Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary data

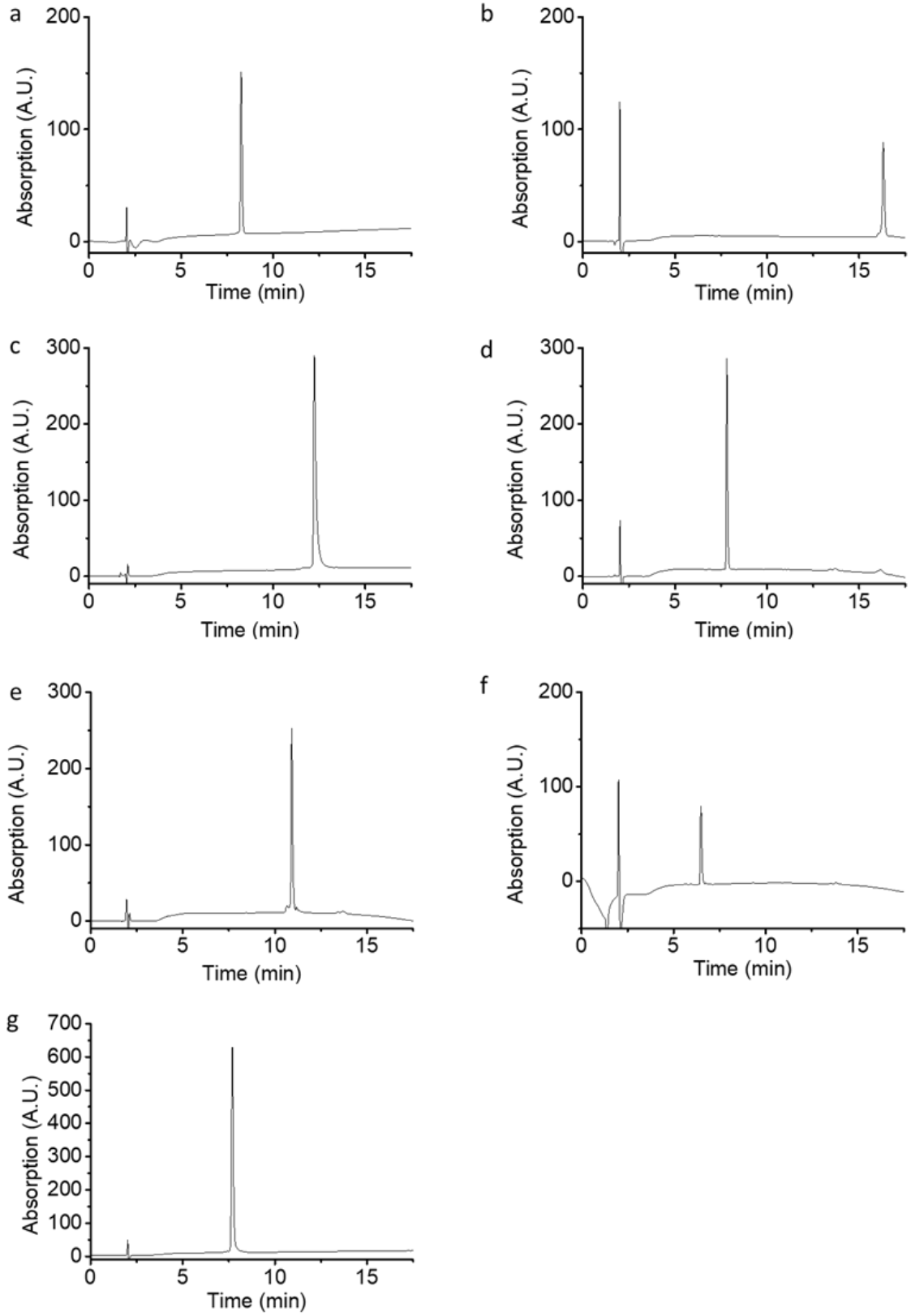


Figure S1: *HPLC chromatogram of purified DAHK (a), SG3 (b), AKH-αR4W5^{NBD} (c), DSIP (d), AKH-LAH4^{NBD} (e), AKHK^{NBD} (f), $A\beta_{1-16}$ (g). The separation was performed using a linear*

gradient of buffer A (TFA 0.1%) and buffer B (TFA 0.1%, ACN 90%) ranging from 5% buffer B to 100% buffer B in 30 minutes, flow 1ml/min, and UV detection at 214 nm.

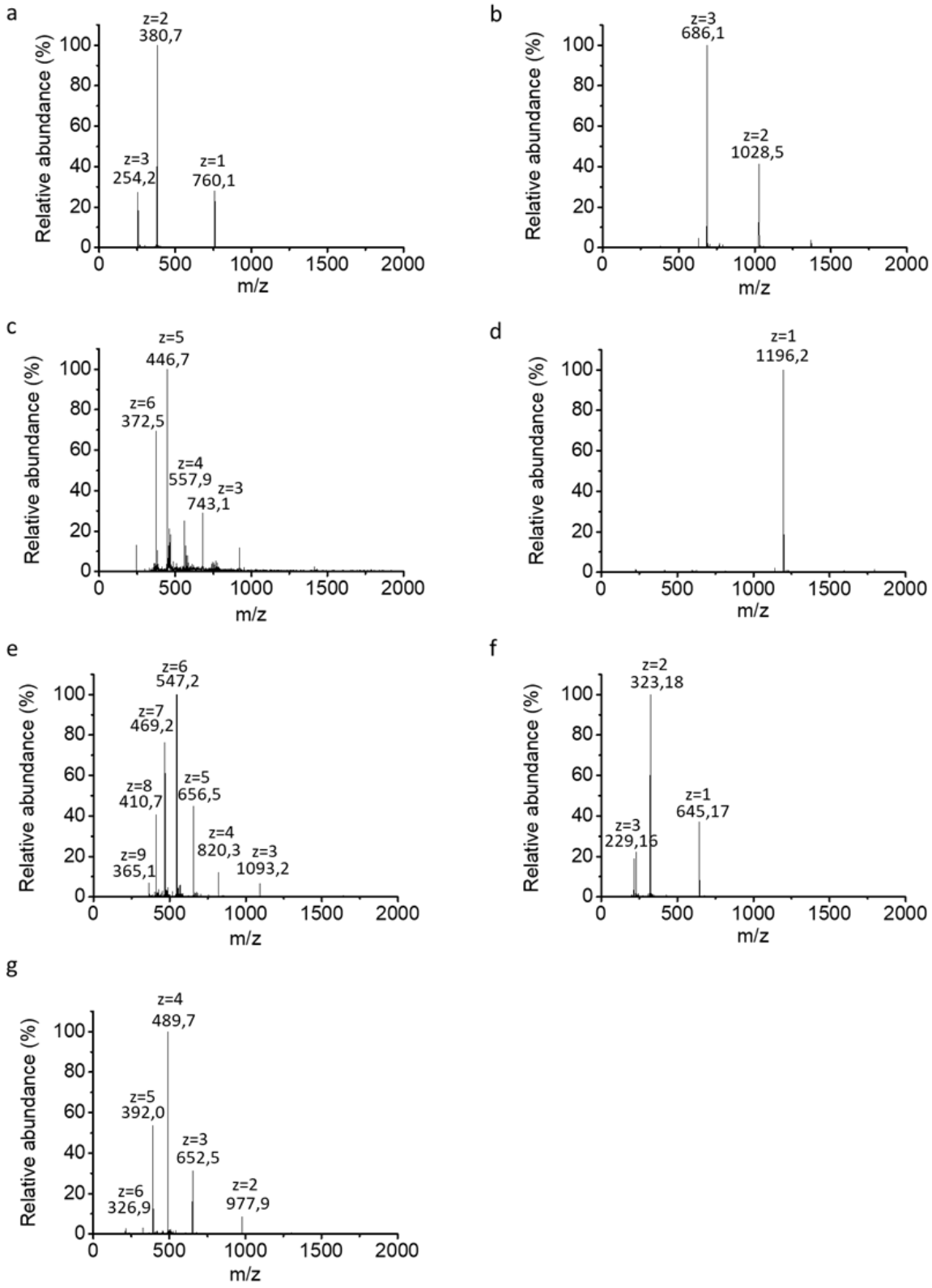


Figure S2: *LC-MS spectra of purified DAHK (a), SG3 (b), AKH-αR4W5^{NBD} (c), DSIP (d), AKH-LAH4^{NBD} (e), AKHK^{NBD} (f), Aβ$_{1-16}$ (g). The separation was performed using a linear gradient of buffer A (Formic acid 0.1%) and buffer B (Formic acid 0.1%, ACN 90%) ranging*

from 5% buffer B to 100% buffer B in 15 minutes, flow 1ml/min, and m/z detection from 0 to 2000 Da.

	Theoretical m/z	Observed m/z	Yield (%)	Purity (%)
DAHKK^{NBD}	759.78	760.10	5.72	99.8
SG3^{NBD}	2055.35	2054.80	1	87.4
AKH-αR5W4^{NBD}	2227.55	2228.14	2	98.95
DSIP^{NBD}	1194.18	1196.08	8	>99
AKH-LAH4^{NBD}	3276.04	3277.22	4.3	88.3
AKHK^{NBD}	644.31	644.57	13.3	94.3
$A\beta_{1-16}$	1954.05	1954.57	65.7	98.2

Table S1: *Comparison between theoretical and observed m/z values of purified peptides as well as their yield and purity*

Peptide	ATCUN Motif	Linker + CPP Sequence	Fluorophore (AA Position)	C-ter
SG3	-	-KGRLSGMNEVLSFRWL	NBD (1)	Amide
αR5W4	-	-KGRRWWRRWWR	NBD (1)	Amide
AKH-LAH4	AKH-	-KKALLALALHHLAHLALHLALALKKA	NBD (2)	Amide
DSIP	-	-KGWAGGDASGE	NBD (1)	Amide

Table S2: *List of peptides screened for their capacity to penetrate PC12 cells: Peptides synthetized contained different CPPs previously tested in the literature, including SG3*[50]*, and αR5W4* [39] *or has been shown to be an effective antimicrobial peptide LAH4 (LAH5 and AKH-LAH4)* [51].

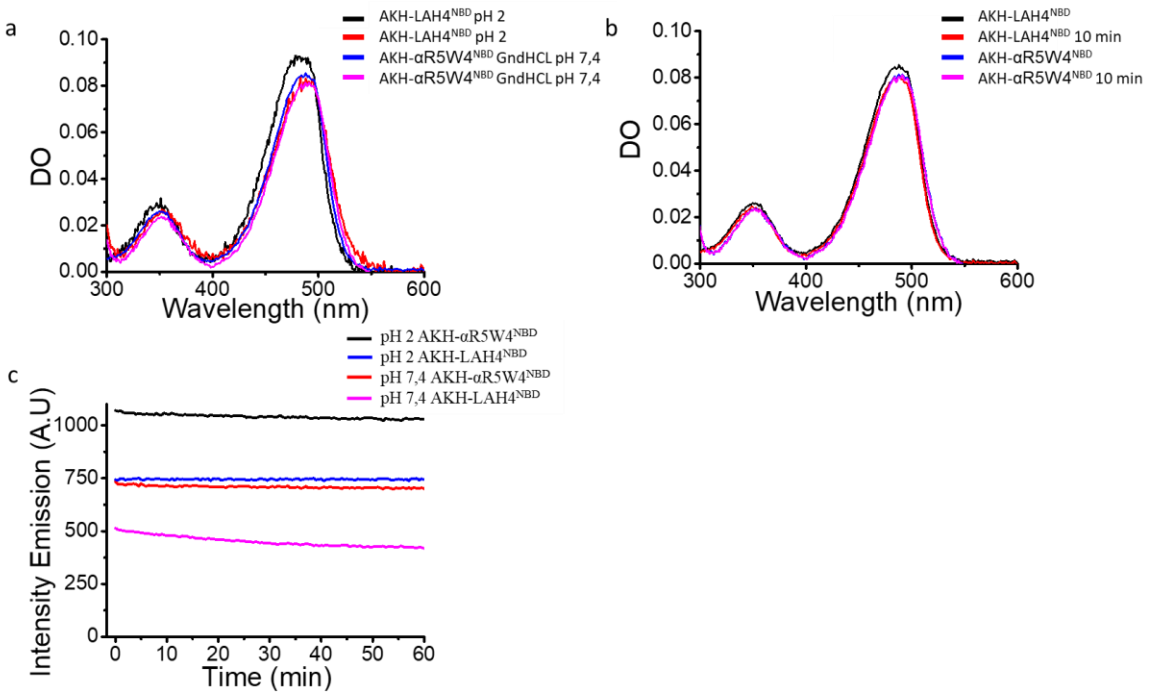


Figure S3: *(a)* Absorption spectra of *AKH-αR5W4^{NBD} versus AKH-LAH4^{NBD} at pH 2 or at pH 7.4 GndHCL 6M. (b)* Absorption spectra of *AKH-αR5W4^{NBD} versus AKH-LAH4^{NBD} at pH 7.4 GndHCL 6M at 0 min vs 10 min incubation. (c) Stability of AKH-αR5W4^{NBD} versus AKH-LAH4^{NBD} at different pH: Each peptide was incubated in solutions at either pH 2 (0.01% TFA) or pH 7.4 (100 mM HEPES) for 60 min. Conditions: AKH-αR5W4^{NBD} = AKH-LAH4^{NBD} = 4 µM; pH 2 was buffered using 0.01% TFA; pH 7.4 was buffered using 100 mM HEPES in the presence (a and b) or absence (c) of GndHCL 6M. Experiments are representative of 3 independent experiments.*

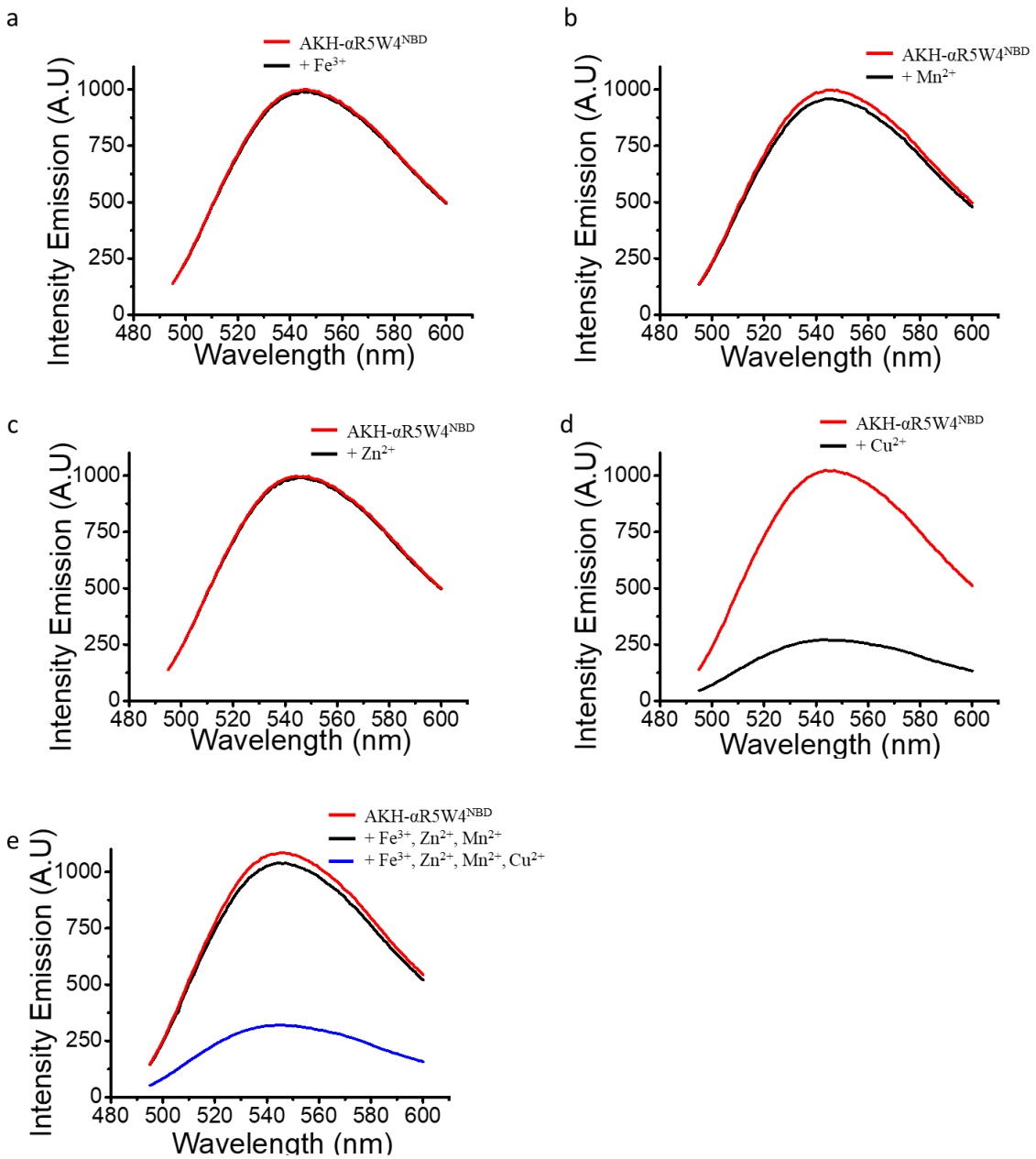


Figure S4: *Spectra depicting the quenching of AKH-αR5W4^{NBD} by different metal ions as plotted in Figure 3a. Conditions: AKH-αR5W4^{NBD}: 7 µM ; Cu(II), Zn(II), Fe(III), Mn(II):5 µM; HEPES 100 mM pH 7.4.*

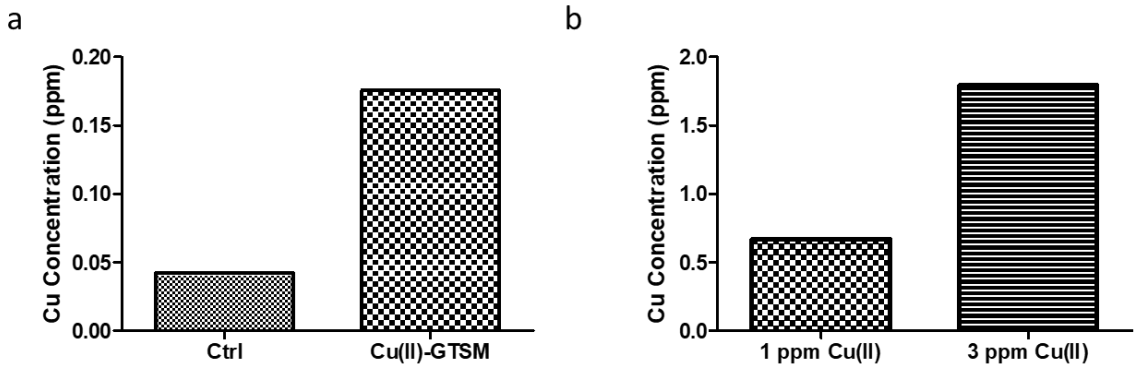


Figure S5: *a)Cu concentration in untreated cells (Ctrl) and after 1h 1 µM GTSM treatment measured by ICP-AES per 2 x 10^6 PC12 cells. b) Detected Cu concentration in medium containing cell lysate after spiking Cu(II) with 1ppm (15.74 µM) and 3ppm (47.2 µM) Cu(II). Values are expressed as in parts per million (ppm)*

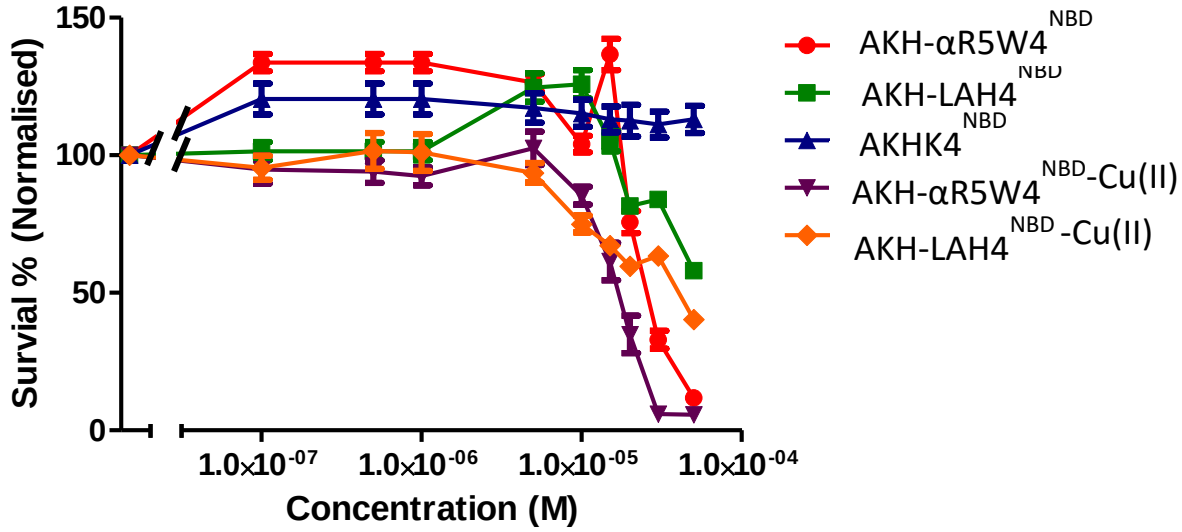


Figure S6: *Viability of PC12 after treatment with AKH-αR5W4^{NBD} or AKH-LAH4^{NBD} in presence and absence of Cu(II). 5 X 10^4 PC12 cells were incubated with the indicated concentration of peptides.*

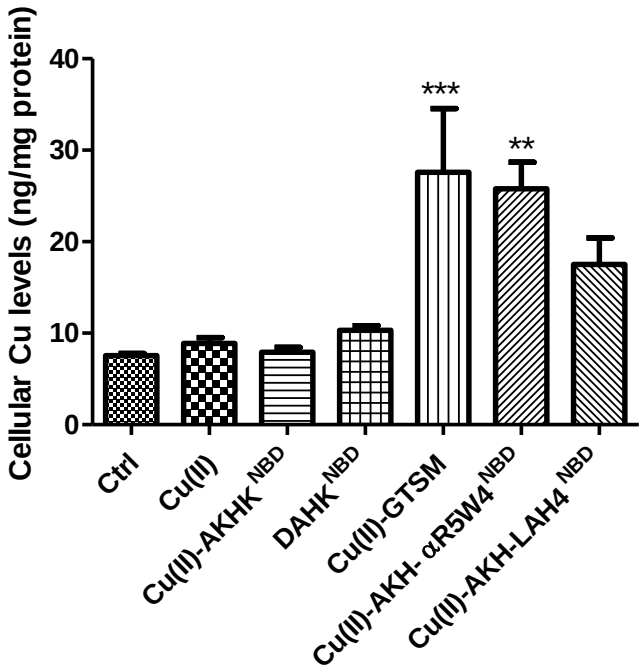


Figure S7: *AKH-αR5W4^{NBD} and AKH-LAH4^{NBD} imports Cu into PC12 cells after 1h incubation at 37°C, measured by ICP-MS. Each condition was done in duplicates, n=3 independent experiments with 2.10^{6} PC12 cells. A parametric ANOVA test was carried out with a Dunnett's Multiple Comparison Test ** p<0,001, *** p<0,0001.*

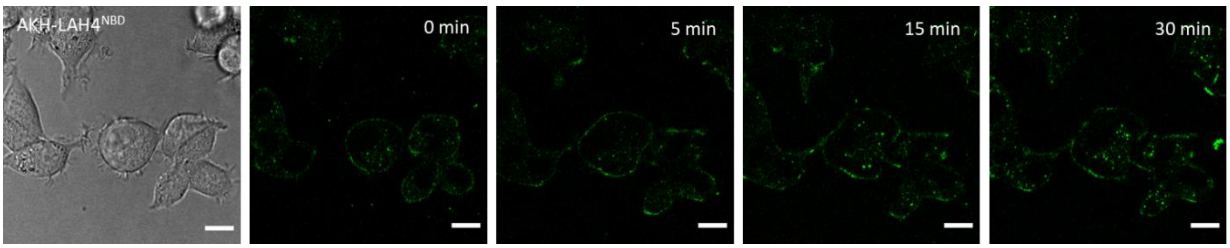
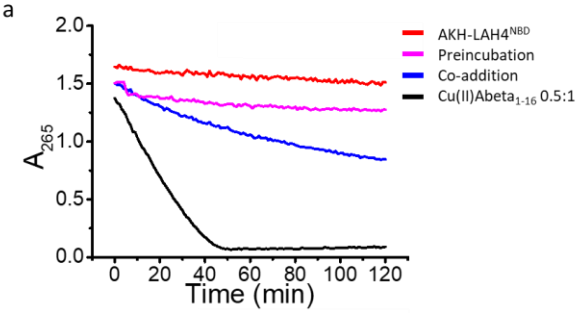
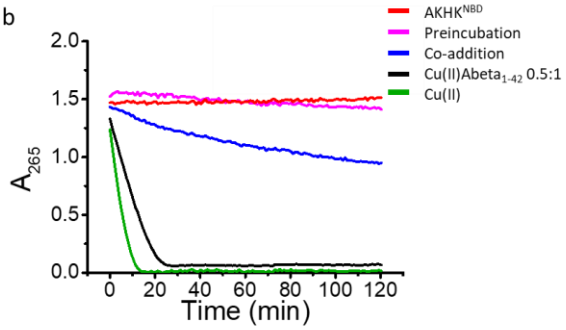


Figure S8: *Penetration of Cu(II)-AKH-LAH4^{NBD} into PC12 cells. Representative images at the indicated time obtained from live imaging performed on 5.10^{4} cells incubated with Cu(II)-AKH-LAH4^{NBD} =1 µM, at 37°C using a Leica TCS SP5 (II) confocal microscope, with an excitation wavelength at 477 nm for 30min. Time point zero is about 1 minute after incubation with the peptides. Bars = 10 µm. Similar observations were obtained with 5 different cell cultures.*

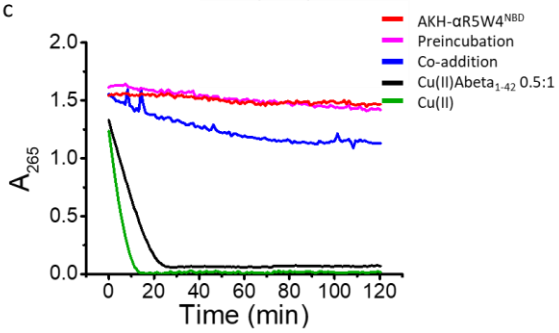
a



b



c



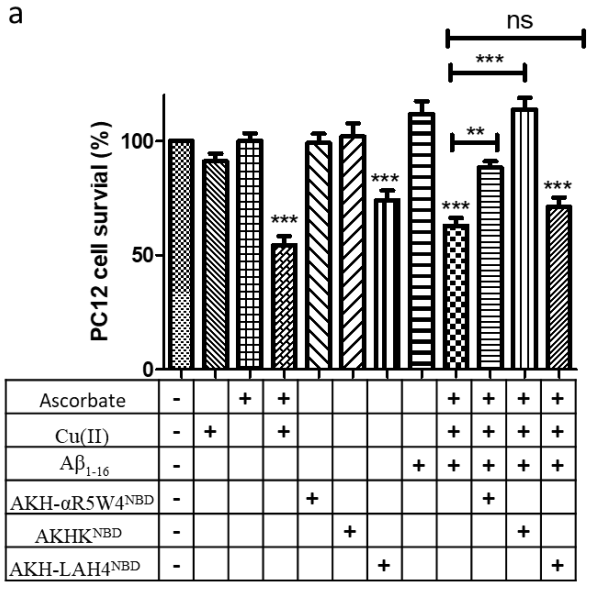
d

Conditions		Asc consumption (µM/min)	SD (+/-)
$Abeta_{1-16}$-Cu(II) 0.5:1		1.51	0.13
AKH-αR5W4^{NBD}	DFO	0.13	0.06
	Pre-Incubation	0.33	0.03
	Co-addition	1.20	0.05
AKH-LAH4^{NBD}	DFO	0.06	0.06
	Pre-Incubation	0.11	0.02
	Co-addition	0.44	0.21
AKHK^{NBD}	DFO	0.02	0.02
	Pre-Incubation	0.06	0.03
	Co-addition	1.30	0.56
$Abeta_{1-42}$-Cu(II) 0.5:1		5.50	1.07
AKH-αR5W4^{NBD}	Pre-Incubation	0.07	0.10
	Co-addition	0.65	0.28
AKHK-αR5W4^{NBD}	Pre-Incubation	0.03	0.15
	Co-addition	0.82	0.28

Figure S9: *Retrieval of Cu(II) from Aβ by AKH-αR5W4^{NBD}, AKHK^{NBD} or AKH-LAH4^{NBD}, reduces ROS production. (a) Inhibition of Cu(II)-Aβ$_{1-16}$ ROS production by AKH-LAH4^{NBD}. Inhibition of Cu(II)-Aβ$_{1-42}$ ROS production by AKH-αR5W4^{NBD}(b), or AKHK^{NBD}(c) monitored by the absorbance at 265 nm of ascorbate. Preincubation: 10 µM Cu(II)-Aβ$_{1-16/42}$ 0.5:1 and 5 µM AKH-αR5W4^{NBD}, AKHK^{NBD} or AKH-LAH4^{NBD} were incubated for 1h before addition of ascorbate followed by absorbance measurement; Co-addtion: 10 µM Cu(II)-Aβ$_{1-16}$ 0.5:1 and 5 µM AKH-αR5W4^{NBD} or AKHK^{NBD} is added together with ascorbate followed by absorbance measurement. (d) Table showing the consumption of ascorbate expressed in µM/min. Conditions: Asc: 100 µM, AKH-αR5W4^{NBD}=AKHK^{NBD}= AKH-LAH4^{NBD} = Cu:5 µM, Aβ$_{1-16}$: 10 µM, DFO: 10 µM; HEPES 100 mM pH 7,4. n=2 independent experiments.*

	(a)Aß$_{1-16}$		(b)GSH	
	Half time (min)	Fluorescence Quench (%)	Half time (min)	Fluorescence Recovery (%)
AKH-αR5W4^{NBD}	10.52 ± 0.6	91.3 ± 10.1	30.9 ± 5.9	79 ± 4.3
AKHK^{NBD}	12.39 ± 1.2	105.2 ± 5	96.4 ± 7.4	86.1 ± 12

Figure S10: *(a) Kinetics of Cu(II) transfer from Aß$_{1-16}$ to AKH-αR5W4^{NBD} or AKHK^{NBD} . Conditions: AKH-αR5W4^{NBD} and AKHK^{NBD}: 5 µM; Aß1-16 :10 µM; Cu:5 µM; DMEM 10% pH 7,4; 25°C; n=3. (b) Reduction kinetics of Cu(II) on AKH-αR5W4^{NBD} or AKHK^{NBD} by 5 mM GSH. Conditions: 4 µM Cu(II)-AKH-CPP^{NBD}, 5 mM GSH, HEPES 100 mM pH 7.4; 37°C; n=3 independent experiments.*



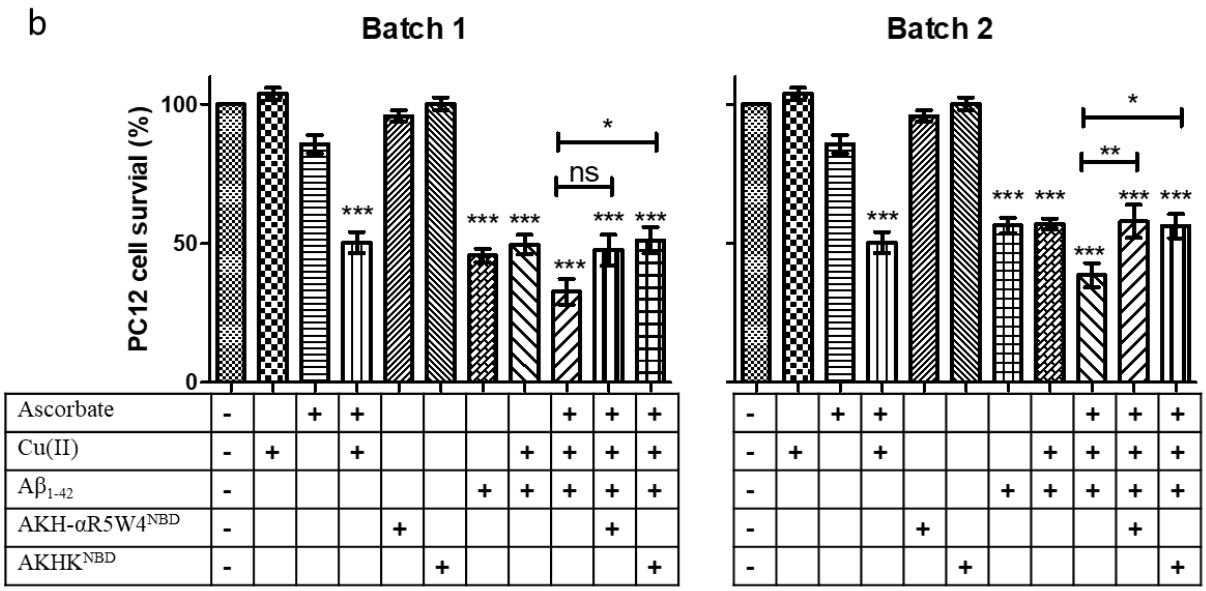


Figure S11: *Transfer of Cu(II) from Aß to ATCUN motif prevents Cu-induced ROS production and toxicity to PC12 cells. (a) $Aß_{1-16}$, (b) $Aß_{1-42}$ (2 different batches). Preincubation of 5 µM AKH-LAH4^{NBD}, AKH-αR5W4^{NBD} or AKHK^{NBD} with 10 µM Cu(II)-$Aß_{1-16/42}$ 0.5:1 in 10% DMEM in a test tube for 1h before addition of 500µM ascorbate and immediate administration on the PC12 cells. Experiments were done in triplicates, n=3. A parametric ANOVA test was carried out with a Tukey Post Test ** p<0,001, *** p<0,0001. Experiments were carried out in DMEM dilutes to 10% (10% DMEM) with salts: 0.2g/L CaCl2, 0.0001g/L Fe(NO3)3, 0.098g/L MgSO4, 0.4g/L KCl, 3.7g/L NaHCO3, 6.4g/L NaCl, 0.11g/L NaH2PO4, 4.5g/L D-Glucose.*