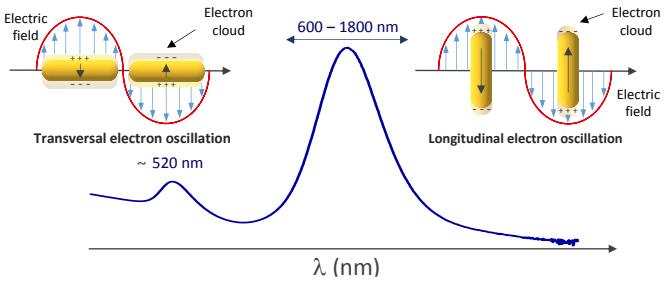
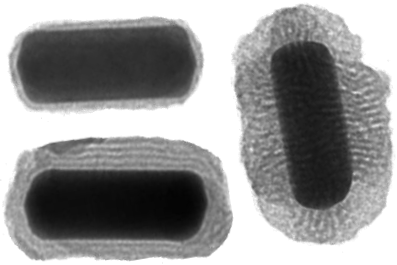
Pulsed Laser Photoinduced Gene Delivery using Tailored Core-Shell Au@SiO_2 NanoRods

1) Study context

Gold nanorods (AuNRs) are now, and over several decades, established as central items for biological applications including biosensing, drug delivery and photothermal therapy. Their ability to absorb light upon irradiation originates from the collective oscillation of electrons in the conduction band of the gold surface; it results in two localized Surface Plasmon Resonance (LSPR) bands called the transversal and longitudinal bands, t-LSPR and l-LSPR, respectively; they correspond to the resonance along the short and long axes of the particle, respectively. [1-2] *The position of l-LSPR band can be finely tuned from 600 and up to 1800 nm by controlling the particle aspect ratio.* [3] *This versatility is of high interest for biomedical applications such as photothermal therapy or drug delivery because of the minimal absorption of blood and human tissues in this region.* [4] *The extensive development of synthesis protocols enables the preparation of particles with desired and controlled properties, mainly using cetyltrimethylammonium bromide (CTAB) as surfactant, surface stabilizer, and shape inducing agent. However, the cytotoxicity of CTAB and its interferences with biological processes, restrict the biomedical applications of AuNRs in photothermal therapy or drug delivery.* [5] *Besides the damaging effect of CTAB, one major drawback of AuNRs application in photothermal therapy, is that they are not stable under irradiation and reshape into spherical nanoparticles.* [6] *In addition, the presence of CTAB limits the accessibility to gold surface and strongly inhibits the grafting of biomolecules such as oligonucleotides for gene therapy.* [1] *To overcome these limitations, the growth of a silica shell on AuNRs, also referred to as silica coating, is the most promising route and research in this topic has expanded tremendously over the last decade as it allows to eliminate or screen CTAB while preserving AuNRs' shape and therefore, their optical properties. The LRS group at Sorbonne University has recently reported a versatile method for the synthesis of core-shell AuNR@SiO_2 nanoparticles with tailored shell thickness and porosity to adapt it to the desired biological application.* [7] *We intend in this project to use this technology for in vitro and in vivo assessments of the intracellular gene delivery by laser-induced photothermal conversion in the context of cancer fight. For this, we will rely on a precise nanoscale thermal management in order to deliver the only desired genetic material while minimizing side effects. This will be insured thanks to our multiscale modeling approach and experimental monitoring with base pair spatial resolution. The co-supervising group at MASCOT has an established expertise in tissue analyses and in murine models for translational research purpose, including preclinical drug development. The PhD project will also benefit from the expertise of LuMIn group in modelling and measuring the stationary and ultrafast transient optical properties of gold nanoparticles, ultrashort laser pulse experiments, light-heat conversion by nanoparticles, and heat transfer at short time and space scales.* [8-9]





In what follows the detailed description of the methodology adopted here to reach the scientific objectives of this multidisciplinary PhD project.

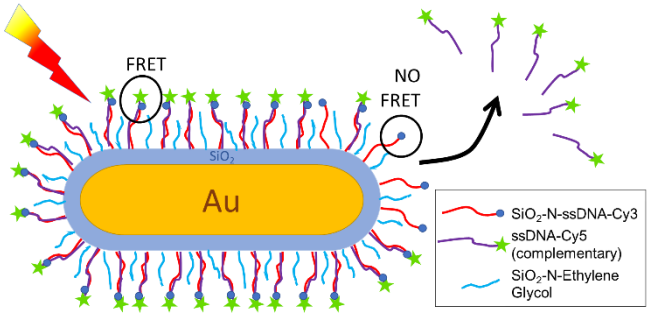
2) Methodology to reach the scientific objectives

This PhD project is co-supervised by Souhir Boujday at Sorbonne University (UMR 7197 LRS) and Guilhem Bousquet at University Sorbonne Paris Nord (UMR_S942 MASCOT), in collaboration with Bruno Palpant at University Paris-Saclay (UMR 9024 LuMIn).

1. Synthesis of gold nanorods, silica coating. *The candidate will synthesize AuNRs with proper aspect ratio by the seed-mediated method. Centrifugation-redispersion cycles will refine the shape distribution. A silica layer (1- 10 nm thick), either dense or porous, will then be initiated and developed on the AuNR surface following the previously mentioned protocols. These protocols lead to the complete removal of CTAB with resulting reproducible and stable colloidal particles. The multiple steps of synthesis and coating with SiO_2 will be assessed and quantified relying on the characterization platforms available at LRS, UV-vis spectroscopy, DLS and zetametry, Raman, XPS and Electron microscopy. The input of porosity to further protect the oligonucleotides from nucleases will be explored.*

2. Biofunctionalization and DNA strand grafting. *Relevant sequences of therapeutic antisense oligonucleotides (ON), single strand DNA (ssDNA) and the complementary sequence leading to the double strand DNA (dsDNA), selected and provided by our collaborator expert in DNA biophysics, Brahim Heddi at LBPA in Paris Saclay, will be used in this project. Once the synthesis and silica coating protocols mastered by the PhD student, the AuNR@SiO_2 surface will be functionalized to optimize ssDNA and/or dsDNA grafting by condensation of silanes terminated by aldehyde or thiol groups. Subsequently single and double DNA strands will be attached and/or embedded within the porosity of silica. The coverage, density and accessibility of these strands will be assessed at the LRS by the techniques mentioned above.*

3. Photo-induced oligonucleotide release: analysis and optimization. *The cellular toxicity and cellular uptake of AuNR@SiO_2 will be assessed and optimized, using two different triple negative breast cancer cell lines. The laser-induced release of ssDNA from dsDNA grafted onto AuNPs will be quantified. For this, we will carry out at LuMIn fluorescence imaging experiments based on the FRET energy transfer between two fluorophore tags labeling each of the ssDNA in a duplex. We will study the complete denaturation of dsDNA strands thanks to the dependence of the emitted intensity on the fluorophore-AuNP distance, in vitro and then in cellulo.*



4. In vivo assessment of the therapeutic relevance of the functionalized nano-objects. For in vivo experiments, we will use patient-derived xenografts (PDX) obtained from metastases of women with triple negative breast cancers. *MASCOT* has already implemented various models of PDX and has demonstrated their relevance for preclinical development of drugs, including nano-objects [10] [11] [12] [13]. The validation of our NPs for targeted gene delivery will start with assessing their biodistribution *in blood and different tissues (liver, spleen, kidney) including tumors 24h, 48h, and 72h after intravenous injection. Nanoparticles biodistribution will be determined by the absolute quantification of gold in tissues using ICP-MS, and by their cell and sub-cellular localization using two complementary methods mastered by MASCOT team, dark-field microscopy and electron microscopy. Through systematic analysis of all organs obtained at the time of euthanasia, we will also assess the potential toxic effects of the nanoparticles on normal tissues. Data obtained from the pharmacokinetic/biodistribution study will enable to determine the time nanoparticles accumulate the most in the tumor after intravenous injection, and will be a requisite for further irradiation study. MASCOT has determined optimal irradiation conditions using a subpicosecond pulsed laser (100 mW/cm², 60 kHz, 10 minutes of irradiation) not to induce toxic effects on normal tissues in the absence of nanoparticles (manuscript submitted). After irradiation of the same breast cancer xenografts, we will then demonstrate and improve the efficiency of the light-induced ON release in vivo, through detection of fluorescence-labeled DNA on tissue section. Using digital-droplet PCR combined with laser-microdissection of fluorescent cancer cells on frozen sections obtained at the time of euthanasia, we will then quantify the specific ON delivered.*

3) References (colored for the supervising groups)

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