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Evaluation of Cobalt Complexes with Tripod Ligands for Zinc Finger Targeting

Heidar Mar Adalsteinsson[a], Frederico A. Lima [b], Carolina Galuppo[a] and Camilla Abbehausen [a*]

[a.] University of Campinas, P.O. Box 6154, 13083-970, Brazil

[b.] European X-Ray Free-Electron Laser Facility GmbH, Holzkoppel 4, 22869 Schenefeld, Germany

**camilla@unicamp.br*

Synthetic procedures

Synthesis and Characterization of Ligands and Complexes

The synthesis of the tri(2-pyridylmethyl)amine (NNN) and N,N-di(2-pyridylmethyl)glycinate (NNO) ligands were adapted from methods previously described in the literature.1,2

Synthesis of bis(2-pyridylmethyl)amine (NN intermediate)

In a round bottom flask, 40 mL of methanol were mixed with 1.5 mL (15.8 mmol) of freshly distilled picolinaldehyde and 1.55 mL (15.8 mmol) of picolylamine. The solution was stirred at RT for 1 hour and then cooled to 0 °C. Then 1.44 g (38 mmol) $NaBH_4$ were added slowly followed by overnight stirring at RT. Solvent was removed under reduced pressure, and 20 mL of H_2O was added to the resulting oil. The solution was extracted with 4 x 20 mL of chloroform. The extracts were then combined and the solution dried using $NaSO_4$ followed by rota-evaporation, resulting in a light yellow oil which was analyzed by ^1H NMR. Yield = 95%. ^1H NMR ($CDCl_3$): d 3.70 (s, 2H, CH_2N); d 6.85 (t, H, pyr); d 7.08 (d, H, pyr); d 7.33 (t, H, pyr); d 8.28 (d, H, pyr)

Synthesis of tris(2-pyridylmethyl)amine (NNN ligand)

To a round bottom flask containing 30 mL of methanol were added 1.44 mL (15.1 mmol) of freshly distilled picolinaldehyde and 2.57 mL (45 mmol) glacial acetic acid. To this mixture were added 3.07 g (15.1 mmol) of NN intermediate and the solution stirred for 1 hour at RT. The solution was then brought to 0 °C and 1.9741 g (31.4 mmol) $NaBH_3CN$ were slowly added while stirring. The solution was stirred at RT overnight and then rota-evaporated. The resulting oil was filtered through silica loaded on a fritter using a solution of 5% methanol in chloroform as a mobile phase, followed by drying of the resulting solution using K_2CO_3 and removal of the solvent under reduced pressure. The product is light yellow oil. Yield 90%. ^1H NMR ($CDCl_3$): d 3.88 (s, 2H, CH_2N); d 7.15 (t, H, pyr); d 7.56 (d, H, pyr); d 7.66 (t, H, pyr); d 8.54 (d, H, pyr).

Synthesis of bis(2-pyridylmethyl)glycine ethylester (NNO-Et ligand)

To a round bottom flask containing dry THF, 3.01 g (15.1 mmol) of NN intermediate and 2.10 mL (15.1 mmol) of Et_3N were added. The solution was brought to 0 °C before slowly adding ethylbromoacetate under stirring. The solution was then stirred at RT overnight followed by filtration through a fritter loaded with Celite®. The solvent was then removed under reduced pressure. The product is a dark orange, almost brown, oil. Yield 82%. ^1H NMR (DMSO–d_6): d

1.25 (t, 3H, OEt); d 3.46 (s, 2H, CH2N); d 3.99 (s, 4H, CH2N); d 4.16 (q, 2H, OEt); d 7.14 (t, 2H, pyr); d 7.56 (d, 2H, pyr); d 7.65 (t, 2H, pyr); d 8.52 (d, 2H, pyr).

The synthesis of $[Co^{III}(NNN)Cl_2]ClO_4$ (1), $[Co^{III}(NNN)(NO_2)_2]ClO_4$ (2) and $[Co^{II}(NNO)Cl]$ (5) was carried out using methods already described in the literature.[3–5] Slightly modified methods from the literature were used in the synthesis of $[Co^{II}(NNN)Cl]Cl$ (3) and $[Co^{III}(NNO)(NO_3)_2]$ (4).[5–7]

Synthesis of $[Co^{III}(NNN)(NO_2)_2]ClO_4$.

In a round bottom flask, 0.14 g (0.50 mmol) NNN ligand was dissolved in a mixture of 10 mL H_2O and 2 mL $HClO_4$ (2 $molL^{-1}$). Then, 0.20 g of $Na_3[Co(NO_2)_6]$ (0.50 mmol) were dissolved in 10 mL H_2O and added dropwise to the ligand solution while stirring. The mixture was then heated to 80 °C in a water bath for 5 minutes, during which a yellow precipitate formed. The precipitate was filtered and recrystallized in hot water and formed yellow needles. Yield = 65.5%. Selected IR bands: ν (Cl-O)(ClO_4^-), 1120, 1094 and 1077 cm^{-1}; ν (N-O)(NO_2^-), 1318 and 1286 cm^{-1}; ν (C-H)(pyr), 3087, 3050 and 2979 cm^{-1}. ESI-MS: m/z 421.09 $[Co^{III}(NNN)(NO_2)CN]^+$; m/z 426.07 $[Co^{III}(NNN)(NO_2)OMe]^+$; m/z 441.08 $[Co^{III}(NNN)(NO_2)_2]^+$.

Synthesis of $[Co^{III}(NNN)Cl_2]ClO_4$

A suspension of $[Co^{III}(NNN)(NO_2)_2]ClO_4$ 0.54 g (1.23 mmol) in a 15 mL HCl solution (6 mol L^{-1}) was heated to 75 °C in a water bath. The solution started changing colour to a dark purple. The solution was kept hot for approximately one hour when no further colour change was observed. The volume was reduced to approximately 5 mL under vacuum and stored in a freezer for 48 hours. Purple crystals formed which were filtered and washed with isopropanol followed by diethyl ether. Due to the low yield (12.5%), A mixture of iso-4-propanol and diethyl ether (10:1) was added to the mother solution, and it was stored in the freezer for another 48 h. From this solutionprecipitated more crystals, which were analyzed by X-ray diffraction. Total yield = 52 %. ^1H NMR (D_2O): d 4.78 (half of AB, 2H, CH_2N); d 5.14 (s, 2H, CH_2N); d 5.34 (half of AB, 2H, CH_2N); d 7.10 (d, H, pyr); d 7.37 (t, H, pyr); d 7.53 (t, 2H, pyr); d 7.65 (d, 2H, pyr); d 7.70 (t, H, pyr); d 8.03 (t, 2H, pyr); d 9.12 (d, 2H, pyr); d 9.15 (d, H, pyr). ESIMS: m/z 419.02 $[Co^{III}(NNN)Cl_2]^+$; m/z 410.06 $[Co^{III}(NNN)(CN)Cl]^+$; m/z 401.06 $[Co^{III}(NNN)Cl(OH)]^+$, m/z 383.05 $[Co^{III}(NNN)(OH)_2]^+$.

Synthesis of $[Co^{II}(NNN)Cl]Cl$

In a round bottom flask, 0.61g (2.58 mmol) of NNN was dissolved in 10 mL of MeOH. A solution of 0.75g (2.58 mmol) of $CoCl_2 \cdot 6H_2O$ in 10 mL MeOH was added dropwise and stirred for 30 minutes at RT. The mixture turned green instantly. Addition of excess diethyl ether precipitates a green powder. The precipitate was washed with ice-cold EtOH and diethyl ether. Product was recrystallized with vapour diffusion of diethyl ether into a methanol solution of the complex. Yield = 40 %. ESI-MS: m/z 384.06 $[Co^{II}(NNN)Cl]^+$; m/z 394.09 $[Co^{II}(NNN)OMe]^+$. %Found: C, 41.8; H, 4.0; N, 11.0. %Calc ($[Co^{II}(NNN)Cl]Cl \cdot 5H_2O$): C,42.5; H,5.3; N,11.0.

Synthesis of $[Co^{III}(NNO)(NO_3)_2]$.

In a round bottom flask, 0.40 g (1.37 mmol) of $Co(NO_3)_2 \cdot 6H_2O$ was dissolved in 15 mL of a buffer solution containing 0.60 g NaOH and 1.75 mL glacial acetic acid in H_2O. An amount of 0.3520 g (1.30 mmol) of NNOEt ligand was dissolved in a minimal volume of MeOH and added to the buffer solution. After stirring the solution at RT for 1 hour, 1 mL of 30% H_2O_2 was added. Air was bubbled through the solution for 8 hours/day for five days adding water to compensate for solvent evaporation. Yellow powder started precipitating during this process, which was then filtered, washed successively with ice-cold ethanol and diethyl ether. Yield = 20%. ^1H NMR (DMSO–d_6): d 9.51 (d, H, pyr); d 8.68 (t, H, pyr); d 8.51 (t, H,pyr); d 8.18 (m, 3H, pyr); d 7.82 (d, 2H, pyr); d 5.35 (AB, 4H, CH_2N); d 5.14 (s,2H,CH_2N); Selected IR bands: n (CH)(pyr), 3115 and 3076 cm^{-1}; n (C-H)(CH2N), 2968 and 2936 cm^{-1}; n (C=O)(carboxyl)), 1655 cm^{-1}; va $(NO_2)(NO_3)$, 1609 and 1571 cm^{-1}; vs (NO_2)(NO3), 1476 and 1445 cm–1. ESI-MS: m/z 384.06 $[Co(II)(NNN)(Cl)]^+$;

Synthesis of $[Co^{II}(NNO)Cl]$.

A solution of 1.31 g (5.53 mmol) $CoCl_2 \cdot 6H_2O$ in 12.5 mL EtOH was added to a 12.5 mL solution of 1.50 g (5.53 mmol) NNOEt ligand. The mixture was refluxed for 1 hour. The solution was then brought to RT, and 0.77 mL of freshly distilled Et_3N were added. The solution was refluxed for an additional 6 hours. The solution was filtered through a fritter and left at room temperature until a dark blue precipitate formed. The precipitate was collected and washed with diethyl ether. Yield = 20%. %Found: C,41.7; H,4.5; N,10.5. %Calc. for $[Co(NNO)Cl] \cdot 3H_2O$: C,41.6; H,5.0; N,10.4. ESI-MS: m/z 315.05 $[Co^{II}(NNO)]^+$; m/z 351.02 $[Co^{II}(NNO)Cl + H^+]^+$; m/z 361.05 $[Co^{II}(NNO)(CH_3CH_2OH)]^+$.

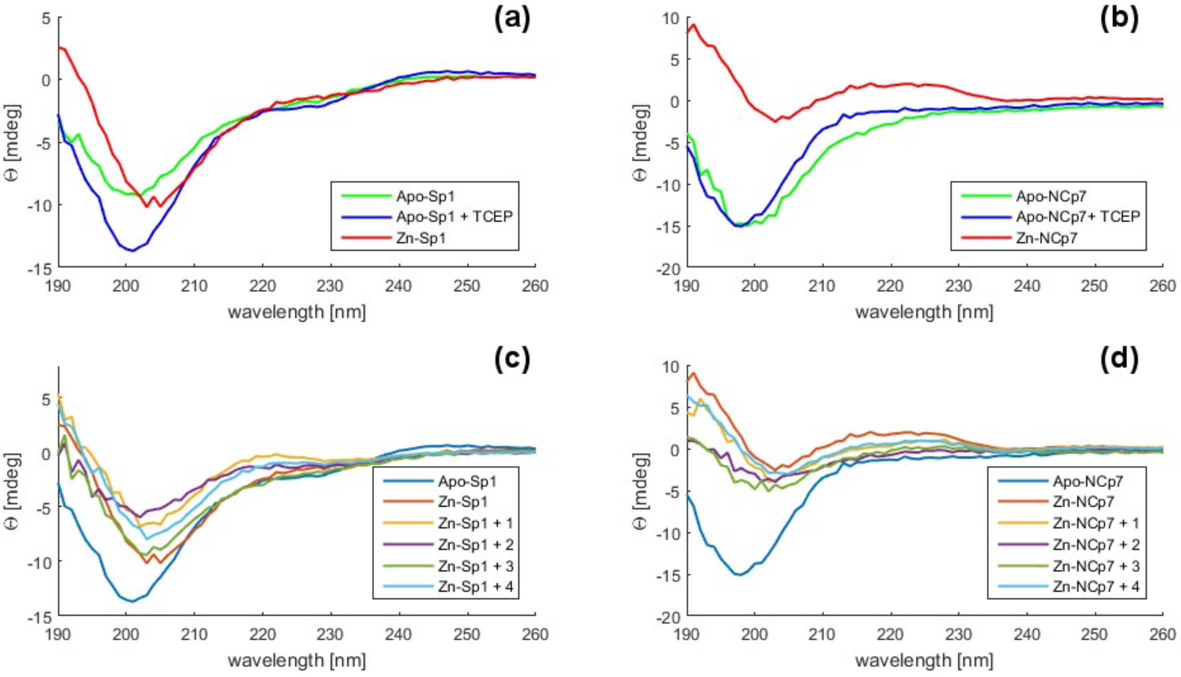


Figure S1. Circular dichroism spectra of ZF models a) CD spectra demonstrating Sp1 formation from apo-Sp1, b) CD spectra demonstrating NCp7 formation from apo-NCp7, c) Interaction of Co complexes 1-4 with Sp1, d) Interaction of Co complexes 1-4 with NCp7.

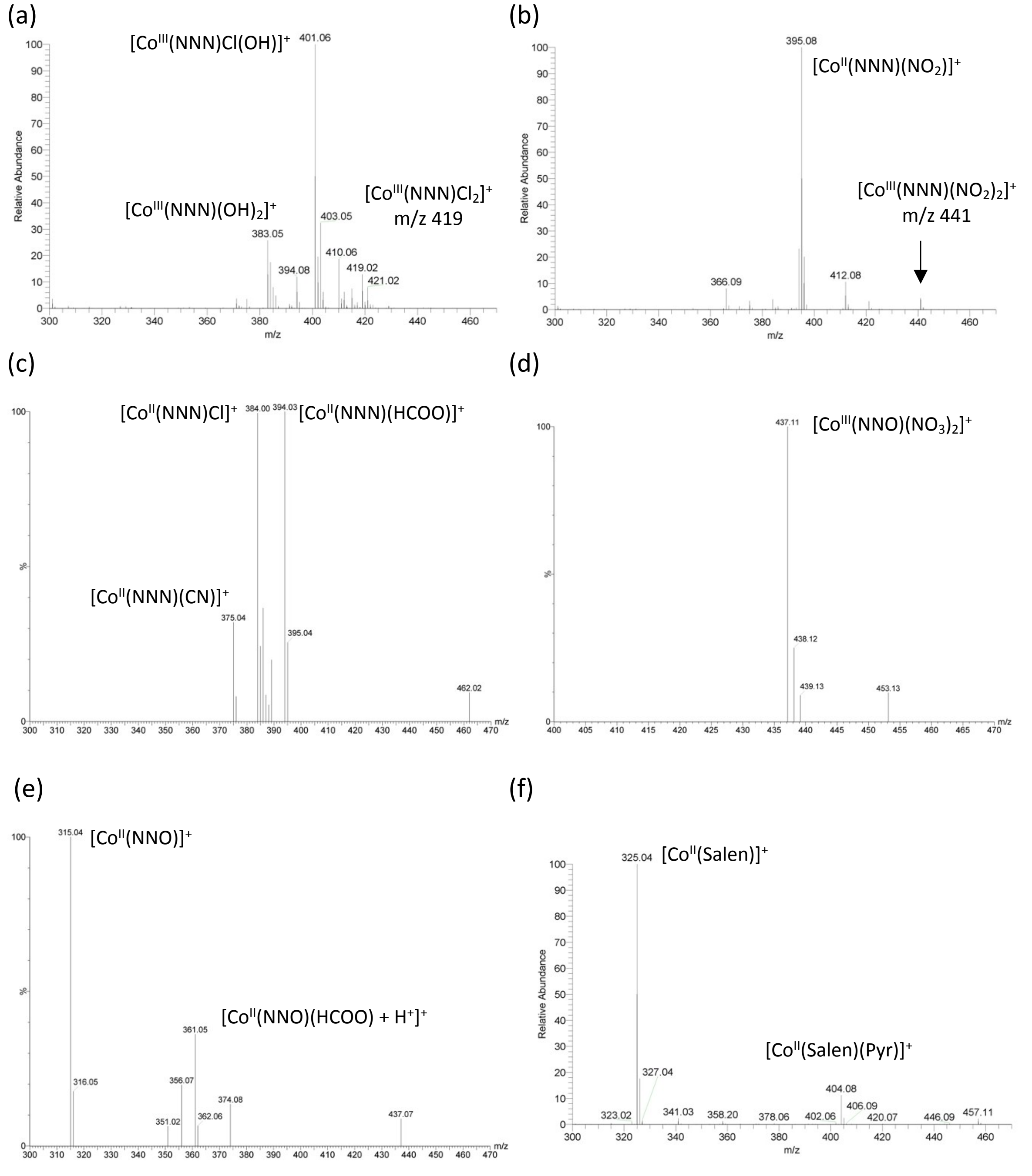


Figure S2. ESI-MS spectra of the compounds (a) $[Co^{III}(NNN)Cl_2]^+$, (b) $[Co^{III}(NNN)(NO_2)_2]^+$, (c) $[Co^{II}(NNN)Cl]^+$ (d) $[Co^{III}(NNO)(NO_3)_2]^+$ (e) $[Co^{II}(NNO)Cl]^+$ (f) $[Co^{II}(Salen)]$. CN^- is generated from acetonitrile used as solvent. $HCOO^-$ ligand is due to the use of formic acid to ionize sample and Pyr is the pyridine used as solvent in $[Co^{II}(Salen)]$ sample preparation.

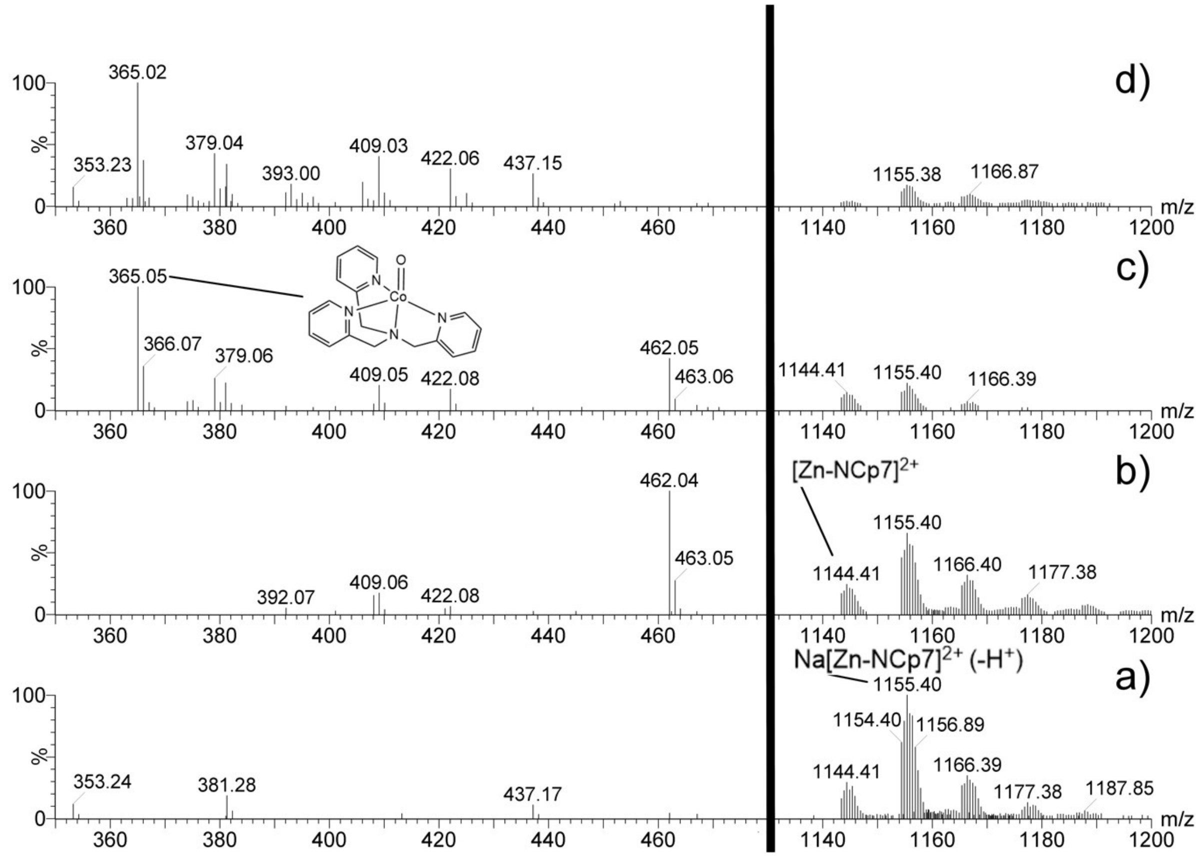


Figure S3. ESI-MS spectra after incubation of the NCp7 ZF with a) no inhibitor, b) $[Co^{III}(NNN)Cl_2]Cl$ 1 molar equivalent, 2h, c) $[Co^{III}(NNN)Cl_2]Cl$ 1 molar equivalent, 24 h, d) $[Co^{III}(NNN)Cl_2]Cl$ 2 molar equivalent, 24 h. where the line separates the low mass region of the complex and the strongest ZF signal of $[NCp7 + Na^+]^{2+}$ at 1155 m/z.

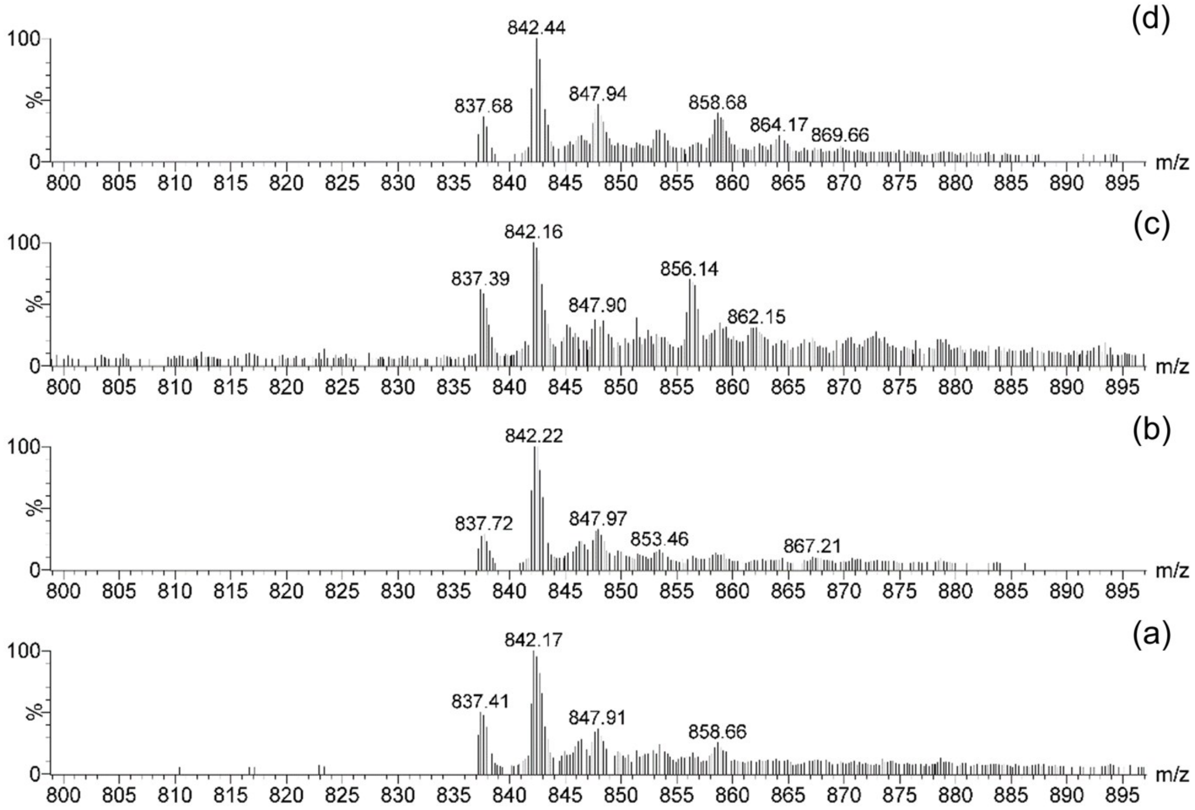


Figure S4. ESI-MS spectra (m/z 800 – 900) after incubation of the Sp1 ZF with 2 equivalents of inhibitor for 24 hours at 37 °C a) $[\mathrm{Co}^{III}(\mathrm{NNN})\mathrm{Cl}_2]^+$, b) $[\mathrm{Co}_{III}(\mathrm{NNN})(\mathrm{NO}_2)_2]^+$, c) $[\mathrm{Co}^{II}(\mathrm{NNN})\mathrm{Cl}]^+$, d) $[\mathrm{Co}^{III}(\mathrm{NNO})(\mathrm{NO}_3)_2]$.

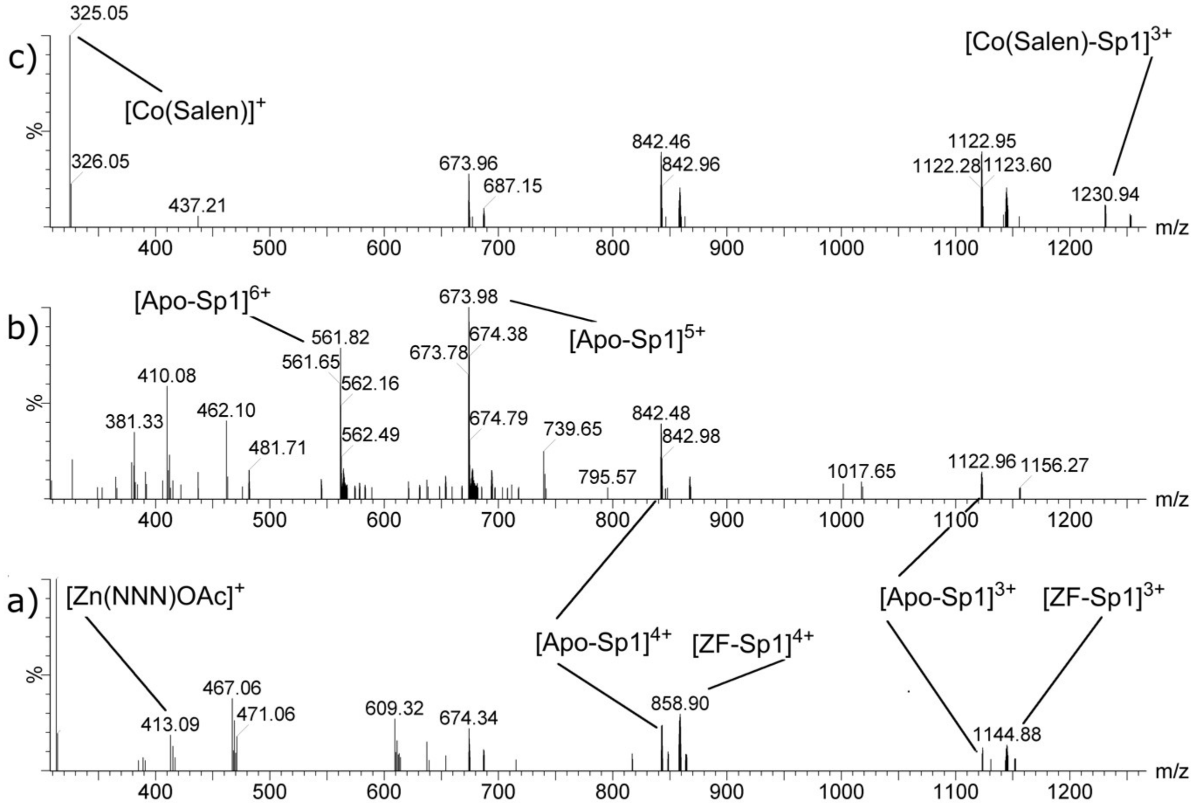


Figure S5. a) ESI-MS of an equimolar solution of the NNN ligand and Sp1 after 2 hours of incubation. b) ESI-MS of an equimolar solution of apo-Sp1 and [Co(III)(NNN)Cl_2] after 2 hours of incubation. c) ESI-MS of an equimolar solution of [Co(Salen)] and Sp1 after 24 hours of incubation

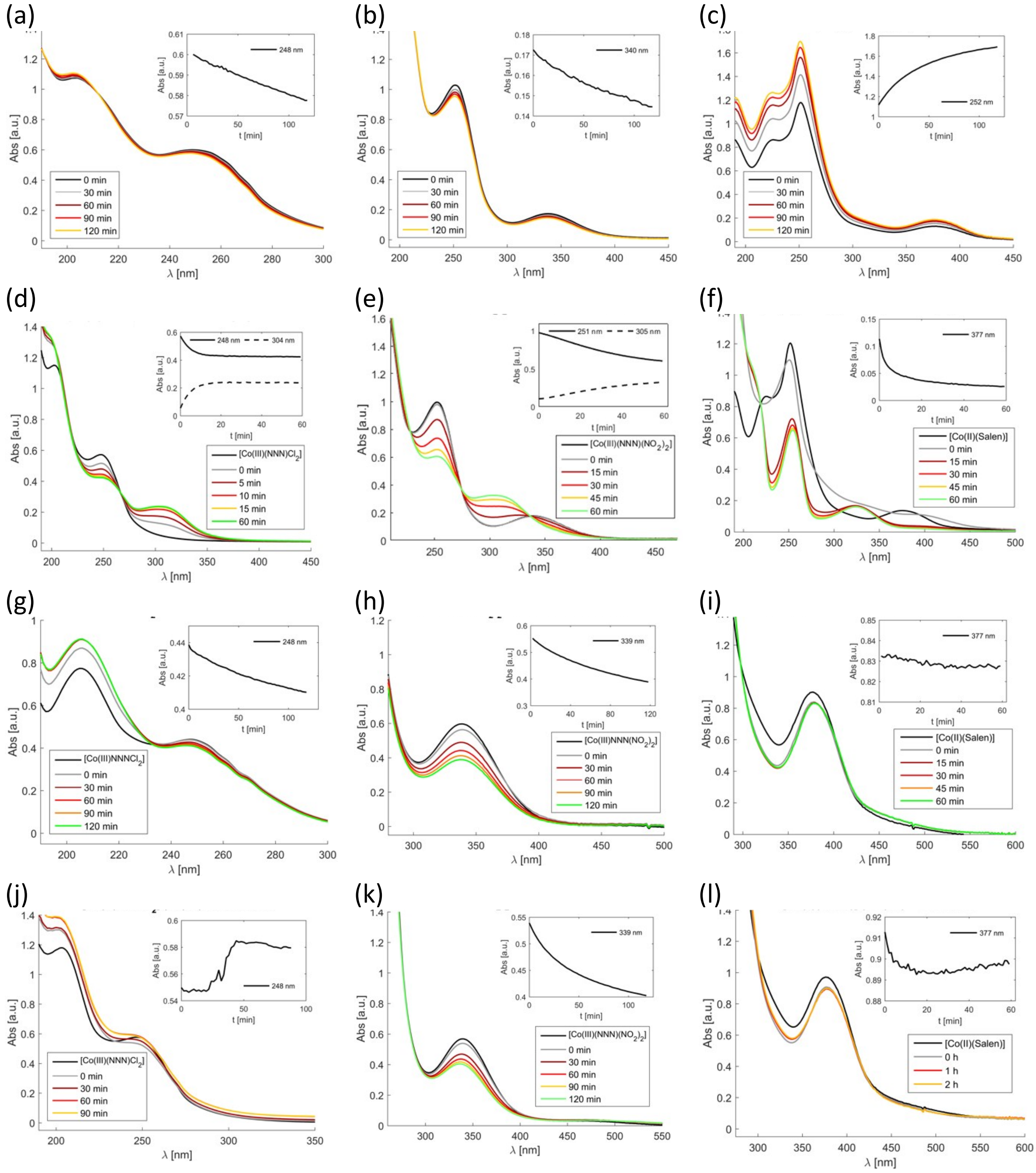


Figure S6. UV-Vis spectra of the complexes $[Co^{III}(NNN)Cl_2]^+$(1), $[Co(NNN)(NO_2)_2]^+$(2) and $[Co^{II}(Salen)]$(6) acquired in the kinetic mode reacting with model amino acids. Insets represents absorbance at definite wavelength with time. (a) 1 in aqueous solution (b) 2 in acetonitrile/water solution (c) 6 in acetonitrile water solution (d) 1 with nac (e) 2 with nac (f) 6 with nac (g) 1 with BOC-His (h) 2 with BOC-His (i) 6 with BOC-His (j) 1 with L-His (k) 2 with L-His (l) 6 with L-His.

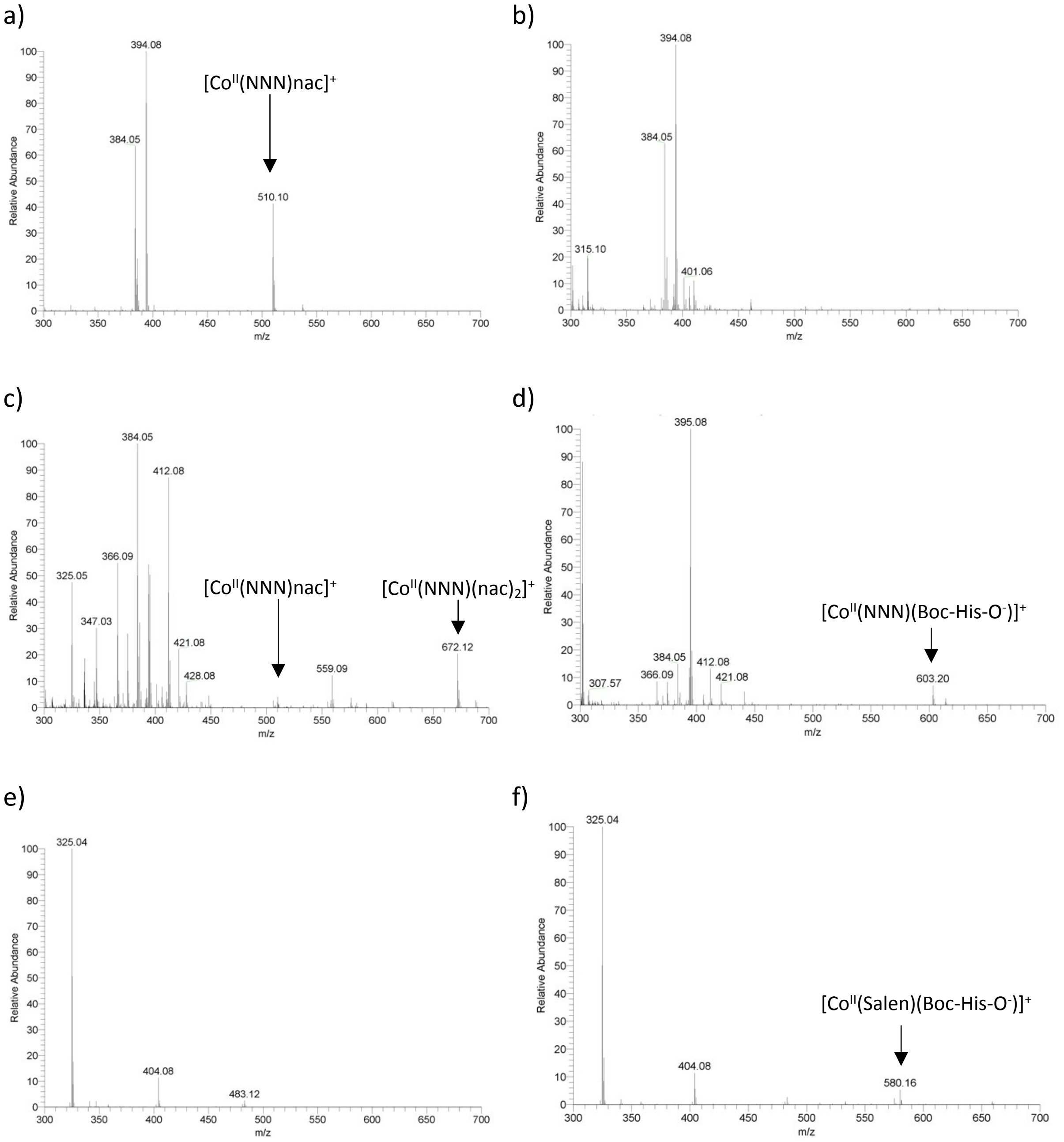
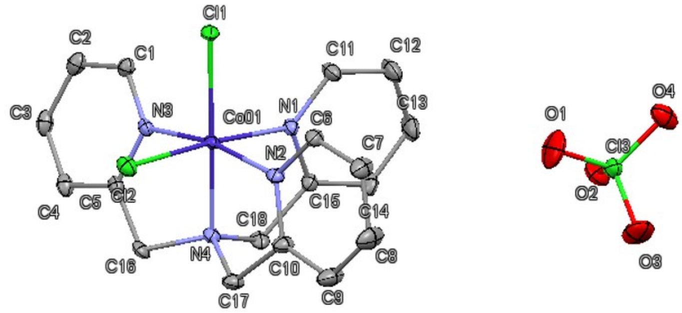
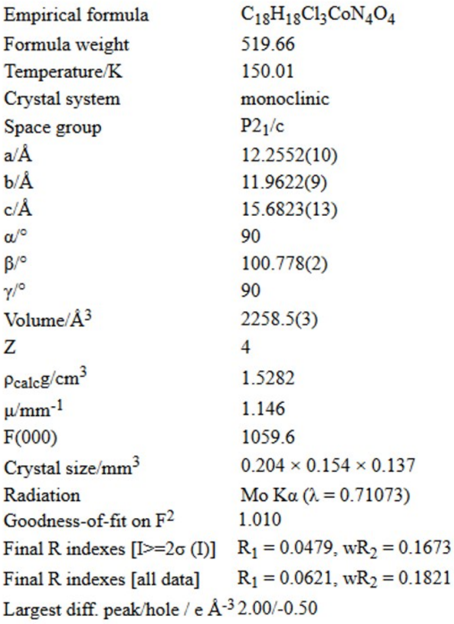


Figure S7. ESI-MS of compounds 1, 2 and 6 ([Co^{II}(Salen)] with model amino acids nac and Boc-L-His. a) 1+nac; b) 1+Boc-L-His; c) 2+nac; d) 2+Boc-L-His; e) 6+nac; f) 6+Boc-L-His





Empirical formula	$C_{18}H_{18}Cl_3CoN_4O_4$
Formula weight	519.66
Temperature/K	150.01
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	12.2552(10)
b/Å	11.9622(9)
c/Å	15.6823(13)
α/°	90
β/°	100.778(2)
γ/°	90
Volume/Å^3	2258.5(3)
Z	4
ρ_{calc}g/cm^3	1.5282
μ/mm^{-1}	1.146
F(000)	1059.6
Crystal size/mm^3	0.204 × 0.154 × 0.137
Radiation	Mo Kα (λ = 0.71073)
Goodness-of-fit on F^2	1.010
Final R indexes [I>=2σ (I)]	R_1 = 0.0479, wR_2 = 0.1673
Final R indexes [all data]	R_1 = 0.0621, wR_2 = 0.1821
Largest diff. peak/hole / e Å^{-3}	2.00/-0.50

Figure S8. ORTEP diagram of [Co^{III}(NNN)Cl$_2$]ClO$_4$ and crystallographic parameters.

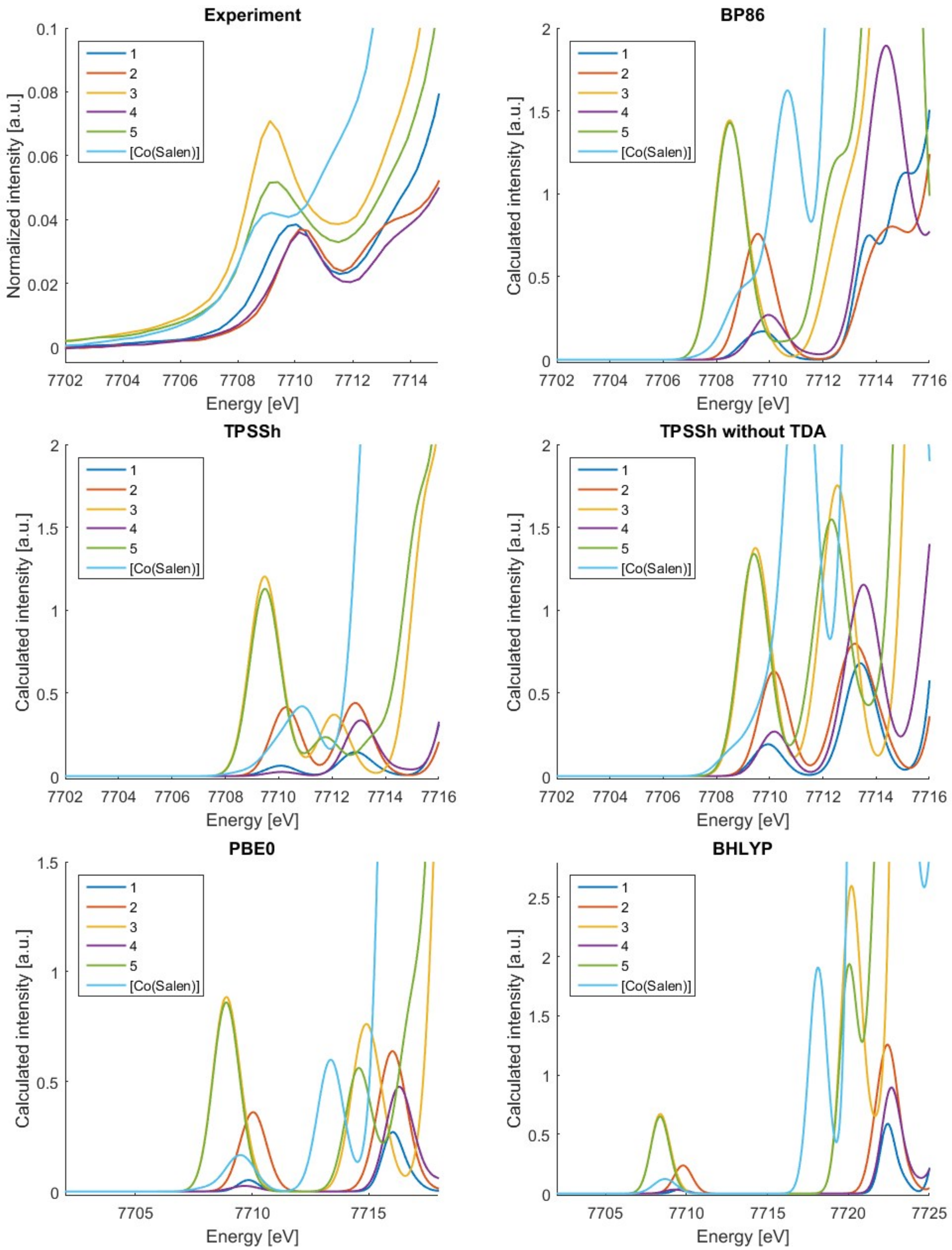


Figure S9. A comparison of the experimental and calculated XAS spectra using functionals of different Hartree-Fock exchange.

Table S1.Experimental and calculated pre-edge energies of Co-K-edge XAS spectra of Co complexes. The simulated spectra were performed by TDDFT with different functionals (chosen with variable amounts of HF exchange). The p1 represents 1s ⟶ 3d transition, p2 1s ⟶ π* and Δ = Ep2 – Ep1

		Experimental	**BP86**	**TPSSh**	**TPSSh (Without**	**PBE0**	**BHLYP**
[Co^{III}(NNN)Cl$_2$]ClO$_4$	**p1**	7709.9	7710.2	7709.7	7710.0	7710.1	7709.5
	p2	7714.1		7712.5	7713.5	7716.3	7723.3
	Δ	4.2		2.8	3.5	6.2	13.8
[Co^{III}(NNN)(NO$_2$)$_2$]ClO$_4$	**p1**	7710.3	7710.0	7709.9	7710.2	7710.3	7710.6
	p2	7713.4		7712.5	7713.3	7716.3	7723.2
	Δ	3.1		2.6	3.1	6.0	12.6
[Co^{II}(NNN)Cl]Cl	**p1**	7709.2	7709.0	7709.1	7709.5	7709.2	7709.2
	p2	7714.3		7711.7	7711.8	7715.2	7721.0
	Δ	5.1		2.6	2.3	6.0	11.8
[Co^{III}(NNO)(NO$_3$)$_2$]	**p1**	7710.2	7710.4	7709.7	7710.2	7710.0	7710.1
	p2	7713.8		7712.7	7713.6	7716.6	7723.5
	Δ	3.6		3.0	3.4	6.6	13.4
[Co^{II}(NNO)Cl]	**p1**	7709.3	7709.0	7709.1	7709.5	7709.1	7709.1
	p2	7714.3		7711.4	7712.4	7714.0	7719.9
	Δ	5.0		2.3	2.9	4.9	10.8
[Co^{II}(Salen)]	**p1**	7709.2	7709.4	7710.5	7708.6	7709.3	7709.5
	p2	7711.8		7713.2	7711.2	7713.6	7719.0
	Δ	2.6		2.7	2.6	4.3	9.5