

Supporting information
for

**DICARBOXYL-TERMINATED IRON(II) CLATHROCHELATES AS CD-
REPORTERS FOR A SERIES OF GLOBULAR PROTEINS**

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1. NMR spectra

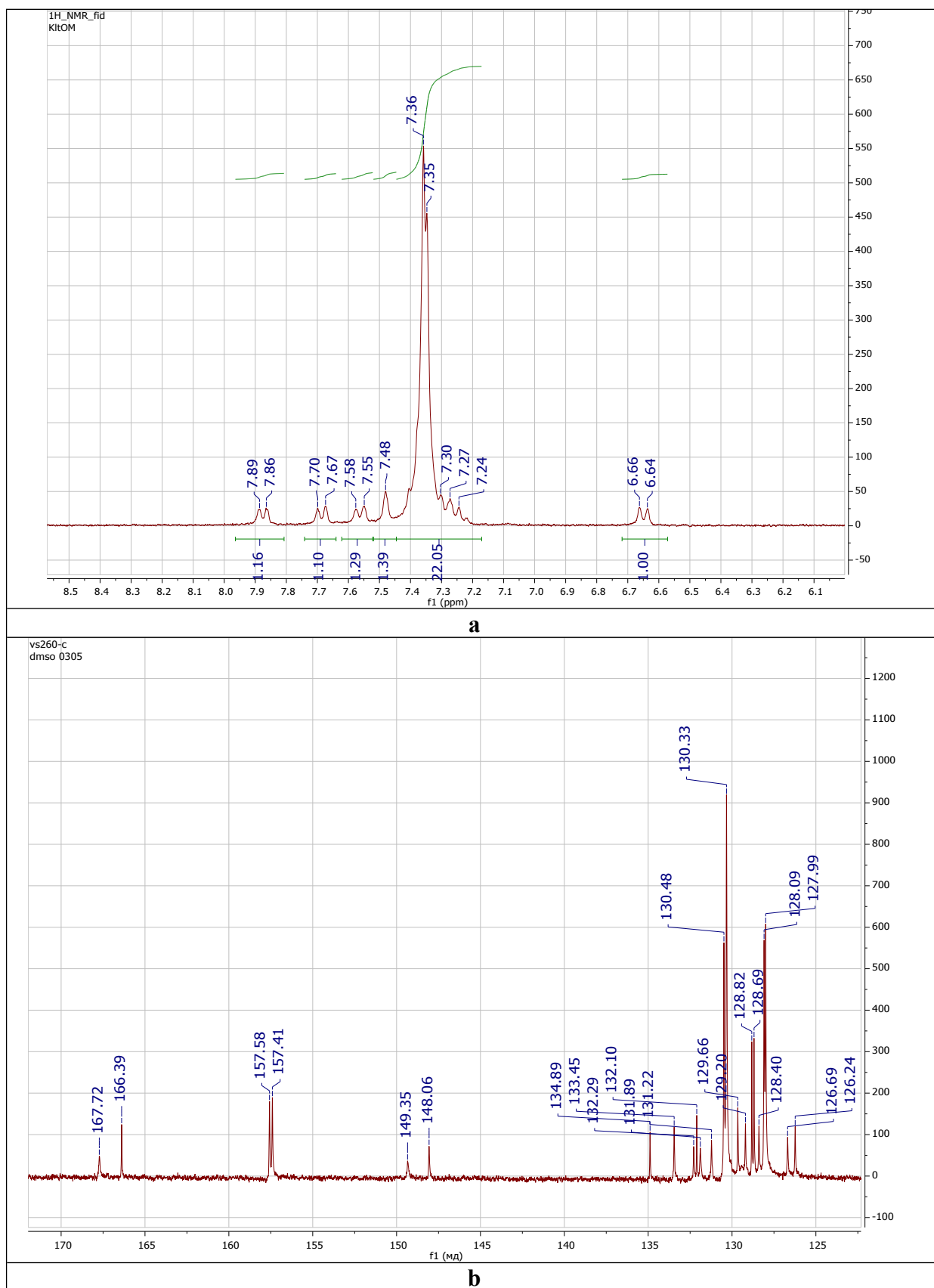
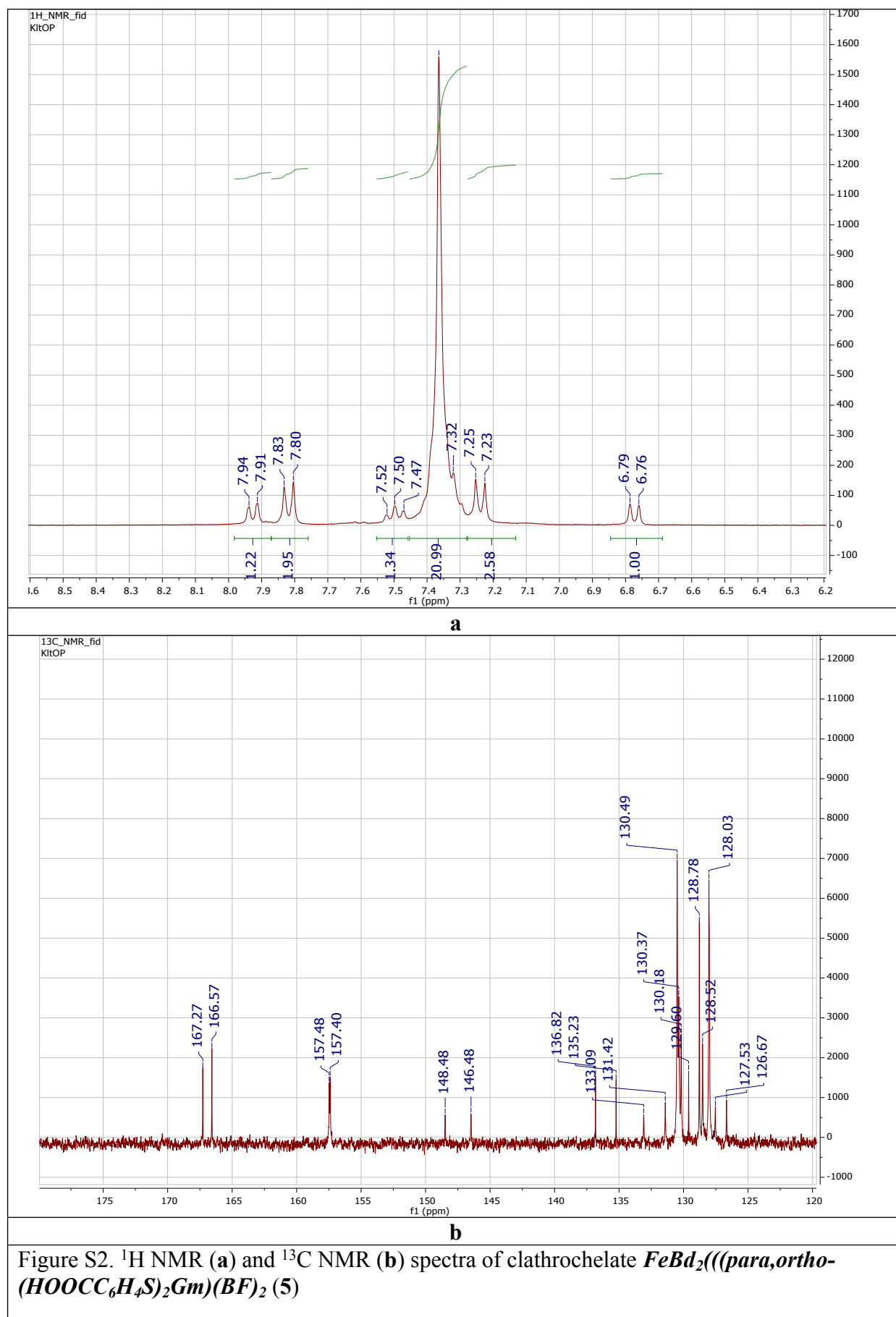
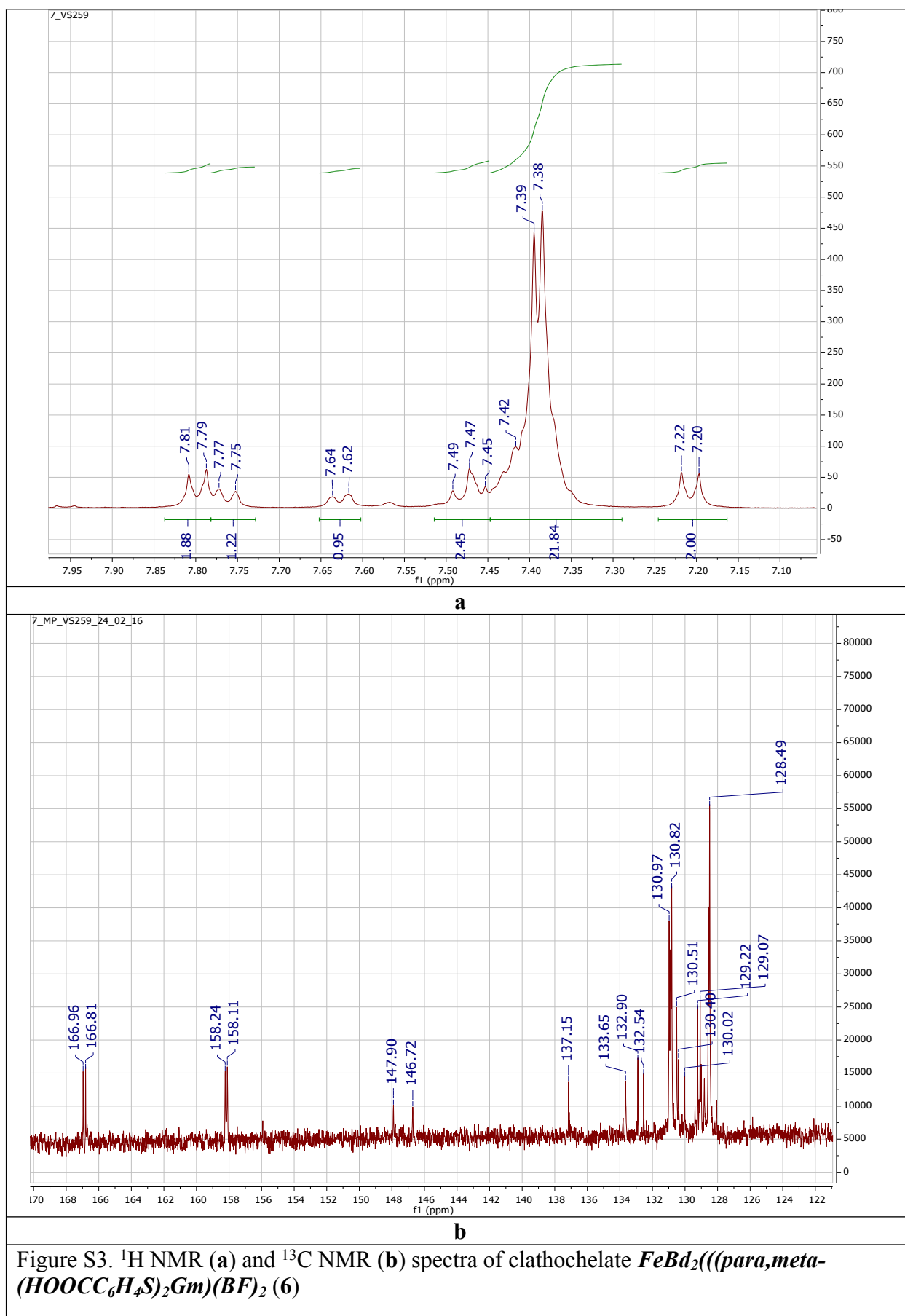


Figure S1. ^1H NMR (a) and ^{13}C NMR (b) spectra of clathrochelate $\text{FeBd}_2(((\text{meta},\text{ortho}-(\text{HOCC}_6\text{H}_4\text{S})_2\text{Gm})(\text{BF})_2$ (4)





2. Protein fluorescence quenching curves

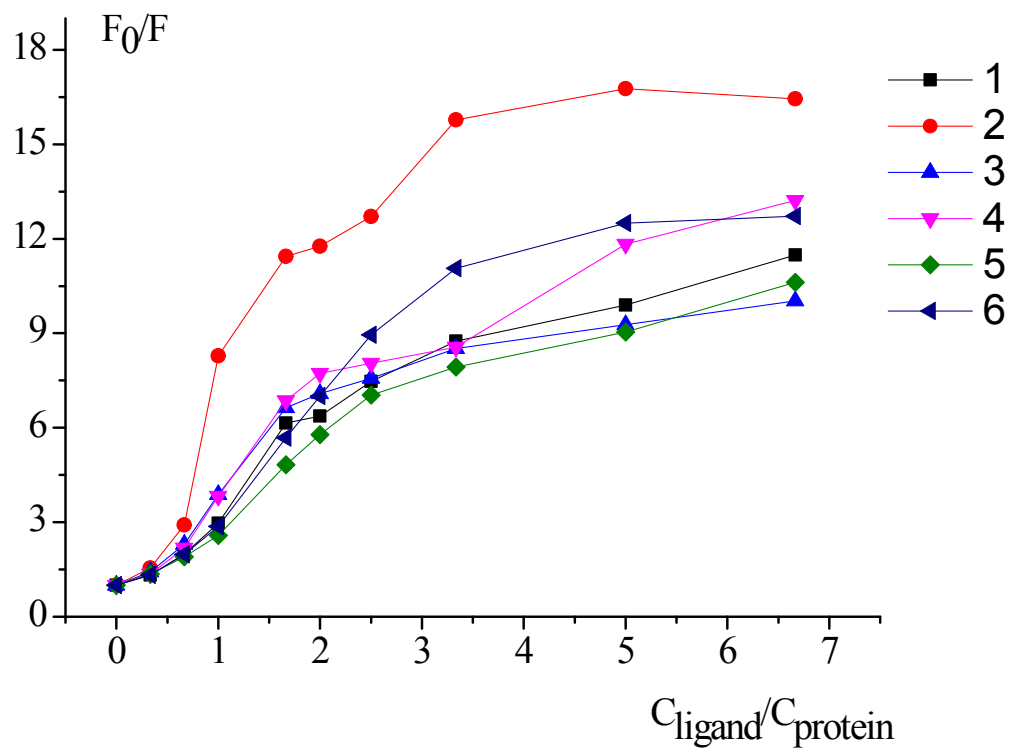


Figure S4. Stern–Volmer plots of a fluorescence quenching of **BSA** by the complexes **1** – **6**, excitation at 280 nm, emission at 344 nm, $c_{\text{protein}} = 3 \mu\text{M}$, 0.05M Tris–HCl buffer with pH 7.9 used as a solvent at 25° C

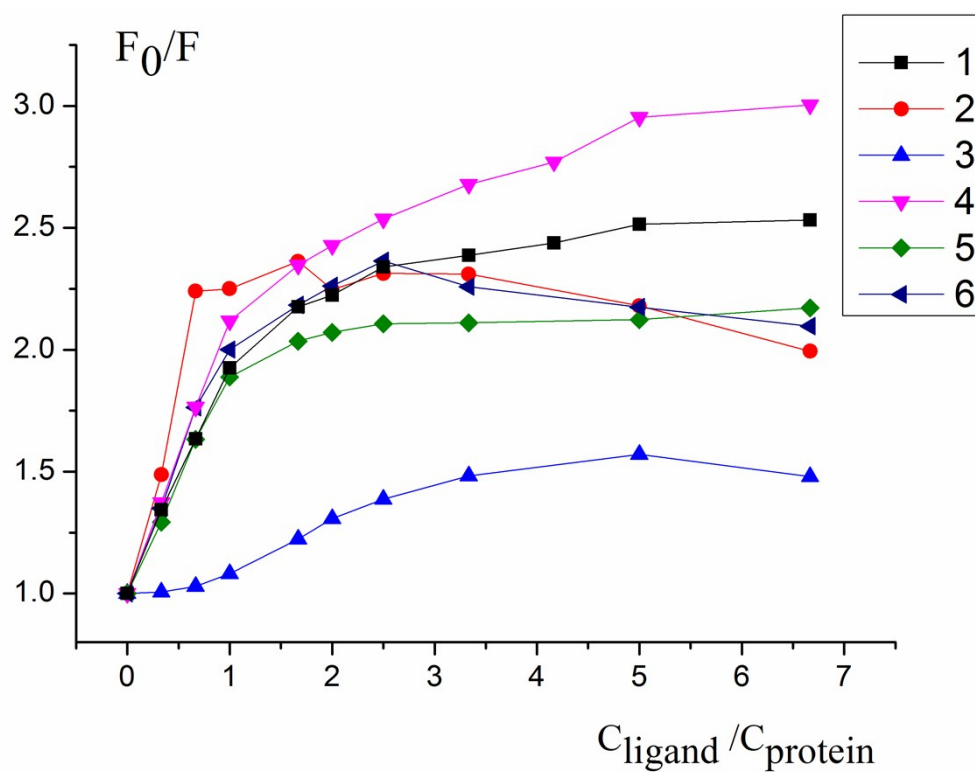


Figure S5. Stern–Volmer plots of a fluorescence quenching of **HSA** by the complexes **1** – **6**, $\text{ex}=280\text{ nm}$, emission at 353 nm , $c_{\text{protein}} = 3\text{ }\mu\text{M}$, 0.05M Tris–HCl buffer with pH 7.9 used as a solvent at 25°C

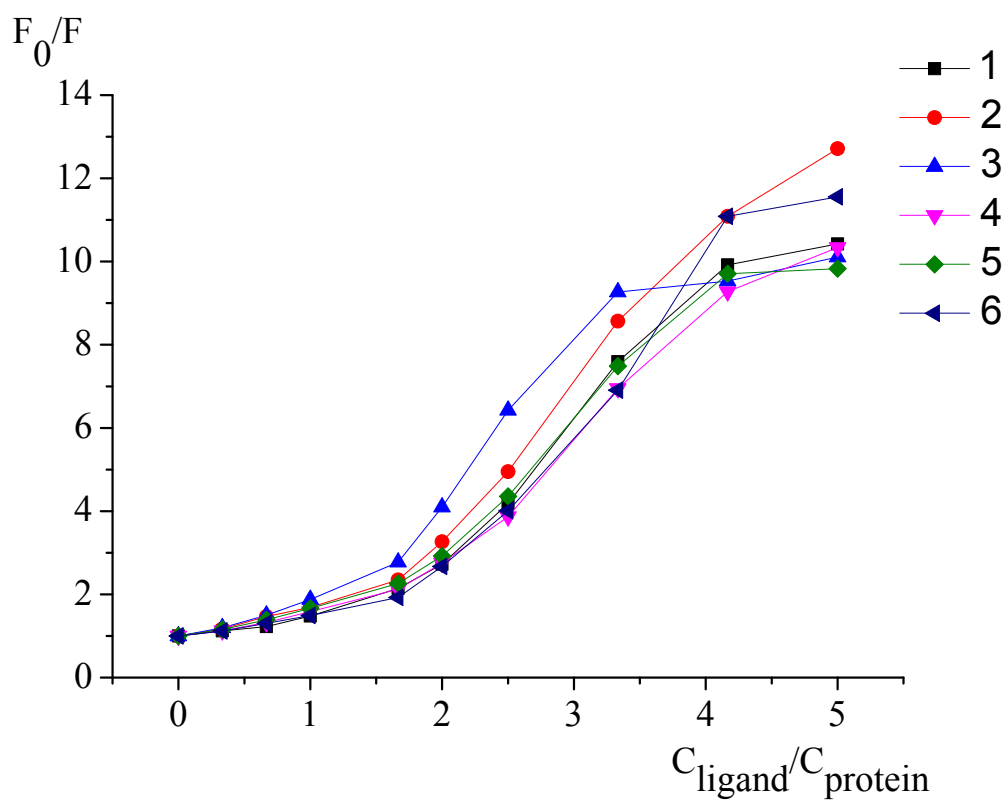


Figure S6. Stern–Volmer plots of a fluorescence quenching of **LYZ** by the complexes **1** – **6** λ_{ex} =280 nm, emission at 344 nm, $c_{\text{protein}} = 3 \mu\text{M}$, 0.05M Tris–HCl buffer with pH 7.9 used as a solvent at 25° C.

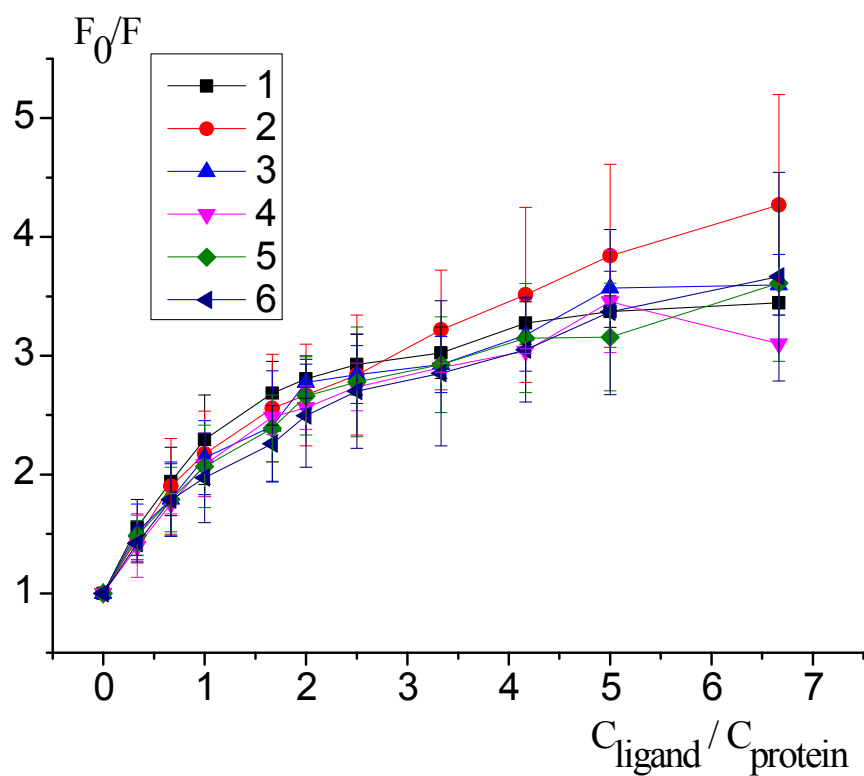


Figure S7. Stern–Volmer plots of a fluorescence quenching of **BLG** by the complexes **1** – **6**, $\text{ex}=280\text{ nm}$, emission at 336 nm , $c_{\text{protein}} = 3\text{ }\mu\text{M}$, 0.05M Tris–HCl buffer with pH 7.9 used as a solvent at 25°C .

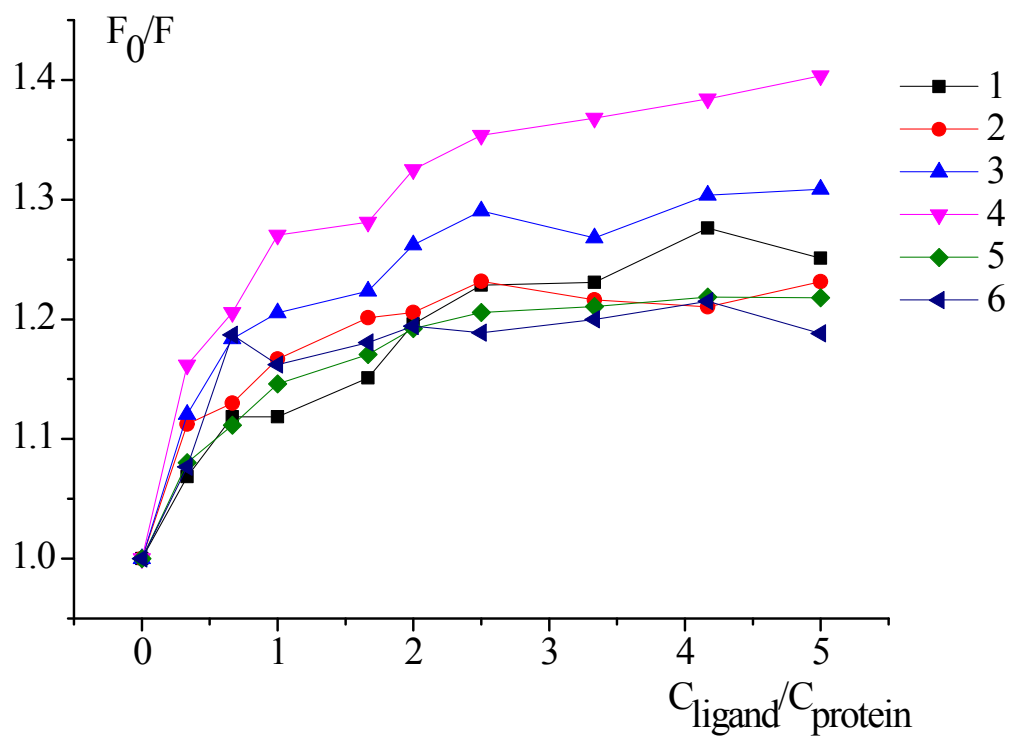
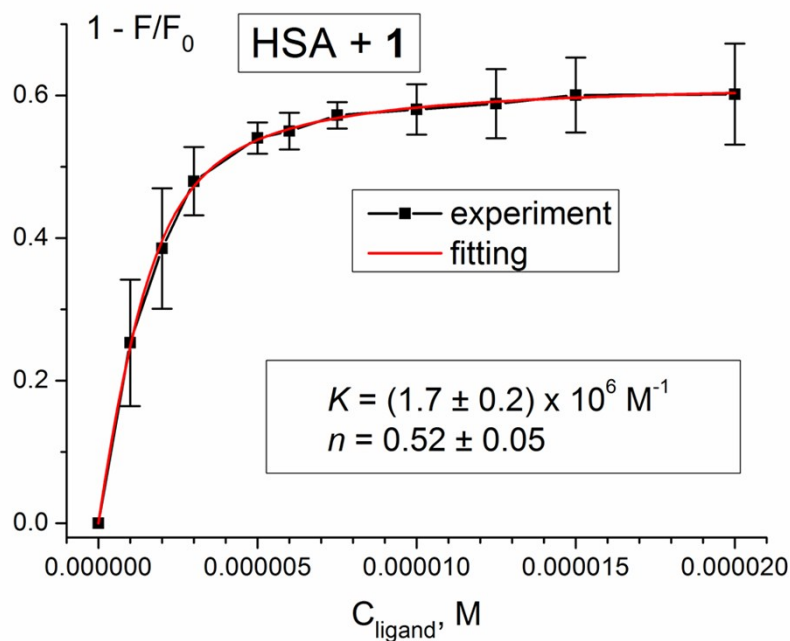
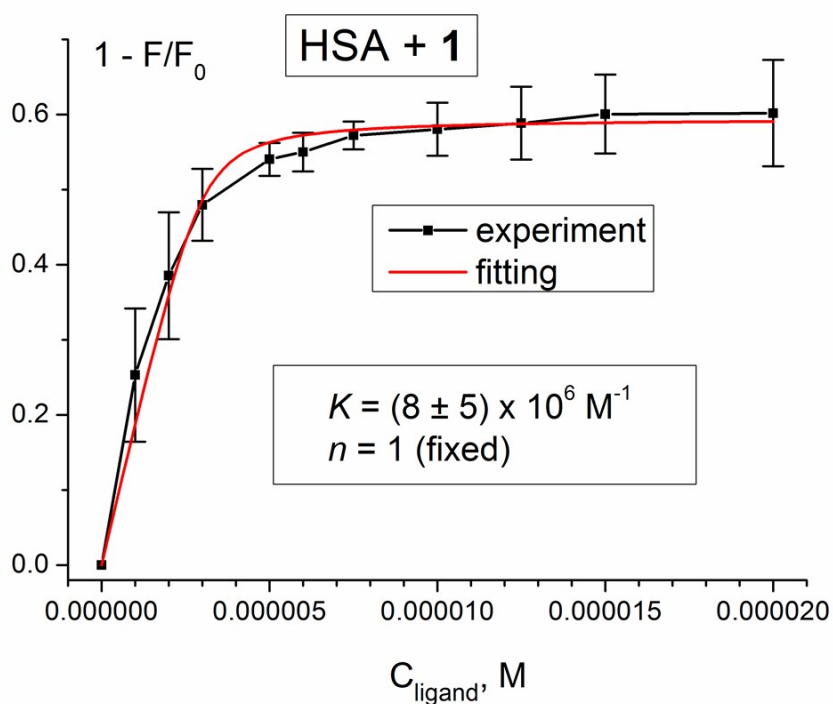


Figure S8. Stern–Volmer plots of a fluorescence quenching of **trypsin** by the complexes **1** – **6** $\text{ex}=280\text{ nm}$, emission at 343 nm , $c_{\text{protein}} = 3\text{ }\mu\text{M}$, 0.05M Tris–HCl buffer with pH 7.9 used as a solvent at 25°C



a



b

Fig. S9. The plots of $(1 - F/F_0)$ on the concentration of the clathrochelate (C_{ligand}) for the titration of HSA (3 μM) with the compound 1, and the fit of this plot with the dependence (5) (see below, Section 5 of the SI). 0.05M Tris-HCl buffer with pH 7.9 used as a solvent. Experiment performed three times, average values of $(1 - F/F_0)$ are presented; error bars correspond to confidence level of 0.95. K is found as the parameter of fitting; n is found as the parameter of fitting (a), and is fixed as 1 (b).

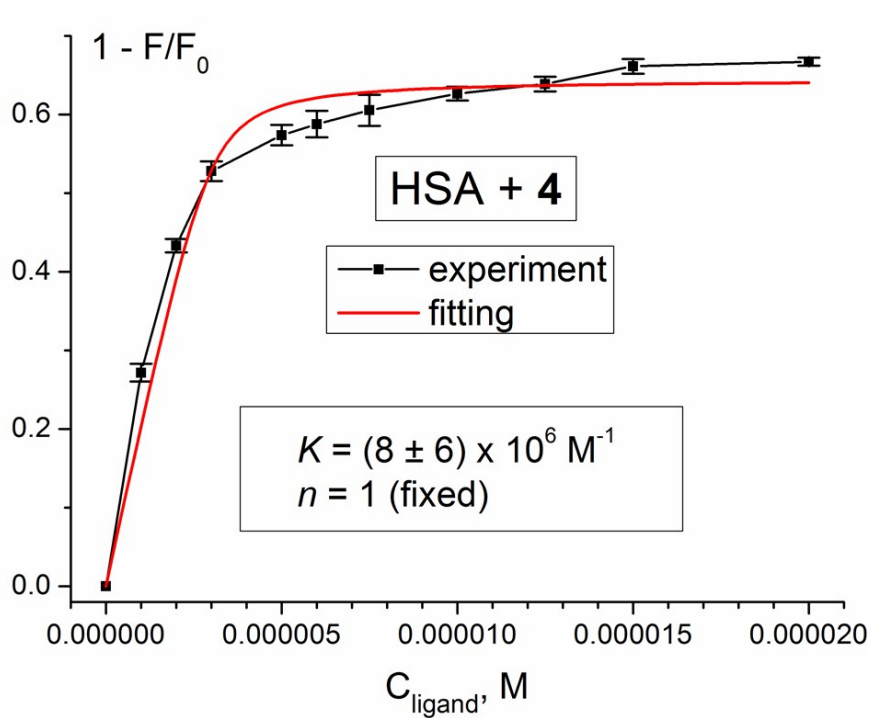
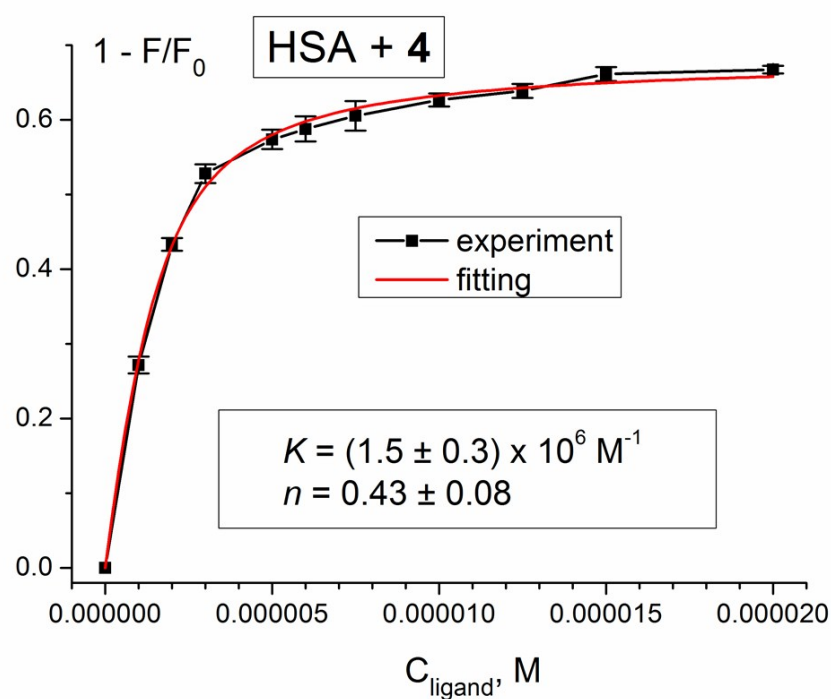
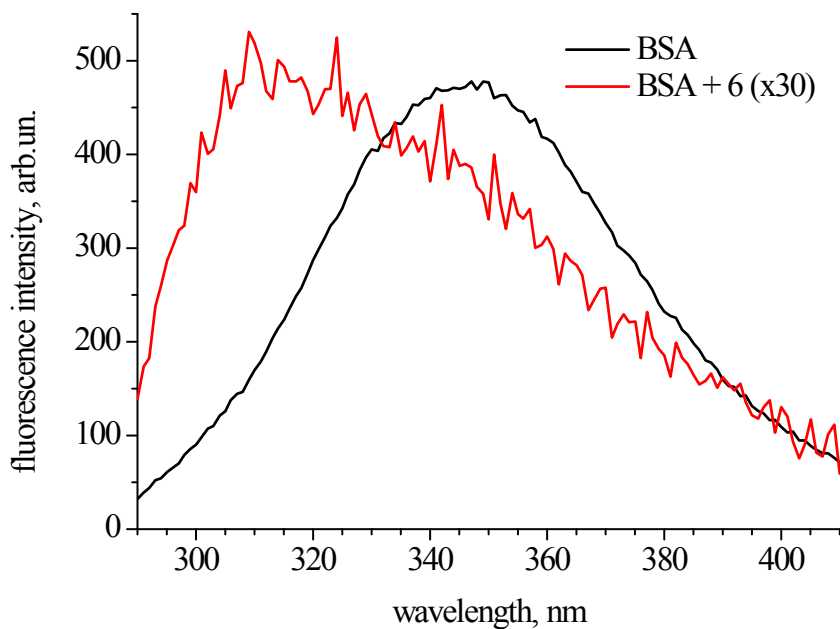
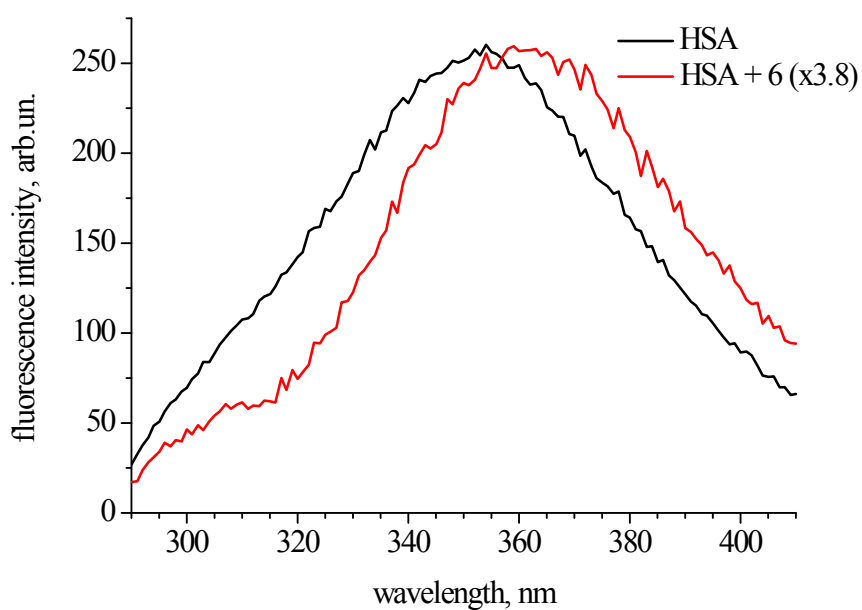


Fig. S10. The plots of $(1 - F/F_0)$ on the concentration of the clathrochelate (C_{ligand}) for the titration of HSA (3 μM) with the compound **4**, and the fit of this plot with the dependence (5) (see below, Section 5 of the SI). 0.05M Tris-HCl buffer with pH 7.9 used as a solvent. Experiment performed three times, average values of $(1 - F/F_0)$ are presented; error bars correspond to confidence level of 0.95. K is found as the parameter of fitting; n is found as the parameter of fitting (a), and is fixed as 1 (b).

3. Fluorescence spectra



a



b

Figure S11. Fluorescence spectra of BSA at excitation wavelength of 280 nm (**a**) and of HSA (**b**) in the absence and in the presence of the clathrochelate **6**. $c_{protein} = 3 \cdot 10^{-5} \text{ mol} \cdot \text{l}^{-1}$, $c_{clt} = 10 \cdot 10^{-5} \text{ mol} \cdot \text{l}^{-1}$, 0.05M Tris-HCl buffer with pH 7.9. The spectra quenched by **6** were normalized to the initial, non-quenched, spectra.

4. CD spectra

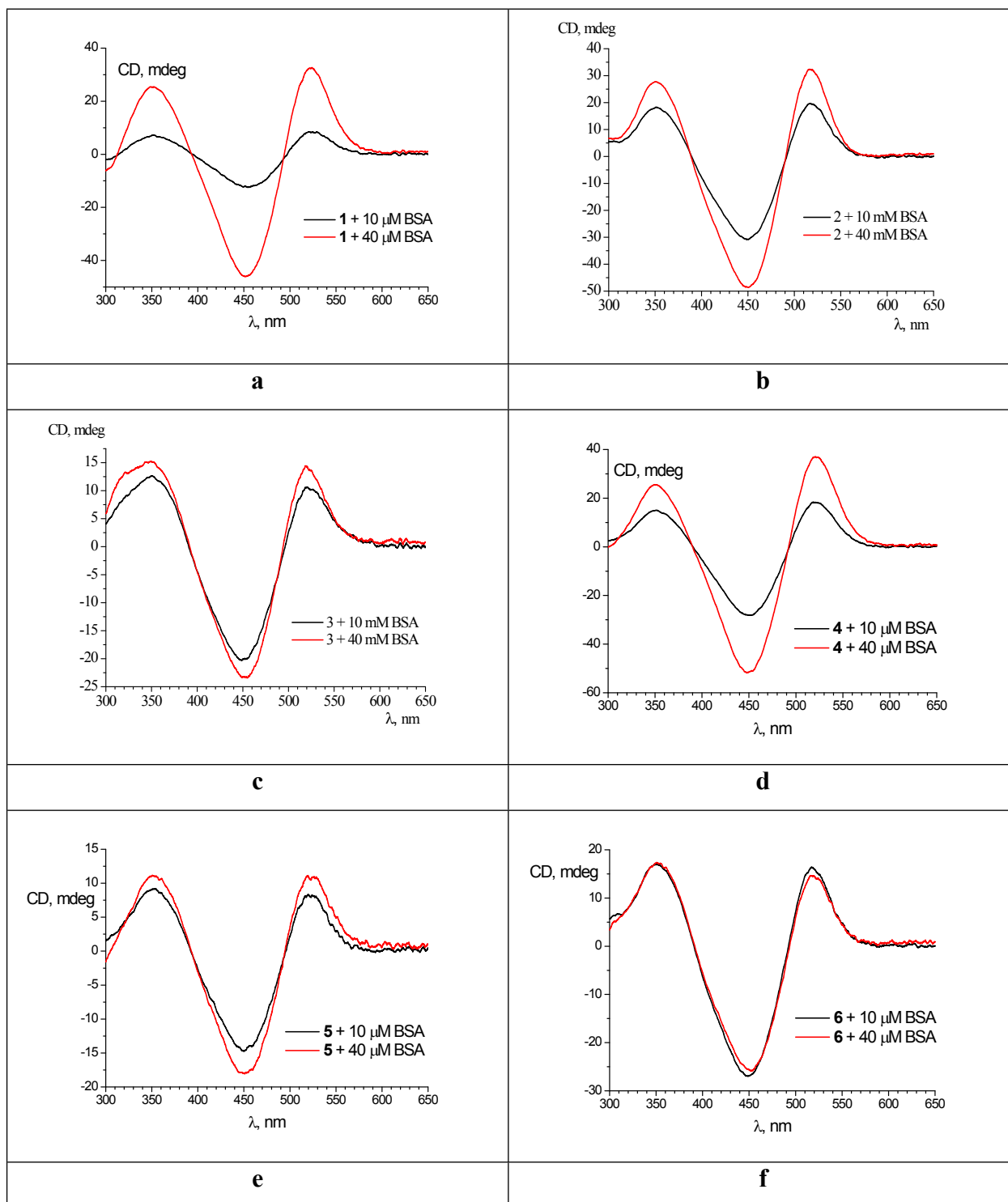


Figure S12. CD spectra of the protein – clathrochelate assemblies of BSA with clathrochelates **1** (a), **2** (b), **3** (c), **4** (d), **5** (e), and **6** (f) which are recorded at $c_{protein} = 4 \cdot 10^{-5}$ and $1 \cdot 10^{-5} \text{ mol} \cdot \text{l}^{-1}$, $c_{clt} = 2 \cdot 10^{-5} \text{ mol} \cdot \text{l}^{-1}$ in 0.05M Tris-HCl buffer with pH 7.9.

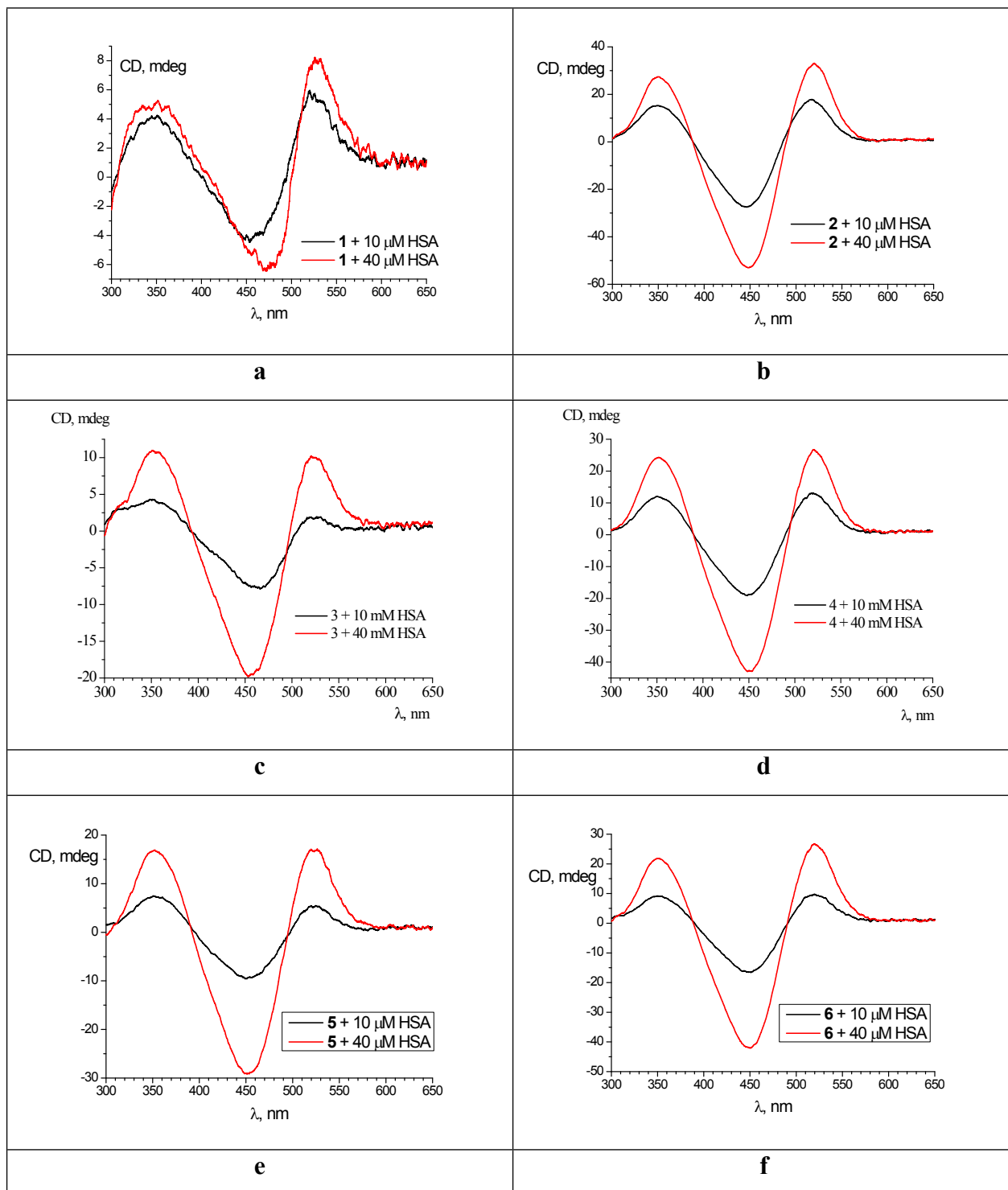


Figure S13. CD spectra of the protein – clathrochelate assemblies of HSA with clathrochelates **1** (a), **2** (b), **3** (c), **4** (d), **5** (e), and **6** (f) which are recorded at $c_{protein} = 4 \cdot 10^{-5}$ and $1 \cdot 10^{-5} \text{ mol} \cdot \text{l}^{-1}$, $c_{clt} = 2 \cdot 10^{-5} \text{ mol} \cdot \text{l}^{-1}$ in 0.05M Tris-HCl buffer with pH 7.9.

5. Estimation of affinity and stoichiometry of clathrochelates binding to HSA

For HSA that contains single Trp residue, we expected the relation of fluorescence quenching curve to binding isotherm to be less complicated as compared to other proteins studied (containing multiple Trp residues). Hence, binding stoichiometry and binding affinity were estimated for the studied clathrochelates; for those where the fitting was the most adequate, the values were provided. Starting with the assumption that each protein globule has n sites for clathrochelate binding with the equal values of the binding constant K , the following expression could be written:

$$K = \frac{C_{BL}}{(C_L - C_{BL}) \times (n \times C_P - C_{BL})} \quad (1)$$

where K is the binding constant; n is the number of clathrochelate molecules bound per HSA globule; C_P , C_{BL} and C_L are the concentrations of protein globules, HSA-bound ligand (i.e. clathrochelate) molecules and total ligand concentration respectively. Solving the quadratic equation, we obtain:

$$C_{BL} = \frac{n \times C_P}{2} + \frac{C_L}{2} + \frac{1}{2 \times K} - \sqrt{\left(\frac{n \times C_P}{2} + \frac{C_L}{2} + \frac{1}{2 \times K}\right)^2 - n \times C_P \times C_L} \quad (2)$$

To obtain the relation between C_{BL} and the observed fluorescence intensity of the protein (F), we supposed that the protein fluorescence intensity is proportional to the number of non-occupied binding sites. In this case, the observed fluorescence intensity of the protein could be expressed as:

$$F = \frac{n - C_P - C_{BL}}{n - C_P} F_0 + \frac{C_{BL}}{n - C_P} F_{min} \quad (3)$$

where F_0 and F_{min} are fluorescence intensities of the protein in the absence of clathrochelates, and protein with all binding sites occupied with clathrochelate molecules, respectively. It could be obtained from (3):

$$\frac{C_{BL}}{n - C_P} = \frac{F_0 - F}{F_0 - F_{min}} \quad (4)$$

Together, (2) and (4) result in the final equation:

$$Y = A \times \left[\frac{1}{2} + \frac{x}{2 \times C_P \times n} + \frac{1}{2 \times K \times C_P \times n} - \sqrt{\left(\frac{1}{2} + \frac{x}{2 \times C_P \times n} + \frac{1}{2 \times K \times C_P \times n}\right)^2 - \frac{x}{C_P \times n}} \right] \quad (5)$$

where $x = C_L$, $Y = (1 - F/F_0)$, $A = (1 - F_{\min}/F_0)$. Thus, the experimentally obtained curve of protein fluorescence quenching is presented as the dependence of $(1 - F/F_0)$ on C_L , and further fitted with the dependence (5). As a result of this fitting, the values of K , n and A are obtained as fitting parameters.