

## SUPPORTING INFORMATION

### Synthesis of cyclic, multivalent Arg-Gly-Asp using sequential thiol-ene/thiol-yne photoreactions.

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#### **Materials:**

All reagents used were peptide synthesis grade unless otherwise noted. Fmoc-Cys(Mmt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-D-Phe-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Lys(Alloc)-OH were obtained from Anaspec (San Jose, CA). Fmoc-Ahx-OH and Rink Amide MBHA resin (0.56 mmol/g resin) were purchased from Novabiochem (La Jolla, CA). Piperidine, 1,8-diazabicyclo [7] undec-7-ene (DBU), and 2,6-lutidine were obtained from Sigma-Aldrich (St. Louis, MO). N-methylmorpholine (NMM) and acetic anhydride were purchased from Fisher Scientific (Pittsburgh, PA). N-methylpyrrolidone (NMP), dimethylformamide (DMF), and diisopropylethylamine (DIPEA) were purchased from Applied Biosystems (Foster City, CA). O-Benzotriazole-N,N,N',N'-tetramethyluronium-hexafluoro-phosphate (HBTU) and 2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU) were obtained from Anaspec and ChemPep, Inc. (Wellington, FL), respectively. Trifluoroacetic acid (TFA), triisopropylsilane (TIPS), phenol, 4-pentynoic acid, and (human) fibrinogen were purchased from Sigma-Aldrich. 2,2-dimethoxy-2-phenylacetophenone (DMPA) photoinitiator was obtained from Ciba (Tarrytown, NY). GPIIb/IIIa was obtained from Enzyme Research Laboratories (South Bend, IN). Fibrinogen antibody (HRP) was purchased from Abcam (Cambridge, MA).

#### **General peptide synthesis and purification:**

Peptides were built on the solid phase using an automated Tribute Peptide Synthesizer (Protein Technologies, Tucson, AZ). Peptides were synthesized on the 0.25-0.5 mmol scale. Fmoc deprotection was achieved using 20% piperidine in NMP (5min x 2). 4 eq.

of Fmoc-protected amino acids were activated using HBTU/NMM (1:2, molar ratios of HBTU and NMM, respectively, in relation to amino acid) and added to the resin and allowed to react for 35 min. Any unreacted sites were acetylated using 5% (v/v) acetic anhydride/6% (v/v) 2,6-lutidine in NMP (10 min). Peptides were cleaved from their solid support using TFA/TIPS/H<sub>2</sub>O (95:2.5:2.5 (v/v)) and phenol (50mg mL<sup>-1</sup> cleavage solution) and allowed to react for 2 h. Peptides were precipitated in chilled diethyl ether and washed (3x). Product was allowed to dry in a desiccator for 2h prior to HPLC purification. Peptides were purified using RP-HPLC (Waters Delta Prep 4000) with a C<sub>18</sub> prep column (Sunfire 30mm x 150mm) using a 70-min linear (5-95%) gradient of acetonitrile in 0.1% trifluoroacetic acid. Peptides were characterized using analytical scale RP-HPLC (XBridge 4.6mm x 50mm), MALDI-TOF MS (Applied Biosystem DE Voyager), and <sup>1</sup>H NMR.

#### **Synthesis of H-GK(4-pa)G-NH<sub>2</sub> (Core 1):**

H-GK(Mtt)G-resin was synthesized as described in the General Peptide Synthesis section. The Mtt group was selectively deprotected on resin as described previously.<sup>1</sup> Briefly, 1.5% TFA in dichloromethane was added to the resin (30 sec. x 9). A positive Kaiser test confirmed the availability of the free amine. 4-pentynoic acid (4-pa; 5 eq. to resin) was then coupled to the ε-amino group using HATU/DIPEA in DMF and allowed to react for 2 hr under Ar. A negative Kaiser test confirmed successful coupling. Product was characterized using MALDI-TOF and <sup>1</sup>H NMR. MALDI: Calculated [M+H]<sup>+</sup>=340.4 g/mol, found [M+H]<sup>+</sup>=340.7 g/mol. δ <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) 4.29 (1 H, t, J 6.8), 3.92-3.83 (2 H, m, J 17.2), 3.84 (2 H, s), 3.17 (2 H, t, J 6.3), 2.45 (2 H, d, J 6.2), 2.39 (2 H, t, J 6.7), 2.32 (1 H, s), 1.85 – 1.66 (2 H, m), 1.56 – 1.46 (2 H, m), 1.37 (2 H, d, J 7.8).

#### **Synthesis of H-GK(4-pa)G-Ahx-GK(4-pa)G-NH<sub>2</sub> (Core 2):**

H-GK(Mtt)G-Ahx-GK(Mtt)G-resin was synthesized and 4-pentynoic acid was coupled through the Lys residues as described previously. MALDI: Calculated [M+H]<sup>+</sup>=775.9 g/mol, found [M+H]<sup>+</sup>=775.6 g/mol. δ <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) 4.32 – 4.22 (2 H, m), 3.90 (2 H, s), 3.87 (2 H, d, J 8.5), 3.84 (4 H, d, J 4.7), 3.17 (6 H, t, J 6.5), 2.45 (4 H, d, J

6.4), 2.39 (4 H, t, J 6.6), 2.32 (2 H, s), 2.28 (2 H, t, J 7.5), 1.75 (4 H, d, J 33.8), 1.62 – 1.54 (2 H, m), 1.49 (6 H, d, J 7.0), 1.33 (6 H, dd, J 7.3, 38.6).

### **Synthesis of H-GK(4-pa)G-Ahx-GK(4-pa)G-Ahx-GK(4-pa)G-NH<sub>2</sub> (Core 3):**

H-GK(Mtt)G-Ahx-GK(Mtt)G-Ahx-GK(Mtt)G-resin was synthesized and 4-penytnoic acid was coupled through the Lys residues as described previously. MALDI: Calculated  $[M+H]^+ = 1211.4$  g/mol, found  $[M+H]^+ = 1211.1$  g/mol.  $\delta$  <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) 4.32 – 4.18 (3 H, m), 3.92 (4 H, d, J 18.3), 3.87 (2 H, d, J 8.9), 3.83 (6 H, t, J 4.6), 3.17 (6 H, d, J 6.8), 3.15 (4 H, d, J 7.2), 2.45 (6 H, dd, J 5.2, 7.5), 2.38 (6 H, t, J 6.6), 2.32 (3 H, t, J 2.4), 2.28 (4 H, t, J 7.4), 1.75 (6 H, d, J 34.4), 1.56 (4 H, dd, J 7.7, 15.2), 1.49 (10 H, d, J 6.7), 1.42 – 1.21 (10 H, m).

### **Synthesis of H-CGGRGDS-NH<sub>2</sub> (1):**

**1** was synthesized according to the General Peptide Synthesis procedure. MALDI: Calculated  $[M+H]^+ = 650.7$  g/mol, found  $[M+H]^+ = 650.6$  g/mol. Yield: 82%.

### **Synthesis of H-CGGc[CRGDSfK(Alloc)]-NH<sub>2</sub> (2):**

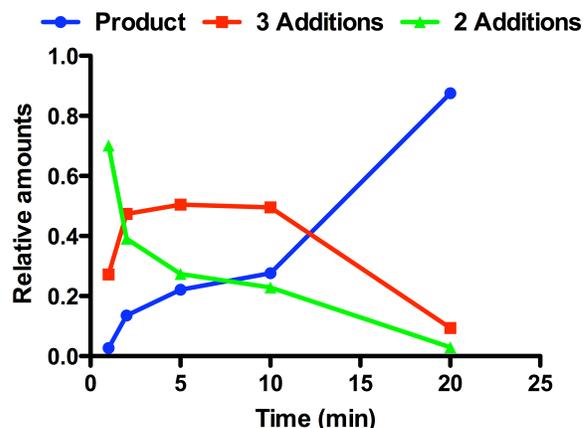
Fmoc-C(Mmt)RGDSfK(Alloc)-resin was synthesized as described previously. The monomethoxytrityl (Mmt) group was selectively deprotected on resin according to protocol.<sup>2</sup> An on-resin modified Ellman's assay was used to qualitatively determine the presence of free thiol.<sup>3</sup> Peptide cyclization was achieved using thiol-ene photochemistry as described elsewhere.<sup>4</sup> Briefly, the resin was swollen in DMF (10 min) and the vessel purged with Ar. DMPA (1 eq. to resin) was added and irradiated with UV light (365nm, 20 mW cm<sup>-2</sup>) for 3 hr. DMPA was supplemented every 10 min to account for initiator consumption due to photolysis. A negative Ellman's test (indicating no thiols) was used to determine when the reaction was complete. The remaining amino acids (Cys(Trt), Gly, Gly) were coupled (HATU/NMM) using the Tribute synthesizer. MALDI: Calculated  $[M+H]^+ = 1112.3$  g/mol, found  $[M+H]^+ = 1112.2$  g/mol. <sup>1</sup>H NMR confirmed the disappearance of vinyl protons. Yield: 15%.

**General procedure for the synthesis of multivalent RGD using thiol-yne photochemistry:**

Cys-containing peptides (**1** or **2**) were dissolved in an appropriate solvent (0.2 M). At this concentration, **1** was solubilized in H<sub>2</sub>O while **2** was dissolved in DMF. The appropriate amount of core molecule was added to achieve a [SH]:[alkyne] ratio of 4:1 which was held constant for all reactions. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was used as a water soluble (used with **1**) Type I photoinitiator.<sup>5</sup> 2,2-dimethoxy-2-phenyl acetophenone (DMPA) was solubilized in DMF (used with **2**). Photoinitiators were added at the following molar ratios [SH]:[initiator]=50:1. The reaction mixture was purged with Ar and irradiated with UV light (365nm, 15mW cm<sup>-2</sup>) for 20 minutes. Photoinitiator was supplemented at 10 min to account for photolysis. Reactions in DMF were precipitated in chilled diethyl ether and washed (2x) and then purified using RP-HPLC. Reactions in H<sub>2</sub>O were diluted and directly purified with RP-HPLC.

### **Kinetics of photoinitiated thiol-yne peptide clustering:**

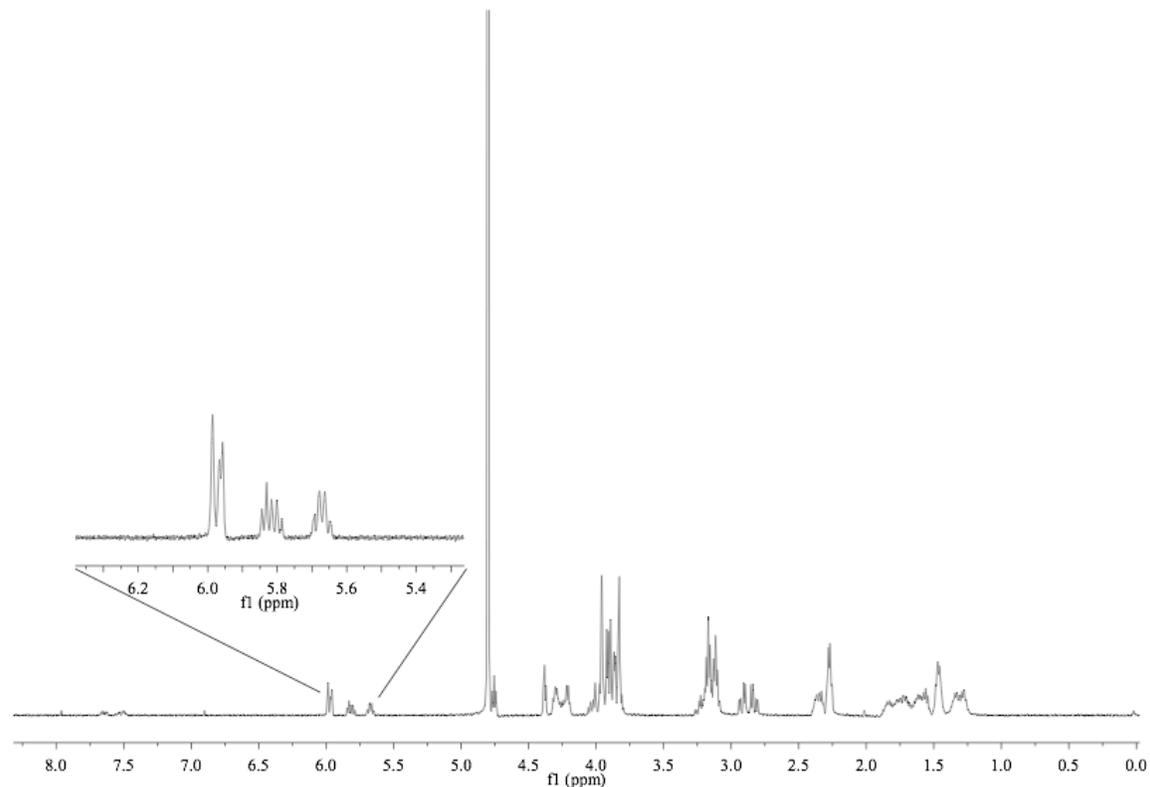
Model System: Core 2 + 1 to form 4 (Linear RGD tetramer).



**Figure S1** Relative amounts of varying species present during the thiol-yne induced formation of a linear RGD tetramer. Determined by integration of the corresponding HPLC peaks.

### **Evaluation of multivalent RGD peptides:**

A competitive binding ELISA was used to determine the potency of the multivalent RGD peptides. GPIIb/IIIa is known to contain the integrins  $\alpha$ IIb and  $\beta$ 3, which bind RGD peptide sequences. Fibrinogen is also known to bind to these integrins. The ELISA was performed to quantitate the inhibition of bound fibrinogen in the presence of RGD peptide. The ELISA was performed as described previously.<sup>4</sup> Briefly, GPIIb/IIIa was incubated in a 96 well Maxisorp plate (Nunc) ( $10\mu\text{g ml}^{-1}$ ) overnight at  $4^\circ\text{C}$ . The plate was blocked for non-specific interactions using BSA (3.5 wt%, 3hr). Varying peptides concentrations and fibrinogen (40nM) was incubated in the plate for 3hr at room temperature. Fibrinogen antibody (HRP) (1:20,000 dilution) as added (1hr). The substrate (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]- diammonium salt-ABTS) was added and the absorbance measured at 405nm. Peptide concentrations are reported as total molecule molarity as opposed to normalized [RGD].



**Figure S2**  $^1\text{H}$  NMR corresponding to Peak C in Figure 1. Highlighted peaks ( $^1\text{H}$   $\delta$  6.02 – 5.63 ppm) correspond to vinyl sulfide proton peaks.

#### Supporting Information References:

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