Supporting Information

Peptide Functionalized Oxime Hydrogels with Tunable Mechanical Properties and Gelation Behavior

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Materials and Methods

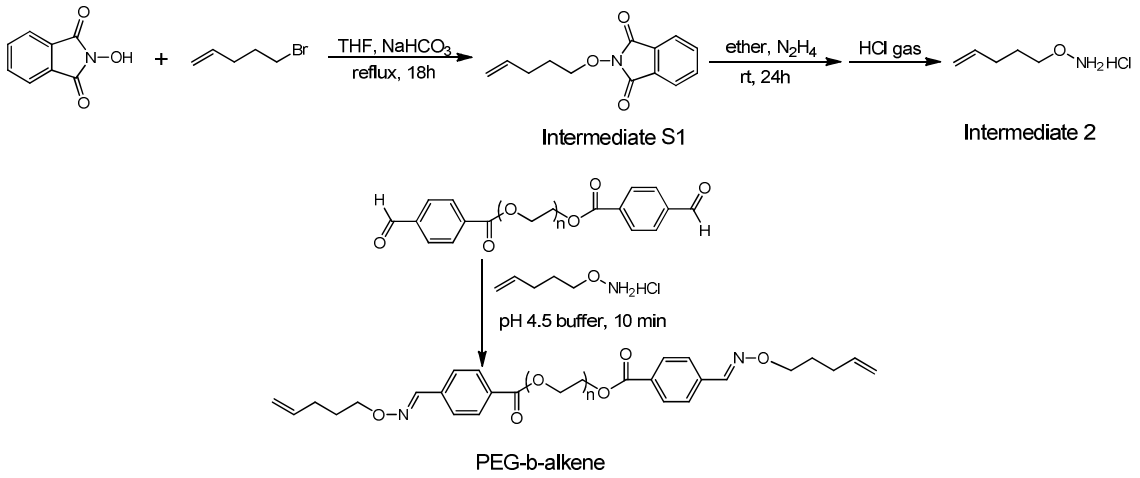
Materials: All commercial reagents and solvents were purchased from Aldrich or Fisher Scientific and used without further purification unless noted otherwise. All reactions were performed under a blanket of nitrogen unless noted otherwise.

Instrumentation: NMR spectra were obtained by Varian NMRS 300 MHz spectrometer. All chemical shifts are reported in ppm (δ), and referenced to the chemical shifts of residual solvent resonances (^{1}H NMR $CDCl_3$ 7.27 ppm, D_2O 4.80 ppm, DMSO-d_6 2.50 ppm); ^{13}C $CDCl_3$ 77.00 ppm, DMSO-d_6 39.50 ppm). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, br = broad singlet, m = multiplet. FT-IR spectroscopy was recorded on a SHIMADZU MIRacle 10 ATR-FTIR. Raman spectra were recorded on a LabRAM HR 800 Spectrophotometer. Fluorescence microscopy images were recorded on OLYMPUS IX 81 Microscope and are unaltered. Size exclusion chromatographic analyses (SEC) were performed using a Waters 150-C Plus instrument equipped with three HR-Styragel columns [100 Å, mixed bed (50/500/103/104 Å), mixed bed (103, 104, 106 Å)], and a differential refractometer (Waters 410) detector. THF was used as eluent with a flow rate of 1.0 mL/min at 30 °C. The molecular mass and mass distribution were calculated from polystyrene standards. Electrospray ionization (ESI) was performed using a HCT Ultra II quadrupole ion trap mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with an electrospray ionization source. MALDI-TOF mass spectra were carried out on a Bruker Ultraflex-III TOF/TOF mass spectrometer (Bruker Daltonics, Inc., Billerica, MA) equipped with a Nd:YAG laser (355 nm). All spectra were measured in positive reflection mode.

Trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]-malononitrile (DCTB, Aldrich, >98%) and sodium trifluoroacetate served as matrix and cationizing salt, respectively.

Experimental Section.

1. Model oxime ligation reaction.



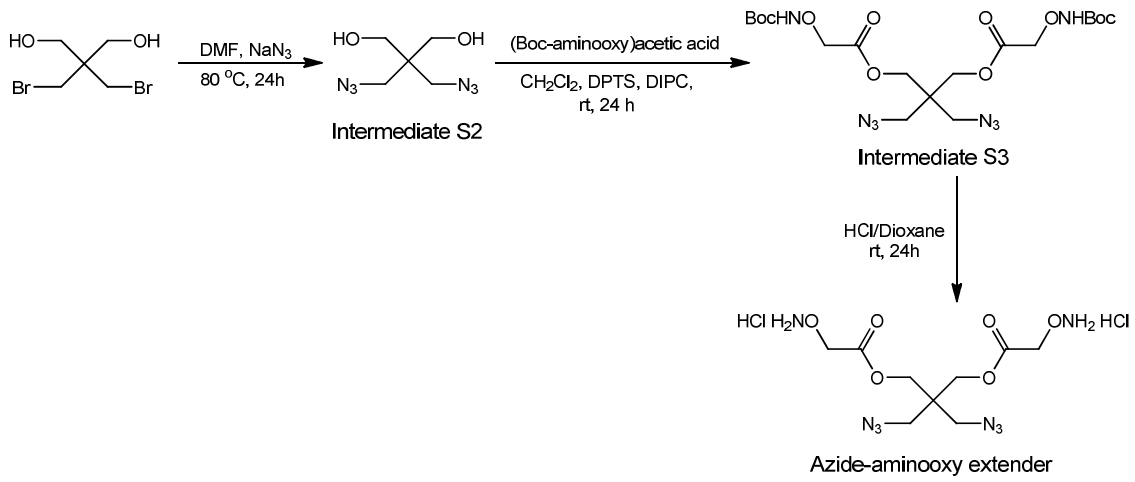
Scheme S1. Model reaction of oxime ligation between PEG-bCHO and intermediate 2.

Intermediate S1. In a 250 mL round bottom flask, *N*-hydroxyphthalimide (8.2 g, 50 mmol), 5-bromo-1-pentene (11.3 g, 75 mmol), and $NaHCO_3$ (8.0 g, 75 mmol) were mixed in 80 mL of THF. The red suspension was allowed to reflux for 24 h. After filtration to remove the solid, the collected solution was concentrated and re-diluted with 100 mL CH_2Cl_2. The solution was then washed with saturated $NaHCO_3$ solution until the aqueous layer was colorless. After drying with $MgSO_4$, the organic layer was collected and concentrated for chromatography purification on silica gel with CH_2Cl_2 as elute fluid. The resulting product was a white solid (9.2 g, yield 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.70-7.90 (m, 4H, aromatic H), 5.75-6.60 (m, 1H, CH_2=C***H***CH_2-), 4.95-5.35 (m, 2H, C***H*****$_2$**=CHCH_2-), 4.23 (t, 2H, -CH_2C***H*****$_2$**O-), 2.25-2.35 (m, 2H, CH_2=CHC***H*****$_2$**CH_2CH_2O-), 1.83-1.97 (m, 2H, CH_2=$CHCH_2$C***H*****$_2$**CH_2O-).

O-(pent-4-en-1-yl) hydroxylamine (2). In a 250 mL round bottom flask, intermediate **2** (2.3 g, 10 mmol) was dissolved in 80 mL of ether, followed by the addition of hydrazine monohydrate. White solid was observed within minutes. This reaction mixture was allowed to stir at room temperature 24 h. The white solid byproduct was removed via filtration. Dry HCl gas was purged into the collected organic solution for 30 min. The white solid was collected, dried in vacuum, and stored at room temperature (1.1 g, yield 78 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ=11.11 (s, 3H, $^{+}$N***H*****$_3$**O-), 5.75-6.60 (m, 1H, CH_2=C***H***CH_2-), 4.95-5.35 (m, 2H, C***H*****$_2$**=$CHCH_2$-), 4.02 (t, 2H, -CH_2C***H*****$_2$**O-), 1.95-2.15 (m, 2H, CH_2=CHC***H*****$_2$**CH_2CH_2O-), 1.55-1.75 (m, 2H, CH_2=$CHCH_2$C***H*****$_2$**CH_2O-). ^{13}C NMR (300 MHz, DMSO-d_6): δ=136.5, 115.4, 73.4, 29.2, 26.4.

Bi-alkene derived PEG (PEG-b-alkene). In a 4 mL glass vial, PEG-bCHO (100 mg, 0.02 mmol) and intermediate **3** (8.4 mg, 0.06 mmol) was dissolved in acetic buffer (pH 4.5) and stirred at room temperature for 10 min. The reaction mixture was subjected for MALDI-TOF characterization without purification. Pure sample was obtained by dialyzation in deionized water (molecular weight cut off (MWCO) 3000 g/mol, cellulose membrane, Pierce), followed by lyophilization. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.05-8.10 (m, 2H, aromatic H), 7.58-7.68 (m, 2H aromatic H), 5.75-5.95 (m, 1H, CH_2=C***H***CH_2-), 4.93-5.13 (m, 2H, C***H*****$_2$**=$CHCH_2$-), 4.40-4.55(t, 2H, -COOC***H*****$_2$**CH_2O-), 4.15-4.25 (t, 2H, -CH_2C***H*****$_2$**ON=C-), 3.80-3.90(m, 2H, -$COOCH_2$C***H*****$_2$**O-), 3.55-3.75 (s, ~220H, -C***H*****$_2$**C***H*****$_2$**O-, PEG main chain), 2.10-2.25 (m, 2H, CH_2=CHC***H*****$_2$**CH_2CH_2O-), 1.75-1.90 (m, 2H, CH_2=$CHCH_2$C***H*****$_2$**CH_2O-).

2. Azide-aminooxy extender (azide-ONH_2)



Scheme S2. Synthesis route of azide-aminooxy extender.

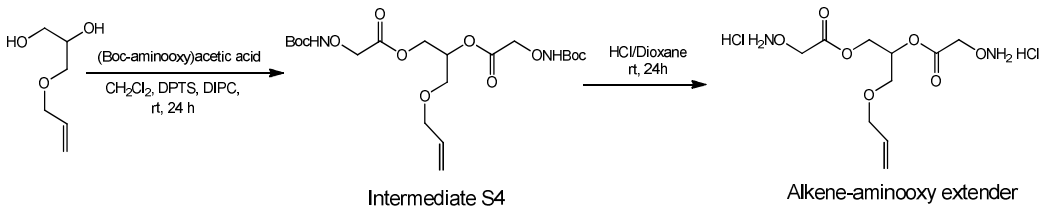
Intermediate S2 was synthesized as reported previously.[1]

Intermediate S3 was synthesized via general esterification catalyzed by DPTS/DIPC. ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.87 (s, 2H, -N***H***-), 4.47 (s, 4H, -NHOC***H₂***CO), 4.15 (s, 4H, -COOC***H₂***C-), 3.46 (s, 4H, -CC***H₂***N_3), 1.49 (s, 18H, (C***H₃***)$_3$CCO-). ^{13}C NMR (300 MHz, $CDCl_3$): δ= 169.0, 156.2, 82.4, 72.4, 63.1, 51.2, 43.0, 28.2.

Azide-aminooxy extender was obtained by the Boc deprotection of intermediate **S3**.

^{1}H NMR (300 MHz, D_2O): δ= 4.67-4.73 (m, 4H, $^{+}NH_3OC$***H₂***COO-), 4.154.25 (m, 4H, -CH_2COOC***H₂***C-), 3.42-3.52 (m, 4H, -CC***H₂***N_3).

3. Alkene-aminooxy extender (alkene-ONH_2)



Scheme S3. Synthesis route of alkene-aminooxy extender.

Intermediate S4 was synthesized via general esterification catalyzed by DPTS/DIPC. ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.87 (s, 2H, -N***H***-), 5.75-6.00 (m, 1H, -C***H***=CH_2), 5.15-5.40 (m, 3H, -$COOCH_2C$***H***OOC-, -CH=C***H*****$_2$**), 4.47-4.55 (m, 1H,-$COOC$***H*****$_2$**$CHOOC$-), 4.45 (d, 4H, -$NHOC$***H*****$_2$**COO-), 4.28-4.38 (m, 1H, -$COOC$***H*****$_2$**$CHOOC$-), 4.00 (d, 2H, -OC***H*****$_2$**$CH=CH_2$), 3.60 (d, 2H, -CHC***H*****$_2$**$OCH_2CH=CH_2$), 1.48 (s, 18H, $(CH_3)_3CCO$-).

Alkene-aminooxy extender was obtained by the Boc deprotection of intermediate S4.

^{1}H NMR (300 MHz, D_2O): 5.75-6.05 (m, 1H, -C***H***=CH_2), 5.10-5.50 (m, 3H, -$COOCH_2C$***H***OOC-, -CH=C***H*****$_2$**), 4.55-4.80 (m, 4H, $^+NH_3OC$***H*****$_2$**COO-), 4.30-4.50 (m, 2H, -$COOC$***H*****$_2$**$CHOOC$-), 3.95-4.15 (m, 2H, -OC***H*****$_2$**$CH=CH_2$), 3.78(d, 2H, -CHC***H*****$_2$**$OCH_2CH=CH_2$).

Reference

(1) Grandjean, C.; Boutonnier, A.; Guerreiro, C.; Fournier, J.-M.; Mulard, L. A. *The Journal of Organic Chemistry* **2005**, *70*, 7123.

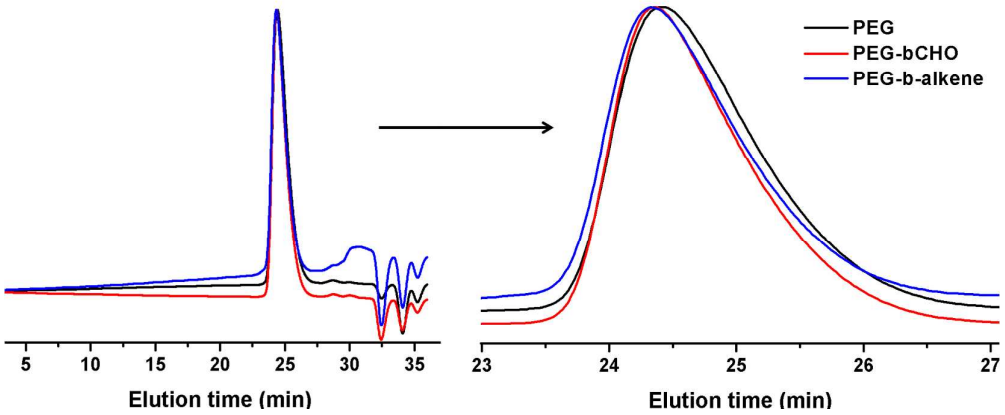


Figure S1. SEC elution curves of PEG raw materials (black), PEG-bCHO (red), and PEG-b-alkene (blue). THF was used as eluent with a flow rate of 1.0 mL/min at 30 °C.

Table S1. Molecular Mass Data of Polymers. M_n: number average molecular weight; PDI: polydispersity index. M_n and PDI were calculated from polystyrene standards.

	PEG	PEG-bCHO	PEG-b-alkene
M_n (Da)	4860	5200	5390
PDI	1.10	1.08	1.12

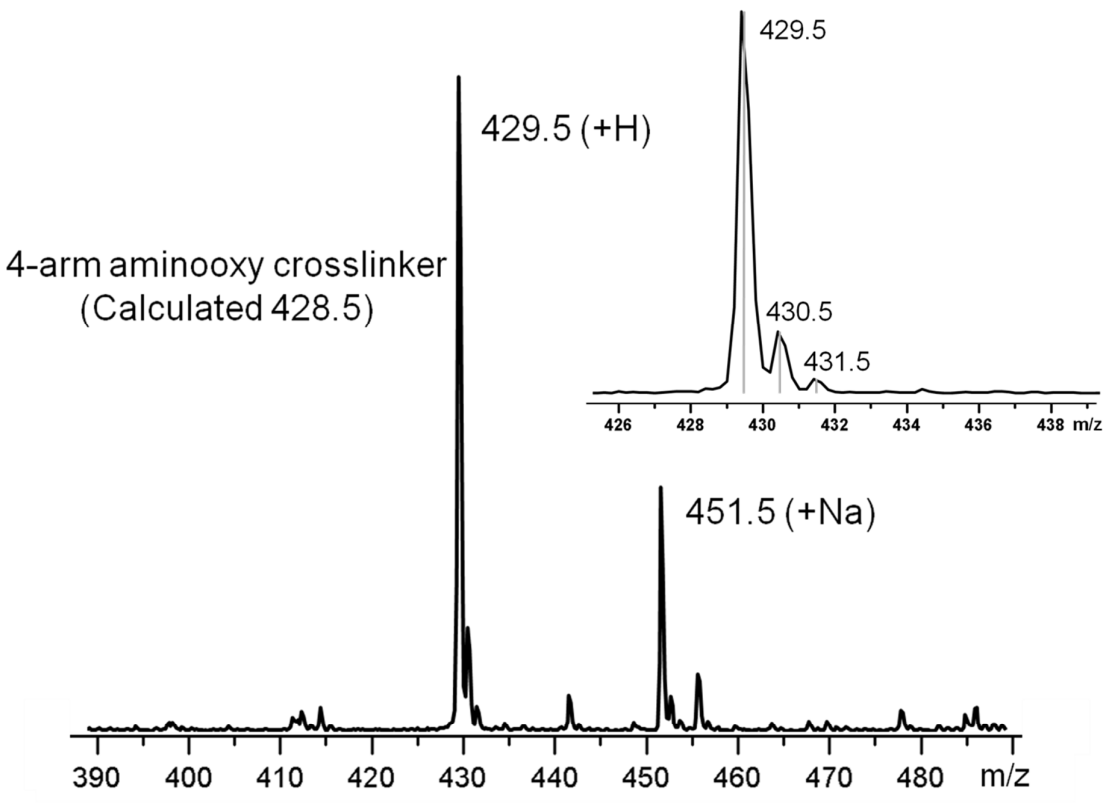


Figure S2. ESI spectrum of 4-arm aminooxy crosslinker.

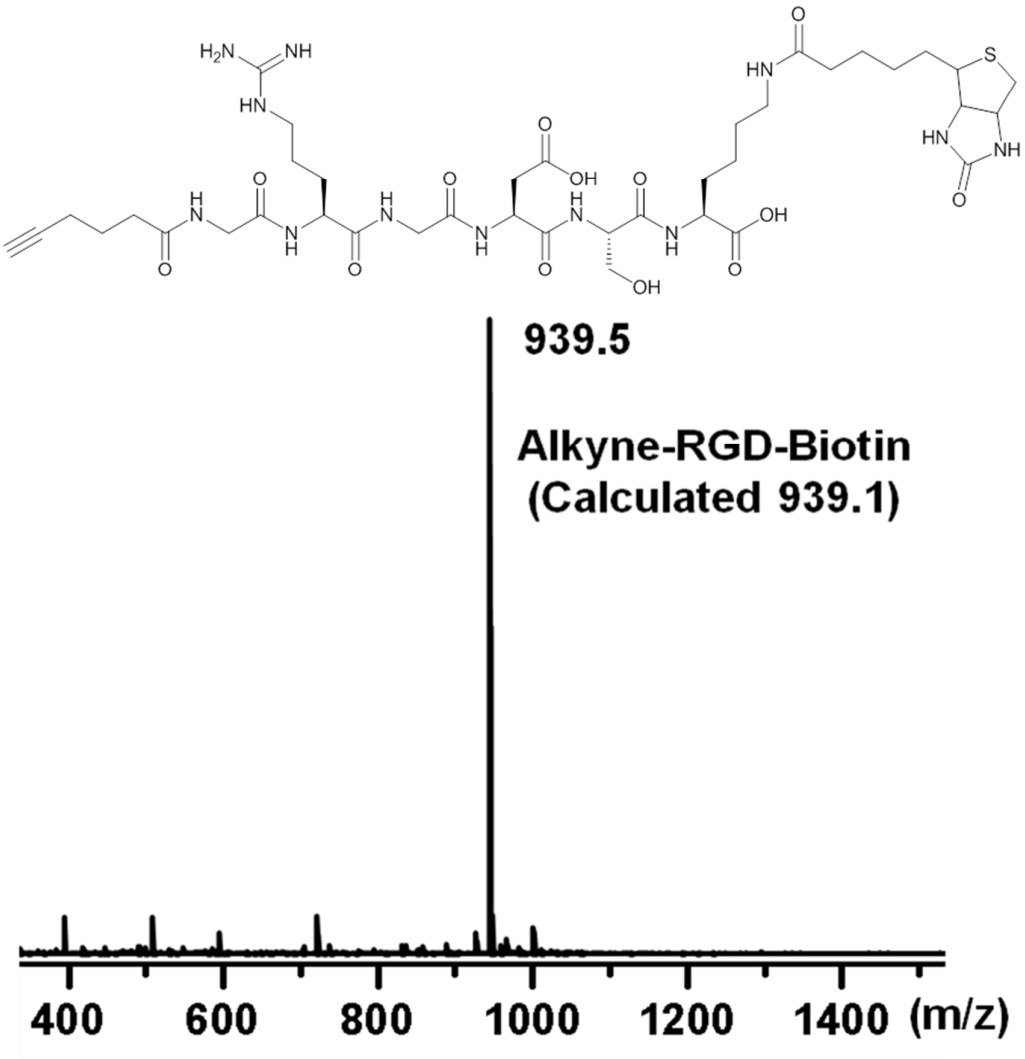


Figure S3. ESI spectrum of Alkyne-RGD-Biotin.

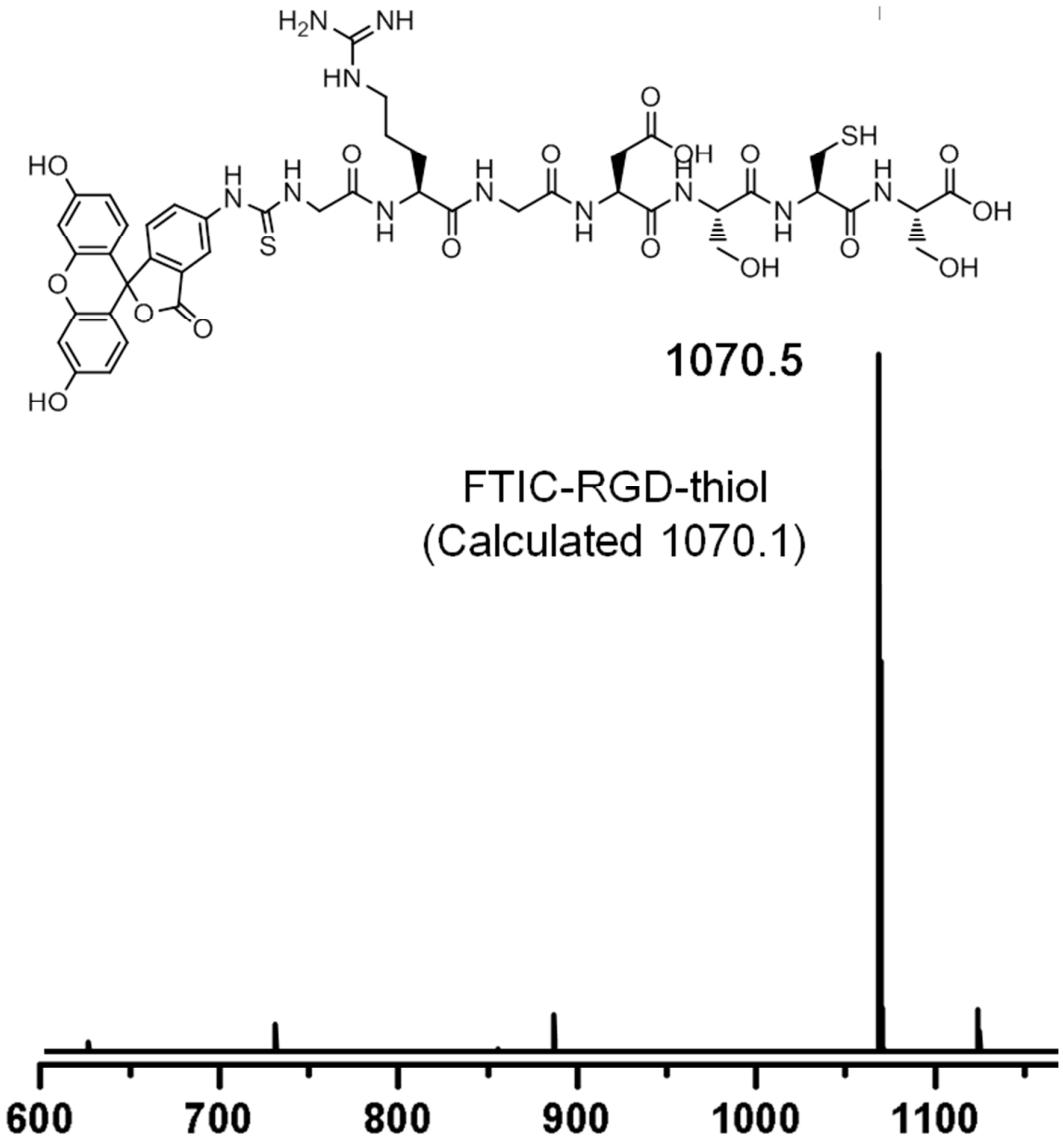


Figure S4. MALDI-TOF spectrum of FTIC-RGD-thiol.