SCALE
A new team
in Department of Analytical Sciences

SCIENTIFIC COUNCIL, MARCH 31, 2021

The involved UL members

Igor Clarot (Pr.)
Chimie Analytique

Ariane Boudier (Pr.)
Physico-Chimie des Matériaux

Thomas Chaigneau (Ing.Et.)
Sciences séparatives - Qualité

Benjamin Creusot (Adj.Tech.)
Physico-Chimie - Synthèse

Arnaud Pallotta (MCF)
Chimie Analytique

MCF 2021
Chimie Analytique

EA 3452 CITHEFOR

L^2CM, UMR CNRS-UL 7053

Raphaël E. Duval (Pr.)
Microbiologie Clinique

Emmanuel Lamouroux (MCF)
Nanochimie

UMR 7178 CNRS – Unistra

EA 3452 CITHEFOR

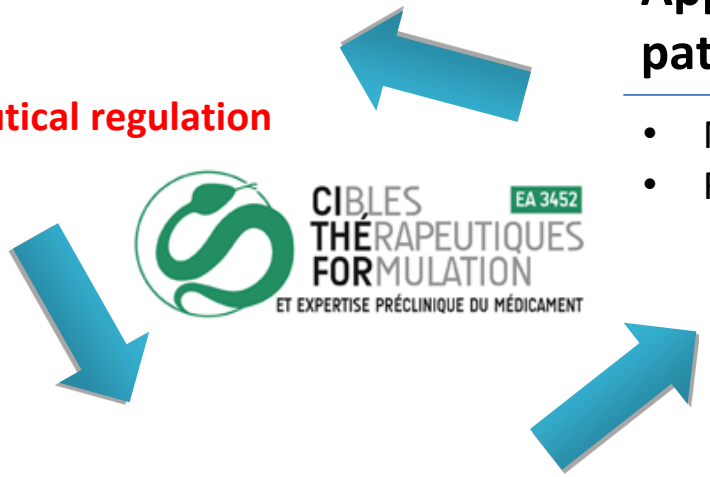
EA 3452 is a single multidisciplinary team : Innovative molecules and nanoformulations of nitric oxide (NO) donors.

Synthesis and control

- Synthesis of NO donors
- Molecular and macromolecular scale
- **Quality Control according to the pharmaceutical regulation**

Application in vascular ischemic pathologies

- New therapeutic targets
- Redox signaling

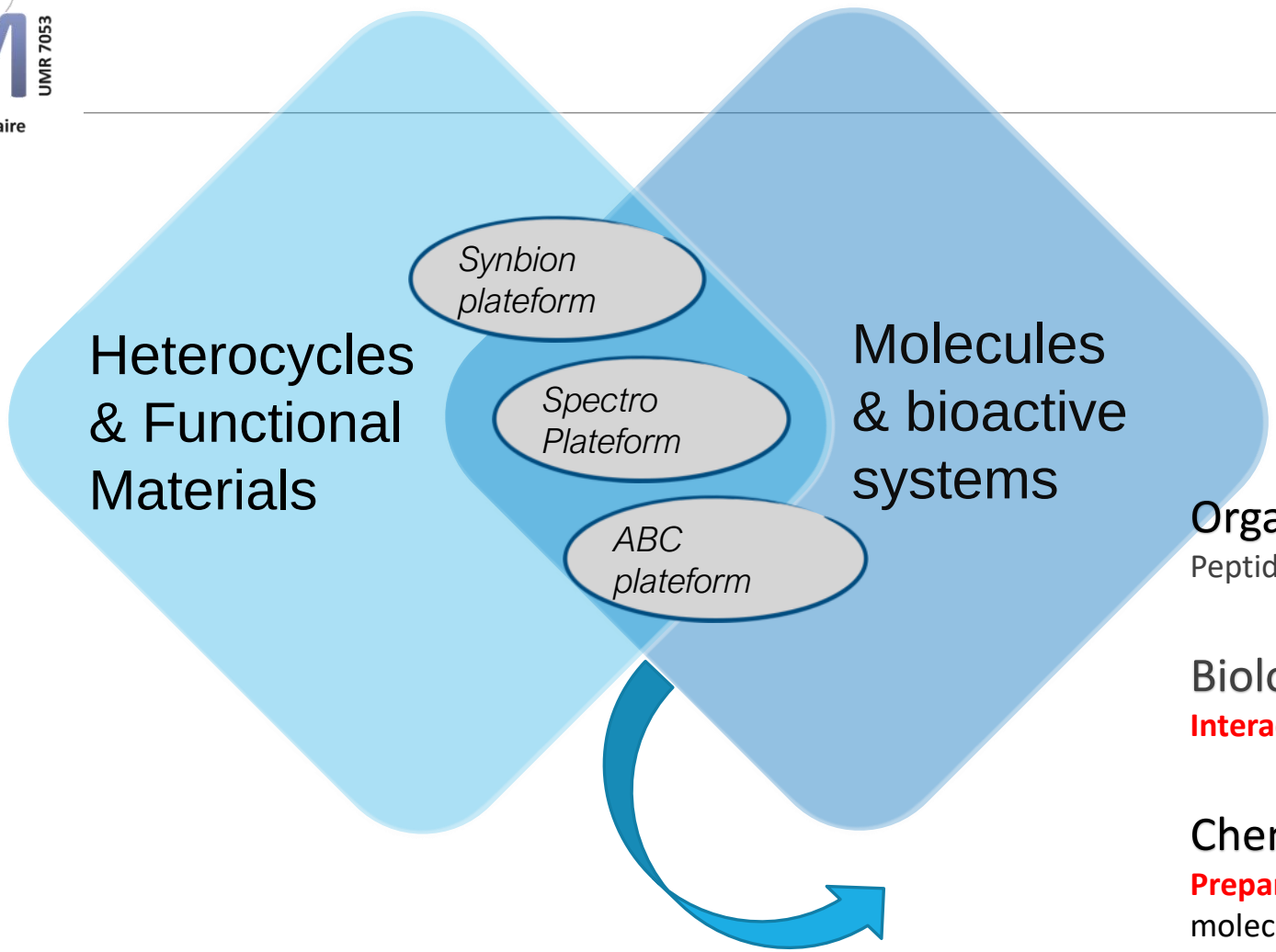


Multi-scale formulation

Nanoparticles
Control of assay content, impurity profile
Nanostructured surfaces

UMR 7053 L²CM

Laboratoire Lorrain de Chimie Moléculaire



Organic and bioorganic Chemistry

Peptides, sugars, heterocycles, calixarenes

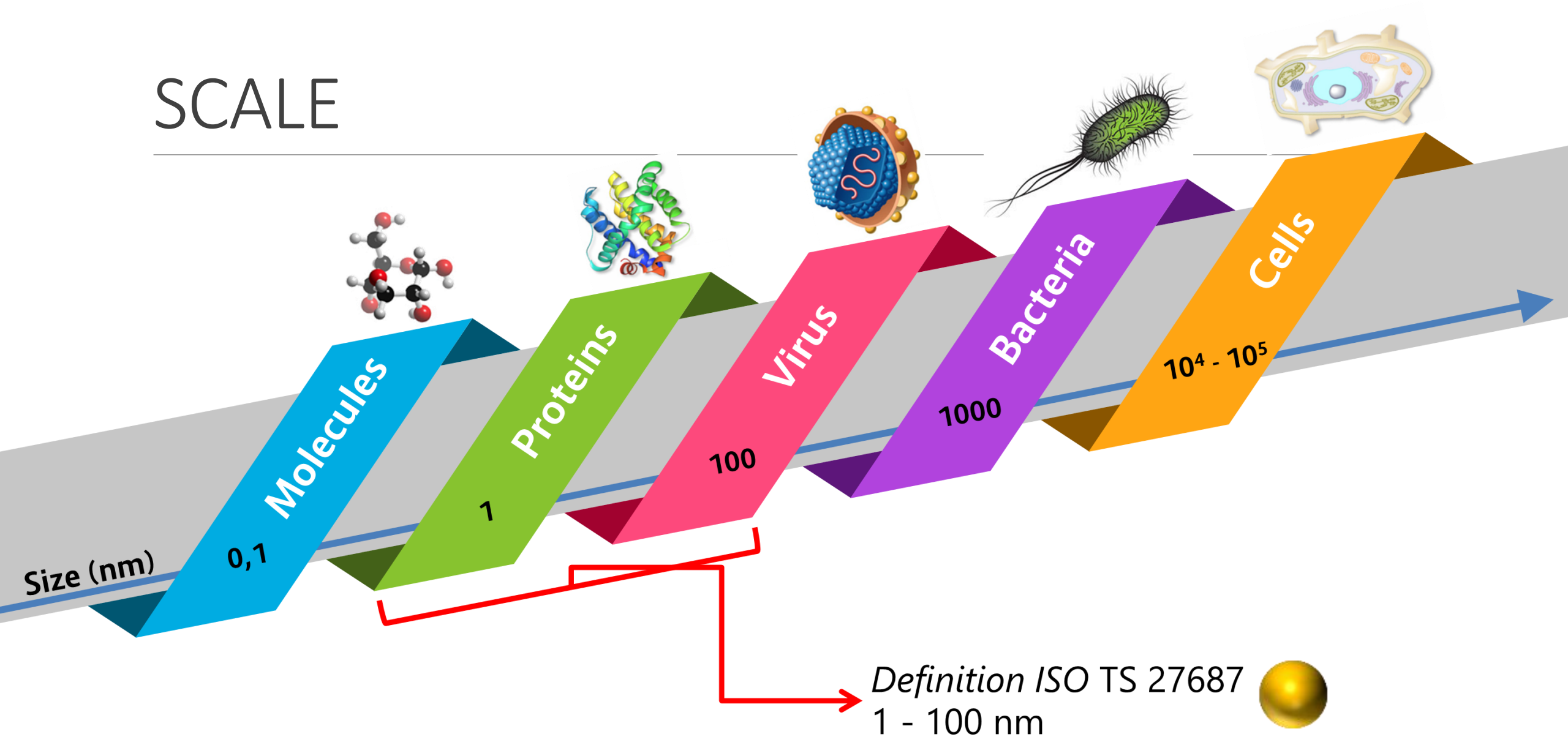
Biology

Interactions with the biological elements

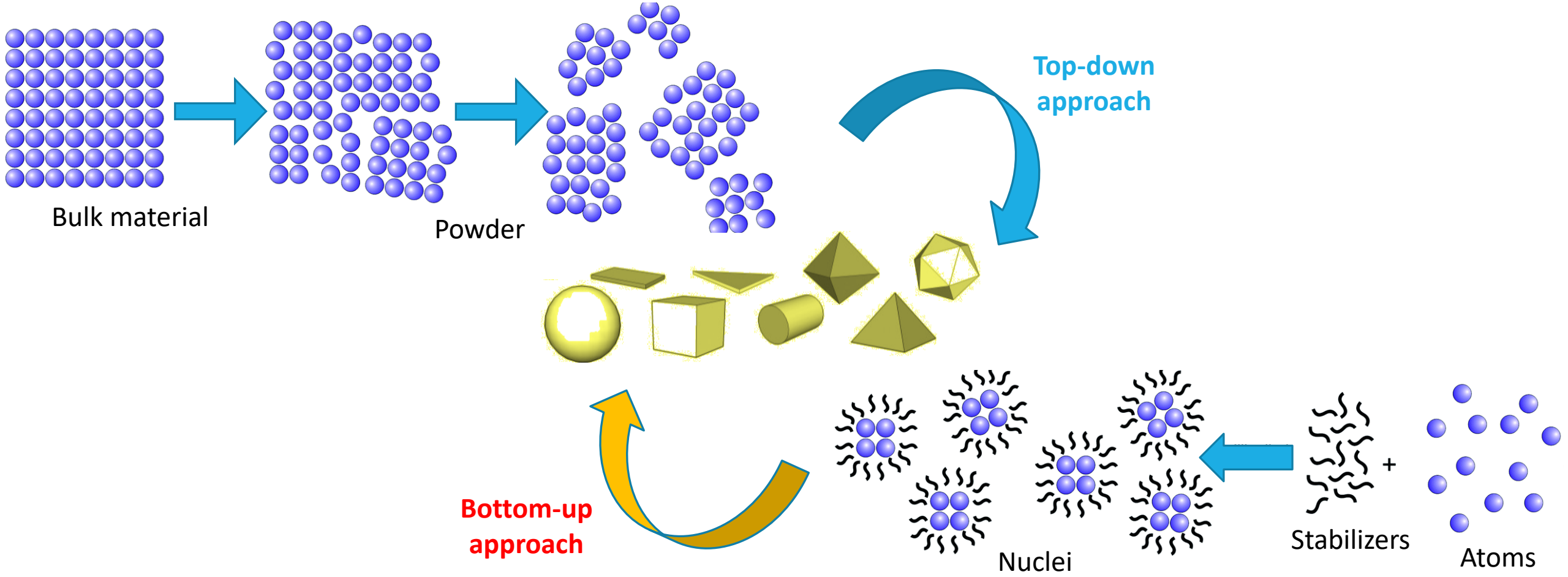
Chemistry of interfaces

Preparation, characterization of organised molecular systems, **Nano and micro-particles**, capsules and emulsions

SCALE


Size (nm)
0,1
Molecules
1
Proteins
100
Virus
1000
Bacteria
10^4 - 10^5
Cells
Definition ISO TS 27687
1 - 100 nm

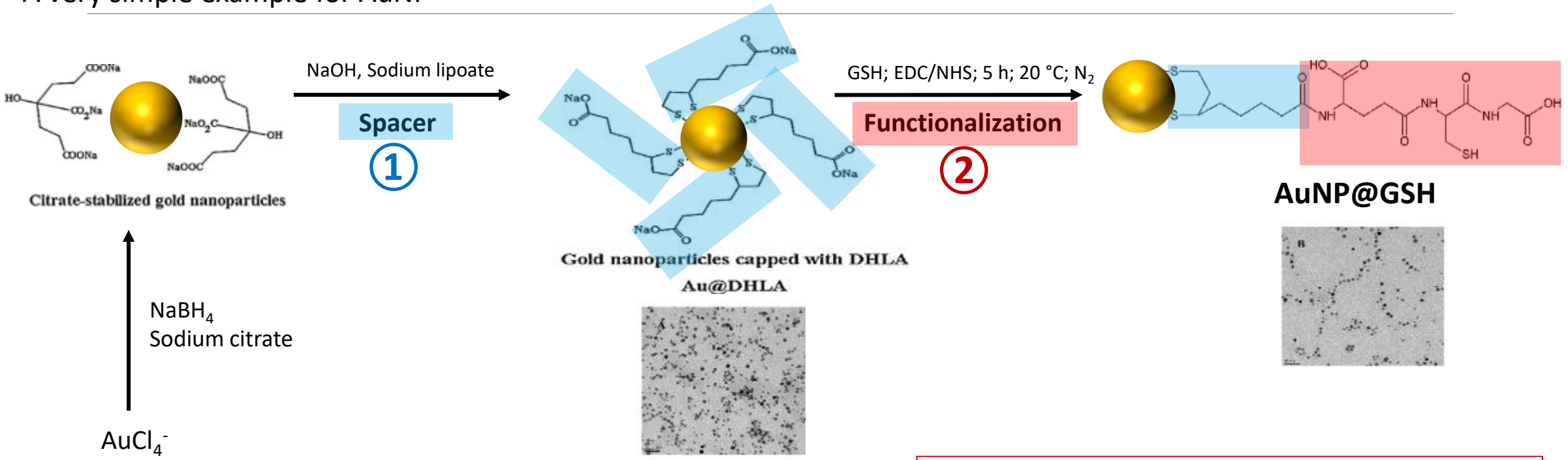
Inorganic nanoparticles synthesis



Collab. with : Institut de Chimie Physique

Synthesis and Fonctionnalisation

A very simple example for AuNP

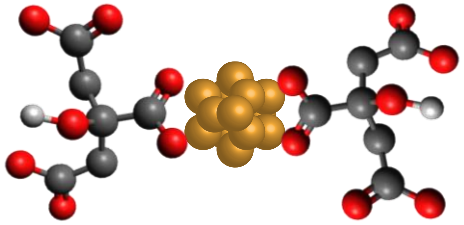


Loading capacity ++
AuNP@GSH : 7500 GSH per AuNP

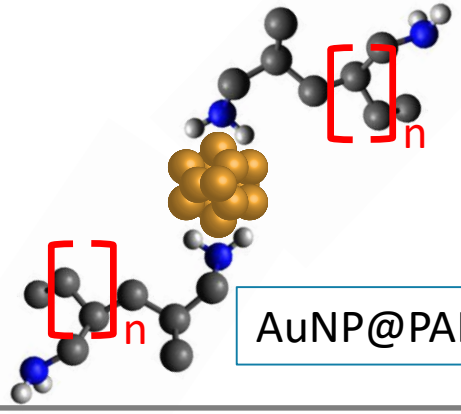
Luo et al, Colloid and Interface Science Communications, 2016, 14, 8-12.

GSH: reduced glutathion; EDC: 1-ethyl-3-(3-diméthylaminopropyl)carbodiimide; NHS: *N*-hydroxy-succinimide

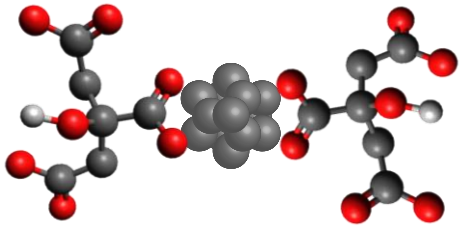
NANOLIBRARY



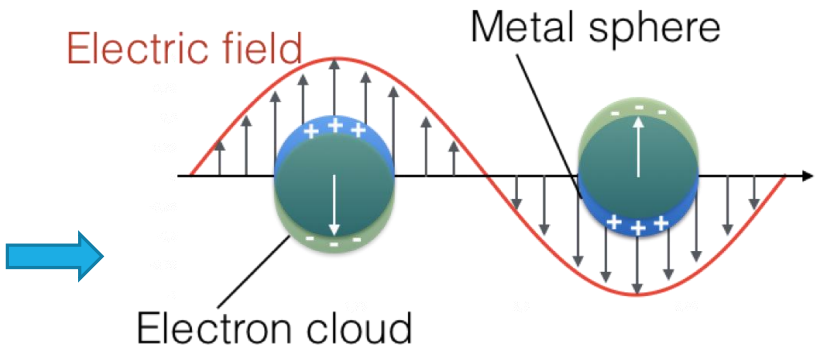
AuNP@citrate



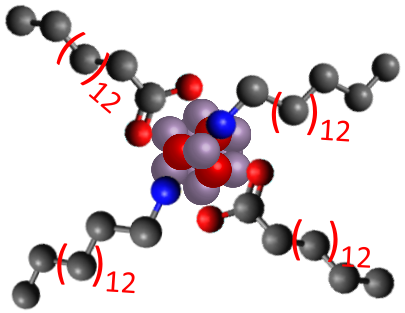
AuNP@PAH



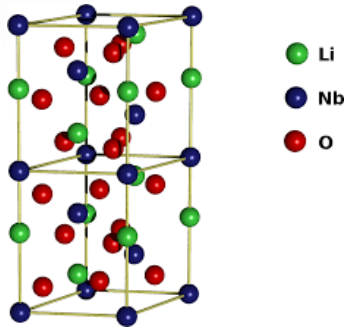
AgNP@citrate



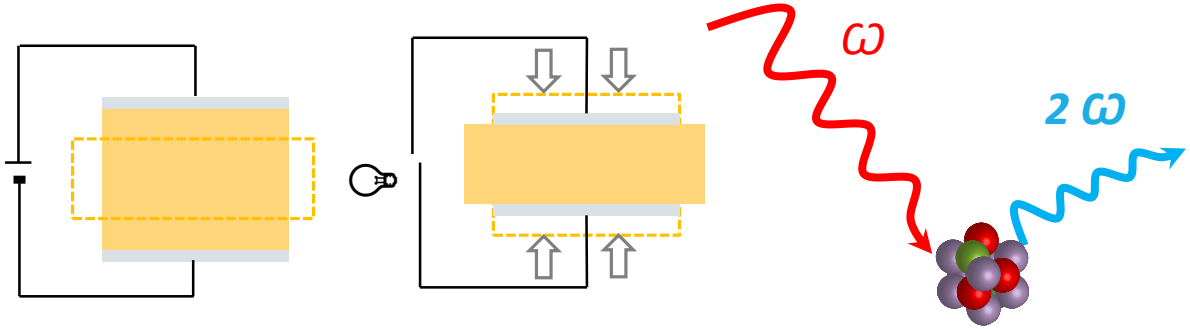
Spectral properties
(Localized surface plasmon resonance)



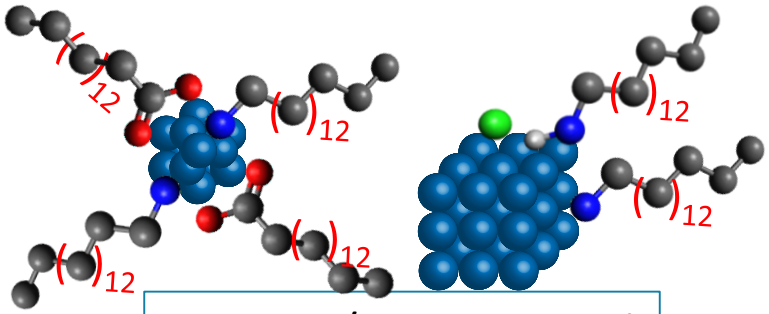
ZnO@HDA/PA



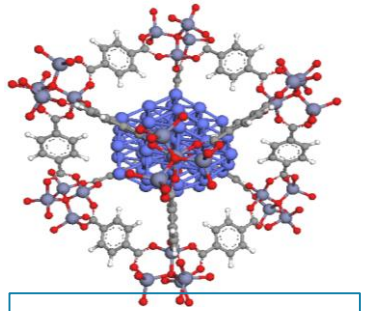
$LiNbO_3NP$@-OH



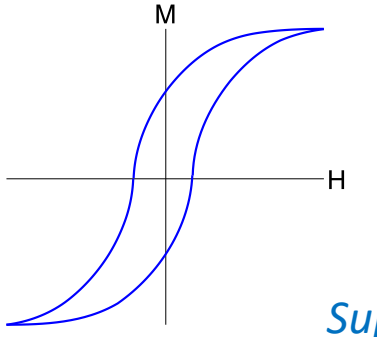
Piezoelectricity / Second Harmonic Generation

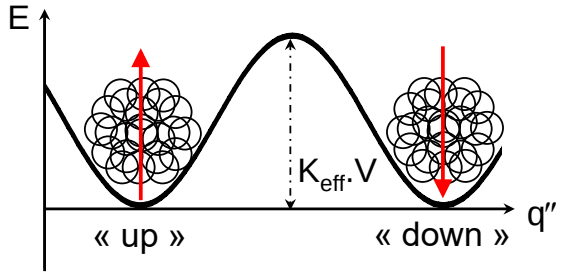


Fe@HDA/PA or HDA.HCl



$Co@Zn_4O(BDC)$





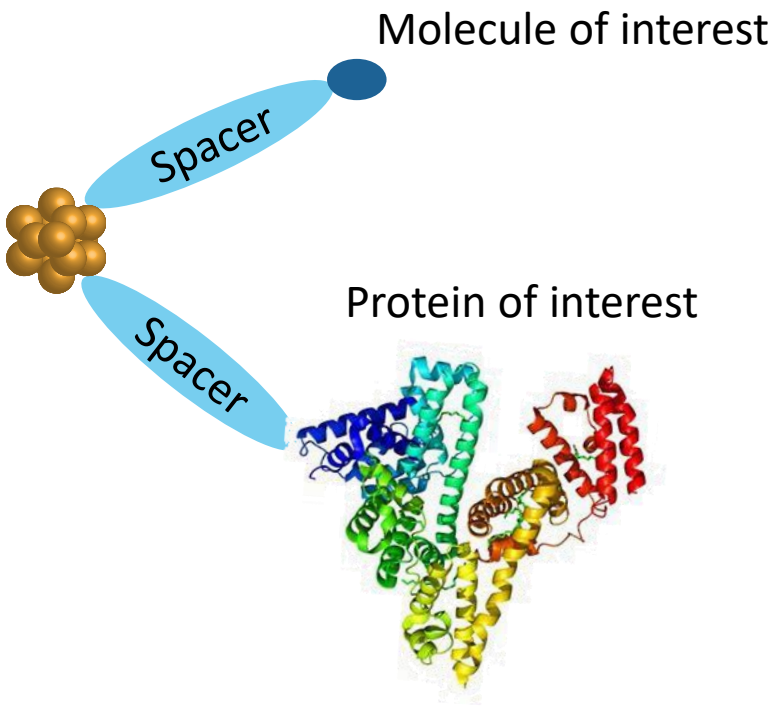
Superparamagnetism

PA: palmitic acid; HDA: hexadecylamine; BDC: benzene-1, 4-dicarboxylate

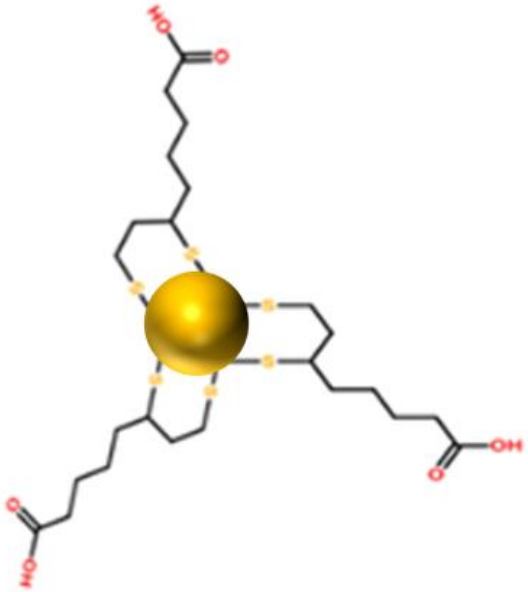
Nanolibrary

Functionalisation of the nanoparticle surface

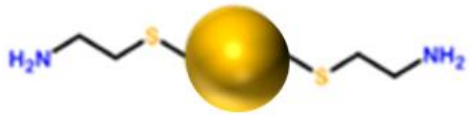
core/spacer strategy



Examples core/spacer



AuNP@DHLA

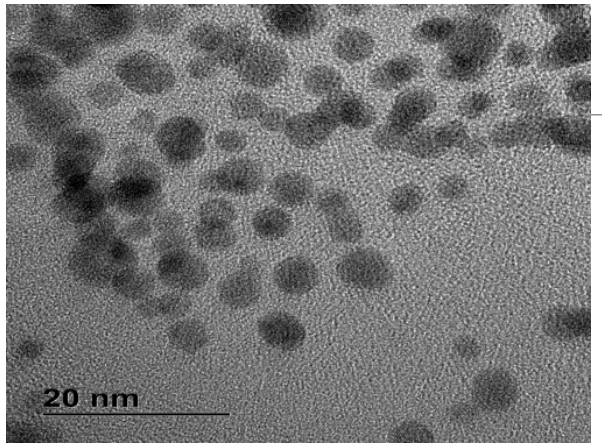


AuNP@Cysteamine

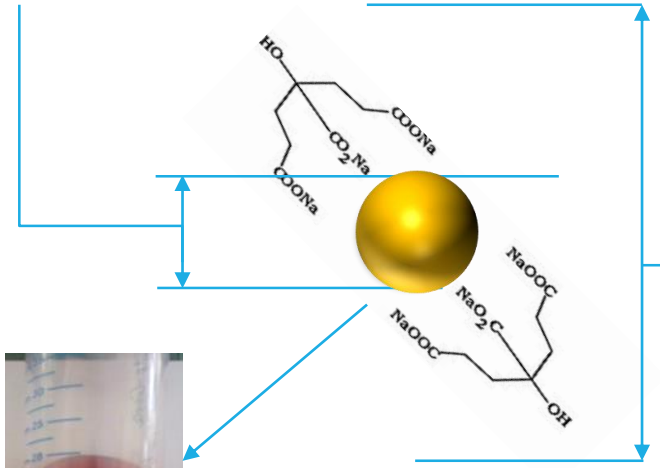
Physicochemical characterisation

Conventional methods

Collab. with : Institut de Chimie Physique

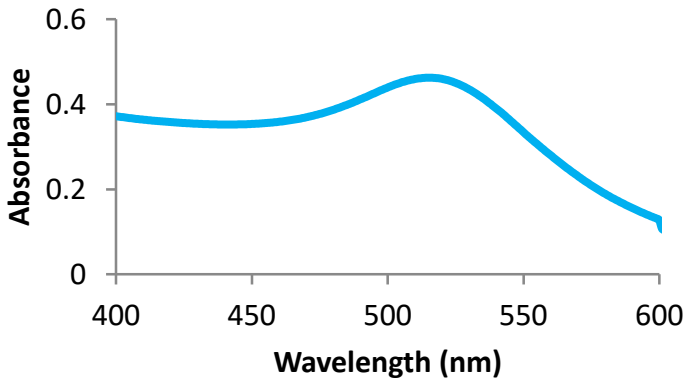


Diameter of the metal core :
5.3 ± 1.1 nm (TEM)



Hydrodynamic diameter :
6.5 ± 0.9 nm (DLS)

Surface charge (ζ potential) : - 33 ± 1 mV



Plasmonic resonance band
λ_{max} : 514 ± 1 nm

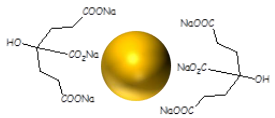
Towards standardized parameters

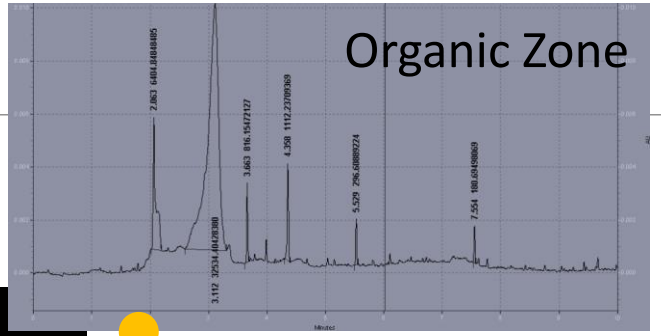
TEM: Transmission Electronic Microscopy; DLS :Dynamic light scattering

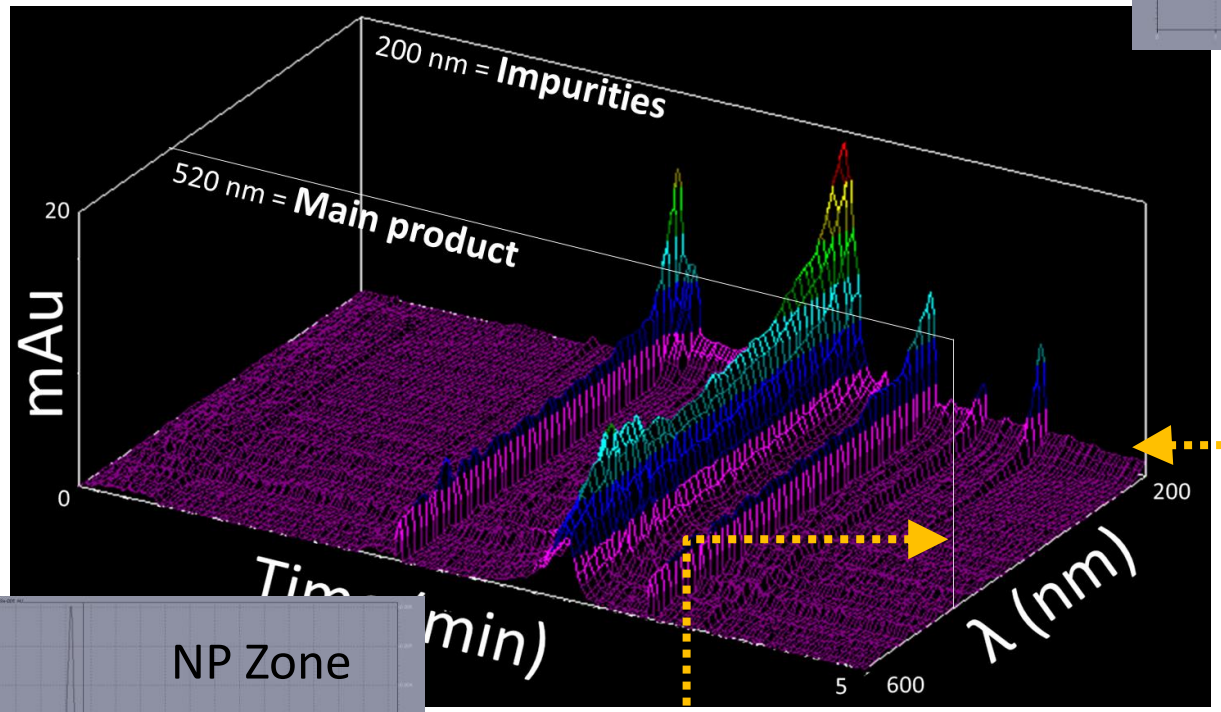
Tournebize et al, ACS Appl. Mater. Int. 2012, 4:5790-99 ; Tournebize et al, Int. J.Pharmaceut. 2012, 438:107-116
Pallotta et al, J. Chromatogr. A 2016, 1461:179-84

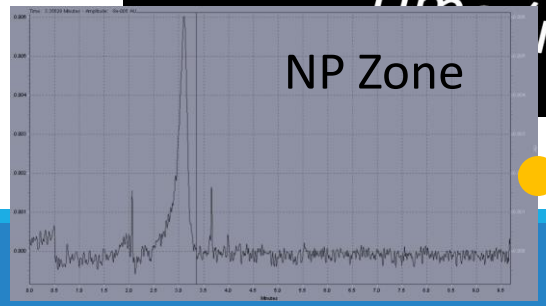
Nanoparticle Quality Control

Capillary Electrophoresis









Development of dedicated electrophoretic methods

Lot	Assay (%)	Impurities	
		Organic (%)	Nanoparticular (%)
#1	79.8	6.1	14.1
#2	76.8	23.2	0.0
#3	90.5	9.5	0.0
#4	**68.3**	11.6	20.1

CZE Results for AuNP-Cit commercial batches (Sigma-Aldrich and Nanocomposix)

Pallotta et al, J. Chromatogr. A 2016, 1461:179-84 ; Pallotta et al, Int J Pharmaceut. 2019, 569:118583

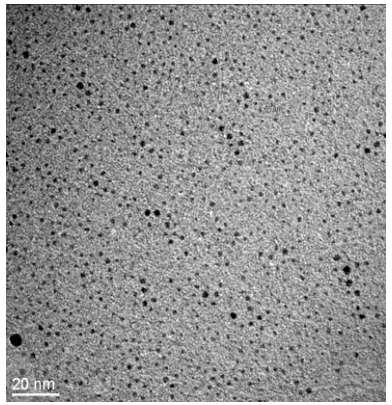
Nanoparticle Quality Control

Capillary Electrophoresis

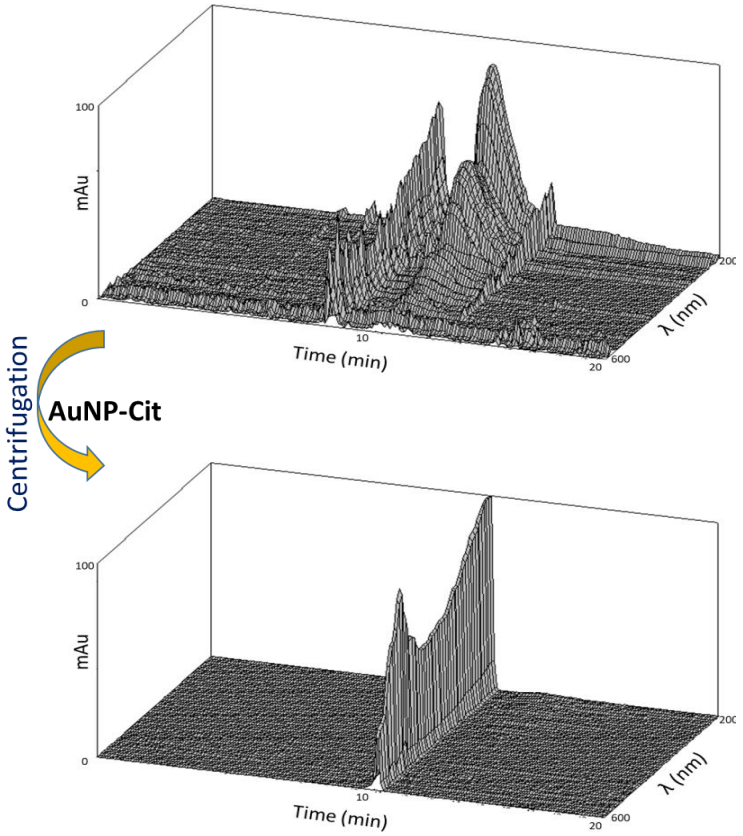
Collab. with :

Size by Taylor Diffusion Analysis (TDA)

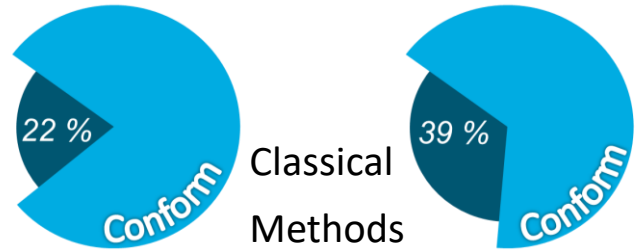
NP	DLS	TDA
AgNP	1.9 nm	2.1 nm
AgNP	14.9 nm	14.8 nm



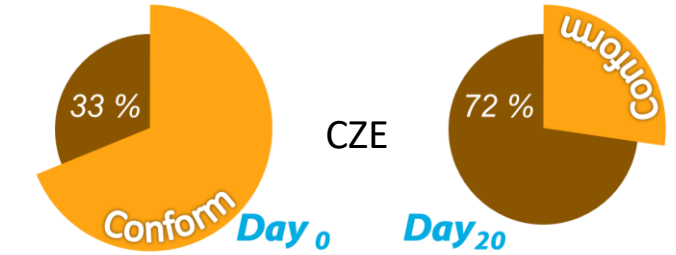
Process monitoring



Stability evaluation (on 18 batches)



Distribution NC - C



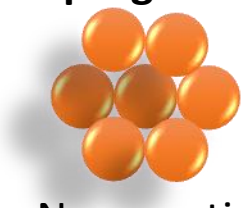
… (3 weeks max for AuNP-Cit)

Pallotta et al, Int J Pharmaceut 2019, 569:118583.

DLS: dynamic light scattering; CZE: Capillary Zone Electrophoresis

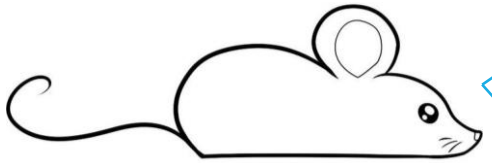
Metallic particles to treat an orphan disease

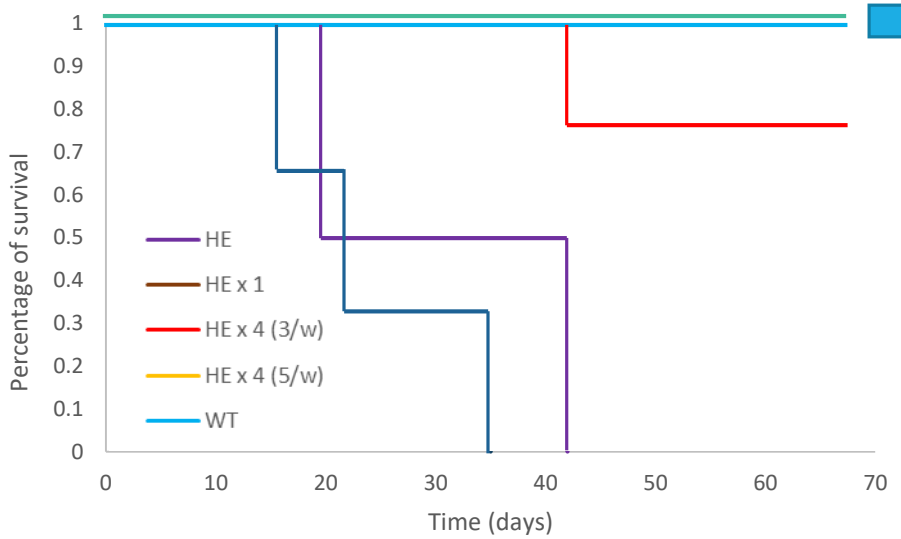
Patent in progress



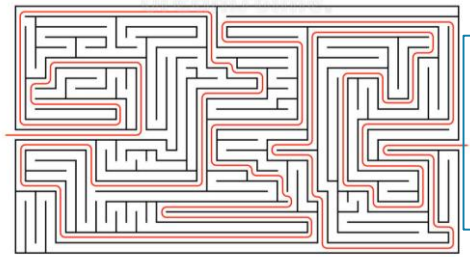
Nanoparticle

Treated mice
(HE +/- particle at various dosages)





No toxicity, no adverse reaction



Correction of behavioral troubles => treated HE = WT
Still ongoing

Biodistribution studies
CopperNiC project (**MITI 2021**) with DRHIM (**accepted**)

Discussions with industrial pharmaceutical partners

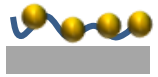
HE: hemizygote; WT: wild type; w: weeks

MITI: mission pour les initiatives transverses et interdisciplinaires

Nanostructuration

Specific properties coming from nanoparticles

2D Surfaces



Monolayer systems

(a PhD ongoing)

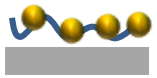
Packaging for preservation

Nanostructuration

Collab :

DSA

Specific properties coming from nanoparticles : Antioxydant activity

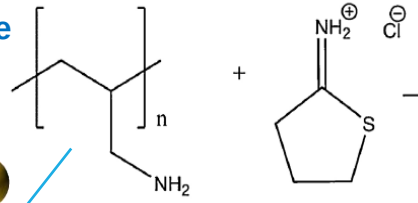


Polyallylamine

Electrostatic interaction

+ AuNP

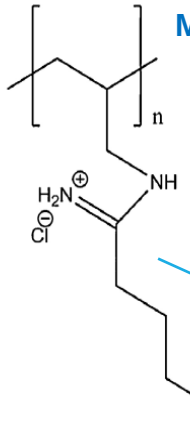
3.8 x 10^{13} AuNP/cm²
after 15 layers of AuNP



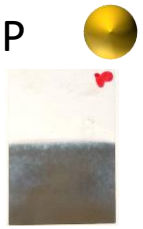
2-iminothionale

Modified Polyallylamine
PAH-(SH)n

Electrostatic interaction + –SH groups

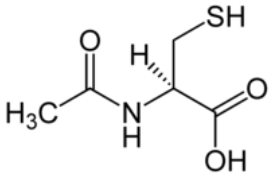


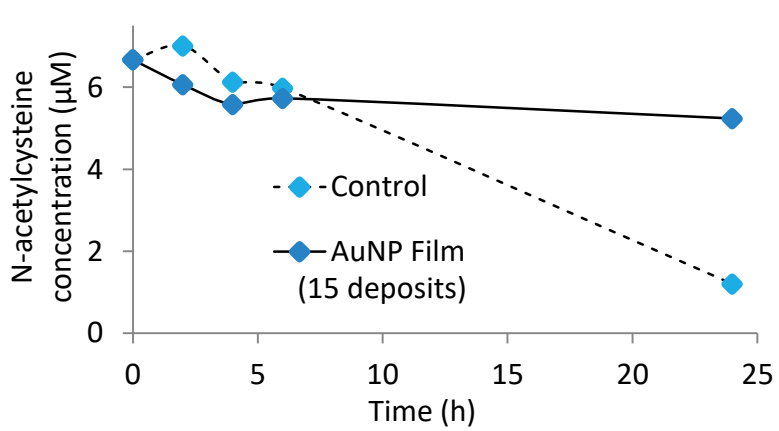
+ AuNP



↗ AuNP density

Half-life of N-acetylcystein
is ten fold increased





Work in progress test on the preservation of vitamins
Effect vs BHA and BHT
(controversial substances)

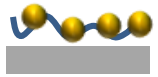
Colloid and Surface B: Biointerfaces 176 (2019) 439-448, Ph D of Célia Boukoufi ongoing

NAC: N-acetylcysteine

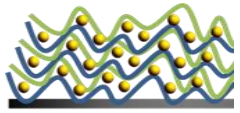
Nanostructuration

Specific properties coming from nanoparticles

2D Surfaces



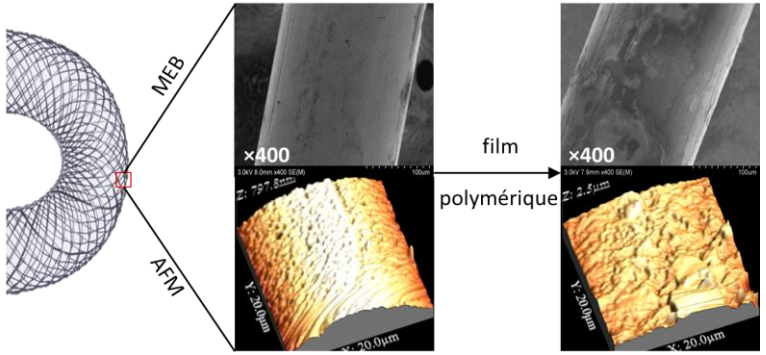
Monolayer systems



Multilayer (Layer-by-layer systems)

(a PhD ongoing)

Medical devices for the delivery of active substances (functionalized AuNP)



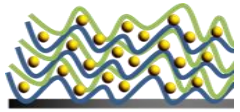
Nanostructuration

Specific properties coming from nanoparticles

Collab.
INSERM UMR 1121
BIOMATÉRIAUX BIOINGÉNIERIE

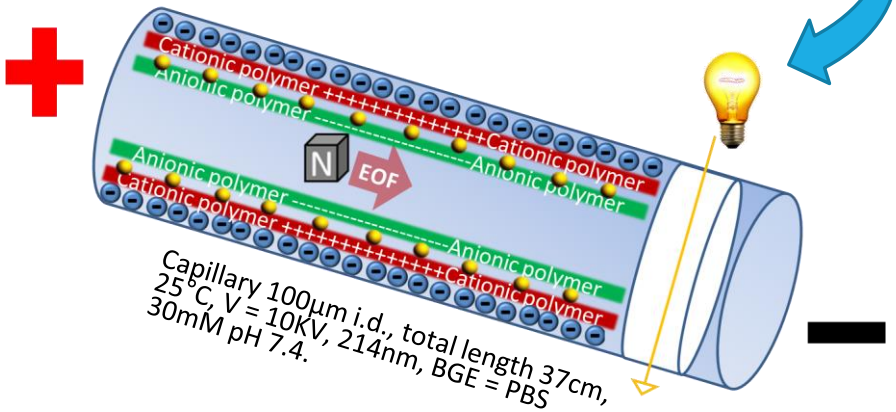
2D Surfaces

Anionic polymer
AuNP
Cationic polymer

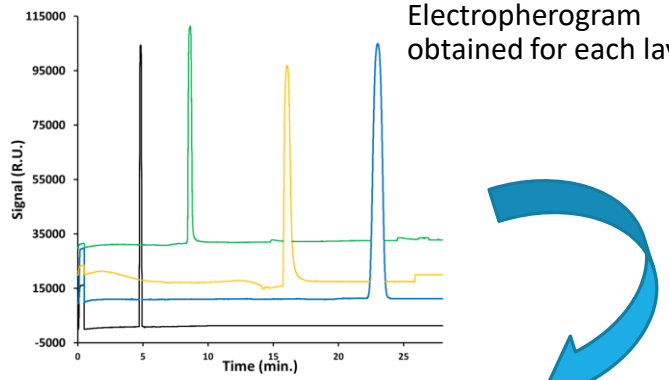


Multilayer (Layer-by-layer systems)

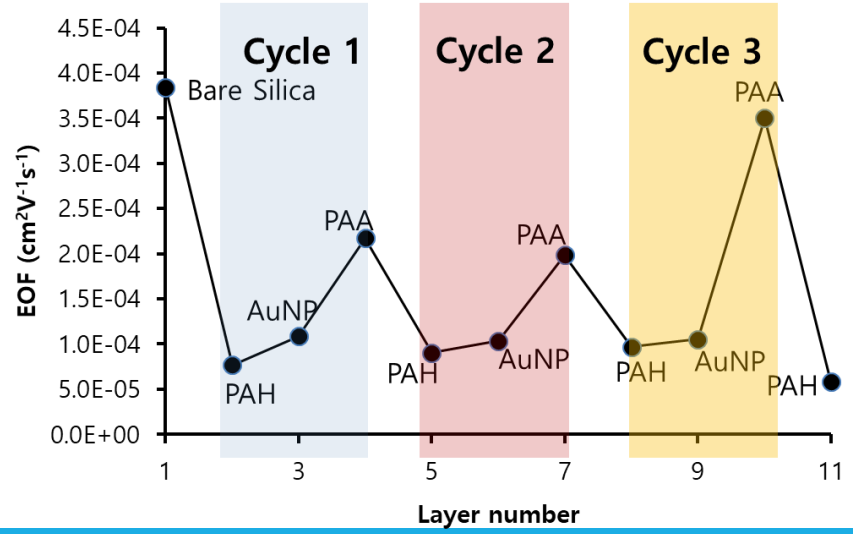
Capillary Electrophoresis



Surface charge evaluation



Electropherogram obtained for each layer



Analytical development for film design modelization and stability evaluation

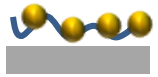
Pallotta et al, Part. & Part. Sys. Char. 2017, 34, 1600184 ; Pallotta et al, Anal. Bioanal. Chem. 2021, 413(5):1473-83, Ph D Jordan Beurton ongoing
PAH: polyallylamine ; PAA: polyacrylic acid

PAA, PAH

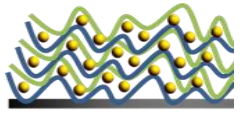
Nanostructuration

Specific properties coming from nanoparticles

2D Surfaces

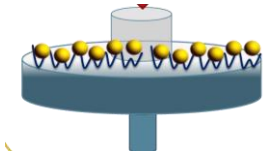


Monolayer systems



Multilayer (Layer-by-layer systems)

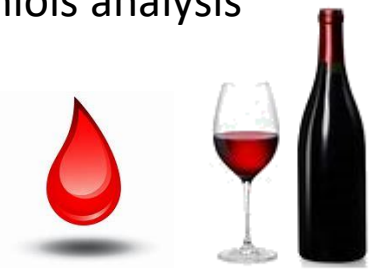
3D materials



Nanostructured membranes

(a PhD ongoing)

Sniffers for volatil thiols analysis



Covidog Project : a PhD coming soon (09/2021) for olfactive Covid-19 evaluation

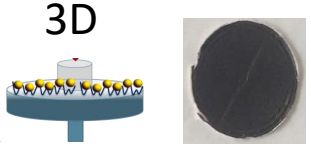
Twistaroma

Nanostructuration

Collab

DSA

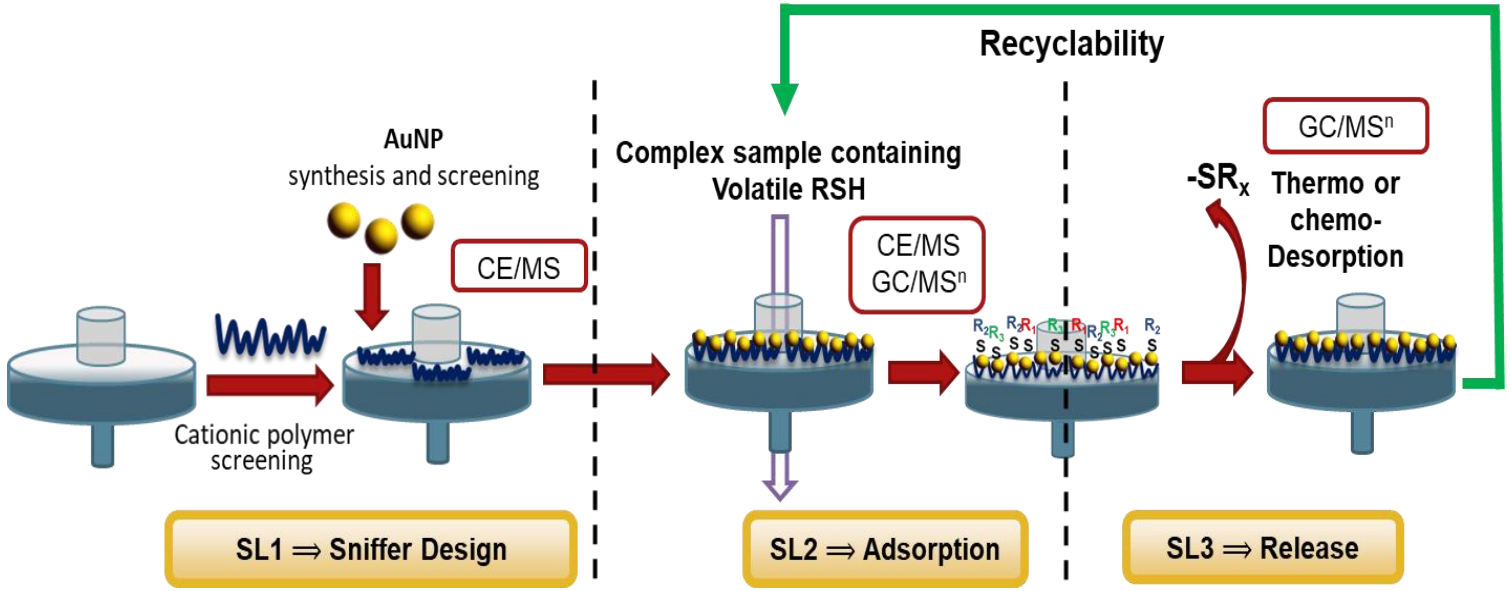
3D



Sniffers for volatil thiols analysis

Proposed to FRCR and ANR:

Design of nanostructured sniffers including immobilized gold nanoparticles (AuNP) to investigate the olfactory signature of beverages



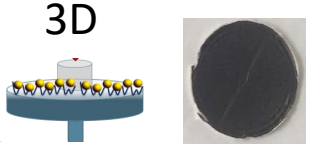
FRCR: Fonds Régional de Coopération pour la Recherche; CE: Capillary Electrophoresis; MS: Mass spectrometry, GC: Gas Chromatography

Collab

DSA

Nanostructuration

3D

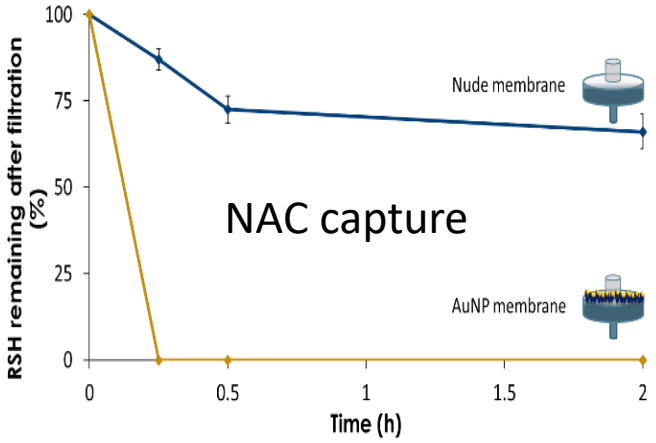


Sniffers for volatil thiols analysis

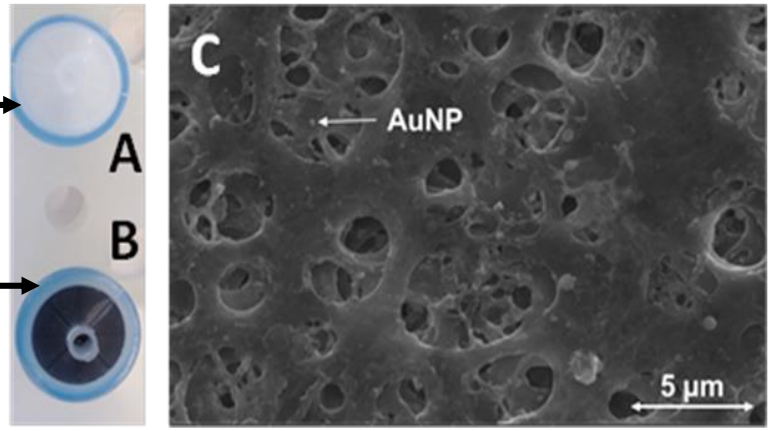
Preliminary results :

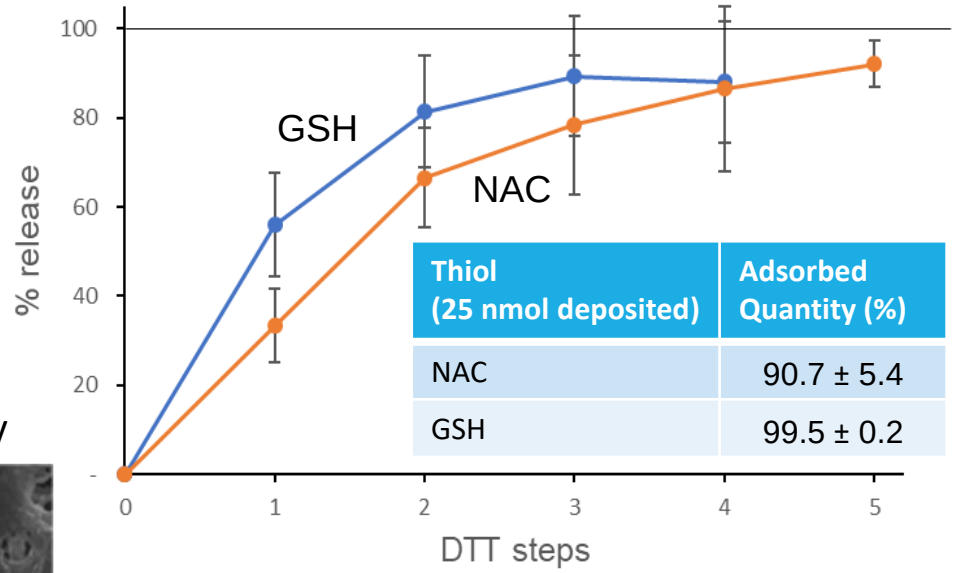
concentration effect of 300 000

(for example : 2-furfuryl thiol at 0.1 nM (initial concentration), membrane capacity 60 nmol, release with 2 mL)



Scanning Electron Microscopy





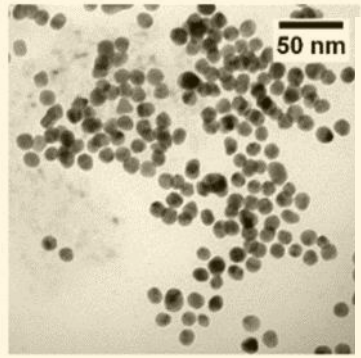
Thiol (25 nmol deposited)	Adsorbed Quantity (%)
NAC	90.7 ± 5.4
GSH	99.5 ± 0.2

Ph D Margaux Berthou ongoing, publication in preparation

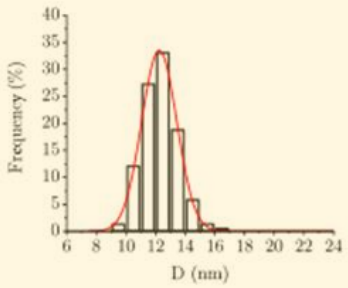
GSH: reduced glutathion ; NAC: N-acetylcysteine ; DTT: dithiothreitol

Collab.

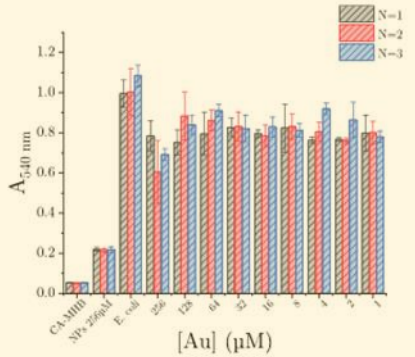
Antibacterial activity of nanoparticles



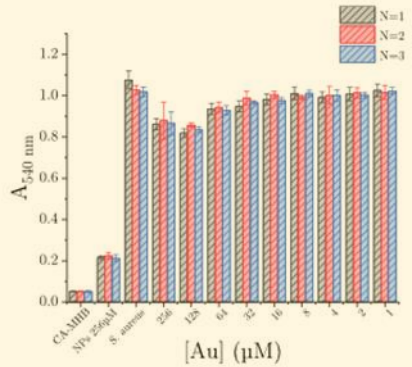
(a)



(c)



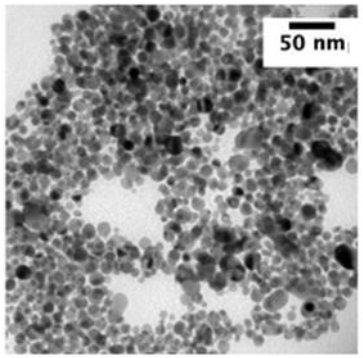
(a)



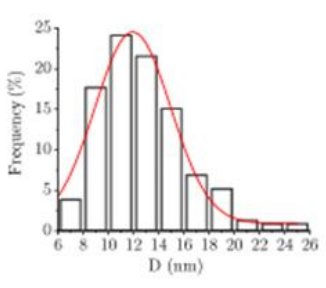
(c)

AuNPs

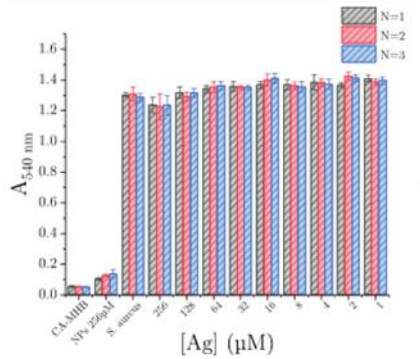
Weak antibacterial activity against *S. aureus* and *E. coli*



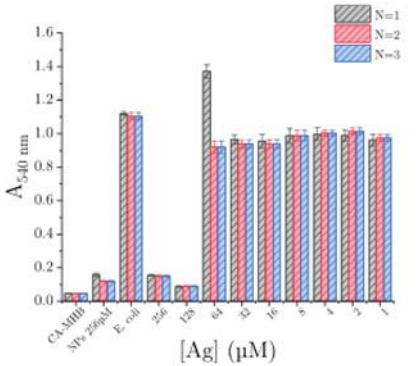
(b)



(d)



(b)



(d)

AgNPs

High antibacterial activity against *E. coli* (MIC = 128µmol/L) no activity on *S. aureus*

TEM micrographs and size distributions of AuNPs (a,c) and AgNPs (b,d) in culture media.

AgNPs and AuNPs antibacterial activity evaluation against (a,b) *Staphylococcus aureus* ATCC 29213 and (c,d) *Escherichia coli* ATCC 25922

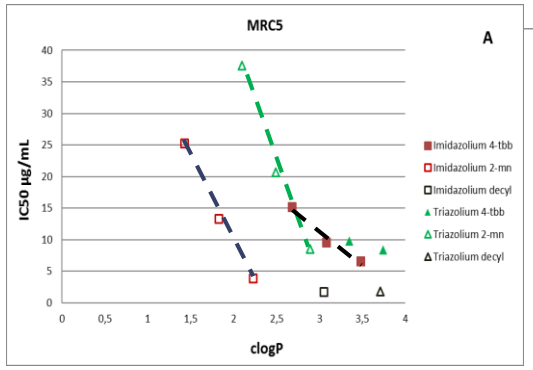
Next step: resistant bacteria

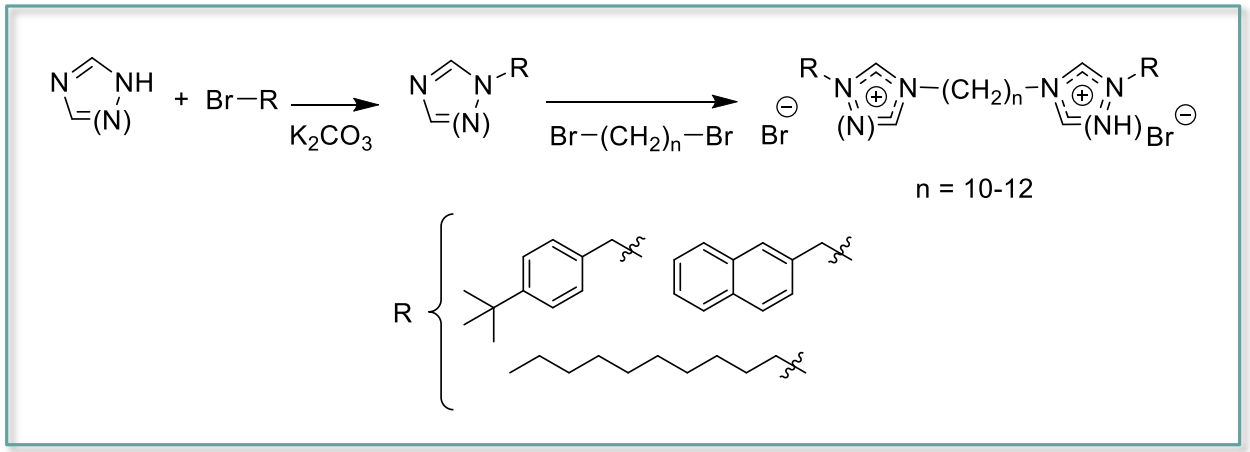
Gouyau et al, Int. J. Mol. Sci. 2021, 22, 1905

MIC: Minimum Inhibitory Concentration

Research of new anti-infectious molecules

Synthetic Chemistry

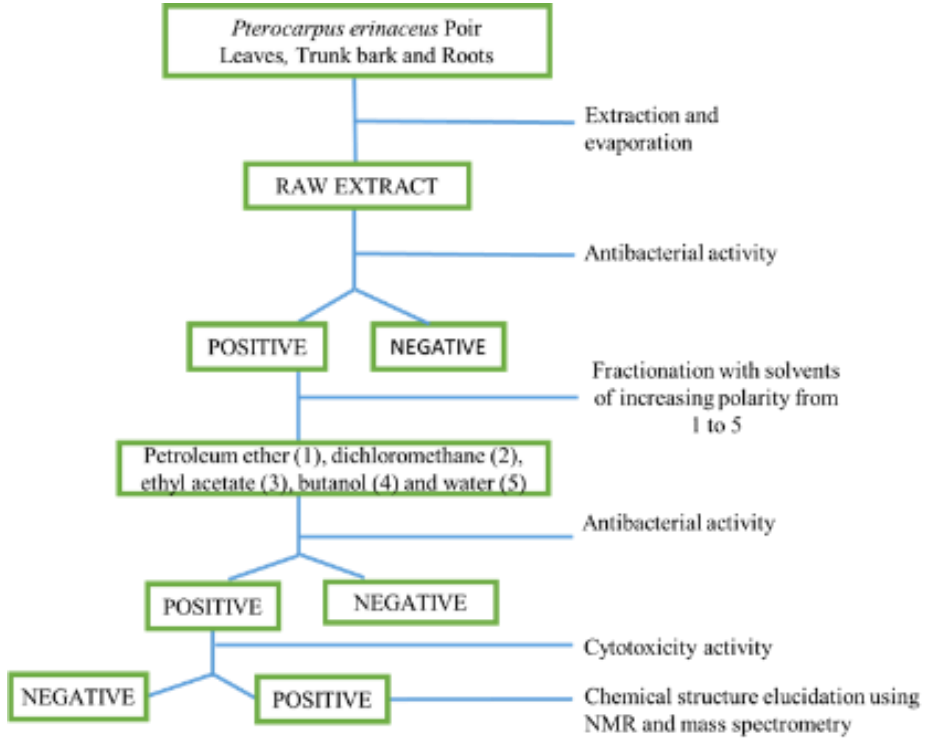




- ✓ MIC # 1 µg/mL on various bacterial strains (including multi-resistant)
- ✓ Low toxicity on eukaryotic cells
- ✓ Linear relationship toxicity vs logP

Chemistry of Natural Substances

Exploratory work by "bio-guided assay" approach on *Pterocarpus erinaceus*



Tittikpina, *J. Ethnopharmacol.*, **2018**, *212*, 200

Tittikpina, *Phytochem. Lett.*. **2019**, *32*, 110

Thomas *ChemMedChem* 2019, *14*, 1232-1237

MIC: minimun inhibitory concentration

ABC Platform
AntiBacterials Chemistry

Collab
Djibouti Research Center
DYNAMIC
UMR A 1128

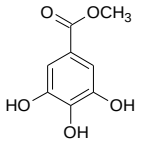
Research of new anti-infectious molecules

From Plants

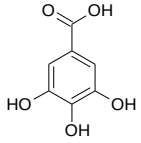
Dried fruits
Indigofera caerulea

Isolation and identification

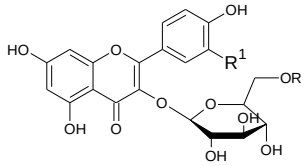
CMI Methanolic extract :
128 µg/mL (*S. aureus*)



Méthyl gallate



Acide gallique



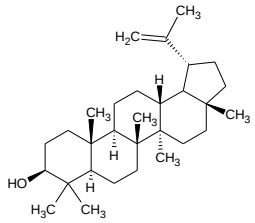
R=Rha, R^1=H, Kaempferol-3-rutinoside
R=H, R^1=OH, Quercetine
R=Rha, R^1=OH, Rutine

CMI = 64 µg/mL (*S. aureus*)

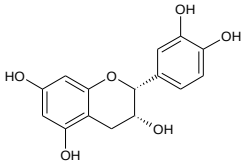
Bark
Acacia seyal

Phytochemical study

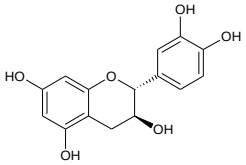
Methanolic extract :
CMI: 32 µg/mL (*S. aureus*)



Lupéol



Epicatéchine



Catéchine

From Microorganisms

High species diversity (>125)
Antibacterial & antifungal activities on clinical isolates

Elmi et al, *Molecules,* 2020, *25*, 2392 ; Elmi et al, *Ind. Crops Prod.*, 2018,*125*, 123

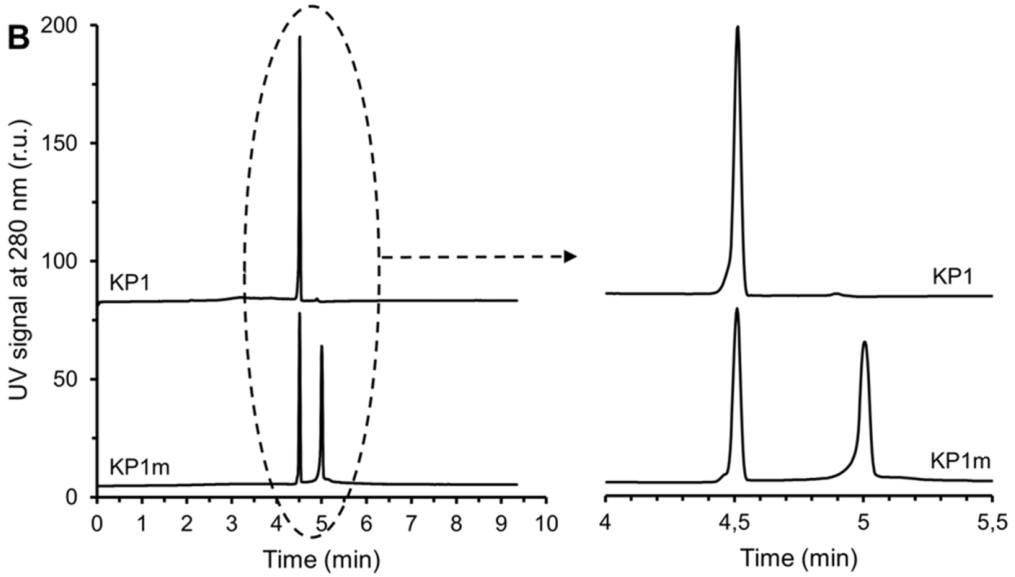
Benhadj et al, *Ann. Biol. Clin.*, 2018, *76*, 81

Collab.

Antibacterial activity

Development of analytical methods for the quantification and identification of bacteria

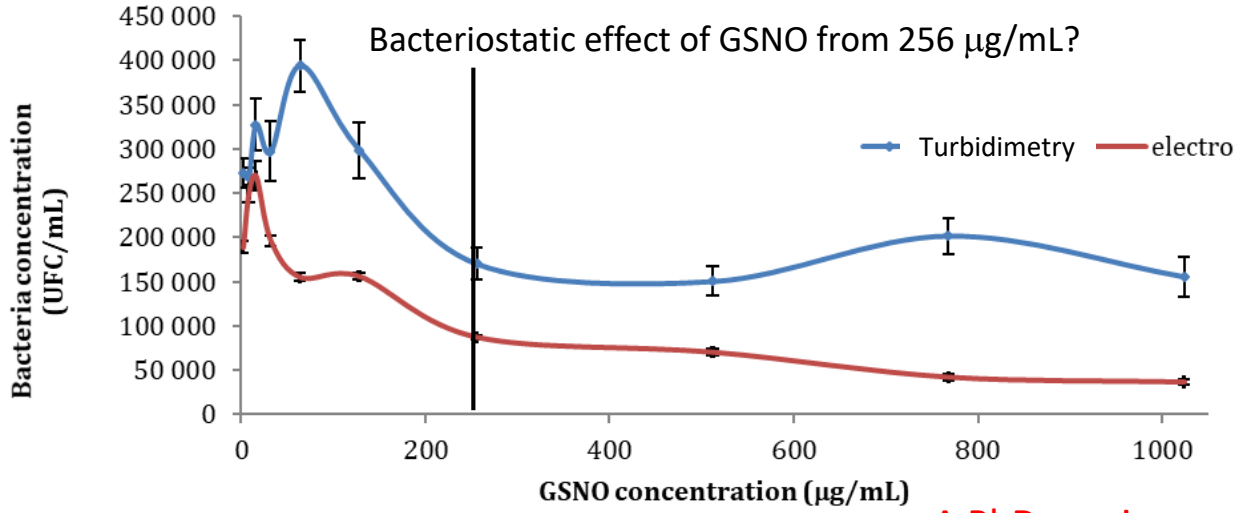
Mutation vs morphology ?



Comparison between a reference and mutated (m) *Klebsiella pneumoniae (KP)* culture shows significant changes in morphology and/or membrane and/or charge.

Towards the determination of MIC by CE ?

Comparison of *Staphylococcus aureus* quantification by capillary electrophoresis and turbidimetry



A PhD coming soon (2022)

Sautrey et al, Electrophoresis 2015, 36(20), 2630–33.

MIC: minimun inhibitory concentration

GSNO: *S*-nitrosoglutathion

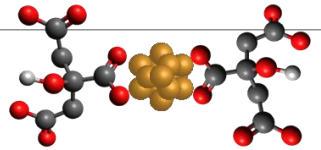
Towards the SCALE project

Current activities =

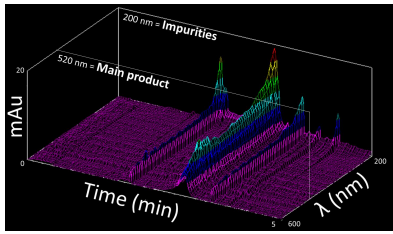
→ Development of nanoparticles and nanostructured materials

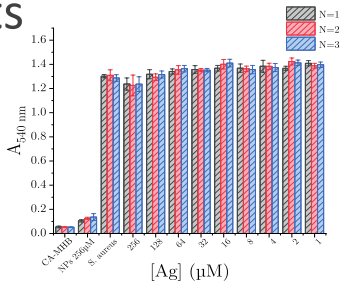
→ Development of analytical methods specific to nano and micro-objects

→ Evaluation of antioxidant and antibacterial activities of nano-objects



AuNP@citrate





SCALE = Analytical Sciences + Diagnostic / Nanostructures

= discussion since August 2019 with IPHC because scientific relevance + complementary to current DSA teams

SCALE

Some Questions ?