Supporting Information

Self-Assembly of Minimal Peptoid Sequences

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1. Experimental

1.1. Materials.

N,*N*-Diisopropylcarbodiimide (DIC) and bromoacetic acid (BAA) were procured from Apollo Scientific. Novabiochem MBHA low-loading (LL) Rink amide resin (ca. 0.3 mmole/g) and benzylamine (submonomer for Nphe) were purchased from Sigma-Aldrich (Dorset, UK). *Tert*-butyl 2-aminoethylcarbamate (submonomer for Nae) and *tert*-butyl 4-aminobutylcarbamate (submonomer for Nlys) were purchased from Fluorochem (Derbyshire, UK). ACS or higher grades of trifluoroacetic acid (TFA), triisopropylsilane (TIPS), piperidine, *N*,*N*-dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich, Rathburn (Walkerburn, Scotland) or Fisher Scientific (Acros Organics) and used without further purification.

1.2. Synthesis of Peptoids:

All peptoids were synthesized manually by typical solid-phase sub-monomer protocol at 0.1 to 0.3 mmole scale.[1] The Fmoc-protected resin was deprotected using 20% piperidine in DMF for 20 min, applied twice. Each monomer addition proceeded first with a 20 min bromoacetylation step at r.t. using BAA with DIC as coupling agent (20-equivalent BAA at 1.5 M in DMF and 19.5 equivalent DIC as 1:1 DIC:DMF solution). This was followed by a 2 h amine displacement step at r.t. (20-equivalent of submonomer benzylamine, *tert*-butyl 2-aminoethylcarbamate, and *tert*-butyl 4-aminobutylcarbamate for residues of, respectively, Nphe, Nae, and Nlys, all dissolved in NMP at 1 M). All steps received 5x DMF washing of the resin after each reaction step. N-terminal acetylation was performed using acetic anhydride (1 h r.t. shaking in 1 M acetic anhydride in DCM; amount used was a few times the volume of resin giving ca. 10-equivalent). The sequences and sidechain protection groups were cleaved by swelling the resin in 95% TFA with 2.5% water and 2.5% TIPS for at least 20 min. Before cleaving, the resin was washed in 3 repeated swelling-

deswelling cycles in DCM followed by methanol, and by a final wash in DCM, before drying by aspiration.

The cleaved peptoid was collected by filtering and rinsing the resin with more TFA cocktail, and excess TFA was removed using a rotary evaporator. The peptoid was precipitated from the oily product using diethyl ether. The collected material was dissolved in small amounts of 1:1 ACN:H_2O to ease sample transfer and further dried in a lyophilizer. The dried crude products were weighed and dissolved in ACN-H_2O mixtures (up to 100 mg/mL, depending on quantity available) for preparative RP-HPLC.

1.3. Peptoid Purification and Characterization.

The peptoids were purified by preparative RP-HPLC (Dionex Ultimate 3000 with an UltiMate 3000 Fraction Collector) using acetonitrile-water gradients containing 0.1% TFA at 5 mL/min flow rate with a semi-prep 100x21.2 mm Phenomenex Luna C18 column. Fractions containing the pure product were identified by ESI-LC-MS analysis (Agilent 1200 with a Poroshell C18 column coupled to an Agilent 6130 Mass Spectrometer) and by analytical RP-HPLC (Dionex P680) using a 250x4.6 mm "Nucleosil" C18 column (Macherey-Nagel) with a 30 min gradient of 5−95% acetonitrile and water containing 0.1% TFA at 1 mL/min flow rate.

The purified fractions in ACN-H_2O collected from preparative HPLC were aliquoted into glass vials and lyophilized. The exact weights of each aliquot were measured with a microbalance after lyophilization. Each aliquot typically contained 6-10 mg of purified product.

The MS and HPLC characterization data of the purified peptoids are shown in Figures S1-3. As customary for solid phase synthesized peptoids (and peptides), additional NMR characterization was not performed. This is because the residues and their monomers used in our sequences are quite common and the set of two iterative coupling reactions used closely followed common solid

phase protocols—the desired sequence and potential side products would only differ by specific combinations of monomers that are readily isolated by RP-HPLC and identified by MS.

1.4. Peptoid Self-Assembly.

Suitable volumes of milliQ deionized water at room temperature were pipetted directly into vials containing known weights of aliquoted peptoid dried products (see "Purification" above) to prepare concentrations of typically 10 or 20 mg/mL (i.e. 1 or 2 wt.%). In experiments where a range of concentrations were required (e.g. fluorescence measurements), the solution at the highest concentration was first prepared and then solutions at lower concentrations were obtained by serial dilution. The solutions were vortexed to ensure homogenization. No particular agitation or other steps were required for observing the assemblies.

The peptoid solutions were acidic as prepared since they contained some TFA lyophilized together with the peptoids. This is typical of preparative HPLC purified samples (using 0.1% TFA in the mobile phase). The pH of 20 mg/mL solutions (2 wt.%) was approximately ~3, as measured by universal pH indicator strips. It was not feasible to measure pH more precisely with a pH meter, using the pH probe sizes available, since generally only 0.3-0.5 mL were available. At 1 mg/mL (0.1 wt.%), the pH was ~6. At these pH values, the Nlys/Nae sidechains as well as the N-amine are expected to be protonated and positively charged.

1.5. Fluorescence Assays.

Regular measurements of the phenyl fluorescence were performed at r.t. with a Jasco FP-8500 instrument at an excitation wavelength of 265 nm (data obtained at 1 nm intervals using 5 nm excitation and 3 nm emission bandwidths; the scan speed was 200 nm/min). The pathlength of the quartz cuvette used was 2 mm. The highest concentration used was 20 mg/mL peptoid dissolved in DI water. This solution was first prepared and then other solutions were obtained by serial dilution. No particular agitation or other steps were required for observing the reported

spectroscopic features. Measurements at 20 mg/mL were taken from 30 min onwards, counting from initial dissolution. No significant changes were observed up to 4 months' storage at 4°C (the longest time tested). See Figures S6 and 7.

Critical aggregation concentration (CAC) measurements were performed using the sensitivity of the fluorescence intensity of ANS (8-anilo-1-naphthalenesulfonic acid) in environments of different polarities,[2] making it suitable to determine the CAC due to hydrophobic collapse. ANS assays were performed with quartz cell in a Varian Cary Eclipse spectrofluorimeter, measuring spectra from 400 to 670 nm (λ_{ex} = 356 nm) at a fixed 0.002 wt.% concentration of ANS. The highest peptoid concentration used was 1 wt.% in DI water (i.e. 10 mg/mL peptoid). Measurements of CAC were taken 2 h after the solutions were prepared.

Results from the ANS assays are plotted as I/I_0 *vs* log(c) (I and I_0: peak ANS fluorescence intensities with and without dissolved peptoid, respectively; c: peptoid concentration). The peak emission wavelength blue-shifted from ca. 525 nm to 510 nm as the content of TFA in the solution decreased concomitant with a decrease in peptoid concentration.

To obtain samples at different pH, the original 1 wt.% sample was diluted with DIW. This caused a corresponding increase in pH, as the TFA contained with the peptoid also decreased. For example, for N(FkF), the pH decreased from pH~3 at 2 wt.% to pH~4 at 0.4 wt.%, pH~5.5 at 0.1 wt.%, and pH~6.5 at 0.01 wt.%. Ideally, it would have been preferable to maintain the same pH across all concentration. However, we did not wish to introduce a different buffer system in case it interfered with assembly, and in any case, the pH was still similarly acidic (pH ~ 4) near the measured CAC of 0.3 wt.% as the initial pH at 2 wt.%. Moreover, we show in our manuscript that our structures are insensitive to pH from acidic to mildly basic (e.g. from pH 3 to 11 for N(FkF)). This is further corroborated by an additional CAC measurement we have performed for N(FkF) at pH 9 (see Figure S10), showing a CAC of ~0.4 wt.%, essentially identical, within uncertainty, to

our data shown in Figure 2. The pH was maintained at all concentrations by addition of small amounts of NaOH.

1.6. Absorbance Spectroscopy.

UV-vis absorbance measurements were performed in parallel with the phenyl fluorescence measurements using the same solutions in the same 2 mm pathlength quartz cuvette. The data were obtained using a Jasco V-660 instrument (data taken at 0.2 nm interval and bandwidth of 0.5 nm; scan speed was 400 nm/min).

1.7. Cryogenic-Transmission Electron Microscopy (Cryo-TEM).

As with other measurements, 2 wt.% (20 mg/mL) peptoid solutions in DI water were prepared for cryo-TEM. Imaging was carried out using a field emission cryo-electron microscope (JEOL JEM-3200FSC), operating at 200 kV. Images were taken in bright field mode and using zero loss energy filtering (omega type) with a slit width of 20 eV. Micrographs were recorded using a Gatan Ultrascan 4000 CCD camera. The specimen temperature was maintained at -187 °C during the imaging. Vitrified specimens were prepared using an automated FEI Vitrobot device using Quantifoil 3.5/1 holey carbon copper grids with a hole size of 3.5 μm. Just prior to use, grids were plasma cleaned using a Gatan Solarus 9500 plasma cleaner and then transferred into the environmental chamber of a FEI Vitrobot at room temperature and 100% humidity. Thereafter 3 μl of sample solution was applied on the grid and it was blotted twice for 5 seconds and then vitrified in a 1/1 mixture of liquid ethane and propane at temperature of -180 °C. The grids with vitrified sample solution were maintained at liquid nitrogen temperature and then cryo-transferred to the microscope.

1.8. Dynamic Light Scattering (DLS).

Samples containing 2 wt.% (20 mg/mL) peptoid in DI water were prepared and filtered through 0.20-μm Anotop filters from Whatman into standard 0.5 cm diameter cylindrical glass cells. Experiments were performed using an ALV CGS-3 system with a 5003 multi-digital correlator.

The light source was a 20 mW He-Ne laser, linearly polarized, with $\lambda = 633$ nm. Scattering angles in the range $30 \leq \theta \leq 150^{o}$ were used for all the experiments. DLS experiments measured the intensity correlation function. A general purpose constrained regularization method,[3] integrated in the software to control the ALV CGS-3 system with 5003 multi-digital correlator, was used to calculate the hydrodynamic radii of gyration, R_H, from the fitting to the intensity correlation function.

2. Supplementary Data

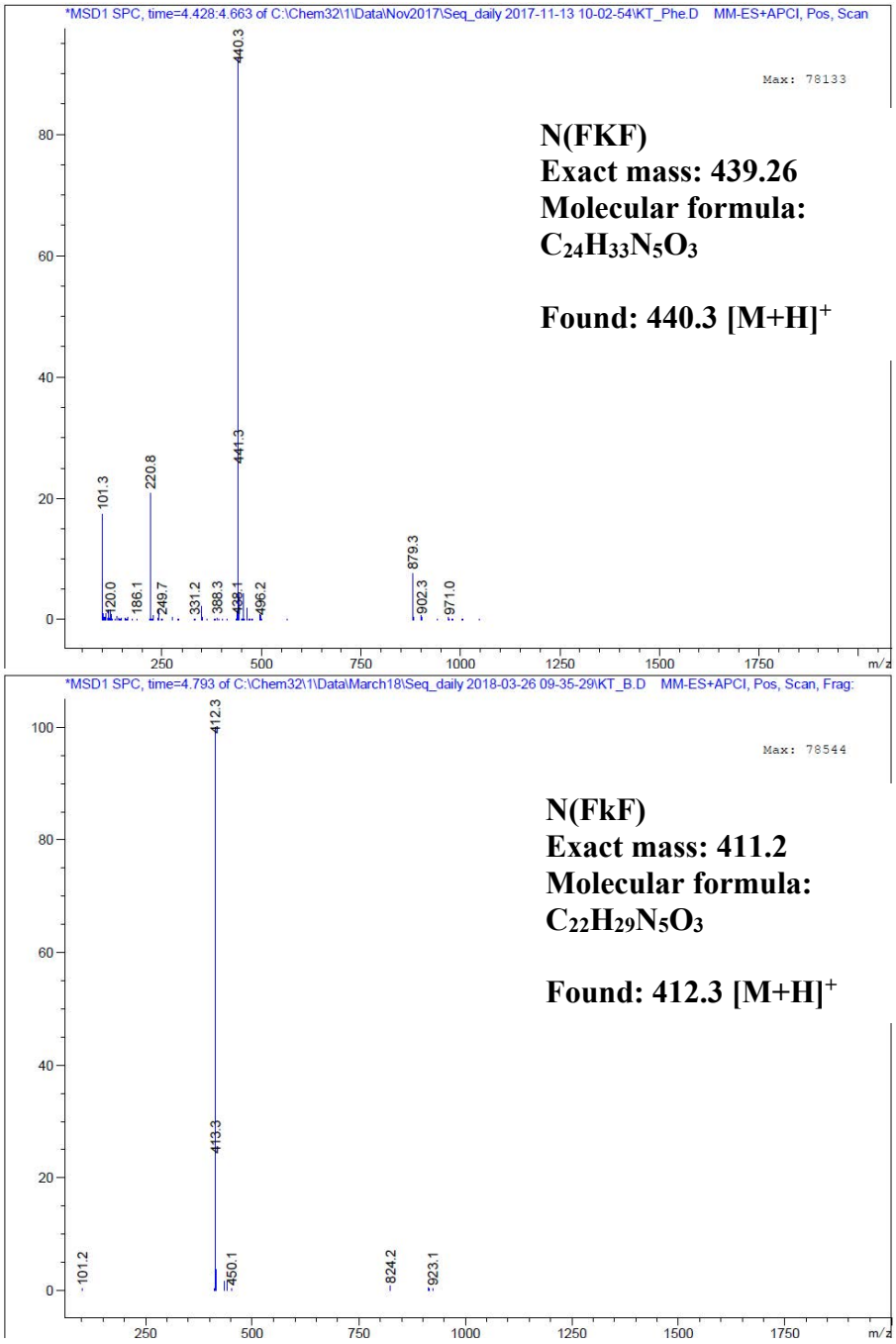

Figure S1. ESI MS data for peptoids N(FKF) and N(FkF).

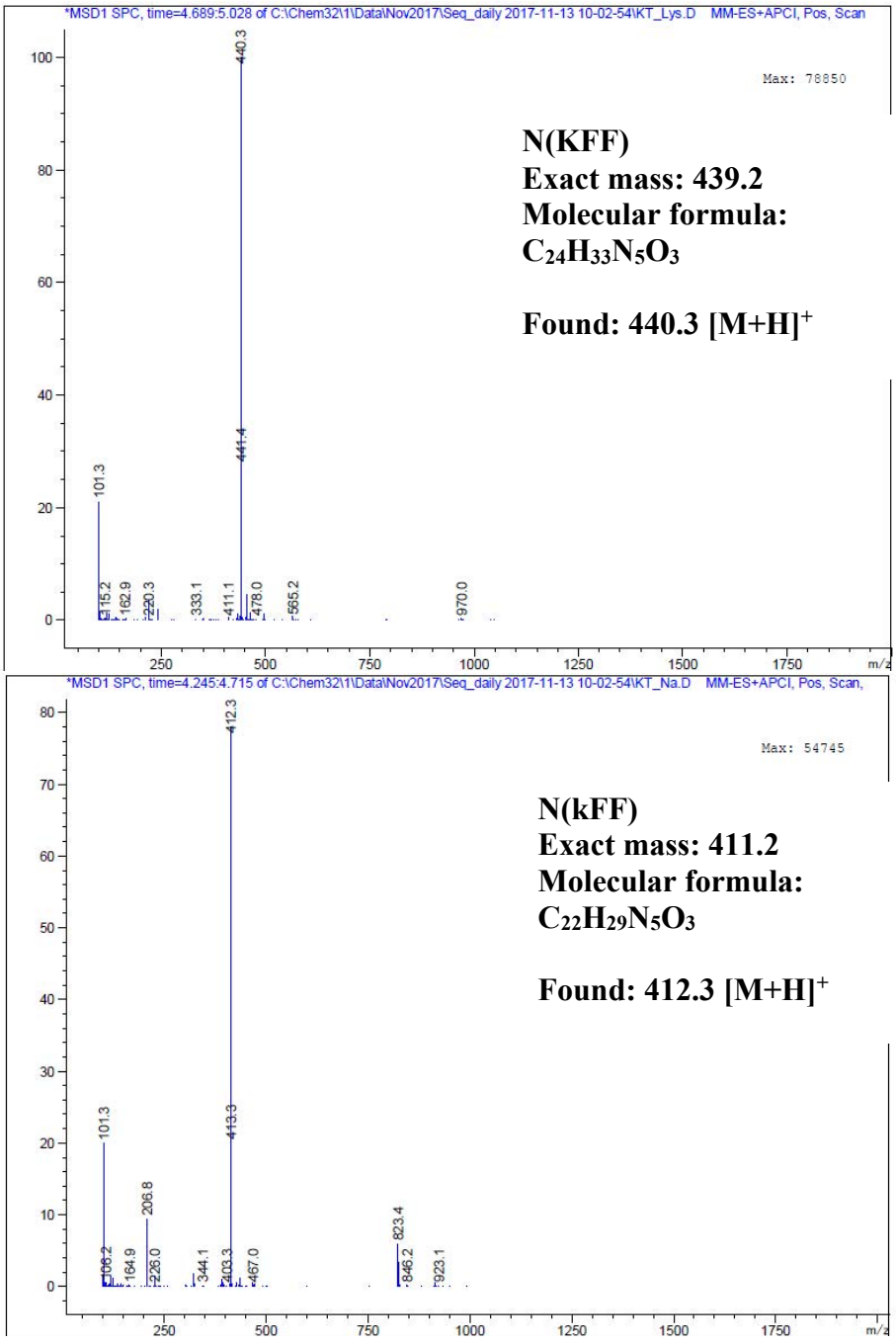

Figure S2. ESI MS data for peptoids N(KFF) and N(kFF).

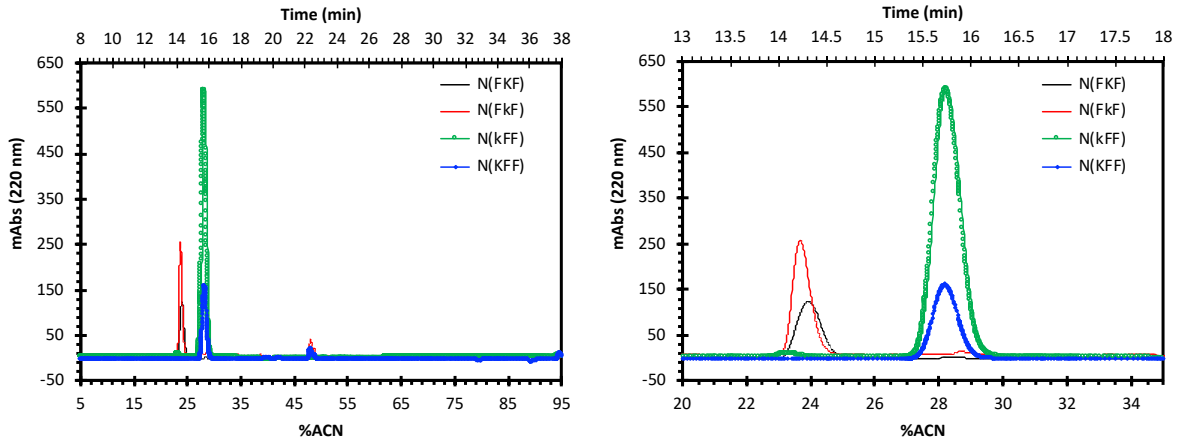

Figure S3. HPLC of purified tripeptoids (absorbance measured at 220 nm).

The elutions were obtained using a C18 column (see Experimental) and the chromatograms were baseline-subtracted by the HPLC software against a pure mobile phase reference measured in the same batch run. A) shows the full elution from 5 %ACN (8 min) to 95 %ACN (38 min). The column was equilibrated at 5% ACN before the gradient (0-8 min) and washed at 95% ACN afterwards (38-43 min). B) shows the region where the tripeptoids eluted. The different peak intensities were due to the different concentrations of peptoids collected in the different purified fractions. The disturbance around 22.5 min (48 %ACN) was an impurity in the column

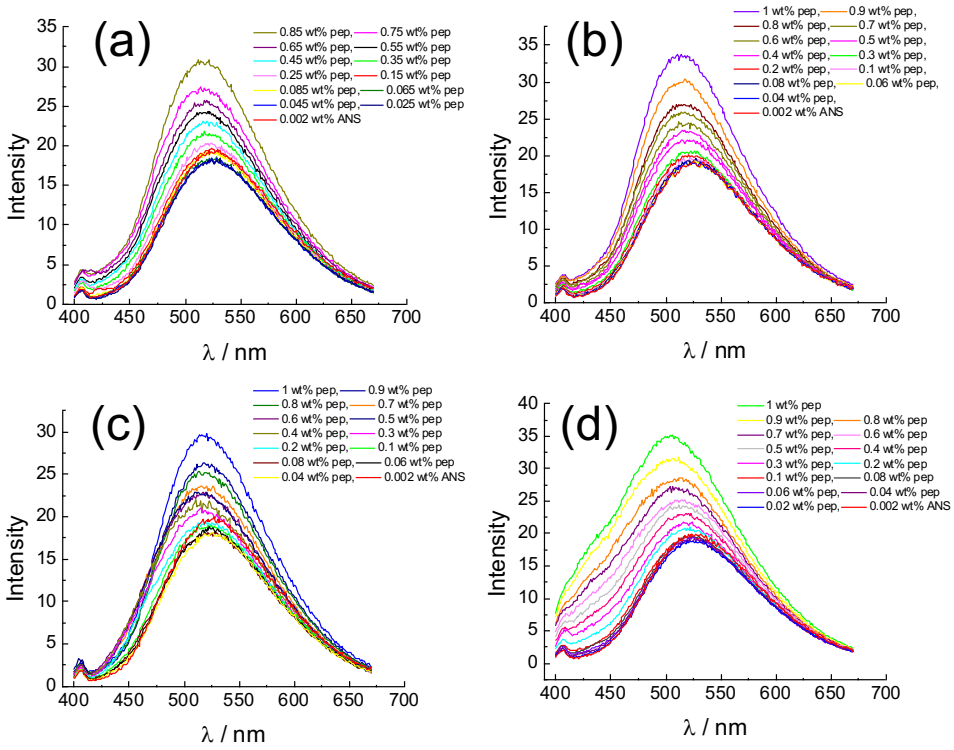

Figure S4. Original ANS fluorescence spectra at λ_{ex} = 356 nm. (a) N(FKF), (b) N(FkF), (c) N(KFF), (d) N(kFF).

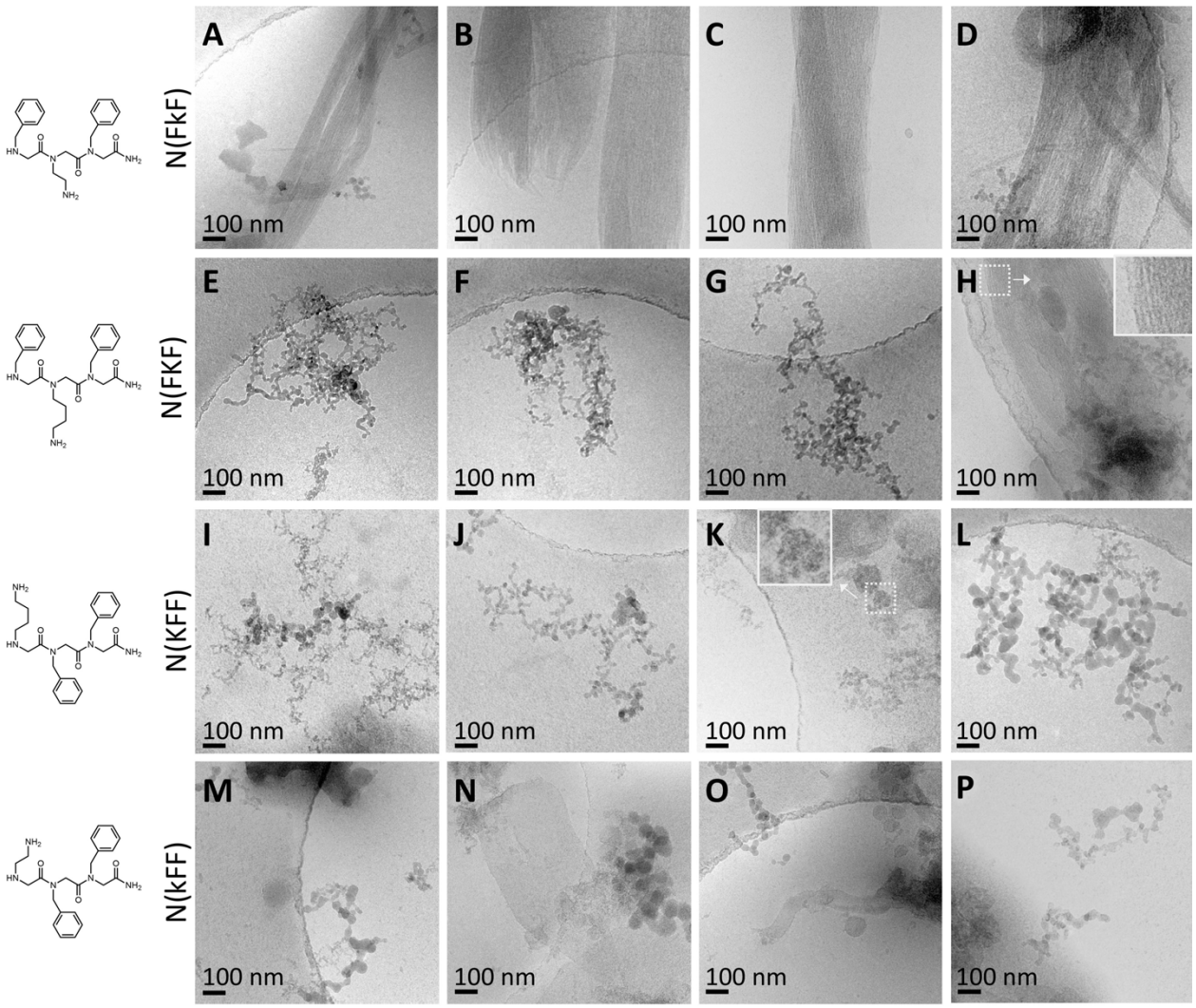

Figure S5. Overview of cryo-TEM observations.

The sequences N(FkF), N(FKF), N(KFF), and N(kFF) are arranged by row. All samples were prepared from 2 wt.% (20 mg/mL) solutions. The two columns on the left are duplicated from Figure 2 of the main text, while the two columns on the right show additional areas. The imaged features include both typical some less often observed structures, including fiber bundles of N(FKF) in (H), and sheet-like structures for N(kFF) in (N) that spans ca. 150 nm x 500 nm.

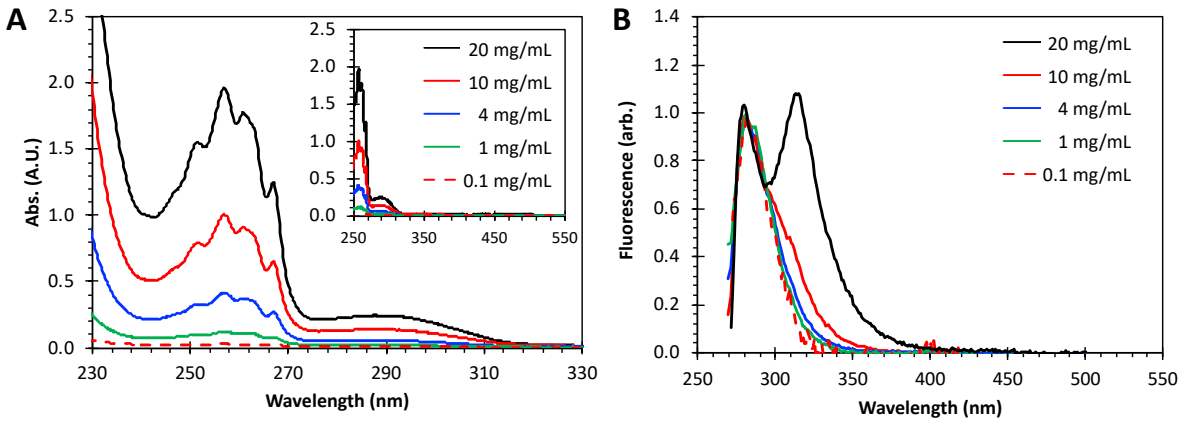

Figure S6. Absorbance (A) and fluorescence (B) measurements of N(FkF) at different concentrations.

The peptoid concentrations indicated—0.1 to 20 mg/mL—are equivalent to 0.01 to 2 wt.% (i.e. 24.3 μM to 48.6 mM).

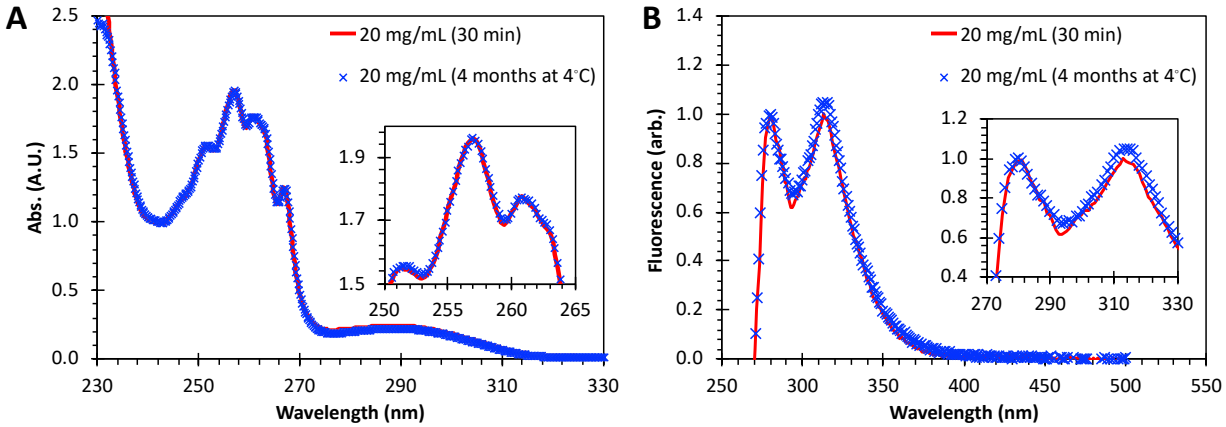

Figure S7. Comparison of N(FkF) absorbance (A) and fluorescence (B) after long-term storage.

The spectra were essentially unchanged before and after 4 month's refrigeration of the samples at 4^0C. The absorbances were normalized to the peak at 258 nm; the fluorescence traces were normalized to the 280 peak. Only minor differences were observed (see insets). There was a slight increase in the 312 nm excimer fluorescence peak intensity after storage, consistent with its assignment to an extended assembly structure that could become more ordered over time.

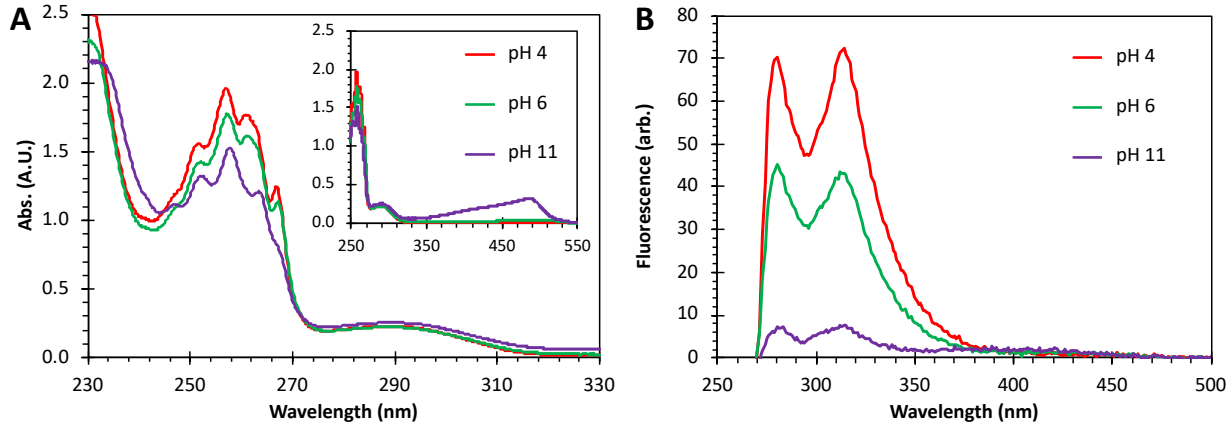

Figure S8. Absorbance (A) and fluorescence (B) measurements of N(FkF) at different pH.

The pH 4 sample was obtained by addition of 10 µL 0.1 M NaOH to 500 µL of a 20 mg/mL sample at pH 3, for which the data is shown in Figure S6 above. pH 6 and then pH 11 were obtained by further addition 0.1 M NaOH and 1 M NaOH.

In (A), the absorbance was used to estimate the concentrations after NaOH addition by the proportional decrease in the 270 nm peak absorbance of the phenyl fingerprint region. These were 20 mg/mL, 18 mg/mL, and 15.3 mg/mL for the pH 4, 6, and 11 samples, respectively.

In (B), the relative intensities of the fluorescence peaks of the N(FkF) monomer at 280 nm and red-shifted excimer at 312 nm remained essentially unchanged from acidic to basic pH. This is in remarkable contrast to the reduction in the 312 nm excimer peak with decreases in concentration shown in Figure S6B, indicating instead that an increase in pH does not affect the overall interactions between phenyl sidechains of the original nanofibers. However, the overall fluorescence intensity appeared to have been strongly attenuated above pH 6 by a color complex that showed absorbance from 280 to 480 nm (inset in panel A). The phenyl absorbance fingerprint pattern was also altered at pH 11 (but preserved up to pH 6). Further detailed investigations, beyond the scope of the present initial study, will be required to more fully understand the changes in molecular and assembled structures with changes in pH.

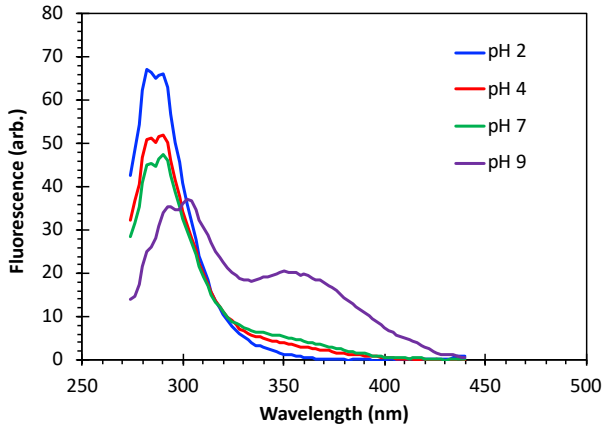

Figure S9. Fluorescence measurements of N(FKF) at different pH.

The sample preparation methods and conditions for N(FKF) are analogous to those for the N(FkF) peptoid shown in Figure S8 above. In the case of N(FKF), again, the phenyl fluorescence is largely unchanged over a wide range of pH from 2 to <9, from acidic to basic values. However, there is an additional peak around 360 nm starting to appear towards higher pH.

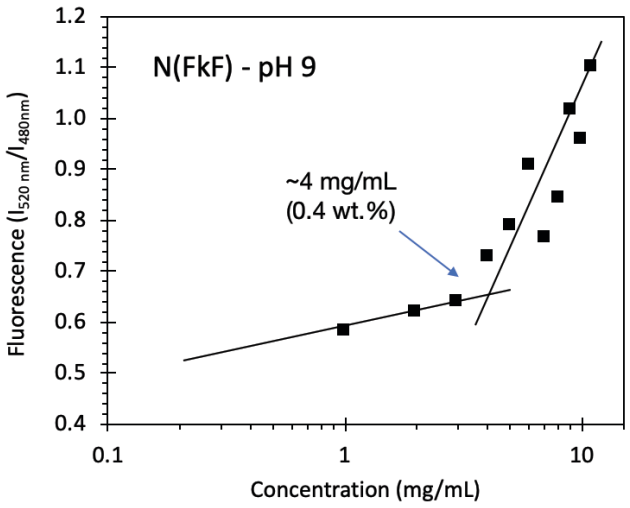

Figure S10. CAC measurement for N(FkF) at pH 9.

CAC was determined to be ~0.4 wt.%, essentially identical, within uncertainty, to the CAC measured at pH ~3 (data shown in Figure 2). The ANS fluorescence peak was shifted at this higher pH, and hence the ratio of I_{520nm}/I_{480nm} was instead plotted. DIW was added directly to pre-weighed lyophilized peptoids to obtain the highest concentrations shown and the pH was adjusted by addition of NaOH. The samples were diluted further for measurements at lower concentrations with water of pH already adjusted to 9.

References

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(3) Provencher, S. W. CONTIN: A general purpose constrained regularization program for inverting noisy linear algebraic and integral equations. *Computational Physics Communications* **1982,** *27*, 229-242.