

Tech Session 2: Nanomedicine and Nanoscale Delivery

Juan C. Fraire

“LIGHT-TRIGGERED NANOBOMBS FOR NANOPROJECTILE-MEDIATED DELIVERY OF BIG MACROMOLECULES”

CRS 2022 Annual Meeting & Expo

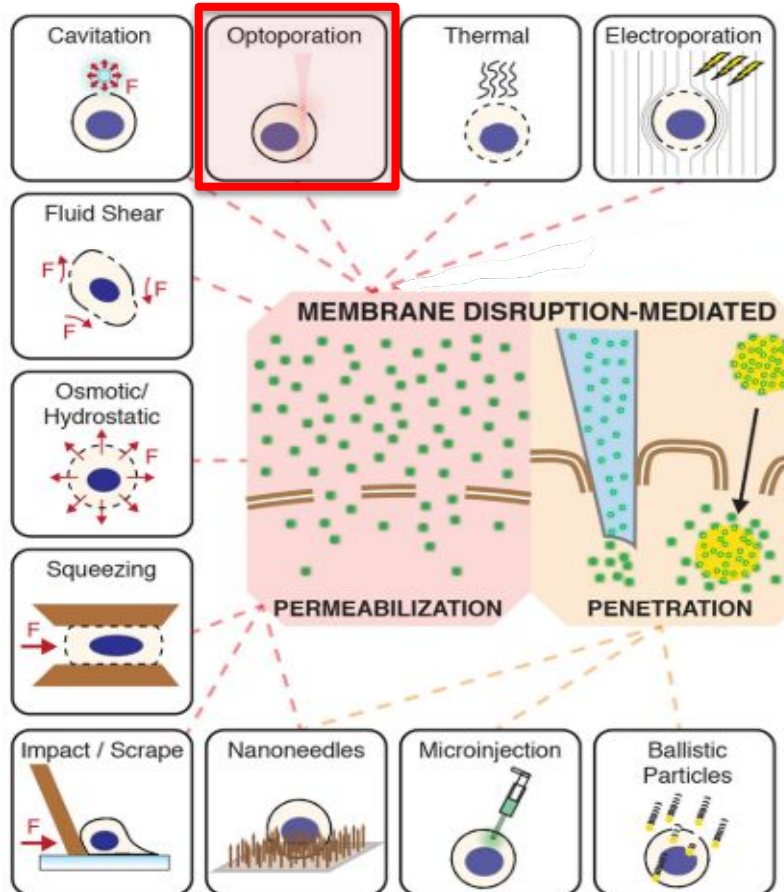
July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Advanced Delivery Science

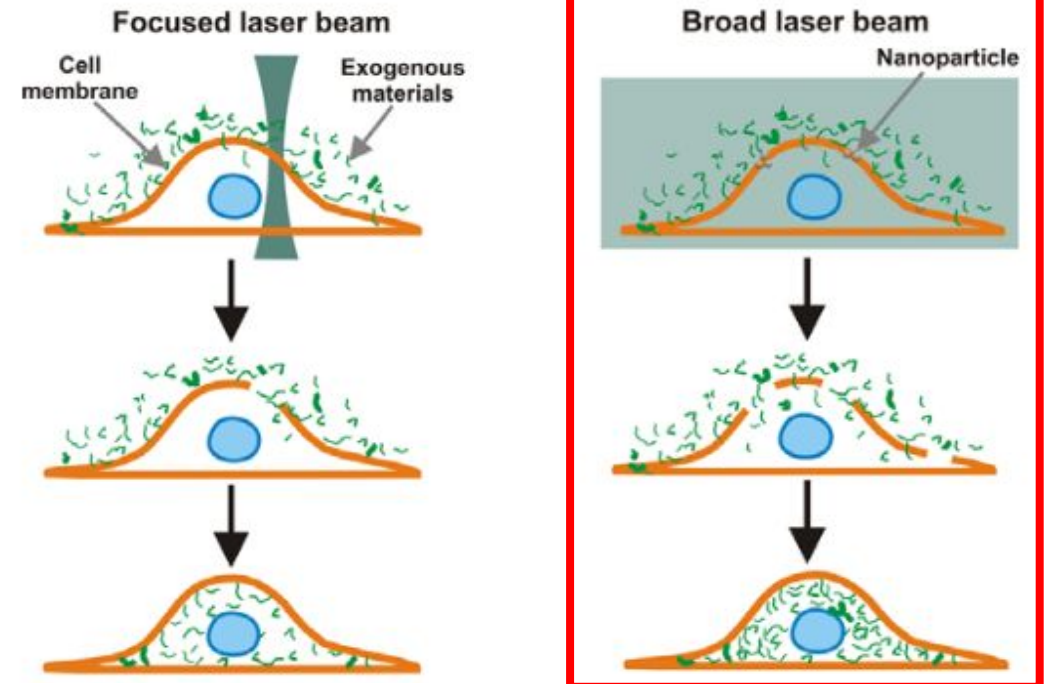


Membrane disruption-mediated

Physical Methods



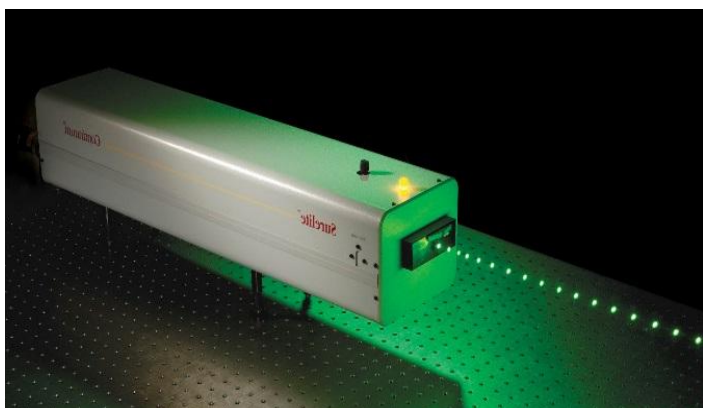
Photoporation



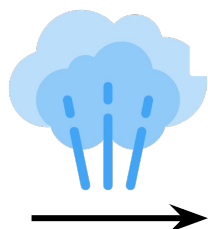
Xiong, R.; et al. Adv. Phys. X 2016, 1 (4), 596.



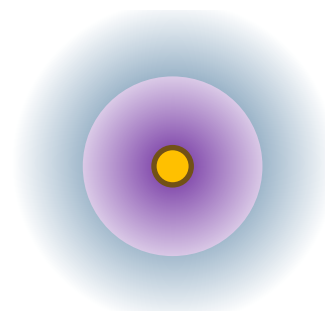
Pulsed Laser Light



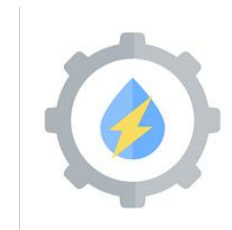
Heat



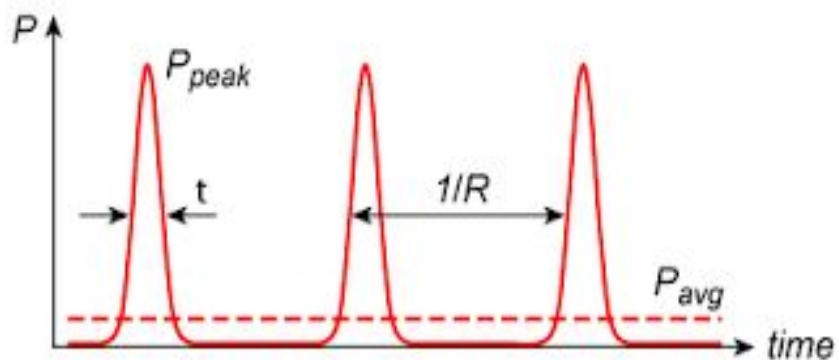
Vapor nanobubble
(VNB)



Photothermal Nanoparticle
in aqueous environment



MECHANICAL
ENERGY
(@ nano-scale)

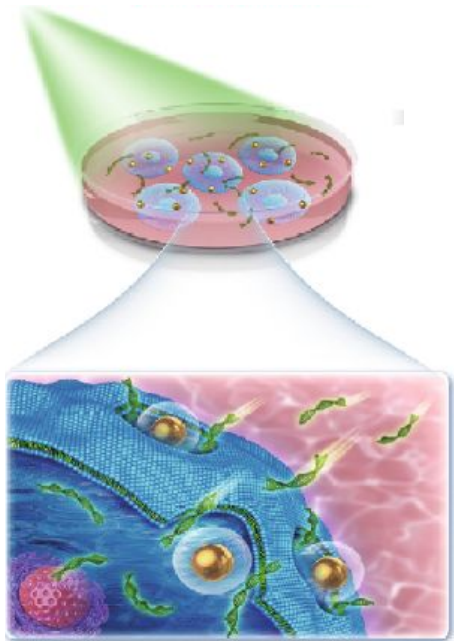


Nanoparticle-Mediated Photoporation

Mechanical
energy

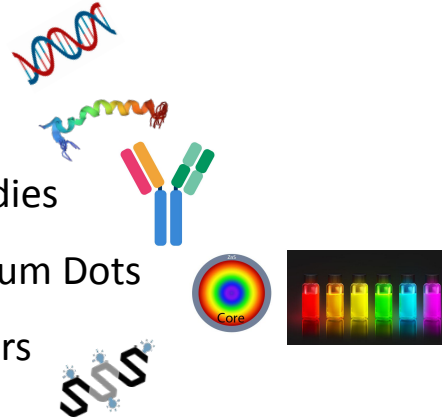


Transient
pores



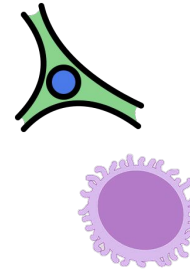
Direct cytosolic delivery :

- Nucleic acids
- Peptides
- (Labeled) Antibodies
- (Targeted) Quantum Dots
- (Labeled) Polymers

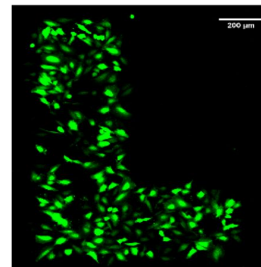


Into living :

- Adherent Cells
- Suspension Cells
- Difficult to transfect cells

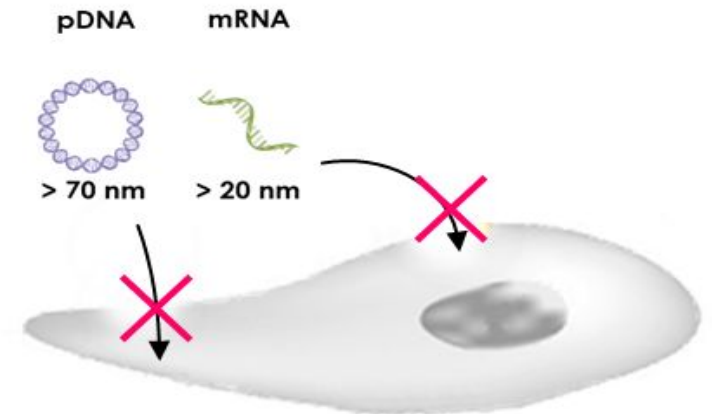


Spatio-temporal control:



Challenges:

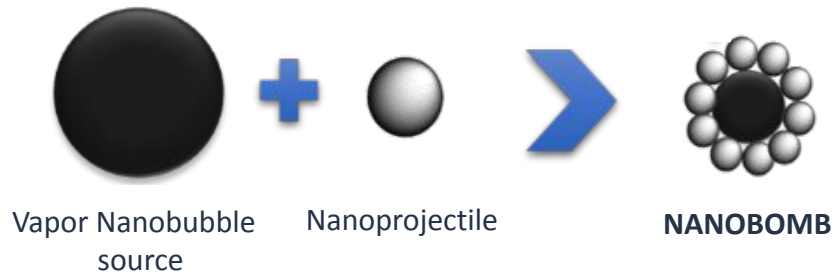
Small pores



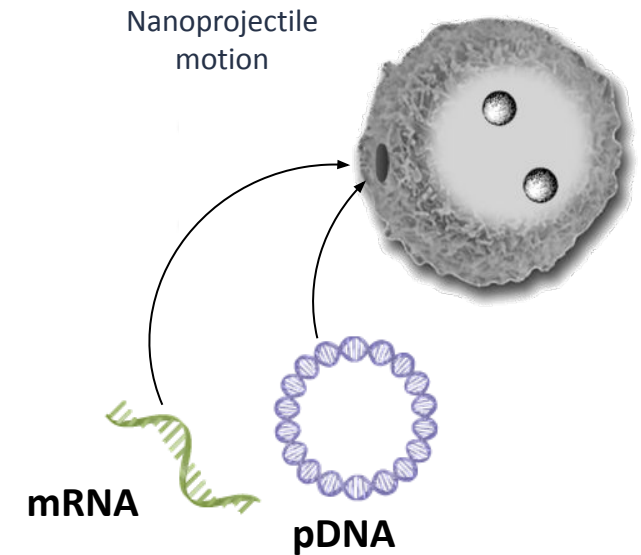


Nanobomb concept

Nanobomb Synthesis

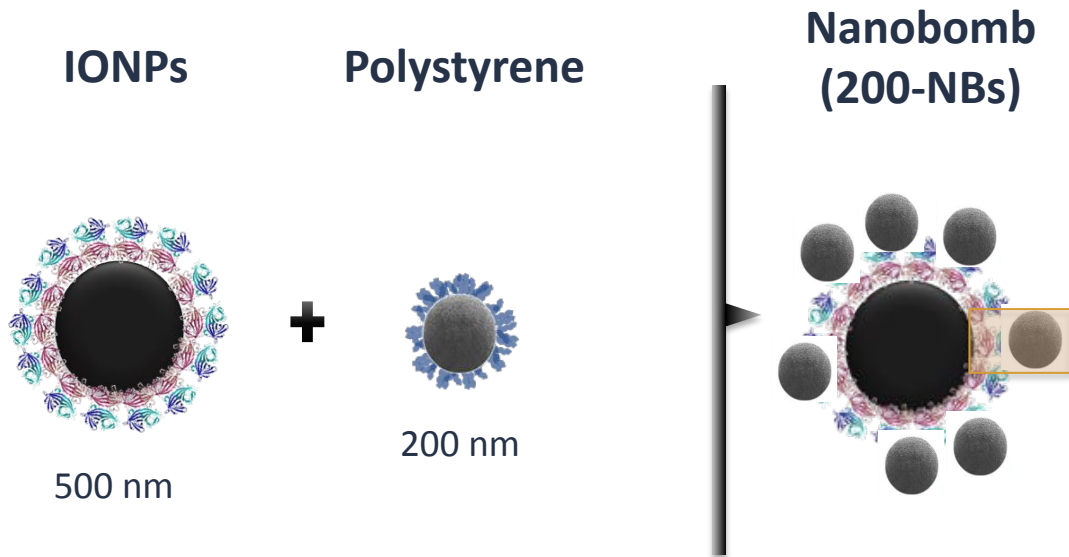


Delivery of **big macromolecules** like **mRNA** and **pDNA**

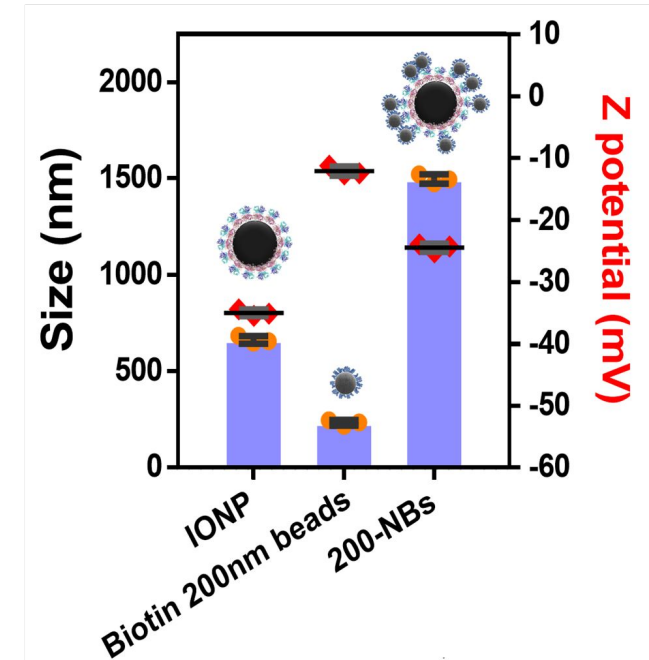
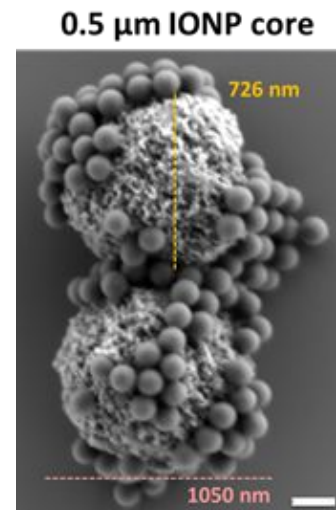
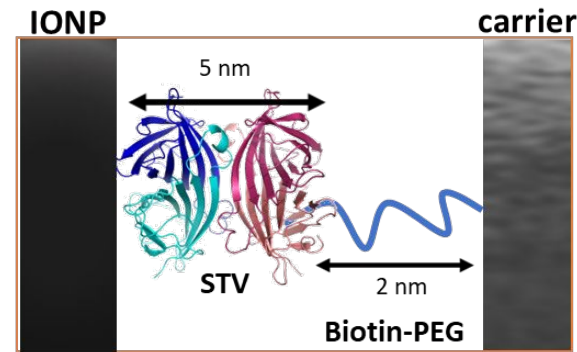




Nanobombs synthesis



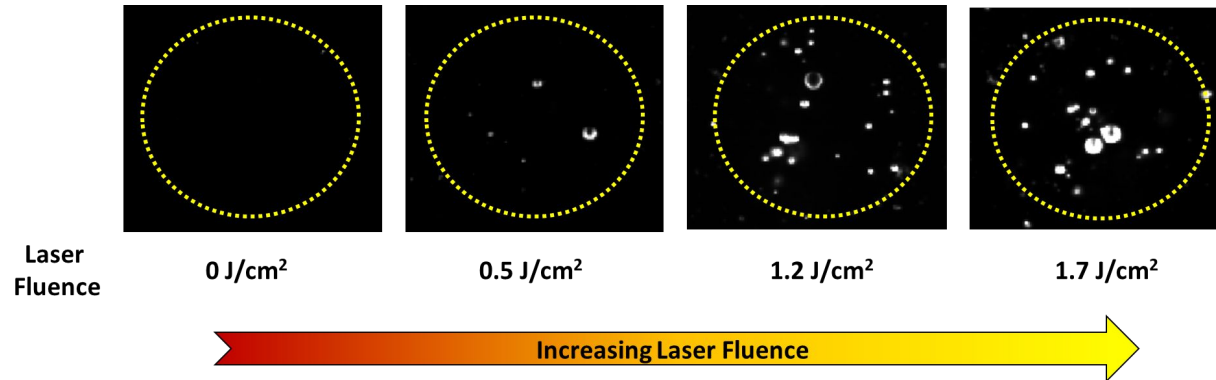
Streptavidin-biotin



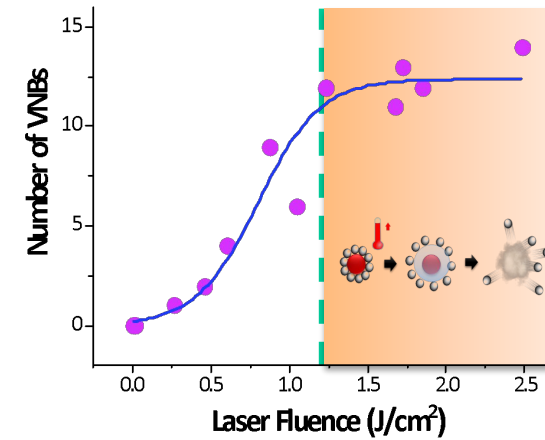


Activation – Vapor Nanobubble (VNB) formation

- VNB formation

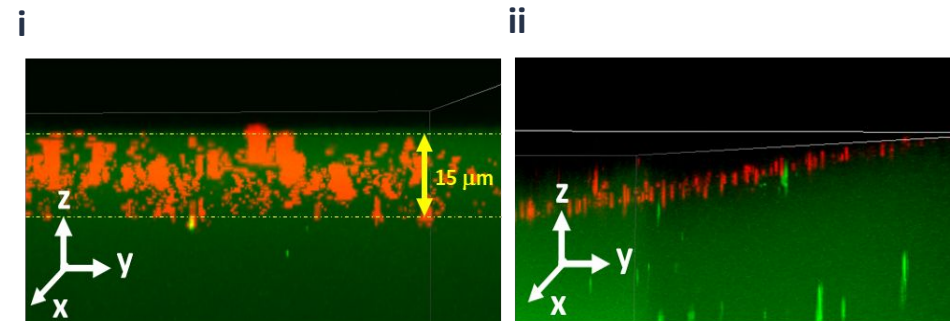
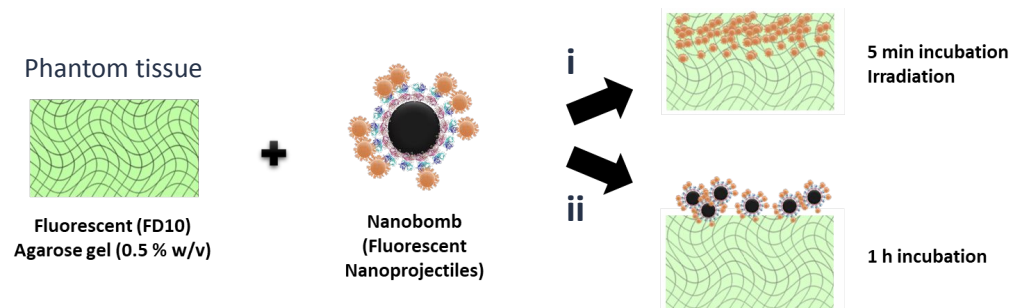


- VNB Threshold



- VNB formation
- Projectiles release
- Penetration of projectiles

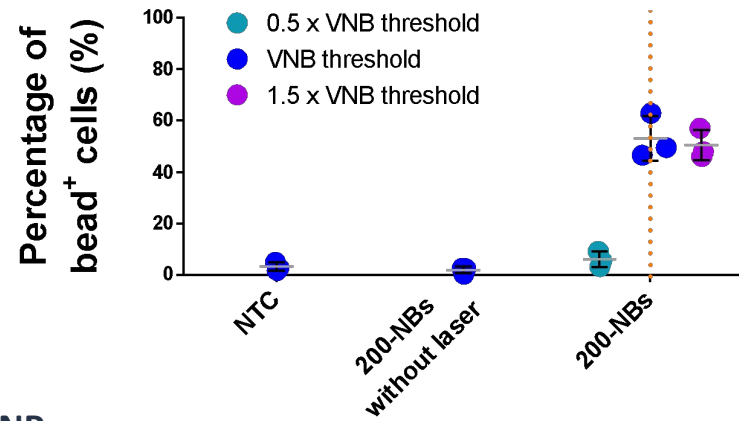
- Tissue penetration



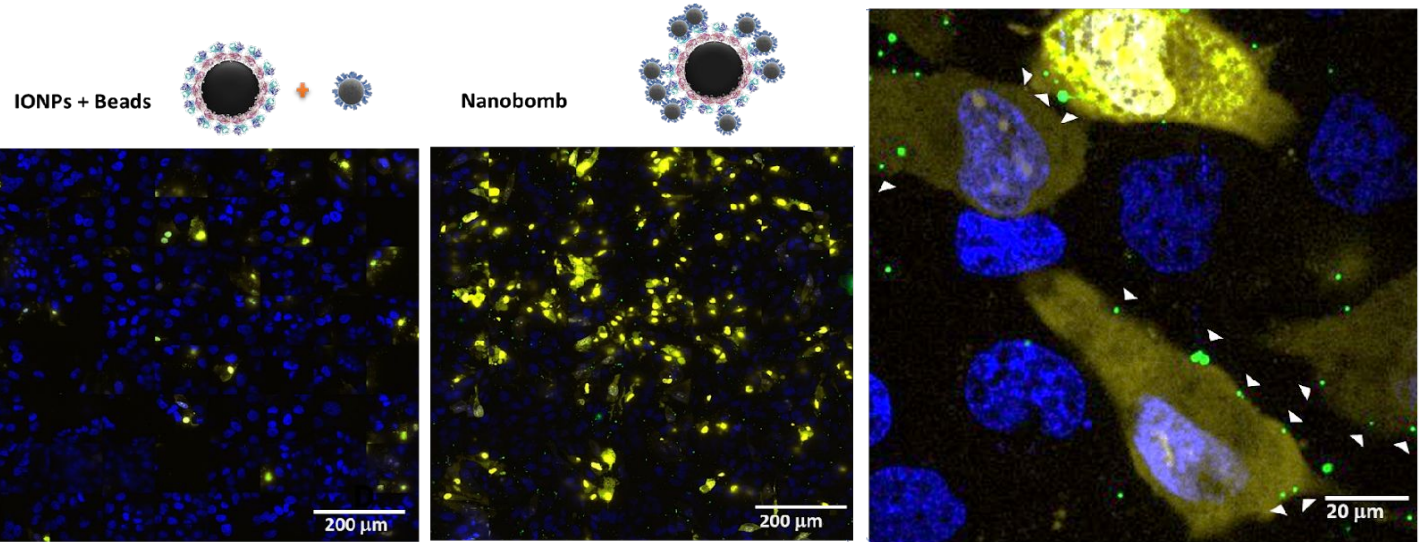
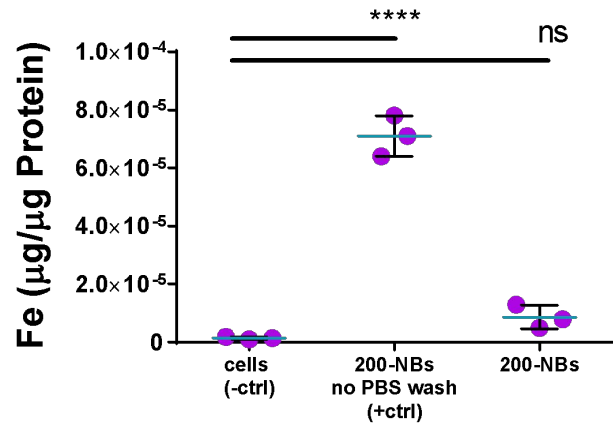


Activated NBs/cell interactions

• Projectile



• IONP core

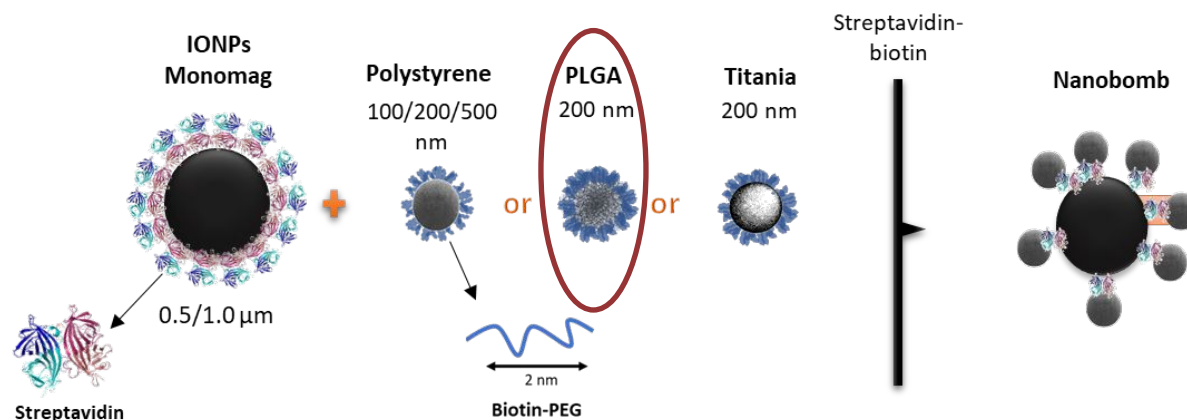


- Penetration of projectiles
- IONP Core remains out of the cell



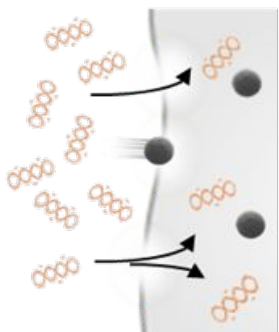
Cargos and Cells

Synthesized Nanostructures



Intracellular Delivery

Creating membrane pores



- FD500 (optimization) – 2mg/mL
- GFP mRNA (996 nts) – 0.1μg/μL
- GFP pDNA (5757 bps) – 0.1μg/μL
- CRE mRNA (1350 nts) – 0.1μg/μL



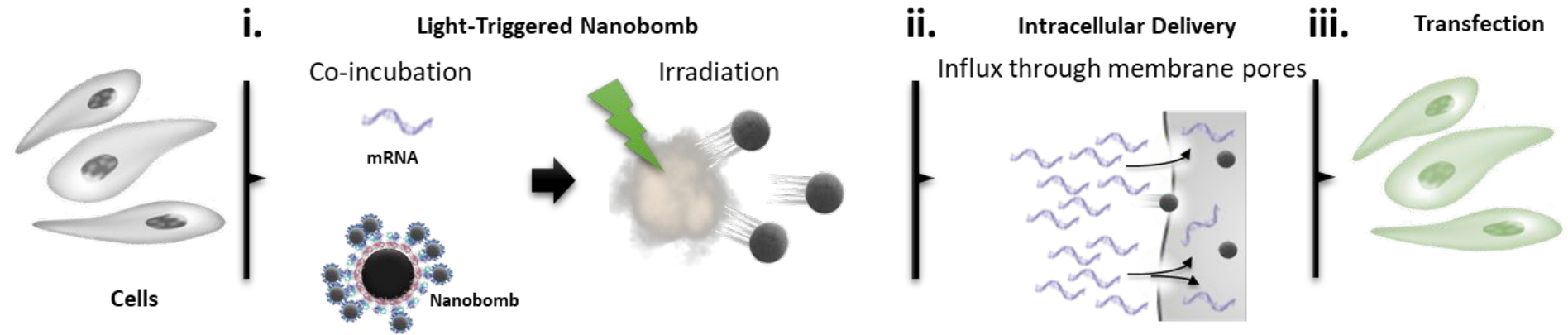
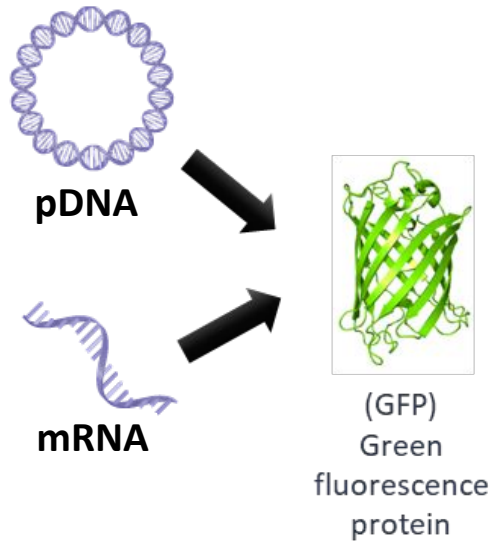
Hela cells / Hela DsRed-GFP



Jurkat cells



Transfection



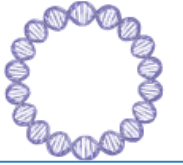


GFP mRNA and pDNA / Jurkat cells

mRNA

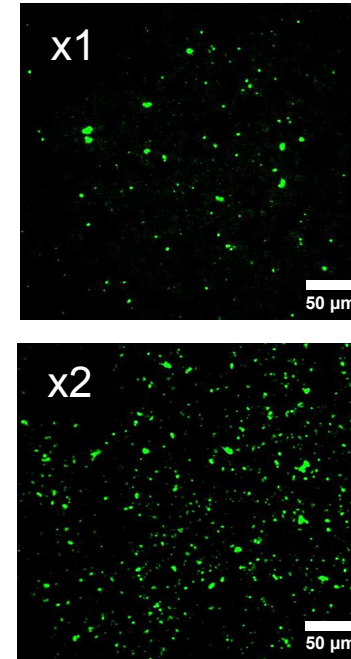
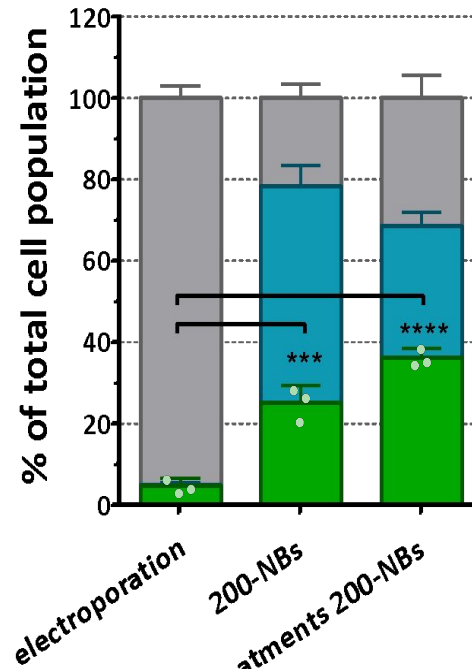


pDNA

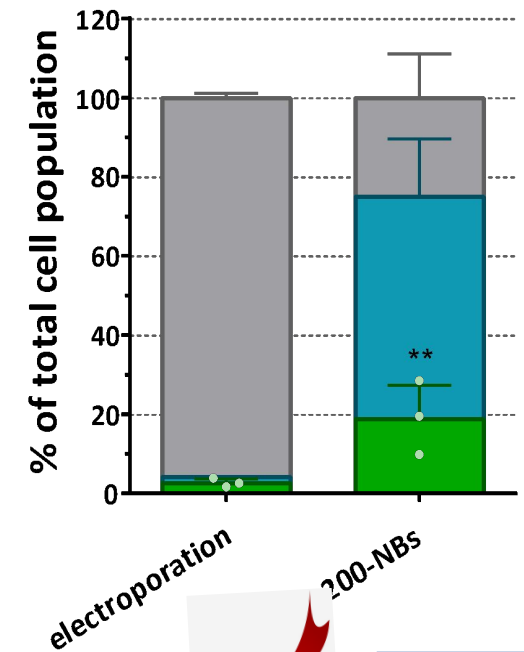


- 200 nm PLGA
- 1.3×10^8 NBs/mL
- 0.1 $\mu\text{g}/\mu\text{L}$ mRNA or pDNA (per treatment)
- 20 min incubation
- VNB threshold

Dead
Viable - Untransfected
Viable - Transfected



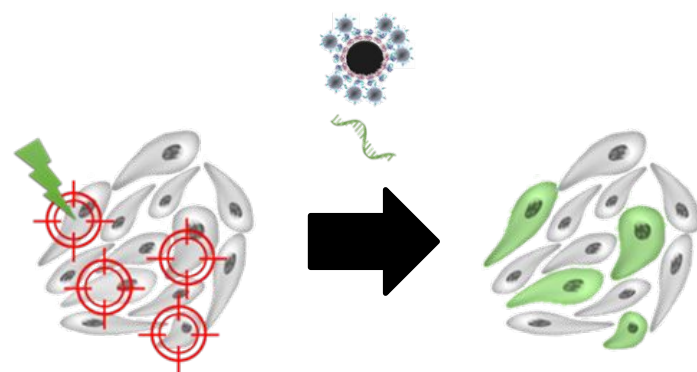
• Transfec ~ 53%
• Viability ~ 69%



• Transfec ~ 27%
• Viability ~ 76%

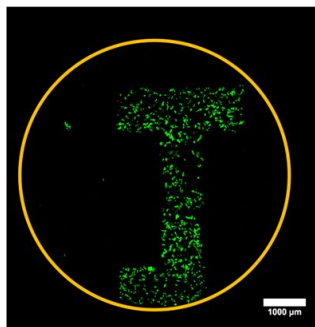
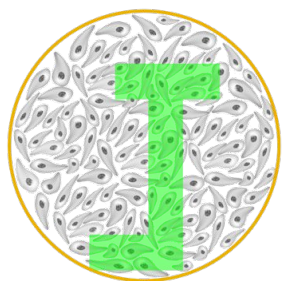
GFP mRNA in Hela

Spatial-selective transfections

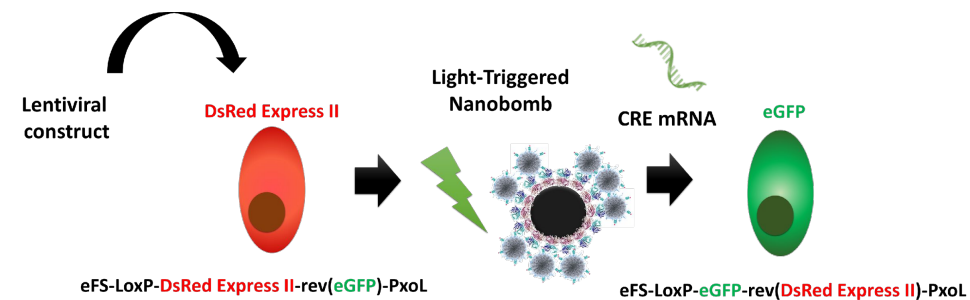


Irradiation
pattern

eGFP



Spatial-selective reprogramming

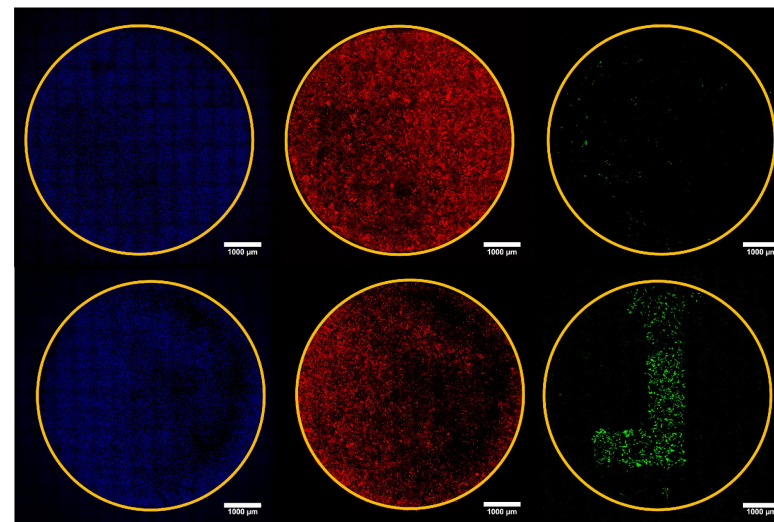
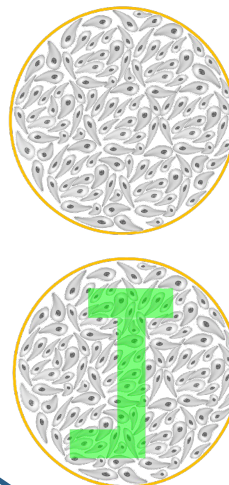


Irradiation
Pattern

Hoechst

DsRed Express II

eGFP





Conclusions



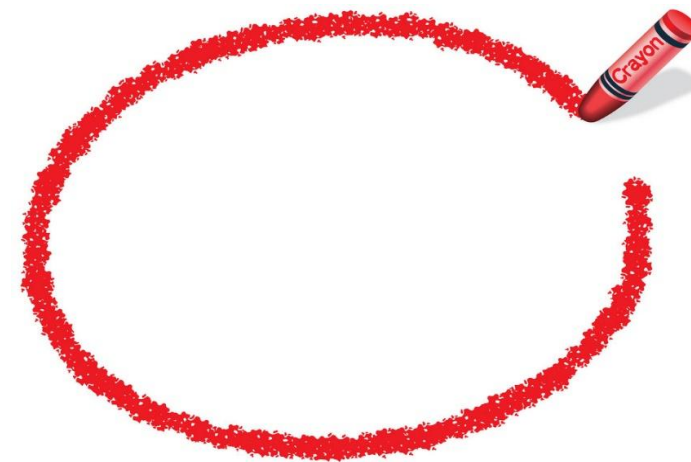
Design of **Self-assembled Nanobombs** for disrupting biological membranes by **propelling parts of the structure (nanoprojectiles)**



Enhanced **delivery of big macromolecules:** (mRNA and pDNA)



Spatial-selective mRNA delivery



Fraire, J.C., et al. Nat Commun 13, 1996 (2022). <https://doi.org/10.1038/s41467-022-29713-7>



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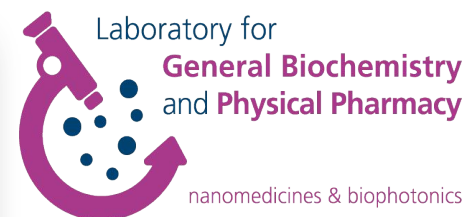
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