Supplementary Information

Design of a selenylsulfide-bridged EGFR dimerization arm mimic

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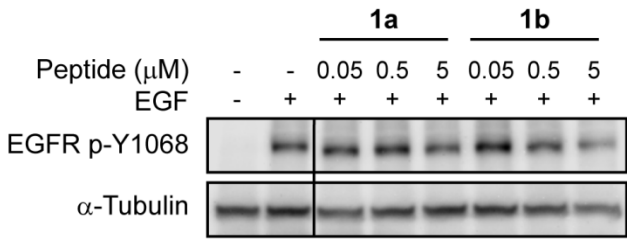
S1. Synthesis of peptides 1a-b

Peptides were synthesized on a 25 μmol scale using rink amide MBHA resin. Deprotections were performed in 25% piperidine in NMP for 25 min. All Fmoc-protected amino acids were coupled using 0.5 M amino acid (0.5 mL, 250 μmol, 10 equiv.), 0.5 M HCTU (0.495 mL, 247.5 μmol, 9.9 equiv.) and DIPEA (87 μL, 0.5 mmol, 20 equiv.) for at least 45 min. unless otherwise noted. Fmoc-Sec(PMB)-OH and Fmoc-12-amino-4,7,10-trioxadodecanoic acid were coupled using 0.5 M amino acid (0.2 mL, 0.1 mmol, 4 equiv.), 0.5 M HCTU (0.248 mL, 124 μmol, 4.96 equiv) (Peptides International), and DIPEA (43.5 μL, .250 μmol, 10 equiv.) for at least 1 hr. Peptides were labeled with 5(6)-carboxyfluorescein (19 mg, 50 μmol, 2 equiv.), HCTU (19 mg, 45 μmol, 1.8 equiv.) and DIPEA (20 μL, 115 μmol, 4.6 equiv.) in DMF overnight. The disulfide peptide was cleaved in a solution of 95% TFA, 2.5 % triisopropylsilane, and 2.5% water for 4 hours. The selenylsulfide was cleaved in a solution of 97.5% TFA, 2.5% thioanisole, and DTNP (4 mg, 12.5 μmol, 0.5 equiv.) for 1.5 hr. Peptides were characterized by LC-MS and purified using reverse-phase HPLC with a gradient of 10-100% acetonitrile in water with 0.1% TFA. Disulfide **1a** molecular weight = 1765.4 (expected = 1763.9). Selenylsulfide **1b** products: selenylsulfide **1b** molecular weight = 1806.6 (expected 1810.8), methylated, reduced peptide molecular weight = 1827.6 (expected = 1826.8) and methylated dehydroalanine peptide = 1744.6 (expected = 1744.9).

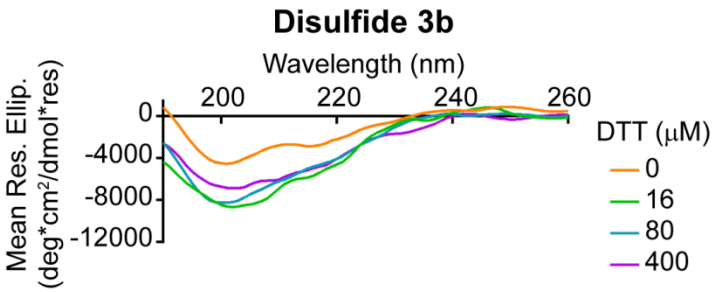
S2. EGFR phosphorylation assay with peptides 1a-b

MDA-MB-231 cells were seeded into 24 well plates in RPMI-1640 supplemented with 10% FBS and penicillin/streptomycin. Cells were allowed to adhere and grow to approximately 70% confluence then serum starved in RPMI-1640 containing 0.1% BSA and penicillin/streptomycin for 23.5 hours. Cells were pretreated with peptide for approximately 30 min. then stimulated with 50 ng/mL EGF for 5 min. Cells were immediately lysed in 1x Laemmli. Lysates were boiled 10 min. and proteins were separated by SDS-PAGE on an 8% gel. Western blotting was performed using PVDF membrane, and membranes were probed for EGFR p-Y1068 (Abcam) and α-Tubulin (University of Iowa). Bands were quantified using Licor Image Studio Lite.

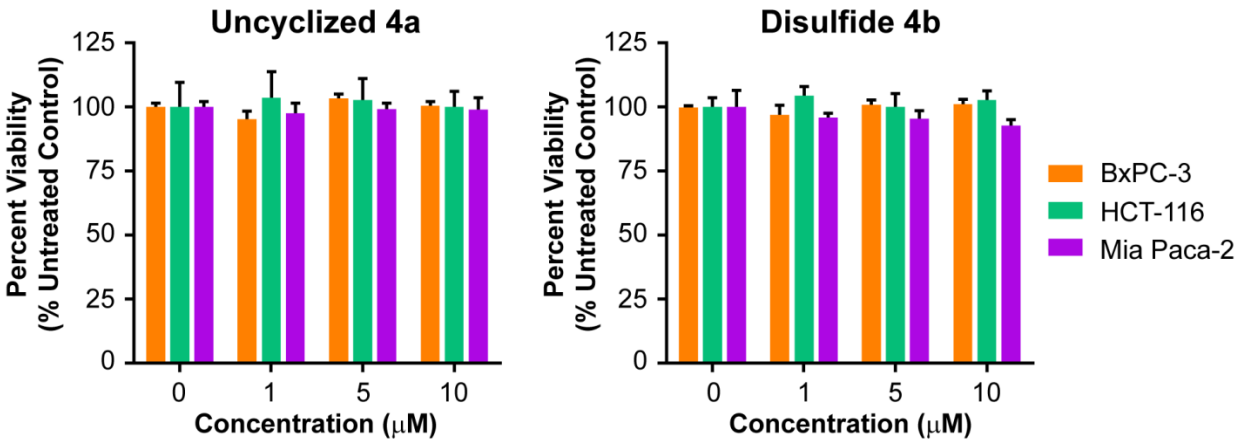
Peptide	Sequence
1a	**FAM-PEG-**C Y N P T T Y Q Nle C
1b	**FAM-PEG-**U Y N P T T Y Q Nle C



Supplementary Figure S1. Disruption of phosphorylation by dimerization arm mimics **1a** and **1b**. Sequences of peptides **1a** and **1b** are listed in the top panel (U = selenocysteine, FAM = 5(6)-carboxyfluorescein, and PEG = 12-amino-4,7,10-trioxadodecanoic acid). Serum-starved MDA-MB-231 cells were pretreated with peptide **1a** or **1b** for 30 min, then stimulated with 50 ng/mL EGF for 5 min. Proteins were separated by SDS-PAGE on an 8% gel and western blot analysis was performed. Treatment with both the disulfide and selenylsulfide products resulted in a notable decrease in phosphorylated EGFR. Vertical lines indicate non-adjacent bands from the same membrane image. Images are representative of three experiments.



Supplementary Figure S2. Disulfide **3b** CD Spectra. Circular dichroism spectra were obtained for the disulfide peptide with different concentrations of DTT in 10 mM sodium phosphate buffer, pH 7.0 at 25 ºC.



Supplementary Figure S3. MTT toxicity assays for uncyclized **4a** and disulfide **4b**. BxPC-3, HCT-116 or MiaPaca-2 cells were treated with peptide for 6 hours then incubated with MTT reagent for 2 hours. The formazan crystals were solubilized in DMSO for 15 min and absorbance was measured at 570 nm. There was no apparent toxicity for either peptide over the range of concentrations tested. For each peptide a two-way ANOVA was performed across all cell lines with Tukey's multiple comparisons test. While none of the means differed significantly from the untreated control ($p > 0.5$), there was a significant difference ($p < 0.5$) between HCT-116 and Mia Paca-2 cells treated with 1 and 10 μM disulfide **4b**.