Supporting Information

Relationship Investigation of Molecular Structure–Binding Affinity of Antibiotics to Bovine Serum Albumin Using Flow Injection Chemiluminescence Analysis and Molecular Docking

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1. Fluorescence measurements

The utilized F-4500 fluorophotometer in this work equipped with a 150 W xenon lamp and a 3.0 mL quartz cuvette with a path length of 1.0 cm. The slit widths of Ex/Em were fixed at 5.0 nm/5.0 nm. The negative high voltage of the PMT and the scanning rate were 700 V and 240 nm·min^{-1}, respectively. A series of antibiotic solutions (within the range of 5.0×10^{-8} ~ 5.0×10^{-6} mol·L^{-1}, **Table S1**) was fortified into BSA solution (5.0×10^{-7} mol·L^{-1}). After the mixed solution was well homogenized, the fluorescence spectra of the mixture was recorded in the wavelength range of 290–450 nm with the Ex/Em of 280 nm/340 nm. Herein, the fluorescence intensity of BSA in the absence and presence of antibiotics were F_0 and F, respectively.

It was found that all the studied antibiotics had quenching effect on the intrinsic fluorescence intensity of BSA, and the data were analyzed using double-logarithmic equation[38,49–51] of $\log[(F_0 - F)/F] = \log K + n\log C_{\text{Antibiotic}}$, The binding parameters K and n of antibiotics to BSA were given from the linear plots of $\log[(F_0 - F)/F]$ *vs* $\log C_{\text{Antibiotic}}$, with results listed in **Table 2** (298 K) and **Table S1** (288/308 K).

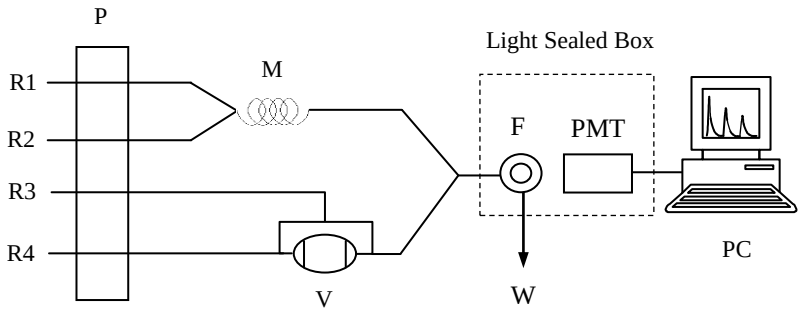

Fig. S1

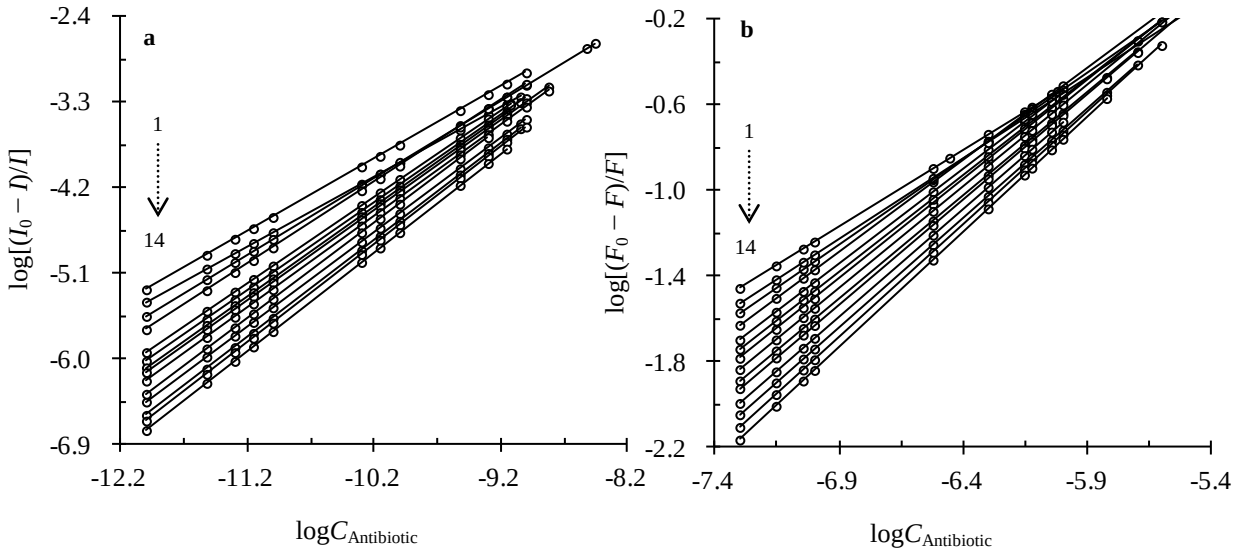

Fig. S2

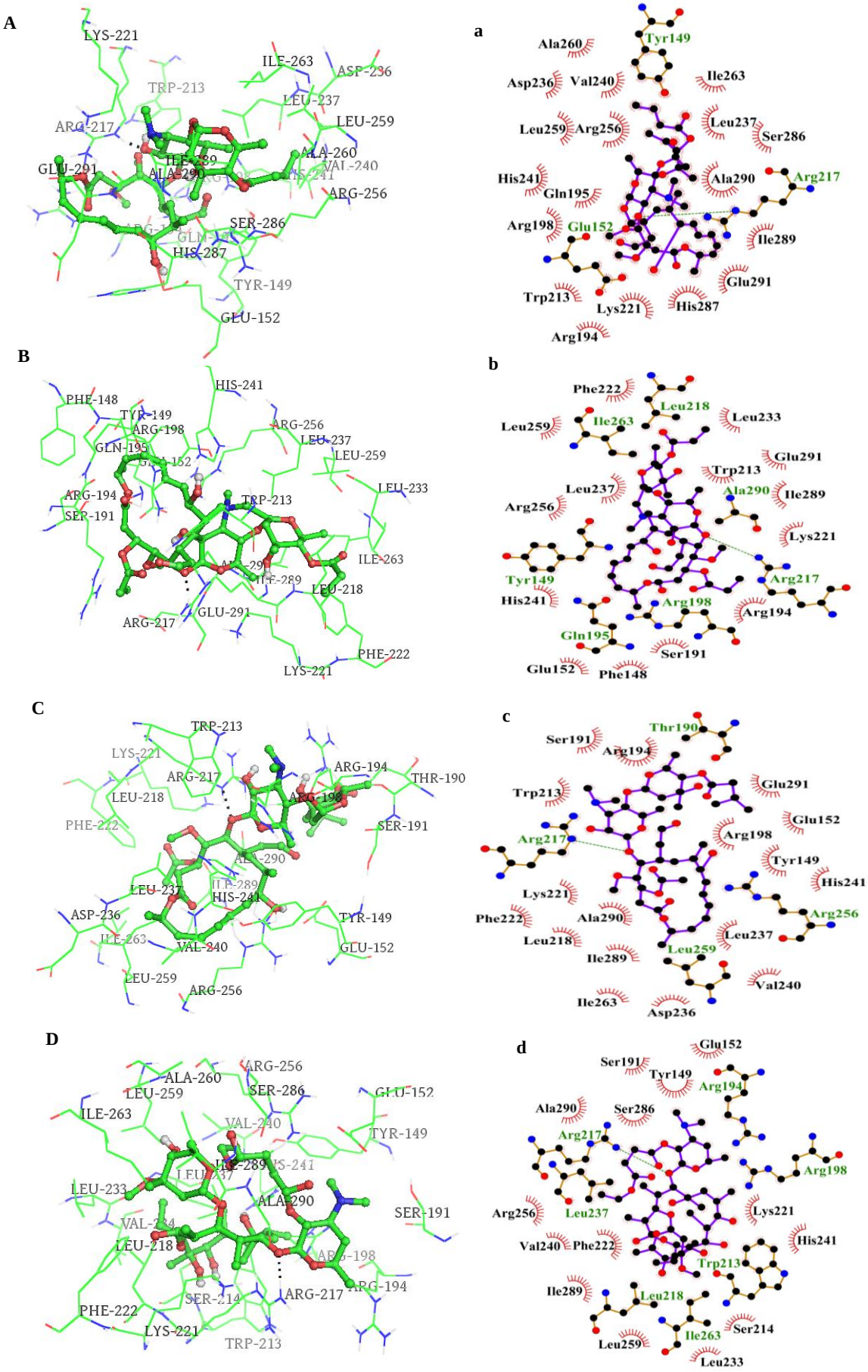
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Fig. S3–1

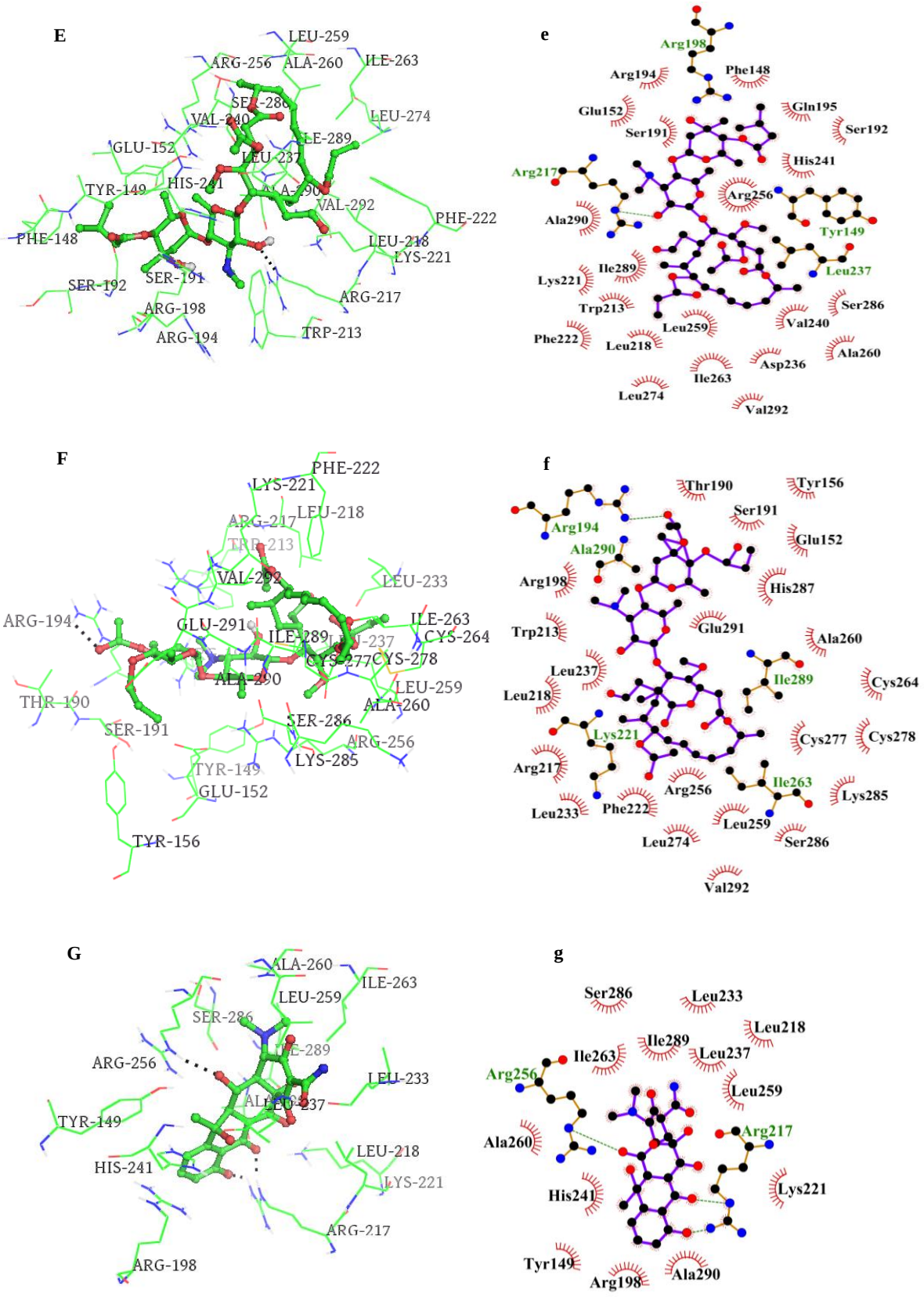
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Fig. S3–2

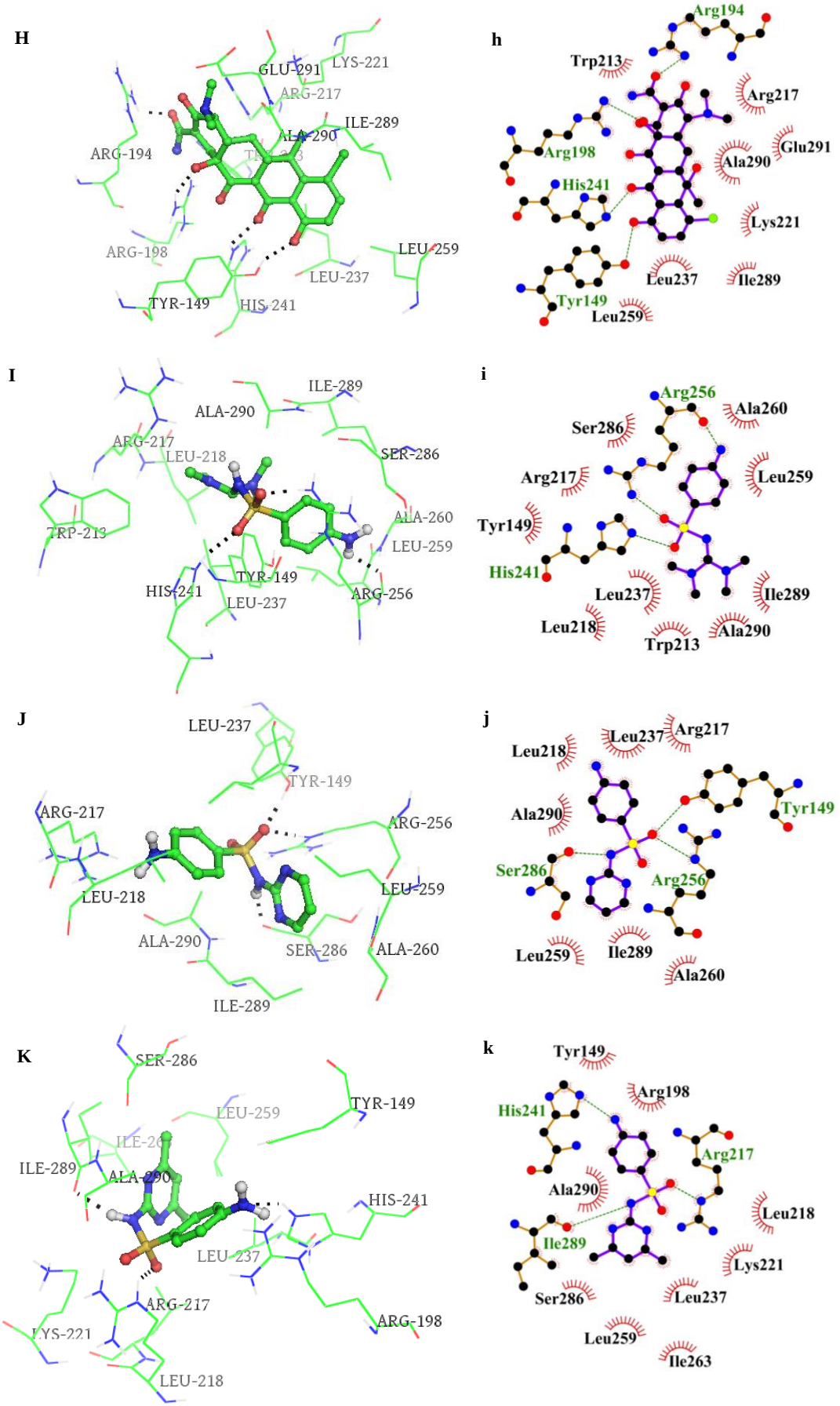
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Fig. S3–3

Table S1 Binding parameters of antibiotic to BSA at 288/308 K by FI-CL and FQ method

Antibiotics	K (L·mol^{-1})		n		Linear range [a]
	FI-CL / FQ (288 K)	FI-CL / FQ (308 K)	FI-CL / FQ (288 K)	FI-CL / FQ (308 K)	(μmol·L^{-1})
ERY	2.13×10^{3} / 2.15×10^{3}	3.49×10^{3} / 3.52×10^{3}	0.71 / 0.72	0.74 / 0.75	0.05 ~ 1.0
KIT	6.06×10^{3} / 5.96×10^{3}	1.02×10^{4} / 9.90×10^{3}	0.75 / 0.74	0.79 / 0.77	0.05 ~ 3.0
MID	1.04×10^{4} / 1.01×10^{4}	1.86×10^{4} / 1.81×10^{3}	0.80 / 0.78	0.83 /0.82	0.05 ~ 3.5
JOS	2.48×10^{4} / 2.50×10^{4}	4.50×10^{4} / 4.72×10^{3}	0.82 / 0.83	0.89 / 0.90	0.05 ~ 5.0
ERE	4.79×10^{4} / 4.62×10^{4}	8.93×10^{4} / 8.83×10^{3}	0.88 / 0.87	0.92 / 0.91	0.05 ~ 1.0
JOP	5.26×10^{4} / 5.06×10^{4}	9.99×10^{4} / 9.79×10^{4}	0.90 / 0.88	0.93 / 0.92	0.05 ~ 2.5
MIA	5.88×10^{4} / 5.58×10^{5}	1.25×10^{5} / 1.12×10^{5}	0.92 / 0.90	0.95 / 0.93	0.05 ~ 2.5
TET	3.57×10^{5} / 3.17×10^{5}	1.29×10^{5} / 1.17×10^{5}	0.97 / 0.96	0.94 / 0.94	0.05 ~ 3.5
OXY	4.11×10^{5} / 4.01×10^{5}	1.39×10^{5} / 1.36×10^{5}	0.98 / 0.97	0.95 / 0.95	0.05 ~ 1.5
CHL	4.28×10^{5} / 4.45×10^{5}	1.42×10^{5} / 1.49×10^{5}	0.99 / 1.00	0.96 / 0.96	0.05 ~ 3.0
SNM	4.35×10^{5} / 4.51×10^{5}	3.72×10^{5} / 3.76×10^{5}	0.99 / 1.02	0.97 / 0.99	0.05 ~ 1.0
SGD	5.35×10^{5} / 5.05×10^{5}	4.41×10^{5} / 4.11×10^{5}	1.02 / 1.03	0.99 / 1.00	0.05 ~ 2.0
SDZ	6.32×10^{5} / 6.48×10^{5}	5.05×10^{5} / 5.15×10^{5}	1.05 / 1.07	1.02 / 1.04	0.05 ~ 2.5
SMZ	6.89×10^{5} / 7.14×10^{5}	5.42×10^{5} / 5.52×10^{5}	1.06 / 1.10	1.03 / 1.05	0.05 ~ 1.5

[a] The shown linear ranges of antibiotics were used in FQ study.

Table S2 Results of ΔG values of antibiotic to BSA at 288/308 K by FI-CL and FQ method [a]

Antibiotics	FI-CL		FQ	
	288 K	308 K	288 K	308 K
ERY	−18.35	−20.89	−18.37	−20.91
KIT	−20.85	−23.64	−20.81	−23.56
MID	−22.15	−25.18	−22.08	−25.10
JOS	−24.23	−27.44	−24.24	−27.56
ERE	−25.81	−29.19	−25.72	−29.16
JOP	−26.03	−29.46	−25.93	−29.43
MIA	−26.30	−30.05	−26.17	−29.77
TET	−30.61	−30.13	−30.33	−29.88
OXY	−30.95	−30.32	−30.89	−30.27
CHL	−31.05	−30.38	−31.14	−30.50
SNM	−31.09	−32.84	−31.17	−32.87
SGD	−31.58	−33.28	−31.44	−33.10
SDZ	−31.98	−33.63	−32.04	−33.68
SMZ	−32.19	−33.82	−32.27	−33.85

[a] The unit of ΔG is kJ·mol^{-1}.

Table S3 Residues of BSA towards functional groups at R_1 ~ R_4 in macrolides [a]

Antibiotics	Residues of BSA
ERY	–
KIT	Val240, Arg256, Leu237and Ile263
MID	Arg194, Phe222, Leu233, Glu291 and Trp213
JOS	Thr190, Leu218, Lys221, Phe222, Ala290 and Glu291
ERE	Ser191, Phe222, Leu237, Val240, Arg256, Ser286 and Ala290
JOP	Phe148, Ser192, Gln195, Trp213, Leu218, Lys221, Phe222, His241, Leu259, Ser286 and Ile289
MIA	Glu152, Tyr156, Thr190, Ser191, Phe222, Leu233, Arg256, Leu29, Leu274, His287, Ala290, Glu291 and Val292

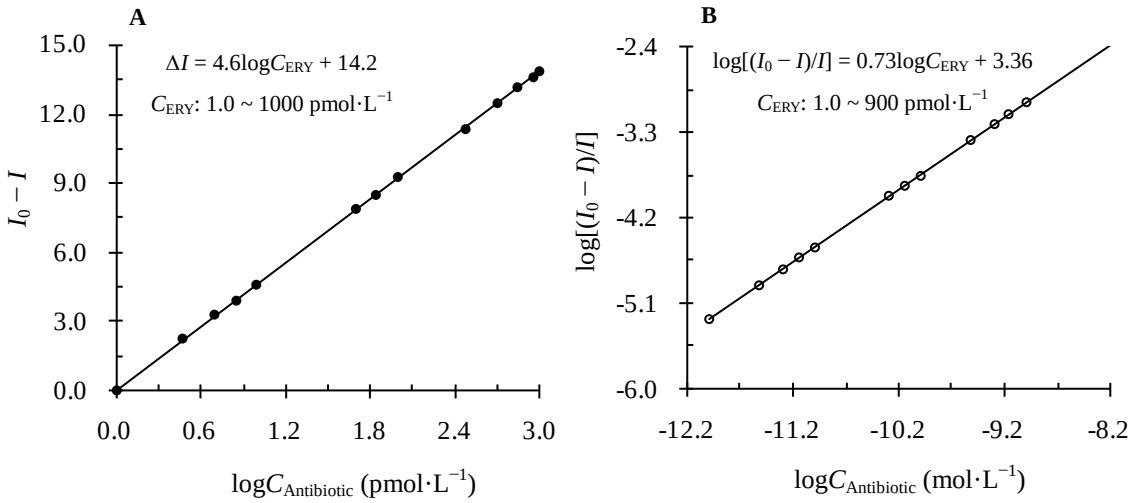
[a] Residues involved in hydrophobic interaction.

Referees' concerns

Concern 1:

In this work, the linear equation is $\Delta I = A\log C_{\text{Antibiotic}} + B$, with corresponding antibiotic concentration linear ranges from 1.0 to 5000 pmol·L^{-1} (see **Table 1**); the homemade FI-CL model, the linear equation is $\log[(I_0 - I)/I] = \log K + n\log C_{\text{Antibiotic}}$, with corresponding antibiotic concentration linear ranges from 1.0 to 3500 pmol·L^{-1} (see **Table 2**), and the binding constant K could be obtained from the intercept log$K$.

By taking ERY to BSA as an example, the linear plots of ΔI *vs* $\log C_{\text{Antibiotic}}$ (pmol·L^{-1}) and $\log[(I_0 - I)/I]$ *vs* $\log C_{\text{Antibiotic}}$ (mol·L^{-1}) are shown in the following figures **A** and **B** (correlation coefficient r > 0.99), respectively; and the K of 2.30×10^3 L·mol^{-1} for ERY to BSA could be obtained from the intercept (log$K$: 3.36).



Concern 2:

It is known that AutoDock 4.2 is a popular tool to predict and model protein–ligand interaction offering visual results with good docking accuracy and reliability (the evaluation about AutoDock 4.2 can be got by the link of http://autodock.scripps.edu/ and refs of A. P. Norgan, et al., *J. Cheminformatics*, **2011**, 3, 12; S. F. Sousa, et al.,

Proteins, **2006**, 65, 15). For example, the binding sites and modes of BSA, marine alkaline protease and human serum albumin–ligands interactions have been reported by the refs: N. Dash, et al., *J. Pharmaceut. Biomed. Anal.*, **2013**, 77, 55; X. F. Ji, et al., *J. Mol. Graph. Model.*, **2013**, 46, 125; A. Garg, et al., *PLoS One*, **2013**, 8, e63805; D. Xiao, et al., *J. Lumin.*, **2014**, 146, 218; the description about the H-bond about BSA–ligand interaction has been discussed by the refs: M. Sarkar, et al., *J. Lumin.*, **2013**, 142, 220; X. Zhang, et al., *PLoS One*, **2013**, 8, e59106; H. L. Xu, et al., *Int. J. Mol. Sci.*, **2013**, 14, 14185; N. Shahabadi and S. M. Fili, *Spectrochim. Acta A: Mol. Biomol. Spectrosc.*, **2014**, 118, 422.

Concern 3:

It is a fact that chemiluminescence analysis and fluorescence spectroscopy are very different.

Based on the **intrinsic fluorescence of BSA** ($\lambda_{Ex}/\lambda_{Em}$ = 280 nm/340 nm), the FQ equation, $\log[(F_0 - F)/F] = \log K + n\log C_{Antibiotic}$, corresponded to the antibiotic concentration linear ranges between **0.05 and 5.0 μmol·L^{-1}** (see **Table S1**).

Based on the **chemiluminescence from luminol with BSA reaction**, the linear equation, $\log[(I_0 - I)/I] = \log K + n\log C_{Antibiotic}$, corresponded to the antibiotic concentration linear ranges between **1.0 and 3500 pmol·L^{-1}** (see **Table 2**).

It is clear that the **binding constants *K*** of antibiotics to BSA by FQ and the proposed method have no significant differences; and the sensitivity of this proposed FI-CL procedure is higher than that of FQ method at least three orders of magnitudes.

The details about the derivation of the homemade FI-CL model are given as follows:

Luminol and protein form the complex of $P^{\cdots}L^{*}$ (*P*: protein, *L*: luminol) which produces the steady CL

intensity; the process is expressed as

$$P + L \underset{}{\overset{K_P}{\rightleftharpoons}} P\cdots L^* \longrightarrow P\cdots L + h\upsilon \quad (\lambda_{max}: 425\ nm) \tag{1}$$

where K_P is the binding constant of protein with luminol.

In the presence of ligand, the interaction of protein and ligand leads to the formation of a ternary complex D_n···P···L (D refers to the ligand), with the binding process described as

$$n\ D + P\cdots L^* \underset{}{\overset{K}{\rightleftharpoons}} D_n\cdots P\cdots L \tag{2}$$

where K and n are the binding constant and the number of binding sites of ligand to protein, respectively.

Herein, the K can be expressed as

$$K = \frac{[D_n\cdots P\cdots L]}{[D]^n[P\cdots L^*]} \tag{3}$$

which equals to

$$K[D]^n = \frac{[D_n\cdots P\cdots L]}{[P\cdots L^*]} \tag{4}$$

According to chemical reaction equilibrium theory

$$[P\cdots L^*]_0 = [D_n\cdots P\cdots L] + [P\cdots L^*] \tag{5}$$

Using equations (4) and (5), there is

$$K\ [D]^n = \frac{[P\cdots L^*]_0 - [P\cdots L^*]}{[P\cdots L^*]} \tag{6}$$

Taking logarithms of both sides, the following equation is given

$$\log K + n\log[D] = \log\frac{[P\cdots L^*]_0 - [P\cdots L^*]}{[P\cdots L^*]} \tag{7}$$

D_n···P···L does not emit photon, the mole fraction of unassociated luminol–protein is then expressed by the corresponding CL intensity

$$\frac{I}{I_0} = \frac{[P\cdots L^*]}{[P\cdots L^*]_0} \tag{8}$$

Equation (8) equals to

$$\frac{I_0 - I}{I} = \frac{[P\cdots L^*]_0 - [P\cdots L^*]}{[P\cdots L^*]} \tag{9}$$

By substituting equation (9) into equation (7), the homemade FI-CL model is obtained

$$\log\frac{I_0 - I}{I} = \log K + n\log[D] \quad (10)$$

where the K and n are the binding constant and the number of binding sites of protein with ligand, the I_0 and I are the CL intensity from luminol–protein system in the absence and presence of ligand, and [D] is the ligand concentration.

By plotting log[(I_0 − I)/I] against log[D], the binding constant K could be obtained from the intercept log$K$.

This work with homemade FI-CL model was published in *Analyst*, **2010**, 135, 2546 and *Talanta*, **2010**, 83, 312.