# CD44-targeted PLGA-based nanoparticles for the delivery of siRNA to cancer cells

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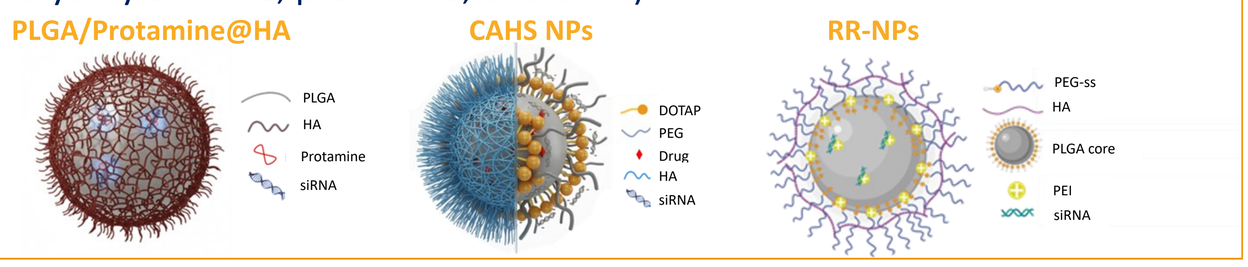
## INTRODUCTION

siRNA therapy, alone or in association with chemotherapy, represents a promising strategy for the treatment of cancer [1]. However, clinical translation of siRNA can be improved by its delivery through polymeric nanoparticles (NPs) able to overcome different biological barriers, thus ameliorating siRNA efficacy and reducing off-target effects [2]. Furthermore, in order to improve NP accumulation in solid tumors, it is recognized that Hyaluronic Acid (HA) is an important targeting ligand that promotes the cellular uptake of NPs by CD44-presenting cancer cells [3]. Therefore, we propose novel CD44-targeted polymeric NPs decorated on the surface with HA intended for siRNA delivery to solid tumors.

Different HA-coated poly (D, L-lactide-co-glycolide) (PLGA) NPs with or without a poly(ethylene glycol) (PEG) surface were prepared through nanoprecipitation or emulsion/diffusion technique, depending on the type of PLGA as well as the cationic moiety employed for siRNA delivery (Polyethylenimine, protamine, or DOTAP).

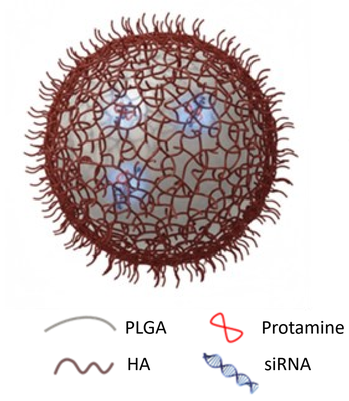



## RESULTS AND DISCUSSION

Colloidal properties of NPs were measured on a Zetasizer Nano ZS. siRNA was loaded inside NPs at different N/P ratios depending on the cationic agent added into the formulations, and the loading was determined by RiboGreen assay and agarose gel electrophoresis. Cytotoxicity, cellular uptake, and transfection studies were performed in different cancer cell lines (colon, breast, gastric cancer) which overexpress the CD44 receptor.

### NP sketch

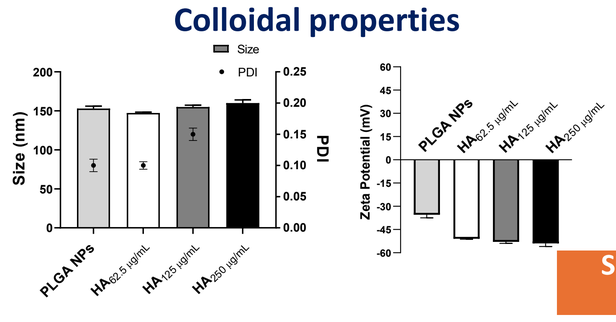
### PLGA/Protamine@HA



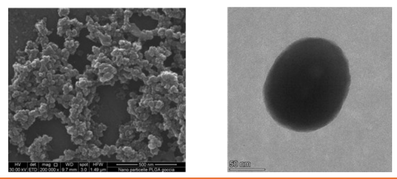

Technological characterization

Biological characterization

### Colloidal properties

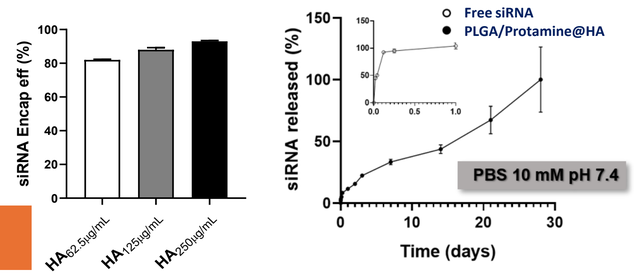



### SEM/TEM images

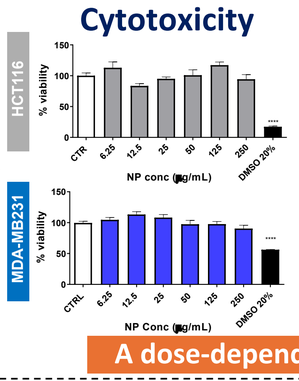


Spherical morphology and uniform size distribution

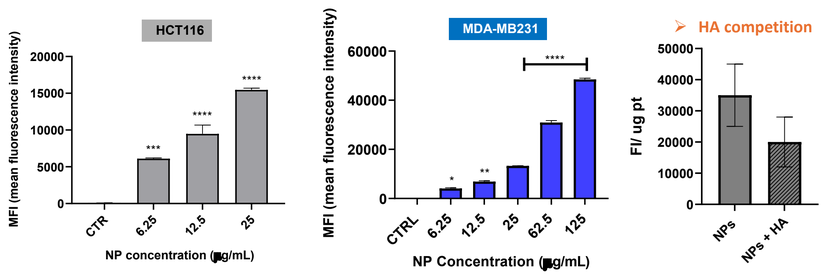
### siRNA entrapment and release



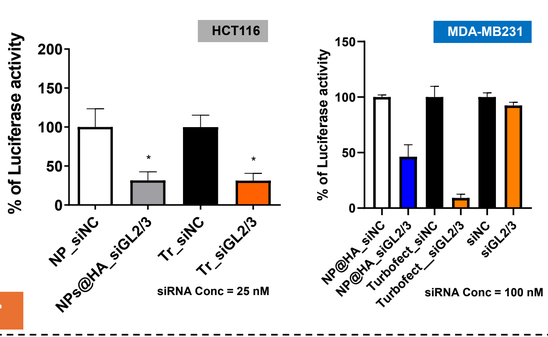

### Cytotoxicity




### Uptake

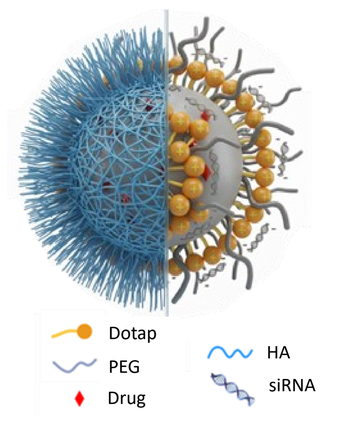



### siRNA transfection




A dose-dependent internalization into cancer cells mediated by the CD44 receptor

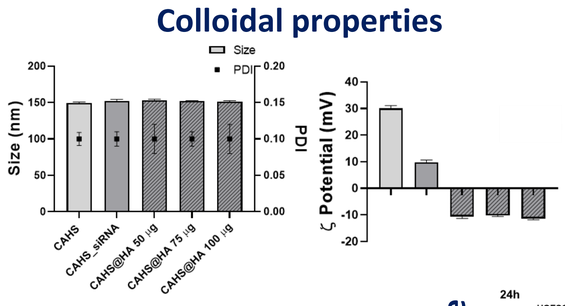
### CAHS NPs



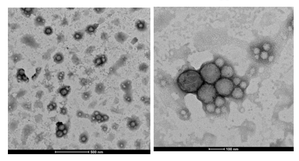

Technological characterization

Biological characterization

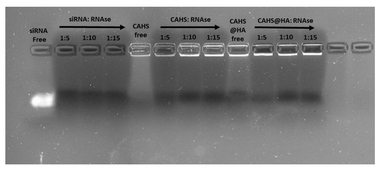
### Colloidal properties



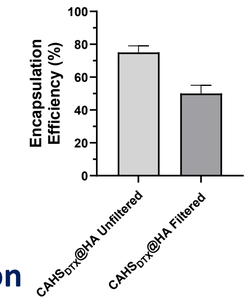

### TEM images



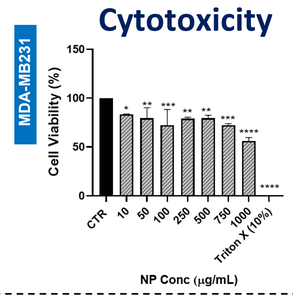
### Gel retardation assay



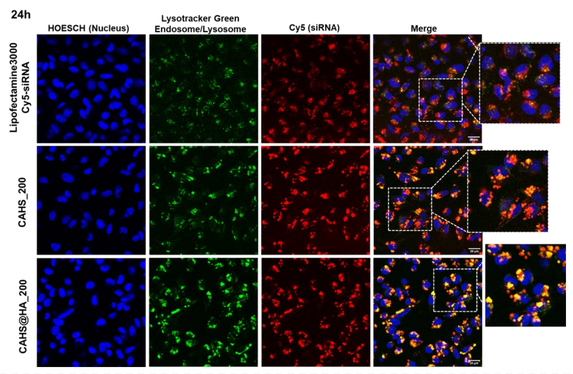

### Docetaxel entrapment



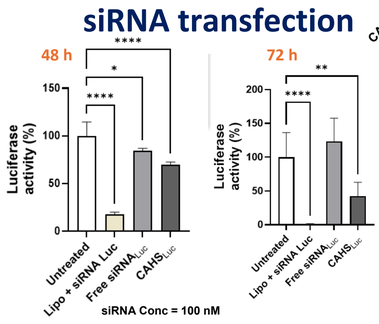

### Cytotoxicity




### Cell uptake and endolysosomal escape




### siRNA transfection

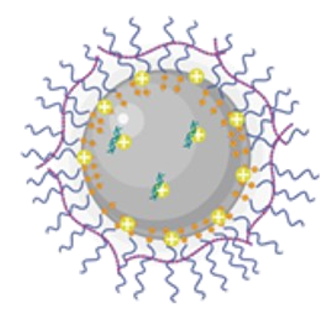



Safe NPs highly uptaken by cells and with a strong transfection efficiency of the delivered siRNA

### Colloidal properties

| Size ±SD (nm) | PDI | ζ Pot ±SD (mV) | siRNA actual loading | Entrap. Efficiency (%) |
|---|---|---|---|---|
| 157.1 | 0.168 | -19.8 | 1 nmol/10 mg NPs | 100 |

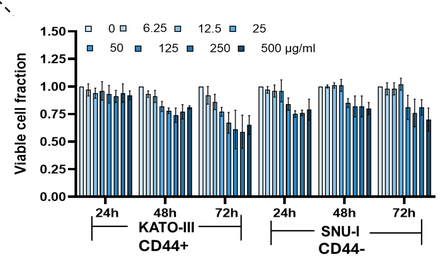

### RR-NPs




Technological characterization

Biological characterization

### Cytotoxicity

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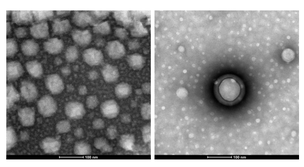
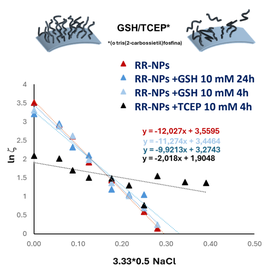


Spherical morphology and high siRNA entrapment

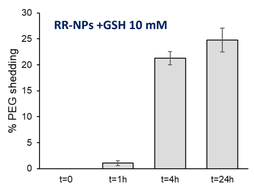
### FALT measurements



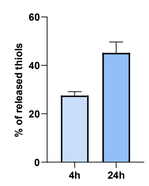

### Redox-responsiveness of RR-NPs and PEG shedding

PEG shedding of around 30% after 24h of treatment with Glutathione 10 mM

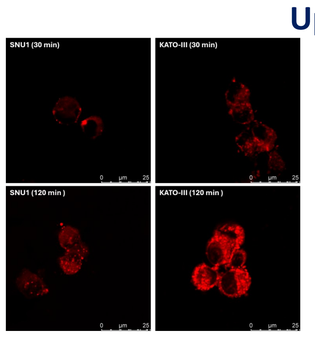
### PEG iodine assay



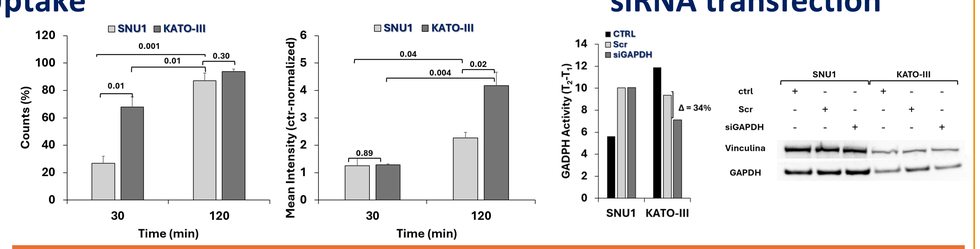

### Ellman assay




### Uptake




### siRNA transfection




Higher internalization and gene silencing efficacy in KATO III gastric cancer cells overexpressing the CD44 receptor

## CONCLUSIONS

We have developed three different systems decorated with HA for the delivery of siRNA to cancer cells overexpressing the CD44 receptor. Although some cell studies are still in progress, all the formulations can be ideal candidates for siRNA delivery to cancer. Next steps will be devoted to the co-encapsulation of conventional anticancer drugs with a therapeutic siRNA for an eventual combined anticancer therapy.

References: [1] Kumar K, Rani V, Mishra M, Chawla R. Curr Res Pharmac Drug Discov. 2022:28-3. [2] Cavallaro G, Sardo S, Craparo EF, Porsio B, Giammona G. Int J of Pharm. 2017: 313-333. [3] Harshvardhan R. and Bhattacharya S. Cur Med Chem. 2025:3960-3990.

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