

Functional RNA-AuNP conjugates for gene expression regulation based on the GFP example

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Gene expression is precisely regulated in all cells. One of the mechanisms responsible for this process is RNA interference, which can suppress protein expression by directed recognition of mRNA with short interfering fragments. On the other hand, RNA can occur in tertiary form and many RNA structural motifs have been defined. This is a foundation for RNA architectonics, which focuses on rational design and synthesis of structural and functional RNA nanoparticles (tectoRNA). Owing to development of bioinformatics and bionanotechnology, RNA motifs can be turned into the functional tectoRNAs with regulatory sequences to target a gene of interest. Scientists are keen to use gold nanoparticles (AuNPs), due to their optical properties, biocompatibility and the fact that they can penetrate cells or be a carrier of other molecules, such as: drugs, peptides, antibodies or nucleic acids. The conjugates of nucleic acids and AuNP, referred as spherical nucleic acids, were described previously and applied as potential therapeutics and biosensors.

In our research the merger of the RNA and AuNP nanotechnologies has led to creation of spherically organized, structural RNAs. Here, for the first time, we present a structural RNA conjugated with spherical AuNP. The main advantage of RNA-AuNP is the increased local concentration and controlled composition of regulatory fragments in tectoRNA, which cannot be achieved with dsRNAs. To study this system, we designed, synthesized and assembled a set of siRNAs and tectoRNA trimers targeting CopGFP gene in the model cell line. The RNA structures were hybridized with AuNPs and applied in the MDA-MB-231 cell line, stably expressing GFP. Our studies prove that the tectoRNA-AuNP nanoparticles penetrate cells, which was observed by TEM analysis; and can regulate gene expression indicated by reduction of cells' GFP fluorescence measured with plate reader, flow cytometry and under fluorescent microscope.