Electronic Supplementary Material (ESI) for ChemComm.

Proximity-enhanced synthesis of DNA-peptide-DNA triblock molecules

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Supporting Information

S1. Materials and Methods

Peptide Synthesis. All peptides were synthesized on a CEM Liberty Blue microwave-assisted synthesizer at a 0.1 mmol scale, using a Rink amide resin and standard Fmoc chemistry according to previously reported protocols(1). Briefly, a 20% piperidine solution was used for deprotection, 0.5 M diisopropylcarbodiimide was used as an activator, and a solution of 1 M oxyma with 0.1 M diisopropylethylamine was used as an activator base. Amino acids were added to the resin at a concentration of 0.2 M and coupled for 4 min. The peptide was cleaved from the resin at room temperature for 4 h using a 95:2.5:2.5 mixture of trifluoroacetic acid (TFA):triisopropyl silane (TIPS):water. The crude peptide was precipitated into cold diethyl ether, and resuspended in water + 0.1 M TFA. Peptides were purified on a Waters HPLC instrument using a gradient of 0-80% acetonitrile with 0.1% TFA. Fractions with an absorbance (230 nm) reaching a threshold of 300 mAU were collected and analyzed using matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). All pure fractions with the corresponding peptide mass were pooled and lyophilized.

DNA purification. All oligonucleotides used were purchased from Integrated DNA technologies (Coralville, Iowa) and purified using a 14% urea-based denaturing polyacrylamide gel electrophoresis (PAGE). The gel was run in 1x Tris, boric acid, EDTA running buffer at 45 volts for 2 h. The desired band was detected using UV shadowing, excised, and eluted using a buffer of ammonium acetate, magnesium acetate, and EDTA. The DNA was precipitated using 100% ethanol followed by centrifugation at 16,000 RPM for 5 min. The pellet was resuspended in Nanopure water and the concentration was obtained from the 260 nm absorbance using a Nanodrop instrument.

Synthesis of peptide-DNA conjugates using SPAAC. Amine modified oligonucleotides were dissolved in 1xPBS (pH 7.5) buffer to a concentration of 1 μM. To the oligonucleotide solution

was 5 equivalents of NHS-Sulfo-DBCO ester (as a solution in DMSO). The mixture was incubated at RT for 4 h, after which a second aliquot (5 equivalents) of the NHS-sulfo-DBCO was added and the mixture was incubated at RT overnight. The DBCO-modified DNA was purified from unreacted DNA using reverse phase HPLC on an Agilent 1220 instrument, using a Zorbax Eclipse C18 column with 50 mM triethylammonium acetate and methanol as the running buffers. A gradient of 0-70% methanol was applied over 45 min while monitoring the absorbance at both 260 and 309 nm, the peak absorbance wavelengths of the DNA and the DBCO, respectively. The peak displaying an absorbance at both wavelengths was collected and exchanged into water using a 3 kDa molecular weight cut off (MWCO) filter. The DBCO-modified DNA was then mixed with the azidolysine containing peptide in a 1:4 ratio (DNA:peptide) in 1x PBS (pH 7.5) buffer, and incubated at RT overnight. The DNA-peptide conjugate was purified away from the DNA-DBCO using the same HPLC method as for the DNA-DBCO conjugate. All fractions were characterized by MALDI-TOF MS to identify the desired peak.

Synthesis of a DNA-peptide-DNA triblock via proximity-aided CuAAC. Amine-modified DNA was reacted with NHS-PEG_4-azide (Click Chemistry Tools) and purified using the same method as for DBCO-DNA. Fractions containing the azide-DNA were identified using MALDI-TOF MS. The peptide-oligonucleotide conjugate, obtained by SPAAC conjugation, and the azide modified DNA strand were mixed in a 1:1 stoichiometry in 2x PBS (pH 7.5) at a concentration of 15 μM and annealed using a thermal gradient of 95 - 4 °C over 1 h. Cu(I)-catalyzed click was used to conjugate the DNA-peptide (containing propargylalanine) to the azide modified DNA according to the following procedure: a mixture was made containing (final concentrations): 10 μM of the annealed DNA mixture, 1 mM aminoguanidine, 10 μM of a 1:5 mixture of copper [$CuSO_4$]: tris-hydroxypropyltriazolylmethylamine ligand (THPTA), and 20 mM PBS. 100 mM sodium ascorbate was added to the mixture and the reaction was carried out for 3 h at RT, after which point it was quenched with 250 mM EDTA.

Cleavage of peptide using MMP. DNA nanostructures linked by the MMP cleavable peptide were incubated in with 10 ng of human matrix metalloproteinase 8 (PerkinElmer) for 48 h in 1x PBS buffer (pH 7.4).

MALDI-TOF MS Characterization. All samples were characterized using a Bruker Microflex LRF MALDI. Peptides were analyzed in positive reflector mode using α-cyanohydroxycinnaminic acid as a matrix. DNA and DNA-peptide conjugates analyzed shot using positive linear mode with either hydroxypicolinic acid or 6-aza-2-thiothymine with 10 mM ammonium citrate.

Polyacrylamide gel electrophoresis (PAGE). DPD conjugates were probed via urea-based denaturing polyacrylamide gel (8%) electrophoresis at 45 V for 90 min. DX tiles were analyzed using 6% native polyacrylamide gels, with 1x TAE containing 12.5 mM Mg^{2+} as a running buffer, at 200 V for 160 min.

Failed synthesis routes for the DNA-peptide-DNA triblock. Prior to utilizing the proximity aided CuAAC, we attempted a number of other experimental conditions that ultimately proved unsuccessful. Initially, we used a 2:1:10 mixture of azide-DNA:DNA-peptide-alkyne:aminoguanidine solution, which was reduced using a 10-fold excess of sodium ascorbate. The reaction was carried out for 1 hour before the copper was chelated out by 3 washes with ethylenediaminetetraacetic acid (EDTA). When this failed, the azide-DNA was added at higher equivalents (up to 5x) with respect to the DNA-peptide, the Cu(THPTA) was added at higher quantities (ranging up to 1:1 stoichiometry of the DNA-peptide), and the time for

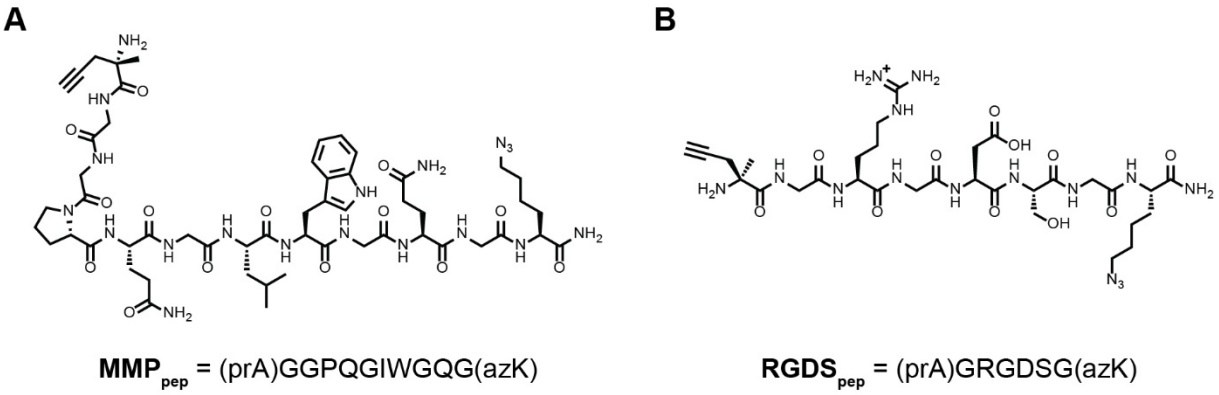
the reaction was extended up to 12 hours. While we observed some minimal success in producing a DNA-peptide-DNA triblock following these routes, the yield was very low and purification (we attempted both size exclusion and ion exchange chromatography, as well as gel extraction) proved difficult and further reduced the yield, resulting in little to no final product. Another synthesis route we explored was assembling the DX tile with both the azide-modified and DNA-peptide conjugate on neighboring duplexes and then carry out the CuAAC. While this led to a higher conversion of DNA-peptide and DNA-azide to DPD than the in-solution attempts, the yield was still below 50% and again proved difficult to purify, especially in the presence of remaining strands that made up the DX tile. We note that this approach *does* work when the two modified DNA strands are on the *same* duplex within the tile. We also attempted to use a shorter complementary region (5 bp), but this made the duplex unstable (see Supplementary Figure 9) at room temperature in the PBS buffer, precluding any proximity enhancement.

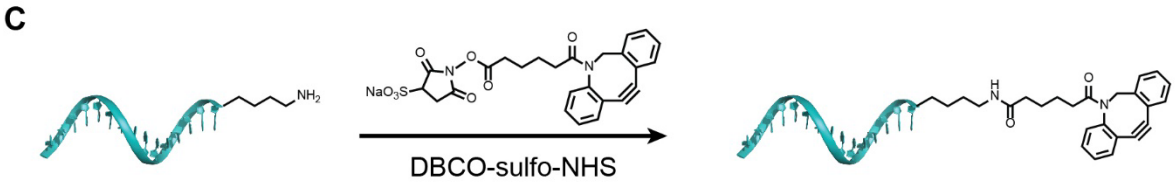
S2. DNA Sequences

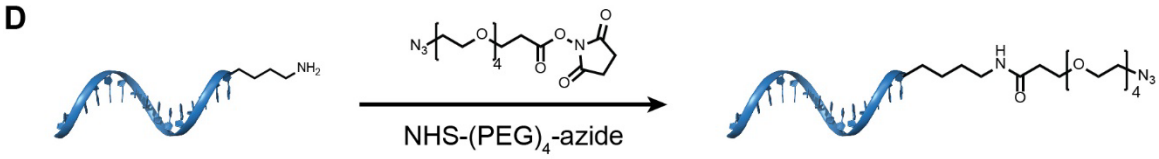
MMP DX Tile 1	
Top	CAGAAATACCGTATTGTGGACGTCATGCAGTG
Bottom	AAGTGACCGCTGAAGAGCAGATCCAACCCTAA
Center	TCTTCGTCCACAATATCTGC
DNA1	NH_2-CACTGCATGACAGCGGTCACTT
Right	TTAGGGTTGGACGGTATTTCAG
MMP DX Tile 2	
Top	CACTGCATGCATCGCTCCGTTAATGCTTTCCC
Bottom	AGCGAGACCTCCCTGACAGTAATTTGAGGGCT
Center	TCAGGAACGGAGCGATACTG
Left	GGGAAAGCATTGAGGTCTCGCT
DNA2	AGCCCTCAAATTGCATGCAGTG-NH_2
RGDS DX Tile	
Top	CGTATTCGACTGGGTG
Bottom	AAGTGACCGCTCAGTCCGAAGAGAATCGAGCC
Center	GACTGATACGAAGGCCTTCG
Left	CACCCAGTCGAAGCGGTCACTT
DNA3	GGCTCGATTCTACCGACGTCCG-NH_2
DNA4	NH_2-CGGACGTCGGTGCCTT

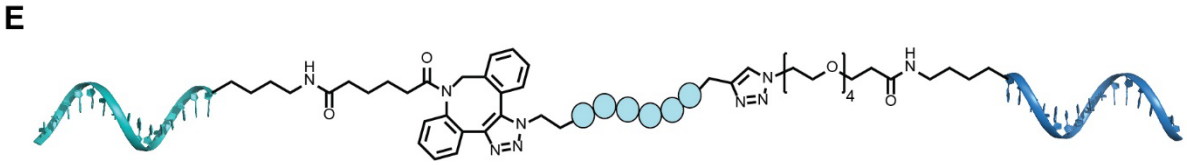
Supplementary Table S1. Sequences of all DNA oligonucleotides used to synthesize the DNA-peptide-DNA conjugates, as well as the strands that comprise the DX tiles. "NH_2" denotes an amine linked by a C6 alkyl linker.

S3. Supplementary Figures

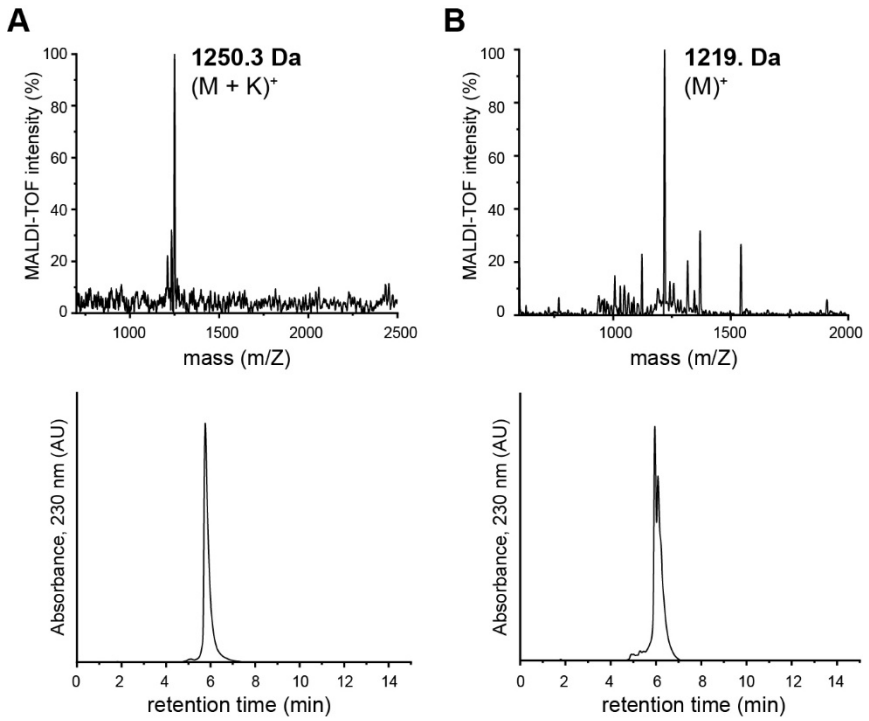




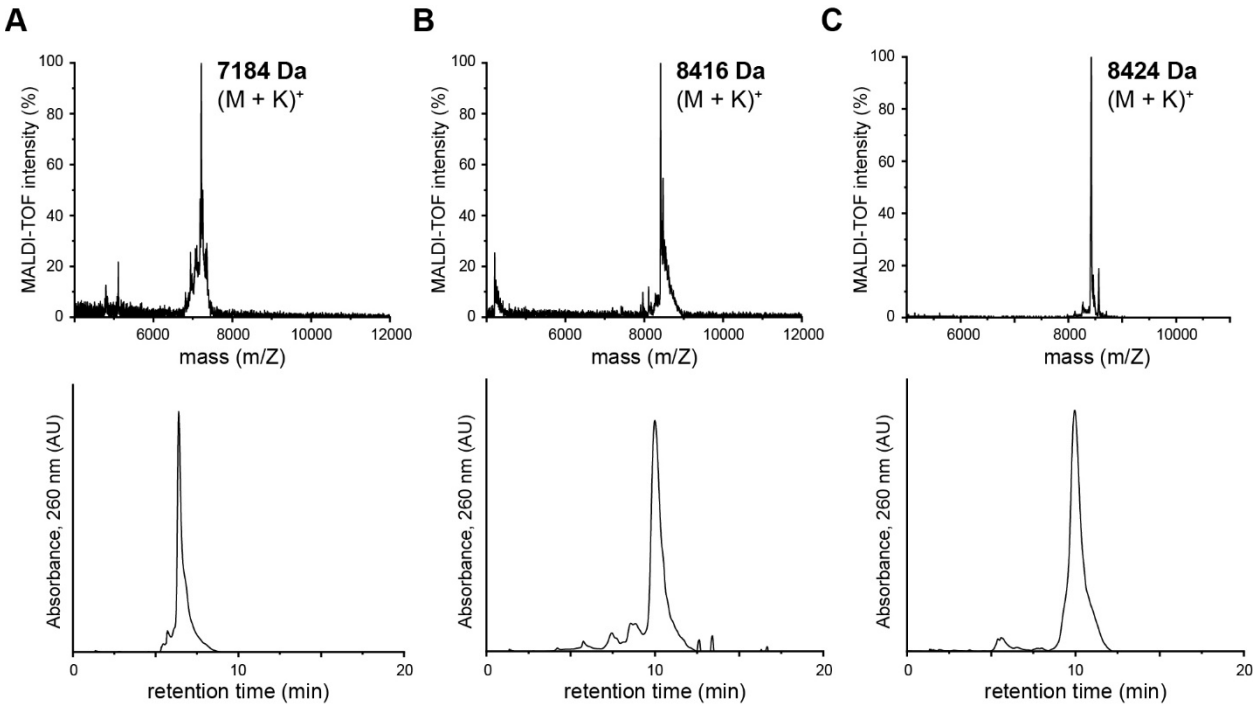




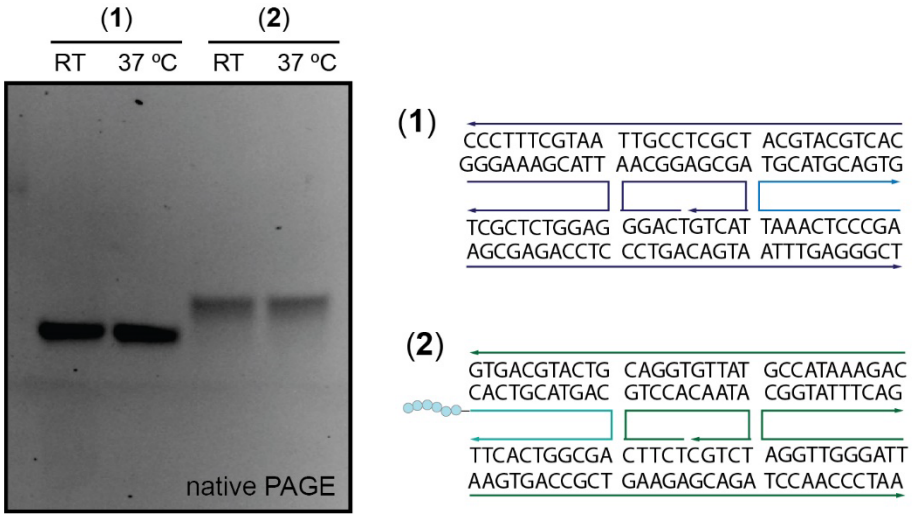
Supplementary Figure S1. Full chemical structures of MMP$_{pep}$ (**A**) and RGDS$_{pep}$ (**B**). The noncanonical amino acids propargylalanine (prA) and azidolysine (azK) are indicated. **C,D)** Chemical synthesis of DNA-DBCO and DNA-azide, respectively. **E)** Chemical structure of DPD triblock molecule.



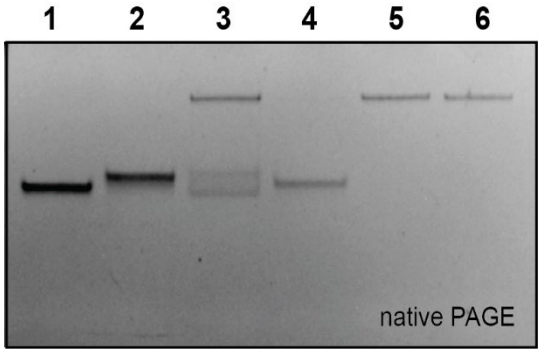
Supplementary Figure S2. Characterization confirming the synthesis and purity of (A) MMP_{pep} and (B) $MMP_{pep\text{-}scram}$ (prA-GQGIPQGWGG-azK) using MALDI-TOF mass spectrometry (top) and RP-HPLC (bottom). The expected mass of both peptides is 1216.6 Da.



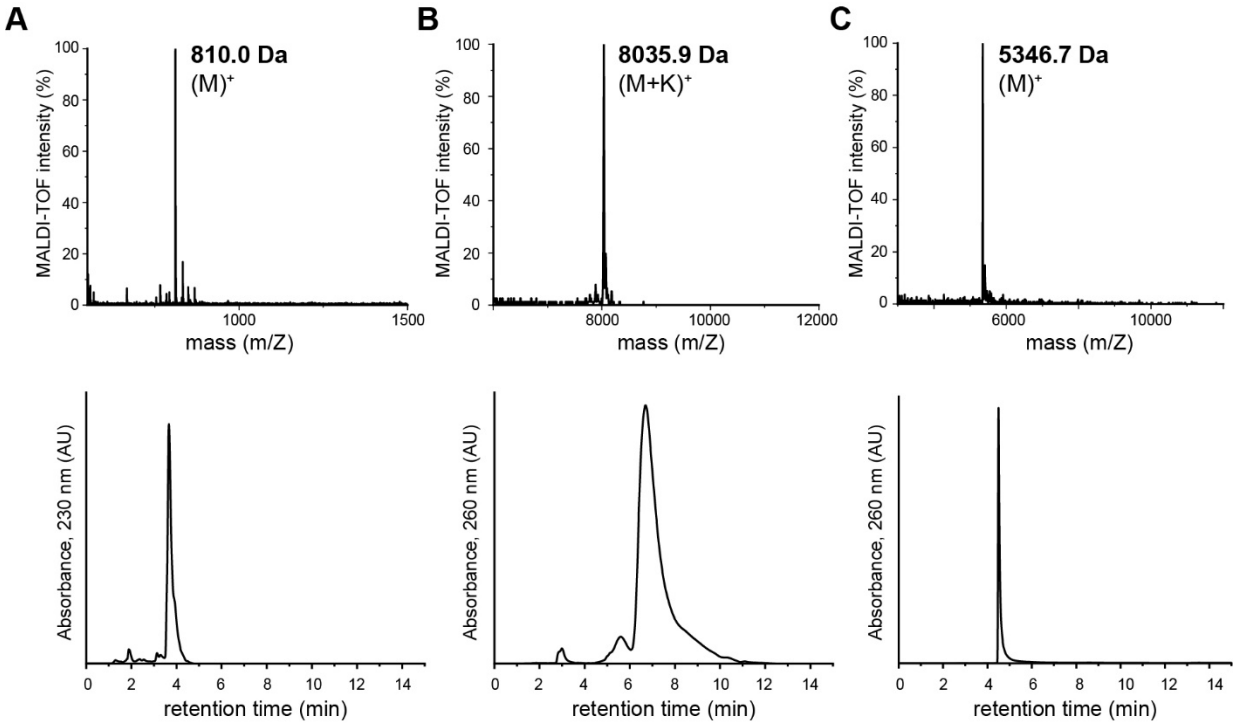
Supplementary Figure S3. Characterization confirming the synthesis and purity of (A) DNA2-azide, (B) DNA1-MMP_{pep} , and (C) DNA1-$MMP_{pep\text{-}scram}$ using MALDI-TOF mass spectrometry (top) and RP-HPLC (bottom). The expected masses are 7148 Da (A) and 8366 Da (B and C).



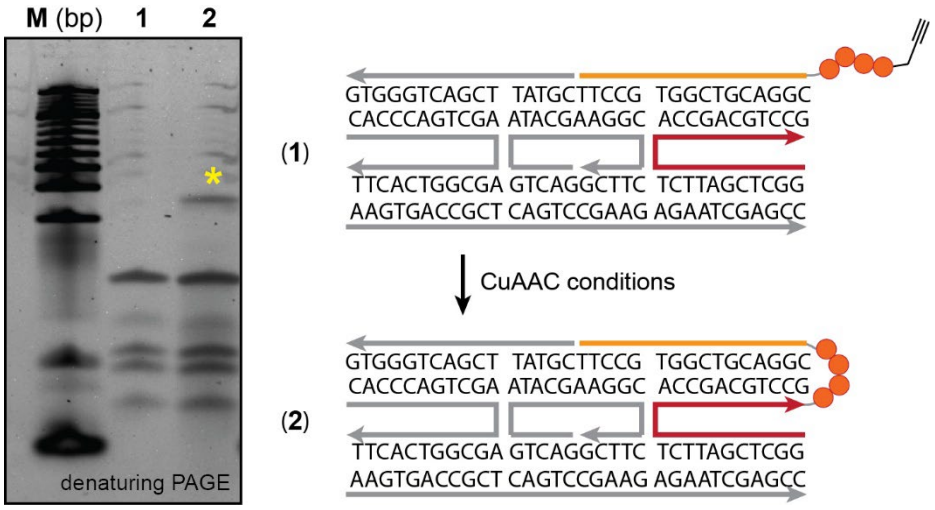
Supplementary Figure S4. Confirmation that the DX tiles are stable at the optimal incubation temperature of matrix metalloproteinase (37° C). The bands run at the same retention as tiles incubated at RT, so the elevated temperature does not affect them. Sample (**1**) is an all-DNA tile, and (**2**) contains the DNA1-MMP_{pep} conjugate. Once again, we note that after incorporation of the peptide, the DX tile intensity drops, despite having the same concentration as the all-DNA tiles.



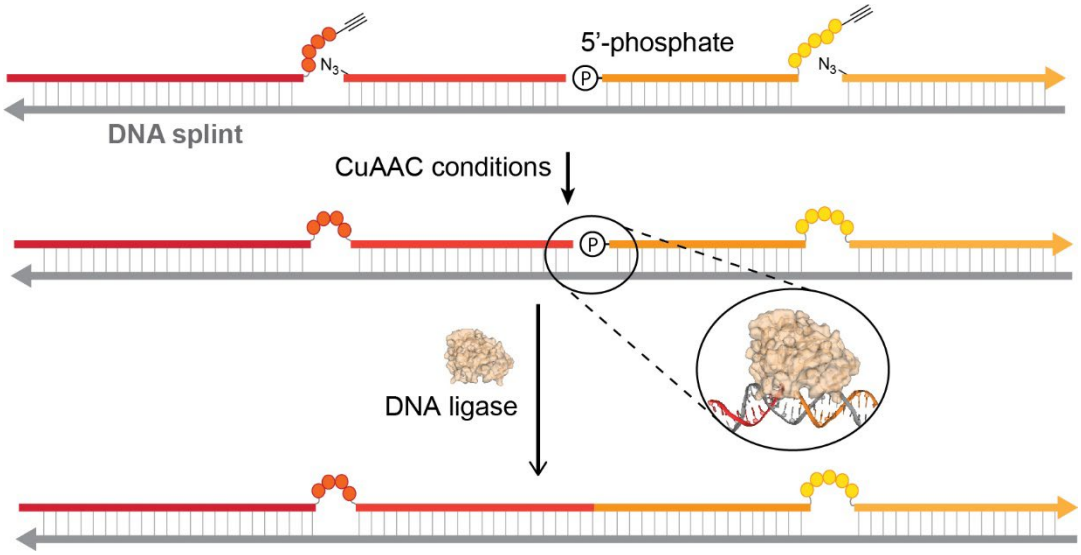
Supplementary Figure S5. Confirmation that the MMP_{pep} sequence is required for MMP8 cleavage of a DX tile dimer. Lanes 1-4 correspond to the same samples as Figure 3A in the main text: individual tiles (lanes 1 and 2), the tile dimer before (lane 3) and after (lane 4) exposure to MMP8. Lanes 5 and 6 are a tile dimer linked by $MMP_{pep\text{-}scram}$ before (lane 5) and after (lane 6) exposure to MMP8.



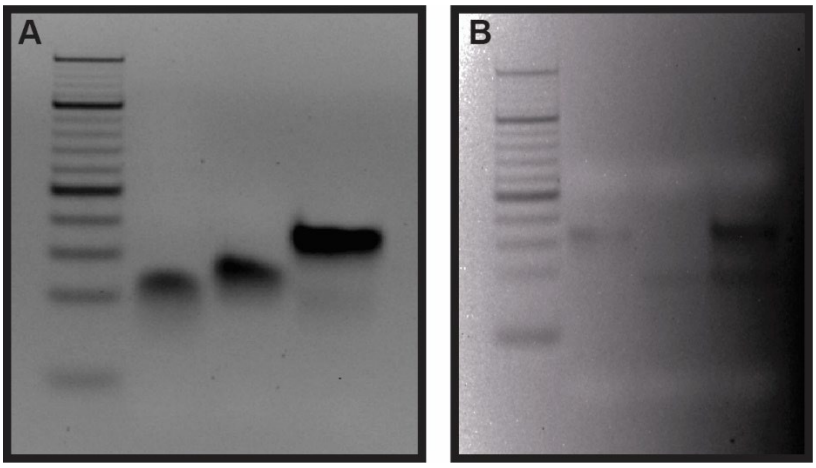
Supplementary Figure S6. Characterization confirming the synthesis and purity of (A) RGDS peptide (expected mass 809.39), (B) DNA-RGDS (expected mass 7992), and (C) DNA-azide (expected mass 5346) using MALDI-TOF mass spectrometry and RP-HPLC chromatography.



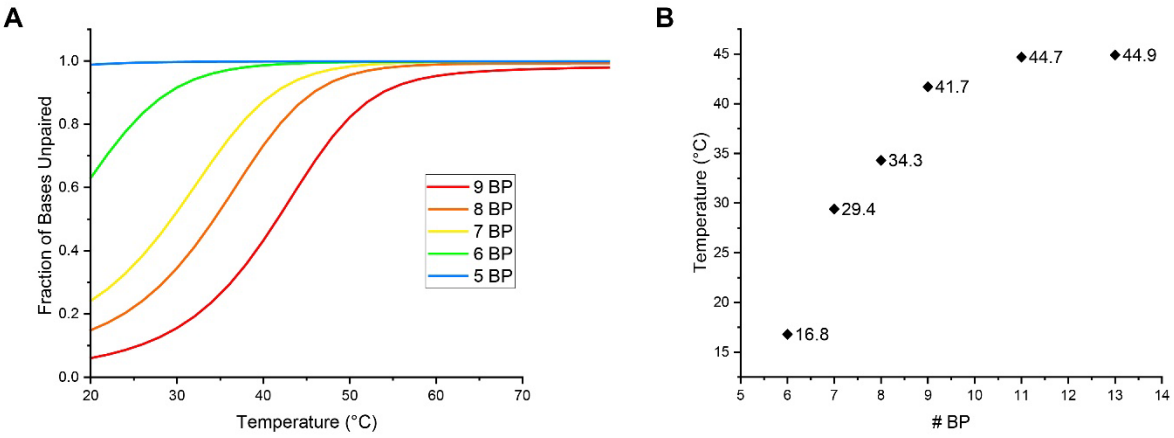
Supplementary Figure S7. Synthesis of a DPD triblock on an assembled nanostructure. Denaturing PAGE showing the annealed tile before exposure to CuAAC conditions (**1**) and after (**2**). The appearance of an upper band (yellow star) corresponds to the complete DPD conjugate. However, it is difficult to estimate the relative yield of the reaction because of the multiple strands of varying length, which do not all stain equally.



Supplementary Figure S8. Proposed route to produce DNA-peptide-DNA-peptide-DNA pentablock molecules (or, in principle, higher order copolymers). Two DPD conjugates can be further linked by splint ligation by incorporating a 5'-phosphate into one of the DNA handles and using DNA ligase.



Supplementary Figure S9. 8% native PAGE comparison of the stability of the duplex at 11° C for (A) the longer 9-bp duplex used in the synthesis vs a (B) shorter 5-bp complementary region. Both gels contain a 10 bp ladder in the first lane, the azide functionalized DNA strand in lane 2, the DNA-peptide conjugate in lane 3, and the "duplex" after being annealed using a thermal gradient of 95 - 4 °C over 1 hour. Lane 4 in panel (B) highlights the lack of stability of the shorter duplex, as indicated by two bands; lane 4 in panel (A) clearly shows hybridization of the two strands.



Supplementary Figure S10. Modeling the melting temperature of varying lengths of duplexes to determine the minimal number of complementary bases for stability of the duplex for proximity-aided reaction. (A) Melting curves with varying lengths of complementary regions and (B) the melting temperature, defined as the temperature which a fraction of bases unpaired= 0.5, vs. the length of the complementary region. These values were determined using the Nupack software(*2*), where the melting temperatures were determined between 20 to 80 degrees with intervals of 2 °C and both strands at a concentration of 1 μM, in a buffer containing only Na^+ ions and no Mg^{2+} in order to mimic the reaction conditions of the CuAAC. The DNA1 sequence and its complement were modeled, with truncation from the 3' end to model the number of bases.

References

1. A. Buchberger, C. R. Simmons, N. E. Fahmi, R. Freeman, N. Stephanopoulos, Hierarchical Assembly of Nucleic Acid/Coiled-Coil Peptide Nanostructures. *Journal of the American Chemical Society* **142**, 1406-1416 (2020).
2. J. N. Zadeh *et al.*, NUPACK: analysis and design of nucleic acid systems. *J Comput Chem* **37**, 170-173 (2011).