Single Gold Nanoparticles as Real-Time Optical Probes for the Detection of NADH-Dependent Intracellular Metabolic Enzymatic Pathways

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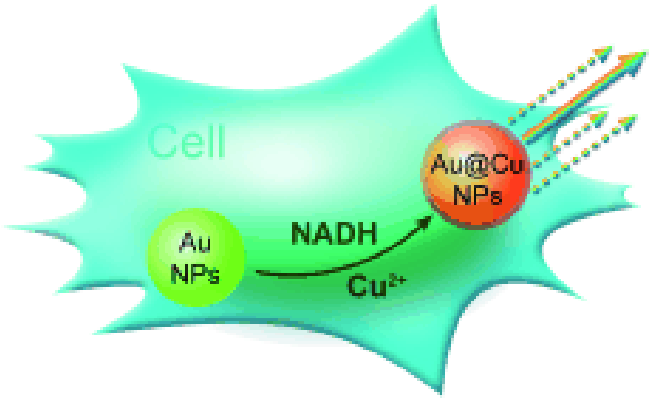

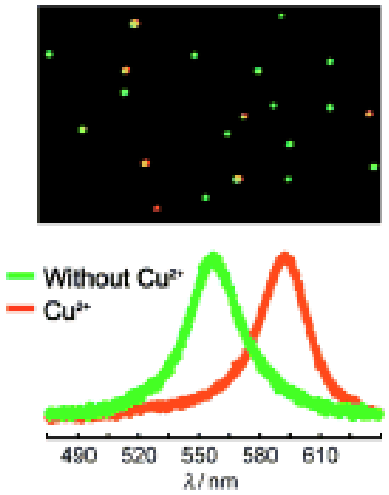

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M. S. Bootharaju

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Introduction

- Plasmonics, is an emerging subfield of nanophontonics, and it attracts increasing attention because of its potential applications in controlling and manipulating light at nanoscale dimensions.
- Single particle spectroscopy has become important which will give scattering spectrum of single nanoparticles affected by their size, shape, composition as well as the local environment, which further facilitate its use in biological-labeling and detection.
- DFM has been used for optical sensing, probing redox reactions, in vivo imaging of cancer cells, etc. in real time.
- Every individual nanoparticle (NP) in the assembly could potentially act as an independent probe.
- Single nanoparticle probes offer improved absolute detection limits and also enable higher spatial resolution.

• NAD^+/NADH plays an important role as cofactor in numerous biocatalyzed processes, including energy metabolism, mitochondrial responses, immunological functions, aging and cell death.

• In this paper, The catalytic deposition of copper on gold nanoparticles (AuNPs) by the NADH cofactor has been applied for the optical and electrochemical detection of NADH and NAD^+-dependent biocatalytic processes.

• Here, they monitor the intracellular metabolism and the effect of anticancer drugs on the cell metabolism using copper growth on the AuNP probes.

Experimental Section

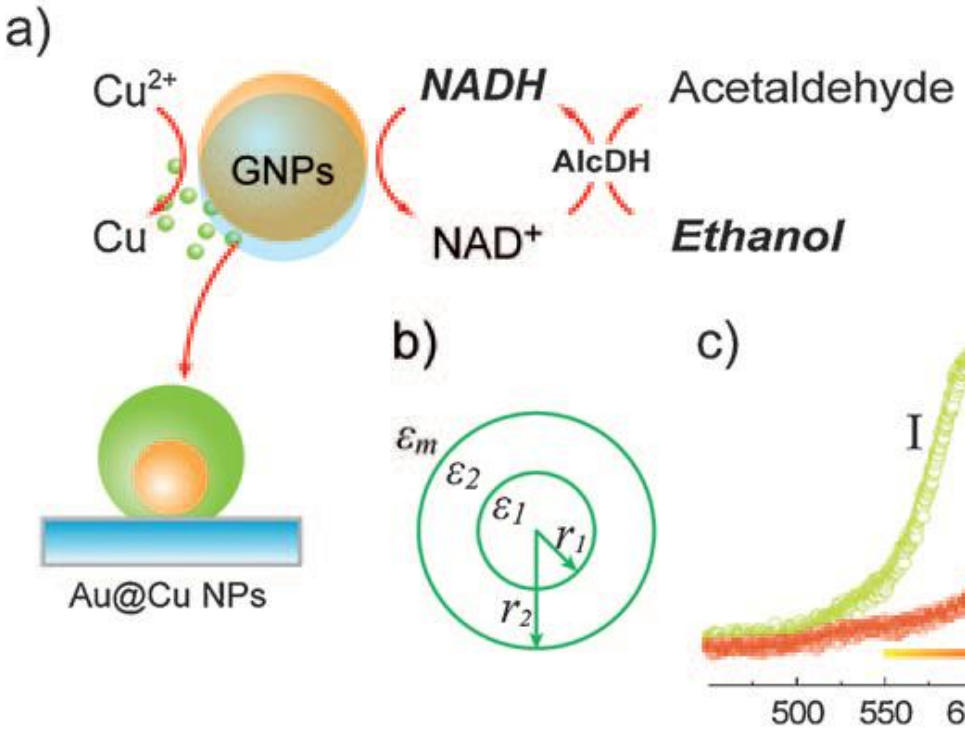
$$\text{Au@citrate NP seeds} + Au^{3+} \xrightarrow{NH_2OH.\ HCl} > 20\ \text{nm particles}$$

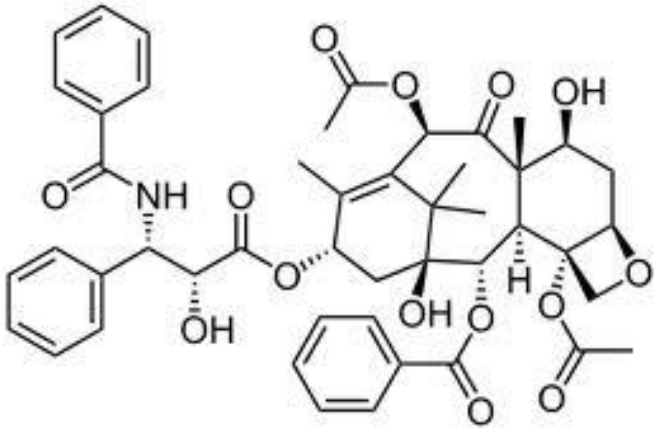
For cell culture, Tween 20 was added for stability followed by centrifuged for 30 min., at 5000 rpm

Cell culture

HeLa cells were incubated with freshly prepared Au NPs for 24 h. 10 μM of taxol solution was added to the culture medium, followed by incubation at 37 °C for 5 h. Rinsed with tris buffered-saline followed by addition of 20 μL $CuCl_2$ solution.

Results and discussions





bark of the pacific yew (Taxus brevifolia Nutt)

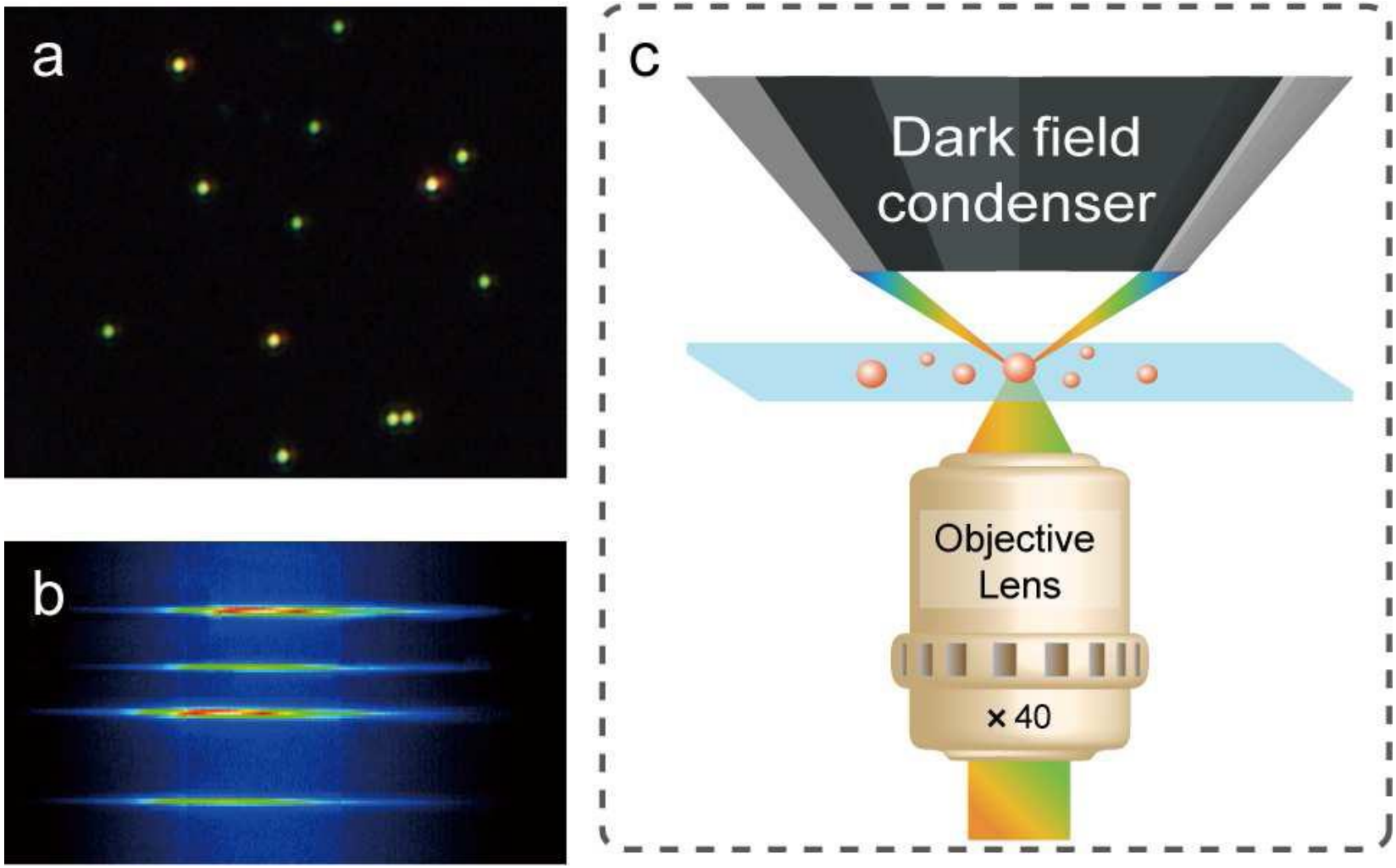


Figure. a, A typical dark-field Rayleigh scattering image of GNPs modified on a microscopy slide; b, Corresponding scattering spectra of single GNPs in slit. c, Detailed experimental configuration.

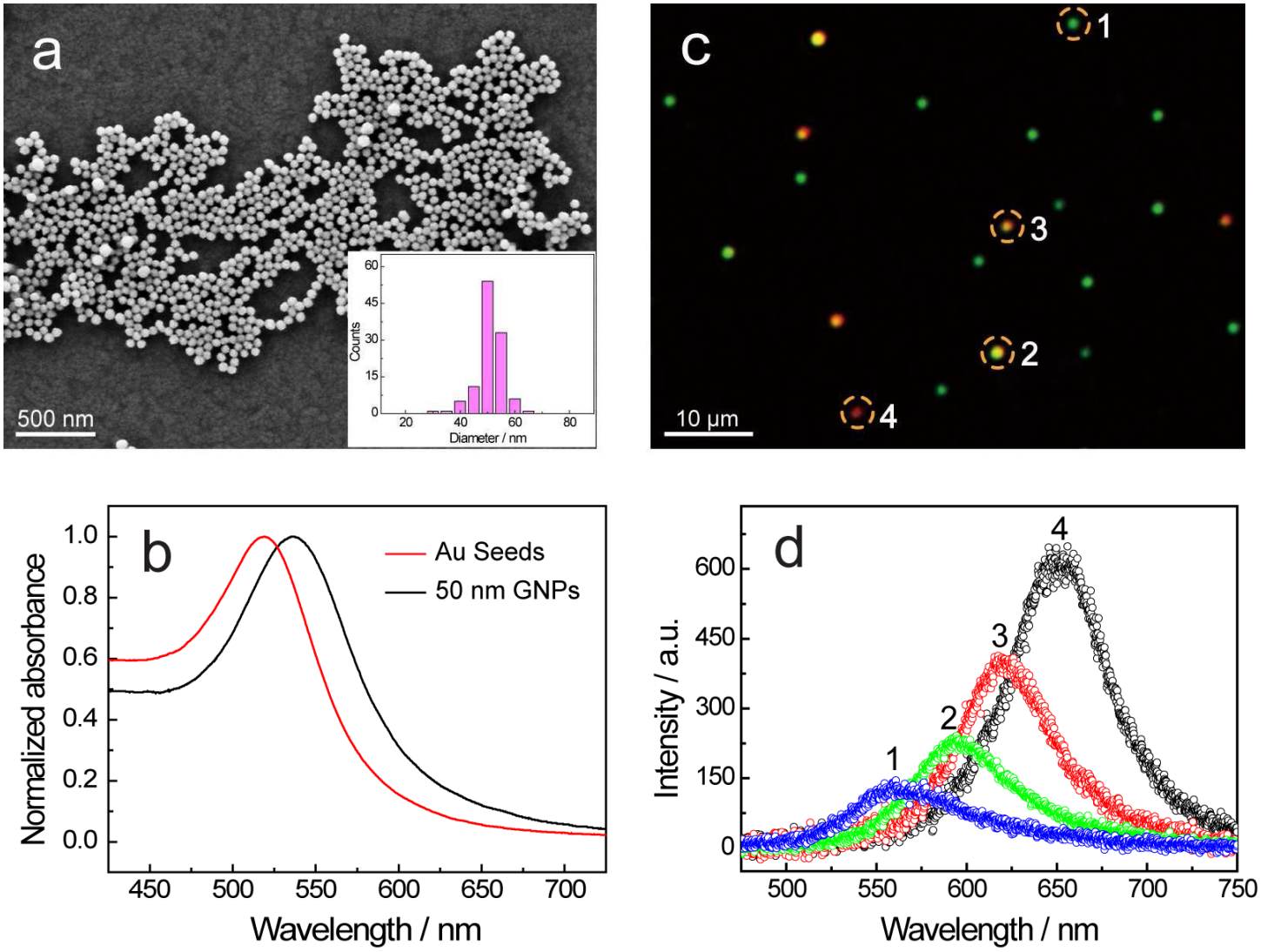


Figure. a, SEM images of 50 nm GNPs. b, UV-vis spectra of seed GNPs and 50 nm GNPs. c, Dark-field color images of GNPs immobilized on microscopy slide. d, Corresponding PRRS spectra of single GNP with different size

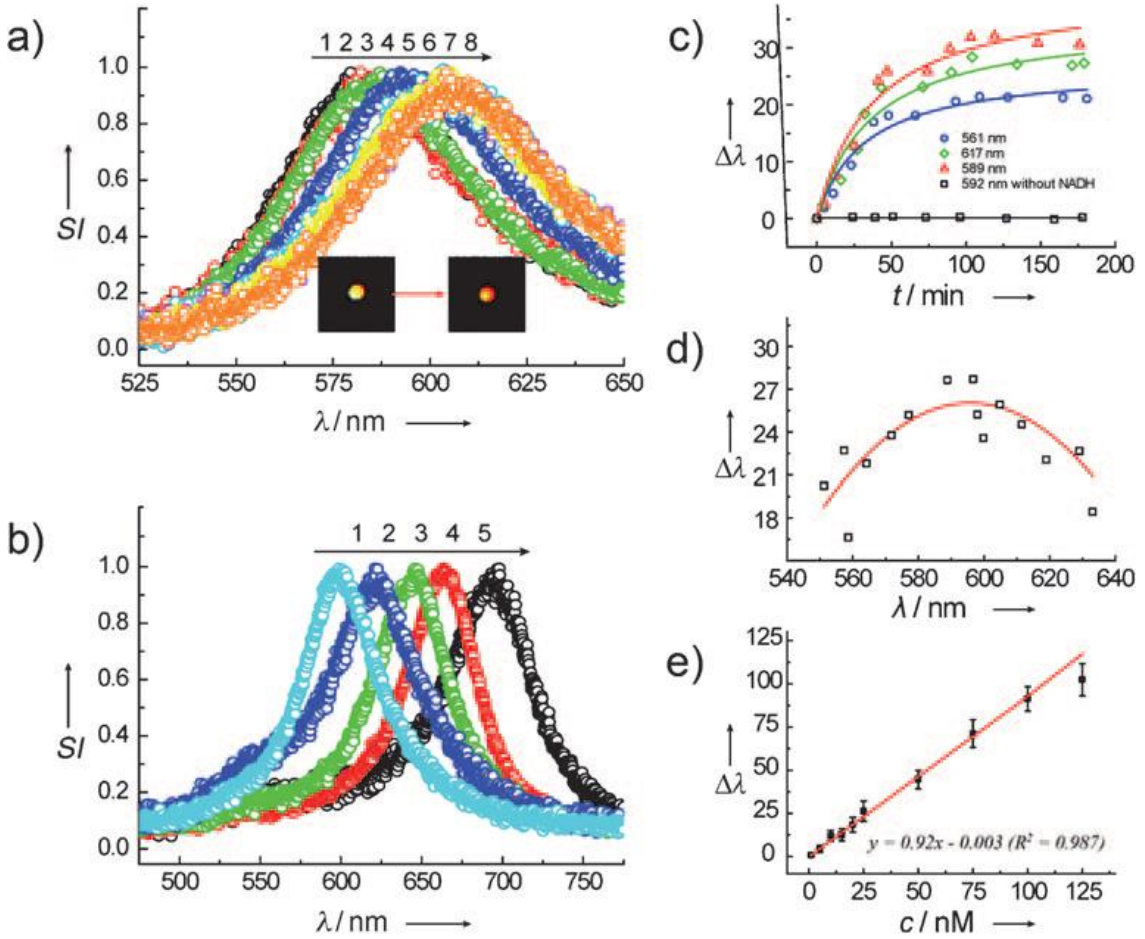


Figure. a) Representative time-dependent single AuNP scattering spectra upon treating the AuNPs with $CuCl_2$ 20 μM and NADH 30 nM, showing that the λ_{max} of the PRRS spectra are red shifted, spectra 1–8: 0, 5, 13, 24, 41, 59, 87, 131 min. The insets shows the color image of a typical AuNP before and after being covered by the Cu shell, demonstrating the color transition from yellow to red.b) The PRRS spectra of Au@Cu core–shell nanoparticles upon interaction with different concentrations of NADH for a fixed time interval of 2 h, spectra 1–5: 0, 25, 50, 75, 100 nM of NADH. c) Time-dependent $\Delta\lambda_{max}$ changes of three different sized AuNPs with $\Delta\lambda_{max}$ values of 561 nm (*), 589 nm (~) and 617 nm (^) upon treatment with $CuCl_2$/NADH. d) Distribution curve corresponding to the $\Delta\lambda_{max}$ values of a collection of different AuNPs treated with $CuCl_2$/NADH for 2 h. The red line is the Gaussian fit of the experimental data. e) Calibration plot corresponding to the $\Delta\lambda_{max}$ shifts of the PRRS spectra at different concentrations c of NADH for a single AuNP. (The scattering intensities (SI) of the all the spectra have been normalized.)

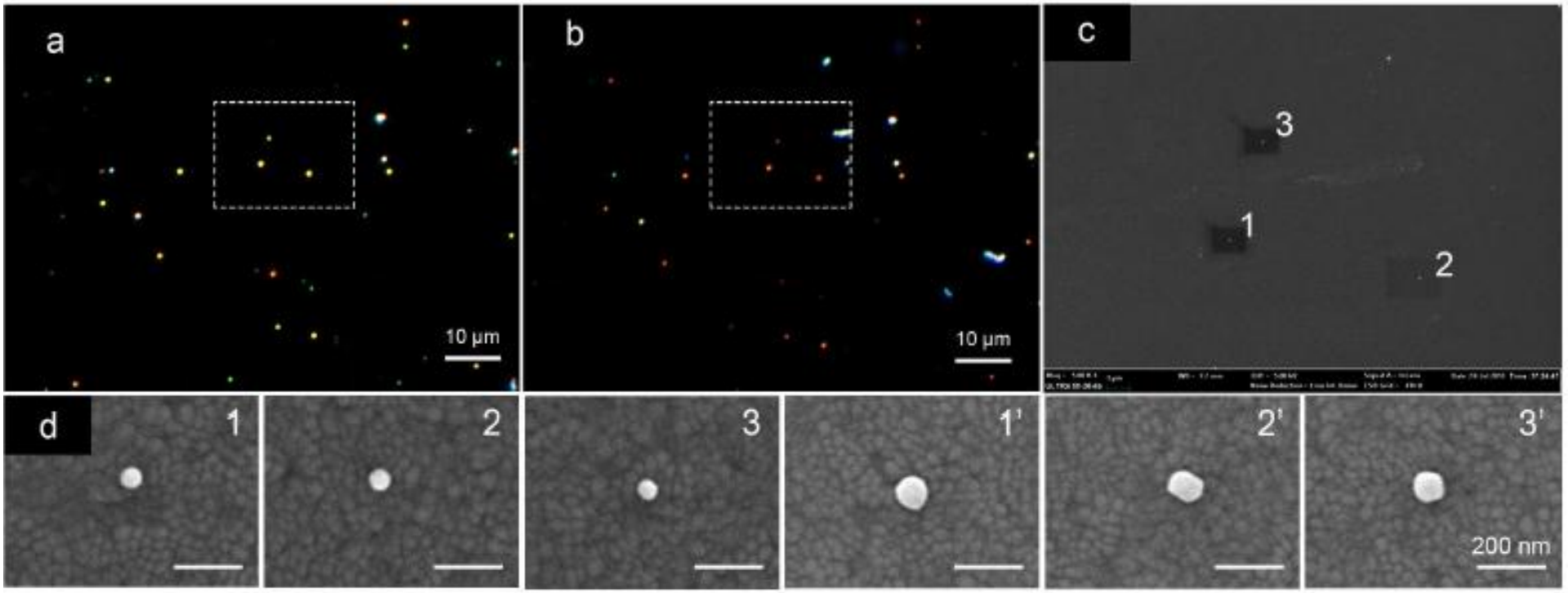


Figure. True color images of GNPs Plasmon resonance Rayleigh scattering before (a) and after (b) the formation of Au@Cu core-shell nanostructure. c, Corresponding SEM image of selected region in a. d, Enlargement SEM images of each single GNP with corresponding number (1~3: original GNPs, 1'~3': Au@Cu NPs). GNPs in 20 μM $CuCl_2$ containing 25 nM NADH for 3 h.

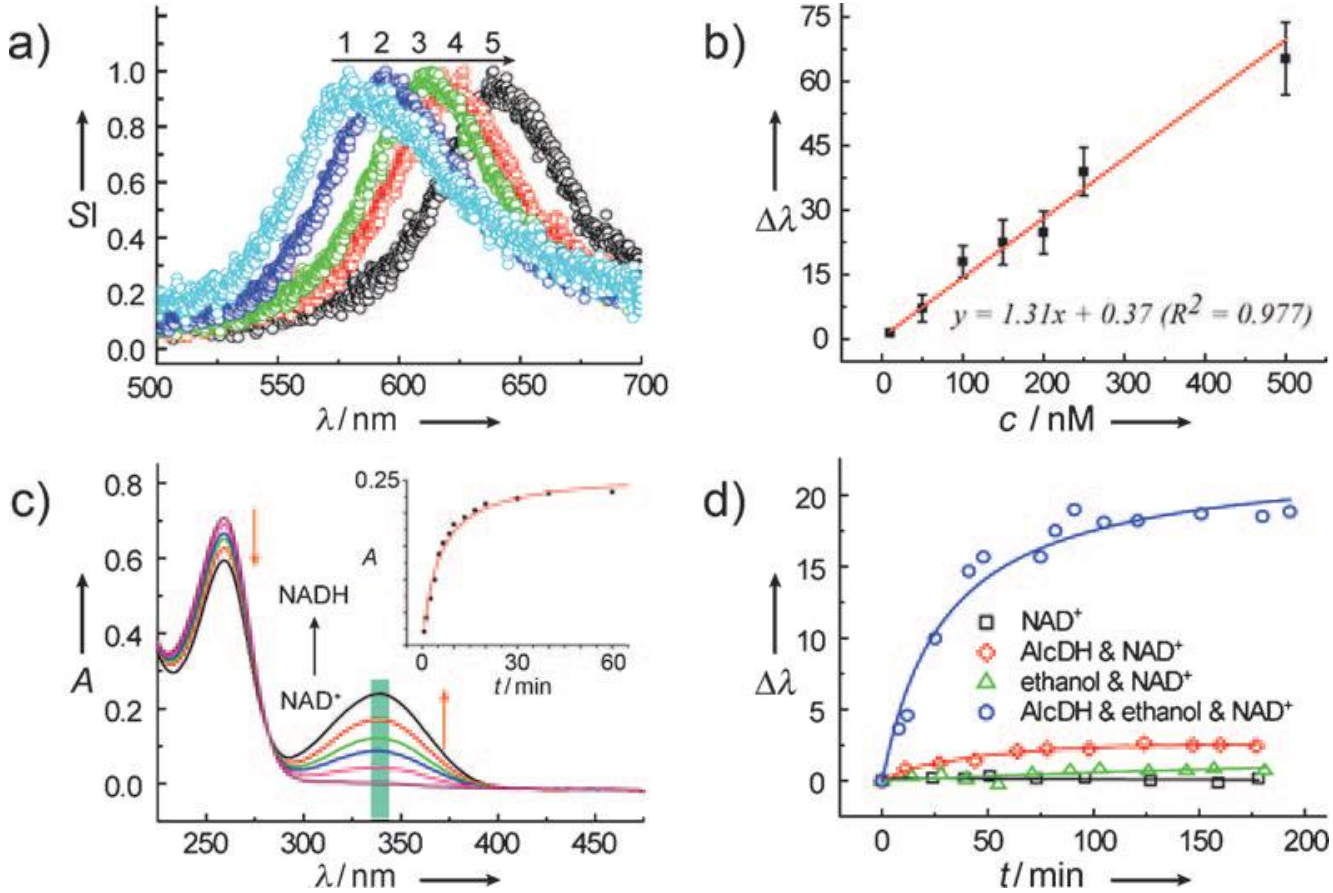


Figure. a) Representative scattering spectra of a typical single AuNP in developing solution containing a varying concentration of mixture (spectra 1–5: 0, 100, 200, 250, 500 nM of ethanol; after a fixed reaction time of 30 min, medium: 1 mM NAD^+, 0.1 mg/mL AlcDH, and 20 μM $CuCl_2$). b) Calibration plot corresponding to the PRRS spectra $\Delta\lambda_{max}$ shift with developing solution containing different concentrations of ethanol. c) Real-time UV/Vis spectra of the mixture of NAD^+ (1 mM), ethanol (1 μm) and AlcDH (0.1 mg/mL) at different times; Inset: the relationship between intensity (340 nm; green bar in the main spectrum) and time. d) The plots of scattering spectra $\Delta\lambda_{max}$ versus time for AuNPs under different conditions.

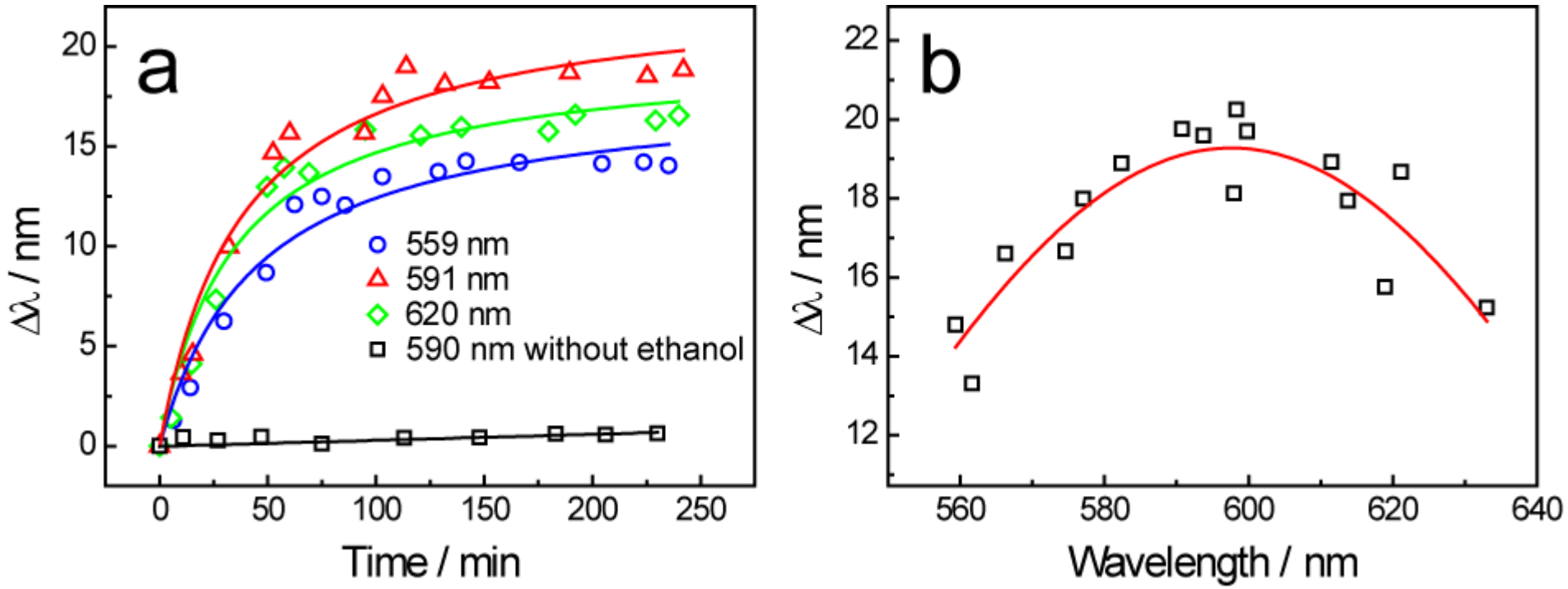


Figure. a, Time-dependent $\Delta\lambda_{max}$ changes of three different sized GNPs exhibition λ_{max} values corresponding to 559 nm (○), 591 nm (△) and 620 nm (◇) upon treatment with the mixer of $CuCl_2$/NAD^+/AlcDH/ethanol. $\Delta\lambda_{max}$ shifts of up to 19 nm are observed, upon the formation of the Au@Cu nanostructure in the presence of ethanol. b, Distribution curve corresponding to the $\Delta\lambda_{max}$ values of a collection of different GNPs interacted with the mixer of $CuCl_2$/NAD^+/AlcDH/ethanol for 2 hours. The red line is the Gaussian fit of the experimental data.

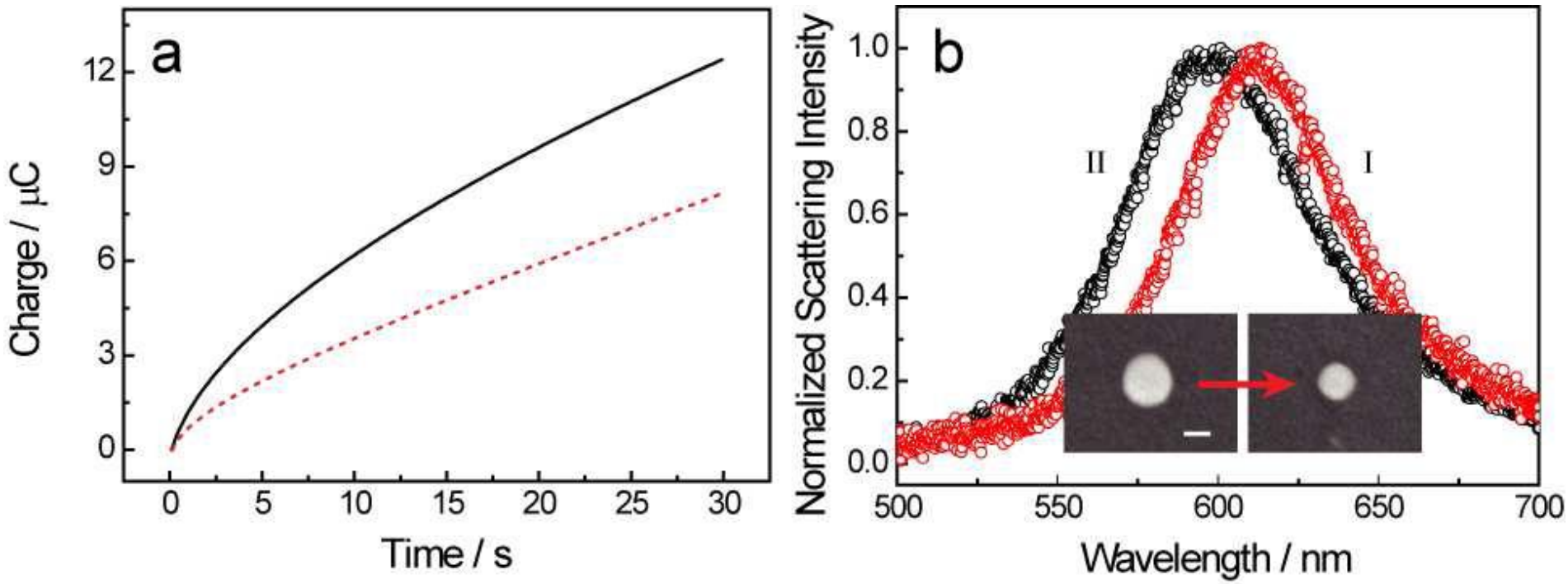
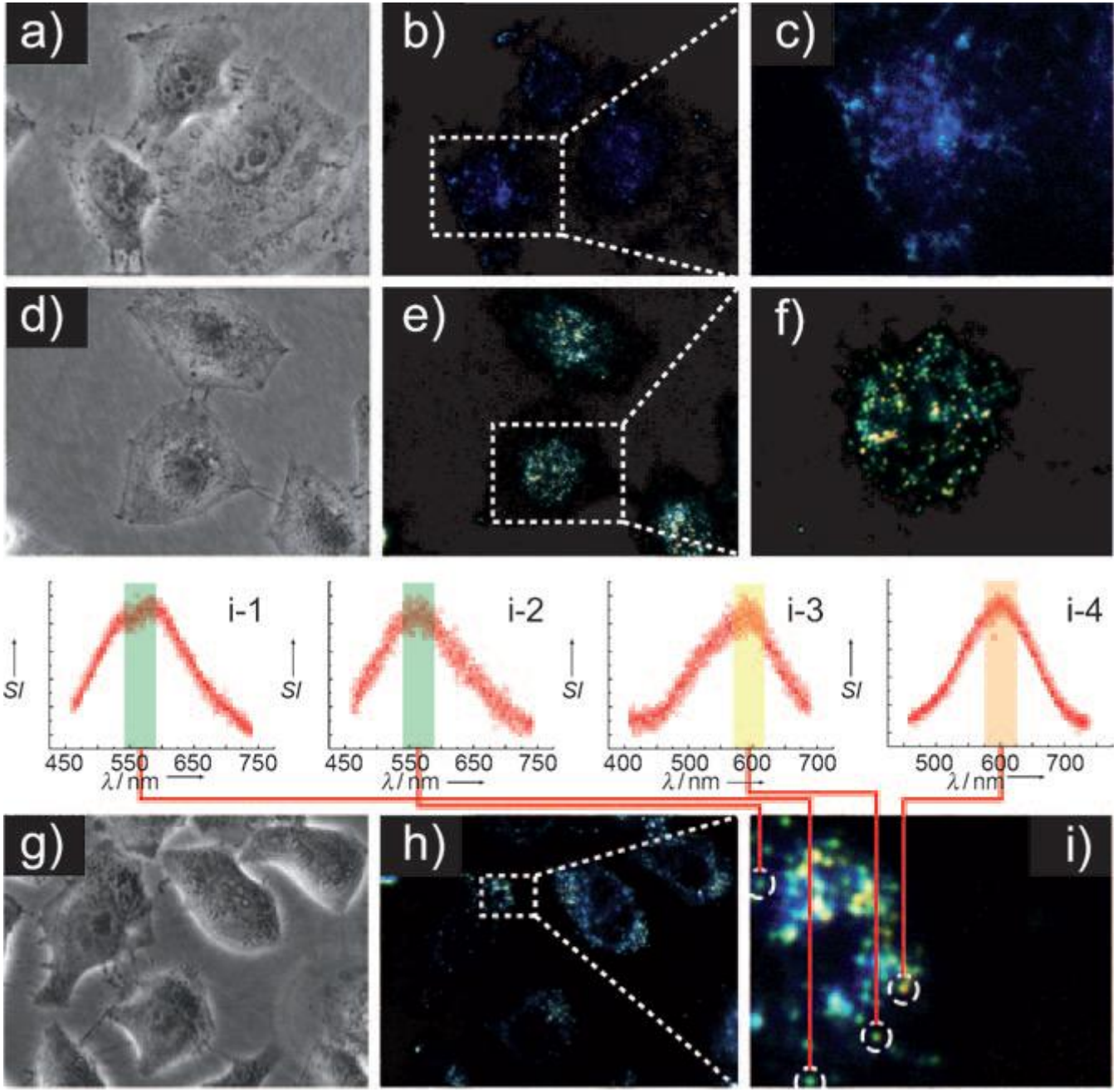


Figure. a, Chronocoulometric transients measured upon application of a potential step of -0.4 V to 0.1 V on the GNP-modified ITO electrode after deposition of Cu^0 in the absence (dot line) and in the presence of 25 nM NADH (solid line). b, PRRS spectra of a single GNP: I) Au@Cu core-shell with 25 nM NADH, II) GNPs after electrochemical stripping for 10 min. The obtained intensities of the all the scattering spectra have been normalized. The insert is SEM images of a typical individual nanoparticle before and after electrochemical stripping experiment, scale bar, 50 nm.


a)
b)
c)
d)
e)
f)
i-1
i-2
i-3
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SI
SI
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450 550 650 750
λ / nm
450 550 650 750
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400 500 600 700
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g)
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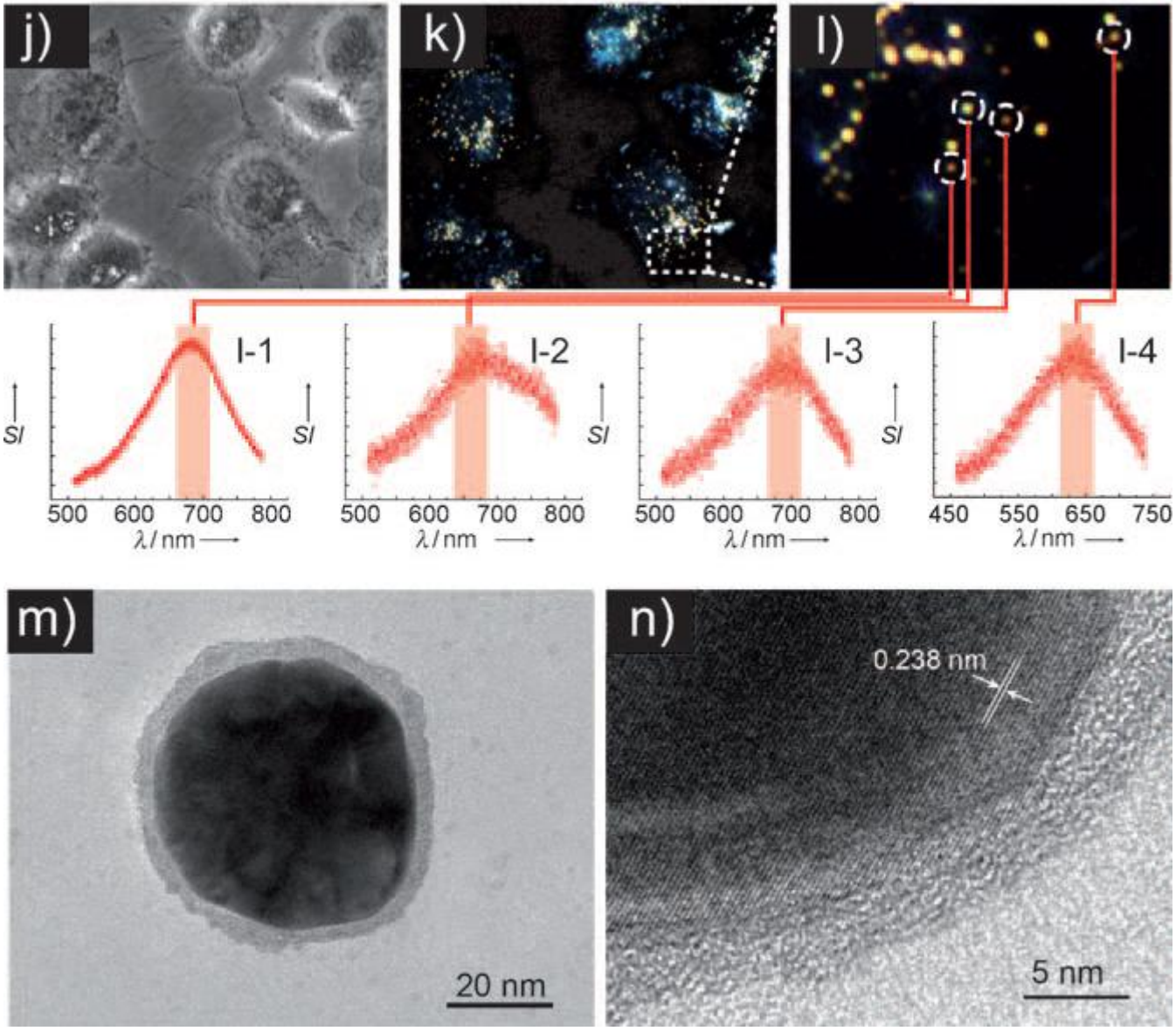
j)
k)
l)
I-1
I-2
I-3
I-4
SI
500 600 700 800
450 550 650 750
λ / nm
m)
20 nm
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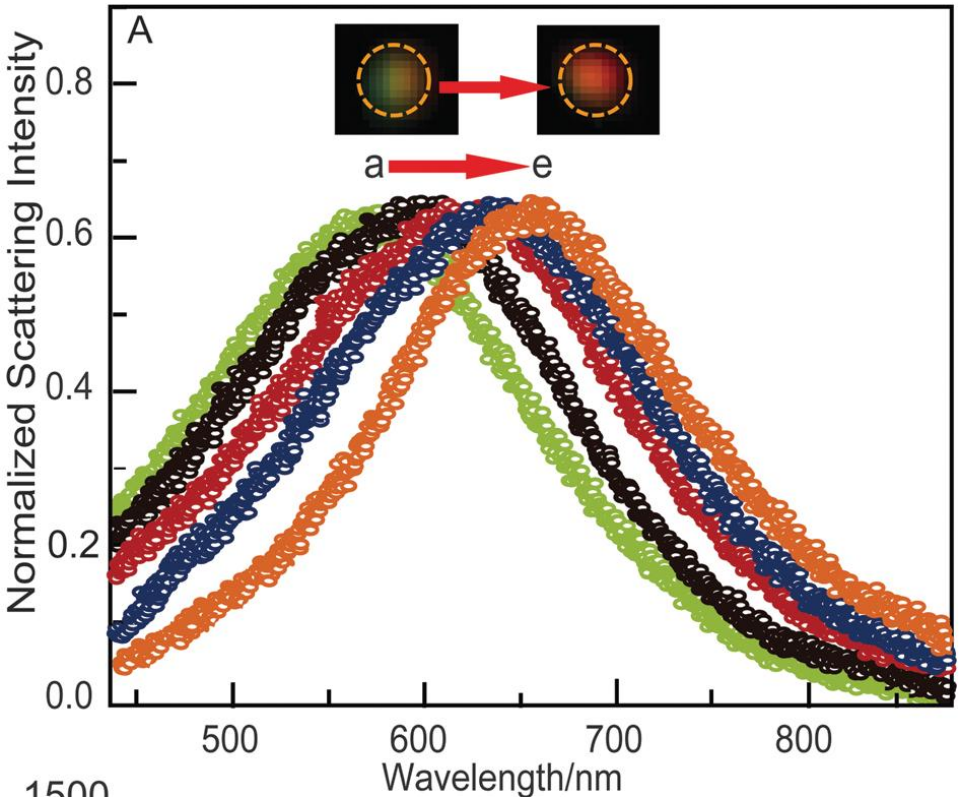

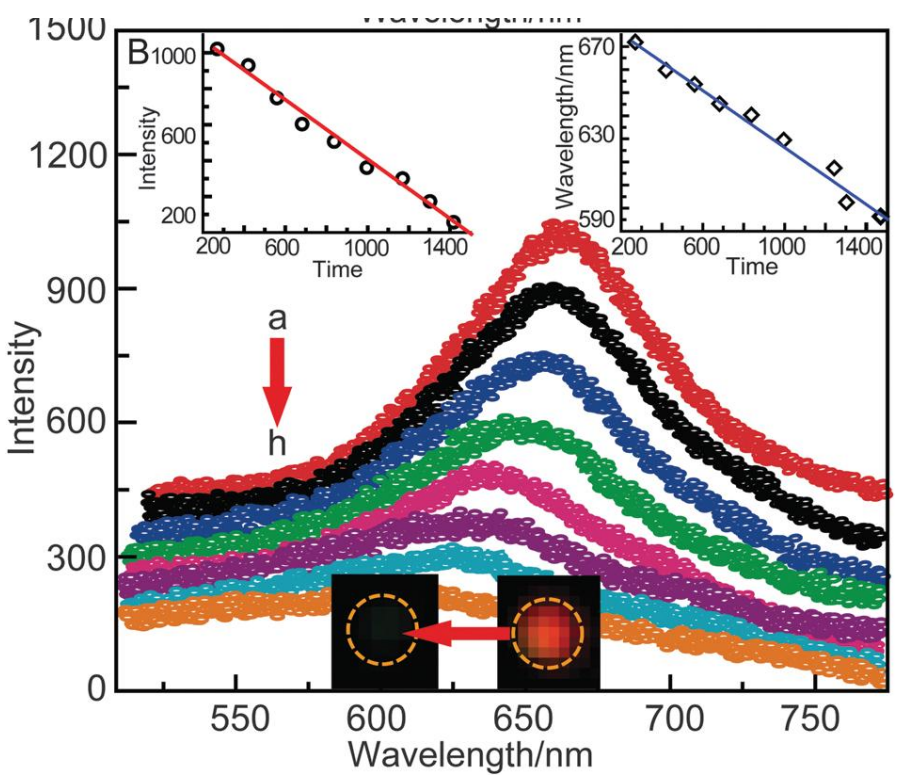

http://pubs.rsc.org/en/content/articlepdf/2012/cc/c1cc14326c

Thank you

May be possible to study,

- Interactions of nanoparticles with biologically relevant molecules and ions (urea, glucose, sucrose, I^-, etc.).
- We know that glucose acts as reducing agent for nanoparticles synthesis. We can monitor nanoparticles evolution in real time.