Supporting Information For:

Fine Customization of Calcium Phosphate Nanostructures with Site-Specific Modification by DNA Templated Mineralization

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1. Materials and instruments. All solvents and chemicals were purchased from commercial sources and were used without further purification. DNA staple strands were purchased from Wuhan Genecreate Biological Engineering Co., LTD. Agarose gel electrophoresis was performed using Bio-Rad Mini-Sub Cell GT horizontal electrophoresis system. Bio-Rad MyCycler™ Thermal Cycler was used for annealing of MP13mp18 phage DNA and DNA staple strands to form DNA origami. Concentration of DNA origami was determined by Spark ® 20M with Nanoquant plateTM.

2. Experimental section

Fabrication of DNA origami nanostructures. DNA origami templates with different structures were prepared according to the published works[1-3]. In short, for l-origami 5 nM M13MP18 phage DNA of 7560 nt for scaffold strand with 50 nM desired 210 staple strands in 1 × TE / Mg buffer (5 mM Tris, 1 mM EDTA, 5 mM NaCl, and 10 mM $MgCl_2$, pH 8.2) and annealing from 65 °C to 20 °C over 2 h. For r-origami the origami structures were folded in a 27 h annealing process from 65 °C to 4 °C, with 5 nM of scaffold strand (7560 nt, M13mp18 phage DNA), 50 nM desired 173 staple strands in 1 × TE / Mg buffer. And for t-origami, l-origami (0.5 pmol) solution was added a set of 16 folding DNA strands (250 pmol each) and the mixture was incubated at 32 °C for overnight. Followed by purification with polyethylene glycol (PEG) precipitation method.

Preparation of supersaturated solutions. For preparation of supersaturated solutions, a range of supersaturated solutions (σ_{OCP} = 1.77–2.24, pH = 6.50, and an ionic strength (IS) of 0.15 M) were prepared. The experimental conditions are summarized in Table 1.

Table 1. Conditions in OCP Nucleation and Growth Experiments

	final solution concentration (mM L^{-1})[a]		
$\sigma_{Ca\text{-}P}$	NaCl	$CaCl_2$	KH_2PO_4
1.77	133 mM	4.1 mM	3.10 mM
1.98	133 mM	4.5 mM	3.40 mM
2.24	130 mM	5.0 mM	3.76 mM

a The solutions were freshly prepared using the stock solutions (1.0 M NaCl, 0.04 M $CaCl_2$, and 0.04 M KH_2PO_4).

Atomic force Microscopy (AFM). Imaging was performed with a Bruker Multi mode 8 AFM (Bruker, Santa Barbara, CA, USA) equipped with the ScanAsyst mode. The sample solution was deposited onto freshly cleaved mica surface, and left for 5 min at room temperature to allow adsorption of the DNA origami structures. After addition of 70 µL of 1 × TAE / Mg buffer, the sample was scanned with the scan rates between 1 and 3 Hz, all of the AFM images were captured using ScanAsyst mode. For *in situ* AFM experiment, the rate of addition of solutions was 0.3 ml/min for 2 hours, using ScanAsyst in fluid+ probe, the solution used during the experiment was prepared according to the

solutions in DNA origami mineralization section. Several AFM images were acquired at different areas of the mica surface to ensure the reproducibility of the results. All images were analyzed by using the NanoScope Analysis 1.90 software.

3. **Mineralization of DNA origami.**

In each experiment, we carefully added a certain volume of DNA origami nanostructures to the Ca-P supersaturated solution. To ensure that the concentration of Ca^{2+} and PO_4^{3-} in the solution do not change drastically, we set the concentration of DNA origami in the final reaction solution to 3 -5 nM, and the final volume of the solution is 50 uL. DNA origami was added into Ca-P supersaturated solutions after pH adjusted to pH 6.5. In each experiment, DNA origami stock solution was prepared and purified following the method which was mentioned above, and the DNA origami was dissolved in 1 × TE / Mg buffer. The DNA origami was incubated into different supersaturated Ca-P solutions (Figure 1) at a series of incubation time (4, 8 and 12 h), incubation temperatures (0, 25 and 37 ^{o}C) for optimization of preparing mineralized DNA origami (See table 2 and table 3).

Table 2. Conditions of preparing mineralized DNA origami at 37 ^{o}C

Incubation time (h) / **Supersatruation (σ)**	4	8	12
1.77	√	√	×
1.98	×	×	×
2.24	×	×	×

√ Under these conditions, the desired calcium phosphate mineralized DNA origami nanostructures can be obtained.

× Under these conditions, it is difficult to obtain the desired calcium phosphate mineralized DNA origami nanostructures.

Table 3. Conditions of preparing mineralized DNA origami at σ=1.77

Incubation time (h) / Temperature (°C)	4	8	12
4	√	√	√
25	√	√	√
37	√	√	√

√ Under these conditions, the desired calcium phosphate mineralized DNA origami nanostructures can be obtained.

Since the nucleation and growth of calcium phosphate is based on the selection of Ca/P for the supersaturation and Ca/P molar ratio selected in this study, we calculated, the relative supersaturation of different phases of calcium phosphate, according to different Ca/P molar ratios under the same conditions (SI = 0.15 M, pH=6.5, Ca^{2+} concentration is 4.1 mM). It can be seen from Figure S1 that the relative supersaturation of the same crystal phase obtained under the same conditions according to the different Ca/P molar ratio is different, and under the same Ca/P molar ratio, the corresponding supersaturation of different crystal phases is also different. Therefore, in this experiment, it is necessary to select the appropriate growth rate, that is, the Ca/P ratio of the required Ca^{2+} and PO_4^{3-} concentration is relatively low, but does not affect the crystal growth rate. The results in Figure S1 shows that the lower the Ca/P molar ratio, the higher the supersaturation corresponding to different crystals, and the faster the nucleation rate in the corresponding solution; and when the Ca/P molar ratio increases and the Ca^{2+} concentration remain the same, the PO_4^{3-}concentration needs to be gradually reduced, so the nucleation rate in the solution will also be reduced, so as to achieve the purpose of easily regulating the nucleation and growth of Ca-P in the solution. Although the nucleation rate is too fast or too slow, it can achieve the purpose of preparing mineralized DNA origami, but too fast or too slow nucleation rate is not the preferred condition for this experiment (for example, the Ca/P molar ratio is 1 or 2), and based on previous experience on crystal nucleation and growth[4-6], we chose a Ca/P molar ratio of 1.33, which is the Ca/P molar ratio used in the experiment. This happens to be the theoretically Ca/P molar ratio of the OCP in calcium phosphate crystal phases, so the supersaturation mentioned in the article is based on the OCP phase to calculate the relative supersaturation.

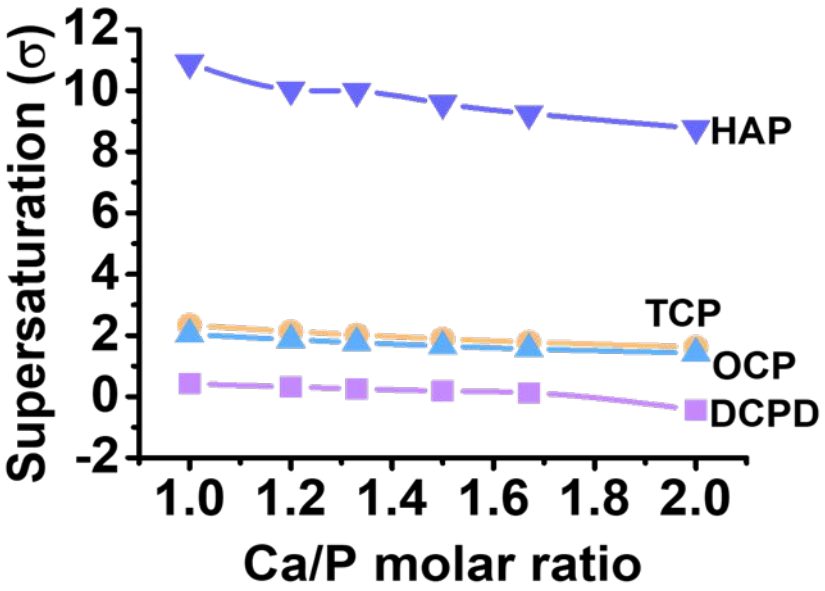

Figure S1 Selection of supersaturation and Ca/P molar ratio used in the experiment. The relative supersaturation of different calcium phosphate crystal phases (DCPD, TCP, OCP and HAP) calculated under different Ca/P ratios under the same conditions (IS=0.15 M, pH =6.5, Ca2+ concentration of 4.1 mM).

A controled mineralization experiment under identical conditions obviating DNA template was also conducted, and typical CPA phenomena were observed. Nanoparticles with a diameter of *ca.* 50 nm were visible in the supersaturated solution after 8 h (Figure S7). In contrast, the presence of DNA nanostructures
led to a clear accumulation around their frameworks and significantly inhibited the crystal growth in the bulk solution (Figure S7). It was until 12 h later that the free nanoparticles were noticeable in solution (Figure 2E). These observations also support the CPA process discussed above.

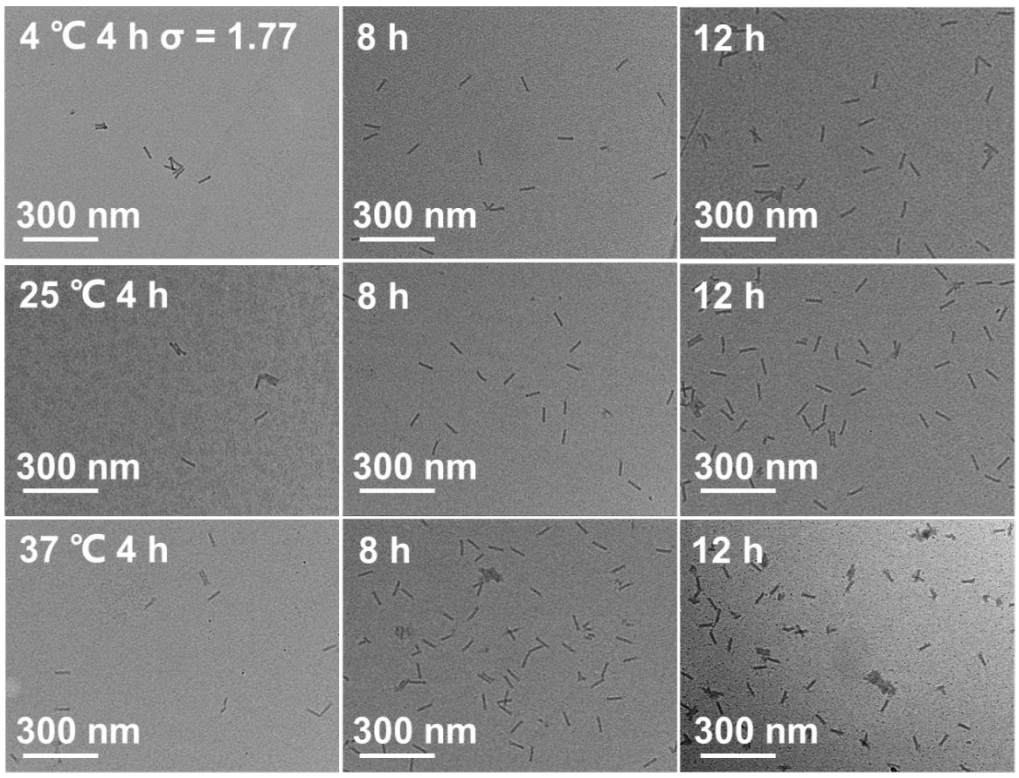

Figure S2 TEM images of optimization of r-origami mineralization condition, at different temperatures (4, 25, 37 ^{o}C) and at different incubation time (4, 8, 12 h) using the best supersaturation condition (σ= 1.77). TEM images result show that different temperature tested did not apparently affect the mineralization process within 8h, therefore, the mineralization time of 8h was considered the best.

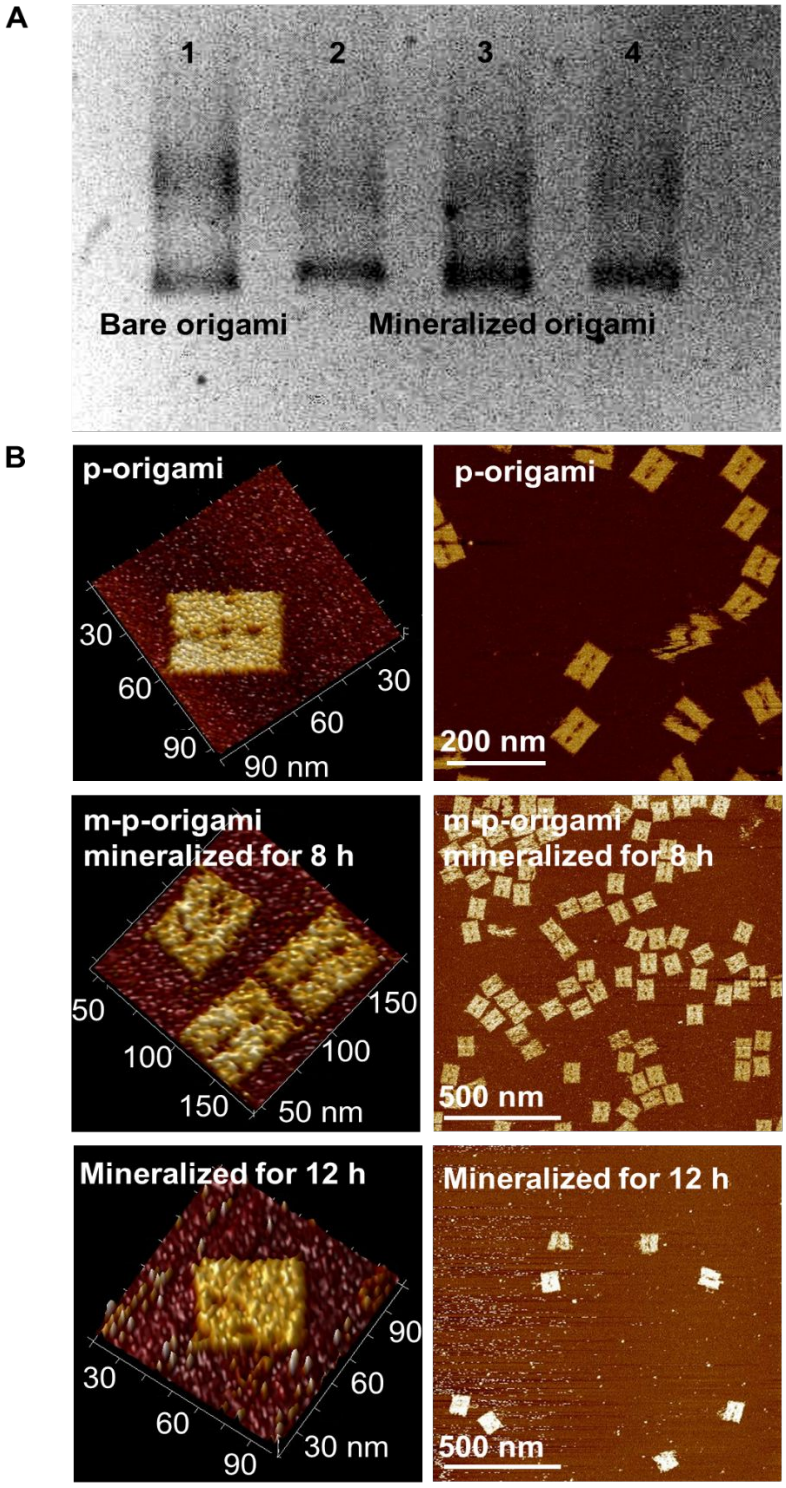

Figure S3 Agarose gel electrophoresis and AFM images of bare origami and m-origami structures. A is electrophoresis image, 1 is band for bare l-origami, 2-4 are bands for m-l-origami, which shows that there is no significant difference in terms of chargeability; B is AFM images for bare *p*-origami and m-*p*-origami incubated for different times (8 h and 12 h). AFM images show that incubated for 8 h, the defined pore structure on *p*-origami could still be clearly observed; but after 12 h incubation, the pore structure on *p*-origami disappears.

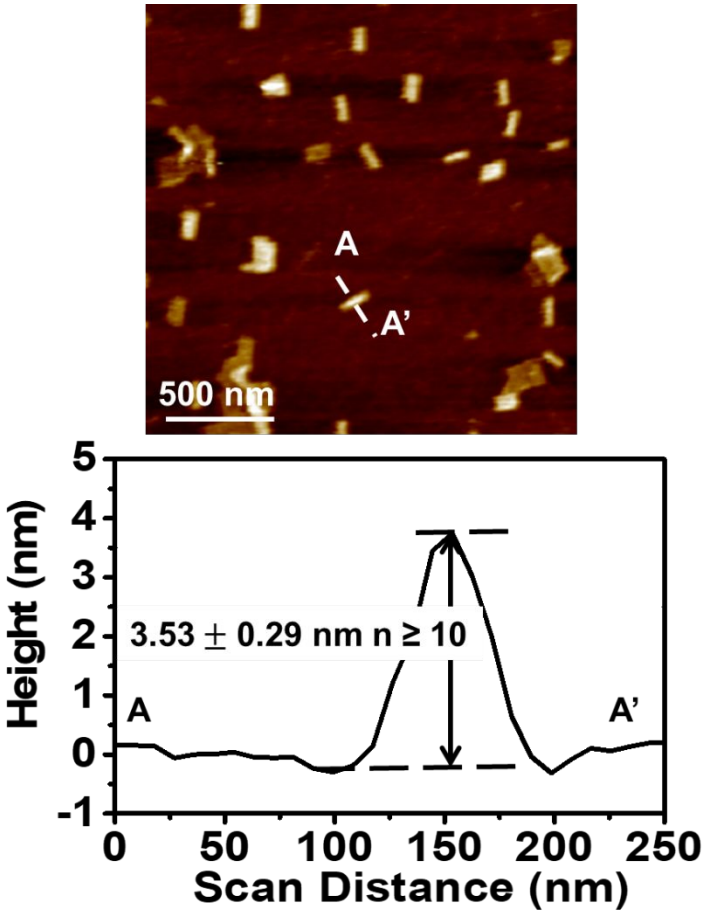

Figure S4 Height statistics of t-origami using AFM image. And average height of t-origami is 3.53 ± 0.29 nm (n ≥ 10).

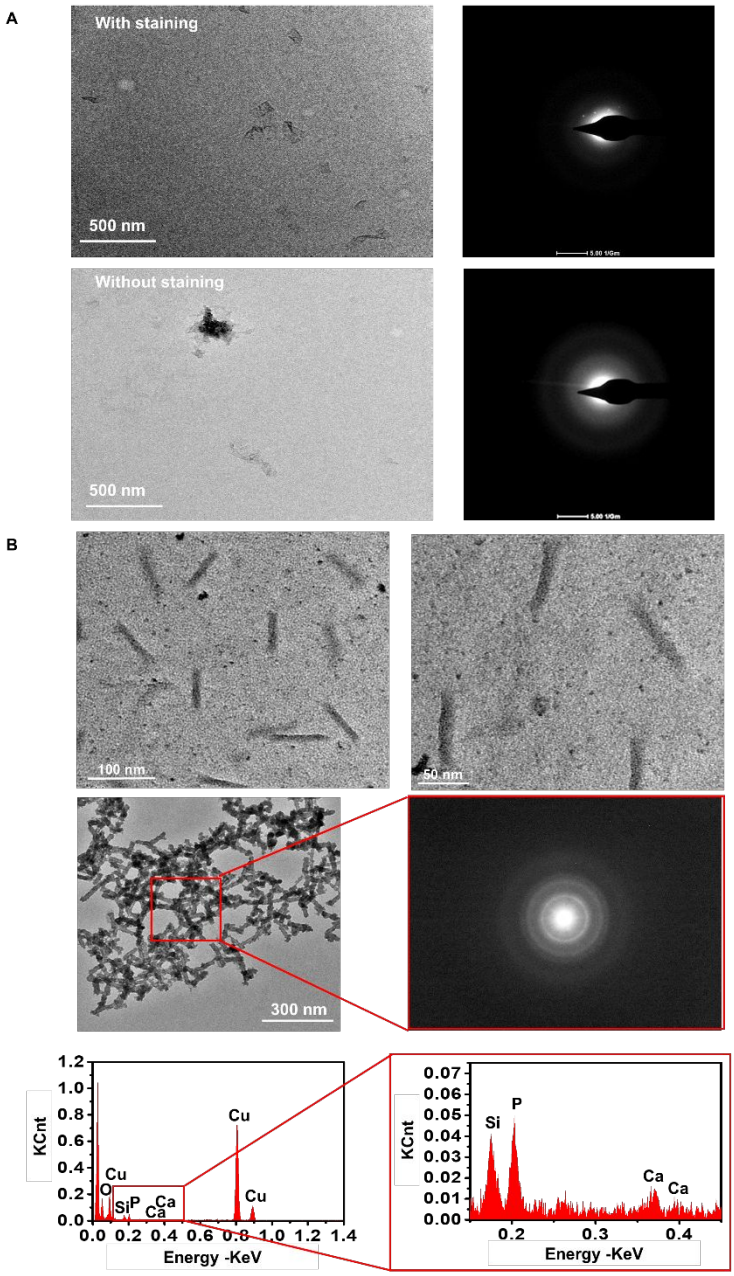

Figure S5 TEM images of mineralized DNA origami structures with different shapes, as well as SAED and EDXA characterization results. A is the TEM images of l-origami and m-l-origami and their EDXA images. Since the sample is a DNA molecule, no diffraction ring appears in bare l-origami SAED image. And because the content of Ca-P crystals on the surface of a single DNA origami structure is very small, only a weak SAED signal can be seen in the place where origami gathers after mineralization. B TEM image of m-r-origami, as well as SAED and EDXA characterization results. The TEM result shows that particles which attached on the r-origami surface, are amorphous calcium phosphate and a small amount of Ca element also appears in the EDXA spectrum. Therefore, it can be proved that a layer of amorphous Ca-P is adhered on to the DNA origami surface.

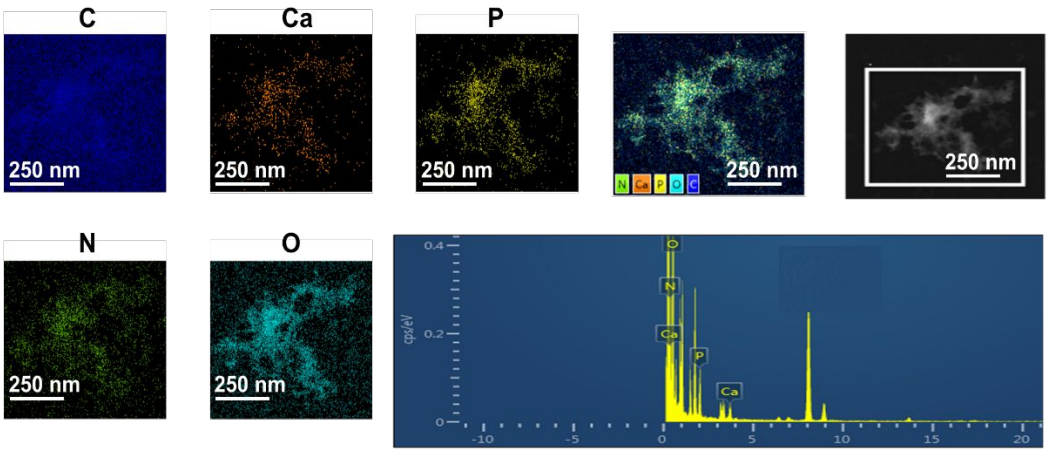

Figure S6 HAADF image of l-origami structure and results of EDXA mapping. Element analysis of HAADF gives the same result, indicating that Ca element signals can be observed on the DNA origami. But because the amount of Ca element adsorbed on the surface of the DNA origami structure is too small, it is difficult to get the exact positional relationship between Ca element and DNA origami by EDXA mapping.

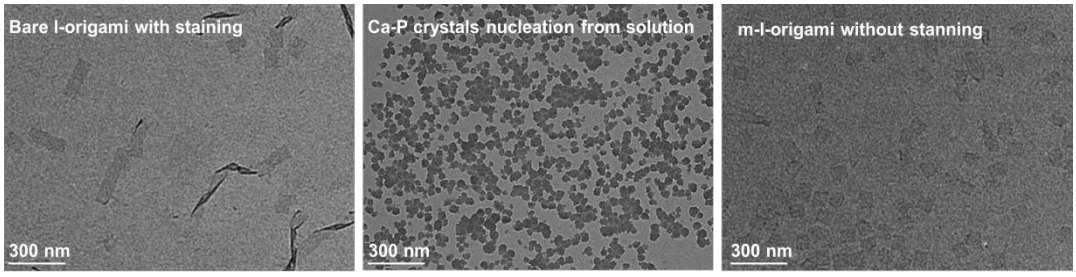

Figure S7 TEM images of bare l-origami, Ca-P crystals nucleation from supersaturated solution (σ= 1.77) incubated for 8 h and mineralized origami without staining. TEM images showed that the morphology of Ca-P particles could be regulated by the DNA origami structure when incubated in Ca-P supersaturated solution for 8 hours.

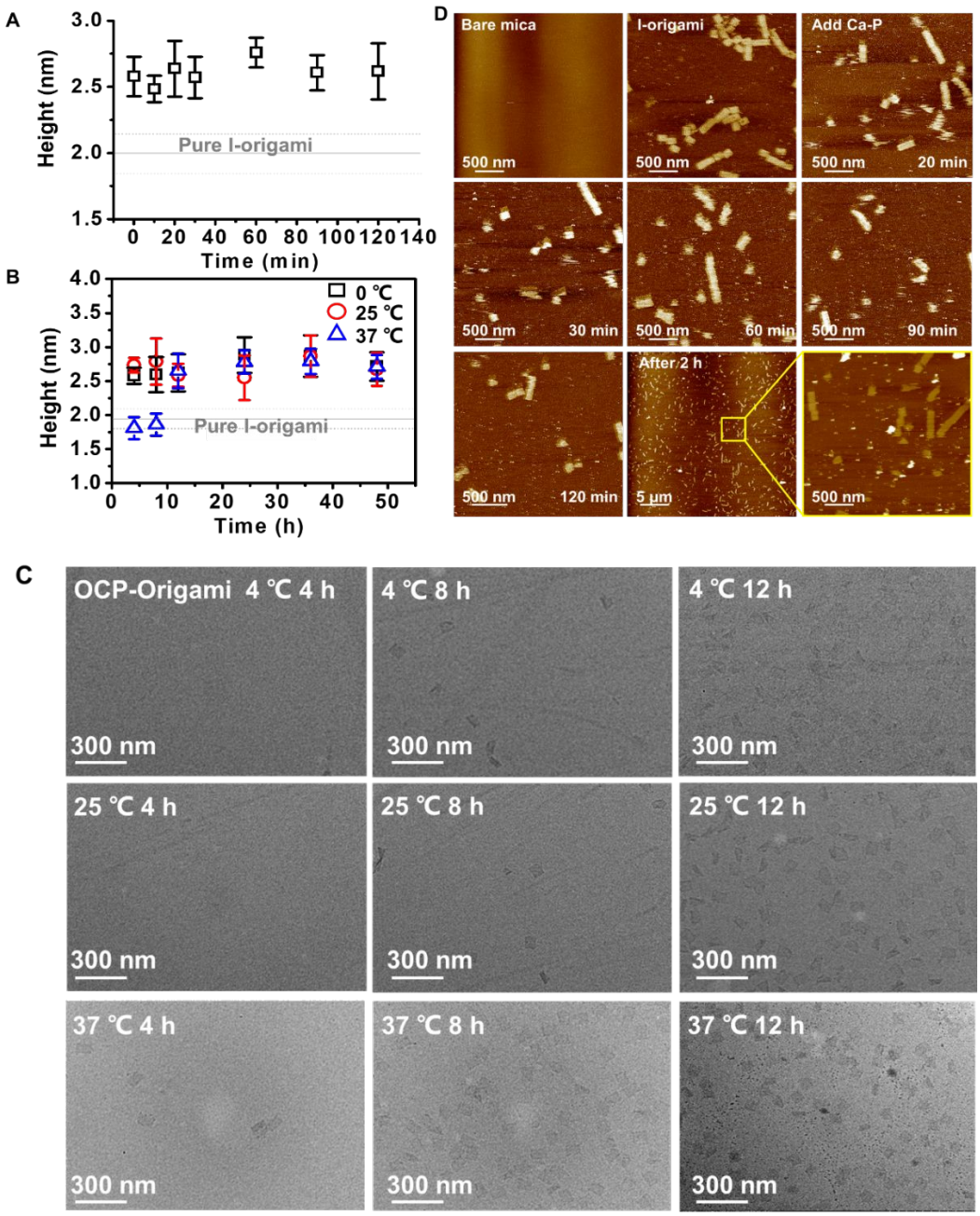

Figure S8 (A) The height statistics of in situ AFM images of mineralized I-origami with 2 h. (B) TEM images of optimization of I-origami mineralization condition, at different temperatures (4, 25, 37 ^{o}C) and at different incubation time (4, 8, 12 h) using supersaturated solution (σ= 1.77). (C) The height statistics of calcium phosphate mineralization of DNA origami at different temperatures and different times using the same supersaturation (σ= 1.77) condition within 48 h. (D) Is *in situ* AFM images of Ca-P mineralization on I-origami (pH = 6.5, *IS* = 0.15 M). Time sequence of AFM height images.

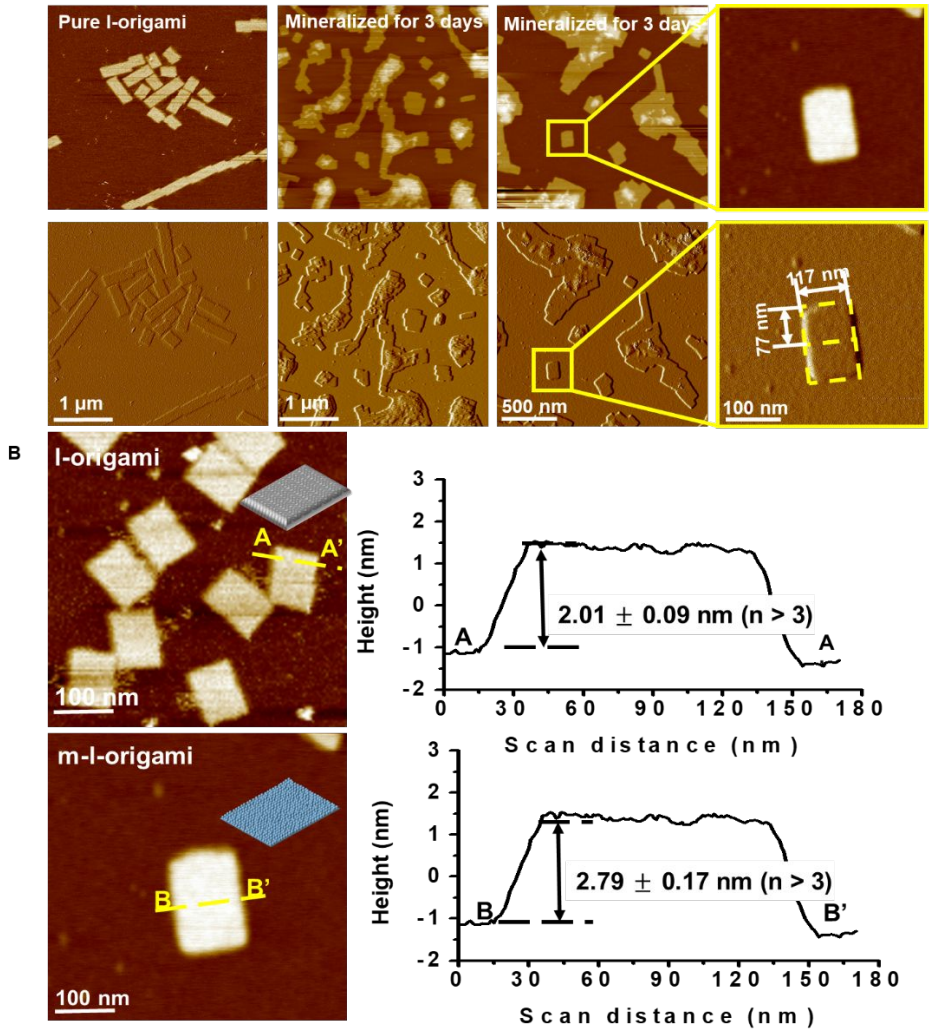

Figure S9 AFM images and height statistics of mineralized l-origami structures prepared under the same experimental conditions of mineralized l-origami (σ= 1.77, 37 °C). (A) AFM images shows that, after 3 days of mineralization, a lot of aggregated m-l-origami appeared. But the single structure shows that the length and width remain the same which is 117 nm in length and 77 nm in width, respectively. (B) AFM images and shows that the height of bare l-origami is 2.01 ± 0.09 nm (n > 3) on average, after 3-day-mineraliztion, the height increased to 2.79 ± 0.17 nm (n > 3).

4. Modulus measurement

For sample analysis, the peak force set point was set at 0.025 V, amplitude at 50 nm, scan rate at 0.977 Hz and Poisson's ratio at 0.25. The latter is the standard value used bare mica. These parameters were chosen as they proved to be optimum values for obtaining the nominal value of the modulus of DNA origami. Force curves and images with scan dimensions of 5 × 5 μm were obtained using the Peakforce Capture function to probe microscale and nanoscale morphology and mechanical properties. Average moduli were obtained using the DMT model. Height images were also obtained to assess morphological features.

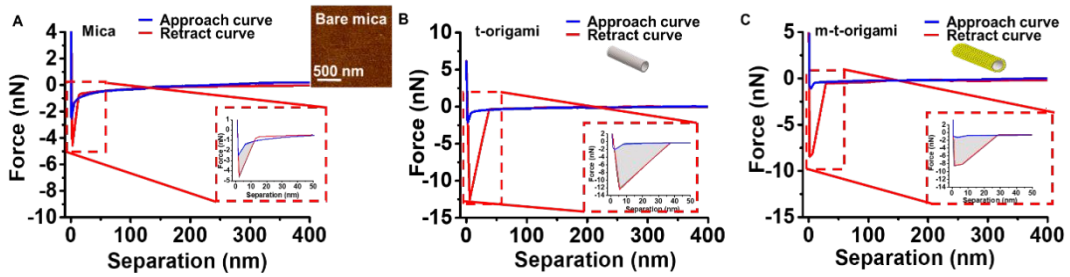

Figure S10 A is typical force-distance curves of mica and AFM image on bare mica. the insert shows that both of the freshly cleaved mica surface and the AFM probe are clean. B is typical force-distance curves of bare t-origami. zoom in graph shows the detail part of the force-distance curves. C is typical force-distance curves of m-t-origami. zoom in graph shows the detail part of the force-distance curves.

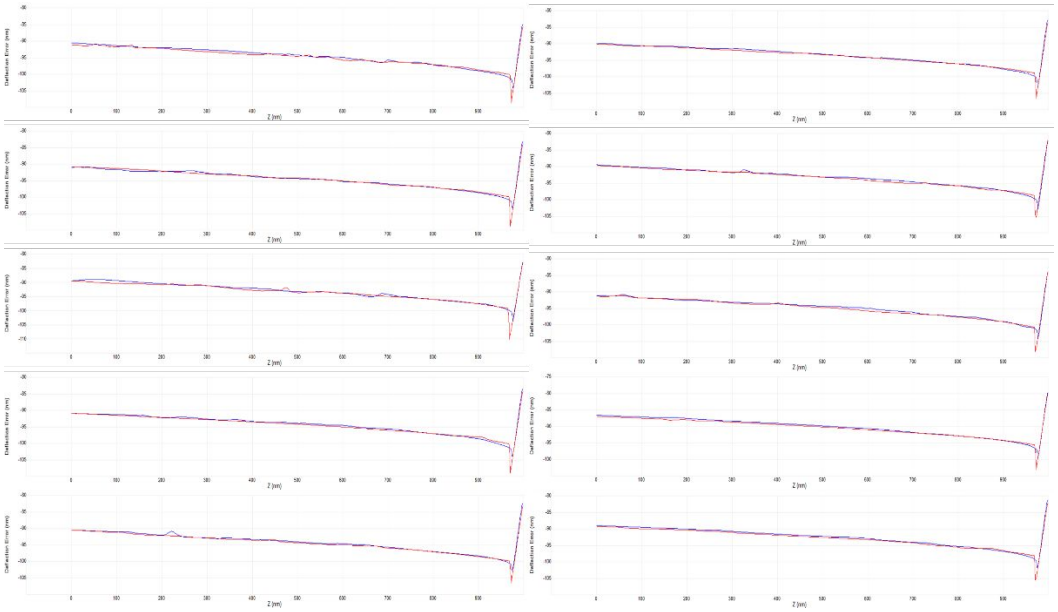

Figure S11 Force-distance curves of mica. The force-distance curves at different positions of the freshly cleaved mica surface obtained in AFM are consistent, indicating that the surface of the mica substrate is clean, and the reliability of mechanical experiments.

5. The thermal stability experiment

For the thermal stability experiment of DNA origami, we separately deposited the prepared bare DNA origami and mineralized DNA origami on the freshly cleaved mica, and then heated them at 70 °C to observe the thermal stability of DNA origami after mineralization.

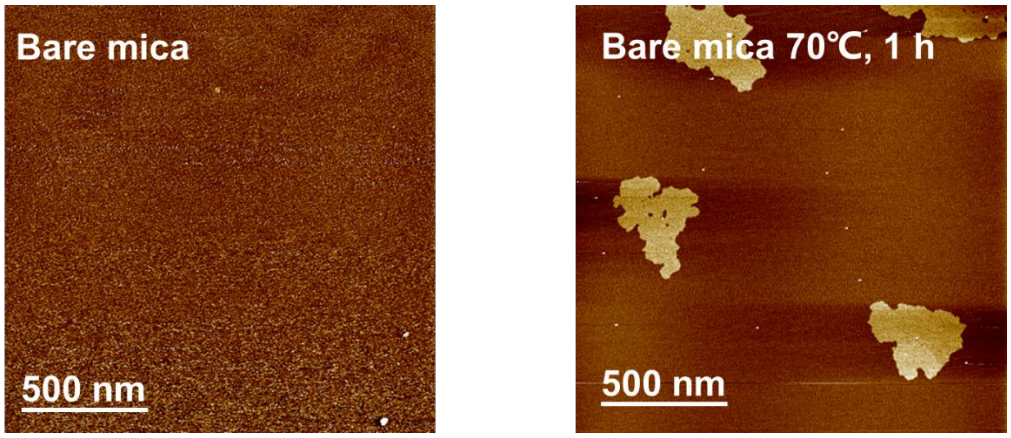

Figure S12 AFM images of freshly cleaved bare mica surface before and after 70 °C heating for 1 h. The impurities appeared on mica, which looked like the impurities that appear after heating at 70 °C for 1 hour, in thermal stability test of origami after mineralization.

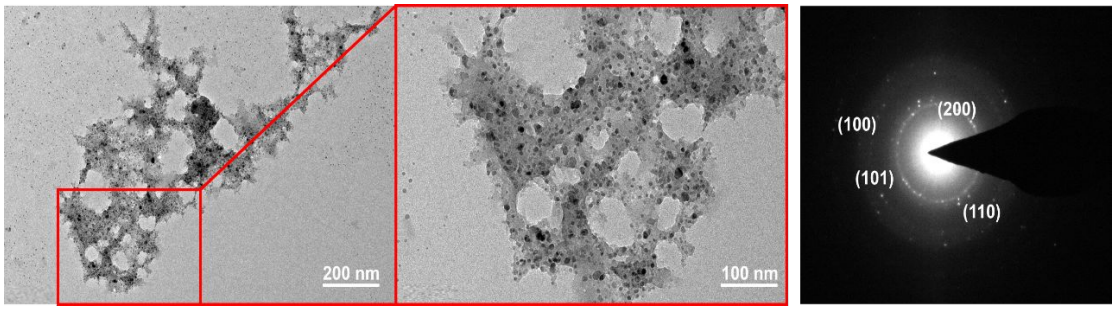

Figure S13 TEM images of the heated r-origami and the corresponding SAED image. The purpose of selecting a large number of r-origami aggregated sites is to make it easier to obtain SAED diffraction result. The results show that after heating, amorphous Ca-P particles attached onto the r-origami surface are changed into HAP.

6. Addressability of mineralized DNA origami.

For sample preparation, the streptavidin labeled DNA origami nanostructure were mineralized following the mineralization method above, and then the mineralized DNA origami nanostructure were incubated overnight with transferrin at 37 °C. The molar ratio of transferrin/streptavidin = 10:1. And the addressability of mineralized DNA origami were characterized using AFM and TEM.

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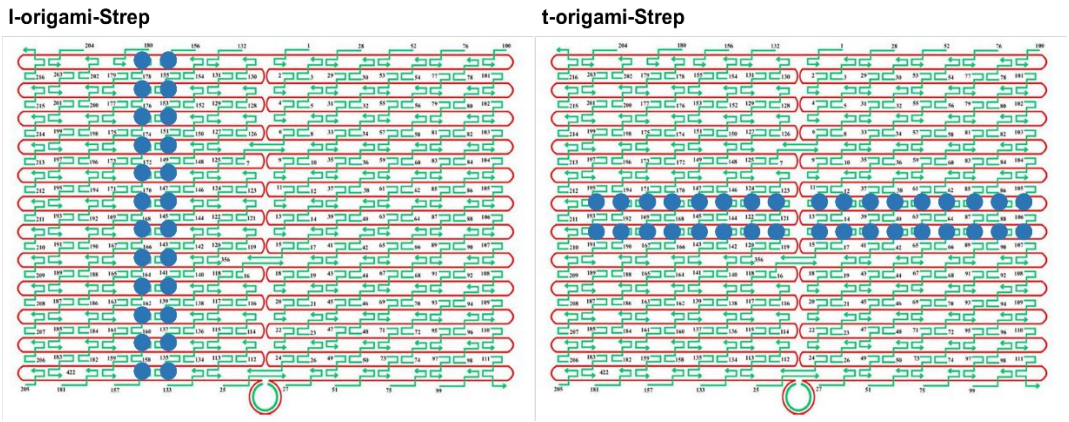

Figure S14 Design of the pre-immobilized streptavidin on l-origami and t-origami, the schematic of DNA nanotile and the blue dots indicate the positions of streptavidin immobilized stable strands.

Table1 The list of staple DNA sequences

No.	Sequence (5'-3')
1	CAAGCCCAATAGGAACCCATGTACAAACAGTT
2	AATGCCCCGTAACAGTGCCCGTATCTCCCTCA
3	TGCCTTGACTGCCTATTTCGGAACAGGGATAG
4	GAGCCGCCCCACCACCGGAACCGCGACGGAAA
5	AACCAGAGACCCTCAGAACCGCCAGGGGTCAG
6	TTATTCATAGGGAAGGTAAATATTCATTCAGT
7	CATAACCCGAGGCATAGTAAGAGCTTTTTAAG
8	ATTGAGGGTAAAGGTGAATTATCAATCACCGG
9	AAAAGTAATATCTTACCGAAGCCCTTCCAGAG
10	GCAATAGCGCAGATAGCCGAACAATTCAACCG
11	CCTAATTTACGCTAACGAGCGTCTAATCAATA
12	TCTTACCAGCCAGTTACAAAATAAATGAAATA
13	ATCGGCTGCGAGCATGTAGAAACCTATCATAT
14	CTAATTTATCTTTCCTTATCATTCATCCTGAA
15	GCGTTATAGAAAAAGCCTGTTTAGAAGGCCGG
16	GCTCATTTTCGCATTAAATTTTTGAGCTTAGA
17	AATTACTACAAATTCTTACCAGTAATCCCATC
18	TTAAGACGTTGAAAACATAGCGATAACAGTAC
19	TAGAATCCCTGAGAAGAGTCAATAGGAATCAT
20	CTTTTACACAGATGAATATACAGTAAACAATT
21	TTTAACGTTCGGGAGAAACAATAATTTTCCCT
22	CGACAACTAAGTATTAGACTTTACAATACCGA
23	GGATTTAGCGTATTAAATCCTTTGTTTTCAGG
24	ACGAACCAAAACATCGCCATTAAATGGTGGTT

25	GAACGTGGCGAGAAAGGAAGGGAACAAACTAT
26	TAGCCCTACCAGCAGAAGATAAAAACATTTGA
27	CGGCCTTGCTGGTAATATCCAGAACGAACTGA
28	CTCAGAGCCACCACCCTCATTTTCCTATTATT
29	CTGAAACAGGTAATAAGTTTTAACCCCTCAGA
30	AGTGTACTTGAAAGTATTAAGAGGCCGCCACC
31	GCCACCACTCTTTTCATAATCAAACCGTCACC
32	GTTTGCCACCTCAGAGCCGCCACCGATACAGG
33	GACTTGAGAGACAAAAGGGCGACAAGTTACCA
34	AGCGCCAACCATTTGGGAATTAGATTATTAGC
35	GAAGGAAAATAAGAGCAAGAAACAACAGCCAT
36	GCCCAATACCGAGGAAACGCAATAGGTTTACC
37	ATTATTTAACCCAGCTACAATTTTCAAGAACG
38	TATTTTGCTCCCAATCCAAATAAGTGAGTTAA
39	GGTATTAAGAACAAGAAAAATAATTAAAGCCA
40	TAAGTCCTACCAAGTACCGCACTCTTAGTTGC
41	ACGCTCAAAATAAGAATAAACACCGTGAATTT
42	AGGCGTTACAGTAGGGCTTAATTGACAATAGA
43	ATCAAAATCGTCGCTATTAATTAACGGATTCG
44	CTGTAAATCATAGGTCTGAGAGACGATAAATA
45	CCTGATTGAAAGAAATTGCGTAGACCCGAACG
46	ACAGAAATCTTTGAATACCAAGTTCCTTGCTT
47	TTATTAATGCCGTCAATAGATAATCAGAGGTG
48	AGATTAGATTTAAAAGTTTGAGTACACGTAAA
49	AGGCGGTCATTAGTCTTTAATGCGCAATATTA
50	GAATGGCTAGTATTAACACCGCCTCAACTAAT
51	CCGCCAGCCATTGCAACAGGAAAAATATTTTT
52	CCCTCAGAACCGCCACCCTCAGAACTGAGACT
53	CCTCAAGAATACATGGCTTTTGATAGAACCAC
54	TAAGCGTCGAAGGATTAGGATTAGTACCGCCA
55	CACCAGAGTTCGGTCATAGCCCCCGCCAGCAA
56	TCGGCATTCCGCCGCCAGCATTGACGTTCCAG
57	AATCACCAAATAGAAAATTCATATATAACGGA
58	TCACAATCGTAGCACCATTACCATCGTTTTCA
59	ATACCCAAGATAACCCACAAGAATAAACGATT
60	ATCAGAGAAAGAACTGGCATGATTTTATTTTG
61	TTTTGTTTAAGCCTTAAATCAAGAATCGAGAA
62	AGGTTTTGAACGTCAAAAATGAAAGCGCTAAT
63	CAAGCAAGACGCGCCTGTTTATCAAGAATCGC
64	AATGCAGACCGTTTTTATTTTCATCTTGCGGG

65	CATATTTAGAAATACCGACCGTGTTACCTTTT
66	AATGGTTTACAACGCCAACATGTAGTTCAGCT
67	TAACCTCCATATGTGAGTGAATAAACAAAATC
68	AAATCAATGGCTTAGGTTGGGTTACTAAATTT
69	GCGCAGAGATATCAAAATTATTTGACATTATC
70	AACCTACCGCGAATTATTCATTTCCAGTACAT
71	ATTTTGCGTCTTTAGGAGCACTAAGCAACAGT
72	CTAAAATAGAACAAAGAAACCACCAGGGTTAG
73	GCCACGCTATACGTGGCACAGACAACGCTCAT
74	GCGTAAGAGAGAGCCAGCAGCAAAAAGGTTAT
75	GGAAATACCTACATTTTGACGCTCACCTGAAA
76	TATCACCGTACTCAGGAGGTTTAGCGGGGTTT
77	TGCTCAGTCAGTCTCTGAATTTACCAGGAGGT
78	GGAAAGCGACCAGGCGGATAAGTGAATAGGTG
79	TGAGGCAGGCGTCAGACTGTAGCGTAGCAAGG
80	TGCCTTTAGTCAGACGATTGGCCTGCCAGAAT
81	CCGGAAACACACCACGGAATAAGTAAGACTCC
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83	TTATTACGGTCAGAGGGTAATTGAATAGCAGC
84	TGAACAAACAGTATGTTAGCAAACTAAAAGAA
85	CTTTACAGTTAGCGAACCTCCCGACGTAGGAA
86	GAGGCGTTAGAGAATAACATAAAAGAACACCC
87	TCATTACCCGACAATAAACAACATATTTAGGC
88	CCAGACGAGCGCCCAATAGCAAGCAAGAACGC
89	AGAGGCATAATTTCATCTTCTGACTATAACTA
90	TTTTAGTTTTTCGAGCCAGTAATAAATTCTGT
91	TATGTAAACCTTTTTTAATGGAAAAATTACCT
92	TTGAATTATGCTGATGCAAATCCACAAATATA
93	GAGCAAAAACTTCTGAATAATGGAAGAAGGAG
94	TGGATTATGAAGATGATGAAACAAAATTTCAT
95	CGGAATTATTGAAAGGAATTGAGGTGAAAAAT
96	ATCAACAGTCATCATATTCCTGATTGATTGTT
97	CTAAAGCAAGATAGAACCCTTCTGAATCGTCT
98	GCCAACAGTCACCTTGCTGAACCTGTTGGCAA
99	GAAATGGATTATTTACATTGGCAGACATTCTG
100	TTTTTATAAGTATAGCCCGGCCGTCGAG
101	AGGGTTGATTTTATAAATCCTCATTAAATGATATTC
102	ACAAACAATTTTAATCAGTAGCGACAGATCGATAGC
103	AGCACCGTTTTTTAAAGGTGGCAACATAGTAGAAAA
104	TACATACATTTTGACGGGAGAATTAACTACAGGGAA

105	GCGCATTATTTTGCTTATCCGGTATTCTAAATCAGA
106	TATAGAAGTTTTCGACAAAAGGTAAAGTAGAGAATA
107	TAAAGTACTTTTCGCGAGAAAACTTTTTATCGCAAG
108	ACAAAGAATTTTATTAATTACATTTAACACATCAAG
109	AAAACAAATTTTTTCATCAATATAATCCTATCAGAT
110	GATGGCAATTTTAATCAATATCTGGTCACAAATATC
111	AAACCCTCTTTTACCAGTAATAAAAGGGATTCACCAGTCACACGTTTT
112	CCGAAATCCGAAAATCCTGTTTGAAGCCGGAA
113	CCAGCAGGGGCAAAATCCCTTATAAAGCCGGC
114	GCATAAAGTTCCACACAACATACGAAGCGCCA
115	GCTCACAATGTAAAGCCTGGGGTGGGTTTGCC
116	TTCGCCATTGCCGGAAACCAGGCATTAAATCA
117	GCTTCTGGTCAGGCTGCGCAACTGTGTTATCC
118	GTTAAAATTTTAACCAATAGGAACCCGGCACC
119	AGACAGTCATTCAAAAGGGTGAGAAGCTATAT
120	AGGTAAAGAAATCACCATCAATATAATATTTT
121	TTTCATTTGGTCAATAACCTGTTTATATCGCG
122	TCGCAAATGGGGCGCGAGCTGAAATAATGTGT
123	TTTTAATTGCCCGAAAGACTTCAAAACACTAT
124	AAGAGGAACGAGCTTCAAAGCGAAGATACATT
125	GGAATTACTCGTTTACCAGACGACAAAAGATT
126	GAATAAGGACGTAACAAAGCTGCTCTAAAACA
127	CCAAATCACTTGCCCTGACGAGAACGCCAAAA
128	CTCATCTTGAGGCAAAAGAATACAGTGAATTT
129	AAACGAAATGACCCCCAGCGATTATTCATTAC
130	CTTAAACATCAGCTTGCTTTCGAGCGTAACAC
131	TCGGTTTAGCTTGATACCGATAGTCCAACCTA
132	TGAGTTTCGTCACCAGTACAAACTTAATTGTA
133	CCCCGATTTAGAGCTTGACGGGGAAATCAAAA
134	GAATAGCCGCAAGCGGTCCACGCTCCTAATGA
135	GAGTTGCACGAGATAGGGTTGAGTAAGGGAGC
136	GTGAGCTAGTTTCCTGTGTGAAATTTGGGAAG
137	TCATAGCTACTCACATTAATTGCGCCCTGAGA
138	GGCGATCGCACTCCAGCCAGCTTTGCCATCAA
139	GAAGATCGGTGCGGGCCTCTTCGCAATCATGG
140	AAATAATTTTAAATTGTAAACGTTGATATTCA
141	GCAAATATCGCGTCTGGCCTTCCTGGCCTCAG
142	ACCGTTCTAAATGCAATGCCTGAGAGGTGGCA
143	TATATTTTAGCTGATAAATTAATGTTGTATAA
144	TCAATTCTTTTAGTTTGACCATTACCAGACCG

145	CGAGTAGAACTAATAGTAGTAGCAAACCCTCA
146	GAAGCAAAAAAGCGGATTGCATCAGATAAAAA
147	TCAGAAGCCTCCAACAGGTCAGGATCTGCGAA
148	CCAAAATATAATGCAGATACATAAACACCAGA
149	CATTCAACGCGAGAGGCTTTTGCATATTATAG
150	ACGAGTAGTGACAAGAACCGGATATACCAAGC
151	AGTAATCTTAAATTGGGCTTGAGAGAATACCA
152	GCGAAACATGCCACTACGAAGGCATGCGCCGA
153	ATACGTAAAAGTACAACGGAGATTTCATCAAG
154	CAATGACACTCCAAAAGGAGCCTTACAACGCC
155	AAAAAAGGACAACCATCGCCCACGCGGGTAAA
156	TGTAGCATTCCACAGACAGCCCTCATCTCCAA
157	GTAAAGCACTAAATCGGAACCCTAGTTGTTCC
158	AGTTTGGAGCCCTTCACCGCCTGGTTGCGCTC
159	AGCTGATTACAAGAGTCCACTATTGAGGTGCC
160	ACTGCCCGCCGAGCTCGAATTCGTTATTACGC
161	CCCGGGTACTTTCCAGTCGGGAAACGGGCAAC
162	CAGCTGGCGGACGACGACAGTATCGTAGCCAG
163	GTTTGAGGGAAAGGGGGATGTGCTAGAGGATC
164	CTTTCATCCCCAAAAACAGGAAGACCGGAGAG
165	AGAAAAGCAACATTAAATGTGAGCATCTGCCA
166	GGTAGCTAGGATAAAAATTTTTAGTTAACATC
167	CAACGCAATTTTTGAGAGATCTACTGATAATC
168	CAATAAATACAGTTGATTCCCAATTTAGAGAG
169	TCCATATACATACAGGCAAGGCAACTTTATTT
170	TACCTTTAAGGTCTTTACCCTGACAAAGAAGT
171	CAAAAATCATTGCTCCTTTTGATAAGTTTCAT
172	TTTGCCAGATCAGTTGAGATTTAGTGGTTTAA
173	AAAGATTCAGGGGGTAATAGTAAACCATAAAT
174	TTTCAACTATAGGCTGGCTGACCTTGTATCAT
175	CCAGGCGCTTAATCATTGTGAATTACAGGTAG
176	CGCCTGATGGAAGTTTCCATTAAACATAACCG
177	TTTCATGAAAATTGTGTCGAAATCTGTACAGA
178	ATATATTCTTTTTTCACGTTGAAAATAGTTAG
179	AATAATAAGGTCGCTGAGGCTTGCAAAGACTT
180	CGTAACGATCTAAAGTTTTGTCGTGAATTGCG
181	ACCCAAATCAAGTTTTTTGGGGTCAAAGAACG
182	TGGACTCCCTTTTCACCAGTGAGACCTGTCGT
183	TGGTTTTTAACGTCAAAGGGCGAAGAACCATC
184	GCCAGCTGCCTGCAGGTCGACTCTGCAAGGCG

185	CTTGCATGCATTAATGAATCGGCCCGCCAGGG
186	ATTAAGTTCGCATCGTAACCGTGCGAGTAACA
187	TAGATGGGGGGTAACGCCAGGGTTGTGCCAAG
188	ACCCGTCGTCATATGTACCCCGGTAAAGGCTA
189	CATGTCAAGATTCTCCGTGGGAACCGTTGGTG
190	TCAGGTCACTTTTGCGGGAGAAGCAGAATTAG
191	CTGTAATATTGCCTGAGAGTCTGGAAAACTAG
192	CAAAATTAAAGTACGGTGTCTGGAAGAGGTCA
193	TGCAACTAAGCAATAAAGCCTCAGTTATGACC
194	TTTTTGCGCAGAAAACGAGAATGAATGTTTAG
195	AAACAGTTGATGGCTTAGAGCTTATTTAAATA
196	ACTGGATAACGGAACAACATTATTACCTTATG
197	ACGAACTAGCGTCCAATACTGCGGAATGCTTT
198	CGATTTTAGAGGACAGATGAACGGCGCGACCT
199	CTTTGAAAAGAACTGGCTCATTATTTAATAAA
200	GCTCCATGAGAGGCTTTGAGGACTAGGGAGTT
201	ACGGCTACTTACTTAGCCGGAACGCTGACCAA
202	AAAGGCCGAAAGGAACAACTAAAGCTTTCCAG
203	GAGAATAGCTTTTGCGGGATCGTCGGGTAGCA
204	ACGTTAGTAAATGAATTTTCTGTAAGCGGAGT
205	TTTTCGATGGCCCACTACGTAAACCGTC
206	TATCAGGGTTTTCGGTTTGCGTATTGGGAACGCGCG
207	GGGAGAGGTTTTTGTAAAACGACGGCCATTCCCAGT
208	CACGACGTTTTTGTAATGGGATAGGTCAAAACGGCG
209	GATTGACCTTTTGATGAACGGTAATCGTAGCAAACA
210	AGAGAATCTTTTGGTTGTACCAAAAACAAGCATAAA
211	GCTAAATCTTTTCTGTAGCTCAACATGTATTGCTGA
212	ATATAATGTTTTCATTGAATCCCCCTCAAATCGTCA
213	TAAATATTTTTTGGAAGAAAAATCTACGACCAGTCA
214	GGACGTTGTTTTTCATAAGGGAACCGAAAGGCGCAG
215	ACGGTCAATTTTGACAGCATCGGAACGAACCCTCAG
216	CAGCGAAAATTTTACTTTCAACAGTTTCTGGGATTTTGCTAAACTTTT
217	AACATCACTTGCCTGAGTAGAAGAACT
218	TGTAGCAATACTTCTTTGATTAGTAAT
219	AGTCTGTCCATCACGCAAATTAACCGT
220	ATAATCAGTGAGGCCACCGAGTAAAAG
221	ACGCCAGAATCCTGAGAAGTGTTTTT
222	TTAAAGGGATTTTAGACAGGAACGGT
223	AGAGCGGGAGCTAAACAGGAGGCCGA
224	TATAACGTGCTTTCCTCGTTAGAATC

225	GTACTATGGTTGCTTTGACGAGCACG
226	GCGCTTAATGCGCCGCTACAGGGCGC
Biotin-DNA	Biotin-TTTTCTCTACCACCTACTA

Table2 List of modified sequences of l-origami

No.	Sequence (5'-3')
Strep-135	GAGTTGCACGAGATAGGGTTGAGTAAGGGAGCTTTTTTTAGTAGGTGGTAGAG
Strep-137	TCATAGCTACTCACATTAATTGCGCCCTGAGATTTTTTTAGTAGGTGGTAGAG
Strep-139	GAAGATCGGTGCGGGCCTCTTCGCAATCATGGTTTTTTTAGTAGGTGGTAGAG
Strep-141	GCAAATATCGCGTCTGGCCTTCCTGGCCTCAGTTTTTTTAGTAGGTGGTAGAG
Strep-143	TATATTTTAGCTGATAAATTAATGTTGTATAATTTTTTTAGTAGGTGGTAGAG
Strep-145	CGAGTAGAACTAATAGTAGTAGCAAACCCTCATTTTTTTAGTAGGTGGTAGAG
Strep-147	TCAGAAGCCTCCAACAGGTCAGGATCTGCGAATTTTTTTAGTAGGTGGTAGAG
Strep-149	CATTCAACGCGAGAGGCTTTTGCATATTATAGTTTTTTTAGTAGGTGGTAGAG
Strep-151	AGTAATCTTAAATTGGGCTTGAGAGAATACCATTTTTTTAGTAGGTGGTAGAG
Strep-153	ATACGTAAAAGTACAACGGAGATTTCATCAAGTTTTTTTAGTAGGTGGTAGAG
Strep-155	AAAAAAGGACAACCATCGCCCACGCGGGTAAATTTTTTTAGTAGGTGGTAGAG
Strep-156	TGTAGCATTCCACAGACAGCCCTCATCTCCAATTTTTTTAGTAGGTGGTAGAG
Strep-158	AGTTTGGAGCCCTTCACCGCCTGGTTGCGCTCTTTTTTTAGTAGGTGGTAGAG
Strep-160	ACTGCCCGCCGAGCTCGAATTCGTTATTACGCTTTTTTTAGTAGGTGGTAGAG
Strep-162	CAGCTGGCGGACGACGACAGTATCGTAGCCAGTTTTTTTAGTAGGTGGTAGAG
Strep-164	CTTTCATCCCCAAAAACAGGAAGACCGGAGAGTTTTTTTAGTAGGTGGTAGAG
Strep-166	GGTAGCTAGGATAAAAATTTTTAGTTAACATCTTTTTTTAGTAGGTGGTAGAG
Strep-168	CAATAAATACAGTTGATTCCCAATTTAGAGAGTTTTTTTAGTAGGTGGTAGAG
Strep-170	TACCTTTAAGGTCTTTACCCTGACAAAGAAGTTTTTTTTAGTAGGTGGTAGAG
Strep-172	TTTGCCAGATCAGTTGAGATTTAGTGGTTTAATTTTTTTAGTAGGTGGTAGAG
Strep-174	TTTCAACTATAGGCTGGCTGACCTTGTATCATTTTTTTTAGTAGGTGGTAGAG
Strep-176	CGCCTGATGGAAGTTTCCATTAAACATAACCGTTTTTTTAGTAGGTGGTAGAG
Strep-178	ATATATTCTTTTTTCACGTTGAAAATAGTTAGTTTTTTTAGTAGGTGGTAGAG

Table3 List of modified sequences of t-origami

No.	Sequence (5'-3')
Strep-11	CCTAATTTACGCTAACGAGCGTCTAATCAATATTTTTTTAGTAGGTGGTAGAG
Strep-12	TCTTACCAGCCAGTTACAAAATAAATGAAATATTTTTTTAGTAGGTGGTAGAG
Strep-13	ATCGGCTGCGAGCATGTAGAAACCTATCATATTTTTTTTAGTAGGTGGTAGAG
Strep-14	CTAATTTATCTTTCCTTATCATTCATCCTGAATTTTTTTAGTAGGTGGTAGAG
Strep-37	ATTATTTAACCCAGCTACAATTTTCAAGAACGTTTTTTTAGTAGGTGGTAGAG
Strep-38	TATTTTGCTCCCAATCCAAATAAGTGAGTTAATTTTTTTAGTAGGTGGTAGAG
Strep-39	GGTATTAAGAACAAGAAAAATAATTAAAGCCATTTTTTTAGTAGGTGGTAGAG
Strep-40	TAAGTCCTACCAAGTACCGCACTCTTAGTTGCTTTTTTTAGTAGGTGGTAGAG

Strep-61	TTTTGTTTAAGCCTTAAATCAAGAATCGAGAATTTTTTTTAGTAGGTGGTAGAG
Strep-62	AGGTTTTGAACGTCAAAAATGAAAGCGCTAATTTTTTTTTAGTAGGTGGTAGAG
Strep-63	CAAGCAAGACGCGCCTGTTTATCAAGAATCGCTTTTTTTTAGTAGGTGGTAGAG
Strep-64	AATGCAGACCGTTTTTATTTTCATCTTGCGGGTTTTTTTAGTAGGTGGTAGAG
Strep-85	CTTTACAGTTAGCGAACCTCCCGACGTAGGAATTTTTTTTAGTAGGTGGTAGAG
Strep-86	GAGGCGTTAGAGAATAACATAAAAGAACACCCTTTTTTTTAGTAGGTGGTAGAG
Strep-87	TCATTACCCGACAATAAACAACATATTTAGGCTTTTTTTTAGTAGGTGGTAGAG
Strep-88	CCAGACGAGCGCCCAATAGCAAGCAAGAACGCTTTTTTTTAGTAGGTGGTAGAG
Strep-105	GCGCATTATTTTGCTTATCCGGTATTCTAAATCAGATTTTTTTTAGTAGGTGGTAGAG
Strep-106	TATAGAAGTTTTCGACAAAAGGTAAAGTAGAGAATATTTTTTTTAGTAGGTGGTAGAG
Strep-121	TTTCATTTGGTCAATAACCTGTTTATATCGCGTTTTTTTTAGTAGGTGGTAGAG
Strep-122	TCGCAAATGGGGCGCGAGCTGAAATAATGTGTTTTTTTTTAGTAGGTGGTAGAG
Strep-123	TTTTAATTGCCCGAAAGACTTCAAAACACTATTTTTTTTTAGTAGGTGGTAGAG
Strep-124	AAGAGGAACGAGCTTCAAAGCGAAGATACATTTTTTTTTTAGTAGGTGGTAGAG
Strep-144	TCAATTCTTTTAGTTTGACCATTACCAGACCGTTTTTTTTAGTAGGTGGTAGAG
Strep-145	CGAGTAGAACTAATAGTAGTAGCAAACCCTCATTTTTTTTAGTAGGTGGTAGAG
Strep-146	GAAGCAAAAAAGCGGATTGCATCAGATAAAAATTTTTTTTAGTAGGTGGTAGAG
Strep-147	TCAGAAGCCTCCAACAGGTCAGGATCTGCGAATTTTTTTTAGTAGGTGGTAGAG
Strep-168	CAATAAATACAGTTGATTCCCAATTTAGAGAGTTTTTTTTAGTAGGTGGTAGAG
Strep-169	TCCATATACATACAGGCAAGGCAACTTTATTTTTTTTTTTAGTAGGTGGTAGAG
Strep-170	TACCTTTAAGGTCTTTACCCTGACAAAGAAGTTTTTTTTTAGTAGGTGGTAGAG
Strep-171	CAAAAATCATTGCTCCTTTTGATAAGTTTCATTTTTTTTTAGTAGGTGGTAGAG
Strep-192	CAAAATTAAAGTACGGTGTCTGGAAGAGGTCATTTTTTTTAGTAGGTGGTAGAG
Strep-193	TGCAACTAAGCAATAAAGCCTCAGTTATGACCTTTTTTTTAGTAGGTGGTAGAG
Strep-194	TTTTTGCGCAGAAAACGAGAATGAATGTTTAGTTTTTTTTAGTAGGTGGTAGAG
Strep-195	AAACAGTTGATGGCTTAGAGCTTATTTAAATATTTTTTTTAGTAGGTGGTAGAG

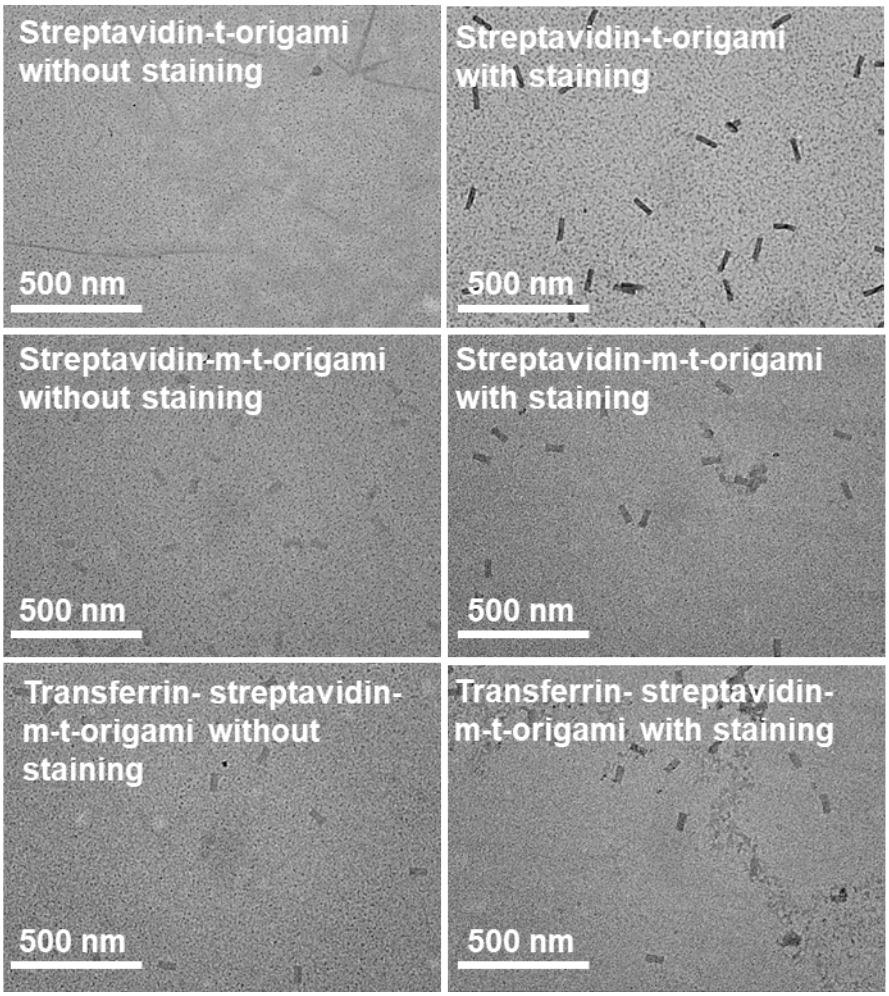

Figure S15 TEM images of mineralized t-origami modified with streptavidin and the images of streptavidin-m-t-origami labeled with transferrin, with and without staining. The successful mineralization was confirmed by TEM images, even after incubated with streptavidin and transferrin solutions.

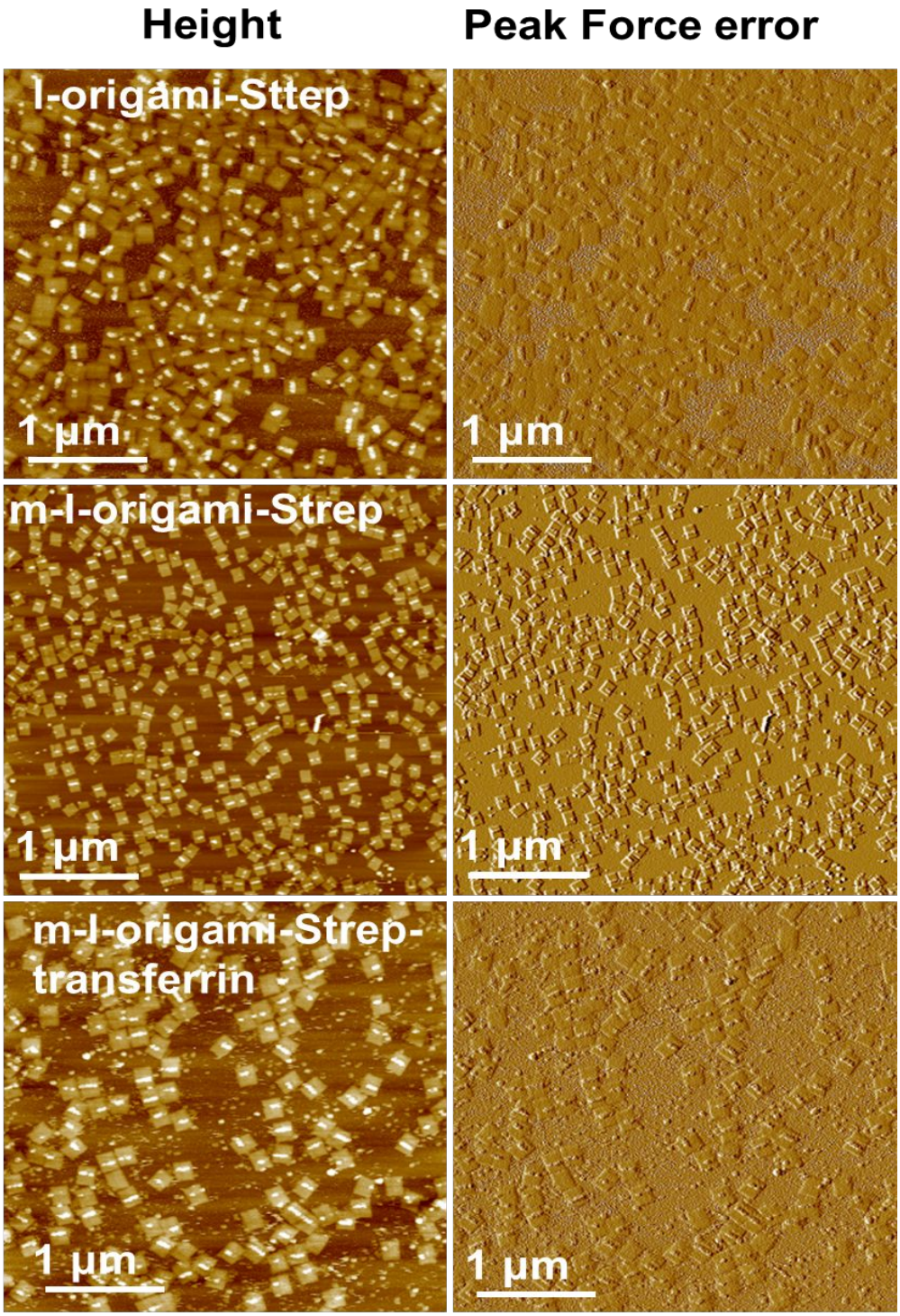

Figure S16 AFM height and peak force error images of the addressability of streptavidin-l-origami, streptavidin-m-l-origami and transferrin-streptavidin-m-l-origami.

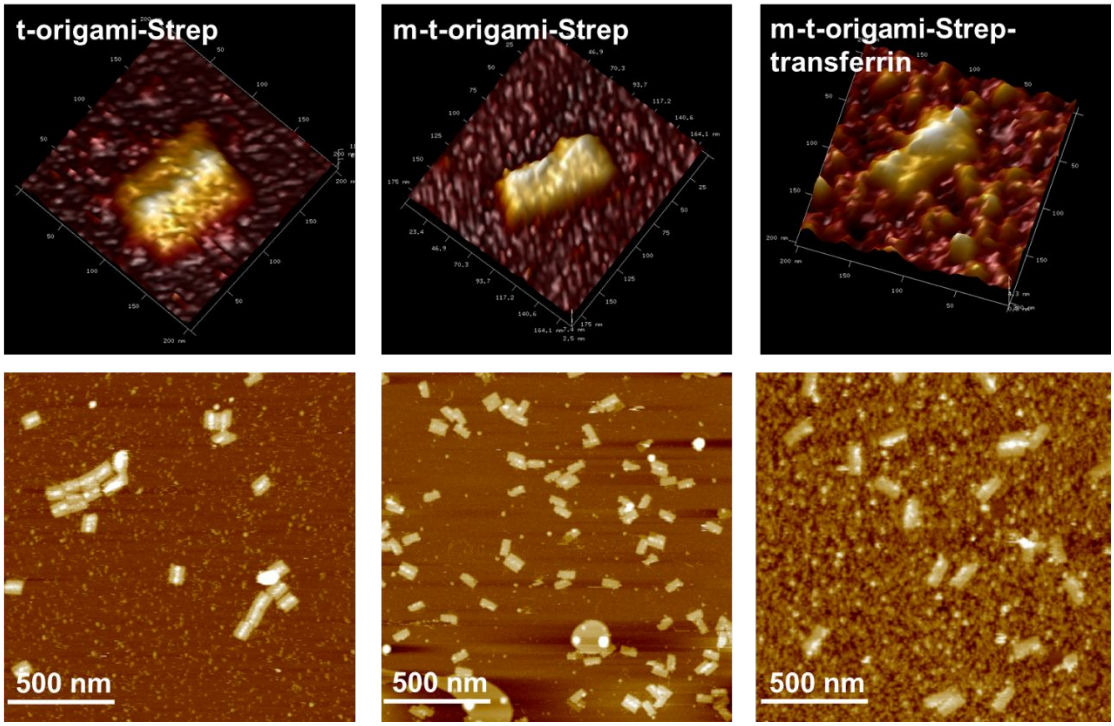

Figure S17 AFM images of the addressability of streptavidin-t-origami, streptavidin-m-t-origami and transferrin-streptavidin-m-t-origami.

7 References

(1) Tokura, Y.; Harvey, S.; Xu, X.; Chen, C.; Morsbach, S.; Wunderlich, K.; Fytas, G.; Wu, Y.; Ng, D. Y. W.; Weil, T. Polymer Tube Nanoreactors *via* DNA-Origami Templated Synthesis. *Chem. Comm.* **2018**, *54*, 2808-2811.

(2) Zhang, T.; Neumann, A.; Lindlau, J.; Wu, Y.; Pramanik, G.; Naydenov, B.; Jelezko, F.; Schüder, F.; Huber, S.; Huber, M.; Stehr, F.; Högele, A.; Weil, T.; Liedl, T. DNA-Based Self-Assembly of Fluorescent Nanodiamonds. *J. Am. Chem. Soc.* **2015**, *137*, 9776-9779.

(3) Tokura, Y.; Harvey, S.; Chen, C.; Wu, Y.; Ng, D. Y. W.; Weil, T. Fabrication of Defined Polydopamine Nanostructures by DNA Origami-Templated Polymerization. *Angew. Chem. Int. Ed.* **2018**, *57*, 1587-1591.

(4) Wu, S.; Yu, M.; Li, M.; Wang, L.; Putnis, C. V.; Putnis, A. *In situ* Atomic Force Microscopy Imaging of Octacalcium Phosphate Crystallization and its Modulation by Amelogenin's C-Terminus. *Cryst. Growth Des.* **2017**, *17*, 2194−2202.

(5) Wu, S.; Zhai, H.; Zhang, W.; Wang, L. Monomeric Amelogenin's C-Terminus Modulates Biomineralization Dynamics of Calcium Phosphate. *Cryst. Growth Des.* **2015**, *15*, 4490-4497.

(6) Li, S.; Wu, S.; Nan, D.; Zhang, W.; Wang, L. Inhibition of Pathological Mineralization of Calcium Phosphate by Phosphorylated Osteopontin Peptides through Step-Specific Interactions. *Chem. Mater.* **2014**, *26*, 5605-5612.