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Intracellular delivery of a catalytic organometallic complex

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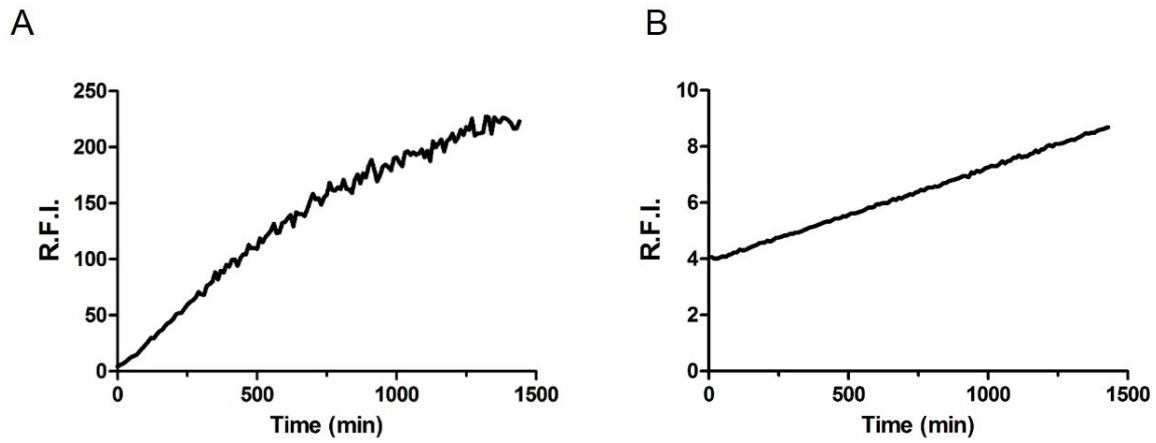


Fig. S1. Catalysed fluorescence "switch-on" of the caged fluorophore **5** (50 μM) with the Pd–peptide **1** (5 μM) in (A) PBS and (B) PC-3 cell lysate for 24 h at 37 °C. Relative increase in fluorescence (RFI) was measured at $\lambda_{Ex/Em}$ 495/520 nm and normalised to a control solution of **5** in the absence of the Pd–peptide catalyst.

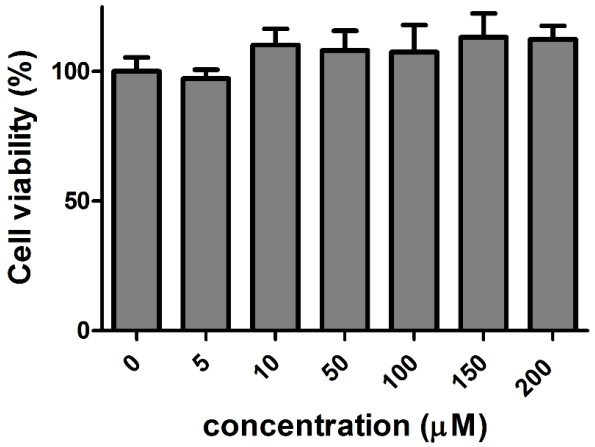
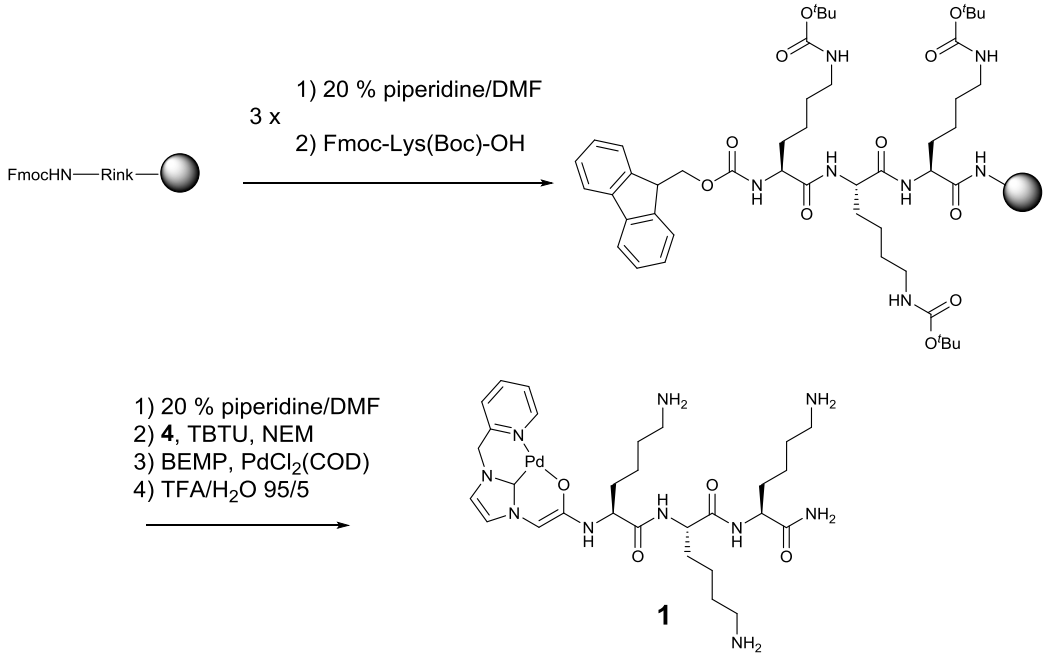


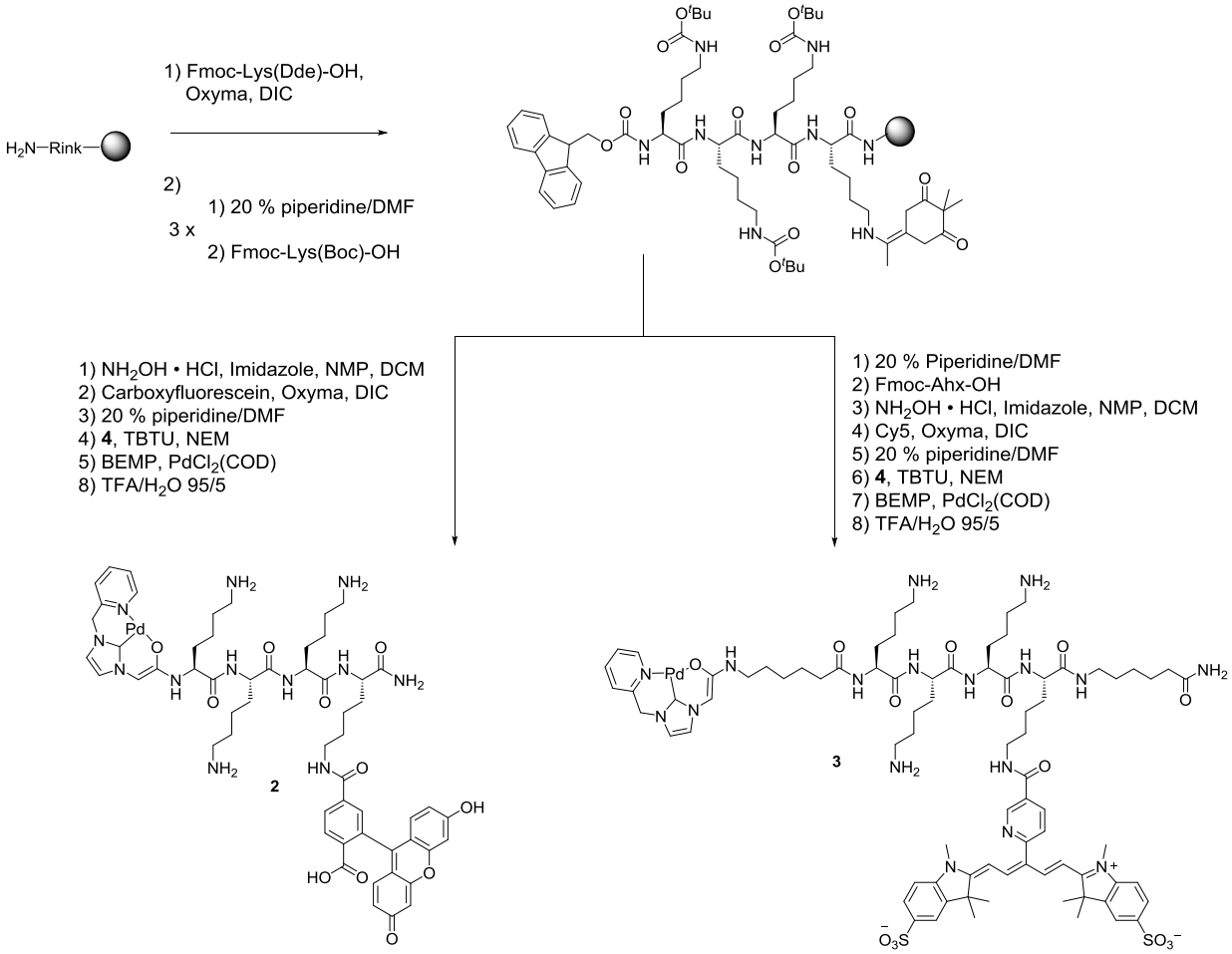
Fig. S2. Evaluation of cytotoxicity of Pd–peptide **1** on PC-3 cells. Cells were incubated with **1** at 0–200 μM for 24 h and cell viability measured using an MTT assay (n = 3).

S2. Experimental

All chemicals were purchased from Sigma Aldrich or Acros and used as received. All amino acids, aminomethyl polystyrene resin, and Fmoc-Rink amide linker were purchased from GL Biochem (Shanghai) Ltd and NovaBiochem. 3-(Carboxymethyl)-1-[(pyridin-2-yl)methyl]-3*H*-imidazol-1-ium trifluoroacetate **4** and *bis*-propargyloxycarbonyl-protected rhodamine 110 **5** were synthesised according to previously reported procedures.[1,2]



Scheme S1. Synthetic route to the preparation of **1**.



Scheme S2. Synthetic route to the preparation of **2** and **3**.

Synthesis of catalysts 1–3

The Pd catalysts were synthesised (Scheme S1 and S2) on aminomethyl polystyrene resin (100–200 mesh, loading 0.745 mmol/g). The Fmoc Rink-linker (*p*-{(*R*,*S*)-α-[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl}-phenoxyacetic acid) was coupled to the resin using the general coupling procedure given below. For **2** and **3** Fmoc-Lys(Dde)-OH was coupled first, followed by Fmoc deprotection and coupling of the relevant amino acid sequence (including εAhx-spacers for **3**). Once the sequences were complete, the Dde protecting group was removed with $NH_2OH \cdot HCl$/imidazole in NMP–DCM (see below) and 5(6)-carboxyfluorescein or sulfonated Cy5 were coupled to the Lys side chain. Ninhydrin tests were used to confirm the completeness after each coupling step. After cleavage from the resin, the compounds were purified by preparative HPLC, and analysed by HRMS and HPLC.

Coupling procedure. The appropriate Fmoc-protected amino acid or 5(6)-carboxyfluorescein (3 eq, 0.1 M) and Oxyma (4 eq) in DMF were stirred for 10 min. DIC (3 eq) was added and the mixture stirred for a further 5 min. The coupling solution was added to the resin (1 eq, ~ 250 mg resin),

preswollen in DCM, and shaken for 1 h. After filtration, the resin was washed with DMF (3 × 10 mL), DCM (3 × 10 mL), and MeOH (3 × 10 mL). Cy5 coupling procedure: sulfonated Cy5 (3 eq, 0.1 M), was activated with dipyrrolidino (*N*-succinimidyloxy)carbenium hexafluorophosphate (HSPyU) (3 eq) and DIPEA (3 eq) in DMF for 1 h at 40 ºC and added to the resin (1 eq, ~ 250 mg resin), preswollen in DCM, and shaken for 3 h. After filtration, the resin was washed with DMF (3 × 10 mL), DCM (3 × 10 mL), and MeOH (3 × 10 mL).

Fmoc deprotection. The resin was preswollen in DCM, and treated with 20 % piperidine in DMF (v/v) (3 mL, 2 × 5 min). The resin was isolated by filtration, and washed with DMF (3 × 10 mL), DCM (3 × 10 mL), and MeOH (3 × 10 mL).

Dde deprotection.[3] $NH_2OH{\cdot}HCl$ (1.25 g, 1.80 mmol) and imidazole (0.92 g, 1.35 mmol) were suspended in 5 mL of NMP and the mixture was sonicated until complete dissolution. Before the reaction, 5 volumes of the deprotection solution were diluted with 1 volume of DCM. The solution was added to the corresponding resin (100–125 mg, 20 mg/mL) and stirred for 2 h. The deprotected resin was isolated by filtration, and washed with DMF (3 × 10 mL), DCM (3 × 10 mL), and MeOH (3 × 10 mL).

Coupling of imidazolium salt and Pd loading. The imidazolium salt **4** (3 eq, 0.1 M), *N*-[(1H-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide (TBTU) (2.9 eq) and *N*-ethylmorpholine (4 eq) were stirred in DMF for 10 min. The coupling solution was added to the resin (1 eq), preswollen in DCM, and heated at 60 °C for 20 minutes under microwave irradiation (Biotage initiator + SP wave). After extensive washing with DMF (5 × 10 mL), DCM (5 × 10 mL), MeOH (5 × 10 mL), DMF (5 × 10 mL), and drying, the resin was treated with 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) (2.5 eq) in anhydrous DMF under a N_2 atmosphere for 15 min, followed by the addition of $PdCl_2(COD)$ (1.5 eq). The reaction mixture was stirred at room temperature for 18 h, the resin filtered and washed DMF (5 × 10 mL), DCM (5 × 10 mL).

Cleavage from the resin. The resin (100–125 mg), preswollen in DCM, was treated with 2–3 mL of TFA/H_2O (95/5) for 3 h. Addition of traditional scavenging agents, such as triisopropylsilane, destroyed the Pd complex. The cleavage solution was isolated from the resin by filtration, and the compounds precipitated by the addition of cold Et_2O and the product isolated by centrifugation.

Purification and characterisation of 1–3

Preparative HPLC was performed using an Agilent Technologies 1100 instrument with UV/Vis detection fitted with an Agilent Eclipse XDB-C18 (5 µm, 9.4 × 250 mm) column. Samples were eluted with a gradient of H_2O–CH_3CN with 0.1% formic acid, flow rate 2 mL/min. Gradient for **1** (collection at 254 nm): 2 to 15 % CH_3CN in 10 min; 15 to 95 % CH_3CN in 3 min; isocratic 95 % CH_3CN for 2 min. Gradient for **2** (collection at 495 nm): 2 to 35 % CH_3CN in 10 min; 35 to 95 % CH_3CN in 3 min; isocratic 95 % CH_3CN for 2 min. Gradient for **3** (collection at 650 nm): 2 to 5 % CH_3CN in 15 min; 15 to 95 % CH_3CN in 3 min; isocratic 95 % CH_3CN for 2 min.

Analytical HPLC was performed using an Agilent 1100 ChemStation equipped with a Zorbax Eclipse C18 reverse phase column (4.6 × 100 mm, 3.5 µm) eluting with a gradient of water/formic acid (0.1%) to MeCN/formic acid (0.1%) over 10 minutes or 15 minutes with a flow rate of 1 mL/min, and compounds were detected using an ELS detector and a multi-wavelength detector.

High Resolution Mass Spectra (HRMS) of the peptides was recorded on a Bruker 3.0 T Apex II spectrometer.

ICP-OES data was obtained on a Perkin Elmer Optima 5300 DV ICP-OES suitable for the trace analysis of metals between 0.0002–1000 ppm. Peptides **1**–**3** (0.003 mmol) were dissolved in HNO_3 (5%, 3 mL), sonicated for 30 min and analysed by ICP-OES. Pd content is displayed as mol% of analysed peptide.

Table S1. HR-MS, HPLC and ICP-OES data of Pd-peptides **1**, **2**, and **3**.

		HR-MS		**HPLC**	**ICP-OES**
		calc.	found	t_r (min), purity	Pd content of peptide (mol%)
1	$C_{29}H_{47}O_4N_{10}Pd$	705.2811	705.2811	2.47, (>95%)	27%
2	$C_{56}H_{68}O_{11}N_{12}Pd$	1191.4257	1191.4240	3.87, (>97%)	25%
3	$C_{74}H_{103}N_{16}O_{13}PdS_2^+$	1594.646	1594.341	2.29, (>95%)	23%

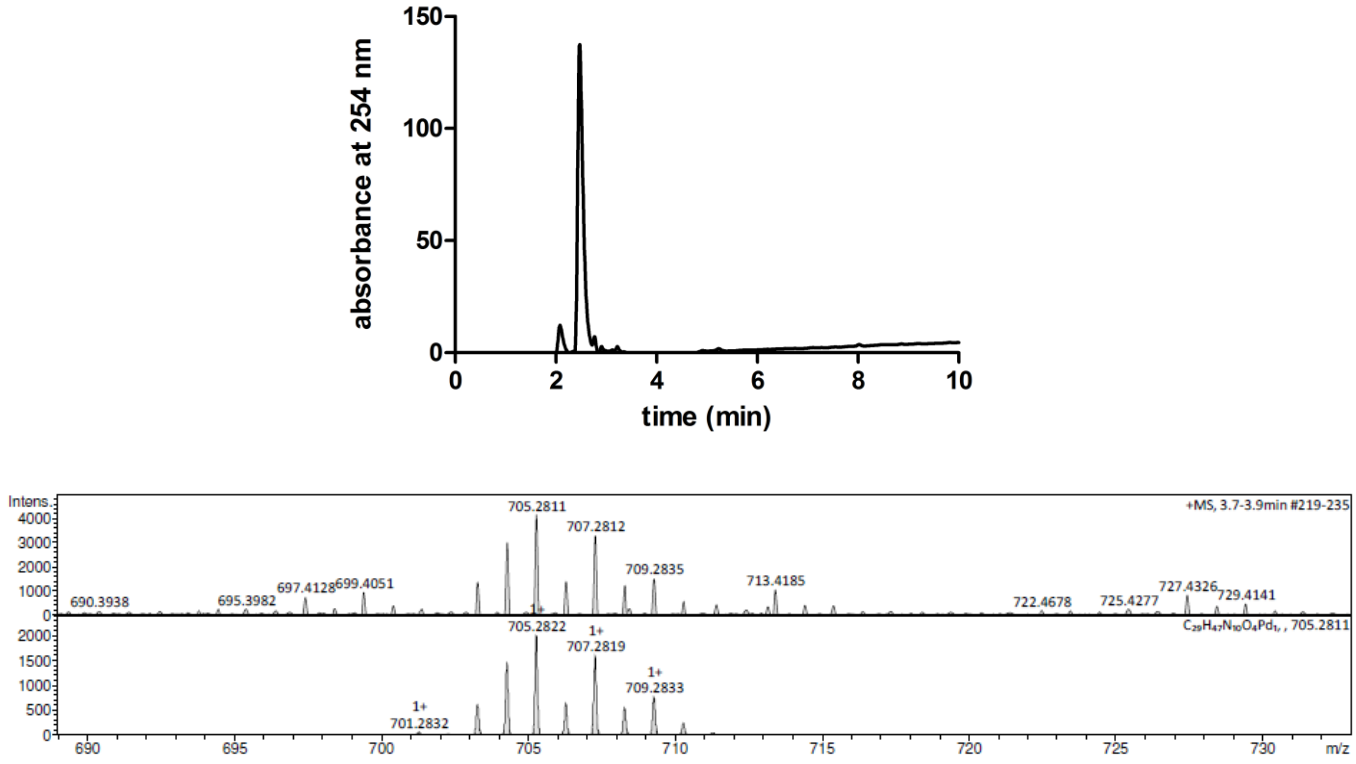


Figure S3. HPLC trace (top) and HR-MS spectrum (bottom) of Pd-peptide **1**.

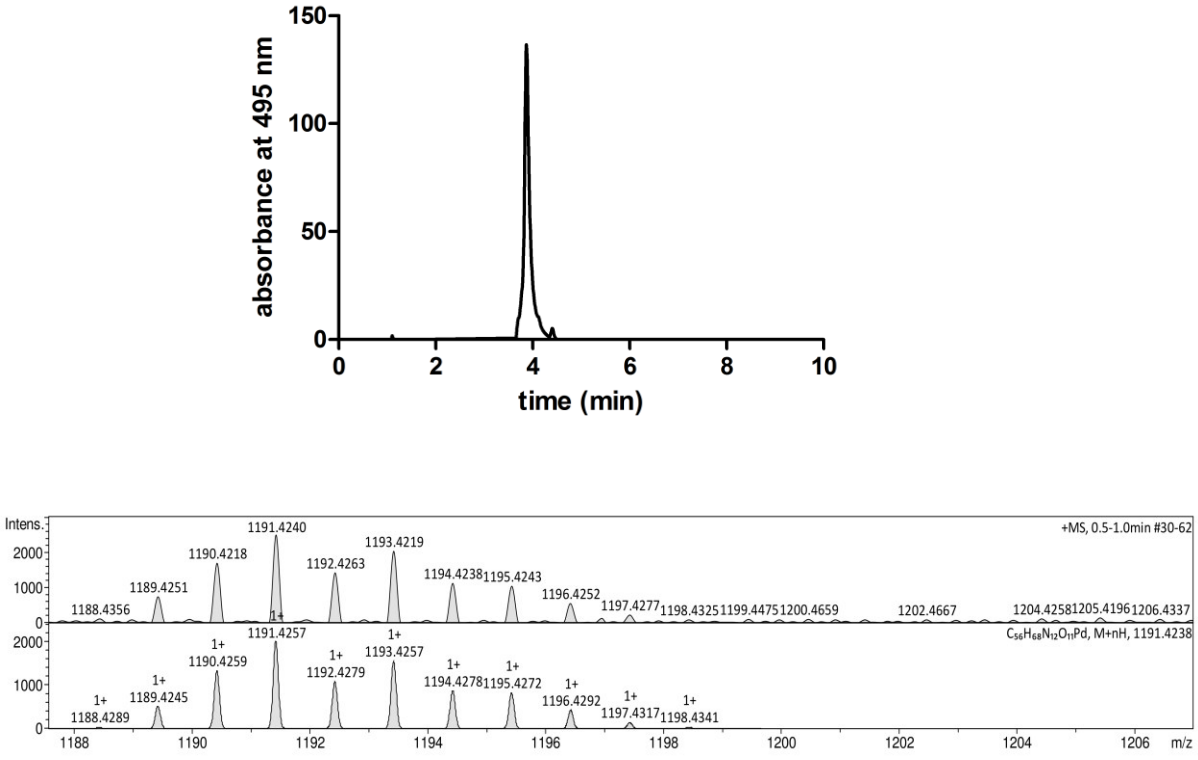


Figure S4. HPLC trace (top) and HR-MS spectrum (bottom) of Pd-peptide **2**.

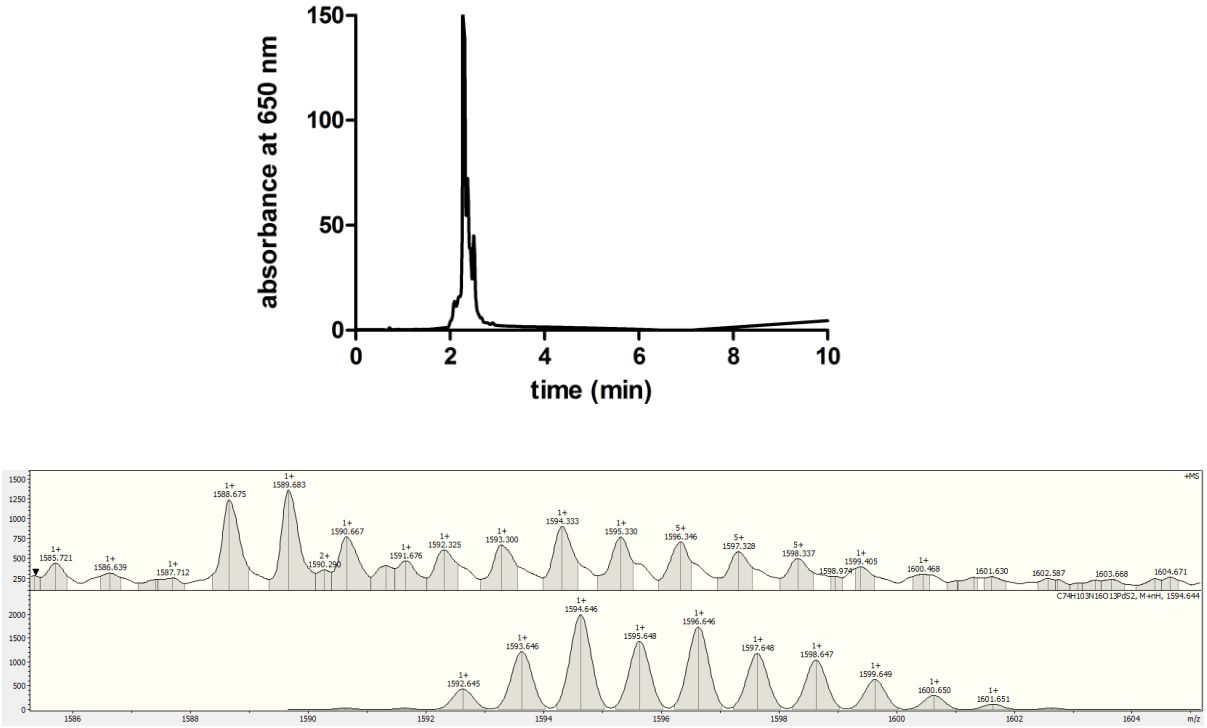


Figure S5. HPLC trace (top) and HR-MS spectrum (bottom) of Pd-peptide **3**.

***In vitro* evaluation of proc-rhodamine cleavage**

To 500 µL of PBS (pH 7.3, 10 mM) or PC-3 cell lysate, 10 µL of **5** (10 mM in DMSO) was added to give a final concentration of 50 µM. Pd catalyst **1** (5 µM Pd, from 10 mM stock solution in H_2O) was added and the mixture incubated at 37 °C for 18 h and fluorescence recorded every 10 min ($\lambda_{Ex/Em}$ 495/520 nm, BioTek HT Synergy multi-mode reader).

Cell culture

PC-3 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), *L*-glutamine (4 mM) and antibiotics (penicillin and streptomycin, 100 units/mL). Cell culture was performed in a 5% CO_2 atmosphere at 37 °C in a SteriCult 200 (Hucoa-Erloss) incubator. The day before the assays, the cells were washed with PBS, detached with trypsin/EDTA (0.25% trypsin, 1 mM EDTA), counted, and diluted with DMEM to the appropriate concentration.

Preparation of cell lysate

PC-3 cells (3 million) were washed with PBS, detached with Trypsin/EDTA, harvested with water (2 mL) and sonicated in an ultrasonic bath (Fisherbrand F15053). The lysate was centrifuged for 10 min at 13,000 rpm. The supernatant was collected and used in the *in vitro* studies.

MTT assay

PC-3 cells were plated in a 96-well plate with a density of 5,000 cells/well and left to grow for 24 h. A solution of the Pd catalyst **1** was added to the wells at desired concentrations and incubated with the cells for 24 h. The media was then replaced with 100 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT) solution (1 mg/mL) in PBS and the cells incubated for 3 h at 37 °C. After incubation, the resulting formazan crystals were dissolved by adding 100 µL of MTT solubilisation solution (10 % Triton-X 100 in 0.1 N HCI in isopropanol). The absorbance was measured at a wavelength of 570 nm (BioTek HT Synergy multi-mode reader using the Gen5 microplate and imaging software 2.0.) and the results compared to untreated cells.

ICP-OES of cell lysate

PC-3 cells were grown in a 6 well-plate until confluent and then treated with the Pd catalyst **3** (3 and 30 µM) or $Pd(OAc)_2$ (10 µM) for 3 h. The media was removed, the cells washed with PBS (3×), detached with trypsin, and counted (hemocytometer). The cells were centrifuged at 7000 rpm for 5 min after which the cell pellet was suspended in aqueous HNO_3 (5%), sonicated for 1 h and analysed by ICP-OES, to give the final results as ng of Pd per million cells.

Live cell flow cytometry

PC-3 cells were plated in a 24-wellplate (2×10^4 cells/well) and grown for 24 h at 37 °C. For the uptake studies, the media was removed and a solution of Pd catalyst **2** (30 µM) in media was added and the cells incubated for 2 h at 37 °C. For proc-rhodamine decaging studies, the media was removed and a solution of Pd catalyst **3** (30 µM) in media was added and incubated for 2 h at 37 °C. Cells were washed twice with PBS and incubated with a solution of **5** (50 µM) for 18 h at 37 °C. After incubation, cells were washed twice with PBS, harvested with Trypsin/EDTA and resuspended in DMEM. The cells were analysed by flow cytometry (Becton Dickinson (BD) FACSAriaTM) using a 530/30 nm (compound **2** and **6**) or 660/20 nm (compound **3**) band pass filter, and the data analysed using FlowJo.

Confocal microscopy imaging

PC-3 cells were cultured on sterilised glass cover slips (24 mm diameter). The coverslips were placed in 6 well-plates and seeded with 9×10^4 cells/well in 1.6 mL of DMEM and incubated for 24 h. The media was removed and a solution of Pd catalyst **2** or **3** (30 µM) was added and the cells incubated for 2 h at 37 °C. For uptake studies with **2**, cells were washed twice with PBS, fixed with 4 % paraformaldehyde in PBS for 15 min, and nuclei stained by incubation with a 10 µM solution of DAPI in PBS for 15 min. For proc-rhodamine decaging studies with **3**, the cells were washed twice with PBS and incubated with a solution of **5** (50 µM) for 18 h, washed twice with PBS, fixed with 4 % paraformaldehyde in PBS for 15 min, and nuclei stained by incubation with a 10 µM solution of DAPI in PBS for 15 min. Confocal images were taken on a Leica SP5 confocal microscope and ImageJ was used for digital analysis.

S3. References

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