

Supplementary material for the article:

# **A multi-spectroscopic and molecular docking analysis of the biophysical interaction between food polyphenols, urolithins, and human serum albumin**

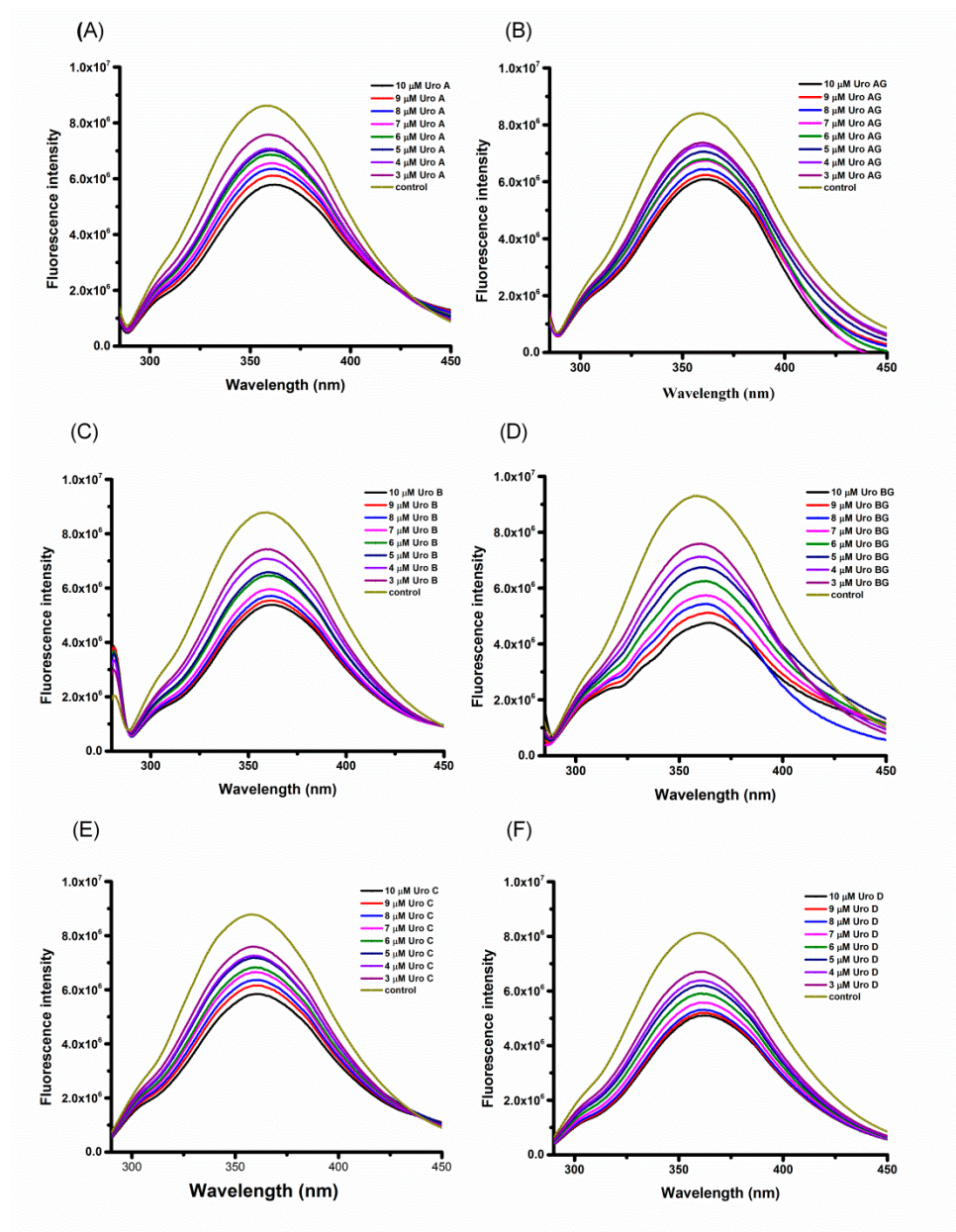
Nevena Zelenović<sup>1</sup>, Predrag Ristić<sup>2</sup>, Natalija Polović<sup>2</sup>, Tamara Todorović<sup>2</sup>, Milica Kojadinović<sup>3</sup>, Milica Popović<sup>2\*</sup>

<sup>1</sup> University of Belgrade-Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, 11000, Belgrade, Serbia;

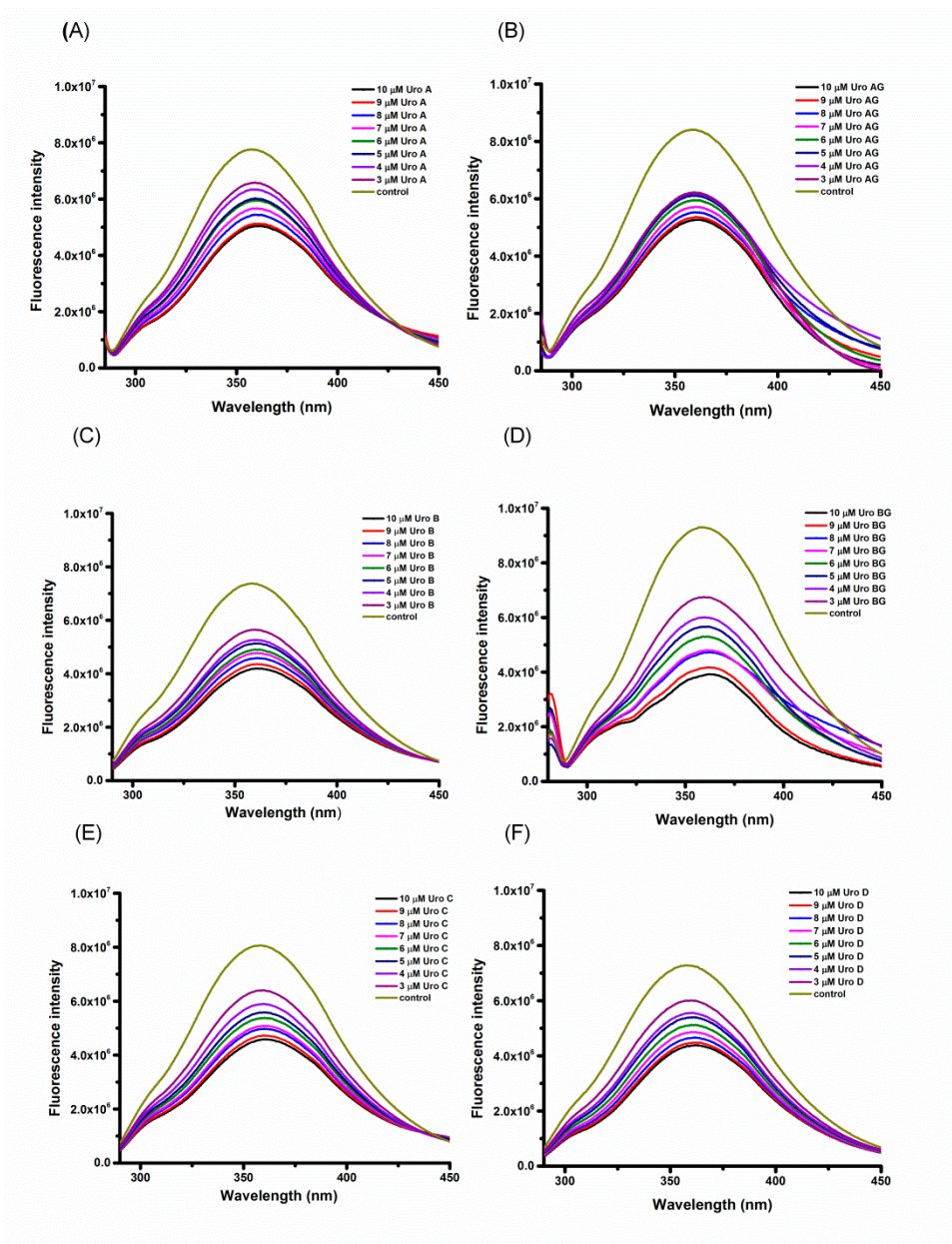
<sup>2</sup> University of Belgrade-Faculty of Chemistry, Studentski trg 12-16, 11000, Belgrade, Serbia;

<sup>3</sup> University of Belgrade-Institute of Medical Research, National Institute of the Republic of Serbia, Tadeuša Košćuška 1, 11000, Belgrade, Serbia

\* Correspondence: [la\\_bioquimica@chem.bg.ac.rs](mailto:la_bioquimica@chem.bg.ac.rs);



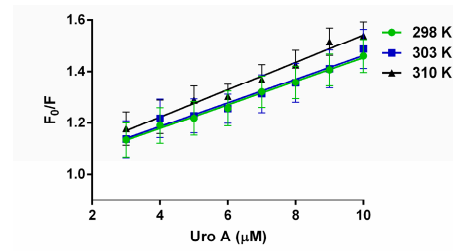
**Figure S1.** The fluorescence emission spectra of HSA in the presence of increasing concentration of URO A) URO A B) URO AG C) URO B D) URO BG E) URO C F) URO D at  $\lambda_{\text{ex}} = 280$  under conditions: pH = 7.4, T = 303 K, respectively. The HSA concentration was  $3 \times 10^{-6} \text{ mol L}^{-1}$  while the URO concentration was increased from  $3 \times 10^{-6} \text{ mol L}^{-1}$  to  $10 \times 10^{-6} \text{ mol L}^{-1}$  at increment of  $1 \times 10^{-6} \text{ mol L}^{-1}$ .



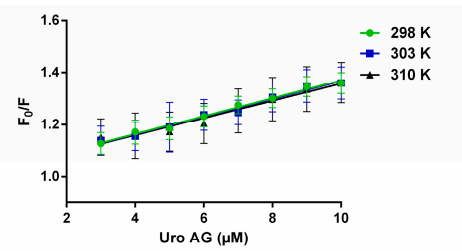


**Figure S2.** The fluorescence emission spectra of HSA in the presence of increasing concentration of URO A) URO A B) URO AG C) URO B D) URO BG E) URO C F) URO D at  $\lambda_{\text{ex}} = 280$  under conditions: pH = 7.4, T = 310 K, respectively. The HSA concentration was  $3 \times 10^{-6} \text{ mol L}^{-1}$  while the URO concentration was increased from  $3 \times 10^{-6} \text{ mol L}^{-1}$  to  $10 \times 10^{-6} \text{ mol L}^{-1}$  at increment of  $1 \times 10^{-6} \text{ mol L}^{-1}$ .

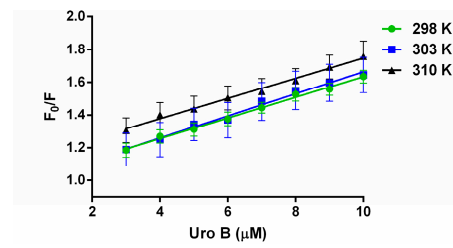
(A)



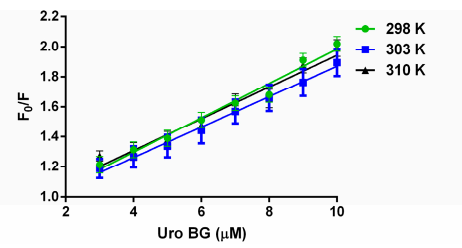
(B)



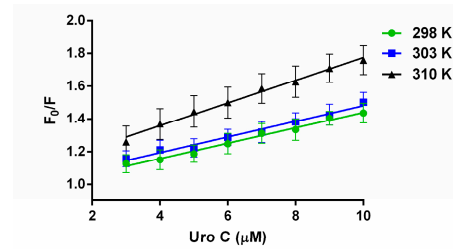
(C)



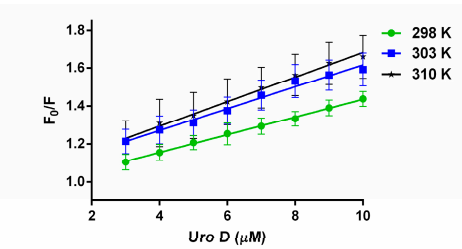
(D)



(E)

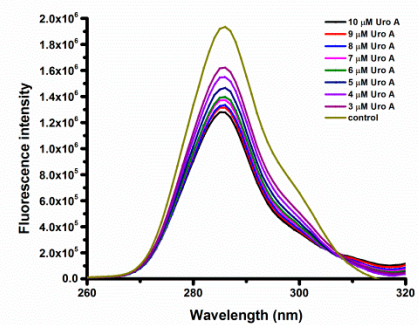


(F)

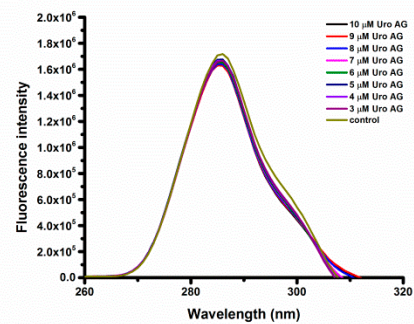


**Figure S3.** Stern-Volmer plots were generated to analyze the quenching of HSA by several compounds, namely **A)** URO **A** **B)** URO AG **C)** URO B **D)** URO BG **E)** URO C **F)** URO D, at temperatures of 298 K, 303 K and 310 K, and a pH value of 7.4. The HSA concentration was  $3 \times 10^{-6} \text{ mol L}^{-1}$  while the URO concentration was increased from  $3 \times 10^{-6} \text{ mol L}^{-1}$  to  $10 \times 10^{-6} \text{ mol L}^{-1}$  at increment of  $1 \times 10^{-6} \text{ mol L}^{-1}$ . Error bars indicate standard errors of triplicate measurements

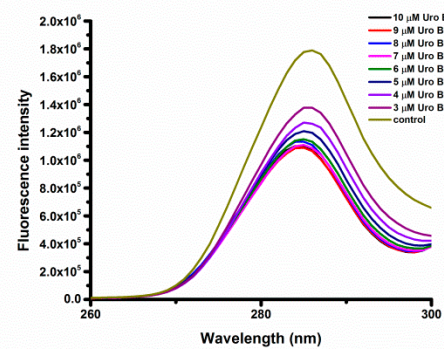
(A)



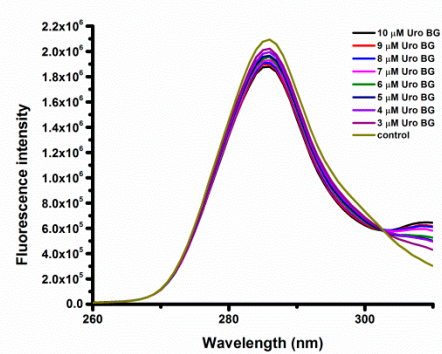
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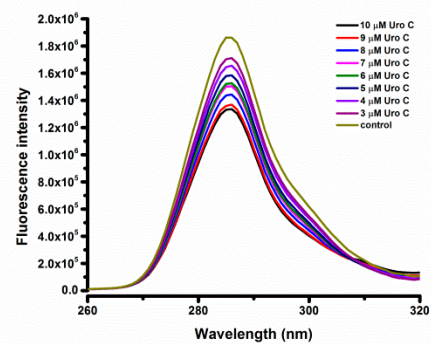
(C)



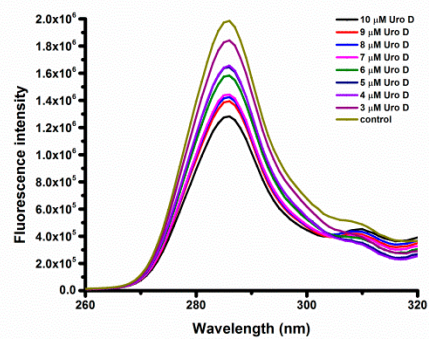
(D)



(E)

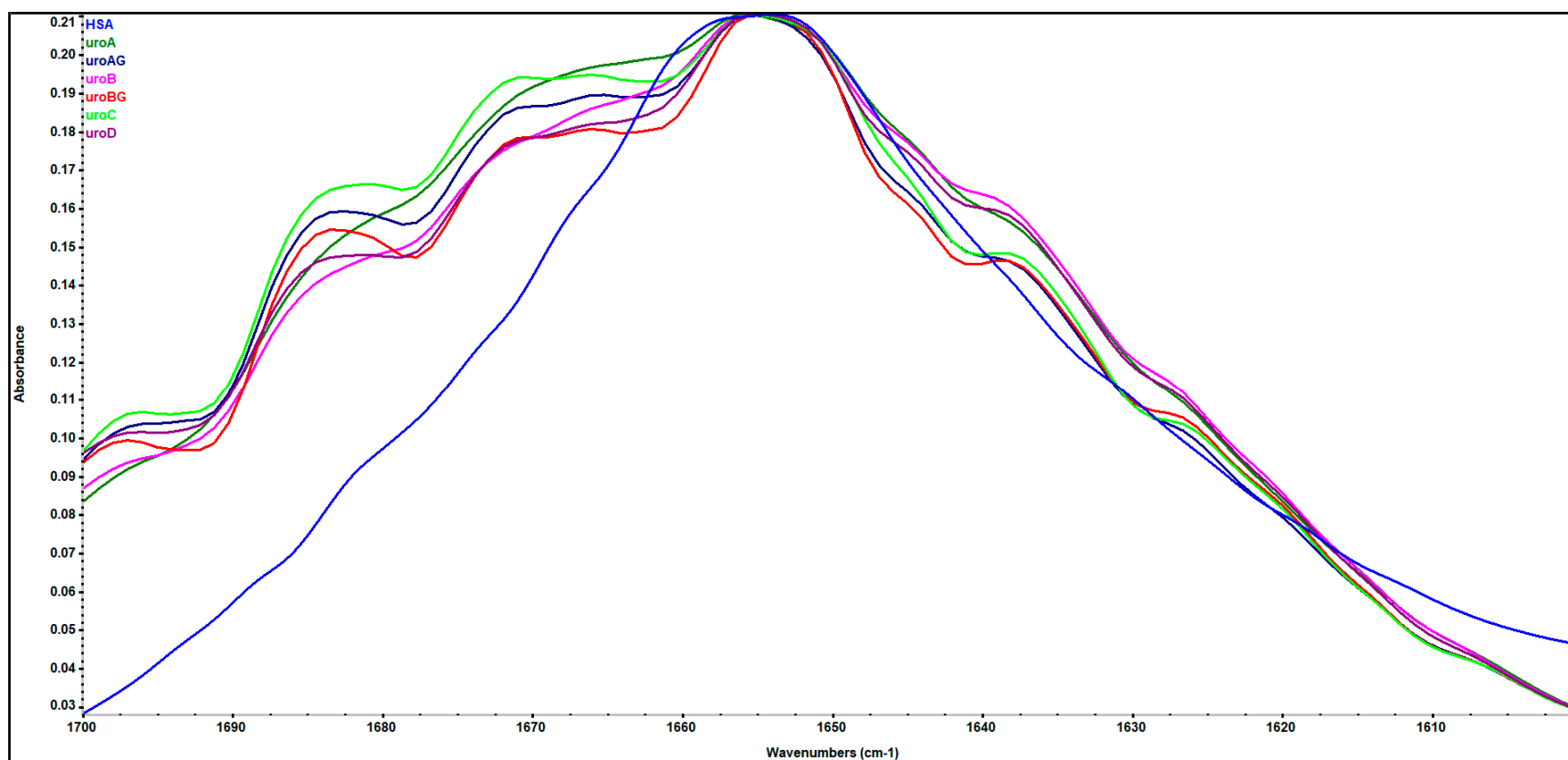


(F)

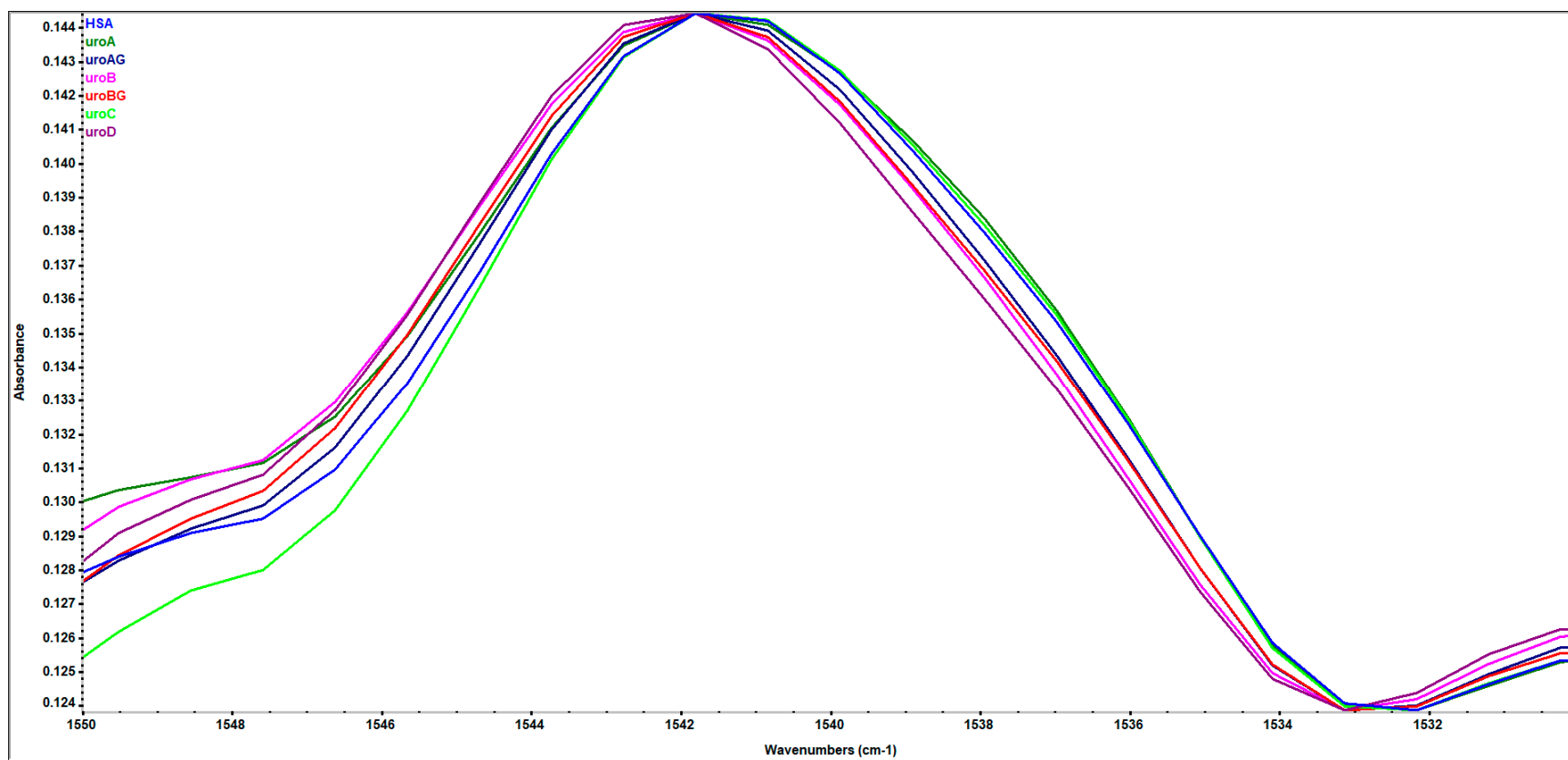




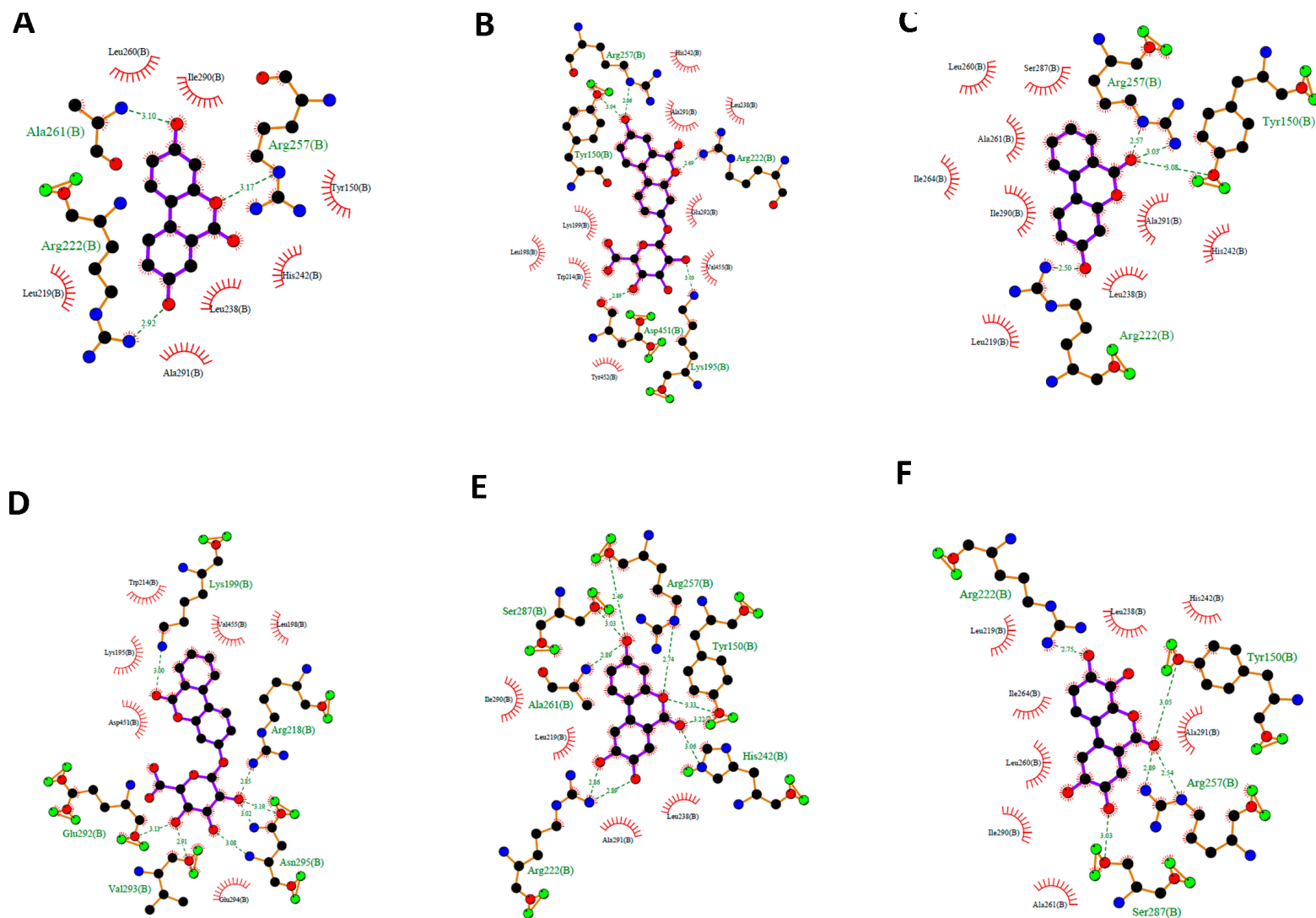
**Figure S4.** The impact of addition of increasing URO concentration on the synchronous fluorescence spectra of HSA at  $\Delta\lambda = 15$  nm: A) URO A; B) URO AG; C) URO B; D) URO BG; E) URO C; F) URO D. The HSA concentration was  $3 \times 10^{-6}$  mol L<sup>-1</sup>, while the URO concentrations ranged  $3 \times 10^{-6}$  -  $10 \times 10^{-6}$  mol L<sup>-1</sup> from top to bottom.



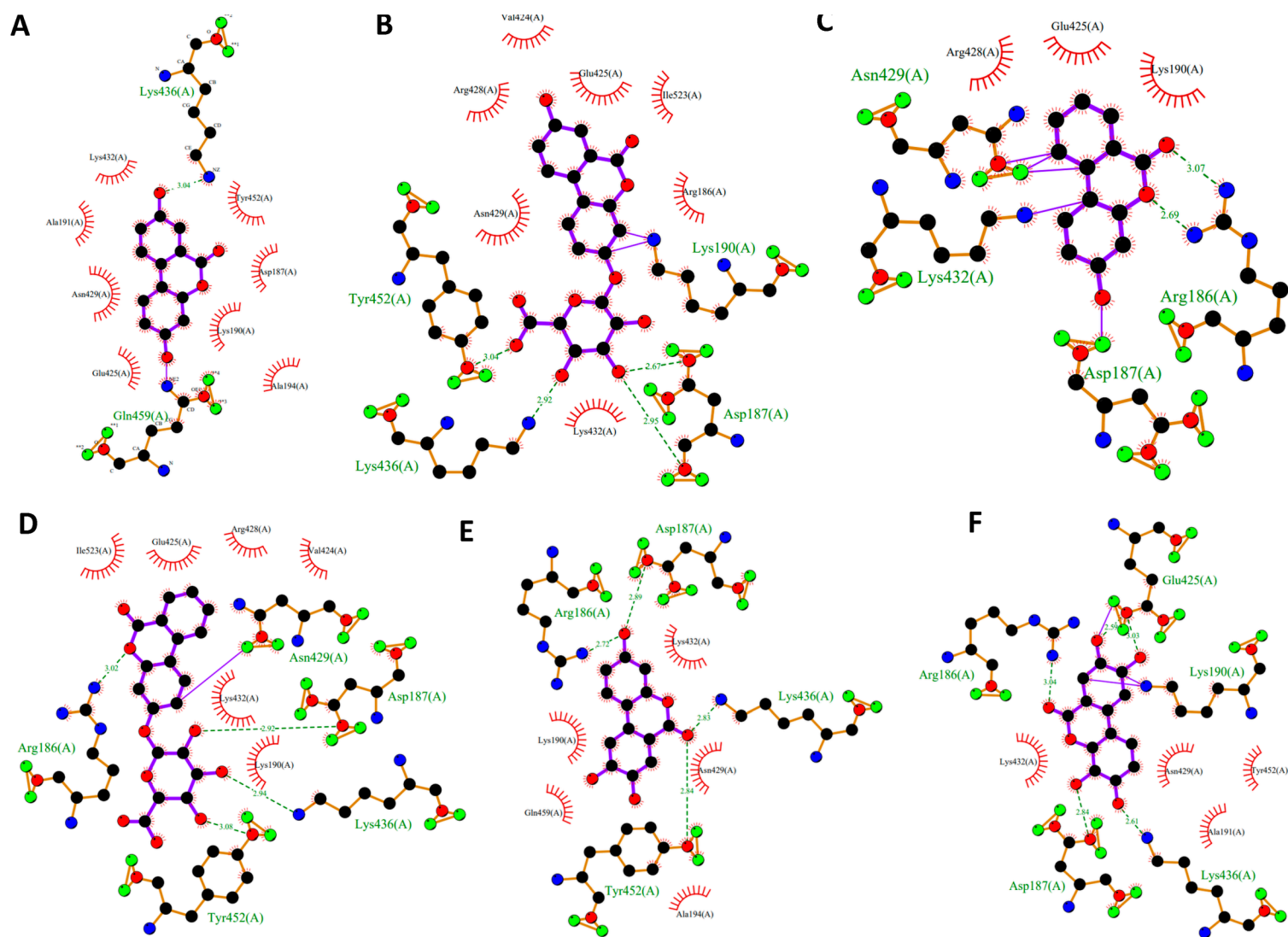
**Figure S5.** Amide I region of FTIR spectra of HSA in the absence and presence of URO (at pH 7.4)



**Figure S6.** Amide II region of FTIR spectra of HSA in the absence and presence of URO (at pH 7.4).

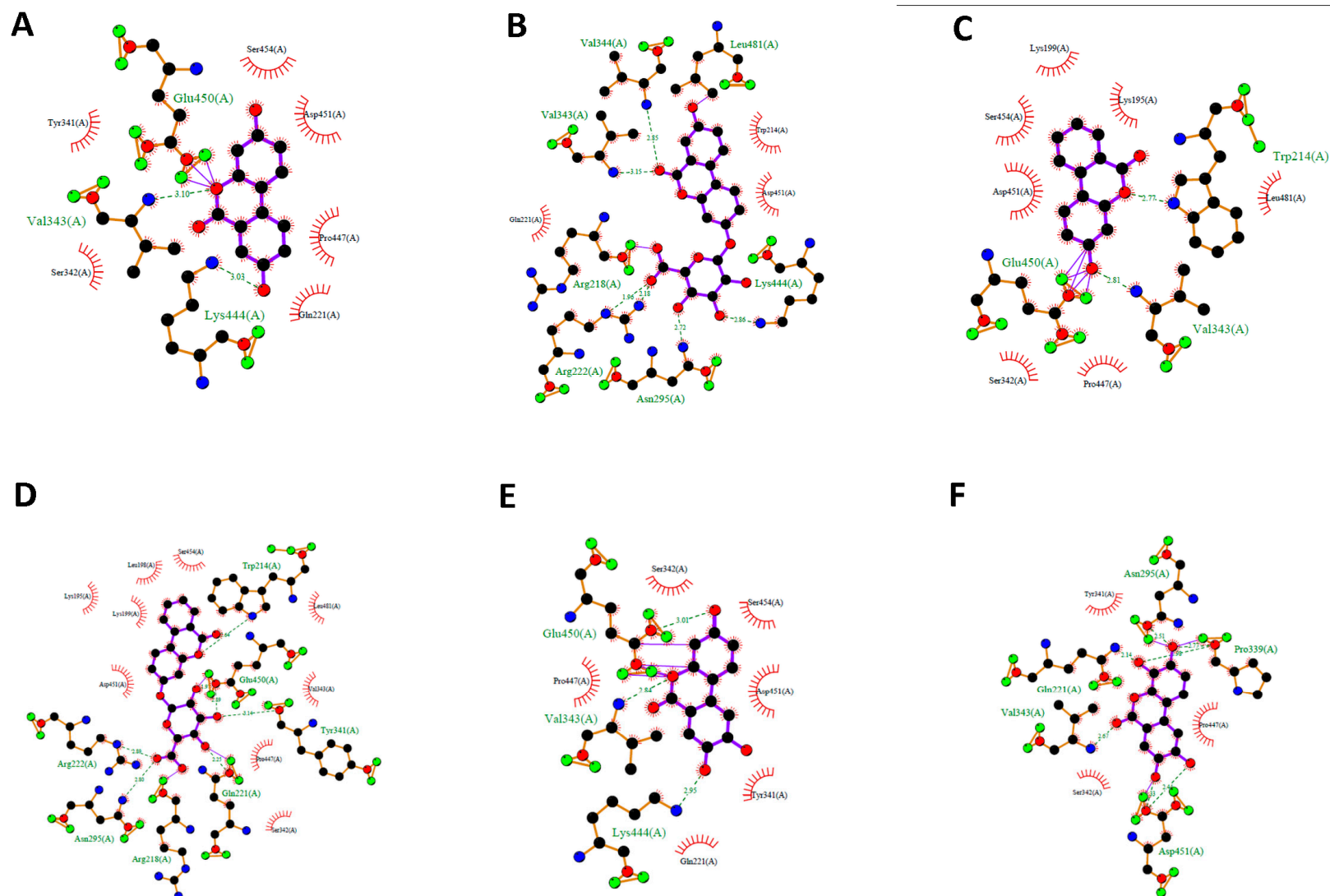


**Figure S7.** Schematic drawings of the interactions of the first GOLD cluster docked solutions of URO A (A), URO AG (B), URO B (C), URO BG (D), URO C (E) and URO D (F) @ Sudlow's site I of ligand-free HSA (PBD ID 1BM0) generated using LIGPLUS. Dashed lines are hydrogen bonds and 'eyelashes' show residues involved in hydrophobic interactions.

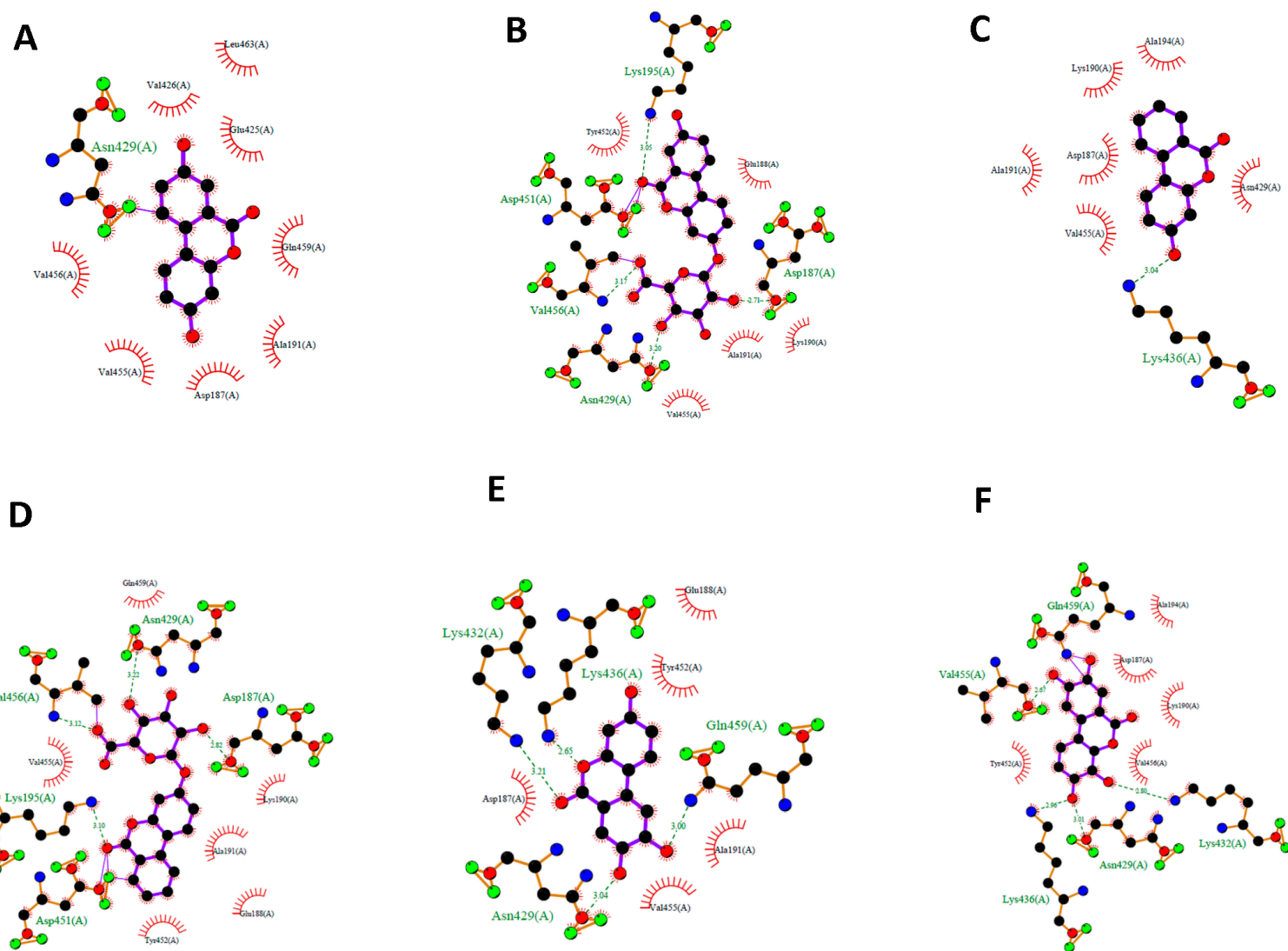


**Figure S8.** Schematic drawings of the interactions of the first GOLD cluster docked solutions of URO A (A), URO AG (B), URO B (C), URO BG (D), URO C (E) and URO D (F) @ FA9/cleft site of ligand-free HSA (PBD ID 1BM0) generated using LIGPLUS. Dashed lines are hydrogen bonds and ‘eyelashes’ show residues involved in hydrophobic interactions.

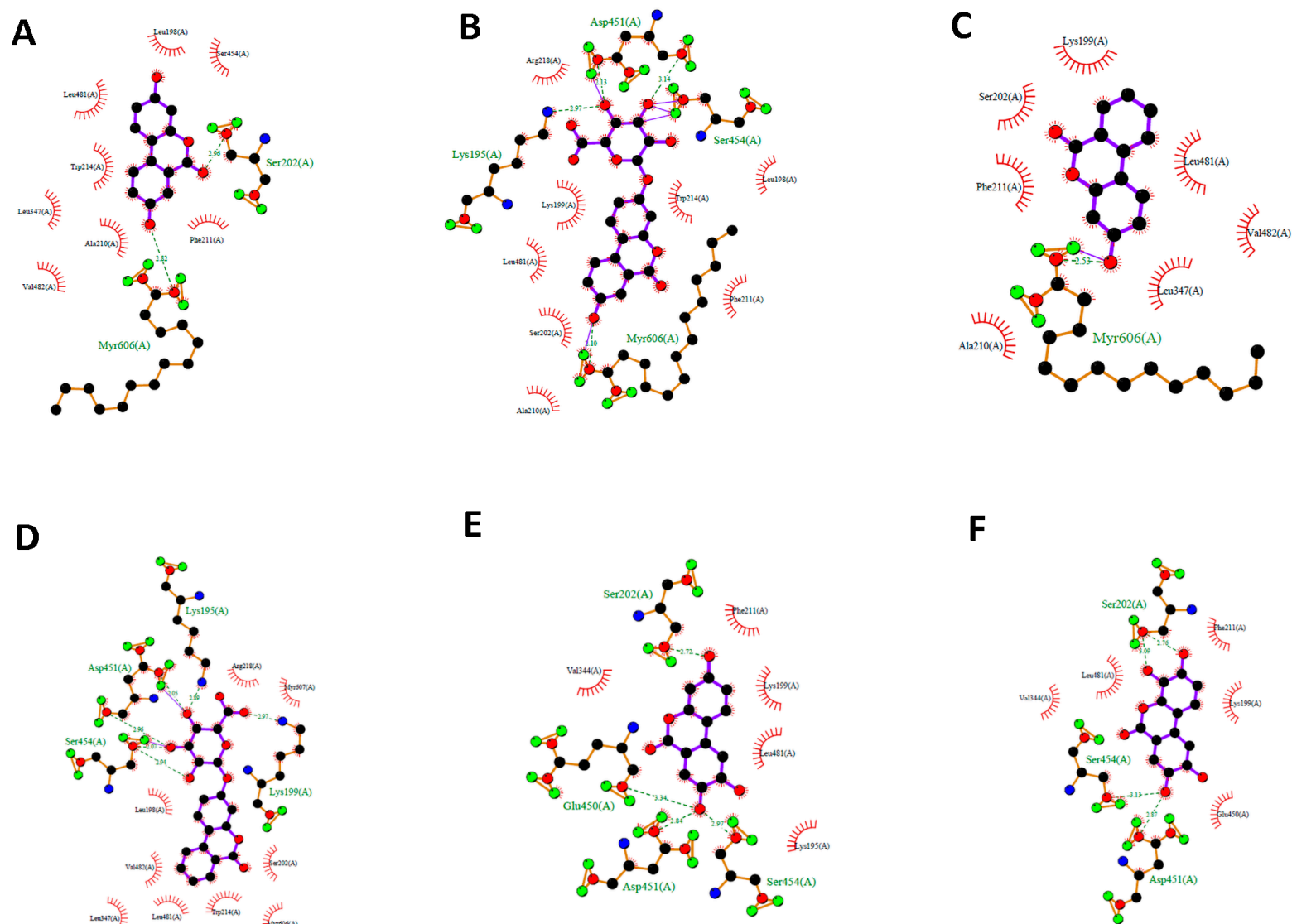




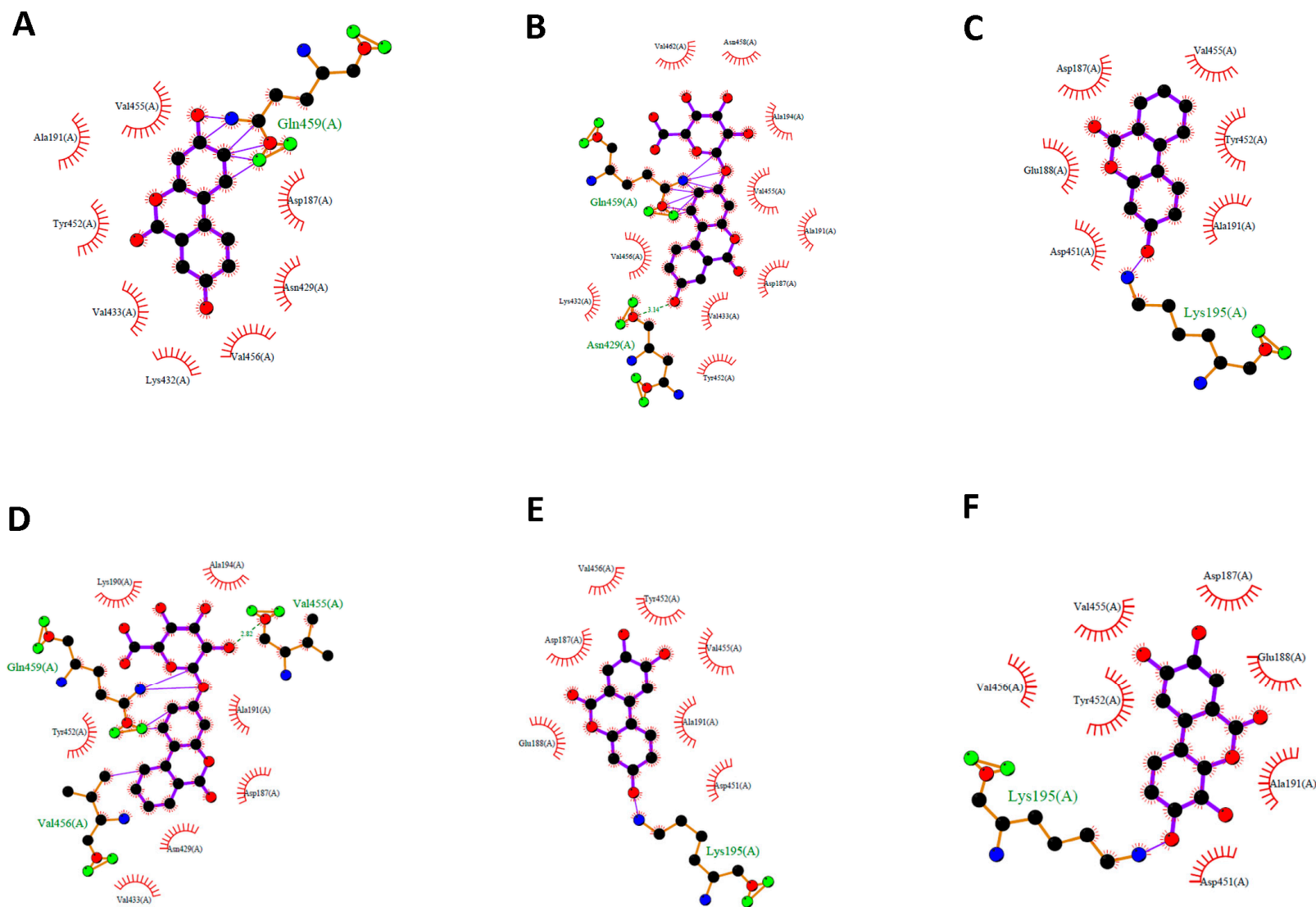
**Figure S9.** Schematic drawings of the interactions of the first GOLD cluster docked solutions of URO A (A), URO AG (B), URO B (C), URO BG (D), URO C (E) and URO D (F) @ FA8 site of heme-HSA (PBD ID 1N5U) generated using LIGPLUS. Dashed lines are hydrogen bonds and ‘eyelashes’ show residues involved in hydrophobic interactions.



**Figure S10.** Schematic drawings of the interactions of the first GOLD cluster docked solutions of URO A (A), URO AG (B), URO B (C), URO BG (D), URO C (E) and URO D (F) @ FA9/cleft site of heme-HSA (PBD ID 1N5U) generated using LIGPLUS. Dashed lines are hydrogen bonds and 'eyelashes' show residues involved in hydrophobic interactions.

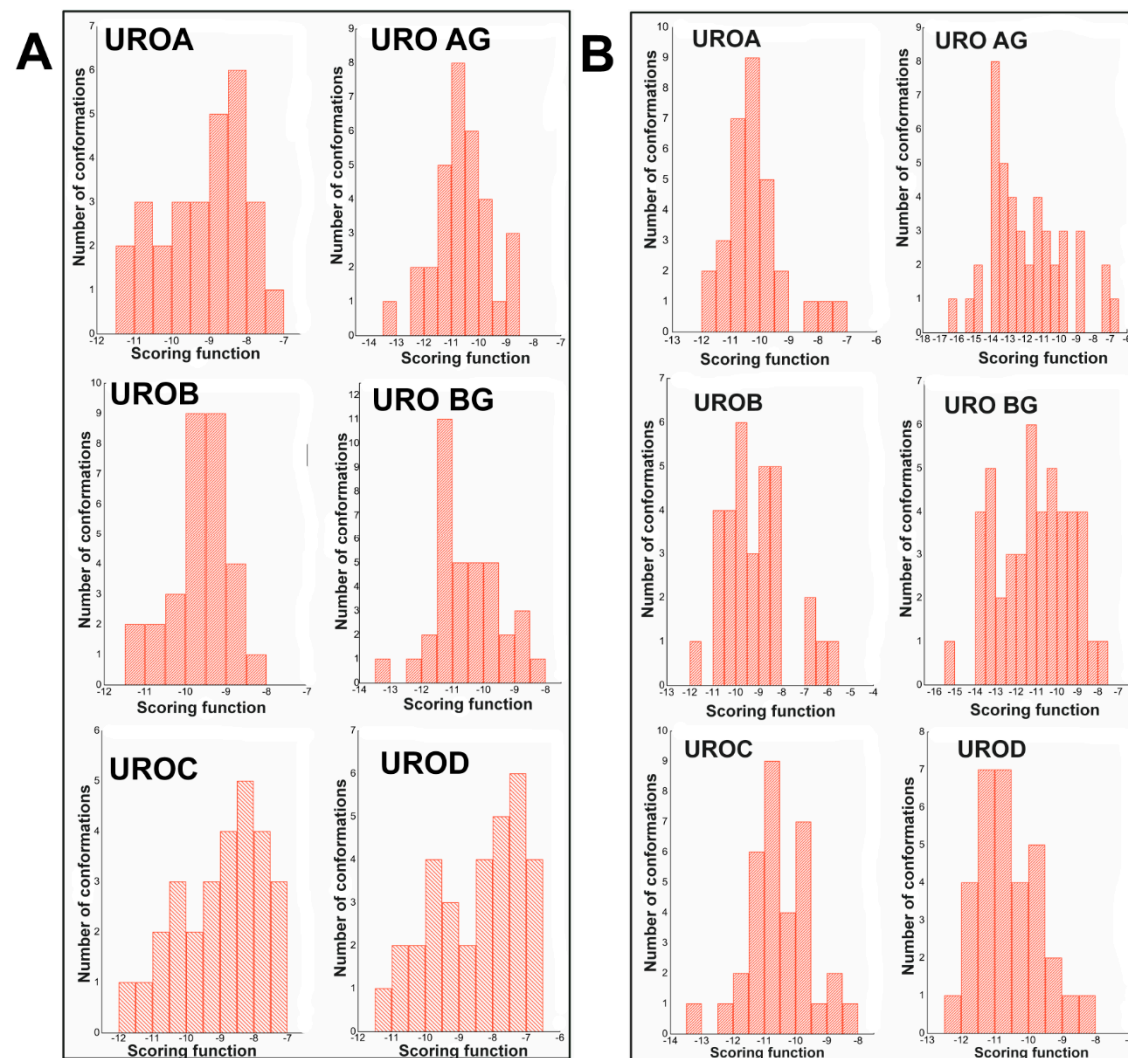


**Figure S11.** Schematic drawings of the interactions of the first GOLD cluster docked solutions of URO A (A), URO AG (B), URO B (C), URO BG (D), URO C (E) and URO D (F) @ FA8 site of FA-HSA (PBD ID 8RCF) generated using LIGPLUS. Dashed lines are hydrogen bonds and 'eyelashes' show residues involved in hydrophobic interactions.

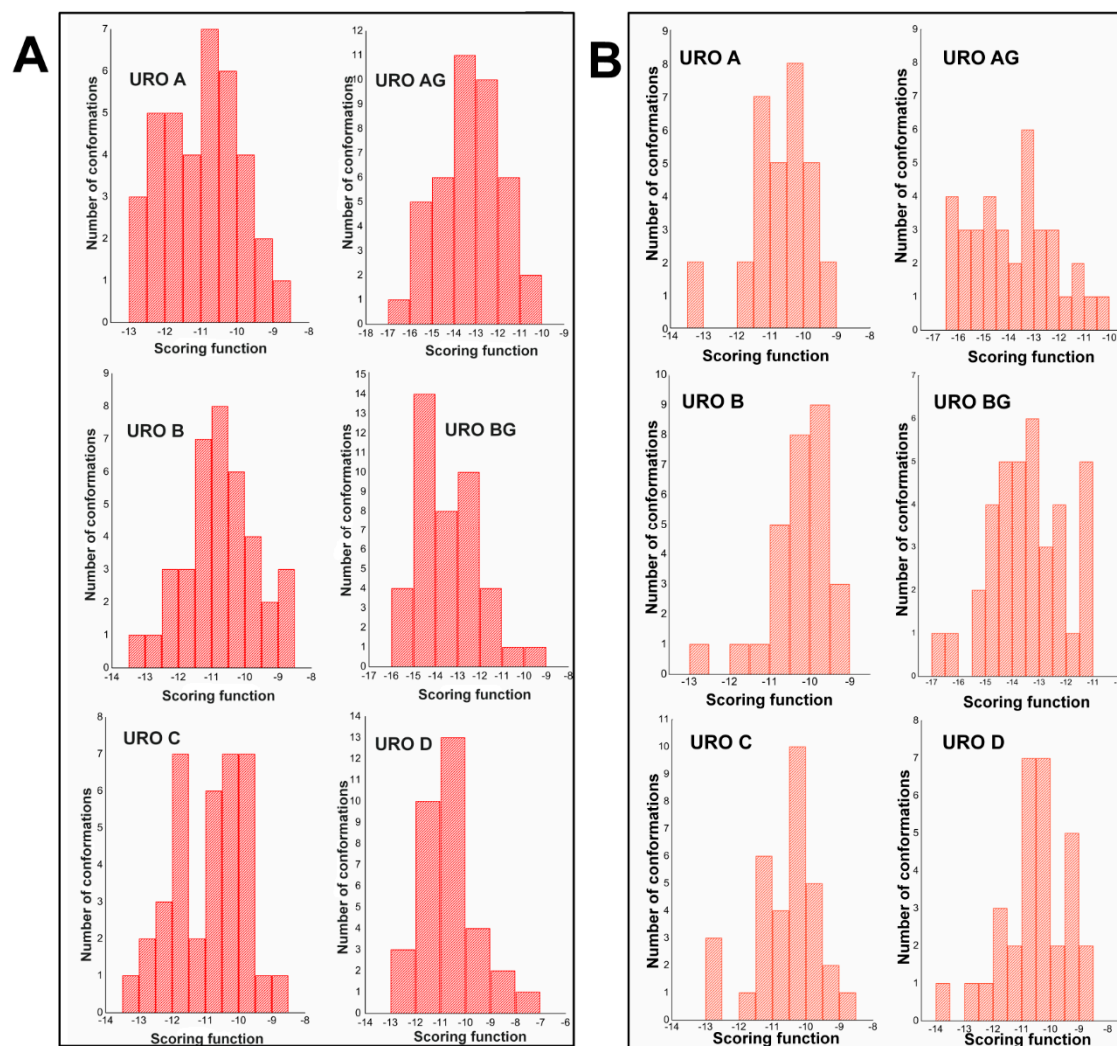


**Figure S12.** Schematic drawings of the interactions of the first GOLD cluster docked solutions of URO A (A), URO AG (B), URO B (C), URO BG (D), URO C (E) and URO D (F) @ FA9/cleft site of FA-HSA (PDB ID 8RCP) generated using LIGPLUS. Dashed lines are hydrogen bonds and ‘eyelashes’ show residues involved in hydrophobic interactions.

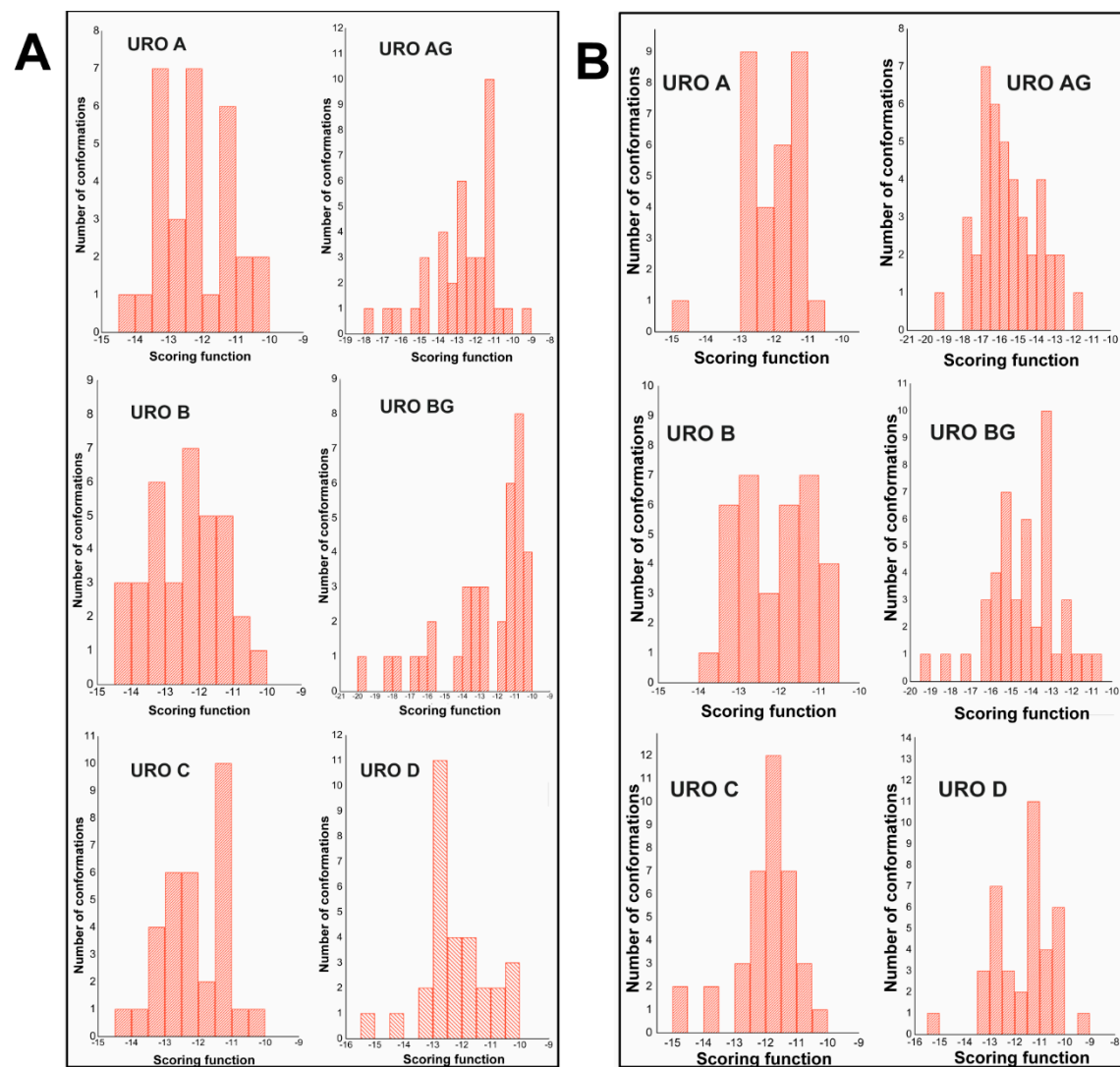




**Figure S13.** Cluster analysis of docking results of URO @ Sudlow's site I (A) and FA9/cleft site (B) of ligand-free HSA (PBD ID 1BM0) using a 1.0 Å RMSD.



**Figure S14.** Cluster analysis of docking results of URO @ FA8 (A) and FA9/cleft site (B) of heme-Fe(III)- and myristic acid-bound HSA HSA (PBD ID 1N5U) using a 1.0 Å RMSD.



**Figure S15.** Cluster analysis of docking results of URO @ FA8 (A) and FA9/cleft site (B) of myristic acid-bound HSA (PDB ID 8RCP) using a 1.0 Å RMSD.