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Supporting Information

Improved chemical and mechanical stability of peptoid nanosheets by photo-crosslinking the hydrophobic core

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1. Synthesis of B28-*p*Cl_7 peptoid oligomer

Materials: Bromoacetic acid, N,N'-diisopropylcarbodiimide (DIC), trifluoroacetic acid, triisopropylsilane (TIPS), and all amine reagents were purchased from commercial sources and used without further purification. Solvents were purchased from commercial sources.

General: B28-pCl_7 peptoid was synthesized by the solid-phase submonomer method as previously described,[1,2] using polystyrene Rink Amide resin (0.57 mmol/g) on a Protein Technologies Prelude peptide synthesizer. After the synthesis, the peptoid-resin was washed with DCM (3 × 1 mL) and treated with a cleavage cocktail of 95:5 TFA/H_2O (v/v) mixture for 2 h. The TFA cleavage solution was collected by filtering the resin through a disposable, polypropylene fritted cartridge, and the filtrate was evaporated to dryness using a Biotage V10 evaporator. The crude peptoid was redissolved in 35% MeCN in H_2O and purified by reversed-phase HPLC using a semipreparative column (5 µm, 250 mm × 21.2 mm, C18 Vydac column™) and a 40-75% gradient MeCN in H_2O over 60 min with a 15 mL/min flow rate, affording B28-pCl_7 peptoid as a white powder after lyophilization in 79% purity (13 mg, 6.3% yield). B28-pCl_7: analytical HPLC (20–80% MeCN, 30 min) retention time = 18.15 min (Sure 1a). The peptoid was characterized by MALDI mass spectrometry, using 10 mg/mL *alpha*-cyano-4-hydroxycinnamic acid in 1:1 MeCN/H_2O v/v with 0.1% TFA as matrix (Figure S1b). The molecular ion was found at 4119.6 m/z (calculated = 4119.6).

The lyophilized peptoid was re-dissolved in 2:1 (v:v) DMSO:H_2O to create a 2 mM stock solution (100X).

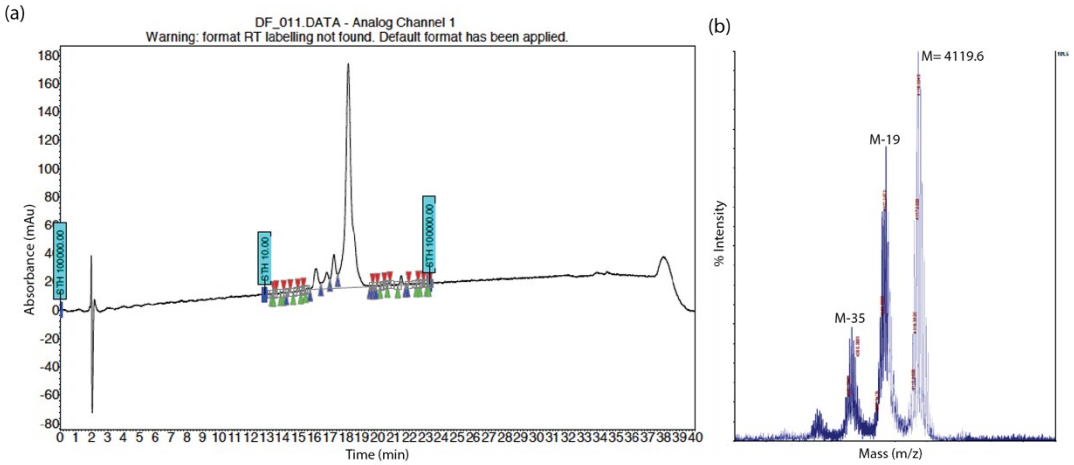

Figure S1. (a) RP-HPLC Chromatogram of purified B28-pCl_7 on C18 Column, using a gradient of 20-80% MeCN in water with 0.1% TFA, detecting at 214 nm (b) MALDI mass spectrum of purified B28-pCl_7 with *alpha*-cyano-4-hydroxycinnamic acid matrix.

2.Assembly of B28-*p*Cl_7 into nanosheets

General: Peptoid nanosheets were created using the previously reported vial rocking method.[2] Briefly, 500 µL of a 20 µM B28-pCl_7 solution in 10 mM Tris Buffer (pH 8) was placed in a 4 mL vial and rocked with a 900 second wait time for up to 3 days (288 cycles) to produce the nanosheets, using a custom-built rocking device. 2 µL of sheet solution with 1 µM Nile Red were deposited onto 1% agarose gel pads (1 cm^2) and visualized using florescence or differential interfering contrast microscopy.

3. Nanosheet UV crosslinking

General: 500 µL of freshly prepared peptoid nanosheet solution, without further processing, was irradiated directly in 4 mL (5 mm deep) open glass vials in a Spectrolinker XL-1500 UV Crosslinker (6 x 15 W lamps) for up to three hours, at 254 nm. Samples were placed 7.5 cm from the source. Samples were removed at various times for analysis. Highly-crosslinked nanosheets (irradiated for 3 hours) were used for characterization in sections 5-8 below.

4. SDS-PAGE analysis

General: 200 µL aliquots of nanosheet solution were concentrated in Amicon 100 kD centrifugal filter units at 8k rpm for 8 minutes to yield 20 µL of concentrated nanosheet solution. 10 µL of concentrated sheet solution was mixed with 20 µL Bio-Rad Laemmli sample buffer (161-0737). The sample was not heated as SDS was shown to sufficiently denature sheets. A ladder was created by adding 2 µL of Bio-Rad Precision Plus Protein™ Unstained Protein Standards (#161-0363) that ranged from 10 kD to 250 kD.These samples were then loaded onto Bio-Rad 4-20% precast gels, submerged in Tris-HCl buffer, and run at 120V for 90 minutes. The gel was then stained with Coomassie blue for 1.5 hours and destained in water overnight.

5. Raman spectroscopy analysis

General: The nanosheet solutions (2 mL) were washed with water (3x) to remove salts, then concentrated tenfold to 200 µL in Amicon 100k centrifugal filter units at 8k rpm for 10 minutes. The concentrated nanosheet solutions were then spun at 12.3k for 20 minutes in a microcenterfuge to form a solid pellet. This pellet was transferred *via* pipett to a glass cover slip that was plasma cleaned at 200 mTorr for 3 minutes and set to dry. Raman spectra were obtained on a Witec Alpha 300R confocal Raman microscope with 100x .095 NA objective, using a 532 nm laser (1mW) with an integration time of 0.1 sec/point.

6. Powder X-ray diffraction (XRD) analysis

General: The nanosheet solutions (2.5 mL) were washed, concentrated and pelleted as described before (section 5). The pelleted nanosheets were transferred to a kapton grid and the samples were then allowed to dry. Powder X-ray diffraction data were collected at beamline 8.3.1 at the Advanced Light Source located at Lawrence Berkeley National Laboratory, which is equipped with a 5 T single pole superbend source with an energy range of 5-17 keV. Data were collected with a 3 x 3 CCD array (ADSC Q315r) detector at a wavelength of 1.1159 Å. Data sets were collected with the detector 200 mm from the sample.

7. Atomic Force Microscopy (AFM) analysis

General: The nanosheet solutions (0.5 mL) were dialyzed against water overnight using 100 kD Float-A-Lyzer dialysis kits. 2 µL of dialyzed nanosheet solution was deposited onto a mica surface and allowed to dry. AFM micrographs were obtained on an Asylum MFP-3D AFM. The heights of three individual sheets in each sample were measured and their values averaged.

8. Mechanical and chemical stability analysis

Sonication: The nanosheet solutions (0.5 mL) were sonicated in a bath sonicator for up to 30 minutes. Periodically, small aliquots of sheet solution were removed, dyed with 1 μM Nile red, dropped onto a 1% agarose pad (1 cm^2) and imaged *via* fluorescence microcopy.

Lyophilization: Nanosheet solutions (0.5 mL) were dialyzed against water, overnight using 100 kD Float-A-Lyzer dialysis kits. The sample was then frozen at -80 °C and lyophilized overnight. The resulting powder was resuspended in 10 mM Tris Buffer (pH 8). The sheets were imaged by fluorescence microscopy as stated above (section 8).

Pelleting: Nanosheet solutions (0.5 mL) were transferred to a 1.5 mL Eppendorf tube and spun at 13.2k rpm for 20 minutes to produce a solid pellet. The supernatant was decanted, and the solid pellet was resuspended in fresh 10 mM Tris Buffer (pH 8) through pipette mixing. The sheets were imaged by fluorescence microscopy as stated above (section 8).

Acetonitrile Exposure: Acetonitrile was added to 20 μL of nanosheet solution in a 1.5 mL Eppendorf tube to yield a solution with the desired MeCN content. The volatiles were then removed using an Eppendorf Vacufuge concentrator at 60 °C for 20 minutes. The dry sample was resuspended in 20 μL of fresh 10 mM Tris Buffer (pH 8) thorough pipette mixing. The sheets were imaged by fluorescence microscopy as stated above (section 8).

9. References

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2. B. Sanii, R. Kudirka, A. Cho, N. Venkateswaran, G. K. Olivier, A. M. Olson, H. Tran, R. M. Harada, L. Tan and R. N. Zuckermann, *J. Am. Chem. Soc.*, 2011, **133**, 20808-20815.