SUPPLEMENTARY INFORMATION

A Multi-Stimuli Responsive, Self-Assembling, Boronic Acid Dipeptide

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Experimental Methods

Materials

Fmoc-Phe-OH, Fmoc-Phe-Wang resin (100-200 mesh), and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) were purchased from EMD Millpore. 4-carboxyphenylboronic acid (4-CPBA) and acetonitrile (MeCN, HPLC grade) were purchased from Alfa Aesar. Acetic acid, ammonium hydroxide, dichloromethane (DCM), dimethylformamide (DMF), hydrochloric acid, monobasic potassium phosphate, dibasic potassium phosphate, potassium chloride, and sodium chloride were purchased from Fisher Scientific. Catechol, deuterium oxide, diethyl ether, *N*-ethyldiisopropylamine (DIEA), hexafluoroisopropanol (HFIP), sorbitol, trifluoroacetic acid (TFA), and triisopropylsilane (TIS) were purchased from Sigma-Aldrich. All materials were used as received without further purification. Water was used freshly deionized from a Cascada LS purification system (Pall Corporation). Stock solutions of various pH were prepared by dissolving monobasic and dibasic potassium phosphate in H_2O or D_2O at 0.05 M total phosphate concentration.

Equivalent results are observed when the corresponding sodium phosphates are used to prepare these buffer solutions.

Peptide Synthesis

The linear dipeptide BFF (Fig. 1a of the main text) was synthesized on a 0.25 mmol scale using standard, Fmoc-based, solid-phase protocols.[1] The boronic acid functionality was incorporated by coupling 4-CPBA to the *N*-terminus of resin-bound di(phenylalanine). This coupling was achieved by shaking the resin in 0.75:0.75:1.5 mmol of 4-CPBA:PyBOP:DIEA dissolved in 10 mL DMF for 2 hr at room temperature. After a subsequent wash of the resin with DMF (3 x 15 mL) and DCM (3 x 15 mL), the peptide was cleaved from the resin by shaking in 95:2.5:2.5 (vol:vol:vol) TFA:TIS:H_2O for 3 hr. The peptide solution in TFA was then separated from the resin by filtration, and the peptide precipitated in cold (−40 °C) ether. The precipitated peptide was recovered by centrifugation (5,000 rpm, 10 min) and decantation of the supernatant. This process was repeated two additional times to wash the peptide powder with ether, after which the powder was dried to constant weight under vacuum. The purity of the peptide thus obtained was assessed by reverse phase-high performance liquid chromatography (RP-HPLC) and found to be 98%. Yield: 67 mg (58%). Melting point: 168 °C. ^{1}H-NMR (500 MHz, in D_2O + 0.25 vol. % ND_4OD): δ 7.63 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.04-7.18 (m, 10H), 4.66 (dd, *J* = 5.4, 10 Hz, 1H), 4.34 (dd, *J* = 4.8, 8.2 Hz, 1H), 3.08 (m, 2H), 2.82 (m, 2H). ^{11}B-NMR (160 MHz, in D_2O + 0.25 vol. % ND_4OD): δ 6.3. ^{13}C-NMR (125 MHz, in D_2O + 0.25 vol. % ND_4OD): δ 177.5, 171.9, 170.4, 137.4, 136.9, 134.1, 133.3, 129.3, 129.1, 128.6, 128.4, 126.9, 126.7, 126.4, 56.1, 55.2, 37.8, 36.6. HR-MS (ESI): calculated for $C_{25}H_{25}BN_2O_6$ $[M–H]^-$: 459.1727; found: 459.1725.

Preparation of Self-Assembled Peptide Materials

To demonstrate pH-responsive self-assembly, 0.5-2 mg BFF was added to deionized H_2O at 2 mg/mL. Concentrated NH_4OH was then added in 0.5 μL aliquots to raise the

pH to 10, yielding a visually homogenous solution. The pH was then lowered by two different methods: i) concentrated HCl was added to the peptide in 0.5 μL aliquots; or ii) the peptide solution was placed in a sealed container with, but physically separated from, a 20 mL reservoir of 5 vol. % acetic acid in H_2O. When acidification was effected by addition of concentrated HCl, a fine precipitate formed rapidly upon reaching pH ≤ 5. When acidification was effected by diffusion of acetic acid vapor, a single, fibrous mass precipitated after several days upon reaching pH ≤ 5. For comparison, 0.5-2 mg BFF was also dissolved in HFIP at 100 mg/mL. This concentrated solution was diluted to 2 mg/mL with deionized water, causing the rapid formation of a fine precipitate.

To demonstrate salt-responsive self-assembly, 0.5-2 mg BFF was first dissolved in pH 7 phosphate buffer at 2 mg/mL or 10 mg/mL. NaCl or KCl was then added in an amount corresponding to 1.5-3 M concentration and quickly vortexed to dissolve. Shortly thereafter, a hazy suspension or a highly opaque, self-supporting hydrogel is formed at 2 or 10 mg/mL, respectively. Equivalent results are obtained when salt is introduced via liquid phase. For example, BFF can be dissolved at 4 mg/mL in pH 7 phosphate buffer, while NaCl can be dissolved at 6 M in a separate portion of buffer. Mixing equal volumes of peptide and NaCl solution produces equivalent behavior as when 3 M NaCl is dissolved directly in a 2 mg/mL peptide solution.

To demonstrate polyol-responsive disassembly, salt-induced self-assembled materials were first prepared as described above. The desired polyol, *e.g.*, catechol, was then introduced by direct dissolution of small portions in the peptide suspension or gel. As greater and greater amounts of polyol were added, the peptide suspension or gel gradually transitioned to a clear, visually homogeneous solution. Equivalent results are again obtained when the polyol is introduced via liquid phase, specifically, by injecting a 1.5 M solution (with an appropriate amount of NaCl and buffer) directly into the suspension or gel, followed immediately by vortexing.

High Performance Liquid Chromatography

RP-HPLC was conducted using an Agilent 1200 series preparative-scale system equipped with two model G1361A pumps, a model G1315D diode array detector, a model G1315D fraction collector, a Rheodyne model 3725i-038 manual injector, and a Zorbax 300SB C18 column. A mobile phase gradient between H_2O with 0.05 vol. % TFA (A) and MeCN with 0.045 vol. % TFA (B) was employed, starting from 99:1 (vol:vol) A:B with a linear increase of 1 %B/min. Elution was monitored at 206 nm (10 nm bandwidth) from which a 450 nm reference (80 nm bandwidth) was subtracted.

Nuclear Magnetic Resonance Spectroscopy

Solution NMR spectroscopy was performed using a Bruker Avance-III 500 MHz spectrometer. Quartz sample tubes were used to limit background signal from boron present in standard borosilicate glass tubes. ^{1}H spectra were acquired over 32 scans using a 5 s delay time. Chemical shifts were referenced to residual hydrogenated solvent as an internal standard, H_2O δ 4.8 ppm (2H, s). ^{11}B spectra were acquired over 512 scans using a 5 s delay time. Chemical shifts were referenced to $BF_3{\cdot}Et_2O$ as an external standard (δ 0 ppm). ^{13}C spectra were acquired over 50000 scans using a 5 s delay time. Chemical shifts were referenced to tetramethylsilane as an external standard (δ 0 ppm).

Mass Spectrometry

Mass spectrometry (MS) was performed using a Waters Corporation (Manchester, UK) Synapt G2-HDMS™ time-of-flight (TOF), high-definition spectrometer with direct infusion in negative ionization mode (electrospray ionization, ESI). Mass analysis was performed in sensitivity mode with the following TOF-MS settings: source temperature of 125°C, sampling cone at 40.0 V, extraction cone at 5.0 V, acquisition mass range of 50-2000 Da, scan time of 0.5 seconds, detector at 2000 V, and data format set to continuum.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectra were collected on a Scimitar 2000 series spectrometer (Varian, Inc.) equipped with a Pike MIRacle attenuated total reflectance (ATR) accessory having a diamond cell. Spectra were collected with 2 cm^{-1} resolution over an average of at least 40 scans, from which a background spectrum was subsequently subtracted. Solution spectra were recorded by placing a 5 µL drop of a D_2O solution directly on the ATR crystal, followed immediately by the collection of data. For gels and suspensions, a 5 uL volume of material was placed directly on the ATR crystal and allowed to dry, followed subsequently by the collection of data on the resultant dried film.

Circular Dichroism Spectroscopy

Circular dichroism (CD) spectra were collected on a J-815 spectropolarimeter (Jasco, Inc.) using a quartz cell with a 0.01 mm path length. A typical spectrum consisted of an average of at least eight scans, from which a corresponding background spectrum was subsequently subtracted. Data were collected over wavelengths of 300 nm to 180 nm, with a pitch of 0.2 nm, integration time of 1 s, bandwidth of 1 nm, and scanning speed of 100 nm/min. No dilution of peptide solutions, suspensions, or gels relative to the concentrations of interest was necessary to obtain satisfactory CD, high tension, and absorbance values at wavelengths > 190 nm.

Ultraviolet-Visible Spectroscopy

Ultraviolet-visible (UV-vis) spectroscopy was performed using a DT-MINI-2-GS light source (Ocean Optics), with a deuterium lamp and a tungsten halogen lamp for UV and visible/near infrared light over a spectral range of 215-2500 nm. UV-vis spectra were collected and analyzed using a quartz cell with a 10 mm path length and

OOIBase32 software. The peptide concentration in solution was 0.2 mM and appropriate background spectra were subtracted to give the absorption characteristics of the peptide.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was performed using a Supra 55VP Field Emission microscope (Carl Zeiss AG) operating at 3 kV. Samples for imaging consisted of 5 μL volumes of peptide suspensions or gels that were placed on a silicon substrate and allowed to dry under ambient conditions. For salt-containing materials, excess solution was wicked away by touching a piece of filter paper to the deposited material. All samples were sputter-coated with Au/Pd prior to imaging to reduce charging under the electron beam. Topographical images were collected with an in-lens secondary electron detector.

Atomic Force Microscopy

Atomic force microscopy (AFM) was performed in contact mode using a ThermoMicroscopes Autoprobe CP microscope (Veeco Instruments). As with SEM, samples for imaging consisted of 5 μL volumes of peptide suspensions or gels that were placed on a silicon substrate and allowed to dry under ambient conditions. For salt-containing materials, excess solution was wicked away by touching a piece of filter paper to the deposited material. After scanning, the collected height images were flattened using a parabolic fit.

Small Angle X-ray Scattering

Small angle x-ray scattering (SAXS) was performed using a NANOSTAR instrument (Bruker Corporation) equipped with a Cu microfocus source, a pinhole collimation system, and a VÅNTEC-2000 area detector. Samples were loaded into glass capillary tubes of 2 mm outer diameter (Charles Supper Company), which were subsequently

sealed with a fast-cure epoxy. Two-dimensional scattering patterns were accumulated over a minimum of 4 hr of exposure. All collected patterns were isotropic and were azimuthally integrated to yield one-dimensional patterns of scattered intensity as a function of the magnitude of the scattering wavevector, q. Scattering patterns of the corresponding backgrounds were collected and processed under identical conditions, and then subtracted from the sample scattering patterns to give the scattering contribution arising from only the peptide component.

Additional Characterization Results

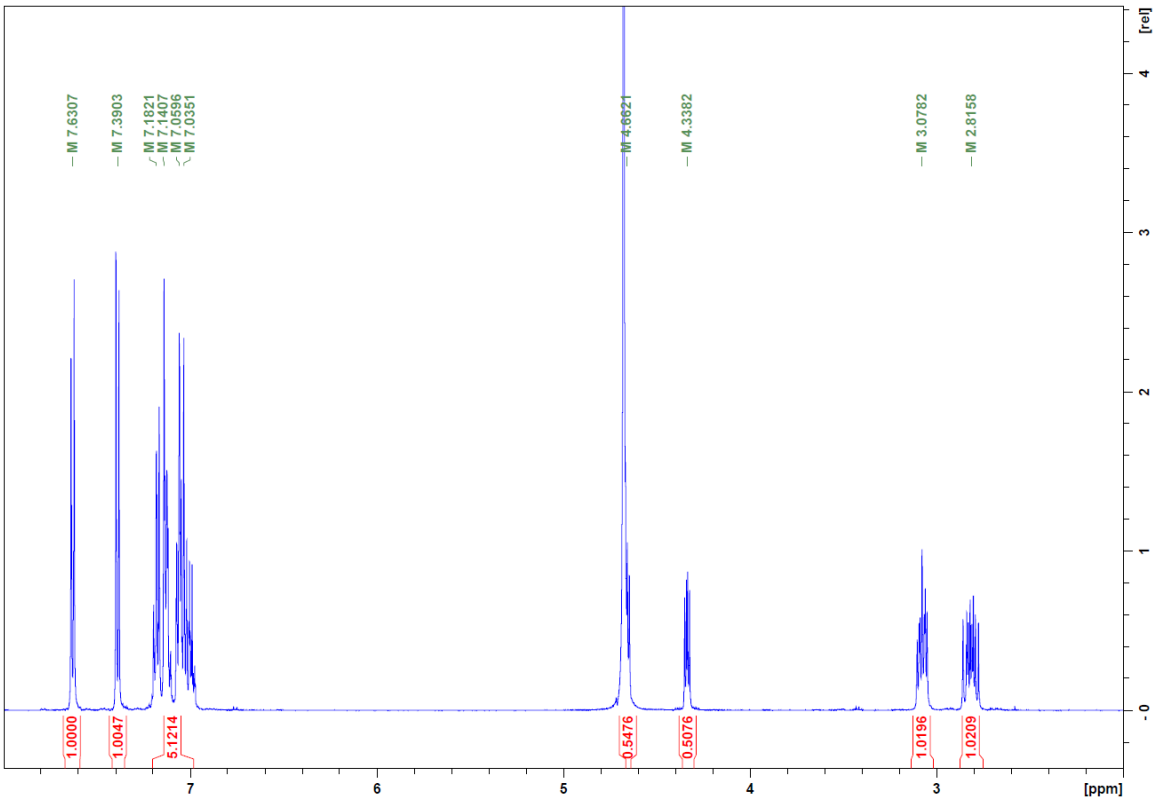

Fig. S1 ^{1}H-NMR spectrum of as-synthesized BFF dissolved in D_2O + 0.25 vol. % ND_4OD.

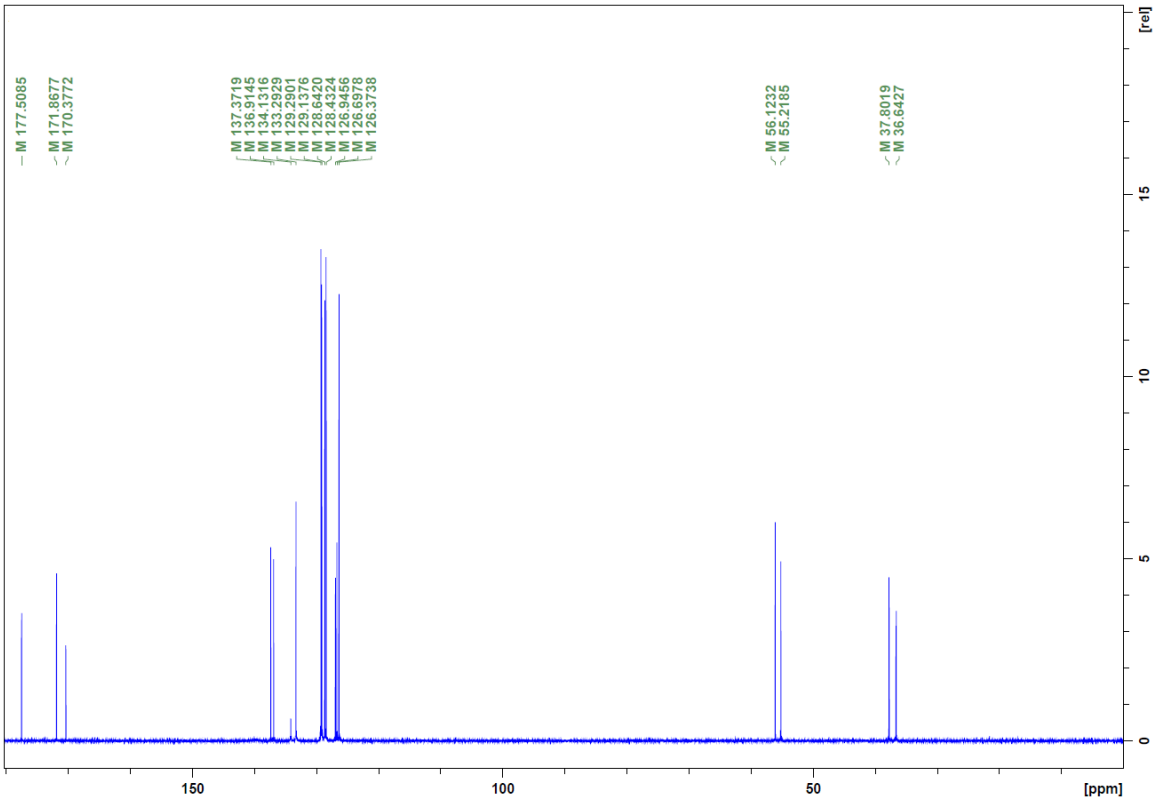

Fig. S2 ^{13}C-NMR spectrum of as-synthesized BFF dissolved in D_2O + 0.25 vol. % ND_4OD.

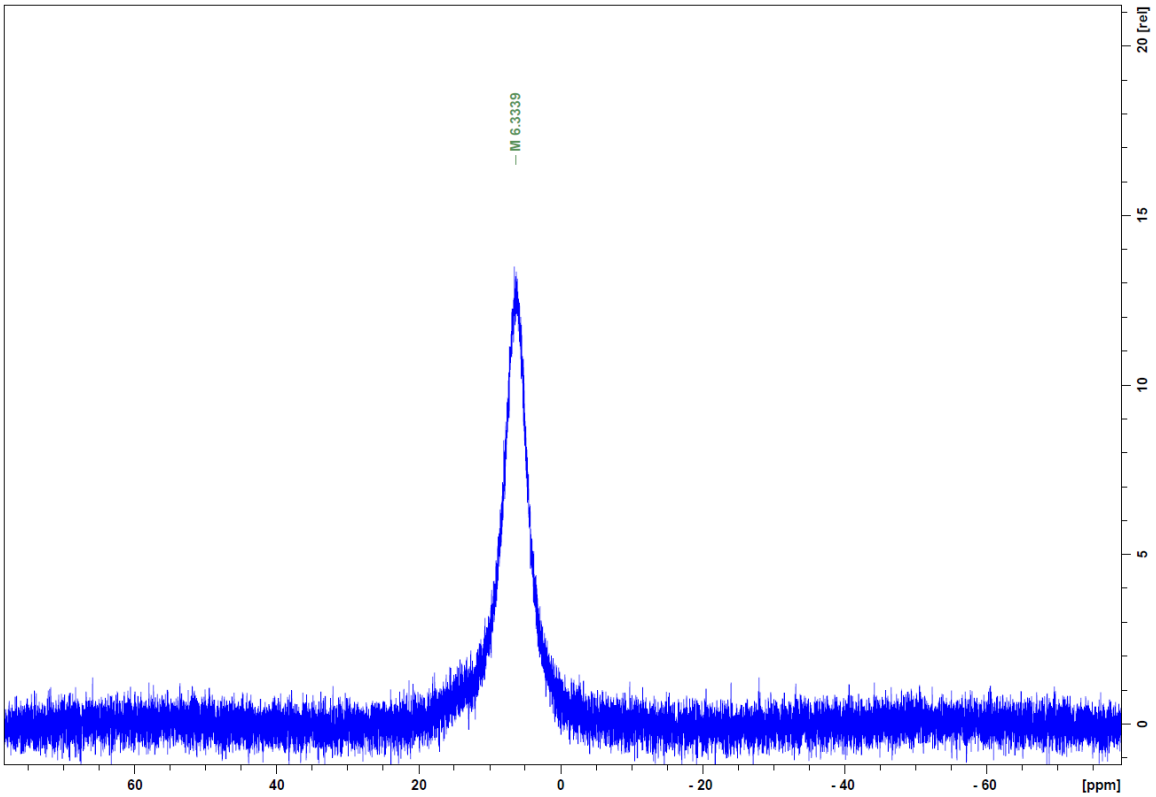

Fig. S3 ^{11}B-NMR spectrum of as-synthesized BFF dissolved in D_2O + 0.25 vol. % ND_4OD. The single resonance at 6.3 ppm is consistent with the installation of an aryl BA moiety onto the *N*-terminus of the dipeptide.

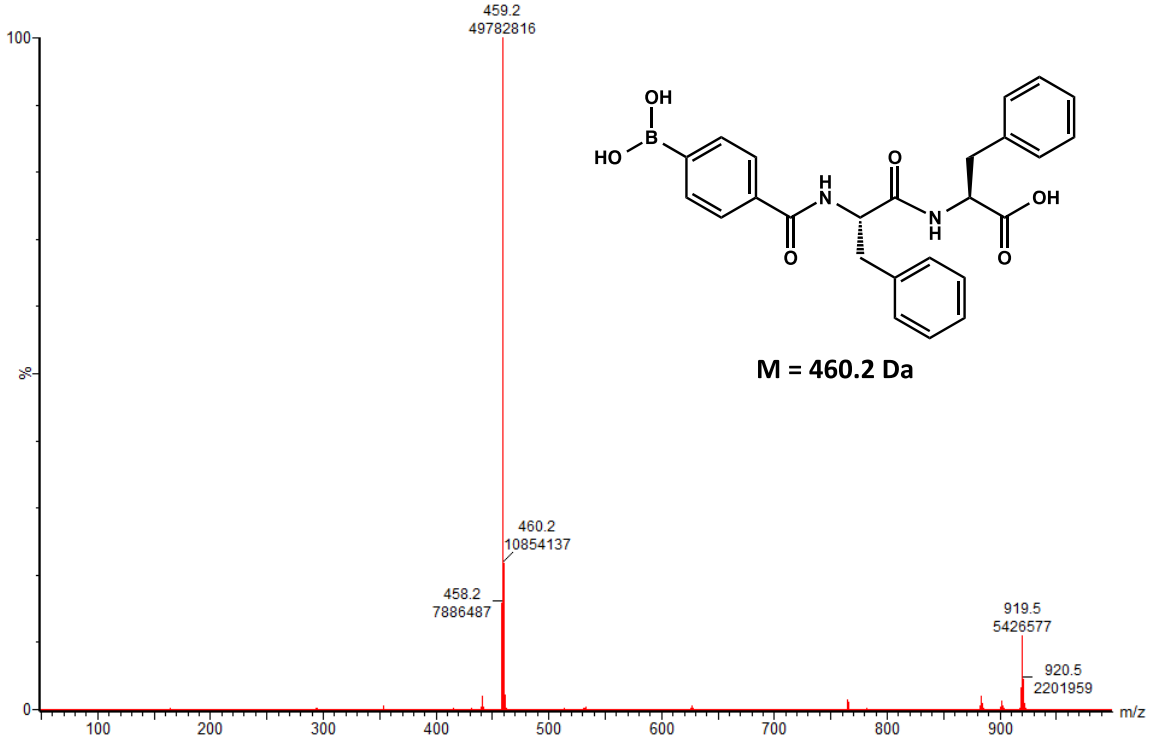

Fig. S4 Negative mode ESI-MS spectrum of as-synthesized BFF dissolved in pH 10 NH_4OH in H_2O, confirming the successful synthesis of BFF in high purity. The principal peak at *m/z* = 459.2 is consistent with a singly charged ion of BFF (molar mass 460.2 Da). Moreover, it exhibits the isotopic signature of a boron-containing compound;[2] namely, the peak at *m/z* = 458.2 arises from the natural presence of ^{10}B. The peak at *m/z* = 919.5 is attributed to a dimerized species of BFF.

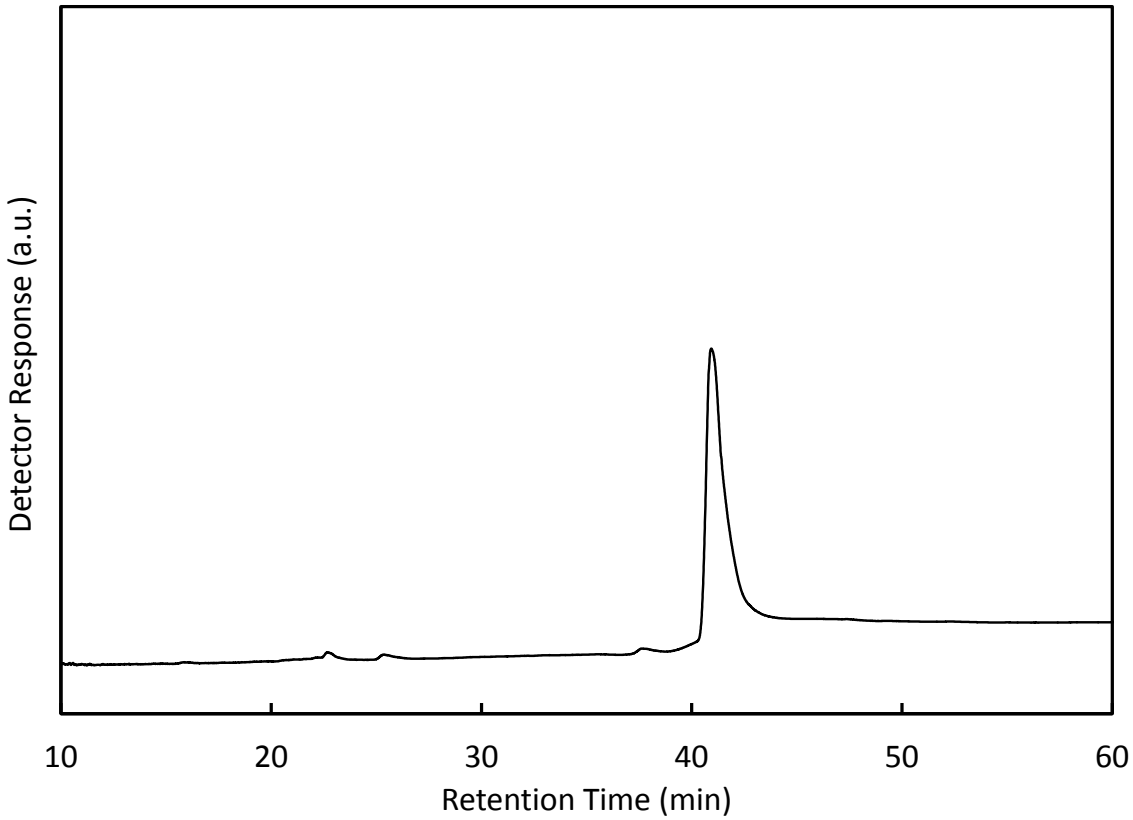

Fig. S5 RP-HPLC trace of as-synthesized BFF. Integration of the main peak at 41 min relative to the total area under the curve indicates a purity of 98%.

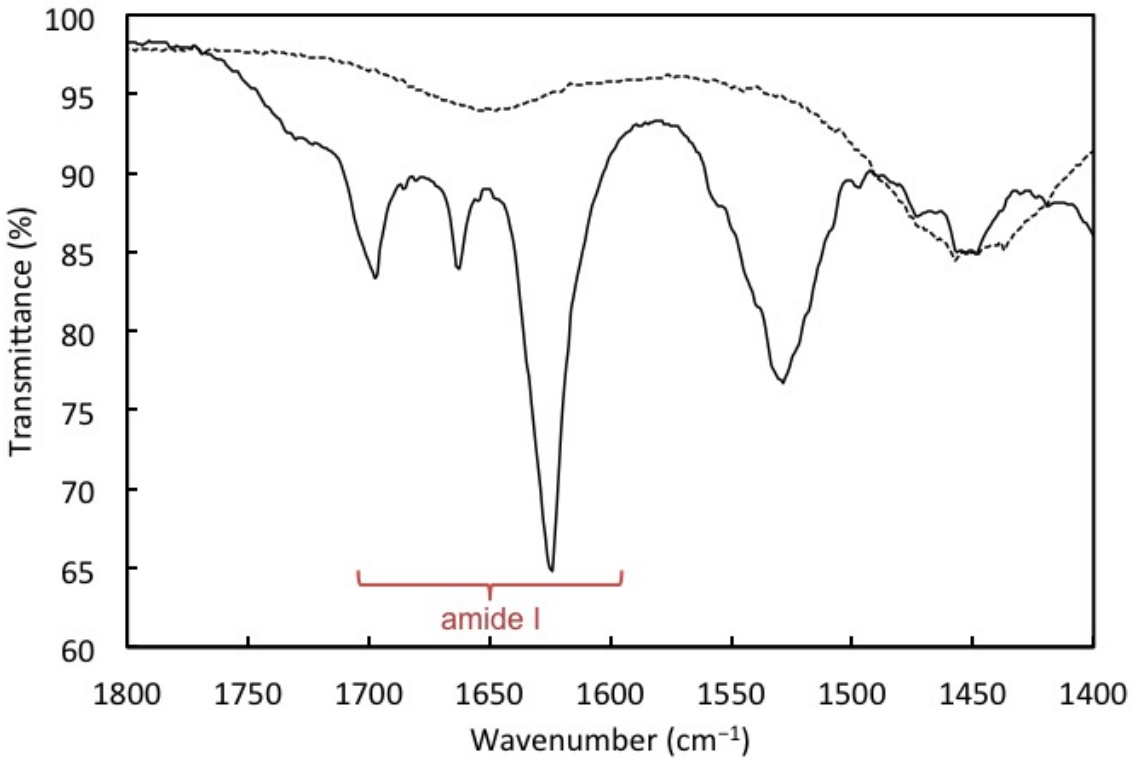

Fig. S6 FTIR spectra of (dotted) 2 mg/mL solution of BFF in D_2O. The pH of the solution was adjusted to 10 by addition of NH_4OH. The broad absorbance from the amide I vibration, centered at 1644 cm^{-1}, is consistent with a disordered peptide conformation.[3] (solid) Precipitate formed by acidification of a 2 mg/mL BFF solution. Equivalent spectra are obtained regardless of acidification method. The amide I vibration shows three primary contributions to the absorbance of the peptide, at 1624, 1661, and 1696 cm^{-1}. All three contributions are indicative of an ordered conformation of BFF, with the former typically being ascribed to a β-sheet conformation and the latter two typically being ascribed to β-turn conformations.[3]

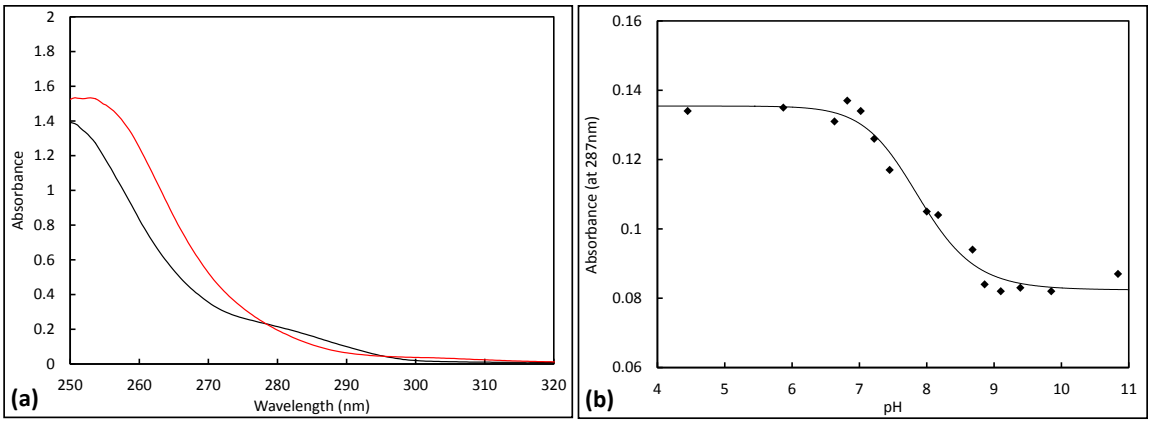

Fig. S7 Spectrophotometric determination of pK_{BA}, adapted from the method described by Yan, *et al.*[4] (a) UV-vis spectra of 0.2 mM BFF in 0.05 M phosphate buffer. The black curve shows a typical spectrum well below pK_{BA} (pH = 5.87), while the red curve shows a typical spectrum well above pK_{BA} (pH = 10.84). The distinguishing feature between these two regimes is the broad shoulder at 280-290 nm, which is only observed below pK_{BA}. (b) UV-vis absorbance monitored at 287 nm as a function of pH. The solid line shows a sigmoid fit (R^2 = 0.98) to the data, from which pK_{BA} = 7.9 is extracted as the midpoint of the sigmoid.

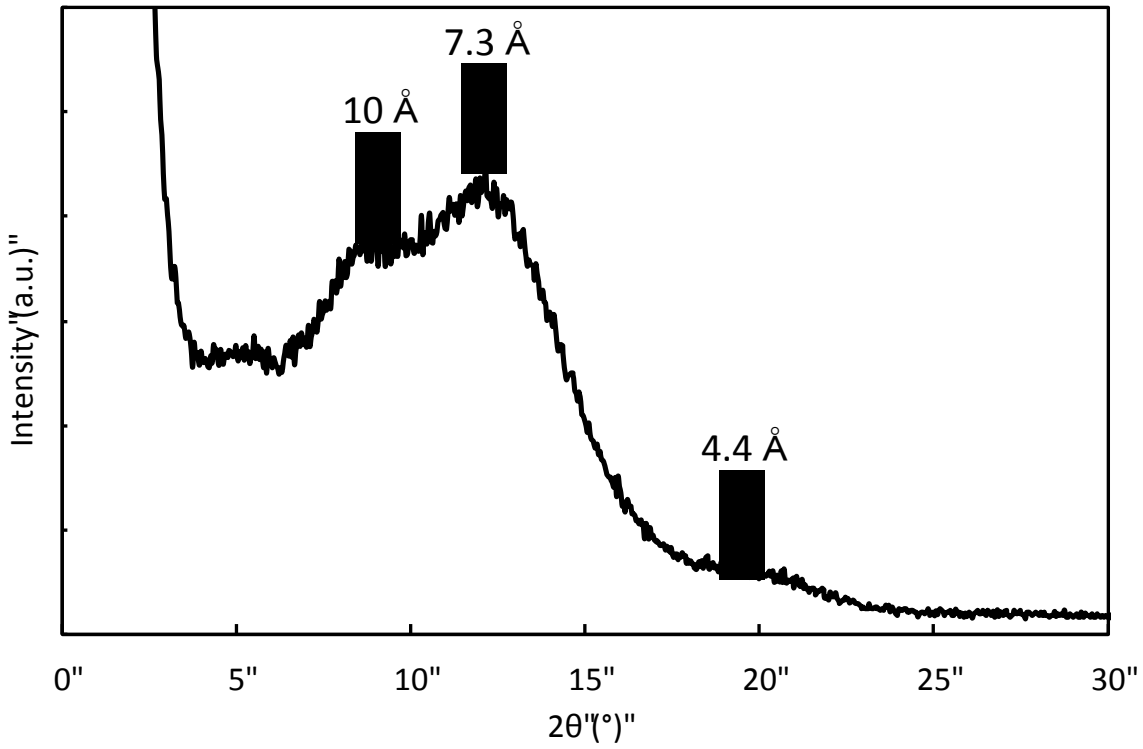

Fig. S8 Powder x-ray diffractogram of dried BFF suspension. The suspension was produced by acidification of a 2 mg/mL aqueous solution. At least three reflections are apparent, with approximate *d*-spacings indicated above each arrow. These reflections indicate a fundamentally ordered molecular packing of BFF upon self-assembly, and likely correspond to characteristic length scales such as molecular size, hydrogen bond lengths, and separation distances of aromatic moieties. The broad nature of the reflections suggests a hierarchical structure of BFF, whereby the larger, self-assembled nanoribbons are comprised of smaller nanocrystallites.

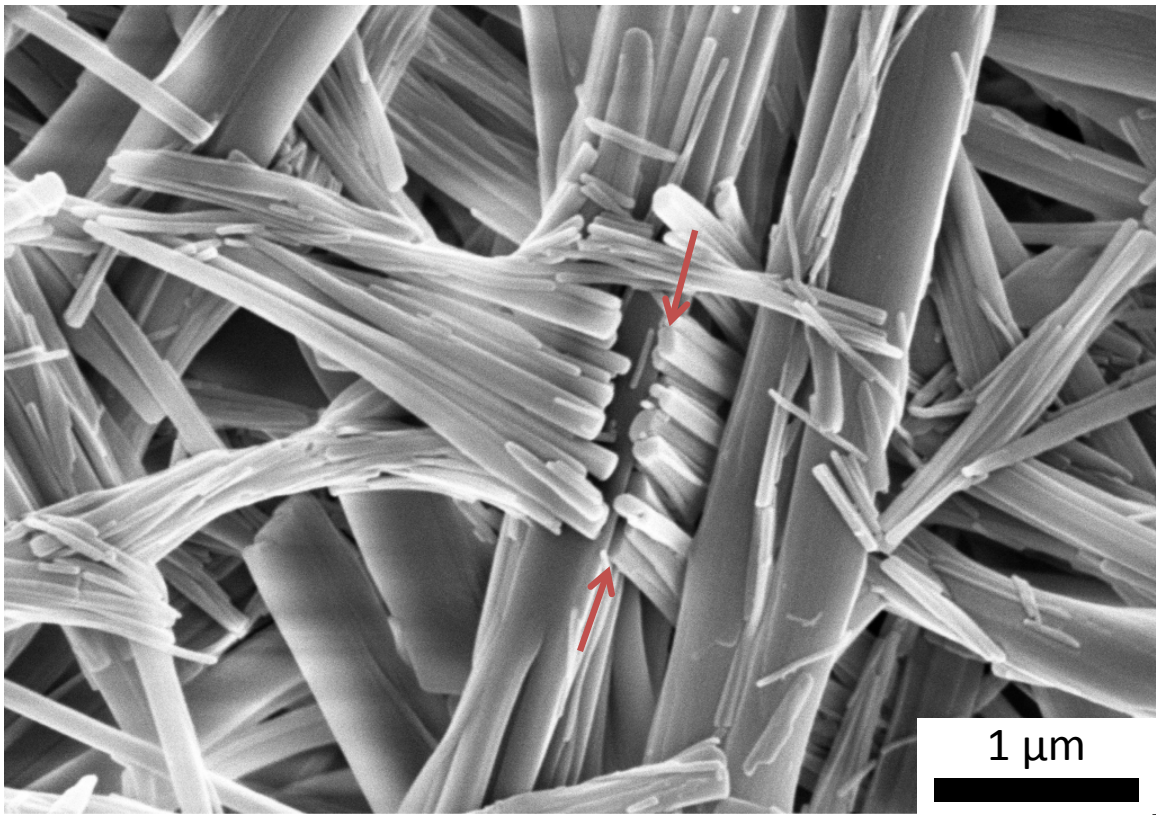

Fig. S9 Additional SEM image of peptide nanoribbons produced by pH-induced self-assembly of BFF. The red arrows demarcate a region where the nanoribbons can be viewed edge-on. The roughly rectangular cross-section (*i.e.*, a width that is substantially greater than the thickness) seen in these examples is further evidence of the ribbon-like morphology.

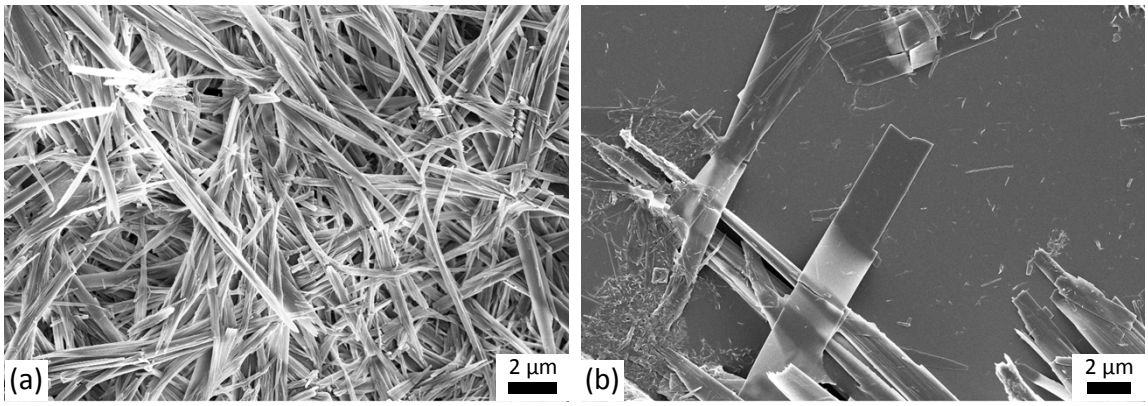

Fig. S10 SEM images of peptide nanoribbons produced by acidification of a 2 mg/mL BFF solution: (a) rapid acidification with concentrated HCl solution, (b) slow acidification with acetic acid vapor. The images were taken at nearly identical magnifications and highlight the larger width of the nanoribbon aggregates that result from slow acidification.

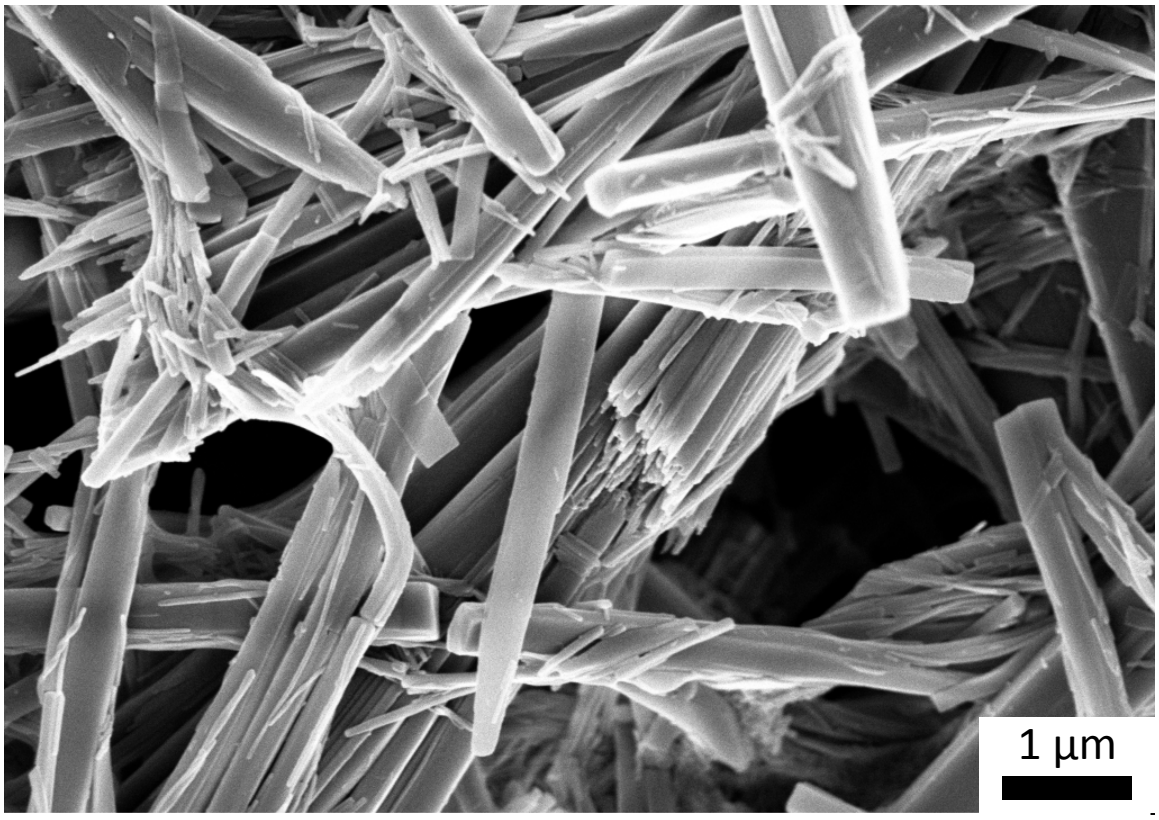

Fig. S11 SEM image of peptide nanoribbons produced by dilution of a concentrated (100 mg/mL) solution of BFF in HFIP with a 50-fold excess of H_2O.

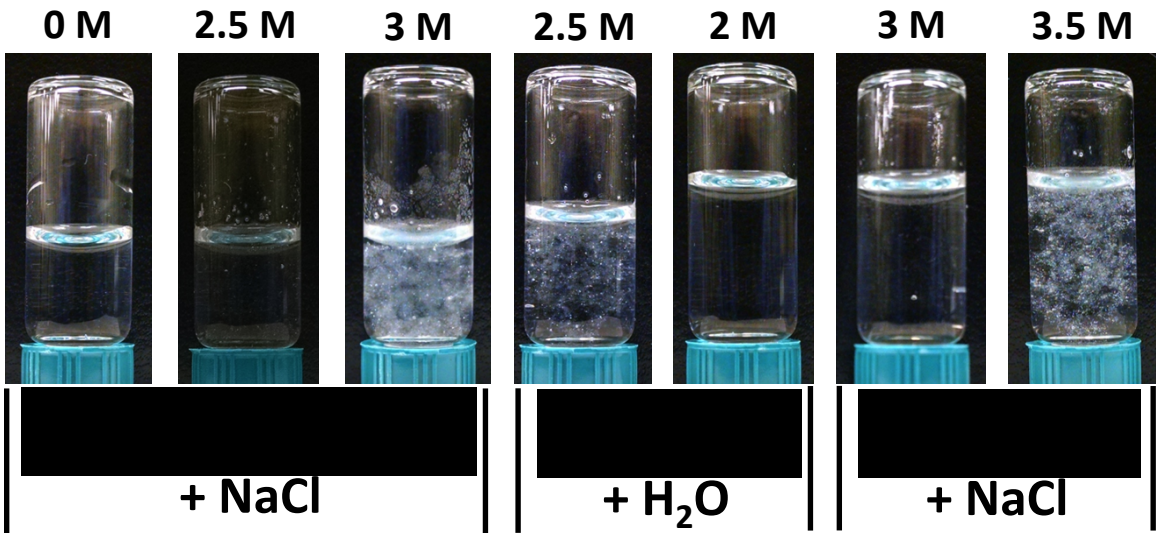

Fig. S12 Macroscopic observation of reversible salt-responsive BFF assembly. The salt concentration is indicated above each image. The leftmost image shows 2 mg/mL BFF dissolved in pH 7.0 buffer. Working from left to right, the salt concentration is first increased to trigger assembly of BFF and the formation of a hazy suspension. Then, additional buffer is added to reduce the salt concentration, thereby triggering disassembly of BFF and the formation of a homogeneous solution. Finally, the salt concentration is increased to once again trigger assembly of BFF and the formation of a hazy suspension. As mentioned in the main text, the amount of salt required to initiate assembly increases with decreasing peptide concentration. Because the peptide concentration is also reduced in the middle stage by the addition of buffer, the amount of salt required to subsequently reinitiate assembly in the final stage is increased.

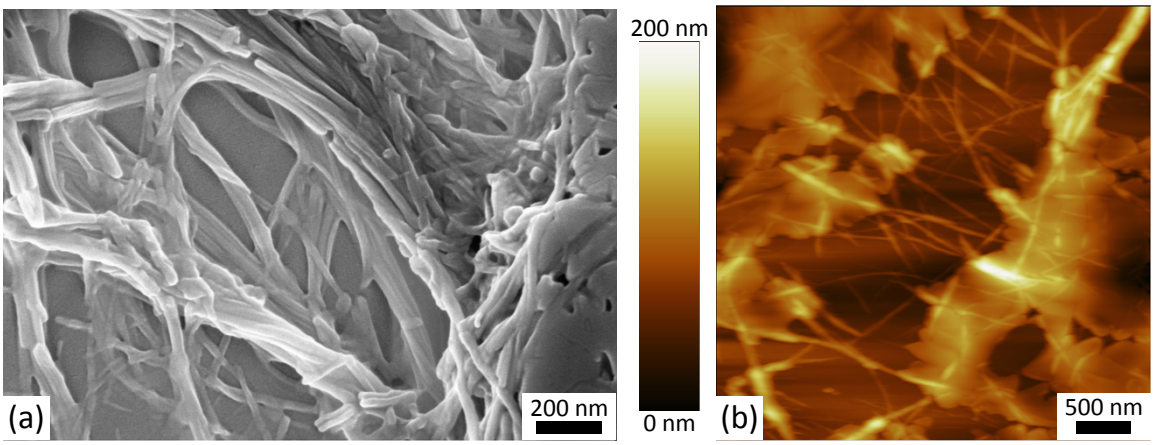

Fig. S13 (a) SEM image and (b) AFM height image of self-assembled fibrillar networks formed by addition of 3 M NaCl to a solution of BFF in pH 7.0 phosphate buffer. The peptide concentration in (a) and (b) was 10 mg/mL and 2 mg/mL, respectively.

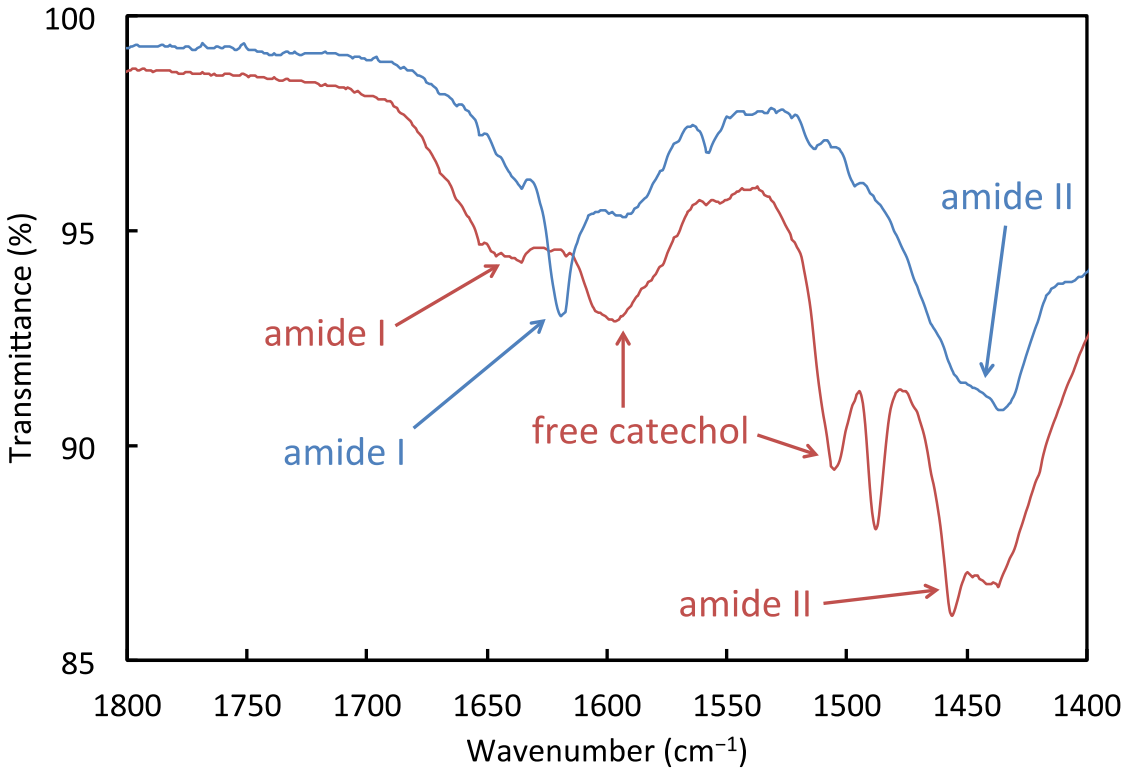

Fig. S14 FTIR spectra of 2 mg/mL BFF in D_2O after the addition of (blue) 3 M NaCl, followed by (red) a 6-fold excess of catechol relative to the amount of BFF. The amide I vibration at 1617 cm^{-1} in NaCl is attributed to a β-sheet conformation of the peptide,[3] and reflects the salt-induced self-assembly of BFF. This vibration shifts to 1640 cm^{-1} upon addition of catechol, indicating a disordered conformation of the peptide,[3] consistent with dissociation of the self-assembled structure.

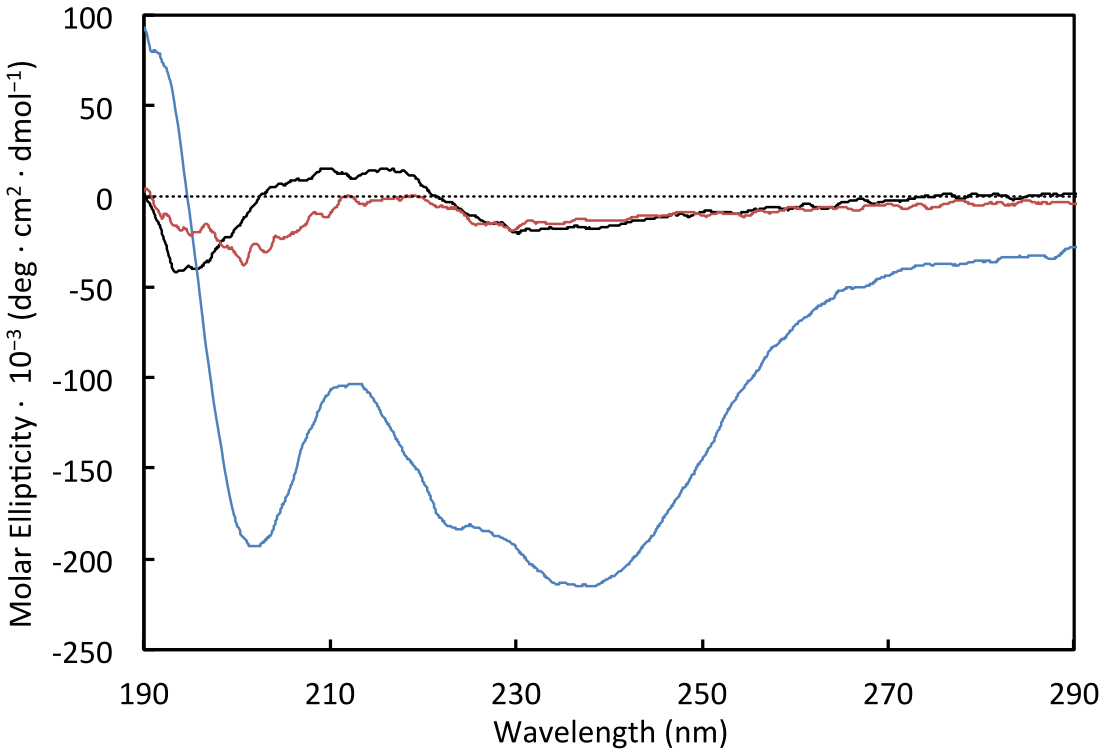

Fig. S15 CD spectra of 2 mg/mL BFF: (black) dissolved in pH 7.0 phosphate buffer, (blue) after addition of 3 M NaCl, followed by (red) a 6-fold excess of catechol relative to the amount of BFF. The dotted line is a guide to the eye, drawn at zero ellipticity. At a cursory level, the transition of the magnitude of the CD signal from weak to strong upon addition of salt and from strong to weak upon subsequent addition of catechol reflects conformational disorder-order and order-disorder transitions,[5] respectively, in agreement with the salt-induced self-assembly and catechol-induced disassembly behavior discussed in the main text. More specifically, the CD spectra of BFF in buffer and after catechol addition exhibit weak minima below 200 nm, consistent with disordered peptide conformations;[5] the weak maxima and minima around 212 nm and 238 nm, respectively, are attributed to a universal contribution of phenyl chromophores to the CD signal.[6] The CD spectrum of BFF after salt addition also shows a local maximum and a local minimum at 212 and 238 nm, respectively, due to the contribution of the phenyl groups of the peptide.[6] However, the additional minimum at 224 nm, as well as the positive CD signal below 195 nm, indicates an ordered conformation of the peptide, most likely that of a β-sheet.[5]

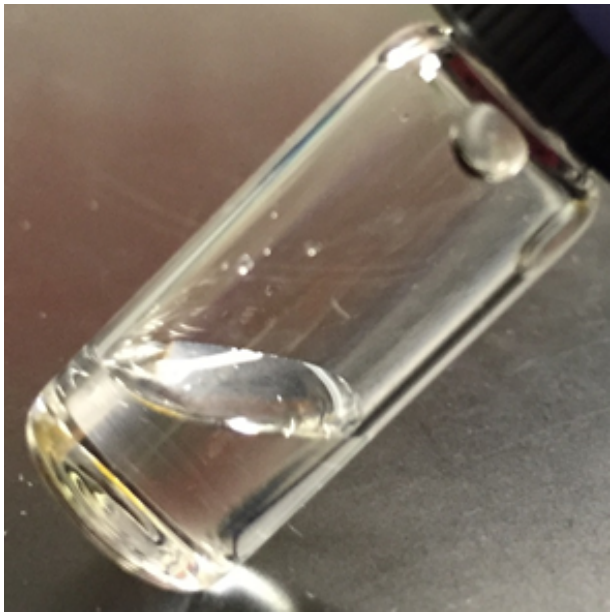

Fig. S16 Image of BFF dissolved in pH 10 NH_4OH/H_2O to which 6 M NaCl has been added. The clear solution indicates the inability of salt to induce self-assembly of BFF above pK_{BA}.

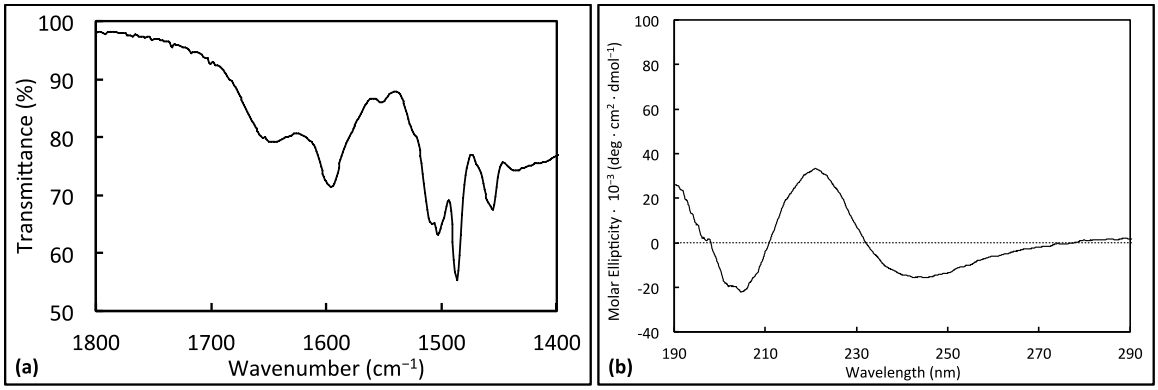

Fig. S17 (a) FTIR and (b) CD spectra of 10 mg/mL BFF dissolved in pH 7 phosphate buffer, to which 3 M NaCl was added, followed by a 6-fold excess of catechol. These spectra show the same relevant features as discussed in the corresponding spectra for 2 mg/mL BFF (Fig. S14 and Fig. S15), indicating that catechol similarly triggers a conformational order-disorder transition of BFF at 10 mg/mL.

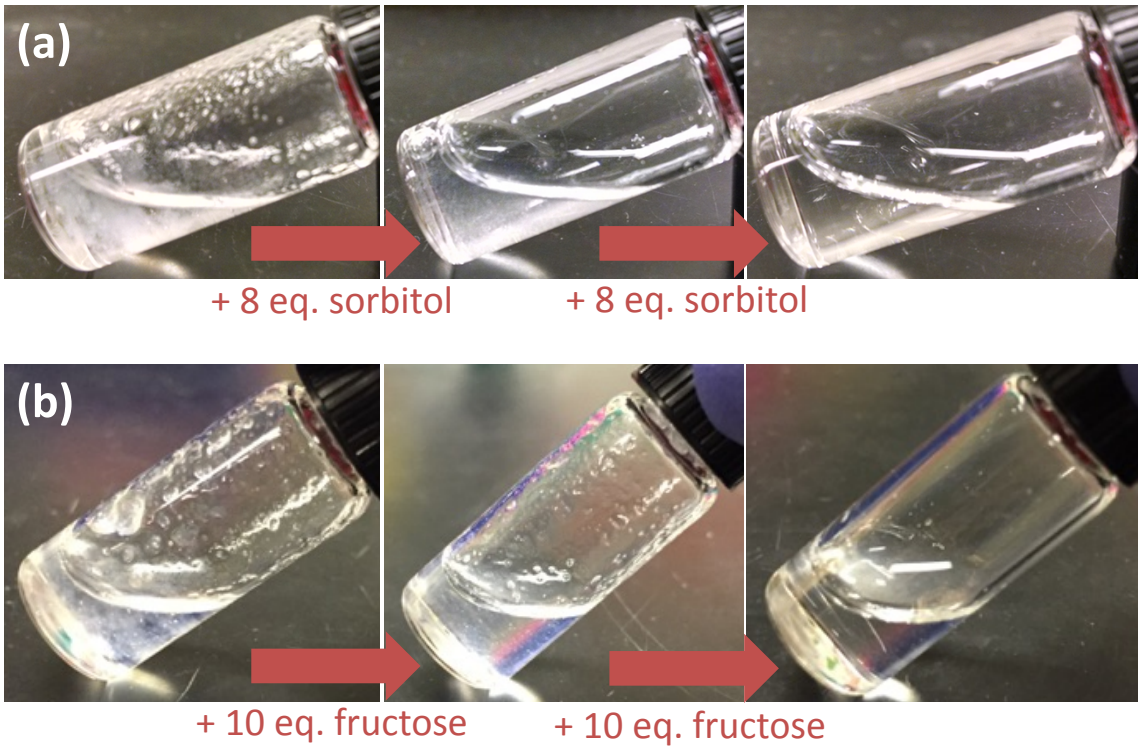

Fig. S18 Macroscopic observation of (a) sorbitol-triggered and (b) fructose-triggered disassembly of BFF. The leftmost image in both panels shows 2 mg/mL BFF self-assembled in pH 7.0 buffer by the addition of 3 M NaCl. Addition of 8-fold or 10-fold molar excesses (center) of sorbitol or fructose, respectively, causes substantial clarification of the solution, while addition of 16-fold or 20-fold molar excesses (right) of sorbitol or fructose, respectively, removes all visual trace of precipitate.

References

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(2) D. G. Hall, *Boronic Acids: Preparation and Applications in Organic Synthesis and Medicine*, ed. D. G. Hall, Wiley-VCH, Weinheim, Germany, 2005, pp. 1-133.

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