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Electronic Supplementary Information

Dynamic nanoproteins: Self-assembled peptide surfaces on monolayer protected gold nanoparticles

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1. Instrumentation and reagents

Instrumentation:

Fluorescence measurements were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with thermostatted cell holders.

HPLC purifications were performed on a preparative HPLC Shimadzu LC-8A equipped with a Shimadzu SPD-20A UV detector. HPLC analyses were performed on an analytical Shimadzu LC-10AT equipped with a Shimadzu SPD-10A UV detector. The UHPLC analysis were performed on an Agilent Technologies 1290 Infinity equipped with a DAD detector and a Quadrupole LC/MS.

An AB SCIEX MALDI TOF/TOF mass spectrometer was used for the characterization of the peptide library using α-cyano-4-hydroxycinnamic acid (CHCA) as matrix.

For the centrifugation of the samples an Eppendorf MiniSpin centrifuge was used.

Reagents:

All compounds were purchased from Sigma Aldrich. L-Amino acids were purchased from Sigma Aldrich or Iris Biotech. $Zn(NO_3)_2$ and $Cu(NO_3)_2$ were analytical grade products. In all cases, stock solutions were prepared using deionized water filtered with a MilliQ-water-purifier (Millipore) and stored at 4 °C.

The filtration experiments were performed using VIVASPIN 500 ultracentrifugation filters (500 µL, Sigma Aldrich, Z614025) with a PES (polyethersulfone) membrane having a 10 kDa of cut-off.

2. Synthesis and characterization of peptides I-VII

The synthesis of peptides **I-VII** was carried out by Fmoc-based SPPS (Solid Phase Peptide Synthesis) using 2-chlorotrityl resin (CTC resin) (f=1.6 mmol/g). The swelling was performed using DCM and DMF (30 min each). After coupling of the first amino acid, unreacted sites of the resin were deactivated using DCM/MeOH/DIEA 17:2:1 (3 x 10 min). The coupling steps were performed using 5 eq. of Fmoc-AA-OH, 5 eq HOBt and 5 eq DIPCDI. Deprotection of the Fmoc-group was performed using 20% piperidine in DMF (2 x 10 min). Final cleavage of the peptide from the resin was carried out using a mixture TFA:H_2O:TIS = 95:2.5:2.5. Cold diethyl ether (x 4) was used to precipitate the peptides.

The purification of the peptide library was performed by RP-HPLC using a C18 Jupiter 4 µ Proteo 90 Å, 250 x 21,1 mm reverse phase column. A binary eluent mixture of A: H_2O + TFA 0.1% and B: ACN + TFA 0.1% was used to create gradients as indicated in Table S1. After purification the peptides were lyophilized, tested for purity using UHPLC and characterized by MALDI-TOF (Table S1).

Table S1. Gradient, purity and mass of the peptides.

Peptide	Purification Gradient	Yield (%)	RT (min)	Purity* (%)	MALDI-TOF (m/z)	MALDI-TOF calc. (m/z)
I	40%-55% B in 30 min	2.4	8.2	≥ 98	763.4	$[M-H]^-$ = 763.3
II	20%-80% B in 30 min	1.3	16.3	≥ 99	695.4	$[M-H]^-$ = 695.3
III	20%-35% B in 30 min	20.1	3.8	≥ 97	697.3	$[M-H]^-$ = 697.2
IV	20%-60% B in 30 min	1.8	4.2	≥ 99	643.2	$[M-H]^-$ = 643.2
V	20%-35% B in 30 min	8.6	4.1	≥ 99	699.3	$[M-H]^-$ = 699.2
VI	20%-35% B in 30 min	19.9	3.8	≥ 97	725.4	$[M-H]^-$ = 725.3
VII	20%-35% B in 30 min	26.4	10.1	≥ 99	781.4	$[M-H]^-$ = 781.3

* Determined by UHPLC (Figure **S1**-**S7**)

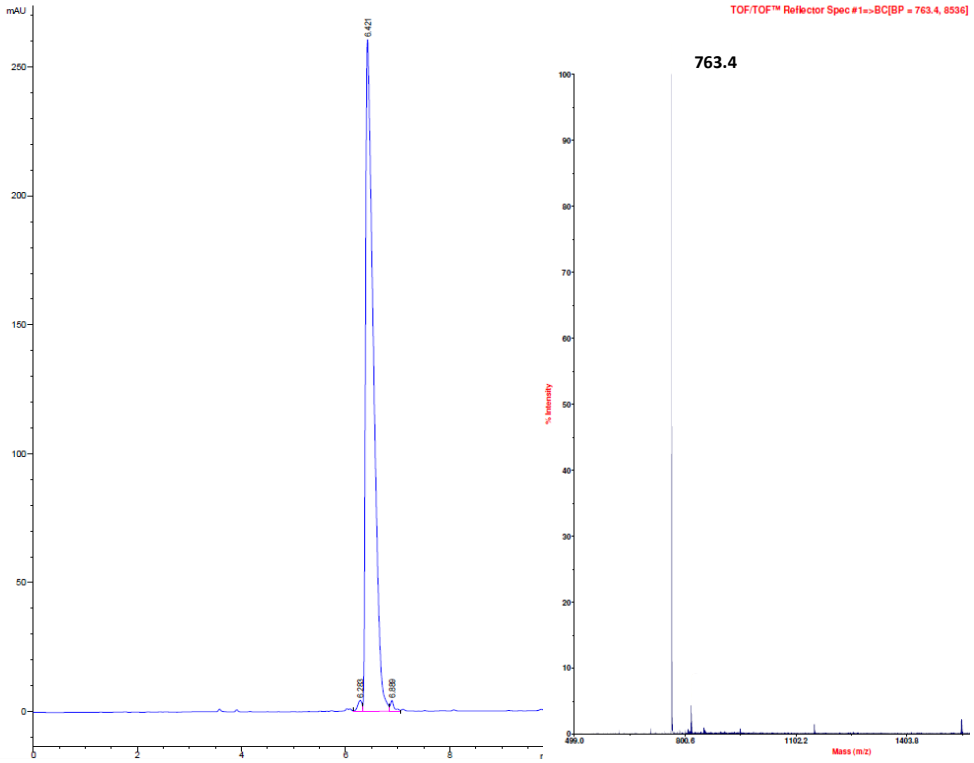


Figure S1. UHPLC chromatogram and MALDI-TOF spectrum of peptide **I**

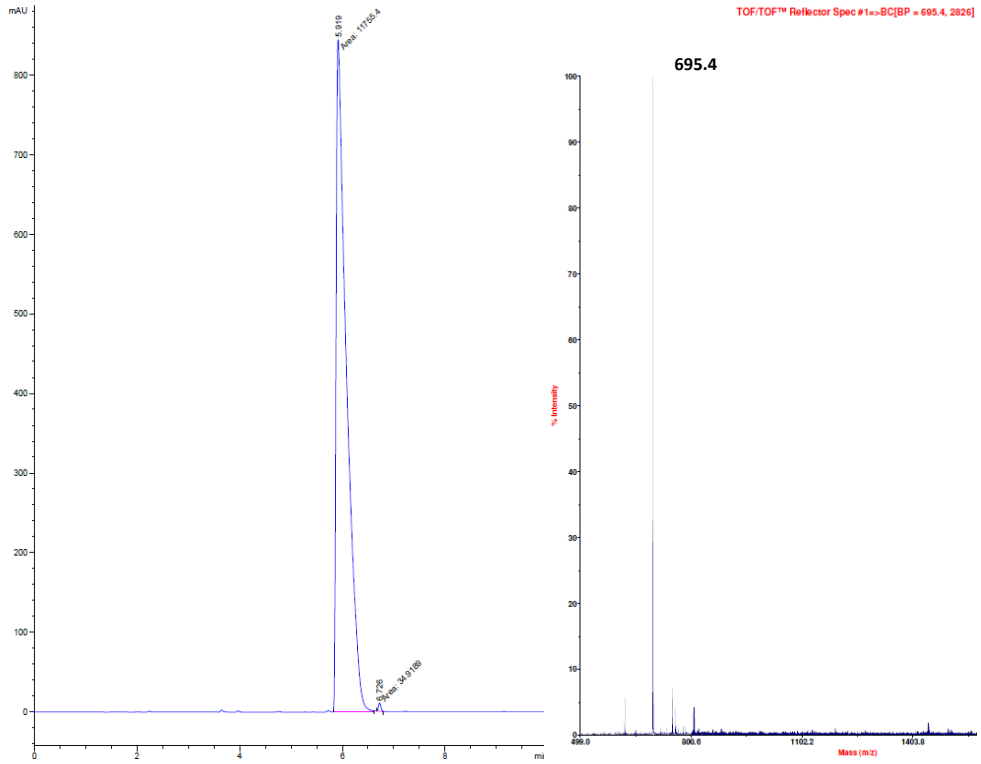


Figure S2. UHPLC chromatogram and MALDI-TOF spectrum of peptide **II**

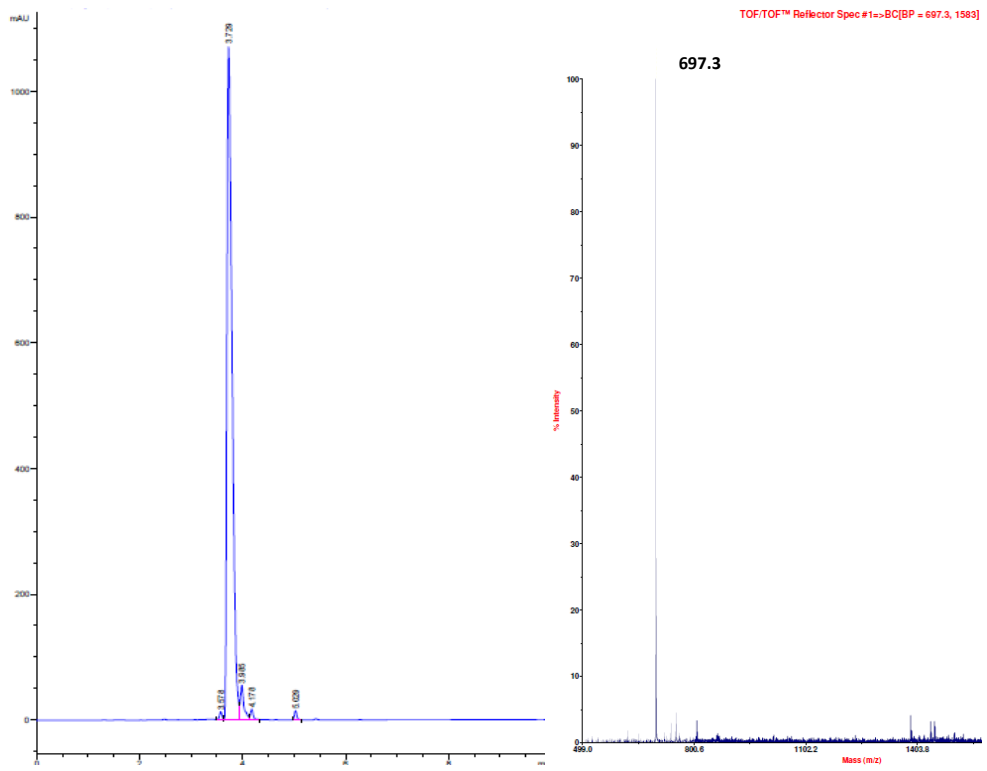


Figure S3. UHPLC chromatogram and MALDI-TOF spectrum of peptide **III**

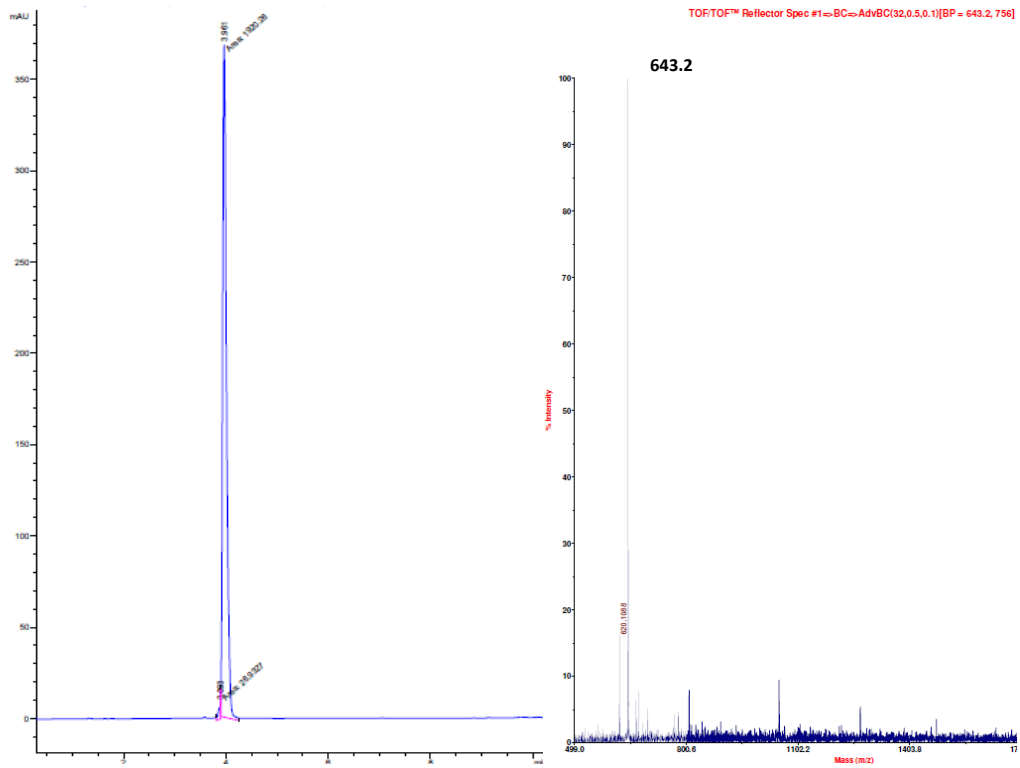


Figure S4. UHPLC chromatogram and MALDI-TOF spectrum of peptide **IV**

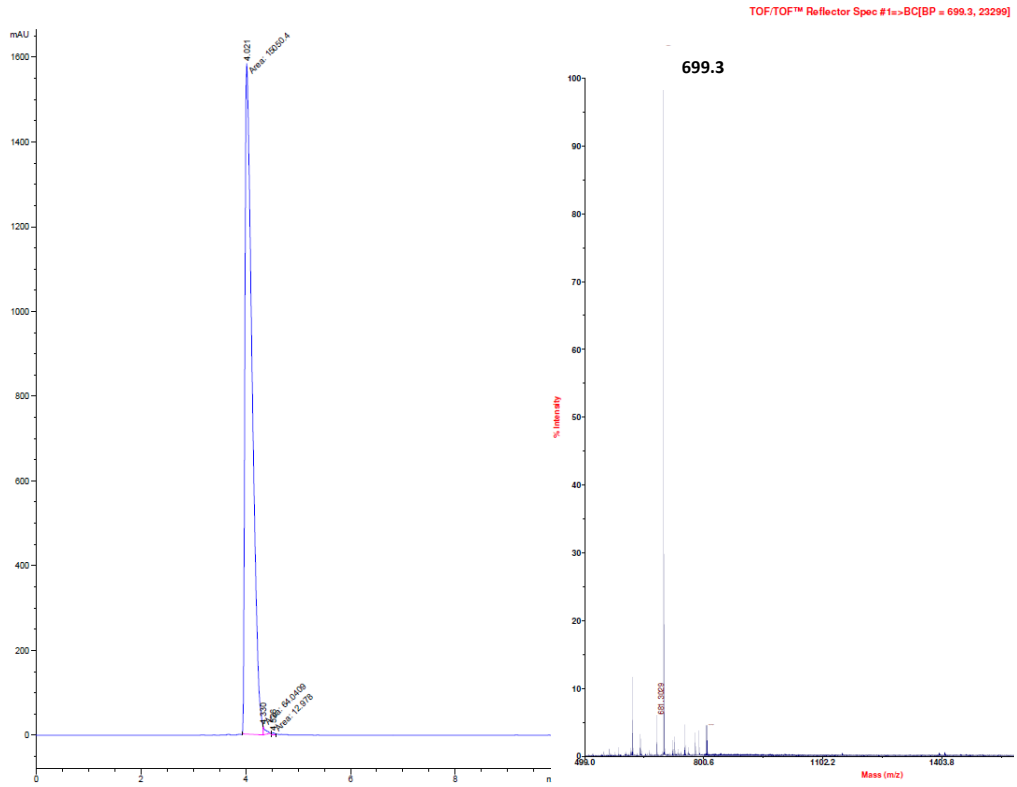


Figure S5. UHPLC chromatogram and MALDI-TOF spectrum of peptide **V**

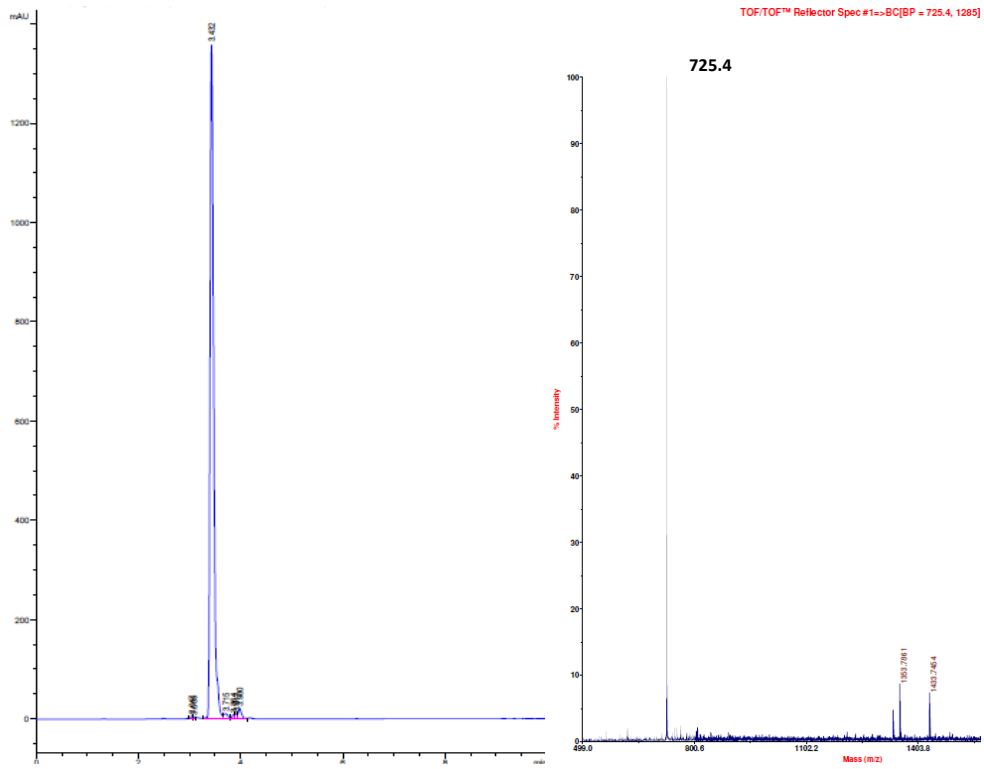


Figure S6. UHPLC chromatogram and MALDI-TOF spectrum of peptide **VI**

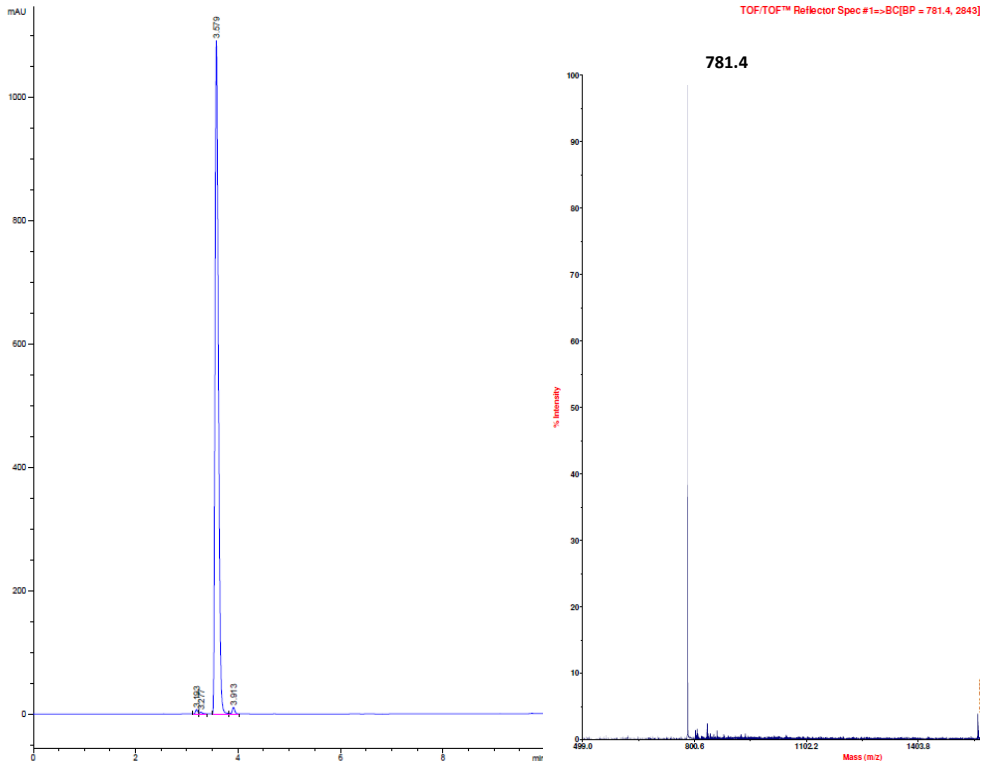


Figure S7. UHPLC chromatogram and MALDI-TOF spectrum of peptide **VII**

3. Binding and displacement experiments

The surface saturation concentrations (SSCs) of peptides **I - V** and the peptide mixture were determined as described previously (G. Pieters, A. Cazzolaro, R. Bonomi and L. J. Prins, *Chem. Commun.*, 2012, **48**, 1916-1918). The fluorescence intensities (λ_{ex}=280 nm, λ_{em}=360 nm, slits: 20/10 nm) were measured upon subsequent additions of the peptide after the signal had stabilized (2-3 minutes) (Fig. 2a, 2c). The SSC-values of peptides are summarized in Table S2 for [TACN·Zn^{2+}] = 10 ± 1 µM; [HEPES] = 10 mM, pH 7.0. The SSCs were determined via extrapolation of the linear part of the curves to the y-axis. For the peptide mixture the SSC was also determined at a Au NP **1** - concentration of 50 µM (Figure S8), because the filtration experiments were performed at this concencentration.

Table S2. SSC of the peptides and ADP-concentrations required to displace 50% of the peptide.

	SSC (µM)	$[ADP]_{50\%}$ (µM)
I	3.6	5.3
II	4.3	4.8
III	3.6	3.9
IV	3.1	3.0
V	3.1	17.3
Peptide Mixture	2.4	-

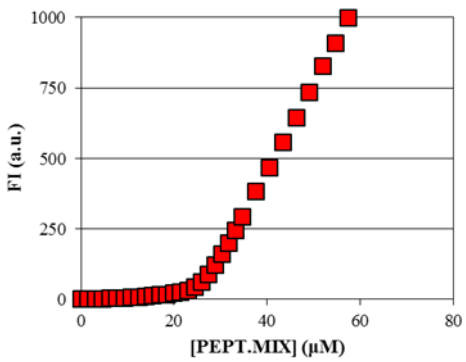


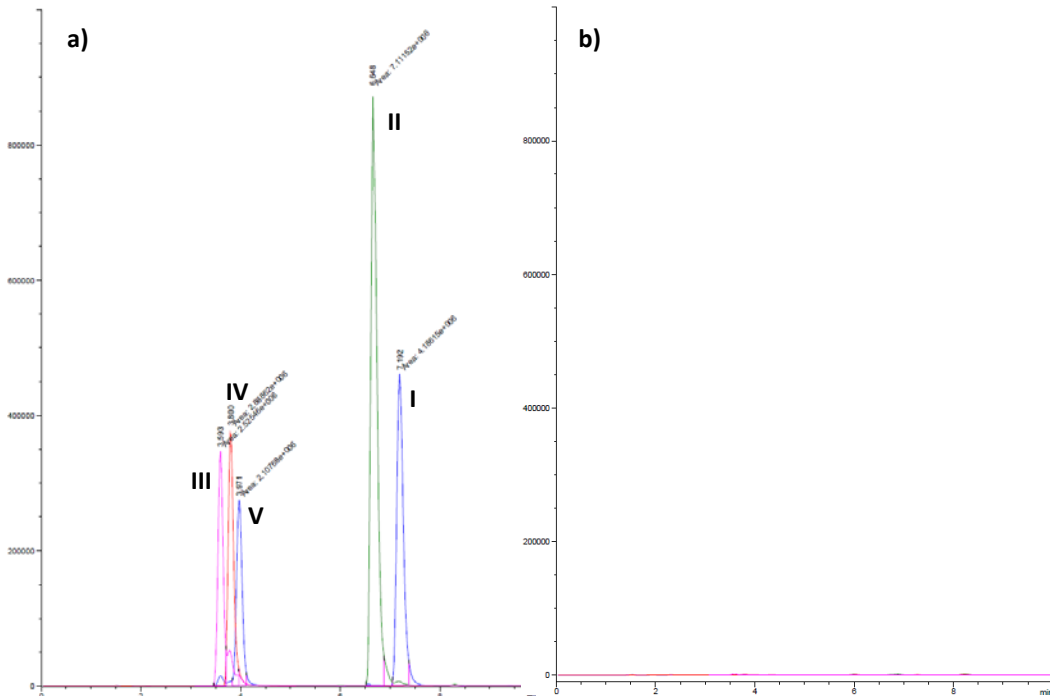
Figure S8. Binding assay of the peptide mixture at [TACN] = 50 ± 5 µM; [Zn^{2+}] = 10 µM; [HEPES] = 10 mM, pH 7.0.

Displacement experiments were performed using ADP as a non-fluorescent competing probe. Displacement experiments were carried out by measuring the fluorescent intensity of Trp (λ_{ex} = 280 nm, λ_{em} = 360 nm) upon addition of increasing amounts of ADP to Au NP **1** covered with peptides **I-V** at 80% of the SSC. To ensure that equilibrium was reached after each addition of ADP, the fluorescence intensity was measured in time until a constant value was obtained. Experimental conditions: [TACN·Zn^{2+}] = 10 ± 1 µM; [HEPES] = 10 mM, pH 7.0. The ADP-concentrations required to displace 50% of the peptide are given in Table S2. In each titration, the fluorescence intensities (after complete displacement) were normalized to the fluorescence intensity expected for a full displacement. This value was calculated based in the linear parts of the titration curves given in Figure 2a.

4. Ultracentrifugation experiments

The ultracentrifugation experiments were performed by preparing five samples containing equimolar amounts of peptides **I** - **V** at different concentrations (25, 50, 75, 100 and 125 µM) and Au NP **1** [TACN·Zn^{2+}] = 50 µM in aqueous buffer ([HEPES] = 30 mM at pH = 7 and 25 ^{o}C) reaching a final volume of 500 µL. Next, the solutions were equilibrated for 15 min before centrifugation (12.000 r.p.m.) was started with a duration of 15 sec. The total volume was reduced by around 20 %.

A 20 µl-sample (using glass insert) was injected in the UHPLC equipped with a Zorbax Eclipse Plus C18 column at a flow rate of 0.2 mL/min. A solvent gradient from 10% (v/v) ACN+0.1% HCOOH in H_2O+0.1% HCOOH to 80% (v/v) ACN+0.1% HCOOH in H_2O+0.1% HCOOH (10 min) was used. This concentration was kept constant for 12 min after which a gradient to 10% (v/v) ACN+0.1% HCOOH in H_2O+0.1% HCOOH was imposed (15 min). The column temperature was set to 40 ^{o}C. The peptides were monitored using SIM mode (selected-ion monitoring mode) with a separate channel dedicated to each of the peptides (Figure S9). The intensities were normalized to those obtained for the peptide mixture at the same concentration, but centrifuged in the absence of Au NP **1**. All measurements were repeated five times, yielding the average values given in Figure 3b.



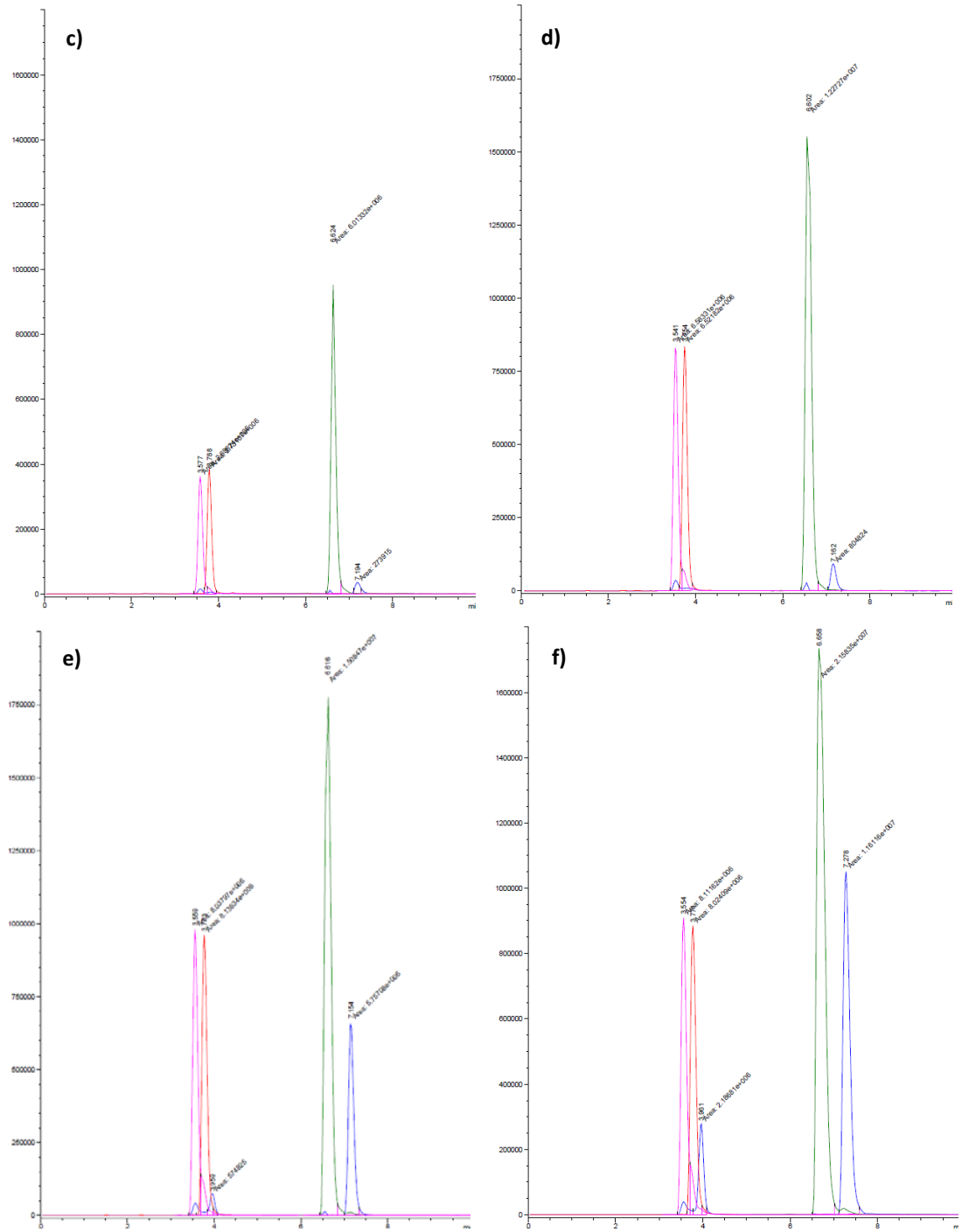
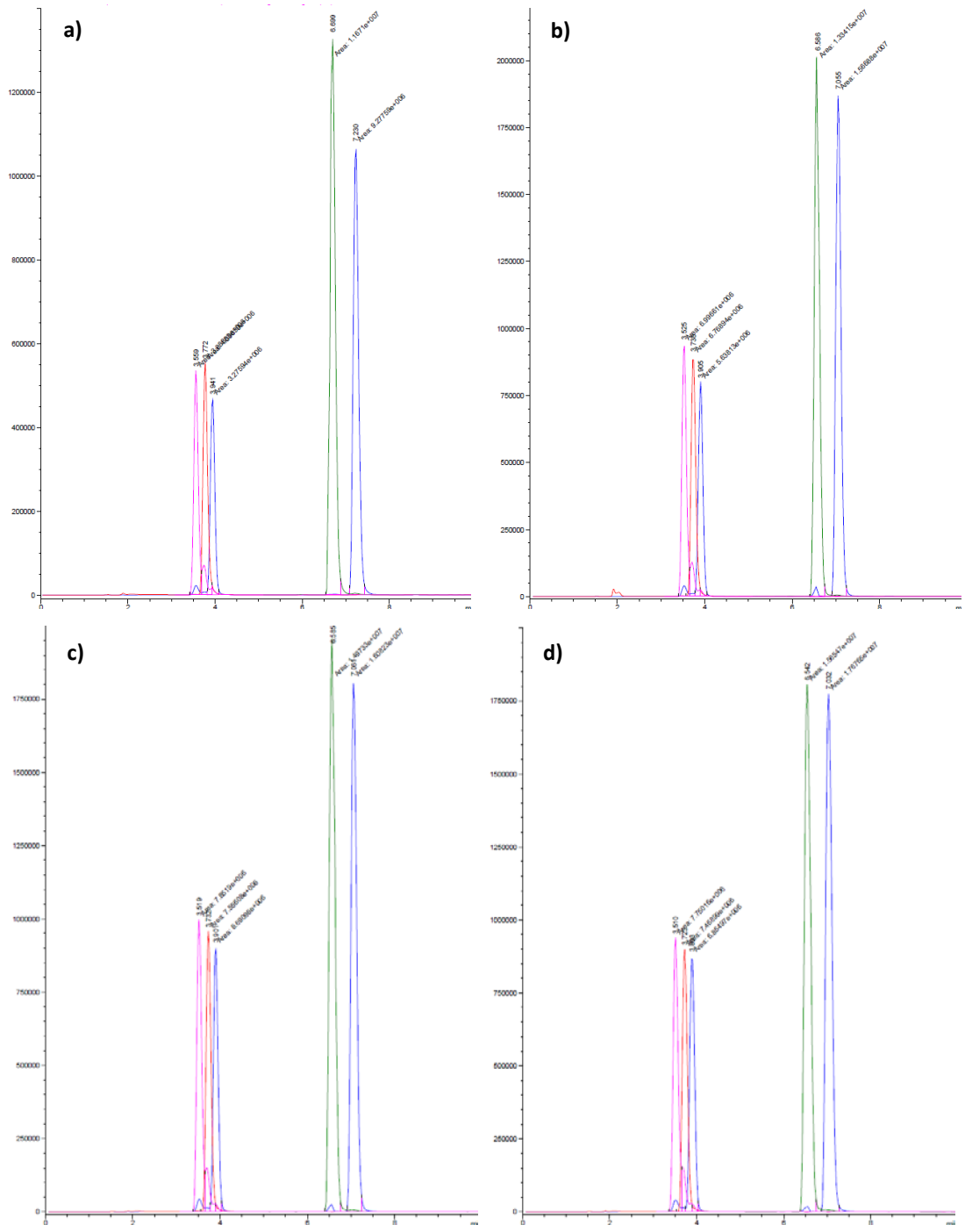


Figure S9. Representative chromatograms of the filtrates obtained from: (**a**) Control (without Au NP **1**) at 25 µM, (**b**) 25 µM, (**c**) 50 µM, (**d**) 75 µM, (**e**) 100 µM, (**f**) 125 µM of the peptide mixture.

The same experiments were repeated in the presence of the strong competitor [ATP] = 200 µM in order to demonstrate that the absence of peptides in the filtrate indeed originated from their interaction with Au NP **1** (Figure S10).



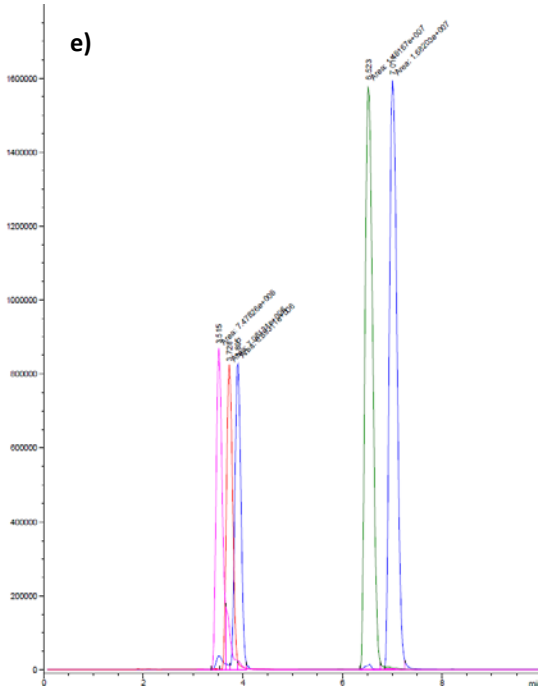


Figure S10. Representative examples of the chromatograms obtained at different concentrations of the peptide mixture: (**a**) 25 µM, (**b**) 50 µM, (**c**) 75 µM, (**d**) 100 µM, (**e**) 125 µM in the presence of [ATP]= 200 µM.