Nanoquímica: aplicacions en Biomedicina

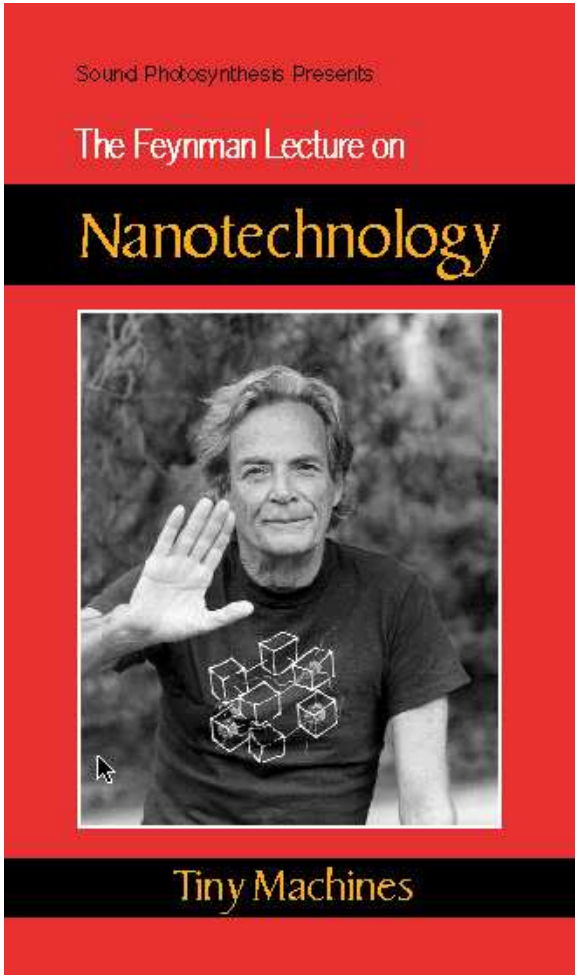
Ma Lluïsa Pérez-García

Department of Pharmacology and Therapeutical Chemistry, Faculty of Pharmacy
Institute of Nanoscience and Nanotechnology UB (IN2UB)
University of Barcelona

Seminaris de Recerca- Facultat de Farmàcia (UB)- 6 de març 2012

Feynman's Vision

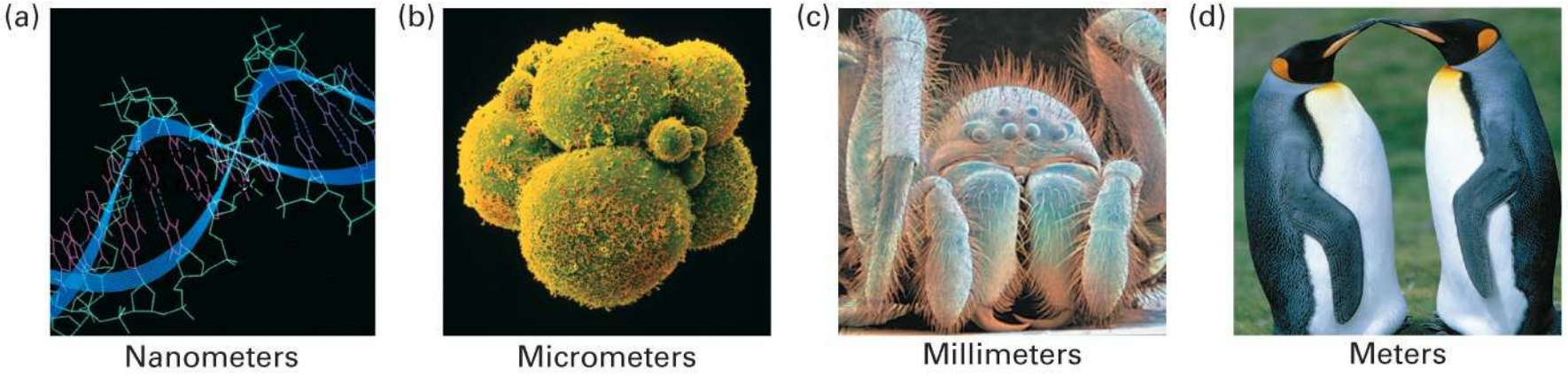
"There's Plenty of Room
at the Bottom,
An Invitation to
Enter a
New Field of
Physics"

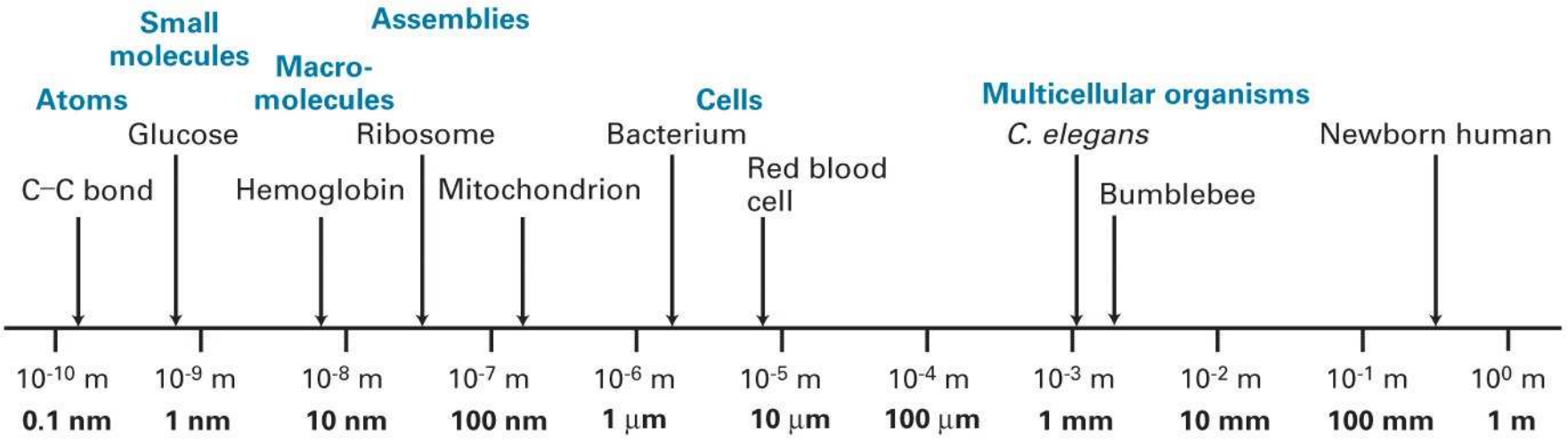


http://www.zyvex.com/nanotech/feynman.html

Dec 1959 Talk at APS / CalTech

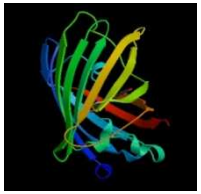
http://www.photosynthesis.com/RICHARD_F.html

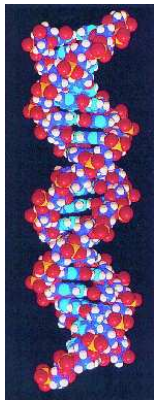


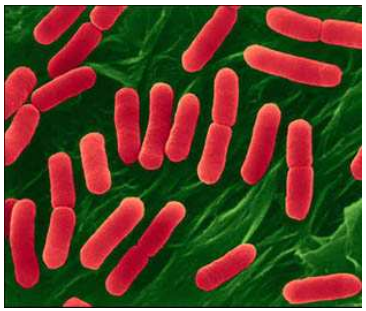


Simple molecules <1nm

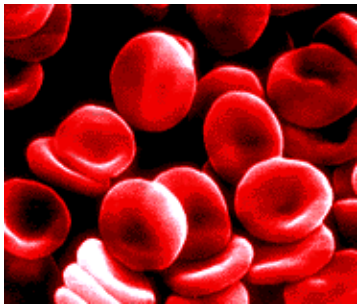
DNA proteins nm



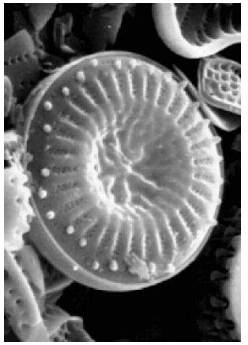




red blood cell ~5 μm (SEM)



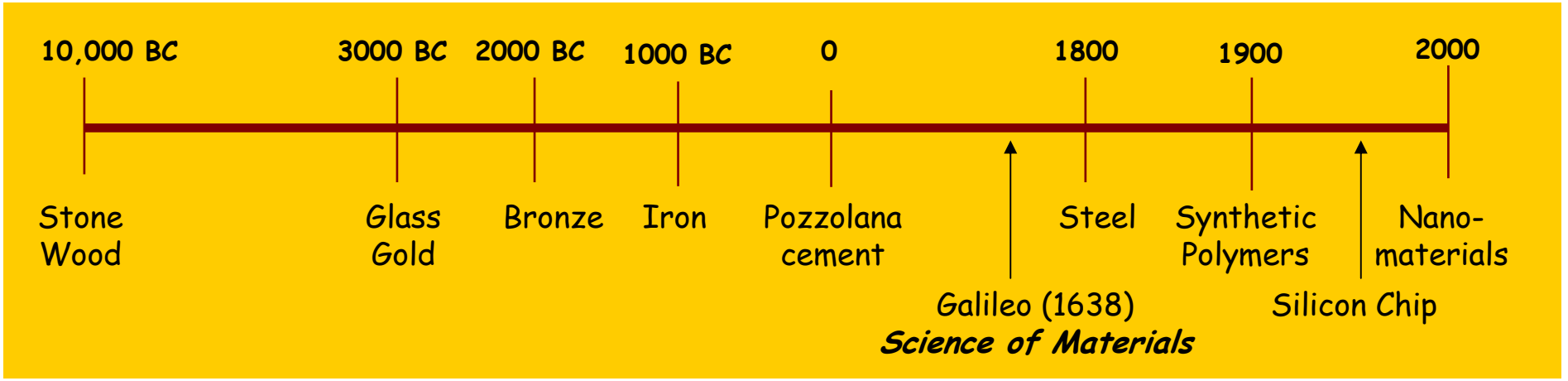
diatom 30 μm



Materials

Material: things needed for doing or making something; "writing materials"; "useful teaching materials"

Material, stuff: the tangible substance that goes into the makeup of a physical object; "coal is a hard black material"



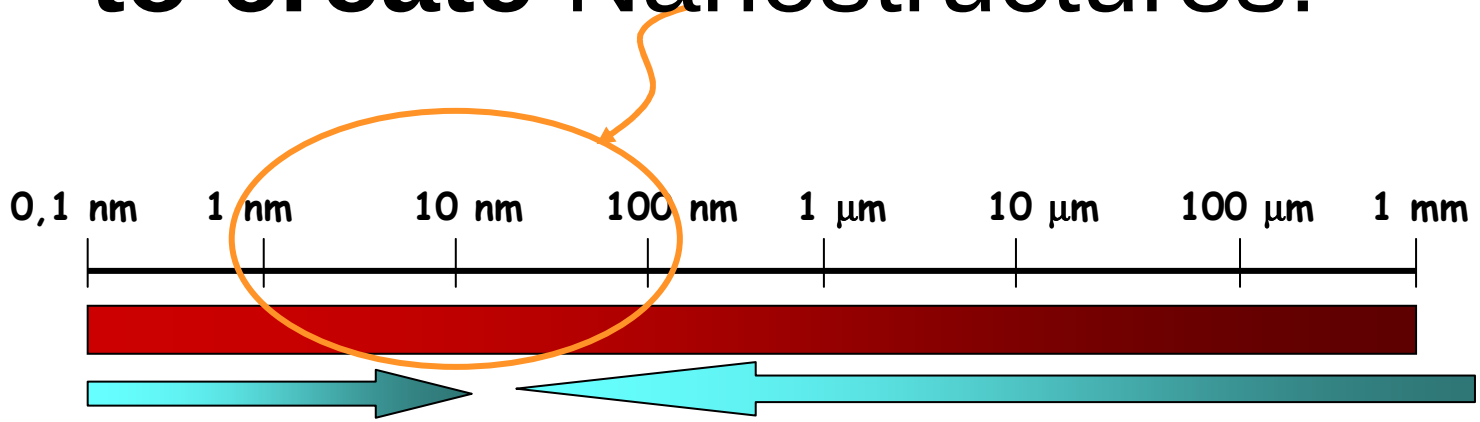
Nanomaterials

"A nanomaterial is a material consisting of a substance or structure which has at least one dimension less than 100 nm"

http://www.zyvex.com/AboutUs/Profiles/Sabbio.html

Size-dependent properties make the nanometre regime special

Two contrasting approaches to create Nanostructures:



"Bottom-up"

Individual atoms and molecules are placed or are driven to precisely where they are needed by:

Chemical synthesis
Self-assembly
Self-organisation
Deposition

Mixed approaches

e.g. Lithography and Self-Assembly

"Top-down"

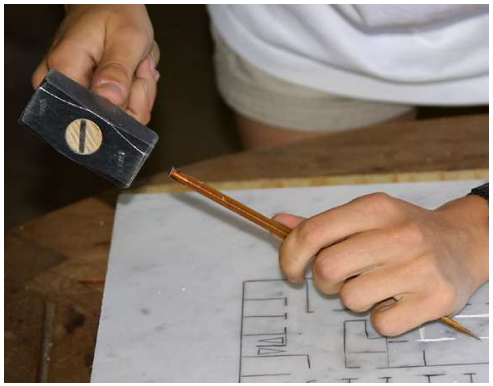
Take a block of material and carve it away until the object that is wanted is reached by:

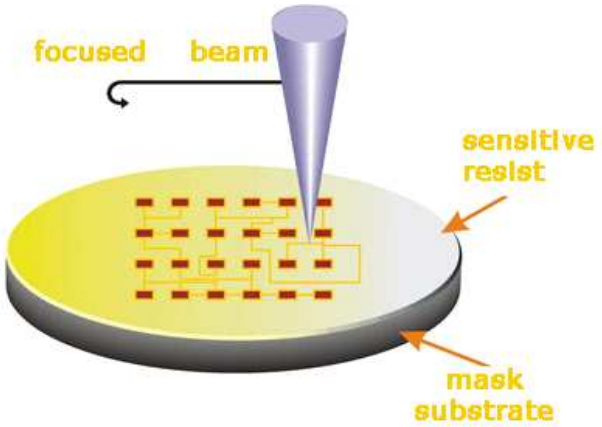
Engraving
Photolithography
Milling
Destruction!

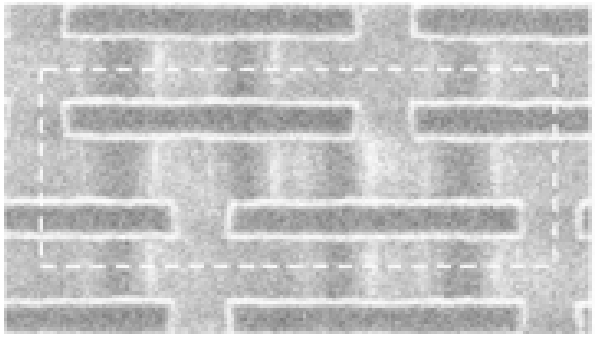
Top-Down Nanoscience and Technology

Take a block of material and

carve it away until the object that is wanted is reached



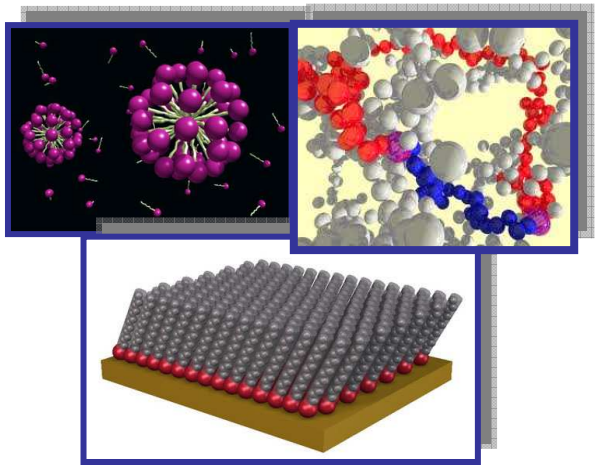




Intel® 45nm, six transistor SRAM cell.

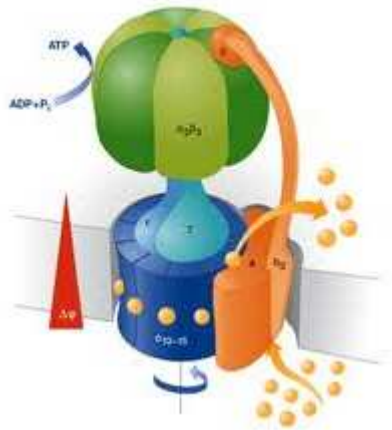
Non-covalent interactions

Molecular chemistry



Bottom-up

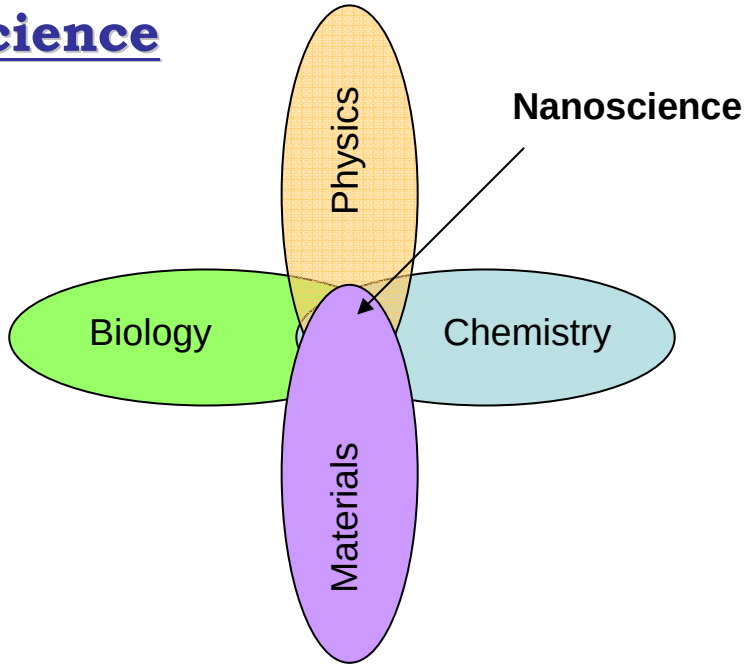
Supramolecular Chemistry



Nanomaterials

- **Molecular recognition**
- **Self-assembly**
- **Self-organisation**

Interdisciplinary science



Nanomedicine

Nanotechnology

(Nanomaterials)

Medicine

Nanomedicine

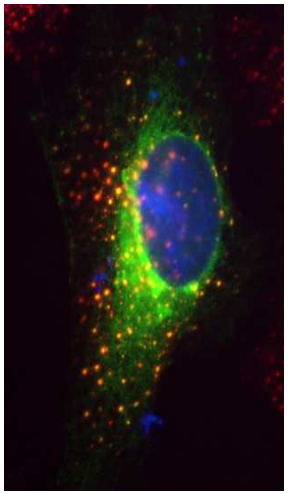
Diagnosis

Imaging

Tissue regeneration

Treatment

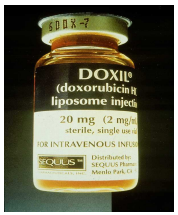
Applications of Nanotechnology in Medicine



Analytical tools, diagnostics and sensors
- **for use outside the patient**
- **with surgical access**

Imaging molecules and patients

Therapeutics and Drug Delivery Systems



Professor Alan Hill-Oxford Biosensors

- Direct patient exposure so technology must be safe

Technology must be cost effective and convenient to use

" Nanopharmaceuticals" or "Nanomedicines"

" Nanopharmaceuticals can be developed either as drug delivery systems or biologically active drug products".

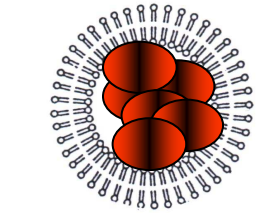
……. nanometre size scale complex systems, consisting of at ***least two components***, one of which being the active ingredient…….

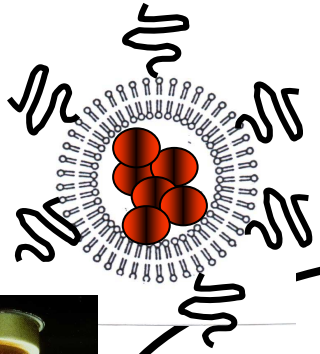
European Science Foundation (Dec 2005) www.esf.org

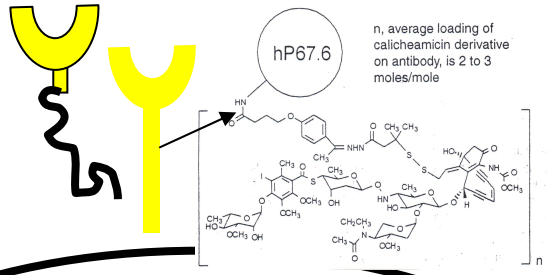
Liposomes

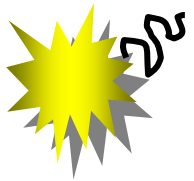
antibodies
conjugates

vectors for gene therapy





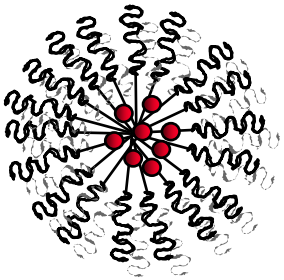




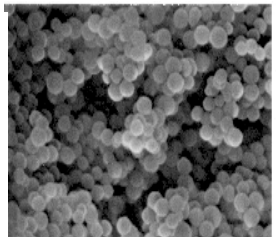
polymer
micelles

Myelotarg, Rituximab

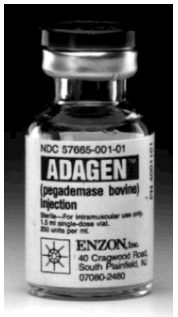
Nanomedicines

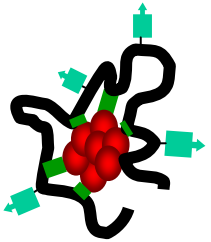


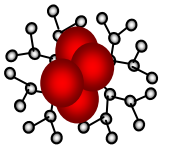
nanoparticles



Polymer-protein
conjugates

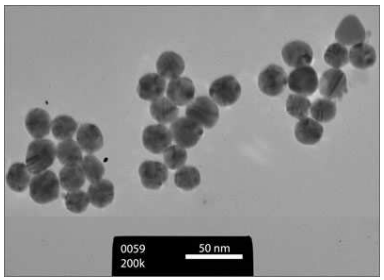






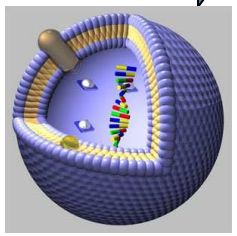
Polymer Therapeutics

Nanoscience → Nanomaterials → Nanotechnology

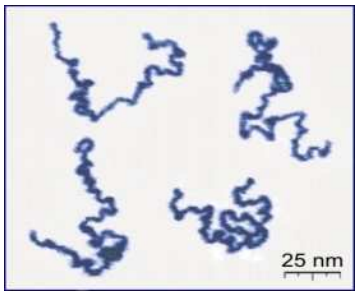


PARAMAGNETIC NANOPARTICLES

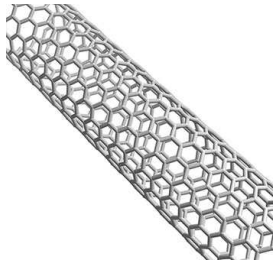
LIPOSOMES



POLYMERS

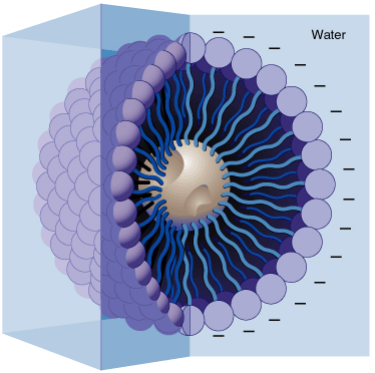


NANOMATERIALS

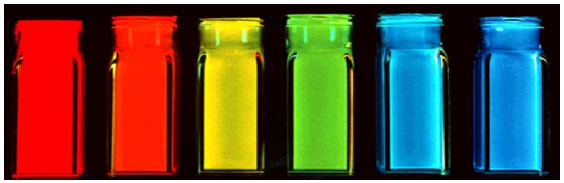


CARBON NANOTUBES

MICELLES

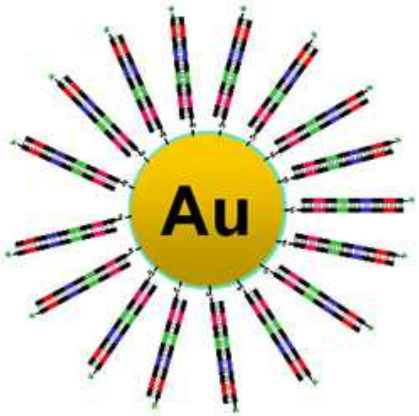


QUANTUMDOTS



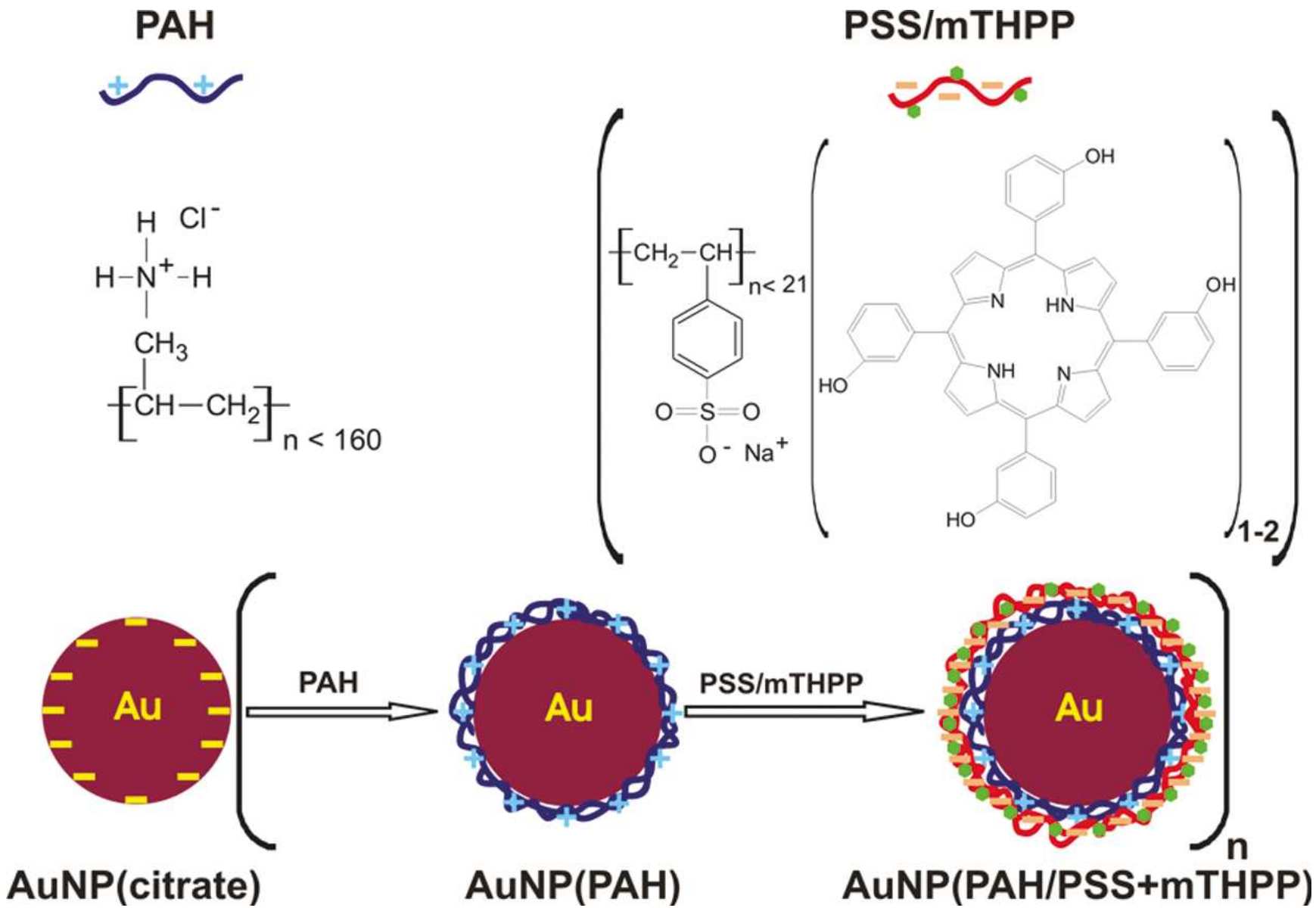
Gold nanoparticles

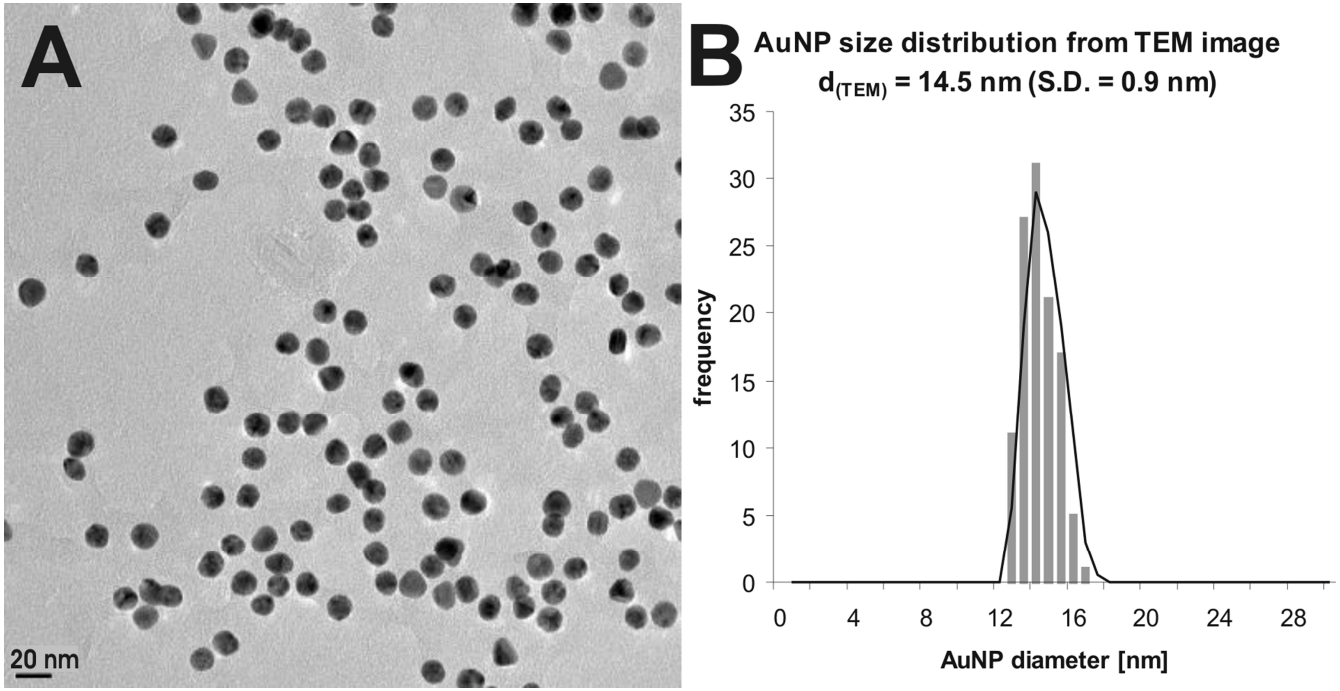
- ✓ Easy to synthesize
- ✓ Easy control of their properties (size, morphology, surface functionalization…)
- ✓ Biocompatible
- ✓ SPR
- ✓ Scattering



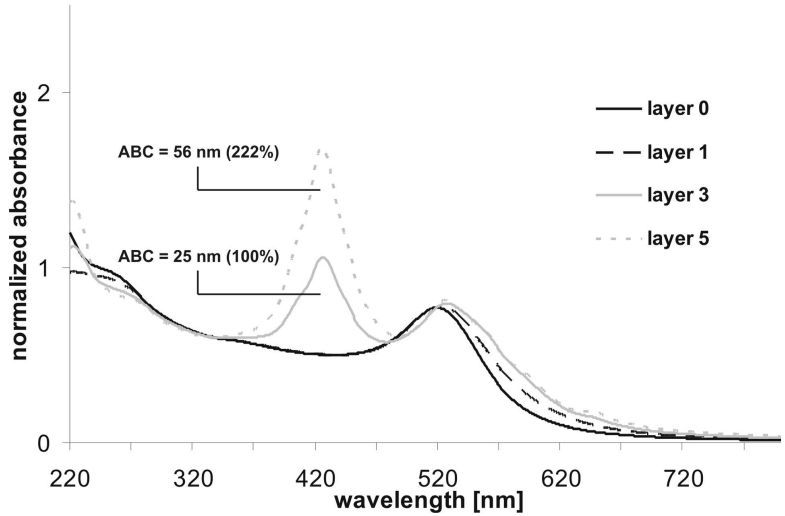
Photothermal therapy

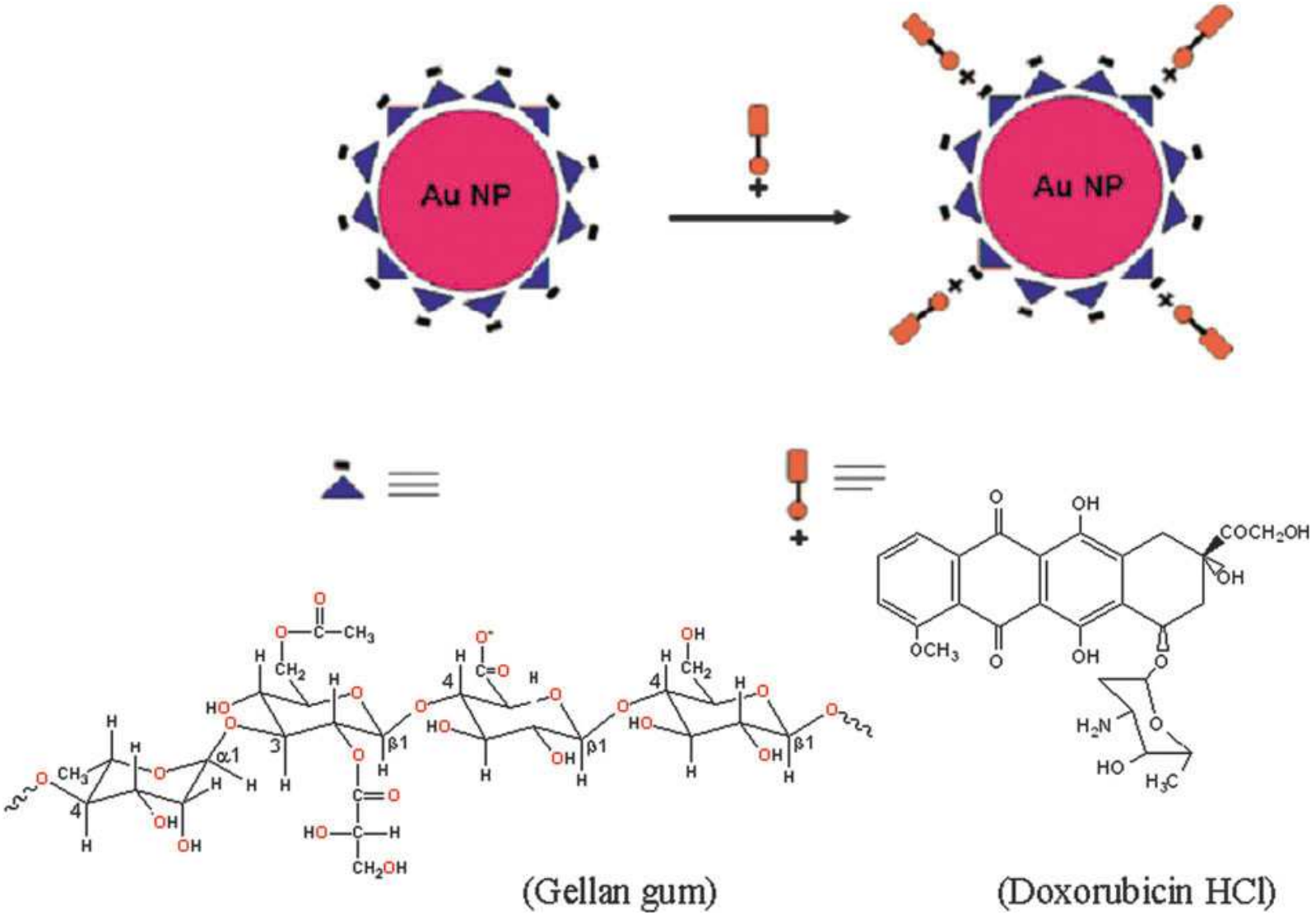
Sensing and delivery


PAH
PSS/mTHPP
n < 160
n< 21
1-2
PAH
PSS/mTHPP
Au
Au
Au
n
AuNP(citrate)
AuNP(PAH)
AuNP(PAH/PSS+mTHPP)



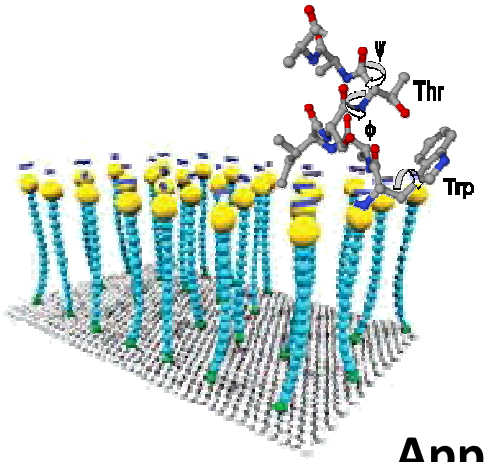
From AuNP-citrate to AuNP(PAH/PSS+mTHPP)$_2$PAH




Au NP
Au NP
(Gellan gum)
(Doxorubicin HCl)

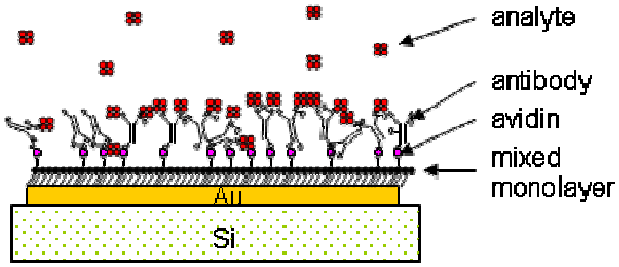
Self assembled monolayer

A spontaneously organised molecule layer in which a suitable functionalised molecule assembles through chemisorption on metal or glass surfaces.

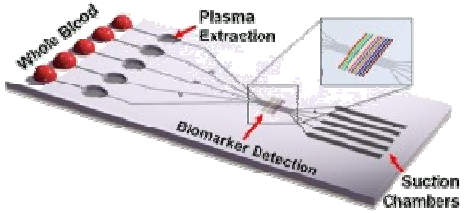


- Immobilize (bio)active molecules
- sense important chemical or biological parameters
- detect pathogens and metabolites

Applications : DIAGNOSTIC TOOLS

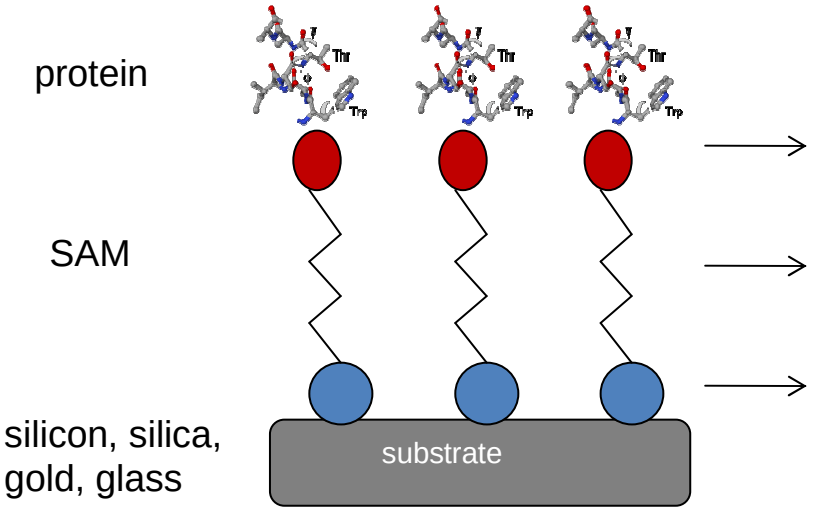


(BIO)SENSORS



(BIO)CHIPS

Self Assembled Monolayers (SAMs)



Parts of a SAM

Receptor: Terminal functional group (binds (bio)molecules)

Alkyl chain: linker and gives stability

Head group (binds to the substrate)

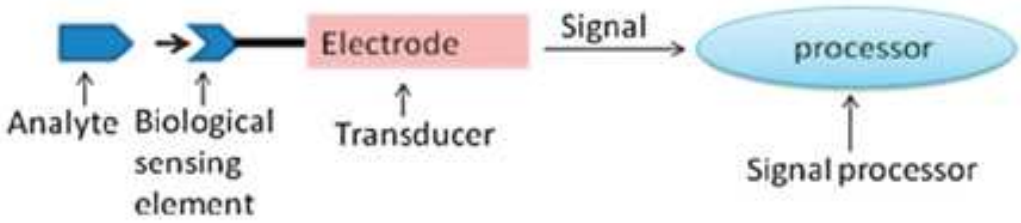
✓excellent biosensor stability

✓easy preparation in the Laboratory

✓ economical viable

Sensor Applications

➢ Electrochemical biosensors



- Glucose sensor for monitoring blood glucose levels

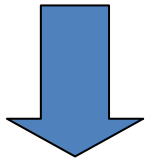
  - Immobilization of GLUCOSE OXIDASE (GOx-FAD)

$$\beta\text{-D-Glucose} + \text{GOx-FAD} \rightarrow \text{GOx-FADH}_2 + \delta\text{-D-gluconolactone} \quad (1)$$

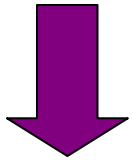
$$\text{GOx-FADH}_2 + O_2 \rightarrow \text{GOx-FAD} + H_2O_2 \quad (2)$$

$$H_2O_2 \rightarrow 2e^- + O_2 + 2H^+ \quad (3)$$

Supramolecular chemistry



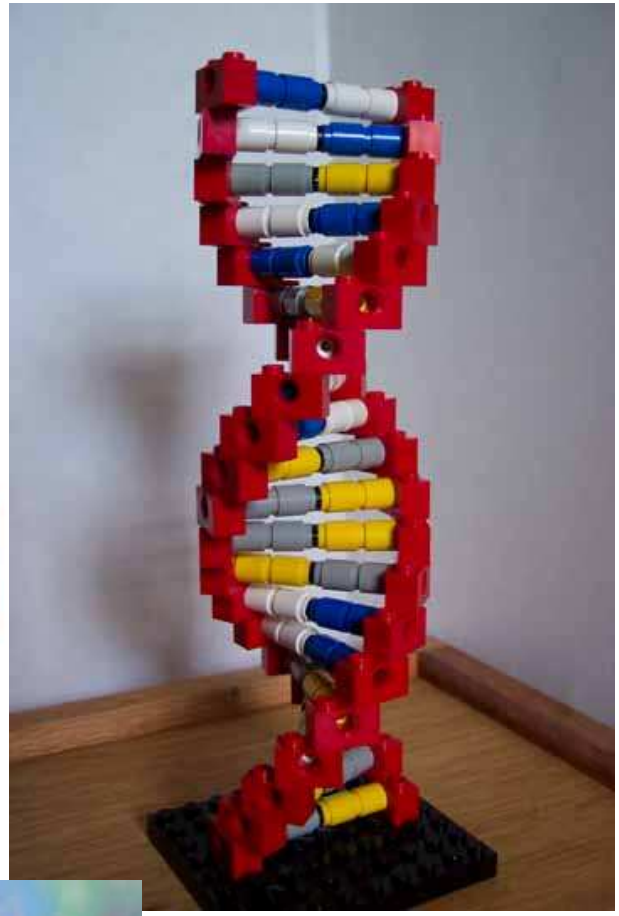
Self-assembly



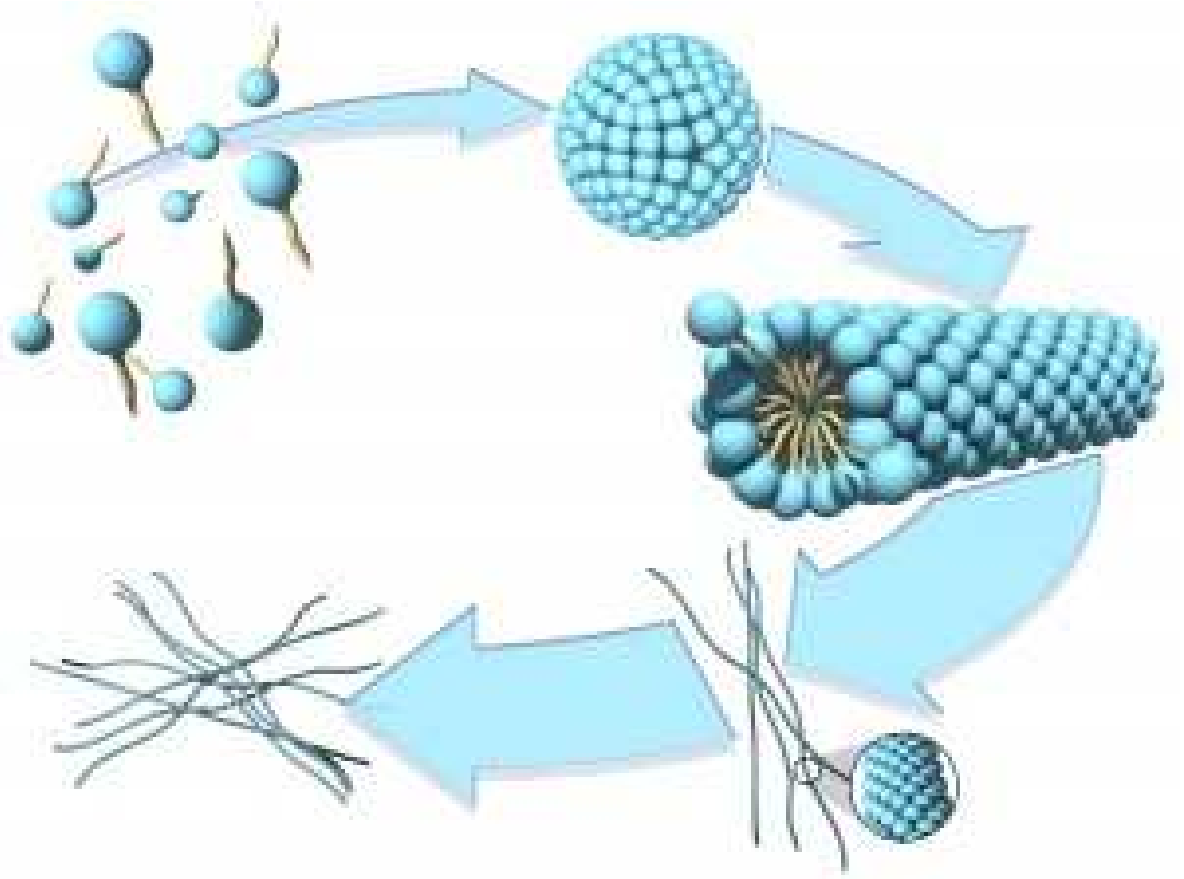
Functional Nano-Materials

CHEMISTRY

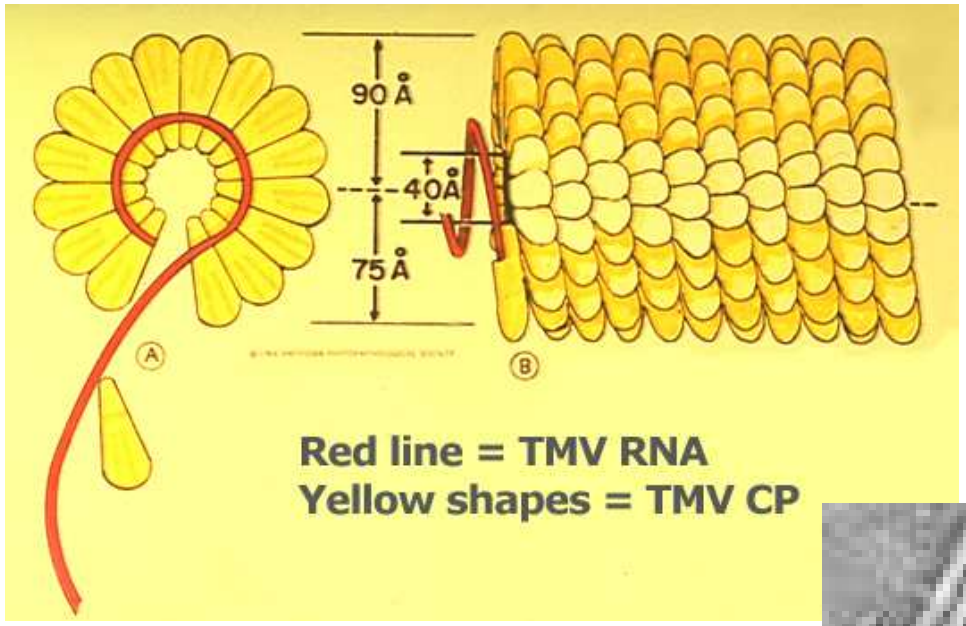
It's all about building blocks

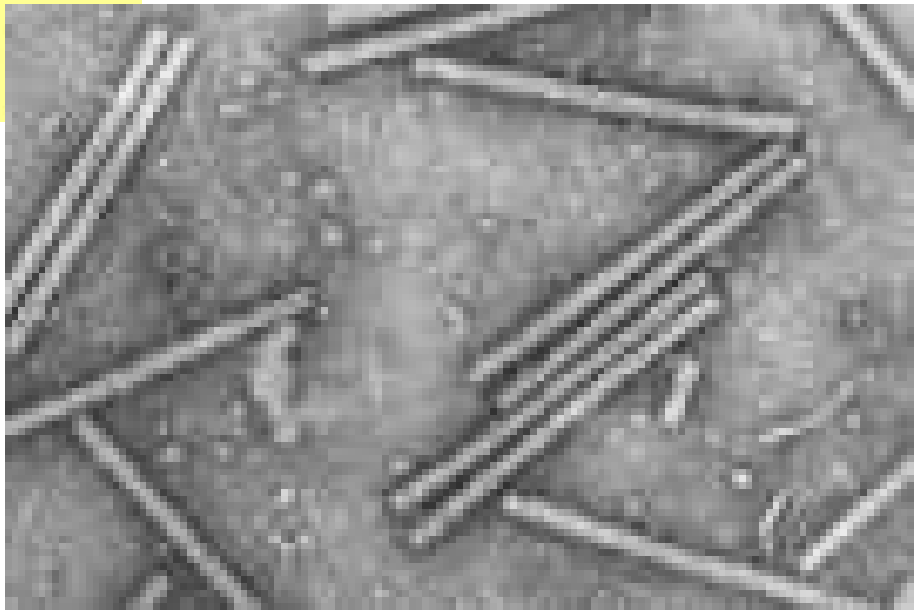


Self-assembly: surfactants



Self-assembly: proteins

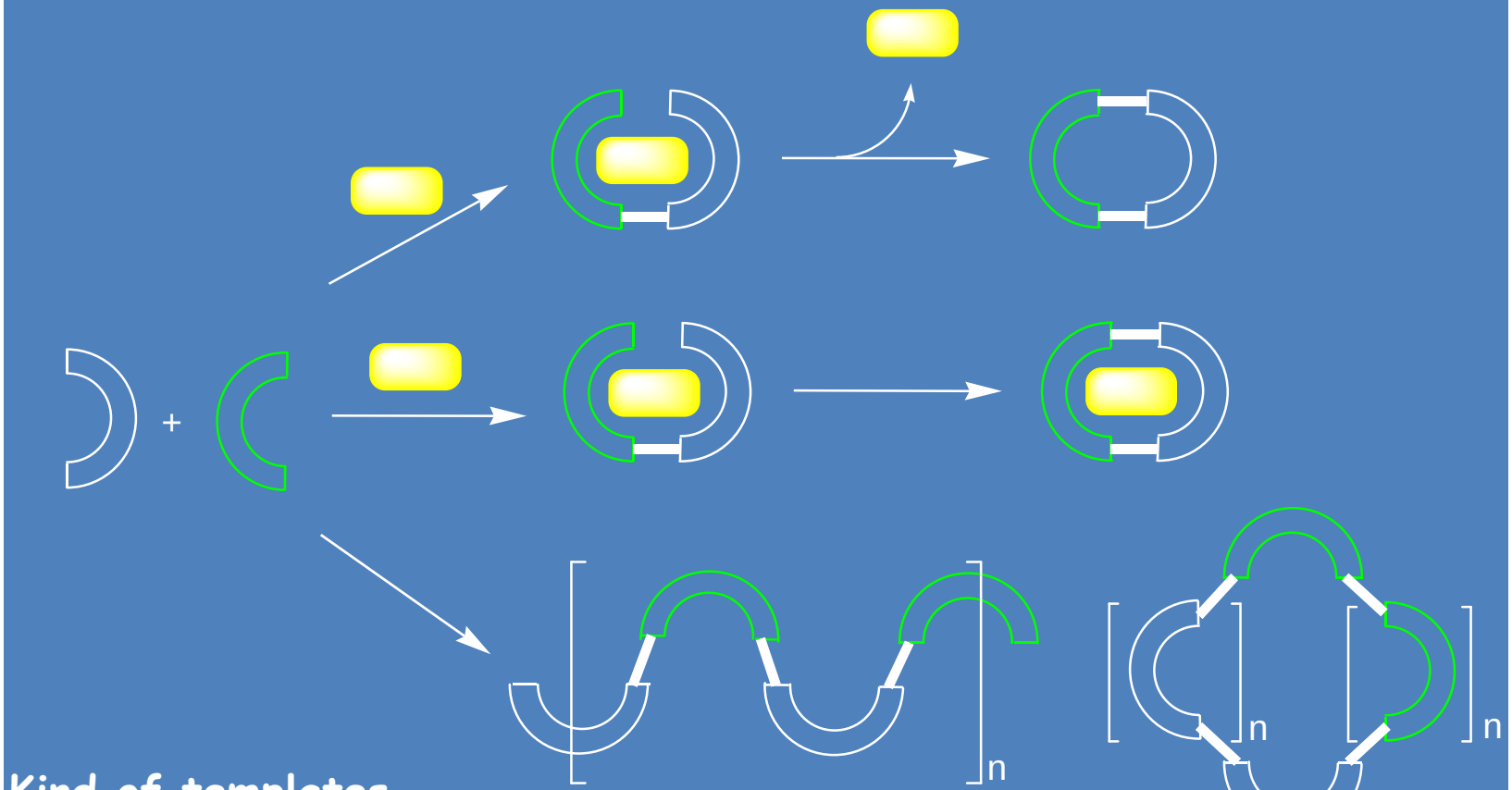




Template based self-assembly

Anion templated-synthesis of anion receptors

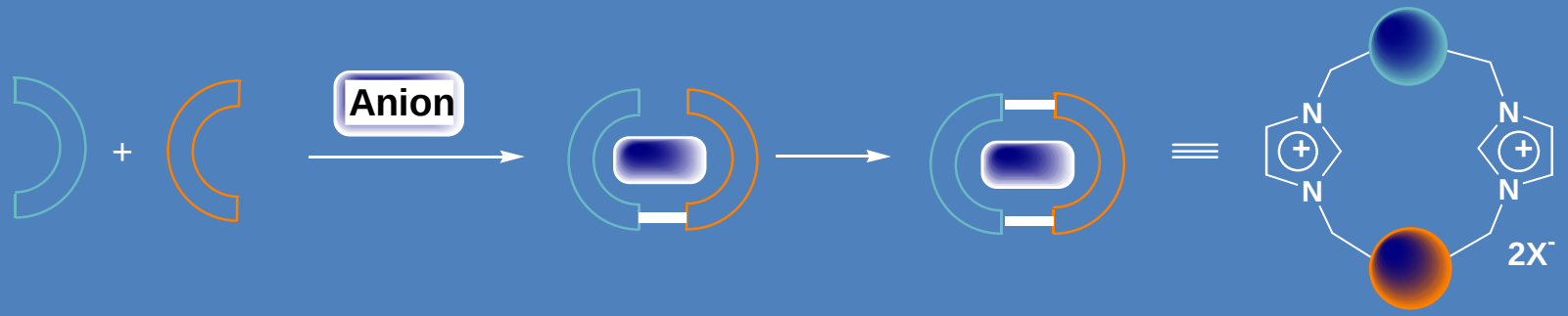
Template-Directed synthesis



Kind of templates

- Cations
- Neutral molecules
- Anions

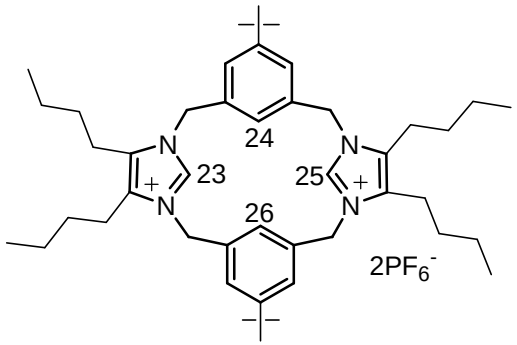
Anionic Template-Directed Synthesis of Dicationic [1_4]Imidazoliophanes

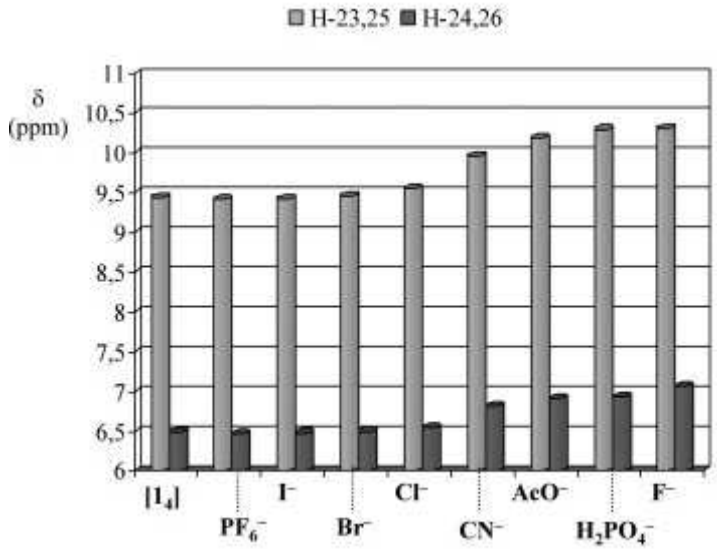


- Assessment of the anion control
- Quantification of the template effect

Imidazolium derived macrocycles for anion recognition

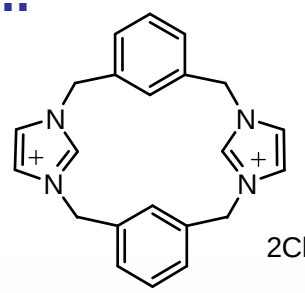
In solution…

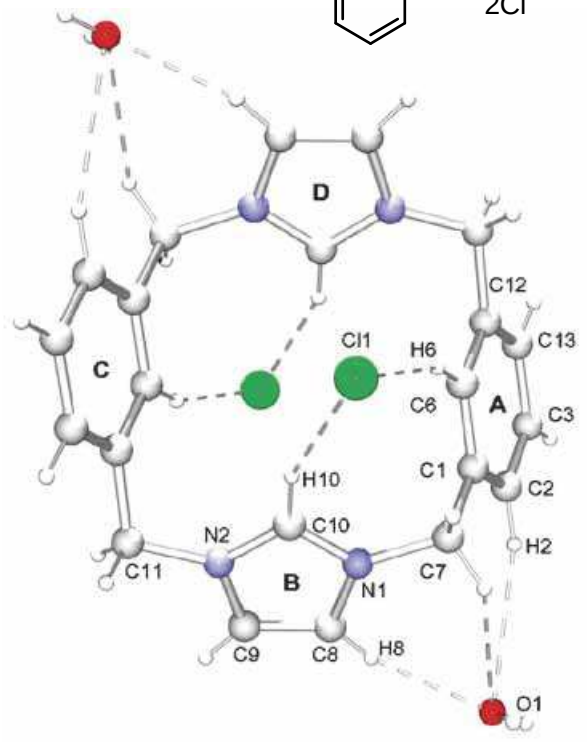




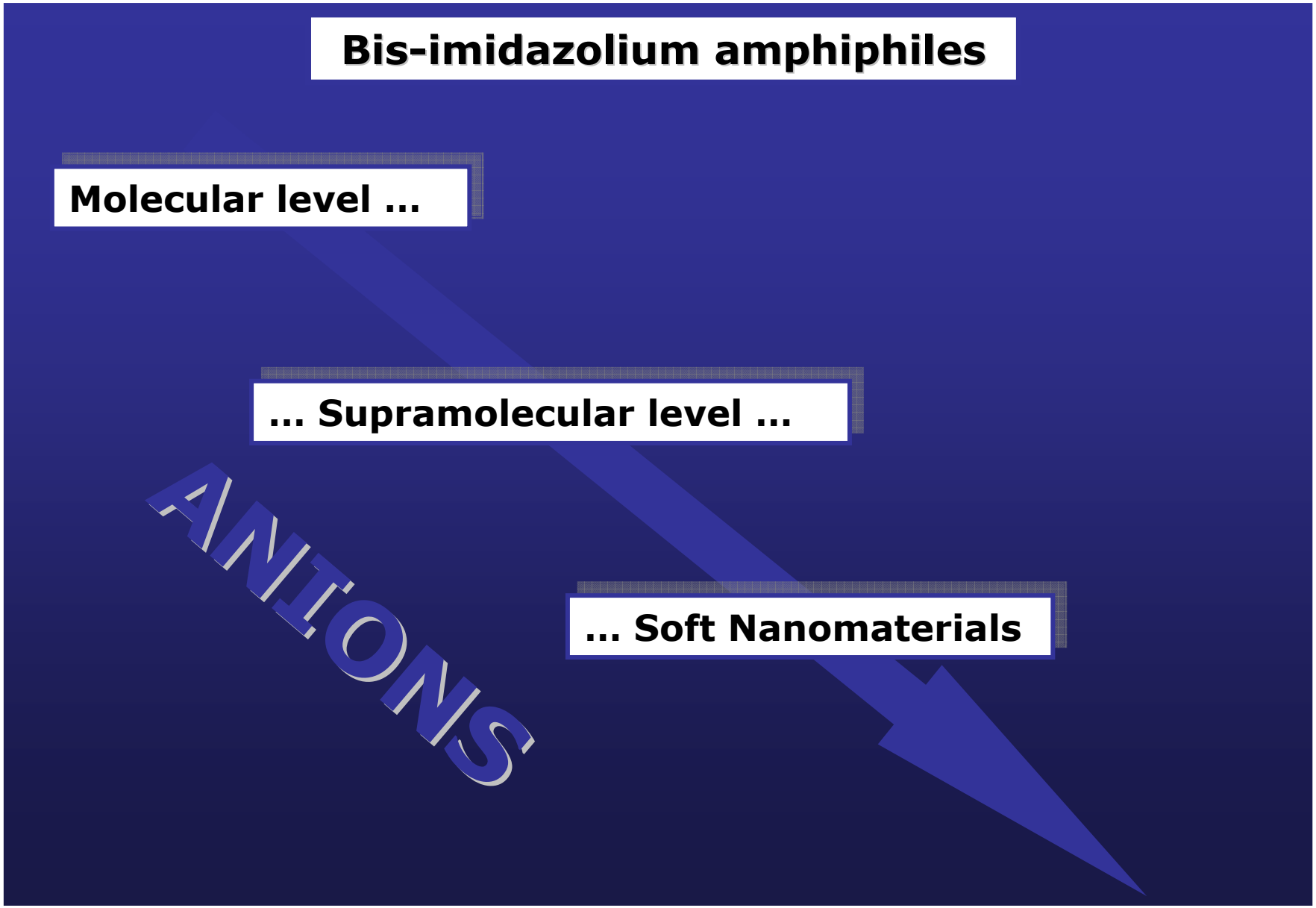
E. Alcalde, N. Mesquida, L. Pérez-García, *Eur. J. Org. Chem.* **2006**, 3988-3996.

In solid state…





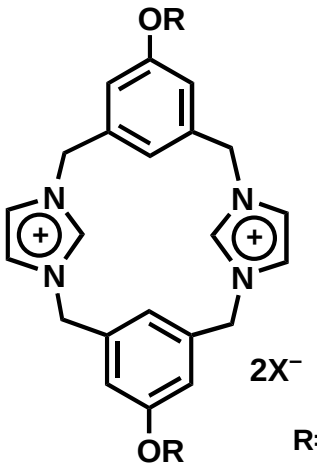
E. Alcalde, C. Alvarez-Rúa, S. García-Granda, E. García-Rodriguez, N. Mesquida, L. Pérez-García, *Chem. Commun*. **1999**, 395-396

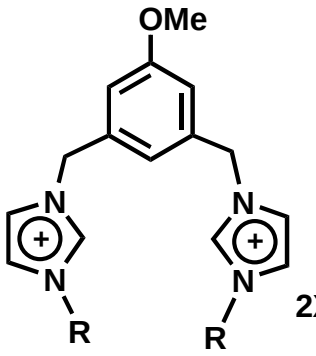

Bis-imidazolium amphiphiles
Molecular level …
… Supramolecular level …
… Soft Nanomaterials
ANIONS

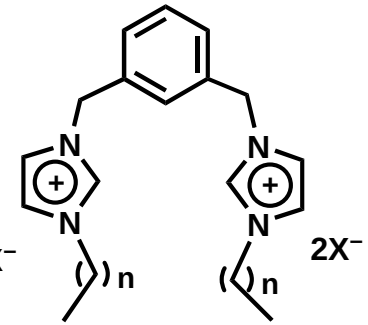
Self-assembly and self-organization

Dicationic amphiphiles

- Liquid crystals
- surfactants
- sensors





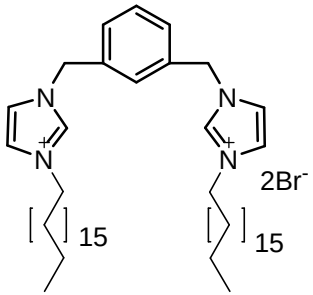


R= $-(CH_2)_{17}-CH_3$;
$-(CH_2)_{11}-S-(CH_2)_{11}-CH_3$

n= 5, 7, 9, 15

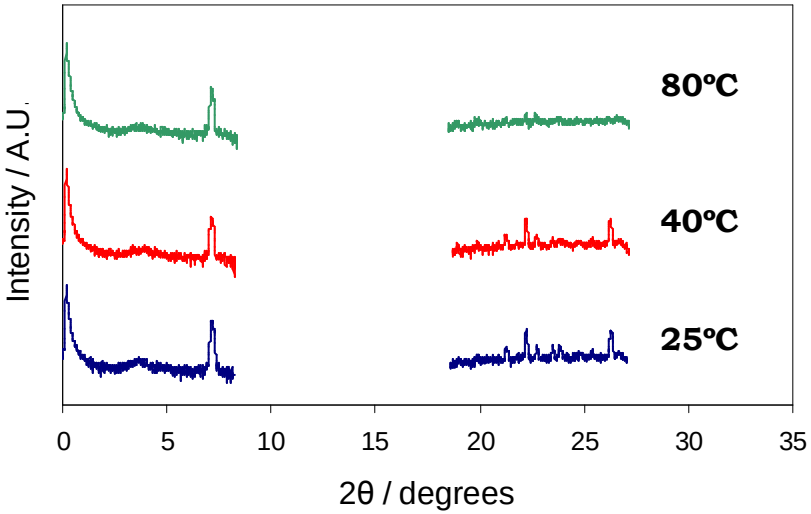
Bis-imidazolium amphiphiles: liquid crystal behaviour

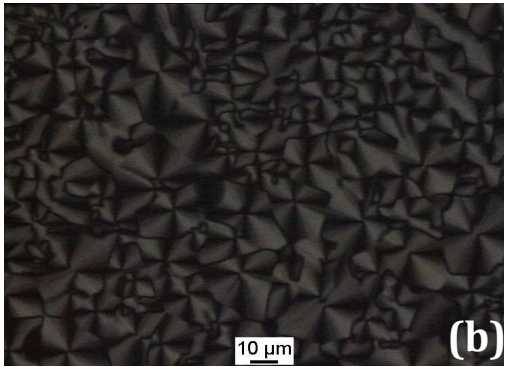
Thermotropic LC



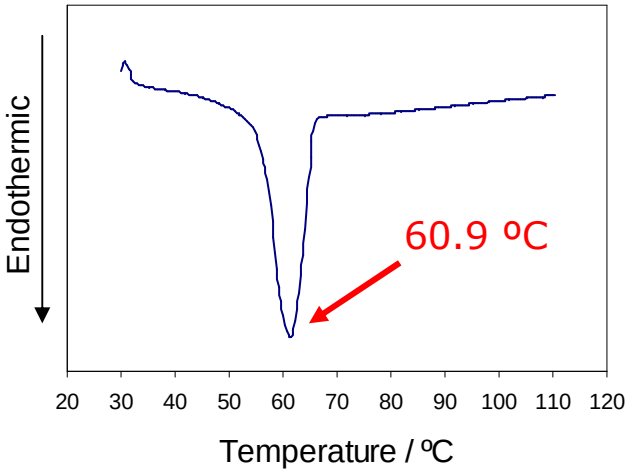
PpC18SIII·2Br

XRD

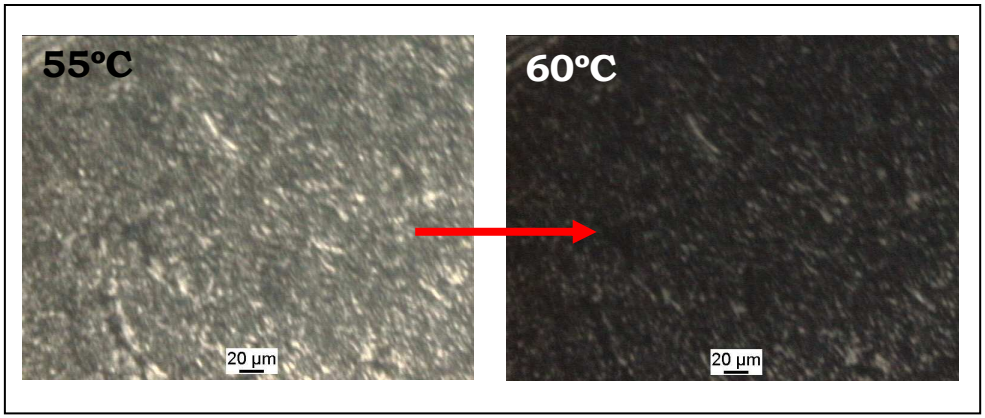




DSC



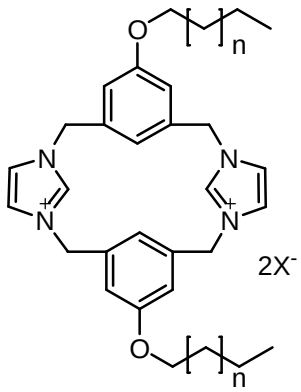
POM - HT



L. Casal-Dujat, O. Penón, C. Rodríguez-Abreu, C. Solans and L. Pérez-García, *New. J. Chem.*, **2012**, *36*, 558–561

Bis-imidazolium amphiphiles: liquid crystal behaviour

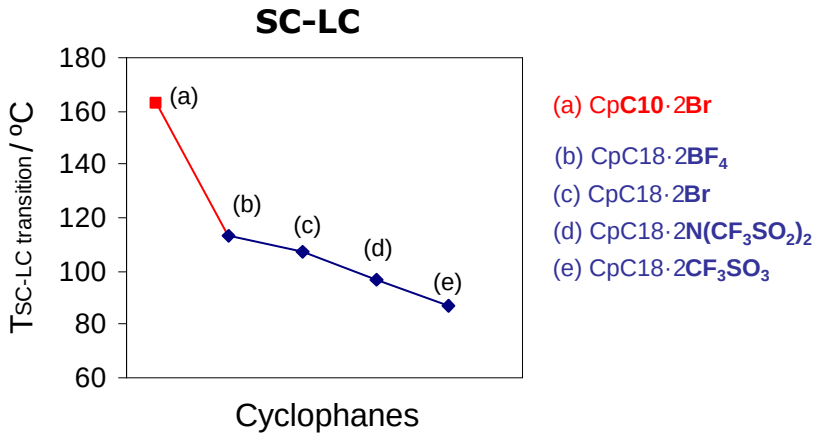
Thermotropic LC



CpC10SI·2Br: n=7

CpC18SI·2X: n=15 (X=Br, BF_4, CF_3SO_3, $N(CF_3SO_2)_2$

Transition temperatures:



(a) Cp**C10**·2**Br**

(b) CpC18·2$\mathbf{BF_4}$

(c) CpC18·2**Br**

(d) CpC18·2$\mathbf{N(CF_3SO_2)_2}$

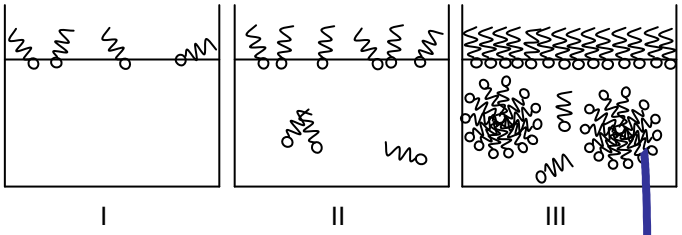
(e) CpC18·2$\mathbf{CF_3SO_3}$

➢ The **increase** of **carbon atoms** in the alkyl chain induce **decrease** in SC-LC transition **temperature**

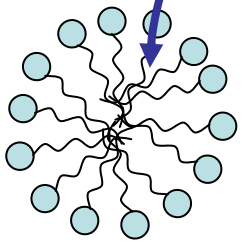
➢ The **counter-ion modulates** the SC-LC transition temperature in CpC18 and decrease in the order: BF_4 > Br > $N(CF_3SO_2)_2$ > CF_3SO_3

Aggregation systems:

In the bulk:

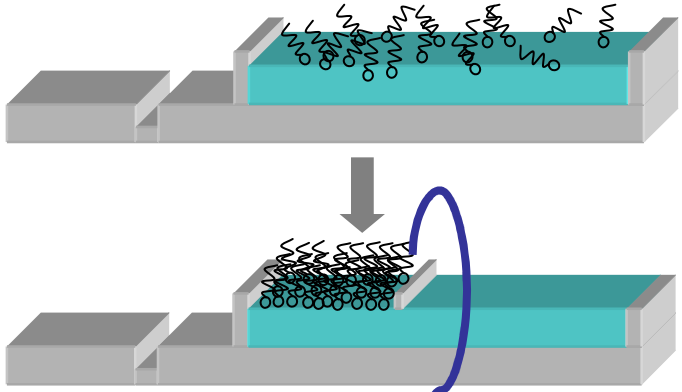


Self-assembly

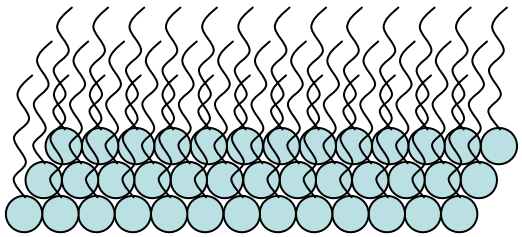


Micelles

At the air-water interface:



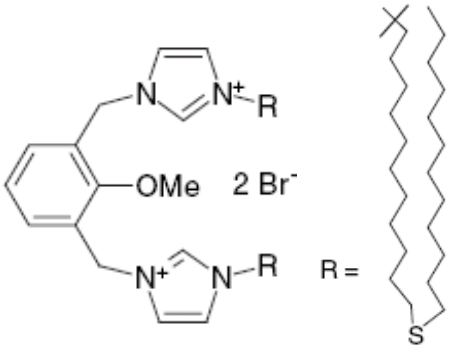
Self-organisation



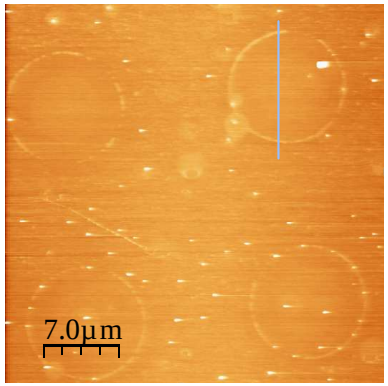
Monolayer

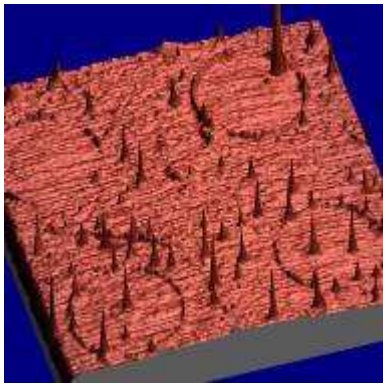
Dicationic amphiphiles — **Organization**

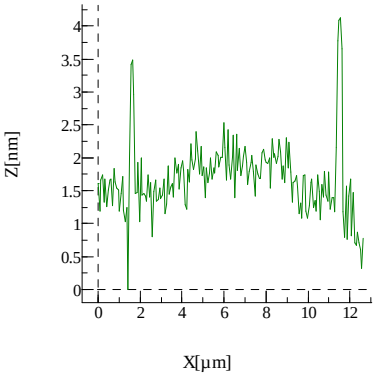
On gold surfaces



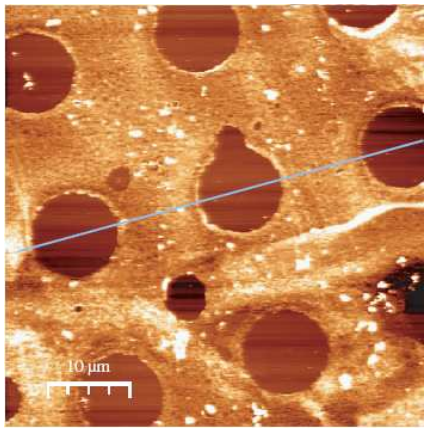
Tapping mode Topography scan images

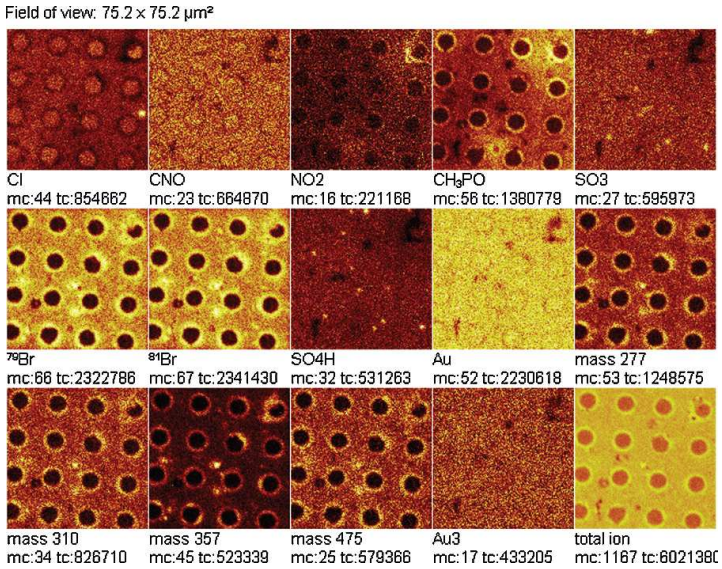






5mM en CH_2Cl_2 / CCl_4
10µm, round negative

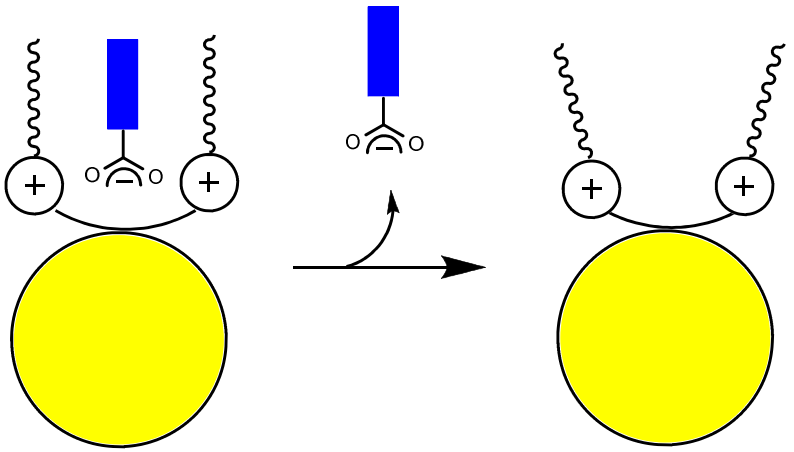


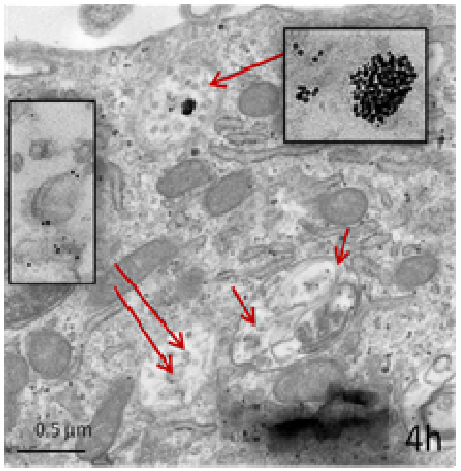


C. Sporer, L. Casal, D. Caballero, J. Samitier, A. Errachid, L. Pérez-García, *Sensor Lett.,* **2009**, *7*, 757-764

Gold nanoparticles for drug delivery

- Synthesis
- Characterisation
- Toxicity studies
- Internalization in cells
- Incorporation of anionic drugs





Synthesis of gold nanoparticles

Brust-Schiffrin
(AuNP in organic solvent)

$HAuCl_4$ (aq)
+
tetraoctylammonium (TA)

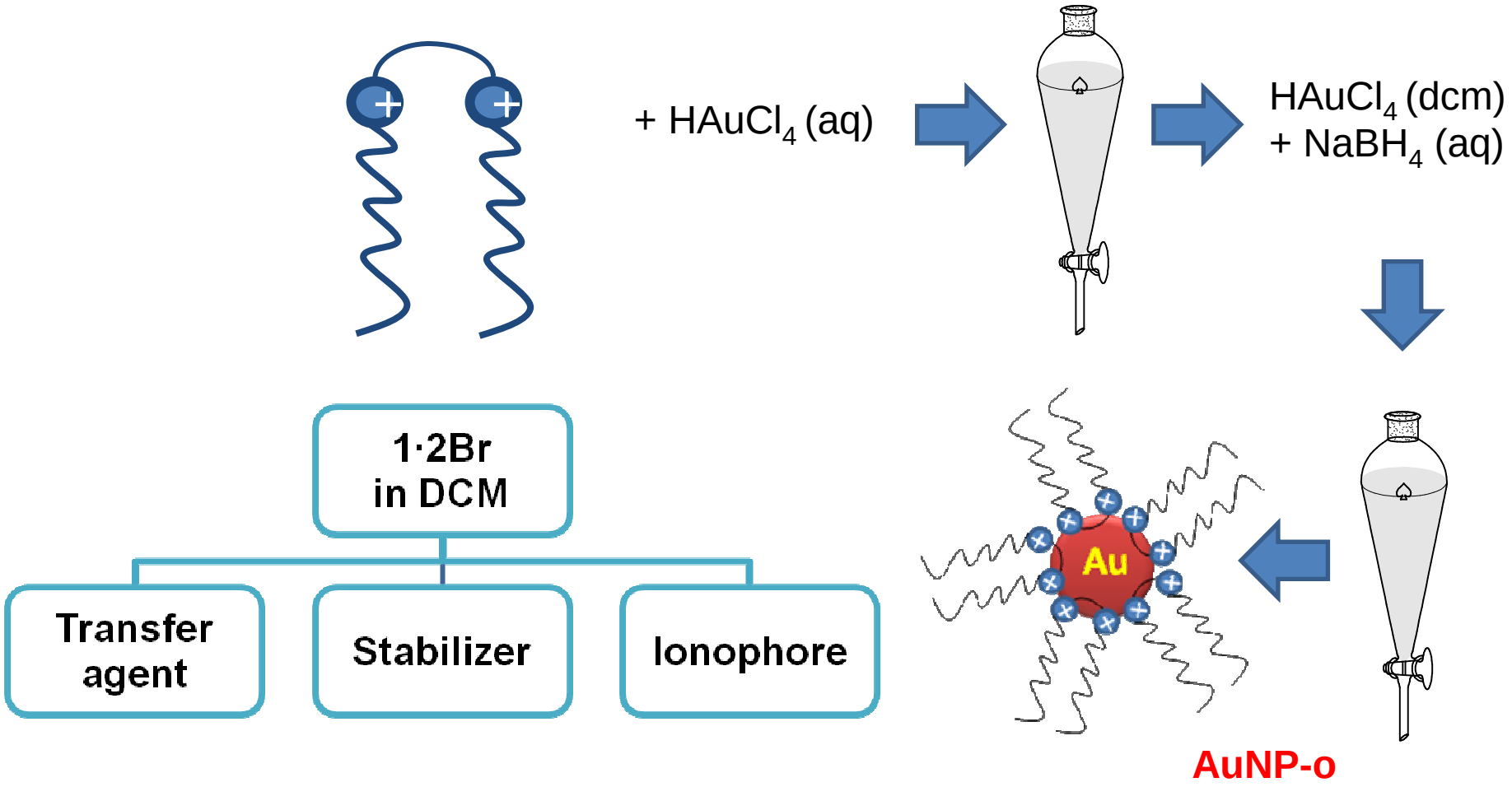
$NaBH_4$ (reducing agent)
+
dodecanethiol (stabilizer)

Turkevitch
(AuNP in water)

$HAuCl_4$

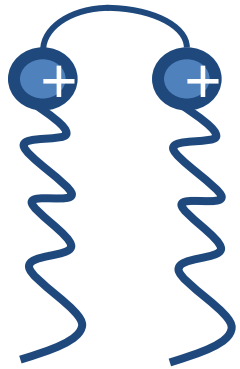
boiling
+
citrate (stabilizer)

Synthesis of gold nanoparticles soluble in organic media

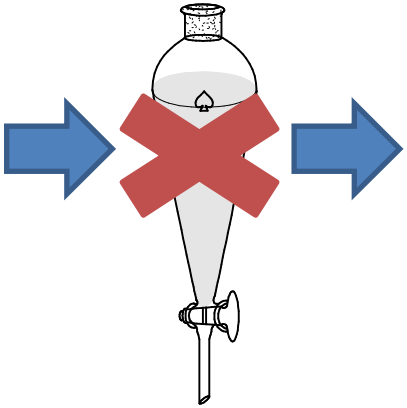


L. Casal-Dujat, M. Rodrigues, A. Yagüe, A. C. Calpena, D. B. Amabilino, J. González-Linares, M. Borràs, and L. Pérez-García, *Langmuir*, **2012**, *28*, 2368–2381

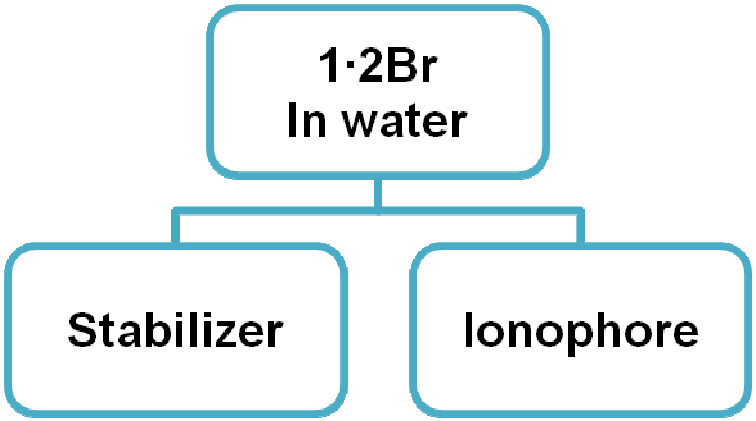
Synthesis of gold nanoparticles soluble in water

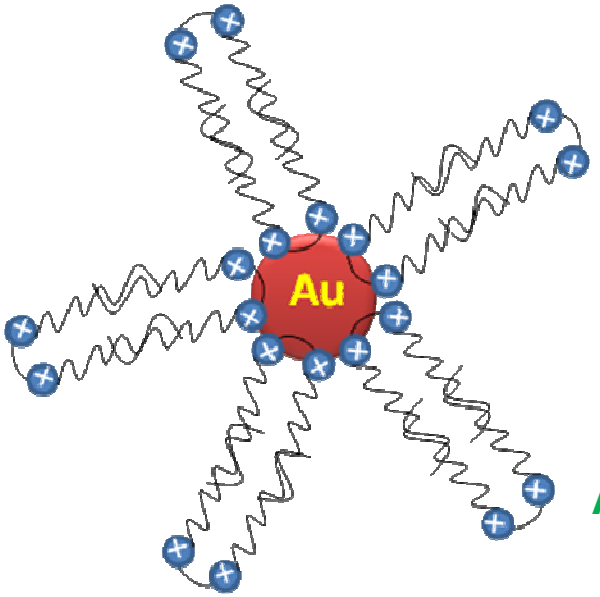


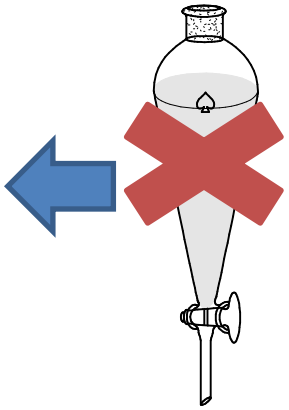
+ $HAuCl_4$ (aq)



+ $NaBH_4$ (aq)

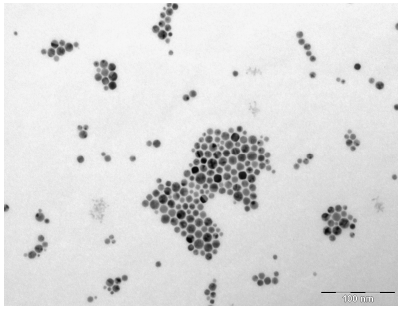


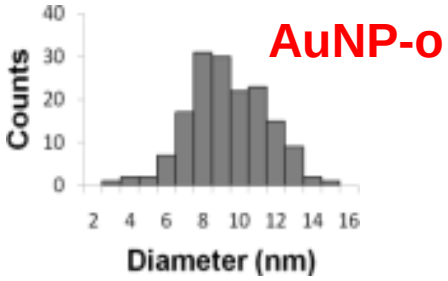


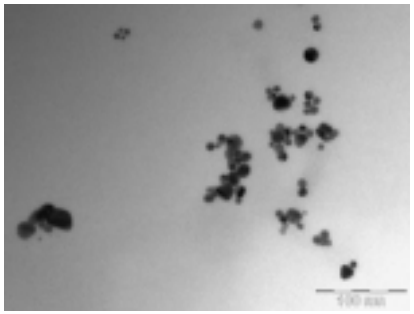


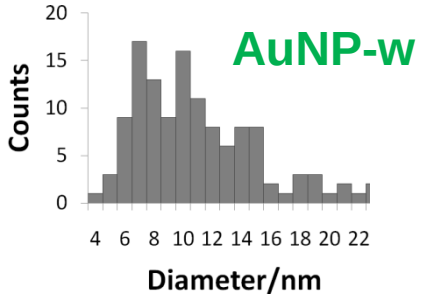
AuNP-w

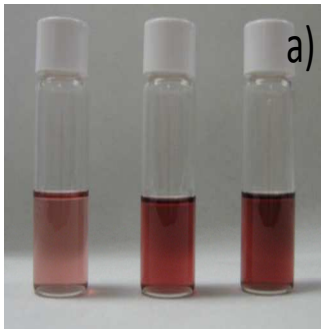
Characterization UV-vis – size (TEM)

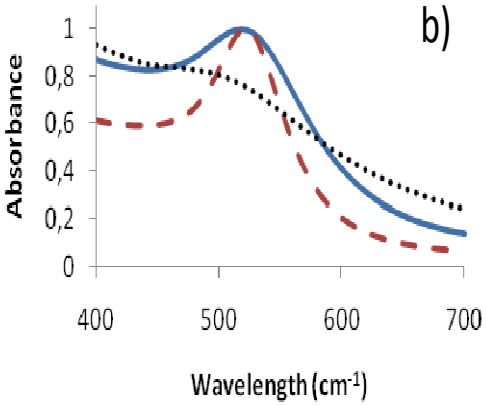




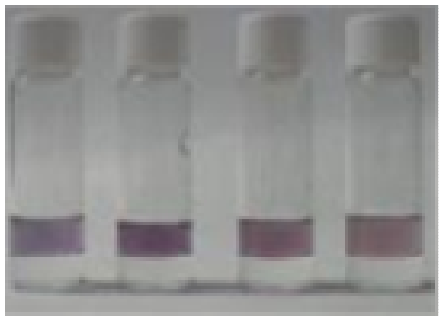






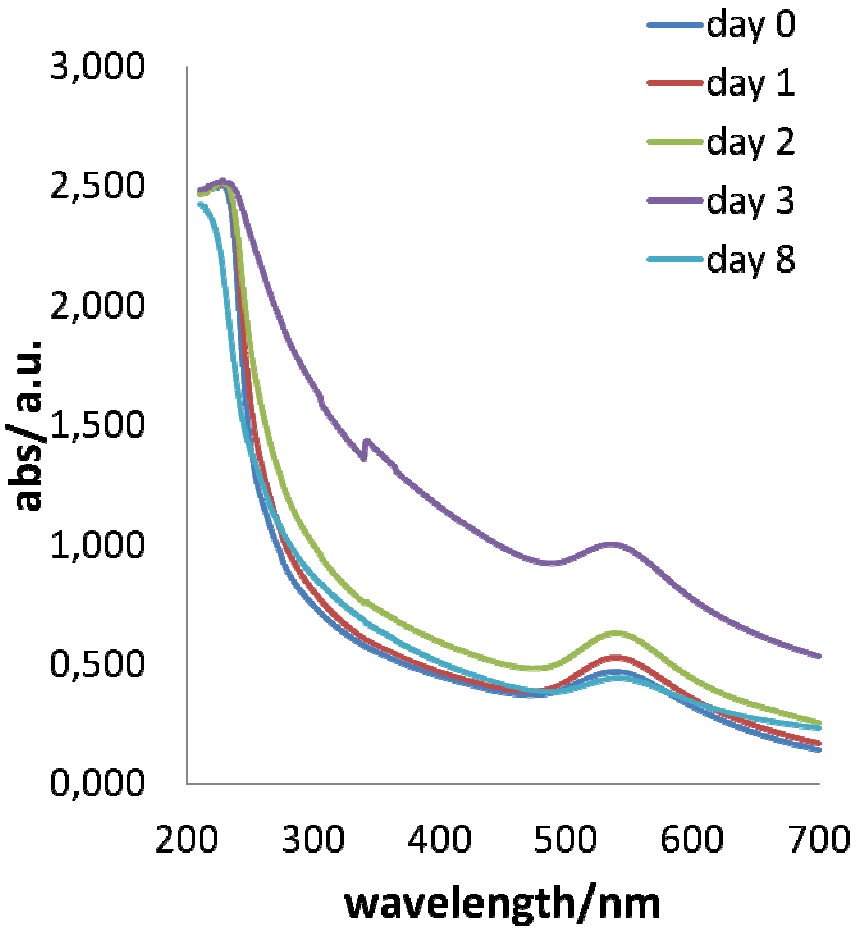


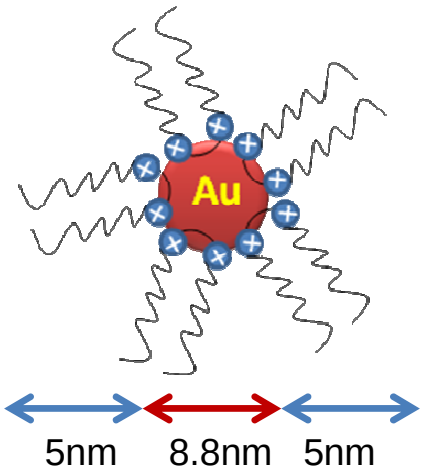
	Size/nm
AuNP-o	8.8 ± 2.2
AuNP-w	10.4 ± 4.2



TEM micrographs of **NP-a**, **NP-b**, and respective size distribution histograms.

Stability - UV-Vis and DLS

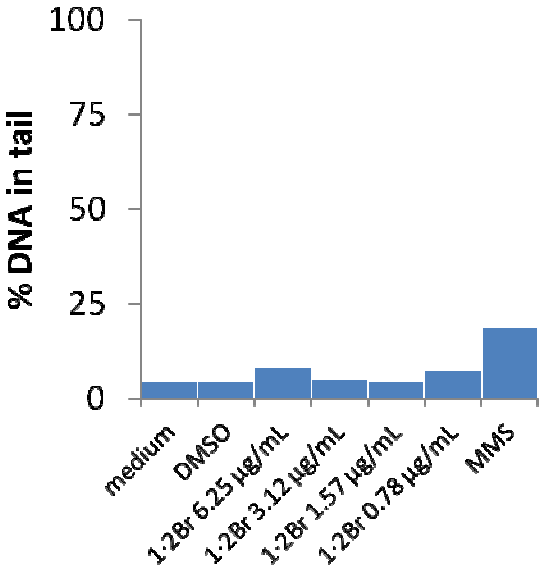


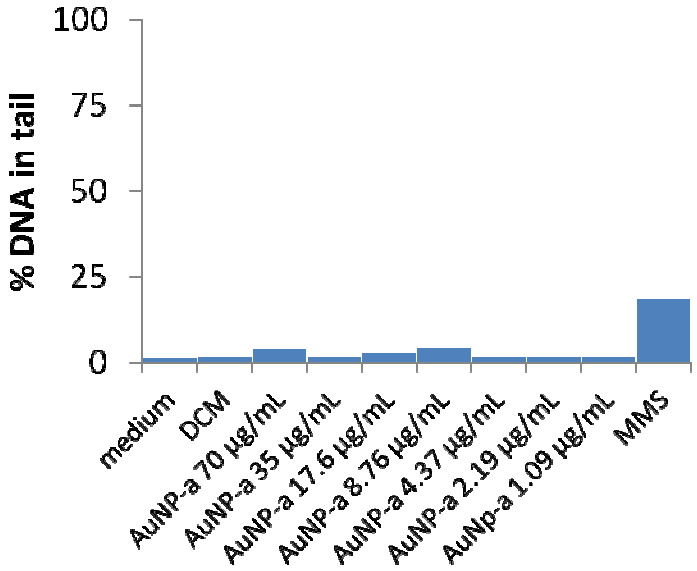


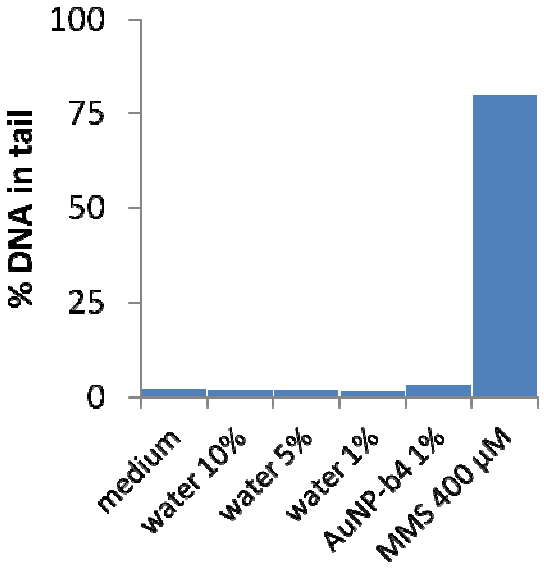
Temperature/℃	Size/nm
25	18.28±0.14
37	19.52±0.29
4	19.89±0.09

Toxicity studies

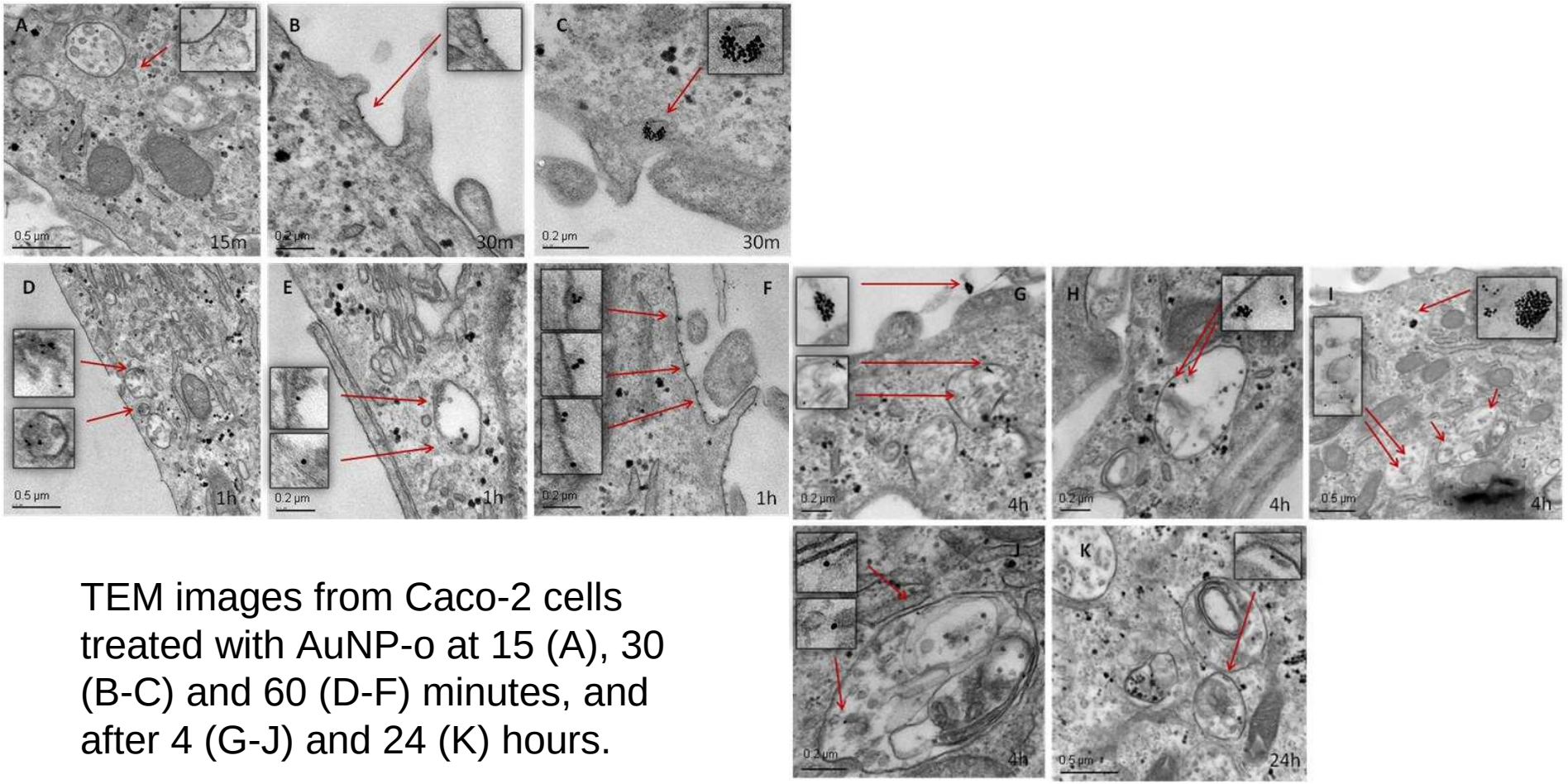
EC_{50}	$\mu g.mL^{-1}$	nM
1·2Br	13.18	14600
AuNP-o	> 70	-----
AuNP-w	-----	0.96





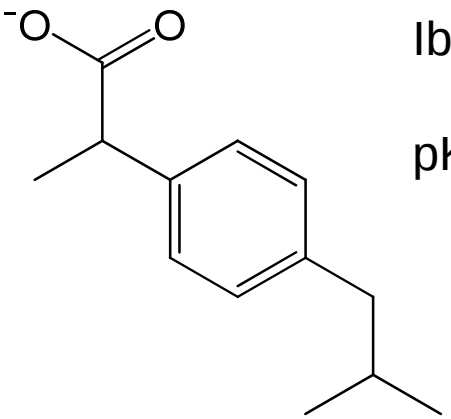


Internalization studies



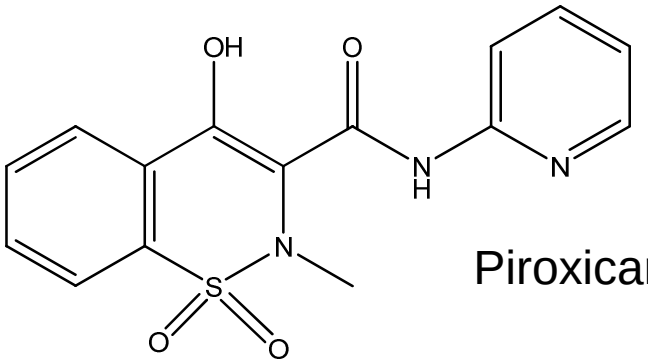
TEM images from Caco-2 cells treated with AuNP-o at 15 (A), 30 (B-C) and 60 (D-F) minutes, and after 4 (G-J) and 24 (K) hours.

Release of model drug from AuNP



Ibuprofenate

pK_a 4.45

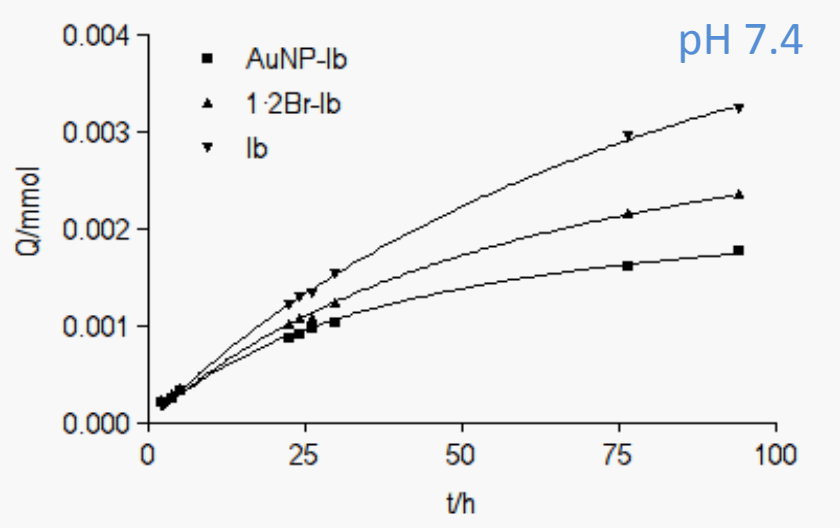


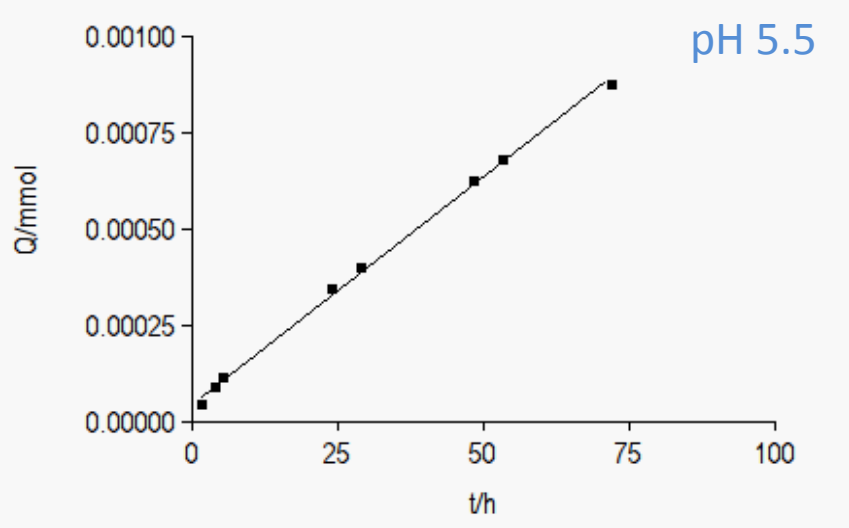
Piroxicam

pK_a 5.1

pH	T/℃
7.4	37
5.5	32

Release of ibuprofenate from AuNP-a

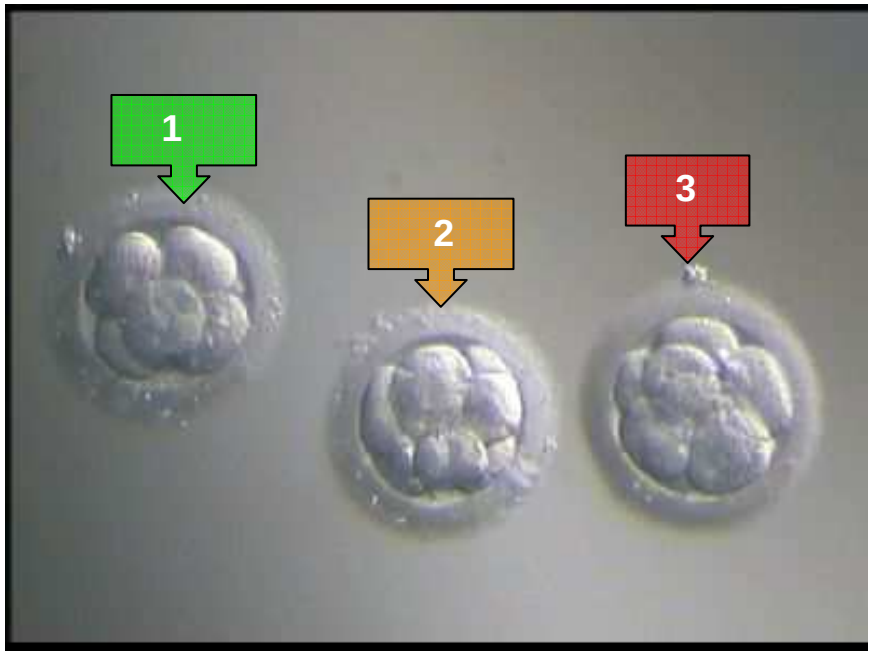




pH		K_D
7.4	Ib-AuNP	39.98 h^{-1}
	Ib-1·2Br	65.47 h^{-1}
	Ibuprofenate	106.10 h^{-1}
5.5	Ib-AuNP	0.00001265 mmol h^{-1}

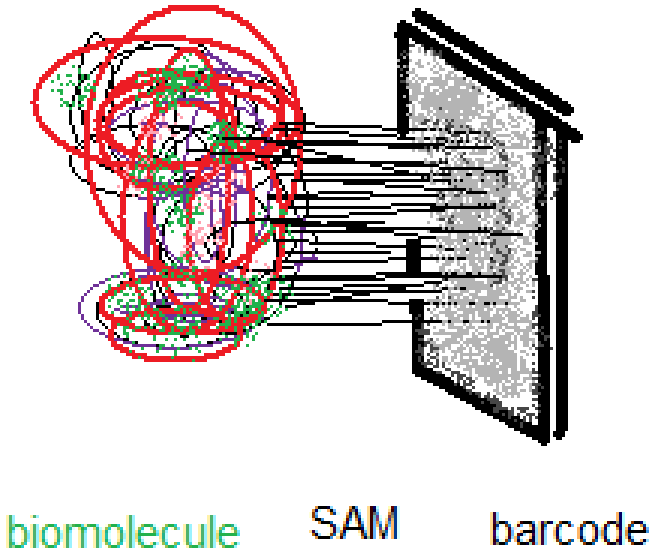
Biofunctionalization of micronanotools to tag, sense and actuate in living cells

- Synthesis
- Characterisation
- Cell tagging



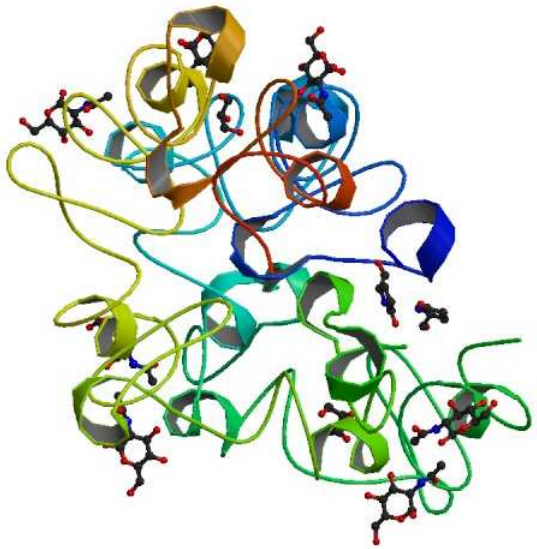
Micronanotool elements

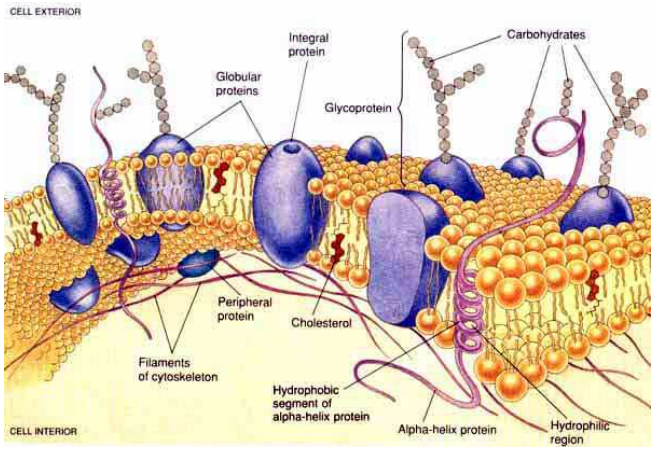
- (Bio)molecule
- Biocompatible material
- Self assembled monolayer



Biomolecule

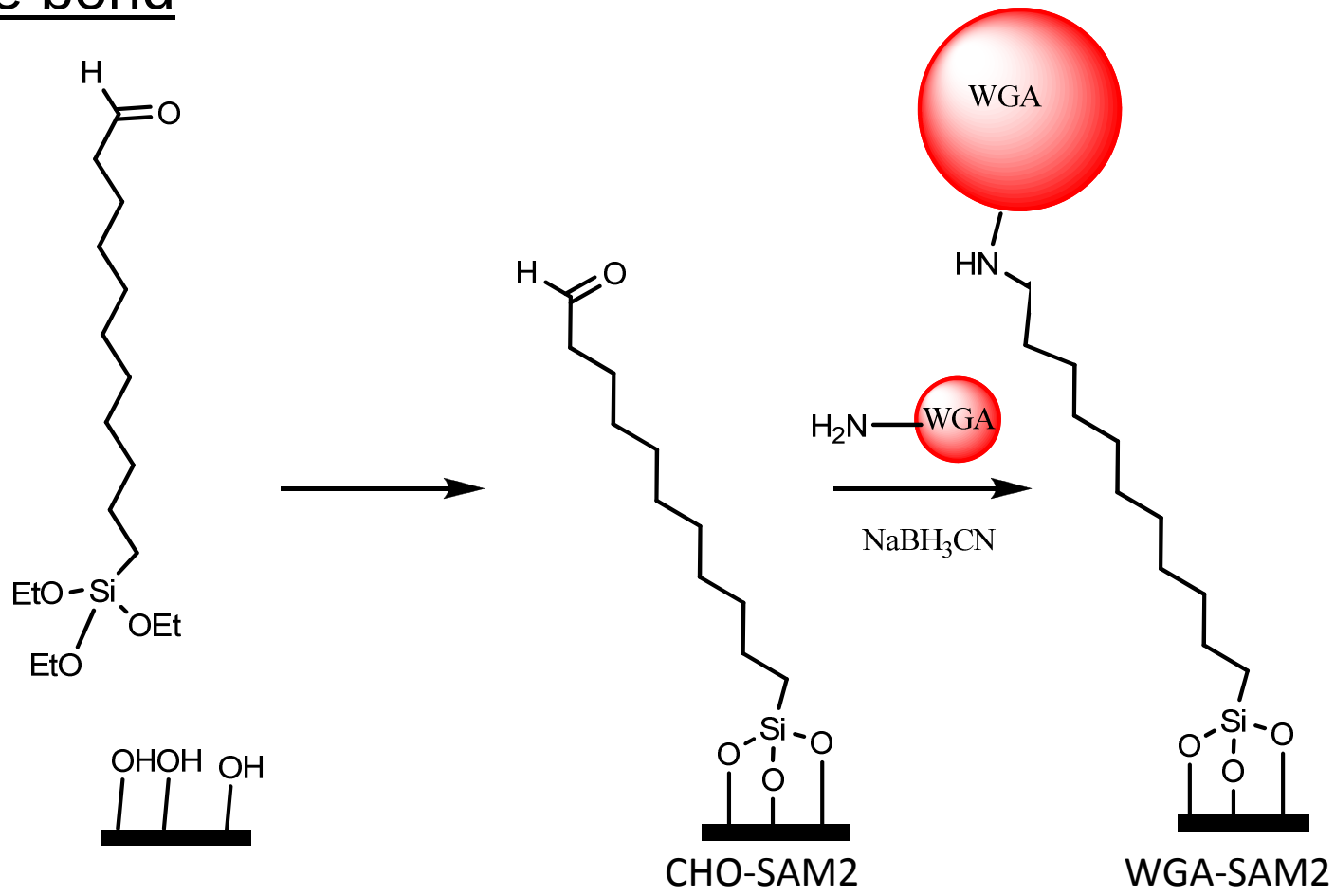
- Wheat Germ Agglutinin (WGA) is used as a biomolecule
- Capacity to recognize specific sugars (*N*-acetylglucosamine and sialic acid)





SAM

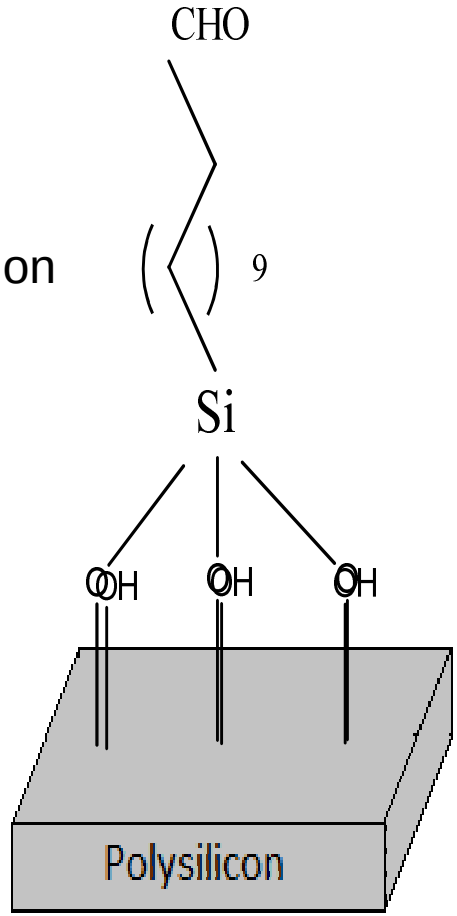
- Amine bond



TESUD SAM FORMATION

Contact Angle Pictures

3. TESUD Deposition



1. H_2SO_4/H_2O_2
2. $NH_4OH/H_2O_2/H_2O$

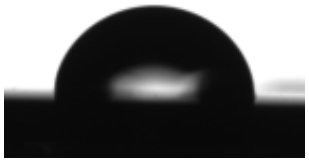
Hydrophobic

100°

Hydrophilic

15 °

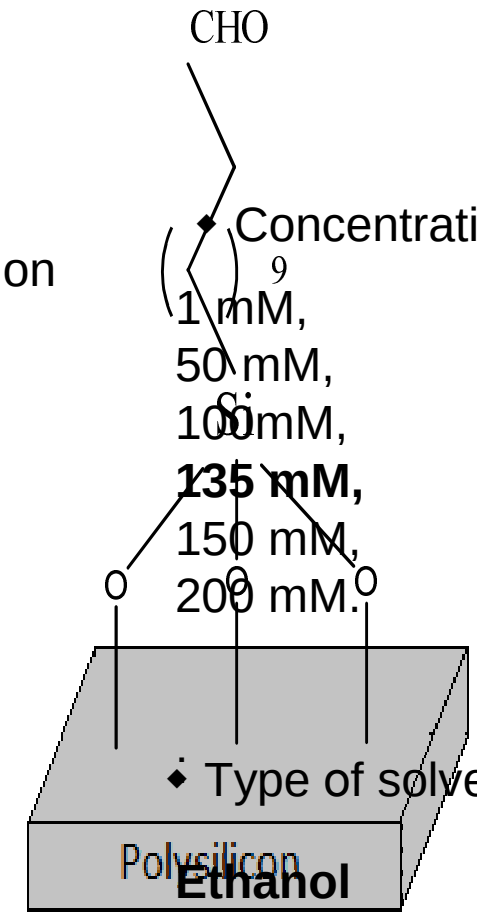
Hydrophobic



94°

PROTOCOL FOR TESUD SAM FORMATION

3. TESUD Deposition



- Concentration
  1 mM,
  50 mM,
  100 mM,
  135 mM,
  150 mM,
  200 mM.
- Type of solvent
  Ethanol
  Toluene
  Water
- Method

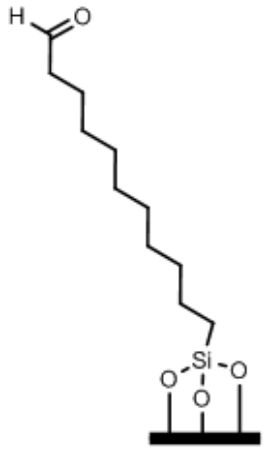
Solution-phase Vapor phase

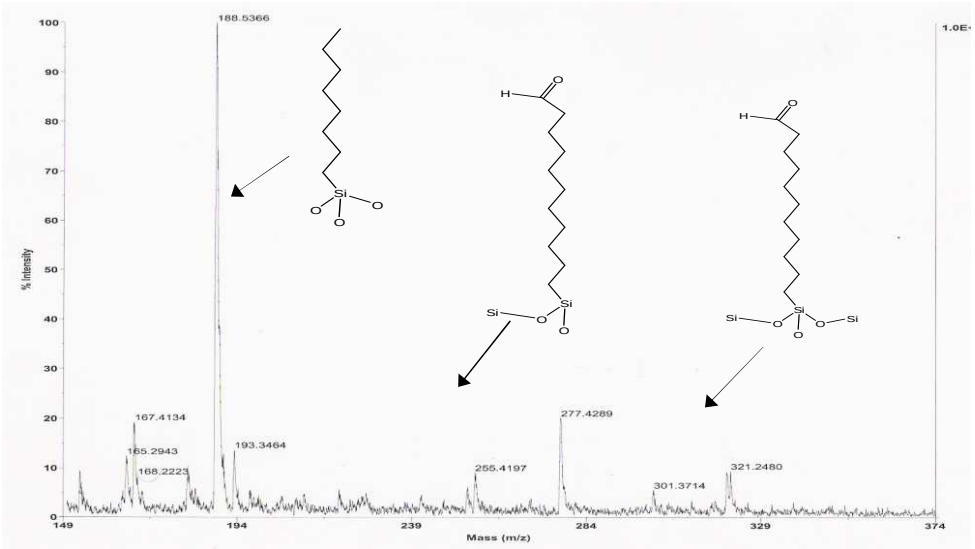
- Deposition time

  60 min
  120 min
  180 min
  overnight

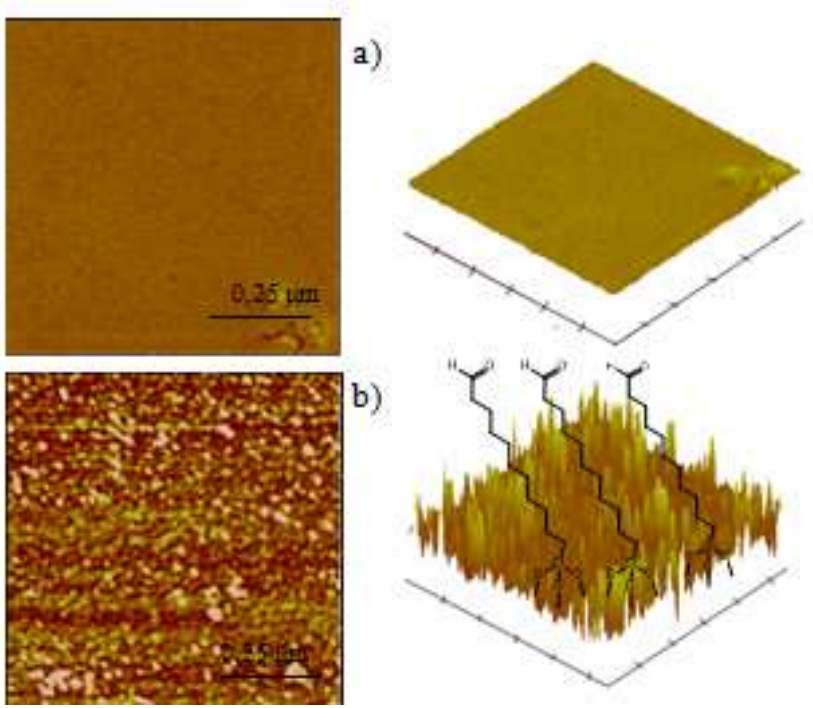
– Contact Angle Characterization

Laser desorption/ionization time of flight mass spectrometry (LDI-TOF MS)



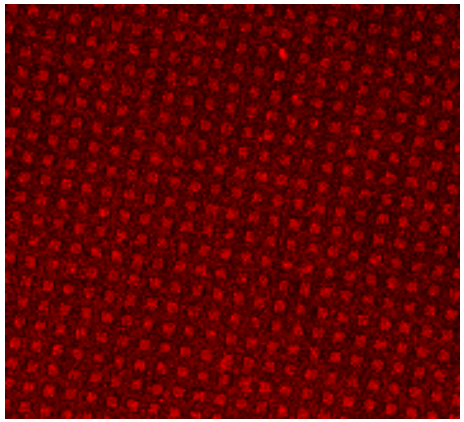


Atomic Force Microscopy (AFM)

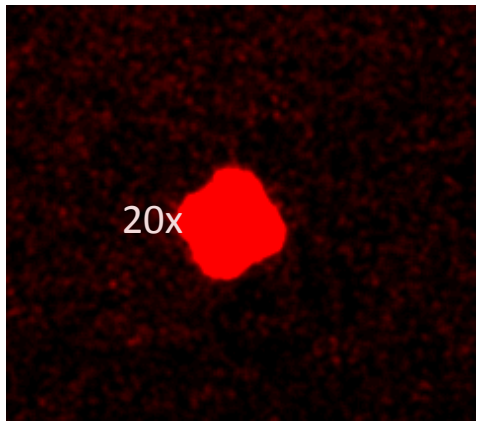


Fluorescence microscopy

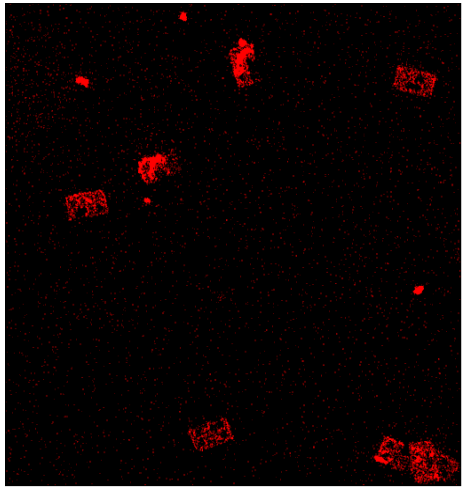
- Silicon wafer

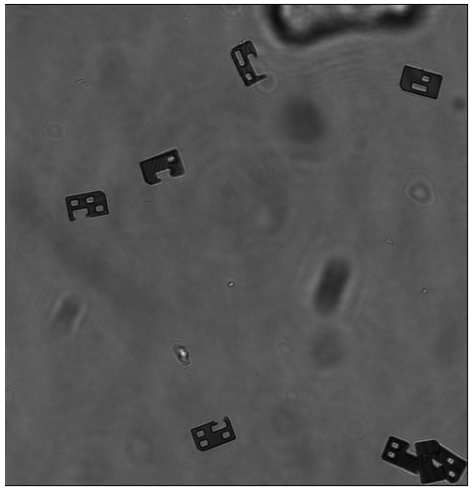


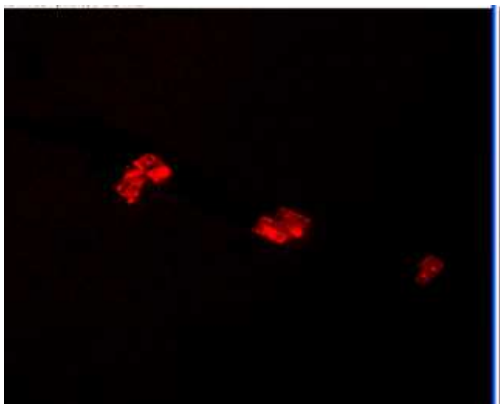
- Particles

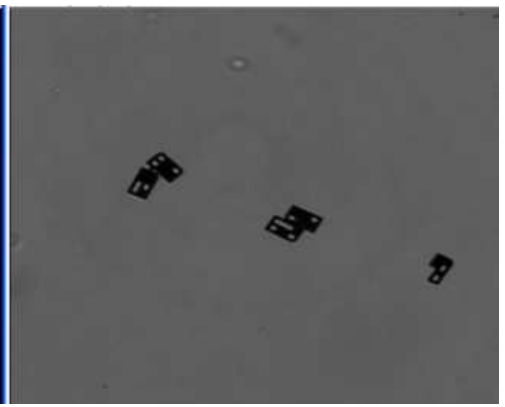


- Barcodes

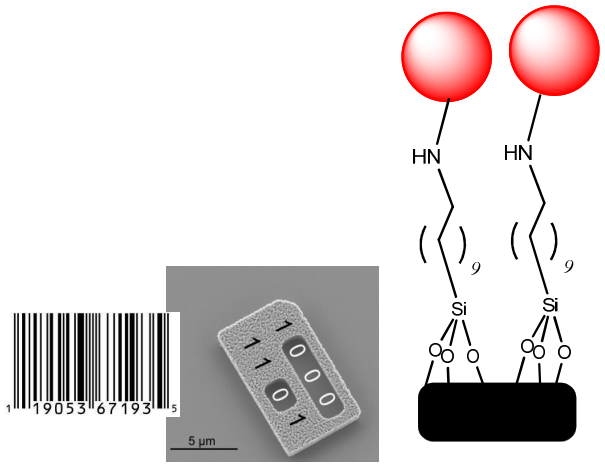




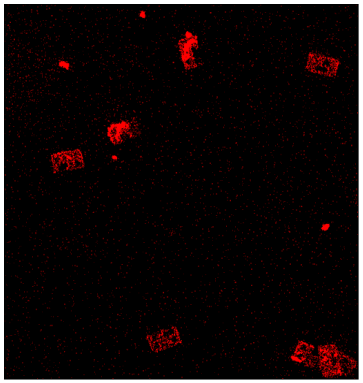




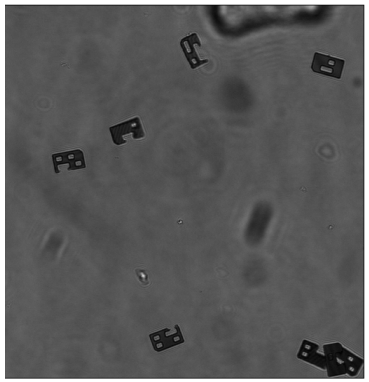
Cell Tagging: *Barcodes adhesion*



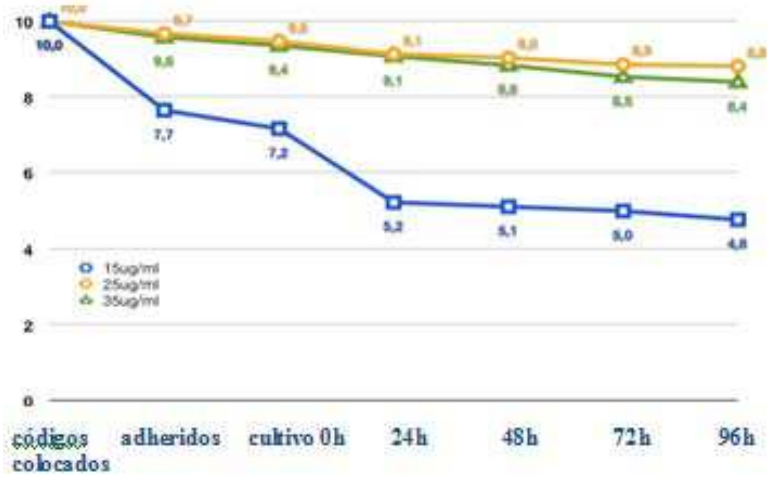
Silicon barcode 10x6um



Fluorescence microscopy

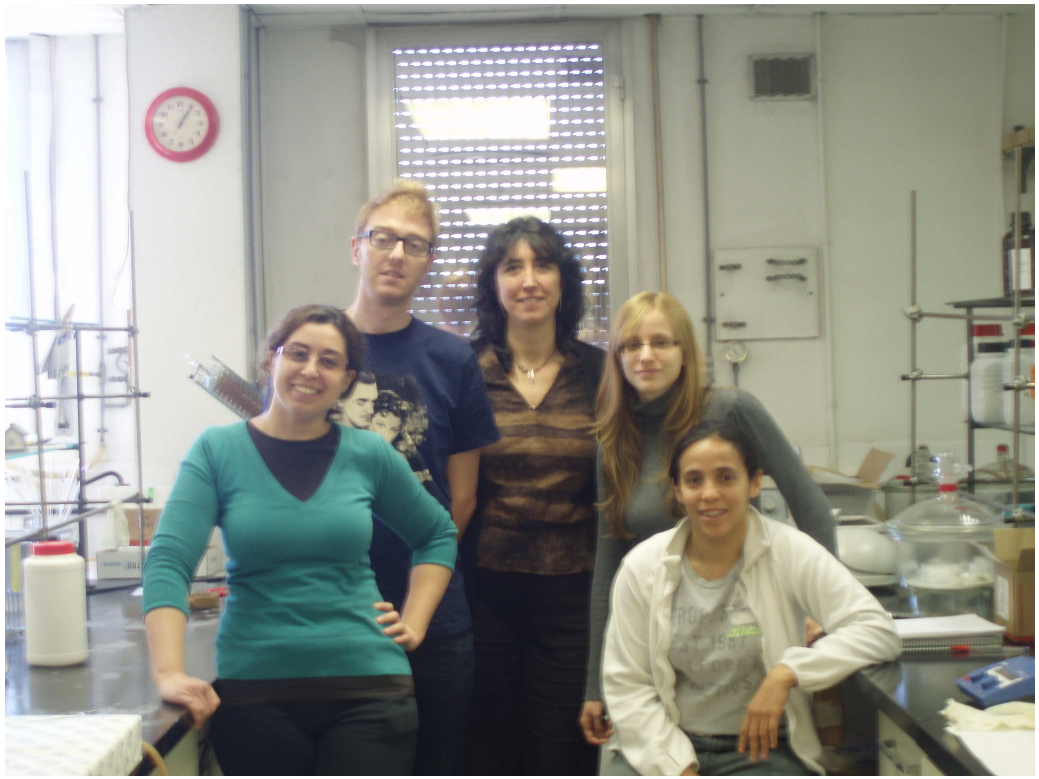


Optical microscopy



Supramolecular systems in Nanomedicine

Dra. Susana Ramos
Dra. Lucia Casal-Dujat
Dr. Christian Sporer
Oriol Penón
Mafalda Rodrigues
Paula García
Aniuska Bolívar
Alex Yagüe
Davide Santucci
Dimitris Siapkas



Dra. Carme Nogués. UAB
Dr. José Antonio Plaza. IMB-CSIC

Dra. Laura Rodriguez-UB
Dra. Anna Calpena-UB
Dr. Miquel Borras-CERETOX
Dr. Jordi Hernández-UB
Dra. Teresa Montero-UB
Dra Conxita Solans, IQAC-CSIC
Dr. Carlos Rodriguez-Abreu, INL, Portugal
Dr. D. Amabilino, ICMAB-CSIC
Dr. Peter Griffiths, University of Cardiff

MICINN TEC2008-06883-C03-02
TEC2011-29140-C03-02
Generalitat de Catalunya (2009SGR158)