SUPPLEMENTARY INFORMATION

Complex formation and cytotoxicity of Triapine derivatives: a comparative solution study on the effect of the chalcogen atom and NH-methylation

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Table S1. Calculated absorption maxima (λ_{max}), molar absorptivity (ε) and chemical shifts (δ) of the ligand species in the different protonation states. {T = 25 °C, I = 0.10 M (KCl)}

	O-Triapine	**Triapine**	**Se-Triapine**	**Me-Triapine**
λ_{max} / nm (ε / $M^{-1}cm^{-1}$)				
H_2L^+	382 (13700)	402 (20600)	412 (19400)	
		290 (10800)	288 (9400)	
$HL^{+/o}$	352 (11300)	368 (15600)	372 (16480)	406 (22100)
		290 (11500)	300 (7400)	
$L^{o/-}$		376 (17200)	376 (16060)	364 (18160)
δ / ppm				
CH=N HL^+ / L	8.21 / 8.14			8.07 / 8.16
CH(4) HL^+ / L	7.81 / 7.31			7.83 / 7.36
CH(5) HL^+ / L	7.64 / 7.25			7.67 / 7.31
CH(6) HL^+ / L	7.99 / 7.94			8.02 / 8.00

Table S2 Overall stability constants (logβ) of the Cu(II) complexes of the studied ligands determined by various methods in 30% (w/w) DMSO/H_2O. {T = 25 °C, I = 0.10 M (KCl)} [a]

logβ	**O-Triapine** [b]		
	UV-vis	*pH-pot.*	*EPR*
$[CuLH]^{2+}$	4.76(4)	4.80(2)	4.83(2)
$[CuL]^{+}$	0.29(2)	0.22(2)	0.28 [c]
$[CuLH_{-1}]$	–7.56(5)	–8.56(2)	–8.50 [d]
$[CuLH_{-2}]^{-}$	–18.21(6)	–19.48(3)	–19.65(3)
$[CuL_2]$	–	–	–5.63(6)
	Triapine [e]		
	pH-pot.	*EPR*	
$[CuLH]^{2+}$	–	16.69	
$[CuL]^{+}$	13.89	14.35	
$[CuLH_{-1}]$	5.89	4.68	
$[CuLH_{-2}]^{-}$	–5.98	–7.57	
$[CuL_2H]^{+}$	27.16	28.67	
$[CuL_2]$	20.32	20.95 [f]	
$[Cu_2L_3]^{+}$	38.79	39.50	
	Se-Triapine		**Me-Triapine**
	UV-vis		*UV-vis*
$[CuLH]^{2+}$	≥ 20.3	**$[CuL]^{2+}$**	≥ 11
$[CuL]^{+}$	≥ 18.55(6)	**$[CuLH_{-1}]^{+}$**	≥ 4.64(4)
$[CuLH_{-1}]$	≥ 9.12(15)	**$[CuLH_{-2}]$**	≥ *c.a.*–4.8

[a] Uncertainties (SD) are shown in parentheses for the complexes determined in the present work. [b] O-Triapine possesses only one dissociable proton in the studied pH range (pyridinium NH^+) and only pK_1 could be determined accurately (pK_2 was too high). Thus, during the computation of the logβ values of the complexes the ligand was considered as a monoprotic ligand: HL^+ was used as the fully protonated form instead of H_2L^+. [c] Two isomers were detected. Isomer H (with higher g_0 value): logβ = 0.21(2) and isomer L (with lower g_0 value): logβ = 0.53(8). [d] Two isomers were detected. Isomer H: logβ = –8.73(2) and isomer L: logβ = –8.90(3). [e] Data taken from Ref. [30] [f] Two isomers were detected. Isomer L (with lower g_0 value): logβ = 20.66 and isomer H (with higher g_0 value): logβ = 20.64, reported in Ref. [30]

[30] É. A. Enyedy, N. V. Nagy, É. Zsigó, C. R. Kowol, V. B. Arion, A. Roller, B. K. Keppler and T. Kiss, Eur. J. Inorg. Chem., 2010, 2010, 1717, DOI: 10.1002/ejic.200901174

Table S3 Overall stability constants (logβ) of the Fe(III) and Fe(II) complexes of the studied ligands determined by pH-potentiometry in 30% (w/w) DMSO/H_2O. {T = 25 °C, I = 0.10 M (KCl)} [a]

logβ	O-Triapine [b]	Triapine [c]	Se-Triapine	logβ	Me-Triapine
[Fe(II)LH]$^{2+}$		15.91			
[Fe(II)L]$^{+}$	-4.96(3)	12.29	10.56(9)	**[Fe(II)L]$^{2+}$**	7.05(9)
[Fe(II)L_2H]$^{+}$		27.70			
[Fe(II)L_2]	-12.49(5)	22.55	19.9(1)	**[Fe(II)L_2]$^{2+}$**	11.96(9)
[Fe(II)L_2H_{-1}]$^{-}$		10.83			
[Fe(III)LH]$^{3+}$	10.55(3) [d]				
[Fe(III)L]$^{2+}$	6.43(3) [d]	14.03	11.02(10)		
[Fe(III)L_2]$^{+}$		26.25	22.31(9)		
				[Fe(III)LH_{-1}]$^{2+}$	1.96(9)

[a] Uncertainties (SD) are shown in parentheses for the complexes determined in the present work. [b] O-Triapine possesses only one dissociable proton in the studied pH range (pyridinium NH^+) and only pK_1 could be determined accurately (pK_2 was too high). Thus, during the computation of the logβ values of the complexes the ligand was considered as a monoprotic ligand: HL^+ was used as the fully protonated form instead of H_2L^+. [c] Data taken from Ref. [31] [d] Determined by UV-vis spectrophotometry.

[31] É. A. Enyedy, M. F. Primik, C. R. Kowol, V. B. Arion, T. Kiss and B. K. Keppler, Dalton Trans., 2011, 40, 5895, DOI: 10.1039/C0DT01835J

Table S4. Isotropic EPR parameters of the components obtained for Cu(II) complexes of O-Triapine. (Uncertainties (SD) are shown in parentheses.)

	g_o	A_o /G	a_o^N /G	α /G	β /G	γ /G
Cu^{2+}	2.192(1)	33.2(8)		45(1)	-2.4(6)	0.1(5)
O-Triapine						
$[CuLH]^{2+}$	2.1431(4)	50.15(4)	14.4(7)	45.6(6)	-16.7(3)	1.2(1)
$[CuL]^{+}$						
isomer 1	2.1240(2)	61.4(2)	12.2(4), 13.9(3)	30.6(3)	-15.5(1)	2.5(2)
isomer 2	2.104(2)	64(2)	15(1), 15(1), 17(2)	33(2)	-15(1)	1.5(8)
$[CuLH_{-1}]$						
isomer 1	2.1243(5)	38.1(5)	12.8(7), 13(1)	33.8(9)	-17.5(3)	2.9(1)
isomer 2	2.0915(5)	54.0(8)	17.4(8), 14(1), 14(1)	33(1)	-17.0(1)	2.1(5)
$[CuLH_{-2}]^{-}$	2.0959(1)	84.8(1)	16.7(2), 15.3(2)	24.0(1)	-15.9(1)	3.7(1)
$[CuL_2]$	2.125(2)	12(3)		29(2)	-9(2)	5.3(8)
Triapine [a]						
$[CuLH]^{2+}$	2.1069(3)	73.7(4)	15(1), 10(1)	34.8(5)	-18.0(1)	4.8(3)
$[CuL]^{+}$	2.0958(1)	72.6(1)	16.7(5), 9.8(5)	23.9(2)	-12.1(1)	2.0(1)
$[CuLH_{-1}]$	2.0865(4)	70.7(5)	14.1(4), 10.8(4)	28.6(1)	-17.9(1)	4.0(1)

[a] Dara are taken from Ref. [30]

[30] É. A. Enyedy, N. V. Nagy, É. Zsigó, C. R. Kowol, V. B. Arion, A. Roller, B. K. Keppler and T. Kiss, Eur. J. Inorg. Chem., 2010, 2010, 1717, DOI: 10.1002/ejic.200901174

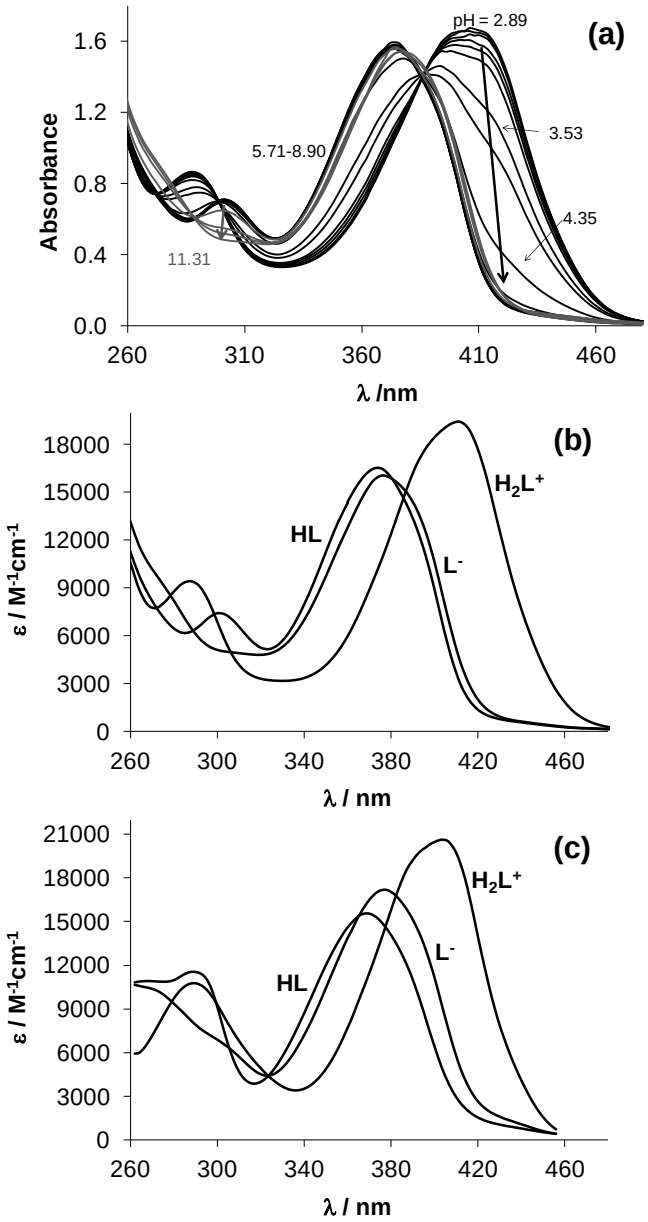

Figure S1. a) UV-vis absorption spectra of Se-Triapine recorded at various pH values under strictly O_2-free condition. Calculated individual absorption spectra of ligand species in the case of b) Se-Triapine and c) Triapine. {c_L = 95 μM; 30% (w/w) DMSO/H_2O; pH = 2 – 12.2; T = 25 °C; I = 0.10 M (KCl); ℓ = 1.0 cm}.

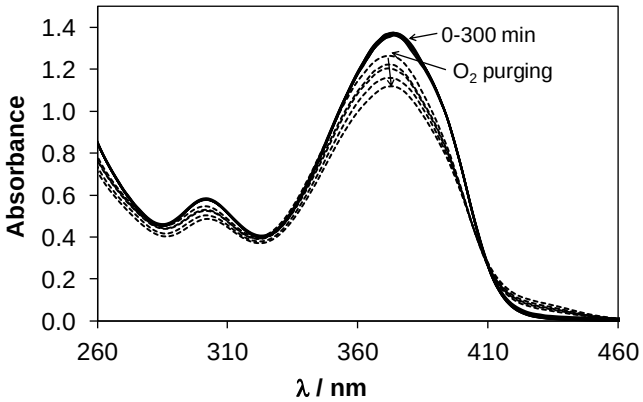

Figure S2. UV-vis absorption spectra of Se-Triapine recorded at pH 7.4 under strictly O_2-free condition in the period 0-300 min and effect of O_2 purging. {c_L = *85 μM; 30% (w/w) DMSO/H_2O; pH = 7.4; T = 25 ˚C; I = 0.10 M (KCl); ℓ = 1.0 cm*}.

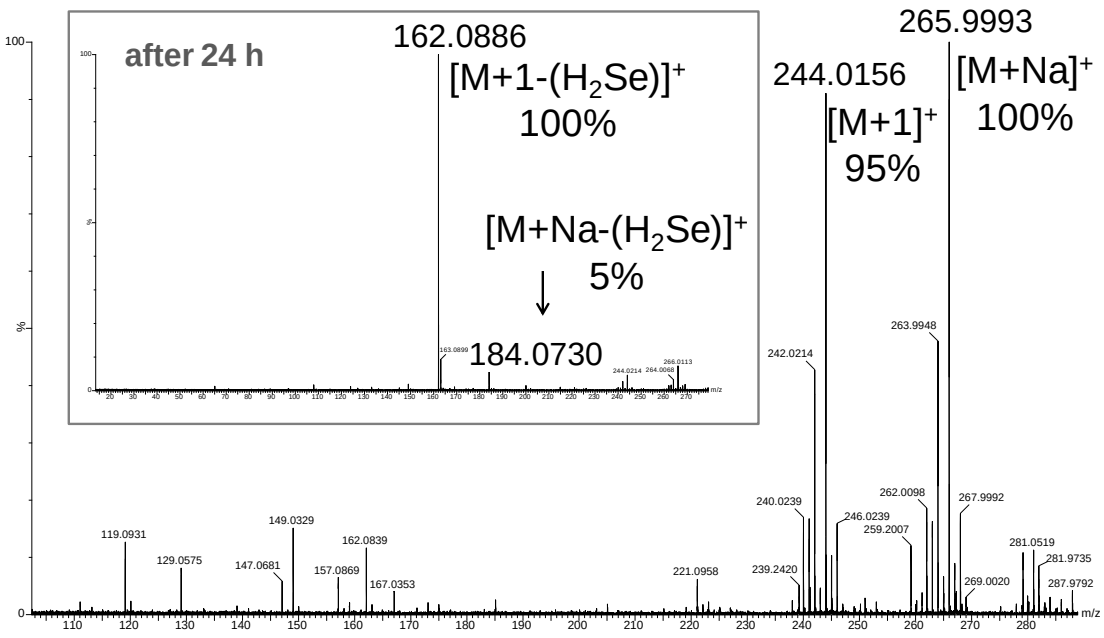

Figure S3. ESI-MS spectrum of Se-Triapine at pH 3.0 immediately after dissolution in water and after 24 h (the framed spectrum). {c_L ~ *25 μM; positive mode*}.

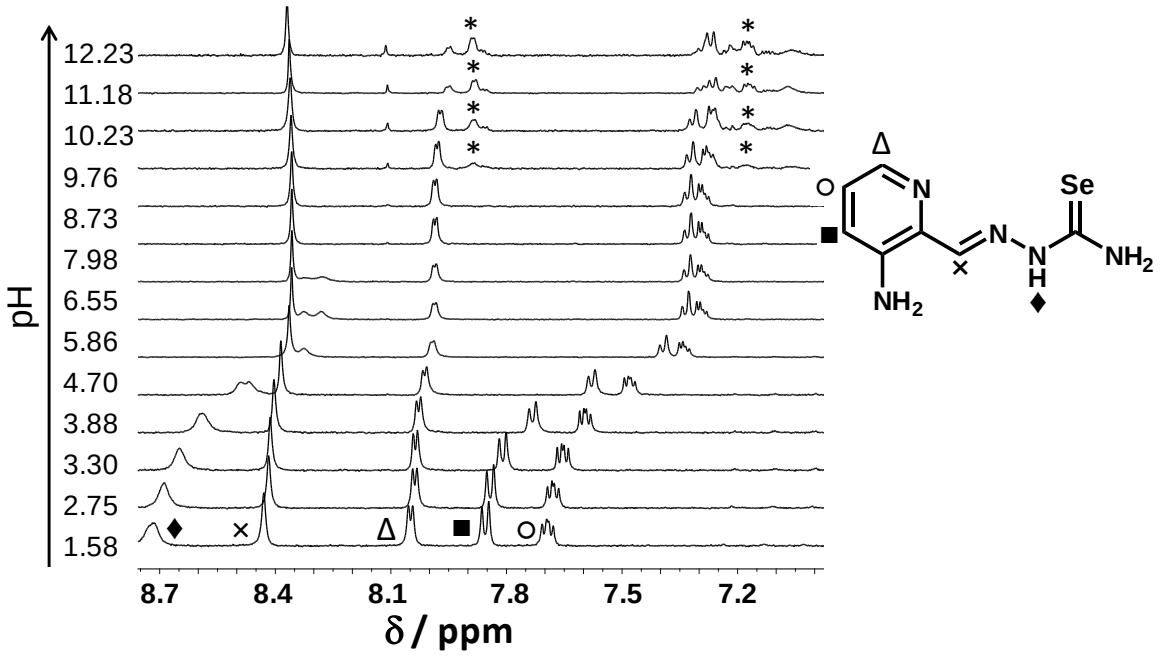

Figure S4. ^{1}H NMR spectra of Se-Triapine recorded at indicated pH values together with the notation of the symbols at the various peaks. The symbol * indicates the peaks of the decomposition product. {*c_L = 1.0 mM; 30% (v/v) d_6-DMSO/H_2O; pH = 1.6– 12.2; T = 25 ˚C; I = 0.10 M (KCl)*}.

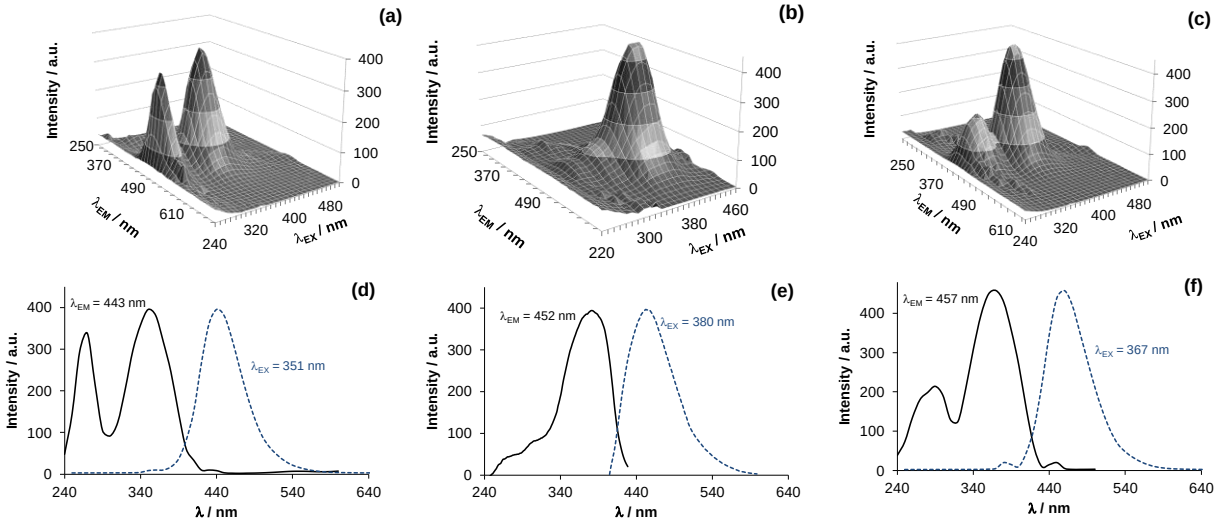

Fig. S5. 3D fluorescence spectra of a) O-Triapine, b) Se-Triapine and c) Me-Triapine recorded at pH 7.4. Excitation (black lines) and emission (blue dashed lines) spectra of d) O-Triapine, e) Se-Triapine and f) Me-Triapine. {c_L = 10 μM; 30% (w/w) DMSO/H_2O; pH = 7.4; T = 25 ˚C; I = 0.10 M (KCl); ℓ = 1.0 cm}

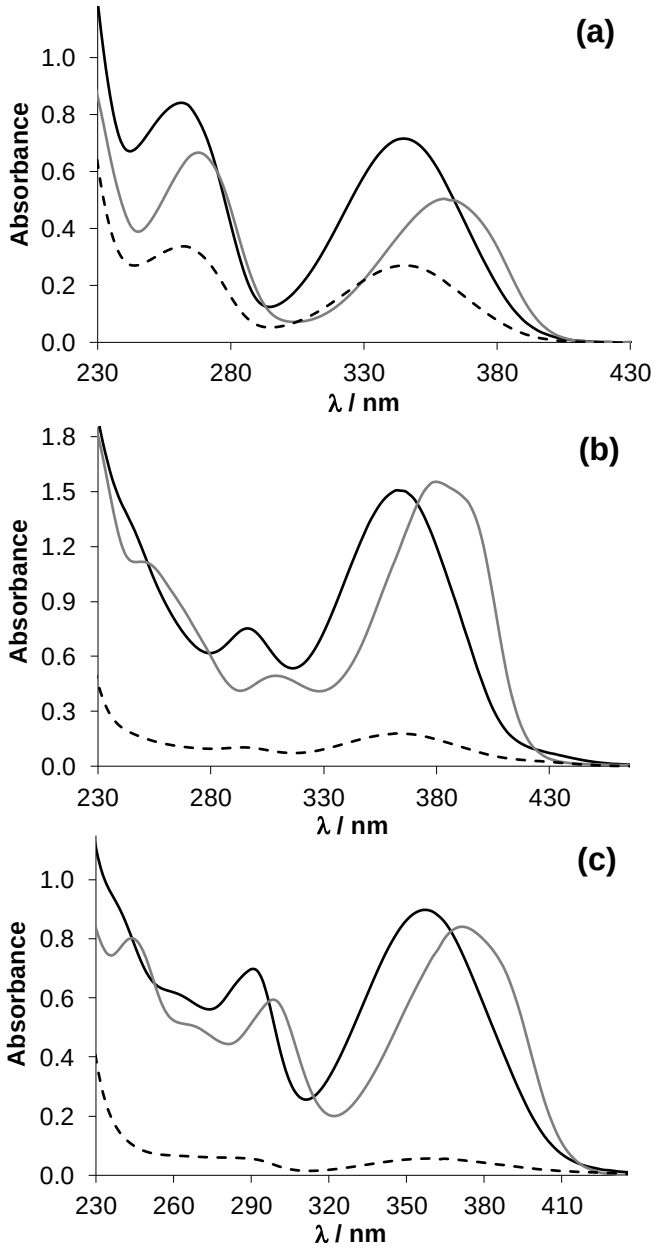

Figure S6. UV-vis absorption spectra of a) O-Triapine, b) Se-Triapine and c) Me-Triapine recorded for the original solution before partitioning (black solid line), in the aqueous phase (black dashed line) and in the *n*-octanol phase (grey solid line) following the separation. {$c_{O\text{-}Triapine}$ = 63 μM; $c_{Se\text{-}Triapine}$ = 100 μM; $c_{Me\text{-}Triapine}$ = 50 μM; pH = 7.40 (20 mM HEPES); T = 25 ˚C; I = 0.10 M (KCl); ℓ = 1.0 cm}.

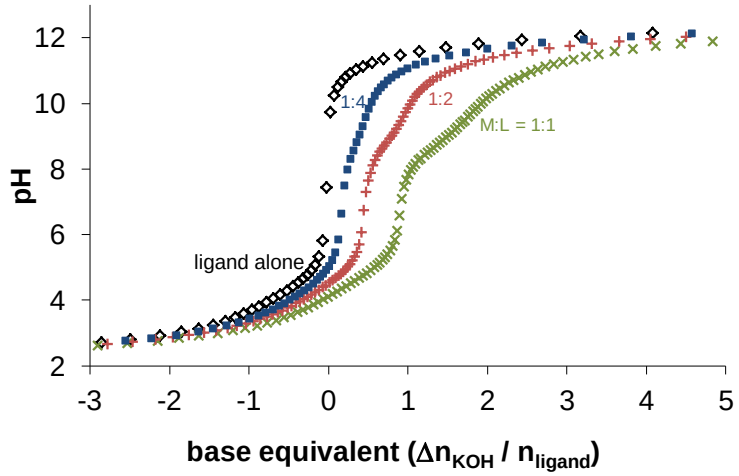

Figure S7. pH-potentiometric titration curves for O-Triapine (ligand alone) and for Cu(II)–O-Triapine system at various metal-to-ligand ratios. {$c_{O\text{-}Triapine}$ *= 1 mM; 30% (w/w) DMSO/*H_2O*; T = 25 °C; I = 0.10 M (KCl)*}.

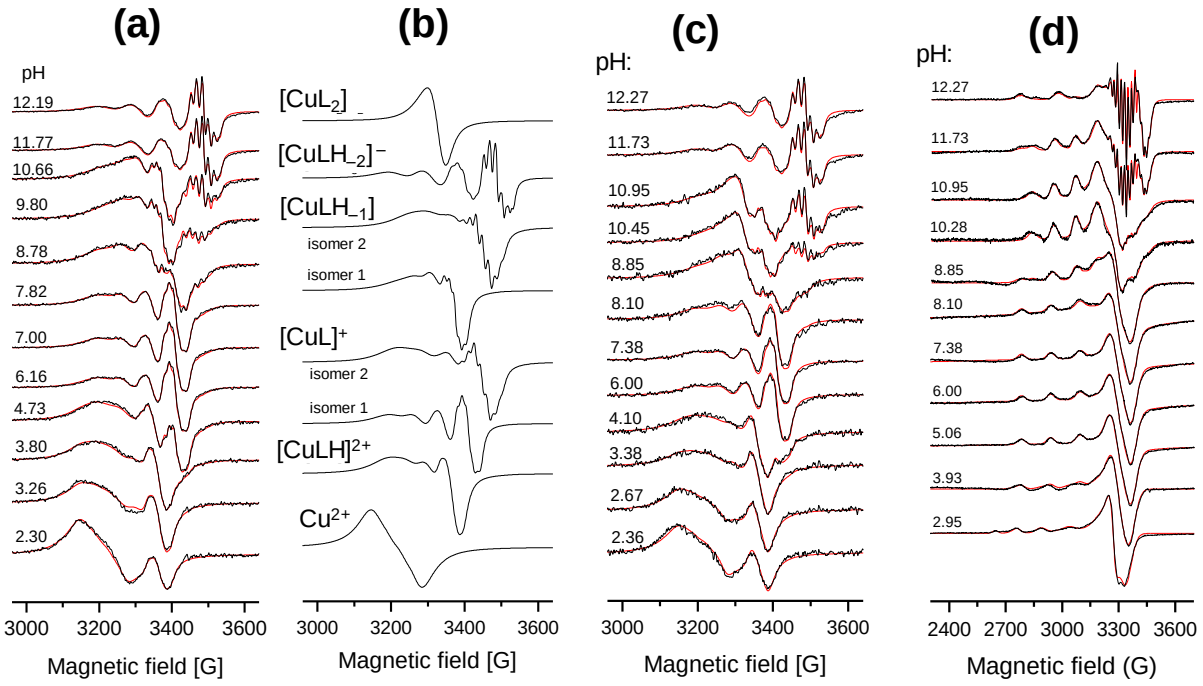

Figure S8. Experimental (black) and simulated (red) solution EPR spectra recorded for the Cu(II)–O-Triapine a) (1:1) system and b) the calculated component EPR spectra of the species, and the (1:2) system at c) 295 K and d) 77 K at various pH values in 30% (v/v) DMSO-water solution. {c_L *= 1 mM;* c_{Cu} *= 1.0 or 0.5 mM; 30% (w/w) DMSO/*H_2O*; T = 25 °C; I = 0.10 M (KCl).*

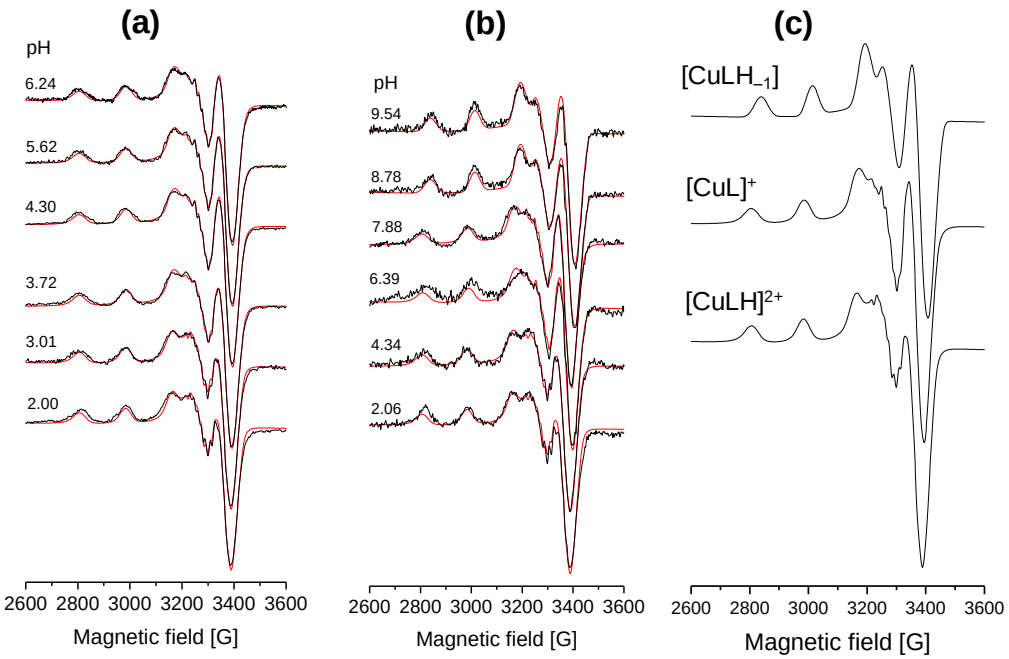

Figure S9. Experimental (black) and simulated (red) EPR spectra recorded for the Cu(II)–Se-Triapine system in 30% (v/v) DMSO-water solution at a) 1:1 and b) 1:2 metal-to-ligand ratio at 77 K at various pH values. {*c_L = 0.5 mM; c_{Cu} = 0.5 or 0.25 mM; 30% (w/w) DMSO/H_2O; T = 25 ˚C; I = 0.10 M (KCl)* c) Calculated component EPR spectra obtained for the Cu(II)–Se-Triapine complexes.

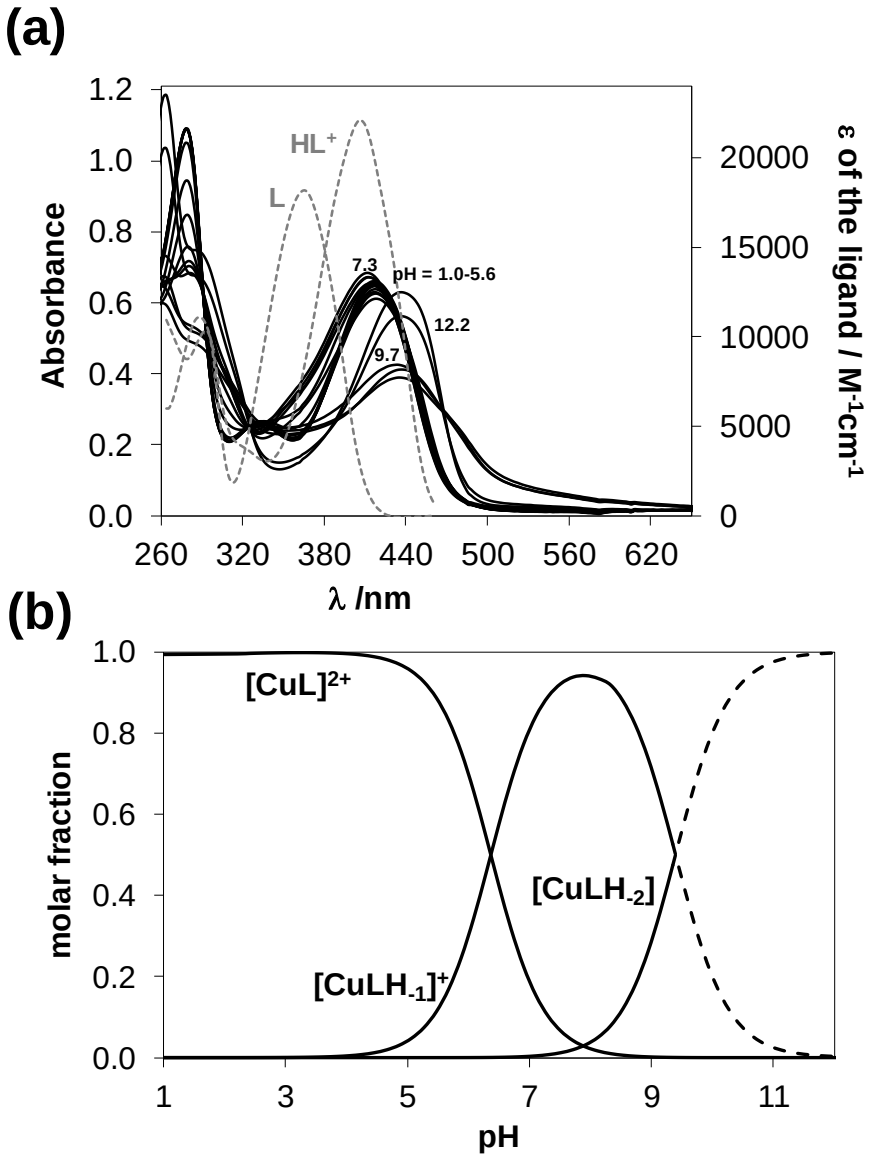

Fig. S10. a) UV-vis absorption spectra of Cu(II)–Me-Triapine (1:1) system recorded at various pH values (black solid lines) together with the molar absorbance spectra of the ligand species (dashed lines). b) Concentration distribution curves for the same system. {c_L = c_{Cu} = 50 μM; 30% (w/w) DMSO/H_2O; T = 25 ˚C; I = 0.10 M (KCl); ℓ = 1.0 cm}

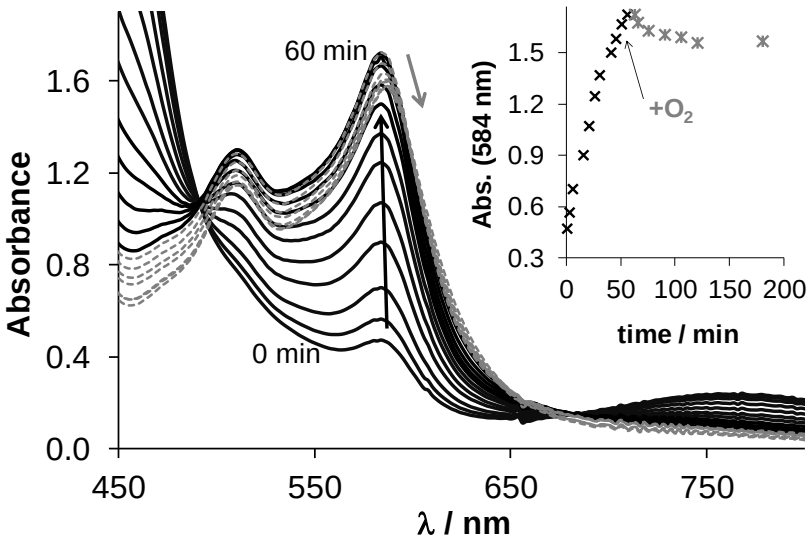

Fig. S11. Time-dependence of UV-vis absorption spectra of Fe(III)–Me-Triapine (1:2) system recorded at pH 7.4 revealing the decomposition with time. {c_L = 1 mM; 30% (w/w) DMSO/H_2O; T = 25 ˚C; I = 0.10 M (KCl); ℓ = 1.0 cm}

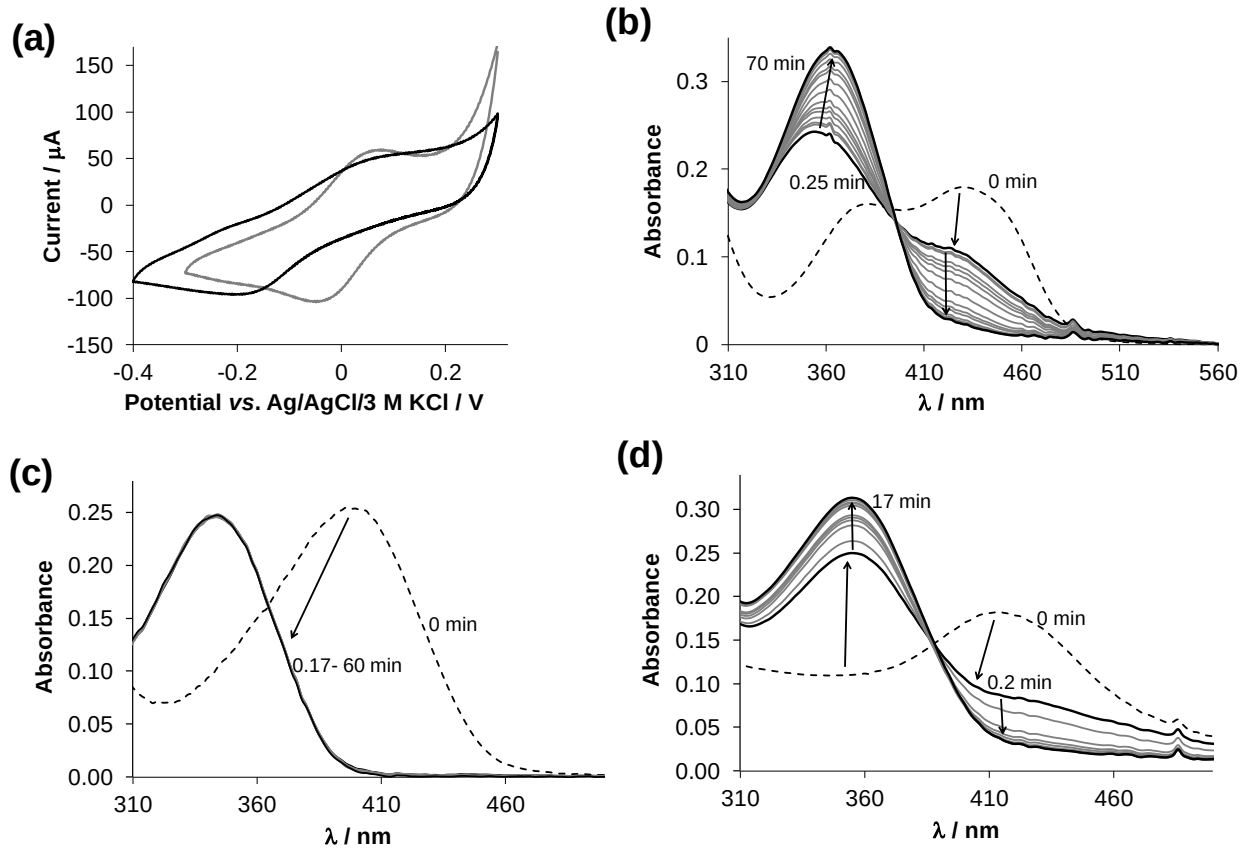

Fig. S12. a) Cyclic voltammograms of Cu(II)–Se-Triapine (black line) and Cu(II)–Me-Triapine (grey line) system at 1:1 metal-to-ligand ratio. {c_L = c_{Cu} = 0.5 mM; 70-30% (v/v) DMF/0.2 M HEPES (pH = 7.4); T = 25 ˚C; I = 0.10 M (KNO_3)} Time dependent changes of the UV-vis absorption spectra of b) Cu(II)–Se-Triapine, c) Cu(II)–O-Triapine and d) Cu(II)–Me-Triapine systems in the presence of 50 equiv. GSH at pH 7.4 in buffered aqueous solution under argon. {c_L = c_{Cu} = 25 µM; c_{GSH} = 1.25 mM; c_{HEPES} = 50 mM; T = 25 ˚C; I = 0.10 M (KCl); ℓ = 1.0 cm}

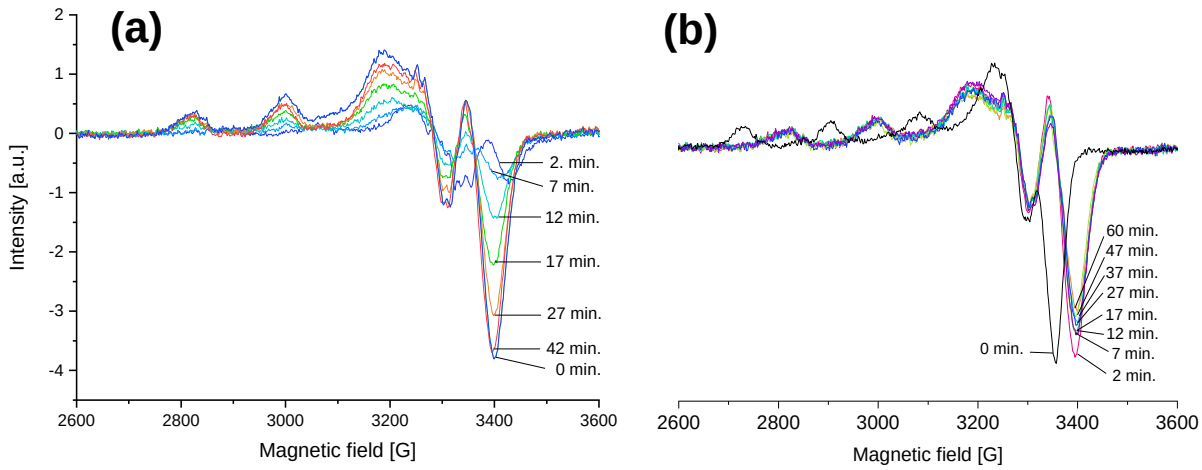

Figure S13. Anisotropic EPR spectra recorded for the Cu(II)–Triapine system in the presence of a) 5 equiv. GSH and b) 20 equiv. ascorbic acid at pH 7.4 under aerobic conditions. {c_{HEPES} = *50 mM; T = 25 ˚C; I = 0.10 M (KCl)*} 0 min: before the addition of the reducing agent. 2 min: the first spectrum recorded after the addition of GSH/ascorbic acid. Addition of GSH results in immediately low intensities, which was increased in time due to the re-oxidation of the Cu(II) centre. Addition of the ascorbic acid results in the shift of the peaks due to the formation of a ternary Cu(II)-Triapine-ascorbate complex, but then the signal intensity was not changed due to the lack of the reduction of Cu(II).

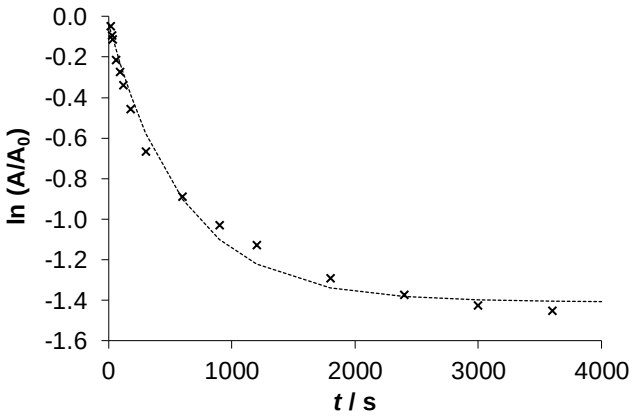

Figure S14. The ln A/A_0 values recorded at 370 nm plotted against the time (×) and the simulated curve (dashed line) for the Cu(II)–Se-Triapine system in the presence of 50 equiv. GSH at pH 7.4 in pure water under argon (A: actual absorbance, A_0: initial absorbance). k_{obs} = 0.113 min^{-1} (k_{obs} = 0.110 min^{-1} for Cu(II)-Triapine complex [23]). {$c_L = c_{Cu}$ = *25 μM; c_{GSH} = 1.25 mM; c_{HEPES} = 50 mM; T = 25 ˚C; I = 0.10 M (KCl); ℓ = 1.0 cm*}

[23] S. Hager, V. F. S. Pape, V. Pósa, B. Montsch, L. Uhlik, G. Szakács, S. Tóth, N. Jabronka, B. K. Keppler, C. R. Kowol, É. A. Enyedy and P. Heffeter, Antioxid. Redox Signal., 2020, 33, 395, DOI: 10.1089/ars.2019.7854

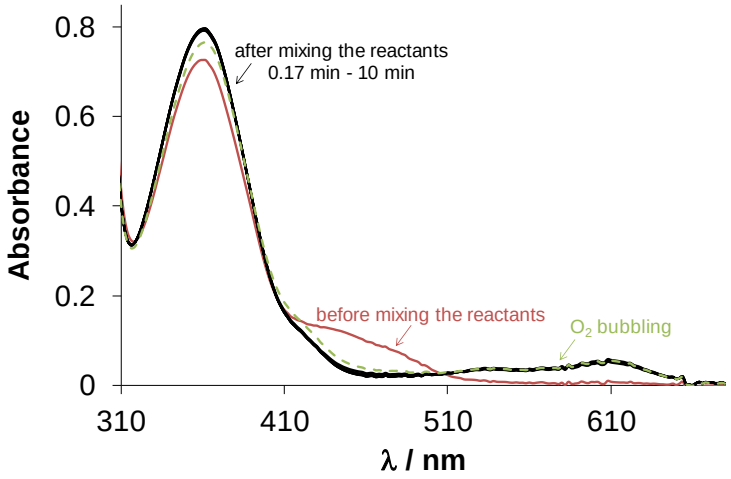

Figure S15. Time dependent changes of the UV-vis absorption spectra of Fe(III)–Triapine (1:2) system in the presence of 100 equiv. ascorbate at pH 7.4 in pure water under argon and effect of O_2 bubbling (green dashed lines). {*c_L = 50 μM; $c_{Fe(III)}$ = 25 μM; $c_{ascorbic\ acid}$ = 2.5 mM; c_{HEPES} = 50 mM; T = 25 °C; I = 0.10 M (KCl); ℓ = 1.0 cm*}