Unbiased selection of peptide-peptoid hybrids specific for lung cancer compared to normal lung epithelial cells

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Supporting Information

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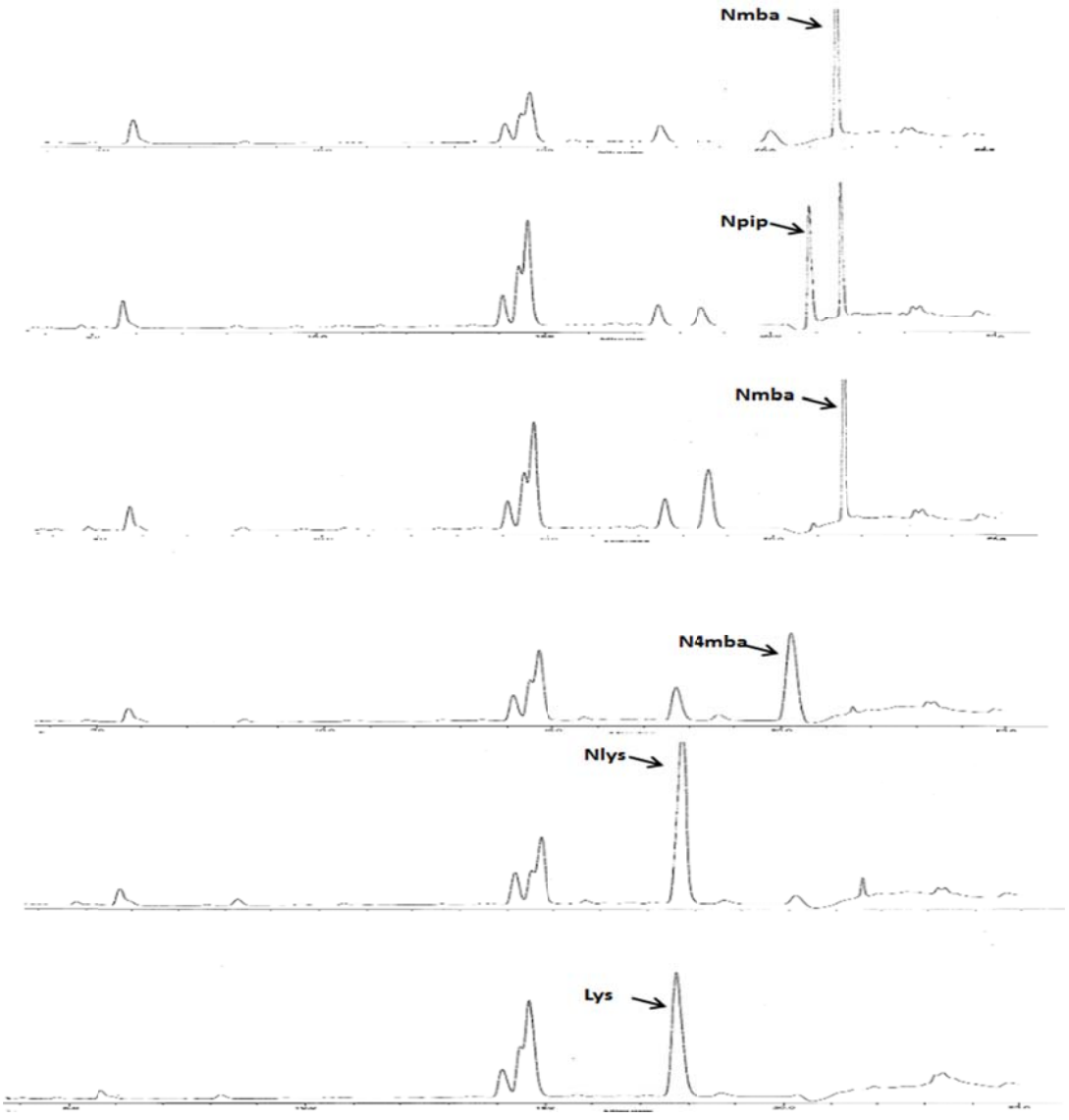
S1. List of 12 amino acids used in the library

1. Lysine
2. Leucine
3. Valine
4. Phenylalanine
5. Asparagine
6. Glutamine
7. Aspartic acid
8. Glutamic acid
9. Histidine
10. Serine
11. Threonine
12. Glycine

S2: Edman sequencing graphs of PPS1 structure elucidation



S3. Genetic data profiles of HCC4017 & HBEC30KT

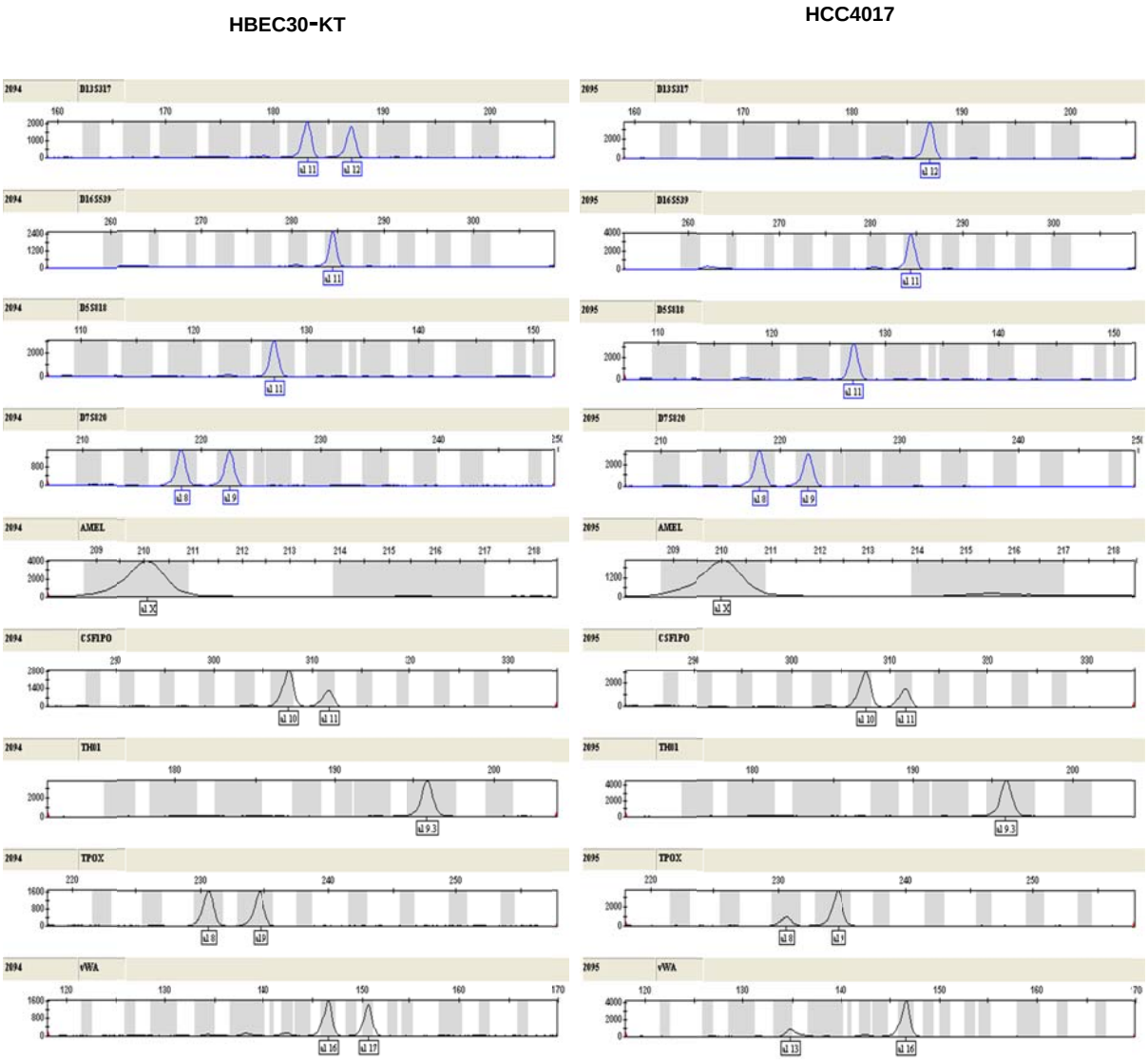


Figure S3 PowerPlex 1.2 STR Fingerprinting results for HBEC30-KT and HCC4017 showing identity at 7/9 markers. The remaining two markers DS13S317 and vWA show loss of heterozygosity (red lines) in the tumor derived cell line (HCC4017)

S4: Synthesis and characterization of control compound PC462

NovaSyn TGR resin (EMD Millipore, MA) 150 mg were swelled in dimethylformamide (DMF, Acros Organics, NJ) for 30 min at room temperature in a 5 ml reaction vessel (intavis AG, Germany). The reaction vessels were drained and treated with 2M Fmoc-Met-OH amino acid (with coupling agents HBTU, HOBt and DIPEA) in anhydrous DMF (Sigma-Aldrich, MO).Then the reaction vessel was placed on a shaker overnight, drained and washed with DMF (5 ml X 10 times). Fmoc group was removed by treating the beads with 20% piperidine (Sigma-Aldrich, MO) for 10 minutes twice on the shaker. After washing the reaction vessel, subsequent amino acids Fmoc-D-Lys(Boc)-OH and Fmoc-Gly-OH were added (for 2h reaction time) after removing Fmoc group as described above. Then the 5-mer peptoid region was synthesized using microwave assisted peptoid synthesis protocol. Reaction vessels were treated with 2M Bromoacetic acid in anhydrous DMF (1ml) and 2M DIC in anhydrous DMF (1ml), gently shaken for 30 seconds and microwaved (1000W) for 15 seconds with the power set at 10%. The beads were shaken again for about 15 seconds and microwaved another round as described above. The reaction vessel was then drained and washed with DMF (2ml x 10 times). Then the reaction vessel was treated with 1M solution of the primary amine (2 ml) and microwaved two times for 15 seconds after gentle shaking. The primary amines used were Allyamine and 2-Methoxyethylamine. At the end of synthesis the beads were washed with Dichloromethane (DCM) (2ml x 10 times), and the compound was cleaved with 2.5 ml of cleavage cocktail containing 95% Triflouoroacetic acid (TFA), 2.5% water and 2.5% Triisopropylsialine(TIS) on the shaker for 2 hours and compound was purified using HPLC. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems, MA).

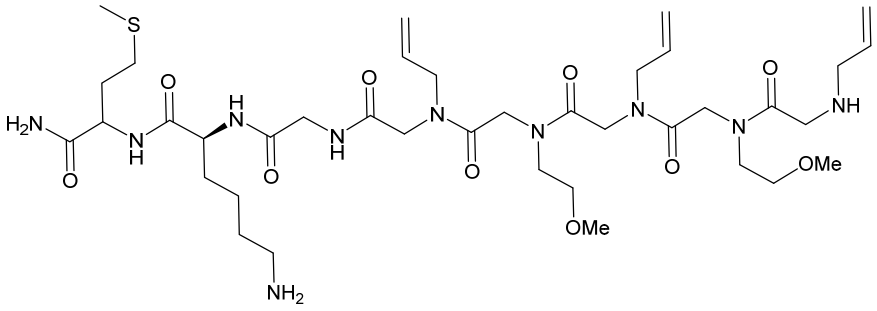


Figure S4.1 Chemical structure of PC462

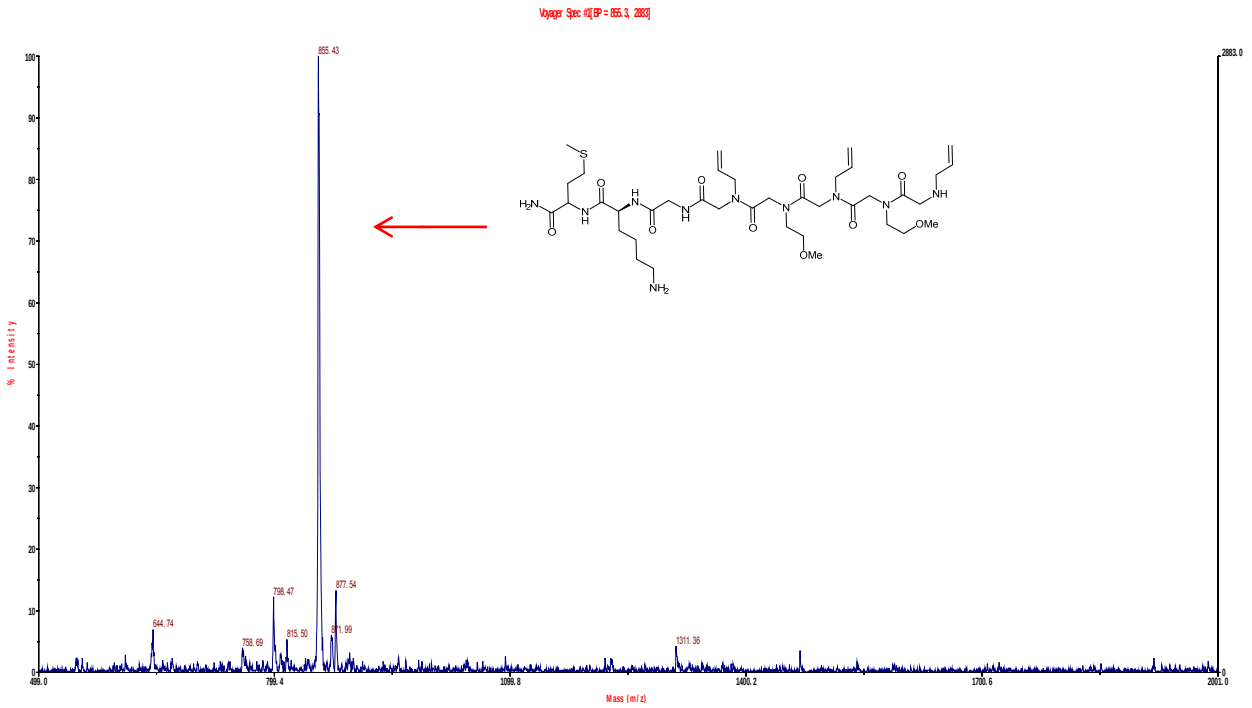


Figure S4.2 MALDI-TOF spectrum of PC462

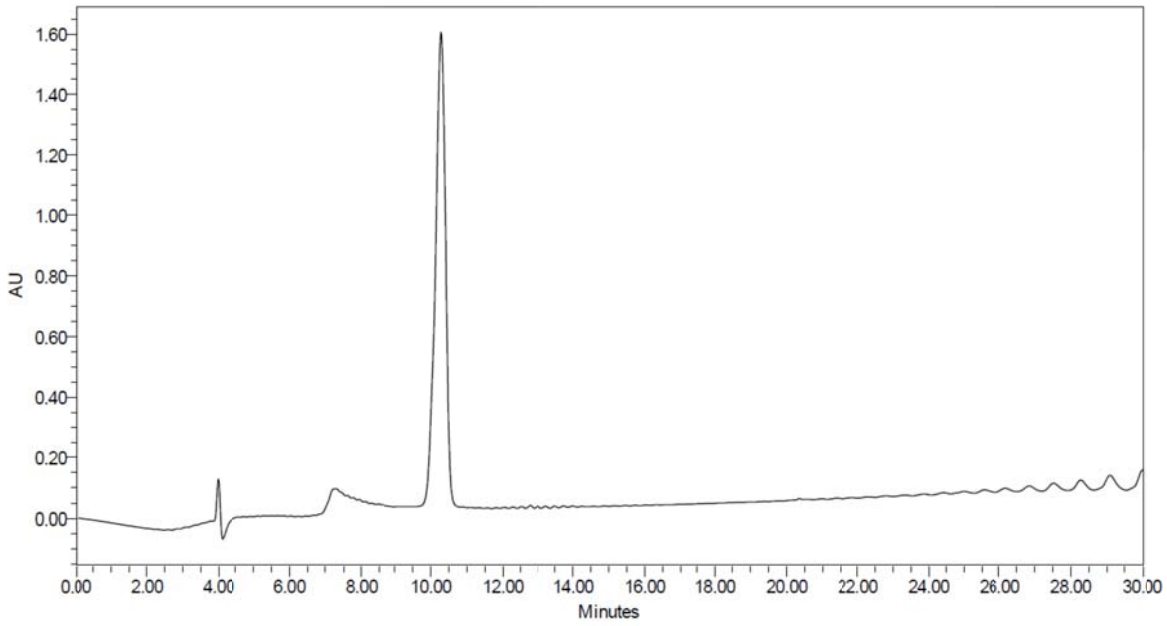


Figure S4.3 Analytical HPLC of PC462

S5: Synthesis and characterization of control compound PC462D1

PC462D1 was synthesized on NovaSyn TGR resin (EMD Millipore, MA).First, Fmoc-Lys(Fmoc)-OH was coupled overnight as the central linker, and both Fmoc groups were removed simultaneously allowing two copies of the sequence to be built on two amine groups of this central Lys. Then first three amino acids Fmoc-Met-OH, Fmoc–D-Lys(Boc)-OH and Fmoc-Gly-OH were loaded to the resin with removing Fmoc group each time. Then the 5-mer peptoid region containing Allyamine and 2-Methoxyethylamine was synthesized using microwave assisted peptoid synthesis protocol as described previously. At the end of synthesis the beads were washed with Dichloromethane (DCM) (2ml x 10 times), and peptoid was cleaved with 2.5 ml of cleavage cocktail containing 95% Triflouoroacetic acid (TFA), 2.5% water and 2.5% Triisopropylsialine(TIS) on the shaker for 2 hours and compound was purified using HPLC. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems).

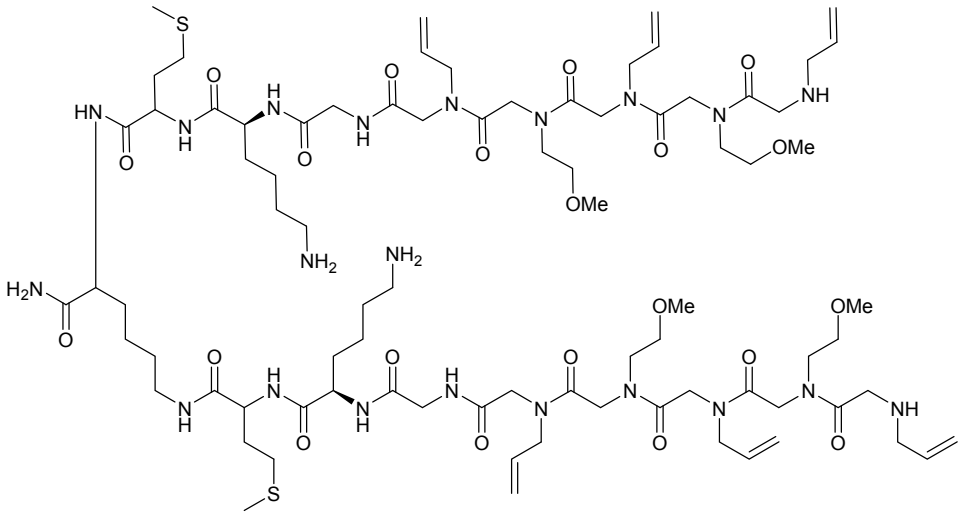


Figure S5.1 Chemical structure of PC462D1

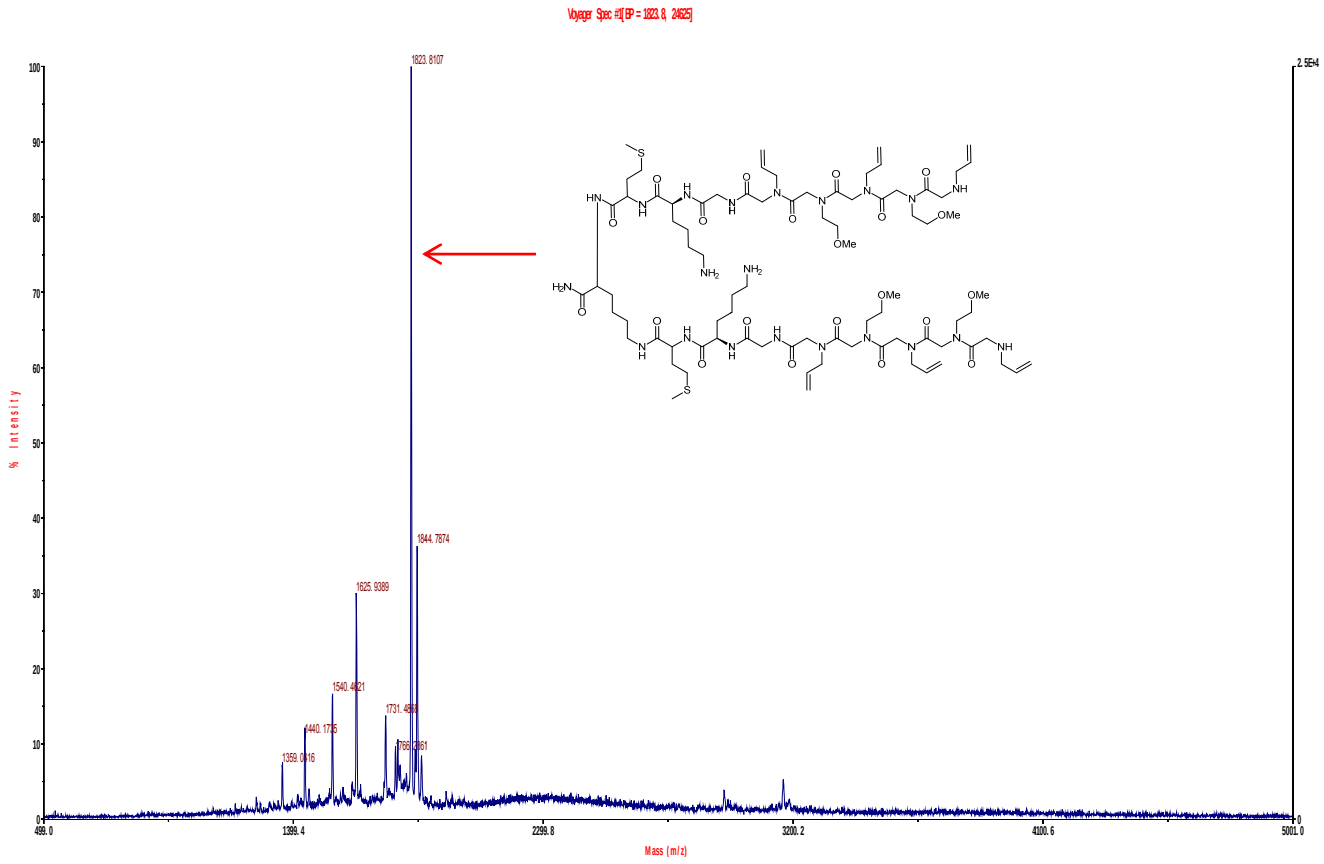


Figure S5.2 MALDI-TOF spectrum of PC462D1

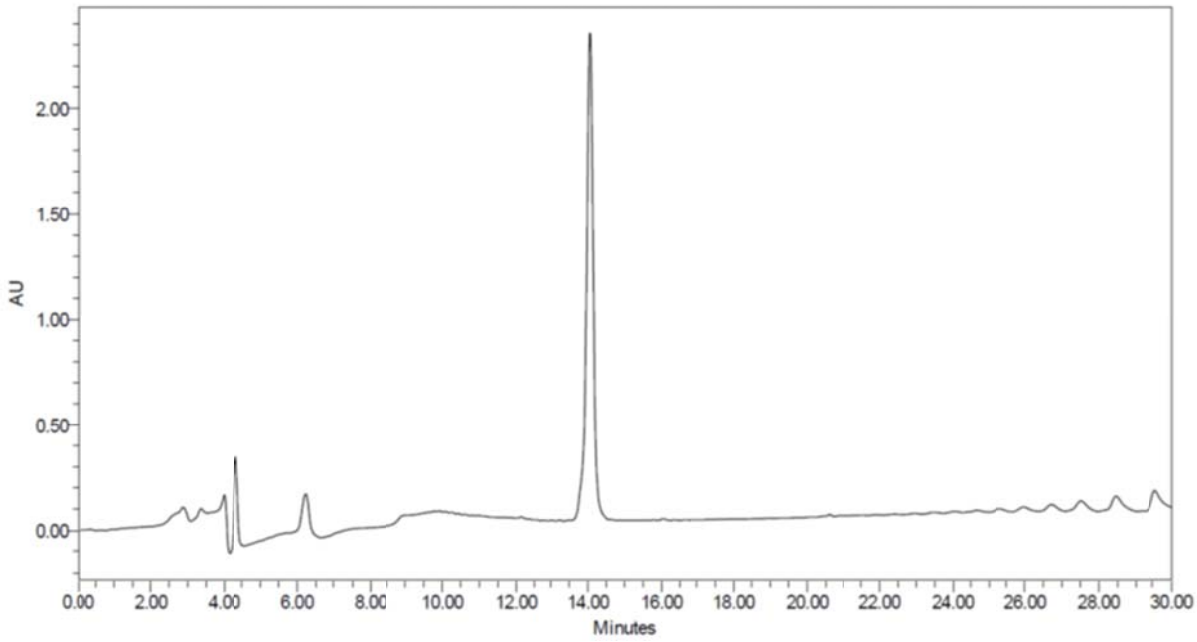


Figure S5.3 Analytical HPLC of PC462D1

S6. Synthesis and characterization of PPS1

PPS1 was synthesized on: (I) TentaGel beads (Rapp polymere, Germany) for on-bead cell binding assay, and (II) NovaSyn TGR resin (EMD Millipore, MA) for all the other assays. First three amino acids, Fmoc-Met-OH, Fmoc–D-Lys(Boc)-OH and Fmoc-Lys(Boc)-OH were loaded to the resin after Fmoc removal each time. Then 5-mer peptoid region containing Boc-Diaminobutane, 4-methoxybenzylamine, (R)-Methylbenzylamine, Piperonylamine and (R)-Methylbenzylamine was completed using microwave assisted peptoid synthesis protocol. At the end of synthesis the beads were washed with Dichloromethane (DCM) (2ml x 10 times), and the compound was cleaved with 2.5 ml of cleavage cocktail containing 95% Triflouoroacetic acid (TFA), 2.5% water and 2.5% Triisopropylsialine(TIS) on the shaker for 2 hours and compound was purified using HPLC. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems, MA).

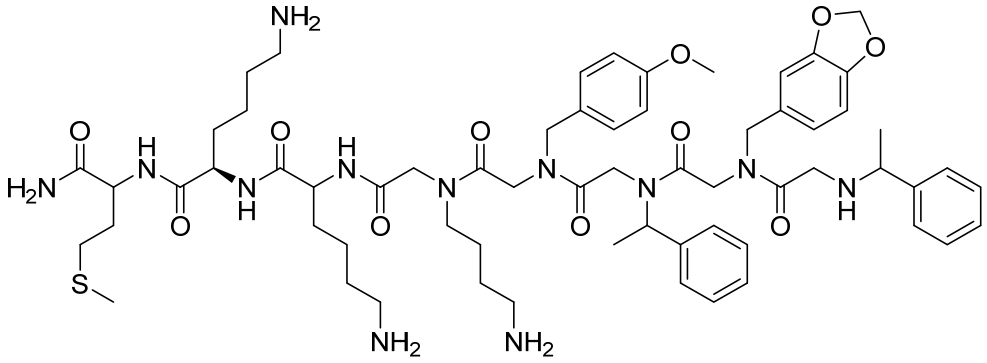


Figure S6.1 Chemical structure of PPS1

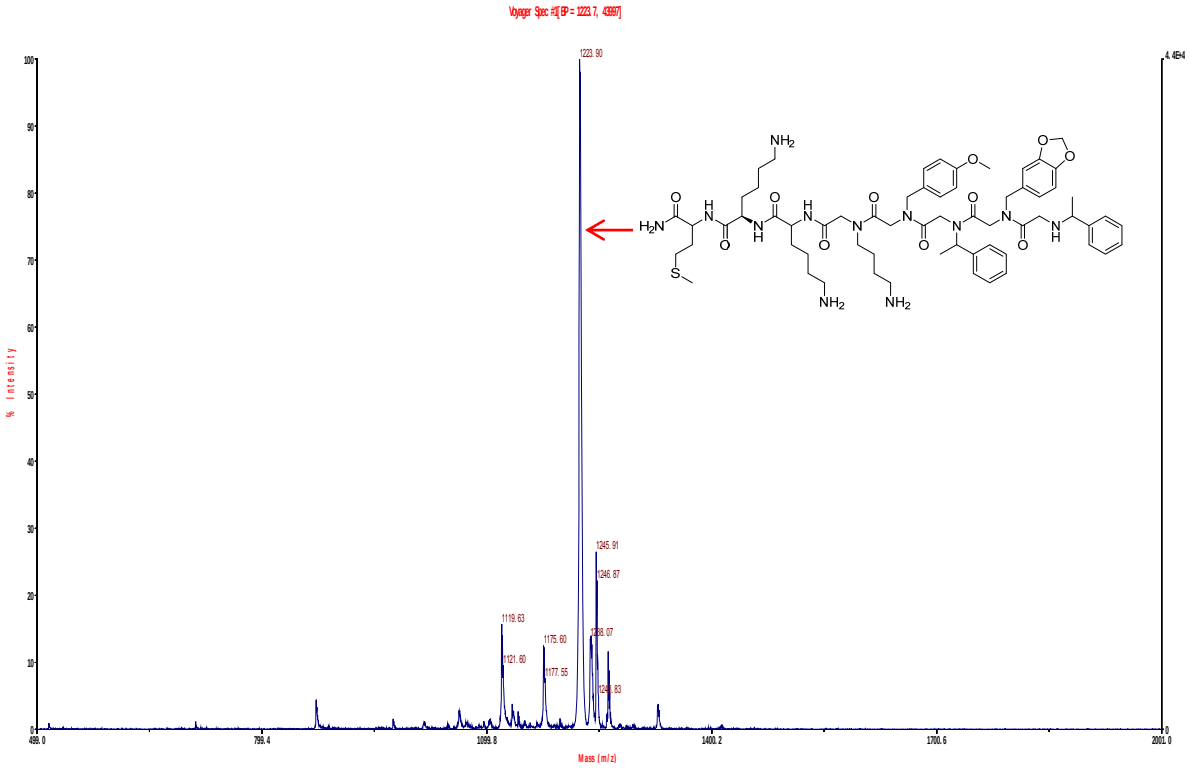


Figure S6.2: MALDI-TOF spectrum of PPS1

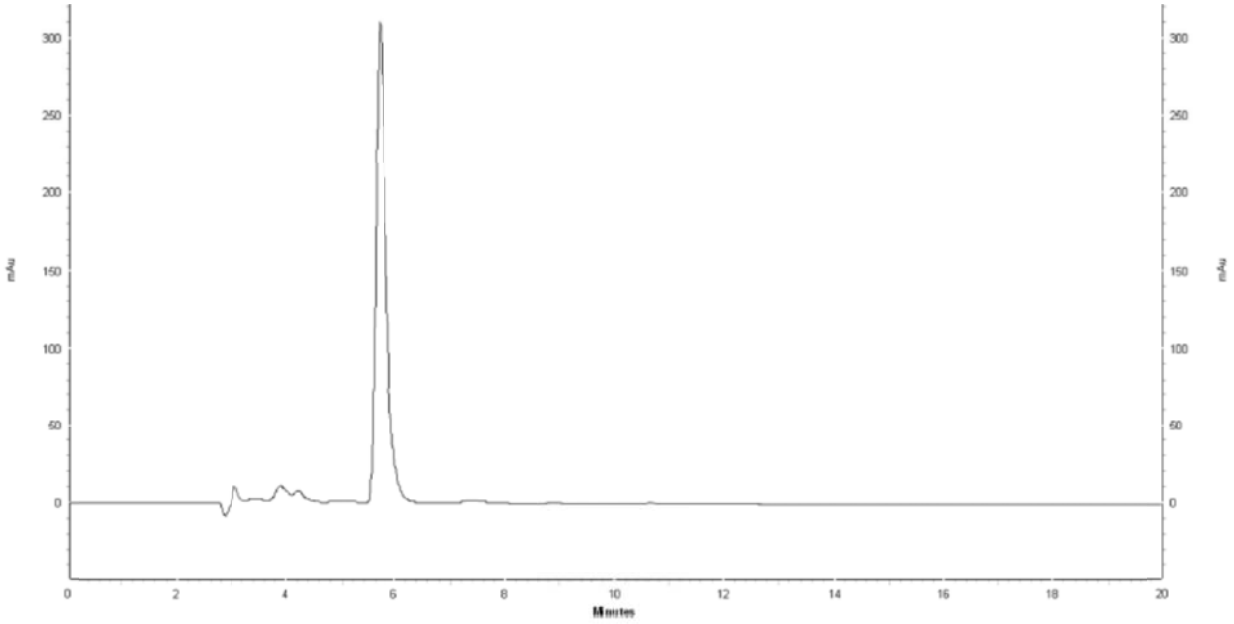


Figure S6.3: Analytical HPLC of PPS1

S7. Synthesis and characterization of PPS1D1

PPS1D1 was synthesized on NovaSyn TGR resin (EMD Millipore, MA). First, Fmoc-Lys(Fmoc)-OH was coupled overnight as the central linker, and both Fmoc groups were removed simultaneously allowing two copies of the sequence to be built on two amine groups of this central Lys. First three amino acids, Fmoc-Met-OH, Fmoc–D-Lys(Boc)-OH and Fmoc-Lys(Boc)-OH were loaded to the resin after Fmoc removal each time. Then 5-mer peptoid region containing Boc-Diaminobutane, 4-methoxybenzylamine, (R)-Methylbenzylamine, Piperonylamine and (R)-Methylbenzylamine was completed using microwave assisted peptoid synthesis protocol. At the end of synthesis the beads were washed with Dichloromethane (DCM) (2ml x 10 times), and the compound was cleaved with 2.5 ml of cleavage cocktail containing 95% Triflouoroacetic acid (TFA), 2.5% water and 2.5% Triisopropylsialine(TIS) on the shaker for 2 hours and compound was purified using HPLC. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems, MA).

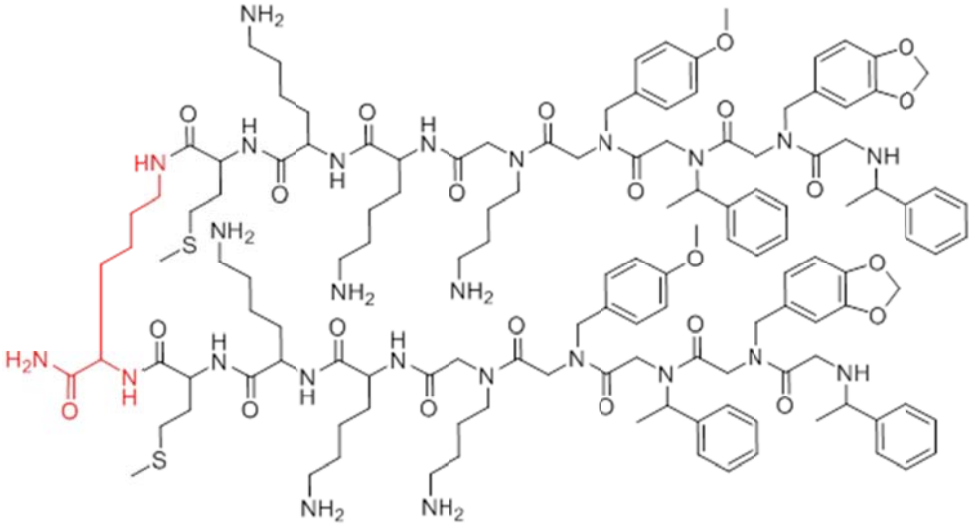


Figure S7.1 Chemical structure of PPS1D1

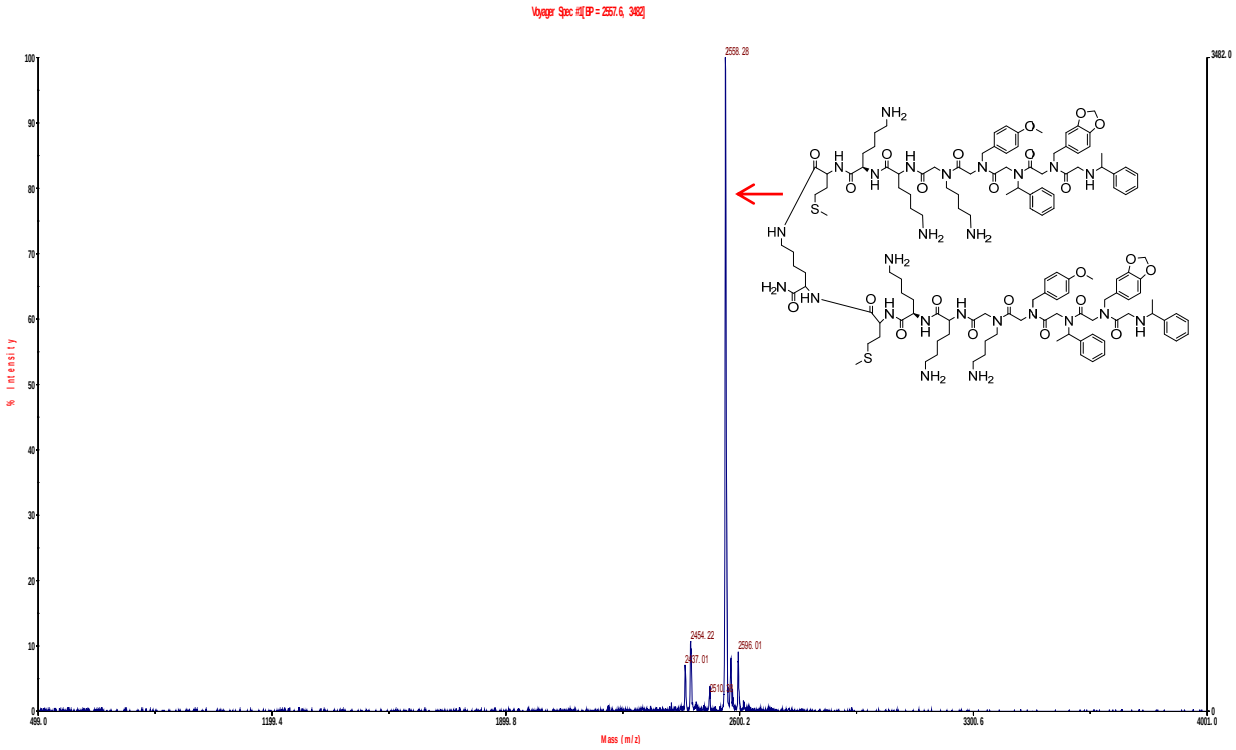


Figure S7.2: MALDI-TOF spectrum of PPS1D1

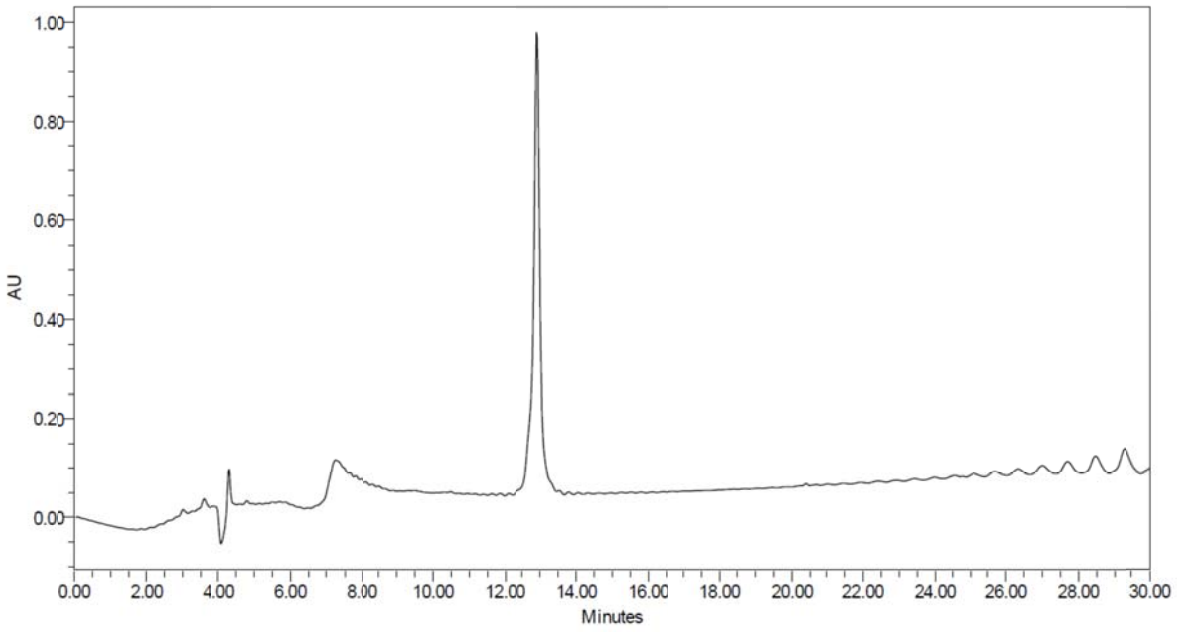


Figure S7.3 Analytical HPLC of PPS1D1

S8. Synthesis and characterization of FITC-PPS1

This synthesis was carried out on NovaSyn TGR resin (EMD Millipore, MA). Fmoc-Cys(Trt)-OH (HOBt, HBTU,DIPEA) was loaded as first amino acid on to the resin and the rest of the PPS1 synthesis was conducted as described previously. At the end 95% TFA, 2.5% water and 2.5% TIS mixture was used to cleave the compound from resin and to remove the side chain protection. Then the TFA was evaporated and resulting solid compound was dissolved in 1:1 water: Acetonitrile (ACN) mixture. This solution was subjected to HPLC purification using the solvent conditions starting from 100:0 water: ACN to 50:50 water: ACN. The purified compound was lyophilized to obtain the dry product. Fluorescein-5–maleimide (Thermofisher, MA) dissolved in DMSO was coupled to this compound (1M: 1 M ratio) in buffer solution at pH 7. The coupled FITC-PPS1D1 compound was purified with HPLC. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems, MA).

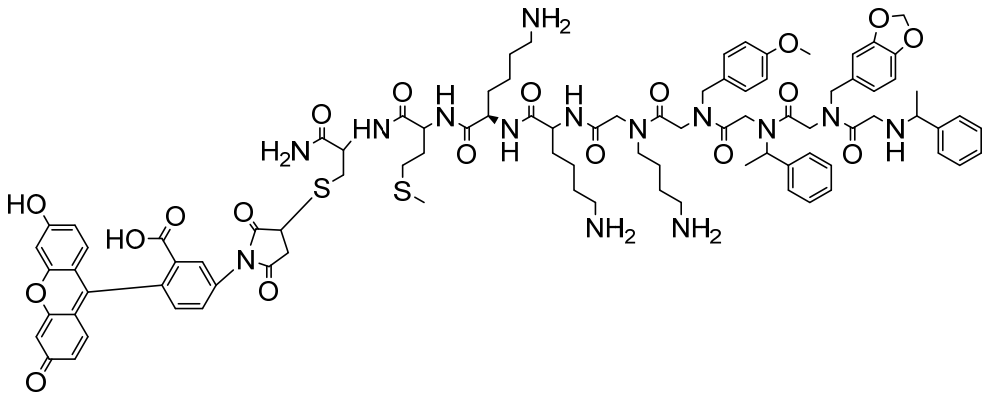


Figure S8.1 Chemical structure of FITC-PPS1

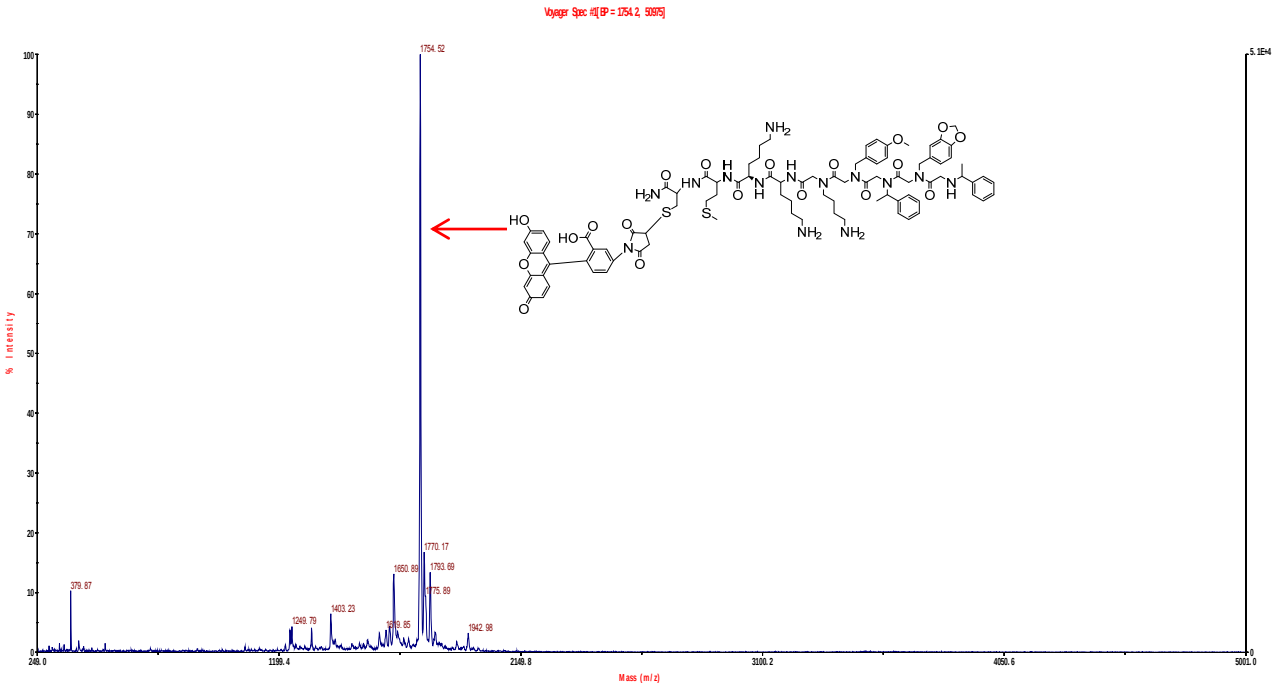


Figure S8.2: MALDI-TOF spectrum of FITC-PPS1

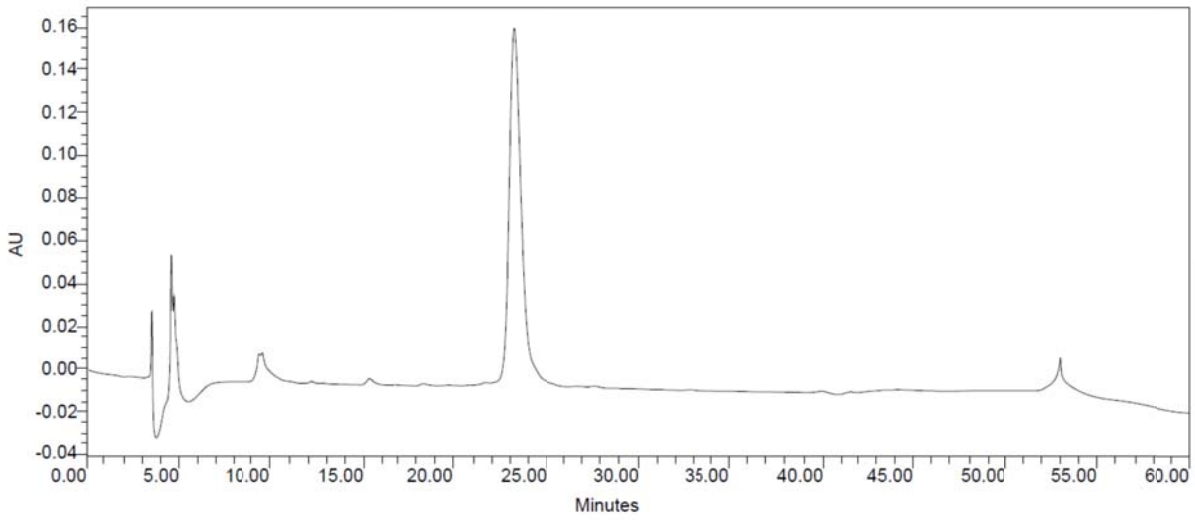


Figure S8.3: Analytical HPLC of FITC-PPS1

S9.Synthesis and characterization of biotinylated PPS1

This synthesis was carried out on NovaSyn TGR resin (EMD Millipore, MA). Fmoc-Cys(Trt)-OH (HOBt, HBTU,DIPEA) was loaded as first amino acid on to the resin and the rest of the PPS1 synthesis was conducted as described previously. At the end 95% TFA, 2.5% water and 2.5% TIS mixture was used to cleave the compound from resin and to remove the side chain protection. Then the TFA was evaporated and resulting solid compound was dissolved in 1:1 water: Acetonitrile (ACN) mixture. This solution was subjected to HPLC purification using the solvent conditions starting from 100:0 water: ACN to 50:50 water: ACN. The purified compound was lyophilized to obtain the dry product. Biotin-5–maleimide (Thermofisher, MA) dissolved in DMSO was coupled to this compound (1M: 1 M ratio) in buffer solution at pH 7. The coupled Biotinylated PPS1 compound was purified with HPLC. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems, MA).

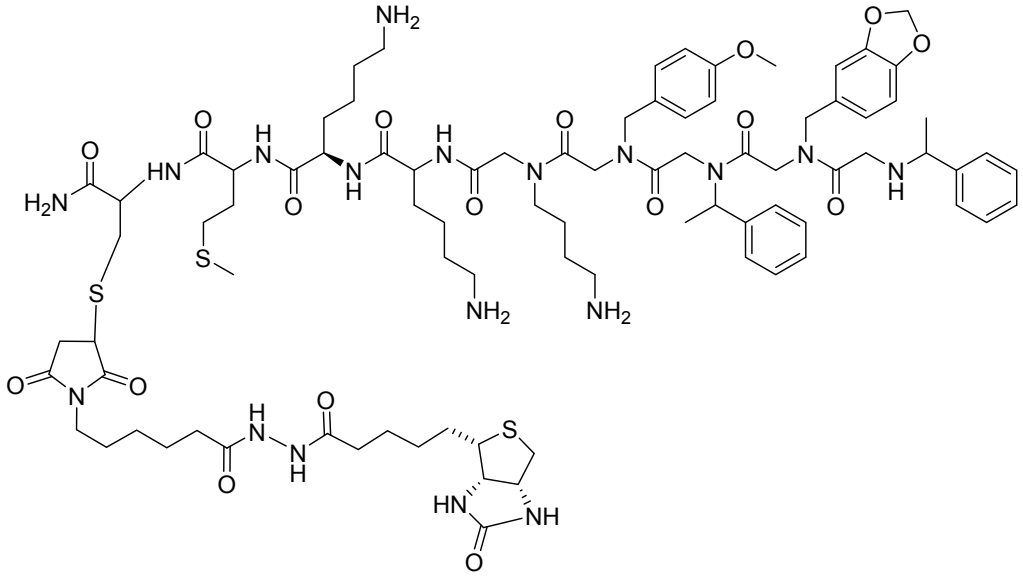


Figure S9.1 Chemical structure of biotinylated PPS1

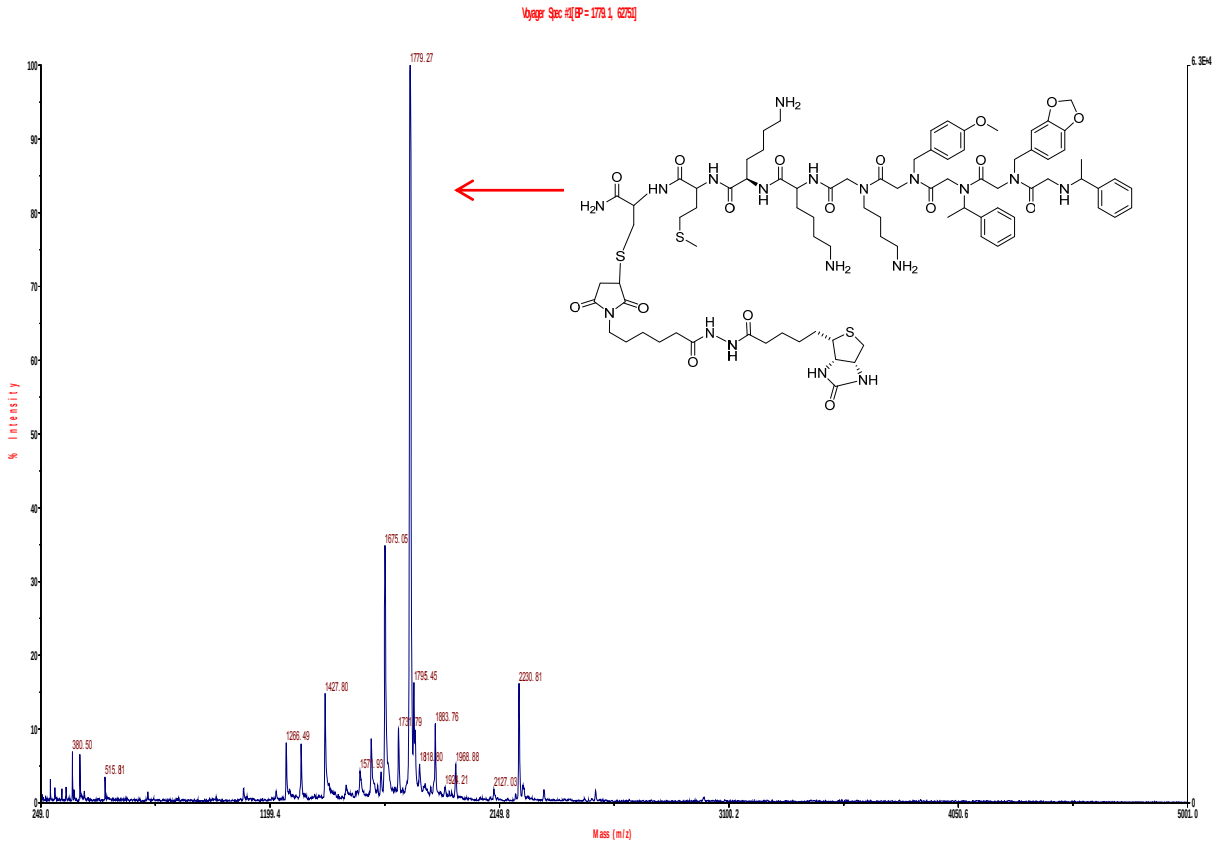


Figure S9.2: MALDI-TOF spectrum of biotinylated PPS1

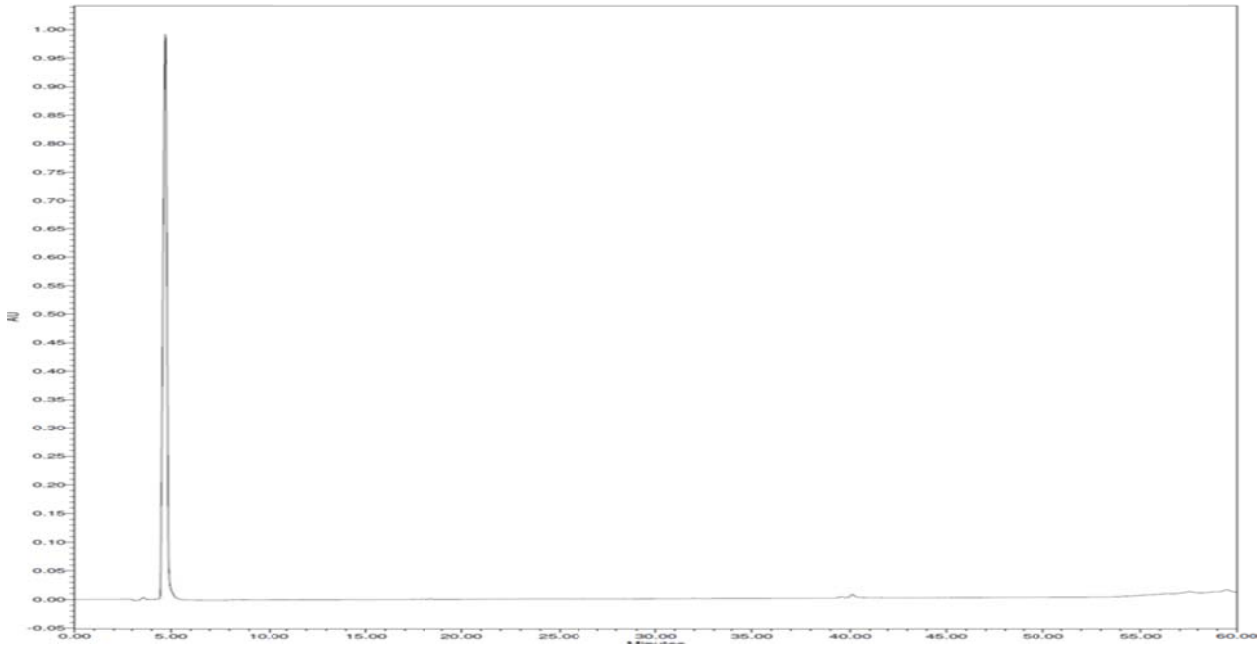


Figure S9.3: Analytical HPLC of biotinylated PPS1

S10. Synthesis and characterization of PPS1-(Eu^{3+})-DTPA

PPS1-(Eu^{3+})-DTPA was synthesized on NovaSyn TGR resin (EMD Millipore, MA). The PPS1 synthesis was conducted as described previously. The beads with compound PPS1 having secondary amine at the terminal were then coupled with 0.2M Fmoc-amino-ethyloxy-ethyloxy-acetyl (Fmoc-AEEAc-OH) using coupling reagent HBTU (0.2M) and HOBt (0.2 M) in presence of DIPEA (0.4M) in DMF (2 mL) at room temperature for overnight. After washing with DMF (2 mL x 10times), Fmoc group was removed by 20% piperidine solution in DMF [2 times x (2 mL x 10 min)]. The resulting free terminal amine was then coupled to DTPA using same peptide coupling reaction conditions. The beads were then treated with 95% TFA, 2.5% triisopropylsilane, and 2.5% water mixture for 4 h. This solution was subjected to HPLC purification using the solvent conditions starting from 100:0 water: ACN to 50:50 water: ACN. The purified compound was lyophilized to obtain the dry product. The metal complexation was then performed overnight with a 0.2 M EuCl3 solution at pH 6.3. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems, MA).

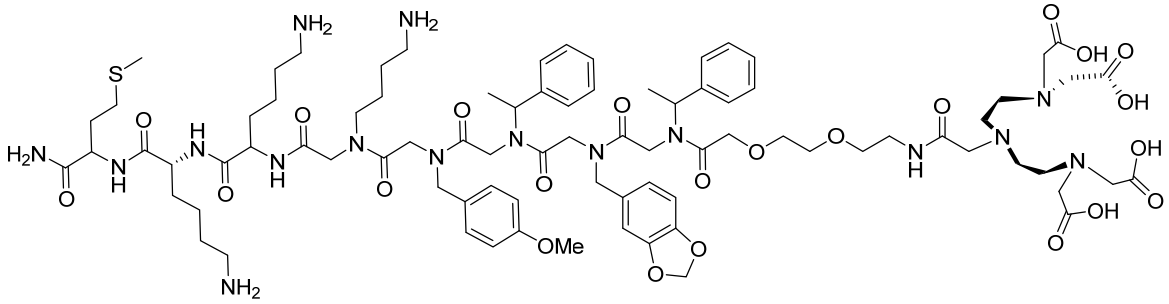


Figure S10.1 Chemical structure of PPS1-(Eu^{3+})-DTPA

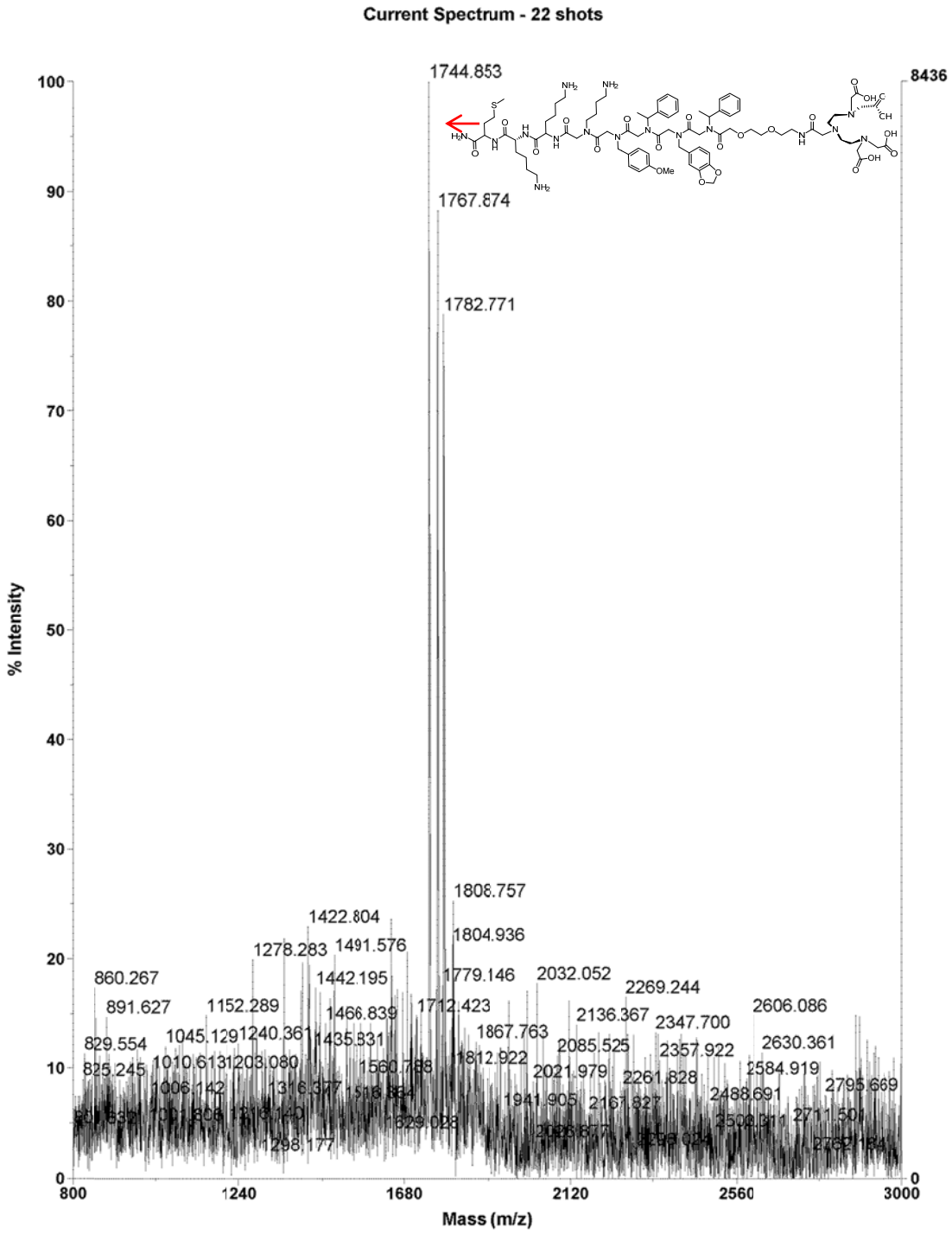


Figure S10.2: MALDI-TOF spectrum of PPS1-(Eu^{3+})-DTPA

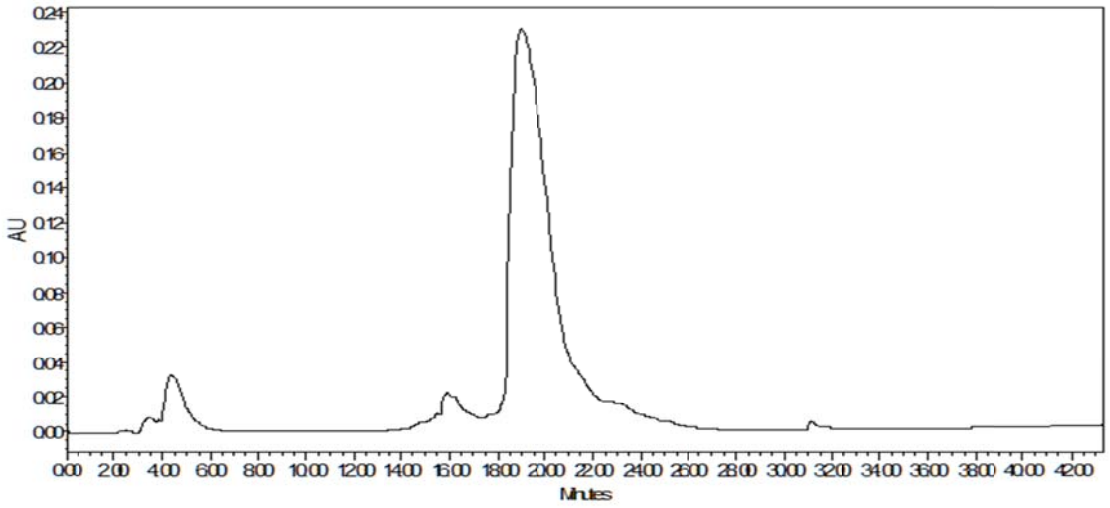


Figure S10.1 Analytical HPLC of PPS1-(Eu3+)-DTPA

S11. Synthesis and characterization of scramble PC2 on tentagel beads

This synthesis was carried out on TentaGel beads (Rapp polymere, Germany) for on-bead cell binding assay. First, amino acid Fmoc-Met-OH was coupled overnight and Fmoc group was removed. Then the 4-mer peptoid region containing 4-Methoxybenzylamine, (R)-Methylbenzylamine, Boc-Diaminobutane and Piperonylamine was synthesized using microwave assisted peptoid synthesis protocol as described previously. Then amino acid Fmoc-D-Lys(Boc)-OH was coupled overnight followed by Fmoc-Lys(Boc)-OH after Fmoc removal. In the end, peptoid (R)-Methylbenzylamine was added through microwave assisted synthesis described previously. At the end of synthesis, the beads were washed with Dichloromethane (DCM) (2ml x 3 times), and treated with 2.5 ml of 95% Trifluoroacetic acid (TFA), 2.5% water and 2.5% Triisopropylsilane (TIS) on the shaker for 2 hours to remove the side chain protection. Reaction vessel was drained, washed with DMF (2ml x 3 times) and stored in anhydrous DMF at 400C. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems) after cyanogen bromide cleavage.

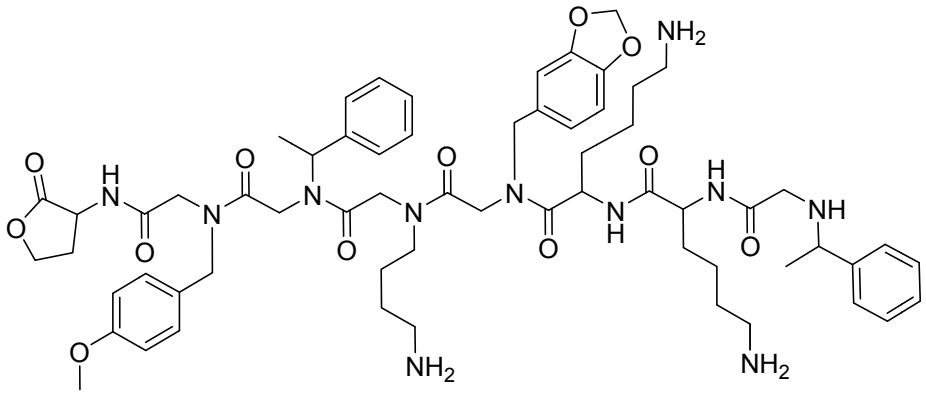


Figure S11.1 Chemical structure of PC2

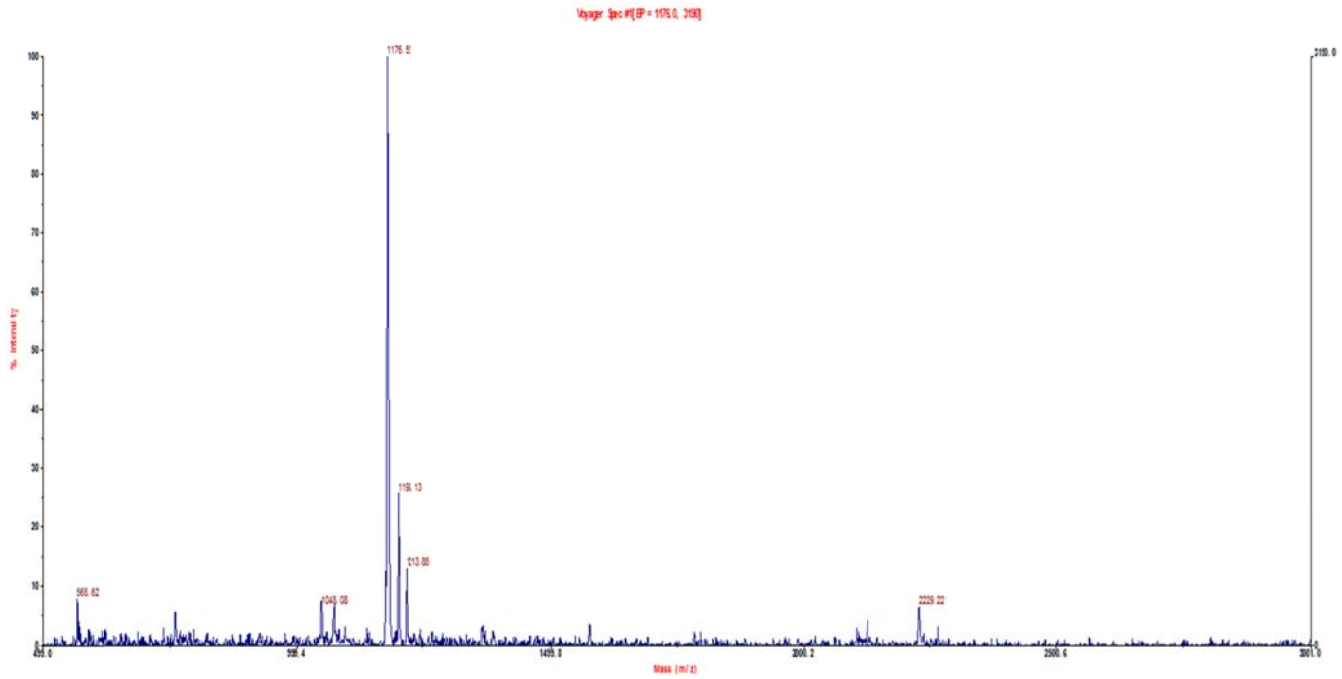


Figure S11.2 MALDI-TOF spectrum of PC2

S12. Synthesis and characterization of scramble PC2D1 for MTS

PC2D1 was synthesized on NovaSyn TGR resin (EMD Millipore, MA).First, Fmoc-Lys (Fmoc)-OH was coupled overnight as the central linker, and both Fmoc groups were removed simultaneously allowing two copies of the sequence to be built on two amine groups of this central Lysine. Then first amino acid Fmoc-Met-OH was loaded to the resin after removing Fmoc group. Then the 4-mer peptoid region containing 4-Methoxybenzylamine, (R)-Methylbenzylamine, Boc-Diaminobutane and Piperonylamine was synthesized using microwave assisted peptoid synthesis protocol as described previously. Then amino acid Fmoc-D-Lys(Boc)-OH was coupled overnight followed by Fmoc-Lys(Boc)-OH after Fmoc removal. In the end, peptoid (R)-Methylbenzylamine was added through microwave assisted synthesis described previously. At the end of synthesis the beads were washed with Dichloromethane (DCM) (2ml x 10 times), and peptoid was cleaved with 2.5 ml of cleavage cocktail containing 95% Trifluoroacetic acid acid (TFA), 2.5% water and 2.5% Triisopropylsilane (TIS) on the shaker for 2 hours and compound was purified using HPLC. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems).

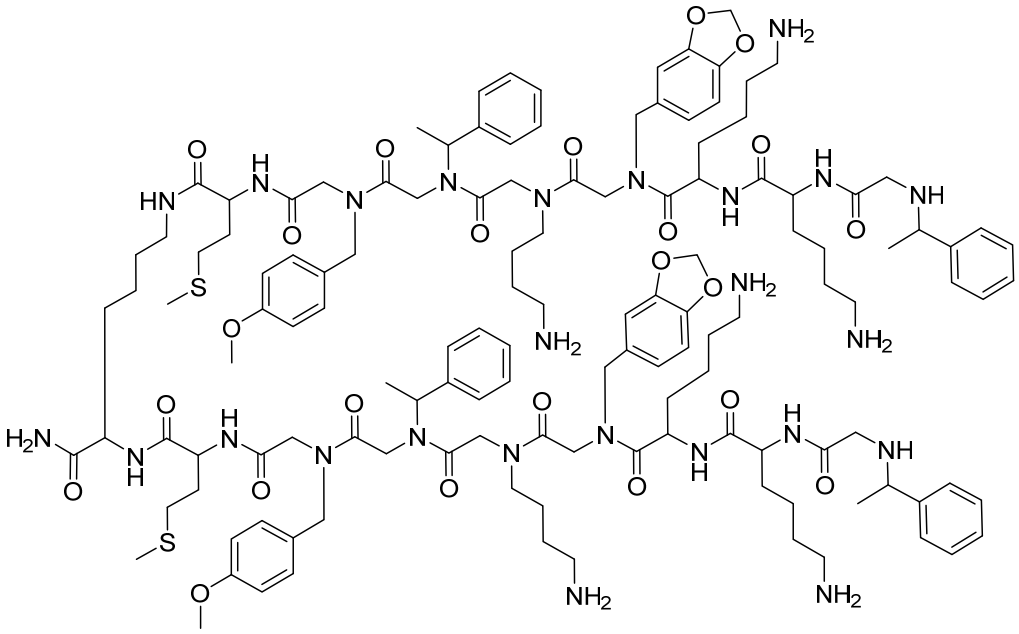


Figure S12.1 Chemical structure of PC2D1

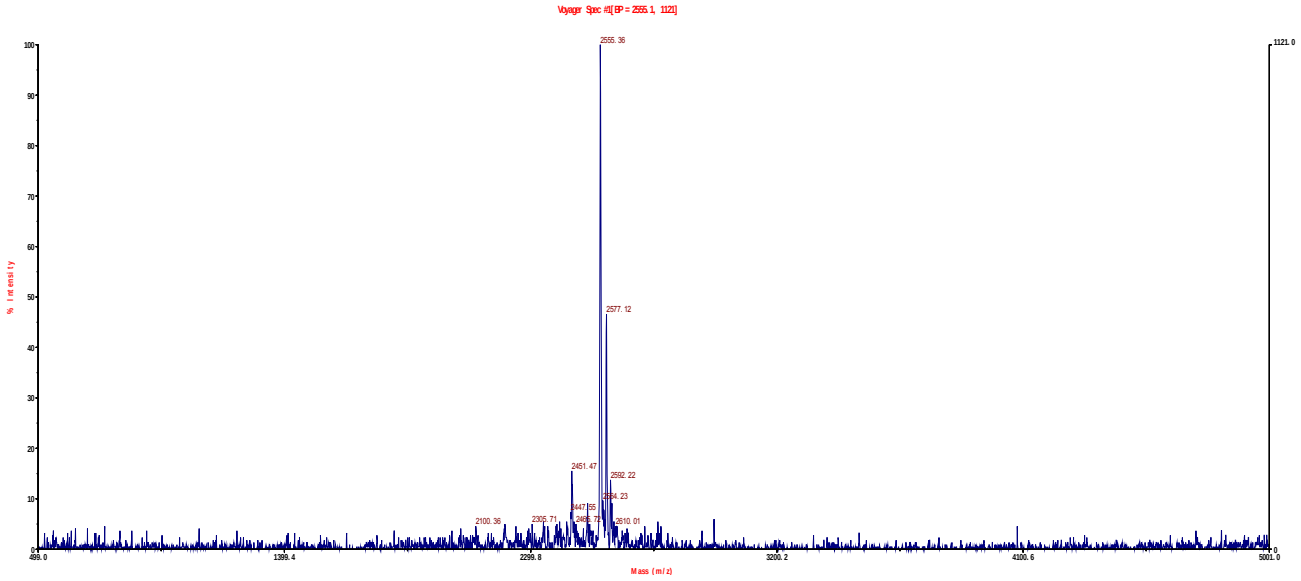


Figure S12.2 MALDI-TOF spectrum of PC2D1

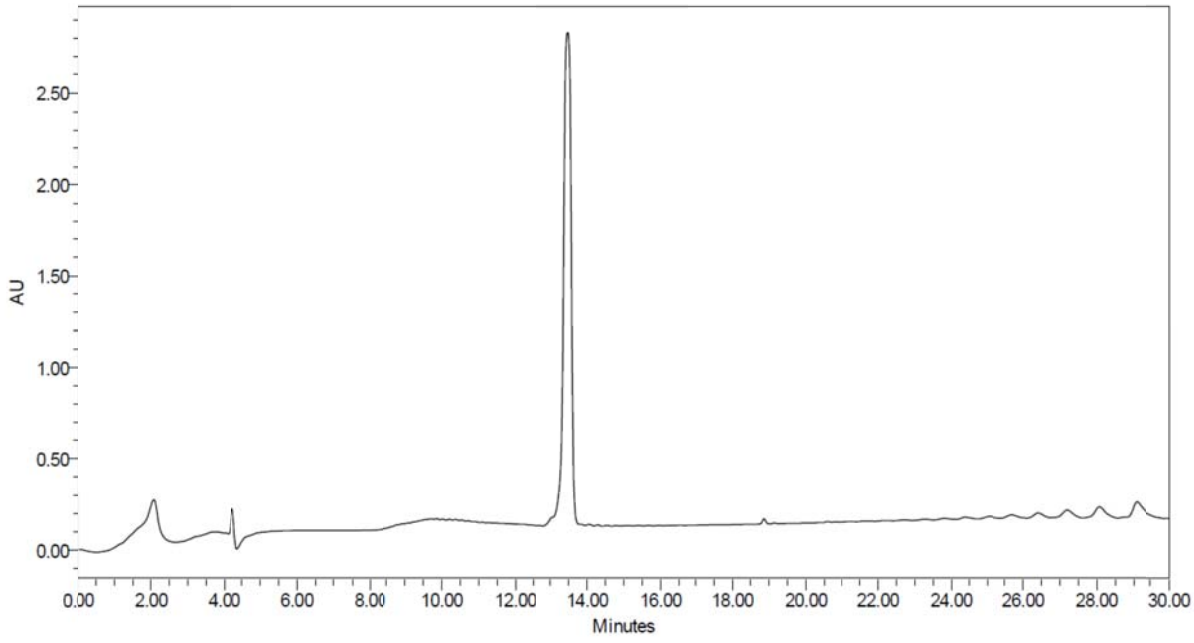


Figure S12.3 Analytical HPLC of PC2D1

S13: Compound synthesis

All the amino acids were purchased from EMD Millipore, MA and all the primary amines from Sigma-Aldrich, MO. O-Benzotriazole-N, N, N', N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and N – Hydroxybenzotriazole H_2O (HOBt) were purchased from AnaSpec, CA. Applied Biosystems Voyager DePro MALDI mass spectrometer was used in positive reflector mode to acquire MALDI-TOF mass spectra. Alpha-Cyano-4-hydroxycinnamic acid (Sigma Aldrich, MO) was used as matrix. HPLC purification was performed in a Waters 1525 Binary HPLC pump connected to Waters 2487 Dual λ Absorbance Detector using Protein & Peptide C18 300A°, 22 X 250 mm, 10 micron column from Grace Davison Discovery Sciences. Compound separation was carried out at room temperature using Acetonitrile (ACN; Honeywell, NJ) and water containing 0.1% Trifluoroacetic acid (TFA; Sigma Aldrich, MO).

S14: Library synthesis

The basic structure of the library consists of three amino acids followed by 5-mer diversified peptoid region. TentaGel macrobeads 2g (140-170μm; substitution: 0.48mmol/g resin; Rapp Polymere, Germany) were swelled in extra pure dimethylformamide (DMF, Acros Organics, NJ) for 30 min at room temperature in a 5 ml reaction vessels (intavis AG,Germany). The reaction vessels were drained and treated with premixed 0.4M Fmoc-Met-OH amino acid (Sigma-Aldrich, MO) and 0.4M HBTU in anhydrous DMF containing 0.8M N-methyl morphaline (NMM, 12ml, Sigma–Aldrich, MO). Then the reaction vessels were placed on a shaker for overnight, after which they were drained and washed with DMF (5 ml X 10 times). Fmoc group was removed by treating the beads with 20% piperidine (Sigma-Aldrich, MO) for 10 minutes twice on the shaker. After washing the resins, subsequent amino acid Fmoc-D-Lys(Boc)OH was added (for 2h reaction time) and Fmoc group was removed as described previously. The rest of the

synthesis was achieved using the split-pool synthesis protocol.[1] The beads were equally distributed into 12 reaction columns and each of the following amino acids was added to each one of them: Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Asn-OH, Fmoc-Glu(Trt)-OH, Fmoc-Gln(Ot-Bu)-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-His(Trt)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Lys(Boc)-OH. The beads from all 12 reaction columns were pooled together, Fmoc group was removed and the beads were equally divided into 8 reaction columns for microwave (1000W) assisted peptoid synthesis steps[2].

Each of the reaction vessels were treated with 2M Bromoacetic acid in anhydrous DMF (1ml) and 2M DIC in anhydrous DMF (1ml), gently shaken for 30 seconds and microwaved (1000W) for 15 seconds with the power set at 10%. The beads were shaken again for about 15 seconds and microwaved another round as described above. The reaction vessels were drained and washed with DMF (2ml x 10 times). Then each of the reaction vessels was treated with 1M solution of the primary amines (2 ml) and microwaved two times for 15 seconds after gentle shaking. The beads were washed, pooled and divided equally into 8 reaction columns and subjected to addition of next peptoid residue. This procedure was repeated until 5-mer peptoid region was completed.

At the end of synthesis, the beads were washed with Dichloromethane (DCM) (2ml x 3 times), and treated with 2.5 ml of 95% Triflouoroacetic acid (TFA), 2.5% water and 2.5% Triisopropylsialine (TIS) on the shaker for 2 hours to remove the side chain protection and were neutralized with 10% diisopropylethylamine in DMF. Reaction vessel was drained, washed with DMF (2ml x 3 times) and stored in anhydrous DMF at 4^0C.

S15: On bead two color binding assay for combinatorial library screen using HCC4017 cells and HBEC30KT cells[2]

Nearly 100,000 peptoid library beads were washed two times in RPMI medium (Sigma-Aldrich, MO) with 5% fetal bovine serum (FBS) and equilibrated in the same medium containing 2% Bovine Serum Albumin (BSA, Sigma- Aldrich, MO) for 1 hour in three polypropylene tubes.

HCC4017 and HBEC30KT cells were removed from culture plates with GIBCO enzyme free cell dissociation buffer (Invitrogen, NY) 2ml per plate (5 minutes for HCC4017 and 20 minutes for HBEC30KT) at 37^0 C. HCC4017 cells were washed and suspended in RPMI medium with 5% FBS. HBEC30KT cells were washed and suspended in KSFM medium (Life technologies, CA). Cells were counted and distributed in three 1.5ml microcentrifuge tubes (total of six tubes for both cell lines) with 1 x 10^6 cells in 1ml of media per each tube.

Then the cell labeling procedure was conducted as follows: to prepare 10nM labeling solution (typical working concentration is 2-15nM), pre-mix 1µl each of Qtracker reagent (Invitrogen) A and B in a 1.5ml microcentrifuged tubes (prepared three tubes for each color) and incubated for 5 minutes at room temperature. 0.2ml of respective medium was added to each tube and vortexed for 30 seconds. 1 X 10^6 cells were added to each tube (three HCC4017 and three HBEC30KT) containing the labeling solution and incubated at 37°C for 60 minutes. HCC4017 cells were labeled with Qtracker 655 (red color) and HBEC30KT cells labeled with Qtracker 565 (green color). Cells were twice washed with RPMI medium with 5% FBS and re-suspended in RPMI media with 5% FBS and 1% BSA (3mL for each type). Labeled cells were visualized with DAPI filter of BX-51 fluorescence microscope (Olympus, PA) with a color camera.

Both cell types were mixed thoroughly and pipetted up and down several times to break the clumps. 2ml of cell suspension mixture was added to each of the beads containing (approximately 33,000 beads) three polypropylene tubes and incubated at room temperature with gentle shaking for 30 minutes. (Final cell density for each cell type was 0.5 x 10^6 and the total cell density was 1 x 10^6). During incubation, cell binding to the beads were checked time to time at about 10 minutes intervals to make sure not to over equilibrate, which could increase non-specific binding of cells to the beads. The beads were gently washed two times with RPMI medium and visualized under the fluorescent microscope using DAPI filter.

S16: Isolation and preparation of beads for sequencing

Single positive bead containing fluorescently tagged cells (red color) was identified using a fluorescent microscope under 2.5 x objective magnification and removed manually with a 20 µl pipette with medium size pipette tips. Care was taken to avoid picking up of the beads with both red and green cells. Selected beads were washed three times with 1% SDS and boiled in the same solution for 45 minutes to strip off bound cells and proteins. Finally the beads were washed three times with water. Single bead placed on Edman sequencing cartridges was used for sequencing.

S17: Cyanogen Bromide (CNBr) cleavage of beads

Small amounts of beads were removed from reaction vessels before storage and washed with DCM (2ml x 3 times). 30mg/ml CNBr solution (1ml) was prepared in 5:4:1 Acetonitrile: Acetic acid: water. 50µl from this solution was added to the beads and kept on the shaker overnight. CNBr solution was allowed to evaporate and 1:1 mixture of acetonitrile and water was added to the beads and resulting solution was used to confirm mass of the compound.

S18: On bead cell binding assay for qualitative binding confirmation of HCC4017 cells to PPS1 compound

200 µl of TentaGel beads containing PPS1 compound were transferred into each of three 1.5 microcentrifuge tubes. The beads were washed 2 times in RPMI medium with 5% FBS and equilibrated in same medium containing 2% BSA for 1 hour. HCC4017 and HBEC30KT cells were removed from culture plates, counted and 0.4 x10^6 cells from each cell types were distributed in 1.5ml microcentrifuge tube and subjected to labeling procedure as described in library screening. At the end of the labeling procedure cells were suspended in 0.5ml of RPMI medium containing 5% FBS or KSFM media with supplements and pipetted several times to break cell clumps. Red and green cells were separately added to two tubes and 1:1 mixture to third tube. Cell density for each cell type was kept as 0.4 x 10^6 cells in each tube. The beads were incubated at room temperature with gentle shaking for 20 minutes. During incubation cell binding to the beads were checked time to time at about 5minutes intervals and it was observed that HCC4017 cells demonstrated significant binding within 10 minutes. Finally, the beads were gently washed and visualized under the fluorescent microscope equipped with the DAPI filter.

S19: Magnetic bead binding assay

This assay was performed with Dynabeads M-280 Streptavidin (Life technologies, CA). First the beads were re-suspended in the original vial by vortexing. From this 14µl of beads (approximately 9 x 10^6 beads) were transferred to a microcentrifuge tube and 500µl of PBS with 0.1%BSA was added. The microcentrifuge tube containing the beads was placed on the magnet

for 2 minutes and the supernatant was removed by aspiration. The beads were washed three times with 500µl of PBS with 0.1%BSA. Then biotinylated PPS1 or PC462 was added to each vial and the reaction was incubated for 30 minutes at room temperature with gentle shaking. Then the beads were washed 3 times with 500µl of PBS with 0.1% BSA. HCC4017, HBEC30KT and HBEC3KT cells (0.5×10^6 cells in 1ml of RPMI with 1% BSA) were added to each vial and incubated for 30 minutes at room temperature with gentle shaking. The bead bound cells were isolated by placing the vial on the magnet and after removing supernatant, cells were counted with hemocytometer.

S20: ELISA-like binding assay

5,000 of HCC4017 cells were grown in each well of a white clear bottom 96 well plate (Corning Inc, NY) 24 hours prior to the experiment. Each well was blocked with 100 µl of 5% BSA in Phosphate Buffered Saline (PBS; Life technologies, NY) for 15 minutes at room temperature. Then the BSA was removed from wells and each well was treated with 50µl of graded concentrations of FITC-PPS1/PPS1-(Eu3+)-DTPA compound prepared in 1% BSA containing PBS and incubated for 45 minutes at room temperature. Wells were washed with PBS and fluorescence was measured at 520 nm (for FITC) and 610 nm [for (Eu3+)-DTPA] using the plate readers (Fluostar Optima, BMG Laboratories, NC and Spectra max i3, Molecular Devices, CA).

S21: MTS viability assay on HCC4017 cells

5,000 of HCC4017 and HBEC30KT cells were grown in each well of a white clear bottom 96 well plates (Corning Inc, NY) on day 1 of the experiment. On day 2, wells were treated with graded concentrations of PPS1, PPS1D1, PPS1-Cys, control PC462D1 and PC462 in RPMI medium with 5% FBS containing 3% BSA. For HBEC30KT, Keratinocyte-SFM with 3% BSA media was used. Eight graded concentrations ranging from 0.01 µM – 50 µM were used from both compounds and each concentration was done in triplicates. 6 wells were left untreated as controls. On day 4, media was removed from each well and treatment was repeated as described previously. On day 5, 20 µl of CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, WI) was added to each well and absorbance was measured at 490 nm using a plate reader (Fluostar Optima, BMG Laboratories, NC and Spectra max i3, Molecular Devices, CA) 2 hours after treatment.

References:

[1] Lam, K. S., Salmon, S. E., Hersh, E. M., Hruby, V. J., Kazmierski, W. M., and Knapp, R. J. (1991) A new type of synthetic peptide library for identifying ligand-binding activity, *Nature 354*, 82-84.

[2] Udugamasooriya, D. G., Dineen, S. P., Brekken, R. A., and Kodadek, T. (2008) A peptoid "antibody surrogate" that antagonizes VEGF receptor 2 activity, *Journal of the American Chemical Society 130*, 5744-5752.