FINAL PUBLISHABLE SUMMARY REPORT

The project objectives for the TNP-HGNs project can be summarized in two sections; 1) Mobility and Integration objectives, and 2) Experimental objectives. Experimental objectives are; a) Synthesis of dye/drug containing thermolabile nanoprobes (TNP), b) Fabrication of gold nanostructures (HGNs), c) Conjugation of TNP onto the HGNs, d) Heat- & Laser-triggered release profiles of dye/drug containing TNP-HGNs, e) Investigation of the in vitro performance of drug containing TNP-HGNs.

In the duration of IRG project, it can be easily concluded that the mobility and integration objectives of the TNP-HGNs project are fulfilled. The researcher made three US university visits, participated in three international conferences/meetings and five national conferences, and made several presentations. He made various collaborations (detailed in section 7) which yielded 13 journal publications within the project period. The direct results of the TNP-HGNs project are in preparation for a full journal article which will be submitted early Fall 2013. The project also greatly enhanced the reintegration of the researcher. He is currently a faculty member at Materials Engineering Department as an Associate Professor and vice chair at the top advanced technology research center in Turkey.

In terms of experimental progress, metal nanostructures (HGNs) such as silver nanocubes, gold nanocages and gold nanospheres were successfully fabricated (Figure 1). Ag nanocubes were fabricated from a modified polyol procedure as addition of Ag salt in water instead of in ethylene glycol. Using these Ag nanocubes, Au nanocages were easily synthesized via galvanic reaction. Moreover, the Au nanospheres were synthesized by using Turkevich method which is using sodium citrate as both the stabilizer and the reductant.

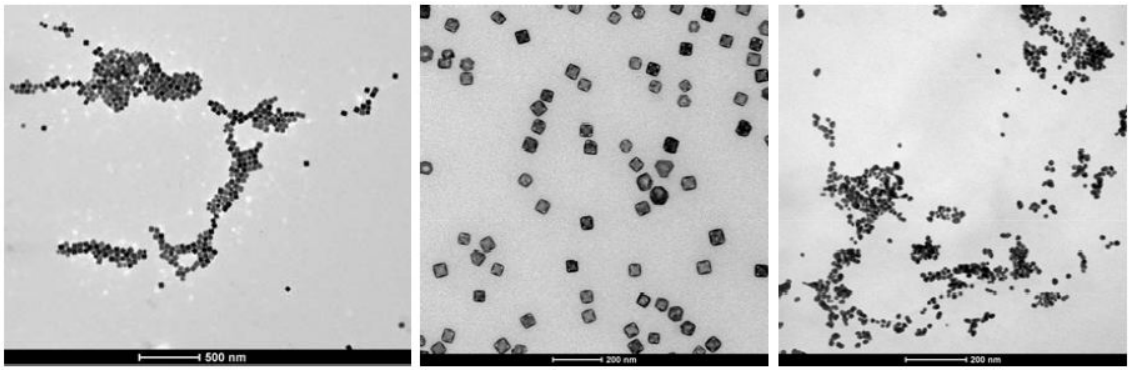
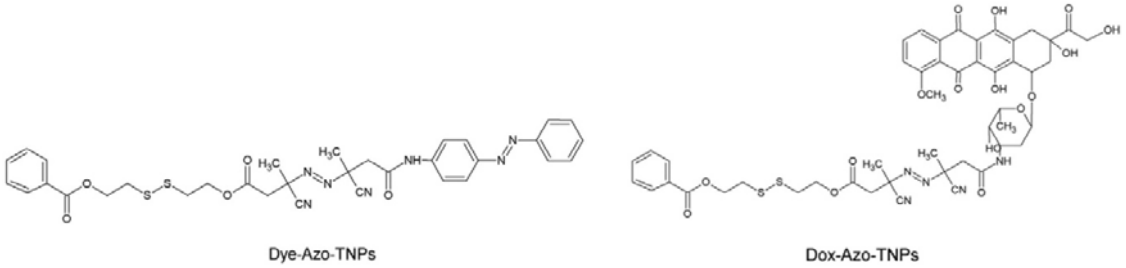


Figure 1. TEM images of Ag nanocubes (left), Au nanocages (middle), and Au nanospheres (right)

To synthesize dye containing TNP (**Dye-TNP**), one alcohol side of disulfide reactant was blocked with benzoyl ester. After this blocking step, the azo and disulfide groups containing first step product was successfully synthesized and characterized by NMR. In the second step, the dye molecules were coupled to synthesize Dye-TNP. The 4-phenylazoaniline containing TNP (Dye-TNP) as shown in Scheme 1 (left) were successfully synthesized and characterized by NMR. Dye-TNP was added to plain AuNPs for exchanging process. After washing steps, the Dye-TNP coated AuNPs (**Au-DyeTNP**) were successfully fabricated and characterized by using UV-Vis spectrophotometer. Then, the dye release profiles of Au-DyeTNP were studied using heat and laser irradiation.

After successful dye release studies from Au-DyeTNP, drug (Dox in this case) containing TNP (**Dox-TNP**) as the chemical structure shown in Scheme 1 (right) were planned to synthesize. The synthesis route for Dox-TNP was the same as the synthesis of Dye-TNP. The product was successfully synthesized and characterized. The Dox-TNP crude solution was added to the AuNPs for exchange process. After conjugation and washing steps, the Dox-TNP coated AuNPs (**Au-DoxTNP**) were successfully were fabricated and characterized by using UV-Vis spectrophotometer. The Dox release

profiles of Au-DoxTNP were studied using heat and laser irradiation. Also, the heat-triggered Dox release profiles of Au-DoxTNP were studied in the presence of Breast Cancer Cells (MCF7 cell line).



Scheme 1. The general chemical structure of Dye-TNP (left) and Dox-TNP (Right)

For the in-vitro cellular studies, continuous wave laser-triggered DOX release experiments were applied in the presence of MCF7 breast cancer cells. As shown in Figure 2 (left), there is a negligible experimental error in the control experiments which shows no effect (as destruction or killing effect) on cancer cells when only laser irradiation or only Au-DoxTNP addition were applied to the cancer cells. When irradiating with 50 mW CW laser, there was no significant decrease in cell viability which could be due to the lack of enough heat generation in 30 seconds in order to break the azo linkages which results the release of DOX from Au-DoxTNP species. In case of 100 mW and 500 mW, a small decrease in cell viability was observed. But, when irradiated with high power (2 W) laser, there is clear decrease in cell viability and almost half of the cancer cells were killed by the DOX released from the Au-DoxTNP.

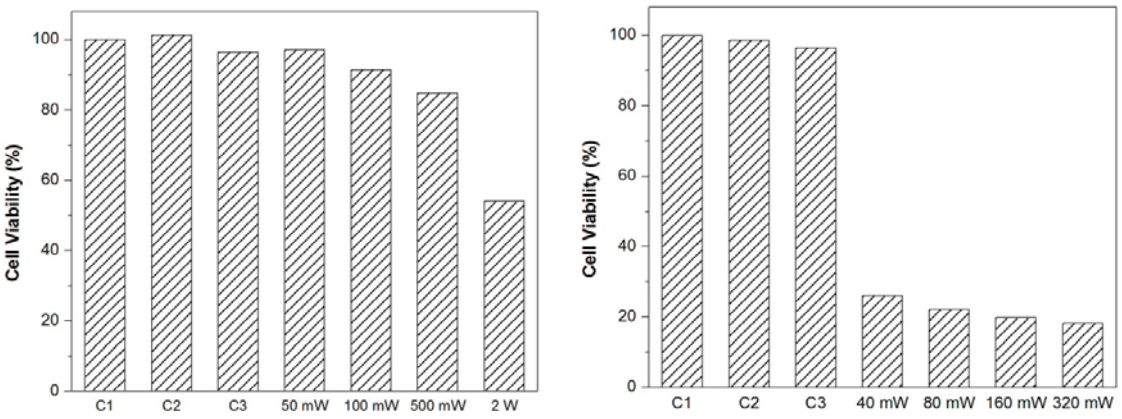


Figure 2. Cell viability of MCF7 cancer cells after CW (left) and NS (right) irradiation.

For the treatment of nanosecond (NS) pulse laser source, the same procedure was applied as CW laser experiments. As shown in Figure 2 (right), in the absence of Au-DoxTNP, the irradiation of cancer cells with a power of 40 mW was not effect to the cells. In the presence of Au-DoxTNP, powers of 40 mW, 80 mW, 160 mW, and 320 mW NS laser were irradiated to the breast cancer cells. The irradiation with high powered NS laser was killed more than 80 % cancer cells.

In conclusion, different shaped Au nanoparticles (AuNPs) and dye/drug containing thermolabile nanoprobes (TNP) were successfully synthesized. After conjugation of TNP on AuNPs, the heat- and laser-triggered release of dye/drug profiles were studied in acellular environment. Furthermore, in the presence of breast cancer cells (MCF7), the irreversible cleavage of azo-thermolabile groups on the Au-DoxTNP via laser irradiation (CW or NS lasers) resulted the release of Dox from the surface of Au nanoparticles which was triggered the deaths of the breast cancer cells.