

## Supporting Information

# Electron-Equivalent Valency through Molecularly Well-Defined Multivalent DNA

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# Table of Contents

Section 1: Oligonucleotide sequences and synthesis.....	S03
Section 2: DNA functionalization of nanoparticles.....	S04
Section 3: Synthesis of multi-azide cores.....	S05
Section 4: NMR spectra of multi-azide cores .....	S12
Section 5: Synthesis and purification of SMDHs.....	S24
Section 6: PAGE purification and characterization.....	S26
Section 7: SAXS characterization.....	S28
Section 8: STEM characterization .....	S34
Section 9: UV-vis spectroscopy experiments.....	S39
Section 10: References.....	S41

## Section 1: Oligonucleotide Sequences and Synthesis

Oligonucleotide strands were synthesized using a MerMade 12 oligonucleotide synthesizer (Bio Automation, Texas, USA) or an ABI synthesizer on controlled pore glass (CPG) beads, specifically the Universal UnyLinker Support (1000 Å). All phosphoramidites and oligonucleotide synthesis reagents were purchased from Glen Research or ChemGenes and used as received. All oligonucleotides were deprotected under conditions recommended by the manufacturer and purified by reverse-phase high-performance liquid chromatography (RP-HPLC; Agilent). All oligonucleotides were characterized by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF; AutoFlex-III, Bruker) mass spectrometry (matrix: 3-hydroxypicolinic acid). DNA concentrations were determined by measuring the solution absorbance at  $\lambda = 260$  nm (Cary 5000 UV-vis spectrophotometer, Varian) and using the corresponding extinction coefficients calculated by the OligoAnalyzer tool (Integrated DNA Technologies). A complete list of the oligonucleotides that were synthesized can be found in Table S1.

**Table S1.** DNA sequences used in this study.

	DNA Sequence	Calculated MW	Measured MW
PAE anchor	5' Thiol-(Sp18) <sub>2</sub> -CATCCATCCTTATCAACT 3'	6380	6355
PAE linker	5' AAGGAA-A-AGGTGAGTATGAGTCGTT 3'	7834	7847
EE anchor	5' DBCO-(Sp18) <sub>2</sub> -CATCCATCCTTATCAACT 3'	6613	6618
EE linker	5' TTCCTT-A-AGTTGATAAGGATGGATG 3'	7751	7795
EE linker Cy3	5' TTCCTT-A-AGTTGATAAGGATGGATG-Cy3 3'	8259	8259
EE linker Cy5	5' TTCCTT-A-AGTTGATAAGGATGGATG-Cy5 3'	8285	8287

Sp18: 18-*O*-Dimethoxytritylhexaethyleneglycol,1'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Spacer phosphoramidite 18)

Thiol: 1-*O*-Dimethoxytrityl-hexyl-disulfide,1'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Thiol-modifier C6 S-S)

DBCO: 10-(6-oxo-6-(dibenzo[b,f]azacyclooct-4-yn-1-yl)-capramido-*N*-ethyl)-*O*-triethyleneglycol-1'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (5'-DBCO-TEG phosphoramidite)

Cy3: 1-[3-(4-monomethoxytrityloxy)propyl]-1'-[3-[(2-cyanoethyl)-(N,N-diisopropyl)]

phosphoramidite]propyl]-3,3,3',3'-tetramethylindocarbocyanine chloride (Cyanine 3 phosphoramidite)

Cy5: 1-[3-(4-monomethoxytrityloxy)propyl]-1'-[3-[(2-cyanoethyl)-(N,N-diisopropyl) phosphoramidite]propyl]-3,3,3',3'-tetramethylindocarbocyanine chloride (Cyanine 5 phosphoramidite)

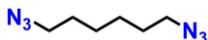
## **Section 2: DNA Functionalization of Nanoparticles**

10-nm gold nanoparticles (AuNPs) were purchased from Ted Pella and used as received. 5' thiolated DNA strands were activated as follows: 0.8 mL of 100 mM dithiothreitol (DTT) in 170 mM Na<sub>3</sub>PO<sub>4</sub> (pH~7.0-8.0) was added to 40 OD of 5' thiolated DNA. After 1 h, excess DTT was removed using a size exclusion column (NAP-10), and the eluent was added to 40 mL of AuNPs. After brief vortexing, the AuNP solution was allowed to stand for 2 h; then, 0.4 mL of 1% sodium dodecyl sulfate (SDS) was added to prevent nanoparticle aggregation. After 20 min, 0.4 mL of 1 M phosphate-buffered saline (PBS, pH = 8) was added to the AuNP solution, which was then vortexed and sonicated briefly. After 30 min, using a stock solution of 5 M NaCl, the AuNP solution was brought up to 0.5 M NaCl over seven additions. The AuNP solution was left for 25 min between each salt addition to enable the functionalization of the nanoparticle surface with a dense coating of DNA strands. After the salt additions, the AuNP solution was shaken at 100 rpm for two days in the dark. The AuNPs were concentrated and purified using centrifugal filters (100 kDa) to remove excess, free DNA strands. The AuNPs were washed thoroughly with 10 mL of 0.02 % SDS (2×) and water (3×), and the concentration of the AuNPs was determined by measuring the absorbance at 520 nm using UV-vis spectroscopy. The DNA-functionalized AuNPs were stored in the dark and in a refrigerator at 4 °C prior to use.

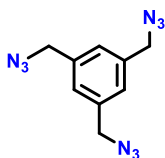
### Section 3: Synthesis of Multi-Azide Cores

All chemicals, reagents, and solvents were purchased from either Sigma-Aldrich, Acros, Alfa Aesar, or BroadPharm and used as received unless otherwise stated. Stir bars and glassware were dried in an oven at 180 °C prior to use. Flash chromatography was performed using silica 60 (230–400 mesh ASTM, 0.040–0.063 mm; Fluka). Deuterated solvents were purchased from Cambridge Isotope Laboratories or Sigma Aldrich and used as received. A Bruker Avance 400 MHz NMR spectrometer were used to record  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, and chemical shifts ( $\delta$ ) are reported in parts per million.  $^1\text{H}$  NMR spectra were referenced to residual proton resonances in the deuterated solvents (dichloromethane- $d_2 = \delta$  5.32; chloroform- $d_3 = \delta$  7.26; methanol- $d_4 = \delta$  3.31), while absolute referencing was applied for heteronuclear NMR spectra ( $\Xi_{\text{C}} = 25.145020$ ). High resolution mass spectra (HRMS) were recorded on an Agilent 6120 LC-TOF instrument in positive ion mode, and MALDI spectra were recorded on a Bruker AutoFlex-III. **Caution:** *Sodium azide and multi-azide organic molecules are potentially explosive, and thus the heating, drying, and transferring processes should be done with caution and using appropriate personal protective equipment (PPE). Hydrazoic acid can potentially evolve in mildly acidic solutions of inorganic azides. All procedures should be conducted in a fume hood.*

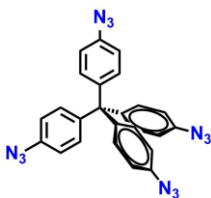




**1,6-diazidohexane** was synthesized by adapting a previously reported procedure.<sup>1</sup> 1,6-dibromohexane (4.76 g, 3 mL, 19.5 mmol, 1 equiv.) and sodium azide (3.8 g, 58.5 mmol, 3 equiv.) were added to 100 mL of DMF/water (v/v 9:1). The reaction mixture was heated at 80 °C. After 26 h, the reaction mixture was cooled to room temperature. The mixture was extracted with brine/diethyl ether (3×), and the organic layers were combined, dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo* to give a light brown oil (2.99 g, 17.8 mmol, isolated yield = 91%). <sup>1</sup>H NMR (400 MHz, chloroform-*d*) δ 3.28 (t, J = 6.9 Hz, 4H), 1.68 – 1.51 (m, 4H), 1.49 – 1.35 (m, 4H). <sup>13</sup>C NMR (101 MHz, chloroform-*d*) δ 51.30, 28.71, 26.28. HRMS (ESI+) *m/z* Calcd for [M–Cl]<sup>+</sup>: 377.0766 *m/z*. Found: 377.0770 *m/z*.

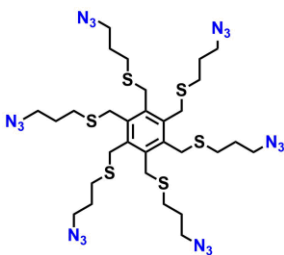


**1,3,5-tris(azidomethyl)benzene** was synthesized by adapting a previously reported procedure.<sup>2</sup> To a solution of 1,3,5-tris(bromomethyl)benzene (1.5 g, 4.2 mmol, 1 equiv.) in 11 mL DMF, sodium azide (4.1 g, 63 mmol, 15 equiv.) was added under a nitrogen atmosphere, and the reaction was stirred at 85 °C for 20 h. After cooling to room temperature, 100 mL of saturated sodium chloride solution (aq) was added to the reaction mixture, which was then extracted with brine/dichloromethane three times. The combined organic layers were dried with magnesium sulfate, filtered, and concentrated to give a light brown oil (0.96 g, 3.95 mmol, isolated yield = 94%). <sup>1</sup>H NMR (400 MHz, chloroform-*d*) δ 7.25 (s, 3H), 4.40 (s, 6H). <sup>13</sup>C NMR (101 MHz, chloroform-*d*) δ 136.97, 127.44, 54.28.



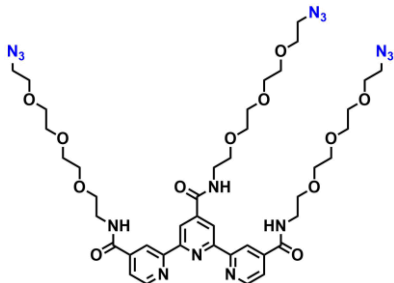
**Tetrakis(4-azidophenyl)methane** was synthesized by adapting a previously reported procedure.<sup>3</sup> Tetrakis(4-aminophenyl)methane (0.99 g, 2.6 mmol, 1 equiv.) was dissolved in 50 mL of 2 M HCl (aq) in a 500-mL round bottom flask and cooled to 0 °C. An aqueous solution (5 mL) of sodium nitrite (0.8 g, 11.7 mmol, 4.5 mmol) was added with vigorous stirring for 30 min, and the pale-yellow solution turned pale-brown. The solution was then

neutralized with calcium carbonate slowly until gas bubbles no longer formed. Then, 5 mL of a solution of sodium azide (0.9 g, 13.9 mmol, 5.3 equiv.) was added to the reaction mixture, which was pre-cooled in an ice bath. After stirring for 20 min, the resulting mixture was extracted with brine/chloroform (3×), and the organic layers were combined, dried over magnesium sulfate, and dried *in vacuo* to obtain a creamy powder (0.817 g, isolated yield = 65%). <sup>1</sup>H NMR (400 MHz, chloroform-*d*) δ 7.17 – 7.09 (m, 8H), 6.98 – 6.89 (m, 8H). <sup>13</sup>C NMR (101 MHz, chloroform-*d*) δ 142.85, 138.18, 132.04, 118.39, 63.24. HRMS (ESI+) *m/z* Calcd for [M-Cl]<sup>+</sup>: 607.0020 *m/z*. Found: 607.0029 *m/z*.

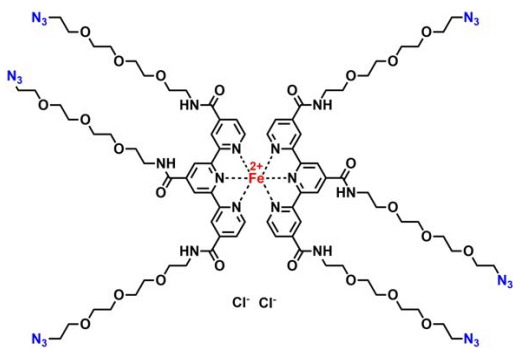


**Hexakis[(3-azidopropyl)thiomethyl]benzene.** 2.59 mL of 25% wt. sodium methoxide (9 equiv.) solution in methanol was transferred to a round-bottom flask and bubbled with nitrogen for 30 min. (3-Azidopropyl)thioacetate (1.803 g, 11.3 mmol, 9 equiv.) was then added dropwise to the sodium methoxide solution and stirred at room temperature for 30 min. The clear solution turned cloudy yellow gradually. Hexakis(bromomethyl)benzene (0.8 g, 1.26 mmol, 1 equiv.) was then added to the round bottom flask, which was then attached to a reflux condenser. After three cycles of freeze-pump-thaw, the reaction was refluxed over two days at 65 °C. After removing methanol using a rotary evaporator, the residue was dissolved in dichloromethane and extracted with brine/dichloromethane (3×). The organic layers were combined, dried over sodium sulfate, and purified over a silica column (eluent: hexane/dichloromethane). The product eluted at 100% dichloromethane (5% methanol might be needed to get the product off the column) was dried to give an orange oil (0.449 g, 0.526 mmol, isolated yield = 42%). <sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 4.04 (s, 12H), 3.47 (t, J = 6.6 Hz, 12H), 2.76 (t, J = 7.1 Hz, 12H), 1.97 (p, J = 6.9 Hz, 12H). <sup>13</sup>C NMR (101 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 135.80, 50.17, 30.96, 30.75, 28.88. HRMS (ESI+) *m/z* Calcd for [M+Na]<sup>+</sup>: 875.253 *m/z*. Found: 875.039 *m/z*.



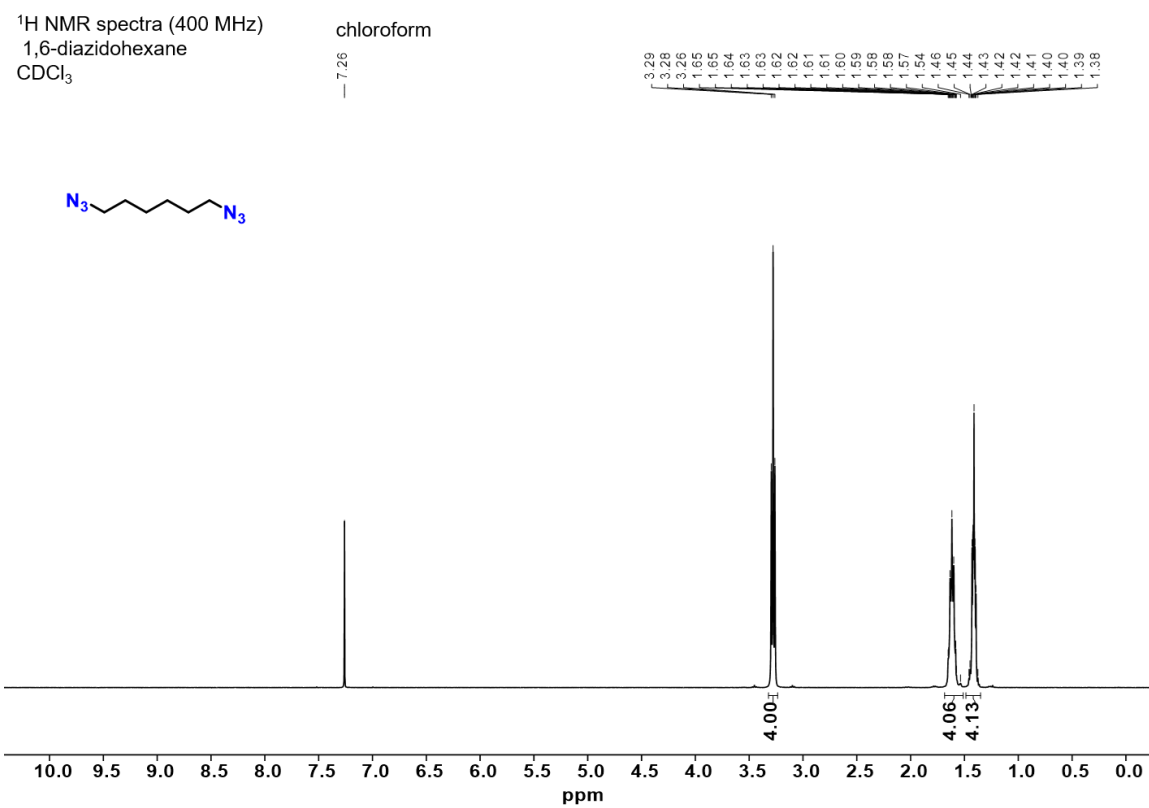


**Tri(11-azido-3,6,9-trioxaundecanyl) 2,2':6',2''-terpyridine-4,4',4'''-tricarboxamide** was synthesized by adapting a previously reported procedure.<sup>4</sup> Trimethyl 2,2':6',2''-terpyridine-4,4',4'''-tricarboxylate (0.2 g, 0.49 mmol, 1 equiv.) was added to 37 mL of 1 M sodium hydroxide solution in methanol/water (v/v 3:1) and refluxed at 95 °C for 21 h. After cooling the reaction mixture to room temperature, the precipitate was centrifuged and washed with methanol/water (v/v 3:1). The precipitates were then dissolved in 20 mL of water, and the resulting solution was acidified with 3 M HCl, and the newly formed precipitates were collected by filtration and washed with water (3×) and methanol (3×). After drying, the precipitate (2,2':6',2''-terpyridine-4,4',4'''-tricarboxylic acid, 0.11 g, 0.301 mmol, isolated yield = 63%) was used directly for the next step. Thionyl chloride (3 mL) was added to the precipitate, and the resulting mixture was refluxed at 83 °C. After 6 h, thionyl chloride was removed *in vacuo* (and collected in a liquid nitrogen trap), and the yellow precipitate formed was dissolved in 3 mL of dry dichloromethane. Azido-PEG<sub>3</sub>-amine (0.59 g, 2.71 mmol, 9 equiv.) dissolved in 3 mL of dry dichloromethane was added to the reaction mixture and stirred at room temperature overnight. The solvent was removed by rotary evaporation, and the residue was purified with an alumina column. The product eluted out at 100% dichloromethane and was dried *in vacuo* to yield a white solid (0.198 g, 0.205 mmol, isolated yield = 68%). <sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 8.96 (s, 2H), 8.83 (dd, J = 5.0, 0.8 Hz, 2H), 8.81 (s, 2H), 7.76 (dd, J = 5.0, 1.6 Hz, 2H), 7.56 (s, 2H), 7.20 (s, 1H), 3.76 – 3.64 (m, 20H), 3.63 (tt, J = 6.0, 1.9 Hz, 6H), 3.63 – 3.44 (m, 16H), 3.30 (t, J = 5.0 Hz, 2H), 3.23 (t, J = 5.0 Hz, 4H). MALDI-TOF (matrix: 2,5-dihydroxybenzoic acid). Calcd for [M+H]<sup>+</sup>: 966.455 *m/z*. Found: 966.712 *m/z*.



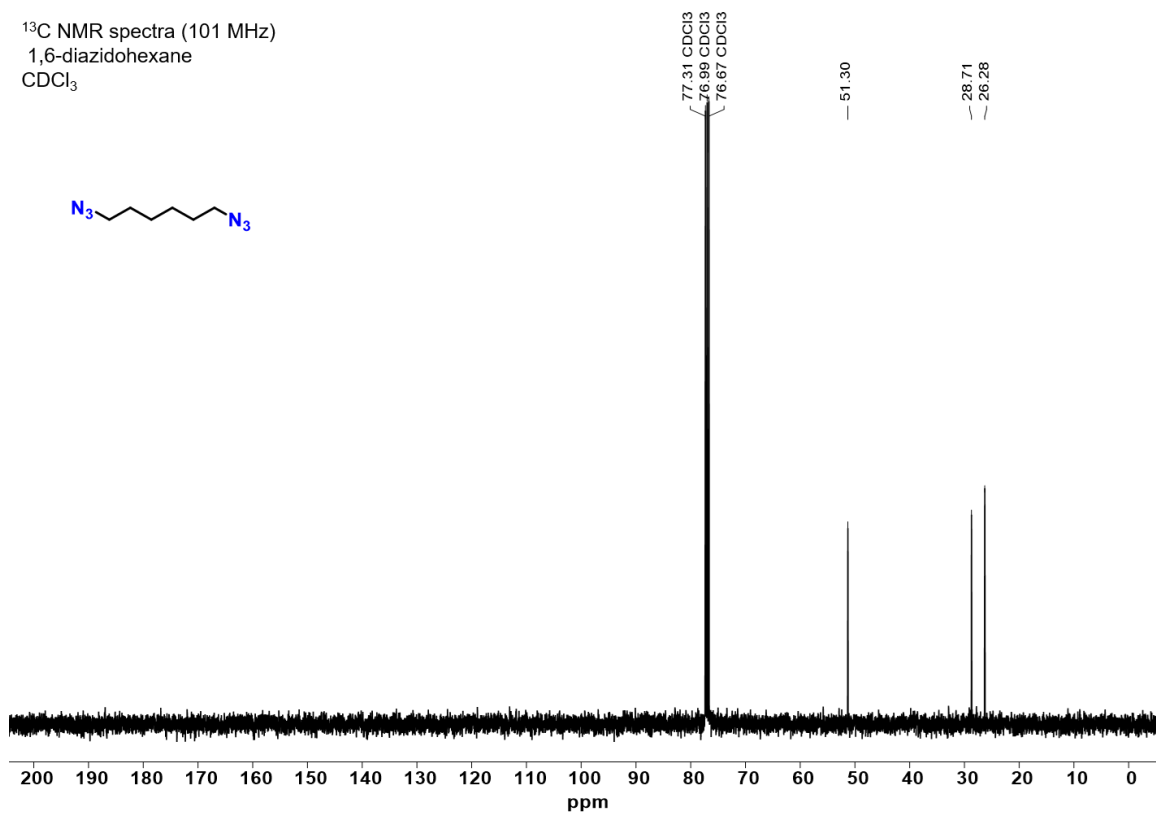
**Bis[tpy-(N<sub>3</sub>)<sub>3</sub>]FeCl<sub>2</sub>** was synthesized by adding a methanol solution of tri(11-azido-3,6,9-trioxaundecanyl) 2,2':6',2''-terpyridine-4,4',4''-tricarboxamide (0.0194 g, 0.02 mmol, 2 equiv.) to a methanol solution of FeCl<sub>2</sub>·4H<sub>2</sub>O (0.002 g, 0.01 mmol, 1 equiv.). Removing solvent *in vacuo* gave a purple solid (0.0214 g, 0.01 mmol, quantitative yield). <sup>1</sup>H NMR (400 MHz, methanol-*d*<sub>4</sub>) δ 9.70 (s, 2H), 9.17 (d, J = 1.8 Hz, 2H), 7.56 (dd, J = 5.8, 1.8 Hz, 2H), 7.38 (d, J = 5.8 Hz, 2H), 3.91 (s, 4H), 3.80 (s, 4H), 3.78 – 3.67 (m, 4H), 3.61 (dddd, J = 16.4, 11.4, 8.2, 3.2 Hz, 32H), 3.30 – 3.23 (m, 4H). MALDI-TOF (matrix: dithranol). Calcd for [M-2Cl]<sup>+</sup>: 1986.828 *m/z*. Found: 1987.284 *m/z*.

## Section 4: NMR Spectra of Multi-Azide Cores



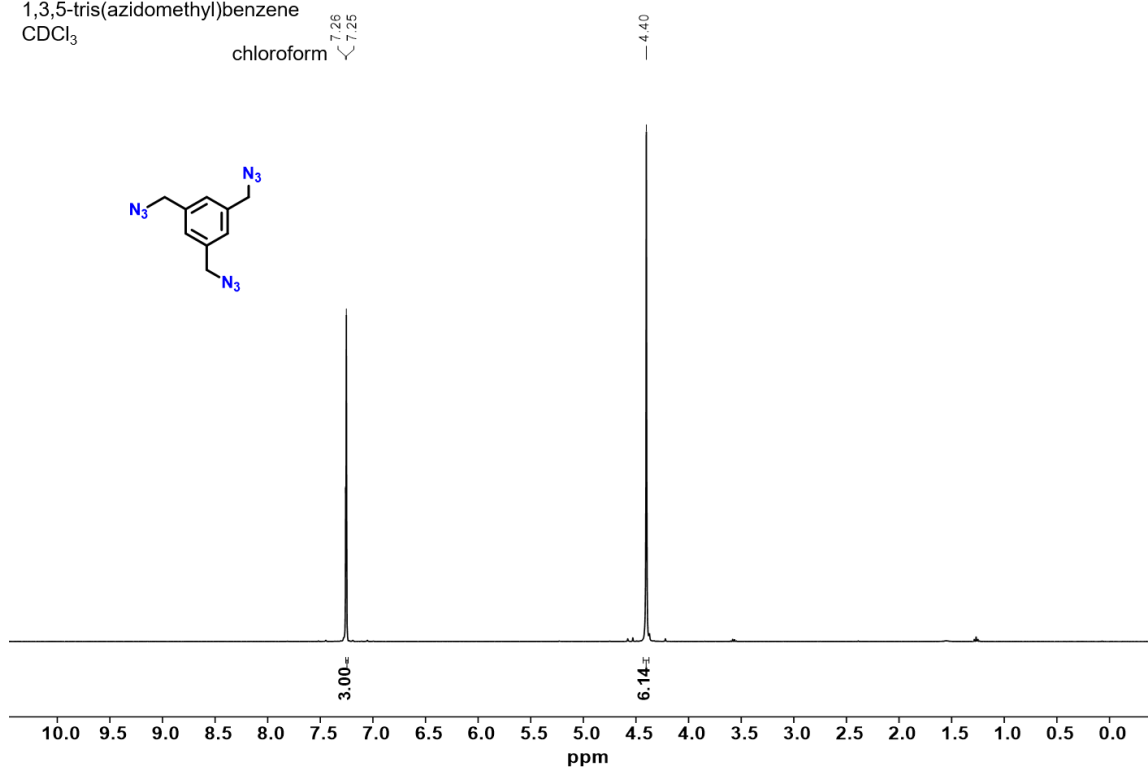
**Figure S1.** <sup>1</sup>H NMR (400 MHz, chloroform-*d*, 298 K) spectrum of 1,6-diazidohexane.

$^{13}\text{C}$  NMR spectra (101 MHz)  
1,6-diazidohexane  
 $\text{CDCl}_3$



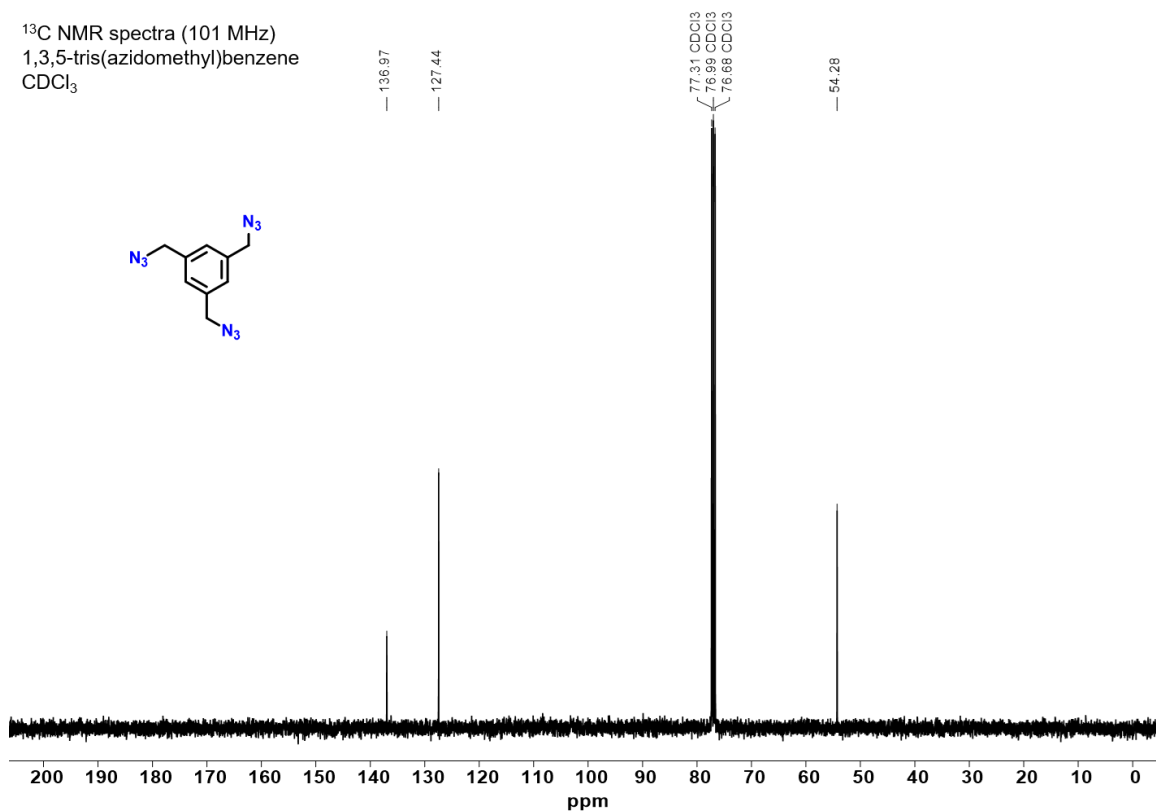
**Figure S2.**  $^{13}\text{C}$  NMR (101 MHz, chloroform-*d*, 298 K) spectrum of 1,6-diazidohexane.

<sup>1</sup>H NMR spectra (400 MHz)  
1,3,5-tris(azidomethyl)benzene  
CDCl<sub>3</sub>



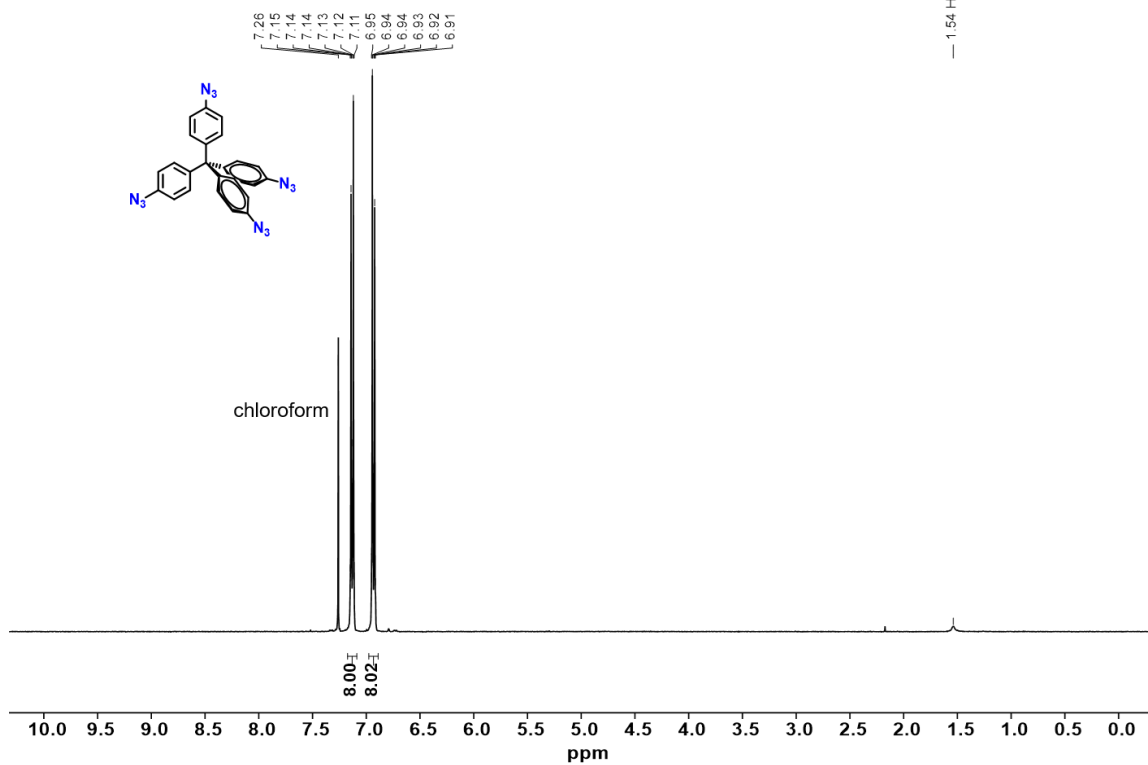
**Figure S3.** <sup>1</sup>H NMR (400 MHz, chloroform-*d*, 298 K) spectrum of 1,3,5-tris(azidomethyl)benzene.

$^{13}\text{C}$  NMR spectra (101 MHz)  
1,3,5-tris(azidomethyl)benzene  
 $\text{CDCl}_3$



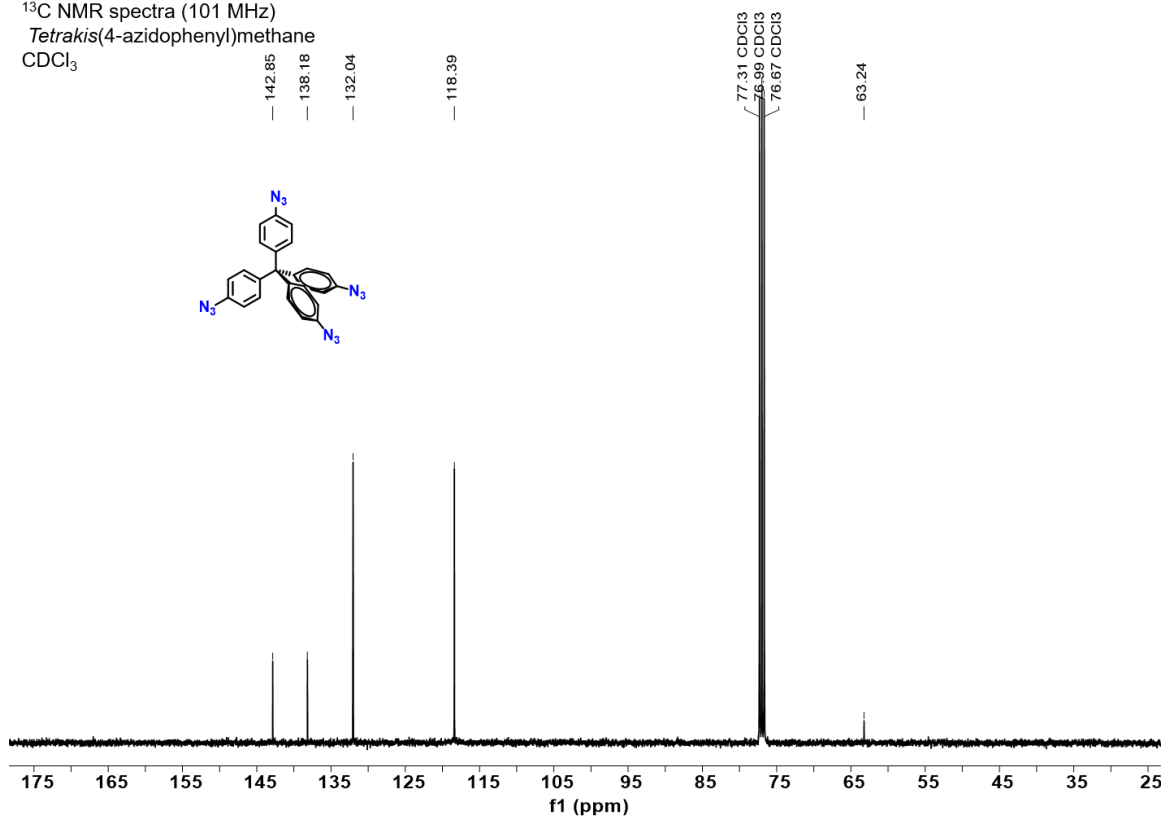
**Figure S4.**  $^{13}\text{C}$  NMR (101 MHz, chloroform-*d*, 298 K) spectrum of 1,3,5-tris(azidomethyl)benzene.

<sup>1</sup>H NMR spectra (400 MHz)  
*Tetrakis(4-azidophenyl)methane*  
CDCl<sub>3</sub>



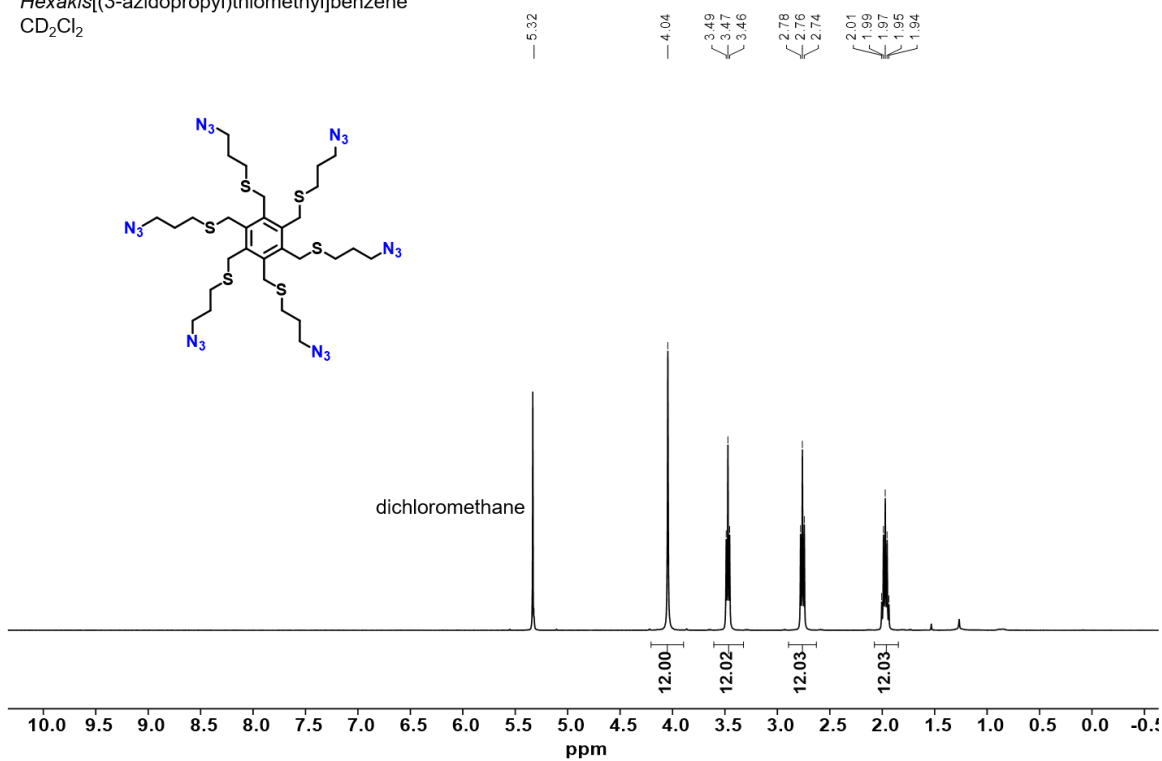
**Figure S5.** <sup>1</sup>H NMR (400 MHz, chloroform-*d*, 298 K) spectrum of *tetrakis(4-azidophenyl)methane*.

<sup>13</sup>C NMR spectra (101 MHz)  
*Tetrakis(4-azidophenyl)methane*  
CDCl<sub>3</sub>



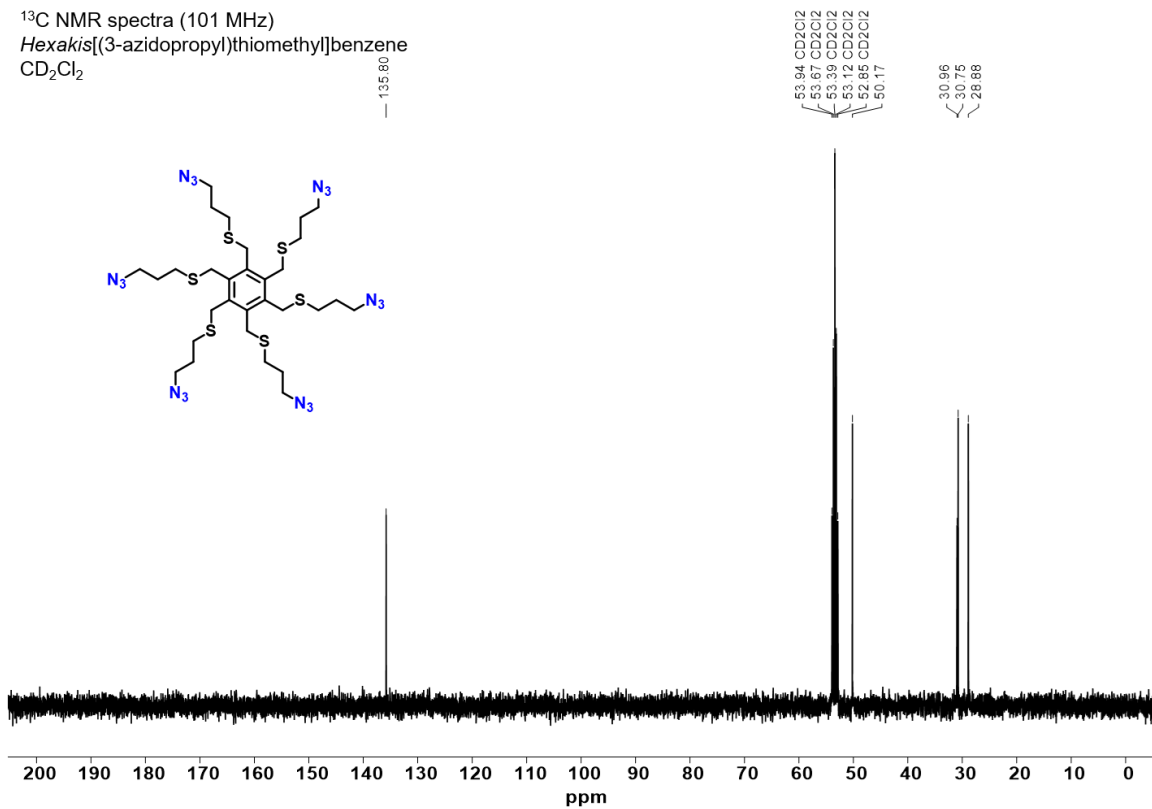
**Figure S6.** <sup>13</sup>C NMR (101 MHz, chloroform-*d*, 298 K) spectrum of *tetrakis(4-azidophenyl)methane*.

<sup>1</sup>H NMR spectra (400 MHz)  
*Hexakis*[(3-azidopropyl)thiomethyl]benzene  
CD<sub>2</sub>Cl<sub>2</sub>

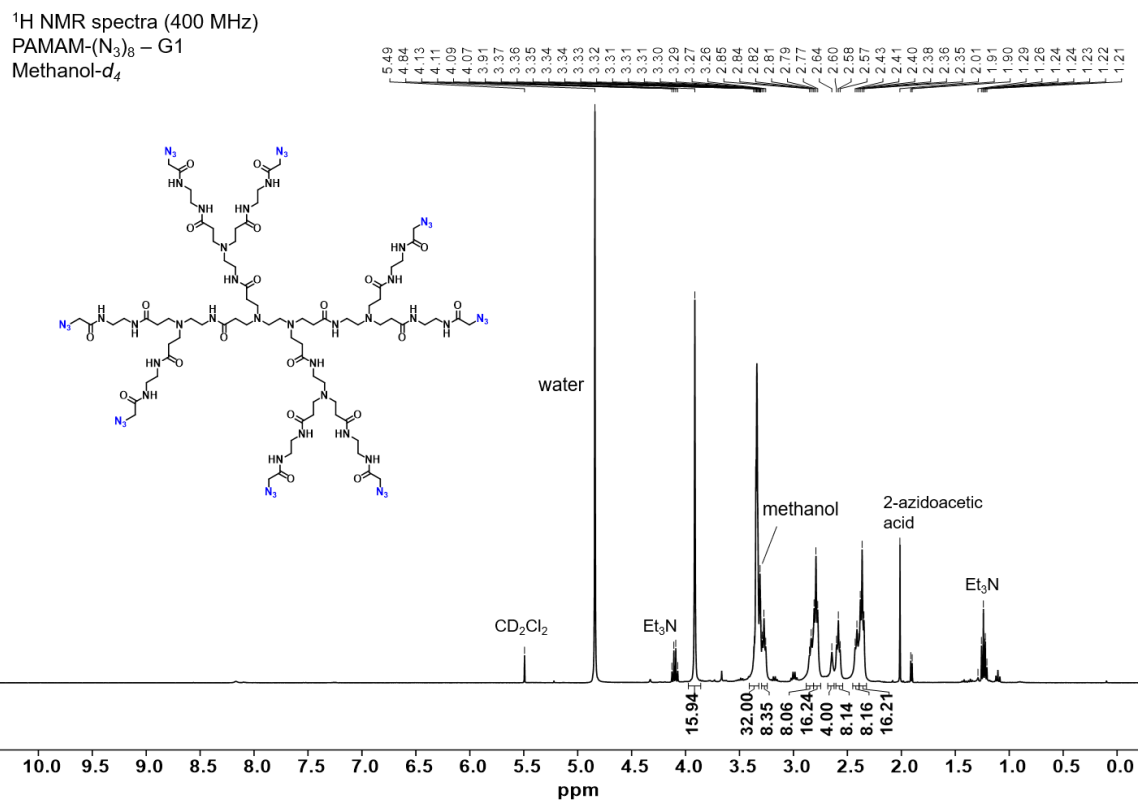


**Figure S7.** <sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>, 298 K) spectrum of *hexakis*[(3-azidopropyl)thiomethyl]benzene.

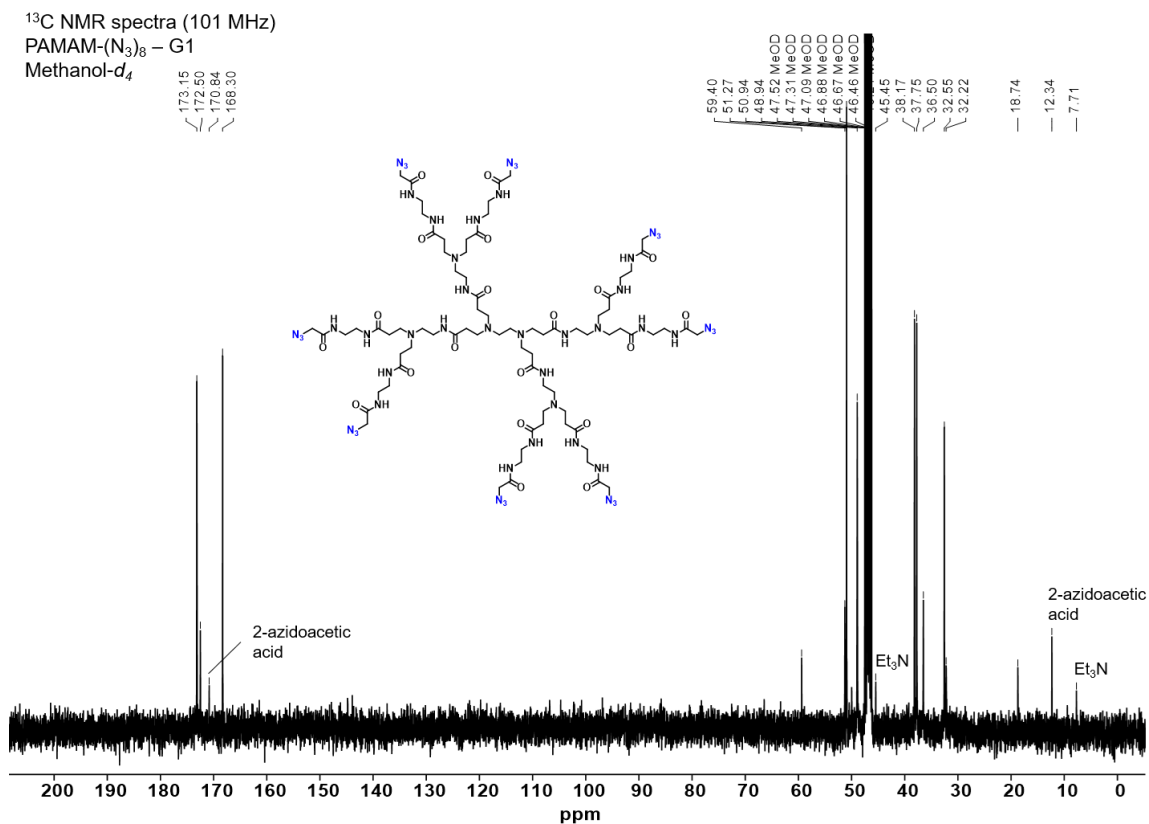
$^{13}\text{C}$  NMR spectra (101 MHz)  
*Hexakis*[(3-azidopropyl)thiomethyl]benzene  
 $\text{CD}_2\text{Cl}_2$



**Figure S8.**  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_2\text{Cl}_2$ , 298 K) spectrum of *hexakis*[(3-azidopropyl)thiomethyl]benzene.

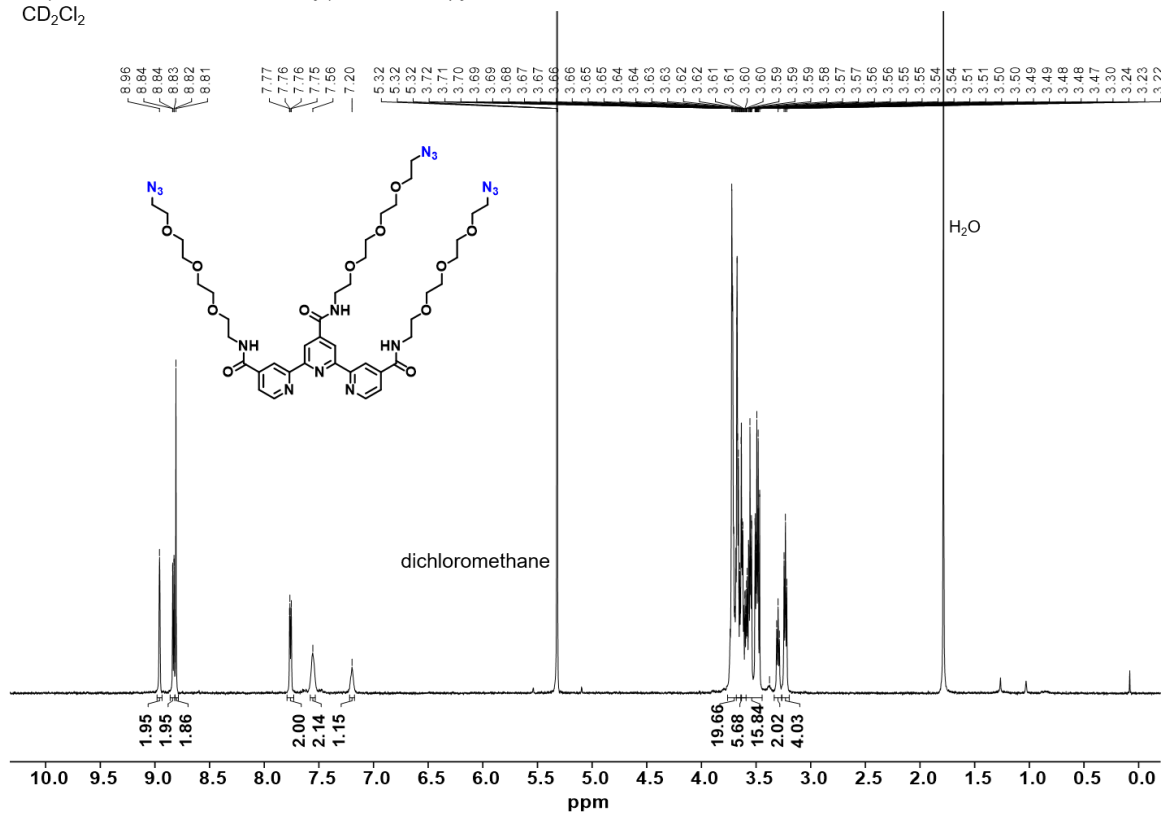


**Figure S9.** <sup>1</sup>H NMR (400 MHz, methanol-*d*<sub>4</sub>, 298 K) spectrum of PAMAM-(N<sub>3</sub>)<sub>8</sub> G-1. Note the residual 2-azidoacetic acid and Et<sub>3</sub>N, which are eliminated at the post-DNA modification step.

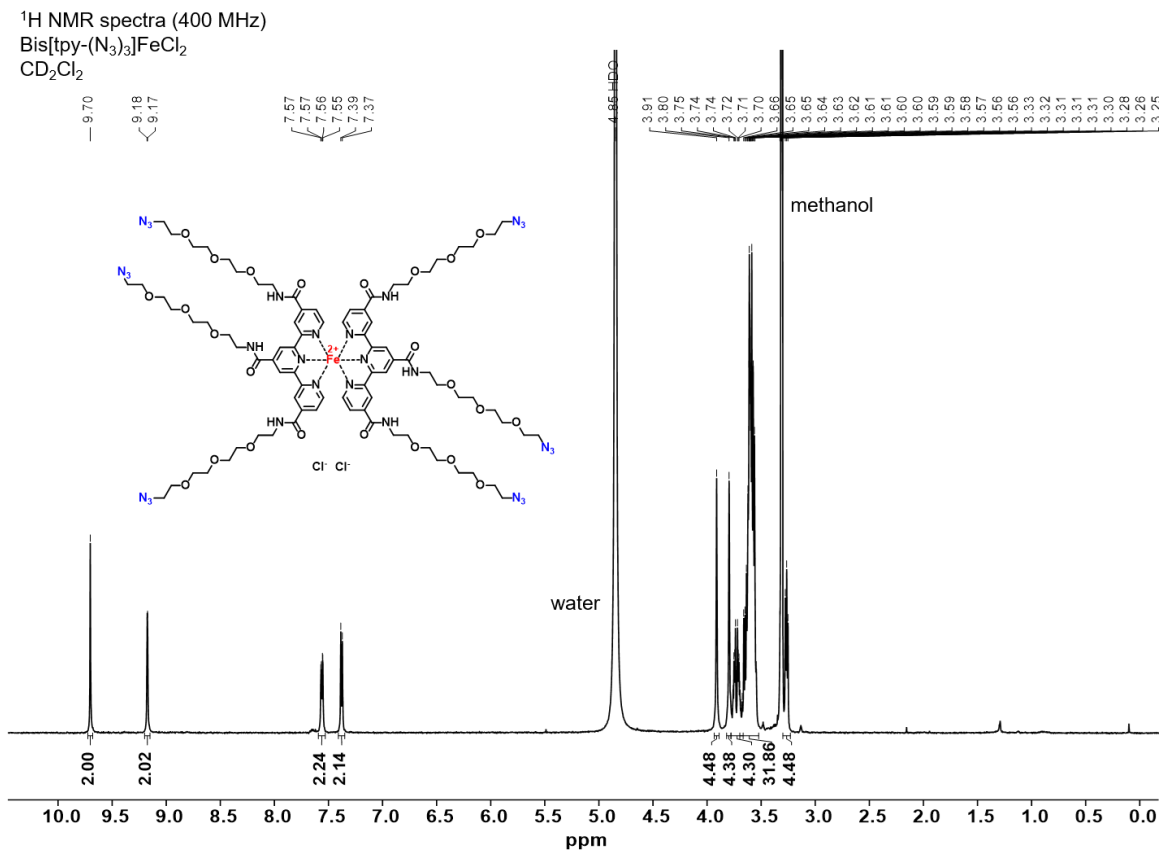


**Figure S10.** <sup>13</sup>C NMR (101 MHz, methanol-*d*<sub>4</sub>, 298 K) spectrum of PAMAM-(N<sub>3</sub>)<sub>8</sub> G-1. Note the residual 2-azidoacetic acid and Et<sub>3</sub>N, which are eliminated at the post-DNA modification step.

<sup>1</sup>H NMR spectra (400 MHz)  
Tri(11-azido-3,6,9-trioxaundecanyl) 2,2':6',2''-terpyridine-4,4',4''-tricarboxamide  
CD<sub>2</sub>Cl<sub>2</sub>



**Figure S11.** <sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>, 298 K) spectrum of Tri(11-azido-3,6,9-trioxaundecanyl) 2,2':6',2''-terpyridine-4,4',4''-tricarboxamide. Note the residual H<sub>2</sub>O, which is difficult to remove but does not affect post-functionalization of DNA, which is carried out in H<sub>2</sub>O.



**Figure S12.** <sup>1</sup>H NMR (400 MHz, methanol-*d*<sub>4</sub>, 298 K) spectrum of bis[tpy(N<sub>3</sub>)<sub>3</sub>]FeCl<sub>2</sub>. Note the residual H<sub>2</sub>O, which is difficult to remove but does not affect post-functionalization of DNA, which is carried out in H<sub>2</sub>O.

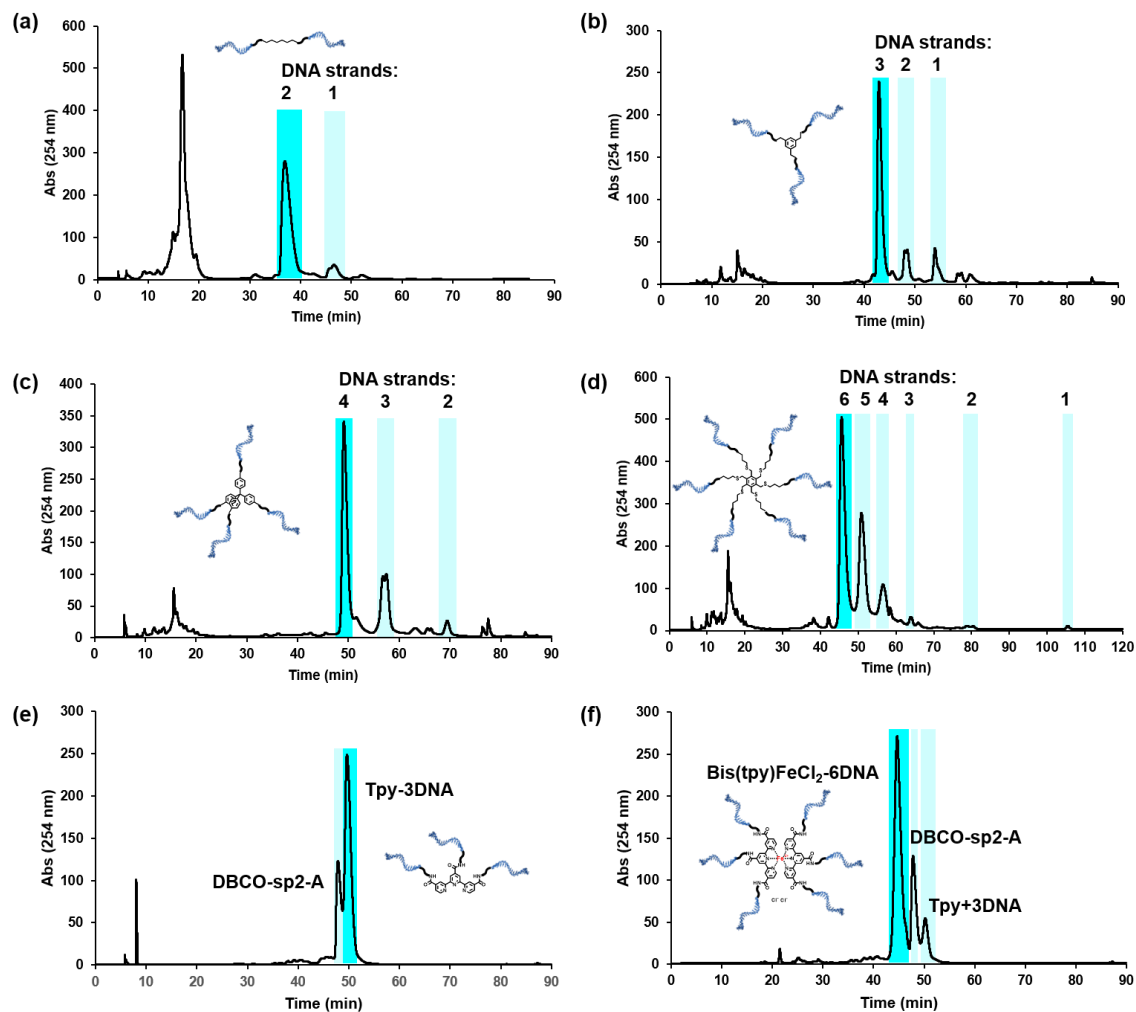
## Section 5: Synthesis and Purification of SMDHs

General solid-phase coupling procedure (modified from reported procedure)<sup>5</sup>: A solution of a multi-azide molecule (see Table S2 for conditions) dissolved in 1.8 mL dimethylformamide was added to ~ 20 mg of 1000 Å CPG beads (48 μmol g<sup>-1</sup>) that had been functionalized with DNA strands containing a 5' DBCO moiety. The suspension was shaken at room temperature at 1,500 rpm. After 48 h, the suspension was centrifuged, and the supernatant was discarded. The beads were then washed with 1.8 mL of dimethylformamide (2×) and acetone (3×) to remove residual, unreacted multi-azide molecules. The beads were then subjected to ~ 1 mL of 30% ammonium hydroxide for 17 h at room temperature for deprotection and cleavage. After removing ammonia by blowing nitrogen gas over the solution, the SMDHs were purified by RP-HPLC or denaturing PAGE.

General solution phase coupling procedure: A solution of a multi-azide molecule in dimethylformamide was added to a solution of 5' DBCO-functionalized DNA (2 equiv. of DNA per azide) dissolved in 1.5 M NaCl. The mixture was shaken at room temperature at 1,500 rpm. After 72 h, the mixture was lyophilized and concentrated and purified using RP-HPLC or denaturing PAGE.

**Table S2.** Coupling conditions, yields, and MALDI-MS characterization. Yields were calculated after taking into consideration that a 1-μmol solid-phase synthesis results in approximately 0.22 μmol of 18-base DNA.

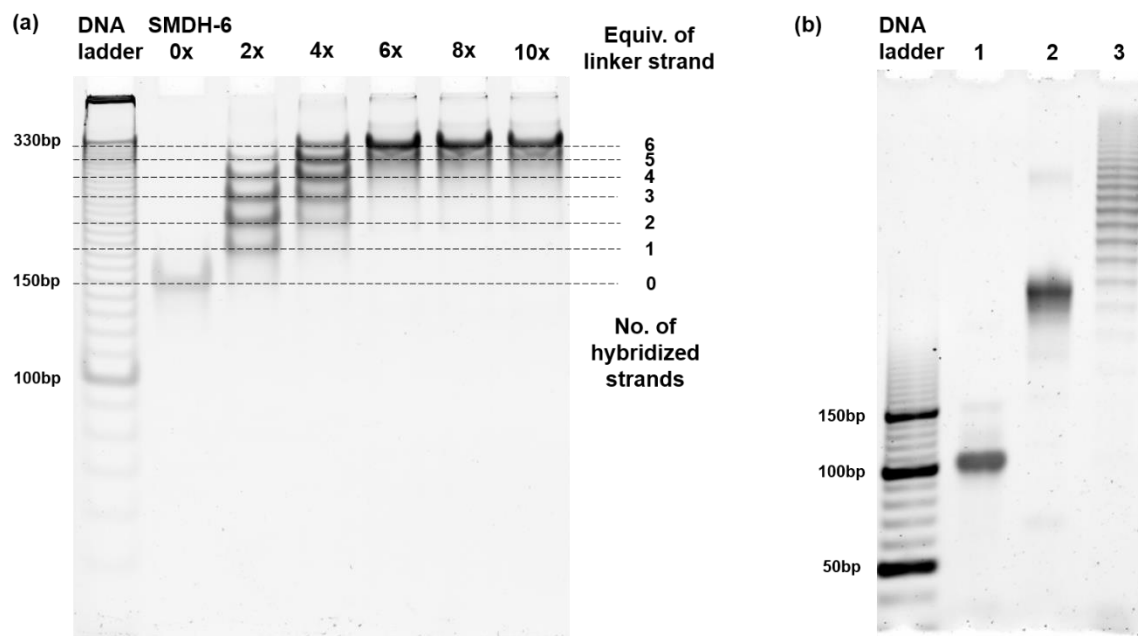
SMDH	Solid-phase coupling					Solution-phase coupling			
	2	3	4	5	6	7	8	Tpy	Bis(tpy)FeCl <sub>2</sub>
[Multi-azide molecule]	10 mM	6 mM	4 mM	N/A	3 mM	N/A	0.17 mM	0.33 mM	0.17 mM
[DBCO-DNA]	N/A	N/A	N/A	N/A	N/A	N/A	2.7 mM	2 mM	2 mM
Yield (%)	50	38	54	14	23	10	8	61	33
Calc'd (kDa)	13.4	20.1	26.9	33.9	40.5	48.5	55.1	20.8	41.7
Exp't (kDa)	13.7	20.3	27.1	34.8	41.0	49.2	55.8	20.9	42.8



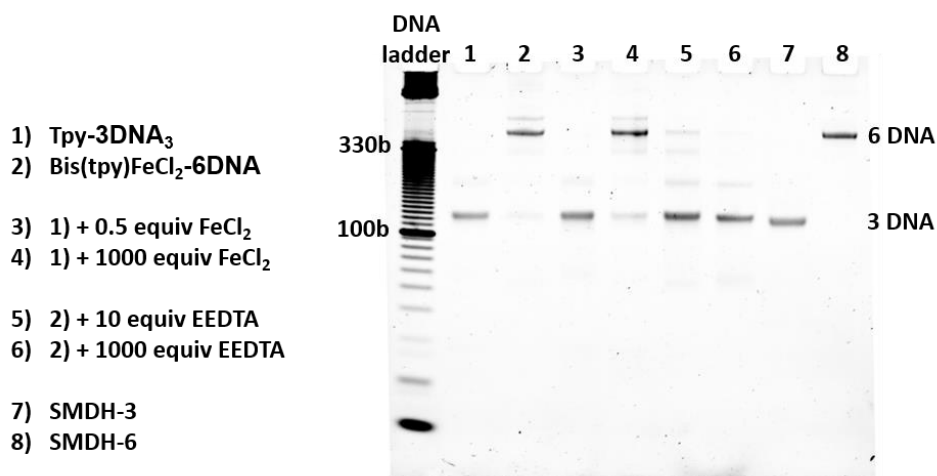
**Figure S13.** Representative RP-HPLC chromatograms of (a) SMDH-2, (b) SMDH-3, (c) SMDH-4, (d) SMDH-5, and SMDH-6, (e) tpy-3DNA, and (f) bis(tpy)FeCl<sub>2</sub>-6DNA. Elution gradient: 95:5 v/v 0.1 M TEAA (aq) : MeCN to 60:40 v/v 0.1 M TEAA (aq) : MeCN (+ 0.5 vol % MeCN / min).

## Section 6: PAGE Purification and Characterization

Polyacrylamide gel was cast by pouring a gel stock solution between two pre-assembled glass plates and adding at the top a comb before the gel fully polymerized. Gel stock solution was prepared at 1× Tris/Borate/EDTA (TBE) and diluted to the desired acrylamide concentration (from 5 to 12%) with urea added (to 6 M in urea) to produce a denaturing environment. Approximately 2 mg of ammonium persulfate and 15 μL of tetramethylethylenediamine was added for every 15 mL of gel stock solution just before casting. The samples were loaded into the wells in amounts that corresponded to ~ 0.015 optical density (OD), and the solution was made up to 6 M urea to produce a denaturing environment; glycerol was added instead of urea for non-denaturing conditions to create a dense solution suitable for loading. Submerged in 1× TBE buffer, the gel was run at 4 V/cm and then ramped up to 8 V/cm once all the samples entered the gel. A loading dye (e.g., xylene cyanol and bromophenol blue) can be added to track the gel. For purification gels, the gel bands were visualized under a UV lamp, and then the relevant bands were excised, crushed, frozen, and extracted into water over two days at room temperature. The sample solution was then desalted, lyophilized, quantified, and characterized. For analytical gels, the gel was visualized using GelRed stain and imaged on a ChemiDoc imager.



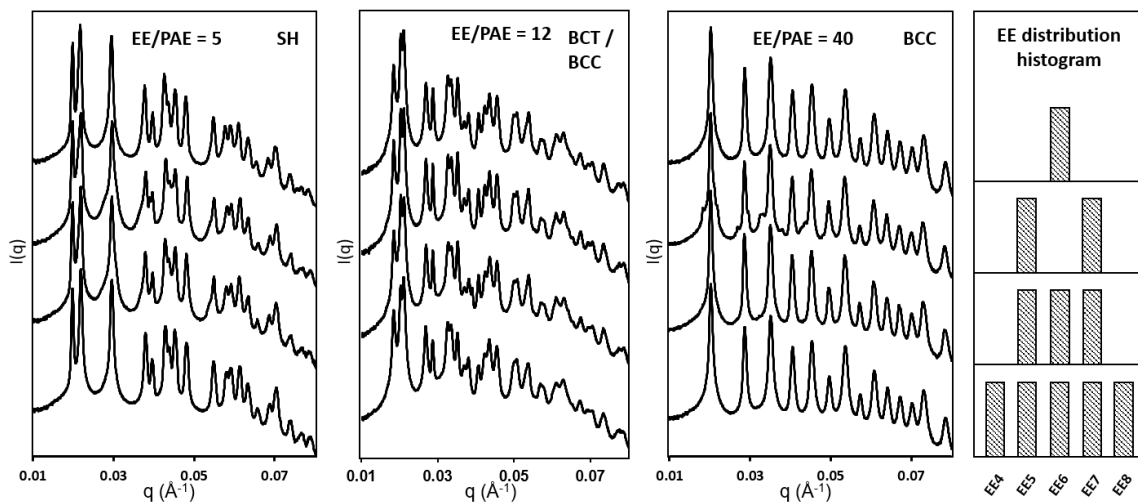
**Figure S14.** (a) Image of a native PAGE (8 %) of SMDH-6 (sequence: 5' DBCO -(Sp18)<sub>2</sub>-CATCCATCCTTATCAACT 3') added with increasing equivalents of linker strand (sequence: 5' TTCCTT-A-AGTTGATAAGGATGGATG 3'). (b) Image of a denaturing PAGE (5%) of SMDH-3 (lane 1), SMDH-7 (lane 2), and an AuNP-templated with an average of seven DNA strands/particle (lane 3).



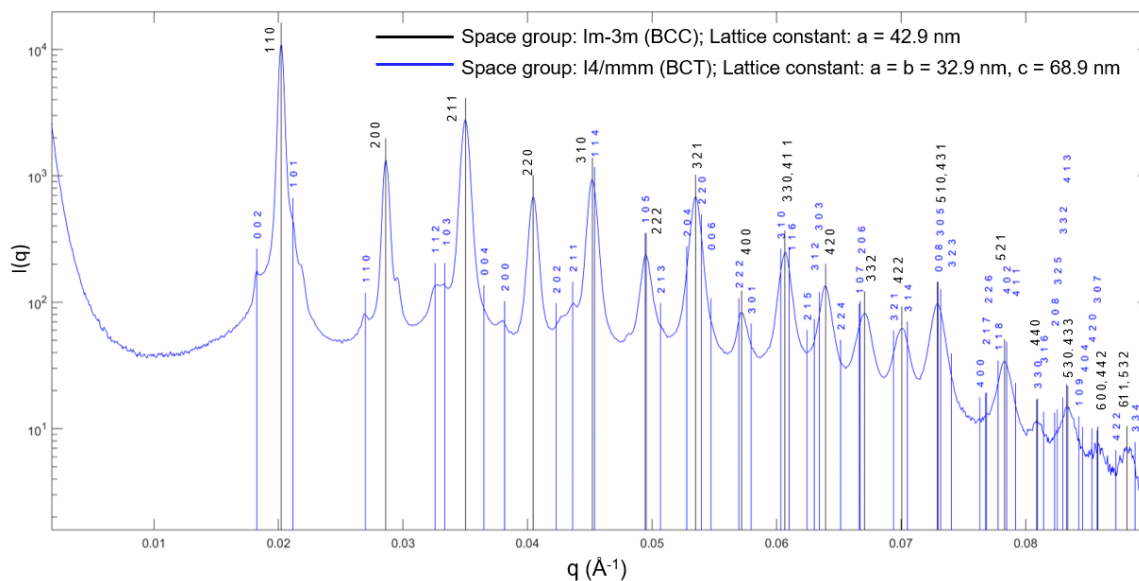
**Figure S15.** Image of a denaturing PAGE (5 %, 6 M urea) of tpy-3DNA and bis(tpy)FeCl<sub>2</sub>-6DNA, showing the corresponding change in size upon the addition and removal of ferrous ion. Their sizes are very similar to SMDH-3 (lane 7) and SMDH-6 (lane 8) respectively.

## Section 7: SAXS Characterization

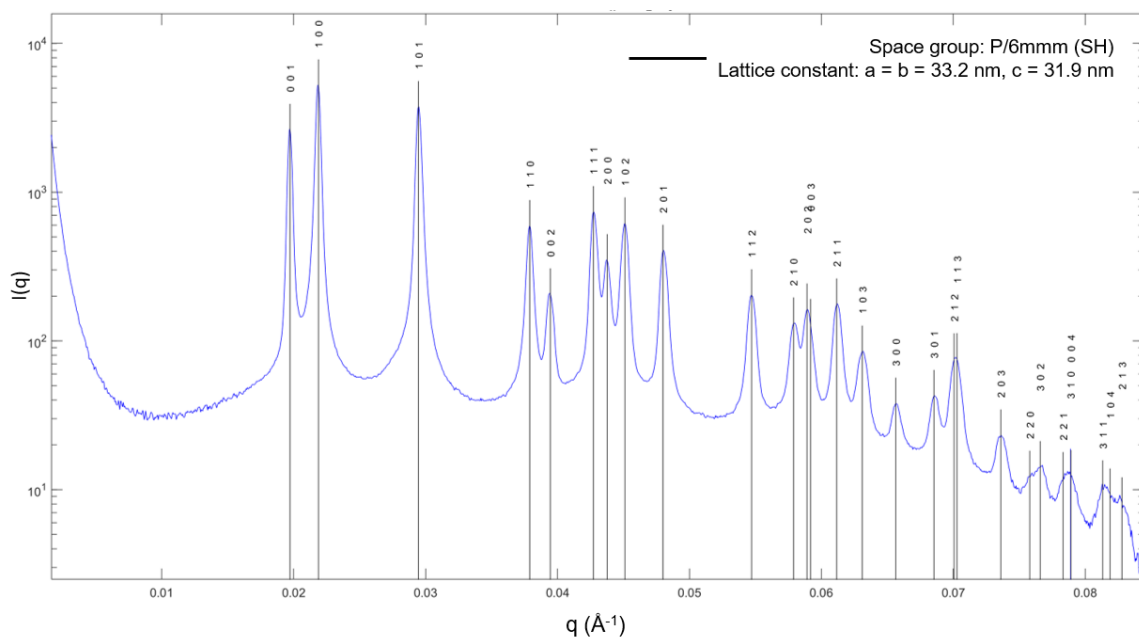
All SAXS experiments were performed in Sector 5 of the DuPont-Northwestern-Dow Collaborative Access Team of the Advanced Photon Source at Argonne National Laboratory. Using X-rays with wavelengths of 1.24 Å (10 keV), the samples were exposed for 0.1 to 0.2 s, and the scattered beam was collected on a CCD detector. By radially averaging the images, one-dimensional scattering spectra were obtained and plotted as scattering intensities  $I(q)$  against the scattering vector  $q$ . One-dimensional SAXS spectra were simulated and indexed to determine crystallographic symmetries and lattice parameters (programs available at <https://sites.google.com/site/byeongdu/software>).<sup>6</sup> Included in this section are representative, indexed SAXS data (Figure S18 to S25) obtained from samples with tpy-3DNA or bis(tpy)FeCl<sub>2</sub>-6DNA as EEs.



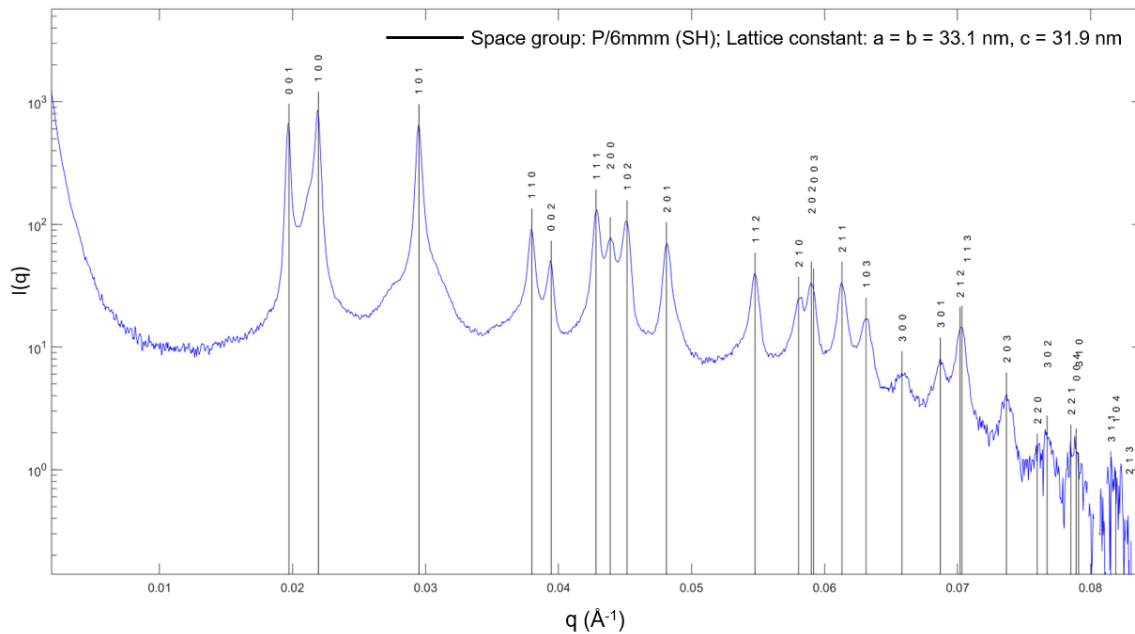
**Figure S16.** SAXS characterization of colloidal crystals prepared from EE input with precisely engineered polydispersity. SAXS data for EE/PAE = 5 and EE/PAE = 40 are identical to those presented in Figure 4.



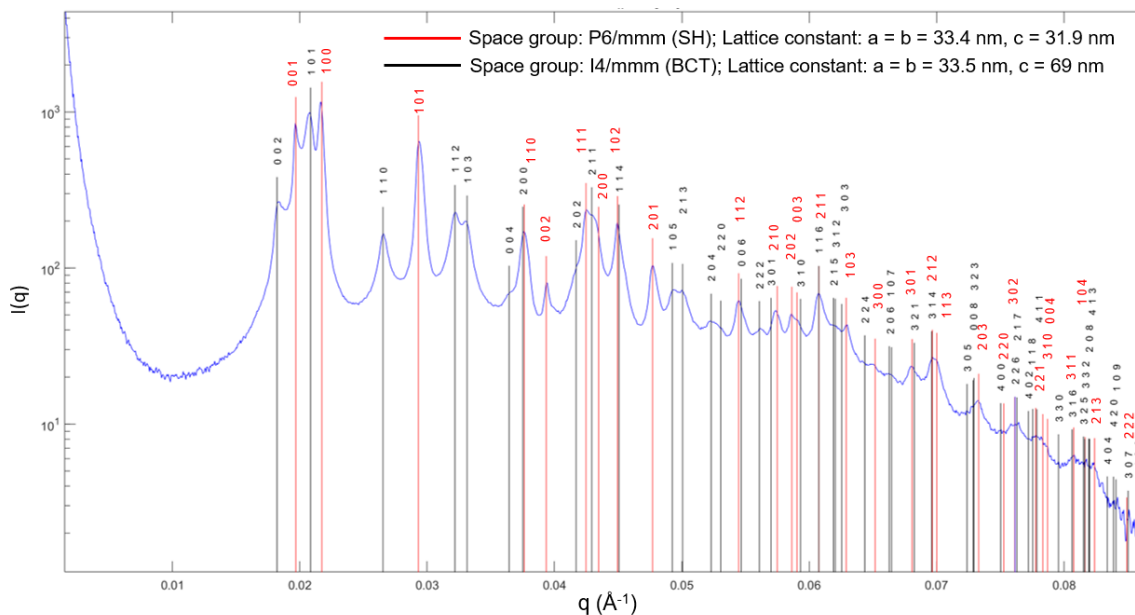
**Figure S17.** Indexed SAXS data of colloidal crystals prepared from EE-5 : EE-7 = 1 : 1; EE/PAE = 40, illustrating the decrease in phase purity observed with disjointed EE distributions (major: BCC phase; minor: BCT phase).



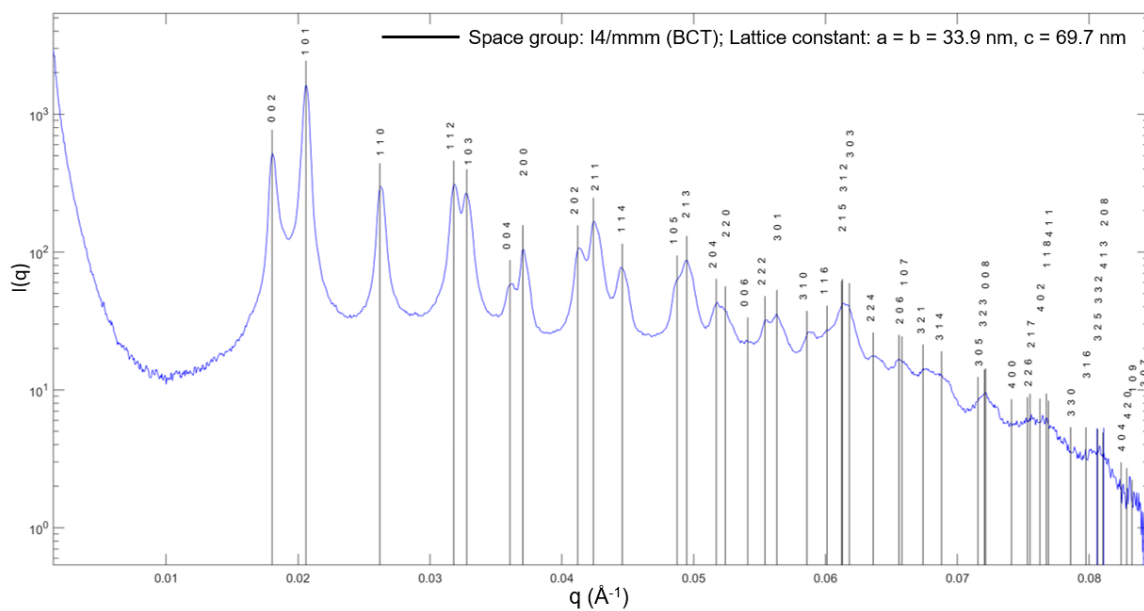
**Figure S18.** Indexed SAXS data of colloidal crystals prepared using bis(tpy)FeCl<sub>2</sub>-6DNA; EE/PAE = 5 (SH phase).



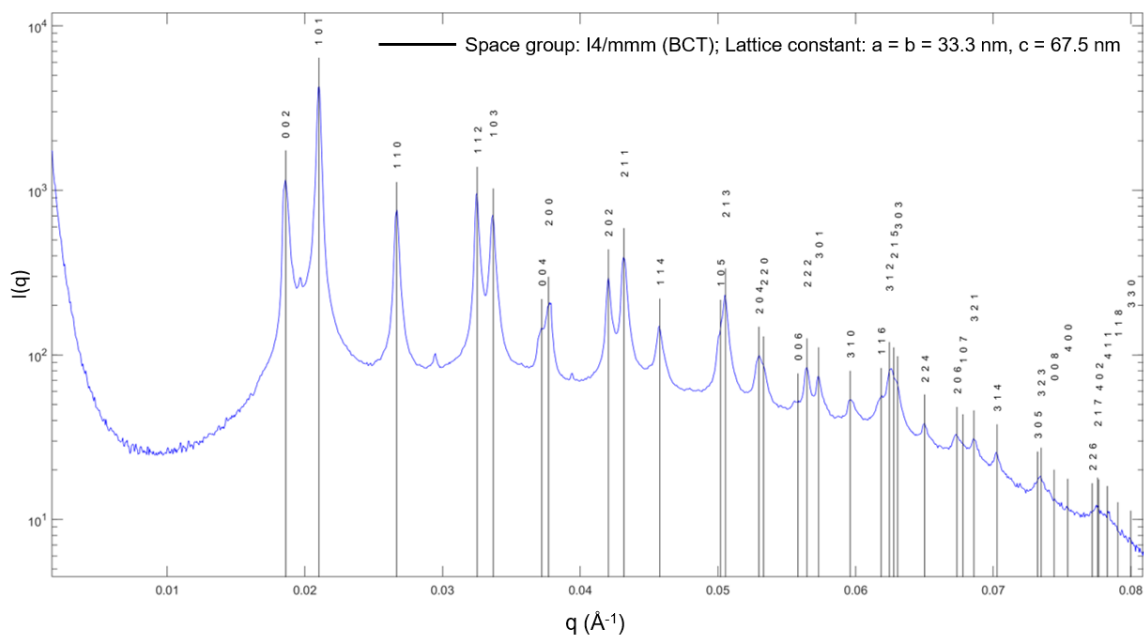
**Figure S19.** Indexed SAXS data of colloidal crystals prepared using tpy-3DNA; EE/PAE = 10 (SH phase).



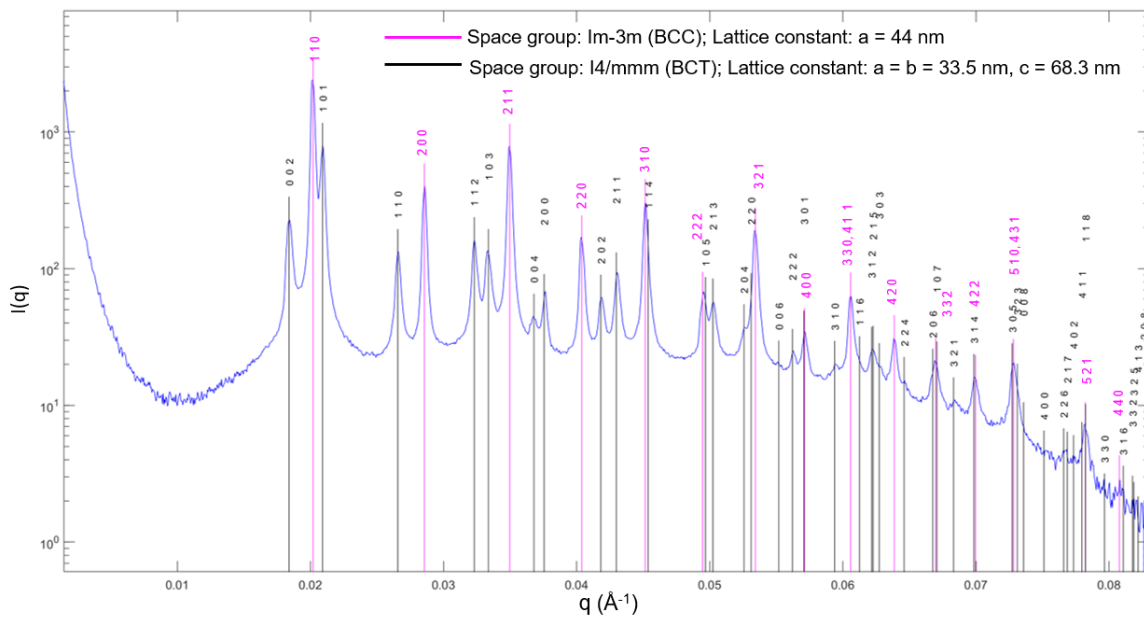
**Figure S20.** Indexed SAXS data of colloidal crystals prepared using bis(tpy)FeCl<sub>2</sub>-6DNA; EE/PAE = 10 (SH and BCT phases).



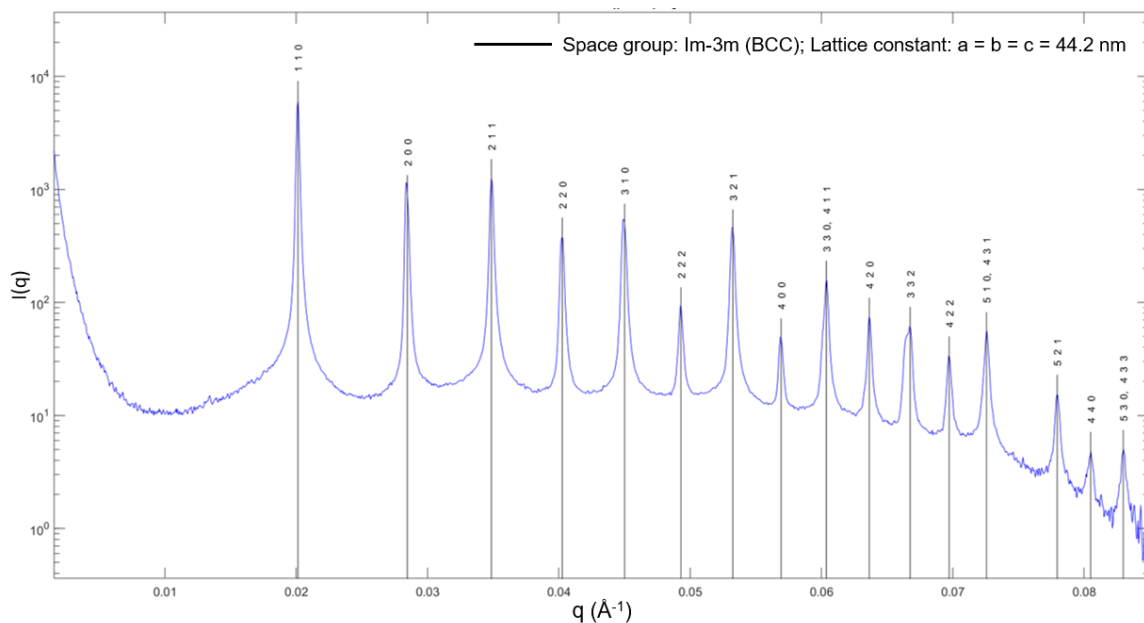
**Figure S21.** Indexed SAXS data of colloidal crystals prepared using tpy-3DNA; EE/PAE = 20 (BCT phase).



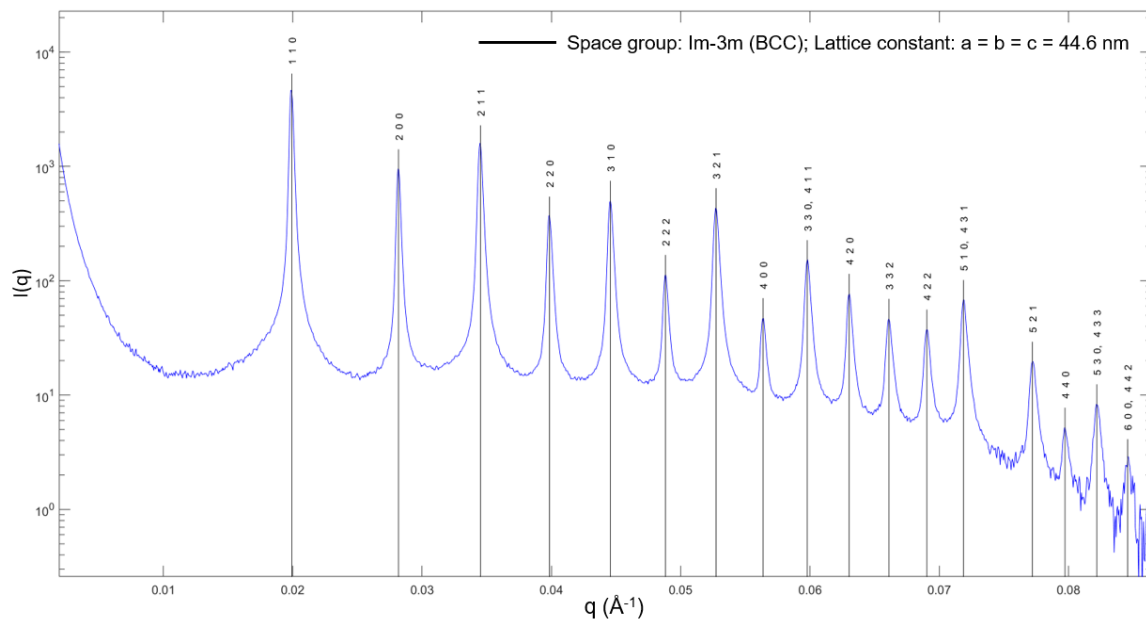
**Figure S22.** Indexed SAXS data of colloidal crystals prepared using bis(tpy)FeCl<sub>2</sub>-6DNA; EE/PAE = 25 (BCT phase, trace BCC phase).



**Figure S23.** Indexed SAXS data of colloidal crystals prepared using tpy-3DNA; EE/PAE = 50 (BCC and BCT phases).



**Figure S24.** Indexed SAXS data of colloidal crystals prepared using bis(tpy)FeCl<sub>2</sub>-6DNA; EE/PAE = 50 (BCC phase).



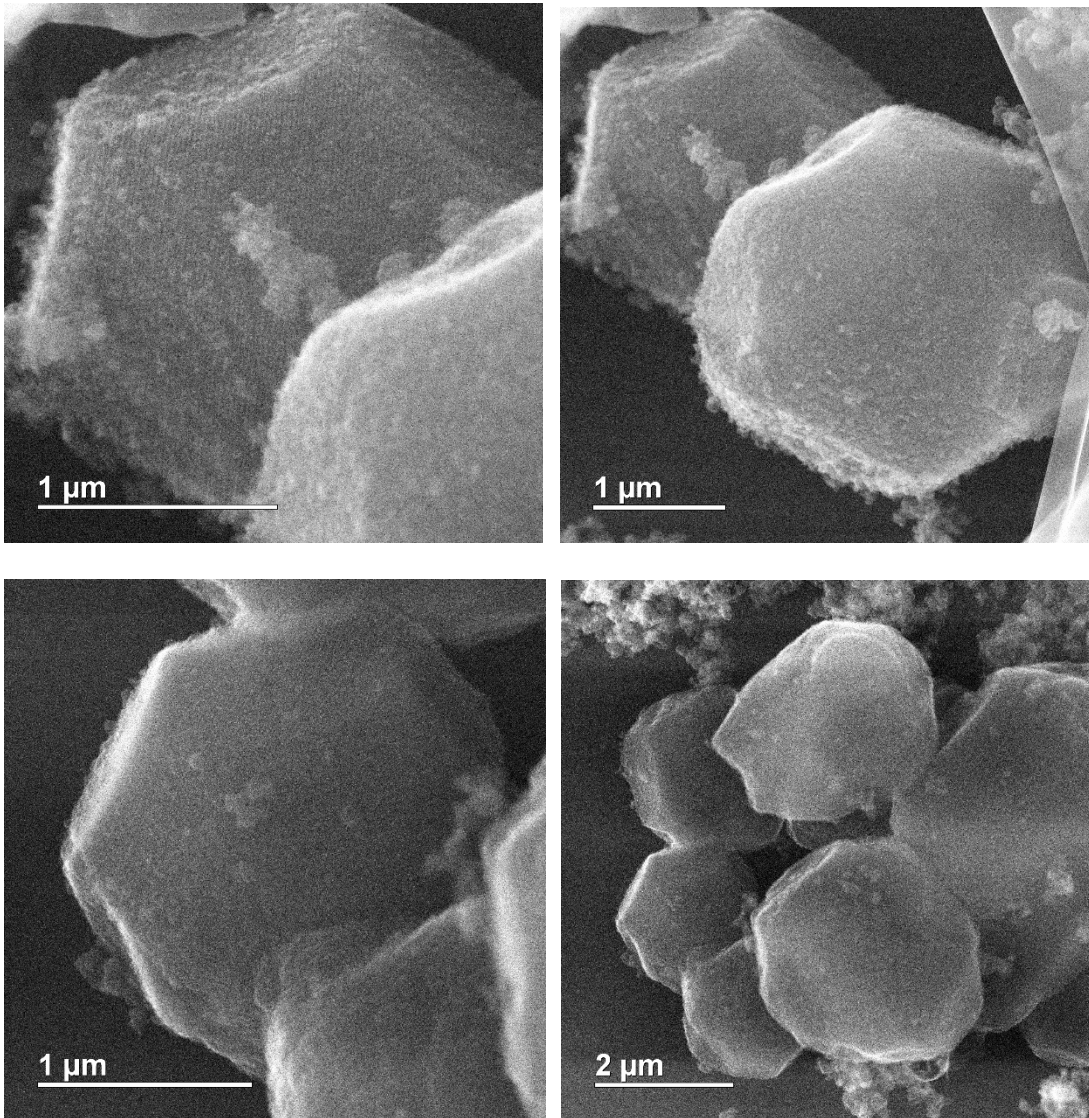
**Figure S25.** Indexed SAXS data of colloidal crystals prepared using tpy-3DNA; EE/PAE = 100 (BCC phase).

## Section 8: STEM Characterization

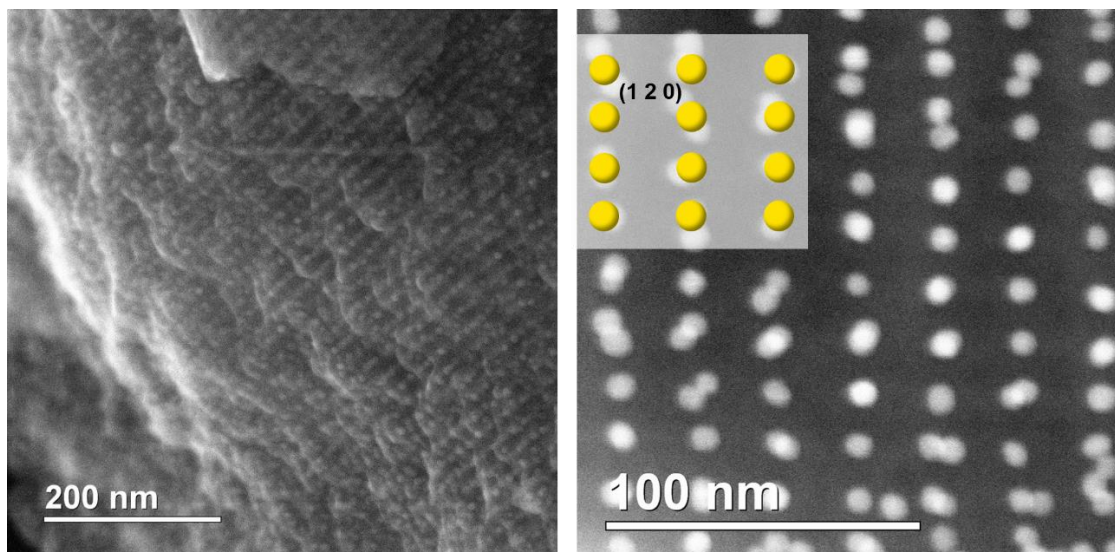
Colloidal crystal samples were encapsulated in silica using a modified procedure from a previously reported protocol.<sup>7</sup> Briefly, superlattice samples were transferred in a minimal amount of assembly buffer to an Eppendorf tube with 0.45 mL of 0.5 M NaCl, 10 mM phosphate buffer that was pre-cooled to 18 °C. This step was performed to remove excess SDS and prevent silica overgrowth. Then, 2  $\mu$ L of *N*-trimethoxysilylpropyl-*N,N,N*-trimethylammonium chloride was added, and the solution was shaken at 850 rpm at 18 °C for 1 h. 4  $\mu$ L of triethoxysilane was then added, and the solution was shaken at 850 rpm at 18 °C overnight. Silica growth usually begins in  $\sim$  10 min. The assemblies should remain intact throughout the procedure. Afterwards, excess amorphous silica was pipetted out carefully to avoid removing any encapsulated superlattices (appearance: dark red/black dots). The samples were suspended and washed with 200  $\mu$ L of water (3 $\times$ ), and then ethanol (2 $\times$ ), and stored in 50  $\mu$ L of ethanol.

To obtain cross-sections of the superlattices, the silica-encapsulated samples were embedded in resin and ultramicrotomed into thin slices using the following procedure modified from the previously reported protocol.<sup>7</sup> Briefly, 0.15 mL of 4% gelatin solution was added to thoroughly dried, silica-encapsulated samples. The gelatin block was frozen and solvent exchanged (volume: 1.5 mL) through the following gradient: 30% EtOH (2 $\times$ )  $\rightarrow$  50% EtOH (2 $\times$ )  $\rightarrow$  70% EtOH (2 $\times$ )  $\rightarrow$  80% EtOH (2 $\times$ )  $\rightarrow$  90% EtOH (2 $\times$ )  $\rightarrow$  100% EtOH  $\rightarrow$  acetone (2 $\times$ ). EMBed-812 resin (Electron Microscopy Sciences) was prepared by mixing the following: 9 g of dodecenyl succinic anhydride, 22.6 g of EMBed-812, and 14.7 g of nadic methyl anhydride. After removing the acetone, 1.5 mL of 1:1 acetone/resin was added. After shaking the sample overnight at 100 rpm, the solvent was exchanged into 1:3 acetone/resin, and the sample was shaken at 100 rpm for 1 h; the acetone was then allowed to evaporate for 2 h. The gelatin block was then submerged and shaken in fresh, pure resin for two days, after which the resin is cured by exchanging into a resin mixture with crosslinker added (0.7 g DMP-30) and heating in an oven overnight at 60 °C.

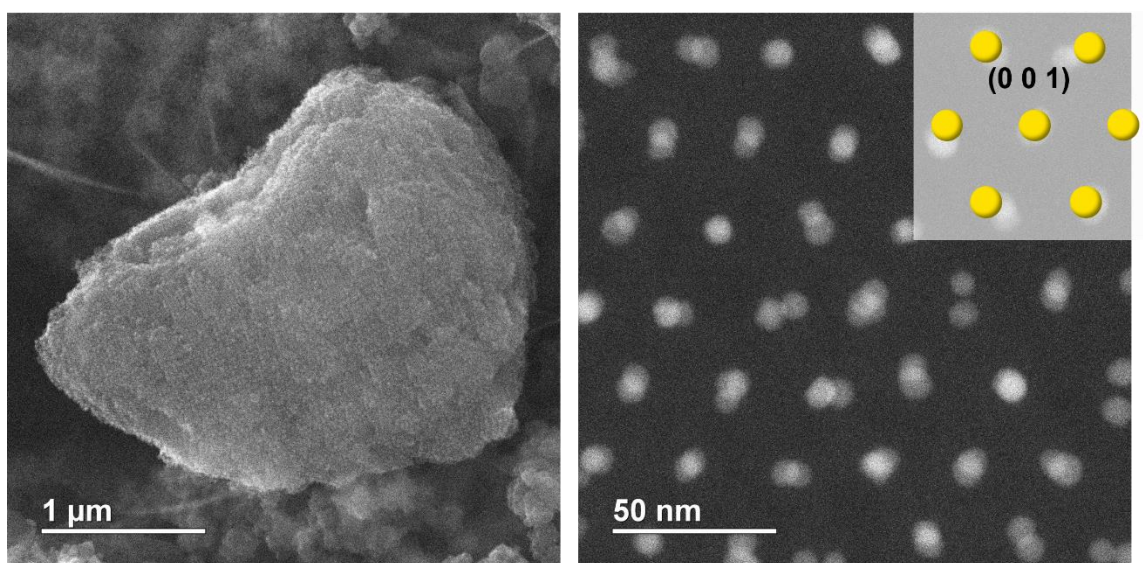
Silica-encapsulated samples or ultramicrotome-sectioned samples were deposited on carbon film-coated copper grids (Ted Pella) and loaded onto a double-tilt sample holder for STEM imaging. Both annular dark field (ADF) and secondary electron (SE) imaging were performed on a Hitachi HD-2300A with a field emission gun operated at 200 kV.



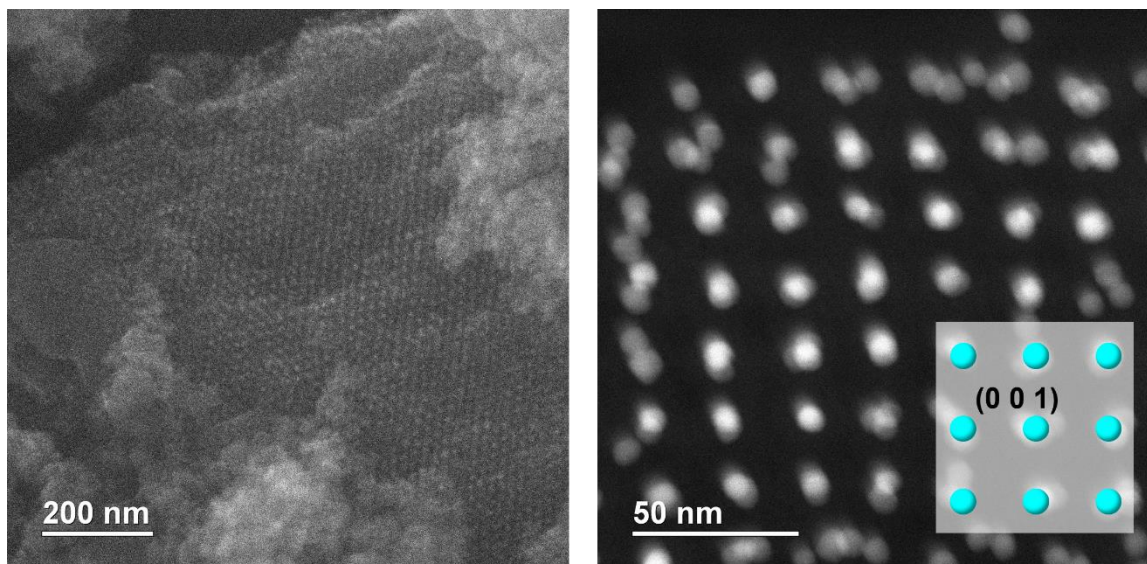
**Figure S26.** Representative SE-STEM images of silica-encapsulated colloidal crystals with BCC phase symmetry showing characteristic rhombic dodecahedron crystal facets.



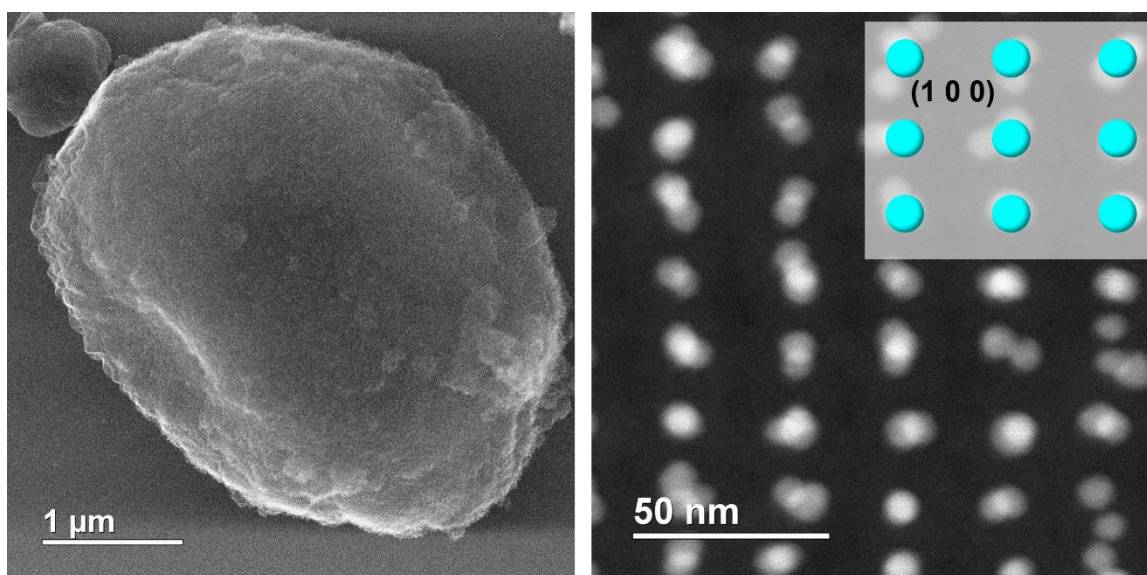
**Figure S27.** Representative SE-STEM image (left) and cross-sectional ADF-STEM image (right, along  $[1\ 2\ 0]$  zone axis) of a SH phase superlattice (lattice parameter  $a = b = 33.9\text{ nm}$ ,  $c = 32.6\text{ nm}$ ) synthesized from 5 equiv. EE-6 / AuPAE.



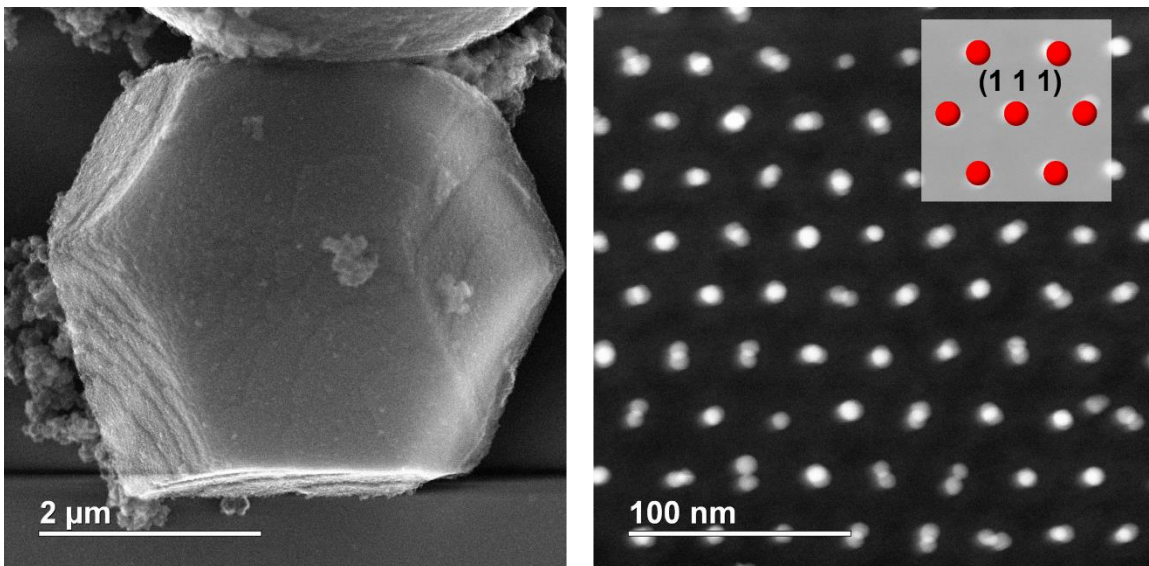
**Figure S28.** Representative SE-STEM image (left) and cross-sectional ADF-STEM image (right, along  $[0\ 0\ 1]$  zone axis) of a SH phase superlattice (lattice parameter  $a = b = 33.1\text{ nm}$ ,  $c = 31.8\text{ nm}$ ) synthesized from 5 equiv. EE-8 / AuPAE.



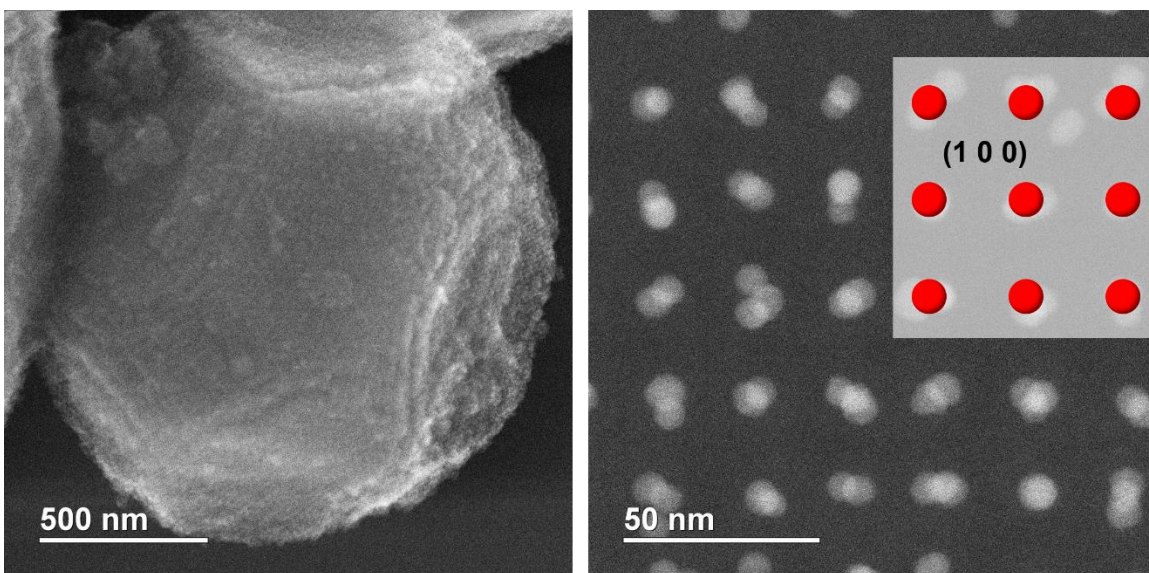
**Figure S29.** Representative SE-STEM image (left) and cross-sectional ADF-STEM image (right, along  $[0\ 0\ 1]$  zone axis) of a BCT phase superlattice (lattice parameter  $a = b = 33.3$  nm,  $c = 67.5$  nm) synthesized from 50 equiv. EE-4 / AuPAE.



**Figure S30.** Representative SE-STEM image (left) and cross-sectional ADF-STEM image (right, along  $[1\ 0\ 0]$  zone axis) of a BCT phase superlattice (lattice parameter  $a = b = 33.3$  nm,  $c = 67.5$  nm) synthesized from 25 equiv. bis(tpy)FeCl<sub>2</sub>-6DNA / AuPAE.



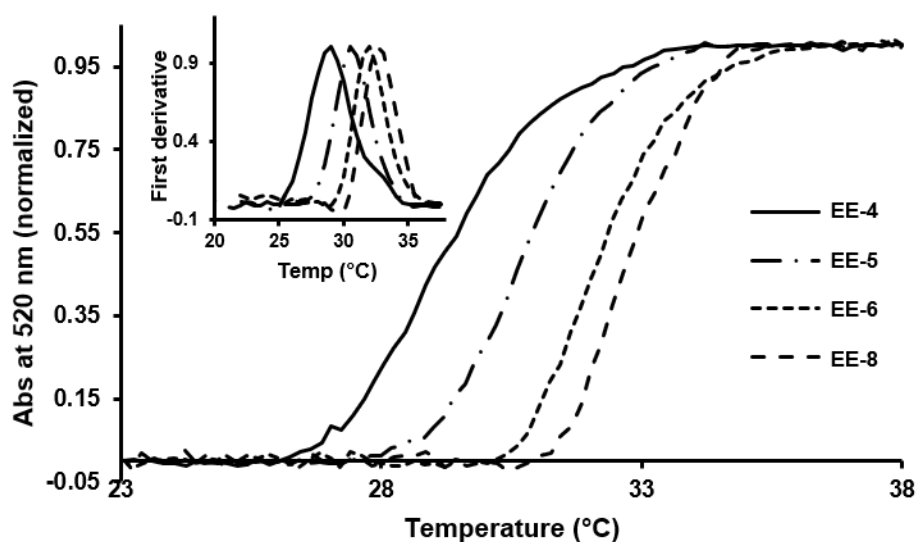
**Figure S31.** Representative SE-STEM image (left) and cross-sectional ADF-STEM image (right, along  $[1\ 1\ 1]$  zone axis) of a BCC phase superlattice (lattice parameter  $a = b = c = 44.4\text{ nm}$ ) synthesized from 200 equiv. EE-5 / AuPAE.



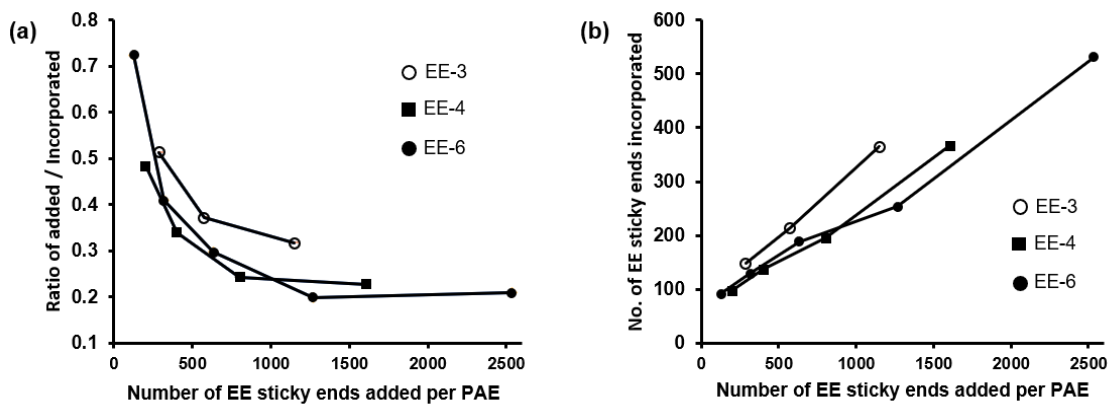
**Figure S32.** Representative SE-STEM image (left) and cross-sectional ADF-STEM image (right; along  $[1\ 0\ 0]$  zone axis) of a BCC phase superlattice (lattice parameter  $a = b = c = 44.5\text{ nm}$ ) synthesized from 200 equiv. EE-6 / AuPAE.

## Section 9: UV-vis Spectroscopy Experiments

All UV-Vis measurements were taken using an Agilent Cary5000 UV-vis spectrometer. In a typical experiment to obtain the melting temperature of a sample, 100  $\mu\text{L}$  of the superlattice suspension was diluted to 0.75 mL in 0.5 M NaCl, 10 mM PBS buffer (pH = 7.4) in a UV-vis cuvette with a magnetic stir bar and a cap. The sample was then heated at a ramp rate of 0.2  $^{\circ}\text{C}/\text{min}$  while the absorbance at 520 nm was measured. Alpha ( $\alpha$ ) curves, first derivative plots and melting temperatures ( $T_m$ ) were generated from the data using the Varian Thermal Application software.



**Figure S33.** Representative melting curves of EE-PAE assemblies prepared from EEs with increasing valency at a constant EE/PAE = 50. When EEs with valency were used, higher melting temperatures ( $T_m$ ) and narrower transitions were observed. Insert: A plot of the first derivatives of the melting curves (normalized) versus temperature.



**Figure S34.** (a) A plot of the number of EE sticky ends added per PAE versus the ratio of EEs incorporated in EE-PAE superlattices. (b) A plot of the number of EE sticky ends added per PAE versus the number of EE sticky ends incorporated in EE-PAE superlattices. Cy5-labeled EEs were utilized to enable quantification of EE incorporation, via measuring the unique and non-overlapping Cy5 absorbance at 648 nm of dissolved superlattice samples.

## Section 10: References

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