Ratiometric and Turn-On Fluorescent Sensors: Tools for the Detection of Biological Ions

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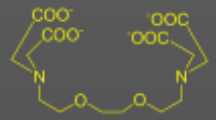
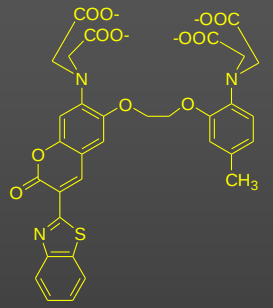
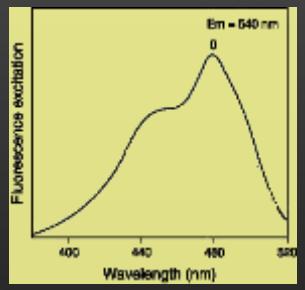
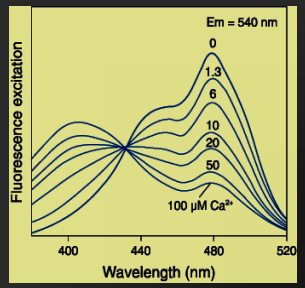
HOW DOES A FLUORESCENT ION PROBE FUNCTION?

- THE NEED OF AN IONOPHORE
- THE NEED OF A CHROMOPHORE
- THE NEED OF A FLUORESCENCE SIGNAL
- THE NEED OF A RESPONSE TO THE ION





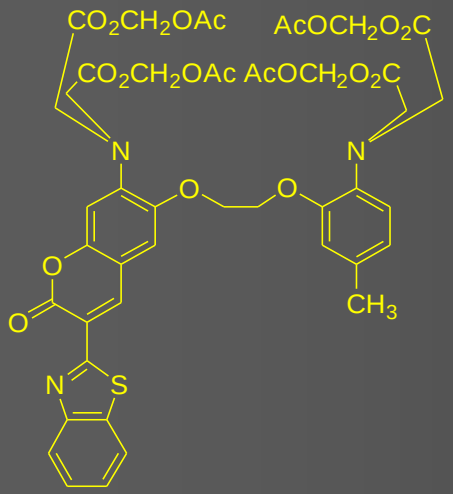


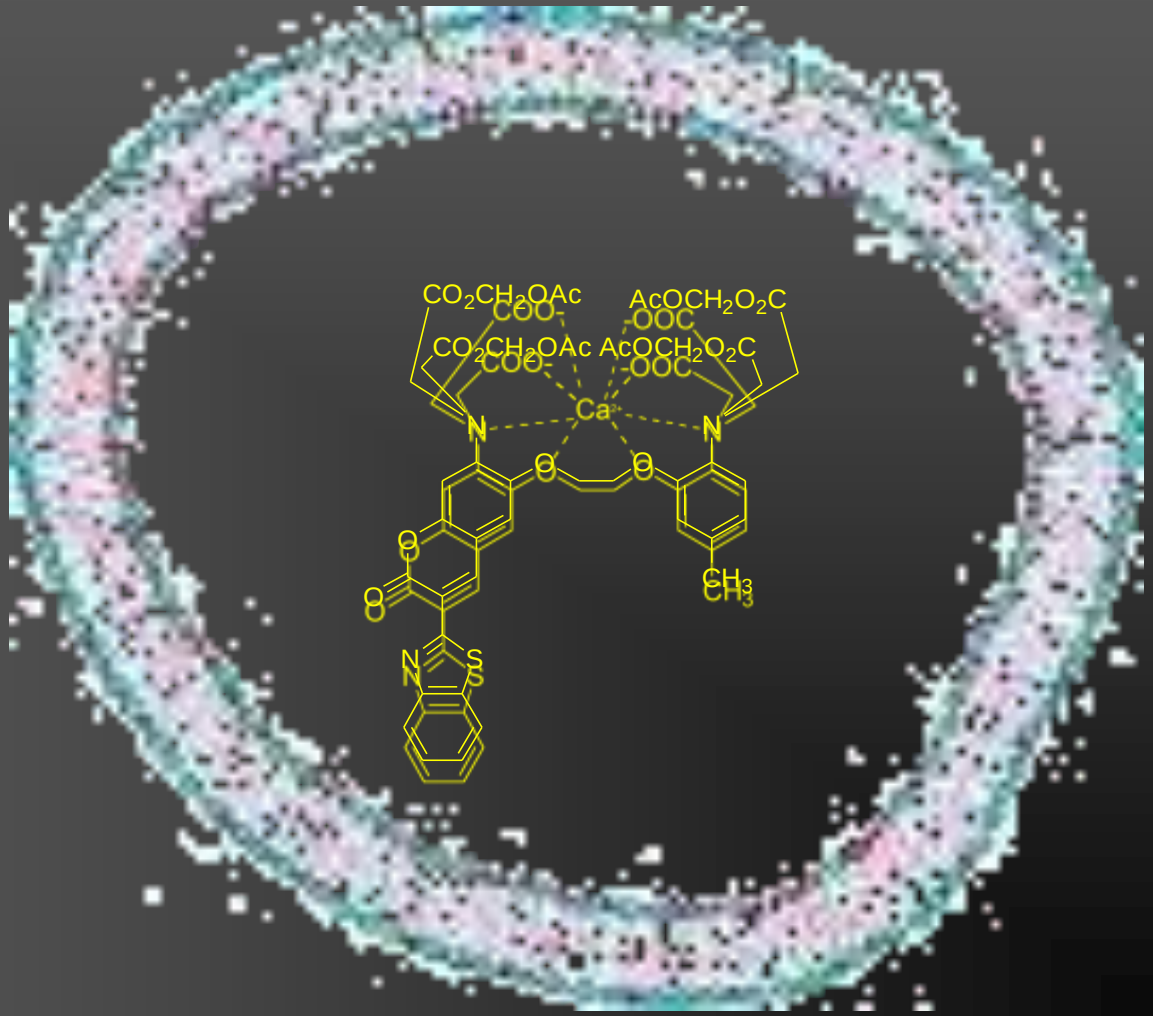


Desired Properties for Intracellular Ion Indicators

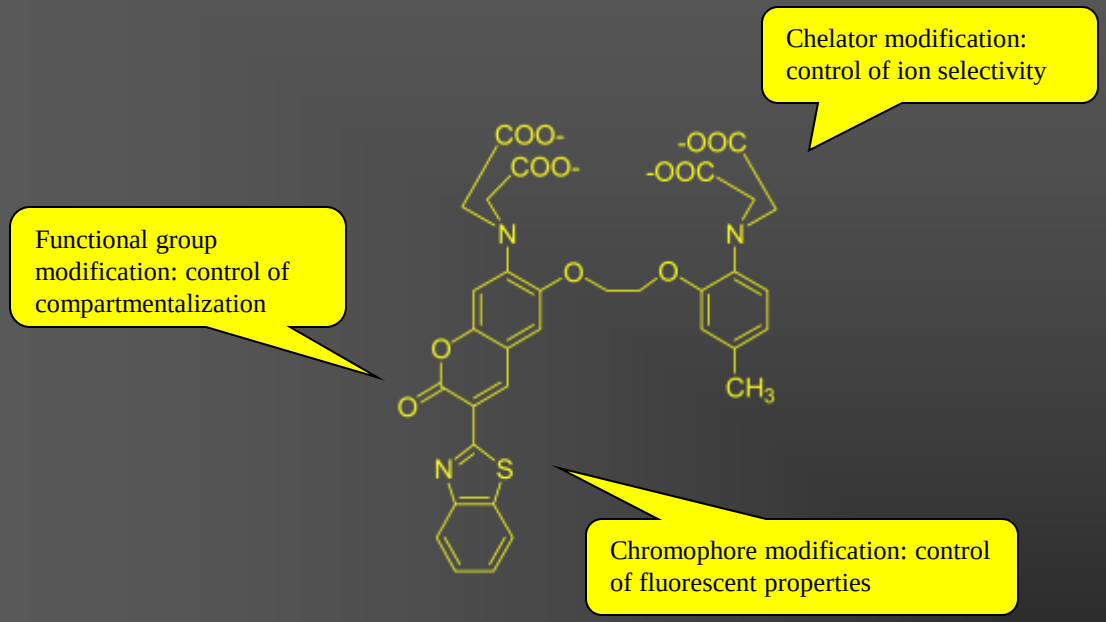
- High selectivity for the ion being studied.
- A binding constant adjusted to the mid-point of the physiological concentration range of the ion: $0{,}1 \cdot Kd < [Ca2+]_i < 10 \cdot Kd$.
- A significant fluorescence Stokes shift to avoid the overlap of excitation and emission peaks.
- A large extinction coefficient, meaning high absorbance.
- A large fluorescence quantum yield.
- An excitation wavelength above 400 nm to minimize "background" fluorescence,
- Non-toxicity, and
- In the case of measurements of intracellular components, increased permeability to the cell membrane.

How do these indicators become cell permeable?

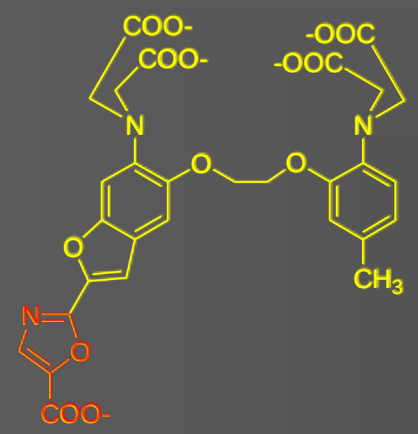


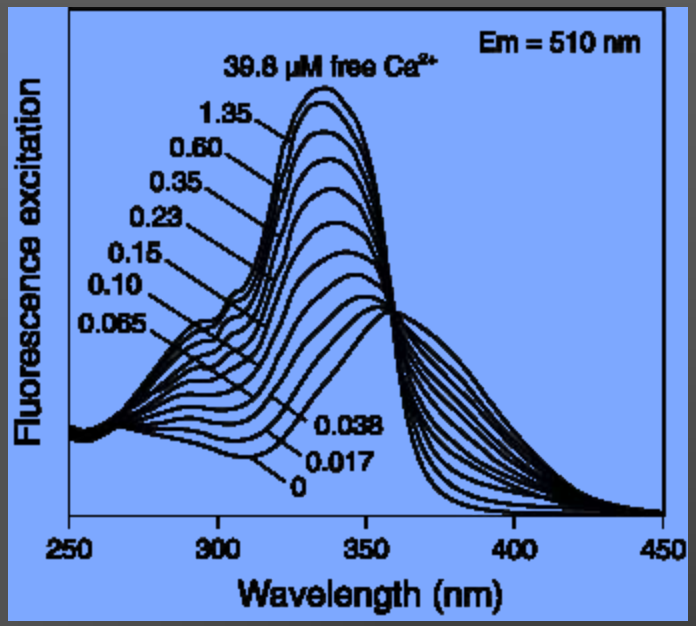


Which are the factors that influence the properties of fluorescent ion probes?



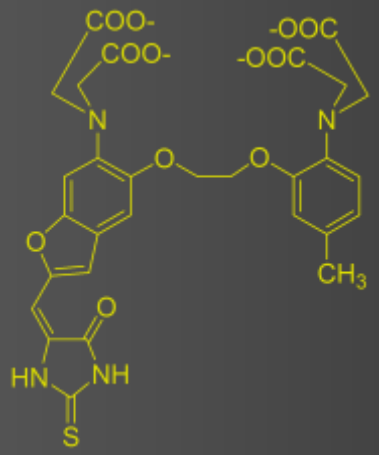
Influence of chromophore modification in the fluorescence profile of Fura-2 and Fura-Red indicators



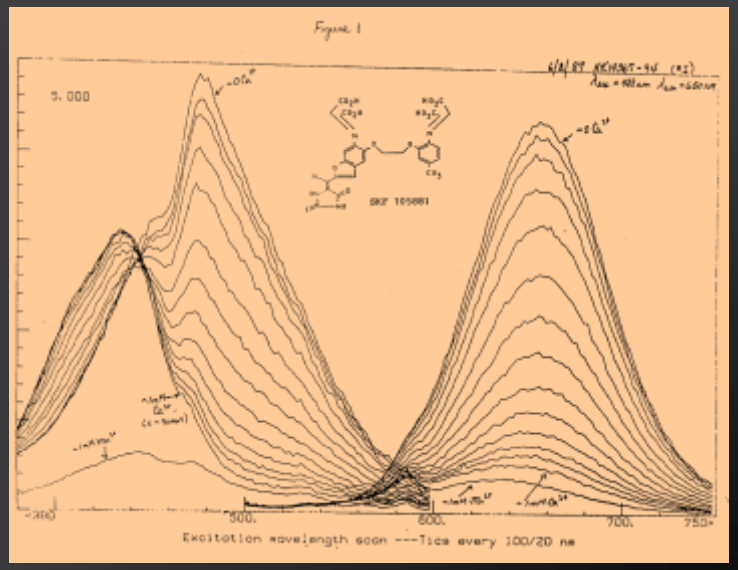


$$[Ca^{2+}] = K_d \frac{R - R_{\min}}{R_{\max} - R} \left(\frac{S_{f(\lambda_1)}}{S_{b(\lambda_1)}}\right)$$

Fura 2 (UV excited)
K_d= 145 nM, Φ = 0.49

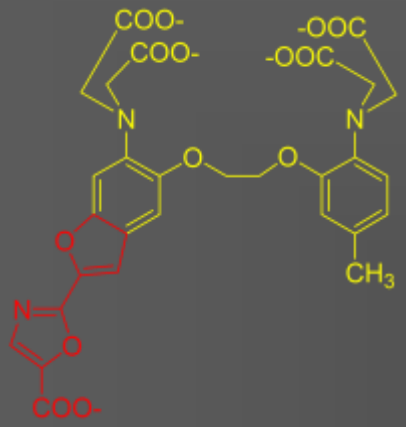


FuraRed (visible excited)
K_d= 140 nM, Φ = 0.013



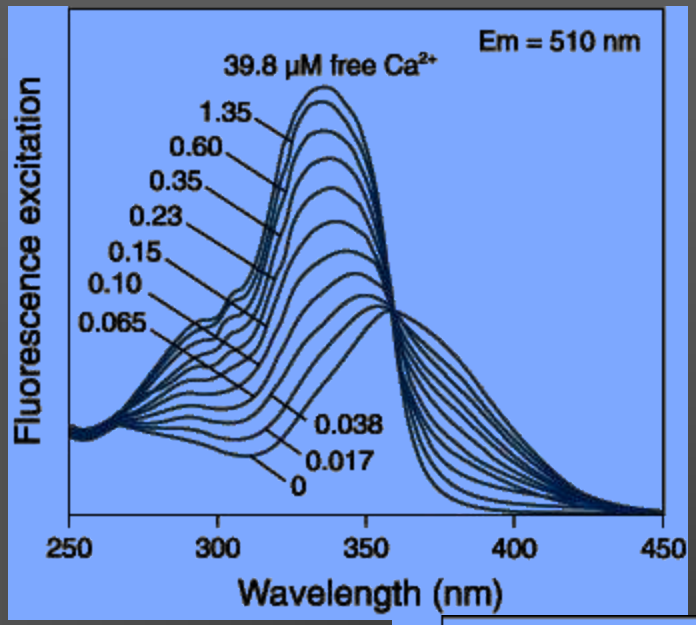
R.M. DeMarinis, H.E. Katerinopoulos, K. Muirhead, U. S. Patent 196654, **1989**.

Introduction of the coumarin moiety as a chromophore group: The case of the BTC Indicator



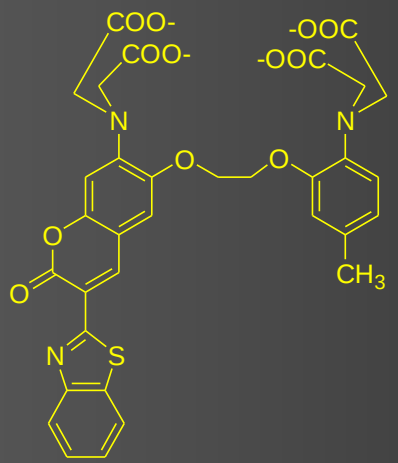
K_d= 145 nM

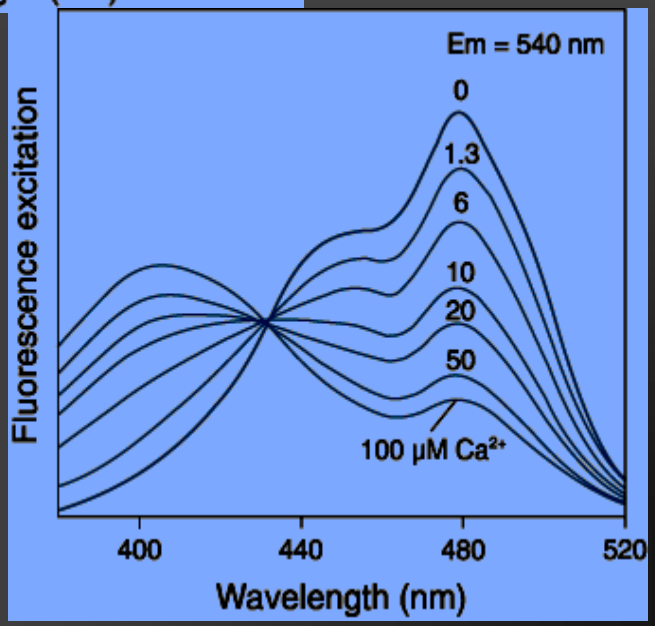
Φ = 0.49



K_d= 7000 nM

Φ = 0.12

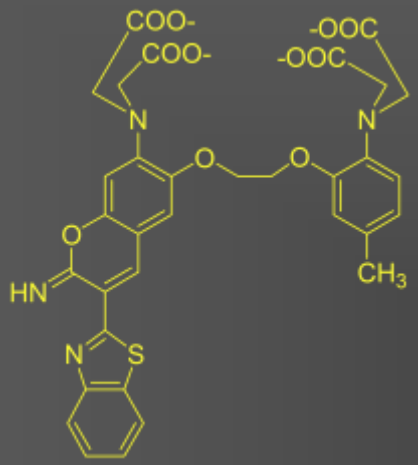


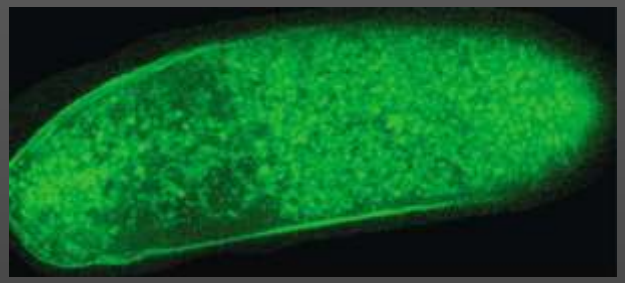


G. Grynkiewicz, M. Poenie and R. Y. Tsien, *J. Biol. Chem.*, 1985, **260**, 3350.

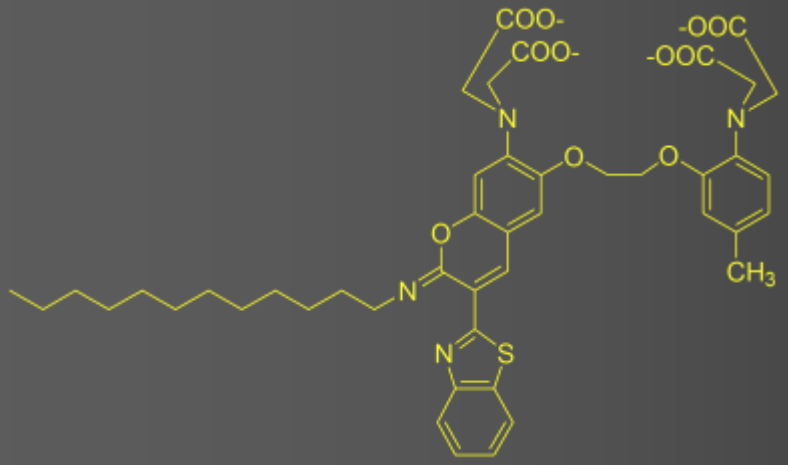
H. Iatridou, E. Foukaraki, M.A. Kuhn, E.M. Marcus, R.P. Haugland and H.E. Katerinopoulos *Cell Calcium* **1994**, *15*, 190-198.

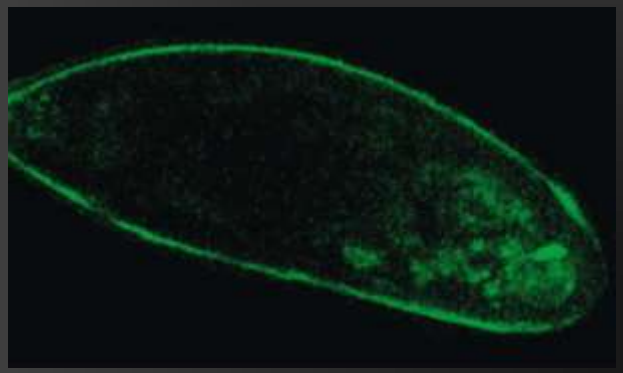
Functional group modification
The potential of the iminocoumarin moiety: C-12 BTIC





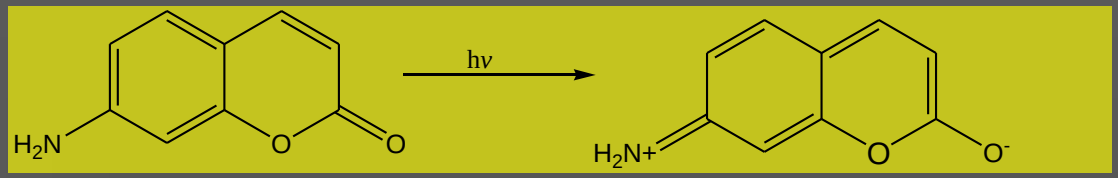
The iminocoumarin probe stains the entire cell (upper figure) whereas the alkyl group acts as an anchor that docks in the inner membrane region (lower figure).





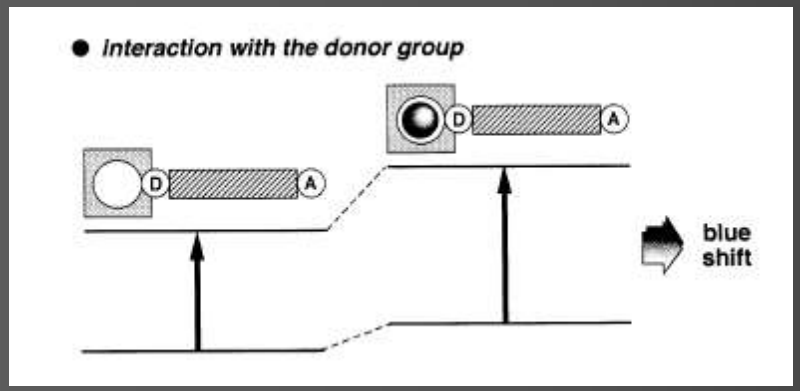
F. Liepouri, T. G. Deligeorgiev, Z. Veneti, C. Savakis, H. E. Katerinopoulos *Cell Calcium*, **2002**, *31*, 221-227.

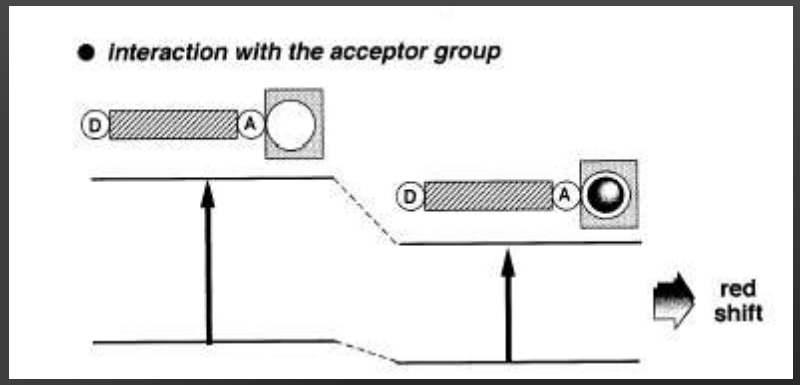
Looking from the point of view of the fluorescence profile: photoinduced charge transfer (PCT) or ratiometric probes

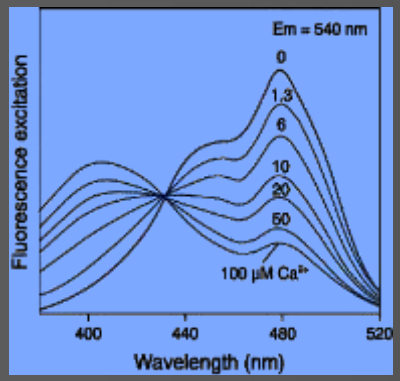


CATION INTERACTION DESTABILIZES SYSTEM

CATION INTERACTION STABILIZES SYSTEM

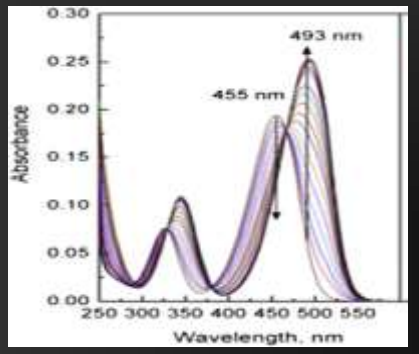




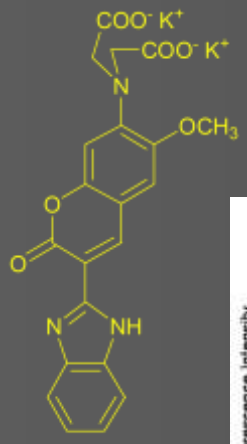


A case of a blue shift

A case of a red shift

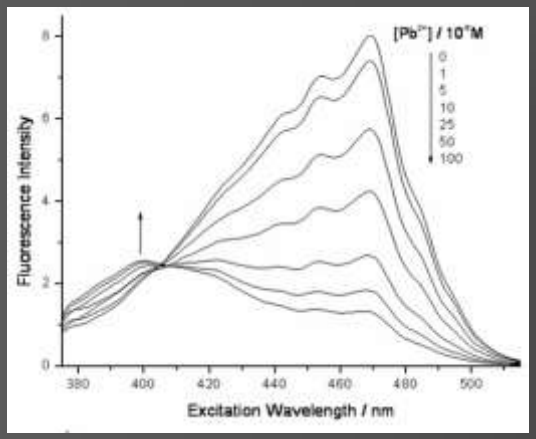


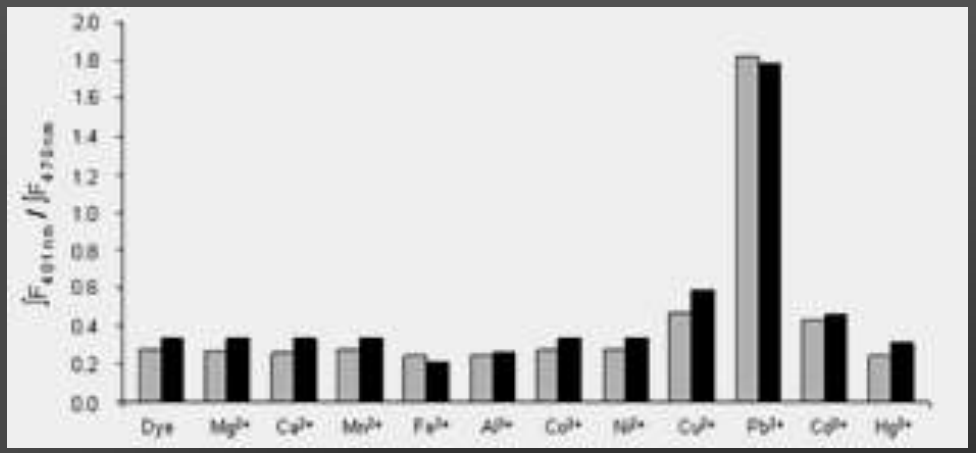
Combining modifications in the chromophore and the ionophore moieties: Synthesis of a coumarin-type Pb^{2+} probe



1

BICPb

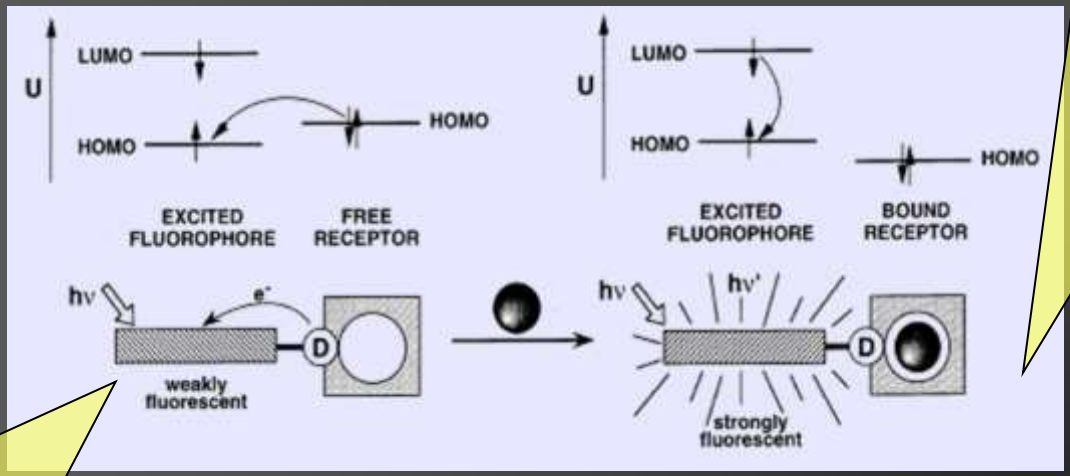




Roussakis, E., Pergantis, S.A., Katerinopoulos, H.E. *Chem. Commun.* **2008,** *46*, 6221-6223.

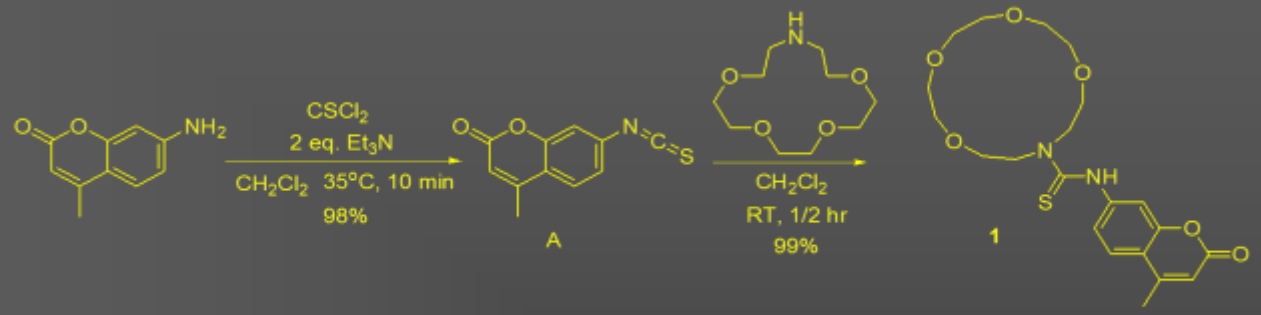
Photoinduced Electron Transfer (PET) or “Turn-On” Indicators

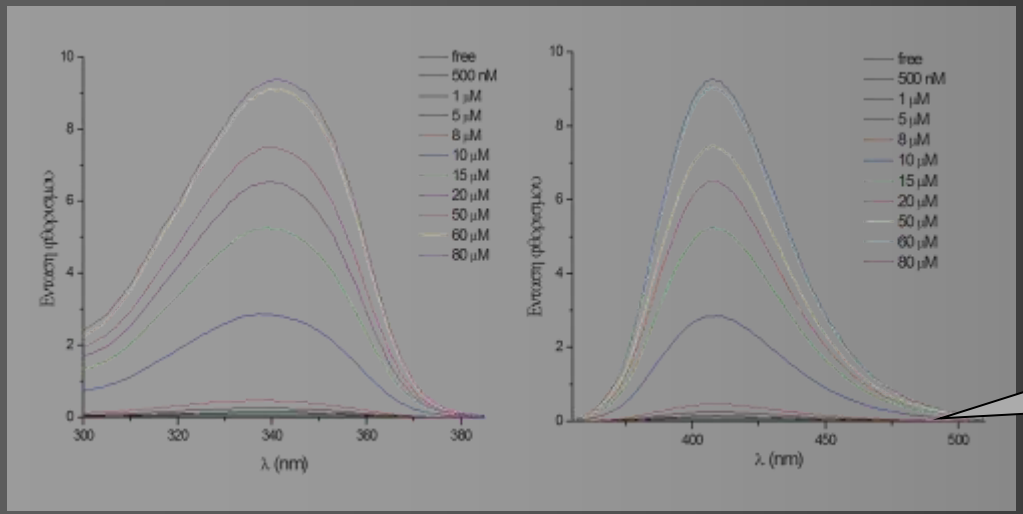
In the ion bound form, the redox potential of the donor changes and its HOMO becomes lower in energy than that of the fluorophore. As a result, PET is not possible any more and an increase in fluorescence intensity is observed upon cation binding.



In the photoinduced electron transfer (PET) , in the ion-free probe excitation of the fluorophore promotes an electron of the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). Consequently a PET takes place from the HOMO of the donor to that of the fluorophore causing fluorescence quenching of the latter.

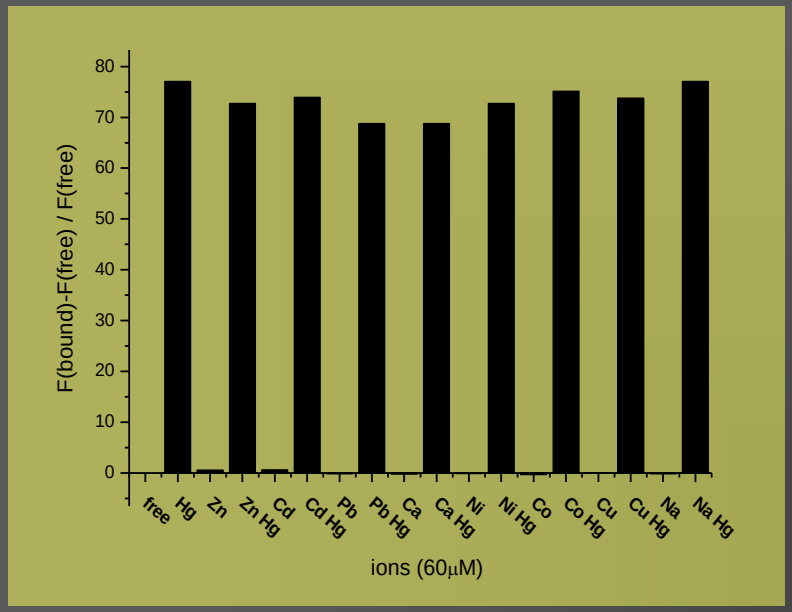
Combining modifications in the chromophore and the ionophore moieties: Synthesis of a monoaza crown ether-type Hg^{2+} probe



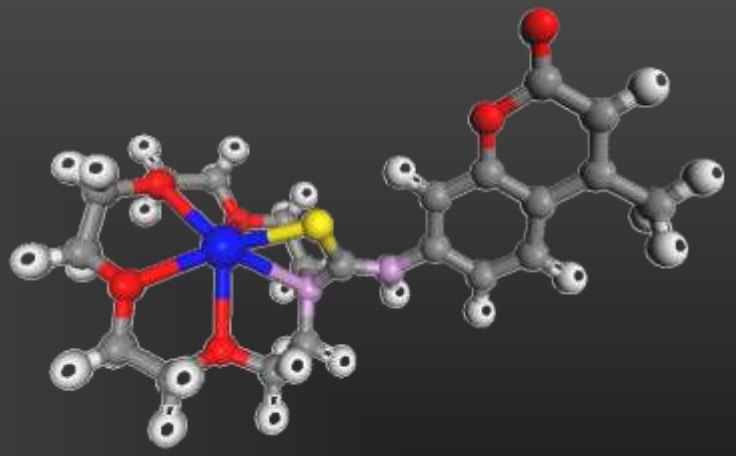


This is the case of a PET probe. The free probe exhibits almost zero fluorescence. Addition of metal ions enhances fluorescence

Synthesis of a monoaza crown ether-type Hg^{2+} probe: Ion Selectivity studies



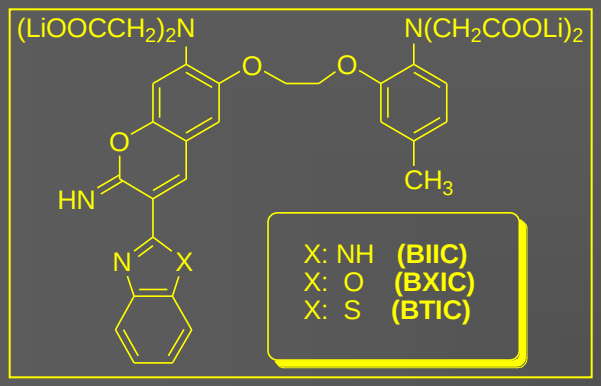
$K_d\,(Hg^{2+}) = 13.1\mu M$

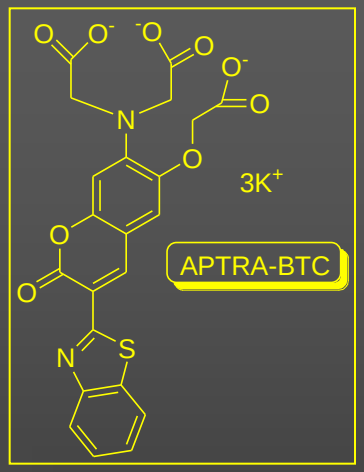


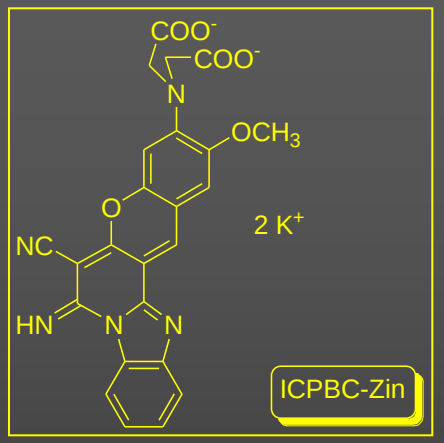
DFT geometry optimization for the **3**-Hg2+ complex.
Atoms are represented in colours; mercury: blue, sulphur: yellow, nitrogen: pink, oxygen: red, carbon: grey, hydrogen: white.

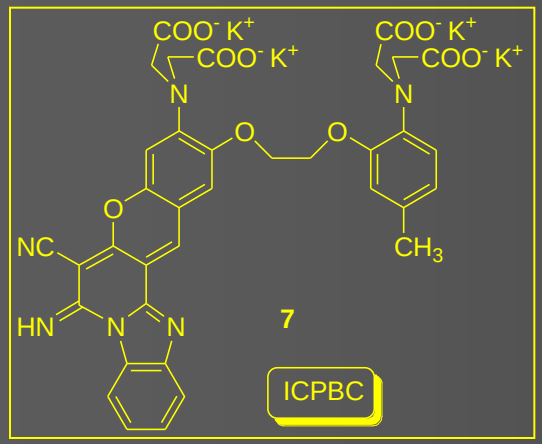
S. Voutsadaki, G. K. Tsikalas, E. Klontzas, G. E. Froudakis, H. E. Katerinopoulos *Chem. Commun.*, **2010**, DOI: 10.1039/B926384E

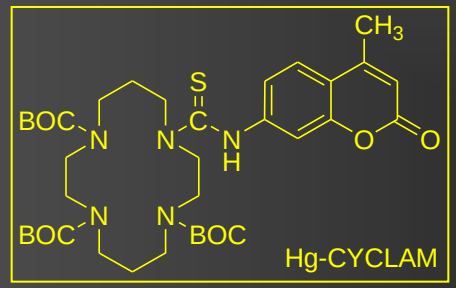
Additional Fluorescent Ion Probes from our Laboratory

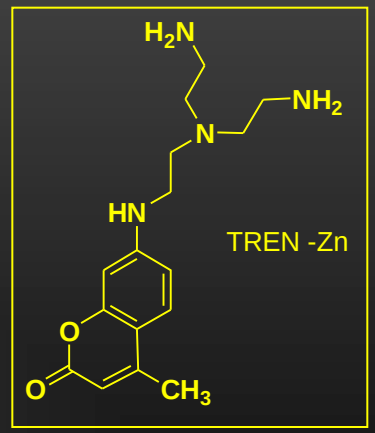


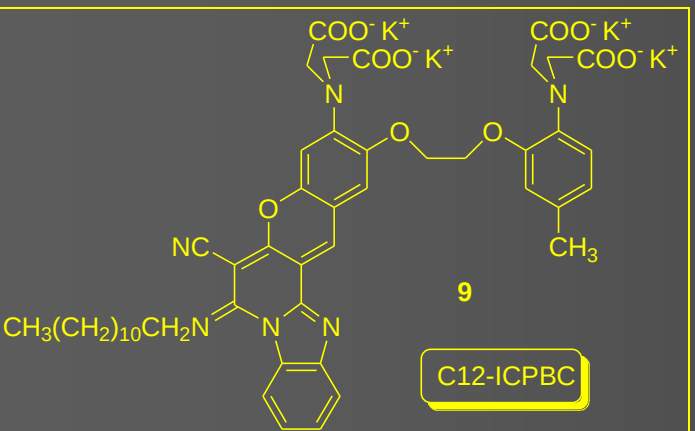












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