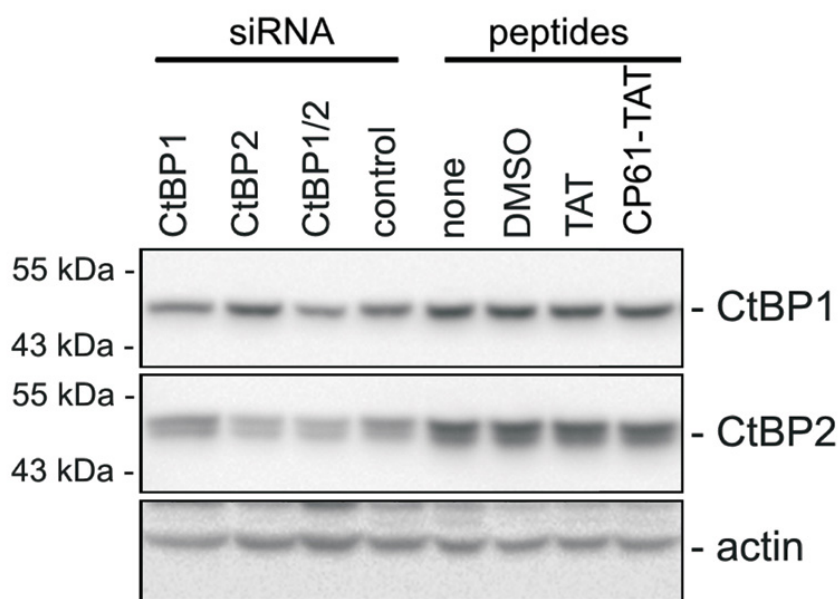
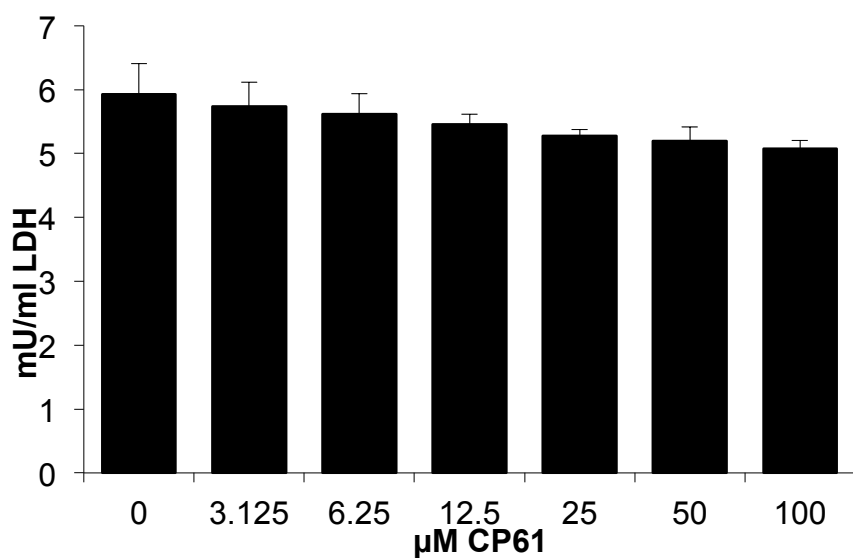


## Supplementary Information



**Figure S1. Assays to determine CtBP abundance in CP61-treated cells.** TAT-tagged cyclic peptides do not affect CtBP protein abundance. MCF-7 cells were transfected with siRNA, or exposure to the indicated peptide (50  $\mu$ M) for 48 h prior to the analysis of CtBP1 and CtBP2 by western blot.



**Figure S2. CP61 does not inhibit lactate dehydrogenase (LDH) activity.** LDH activity was assessed using the standard LDH enzyme from a colorimetric LDH cytotoxicity kit (Cayman Chemicals). 5 mU/ml LDH was incubated with increasing concentrations of CP61 for 30 min and final abs read at 490 nm. Increasing concentrations of CP61, up to 100  $\mu$ M does not significantly affect LDH activity. Bars show mean  $\pm$  SEM from three independent experiments.

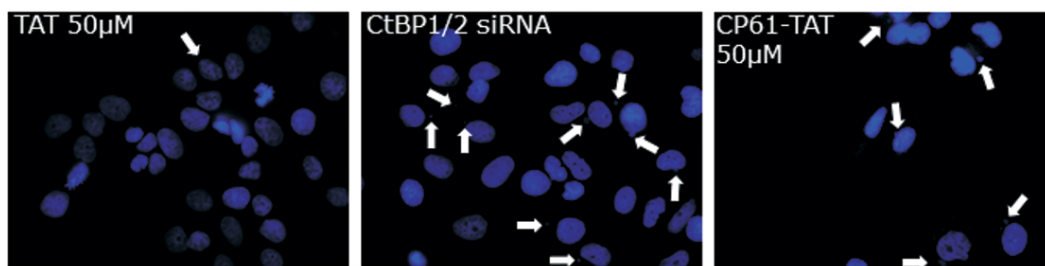


Figure S3. Representative images of induced micronuclei in MCF-7 cells. Micronuclei are quantified in Fig. 6B.

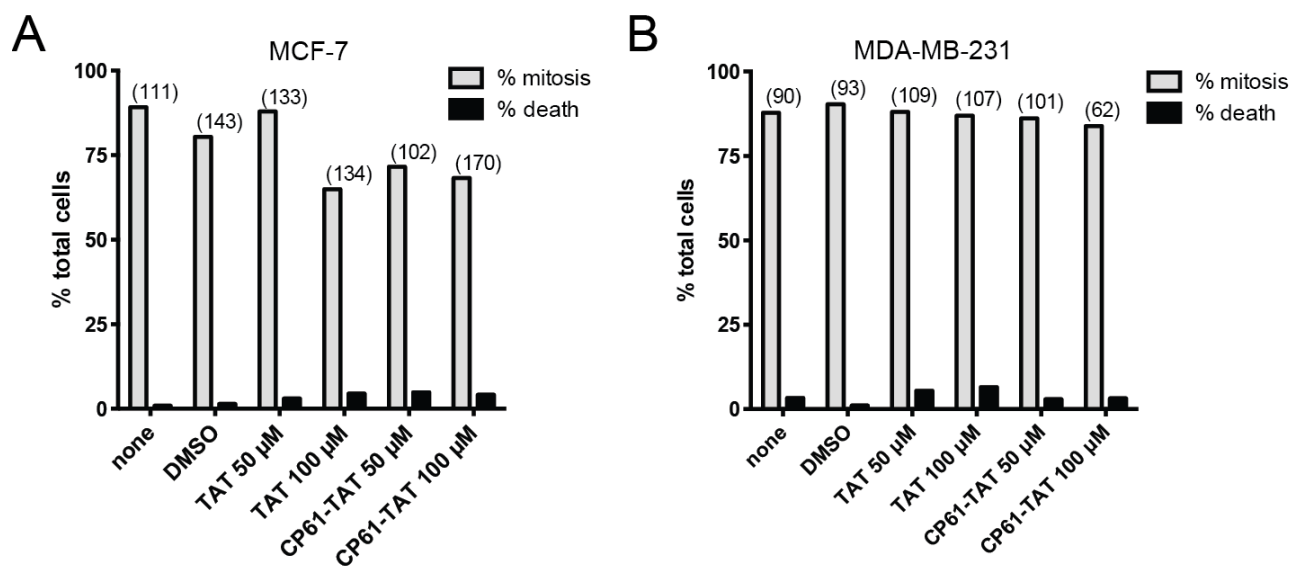
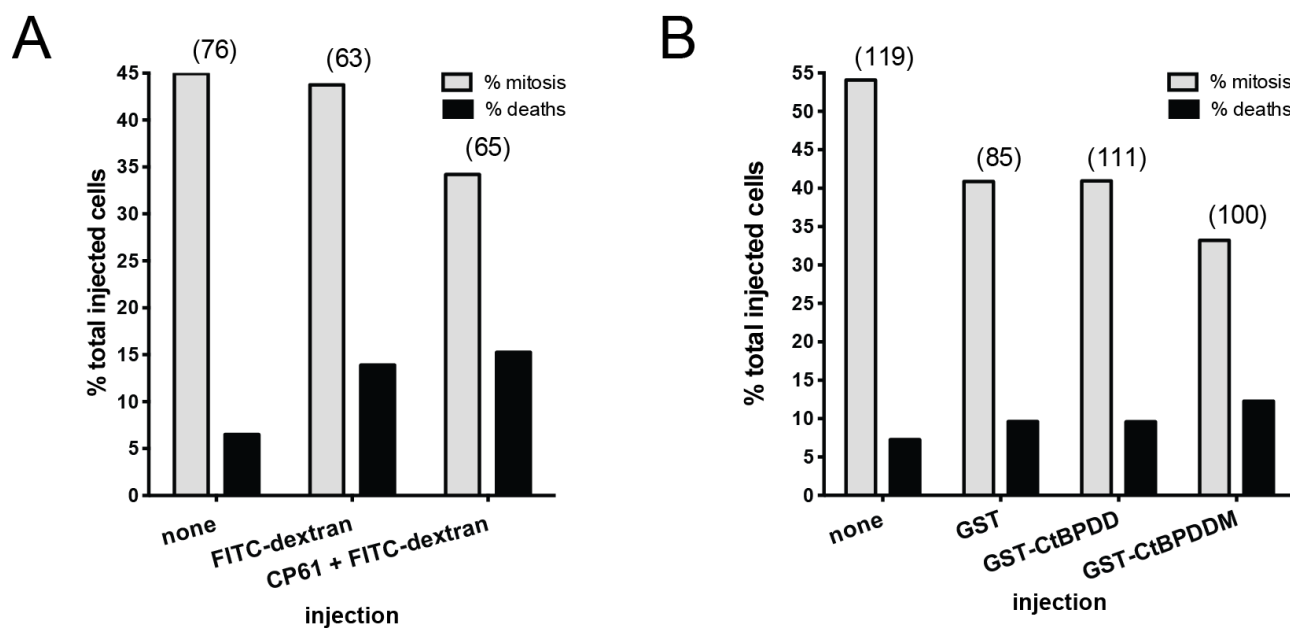


Figure S4. Effect of CP61-TAT on cell viability. Following treatment with the indicated reagent the proportion of cells in which the first event was either mitosis or death was scored for (A) MCF-7 cells and (B) MDA-MB-231 cells. Numbers of scored cells are shown in parentheses.



**Figure S5. Effect of microinjecting CtBP dimerization inhibitors on MCF-7 viability.** Following microinjection with the indicated reagents (CP61 in (A), GST-CtBP<sup>DD</sup> in (B)), the proportion of cells (from experiment shown in Figure 7) in which the first event was either mitosis or death was scored. Numbers of scored cells are shown in parentheses.

### Legends To Supplemental Videos

**Video 1\_TAT only** MCF-7 cells were incubated with TAT peptide (50  $\mu$ M). 30 min per frame. 7 frames per second.

**Video 2\_CP61-TAT** MCF-7 cells were incubated with CP61-TAT peptide (50  $\mu$ M). 30 min per frame. 7 frames per second.

**Video 3\_FITC control** Video microscopy of MCF-7 cells injected with FITC-dextran. 30 min per frame. 7 frames per second.

**Video 4\_CP61 field a** Video microscopy of MCF-7 cells co-injected with FITC-dextran and CP61, 30 min per frame. 7 frames per second.

**Video 5\_CP61 field b** Video microscopy of MCF-7 cells co-injected with FITC-dextran and CP61. 30 min per frame. 7 frames per second.

### Supplementary methods

Escherichia coli cultures were maintained in LB broth. DNA manipulations were performed with E. coli DH5 $\alpha$ -E (Invitrogen), GM2929 (CGSC), or DH5 $\alpha$ -pir (CGSC) cells. Plasmids were transformed into E. coli by heat shock or electroporation. Restriction and DNA-modifying enzymes were purchased from New England BioLabs. Oligonucleotides were purchased from Eurofins MWG Operon (Germany). PCR purification and gel extraction kits were purchased from Qiagen. DNA sequencing was performed by Eurofins MWG Operon (Germany). All peptides were synthesized using a Liberty 1 microwave peptide synthesizer (CEM), and purified on a Waters HPLC system using a Waters C18 Atlantis T3, or a Waters C18 Atlantis Prep OBD column, with traces measured at 280 nm. HPLC solvent A was 0.1 % TFA in H<sub>2</sub>O and solvent B was 0.1 % TFA in acetonitrile; HPLC gradient used for peptide purification: 0 mins 5 % B, 10 min 45% B, 15 mins 45 % B, 15.5 mins 5 % B, 20 mins 5 % B.

### Construction of CtBP1 and CtBP2 RTHS

All CtBP RTHS were constructed as previously described for the ATIC RTHS <sup>1</sup>.

### Genetic selection of CtBP inhibitors

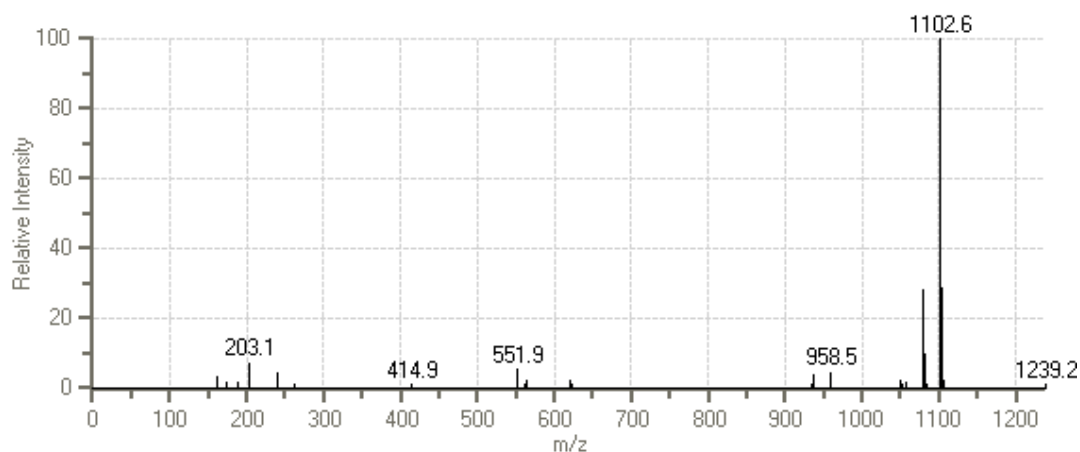
SICLOPPS libraries were constructed as described,<sup>2</sup> and transformed into the CtBP1 RTHS by electroporation. The number of transformants was measured by plating 10-fold serial dilutions of 1  $\mu$ l of the recovery mixture on to LB-agar plates containing chloramphenicol, and was typically in the order of 10<sup>9</sup>. Transformants were washed with minimal media A and plated on minimal media A supplemented with 13  $\mu$ M L-(+)-arabinose, 2.5 mM 3-amino-1,2,4-triazole (3-AT), 25  $\mu$ g/ml kanamycin, 30  $\mu$ g/ml chloramphenicol and 50  $\mu$ M IPTG. After incubation at 37 °C for 2-4 days, surviving colonies were picked

and streaked onto LB-agar plates containing 25  $\mu\text{g/ml}$  spectinomycin and 30  $\mu\text{g/ml}$  chloramphenicol. Surviving colonies from these plates were grown overnight and assessed by drop spotting 10-fold serial dilutions onto minimal media A plates, supplemented with antibiotics, IPTG and 3-AT as above, with and without 13  $\mu\text{M}$  L-(+)-arabinose. Plasmids from strains showing a growth advantage in the presence of arabinose were isolated and retransformed into the original selection strain and reassessed for IPTG-dependent inhibition of growth, and arabinose growth rescue. SICLOPPS plasmids from colonies demonstrating the expected phenotypes were assessed for their CtBP-specificity by transformation into an identical RTHS, except for the replacement of CtBP with an unrelated homodimeric protein (ATIC). Plasmids that caused a growth-advantage in the ATIC RTHS were discarded for being non-specific. The remaining plasmids were transformed into the CtBP2 RTHS to assess the CtBP1 specificity of the selected cyclic peptides. The activity of the remaining SICLOPPS plasmids was ranked by drop spotting, and the identity of the variable insert regions (encoding the cyclic peptide) was revealed by DNA sequencing.

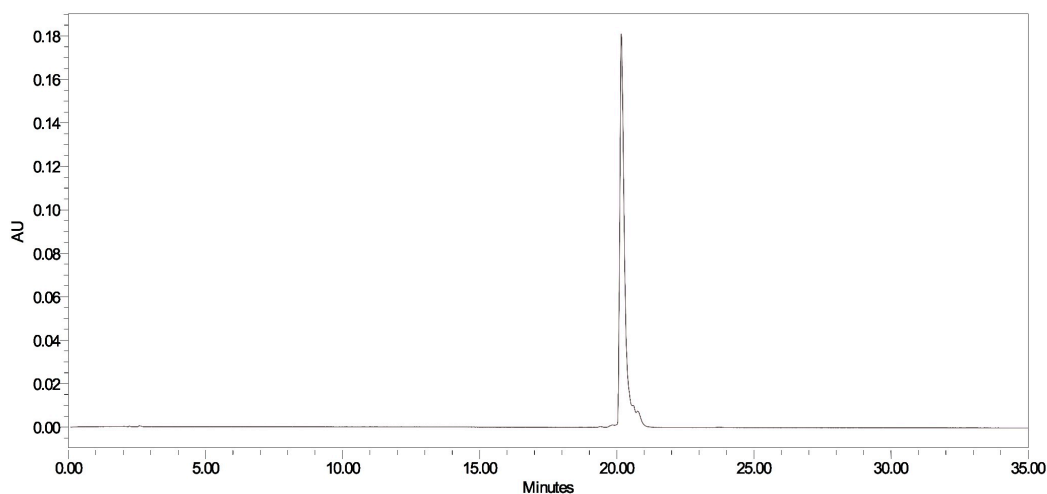
### Synthesis of CP61 (SGWTVVRMY)

The linear peptide GWTVVRMYS was synthesized using a Liberty One peptides synthesizer (CEM). The crude product was purified using reverse-phase HPLC (retention time 8.6 minutes) and characterized by ESI+ mass spectrometry: (M+H)+1099.1.

To a mixture of linear GWTVVRMYS (1eq, 0.128 mmol, 0.141g) in DMF (150 mL), EDC (3eq, 0.39 mmol, 76 mg) and HOBt (6eq, 0.768 mmol, 103 mg) were added. The reaction mixture was stirred for 24 h, concentrated *in vacuo*. The resulting oil was added dropwise to 50 mL cold diethyl ether. The resulting white solid was isolated and purified by reverse phase HPLC. CP61 (36.7 mg, 26% yield) was stored at  $-80\text{ }^\circ\text{C}$ . HM ESI+ Mass Spectrometry: (M+Na)+ 1102.6, (M+H)+ 1080.5, (Calculated mass: 1079.5).



Mass Spectrum of CP61

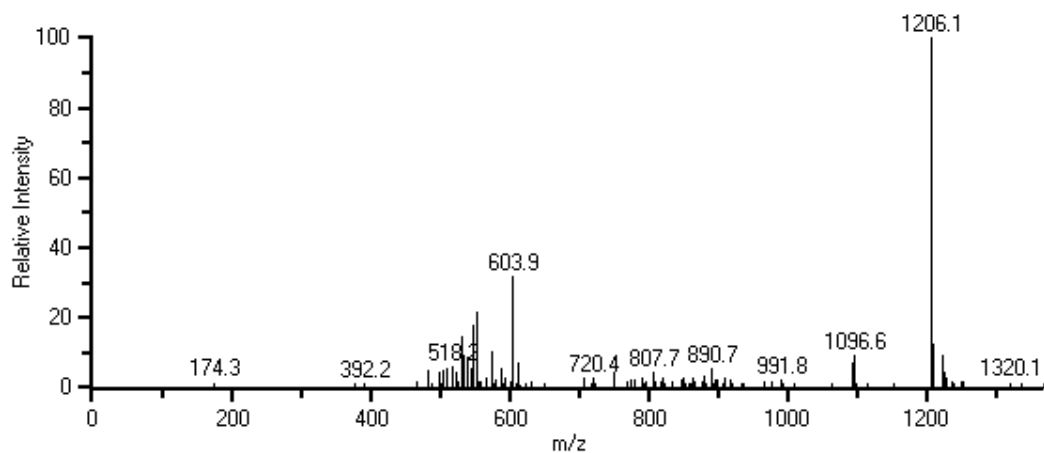


Analytical HPLC of CP61

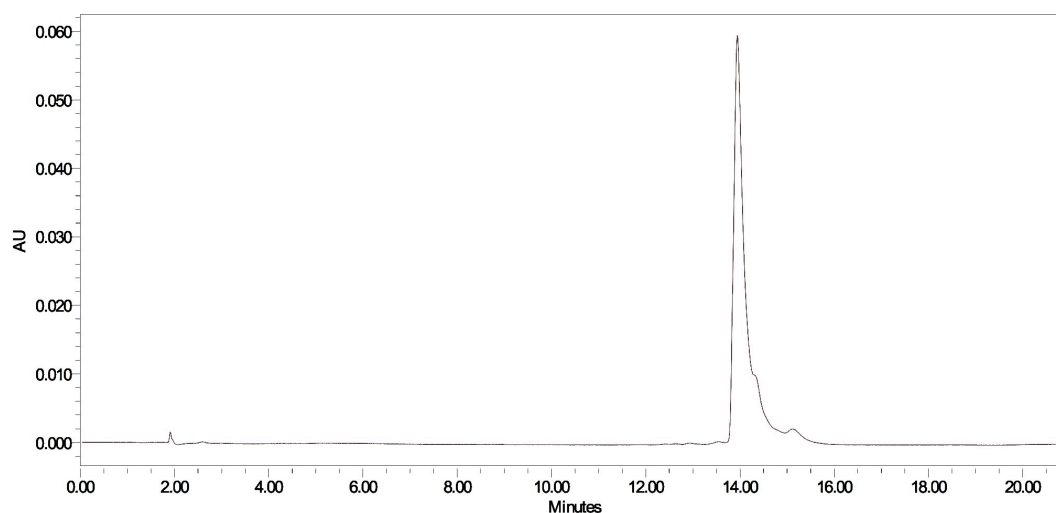
### Synthesis of CP61-TAT

Linear CGWTVVRMY was synthesized using a Liberty One peptide synthesizer (CEM). The resulting peptide was dissolved in DMF. A ten-fold excess of Aldrithiol-2 was added and the reaction was stirred overnight. The mixture was concentrated *in vacuo* and the resulting oil added to 50 mL of cold diethyl ether. The resulting precipitate was purified via HPLC (retention time 7.76 minutes). The presence of the product was confirmed by HM ESI+ mass spectrometry: (M+H)<sup>+</sup> 1224.1.

Aldrithiol-protected CGWTVVRMY (1eq, 0.173 mmol 0.212g) was added to a solution of EDC (3eq, 0.52 mmol, 99 mg) and HOBt (9eq, 1.56 mmol, 210 mg) in DMF (200 mL). The reaction mixture was stirred for 24 h, followed by concentration *in vacuo* and ether precipitation. The crude product was purified via HPLC to give a white solid (91 mg, 43%) whose identity was confirmed by HM ESI+ mass spectrometry: (M+H)<sup>+</sup> 1206, (M+2H)<sup>+</sup> 603.9. HM ESI+ Mass Spectrometry: (M+H)<sup>+</sup> 1206.1 (M+2H)<sup>+</sup> 603.9, (Calculated mass: 1205.5).

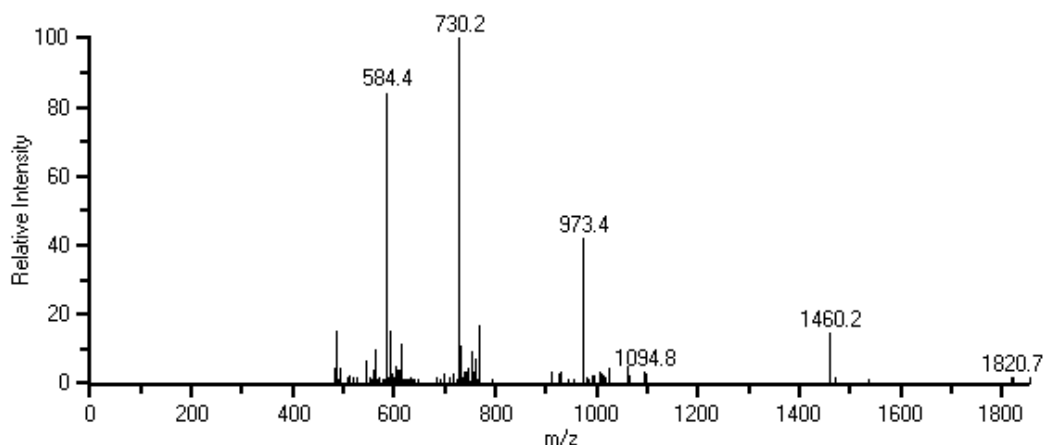


Mass Spectrum of CGWTVVRMY

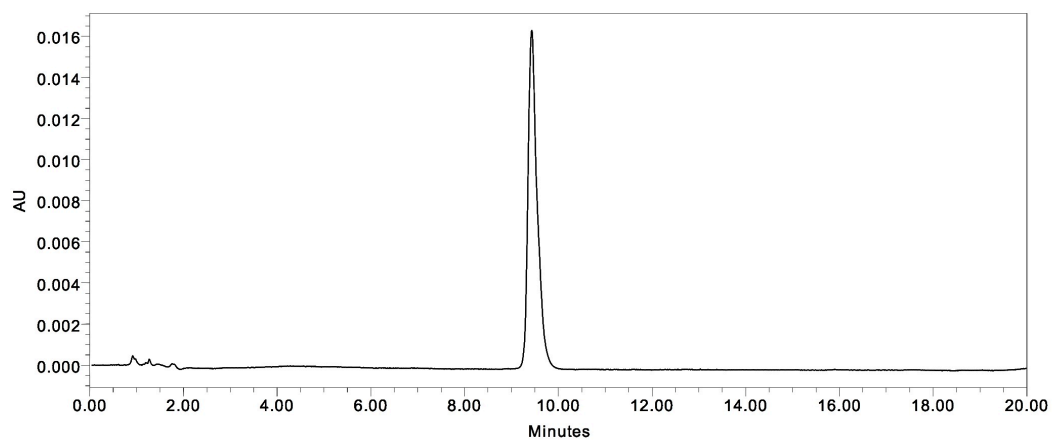


Analytical HPLC of CGWTVVRMY

TCEP-HCl (1eq, 0.075 mmol, 21.5 mg), DMF (9 mL) and H<sub>2</sub>O (0.05mL) were added to the above cyclic peptide (1eq, 0.075 mmol, 91 mg) and the mixture stirred for 1 hr. The deprotected peptide was purified by ether precipitation and added to aldrithiol-protected Cystein-TAT (2eq, 290 mg) in DMF (2 mL). The mixture was stirred for 3 hr and concentrated *in vacuo*. The desired product was purified by reverse phase HPLC (retention time 9.4 minutes) to give a white solid (75 mg, 68%) whose identity was confirmed by HM ESI+ Mass Spectrometry: (M+2H)<sup>+</sup> 1460.2, (M+3H)<sup>+</sup> 973.4, (M+4H)<sup>+</sup> 730.2, (M+5H)<sup>+</sup> 584.4 (Calculated mass: 2914.5).



Mass Spectrum of CP61-TAT



Analytical HPLC of CP61-TAT

### References

- (1) Tavassoli, A.; Benkovic, S. J. *Angew Chem Int Ed Engl* **2005**, *44*, 2760.
- (2) Tavassoli, A.; Benkovic, S. J. *Nat Protoc* **2007**, *2*, 1126.