Communications

Gold Nanoclusters

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# Insulin-Directed Synthesis of Fluorescent Gold Nanoclusters: Preservation of Insulin Bioactivity and Versatility in Cell Imaging

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# Content:

- *Introduction*
- *Experimental section*
- *Results and Discussion*
- *Summary*

# i) Background:

- *Fluorescent nanomaterials have been studied extensively because of their unique optical and photo physical properties. Among various NCs, maximum effort has been dedicated to the study of* ***fluorescent Au-NCs.***

- *The fluorescent Au NCs with their ultrafine size do not disturb the biological functions……* ***Can be used as potential new luminescent level.***

- *Proteins as a green-chemical reducing and stabilizing agent is advantageous due to their complex 3D structure……* ***have been used for sensing*** $Hg^{2+}$ , $CN^{-}$, $H_2O_2$.

- *BSA-Au NCs have been used for* ***cancer cell imaging*** *through the conjugation to folic acid.*

## ii) Aim:

❑ *Protein directed growth of **fluorescent Au- NCs for retaining biological activity** so that the associated biological role can be pursued by various imaging techniques.*

❑ *Insulin, a polypeptide hormone, having 51 amino acid residues function in the regulation of insulin-responsive tissues and many other diseases, and hence exerts a profound influence on metabolism and reproduction……. **So Insulin was of prime interest.***

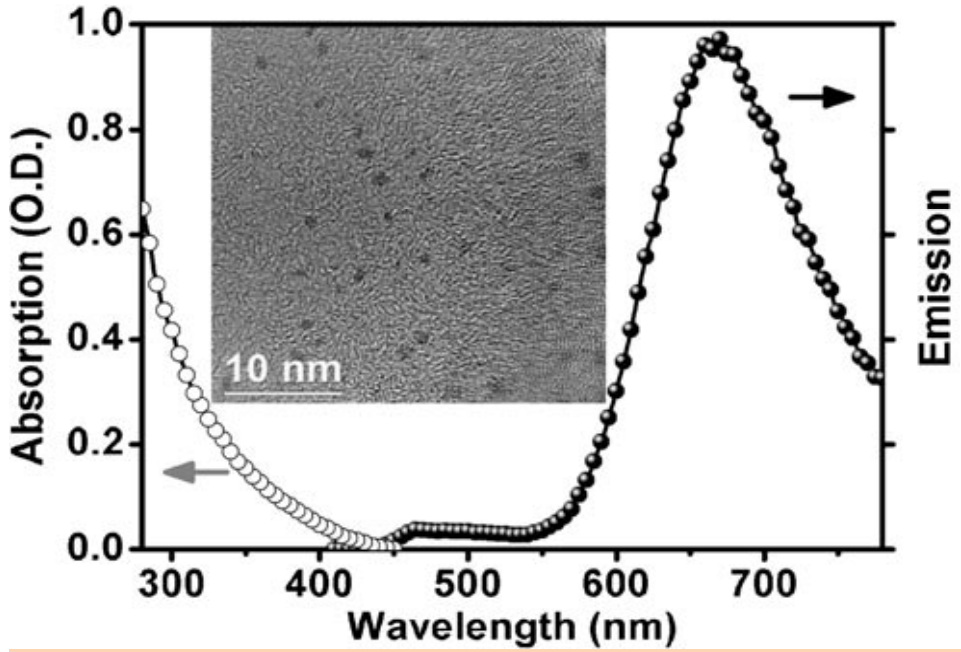
***……..They reported first time the synthesis of Au-NCs using Insulin as template.***

# Synthesis and characterization:

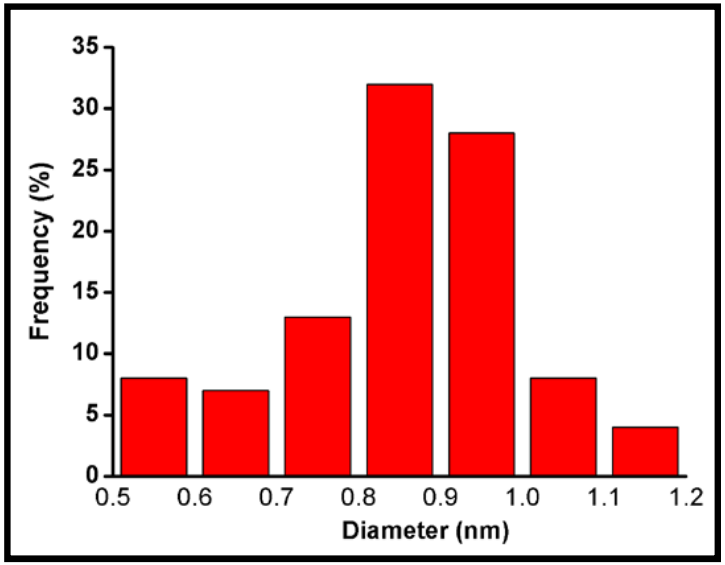
Insulin + $HAuCl_4, 3H_2O$ → ($Na_3PO_4$/ pH 10.4; 4°C / 12 hr in dark) → Au-NCs

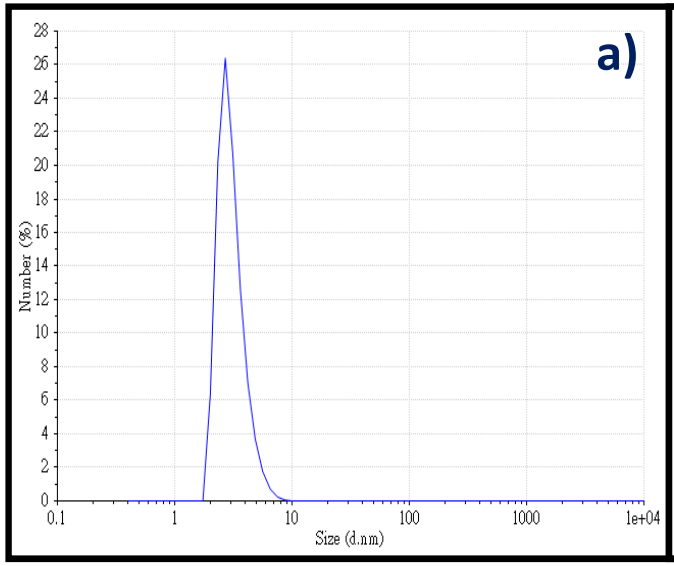
Centrifugal filtration

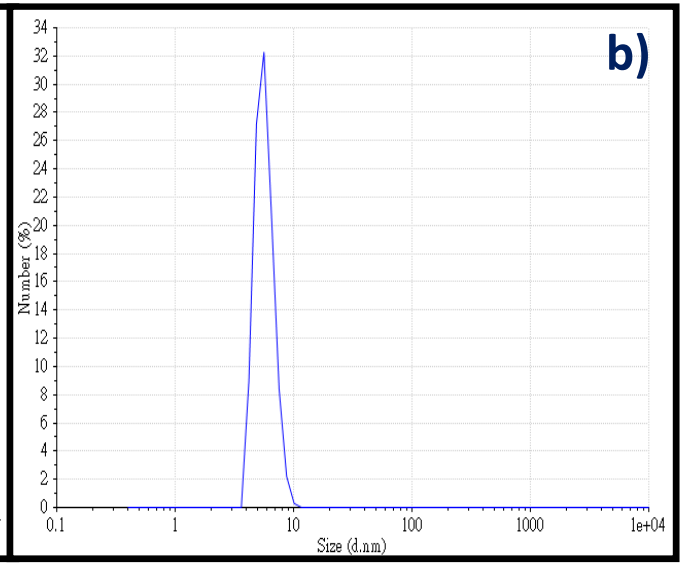
Purified Insulin-Au NCs




*Absorption and emission spectra of aqueous solutions of insulin–Au NCs. The excitation wavelength is at 400 nm. Inset: Representative HRTEM image*

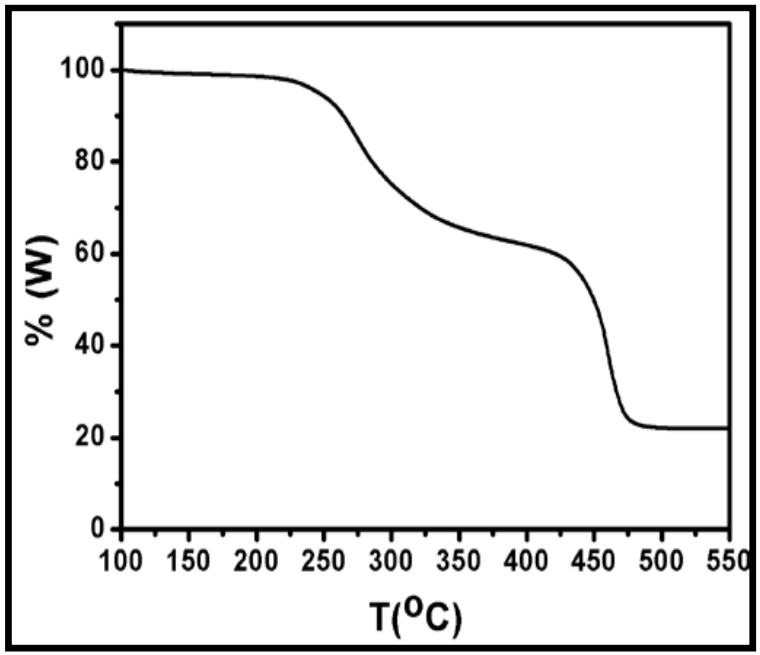


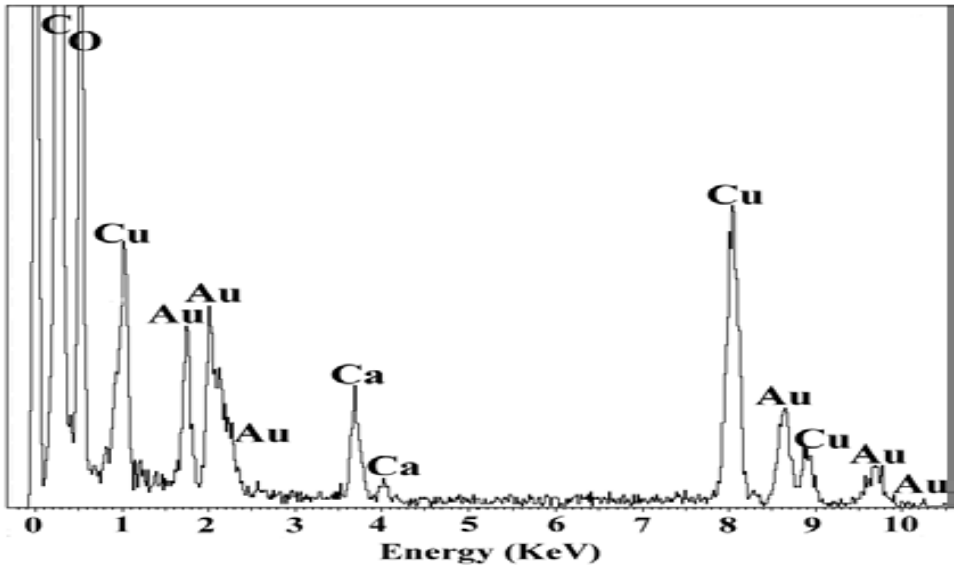




***The histogram analysis of the insulin-Au NCs.***

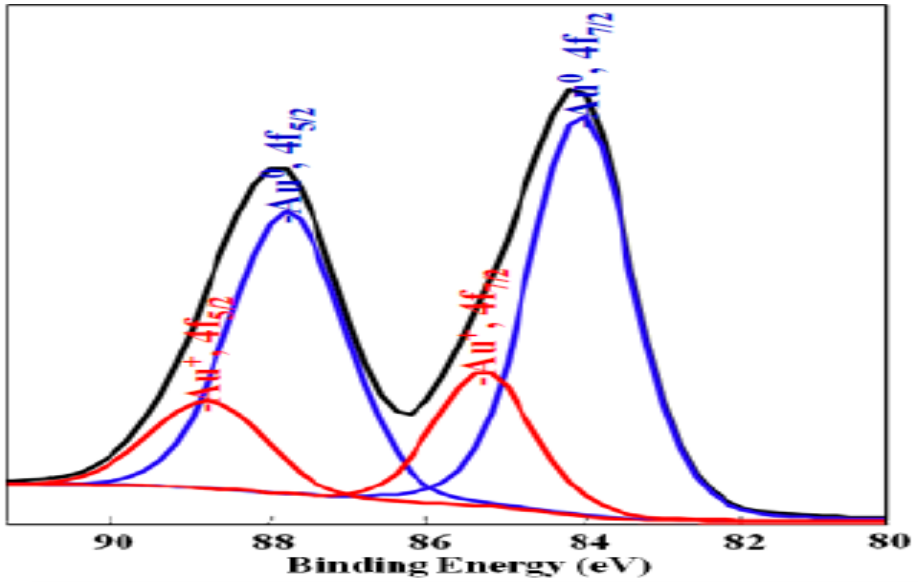
**Dynamic light scattering (DLS) shows that the mean sizes of a) insulin and b) insulin-Au NCs are 2.5±0.7 nm and 3.5±0.4 nm, respectively.**



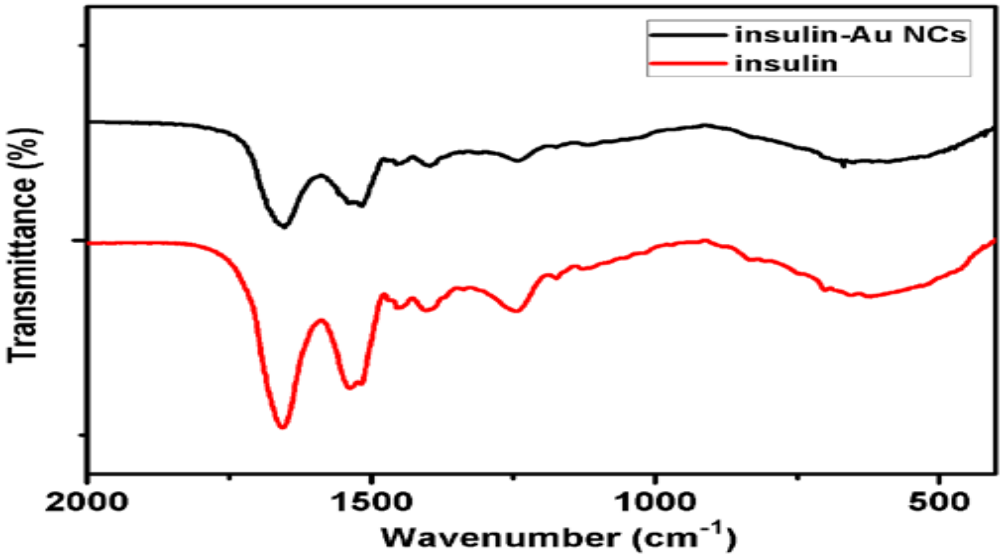

**Thermal gravimetric analysis (TGA) of insulin-Au NCs powder in air shows a ~80% weight loss with heating above 470°C.**



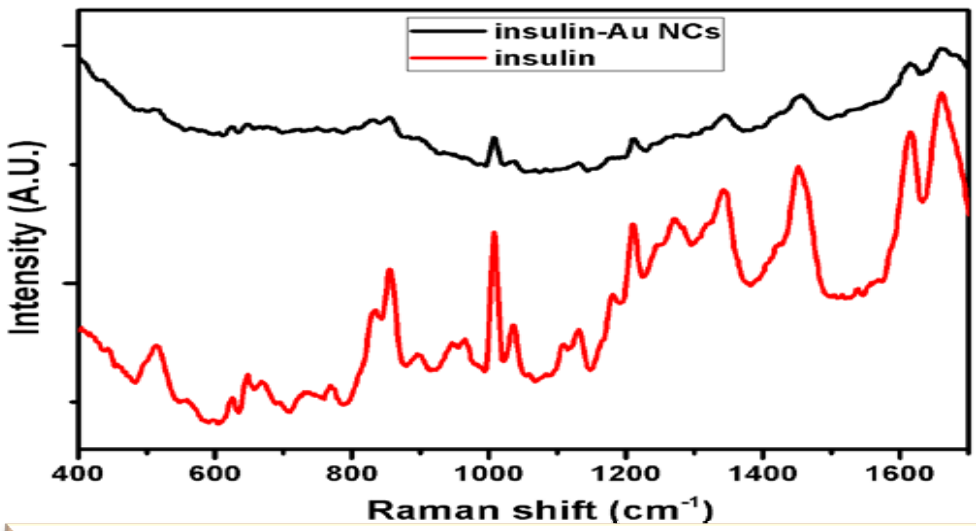

EDX spectra of insulin-Au NCs on a carbon-coated copper mesh grid



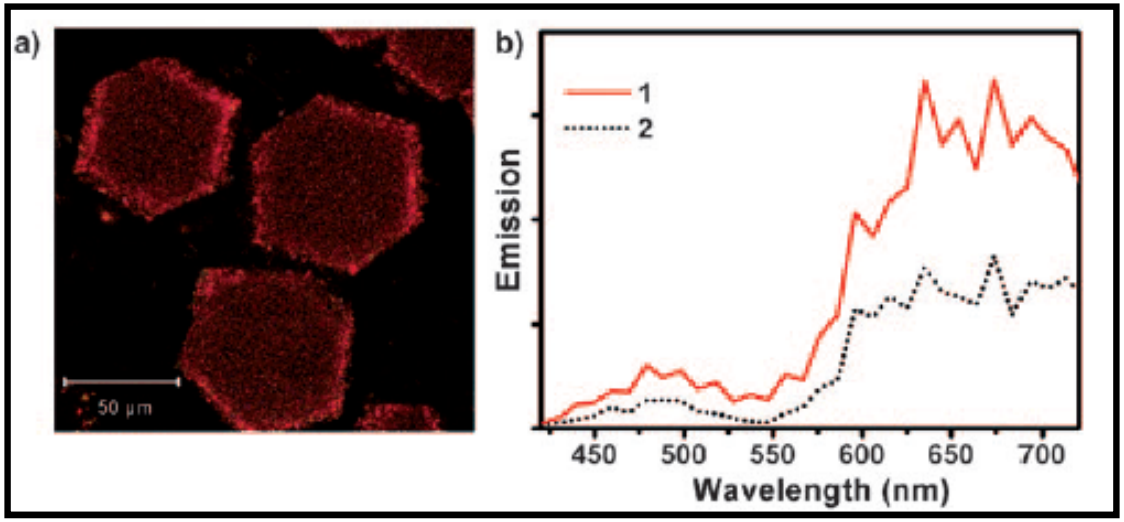

XPS spectra of atomic Au for insulin-Au NCs



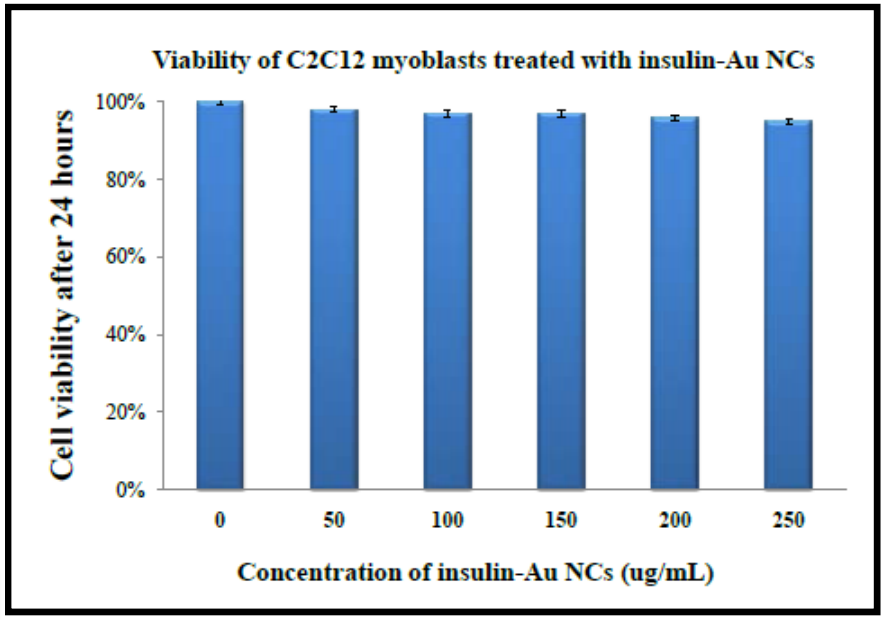

IR spectra of insulin-Au NCs and insulin



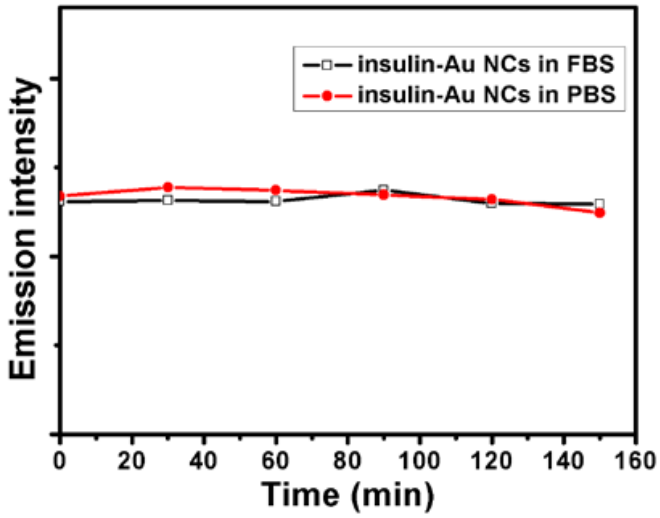

Raman spectra of insulin-Au NCs and insulin (λex: 413.1 nm).



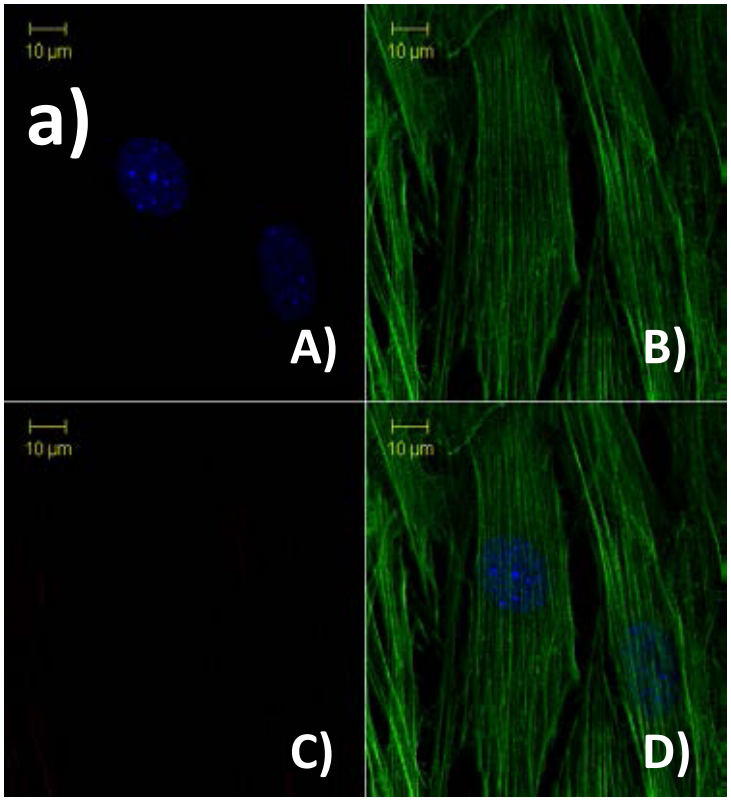

Two-photon fluorescence image of insulin–Au NC crystals. The excitation wavelength is 800 nm. b) Emission spectra of different depths of the crystal: 1) on the surface and 2) 5.0 mm below the incident surface.

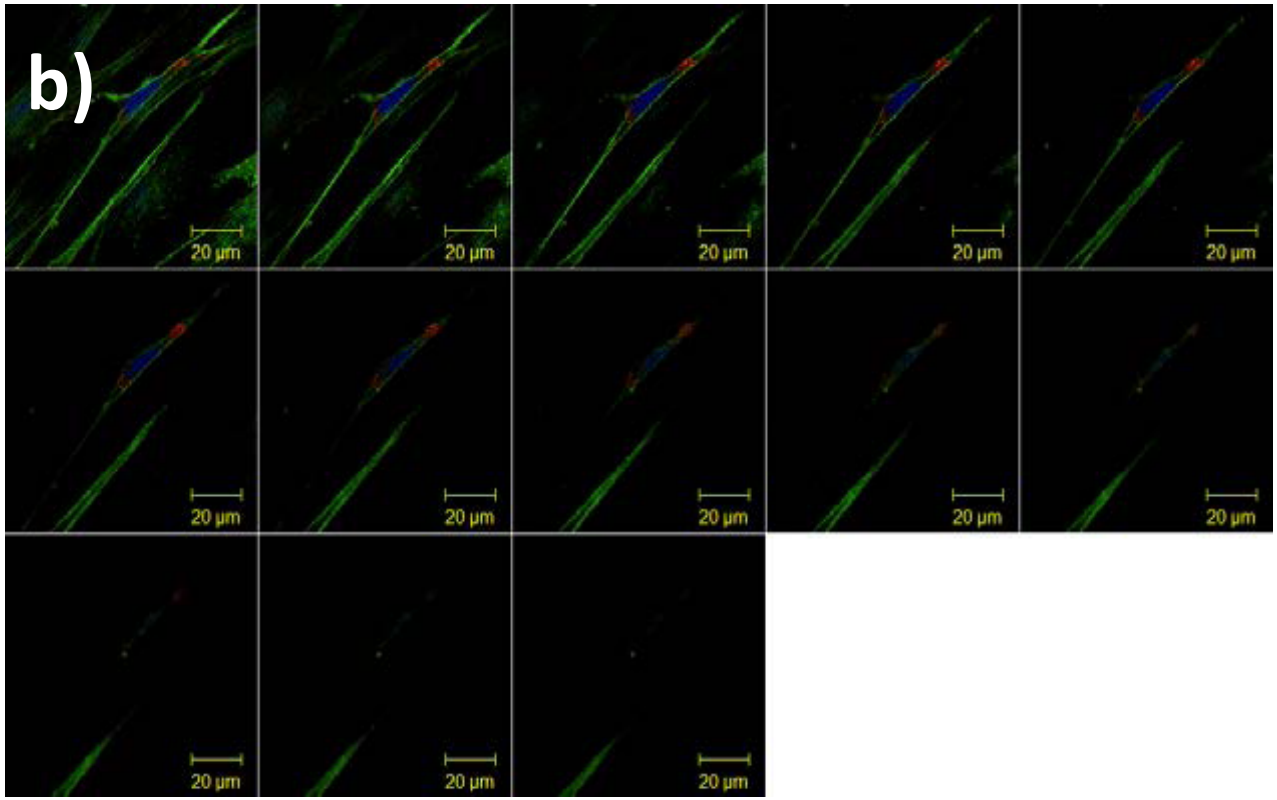



The viability of C2C12 myoblasts after treating with different conc. of insulin-Au NCs (0, 50,100, 150, 200, and 250 μg/mL) for 24 hours.



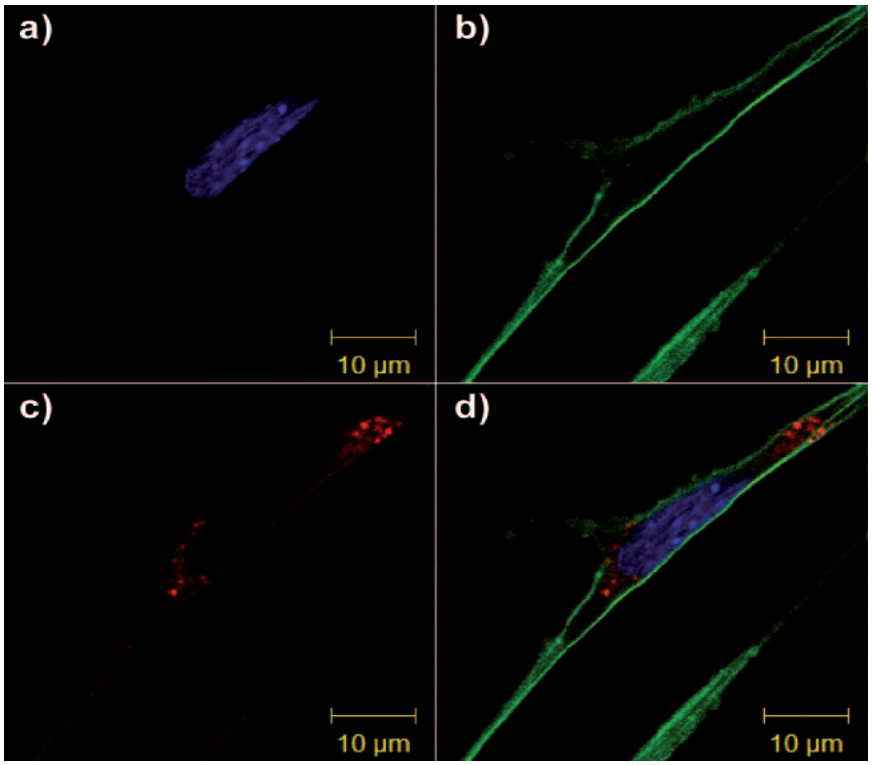

The fluorescence intensity as a function of time of insulin-Au NCs in FBS and PBS



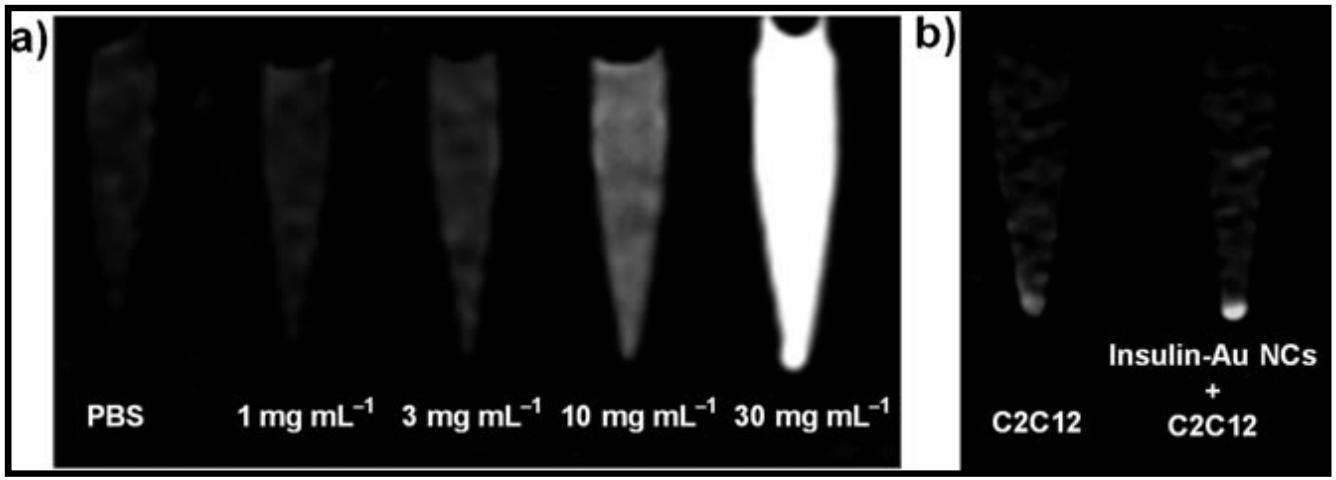




a) Control experiment of differentiated C2C12 myoblasts untreated with insulin-Au NCs. A) Cell nucleus was stained with DAPI (blue color). B) Actin fiber was stained with Alexa Fluor® 488 phalloidin to confirm the cell boundary (green color). C) No obviously red emission detected. D) Fluorescence image overlay of the three images.

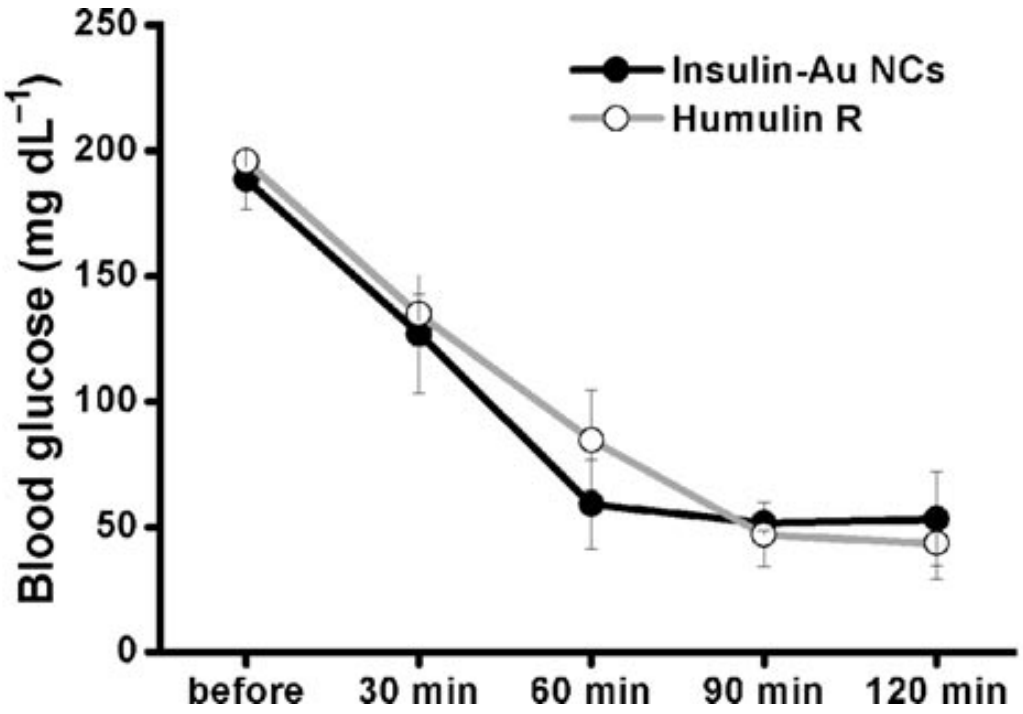
b) Z-stacking shows the insulin-Au NCs inside the C2C12 myoblasts



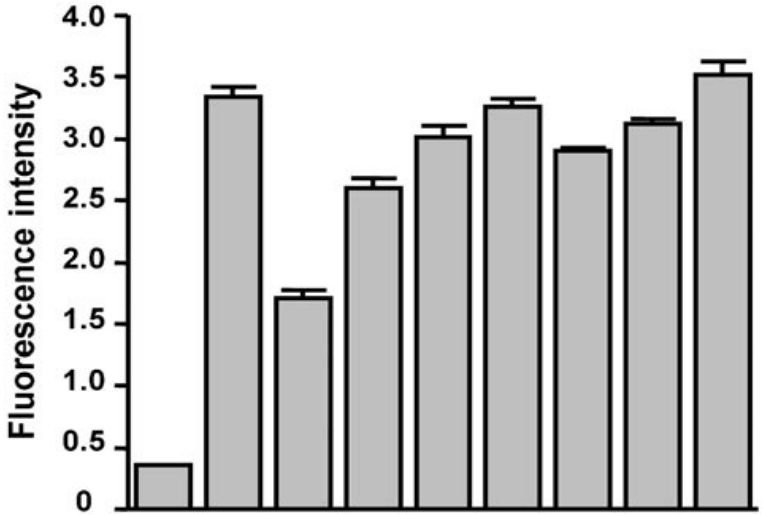

Microscopic observation of internalization of the insulin–Au NCs. Differentiated C2C12 myoblasts were treated with insulin–Au NCs for 2 h. a) Cell nucleus stained with 4',6-diamidino-2-phenylindole (DAPI, blue). b) Actin fiber stained with Alexa Fluor 488 phalloidin to confirm the cell boundary (green). c) Insulin–Au NCs exhibit red luminescence. d) Fluorescence image overlay of the three images.




a) CT imaging of insulin–Au NCs in sequential dosage and b) differentiated C2C12 myoblasts with (20 mgmL1, right) and without (left) insulin–Au NCs.




Blood glucose versus elapsed time of treatments with insulin–Au NCs and Humulin R of Wistar rats.




| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Brain homogenate | + | - | + | + | + | + | + | + | + |
| Insulin-Au NCs | - | + | + | + | + | + | + | + | + |
| Racecadotril (mM) | - | - | - | 0.01 | 0.1 | 1 | - | - | - |
| Thiorphan (mM) | - | - | - | - | - | - | 0.01 | 0.1 | 1 |

Fluorescence quenching (monitored at 670 nm) of insulin–Au NCs by brain homogenate and brain homogenate inhibited by racecadotril and thiorphan.

# Summary:

❖*They have reported* ***for the first time*** *the insulin-directed synthesis of fluorescent gold NCs.*

❖*The as prepared insulin–Au NCs show* ***excellent biocompatibility and retain the insulin bioactivity.***

❖*Versatility in applications such as* ***fluorescence imaging, CT, and in vivo blood-glucose regulation*** *has been successfully demonstrated.*

❖*The insulin–Au NC imaging techniques may provide innovative and supplementary methods in addition to conventional isotope* ***I-insulin and anti-insulin antibody conjugated to chemiluminescent enzyme, which should be highly attractive to biolabeling and bioimaging applications in the future.***

# ***Future Outcome:***

❖*In our Lysozyme work also we got similar kind of situation……* ***no mass shift in the protein molecular mass region. Similar kind of study can be done to prove*** $Au_{25}$***(?) cluster is forming outside the protein.***

❖*Lysozyme is also having some biological activity……* ***retention of that can be checked by some hydrolysable substance.***

❖*Extent of* ***accessibility*** *can be checked to perform better* ***catalytic reactions****.*

Thank you.....