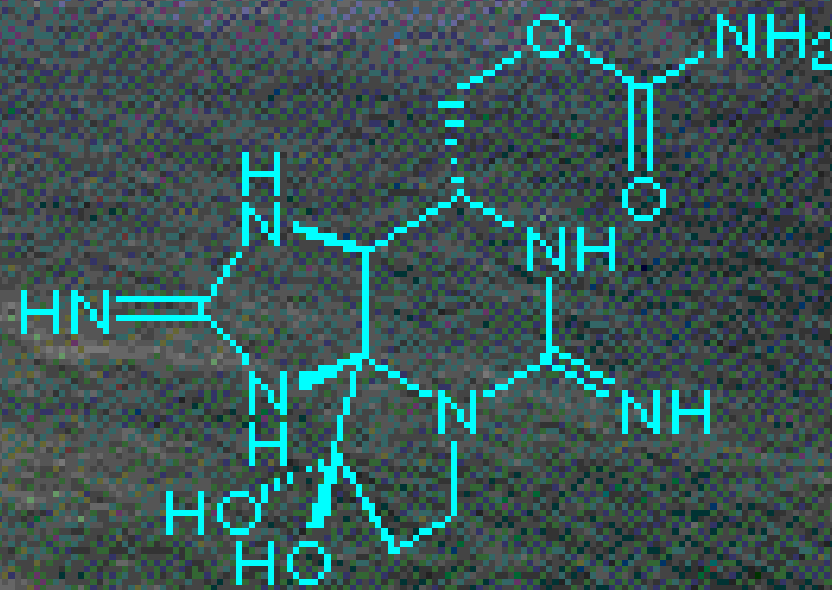
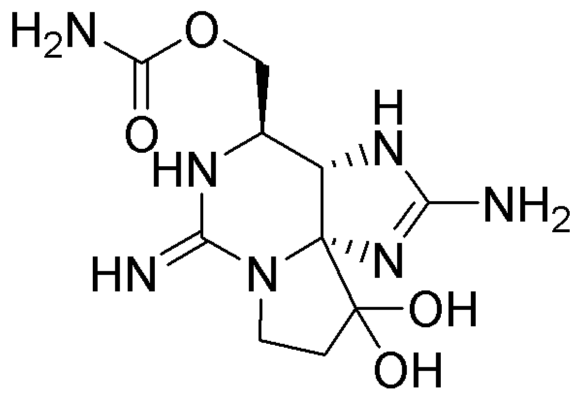


A case study – detection of saxitoxin

Saxitoxin

- One of the most toxic non-protein substances
- 0.2 mg is lethal to an average weight human
- Produced by certain algae species (dinoflagellates, cyanobacteria)
- The toxin accumulates in shellfish (mussels, clams and scallops)



Saxitoxin, STX

Saxitoxin

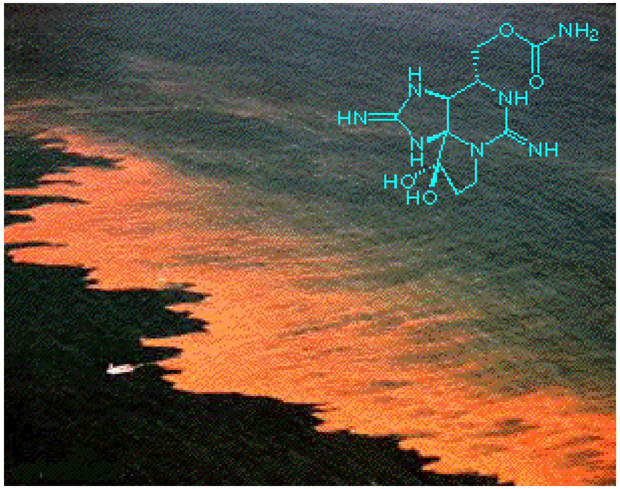
- Harmful Algal Blooms
- The toxin accumulates in shellfish
- Paralytic shellfish poisoning (PSP)
- Blocks neuronal sodium channels and mostly causes respiratory failure (artifical respiration is essential)
- Military interest (1000 times more toxic than nerve gas sarin)
- Suicide capsules (U2 pilot Francis G. Powers)
- In 1970 ”all” stx was destroyed

Saxitoxin

- Harmful Algal Blooms – e.g. red tides

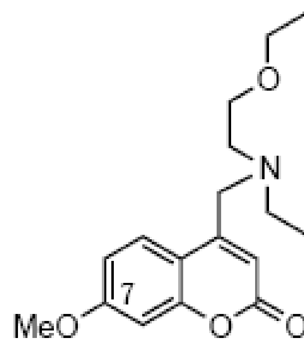
...and the waters that were in the river were turned to blood. And the fish that were in the river died; and the river stank and the Egyptians could not drink of the water of the river... Exodus 7: 20-21

- Official test: mouse ”assay” (LD_{50} = 8 μg/kg)
- A more sensitive and friendly assay is needed

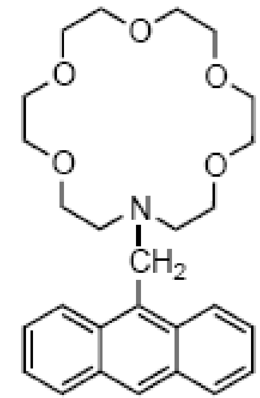


Saxitoxin detection by PET sensors

- aza-18-crown 6 ethers
- coumaryl and anthracyl signaling units



1



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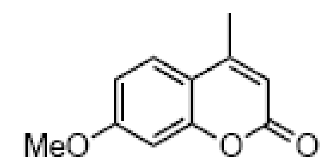

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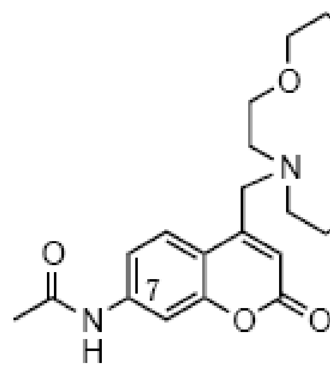

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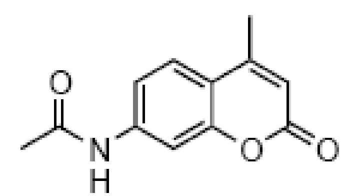
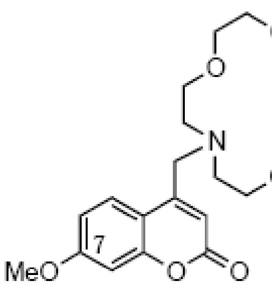
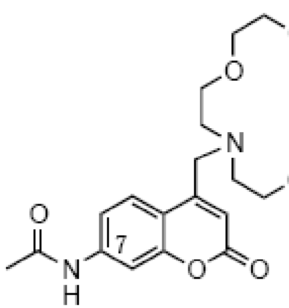


5

Saxitoxin detection by PET sensors



1



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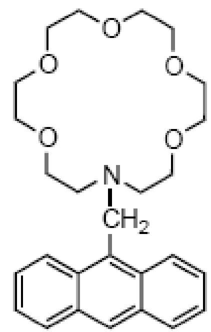
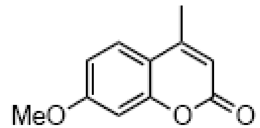


3



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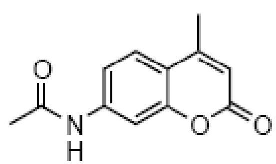
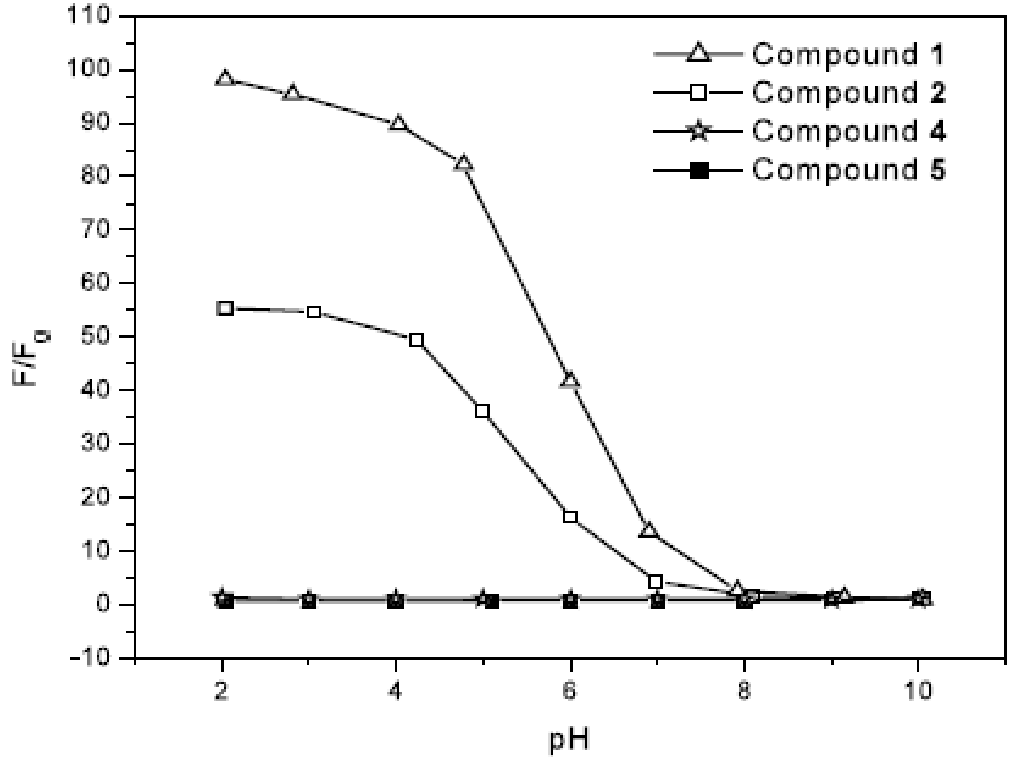
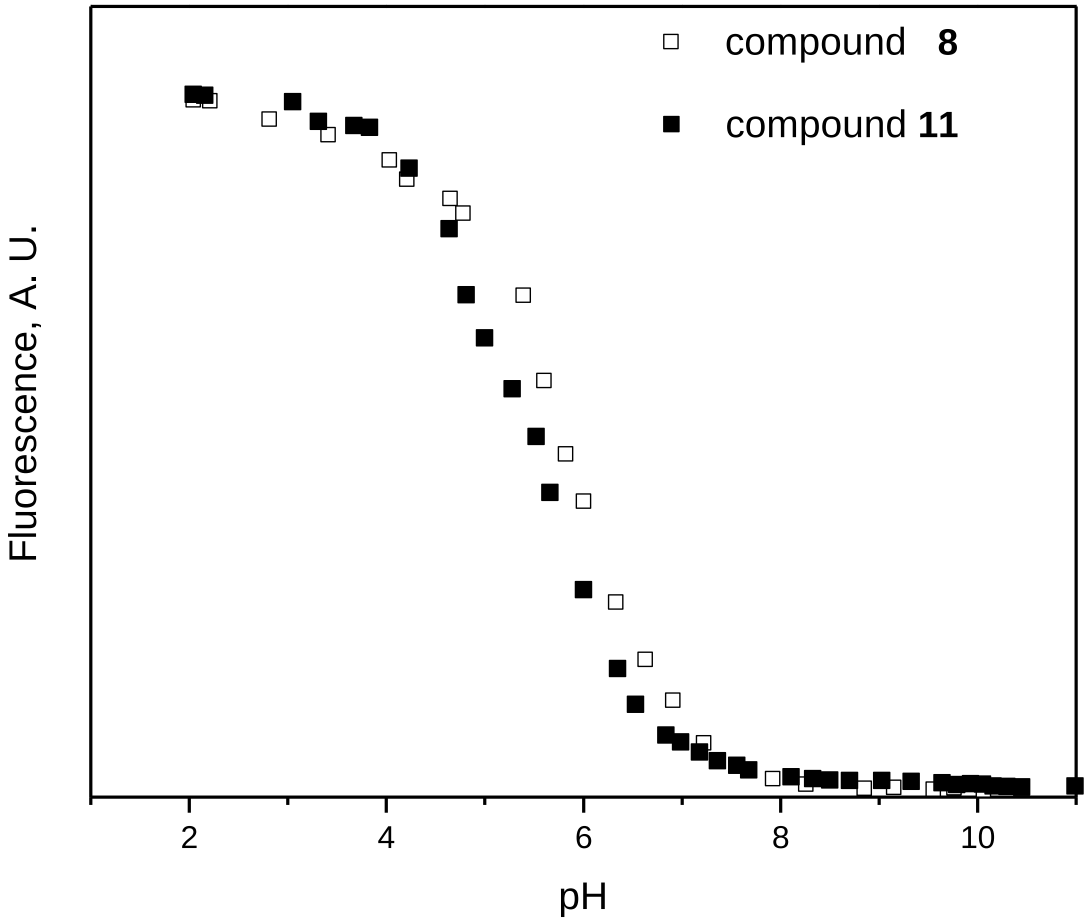


5

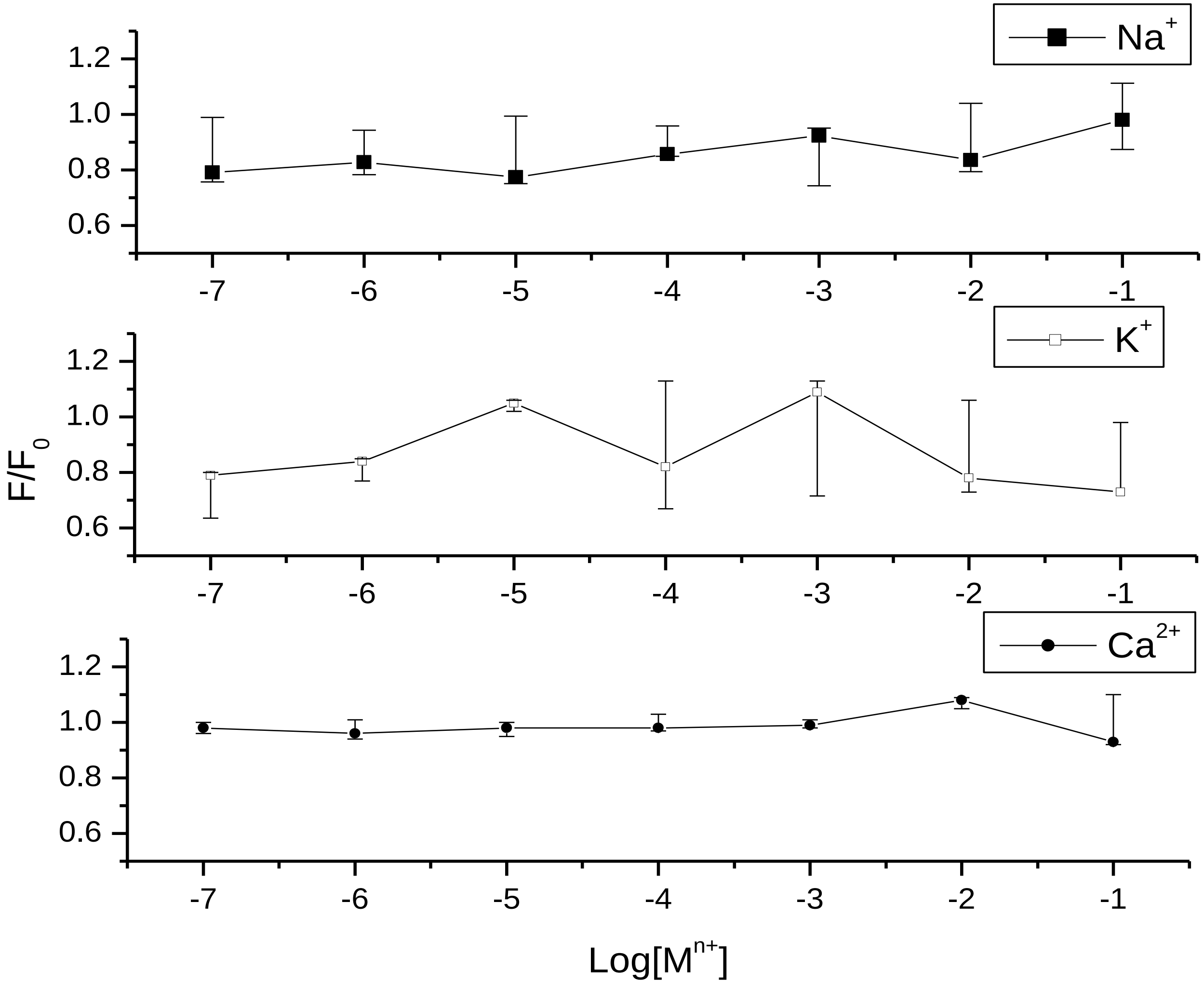


pH dependency of PET sensors

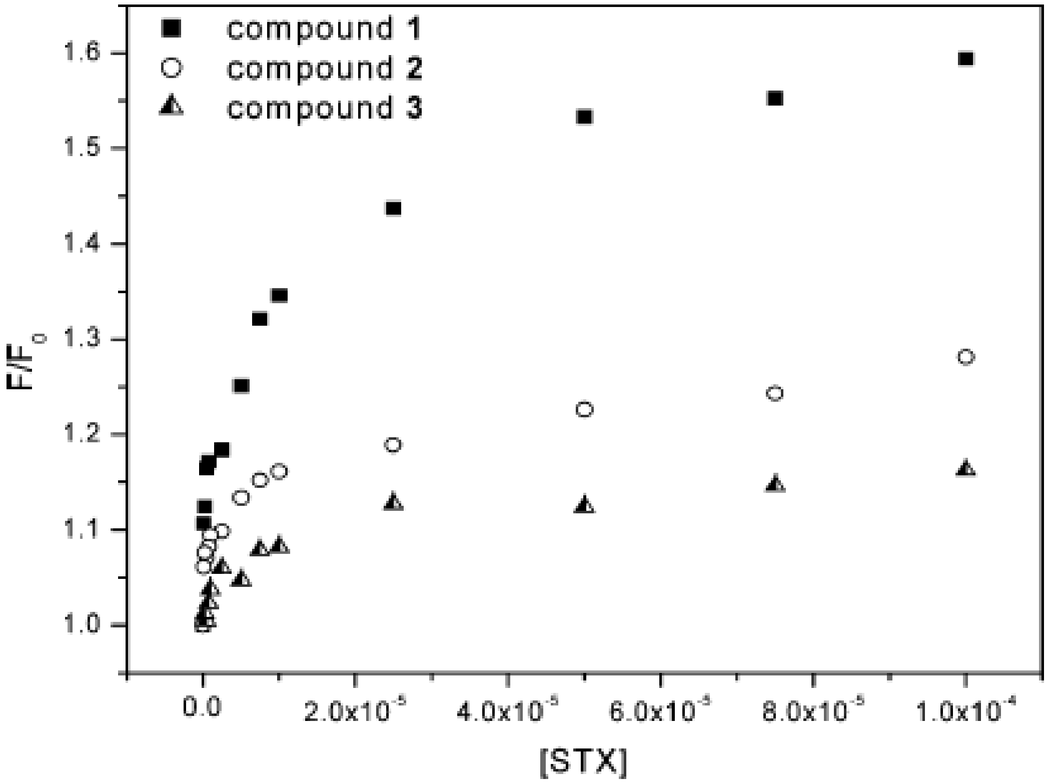


- pKa of comaryl sensors are: 5.8 and 6.0
- pKa of anthracy sensor: 8.0

Presence of cations



Presence of STX



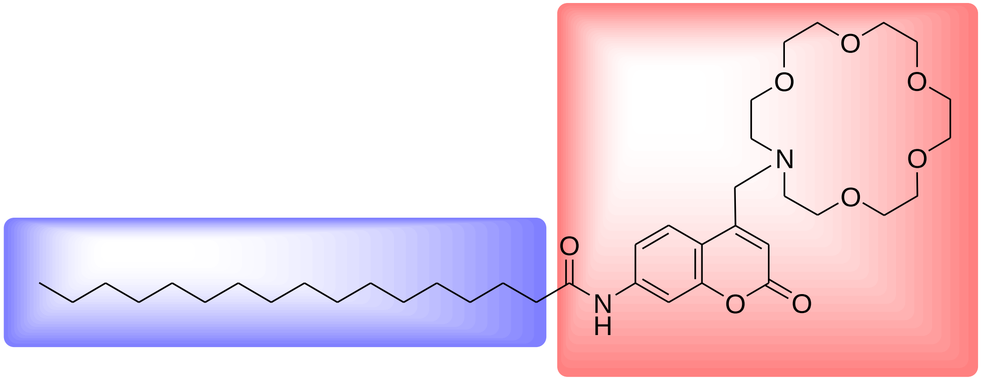
Compound	K_b (M^{-1})	Correlation coefficient
1	$1.35 \pm 0.72 \times 10^5$	0.96
2	$4.15 \pm 0.12 \times 10^5$	0.92
3	$1.25 \pm 0.22 \times 10^5$	0.98

- 1 is the most water soluble and gives the largest signal
- 2 binds STX the strongest
- 1:1 complex is assumed

Kele et al. Tetrahedron Lett. 2002, 43, 4413-4416.

Sensor fabrication

- Sensory layer on a support (physically adsorbed, chemically bound)
- Surface properties should be characterized first (photophysical and sensing properties)
- Surface chemistry (Langmuir and Langmuir-Blodgett films)
- Air / water interface using sensor amphiphile

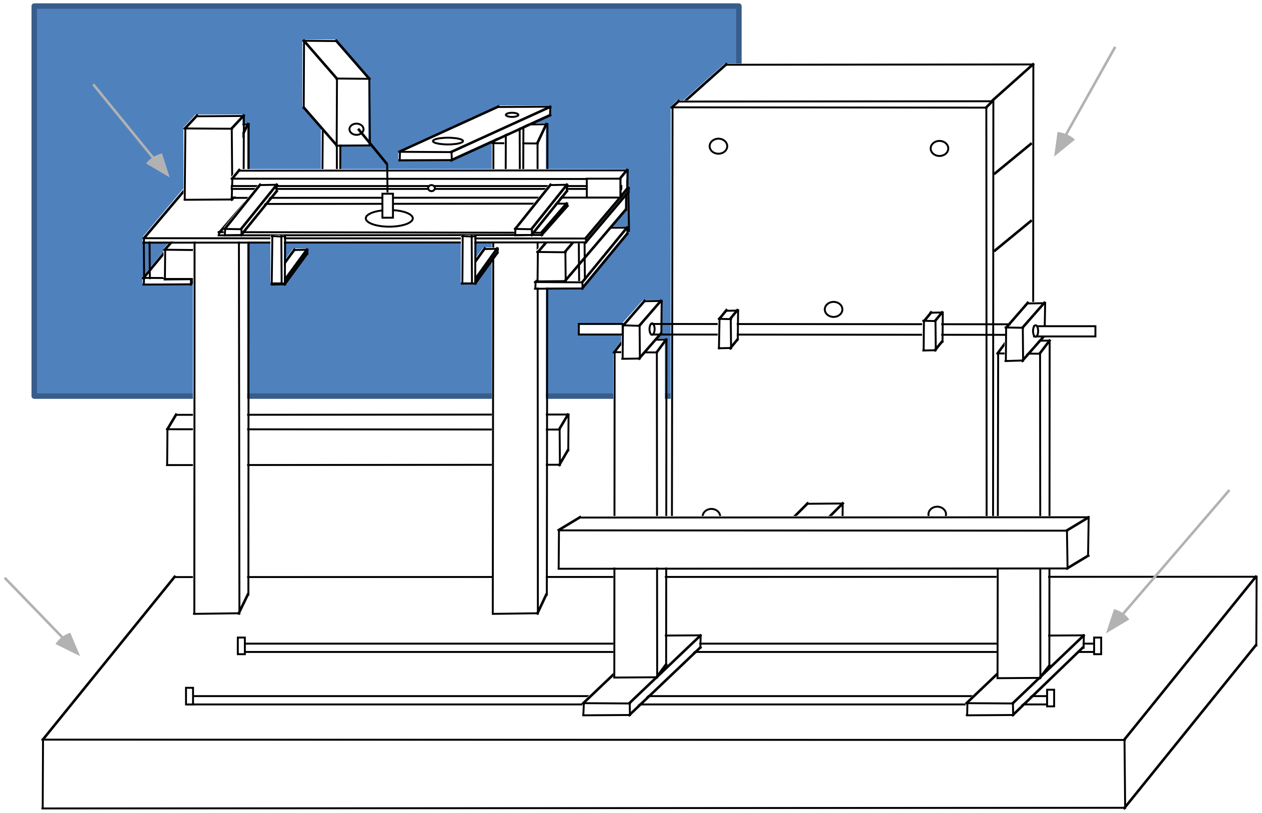


hydrobhobic tale

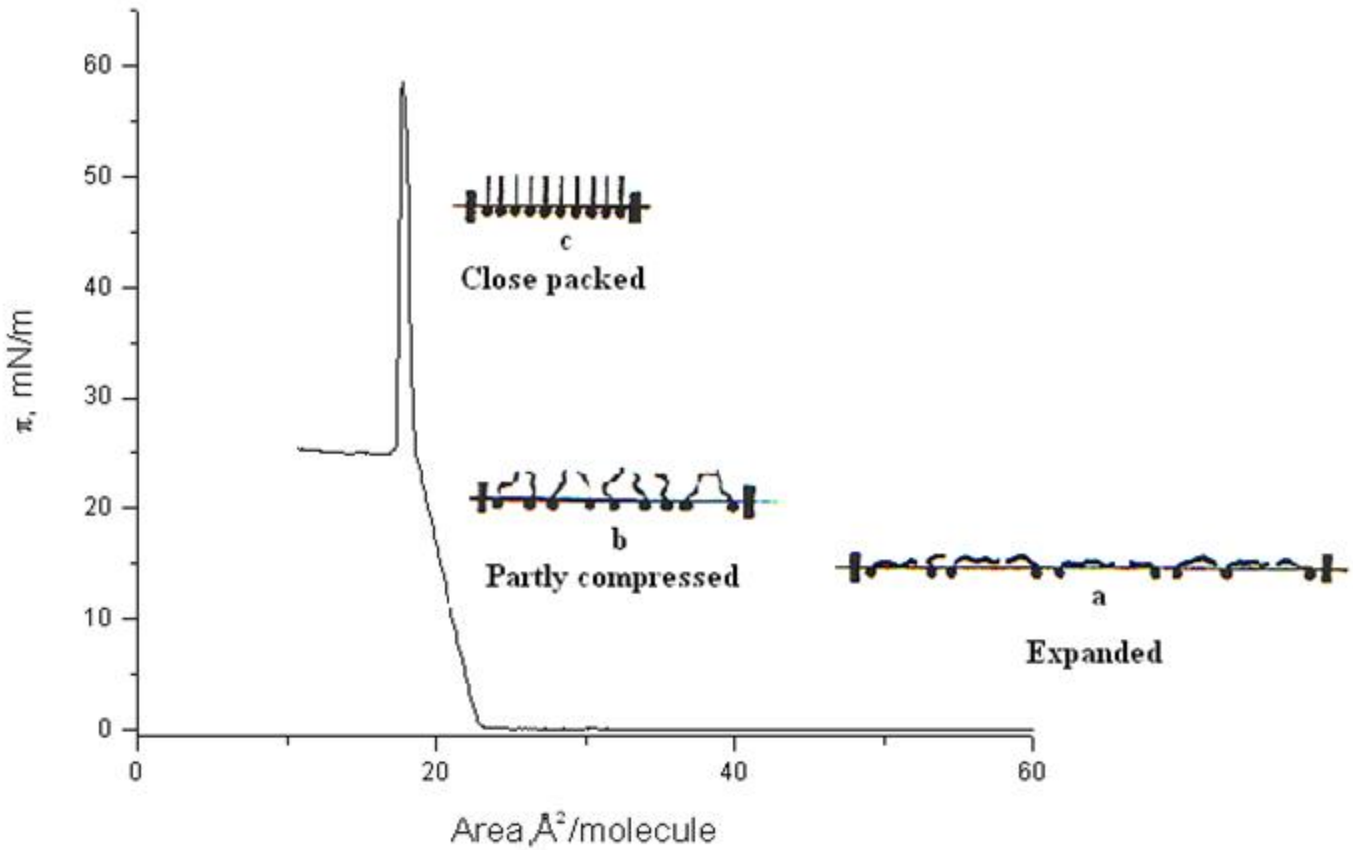
hydrophylic head

Langmuir film

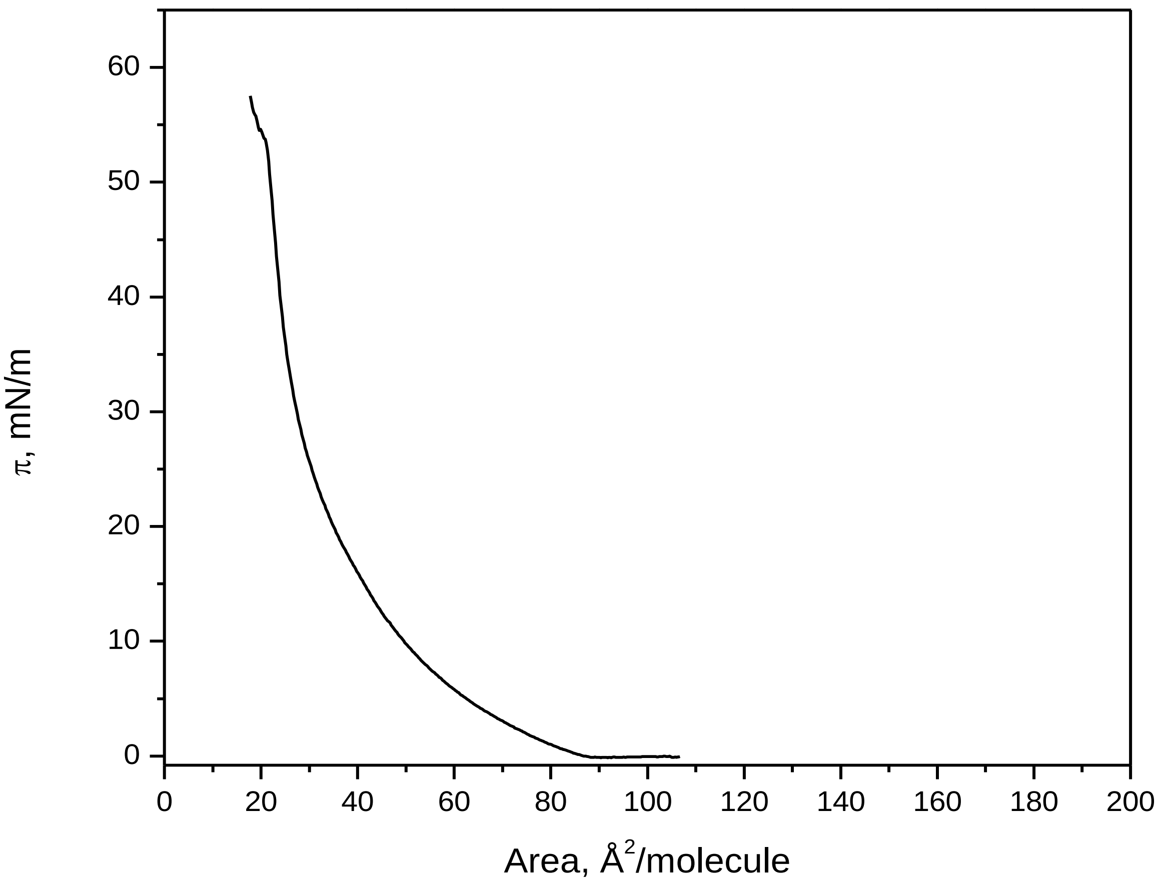
- An amphiphil monolayer at the air/water interface
- The surface presure – area curve shows where it forms a stable compact film



Surface pressure – area curve of stearic acid

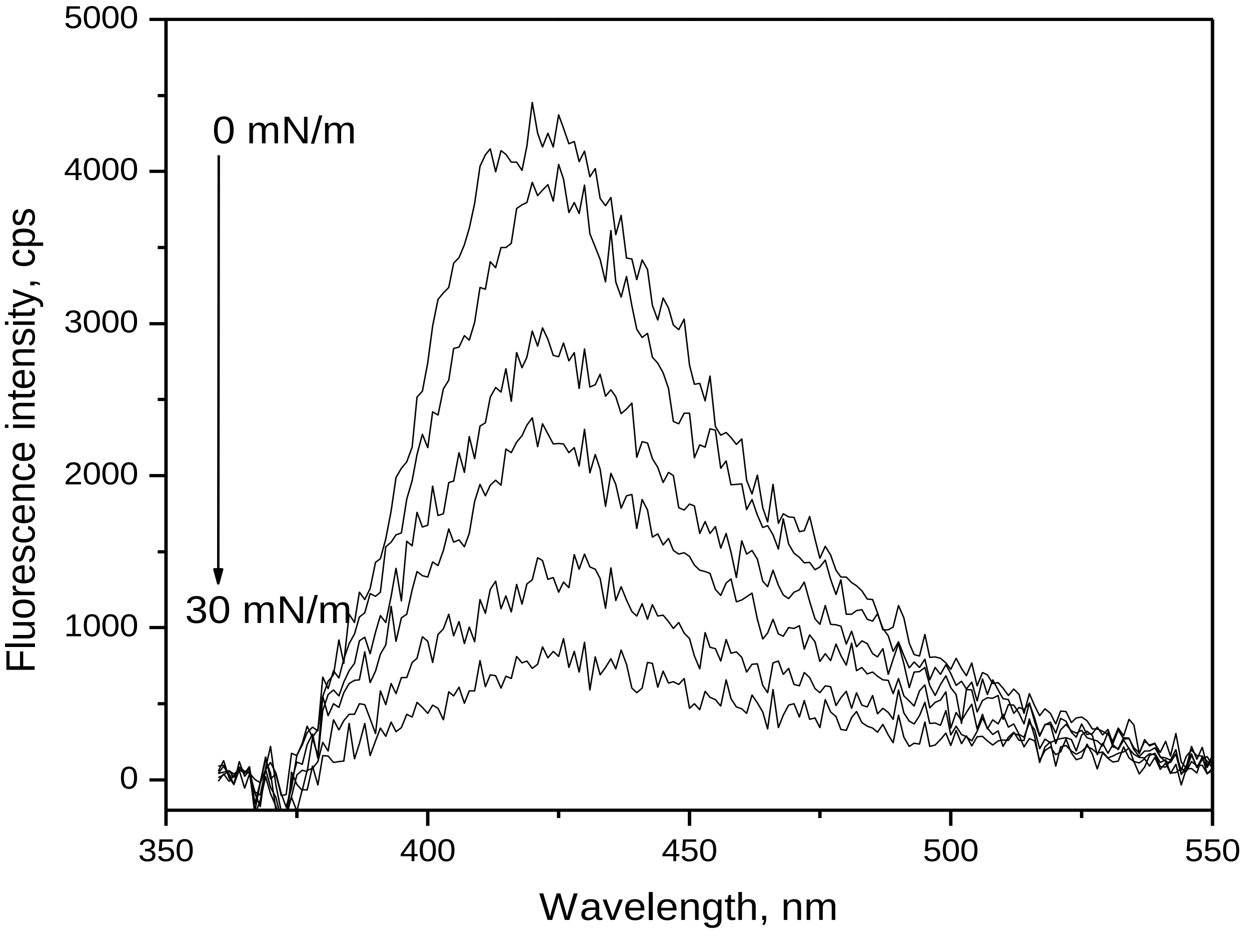


Surface pressure – area curve of sensor amphiphile

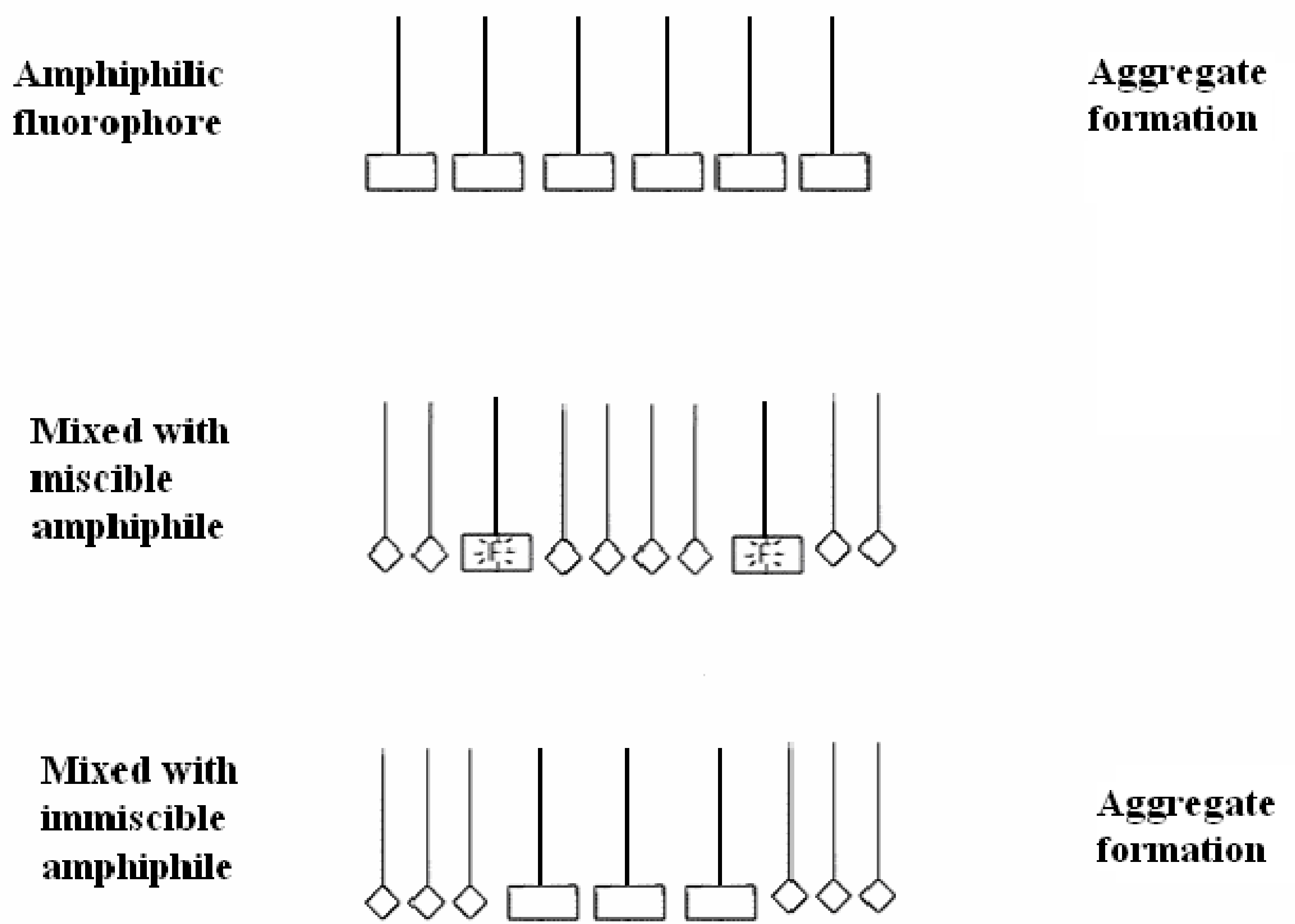


Limiting molecular area: 38 $Å^2$/molecule

Fluorescence is quenched when fluorphores are compressed



Mixed monolayers – restored fluorescence

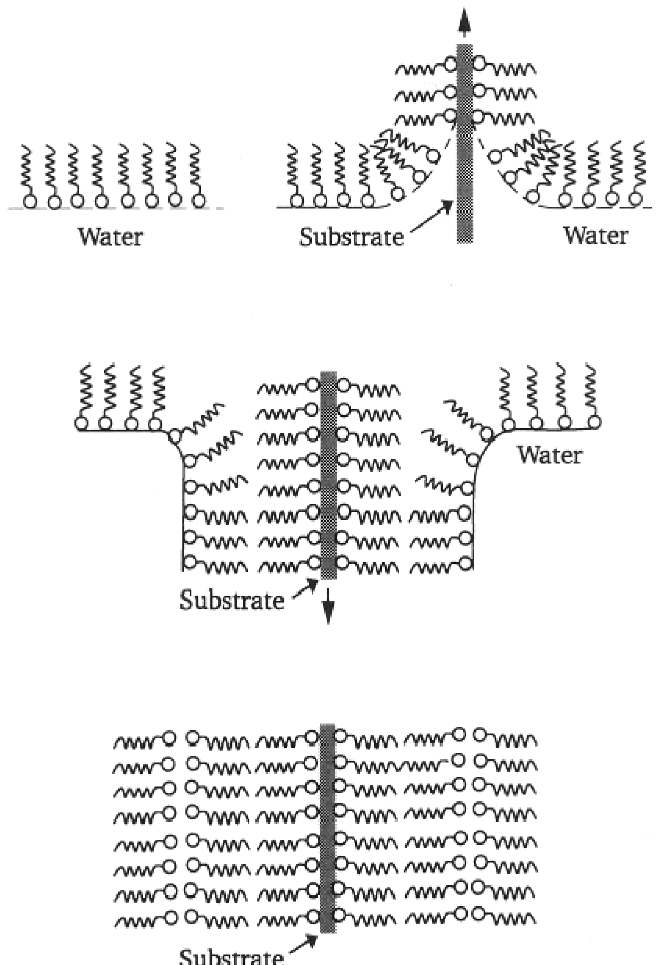


- Peptidolipid as two-dimensional solvent

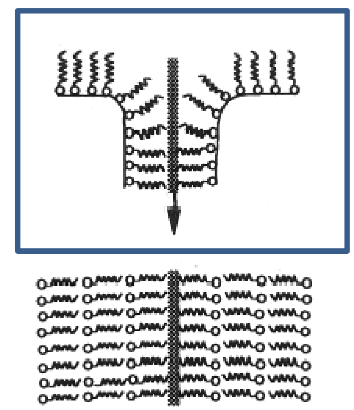
$C_{17}H_{35}CO$-Gly-Gly-Ala-Gly-NH_2

- Fluorescence intensity increased until 15 mN/m

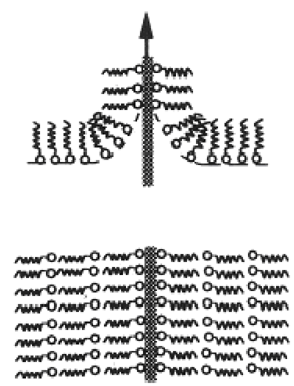
Transfer of the monolayer onto a solid support – Langmuir-Blodgett films



Y-deposition

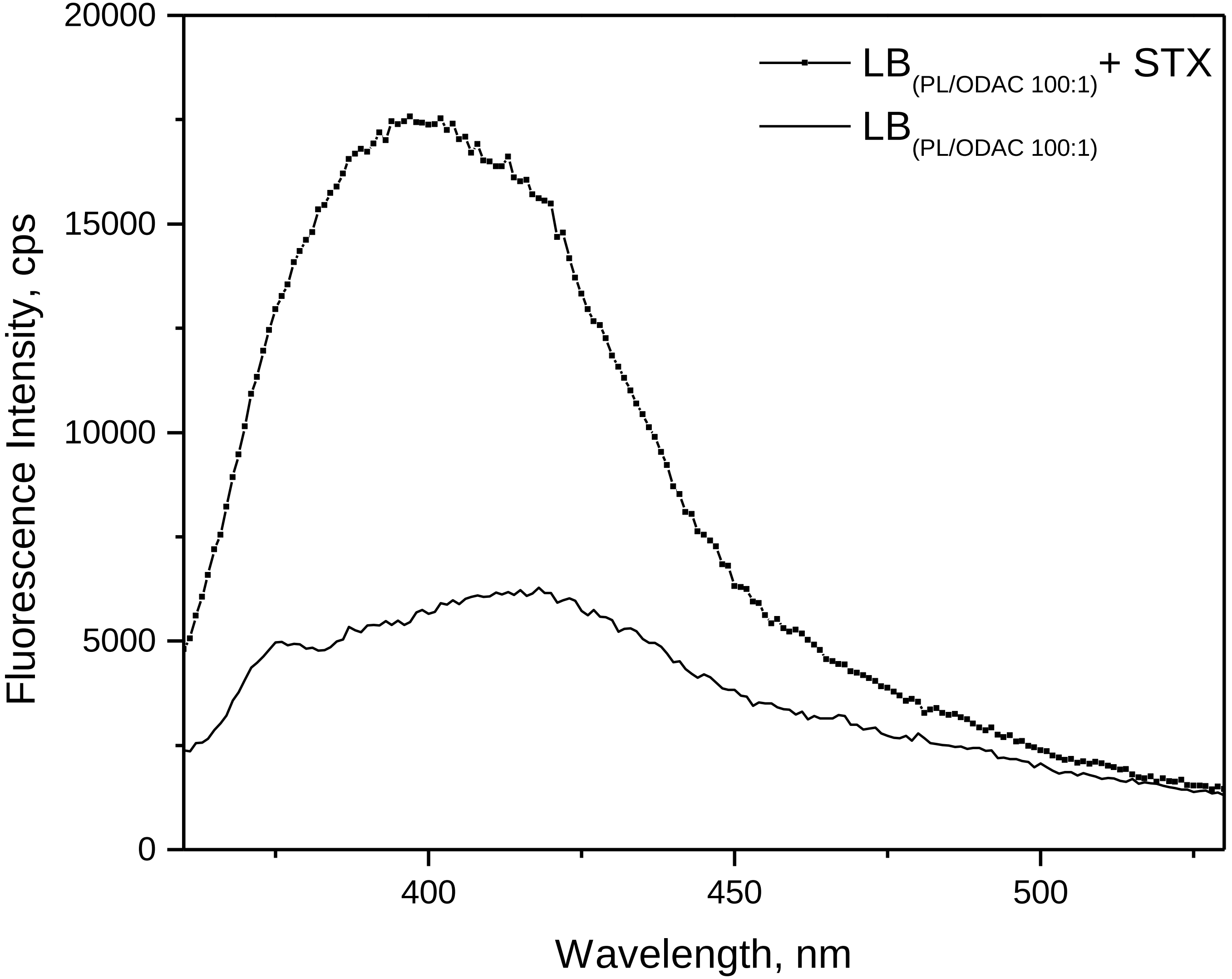


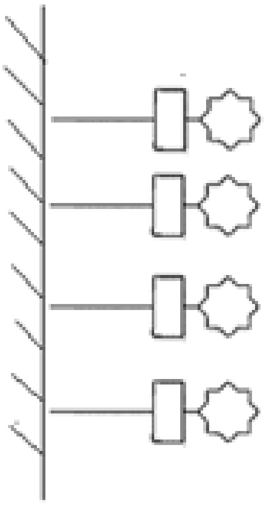
X-deposition



Z-deposition

Langmuir-Blodgett film detects STX

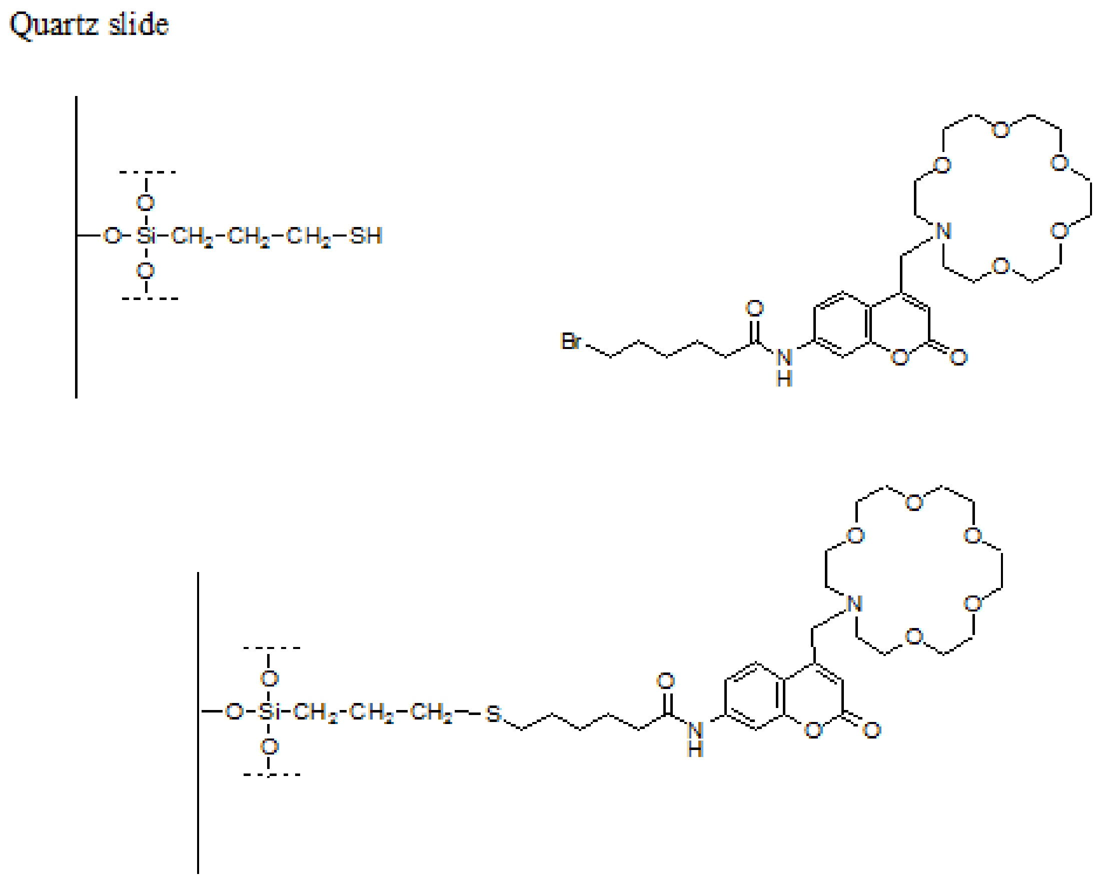




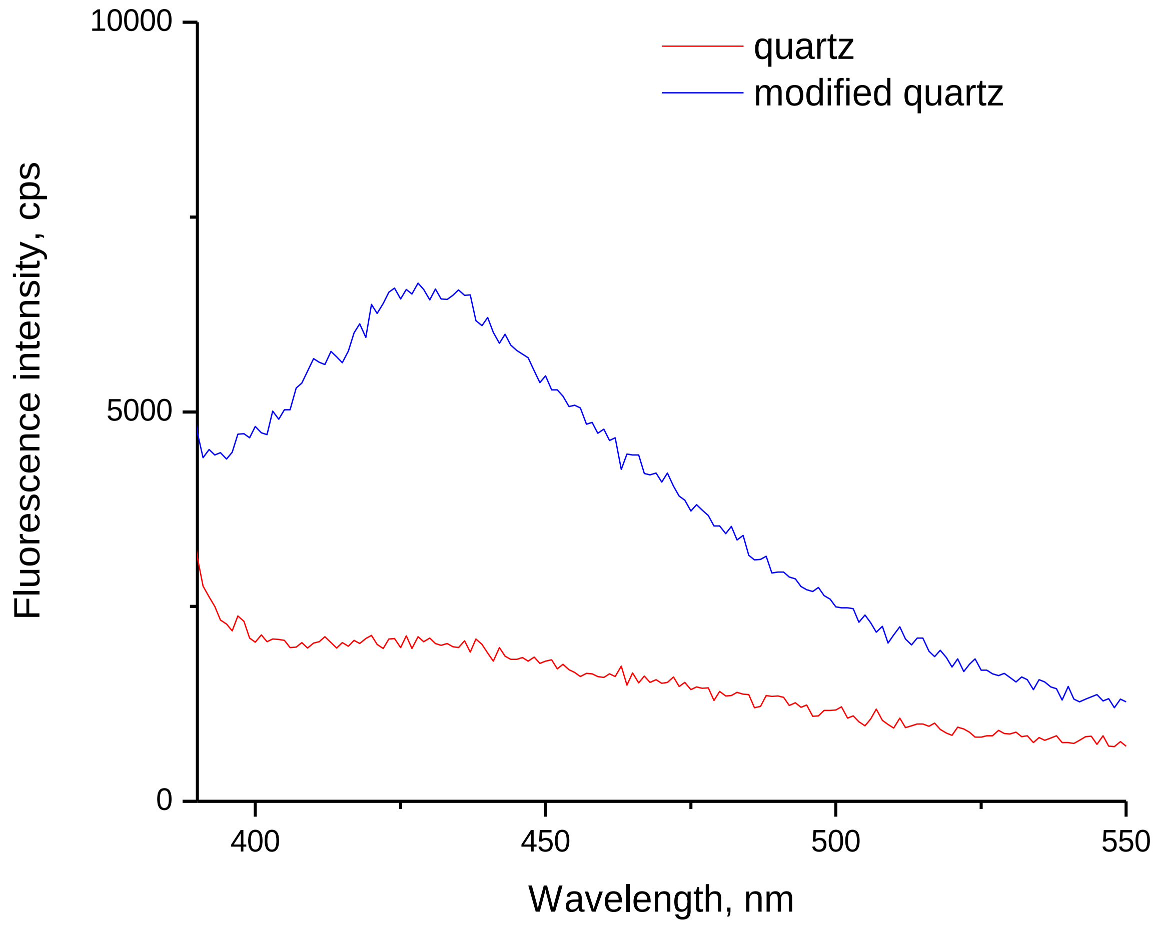
Kele et al. Langmuir 2002, 18, 8523-8526.

Covalent attachment of sensor film onto support – Self assembling monolayer (SAM)

- No leaching from the support
- Reusable sensor

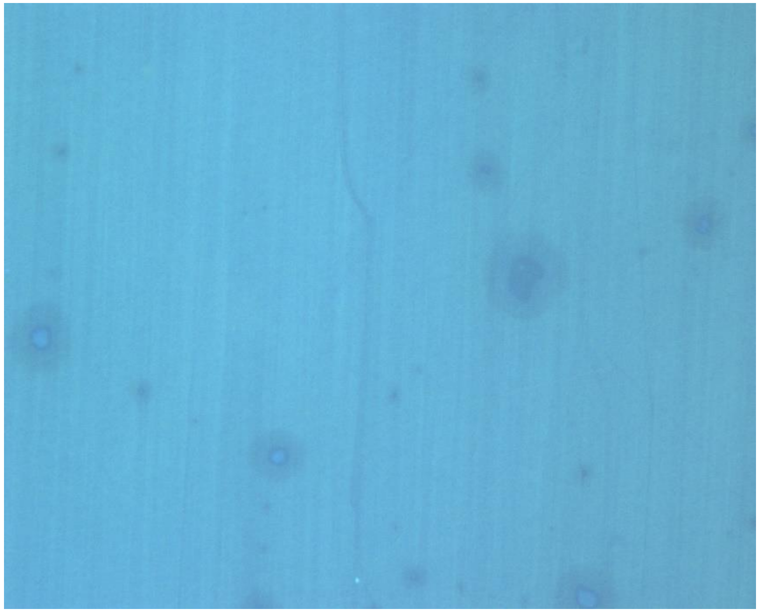


Fluorescence of modified quartz slide



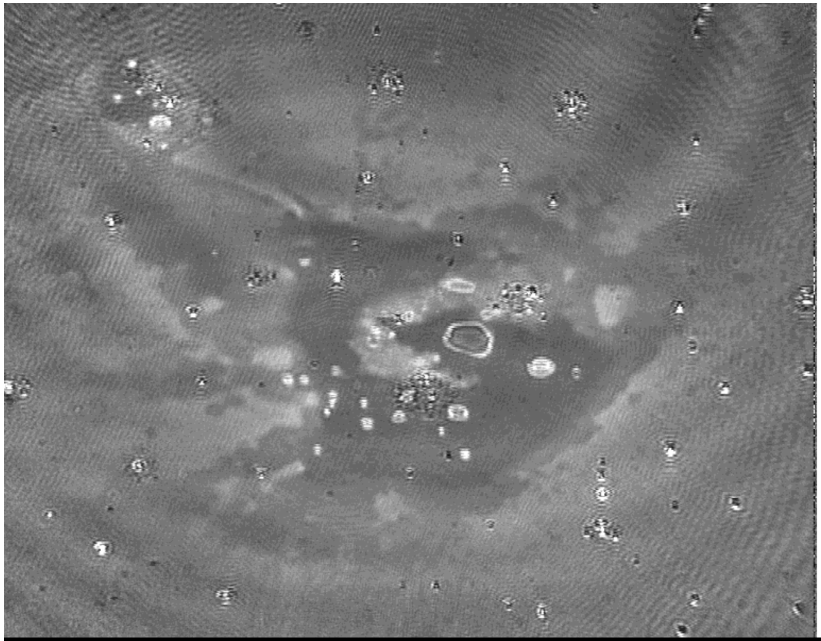
Epifluorescence microscopy of quartz slides

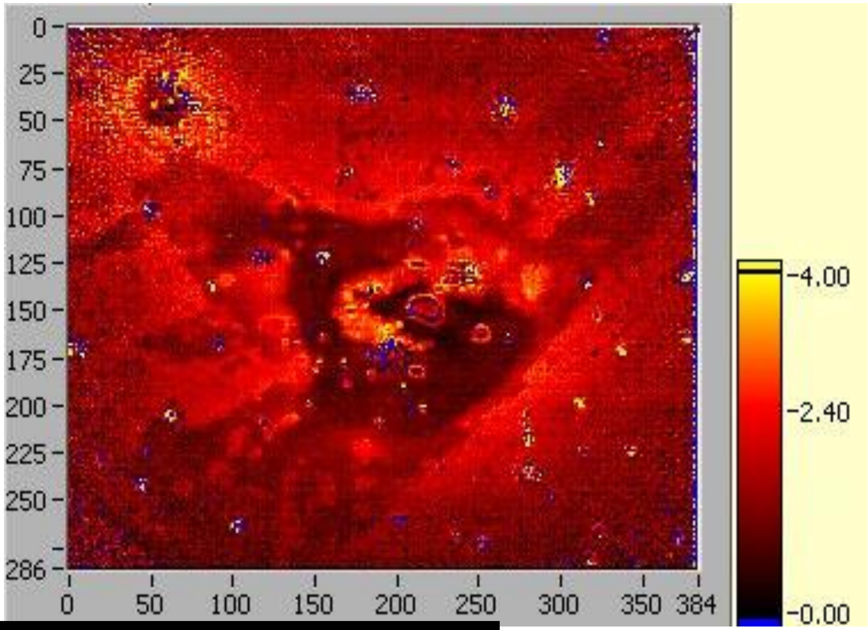
Modified

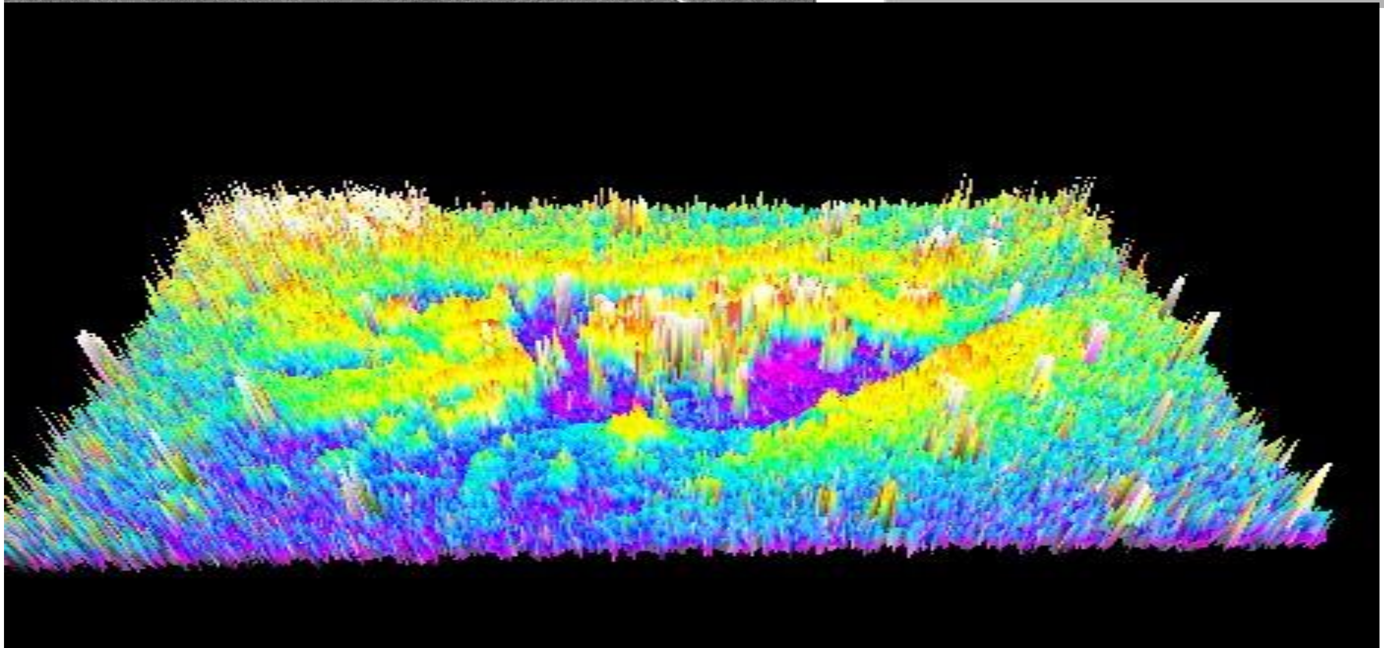


Unmodified

Imaging ellipsometry of modified quartz slide

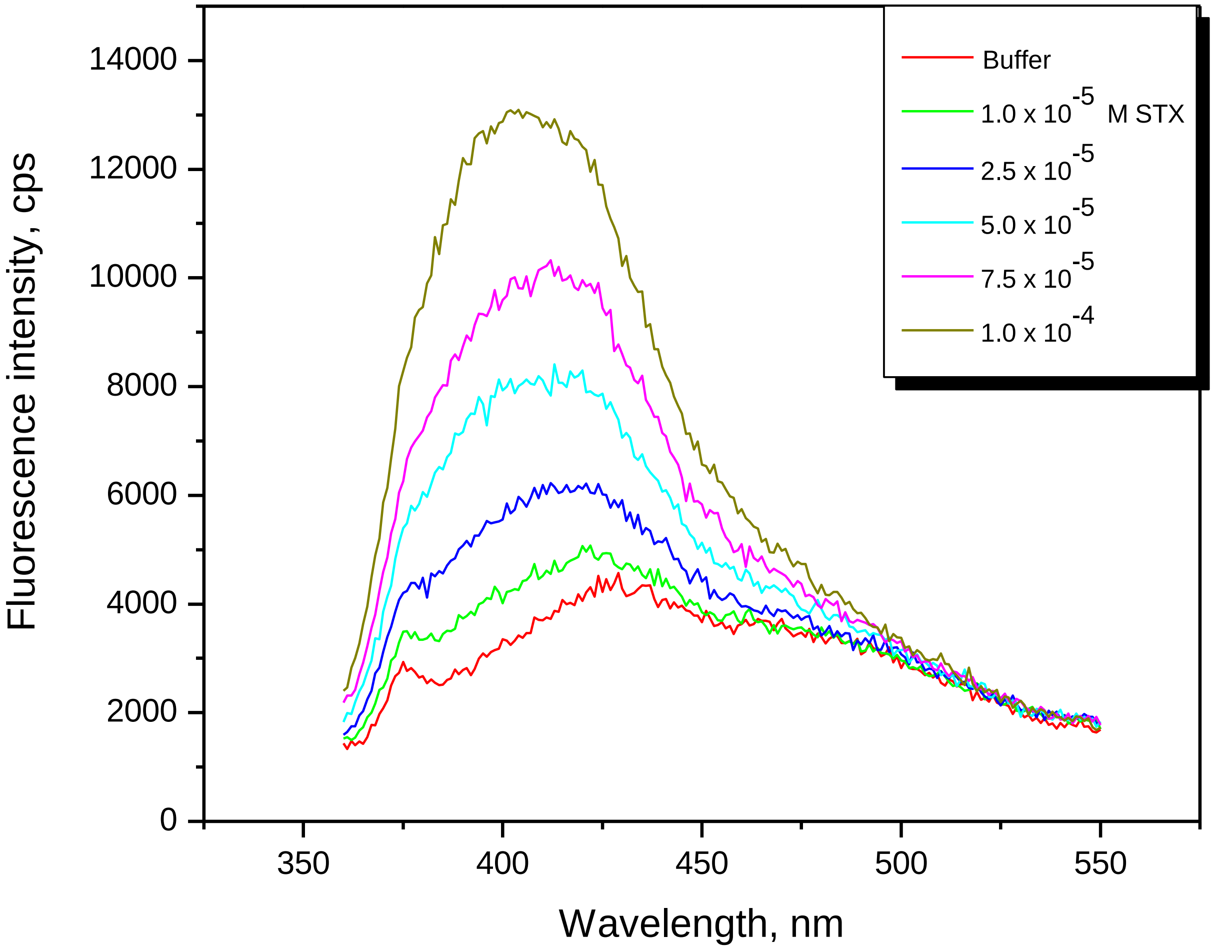






~72% Surface coverage

Detection of STX with coumaryl-crown modified quartz slide



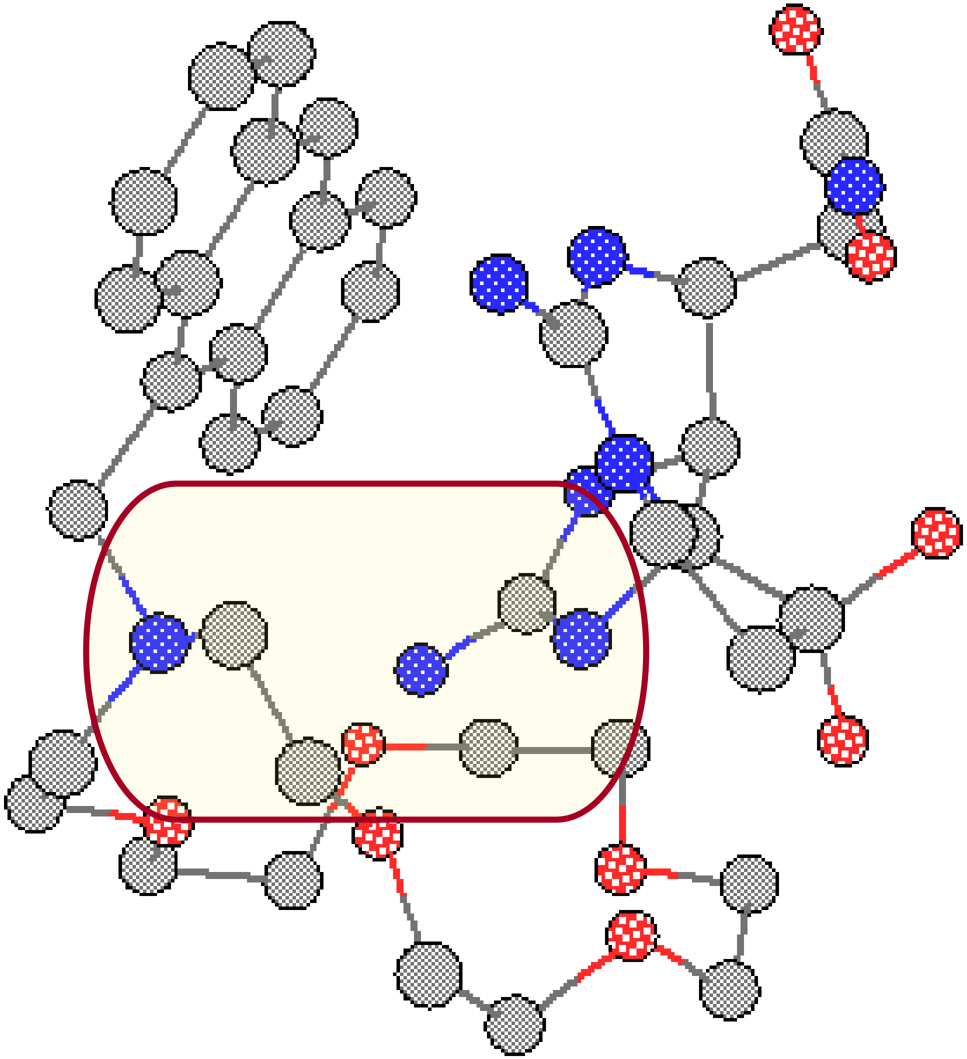
Kele et al. Chem. Commun. 2006, 14, 1494-1496.

Afterparty

- Docking experiments revealed that the guanidino function is not at the right orientation to H-bond with the quencher N

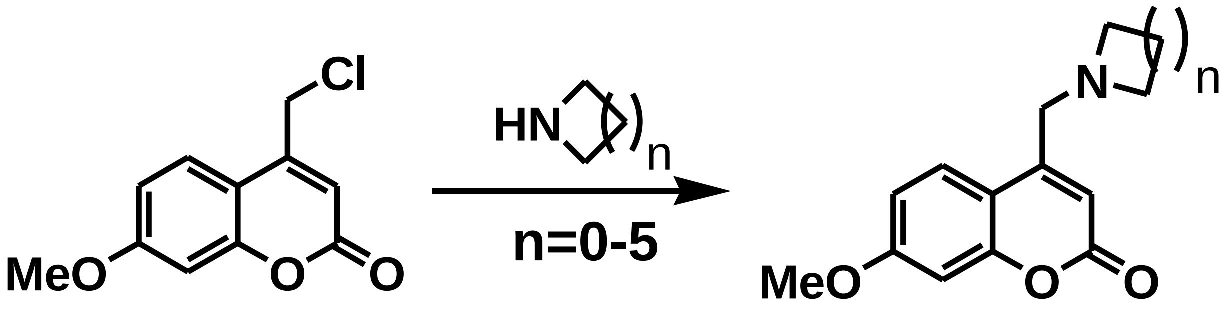
- Only the 7th lowest energy conformation showed close proximity of this two groups

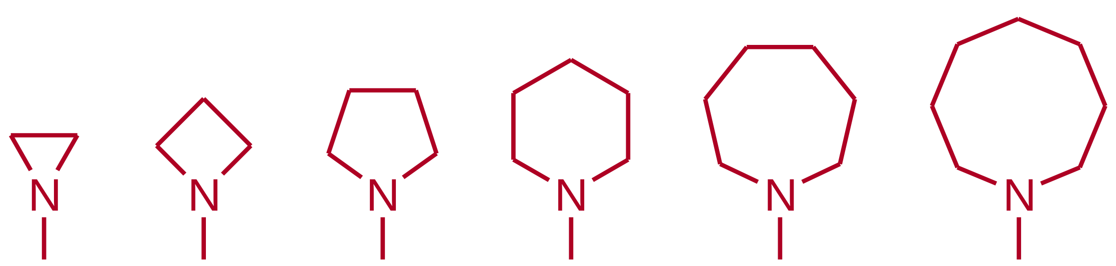
- Why does it work?



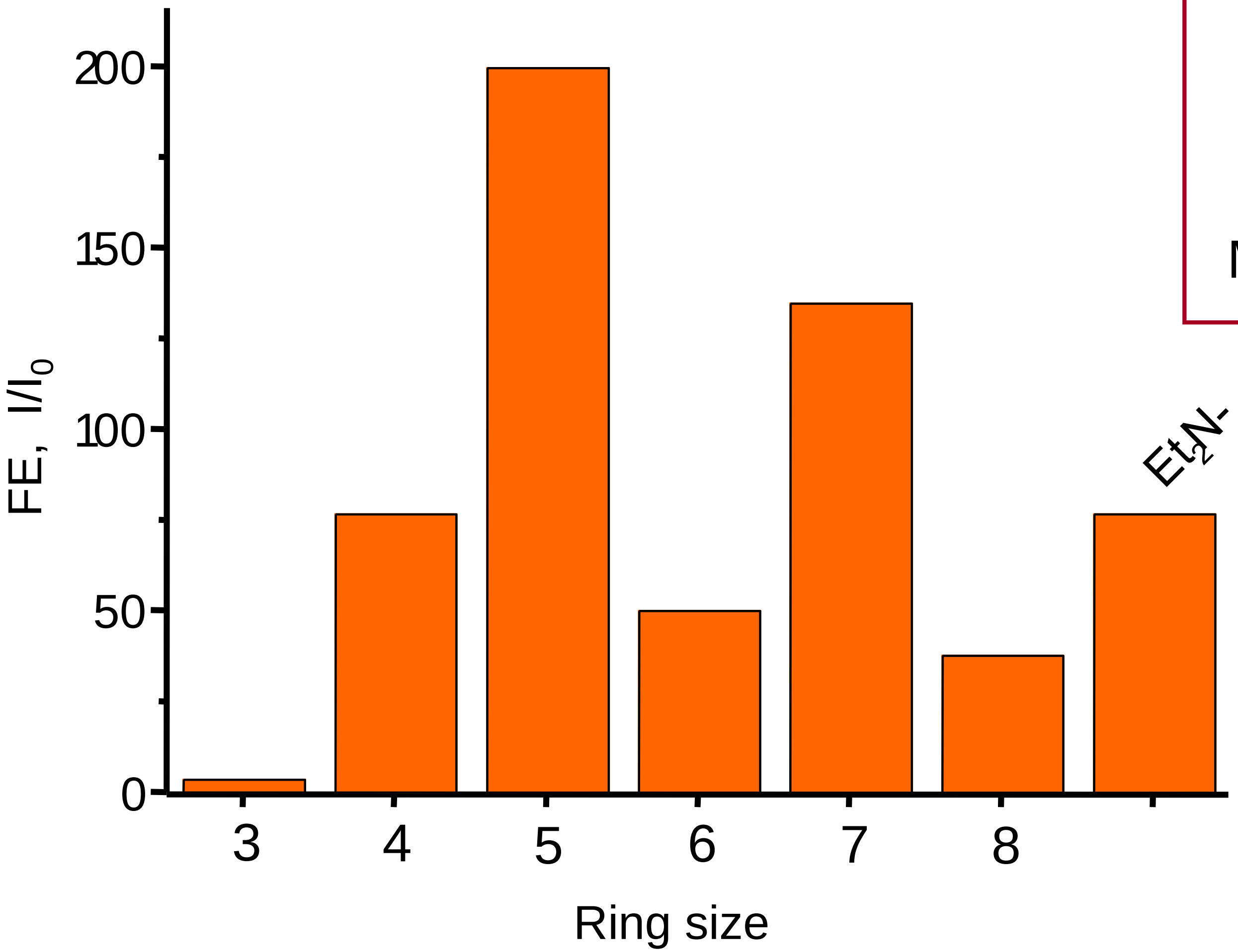
The effect of conformational mobility on PET

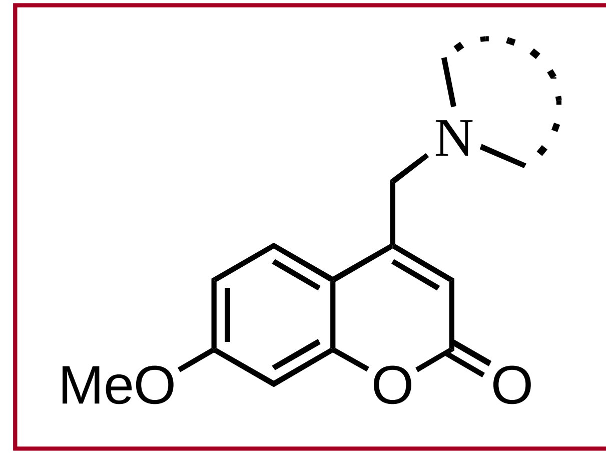
- Efficiency of PET in case of different membered rings





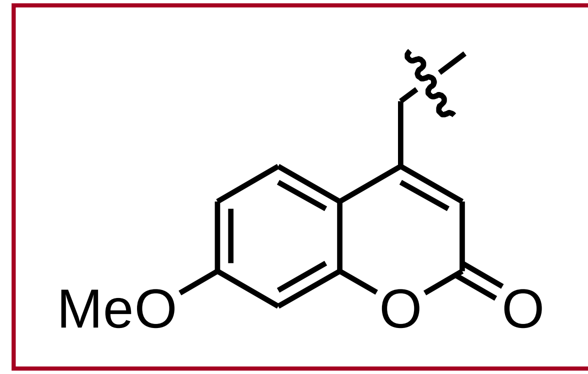
PET efficiency = protonation induced FE

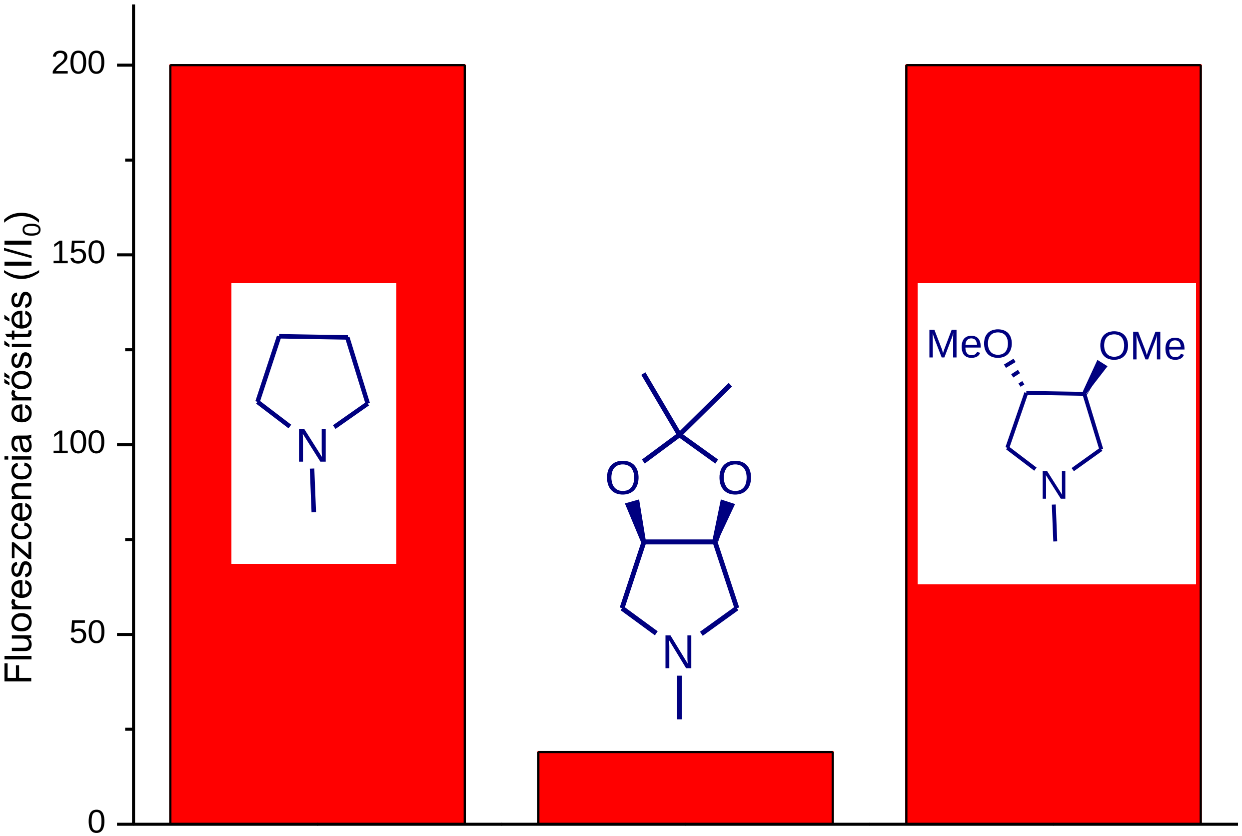




10^{-6} M sensor
10^{-3}M HBF_4
MeCN

Electronic effects



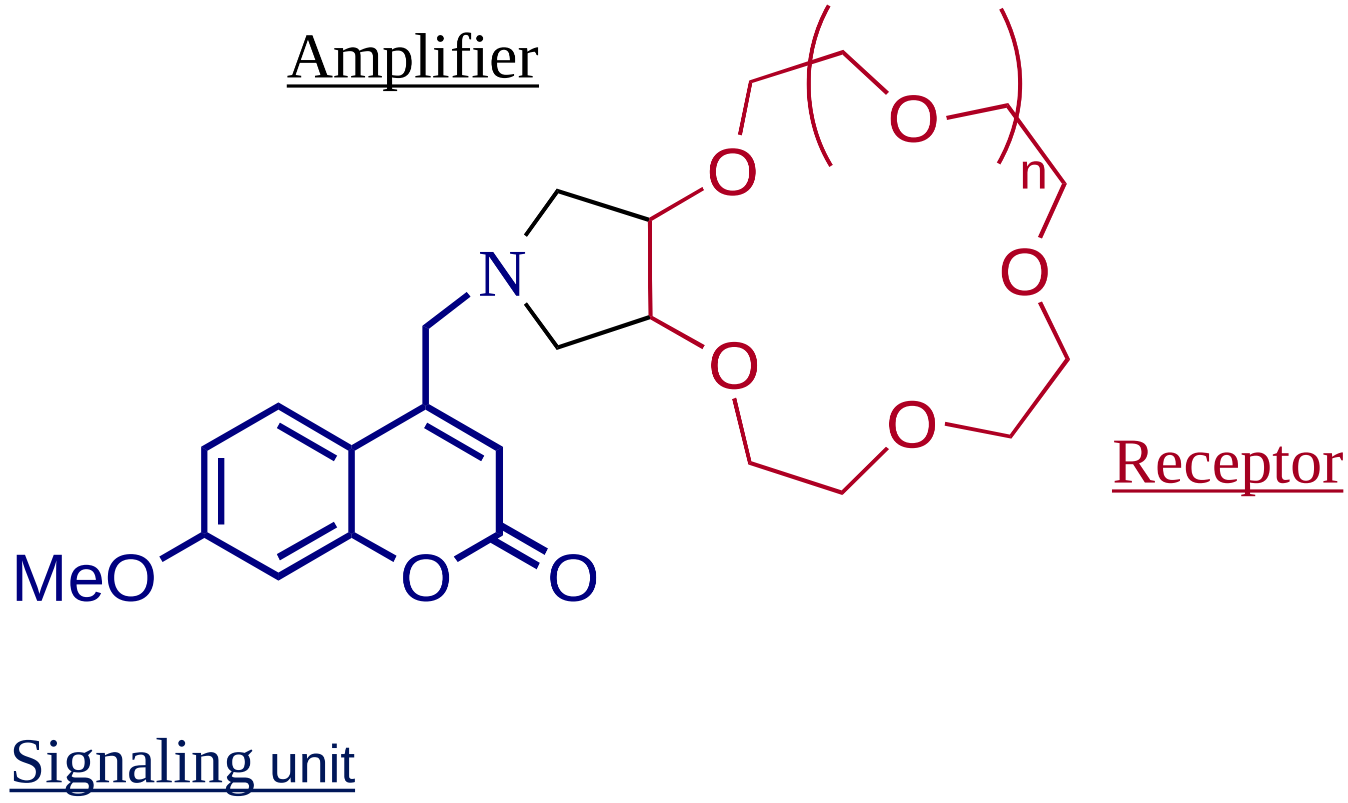


Effect of substituents

- No electronic effect (dimethoxy derivative)

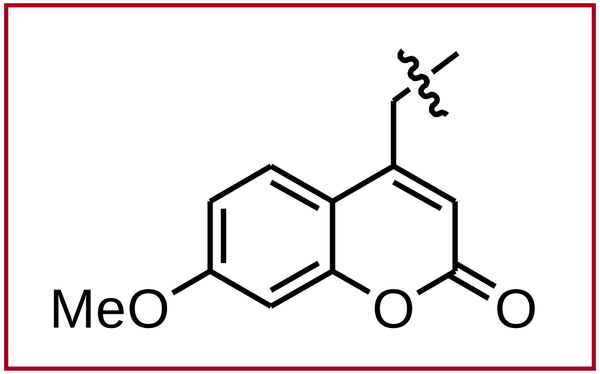
- Large conformational effect (rigid acetonide)

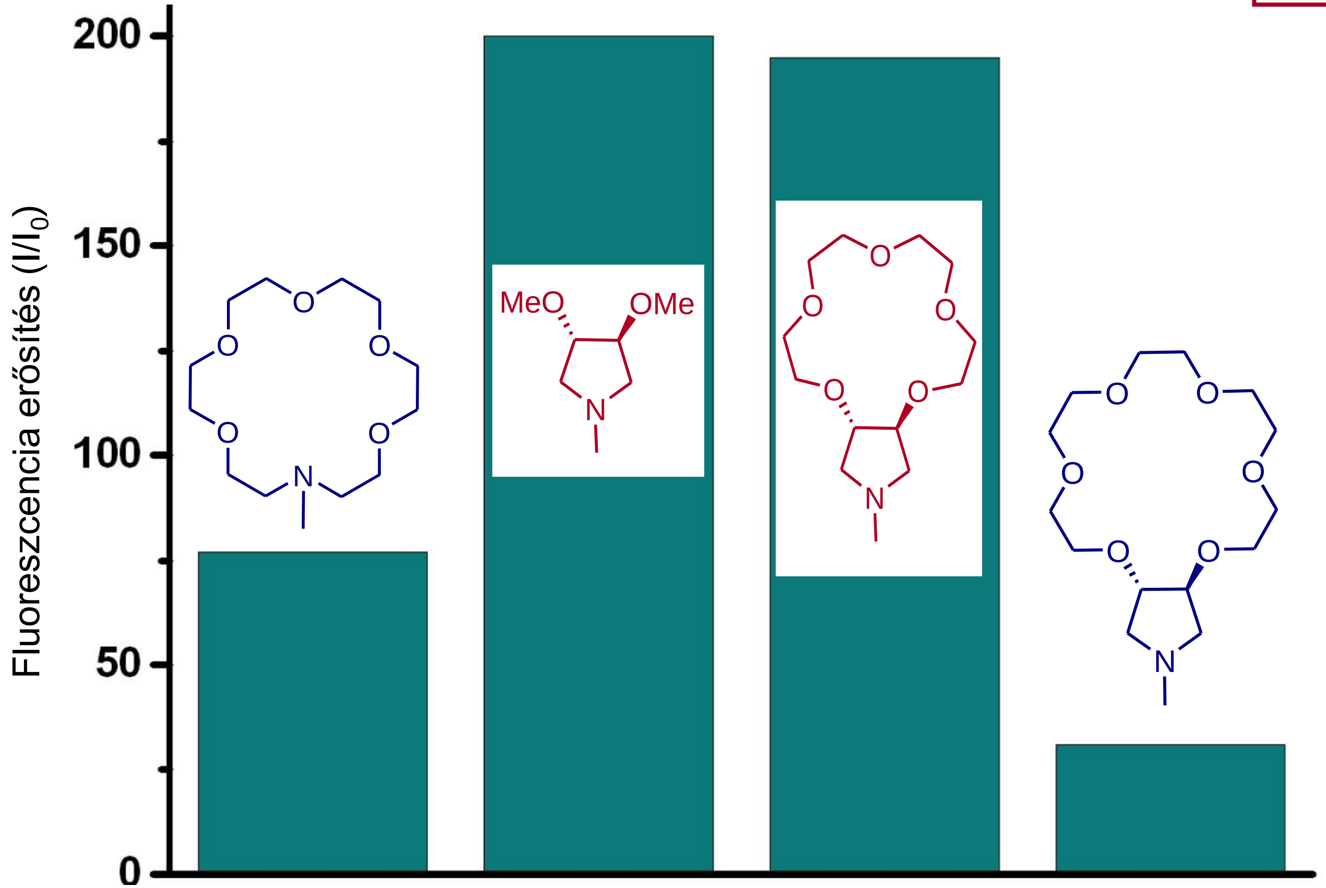
Further elaboration of conformational effects



Effect of protonation

10^{-6} M sensor
10^{-3}M HBF_4
MeCN



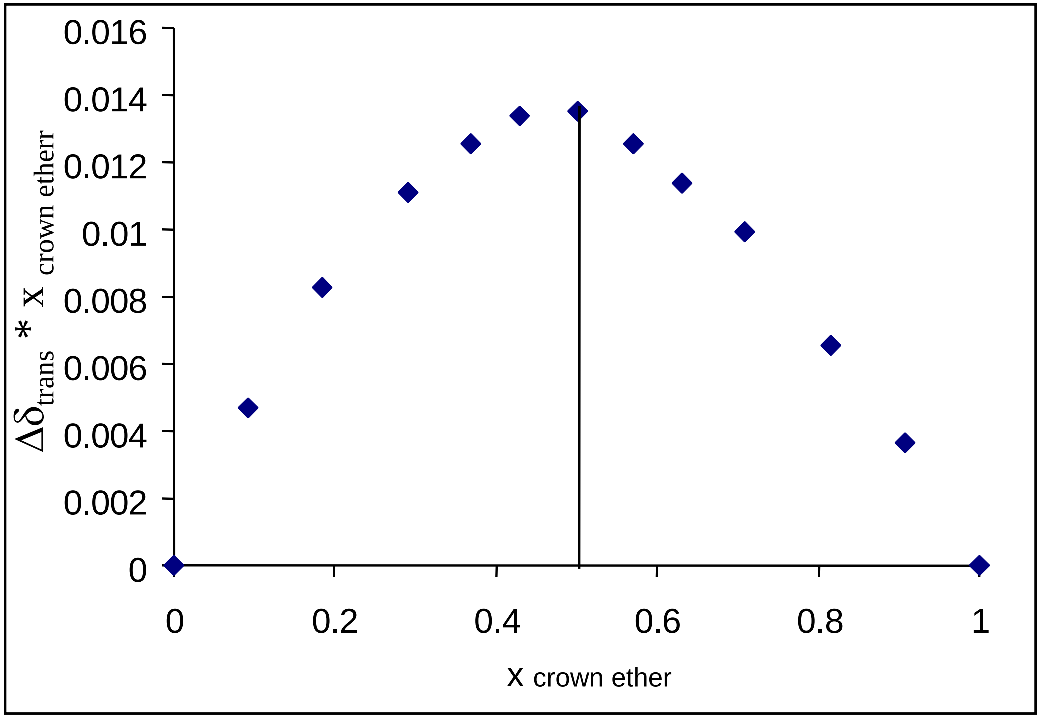


- the 18-crown moiety distorts the 5 membered ring – less eficient PET

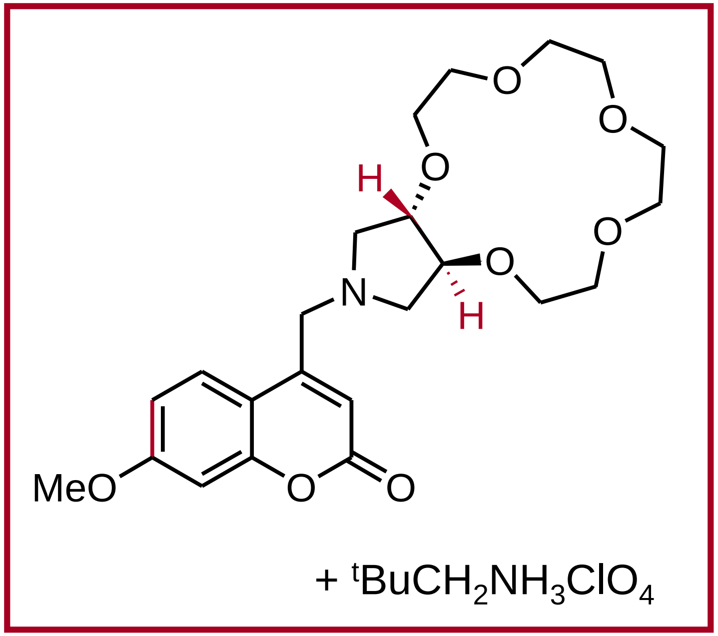
- the 15-crown has larger signaling potential (small changes in conformation results in lage increase

Stoichiometry of the Crown-neopentyl-ammonium complex

^{1}H NMR

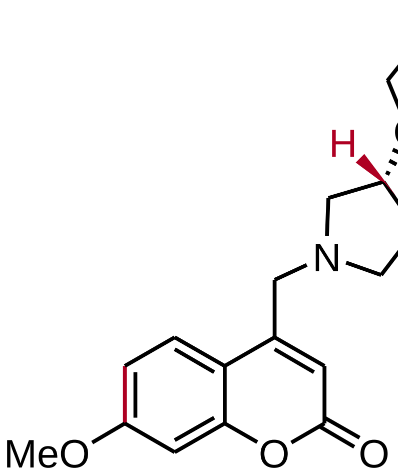


Job-plot

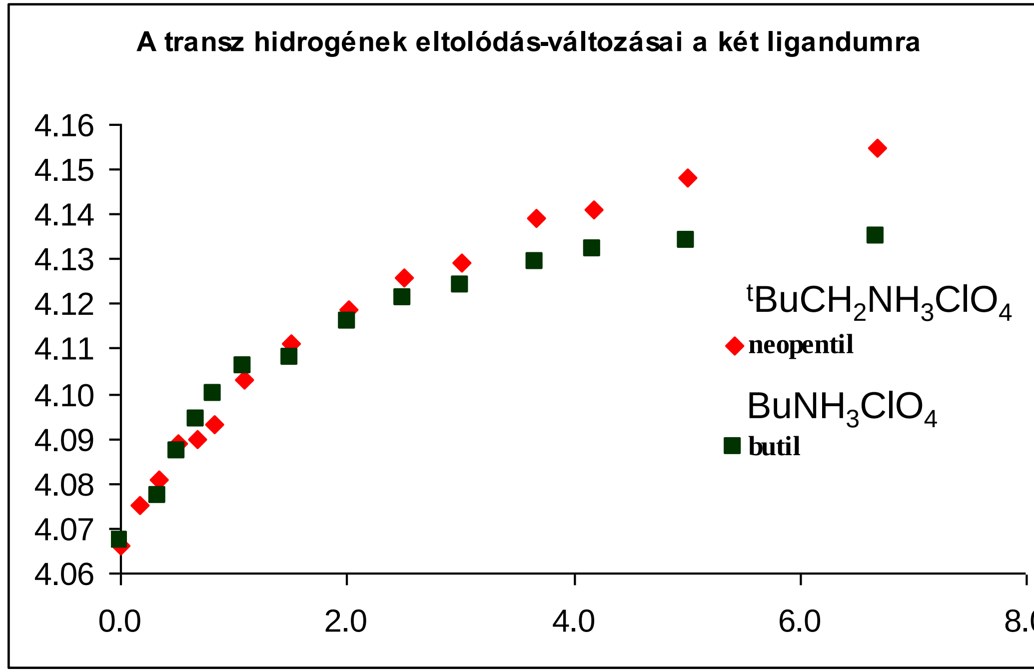


only 1:1 complex was formed

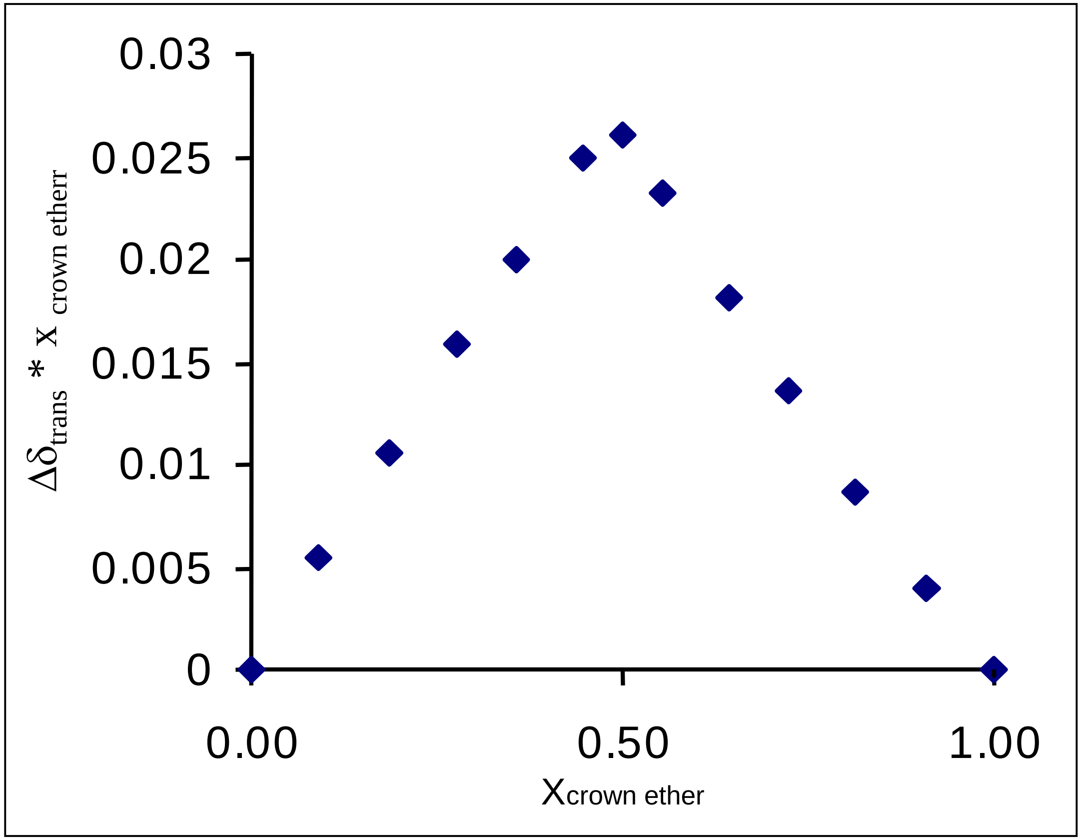
Stability of different complexes

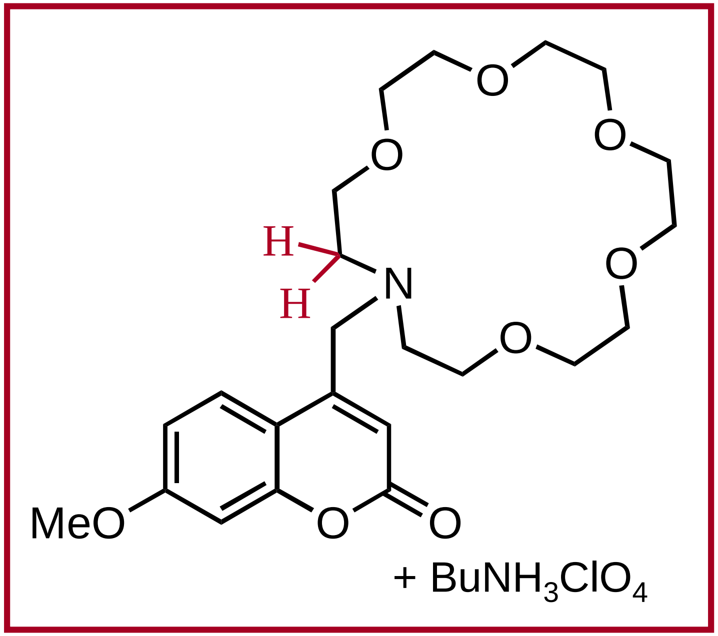


$CH_2Cl_2 : CDCl_3 : CD_3OD$ 90 : 9 : 1



	$\Delta\delta_{max}$ **H_{trans}**	**log*K***	***K***
butyl	0,074 ppm	3,30 ± 0,02	**4 000**
neopentyl	0,114 ppm	3,00 ± 0,02	**1 000**

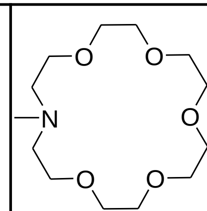
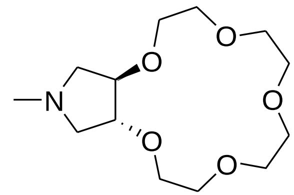

0.03
0.025
0.02
0.015
0.01
0.005
0
0.00
0.50
1.00
Δδtrans * x crown etherr
Xcrown ether


MeO
O
O
N
H
H
+ BuNH3ClO4

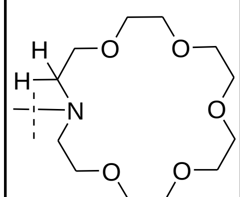
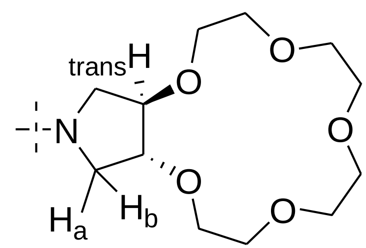
Job-plot

NMR studies

Binding constants logK

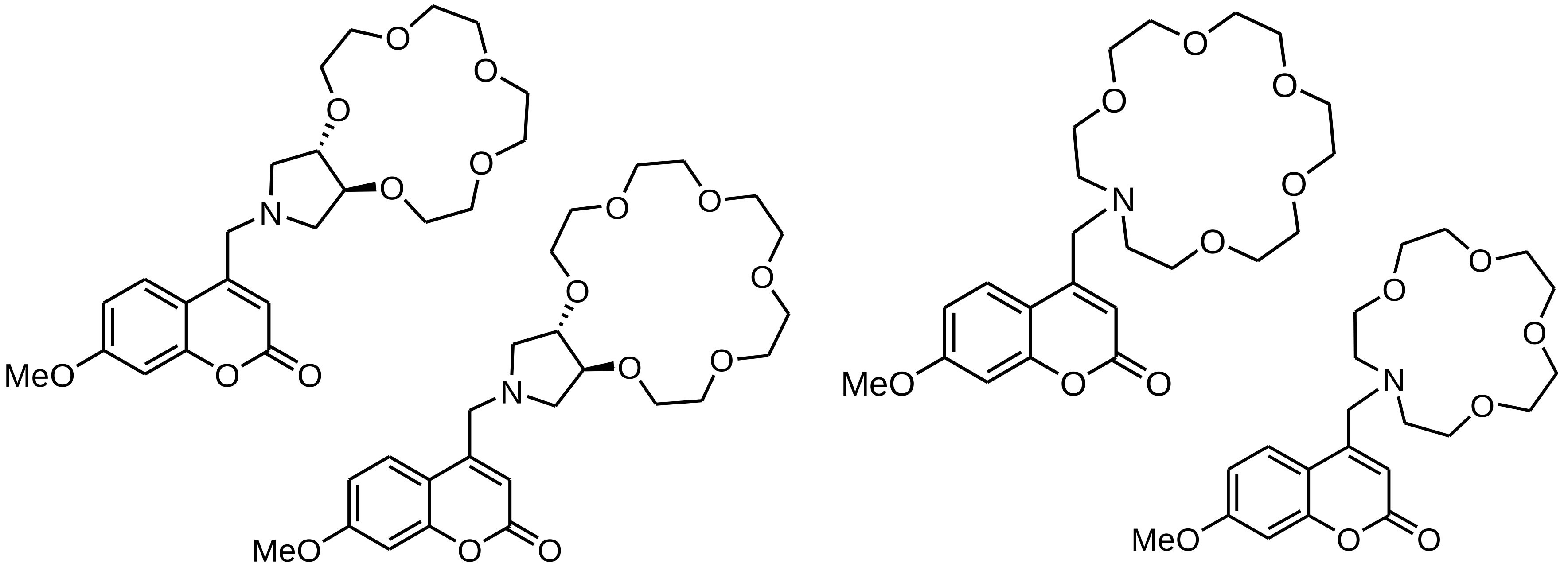
		
butyl	6.00 (1)	3.30 (3)
neopentyl	5.23 (8)	3.00 (8)

Changes in chemical shifts (Δδ)

				
	CH_2	**CH_{2a}**	**CH_{2b}**	**CH_{trans}**
nBu	0.052	0.069	0.096	0.077
nP	0.061	0.096	0.13	0.116

Systems elaborated

- Sensors

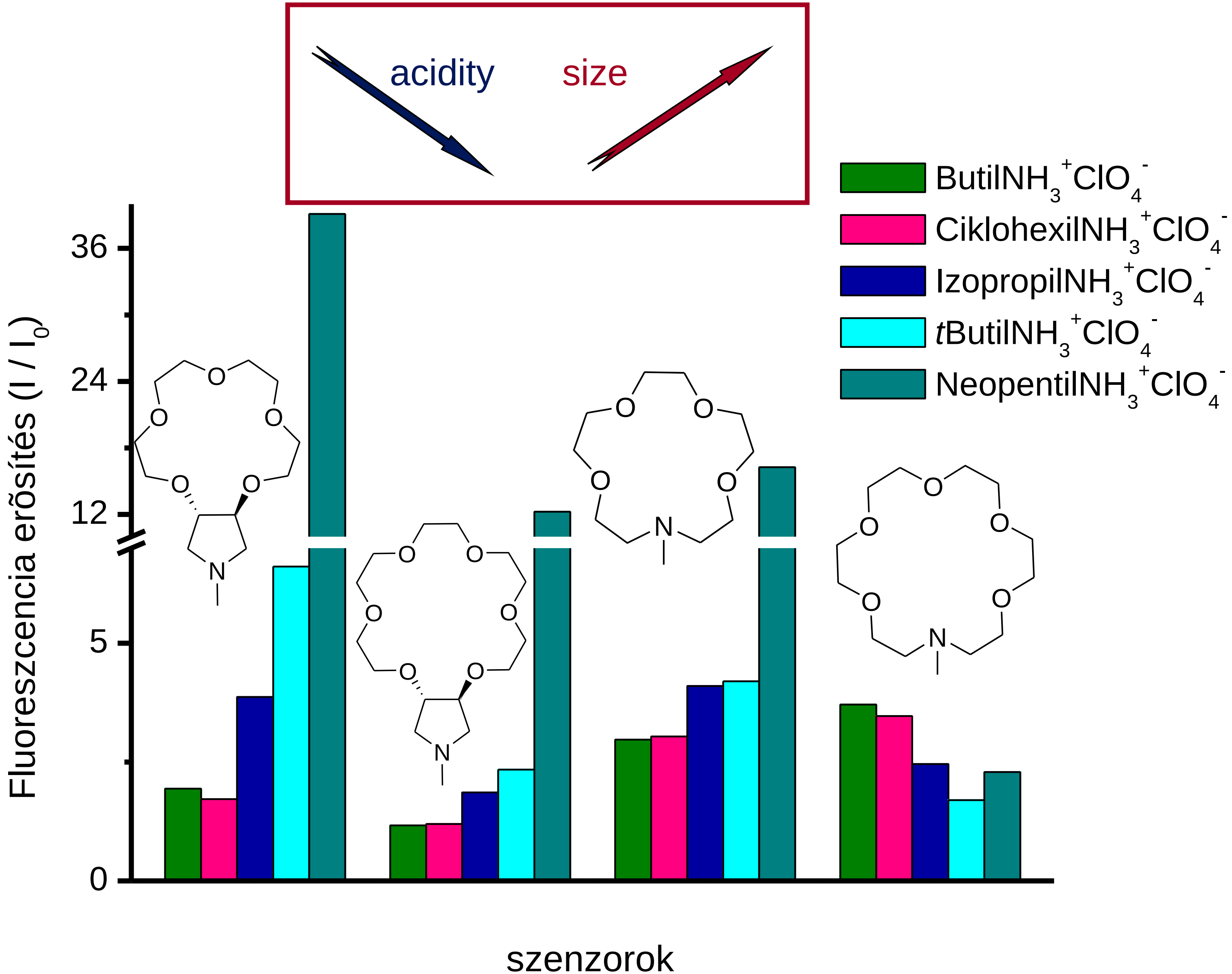


Guests

$BuNH_3ClO_4$ $CyNH_3ClO_4$ $^iPrNH_3ClO_4$ $^tBuNH_3ClO_4$ $^tBuCH_2NH_3ClO_4$

size →

← H-donor ability



Kele et al. Angew. Chem., Intl. Ed. 2006, 45(16), 2565-2567.

Summary

-Conformational freedom / rigidity can have a profound effect on PET

- There are cases where PET is dependent on :
    - purely direct coordination
    - purely on conformation
    - both