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Fluorescence Study of the Supramolecular Interactions between Coumarins and Serum Albumin

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Abstract: In this work we study the supramolecular interactions between fluorescent dyes of the family of aminocoumarins and a protein which is the main responsible for matter transport in blood, the serum albumin. The experimental data obtained by fluorescent titrations are analysed using Principal Components Global Analysis to obtain the equilibrium binding constants and the fluorescence spectra of the dyes bound to the protein. The results obtained for the different aminocoumarins are then compared.

Introduction: BSA

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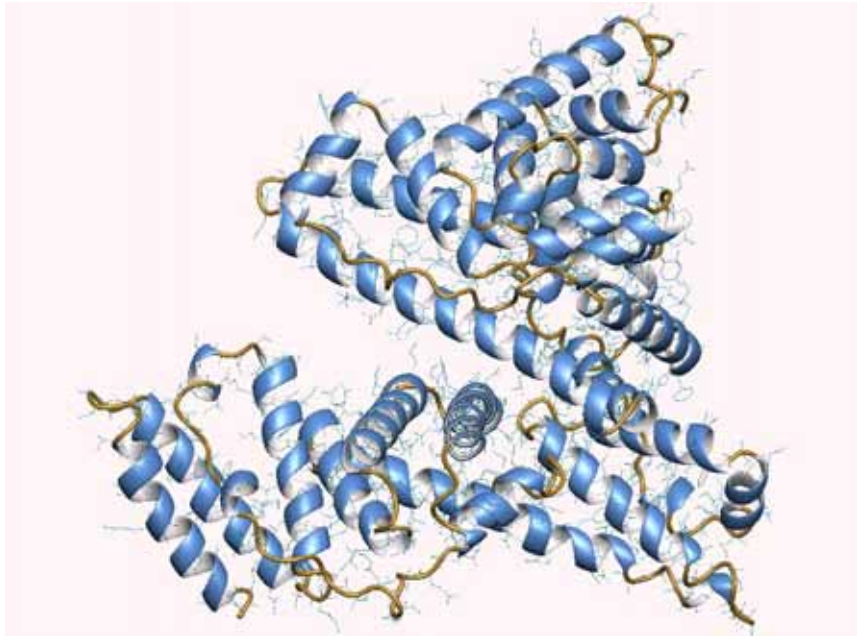
The aim of this work is to study the supramolecular binding between some aminocoumarins and the transport protein BSA using fluorescence techniques.

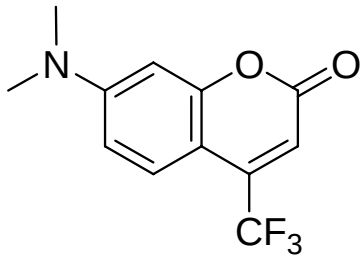
- ✓ Serum albumin is the most abundant protein in plasma with a typical concentration of 5g/100ml.
- ✓ The most outstanding property of albumin is its ability to bind reversibly an incredible variety of ligands.
- ✓ Serum albumin is the principal carrier of fatty acids that are otherwise insoluble in circulating plasma, but it can also carry drugs and other metabolites and can strongly effect the way they are delivered through the body.



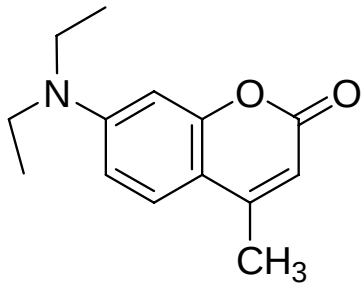
Bovine Serum Albumin (BSA)

Introduction: Aminocoumarins

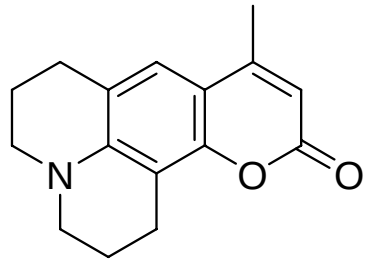
- ✓ Aminocoumarins are natural compounds, some of them are biologically active as analgesics, anticoagulants, etc.
- ✓ They are mainly used in cosmetics and in the food industry because of their ability to bind to proteins.
- ✓ Some aminocoumarins undergo charge transfer processes in the excited state that make their fluorescence highly sensitive to the environment.



C152



C460

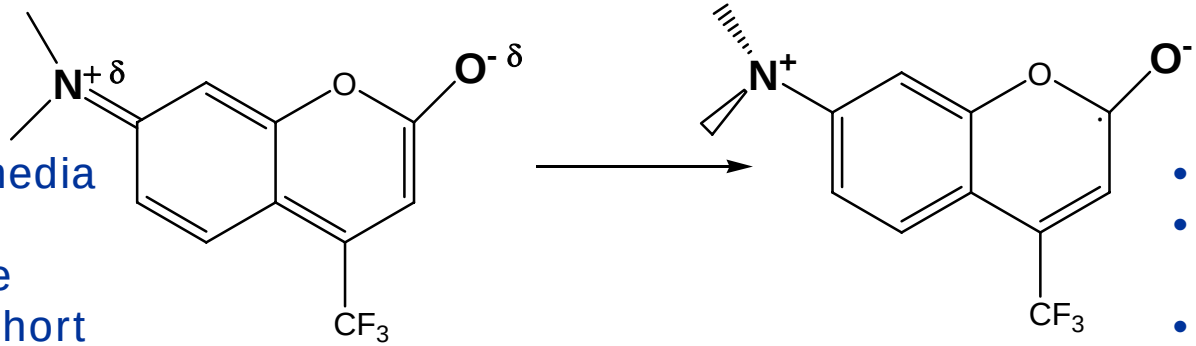


C102

Coumarins used in this work

ICT state

- Non polar media
- Strong fluorescence
- Emision at short wavelengths

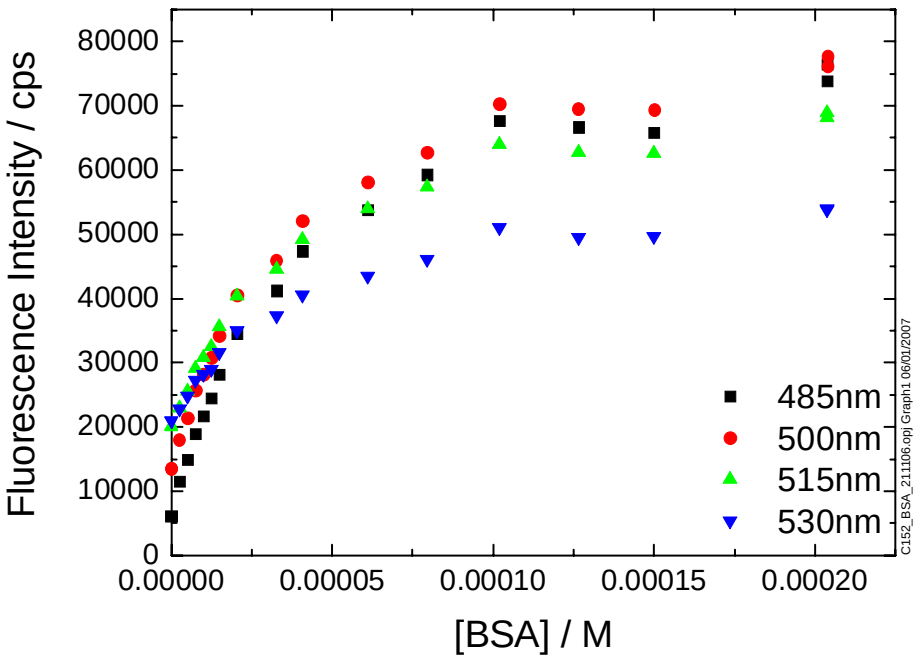


TICT state

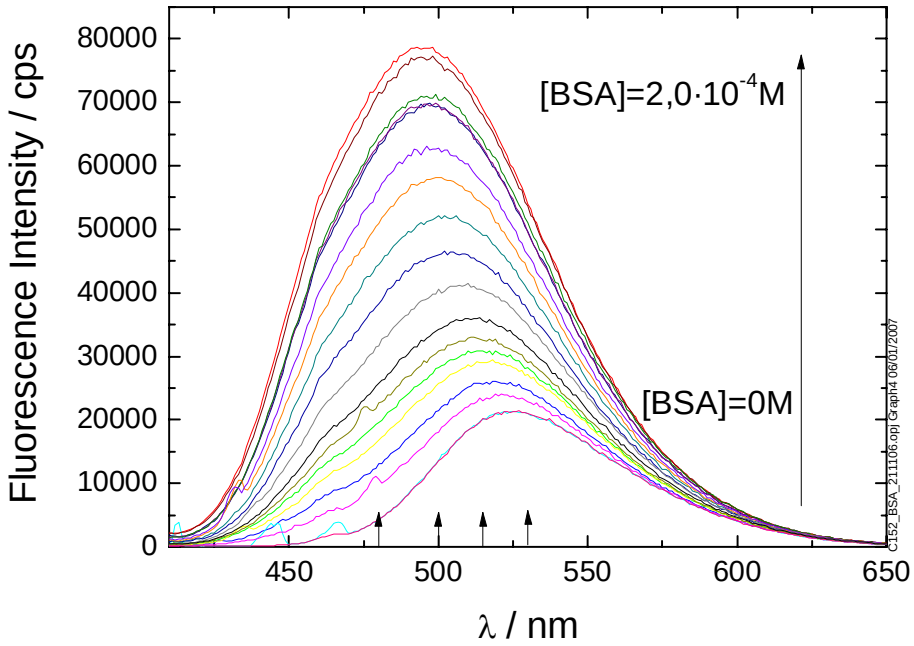
- Polar media
- Weak fluorescence
- Emision at long wavelengths

Results and Discussion: Emission Spectra

- ✓ The fluorescence emission spectrum of the aminocoumarin shifts to lower wavelengths when increasing the concentration of BSA.
- ✓ A huge increase of the fluorescence intensity is observed by increasing the concentration of BSA, specially for the coumarins C152 and C460.



C152 + BSA



- ✓ The plots of fluorescence intensity at a certain wavelength versus BSA concentration shows the existence of binding between the coumarin and the BSA.

Results and Discussion: Stoichiometry of the complexation

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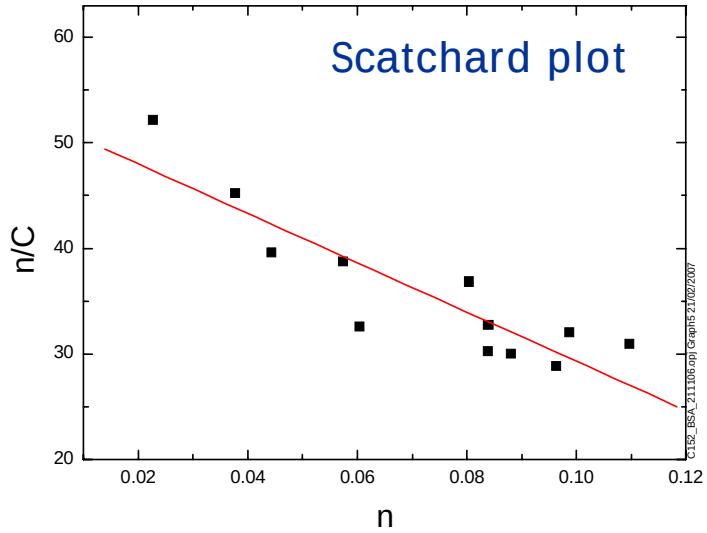
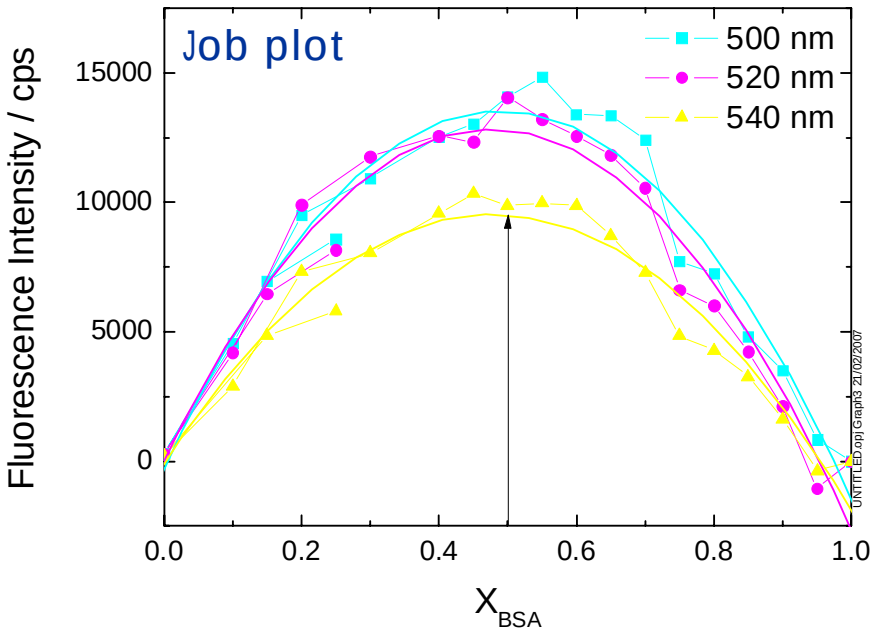
System: C152 + BSA

- ✓ Linearization of the data obtained in the fluorescence titrations with the Scatchard method yields a linear plot. This means that a 1:1 association takes place.
- ✓ Principal Components Analysis of these data yields two structural components, in agreement with a 1:1 complexation.





- ✓ The fluorescence measurements in samples prepared after the method of continuous variations yield Job plots with maximum at a molar fraction of BSA of 0.5, indicating a complexation of stoichiometry 1:1.

Results and Discussion. Model: Association 1:1 coumarin:BSA

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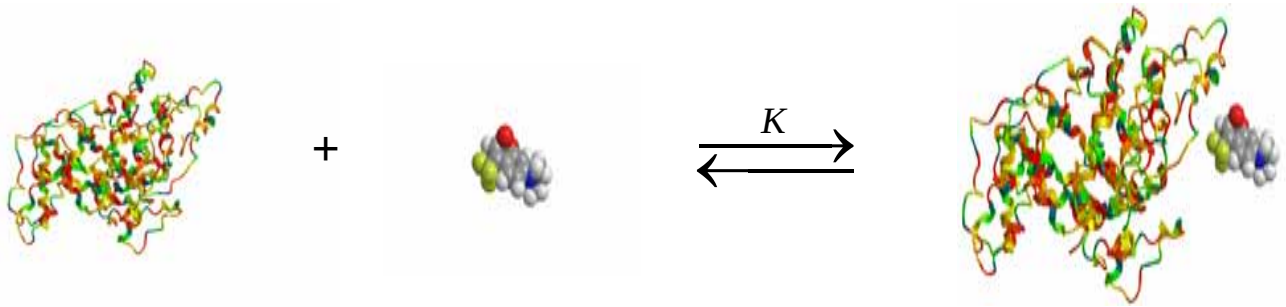
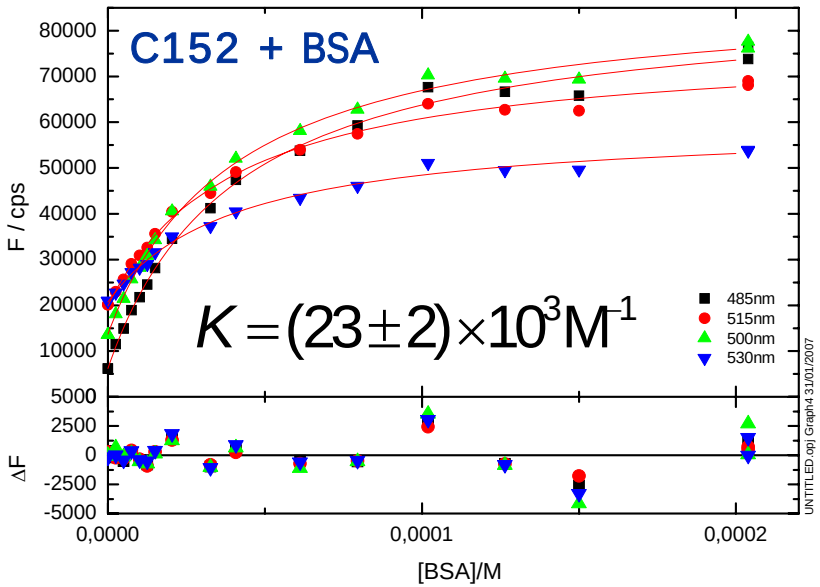
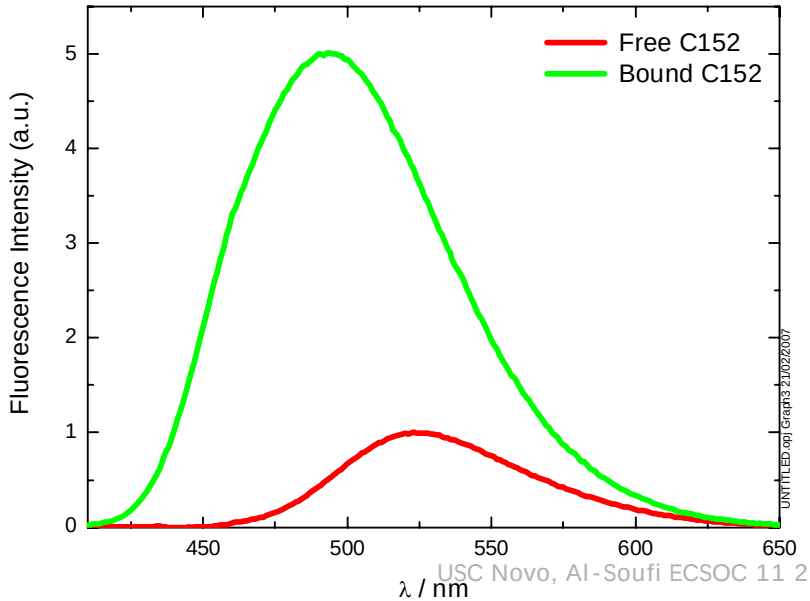
- ✓ Two fluorescent species are involved: free coumarin and bound coumarin.



- ✓ The equilibrium constant K and the fluorescence spectra of free coumarin and coumarin bound to the protein are obtained by global analysis.
- ✓ Fluorescence maxima and quantum yields (Q) are determined.





Summary of results: Fluorescence Maximum

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	Fluorescence maximum /nm		Fluorescence quantum yield		Binding constant
	Free coumarin	Bound coumarin	Free coumarin	Bound coumarin	K / 10^3 M^{-1}
C152	523	494 (-29)	0.06	0.32 (x5)	23 ± 2
C460	469	433 (-36)	0.08	0.66 (x8)	28 ± 3
C102	488	460 (-28)	0.59	0.84 (x1.4)	112 ± 19

- ✓ All three coumarins show a strong shift of the emission spectrum to shorter wavelengths when they are bound to the protein.
- ✓ The observed shift indicates a non-polar environment of the dye bound to the protein, where the ICT state is the main responsible for the fluorescence emission.
- ✓ The binding to the protein is sensitively indicated by the color change of all three coumarins.

Summary of results: Fluorescence quantum yield

	Fluorescence maximum /nm		Fluorescence quantum yield		Binding constant
	Free coumarin	Bound coumarin	Free coumarin	Bound coumarin	K / 10^3 M^{-1}
C152	523	494 (-29)	0.06	0.32 (x5)	23 ± 2
C460	469	433 (-36)	0.08	0.66 (x8)	28 ± 3
C102	488	460 (-28)	0.59	0.84 (x1.4)	112 ± 19

- ✓ The three coumarins show a significant increase of the fluorescence quantum yield when they are bound to the protein, specially the C460 with a 8-fold increase, followed by the C152.
- ✓ This increase is due to the enhance of emission from the ICT state in the complexes, being a much more fluorescent state.
- ✓ The coumarins C460 and C152 are highly sensitive to the binding with BSA with respect to the intensity of the emission, in contrast to C102.

Summary of results: Binding Constant

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	Fluorescence maximum /nm		Fluorescence quantum yield		Binding constant
	Free coumarin	Bound coumarin	Free coumarin	Bound coumarin	K / 10^3 M^{-1}
C152	523	494 (-29)	0.06	0.32 (x5)	23 ± 2
C460	469	433 (-36)	0.08	0.66 (x8)	28 ± 3
C102	488	460 (-28)	0.59	0.84 (x1.4)	112 ± 19

- ✓ All three coumarins bind effectively to the protein BSA as deduced from the high equilibrium binding constants.
- ✓ The coumarins C460 and C152 show similar values of the binding constant as it could be expected from their similar structure.
- ✓ The coumarin C102 shows a much higher binding constant than the other coumarins, probably due to its more hydrophobic structure, and it is therefore the most sensitive to the presence of BSA.

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Acknowledgements

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Students:

Belén Reija
Jorge Bordello
Sonia Freire
Javier Filgueira
Sheila García
Lucía Álvarez

Financial support:
MEC (CTQ2004-07683-C02, HA2005-0063)
Xunta de Galicia (PGIDIT05 PXIC26202PN)

Molecular Photophysics & Photochemistry Group