SHORT TERM MOBILITY PROGRAM – YEAR 2016

Final Report

Title of the program: Synthesis of hybrid magnetoplasmonic nanostructures

***Proposer*:** Dr. Claudio Sangregorio

***Beneficiary*:** Dr. Nikola Knezevic

***Institution of the beneficiary*:** Vinca Institute of Nuclear Sciences, Serbia

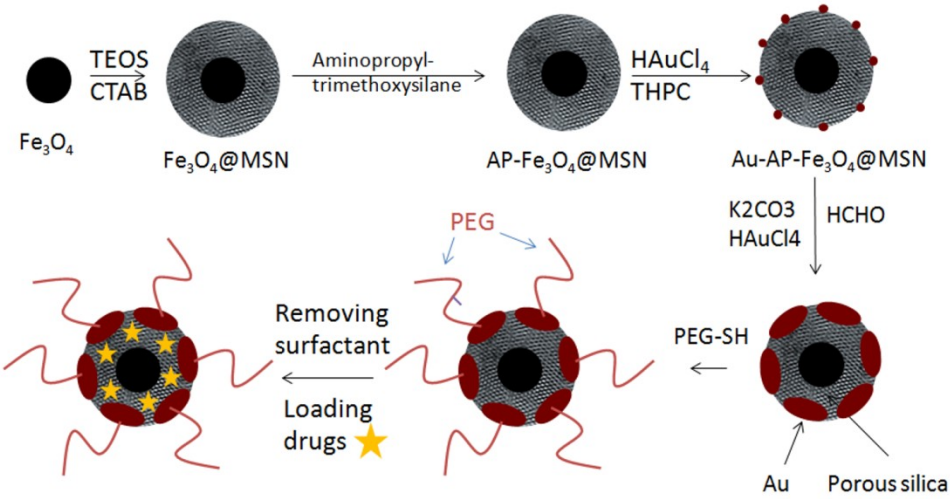
***Host institution*:** CNR-ICCOM, Sesto Fiorentino (Fi), Italy

***Period of stay*:** 16.05.2016. – 01.06.2016.

The goal of the research stay was to construct core/shell nanomaterials for application in diagnostics and treatment of tumors. Nanomaterials, when used as drug carriers, are known to enable preferential accumulation of drugs in tumor tissues due to the enhanced permeability and retention (EPR) effect of cancer, which enables spontaneous accumulation of drug carriers along with other macromolecules of suitable size in the extracellular medium of tumor tissue and hence inhibits occurrence of side effect during chemotherapy.[1] This feature can be further enhanced by structuring nanomaterials which can target the diseased tissues and deliver drugs upon exposure to selectively applied stimuli. For this reason, we proposed the construction of magnetic-plasmonic nanomaterials for cancer therapy exploiting the possibility to induce therapeutic effect by externally applied alternating magnetic field and light irradiation to induce the drug delivery to tumors. In addition, the presence of magnetite nanoparticles will allow direct MRI imaging of the treatment progression. Mesoporous silica nanoparticles (MSNs) are particularly attractive scaffolds for construction of such drug delivery nanodevices,[2] due to high surface area, isostructural mesoporosity and a particle diameter (100–200 nm) in the range needed to enhance the targeting ability of a Drug Delivery System, (DDS), through the EPR effect of cancerous tissue. *In vivo* studies have proven that MSNs are biocompatible, preferentially accumulate in tumors, and effectively deliver drugs to cancer tissues.[3]

The envisioned nanomedicines will be synthesized by the steps depicted on Scheme 1. The final nanosystem would have triple-layer morphology, with the central core composed of magnetite nanoparticles, the middle layer would be mesoporous silica while the outer layer would contain gold nanoparticles. The surface of the triple-layer structure will be finally functionalized with polyethylene (PEG) moieties for improving the stability of the nanocomposite in aqueous

solution and for improving the capabilities of the nanocarrier to evade the removal from biological environment through the immune response.[4] Hence, the drug delivery process would be responsive to two stimuli: alternating magnetic field (AMF) would cause drug release through heating of the local environment by magnetic NPs and infrared light would be capable to cause drug release through plasmonic heating of Au surface as well as to coadiuvate the drugs action by increasing the temperature of the tumor (hyperthermic therapy).



Scheme 1. Synthetic steps for the construction of the proposed magnetic-plasmonic nanosystem

Experimental section

Synthesis of magnetite nanoparticles (I)

All reagents (tris(acetylacetonato) iron(III) ($Fe(acac)_3$) 2 mmol, Oleic acid 10 mmol and Oleylamine 10 mmol) were dissolved in 25 mL of dibenzyl ether in a three neck flask. Nitrogen was first purged for 15 minutes and then the reaction mixture was stirred at 7000 rpm with magnetic stirrer and heated first to 200 ˚C (ramp in 15 minutes), then the temperature was held at 200 ˚C for 90 minutes, ramp again in 10 minutes to 300˚C and held at 300˚C for 1 h, all in nitrogen atmosphere.

After this time the reaction was quenched by rapid cooling to room temperature and then NPs were precipitated with ethanol (250 mL). The magnetite NPs were washed with ethanol twice and redispersed in chloroform.

Two additional types of magnetite nanoparticles were synthesized by the following methodology:

Synthesis of magnetite nanoparticles (II)

All reagents (tris(acetylacetonato) iron(III) ($Fe(acac)_3$) 2 mmol, Oleic acid 15 mmol and Oleylamine 15 mmol) were dissolved in 25 mL of dioctyl ether in a three neck flask. Nitrogen was first purged for 15 minutes and then the reaction mixture was stirred at 7000 rpm with magnetic stirrer and heated first to 200 ˚C (ramp in 15 minutes), then the temperature was held at 200 ˚C for 90 minutes, ramp again in 10 minutes to 300˚C and held at 300˚C for 1 h, all in nitrogen atmosphere.

After this time the reaction was quenched by rapid cooling to room temperature and then NPs were precipitated with ethanol (about 250 mL). The magnetite NPs were washed with ethanol twice and redispersed in dichloromethane.

Synthesis of magnetite nanoparticles (III)

All reagents ($Fe(acac)_3$ 2 mmol, Oleic acid 10 mmol and Oleylamine 10 mmol) were dissolved in 25 mL of dibenzyl ether in a three neck flask. Nitrogen was first purged for 15 minutes and then the reaction mixture was stirred at 7000 rpm with magnetic stirrer and heated first to 200 ˚C (ramp in 15 minutes), then the temperature was held at 200 ˚C for 90 minutes, ramp again in 10 minutes to 300˚C and held at 300˚C for 30 minutes, all in nitrogen atmosphere.

After this time the reaction was quenched by rapid cooling to room temperature and then NPs were precipitated with ethanol (250 mL). The magnetite NPs were washed with ethanol twice and redispersed in chloroform.

Synthesis of core-shell magnetite containing mesoporous silica nanoparticles (MMSN)

Iron oxide nanocrystals dissolved in chloroform (50 mg) were added to a solution of 250 mg cetyl trimethylammonium bromide (CTAB, Aldrich, 95%) in 20 mL of water. The mixture was sonicated for 30 minutes and the chloroform was boiled off from the solution with rapid stirring at 60 ˚C. During this process the reaction mixture changed from milky white to clear solution of magnetite in aqueous CTAB stabilized solution.

Then warm solution containing 875 µL of 2.0 M NaOH in 100 mL miliQ water was added to the solution of magnetite and the reaction solution was heated to 80 ˚C (ramp 30 minutes) and 1.25 mL of tetraethyl orthosilicate (TEOS, Aldrich, 98%) was added dropwise. After two hours of rapid stirring at 80 °C, the magnetic-core silica nanoparticles were collected by filtration and washed twice with water and ethanol. Isolated 320 mg of material.

Attachment of gold nanoparticles to the surface of MMSN (Au-AP-MMSN)

Aminopropyl-functionalized MMSN (AP-MMSN) was obtained by the following method:

150 mg of MMSN was heated at 80 ˚C under vacuum over night and then 25 mL of dry toluene was added, followed by 0.5 mL of 3-aminopropyltrimethoxysilane (AP-TMS). The reaction was heated to 110 ˚C under nitrogen and stirred vigorously for 2 h. The solution was then cooled, centrifuged at 11000 rpm, washed with 2-propanol, methanol and water.

Preparation of gold nanoparticles: 1 ml of KOH solution (1 M) and 13 µl of tetrakis(hydroxymethyl)phosphonium chloride (THPC) (80% in water) was mixed in 45 mL for 10 min. Then solution of $HAuCl_4$ (20 mg in 2 mL of water) was added quickly under vigorous stirring. The solution was stirred for 30 minutes.

The solution of gold nanoparticles was neutralized with HCl (1 M) and the aqueous suspension (previously sonicated) of AP-MMSN was added dropwise. The suspension was stirred gently over night. Then, the product (NK5) was isolated by centrifugation and washed 3 times with water and redispersed in 4 mL of water until further use.

Formation of polyethyleneglycol (PEG)-stabilized nanomaterial with Au shell on MMSN (PEG-Au-AP-MMSN)

K-gold solution was prepared by dissolving 25 mg K_2CO_3 in 100 mL of water and solution of $HAuCl_4$ (15 mg in 1.5 mL of water) was added. The solution was stirred for 10 minutes and left over night.

Into 160 mL of K-gold solution 2 mL of as prepared suspension of Au-AP-MMSN was added. The suspension was purged with nitrogen and stirred for 10 minutes. Then 0.4 mL of HCHO was added and suspension was vigorously stirred for 1h. Then additional 0.4 mL of HCHO was added and stirred additional 10 minutes, then 20 mg of O-(2-Mercaptoethyl)-O'-methylpolyethylene glycol (PEG-SH) (M = 10000) was added and the solution was additionally stirred for 10 minutes. The material was centrifuged, washed twice with water and re-dispersed in water.

Results and Discussion

Synthesis of magnetite nanoparticles were performed by thermal decomposition of tris(acetylacetonato) iron(III) ($Fe(acac)_3$) complex in a high boiling solvent (dibenzyl ether, dioctyl ether). The characteristics of the resulting synthesized magnetite nanoparticles can be seen on XRD and TEM images on Figures 1-3. Analysis of XRD patterns revealed that the nanoparticles have magnetite/maghemite structure and that the crystallite size of the magnetic nanoparticles I, II and III are 15 nm, 4.2 nm and 16.5 nm, respectively. TEM images show that the samples I and III are monodispersed and uniform while the diameter of nanoparticles in the sample II is polydispersed.

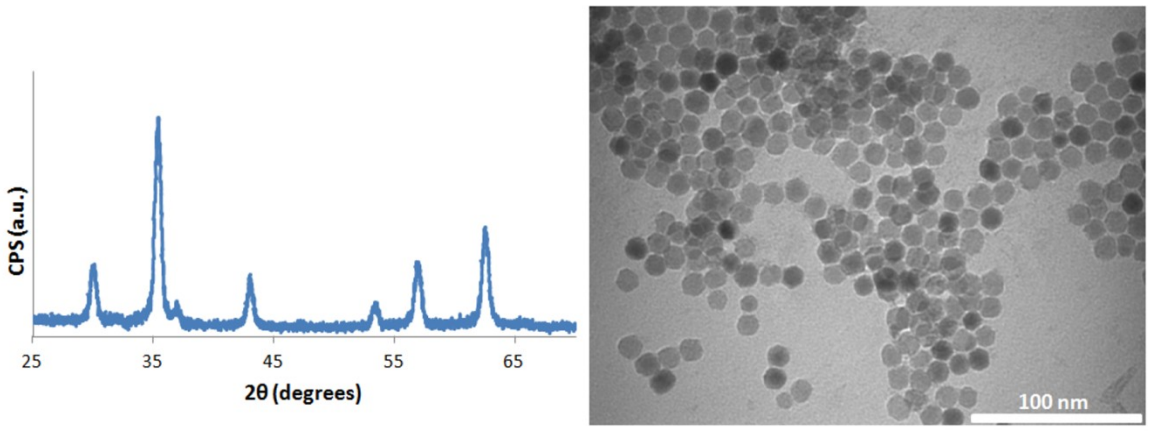


Figure 1. XRD pattern and TEM image of magnetic nanoparticles (I)

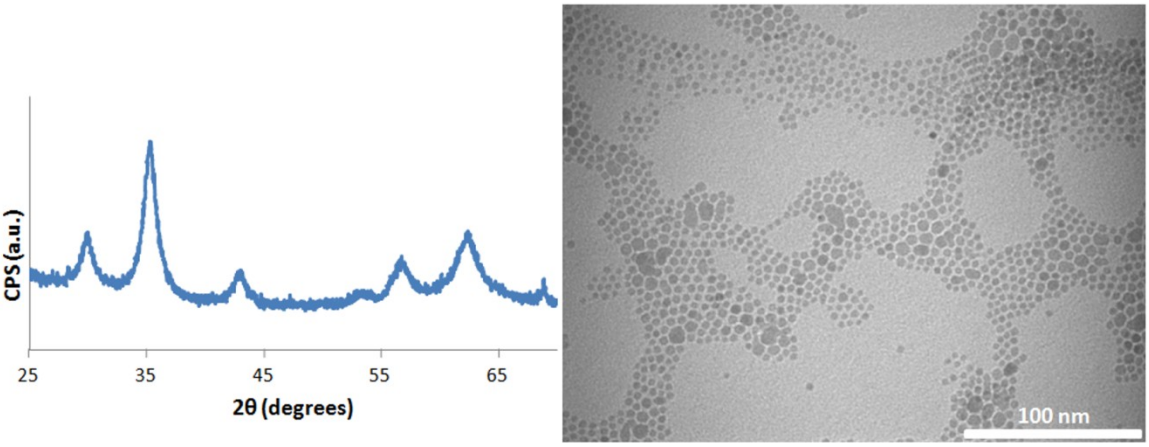


Figure 2. XRD pattern and TEM image of magnetic nanoparticles (II)

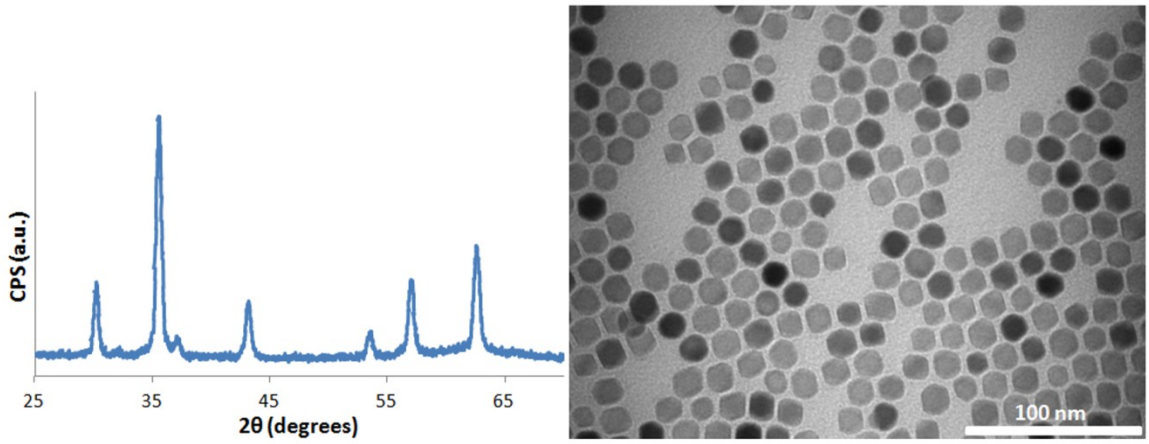


Figure 3. XRD pattern and TEM image of magnetic nanoparticles (III)

Sample I of magnetic nanoparticles was then used for construction of core-shell nanoparticles, with mesoporous silica as the shell in the material. Hydrophobic oleate-capped magnetic NPs were transferred into aqueous environment by addition of cetyltrimethylammonium bromide, which yielded a clear aqueous solution of magnetic NPs after removal of chloroform by heating at 60 ˚C. The hydrolysis and condensation of silica precursors in basic environment was then employed for the formation of mesoporous silica nanoparticles with the embedded magnetic nanoparticles as cores (MMSN). As can be observed on TEM images of the prepared core-shell material (Figure 5), the nanoparticles were successfully formed with a diameter of ca. 150 nm, and each nanoparticle contains one or more magnetic NPs. The presence of mesopores within the silica nanoshell is evidenced on higher magnification TEM images (Fig 5).

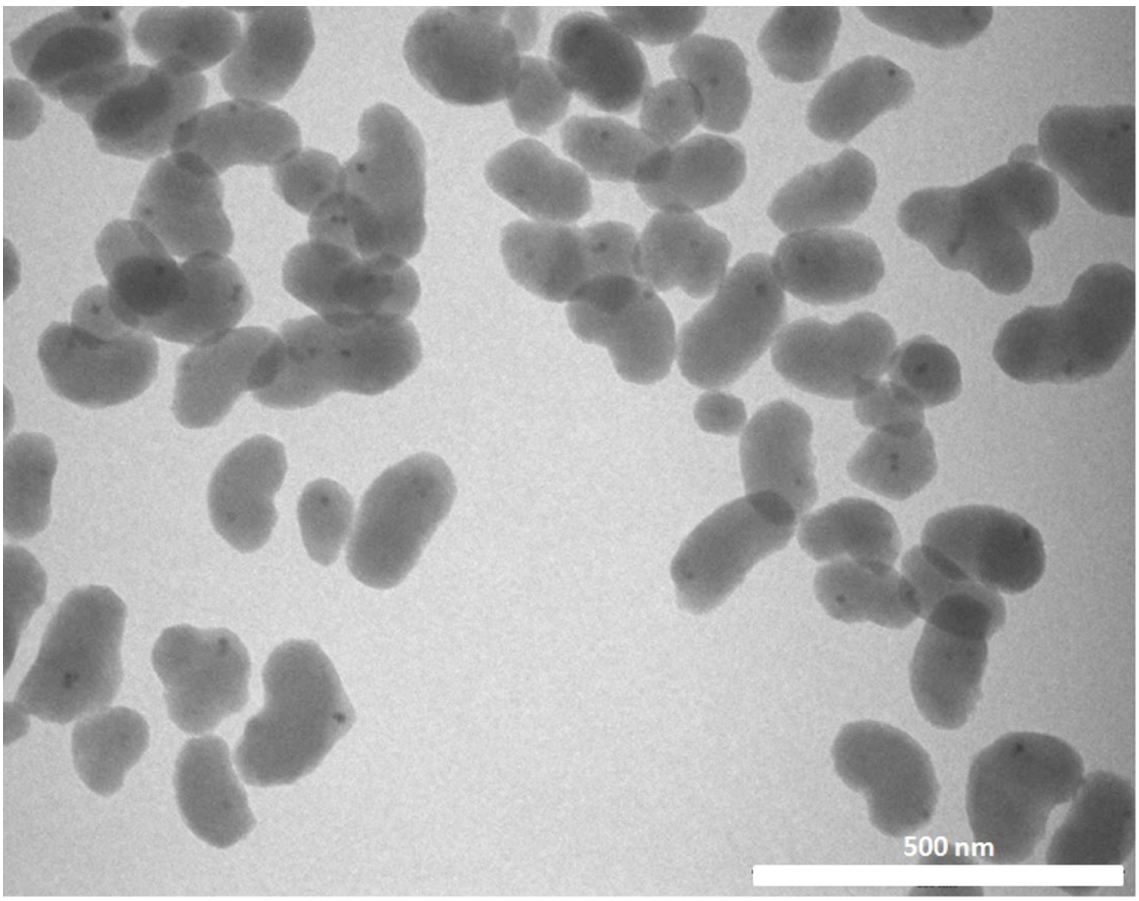


Figure 4. Low magnification TEM of core-shell MMSN material showing mesoporous silica nanoparticles with embedded magnetic nanoparticles (black dots).

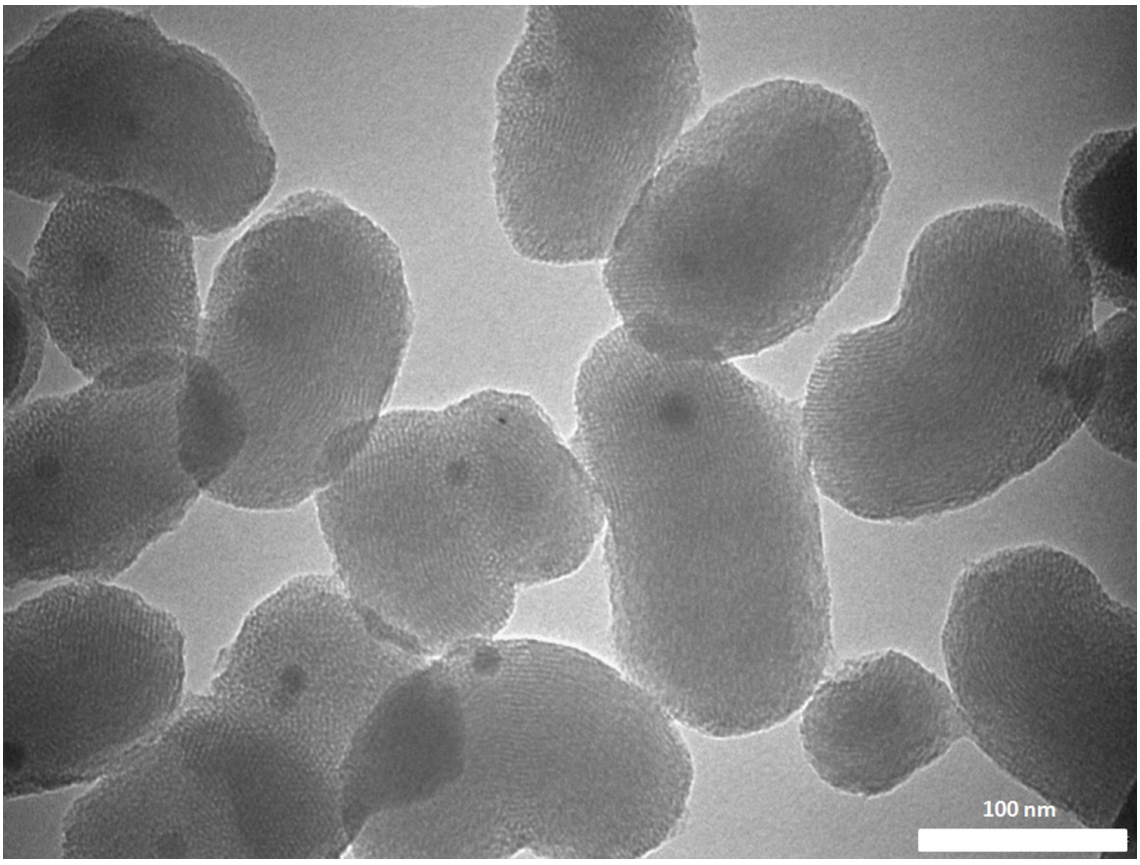


Figure 5. High magnification TEM image of core-shell MMSN showing the presence of mesopores within the silica nanoparticles (brighter lines and dots)

XRD measurements at low angle revealed that the MMSN material contains hexagonally packed mesopores as shown on Figure 6a. High angle XRD evidences the presence of magnetite/maghemite pattern within the MMSN material (Figure 6b).

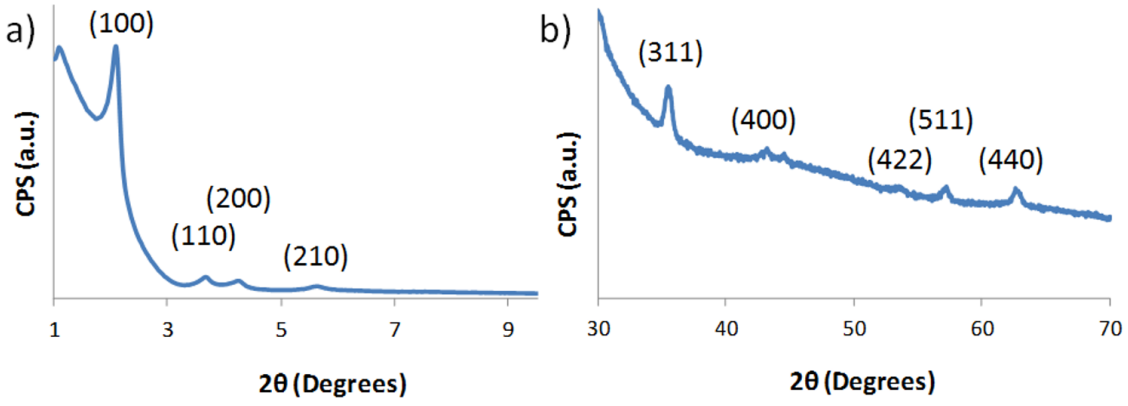


Figure 6. XRD measurement data for MMSN at a) low angle and b) high angle

In order to construct magnetic-plasmonic nanomaterial, the surface of MMSN was functionalized with 3-aminopropyltrimethoxysilane to form the material with positively charged amine moieties (AP-MMSN), which then would be capable to couple with negatively charged tris(hydroxymethyl)phosphine oxide (THPO)-functionalized gold nanoparticles. The reducing agent tetrakis(hydroxymethyl)phosphonium chloride (THPC) served for reduction of $HAuCl_4$ and formation of gold nanoparticles while the product THPO served as a negatively charged stabilizing agent.[5] After formation of THPO-stabilized Au nanoparticles, the solution was neutralized with HCl and mixed with AP-MMSN, which led to functionalization of silica surface with gold nanoparticles through the electrostatic interaction. In the attempt to further grow the Au shell on the silica surface, the obtained material was further subjected to reaction in the aqueous solution of $HAuCl_4$ and K_2CO_3 in the presence of HCHO. The growth of Au layer is desirable in order to increase the plasmonic absorption of gold at longer wavelengths, which would contribute to a deeper penetration ability of light through the tissue.[6] Finally, poleyethyleneglycol (PEG) moieties were functionalizated on Au surface with O-(2-Mercaptoethyl)-O'-methylpolyethylene glycol (PEG-SH) in order to improve the stability of the synthesized nanocomposite in aqueous solution.

The TEM images of the obtained Au-AP-MMSN and PEG-Au-AP-MMSN can be observed on Figure 7. As can be seen, the silica surface was irregularly covered by Au nanoparticles (black spheres): a portion of AP-MMSN nanoparticles are not covered by Au NPs while others display a good Au coverage. This result is surely due to a low Au/AP-MMSN ratio used during the synthesis, which needs to be increased in further attempt. Upon further reaction with gold solution no drastic increase could be observed in the size of Au nanoparticles on TEM images. UV/VIS/IR absorption measurements (Figure 8) show that both Au-AP-MMSN and PEG-Au-AP-MMSN have broad light absorption area which stretches into infrared region. This feature can be explained by the presence of Au nanoparticles of different sizes, which ranges from 2 nm to 45 nm in diameter.

Conclusions

The Short Term Mobility Program stay of Nikola Knezevic at CNR-ICCOM led to successful preparation of magnetic nanoparticles of different diameters and their incorporation into monodisperse core-shell magnetic mesoporous silica nanoparticles, which contain hexagonally packed mesopores. Magnetic-plasmonic nanocomposites were also synthesized by attaching preformed gold nanoparticles on the silica surface. The envisioned research study will be further continued in a continuous feedback between the ICCOM-CNR and Vinca laboratories, to optimize the morphology of the magnetic-plasmonic nanocomposite material, to load

anticancer drugs and to test the drug release and anticancer activity *in vitro* and *in vivo* upon exposure to alternating magnetic field and near infrared irradiation.

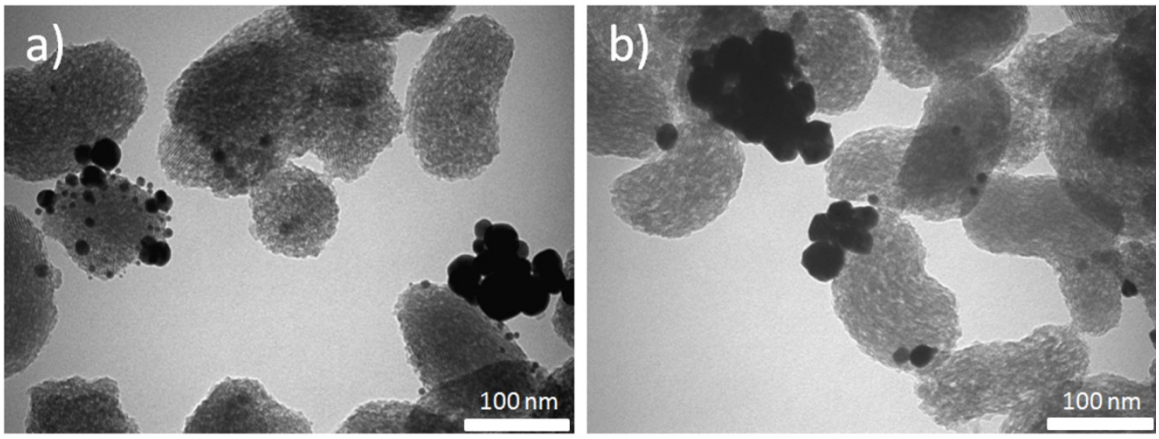


Figure 7. TEM images of a) Au-AP-MSN and b) PEG-Au-AP-MSN

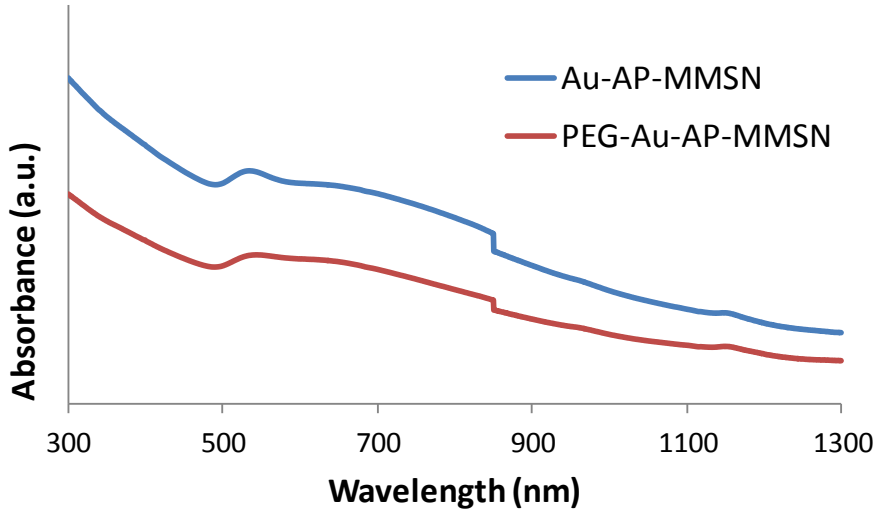


Figure 8. UV/VIS/IR absorption spectra of the prepared gold-containing nanocomposites. The broad band in the visible region arises due to Au plasmonic resonance

Additional activities of Nikola Knezevic at CNR-ICCOM

I

During his stay Nikola held two research seminars, one at CNR-ICCOM entitled “Functionalized silica-based nanoparticles for targeted cancer treatment” and one at the Department of

Chemistry “U. Schiff” – University of Florence, entitled “Magnetic-plasmonic nanomaterials for biomedical applications”

II

He performed alternating magnetic field induced hyperthermia measurements on two samples from Serbia. The two samples were measured in aqueous solution and in the gel form, and further collaboration in this research area is established.

III

Possibilities for future research collaborations between Vinca Institute in Serbia and CNR-ICCOM in various research projects were discussed and concrete research plans are established.

References

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