Inštitut za okoljevarstvo in senzorje, d.o.o.

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Optical chemical sensors for food freshness

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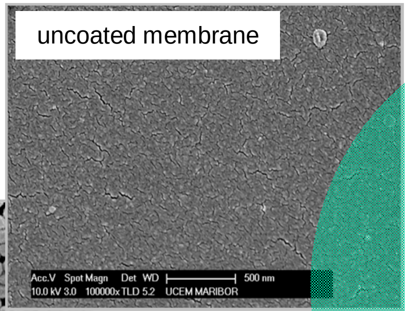
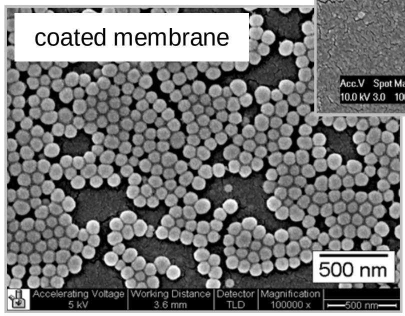
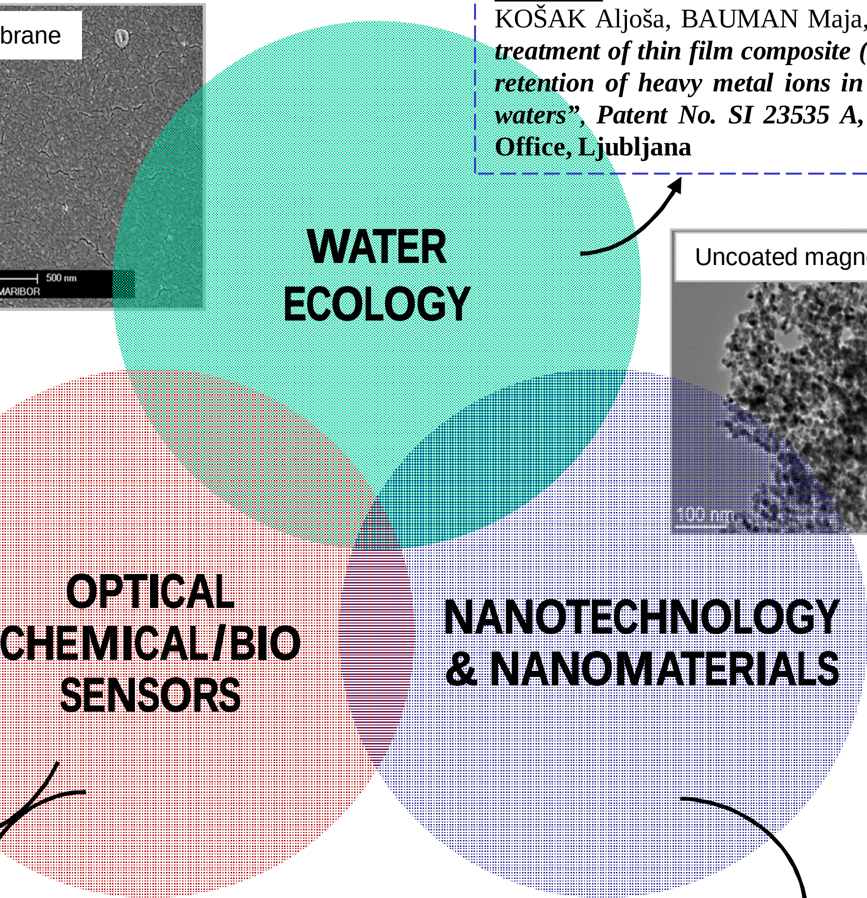
http://www.ios.si/

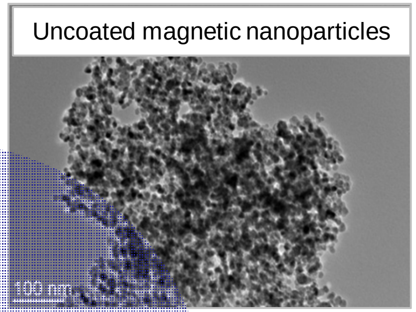
IOS, Institute of Environmental Protection and Sensors

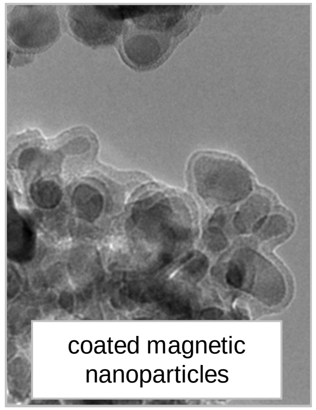




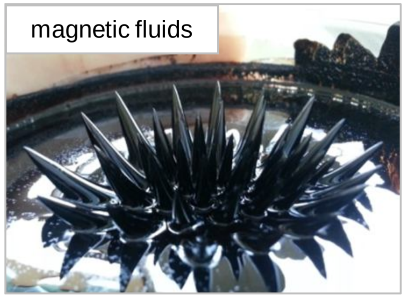
PATENT:
KOŠAK Aljoša, BAUMAN Maja, LOBNIK Aleksandra *“**A method of surface treatment of thin film composite (TFC) membranes with tetraalkoxysilanes for retention of heavy metal ions in the membrane filtration processes of waste waters”, Patent No. SI 23535 A, 2012***, **The Slovenian Intellectual Property Office, Ljubljana**







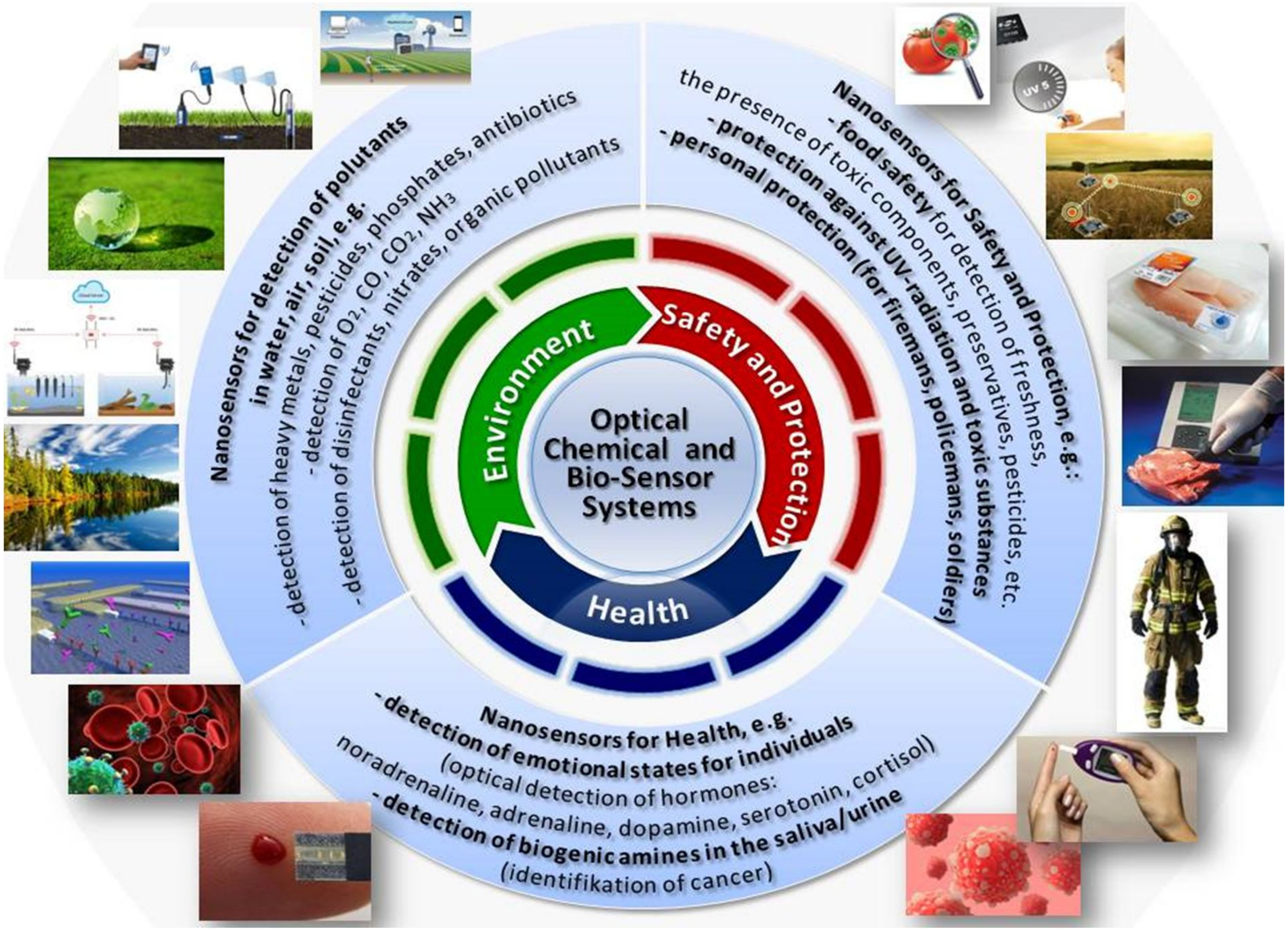
PATENT:
LOBNIK Aleksandra,
KORENT UREK Špela.
“A method and an optical chemical sensor with a sol-gel membrane for the detection of organophosphates”,
Patent No. SI 23556 A, 2012,
The Slovenian Intellectual Property Office, Ljubljana



PATENT:
LOBNIK, Aleksandra ***“Sol-gel based optical chemical sensor for detection of organophosphates and method for preparation thereof”:***
US Patent 2013/0251594A1; Appl. No.: 13/989,529; PCT/SI2011/000068;
United States and International Intelectual Property Law, 2013

PATENT:
KOŠAK Aljoša, LAKIĆ Marijana, LOBNIK Aleksandra, *“**Process for the preparation of superparamagnetic hollow spherical nanostructures**”*, ***application number P-201400120***, **European Patent Office CA G2**, *25 Mar 2015*.

Sensor Applications



DEFINITION OF SENSORS

Chemical Sensors are miniaturized analytical devices that can deliver real-time and on-line information on the presence of specific compounds or ions in complex samples.

Nanosensors – using nanomaterials or nanotechnologies to prepare nano sensor receptors

Chemosensors and Biosensors

Sensing versus Analyzing: the fundamental differences

Chemical Analysis	*Chemical Sensing*
sample treatment possible	**sample treatment not desired or even not possible**
result obtained after some time	**result obtained on-line (= continuously)**
results obtained in laboratory	**results obtained in-situ**
pH can be adjusted	**pH cannot be adjusted usually**
preconcentration possible	**preconcentration not possible**
includes manual operation	**usually fully automatted**
chemical & instrumental	**instrumental only**

Sensor Types

Electrical	*Optical*	*Others*
potentiometry	reflectometry	piezo-electric
coulometry (amperometry)	fluorescence, phosphorescence	quartz micro balance (QMB)
conductometry	surface plasmon resonance (SPR)	acoustic sensing
capacitive (impedimetry)	interferometry	calorimetry
…..	infrared, Raman, evanescent wave, ….	…..

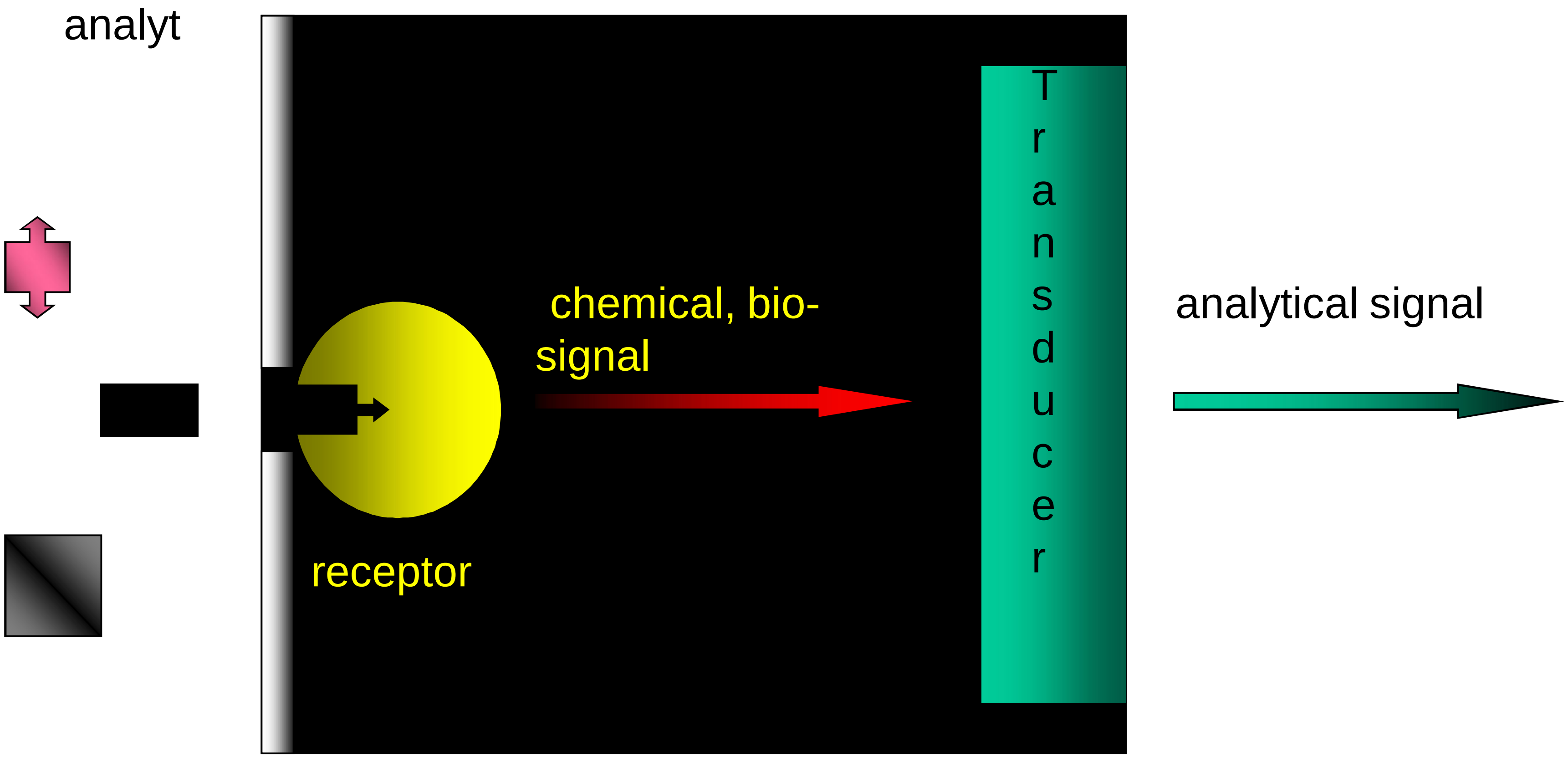
Sensor Technology Involves …

* Spectroscopy, Electrochemistry, etc.
* Polymer Chemistry
* Physical Chemistry
* Organic and Inorganic Materials
* Interface chemistry
* Biochemistry
* Nanotechnology
* Analytical Chemistry
* Computational Chemistry (AANs)

“Optrode” - (from optical electrode) and “optode” (from Greek - the optical way)

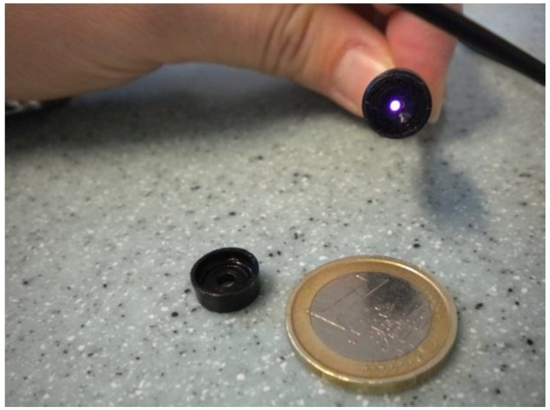
- intrinsic optical property of the analyte is utilized for its detection
- indicator (or label) based sensing is used when the analyte has no intrinsic optical property INDICATOR CHEMISTRY
- (FOCSs) represents a subclass of chemical sensors in which an optical fiber is used aspart of the transduction element.

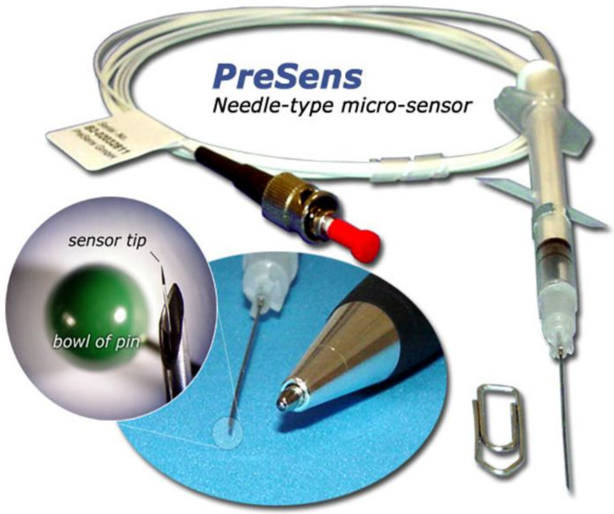
OPTICAL CHEMICAL/BIO- SENSOR system



FIBRE OPTICAL CHEMICAL SENSORS

- optical fiber is used to transmit the EM radiation to and from a sensing region
- remote sensing





The major advantages:

- Optodes do not require a reference cell.
- Miniaturization.
- Remote sensing.
- No intereferences to strong magnetic fields/pressure.
- Multiple analysis with a single instrument.

Disadvantages:

- Ambient light can interfere.
- Limited long-term stability because of photobleaching or wash-out of the immobilized indicator.
- Mass transfer of the analyte from the sample into indicator phase is necessary in order to obtain a steady-state signal.
- Limited dynamic range.
- Selectivity of indicators and the immobilization techniques are to be improved.

ANALYTICAL ASPECTS OF SENSORS

- sensitivity and selectivity for the analyte
- broad dynamic range
- reversibility
- lack of frequent calibration
- fast response
- small size

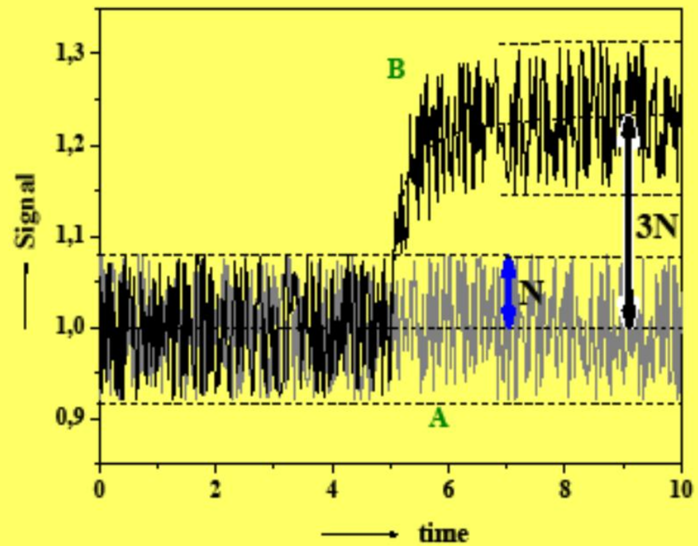
Analytical aspects of sensors

- sensitivity in the range of interest
- selectivity for the analyte
- broad dynamic range
- reversibility
- lack of frequent calibration
- fast response
- inertness to sample matrix
- small size

Figures of Merit of Sensors

* **Sensitivity (ΔS / ΔC);** ***Note: by definition of IUPAC, sensitivity is <u>not</u> the limit of detection!***

* **LOD** **(usually 3x the noise *N*) ➔**



* **Dynamic range**

* **Selectivity**

* **Linearity (linear range)**

* **Resolution** **(smallest difference in concentrations that is detectable)**

The best chemical sensor ...

……is the pH electrode since it

* measures over 10 log concentration units

* acts fully reversibly

* is very stable over time

* is not expensive

* is sterilizable

* has been optimized over 60 years.

Even though

* it could be smaller

* it could be even more selective

Design of optical chemical sensor
„Indicator chemistry“

Indicator

Polymer matrix

Immobilization

→

Sensor characteristics

Indicators

Absorbance based:

Undergo colour change

Detection by “naked "eye

Detection by colorimetry

Luminescence based:

Fluorescence

Phosphorescence

Chemiluminiscence

Electroluminiscence

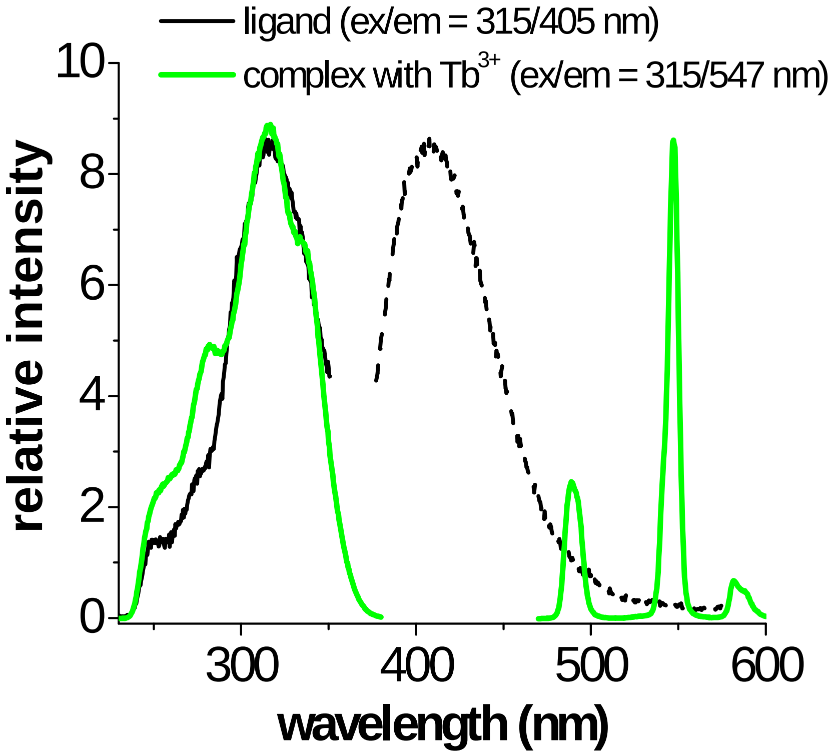
Detection by:

UV lamp

Intensity change

Lifetime measurements

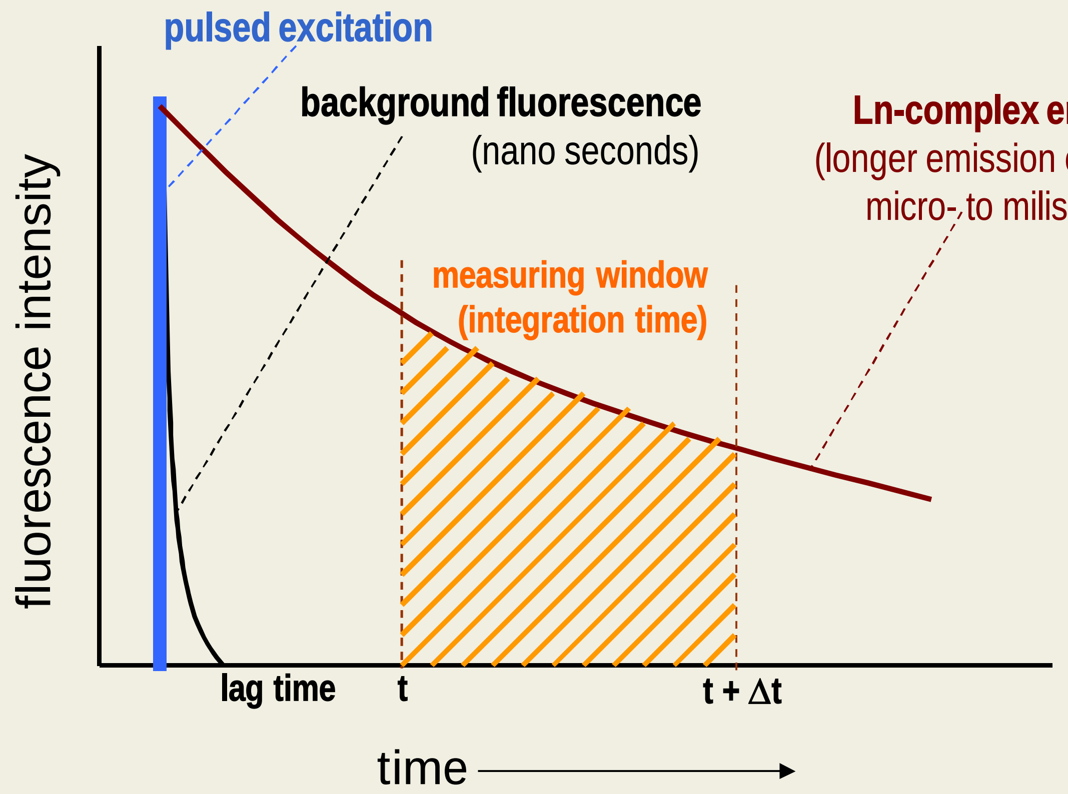
Fluorescent intensity and lifetime based "Lanthanide chelates"



- narrow, line-like emission peaks
- large Stokes' shifts (≥ 200 nm)
- long lifetimes (from micro- to several mili-seconds range)
  → lifetime-based assays advantageous over intensity-based: higly immune to photobleaching, changes in fluorophore concentration, tubidity in the sample, optical misalignment, etc.

- long lifetimes enable gated detection mode
  → time-resolved luminescence

Time-resolved luminescence



After »short-lived« fluorescence has ceased away, only »long-lived « luminescence of Ln-complex is measured.

Polymer carrier

* hydrophobic

PVC, PMMA,
PE, PS, …

Detection of gases

* hydrophilic

polysaccharides,
polyacrilates,
polyamines,
hydrogels…

Ion detection

* hidrophobic-hydrophilic

sol-gel

Detection of ions and gases

Sol-gel process (Silica nanoparticles)

1. *hydrolysis*

$Si(OR)_4 + H_2O \rightarrow HO\text{-}Si(OR)_3 + ROH$

2. condensation*:*

$HO\text{-}Si(OR)_3 + HO\text{-}Si(OR)_3 \rightarrow HO\text{-}Si(OR)_2\text{-}O\text{-}Si(OR)_2 + HOH$

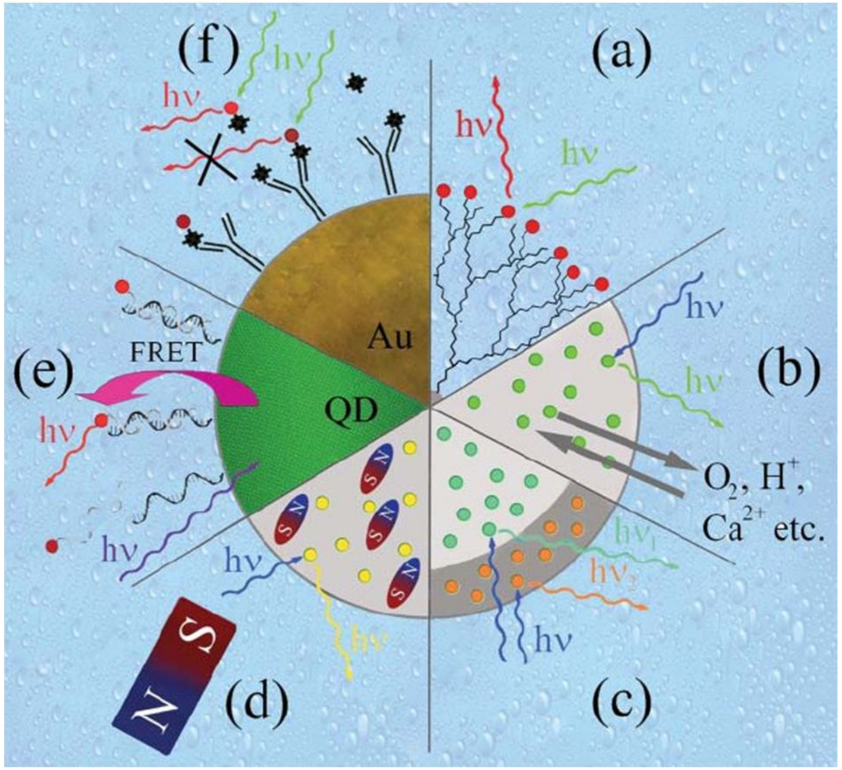
$HO\text{-}Si(OR)_3 + Si(OR)_4 \rightarrow Si(OR)_3\text{-}O\text{-}Si(OR)_3 + ROH$

Monomers	Other metals
$Si(OR')_4$	$Zr(OR')_4$
$R_1\text{-}Si(OR')_3$	$R_1\text{-}Zr(OR')_3$
$R_1\ R_2\text{-}Si(OR')_2$	$R_1\ R_2\text{-}Zr(OR')_2$
R: aliphatic, aromatic	Ti, Sn

ADVANTAGES OF USING NANOMATERIALS FOR SENSORS

- Improved sensor characteristic (response time, sensitivity, etc.)
- In-vivo measurements,
- Small sample volumes,
- Multi-analyte sensing

Design of Optical nanosensor



Borisov SM, Klimant I (2008) Analyst 133:1302-1307

a, macromolecular nanosensors (dendrimers); b, NSs based on polymer materials and sol-gels; c, multi-functional core-shell systems; d, multi-functional magnetic beads; e, NSs based on quantum dots; f, NSs based on metal beads

Silica nanoparticles - Sol-gel process

1. *hydrolysis*

$$Si(OR)_4 + H_2O \rightarrow HO\text{-}Si(OR)_3 + ROH$$

2. condensation*:*

$$HO\text{-}Si(OR)_3 + HO\text{-}Si(OR)_3 \rightarrow HO\text{-}Si(OR)_2\text{-}O\text{-}Si(OR)_2 + HOH$$

$$HO\text{-}Si(OR)_3 + Si(OR)_4 \rightarrow Si(OR)_3\text{-}O\text{-}Si(OR)_3 + ROH$$

Monomers	Other metals
$Si(OR')_4$	$Zr(OR')_4$
$R_1\text{-}Si(OR')_3$	$R_1\text{-}Zr(OR')_3$
$R_1\ R_2\text{-}Si(OR')_2$	$R_1\ R_2\text{-}Zr(OR')_2$
R: aliphatic, aromatic	Ti, Sn

Applications in Food safety: Food freshness

Food freshness depends on microbialogical activity

and

Various Biogenic amines are formed and released

Hrana in pijača

- **Vino**
  (histamin, tiramin, triptamin, feniletilamin, agmatin, kadaverin, putrescin, spermidin)
- **Sir**
  (putrescin, kadaverin, histamin, tiramin, feniletilamin, spermin, spermidin, agmatin)
- **Riba/Tuna**
  (histamin, triptamin, kadaverin, feniletilamin, spermin, spermidin, tiramin, agmatin)
- **Meso/Klobasa**
  (triptamin, feniletilamin, putrescin, kadaverin, histamin, serotonin, tiramin, spermin, spermidin, agmatin)
- **Sadje/Zelenjava**
  (dopamin, tiramin, putrescin, kadaverin, histamin, serotonin, agmatin, feniletilamin, spermidin, spermin, agmatin)

Amino acid — decarboxylation amino acid — biogenic amine

$$H_2N-\underset{\displaystyle R}{\underset{|}{CH}}-\boxed{COO}H \xrightarrow[-\,CO_2]{\text{decarboxylation amino acid}} R-CH_2NH_2 + CO_2$$

METHODS FOR BIOGENIC AMINE DETERMINATION

- **Instrumental or classical methods**
  - High-performance liquid chromatography (HPLC)
  - Thin-layer chromatography (TLC)
  - Gas chromatography (GC)
  - Micellar electrokinetic chromatography (MEKC)
- **Other methods**
  - electrochemical methods (capillary electrophoresis) (CE)
  - enzymatic methods (biosensors)
- **Optical methods**
  - **Optical chemical sensors (OCS)**

Derivational reagents:

- dansyl chloride,
- benzoyl chloride,
- dansyl chloride,
- fluorescein,
- 9-fluorenylmethyl chloroformate,
- naphthalene-2,3-dicarboxaldehyde
- **orthophthalaldehyde (OPA)**

OPTICAL DETERMINATION OF BA BY O-PHTHALDIALDEHYDE (OPA)

Spectral properties

Response time

The impact of the concentration of OPA

Influence of pH medium

OPA sensitivity to other BA

BA determination in real samples

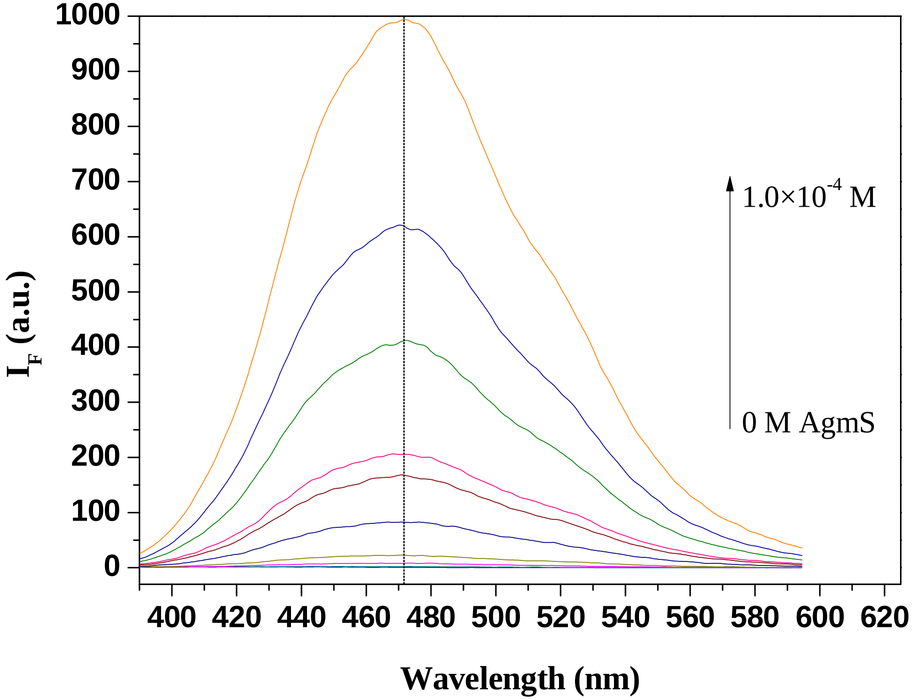
1. Agmatine
2. Serotonin
3. Dopamine
4. Octopamine,
5. **Tyramin**
6. Ethanolamine
7. Spermidine
8. **Cadaverine**
9. methyl-fenilethylamine
10. Noradrenalin
11. Adrenalin
12. Isopenthylamine
13. Melatonin
14. Acetilholin chloride
15. **Putrescine**
16. Isopropanolamine
17. β-alanine
18. Histamine
19. Cysteamine
20. Spermine

OPTICAL DETERMINATION OF BA IN SOLUTION

Spectral properties

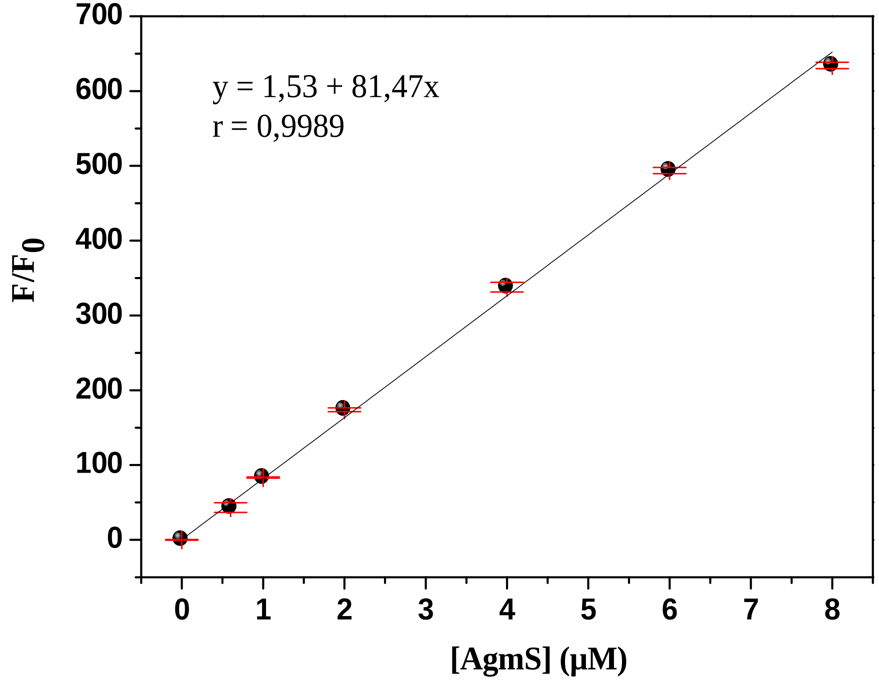
AGMATINE

Response time



Emission spectra of OPA-AgmS product

$6{,}0\times10^{-7}$ M – $1{,}0\times10^{-4}$ M

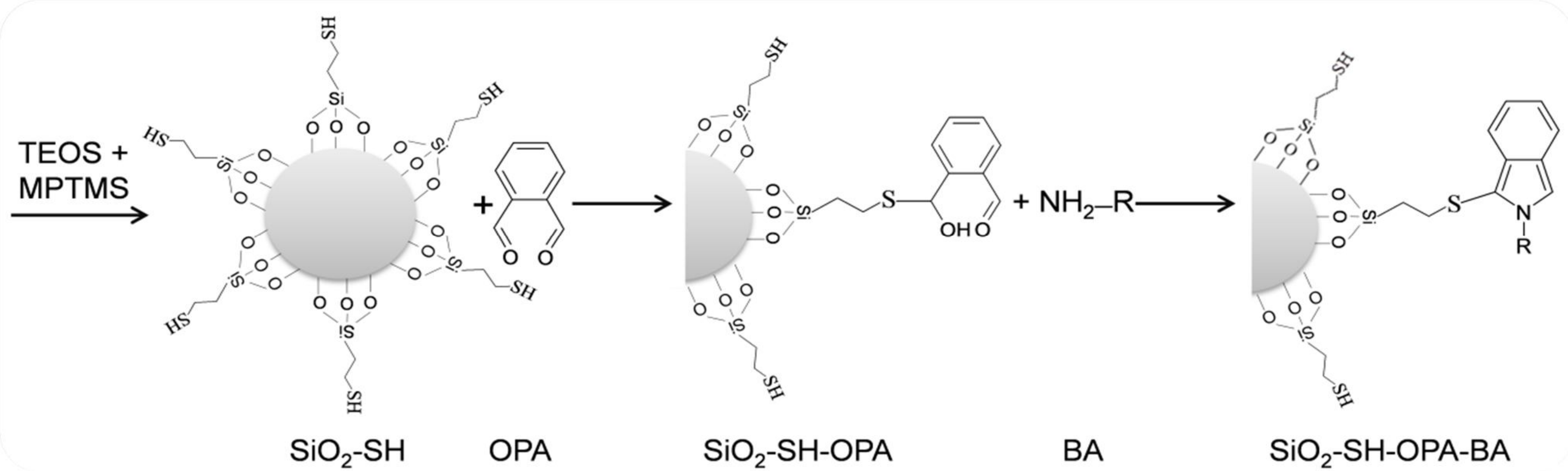


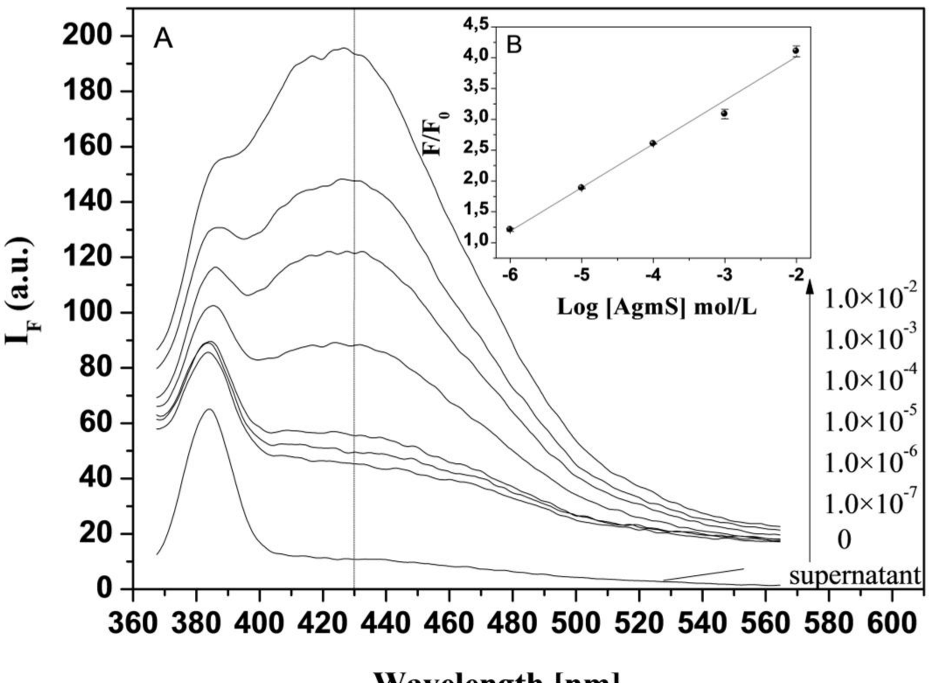
Calibration curve of fluorescent product OPA-AgmS

$6{,}0\times10^{-7}$ M – $8{,}0\times10^{-6}$ M

LOD = $2{,}5\times10^{-7}$ M

OPTICAL DETERMINATION OF BA BY O-PHTHALDIALDEHYDE (OPA)

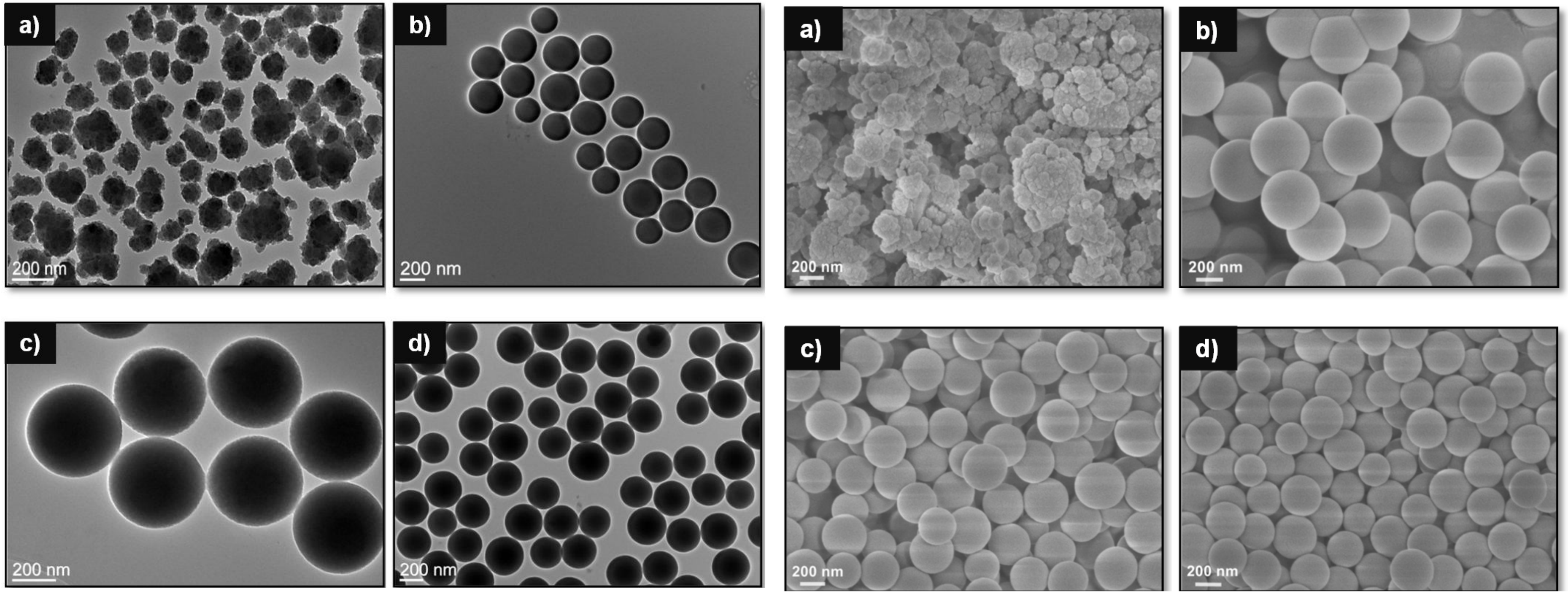




OPTICAL DETERMINATION OF BA BASED ON SiO_2 PARTICLES –

Characterization of SiO_2 particles

TEM, SEM, FT-IR, BET, potentiometric titration, zeta potential



TEM (on the left side) and SEM (on the right side) images of SiO_2 particles which they have been prepared based on precursor TEOS with different molar ratios (R) : (a) R=4, (b) R=20, (c) R=40, (d) R=80.

OPTICAL DETERMINATION OF BA BASED ON SiO_2 PARTICLES

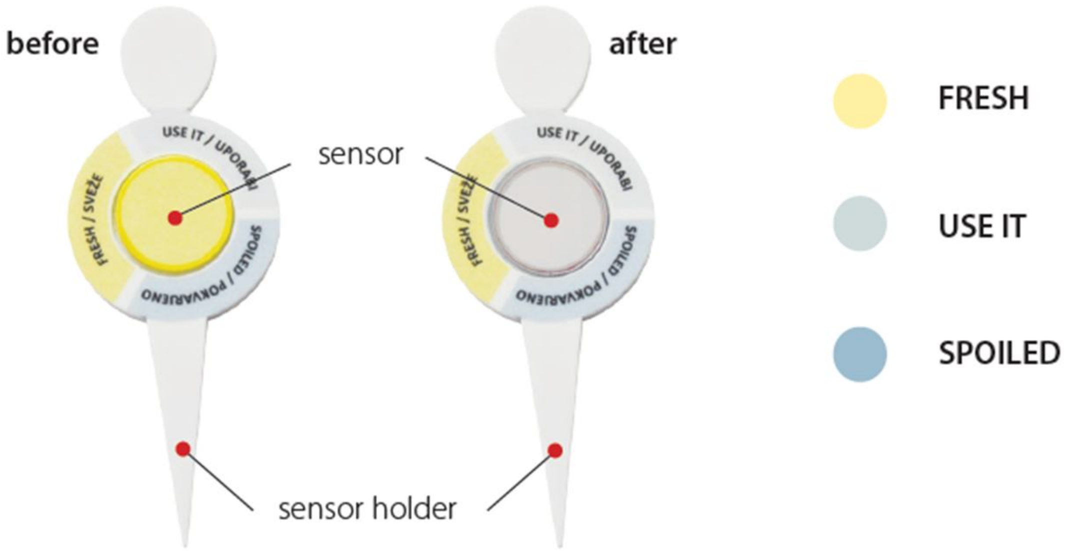
SUMMARY

AGMATINE

Comparison of the results based on the optical determination of AgmS in solution with and without SiO_2-SH-OPA particles at pH 13

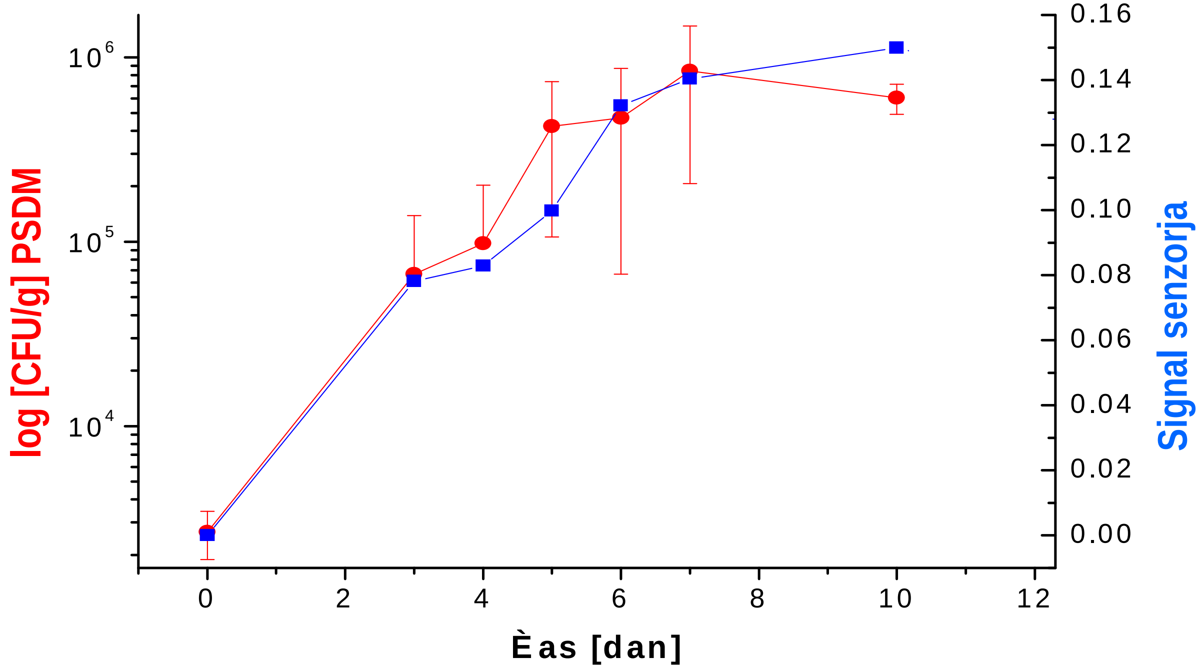
Parameters	Optical determination of AgmS	
	with OPA	with SiO_2-SH-OPA particles
Fluorescent product	OPA-AgmS	SiO_2-SH-OPA-AgmS
Spectral properties ($\lambda_{ex}/\lambda_{em}$)	340 nm / 473 nm	340 nm / 430 nm
Concentration range	6.0×10^{-7} M – $8.,0 \times 10^{-6}$ M	1.0×10^{-6} M – 1.0×10^{-2} M
The correlation coefficient r^2	0.9989	0.9989
Linear equation	$y = 1.53 + 81.47x$	$y = 5.43 + 0.71x$
LOD	2.5×10^{-7} M	7.3×10^{-7} M
Response time	20 min	2 – 3 min
Buffer	pH 13	pH 13

Freshsens



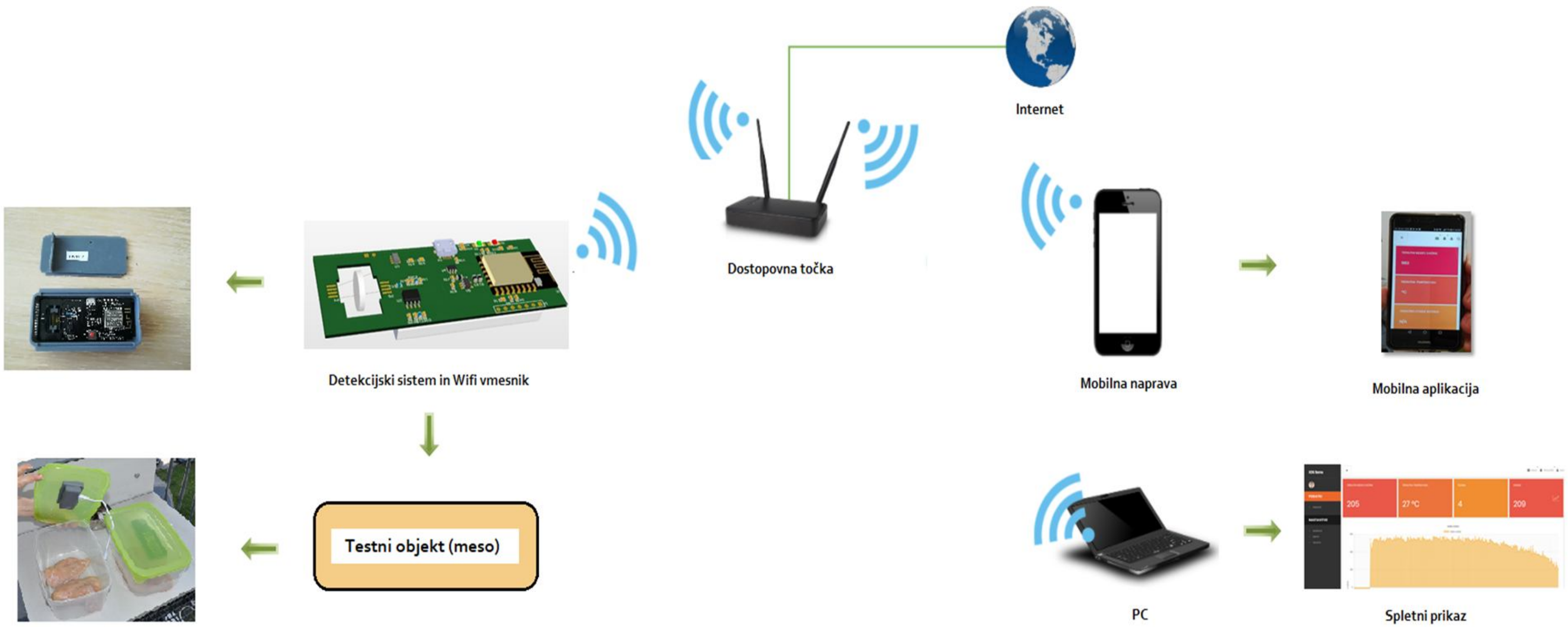
- The sensor is suitable for raw, untreated fish and chicken meat
- Color change is a measure of the usefulness of the meat see color scale)
- Response time is 30 minutes
- The sensor is useful when blue coloration is reached (spoiled meat) and can be used again if the initial color was yellow

Correlation to the microbiological measurements



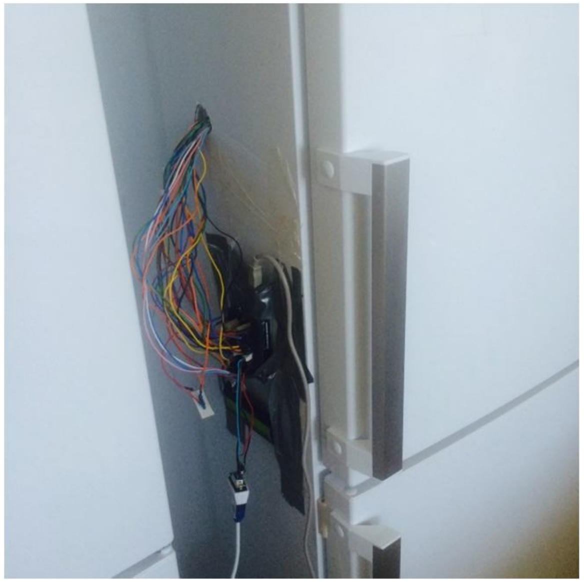
- sensor absorption measurements on spectrophotometer (laboratory)
- monitoring of the activity of microbiological parameters - bacteria Pseudomonas spp. (PSDM)
- signal of the sensor in correlation with the increase in the number of bacteria Pseudomonas spp.

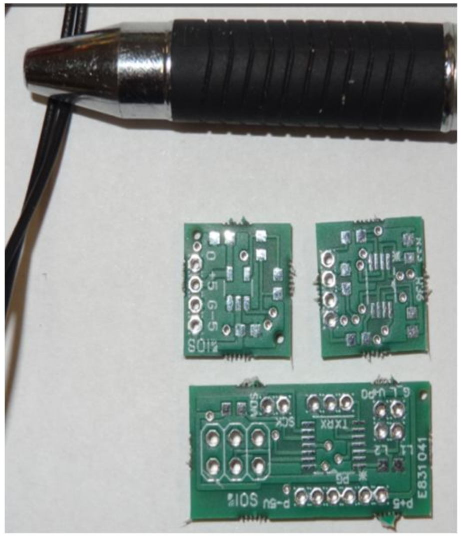
Freshsens

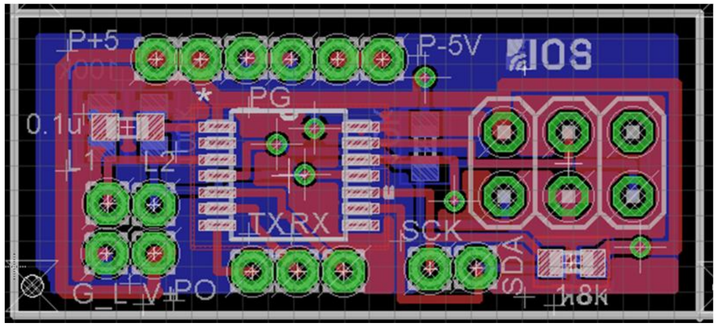

Internet
Dostopovna točka
Detekcijski sistem in Wifi vmesnik
Testni objekt (meso)
Mobilna naprava
Mobilna aplikacija
PC
Spletni prikaz

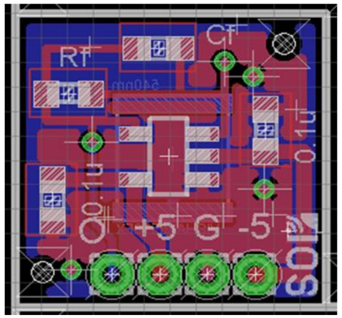
OPTICAL DETERMINATION OF BA BASED

Freshness of the food in fridge



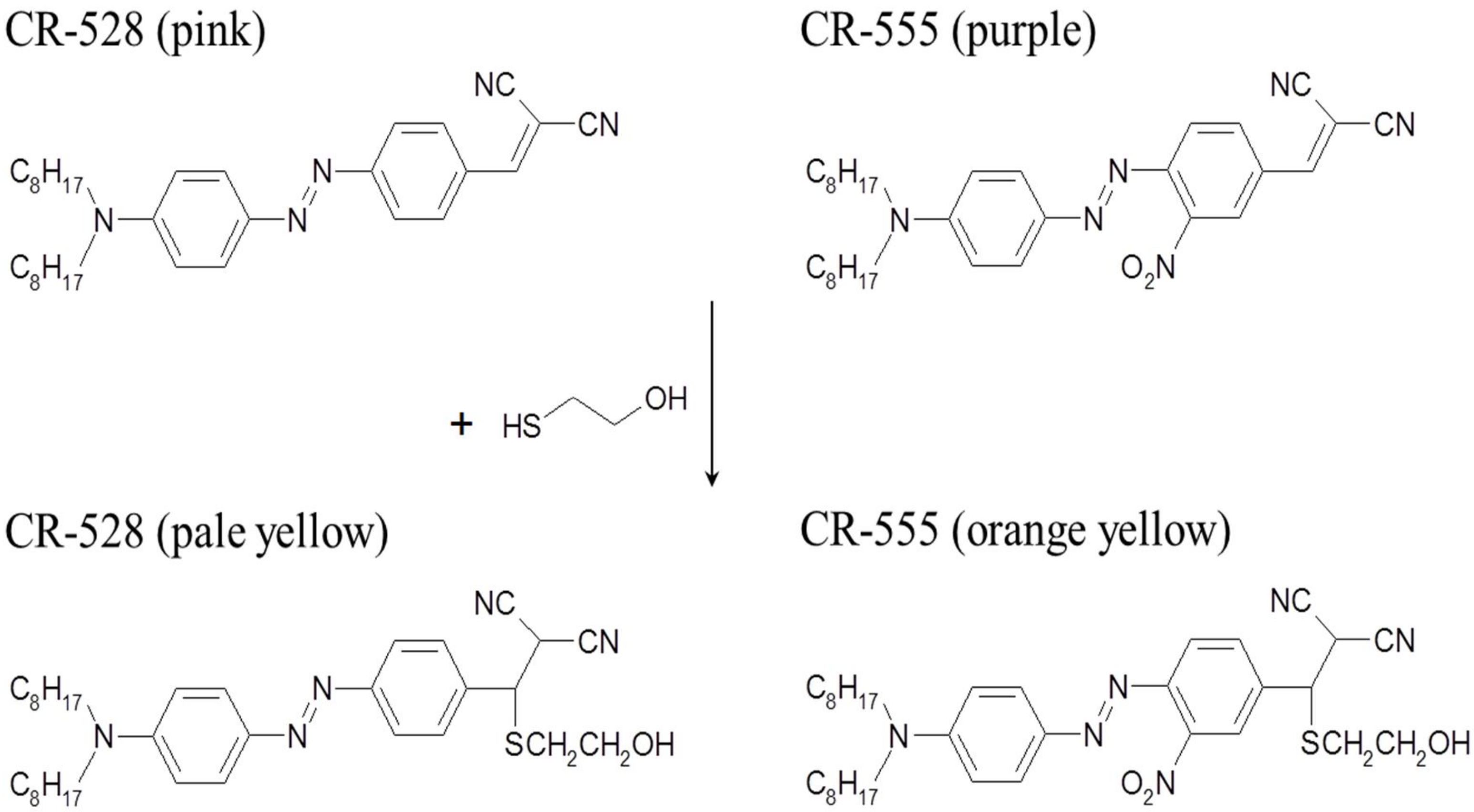






In the meet package

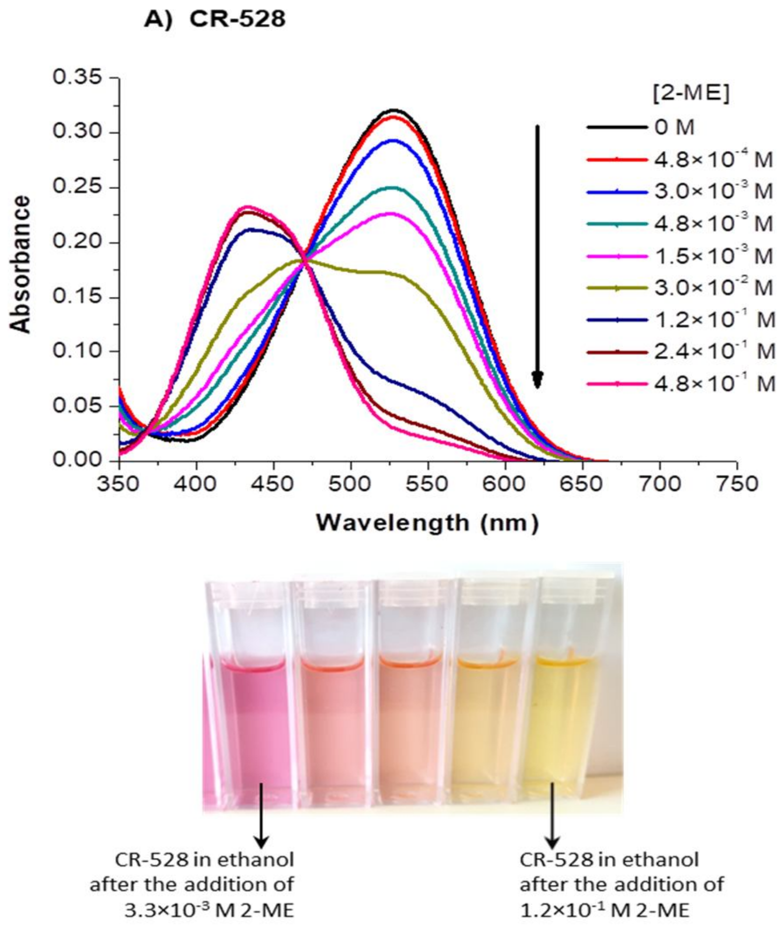
Design and characterization of azo (dicyanovinyl) dyes for the colorimetric detection of thiols and biogenic amines detection

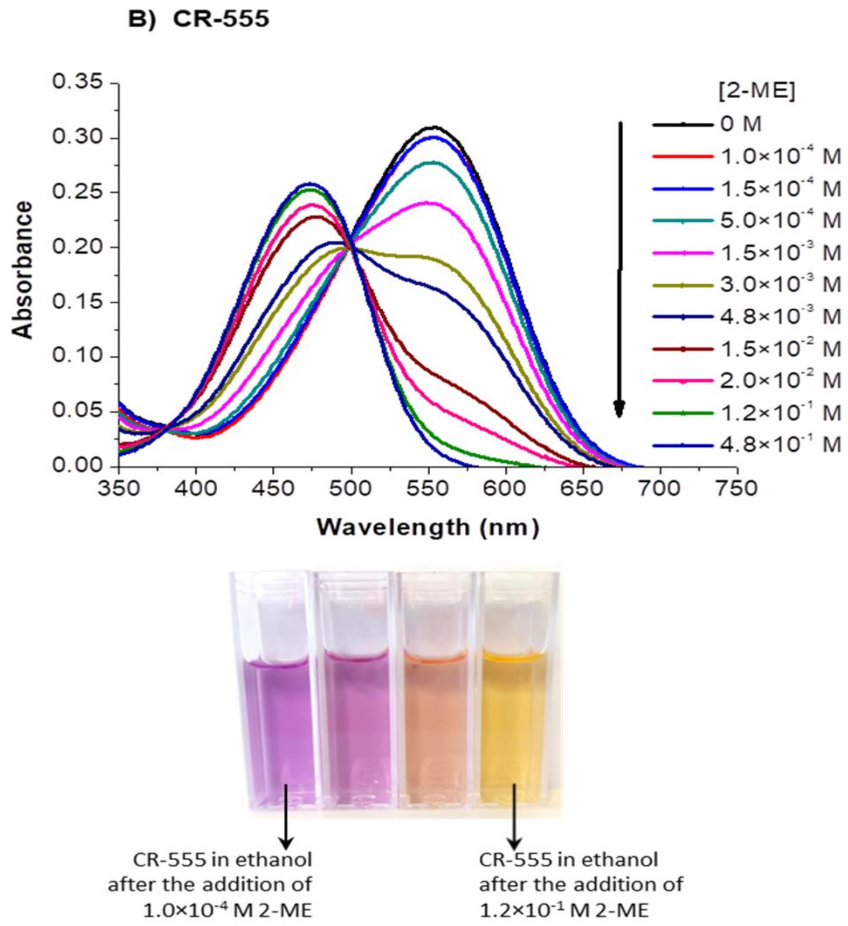


Chemical structures of the azobenzene dyes CR-528 and CR-555 before and after the reaction with 2-mercaptoethanol (2-ME).

T.Mastnak, A. Lobnik, Sensors, 2018

Design and characterization of dicyanovinyl reactive dyes for the colorimetric detection of thiols and biogenic amines detection

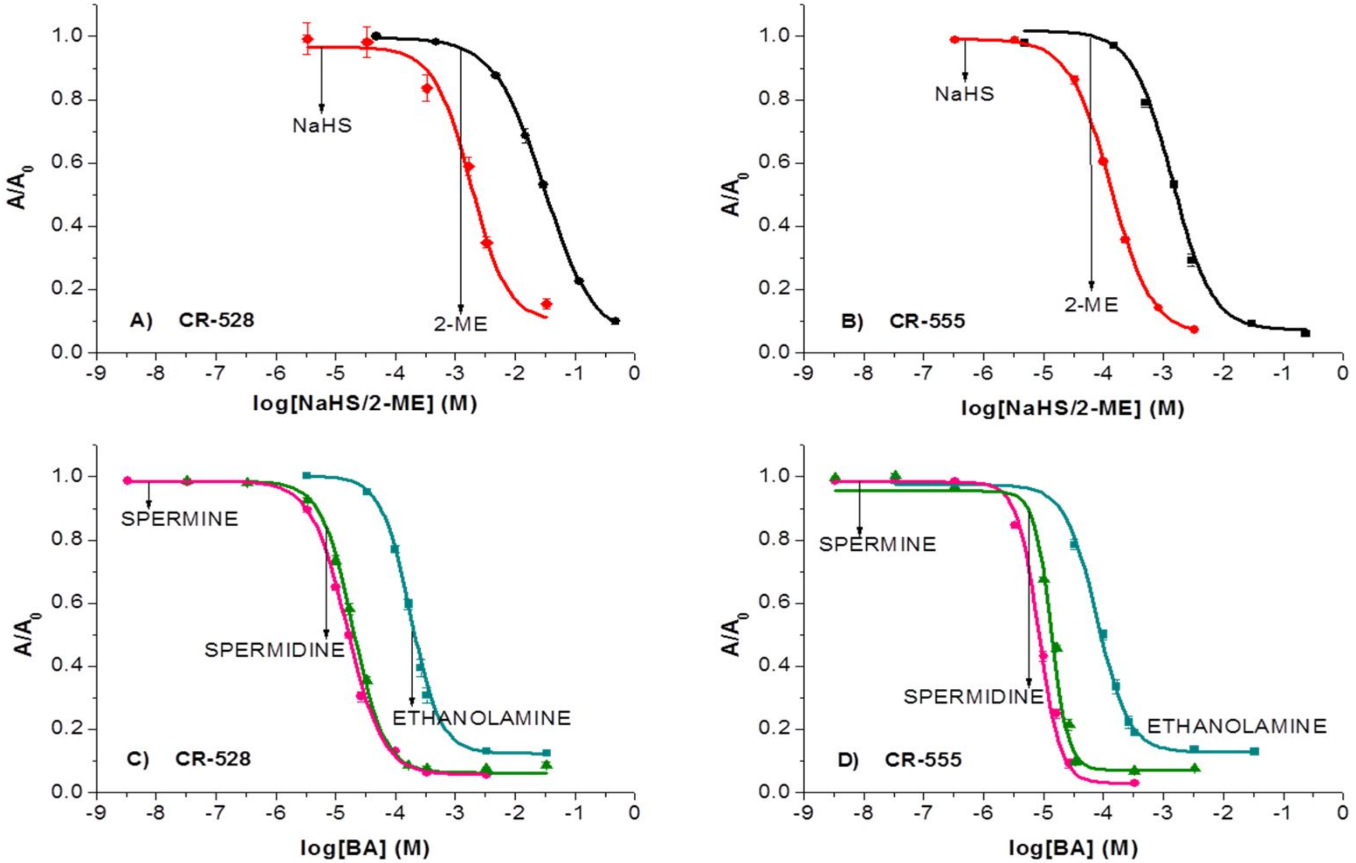




Absorption spectra of CR-528 (7.2×10^{-6} M; A) and CR-555 (7.9×10^{-6} M; B) in the presence of various concentrations of 2-ME (**from 0 to 4.8×10^{-4} M)** in ethanol solution.

T.Mastnak, A. Lobnik, Sensors, 2018

Design and characterization of dicyanovinyl reactive dyes for the colorimetric detection of thiols and biogenic amines detection



Spectrophotometric titrations (calibration curves) for sulfur-based analytes (NaHS, 2-ME; A, B) and for amine-based (BA) analytes (spermine, spermidine, ethanolamine; C, D); n = 3.

T.Mastnak, A. Lobnik, Sensors, 2018

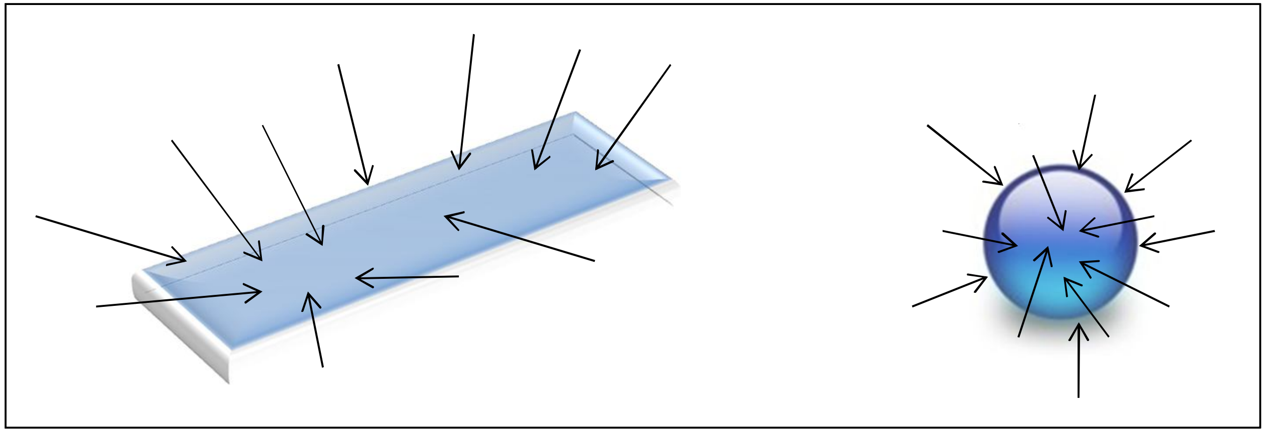
Design and characterization of dicyanovinyl reactive dyes for the colorimetric detection of thiols and biogenic amines detection

Indicator	Analyte	Working Range ($molL^{-1}$)	Response time (min)	Remark	[Ref.]
CR-528	Spermine	$3\times10^{-6} – 1.2\times10^{-4}$	30	A, ethanol solution	our work
	Spermidine	$3\times10^{-6} – 1.2\times10^{-4}$			
	Ethanolamine	$5\times10^{-5} – 1\times10^{-3}$			
	NaSH	$2\times10^{-4} – 3\times10^{-2}$			
	2-ME	$3\times10^{-3} – 3\times10^{-1}$			
CR-555	Spermine	$2\times10^{-6} – 2\times10^{-5}$	30	A, ethanol solution	our work
	Spermidine	$5\times10^{-6} – 2.5\times10^{-5}$			
	Ethanolamine	$2\times10^{-5} – 3.1\times10^{-4}$			
	NaSH	$1.2\times10^{-5} – 2.5\times10^{-4}$			
	2-ME	$1.5\times10^{-4} – 1\times10^{-2}$			

T.Mastnak, A. Lobnik, Sensors, 2018

Organophosphate (pesticide) fluorescence sensor based on thin film and SiO_2 NP

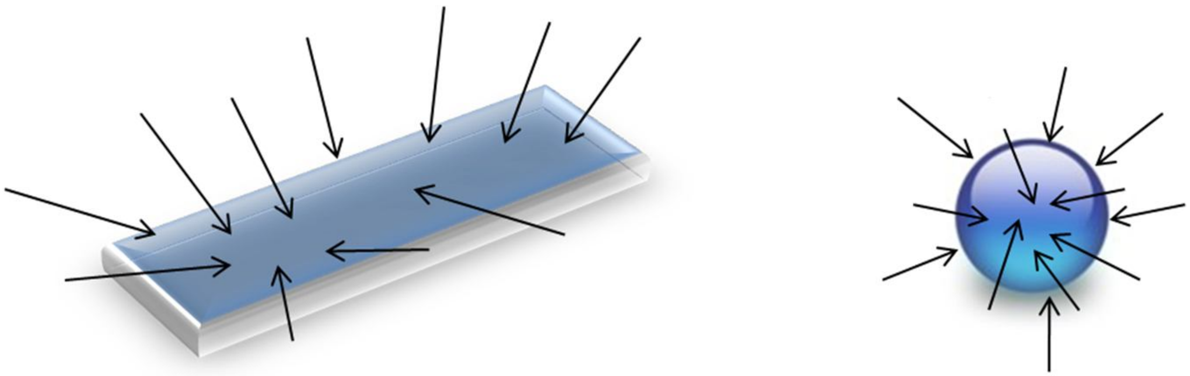
A. Lobnik, EU patent, USA and Russian patent



Sensor configuration	t_{95}
Thin-film	600 s
Silica NP	12 s

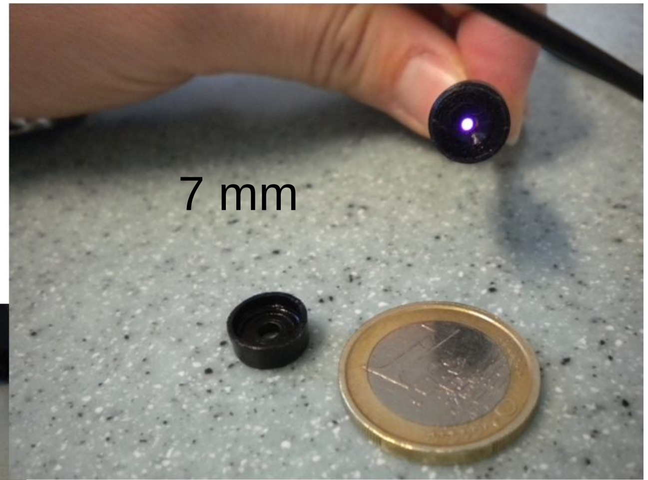
Comparison of OP sensor characteristics

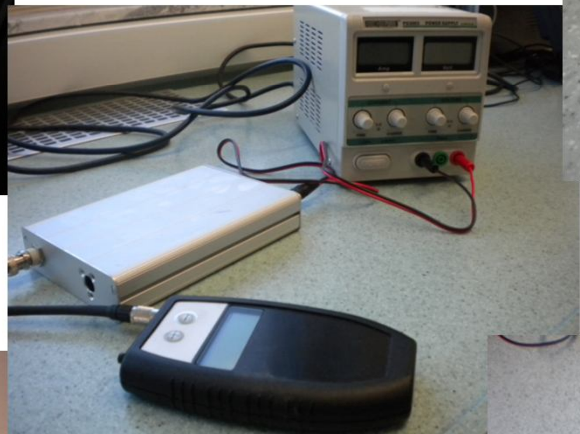
(A. Lobnik, Š. Korent Urek, EU, USA, Russia patents)

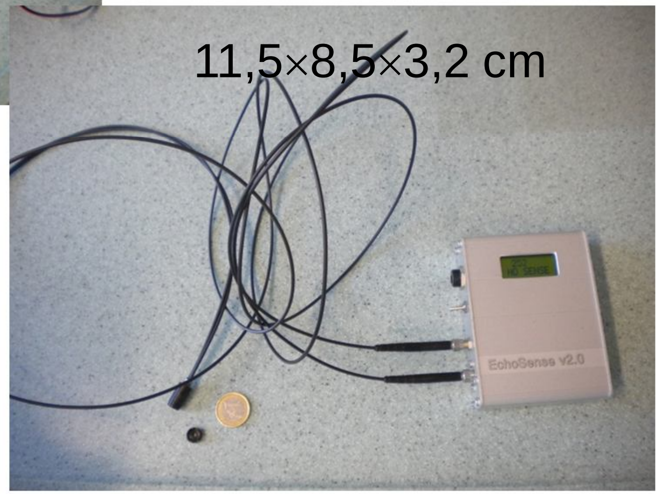


	Dye-doped thin films	Dye-doped nanoparticles
Limit of detection (mol/L)	6.7×10^{-7}	0.17×10^{-9}
Working range (mol/L)	6.9×10^{-7} – 6.9×10^{-3}	0.17×10^{-9} – 2.3×10^{-7}
Response time (s)	600	12

Luminiscence measurements of OP







Biosensing layer for OP determination

(

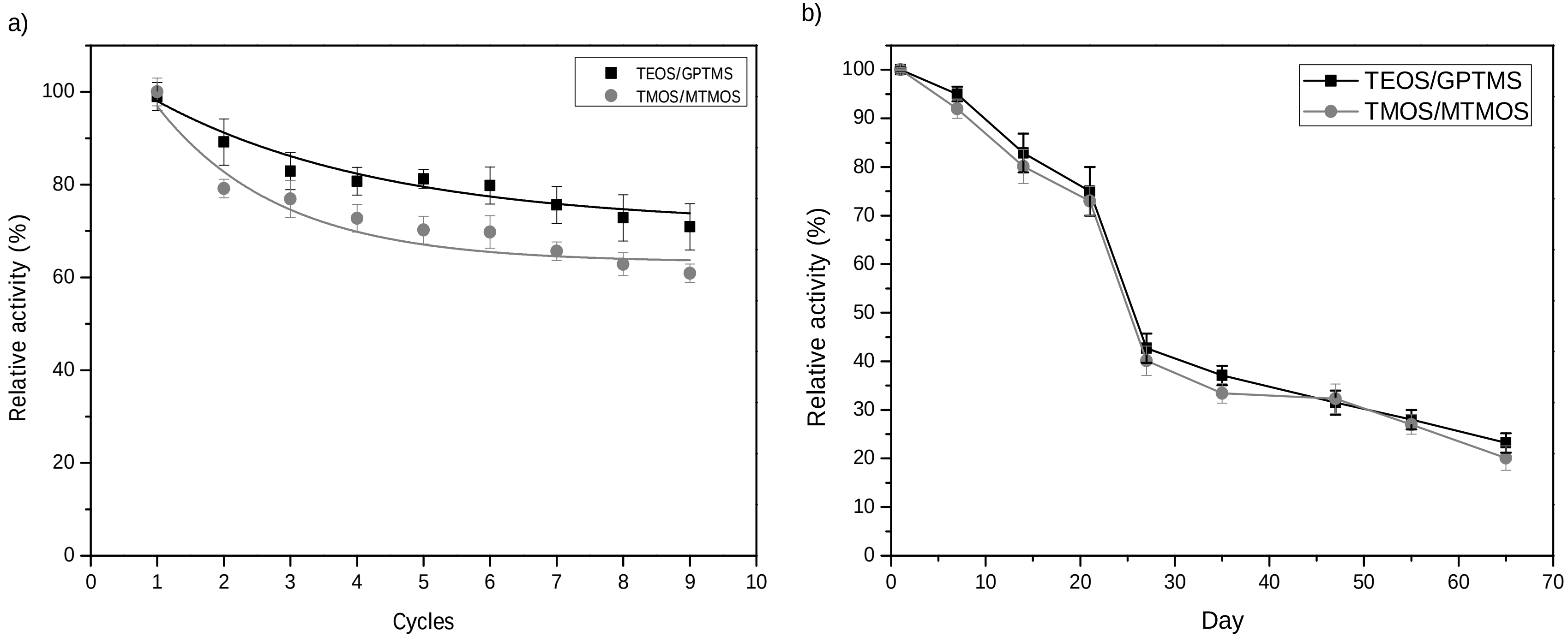
N. Francic, A. Lobnik, E. Efremenko, Bioscience and Technology, 2012)

- **His_6-OPH (EC 3.1.8.1.) – organophosphorous hydrolase**
- Enzyme hydrolyzing a broad spectrum of organophosphorous compounds (OPCs) containing P–O, P–F and P–S bonds in the triesters of orthophosphoric acid
- Metalloenzyme: cofactors are Co^{2+} and other bivalent ions
  - Optimal activity :
    - T = 45 - 53 °C (pH 10.5)
    - pH between 10 in 11.5
  - High specific activity: ~ 5000 U/mg

- **hexahistidine (His_6) tag fused to OPH** → improving the catalytic efficiency, especially towards P–S-containing substrates, and the stability under alkaline hydrolysis conditions compared to native OPH

Votchitseva Y. A., Efremenko E.N., Aliev T.K., Volfomeyev S.D., "Properties of Heksahistidine-Tagged Organopgosphate Hydrolase", Biocemistry (Moscow) 71, 167-172 (2006)

Entraped His_6-OPH within hydrid SiO_2 sol-gel layer

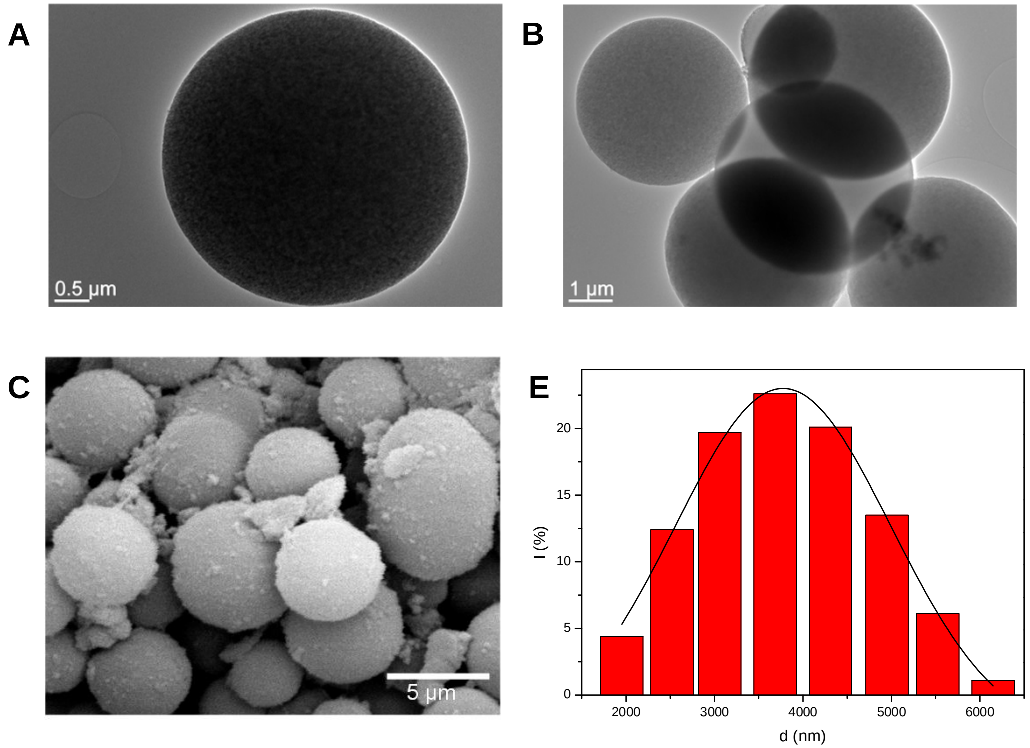


Comparison of two types of biocatalyst films TEOS/GPTMS (R=188, P=5:1) and TMOS/MTMOS (R=148, P=1:2) for a) repeated use in the detoxification of POX. Conditions: 0.675 mM paraoxon, temperature 25 °C, 50-mM Na-carbonate buffer (pH 9.5); and b) stability of SiO_2 thin films with entraped His_6-OPH

Anal Bioanal Chem (2011) 401:2631–2638

J Sol-Gel Sci Technol (2015) 74:387–397

Silica particles with immobilized His_6-OPH for POX determination/detoxification



TEM micrographs of silica particles. (A-B), SEM microgram (C), and particle size distribution (D) of MPS 5 particles.

Silica particles with immobilized His_6-OPH for POX determination/detoxification

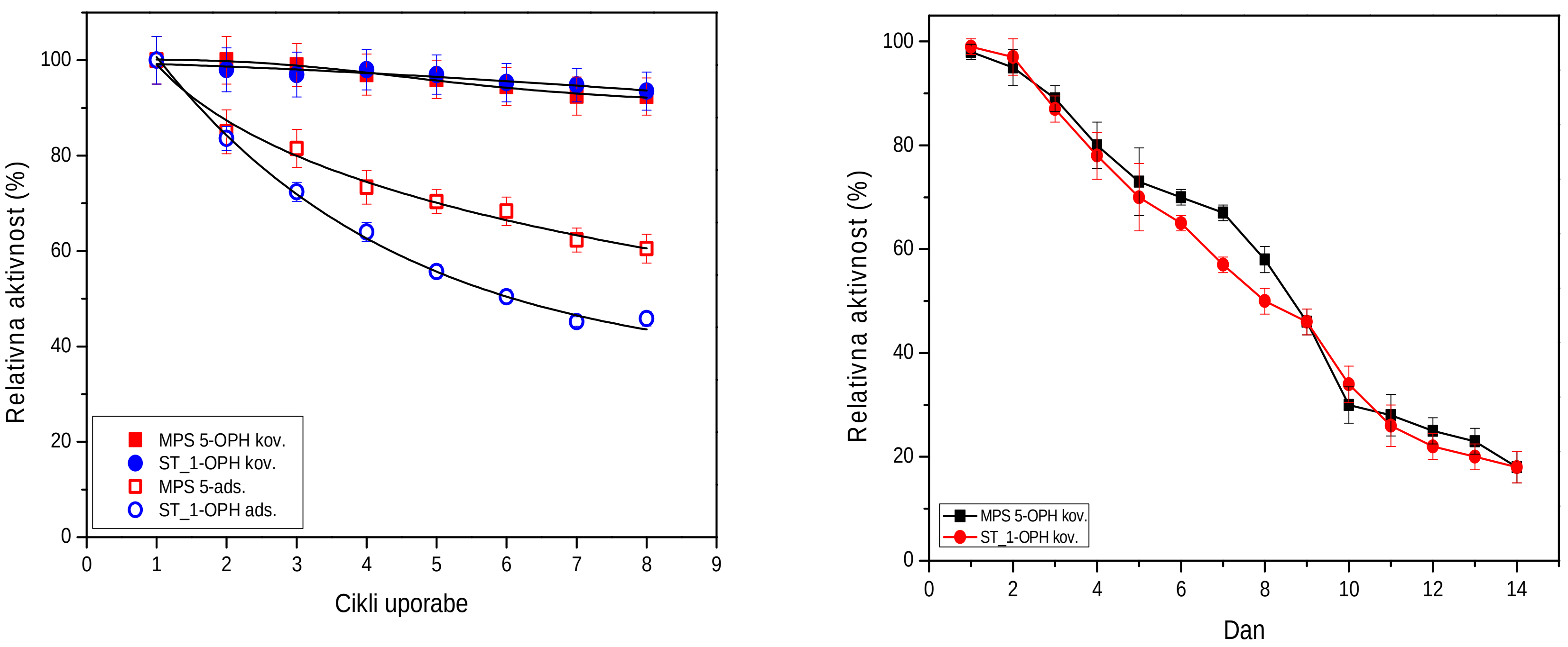
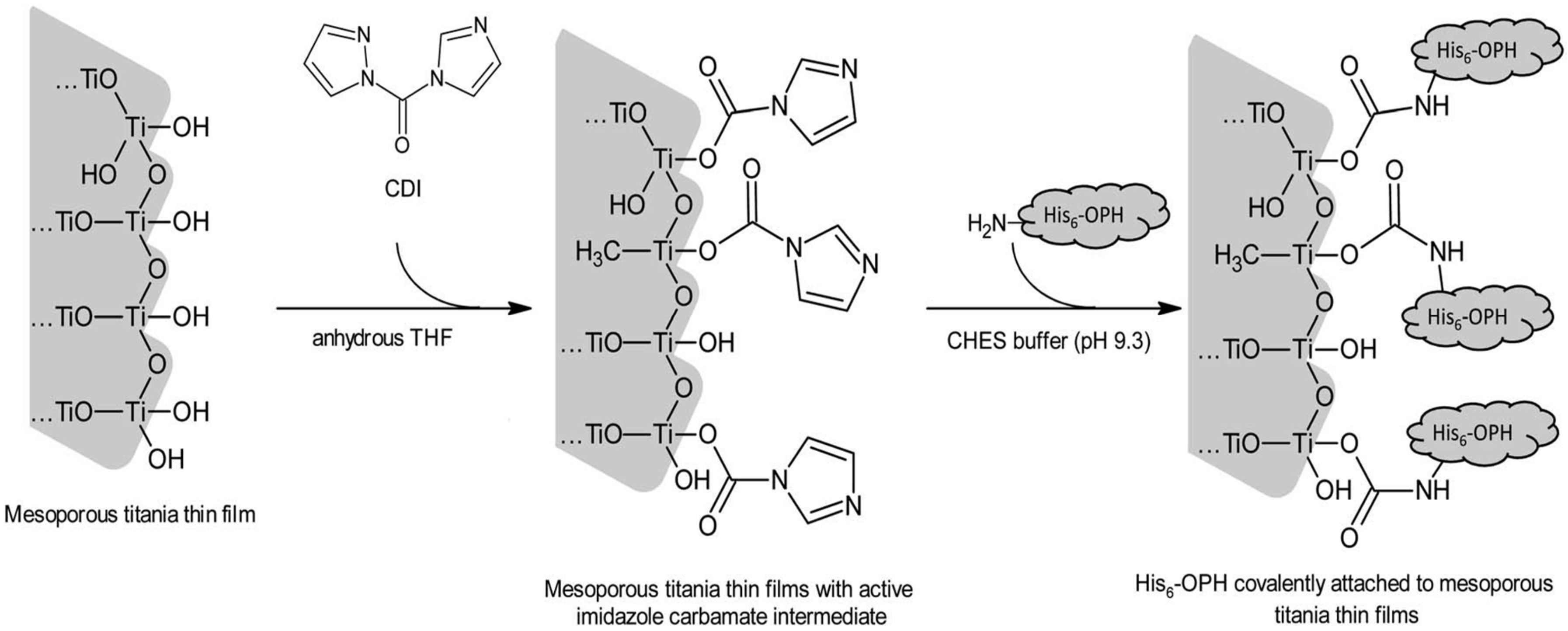


Fig. 4: Cycles of usage (a) and stability (b) of silica particles with immobilized His_6-OPH.

Mesoporous TiO_2 thin films as efficient enzyme carriers for paraoxon determination/detoxification



Schematic representation of the preparation route of His_6-OPH-conjugated mesoporous titania thin films trough CDI mediated reaction.

Analyst, 2014, 139, 3127–3136

Mesoporous TiO_2 thin films as efficient enzyme carriers for paraoxon determination/detoxification

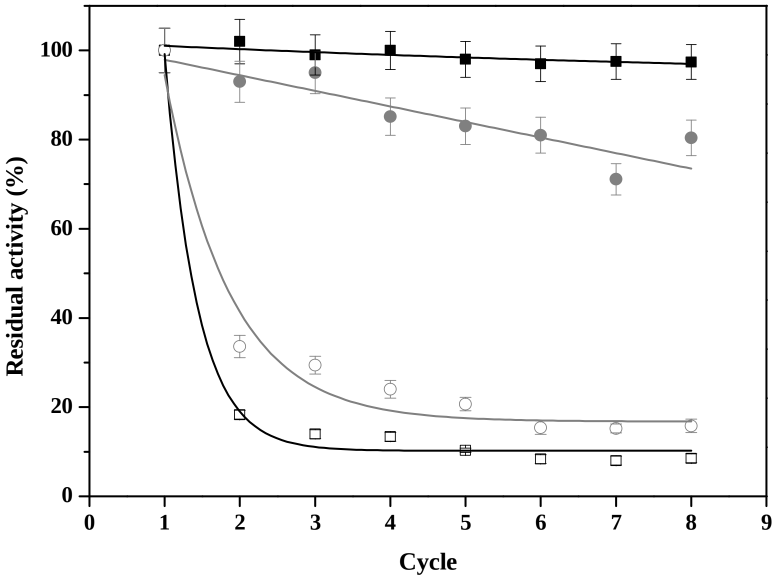


Figure Cycles of usage for covalently attached His_6-OPH, TiF-10 and TiF-bim (black and grey squares), and adsorbed His_6-OPH, TiF-10, TiF-10 and TiF-bim (black and grey circles). Measurements were performed with selected 50 mm^2 bio-functionalized mesoporous titania thin-films with covalently attached His_6-OPH at 20 °C and pH 10.5 (CB, 50 mM). Substrate: 0.3 mM paraoxon.

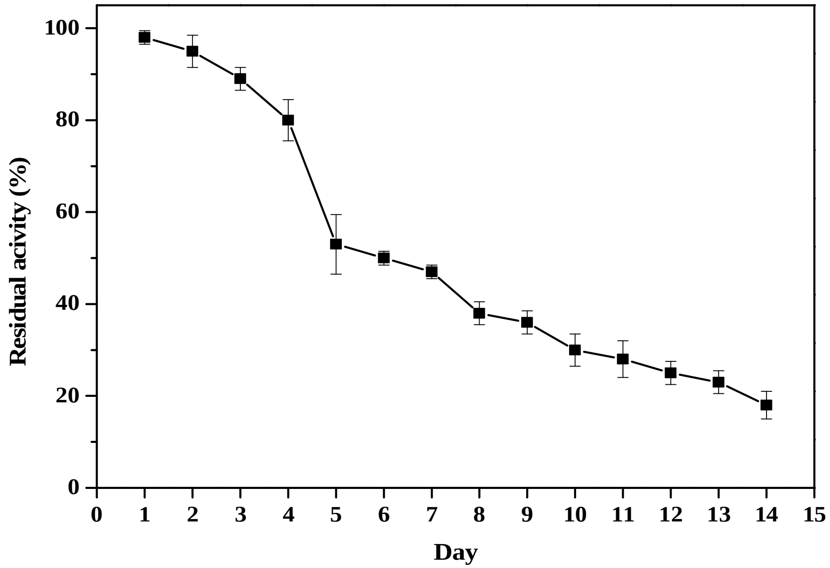
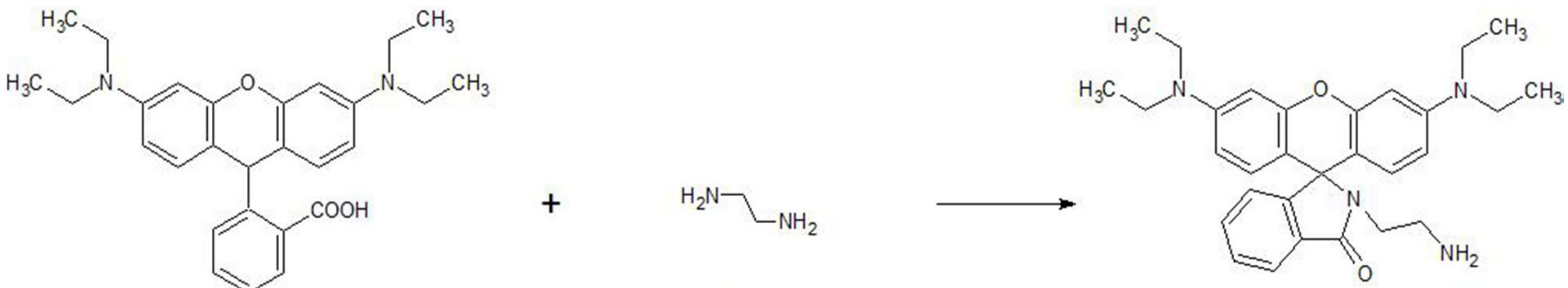


Figure Stability of titania bio-sensing film (TiF-9) with covalently attached enzyme several days after film preparation.

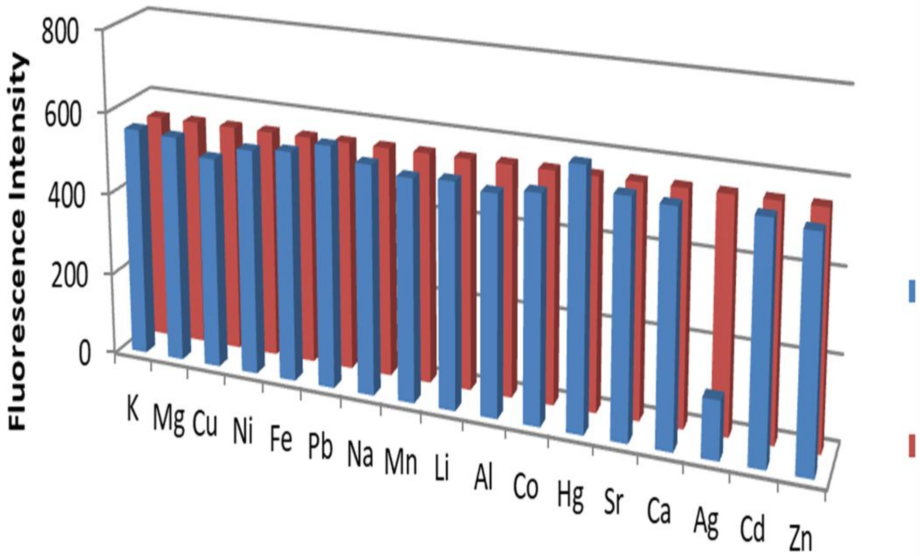
Design and investigation of optical properties of *N*-(Rhodamine-B)-lactam-ethylenediamine (RhB-EDA) fluorescent probe

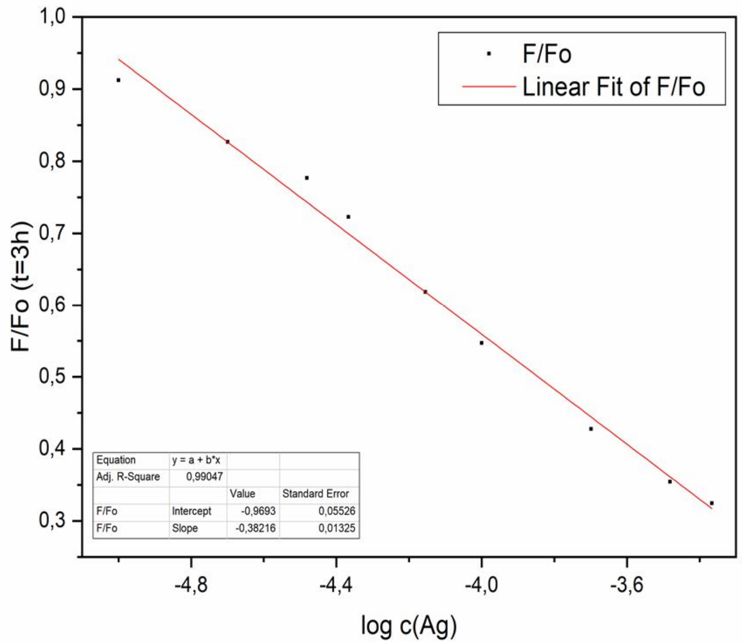
(E. Soršak, A. Lobnik, Sensors 2018)



1 2 3

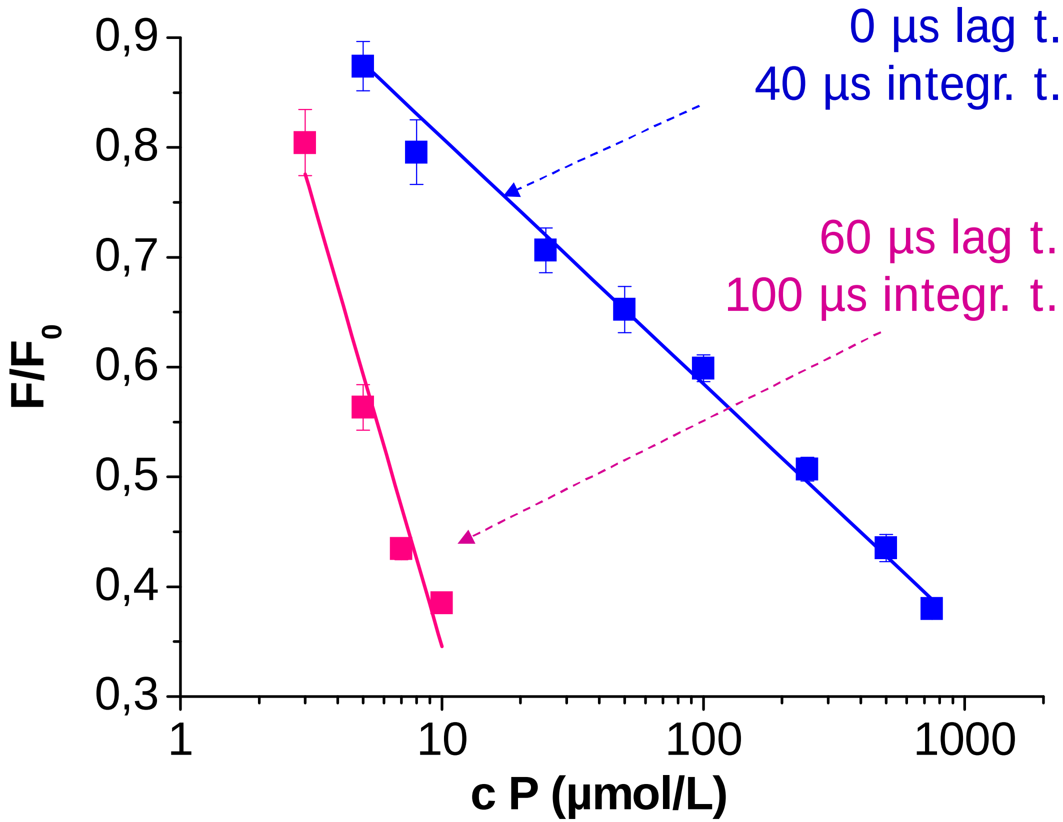
The linear range for Ag^+ ions` detection is from $0.43 \cdot 10^{-3}$ to 10^{-6} M.





Applications in water: Determination of phosphate (P) using europium-tetracycline complex (EuTc)

(A. Durkop, M. Turel, O.S. Wolfbeis, A. Lobnik, Anal. Chim Acta, 2006)



5-750 µmol/L PO_4^{3-}
LOD = 5 µmol/L

3-10 µmol/L PO_4^{3-}
LOD = 3 µmol/L

$$y = A + B \times \log(c_P \times 10^6)$$

	0 µs lag.t. 40 µs int.t.	60 µs lag.t 100 µs int.t.
A	1,033 ±0,018	0,955 ±0,081
B	-0,224 ±0,007	-0,586 ±0,091
r^2	0,993	0,954

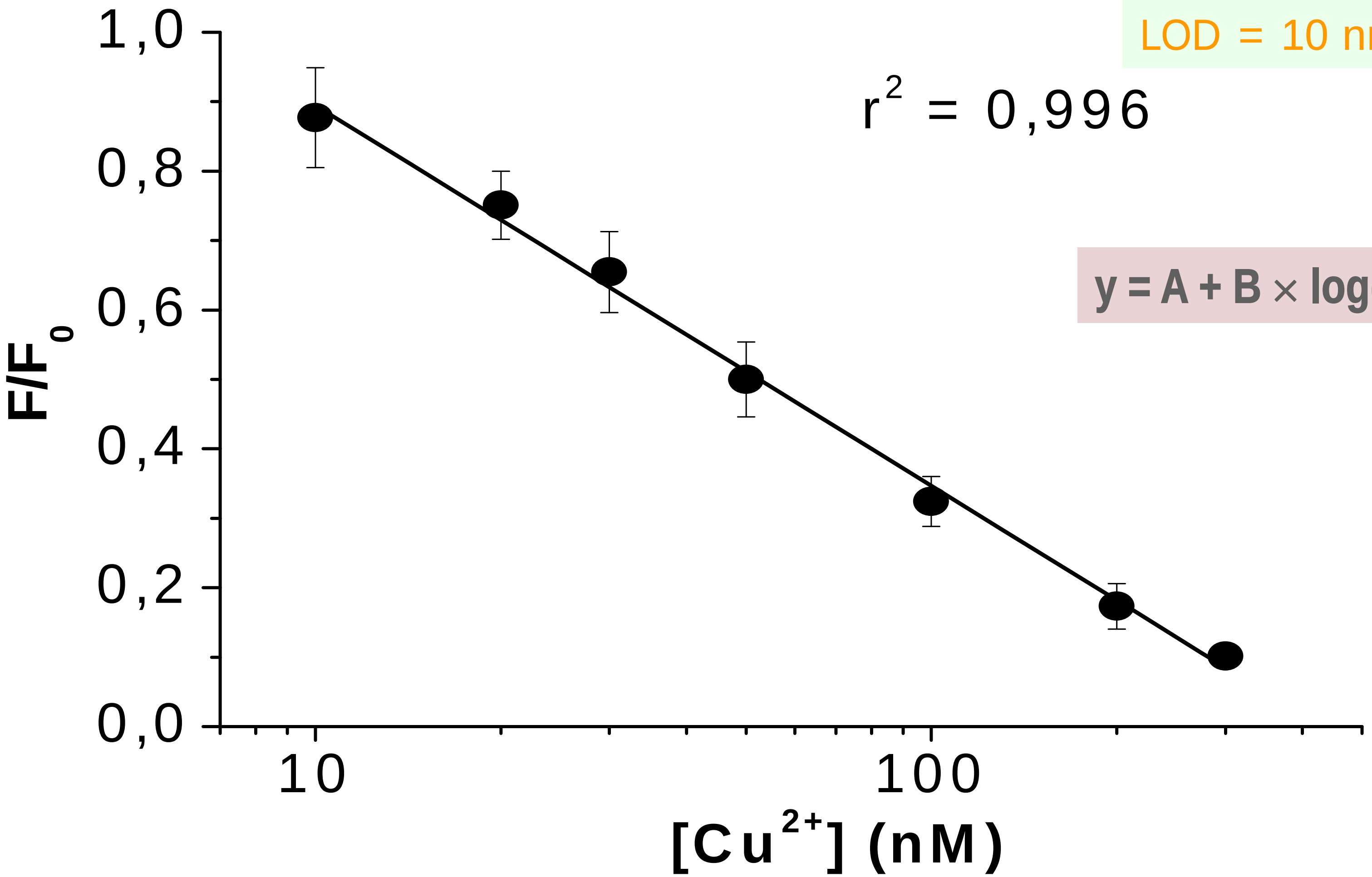
Ex filter = 405±30 nm, Em filter = 612±10 nm

Determination of copper (Cu) using terbium-ligand complex (TbL_2)

Spectral properties of TbL_2 in water solution

lag time = 50 µs, integration time = 1000

10-300 nmol/L Cu^{2+}
LOD = 10 nmol/L



$y = A + B \times \log(c_{Cu} \times 10^9)$

RESYNTEX

A new circular economy concept: from textile waste towards chemical and textile industries feedstock

Prof. dr. Aleksandra Lobnik, UMARI, IOS, Ltd.
Resyntex Co-coordinator, Technical Manager

Resyntex Partners

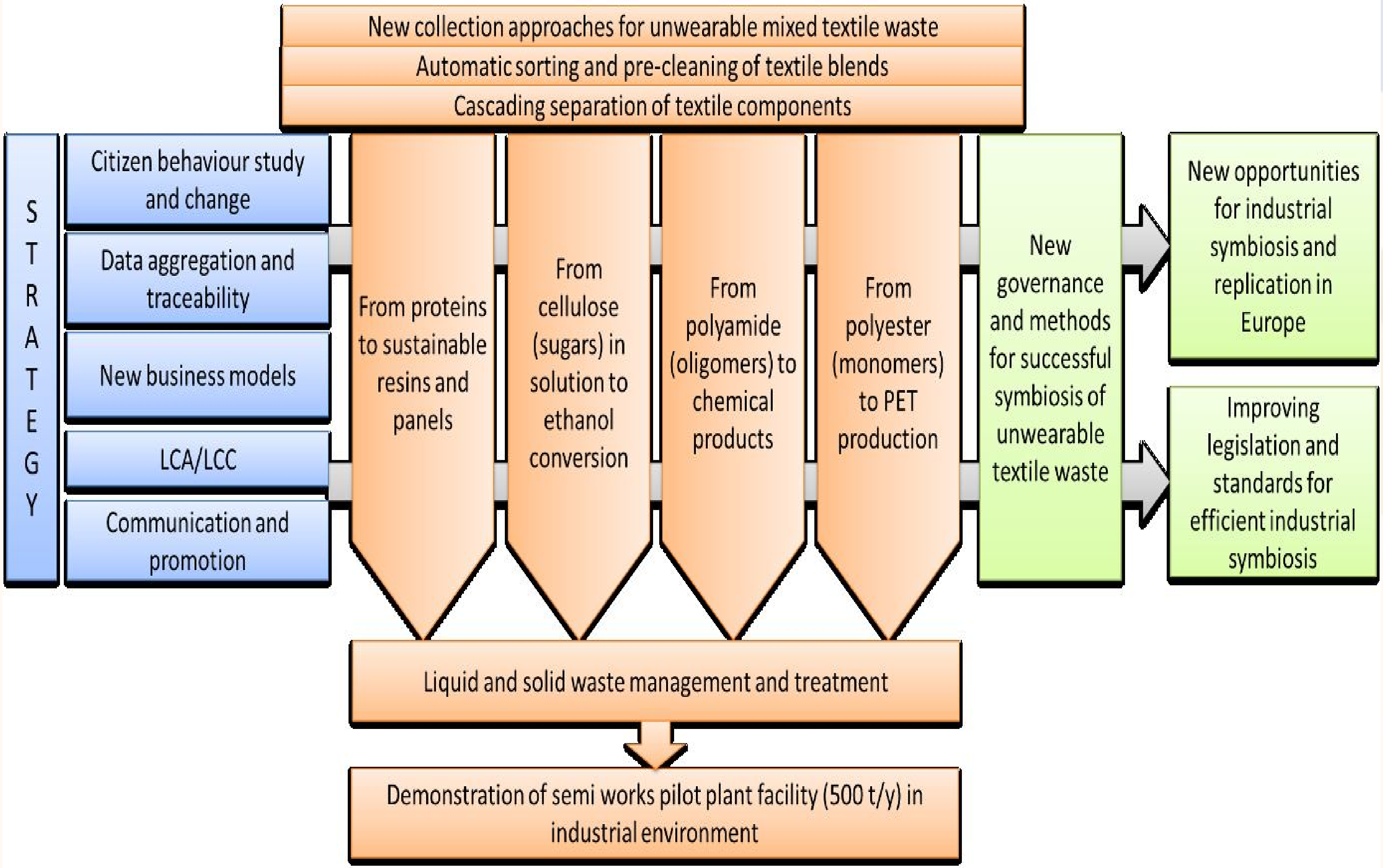
COORDINATOR: SOEX
CO-COORDINATOR: IOS

20 project partners from 10 different EU members:

industrial associations, enterprises, small and medium-sized enterprises, research institutions.

Together, we create an effective model for the whole value chain.

The RESYNTEX project is considering and demonstrating the whole value chain starting from



RESYNTEX

RESYNTEX PROJECT WORK STRUCTURE

What are the Aiams of the Resyntex project

As a result, **economic advantages** can be provided besides prevention of industrial environmental problems

Feedstock from textile waste	Obtained chemical products from symbiosis
Protein hydrolysates	Adhesives used for the manufacturing of wood-based panels (**CHIMAR**)
Depolymerized cellulose (sugars in solution)	Cellulosic ethanol manufacturing based on the PROESA® technology (**CTXI**)
Polyamide oligomers (mainly polyamide 6 and 66)	Hydrogenolysis/hydrolysis of PA6 and PA6,6 leading to hexanoic acid, N-pentylamine (also known as amylamine), aminohexanoic acid, caprolactam and other important chemical intermediates, (**Arkema**)
	Reaction of alcohols and PA6,6 leading e.g. to diesters (e.g. as solvents) and hexamethylenediamine (**Arkema)**
PET monomers (terephthalic acid, ethylene glycol)	Plastic bottles constituted of PET from the reaction of terephthalic acid (PTA) and ethylene glycol (EG) (**CTXI**)

Co-funded by the European Union's Horizon 2020 research and innovation programme

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Nanomaterials & Applications

June / 2019, Ljubljana / Slovenia