A gold-based nano-formulation of the CRISPR/Cas9 ribonucleoprotein for efficient delivery and genome editing

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BACKGROUND

- CRISPR/Cas9 technology, the "genetic scissors", has been awarded the Nobel Prize in Chemistry 2020.
- CRISPR/Cas9 has emerged as a promising tool in cancer treatment.
- Critical barriers related to safety and delivery are persisting.



Here, we propose a gold-based nano-formulation of the Cas9 ribonucleoprotein (AuNP-Cas9) for precise genome editing and efficient delivery.

representation of the goldbased nanoformulation of the CRISPR/Cas9 (AuNP-Cas9)

Synthesis & Characterization of the AuNP-Cas9

12nm Au nanoparticles were produced by ProChimia Surfaces for the purposes of the project. These NPs were functionalized with the Cas9 protein relying on affinity binding.

The functionalized nanoparticles were characterized by Transmission Electron Microscopy (TEM) & Dynamic Light Scattering (DLS).



AuNP-Cas9 cleaves target DNA *in vitro*

The concentration of the bound Cas9 protein onto the nanoparticles was measured by Dot blot analysis.

The AuNP-Cas9 was able to successfully cleave *in vitro* the DNA fragment. Higher protein amounts corresponded to a more efficient DNA digestion.



A: Schematic representation of the DNA cleavage by AuNP-Cas9



AuNP-Cas9 In µM 1.5 1 0.5 0.25 0.13 1.5 1 0.5 0.25 0.13 CTRL 100bp 100bp

> B: A representative 1.5% ethidium bromide-stained agarose gel showing the successful cleavage of the target Tyr1274 by AuNP-Cas9; For all the gels the concentration of DNA was 50nM and the gRNA was in equimolar amount with the Cas9.

> C: Analysis of endonuclease efficiency of the AuNP-Cas9. Statistical analysis was performed by 2-way ANOVA, Row Factor





(*dose*): *p*<0.0001; *Column Factor* (*treatment*): *p*=0.0002. *Data* from 3 independent experiments. Data shown as mean ±SEM.; CTRL, template DNA.

AuNP-Cas9 induces gene editing in zebrafish

The AuNP-Cas9 was injected in zebrafish embryos at the one-cell stage and induced loss of function of the tyrosinase gene resulting in depigmented zebrafish at 3 days post fertilization (3dpf).



A: Schematic representation of the microinjection process

B: Representative images of depigmented gene edited zebrafish at 3dpf. The depigmented phenotype means the gene editing against the tyrosinase gene was successful.

C: Survival test. Statistical analysis was done by Kruskal-Wallis test, p=0,0002 (from 4 independent experiments for CTRL, AuNP-Cas9, Cas9 1125 pg/embryo, and experiments for Cas9 750 pg/embryo).

AuNP-Cas9 enters spontaneously in human melanoma cells

The AuNP-Cas9 internalized spontaneously in A375 human melanoma cells after 2 hours of exposure without any transfection method. It localized as single nanoparticles in the cytoplasm, in organelles, and in the nucleus, as shown by TEM. The nuclear localization efficiency of AuNP-Cas9 was compared with the one using RNAiMAX to deliver the Cas9:gRNA RNP inside the cells.

В





B: TEM images of human melanoma cells A375 incubated with the AuNP-dCas9 (dead-catalytically Cas9, no DNA cleavage) 2x10^11 NPs/ml for 24 hours. Cells were then washed and fixed with 1.5% glutaraldehyde in Na Cacodylate buffer 0.1M pH 7.3. Results show the presence of AuNP-Cas9 in the cytoplasm, mitochondria (black circles), and nucleus (white circle).





E: Melting analysis performed Resolution Melt by High (Applied Biosystems). In the Difference Plots an uninjected control sample

was selected as reference

A: Confocal images of treated A375 cells (treatment stated for each group image). Cells were incubated for 2 hours, and then fixed in 4% formaldehyde.



C: Quantification of the fluorescence in the nucleus of A375 cells by confocal imaging. Statistical analysis was done by Kruskal-Wallis *test, p<0,0001*

Analysis based on the detection of Cas9. Data from 3 independent experiments. Results are shown in boxplot 10-90 percentage

AIM/PERSPECTIVES

- AuNP-Cas9 as a bio-tool for cellular biology applications
- > Exploit the plasmonic properties of gold nanoparticles to induce targeted photothermal therapy in cancer cells
- Produce a totally home-made AuNP-Cas9

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