

## Functionalized DNA nanostructures as scaffolds for guided mineralization

Supplementary Information

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## General

Acetonitrile (HPLC grade), boric acid, bromophenol blue, chloroacetic anhydride, formamide, glycerine, hydroxylamine hydrochloride, N-hydroxysuccinimide, potassium thioacetate, ProteoSilver™ Plus Silver Stain Kit (PROT-SIL2), sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate, toluene, tris(hydroxymethyl)-aminomethane (Tris), urea and xylene cyanole were purchased from Sigma-Aldrich. Glacial acetic acid was purchased from Anachemia. GelRed® Nucleic Acid Stain was purchased from Biotium. Calcium chloride and magnesium chloride were purchased from Fisher Scientific. Hydrochloric acid and 40% acrylamide/bis-acrylamide 19:1 solution were purchased from BioShop. Unmodified synthetic DNA was purchased from Integrated DNA Technologies (IDT DNA). 5'-maleimide DNA strands were purchased from GeneLink. Poly(L-aspartic acid) sodium salt, (MW 27,000 g/mol) was purchased from Alamanda Polymers. Highest grade V1 Mica sheets (2 x 3") were purchased from Ted Pella Inc., while cantilevers (OTESPA-R3) were purchased from Bruker. 400 mesh carbon coated copper grids were purchased from Electron Microscopy Sciences. 1 x TB buffer is composed of 90 mM Tris and 90 mM boric acid, pH 8.0. 1 x TAMg buffer is composed of 40 mM Tris, 12.5 mM MgCl<sub>2</sub>·6H<sub>2</sub>O and 20 mM acetic acid (glacial). The pH was adjusted to 7.8 ± 0.1 using HCl. 1 x TACa buffer is composed of 40 mM Tris and 12.5 mM CaCl<sub>2</sub>·2H<sub>2</sub>O and 20 mM acetic acid (glacial). The pH was adjusted to 7.8 ± 0.1 using HCl. The pH 0.1 M phosphate was adjusted to 7.4 ± 0.1 using NaOH.

## DNA assembly

Gel electrophoresis experiments were carried out on an acrylamide 20 x 20 cm vertical Hoefer 600 electrophoresis unit. Thermal anneal cycles were conducted using Flexigene Techne 96 well thermocycler. AFM was performed with a MultiMode™ SPM connected to a Nanoscope™ IIIa controller, from the Digital Instruments Veeco Metrology Group. Transmission electron microscopy (TEM) images were obtained using a FEI Tecnai 12.

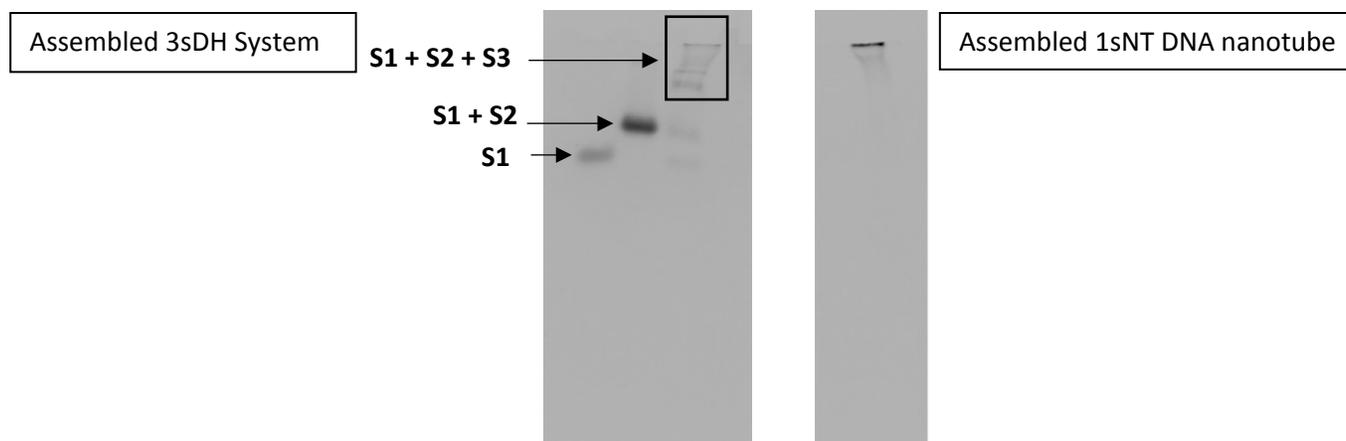
### I. DNA sequences

Strand	Sequence (5' to 3')
<b>1sNT<sup>1</sup></b>	CCAAGCTTGGACTTCAGGCCTGAAGTGGTCATTCGAATGACCTGAGCGCTCA
<b>S1</b>	TGTCAGGTA ACTCAGCAGCGAAAAACCGCTTTACCACATTCGAGGCACGTTGTACGTC CACACTTGGAACCTC CGTCGTAGCAG
<b>S2</b>	GAGGTTCCAAGTGTGGACGTACAACGTGCCTCGAATGTGGTAAAGCGGTTTTTCGCTGCTGA G
<b>S3</b>	TTACCTGACACTGCTACGACG
<b>S3-Mal</b>	Mal-TTACCTGACACTGCTACGACG

**Table S1.** Sequences of DNA strands used for the 1sNT and 3sDH systems.

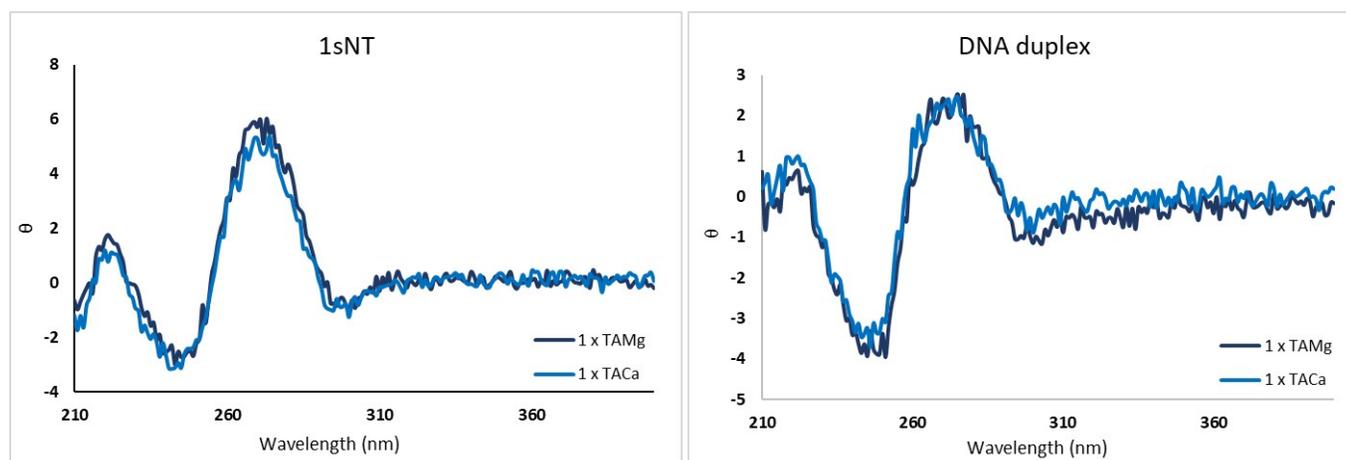
## II. DNA assembly in calcium-containing buffers

DNA assembly in 1x TACa buffer was assembled by a thermal anneal from 90 to 4°C (over 4 hours) for the



1sNT system and 56 to 20°C (over 1 hour) for the 3sDH system.

**Figure S1.** 8% native gels in 1 x TACa, stained with GelRed®, of the assembled 3sDH (left) and the 1sNT (right) systems.



**Figure S2.** Circular dichroism spectra of the 1sNT (left) and of a 20 bp DNA duplex (right) in 1 x TAMg and 1 x TACa. No structural changes are observed in the double helical structure when the counter ion in the buffer is changed.

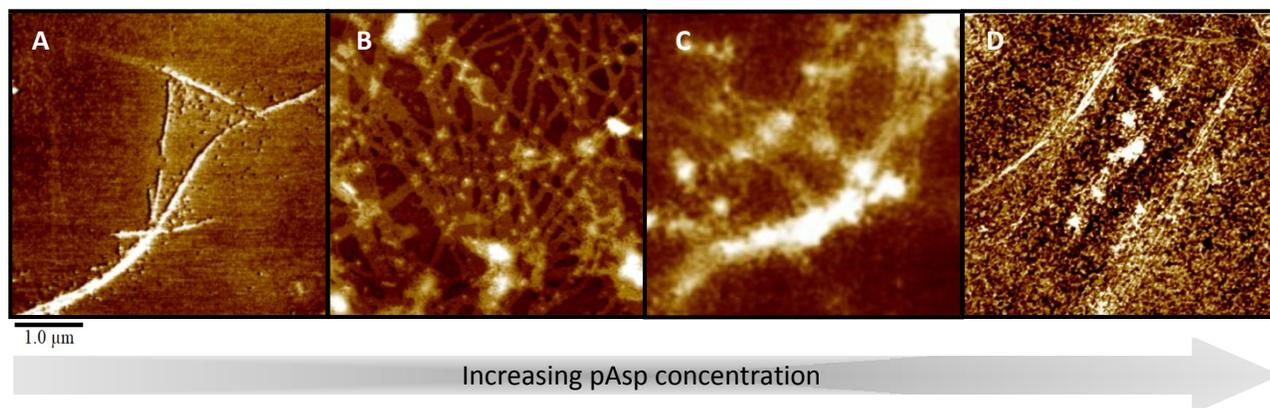
### Passive addition of PAsp to 1sNT

*1sNT assembly:* 1sNT DNA (20 μM) was dissolved in 1x TACa buffer and assembled into nanotubes using a thermal anneal cycle from 90 to 4°C over a period of 4 hours. While most artificial DNA structures are designed to assemble in the presence of Mg<sup>2+</sup>, our lab has demonstrated that successful assembly of 1sNT can be achieved in the presence of Ca<sup>2+</sup> instead of Mg<sup>2+</sup> without resulting in changes to its morphology.

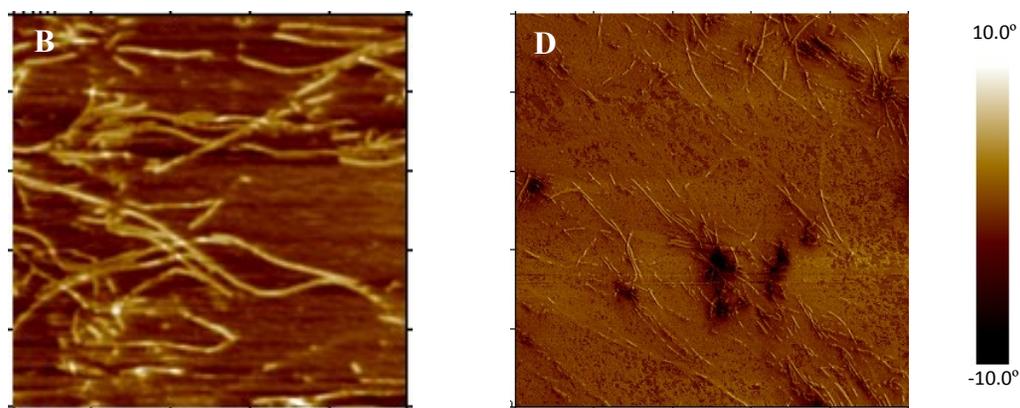
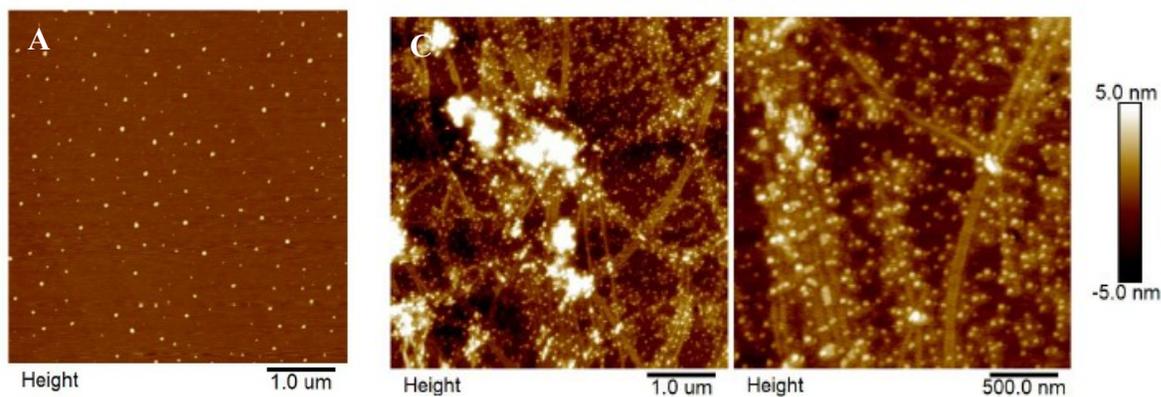
*PAsp assembly:* 1 mg of the PAsp salt was dissolved in 10 mL of MilliQ water (deionized H<sub>2</sub>O) and sonicated for 10 minutes prior to usage.

*Co-assembly of 1sNT and PAsp:* The assembled PAsp (0.25  $\mu\text{L}$ ) and 1sNT DNA (8  $\mu\text{L}$ ) in 1 x TACa buffer were incubated at room temperature for 20 minutes prior to surface deposition and AFM characterization.

*Atomic Force Microscope (AFM):* Mica and AFM specimen discs (12 mm diameter) were purchased from Ted Pella Inc. 8-10  $\mu\text{L}$  of sample solution were dropped onto freshly cleaved mica surface and left for 1 minute to adsorb. The surface was dried with compressed air. Phase and height images were obtained using tapping-mode AFM on a Multimode Nanoscope IIIa microscope (OTESPA-R3 cantilever tips), in



**Figure S3.** Passive addition of pAsp nanospheres in **A** 2.5%; **B** 5%; **C** 7.5%; and **D** 10% w/w to 1sNT assemblies.

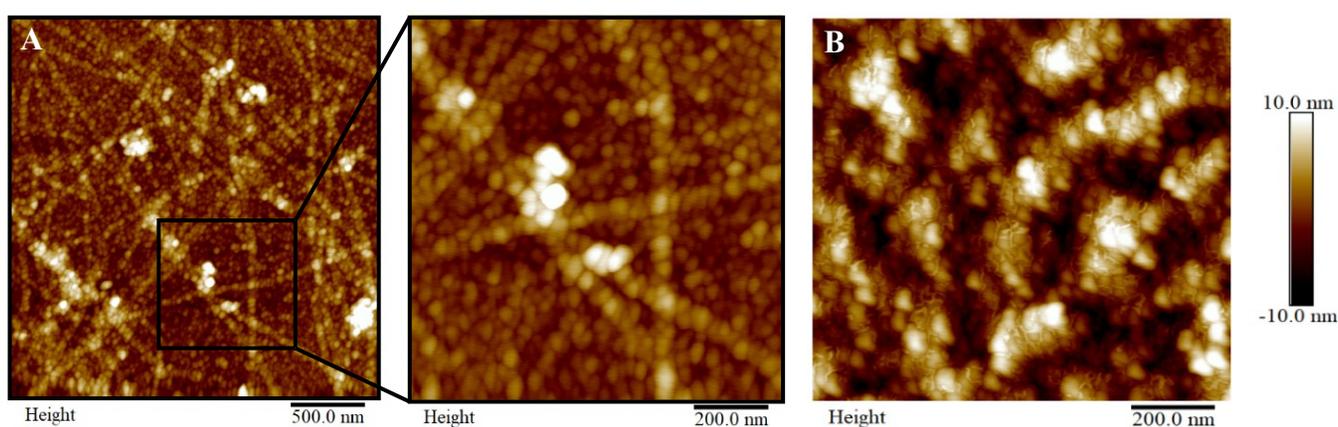


ambient conditions within 24 hours.

**Figure S4.** AFM characterization in 1xTACa of: **A** pAsp nanospheres in ambient conditions; **B** 1sNT in liquid conditions; and 1sNT incubated with 10% pAsp for 20 minutes in **C** ambient and **D** liquid conditions.

Sample	Width (nm)	Height (nm)
1sNT in TACa	$66.7 \pm 7.4$	$4.0 \pm 0.2$
Assembled pAsp in TACa	$56.6 \pm 8.2$	$3.7 \pm 0.3$
1sNT + pAsp in TACa	n/a *	$7.7 \pm 0.4$

**Table S2.** Width and height measurements of one strand nanotubes (1sNT) assembled in 1 x TACa. A noticeable difference in height was observed when pAsp was adsorbed on top of 1sNT assemblies, roughly corresponding to the height of pAsp aggregates themselves. \*Not measured since mostly bundles were observed.



**Figure S5.** AFM images of: **A** Passive addition of pAsp along 1sNT. **B** 1sNT in 1 x TACa incubated with phosphate ions for 20 minutes.

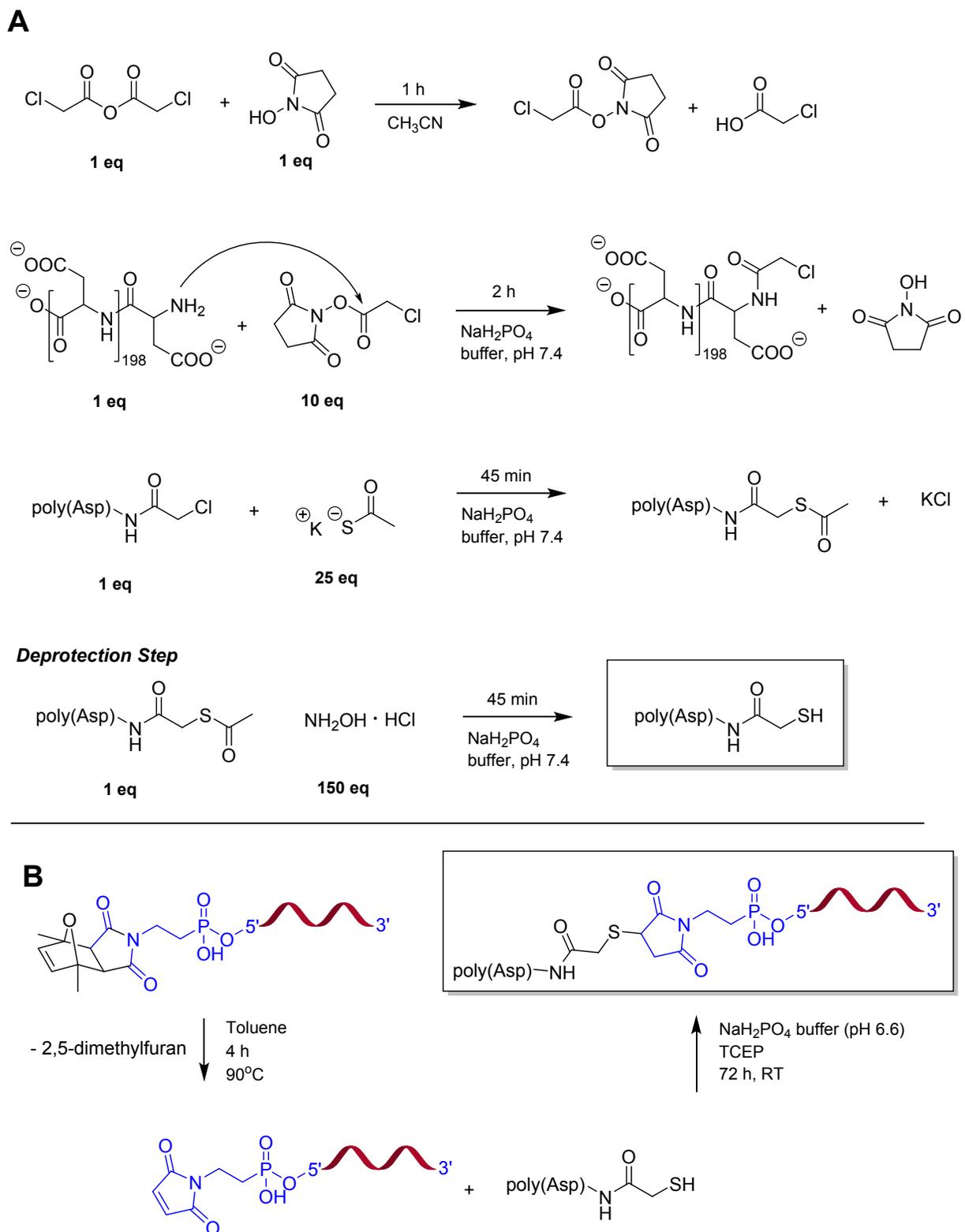
## Covalent attachment of pAsp and DNA

*Functionalization of pAsp with a thiol group:* pAsp (MW 27,000 g/mol) was prepared in sodium phosphate buffer (pH 7.4) and functionalized with a thiol group at the N-terminus in a reaction sequence (figure 3A of the manuscript). The crude products were concentrated using a lyophilizer with the setting on high temperature and desalted using Amicon Ultra centrifugal filters (10,000 MWCO).

*Covalent conjugation of pAsp to DNA:* The thiol-modified pAsp was conjugated to synthetic DNA-maleimide strands using a previously described method of thiol-maleimide chemistry.<sup>11,12</sup> Synthetic DNA strands containing a protected maleimide group at the 5' end (purchased from Gene Link) were suspended in HPLC grade acetonitrile. An aliquot was transferred to a glass vial and the acetonitrile was evaporated with forced air. ~600  $\mu$ L of toluene was added to the DNA under anhydrous conditions. The DNA and toluene were incubated at 90°C for 3.5 to 4 hours. This was done to promote a reverse Diels–Alder reaction that results in deprotection of the maleimide group. pAsp (which was previously functionalized with a thiol group) was deprotected using 10 mg of hydroxylamine hydrochloride in 50  $\mu$ L of 0.1M sodium phosphate buffer (pH 7.4). 1  $\mu$ L of 0.5 M tris(2-carboxyethyl) phosphine (TCEP), a reducing agent, was added to the pAsp to prevent the formation of di-sulfide bonds between the thiol groups. The

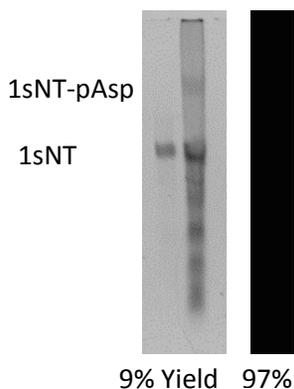
pAsp was added to the deprotected DNA and left to react over 72 hours on a rotator under ambient conditions.

*Purification of pAsp-DNA conjugate product:* The products were separated from any remaining, unconjugated DNA via gel electrophoresis. The upper bands of lower mobility were excised from the gel and collected via electroelution. This process was carried out twice in order to ensure that the product was appropriately purified.



**Figure S6.** Scheme of the reaction conditions for covalent conjugation of pAsp to 1sNT DNA. Reaction conditions to: **A** transform the amino terminal of pAsp into a thiol group; and **B** deprotect the maleimide group in the DNA strand (step 1) and thiol-ene conjugation reaction between pAsp and DNA (step 2).

The conjugation reactions were characterized using polyacrylamide gel. The gels were run at 300 V, 15 mA for 1.5 hours, then stained using GelRed® or a ProteoSilver™ Silver Stain Kit. The product yield was



calculated using ImageJ software.

**Figure S7.** 12% denaturing PAGE of the crude pAsp-DNA conjugate.

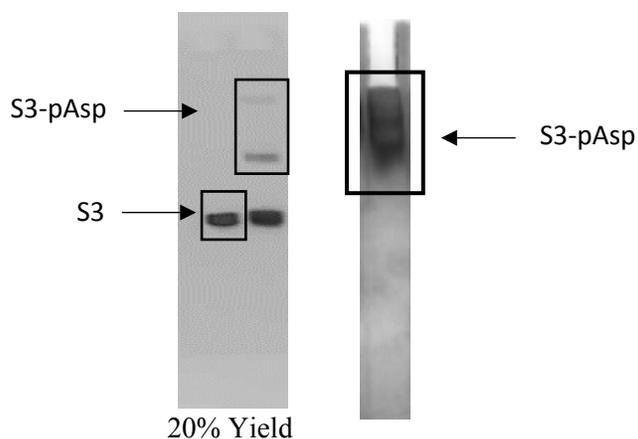
**Figure S8.** Quantification of the reaction yield, as calculated by ImageJ analysis.

For the optimization of the product yield, it is important to note that firstly, the 95% yields were obtained following the use of purified DNA-maleimide while lower product yields (~10% and ~25%) used unpurified DNA-maleimide as a starting material. Secondly, sonication of the product throughout the reaction, as well as prior to imaging improved the yield of the reaction. Thirdly, we found that using polypropylene tubes instead of glass vessels improved the efficiency of the reaction yield; this was likely because pAsp tends to bind to the walls of the glass vial. The reaction yield was also improved when the DNA was reacted under anhydrous conditions, after being washed with acetonitrile and dried with compressed air, rather



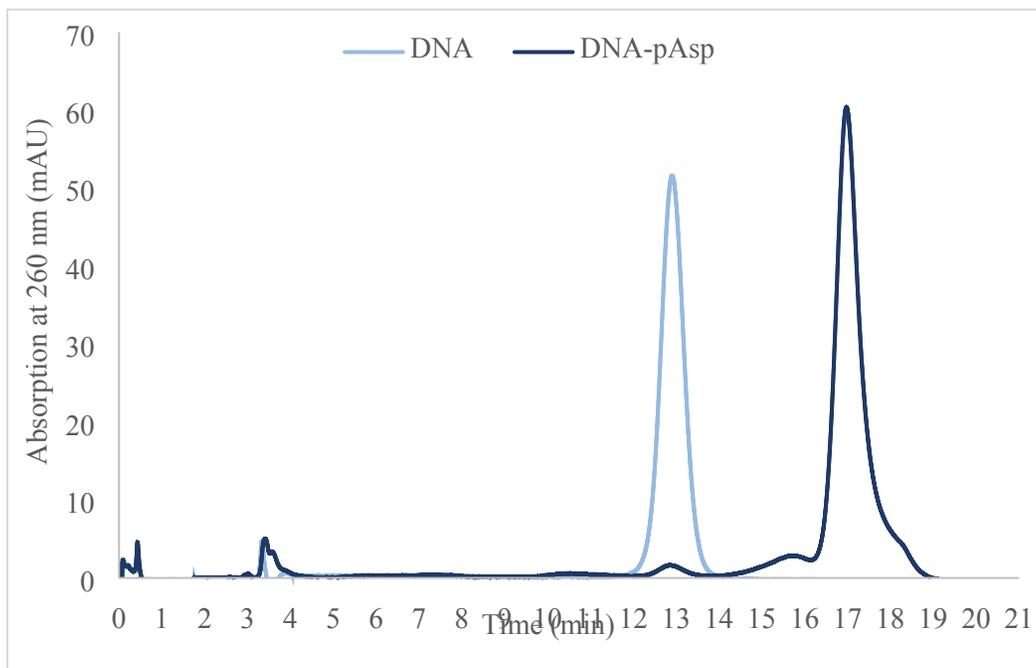
#	AUC (Product)	AUC (Total)	Product / Total	Percentage
1	41849.626 2257.489	44107.115	0.948818	94.88%
2	31467.706 487.477 44.95 80.485 31.657 8.828	32121.103	0.9796583	97.96%
3	24263.827 425.891 30.657	24720.375	0.9815315	98.15%
4	18328.765 425.891 30.657	18785.313	0.9756965	97.57%
5	32530.948 710.033 73.192 47.364 63.243	33424.78	0.9732584	97.32%
<b>Mean % Yield</b>			<b>= (94.88 + 97.96 + 98.15 + 97.57 + 97.57) / 5 = 97.176%</b>	

than in buffer. Additionally, the reaction produced higher yields at room temperature rather than at 5°C.

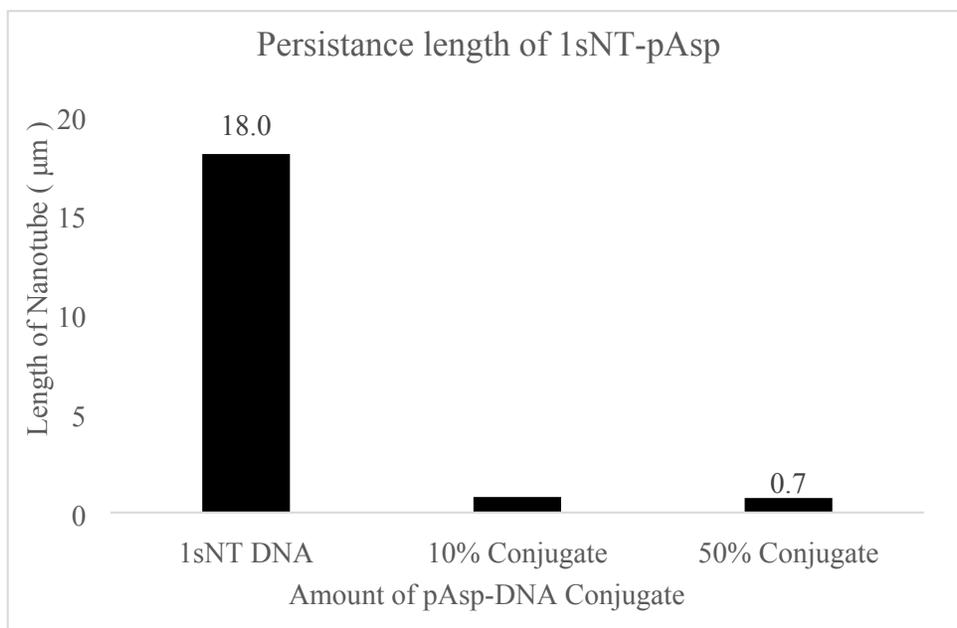


**Figure S9.** Following electroelution, the purified pAsp-DNA conjugate sample was visualized via silver stain. This image shows a polyacrylamide denaturing gel, stained with ProteoStain™.

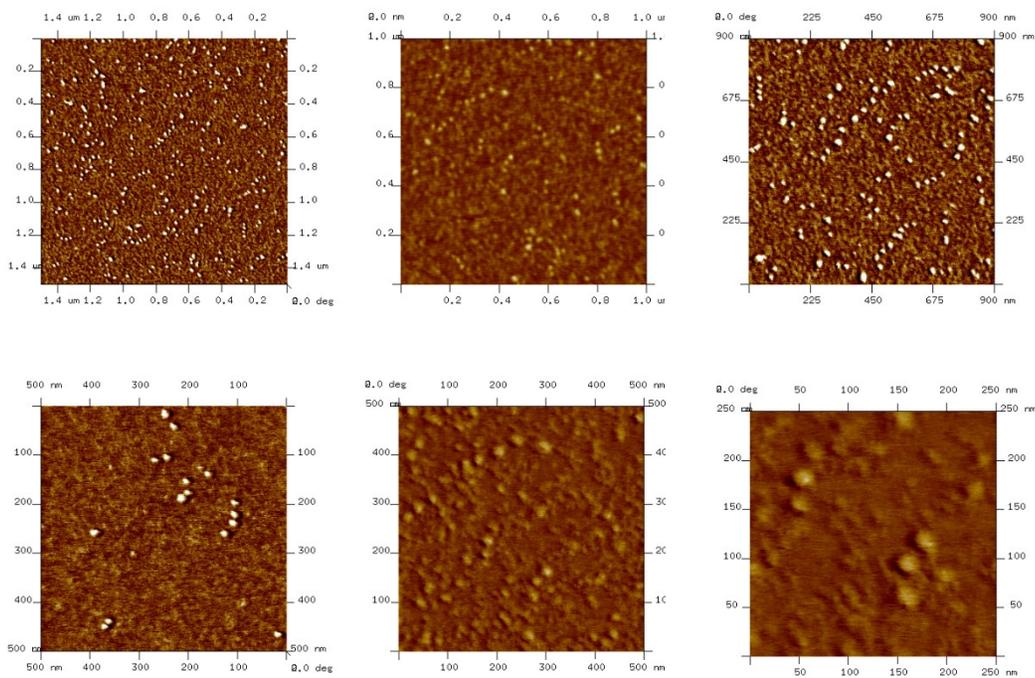
DNA-pAsp conjugate was characterised by anion exchange chromatograph using the ProFIRE instrument (Dynamic Biosensors). The anion exchange column had a matrix of hydrophilic porous polymer beads with a particle size of 30  $\mu\text{m}$  and the functional group  $-\text{R}-\text{N}-(\text{CH}_3)_3$ . The ProFIRE used a 50 mM phosphate buffer (pH 7.2) with a flowrate of 1 mL/min, increasing the ionic strength from minute 3 to 19 from 0.15 M to 0.83 M NaCl. The absorption was measured at 260 nm.



**Figure S10.** Anion exchange chromatogram of unfunctionalized DNA (light blue) and DNA-pAsp conjugate (dark blue).



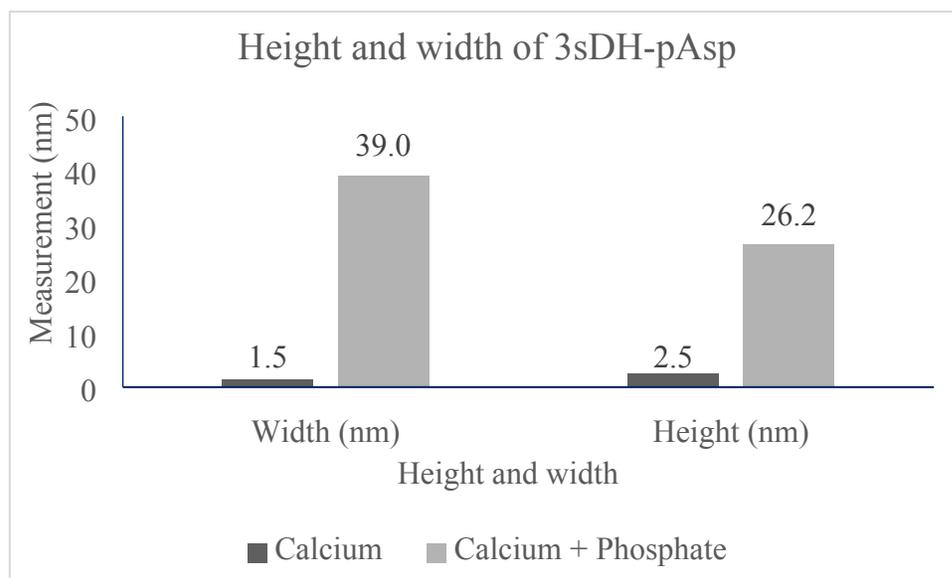
**Figure S11.** Persistence length measurements of assembled 1sNT were obtained using NanoScope Analysis 1.9. The incorporation of pAsp-DNA conjugates yielded the formation of improperly assembled, shorter and “kinked”, tubes.



**Figure S12.** Additional AFM images of the 3sDH-pAsp system in 1xTACa.

## Mineralization Experiments

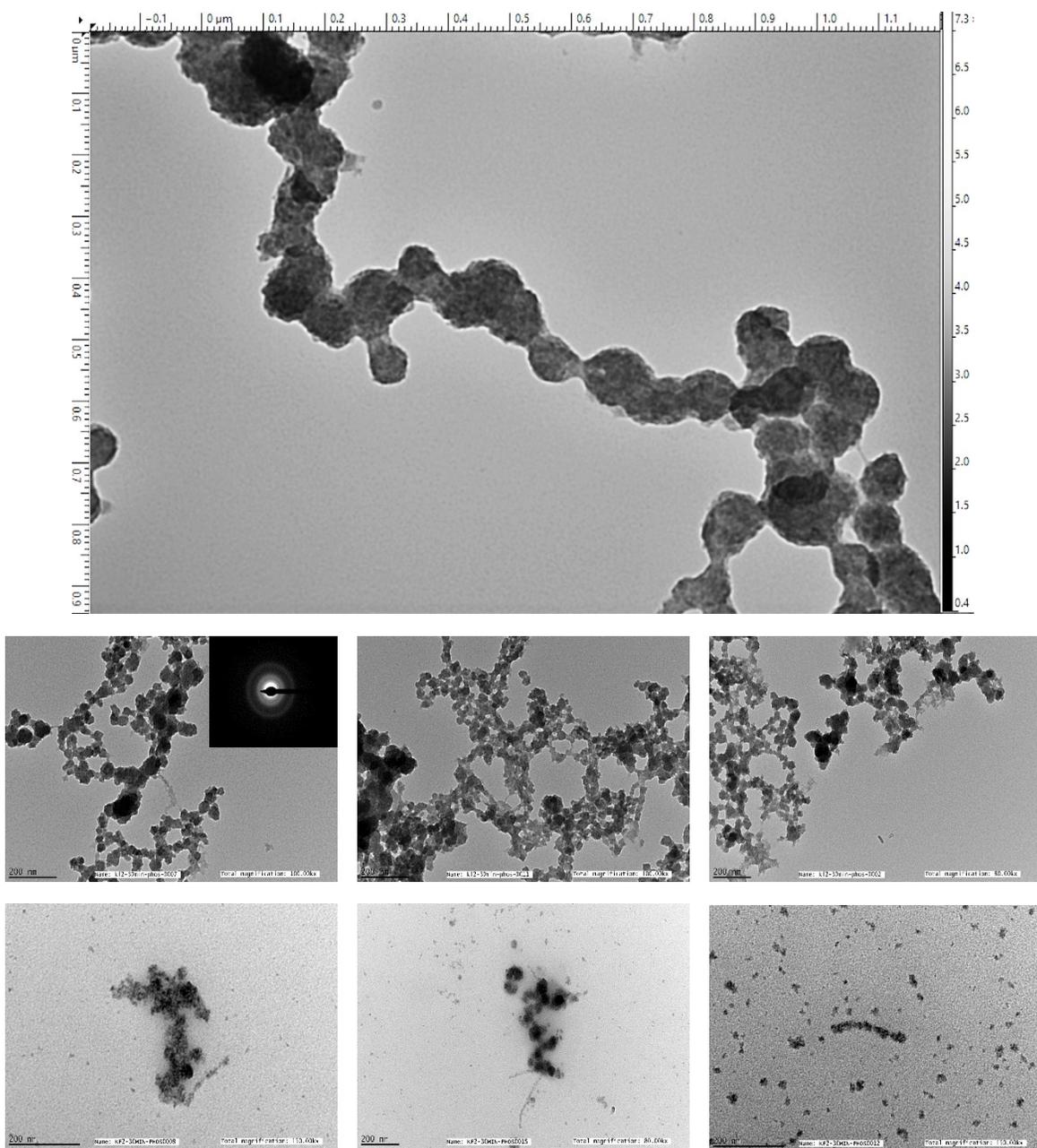
When the 3sDH system was incubated with phosphate, the pAsp spheres seemed to “swell” and mineralization was observed along the spheres. Following the PILP protocol, we hypothesize that pAsp acts as an amorphous mineral carrier along the DNA strand to mimic mineral inducing proteins present *in vivo*. In the presence of calcium and phosphate, mineralization was observed along the DNA scaffolds following 20-minutes to 3-hour incubation period.



**Figure S13.** Following exposure to  $\text{Ca}^{2+}$  and  $\text{PO}_4^-$ , the pAsp nanospheres increased in both height and width. These measurements were obtained using NanoScope Analysis 1.9.

## Transmission Electron Microscopy

5  $\mu\text{L}$  of sample were deposited on a glow discharged carbon-coated copper grid (Ted Pella, Redding, CA, USA) and incubated for 120 seconds. The sample was wicked onto filter paper, washed two times with autoclaved deionized water on an inverted drop and air dried. The grids were imaged with a FEI Tecnai 12 TEM (FEI Company, Hillsboro, OR, USA) at 200 kV. Data were acquired with a 4x4 k Gatan Ultra Scan CCD camera (Gatan, Pleasanton, CA, USA).



**Figure S14.** Guided mineralization achieved by the 3sDH-pAsp. TEM image of the assembled 3sDH-pAsp in 1 x TACa, incubated with  $\text{PO}_4^-$  for 30 minutes.