Bio-templated Fluorescent Metal Nanoclusters: A Multifunctional Platform

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Acknowledgements

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  - Dr. Namas Chandra, NJIT, USA

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  - DoD Blast Injury Research Program Office, USA MRMC
  - ARL, APG

Bio-Mediated Fluorescent Metal Nanoclusters (fMNCs)

Advantages:

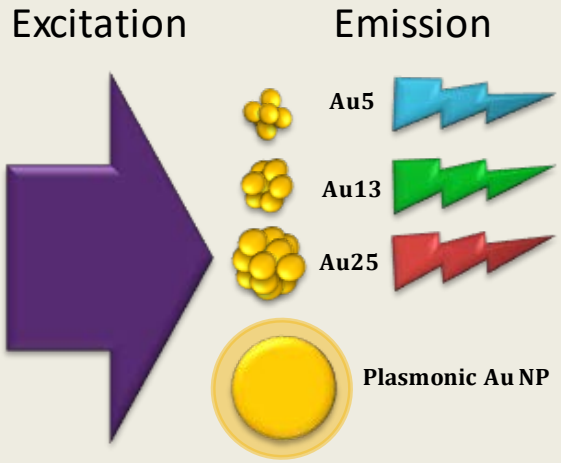
- Bio-Compatible
- Environmentally friendly
- Ease in synthesis, phototunable
- Intense fluorescence Intensity
- Pressure sensitive
- Multifunctional – photophysics of NCs + Bioactivity
    - Real-time sensing, imaging

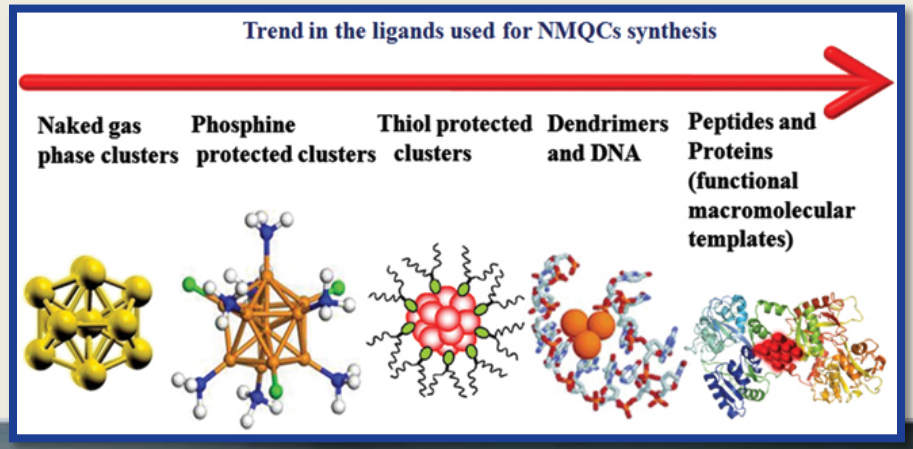
Disadvantages:

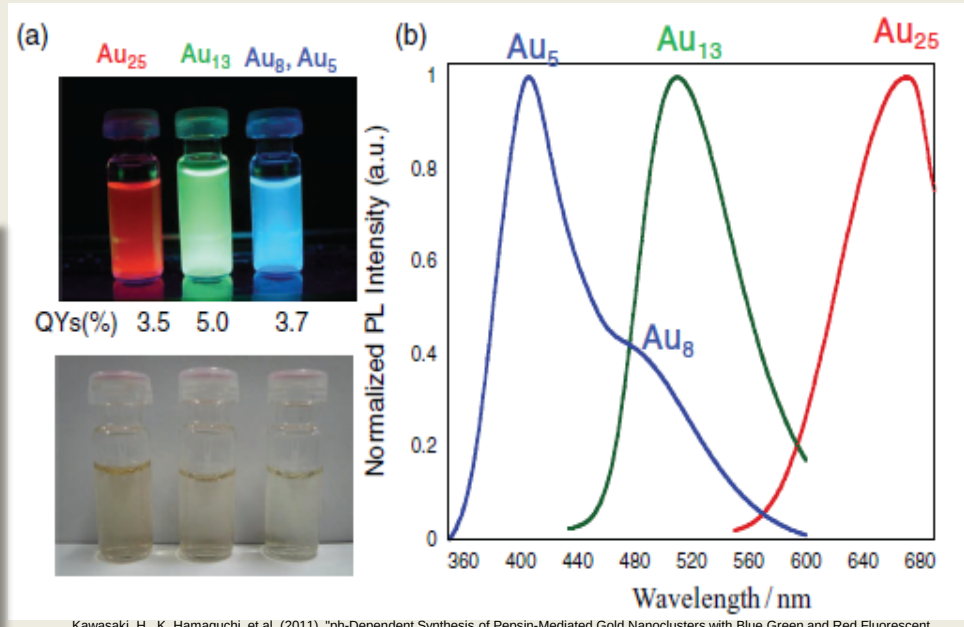
- Sensitive to environment (Light, T, P, chemical exposure, …)
- Often expensive and incompatible with different media

Fluorescent Noble Metal Nanoclusters

- **Ultrasmall clusters of several to tens of atoms**
  - **≤ 2 nm in diameter**
  - **Smaller than the Fermi wavelength of conduction electrons**
- **Exhibit quantized energy levels like molecules or QDs, intense fluorescence, size-tunable**
- **Discrete and size tunable electronic transitions**
  - **E α R^{-1}**
  - **UV to near IR**
- **High photostability**







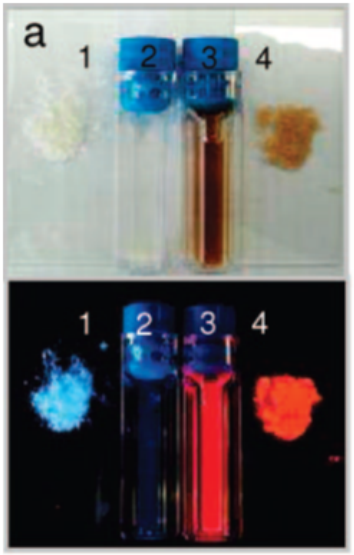
Kawasaki, H., K. Hamaguchi, et al. (2011). "ph-Dependent Synthesis of Pepsin-Mediated Gold Nanoclusters with Blue Green and Red Fluorescent Emission." Advanced Functional Materials 21(18): 3508-3515.

Xavier PL, Chaudhari K, Baksi A, Pradeep T. Protein-protected luminescent noble metal quantum clusters: an emerging trend in atomic precise nanoscience. Nano Today. 2012. 3:17467-83.

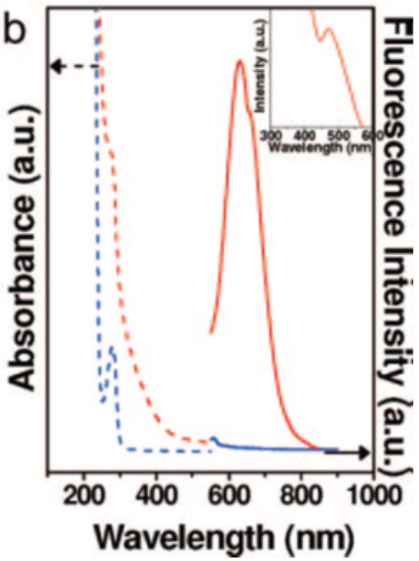
Protein-synthesized Fluorescent Metal Nano Clusters (NCs)

BSA-Directed Au NCs: J. Xie, *et al.*, *J. Am. Chem. Soc.* 2009, *131*, 888–889

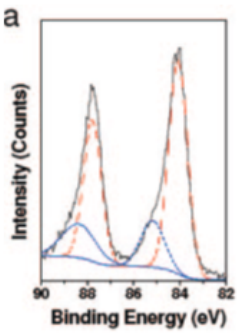
White light



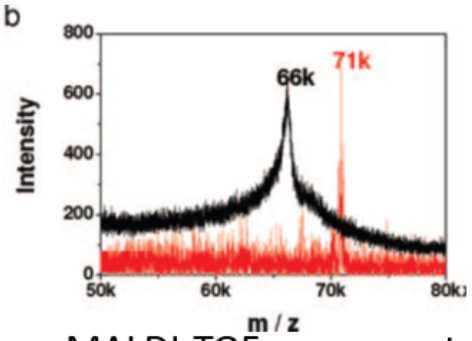
UV-irradiation:
Blue: BSA
Red: BSA-Au NC



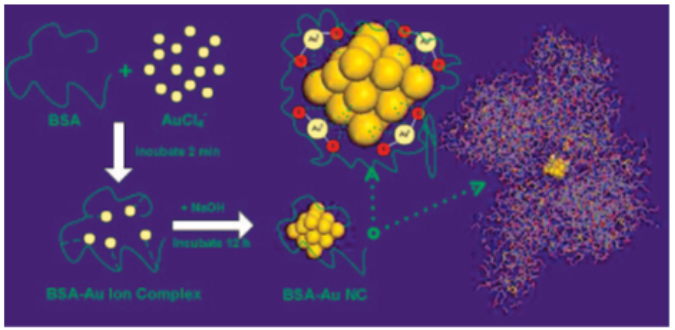
Absorption (blue, red-dotted)
Photo-luminescence (Red)



XPS Spectra



MALDI-TOF mass spectra



Proposed AuNC formation mechanism

BSA-directed synthesis of fluorescent Au Nanoclusters (NCs): First bio-mediated laboratory synthesis of optically active metal NCs

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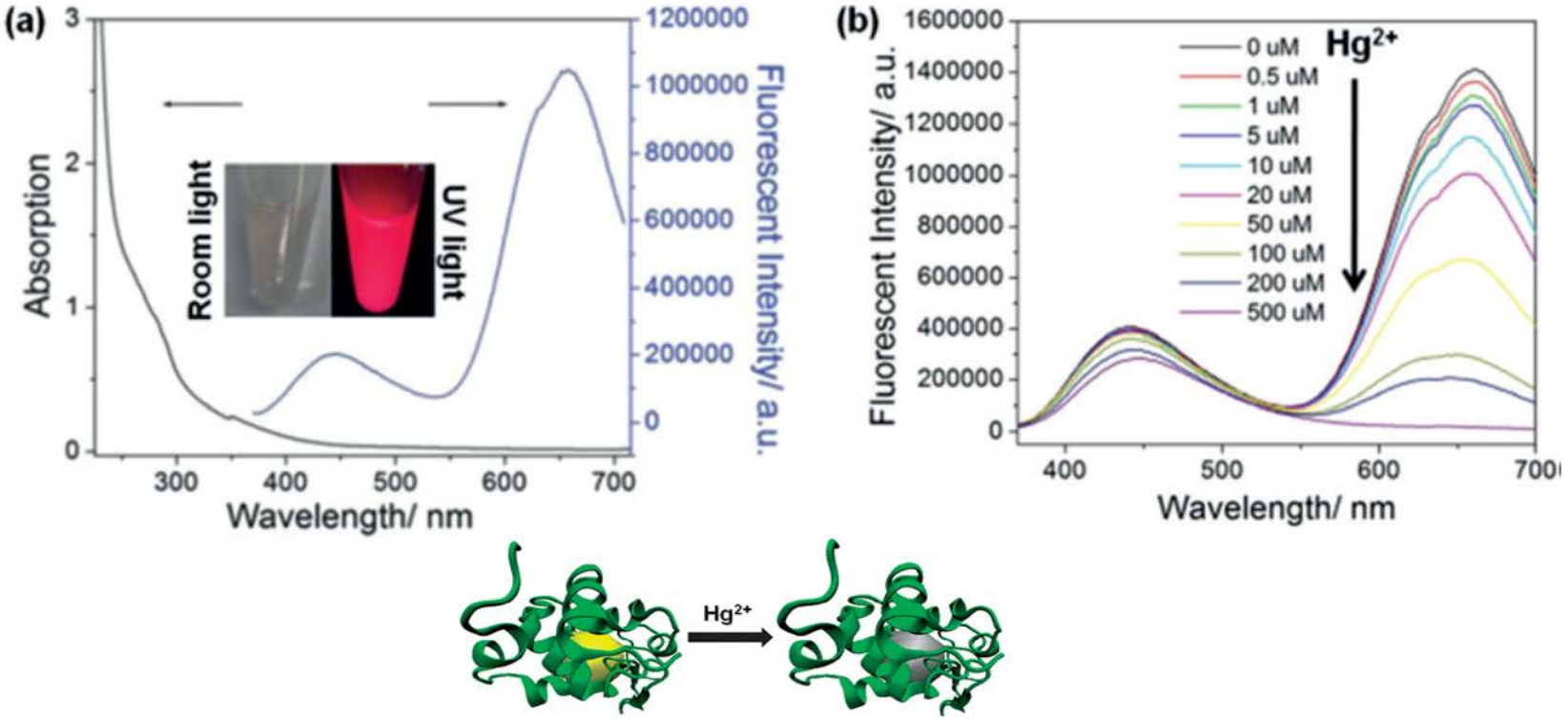
Bio-Templated fMNCs

ARL

Lysozyme-mediated AuNCs: Hg Sensor

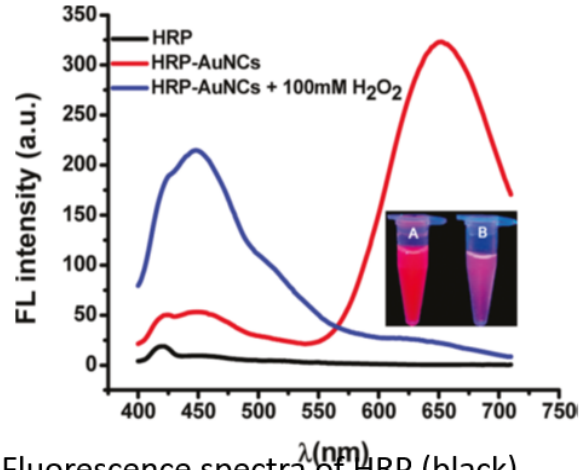
H. Wei et al, *Analyst* 2010, 135, 1406–1410



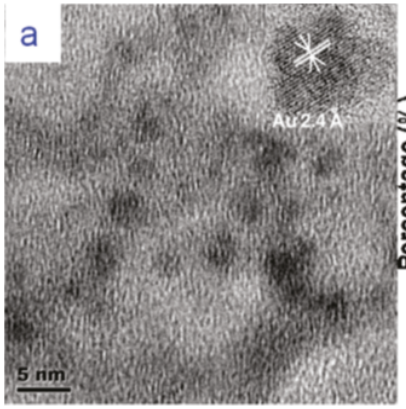
Bio-Templated fMNCs

Horseradish Peroxidase (HRP) Functionalized Fluorescent AuNC: H_2O_2 Sensing

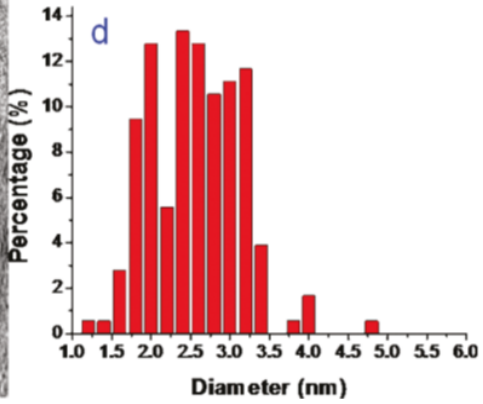
F. Wen et al., *Anal. Chem.* 2011, 83, 1193–1196



Fluorescence spectra of HRP (black), HRP-Au NCs in the absence (red) and presence of 100 mMH_2O_2 (blue line)

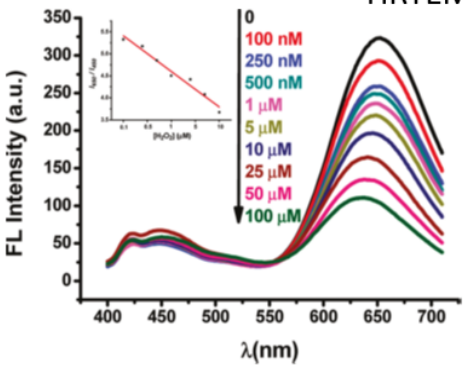


HRTEM Image



Size distribution of AuNCs

Multi-Functional Platform: Fluorescence emission, catalysis, real- time sensing

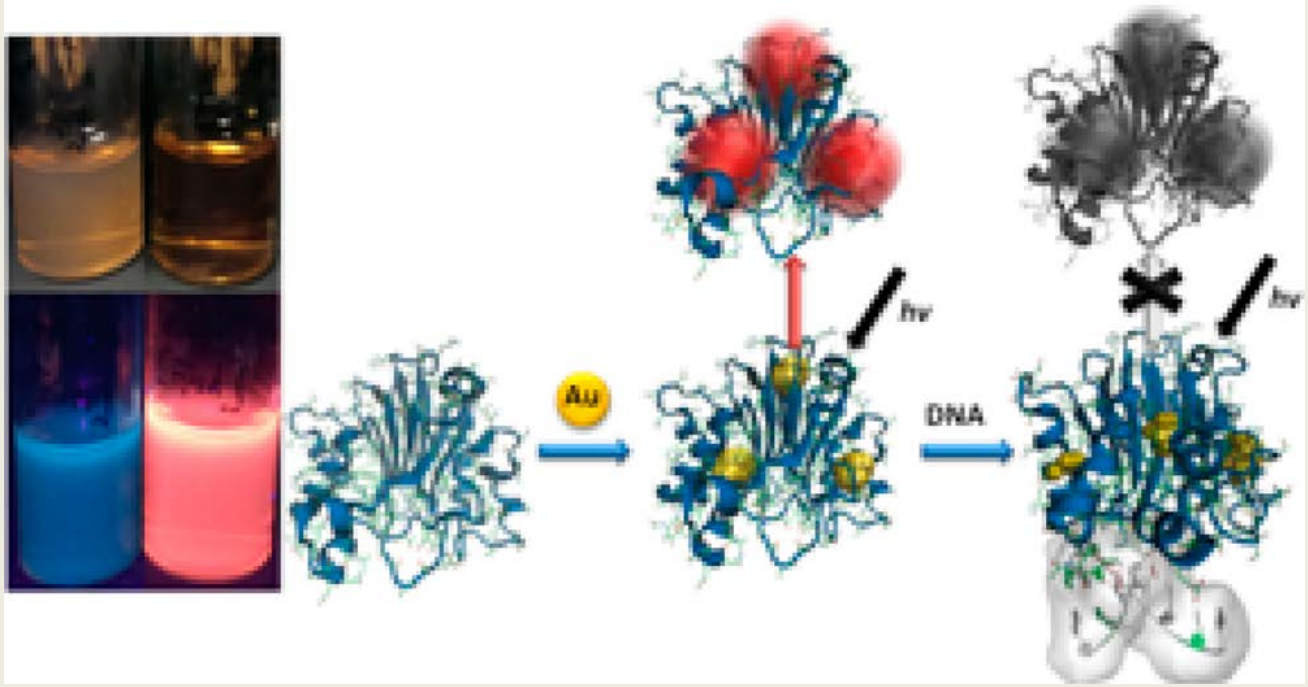


Fluorescence spectra of HRP-PtNC after addition of 0-100 μM of H_2O_2: Real-time sensing

DNase 1 synthesized Au nanoclusters (DNase 1:Au Ncs)

Real-time Biosensing and purification (DNA sensing, RNA purification)

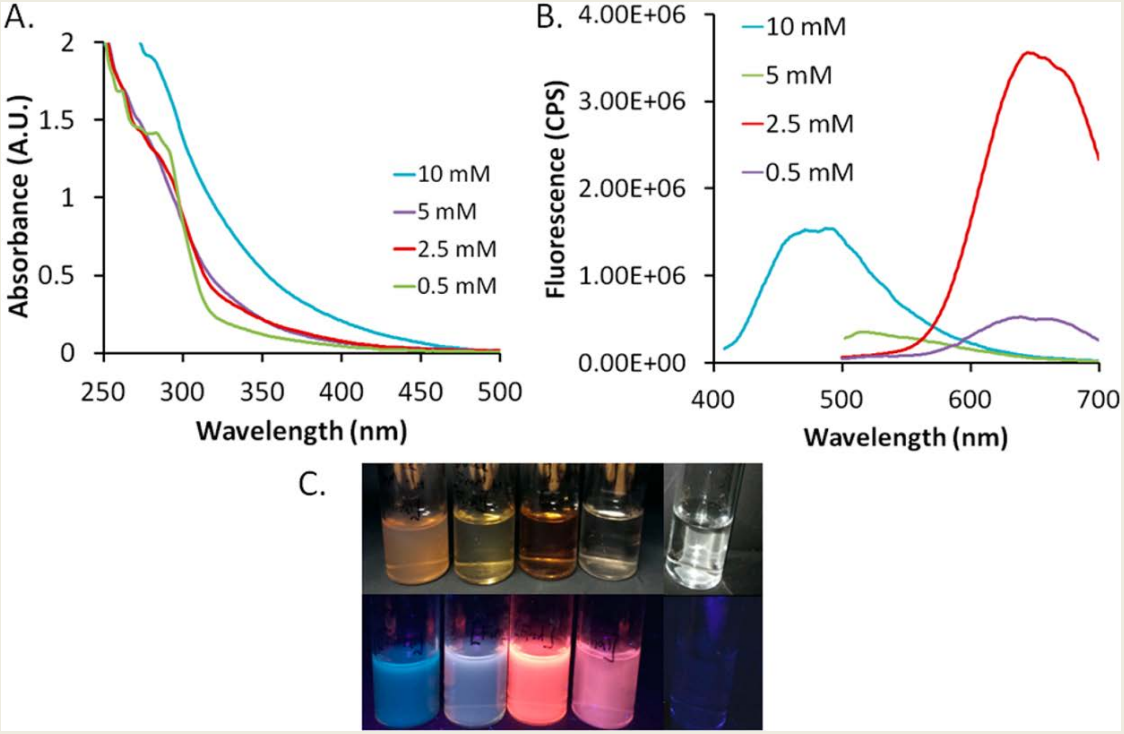
A. L. West et al, *Anal Chem*. **86**, 7077 (2014)



DNase 1-Au NCs

Tunable fluorescence-emission

A. L. West et al, *Anal Chem*. **86**, 7077 (2014)

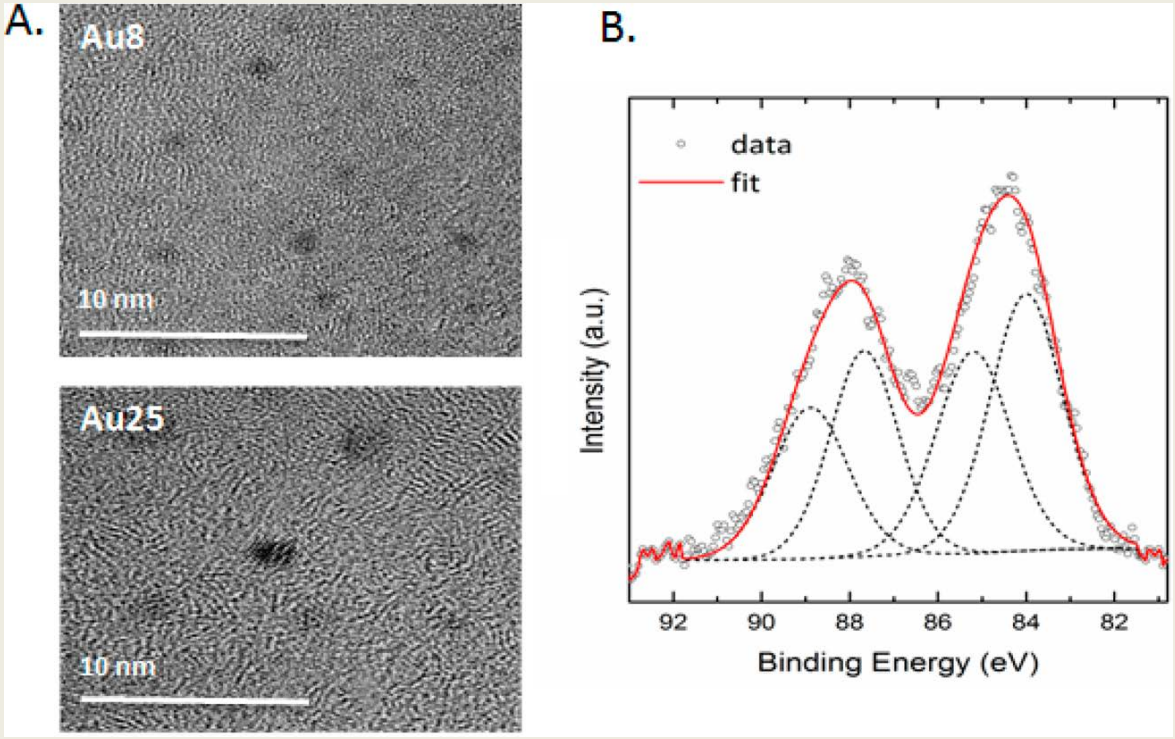


Au-ion concentration-dependent emission

DNase 1-Au NC

Au $4f_{7/2}$ doublet in the XPS: Presence of Au (0) and Au (I) species

A. L. West et al, *Anal Chem*. **86**, 7077 (2014)



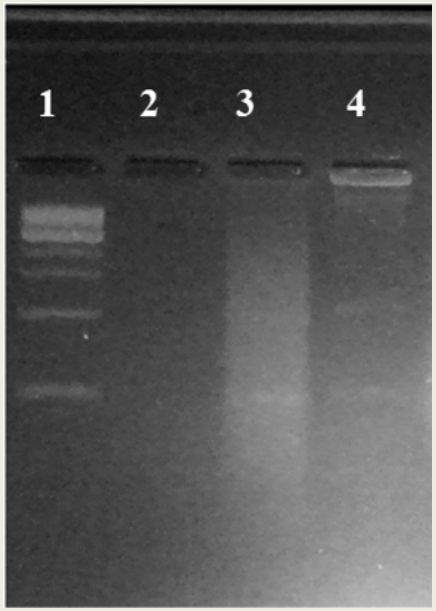
TEM Images of AuNCs
Clusters < 2-4 nm size

XPS Spectra of Dnase 1: Au (25) NC

Dnase 1:AuNCs

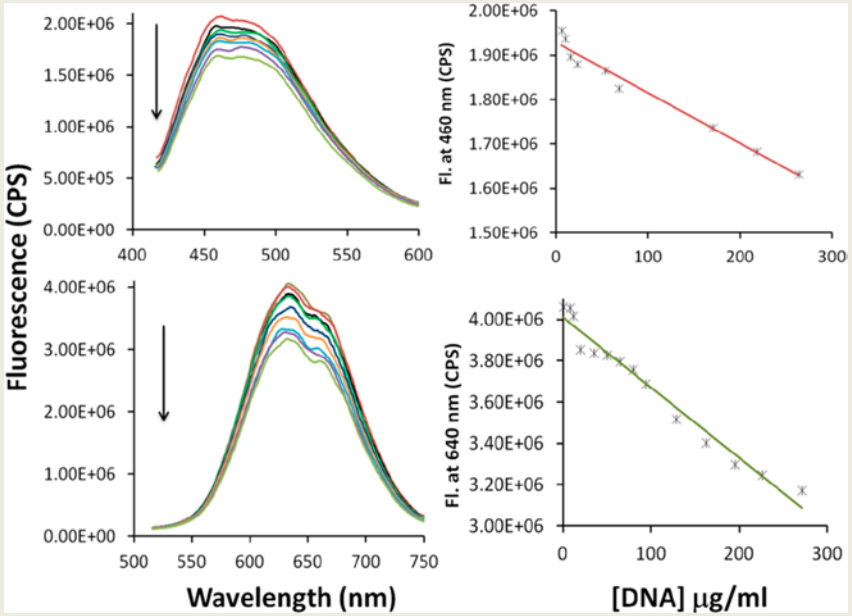
Biocatalytic activity and fluorescence emission: Real-time biosensing

A. L. West et al, *Anal Chem*. **86**, 7077 (2014)



ds DNA+Dnase:AuNC

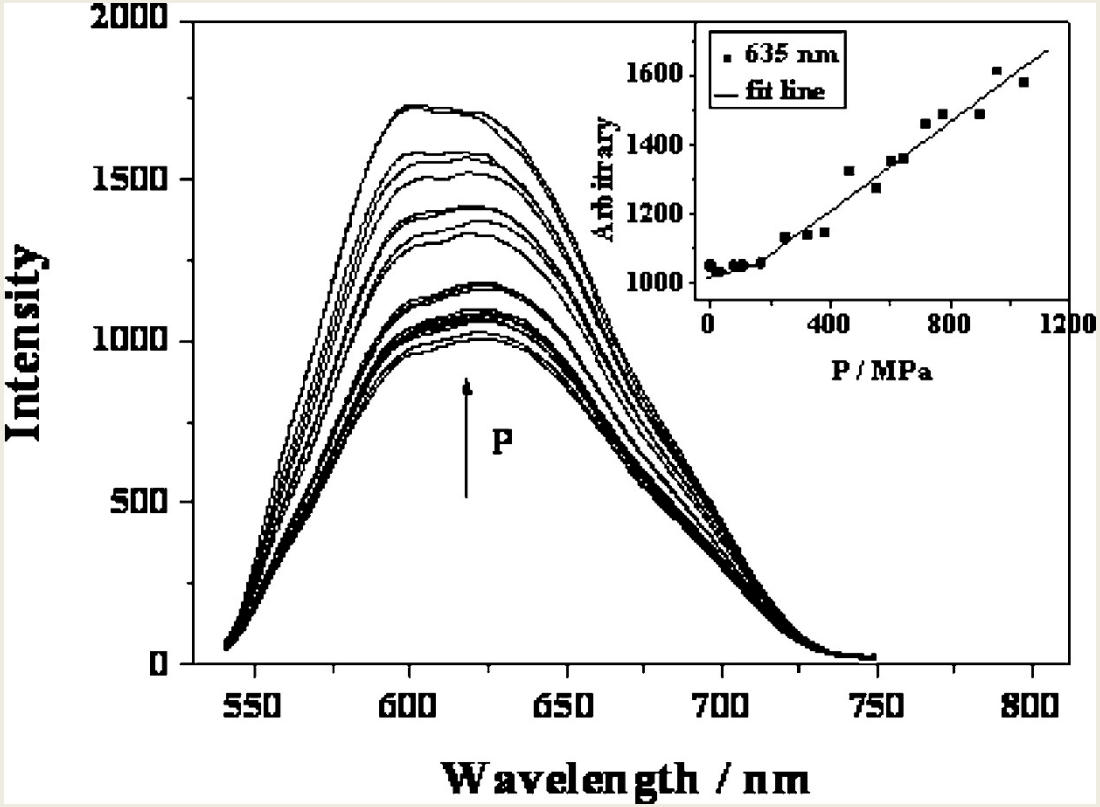
1. DNA
2. DNA+Dnase 1
3. DNA+Au8 NC
4. DNA+Au25 NC



Fluorescence decay with DNA+DNAse1:Au NC

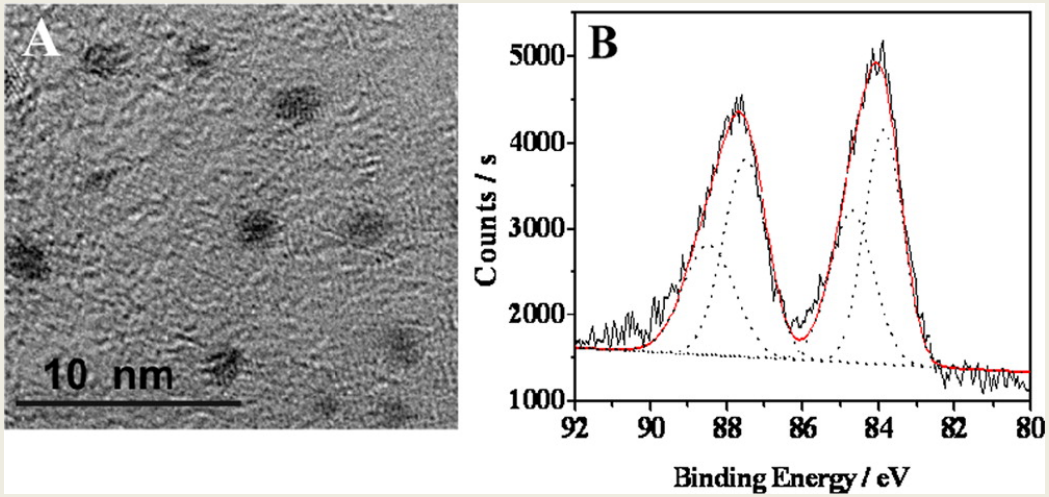
Pressure-sensitive Fluorescent Metal Nanoclusters (p-fMNC)

- Protein-synthesized Gold nanoclusters (~ 2nm diameter)
- Fluorescence in visible
- Fluorescence wavelength tunable
- Fluorescence intensity sensitive to external pressure



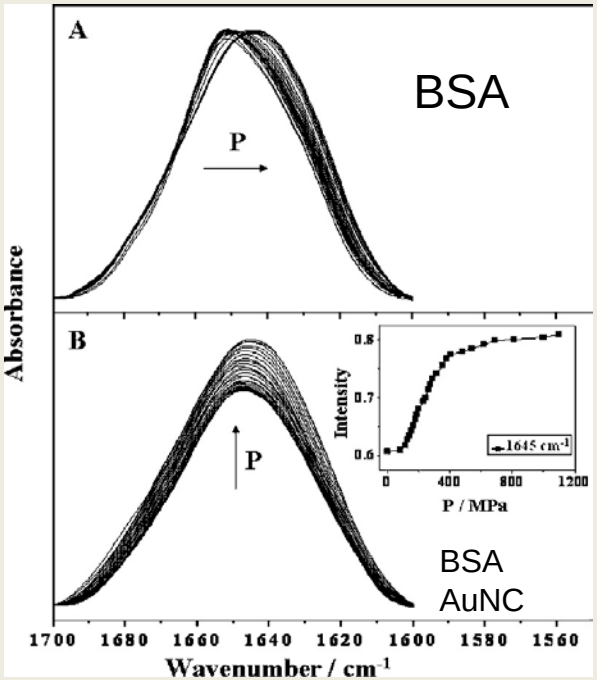
BSA-stabilized Au pNC-
M Zhang et al., *J. Phys. Chem. C* **2013,** 117, 639-647.

BSA-derived Au NCs: Pressure Sensitivity

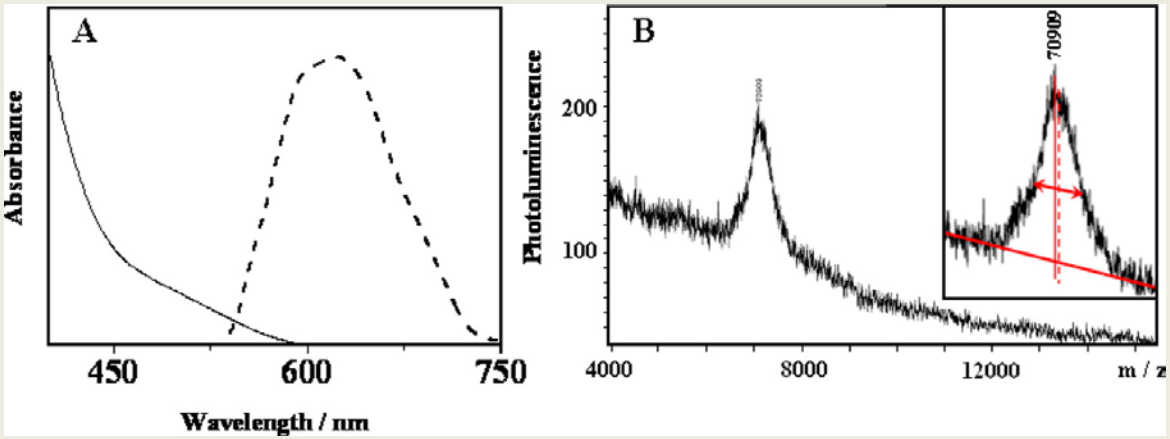


TEM Image

XPS



FT-IR Spectra versus applied Pressure

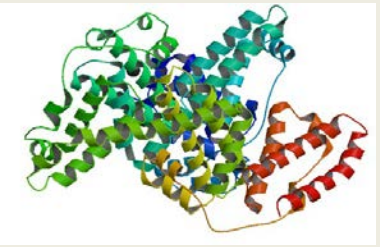


UV -Vis

Raman

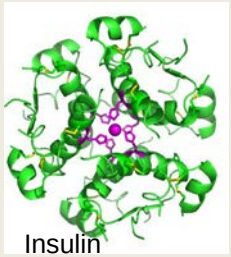
M Zhang et al., *J. Phys. Chem. C* **2013,** 117, 639-647.

Nanocluster Property Tuning via Protein-Host and Metal Composition

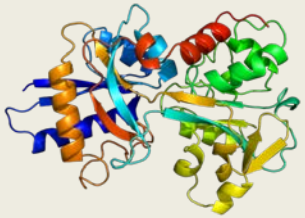


BSA (PDB:3v03)

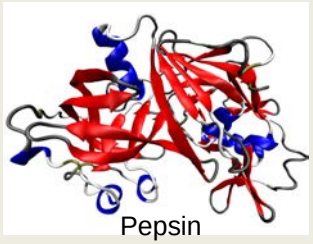
Lysozyme (PBD:132l)



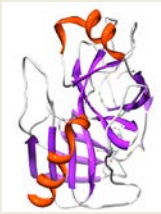
Insulin (PDB:1ai0)



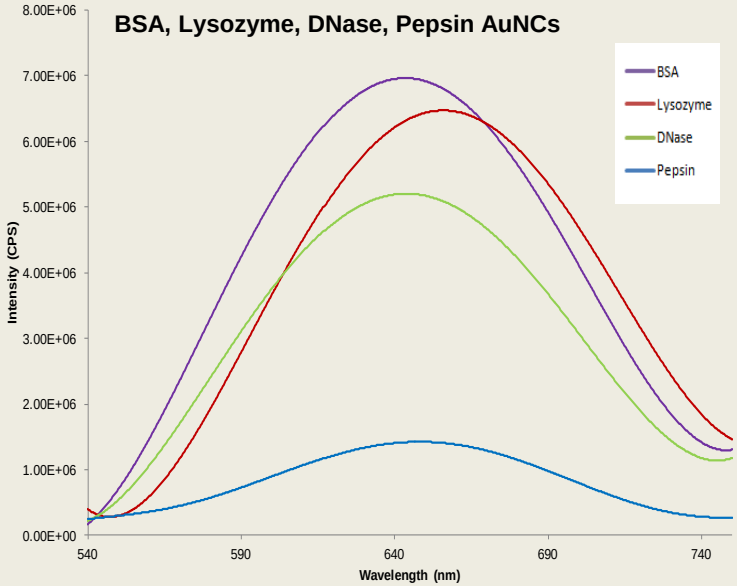
apo-Transferrin (PDB:1a8e)

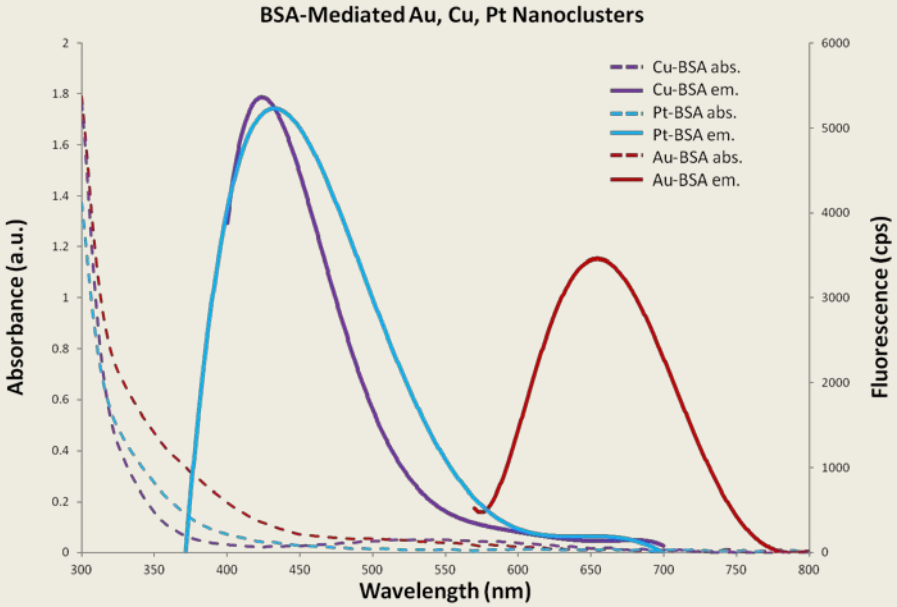


Pepsin (PDB:5PEP)



Trypsin (PDB:1TRY)





Stabilizing proteins differ in:

Fold

Function

Amino acid content

Metals studied:

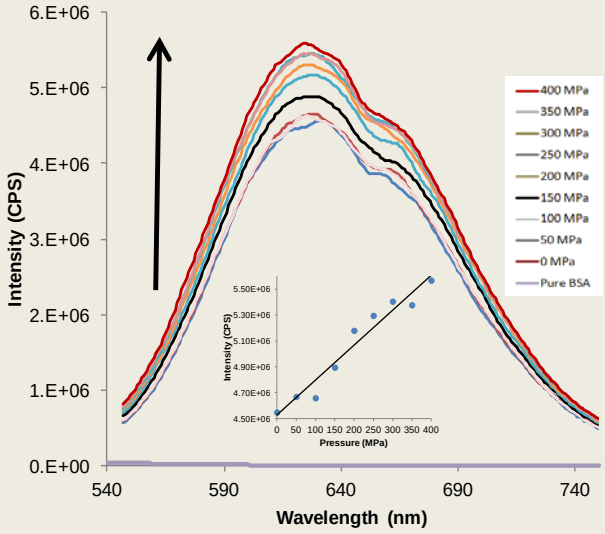
Gold

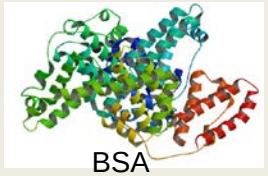
Platinum

Copper

Pressure Responsive Fluorescent NC Tool Box

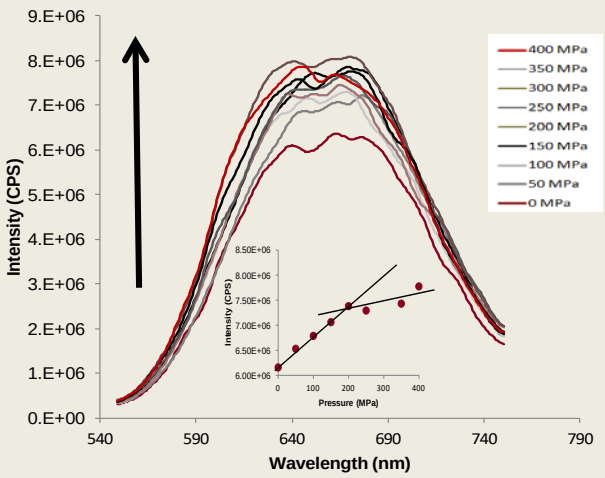
Protein secondary structure dependent low, medium and high pressure-fluorescent response: ability to cover wide pressure range (kPa-GPa)

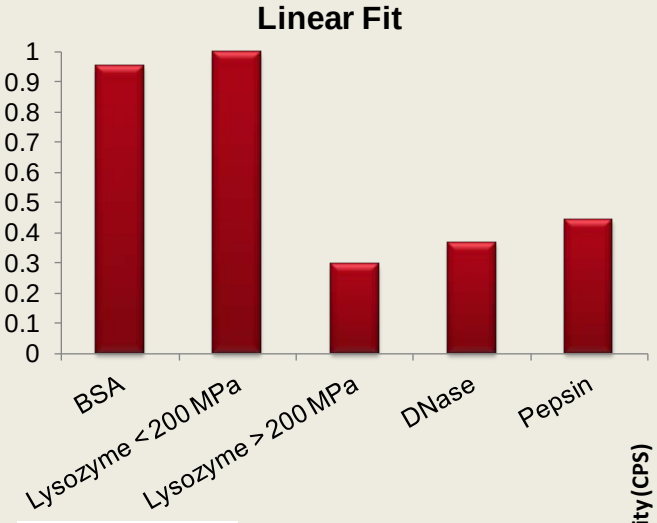


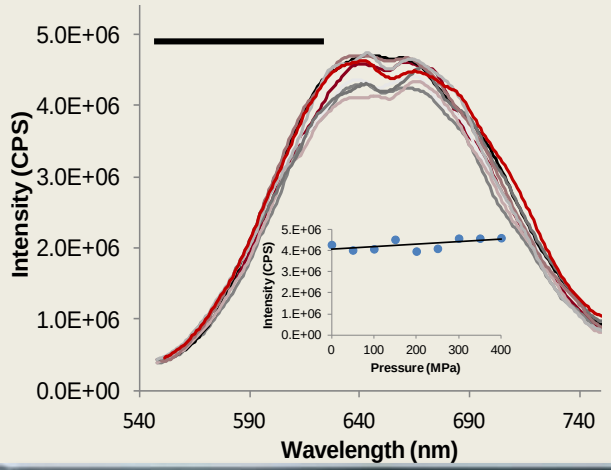


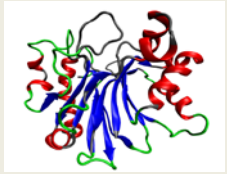
BSA
(PDB:3v03)
100 % α helical

Lysozyme
(PBD:132l)
30 % α helical
60 % random coil

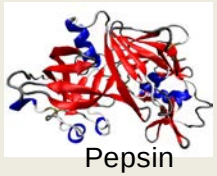




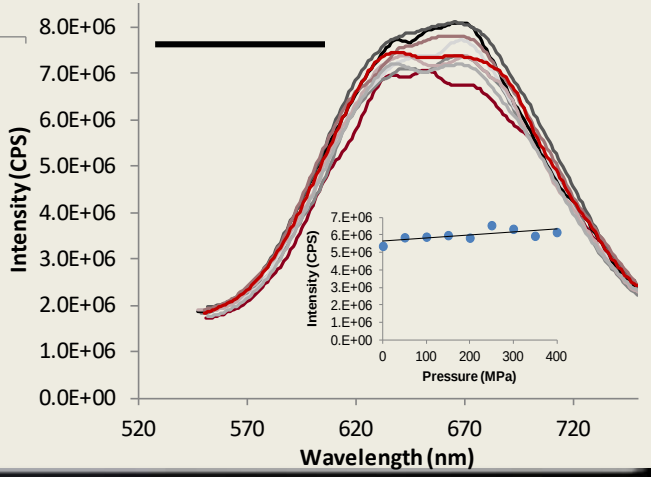




DNase 1
(PDB:1DNK)
22 % α helical



Pepsin
(PDB:5PEP)
3 % α helical



Low-cost Protein: Egg White (EW)

Egg White: Readily available, low cost, composed of 148 different proteins

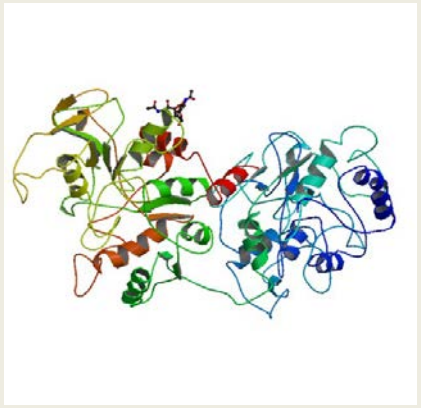
Components:

- Ovalbumin (54%)
- Ovotransferrin (13%)
- Ovomucoid (11%)
- Lysozyme (3.5%)
- Ovomucin (3.5%)
- Others (5%)

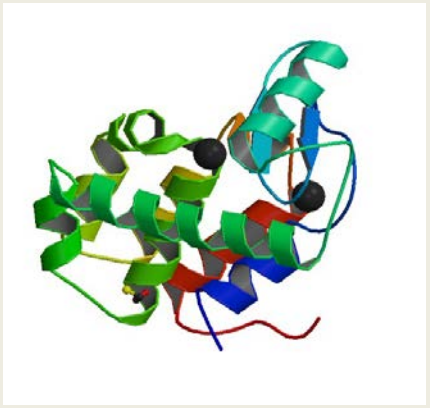
D'Ambrosio, C. et al.,
J. Proteome Res., 2008, 7, 3461-3474

Ovalbumin

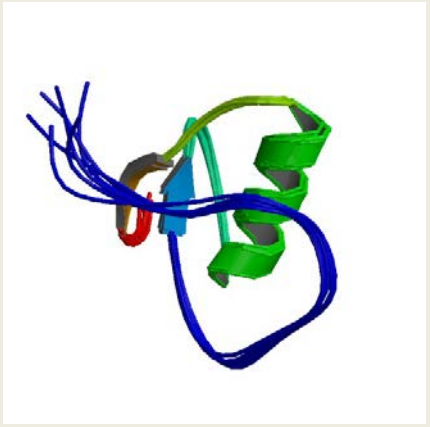
Ovomucoid



Ovotransferrin



Lysozyme



Ovomucin

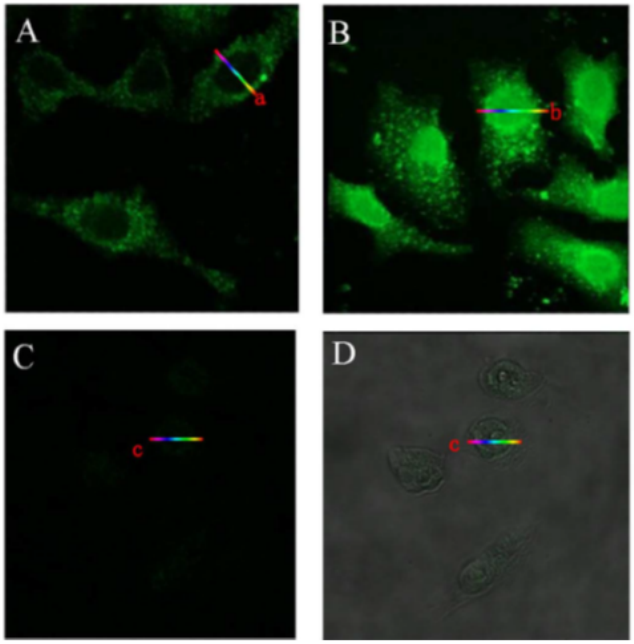
Constitute almost 85% of entire EW proteome

Images taken from RCSB PDB

In situ synthesis of AuNCs in Cancerous Cells: Bio-imaging

J. Wang et al., Sci. Rep., 2013, 3, 1157

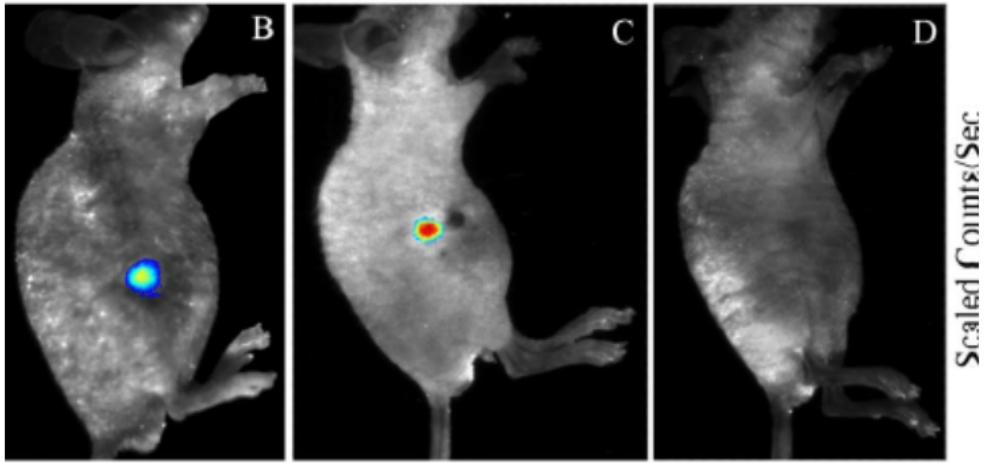
HepG2 (24h) HepG2 (48h)



L02 (24h) L02 (48h)

No NC formation in *healthy* (L02) cells

In situ synthesis of AuNCs in Cancerous (HepG2) cells
2-3 nm diameter, λex=525 nm (Green)



Hepatocellular carcinoma (24h) Chronic myeloid leukemia (24h) Control (healthy) mouse (48h)

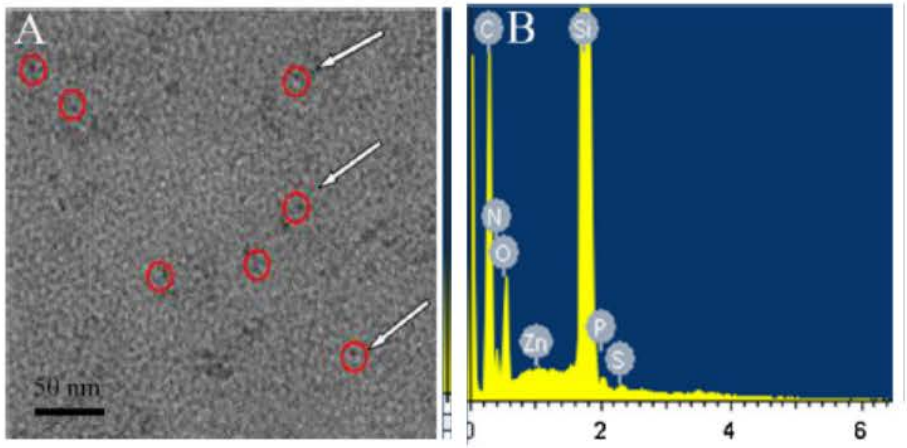
In situ Synthesis of Zn NCs in Hela Cancerous Cells: Imaging

M.-N. Su et al., *Chin. Chem. Lett*, 2015, 26, 1400-1402

$Zn(C_6H_{11}O_7)2$ incubated in Hela and L02 Cell lines

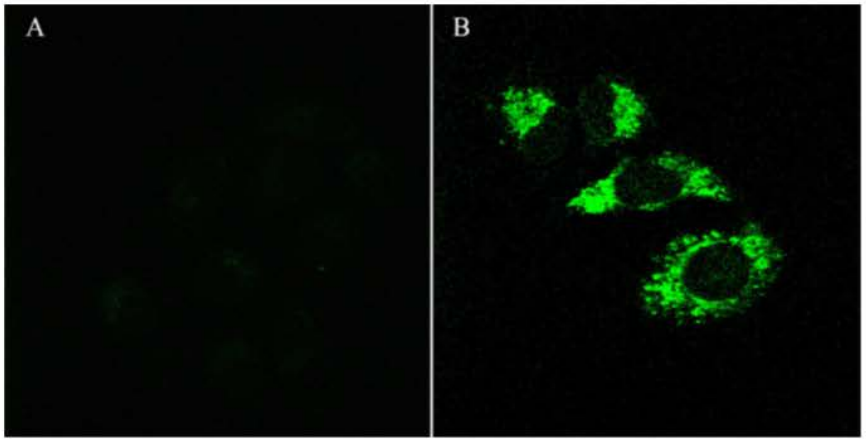
No NC formation in L02 Cells

Zn NCs *ONLY* formed in Hela cell
Fluorescence at λ=640 nm (λex=450 nm)



TEM Image

EDS Spectrum

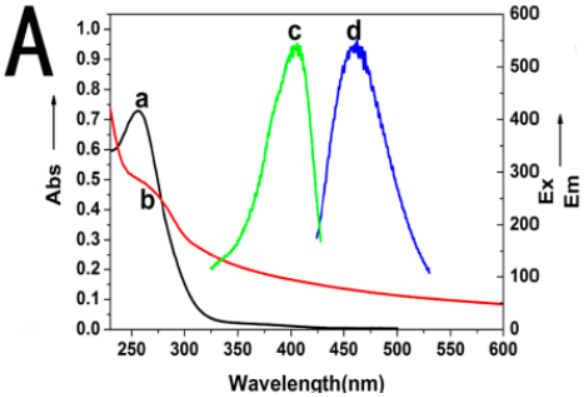


L02 (Healthy) Cell

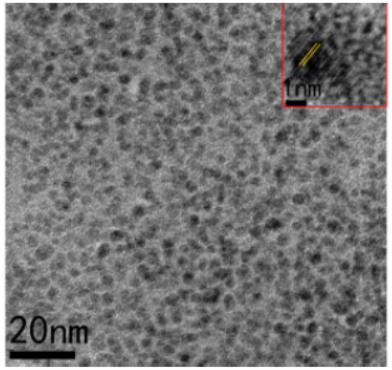
Hela (Cancer) cell

In situ Synthesis of Pt-NCs by Cancerous Cells: In vivo imaging

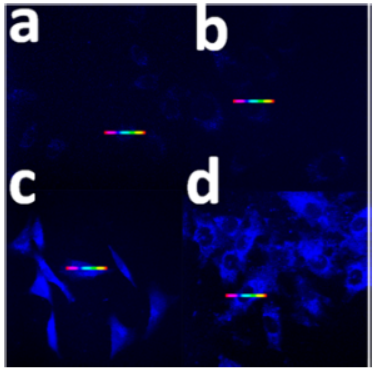
D. Chen et al., *ACS Appl. Mater. Interface* 2015, 7, 1863-18169



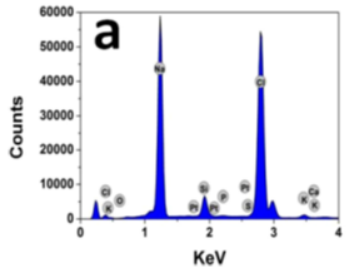
Absorption (blue, red), excitation (green); fluorescence spectra of HepG2-Pt NCs



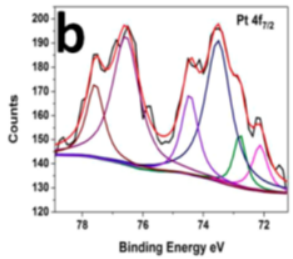
TEM image: 2.3 nm Av Pt NCs



Confocal fluorescence micrograph. a: control L02, b: HepG2, c:b+High Glucose, d:b+PtNC



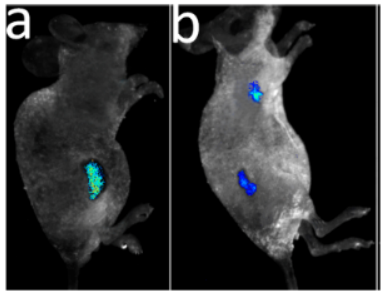
EDS Spectrum



XPS spectra

Xenograft tumor mouse models

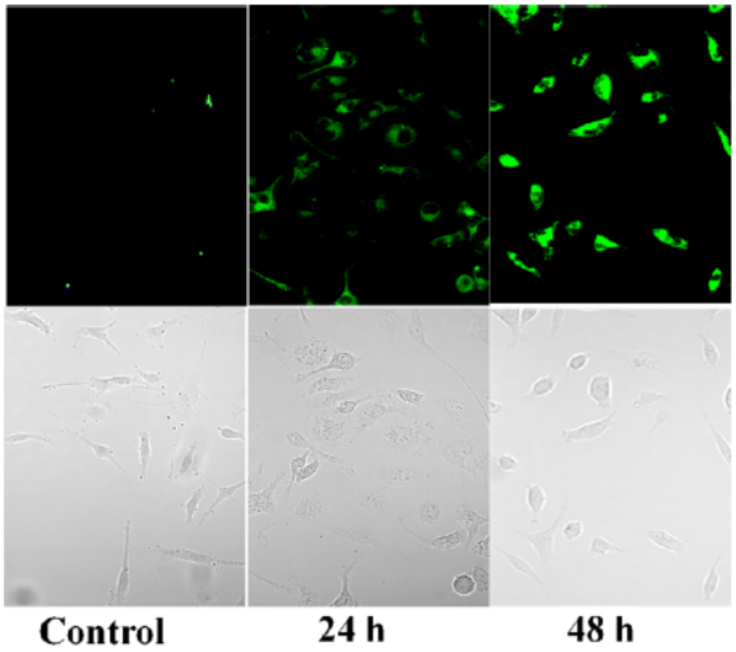
Fluorescence detection of HCT-16 cells



Fluorescence detection of HeLa (upper) HCT-16 (lower) cells

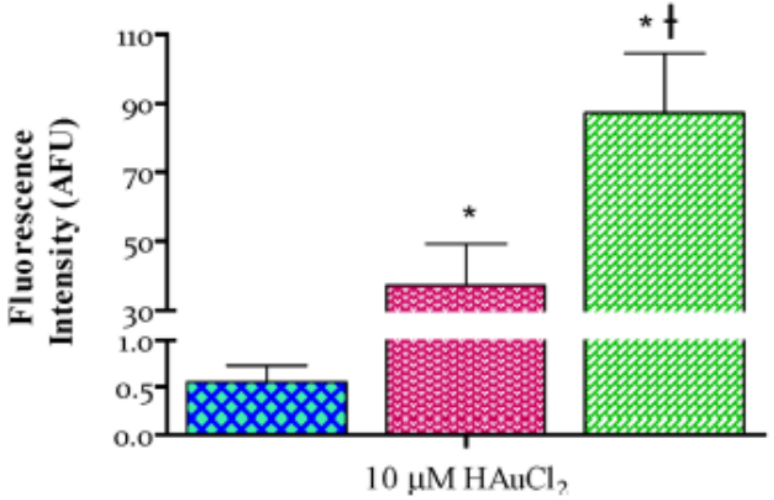
In situ synthesis of AuNCs by *Healthy* Microglial Cell

A. L. West et al., *ACS Appl. Mater. Interfaces* 2016, 8, 21221-21227



Confocal microscopic images of C8B4 cells, C8B4+ $HAuCl_4$ after 24 h, and 48 hrs of incubation, respectively. Dark field (upper panel); bright field (lower) of the same samples

Fluorescent AuNCs are formed in situ in *healthy* (non-tumorigenic) microglial cells



Fluorescence intensity of control (C8B4) cells, C8B4+$HAuCl_4$ after 24 h and after 48 h of incubations, respectively.

In situ synthesis of AuNCs by *Healthy* Microglial Cell

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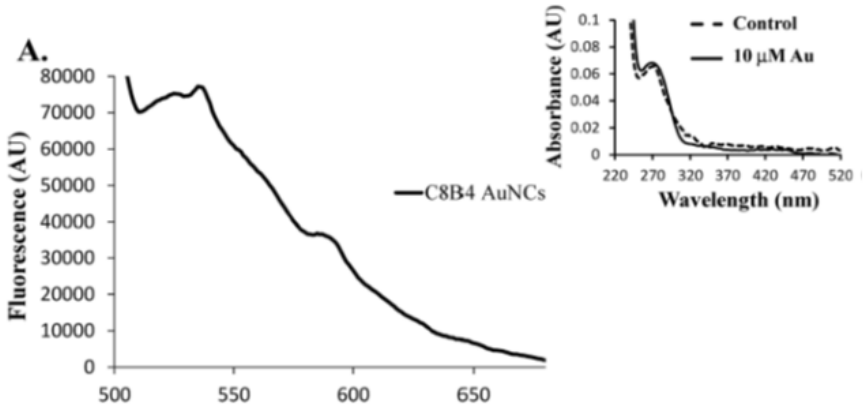
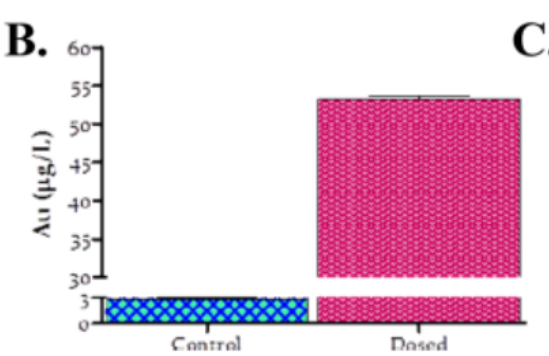
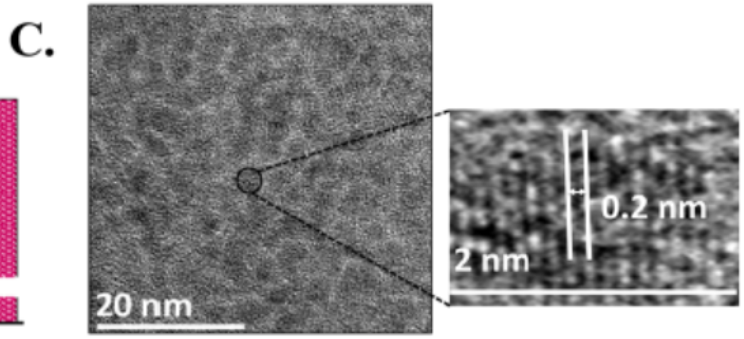


Fluorescence spectrum of AuNC-C8B4 (microglial) cell



Au Concentration



TEM image of Au NCs in Total Protein isolate

In situ synthesis of AuNCs by *Healthy* Microglial Cell

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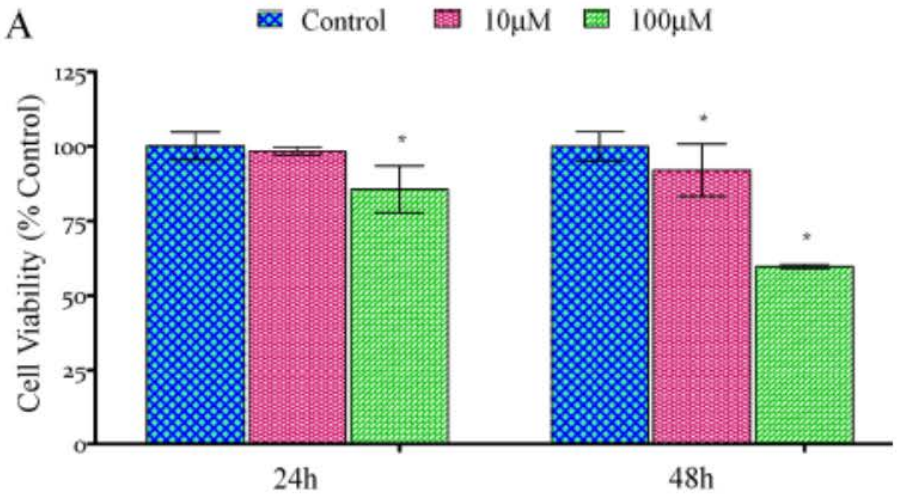
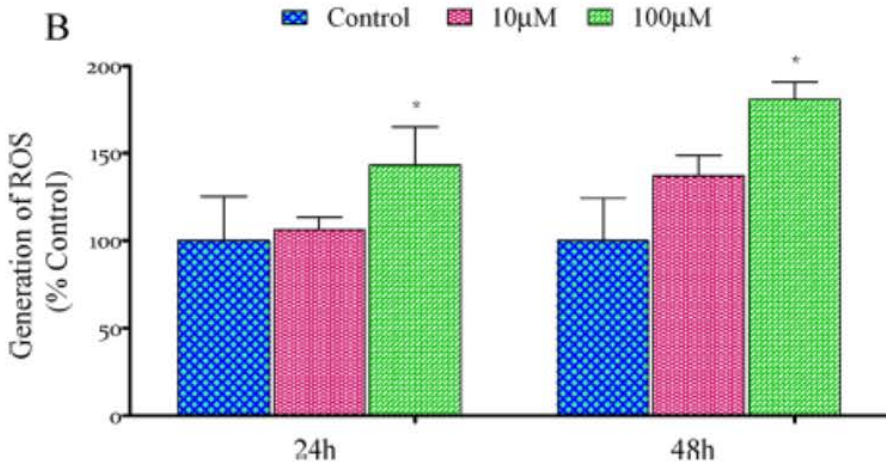
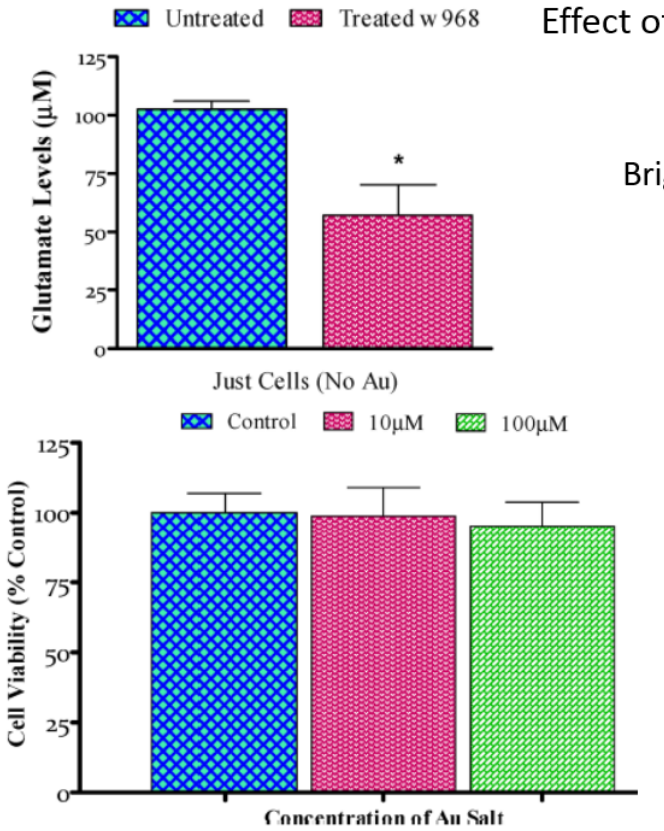




Higher Au concentration reduces cell viability and increases reactive oxygen species in healthy microglial cells

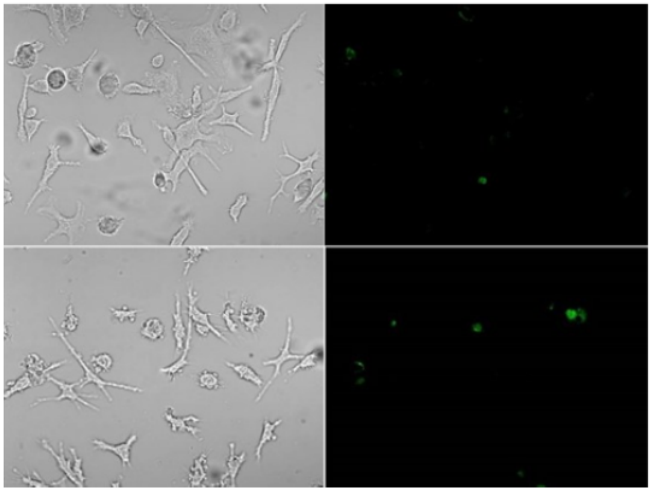
In situ synthesis of AuNCs by *Healthy* Microglial Cell

A. L. West et al., *ACS Appl. Mater. Interfaces* 2016, 8, 21221-21227



Effect of *glutamate inhibitor* on C8B4 cell viability and AuNC formation

Bright Field



Dark Field

Confocal microscopy images: C8B4-cells treated with Glutamate inhibitor (GI) 968. Upper Panel: C8B4+GI968; Lower Panel C8B4+HAuCl4 (10mM)+GI-968

Glutamates present in native proteins in C8B4 cells potentially responsible for Au NC formation

Bio-templated fMNCs: Summary

Protein/cell-templated fluorescent metal nanoclusters

- Synthesis by single, mixed proteins
- Synthesis successful in both tumorigenic and non-tumorigenic cells
- Emission in visible as well as in NIR region
- Multiple applications
  - Biocatalysis, real-time (chem, bio) sensing
  - Biomedical imaging
  - Photodynamic therapy
  - Fe (FeO) nanoclusters superparamagnetic – MR imaging application

Bio-templated fMNCs: Summary

Fundamental scientific questions:

- NC Formation Mechanism?
- NC Formation site (s) in protein? Amino acid residue (s)?
- NC-biomolecular interface – fundamental interactions…
- Emission source: metal NCs? Bio:NC composite? Emergent property?

THANK YOU!

Suggestions Comments Welcome