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FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Life Sciences Department

Gold Nanoparticles for Gene Silencing

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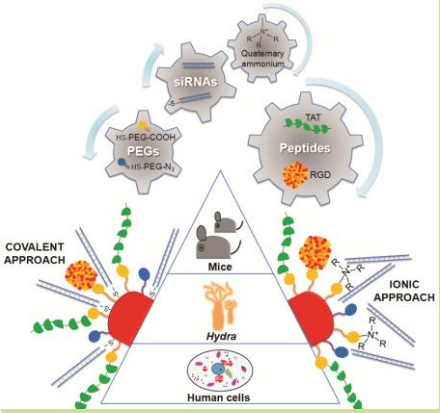
PhD Grant (FCT, SFRH/BD/62957/2009)

Research fellowship (PTDC/BIO/66514/2006)

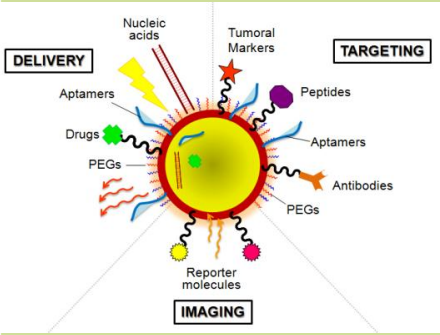
Objectives

The main goal is to find evidence of RNAi triggering, specifically silencing c-myc protooncogene, via the synthesis of a library of novel multifunctional gold nanoparticles (AuNPs).The efficiency of the AuNPs will be demonstrated using a hierarchical approach including three biological systems of increasing complexity: in vitro cultured human cells, in vivo invertebrate (freshwater polyp, Hydra), and in vivo vertebrate (mouse) models. Our synthetic methodology involved fine-tuning of multiple structural and functional moieties. Selection of the most active functionalities was assisted step-by-step through functional testing that adopted this hierarchical strategy. Merging these chemical and biological approaches led to a safe, nonpathogenic, self-tracking, and universally valid nanocarrier that could be exploited for therapeutic RNAi.



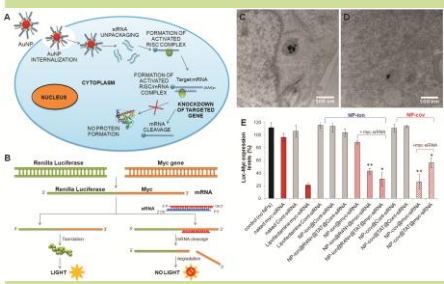
Methodology

Development of effective conjugation strategies to combine, in a highly controlled way, specific biomolecules to the surface of AuNPs such as: a) **Biofunctional spacers**: Poly(ethylene glycol) (PEG) spacers, with a thiol end to bond covalently to the gold nanoparticle and carboxilic acid and azide functional groups in the other end. PEGs are used to increase solubility and biocompatibility; b) **Cell penetrating peptides**: exploit more than one mechanism of endocytosis to overcome the lipophilic barrier of the cellular membranes and deliver large molecules inside the cell. AuNPs functionalize with TAT peptides by EDC/NHS coupling reactions will be used to achieve cytoplasm; c) **Fluorescent Dyes**: TAMRA-cadaverine will be used for tracking the AuNPs into the cells; d) **Tumoral Marker**: RGD peptide; e) **RNA interference**: siRNA complementary to a master regulator gene, the protooncogene c-myc, will be bond covalently and ionically to AuNPs.



Expected Results

In this study, we show that ionic and covalent approaches designed to produce smart multifunctional nanostructures succeeded to generate new innovative and versatile tools for efficient RNA interference in eukaryotic systems. Using the three biological models of increasing complexity, both design and validation of multifunctional nanocarriers capable of selectively and specifically delivering siRNA in vivo were demonstrated. These multifunctional nanocarriers are robust enough to preserve stability without showing acute toxicity or cell viability impairment while simultaneously able to bypass biological barriers to perform RNAi activity without off-target effects. Our universal nanocarrier represents a valid gene delivery platform that can be exploited for clinical application in the near future.



Funding:

FCT grant (SFRH/BD/62957/2009)
ERANET-NANOSCIERA NANOTRUCK project