Supporting Information

Fluorescence probe with a pH-sensitive trigger

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Experimental Procedures

Automated peptide synthesis: Peptide synthesis was performed on an automated peptide synthesizer (ABI 433A, Applied Biosystems, Foster City, CA) employing the traditional Fmoc methodology using FastMoc 0.1 MonPrevPk protocol. Synthesis was started with Fmoc-Gly-wang resin (150 mg, ~0.1 mmol). All amino acids (10 equiv) were attached to the resin by stepwise elongation with HOBt (10 equiv), HBTU (10 equiv), and N,N-diisopropylethylethylamine (DIPEA, 20 equiv) as coupling reagents in the presence of N-methylpyrrolidone (NMP; 15 mL). The Fmoc protecting groups were removed by 20 % (v/v) of piperidine in NMP (15 mL).

Coupling of 3,3'-dithiodipropionic acid: 3,3'Dithiodipropionic acid (0.5 mmol) and HOBt (0.5 mmol) were dissolved in 4 mL 9:1 DCM/DMF. The solution was added to the resin (0.1 mmol, 0.66 mmol/g) and shaken for 10 min. To the resin suspension, solution of DCC in DCM (0.5 mmol in 0.1 mL) was added and the suspension was shaken for 3 h.

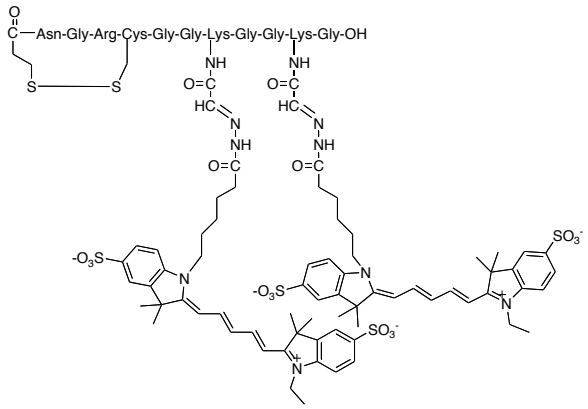
Coupling of Boc-Ser-OH: Boc-Ser-OH was purchased (EMD Biosciences, San Diego, CA) as its dicyclohexylamine (DCHA) salt. Free Boc-Ser-OH was generated by dissolving 1 mmol of Boc-Ser-OH.DCHA in 1.5 mL DCM and extracting this solution (3 × 3mL) with ice-cold aqueous solution (0.3 M) of $KHSO_4$. Following extraction and solvent evaporation, 1 mmol of Boc-Ser-OH, HBTU and HOBt were dissolved in 3 mL of 1:1 DMF/DCM followed by the addition of 2 mmol of DIPEA. The solution was then immediately added to the resin (0.1 mmol) without any preactivation to avoid possible racemization. Four hours later, the ninhydrin test confirmed quantitative coupling.

Periodate oxidation of the diserine peptide: Purified peptide (4.8 mg, 3.84 μmol) was dissolved in 75 μL of 10 mM phosphate buffer (pH 7.0). If required, pH of the solution was further adjusted using 0.1 N NaOH. To this, 120 μL of 140 mM $NaIO_4$ solution (2.2 equivalents) in 10 mM phosphate buffer (pH 7.0) was added. The solution was sonicated for 10 sec and further incubated for 17 min. The reaction mixture was then injected into the preparative RP-HPLC column and the fraction corresponding to the aldehyde peptide was collected.

Coupling of oxidized peptide: The aldehyde Peptide (0.4-0.5 mg, ~ 0.37 μmol) was dissolved in 50 μL of 10 mM phosphate buffer solution of pH 7.0; and 0.7 mg of the Cy5-hydrazide (0.89 μmol, 1.2 equivalents, purchased from Amersham Biosciences NJ, USA) was dissolved in 100 μL of DMSO. The dye solution was mixed with the peptide solution and incubated for 16 h. The reaction mixture was then passed through RP-HPLC (buffer A: 0.004% TFA in water, buffer B: 0.004% TFA in acetonitrile, C18 analytical column, Vydac, 218TP54, flow rate 1 ml/min. 100% buffer A to 100% buffer B in 50 min) and the fraction corresponding to CY5G2 (retention time 23.56 min) was collected.

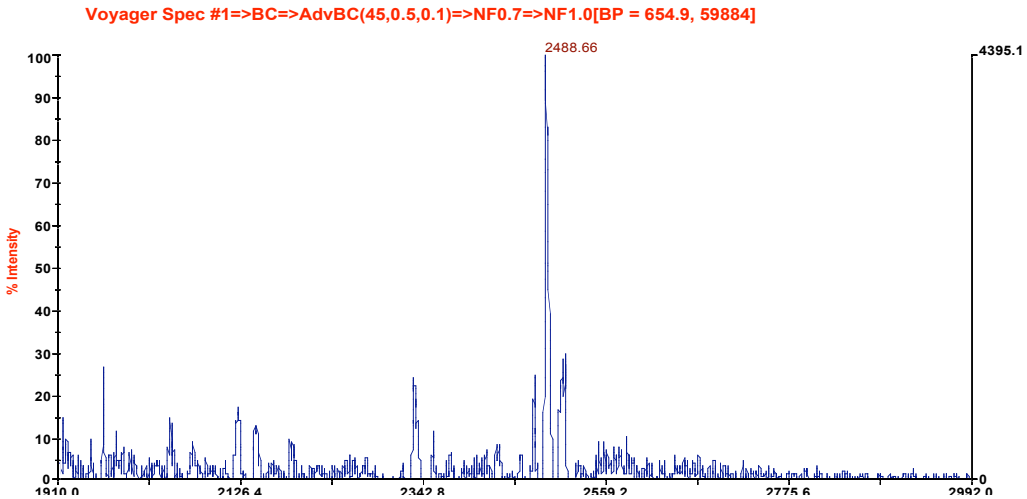
Fluorescence measurement: All the fluorescence measurements were performed in a 96-well assay plate 3603 (Corning Inc. NY USA). Fluorescence measurements were obtained in a fully modular monochromator-based microplate detection system (Safire^2, Tecan, San Jose, CA). Excitation and emission were set at 640 nm and 665 nm, respectively, with the bandwidth of 10 nm and the gain value of 40. Fluorescence emission was monitored at 37^0C.

Compound: CY5G2



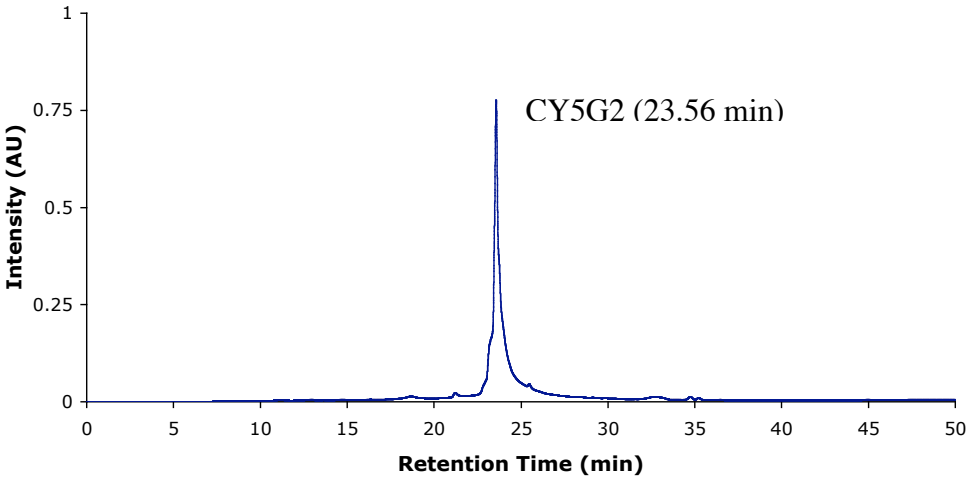
MALDI-TOF mass spectrogram:

MASS EXPECTED: 2488.91
MASS FOUND: 2488.66



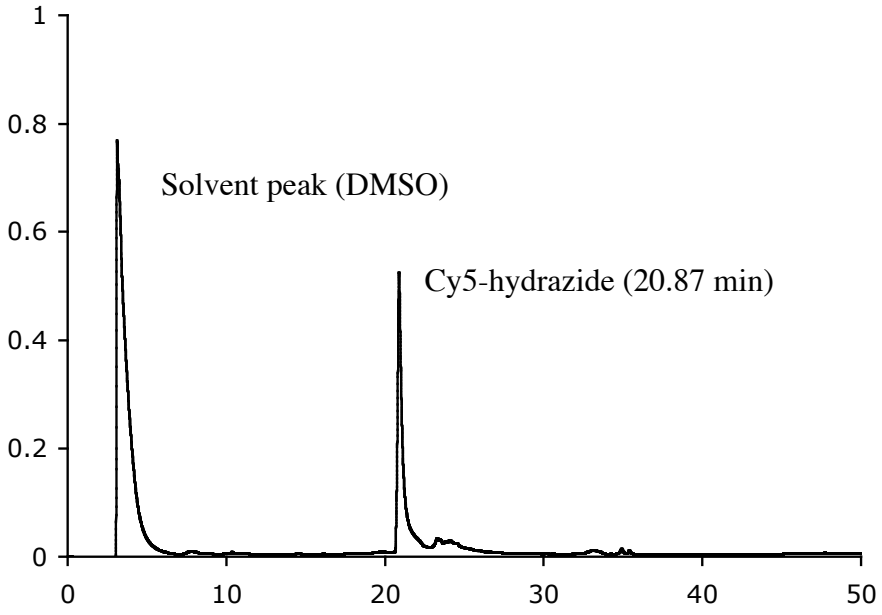
MALDI-TOF conditions: Instrument: Voyager-DE PRO (PerSeptive Biosystems), Instrument mode: Negative, VOLTAGES: Accelerating: 20000, Grid: 95, Guide Wire: 0.05, Delay Time: 100 nsec.
NOTE: Partial cleavage of the hydrazone linkage was observed under MALDI conditions (alpha-cyano-4-hydroxycinnamic acid as a matrix and 0.1% trifuoroacetic acid in 50% acetonitrile) homogeneity and purity of CY5G2 was confirmed using RP-HPLC (see below)

RP-HPLC Chromatogram

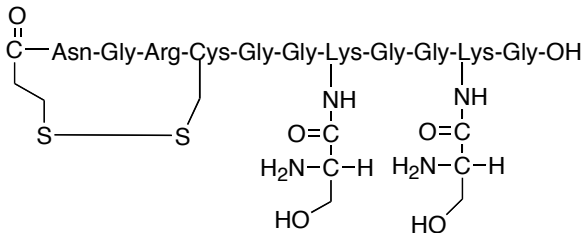


RP-HPLC conditions: Solvent A: 0.004% TFA in water, Solvent B: 0.004% TFA in acetonitrile, C18 analytical column,Vydac, 218TP54, flow rate 1 ml/min. 100% solvent A to 100% solvent B in 50 min.

Under the above-mentioned HPLC conditions, the hydrazide functionalized dye (Cy5-hydrazide) showed retention time of 20.87 min.

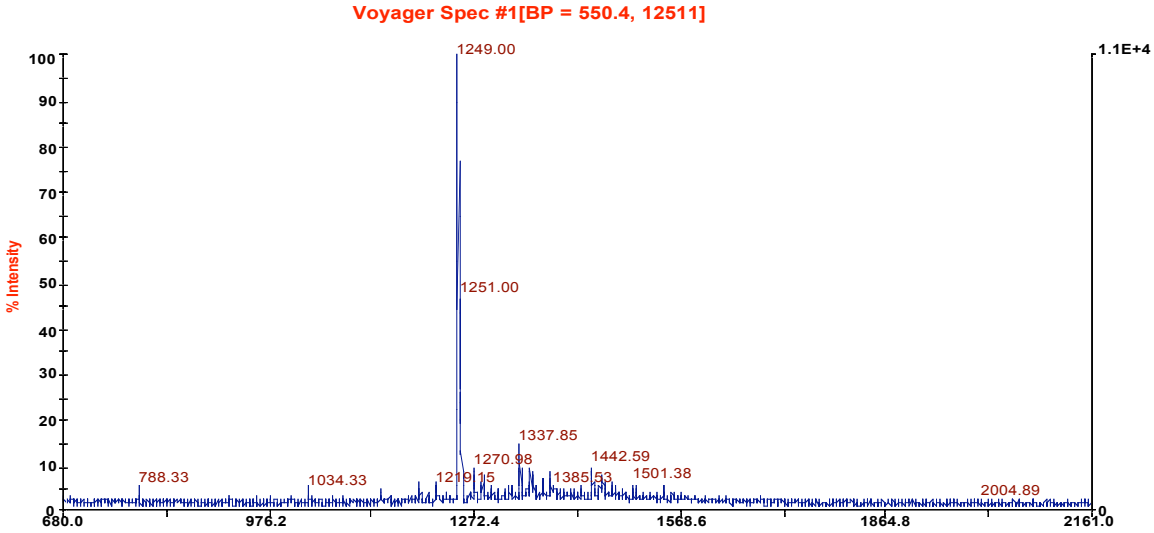


Compound: Diserine peptide

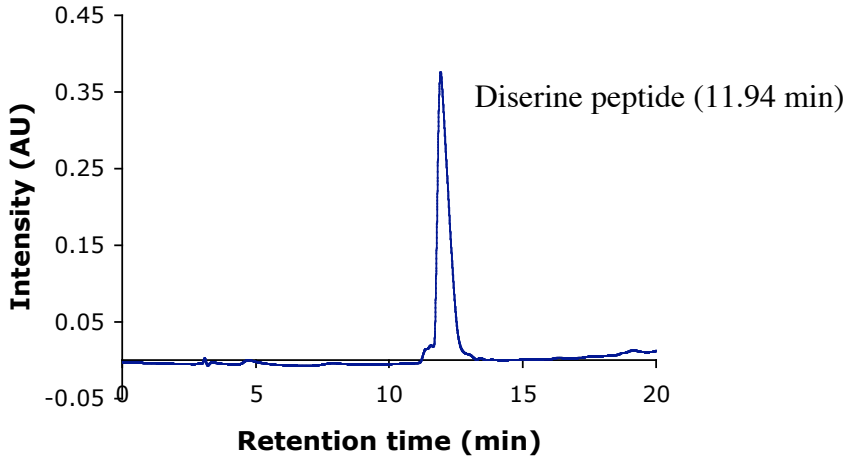


MALDI-TOF mass spectrogram

MASS EXPECTED: 1250.53
MASS FOUND: 1249.00



RP-HPLC Chromatogram



RP-HPLC conditions: Solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in acetonitrile, C18 analytical column,Vydac, 218TP54, flow rate 1 ml/min. 100% solvent A to 100% solvent B in 50 min.