

Controlling Oncogenic KRAS Signaling Pathways with a Palladium-Responsive Peptide

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Supplementary Note 1

CRedit Author attributions

The following table describes the contribution of each author according to the CRediT taxonomy as described in A. Brand, L. Allen, M. Altman, M. Hlava, J. Scott, *Learn. Publ.* **2015**, 28, 151–155. This taxonomy provides a detailed classification of the various roles performed by each author.

The degree of contribution is coded as lead (black), equal (dark grey), or supporting (light grey).

[illegible]

Supplementary Methods

Abbreviations: Ahx: 6-Aminohexanoic acid; CARA: Computer aided resonance assignment; CNS: Crystallography and NMR system; DEDTC: sodium diethyldithiocarbamate; DIC: *N,N'*-Diisopropylcarbodiimide; DIEA: *N,N*-diisopropylethylamine; DMEM: Dulbecco's Modified Eagle Medium; HATU: 2-(1*H*-7-aza-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; NMR: Nuclear Magnetic Resonance; NOESY: Nuclear Overhauser Effect Spectroscopy; SDS: sodium dodecyl sulfate; TFA: trifluoroacetic acid; TIS: triisopropylsilane; TMR: 5-carboxytetramethylrhodamine; TOCSY: Total Correlation Spectroscopy; *p*-ABA: 4-acetamidobenzoic acid

Reagents: *cis*-PdCl₂(en) were purchased from Aldrich. TMR fluorophore were purchased from Carbosynth as a 5-Carboxytetramethylrhodamine succinimidyl ester. The antibodies ERK2 and phospho-ERK1/2 was kindly provided by Dr. José Costoya, from CIMUS—Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas. Avenida Barcelona, s/n, 15782, Santiago de Compostela. Spain. All peptide synthesis reagents and amino acid derivatives were purchased from Sigma Aldrich and Iris Biotech; 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. All other chemicals were purchased from Aldrich or Fluka. All solvents were dry and synthesis grade, unless specifically noted.

Peptide synthesis. The peptide **αH-His₂** was synthesized on a *Liberty Blue Lite* automatic microwave assisted peptide synthesizer from CEM Corporation, following the manufacturer's recommended procedures. The synthesis was on a 0.1 mmol scale using a 0.5 mmol/g load *H-Rink* amide *ChemMatrix* resin. Amino acids were coupled in 5-fold excess using DIC (*N,N'*-Diisopropylcarbodiimide) as activator, Oxime as base, and DMF as solvent. Couplings were conducted for 4 min at 90 °C. Deprotection of the temporal Fmoc protecting group was performed with 20% piperidine in DMF for 1 min at 75 °C. TMR was manually coupled to the N-terminus of the sequence by incubation of the resin-bound peptide with 3 eq. of 5-carboxytetramethylrhodamine (0.15 mmol, 64.5 mg), 3 eq. of HATU, and 5 eq. of DIEA 0.2 M in DMF for 60 min. Cleavage/deprotection was done by treating the resin-bound peptide for 2 h with 900 μL TFA, 50 μL CH₂Cl₂, 25 μL H₂O and 25 μL TIS (1 mL of cocktail/40 mg resin). The resin was filtered, and the filtrate was added onto ice-cold diethyl ether. After ~20 min, the precipitate was centrifuged and washed again with ice-cold ether. The solid residue was dried under argon and redissolved in water/CH₃CN (1:1) for HPLC purification on a semipreparative *Agilent* 1100 series LC using a *Phenomenex Luna-C₁₈* (250 × 10 mm) reverse-phase column and a linear gradient (5 to 75% B over 40 min, 4 mL/min.; A: H₂O 0.1% TFA, B: CH₃CN 0.1% TFA). Collected fractions with pure products were freeze-dried to afford the desired peptide. The peptide was analyzed by analytical UHPLC-MS with an *Agilent* 1200 series LC/MS using a *SB C₁₈* (1.8 μm, 2.1 × 50 mm) analytical column from *Phenomenex* with a linear gradient from 5 to 95% of solvent B for 20 min at a flow rate of 0.35 mL/min (A: water with 0.1% TFA, B: acetonitrile with 0.1% TFA). Compounds were detected by UV absorption at 222, 270, and 330 nm. Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an *Agilent* 6120 *Quadrupole* LC/MS model in positive scan mode using direct injection of the purified peptide solution into the MS detector.

Protein Expression and Purification. The constructs *pDNA2.0 6H-TEV-KRAS G12V* and *pDNA2.0 6H-FLAG-TEV-KRAS G12C* were purchased from *Addgene*. BL21 cells (*Invitrogen*) were transform with the constructs and grown in Luria broth (LB) to OD 600 0.7 and induced with 250 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16 h at 16 °C. Cells were pelleted and resuspended in lysis buffer (20 mM sodium phosphate, pH 8.0, 500 mM NaCl, 10 mM imidazole, 1 mM 2-mercaptoethanol (BME), 5% (vol/vol) glycerol). After centrifugation, the pellet was frozen until use. Then, protease inhibitor (EDTA free) and lysozyme (1mg/mL) were added, and the pellet was sonicated for 6 min. (35 sec. on /10 sec. off). Protein was purified over an IMAC (immobilized metal affinity chromatography) following *HisPur* Ni-NTA protocols (*Thermo Fisher*). Protein was concentrated in a 10-kDa Amicon Ultra-15 (*Millipore*) aliquoted with 15% glycerol, and then flash-frozen and stored at -20 °C.

Nucleotide Release experiment. Loading of fluorescent-labeled GDP (mantGTP) to KRAS^{wt} was conducted following previous reports.^{1,2} Purified KRAS^{wt} was buffer-exchanged in *Zeba* Spin Desalting Columns (*Thermo Fisher*) in loading buffer (20 mM Tris-HCl [pH 7.5], 50 mM NaCl, 4 mM EDTA and 1 mM

DTT). The resulted eluted KRAS^{wt}, after measuring their concentration, was incubated with 10-fold molar excess of mantGTP (*Abcam*) for 1.5 h at 20 °C in the dark. Reactions were supplemented with 10 mM MgCl₂ and incubated for 1 h on ice. Free nucleotide was removed using a *Zeba Spin Desalting Columns* (*Thermo Fisher*) that had been equilibrated with the reaction buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM MgCl₂ and 1 mM DTT). The effect of peptides on the intrinsic rate of nucleotide release was monitored using the decrease in fluorescence with time as mantGTP dissociates from KRAS in a 100 µL reaction mixture (96-well plate) of 1 µM KRAS^{wt}/mantGTP complex and 10 µM of **αH-His₂** or **αH-His₂[Pd]**. Fluorescence was excited at 370 nm and emission was monitored at 430 nm during 30 min, using a *Tecan Infinite M Plex* plate reader. The data was processed using the program *GraphPad Prism*.

Cell internalization and inhibition of ERK1/2. All steps were performed on a sterile clean bench Telstar AV-100 at rt. Solutions stored in a fridge were warmed beforehand in a water bath (37 °C). Unless otherwise specified, all incubations were performed in DMEM containing 5% of fetal bovine serum (FBS-DMEM). Cell Culture: A549 cell line was cultured in DMEM (Dulbecco's modified Eagle's medium), 5 mM glutamine, penicillin (100 units/mL) and streptomycin (100 units/mL), all from *Invitrogen*. Proliferating cultures were maintained in a 5% CO₂ humidified incubator at 37 °C. For all the experiments, cells were seeded in the corresponding well at the indicated concentration two days before treatment. A549 cells were seeded on glass-bottom plates 48 h (150.000 cell/ml) before treatment. Culture medium was removed and DMEM containing 5% fetal bovine serum (FBS-DMEM) and **αH-His₂** or **αH-His₂[Pd]** (10 µM) were added. The metallopeptide was made just before the addition to cells by pre-incubating with metal complexes (1:1) in water for 10 min. After 30 min of incubation with cells, these were washed twice with PBS and replaced with fresh FBS-DMEM to observe under the microscope with adequate filters. Digital pictures of the different samples were taken under identical conditions of gain and exposure. A549 cells were seeded on glass-bottom plates (150.000 cell/ml) 48 h before treatment. Culture medium was removed, the cells was serum-starved and **αH-His₂**, **αH-His₂[Pd]** or *cis*-PdCl₂(en) were added at different concentrations and incubated for 4 h. After two washes with PBS, the cells were lysed in Laemmli buffer containing Tris pH 6,8 1M, Glycerol, SDS, Bromophenol Blue and β-mercaptoethanol, and heated at 95 °C for 10 min. The samples were separated by SDS-12.5% polyacrylamide gel and transferred to nitrocellulose membrane, to probed by Western blotting. Levels of total ERK2 and phosphorylated ERK were detected with anti-ERK1/2 and phospho-ERK1/2) antibodies, respectively and revealed with Luminata™ Classico Western HRP substrate following manufacturer's protocol The visualization was performed with ChemiDoc MP imaging system by *Biorad*, and the processing of the images and densitometry quantification was carried out with ImageLab program.

Fluorescence microscopy. All images were obtained with an *Andor Zyla* mounted on a *Nikon TiE*. LED excitation wavelength 550 nm. Filter cube TRITC-B-000 (Semrock): BP 543/22 nm, LP 593/40 nm and DM 562 nm. Images were further processed with *Image J*.

UHPLC-MS α H-His₂

α H-His₂ TMR-Ahx-RRFFGIHLTNHLKTEEGN, Ahx = aminohexanoic acid; TMN 5-carboxytetramethyl rhodamine.

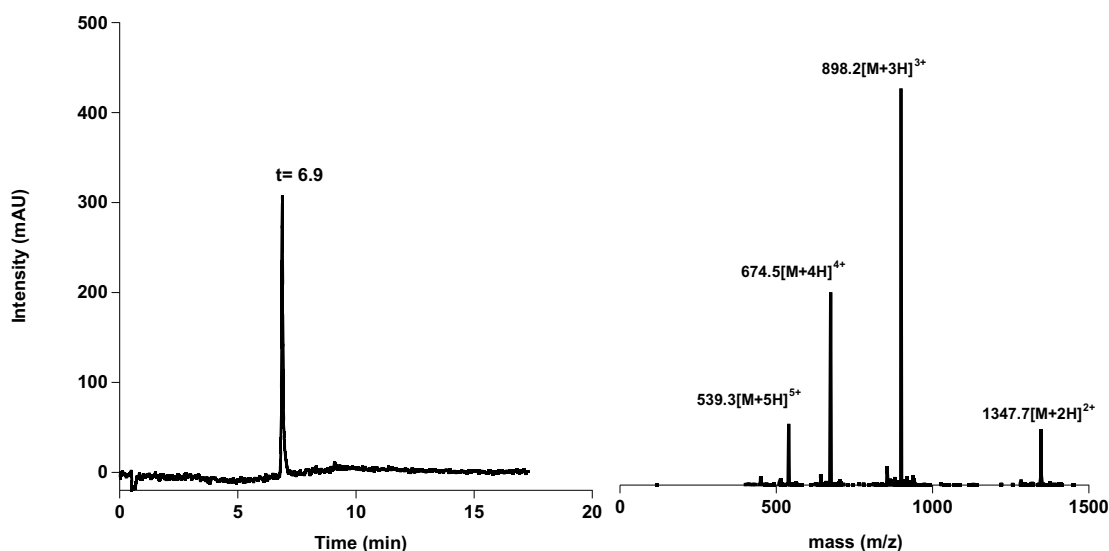


Fig. S1 Left: HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right: Mass spectrum of the purified peptide. EM-ESI⁺ (m/z): Calcd. for $C_{127}H_{181}N_{35}O_{31}$: 2692.4. Found: 1347.7 $[M+2H]^{2+}$; 898.2 $[M+3H]^{3+}$; 674.5 $[M+4H]^{4+}$; 539.3 $[M+5H]^{5+}$

UHPLC-MS α H-His₂[Pd]. The peptide was mixed with *cis*-PdCl₂(en) (1:1) in water for 10 min, and the resulting crude was analyzed by UHPLC-MS to corroborate the formation of metallopeptide.

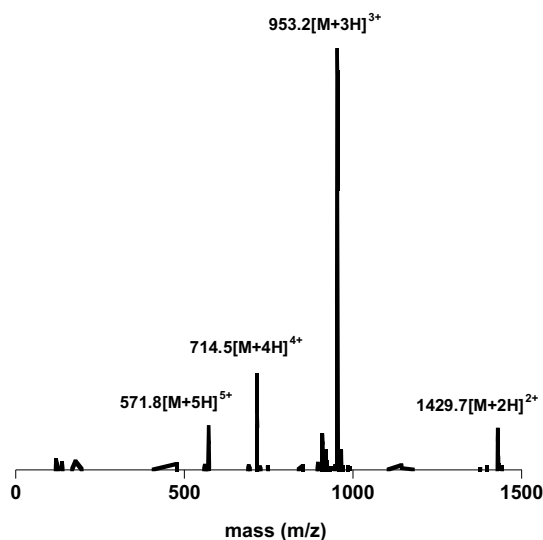


Fig. S2 Mass spectrum of the crude resulting from mixing α H-His₂ + *cis*-PdCl₂(en) (1:1), consistent with the formation of a complex containing Pd and the ethylenediamine ligand. EM-ESI⁺ (m/z): Calcd. for the metallopeptide $C_{129}H_{189}N_{37}O_{31}Pd$: 2858.34. Found: 1429.7 $[M+2H]^{2+}$; 953.2 $[M+3H]^{3+}$; 714.5 $[M+4H]^{4+}$; 571.8 $[M+5H]^{5+}$

Circular Dichroism. Circular Dichroism was measured on a *Jasco-715* coupled with a thermostat *Nestlab* RTE-111 using a 2 mm *Hellma* cuvette at 25 °C and the following settings: 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. The spectra are the average of 5 scans and were processed using the “smooth” macro in the program *KaleidaGraph* (v 3.5 by Synergy Software).

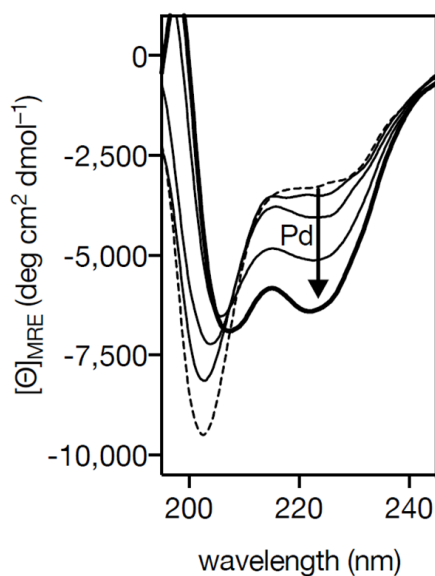


Fig S3. *Left:* CD titration experiment. Circular dichroism of a 20 μ M solution of α H-His₂ (dashed line) and addition of 1, 2, 5 and 10 equivalents of *cis*-PdCl₂(en). The experiments were carried out in 10 mM phosphate buffer pH 7.5, 100 mM of NaCl and 10% TFE, 25 °C. Mean residue molar ellipticity ($[\Theta]_{\text{MRE}}$) was calculated considering the peptide as an 18-mer.

Computational simulations. Ligand-protein docking exploration with GOLD, and GaudiMM, was first performed to discern the possible coordination modes of the Pd(II) complex with the **α H-His₂** peptide. Then, the **α H-His₂**[Pd] cores were parametrized with the Amber tool MCPB.py,³ based on the bonded model approach. QM calculations were performed with the DFT formalism as implemented in Gaussian 09, combined with the hybrid functional B3LYP.⁴ The basis set employed was 6-31g(d,p) for C, H and N atoms, while the SDD basis set with *f*-polarization function was used for the metal ion, with a pseudo-potential for the core electrons.⁵ All these calculations were combined with the Grimme's D3 correction for dispersion.⁶ The complexes were embedded in a solvent-polarizable dielectric continuum model (SMD, water).⁷ Once these QM calculations were obtained, the parameters were created for those atoms coordinating the Pd(II) ion through Seminario's approach.⁸ The point charges were computed based on the RESP protocol.⁹ Once the parameters were obtained, the systems were set up with AMBER18 *tLeap* to build the topology and the coordinates file. The force field used for the protein atoms was the ff14SB,¹⁰ improved force field recommended for the simulation of peptides. For the remaining atoms, the GAFF force field was applied; while the parameters obtained from the MCPB.py calculation were used for the Pd(II) ion. Explicit water TIP3P solvent was also considered in the complexes,¹¹ introducing neutrality by the addition of three and one chloride ions for the metallic and free peptide, respectively. The systems were embedded into a cubic box containing between 2700 and 3000 water molecules, 10 Å from the protein to the edge of the box. The topology and the coordinates files achieved for each complex were then used for a first 10 ns classical Molecular Dynamics (MD) simulation. The coordinates of the last structure were used for the Gaussian accelerated Molecular Dynamics (GaMD) simulations,¹² which allows an extensive exploration of the conformational space. The AMBER ff14SB force field in an NVT ensemble was also used with SHAKE algorithm. A boost on both dihedral a total potential energy was applied, producing GaMDs of at least 2 μ s for each system. To prove the correct exploration of the conformational space, PCA analysis were applied to the trajectories. Despite GaMD allows extensive conformational exploration, simulations were started from different putative folded geometries of the peptide to assure unbiased predictions to evaluate the progression of the system from the two extreme situations (data not shown).

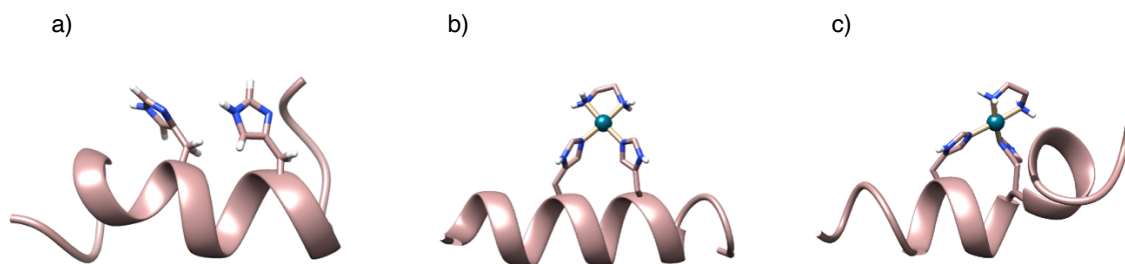
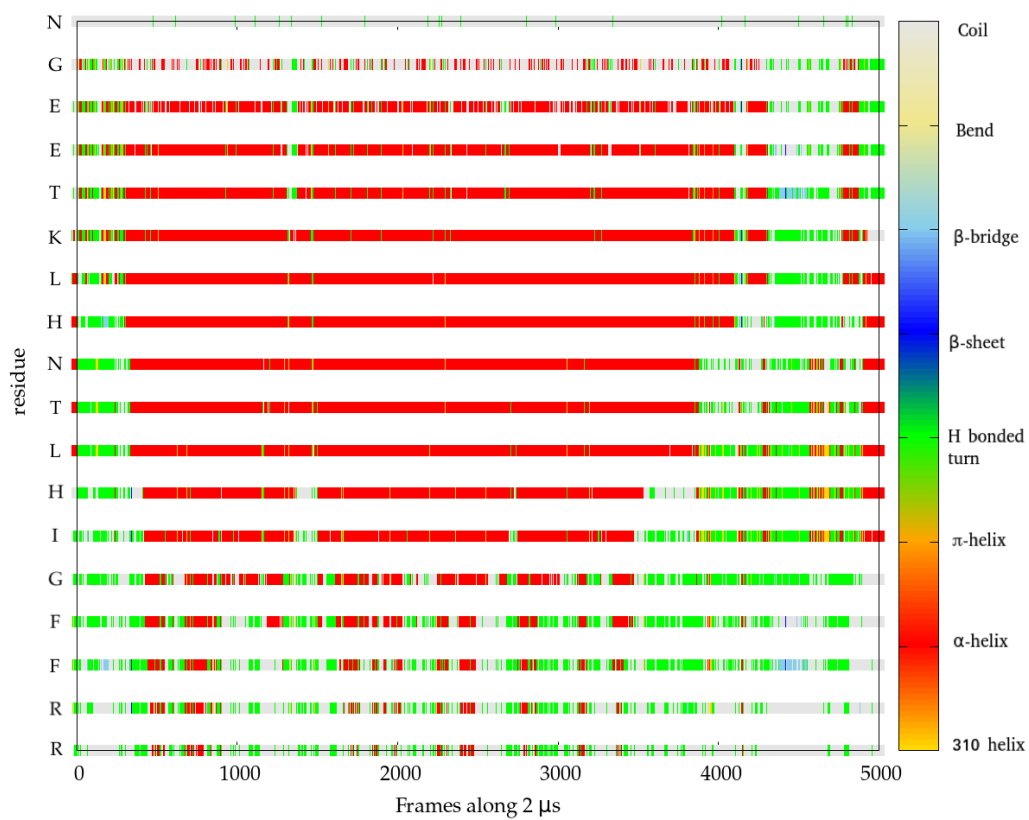
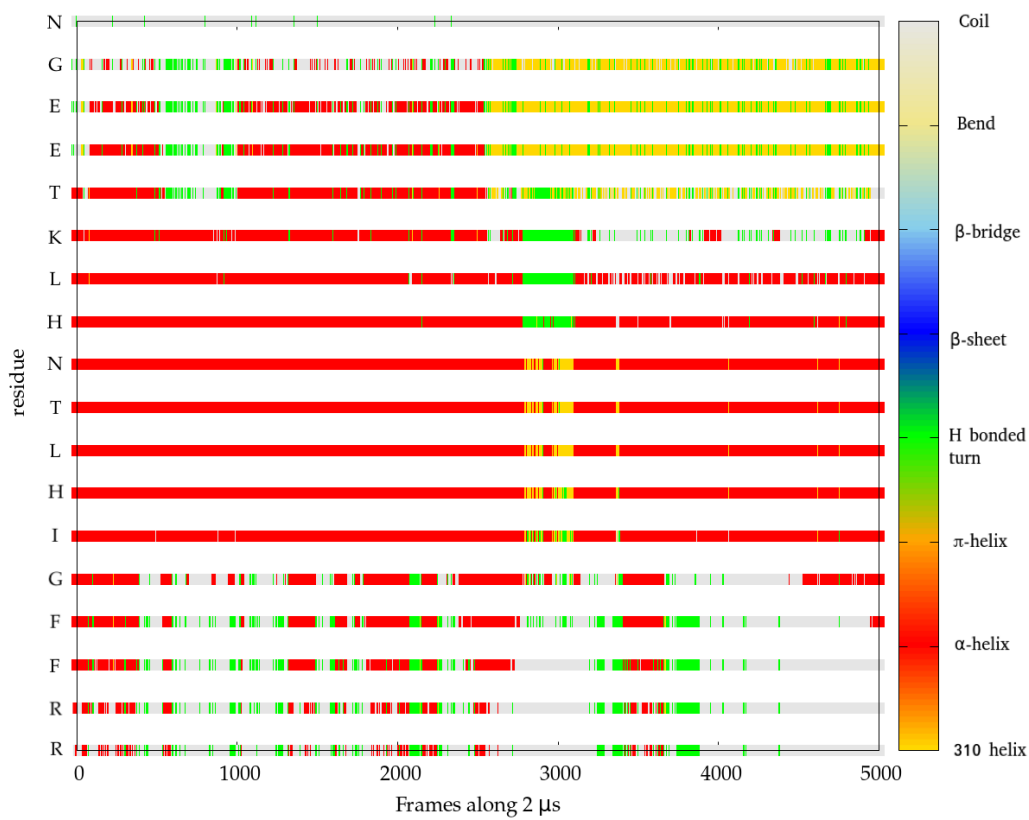


Fig S4. representative structures from each GaMD simulation.

A**B**

c

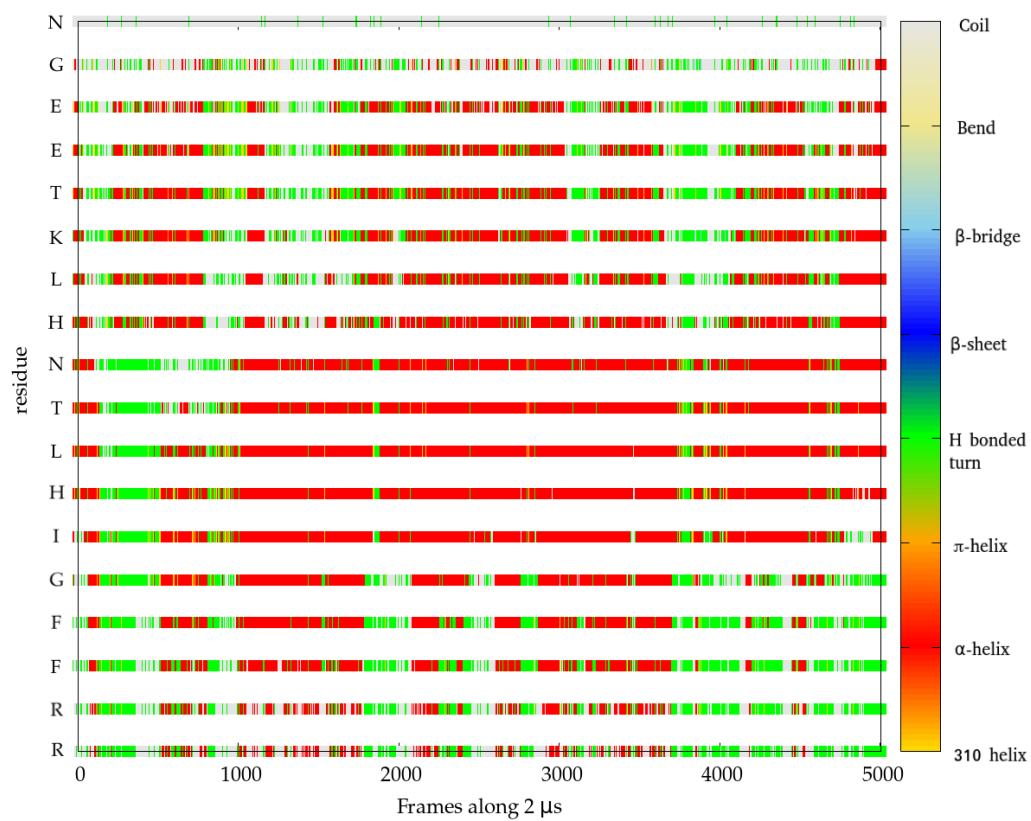


Fig S5. Secondary Structure Analysis along 2 μ s GaMD trajectory: a) α H-His₂; b) α H-His[Pd] *Nr*-His933/*Nr*-His937 complex; c) α H-His[Pd] *Nr*-His933/*Nr*-His937

Fluorescence Anisotropy and curve fitting. Anisotropy measurements were made with a *Jobin-Yvon Fluoromax-3*, (DataMax 2.20), coupled to a *Wavelength Electronics* LFI-3751 temperature controller, and using a 1 mL *Hellma* micro cuvette. 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. Aliquots of a stock solution of protein were added onto 15 nM solutions of **α H-His₂** or **α H-His₂[Pd]** in Tris-HCl buffer 20 mM, pH 7.5, 100 mM NaCl, and the anisotropy was recorded after each addition. Experimental data correspond to the mean of three independent titration experiments and are representative of at least three biological replicates performed with independent preparations of recombinant proteins. All experiments carried out at 25 °C. Before use the corresponding purified protein, the glycerol was removed using a *Zeba Spin Desalting Columns* (*Thermo fisher*) that had been equilibrated with anisotropy buffer (Tris-HCl buffer 20 mM, pH 7.5, 100 mM NaCl). For the switch experiment, after completing the titration at saturating concentrations of the protein, 50 eq. of DEDTC (relative to the metalloprotein) was added to the solution and the anisotropy was recorded. After that, 50 eq. of *cis*-PdCl₂(en) was added (relative to the metalloprotein) and the anisotropy was recorded again.

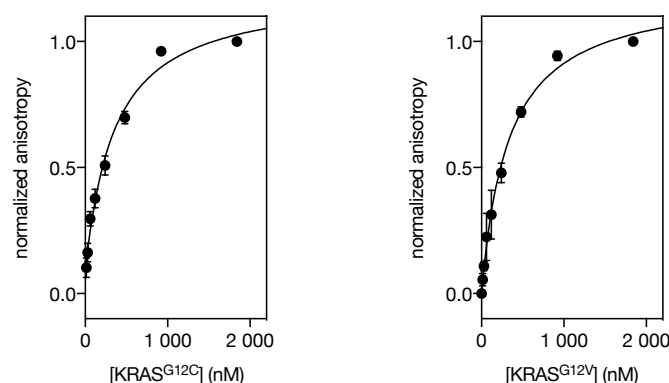


Fig S6. Anisotropy titration with oncogenic mutants KRAS^{G12C} and KRAS^{G12V}. Normalized emission anisotropy at 559 nm of a 15 nM solution of **α H-His₂[Pd]** with increasing concentrations of KRAS^{G12C} (*Left*) and KRAS^{G12V} (*Right*). The best fit to a 1:1 binding model is also shown. Data are mean $\pm$ SEM for experiments performed in technical triplicate and are representative of at least three biological replicates performed with independent preparations of recombinants KRAS. All experiments carried out at 25 °C.

Experimental data were fitted with the *DynaFit 4.0* software, which performs a numerical treatment of the system.^{13,14} The program is available free of charge for academia at <http://www.biokin.com/dynafit/>. *Dynafit* requires plain text files (scripts) that contains information about the chemical model underlying the experimental data, the values of model parameters, such as starting concentrations of reactants, as well as information about location of the files. A typical script used in the analysis titrations is included below. The file has been commented to indicate the purpose of the keywords and sections, but the reader is recommended to review the *DynaFit* scripting manual distributed along the program or available at the *DynaFit* website.

```

[task]                                ;semicolons indicate comments from actual instructions
task = fit                            ;nature of the calculation to be performed
data = equilibria

[mechanism]                           ;Free-form 1:1 binding model with Kd
R + L <==> RL : Kd dissociation      ;to be calculated as dissociation constant

[constants]                           ;Initial Kd value for iteration
Kd = 1.0 ?                            ;the "?" indicates that this will be optimized

[concentrations]                      ;Fixed conc. of the DNA during the peptide titration
R = 2.0

[responses]                           ;contribution to the spectroscopic signal of each
R = 0.1 ?                             ;of the different components of the equilibrium
RL = 1.5 ?                            ;these will be optimized ("?" after the values).

[data]                                ;location of files and information about the data
variable L                            ;the species that changes conc. during the titration
offset auto ?
directory ./exp/brHis                 ;file path (relative to DynaFit program location)
extension txt
file f1                               ;name of the experimental data file

[output]                              ;path indicating location of DynaFit output files
directory ./exp/brHis/out

[settings]                            ;cosmetic settings that control DynaFit graphics
{Output}                              ;fits were exported & finally plotted with
XAxisUnit = uM                        ;GraphPad Prism 7.0c. GraphPad Software,
BlackBackground = n                   ;La Jolla California, www.graphpad.com
XAxisLabel = [peptide]
YAxisLabel = anisotropy
WriteTXT = y

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High resolution NMR experiments. NMR experiments were recorded on a *Bruker Avance III* 600-MHz spectrometer (IRB Barcelona) equipped with a quadruple (^1H , ^{13}C , ^{15}N , ^{31}P) resonance cryogenic probe head and a z-pulse field gradient unit at 298 K using a 1 mM solution of **$\alpha\text{H-His}_2$** peptide—in the presence or absence of *cis*-PdCl₂(en)—1D proton spectra were recorded with a sweep width of 12000 Hz and 32 k data points. A total of 16 scans were accumulated with an acquisition time of 2.05 s. A Watergate w5 composite pulse was used to suppress the water signal. ^1H 2D-TOCSY and NOESY experiments were acquired in 90% H₂O/10% D₂O and used to assign the spin systems corresponding to the peptide resonances.^{15–17} For the 2D-NOESY experiments, mixing times of 300, 150 and 80 ms were acquired to minimize the impact of spin-diffusion in the assignments. Spin-locking fields of 8 kHz and 50 ms mixing time was used for the 2D-TOCSY experiments. All 2D spectral widths were 8000 Hz. The data size was 512 points in F1, indirect dimension, and 2048 points in F2, direct dimension. For each F1 value, 48 transients were accumulated in the NOESY and 32 in the TOCSY experiments respectively. Data were processed with a combination of exponential and shifted sine-bell window functions for each dimension followed by automated baseline and phase correction using *TopSpin* 3.5 (©Bruker 2020). The 512 × 2 k data matrices were zero-filled to 2k×2k (NOESY and TOCSY). Structure Calculation: To prove that the primary structure (composition and connectivity) corresponds to the theoretical sequence, we have followed the sequence assignment strategy.¹⁷ We identified the characteristic spin system of every residue in the sequence, using the 2D TOCSY experiment and every spin system was connected to the following one via NOEs observed from the side-chain of a given residue (*i*) to the amide proton of the following residue (*i*+1) as well as from the amide proton of (*i*) to the amide of (*i*+1). The full spin analysis as well as the assignment of the NOEs were carried out manually using CARS software.^{18,19} Distance restraints derived from the NOESY experiments were used for the NMR-based model building of the peptide in solution using unambiguously assigned peaks exclusively and the program CNS 1.2 (Crystallography and NMR system).²⁰ The protocol consisted of an implicit water simulated-annealing of 120 structures using 8,000 cooling steps followed by an explicit water refinement of the calculated structures using all experimental restraints during 1200 steps. The Pd coordination was not explicitly included in the calculation. To display the metal coordination, we manually optimized the His rotamers to facilitate the coordination and the Pd was added to the final model displayed in Figure 2.

Chemical shift assignments

AlphaHHis2

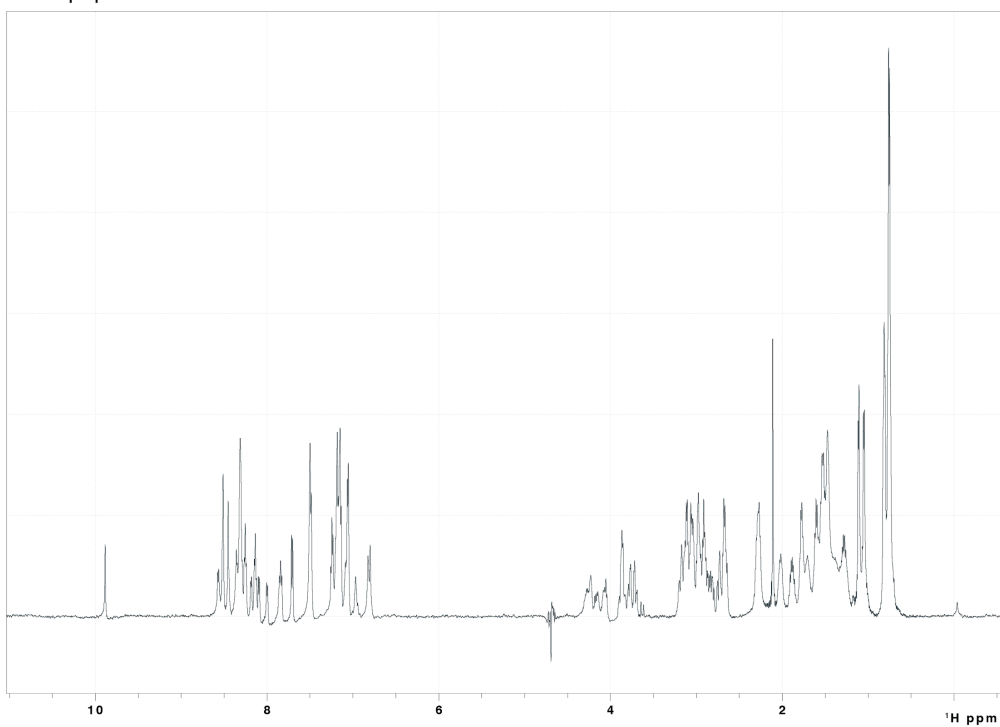
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	8.507		7.841		2.669		8.299
	HA		HA1		HD21		HB2/3
	4.300		3.762		7.502		1.929
	HB2		HA2		HD22		HG2/3
	1.763		3.701		7.062		2.274
	HG2	6 ILE	HN		HN	17 GLY	HN
	1.616		7.815		8.440		8.349
	HG3		HA		HA		HA1/2
	1.529		4.044		4.590		3.861
	HD2/3		HB		HB2		HN
	3.103		1.710		3.184		8.237
	HE		QG2		HB3	18 ASN	HA
	7.084		1.032		3.063		4.634
	HN		HG12		HD2		HB2
	8.249		1.236		7.182		2.737
2 ARG	HA	7 HIS	QD1	12 LEU	HE1		HB3
	4.180		0.739		8.510		2.654
	HB2/3		HN		HN		HD21
	1.522		8.564		8.180		7.477
	HG2/3		HA		HA		HD22
	1.303		4.685		4.252		6.819
	HD2/3		HB2		HB2		
	2.981		3.148		1.549		
	HE		HB3		HB3		
	6.969		3.043		1.470		
3 PHE	HN	8 LEU	HD2	13 LYS	QD1/2		
	8.089		7.149		0.787		
	HA		HE1		HN		
	4.516		8.445		8.188		
	HB2		HN		HA		
	2.957		8.297		4.271		
	HB3		HA		HB2		
	2.807		4.320		1.776		
	HZ/QD		HB2		HB3		
	7.053		1.535		1.696		
4 PHE	QE	9 THR	HB3	14 THR	HG2/3		
	7.160		1.470		1.343		
	HN		QD1/2		HN		
	8.131		0.745		8.126		
	HA		HN		HA		
	4.519		8.134		4.221		
	HB2		HA		HB		
	3.048		4.228		4.138		
	HB3		HB		QG2		
	2.860		4.086		1.106		
	HZ	10 ASN	QG2	15 GLU	HN		
	7.182		1.051		8.312		
	QD		HN		HB2		
	7.137		8.311		2.015		
	QE		HA		HB3		
	7.234		4.582		1.891		
			HB2		HG2/3		
			2.679		2.266		

Chemical shift assignments for AlphaHHis2 + 3 Equiv *cis*-PdCl₂(en)

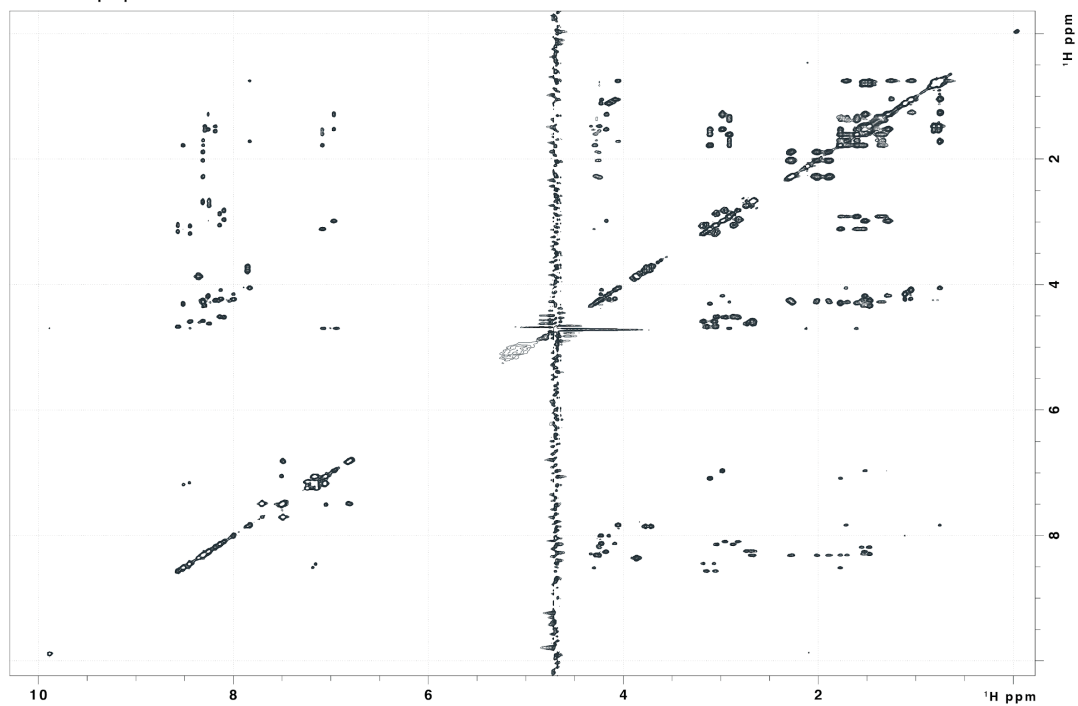
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	HA		7.892		1.057		HZ1
	4.293		HA1		HN		7.425
	HB2/3		3.762		8.385		HN
	1.768		HA2		HA		7.930
	HG2		3.700		4.421		HA
	1.606		HN		2.761		4.238
	HG3		7.811		HB3		HB
	1.524		HA		2.619		4.145
2 ARG	HD2/3	6 ILE	3.944	11 HIS	HD21	15 GLU	QG2
	3.115		HB		7.048		1.100
	HE 7.098		QG2		HD22		HN
	HN		0.667		7.492		8.233
	8.231		HG12		HN		HA
	HA		1.058		8.045		4.275
	4.175		HG13		HA		HB2
	HB2		0.958		4.897		2.030
	1.275		QD1		HB2		HB3
	HB3		0.636		2.775		1.898
3 PHE	1.273	7 HIS	HN	12 LEU	HB3	16 GLU	HG2/3
	HG2/3		8.447		2.727		2.346
	1.519		HA		HD2		HN
	HD2/3		4.371		6.651		8.268
	2.978		HB2		HE1		HB2
	HE		3.031		7.611		2.033
	6.982		HB3		HN		HB3
	HN		2.956		7.953		1.876
	8.041		HD2		HA		HG2/3
	HA		6.649		4.031		2.355
4 PHE	4.512	8 LEU	HE1	13 LYS	HB2	17 GLY	HN
	HB2		7.537		1.556		8.296
	2.960		HN		HB3		HA1
	HB3		7.988		1.475		3.866
	2.791		HA		HG 1.761		HA2
	HZ/ QD		4.228		QD1/2		3.844
	7.035		HB2		0.811		HN
	QE		1.684		HN	18 ASN	8.217
	7.160		HB3		8.448		HA
	HN		1.596		HA		4.447
4 PHE	8.130	9 THR	QD1		4.334		HB2
	HA		0.814		HB2		2.717
	4.527		QD2		1.758		HB3
	HB2		0.776		HB3		2.653
	3.056		HN		1.517		HD21
	HB3		7.636		HG2/3		6.819
	2.886		HA		1.386		HD22
	HZ/QD		4.337		QD		7.470
	7.132		HB		1.602		

NMR Spectra

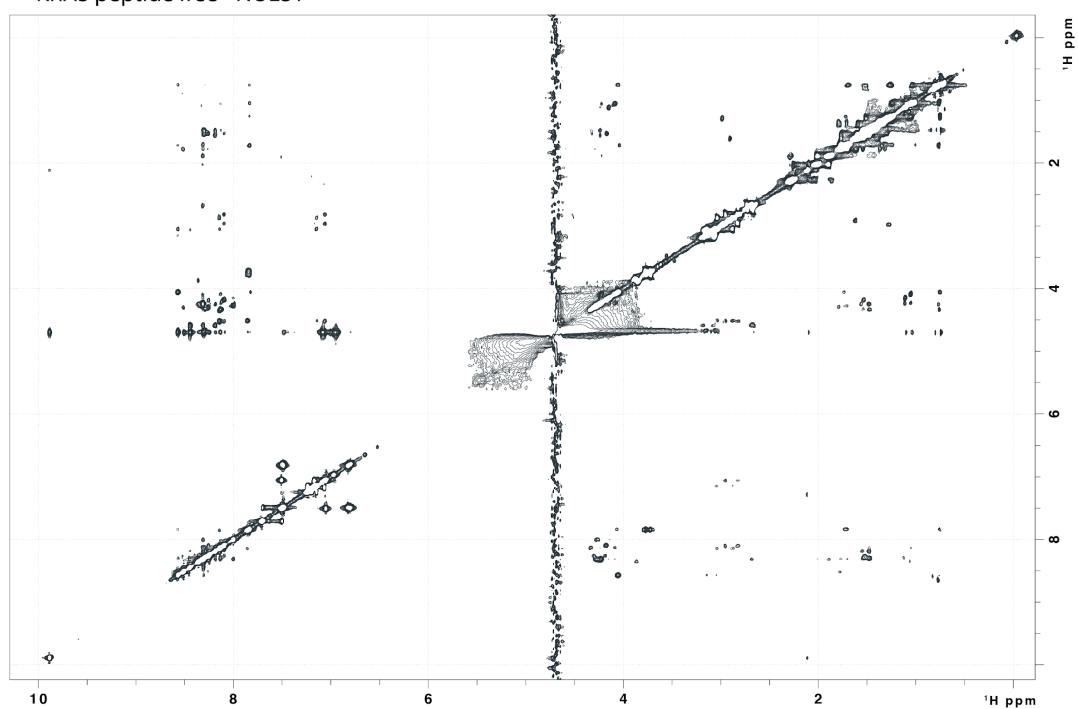
KRAS peptide free



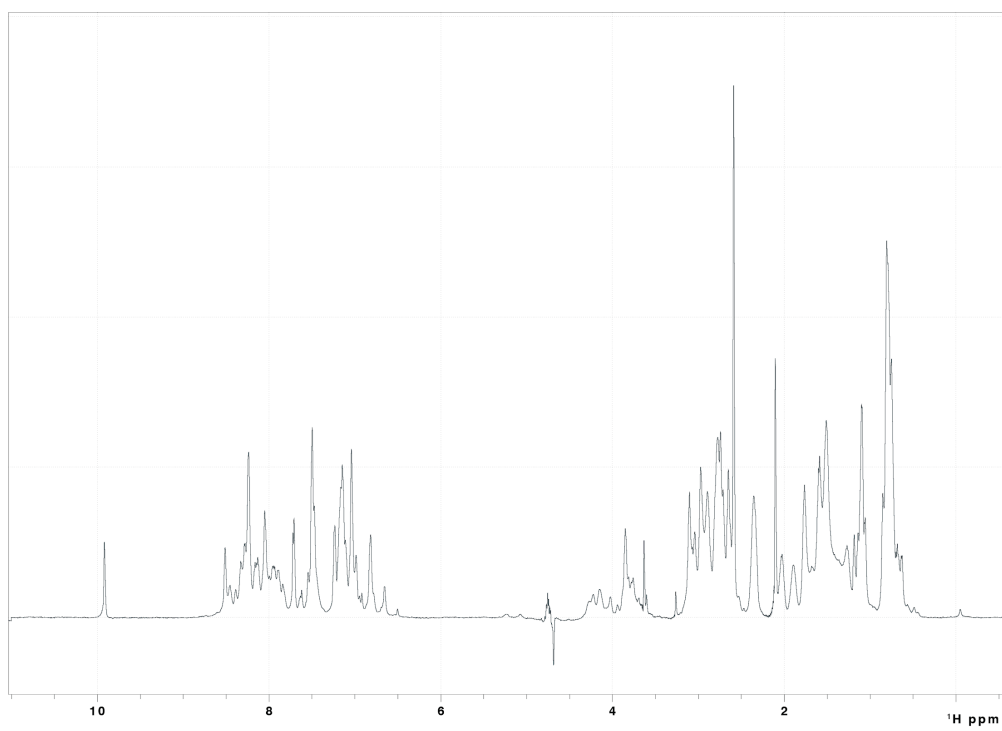
KRAS peptide free - TOCSY



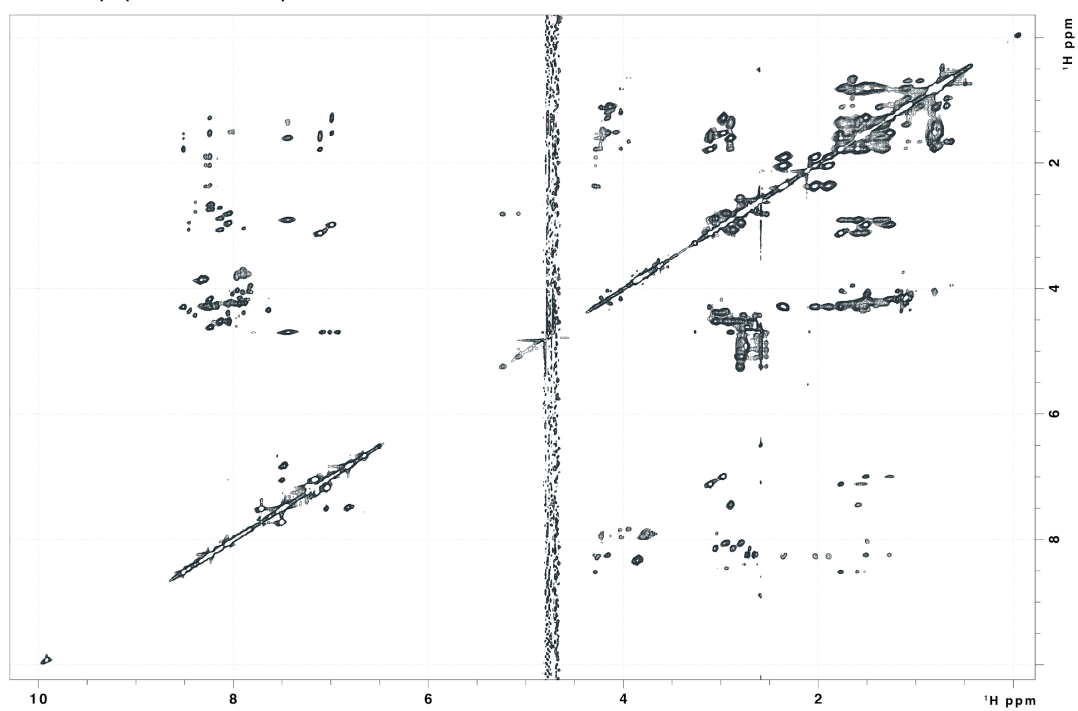
KRAS peptide free - NOESY



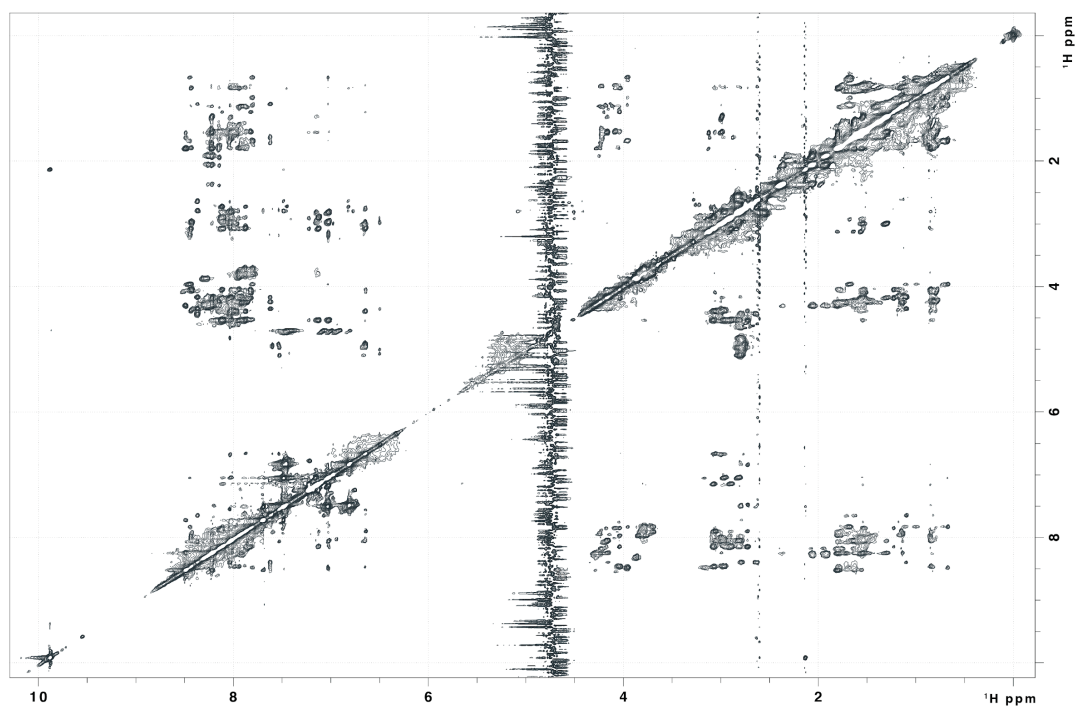
KRAS peptide free + 3eqv Pd



KRAS peptide free + 3eqv Pd - TOCSY



KRAS peptide free + 3eqv Pd - NOESY



Stability studies. $\alpha\text{H-His}_2[\text{Pd}]$ was incubated for 4 h at 37 °C in DMEM + 5%FBS, in HeLa cells lysates and in PBS. The solutions were then analyzed by HPLC-MS, showing that $\alpha\text{H-His}_2[\text{Pd}]$ remains coordinated and stable.

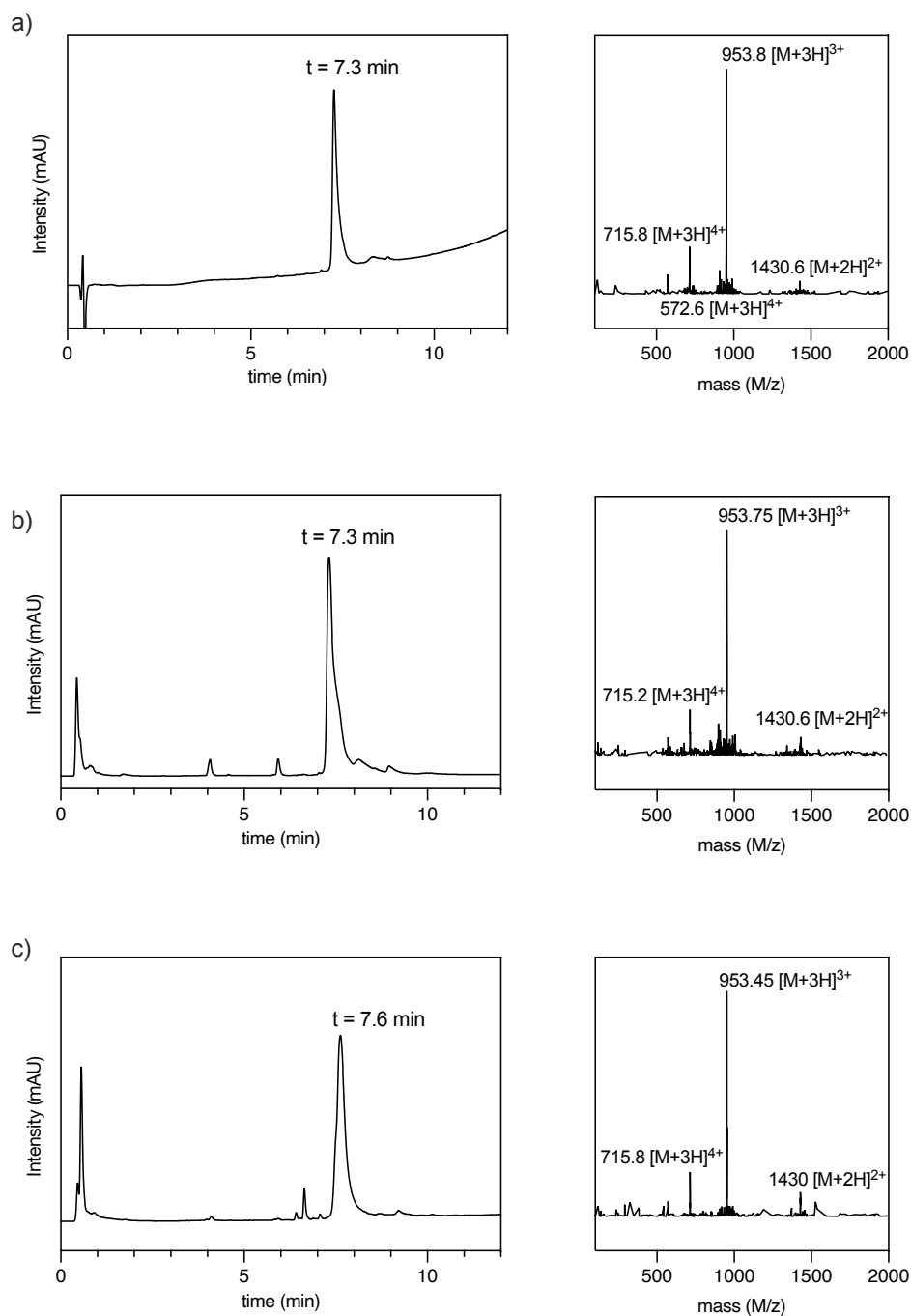


Fig S7. Stability study of $\alpha\text{H-His}_2[\text{Pd}]$. a) incubated with PBS; b) incubated with DMEM + 5% FBS; c) incubated with HeLa cells lysates.

ICP-MS assays. For the ICP measurements, 100.000 cells per well were seeded in 24-well plate two days before treatment with metallopeptide in different concentrations (10 μM and 50 μM) for 4h. Cells were washed twice with PBS and lysed in 70% HNO_3 . The lysates were digested in duplicate with $\text{HNO}_3/\text{H}_2\text{O}_2$ by microwave heating before being analyzed. After performed the ICP-MS we detected only a small percentage of palladium inside the cells.

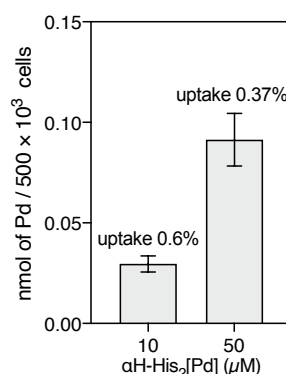


Figure S8. ICP-MS results on the intracellular accumulation of Pd. We incubated HeLa cells with 10 μM or 50 μM of metallopeptide for 4 h at 37 $^\circ\text{C}$. Finally, the cells were washed two time with PBS and digested with HNO_3 . Data are mean $\pm$ SEM for experimental repeated in two independent times.

MTT Assays. The toxicity of metallopeptide, peptide and *cis*-Pd(en)Cl₂ was tested by MTT assays in HeLa cells as follows: 100,000 cells per well were seeded in 96 well plates two days before treatment with different concentrations of the peptides/metal. After 24 h of incubation, HEPES containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added to the cell culture medium to a final concentration of 0.5 mg/ml. Cells were then incubated for 4 h to allow the formation of formazan precipitates by metabolically active cells. A detergent solution of 10% SDS (sodium dodecyl sulphate) and 0.01 M HCl was then added, and the plate was incubated overnight at room temperature to allow the solubilization of the precipitates. The quantity of formazan in each well (directly proportional to the number of viable cells) was measured by recording changes in absorbance at 570 nm in a microtiter plate reading spectrophotometer (*Tecan Infinite 200 PRO*). The MTT assays showed a moderate decrease on cell growth at high concentrations of metallopeptide.

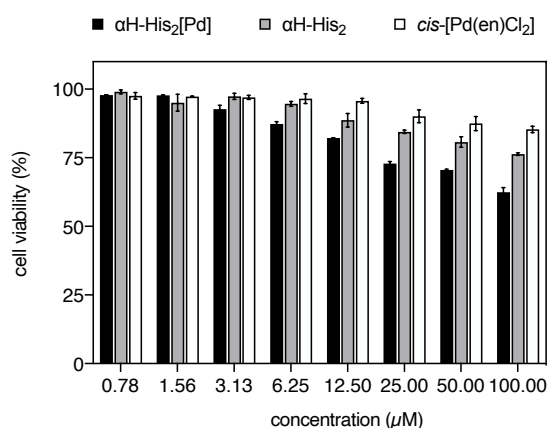


Figure S9. Toxicity assay. HeLa cells were incubated with peptide, metallopeptide and palladium at different concentrations, for 24 h at 37 $^\circ\text{C}$. Then, the cells were washed two time with PBS before carrying out the MTT assay. Data are mean $\pm$ SEM for experimental repeated in two independent times.

Supplementary References

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