DEVELOPMENT OF A TARGETING STRATEGY TO ENABLE SELECTIVE PHOTOPORATION OF THE NUCLEAR ENVELOPE

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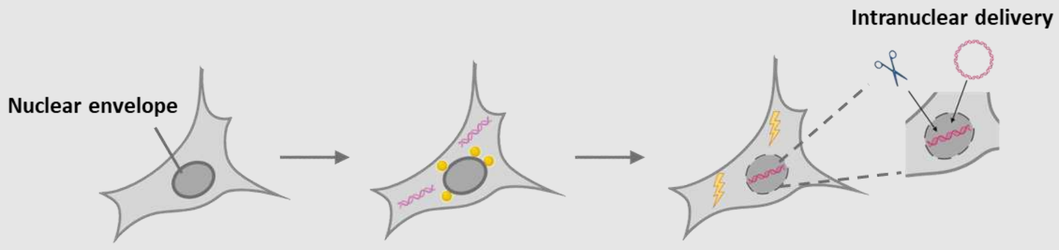
INTRODUCTION



Background

- ✓ Photoporation is a technology relying on laser illumination of photothermal nanoparticles, such as gold nanoparticles (AuNPs), which can permeabilize nearby biological membranes due to heating and mechanical effects
- ✓ Photoporation can be used to induce pore formation in the nuclear envelope (NE) if the sensitizing particles are positioned nearby, offering a promising strategy for intranuclear delivery, a key challenge in genome editing and other nuclear-targeted therapies

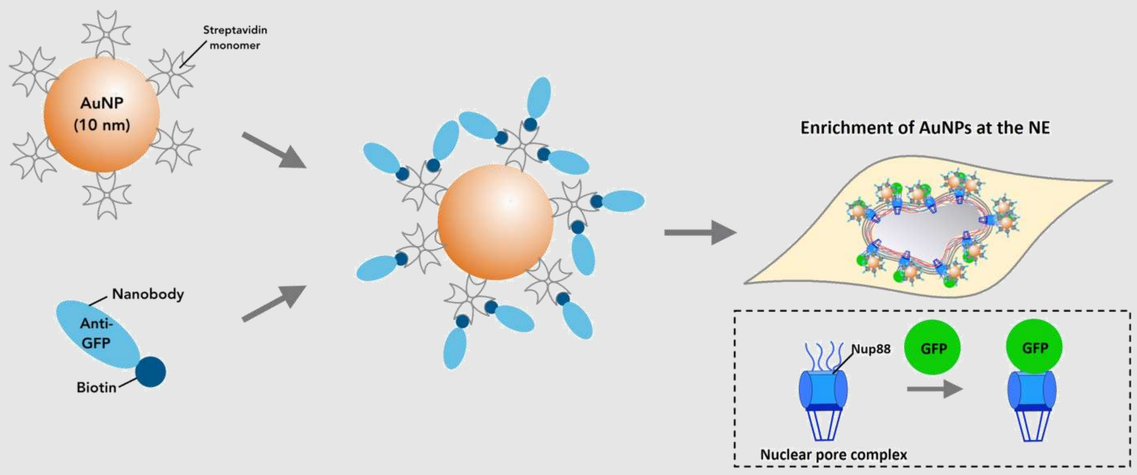
Limits

- When relying on endocytic uptake of the nanoparticles, the efficiency of NE rupture is quite low (around 7%) due to the inability to accurately control the distance between the endocytosed nanoparticles and the NE

How to improve

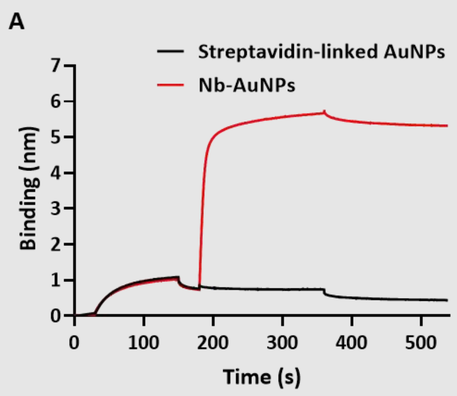
- Cytosolic delivery of AuNPs functionalized with nanobodies targeted to NE-resident proteins, allowing them to bind to the NE and induce NE ruptures upon laser stimulation

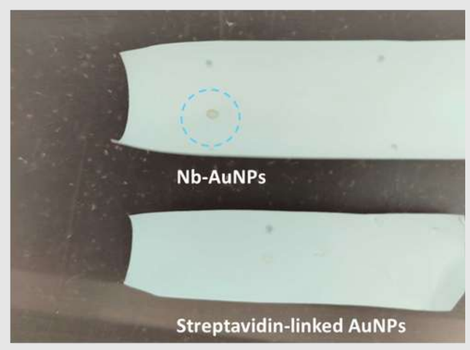
METHODS

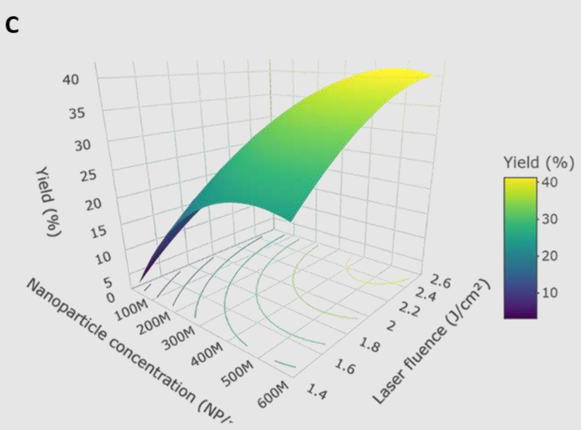


- Ultra-small streptavidin-linked AuNPs (10 nm) are chosen as photosensitizer
- Cell line expressing a GFP-coupled nucleoporin as a proof of concept
- Functionalizing AuNPs with anti-GFP nanobody (Nb-AuNPs) via a biotin-streptavidin link
- NIR photoporation with iron oxide nanoparticles (IONPs) as sensitizers for delivering Nb-AuNPs into the cytosol

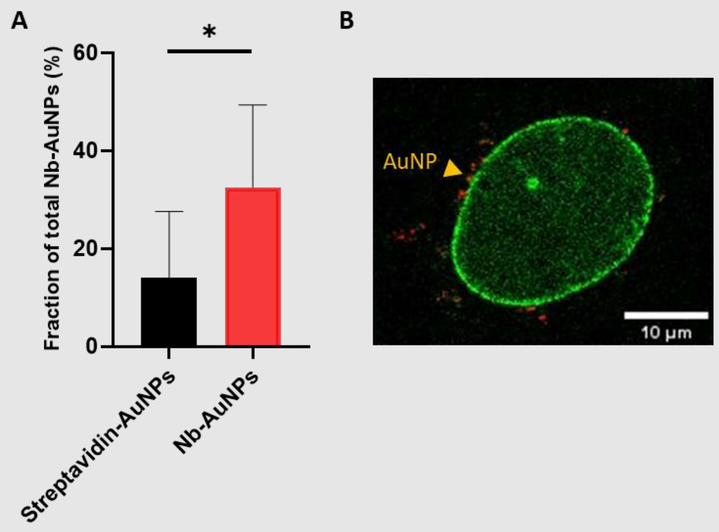
RESULTS







- ✓ Biolayer interferometry confirms successful binding of anti-GFP nanobody to streptavidin-AuNPs
- ✓ Dot blot assay further validates nanobody conjugation, showing strong signal for Nb-AuNPs compared to non-functionalized controls
- ✓ Response surface methodology (RSM) was applied to optimize IONP concentration and laser fluence, identifying optimal parameters for maximal cytosolic delivery



- ✓ The percentage of internalized Nb-AuNPs bound to the NE was quantified using confocal z-stack images analyzed in ImageJ, showing the increase in NE-localized nanoparticles for Nb-AuNPs (n = 133 cells) compared to streptavidin-AuNPs (n = 75 cells) significantly (p = 0.033, Mann–Whitney test)
- ✓ Confocal imaging confirms NE-localization of Nb-AuNPs (red) in U2-OS Nup96-mEGFP cells (green) after cytosolic delivery via plasma membrane photoporation

REFERENCE

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CONCLUSION and FUTURE PERSPECTIVES

We successfully **functionalized AuNPs with nanobodies** that can **target a GFP-coupled nucleoporin**. Cytosolically delivered Nb-AuNPs were found to bind to the NE of Nup96-mEGFP cells. Next steps include validating NE targeting by the Nb-AuNPs using electron microscopy, and evaluating whether the NE-targeted Nb-AuNPs increase the efficiency of **NE photoporation**.

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