SYNTHESIS OF SIZE MONODISPERSE WATER-SOLUBLE METAL NANOCLUSTERS FOR PROTEIN QUANTIFICATION BY ELEMENTAL MASS SPECTROMETRY

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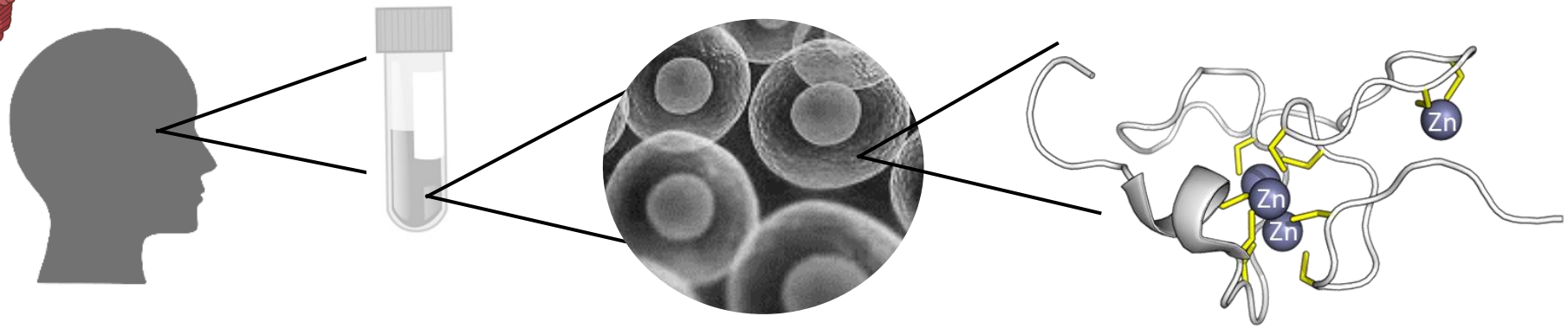
2nd International Online-Conference on Nanomaterials, 15-30 November 2020

STUDY OF PROTEINS AS BIOMARKERS TO UNDERSTAND THEIR BIOLOGICAL FUNCTIONS

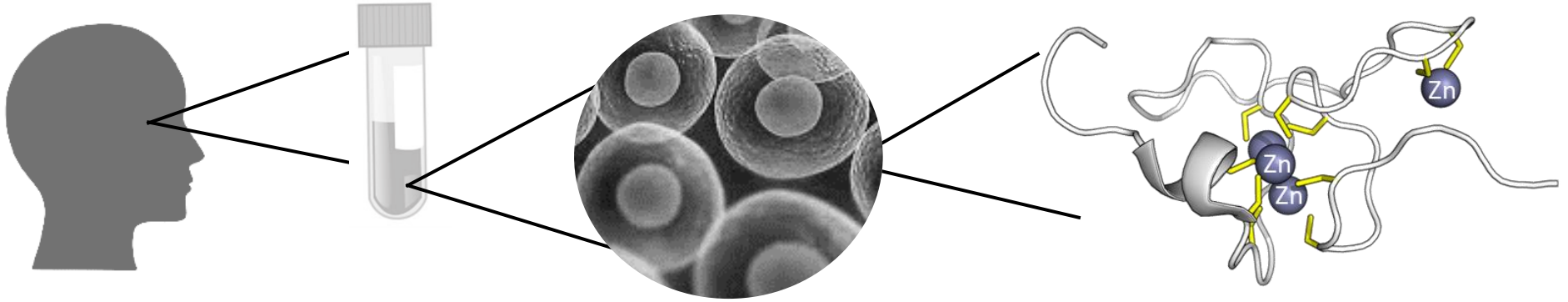


HUMAN DISEASES

BIOLOGICAL SAMPLES

PROTEINS

TOOLS FOR THEIR ACCURATE DETERMINATION



PROTEINS
Biomarkers

ANALYTICAL CHALLENGES

Specificity
(biological samples are complex matrices)

High sensitivity
(the species of interest can be at very low concentrations)

Absolute quantitative information
(<u>not only differential protein levels</u> between samples, <u>but also protein absolute concentrations</u>)

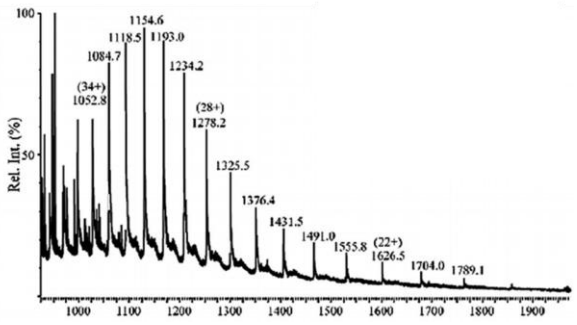
Techniques for the study of proteins

MASS SPECTROMETRY

Molecular MS

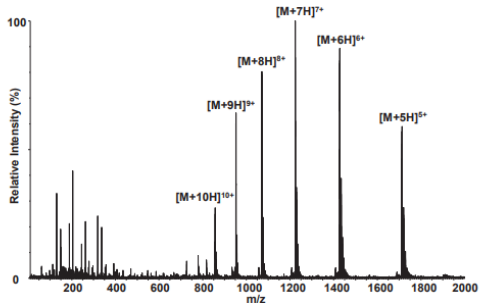
soft ionization sources

Electrospray ionization (ESI)



*Sterba, Jan & Vancová et al., Libor. (2008). Journal of bacteriology. 190. 2619-23.

Matrix assisted laser desorption/ionization (MALDI)

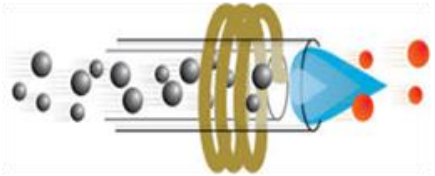


*Fatou, B. et al., (2018). *Molecular & Cellular Proteomics, 17(8), 1637–1649.*

PROTEIN IDENTIFICATION

Elemental MS

(e.g. ICP-MS)



- ✓ Low detection limits (LoDs)
- ✓ Wide linear dynamic range
- ✓ Multi-element (and multi-isotope) analysis
- ✓ Matrix-independent ionization

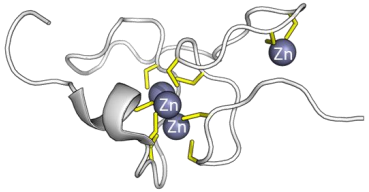
ABSOLUTE PROTEIN QUANTIFICATION

Strategies for the quantification of proteins

ABSOLUTE PROTEIN QUANTIFICATION BY ICP-MS

1

Measurement of naturally present heteroatoms such as S, P and metals



- **Analysis of metalloproteins:**

simultaneous measurement of the protein and the coordinated metals

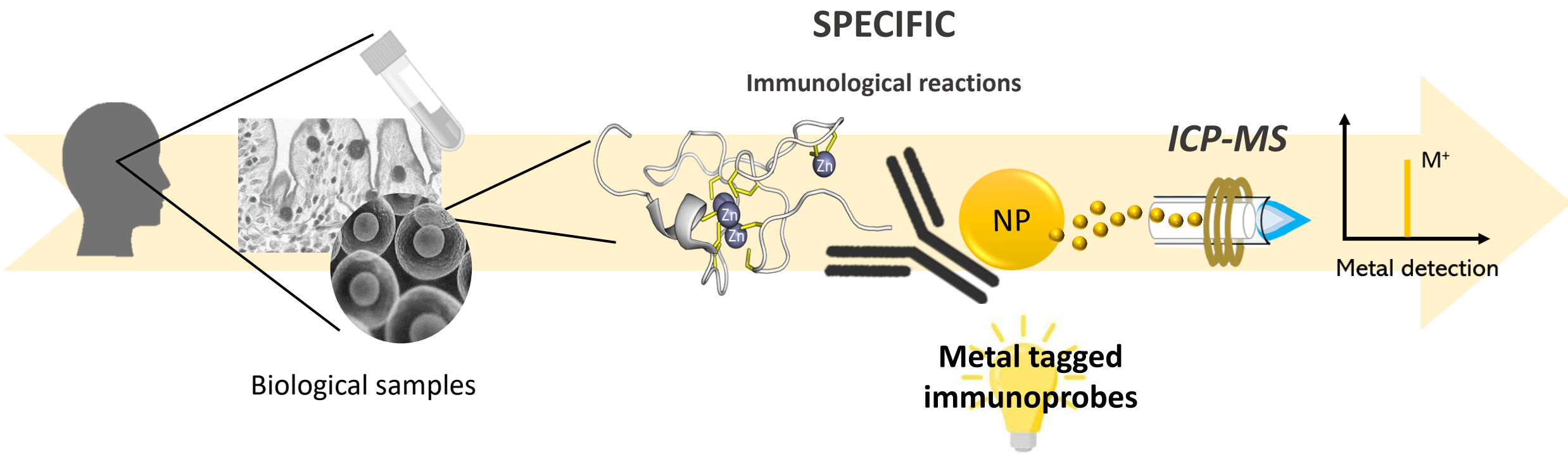
Careful isolation of the target biomolecule

2

Exogenous elemental (or isotopic) <u>direct labelling</u> of biomolecules

Strategies for the quantification of proteins

ABSOLUTE PROTEIN QUANTIFICATION BY ICP-MS



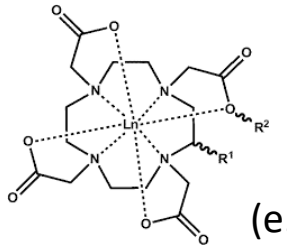
Metal tagged immunoprobes for ICP-MS detection

Metal tagged immunoprobes

1

Metal chelate

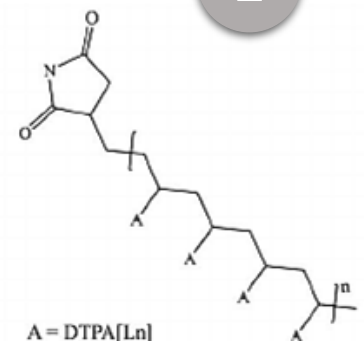
✗ No amplification



(e.g. DOTA)

2

Polymers with several metal chelates



(e.g. MaxPar™)

✗ Their large size can impede an efficient antibody-antigen interaction

✗ No fluorescent

3

Metal NPs (>10 nm)

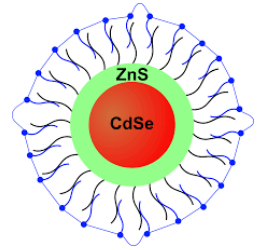
NP

✗ No fluorescent

✗ High size

4

Quantum dots (QDs)

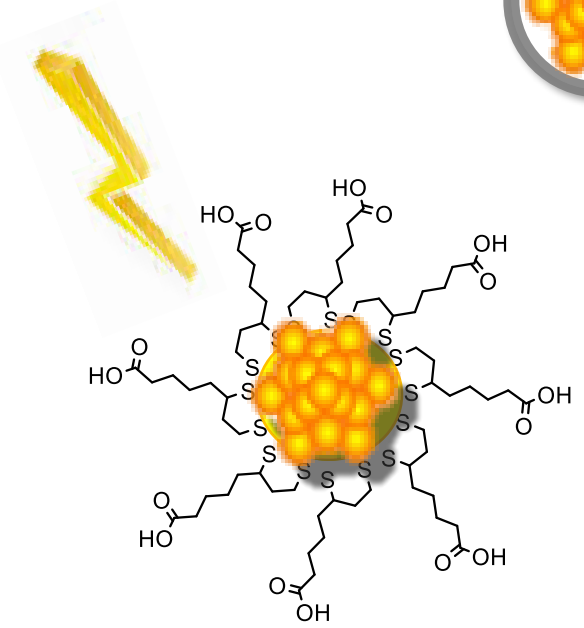


✓ Fluorescence

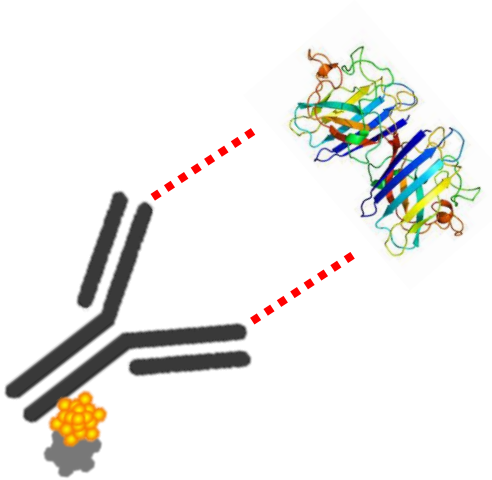
✗ Composed of more than one element

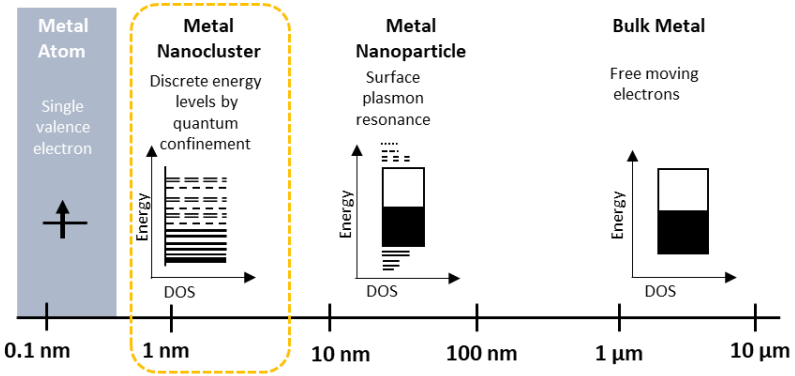
✗ Typical QDs (e.g., CdSe) are toxic

Metal tags for ICP-MS: water-soluble metal nanoclusters

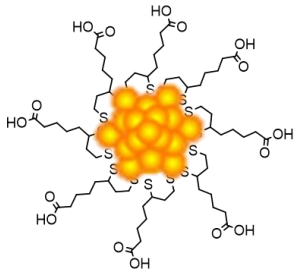


- Composed of just one type of metal (*from a few atoms up to a few **hundred metal atoms**)*
- **Small Size** (*Diameters from 0.2 to 3 nm)*
- High "number of metal atoms/tag size" ratio. **High amplification with a small size**
- **Lower risk of blocking the recognition sites of the antibody compared to NPs**
- **Fluorescent** (*Strong photoluminescence: size & surface ligands)*





Metal tags for ICP-MS: water-soluble metal nanoclusters



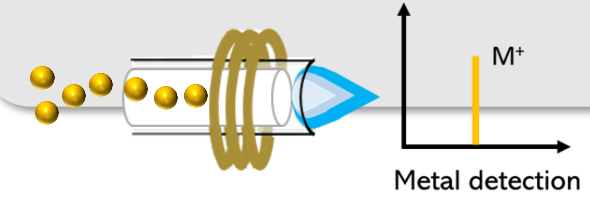
BIMODAL DETECTION

FLUORESCENCE

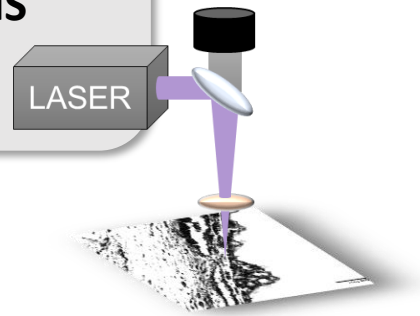
**Alternative to convetional fluorophores*

ELEMENTAL DETECTION

ICP-MS



LA-ICP-MS

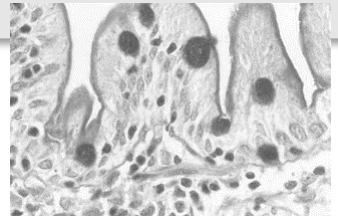


BIOASSAYS

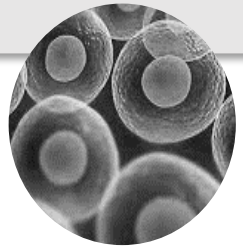
APPLICATIONS

1. IMMUNOASSAY
Determination of analytes in fluids

2. IMMUNHISTOCHEMISTRY
Determination and localization of biomolecules in tissues (bioimaging)



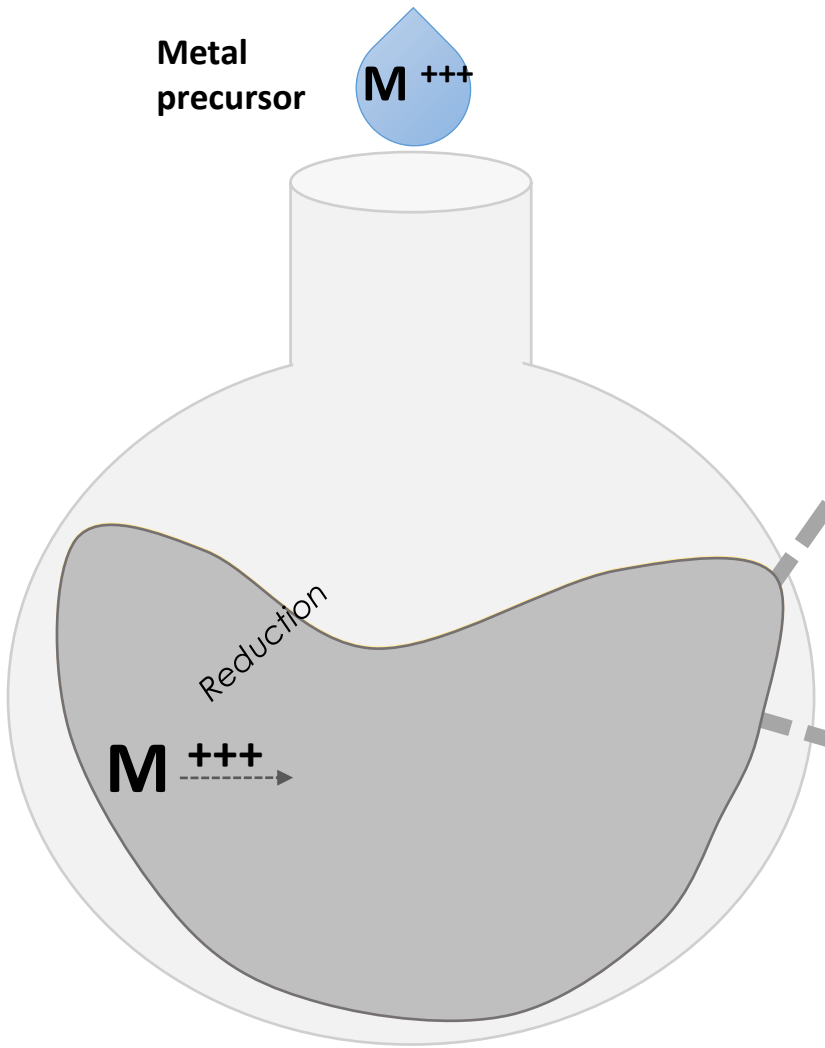
3. IMMUNOCYTOCHEMISTRY
Determination and localization of biomolecules in cell cultures/individual cells



Synthesis of metal nanoclusters

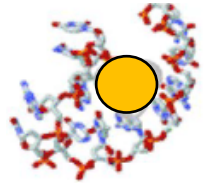
BOTTOM UP APPROACH SYNTHESIS

*K., Zheng, et al.(2014), RSC Adv. 4, 60581-60596.



SCAFFOLDS

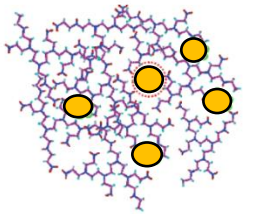
DNA



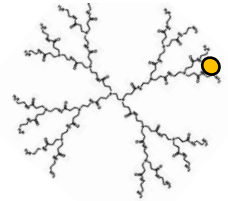
Proteins

Polymers

Polyethylenimine (**PEI**)



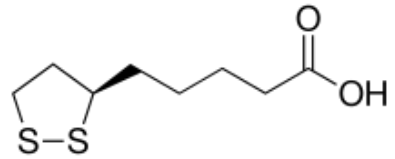
Polyamidoamine (**PAMAM**) dendrimers



Ligands

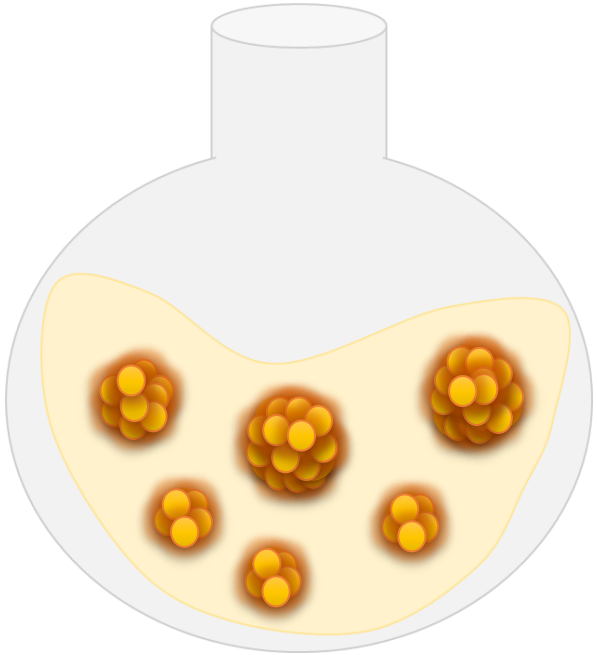
Small Thiolate ligands

Lipoic acid



THE PROBLEM: POLYDISPERSE SIZE

synthesis in progress…

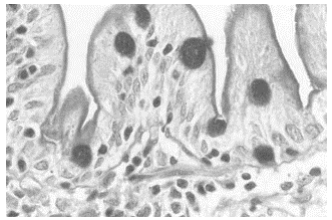


DEVIATION IN SIZE OF NCs

IMPLIES VARIATION IN THE NUMBER OF ATOMS PER TAG

❑ Up to date: Fluids or tissues samples

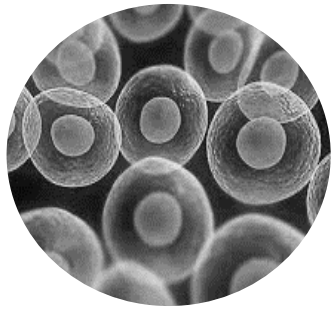
[1]



[2]

- High proteins concentration in the samples
- THE DEVIATION associated to the NCs diameter IS COMPENSATED WITH THE **HIGH NUMBER OF NCs (METAL ATOMS) MEASURED at the same time**

❑ Current challenge: Individual cells



- LOW PROTEINS CONCENTRATION in the samples
- **THE DEVIATION ASSOCIATED TO THE NUMBER OF ATOMS per TAG INCREASES: Direct implications in the accurate determination of proteins concentration**

[1] Lores-Padin, A., Cruz-Alonso, M., Gonzalez-Iglesias, H., Fernandez, B., Pereiro, R.. *Microchim. Acta*, **2019**, 186, 705

[2] Lores-Padín, A., Fernández, B., Álvarez, L., González, H., Lengyel, I., Pereiro R. *Talanta,* **2021**, 221, 121489

AIM OF THE WORK

ACCURATE AND PRECISE DETERMINATION OF BIOMOLECULES

Synthesis of monodisperse AuNCs to reduce the deviation associated with their size (based on previously optimized synthesis of MNCs)

SYNTHESIS APPROACHES

- ❑ Reaction time
- ❑ Decrease the kinetics of the reduction reaction→ **to have a better control of the reaction**
- ❑ Modify the thiolated ligand→ **to get more control of the growth**
- ❑ Post-treatment of polydisperse AuNCs→ **size-focusing**

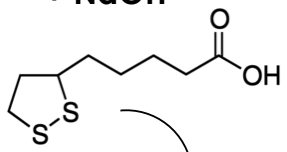
Methodology: General synthesis protocol of AuNCs

Thiolate ligands

Solvent of the reducing agent

LIGAND

Lipoic acid (LA) + NaOH

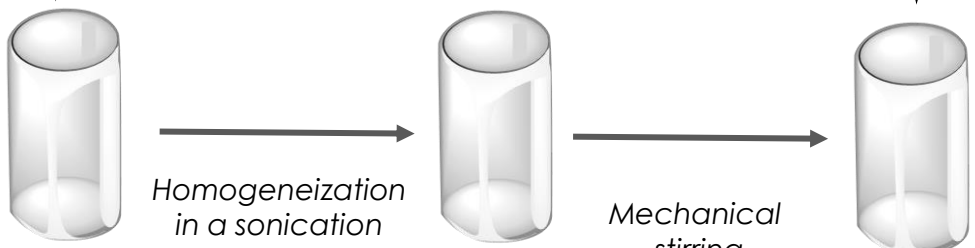


Gold precursor

$AuCl_4^-$

REDUCTANT (organic solvent)

$NaBH_4$



1:3 molar ratio (Au:LA)

$pH_{síntesis}$=11

1:2 molar ratio (Au:$NaBH_4$)

Time

PURIFICATION

Waste

Quality & Size Dispersion

- ❑ Fluorescence (λ_{ex}325/λ_{em}720nm)
- ❑ DLS (PDI)
- ❑ HR-TEM/EDX

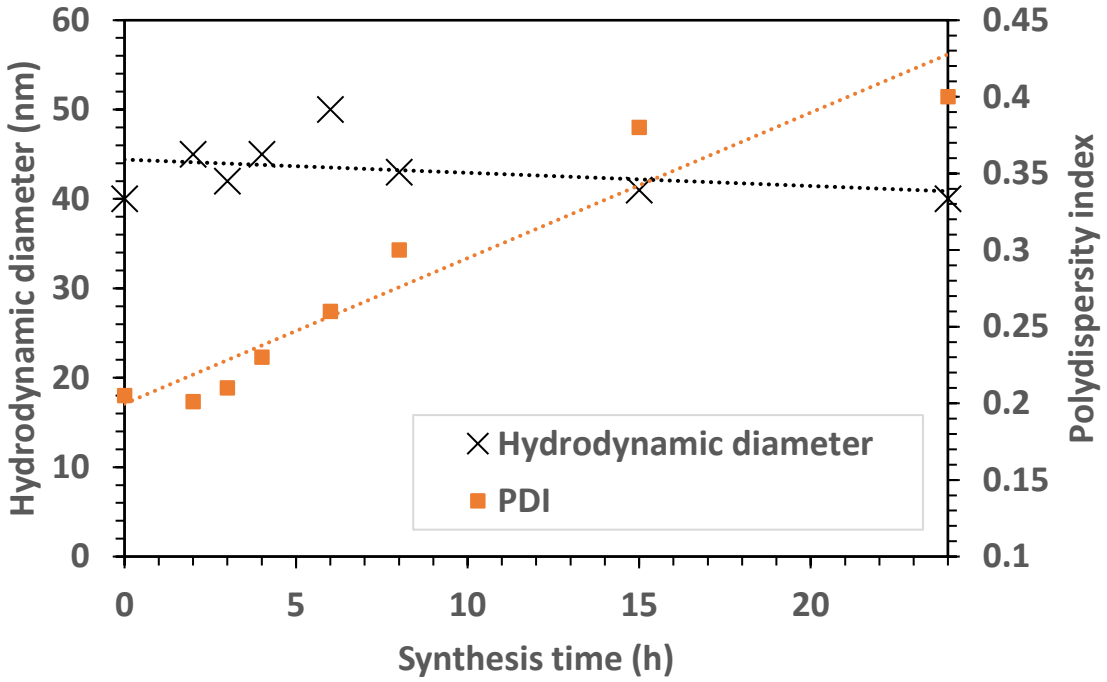
Solvent of the synthesis/pH of the synthesis

*Cruz-Alonso, M. Fernández, B., Navarro, A., Junceda, S., Astudillo, A., Pereiro,R. *Talanta,* **2019**, 197, 413–421

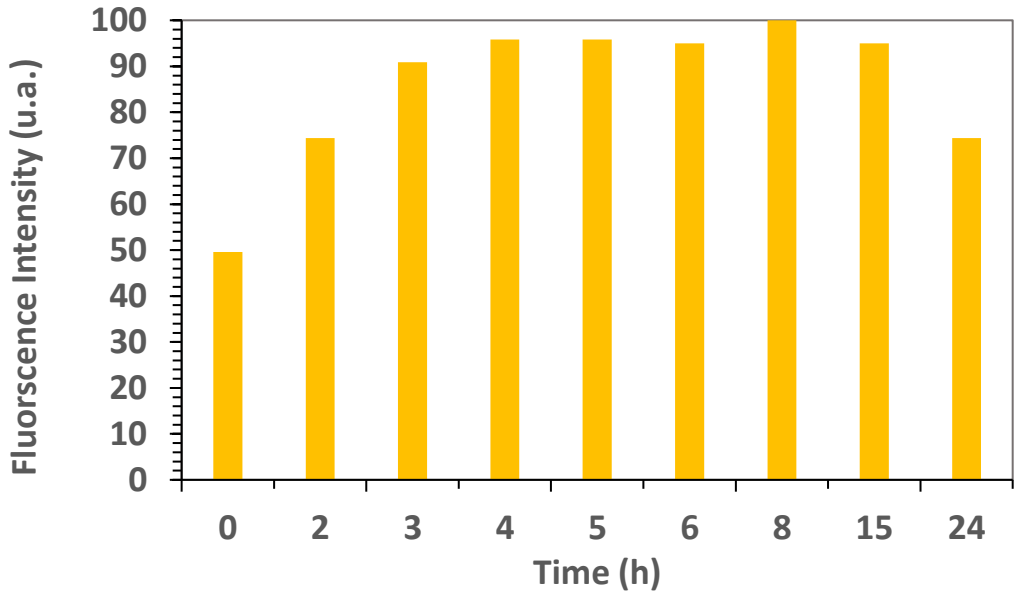
Synthesis optimization: REACTION TIME

Time

DLS



Fluorescence (λ_{ex} 325 nm, λ_{em} 720 nm)



PDI values

Polydispersity: PDI>0.4 # Moderated dispersity: PDI 0.4-0.25

Monodispersity: PDI<0.25 # High monodispersity: PDI<0.1

Synthesis optimization: SOLVENT

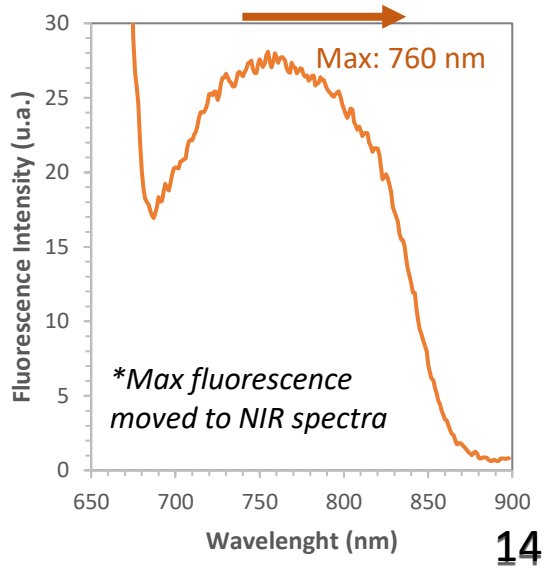
Solvent used with the synthesis reductor

Solvent	Polydispersity Index (PDI)	Hydrodynamic diameter (nm)
Ultrapure water (old original)	0.43	26.8
Isopropanol (original)	0.39	20.3
Methanol	0.38	25.4
Acetone	0.51	>100

Solvent used for the synthesis

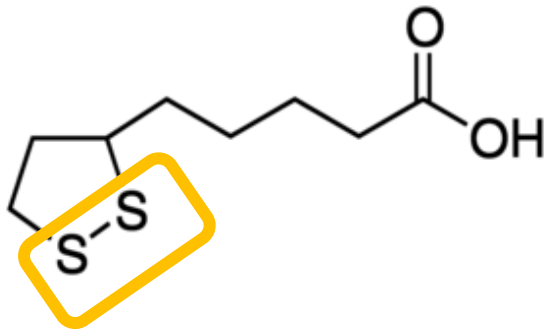
- ❑ Ultrapure water (pH=11)→*Original*
- ❑ Basic media: ultrapure water (pH=13)
- ❑ Methanol

Solvent	PDI
Ultrapure water (pH=11)	0.39
Ultrapure water (pH=13)	0.12
Methanol	0.6

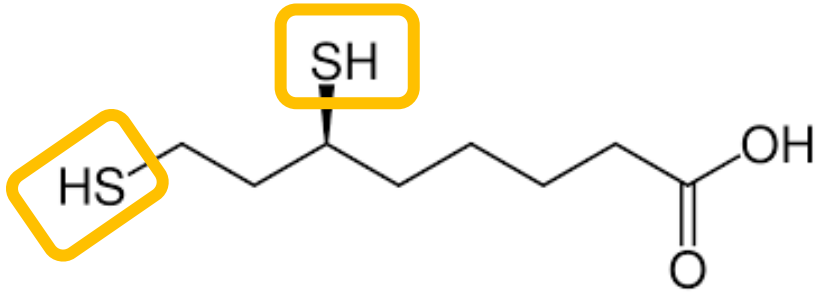


Synthesis optimization: LIGAND

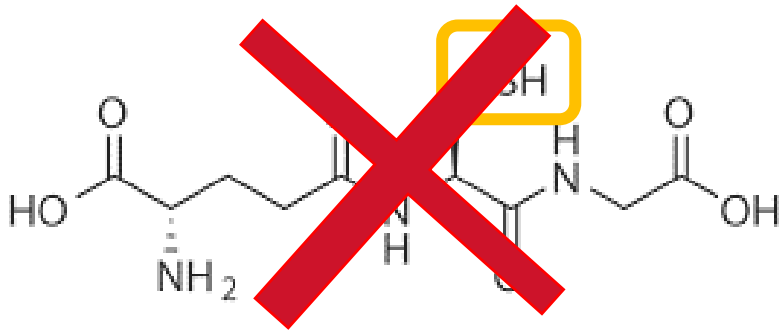
❑ Lipoic acid



❑ Reduced lipoic acid (DHLA)



❑ Glutathione



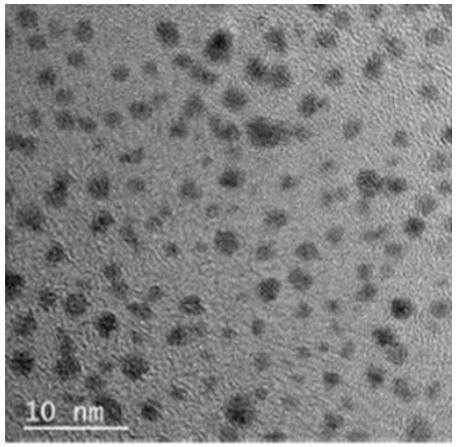
DLS

Ligand	Polydispersity Index (PDI)
Lipoic acid (15 h)	0.38
Lipoic acid (4 h)	0.23
Reduced lipoic acid	0.18
Glutathione	>1

Synthesis optimization: LIGAND

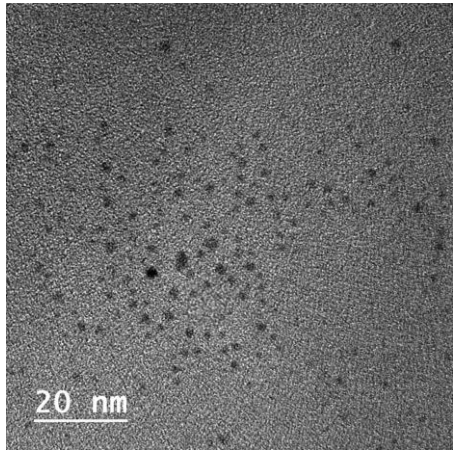
HR-TEM images

- Lipoic acid

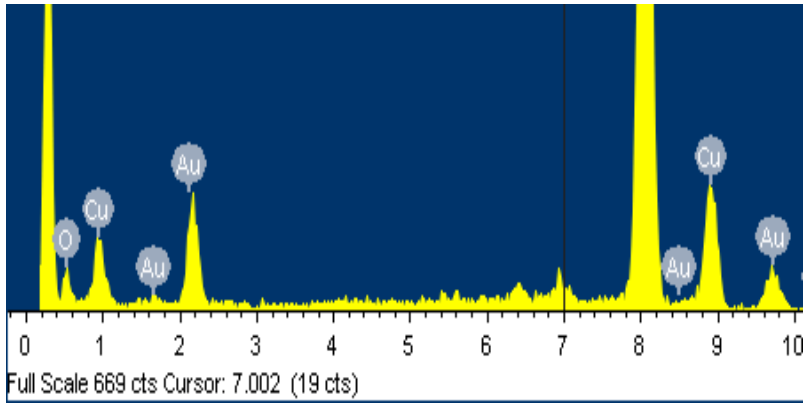


Size= 2.2 ± 0.04 nm
(99% confidence, n=300)

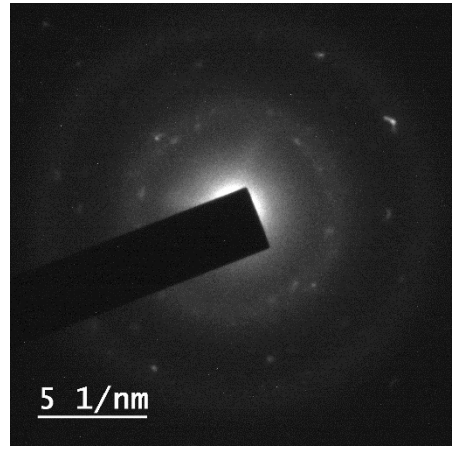
- Reduced lipoic acid



Size= 1.99 ± 0.04 nm
(99% confidence, n=300)

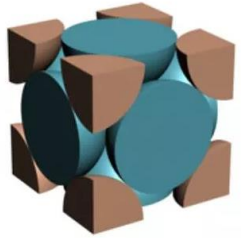


Energy dispersive x-ray (EDX)



X-ray Electron diffraction

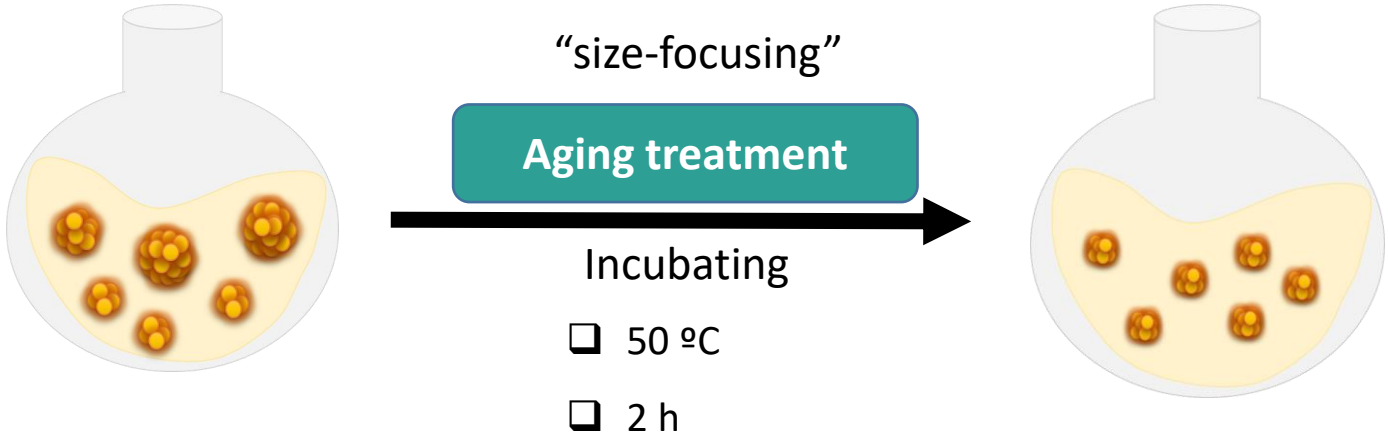
Crystaline structure
Face-centered cubic structure (FCC)



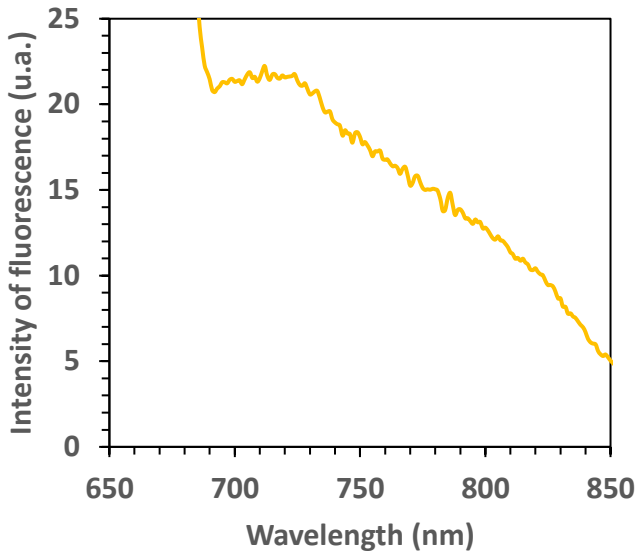
DLS

Ligand	Polydispersity Index	Hydrodinamic size (nm)
Lipoic acid	0.38	10.60 ± 7.89
Reduced lipoic acid	0.18	12.42 ± 5.86

Synthesis optimization: SIZE-FOCUSING

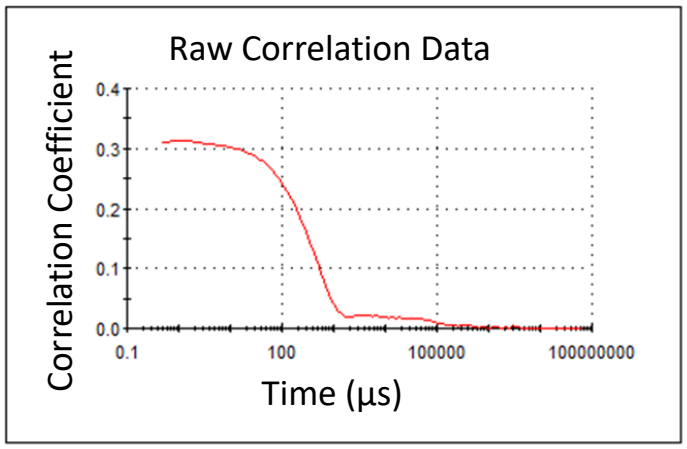


Fluorescence emission

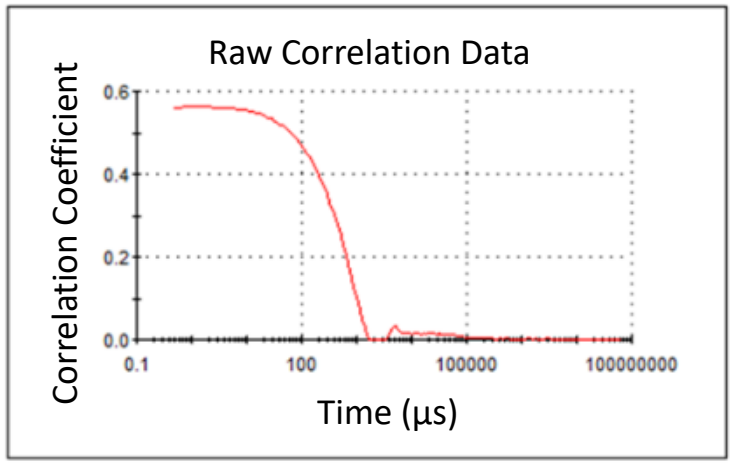


DLS

PDI=0.30



PDI=0.09



CONCLUSIONS

- Higher **reaction times** increase the AuNCs size dispersion → 4 h are enough to obtain high quality AuNCs (by DLS measurements).
- In order to reduce the kinetics of the reaction, **the use of a low pH** (e.g., pH=13) is favourable → improvements in the AuNCs dispersion was observed using basic pH values.
- Several **ligands** were evaluated and experimental results showed that reduced lipoic acid was the best capping agents (lower dispersion values)→ improvement in the control during the AuNCs growth
- The use of a **size focusing treatment** (50 ºC during 2 h) after AuNCs synthesis was found to produce monodisperse AuNCs

AcKnowledgements

- **Organizing Committee**

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