# BIONANOSTRUCTURES

## - SYNTHESIS, NANOMANIPULATION AND BIOMEDICAL APPLICATIONS -

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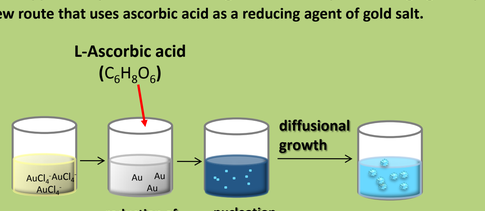
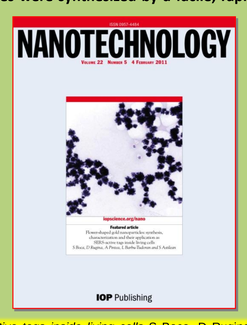
# Synthesis

## Gold Nano-Flowers

**This work*** **presents the synthesis and characterization of flower-shaped gold nanoparticles and demonstrates their applicability as SERS-active tags for cellular spectral imaging. The particles were synthesized by a facile, rapid new route that uses ascorbic acid as a reducing agent of gold salt.**



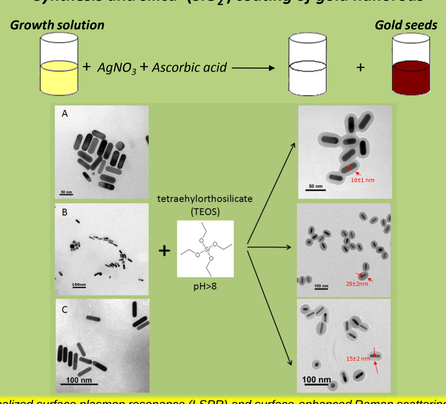




**Flower-shaped gold nanoparticles: synthesis, characterization and their application as SERS-active tags inside living cells* S Boca, D Rugina, A Pintea, L Barbu-Tudoran, S Astilean, **Nanotechnology** *22* **(2011)** 055702.

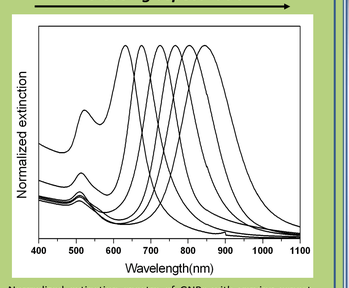
## Gold Nano-Rods

**In our work***, **we currently employ the 2 step CTAB-directed seed-mediated growth method to prepared gold nanorods with well controlled size and shape. Optical properties of GNRs are evaluated using UV-Vis spectroscopy and FDTD simulations. TEM imaging demonstrates the high monodispersity of GNRs.**

***Synthesis and silica*** **($SiO_2$)** ***coating of gold nanorods***




*Increasing aspect ratio*

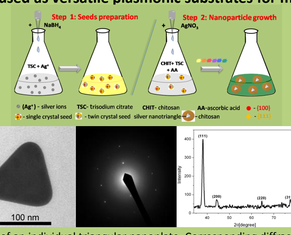


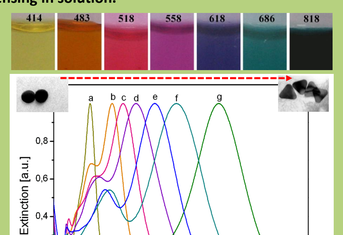

Normalized extinction spectra of GNRs with varying aspect ratios.

**Localized surface plasmon resonance (LSPR) and surface-enhanced Raman scattering (SERS) studies of 4-aminothiophenol adsorption on gold nanorods*, A.M. Gabudean , D. Biro, S. Astilean ,**Journal of Molecular Structure** 993 **(2011)** 420–424

## Silver Nano-Triangles

**In this study*** **we report the formation of chitosan-coated silver nanoparticles of triangular shape in solution by synergistic action of chitosan and trisodium citrate in the presence of silver seeds and ascorbic acid. It has been revealed that these anisotropic silver nanoparticles entrapped in biopolymeric shell are particularly stable and can be successfully used as versatile plasmonic substrates for molecular sensing in solution.**



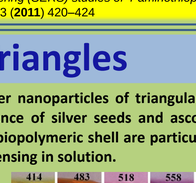

**Up:** TEM image of an individual triangular nanoplate. Corresponding diffraction pattern indicating the single crystal structure of the illustrated nanotriangle and X-Ray diffraction spectrum.
**Right:** Normalized UV-VIS-NIR extinction spectra and corresponding photographs of as-prepared silver colloids by increasing the TSC concentration: from 2.2 mM to 38.8 mM (from left to right) .The numbers on labeled vial correspond to the spectral position of the in-plane dipole resonance band.

**Solution-phase, dual LSPR-SERS plasmonic sensors of high sensitivity and stability based on chitosan-coated anisotropic silver nanoparticles*, Monica Potara, Ana-Maria Gabudean, Simion Astilean, **Journal of Materials Chemistry** 21 **(2011)** 3625-3633.

## Microspheres

The production of glasses by the sol-gel method allows preparation of glasses at far lower temperatures than is possible by using conventional melting. It also makes possible the synthesis of compositions that are difficult to obtain by conventional means because of problems associated with volatilization, high melting temperatures or crystallization. In addition, the sol-gel approach is a high-purity process that leads to excellent homogeneity. The sol-gel process comprises solution, gelation, drying, and densification. The spray drying technique is easy and reproducible to obtain microspheres.



Scanning electron microscopy ofaluminosilicate microspheres showing well defined hollow microspheres, with a spherical shape and Fe2O3 nanocrystals on their surfaces.

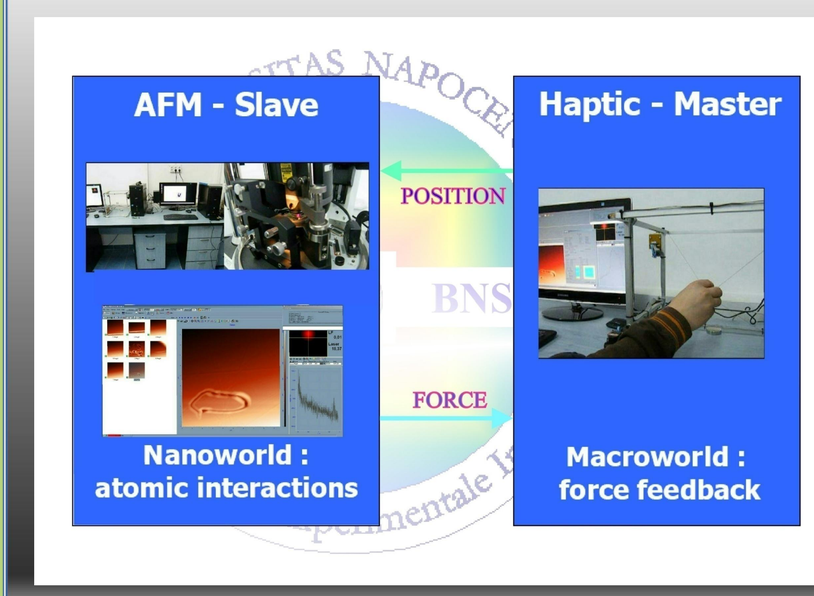


Transmision electron microscope of Si-Ca-P bioactive glass and AuNPs composite after 500° thermal treatment.
Scale bar: **100 nm**.

*M. Todea, B. Frentiu, R.F.V. Turcu and S. Simon*, ***Journal of Phys and Chem of Solids, 2011,*** *72 (3), 164-168*

# Nanomanipulation

## Telepresence and control




**In near upcoming we have much to learn about the nano-scale world, including how properties such as mechanical properties, electrical transport, and dynamics are affected by the atomic scale structure of the nano-objects and their interfaces. At ICEI-BNS, we have combined Scanning Probe Microscope (SPM - AFM) with a SPace Interface Device for Artificial Reality (SPIDAR) in a virtual reality environment to provide the intuitive display of instrument data and natural control of the SPM instrument functions. The significance of the virtual reality interface to the SPM is that it gives the scientist simulated presence on the sample surface – nanoworld telepresence.**

## Controlling molecular response by plasmon resonances

Regular arrays of noble metal nano-half-shells (NHS) are obtained by metal evaporation on 2D arrays of dielectric spheres. These NHS arrays support both localized and propagative surface plasmon excitations, as shown by FDTD electromagnetic (EM) simulations in Fig.(i). We achieve control over the spectral range of these plasmon modes by tuning the NHS size. This tunable plasmon modes can be efficiently used to manipulate the optical/spectroscopic response of molecules in the nanoscopic vicinity of the NHS. As examples NHS arrays were used to increase ($\times 10^6$-$10^7$) the intensity of Raman scattering of molecules adsorbed at the favourable locations of amplified EM fields (Fig.(ii)). The vibrational fingerprint of this molecules can be detected with a sensitivity approaching the single-molecule level, as shown by temporal spectral fluctuations (Fig.(iii)). These plasmonic NHS arrays are equally efficient for the enhancement of molecular fluorescence (Fig.(iv)). Such a control over the plasmon-molecule interactions with noble metal nanostructures is highly useful for developing innovative optical sensors for detection and analysis of bio-chemicals.

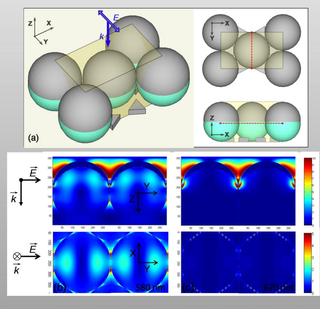



**Fig.(i).** (a) Scheme of the metallic nano-half-shells over dielectric spheres. (b) Simulated electric field maps showing a propagative photonic/plasmonic mode. (c) Simulated electric field maps showing a localized plasmonic mode.

C. Farcau, S. Astilean, *Mapping the SERS Efficiency and Hot-Spots Localization on Gold Film over Nanospheres Substrates*, **J. Phys. Chem. C 2010**, *114*, 11717.

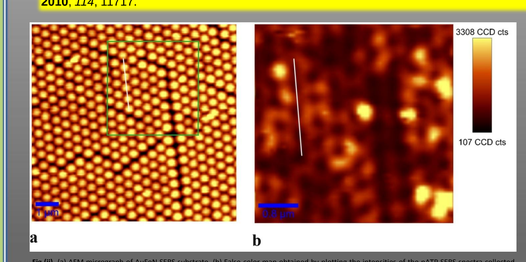
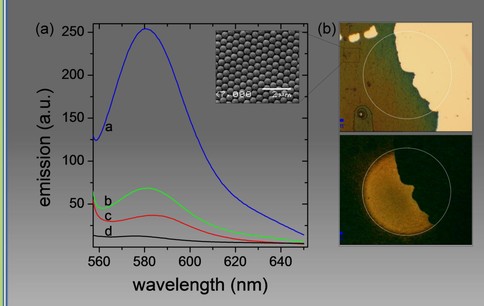



**Fig.(ii).** (a) AFM micrograph of AuFoN SERS substrate. (b) False-color map obtained by plotting the intensities of the pATP SERS spectra collected inside the area marked by the rectangle in A.



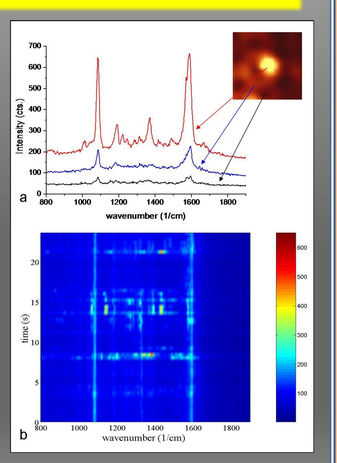




**Fig.(iii).** (a) pATP SERS spectra at three specific points: on top of Au HS (black), in between adjacent HSs (blue) and at hot-spot (red). (b) Waterfall plot of time-series of SERS spectra (integration time 0.4 s / spectrum).

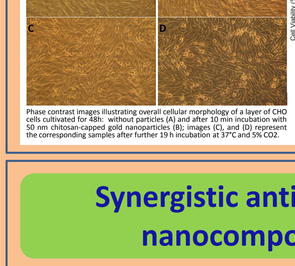
**Fig. (iv).** (a) MEF spectra of RosB on Ag HS arrays of 340 nm (a), 450 nm (b) and 500 nm (c) diameter, and emission of RosB on glass reference (d); inset shows a SEM image of the HS array. (b) top: White-light image of the border between Ag HSs region and mirror-like flat Ag film; bottom: Fluorescence image of the same area viewed through an edge filter under 532 nm laser illumination, revealing a strong contrast between the MEF active surface and the flat film surface.

C. Farcau, S. Astilean, *Silver half-shell arrays with controlled plasmonic response for fluorescence enhancement optimization*, **Appl. Phys. Lett. 2009**, *95*, 193110.

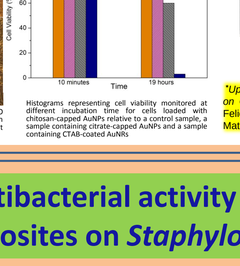
# Biomedical Applications

## Uptake and biological effects of chitosan-capped gold nanoparticles on Chinese Hamster Ovary cells

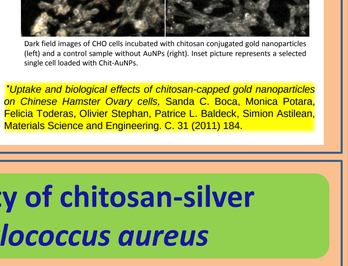
**In this study*** we examine the cytotoxic effect of chitosan capped-gold nanoparticles on Chinese Hamster Ovary (CHO) cells in vitro. The conjugated particles were able to traverse the cell membrane and enter the cells by endocytic pathway, their intracellular presence being clearly revealed by dark field microscopy imaging and light scattering spectra. Gold nanoparticles cytotoxicity was measured and cells were found to be viable more than 85%, even after long time exposure. Our results suggest that chitosan-conjugated gold nanoparticles have minimal impact on cell functions demonstrating great potential to be used in biomedical applications such as cellular imaging or photothermal therapy.




Phase contrast images illustrating overall cellular morphology of a layer of CHO cells cultivated for 48h: without particles (A) and after 10 min incubation with 50 nm chitosan-capped gold nanoparticles (B); images (C), and (D) represent the corresponding samples after further 19 h incubation at 37°C and 5% CO2.




Histograms representing cell viability monitored at different incubation time for cells loaded with chitosan-capped AuNPs relative to a control sample, a sample containing citrate-capped AuNPs and a sample containing CTAB-coated AuNRs

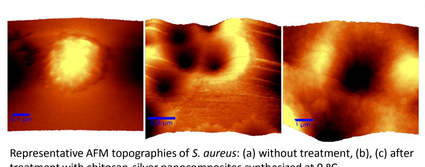


Dark field images of CHO cells incubated with chitosan conjugated gold nanoparticles (left) and a control sample without AuNPs (right). Inset picture represents a selected single cell loaded with Chit-AuNPs.

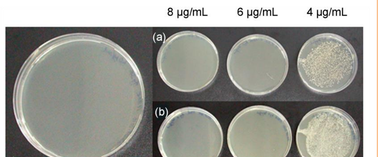
**Uptake and biological effects of chitosan-capped gold nanoparticles on Chinese Hamster Ovary cells*, Sanda C. Boca, Monica Potara, Felicia Toderas, Olivier Stephan, Patrice L. Baldeck, Simion Astilean, Materials Science and Engineering. C. 31 (2011) 184.

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

**In this study*** we evaluate the antibacterial effect of chitosan-silver nanocomposites against two strains of Gram-positive Staphylococcus aureus (S. aureus). We perform comparative quantitative tests and demonstrate that both chitosan and silver nanoparticles exhibit bactericidal activities and, more interestingly, a synergistic activity become operational when both components act together. In particular, we demonstrate by atomic force microscopy (AFM) that chitosan-silver nanocomposites cause considerable morphological changes in bacterial cells, leading to leakage of cell contents. Finally, we find that silver nanoparticles, beside their antibacterial activity, can be used as versatile SERS substrate for directly measuring the Raman signal of bacteria in colloidal solution.



Representative AFM topographies of *S. aureus*: (a) without treatment, (b), (c) after treatment with chitosan-silver nanocomposites synthesized at 0 ºC.

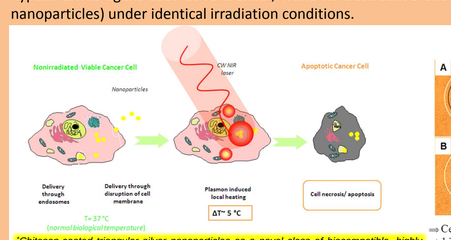


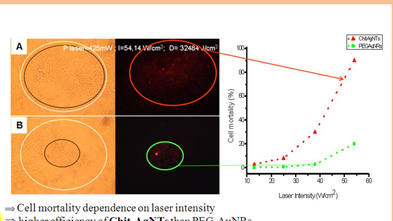

| Strain | Chitosan-silver nanocomposites synthesized at 0 °C | Chitosan-silver nanocomposites synthesized at 35 °C | Chitosan-silver nanocomposites synthesized at 35°C (without chitosan) | Silver nanoparticles synthesized at 0 °C (without chitosan) | Chitosan | | |
|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| UCLA 807/6 | 1.25 ± 0.75 | 6 ± 0 | 4 ± 0 | 15 ± 0 | 4.5 ± 1.5 | 19 ± 3 | 9 ± 4 | >28 | 10 ± 0 | 30 |
| 1190R | 0.75 ± 0.25 | 6 ± 0 | 4 ± 0 | 13.5 ± 1.5 | 6 ± 0 | 22 ± 0 | 10.5 ± 2.5 | >28 | 10 ± 0 | 30 |

**Synergistic antibacterial activity of chitosan-silver nanocomposites on Staphylococcus aureus*, Monica Potara, Endre Jakab, Annette Damert, Octavian Popescu, Valentin Canpean, Simion Astilean, **Nanotechnology 22 (2011) 135101 (9pp).**

## Plasmon assisted photothermal therapy of cancer using NIR- optically active noble metal nanoparticles

One of the relevant directions that nanotechnology is taking nowadays is connected with nanomedicine and specifically related to the use of light and nanoparticles in early diagnosis and effective therapeutics of cancer. Noble-metal nanoparticles can act under laser irradiation as effective photothermal transducers for triggering localized hyperthermia of tumors. **In this work*** we report the performance of newly synthesized chitosan-coated silver nanotriangles (Chit-AgNTs) with strong resonances in near-infrared (NIR) to operate as photothermal agents against a line of human non-small lung cancer cells (NCI-H460*)*. The hyperthermia experiments were conducted by excitation of nanoparticles-loaded cells at 800 nm wavelength from a Ti:Sapphire laser. We found that the rate of cell mortality in the presence of Chit-AgNTs is higher than in the presence of thiolated poly(ethylene) glycol capped gold nanorods (PEG-AuNRs) - a common hyperthermia agent used as reference-, while no destructive effects were noticed on the control sample (cells without nanoparticles) under identical irradiation conditions.



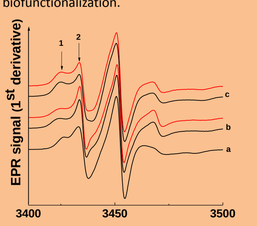

⇒ Cell mortality dependence on laser intensity
⇒ higher efficiency of **Chit-AgNTs** than PEG-AuNRs

**Chitosan-coated triangular silver nanoparticles as a novel class of biocompatible, highly effective photothermal transducers for cancer treatment*, Sanda C. Boca, Monica Potara, Ana-Maria Gabudean, Aurelie Juhem, Patrice L. Baldeck, Simion Astilean, submitted to **Cancer Letters**

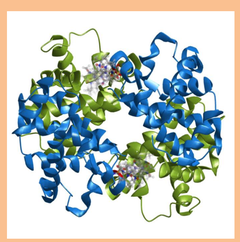
## Protein functionalization of nanostructured bioactive composites and aluminosilicate microspheres

Calcium phosphate and silicate-modified surfaces of gold nanoparticles have potential applications in orthopedic reconstruction and tissue engineering scaffolds because of the great biocompatibility and the variety of surface coatings by biofunctionalization.

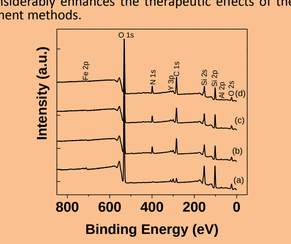
Aluminosilicate glass microspheres containing radioactive yttrium are currently employed with success to treat liver cancer. By addition of iron oxide they could be optimised for hysteresis heating in hyperthermia. The simultaneous application of radiotherapy and hyperthermia considerably enhances the therapeutic effects of the two cancer treatment methods.




X-band cw-EPR spectra of horse methemoglobin spin-labeled in position β-93* recorded at room temperature; the black lines correspond to the spectra recorded in solution (a) and in the adsorbed state, right after immersion on untreated (b) and treated (c) bioglass. The red lines represent the spectra recorded in the same conditions, but after ultrasonication



Structure of methemoglobin obtained by X-ray crystallography (2ZLU from Protein Data Bank). The α-chains are colored in green and the β-chains are colored in blue. The native cysteins from position β-93 are colored in red and for each position β-93 are shown all the 16 possible rotamers for JAA6-SL at room temperature.




X-ray photoemission (XPS) survey spectra of alumino-silicate microsphere system, $60SiO_2$-$20Al_2O_3$-$10Fe_2O_3$-$10Y_2O_3$, before immersion (a), after 5 minutes (b), 2 hours (c) and 24 hours (d) immersion in SBF/BSA. Y 3p (299 eV - 311 eV), Fe 2p (711.3 eV - 725 eV), Si 2p (~ 102.5 eV) and Al 2p (~ 73 eV) peaks were found for all investigated samples. However, for the protein functionalised substrates, detection of these four elements was less obvious due to the outer layer of protein. The N:C ratios of 0.2 and 0.25 established already after 5 minutes immersion time denote the quick attachment of BSA on the microparticles surface.

***C. Gruian, H.-J. Steinhoffs and S. Simon, **Digest Journal of Nanomaterials and Biostructures, 2011,** 6, (2), 373 - 383

**This work involves the contribution from the following authors (in alphabetical order):**
Astilean S., Baldeck P., Barbu-Tudoran L., Biro D., Boca S., Burda I., Canpean V., Chiriac M.T., Damert A., Farcau C., Frentiu B., Gabudean A., Gruian C., Iosin M., Jakab E., Juhem A., Kelemen B., Lucacel R., Lupan I., Pintea A., Ponta O., Popescu O., Potara M., Radu T., Rugina D., Simon S., Simon T., Simon V., Steinhoffs H. J., Stephan O., Todea M., Toderas F., Tunyagi A., Turcu R. V. F., Vanea E.

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1. BIOFUNCTIONAL NANOPARTICLES FOR DEVELOPMENT OF NEW METHODS OF IMAGING, SENSING, DIAGNOSTIC AND THERAPY IN BIOLOGICAL ENVIRONMENT (NANOBIOFUN), PCCE No 129/2008- CNCSIS in the frame of the PN-II research programs.
2. NANOMANIPULATION OF BIOMOLECULES BY ATOMIC FORCE MICROSCOPY, PCCE No 312/2008- CNCSIS in the frame of the PN-II research programs
3. SURFACE AND INTERFACE SCIENCE: PHYSICS, CHEMISTRY, BIOLOGY, APPLICATIONS, PCCE No 76/2008- CNCSIS in the frame of the PN-II research programs