Supplementary Methods

Peptide and Amino Modifications to DNA Origami

The pointed triangle design was selected due to its lower tendency to aggregate and to exhibit blunt end stacking effects[1]. Eighteen peptide and 15 amino anchor modifications were made to the triangle origami design. For the peptide modifications, the original strands that hybridise to the m13m18 backbone were extended with the same sequence (AGTTGTGGATCCTACT). This extended sequence was complementary to the acrydite peptide linker when was conjugated to the peptide. Strands selected for the amino anchors were modified with a poly-T extension, complementary for poly-A 6 carbon amino anchor. All modifications are listed in Supplementary Table 1. During synthesis of the DNA origami, peptide and amino modification were added at a 20x excess to the concentration of the available modification sites.

Supplementary Table 1. Modified strands from the triangular DNA origami design originally published by Rothemund [1]. Mapped strand indicates which strand is modified from Rothemund's original design. Columns 3-5 indicate if the modification is to be included in the 6, 12 or 18 peptide designs.

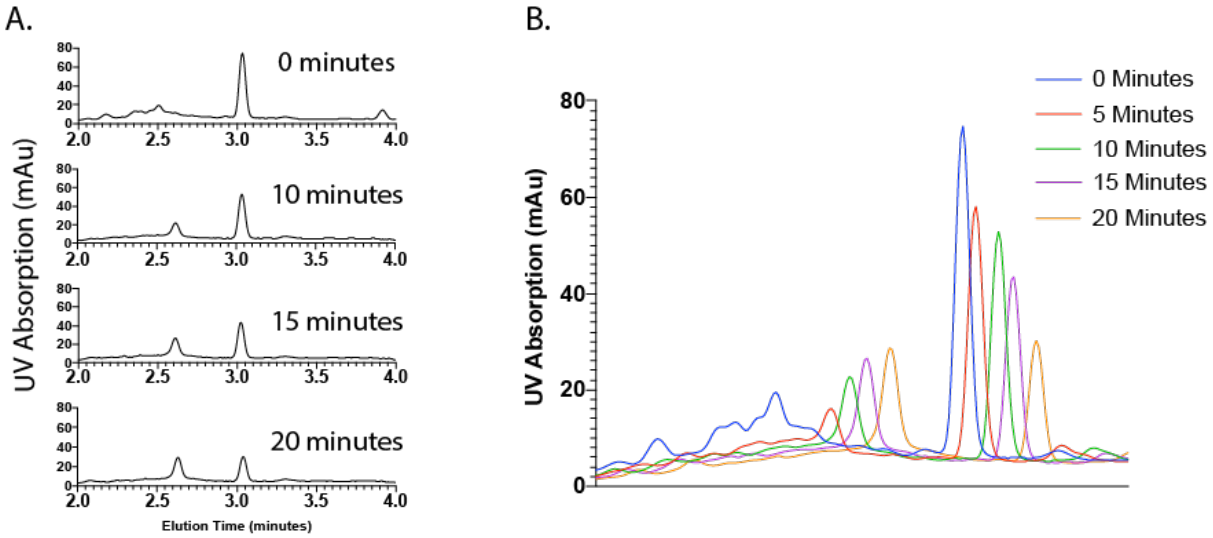
Mapped Strand	Modification	In 18 Pep?	In 12 Pep?	In 6 Pep?	Sequence
b32	Pep	Yes	Yes	Yes	AATACTGCGGAATCGTAGGGGGTAATAGTAAAATGTTTAGACTAGTTGTGGATCCTACT
c05	Amino	Yes	Yes	Yes	TGGCAATTTTTAACGTCAGATGAAAACAATAACGGATTCGTTTTTTTTTTTTTTTTTT
c13	Amino	Yes	Yes	Yes	GATTATACACAGAAATAAAGAAATACCAAGTTACAAAATCTTTTTTTTTTTTTTTTTT
c32	Pep	Yes	Yes	Yes	TCTTTGATTAGTAATAGTCTGTCCATCACGCAAATTAACCGTTAGTTGTGGATCCTACT
b20	Pep	Yes	Yes	No	TAATTGCTTTACCCTGACTATTATGAGGCATAGTAAGAGCAGTTGTGGATCCTACT
a20	Pep	Yes	Yes	No	TTGACGGAAATACATACATAAAGGGCGCTAATATCAGAGAAGTTGTGGATCCTACT
b30	Pep	Yes	No	No	TGCTGTAGATCCCCCTCAAATGCTGCGAGAGGCTTTTGCAAGTTGTGGATCCTACT
a32	Pep	Yes	Yes	Yes	CAGAAGGAAACCGAGGTTTTTAAGAAAAGTAAGCAGATAGCCGAGTTGTGGATCCTACT
b05	Amino	Yes	Yes	Yes	ACAGTCAAAGAGAATCGATGAACGACCCCGGTTGATAATCTTTTTTTTTTTTTTTTTT
a05	Amino	Yes	Yes	Yes	TTTGATGATTAAGAGGCTGAGACTTGCTCAGTACCAGGCGTTTTTTTTTTTTTTTTTT
b13	Amino	Yes	Yes	Yes	CGTTCTAGTCAGGTCATTGCCTGACAGGAAGATTGTATAATTTTTTTTTTTTTTTTTT
a13	Amino	Yes	Yes	Yes	TTTAACGGTTCGGAACCTATTATTAGGGTTGATATAAGTATTTTTTTTTTTTTTTTTT
c30	Pep	Yes	No	No	TAAAACATTAGAAGAACTCAAACTTTTTATAATCAGTGAGAGTTGTGGATCCTACT
c20	Pep	Yes	Yes	No	GAATACGTAACAGGAAAAACGCTCCTAAACAGGAGGCCGAAGTTGTGGATCCTACT
A37	Pep	Yes	No	No	AGAGAATAACATAAAAACAGGGAAGCGCATTAAGTTGTGGATCCTACT
b37	Pep	Yes	No	No	ACAGGTAGAAAGATTCATCAGTTGAGATTTAGAGTTGTGGATCCTACT
a33	Amino	Yes	Yes	Yes	CCTTTTTTCATTTAACAATTTCATAGGATTAGTTTTTTTTTTTTTTTTTT
a30	Pep	Yes	No	No	GAGCCAGCGAATACCCAAAAGAACATGAAATAGCAATAGCAGTTGTGGATCCTACT
b33	Amino	Yes	Yes	Yes	AGGGATAGCTCAGAGCCACCACCCCATGTCAATTTTTTTTTTTTTTTTTT
c33	Amino	Yes	Yes	Yes	CGCGTCTGATAGGAACGCCATCAACTTTTACATTTTTTTTTTTTTTTTTT
c37	Pep	Yes	No	No	CGAGAAAGGAAGGGAAGCGTACTATGGTTGCTAGTTGTGGATCCTACT
b42	Amino	Yes	Yes	Yes	AGACGTTACCATGTACCGTAACACCCCTCAGAACCGCCACTTTTTTTTTTTTTTTTTT
a42	Amino	Yes	Yes	Yes	AGAGTCAAAAATCAATATATGTGATGAAACAAACATCAAGTTTTTTTTTTTTTTTTTT
b50	Amino	Yes	Yes	Yes	AGCGTAACTACAAACTACAACGCCTATCACCGTACTCAGGTTTTTTTTTTTTTTTTTT
a50	Amino	Yes	Yes	Yes	ACATAGCGCTGTAAATCGTCGCTATTCATTTCAATTACCTTTTTTTTTTTTTTTTTT
c61	Pep	Yes	Yes	No	TTCCAGTCCTTATAAATCAAAAGAGAACCATCACCCAAATAGTTGTGGATCCTACT
c49	Pep	Yes	No	No	GTTTGCGTCACGCTGGTTTGCCCCAAGGGAGCCCCCGATTAGTTGTGGATCCTACT
B41	Pep	No	Yes	Yes	CGACCTGCGGTCAATCATAAGGGAACGGAACAACATTATTAGTTGTGGATCCTACT
C41	Pep	No	Yes	Yes	TTTCACCAGCCTGGCCCTGAGAGAAAGCCGGCGAACGTGGAGTTGTGGATCCTACT
A41	Pep	No	Yes	Yes	TTTCCTTAGCACTCATCGAGAACAATAGCAGCCTTTACAGAGTTGTGGATCCTACT
c42	Amino	Yes	Yes	Yes	GTAACCGTCTTTCATCAACATTAAAATTTTTGTTAAATCATTTTTTTTTTTTTTTTTT
c50	Amino	Yes	Yes	Yes	GGATAGGTACCCGTCGGATTCTCCTAAACGTTAATATTTTTTTTTTTTTTTTTTTTT
b49	Pep	Yes	No	No	TATCATCGTTGAAAGAGGACAGATGGAAGAAAAATCTACGAGTTGTGGATCCTACT
a49	Pep	Yes	No	No	AGCATGTATTTCATCGTAGGAATCAAACGATTTTTTGTTTAGTTGTGGATCCTACT
b61	Pep	Yes	Yes	No	AAAACACTTAATCTTGACAAGAACTTAATCATTGTGAATTAGTTGTGGATCCTACT
a61	Pep	Yes	Yes	No	GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTTAGTTGTGGATCCTACT
Acrydite Peptide linker	-	Yes	Yes	Yes	5ACryd/AGTAGGATCCACAACT
Amino Anchor	-	Yes	Yes	Yes	5AmMC6/AAAAAAAAAAAAAAAAAA

Supplementary References:

1. P. W. K. Rothemund, *Nature*, 2006, **440**, 297-302.

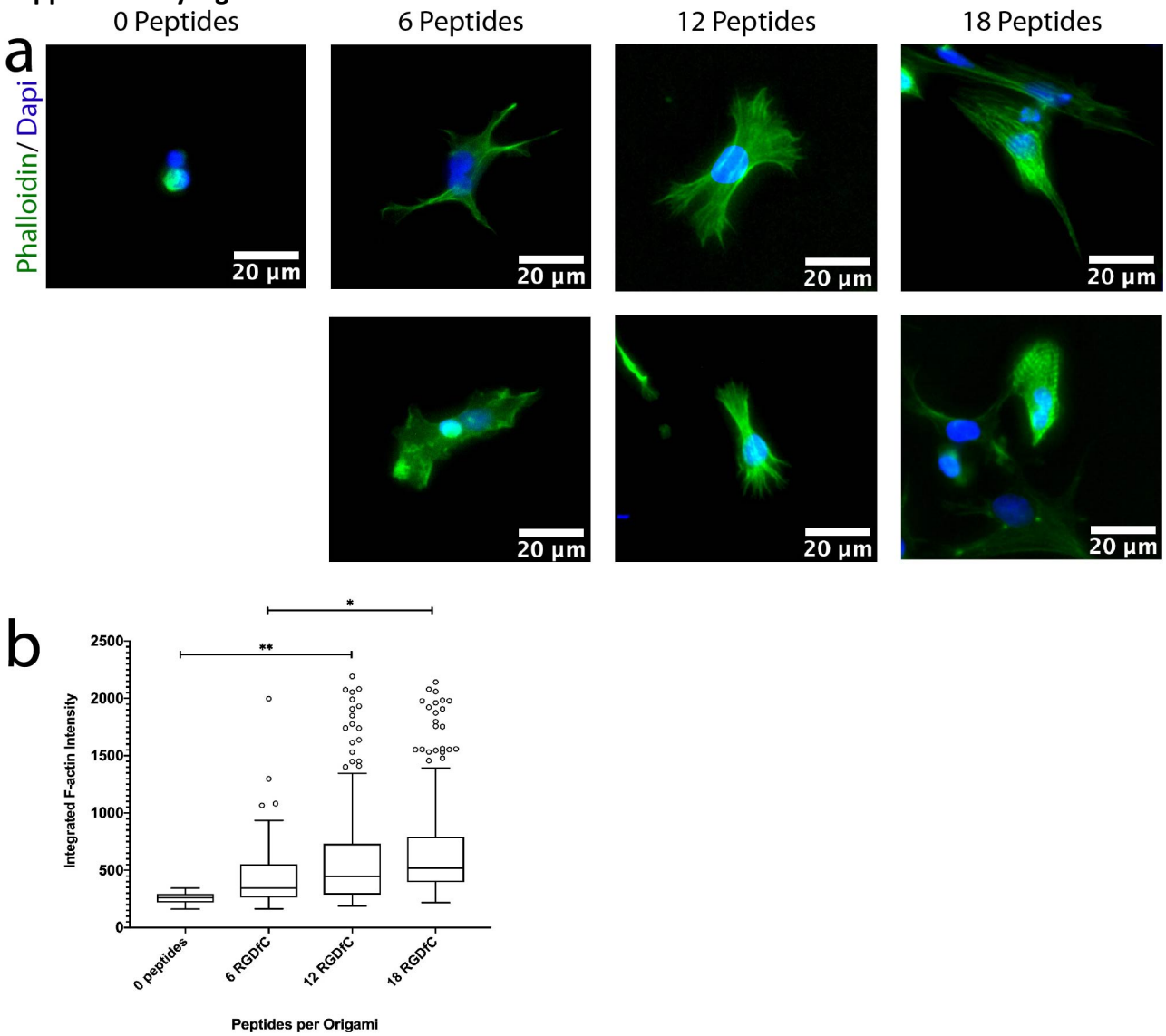
Supplementary Figures:

Supplementary Figure 1



Supplementary Figure 1: HPLC verification of conjugation between cRGDfC peptide and acrydite modified ssDNA. A) HPLC elution time of unconjugated ssDNA (1) and conjugated product (2) with increasing UV exposure time from 0 to 20 minutes. B) HPLC traces have been overlaid and shifted laterally for visual clarity of increasing conjugate yield with increasing UV exposure.

Supplementary Figure 2



Supplementary Figure 2: Increasing the number of peptides on randomly positioned DNA origami promotes myofibril assembly and maturation. a) Only diffuse actin staining or stress-fibre like structures are detected when less than 12 peptides are attached per origami, but sarcomeric striations become detectable at higher peptide densities. b) Increasing actin organisation is also evident from the phalloidin staining intensities. p values were calculated using a one-way ANOVA and Tukey correction for multiple comparisons. * = $p<0.05$; **= $p< 0.01$