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Europium-Doped Ceria Nanocrystals as Nanozyme Fluorescent Probes for Biosensing

Ali Othman

Department of Chemistry & Biomolecular Science

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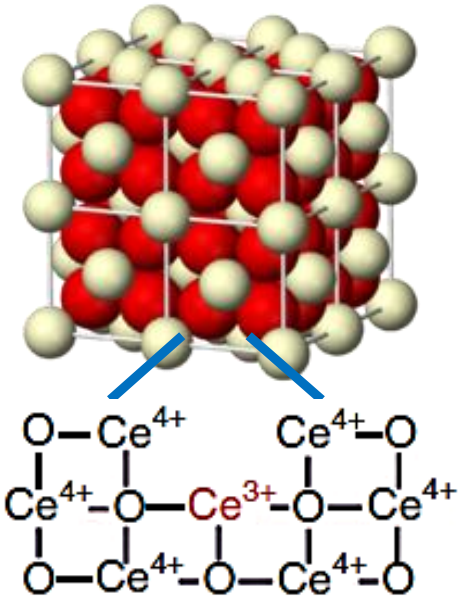
Unique properties of CeO_2 NPs

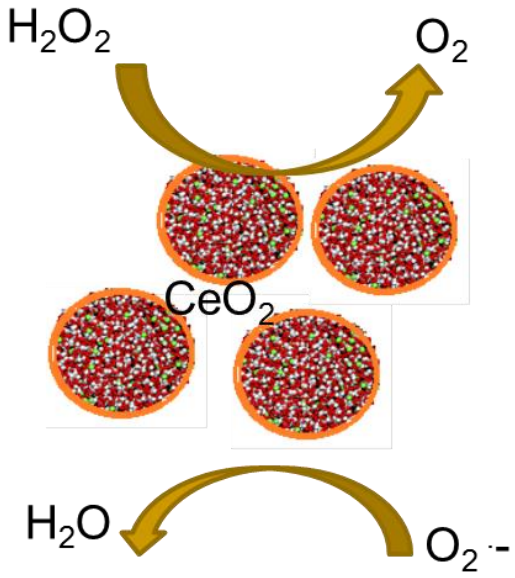
CeO_2 (ceria) NPs, nanoceria

Dual oxidation state



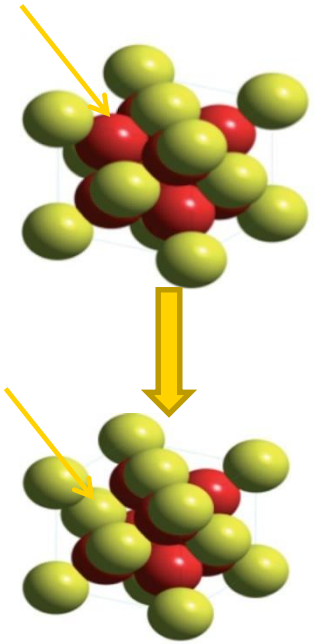
Inter-changeable oxidation states Ce^{3+}/Ce^{4+}
Surface reactivity for ox/red reactions

Recyclable ROS-scavenging activity



Rich surface functionality
Inorganic antioxidant

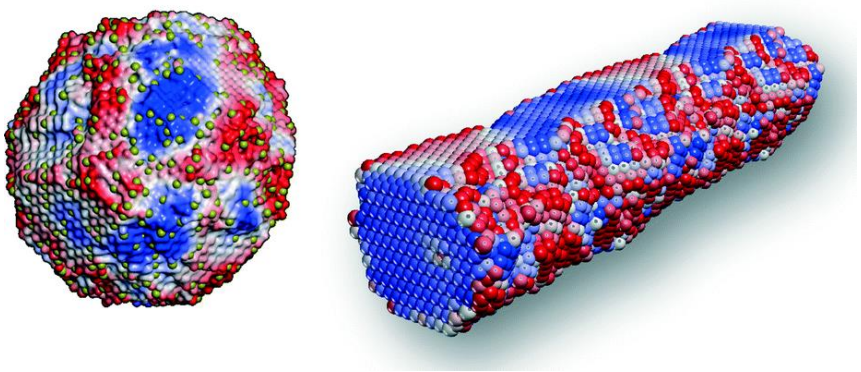
Oxygen vacancy



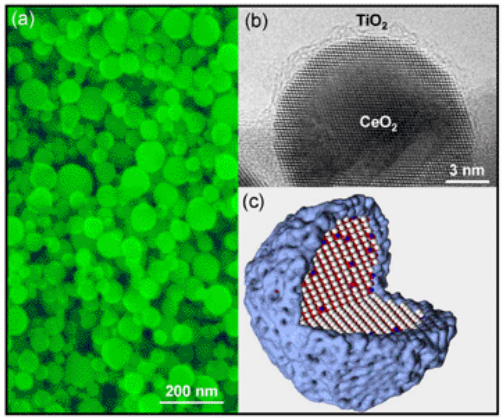
Mobile oxygen under reducing/oxidizing environments
Oxygen release/buffering capacity

- Kwon et. al., *ACS Nano*, **2016**, *10* (2), 2860–2870
- Nelson et. al., *Antioxidants,* **2016**, *5*(2), 15

Unique surface reactivity

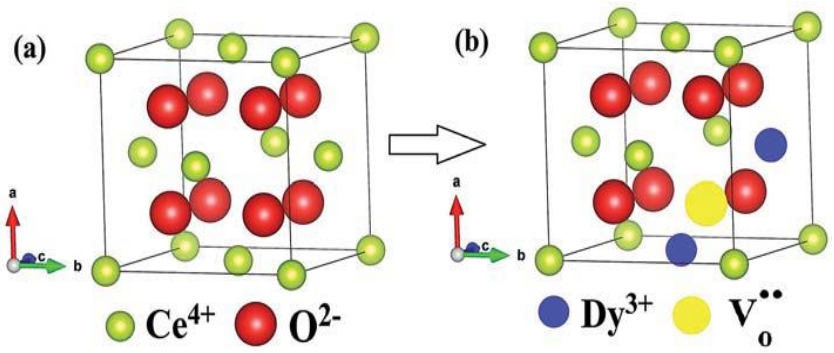


Reactivity hot spots



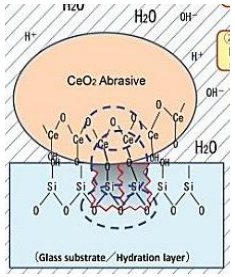
Enhanced properties by doping/surface coatings:

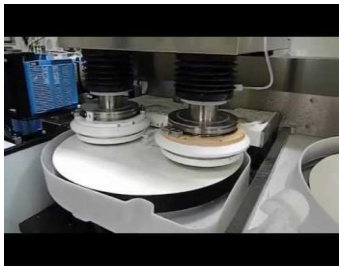
- *Catalysis Pt, Ti*
- *Fluorescence, Eu*
- *Mechanical – coating*
- *Bio-functionalization*



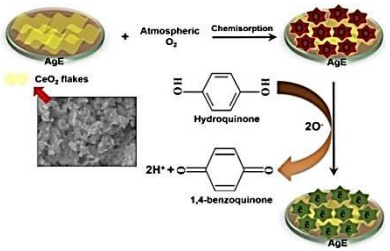
- Sayle et. al., *Nanoscale,* **2013**, *5,* 6063–6073
- Feng et. al., *Science,* **2006**, *312, 1504-1508*

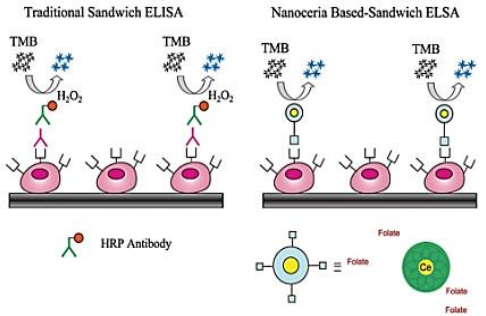
Emerging applications



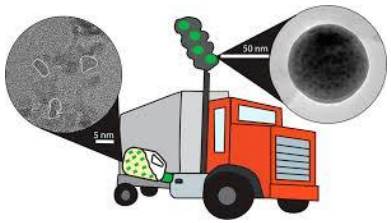


Semiconductors industry





Sensing

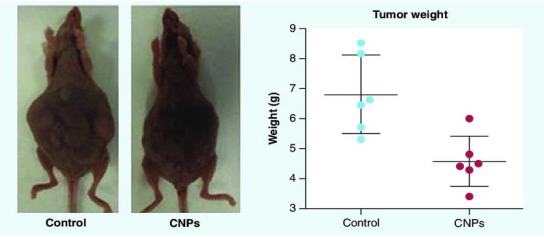


Diesel fuel-borne catalyst (FBC)

Industrial

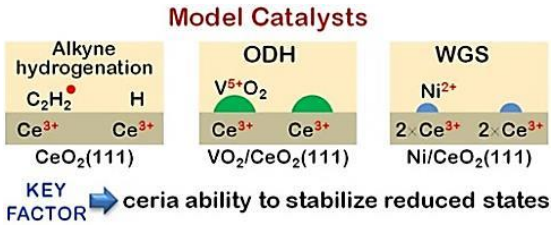
Biomedical

CeO_2-based Materials

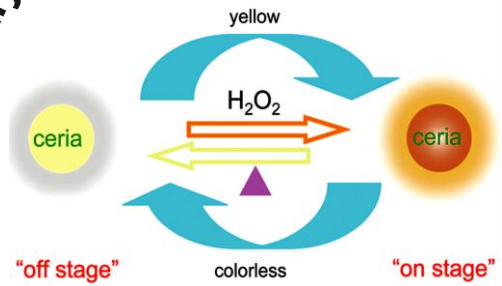


Catalysis

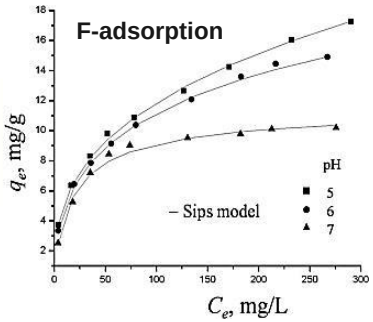
Bioanalytical



Environmental

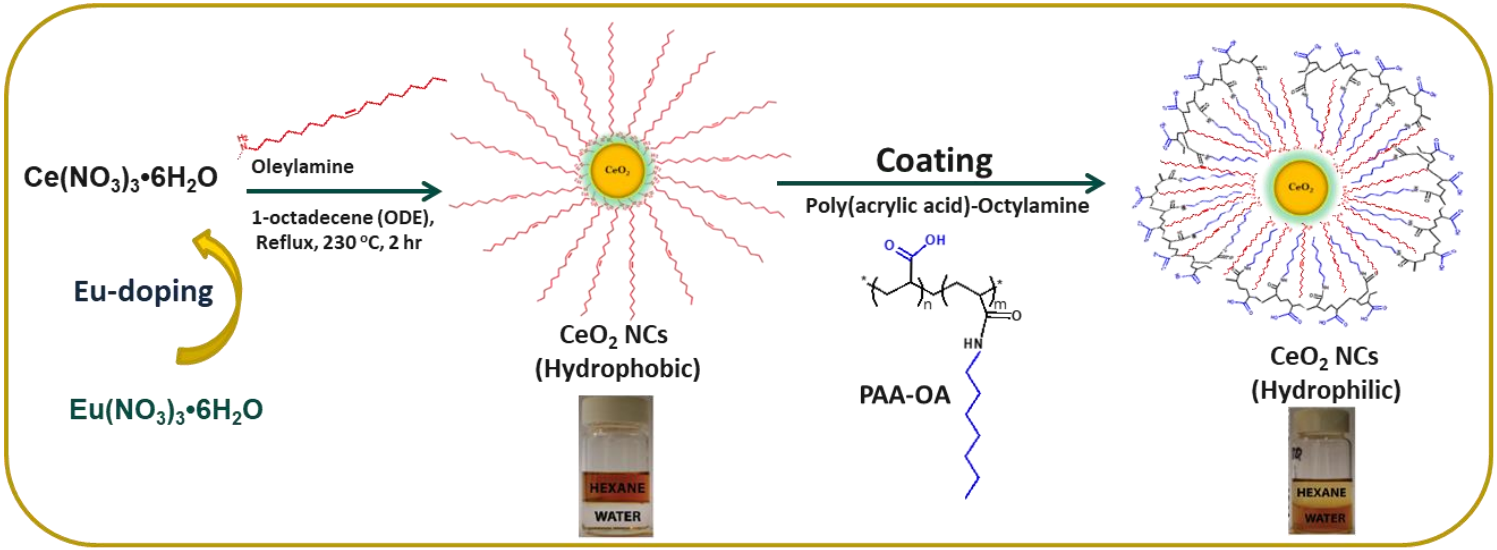


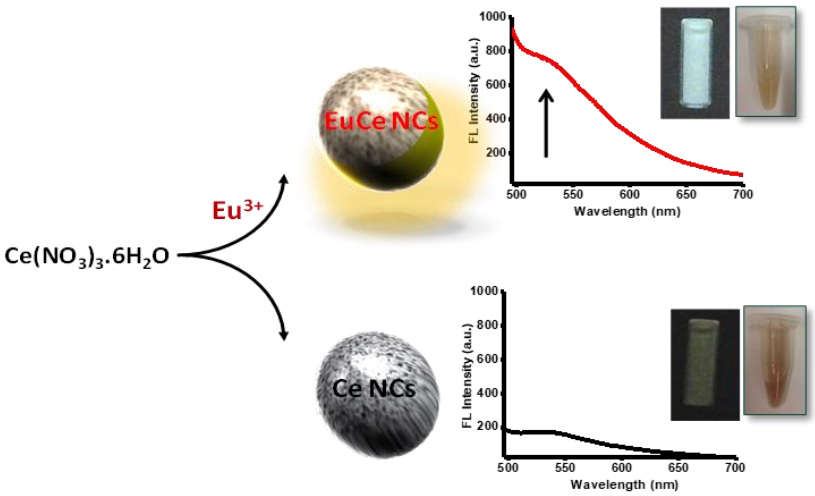
Ce^{4+} – Bone char

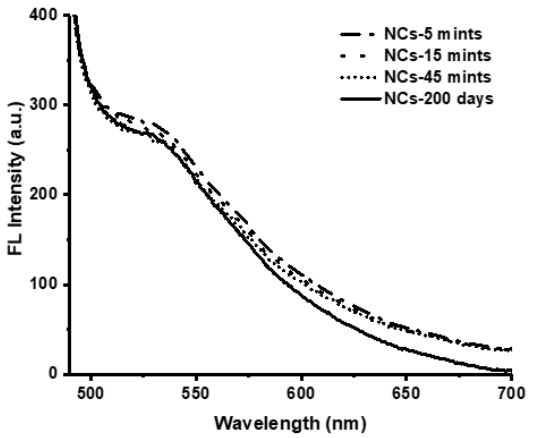


Synthesis of the Eu-doped fluorescence probe

To synthesize and characterize a novel and well-dispersed europium-doped ceria nanocrystals (EuCe NCs) with self-integrated catalytic and fluorescence sensing functions



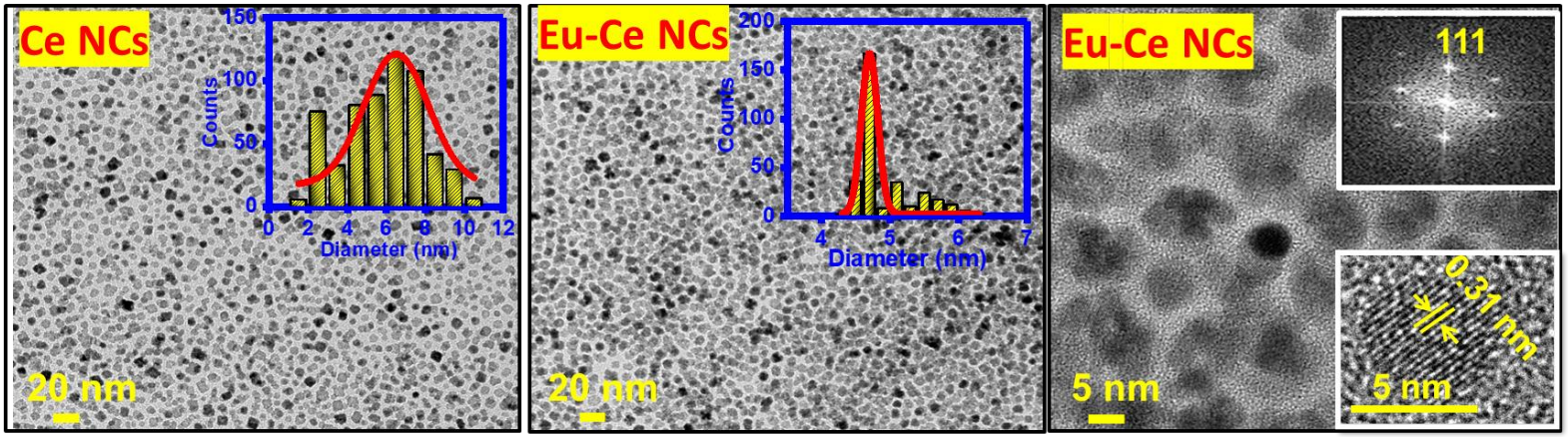




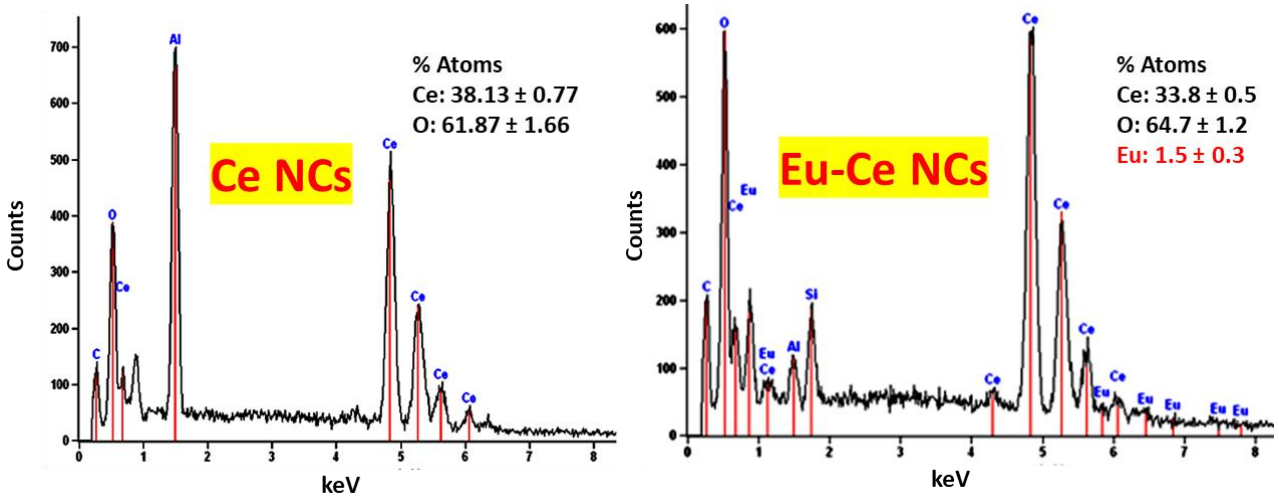
- Othman et. al., *ACS Appl. Nano Mater.*, **2018**, 1, 10, 5722–5735

The NCs are stable (in PBS, pH 7.4) over several months

Morphological characterization



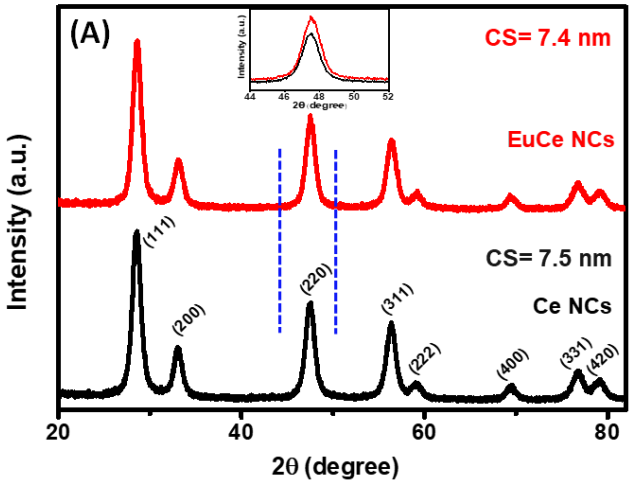
- A highly uniform nanocrystalline (NCs) with average size distribution of 6.5 ± 2 nm.
- Introducing Eu^{3+} into the ceria host resulted in uniform spherical shape NCs with a slightly smaller average size distribution of 4.7 ± 0.1 nm as compared to Ce NCs.
- A diffraction pattern of fcc crystals in the (111) planes.



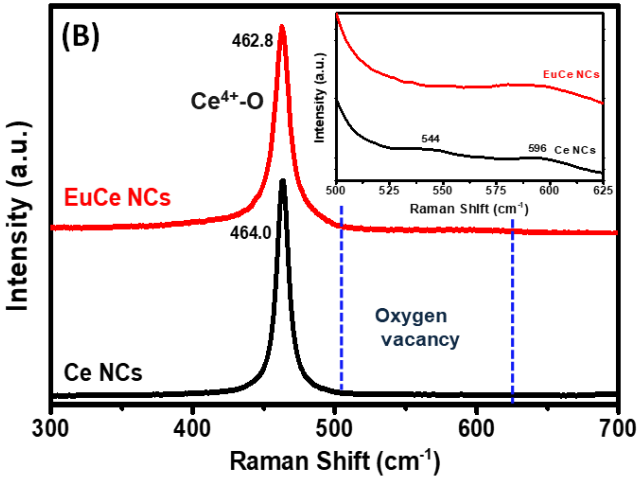
- EDS analysis confirms the presence of Eu atoms in the Eu-Ce NCs.

Structural characterization

Powder X-ray diffraction (PXRD)

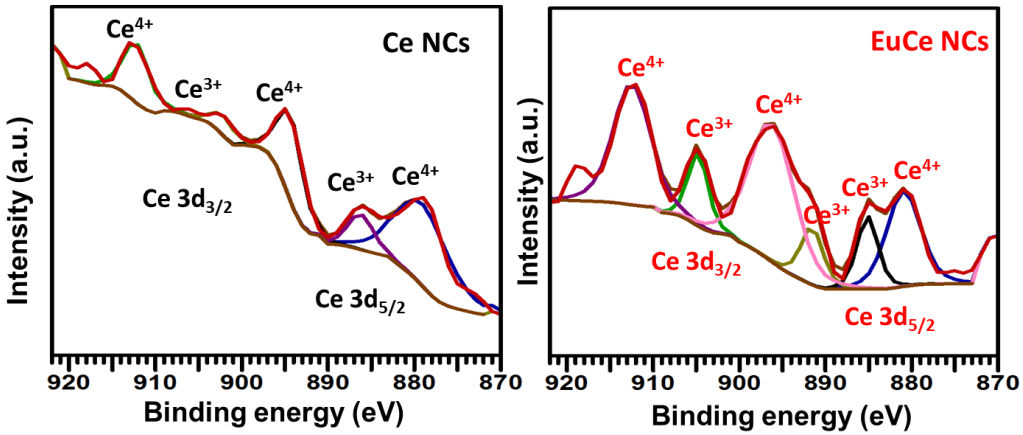


Raman spectroscopy



- Diffraction peaks reveal formation of a face centered cubic (fcc) structure. After incorporation of Eu^{3+} in the ceria lattice the intensity of the diffraction peaks is enhanced.
- Raman spectroscopy analysis demonstrates changes in the vibrational structure caused by doping with Eu^{3+}.

XPS Analysis

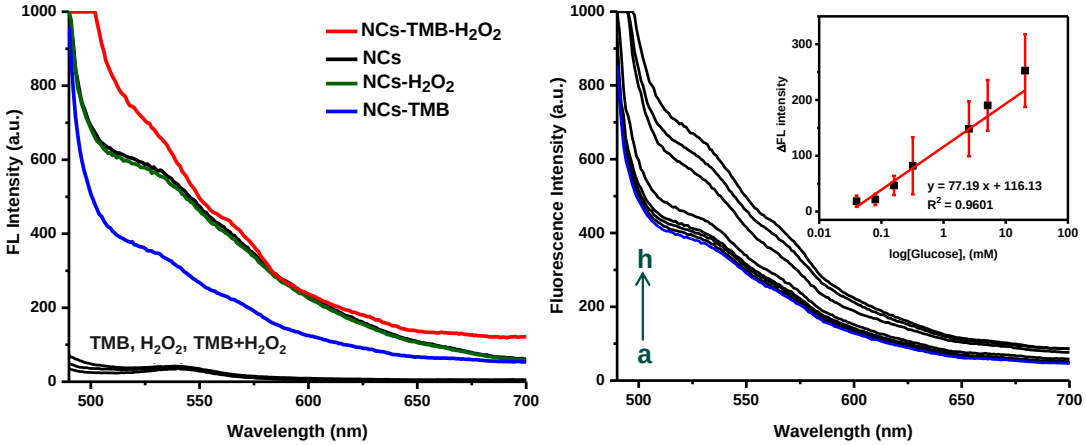


$[Ce^{3+}]$ = Ce NCs: 18.9% EuCe NCs: 29.3%

- All peaks changed significantly suggesting changes in Ce^{3+} concentration due to doping.

Bioanalytical applications

❖ Detection of H_2O_2, Glucose, and Lactate



$Glucose + GOx + O_2 + H_2O \rightarrow gluconic\ acid + H_2O_2$

$2CeO_2 + H_2O_2 \rightarrow Ce_2O_3 + O_2 + H_2O$

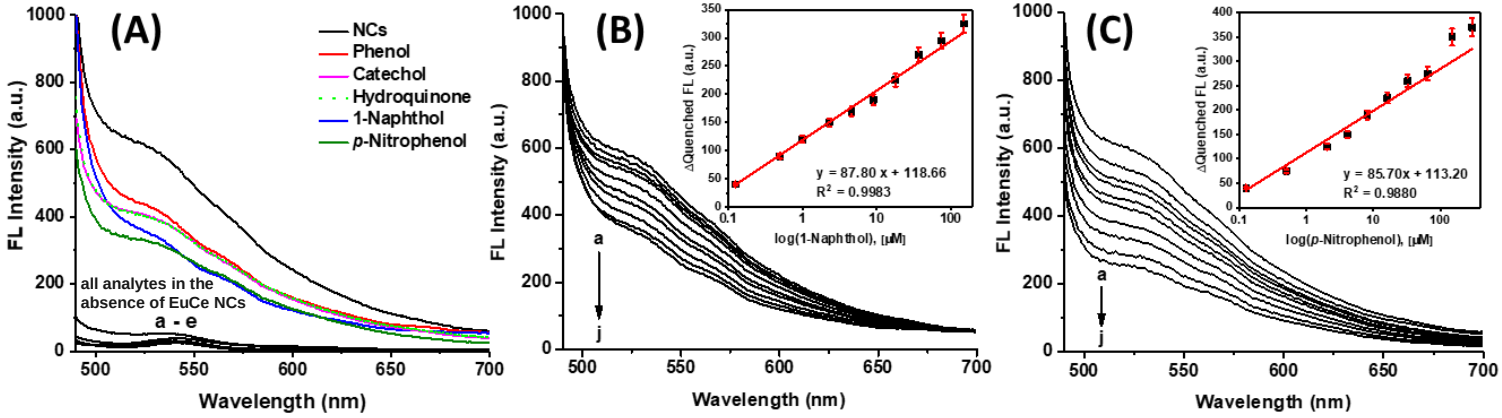
- The range of 0.04−20.48 mM
- LOD of 175 μM

Fluorescent response versus concentration of glucose: (a−h: 0, 0.04, 0.08, 0.16, 0.32, 2.56, 5.12, and 20.48 mM)

λ_{ex} = 466 nm.

- Upon addition of TMB to EuCe NCs, the FL intensity decreased. After addition of H_2O_2, the FL response was significantly enhanced and recovered, which suggests a catalytic effect of the NCs.

❖ Detection of Phosphatase Activity



λ_{ex} = 466 nm.

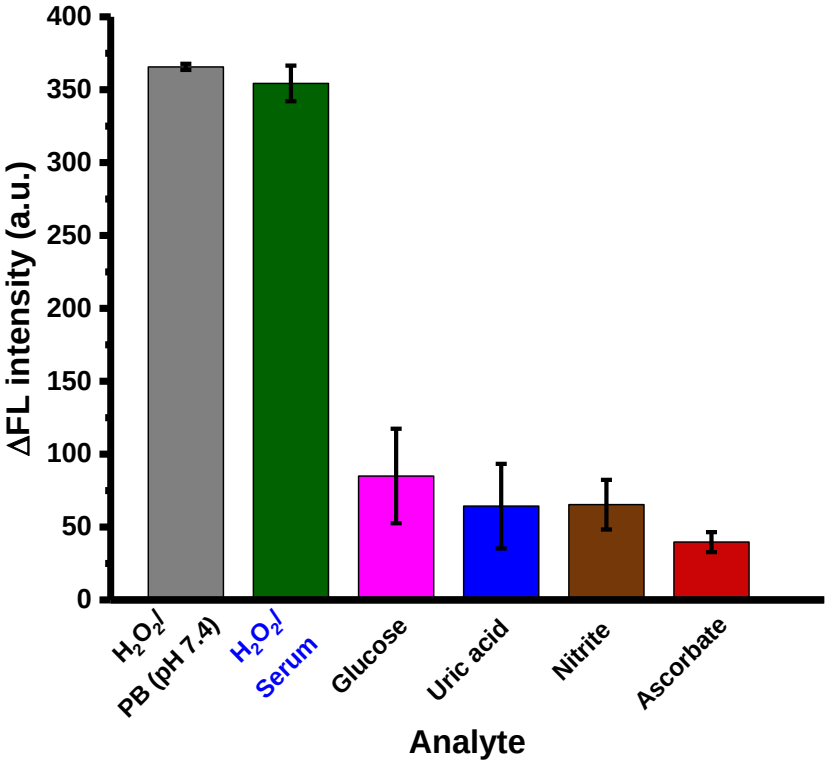
Fluorescence spectra of EuCe NCs in the presence of 1-naphthol; (a−j) 0.125, 0.5, 1, 2.3, 4.6, 9, 18, 37, 75, and 150 μM.

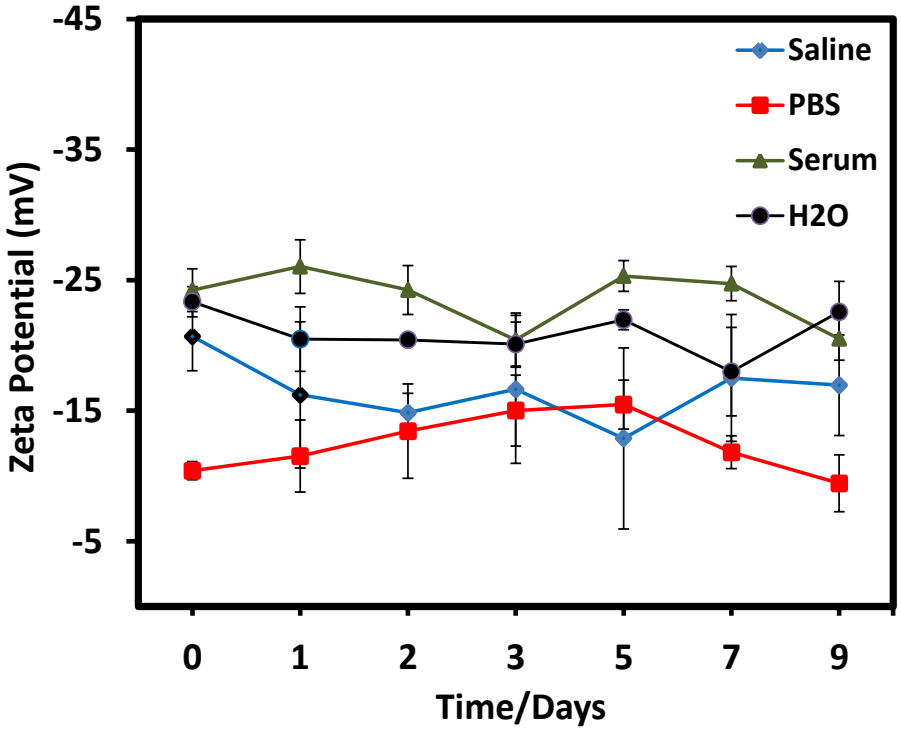
Fluorescence spectra of EuCe NCs in the presence of p-nitrophenol. (a−j) 0.25, 0.5, 2.3, 4.6, 8, 16, 32, 65, 150, and 300 μM.

- The EuCe NCs have also provided an excellent platform for measuring phosphatase activity.
- The detection mechanism is based on the FL quenching of EuCe NCs by the hydrolysis products of phosphatase.

Selectivity & stability of the probe

❖ Determination of H_2O_2 in Serum Samples and Selectivity Study



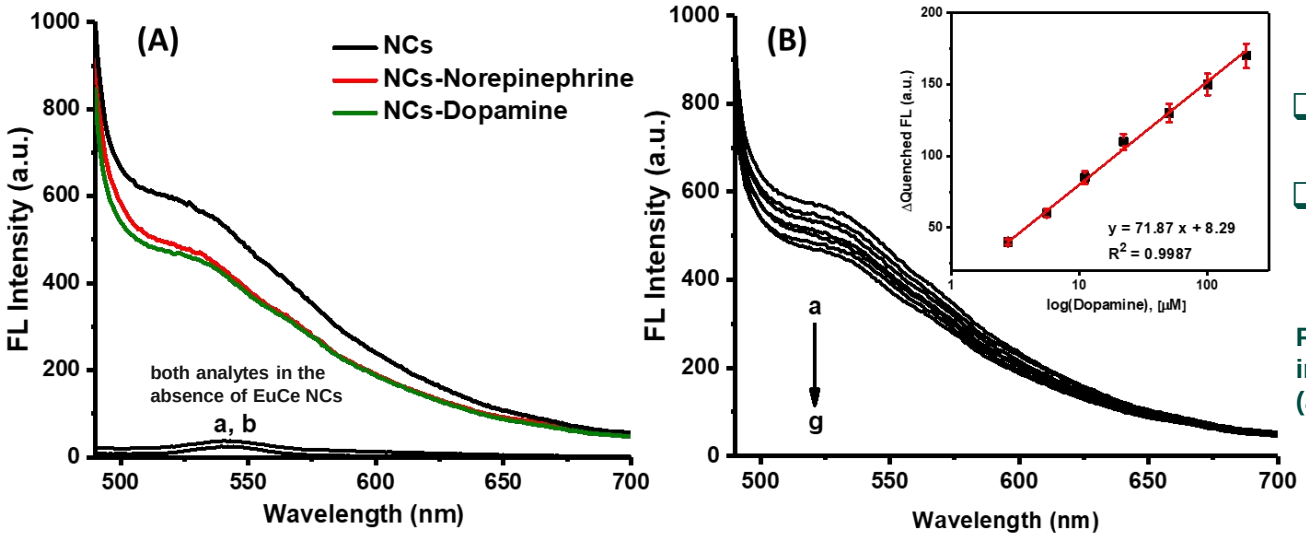


- ❑ The probe works well in a more complex sample (human serum)
- ❑ None of these species produced a significant fluorescent response

Comparison with the literature

❖ Detection of Neurotransmitters (NTs)

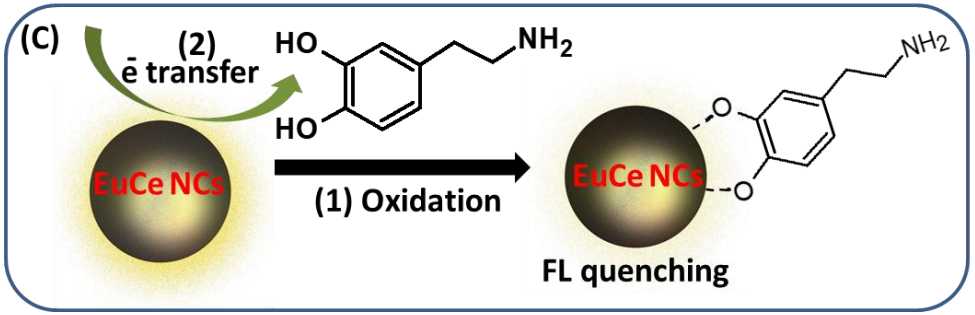
- The sensing capabilities of this method were further extended to the detection of catecholamine.
- Using the oxidase like properties of the EuCe NCs to induce in situ oxidation and measurement of NTs.



- Linear range of 2.75−200 μM for dopamine.
- LOD : 1.0 μM

Fluorescent intensity of EuCe in DI water in the presence of dopamine; (a−g) 2.75, 5.5, 11, 22, 50, 100, and 200 μM.

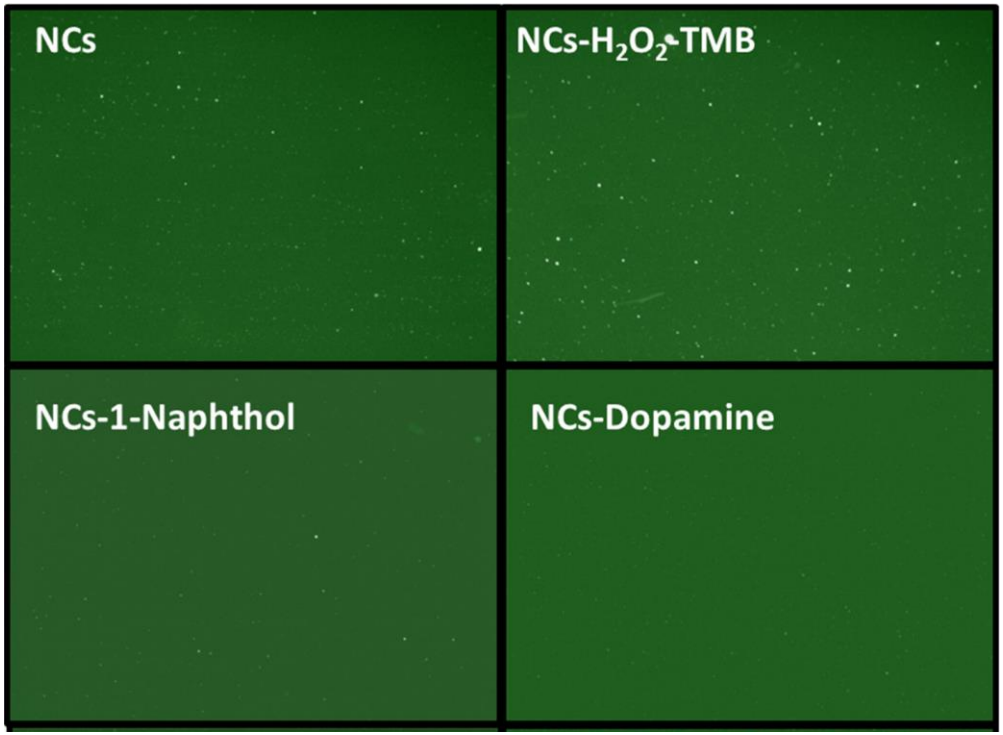
λ_{ex} = 466 nm.



- Ceria NPs induce oxidation of the catechol ring in catechol-containing NTs, followed by surface binding of the reactive intermediates

Fluorescence Imaging

The fluorescence images of EuCe NCs in the presence of H_2O_2, 1-naphthol, and dopamine



- The fluorescent intensity of EuCe NCs + TMB + H_2O_2 assay was enhanced, while that of EuCe NCs-1-naphthol and EuCe NCs-dopamine was quenched when compared to EuCe NCs alone.
- This correlates well and confirms the fluorescence spectroscopy data.
- These results are promising and suggest that these EuCe NCs have potential as an imaging probe for diagnostics, and therapeutic applications
- NCs : Nanocrystals (Nanoparticles with high crystalline structure)

Fluorescence microscopy images. λex = 466 nm. Magnification of the objective is 63X; the size of the particle has an average of 4.7 ± 0.1 nm.

- Othman et. al., *ACS Appl. Nano Mater.,* **2018***,* 1, 10, 5722–5735

Conclusion

- This study described a convenient methodology for the synthesis of highly stable, uniform, water dispersed, and strongly fluorescent lanthanide-doped EuCe NCs.
- The EuCe NCs have an average size of ~5 nm and exhibit excellent fluorescence emission characteristics and stability for several months under different buffer and pH conditions.
- The fabricated new fluorescent Eu-doped CeO_2 NPs was demonstrated with imaging and sensing capabilities for applications in the bioanalytical/sensing & biomedical field.
- The EuCe NCs was demonstrated as a nanoenzyme fluorescent probe to monitor H_2O_2 and quantify enzymatic transformation of oxidase enzyme reactions (i.e., glucose and lactate).
- We also developed a approach for measuring ALP activity by monitoring the quenching fluorescent intensity of the NCs in the presence of 1-naphthyl phosphate.
- Biologically relevant NTs (dopamine and norepinephrine) was also quantified using the oxidative capability of the EuCe NCs as fluorescent changes in the NC's properties upon oxidative induced reconfiguration of the NC's surface.
- We expect the promising potential of this material to open new ways to design nanobiosensors for bioimaging and biocatalytic applications.

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Ali Othman, PhD
Department of Chemistry and Biomolecular Science,
Clarkson University, Potsdam, NY, USA

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