SUPPORTING INFORMATION

Metal-dependent DNA recognition and cell internalization of designed, basic peptides

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1. Abbreviations

DEDTC: sodium diethyldithiocarbamate

DIC: N,N-diisopropyl-carbodiimide

DIEA: diisopropylethylamine

DMEM: Dulbecco's Modified Eagle Medium

HATU: 2-(1H-7-aza-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate

TFA: trifluoroacetic acid

TIS: triisopropylsilane

TMR: tetramethylrhodamine

p-ABA: 4-acetamidobenzoic acid

FITC: Fluorescein-5-Isothiocyanate

Ahx: 6-Aminohexanoic acid

2. Reagents

$Pd(en)Cl_2$, $Pd(PPh_3)_2Cl_2$, $Pd(bpy)Cl_2$, K_2PdCl_6, K_2PdCl_4, *trans*-$Pd(dppe)Cl_2$, $Pt(bpy)Cl_2$, $Pt(en)Cl_2$, K_2PtCl_4, $NiCl_2$, $ZnSO_4$, $CuSO_4$, $Co(ClO_4)_2$ and $Fe(NH4)_2(SO_4)_2$ were purchased from *Aldrich*. $Cu(bpy)Cl_2$,[1] $Ni(bpy)Cl_2$,[2] $Pd(bpy)(NO_3)_2$,[3] $[Au(en)Cl_2]Cl$,[4] and $[Au(bpy)Cl_2]PF_6$,[5] were synthesized according to the literature procedures.

3. Synthesis and characterization of peptides

All peptide synthesis reagents and amino acid derivatives were purchased from *GL Biochem* (Shanghai) and *Novabiochem*; amino acids were purchased as protected Fmoc amino acids with the standard side chain protecting scheme: Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-Glu(O*t*-Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ile-OH, Fmoc-Thr(*t*-Bu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-His(Trt)-OH and Fmoc-Asp(O*t*-Bu)-OH except for the orthogonally protected Fmoc-Lys(Alloc)-OH, which was purchased from *Bachem*. All other chemicals were purchased from *Aldrich* or *Fluka*. All solvents were synthesis grade, unless specifically noted.

Peptides were synthesized using an automatic microwave peptide synthesizer *CEM Liberty Lite*. following the recommended procedures by the manufacturer: Peptide syntheses was performed using Fmoc strategy on a PAL-PEG-PS resin (0.19 mmol/g) using DIC as activator, Oxime as base, and DMF as solvent. The deprotection of the temporal Fmoc protecting group was performed by treating the resin with 20% piperidine in DMF.

br, **brHis**, **brHis$_2$** and **br(S**) included the p-acetamidobenzoic acid (p-ABA) at their N-terminal site for quantification.

Coupling of TMR and fluorescein: Fmoc-6-aminohexanoic acid was added as a spacer between the peptide amino terminal side, and the fluorophore, and, after standard removal of the Fmoc protecting group, the corresponding fluorophore was added: 5(6)-carboxytetramethylrhodamine was coupled using 3 equiv e (0.15 mmol, 64.5 mg), 3 equiv of HATU and 5 equiv of DIEA 0.2 M in DMF for 60 min; Fluorescein-5-Isothiocyanate was coupled using 4 equiv, and 5 equiv of DIEA 0.2 M in DMF for 120 min.

Cleavage/deprotection step was performed by treatment of the resin-bound peptide for 2 h with the following cleavage cocktail: 900 µL TFA, 50 µL CH_2Cl_2, 25 µL H_2O and 25 µL TIS (1 mL of cocktail/40 mg resin).

High-Performance Liquid Chromatography (HPLC) was performed using an *Agilent 1100* series Liquid Chromatograph Mass Spectrometer system. Analytical HPLC was carried out using an *Eclipse XDB-C18* analytical column (4.6 x 150 mm, 5 μm), 1 mL/min, gradient 5 to 75% B over 30 min. Purification of the peptides was performed on a semipreparative *Phenomenex Luna–C18* (250 × 10 mm) reverse-phase column.

The crude products were purified by RP–HPLC, 4 mL/min, gradient 5 to 75% B over 40 min. (A: H_2O 0.1% TFA, B: CH_3CN 0.1% TFA) and identified as the desired peptides.

Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an *Agilent 1100* Series LC/MSD VL G1956A model in positive scan mode.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI/MS) was performed with a Bruker Autoflex MALDI/TOF model in positive scan mode by direct irradiation of the matrix-absorbed peptide.

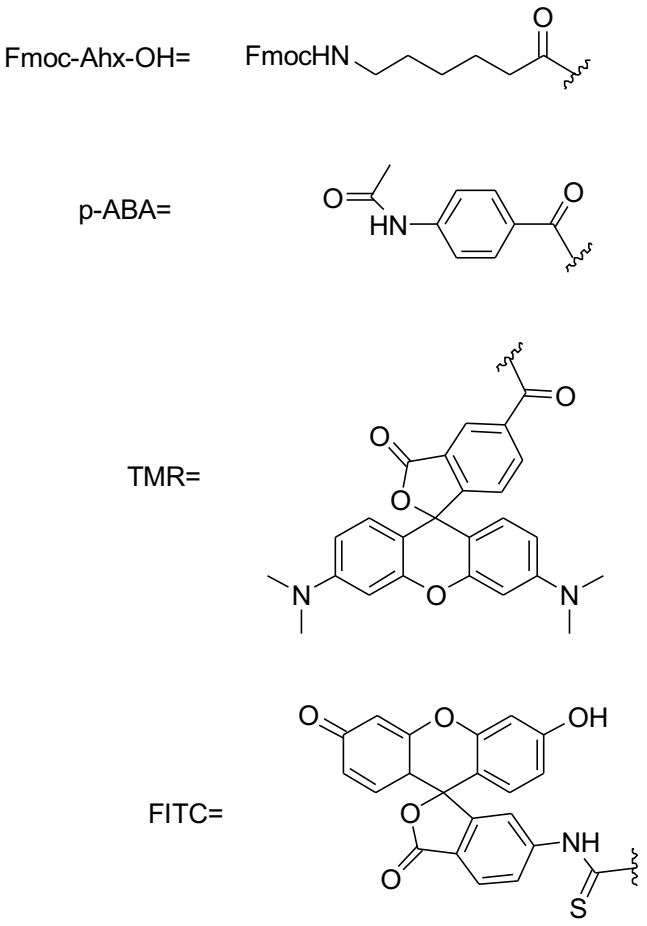

Fig. S1 Structures of Fmoc-Ahx-OH, p-ABA, TMR and FITC.

brHis$_2$ pABA-D-P-A-A-**H**-K-R-A-**H**-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-R-CONH$_2$

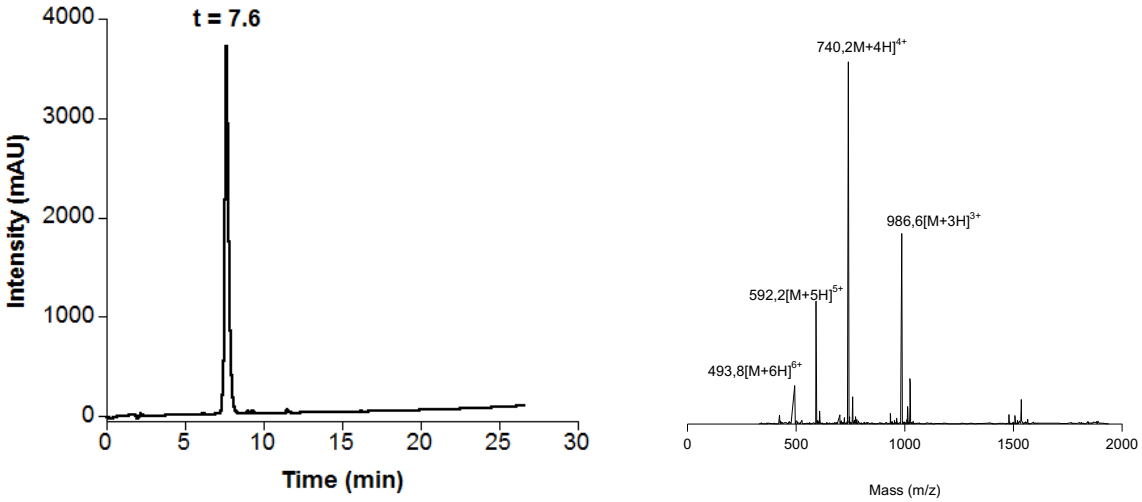

Fig. S2 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI$^+$ (m/z): Calcd. for $C_{123}H_{205}N_{51}O_{35}$: 2956.6. Found: 986.6 $[M+3H]^{3+}$; 740.2 $[M+4H]^{4+}$; 592.2 $[M+5H]^{5+}$; 493.8 $[M+6H]^{6+}$

br pABA-D-P-A-A-L-K-R-A-R-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-R-CONH$_2$

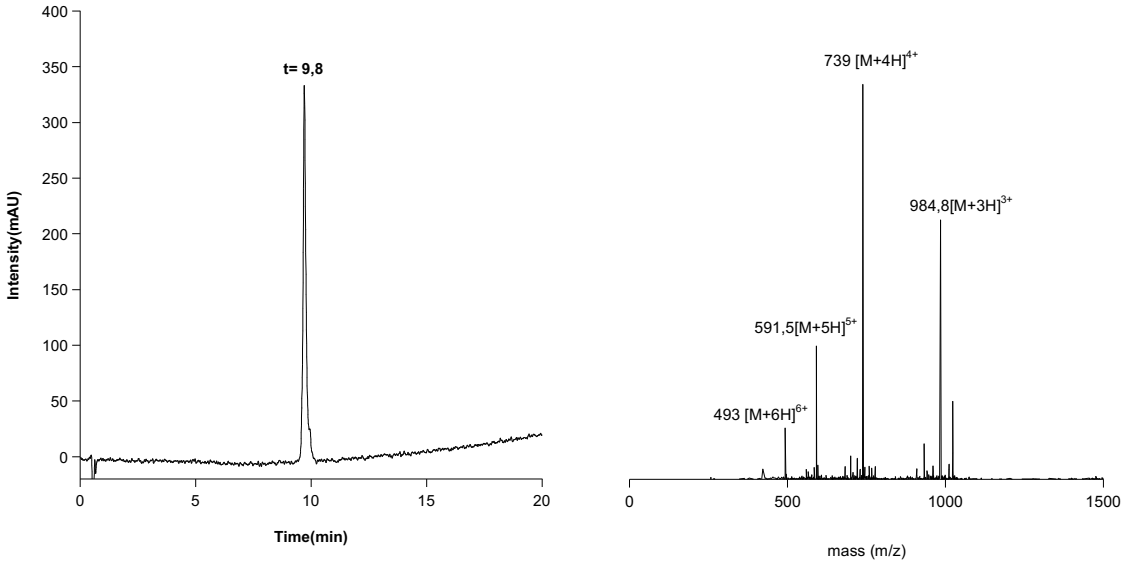

Fig. S3 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI$^+$ (m/z): Calcd. for $C_{123}H_{214}N_{50}O_{35}$: 2951.65. Found: 984.8 $[M+3H]^{3+}$; 739 $[M+4H]^{4+}$; 591.5 $[M+5H]^{5+}$; 493 $[M+6H]^{6+}$

brHis pABA-D-P-A-A-L-K-R-A-**H**-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-R-$CONH_2$

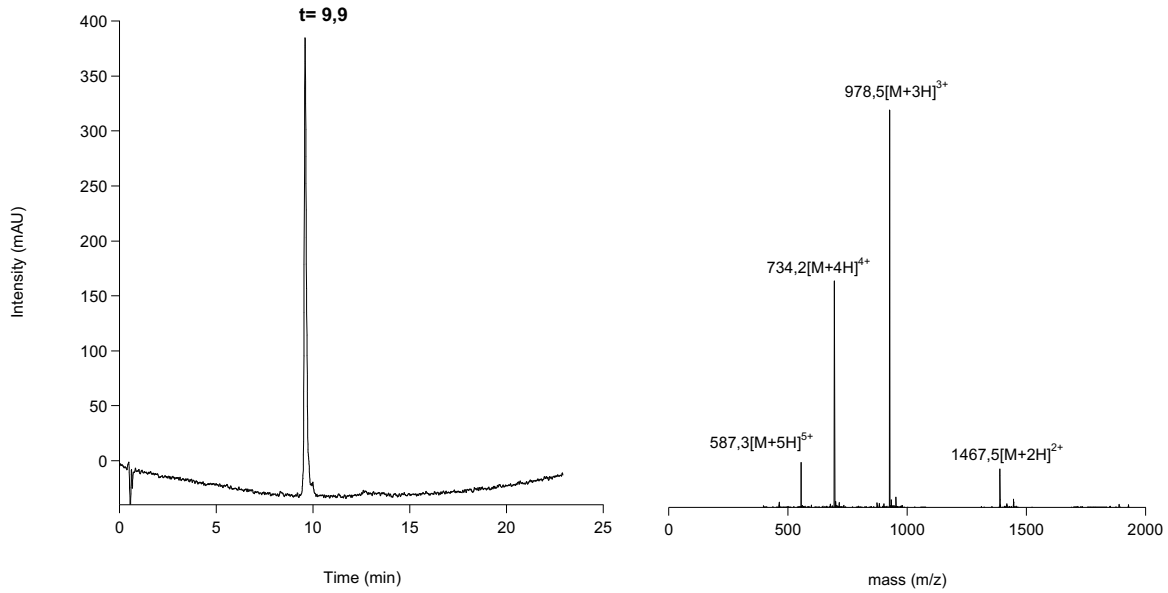

Fig. S4 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI^+ (m/z): Calcd. for $C_{123}H_{209}N_{49}O_{35}$: 2932.51. Found: 1467.5 $[M+2H]^{2+}$; 978.5 $[M+3H]^{3+}$; 734.2 $[M+4H]^{4+}$; 587.3 $[M+5H]^{5+}$

TMR-brHis$_2$ TMR-Ahx-D-P-A-A-**H**-K-R-A-**H**-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-R-$CONH_2$

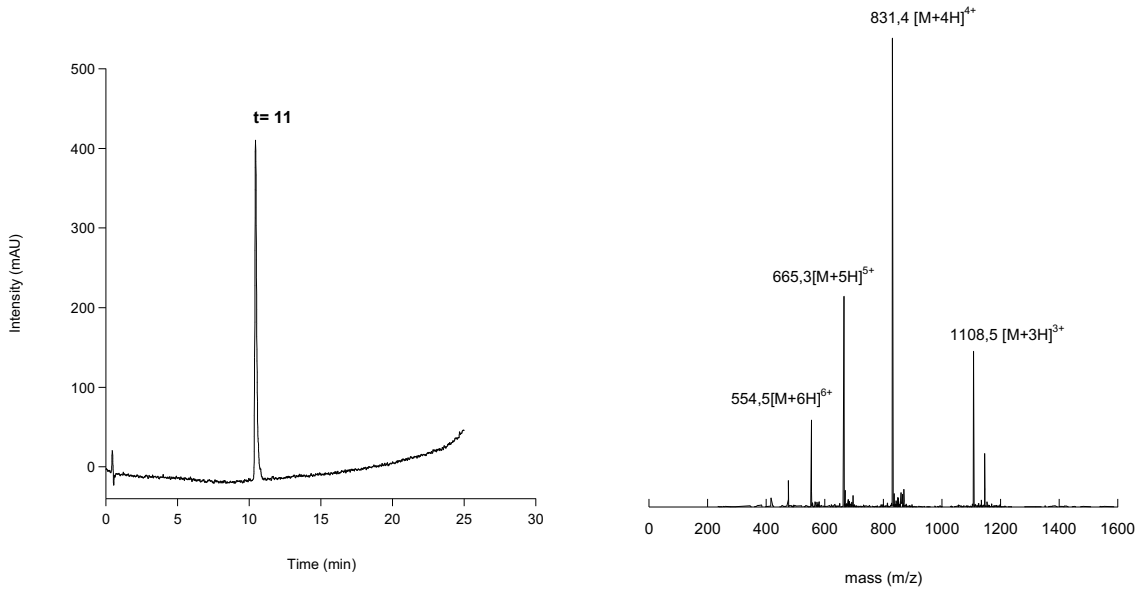

Fig. S5 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI^+ (m/z): Calcd. For $C_{146}H_{224}N_{53}O_{38}$: 3324. Found: 1108.5 $[M+3H]^{3+}$; 831.4 $[M+4H]^{4+}$; 665.3 $[M+5H]^{5+}$; 554.5 $[M+6H]^{6+}$;

TMR-br TMR-Ahx-D-P-A-A-L-K-R-A-R-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-R-$CONH_2$

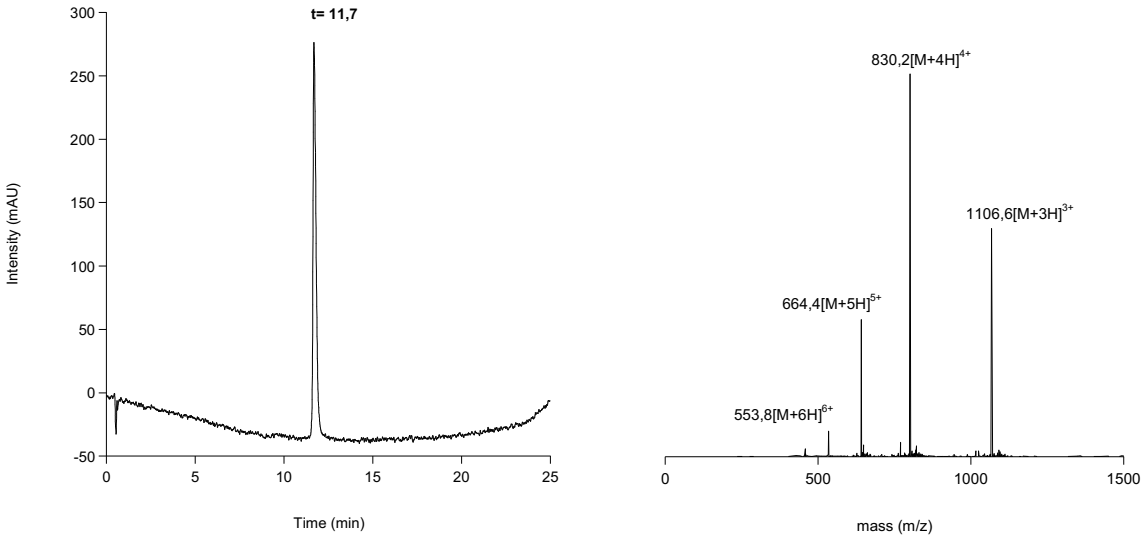

Fig. S6 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI^+ (m/z): Calcd. For $C_{146}H_{230}N_{52}O_{38}$: 3317. Found: 1106.6 $[M+3H]^{3+}$; 830.2 $[M+4H]^{4+}$; 664.4 $[M+5H]^{5+}$; 553.8 $[M+6H]^{6+}$;

TMR-brHis TMR-Ahx-D-P-A-A-L-K-R-A-**H**-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-R-$CONH_2$

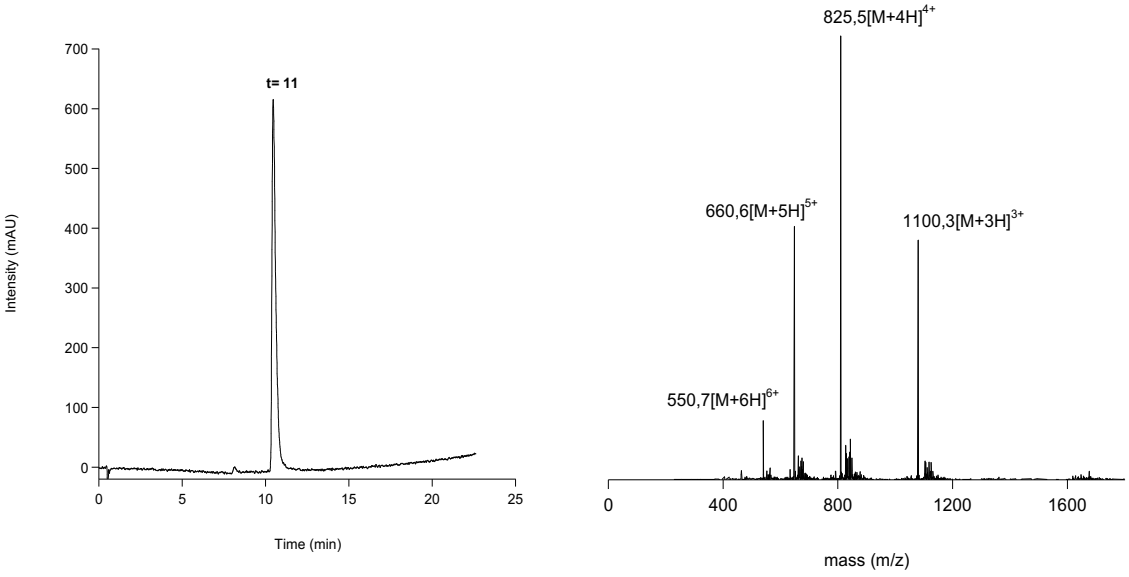

Fig. S7 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI^+ (m/z): Calcd. For $C_{146}H_{226}N_{51}O_{38}$: 3298. Found: 1100.3 $[M+3H]^{3+}$; 825.5 $[M+4H]^{4+}$; 660.6$[M+5H]^{5+}$; 550.7 $[M+6H]^{6+}$;

br(S) pABA-D-P-A-A-L-K-R-A-R-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-G-G-C-CONH

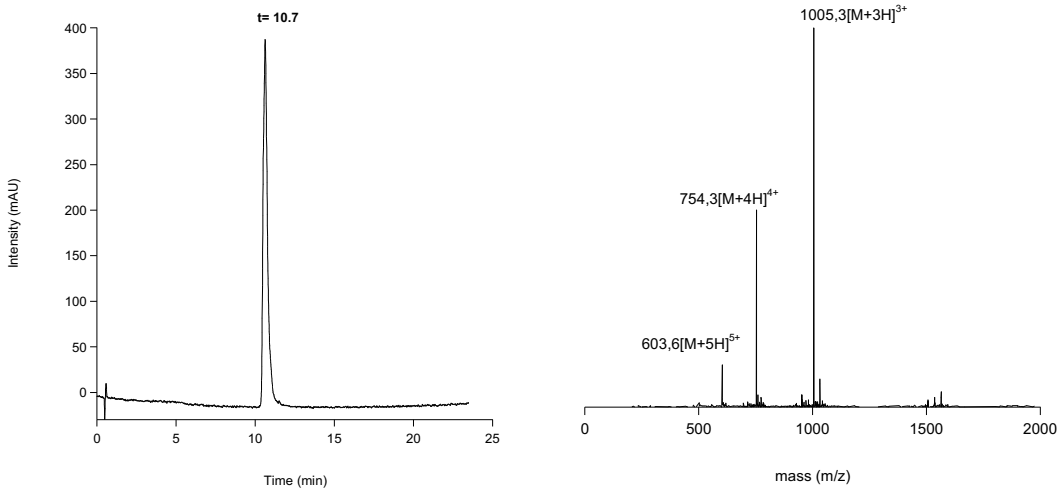

Fig. S8 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI$^+$ (m/z): Calcd. For $C_{124}H_{215}N_{50}O_{36}S$: 3012.6. Found: 1005.3 $[M+3H]^{3+}$; 754.3 $[M+4H]^{4+}$; 603.6 $[M+5H]^{5+}$

Synthesis of the disulfide dimer br$_2$(SS). The monomeric thiol-containing peptide **br(S)** was dissolved in 100 mM phosphate buffer (pH 8.3, 2 mM), and an air current was passed through the solution. Ellmann's reagent[6] (DTNB, 0.8 mM, 0.4 equiv) was added, and the resulting mixture was stirred for 1.5 h at rt. The reaction was quenched using 0.1% aqueous TFA, and the crude purified by RP-HPLC. The collected fractions were lyophilized and stored at –20 °C, after characterization.

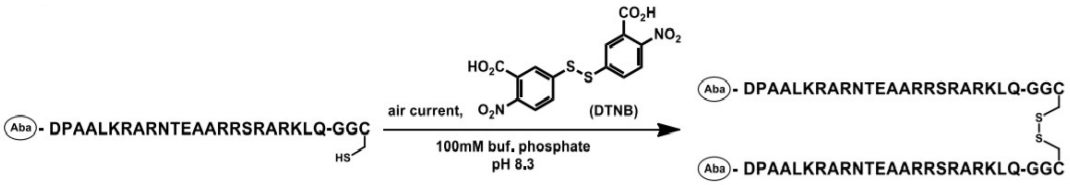

Fig. S9 Synthesis of the disulfide dimer **br$_2$(SS)**.

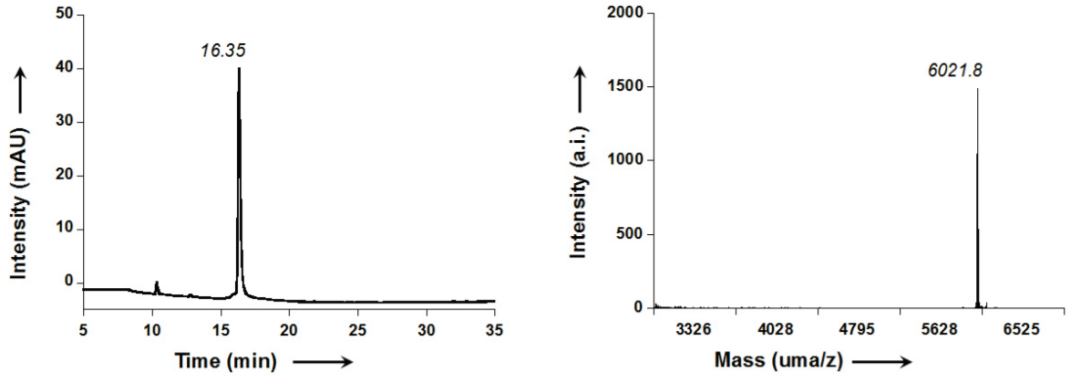

Fig. S10 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the peptide **br$_2$(SS).**

EM–ESI$^+$ (m/z): Calcd. For $C_{248}H_{427}N_{100}O_{72}S_2$: 6025.8. Found: 6026.4.

4. Oligonucleotide Sequences

Double stranded (only one strand is shown) oligonucleotides used for EMSA experiments were supplied by *Thermo Fischer* and their sequences were:

GTCAT	5'-CGC**GTCAT**AATTGAGAGCGC-3'
MUT	5'-CGC**GT**G**AT**AATTGAGAGCGC-3'
FULL MUT	5'-CGACGCTCTCAAATTCCGTC -3'
TMR-GTCAT	5'-(TMR)-CGC**GTCAT**AATTGAGAGCGC-3'
AP-1	5'-TGGAG**ATGAcgTCAT**CTCGT-3'

5. EMSA experiments

EMSA experiments were performed with a *BioRad Mini Protean* gel system, powered by an electrophoresis power supplies *PowerPac* Basic model, maximum power 150 V, frequency 50-60 Hz at 140 V (constant V). Binding reactions were performed over 30 min in 18 mM Tris-HCl (pH 7.5), 90 mM KCl, 1.8 mM $MgCl_2$, 0.2 mM TCEP, 9% glycerol, 0.11 mg/mL BSA and 2.2% NP40. For the experiments we used 75 nM of the dsDNAs, and a total incubation volume of 20 µL. After incubation for 30 min at rt, products were resolved by PAGE using a 10% non-denaturing polyacrylamide gel and 0.5× TBE buffer (0.445M Tris, 0.445 M Boric acid) for 40 min at 25 ºC, and analyzed by staining with SyBrGold (Molecular Probes: 5 µL in 50 mL of 1× TBE) for 10 min. and visualized by fluorescence.

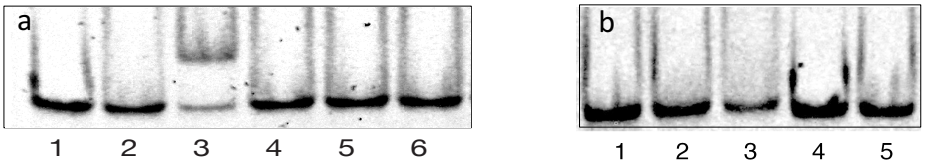

Fig. S11 The DNA binding profile was further confirmed using three different peptides: **br** (no mutations), **brHis** (single His mutated) and **brHis$_2$**. Gel **a**: Lanes 1-3: 75nM ***GTCAT***; Lanes 2-3: 2 μM **brHis$_2$**; Lane 3: 20 μM Pd(en)Cl_2; Lanes 4-6: 75nM ***FULL MUT***; Lanes 5-6: 2 μM **brHis$_2$**; Lane 6: 20 μM Pd(en)Cl_2. Gel **b**: Lanes 1-5: 75nM ***GTCAT***; Lanes 2-3: 2 μM **br**; Lane 3: 20 μM Pd(en)Cl_2; Lanes 4-5: 2 μM **brHis**; Lane 5: 20 μM Pd(en)Cl_2.

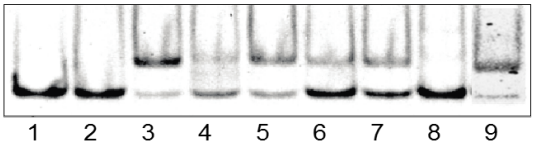

Fig. S12 Screening of different Pd complexes as stapling agents: Lanes 1-9: 75 nM ***GTCAT***; lanes 2-9: 2 μM **brHis$_2$**; Lane 3: 20 μM Pd(en)Cl_2; lane 4: 20 μM Pd(dppe)Cl_2; lane 5: 20 μM Pd(bpy)Cl_2; lane 6: 20 μM K_2PdCl_6; lane 7: 20 μM K_2PdCl_4; lane 8: 20 μM *trans*-Pd$(PPh_3)_2Cl_2$; lane 9: 20 μM Pd(bpy)$(NO_3)_2$. All the metal complexes were dissolved in H_2O/DMSO (1:1) with a concentration of 10mM, to improve the solubility. Then, we prepared stock aliquots of 1 mM, in H_2O, for each of them.

6. Fluorescence Anisotropy

Measurements were made with a *Jobin-Yvon Fluoromax-3*, (DataMax 2.20) coupled to a Wavelength Electronics LFI–3751 temperature controller, using the following settings: integration time: 2.0 s; excitation slit width: 5.0 nm; emission slit width: 20.0 nm; excitation wavelength 559 nm; emission wavelength 585 nm.

Tritation of $Pd(en)Cl_2$ with TMR-$brHis_2$

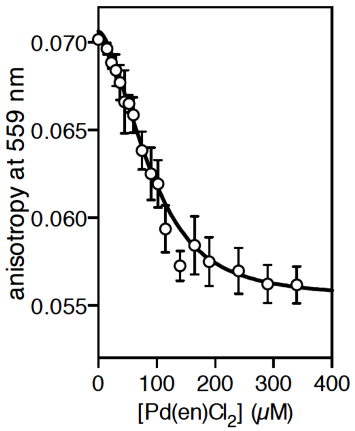

Fig. S13 5 µL of a water solution of **TMR-$brHis_2$** (5 µM) were added to 995 µL of Tris-HCl buffer 20 mM pH 7.5 100 mM NaCl (final concentration 25nM), and the anisotropy was measured at 559 nm, at rt. Aliquots of a stock solution in water of $Pd(en)Cl_2$ were successively added to this solution, starting with 1 nM, and the anisotropic value was recorded after each addition. Experimental data correspond to the mean of three independent titration experiments. We calculated an apparent dissociation constant of 3 μM at 25°C.

7. Circular Dichroism Measurements (CD)

Circular Dichroism experiments were performed on a *Jasco-715* coupled with a thermostat *Nestlab* RTE-111. The settings used were: Acquisition range: 300-195 nm; band width: 2.0 nm; resolution: 0.2 nm; accumulation: 5 scans; sensitivity 10 mdeg; response time: 0.25 s; speed: 100 nm/min. Measurements were carried out in a 2 mm cuvette at 25 ºC. Samples contained 10 mM phosphate buffer pH 7.5 and 100 mM of NaCl, 5 μM peptide, and 5 μM of corresponding dsDNA (when present).

The mixtures were incubated for 5 min before registering. The CD spectra of the peptides or peptides with metal complexes (when measured in the presence of DNA) were calculated as the difference between the spectrum of the peptide/DNA mixture and the measured spectrum of a sample of the DNA oligonucleotide. The spectra are the average of 5 scans and were processed using the "smooth" macro implemented in the program *Kaleidagraph* (v 3.5 by Synergy Software).

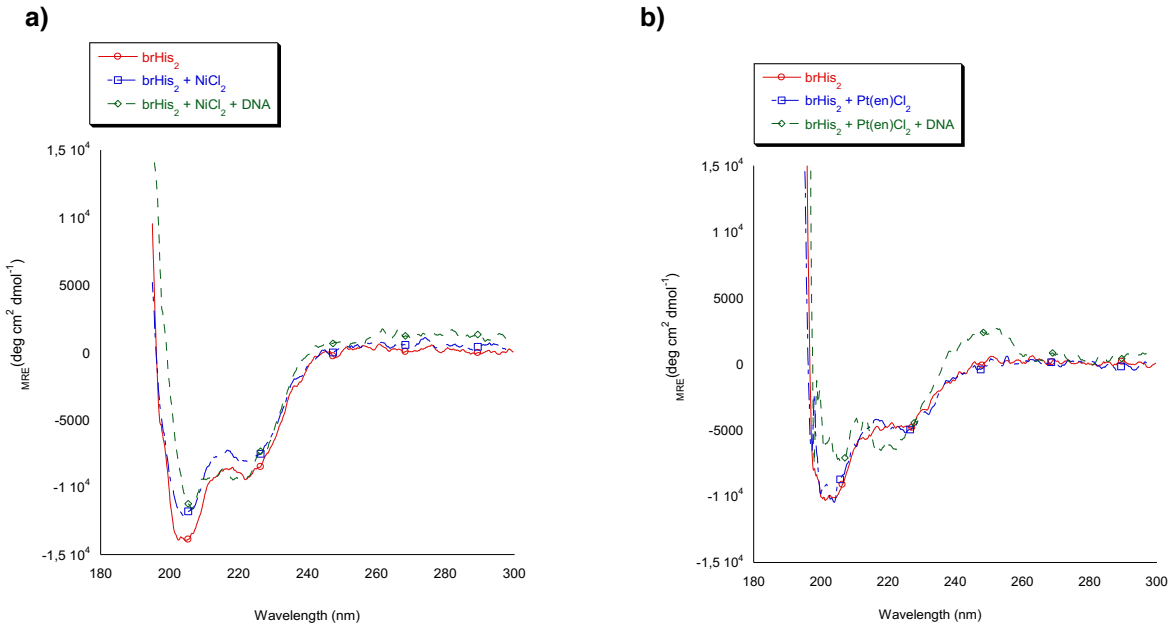

Fig. S14 CD experiments with $NiCl_2$ (**a**) and $Pt(en)Cl_2$ (**b**). Red line: peptide in phosphate buffer; Blue line: after addition of the corresponding metal; Green line: after addition of the ***GTCAT***.

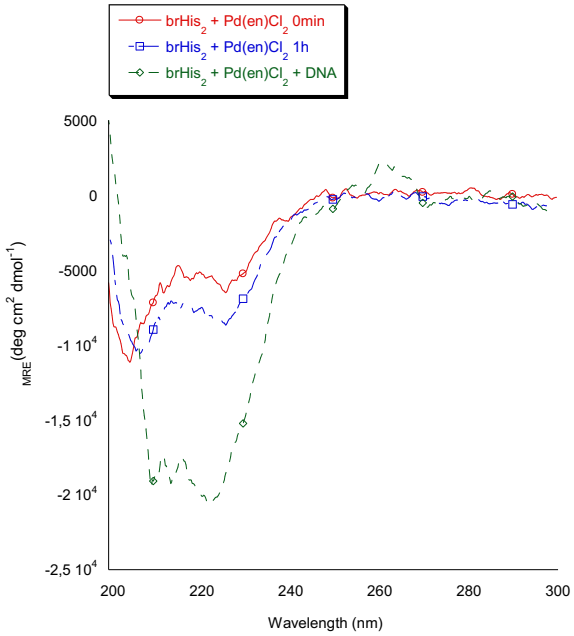

Fig. S15 CD results considering only **brHis$_2$** and **Pd(en)Cl$_2$**. Red line: peptide (5 μM) and one equivalent of the metal complex, measurement at time zero; Blue line: measurement 1h after the mixing; Green line: after addition of ***GTCAT*** (5 μM).

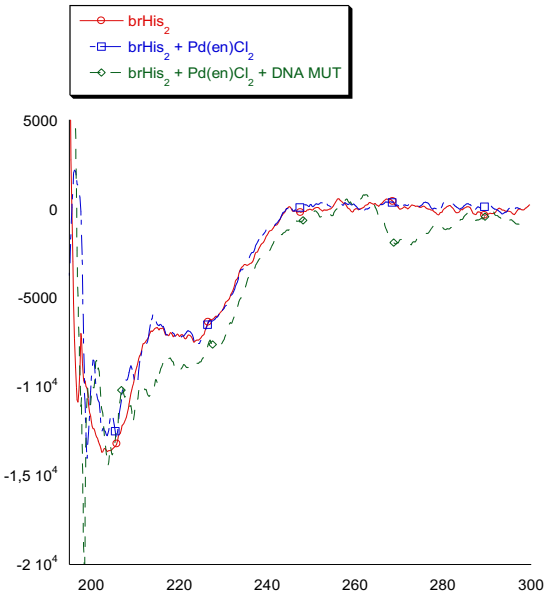

Fig. S16 CD experiments with ***MUT***. Red line: peptide in phosphate buffer; Blue line: after addition of the metal complex; Green line: after addition of ***MUT*** .

8. UV-Vis Spectra

UV-Vis experiments were carried out with a Jasco-V630 spectrophotometer. Measurements were performed in a 2 mm cuvette at 25 ºC. Samples contained 10 mM phosphate buffer pH 7.5 and 100 mM of NaCl, 5 μM peptide, 5 μM of corresponding dsDNA (when present), and one equivalent of either metal complex [Pd(bpy)Cl_2] or [Ni(bpy)Cl_2].

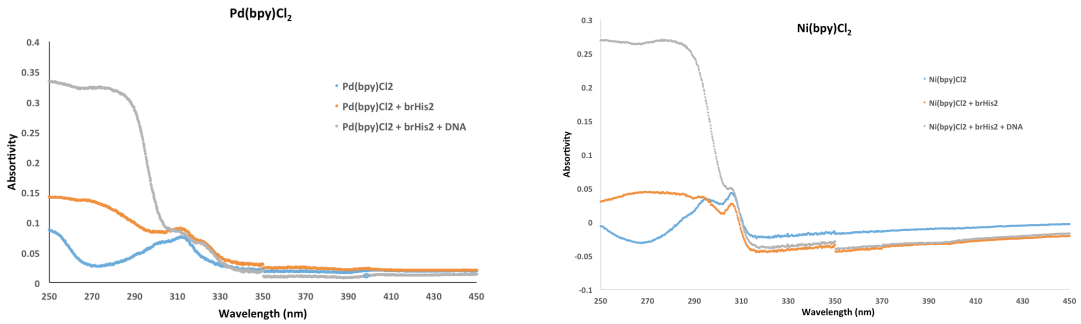

Fig. S17 UV-Vis experiments for compounds [Pd(bpy)Cl_2] (left) and [Ni(bpy)Cl_2] (right). Blue line: just the metal complex; Orange line: after addition of the peptide **brHis$_2$**. Grey lines: after addition of ***GTCAT***. A new band can be observed when the Pd(bpy)Cl_2 is mixed with the peptide; this band shows the presence of the bipy chromophore, and is not observed in the case of Ni(bpy)Cl_2.

9. Dynamic Light Scattering

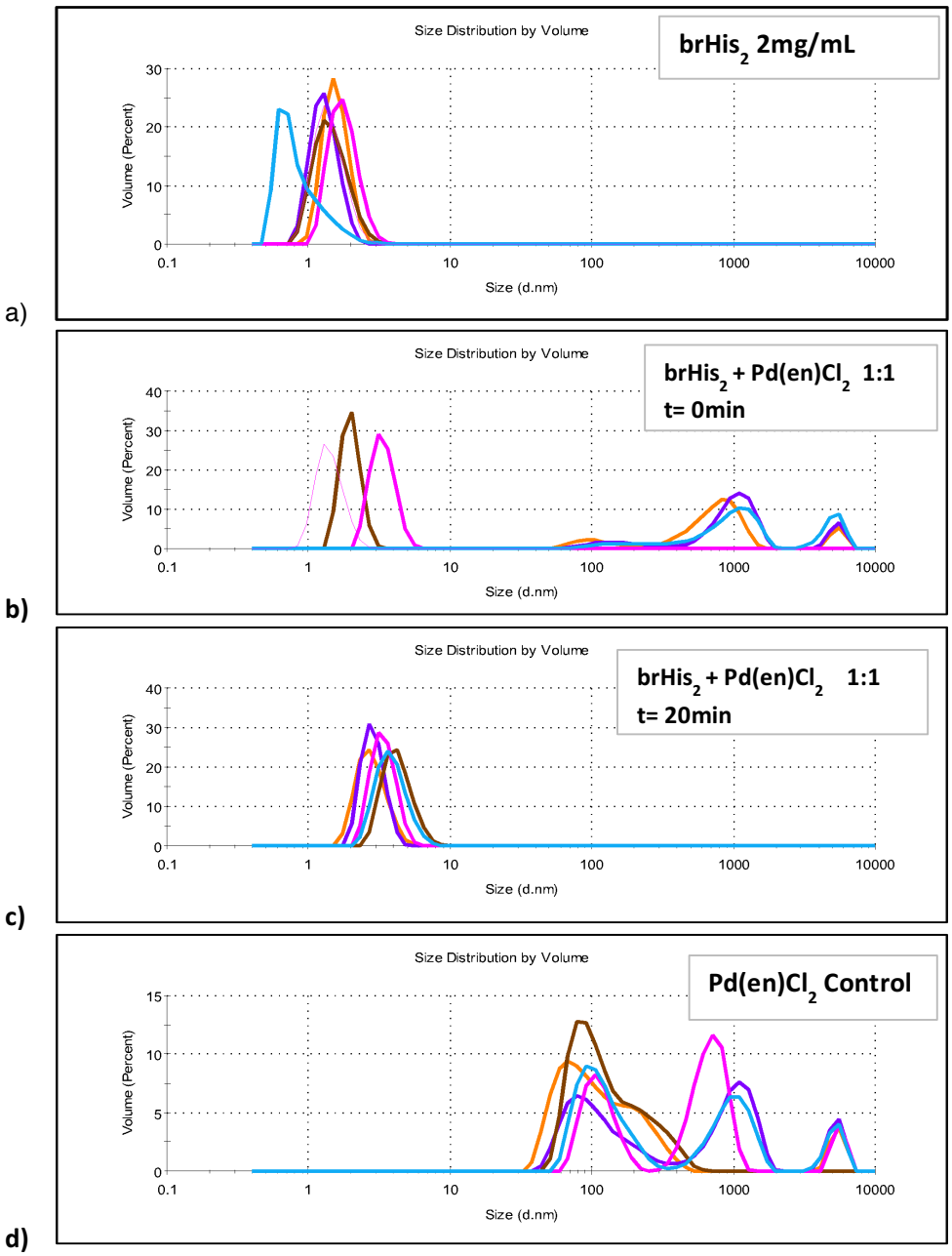

Fig. S18 Peptide aggregation monitored by dynamic light scattering. The experiments were carried out with a Malvern Nano ZS (Malvern Instruments, U.K.) operating at 633 nm with dispersion angle of 173 deg. Measurements were performed in a 2 mm cuvette at 25 ºC and attenuator 11. Samples contained: a) 2 mg/ml of **brHis$_2$** in water; b) 2 mg/ml of **brHis$_2$** in water + Pd(en)Cl$_2$ (1:1), without any incubation; c) 2 mg/ml of **brHis$_2$** in water + Pd(en)Cl$_2$ (1:1), pre-mixed and incubated 20 min before measuring; d) control of Pd(en)Cl$_2$ (2mg/ml). Traces in a) at size c.a. 1 nm, indicate that the peptide does not aggregate; although the mixture with Pd(en)Cl$_2$ showed in b) initially appears to contain some aggregates, those are already present in the Pd(en)Cl$_2$ solution in d), and disappear after a few minutes, when the complexation takes place, as shown in trace c).

The same experiments were performed at 5 μM of **brHis$_2$** with similar results.

10. LC-MS of Palladium Complexes

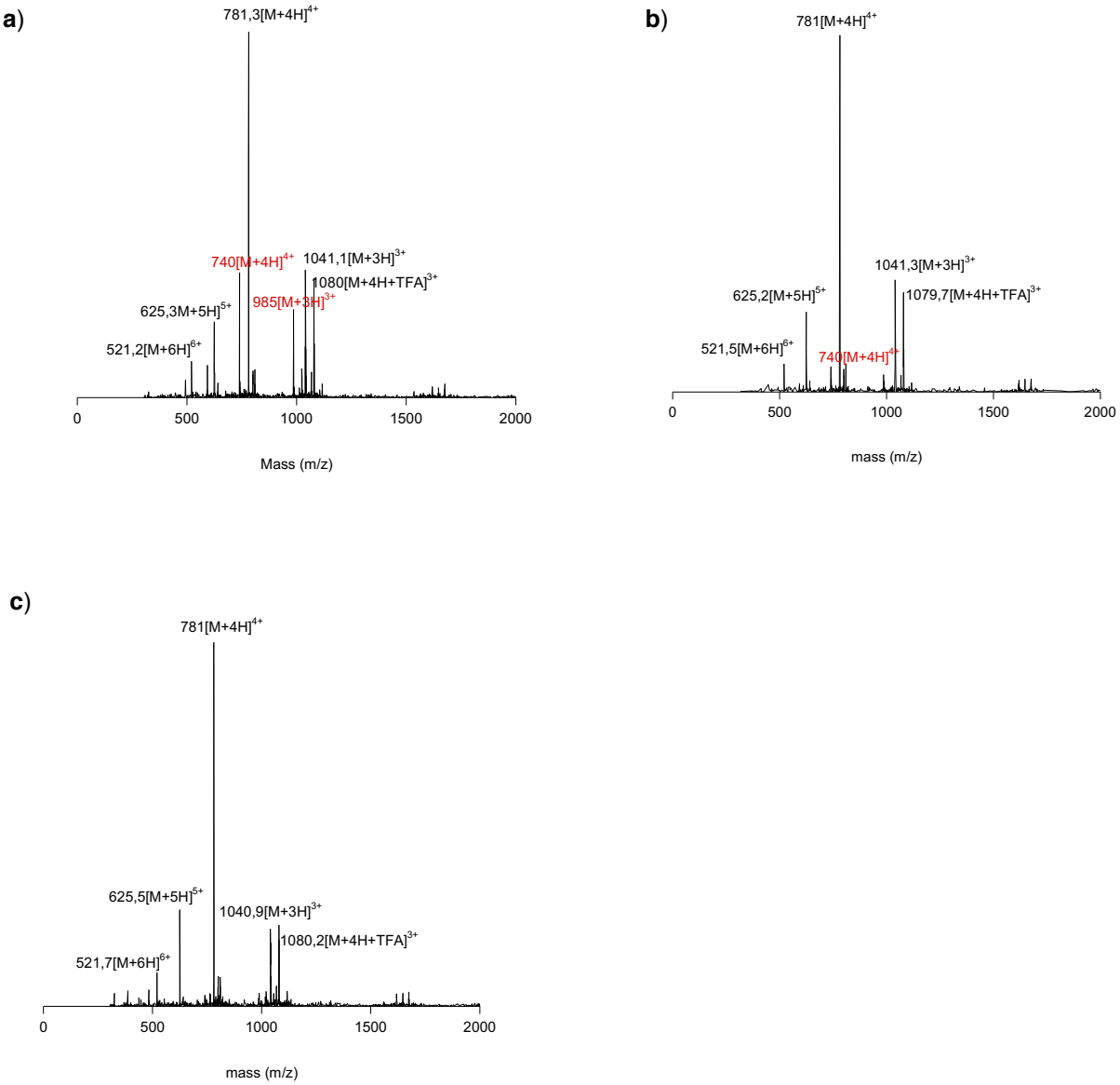

Fig. S19 HPLC-MS experiments of **brHis$_2$** + Pd(en)Cl$_2$ (1:1). A solution containing **brHis$_2$** and Pd(en)Cl$_2$ (1:1) in water was prepared and measured at different time points. **a**) t = 0 min. after incubation; **b**) t = 10 min. after incubation; **c**) t = 20 min. after incubation.

EM–ESI$^+$ (m/z): Calcd. for the complex $C_{125}H_{211}N_{53}O_{35}Pd$: 3122; Calcd. for the peptide $C_{123}H_{205}N_{51}O_{35}$: 2956.

Found a): 1080.2 $[M+4H+TFA]^{3+}$; 1040.9 $[M+3H]^{3+}$; 985 $[M+3H]^{3+}$; 781 $[M+4H]^{4+}$; 740 $[M+4H]^{4+}$; 625.5 $[M+5H]5^+$; 521.7 $[M+6H]^{6+}$ (The red colour corresponds to the peptide´s mass while the black colour it's the complex mass).

Found b): 1079.7 $[M+4H+TFA]^{3+}$; 1041.3 $[M+3H]^{3+}$; 781 $[M+4H]^{4+}$; 740 $[M+4H]^{4+}$;625.2 $[M+5H]5^+$; 521.5 $[M+6H]^{6+}$ (The red colour corresponds to the peptide´s mass while the black colour it's the complex mass).

Found c): 1080.2 $[M+4H+TFA]^{3+}$; 1040.9 $[M+3H]^{3+}$; 781 $[M+4H]^{4+}$; 625.5 $[M+5H]5^+$; 521.7 $[M+6H]^{6+}$

The patterns show the mass for the complex between the peptide and Pd(en)$^{2+}$.

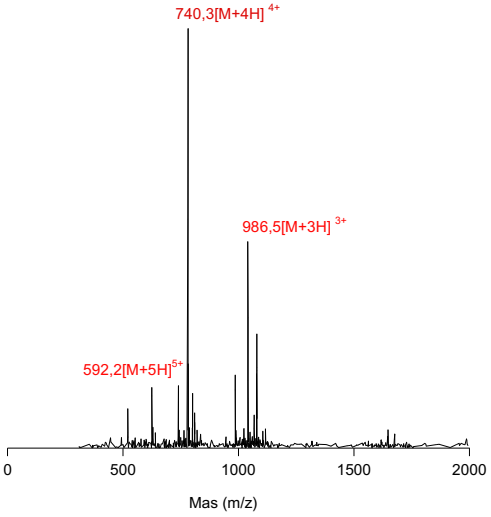

Fig. S20 HPLC-MS spectrum of **brHis$_2$** + Pt(en)Cl$_2$. A solution containing **brHis$_2$** and Pt(en)Cl$_2$ (1:1) in water was prepared and measured at different incubation times (0, 10 and 20 min).

EM–ESI$^+$ (m/z): Calcd. for the complex $C_{125}H_{213}N_{53}O_{35}Pt$: 3212; Calcd. for the peptide $C_{123}H_{205}N_{51}O_{35}$: 2956.

Found: 986.6 $[M+3H]^{3+}$; 740.2 $[M+4H]^{4+}$; 592.2 $[M+5H]^{5+}$

11. Cell internalization studies

Images were obtained with an Andor Zyla digital camera mounted on a Nikon TiE. Images were further processed with Image J or NIS software. The parameters of the fluorescent channels were for the Nikon (Semrock): filter cube DAPI-1160B-000: BP 387/11 nm, LP 447/60 nm and DM 409 nm; filter cube FITC-3540C-000: BP 482/35 nm, LP 536/40 nm and DM 506 nm; filter cube TRITC-B-000: BP 543/22 nm, LP 593/40 nm and DM 562 nm.

All the internalization studies were carried out according to the following protocol: Cells growing on glass coverslips were incubated at 37 ºC in DMEM (completed with 10% Fetal Bovine Serum) with 5 μM of the corresponding peptide, or with the mixture of the peptide under study and 5 μM Pd(en)Cl_2 (incubated for 10 min before addition to the cell culture). After 30 min, cells were washed twice with PBS and observed under the fluorescence microscope. Digital pictures of the different samples were taken under identical conditions of gain and exposure. The assays were carried with HeLa cells.

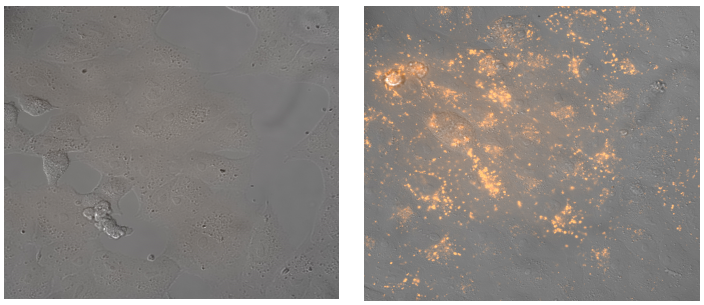

Fig. S21 Fluorescence micrographies of Vero cells, brightfield images are superimposed to the red emission channel; after incubation with 5 μM **TMR-brHis$_2$** for 30 min at 37 ºC (left) and 5 μM **TMR-brHis$_2$** premixed with 5 μM Pd(en)Cl_2 for 10 min, and then incubated with the cells for 30 min at 37ºC (right). NOTE: the results with HeLa cells are similar, and have been included in the main text.

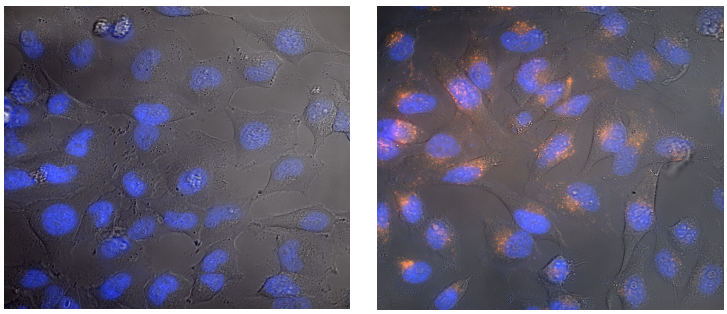

Fig. S22 Fluorescence micrographies of Hela cells, brightfield images are superimposed to the red and blue emission channel after incubation with 5 μM **TMR-brHis$_2$** co-incubated with 10 μM Hoechst, for 30 min at 37 ºC. *Left*: without further additives. *Right*: premixed with 5 μM Pd(en)Cl_2.

Internalization controls with mutant peptides lacking histidines

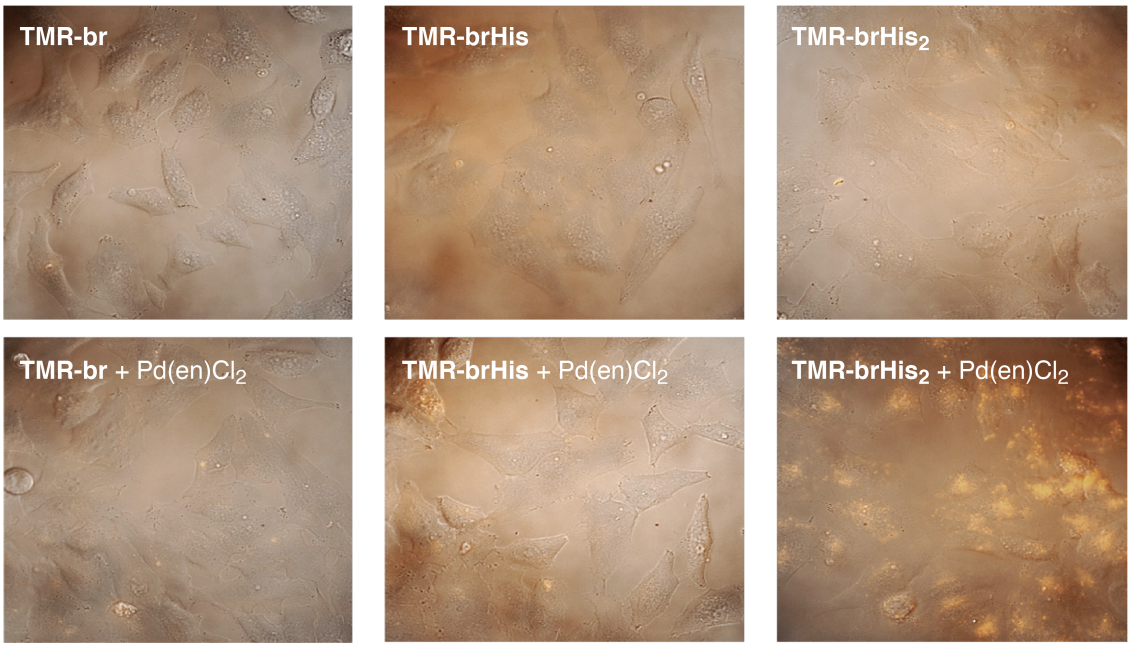

Fig. S23 Fluorescence micrographies of Hela cells, brightfield images are superimposed to the red emission channel after incubation. Top row: incubation with 5 μM of each peptide incubated for 30 min at 37 ºC; bottom row: 5 μM of a mixture of each peptide premixed with 5 μM $Pd(en)Cl_2$ for 10 min, and then incubated with the cells for 30 min at 37 ºC.

Additional Controls

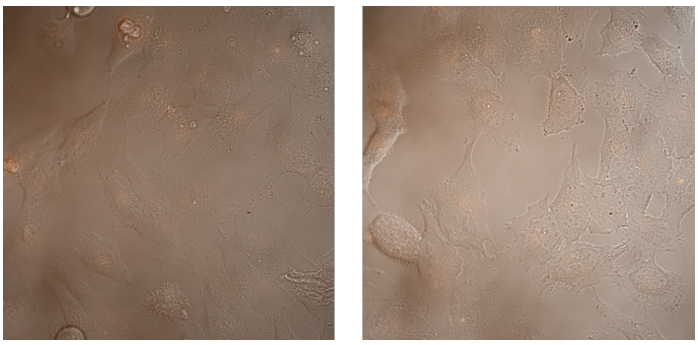

Fig. S24 Fluorescence micrographies of Hela cells, brightfield images are superimposed to the red emission channel. *Left:* 5 μM **brHis$_2$** premixed with 5 μM $Pd(en)Cl_2$ for 10 min, and incubated with the cells for 30 min at 37 ºC. Then, **TMR-br** (5 μM) was added, and incubated for another 30 min. *Right:* mixture of 5 μM **brHis$_2$**, 5 μM $Pd(en)Cl_2$, and 5 μM **TMR-br** incubated for 10 minutes and then for another 30 min at 37 ºC in the presence of the cells.

Cytotoxicity

A cytotoxicity test determined as a function of the metabolic activity using the MTT assay was carried out with 8000 cells seeded in quadruplicates into the wells of 96 well plates and incubated for 24 hours prior to the treatment with different compound concentrations for 48 hours. **TMR-brHis$_2$**, the mixture of **TMR-brHis$_2$** + Pd(en)Cl$_2$ (1:1, premixed for 10 min) and Pd(en)Cl$_2$ were tested. After the treatment, the MTT reagent was added to the cells evaluated according to the manufacturer's protocol. The absorbance was measured with the plate reader Infinite 2000pro.

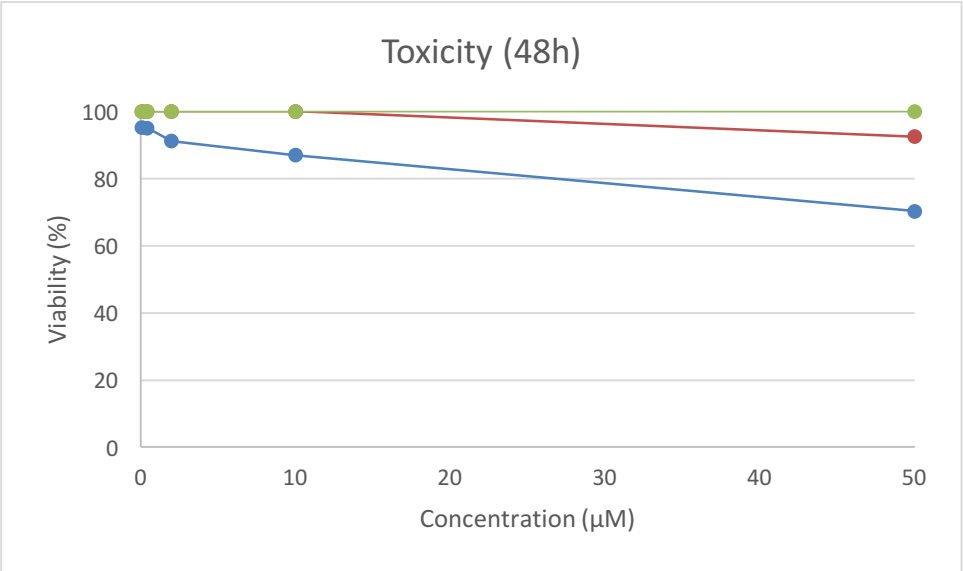

Fig. S25 Toxicity of **TMR-brHis$_2$** (green line), **TMR-brHis$_2$** + Pd(en)Cl$_2$ (1:1) (red line) and Pd(en)Cl$_2$ (blue line). After 48h of treatment we can conclude that only in the case of the metal chloride alone we can observe some toxicity.

12. Mutated peptides. Characterization and internalization

TMR-br(R249A,R245S)His$_2$: TMR-Ahx-D-P-A-A-**H**-K-R-A-**H**-N-T-E-A-A-R-R-S-R-A-**S**-K-L-Q-**A**-$CONH_2$

Arginines 249 and 245 were mutated to Alanine and Serine (in blue), respectively.

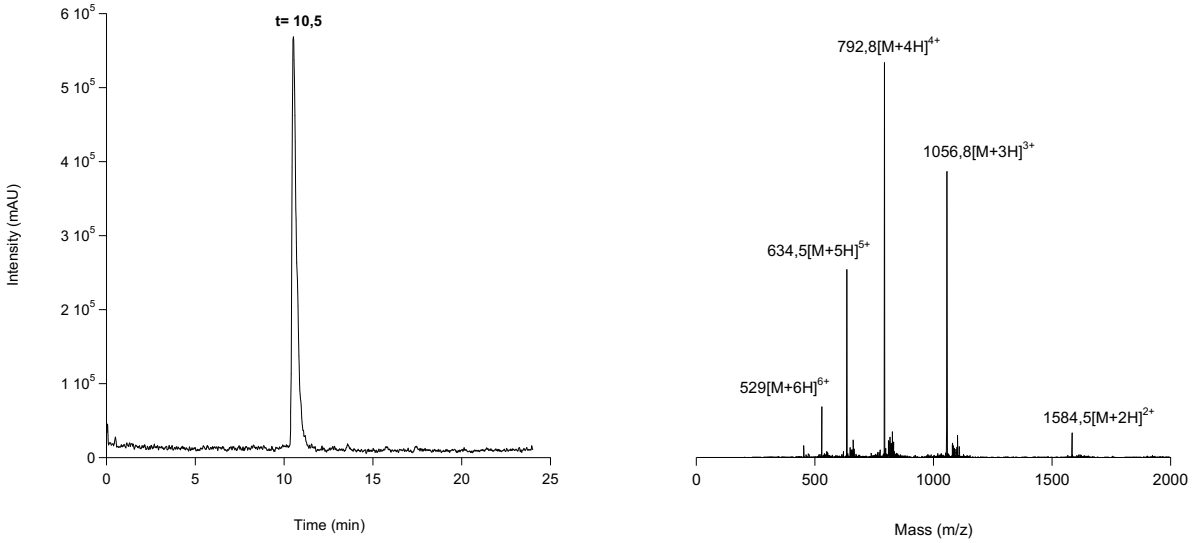

Fig. S26 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI$^+$ (m/z): Calcd. For $C_{140}H_{210}N_{47}O_{39}$: 3168. Found: 1584.5 $[M+2H]^{2+}$; 1056.8 $[M+3H]^{3+}$; 792.8 $[M+4H]^{4+}$; 634.5 $[M+5H]^{5+}$; 529 $[M+6H]^{6+}$;

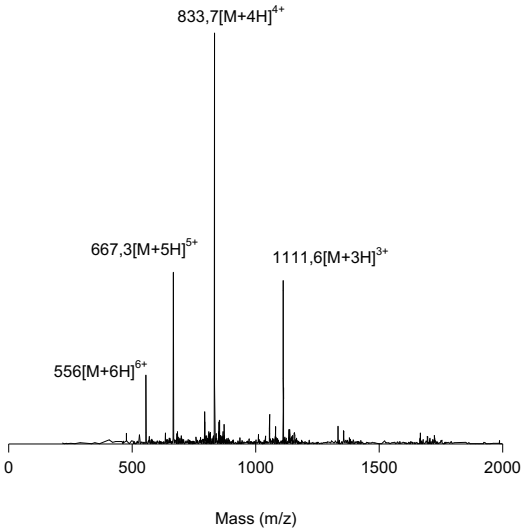

Fig. S27 HPLC-MS spectrum of **TMR-br(A_{249},S_{245})His$_2$** + $Pd(en)Cl_2$. A solution containing **TMR-br(A_{249},S_{245})His$_2$** and $Pd(en)Cl_2$ (1:1) in water was prepared and measured 20 min after incubation.

EM–ESI$^+$ (m/z): Calcd. the complex $C_{142}H_{216}N_{49}O_{39}Pd$: 3334. Found: 1111.6 $[M+3H]^{3+}$; 833.7 $[M+4H]^{4+}$; 667.3 $[M+5H]5^+$; 556 $[M+6H]^{6+}$

The patterns confirm the mass for the complex between the peptide and $Pd(en)^{2+}$.

TMR-br(Q248A,L247A)His$_2$: TMR-Ahx-D-P-A-A-**H**-K-R-A-**H**-N-T-E-A-A-R-R-S-R-A-R-K-**A-A**-R-$CONH_2$

Glutamine 248 and Leucine 247 were mutated to Alanine

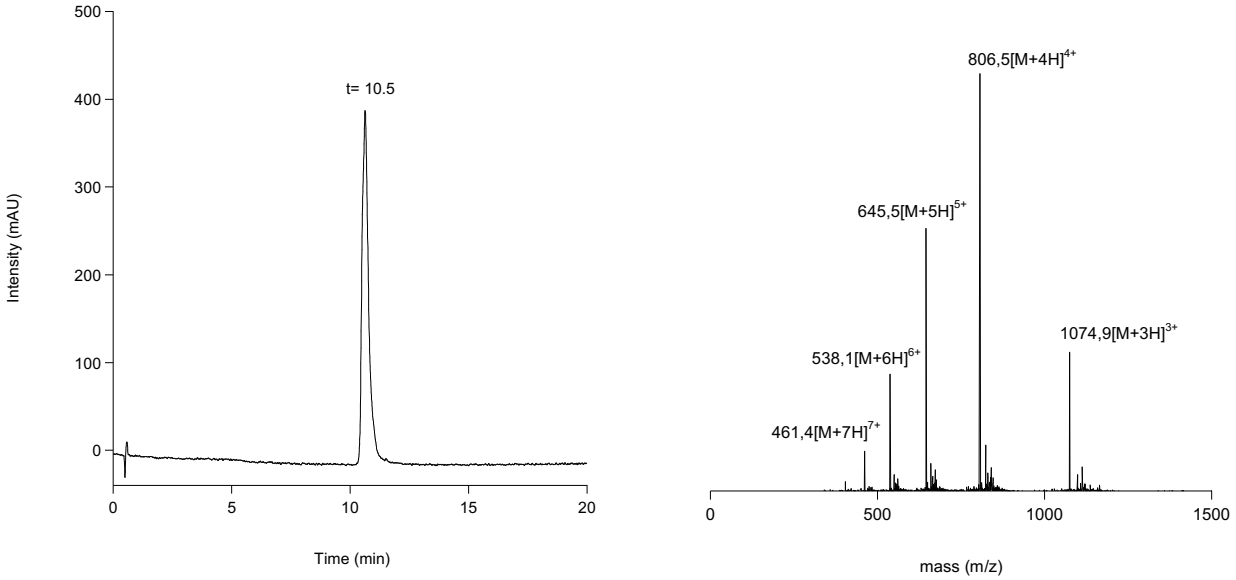

Fig. S28 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI$^+$ (m/z): Calcd. For $C_{141}H_{215}N_{52}O_{37}$: 3222. Found: 1774.9 $[M+3H]^{3+}$; 806.5 $[M+4H]^{4+}$; 645.5 $[M+5H]^{5+}$; 538.1 $[M+6H]^{6+}$; 461.4 $[M+7H]^{7+}$;

FITC-brHis$_2$ **FITC**-Ahx-D-P-A-A-**H**-K-R-A-**H**-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-R-$CONH_2$

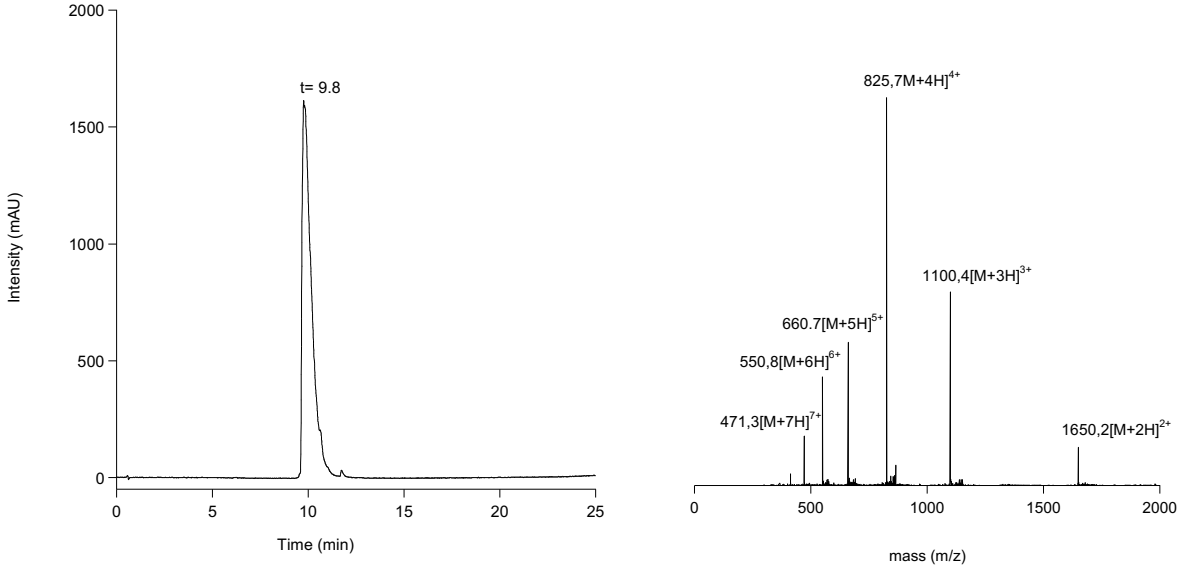

Fig. S29 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI^{+} (m/z): Calcd. For $C_{142}H_{215}N_{52}O_{39}S$: 3298. Found: 1774.9 $[M+3H]^{3+}$; 806.5 $[M+4H]^{4+}$; 645.5 $[M+5H]^{5+}$; 538.1 $[M+6H]^{6+}$; 461.4 $[M+7H]^{7+}$;

TMR-br(T236A,A238T)His$_2$: TMR-Ahx-D-P-A-A-**H**-K-R-A-**H**-N-**A**-E-**T**-A-R-R-S-R-A-R-K-L-Q-R-$CONH_2$

Threonine 236 was mutated to Alanine, and Alanine 238 was mutated to Threonine.

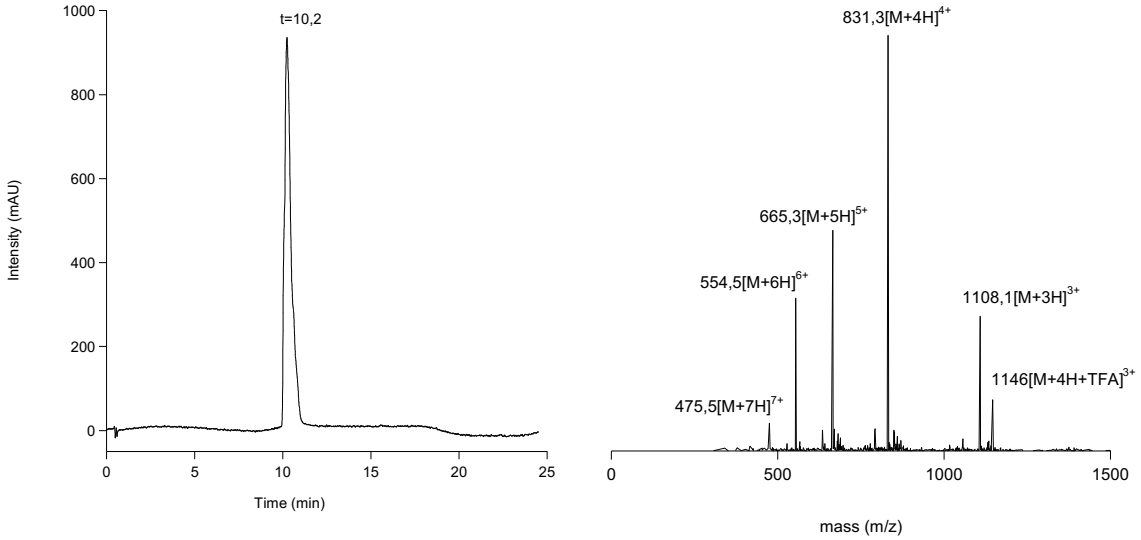

Fig. S30 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI^{+} (m/z): Calcd. For $C_{146}H_{224}N_{53}O_{38}$: 3324. Found: 1146$[M+4H+TFA]^{3+}$; 1108.1 $[M+3H]^{3+}$; 831.3 $[M+4H]^{4+}$; 665.3 $[M+5H]^{5+}$; 554.5 $[M+6H]^{6+}$; 475,5 $[M+7H]^{7+}$;

TMR-br(A238T)His$_2$:TMR-Ahx-D-P-A-A-**H**-K-R-A-**H**-N-T-E-**T**-A-R-R-S-R-A-R-K-L-Q-R-$CONH_2$

Alanine 238 was mutated to Threonine.

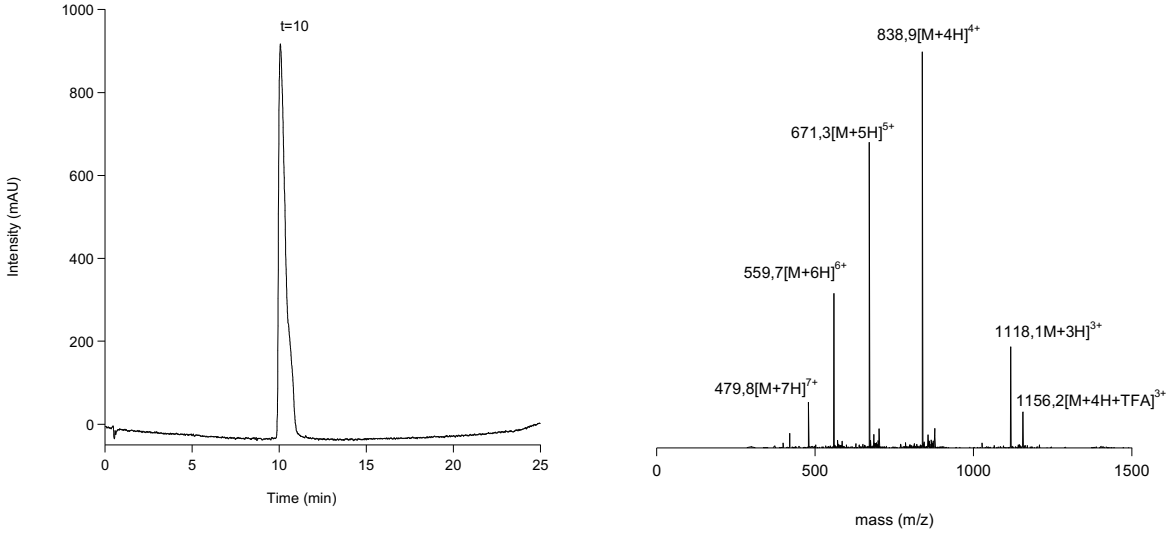

Fig. S31 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI^{+} (m/z): Calcd. For $C_{147}H_{226}N_{53}O_{39}$: 3354. Found: 1156,2[M+4H+TFA]$^{3+}$; 1118,1[M+3H]$^{3+}$; 838,9 [M+4H]$^{4+}$; 671,3[M+5H]$^{5+}$; 559,7 [M+6H]$^{6+}$; 479,8 [M+7H]$^{7+}$;

TMR-br(LS)His$_2$: TMR-Ahx-D-P-A-A-**L-S**-**H**-K-R-A-**H**-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-R-CONH$_2$

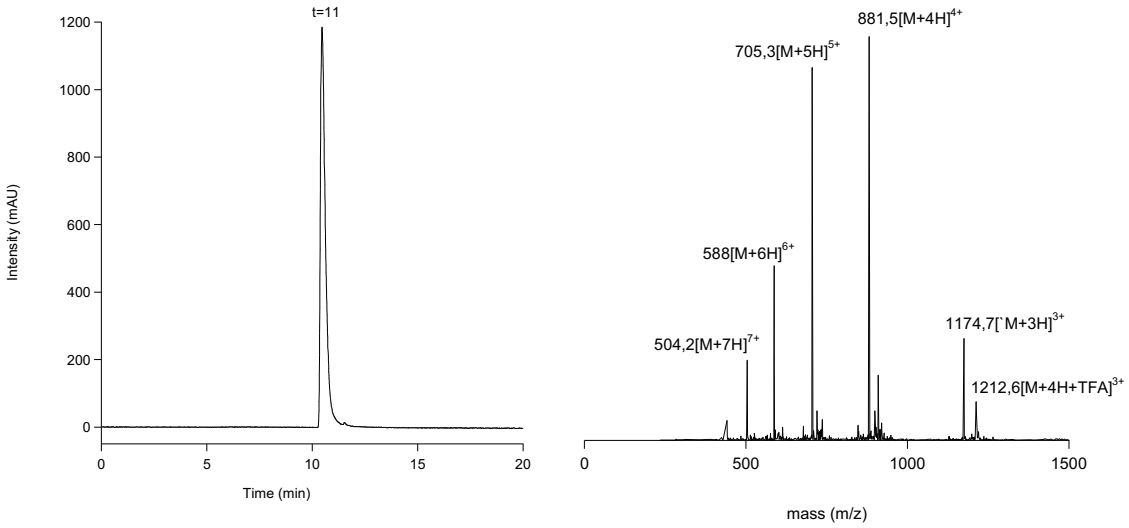

Fig. S32 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI^{+} (m/z): Calcd. For $C_{155}H_{240}N_{55}O_{41}$: 3524. Found: 1212,6[M+4H+TFA]$^{3+}$; 1174,7[M+3H]$^{3+}$; 881,5 [M+4H]$^{4+}$; 705,3[M+5H]$^{5+}$; 588[M+6H]$^{6+}$; 504[M+7H]$^{7+}$;

Internalization controls with the new, mutated peptides

The assays were accomplished using the same conditions indicated in section 11.

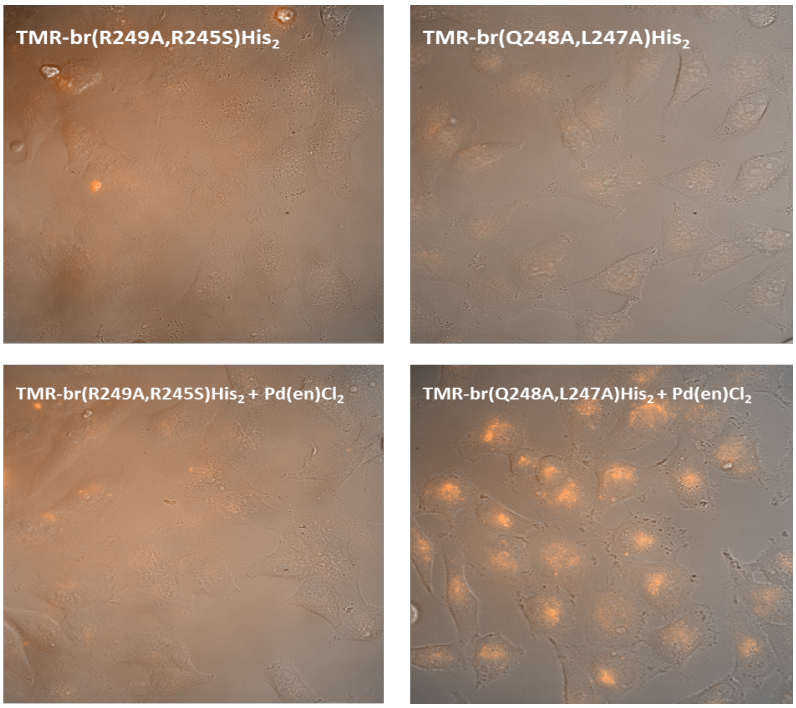

Fig. S33 Fluorescence micrographies of Hela cells, brightfield images are superimposed to the red emission channel; after incubation with the three peptides at 5 μM,for 30 min at 37 ºC (top row), or with the peptides premixed with 5 μM $Pd(en)Cl_2$ for 10 min, and then incubated with the cells for 30 min at 37 ºC (bottom row).

The above pictures demonstrate that peptides lacking the two Arginine residues (left column), undergo a poor internalization, even in presence of the palladium complex. In contrast, the mutant featuring two Alanines, in place of Leu^{247} and Gln^{248}, it is still able to across de cellular membrane in presence of palladium, at comparable levels than **brHis$_2$** (right column).

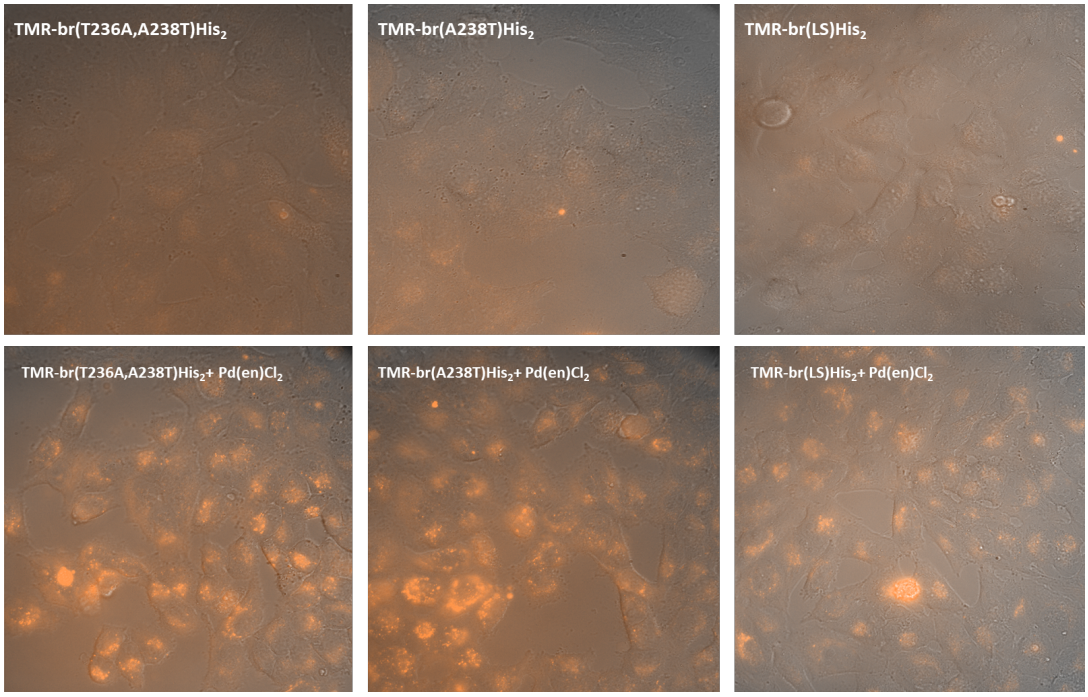

Fig. S34 Fluorescence micrographies of Hela cells, brightfield images are superimposed to the red emission channel; after incubation with the three peptides at 5 μM, for 30 min at 37 ºC (top row), or with the peptides premixed with 5 μM Pd(en)Cl_2 for 10 min, and then incubated with the cells for 30 min at 37 ºC (bottom row).

These pictures demonstrate that the switch strategy is effective with other mutated peptides.

Internalization of FITC-brHis_2 and TMR

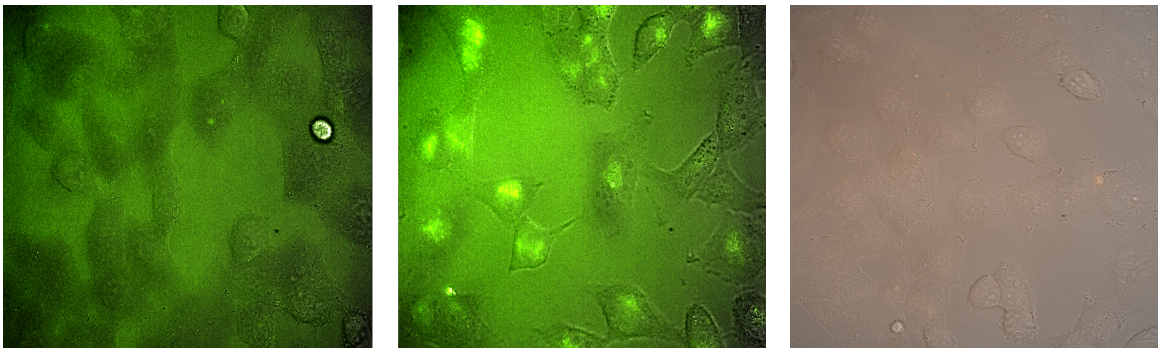

Fig. S35 Fluorescence micrographies of Hela cells. *Left and center:* 5 μM **FITC-brHis_2** (*left*) and the same peptide premixed with 5 μM Pd(en)Cl_2 for 10 min (*center*), and incubated with the cells for 30 min at 37 ºC. Brightfield images are superimposed to the green emission chanel; *Right:* 5 μM of 5(6)-carboxytetramethylrhodamine incubated for 30 min at 37 ºC in the presence of the cells. Brightfield images are superimposed to red emission channel.

13. References

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