

Single cell and single particle ICP-MS: latest developments

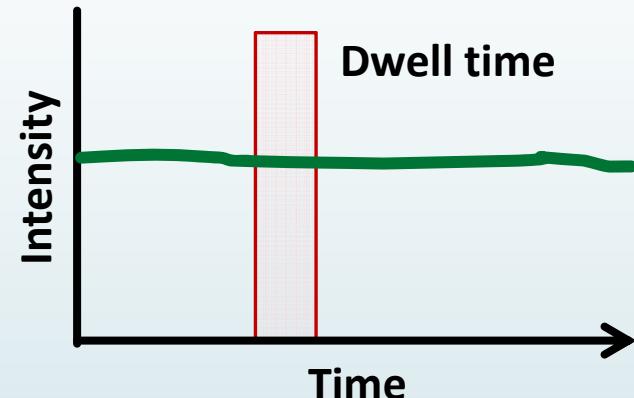
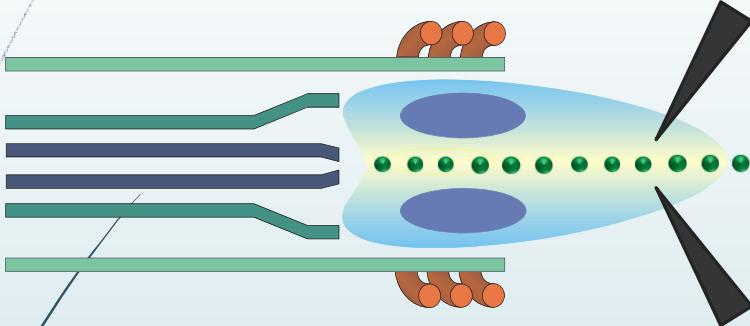
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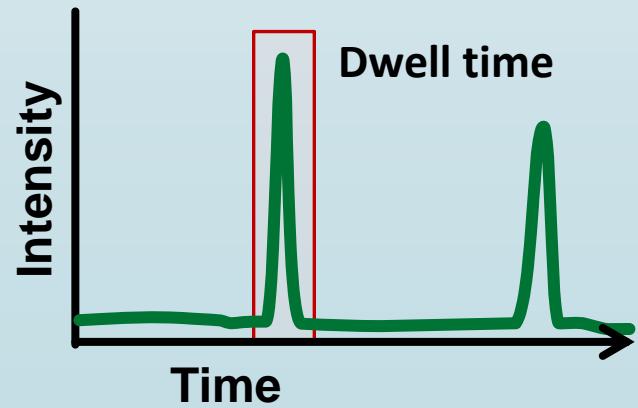
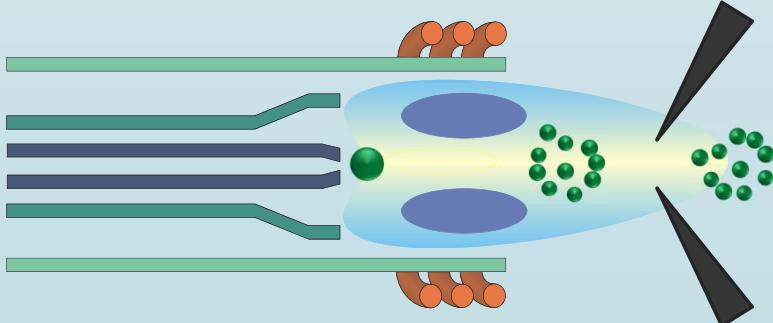
University of Oviedo

Principle of single particle/cell (SC)-ICP-MS

- Dissolved analyte: continuous introduction of analyte results in a constant ICP-MS signal.



- Solution of cells: a single cell produces an ion plume resulting in a single, short ICP-MS signal.



Actual projects in our group in SC/SP-ICP-MS

- **TiO₂ NPs** in candy products
- Evaluation of transport efficiencies of **Au NPs**.

- **CisPt uptake** in cell models
- Cu in **single spores** of Streptomyces.
- Breast cancer **cell biomarkers**

Single Particle

Single Cell

- **Se NPs and Se in single cells** in selenized yeast exposed to different Se media.

ICP-Triple Quadrupole-MS

Mode (Isotope)	Sensitivity [cps fg ⁻¹ Ti]*	Size detection limit TiO ₂ [nm]**	Number of events***
SQ-KED (⁴⁷ Ti)	195	65	193
SQ-NH ₃ (⁴⁷ Ti)	1,066	76	602
SQ-O ₂ (⁴⁷ Ti)	6,548	107	616
TQ-O ₂ (⁴⁷ Ti)	1,990	38	858
TQ-NH ₃ (⁴⁸ Ti)	1,330	32	1,703
TQ-O ₂ (⁴⁸ Ti)	20,310	26	1,386

* Considering same transport efficiency for Au and TiO₂ particles.

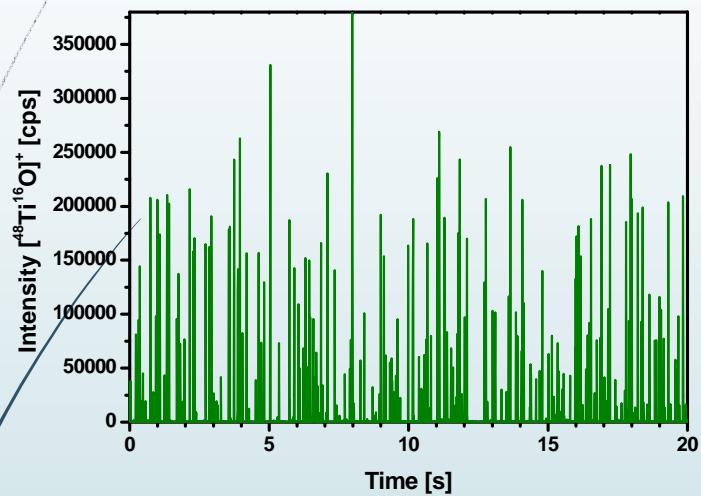
** Considering spherical particles.

*** Registered during 180 s measurement analysing TiO₂ standard (Sigma) after 10⁹-fold dilution and using 3 σ-criterion.

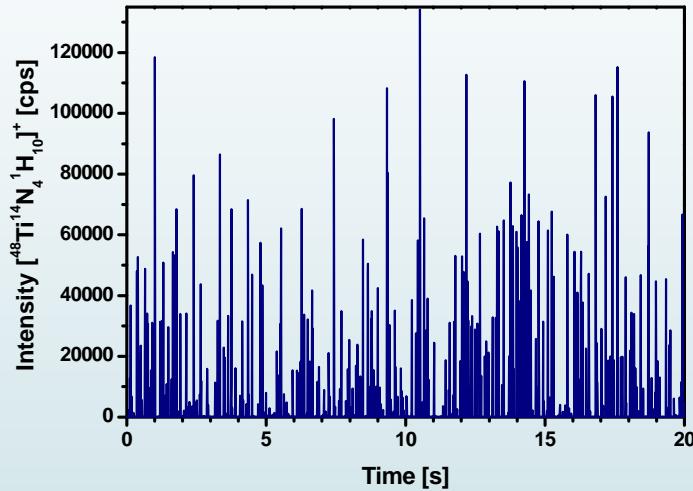


spICP-MS of Suspensions from Chewing Gums

- Comparison of TQ-O₂ and TQ-NH₃ modes using ⁴⁸Ti.



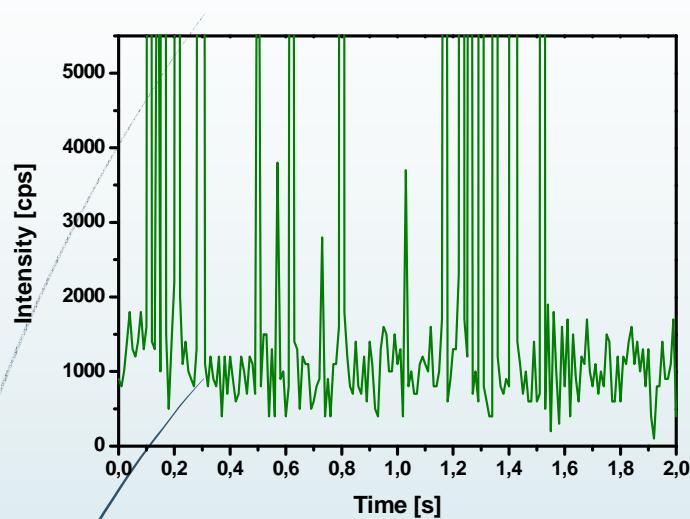
TQ-O₂



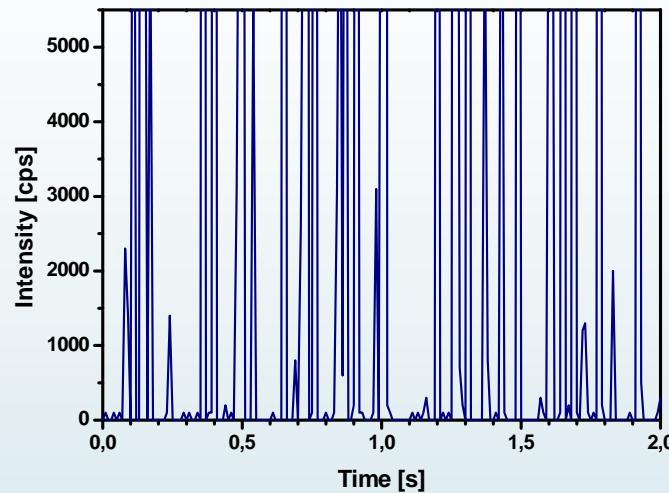
TQ-NH₃

- Multi-phase products were analysed: Predominant amount of TiO₂ is located in the gum shell.
- One chewing gum pellet was immersed in 40 mL H₂O and sonicated for 25 - 30 min, then diluted 1 to 500,000.

spICP-MS of Suspensions from Chewing Gums



TQ-O₂



TQ-NH₃

Mode (Isotope)	TQ-O ₂ (⁴⁷ Ti ⁺)	TQ-O ₂ (⁴⁸ Ti ⁺)	TQ-NH ₃ (⁴⁸ Ti ⁺)
Determined size range [nm]	43-200	31-200	32-186
Particles below 100 nm [%]	42	42	40
Detected particles per chewing gum	$6.75 \cdot 10^{11}$	$7.25 \cdot 10^{11}$	$7.28 \cdot 10^{11}$

ICP-Triple Quadrupole-MS for NPs

- Comparison of transport efficiencies for Au NPs (NIST 30nm) using different configurations.

Enya Mist-TCSC (elbow)



48% Transport Efficiency

DS-5-TCSC (straight)



20% Transport Efficiency

HPCN (High Efficiency Cell Introduction System)



70% Transport Efficiency

Challenges in Metallomics

Analytical Challenge

- Small systems (e.g. single cells)
 - Multielemental
 - Simultaneous

Mass balanced

Diversity of metallo-species

- Unknown (lability?)
- Concentration levels

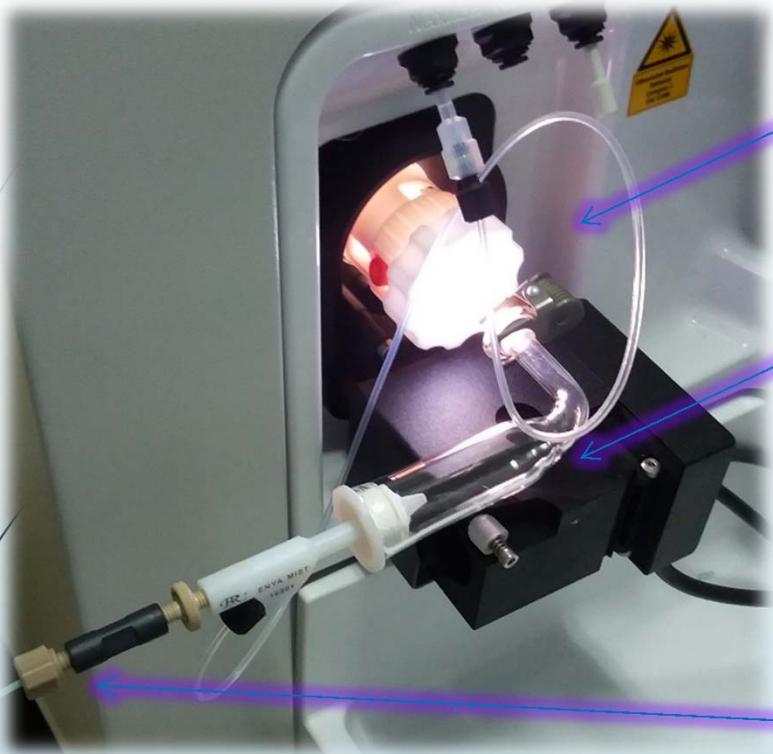
Mass balanced

Metallomic Challenge

multielement quantitative
fingerprint

(multi)element species
quantitative fingerprint

The first instrumental set-up



Thermo Scientific™
iCAP™ TQ ICP-MS

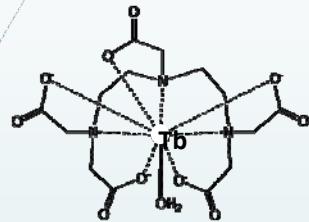
Total consumption
nebulizer/spray chamber

Syringe pump

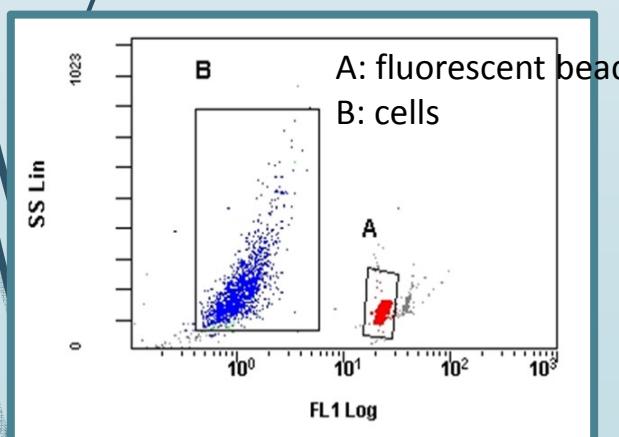


Sample Introduction Efficiency

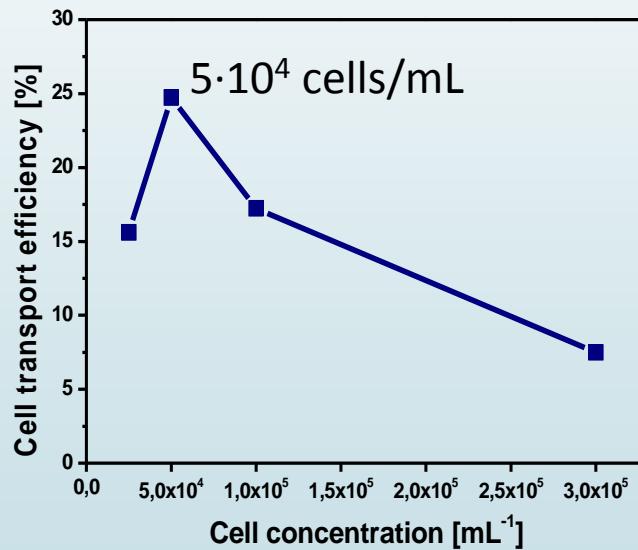
- Tb-DTPA as cell marker to detect cells:



- In combination with **flow cytometry**, to obtain the “real” number of cells in the sample:

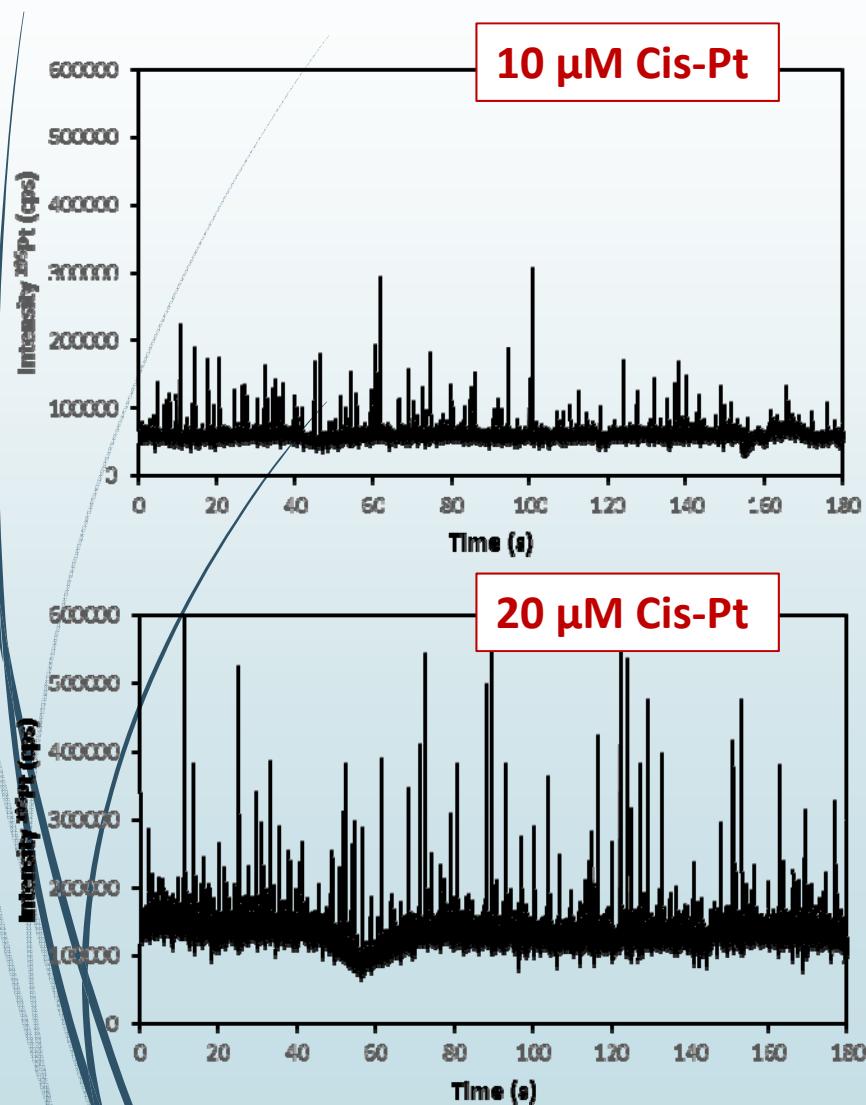


$$\text{Transport efficiency} = \frac{\text{Cells detected in ICP - MS}}{\text{Cells counted in Flow Cytometry}}$$



25% efficiency

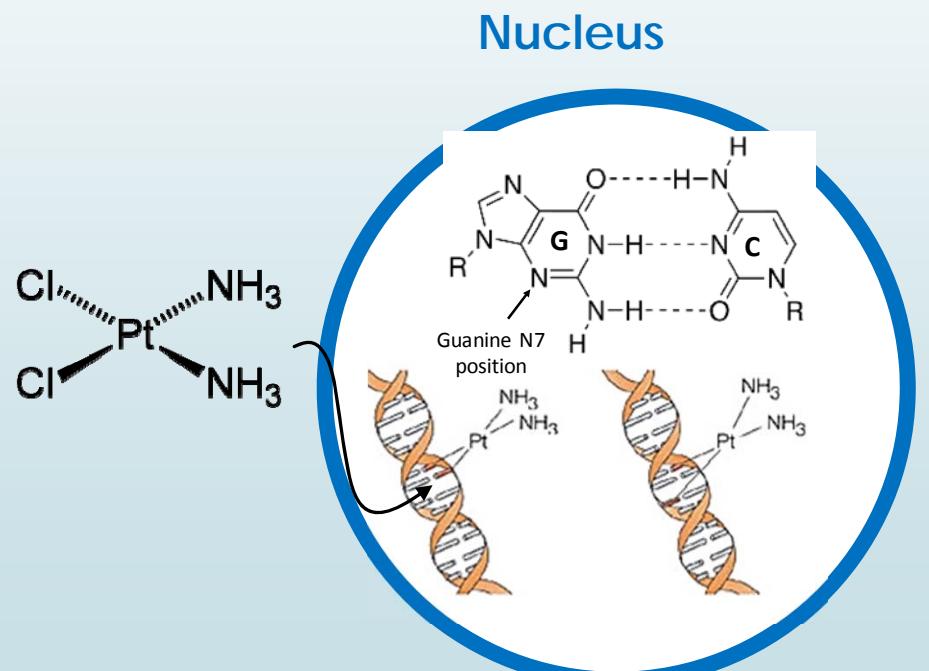
Cis-Pt in individual cells (A-2780)



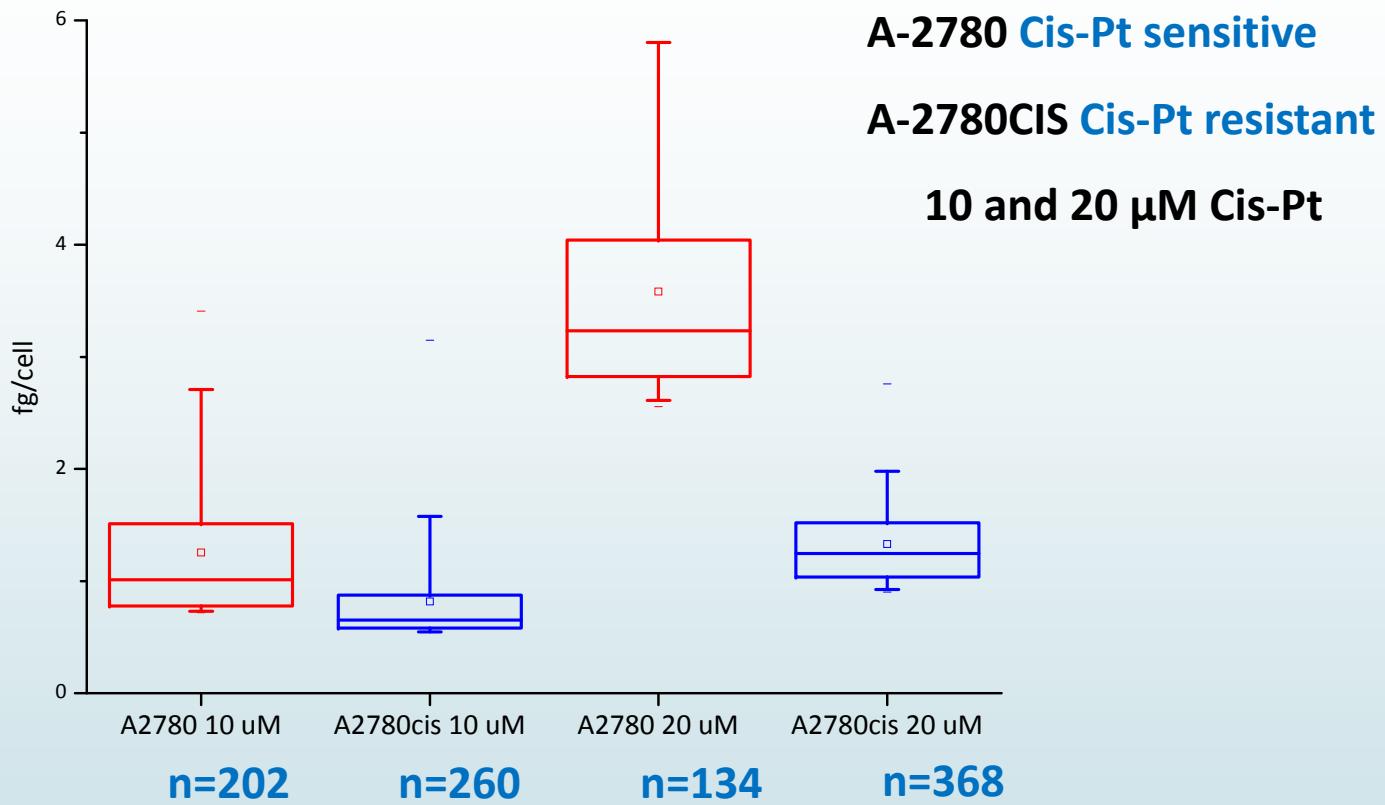
A-2780 Ovarian cancer cell line

5×10^5 cells per mL

Exposed to 10 and 20 µM Cis-Pt

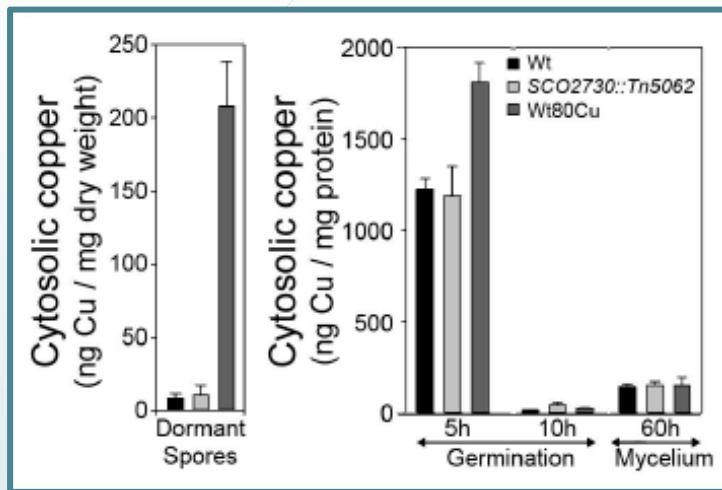


Cis-Pt uptake in individual cells



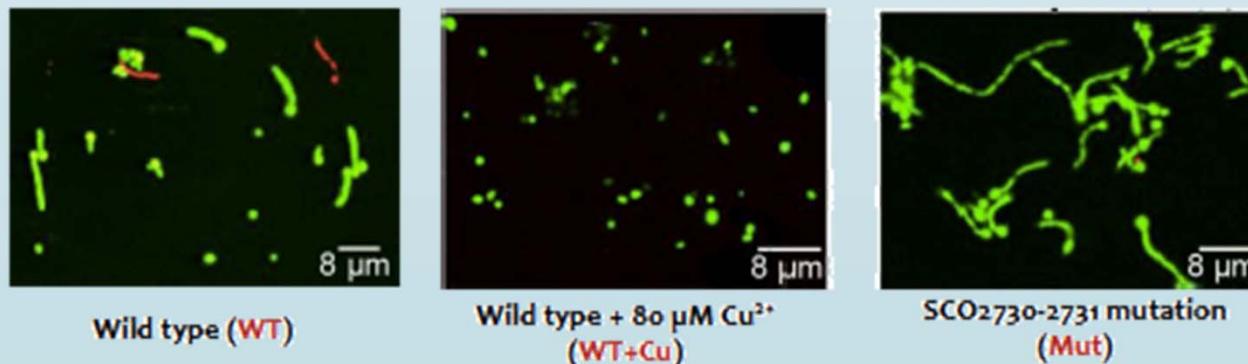
- ✓ **Significant uptake differences** have been found among cell lines with the proposed strategy (sensitive and resistant to cisplatin, respectively).
- ✓ **Higher individual cell** variation has been observed in the sensitive line.

Cu in “single spores” of Streptomyces



- ▶ Streptomyces is a large genus of actinobacteria, widely used for antibiotic production.
- ▶ Cu may be an unknown regulator of germination
- ▶ Collaboration: determination of cytosolic copper in Streptomyces with different mutation status in a gene coding a copper transporter.

Streptomyces coelicolor spore germination: Confocal microscope images

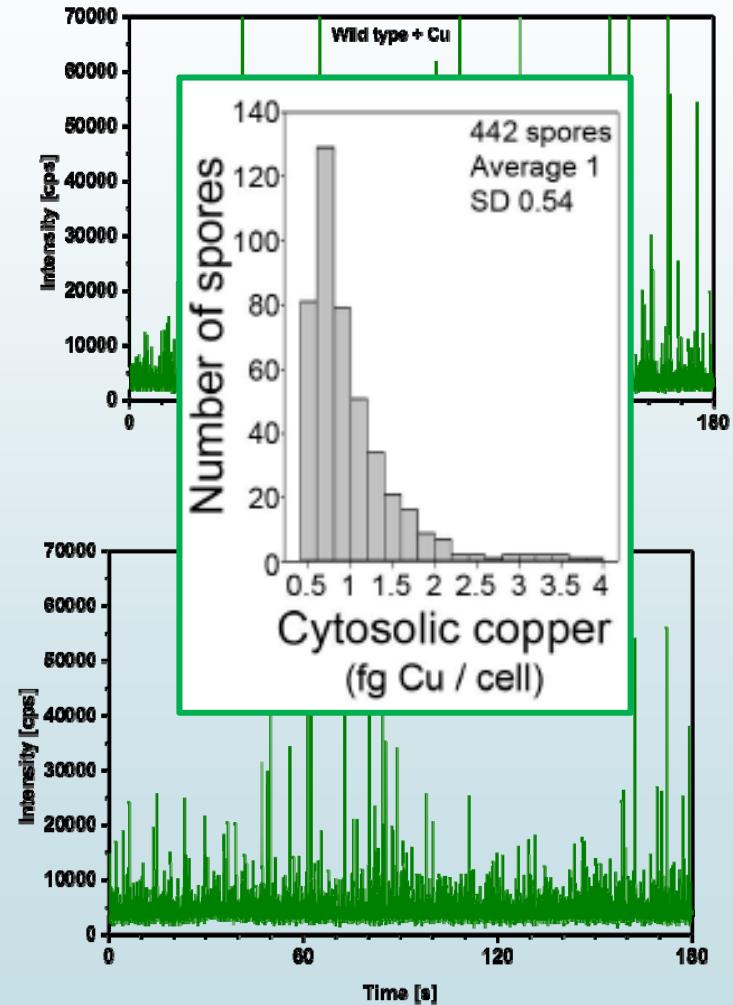
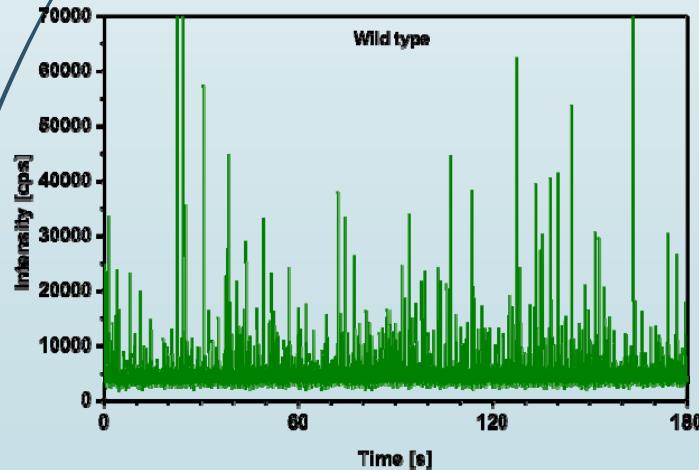


Cu in “single spores” of Streptomyces

SQ-He mode

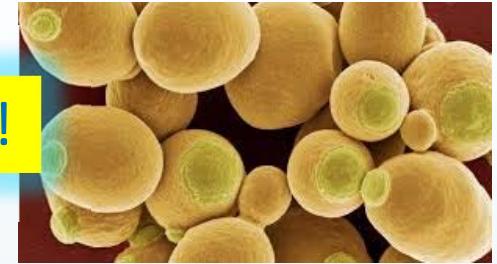
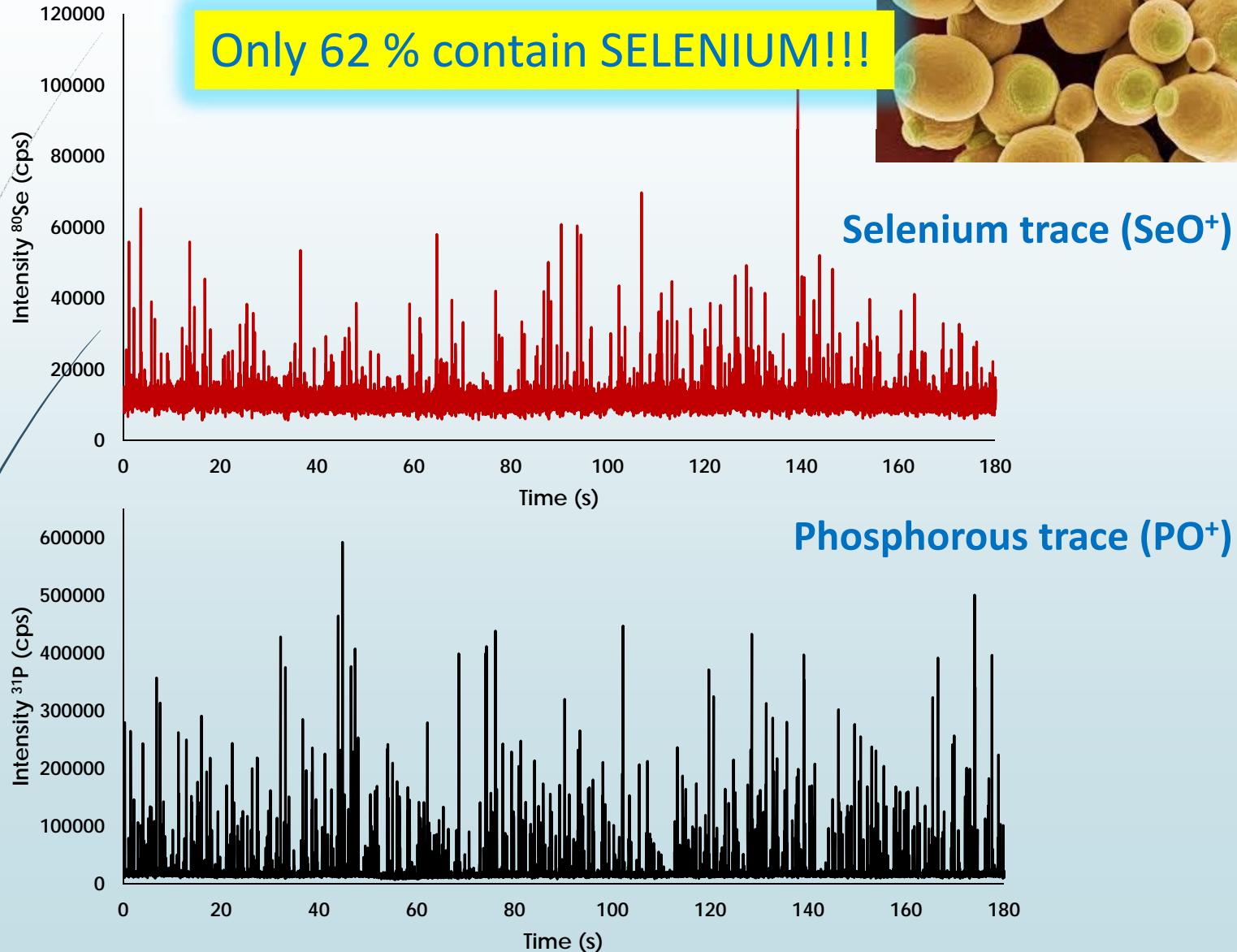
Streptomyces spores in different Cu metabolism status

- ✓ Different mutants are affected in a different way by Cu: can germination be controlled by Cu transporters and be “synchronized” in the spores?



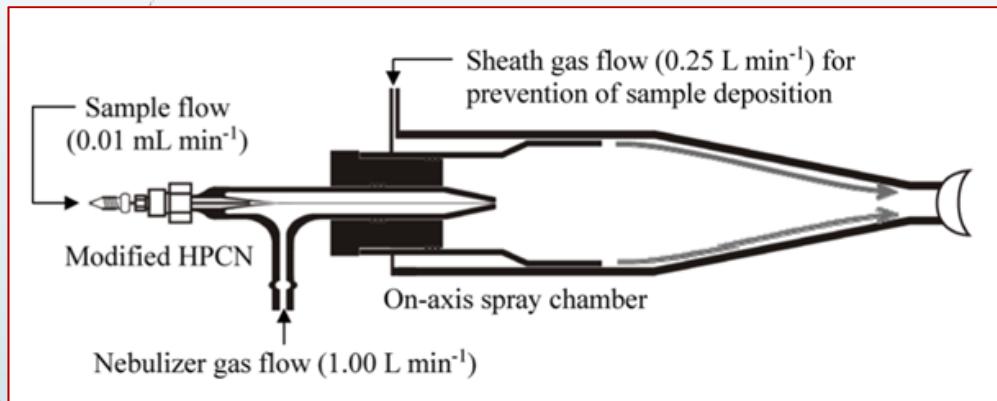
Selenium in individual yeast cells

Selenized yeast cells analysis (Exp-1)



New Sample Introduction System

- **High Efficiency Cell Introduction System (HECIS):** High Performance Concentric Nebulizer (HPCN) + low-volume spray chamber using a **sheath gas flow**.

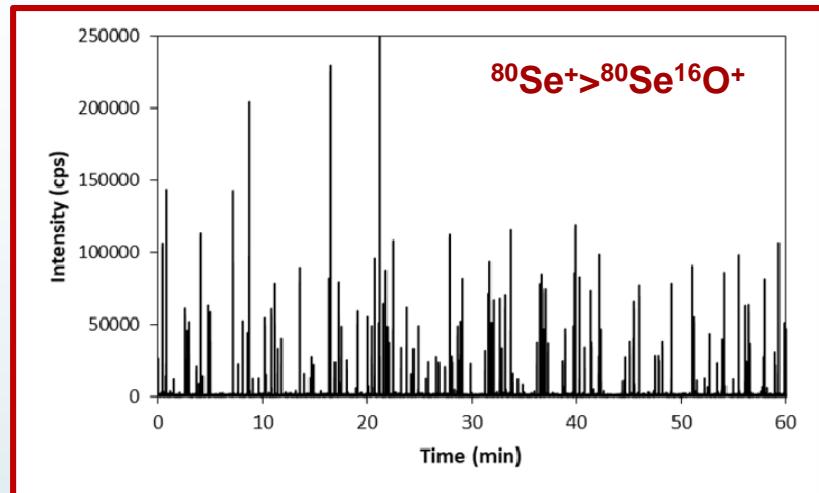
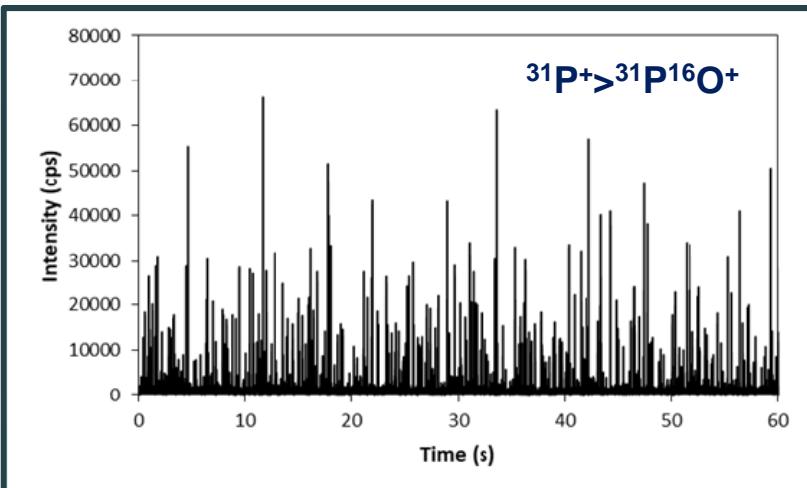


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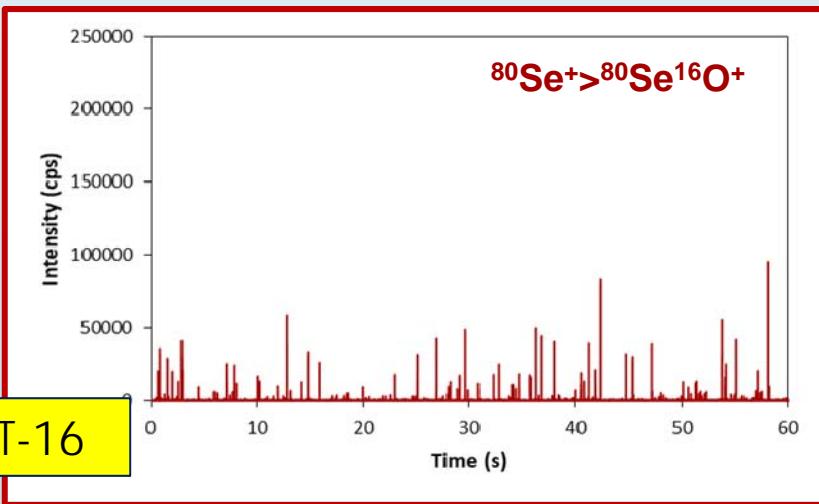
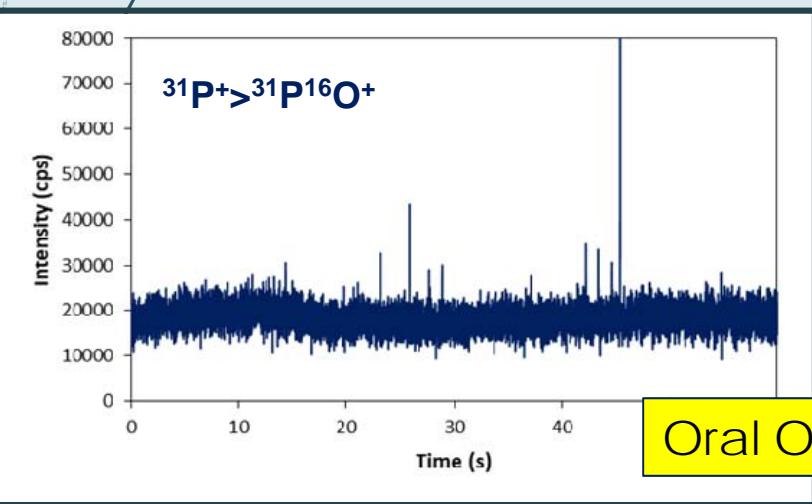
- Sample delivery by **MVX-7100 μL workstation** (Teledyne Cetac Technologies):
Introduces sample volumes of less than $5 \mu\text{L}$ @ flow rates $> 5 \mu\text{L min}^{-1}$.

Selenium NPs in yeast cells (NRC)

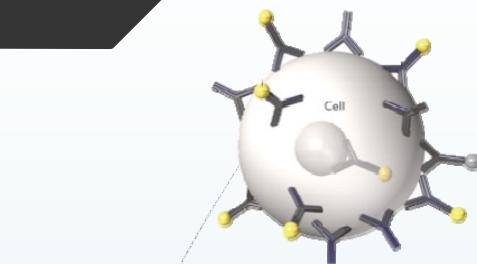
Before cell lysis



After cell lysis



State-of-the-art: Transferrin receptor 1 (TfR1)



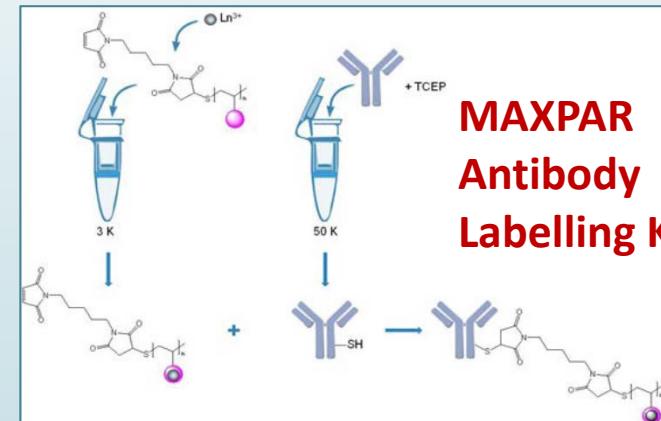
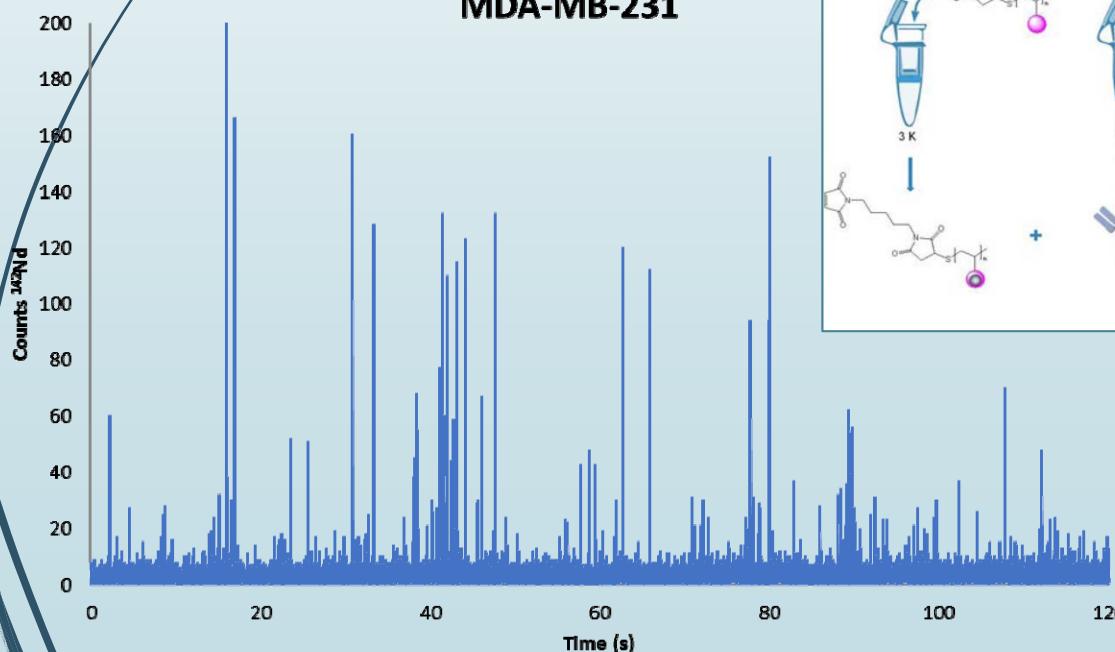
Nd-labelled AB

ICP-MS
(single cell)
HECIS

5 ms dwell time
 PO^+ and Nd

- MDA-MB-231 (highly invasive and metastatic)
- MCF-7 (non metastatic)

MDA-MB-231



**MAXPAR
Antibody
Labelling Kit**

Poster WP-48

SUMMARY

- **Single Particle-ICP-MS:** the combined use of single particle and specific chromatography for NPs (data not shown) with ICP-MS permits the analysis of a greater variety of NPs of different size and composition. More tools, more fun!!!
- **Single cell-ICP-MS:** a new multidisciplinary world of applications is still to be discovered once the sample introduction is totally optimized.



MVX-7100 μL workstation