Supplementary Information

Materials

Dithiothreitol (DTT), ammonium acetate, acetic acid, ammoniac, Tris(2-CarboxyEthyl) phosphine (TCEP), Auranofin and acetonitrile (AcN) were purchased from Sigma-Aldrich. Formic acid was purchased from Thermo Fisher Scientific.

AVP was purchased from Eurogenetec. [Se-Se]-AVP and analogues of auranofin were synthesized as previously described (1,2).

Methods

Sample preparation

All stock solutions were diluted by using ultrapure water, except for Au(I) compounds solutions which were prepared in DMSO. Ammonium acetate buffer solution (2 mM, pH 7.0) was prepared by weighing ammonium acetate and dissolving it in water, pH adjustment was carried out with acetic acid and ammoniac commercial solutions.

Stock solutions of AVP 0.92 mM, [Se-Se]-AVP 0.85mM, DTT 0.4 M and TCEP 0.1 M were prepared by dissolving the samples in ultrapure water.

For the reduction of AVP, aliquots of its stock solution were diluted with ammonium acetate solution 2 mM (pH 7.0) to 0.1 mM final peptide concentration. Then, aliquots of DTT or TCEP stock solution were added in peptide to reducing agent ratios 1:10 (final concentration of 1 mM of reducing agent). The mixtures were incubated for 30 minutes at 37 °C in a water bath under stirring.

For the reduction of [Se-Se]-AVP, aliquots of its stock solution were diluted with ammonium acetate solution 2 mM (pH 7.0) to 0.1 mM final peptide concentration. Then, aliquots of DTT or TCEP stock solution were added in peptide to reducing agent ratios 1:10, 1:100, 1:200 and 1:400 (1 mM, 10 mM, 20 mM, and 40 mM, reducing agent concentration respectively). Those mixtures were explored over different T (from 37 °C up to 70 °C) and at incubation time (from 30 min up to 5 h).

[Se-Se]-AVP aliquots were also incubated, without reducing agents, at 70 °C overnight in a water bath under stirring.

For the incubation of gold(I) compounds, stock solutions at 10mM of AF, Et_3PAuCl, Et_3PAuBr, Et_3PAuI, and $[Au(PEt_3)_2]Cl$ were prepared by dissolving the samples in DMSO.

Then, aliquots of peptide stock solution were diluted with 2 mM ammonium acetate solution pH 7.0 to 0.1 mM final peptide concentration. Then, 3 equivalent of gold (I) compounds (AF, Et_3PAuCl, Et_3PAuBr, Et_3PAuIor $[Au(PEt_3)_2]Cl$ were added to peptide solution (0.3 mM of final gold (I) compound concentration). The mixtures were left under stirring overnight at 37 °C or 70 °C in a water bath.

After the incubation, all solutions were sampled and diluted to a final peptide concentration of 6 µM using 2 mM ammonium acetate pH 7, 2% (v/v) of AcN and used for LC-MS analysis.

LC-MS Methods

Separation and identification of our samples was performed by LC-MS. Liquid chromatography separations were performed using Dionex ultimate 3000 series UHPLC from Thermo Fisher Scientific coupled to an Orbitrap Q-Exactive Plus Mass Spectrometer from Thermo Fisher Scientific.

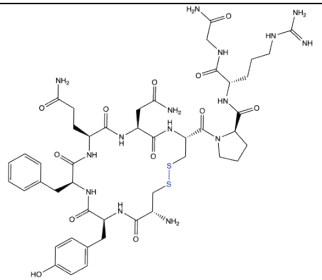
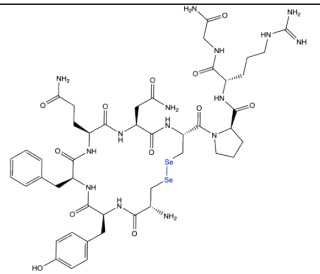
The column AcclaimTM 120 was used: C18, 5 µm, 120 Å, 4.6 mm × 100 mm, Dionex Bonded Silica Products from Thermo Fisher Scientific.

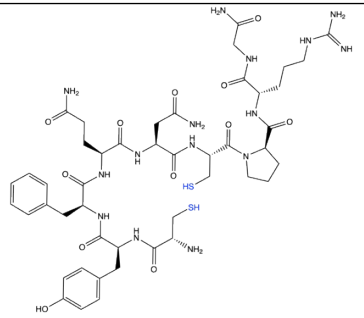
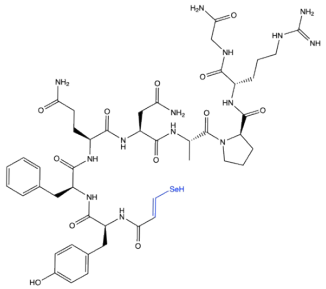
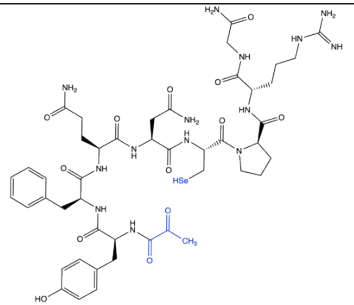
The mobile phases were A, H_2O and B, AcN, both with 0.1% formic acid (FA). The flow rate used in all LC-MS experiments was 1 mL min^{-1}, and sample elution was performed by using the gradient from 0% to 95% of B over 6.5 min. 10 µL of sample injection was set.

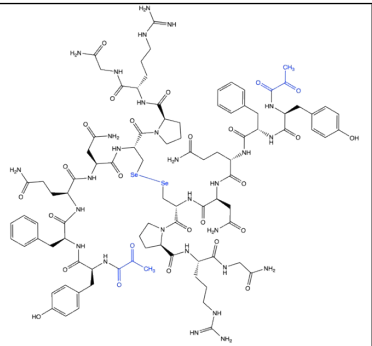
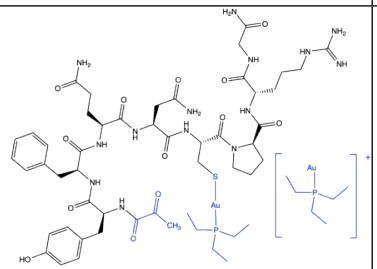
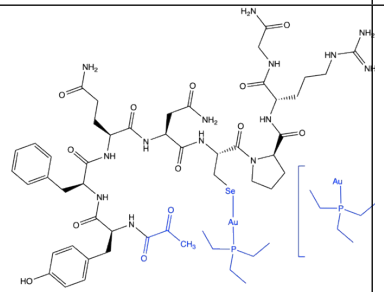
Ionization was performed using an Electrospray ion source operating in positive ion mode with a capillary voltage of 3.80 kV and capillary temperature 400 °C. Sheath gas, auxiliary gas and sweep gas flow rate were set at 75, 20 and 1 (arbitrary units), respectively. Auxiliary gas temperature was set at 500 °C.

The MS/MS were acquired, for each mass studied, with an isolation width 1.0 m/z or 7 m/z and a screening of collision energies was performed from 10 to 40 HCD. All MS data were analysed using Thermo Fisher Scientific Xcalibur Qual Browser software.

Table 1. Observed MS products

Entry, nº	Peptide code	Chemical structure	Formula	Theoretical mass	Experimental mass	Observed ions, m/z
1	AVP		$C_{46}H_{65}N_{15}O_{12}S_2$	1083.438	1083.440	1084.440 (z=1) 542.724 (z=2)
2	[Se-Se]-AVP		$C_{46}H_{65}N_{15}O_{12}Se_2$	1179.326	1179.330	1180.330 (z=1) 590.670 (z=2)

3	Reduced AVP		$C_{46}H_{67}N_{15}O_{12}S_2$	1085.454	1085.457	1086.457 (z=1) 543.733 (z=2)
4	Reduced [Se-Se]-AVP 1		$C_{46}H_{64}N_{14}O_{12}Se$	1084.399	1084.406	1085.406 (z=1) 543.206 (z=2)
5	Reduced [Se-Se]-AVP 2		$C_{46}H_{64}N_{14}O_{13}Se$	1100.394	1100.400	1101.400 (z=1)

6	[Se-Se]-AVP dimer		$C_{92}H_{126}N_{28}O_{26}Se_2$	2198.77	2198.78	1100.389 (z=2) 733.927 (z=3)
7	AVP-Au(PEt_3)		$C_{58}H_{93}Au_2N_{14}O_{13}P_2S$	1681.557	1681.558	841.279 (z=2)
8	[Se-Se]-AVP-Au(PEt_3)		$C_{58}H_{93}Au_2N_{14}O_{13}P_2Se$	1729.501	1729.502	865.251 (z=2)

LC-MS

Unreacted peptides

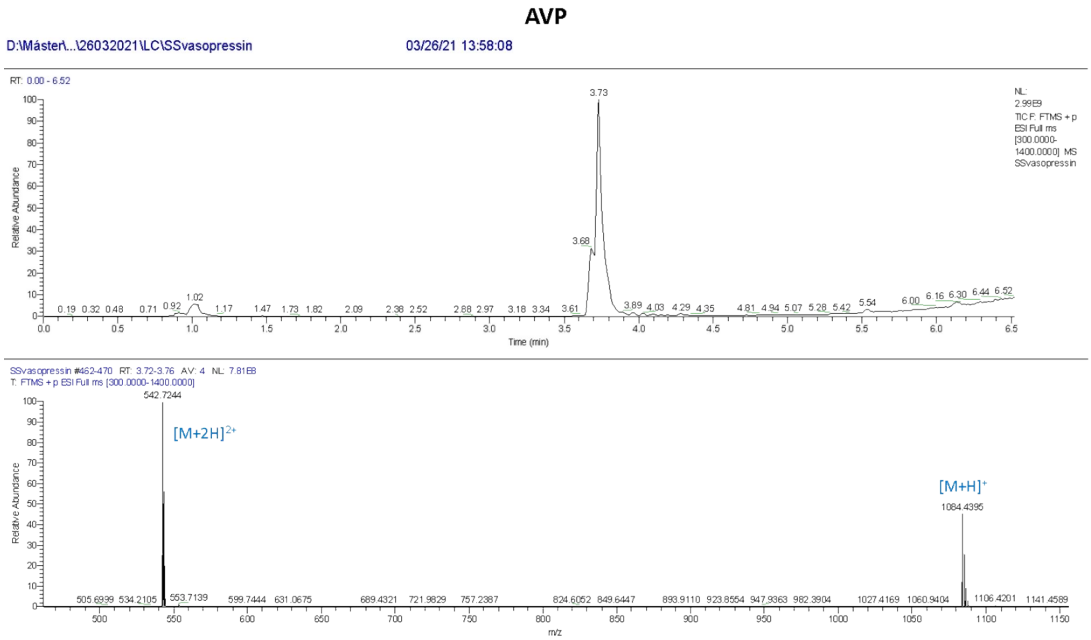


Figure S1. *LC-MS of unreacted AVP. (A) TIC. (B) MS spectrum of peak at t_R = 3.73 min.*

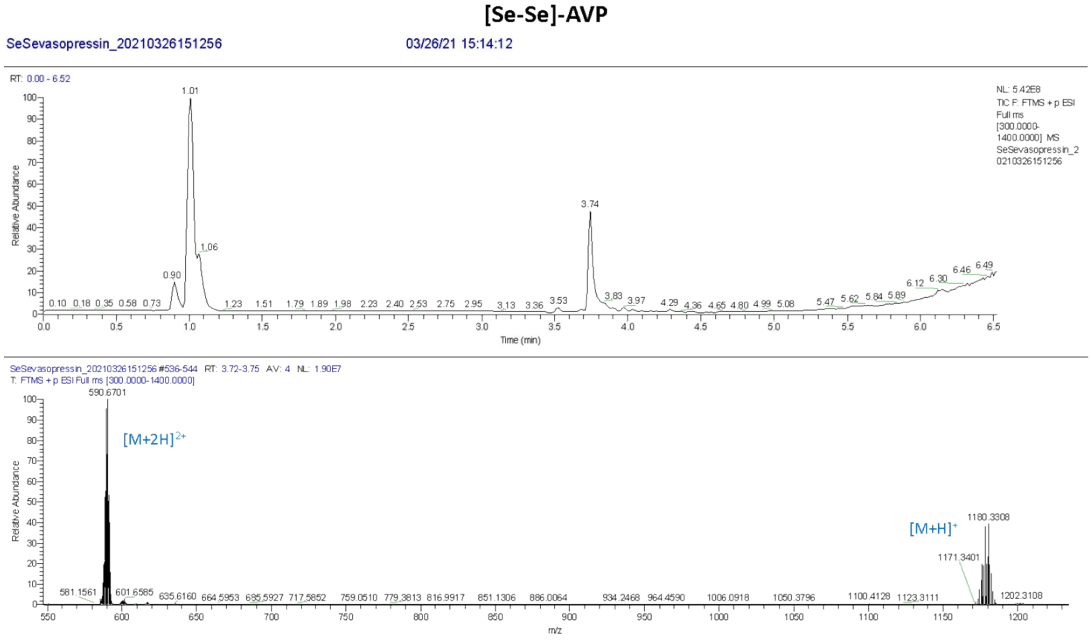


Figure S2. *LC-MS of unreacted [Se-Se]-AVP. (A) TIC. (B) MS spectrum of peak at t_R = 3.74 min.*

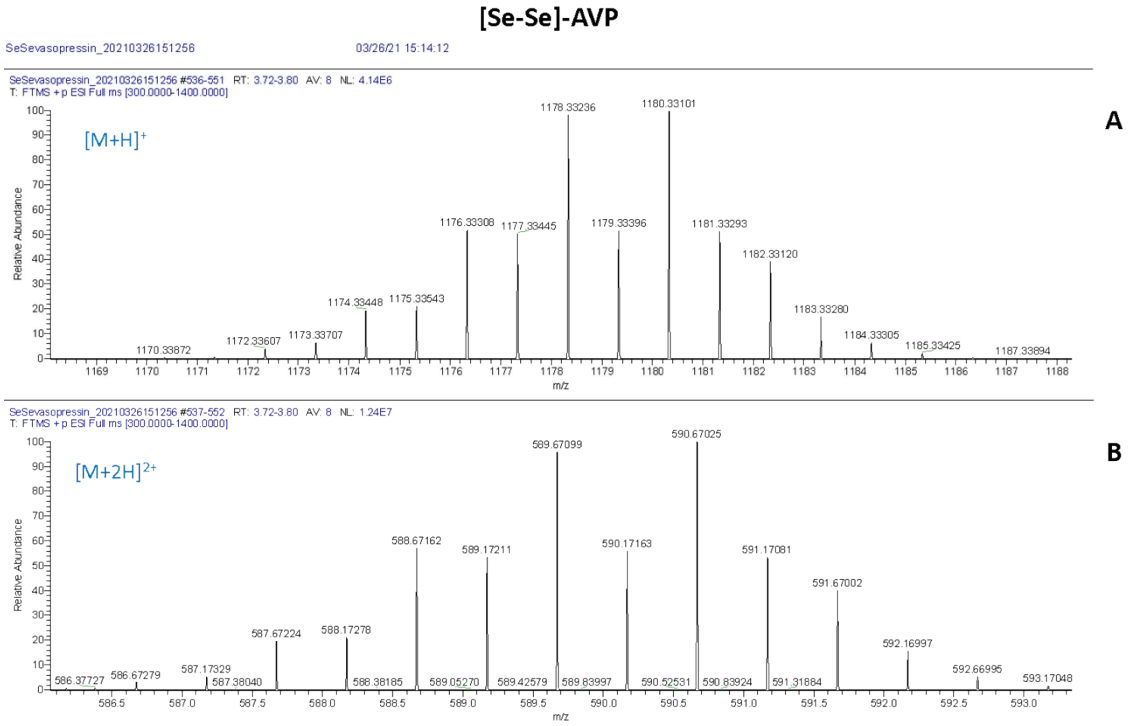


Figure S3. MS isotopic pattern of unreacted [Se-Se]-AVP for the di-charged (m/z 590.6702) (A) and mono-charged ion (m/z 1180.3310) (B).

Reduced peptides

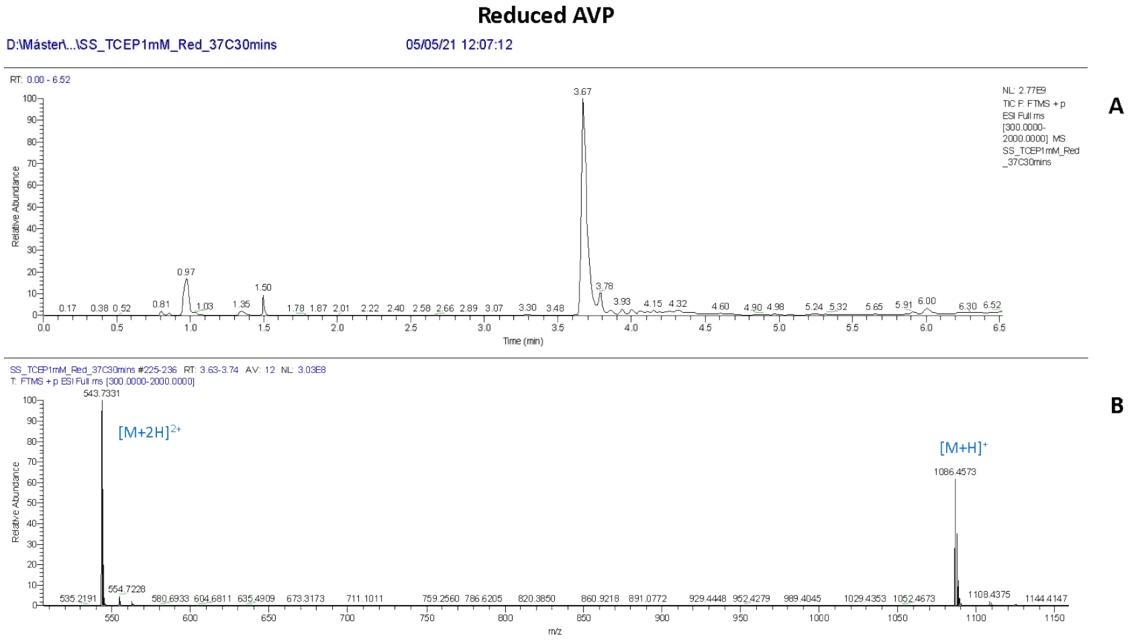


Figure S4. LC-MS of reduced AVP. (A) TIC. (B) MS spectrum of peak at t_R = 3.67 minutes.

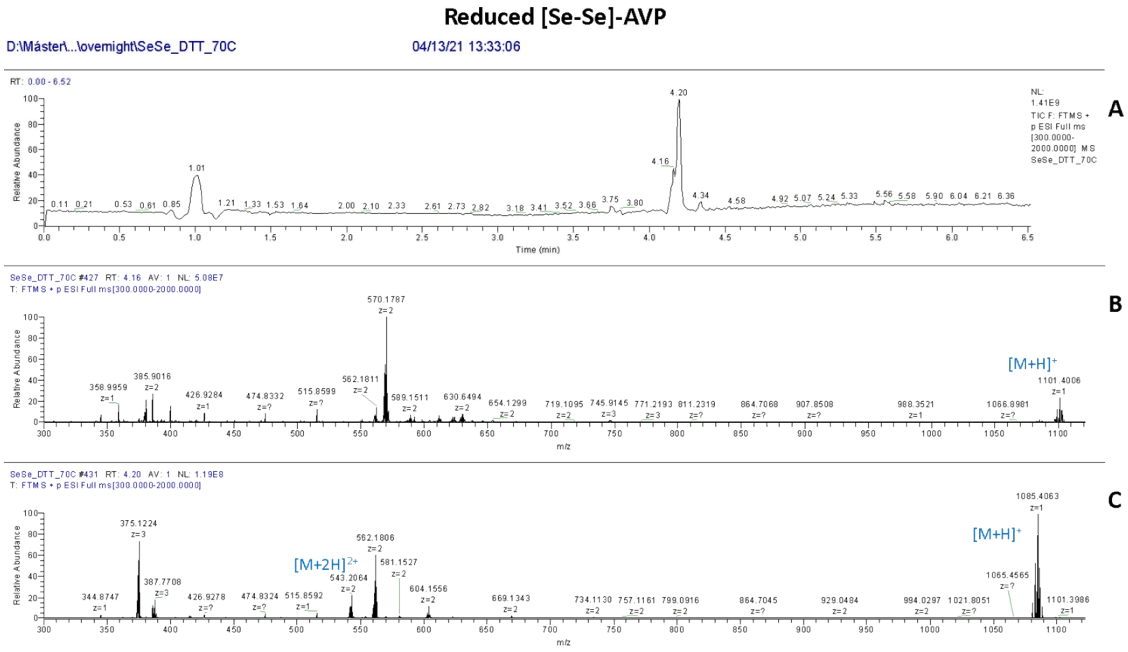


Figure S5. LC-MS of reduced [Se-Se]-AVP. (A) TIC. (B) MS spectrum of peak at t_R = 4.16 min. (C) MS spectrum of peak at t_R = 4.20 min.

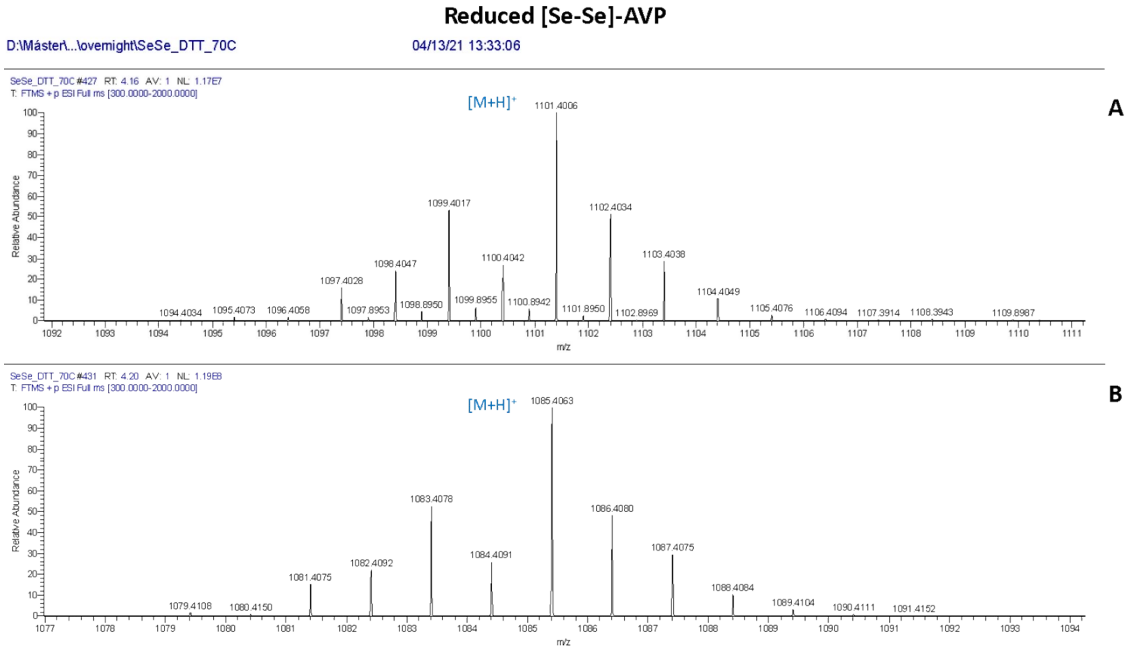


Figure S6. LC-MS of reduced [Se-Se]-AVP. (A) MS spectrum of peak at t_R = 4.16 min. (B) MS spectrum of peak at t_R = 4.20 min.

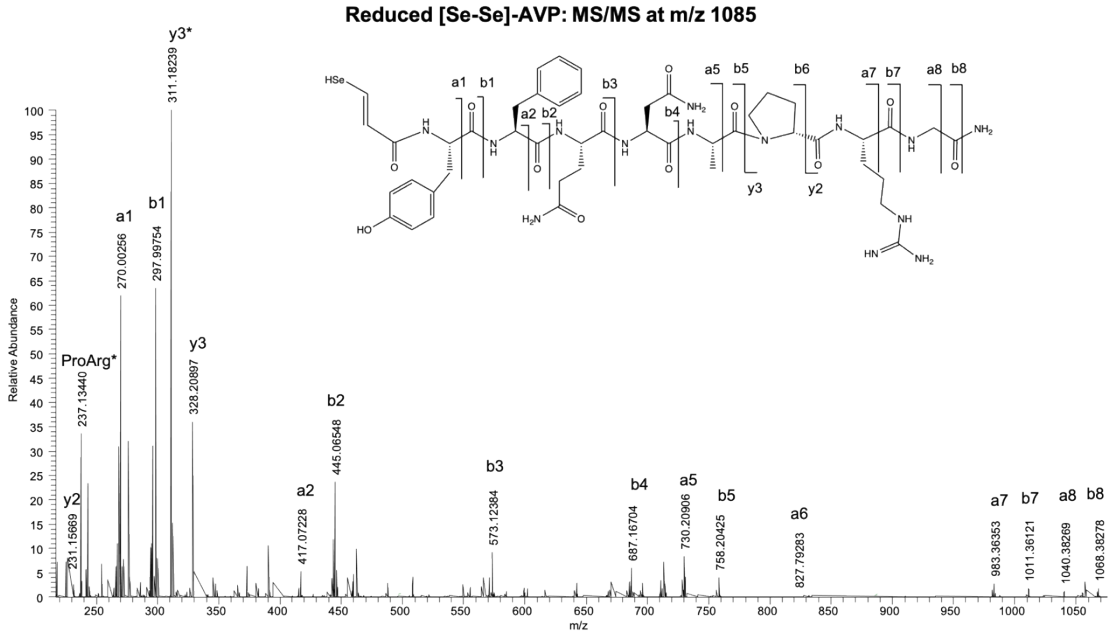


Figure S7. *MS/MS of reduced [Se-Se]-AVP at m/z 1085 (z=1). Principal fragments at HCD = 30.*

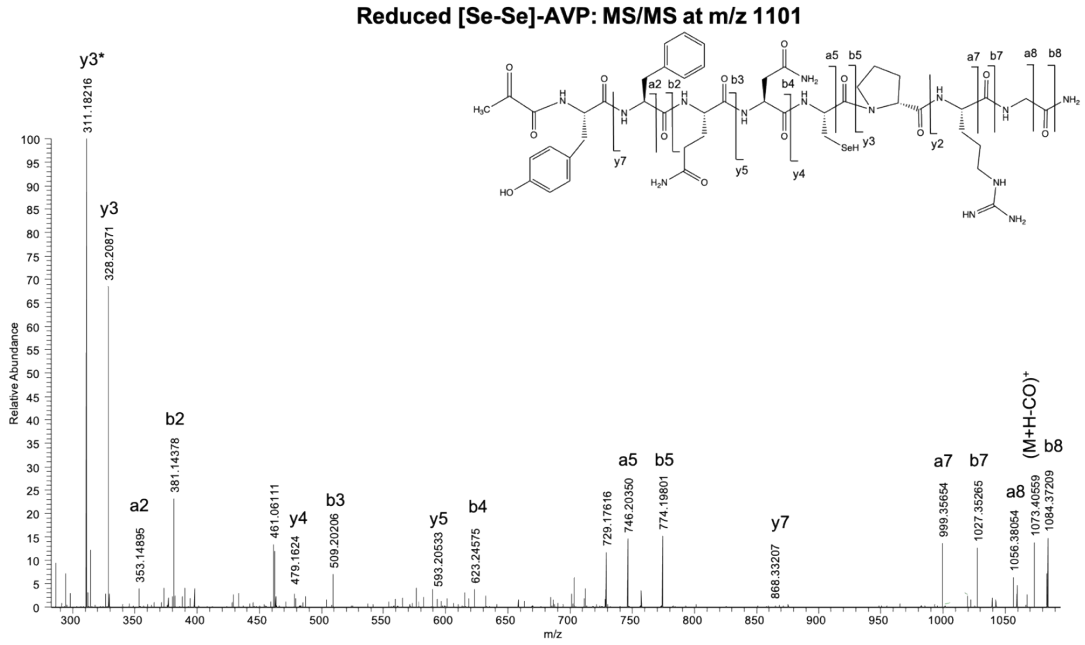


Figure S8. *MS/MS of reduced [Se-Se]-AVP at m/z 1101.3960 (z=1). Principal fragments at HCD = 30.*

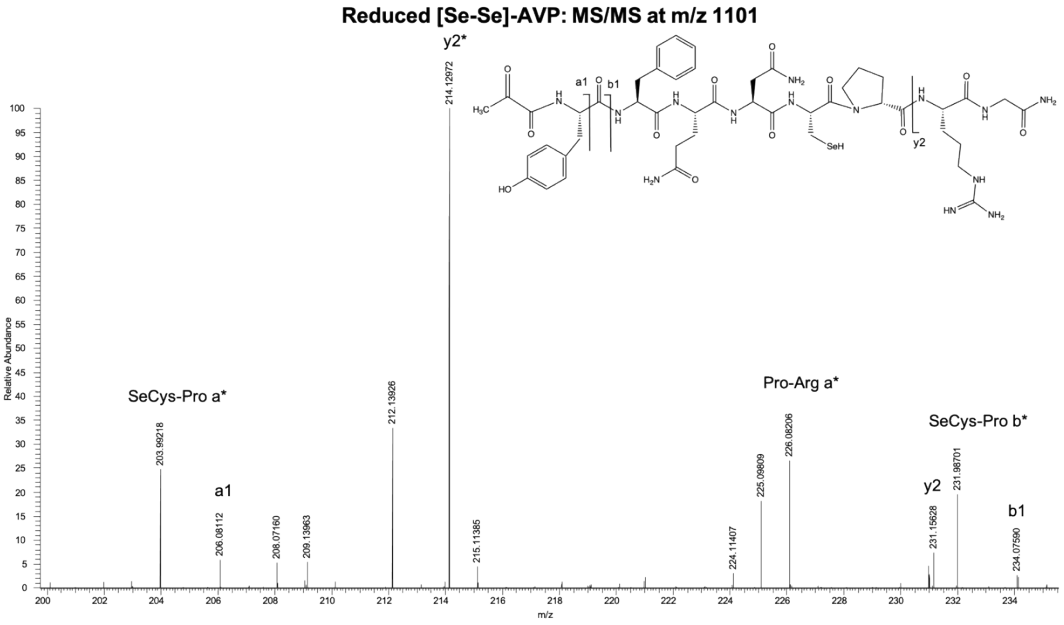


Figure S9. *MS/MS of reduced [Se-Se]-AVP at m/z 1101.3960 (z=1). Principal fragments at HCD = 35.*

Peptide incubation at 70°C

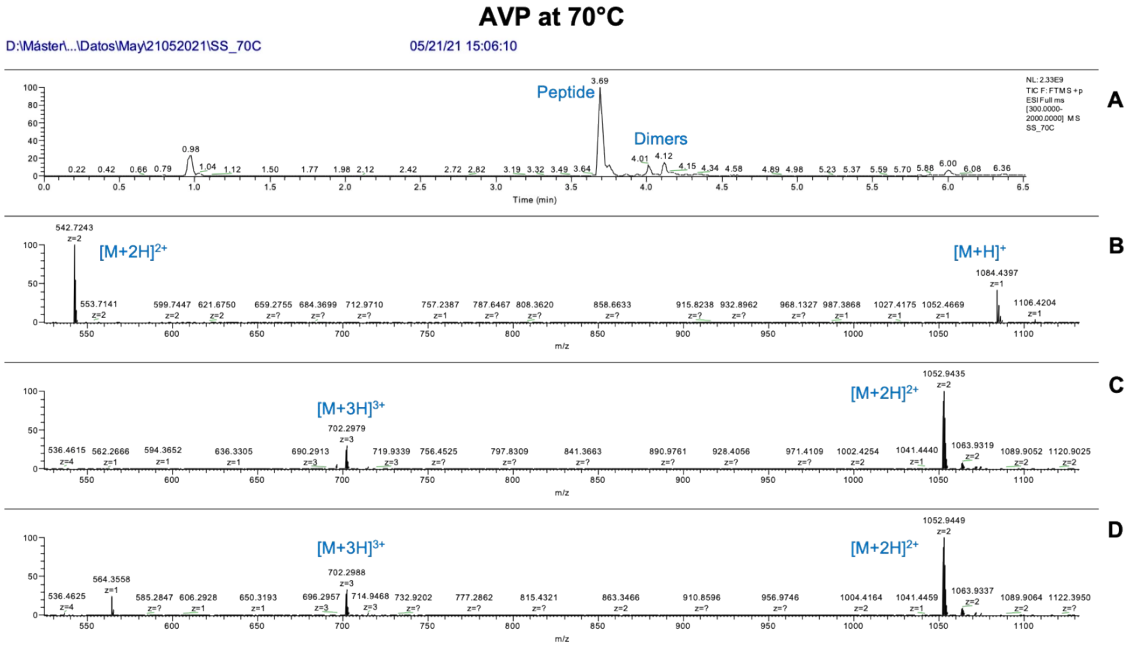


Figure S10. *LC-MS of AVP incubated overnight at 70 °C in the absence of reducing agents. (A) TIC. (B) MS spectrum of peak at t_R = 3.69 minutes. (C) MS spectrum of peak at t_R = 4.12 minutes. (D) MS spectrum of peak at t_R = 4.12 minutes.*

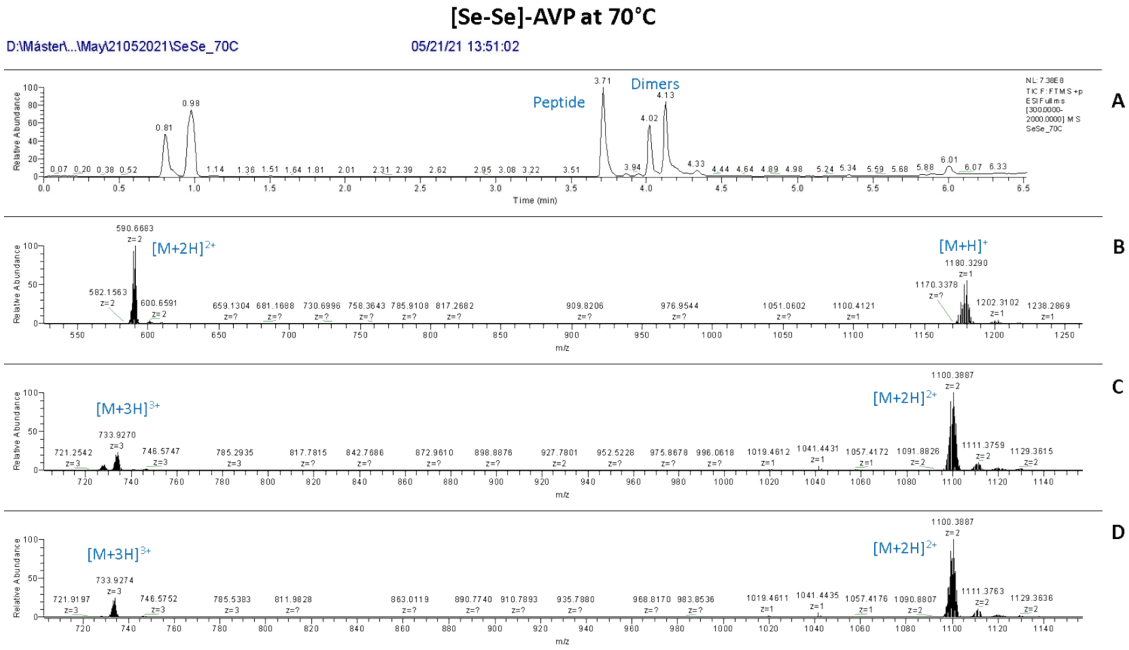


Figure S11. LC-MS of [Se-Se]-AVP incubated overnight at 70 °C in the absence of reducing agents. (A) TIC. (B) MS spectrum of peak at t_R = 3.71 minutes. (C) MS spectrum of peak at t_R = 4.02 minutes. (D) MS spectrum of peak at t_R = 4.13 minutes.

Peptides incubated with gold(I) compounds

AVP

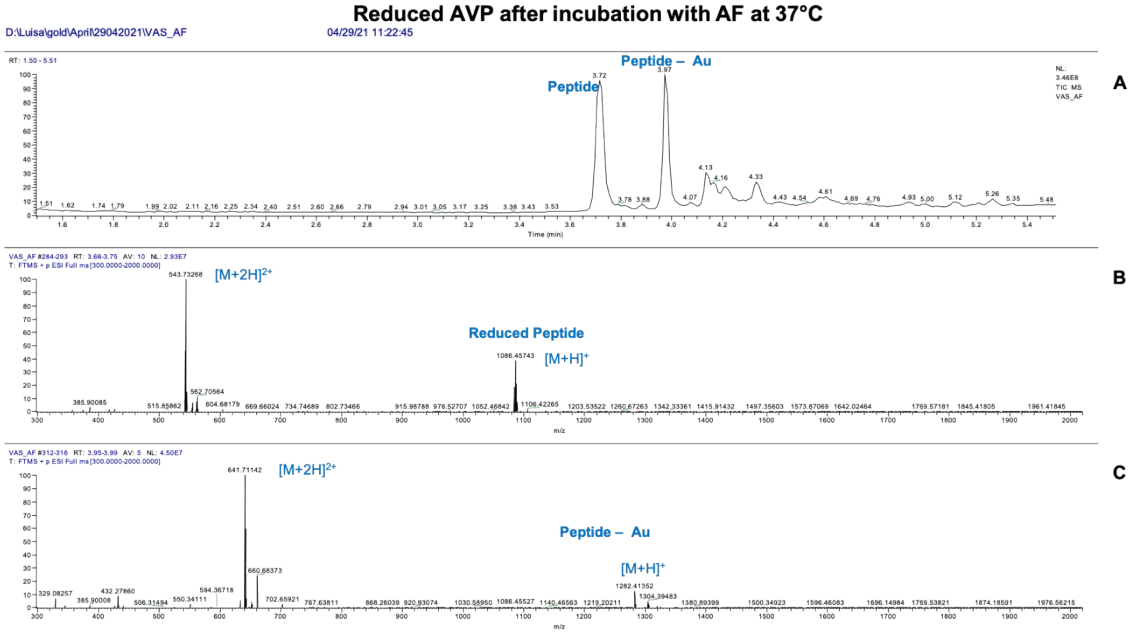


Figure S12. LC-MS of reduced AVP incubated with AF overnight at 37 °C. (A) TIC. (B) MS spectrum of peak at tR = 3.72 minutes. (C) MS spectrum of peak at t_R = 3.97 minutes.

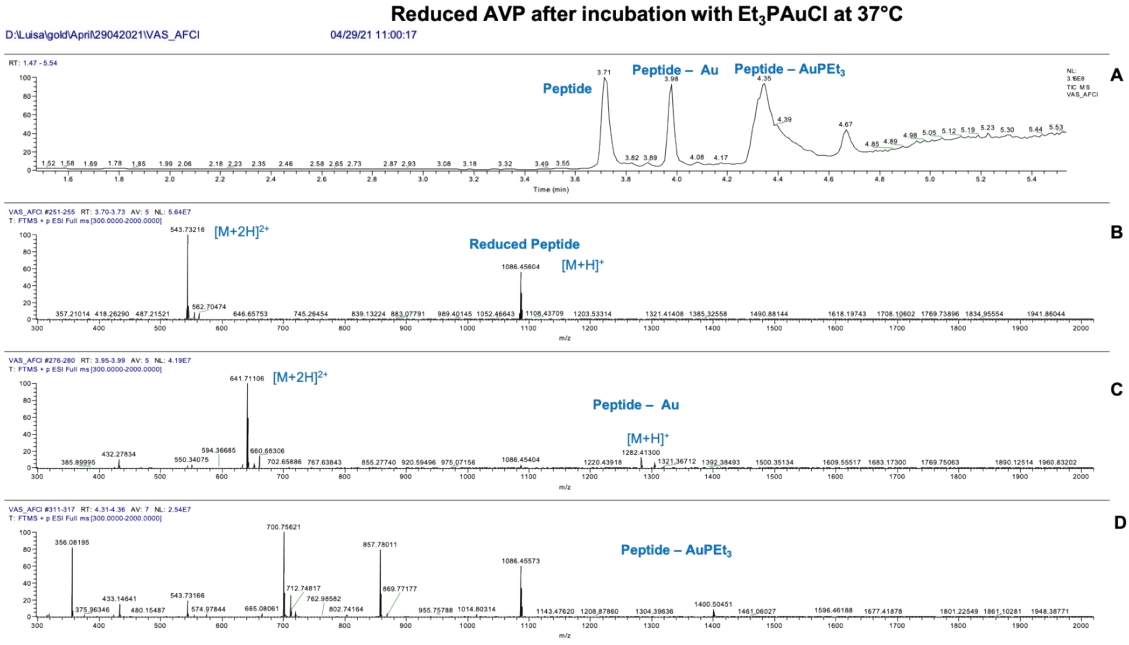


Figure S13. LC-MS of reduced AVP incubated with Et_3PAuCl overnight at 37 °C. (A) TIC. (B) MS spectrum of peak at t_R = 3.72 minutes. (C) MS spectrum of peak at t_R = 3.97 minutes. (D) MS spectrum of peak at t_R = 4.35 minutes.

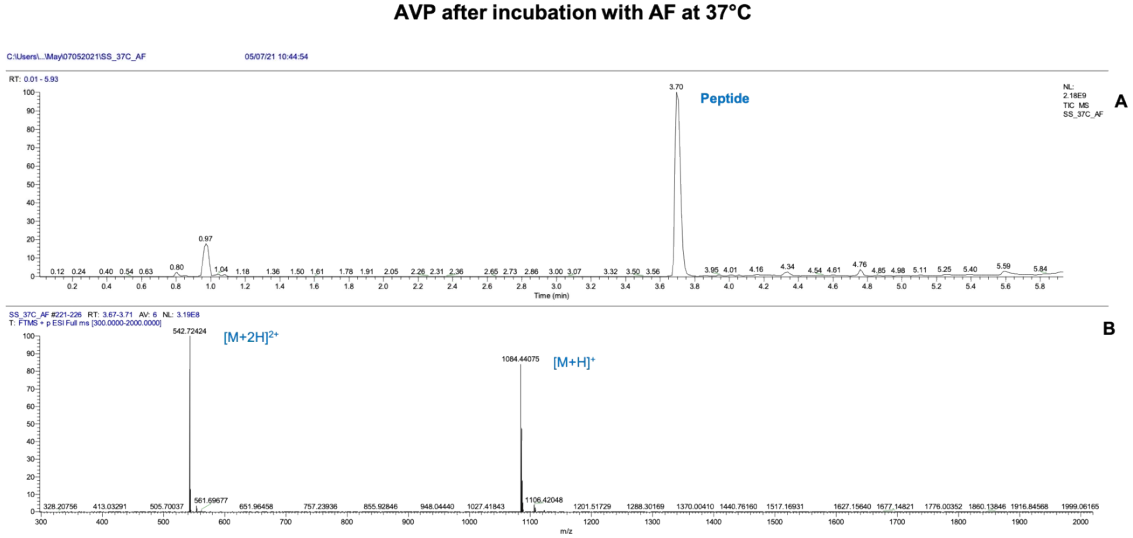


Figure S14. LC-MS of AVP incubated with AF overnight at 37 °C. (A) TIC. (B) MS spectrum of peak at t_R = 3.70 minutes.

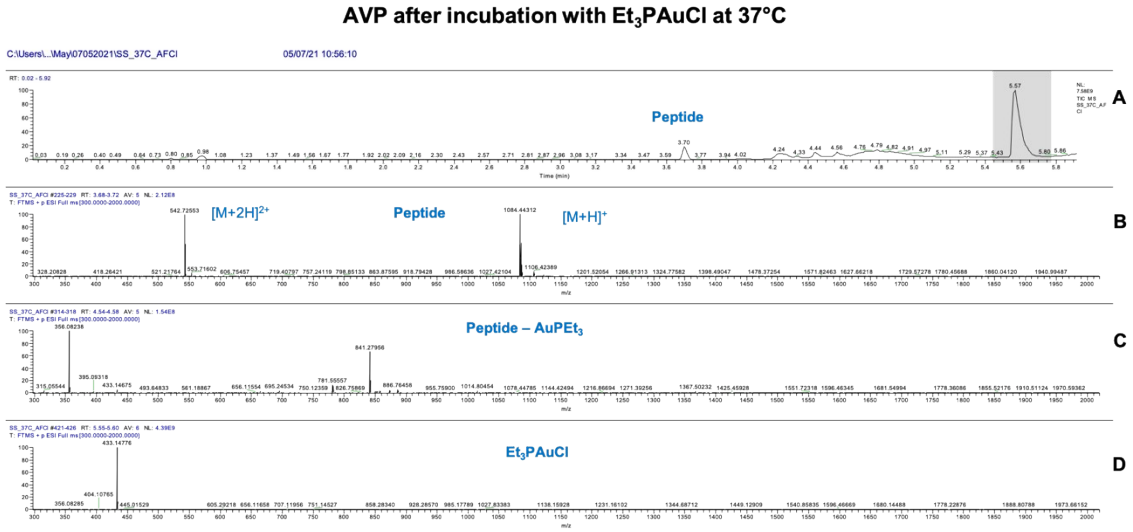


Figure S15. LC-MS of AVP incubated with Et_3PAuCl overnight at 37 °C. (A) TIC. (B) MS spectrum of peak at t_R = 3.70 minutes. (C) MS spectrum of peak at t_R = 4.56 minutes. (D) MS spectrum of peak at t_R = 5.56 minutes. The dark part corresponds to the elution of Et_3PAuCl.

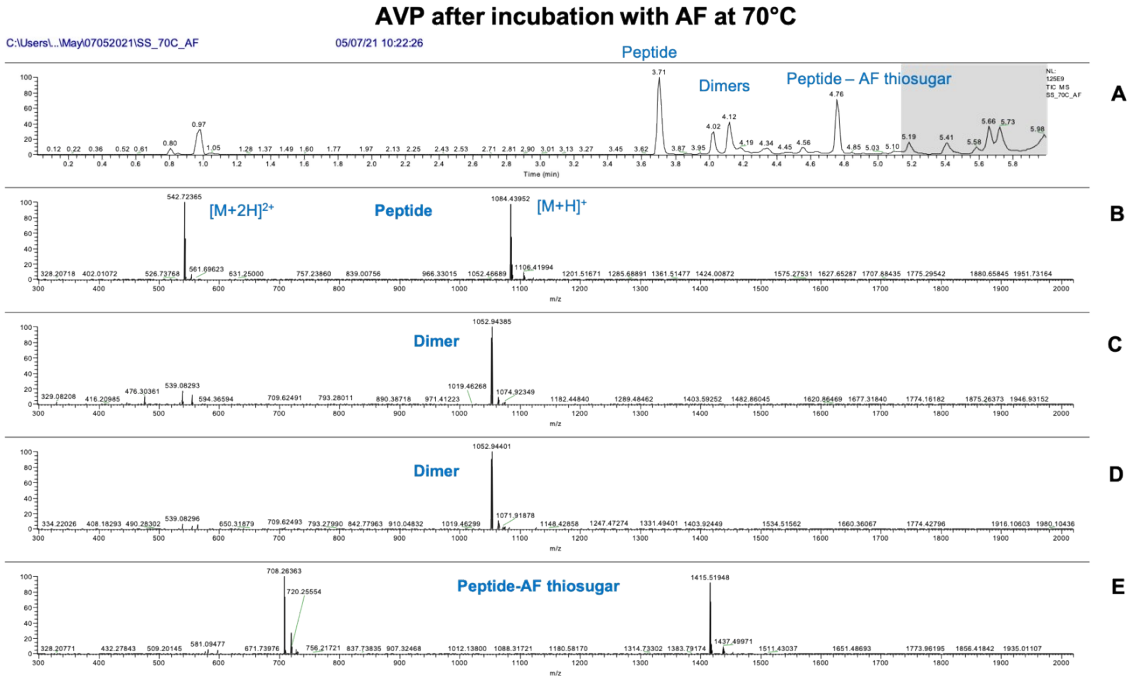


Figure S16. LC-MS of AVP incubated with AF overnight at 70 °C. (A) TIC. (B) MS spectrum of peak at t_R = 3.71 minutes. (C) MS spectrum of peak at t_R = 4.702 minutes. (D) MS spectrum of peak at t_R = 4.12 minutes. (E) MS spectrum of peak at t_R = 4.76 minutes The dark part corresponds to the elution of AF and its rearrangement products (3).

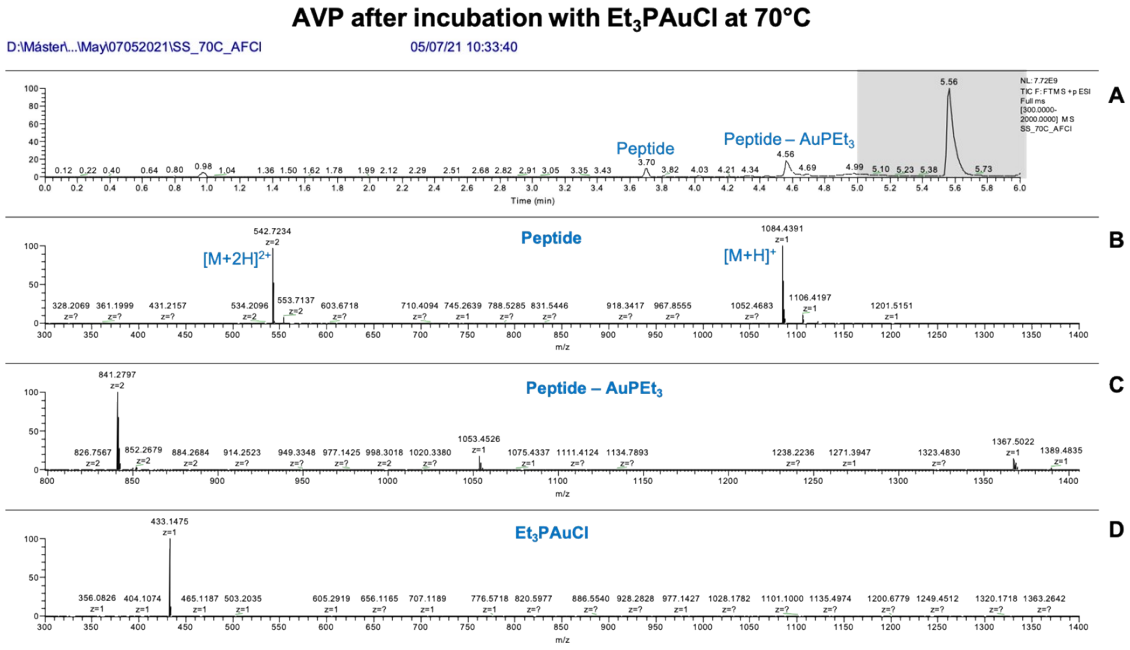


Figure S17. LC-MS of AVP incubated with Et_3PAuCl overnight at 70 °C. (A) TIC. (B) MS spectrum of peak at t_R = 3.7 minutes. (C) MS spectrum of peak at t_R = 4.56 minutes. (D) MS spectrum of peak at t_R = 5.56 minutes. The dark part corresponds to the elution of Et_3PAuCl.

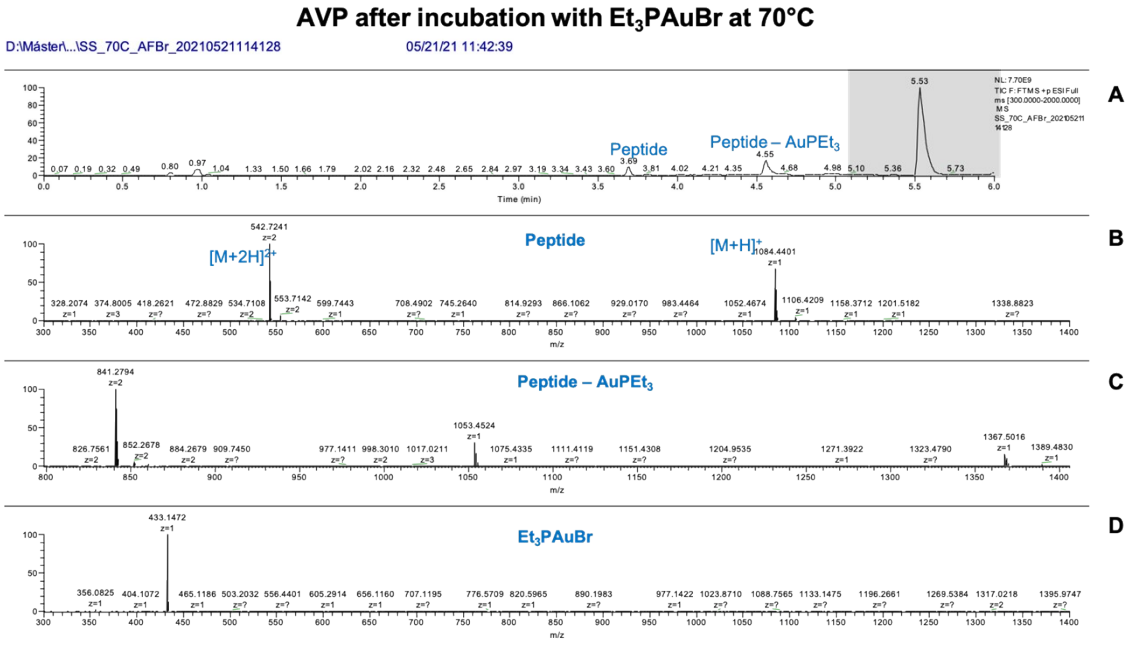


Figure S18. LC-MS of AVP incubated with Et_3PAuBr overnight at 70 °C. (A) TIC. (B) MS spectrum of peak at t_R = 3.69 minutes. (C) MS spectrum of peak at t_R = 4.55 minutes. (D) MS spectrum of peak at t_R = 5.53 minutes. The dark part corresponds to the elution of Et_3PAuBr.

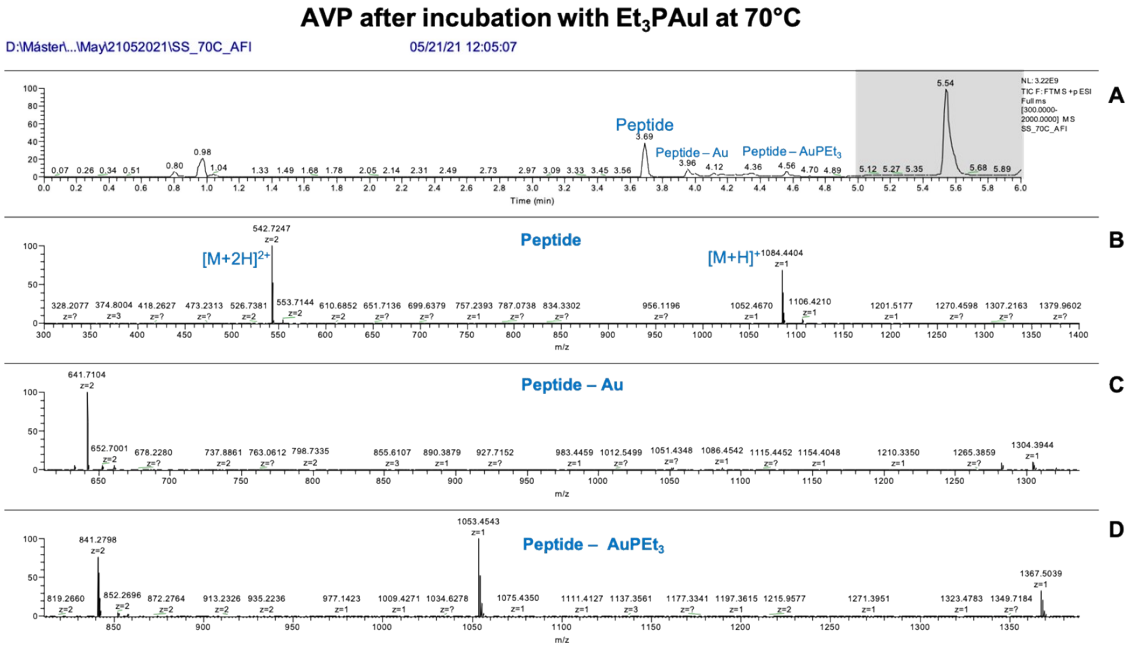


Figure S19. LC-MS of AVP incubated with Et_3PAuI overnight at 70 °C. (A) TIC. (B) MS spectrum of peak at t_R = 3.69 minutes. (C) MS spectrum of peak at t_R = 3.96 minutes. (D) MS spectrum of peak at t_R = 4.56 minutes. The dark part corresponds to the elution of Et_3PAuI.

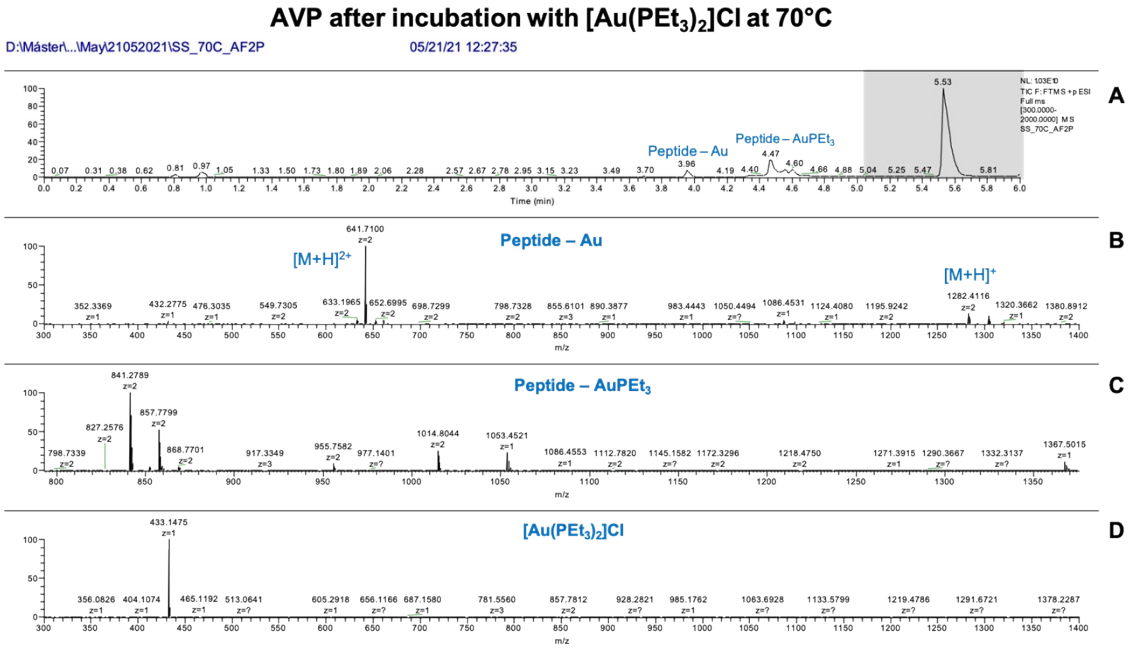


Figure S20. LC-MS of AVP incubated with $[Au(PEt_3)_2]Cl$ overnight at 70 °C. (A) TIC. (B) MS spectrum of peak at t_R = 3.96 minutes. (C) MS spectrum of peak at t_R = 4.47 minutes. (D) MS spectrum of peak at t_R = 4.60 minutes. The dark part corresponds to the elution of $[Au(PEt_3)_2]Cl$.

[Se-Se] AVP

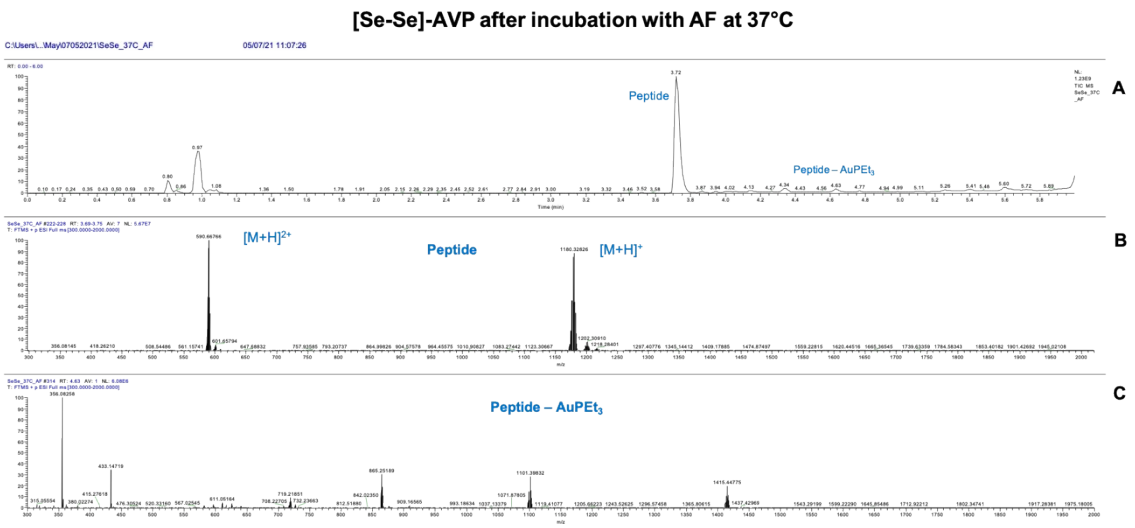


Figure S21. LC-MS of [Se-Se]-AVP incubated with AF overnight at 37°C. (A) TIC. (B) MS spectrum of peak at t_R = 3.72 minutes. (C) MS spectrum of peak at t_R = 4.6 minutes.

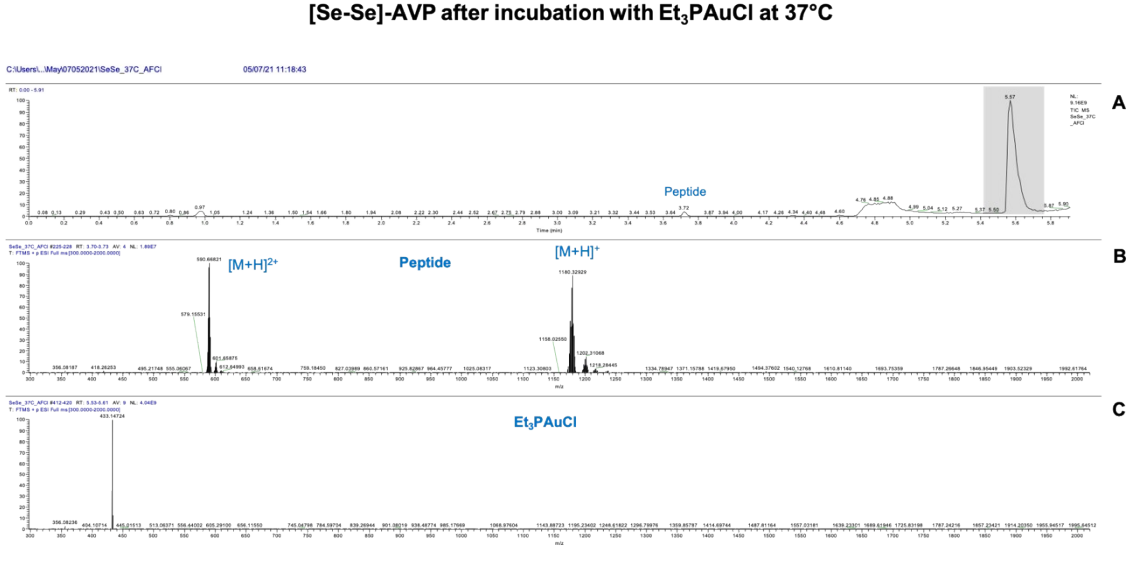


Figure S22. LC-MS of [Se-Se]-AVP incubated with Et_3PAuCl overnight at 37°C. (A) TIC. (B) MS spectrum of peak at t_R = 3.7 minutes. (C) MS spectrum of peak at t_R = 5.5 minutes. The dark part corresponds to the elution of Et_3PAuCl.

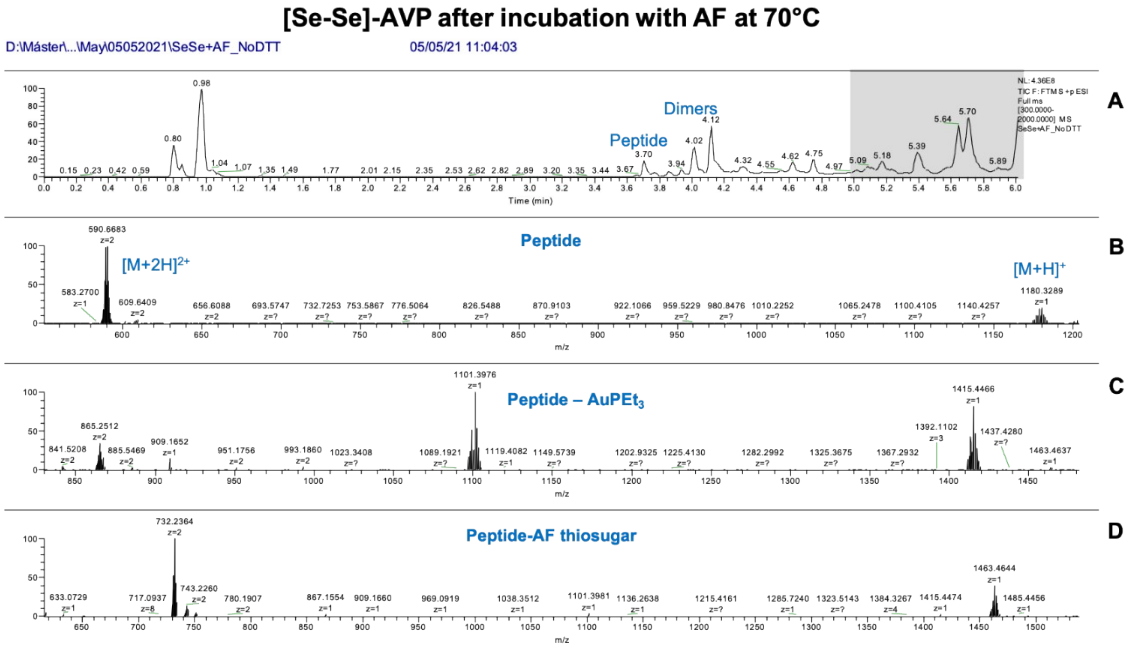


Figure S23. LC-MS of [Se-Se]-AVP incubated with AF overnight at 70°C. (A) TIC. (B) MS spectrum of peak at t_R = 3.70 minutes. (C) MS spectrum of peak at t_R = 4.62 minutes. (D) MS spectrum of peak at t_R = 4.75 minutes. The dark part corresponds to the elution of AF and its rearrangement products (3).

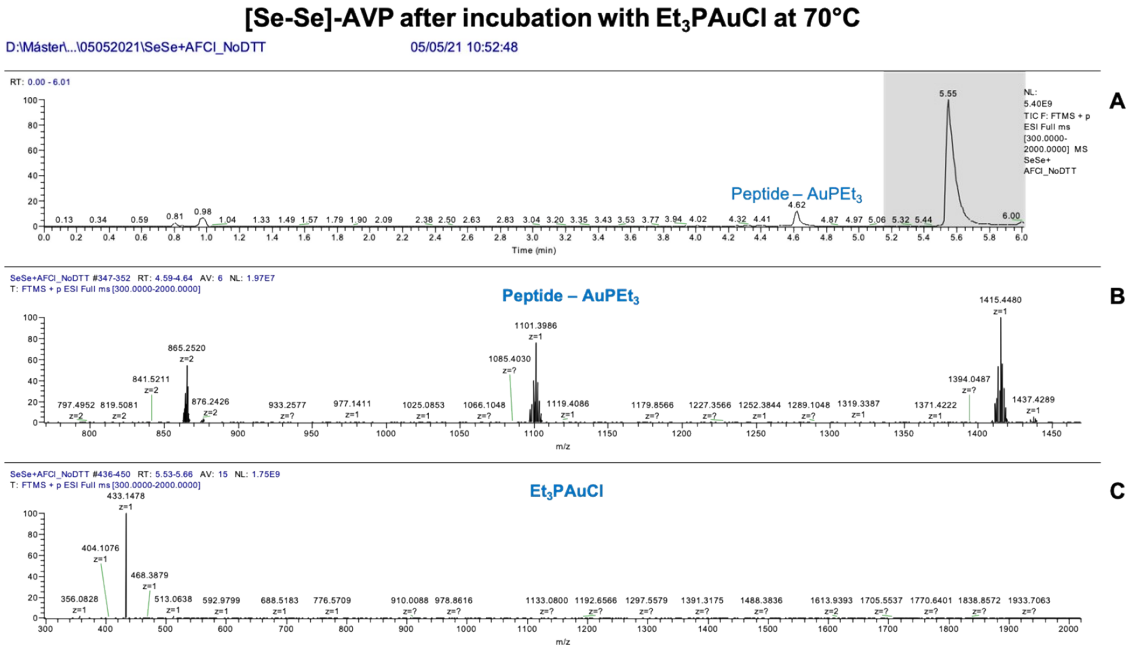


Figure S24. LC-MS of [Se-Se]-AVP incubated with Et_3PAuCl overnight at 70 °C. (A) TIC. (B) MS spectrum of peak at t_R = 4.62 minutes. (C) MS spectrum of peak at t_R = 5.55 minutes. The dark part corresponds to the elution of Et_3PAuCl.

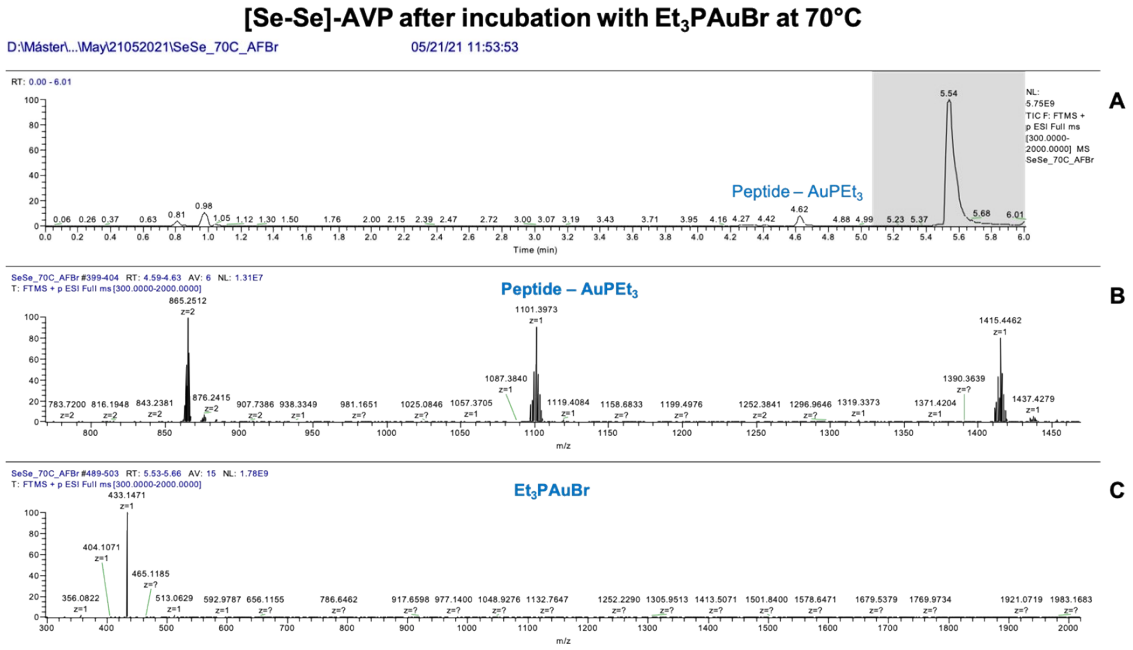


Figure S25. LC-MS of [Se-Se]-AVP incubated with Et_3PAuBr overnight at 70 °C. (A) TIC. (B) MS spectrum of peak at tR = 4.62 minutes. (C) MS spectrum of peak at tR = 5.54 minutes. The dark part corresponds to the elution of Et_3PAuBr.

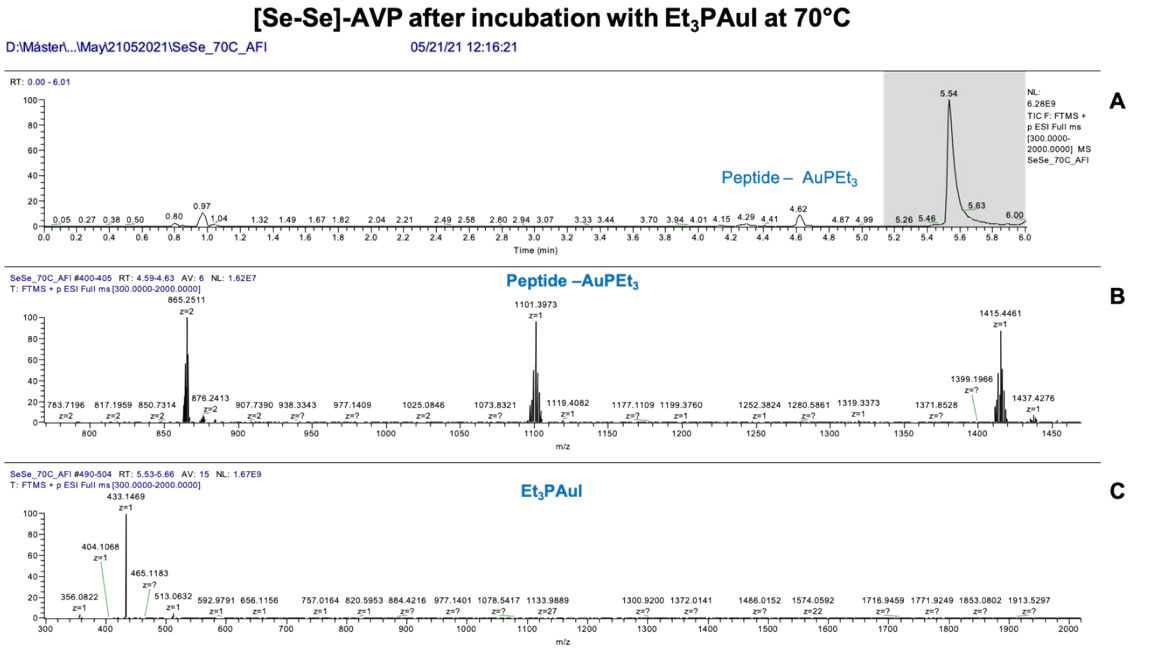


Figure S26. LC-MS of [Se-Se]-AVP incubated with Et_3PAuI overnight at 70 °C. (A) TIC. (B) MS spectrum of peak at t_R = 4.62 minutes. (C) MS spectrum of peak at t_R = 5.54 minutes. The dark part corresponds to the elution of Et_3PAuI.

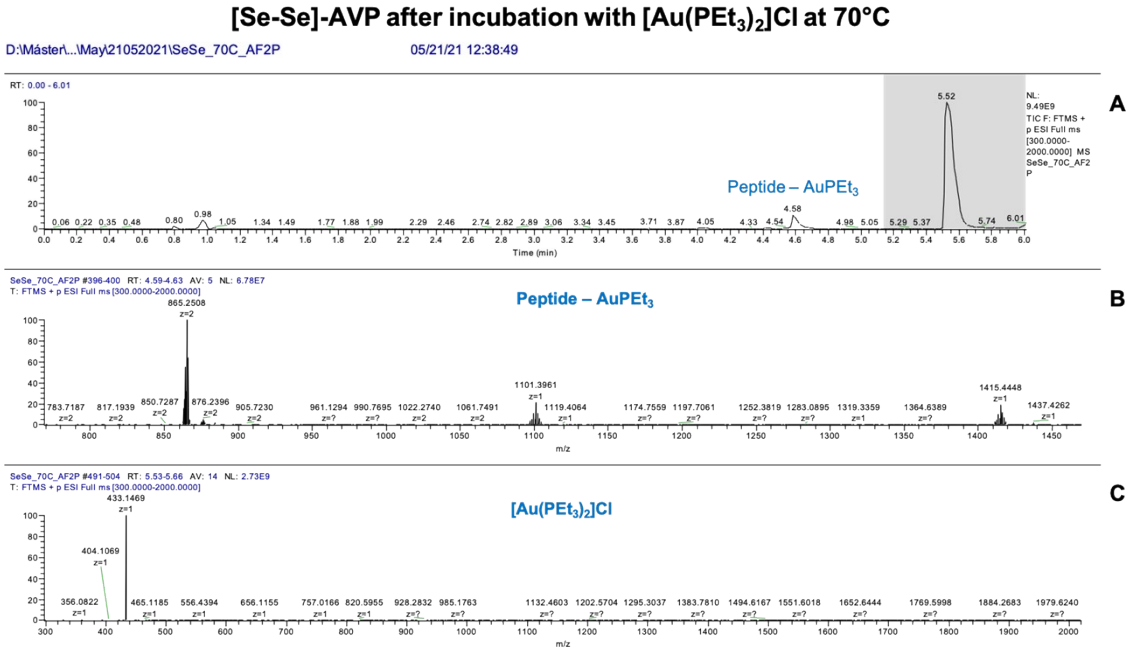


Figure S27. LC-MS of [Se-Se]-AVP incubated with $[Au(PEt_3)_2]Cl$ overnight at 70 °C. (A) TIC. (B) MS spectrum of peak at t_R = 4.58 minutes. (C) MS spectrum of peak at t_R = 5.52 minutes. The dark part corresponds to the elution of $[Au(PEt_3)_2]Cl$.

References

1 E. Cordeau, C. Arnaudguilhem, B. Bouyssiere, A. Hagège, J. Martinez, G. Subra, S. Cantel, C. Enjalbal, *PLOS one*, 2016, 11(6): e0157943.

2 T. Marzo, D. Cirri, S. Pollini, M. Prato, S. Fallani, M.I. Cassetta, A. Novelli, G.M. Rossolini, L. Messori, *Chemical Medicinal Chemistry*, 2018, **13**, 2448.

3 T. Shoeib, D. W. Atkinson, B. L. Sharp, *Inorganica Chimica Acta*, 2010, **363**, 184-192.