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Spectroscopic and molecular docking studies on the interaction of Pd(II) & Co(II) Schiff base complexes with β -lactoglobulin as a carrier protein

Monireh Dehkhodaeia, Mehdi Sahihi^{a*} and Hadi Amiri Rudbaria

^a *Department of Physical Chemistry, University of Isfahan, Isfahan, 81746-73441, Iran*

***Corresponding author**

Dr. Mehdi Sahihi

Assistant Professor of Biophysical Chemistry

University of Isfahan

Isfahan 81746-73441

Iran

Tel.: +98-313-7934929

Fax: +98-313-36689732

E-mail: m.sahihi@chem.ui.ac.ir

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Introduction

The milk is one of the most popular and of course the most essential beverage. This food source is rich of proteins, vitamins and the other necessary ions for body healthy. The protein part of milk is contained of casein (78.3%), whey proteins (19%) and the other proteins (2.7%). Whey proteins are significantly, used in the food and pharmaceutical industry due to their high nutritional value and excellent functional properties. The most important and abundant part of whey proteins is beta-lactoglobulin (BLG) with molecular weight of 18.400 Da (Papiz et al., 1986). This globular protein is contained of 162 amino acids that they are folded in structure including one α -helix, nine antiparallel β -strand with eight β -sheet forming a cone barrel shape with a hydrophobic pocket. This hydrophobic pocket is known as calyx and it is a very convenient location for binding ligands and drugs with very low solubility in water. Generally, BLG has three binding sites for suchlike ligands. Results of previous studies show that vast range of hydrophobic molecules such fatty acids, cholesterol, porphyrin, bilirubin, aromatic hydrocarbons, vitamin D₃, anticancer and chemotherapy drugs can bind to BLG for transporting in body. Also, BLG is known as a carrier protein with controlled release ability (Reddy, Kella, & Kinsella, 1988).

On the other hand, finding novel compounds with therapeutic properties, especially anticancer compounds, for binding to carrier proteins has biological and pharmacological importance (Moghaddam, Pirouzi, Saberi, Chamani, 2014; Omidvar et al., 2011; Omidvar, Asoodeh, Chamani, 2013; Hosainzadeh, Gharanfoli, Saberi, Chamani, 2012). Recently, attention to synthesis of metal based drugs has been enhanced. Schiff-bases are compounds with imine (C=N) functional group, which are formed from condensation reaction between aldehyde and amine. Researchers have focused on these compounds due to their biological aspects like antifungal, antitumor, antiviral, antioxidant and so on (da Silva et al., 2011). Biochemists believe that the existence of imine group in Schiff-bases ligands is responsible for these biological

effects. Also, the metal ions play important role in pharmaceutical properties of these low water soluble compounds, *e.g.* importance of Co(II) in vitamin B₁₂ or Pd(II) role in anticancer and antitumor agents.

With consideration the above-mentioned points, studying on the interaction of Schiff base metal complexes and carrier proteins is very attractive for researches. In this study we investigated the interaction of PdL₂ and CoL₂ Schiff-base complexes which are synthesized from condensation reaction of salicylaldehyde and tert-butylamine (**Scheme S1**), (these complexes are prepared and characterized in our research group recently (Khorshidifard et al., 2015)) with BLG using UV-Vis absorption, fluorescence spectroscopic and molecular docking methods.

Experimental.

Material

All of the materials for synthesis of the complexes were purchased from Merck-Co and were used without further purification. Bovine BLG type A was purchased from Sigma-Aldrich. All salts used for buffer preparation were analytical grade and were dissolved in double distilled water. All of the solutions were used freshly after preparation.

Preparation of compounds and protein stock solutions.

For optical measurements, all solutions were prepared in phosphate buffer (50 mM) at pH=7. A stock solution of BLG was prepared by dissolving the desired amount of BLG in phosphate buffer. The concentration of BLG was determined using the value of 17,600 M⁻¹.cm⁻¹ for the molar absorption coefficient (ϵ) at 279 nm. The solutions of the compounds were first prepared in methanol and DMSO for CoL₂ and PdL₂, as co-solvents, respectively. And then diluted suitably with phosphate buffer to required concentrations. The volume of co-solvents never exceeded 3% (v/v), so their effects were negligible. All the solutions were used freshly after preparation.

Fluorescence spectroscopy study

The fluorescence spectra were obtained at room temperature on a Shimadzu RF-5000 spectrofluorometer in quartz cell. For studying the fluorescence quenching of BLG by adding PdL₂ and CoL₂ complex solutions, 2 mL of BLG solution (10⁻⁵ M) was placed in the cell and various amounts of the compounds (2×10⁻⁴ M) were added to the cell. In each measurement, the mixture was allowed to incubate for 2 min. The fluorescence intensity was measured with excitation wavelength at 295 nm and emission wavelength range of 300-450 nm. Moreover, the measured fluorescence intensities were corrected for the dilution and the inner-filter effects. To eliminate the inner filter effect, which is happen in high concentrations of protein, absorption measurements were carried out at the fluorescence excitation and emission wavelengths. The extent of this effect can be roughly evaluated with the following relationship:

$$F_{corr} = F_{obs} \times e^{(A_{ex} + A_{em})/2}$$

Where, F_{corr} and F_{obs} are the corrected and observed fluorescence intensities, respectively, and A_{ex} and A_{em} are the absorption of compound at excitation and emission wavelengths, respectively.

UV-Vis absorption spectroscopy study

The UV-Vis absorption spectroscopy measurements are carried out to more investigate BLG-binding of the compounds at room temperature. Electronic absorption spectra are recorded on a PERKIN ELLMER Lambda 265 spectrophotometer. For obtaining the UV-Vis absorption spectra 2 mL of BLG solution (2×10^{-5} M) was placed in the cell and various amounts of the compounds (2×10^{-4} M) were added and spectrum changes were followed in the absence and presence of the compounds on 279 nm. All intensities were corrected for dilution effect in fluorescence and UV absorption experiments.

Molecular docking procedure

Molecular docking is a valuable method to study the interaction of protein and ligand and also, is one of the original tools in computational design of drugs. In this work, we docked the complexes with BLG as an important carrier protein. The 3D structures of the metal complexes were obtained using the CIF files of their X-ray crystal structures (Khorshidifard et al., 2015). The CIF files were converted to the PDB format by using the Mercury software (<http://www.ccdc.cam.ac.uk/>). The crystal structure of BLG (PDB ID: 1CJ5) was taken from the Brookhaven Protein Data Bank (<http://www.rcsb.org/pdb>). The resolution of this file was 2.28 Å. Water molecules of the .pdb files were deleted, missing hydrogen atoms and Gasteiger charges were added. Flexible-ligand docking was carried out by AutoDock 4.2.5.1 molecular-docking program using the implemented empirical free energy function and the Lamarckian Genetic Algorithm. The Gasteiger charges were added to prepare the macromolecule input file for docking and the Auto Grid was used to calculate Grid. For the docking of metal complexes with BLG, a blind docking with 126 lattice points along X, Y, and Z axes was performed to find the binding site of complexes on BLG with a grid point spacing of 0.375 Å, to allow the complex to rotate freely. In the next step, the centre of the grid box was located at the binding site and the second docking was performed using a cubic box with 60×60×60 dimensions. 250 docking runs with 25,000,000 energy evaluations for each run were performed.

Result & discussion

Fluorescence quenching analysis

Whereas, BLG known as a carrier protein, understanding of its physicochemical and pharmaceutical features are important steps in investigation of its biological activity. This major bovine whey protein contains remarkable properties that have been previously pointed out. Moreover, its thermodynamic stability in the presence of some surfactants has been investigated, recently. (Marouzi, et al., 2017; Khorsand Ahmadi, et al., 2015; Chamani, et al., 2006; Chamani, 2006; Chavoshpour, Sahihi, & Gharaghani, 2017).

Fluorescence spectroscopy is one of the trusty methods to check the interaction of ligand and protein. This high sensitive technique can indicate accessibility of quencher to fluorophores of protein. Fluorescence quenching could proceed through different mechanisms, usually classified as dynamic and static quenching. Static quenching mode refers to the formation of a non-fluorescent ground state complex, whereas, dynamic quenching mode is resulted from the collision between the fluorophore and quencher in

excited state. Both static and dynamic processes are described by the Stern–Volmer equation as follow:

$$\frac{F_0}{F} = 1 + K_{sv}[Q] = 1 + k_q\tau[Q] \quad (1)$$

In this equation, F_0 and F are the fluorescence intensities of BLG in the absence and presence of the compounds. K_{sv} is the Stern-Volmer quenching constant, k_q is the quenching rate constant of BLG, τ is the average lifetime of BLG without quencher which is typically equal to 10^{-8} s for biomacromolecules and $[Q]$ is the concentration of quencher that quencher is the synthesized compounds here. K_{sv} is determined from the plot of F_0/F vs $[Q]$. The fluorescence spectra of BLG in the presence of different amounts of PdL2 and CoL2 complexes at T=298 K were shown in **Figures 1a** and **1d**. Also these spectra at T=308 and 318 K are shown in **Figures S2** and **S3**. The fluorescence intensity of BLG gradually decreased by adding different volumes (μL) of compounds in each step. Moreover, the appearance of isosbestic points signifies that equilibrium is established between BLG and the compounds and reflects the formation of the ground state complex between them. Whereas, estimating the binding constant of ligand to protein is an important parameter in investigation of interaction of, it could be given by the following equation that it is known as Scatchard equation:

$$\ln\left(\frac{F_0 - F}{F}\right) = \ln(K_b) + n \ln [Q] \quad (2)$$

Where F_0 , F and $[Q]$ are the same as the parameters in the Stern–Volmer equation. K_b is binding constant that is obtained from exponential y-intercept. In this equation (n) is the number of binding site of the compound on the protein that it is calculated from the plot of $\ln((F_0 - F)/F)$ versus $\ln[Q]$ as plot slop. This plots at T=298 K are presented in **Figures 1b** and **1e**. Also, these plots for other temperatures are presented in **Figures S2** and **S3**. The estimated values of K_b , n , K_{sv} and k_q are listed in **Table 1**.

The Stern–Volmer relationship, allows us to explore the kinetics of a photophysical intermolecular deactivation process. However, the binding constant, or association constant, is a special case of the equilibrium constant K . It is associated with the binding and unbinding reaction of receptor and ligand molecules and allows us to explore the thermodynamic of binding process. Hence, the quantities of these constants are different.

Also, fluorescence spectroscopy is more sensitive than the absorption spectroscopy to assess the binding of drugs to proteins. Hence, the obtained binding constants using fluorescence titration method are more accurate.

Generally, binding constant should not be too high which drug cannot separate hereinafter binding to carrier protein and it should not be too low which cannot bind well to carrier protein and achieve to destination. BLG-binding constants for both of the compounds are in a good range ($2-6 \times 10^3$). Also, in this study, the values of k_q were obtained about $10^{10} \text{ M}^{-1} \text{ s}^{-1}$ at room temperature. This observation supports that

fluorescence quenching of BLG occurs by static mechanism. The charts of F_0/F vs $[Comp]$ for both of the compounds in 298 K are present in **Figure S1**.

Analysis of UV-Vis absorption of BLG

UV-Vis absorption spectroscopy is another technique to investigate the interaction of ligand-protein systems. Although sensitivity of this method is not comparable with fluorescence spectroscopy, but it can be employed as a supplement technique.

Concentrations of compounds and BLG are chosen somehow, to cover mole ratio the ($[Comp]/[BLG]=0-5$). By using Benesi–Hildebrand plot and drawing the plot of $1/(A-A_0)$ versus $1/[M]$, binding constant can be estimated as ratio of y-intercept to slope:

$$\frac{1}{A - A_0} = \frac{1}{A_{max} - A} + \frac{1}{K_b(A_{max} - A)} \times \frac{1}{[M]} \quad (3)$$

Where A_0 and A are the absorbance of BLG in the absence and presence of the compounds, respectively. A_{max} is the obtained absorbance at saturation and $[M]$ is the concentration of the compound. The estimated values of K_b for PdL₂ and CoL₂ complexes at T=298 K are 1.73×10^4 and 1.72×10^4 , respectively. The UV-Vis spectra of BLG in the presence of different amounts of PdL₂ and CoL₂ complexes are shown in **Figures 2a** and **2c**.

Energy transfer between BLG and complexes.

Fluorescence resonance energy transfer (FRET) or Förster's theory is a non-destructive spectroscopic method that can estimate distance from the bound ligand to the fluorophore groups of biomacromolecule. The energy transfer could take place through direct electrodynamic interaction between the primarily excited molecule and its neighbours. The following conditions determine the occurrence of energy transfer between a donor and an acceptor: (i) the relative orientation of the donor and acceptor electric dipole moments; (ii) the overlap between the emission spectrum of the donor and the UV–Vis spectrum of the acceptor; and (iii) the distance between the donor and the acceptor (Bai et al., 2015). According to the Förster's dipole–dipole non-radiative energy transfer theory, the efficiency of energy transfer (E) is related not only to the distance between the acceptor and the donor, but also to the critical energy transfer distance (R_0), as described by the following equation:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (4)$$

Where F and F_0 are the fluorescence intensity of the donor in the absence and presence of equal amount of acceptor, r is the distance from the bound ligand to the tryptophan residue, and R_0 is the Förster critical distance at which 50% of the excitation energy is transferred to the acceptor and can be calculated by the following equation:

$$R_0^6 = 8 \cdot 79 \times 10^{-25} K^2 N^{-4} J \phi \quad (5)$$

Where, K_2 is factor describing the relative orientation of the transition dipoles of the

donor and acceptor (for a random orientation as in fluid, $K_2 = 2/3$), N is the average refractive index of the medium in the wavelength range where spectral overlap is significant (N is 1.3598), ϕ is the fluorescence quantum yield of the donor (ϕ is 0.08), and overlap integral J expresses the extent of overlap between the normalized fluorescence emission spectrum of the donor and the acceptor absorption spectrum. J is given by the following equation:

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda}{\sum F(\lambda)\Delta\lambda} \quad (6)$$

Where, $F(\lambda)$ is the fluorescence intensity of the fluorescent donor at wavelength λ and is dimensionless, $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength λ . As it is shown in **Figures 1c** and **1f**, the overlap between the fluorescence emission of BLG and the absorption spectra of the compounds is considerable. The calculated values for E , r and R_0 parameters are collected in **Table 1**.

Analysis of thermodynamic parameters

Results of the reports in literature show that there are four non-covalent effective forces between compounds and biomacromolecules, i.e. hydrophobic, H-bond, Vander Walls and electrostatic interactions. According to the Van't Hoff equation, thermodynamic parameters are estimated by the following equation:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT \ln K \quad (7)$$

Also,

$$\ln \frac{K_2}{K_1} = \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \frac{\Delta H^\circ}{R} \quad (8)$$

In both of these equations, R is gas public constant, T is the experimental temperature, K is binding constant at the corresponding temperature, ΔG° , ΔH° and ΔS° are the standard Gibbs free energy, the enthalpy and the entropy changes, respectively. ΔH° and ΔS° are estimated from the plot $\ln K$ vs $1/T$ as slop and y-intercept, respectively. These plots for CoL₂ and PdL₂ complexes are present in **Figures S4a** and **S4b**, respectively. Also ΔG° is obtained by using equation (7). According to the Rass and Subramanian report (Ross & Subramanian, 1981):

$\Delta H^\circ > 0$ and $\Delta S^\circ > 0$	→	Hydrophobic force
$\Delta H^\circ < 0$ and $\Delta S^\circ < 0$	→	Vander vales
$\Delta H^\circ < 0$ and $\Delta S^\circ > 0$	→	Electrostatic interaction

The obtained results in **Table 2** show that, both of ΔH° and ΔS° parameters are positive. Hence, hydrophobic interactions have main role in the stability of BLG complexes systems. Also, negative values of ΔG° at all temperatures indicates that the interaction of BLG and PdL₂ or CoL₂ is spontaneous.

Molecular docking study

Studies on the interaction between BLG and various compounds show that there are three potential binding sites on BLG: the internal cavity of the β -barrel that is known as main binding site, the surface hydrophobic pocket in a groove between α -helix and β -barrel and the outer surface near TRP19-ARG124. **Figures 3a** and **3b** indicate the binding site position for PdL₂ and CoL₂ with their chosen clusters, respectively. The obtained results showed that PdL₂ complex has located in secondary binding site of BLG, between α -helix and β -barrel with standard binding free energy (ΔG°) of -6.3 kcal.mol⁻¹ ($K_b = 4.17 \times 10^4$ M⁻¹). This score is related to the docking cluster with minimum ΔG° (see **Figure 3a**). Also, three π -cation interactions have been formed between N and H atoms of LYS138 residue with C atoms of phenyl ring of PdL₂ complex. The residues around of this complex are GLN5, THR4, VAL3, TYR2, LEU95, LEU104, LYS138, TYR102, ALA139, ALA142, LEU140 and LEU143. Also, intermolecular energy including H-bonding interactions, van der Waals, desolvation and electrostatic energies, was estimated as -6.85 kcal.mol⁻¹. **Figure 3b** shows the position of CoL₂ complex on BLG. It is placed in entrance of the calyx and is stabilized by one hydrogen bond with SER116 residue of BLG. The amino acids with major role in the stability of CoL₂-BLG system are GLU112, GLN115, PRO113, ASN109, SER116, ASN88, LEU31, PRO38 and LEU39. The intermolecular energy and standard binding free energy (ΔG°) for CoL₂ complex are -6.42 and -5.87 kcal.mol⁻¹ ($K_b = 2.02 \times 10^4$ M⁻¹), respectively. These energies are related to the docking cluster with minimum energy (see **Figure 3b**).

Supportingly, differences between the obtained binding constants using fluorescence spectroscopy and molecular docking methods are related to their different natures.

However, PdL₂ shows more affinity than CoL₂ for binding to BLG in all of the experimental and theoretical methods.

Furthermore, the distances between PdL₂ and CoL₂ with Trp 19 residue are 1.21 and 1.63 nm, respectively. These obtained data are in consistent with obtained efficiency of energy transfer (E) using FRET experiments (Table 1).

Conclusion

Two metal Schiff-base complexes (CoL₂ and PdL₂) have been investigated to obtain

binding affinity to BLG using UV-Vis, fluorescence spectroscopy and molecular docking methods. The obtained results of the experimental section showed that the compounds bind to BLG as PdL₂>CoL₂. Also, to calculate thermodynamic parameters, fluorescence spectroscopy was done at three temperatures (298, 308 and 318 K). The obtained results indicated negative values for ΔG° and positive values for ΔS° and ΔH° , at all temperatures. Also, molecular docking studies revealed that hydrogen bond, hydrophobic and π -cation interactions have the important role in the interaction of these compounds with BLG.

Reference:

- Bai, Z., Liu, Y., Zhang, P., Guo, J., Ma, Y., Yun, X., & Zhang, F. (2015). Fluorescence resonance energy transfer between bovine serum albumin and fluoresceinamine. *Luminescence*, 31(3), 688–693. DOI: 10.1002/bio.3012.
- Chamani, J. (2006). Comparison of the conformational stability of the non-native α -helical intermediate of thiol-modified β -lactoglobulin upon interaction with sodium n-alkyl sulfates at two different pH. *Journal of colloid and interface science*, 299(2), 636-646. DOI: 10.1016/j.jcis.2006.02.049.
- Chamani, J., Moosavi-Movahedi, A.A., Rajabi, O., Gharanfoli, M., Momen-Heravi, M., Hakimelahi, G.H., Neamati-Baghsiah, A. & Varasteh, A.R. (2006). Cooperative α -helix formation of β -lactoglobulin induced by sodium n-alkyl sulfates. *Journal of colloid and interface science*, 293(1), 52-60. DOI: 10.1016/j.jcis.2005.06.015.
- Chavoshpour-Natanzi, Z., Sahihi, M., & Gharaghani, S. (2017). Structural stability of β -lactoglobulin in the presence of cetylpyridinium bromide: spectroscopic and molecular docking studies. *Journal of Biomolecular Structure and Dynamics*, 1-8. DOI:10.1080/07391102.2017.1297254.
- da Silva, C. M., da Silva, D. L., Modolo, L. V., Alves, R. B., de Resende, M. A., Martins, C. V., & de Fátima, Â. (2011). Schiff bases: A short review of their antimicrobial activities. *Journal of Advanced research*, 2(1), 1-8. DOI:10.1016/j.jare.2010.05.004.
- Hosainzadeh, A., Gharanfoli, M., Saberi, M.R. & Chamani, J. (2012). Probing the interaction of human serum albumin with bilirubin in the presence of aspirin by multi-spectroscopic, molecular modeling and zeta potential techniques: Insight on binary and ternary systems. *Journal of Biomolecular Structure and Dynamics*, 29(5), 1013-1050. DOI: 10.1080/073911012010525029.
- Khorshidifard, M., Rudbari, H. A., Askari, B., Sahihi, M., Farsani, M. R., Jalilian, F., & Bruno, G. (2015). Cobalt (II), copper (II), zinc (II) and palladium (II) Schiff base complexes: Synthesis, characterization and catalytic performance in selective oxidation of sulfides using hydrogen peroxide under solvent-free conditions. *Polyhedron*, 95, 1-13. DOI:10.1016/j.poly.2015.03.041.
- Khorsand Ahmadi, S., Mahmoodian Moghadam, M., Mokaberi, P., Reza Saberi, M. & Chamani, J. (2015). A comparison study of the interaction between β -lactoglobulin and retinol at two different conditions: spectroscopic and molecular modeling approaches. *Journal of Biomolecular Structure and Dynamics*, 33(9), 1880-1898. DOI: 10.1080/07391102.2014.977351.
- Moghaddam, M.M., Pirouzi, M., Saberi, M.R. & Chamani, J. (2014). Comparison of the binding behavior of FCCP with HSA and HTF as determined by spectroscopic

and molecular modeling techniques. *Luminescence*, 29(1), 314-331. DOI: 10.1002/bio.2546.

Marouzi, S., Rad, A.S., Beigoli, S., Baghaee, P.T., Darban, R.A. & Chamani, J. (2017). Study on effect of lomefloxacin on human holo-transferrin in the presence of essential and nonessential amino acids: Spectroscopic and molecular modeling approaches. *International journal of biological macromolecules*, 97, 688-699. DOI: 10.1016/j.ijbiomac.2017.01.047.

Omidvar, Z., Asoodeh, A. & Chamani, J. (2013). Studies on the antagonistic behavior between cyclophosphamide hydrochloride and aspirin with human serum albumin: time-resolved fluorescence spectroscopy and isothermal titration calorimetry. *Journal of Solution Chemistry*, 42(5), 1005-1017. DOI: 10.1007/s10953-013-0009-7.

Omidvar, Z., Parivar, K., Sane, H., Amiri-Tehranizadeh, Z., Baratian, A., Saberi, M.R., Asoodeh, A., & Chamani, J. (2011). Investigations with spectroscopy, zeta potential and molecular modeling of the non-cooperative behaviour between cyclophosphamide hydrochloride and aspirin upon interaction with human serum albumin: Binary and ternary systems from the view point of multi-drug therapy. *Journal of Biomolecular Structure and Dynamics*, 29(4), 181-206. DOI: 10.1080/07391102.2011.10507382.

Papiz, M., Sawyer, L., Eliopoulos, E., North, A., Findlay, J., Sivaprasadarao, R., & Kraulis, P. (1986). The structure of b-lactoglobulin and its similarity to plasma retinol-binding protein. *Nature*, 324(6095), 383-385. DOI: 10.1038/324383a0.

Reddy, I. M., Kella, N. K., & Kinsella, J. E. (1988). Structural and conformational basis of the resistance of. beta.-lactoglobulin to peptic and chymotryptic digestion. *Journal of Agricultural and Food Chemistry*, 36(4), 737-741. DOI: 10.1021/jf00082a015.

Ross, P. D., & Subramanian, S. (1981). Thermodynamics of protein association reactions: forces contributing to stability. *Biochemistry*, 20(11), 3096-3102. DOI: 10.1021/bi00514a017.

Table 1. The obtained results from investigation of fluorescence and electronic absorption spectra for PdL₂ and CoL₂ at 25° C and the energy transfer from BLG to complexes.

Complex	$K_b(M^{-1})$ (Fluorescence)	$K_b(M^{-1})$ (UV-Vis)	n	K_{sv} (M^{-1})	k_q ($M^{-1}.S^{-1}$)	E	R_0 (nm)	r (nm)
CoL ₂	$(2.73 \pm 0.02) \times 10^3$	$1.72 \pm 0.08 \times 10^4$	1.24	$(62.00 \pm 0.01) \times 10^2$	62.0×10^{10}	0.43	3.33	3.49
PdL ₂	$(6.48 \pm 0.02) \times 10^3$	$1.73 \pm 0.05 \times 10^4$	0.75	$(25.00 \pm 0.02) \times 10^2$	25.0×10^{10}	0.46	3.24	3.31

Table 2. The thermodynamic parameters for binding of the compounds to BLG.

complex	Temperature (°C)	$K_{binding}$ (M ⁻¹)	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (J/mol. K)
CoL ₂	25	(2.73±0.02)×10 ³	-19.61	222.3	811.37
	35	(2.22±0.02)×10 ⁵	-31.54	222.3	823.75
	45	(7.42±0.31)×10 ⁵	-35.75	222.3	811.11
PdL ₂	25	(6.48±0.02)×10 ³	-21.75	156.43	597.63
	35	(6.89±0.02)×10 ⁴	-28.54	156.43	600.26
	45	(3.40±0.020)×10 ⁵	-33.68	156.43	597.57

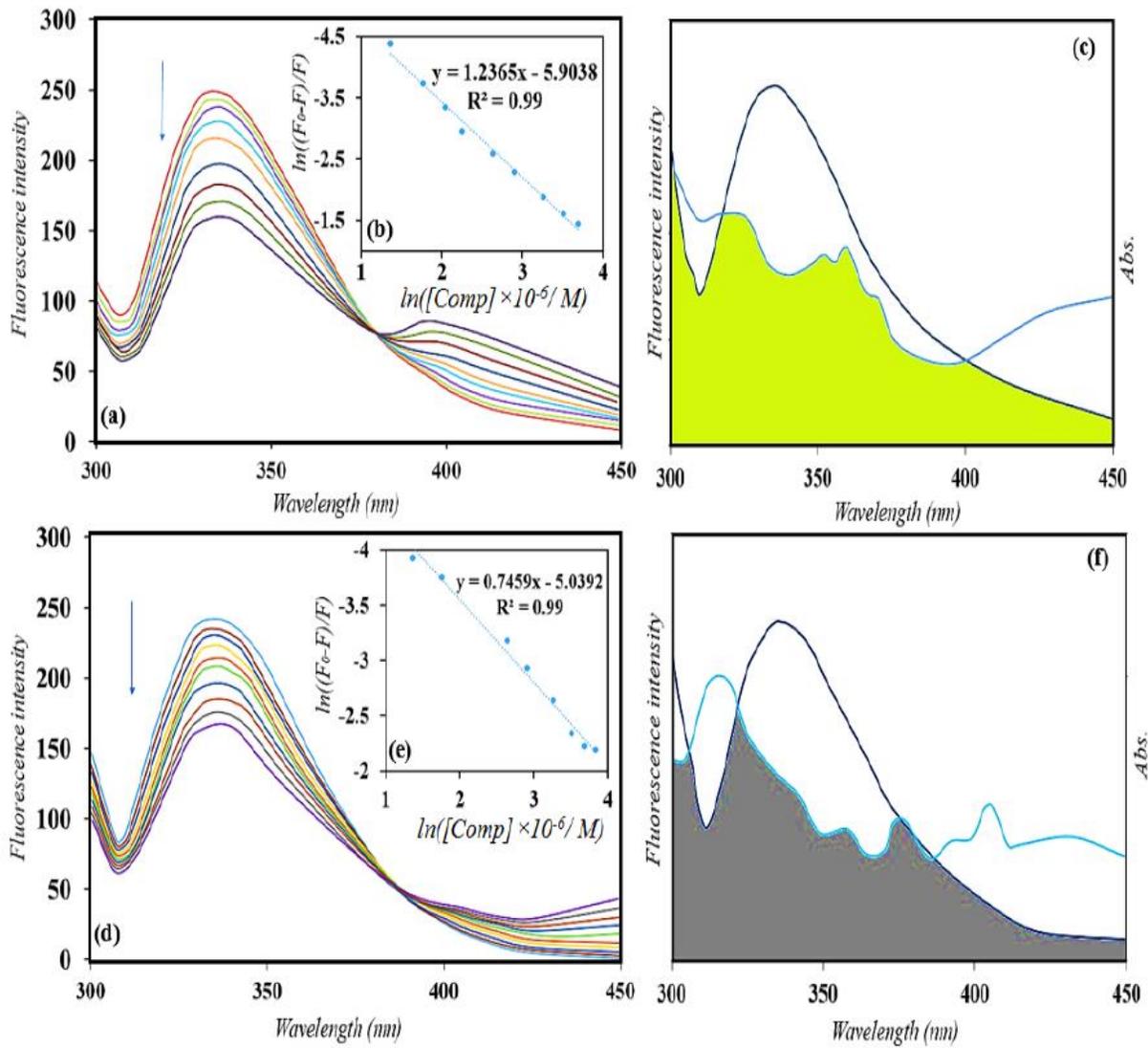


Figure 1. **a and d)** The quenching effect of schiff-base complexes on BLG fluorescence intensity at 25°C for CoL₂ and PdL₂, respectively. [BLG]=10⁻⁵ M, [Comp]=2×10⁻⁴ M and λ_{ex}=295 nm. **b and e)** Plots of ln((F₀-F)/F) versus ln[Comp] for CoL₂ and PdL₂, respectively. **c and f)** Spectral overlap of the BLG fluorescence with the absorption spectra of CoL₂ and PdL₂, respectively.

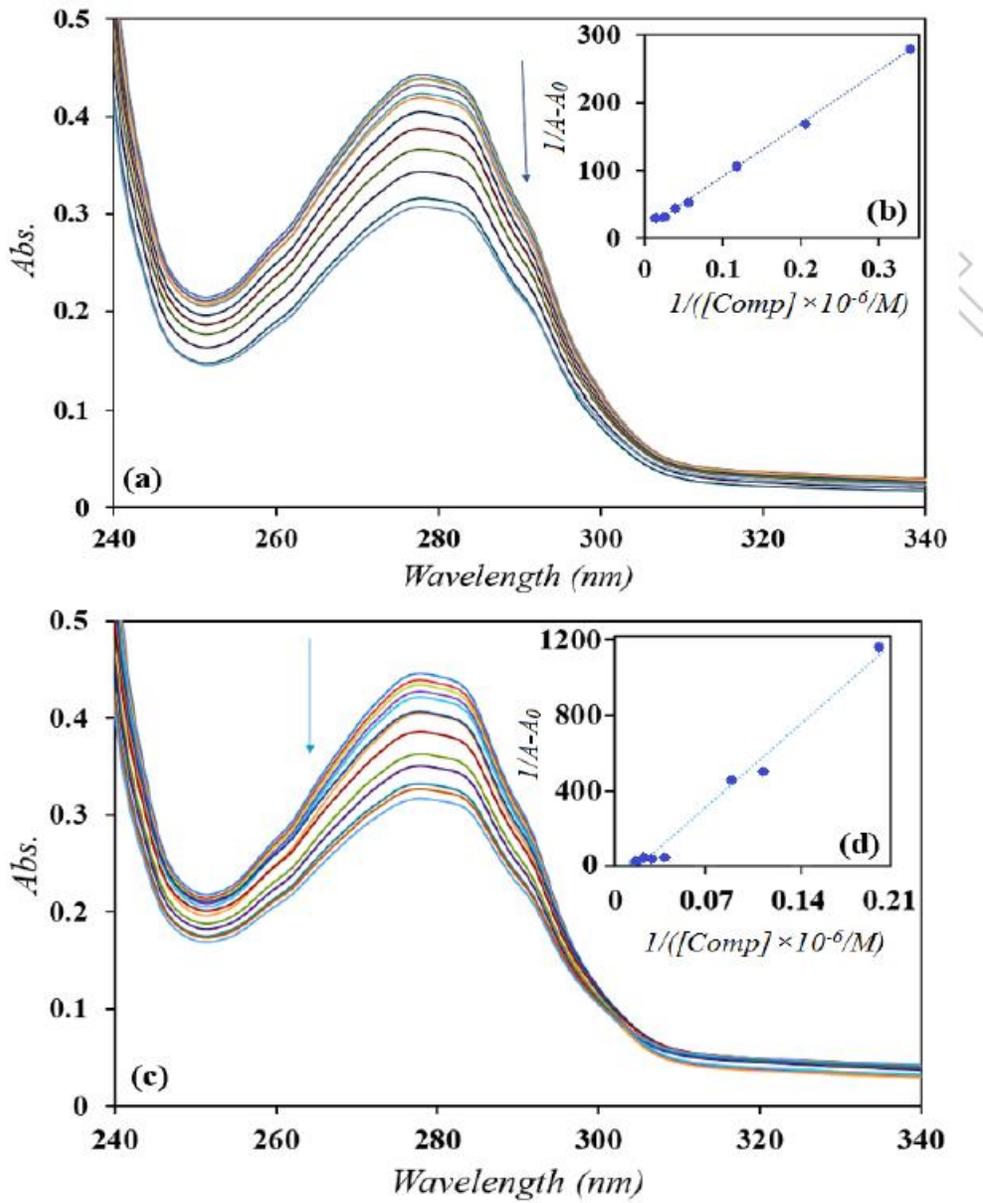


Figure 2. a and c) Changing of UV-Vis spectrum of BLG upon its titration with various amounts of CoL₂ and PdL₂, respectively. [BLG]= 2×10^{-5} M, [Comp]= 2×10^{-4} M. b and d) Plots of $1/A-A_0$ versus $(1/[Comp])$ for CoL₂ and PdL₂, respectively.

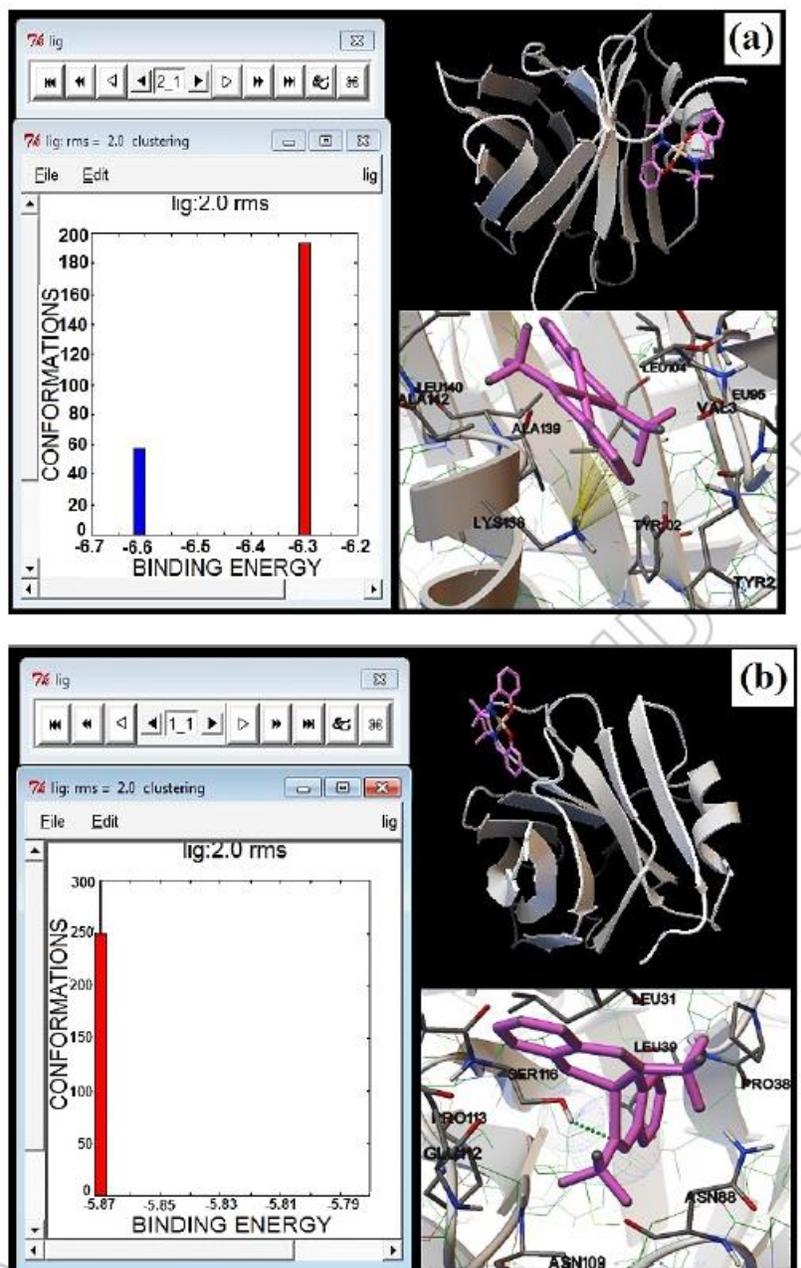


Figure 3. **a)** View of the BLG binding site for PdL2andits selected cluster. **b)** View of the BLG binding site for CoL2andits selected cluster. (Small spheres and yellow cones show H-bond interaction and π -cation interactions, respectively.)

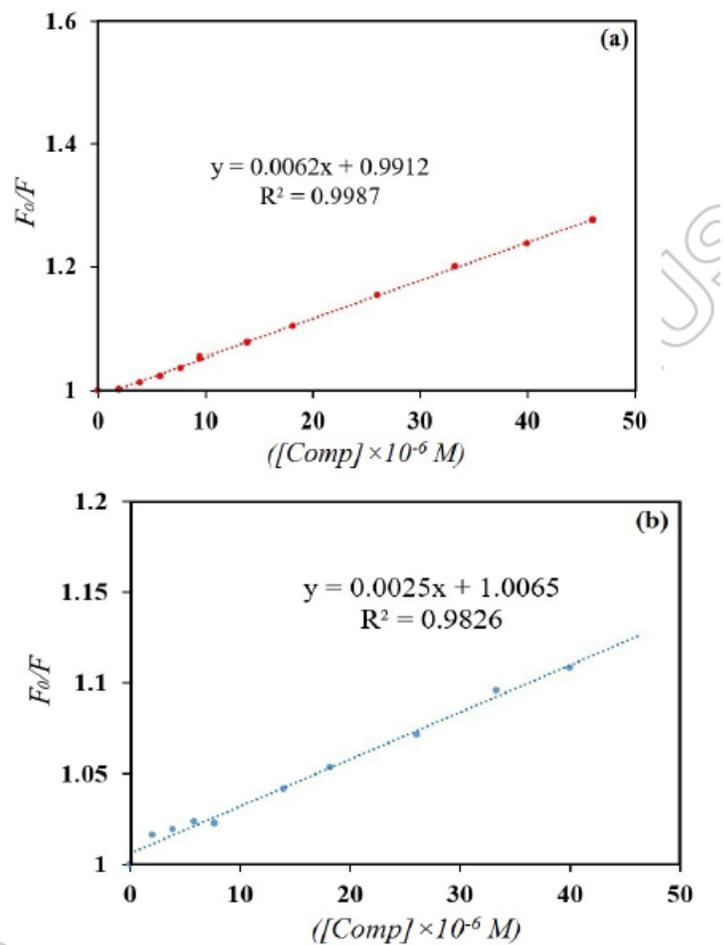


Figure S1. Determination of the complex-BLG Stern-Volmer constant at 25°C for: **a)** CoL2, **b)** PdL2.

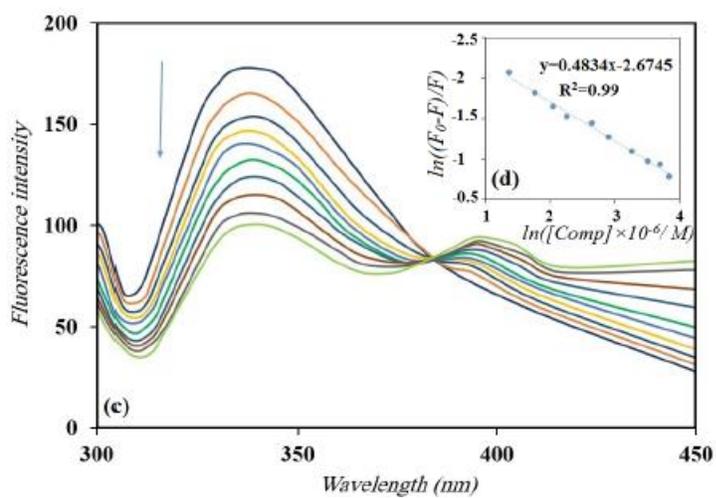
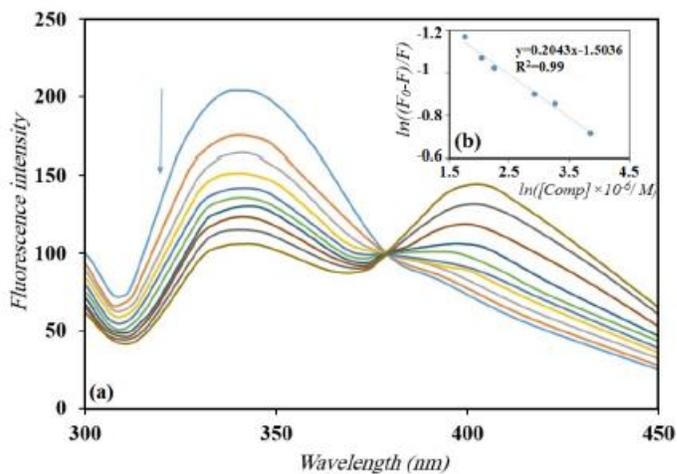


Figure S2.a and c) The quenching effect of schiff-base complexes on BLG fluorescence intensity for a) CoL₂, c) PdL₂, respectively, at 35° C. **b and d)** Plots of $\ln((F_0-F)/F)$ versus $\ln[Comp]$ for CoL₂ and PdL₂, respectively, at 35° C.

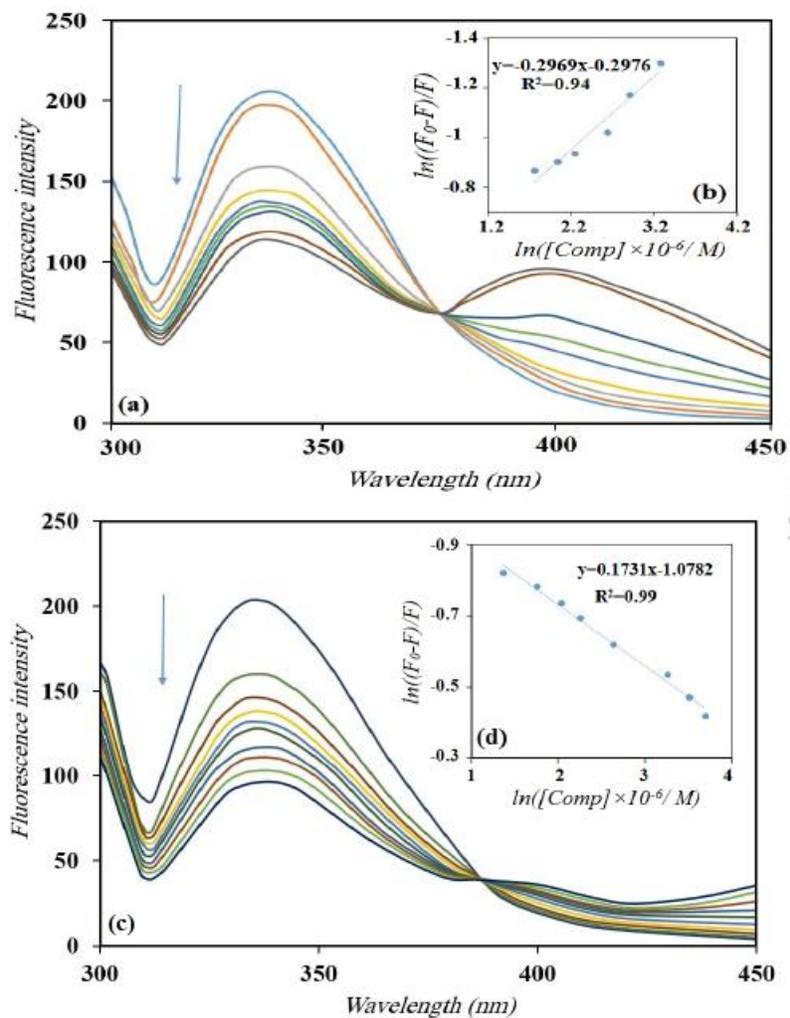


Figure S3.a and c) The quenching effect of schiff-base complexes on BLG fluorescence intensity for **a)** CoL2, **c)** PdL2, respectively, at 45°C. **b** and **d)** Plots of $\ln((F_0-F)/F)$ versus $\ln[Comp]$ for CoL2 and PdL2, respectively, at 45°C.