

Optical and Electrochemical Sensing of Biologically Important Analytes with Novel Receptors

Vladimír Král

***Institute of Chemical Technology,
Department of Analytical Chemistry
Zentiva a.s.***

Definice senzoru

Chemický senzor je převodník, který poskytuje přímo informaci o chemickém složení svého okolí. Je tvořen fyzikálním převodníkem a chemicky citlivou vrstvou.

Janata 1988

Chemical sensors contain two basic functional units: a receptor and a transducer part. IUPAC Orange book 1997

SSS (Selectivity, Sensibility, Stability)

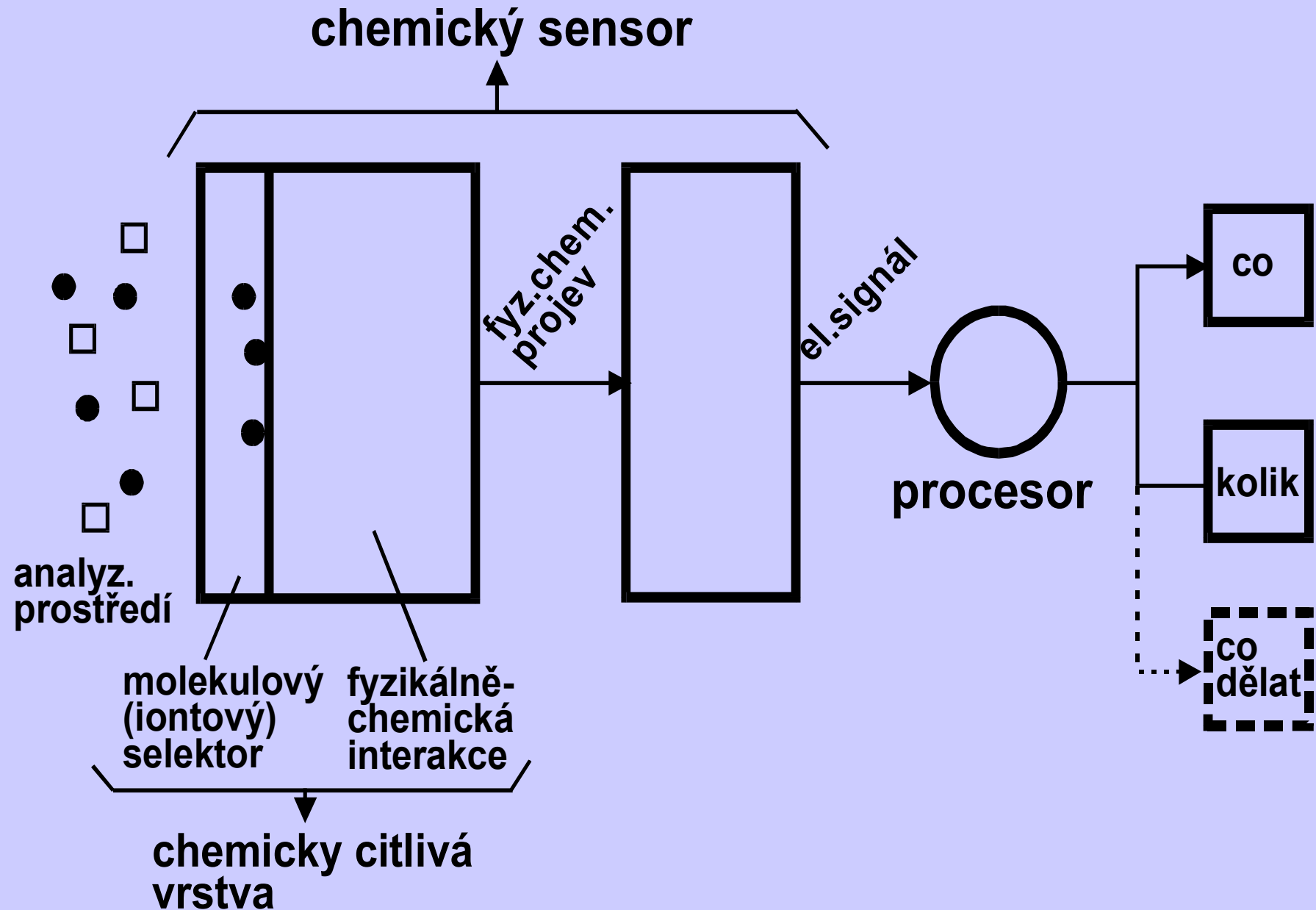
Selectivity by design of receptors

Sensorová analýza:

**Chemické metody analýzy: Klasické
versus**

***Sensorová analýza* jsou založeny na
selektivní interakci analytu s receptorem
Sensory spojují metody chemické a
fyzikální analýzy, citlivá vrstva -
receptor a fyzikální převodník -
zesílení = analytický užitečný signál
(opakovaně použitelné)**

Obecné schema chemického sensoru



Chemické sensory

jsou přístroje (devices), které kvantitativně a reversibilně měří sledovaný analyt. Celý proces je založen na rozpoznání analytu (molecular recognition) a následné transdukci, která tento proces převádí na analyticky užitečný signál.

3. CHEMICKÉ SENSORY - PŘEHLED

EKTROCHEMICKÉ
SENSORY

s kapalným
elektrolytem

s tuhým elektrolytem

SENSORY S MIKROELEKTRO-
NICKOU STRUKTUROU

ISFET

CSFET

work function sensory

SENSORY ZALOŽENÉ NA
MĚŘENÍ ELEKTRONICKÉ
VODIVOSTI

metaloxidové
sensory

chemiresistory

SENSORY ZALOŽENÉ NA
MĚŘENÍ TEPLoty

pelistory, pyroelektrické
sensory

tepelně vodivostní
sensory

SENSORY HMOTNOSTNÍ

křemenné mikrováhy (BAW)

surface acoustic
wave sensory (SAW)

SENSORY OPTICKÉ

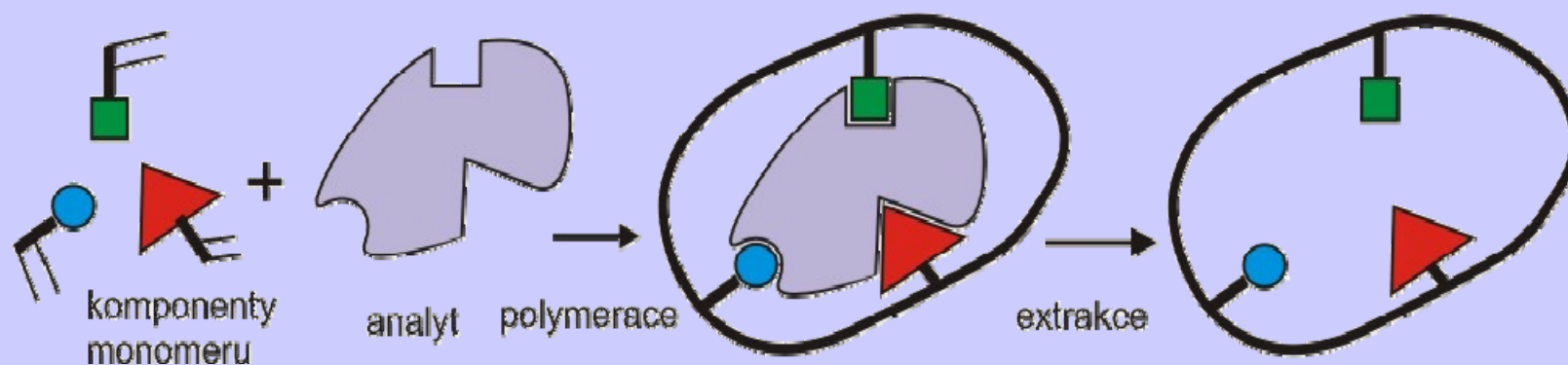
optrody

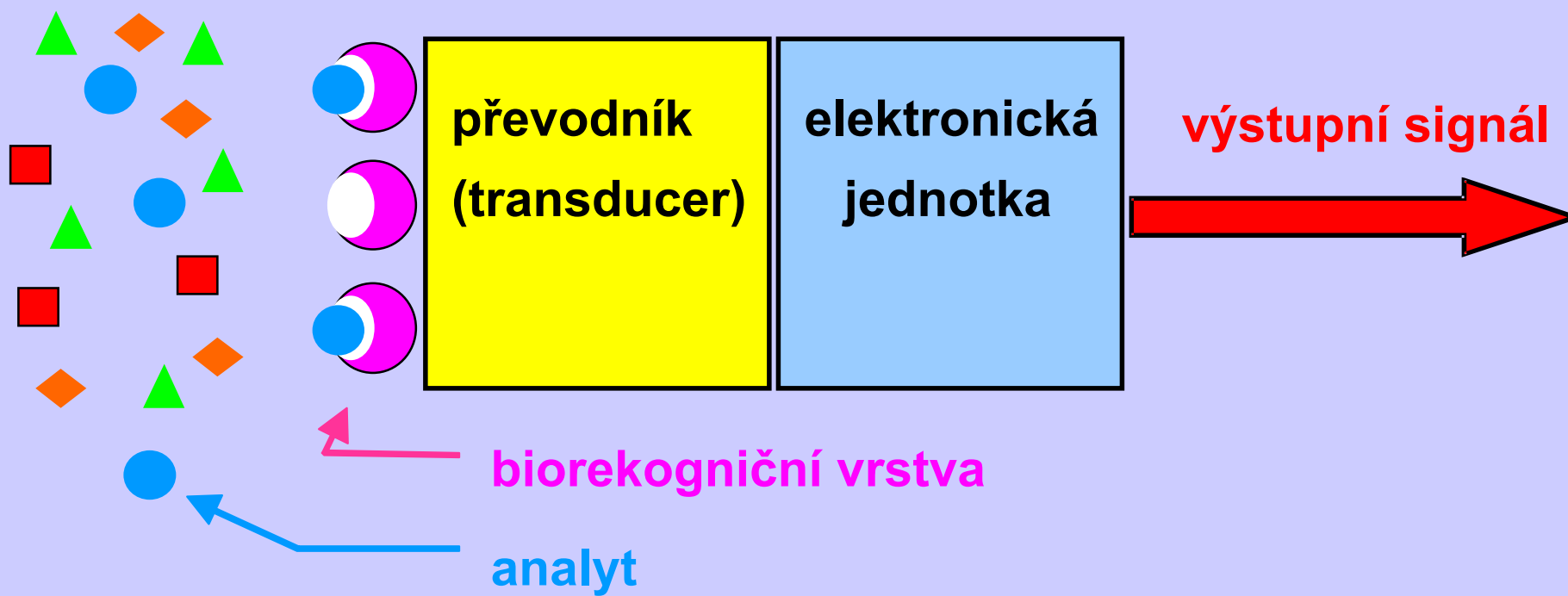
Aplikace supramolekulární chemie pro vývoj senzorů:

Design Receptorů

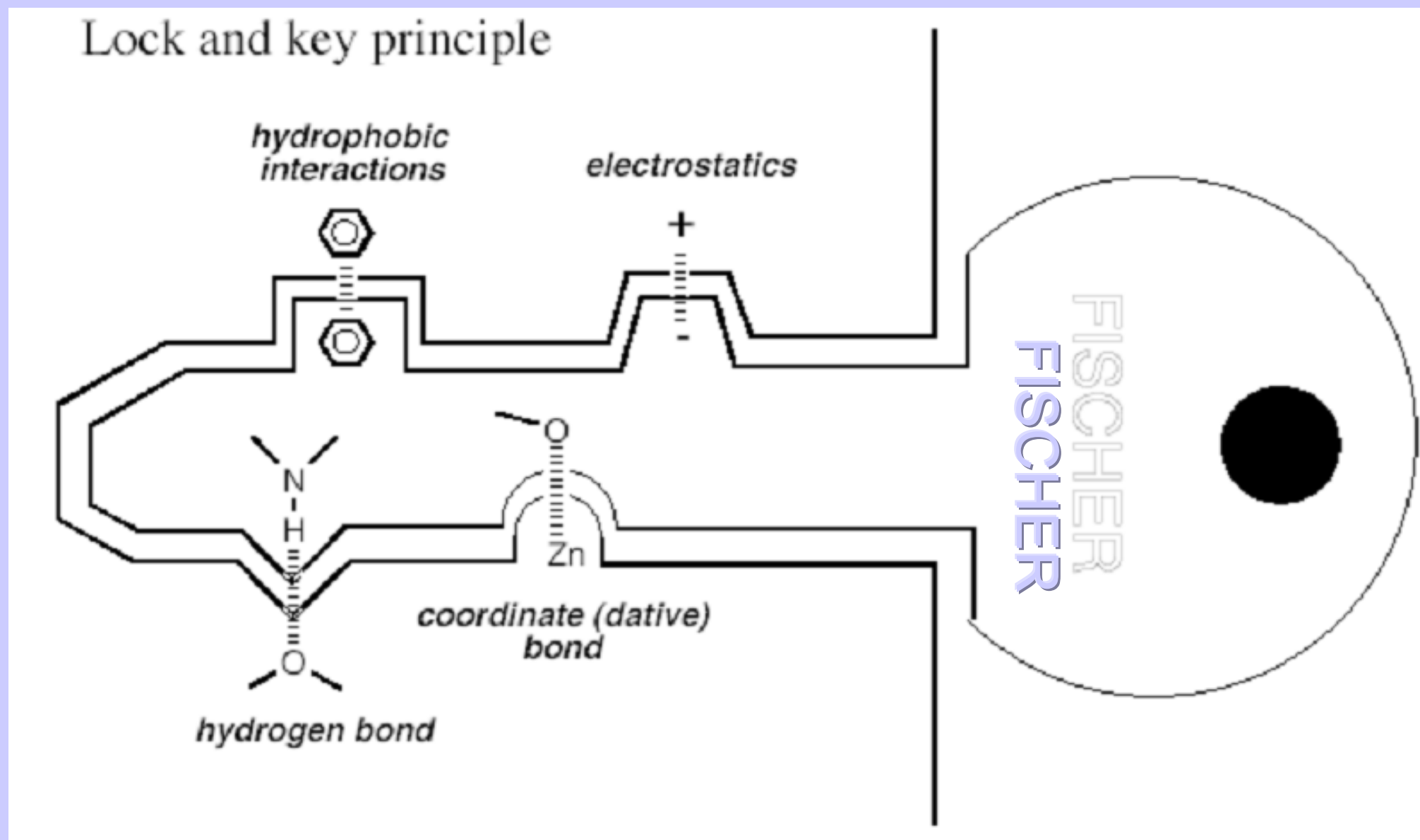
- Kation,**
- anion, polyanion**
- neutrální molekuly
(sacharidy)**

Molekulový (iontový selektor)	Princip selekce
Semipermeabilní membrána	Permeace, $P=Dk$
Molekulové síto	Velikost částic
Iontoměnič, katalyzátor, sorbent	Chemická reakce, nevazebné interakce
Enzymy, protilátky	Biochemické rozpoznávací reakce
Vtištěné (imprinted) polymery	Tvar a velikost molekuly (iontu) analytu

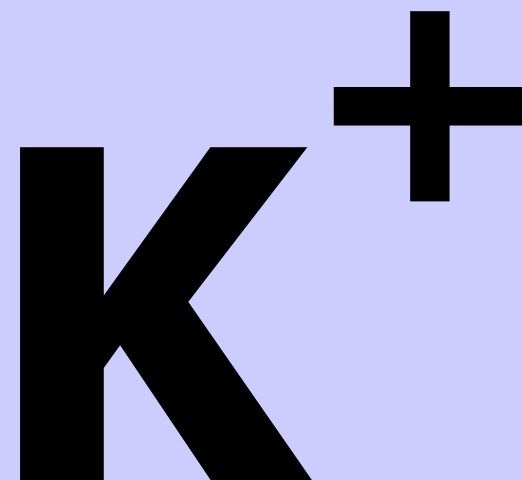
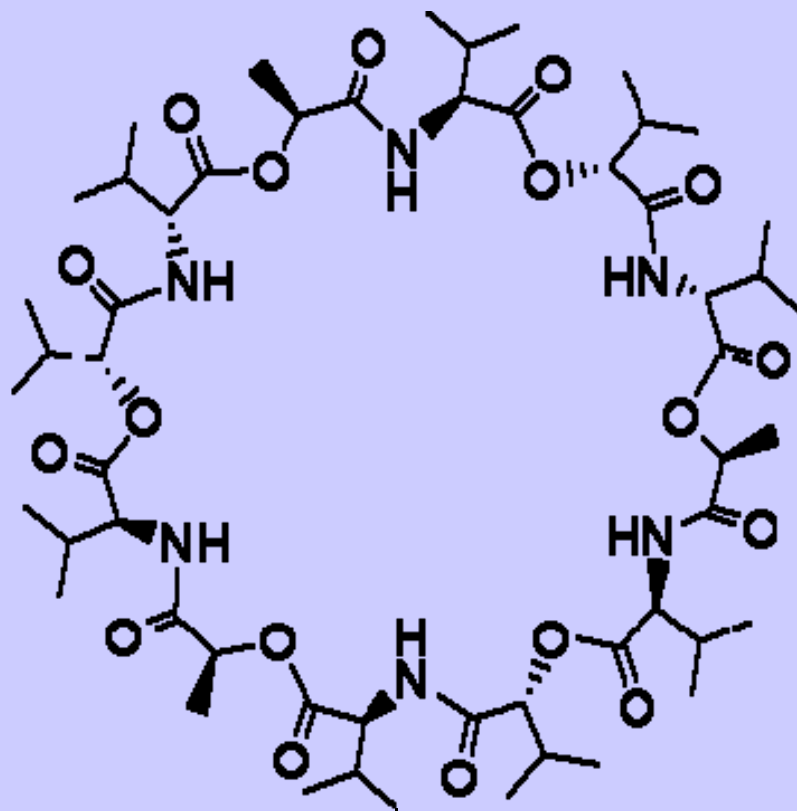




Supramolecular Chemistry: Spatial and charge complementarity



Komerčně nejdůležitější elektrody

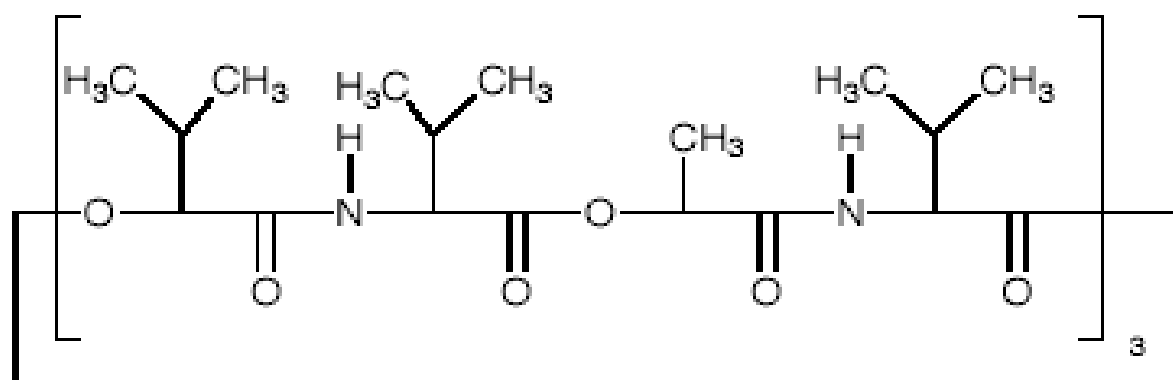


valinomicine, 5.0 wt. %

1,2-dimethyl-3-nitrobenzene, 25.0 wt. %

sebacic acid dibutylester, 68.0 wt. %

potassium tetrakis(4-chlorophenyl)borate, 2.0 wt. %

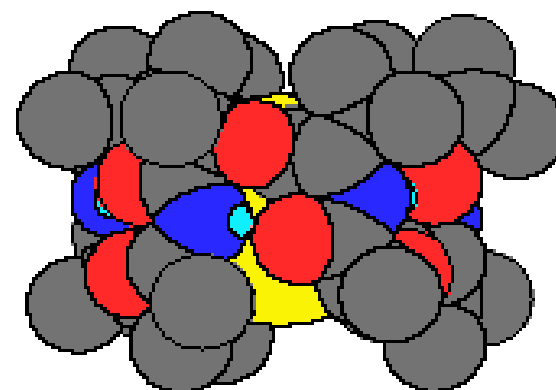


Valinomycin

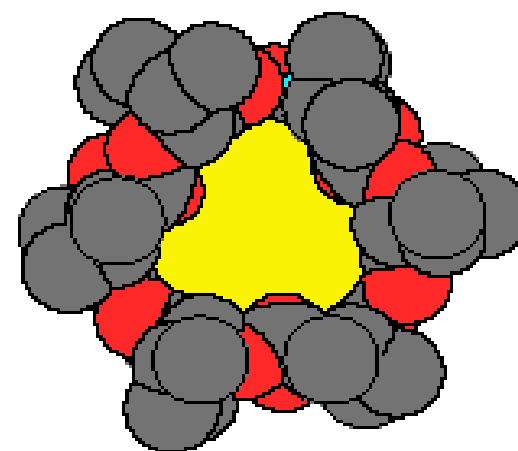


Free receptor

Side view



Top view

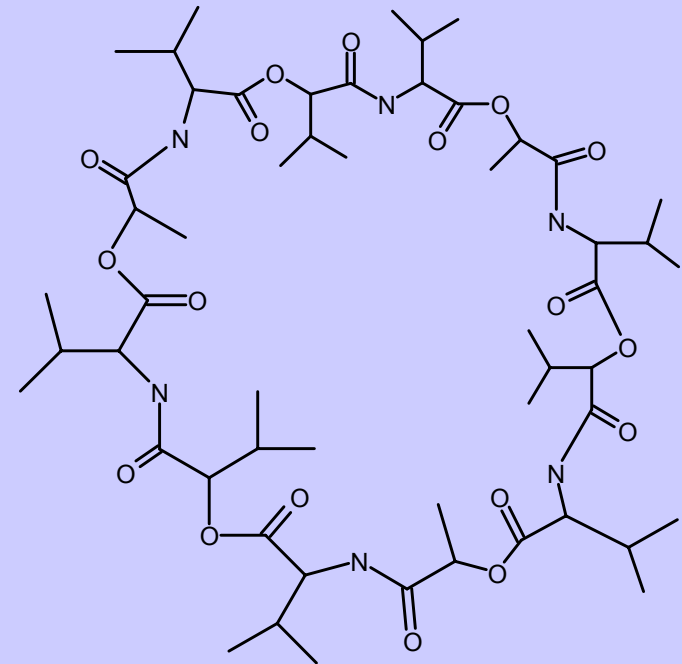
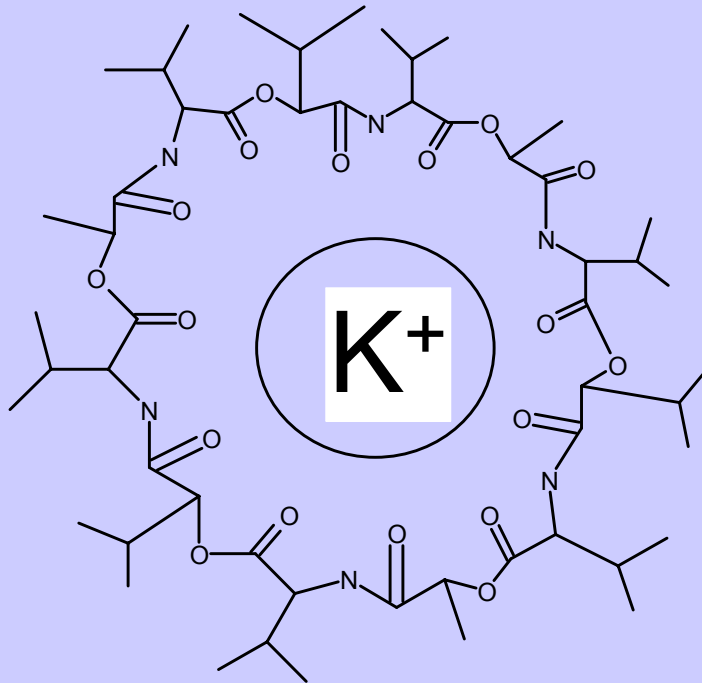


Potassium complex

Sensing and Molecular Recognition

The Most Specific Molecular Receptor for an Ion:

- * cyclo-depsi-peptide, containing 3 of each L-valin, D-valin, L-lactic acid and D- α -hydroxy-isovalerianic acid;
- * 36-ring



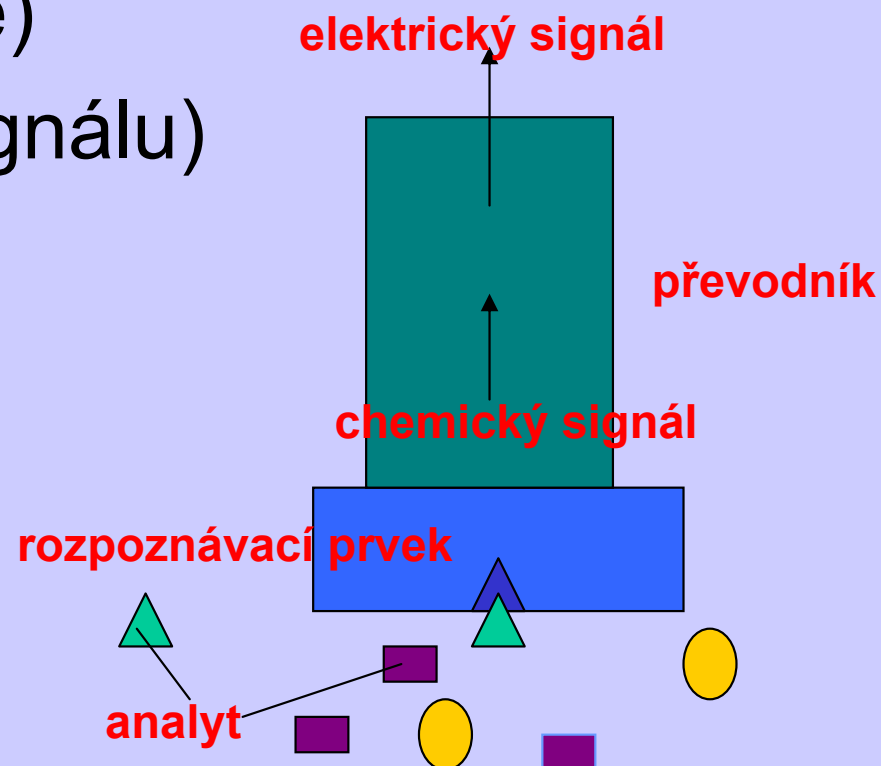
undergoes conformational change on complexation of K^+

ISE=iontově selektivní elektroda

➤ Senzor – reagující na zkoumaný analyt

➤ Část:

- receptorová (v citlivé vrstvě)
- převodníková (přenašeč signálu)

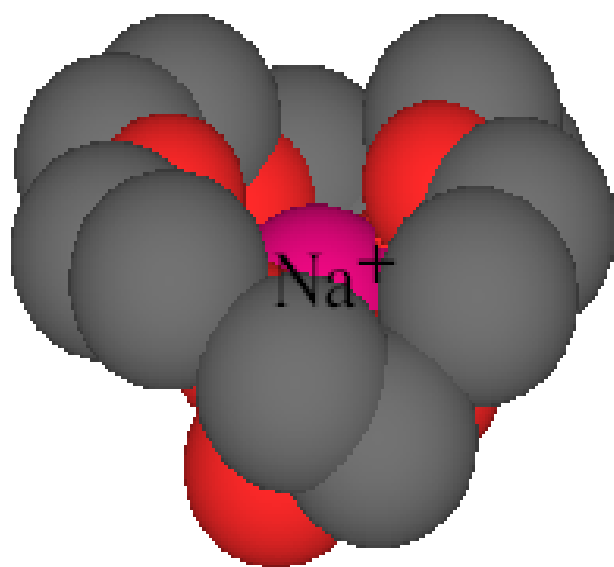


Sensing and Molecular Recognition

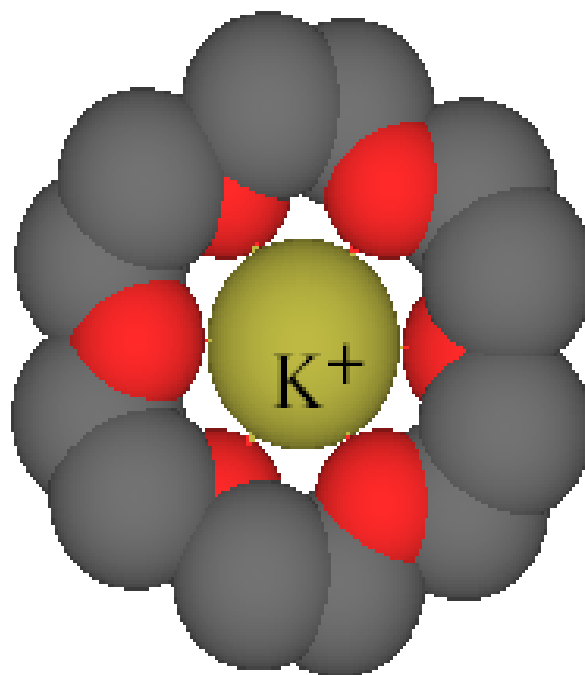


$$K_a = \frac{\text{complex}}{[\text{crown}][\text{cation}]}$$

optimal spacial fit

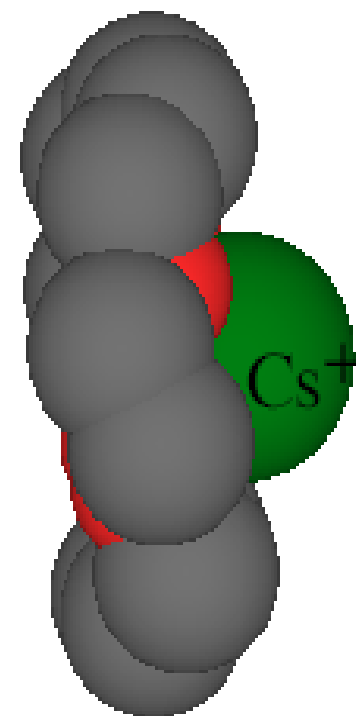


$\text{Log } K_a = 4.32$

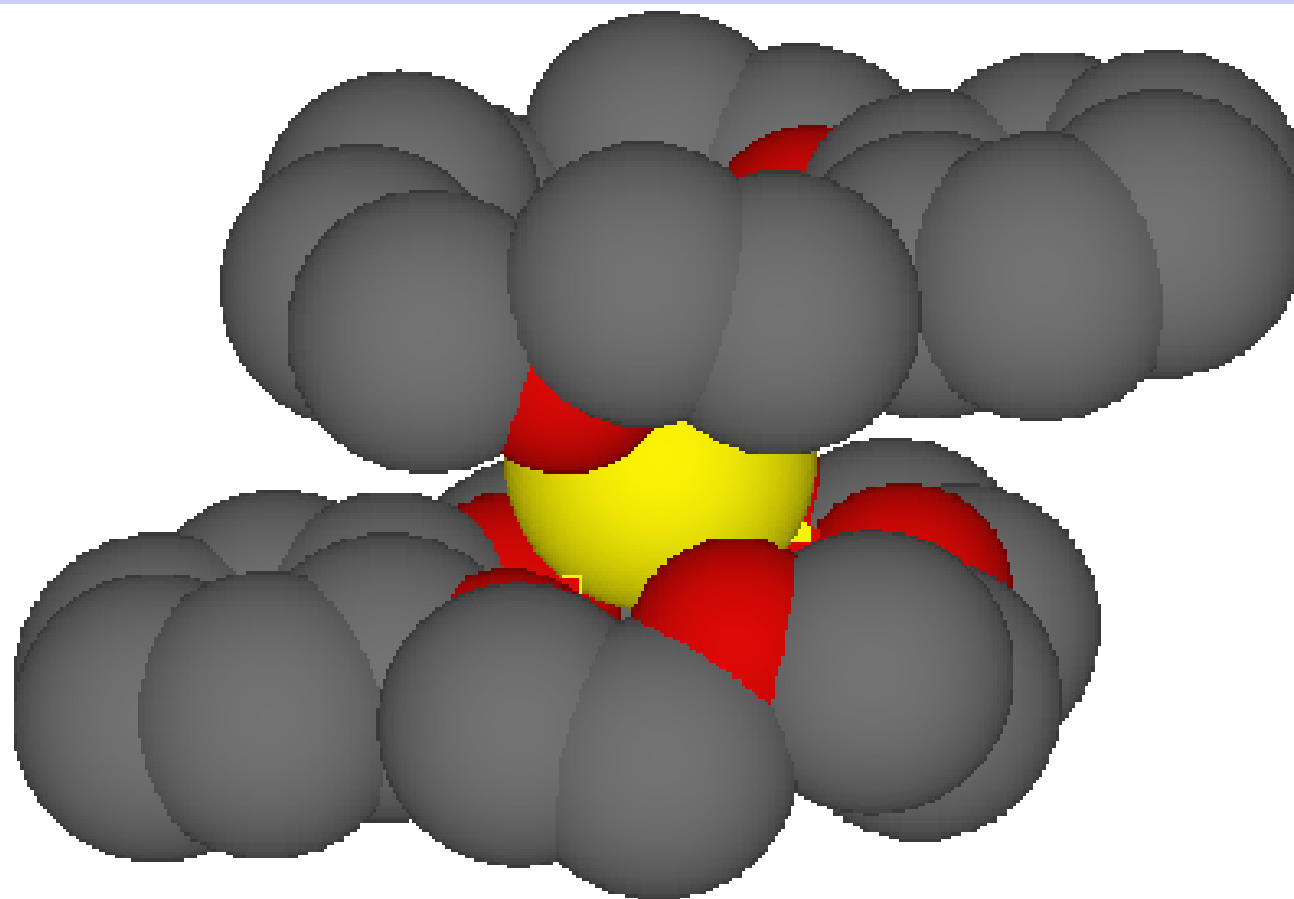


$\text{Log } K_a = 6.10$

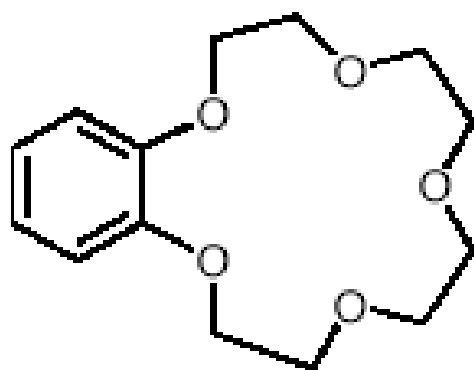
in methanol



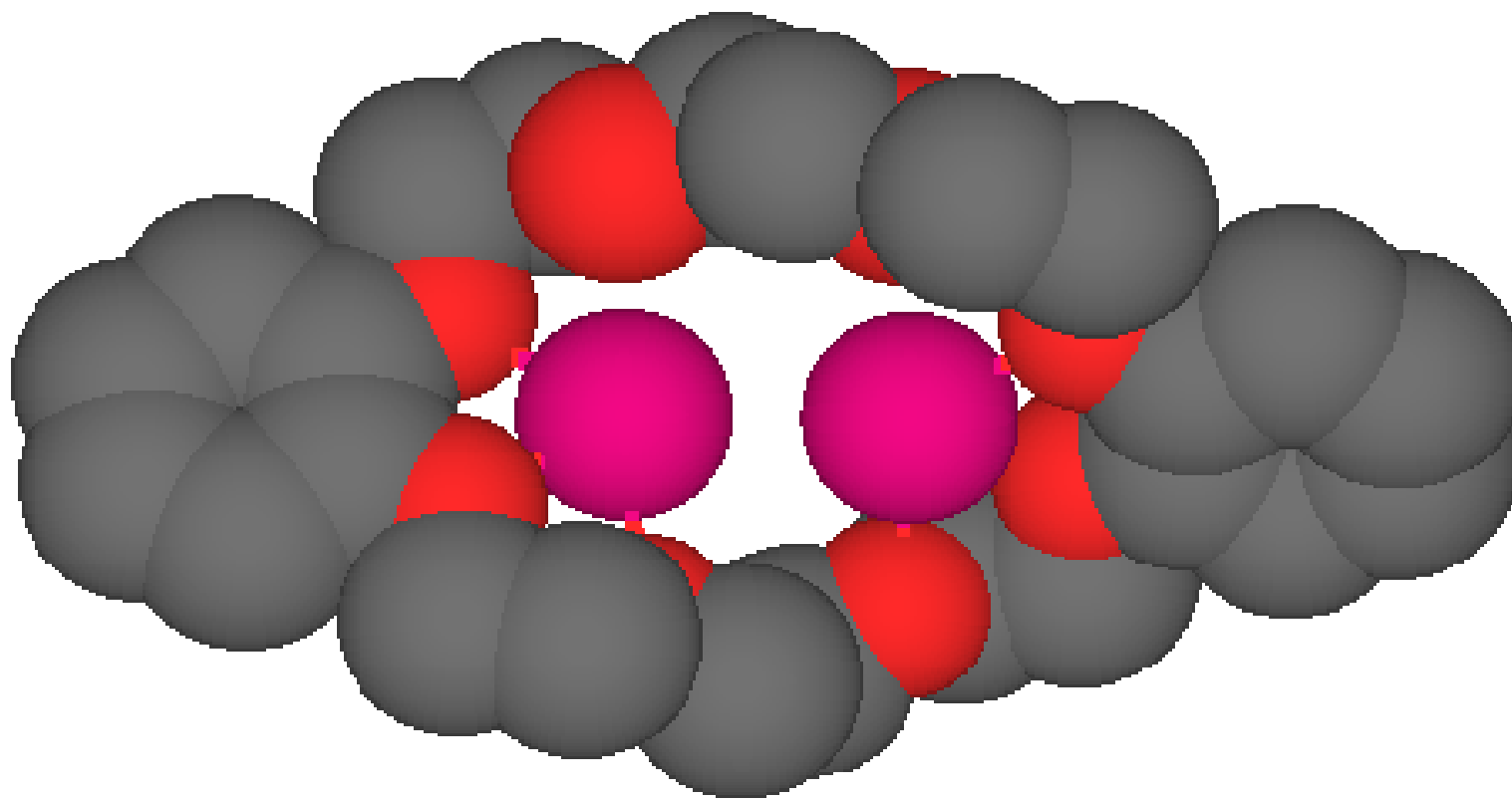
$\text{Log } K_a = 4.62$



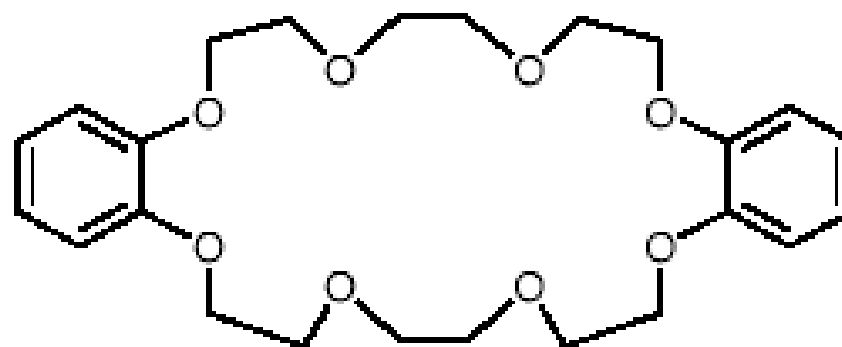
K^+ -(benzo-[15]-crown-5)₂
sandwich complex



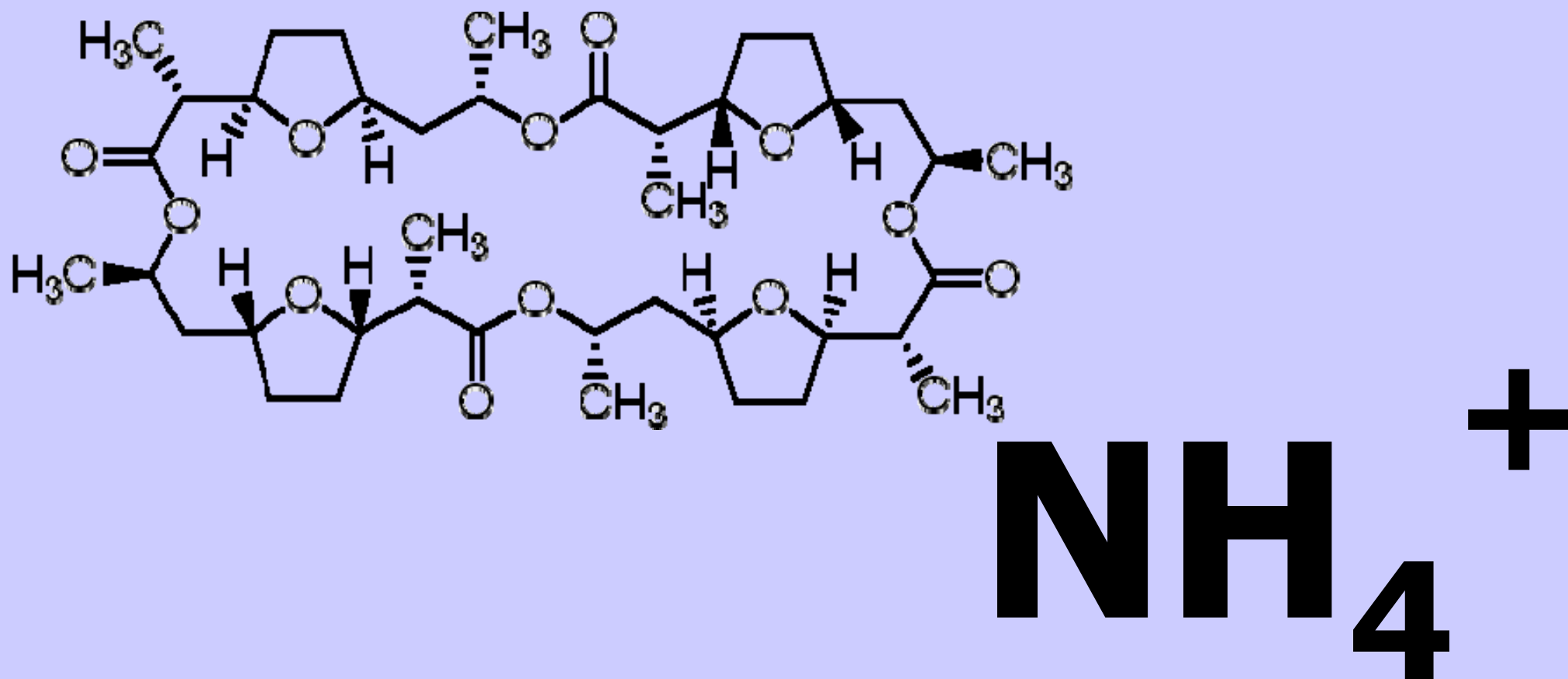
benzo-[15]-crown-5



(Na⁺)₂-dibenzo-[24]-crown-8



Komerčně nejdůležitější elektrody



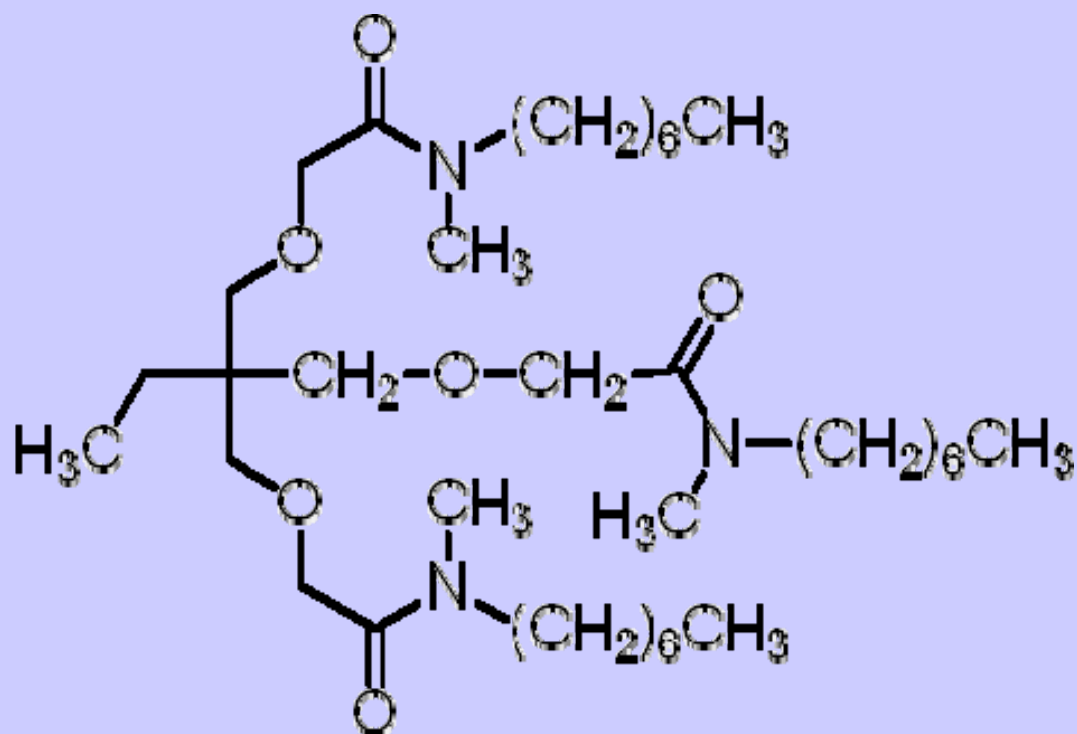
nonactine, 5.0 wt. %

1,2-dimethyl-3-nitrobenzene, 25.0 wt. %

sebacic acid dibutylester, 68.0 wt. %

potassium tetrakis(4-chlorophenyl)borate, 2.0 wt. %

Komerčně nejdůležitější elektrody



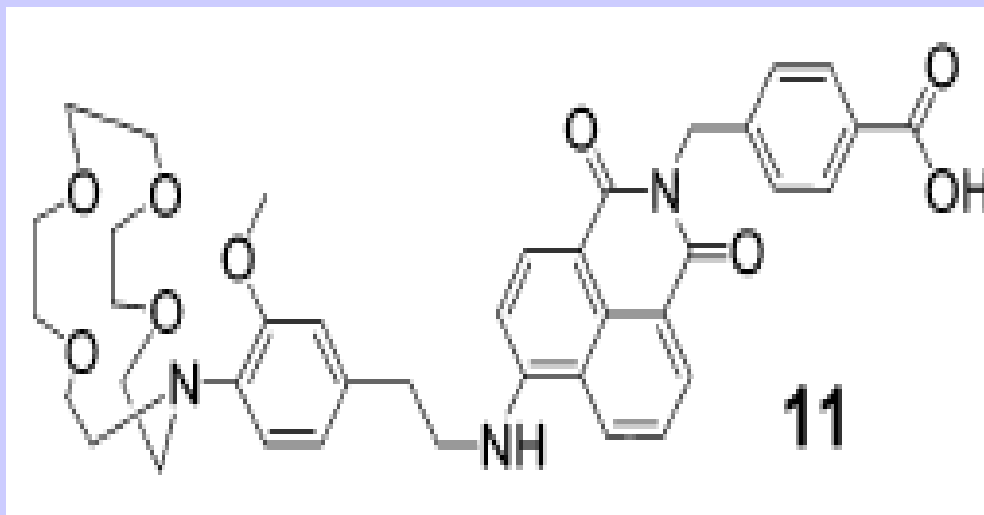
Na⁺

2-nitrophenyl octyl ether (o-NPOE), 89.5 wt. %
sodium ionophore (ETH 227), 10.0 wt. %
sodium tetrakisphenylborate, 0.5 wt. %

Molecular Recognition and Sensing: Na, K

Molecular Sensing of Na⁺ Using an Immobilized Fluoro-Ionophore; H. He et al.; *Anal. Chem.* 75 (2003) 549;
similar for K⁺: JACS, 2003.

Response: ➔



Chemical Structure

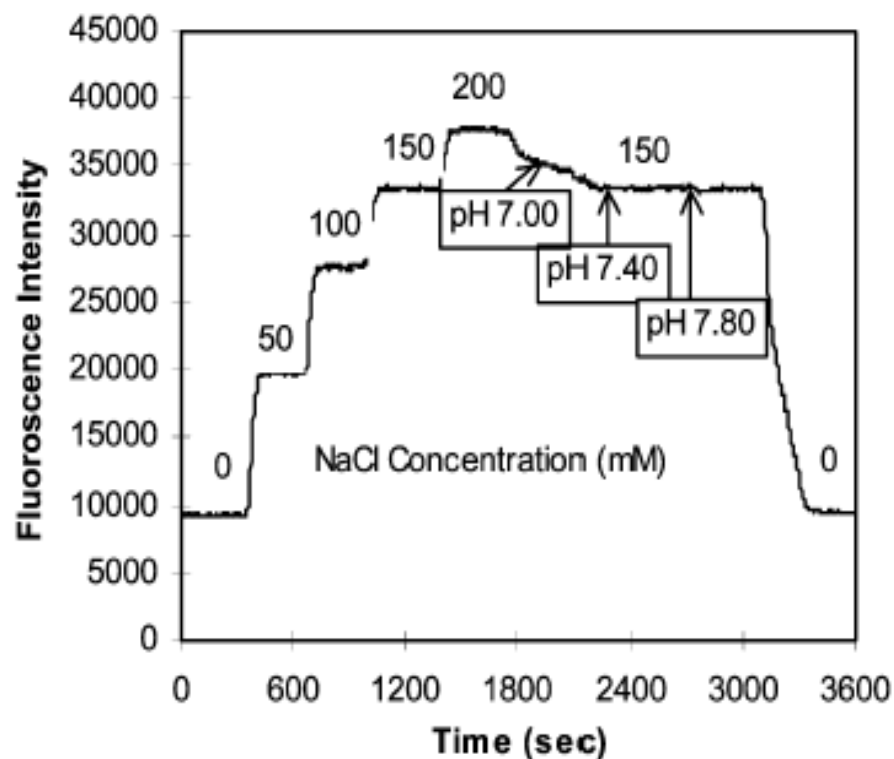
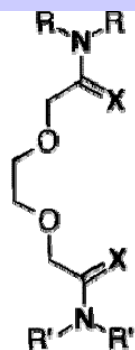
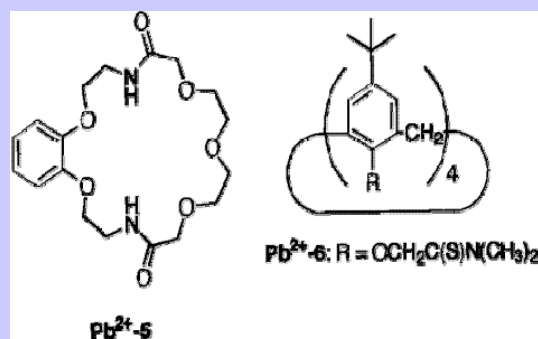
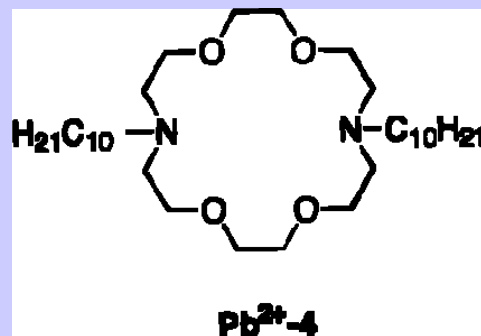
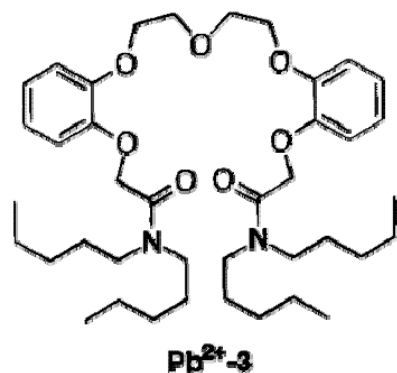


Figure 4. Dynamic response of a sensor disk to various sodium chloride concentrations at pH 7.40 HEPES buffer. Excitation at 470 nm, emission at 540 nm.

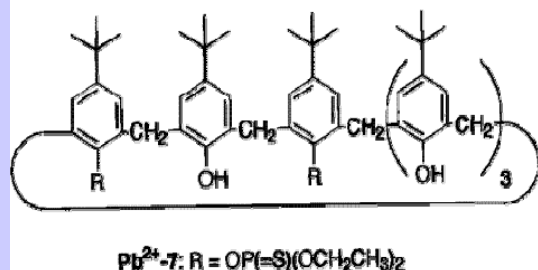
Ionofory senzoru Pb^{2+}



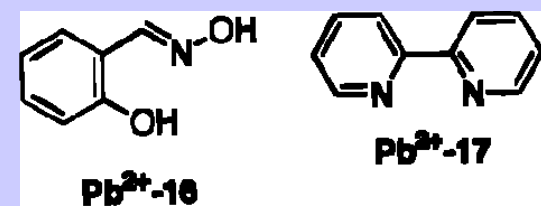
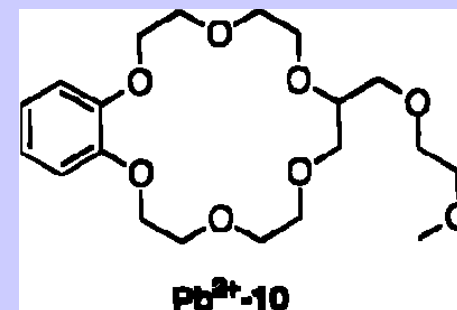
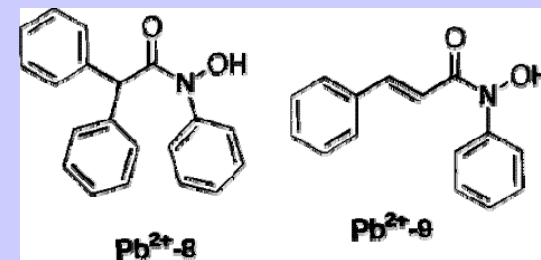
Pb²⁺-1 (ETH 322): $\text{R} = \text{C}_3\text{H}_7$, $\text{R}' = \text{C}_{10}\text{H}_{21}$, $\text{X} = \text{O}$
Pb²⁺-2 (ETH 5435): $\text{R}, \text{R}' = \text{C}_{12}\text{H}_{25}$, $\text{X} = \text{S}$



Pb²⁺-6: $\text{R} = \text{OCH}_2\text{C}(\text{S})\text{N}(\text{CH}_3)_2$

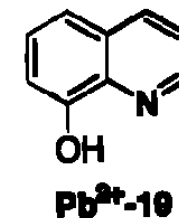
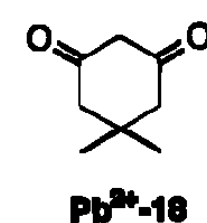


Pb²⁺-7: $\text{R} = \text{OP}(=\text{S})(\text{OCH}_2\text{CH}_3)_2$



Pb²⁺-16

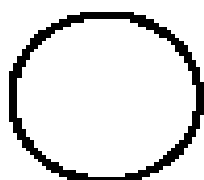
Pb²⁺-17



Pb²⁺-18

Pb²⁺-19

ISE		
detegovaný iont	aktivní membrána	pozn.
H⁺	sklo Corning 015 (22hm% Na₂O+6%CaO, 72%SiO₂), nebo lithné sklo (14,3%Li₂O, 7%BaO, 78,7%SiO₂)	od 10⁻¹ mol/l do 10⁻¹³ mol/l, křehké
Na⁺	sklo NAS 11-18 (11mol%Na a 18 mol% Al)	10⁻¹ až 10⁻⁶, nutno konstantní pH! mezi 6 a 10
NH₄⁺	plastická s nonaktiny	10⁻¹ až 10⁻⁵, analýza krevního séra
K⁺	plastická s valinomicinem	10⁻¹ až 10⁻⁶, analýza krevního séra
Ca²⁺	bud' membrána s iontoměničem typu hydrofobního esteru k fosforečné (srovnej K_s(Ca₃PO₄)) či neutrální ionofor	dtto, stanovení izolovaného Ca²⁺ v krevním séru, součást automatických analyzátorů
NO₃⁻	plastická s organickými dusičnany	dtto, původně firma Orion Research, vadí vysoká koncentrace Cl⁻, stanovení nitrátů v zemědělství
F⁻	LaF₃ + EuF₃	10⁻¹ až 10⁻⁶, bud' s vnitřním elektrolytem nebo s pevným vnitřním kontaktem, vadí OH⁻ - nutno pufovat
Cl⁻	AgCl monokrystalický či polykrystalický lisovaný za zvýšené teploty a tlaku	10⁻¹ až 10⁻⁵
S²⁻	Ag₂S v beta modifikaci	až 10⁻¹⁹ mol/l, extrémně selektivní



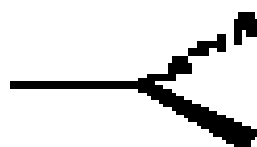
Spherical

F^- , Cl^- , Br^- , I^-



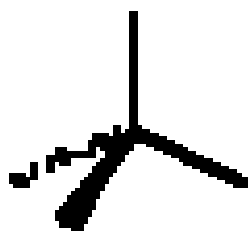
Linear

N_3^- , CN^- , SCN^- , OH^-



Trigonal Planar

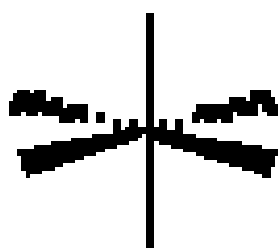
CO_3^{2-} , NO_3^-



Tetrahedral

PO_4^{3-} , VO_4^{3-} , SO_4^{2-} ,
 MoO_4^{2-} , SeO_4^{2-} ,

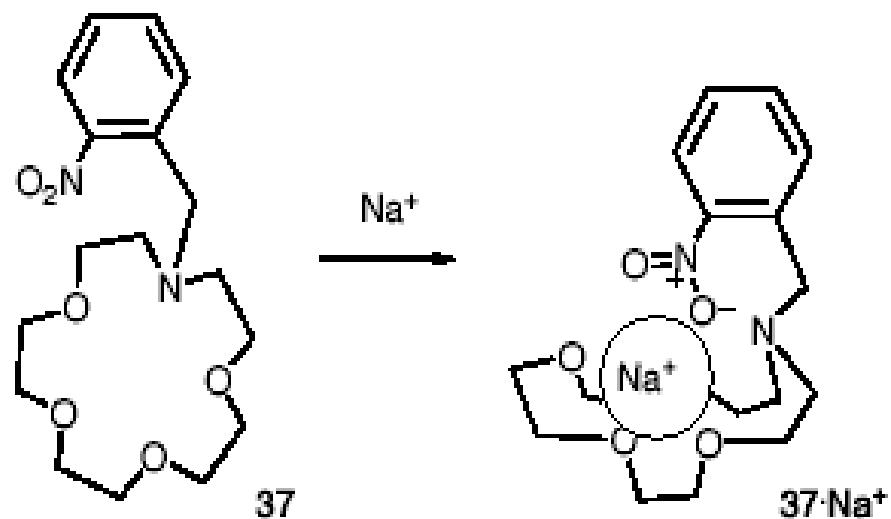
MnO_4^-



Octahedral

$Fe(CN)_6^{4-}$, $Co(CN)_6^{3-}$

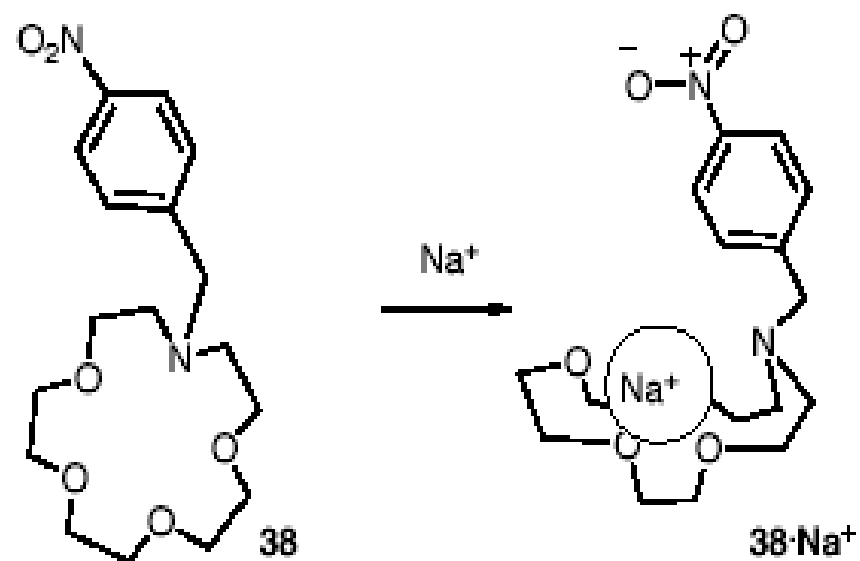
a)



reducible

New wave in CV

b)



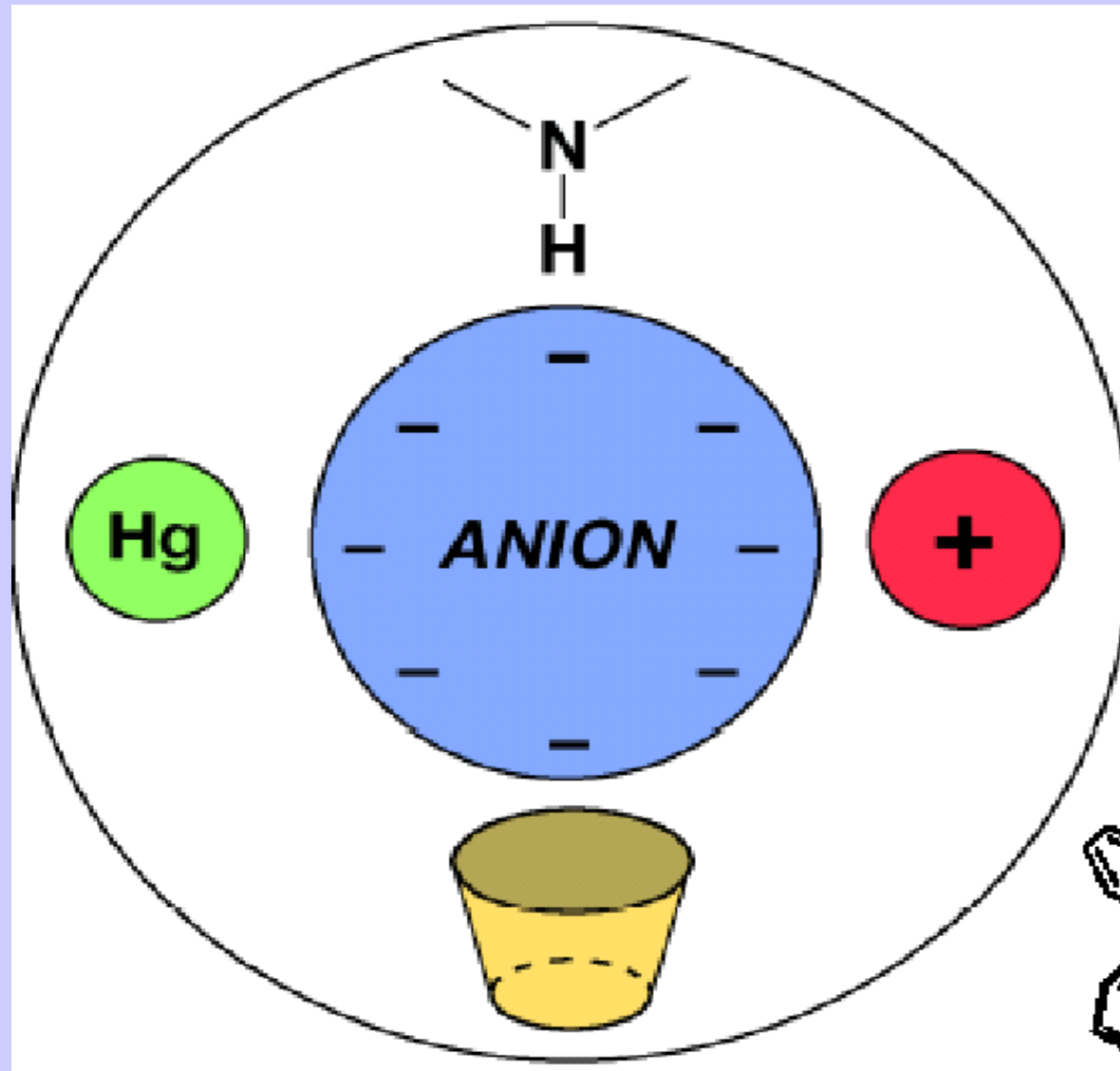
No effect on electrochemistry

Angel Kaifer
J. Am. Chem. Soc. 1983, 105, 7168

Design of receptors:

hydrogen
bonds

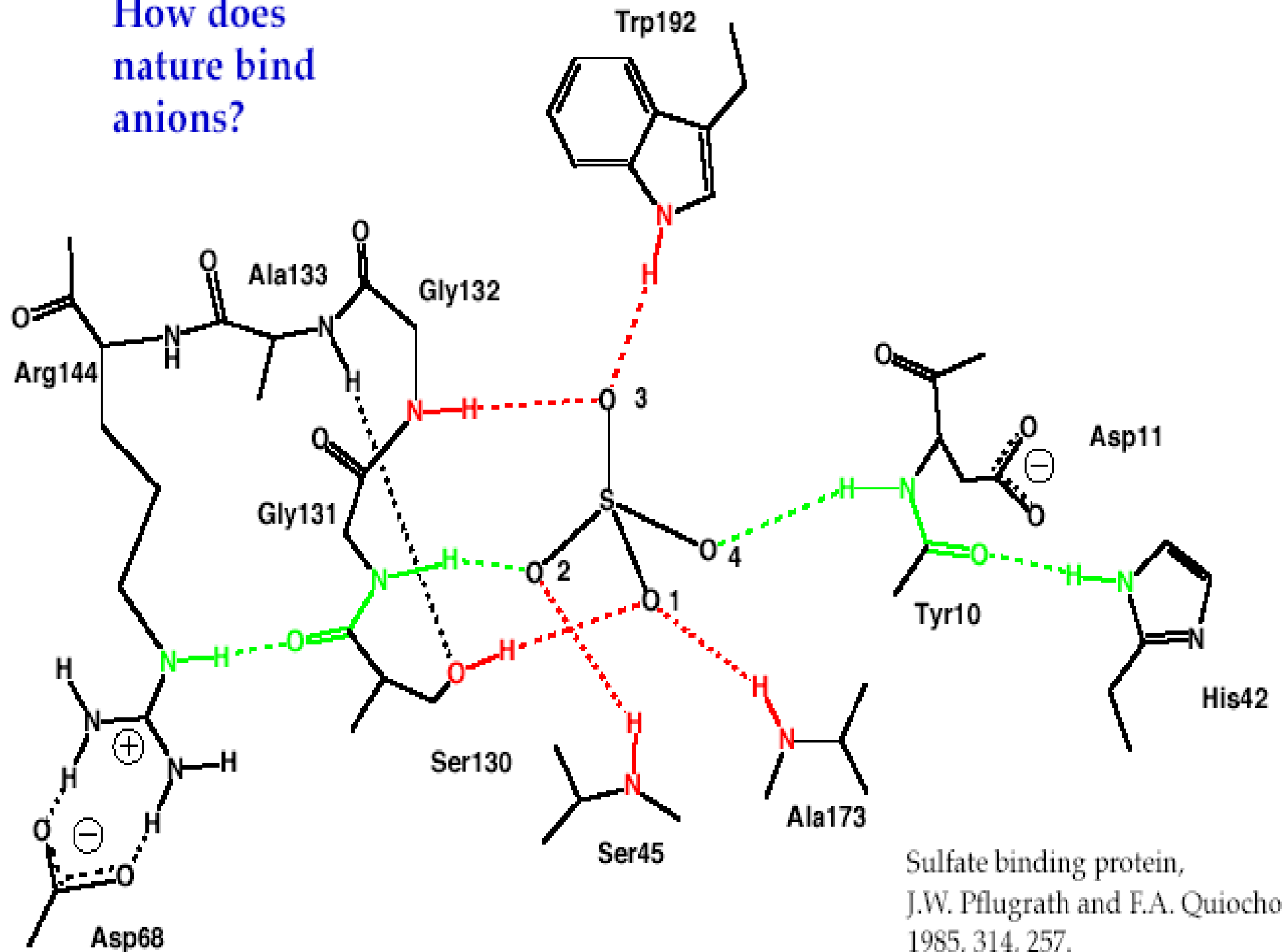
Lewis acids



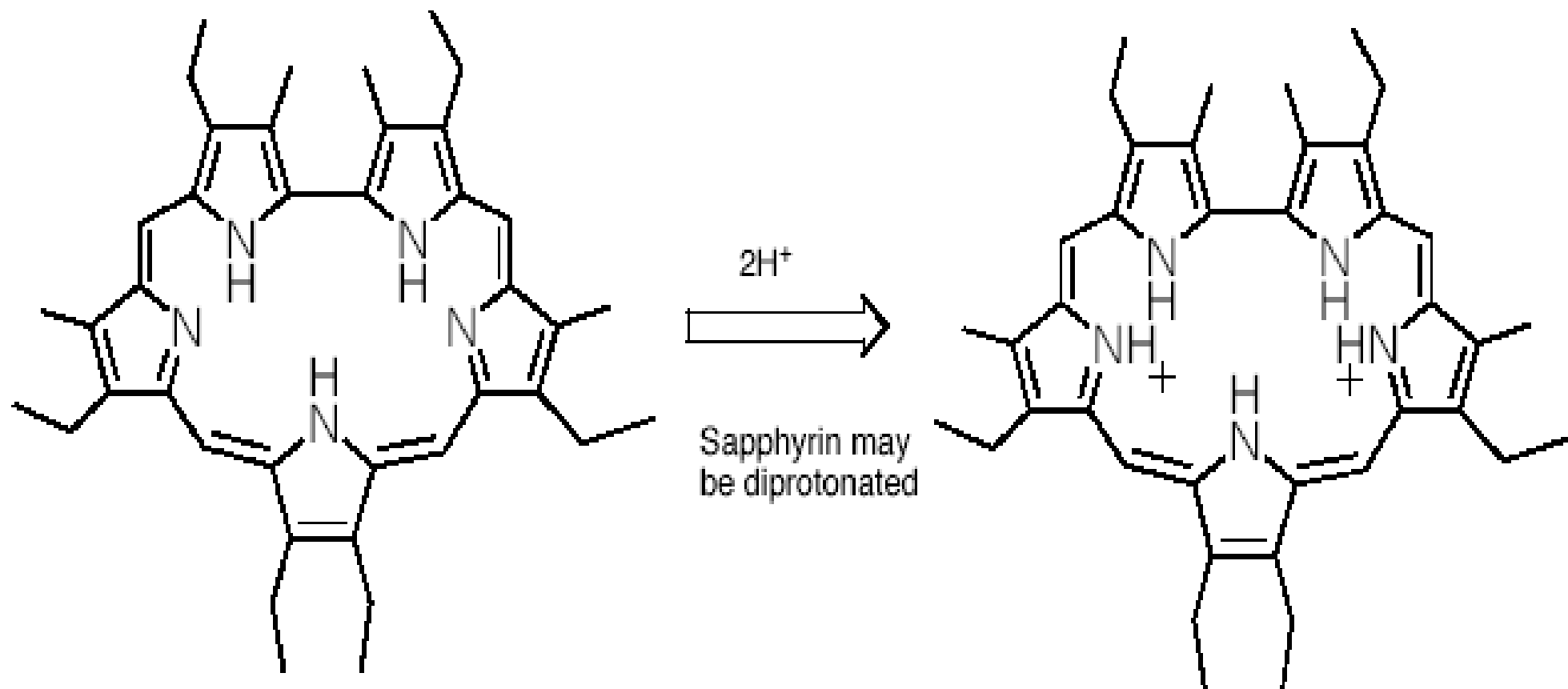
electrostatics

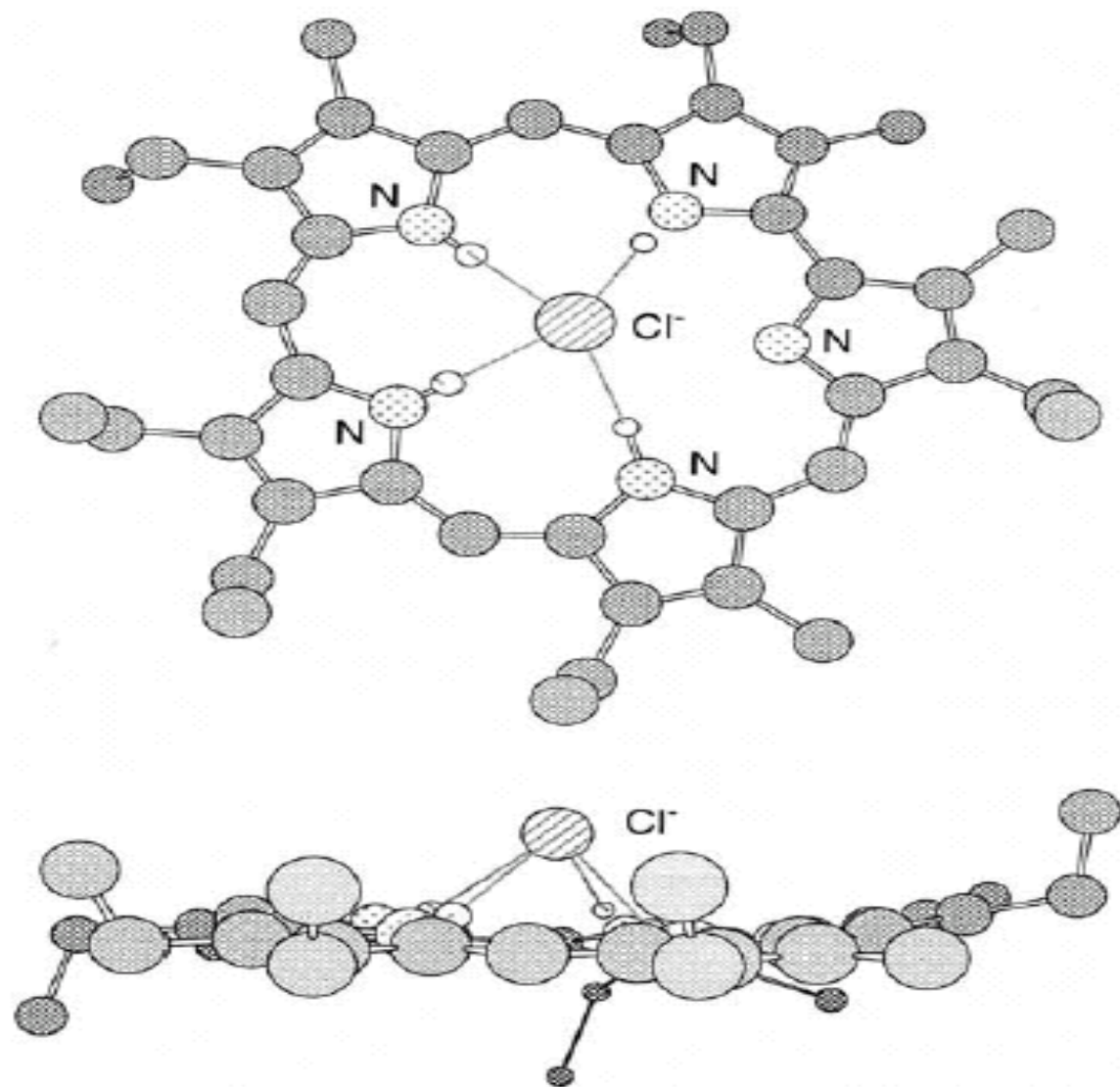
hydrophobic
effect

How does
nature bind
anions?



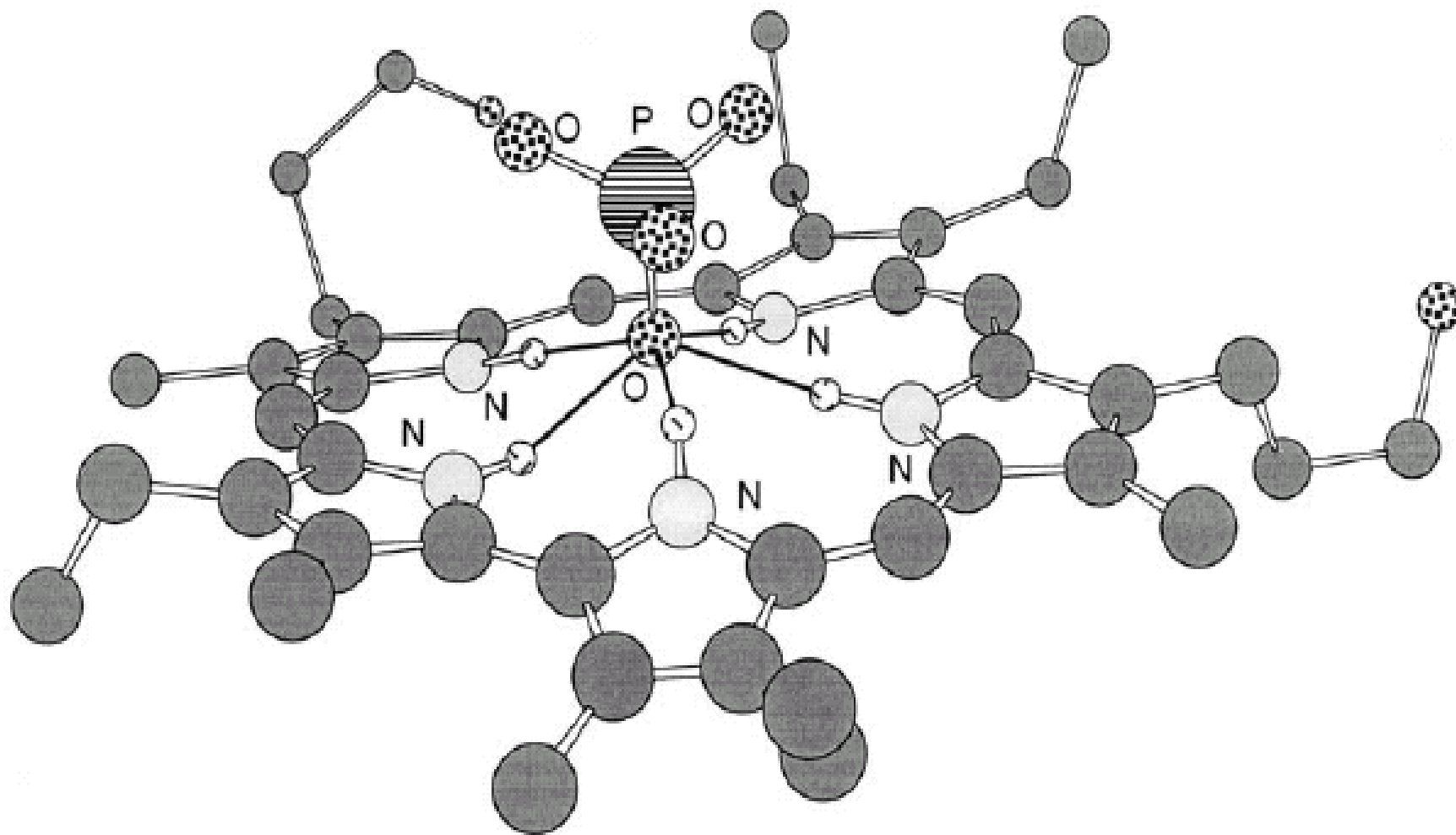
Supramolecular Chemistry



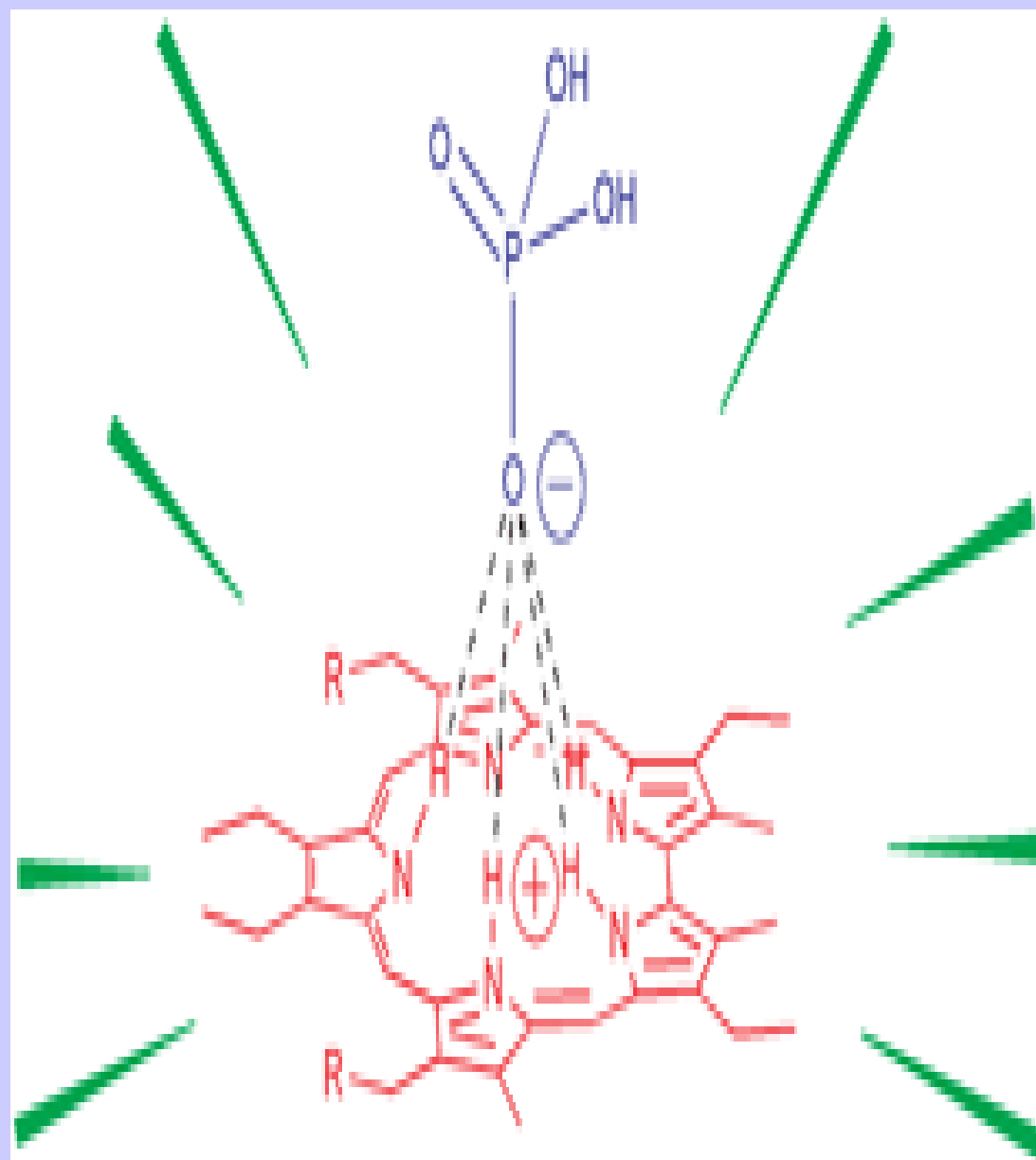
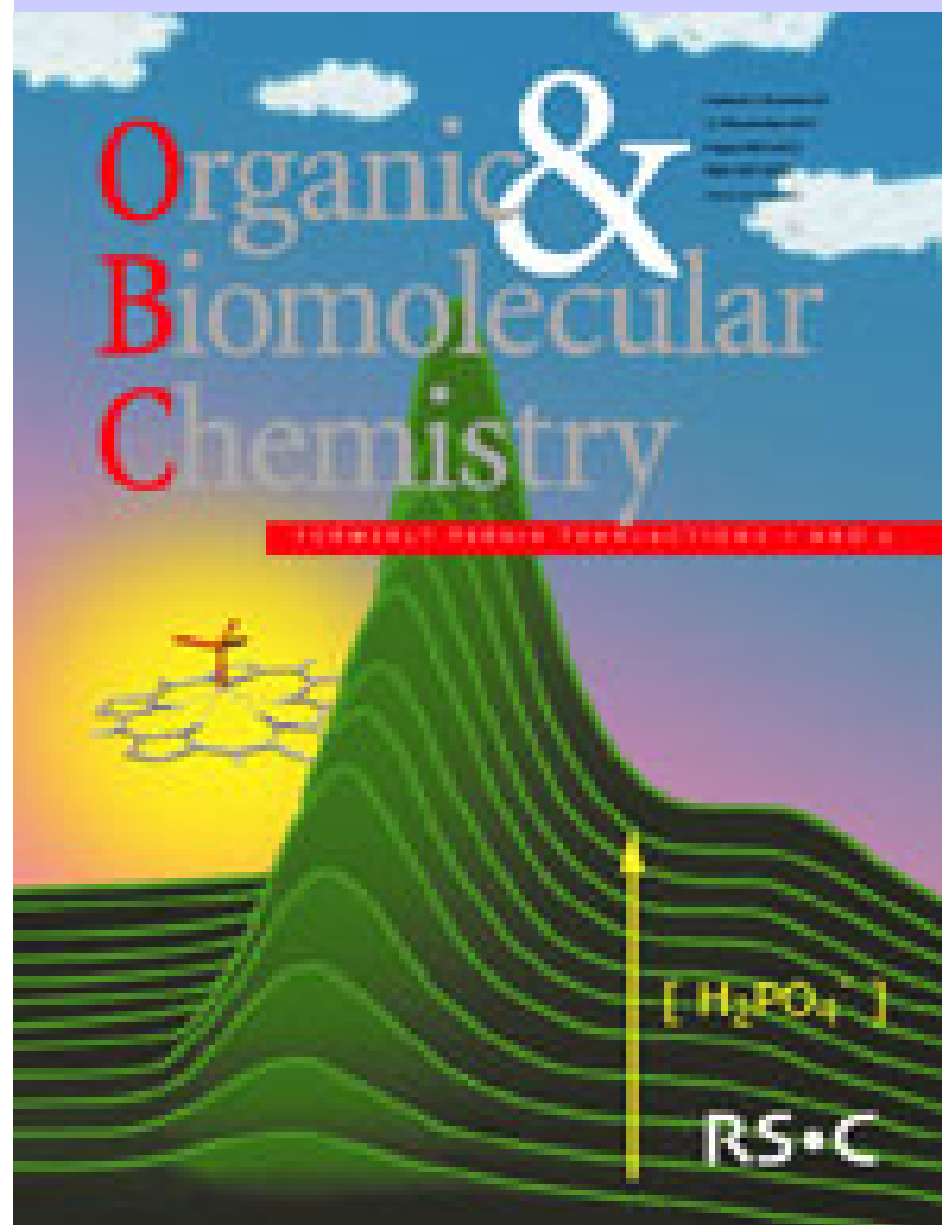


**Anion Binding: New direction in porphyrin research:
PURE AND APPLIED CHEMISTRY
65 (3): 393-398 Times Cited: 87**

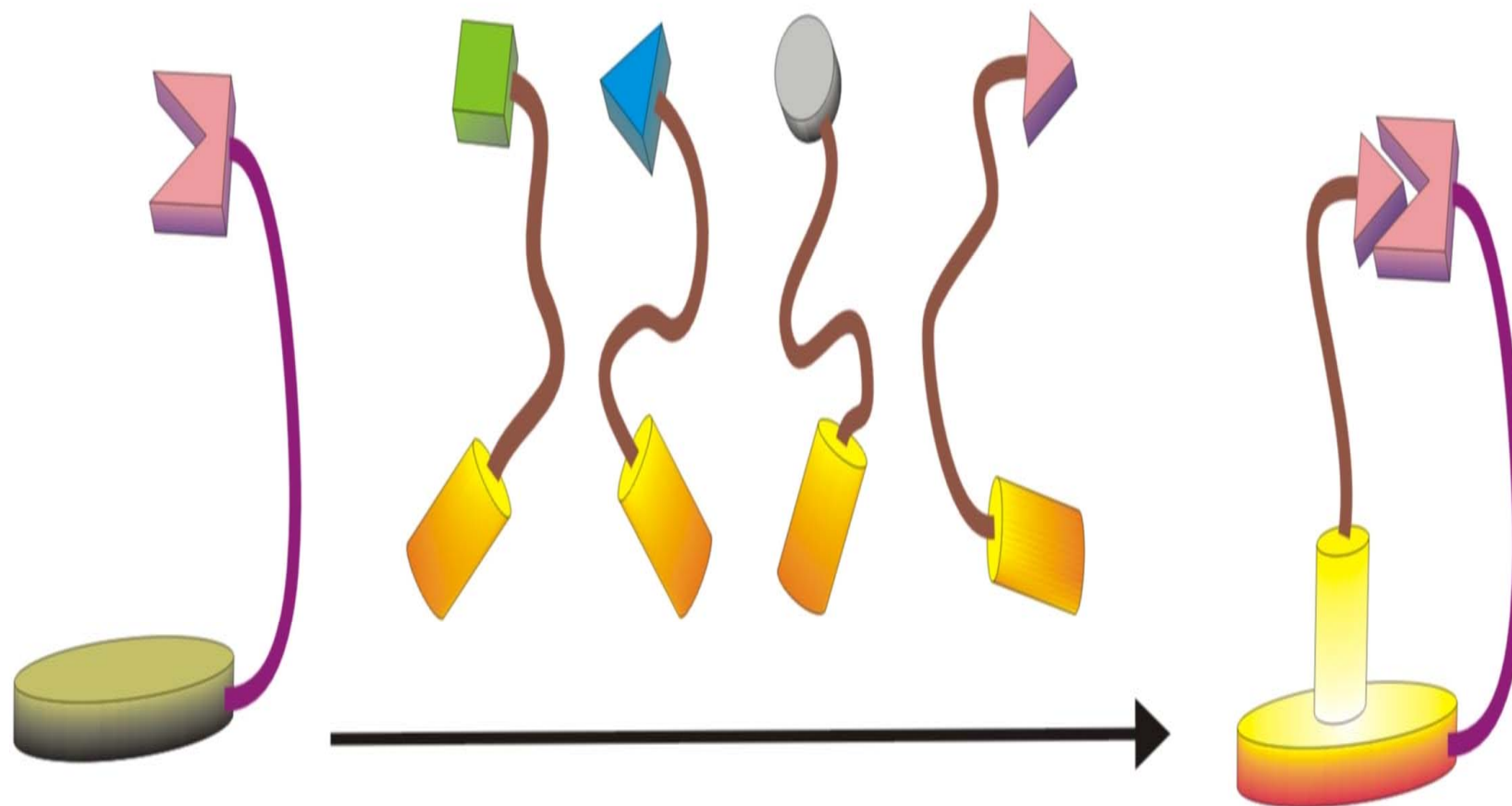
**Kral V, Furuta H, Shreder K, Lynch V, Sessler JL
JACS 1996, 118, 1595-1607: Times Cited: 84**



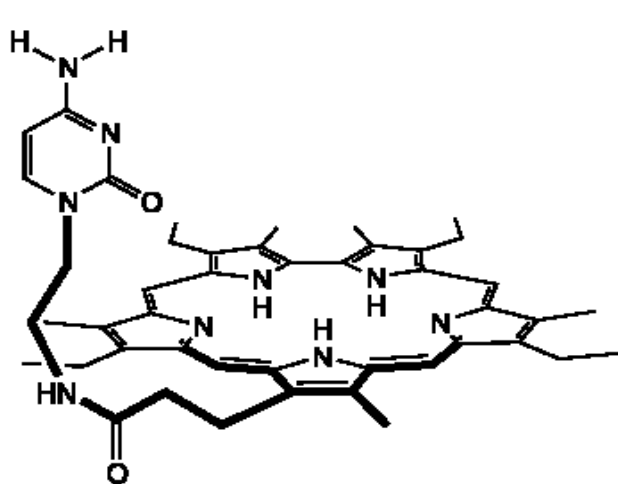
View of the 1:1 complex formed between sapphyrin
and dihydrogenphosphate anion



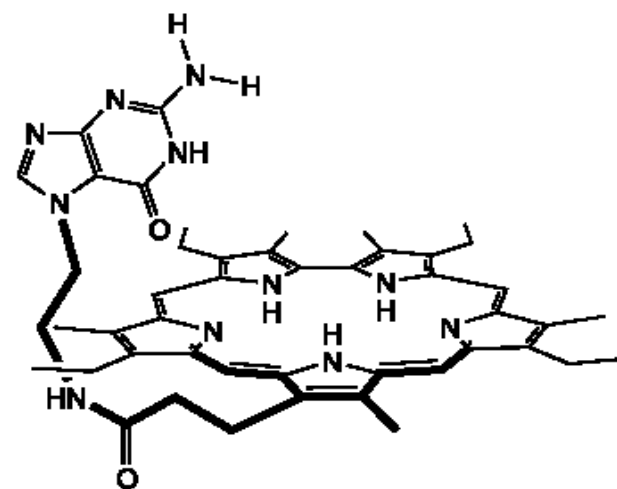
Construction of multitopic receptors



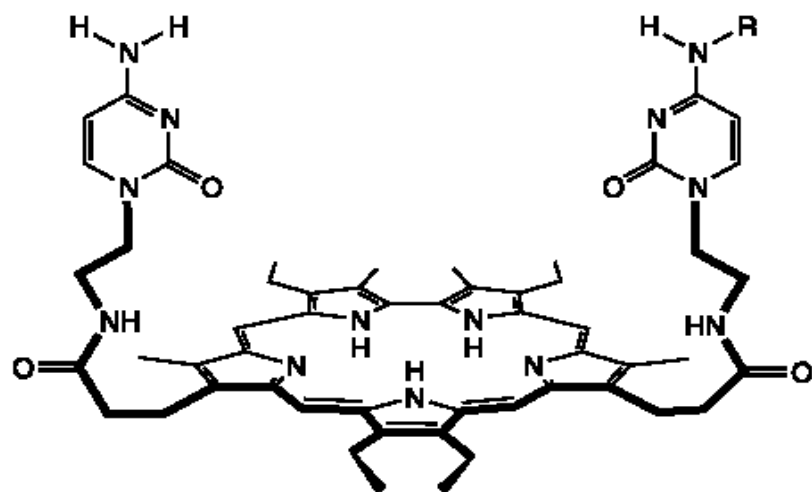
Analytica Chimica Acta 437 (1) 39 – 53, 2001



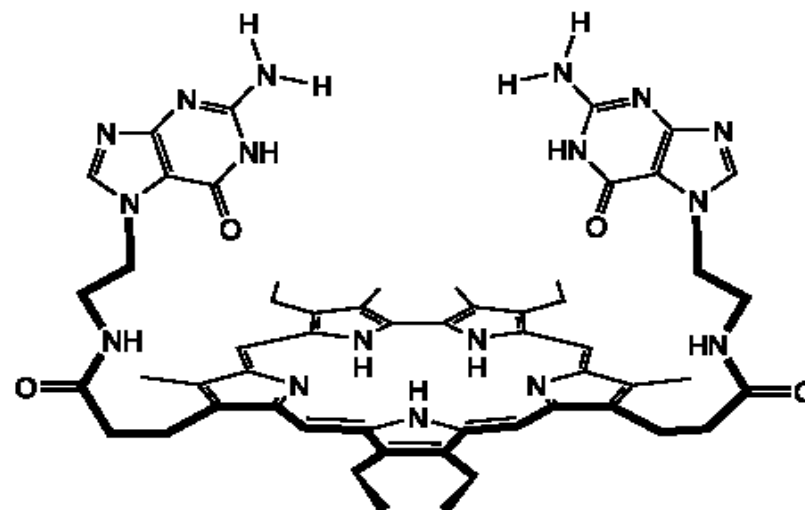
2



3



4



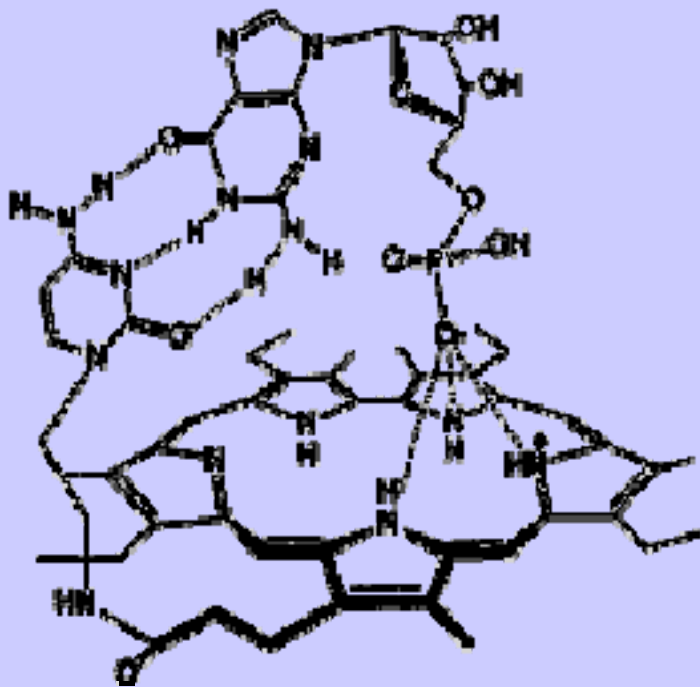
5

Král V.,... Tetrahedron 1995, 51, 539-554

Selective recognition and transport of 5'-GMP

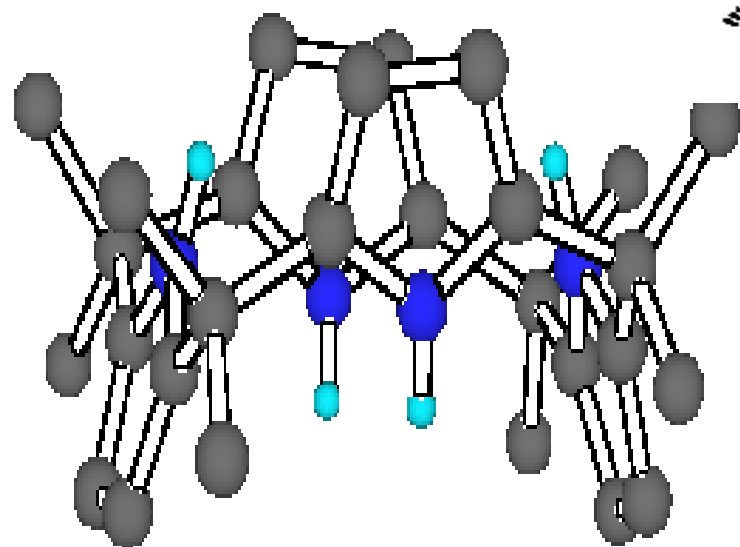
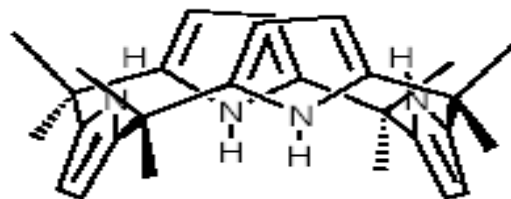
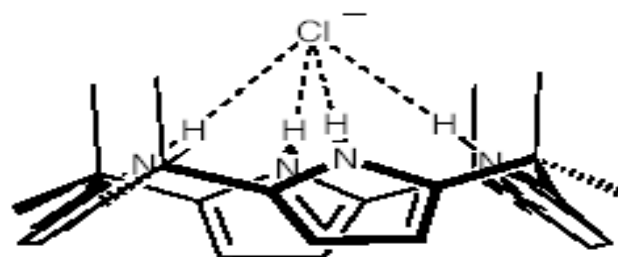
guanosine-5'-monophosphate GMP selective receptor

into-cell transport of nucleotides, MP, TP.

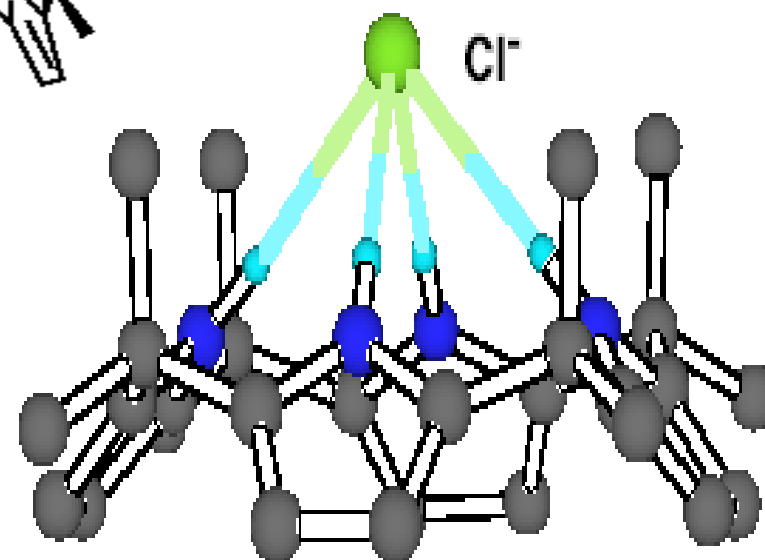


- **guanidine-cytosine Watson-Crick complements**
- **coulombic interaction of phosphate group with cationic motif of receptor, e.g. protonated sapphyrin**

**Král V., Sessler J. L., Furuta H.,
J. Am. Chem. Soc., 1992, *114*, 8704**

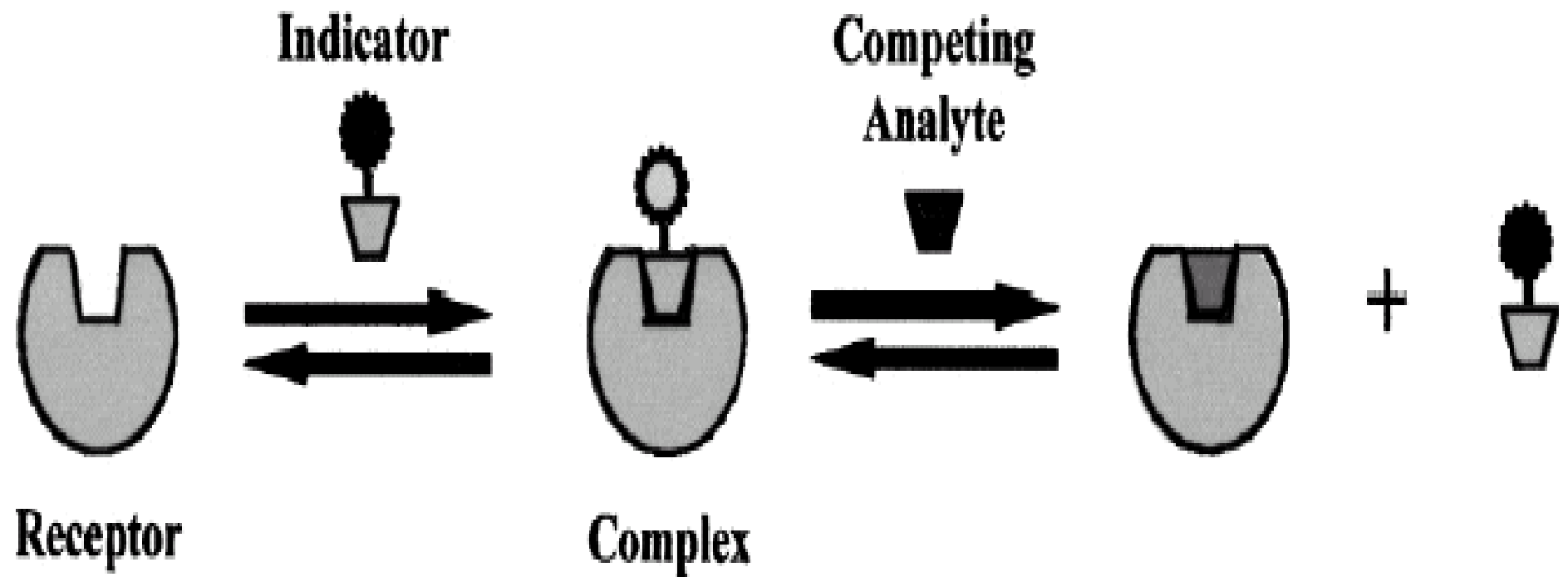


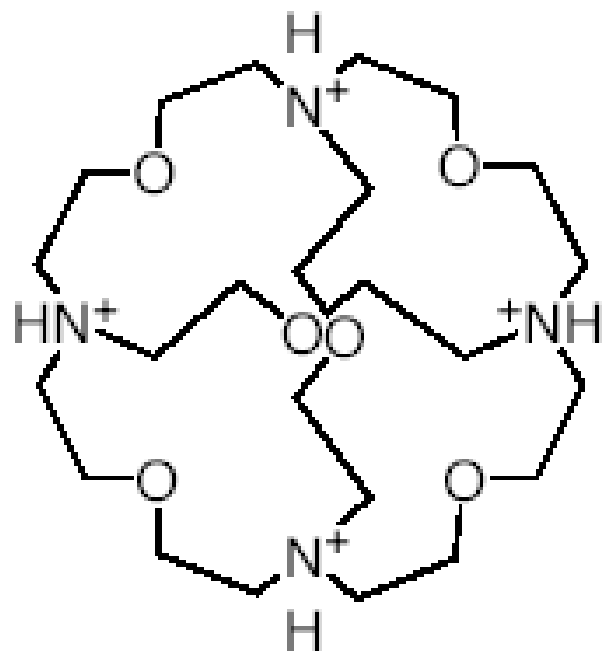
1,3-alternate



cone

JACS 1996, 118 (21): 5140





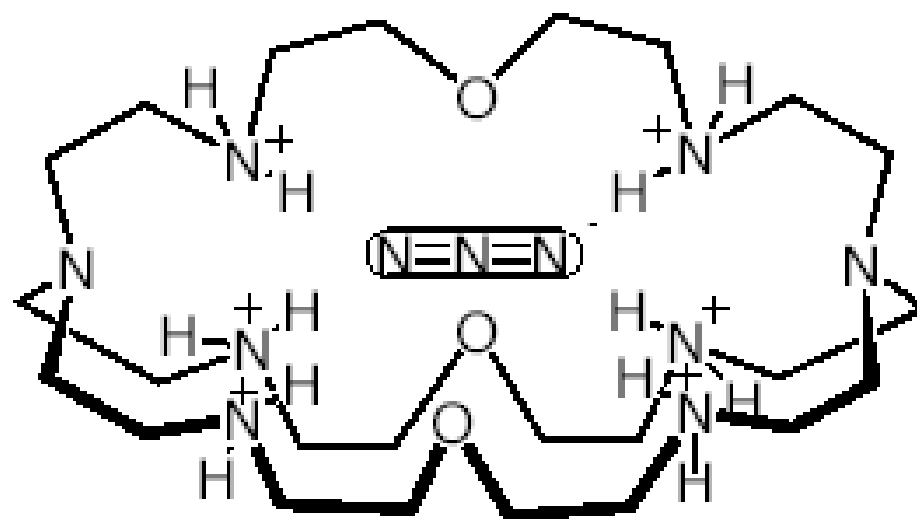
SIZE SELECTIVITY

log K (water)

Cl⁻ >4.0

Br⁻ <1.0

E. Graf and J.-M. Lehn,
J. Am. Chem. Soc., 1976, 98, 6403.



SHAPE SELECTIVITY

log K (water)

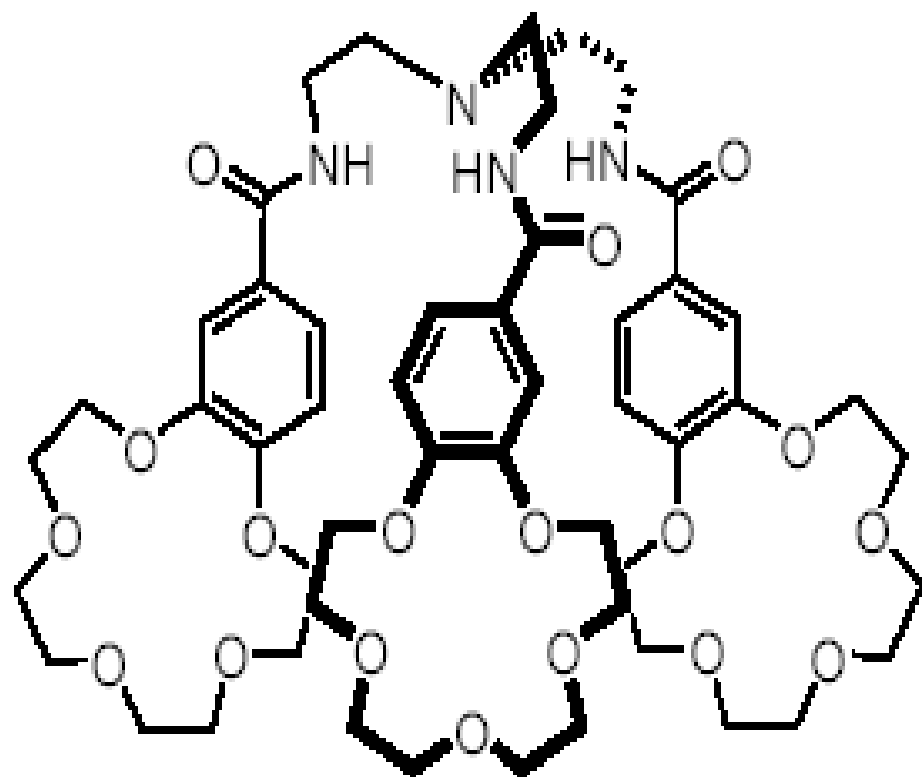
N₃⁻ 4.6

Cl⁻ <1.0

Br⁻ 2.0

J.-M. Lehn, E. Sonveaux and A.K. Willard,
J. Am. Chem. Soc., 1978, 100, 4914.

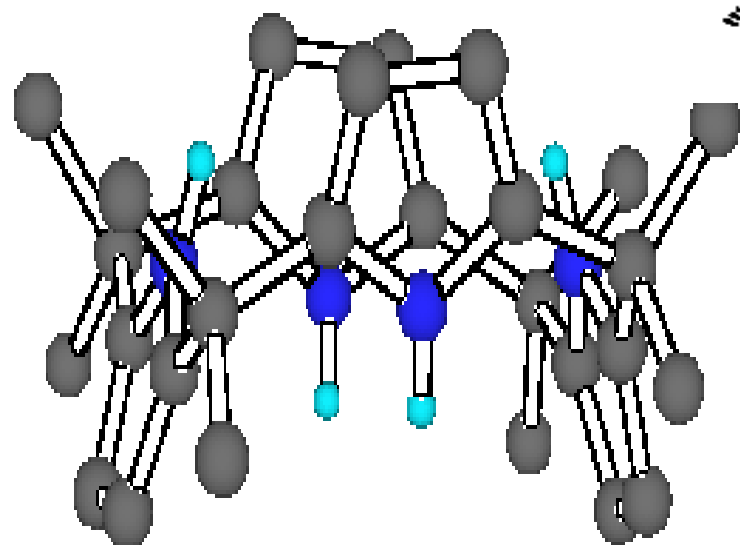
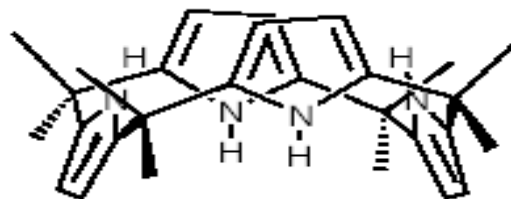
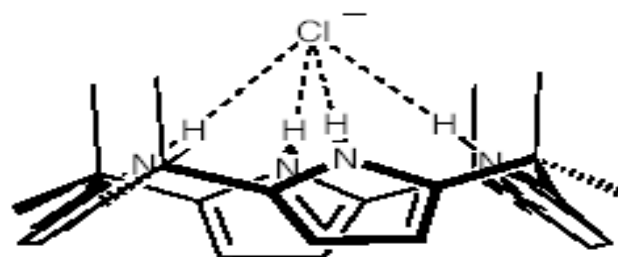
Supramolecular Chemistry



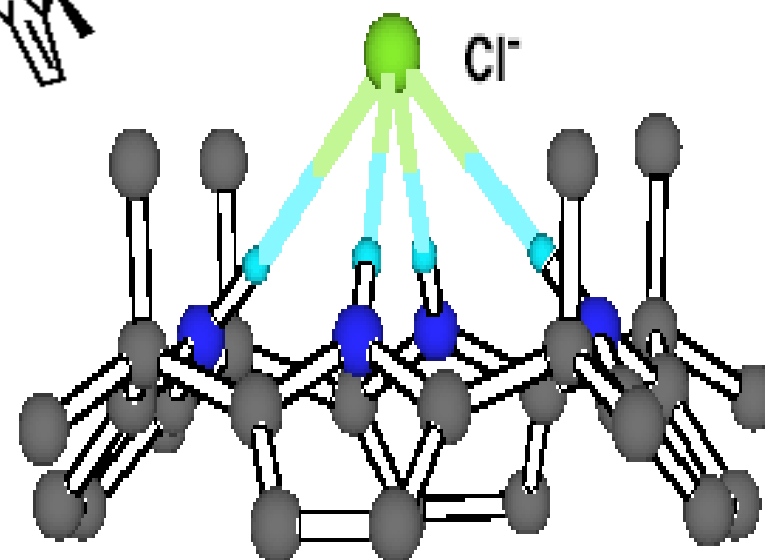
This compound extracts sodium pertechnetate from simulated nuclear waste streams

ISE pro uvedené analyty

<i>Skupina analytů</i>	<i>Iontově-selektivní elektroda</i>
Anorganické kationty	H^+ , Li^+ , Na^+ , Rb^+ , Cs^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} ,
Anorganické anionty	CO_3^{2-} , HCO_3^- , SCN^- , NO_2^- , NO_3^- , OH^- , Cl^- , I^- , ClO_4^- , SeO_3^{2-} , fosfáty,
Organické kationty	1-fenylethylamin, efedrin, norefedrin, amfetamin, lokální anestetika (lidokain, prokain), guanidin, kreatin,
Organické anionty	salicyláty, ftaláty, nukleotidy, heparin,
Neutrální analyty	CO_2 , NH_3 , SO_2 ,



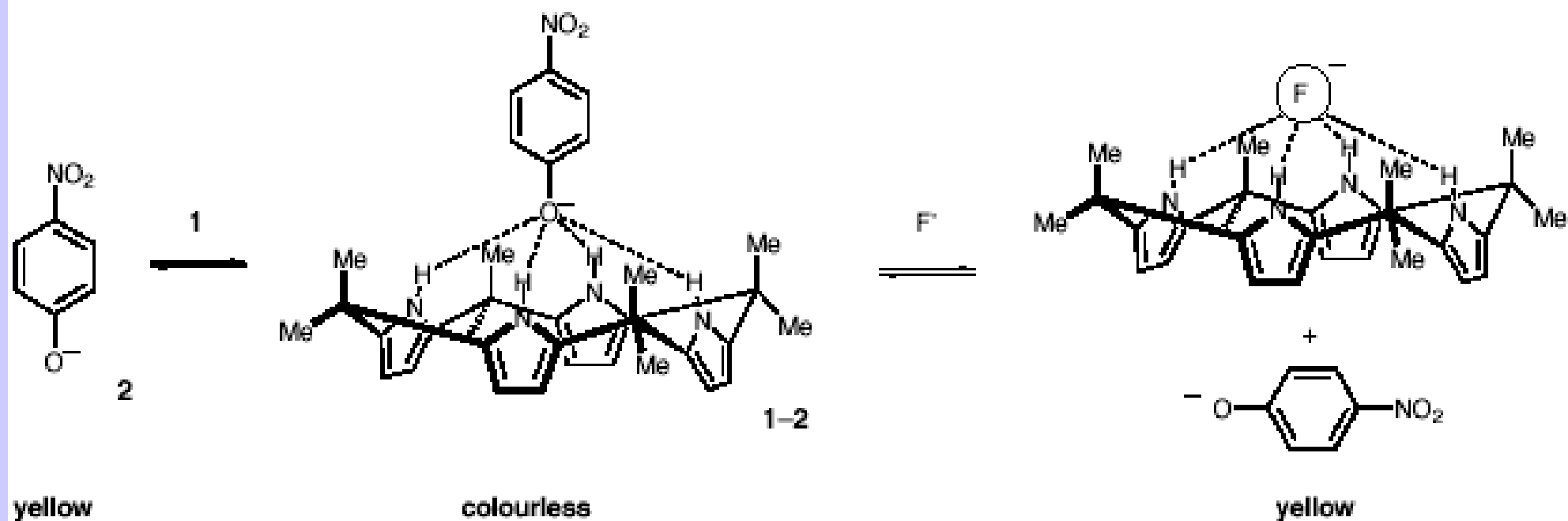
1,3-alternate



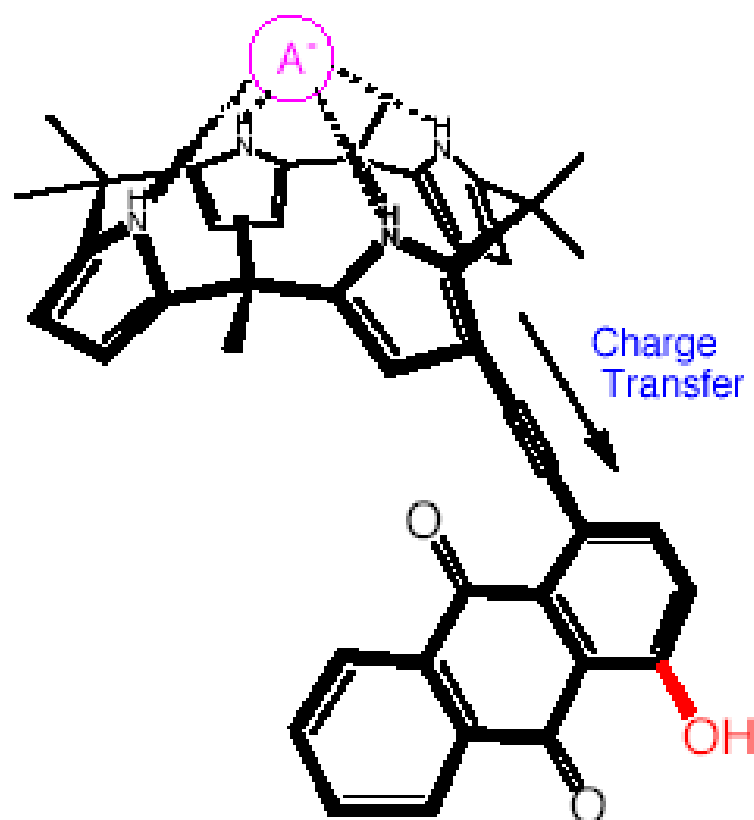
cone

JACS 1996, 118 (21): 5140

Displacement assay



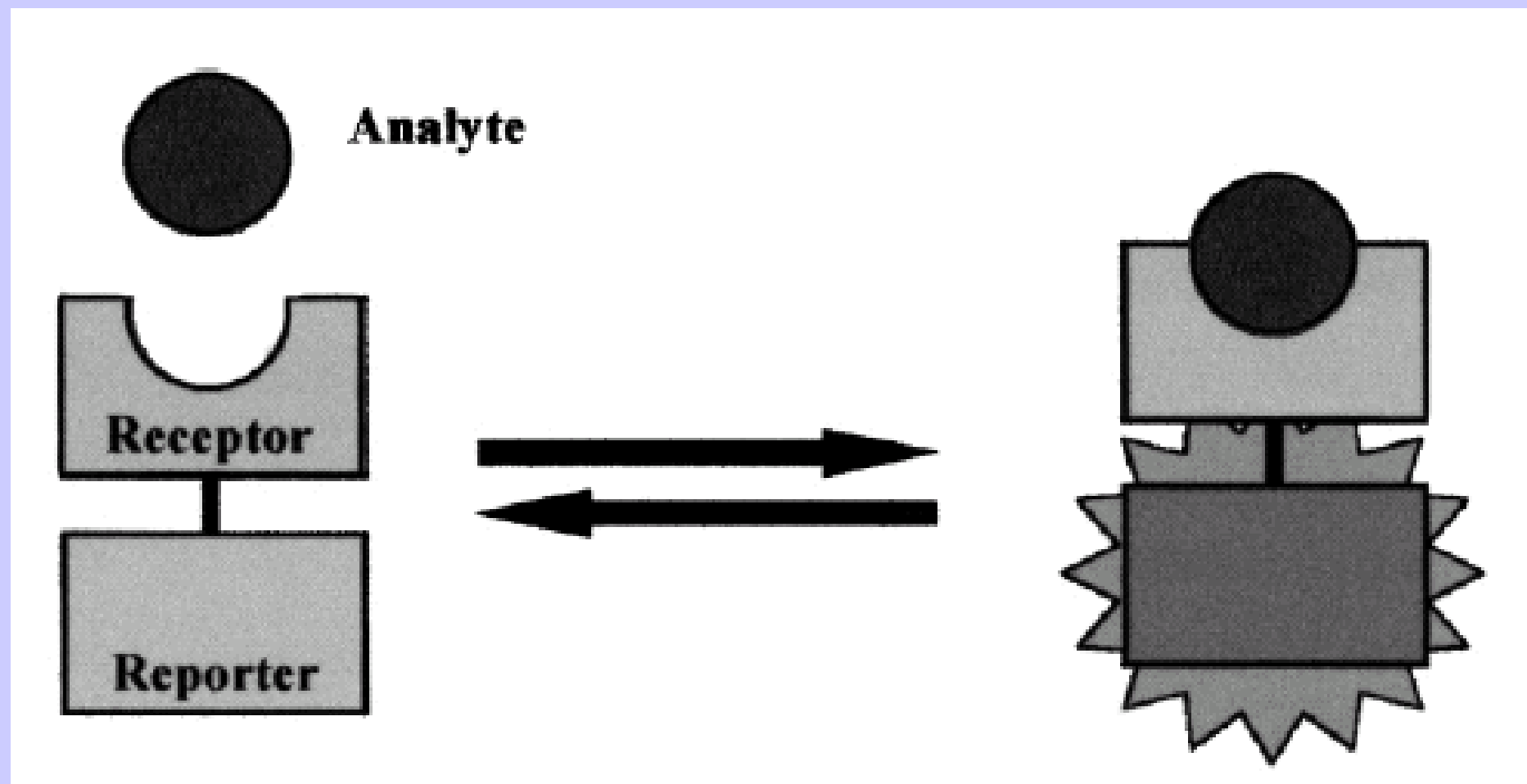
The Higher Affinity For F^- over Cl^- Initially Reported in Apolar Solvents Is Supported



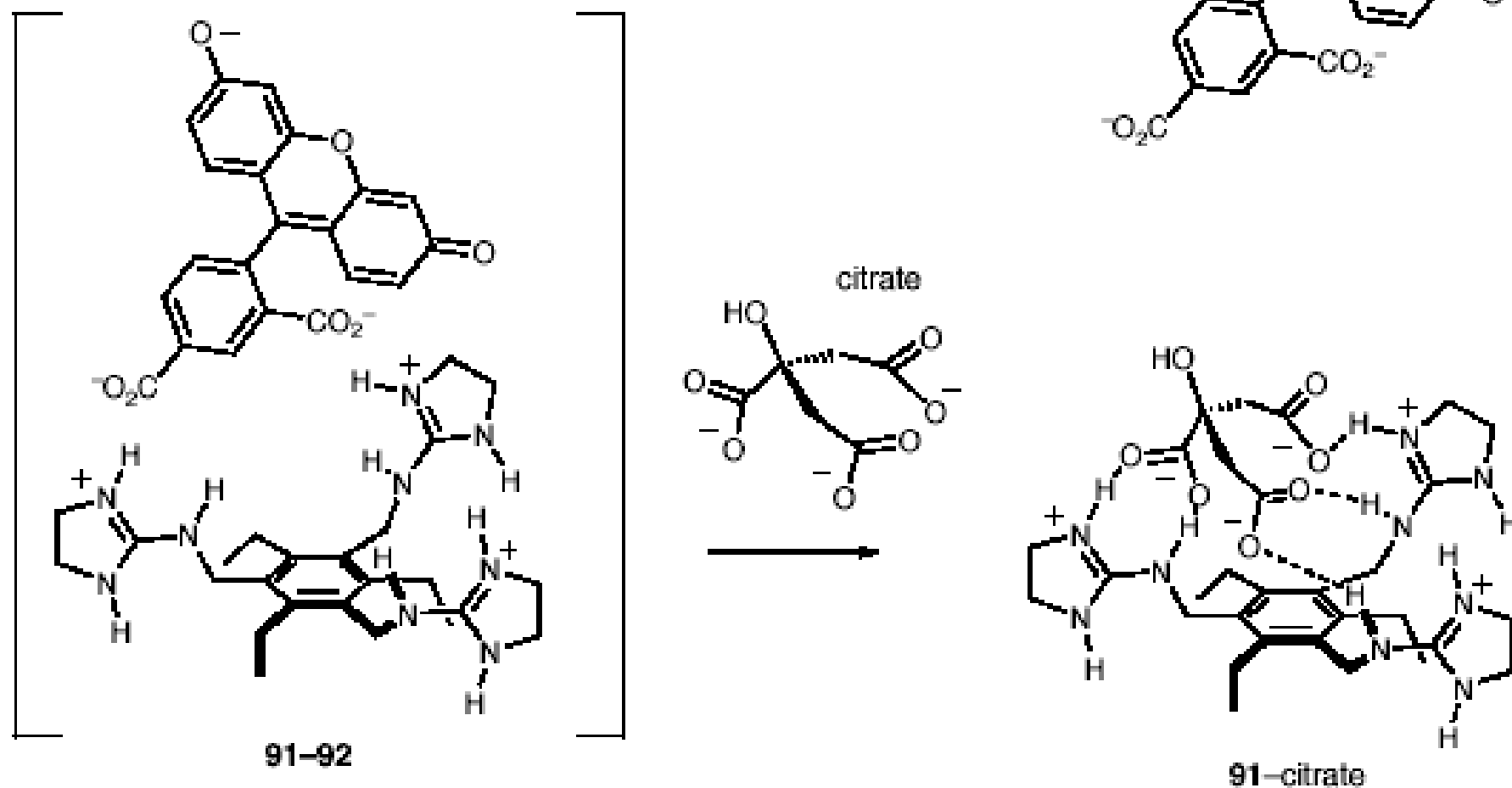
Studies in Dichloromethane with 100 Equiv. TBAF, TBACl, etc.

Angew. Chem. Int. Ed. **2000**, 39, 1777

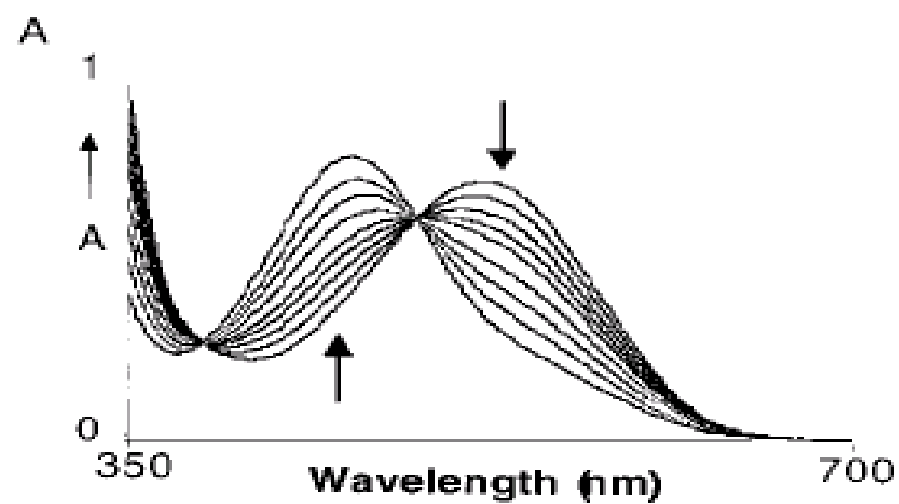
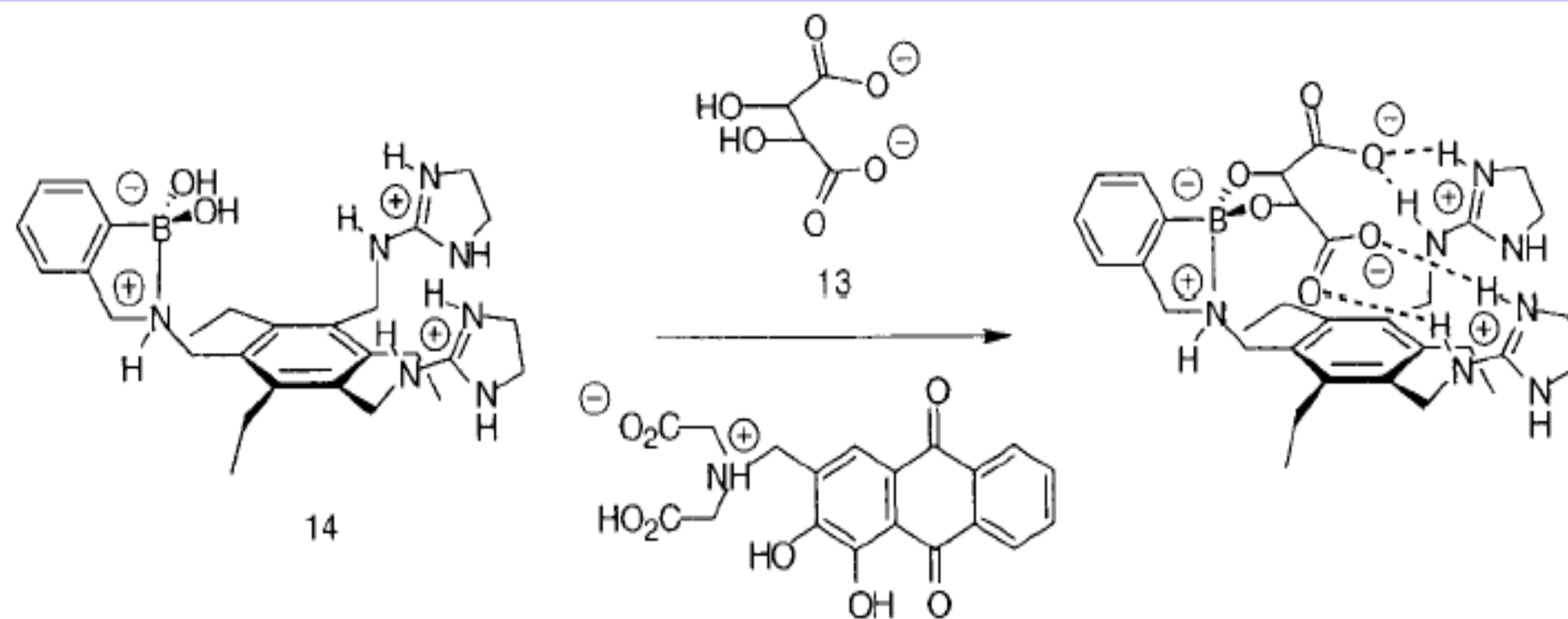
Competitive indicator methods for the analysis of citrate using colorimetric assays

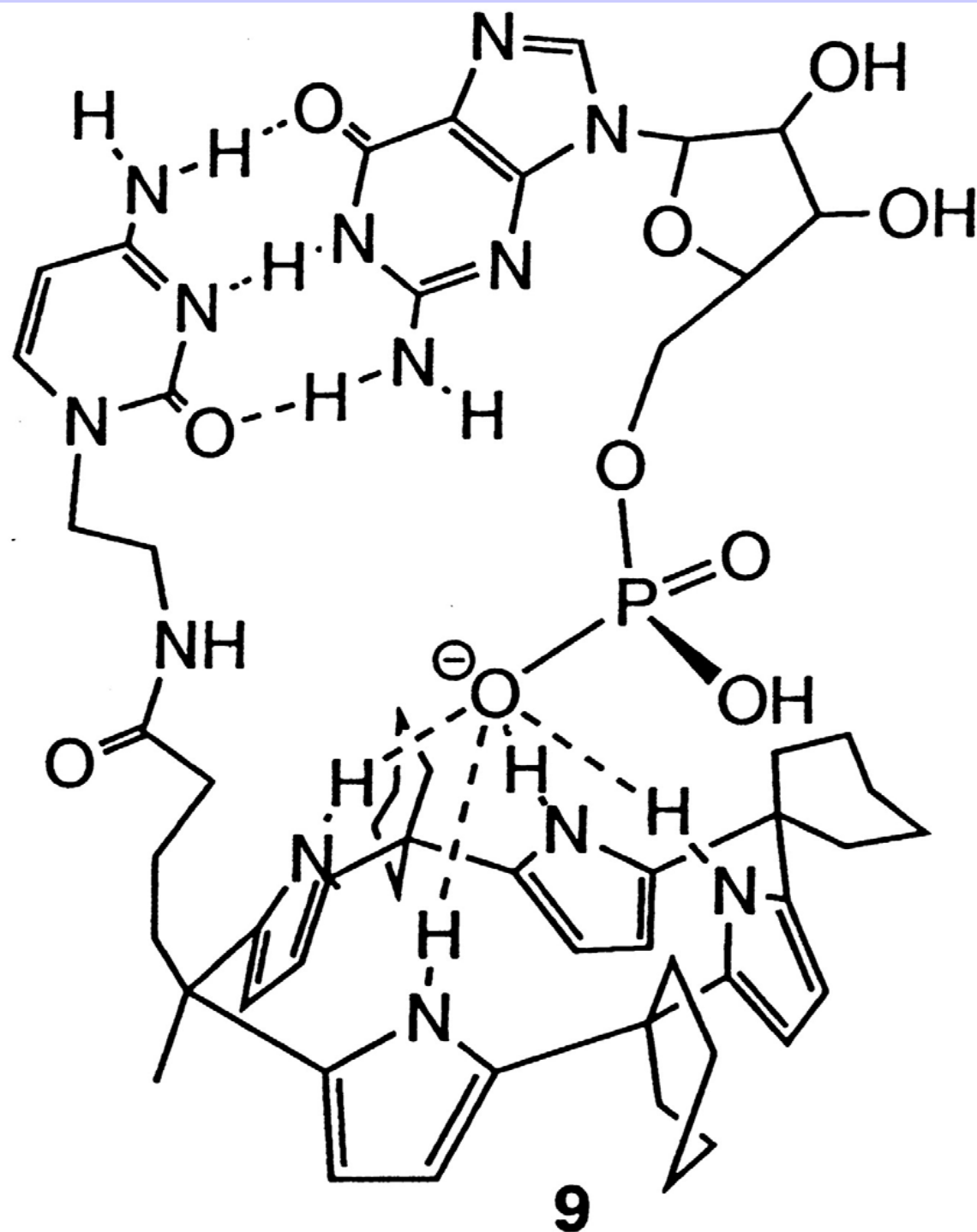


Supramolecular Chemistry



A. Metzger and E.V. Anslyn, *Angew. Chem. Int. Ed.* 1998, 37, 649.



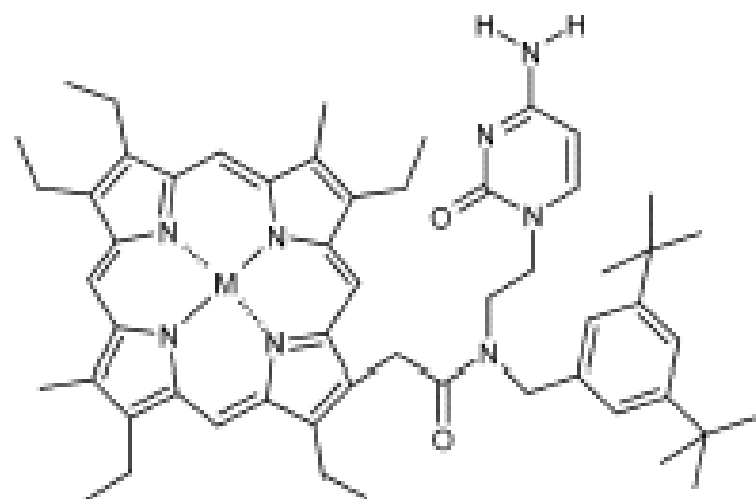


**Cytosine
substituted
calix[4]pyrroles:
Neutral receptors
for 5'-guanosine
monophosphate**

**Chem. Commun.
1998, 1-8**

Times Cited: 97

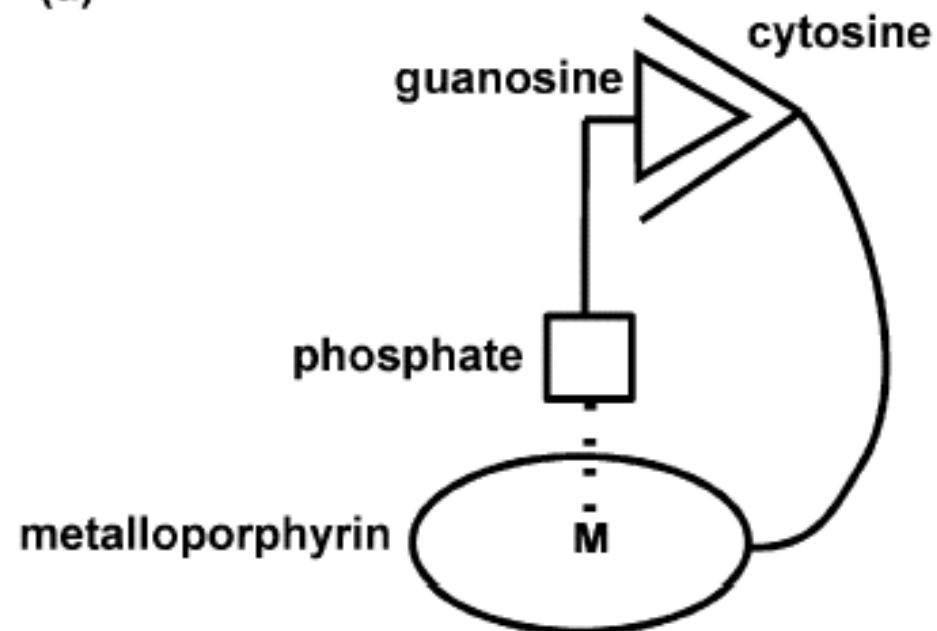
**PNAS 99 (8) 4848 –
4853, 2002**



2: M = Co (III)

3: M = Zn (II)

(a)



Org. Biomol. Chem., 2004, 2, 1169–1175

ARTICLE

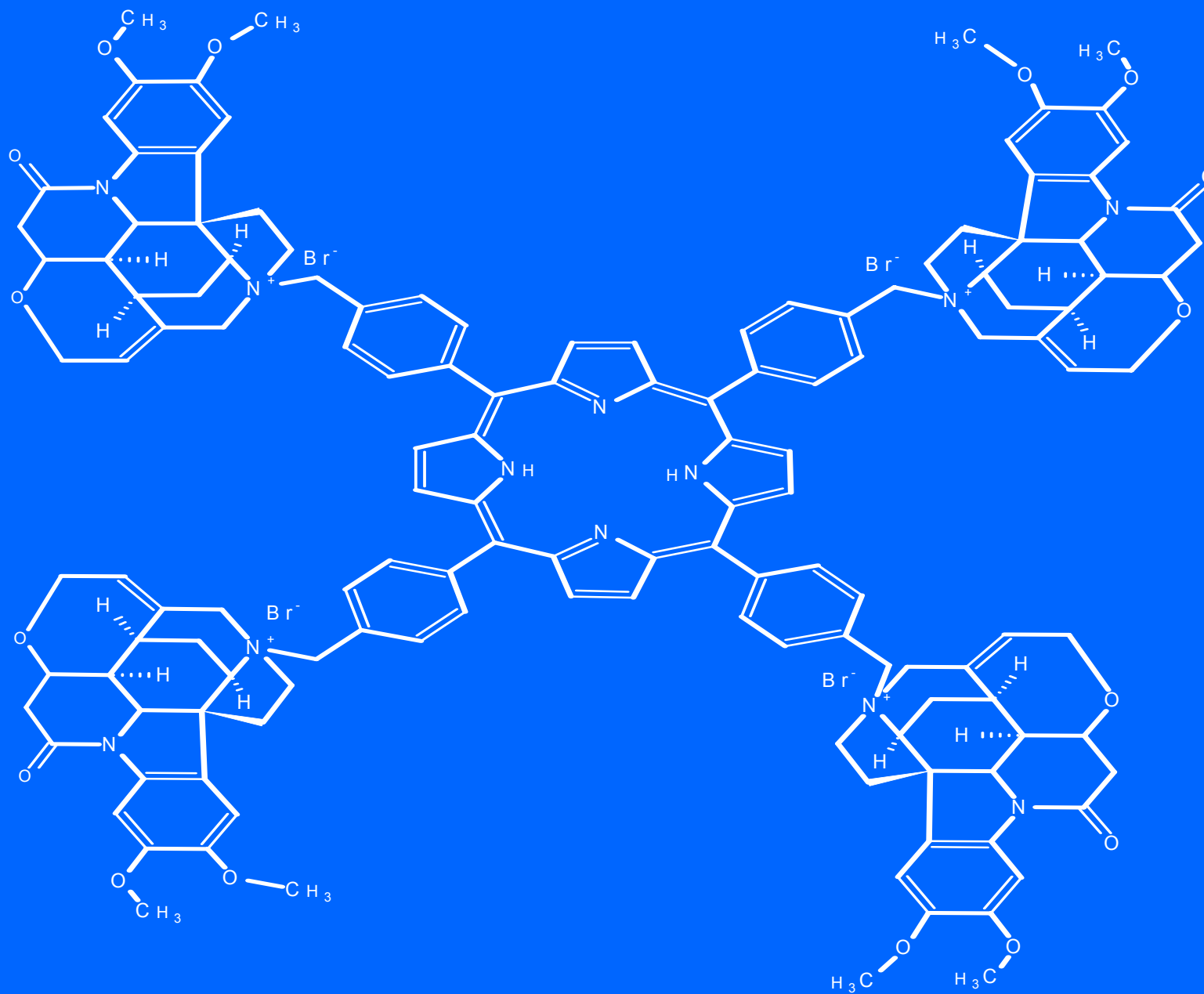
Cytosine-substituted metalloporphyrins: receptors for recognition of nucleotides in ion-selective electrodes

Vladimír Král,^{a*} Tatiana V. Shishkanova,^c Jonathan L. Sessler^{a,b} and Christopher T. Brown^b

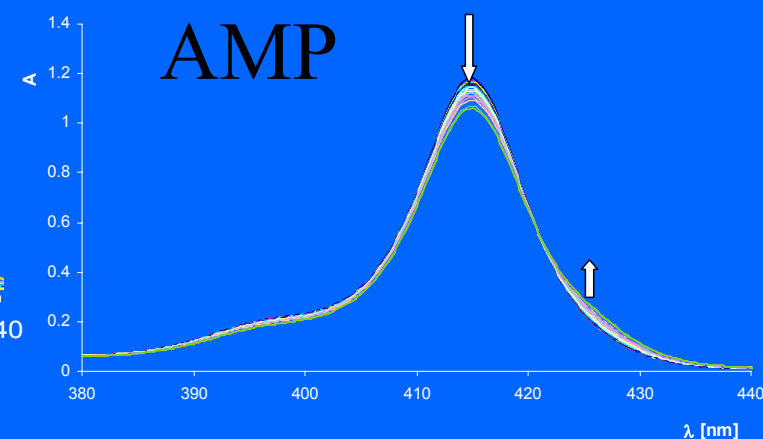
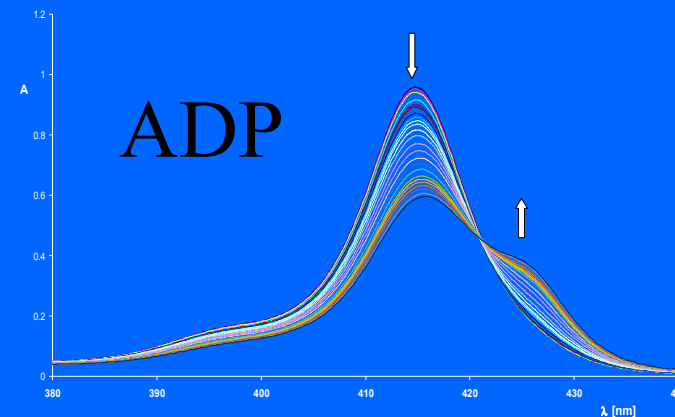
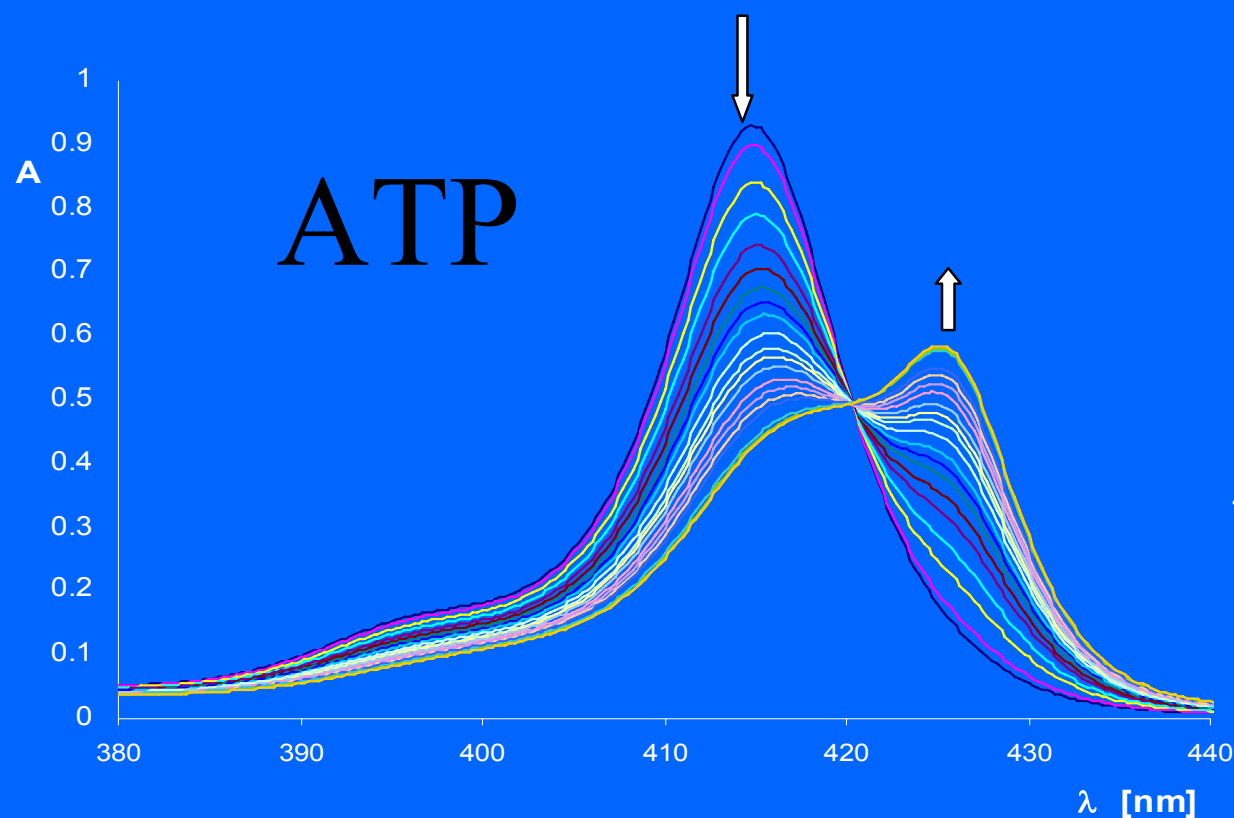
^a Department of Analytical Chemistry, Institute of Chemical Technology, 16628 Prague 6, Technická 5, Czech Republic. E-mail: vladimr.kral@vscht.cz; Fax: +420 2 2431 0352; Tel: +420 2 2435 4227

OBC
www.rsc.org/obc

Receptor Por-alka conjugates

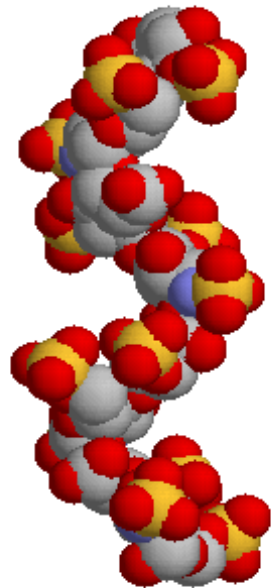


Binding study of por-bru with ATP

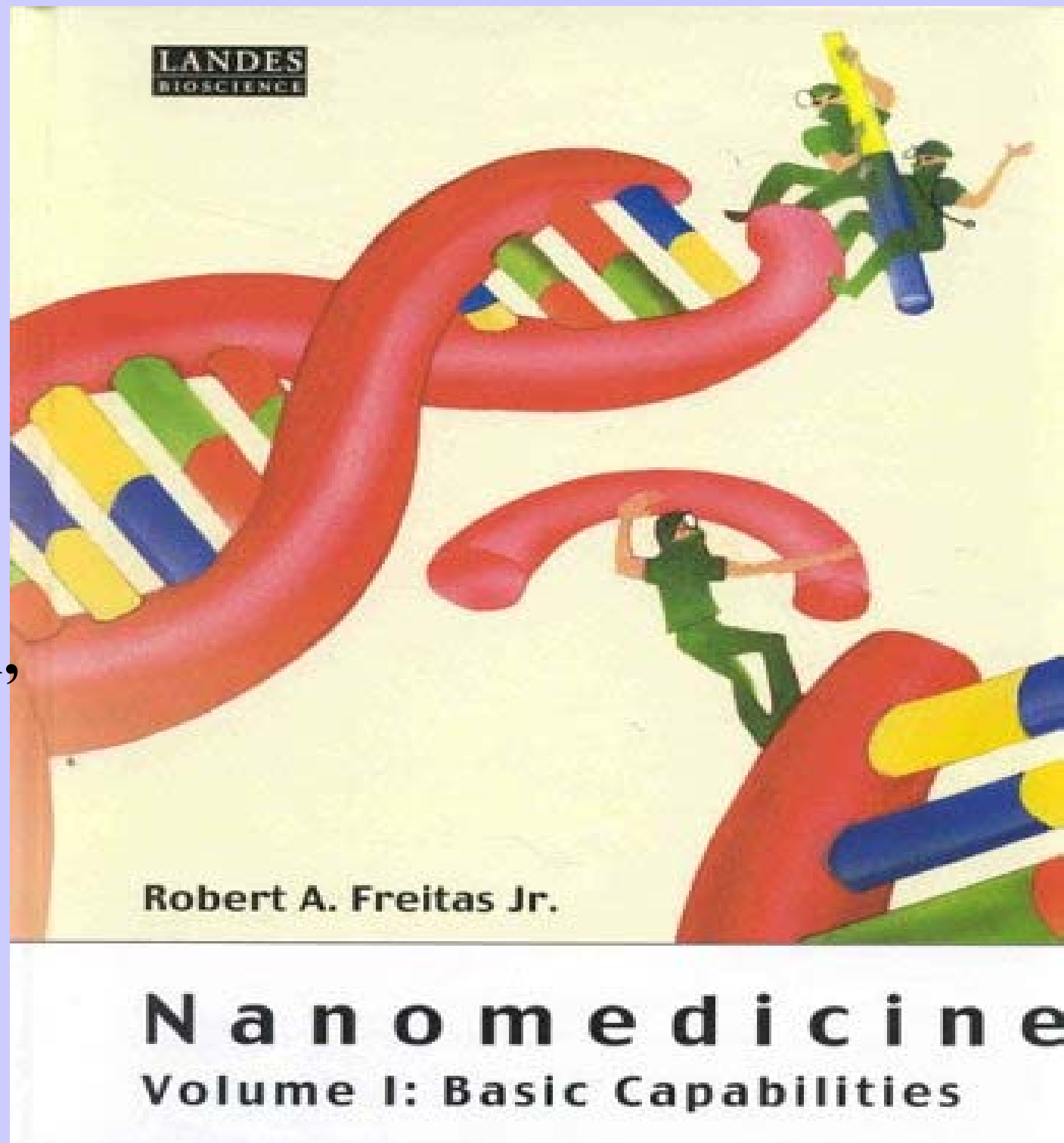


Por-alka receptor ATP (0-150 eq) HEPES, pH 8

Heparine sensing



**Výzvy: NK
sekvence,
Dědičné
choroby,
Mutace p53
Geny nádorů,
infarktu**



Analýza lidského genomu

akademický, firemní výzkum (Celera)

-analyzovány fragmenty DNK lokalizované na jednotlivých chromosomech (50-250 milionů párů bází).

-Analýza sekvence ukazuje, že genom člověka obsahuje asi 40 000 genů.

-celkový počet je asi 3,2 miliardy párů bází

-sekvence lidského genomu není definitivní, protože obsahuje mnoho chyb

-úplná analýza, která bude obsahovat méně než jednu chybu na 10 000 bází, 2004

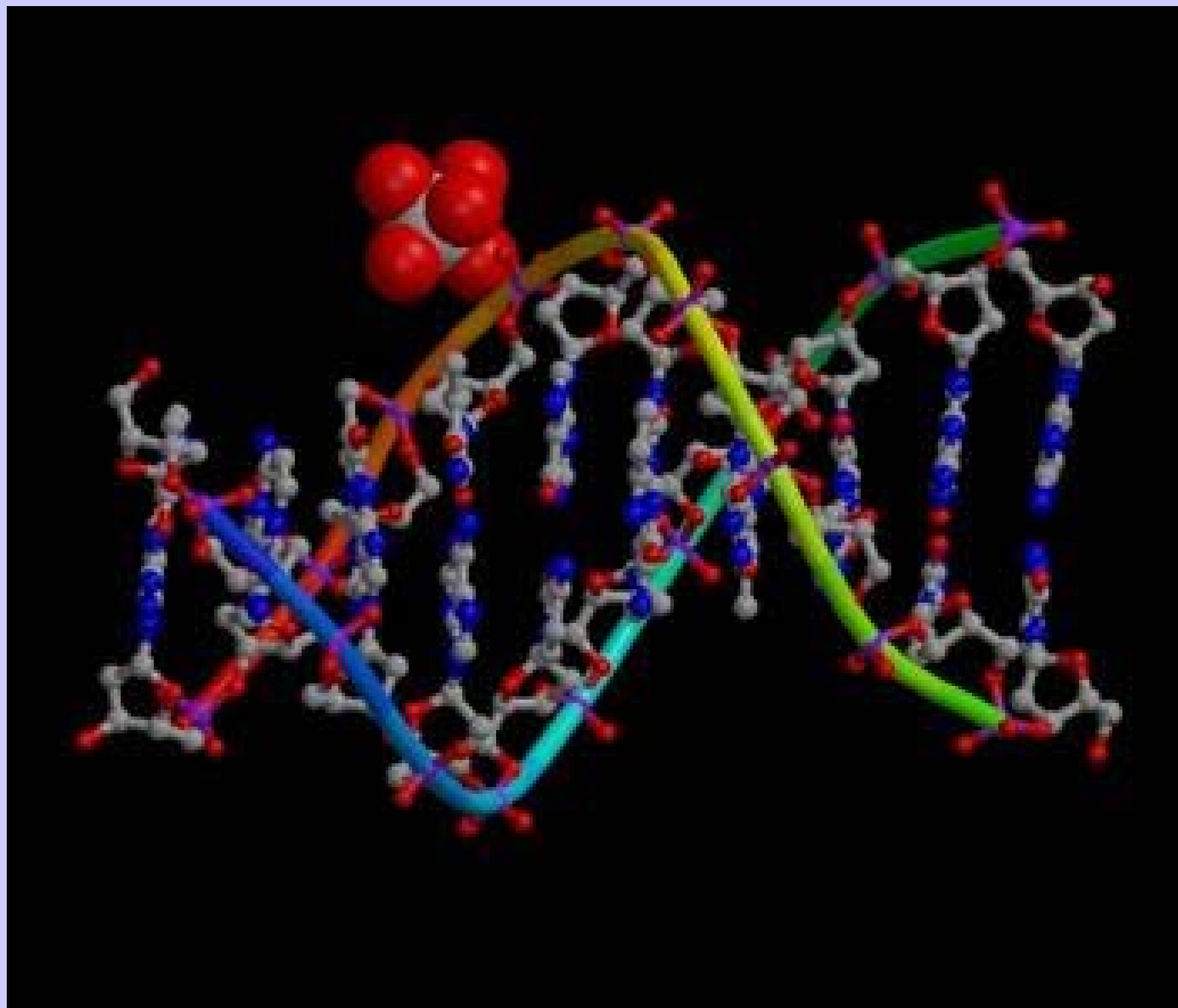
-projekt, byl obrovským přínosem pro vývoj nových metodik analytické a bioanalytické chemie

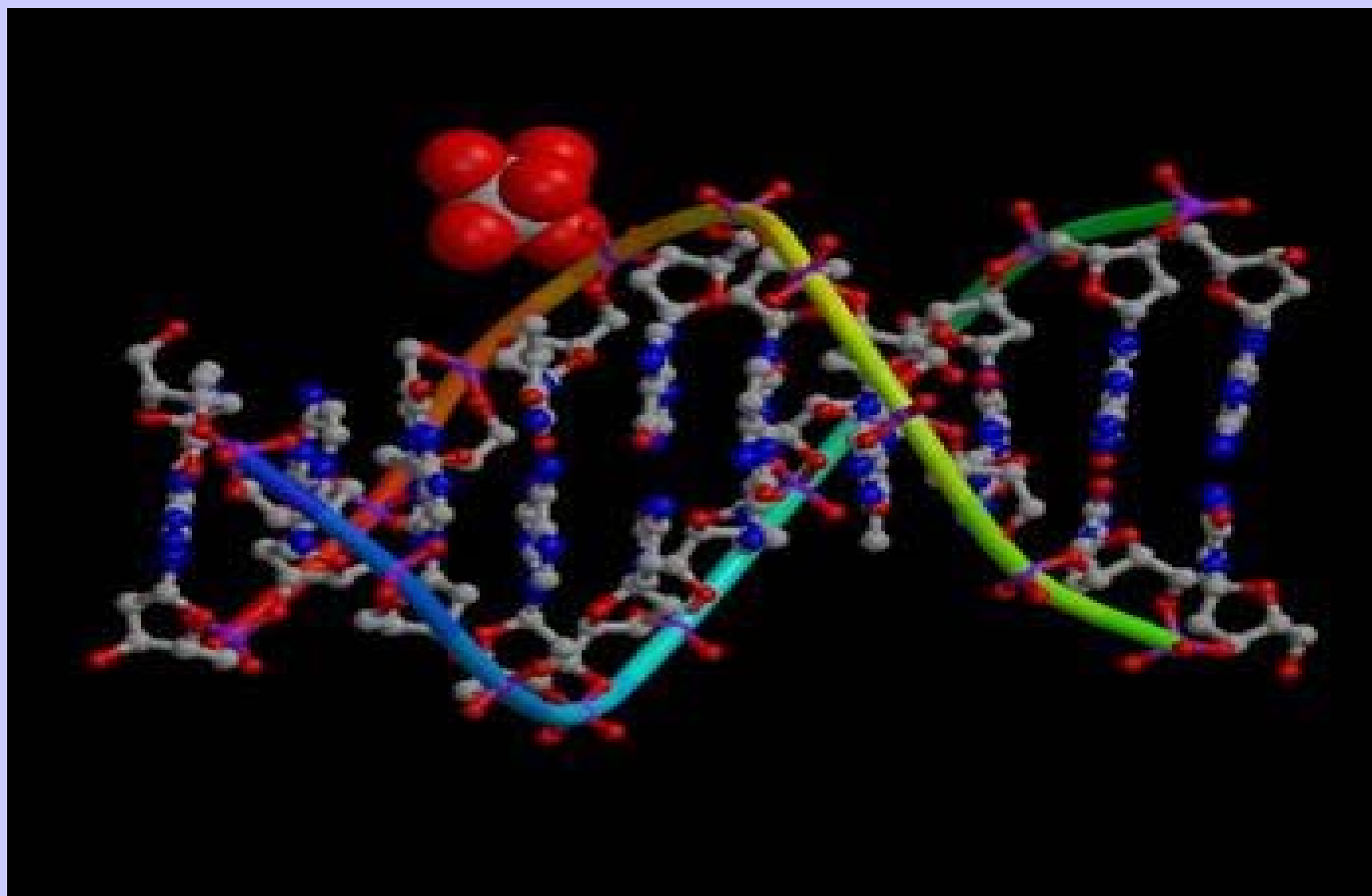
1.Lander, ES., Linton, LM., Birren, B. Nature, 2001, 409, 860-921.

2.Venter, JC., Adams, MD, Myers, EW. Science 2001, 291, 1304-1351.

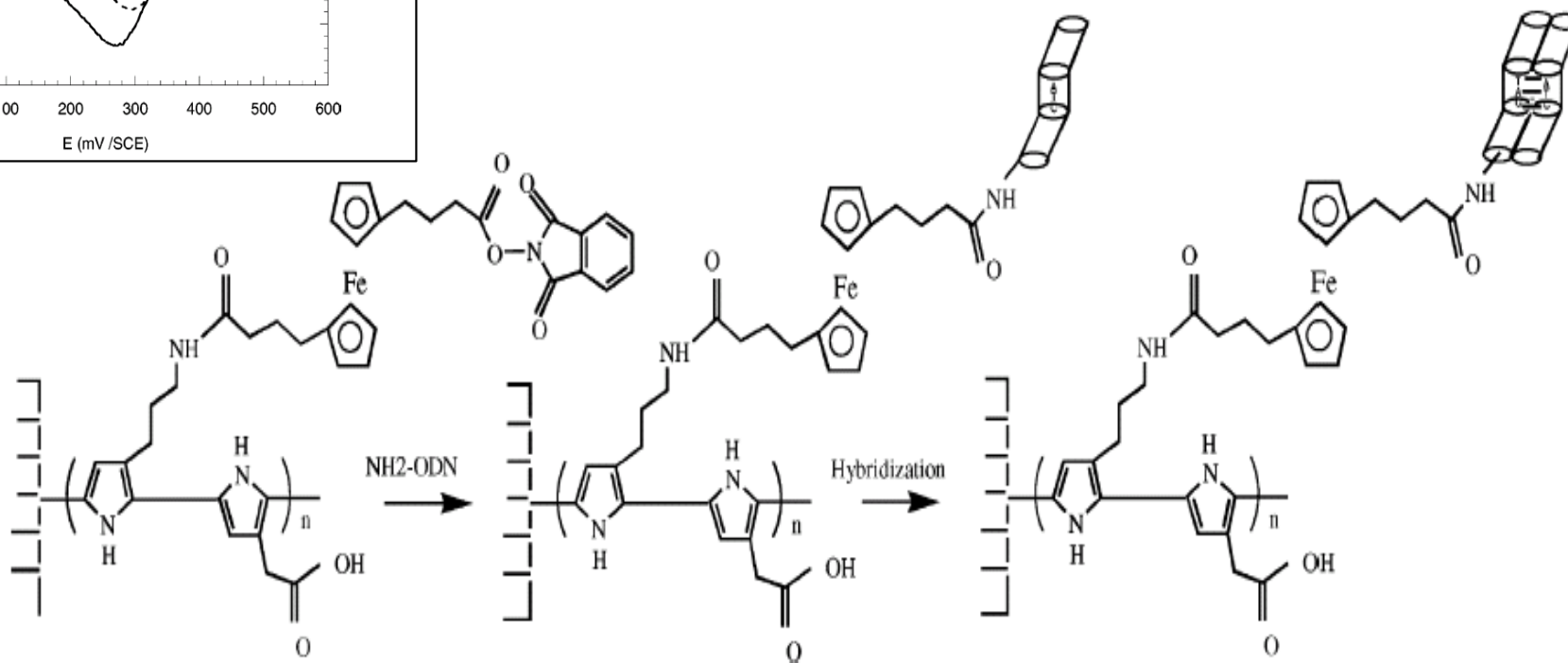
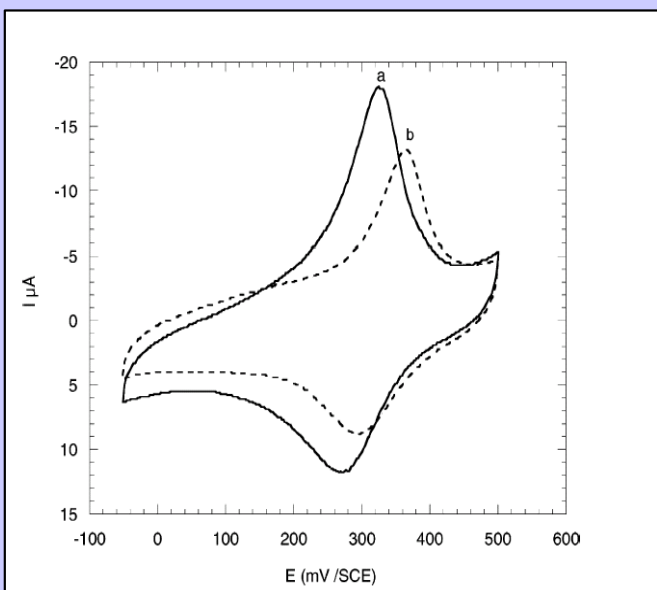
**-nové směry a výzvy postgenomiky
proteomika**

**-analýza mutací, diagnostika,
využití DNK čipů – jednoduchost, rychlost a velká kapacita (místo pracné separace a sekvenace fragmentů**

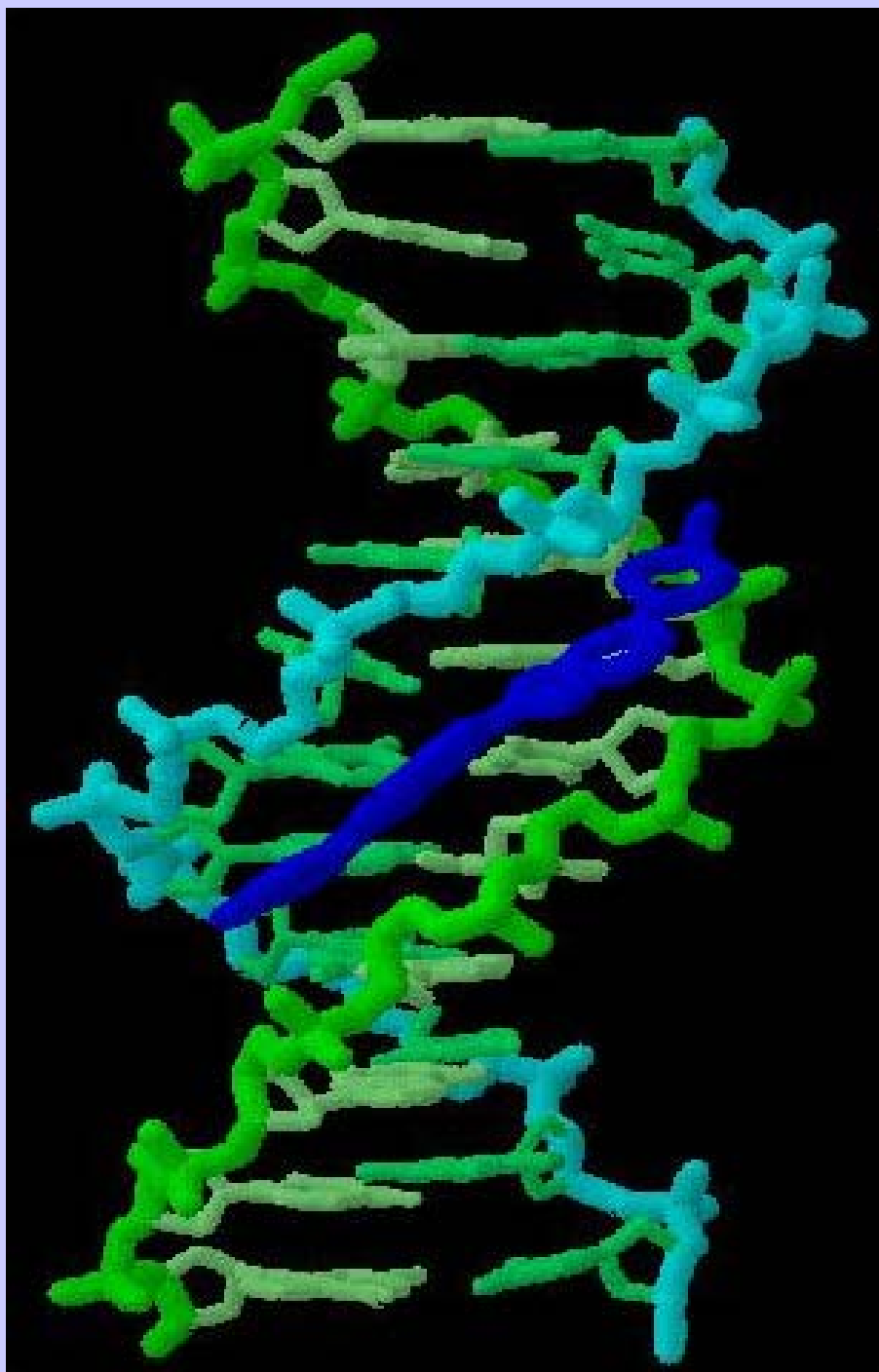




Elektrochemický biosensor



Monitorování DNK hybridizace s použitím ferrocenylové skupiny na funkcionalizovaném polypyrolu (detekční limit je 10^{-14} mol)



***J. Am. Chem. Soc.* 1993, **115**, 11022**

***J. Am. Chem. Soc.* 1996, **118**, 1608**

***J. Am. Chem. Soc.* 1996, **118**, 12322**

Bioorg. Med. Chem. Lett.
1997, **7, 1433**

Photochem. Photobiol. **2000, **57**, 51**

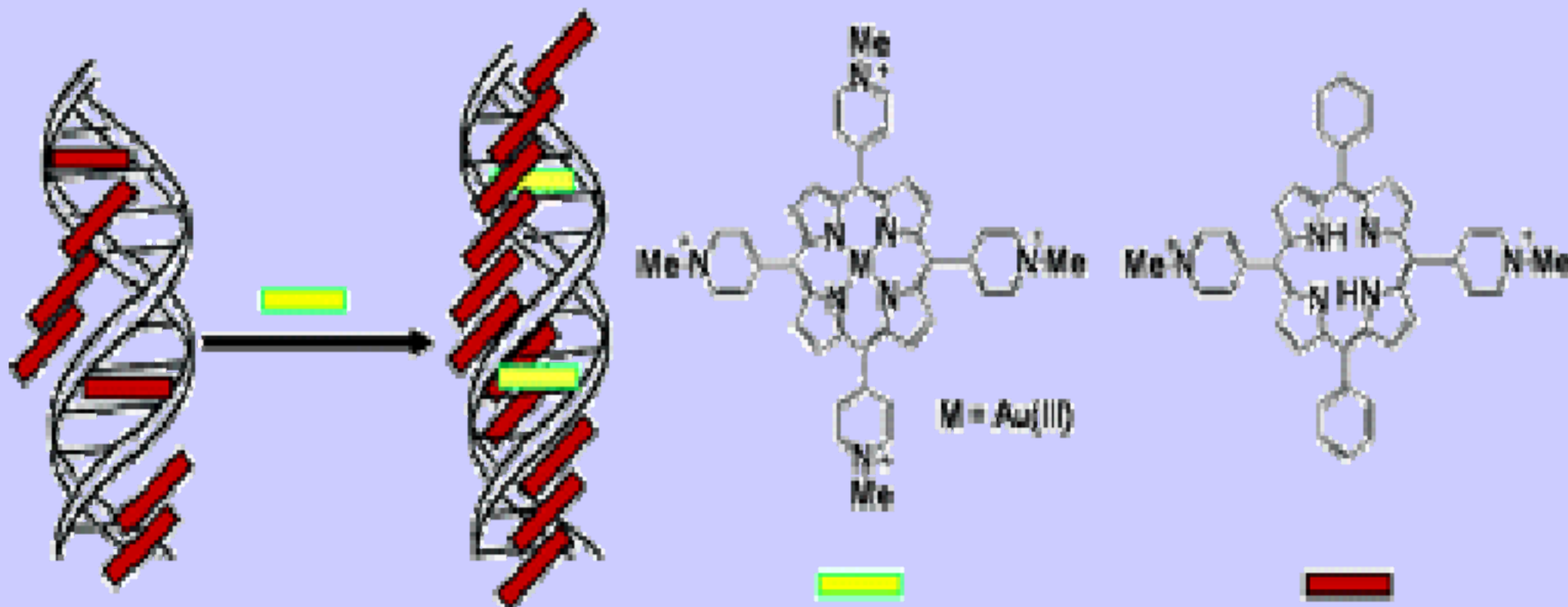
Collect. Czech. Chem. Commun. **65**
2000, **65, 631**

***J. Phys. Chem. B* 2002, **106**, 6784**

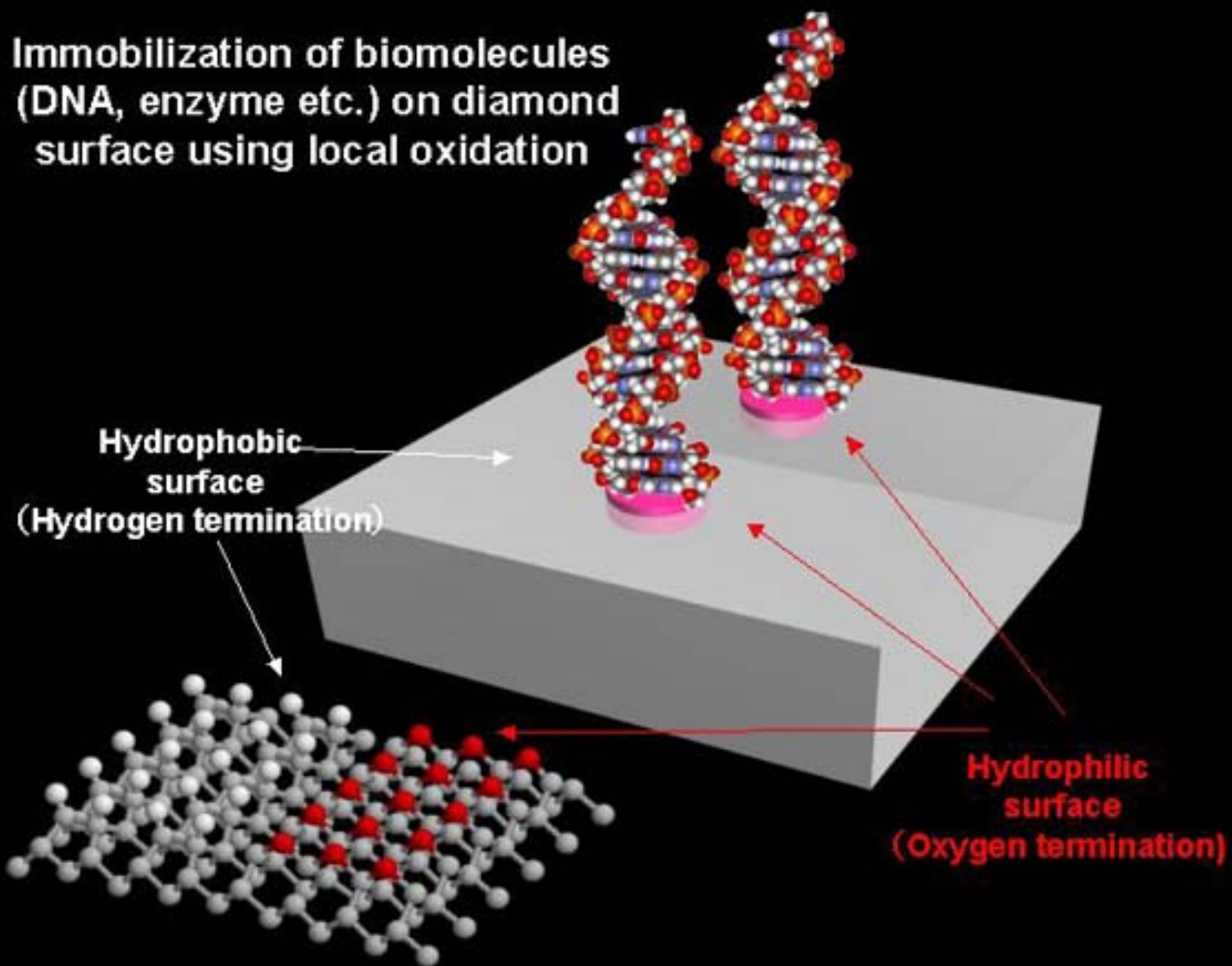
Tetrahedron Letters 2003, **44**, 2083

Tuning Porphyrin/DNA Supramolecular Assemblies by Competitive Binding

J. Am. Chem. Soc.; 2004 ASAP

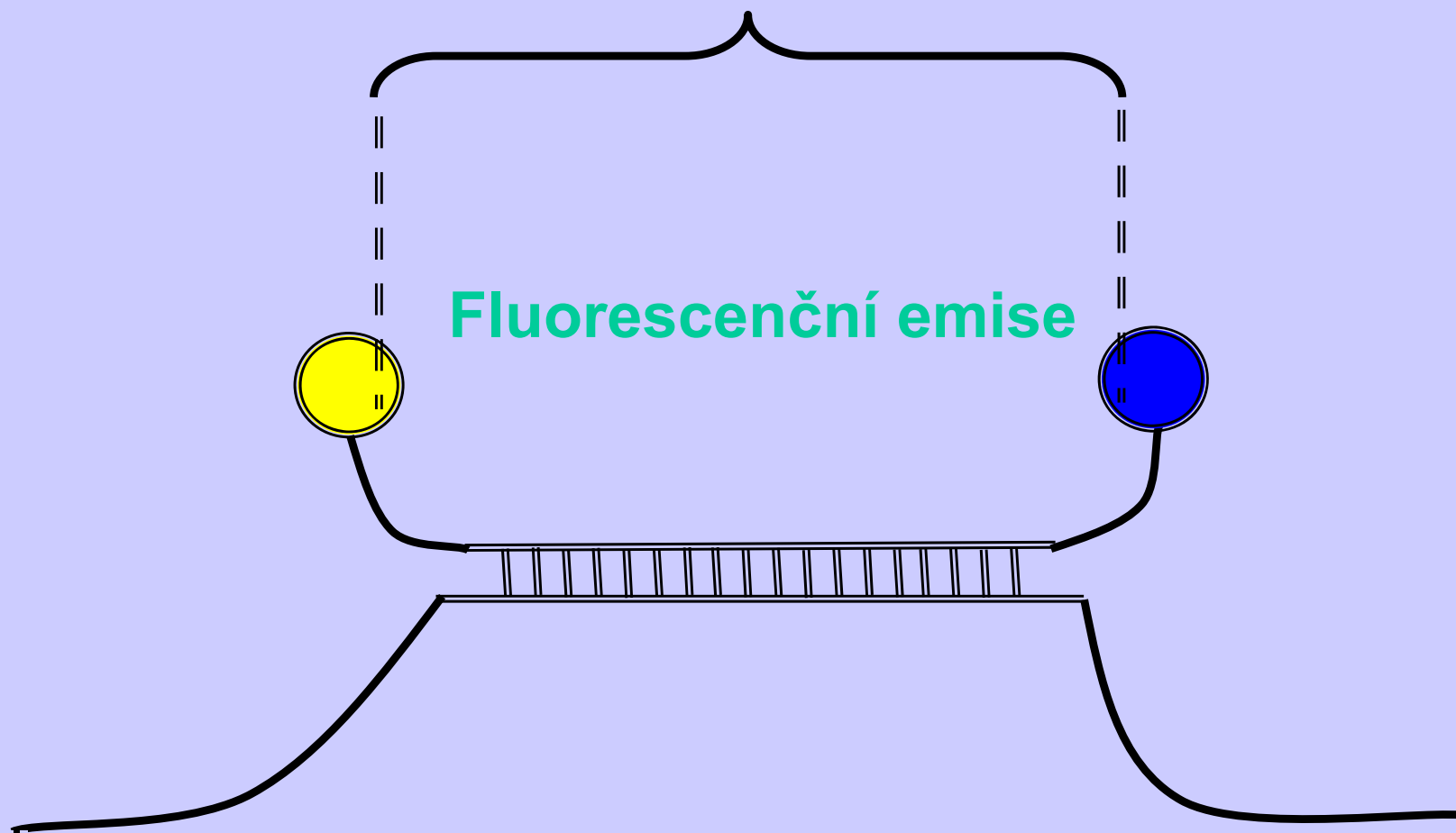


**Immobilization of biomolecules
(DNA, enzyme etc.) on diamond
surface using local oxidation**



**Fig. 1 Immobilization of biomolecules (DNA, enzyme etc.)
on diamond surface using local oxidation**

Molekulární optické značení

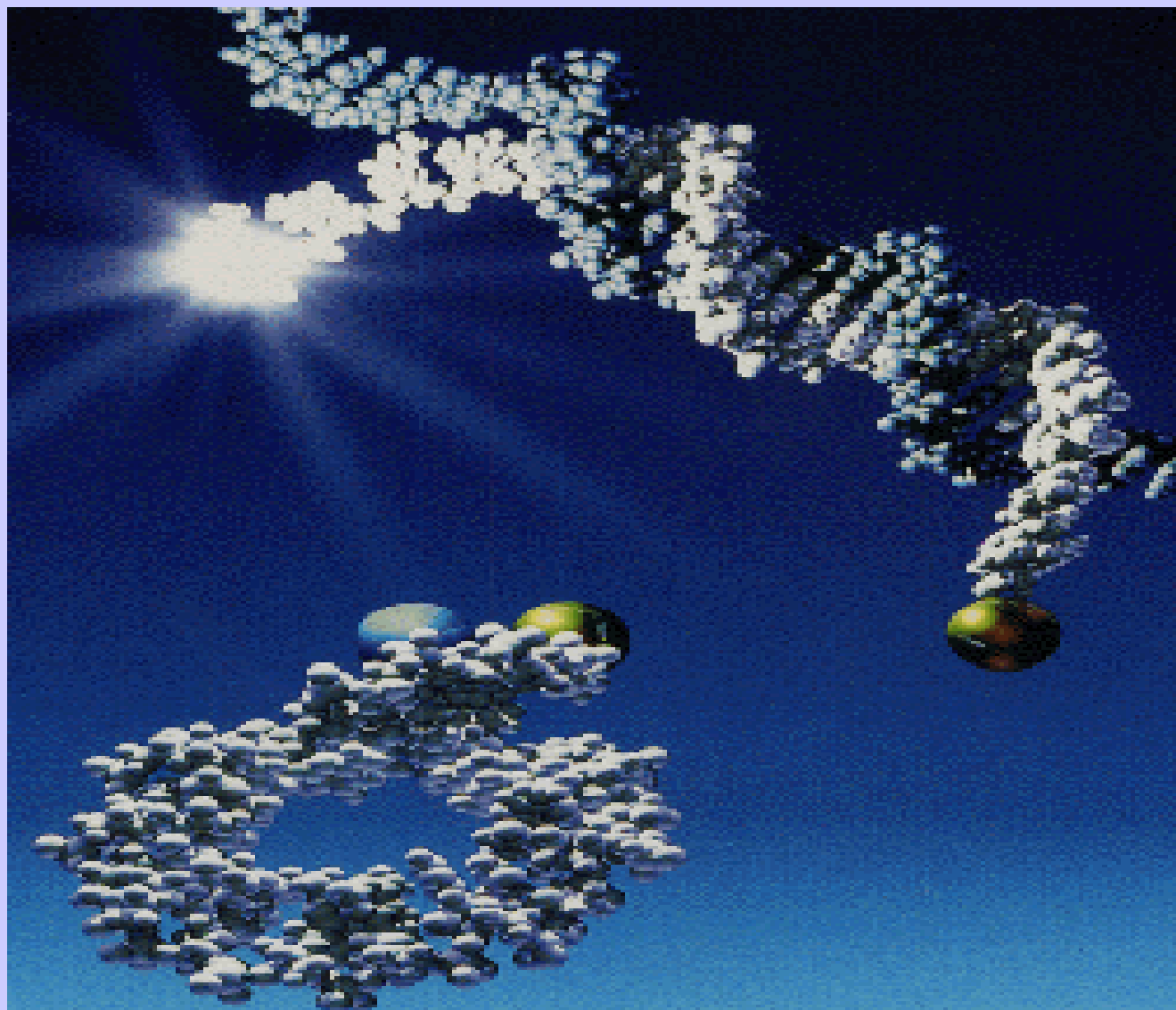


Universální Biosensor na sekvenci NK s nanomolárním-pmol detekčním limitem

Analytical Chemistry A 2004

- kvantitativní biosensor, ODN hybridizace specifické DNA sekvence**
- vysoce rychlý, citlivý a selektivní biosensor (1nM)**
- cíl subpmol detekční limit (bez PCR)**

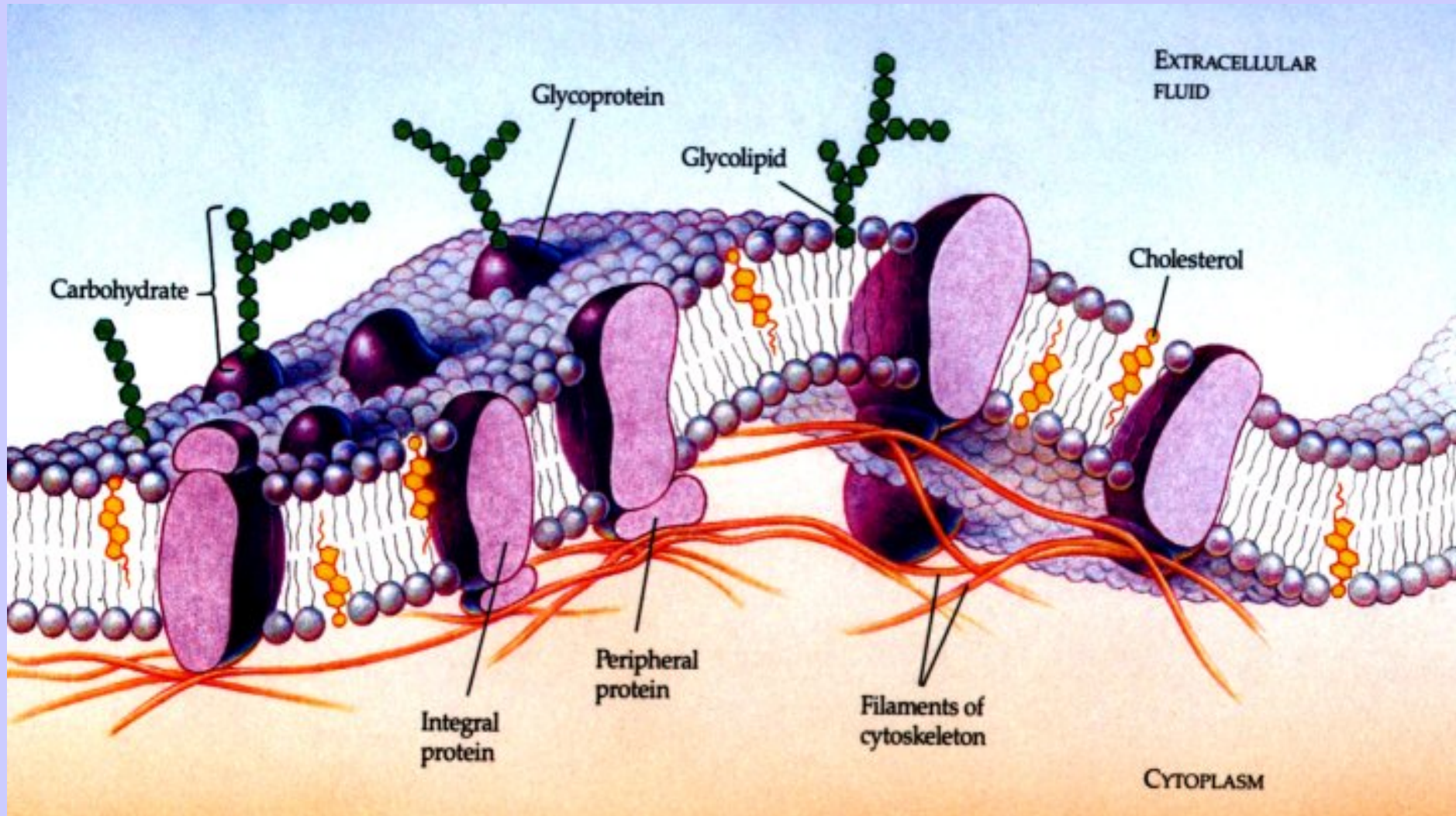
DNA microarray techniky - Jak fungují?



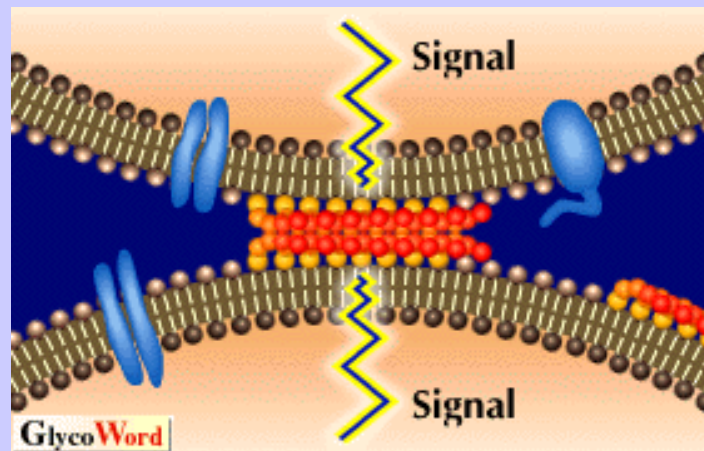
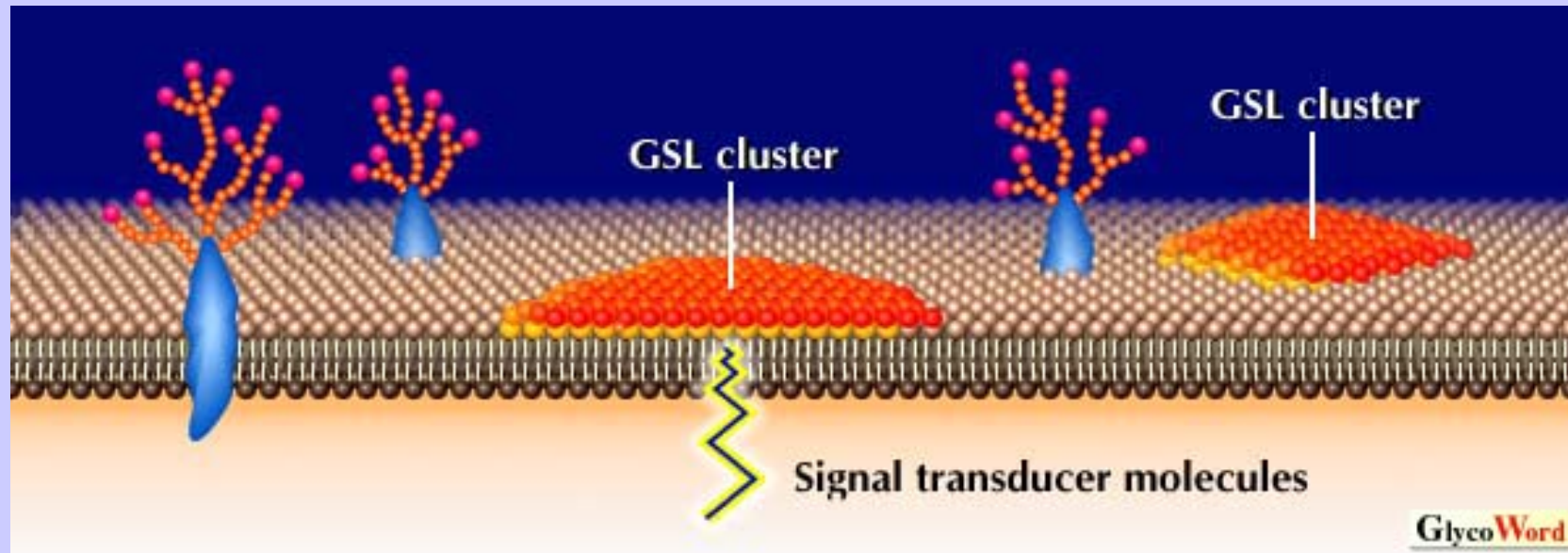
SUPRAMOLECULAR CHEMISTRY AND ANALYTICAL APPLICATION OF FLUORESCENT RECEPTORS FOR SACCHARIDE RECOGNITION



Cell recognition based on oligosaccharide binding

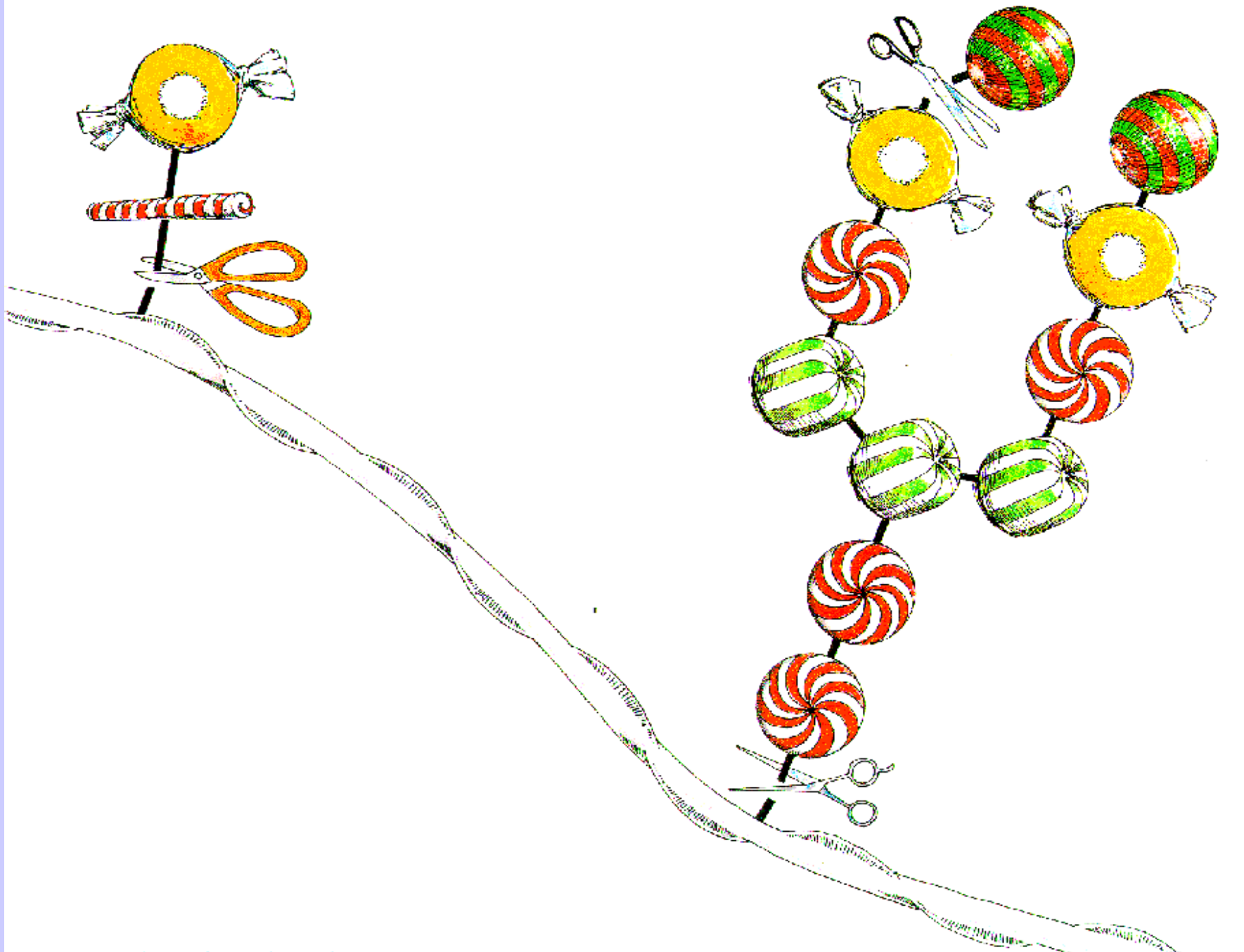


Cell-Cell recognition and communication

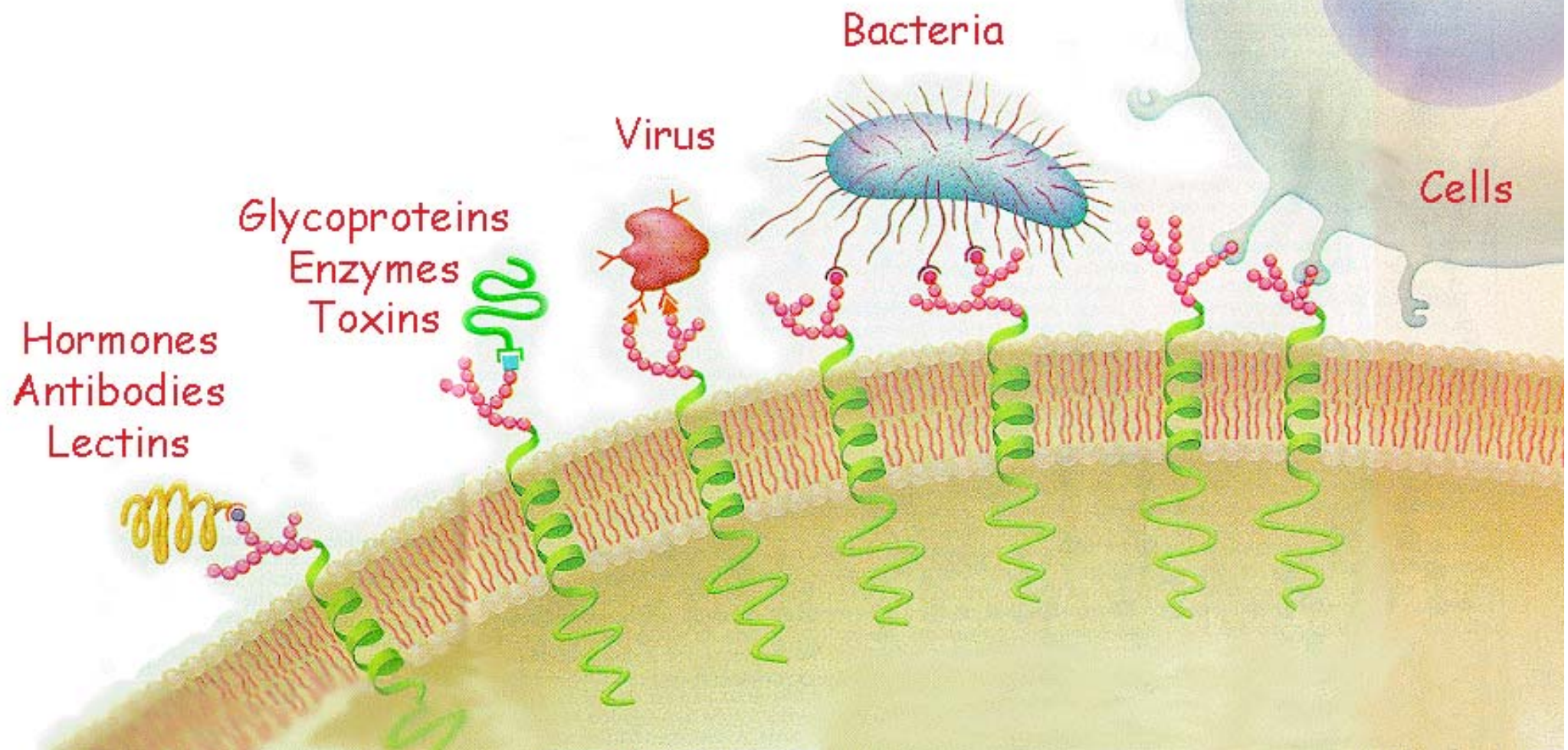


Glykosfingolipids reach regions allow cell recognition and information exchange

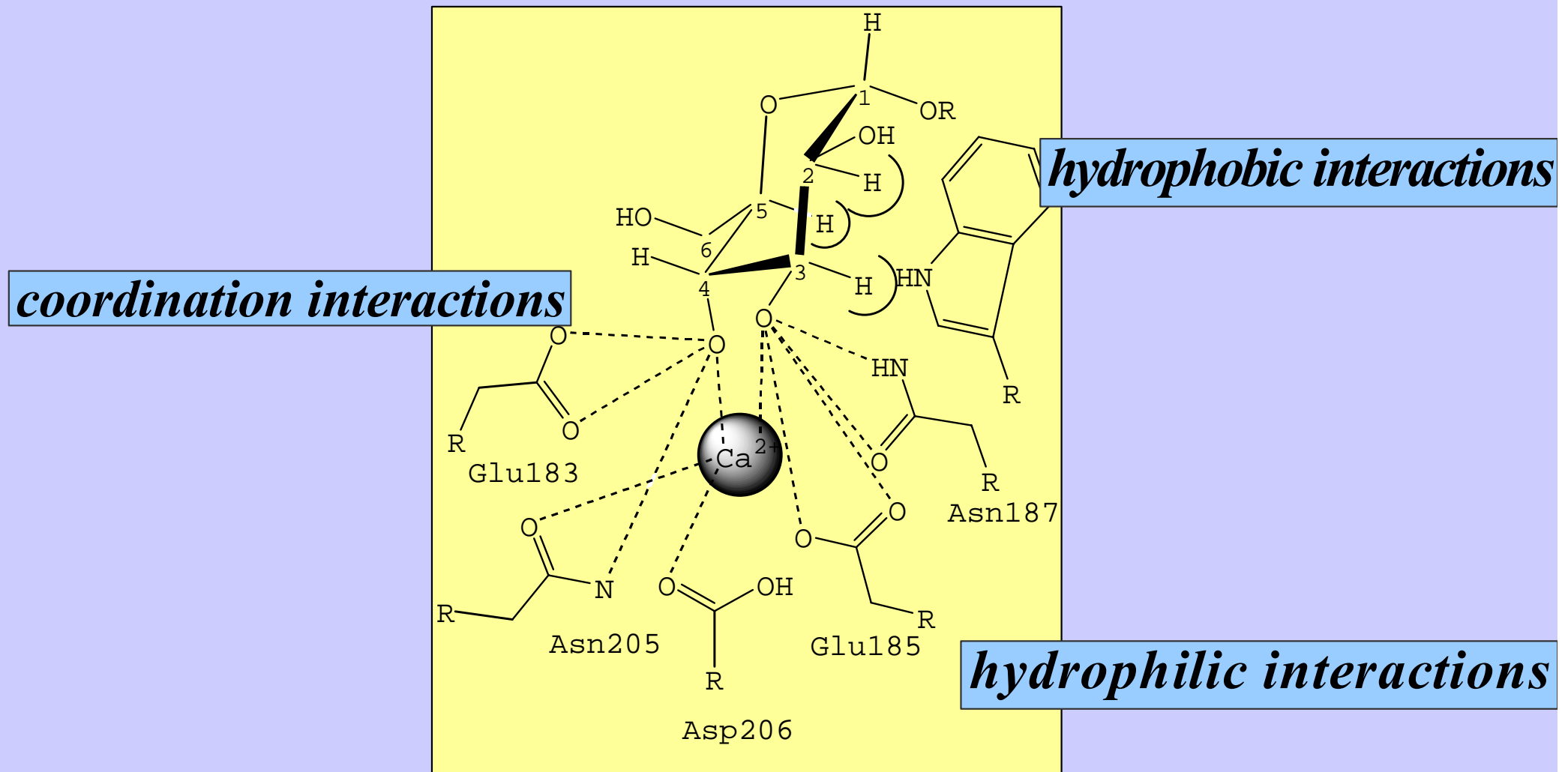
Oligosaccharides : The nice view...



Carbohydrate interactions on the cell surface

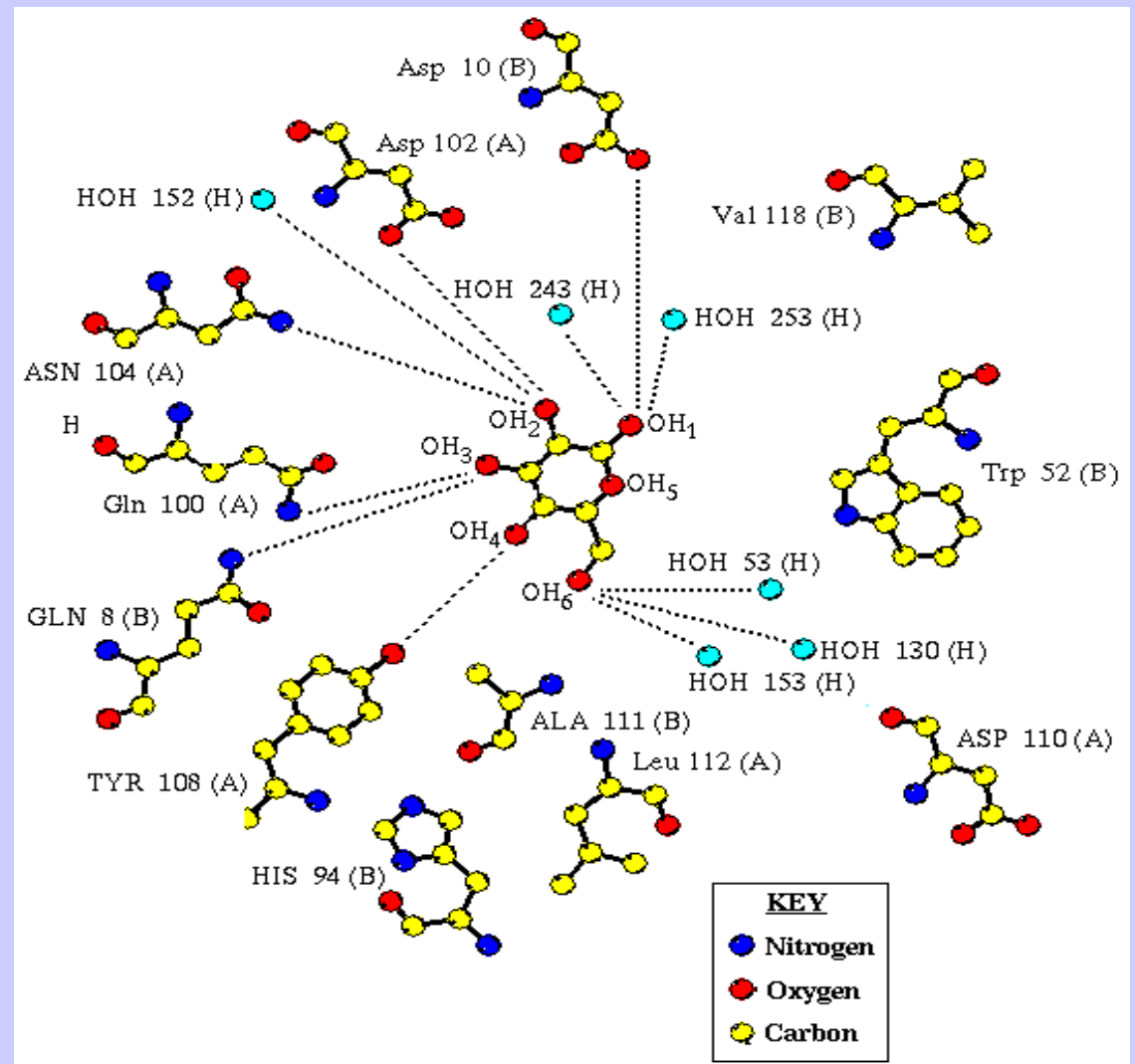
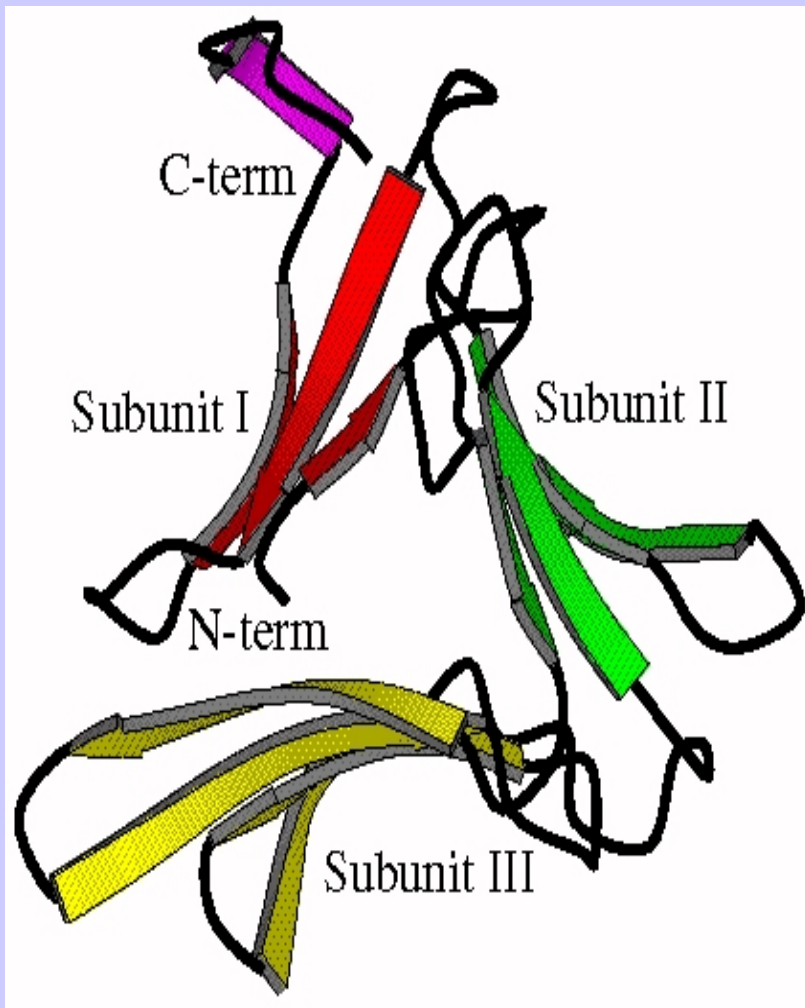


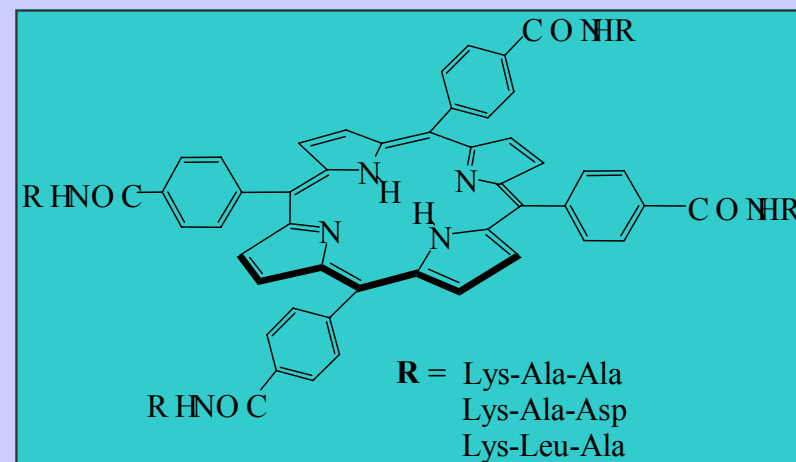
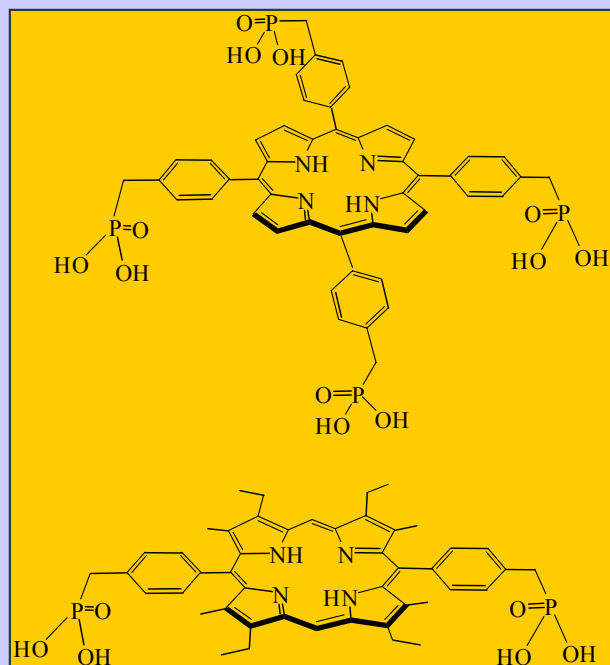
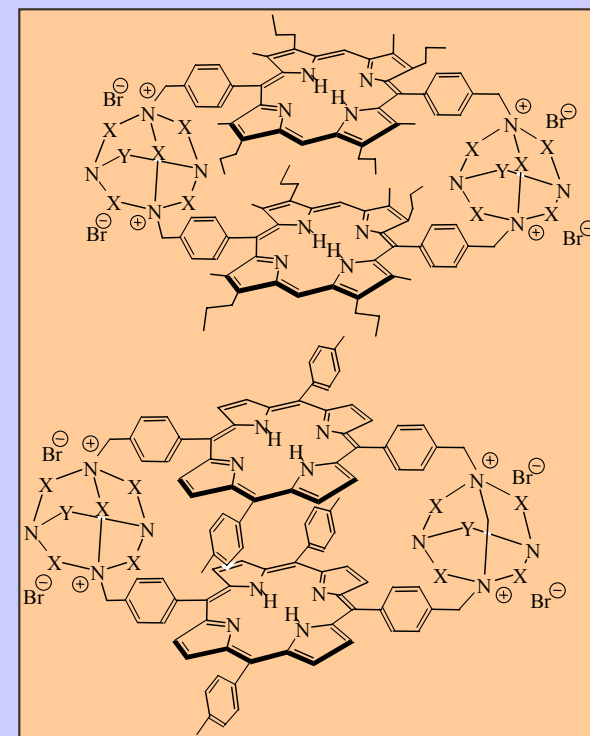
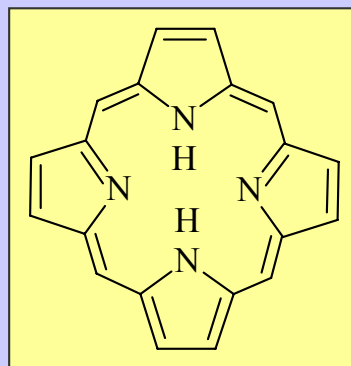
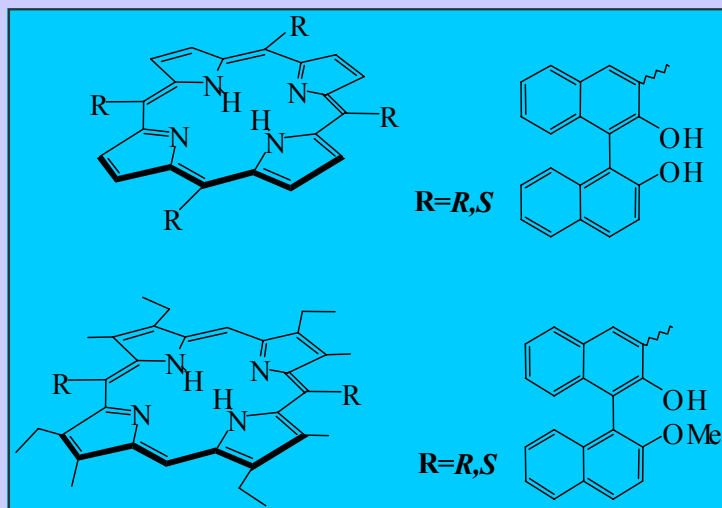
BINDING MODES IN LECTIN-SACCHARIDE COMPLEX



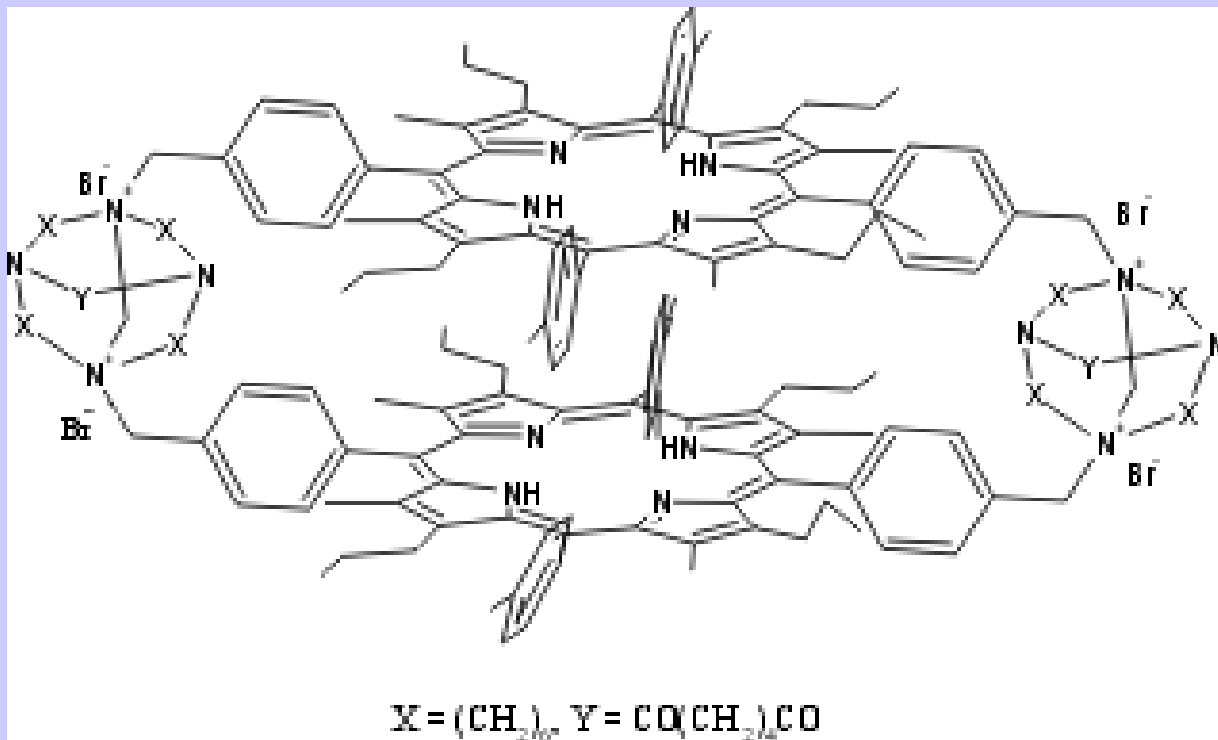
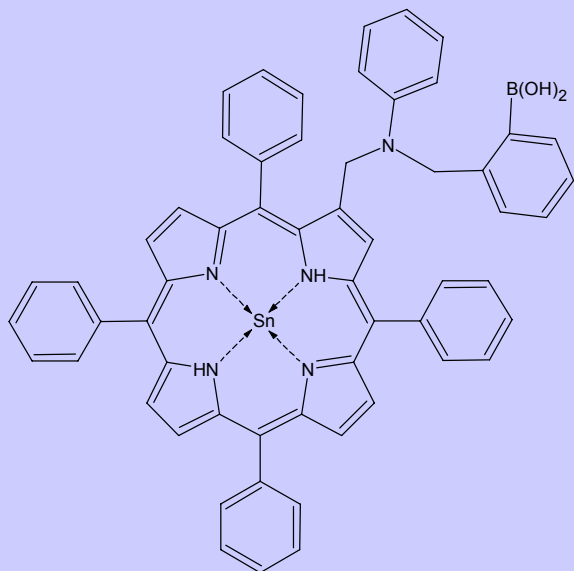
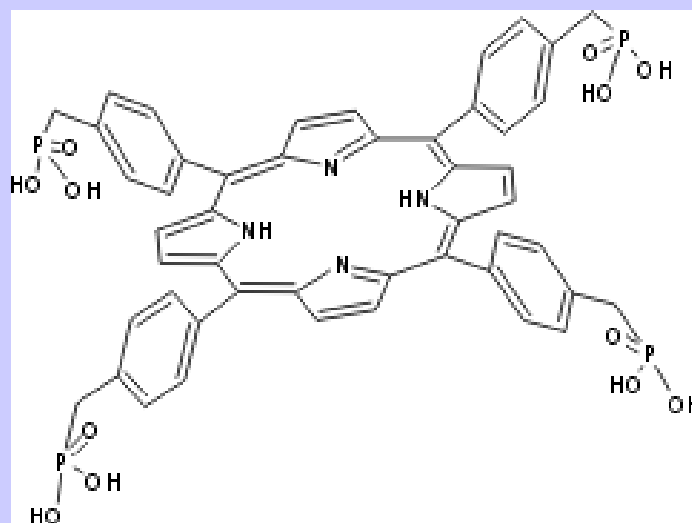
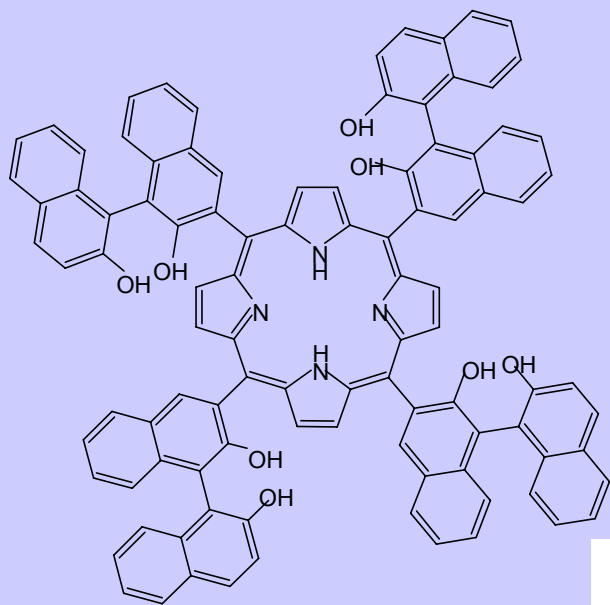
Adapted from: R. U. Lemieux, F. R. S., *Chem. Soc. Rev.* 1989, 18, 347.

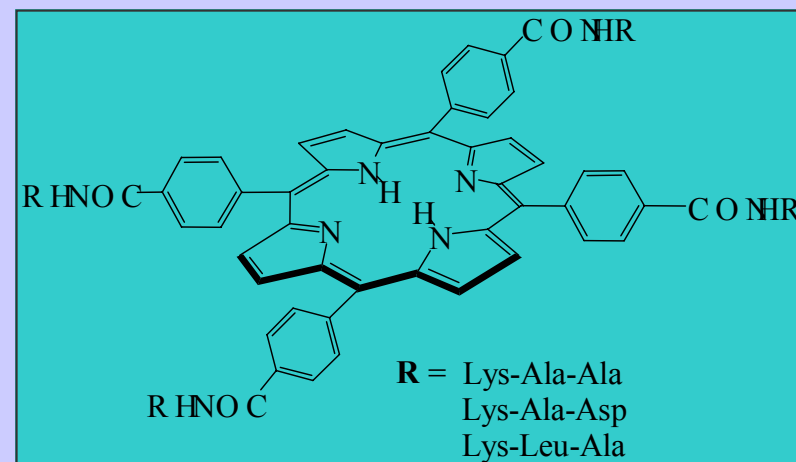
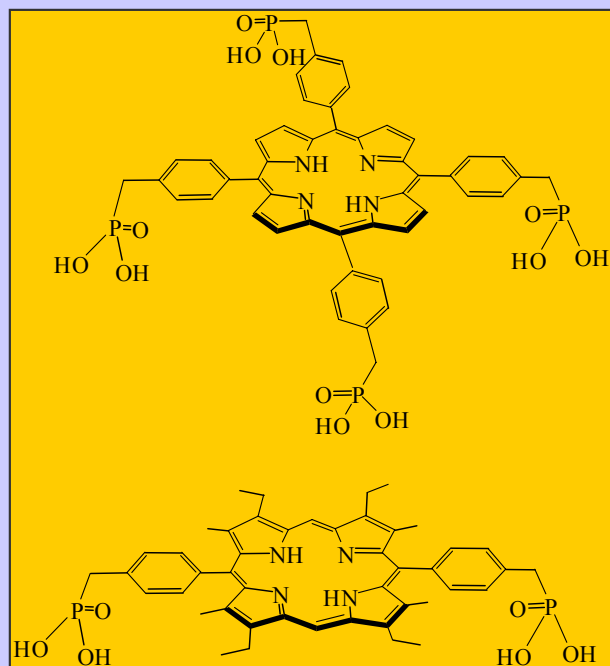
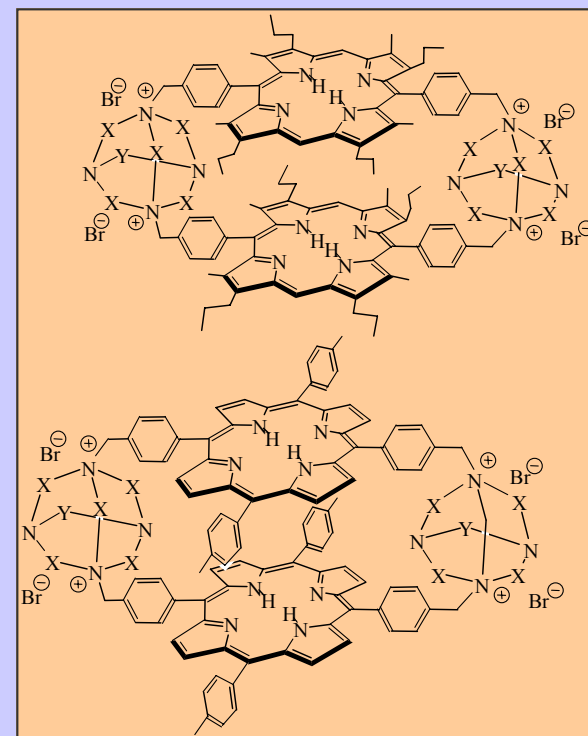
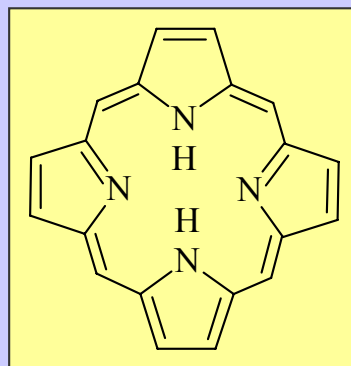
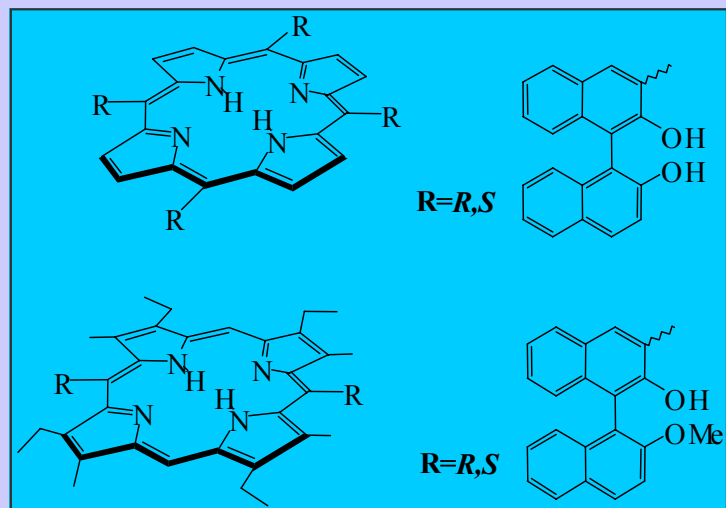
Natural saccharide receptors





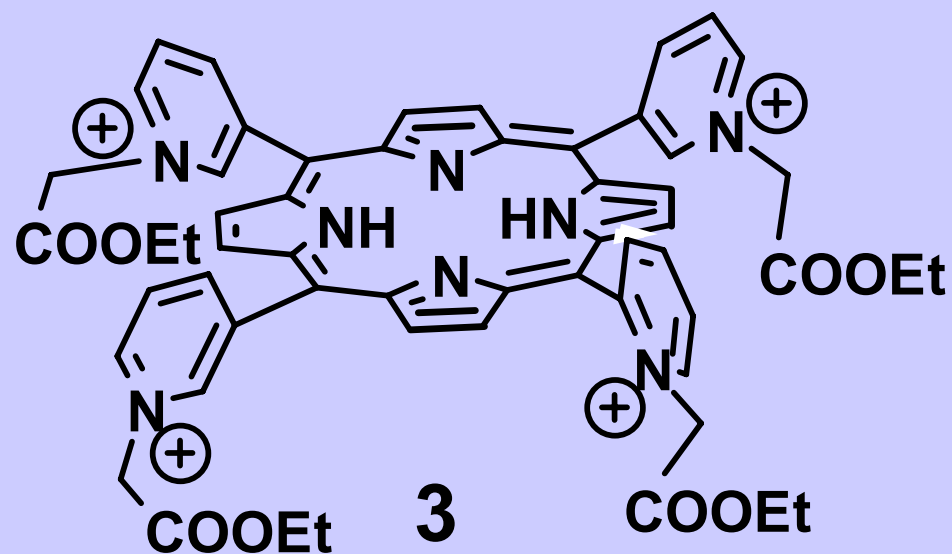
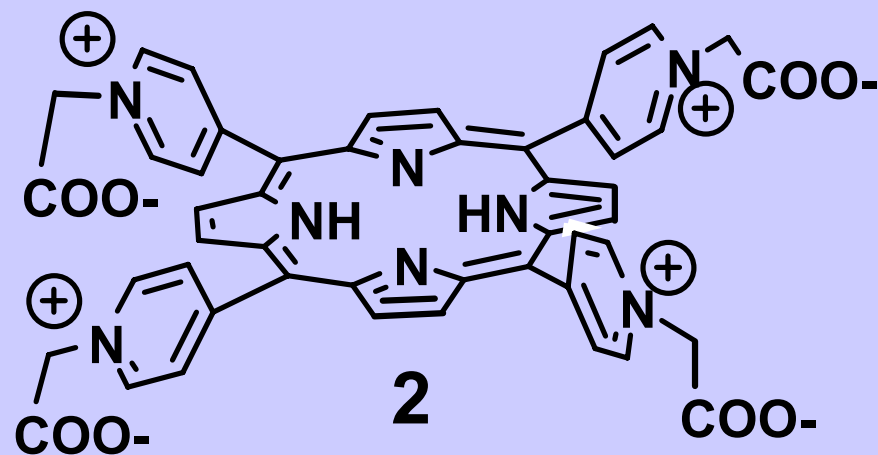
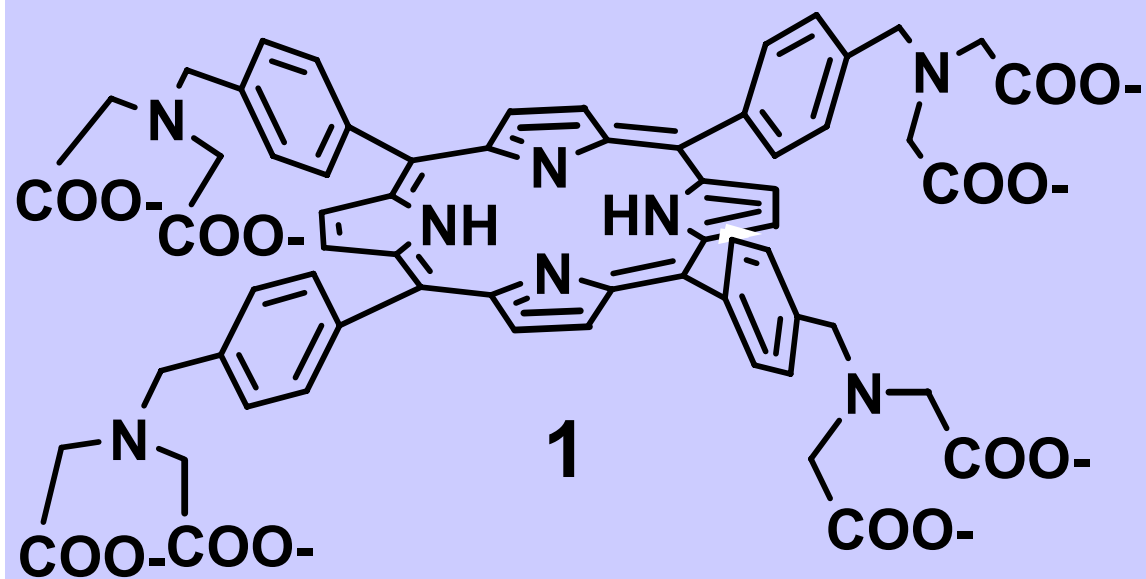
Syntetické sacharidové receptory



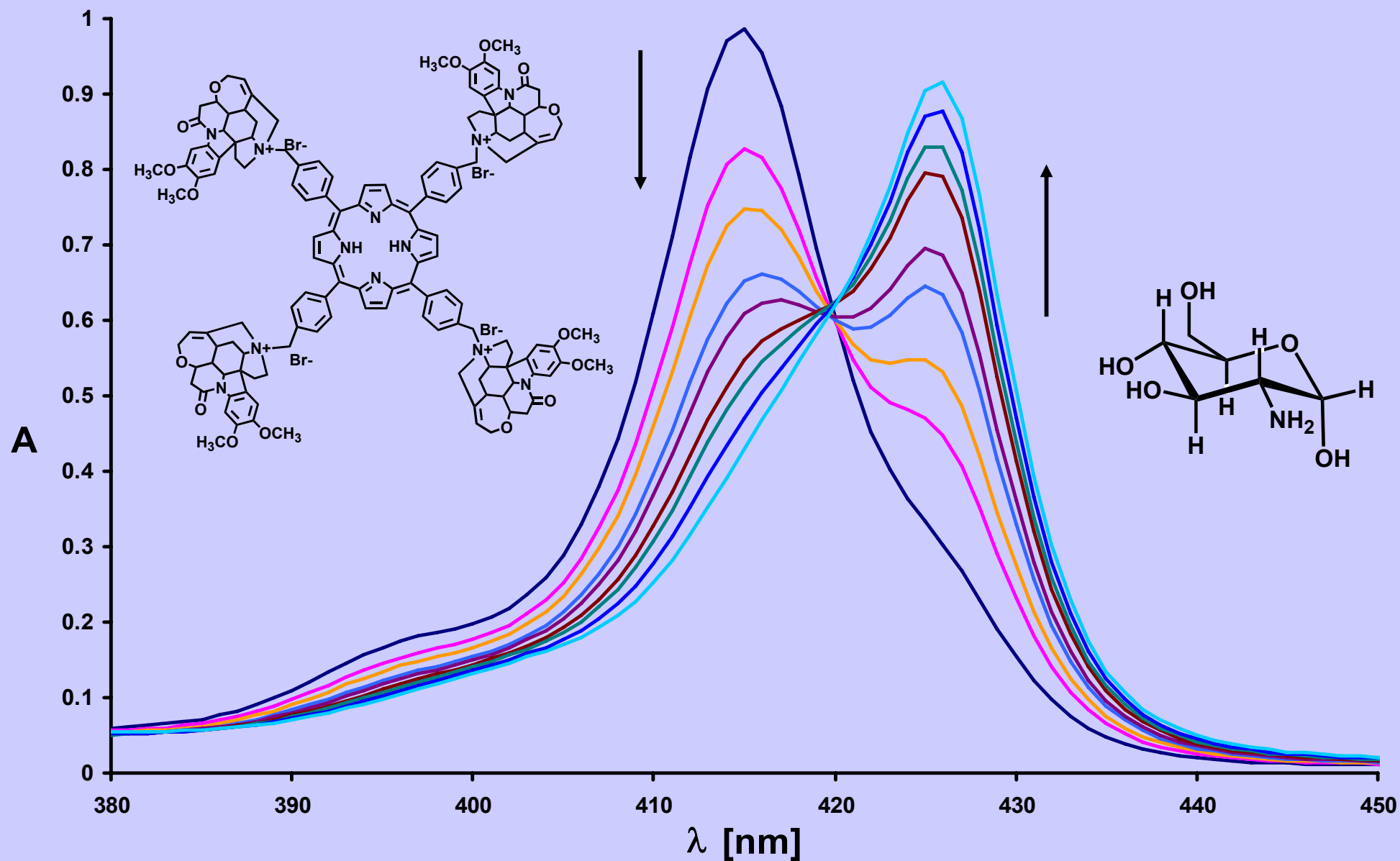


Chemistry Eur. J., 8 (3) 655 – 663, 2002

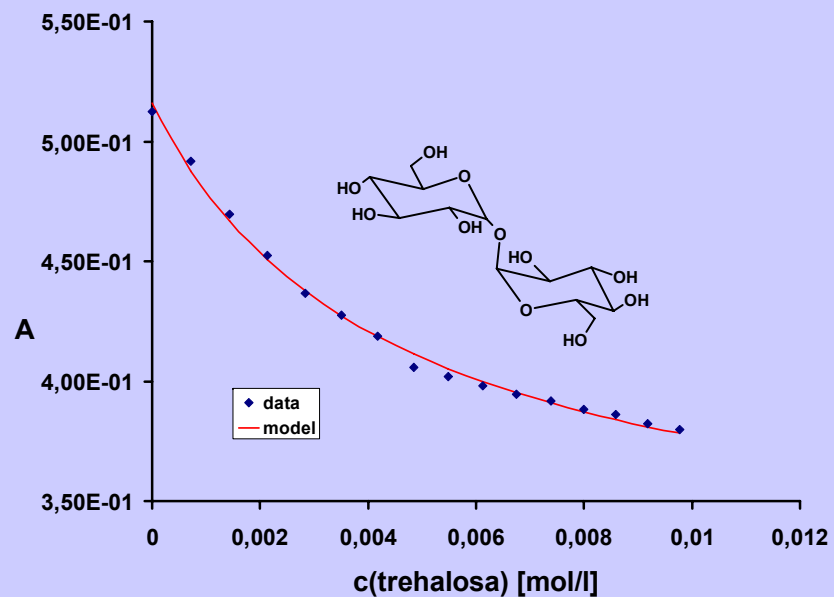
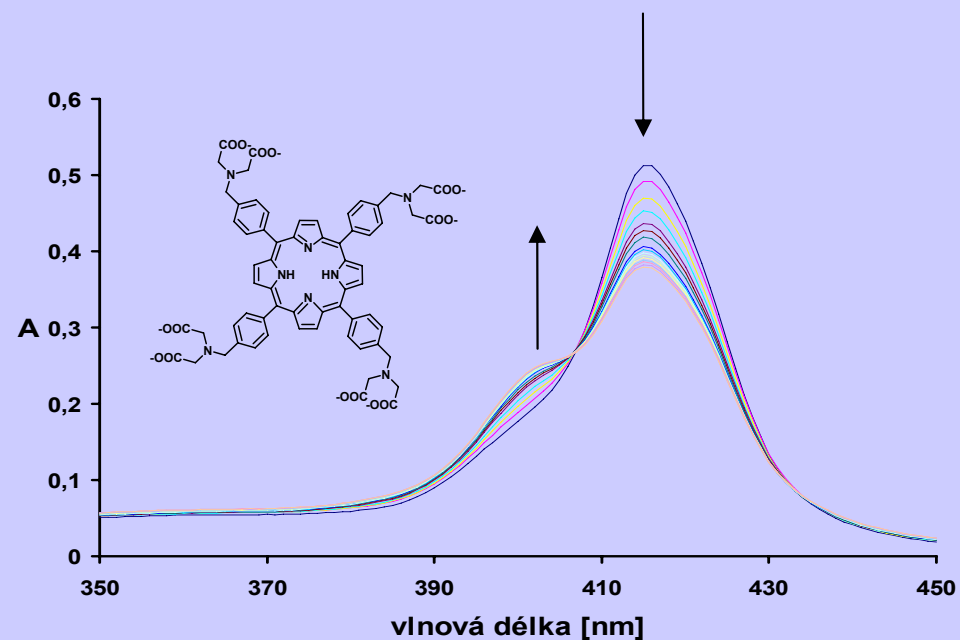
Novel porphyrin receptors



Interaction of tetrabrucine porphyrin derivative with glucosamine

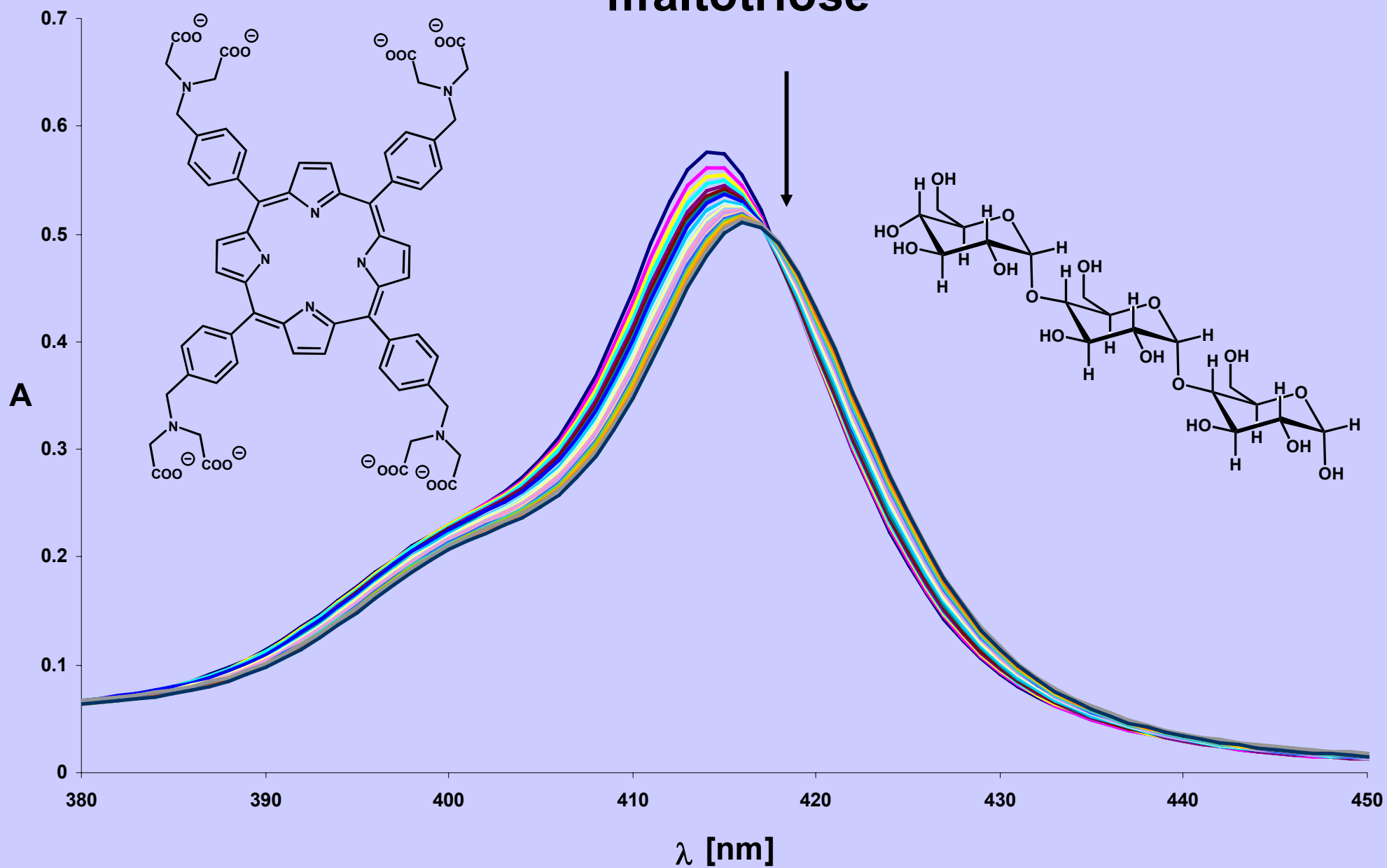


Interaction with neutral saccharides

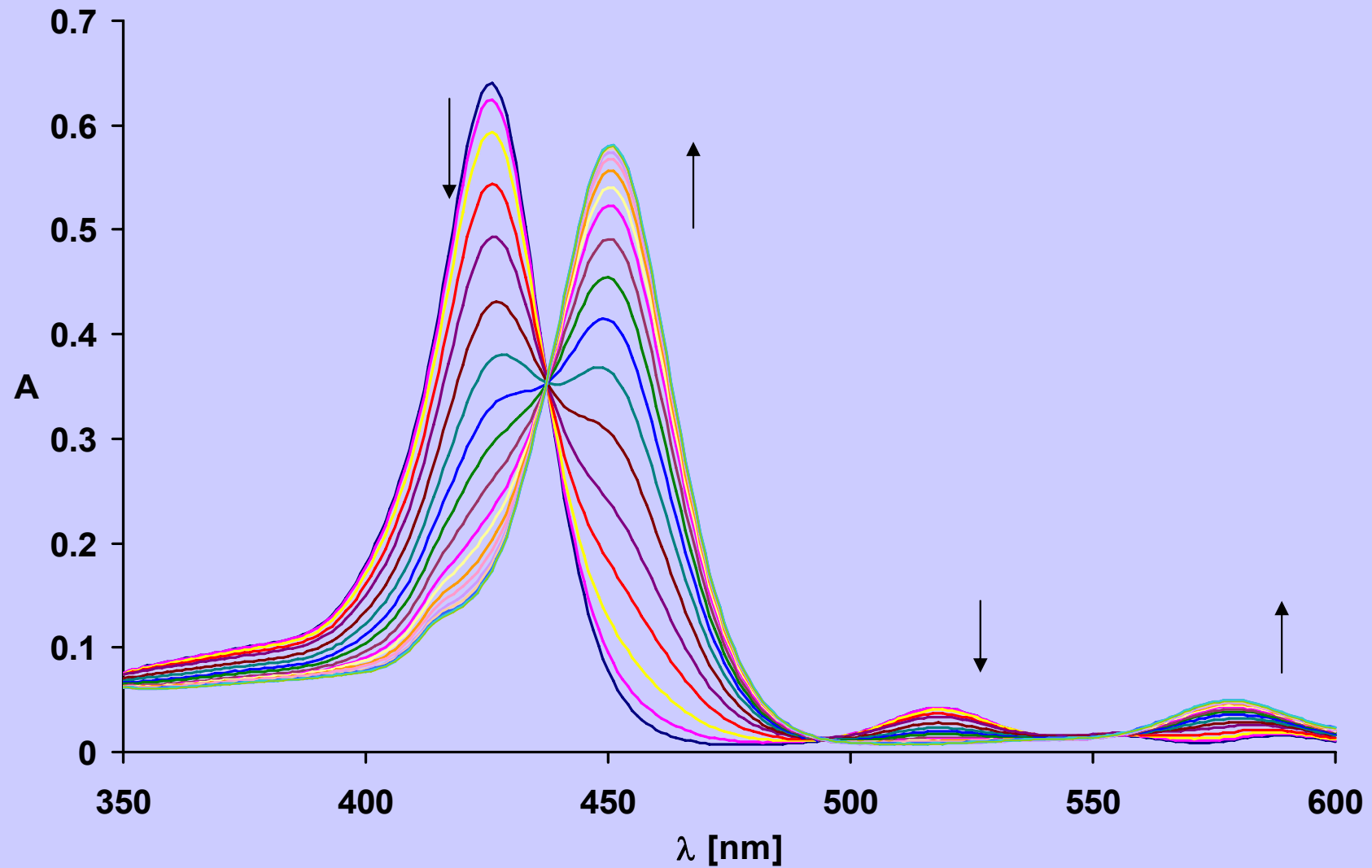


saccharide	logK
ribose	1,4
fructose	2,2
glucose	2,0
galactose	1,6
mannose	1,6
lactose	2,5
maltose	2,4
trehalose	2,4
maltotriose	1,9

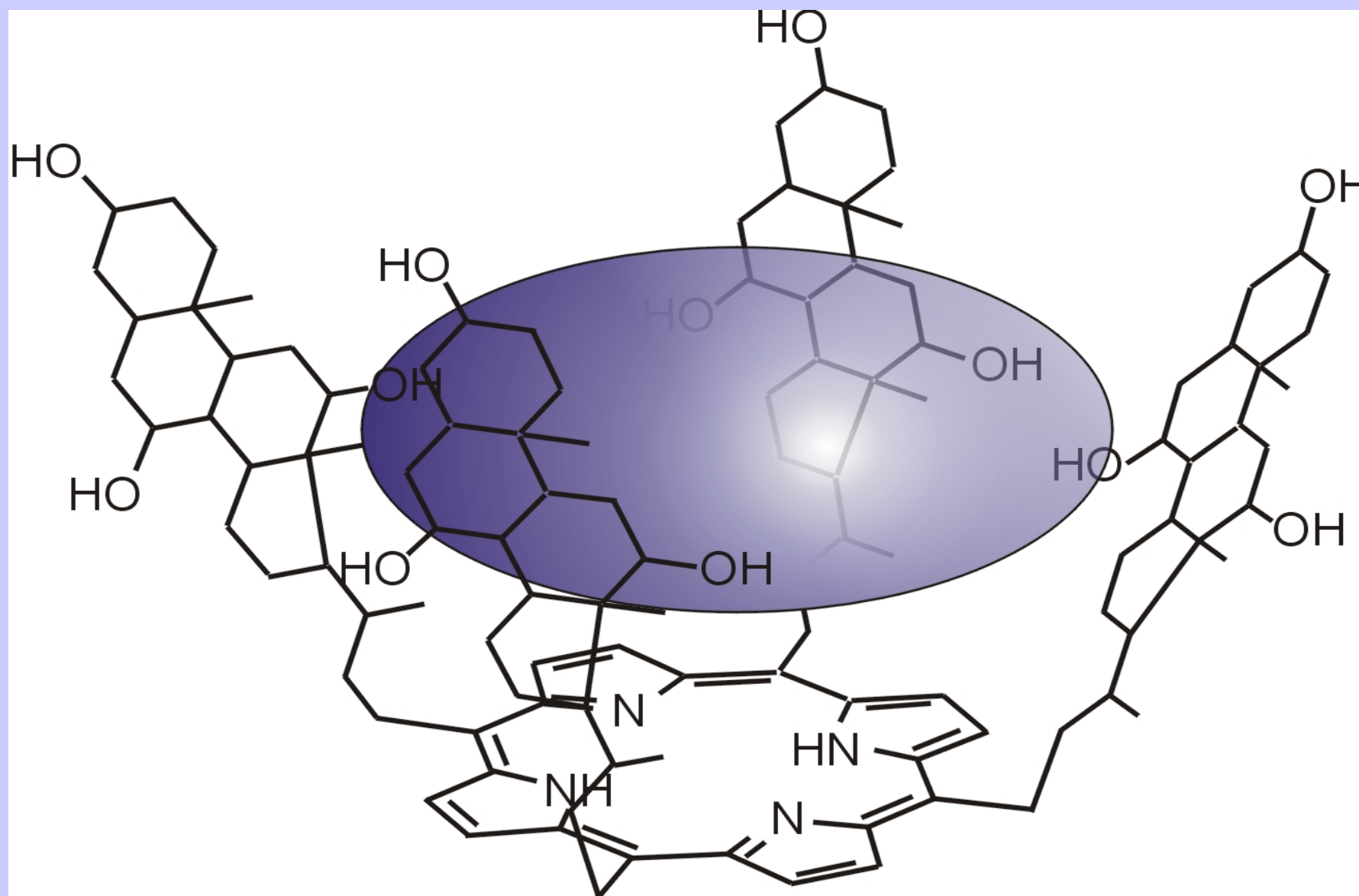
Interaction of porphyrin octacarboxylate with maltotriose



Interaction of octacarboxylated porphyrin with maltotriose and Cd(2+)

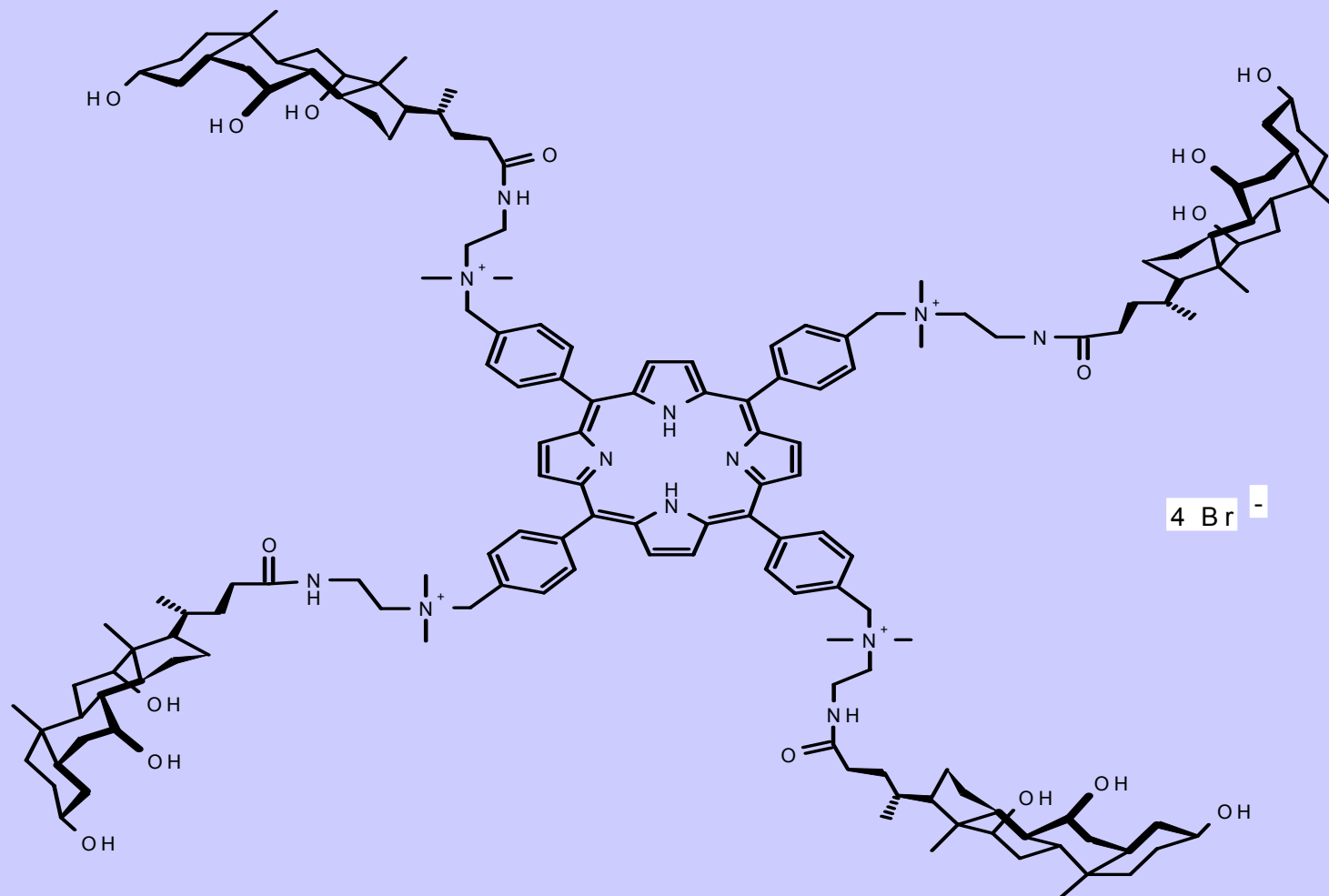


Steroid-porphyrin conjugate for saccharide sensing in protic media



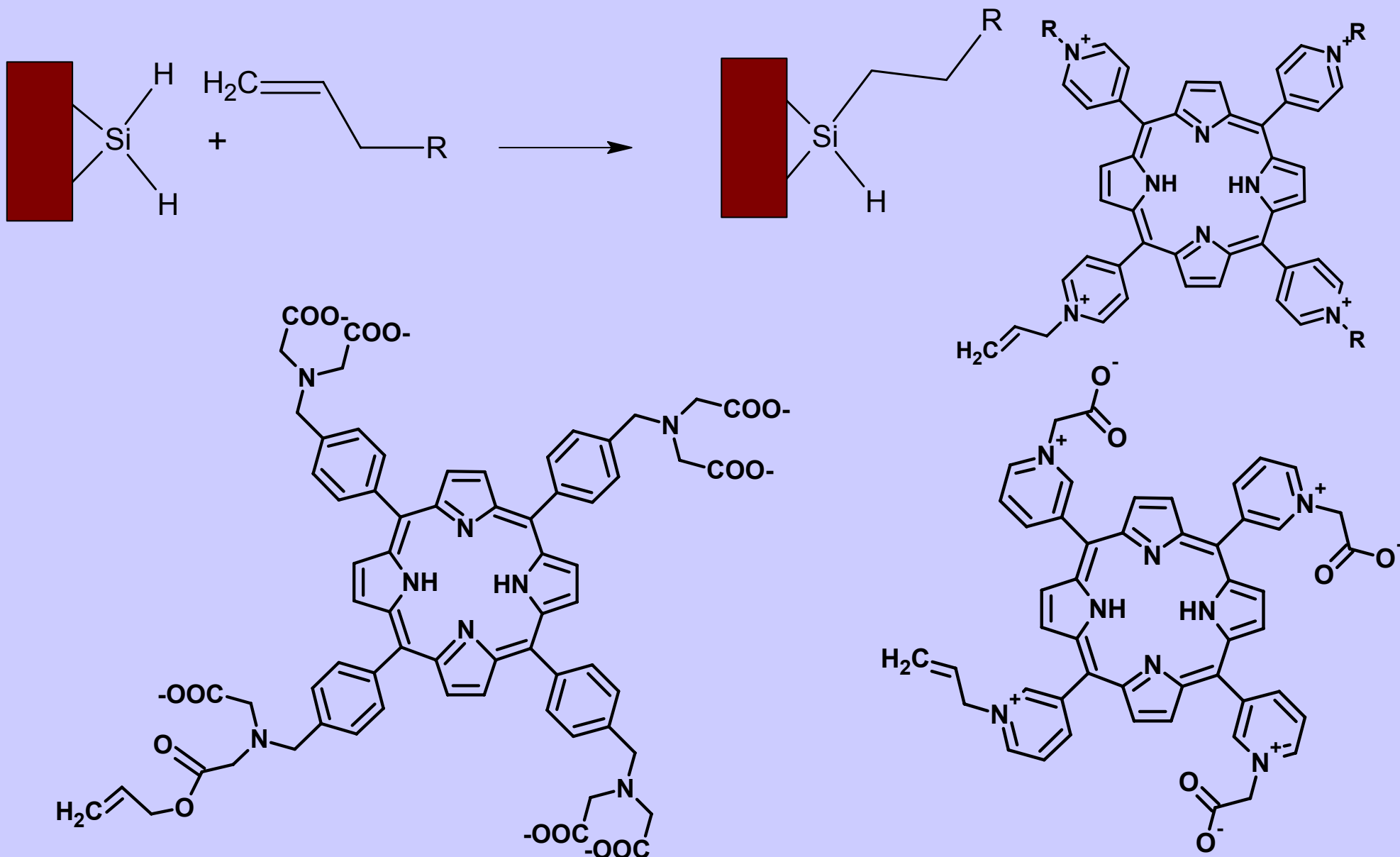
Org. Biomol. Chem., 2003, 1, 3458–3463

SYNTHESIS AND SPECTRAL CHARACTERIZATION OF A NOVEL QUATERNARY ALKYL AMMONIUM PORPHYRIN CHOLIC ACID AMIDE

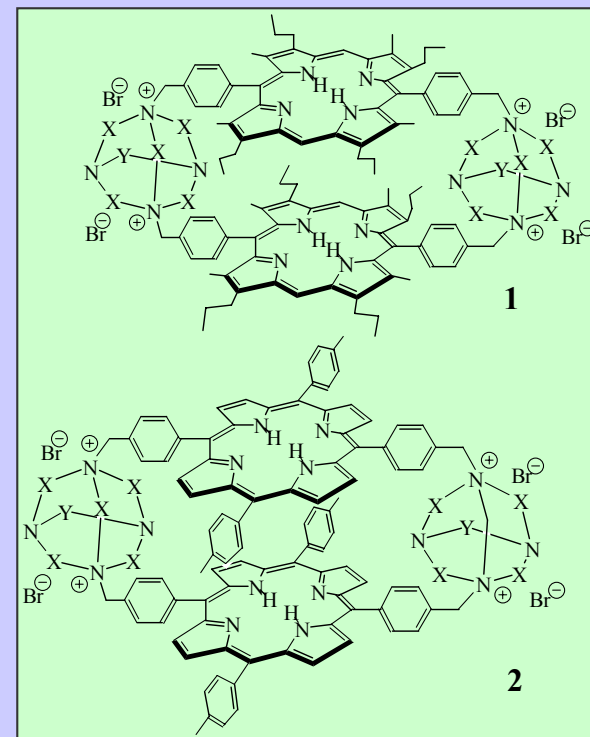
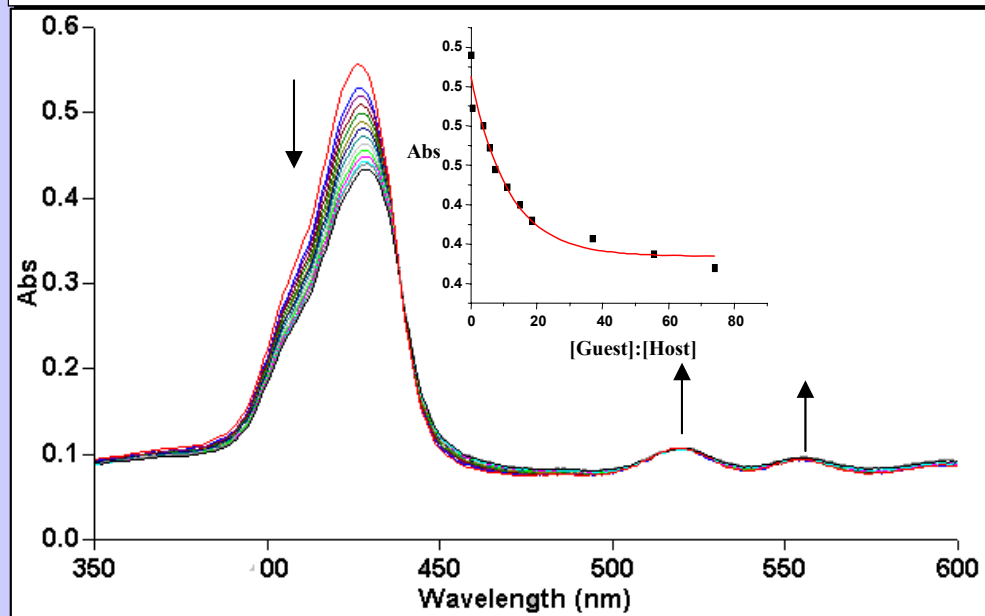
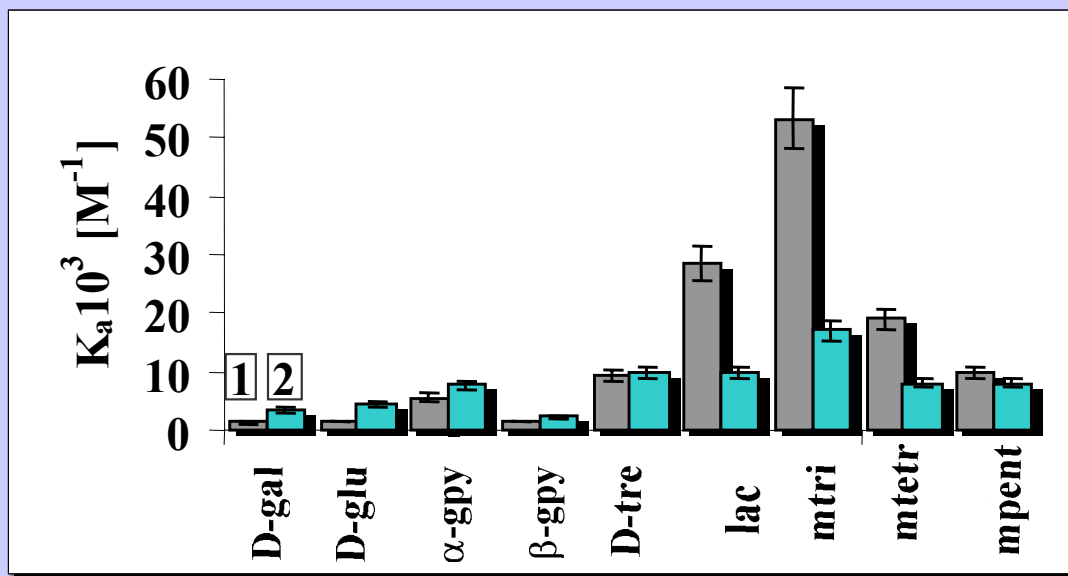


ERKKI KOLEHMAINEN, JUHA KOIVUKORPI and VLADIMIR KRAL

Modification of receptors and their immobilization on the PS surface

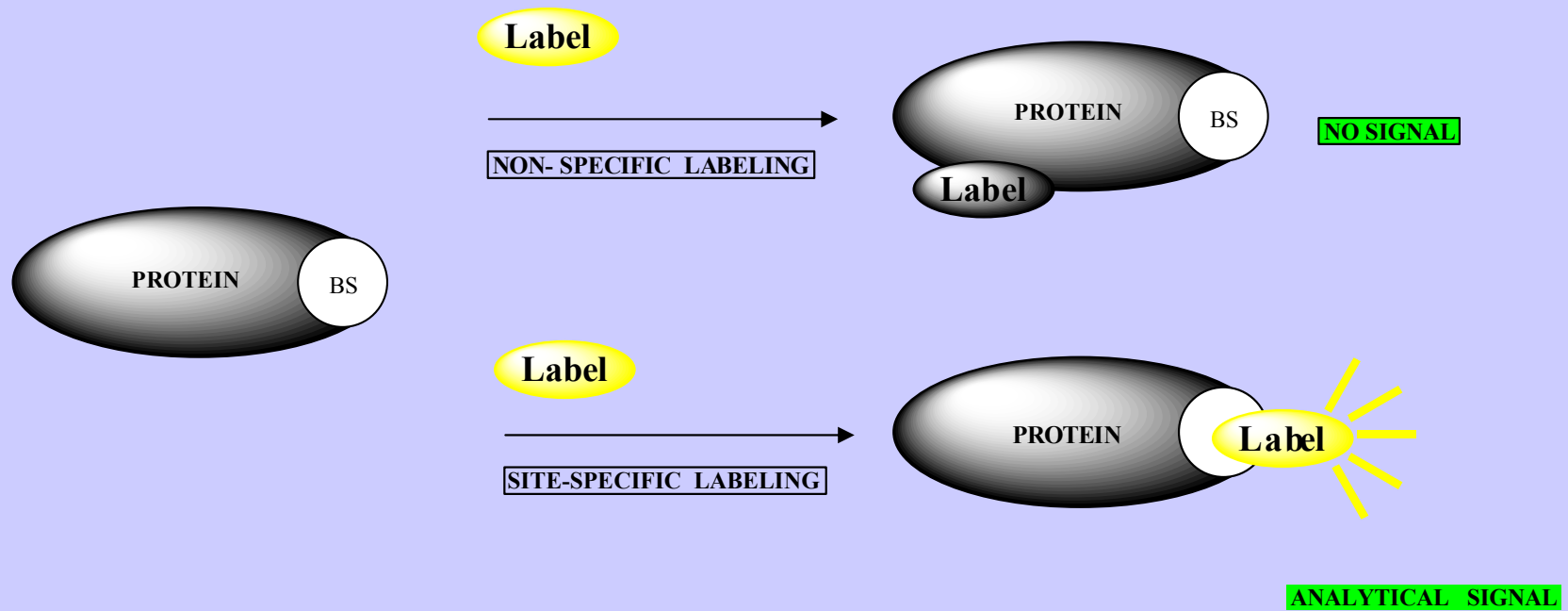


INTERACTION OF CYCLOPORPHYRINS WITH SACCHARIDES II

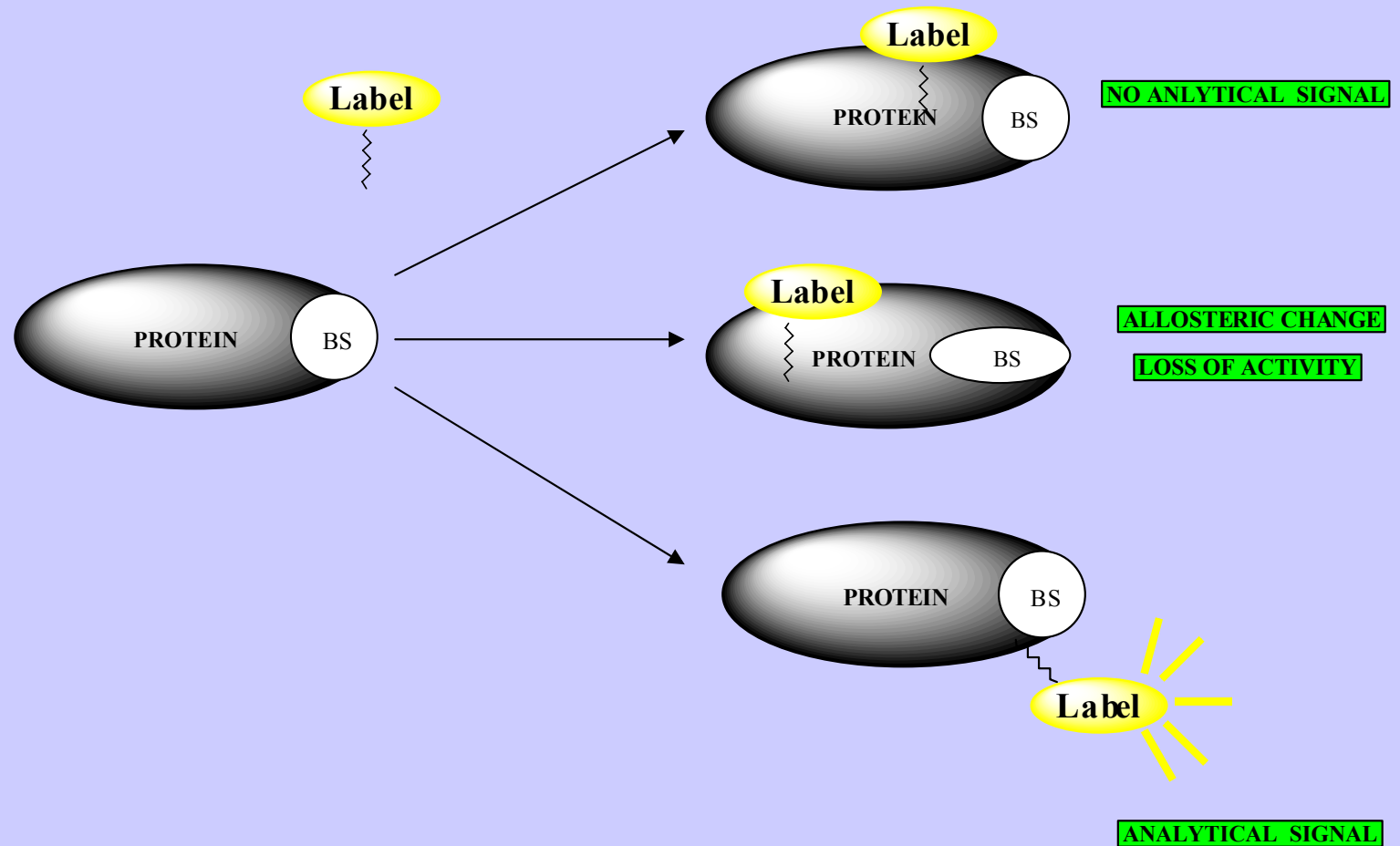


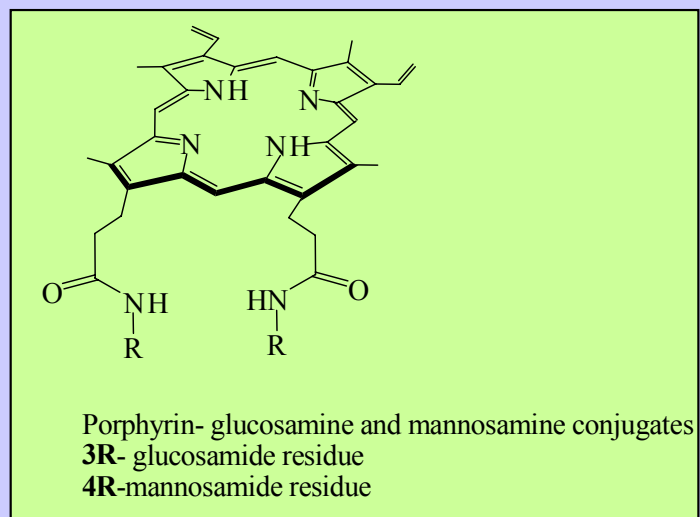
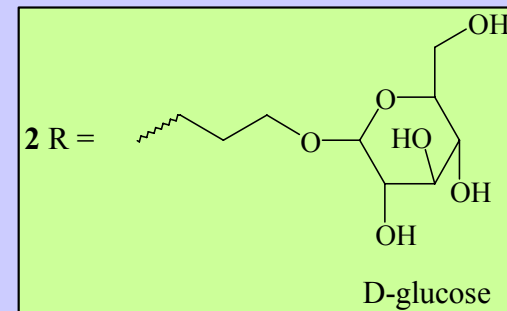
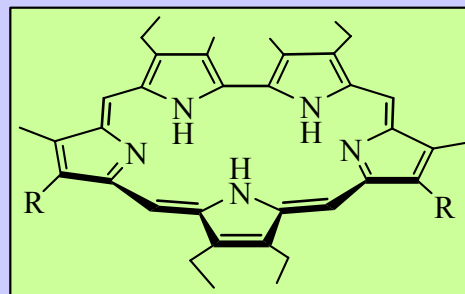
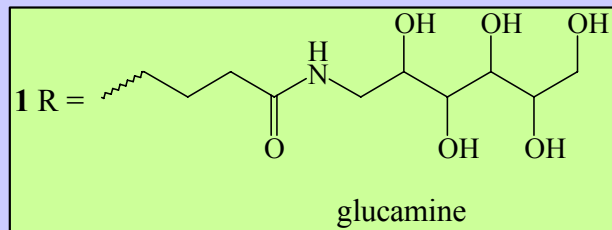
V. Král, O. Rusin, F. P. Schmidtchen,
Org. Letters, 2001, 3, 873.

SCHEMATIC REPRESENTATION OF NON-COVALENT PROTEIN LABELING

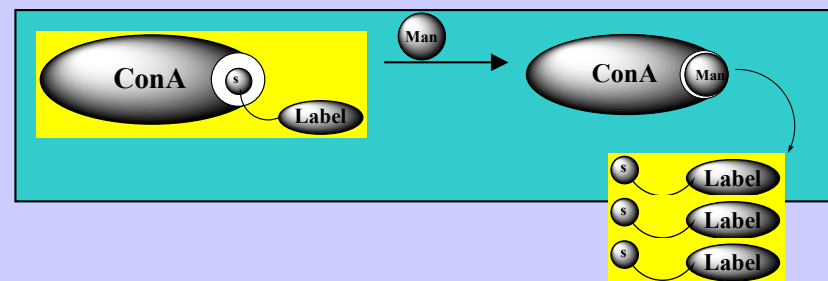
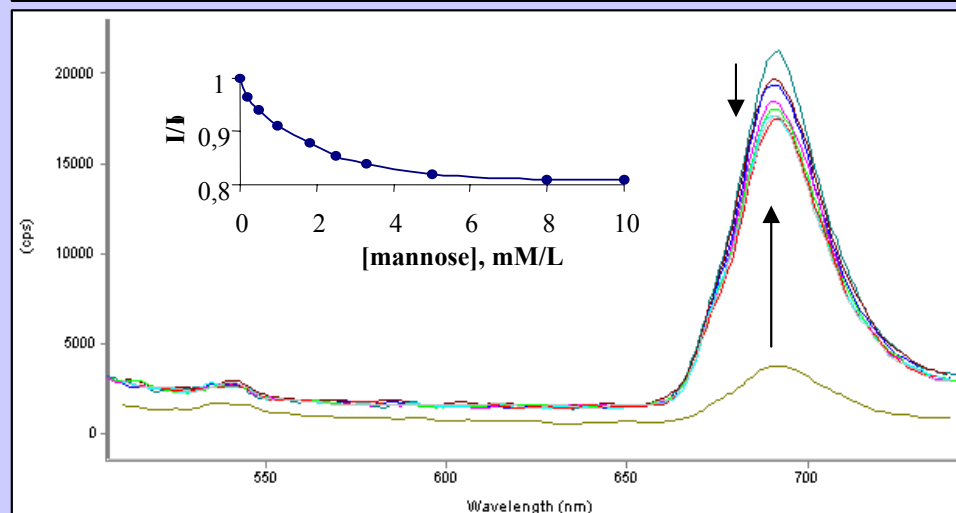
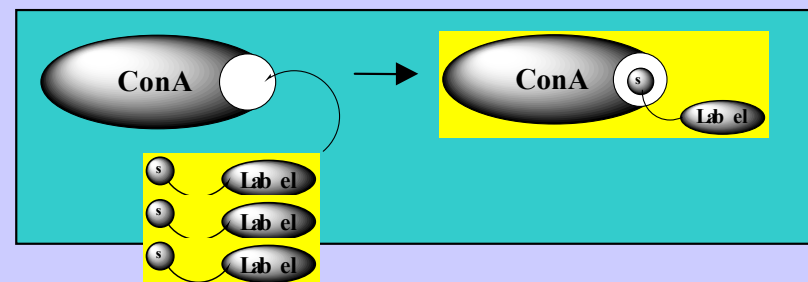
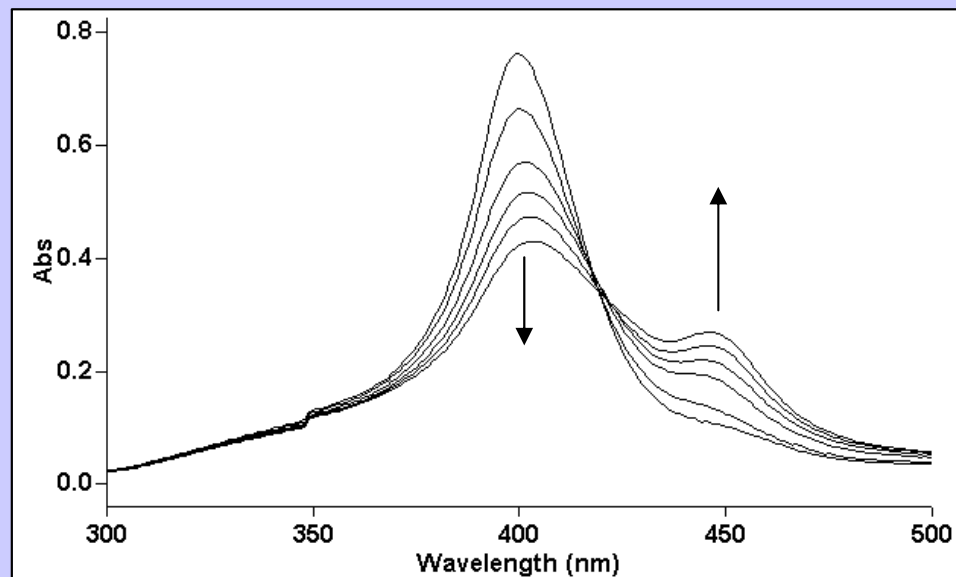


SCHEMATIC REPRESENTATION OF COVALENT PROTEIN LABELING





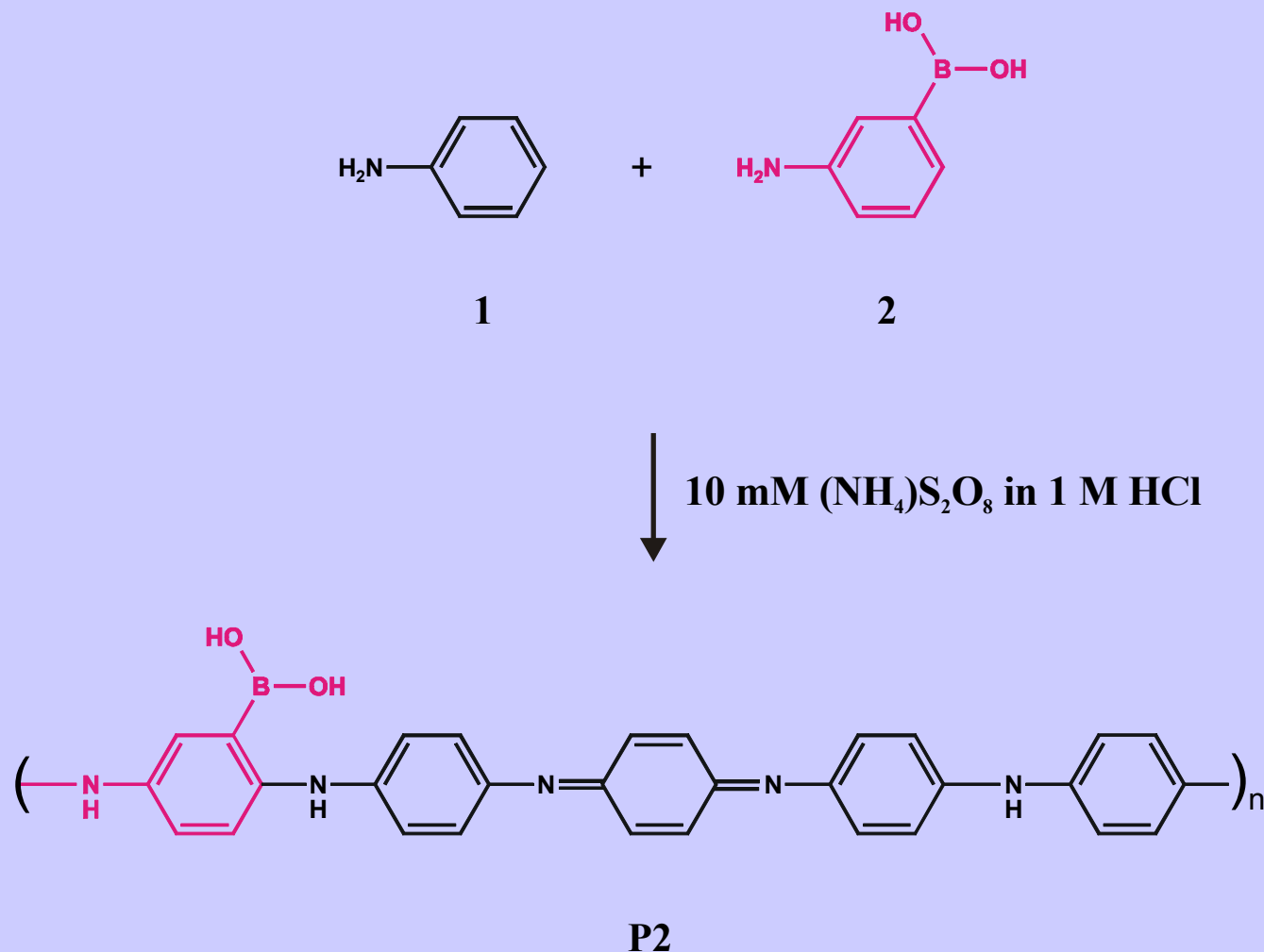
INTERACTION OF LABELED LECTIN ConA WITH D-MANNOSE



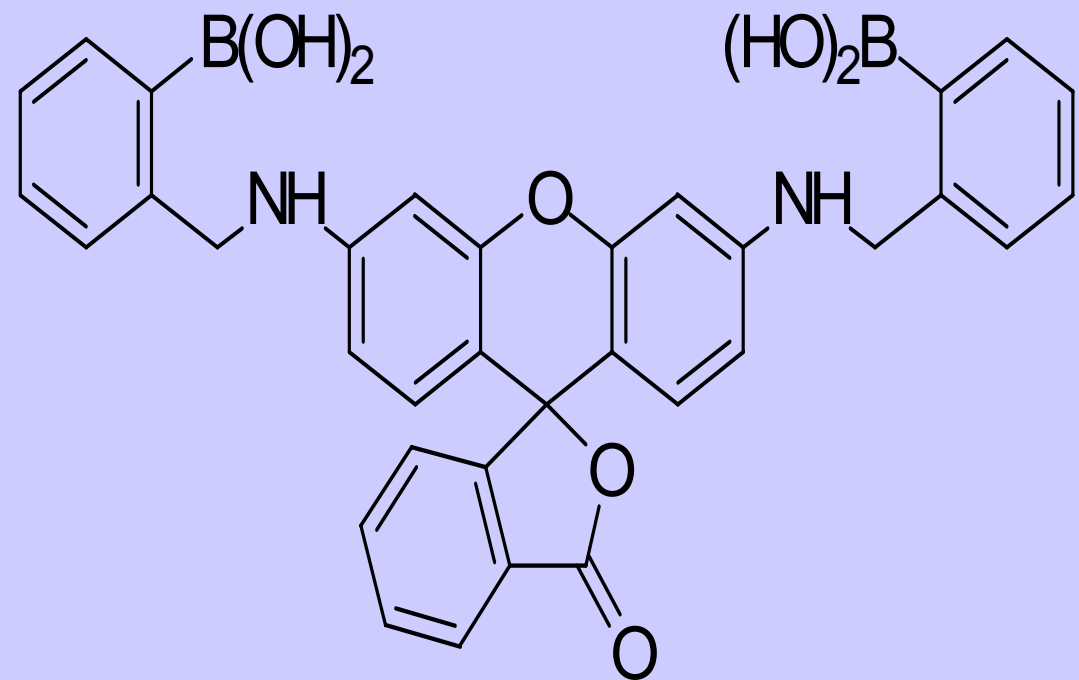
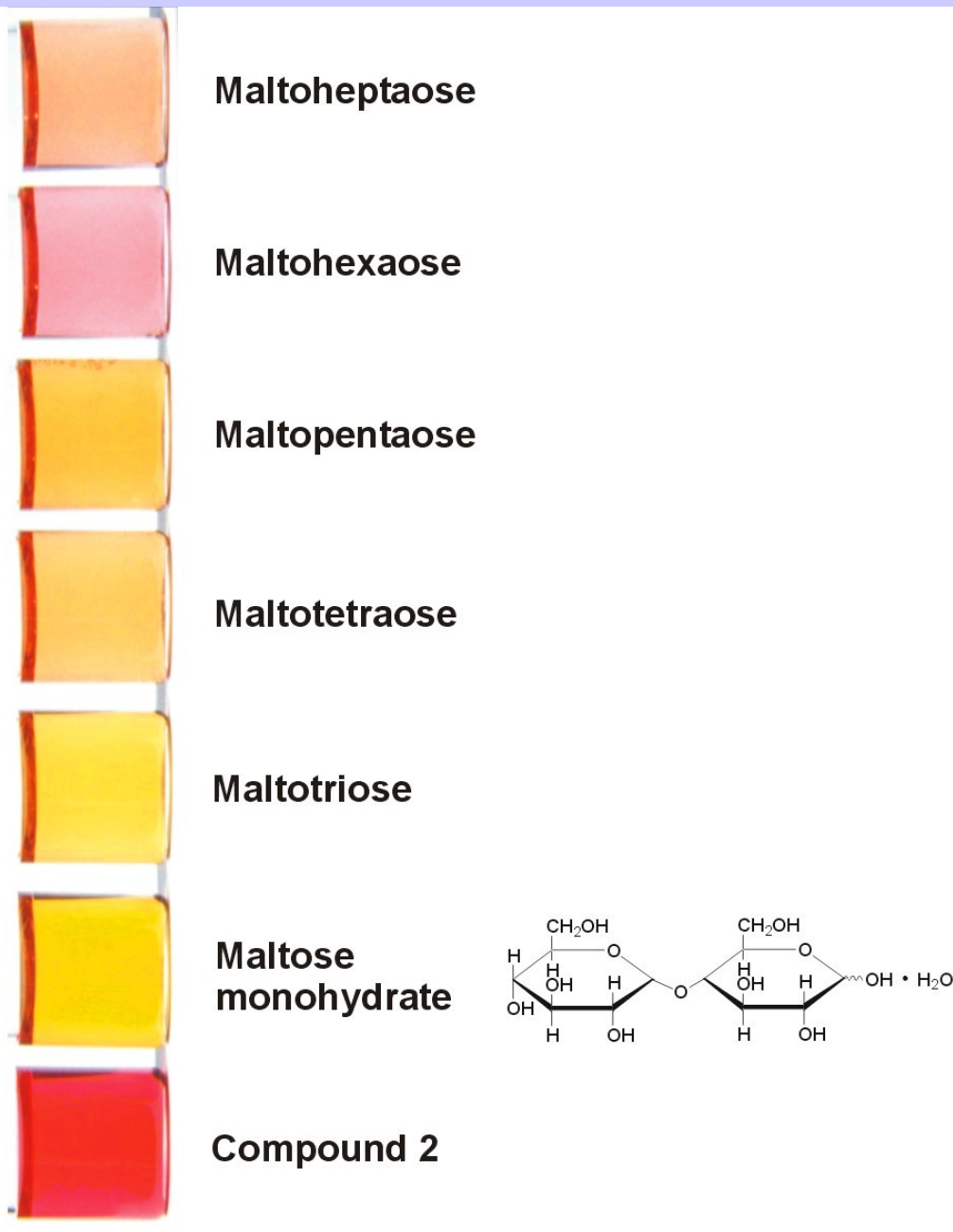
Optical Chemical Sensing Using Conducting Polymers

A Polyaniline with Near- Infrared Optical Response to Saccharides

E. Pringsheim, E.
Terpetschnig, S. A.
Piletsky & O. S.
Wolfbeis, *Adv.
Mater.* 10 (1999) 865

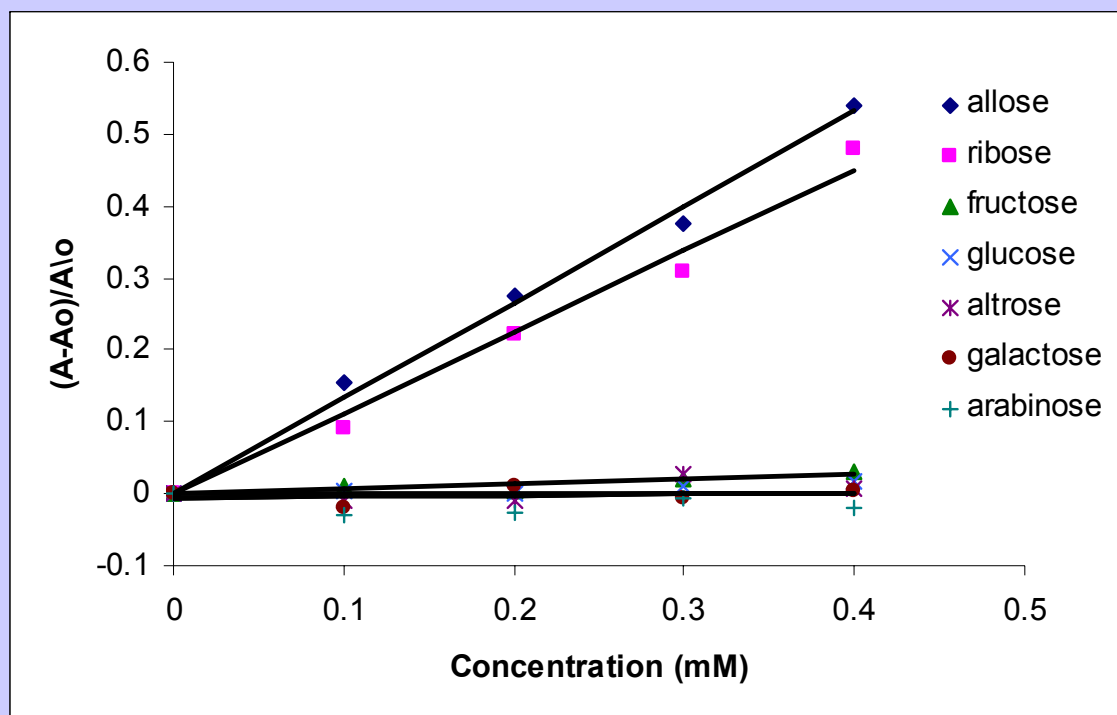


VISUAL DETECTION OF OLIGOSACCHARIDES

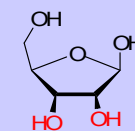


Rhodamine *bis*-boronic acid

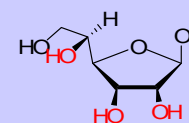
UV-Vis SPECTRA USING ORTHOGONALLY-REACTIVE CHEMOSENSORS



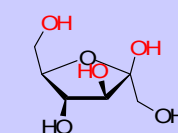
Relative absorbance changes vs. concentration of various monosaccharides in phosphate buffer (0.1 mL, 60 mM, pH 7.4) after incubation with C4BA and then added to RBA (0.04 mM) in DMSO (0.9 mL) at 356 nm



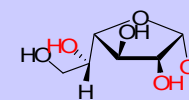
β -D-ribofuranose



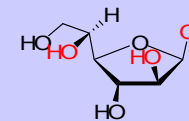
β -D-allofuranose



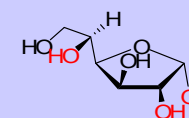
β -D-fructofuranose



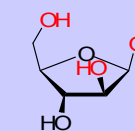
α -D-galactofuranose



β -D-altrofuranose

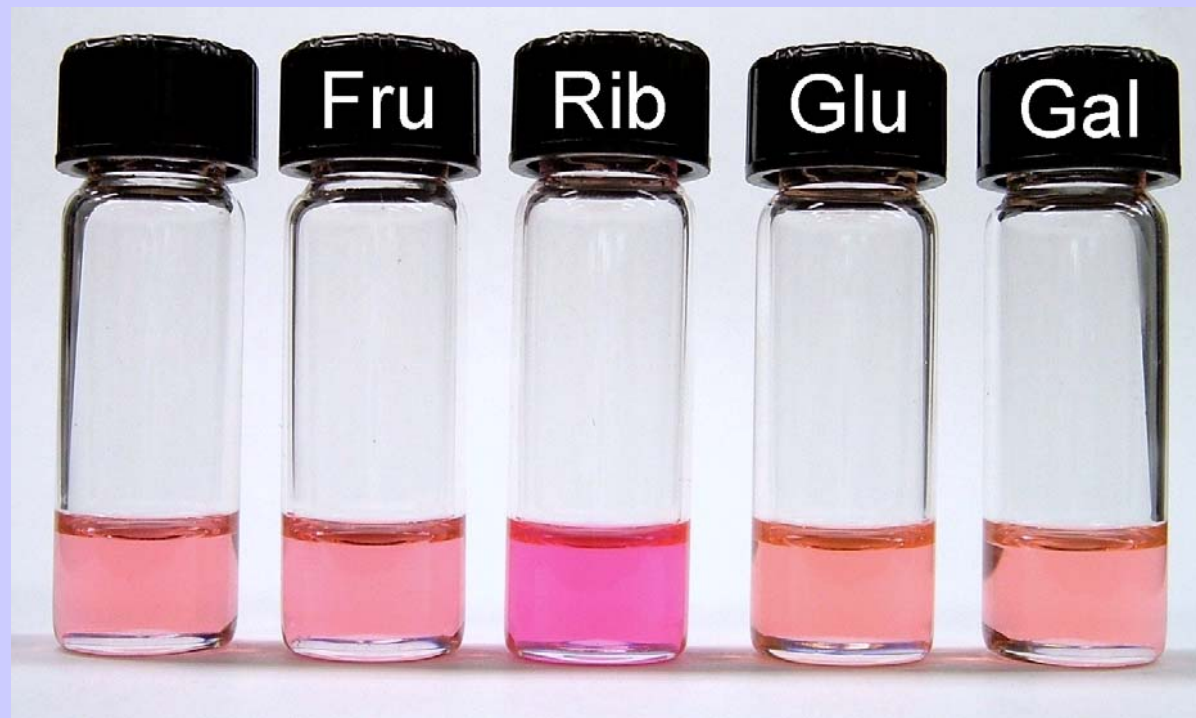


α -D-glucofuranose



β -D-arabinofuranose

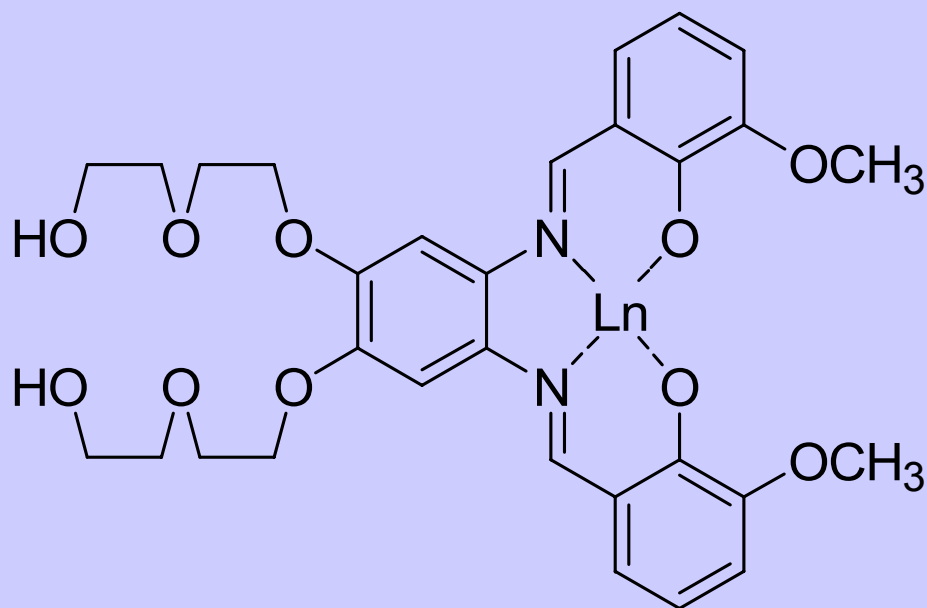
VISUAL DETECTION OF RIBOSE



Challenge:

Neutral saccharide detection using artificial receptors at neutral pH

STRATEGY: Use Water Soluble Salophenes

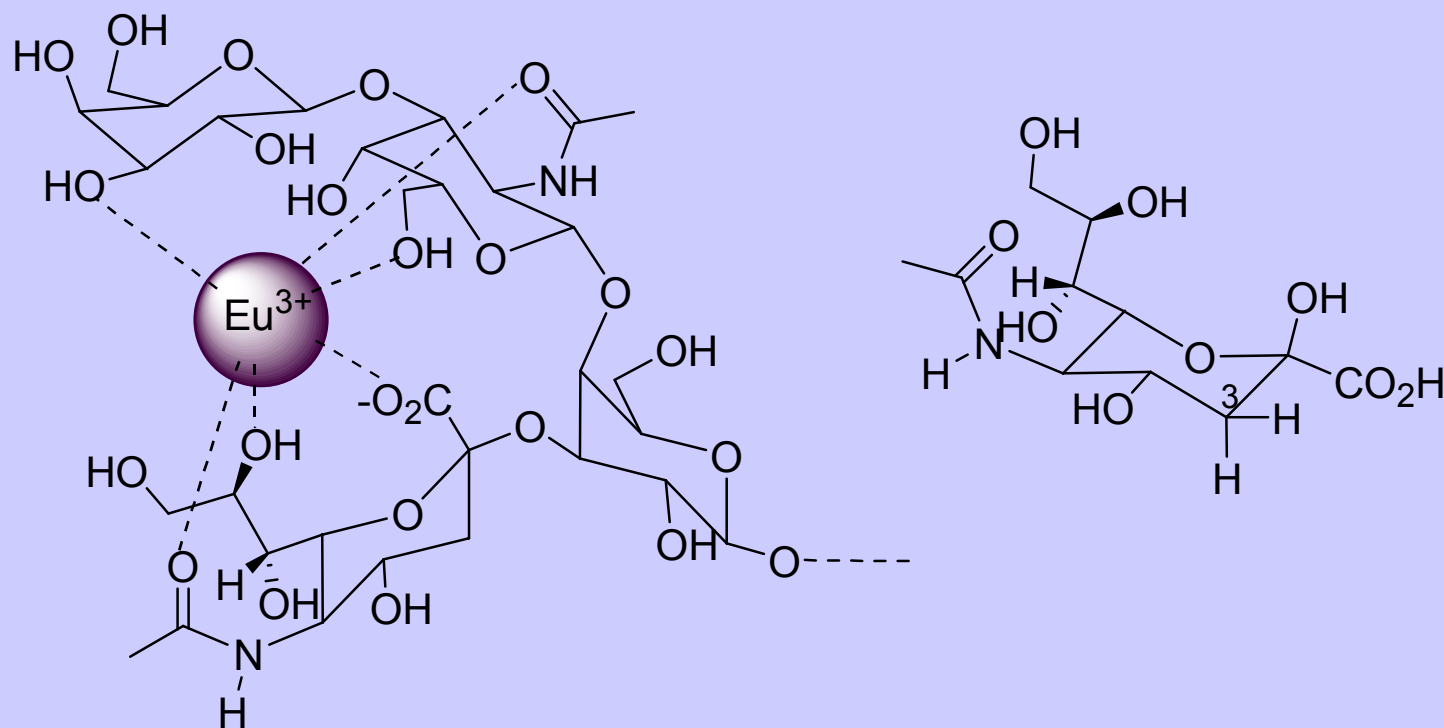


1 Ln = La³⁺

2 Ln = Eu³⁺

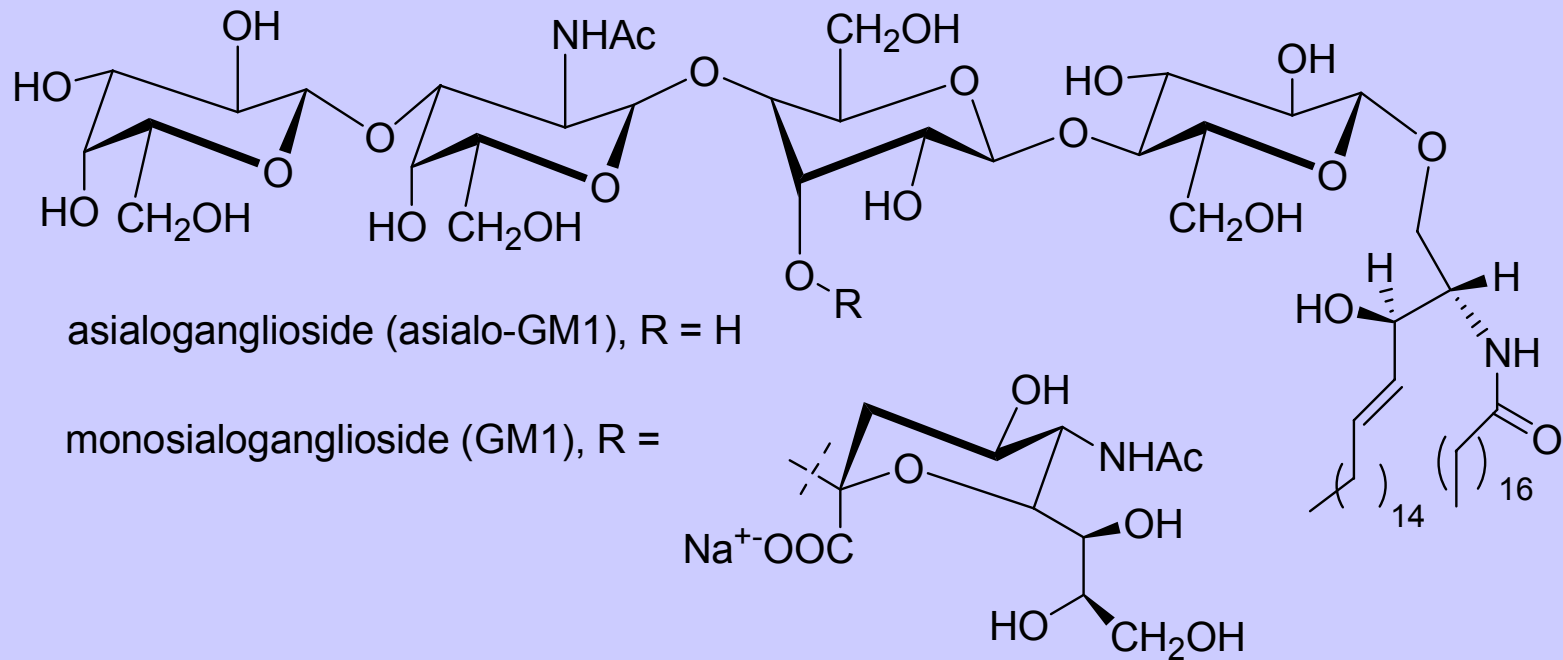
HYPOTHESIS: Lanthanides with smaller ionic radii will detect anions more effectively

Bruce, J. I., et al. (2000) *J. Am. Chem. Soc.* **122**, 9674-9628.

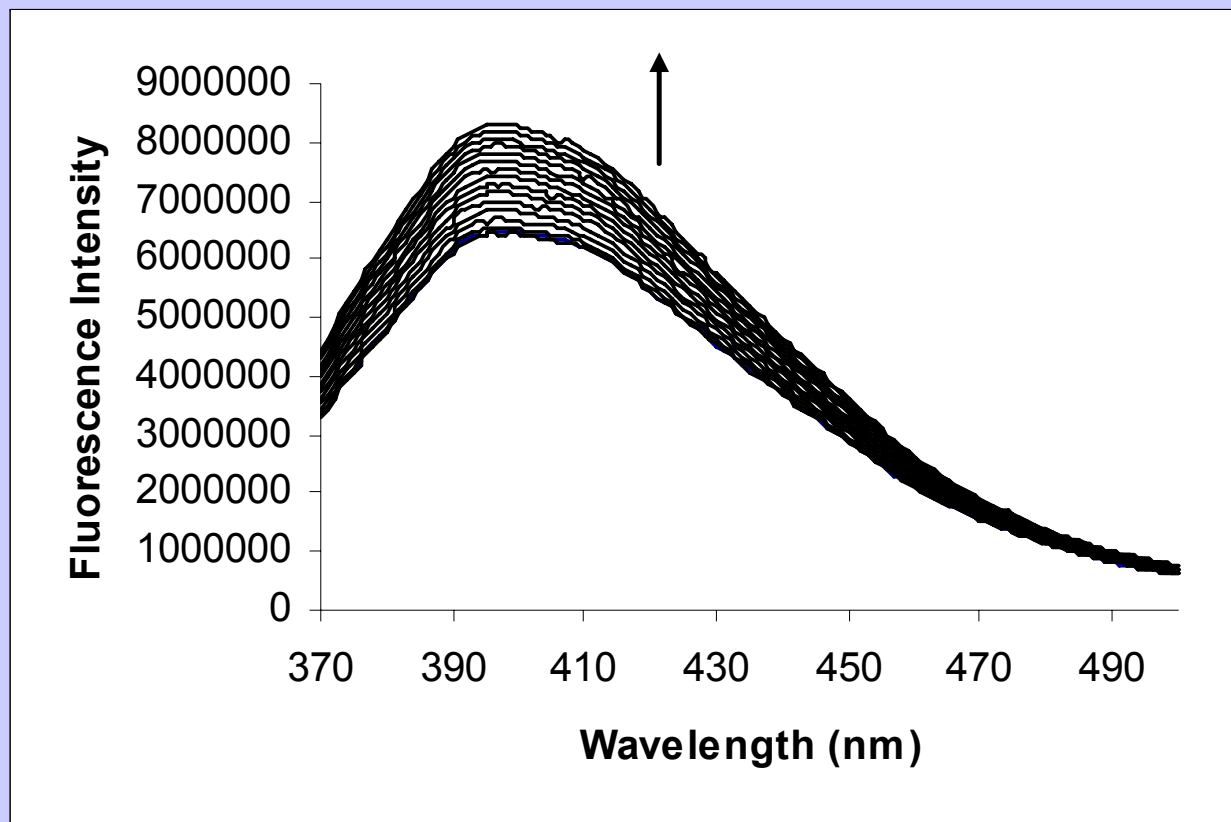


Sillerud, L. O., Prestégard, J. H., Yu, R. K., Schafer D. R. & Konigsberg W. H. (1978) *Biochemistry* **17**, 2619-2628.

Strategy: Test GM1 vs. asialo GM1

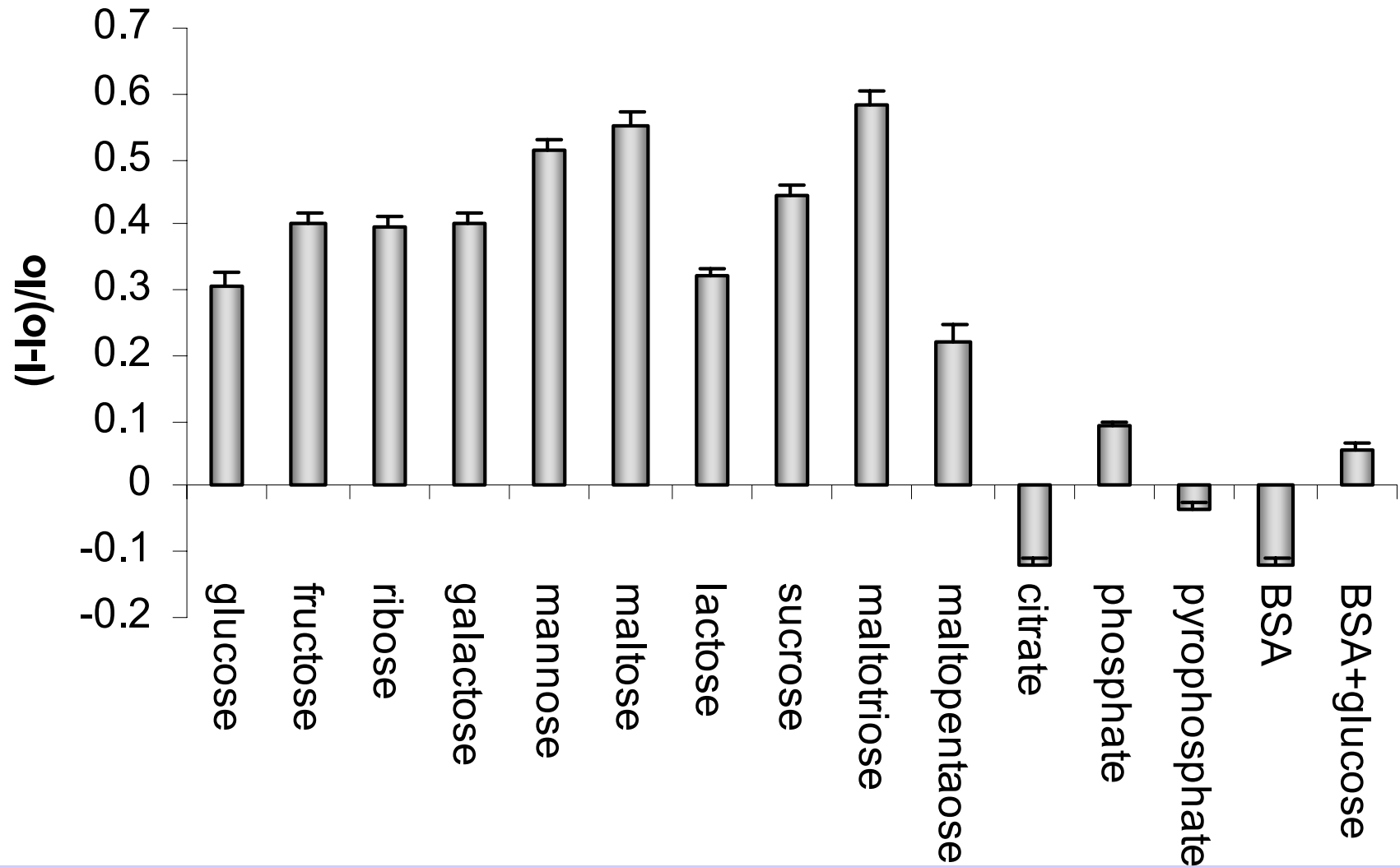


Glucose added to La(III) Salophene: buffer solution pH 7



- Fluorescence changes observed upon titration of **1** with D-glucose in 0.1M HEPES buffer, pH 7.0. The concentration of **La-salophene** = 6×10^{-6} M. The concentration of glucose is increased to 6×10^{-4} M. Excitation is at 360 nm, emission is monitored at 400 nm.

Neutral Sugars vs. Common Anions



Elektronické jazyky a nosy

**Multikomponentí
analýza**

Umělé jazyky



Použití jazyků

Rozpoznávání lidských chutí

- kontrola nápojů

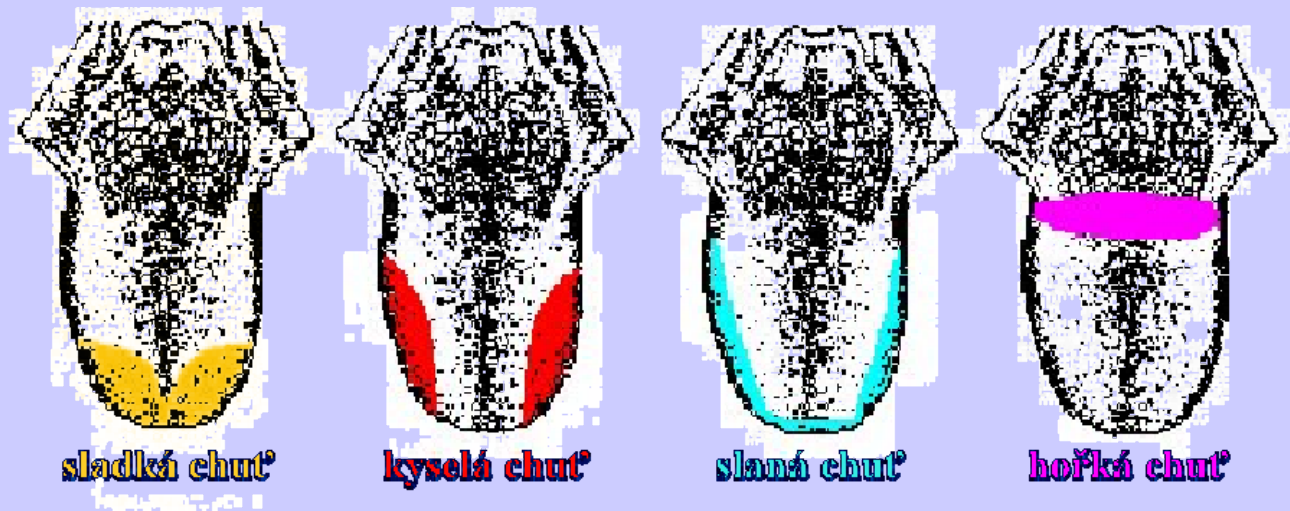
- ...

- kontrola odpadních vod (životní prostředí)

- rozbor moči (medicína)

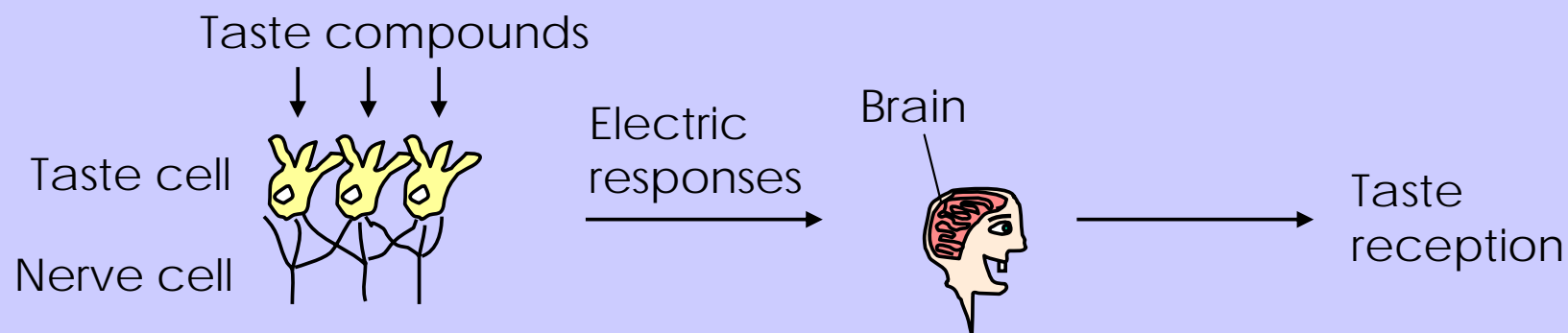
- sledování technologických procesů

- ...

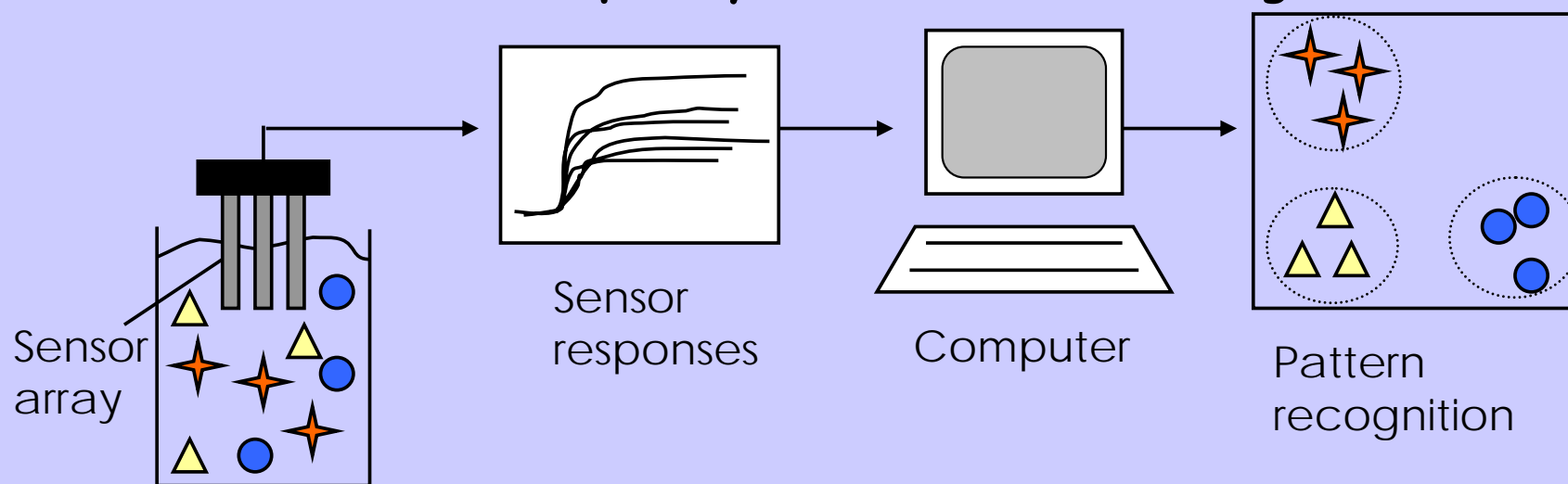


What is an electronic tongue?

Biological taste system



Artificial liquid system - electronic tongue



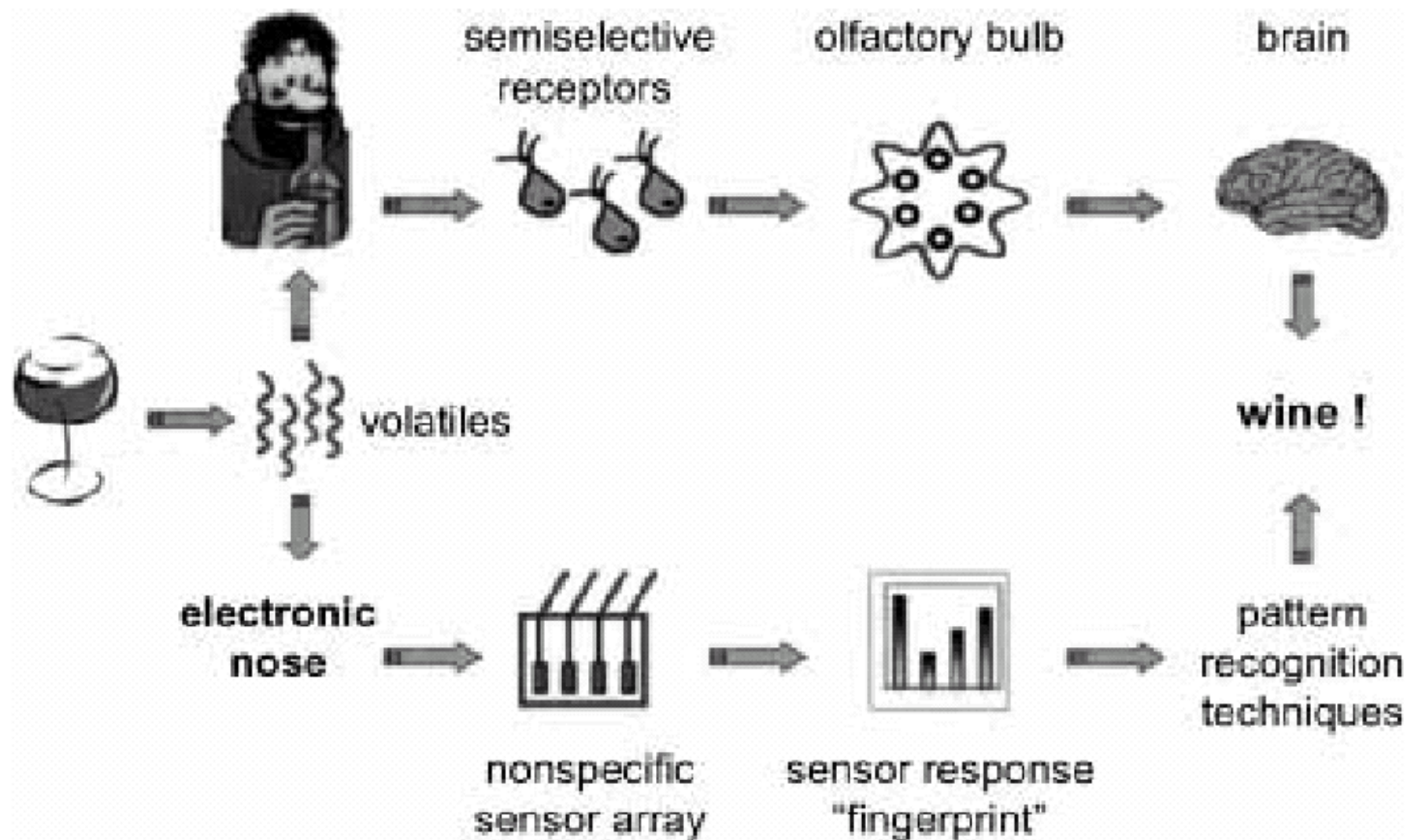
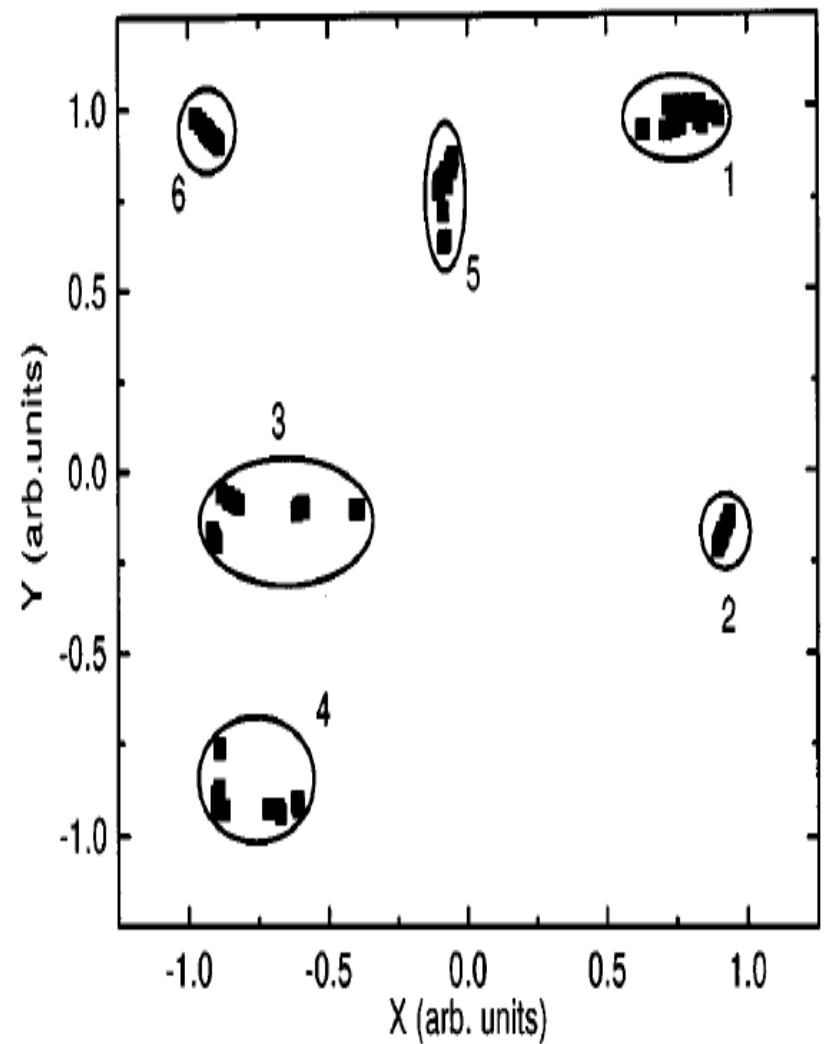
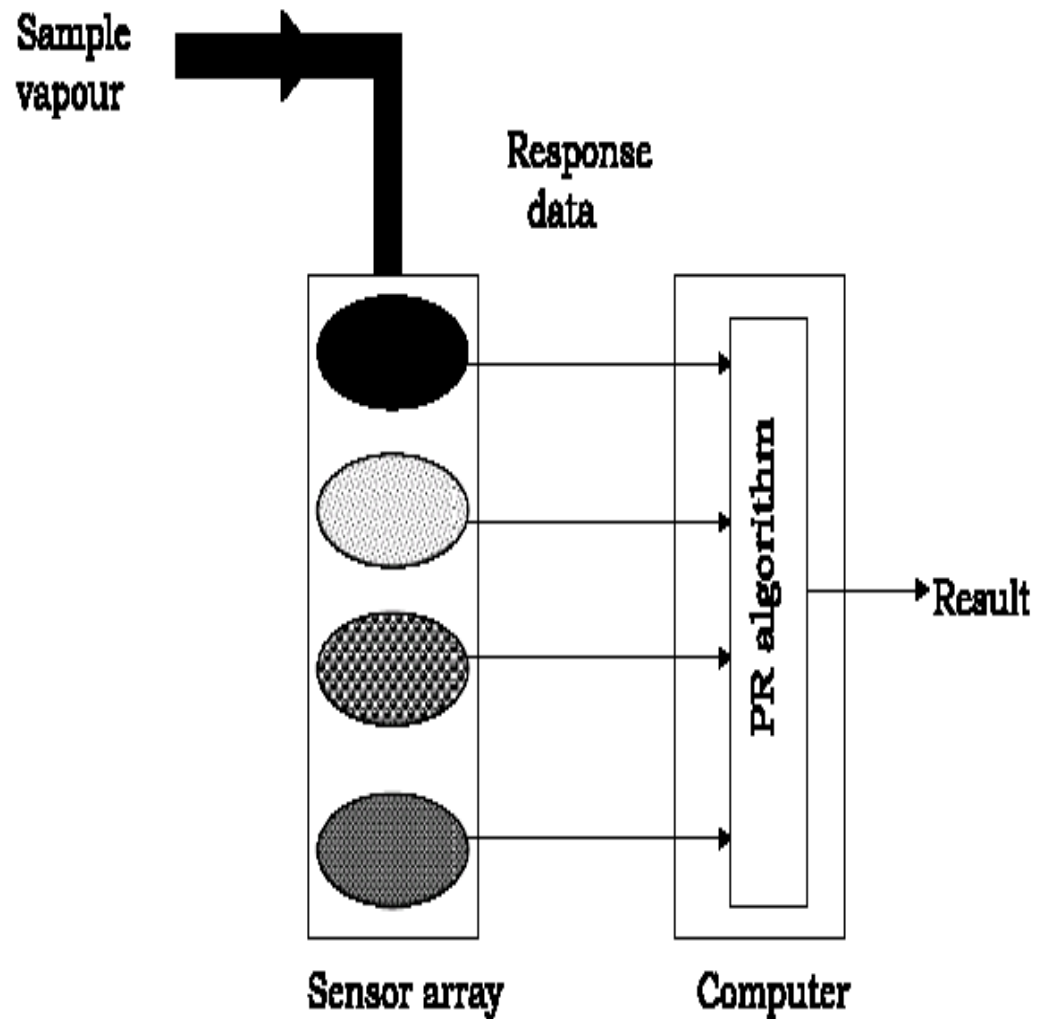


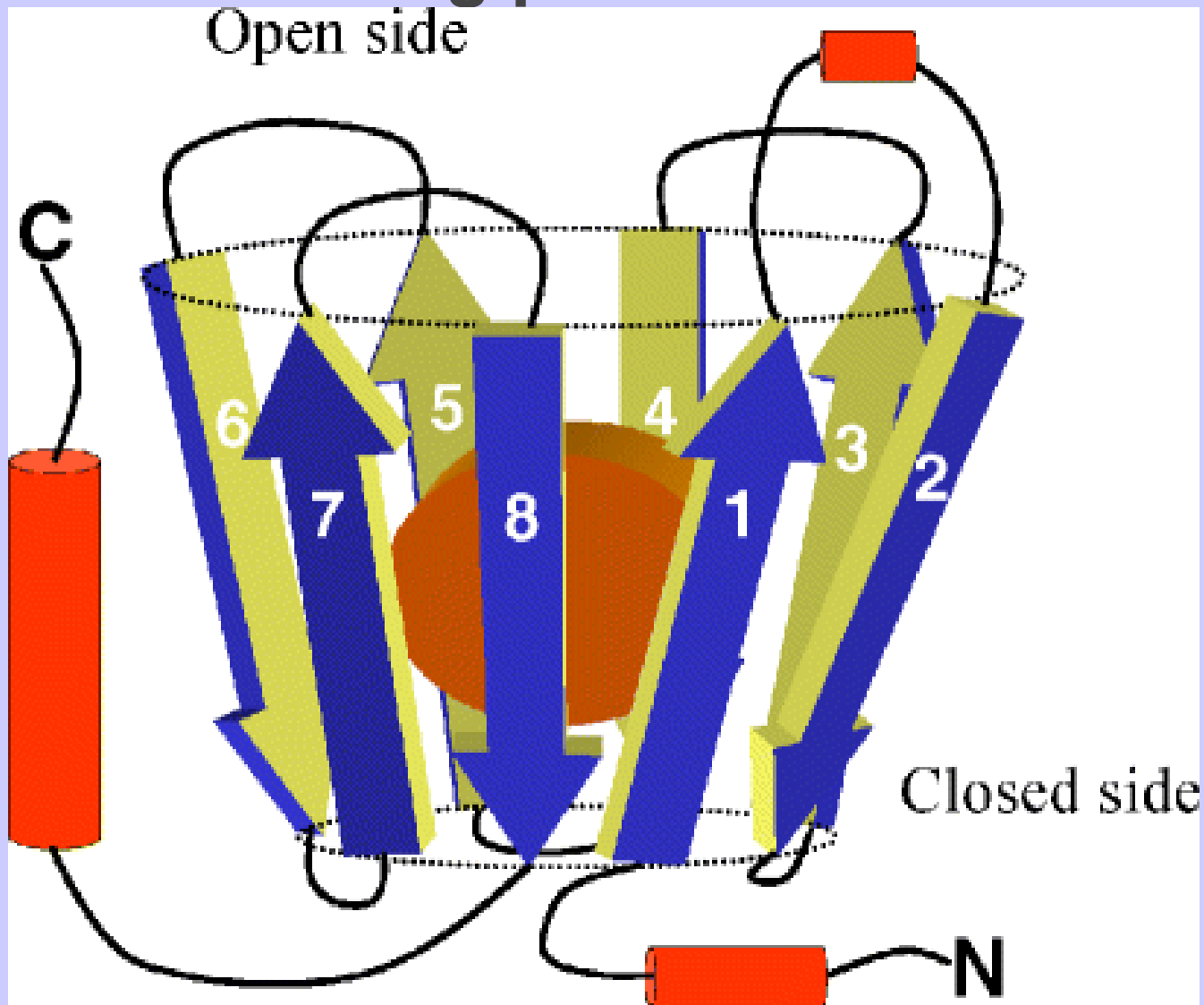
Figure 1. Parallel between the operating principle of the electronic nose and the human olfactory system.

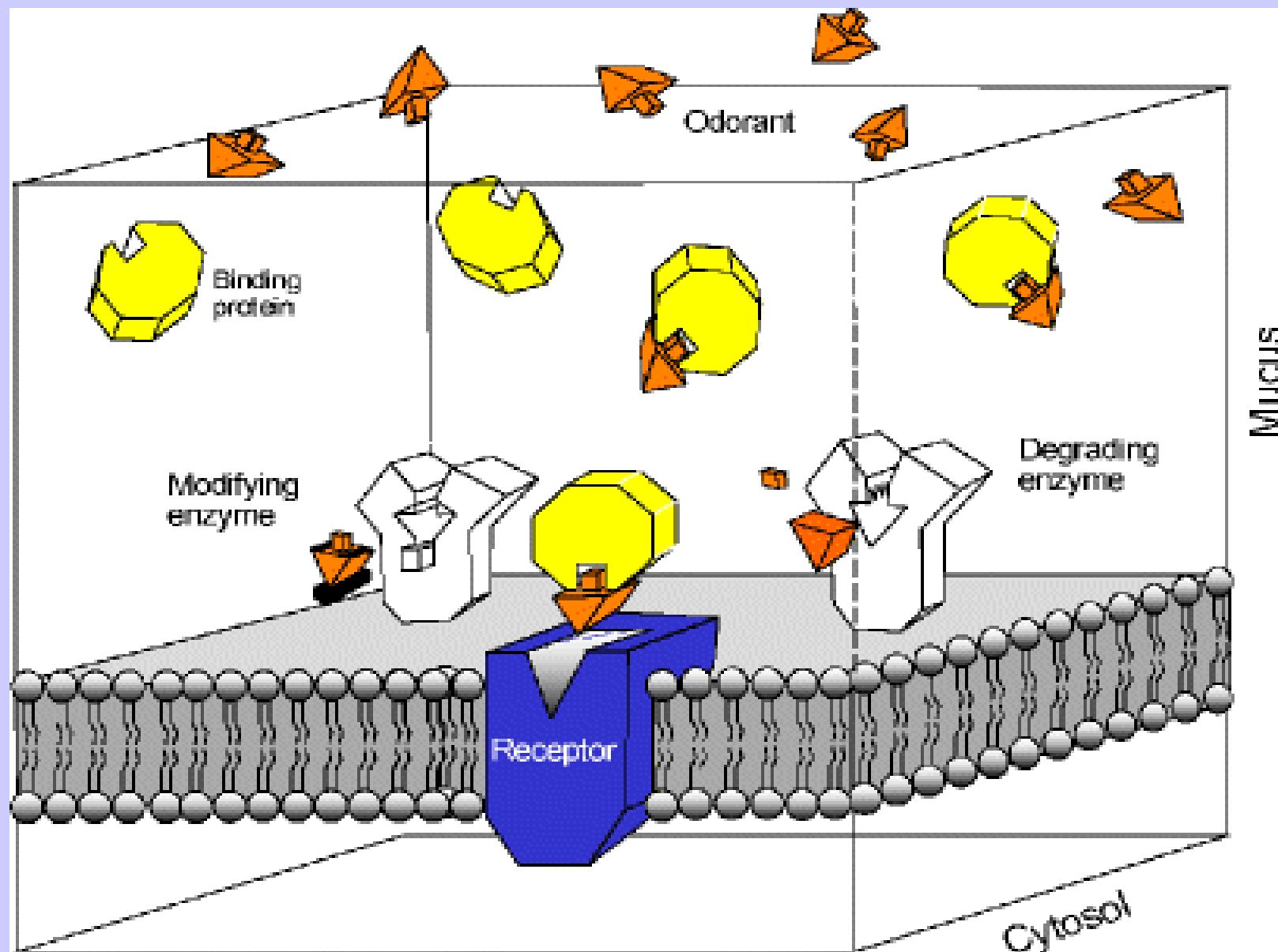


A generic electronic nose.

Olfactory receptors: molecular basis for recognition and discrimination of odors

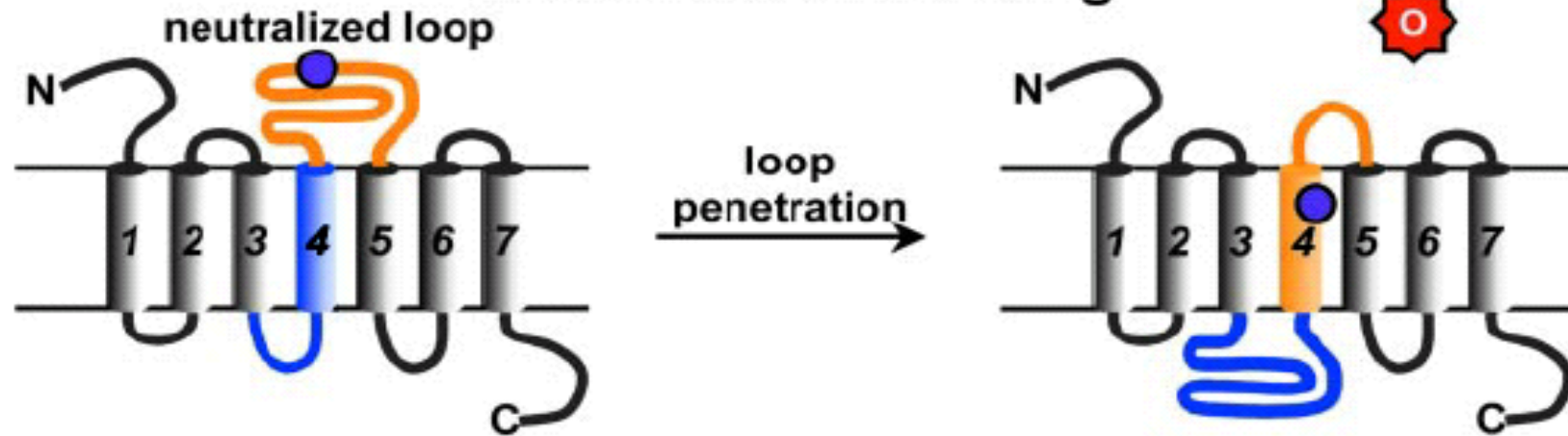
Odorant-binding proteins





Perireceptor events in the mucus layer of the olfactory epithelium influence the entry, exit, or residence time of odorous molecules in the receptor environment. These ancillary processes are integral components of the chemical sensing systems and include the interaction of odorants with specific soluble binding proteins which may act as shuttles for the volatile, lipophilic odorous molecules through the aqueous mucus layer and lead to inactivation of odorants by degrading and/or biotransformation enzymes, thus clearing the system between consecutive sniffs

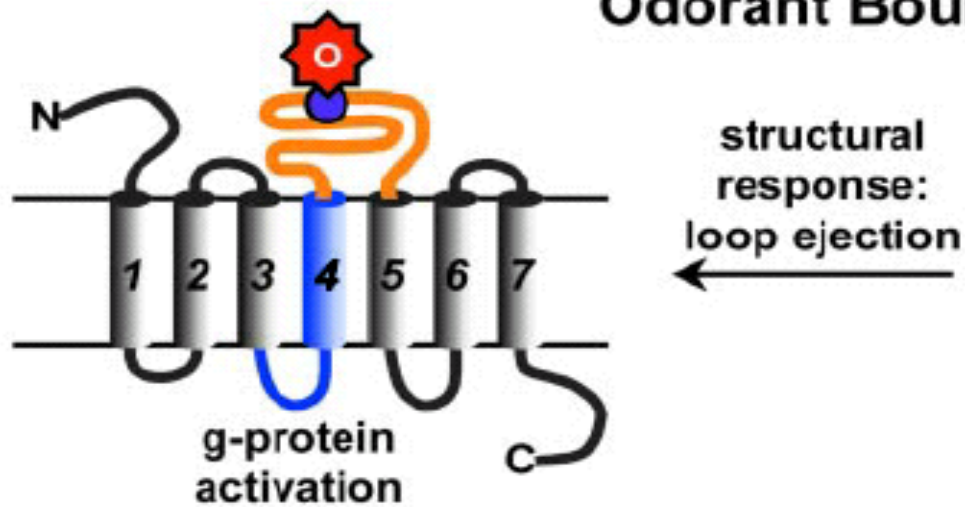
Metalated Active State: Before Odorant Binding

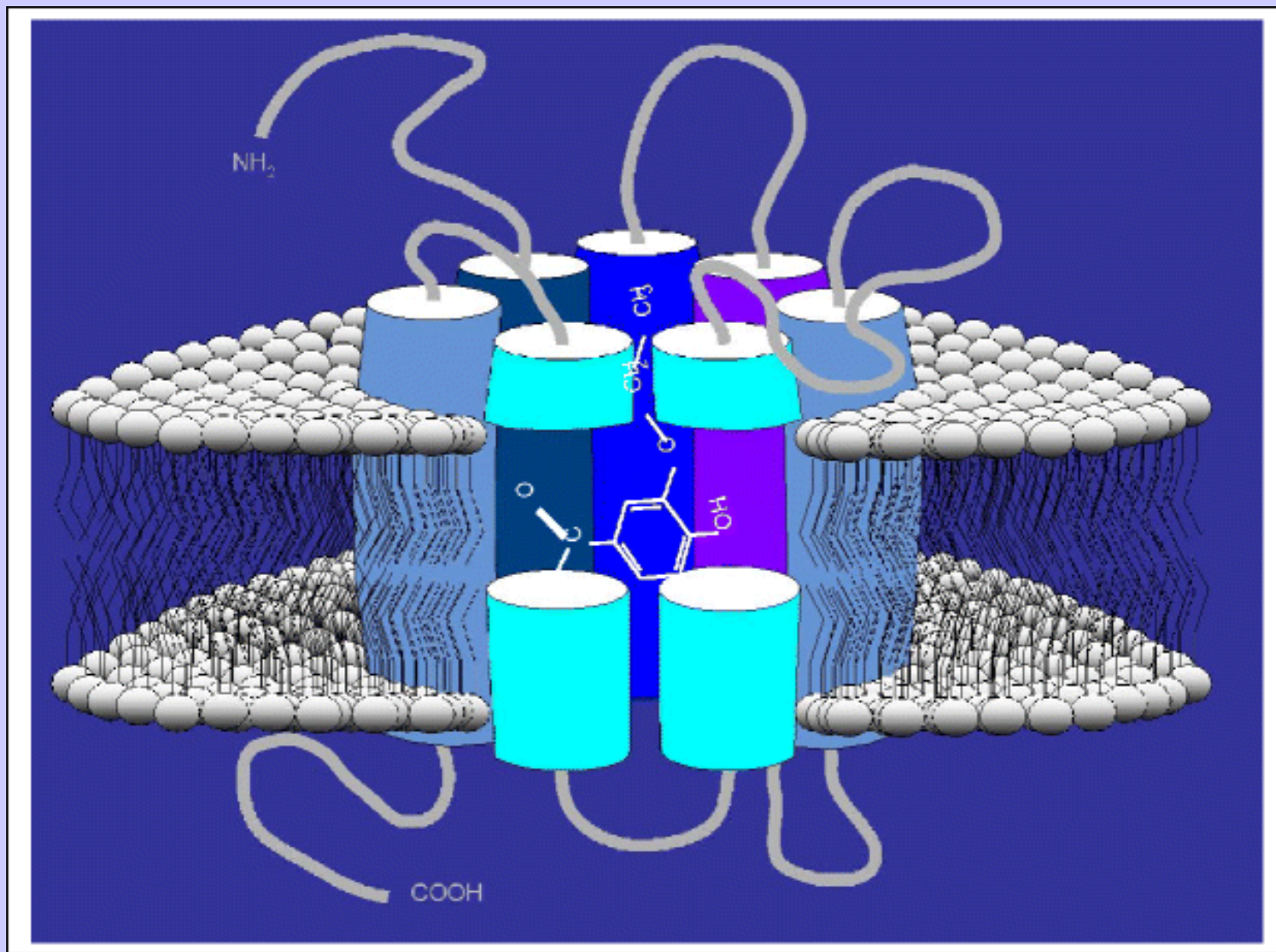


↑
odorant
released
↓

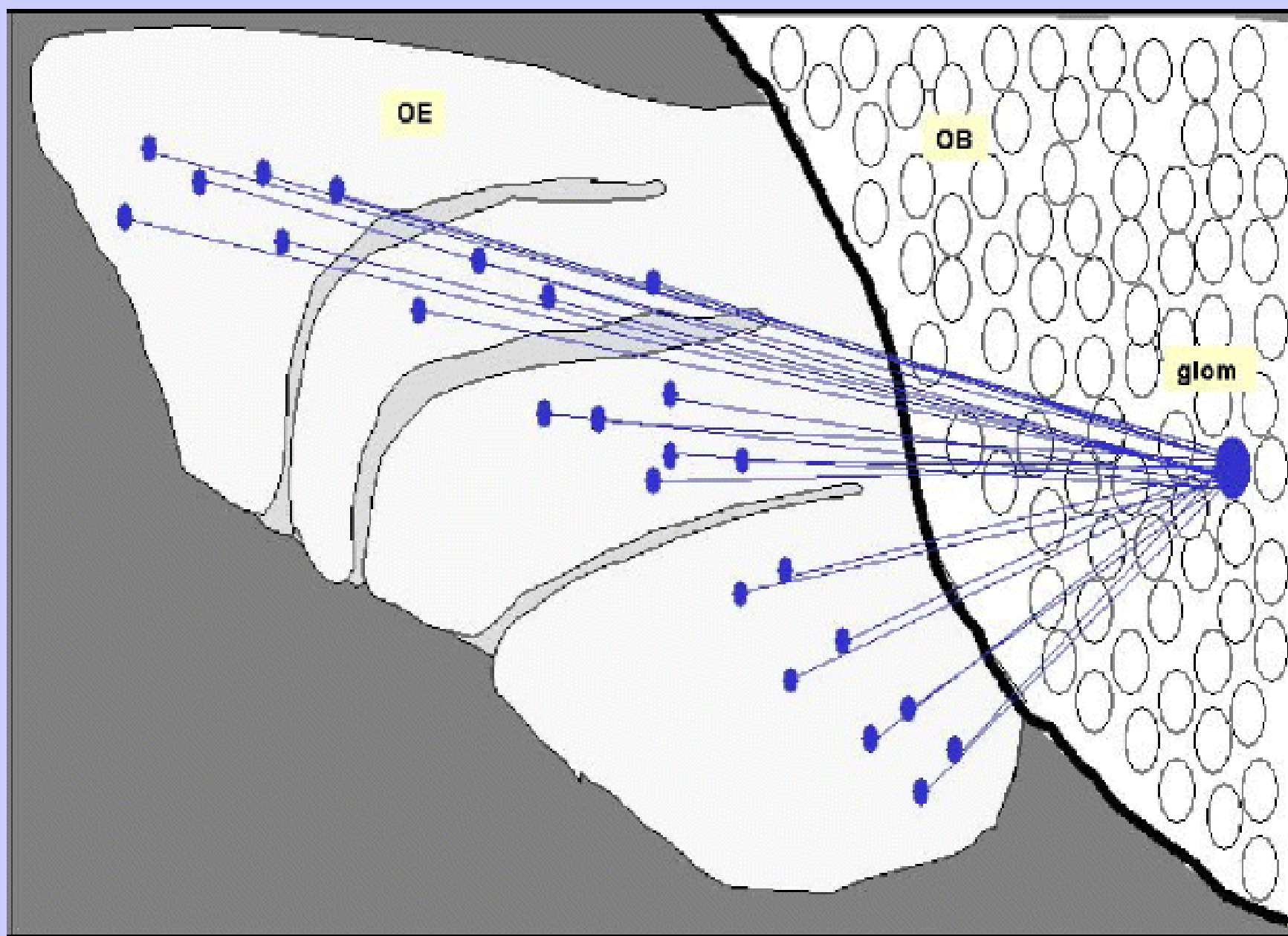
Metalated Active State: Odorant Bound

↑
odorant
binds to
metal ion
↓





Proposed membrane topology of an odorant receptor. The seven transmembrane domains (TMD) are supposed to form a "funnel" which accommodates the odorous ligands. The specificity of the binding site is determined by amino acid side-chains of the central TMD



Distribution and projection patterns of olfactory neurons. Olfactory sensory cells expressing a distinct receptor type are spatially segregated in an anterior-posterior zone of the olfactory epithelium (*OE*) and project their axons to common glomeruli (*glom*) in the olfactory bulb (*OB*)

Applications of electronic noses and tongues in food analysis

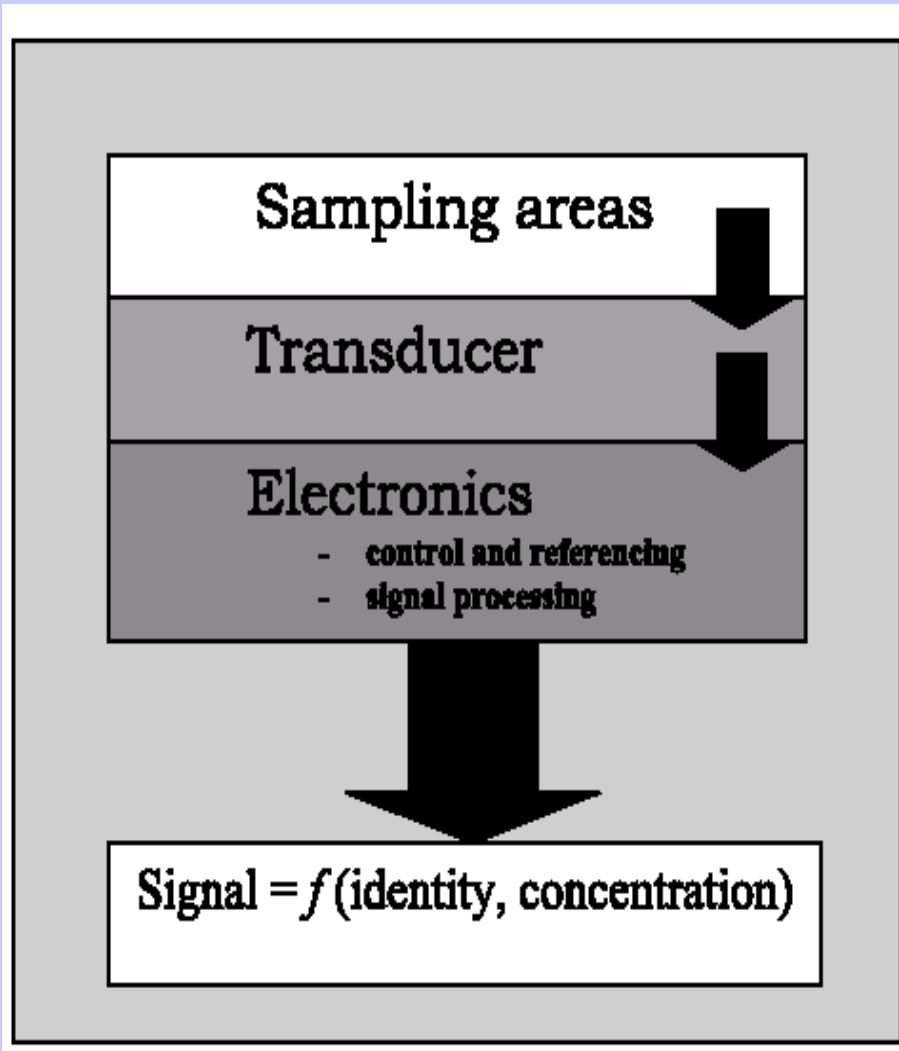


Table 1 Taste sensations (Marieb, 1998)

Sensation	Elicited by these compounds
Sweet	Sugars, amino acids, alcohols
Sour	Acids, e.g. acetic, citric
Salty	Table salt
Bitter	Quinine, caffeine, aspirin, nicotine
Umami	Monosodium glutamate (MSG), disodium inosinate in meat and fish disodium guanylate in mushrooms

Multikomponentní analýza

- Více, téměř 100% selektivních, senzorů integrovaných do jednoho čidla**
- Kolik senzorů - tolik informace o chemickém složení**
- Má všechny klady senzorové analýzy**
- Oproti diskrétním senzorům vede použití senzorového pole k zrychlení analýzy**

Multikomponentní analýza

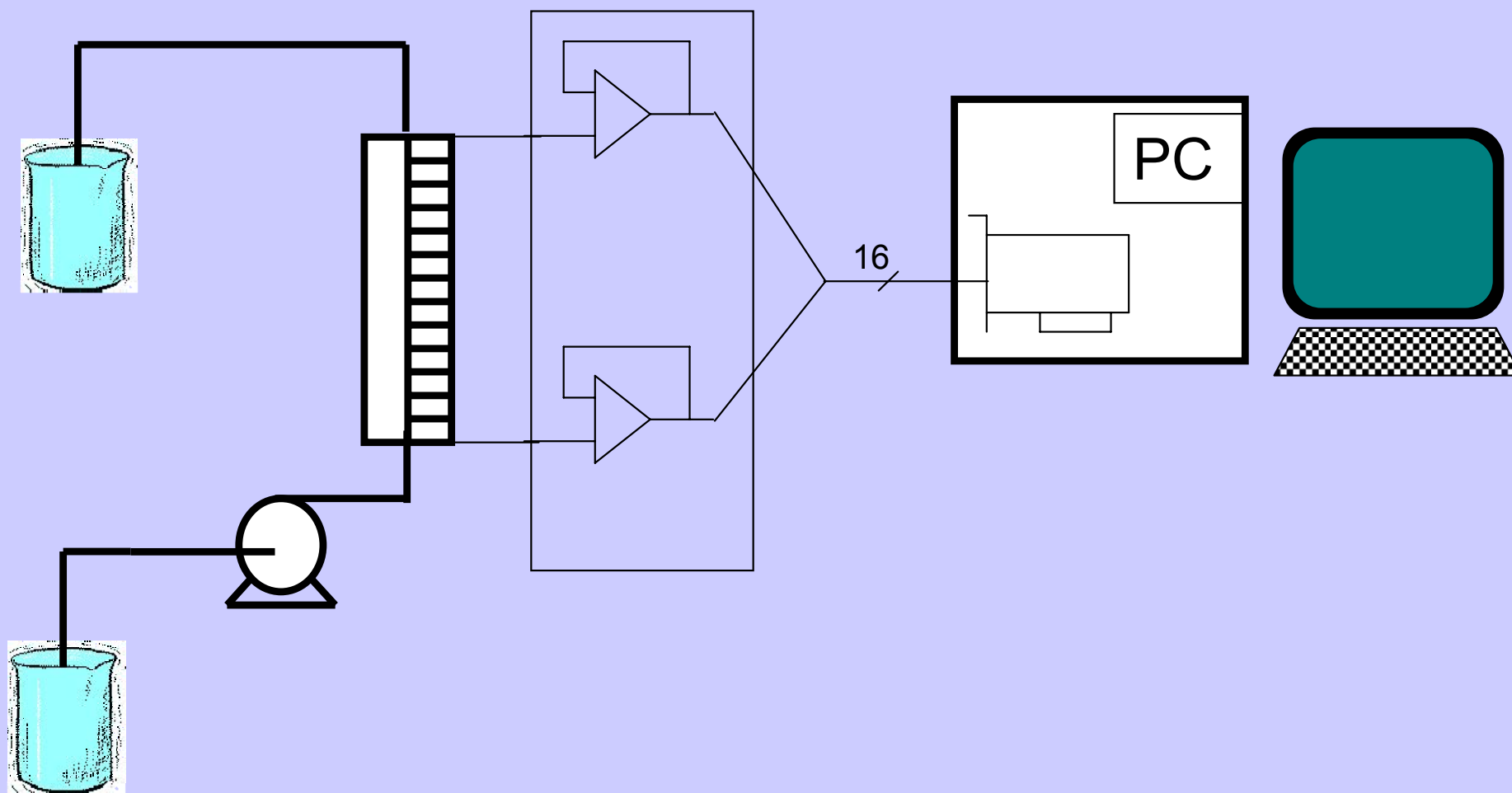
- **Použití neúplně selektivních senzorů**
- **Následuje matematické zpracování dat**
- **PCA analýza**
- **Zpracování dat vytrénovanou neuronovou sítí**

Umělý jazyk

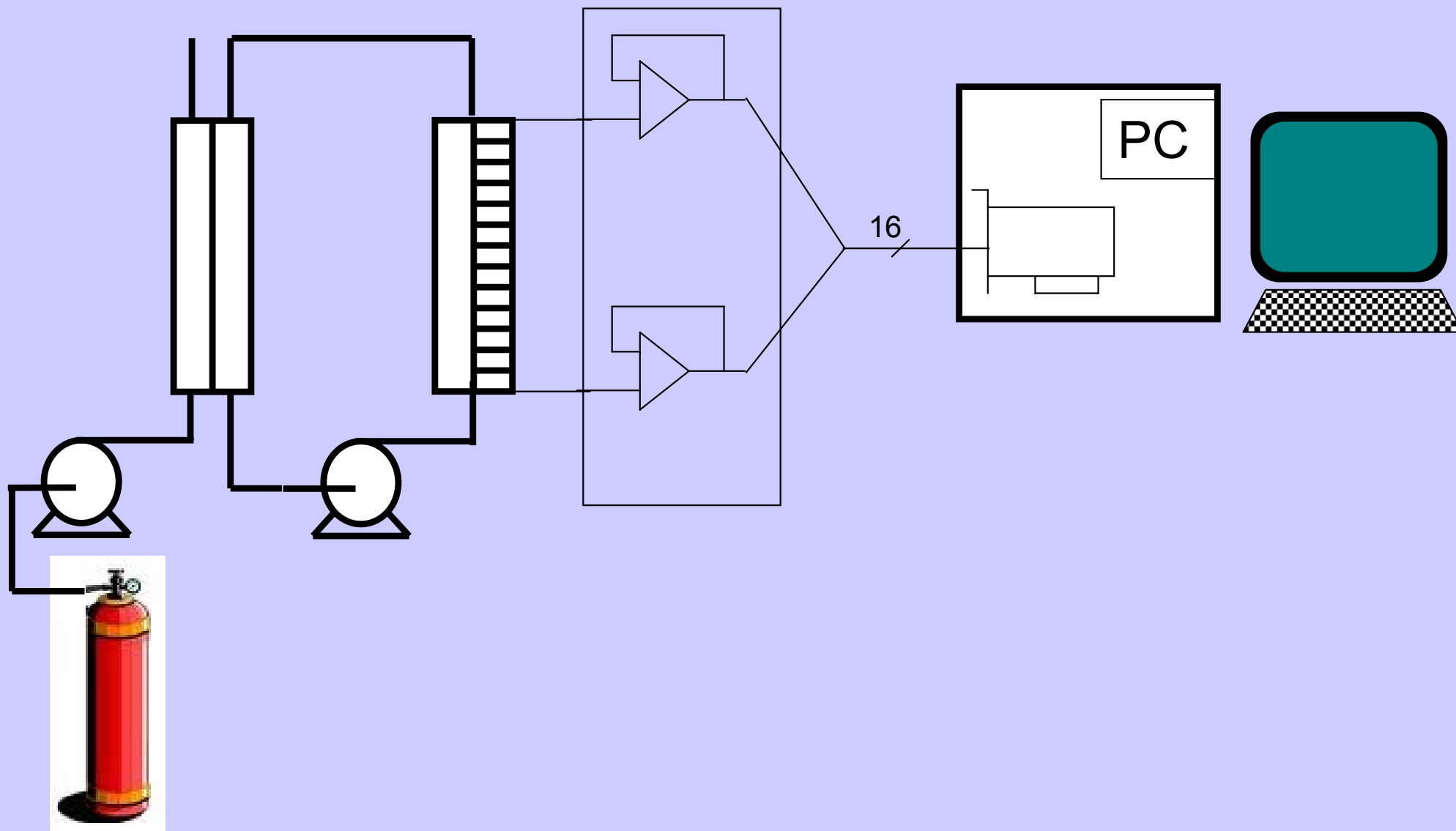
umělý (elektronický jazyk):

- vlastní senzorové pole**
- převodníková elektronika**
- digitalizace signálu**
- software pro řízení měření**
- vyhodnocovací software**

Hardware - język



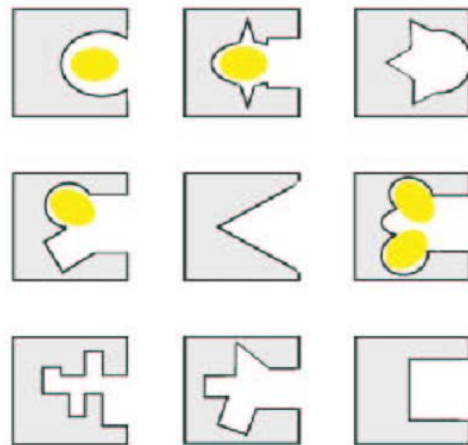
Hardware - nos s meziporpcí



Lock and Key Sensor

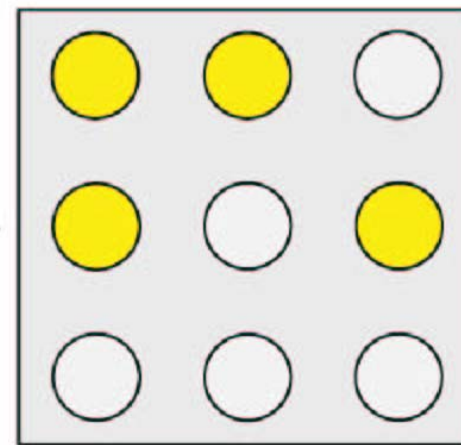


Differential Receptors

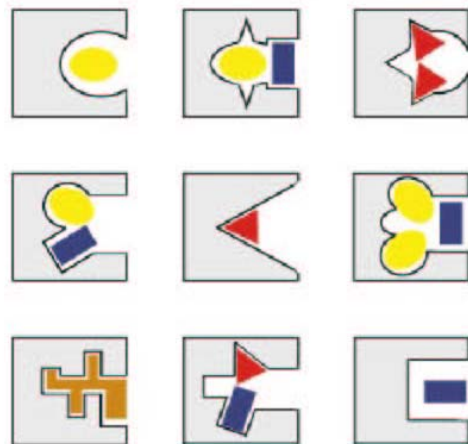


Read out

Single-Analyte Pattern

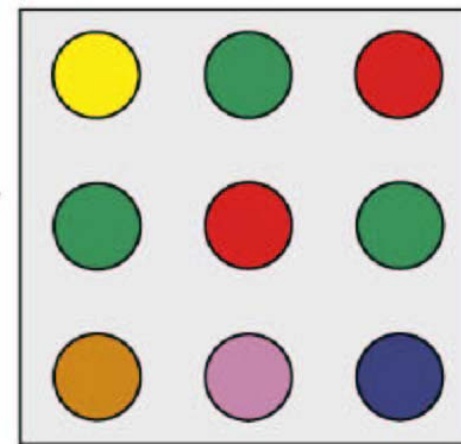


Differential Receptors



Read out

Multi-Analyte Pattern



Digital smells!

An example of the electronic nose is given below, where an array of 8 sensors output different patterns for each gas. If the array is “trained” properly it can recognise the individual gases in mixtures (chemometrics).

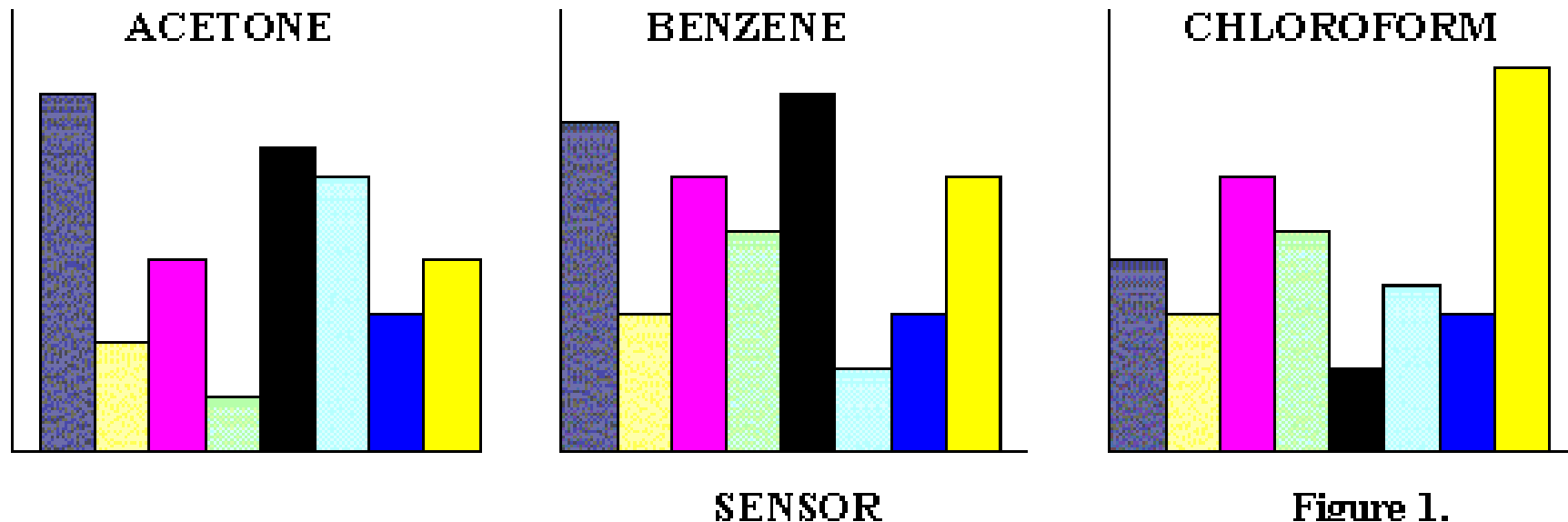


Figure 1.

Klasifikace dle velikosti relativních citlivostí (rozlišení látek se stejným obrazem odezev):

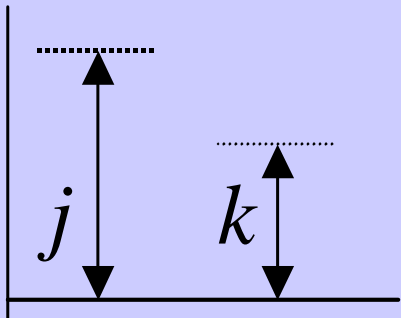
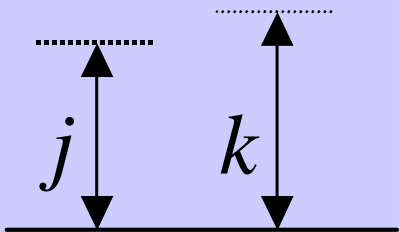
i i -tá látka

j, k sensor j a sensor k v souboru sensorů

R odezva sensoru

a citlivost sensoru

r **relativní citlivost**

látka (i)	A	B
obraz odezev sensorů j a k	$+$ $+$	$+$ $+$
citlivost sensorů j a k	R_A 	R_B 

**Pokud mají sensory různou selektivitu k
podstatným složkám roztoku – získáme
multivariantní data.**

- data z matic sensorových elementů mohou
být dále zpracována:**
- statistické metody, klastrová analýza, NN.**
- Analýza principiálních komponent (PCA),**
- Regrese principiálních komponent (PCR),**
- Analýza klastrů (Cluster Analysis)**
- Artificial Neural Network (ANN).**

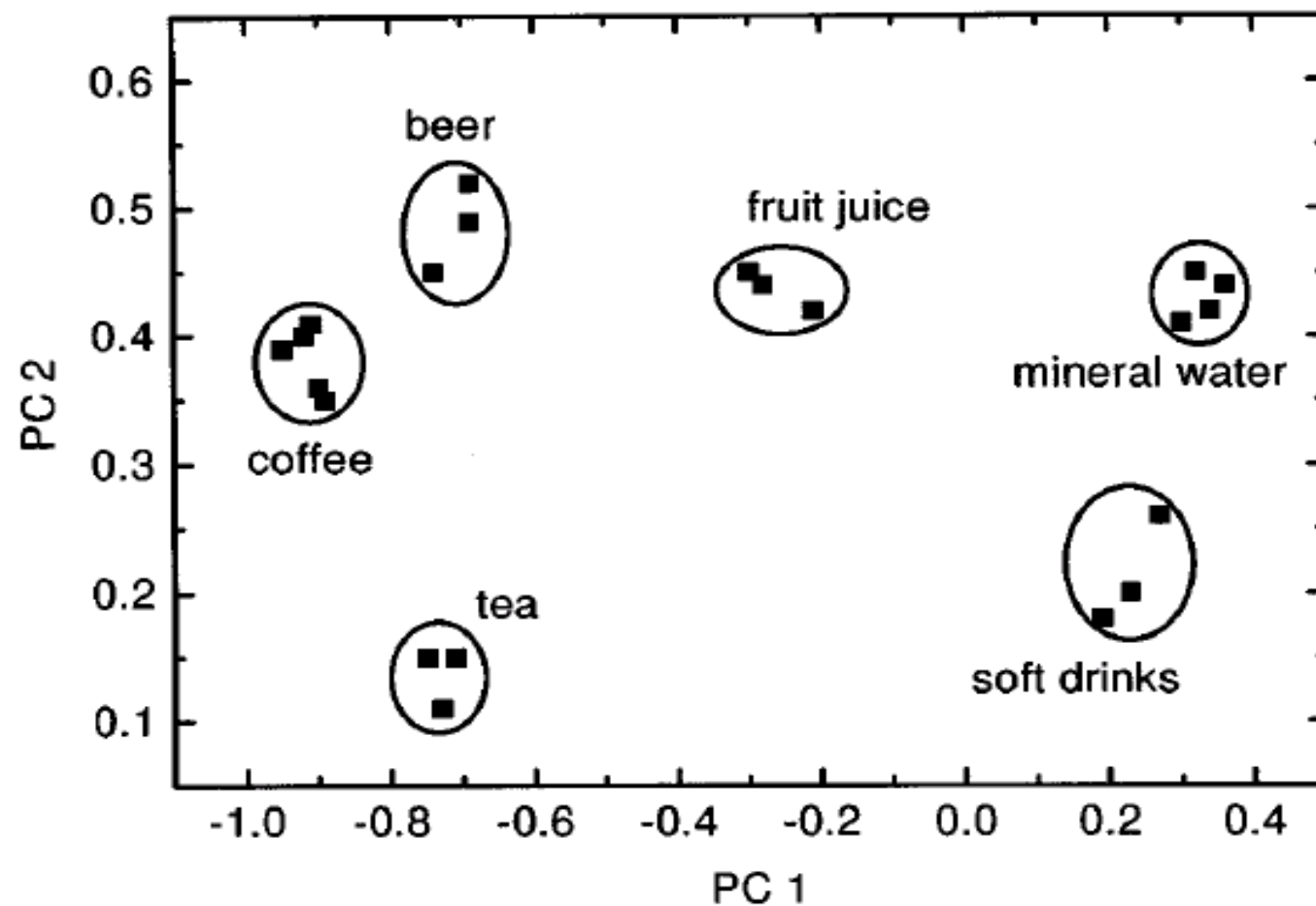
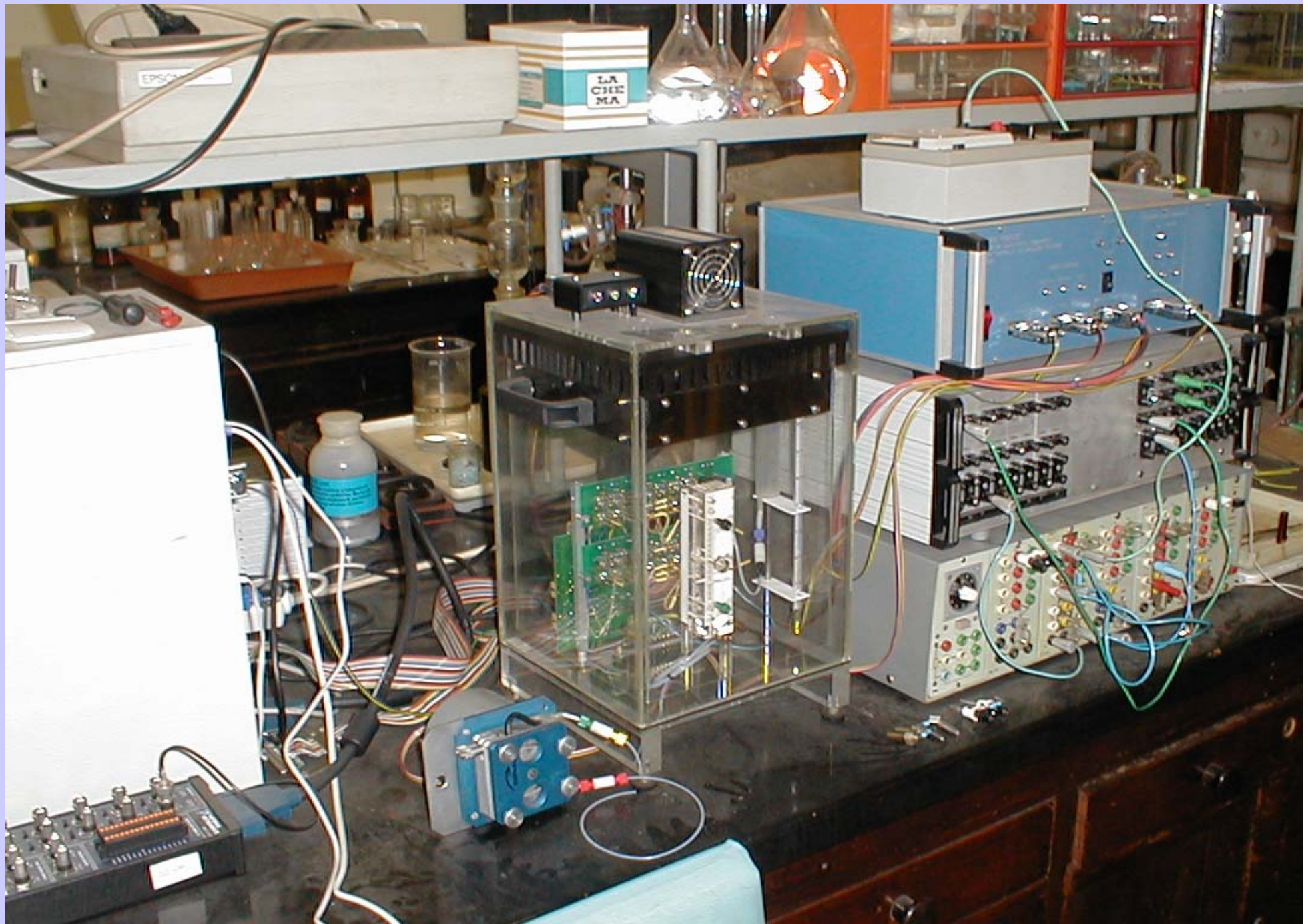


Fig.3 Discriminating abilities of “electronic tongue” in different types of beverages. Data processing has been performed by principal component analysis (PCA). PC 1 and PC 2 are produced during PCA when data dimension is reduced from 30D (number of sensors in the array) to 2D presentation shown in this picture. PC 1 and PC 2 are the most significant components containing the most adequate information about analysed objects

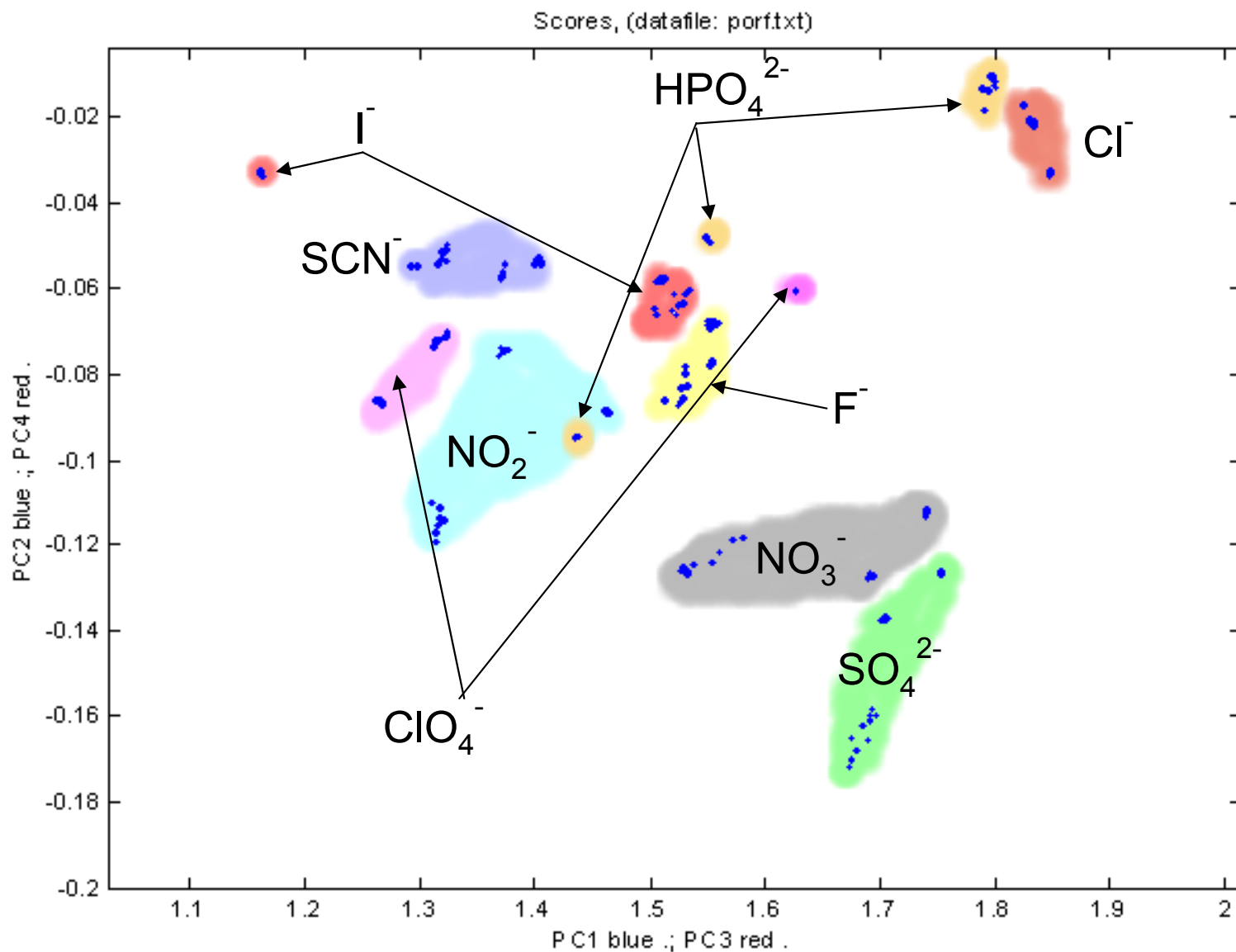
Fotografie sestavy potenciometrického jazyku



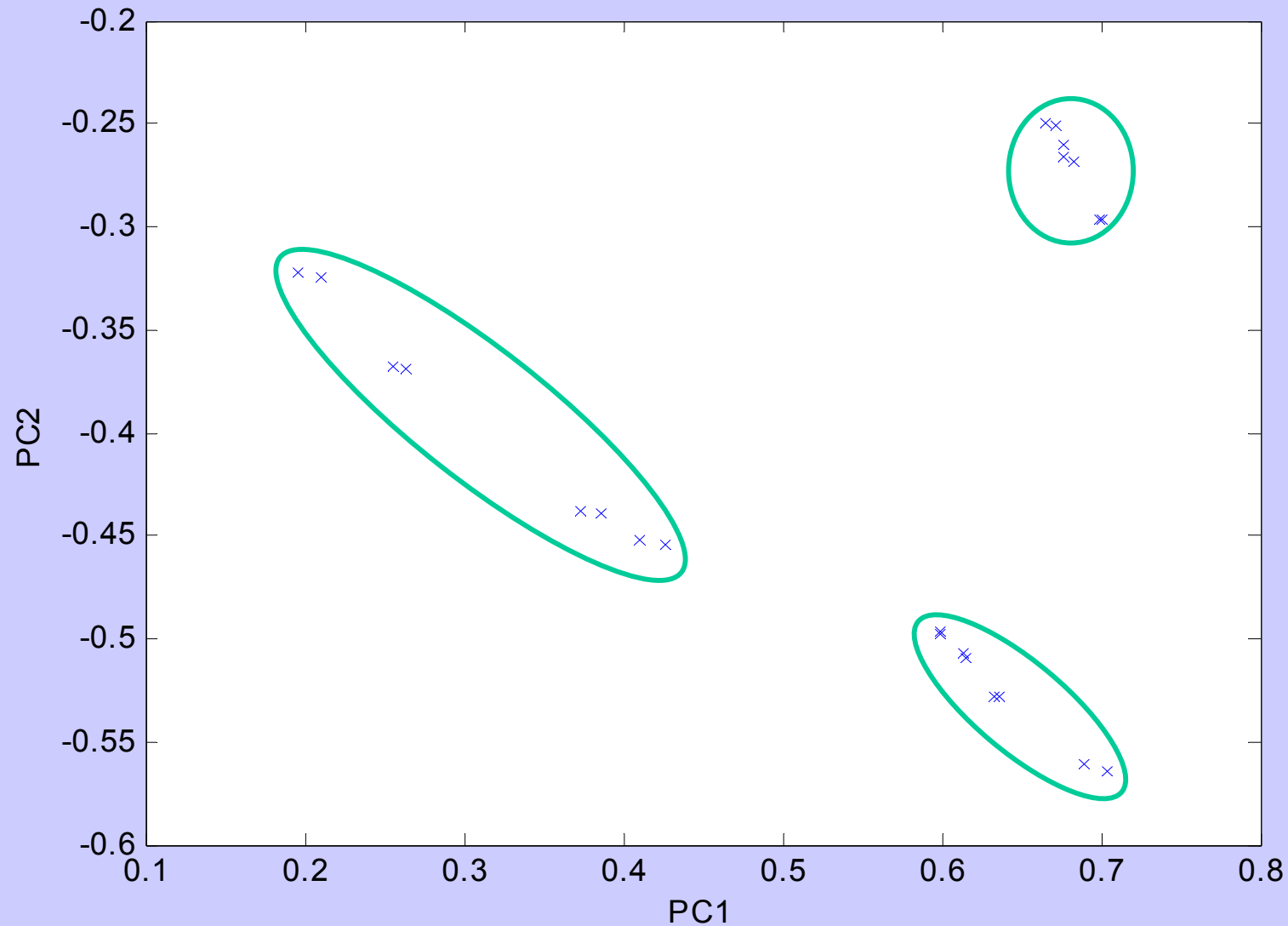
Fotografie sestavy nosu



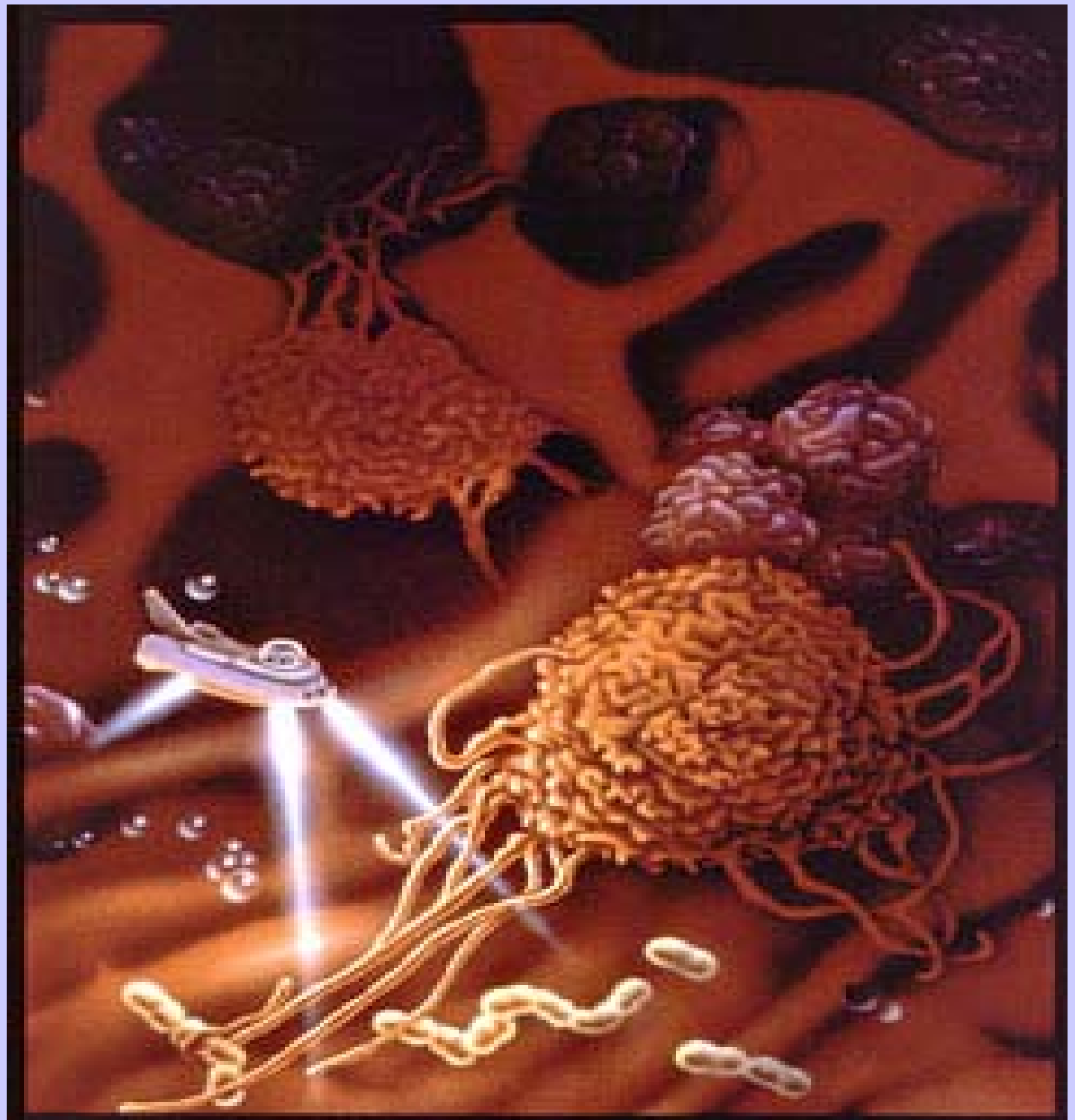
Application of metalloporphyrins for electrochemical anion sensing



System Pt-porf-Co(III)porf



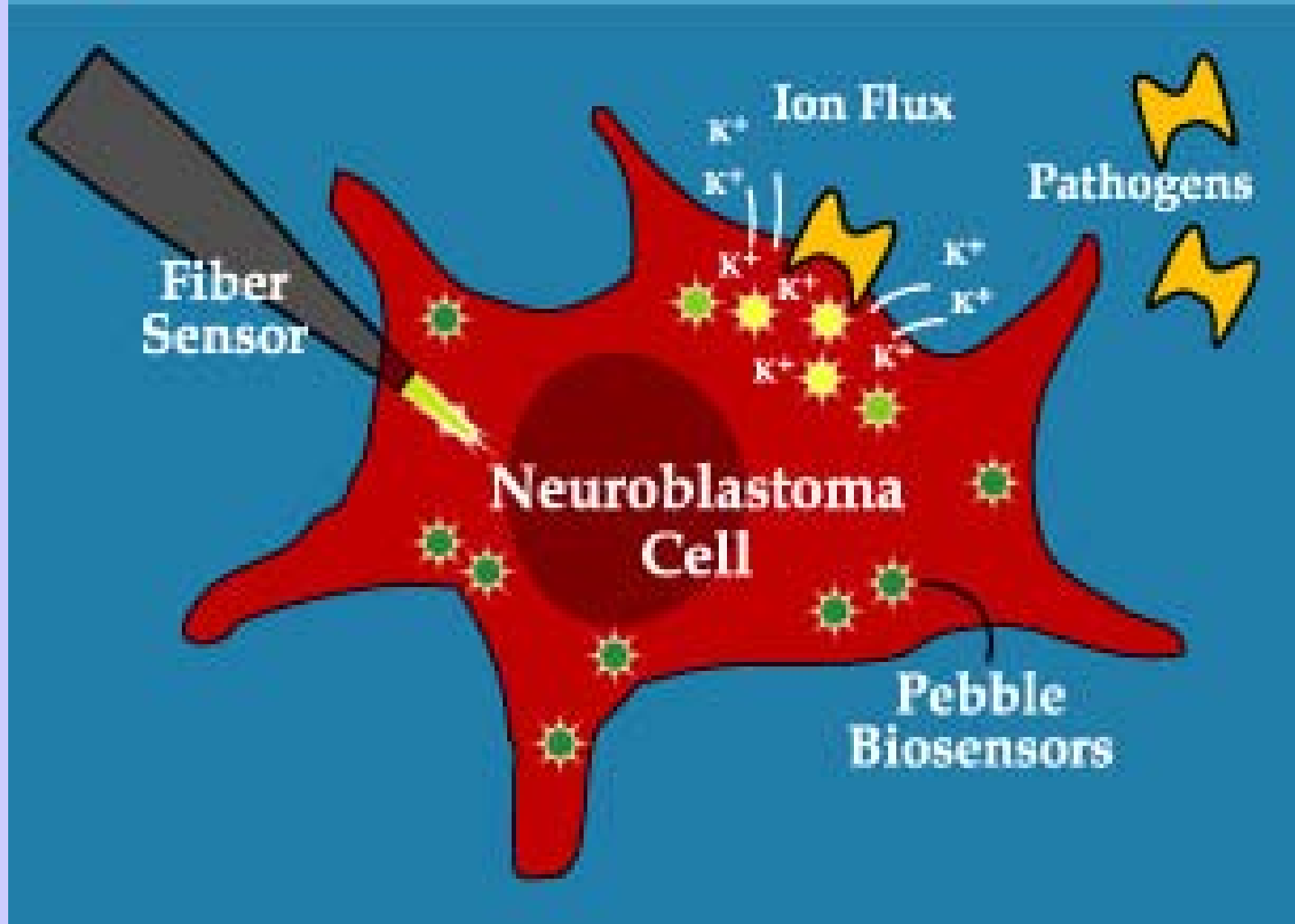
Nano- technology on the cell level





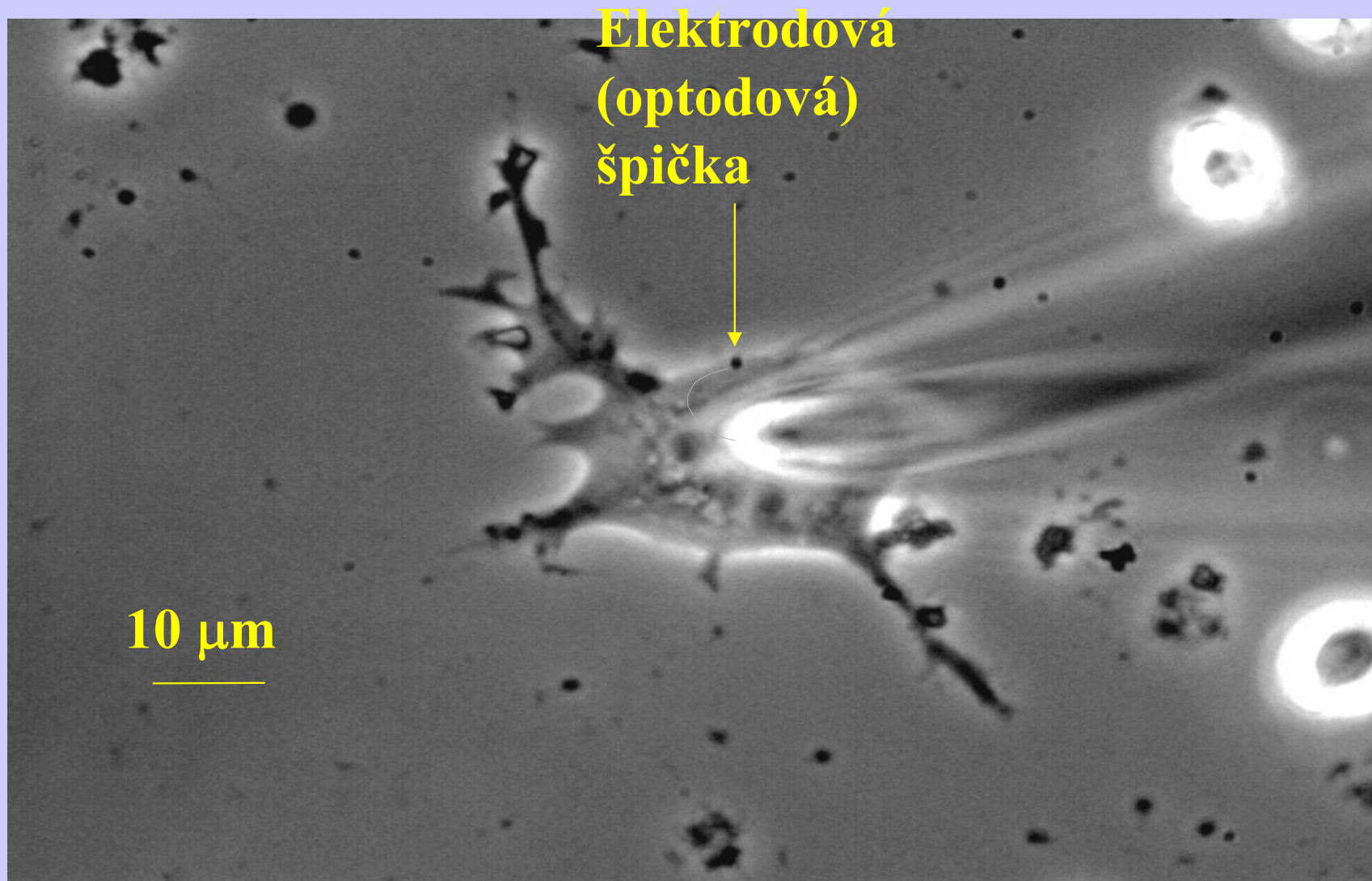
Near-Field Scanning Optical Microscopy led us to use pulled (tapered) fiber optic probes as intracellular sensors.

The tips of these fibers were either coated with a layer of plasticized PVC containing sensor materials, or they had a small bit of sensor material. Optodes can be inserted into single cells to sense analyte levels.



instead of the whole fiber, it would be ideal to take just the tip of the sensor and implant it into the cell. Fluorescent constituents would allow us to probe the signals on a standard microscope or fluorimeter.

Záznam buněčné produkce NO (jednotlivá buňka)



**Stanovení NO v biologických vzorcích - buňkách.
(Poločas života NO v biologických vzorcích je 6-30 sec.)**

Diagnosis:

individual molecular probes (free sensing dyes) are physically small enough

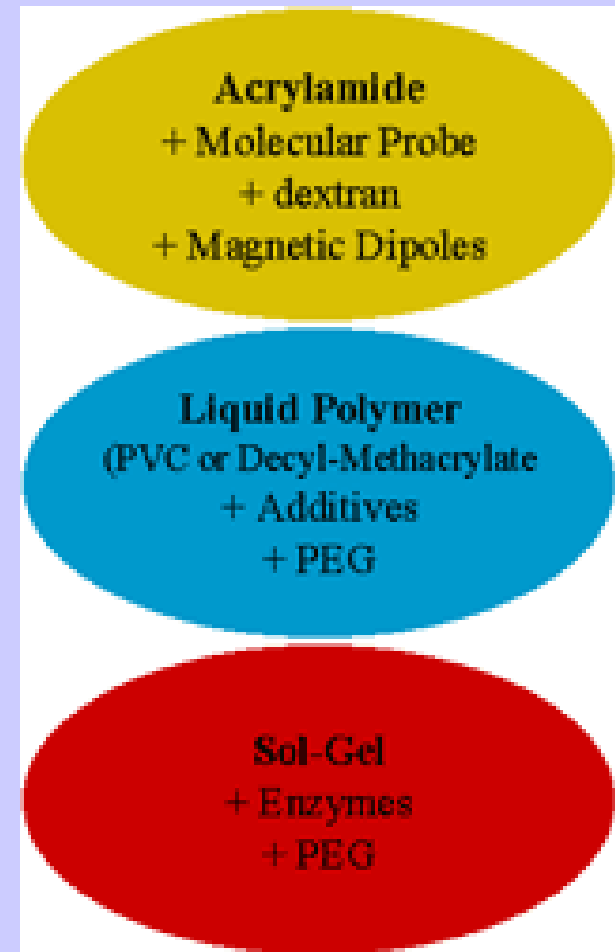
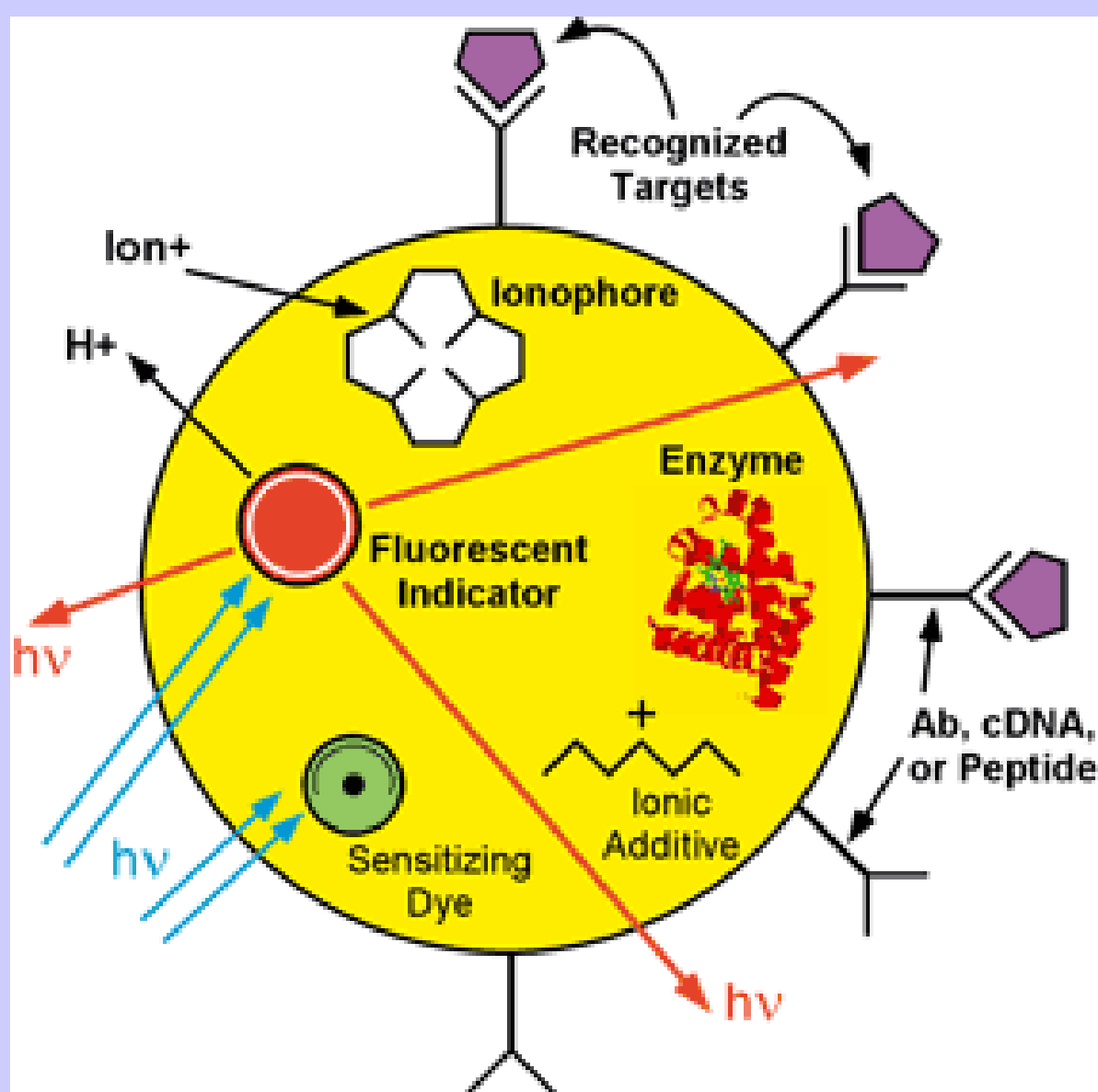
-suffer from chemical interference between probe and cellular components.

PEBBLE sensors (Probes Encapsulated By Biologically Localized Embedding) are nano-scale spherical devices consisting of sensor molecules entrapped in a chemically inert matrix.

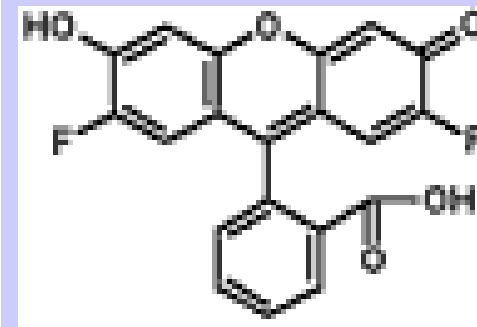
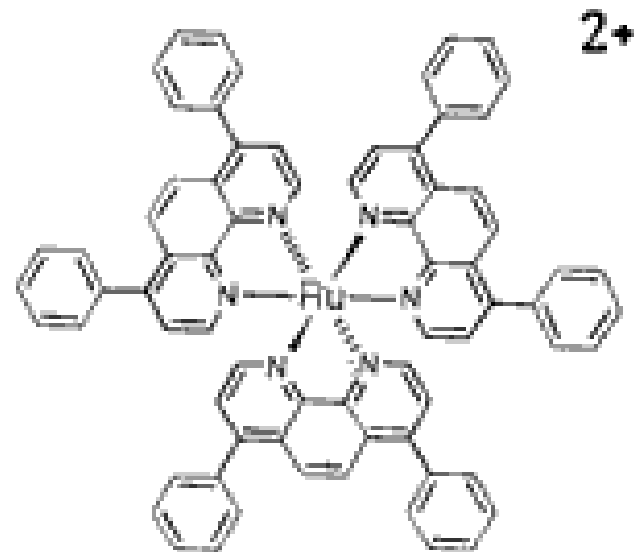
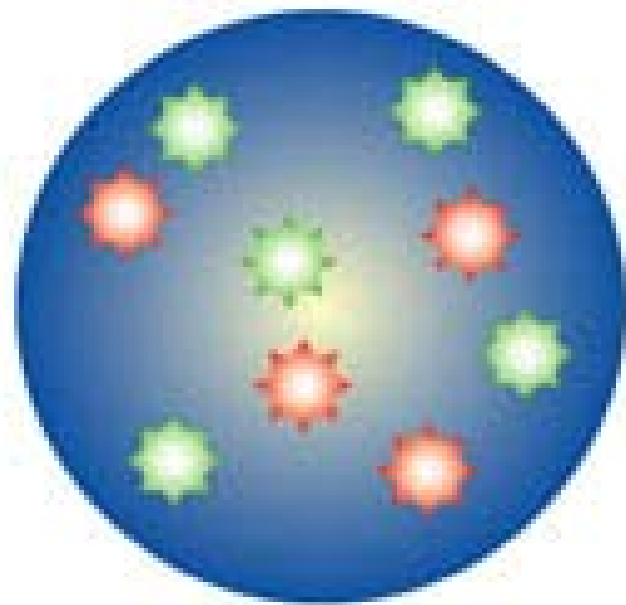
-protective coating eliminates interferences such as protein binding and/or membrane/organelle sequestration which alter dye response.

-the nanosensor matrix also provides protection to the cellular : can be used for intracellular sensing.

- inclusion of reference dyes allows quantitative, ratiometric fluorescence techniques to be used.



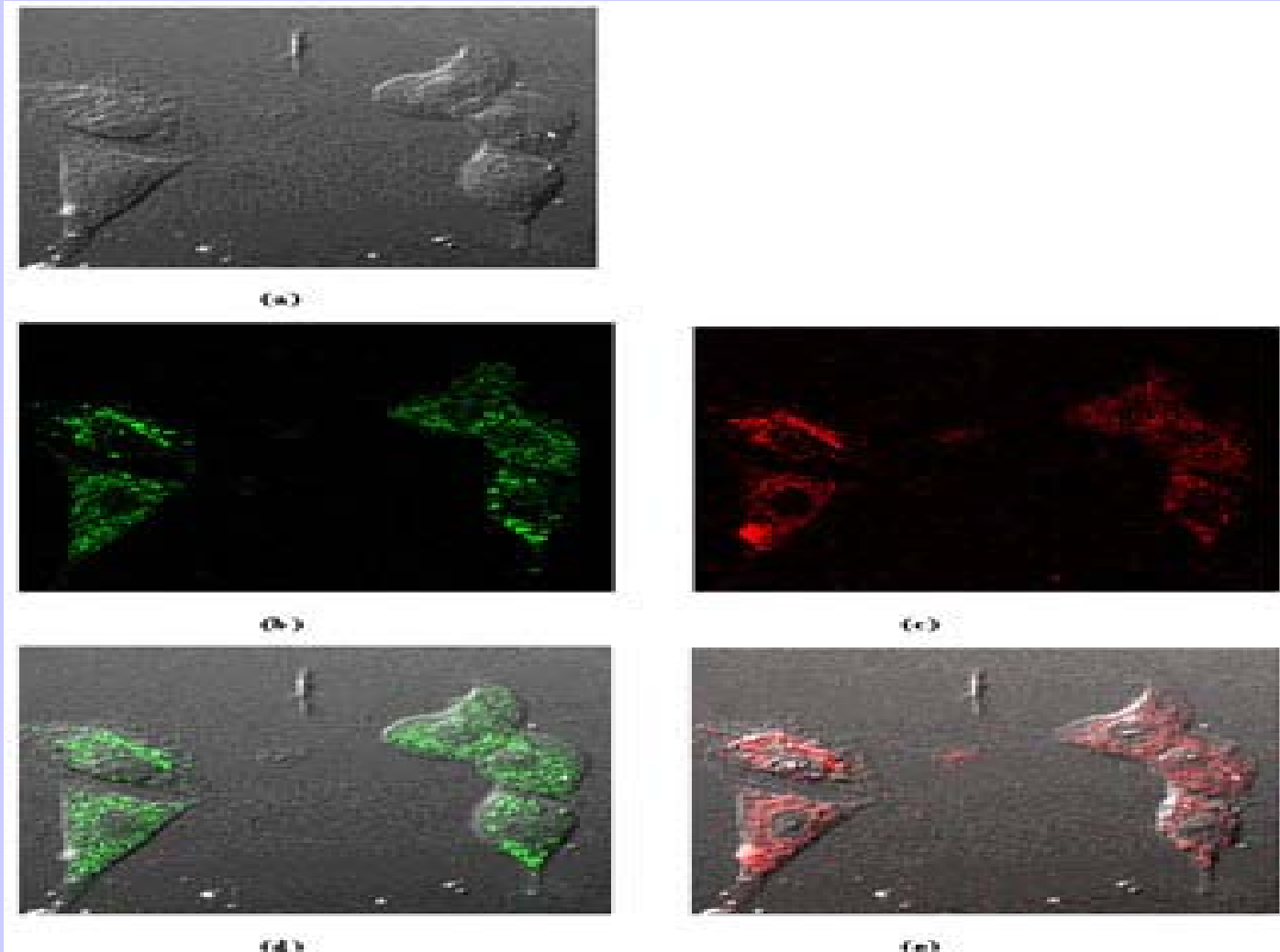
Schematic of a general PEBBLE nanosensor along with three most common matrix materials and options. PEBBLE sizes can range from 20 to 200 nm in diameter.



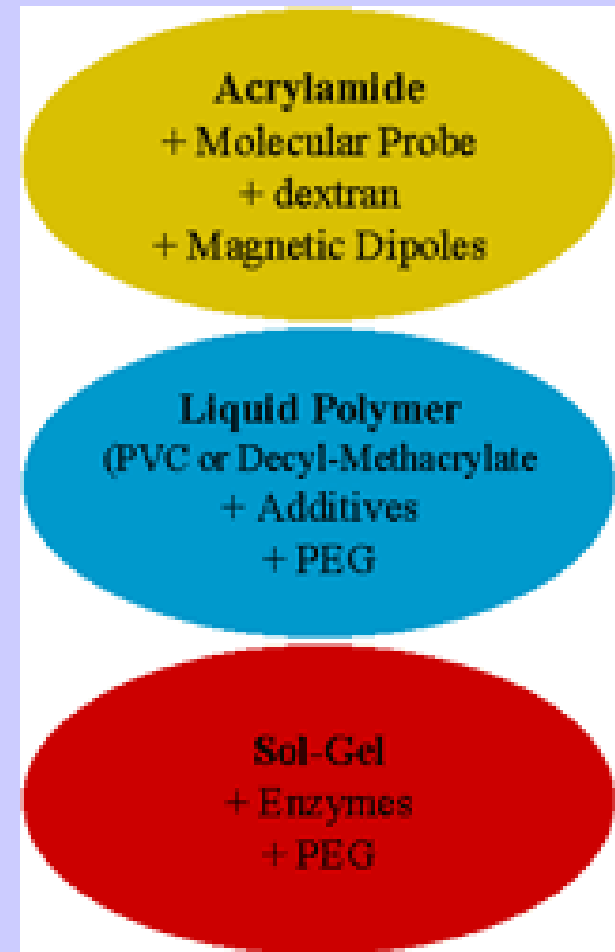
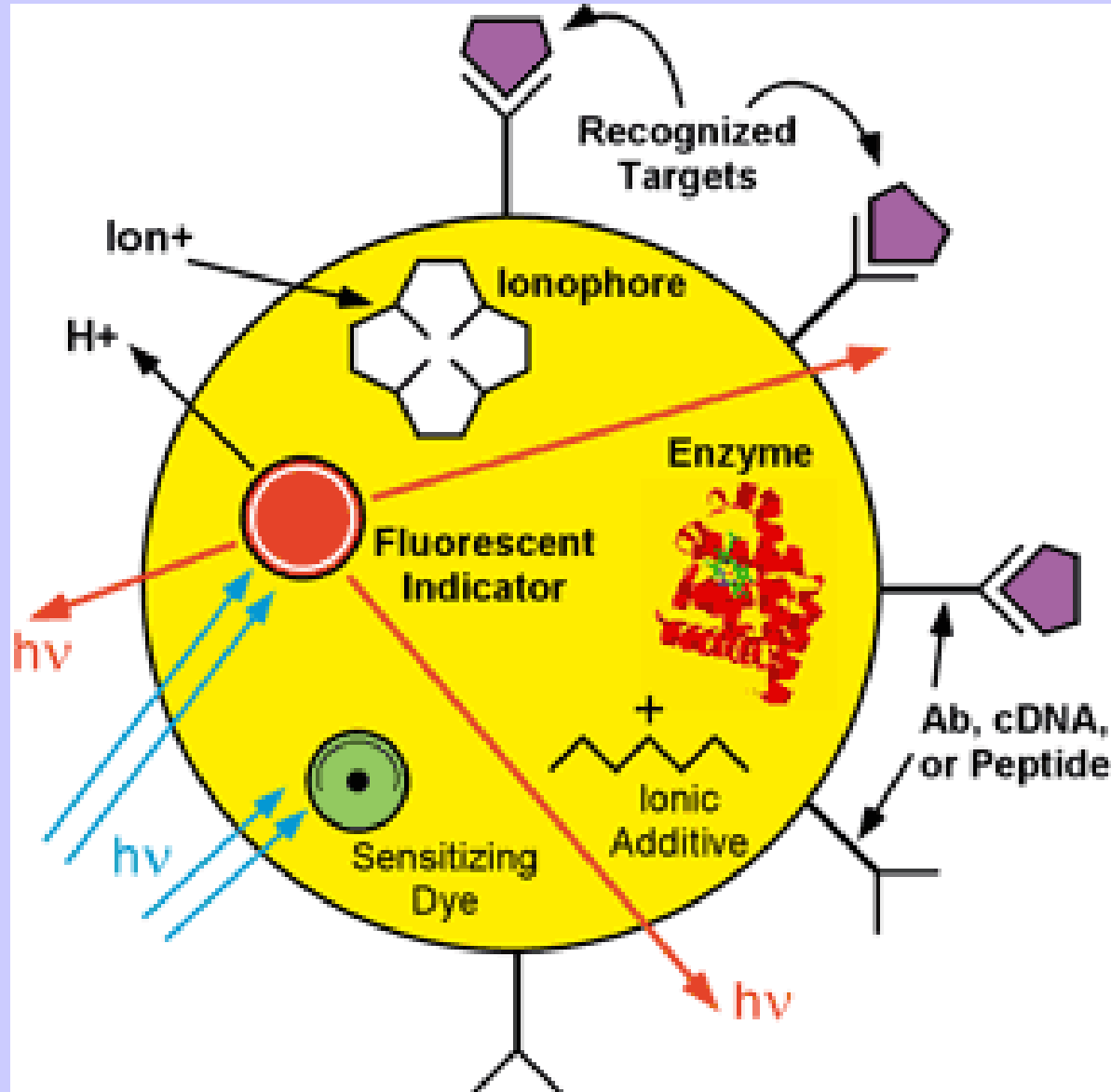
**Example of a ratiometric
PEBBLE using Ru-DPP sensing
dye and Oregon Green as a
reference dye.**

As a first example of a PEBBLE sensor, we take a ratiometric oxygen sensor. This has a Sol Gel (silica glass which is formed at low temperatures in solution) matrix and two different dyes embedded in its core. The Ruthenium dye (orange) fluorescence is quenched very efficiently in the presence of oxygen, so its intensity goes down with more local oxygen concentration. The Oregon Green dye (green) fluorescence is not sensitive to oxygen, so its intensity stays the same and can be used as a reference.

Ratiometric Imaging with Oxygen PEBBLEs



Oxygen PEBBLEs have been used for ratiometric fluorescence imaging in C6 glioma cells. This figure shows a Nomarski (differential phase contrast) image (top - a) of the cultured cells on a coverslip. The next set of images are the green and red channels (b,c) of a confocal image showing the separate fluorescence from the reference dye and the oxygen sensing dye. Computer software has then be used (d,e) to superimpose the images, showing where signals are coming from within the cells. Changes in the red fluorescence can be used to show local changes in oxygen concentration, and ratio images can be generated showing oxygen concentration based on test-tube calibrations of the sensors.



Schematic of a general PEBBLE nanosensor along with three most common matrix materials and options. PEBBLE sizes can range from 20 to 200 nm in diameter.

Materials for Use in Optical pH Sensor

pH indicator	polymer	exc/em	pK_a or pH range
chlorofluorescein octadecylester	polyurethane hydrogel (D4)	470/525 nm	pK _a 6.8
HPTS	ion exchange resin beads im hydrogel	408,468/515 nm	pH 6.2 – 8.8 pK _a 7.75
HPTS	polyacrylamide film	460/520 nm	pH 4 - 9
N9 (azo dye with reactive vinylsulfo group)	cellulose acetate or hydroxy-terminated polyurethanes	460 and 590 nm (2 λ -reflectometry)	5 – 9
phenol red	polyacrylamide beads	540 and 630 nm (2 λ -reflectometry)	physiolog. range;

From: O. S. Wolfbeis, J. Mater. Chem. 15 (2005) 2657

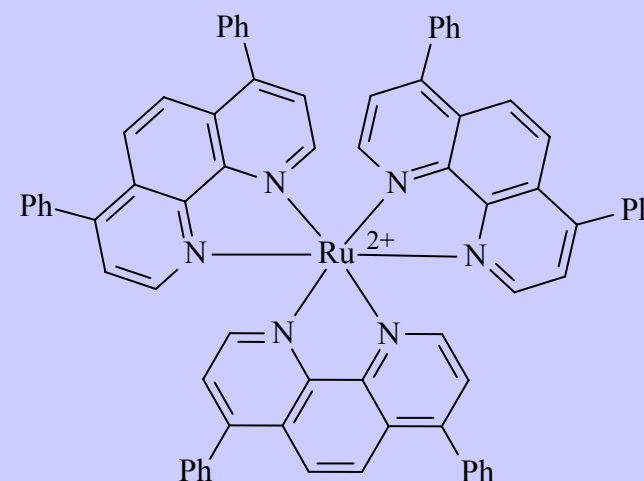
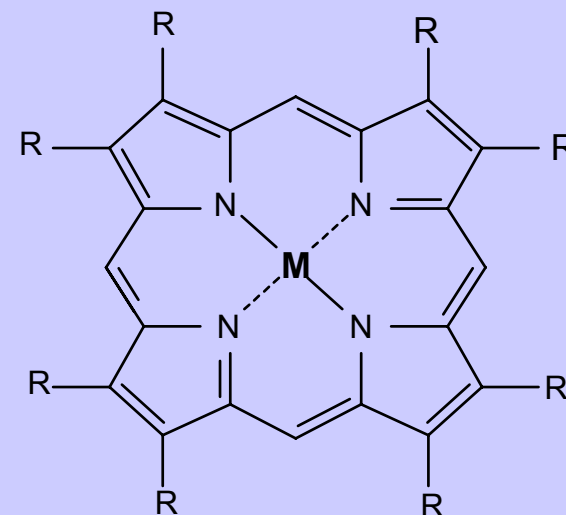
Quenchable Probes for Oxygen

Probes (Indicators) for Oxygen

Metallo-Porphyrins (also fluorinated)

Ruthenium-Diimine Complexes such as Ru(dpp)

Others: decacyclene, pyrene
Al-oxine complex
campher quinone



Dual Sensing of pH and Oxygen

Nanoparticles acting as nanocompartments for indicator chemistries

Oxygen: platinum(II) octaethylporphyrin in poly(acrylonitrile)

pH: McFOE (a fluorescein derivative) in poly(acrylamide)

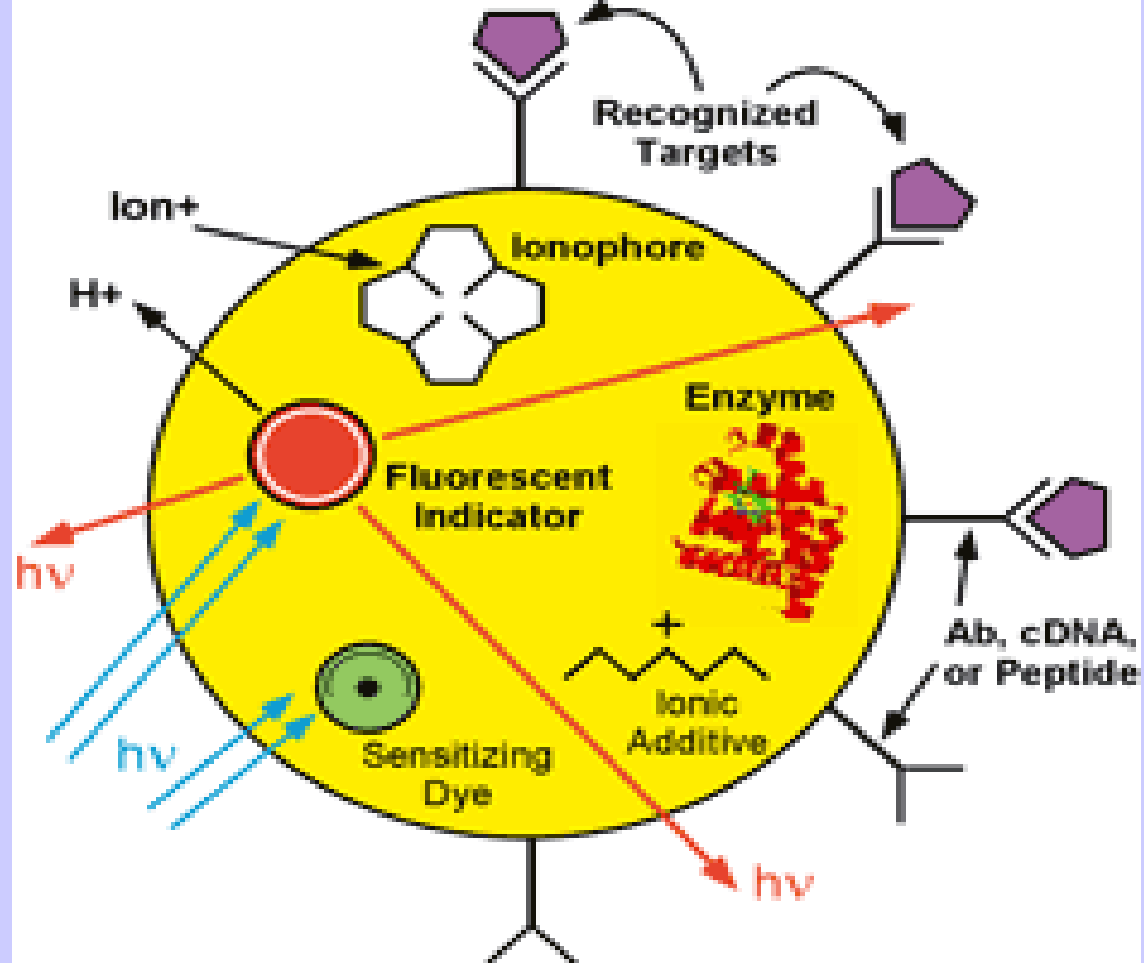
All contained in a hydrogel layer (Hypan)

Signals separated via spectra and decay times

Conclusion

- * Optical sensing enables numerous new sensing schemes**
(from planar sensing to fiber optic systems and to imaging)
- * Optical sensing enables new enzyme-based biosensing schemes**
- * Optical sensor design requires substantial expertise in**
 - material sciences, - spectroscopy, - data processing
- * Implementation of optical sensing schemes requires close interaction with experts in other areas**
(such as biochemistry biology, medicine, electronics, clinical chemistry, environmental analytical chemistry, ...)

Summary of PEBBLE accomplishments:



- 20 nm radius Optochemical Biosensors (1 ppb of cell vol.)
- Non-Invasive Chem Lab inside Live Cell
- Cell Viability 97%
- Response Time < 1 ms.
- H^+ , Ca^{2+} , K^+ , Na^+ , Mg^{2+} , Cl^- , NO_2^- , O_2 , NO, Glucose...
- Targeted Photo-Dynamic & MRI Contrast Platforms using similar technologies

- Redukcí solí kovů



- Nanočástice

Kovů (Au, Ag, Cu, Zn, Ni, Pt, Pd)

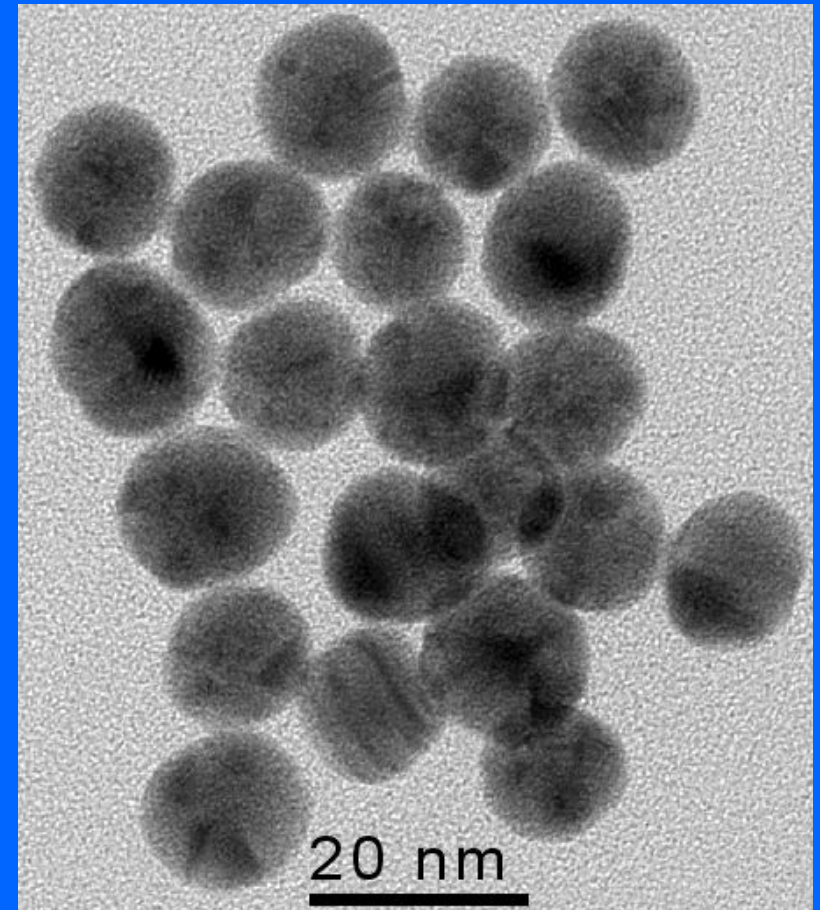
Oxidů (SiO_2 , TiO_2)

Sulfidů (CdS)

Selenidů (CdSe)

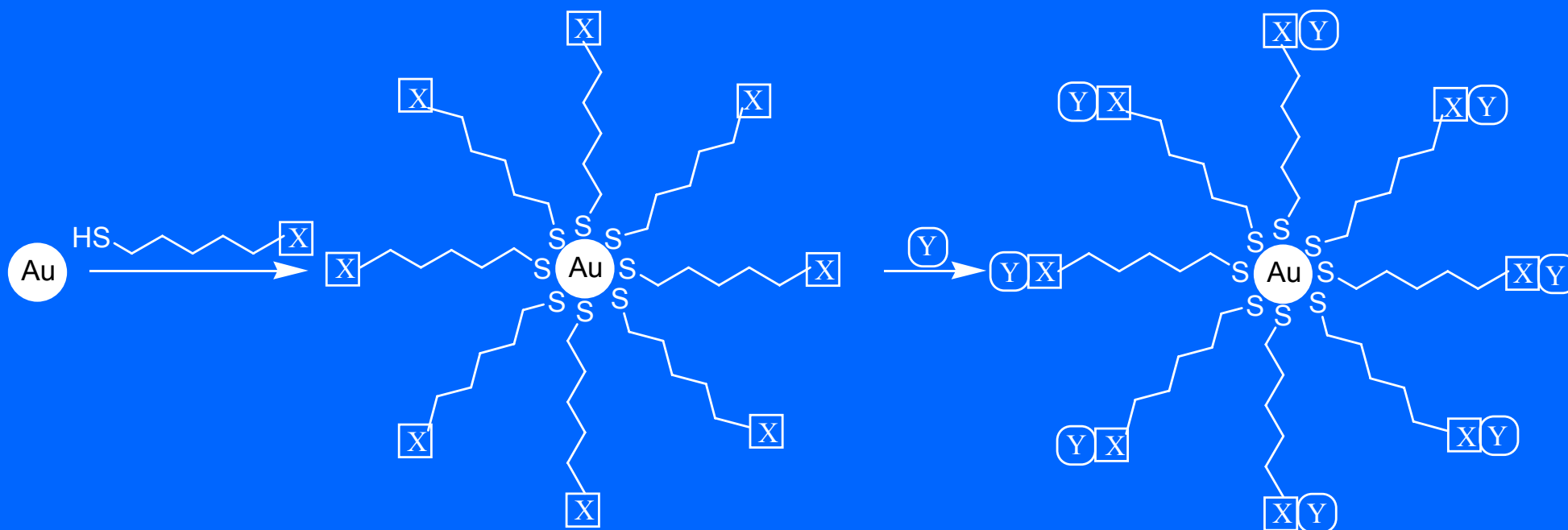
Bimetalické (Ag/Au, Ag/Pt)

Polymerní (kopolymery, dendrimery)



Obrázek 1. Snímek z TEM nanočástic zlata získaných redukcí citrátem

Modifikace



Obrázek 2. Schéma modifikace povrchu nanočástice,
X – funkční skupina, Y – látka mající funkční skupinu interagující s X

Charakterizace

TEM, SEM, AFM, XFS, RLS, DTA, SERS, IR, NMR,
elektrochemické metody

- **Voltametrické metody**

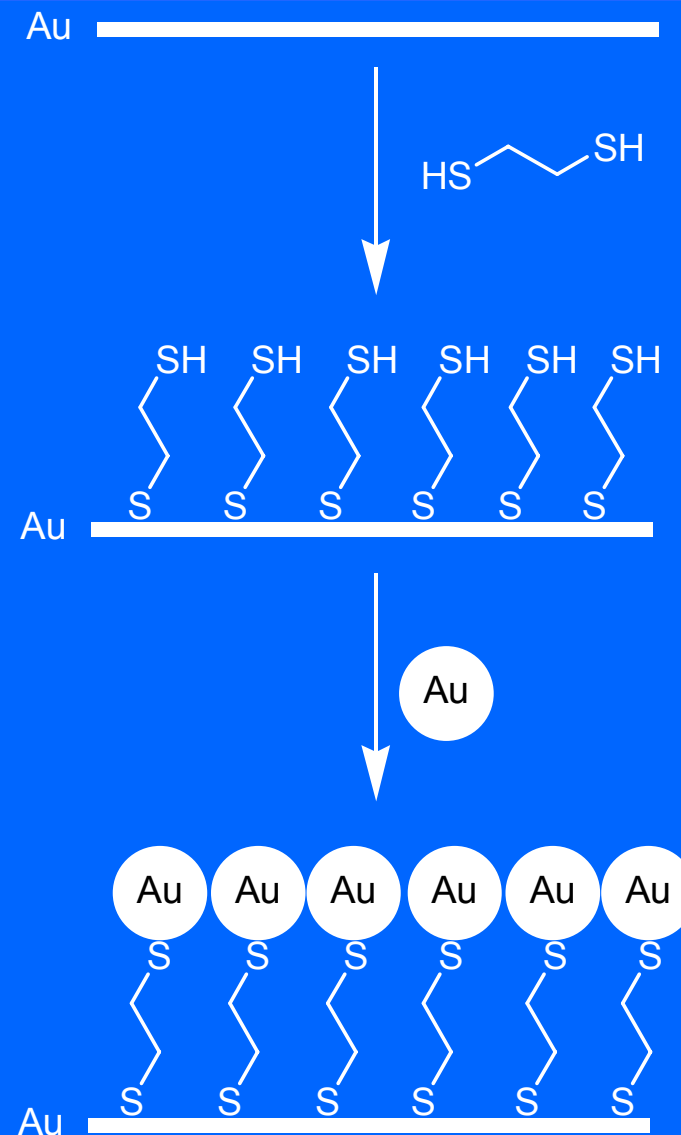
Elektrody a mikroelektrody
s imobilizovanými nanočásticemi

- **Potenciometrie**

Iontově selektivní elektrody,
senzorické pole, elektronický jazyk

- **Použití**

Stanovení As^{3+} (LOD = 0,09 ppb),
 Cu^{2+} , NO_3^- , S^{2-} , pesticidy, herbicidy,
těkavé organické látky, stanovení
koncentrací léčiv, analýza biologických
vzorků (hemoglobin, glukosa),
detekce sekvence DNA



Obrázek 3. Schéma imobilizace nanočástic zlata na povrch modifikované zlaté elektrody

- **Absorpční spektrometrie**

Využití plasmonové rezonance – selektivní stanovení iontů alkalických kovů ve vodném prostředí

Biomolekulární interakce (modifikace peptidy, enzymy, léčivy);
LOD = 1 nmol/l

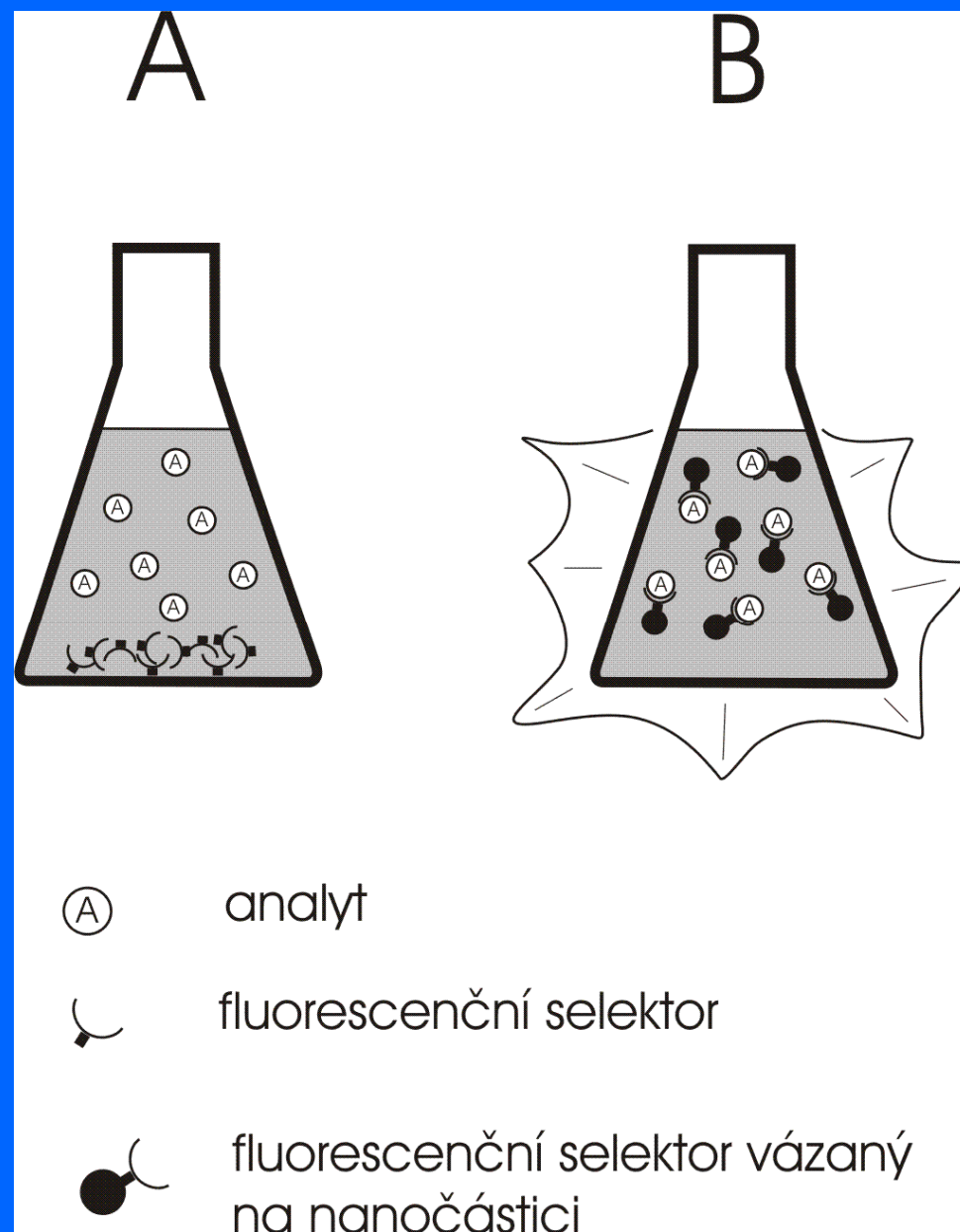
- **Rozptylové techniky**

SERS, RLS (identifikace barviv, detekce biomarkerů rakoviny, detekce léčiv)

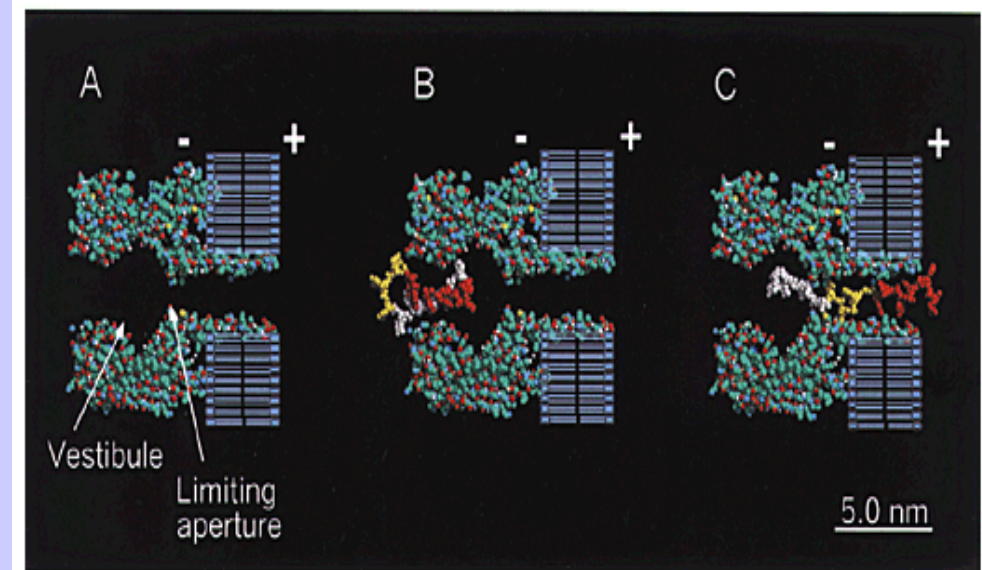
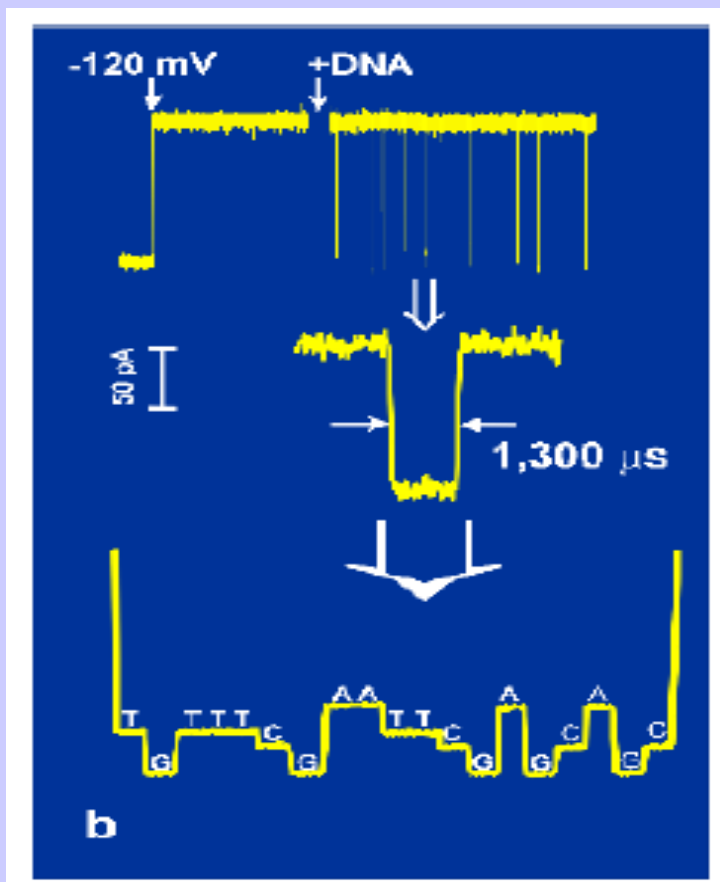
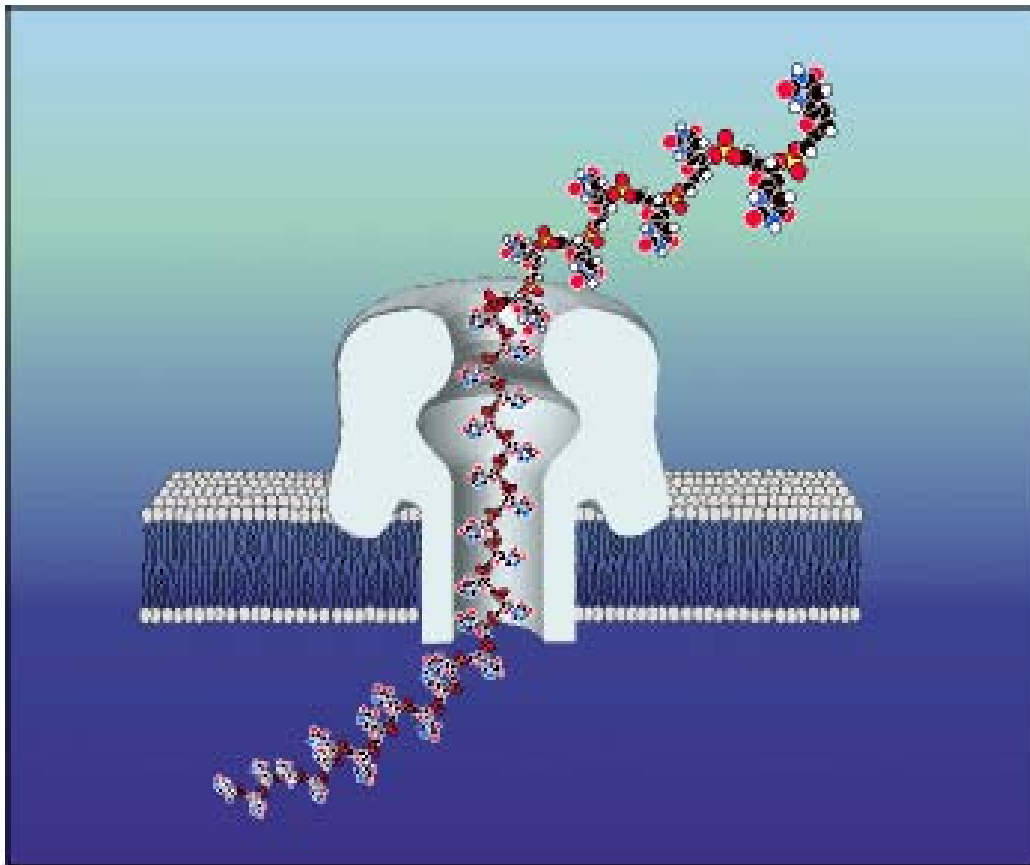
- Luminiscenční techniky**

NP modifikované fluorescenčními látkami (detekce sekvencí DNA, lysozymu, Hg^{2+} , Cr^{6+})

Obrázek 4. Použití ve vodě nerozpustné fluorescenční značky pro stanovení analytu ve vodném prostředí. (A) použití fluorescenční značky bez imobilizace na nanočástice, (B) po imobilizaci na nanočástice.



Single-stranded nucleic acid molecules passing through a nanometer-sized pore modulate the ionic conductance across the membrane. This observation may one day lead to a device for single molecule DNA sequencing.

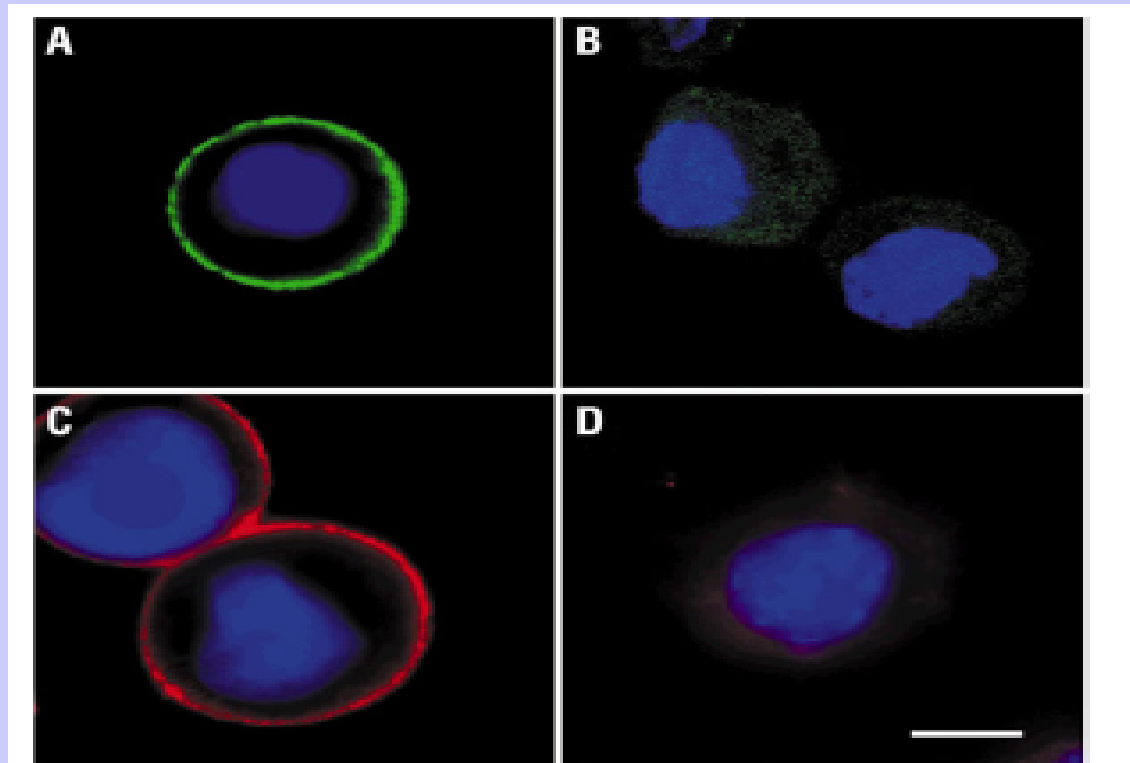


QUANTUM DOTS



Semiconductor quantum dots are being developed for use as probes for intracellular structures.

Use: to label the breast cancer marker Her2 on the surface of fixed and live cancer cells



Nature Biotech., 2003, 21:41-46

The background of the slide is a repeating pattern of green frogs with dark green spots. In the middle-right section of the pattern, one frog is colored red, standing out from the rest.

What is biomarker

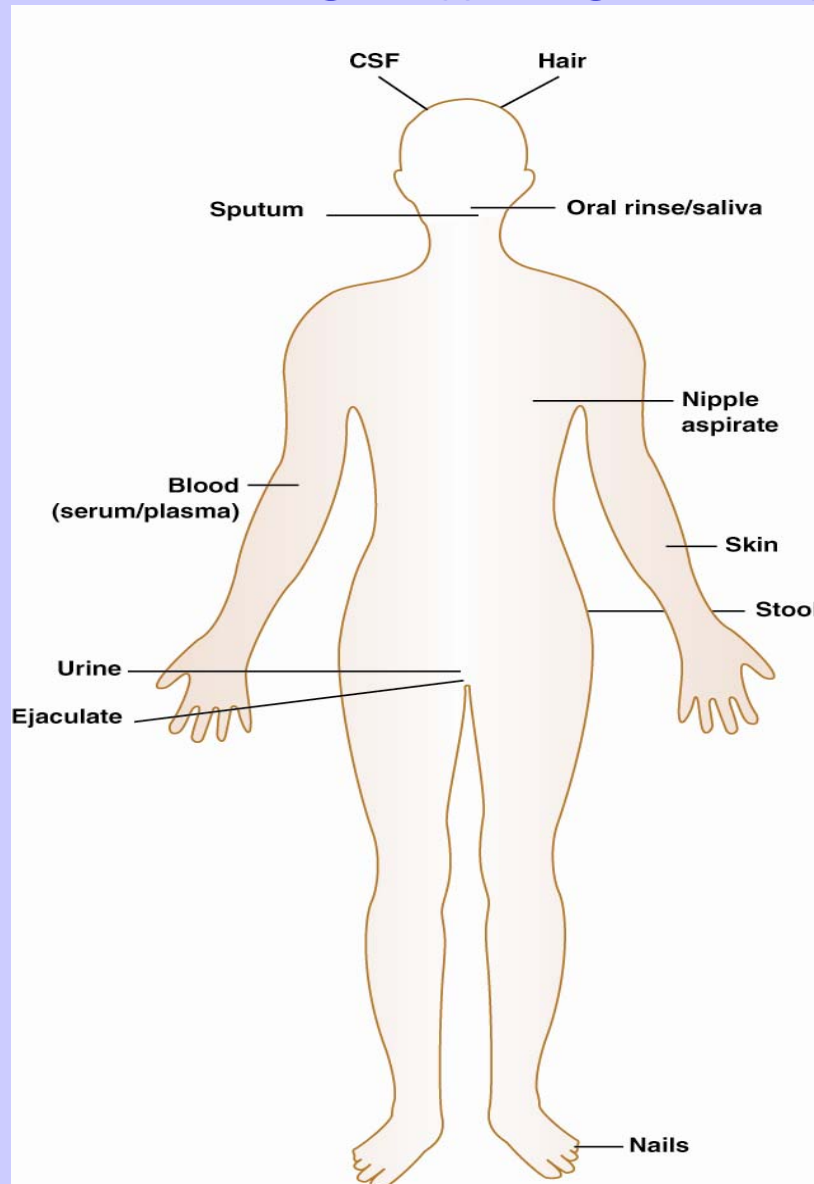
A molecular indicator of a specific biological property; a biochemical feature or facet that can be used to measure the progress of disease or the effects of treatment

Tumor markers are produced either by the tumor itself

or by the body in response to the presence of cancer

or certain benign conditions

Sources of Human Cellular Samples for Biomarker Analyses



Sidransky. *Horizons in Cancer Therapeutics*. 2004;5:13-26.

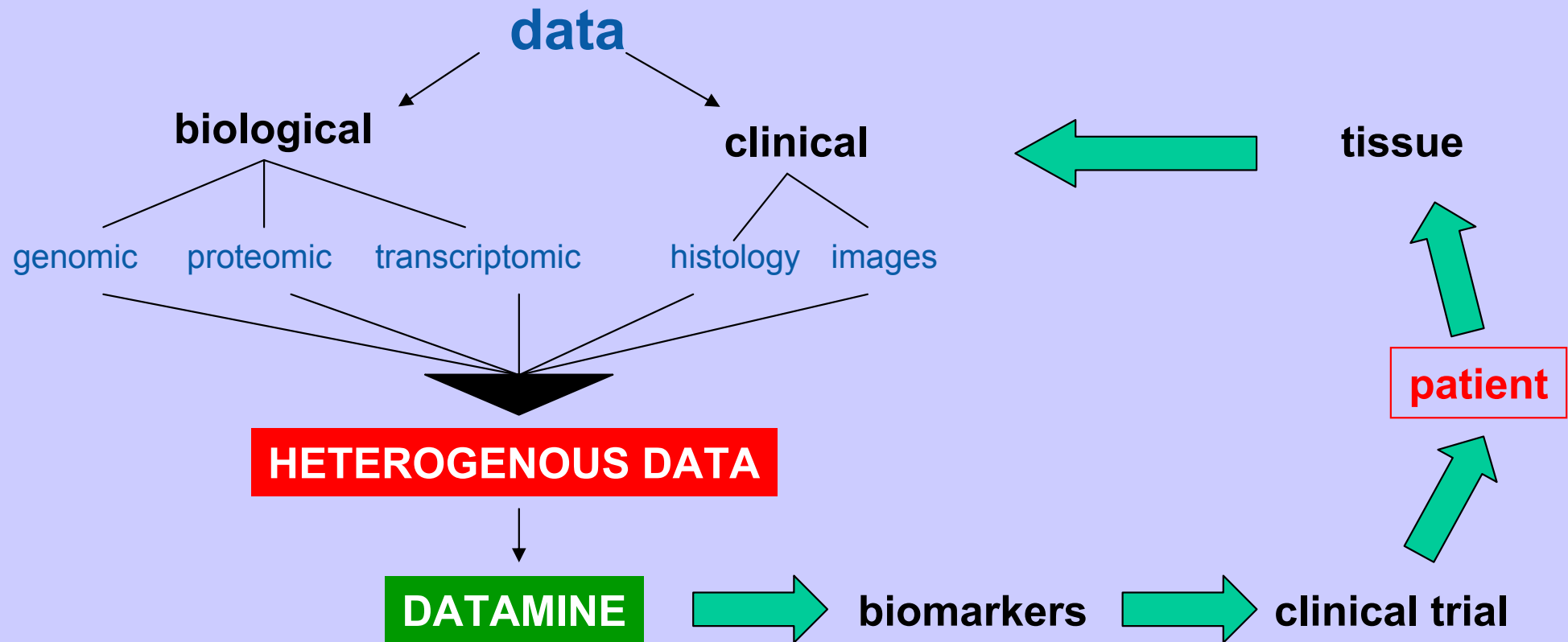
Establishing/validating biomarkers

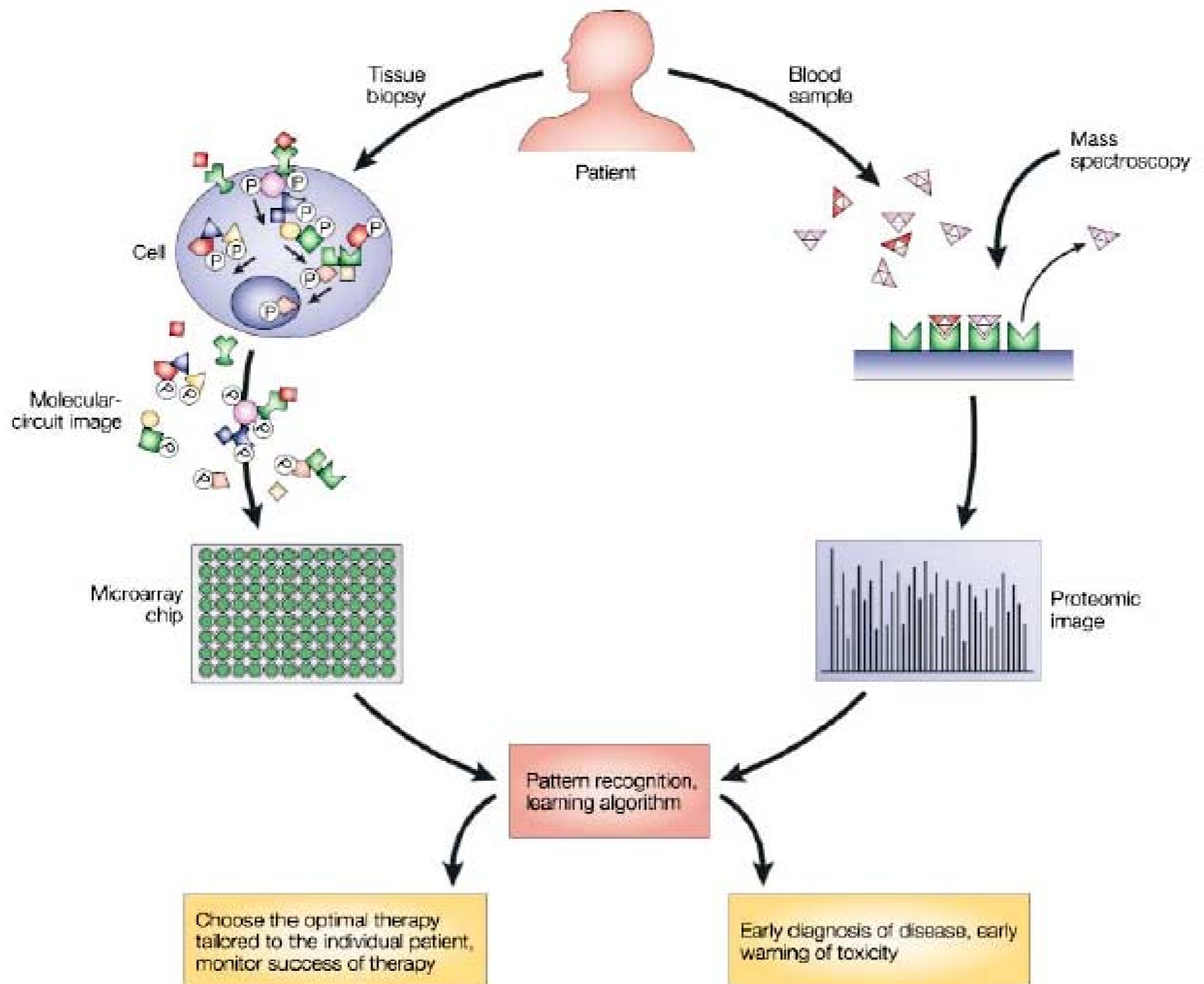
- **Identify candidate**
- **Assays to determine biomarker**
- **Assays to diagnose disease**
- **Detect pre-clinical disease (prediction)**
- **Prospective screening**
- **Quantification of overall impact on disease**

Biomarker examples

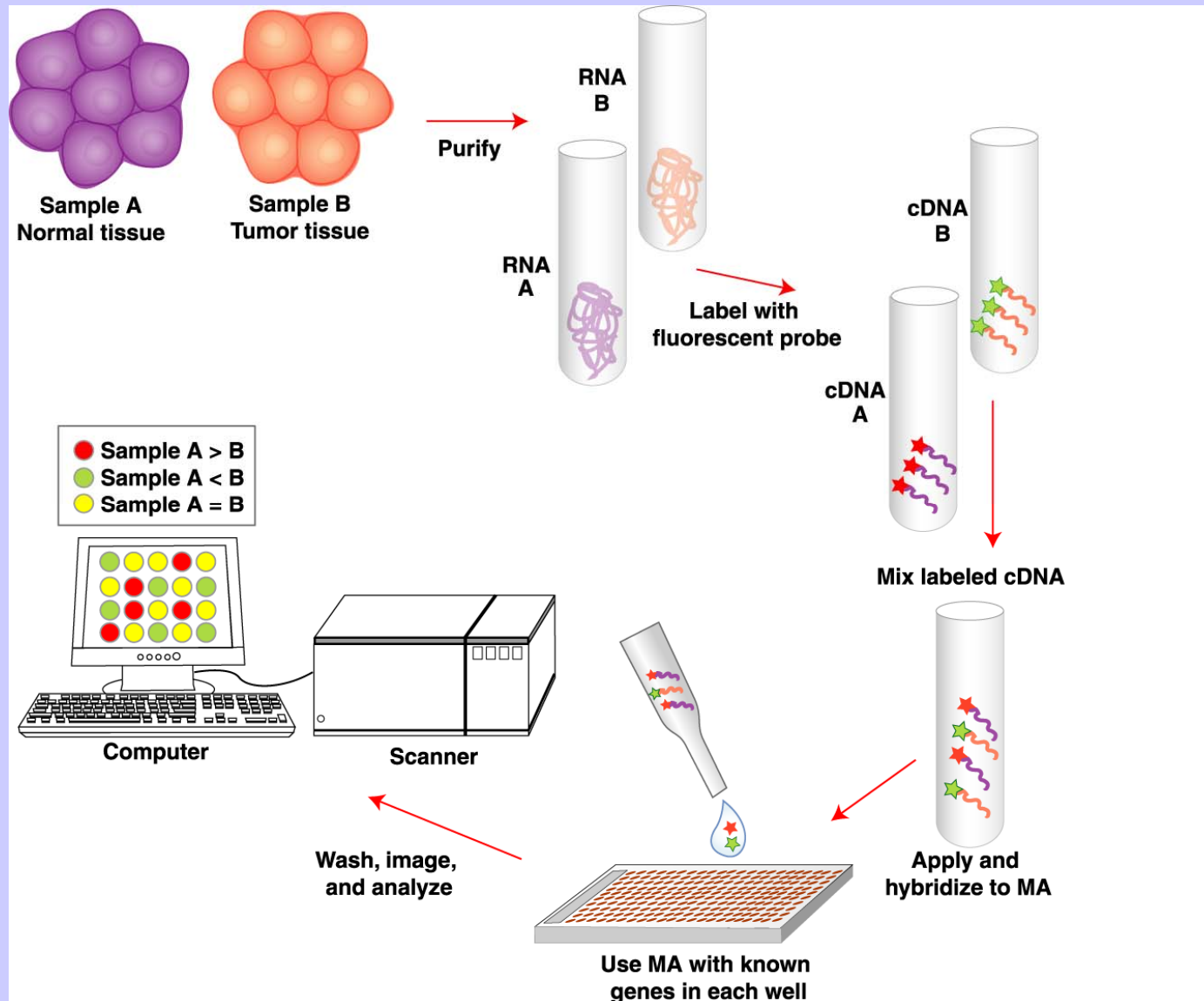
- *P53* mutations
- anti-apoptotic enzymes (e.g. survivin)
- Luteinizing hormone receptor
- Adenosine receptor
- Folic acid receptor
- Cholesterol receptor
- Sialic acid
- Hyaluronic acid
- Heparan sulphate

Translational Cancer Research & Clinical Bioinformatics





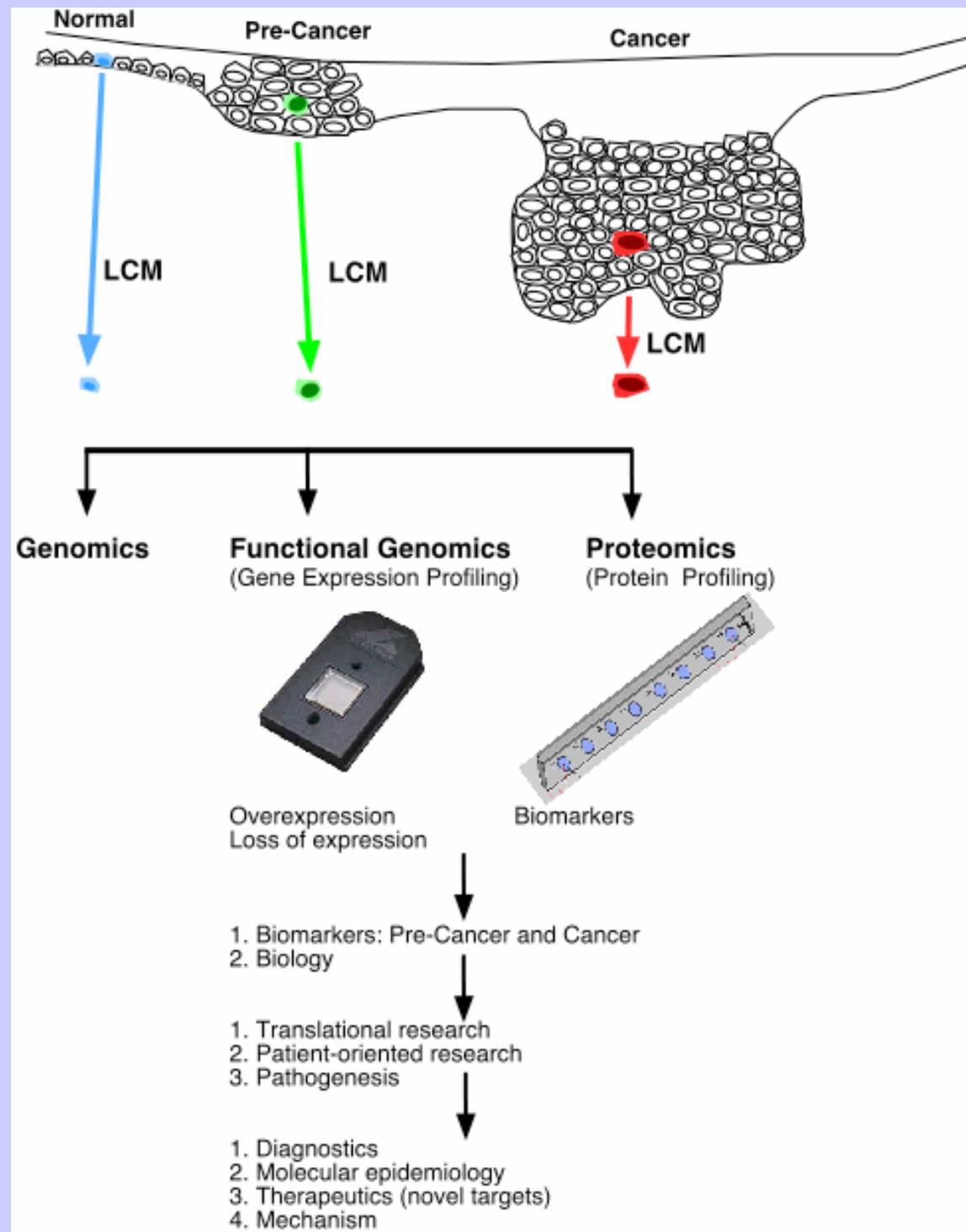
Microarray (MA) Gene Expression Analysis



Trink and Sidransky. *Horizons in Cancer Therapeutics*. 2004;5:4-12.

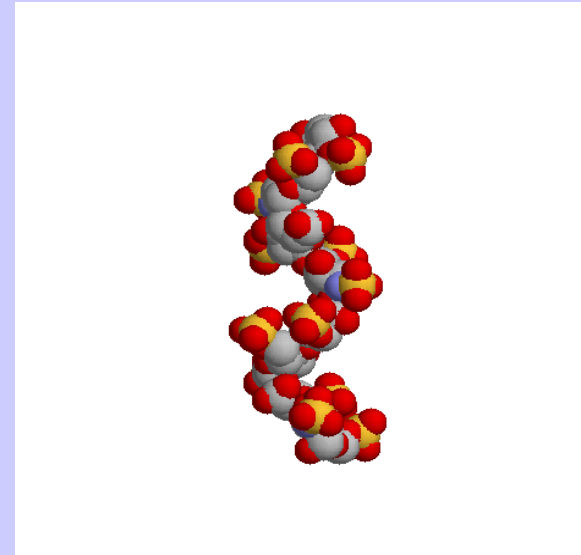
Proteomic techniques and biomarker discovery

Technique	High-throughput	Labour intensive	Cross Experimental comparasion	Cost	Aplication in diagnosis
2D-PAGE/ MALDI-MS	++++	++++	++++	++++	+
LC/MS/MS	++++	+++	++	++++	+
SELDI-TOF	++++	+++	++++	++++	+++
Ab microarray	++++	++	++++	++++	+++
Tissue microarray	++++	++	+	++++	++



Saccharides tumor marker

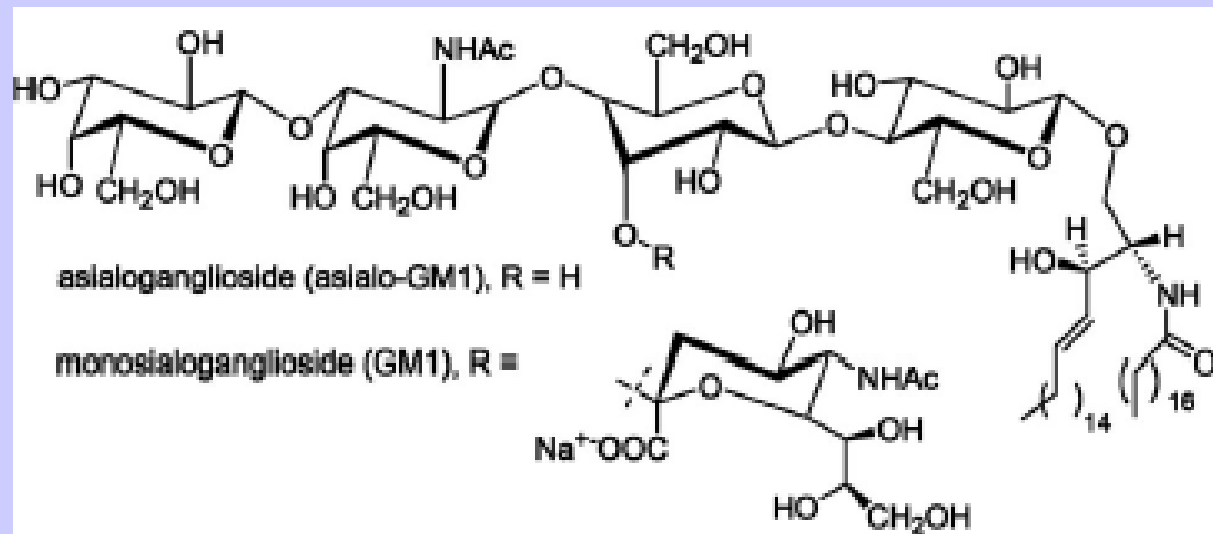
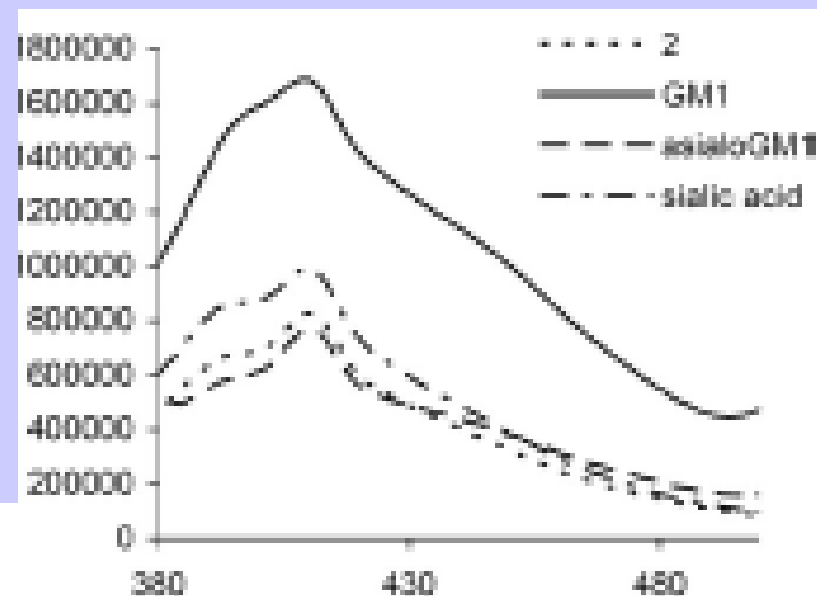
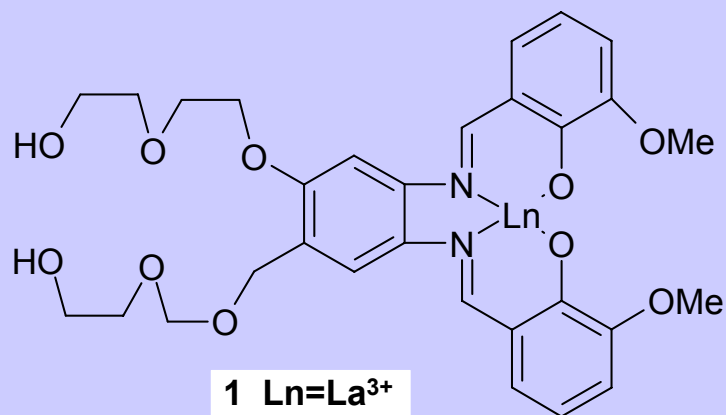
- Sialic acid
- Lewis structure
- glycan
- Hyaluronic acid
- Heparan sulphate



Sensing of cancer saccharides in water

- 1) Boric acid sensor- pH problem
- 2) Sensor of lanthanide
- 3) Sensor with hydrophobic cavities

Lanthanide sensors

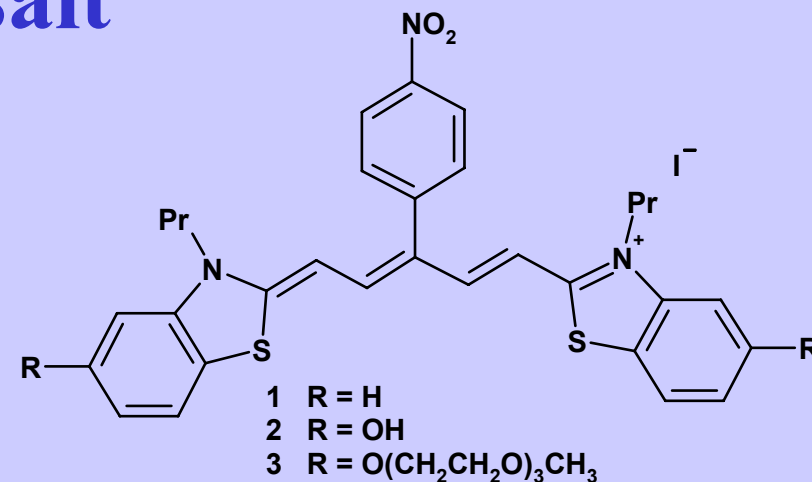
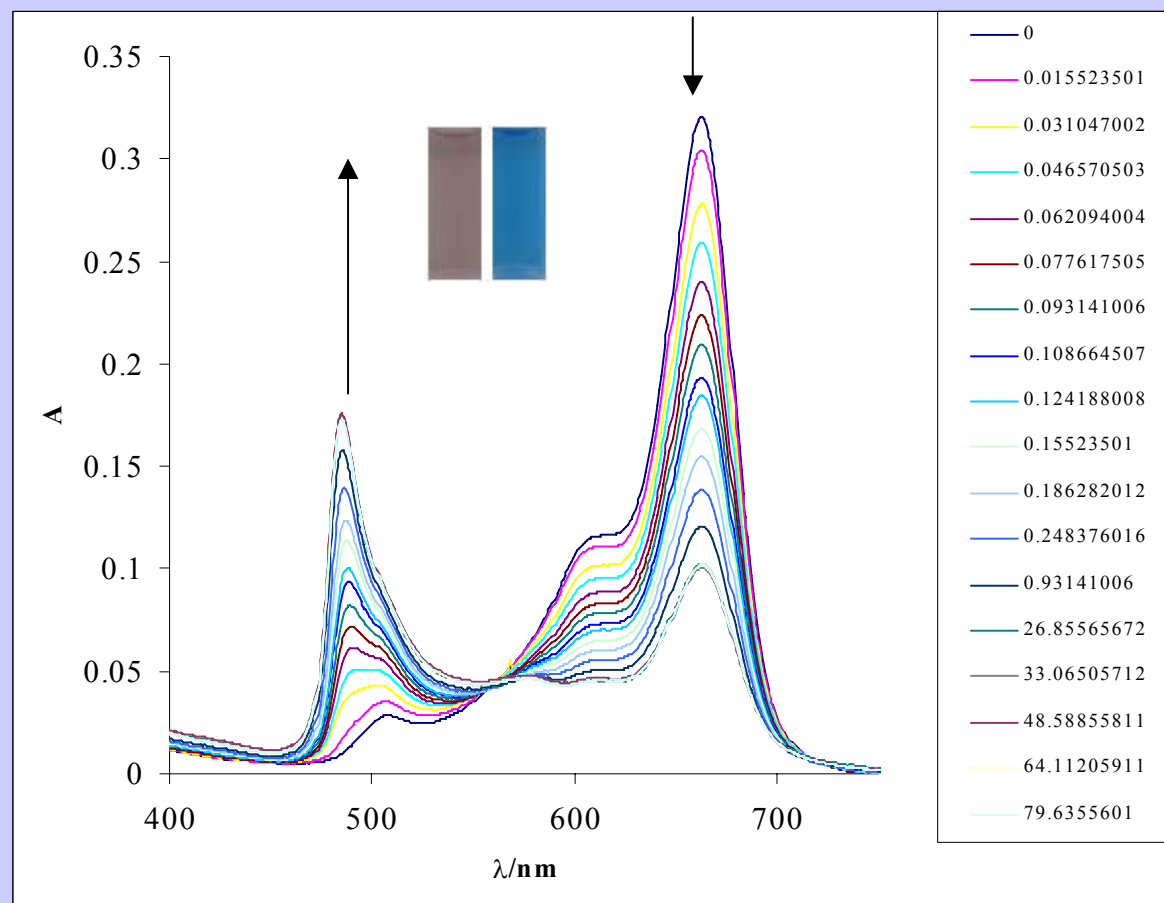


The structures of asialo GM1 and GM1.

Fluorescence intensity change (Ex. $\lambda=360\text{nm}$) of **2** ($5.53 \times 10^{-6}\text{ M}$), GM (10^{-4}M), sialic acid (10^{-3} M), pH 7, 0.1M Hepes

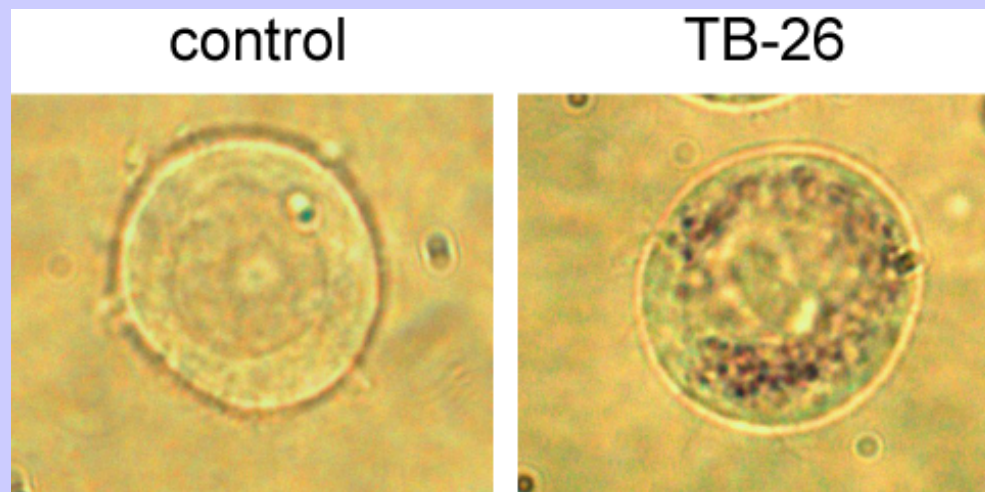
Alpturk, Rusin*, Fakayode, Wang*, Escobedo, Warner, Crowe, Kral, Pruet, Strongin PNAS 2006, 103 9756–9760

Polymethinium salt

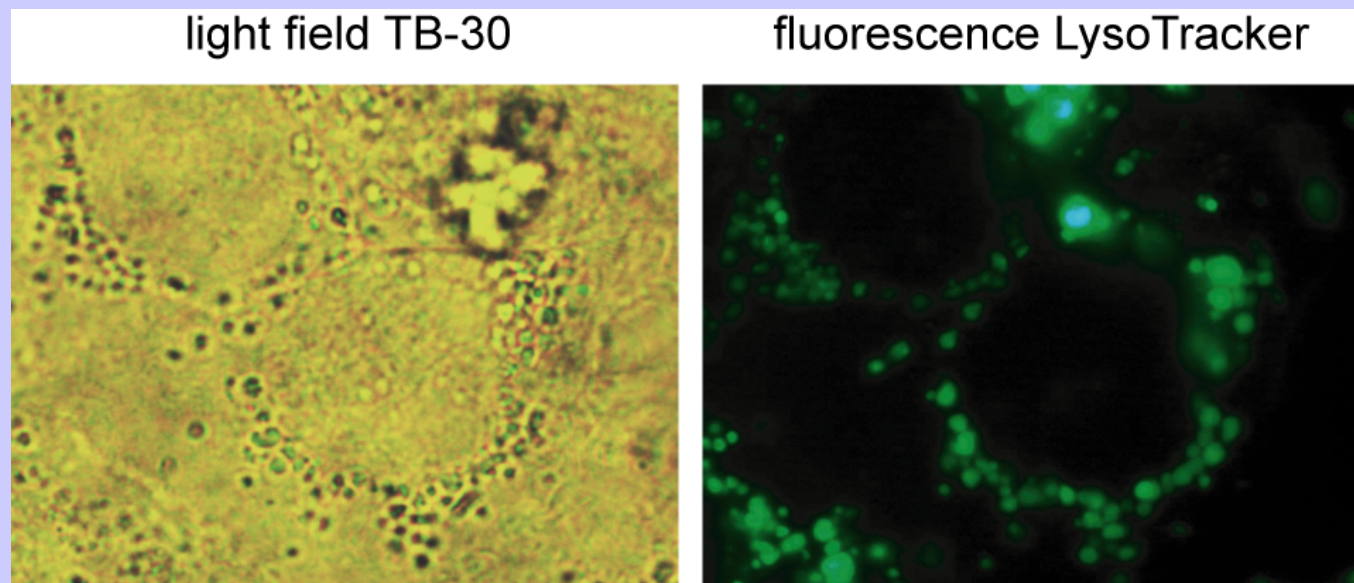


Titration of **3** ($c=4.9 \times 10^{-6} \text{ mol/l}$) by heparan sulfate (HS) in solution of 85% H_2O /13% PEG $n=9$ 2% MeOH, 1mM phosphate buffer pH = 5.53, number of equivalents are included in legend

C of **3** and HS are 1.4×10^{-5} and 5×10^{-6} in cuvette respectively

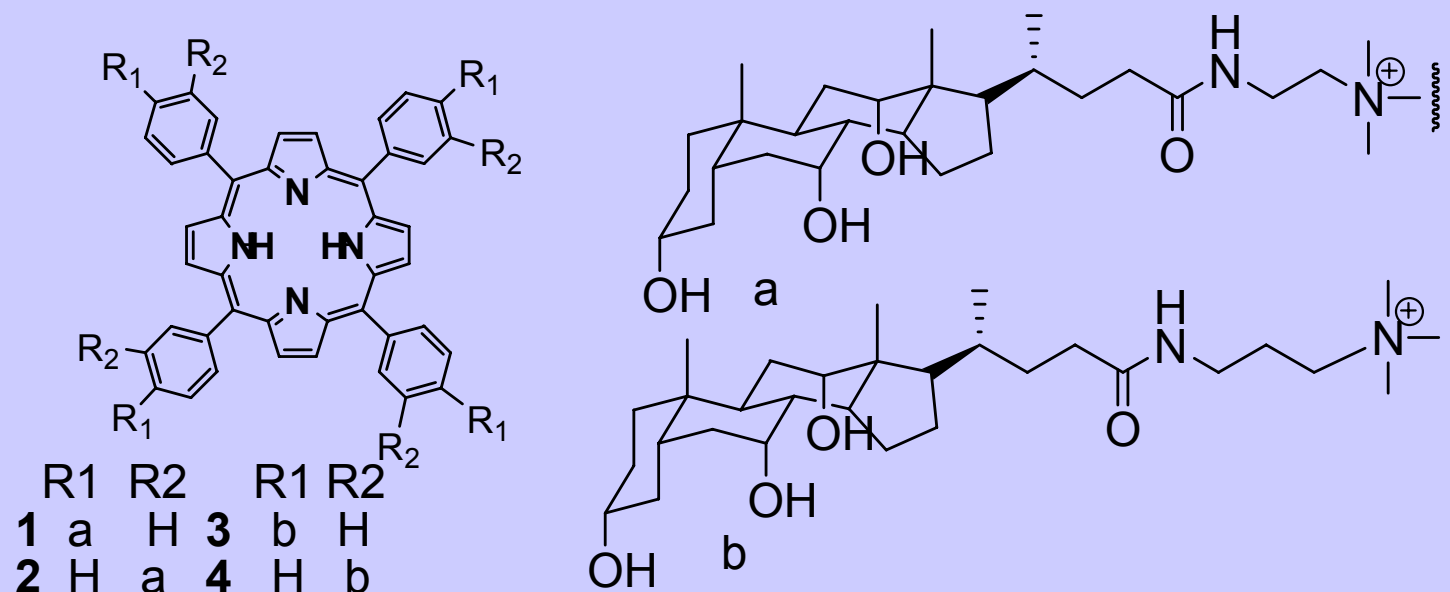


HL60 cells (human promyelotic leukemia) with $2.5\mu\text{M}$ **1**. -1 hour - live



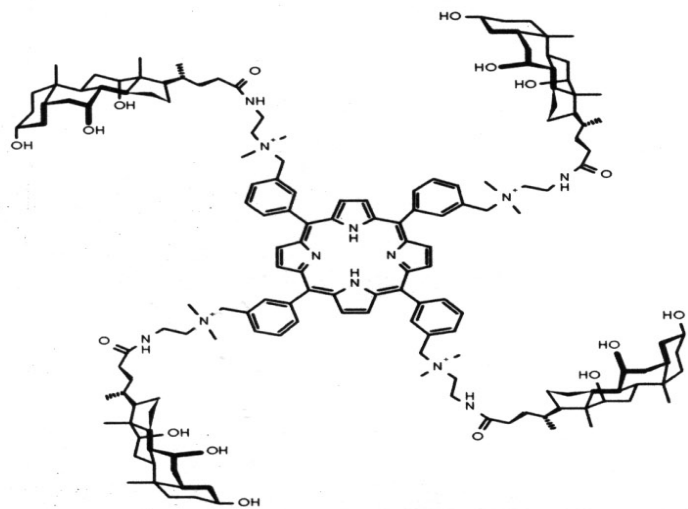
cells localization of **2** (violet),lysosome-specific probe LysoTracker (green fluorescence) in mammary carcinoma cells 4T1

Bile acid porphyrin conjugate

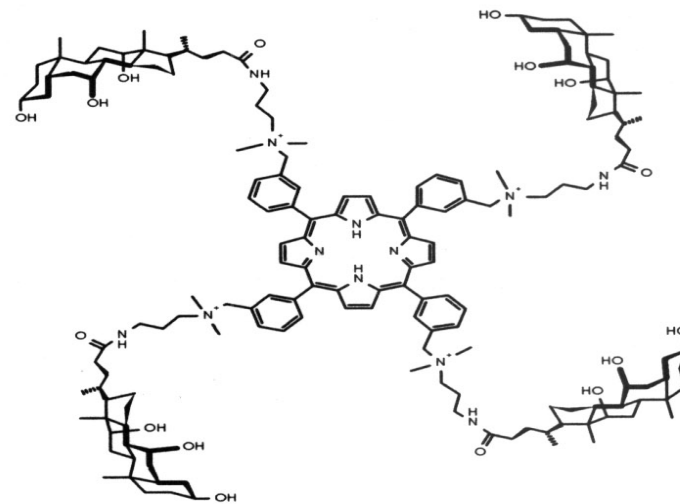


Sensor for saccharides cancer marker(sialic acid, hyaluronic acid, Heparan sulfate)

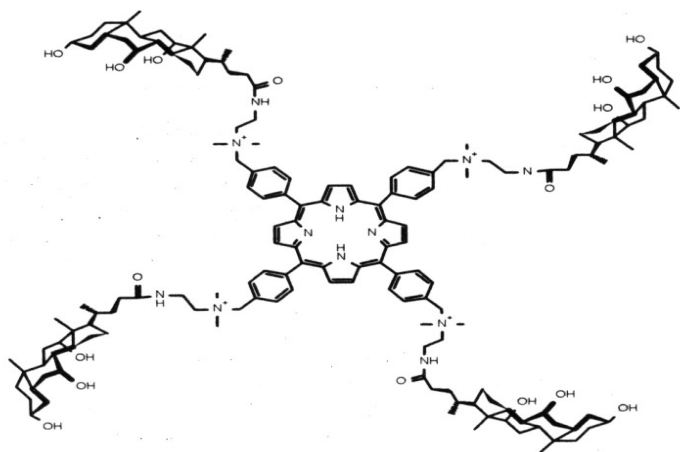
Enhanced Tumor Detection Using Saccharide Receptor-Targeted Fluorescent Conjugates



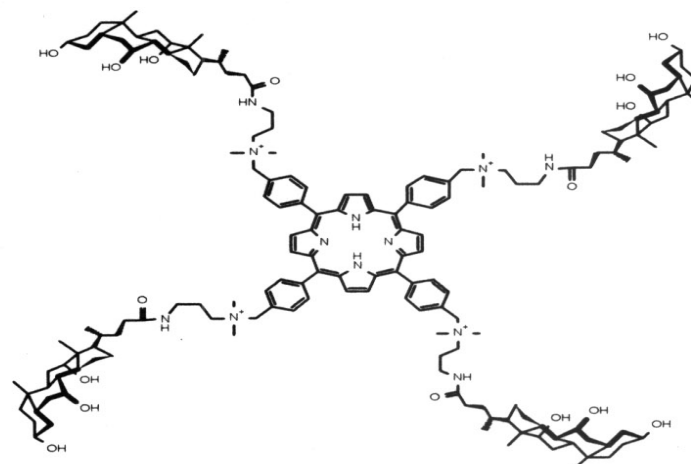
Receptor 2



Receptor 3

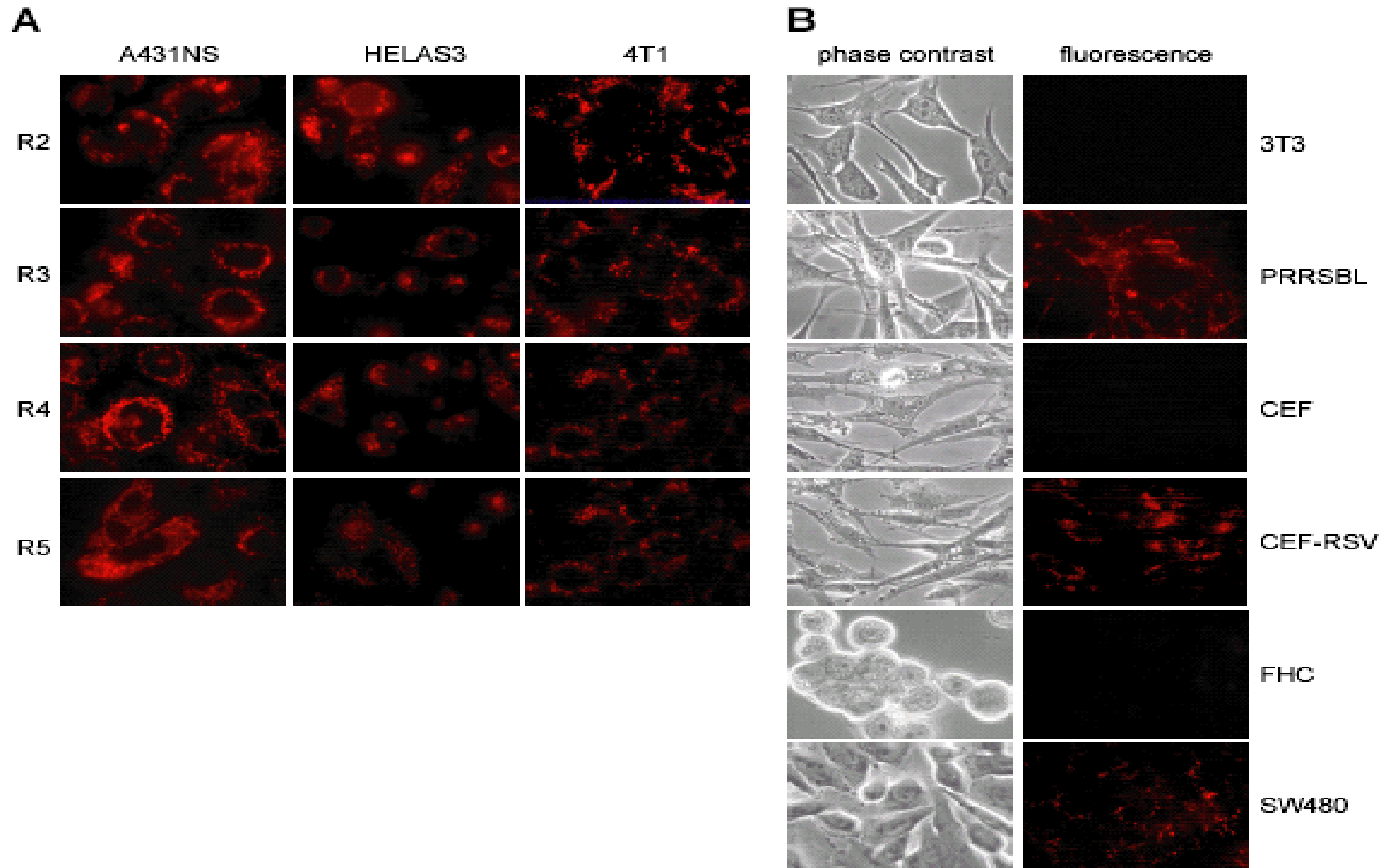


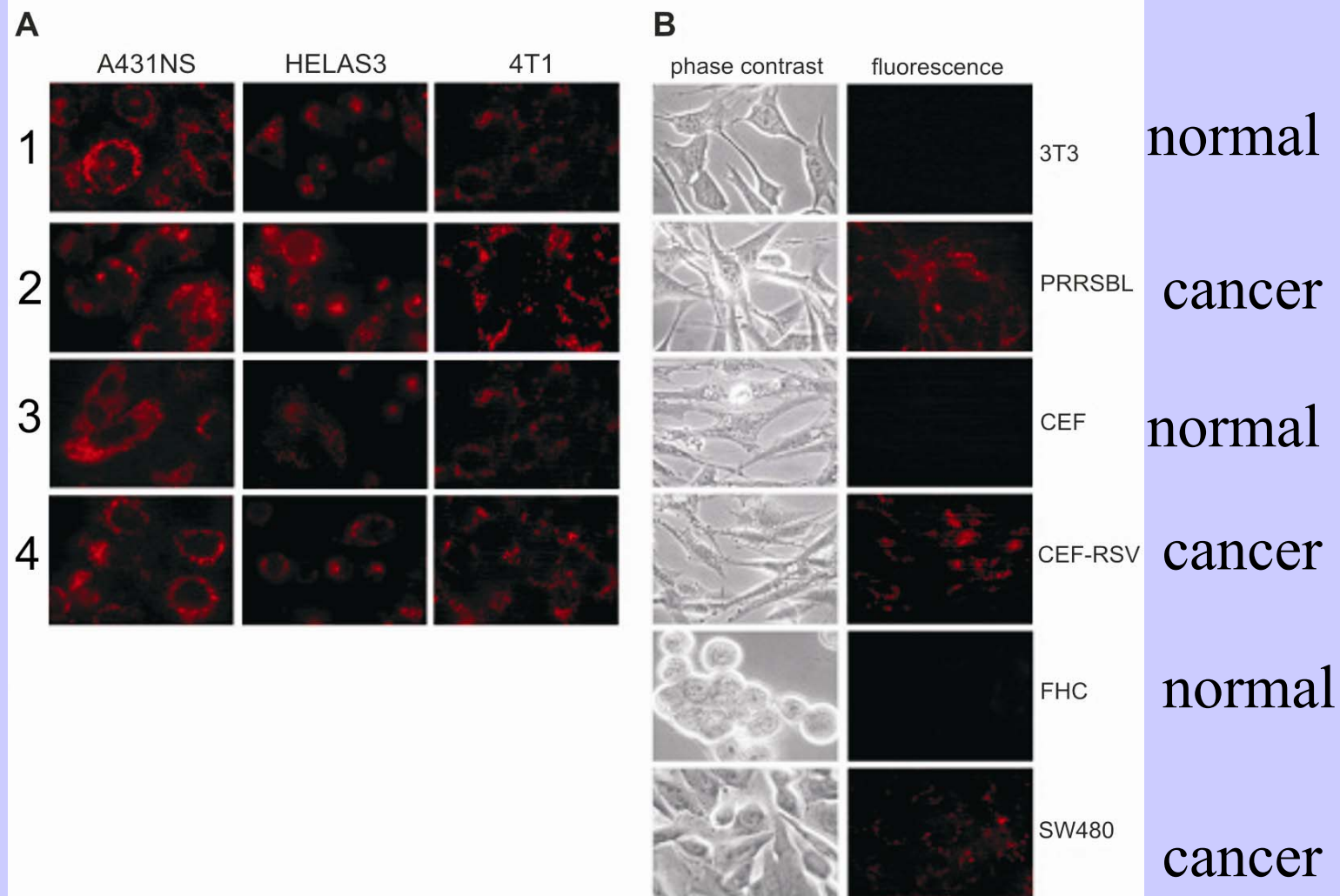
Receptor 4



Receptor 5

Cell surface recognition with porphyrin-cholic acid conjugates





Cellular localization pattern. (A) Fluorescence images of cancer cells (A431NS, HeLaS3, 4T1) (B) tumor cells (PRRSBL, CEF-RSV, SW480) and “normal” cells (3T3, CEF, CHC) with 1 μ M **2**