted by application of six deformations defined in the "Density Functional Theory Calculations" section below. **(A)** C_{11}, C_{22}, C_{33}; **(B)** C_{44}, C_{55}, C_{66}; **(C)** C_{12}, C_{32}, C_{13}

Methods

Boc-FF Crystallization

N-(t-butoxycarbonyl)-L-Phe-L-Phe-COOH (Boc-FF, Bachem) stock solutions were prepared in absolute ethanol, diluted into double distilled water at a 1:1 ratio , and incubated in a polypropylene conical tube at room temperature for several weeks. The resulting colorless, needle-like crystals were visible on the tube-solution interface. The structural morphology was determined using brightfield microscopy and high resolution electron microscopy.

Single Crystal X-ray Diffraction

The crystals were transferred to Paratone oil (Hampton Research) and mounted on a MiTeGen loop and flash frozen in liquid nitrogen. Crystal data for Boc-FF were measured at 100 K on a Bruker KappaApexII diffractometer equipped with [λ (MoK_α) = 0.71073 Å] radiation. The data were processed using Apex2 programs (Bruker). The structure was solved by direct methods with SHELXT-2013 and refined with full-matrix least squares refinement based on F2 with SHELXL-2014.

Morphological orientations *vis a vis* the Si wafer used for the bending measurements (see below) were performed using a Rigaku XtaLabPro diffractometer equipped with [λ (CuK_α) = 1.54184 Å] radiation. The data were processed using CrysAlisPro programs (Rigaku).

Crystallographic data are presented in Table S1 and are available from the CCDC with deposition numbers 1524591.

Bending Test Measurements

A cantilever bending test was performed on individual Boc-FF crystals. First, crystal clusters were removed from the liquid solution, and dried in air in a Petri dish, at room temperature. Individual crystal rods were then carefully separated from the cluster, using fine-tip watchmaker tweezers. The peptide rod was glued onto a 2x5 mm^2 silicon wafer, which was fixed to a nano-manipulator, and an AFM tip was attached to the microscope stage. The beam of the AFM cantilever and the peptide rod were adjusted to

be in the same focus plane, parallel to each other, to ensure that the tip of the AFM cantilever moves perpendicular towards the rod. An AFM cantilever with 3 N m^{-1} spring constant was chosen, so that the movement of the nano-manipulator perpendicular to the cantilever/rod would ensure deflection of both the peptide rod and of the AFM cantilever.

Tensile Test Measurements

Tensile tests were carried out using a tailor-made small-scale testing apparatus (Fig. 2C). All components of this instrument were designed so as to possess high stiffness. The load cell (Kistler, Switzerland) has a load capacity of 0.5 N. The piezo actuator (-216.9S, Physik Instrumente (PI), Germany) has a total travel distance of 180 μm and was used in tension. The setup was mounted on an optical microscope to monitor and record the experiment using a digital camera. One end of the crystal rod was secured to a small stainless steel sample holder using Poxypol adhesive, taking advantage of its relatively short curing time, high viscosity, and stiffness. After mounting the holder to the load cell, the other side of the peptide was fixed on a 'paddle' attached to the actuator. The crystal rod was then pulled by the actuator at a speed of 1 μm sec^{-1} and the load-displacement curve was recorded, and then translated into a stress-strain curve.

High Resolution Scanning Electron Microscopy

High resolution scanning electron microscopy (HRSEM) images were taken under the Leo Supra 55 FEG, Zeiss. The applied voltage was 3 or 5 kV, and working distance was about 5 mm. Prior to the SEM examination, samples were mounted on the aluminum SEM stubs and coated with Au-Pt by sputtering to avoid charging.

Density Functional Theory Calculations

All calculations were performed using the generalized gradient approximation (GGA) exchange-correlation functional of Perdew, Burke, and Ernzerhof (PBE),[28] augmented by the Tkatchenko–

Scheffler[29] dispersive pairwise-correction as implemented in the VASP projector-augmented planewave code.[30] A Brillouin zone sampling of 1x4x2 points was used along the reciprocal of the *a*, *b*, and *c* lattice vectors. A plane-wave cutoff of 750 eV was used to obtain converged stress calculations. The total energy was converged to 10^{-6} eV per unit-cell and all forces in the optimized structure were smaller than 5×10^{-3} eV $Å^{-1}$, with the stress optimized to be lower than the ratio between the minimal force and the lattice face area.

The elastic constant tensor,[24,31] from which Young's moduli were calculated, was computed from stress-strain curves by applying 6 different distortions, given by:

$$\begin{pmatrix} 1+\delta_1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}, \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1+\delta_2 & 0 \\ 0 & 0 & 1 \end{pmatrix}, \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1+\delta_3 \end{pmatrix}, \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & \delta_4 \\ 0 & 0 & 1 \end{pmatrix}, \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ \delta_5 & 0 & 1 \end{pmatrix}, \begin{pmatrix} 1 & \delta_6 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix},$$

where 1-6 are xx, yy, zz, yz, xz, and xy in the Voigt notation. For each distortion, 5 strain (δ) values were applied, namely 0, ±0.005, and ±0.01. Selected stress-strain curves are shown in Fig. S1. In these calculations, the lattice vector *a* was aligned with the *x* axis and *c* was in the *x*-*z* plane for the optimized structure. The off-diagonal elastic constants C_{15}, C_{25}, C_{35}, and C_{46} were found to be very small and in fact lower than the expected accuracy of the computational method. We found that setting these to zero changed the extracted Young's modulus by less than 0.5 GPa, which is about 3% of the values obtained for the pristine Boc-FF crystal using our computational approach.

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