

## Supporting Information

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

*Dillon Flood, Caroline Proulx, Ellen J. Robertson, Alessia Battigelli, Shuo Wang, Adam M. Schwartzberg, and Ronald N. Zuckermann\**

---

The Molecular Foundry, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94720, United States.

#### **Table of Contents**

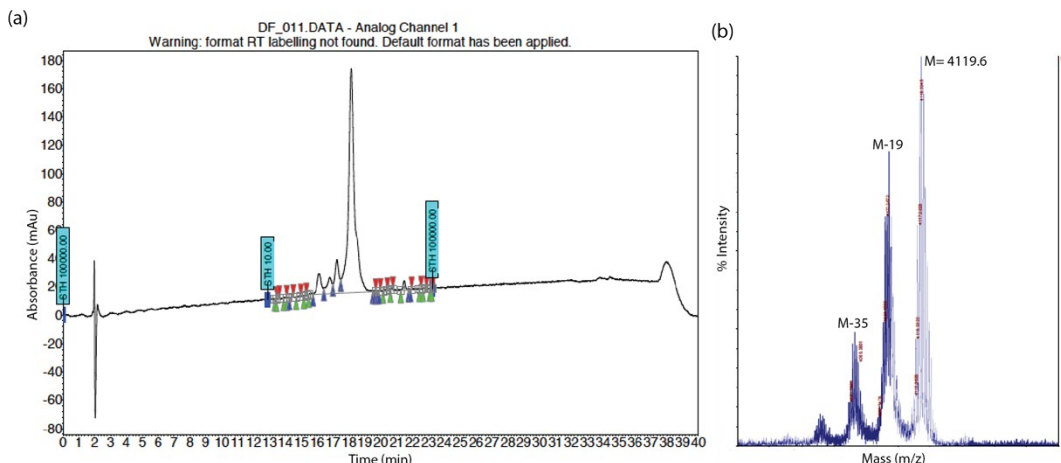
1. Synthesis of B28- <i>p</i> Cl <sub>7</sub> Peptoid .....	2
2. Assembly of B28- <i>p</i> Cl <sub>7</sub> into Nanosheets.....	2
3. UV Crosslinking Procedure .....	3
4. SDS-PAGE Analysis .....	3
5. Raman Spectroscopy Analysis .....	3
6. XRD Analysis .....	3
7. AFM Measurements .....	3
8. Mechanical and Chemical Stability Analysis .....	4
9. References .....	4

## 1. Synthesis of B28-*p*Cl<sub>7</sub> 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-*p*Cl<sub>7</sub> peptoid was synthesized by the solid-phase submonomer method as previously described,<sup>1,2</sup> 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<sub>2</sub>O (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<sub>2</sub>O 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<sub>2</sub>O over 60 min with a 15 mL/min flow rate, affording B28-*p*Cl<sub>7</sub> peptoid as a white powder after lyophilization in 79% purity (13 mg, 6.3% yield). B28-*p*Cl<sub>7</sub>: 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<sub>2</sub>O 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<sub>2</sub>O to create a 2 mM stock solution (100X).



**Figure S1.** (a) RP-HPLC Chromatogram of purified B28-*p*Cl<sub>7</sub> 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-*p*Cl<sub>7</sub> with *alpha*-cyano-4-hydroxycinnamic acid matrix.

## 2. Assembly of B28-*p*Cl<sub>7</sub> into nanosheets

**General:** Peptoid nanosheets were created using the previously reported vial rocking method.<sup>2</sup> Briefly, 500 μL of a 20 μM B28-*p*Cl<sub>7</sub> 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<sup>2</sup>) and visualized using fluorescence or differential interfering contrast microscopy.

### **3. Nanosheet UV crosslinking**

**General:** 500  $\mu$ L 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  $\mu$ L aliquots of nanosheet solution were concentrated in Amicon 100 kD centrifugal filter units at 8k rpm for 8 minutes to yield 20  $\mu$ L of concentrated nanosheet solution. 10  $\mu$ L of concentrated sheet solution was mixed with 20  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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 microcentrifuge 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  $\mu$ L 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  $\mu$ M Nile red, dropped onto a 1% agarose pad (1 cm<sup>2</sup>) and imaged *via* fluorescence microscopy.

**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  $\mu$ L 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  $\mu$ L 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**

1. K. T. Nam, S. A. Shelby, P. H. Choi, A. B. Marciel, R. Chen, L. Tan, T. K. Chu, R. A. Mesch, B.-C. Lee, M. D. Connolly, C. Kisielowski and R. N. Zuckermann, *Nat. Mater.*, 2010, **9**, 454-460.
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.