Coordination Compounds of Nickel(II) with 3,5–Dibromo–Salicylaldehyde: Structure and Interaction with Biomolecules

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Supplementary material

Contents

S1. Protocols for interaction studies with CT DNA

The interaction of the complexes with CT DNA was investigated by UV–vis spectroscopy, and viscosity measurements and *via* the evaluation of their EB–displacing ability studied by fluorescence emission spectroscopy.

S1.1. Binding study with CT DNA by UV–vis spectroscopy

The interaction of complexes **1–3** with CT–DNA was studied by UV–vis spectroscopy in order to investigate the possible binding modes to CT DNA and to calculate the DNA–binding constants (K_b). Control experiments with DMSO were performed and no changes in the spectra of CT DNA were observed. The value of K_b can be obtained by monitoring the changes in the absorbance at the corresponding λ_{max} in the UV–vis spectra of each complex (1×10^{-4} μM) recorded with increasing concentrations of CT DNA (diverse *r* values) and it is given by the ratio of slope to the y intercept in plots [DNA]/($\varepsilon_A-\varepsilon_f$) *versus* [DNA], according to the Wolfe–Shimer equation [1]:

$$\frac{[DNA]}{(\varepsilon_A-\varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b-\varepsilon_f)} + \frac{1}{K_b(\varepsilon_b-\varepsilon_f)} \qquad \text{(Equation S1)}$$

where [DNA] is the concentration of DNA in base pairs, $\varepsilon_A = A_{obsd}/[compound]$, ε_f = the extinction coefficient for the free compound and ε_b = the extinction coefficient for the compound in the fully bound form.

S1.2. CT DNA–binding studies by viscosity measurements

The viscosity of DNA ([DNA] = 0.1 mM) in buffer solution was measured in the presence of increasing amounts of complexes **1–3** (up to the value $r = 0.36$). All measurements were performed at room temperature. The obtained data are presented as $(\eta/\eta_0)^{1/3}$ *versus r*, where η is the viscosity of DNA in the presence of the compound, and η_0 is the viscosity of DNA alone in buffer solution.

S1.3 EB–displacement studies

The competition of complexes **1–3** with EB was investigated by fluorescence emission spectroscopy in order to examine whether the complexes can displace EB from its DNA–EB conjugate. The DNA–EB conjugate was prepared by adding 20 μM EB and 26 μM CT DNA in buffer solution. The possible intercalating effect of the complexes was studied by the addition of a certain amount of a solution of the compound into a solution of the DNA–EB conjugate. The influence of each compound to the DNA–EB complex solution was obtained by recording the changes of the

fluorescence emission spectra with excitation wavelength (λ_{ex}) at 540 nm [2]. Complexes **1–3** do not show any appreciable fluorescence emission bands at room temperature in solution or in the presence of CT DNA or EB under the same experimental conditions (λ_{ex} = 540 nm); therefore, the observed quenching of the EB–DNA solution may be attributed to the displacement of EB from its EB–DNA conjugate.

The Stern–Volmer constant K_{SV} is used to evaluate the quenching efficiency for each compound according to the Stern–Volmer equation (equation S2) [2],

$$\frac{Io}{I} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q] \qquad \text{(Equation S2)},$$

where Io = the initial tryptophan fluorescence intensity of EB-DNA, I = the tryptophan fluorescence intensity of EB-DNA after the addition of the quencher, K_q = the quenching constants of EB-DNA, K_{SV} = the Stern–Volmer constant, τ_o = the average lifetime of EB-DNA without the quencher, [Q] = the concentration of the quencher (i.e. complexes **1–3**). The value of K_{SV} (M^{-1}) can be obtained by the slope of the diagram Io/I *versus* [Q]. Taking τ_o = 23 ns as the fluorescence lifetime of the EB–DNA system [3], the EB–DNA quenching constants (K_q, in $M^{-1}s^{-1}$) of the compounds can be determined according to equation S3:

$$K_{SV} = K_q \tau_o \qquad \text{(Equation S3)}$$

S2. Protocols for interaction studies with serum albumins

The albumin–binding study for complexes **1–3** was performed by fluorescence emission quenching experiments using BSA (3 μM) or HSA (3 μM) in buffer solution. The quenching of the emission intensity of tryptophan residues of BSA at 343 nm or HSA at 340 nm was monitored using complexes **1–3** as quenchers with increasing concentration [2]. The fluorescence emission spectra were recorded in the range 300–500 nm with excitation wavelength of 295 nm. The quantitative studies of the serum albumin fluorescence spectra were performed after correction by subtracting the spectra of the compounds.

The extent of the inner–filter effect can be roughly estimated with the following equation:

$$I_{corr} = I_{meas} \times 10^{\frac{\varepsilon(\lambda_{exc})cd}{2}} \times 10^{\frac{\varepsilon(\lambda_{em})cd}{2}} \qquad \text{(Equation S4)}$$

where I_{corr} = corrected intensity, I_{meas} = the measured intensity, c = the concentration of the quencher, d = the cuvette (1 cm), $\varepsilon(\lambda_{exc})$ and $\varepsilon(\lambda_{em})$ = the ε of the quencher at the excitation and the emission wavelength, respectively, as calculated from the UV–vis spectra of the complexes [4].

The Stern–Volmer and Scatchard graphs are used to study the interaction of a quencher with serum albumins [2]. According to Stern–Volmer quenching equation (Equation S2) [2], where Io =

the initial tryptophan fluorescence intensity of SA, I = the tryptophan fluorescence intensity of SA after the addition of the quencher, K_q = the quenching constants of SA, K_{SV} = the Stern–Volmer constant, τ_o = the average lifetime of SA without the quencher, [Q] = the concentration of the quencher, the value of K_{SV} (M^{-1}) can be obtained by the slope of the diagram Io/I *versus* [Q]. Taking $\tau_o = 10^{-8}$ s as fluorescence lifetime of tryptophan in SA, the value of K_q ($M^{-1}s^{-1}$) is calculated from the equation S3.

From the Scatchard equation [5]:

$$\frac{\Delta I/_{Io}}{[Q]} = nK - K\frac{\Delta I}{Io} \qquad \text{(Equation S5)}$$

where n is the number of binding sites per albumin and K is the SA–binding constant, K (in M^{-1}) is calculated from the slope in plots (ΔI/Io)/[Q] *versus* (ΔI/Io) and n is given by the ratio of y intercept to the slope [5] .

References

1. Wolfe, A.; Shimer, G.H.; Meehan, T. Polycyclic Aromatic Hydrocarbons Physically Intercalate into Duplex Regions of Denatured DNA. *Biochemistry* **1987**, *26*, 6392–6396, doi:10.1021/BI00394A013/ASSET/BI00394A013.FP.PNG_V03.
2. Lakowicz, J.R. *Principles of Fluorescence Spectroscopy*; Springer, 2006; ISBN 0387312781.
3. Heller, D.P.; Greenstock, C.L. Fluorescence Lifetime Analysis of DNA Intercalated Ethidium Bromide and Quenching by Free Dye. *Biophys Chem* **1994**, *50*, 305–312, doi:10.1016/0301-4622(93)E0101-A.
4. Stella, L.; Capodilupo, A.L.; Bietti, M. A Reassessment of the Association between Azulene and [60]Fullerene. Possible Pitfalls in the Determination of Binding Constants through Fluorescence Spectroscopy. *Chemical Communications* **2008**, 4744–4746, doi:10.1039/b808357f.
5. Wu, S.S.; Yuan, W.B.; Wang, H.Y.; Zhang, Q.; Liu, M.; Yu, K.B. Synthesis, Crystal Structure and Interaction with DNA and HSA of (N,N′-Dibenzylethane-1,2-Diamine) Transition Metal Complexes. *J Inorg Biochem* **2008**, *102*, 2026–2034, doi:10.1016/j.jinorgbio.2008.08.005.

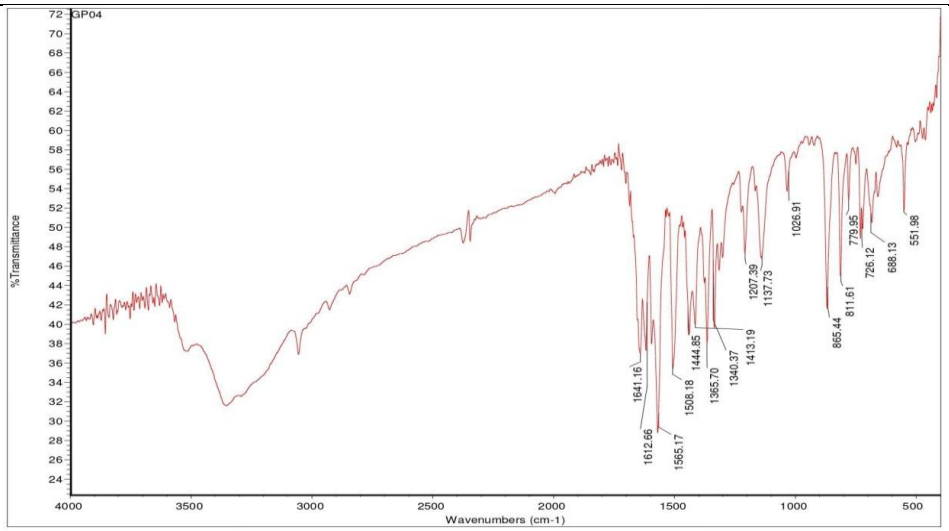
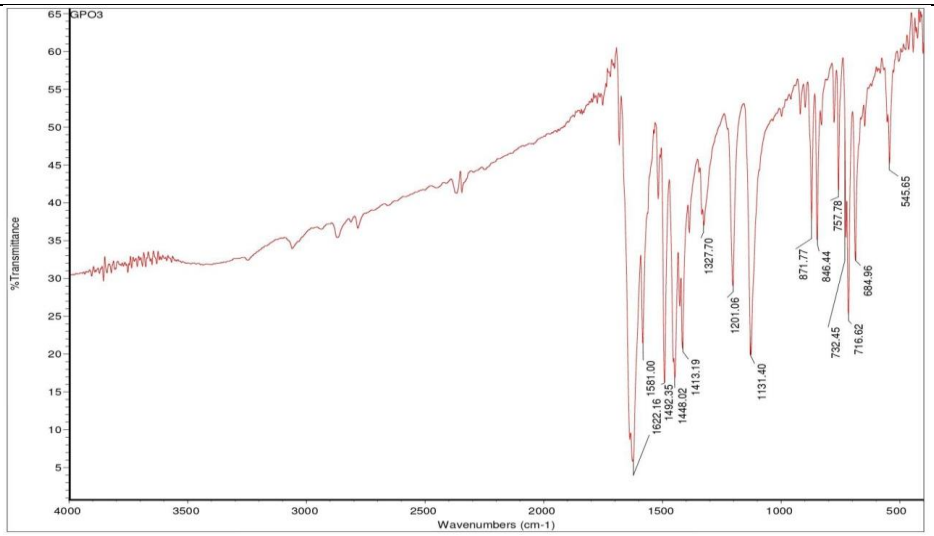
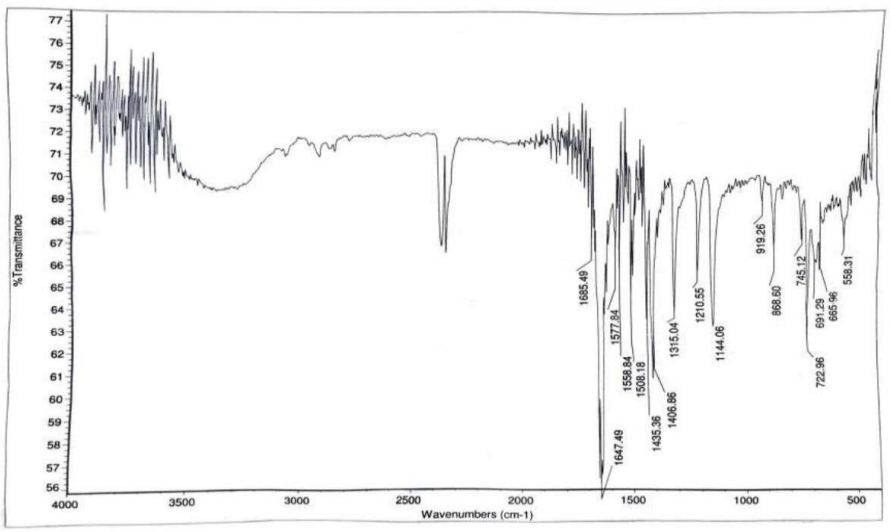
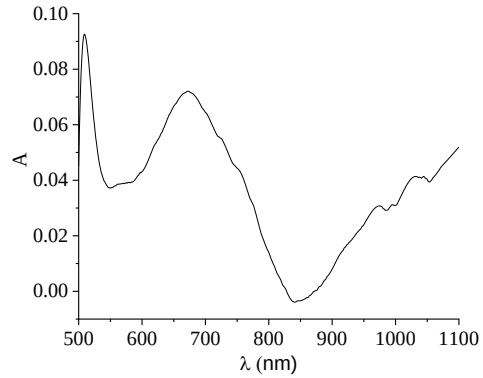
Complex **1**	
Complex **2**	
Complex **3**	

Figure S1. IR spectra of complexes **1–3**.



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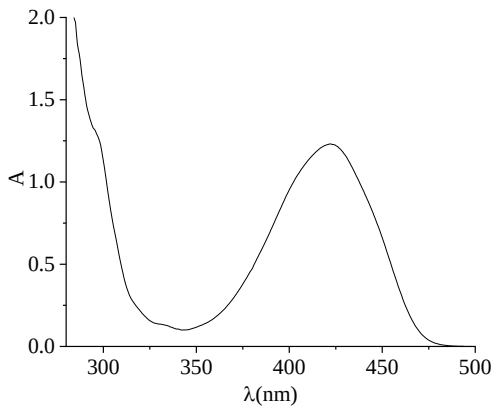

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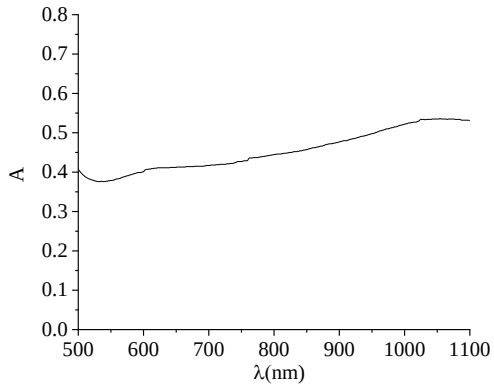

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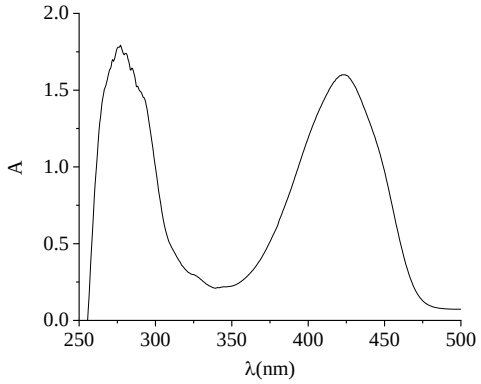

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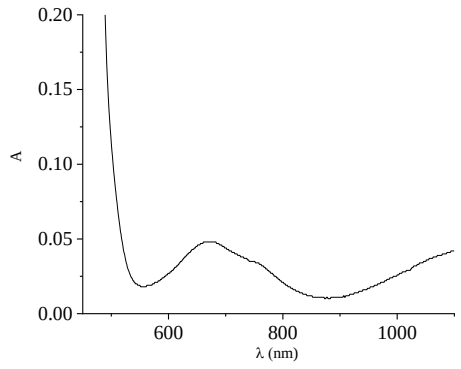
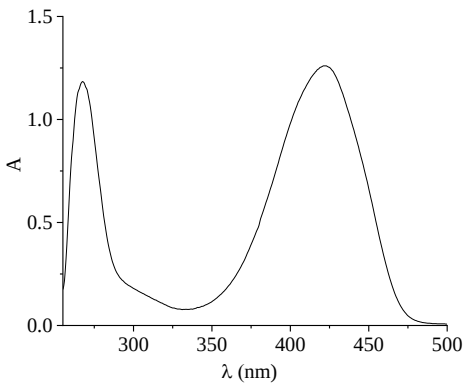
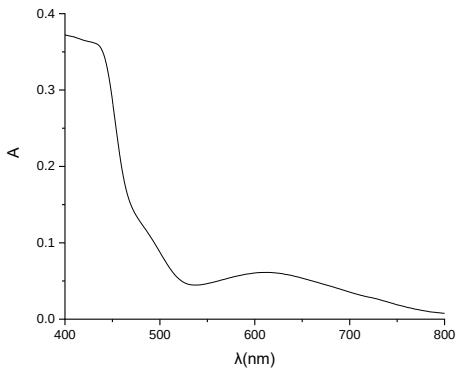


Complex **3 (5×10^{-3} M)**



Complex **3 (10^{-4} M)**

Figure S2. UV-vis spectra of complexes **1–3** in DMSO solution.



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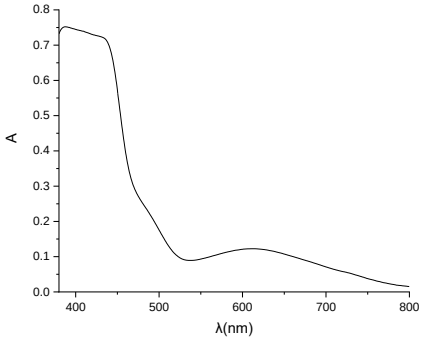

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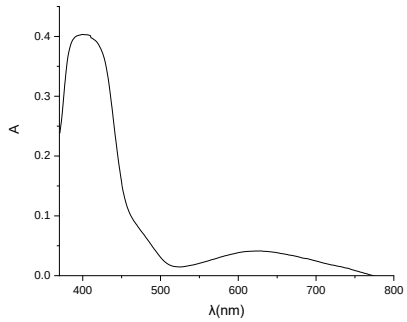
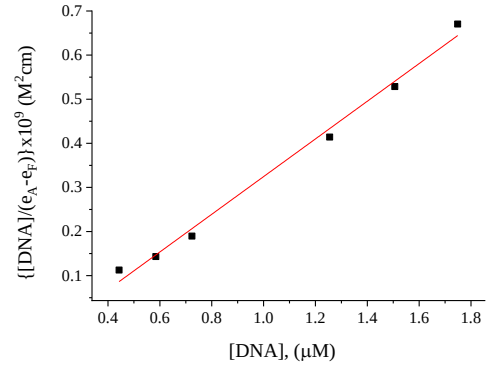


Complex **3**

Figure S3. UV-vis spectra of complexes **1–3** in solid state.



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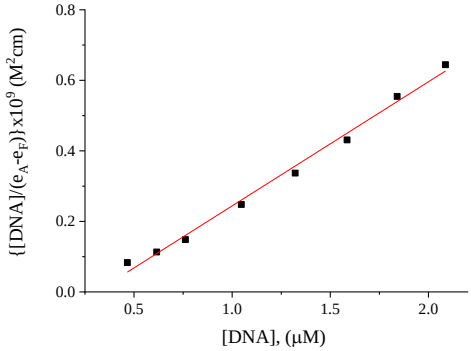

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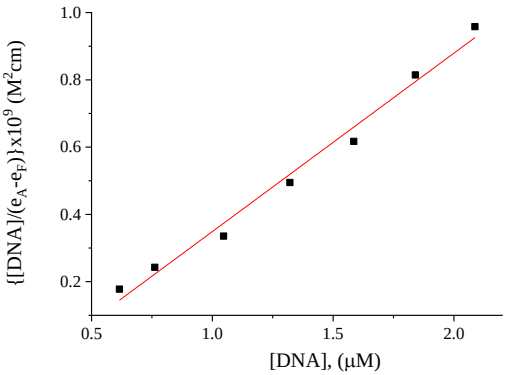
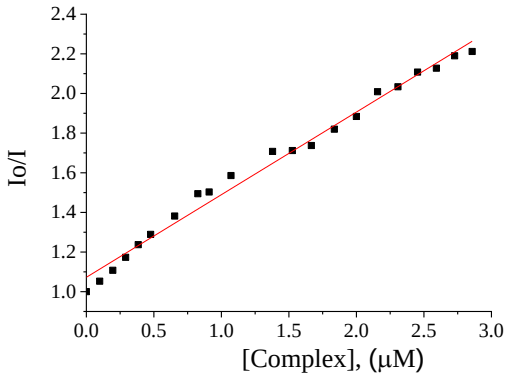


Complex **3**

Figure S4. Plot of $\frac{[DNA]}{(\varepsilon_A - \varepsilon_f)}$ *versus* [DNA] for complexes **1–3**.



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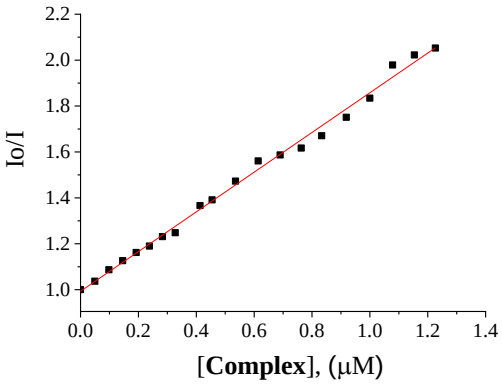

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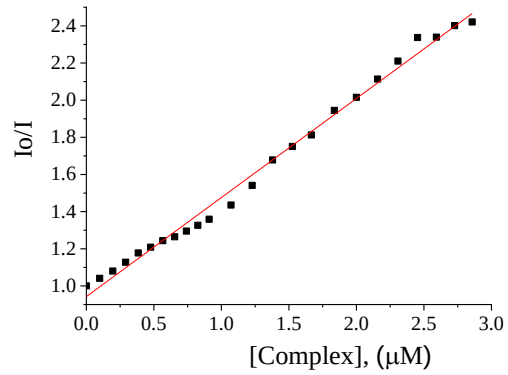
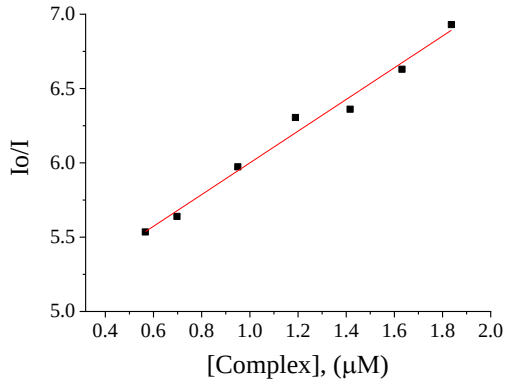


Complex **3**

Figure S5. Stern–Volmer quenching plot of EB–DNA fluorescence for complexes **1–3.**



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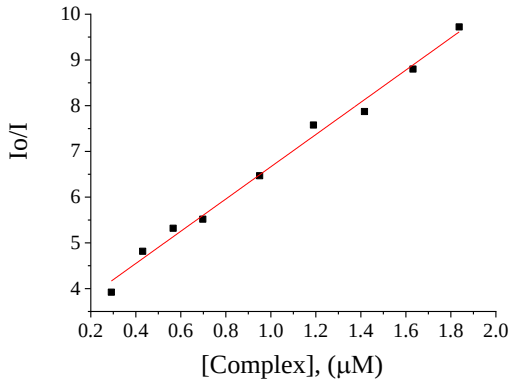

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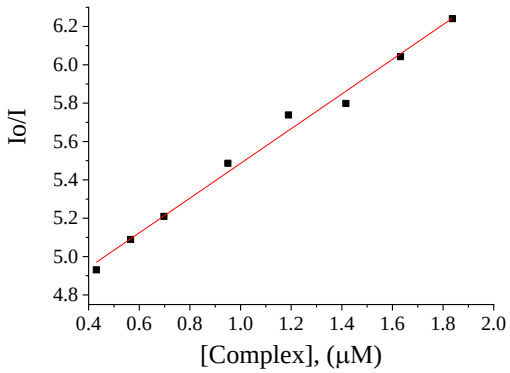

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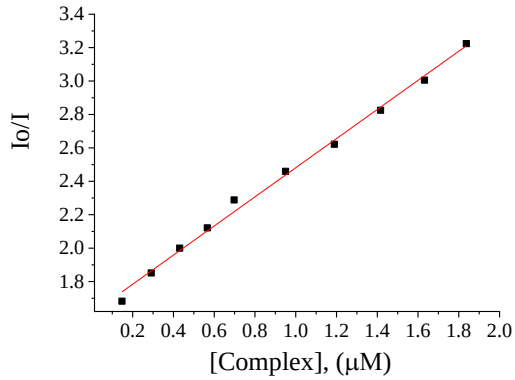
Figure S6. Stern–Volmer quenching plot of BSA fluorescence for complexes **1–3**.



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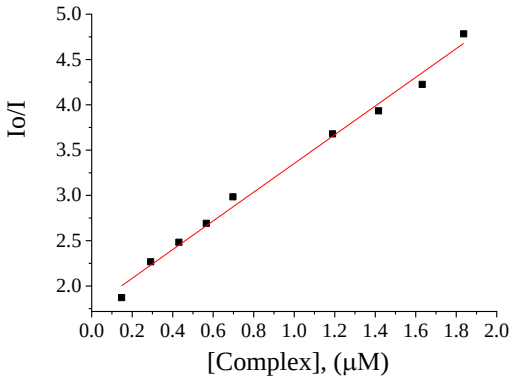

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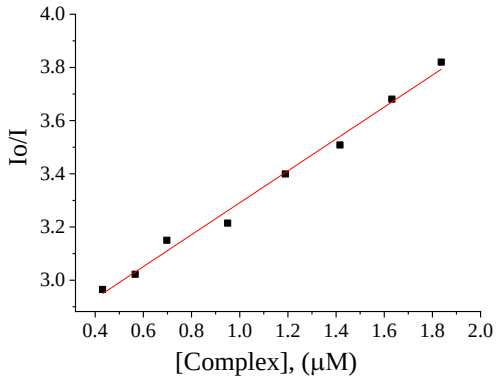
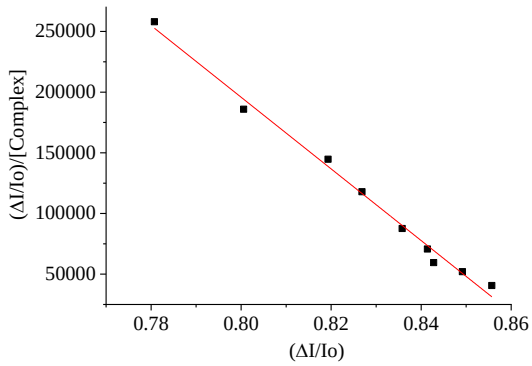


Complex **3**

Figure S7. Stern–Volmer quenching plot of HSA fluorescence for complexes **1–3**.



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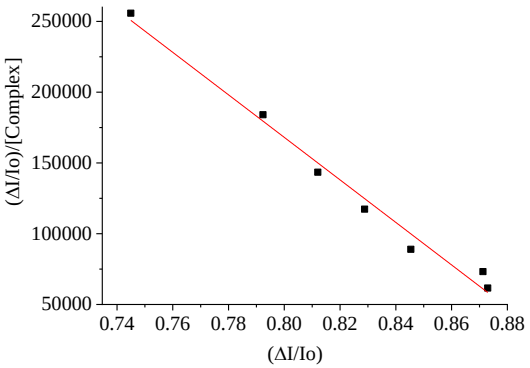

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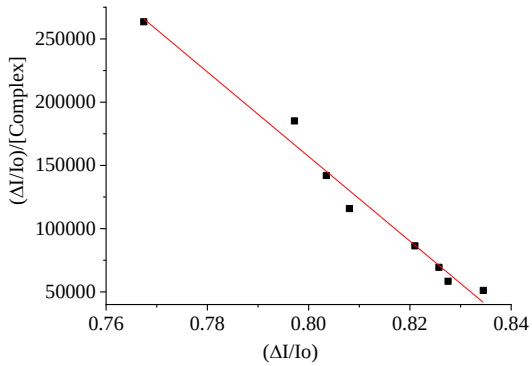
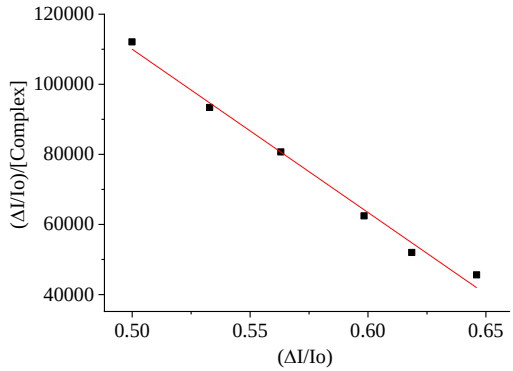


Complex **3**

Figure S8. Scatchard plot of BSA of complexes **1–3.**



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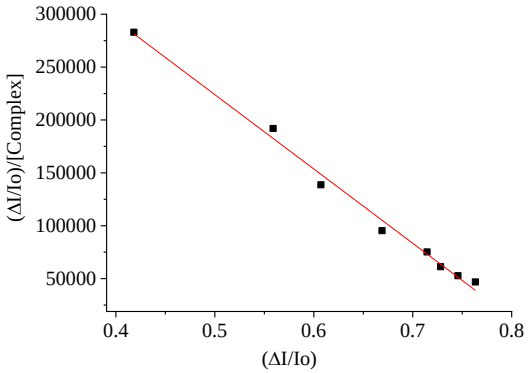

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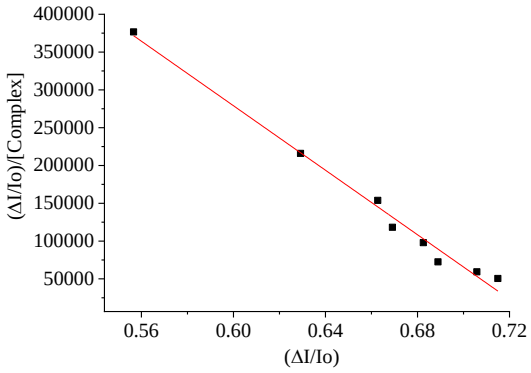

Complex **3**

Figure S9. Scatchard plot of HSA of complexes **1–3.**