ESI

A chiral probe for the acute phase proteins alpha-1-acid glycoprotein and alpha-1-antitrypsin based on europium luminescence

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1. Protein Structure Alignment

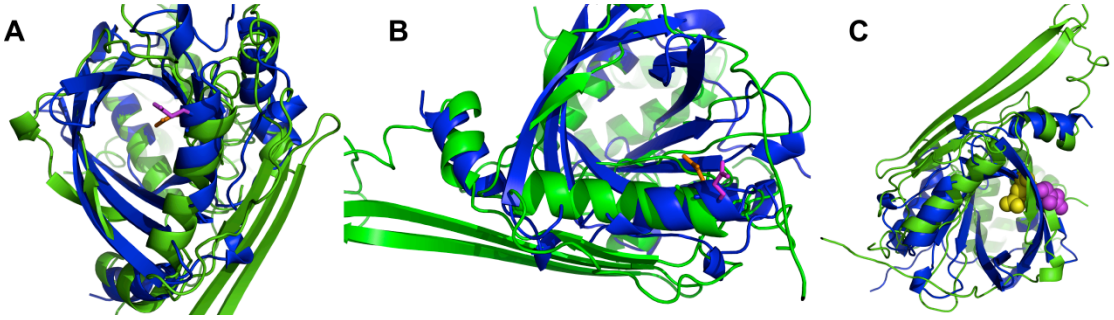
Flexible protein structure alignment was performed on representative PDB entries for α_1-AGP (3KQ0) and α_1-AAT (9API) using the FATCAT algorithm,[1] with the twist parameter *t* set to a maximum of 5.[2]

Although globally the two structures were not significantly similar, local structure alignments yielded 126 equivalent positions with a root mean square deviation of 3.72 Å for a value of $t = 4$. Amongst these, the location of two sets of glutamates is striking.

The first involves the second glutamate in the sequence NEEY of α_1-AGP (residues 34–37), which lies over the entrance to the hydrophobic binding pocket and in close proximity in the modelled structure to the second glutamate of the sequence TEEA from α_1-AAT (residues 150–153, ESI Fig. 1 A, B).

The second involves E64 of α_1-AGP, which lies on the face of the β-sheet in the hydrophobic binding pocket of this protein. In the modelled structure, E206 of α_1-AAT lies in close proximity to this residue, although there are differences in the side-chain orientations of these residues (ESI Fig. 1 C).

[1] Y. Ye and A. Godzik, *Nucleic Acids Res.*, 2004, **32**, W582–5.
[2] Y. Ye and A. Godzik, *Protein Sci.*, 2004, **13**, 1841–1850.



ESI Fig. 1. Structural superposition using FATCAT. In each case, α_1-AGP (3KQ0) is shown in blue, α_1-AAT (9API) is shown in green. (A) and (B), orthogonal views of the superposition of E36 of α_1-AGP (magenta) with E152 of α_1-AAT (orange); (C), showing the position of E64 of α_1-AGP (yellow) and E206 of α_1-AAT (magenta).

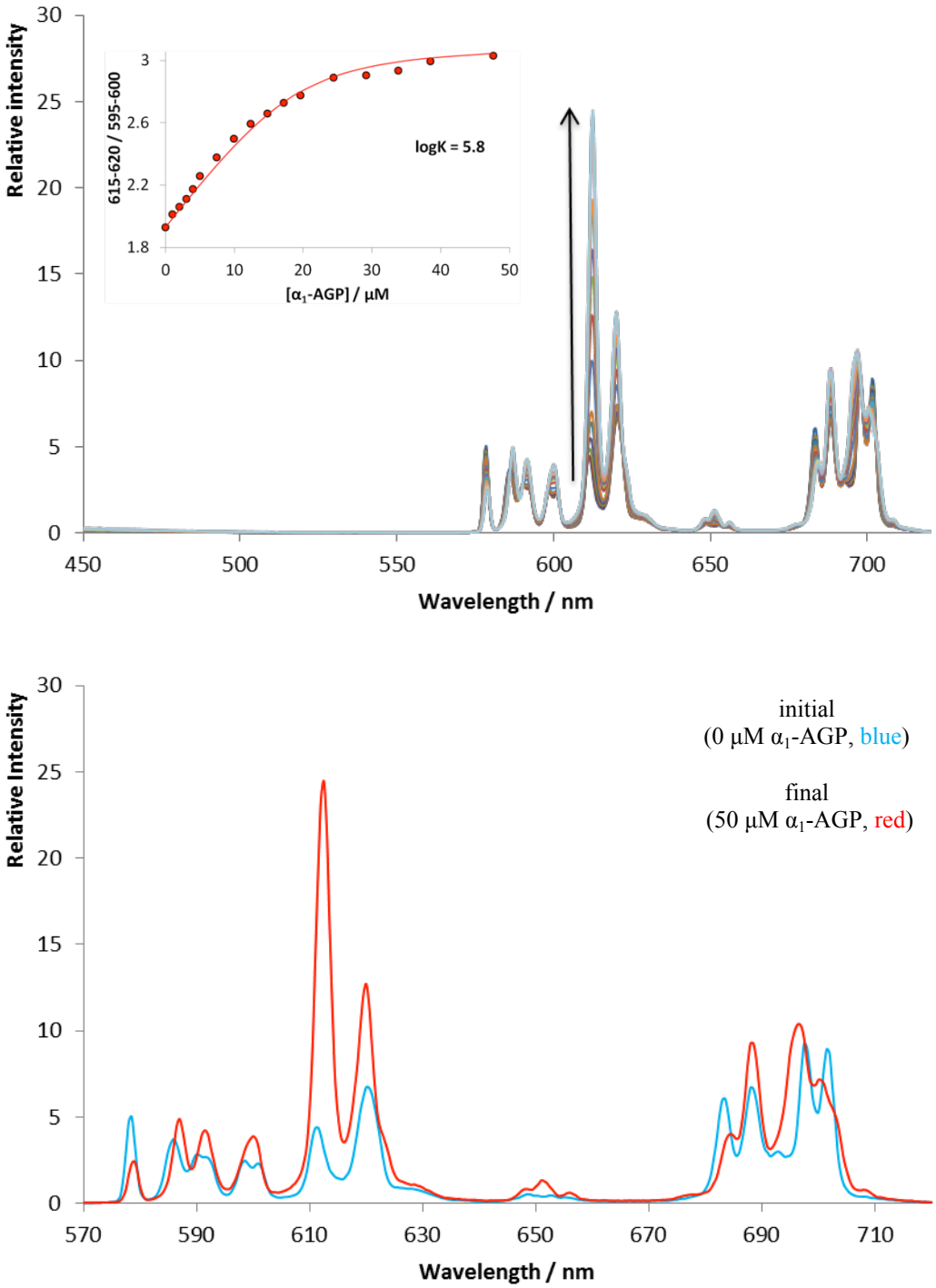
2. Experimental details of CPL and CD acquisition

Circularly polarised luminescence spectra (I_L-I_R) for $[Eu.L^1(OH_2)]^+$ were measured using a homebuilt CPL spectrometer (Glasgow University, UK) based on a Spex-Fluoromax-2-spectrofluorimeter. Samples were prepared using aqueous solutions containing 0.1 M NaCl in disposable UV-grade methacrylate cuvettes. The samples were pH adjusted to 7.4 using c. HCl and c.NaOH and then lyophilised for transportation. Samples were redissolved in D_2O and run at room temperature using indirect excitation at 340 nm.

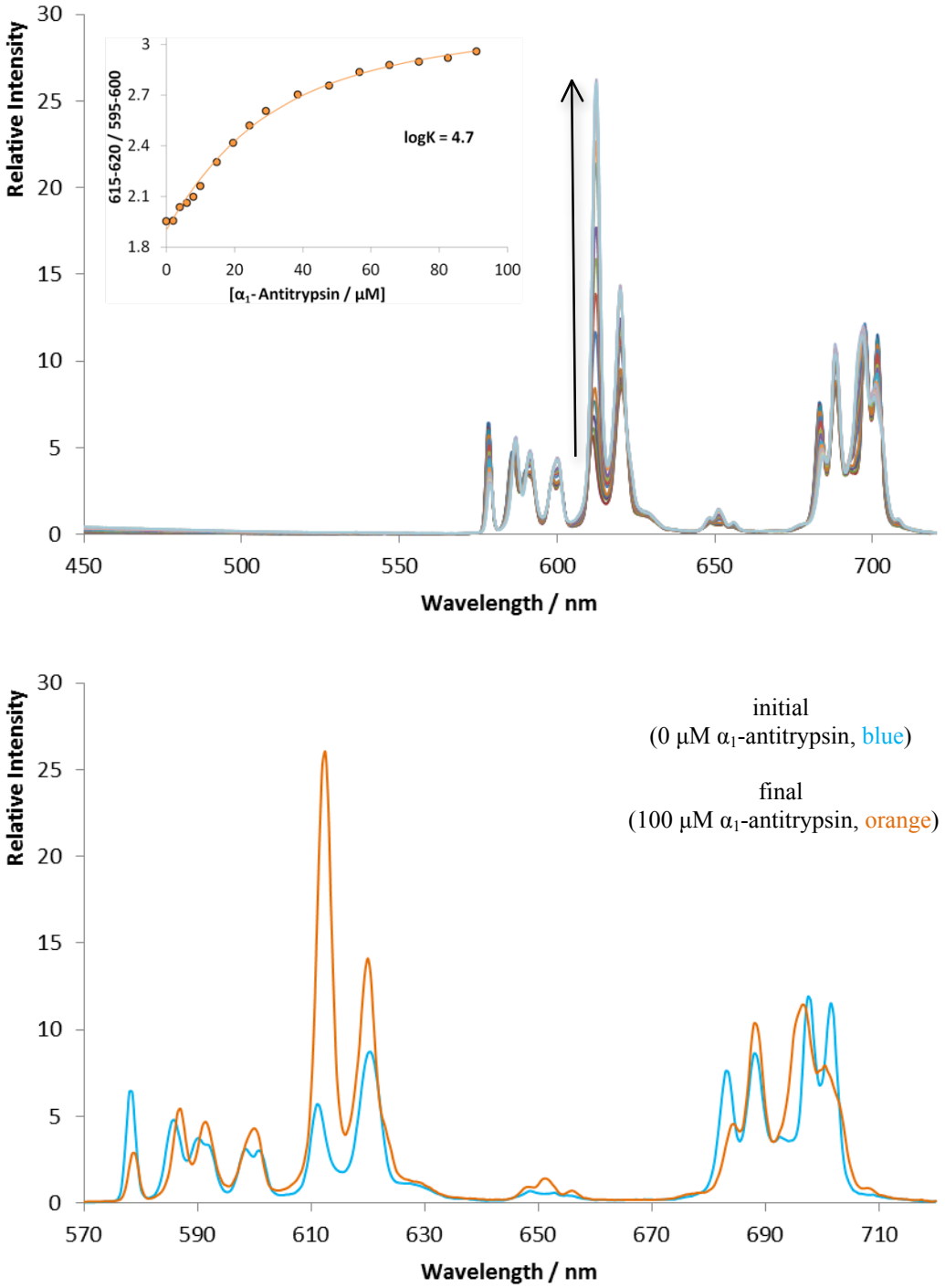
Absorption and ECD spectra obtained at Pisa were acquired on a JASCO V650 spectrophotometer and a JASCO J715 spectropolarimeter respectively. Samples were run in cylindrical quartz cells at room temperature. The band width was set to 2.0 nm, response to 1 sec and scan speed to 50 nm/min. Up to 4 accumulations were averaged to improve S/N. The total concentration of $[Eu.L^1(OH_2)]^+$ alone and in a 1:1 (mol/mol) mixture with α_1-AGP was 41.3 μM. In all cases the absorbance and dichroism data were scaled for the total concentration of $[Eu.L^1(OH_2)]^+$ to yield ε and Δε' (the prime indicates that the real concentration of the ECD-active species is uncertain).

3. Europium emission spectral titrations and data analysis

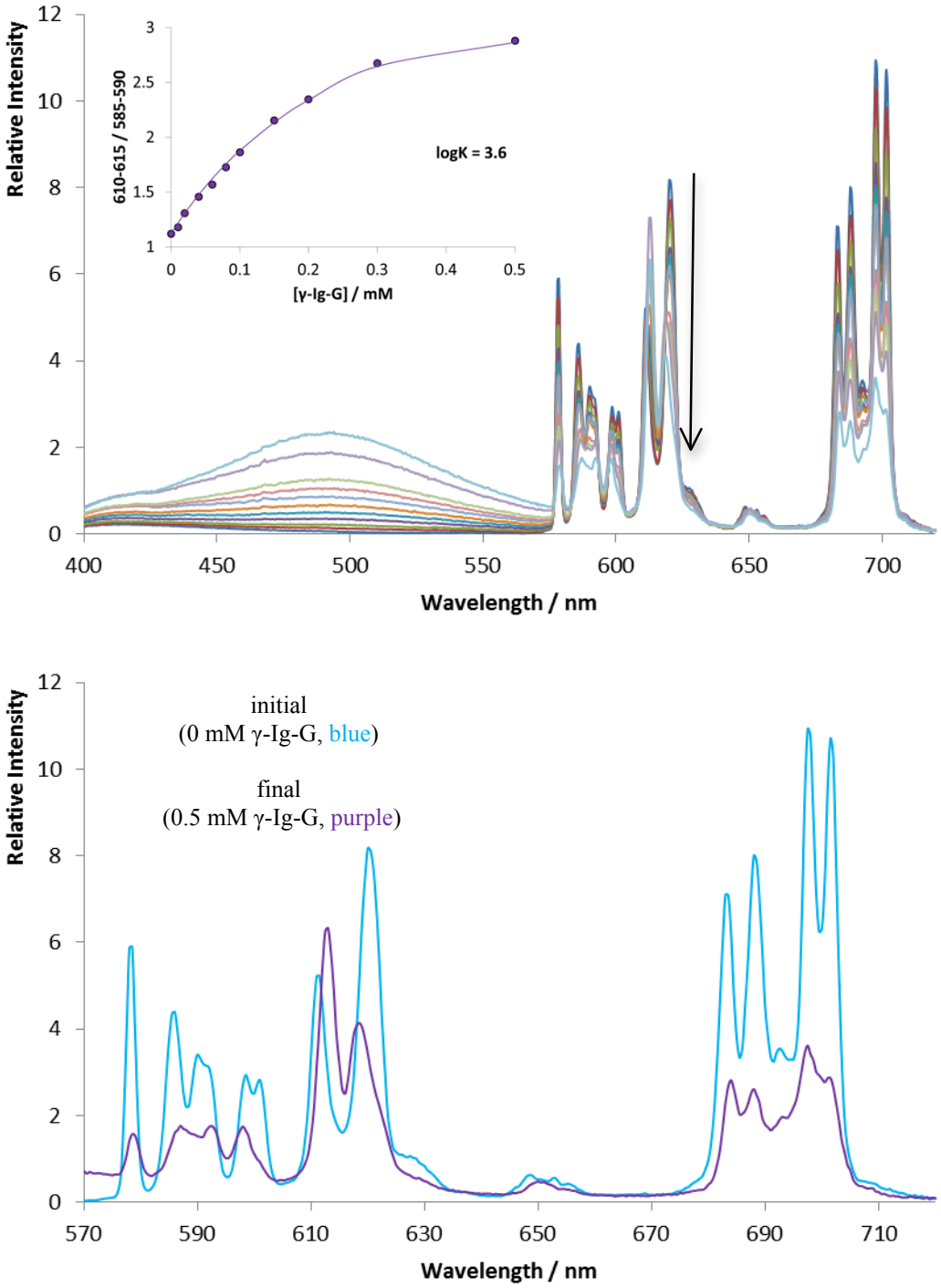
Emission spectra were recorded on an ISA Joblin-Yvon Spex Fluorolog-3 luminescence spectrometer. Quartz cuvettes with a pathlength of 1 cm were used to contain all samples. Luminescence titrations were carried out at pH 7.4 using aqueous solutions containing 0.1 M NaCl in order to maintain a constant ionic background. All proteins were purchased from Sigma. For α_1-AGP, Fibrinogen, apo-Transferrin and α_1-antitrypsin, volumetric addition of stock solutions was carried out. In the case of HSA and γ-Ig-G, the protein was added directly to the solution as a lyophilised solid. In each case, the pH was adjusted following protein addition. Data was analysed as described in reference 21.



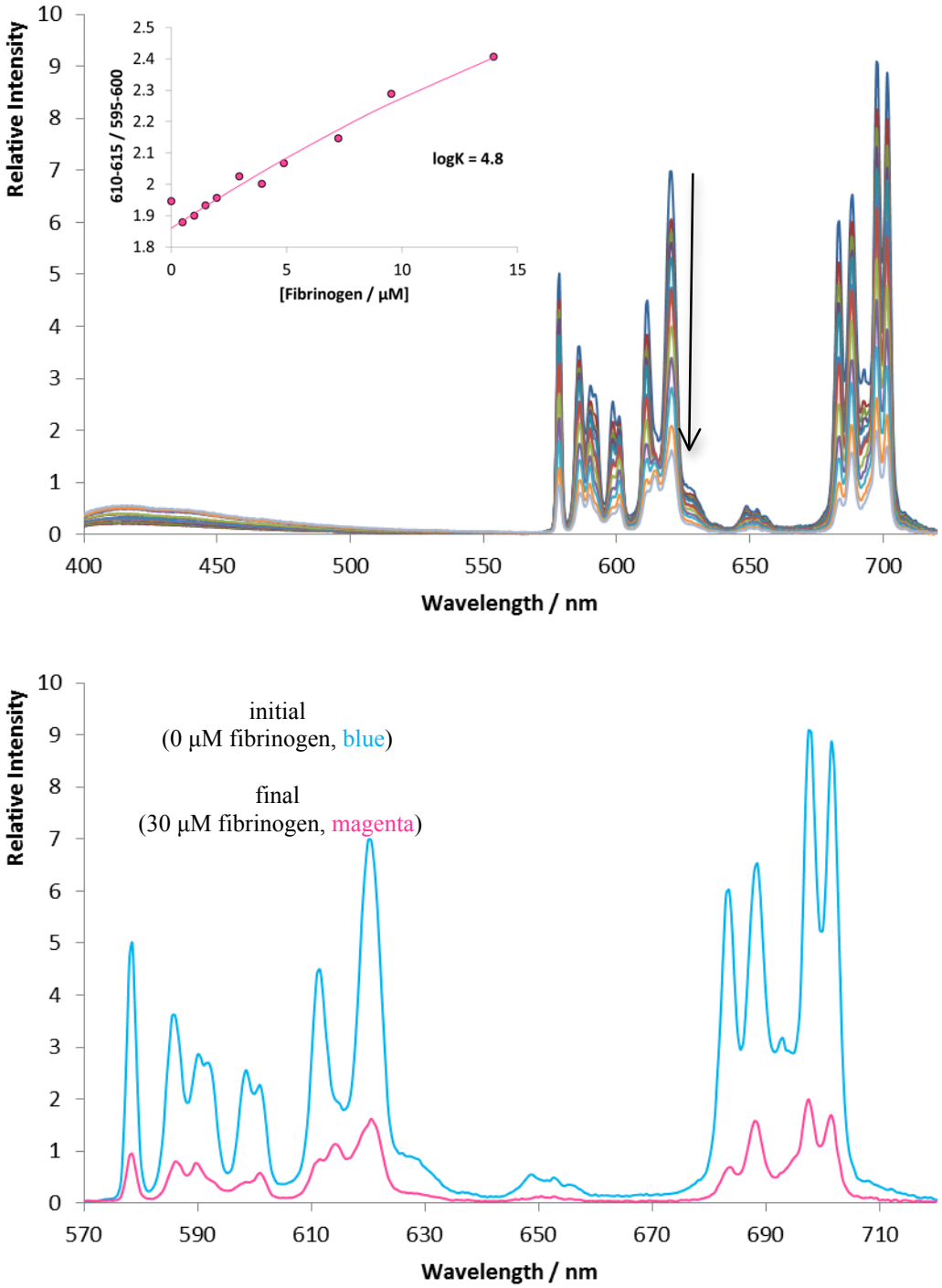
ESI Fig. 2. Europium emission spectral changes following addition of human α_1-AGP to $[Eu.L^1(OH_2)]^+$ (20 μM, 295 K, 0.1 M NaCl, pH 7.4).



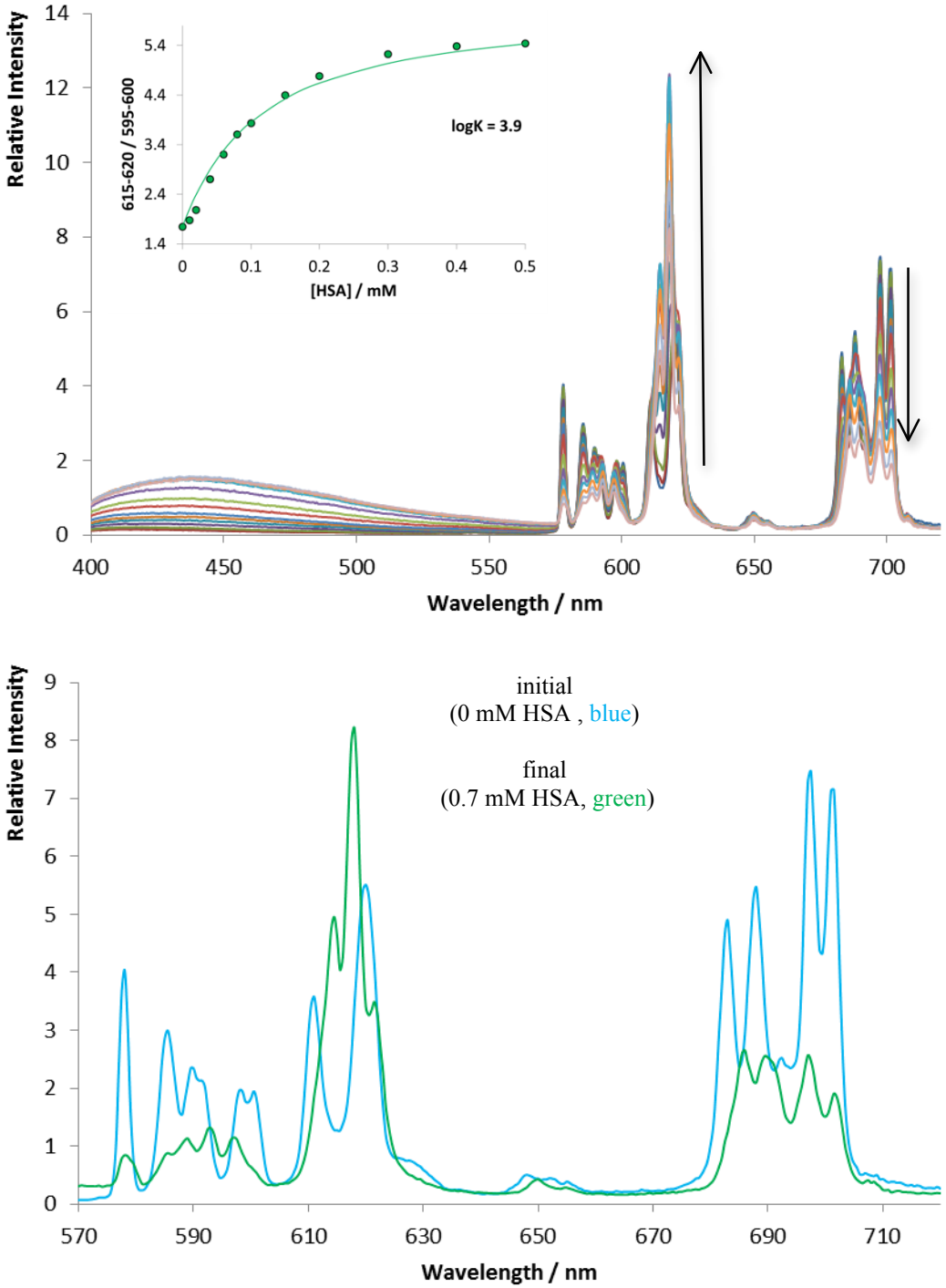
ESI Fig. 3. Europium emission spectral changes following addition of human α_1-antitrypsin to $[Eu.L^1(OH_2)]^+$ (20 μM, 295 K, 0.1 M NaCl, pH 7.4).



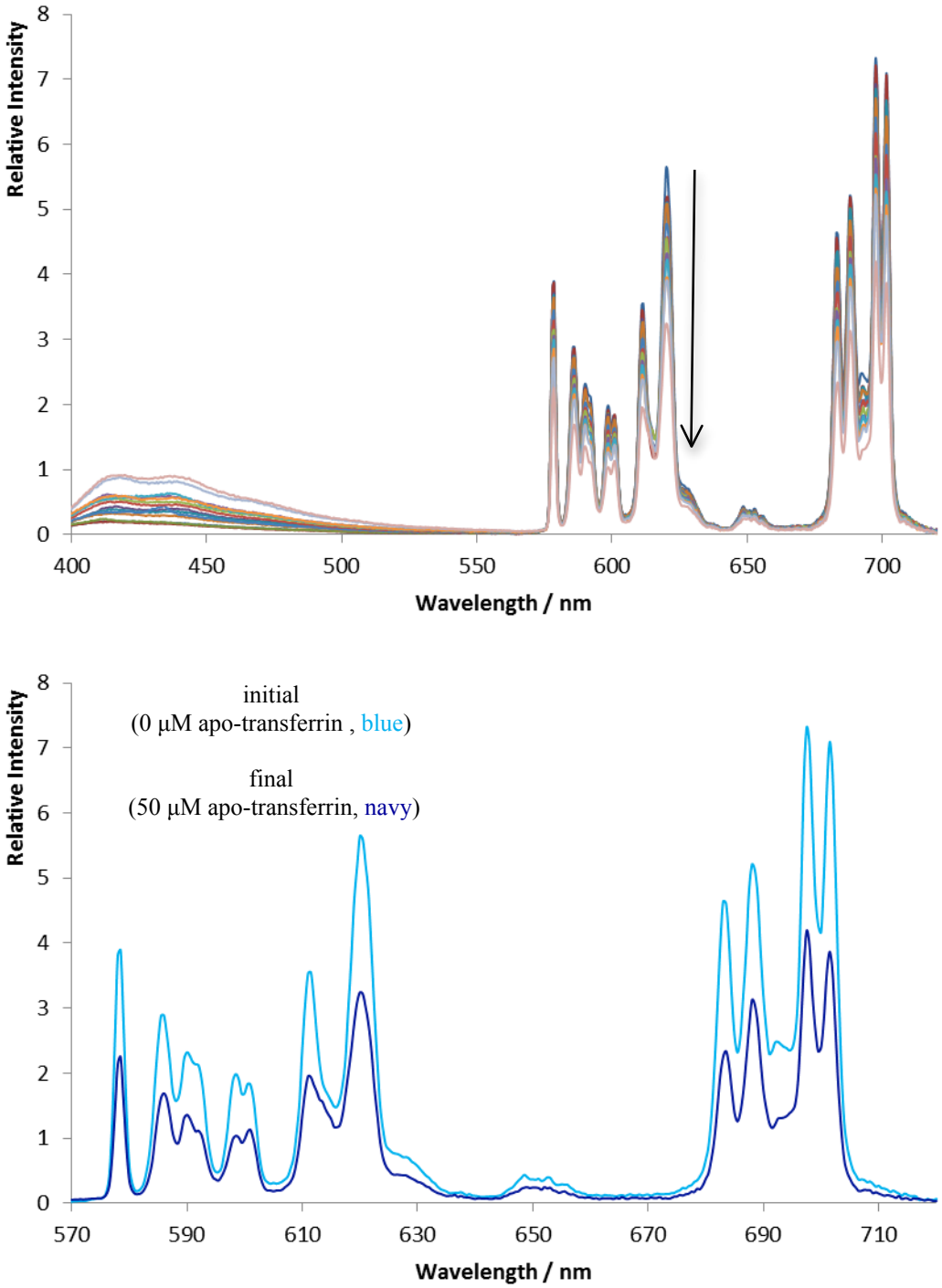
ESI Fig. 4. Europium emission spectral changes following addition of bovine γ-Ig-G to $[Eu.L^1(OH_2)]^+$ (20 μM, 295 K, 0.1 M NaCl, pH 7.4).



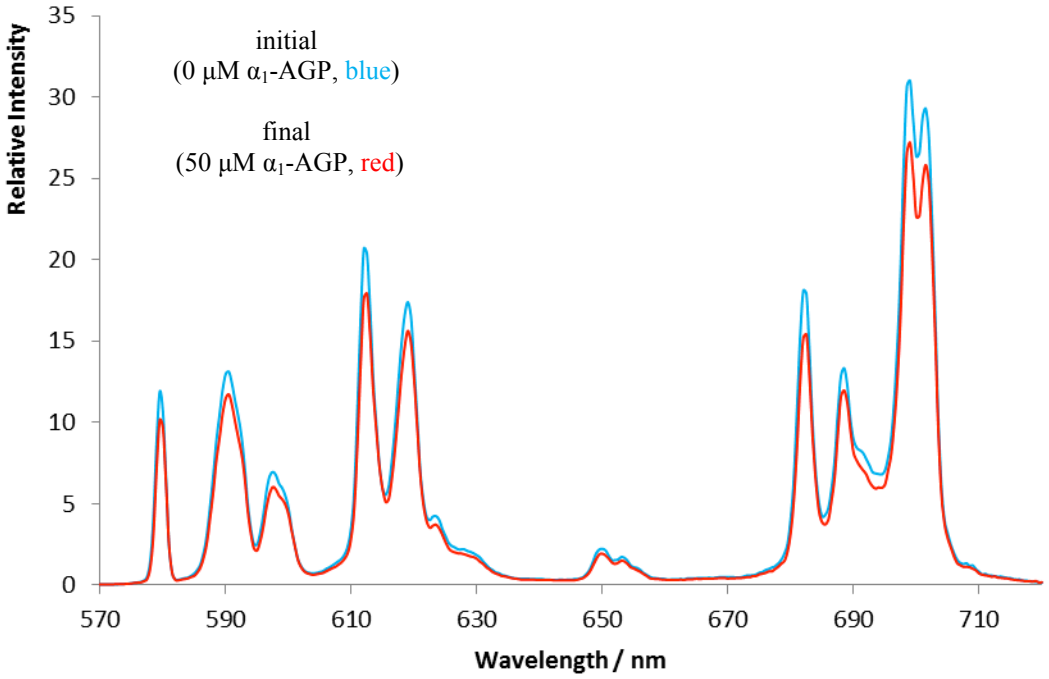
ESI Fig. 5. Europium emission spectral changes following addition of bovine fibrinogen to $[Eu.L^1(OH_2)]^+$ (20 µM, 295 K, 0.1 M NaCl, pH 7.4).



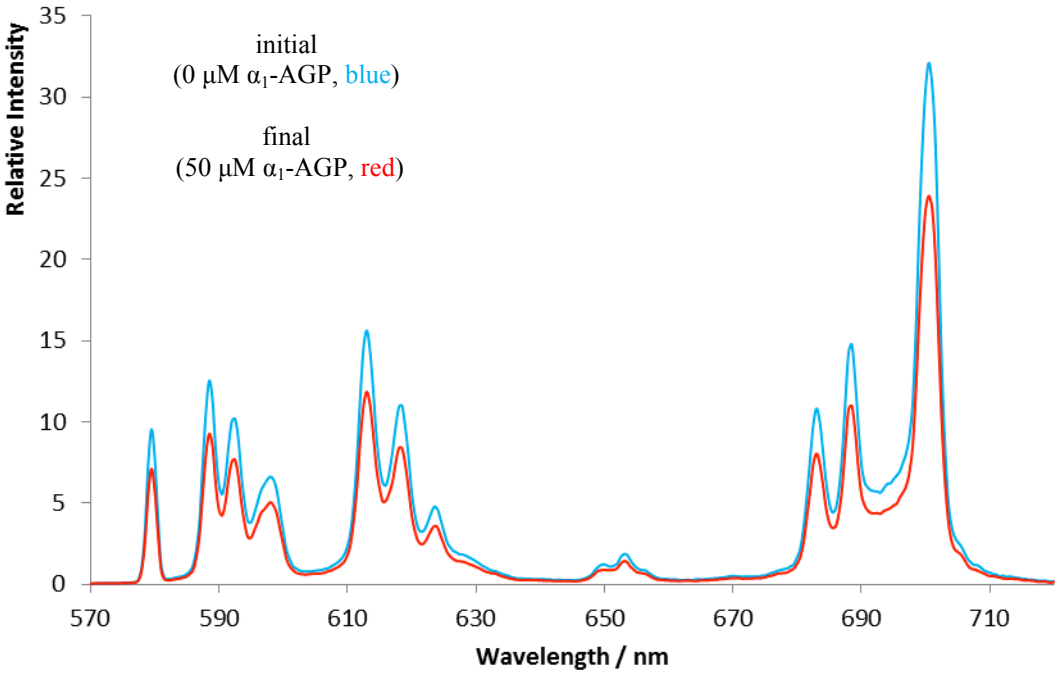
ESI Fig. 6. Europium emission spectral changes following addition of HSA to $[Eu.L^1(OH_2)]^+$ (20 μM, 295 K, 0.1 M NaCl, pH 7.4).



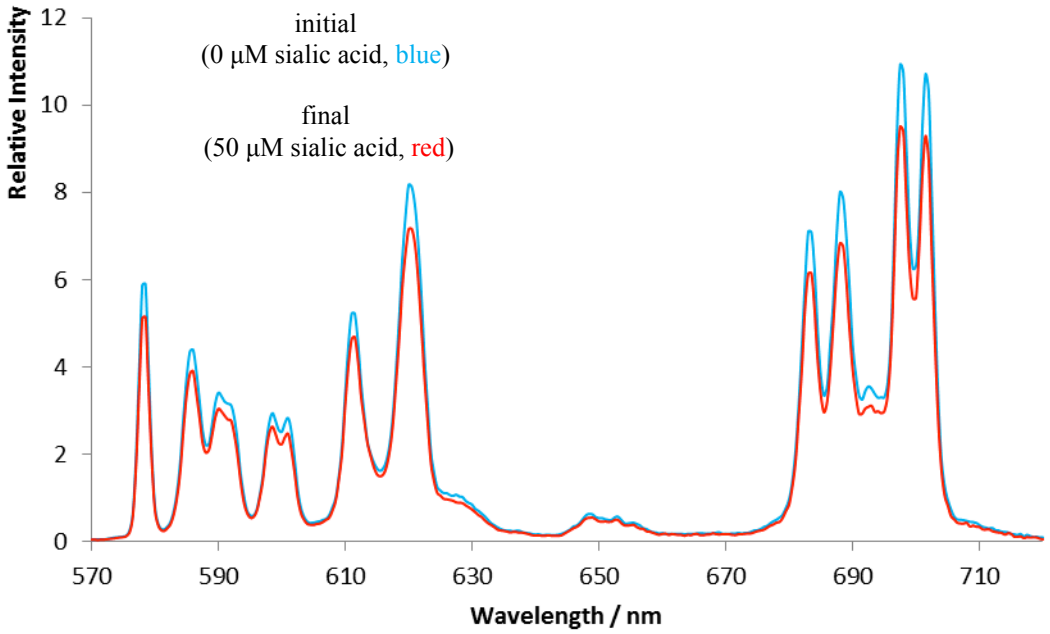
ESI Fig. 7. Europium emission spectral changes following addition of human apo-transferrin to $[Eu.L^1(OH_2)]^+$ (20 µM, 295 K, 0.1 M NaCl, pH 7.4).



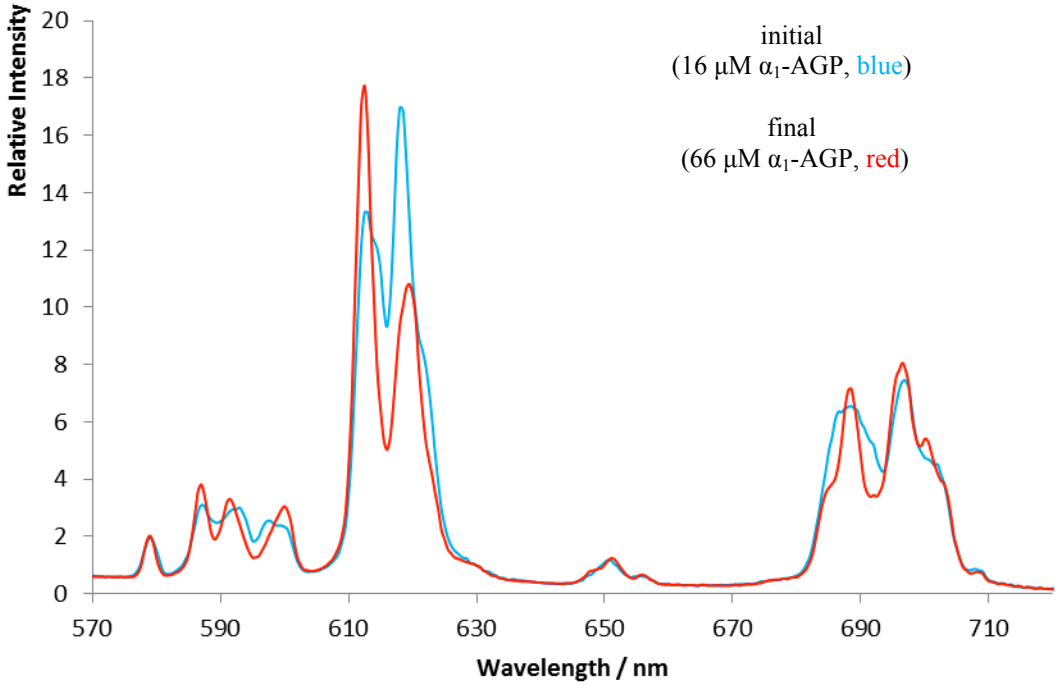
ESI Fig. 8. Europium emission spectral changes following addition of human α_1-AGP to $[Eu.L^2(OH_2)]^+$ (20 μM, 295 K, 0.1 M NaCl, pH 7.4).



ESI Fig. 9. Europium emission spectral changes following addition of human α_1-AGP to $[Eu.L^3(OH_2)]$ (20 μM, 295 K, 0.1 M NaCl, pH 7.4).



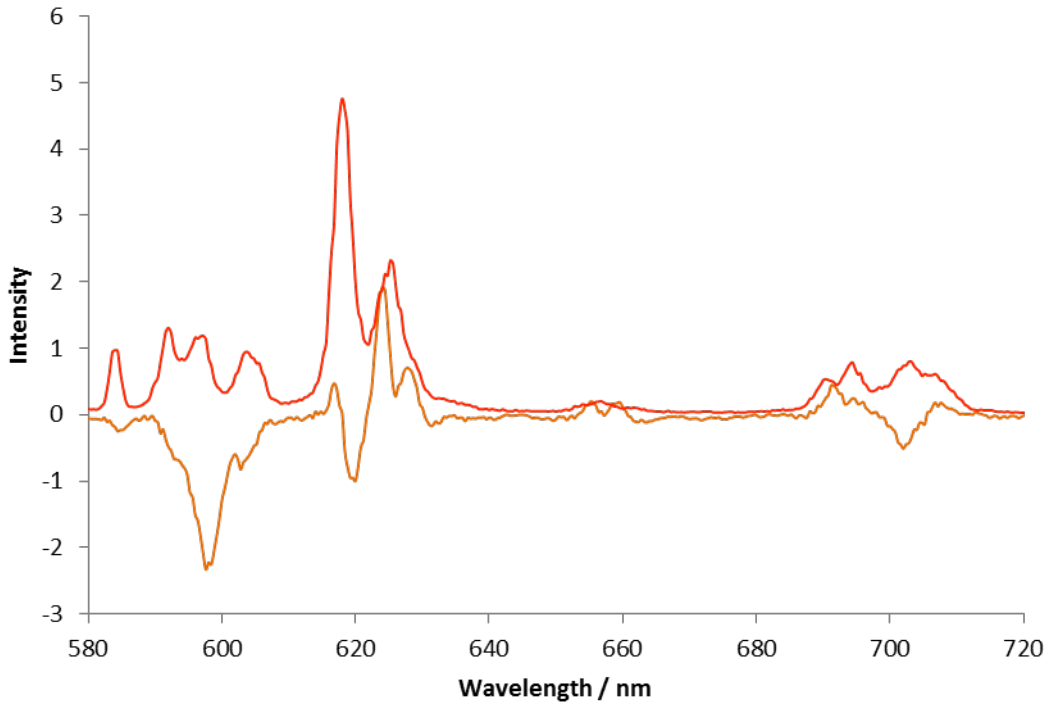
ESI Fig. 10. Europium emission spectral changes following addition of sialic acid to $[Eu.L^1(OH_2)]^+$ (20 µM, 295 K, 0.1 M NaCl, pH 7.4).



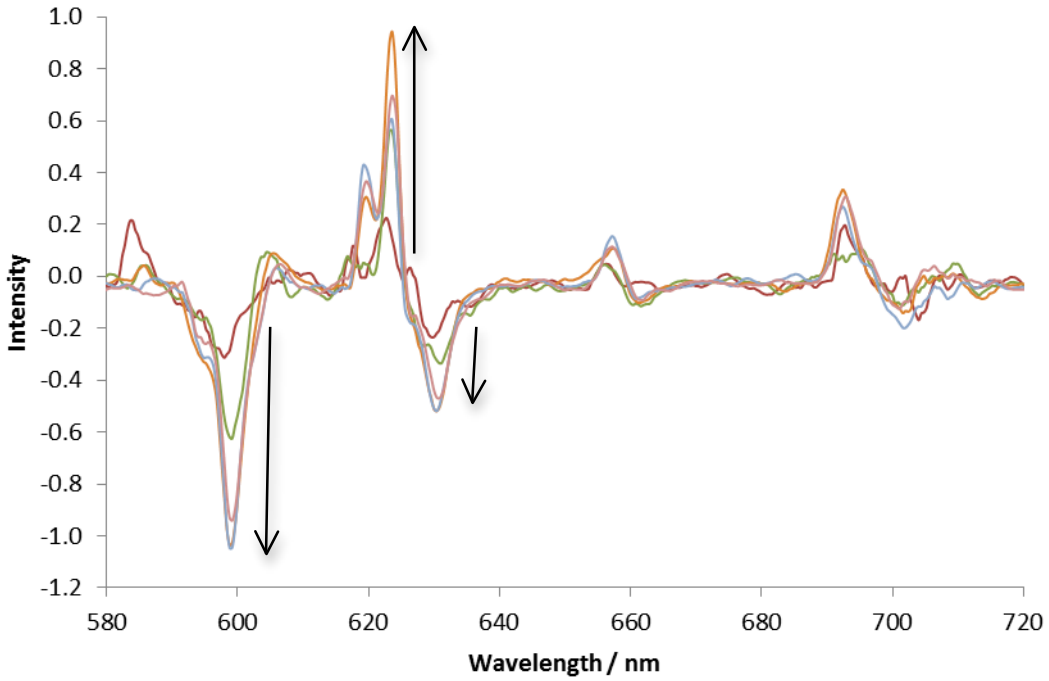
ESI Fig. 11. Europium emission spectral changes following addition of human α_1-AGP to $[Eu.L^1(OH_2)]^+$ in human serum (295 K, pH 7.4).

4. CPL spectra for protein bound complexes and CPL titration of bovine α_1-AGP in human serum

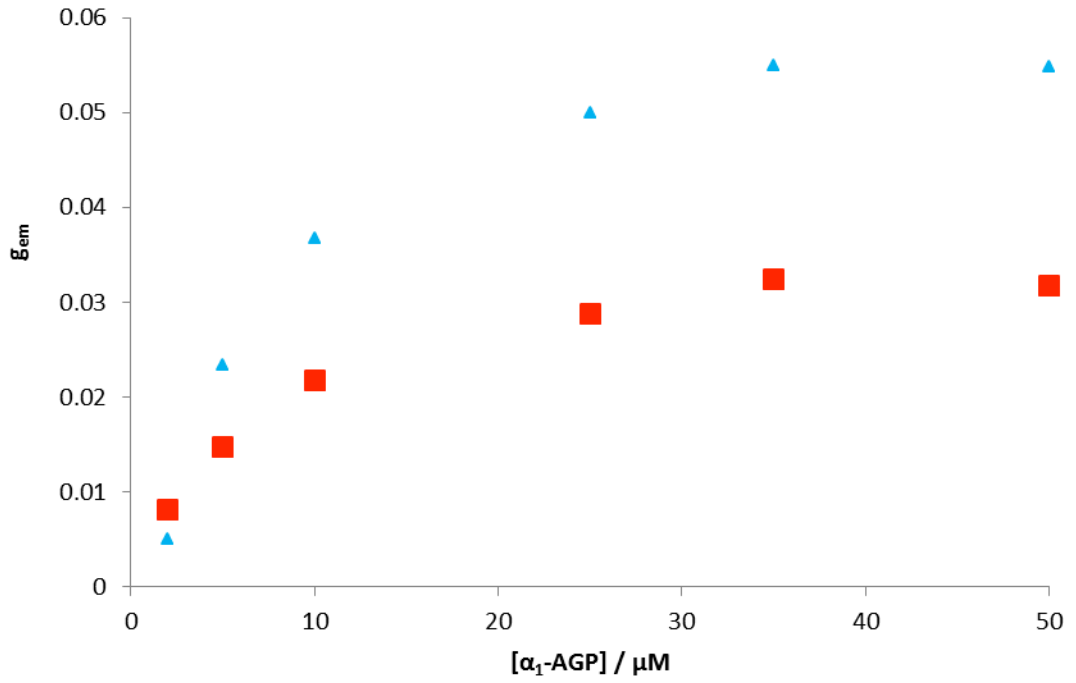
The scale used in each CPL figure in the ESI refers to (I_L- I_R) and is on a scale of x40 with respect to (I_L + I_R). Note that bovine and human α_1-AGP gave similar limiting emission and CPL spectra.



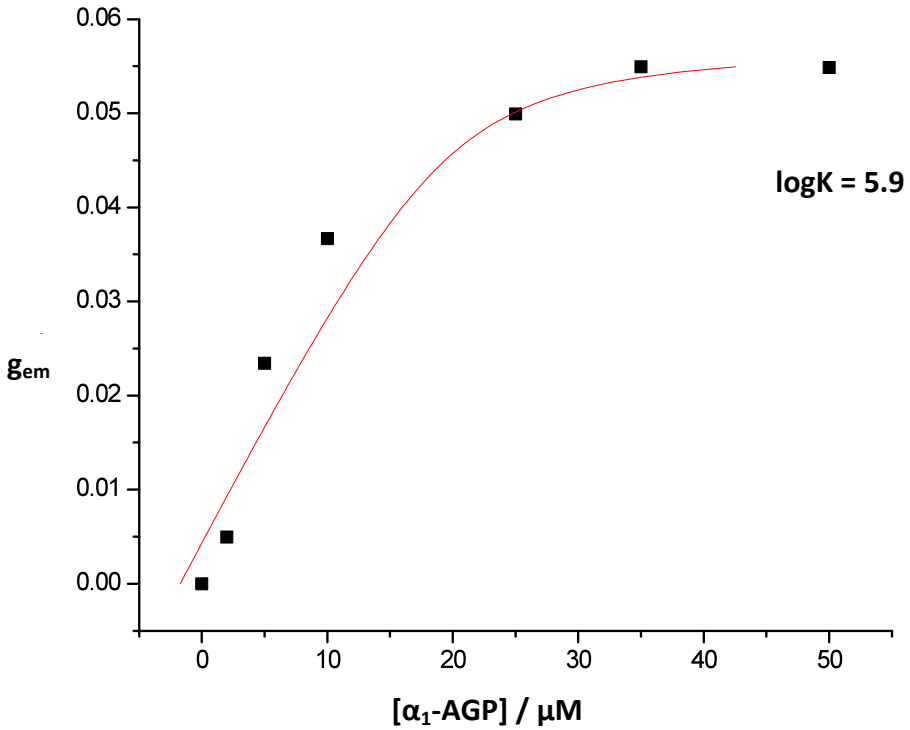
ESI Fig. 12. Europium emission spectrum and CPL spectrum of $[Eu.L^1(OH_2)]^+$ in the presence of 100 μM human α_1-antitrypsin (20 μM, 295 K, 0.1 M NaCl, pH 7.4).



ESI Fig. 13. CPL spectral changes following addition of bovine α_1-AGP to $[Eu.L^1(OH_2)]^+$ (20 μM, 295 K, 0.1 M NaCl, pH 7.4).



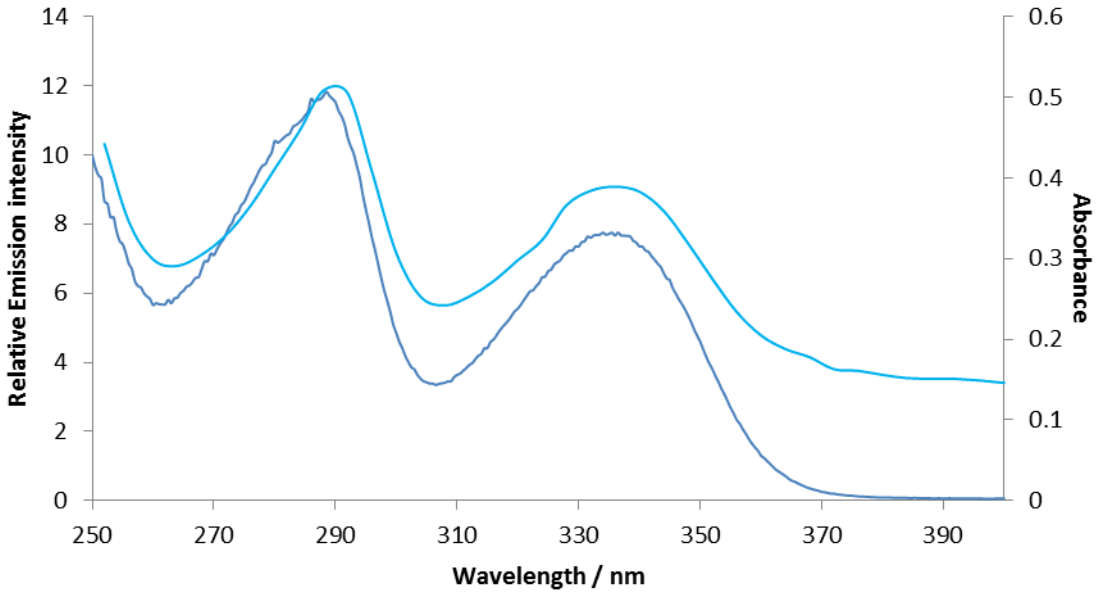
ESI Fig. 14. Variation of the observed dissymmetry factor, g_{em}, with added α_1-AGP for $[Eu.L^1(OH_2)]^+$ (20 μM, 295 K, 0.1 M NaCl, pH 7.4); blue triangles = 600 nm, red squares = 630 nm.



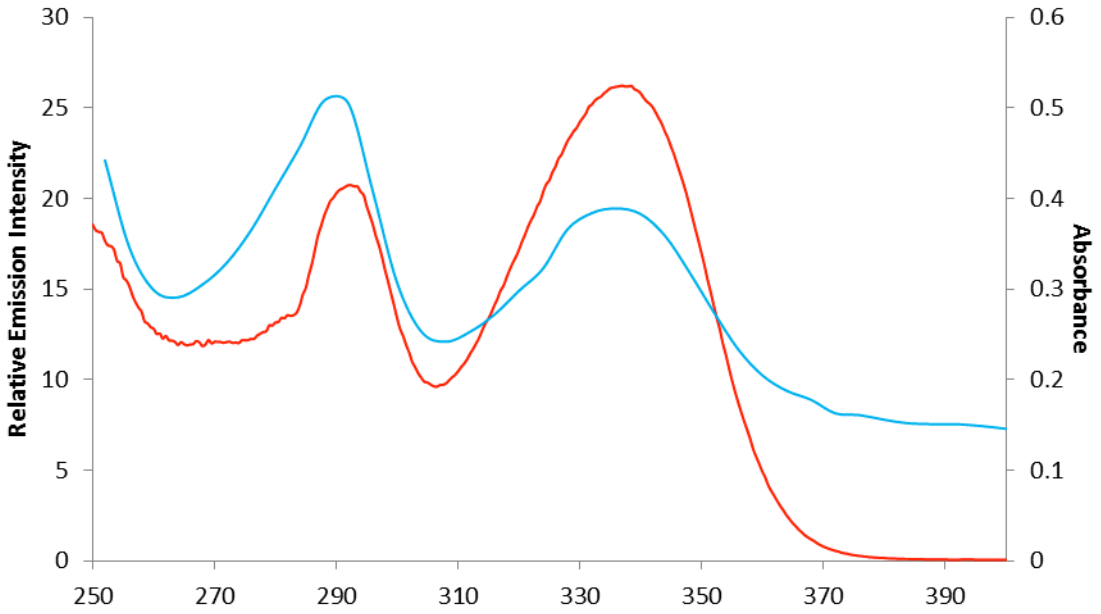
ESI Fig. 15. Variation of the observed dissymmetry factor, g_{em}, with added bovine α_1-AGP for $[Eu.L^1(OH_2)]^+$ (20 μM, 295 K, 0.1 M NaCl, pH 7.4) at 600 nm. Iterative, non-linear least squares data fitting allows a 'global' association constant to be estimated.

5. Excitation spectra in the presence and absence of human α_1-AGP

Excitation spectra were recorded on an ISA Joblin-Yvon Spex Fluorolog-3 luminescence spectrometer. Quartz cuvettes with a path length of 1 cm were used for all samples. Measurements were carried out at pH 7.4 using aqueous solutions containing 0.1 M NaCl, in order to maintain a constant ionic strength.

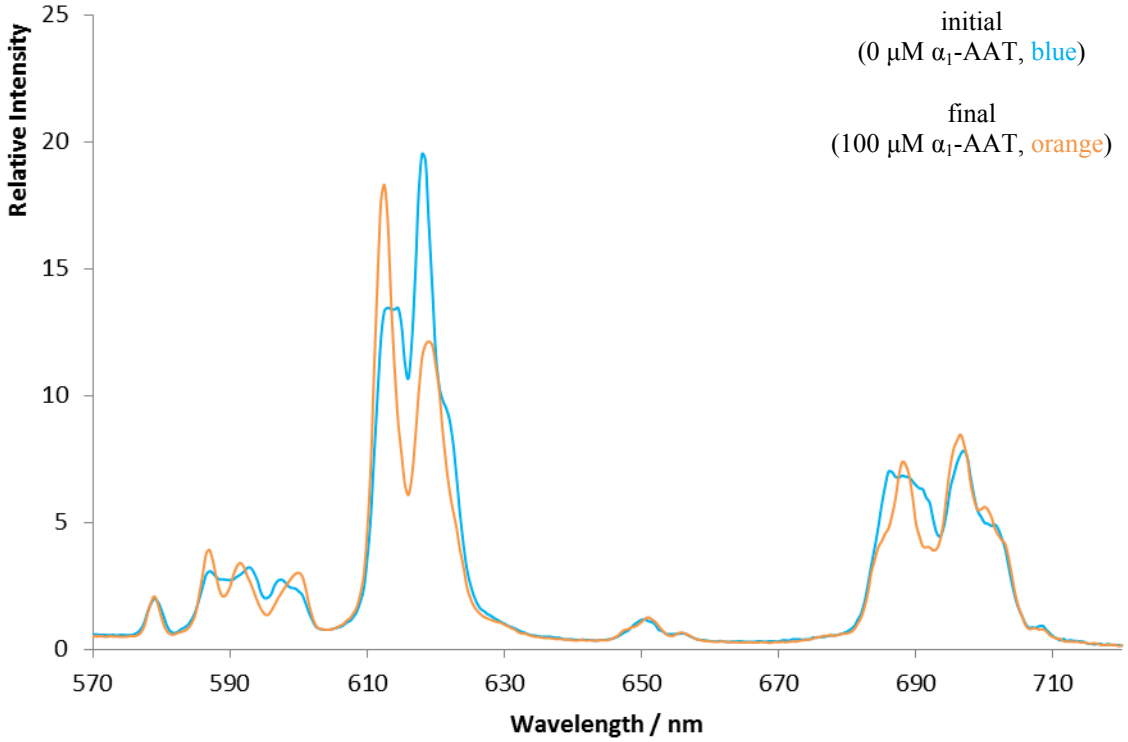


ESI Fig. 16. Absorption spectrum (blue) and excitation spectrum (dark blue, λ_{ems} = 620 nm) of $[Eu.L^1(OH_2)]^+$ (20 µM, 295 K, 0.1 M NaCl, pH 7.4).

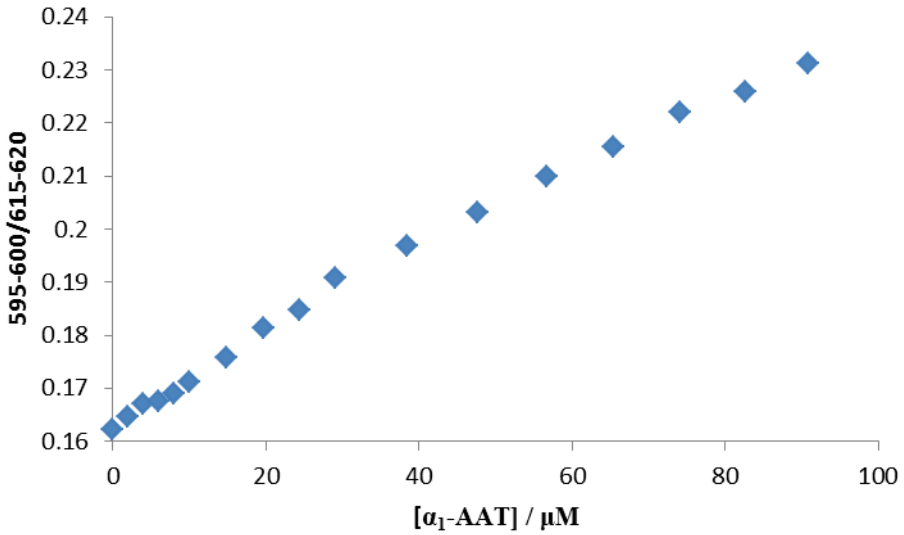


ESI Fig. 17. Absorption spectrum of $[Eu.L^1(OH_2)]^+$ in the absence of protein (blue) and excitation spectrum of $[Eu.L^1(OH_2)]^+$ in the presence of 50 µM human α_1-AGP (red, λ_{ems} = 612 nm) (20 µM, 295 K, 0.1 M NaCl, pH 7.4).

6. Eu emission spectral changes in human serum following addition of α_1-AAT and observed modulation of the Eu emission intensity ratio.



ESI Figure 17 Europium emission spectral changes following addition of human α_1-antitrypsin to $[Eu.L^1(OH_2)]^+$ in human serum (295 K, pH 7.4). The initial concentration of the anti-trypsin is not known, but is assumed to be in the normal range, i.e. 40-60μM.



ESI Figure 18 Variation of the ratio of Eu emission intensity with the concentration of added [α_1-AAT] in human serum; conditions as in ESI Figure 17.