Supporting Information for:

**Surface-Directed Assembly of Sequence-Defined Synthetic Polymers into Networks of Hexagonally-Patterned Nanoribbons with Controlled Functionalities**

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S1. Methods

Peptoid Synthesis
All peptoids and peptoid-peptide hybrids were synthesized on a commercial Aapptec Apex 396 robotic synthesizer on using a solid-phase submonomer cycle as described previously. All amine submonomers and other reagents used for our peptoid synthesis are obtained from commercial sources and used without further purification. Rink amide resin (0.60 mmol/g, Novabiochem, Cat No. 01-64-0013) was used to generate C-terminal amide peptoids. In this method, the Fmoc group on the resin was deprotected by adding 2 mL of 20% (v/v) 4-Methylpiperidine/N,N-dimethylformamide (DMF), agitating for 20 min, draining, and washing with DMF. All DMF washes consisted of the addition of 1 mL of DMF, followed by agitation for 1 min (repeated five times). An acylation reaction was then performed on the amino resin by the addition of 1.0 mL of 1.2M bromoacetic acid in DMF, followed by 0.18 mL of N,N-diisopropylcarbodiimide (DIC, 1.15 mmol, neat). The mixture was agitated for 20 min at room temperature, drained, and washed with DMF. Nucleophilic displacement of the bromide with various primary amines occurred by a 1.0 mL addition of the primary amine monomer as a 1.0 - 1.5 M solution in N-methyl-2-pyrrolidone (NMP), followed by agitation for 60 min at room temperature. The monomer solution was drained from the resin, and the resin was washed with DMF as described above. The acylation and displacement steps were repeated until a polypeptoid of the desired length was synthesized. All reactions were performed at room temperature. The resultant crude were cleaved from the resin by addition of 4.0 mL 95% (v/v) trifluoroacetic acid (TFA) in water for 20 min. Following cleavage, the crude were dissolved in 4.0 mL mixture (v/v = 1:1) of water and acetonitrile for further purification.

For the synthesis of EEEGAPep-1 and DGRG-Pep-1, amino acids such as glycine, aspartic acid, glutamic acid and arginine were incorporated into the peptoid by using a standard peptide synthesis method. Fmoc-Gly-OH (Novabiochem), Fmoc-Asp, Fmoc-Glu and Fmoc-Arg(Pbf)-OH (Novabiochem) in N-methyl-2-pyrrolidone (NMP) (0.8 mmol in 2 mL of NMP) was added to the resin-bound amine with 0.4 M hydroxybenzotriazole in DMF and 137 µL of N,N'-diisopropylcarbodiimide (DIC) (0.92 mmol). The reaction mixture was agitated for 2 hours. When the Fmoc-Gly-OH was added next to a peptoid backbone, the reaction mixture was added twice for efficient coupling. Fmoc group was then deprotected with 20% piperidine in DMF for further synthesis. For these two functionalized peptoids, they were cleaved from resins using the mixture of 92.5:5:2.5 TFA/water/triethylsilane (v/v/v).

All peptoids and peptoid-peptide hybrids were purified by reverse-phase HPLC on a Vydac C4 or C18 column (10 µm, 22 mm × 250 mm), using a gradient of 5-95% acetonitrile in water with 0.1% TFA over 60 min. All final products were analyzed by analytical reverse-phase HPLC (5 - 95% gradient at 1.0 mL/min over 30 minutes at 60°C with a Vydac C18, 5 µm, 4.6 × 150 mm column), and electrospray mass spectrometry (Agilent 1100 series LC/MSD trap system, Agilent Technologies, Santa Clara, CA) or matrix-assisted laser desorption/ionization mass spectrometry (Applied Biosystem/MDS SCIEX 4800 MALDI TOF/TOF Analyzer). The final peptoid or peptoid-peptide hybrid products were lyophilized at least twice from their solution in mixture (v/v = 1:1) of water and acetonitrile. All lyophilized products were finally divided into small portions (3.0 × 10⁶ mol) and stored at -80°C.

For relative hydrophobicity test: purified peptoids were analyzed using Waters ACQUITY reverse-phase UPLC (5–95% CH₃CN in H₂O at 0.4 mL/min over 5 min at 40°C with a ACQUITY®BEH C18, 1.7 µm, 2.1 mm × 50 mm column) that was connected with a Waters SQD2 mass spectrometry system.

Peptoid sequences and their UPLC-MS characterizations
Structures of the synthesized peptoids and molecular weight of each peptoid as determined by mass spectrometry are shown below.
Pep-1: 1965.6 (Molecular weight), 1967.0 (Found:[M+H]+), 983.9 (Found:[M/2+H]+).

MS characterization of Pep-1

UPLC characterization of Pep-1
Pep-2: 2068.9 (Molecular weight), 2070.9 (Found:[M+H]^+), 1035.4 (Found:[M/2+H]^+).

MS characterization of Pep-2

UPLC characterization of Pep-2
Pep-3: 1896.7 (Molecular weight), 1897.9 (Found:[M+H]+), 949.5 (Found:[M/2+H]+).

UPLC characterization of Pep-3

MS characterization of Pep-3
Pep-4: 1896.7 (Molecular weight), 1898.0 (Found:[M+H]^+), 948.9 (Found:[M/2+H]^+).

UPLC characterization of Pep-4

MS characterization of Pep-4
EEEG-Pep-1: 2410.0 (Molecular weight), (Found:[M+H]+), 1206.2 (Found:[M/2+H]+).

UPLC characterization of EEG-PEp-1

MS characterization of EEG-Pep-1
DGRG-Pep-1: 2351.0 (Molecular weight), (Found:[M+H]^+), 1176.1 (Found:[M/2+H]^+).

UPLC characterization of DGRG-Pep-1

MS characterization of DGRG-Pep-1
Mica-directed assembly of peptoids
1.0 mL aqueous solution of CaCl$_2$ (22 mM), NaCl (100 mM), MgCl$_2$ (22 mM), ZnCl$_2$ (22 mM), CoCl$_2$ (100 mM), FeCl$_2$ (22 mM), or FeCl$_3$ (22 mM) was mixed with 1.0 mL 25 mM Tris buffer (pH = 8.0) in 4.0 mL glass vial, then 20 µL 2.0 mM peptoid stock solutions were added and well-mixed. The resultant solutions were incubated with freshly-cleaved mica and then left undisturbed at room temperature for assembly studies.

For self-assembly of Pep-I-Ca$^{2+}$ complexes in the presence of NaCl aqueous solution: Aqueous solutions of CaCl$_2$ (50 mM) and NaCl (1.0 M) were used to make a final assembling solution containing 25 mM CaCl$_2$ and 0.5 M NaCl, while keeping the total solution volume and Pep-I concentration same.

For characterizations of peptoid assemblies on mica surfaces, about 4 - 6 days later, mica surfaces from above incubated solutions were thoroughly washed with water and N$_2$ dried before ex situ AFM imaging.

AFM-based dynamic force spectroscopic study of peptoid-peptoid and peptoid-mica interactions
Microlever Si$_3$N$_4$ AFM tips (Bruker, MSCT, CA) levers B, D or E were used in all experiments. The tip cleaning and cross-linking were done by using a similar method as described previously. Bare tips were first cleaned using a plasma cleaner for 20 seconds. These cleaned tips were immediately used for coating: they were coated with 4 nm Cr followed by ~20 nm Au by thermal evaporation. These coated probes were further plasma cleaned for ~30 seconds before they were immersed for 20 minutes in an anhydrous methanol solution containing 10 mM of the heterobifunctional cross-linker LC-SPDP (Thermo Scientific) consisting of a pyridyl disulfide, which adsorbs to Au, and an N-hydroxysuccinimide (NHS) ester that reacts with the N-terminal amine or lysine of the peptide to form an amide bond. After rinsing well using anhydrous methanol, the tips were immersed in aqueous solution of Pep-I (pH = ~8.0, NaOH was used to adjust solution pH) over night. Functionalized tips were rinsed well with water prior to use.

Force measurements between peptoid-modified tips and freshly-cleaved mica or pre-assembled Pep-I nanoribbons on a mica surface were performed under water with a few drops of Tris buffer (pH 8.0, 25 mM) solutions to adjust pH. Measurements were made with the MFP3D Atomic Force Microscope (Asylum Research, Santa Barbara, CA). To account for any surface heterogeneity, a custom routine was used to randomly sample points on the surface to give a representative average. A constant approach velocity of 200 nm/s was used for every pulling velocity studied. ~ 200 pN trigger forces were used to contact the surface and dwell for 0.99 second before pulling away.
S2. Supporting Figures

Figure S1. Assembly of Pep-I on mica surfaces in the presence of other cations: a) NaCl, b) MgCl$_2$, c) ZnCl$_2$, d) CoCl$_2$, e) FeCl$_2$ and f) FeCl$_3$, which no ribbon structures were formed in the absence of Ca$^{2+}$ cations; we reason that features of Ca$^{2+}$ cations, which include the +2 charge, the size, and the coordination ability to carboxylate, are significant for the formation of hexagonally-patterned Pep-I nanoribbon networks on mica surfaces.

Figure S2. Pep-I self-assembled into nanoribbon networks when even 0.5M NaCl was present with
CaCl₂ (25mM) in the solution used for assembly; the inset is a 2D Fourier transform of the main image showing the six-fold symmetry. Ex situ AFM data show that high ionic strength did not abolish nanoribbon formation, suggesting that hydrophobic interactions contribute significantly to nanoribbon stability.

**Figure S3.** (a-d) Pep-1 self-assembly on four different surfaces: (a) on calcite (104) faces, (b) On Au(111) surface, (c) On silicon (111) surface, (d) On the carbon film of a TEM grid.

**Figure S4.** (a) The proposed model showing the locations of Ca²⁺ cations on the muscovite mica (001) plane, in which the Ca²⁺···Ca²⁺ distance can be either 5.2 or 9.0 Å; Peptoid ribbons are aligned along red-dotted-line directions, and the carboxylate-carboxylate distances of Pep-1 match well with Ca²⁺···Ca²⁺ distances (3.6 nm = 4 × 9 Å), while the flexibility of the peptoid backbone and sidechain N-(2-carboxyethyl)glycines (Nce) groups as well as the potential mobility of absorbed Ca²⁺ cations at the muscovite mica surface make it possible for all other carboxylate groups to coordinate with Ca²⁺ cations; our unpublished high-resolution AFM data further confirmed this model. (b) The proposed model showing Pep-1 with deprotonated carboxylate groups bonded to mica surface through Ca²⁺-carboxylate interactions; the N···N distance of Pep-1 backbone is 3.6 Å, and the carboxylate groups on both two ends
have a distance of 3.6 nm. (c) The proposed model showing the transformation of Pep-1 – Ca\(^{2+}\) discrete nanoparticles to nanoribbons on mica surfaces (nitrogen, blue; oxygen, red; calcium cation, purple; chloride, cyan).

Figure S5. Representative force-distance curves at the retraction velocity 200 nm/s showing Pep-1 functionalized AFM tips interact with mica (a), peptoid nanofibers (b) in the presence of CaCl\(_2\), or with mica in the absence of CaCl\(_2\) (c).

Figure S6. (a) Reverse phase UPLC data showing the relative hydrophobicities of Pep-1 – Pep-4, where longer retention time indicates higher hydrophobicity. (b-d) AFM images showing peptoid assembles from Pep-2 (a), Pep-3 (b) or Pep-4 (c) in the presence of aqueous CaCl\(_2\) solution. We reason that the resulting formation of randomly-packed nanoribbons of Pep-2 and no-assembly of Pep-2 and Pep-4 is due to the change of inter-peptoid hydrophobic interactions that are required to stabilize the nanoribbon formation.

Figure S7. Reverse phase UPLC data showing the relative hydrophobicities of Pep-1, EEEG-Pep-1 and DGRG-Pep-1, where longer retention time indicates higher hydrophobicity.
S3. References


