Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A Terbium(III) luminescent ATCUN-based peptide sensor for selective and reversible detection of Copper(II) in biological media.

Enrico Falcone,[a] Paulina Gonzalez,[a] Lucie Lorusso,[a] Olivier Sénèque,[b] Peter Faller*[a] and Laurent Raibaut*[a]

[a] Institut de Chimie, UMR 7177, CNRS, Université de Strasbourg, 4 Rue Blaise Pascal 67000, Strasbourg, France
[b] Univ. Grenoble Alpes, CNRS, CEA, BIG, LCBM (UMR 5249), F-38000 Grenoble, France

Contents

1. Abbreviations

Fmoc, 9-fluorenylmethoxycarbonyl; *t*Bu, *tert*-Butyl; HBTU, 3-[Bis(dimethylamino)methyliumyl]-3H-benzotriazol-1-oxide hexafluorophosphate; DIEA, N,N-diisopropylethylamine; DMF, N,N-dimethylformamide; TNBS, 2,4,6-Trinitrobenzenesulfonic acid; Boc, tert-butyloxycarbonyl; Alloc, allyloxycarbonyl; Trt, Trityl; DCM, dichloromethane; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; PyBOP, (Benzotriazol-1-yloxy)tripyrrolidino-phosphonium-hexafluorophosphate; TFA, trifluoroacetic acid; TIS, triisopropylsilane; HPLC, High Performance Liquid Chromatography; ESI, electrospray ionization; MS, Mass Spectrometry; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PSA, pig serum albumin; LB, Luria-Bertani; Trt, trityl.

2. Materials and methods

2.1 Peptide Synthesis

ATCUN$^{Trp/Tb}$ (GNHWK$^{DOTA(Tb)}$GK-NH_2) was synthesized manually through a standard Fmoc/*t*Bu solid-phase peptide synthesis. In order to obtain C-terminal-amidated peptides, a Fmoc-Rink amide aminomethyl-polystyrene resin (Fmoc-Rink-Amid AM Resin from Iris Biotech, 0.74 mmol/g loading, 100-200 mesh) was used as solid support. HBTU was used as coupling agent, DIEA as the base and DMF as the solvent. The coupling reactions were performed in a syringe with filter frit by using a 4-fold excess of protected amino acid, a 3.9-fold excess of HBTU and 8-fold excess of DIEA for 1 hour under mixing. After coupling, the presence of unreacted N-terminal free amine was checked with TNBS reagent, and the coupling was repeated in case of a positive test. Capping of the unreacted free amine group was carried out using 5% acetic anhydride and 10% DIEA in DMF for 5 min. N-terminal Fmoc deprotection was carried out using 20% piperidine in DMF. The peptide N-terminus was Boc-protected adding Boc_2O (4 eq.) and DIEA (2 eq.) in DMF for 1 hour. Removal of the N-Alloc protecting group of the Lys5 residue was performed using $Pd(PPh_3)_4$ (0.05 mmol, 0.5 eq., 58 mg) and phenylsilane (2.4 mmol, 24 eq., 0.3 mL) in degassed anhydrous DCM in the dark (2x1h). The resin was then washed with DCM (2×2 min), DMF (2×2 min), 1% H_2O in DMF (2×2 min), DMF (2×2 min), 1% DIEA in DMF (2×2 min), DMF (2×2 min), sodium diethyldithiocarbamate in DMF (0.12 M, 2×5 min) and DMF (2×2 min). DOTA coupling was performed overnight adding DOTA-tris(*t*Bu) ester (0.1 mmol, 57 mg, 1 eq.), PyBOP (0.2 mmol, 104 mg, 2 eq.) and then DIEA (0.8 mmol, 140 µL, 8 eq.) in DMF. Resin cleavage and side-chain deprotection were performed at the same time by treatment with 95% TFA, 2.5% H_2O and 2.5% TIS for 90 min. Crude was precipitated with cold ether and purified by HPLC on a C18 column (XBridge Peptide BEH C18 OBD Prep Column from Waters, 19 mm x 150 mm, pore size 130 Å, particle size 5 µm) using preparative HPLC with UV-vis detection at 214 nm. The Tb^{3+} complex was prepared by overnight incubation of a solution of the peptide in H_2O with a solution of $TbCl_3 \cdot 6H_2O$ in H_2O (5 eq.) at pH ≈ 6.3. Purity and identity of the peptides were assessed by analytical HPLC and ESI-MS ([**ATCUN^{Trp}**+H^+]: experimental m/z = 1211.77, calculated m/z = 1211.63; [**ATCUN$^{Trp/Tb}$**+H^+]: experimental m/z = 1367.55, calculated m/z = 1367.52. Yield: **ATCUN^{Trp}**·$(CF_3COO^-)_3$ = 12%.

2.2 Preparation of stock solutions

All stock solutions were prepared in Milli-Q water. The concentration of peptide solution was determined by tryptophan UV-vis absorption (ε_{280} = 5690 $M^{-1}cm^{-1}$) and UV-vis titration with Cu^{2+}. A stock solution of HEPES buffer (500 mM, pH 7.4) was prepared dissolving free acids in Milli-Q water and adjusting the pH with NaOH. The metal salts used were $CuCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, $ZnSO_4 \cdot 7H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$, $CoCl_2$, $MnCl_2 \cdot 4H_2O$, $MgSO_4$, $CaCl_2$, KCl. The concentration of $CuCl_2 \cdot 2H_2O$ stock solution was verified by UV-Vis spectroscopy (ε_{780} = 12 M^{-1} cm^{-1}). The concentration of the PSA solution was determined by UV-vis absorption spectroscopy (ε_{280} = 43824 $M^{-1}cm^{-1}$).

2.3 UV-vis titration

Peptide solution (500 µM) in HEPES (pH 7.4) was titrated adding 1 µl aliquots of a Cu^{2+} solution. UV-vis spectra were recorded on a Cary 60 spectrophotometer using a 1 cm path quartz cuvette.

2.4 Luminescence Spectroscopy

Luminescence spectra were recorded on a Fluorolog FL3-22 or a FluoroMax-4 spectrometer from HORIBA JobinYvon using a 1 cm path quartz cuvette. All samples were excited at λ_{ex}=280 nm. Time-delayed luminescence spectra were acquired with 100 µs time delay.
Luminescence lifetimes (τ) were determined by fitting a single-exponential decay curve (Eq. 1) to the data.

$$y = A_1 e^{(-x/\tau)} + y_0 \quad \text{(Eq. 1)}$$

The values reported are averages of three independent measurements.
Cu^{2+} titrations were conducted adding small aliquots of Cu^{2+} solution into a peptide solution (10 µM) in HEPES (pH 7.4).
For selectivity experiments, physiologically relevant divalent cations (Zn^{2+}, Fe^{3+}, Co^{2+}, Mn^{2+} 10 µM, Mg^{2+} and Ca^{2+} 2 mM) were individually added to the peptide in HEPES, and also Cu^{2+} was added to the peptide in presence of all the above-mentioned cations and 5 mM K^{+}. Due to slow binding kinetics, the effect of Ni^{2+} binding on the peptide luminescence was measured on different samples obtained by incubating the peptide (10 µM) and $NiCl_2 \cdot 6H_2O$ in HEPES 100 mM (pH 8.2) at different molar ratios for 3 h.
The Cu^{2+} transfer between PSA and the sensor was followed adding the peptide (10 µM) to a solution of Cu-PSA complex (0.9:1, [PSA] = 10 µM) in HEPES 50 mM (pH 7.4) and acquiring luminescence spectra at several time points.
The limit of detection (LOD) was evaluated at 10 µM **ATCUN$^{Trp/Tb}$** in HEPES buffer (100 mM, pH 7.4). Luminescence spectra were acquired with the following parameters: λ_{ex} = 280 nm, excitation slit = 4 nm, emission slit = 3 nm. The LOD was calculated according to Eq. 2

$$LOD = 3\sigma/k \quad \text{(Eq. 2)}$$

where σ is the standard deviation of the blank measurements (over three different samples of **ATCUN$^{Trp/Tb}$**) and k is the slope of the linear regression line between the luminescence quenching ($\Delta I = I_0 - I$) versus Cu^{2+} ion concentration.

3. Figures

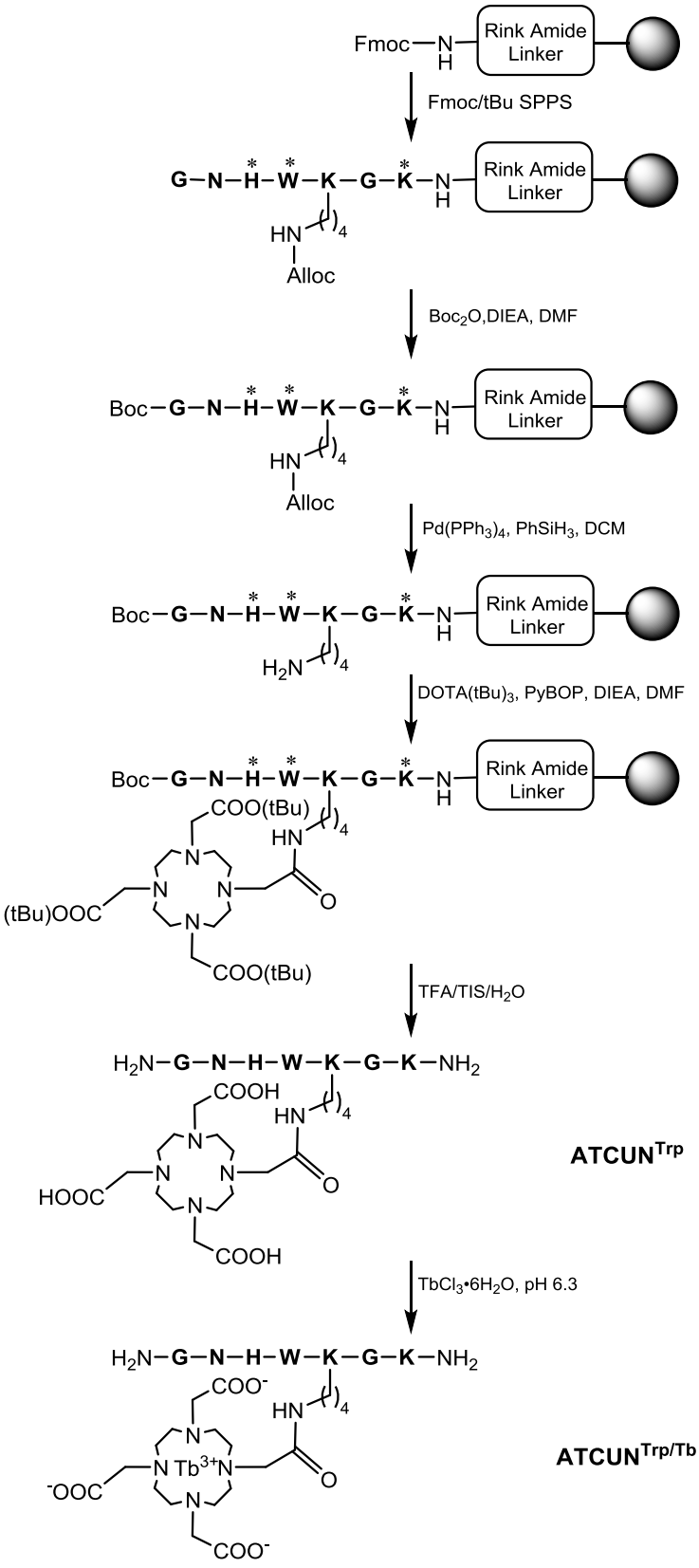


Figure S1. Synthetic pathway of **ATCUN$^{Trp/Tb}$**. Amino acids are labelled in bold. * denotes standard side chain protecting groups (Boc for Lysine and Tryptophan, Trt for Histidine).

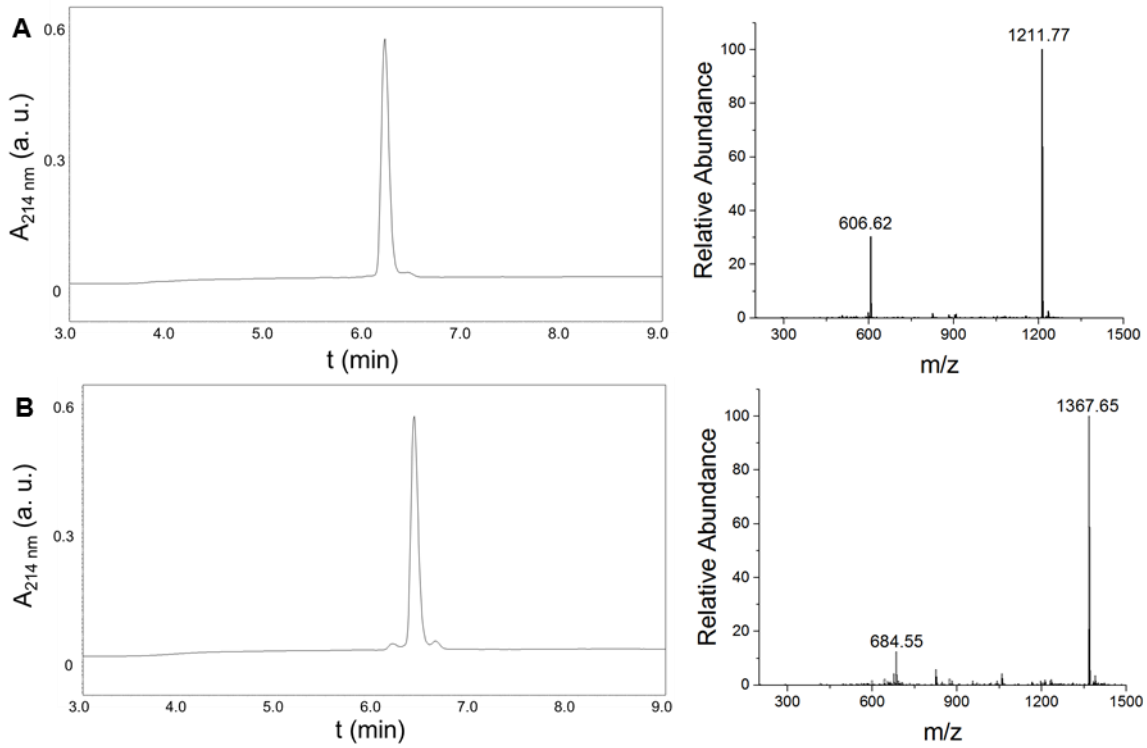


Figure S2. HPLC chromatograms (*left*) and ESI-MS spectra (*right*) of (A) **ATCUN^{Trp}** and (B) **ATCUN$^{Trp/Tb}$**.

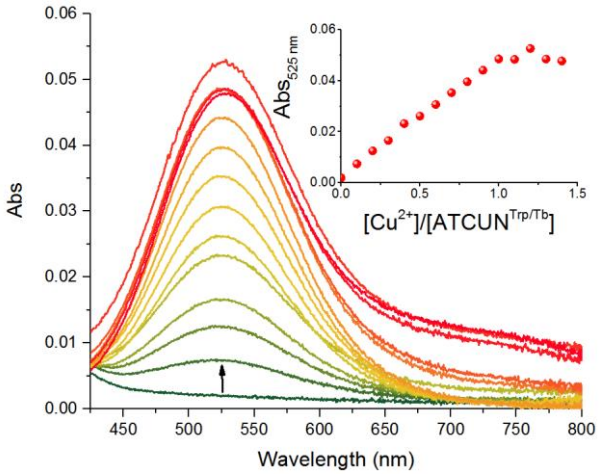


Figure S3. UV-vis titration **ATCUN$^{Trp/Tb}$** with Cu^{2+}. Conditions: [**ATCUN$^{Trp/Tb}$**] = 500µM, HEPES 100 mM pH 7.4. In the inset, $Abs_{800\ nm}$ values were subtracted from $Abs_{525\ nm}$.

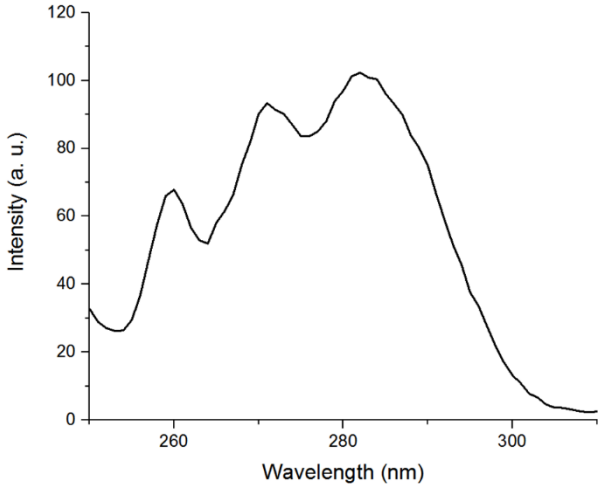
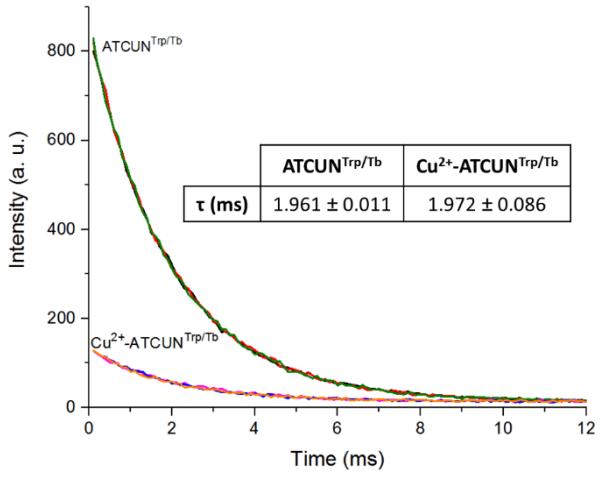


Figure S4. Time-delayed excitation spectrum of **ATCUN$^{Trp/Tb}$** (λ_{em} = 544 nm). Conditions: [**ATCUN$^{Trp/Tb}$**] = 10 µM, HEPES 50 mM pH 7.4.



	ATCUN$^{Trp/Tb}$	Cu^{2+}-ATCUN$^{Trp/Tb}$
τ (ms)	1.961 ± 0.011	1.972 ± 0.086

Figure S5. Luminescence decay curves of **ATCUN$^{Trp/Tb}$** and **Cu^{2+}-ATCUN$^{Trp/Tb}$** complex (three replicates are shown). Conditions: [**ATCUN$^{Trp/Tb}$**] = [Cu^{2+}] = 10 µM, HEPES 50 mM pH 7.4. Inset table shows lifetime values obtained by single-exponential decay fitting.

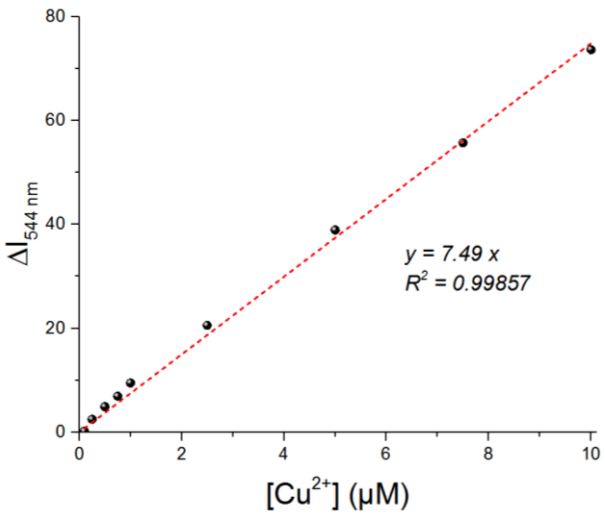


Figure S6. Calibration curve in the range 0.05–10 μM of Cu^{2+} concentration. The derived LOD (3σ/k) was 560 nM.

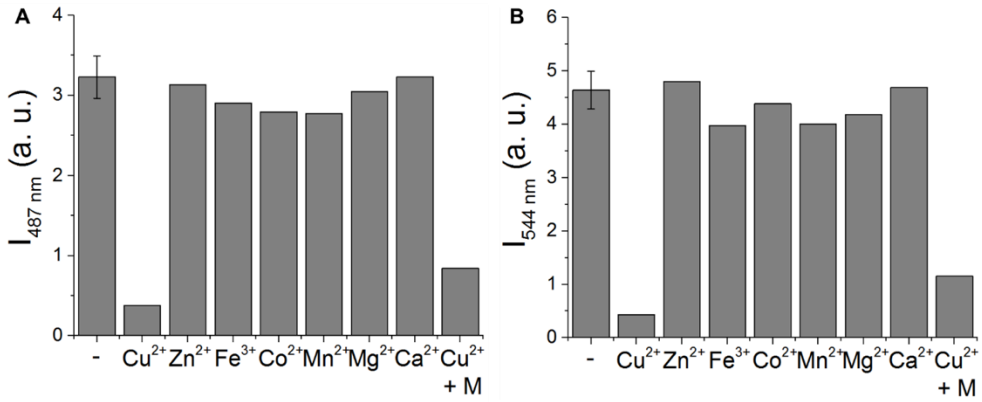


Figure S7. Selectivity diagrams at λ_{em} = 487 nm (A) and 544 nm (B). Conditions: [**ATCUN**$^{Trp/Tb}$] = 10 μM, [Cu^{2+}] = [Zn^{2+}] = [Fe^{3+}] = [Co^{2+}] = [Mn^{2+}] = 10 μM, [Mg^{2+}] = [Ca^{2+}] = 2 mM, HEPES 100 mM, pH 7.4 (M = mix of all the cations).

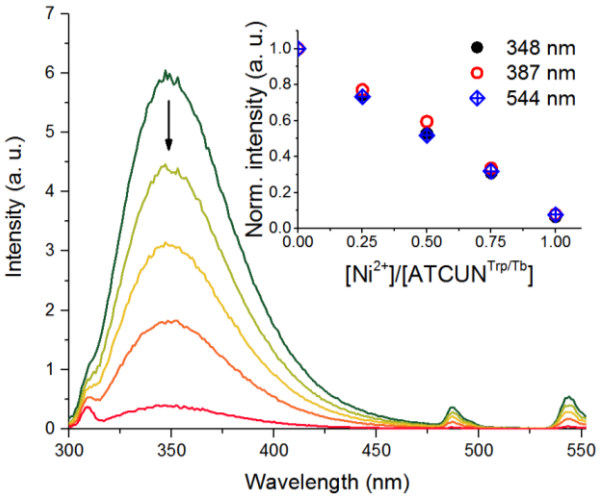


Figure S8. Luminescence of **ATCUN$^{Trp/Tb}$** after 3 h incubation with Ni^{2+} at different molar ratios (**GNH$^{W/Tb}$**:Ni^{2+} 1:0.25, 1.0.5, 1:0.75, 1:1). Conditions: [**ATCUN$^{Trp/Tb}$**] = 10 µM, HEPES 100 mM, pH 8.2.

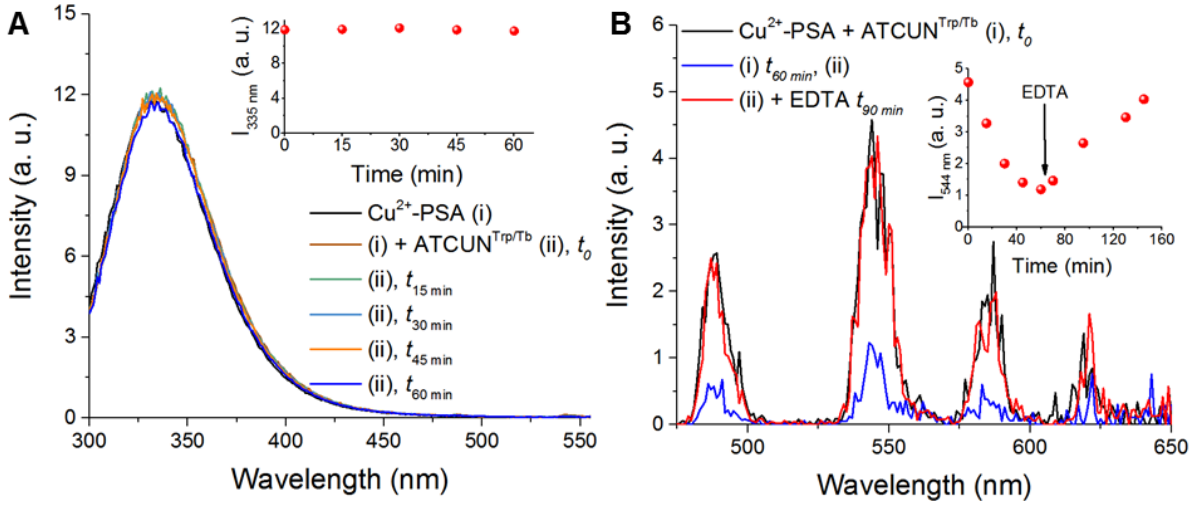


Figure S9. Cu^{2+} transfer from PSA to **ATCUN$^{Trp/Tb}$** and following signal recovery upon EDTA treatment monitored by steady-state (A) and time-delayed (B) luminescence. Conditions: [PSA] = 10 µM, [Cu^{2+}] = 9 µM, [**ATCUN$^{Trp/Tb}$**] = 10 µM, [EDTA] = 35 µM, HEPES 50 mM pH 7.4. Insets: kinetics profile of Cu^{2+} transfer.