Photophysical Properties of Single Core Multimodal Probe for Imaging (SCoMPI) in a Membrane Model and in Cells†

Supporting Information

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1. Table and Figures

Table S1: Spectroscopic properties of the $[Re(CO)_3(X)(PytaCOOMe)]$ complexes and the labelled peptides

	ε_0^{280}($M^{-1}.cm^{-1}$)	ε_0^{350}($M^{-1}.cm^{-1}$)	λ_{max} (ex350) (nm)	Φ (%)
6 (X = Br)	9.9×10^3	1.8×10^3	525	0.044 ± 0.004[1]
7 (X = Py)	12.2×10^3	2.0×10^3	495	0.96 ± 0.05[1]
Cl-R9	9.9×10^3	1.8×10^3	550	0.17 ± 0.02[2]
Cl-R6W3	26.4×10^3	1.8×10^3	550	0.13 ± 0.01[2]
Py-R9	12.2×10^3	2.0×10^3	495	0.69 ± 0.05[2]
Py-R6W3	28.7×10^3	2.0×10^3	495	0.14 ± 0.02[2]

[1] Quantum yields were measured in water containing 2% DMSO. [2] Quantum yields were measured in HEPES buffer (100 mM, pH = 7.4).

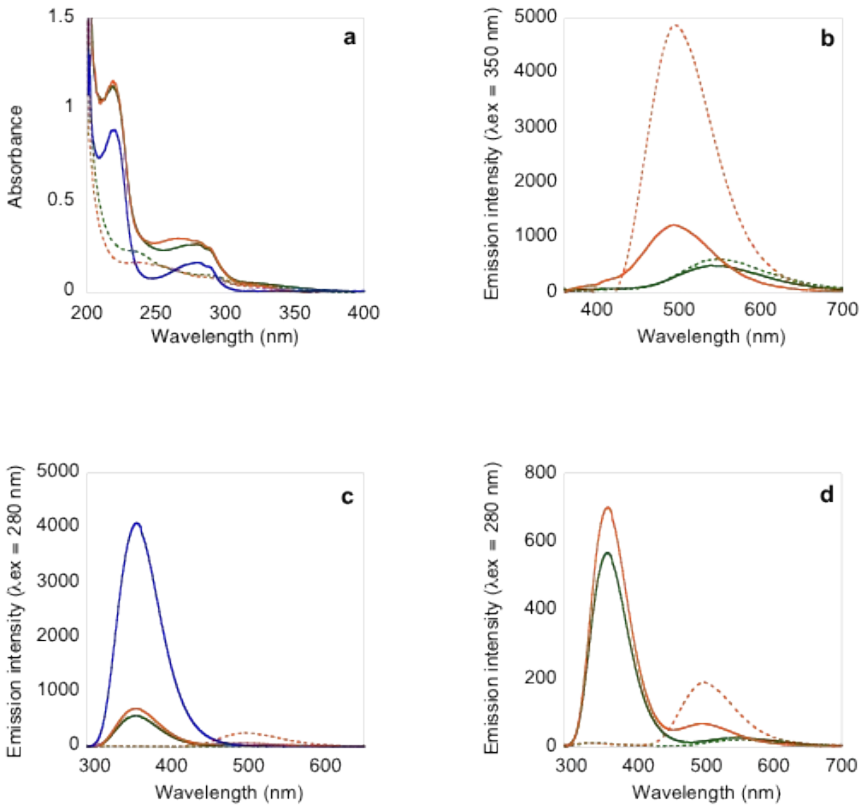


Figure S1. Spectroscopic properties of the labeled and control peptides (10 μM, PBS buffer pH = 7.2, 25°C). R9-PytaPy, orange, dashed line; R6W3-PytaPy, orange, solid line; R9-PytaCl, green, dashed line; R6W3-PytaCl, green, solid line; Ac-R6W3, blue, solid line. (a) Absorption spectra; (b) Emission spectra for excitation at 350 nm (emission and excitation slits = 5 nm); (c) Emission spectra for excitation at 280 nm (emission and excitation slits = 5 nm); (d) Emission spectra of labelled peptides only for excitation at 280 nm (see (c)).

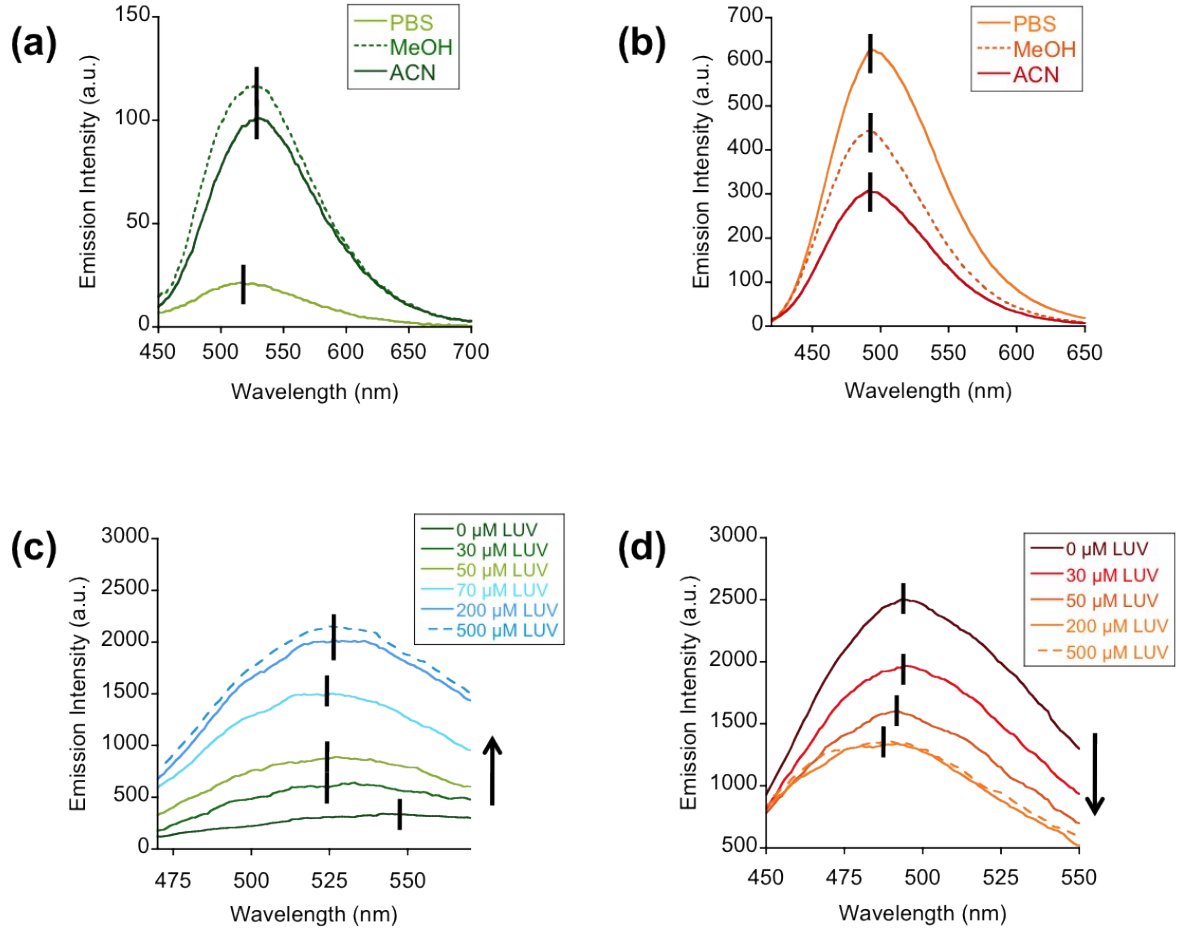


Figure S2. Spectroscopic studies of SCoMPIs in various environments. All the presented spectra are emission spectra for excitation at 350 nm (slits = 5 nm). (a,b) Emission spectra of 10 µM solutions of compound **4** ([Re(CO)$_3$(**Cl**)(PytaCOOMe)]) (a) and compound **7** ([Re(CO)$_3$(**Py**)(PytaCOOMe)]$^+$) (b) in PBS, MeOH and ACN, all containing 0.2 % DMSO. (c,d) Emission spectra of Cl-R9 (c) and Py-R9 (d) in presence of increasing concentrations of LUVs.

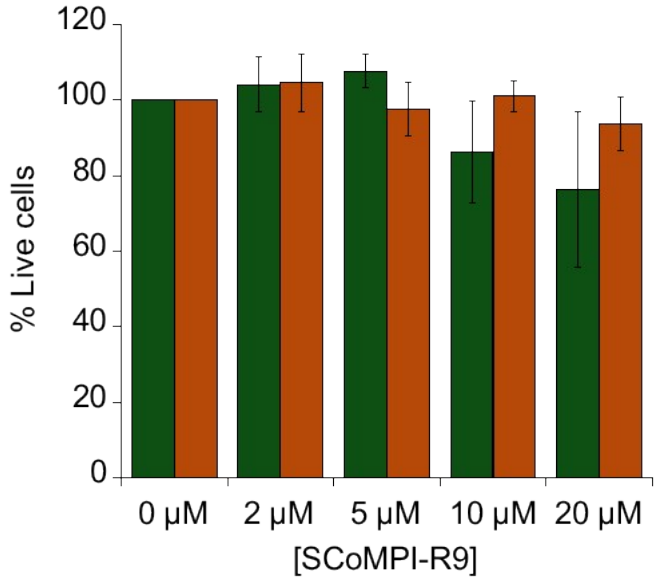


Figure S3. Cytotoxicity of ClR9 and PyR9 on CHO cells. CHO cells were incubated with 10 µM solutions of ClR9 (green) and PyR9 (orange) for 1 h at 37 °C.

2. Synthesis and characterizations of compound 1-8 and labelled peptides

2.1. General

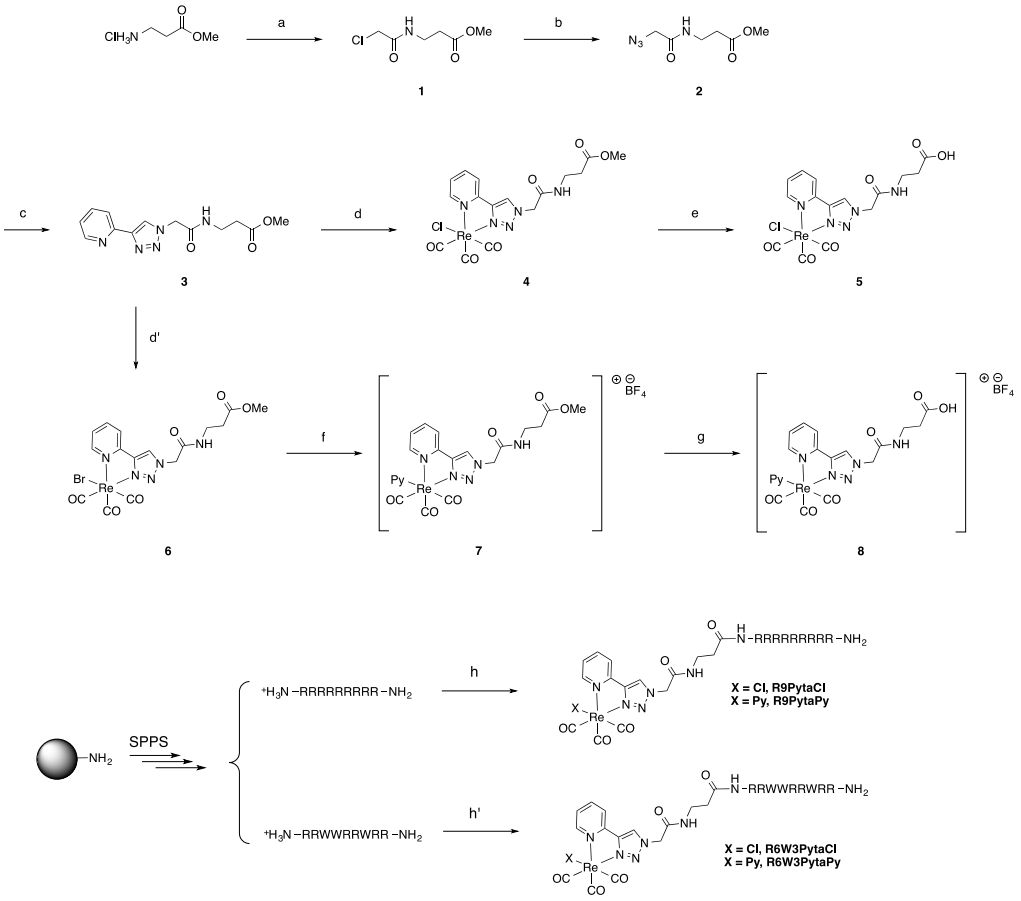
All chemicals and solvents were purchased from commercial sources (Sigma-Aldrich, Acros, Strem, Iris) and were used as received without further purification. Lipids were purchased from Avanti Polar Lipids. Analytical HPLC measurements were run on a Dionex Ultimate 3000 instrument using C18 ACE® column (250 × 4.5 mm) packed with spherical 5 µm particles of 300 Å pore size at 1 mL min^{-1}. Preparative HPLC consisted of a dual wavelength UV-Vis absorbance detector (Waters 2487) and a Waters 600 preparative pump. Purification of crude products was achieved with a C18 Nucleodur® preparative column (250 × 16 mm, 5 µm particles of 300 Å pore size). Experiments were carried out at a flow rate of 14 mL min^{-1} at room temperature. Peptides were characterized by MALDI-TOF-MS in the positive ion reflector mode on an ABI Voyager DE-Pro MALDI-TOF mass spectrometer (Applied Biosystems) using as matrix a saturated solution of CHCA in ACN/H_2O/TFA (50:50:0.1 v:v:v). Preparative flash chromatography was performed using Normasil 60 (40-63 µm) silica. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX300 spectrometer using solvent residual peaks as internal standard. UV−vis absorption spectra were recorded on a Varian Cary 300 Bio spectrophotometer, luminescence emission spectra on a Jasco FP-8300 spectrofluorimeter. FTIR spectra were recorded on a Perkin Elmer Spectrum 100 equipped with an ATR sampling accessory, by evaporation of a solution of SCoMPI or labelled peptide. CaF_2 slides were purchased from Crystran (10×10×0.3 mm^3).

2.2. Peptide synthesis

R9 (RRRRRRRRR-NH_2), R6W3 (RRWWRRWRR-NH_2) and Ac-R6W3 (Ac-RRWWRRWRR-NH_2) were synthesized by solid phase peptide synthesis using a Boc strategy. Side-chain protected peptides were assembled on MBHA resin (loading 0.54 mmol/g) either manually using HBTU/HOBt as coupling agents in basic conditions or using a

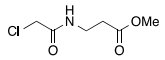
peptide synthesizer (Applied Biosystem 433A) with DCC/HOBt as coupling agents and following standard procedures. Manual coupling was achieved using standard coupling conditions: 3 equivalents of Boc-aa-OH, 3 equivalents of HBTU and HOBt and 6 equivalents of DIEA in NMP were shaken with the resin for 1 hour in a reaction vessel (polypropylene syringe with a frit). The side chains of arginine residues were protected by a tosyl group and that of tryptophan residues were protected by a formyl group. Boc group removal was performed using TFA for 1 min (twice) and followed by washing with 10% DIEA in DCM. Deformylation for R6W3 was performed sequentially with 20% piperidine in NMP: once for 1, 3, 5, 7, 15 and 30 min and finally once for 60 min. Cleavage from the resin and tosyl protecting group removal were performed using pure HF in the presence of dimethyl sulfide and anisole for 2 hours at 0°C. After HF removal, cold diethyl ether was added to precipitate the peptide. The precipitate collected was redissolved using 10% acetic acid in water and freeze-dried. For N-terminus acetylated peptides, the acetylation was performed with 10% acetic anhydride in DCM for 1 hour at room temperature prior to deformylation and cleavage.

2.3. Procedures



Scheme S1 Synthesis of the SCoMPI derivatives. *Reaction conditions*: (a) chloroacetyl chloride, DIEA, dry DCM, 1h, 0°C to RT, 66%, (b) NaN_3, NaI, acetone/water (3:1 v:v), 16h, 35°C, 79%, (c) 2-ethynylpyridine, $CuSO_4$, sodium ascorbate, acetone/water (2:1 v:v), 2h, RT, 70%, (d) $Re(CO)_5Cl$, toluene, 6h, reflux, 100%, (d') $Re(CO)_5Br$, toluene, 6h, reflux, 100%, (e) $LiOH{\cdot}H_2O$, THF/H_2O (2:1 v:v), 45min., RT, 80%, (f) i. $AgBF_4$, acetonitrile, 5h, reflux, Ar; ii. pyridine, THF, 20h, reflux, 79%, (g) $LiOH{\cdot}H_2O$, THF/H_2O (2:1 v:v), 45min., RT, 87%, (h) Compound **5** or **8**, DCC, NHS, DIEA, DMF/H_2O, overnight, RT, (h') Compound **5** or **8**, EDC, DIEA, DMF, overnight.

β-alanine (N-2-chloroacetyl) methyl ester (1)



1

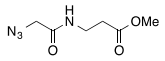
β-alanine methyl ester hydrochloride salt (1.33 g, 9.5 mmol, 1.2 equiv) was suspended in dry DCM (15 mL) under argon. Dry DIEA (3.5 mL, 20 mmol, 2.5 equiv) was added, and the suspension was cooled down in an ice bath. Chloroacetyl chloride (0.64 mL, 8 mmol, 1 equiv) was added dropwise at 0°C, and the reaction mixture was stirred for one hour at room temperature. The organic layer was then diluted with DCM (15 mL), washed once with 0.1N HCl aqueous solution (30 mL), once with 10% $NaHCO_3$ aqueous solution (30 mL) and once with brine (30 mL). It was then dried over $MgSO_4$, filtered, and concentrated to yield the expected compound as a colorless oil (0.947 g, 5.3 mmol, 66%).

^{1}H-NMR (300 MHz, CDCl3): δ 7.17 (s, 1H), 4.03 (s, 2H), 3.72 (s, 3H), 3.58 (q, *J* = 6.0 Hz, 2H), 2.58 (t, *J* = 6.0 Hz, 2H).

^{13}C-NMR (101 MHz, CDCl3): δ 172.40, 166.14, 51.68, 42.40, 35.14, 33.32.

HRMS (ESI+): *m/z* calculated for $[C_6H_{10}ClNO_3+Na]^+$ 202.02414, found 202.02438 error: 1.2 ppm.

β-alanine (N-2-azidoacetyl) methyl ester (2)



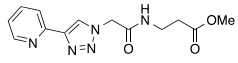
2

Compound 1 (0.947 g, 5.3 mmol, 1 equiv) was dissolved in a 3:1 v:v mixture of acetone (15.6 mL) and water (5.2 mL). Sodium azide (0.69 g g, 10.6 mmol, 2 equiv) and sodium iodide (0.079 g, 0.53 mmol, 0.1 equiv) were then added, and the mixture was stirred at 35°C (bath temperature) for 16 h. Acetone was removed by rotary evaporation and the solution was diluted with DCM (15 mL) and water (5 mL). The mixture was then decanted, the organic layer dried over $MgSO_4$, filtered and concentrated to yield compound 2 as a colorless oil (0.78 g, 4.2 mmol, 79%).

^{1}H-NMR (300 MHz, CDCl3): δ 6.87 (s, 1H), 3.97 (s, 2H), 3.72 (s, 3H), 3.57 (q, *J* = 6.0 Hz, 2H), 2.57 (t, *J* = 6.0 Hz, 2H).

^{13}C-NMR (101 MHz, CDCl3): δ 172.18, 166.94, 52.00, 51.43, 34.67, 33.26.

1H-1,2,3-triazole-1-acetamide-(N-3-methoxy-3-oxopropyl)-4-(2-pyridyl) ***(3, PytaCOOMe)***



3

Compound 2 (0.51 g, 2.74 mmol, 1 equiv) was dissolved in a 2:1 v:v mixture of acetone (36 mL) and water (18 mL). 2-ethynylpyridine (0.28 mL, 2.74 mmol, 1 equiv), copper sulfate (0.17 g, 0.69 mmol, 0.25 equiv) and sodium ascorbate (0.14 g, 0.69 mmol, 0.25 equiv) were then added, and the suspension was sonicated for a few minutes, during which a light brownish precipitate formed. The reaction mixture was then stirred for 2h at room temperature (until the solution turned greenish). The solution was then poured into a 28% ammonia solution and extracted three times with DCM. The organic layers were combined,

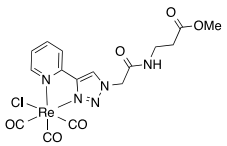
dried over Na_2SO_4, filtered and concentrated. The resulting brown solid was purified by silica gel column chromatography (DCM:EtOAc:MeOH 50:50:0 to 0:98:2 v:v:v) to yield compound 3 (0.56 g, 70%) as a white solid.

^{1}H-NMR (400 MHz, CDCl3): δ 8.56 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1H), 8.28 (s, 1H), 8.10 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.78-7.73 (m, 1H), 7.22 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 6.88 (s, 1H), 5.11 (s, 2H), 3.62 (s, 3H), 3.53 (q, *J* = 6.2 Hz, 2H), 2.53 (t, *J* = 6.2 Hz, 2H).

^{13}C-NMR (101 MHz, CDCl3): δ 172.49, 165.19, 149.58, 136.98, 123.77, 123.16, 120.37, 53.16, 51.97, 35.42, 33.57.

HRMS (ESI+): *m/z* calculated for $[C_{13}H_{15}N_5O_3+H]^+$ 290.12477, found 290.1125527, error: 1.7 ppm.

*1H-1,2,3-triazole-1-acetamide-(N-3-methoxy-3-oxopropyl)-4-(2-pyridyl) rhenium tricarbonyl chloride (**4**)*



4

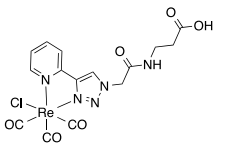
Compound 3 (36.3 mg, 0.10 mmol, 1 equiv) was dissolved in hot toluene (1.3 mL). $Re(CO)_5Cl$ (29 mg, 0.1mmol, 1 equiv) was added, and the suspension was refluxed for 6 h. The reaction mixture was cooled down to room temperature, the resulting yellow precipitate was filtered and washed with cold toluene, to give pure compound 4 (59.5 mg, 0.10 mmol, 100 %) which was used without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 8.64 (d, *J* = 5.4 Hz, 1H), 8.50 (s, 1H), 7.80-7.72 (m, 2H), 7.21-7.16 (m, 1H), 4.93 (q, *J* = 14.5 Hz, 2H), 3.37 (s, 3H), 3.21 (t, *J* = 6.5 Hz, 2H), 2.27 (t, *J* = 6.6 Hz, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 197.55, 196.12, 189.27, 173.12, 165.43, 153.49, 149.87, 149.46, 140.71, 127.06, 126.64, 123.20, 53.88, 52.27, 36.11, 34.04.

HRMS (ESI+): *m/z* calculated for $[C_{16}H_{15}ClN_5O_6Re+Na]^+$ 616.01326, found 616.01333, error: 0.1 ppm.

*1H-1,2,3-triazole-1-acetamide-(N-2-carboxyethyl)-4-(2-pyridyl) rhenium tricarbonyl chloride (**5**)*



5

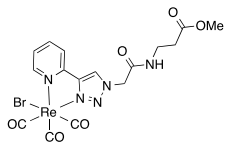
Methyl ester 4 (59.4mg, 0.100 mmol, 1 equiv) was dissolved in a 2:1 mixture of THF/H_2O (1mL). $LiOH·H_2O$ (5.0 mg, 0.119 mmol, 1 equiv) was added, and the reaction mixture was stirred at room temperature for 45 min. THF was removed by rotary evaporation (bath temperature 45°C). A 1M aqueous solution of HCl was added dropwise to the resulting solution, until pH reached 1 (about 0.5 mL). 1 mL of distilled water was added, and the aqueous solution was extracted with EtOAc (15mL). The organic layer was washed once with brine (2 mL). The aqueous layers were extracted twice more with EtOAc (15mL). Organic layers were pooled, dried over Na_2SO_4, filtered and concentrated to give the desired compound 5 as a yellow powder (46.5 mg, 0.080 mmol, 80%).

^{1}H-NMR (400 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 8.65 (d, *J* = 5.4 Hz, 1H), 8.51 (s, 1H), 7.80-7.74 (m, 2H), 7.19 (td, *J* = 6.3, 1.4 Hz, 1H), 4.95 (q, *J* = 19.9 Hz, 2H), 3.22 (t, *J* = 6.6 Hz, 2H), 2.25 (t, *J* = 6.6 Hz, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 196.67, 195.10, 188.44, 173.64, 164.45, 152.79, 149.18, 148.71, 139.74, 125.94, 125.79, 122.21, 52.95, 35.40, 33.17.

HRMS (ESI+): *m/z* calculated for $[C_{15}H_{13}ClN_5O_6Re+Na]^+$ 601.99761, found 601.99786, error: 0.4 ppm.

*1H-1,2,3-triazole-1-acetamide-(N-3-methoxy-3-oxopropyl)-4-(2-pyridyl) rhenium tricarbonyl bromide (**6**)*



6

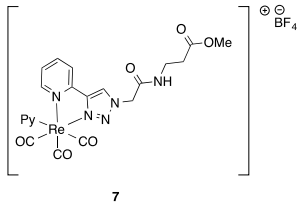
Compound 3 (146 mg, 0.50 mmol, 1 equiv) was dissolved in hot toluene (4 mL). $Re(CO)_5Br$ (205 mg, 0.5 mmol, 1 equiv) was added, and the suspension was refluxed for 6 h. The reaction mixture was cooled down to room temperature, the resulting yellow precipitate was filtered and washed with cold toluene, to give pure compound 6 (323 mg, 0.5 mmol, 100 %).

^{1}H-NMR (300 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 8.99 (d, *J* = 5.5 Hz, 1H), 8.84 (s, 1H), 8.10-8.08 (m, 2H), 7.53-7.48 (m, 1H), 5.27 (q, *J* = 17.4 Hz, 2H), 3.70 (s, 3H), 3.54 (t, *J* = 6.4 Hz, 2H), 2.60 (t, *J* = 6.5 Hz, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 172.10, 164.40, 152.74, 149.02, 148.53, 139.35, 125.73, 125.49, 121.95, 52.69, 51.17, 35.10, 32.99.

HRMS (ESI+): *m/z* calculated for $[C_{16}H_{15}BrN_5O_6Re+Na]^+$ 659.96275, found 659.96321, error: 0.7 ppm.

*1H-1,2,3-triazole-1-acetamide-(N-3-methoxy-3-oxopropyl)-4-(2-pyridyl) rhenium tricarbonyl pyridine tetrafluoroborate salt (**7**)*



7

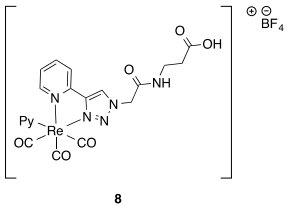
Rhenium tricarbonyl bromide derivative 6 (252.3 mg, 0.394 mmol, 1 equiv) was dissolved in acetonitrile (49 mL) under argon. Silver tetrafluoroborate (78.9 mg, 0.405 mmol, 1 equiv) was added and the mixture was refluxed for 5 h, resulting in the formation of a fine dark suspension. Solvent was evaporated to dryness, and the residue was dissolved in THF (58 mL). Pyridine (96 µL, 1.18 mmol, 3 equiv) was added, and the reaction mixture was refluxed for 20 h. The suspension was filtered over celite and the solvent was removed by rotary evaporation. The crude was co-evaporated three times with toluene, dissolved in the minimal amount of methanol and precipitated in diethyl ether, to give the desired compound as a yellow powder (224.7 mg, 0.310 mmol, 79 %).

^{1}H-NMR (300 MHz, MeOD): δ 8.39 (dd, *J* = 6.5, 1.5 Hz, 2H), 8.26 (td, *J* = 7.8, 1.5 Hz, 1H), 8.17-8.13 (m, 1H), 7.92 (tt, *J* = 7.7, 1.5 Hz, 1H), 7.75 (ddd, *J* = 7.6, 5.6, 1.5 Hz, 1H), 7.43-7.38 (m, 2H), 5.47 (s, 2H), 3.71 (s, 3H), 3.56 (t, *J* = 6.4 Hz, 2H), 2.63 (t, *J* = 6.6 Hz, 2H).

^{13}C-NMR (75 MHz, MeOD): δ 173.67, 166.35, 155.04, 153.40, 150.60, 150.51, 142.75, 141.06, 129.06, 128.68, 127.99, 124.33, 54.72, 52.29, 36.75, 34.45.

HRMS (ESI+): *m/z* calculated for $[C_{21}H_{20}N_6O_6Re]^+$ 637.09684, found 637.09735, error: 0.8 ppm.

*1H-1,2,3-triazole-1-acetamide-(N-2-carboxyethyl)-4-(2-pyridyl) rhenium tricarbonyl pyridine tetrafluoroborate salt (**8**)*



8

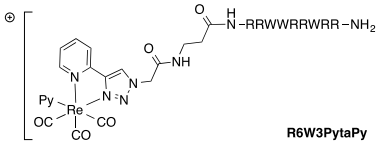
Compound 7 was prepared according to the same procedure as compound 5: compound 7 (148.6 mg, 0.205 mmol, 1 equiv) was dissolved in a 2:1 v:v mixture of THF (1.3 mL) and water (0.65 mL). Lithium hydroxide monohydrate (8.8 mg, 0.210 mmol, 1 equiv) was then added, and the reaction mixture was stirred for 1 h at room temperature. THF was removed by rotary evaporation, and the solution was acidified to pH<1 with 1N HCl aqueous solution. The aqueous layer was extracted three times with EtOAc (5mL). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated to give compound 8 (127.2 mg, 0.179 mmol, 87%) as a yellow powder.

^{1}H-NMR (300 MHz, MeOD): δ 9.31 (d, *J* = 5.4 Hz, 1H), 8.98 (s, 1H), 8.41-8.39 (m, 2H), 8.28 (t, *J* = 7.8 Hz, 1H), 8.18-8.15 (m, 1H), 7.94 (d, *J* = 7.6 Hz, 1H), 7.79-7.74 (m, 1H), 7.42 (t, *J* = 6.7 Hz, 2H), 5.48 (s, 2H), 3.72 (s, 3H), 3.58 (t, *J* = 6.5 Hz, 2H), 2.64 (t, *J* = 6.5 Hz, 2H).

^{13}C-NMR (101 MHz, MeOD): δ 196.64, 195.17, 192.13, 174.95, 166.27, 154.94, 153.32, 150.49, 150.43, 142.69, 140.98, 128.98, 128.65, 127.93, 124.28, 54.71, 36.81, 34.42.

HRMS (ESI+): *m/z* calculated for $[C_{20}H_{18}ClN_6O_6Re]^+$ 623.08119, found 623.08152, error: 0.5 ppm.

Py-R6W3

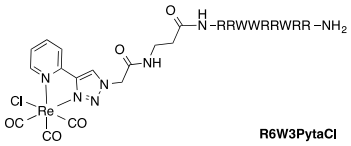


R6W3PytaPy

A solution of EDC in DMF (33.3 mg/mL, 187 µL, 32.5 µmol, 1.5 equiv) were added to compound **8** (15.4 mg, 21.7 µmol, 1 equiv). The mixture was stirred for 20 min before addition of a solution of R6W3 in DMF (462 mg/mL, 108 µL, 21.6 µmol, 1 equiv) and of DIEA (7.5 µL, 43.3 µmol, 2 equiv). The mixture was stirred overnight at room temperature and the crude was purified by HPLC.

MALDI-TOF-MS (CHCA matrix): 2040.48 $[M-Py]^+$

Cl-R6W3

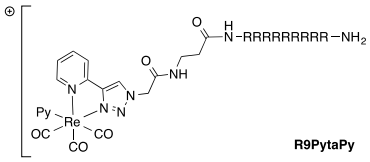


R6W3PytaCl

The procedure is similar to the one used for R6W3PytaPy. A solution of EDC in DMF (33.3 mg/mL, 187 µL, 32.5 µmol, 1.5 equiv) were added to compound **5** (12.6 mg, 21.7 µmol, 1 equiv). The mixture was stirred for 20 min before addition of a solution of R6W3 in DMF (462 mg/mL, 108 µL, 21.6 µmol, 1 equiv) and of DIEA (7.5 µL, 43.3 µmol, 2 equiv). The mixture was stirred overnight at room temperature and the crude was purified by HPLC. The collected pure fraction was frozen and freeze-dried immediately after purification in order to prevent the exchange of the chloride.

MALDI-TOF-MS (CHCA matrix): 2039.8 $[M-Cl]^+$

Py-R9

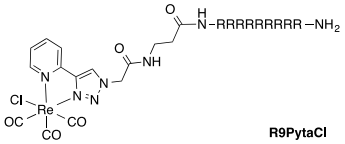


R9PytaPy

Compound **8** (17.7 mg, 24.5 µmol, 1.5 equiv) and N-hydroxysuccinimide (4.8 mg, 42.6 µmol, 2.5 equiv) were dissolved in a solution of DCC in DMF (4.13 mg/mL, 850 µL, 17.7 µmol, 1.1 equiv). The mixture was stirred for 20 min, followed by addition of an aqueous solution of R9 (50 mg/mL, 680 µL, 16.8 µmol, 1 equiv) and DIEA (8.5 µL, 49.1 µmol, 2.9 equiv). The reaction mixture was stirred overnight at room temperature and then purified by HPLC.

MALDI-TOF-MS (CHCA matrix): 1692.9 $[M\text{-}Re(CO)_3Py]^+$

Cl-R9



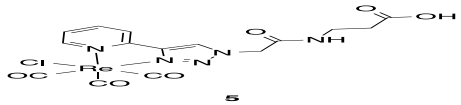
R9PytaCl

Compound **5** (16.1 mg, 27.7 µmol, 1.6 equiv) and N-hydroxysuccinimide (3.9 mg, 33.8 µmol, 2 equiv) were dissolved in a solution of DCC in DMF (4.13 mg/mL, 850 µL, 17.7 µmol, 1.1 equiv). The mixture was stirred for 20 min, followed by addition of an aqueous solution of R9 (50 mg/mL, 680 µL, 16.8 µmol, 1 equiv) and DIEA (8.5 µL, 49.1 µmol, 2.9 equiv). The reaction mixture was stirred overnight at room temperature and then purified by HPLC. Right after purification, the collected pure fraction was frozen and freeze-dried to prevent the exchange of the chloride.

MALDI-TOF-MS (CHCA matrix): 1692.8 $[M\text{-}Re(CO)_3Cl]^+$

3. Measurement of quantum yields

Luminescence quantum yield were measured using a Jasco FP-8300 spectrofluorometer. Emission spectra were recorded upon excitation at 320 nm for both compounds. The quantum yields were calculated according to the following equation[1]:



5

$$\frac{Q_x}{Q_r} = \frac{I(\lambda)}{I(\lambda)} \cdot \frac{n_x^2}{n_r^2} \cdot \frac{D_x}{D_r} \cdot \frac{A_r(\lambda)}{A_x(\lambda)}$$

In this equation, Q_r is the quantum yield of the reference $I(\lambda)$ is the relative intensity of the exciting light at wavelength λ, n is the average refractive index of the solution to the luminescence, D is the integrated area under the emission spectrum, and $A(\lambda)$ is the absorbance of the solution at the exciting wavelength. The subscripts x and r refer to the unknown and reference solutions, respectively. Quinine sulfate in 0.1 N sulfuric acid was used as the standard with a known emission quantum yield of 0.546 (exc 320 nm).

4. Preparation of Large Unilamellar Vesicles

319 µL of a DOPG stock solution in chloroform (25 mg/mL, 10 µmol) were diluted in a few mL of chloroform in a 100 mL round-bottomed flask. Chloroform was slowly removed by rotary evaporation and the resulting lipid film was dried under vacuum for at least 1h. It was then hydrated with 10 mL of PBS (pH 7.4). The resulting lipid suspension was then extruded

7 times through a 200 nm pores filter and 10 times through a 100 nm pores filter. The stock solution was stored at 4°C and used within 15 days.

5. Fluorescence spectroscopy measurements

5.1. Sample preparation

1 mM stock solutions in PBS were prepared for all peptides. Concentration was controlled by UV-visible. Fluorescence measurements were performed using 3 mL quartz cuvettes. For each measurement, 3 mL of a solution of LUV at the desired concentration were prepared and the blank spectra recorded. 3 µL of stock solution of peptide were then added, and the mixture was incubated 5 min before spectra recording. Emission spectra were recorded with 5nm emission and excitation slits. For tryptophan containing peptides (namely Ac-R6W3, R6W3-PytaCl and R6W3-PytaPy), emission spectra were recorded for excitation at 280 nm. Emission spectra for excitation at 350 nm were recorded for all peptides but Ac-R6W3.

5.2. Estimation of Nernst partition coefficient K_P

For each peptide, the maximum intensity of the recorded fluorescence spectra was plotted as a function of the concentration of lipids. The resulting plot was fitted using either the simple partition model (1) or the self-quenching model (2) described by Henriques & Castanho.[2] The Nernst partition coefficient K_P of the labelled peptides between water and the lipid membrane was estimated from these fits. All plot and data processing were done using Kaleidagraph software.

$$\frac{I}{I_W} = \frac{1 + \gamma_L K_P \frac{I_L}{I_W}[L]}{1 + \gamma_L K_P[L]} \quad (1)$$

$$\frac{I}{I_W} = \frac{\gamma_L K_P \frac{I_L}{I_W}[L]}{1 + \gamma_L K_P[L] + kK_P I_L} + \frac{1}{1 + \gamma_L K_P[L]} \text{ with } k = \frac{k_q}{k_f}\frac{1}{\varepsilon l} \quad (2)$$

where I is the emission intensity, I_W the emission intensity in aqueous solution, I_L the emission intensity of the probe in the lipid phase, γ_L the molar volume of lipid and [L] the concentration of lipids in the sample, k_q the kinetic constant of quenching and k_f the radiative fluorescence constant.[2] In the case of DOPG, we estimated $\gamma_L = 0.7572$ L.mol^{-1} from literature.[3]

Peptide	K_P
Ac-R6W3	$4.0 \pm 1.2 \times 10^4$
Cl-R6W3	$4.4 \pm 1.1 \times 10^4$
Py-R6W3	$6.0 \pm 1.7 \times 10^4$

6. Cell experiments

Chinese Hamster Ovarian cells were seeded in 12-well plates containing 10 × 10 × 0.3 mm^3 CaF_2 slides, and grown for 24h. Cells were washed once with HBSS buffer and once with fresh DMEM. Cells were then incubated with 10µM solutions of peptide in DMEM for one hour at 37°C. Cells were washed twice with PBS, and incubated for 8 min with 500 µL of a 3% PFA solution in PBS at room temperature. Finally, cells were washed once with PBS and

twice with milliQ water. CaF_2 slides were left to dry at air prior to examination by microscopy.

7. Cytotoxicity assay

Cytotoxicity was assayed using Dojindo's Cell Couting Kit 8, which is based on the reduction of a tetrazolium (WST-8) into a coloured formazan by hydrogenases from viable cells. CHO-K1 cells were seeded in 96-well plates (10,000 cells per well) one day before treatment. Cells were incubated for one hour at 37°C with 2, 5 10 and 20 µM solutions of Cl-R9 or Py-R9 in DMEM. Cells were then incubated for 3 h at 37°C with the WST-8 solution, prepared according to the manufacturer's indication (10% v:v of stock WST-8 solution in DMEM). Untreated cells were used as negative control (no cytotoxicity) and empty wells as positive control (no cells, maximum cytotoxicity). Measurements were performed in triplicate

8. Fluorescence and IR microscopies

Fluorescence imaging of peptides in CHO cells was performed using an Olympus X71 microscope equipped with a C9100-02 camera (Hamamatsu Corporation, Sewickley, PA), a X20 objective and a Hg lamp (100W) attenuated by a neutral density filter (ND-1). Luminescence signal of SCoMPIs was detected using the following filter set: excitation D350/50x; beam splitter 400DCLP; emission HQ560/80m; Chroma Technology.

Data processing. Bright regions of interest (ROI) were automatically detected using ImageJ software Macro functionality. ROI which areas were inferior to 75 pixels^2 were arbitrarily removed from data set, as they were identified as artefactual. Mean intensities were corrected with background and averaged.

FTIR microspectroscopy was performed on a Nicolet iN10 Infrared Microscope. Aperture was set to 50 x 50 µm^2 aperture. Spectra were recorded from 400 to 4000 cm^{-1} with a 8 cm^{-1} spectral resolution. 256 scans were accumulated for each spectrum. When needed, FTIR interference fringes were removed using the Interference Fringe Removal tool available from http://hines.chem.cornell.edu/FTIR.html and IgorPro. Peak area of SCoMPI A-band (R9PytaPy: 2055-2017 cm^{-1}; R9PytaCl: 2044-2005 cm^{-1}) and Amide-II band (1573-1493) were integrated using Omnic software. The ratio Area(A-band)/Area(Amide I) was calculated for each spectrum.

References

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2. S. T. Henriques and M. A. R. B. Castanho, *Biochim. Biophys. Acta BBA - Biomembr.*, 2005, **1669**, 75–86.

3. J. Pan, F. A. Heberle, S. Tristram-Nagle, M. Szymanski, M. Koepfinger, J. Katsaras and N. Kucerka, *Biochim. Biophys. Acta*, 2012, **1818**, 2135–2148.