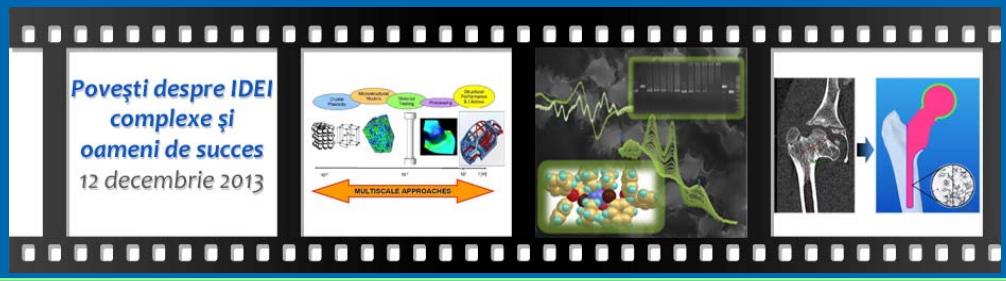


MINISTERUL EDUCAȚIEI NAȚIONALE

Proiect PCCE 129 / 2008

Nanoparticule biofunctionale pentru dezvoltarea unor noi metode de imagistica, senzoristica, diagnostic si terapie moleculara in medii biologice (NANOBIOFUN)

Director proiect: Prof Dr Simion Astilean

Responsabili echipe parteneri:

Prof dr Simion Astilean,	Facultatea de Fizica
Acad Prof dr Octavian Popescu,	Facultatea de Biologie
Prof Dr Simion Simon,	Facultatea de Fizica
Conf dr Gabriela Nemes,	Facultatea de Chimie
Prof dr Onuc Cozar (Prof Dr Vasile Chis),	Facultatea de Fizica
Prof Dr Beu Titus,	Facultatea de Fizica
Prof Dr Mircea Diudea,	Facultatea de Chimie
Prof Dr Catalin Popescu,	Facultatea de Chimie

Instituție coorodonatoare & parteneri — **Universitatea Babes-Bolyai**

Obiective Majore

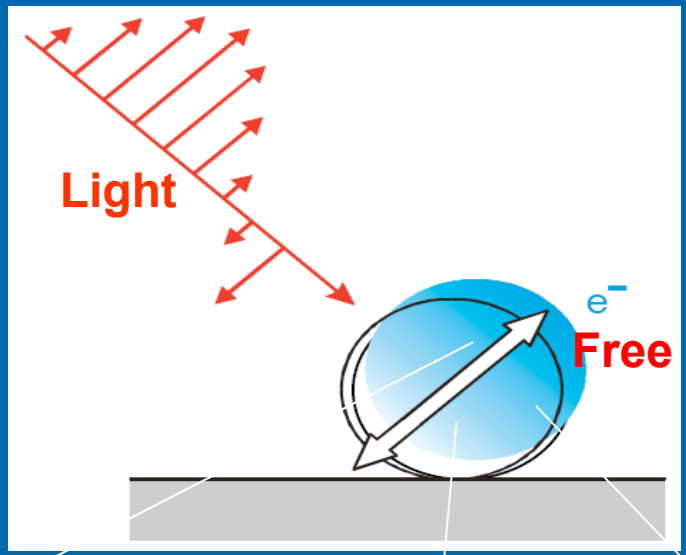
1. Demonstrarea unui model de nanoparticula plasmonica actionata de lumina, capabila sa opereze ca “nano-bisturiu” pentru distrugerea selectiva a celulelor tumorale prin efectul cresterii temperaturii acestora localizate in celule.

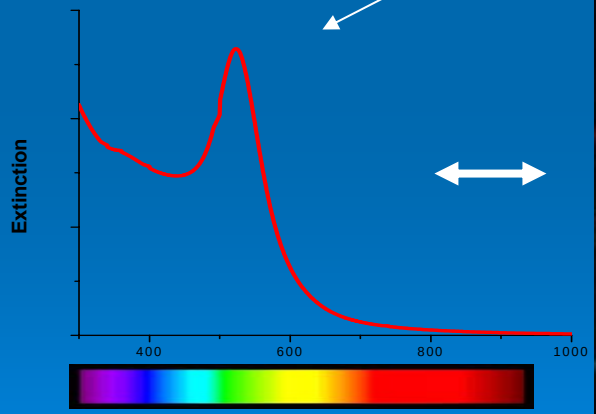
2. Implementarea unor noi metode de senzoristica prin metode optice, spectroscopice si electrochimice pe baza de nanoparticule si nanostructuri plasmonice in vederea detectiei si identificarii unor molecule relevante si biomarkeri

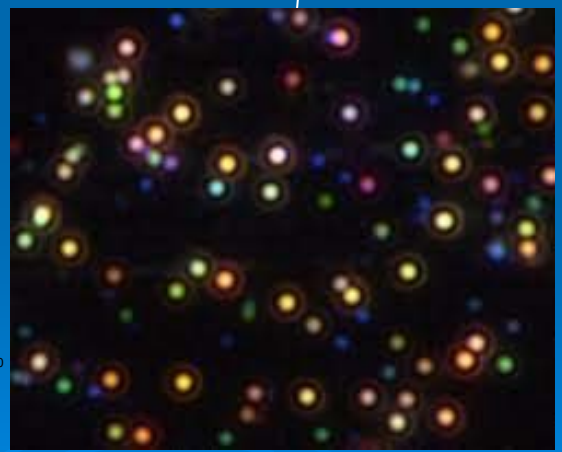
3. Modelarea interactiunilor si structurilor la interfata nano-obiectelor

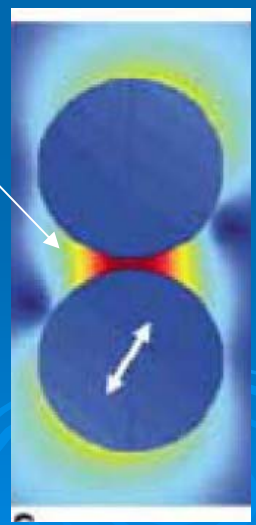
4. Educarea si formarea resursei umane prin activiati de cercetare in domeniului nanotehnologilor

Surface Plasmon Resonances









Resoanant Light Absorption
extinction coefficient of ~ 10^{11} M-1 cm-1

Resonant Light Scattering
~ 10^{6} dye fluorophores

Enhanced Optical Field
~10^{3} times

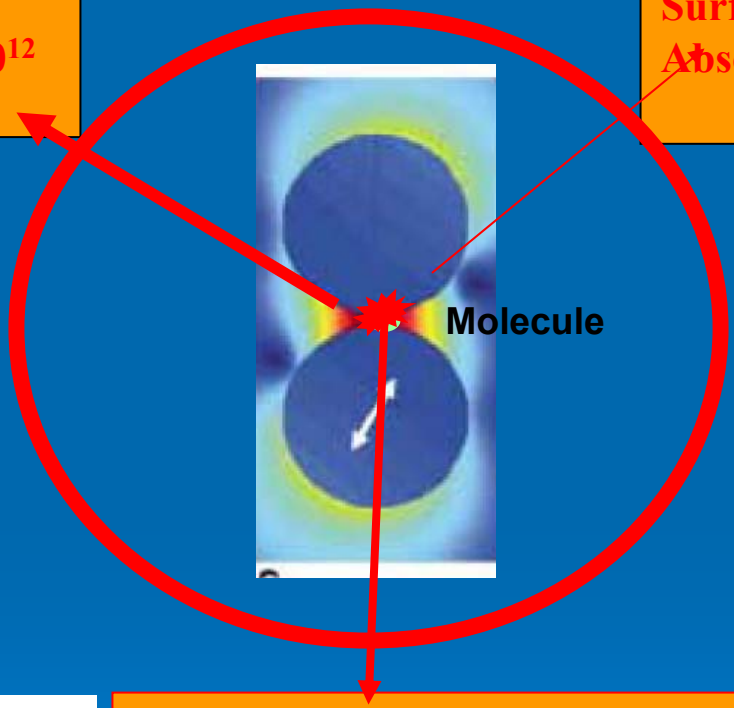
Amplification of Optical Processes (Raman scattering, IR Absorption and Fluorescence Emission) with Plasmonic Nanoparticles

Surface Enhanced Raman Scattering (SERS): 10^{10}-10^{12} single molecule detection!

$$I_{SERS} \approx \left(\frac{E_{Loc}}{E_{Inc}}\right)^4$$

Surface Enhanced Infrared Absorption (SEIRA): 10^{1}-10^{2}

$$A = \left|\frac{\partial \mu}{\partial Q}\right|^2 |E|^2 \cos^2\theta$$

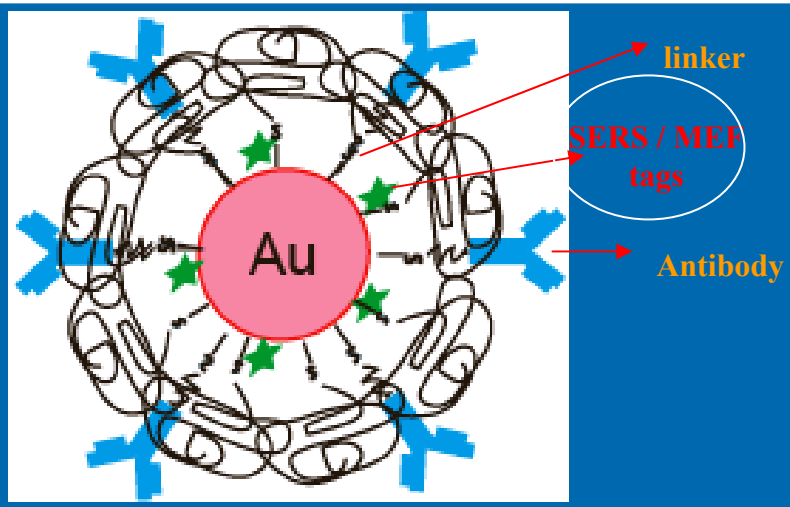


Metal - Enhanced Fluorescence (MEF)

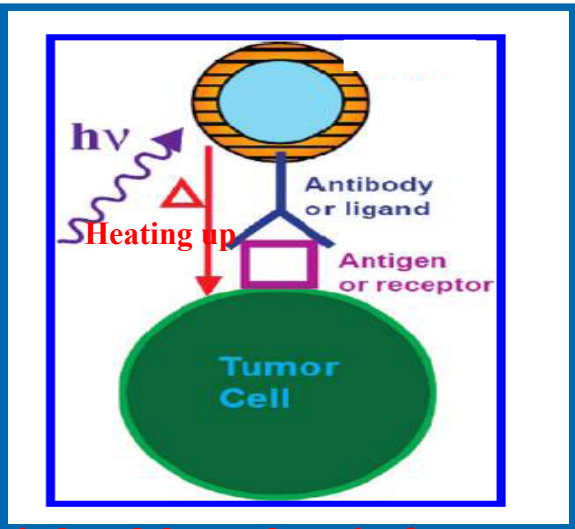
$$\eta = \frac{\gamma_{rad}}{\gamma_{rad} + \gamma_{nrad}^0 + \gamma_\Omega}$$

$$P_{12} = \frac{2\pi}{\hbar} \sum_f \left| \langle \Psi_f | \mu E(r) | \Psi_i \rangle \right|^2 \rho(\omega)$$

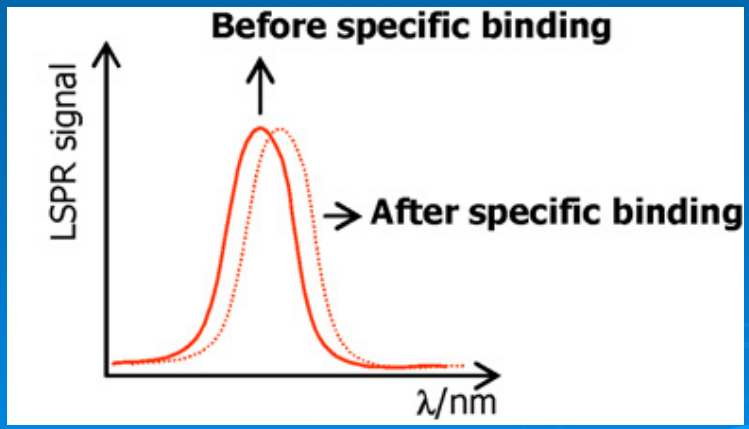
Biomedical Applications of Plasmonic Nanoparticle



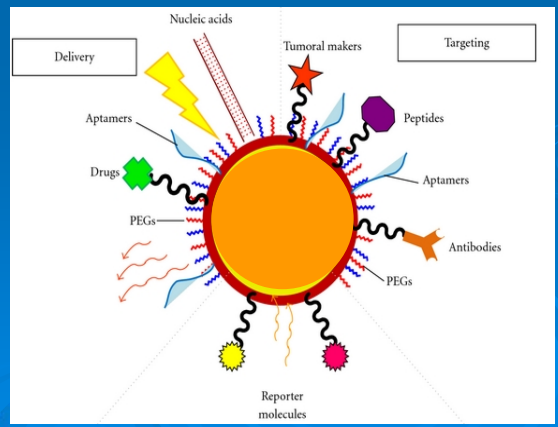
SERS / MEF tags for tracing and imaging



Plasmon-induced hyperthermia for cancer therapy



Localized Surface Plasmon Resonance (LSPR) biosensors



Nanoparticles for Drugs and genes delivery

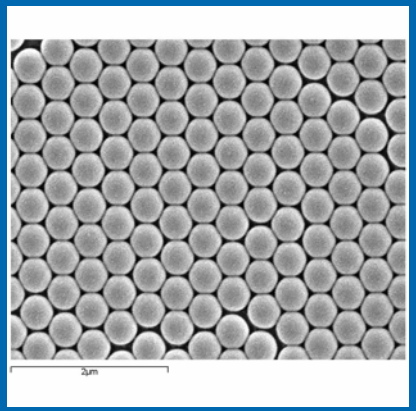
Obiective operationale

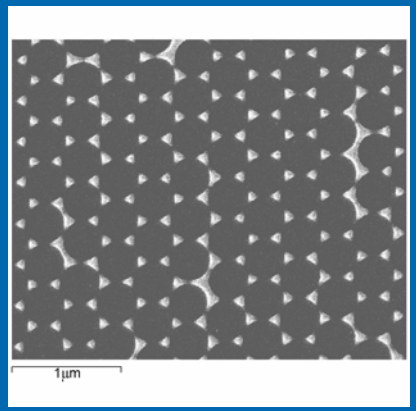
- 1) Sintetizarea / fabricarea unor nanoparticule de metal nobil (aur, argint si hibride polimer/silica/etc.) avand forme si dimensiuni controlabile (2-200nm) si rezonante plasmonice de suprafata ajustate pentru a absorbi in vizibil si NIR.
- 2) Legarea / conjugarea unor biomolecule / proteine / biopolimeri relevanti de nanoparticule metalice.
- 3) Dezvoltarea unor noi senzori optici, spectroscopici si electro-chimici utilizand nanostructuri de metal nobil (aur).
- 4) Evaluarea toxicitatii nanoparticulelor metalice.
- 5) Demonstrarea conceptului de terapie fototermica selectiva indusa laser, in vitro (culturi celulare).
- 6) Atasarea unor medicamente de nanoparticule biofunctionalizate de aur.
- 7) Dezvoltarea unor model si metode de calcul al proprietatilor moleculare si nanoparticulelelor.

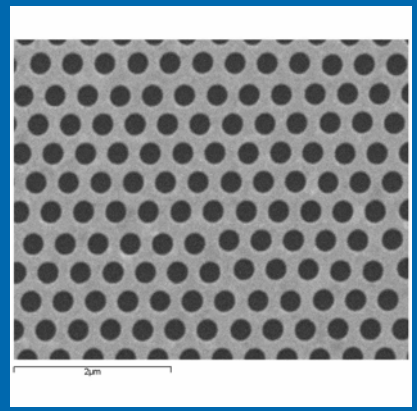
Fabrication of plasmonic nanostructures

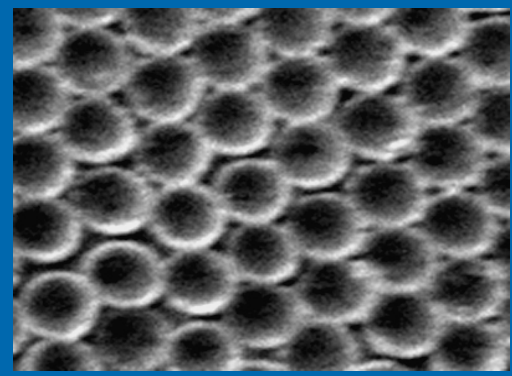
Typical examples of plasmonic nanostructures

- by Nanosphere Lithography

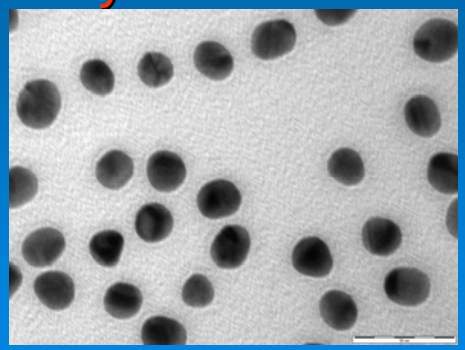
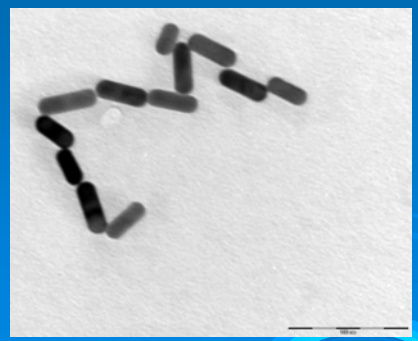
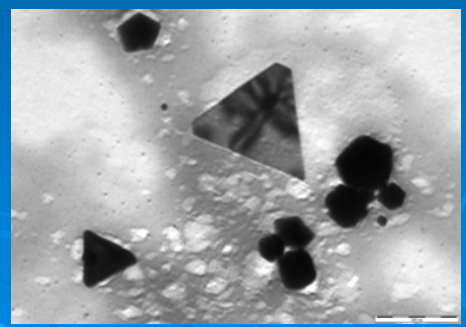
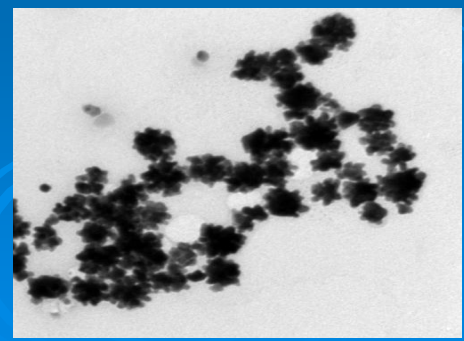








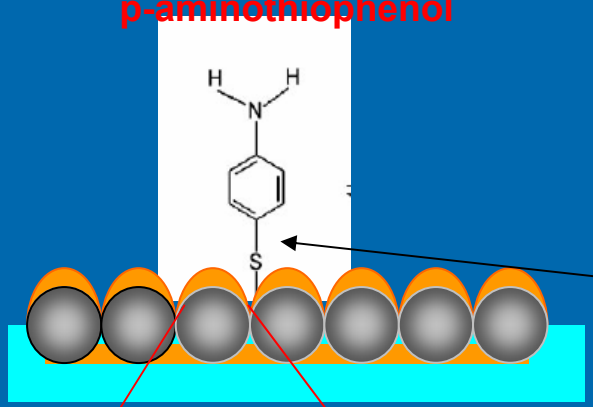
- by Chemical Routes

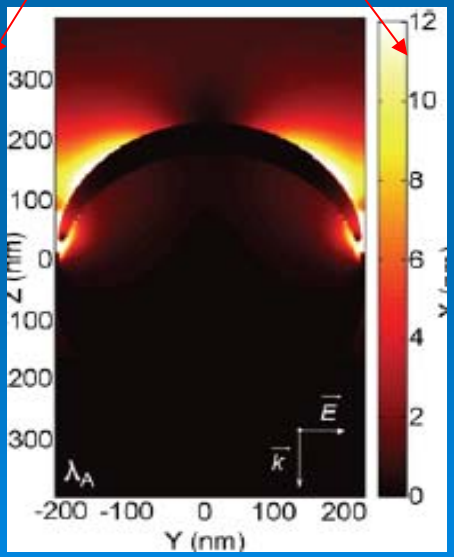
Selected Applications

Mapping "Hot-Spot" on metal-coated colloidal crystal *via SERS Imaging and FDTD simulation*

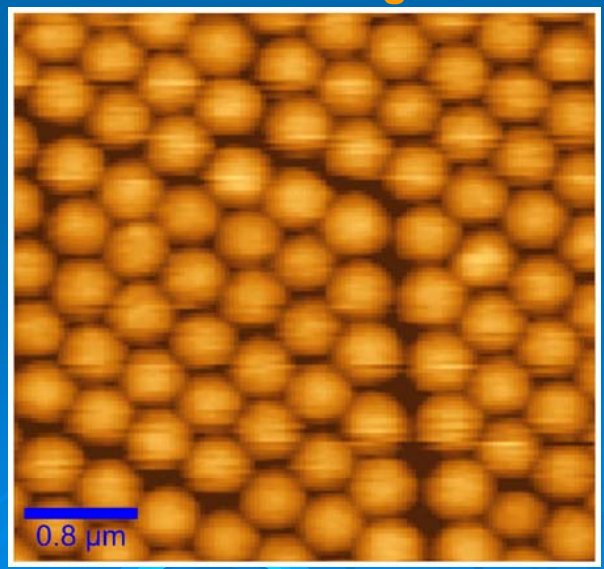
p-aminothiophenol



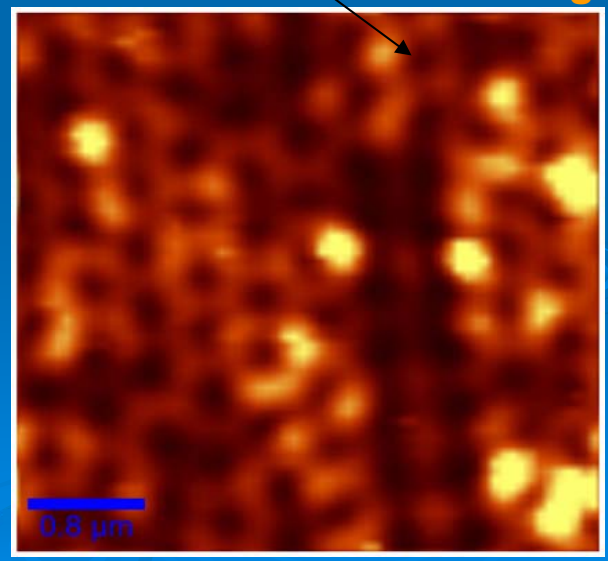
Computed E field



AFM Image



SERS Image

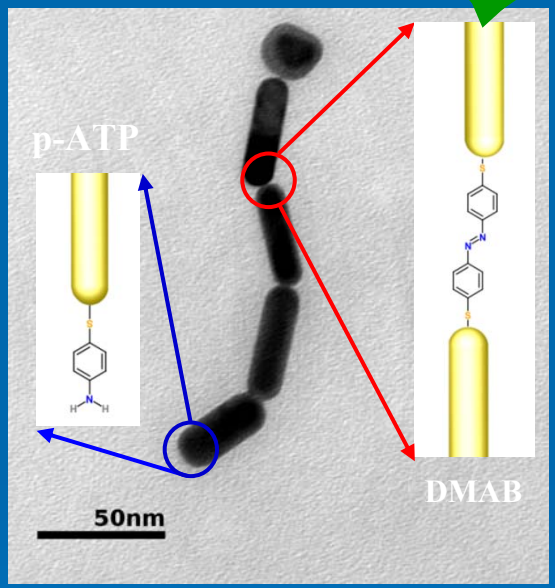


J. Phys. Chem. C, 114, 11717–11722 (2010)

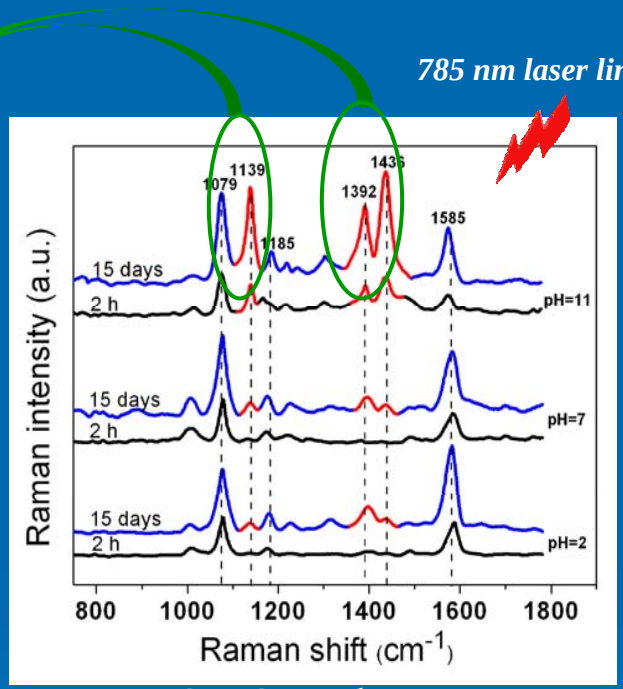
Appl. Phys. B 106:849–856 (2012)

SERS and Fluorescence Molecular Reporters on Gold Nanoparticles

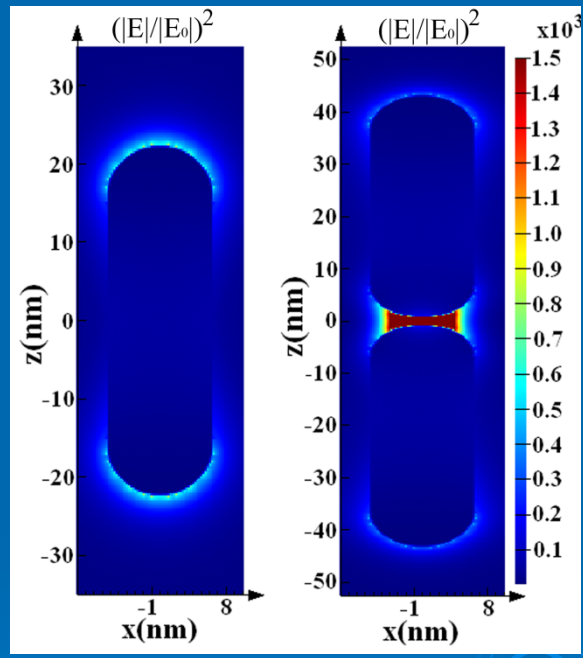
Gold nanorods performing as pH SERS nanoprobes



TEM image of AuNRs at pH=11

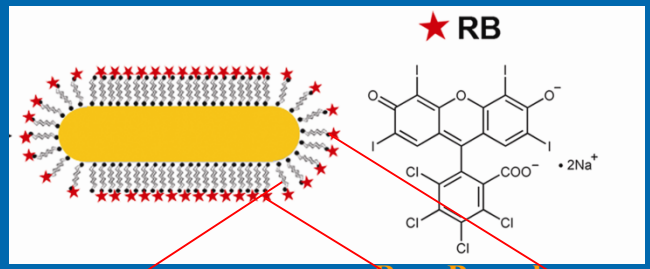


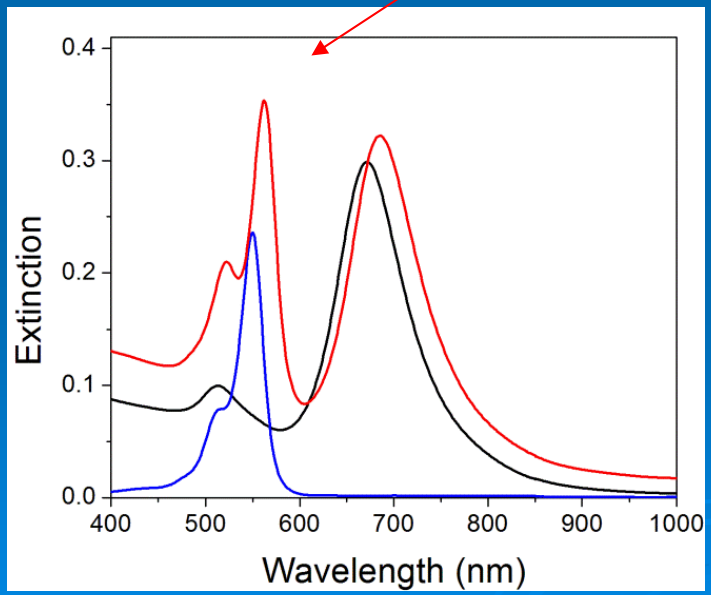
SERS results

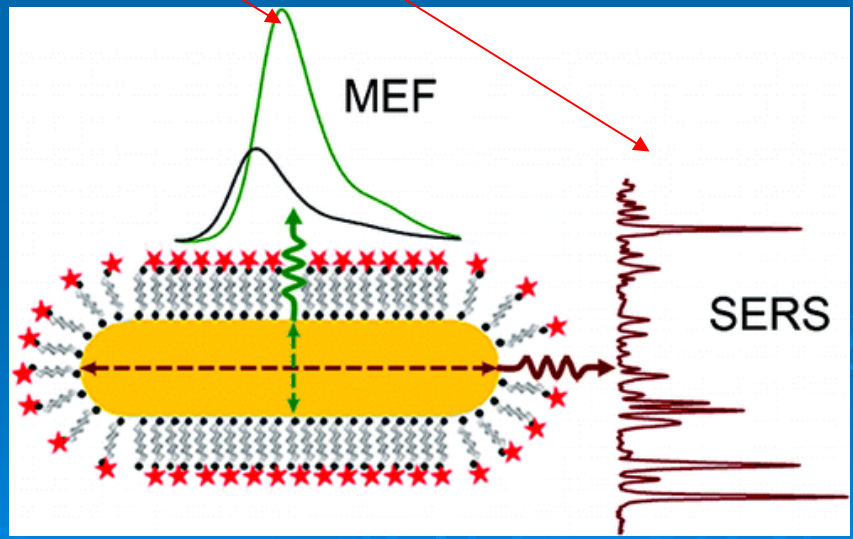


FDTD simulated electric-field enhancement at 785 nm

Gold Nanorods Performing as Dual-Modal Nanoprobes *via* Fluorescence and SERS



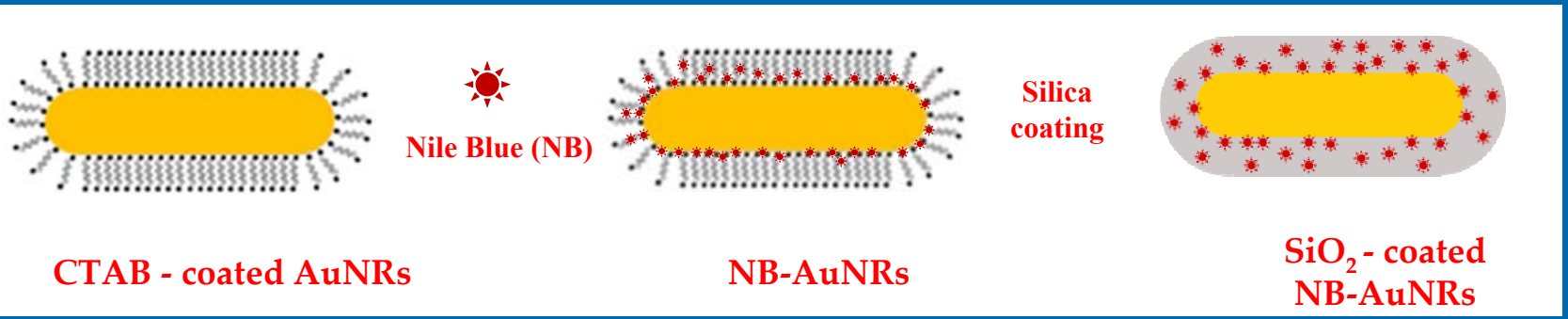


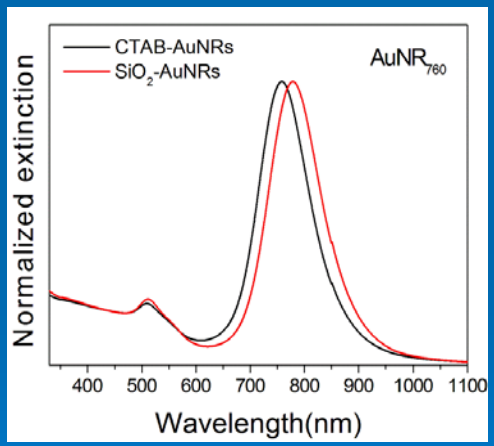


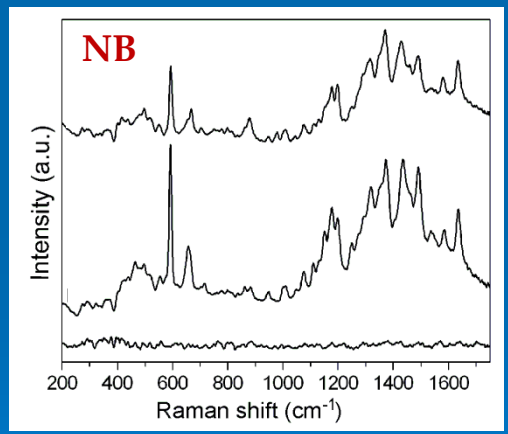
J. Phys. Chem. C. 2012, 116, 12240−12249.

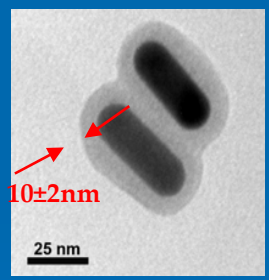
- **Biomedical applications**

Detoxification of gold nanorods and SERS tagging

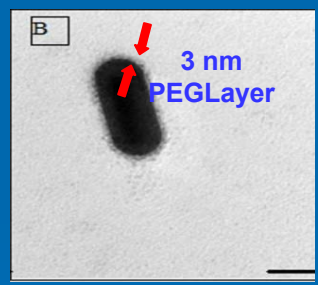








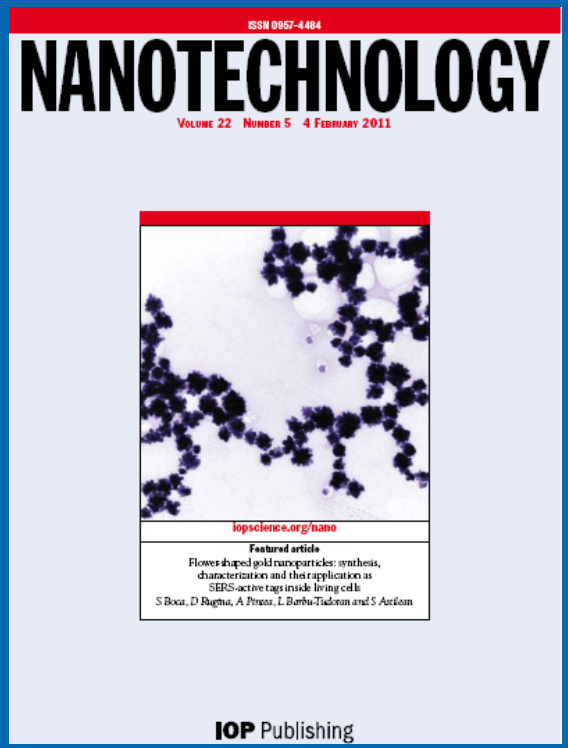
silica shell

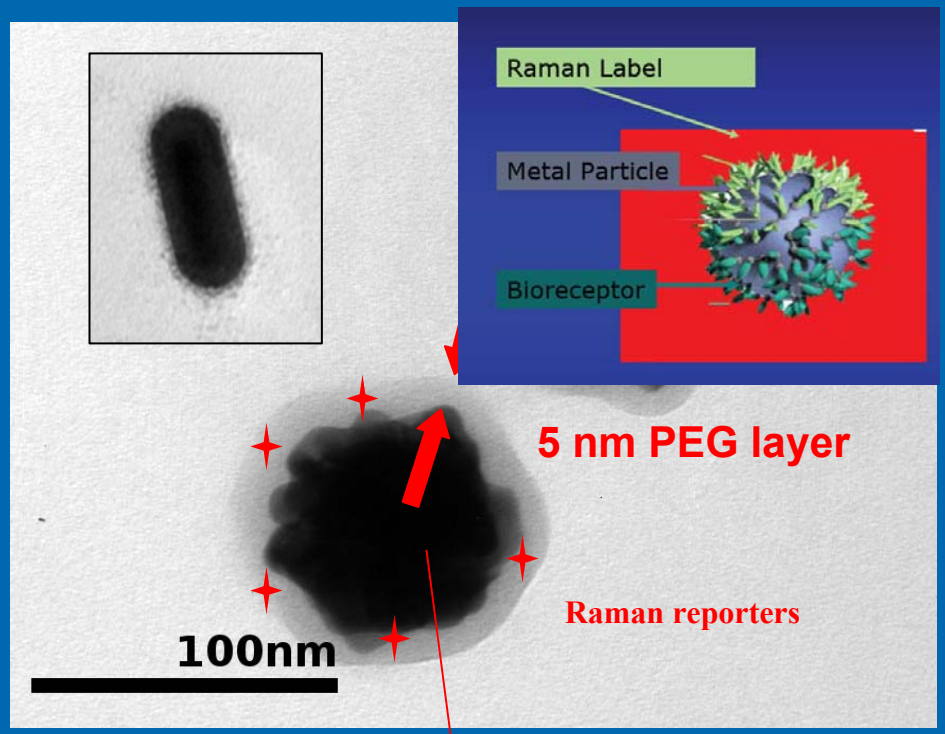


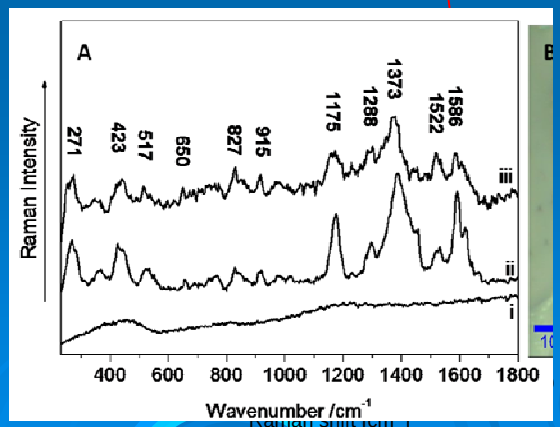
PEG shell

1. Nanotechnology 21, 235601 (2010)
2. Nanotechnology 23 (2012) 485706

SERS-active tags inside living cells



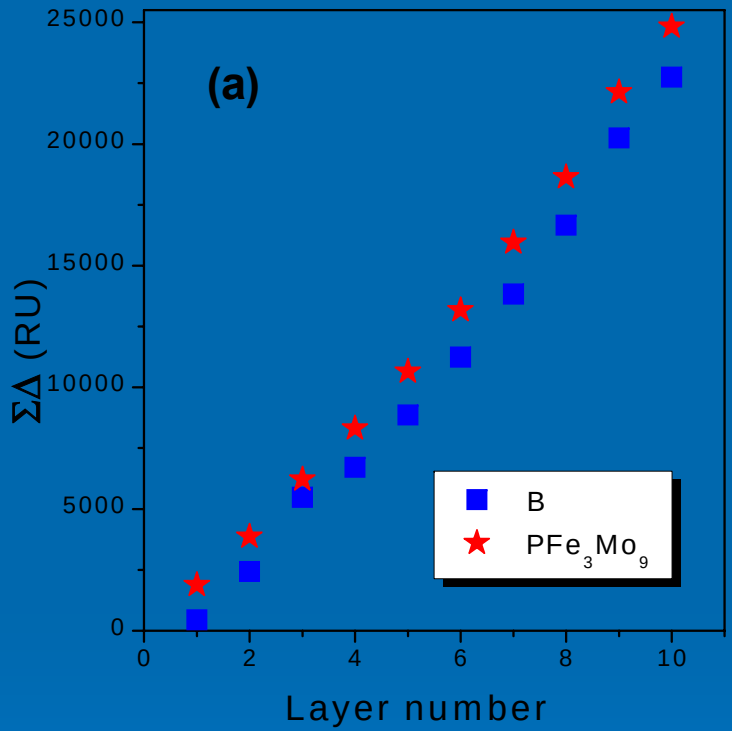


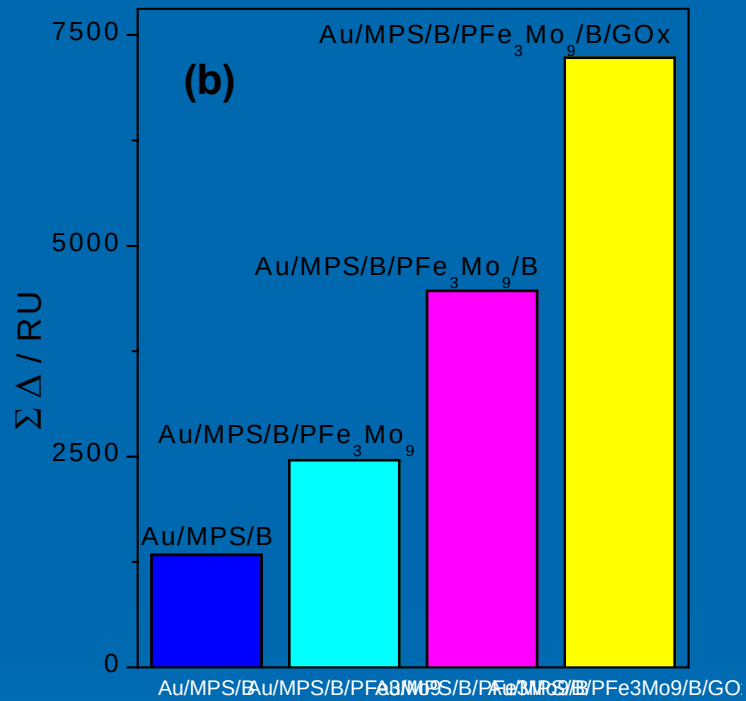


Nanotechnology 22 (2011) 055702

Obtinerea si caracterizarea unor microstructuri de Au biofunctionalizate, cu aplicatii in realizarea de biosenzori

Biosenzor amperometric pentru detectia glucozei / apei oxigenate cu sensibilitate reglabila





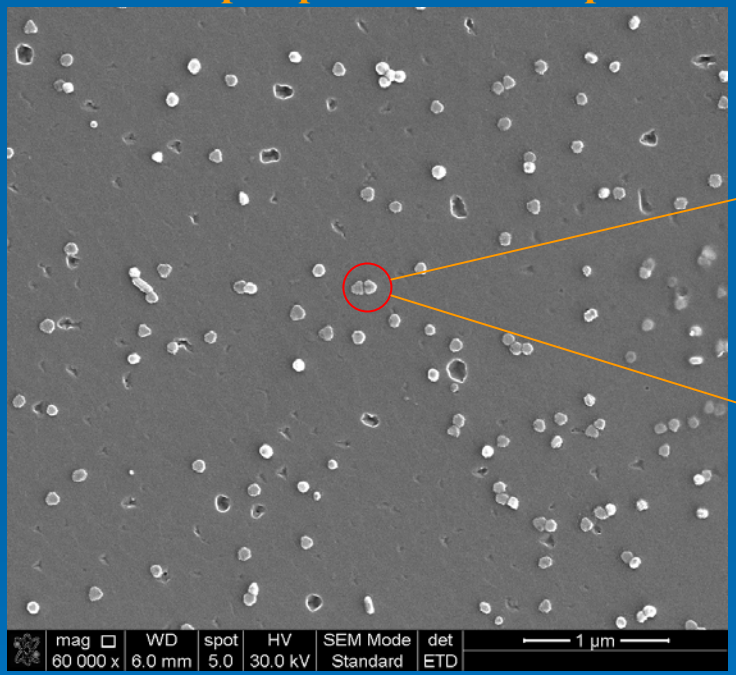
Variatia cumulativa a raspunsului SPR observat pentru nanostructuri realizate succesiv pe suprafata electrodului Au/MPS: (A) $Au/MPS/(B/PFe_3Mo_9)_n/B$; (B) $Au/MPS/B/PFe_3Mo_9/B/GOx$.

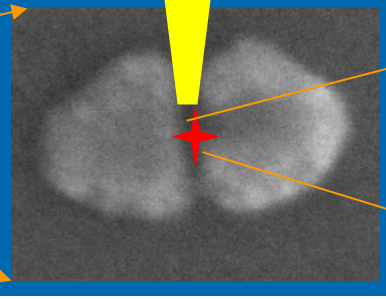
PFe_3Mo_9, $Na_3H_3[A\alpha\text{-}PFe_3^{III}(H2O)_3\text{-}Mo_9O_{37}]$ *14 H_2O; B, poli(4-vinilpiridina); MPS, acid mercapto-propion sulfonic; RU, unitati relative;

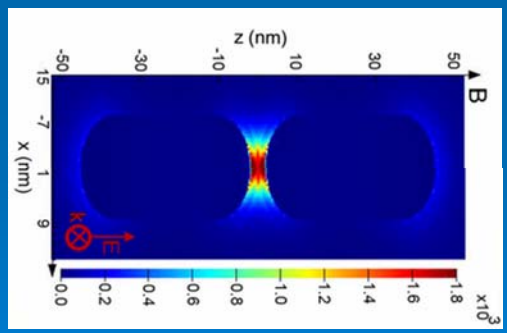
Self-assembled architecture based on triiron-substituted polyoxomolybdate anion and positively charged polymer, G. Turdean and I. C. Popescu, J. Solid State Electrochem ., 16 (2012) 681–687 (P8)

Single-molecule detection *via* SERS

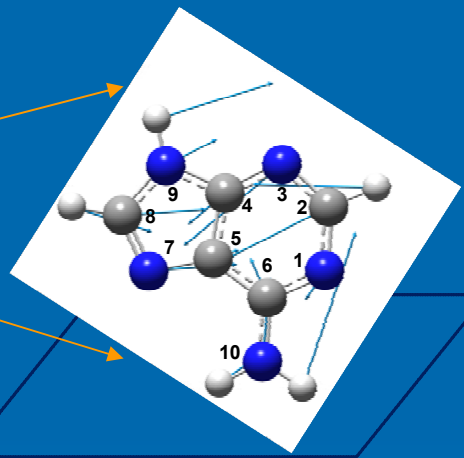
Chitosan-entraped plasmonic nanoparticles







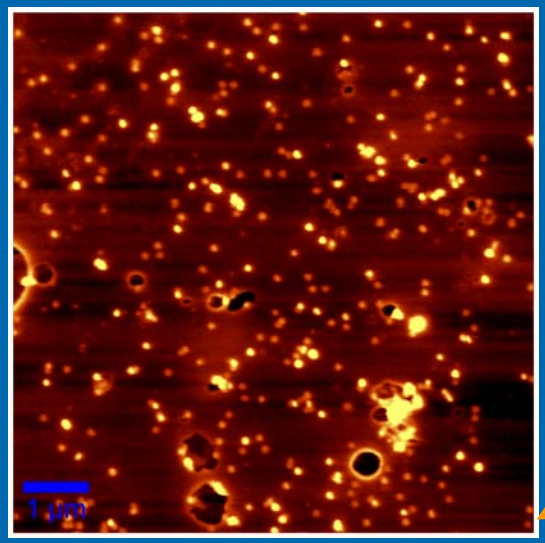
SERS spectrum of analyte molecule



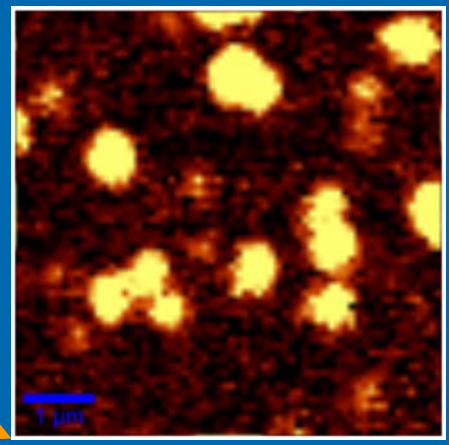
Nanotechnology, Vol: 23 (5) Paper no 055501 (2012)

(highlighted at http://iopscience.iop.org/0957-4484/labtalk-article/48366)

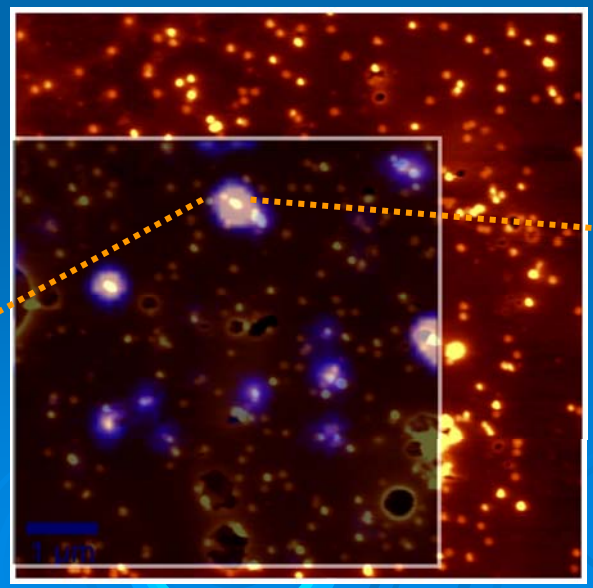
Combining SERS imaging and AFM



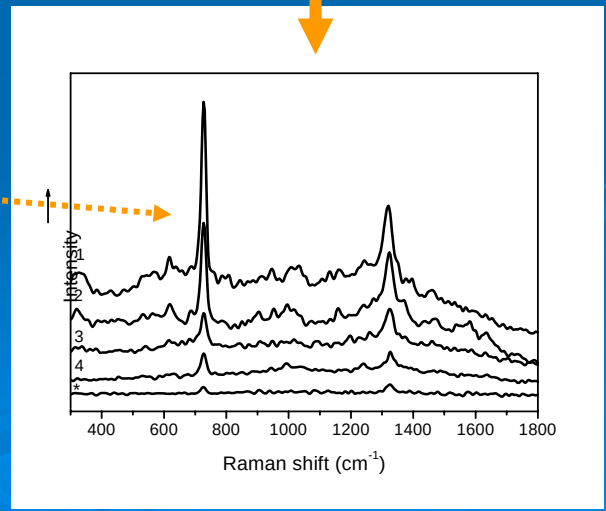
AFM image



SERS image

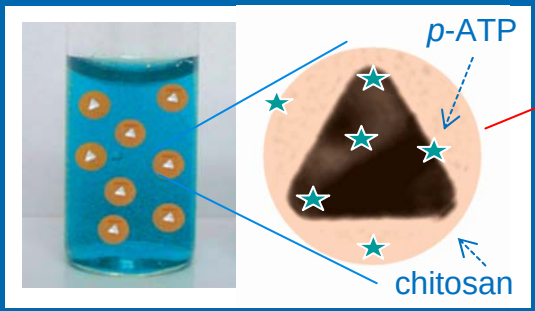


Localization of adenine molecules in "hot-spots"

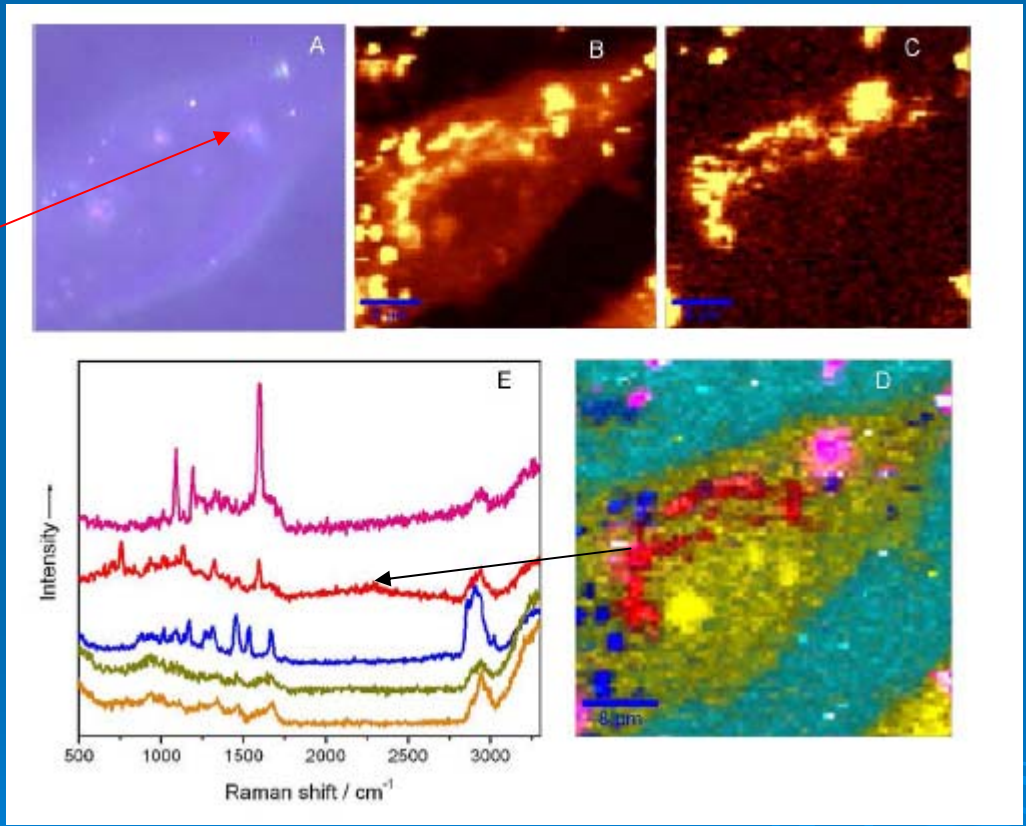


Adenine SERS spectra

SERS imaging of living A549 cells



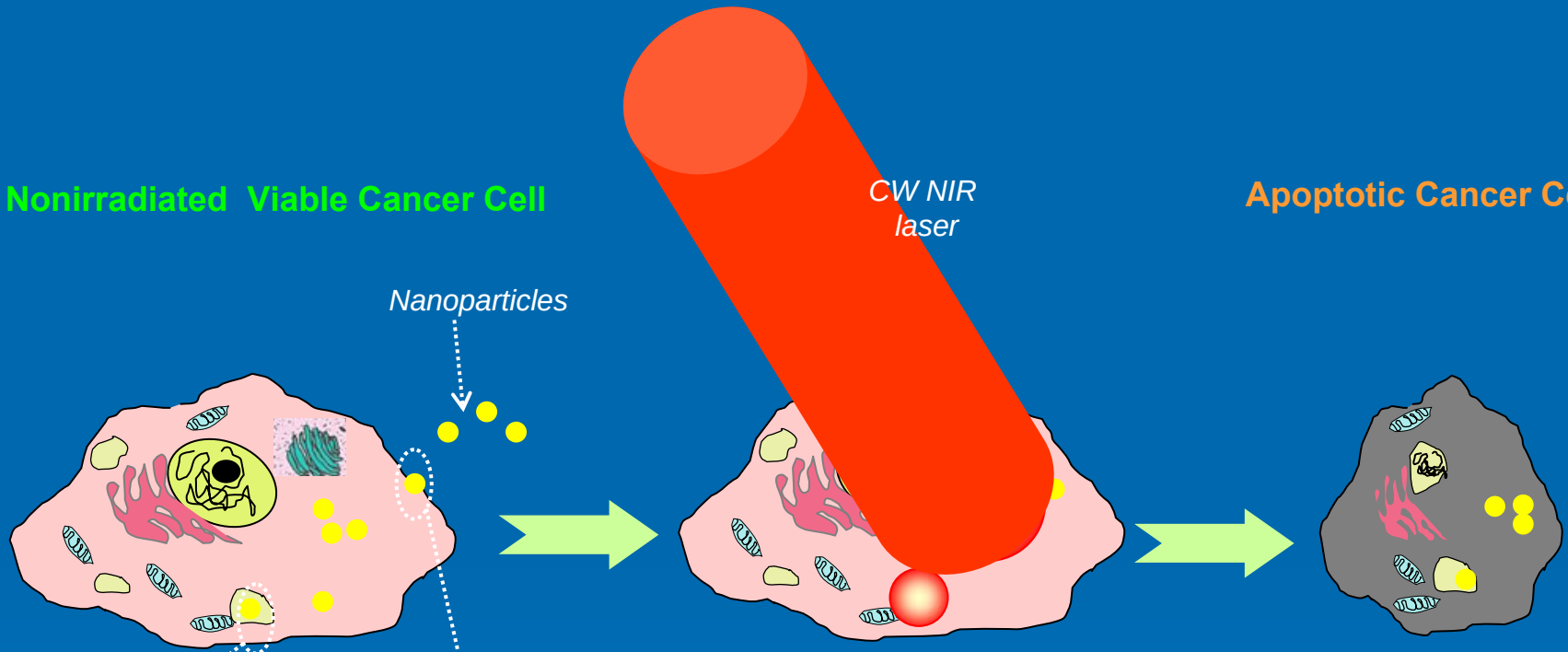
p-ATP labeled chitosan-coated triangular silver nanoparticles



Nanoscale 5, 6013–6022, 2013

Plasmon mediated photothermal therapy of cancer cells

Principle of plasmon-mediated photothermal therapy



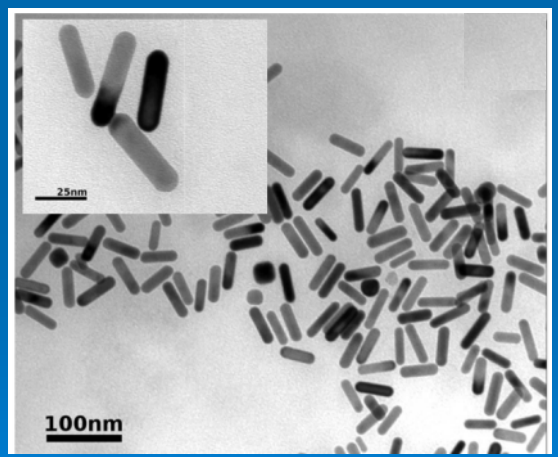
Plasmon mediated photothermal therapy

Cell types used in our experiments:

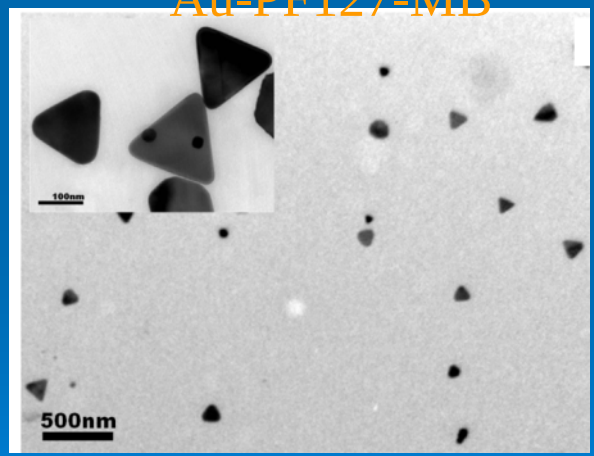
- Human Embryonic Kidney (healthy)
- Human Lung Cancer Cells (tumoral)

Nanoparticles used in our experiments

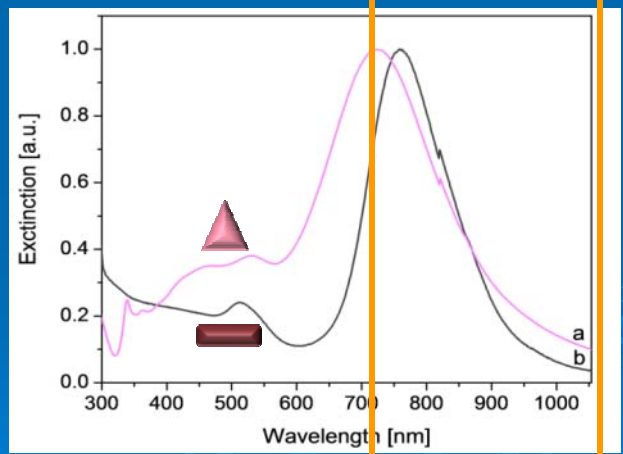
Au-PF127-MB



length: 50 nm
diameter:14 nm



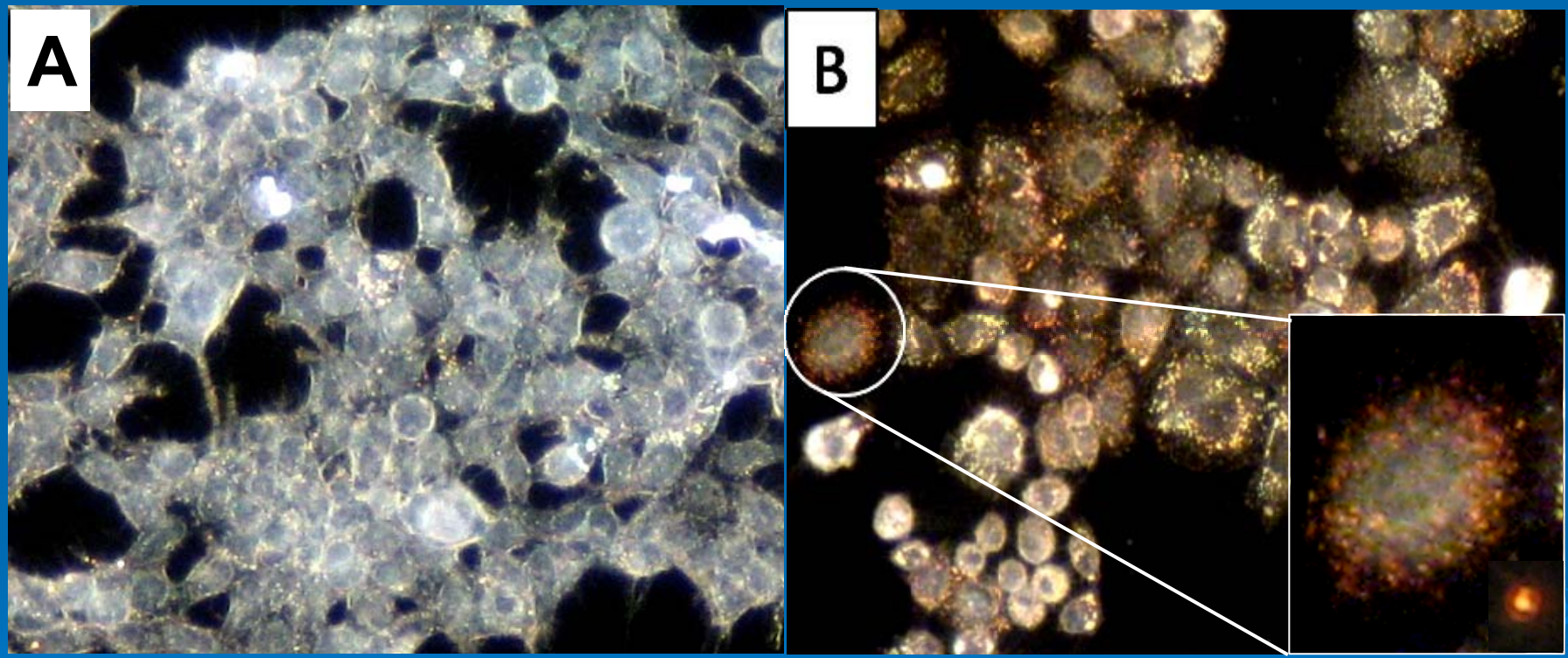
edge length: 120 nm
height: 11 nm



Biomedical applications

S. C. Boca, S. Astilean **Nanotechnology** 2010, *21*, 235601.
M. Potara, A. M. Gabudean, S. Astilean, J. Mater. Chem. 2011, *21*, 3625.

Assesment of nanoparticles uptake by cells
(dark field microscopy imaging)



Cells without nanoparticles

Rod shaped gold nanoparticles inside cells scatter red light

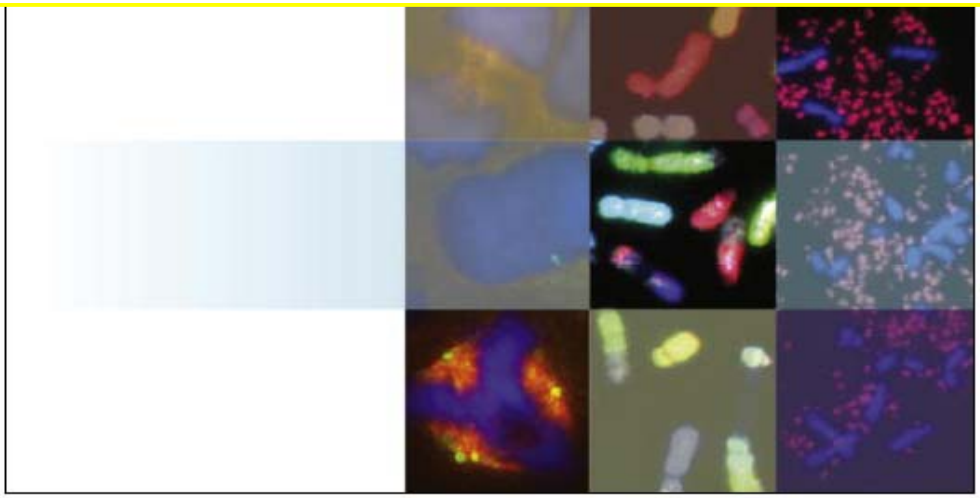
Volume 311, issue 2, 6 December 2011 ISSN 0304-3835

CANCER LETTERS

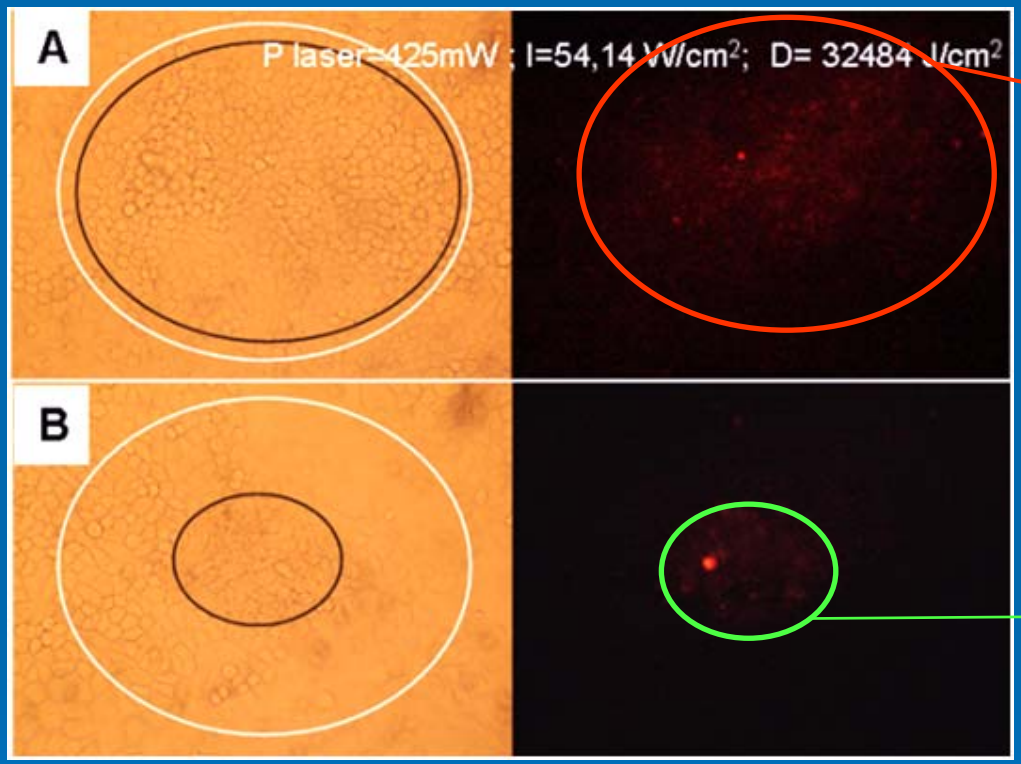
Chitosan-coated triangular silver nanoparticles as a novel class of biocompatible, highly effective photothermal transducers for *in vitro* cancer cell therapy

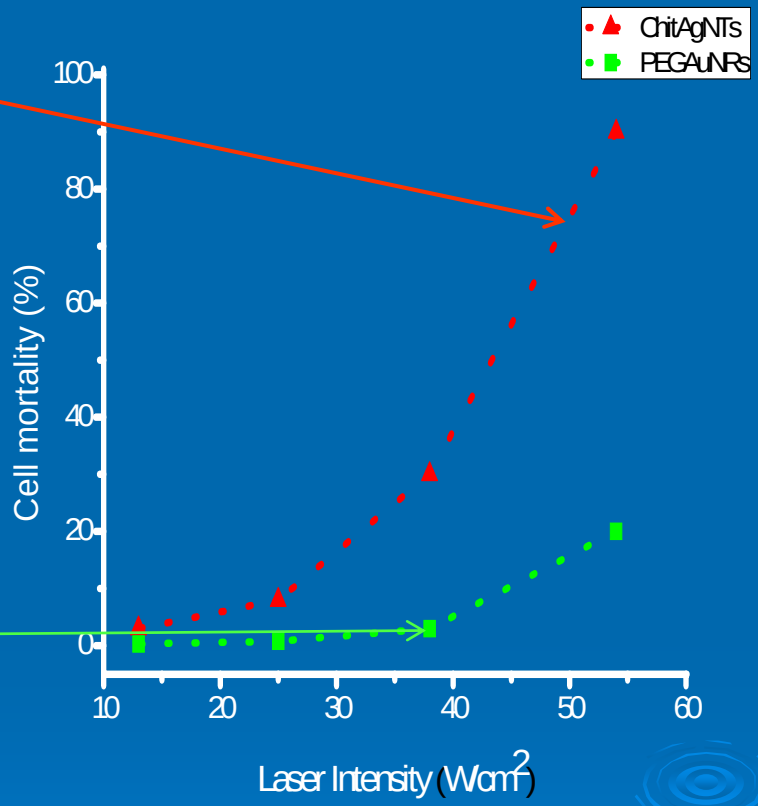
Sanda C. Boca [a], Monica Potara [a], Ana-Maria Gabudean [a], Aurelie Juhem [c], Patrice L. Baldeck [b], Simion Astilean [a,*]

[a] *Nanobiophotonics and Laser Microspectroscopy Center, Interdisciplinary Research Institute on Bio-Nano-Sciences, Faculty of Physics, Babes-Bolyai University, T. Laurian 42, 400271 Cluj-Napoca, Romania*
[b] *Laboratoire interdisciplinaire de Physique, CNRS-UMR 5588, Grenoble Université, 140 rue de la Physique, BP 87, 38402 Saint Martin d'Hères Cedex, France*
[c] *Grenoble Institut de Neurosciences – U836, Equipe 7: Nanomédecine et Cerveau, Université Joseph Fourier, Site Santé, BP 170, 38042 Grenoble Cedex 9, France*



Plasmon mediated photothermal therapy of cancer cells





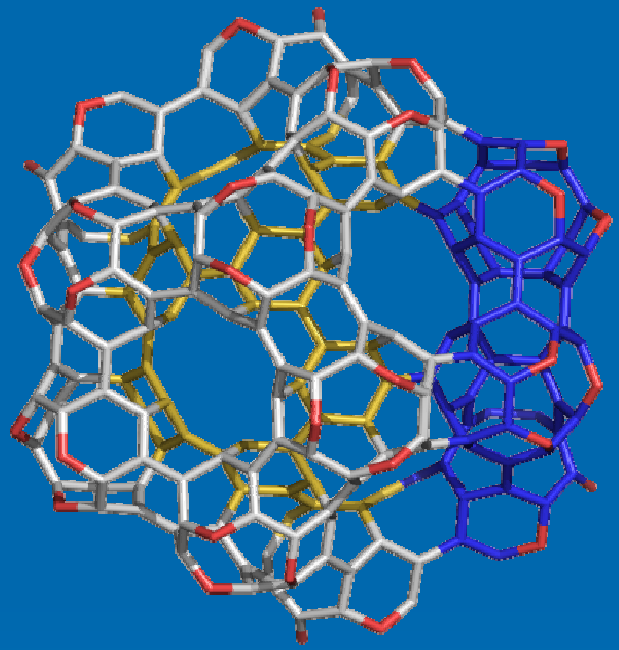
⇒ Cell mortality dependence on laser intensity

⇒ higher efficiency of **Chit-AgNTs** than PEG-AuNRs: -Density
-Morphology
-Silver thermal conductivity

Cancer Letters **. Vol. 311(2):131-40, (2011 Dec. 8).**

- **Nanoparticule Multifunctionale**
- (hipertermie +transport medicament)

Spongy structure of polybenzene, possibly to be used as a drug-carier

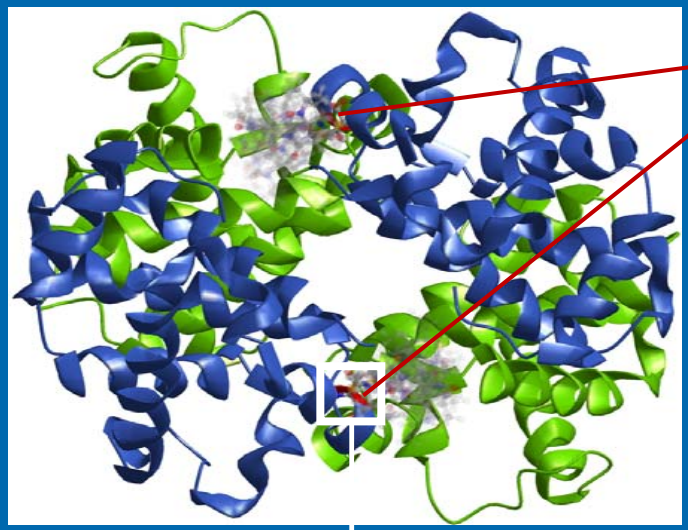


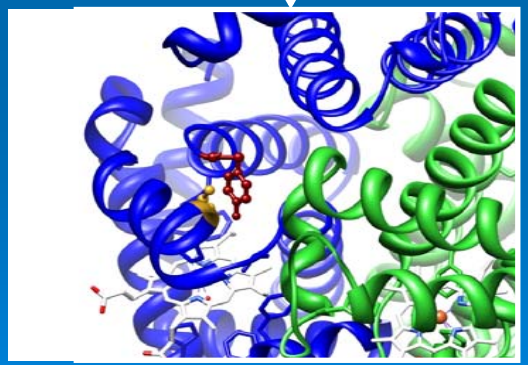
BTZ_{20}: v = 480; e = 690; f_6= 80; f_8= 90; g = 21

B. Szefler, O. Ponta, M. V. Diudea, **Energetics of polybenzene multi-tori,** ***J Mol Structure,*** 2012, 1022, 89-93, **(IF=1,634)**

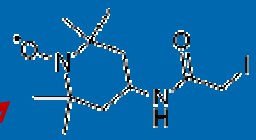
Hemoglobin adsorption onto Si-Ti microspheres

Horse hemoglobin (PDB file 2ZLU) spin labeled in position β-93

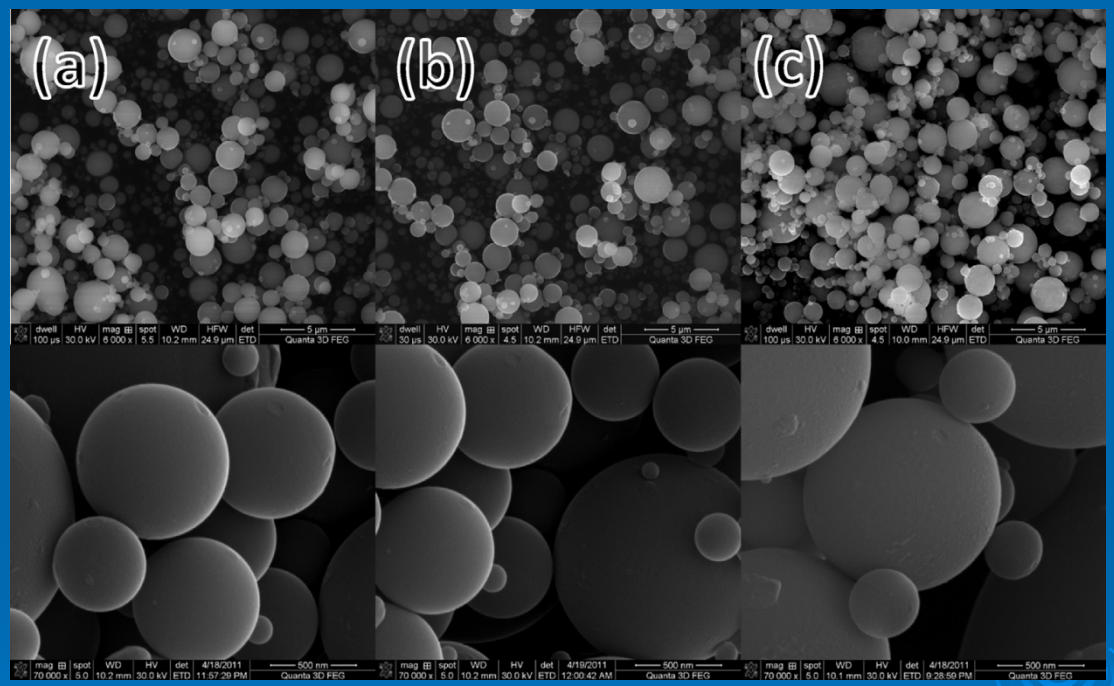




Detailed view onto the surroundings of position β-93 (yellow: cysteine residue, red: tyrosine pocket)



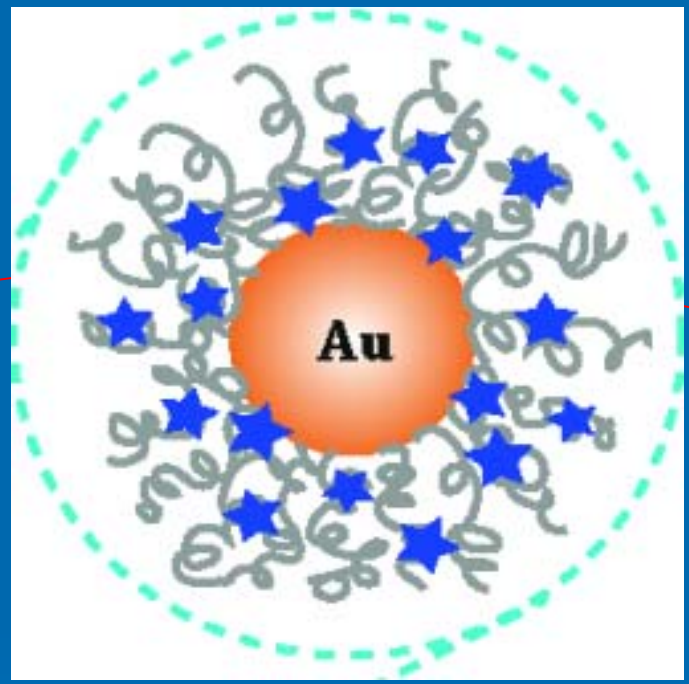
Iodoacetamide SL



O. Ponta, C. Gruian, E. Vanea, B. Oprea, H.J. Steinhoff, S. Simon (2013) Journal of Molecular Structure 1044

E. Vanea, C. Gruian, C. Rickert, H.-J. Steinhoff, V. Simon (2013) Biomacromolecules 14(8), 2582–2592

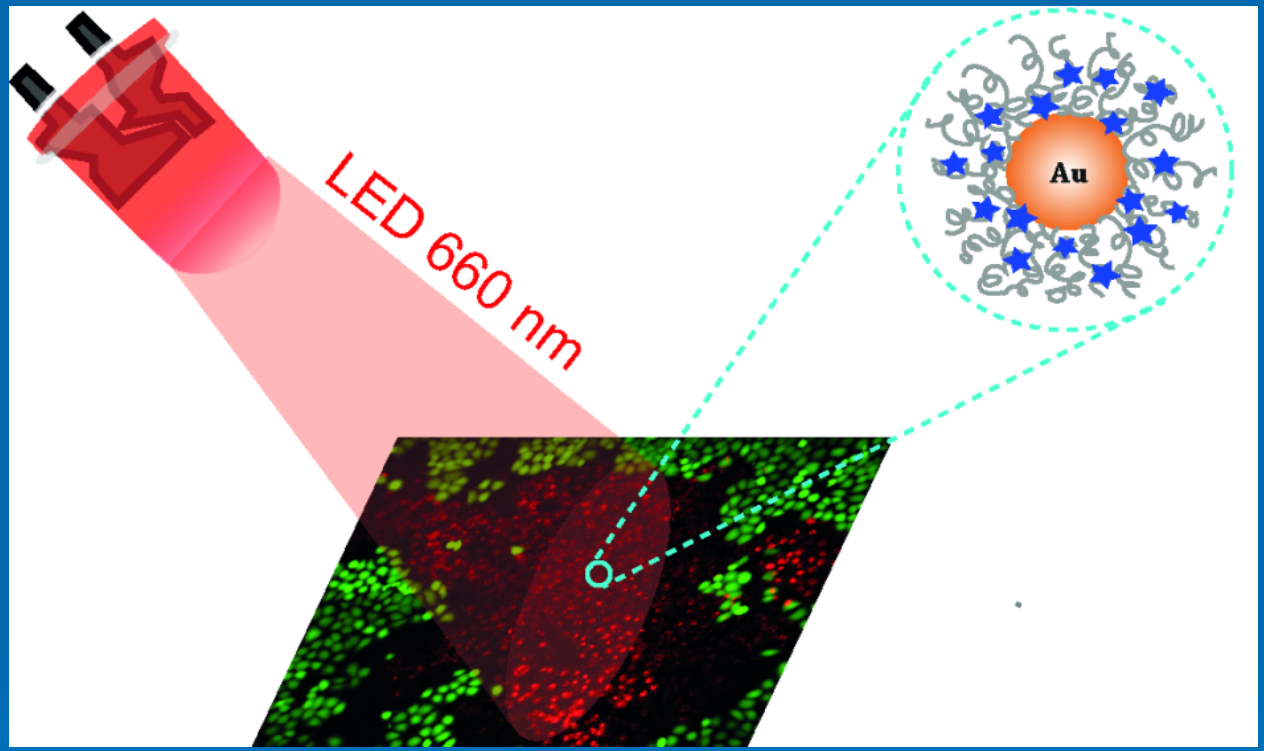
Plasmonic-assisted photodynamic therapy (PDT)



- synergistic treatment of cancer by *combination plasmonic hyperthermia with PDT*
- plasmonic nanoparticles *reduce the photobleaching rate of photosensitizer*
- increase the triplet yield of the conjugated photosensitizer, *enhancing singlet oxygen generation*
-polymer shell can protect the photosensitizer from *enzymatic reduction*

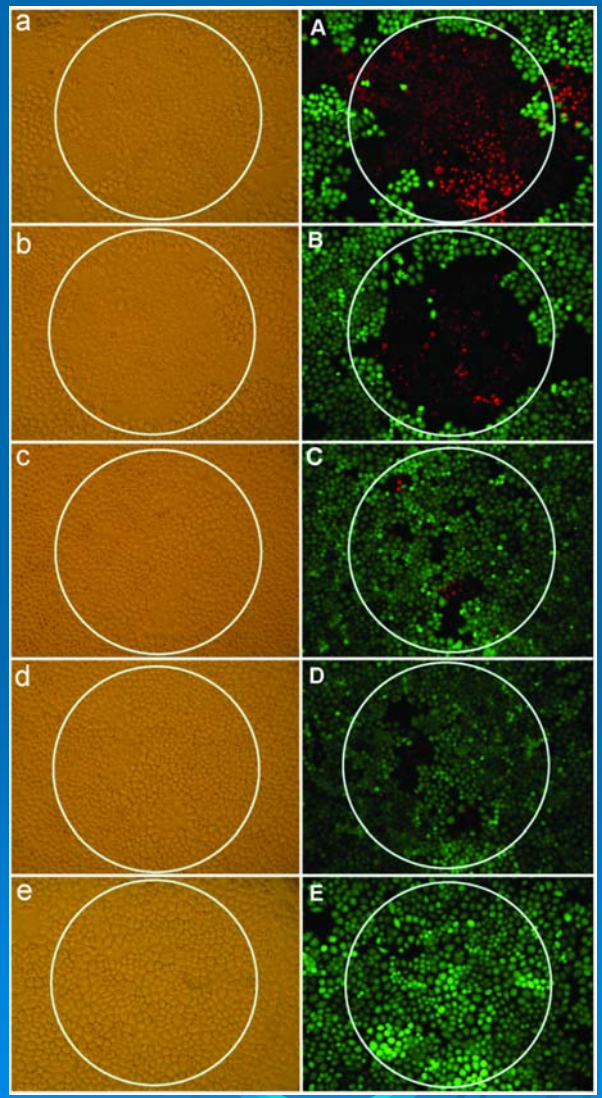
LED-activated methylene blue-loaded Pluronic-nanogold hybrids

(Au-PF127-MB)



T. Simon, S. Boca-Farcau, A-M Gabudean, P. Baldeck, and S. Astilean, (Journal of Biophotonics, 2013)

Fluorescence image illustrating the destruction of human lung carcinoma cells (HTB 177) loaded with Au-PF127-MB upon irradiation with LED.



780 mW/cm2

640 mW/cm2

520 mW/cm2

425 mW/cm2

780 mW/cm2
(control sample)

molecular pharmaceutics

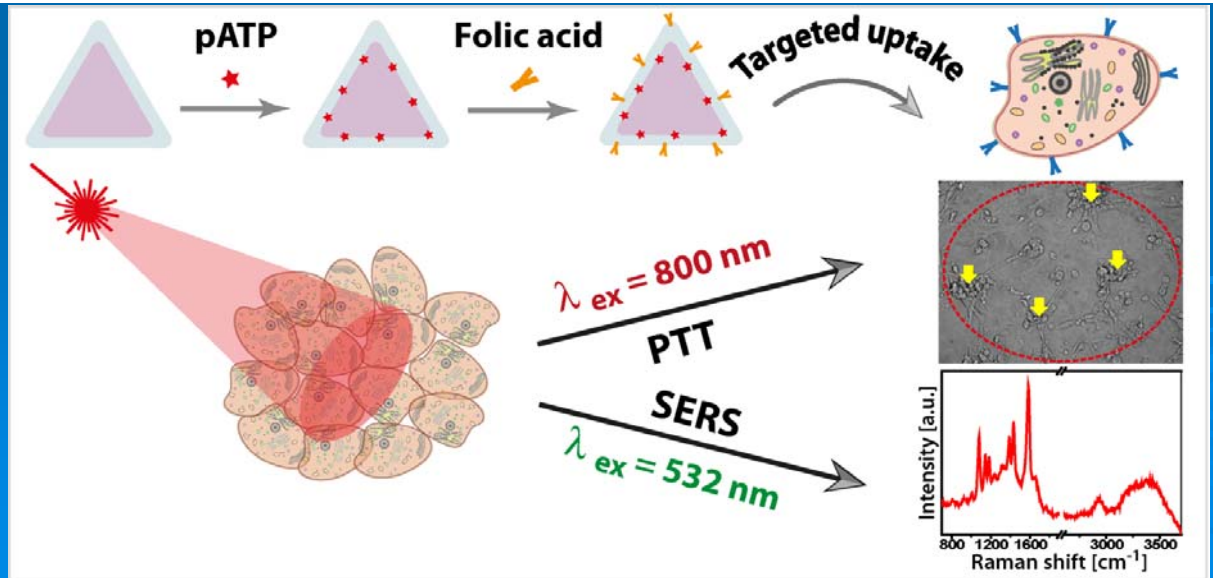
Article

Folic Acid-Conjugated, SERS-Labeled Silver Nanotriangles for Multimodal Detection and Targeted Photothermal Treatment on Human Ovarian Cancer Cells

Sanda Boca-Farcau, Monica Potara, Timea Simon, Aurelie Juhem, Patrice Baldeck, and Simion Astilean

Mol. Pharmaceutics, **Just Accepted Manuscript** • Publication Date (Web): 04 Dec 2013

Just Accepted



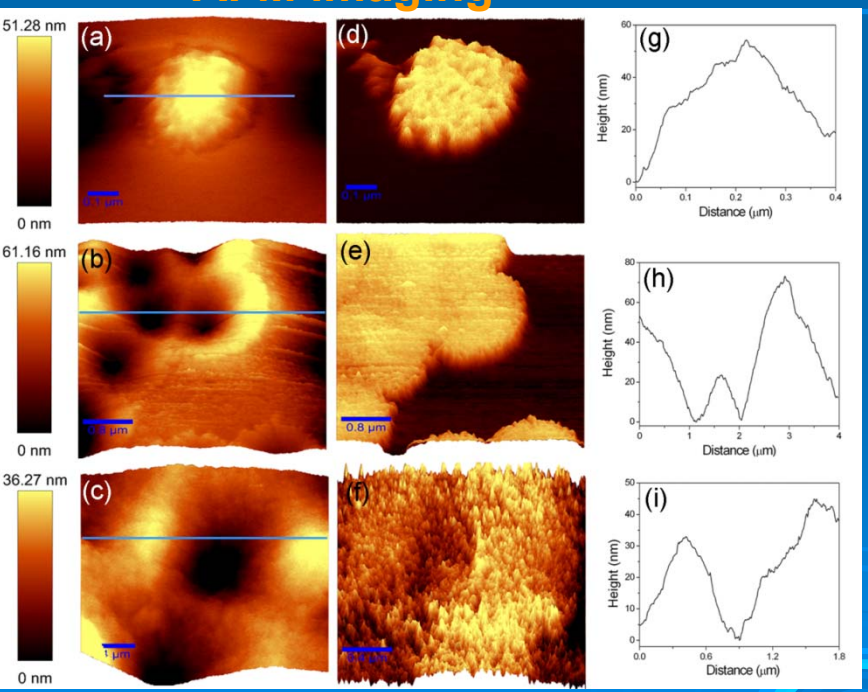
Synergistic antibacterial activity of chitosan-silver nanocomposites on *Staphylococcus aureus*

Bacterial strains used in our experiments

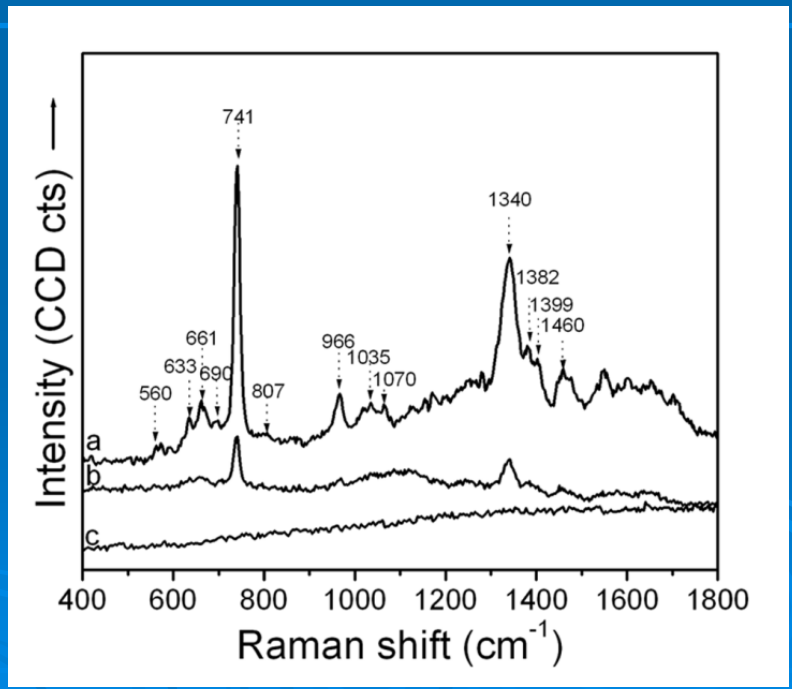
Two strains of Gram-positive methicillin-resistant *Staphylococcus aureus*:

- **UCLA 8076** – **heterogeneous** resistant
- **1190R** – **homogeneously** resistant

AFM imaging



SERS detection



Nanotechnology (2011) 22, 135101

- **Impactul Rezultatelor**

Impactul Rezultatelor

F*actorul de impact* mare al revistelor in care s-au publicat unele rezultate:

- *Nanoscale (IF=6.233), Chem. Commun. (FI: 5,787),*
- *J. Mater. Chem. (FI: 6.101).*
- *Cancer Letters (FI: 4,864), J Phys Chem (FI=4.224), Nanotechnology (FI: 3,644), etc.*

- **68 de articole realizeaza un factor de impact de 191.34 (fata de 80 angajate) si un scor relativ de influenta cumulat: 117,79.**

- **4 conferinte invitate**
- **1 capitol review intr-o carte in curs de aparitie**

- Pina in prezent articolele publicate au inregistrat un numar de 208 citari independente, cu mentiunea ca sunt articole care au peste 20 citari.

- Angajarea unui numar de *58 de tineri*: studenti masteranzi (17 dintre care 7 au devenit doctoranzi), doctoranzi (22 dintre care 7 au sustinut tezele de doctorat) si 19 post-doc.

- Finalizarea unui numar de 8 teze de doctorat pe tematica proiectului
- Organizarea a 2 workshopuri tematice

Vizibilitate internationala

Lab Talk Presentation

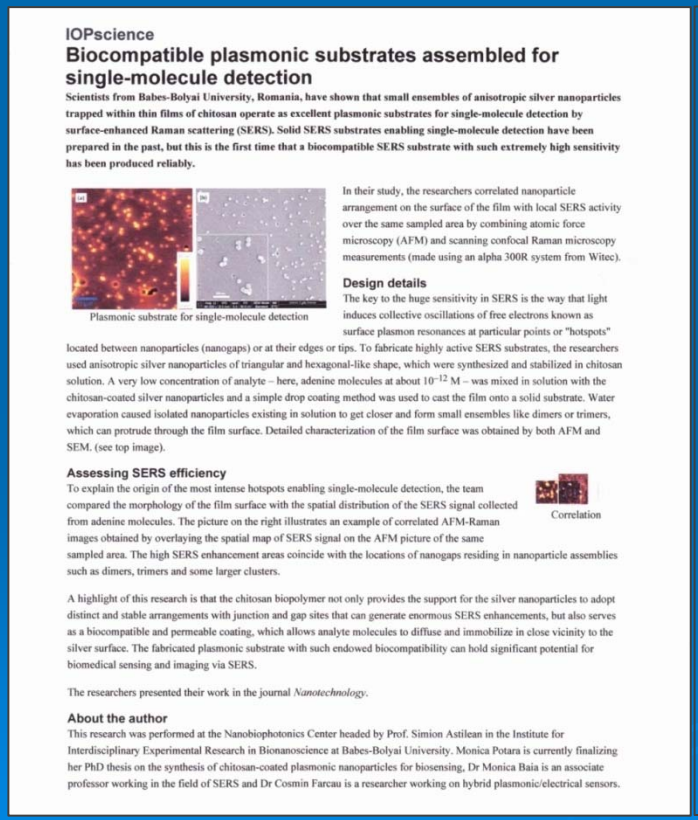
http://iopscience.iop.org/0957-4484/labtalk-article/48366

Nanotechnology- Highlights 2012

http://iopscience.iop.org/0957-4484/page/Highlights_of_2012

Nanotechnology- Cover Article 5/2011

http://ej.iop.org/pdf/nano/vol22/na2205-webcover.pdf



IOPscience

Biocompatible plasmonic substrates assembled for single-molecule detection

Scientists from Babes-Bolyai University, Romania, have shown that small ensembles of anisotropic silver nanoparticles trapped within thin films of chitosan operate as excellent plasmonic substrates for single-molecule detection by surface-enhanced Raman scattering (SERS). Solid SERS substrates enabling single-molecule detection have been prepared in the past, but this is the first time that a biocompatible SERS substrate with such extremely high sensitivity has been produced reliably.

Plasmonic substrate for single-molecule detection

In their study, the researchers correlated nanoparticle arrangement on the surface of the film with local SERS activity over the same sampled area by combining atomic force microscopy (AFM) and scanning confocal Raman microscopy measurements (made using an alpha 300R system from Witec).

Design details

The key to the huge sensitivity in SERS is the way that light induces collective oscillations of free electrons known as surface plasmon resonances at particular points or "hotspots" located between nanoparticles (nanogaps) or at their edges or tips. To fabricate highly active SERS substrates, the researchers used anisotropic silver nanoparticles of triangular and hexagonal-like shape, which were synthesized and stabilized in chitosan solution. A very low concentration of analyte – here, adenine molecules at about 10^{-12} M – was mixed in solution with the chitosan-coated silver nanoparticles and a simple drop coating method was used to cast the film onto a solid substrate. Water evaporation caused isolated nanoparticles existing in solution to get closer and form small ensembles like dimers or trimers, which can protrude through the film surface. Detailed characterization of the film surface was obtained by both AFM and SEM. (see top image).

Assessing SERS efficiency

To explain the origin of the most intense hotspots enabling single-molecule detection, the team compared the morphology of the film surface with the spatial distribution of the SERS signal collected from adenine molecules. The picture on the right illustrates an example of correlated AFM-Raman images obtained by overlaying the spatial map of SERS signal on the AFM picture of the same sampled area. The high SERS enhancement areas coincide with the locations of nanogaps residing in nanoparticle assemblies such as dimers, trimers and some larger clusters.

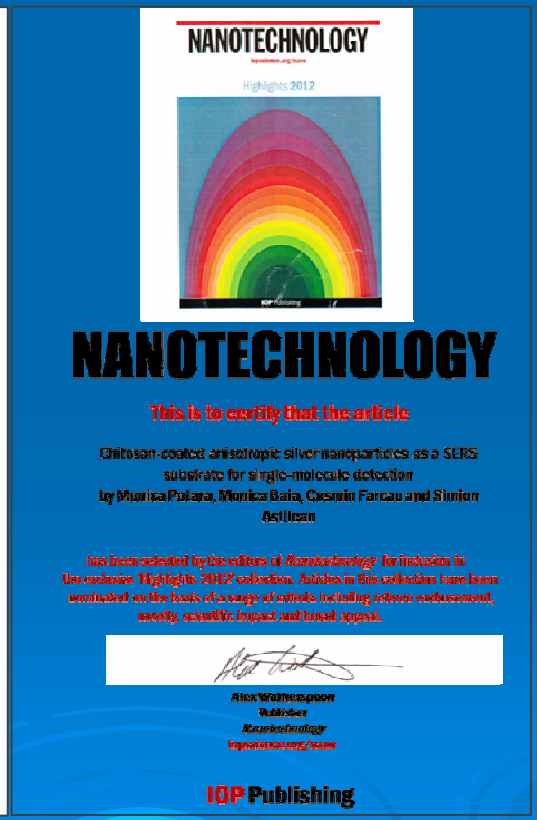
Correlation

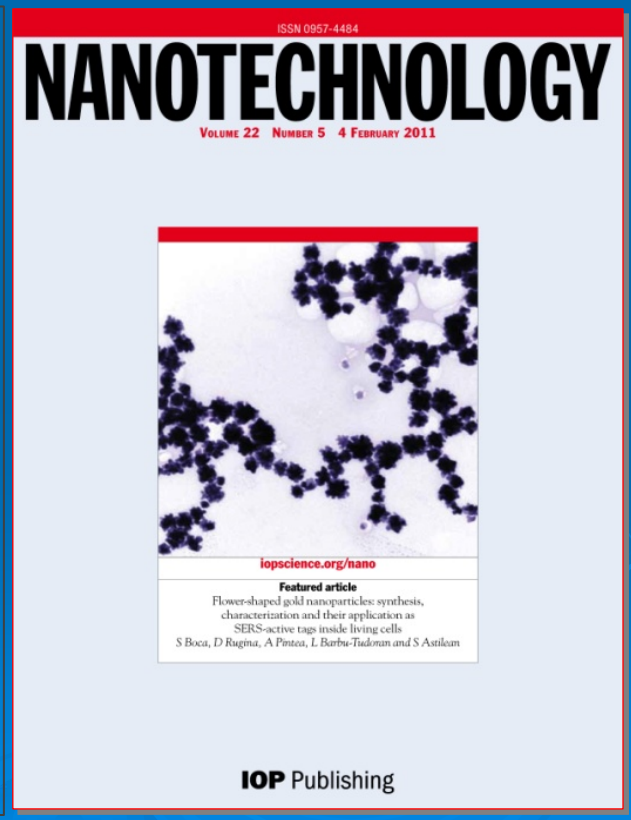
A highlight of this research is that the chitosan biopolymer not only provides the support for the silver nanoparticles to adopt distinct and stable arrangements with junction and gap sites that can generate enormous SERS enhancements, but also serves as a biocompatible and permeable coating, which allows analyte molecules to diffuse and immobilize in close vicinity to the silver surface. The fabricated plasmonic substrate with such endowed biocompatibility can hold significant potential for biomedical sensing and imaging via SERS.

The researchers presented their work in the journal *Nanotechnology*.

About the author

This research was performed at the Nanobiophotonics Center headed by Prof. Simion Astilean in the Institute for Interdisciplinary Experimental Research in Bionanoscience at Babes-Bolyai University. Monica Potara is currently finalizing her PhD thesis on the synthesis of chitosan-coated plasmonic nanoparticles for biosensing, Dr Monica Baia is an associate professor working in the field of SERS and Dr Cosmin Farcau is a researcher working on hybrid plasmonic/electrical sensors.





Monica Potara, Monica Baia, Cosmin Farcau Simion Astilean, **Nanotechnology (**2012), 23, 055501

Sanda Boca, Dumitrita Rugina, Adela Pintea, Lucian Barbu-Tudoran, and Simion Astilean, **Nanotechnology** (2011) 22, 055702.

Perspective

(1) Dezvoltarea unor noi metode de imagistica celulara **prin combinarea microscopiei confocale Raman/SERS cu microscopia confocala de fluorescenta rezolvata temporal (fluorescence lifetime imaging FLIM) pe baza de nanoparticule biofunctionale;**

(2) Producerea de noi clase de agenti antibacterieni **pe baza de nanoparticule**

(3) *Elaborarea de noi nanostructuri/nanoparticule* **in vederea trasportului de medicament la tinta**

Va multumesc