The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

The combined use of AuNPs and NIR radiation enables cytosolic protein delivery

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pharmaceuticals MDPI

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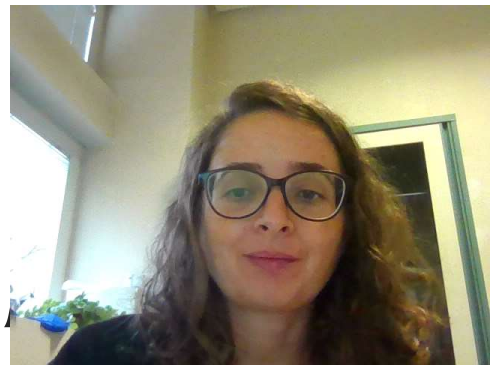
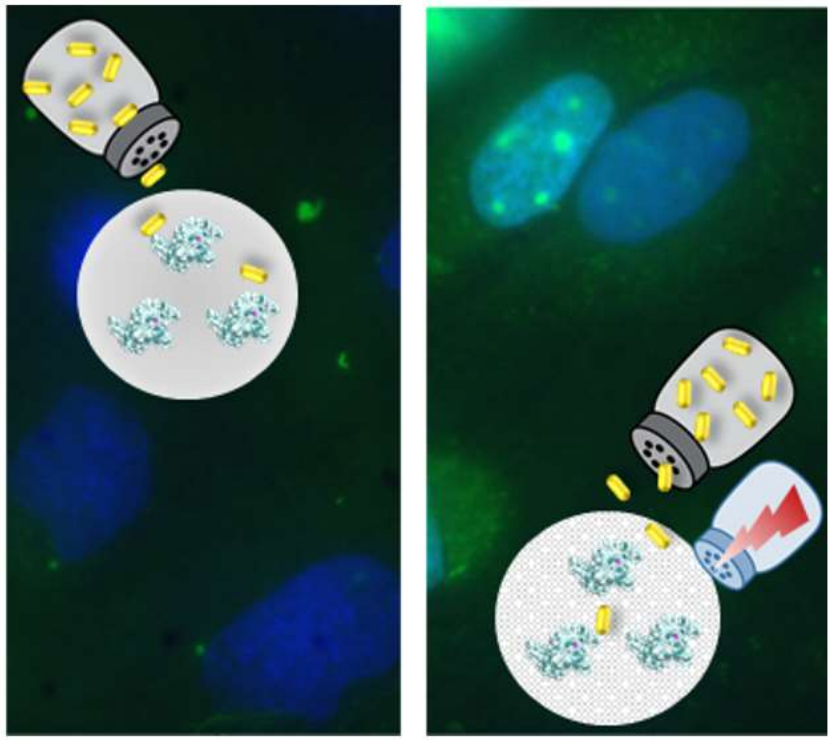


The combined use of AuNP and NIR radiation enables cytosolic protein delivery

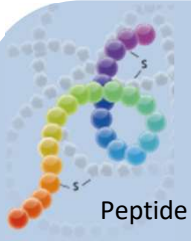


Abstract:
Cytosolic protein delivery remains elusive. The instability of proteins in the endosomal/lysosomal environment and their inability to cross the endosomal membrane are two major bottlenecks. Here we explore the unique photothermal properties of gold nanorods (AuNRs) to trigger cytosolic delivery of proteins. Both partners, protein and AuNRs, are modified with a protease-resistant cell-penetrating peptide with nuclear targeting properties, to induce internalization. Once internalised, spatiotemporal control of protein release is securely achieved by near-infrared laser irradiation in the safe second biological window. Importantly, catalytic amounts of AuNRs are sufficient to trigger cytosolic protein delivery. To the best of our knowledge, this is the first time that AuNRs with their maximum of absorption in the second biological window are used to deliver proteins into the cytosol. This strategy represents a powerful tool for the intracellular delivery of virtually any class of protein.

Keywords: cell-penetrating peptide; cytosolic delivery; Gold nanorod; Near-infrared irradiation.

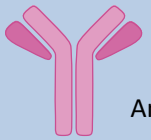
Introduction

Cytosolic delivery of biotherapeutics remains elusive


Peptide


DNA


Protein

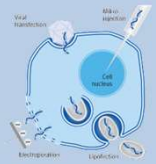

Antibody

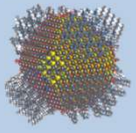

miRNa

- Biotherapeutics interfere with biological processes with great **selectivity** (low [])
- Effect can be exerted both at **intra** and **extra**cellular level
- **Low cell penetration**

- **Strategies to increase cell penetration:**

✓ Electroporation
✓ Protein engineering (to increase pKa)
✓ Nanoparticle formulation
✓ Peptide mediated delivery (CPPs)





- **Limitations**

✓ Endo-lysosomal entrapment
✓ Degradation

- Strategies to **escape** the endosome

✓ Use of chemicals (i.e. chloroquine)
✓ Use of endosomolytic peptides
✓ Use of cyclic CPPs

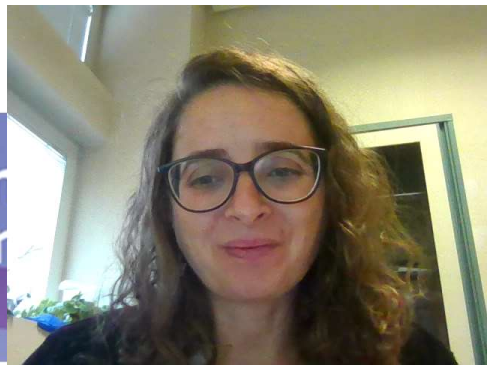
⇩

Lack of spatio-temporal control

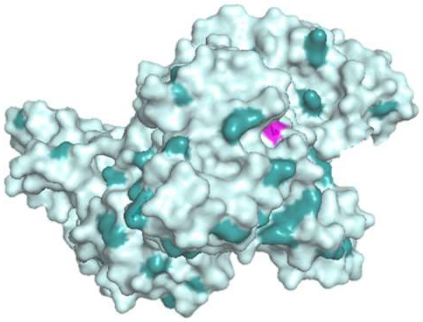
⇩

✓ pH
✓ T[a]
✓ Redox
✓ Magnetic field
✓ Ultrasound
✓ Enzymatic

} Responsive materials

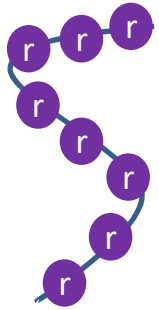


AuNRs are photoresponsive materials

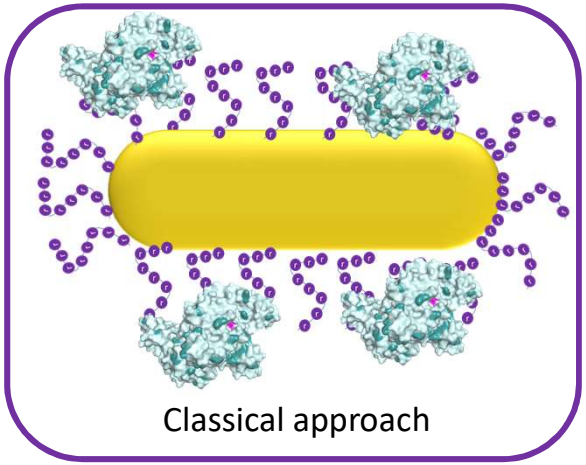


Protein of interest

Gold
NRs

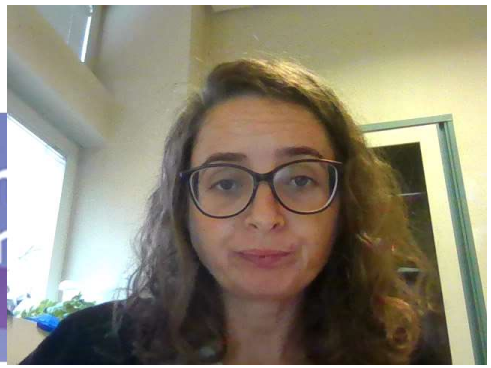


CPP



Classical approach

- The amount of cargo, protein, is related to the amount of NP
- Large concentrations of NPs have to be used to reach the desired amount of protein
- The complex NP/protein has to be stable



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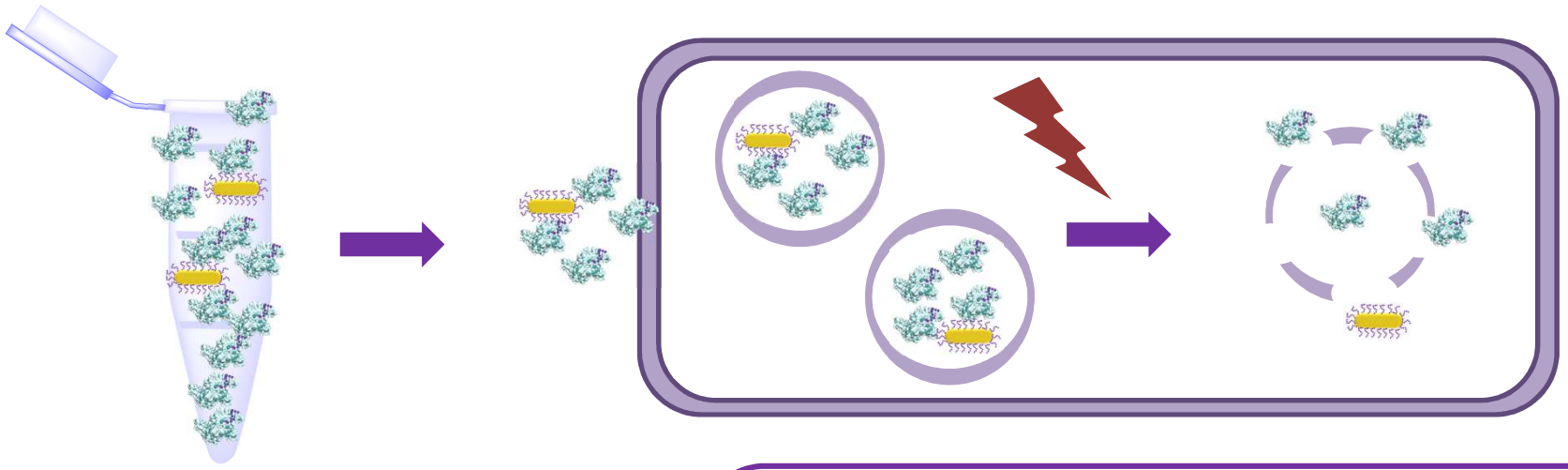
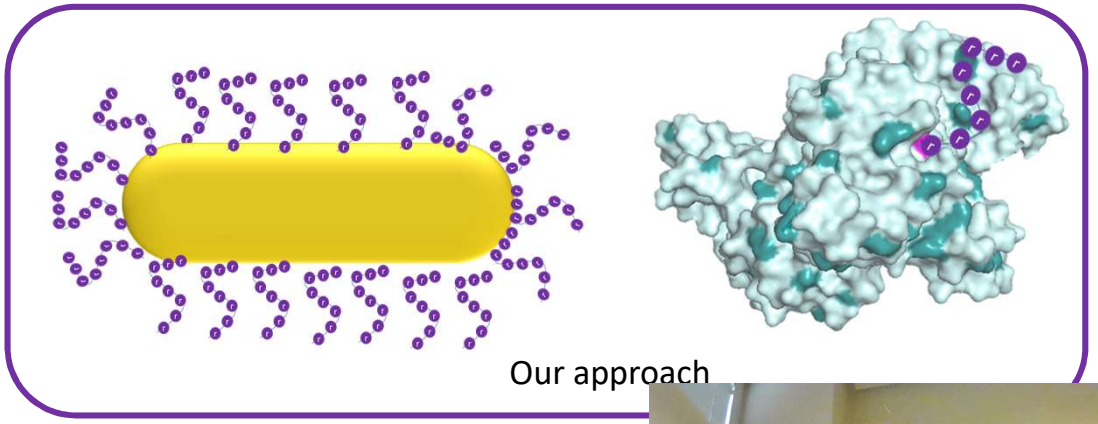
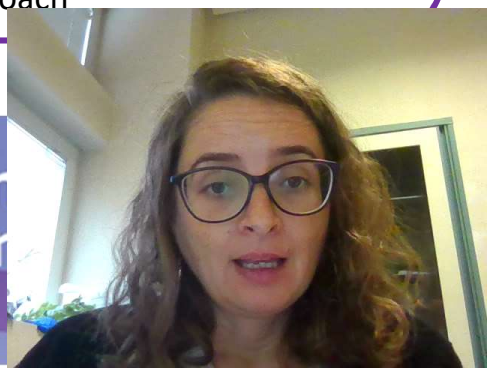


- Both systems are modified with the same CPP (they share the same route of entry)
- The concentration of the NP is much lower than the one fo the protein: less side effects
- Freedom to reach the desired concentration

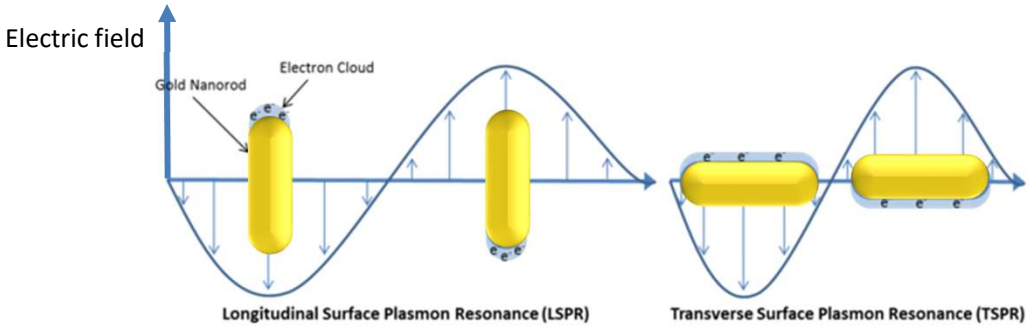


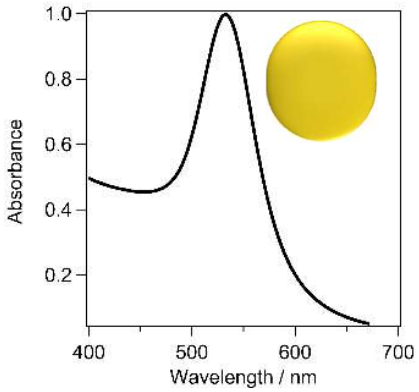
Our approach

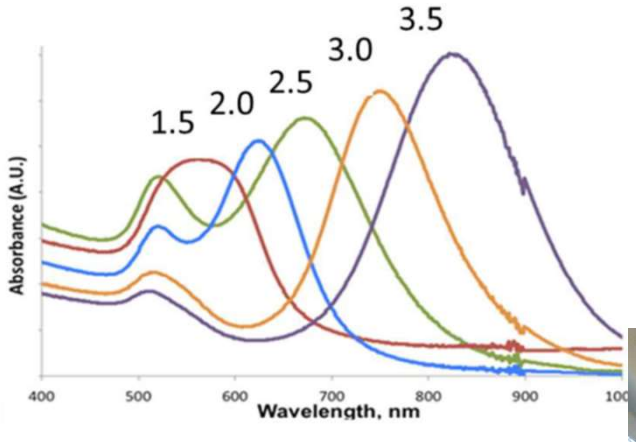


AuNRs are photoresponsive materials

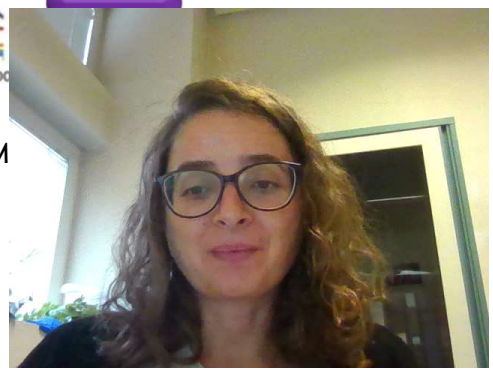
Aspect ratio= l/d



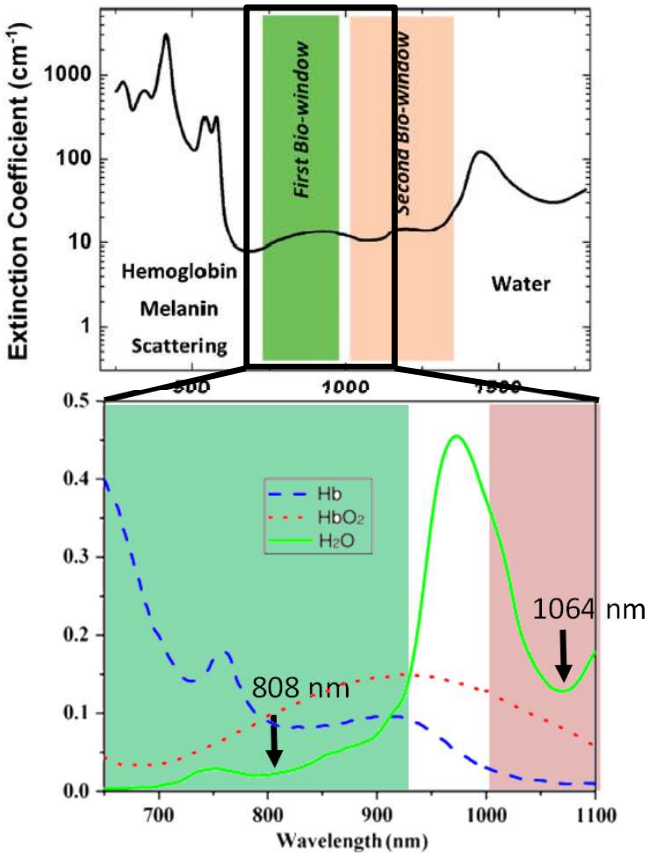




Chemistry of M



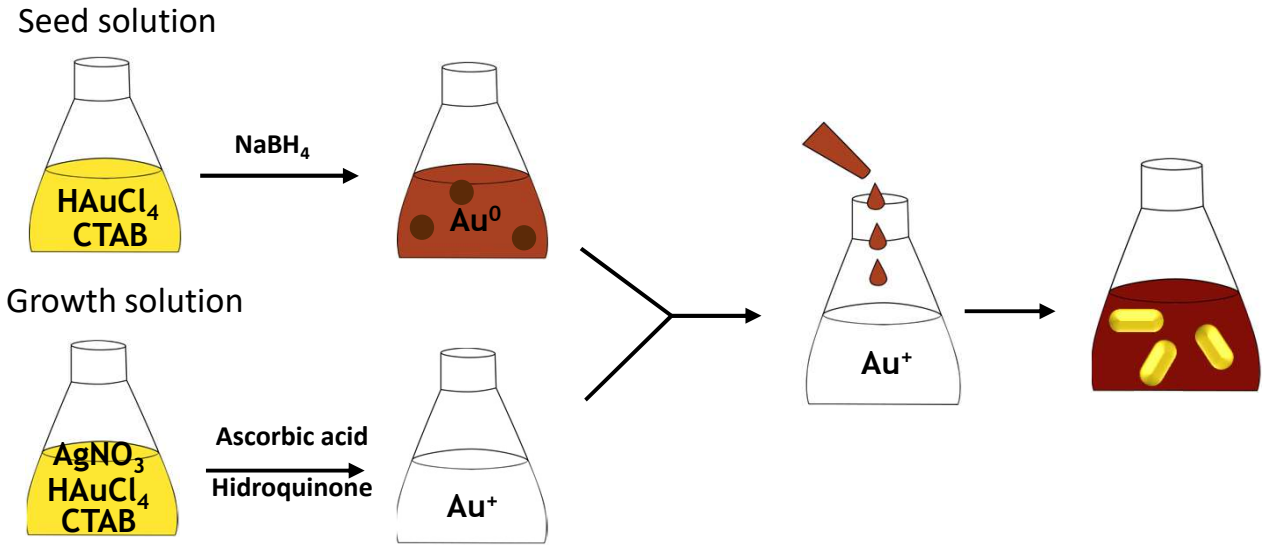
Our AuNRs are active in the second biological window

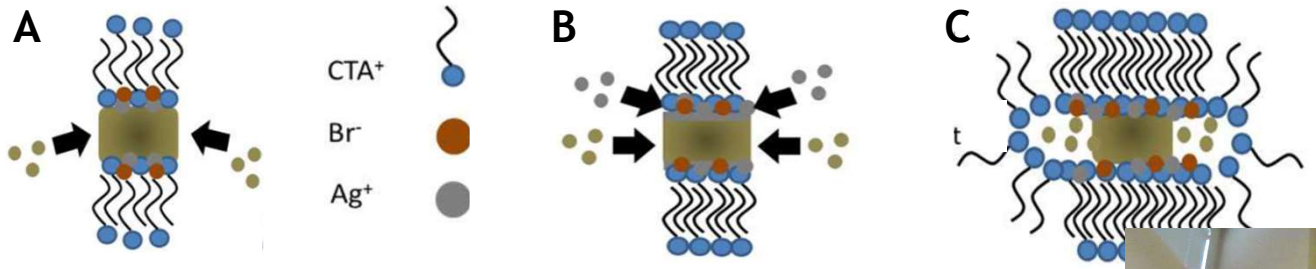


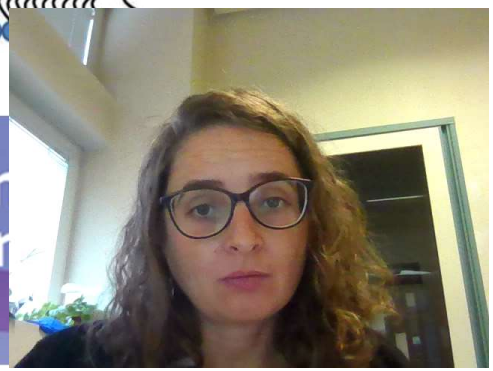
- Biological tissues have a minimal NIR light absorbance in the so-called biological windows:
- ✓ NIR-I: 650–950 nm
- ✓ NIR-II: 1000–1350 nm.
- Water is the most significant component and the major absorber of NIR light in biological tissue.
- The lower water absorption of 808 nm
- 1064 nm laser irradiation in the NIR-I/NIR-II window deeper penetration depth, with minimal tissue overheating.

Results and discussion

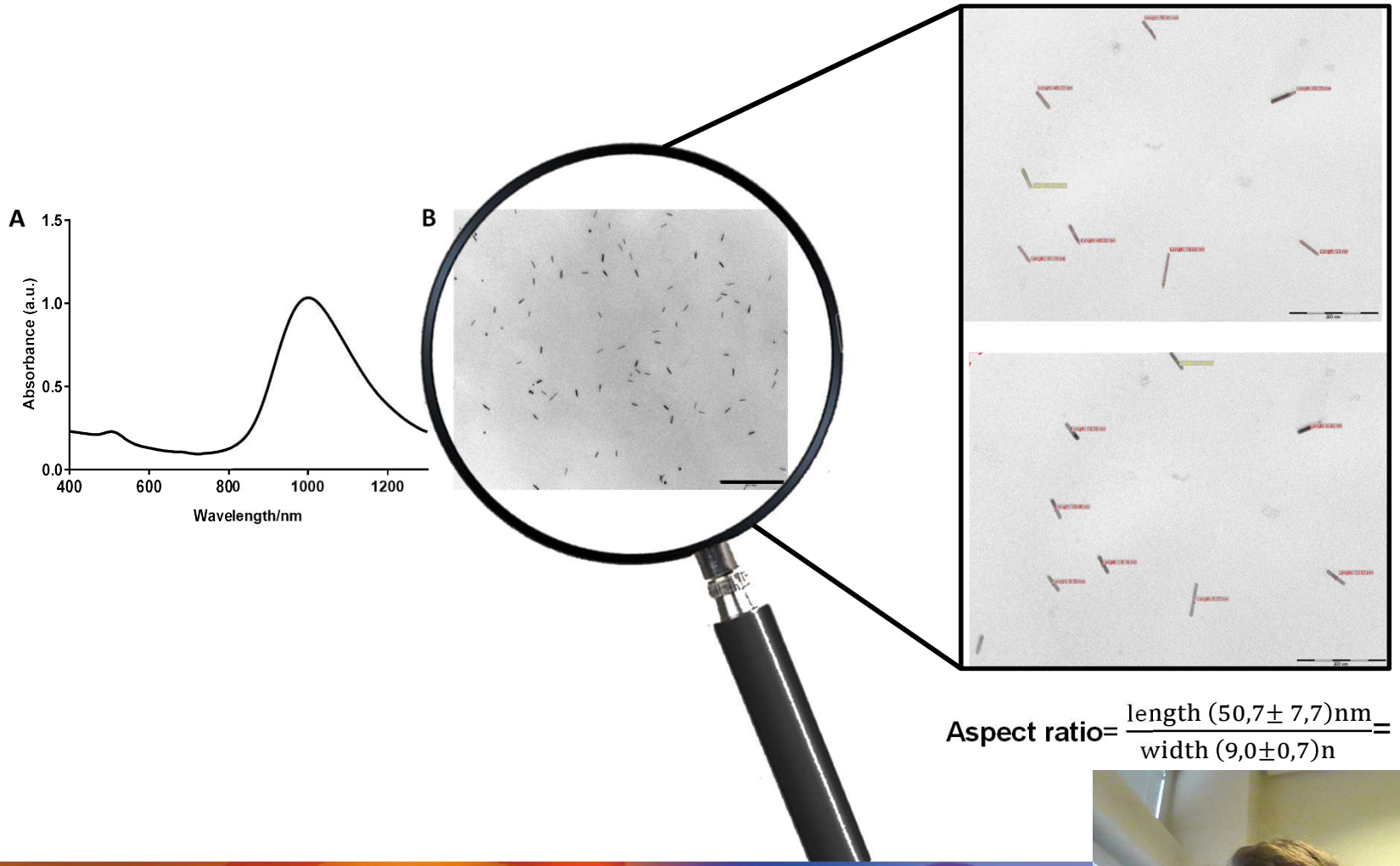
AuNRs were prepared using a seed-mediated growth method





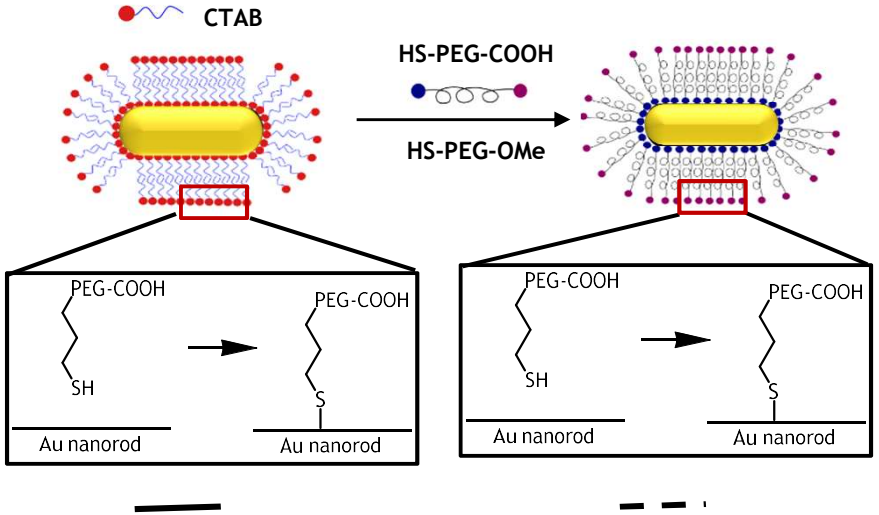


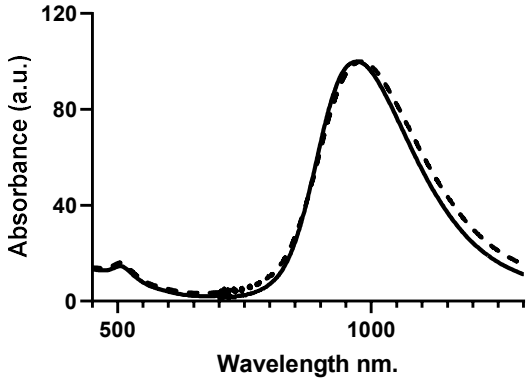
Our AuNRs absorb in the second biological window



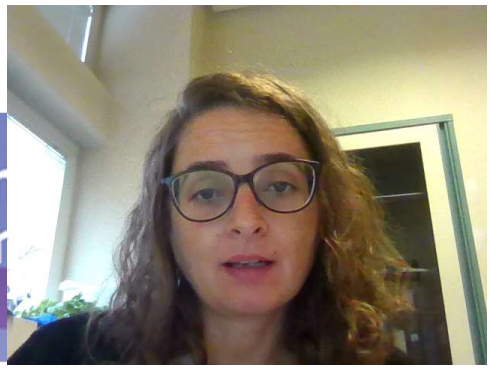
Aspect ratio= $\frac{\text{length } (50{,}7 \pm 7{,}7)\text{nm}}{\text{width } (9{,}0 \pm 0{,}7)\text{n}}$ = 5.6

Our AuNRs absorb in the second biological window





- PEGylation does not affect the Amax



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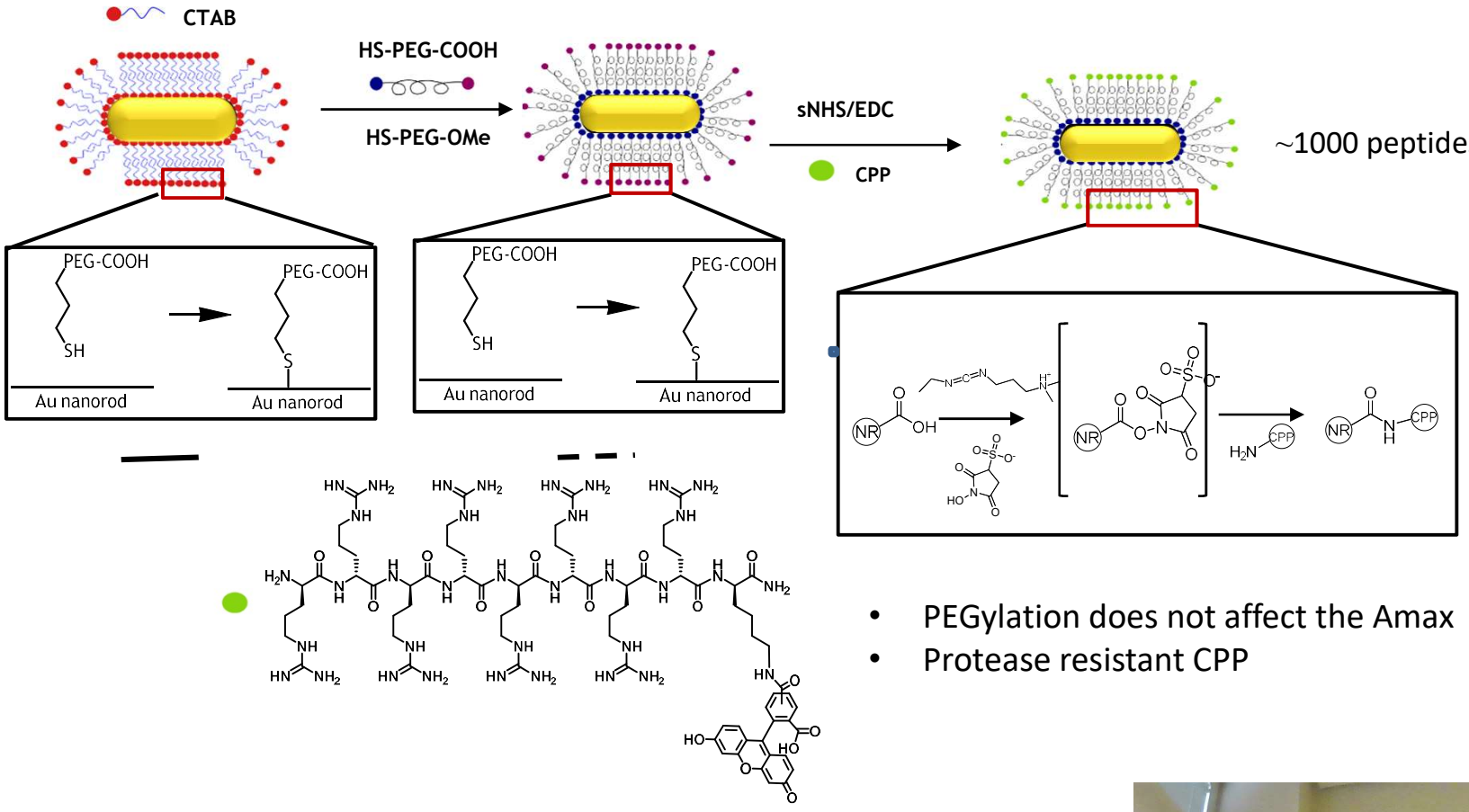
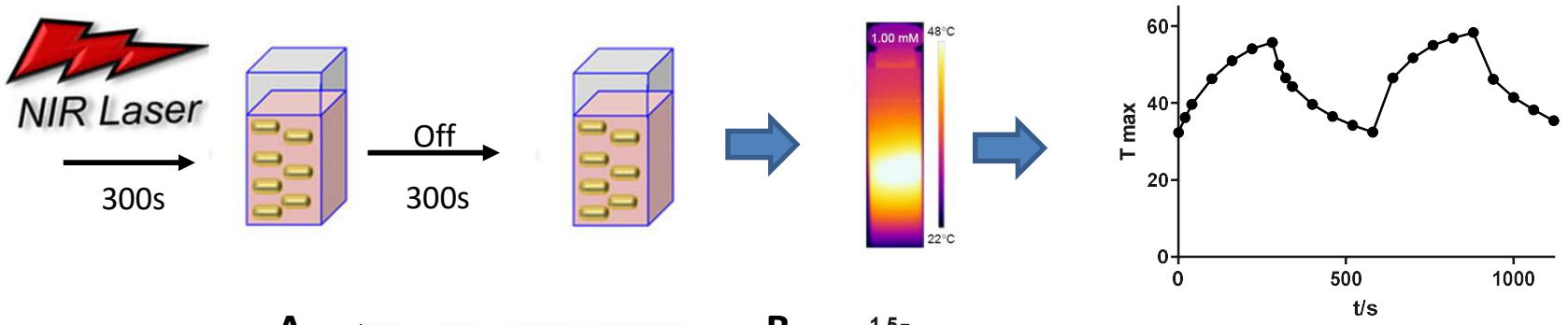
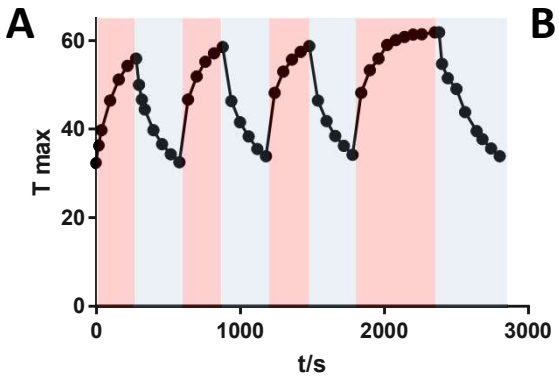
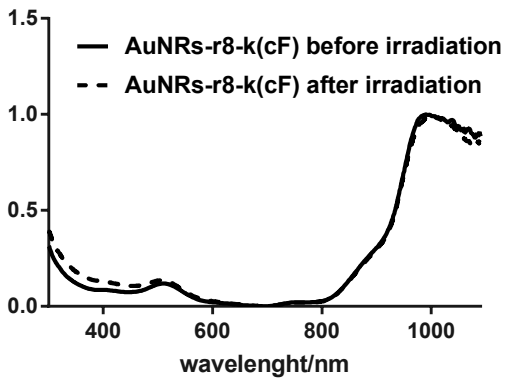


- PEGylation does not affect the Amax
- Protease resistant CPP

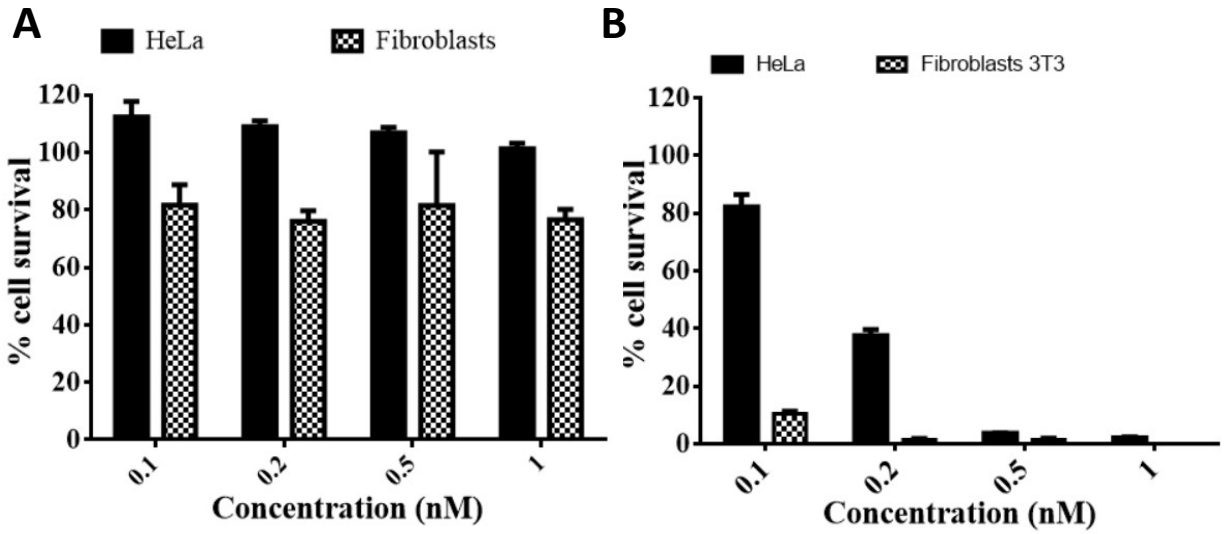
AuNRs are stable to laser irradiation







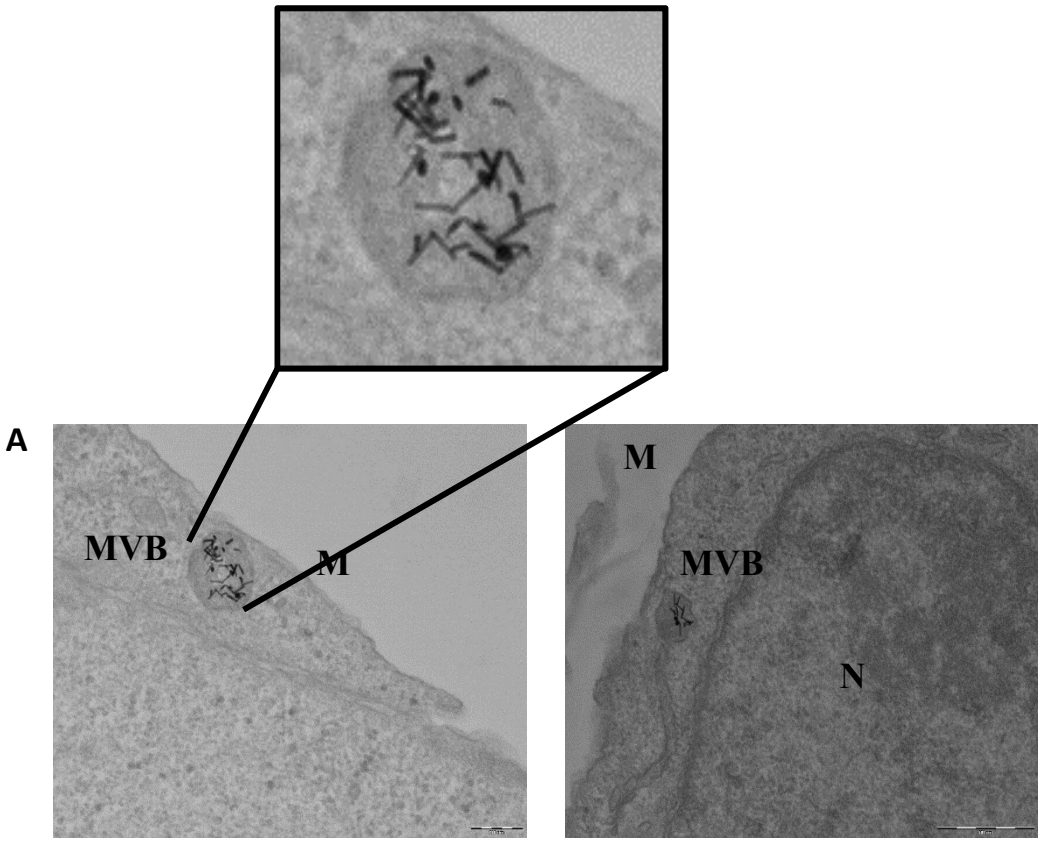
Functionalized AuNRs are not toxic

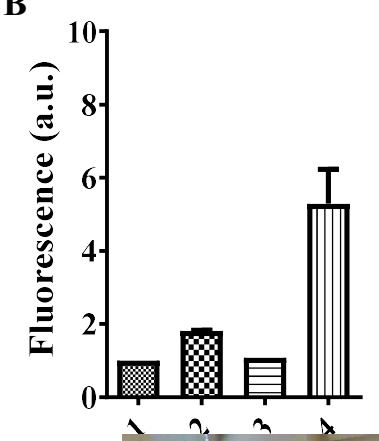


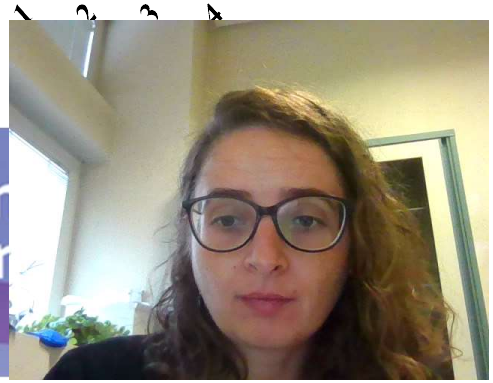
Modified AuNRs

Unmodified AuNRs

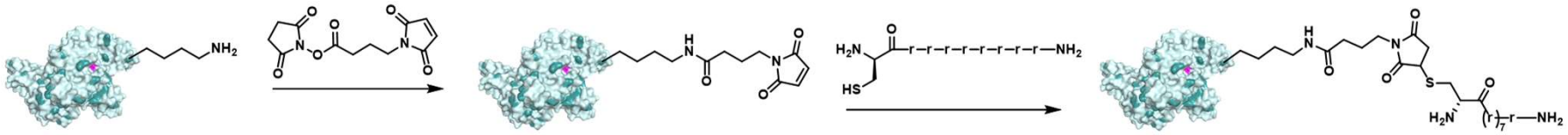
Functionalized AuNRs internalize cells



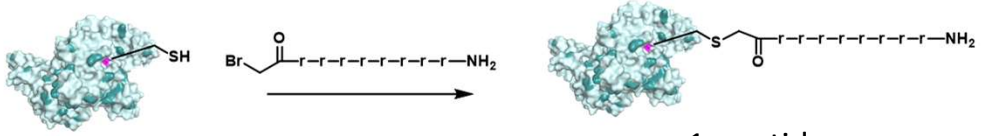




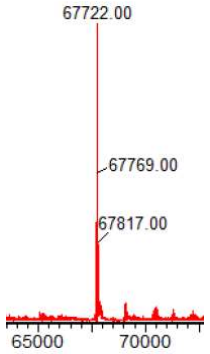
BSA was selected as a model protein

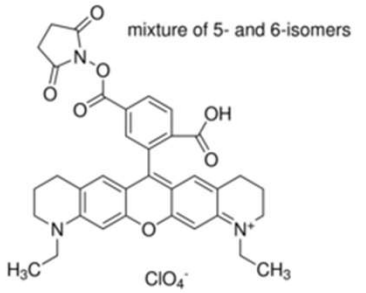


~ 3.4 peptides
(AAA)



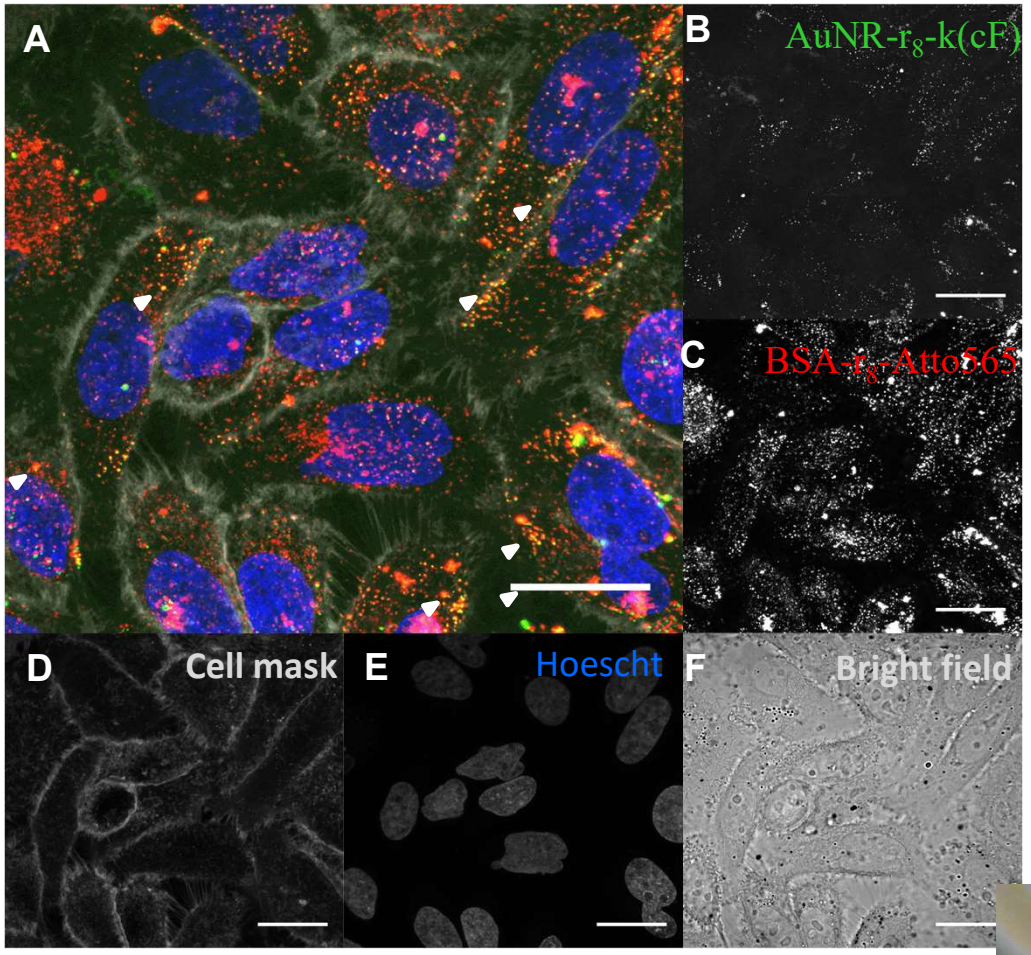
= 1 peptide



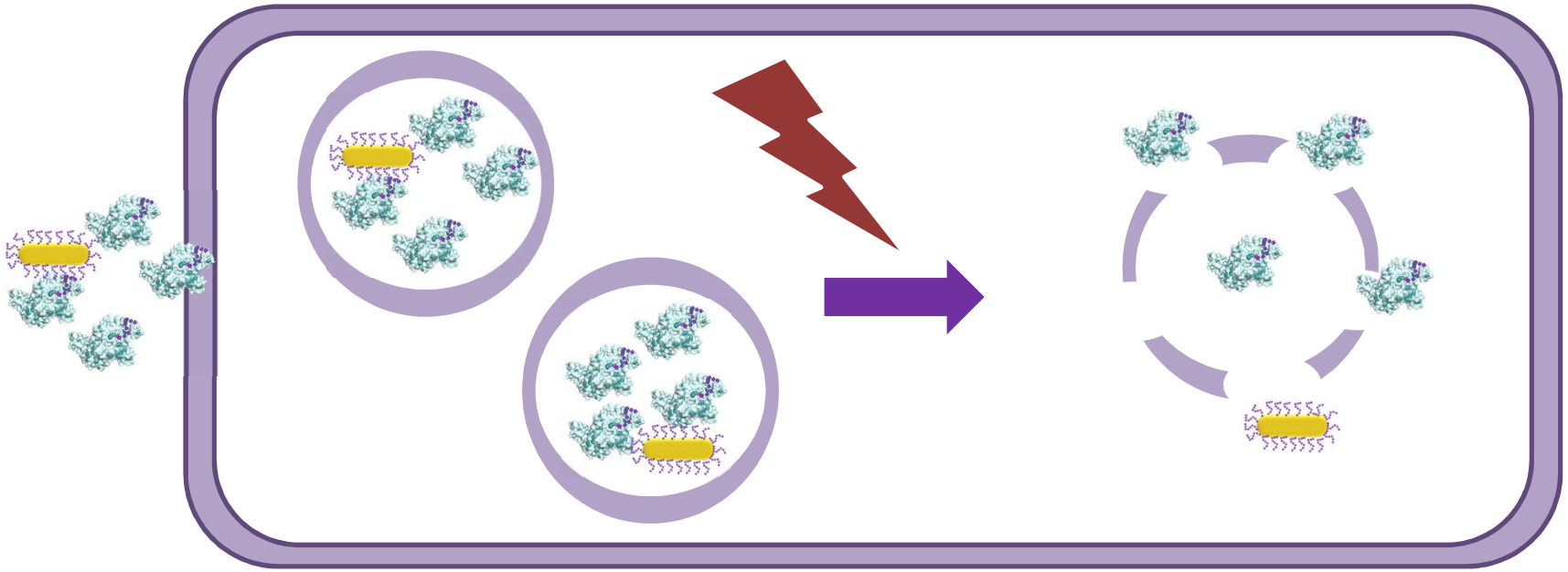


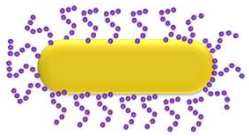
Atto 565

Functionalized AuNRs and BSA internalize cells



Hypothesis





Only AuNRs-r_8k(cF)

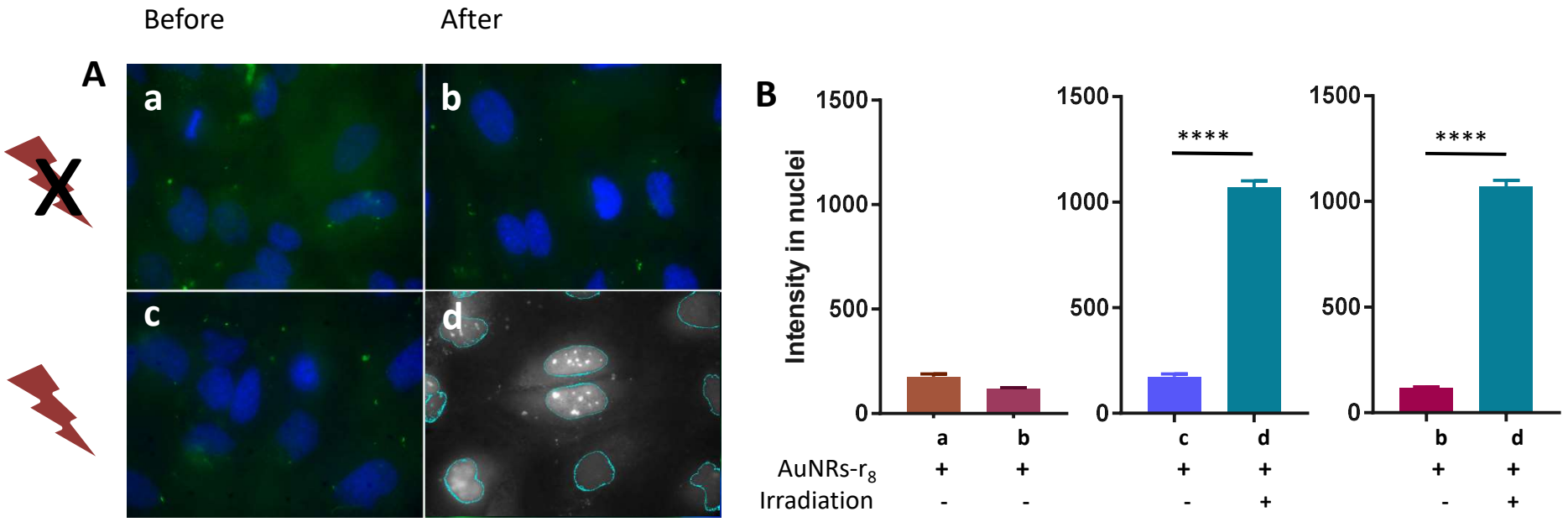
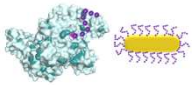


Fig. 5 a) Confocal images of a) AuNRs-r_8k(cF) before irradiation; b) non-irradiated AuNRs-r_8k(cF) after irradiation; c) AuNRs-r_8k(cF) before irradiation ; d) irradiated AuNRs-r_8k(cF) after irradiation. B) Quantification of **a** vs **b**, **c** vs **d** and **b** vs **d**.

Results and discussion

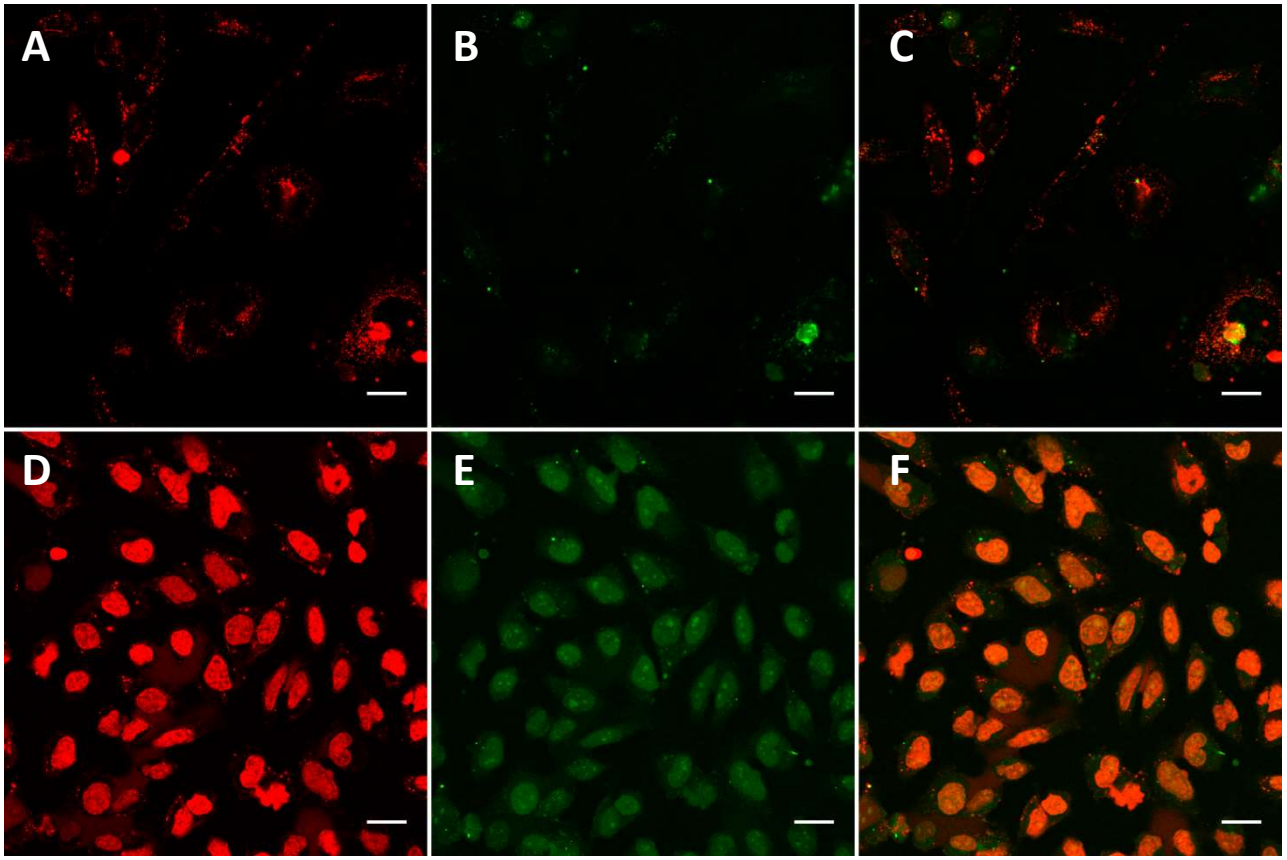
CW NIR laser source
1064 nm
9W/cm^2

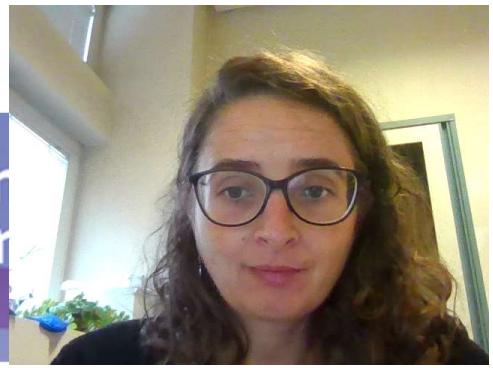
Non-irradiated area



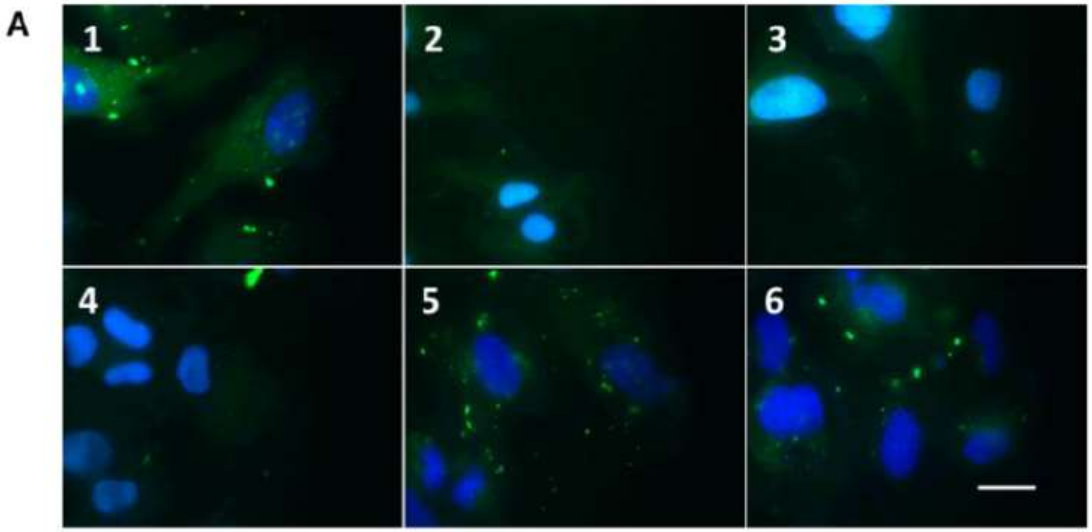
5000 : 1

Irradiated area

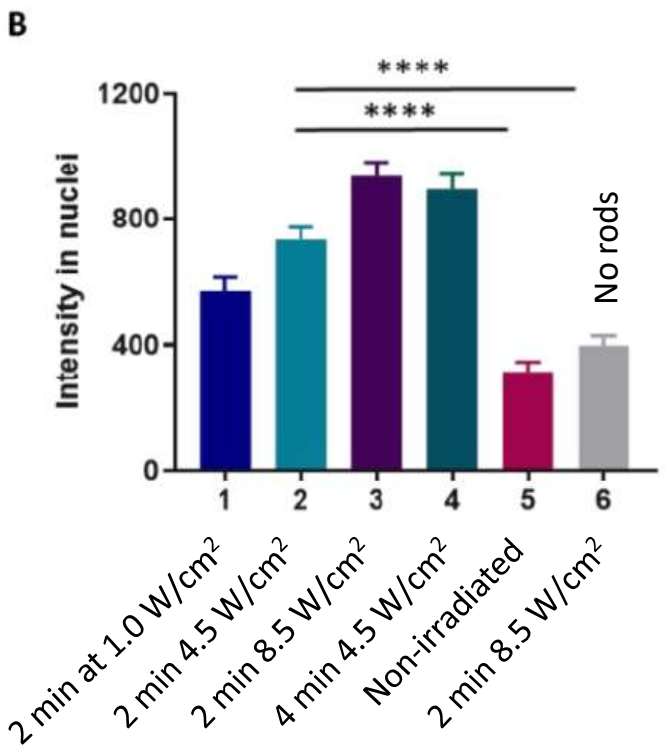




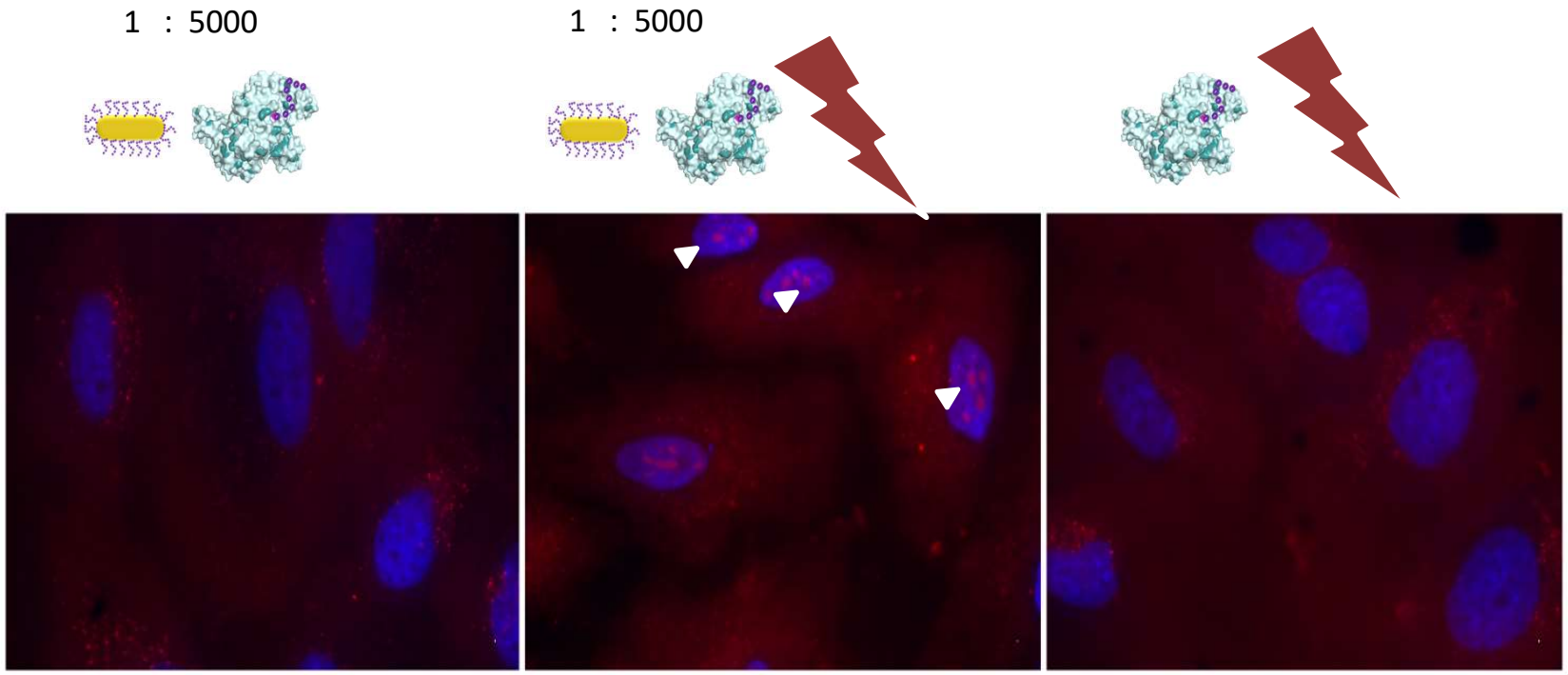
We optimized the laser intensity and irradiation time

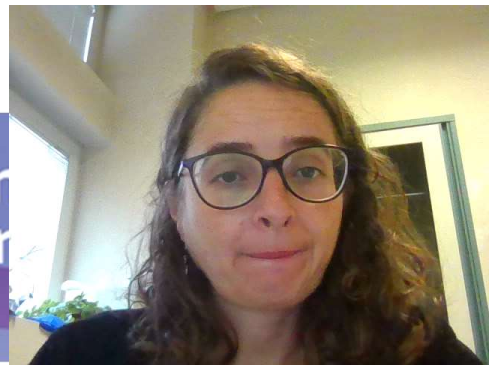


1 nM of AuNR-PEG-r_8-k(cF) and 5µM of r_8-k(cF) (2h)

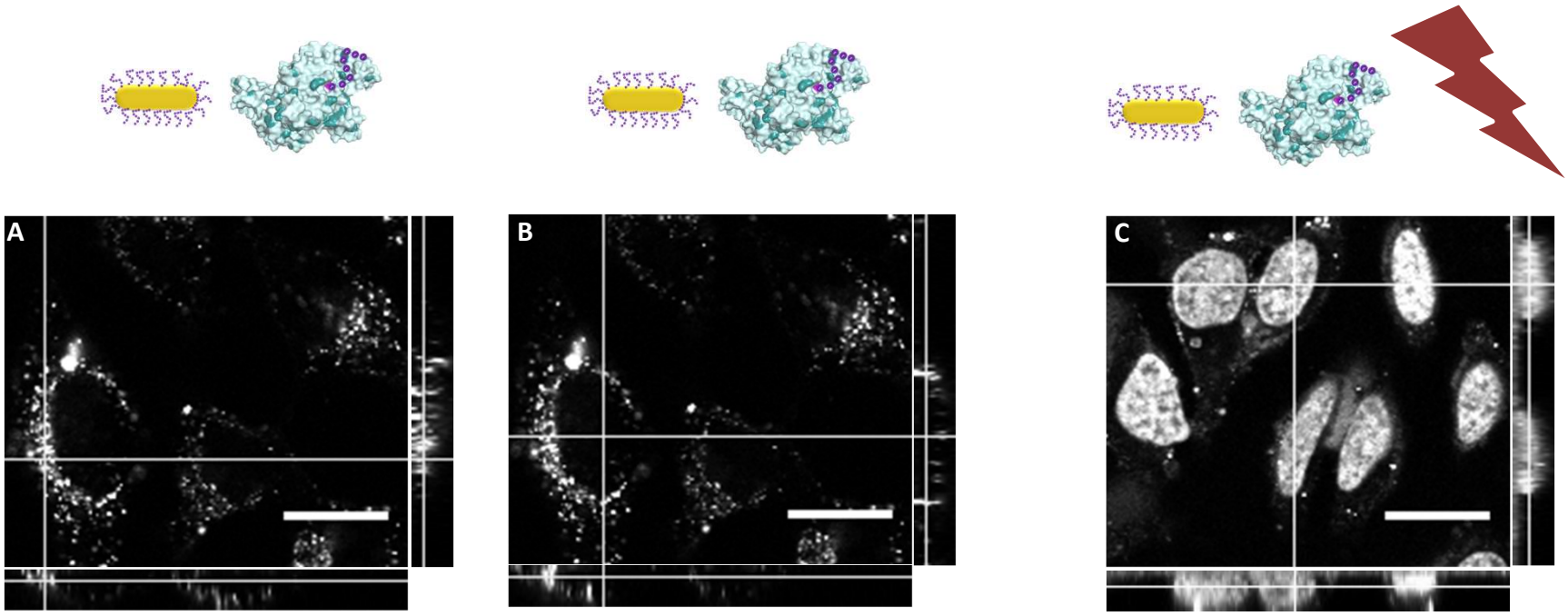


Results and discussion

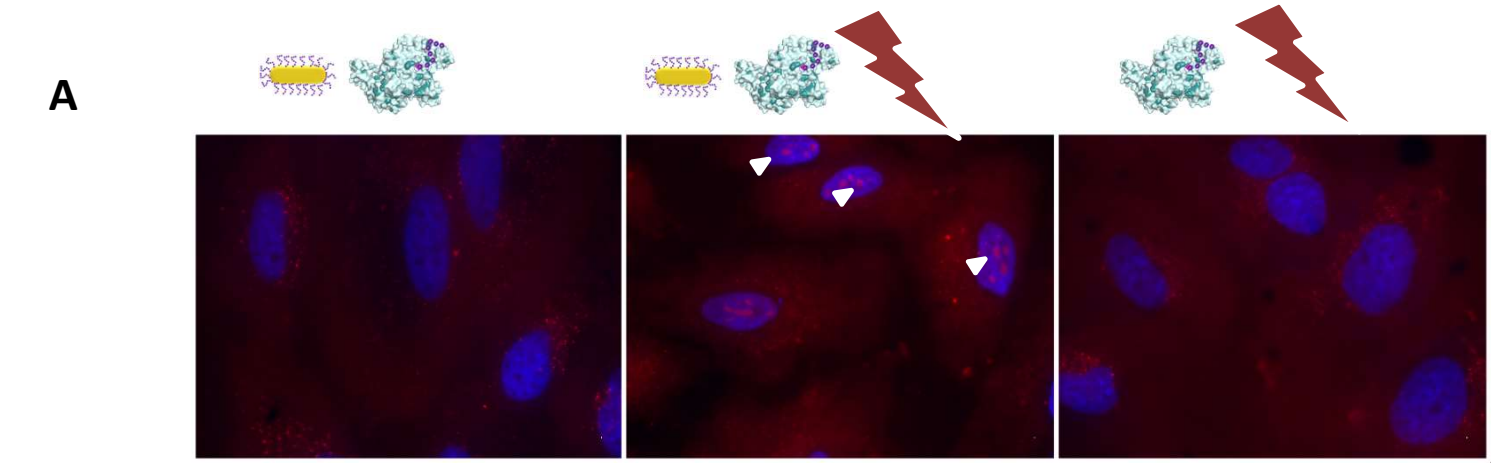


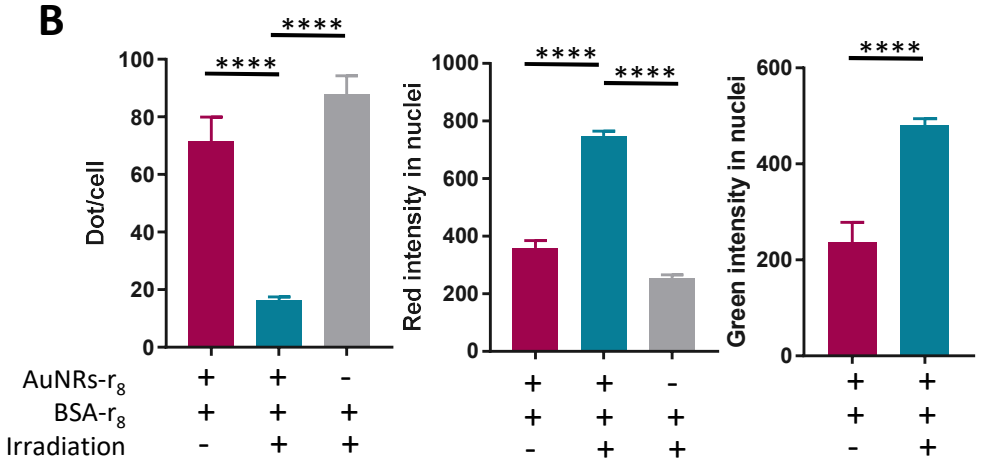


Results and discussion



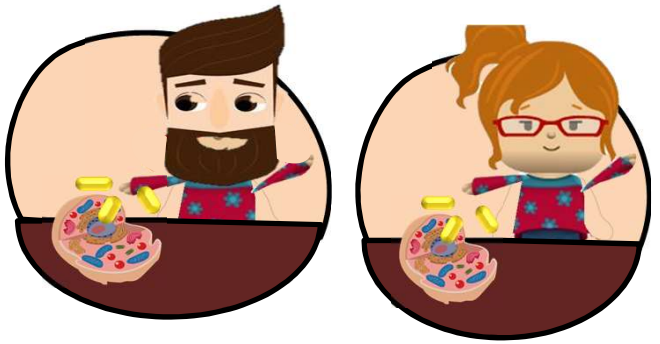
Results and discussion





A) Confocal images of HeLa cells incubated with a) BSA-r_8 and AuNRs-r_8 non-irradiated; b) BSA-r_8 and AuNRs-r_8 after irradiation; quantification of B) number of dots/cell; C) red intensity in nuclei; D) green intensity in nuclei. White arrows mark endosomes in **a** and nucleoli in **b**.

Conclusions



- ✓ AuNRs can be efficiently prepared with a aspect ratio of 5.6 which results in maxima in the second biological window.
- ✓ AuNRs can be functionalized in a biocompatible way. Incorporation of CPP leads to particle internalization.
- ✓ Proteins has been successfully modified to internalise in cells.
- ✓ Both protein and AuNRs modified with r8 share internalization mechanism.
- ✓ CW laser irradiation lead to endosomal disruption: r_8 directs the cargoes to the nucleus (nucleoli).
- ✓ Cells are viable after irradiation.

Chem. Eur. J., **2021**, *27*, 4670 – 4675.

Acknowledgments

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