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Universitat Autònoma de Barcelona

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Cerium (IV) Oxide Nanoparticles: Exploring an alternative to enzymatic labelling for Point-of-Care Diagnostics

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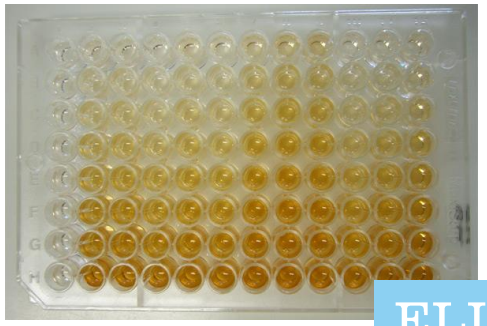
CSIC Consejo Superior de Investigaciones Científicas
CNM Centro Nacional de Microelectrónica IMB
SPEED Self Powered Engineered Devices

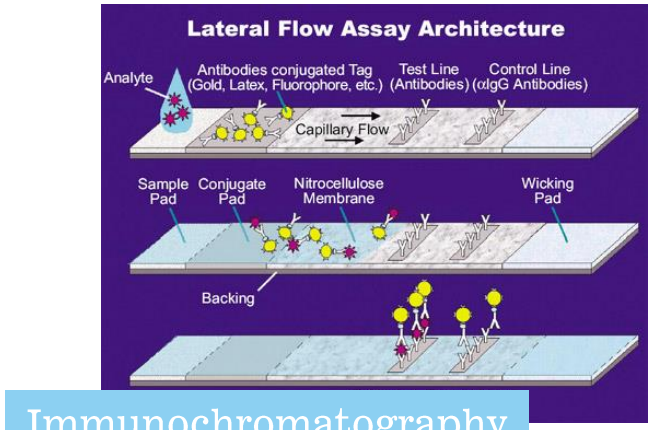
INDEX

A NEW ERA OF CHANGE IN DIAGNOSTICS

But what is essentially a LFT and why is there a need to improve them?



ELISA



Immunochromatography

Electrochemical LFT

Operon, S.A.

Modern days require modern solutions. It is not possible to rely exclusively in colorimetric and analogic signal detection. We must digitalize our medical records!

World Health Organization

ASSURED (**A**ffordable, **S**ensitive, **S**pecific, **U**ser friendly, **R**apid and **R**obust, **E**quipment free and **D**eliverable to end users)

↓

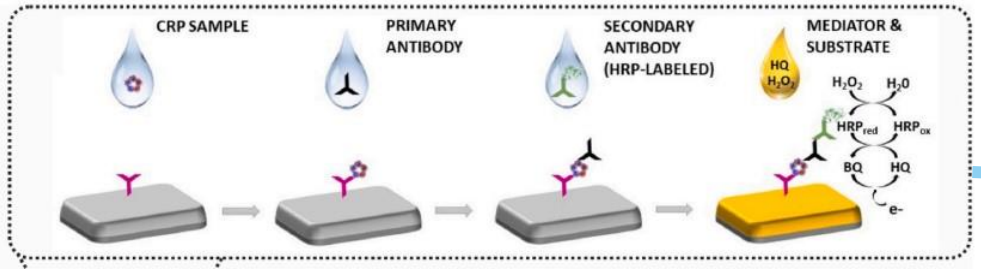
RE (**R**eal-time connectivity & **E**ase of specimen collection/**E**nvironmental friendliness) - **ASSURED**

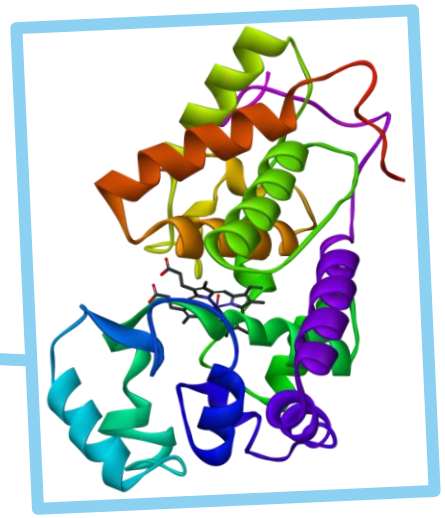
What is this all about?

THE NEED FOR DIFFERENT IMMUNO-LABELS

The origin of the problem:

Fig. 2 from Galyamin, D. et al., 2023





$$Mg \longrightarrow Mg^{2+} + 2\,e^- \quad \text{anode}$$

$$BQ + 2\,e^- + 2\,H^+ \longrightarrow HQ \quad \text{cathode}$$

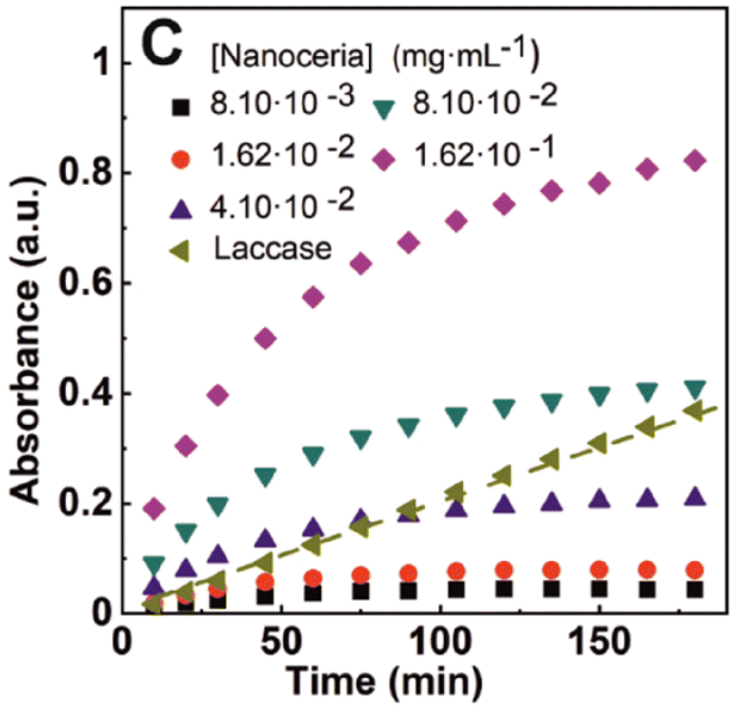
$$BQ + 2\,H^+ + Mg \longrightarrow HQ + Mg^{2+} \quad \text{global}$$

But what is wrong with using this enzyme? Primarily we have that…

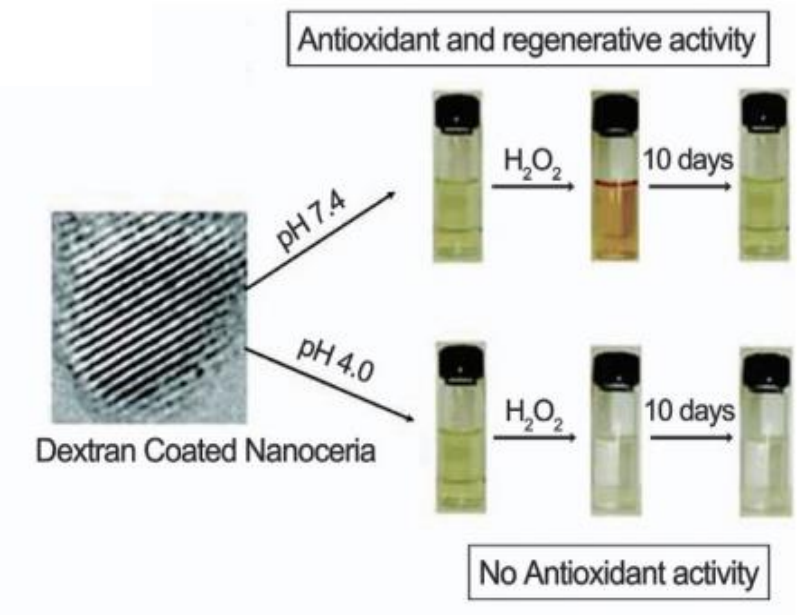
- The use of Horseradish Peroxidase (HRP) relies heavily in batch-driven error (It's an enzyme, it's alive!)
- Hydrogen peroxide is a volatile reactant
- It is not scalable to under-developed countries for mass production & involves many signaling processes!

So, which are the alternatives?

Dmitry Galyamin et al. *Nanoscale*, 2022



Perez, J. M., et al., *Small*, 2008



Nanocerias work as non-reversible enzymes in acidic medium

+ it's a nanoparticle (electrochemical properties are enhanced)

Therefore, are ‘cerias’ a valid proposal for immunoassay labelling?

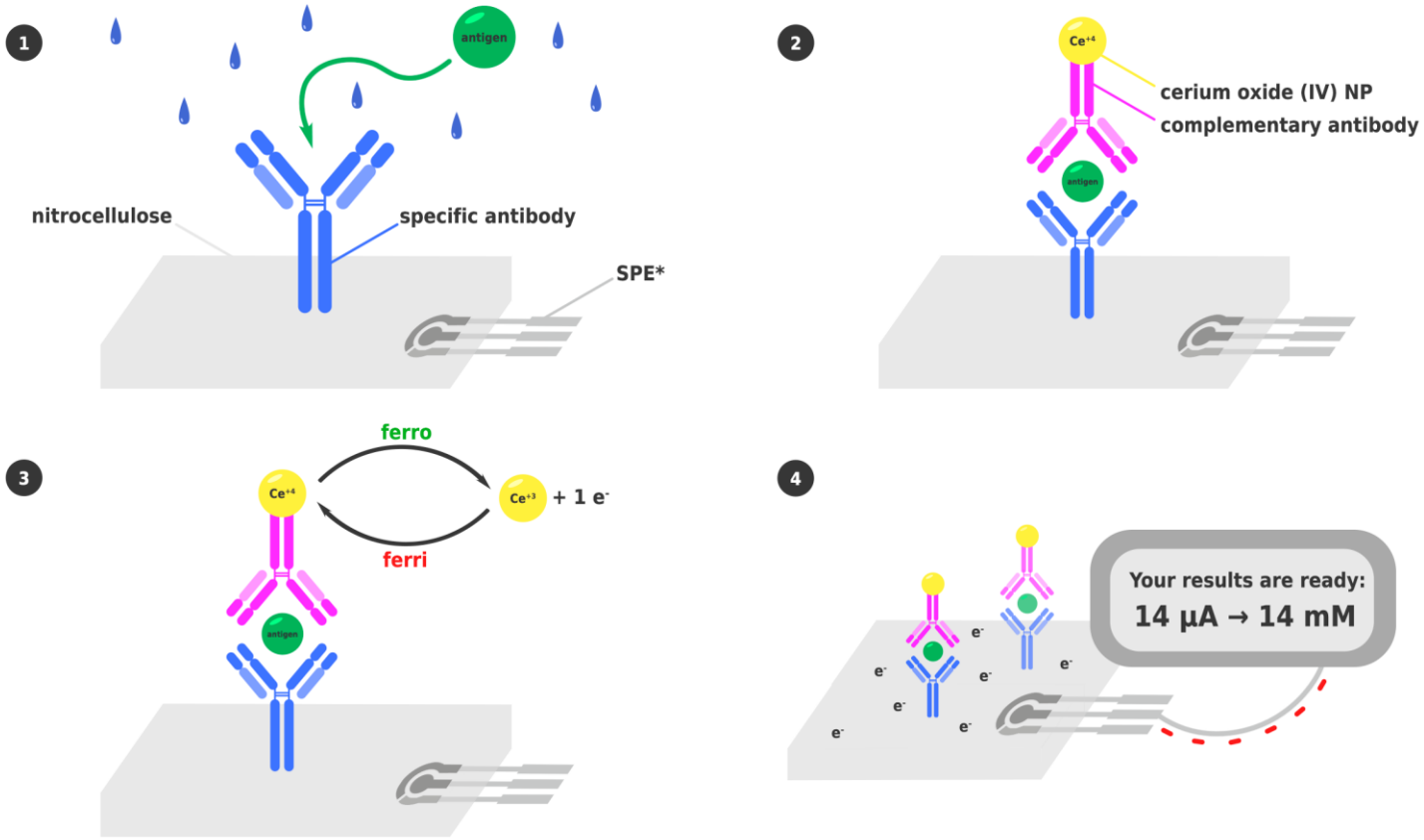
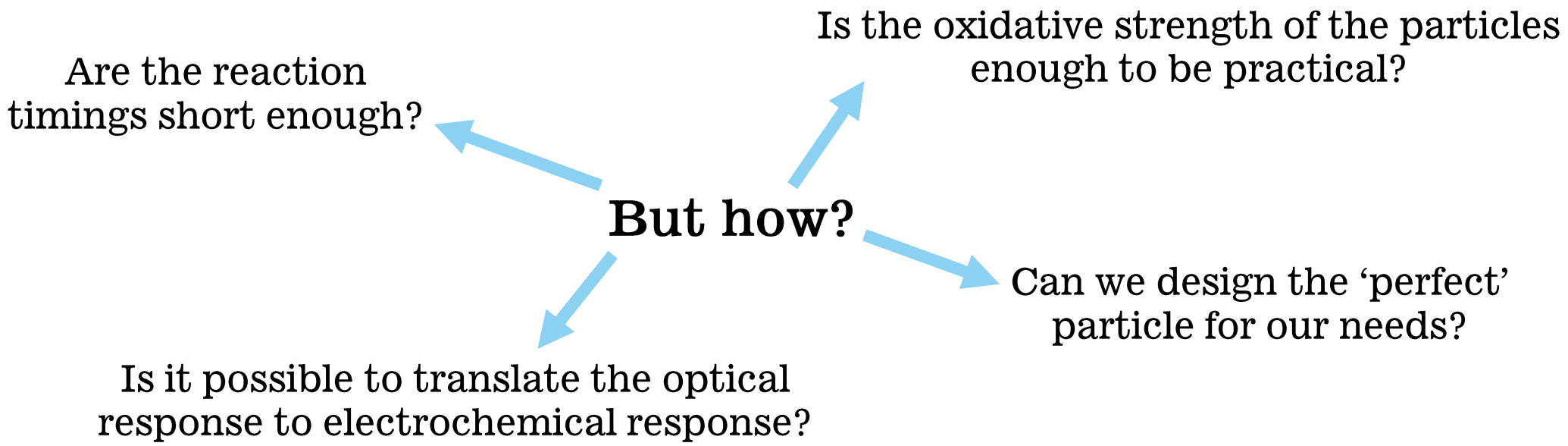


Illustration suited by the author

Let’s find out!

OBJECTIVES

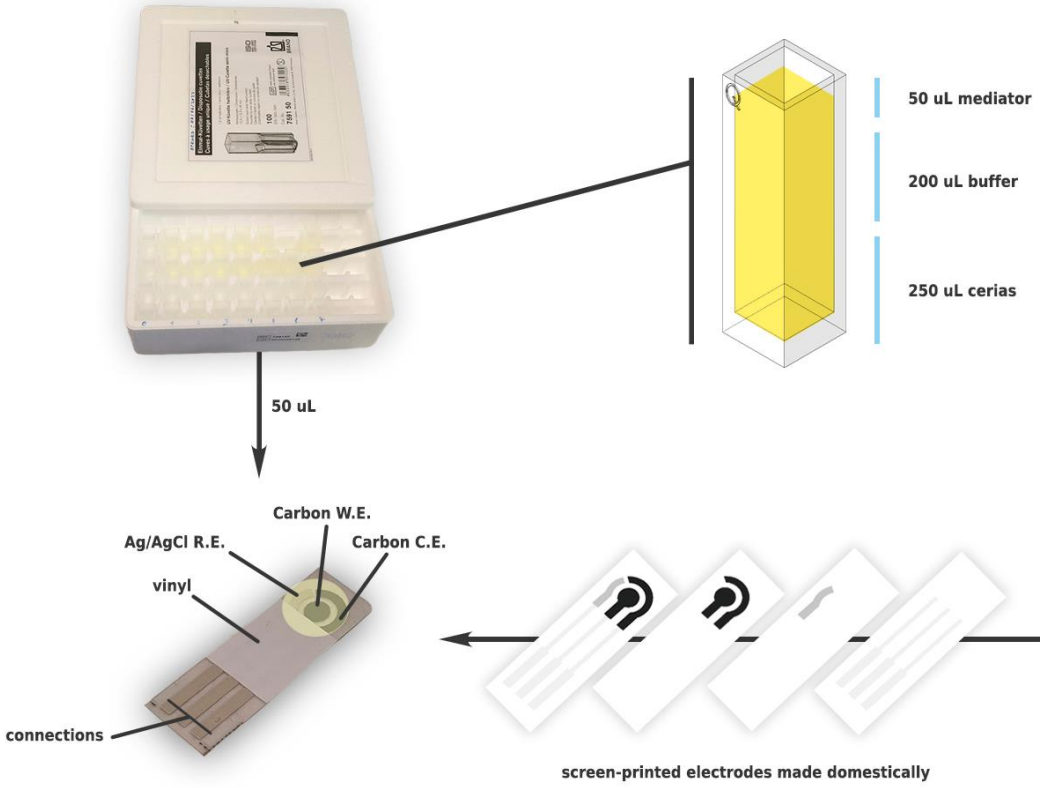
General purpose: Demonstrate the viability of cerium (IV) oxide nanoparticles as immunoassay labels



HANDS ON PRACTICE

First, let's look to both our layout and the chemicals involved:

$$CeO_2 + 4\,H^+ + [Fe(CN)_6]^{4-} \xrightarrow{pH\,4} Ce^{3+} + 2\,H_2O + [Fe(CN)_6]^{3-}$$



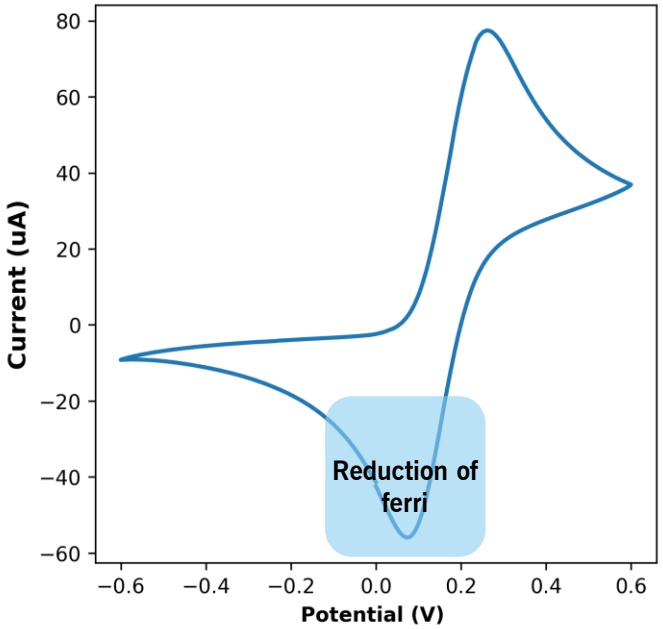
Commercial cerias of 4 nm, %5 wt.

In home cerias of 3 & 7 nm, stabilized with citrate

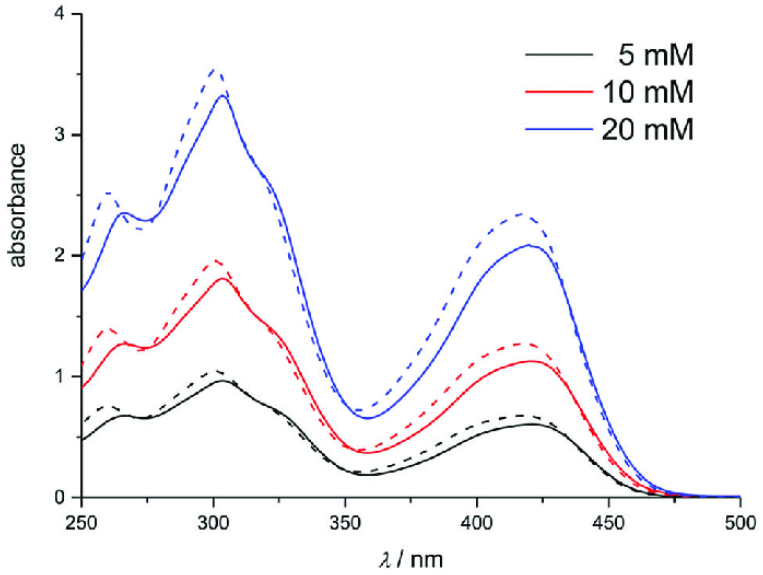
Image taken from pngwing

A short look to our mediator: **potassium hexacyanoferrate (II)/(III)**

Cyclic voltammetry of a ferri/ferro sample immersed in 0.2 M citric/citrate pH 4 buffer



UV/Vis spectra of potassium ferrocyanide (Gäbler, N. et al., *Phys. Chem.*, 2017)

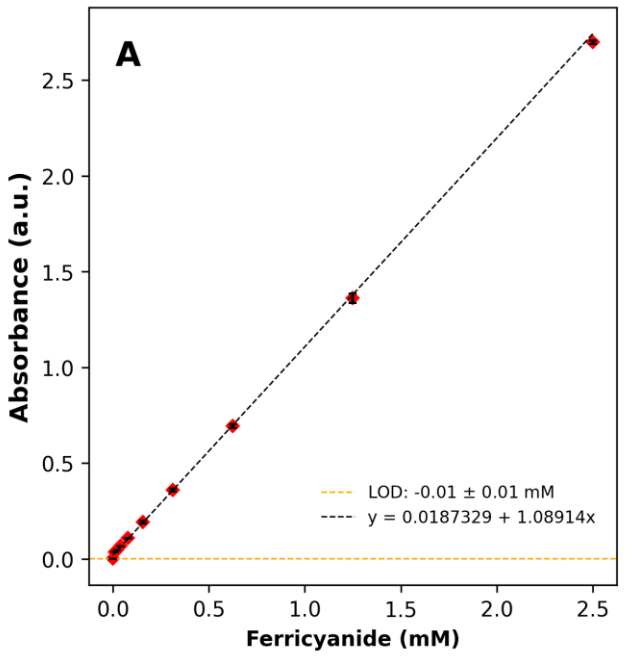


Simultaneous chrono (redox, -0.2V) & absorbance measuring using the oxidized mediator peak at **420 nm**

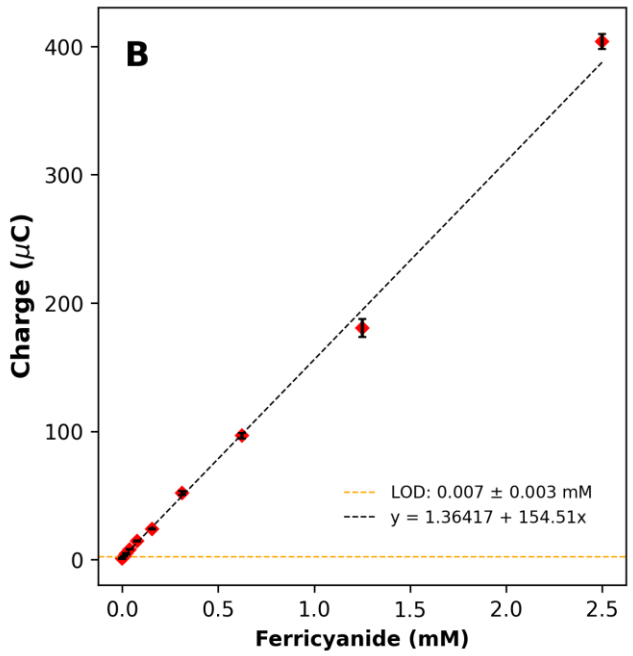
Now it is possible to calibrate the system. How though?

- The limit-of-detection is unreachable for optical studies, meanwhile at electrochemical studies the values are minimal

Optical study



Chronoamperometry



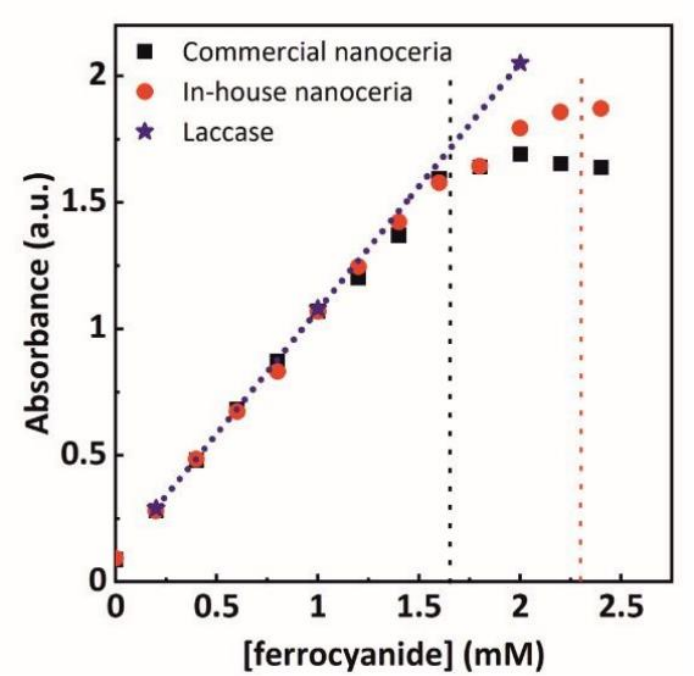
n = 3, except for the blank, which has n = 20.

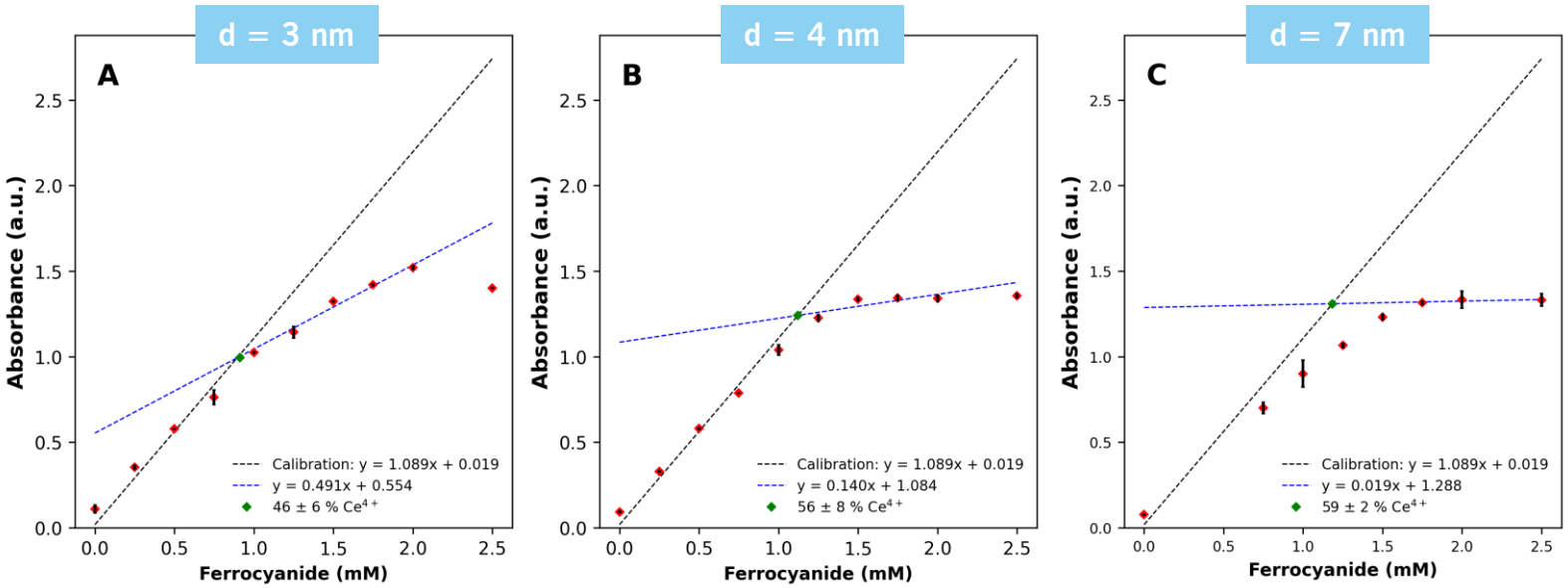
7 ± 3 µM

Other factor to consider: the oxidative strength of cerias

Mols of e- in comparison to mols of compound

Dmitry Galyamin et al. *Nanoscale*, 2022





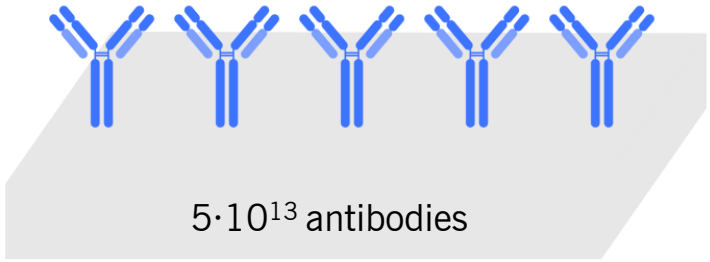
46 ± 6 Ce^{4+} **56 ± 8 Ce^{4+}** **59 ± 2 Ce^{4+}**

Having done the calibration, and knowing the oxidative strength of the particles…

Shouldn't we need a reference to follow?

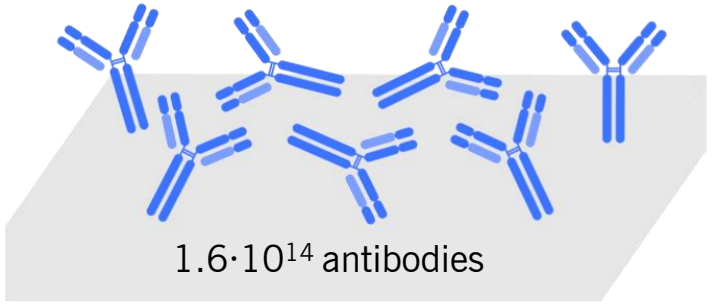
At **maximum values**, we can disguise two possible scenarios:

Size delimitation



$5 \cdot 10^{13}$ antibodies

Weight



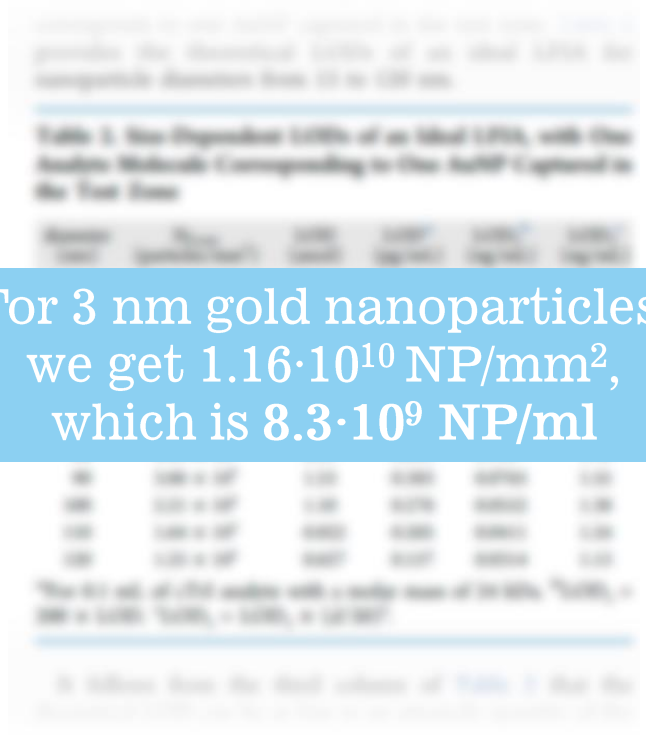
$1.6 \cdot 10^{14}$ antibodies

1 ab = 150 kDa and are 10 nm long (approx.), nitrocellulose strip absorbs from 80 to 100 μg per cm^2

For comparison, 6.3 mM of CeO_2 (3 nm) equals to $1.06 \cdot 10^{16}$ NP/ml

Illustrations suited by the author

At **minimum values**, we need to compare the particles' limit-of-detection (LOD) with other clinical examples:



For 3 nm gold nanoparticles, we get $1.16 \cdot 10^{10}$ NP/mm^2, which is **$8.3 \cdot 10^9$ NP/ml**

N. Khlebtsov, Boris et al. *ACS Applied Nano Materials* 2019

Procalcitonin, has clinical kits with 0.05 ng/ml to 0.01 ng/ml LOD, which range from **$2.3 \cdot 10^9$ to $4.8 \cdot 10^8$ NP/ml**

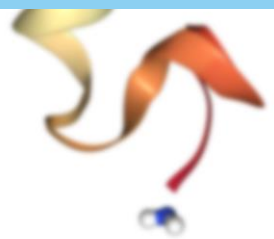


Image granted by Sino Biological

Once we have set the limits…

It is possible to draw a relation between the NP/ml concentration against the particle's size, for a certain limit-of-detection.

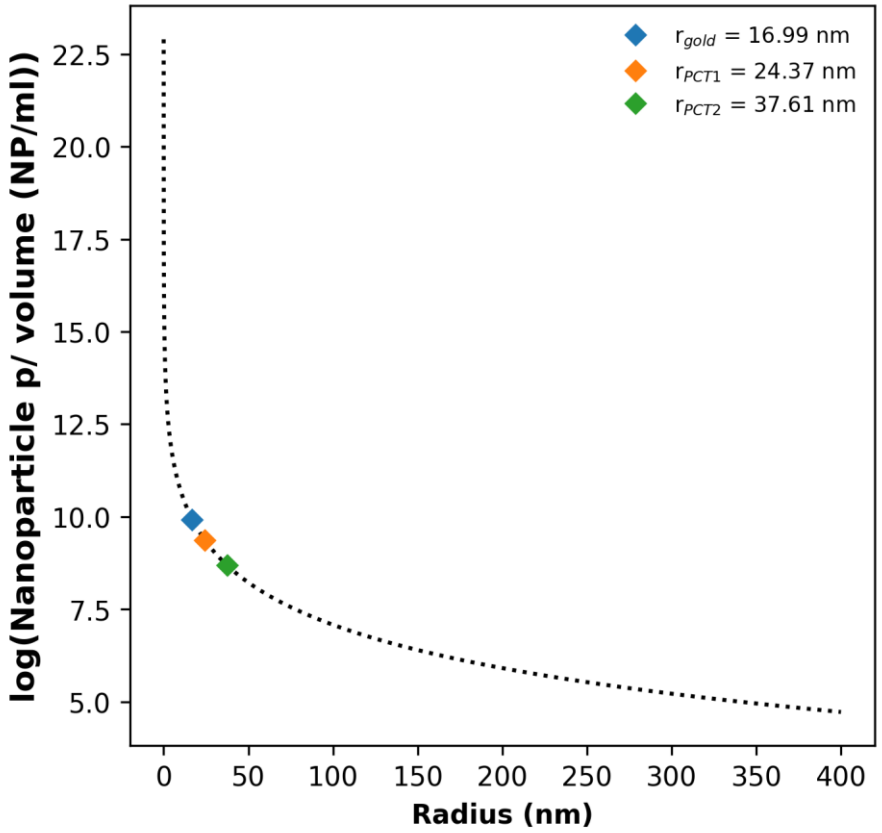
7 ± 3 µM

$$\text{NP concentration} = \text{regression} \cdot \frac{1}{\text{particle volume}} \cdot \frac{1}{\text{density}} \cdot \text{mol. weight} \cdot \text{LOD}$$

$$y = 2.6522 \cdot r + 47.33$$

The radius is reached by:

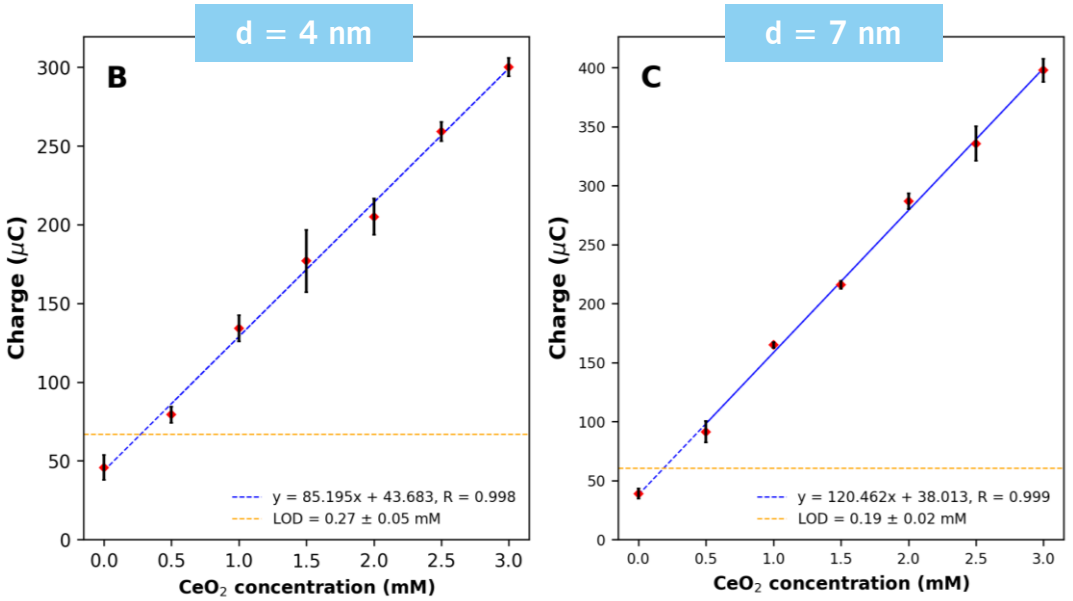
$$r^3 \cdot \frac{2.6522 \cdot r + 47.33}{100} = \left[\frac{1}{4/3\pi(1 \cdot 10^{-9})^3} \cdot \frac{1}{\text{density}} \cdot \text{weight} \cdot \text{LOD} \cdot \frac{1}{\text{conc.}}\right]^3$$



A theoretical **37.61 nm** radius particle is suggested as perfect to maximize the limit-of-detection in the first place

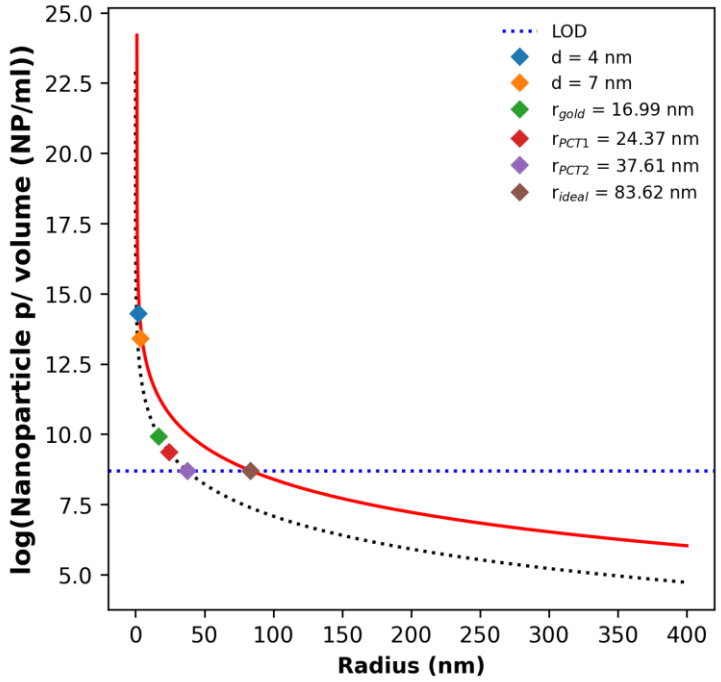
But is this theoretical particle size realistic at all?

We evaluated each particle size we had within their respective limit-of-detection



0.27 ± 0.05 mM **0.19 ± 0.02 mM**

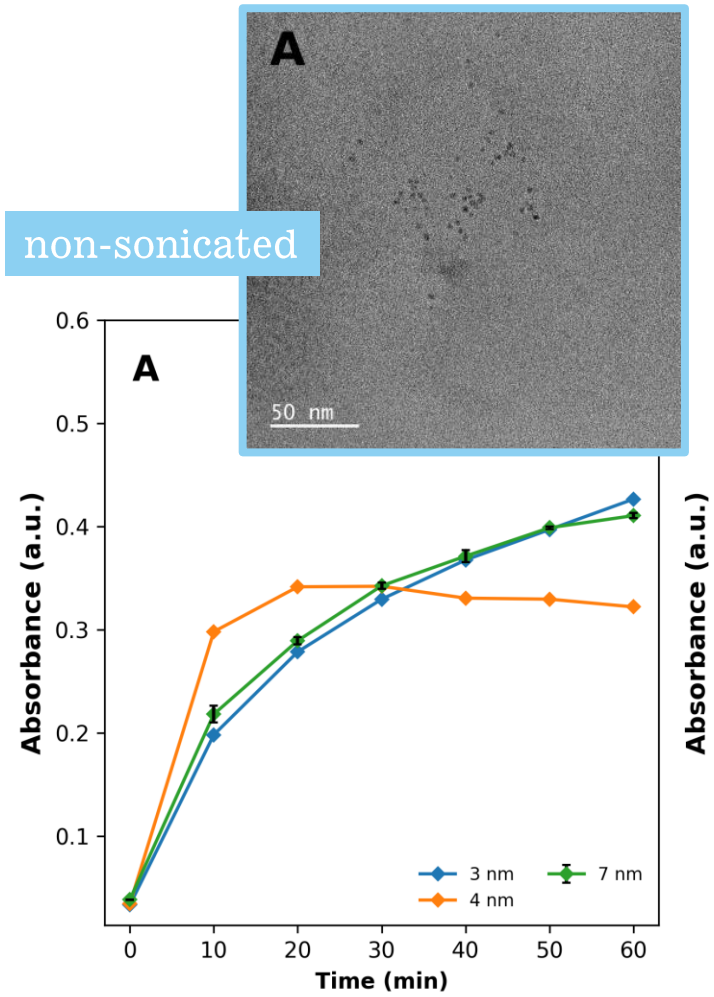
Considering the oxidative strength, values of **154 ± 4 μM** are achieved for 4 nm cerias and **111 ± 13 μM** for 7 nm cerias



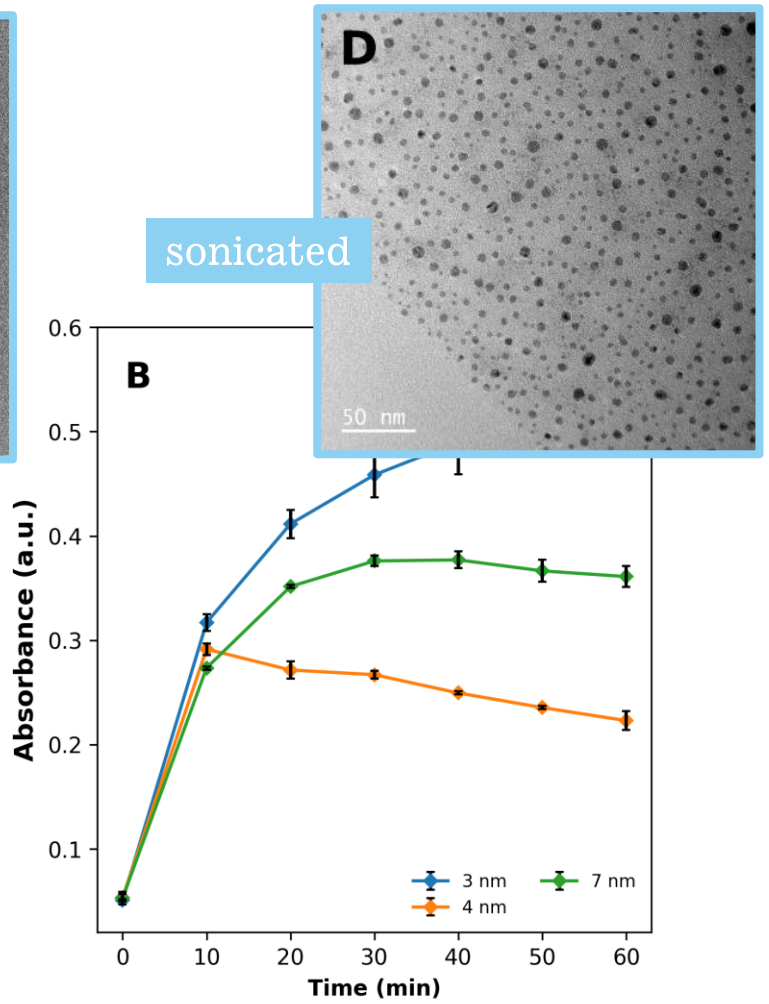
If the real data is implemented, we can suggest a much realistic radius: **83.62 nm (0.01 ng/ml PCT)**

Lastly, what about the operational timings?

non-sonicated



sonicated



Commercial cerias (4 nm) resemble the same activity, meanwhile in-home cerias are enhanced once sonicated.

Both reveal times of saturation between **10 to 20 min**, which is an acceptable standard (remember RATs take 15 min to show a colorimetric signal!)

WHAT HAVE WE ACCOMPLISHED?

Did we accomplish what we were looking for?

Are the reaction timings short enough?

10-20 min seems more than reasonable for a point-of-care device

Is the oxidative strength of the particles enough to be practical?

Higher size ceria have shown to maximize this parameter

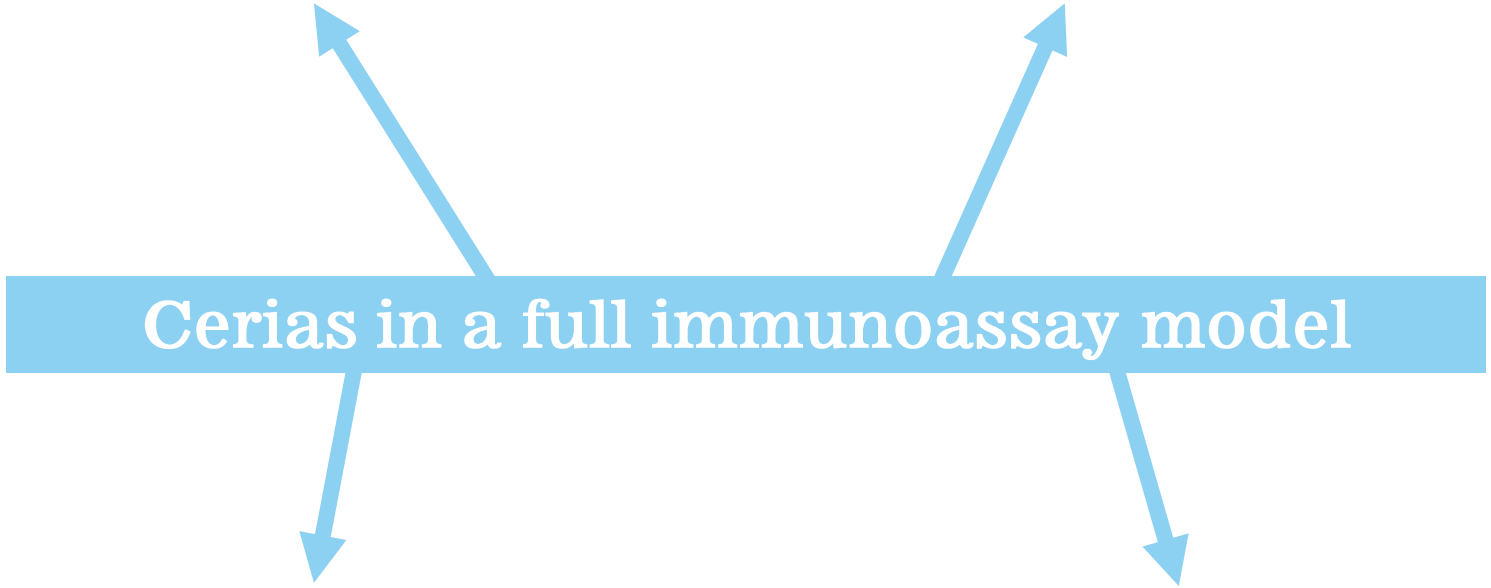
Is it possible to translate the optical response to electrochemical response?

Yes, both experimental tools follow the same reaction with different sensitivities

Can we design the 'perfect' particle for our needs?

Ideal radius is of 83.62 nm. Does this go against 'nano' properties? It's more than enough for LFT LOD parameters

- Design cerias' conjugation strategies that compensate their lack of dimensionality
- Study if its possible to do an immunoassay with cerias



- Examine the same layout with varying conditions: pH, saline solutions…
- Synthetize the 'ideal' particles and corroborate their limits of detections

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Calculations are shown as:

LOD **sample volume**

$$1.16 \cdot 10^{10} \frac{\text{NP}}{\text{mm}^2} \cdot 0.005\text{mm}^2 \cdot \frac{1}{7} \frac{1}{\mu\text{L}} 1000 \frac{\mu\text{L}}{\text{mL}} = 8.286 \cdot 10^9 \frac{\text{particles}}{\text{ml}}$$

nitro's area

LOD **molecular weight** **Avogadro's number**

$$0.05 \frac{\text{ng}}{\text{ml}} \cdot 10^{-9} \frac{\text{g}}{\text{ng}} \cdot \frac{1}{13 \cdot 10^3} \frac{\text{mol}}{\text{g}} \cdot 6.022 \cdot 10^{23} \frac{\text{particles}}{\text{mol}} = 2.316 \cdot 10^9 \frac{\text{particles}}{\text{ml}}$$

Calculations are shown as:

density

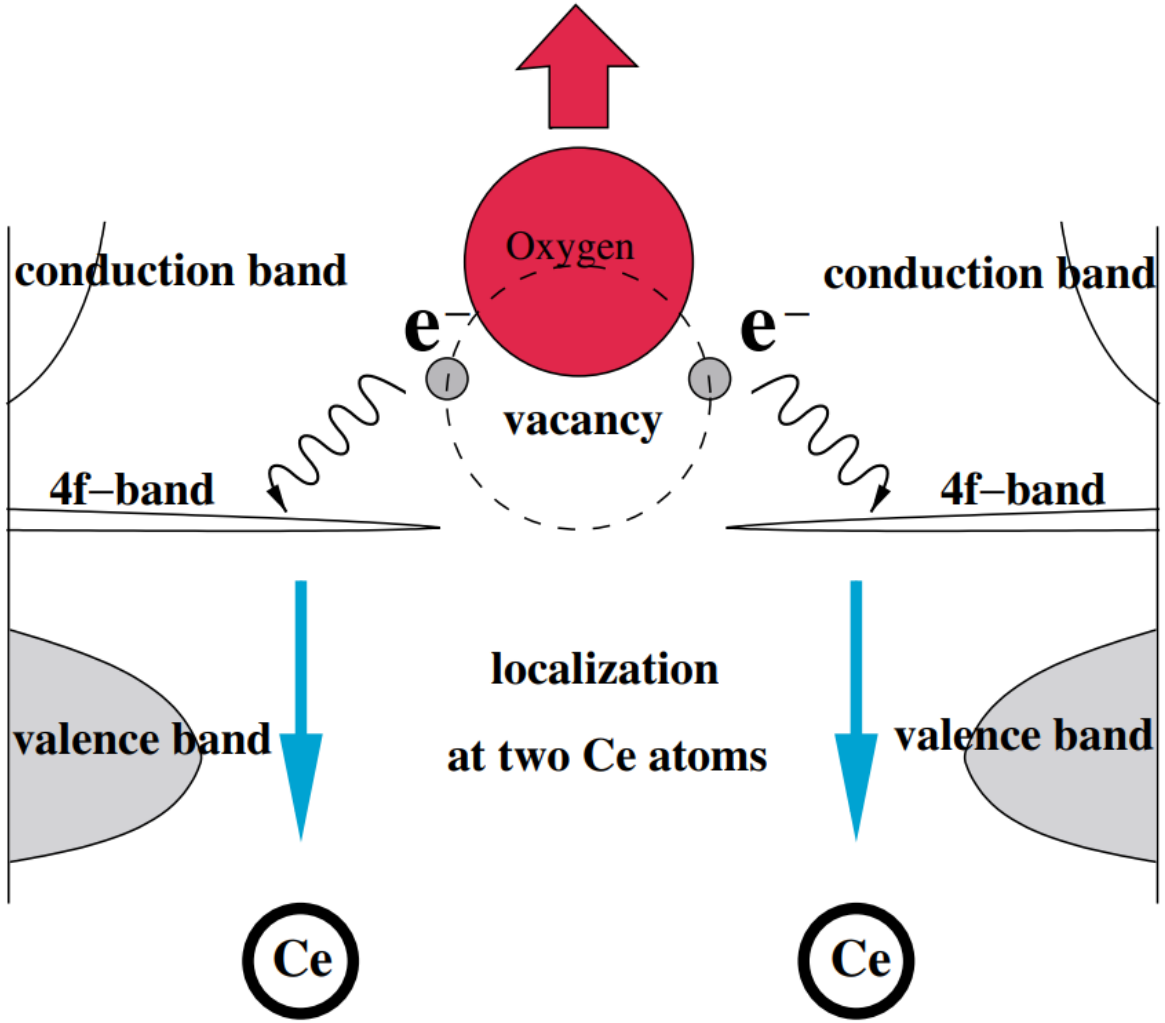
$$[\mathrm{M}] = \mathrm{Particles}\frac{\mathrm{NP}}{\mathrm{mL}} \cdot 1000\frac{\mathrm{mL}}{\mathrm{L}} \cdot (4/3) \cdot \pi \cdot (r)^3 \frac{\mathrm{m}^3}{\mathrm{NP}} \cdot 7220\frac{\mathrm{kg}}{\mathrm{m}^3} \cdot \frac{1}{172.12 \cdot 10^{-3}}\frac{\mathrm{mol}}{\mathrm{kg}}$$

volume's ratio per particle

mol. weight $^{-1}$

$$[\mathrm{Particles}] = \frac{1}{7220 \cdot 10^3}\frac{\mathrm{m}^3}{\mathrm{g}} \cdot \frac{1}{(4/3) \cdot \pi \cdot (r)^3}\frac{\mathrm{NP}}{\mathrm{m}^3} \cdot [\mathrm{Molarity}]$$

Skorodumova, N.V., *Phys. Rev. Lett.*, 2002.



Compression of superficial cerium localizes cerium *p* electrons, making **improbable for them** to be shared with oxygen atoms

A little bit of context:

Lateral flow tests: from their creation to our days have drawn big attention as all-in-one diagnostic tools!

1984

2020

Nevertheless, their fashion has kept the same way for more than 30 years.
It's time to change that!

Images granted by Freepik