Supporting Information

Materials and Methods

Materials

Solvents and reagents obtained from commercial suppliers were used without further purification. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} coated aluminium plates and visualization when required was achieved using UV light. ^{1}H NMR measurements were performed in DMSO-d6 on a Bruker Avance III 300 MHz Spectrometer.

Synthesis of PHP

PHP was obtained following a procedure adapted from the literature:[1] 1-Pyrenecarboxaldehyde (230 mg, 1.0 mmol) and 2-hydrazinopyridine (126 mg, 1.1 mmol) were mixed in 10 mL ethanol solution and the reaction mixture was refluxed overnight. The resulting yellow precipitate was isolated by filtration and washed with ethanol and hot ethyl acetate to give a yellow solid (315 mg, 98% yield). ESI-HRMS: calculated for $C_{22}H_{16}N_3$ ([M+H]$^+$) 322.1344, found 322.1338. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.09 (s, 1H), 9.13 (s, 1H), 8.70 (d, 1H, 9.3 Hz), 8.62 (d, 1H, 8.2 Hz), 8.31-8.34 (m, 4H), 8.16-8.21 (m, 3H), 8.10 (t, 1H, 7.5 Hz), 7.73 (t, 1H, 6.9 Hz), 7.43 (d, 1H, 8.4 Hz), 6.83 (t, 1H, 5 Hz).

Preparation of stock solutions

A stock solution of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (500 mM, pH 7.4) was prepared dissolving HEPES (free acid) in Milli-Q water and adjusting the pH with NaOH. A stock solution of Cu(II) (50 mM) was prepared in Milli-Q water from $CuCl_2 \cdot 2H_2O$ and its concentration was verified by UV-Vis Spectroscopy from the Cu(II) d-d band at 780 nm (ε = 12 $M^{-1}cm^{-1}$). A stock solution of Cu(I) (50 mM) was prepared in MeCN from tetrakis(acetonitrile) copper(I) hexafluorophosphate, $Cu(MeCN)_4PF_6$. Stock solutions of EDTA (10 mM) and BCS (50 mM) were prepared in Milli-Q water. PHP stock solutions were prepared either in MeCN (200 μM) or DMSO (3 mM).

Spectroscopic measurement

Spectroscopic studies of the reaction were performed diluting the stock solutions in a certain solvent. UV-vis spectra were recorded on a Cary 60 spectrophotometer using a 1 cm path cuvette. Fluorescence emission (λ_{ex} = 346 nm) and excitation (λ_{em} = 385 nm) spectra of the reaction mixture were recorded on a HORIBA "Fluorolog FL3-22" fluorimeter. Fluorescence of HPLC fractions was recorded on a Clario Star Plate inside a 384 wells black microplate (λ_{ex} = 340 nm).
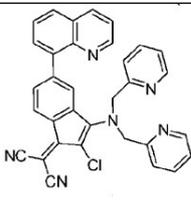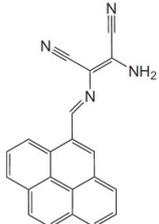
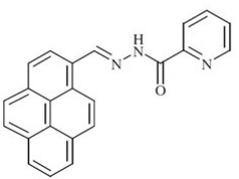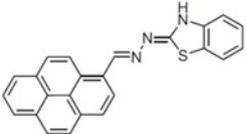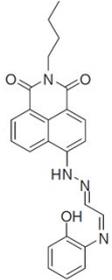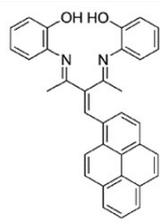### *HPLC-DAD analysis of the reaction mixture*

The reaction mixture was analysed by RP-HPLC on a C18 column (XBridge Peptide BEH C18 column from Waters , 4.6 mm x 150 mm, pore size 300 Å, particle size 3.5 μm) with a step gradient from 95% solvent A (0.1% TFA in water) and 5% solvent B (90% MeCN and 0.1% TFA in water) to 100% solvent B in 30 min, using a Hitachi Primaide instrument with DAD detection.
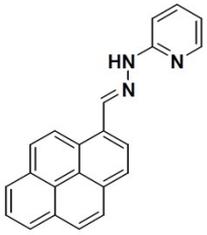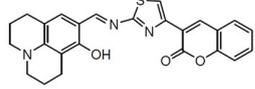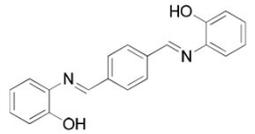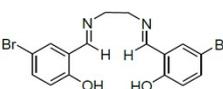
Isolation of oxPHP

PHP (3.5 mg, 11 μmol) was dissolved in MeCN (100 mL) and mixed with a $CuCl_2 \cdot 2H_2O$ solution (300 μL, 16.5 μmol). The mixture volume was reduced under vacuum and the warmed solution was charged on a TLC plate. After elution in MeCN, the oxPHP-containing spot was scratched off and dissolved in hot DMSO. This solution has been directly used for the ESI-MS and HPLC characterizations. And the solvent has been removed by evaporation for the NMR exp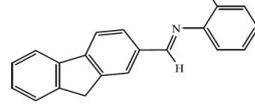eriment. ESI-HRMS: calculated for $C_{22}H_{14}N_3$ ([M+H]$^+$) 320.1182; found 320.1183. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 8.54 (d, 1H, 7.9 Hz), 8.45 (d, 1H,

7.5 Hz), 8.36-8.42 (m, 4H), 8.29 (d, 1H, 9.2 Hz), 8.17-8.20 (m, 2H), 8.00 (d, 2H, 9.2 Hz), 7.52 (dd, 1H, 6.5 Hz, 9.2 Hz), 7.00 (dd, 1H, 6.5 Hz, 6.5 Hz).

Table S1. Structure and properties of some alleged turn-on fluorescent Cu(II) sensors reported in literature.

STRUCTURE	λ_{ex} (nm)	λ_{em} (nm)	SOLVENT	REVERSIBILITY	PROPOSED MECHANISM	REF.
	495	650	MeCN:H$_2$O 1:1	-	-	2
	350	417	MeCN:H$_2$O 1:1 10 mM HEPES pH 7.0	-	PET	3
	360	455	MeOH:H$_2$O 7:3 6 mM HEPES pH 7.0	-	PET	4
	385	468	MeCN:H$_2$O 3:1 5 mM HEPES pH 7.0	-	PET	5
	455	519	MeCN:H$_2$O 7:3 10 mM MOPS pH 7.0	-	PET	6
	330	414	MeCN:H$_2$O 7:3 20 mM HEPES pH 7.5	-	CHEF	7

| Structure						Ref
	346	389	MeCN:H₂O 6:4 10 mM PBS, pH 7.4	Reversible (EDTA) Not reversible in present study (EDTA)	PET	1
	446	488	MeCN:H2O 7:3 10 mM HEPES pH = 7.4	-	CHEF	8
	300	331	MeCN:H2O	-	PET	9
	401	513	MeCN:H2O 9:1 25 mM universal buffer, pH 6.8	Reversible (EDTA)	CHEF	10
	366	480	MeCN:H2O 1:1 pH 7.2	Reversible (EDTA)	CHEF	11
	340	369	MeCN	-	ICTᵃ	12
	380	455	DMSO:H2O 2:1; pH 8.0	Reversible ($C_2O_4^{2-}$, EDTA, CN^-, PO_4^{3-}, S^{2-})	ILCTᵇ	13
	376	439	MeCN	-	-	14
	360	496	MeCN:H₂O 1:1 20 mM Tris-HCl, pH 7.4	Reversible (EDTA)	PET	15
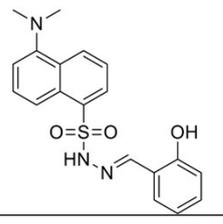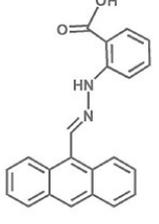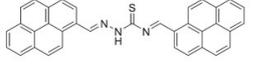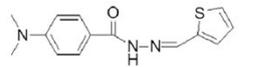
ᵃ Intramolecular Charge Transfer

ᵇ Intra-Ligand Charge-transfer Transition
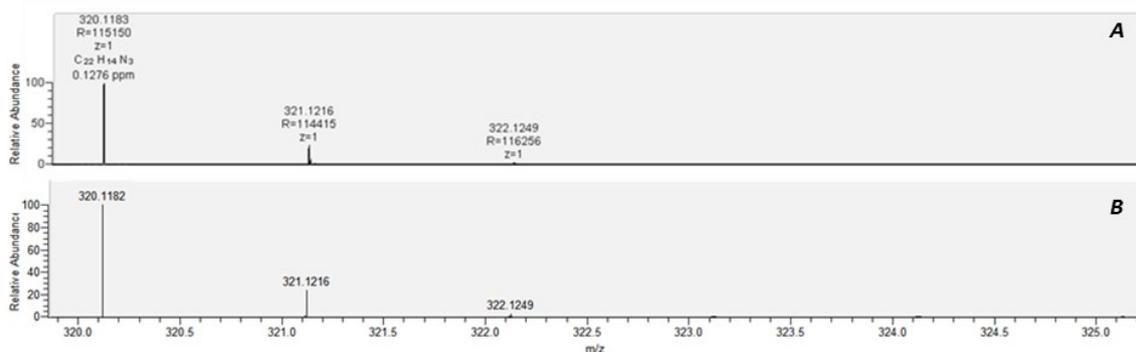
Fig. S1. ESI-HRMS analysis of oxPHP. Experimental isotopic pattern (*A*) is compared with the simulated isotopic pattern (*B*) of the species $[C_{22}H_{13}N_3 + H]^+$.
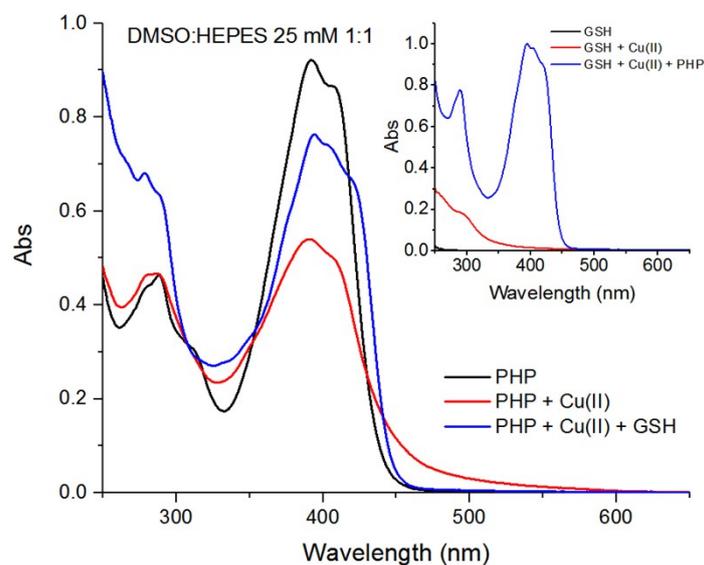
Fig. S2. Effect of the addition of GSH to Cu(II)-PHP solution in DMSO:HEPES 25 mM 1:1. *Inset*: Effect of PHP addition to Cu-GSH solution in DMSO:HEPES 25 mM 1:1. Conditions: 25 µM PHP, 30 µM Cu(II), 5mM GSH.

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