Small Additive Molecules to Enhance the Effective Accessibility of Delivered Nucleic Acid by Cell-Penetrating Peptide

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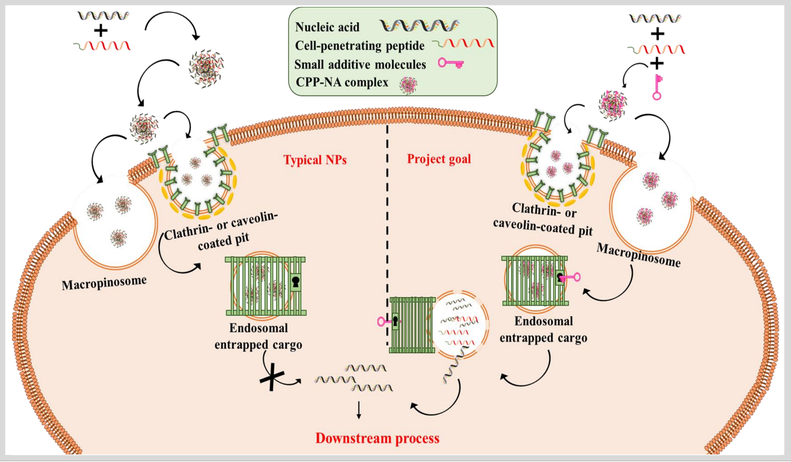
Introduction and Objective

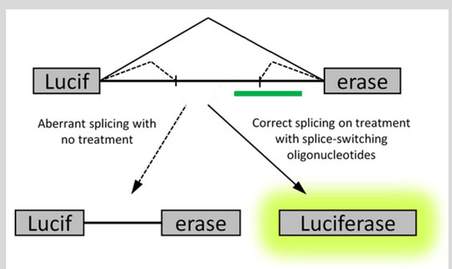
- **Cell-penetrating peptides (CPPs)** are short peptides (up to 30 aa) with cell membrane permeability.
- With **nucleic acids**, CPPs form electrostatic complexes in the nanometer range: nanoparticles (NPs).
- Still, the efficiency of transfection with CPP NPs is usually lower than of lipid-based particles.
- The reason for the inefficient transfection of NAs is predominantly due to the **endosomal entrapment** of the particles.
- In this work, we have introduced several additive molecules in complexes to improve the accessibility of the NAs.
- We screened several FDA-approved compounds, categorized into groups: fusogenic lipids, endosomotropic agents, inhibitors of exocytosis, and autophagy.
- PF14 with sequence stearyl-AGYLLGKLLOOLAAAALOOLL-NH_2 was used as model CPP for our work.



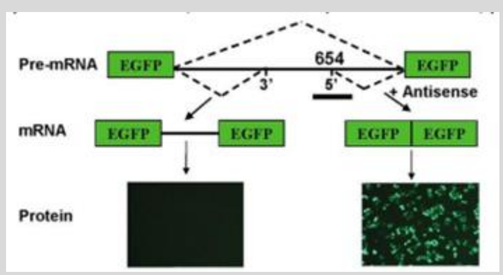
Methodology and Transfection Efficiency Evaluation Assays

Methodology:

1. Mixed CPP with nucleic acid: splice-correcting oligonucleotide (**SCO**) or **siRNA to form NPs**.
2. After 15 min of incubation, **additive**s were added in different concentrations.
3. After 15 min, the complexes were added to the cells for treatment.
4. After 24 hr, **measured** the luminescence or fluorescence.



Restoration of luminescence of luciferase gene with **SCO-705** in HeLa pLuc-705



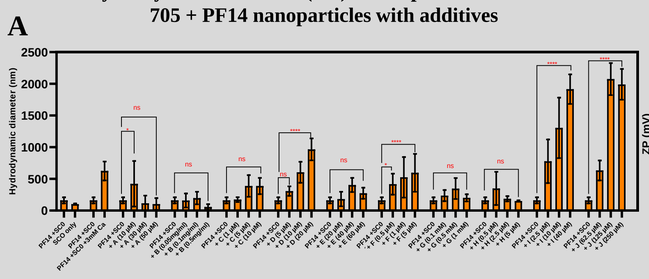
Restoration of fluorescence of EGFP gene with **SCO-654** in HeLa EGFP-654

Reporter cell lines

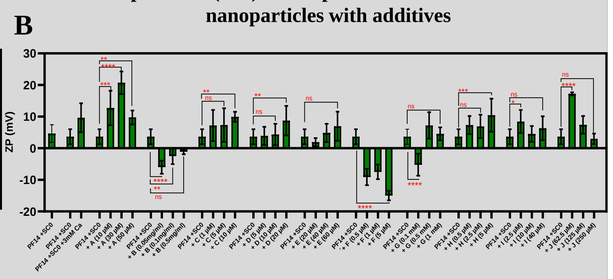
- **HeLa pLuc-705 (**having mutated luciferase gene**)**
- **HeLa EGFP-654 (**having mutated EGFP gene**)**
- **U87 MG-Luc2 (**having luciferase encoding gene**)**

Characteristics of Nanoparticles

Hydrodynamic diameter (nm) of complexes with SCO-705 + PF14 nanoparticles with additives



Zeta potential (mV) of complexes with SCO-705 + PF14 nanoparticles with additives

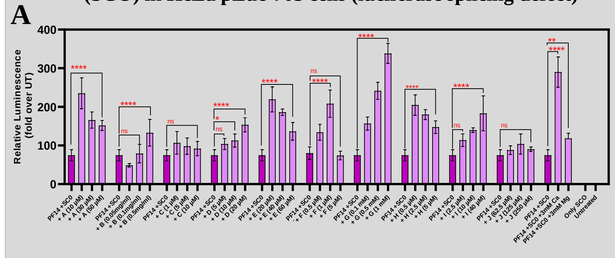


- The inclusion of additives into NPs changed the hydrodynamic diameter of the complexes.
- Compounds I and J significantly increased the size up to 2000 nm at higher concentrations.

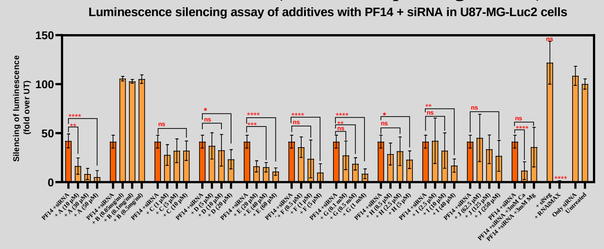
- The addition of additives also affects the zeta potential of the complexes towards a more positive charge.
- Compounds B and F addition resulted in negative zeta potential.

Effect of Additives on Reporter Gene Expression

Increase in luminescence with splice correcting oligonucleotide (SCO) in HeLa pLuc-705 cells (luciferase splicing defect)



Suppression of luminescence with luciferase-targeting siRNA in U87 MG-Luc2 cells (luciferase-expressing cell line)

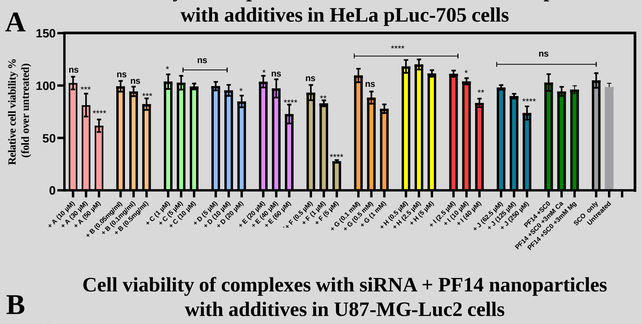


- Expression of **luciferase** was enhanced by supplementing additives along with PF14 + SCO nanoparticles.
- Concentration-dependent effect with high significance can be seen here.

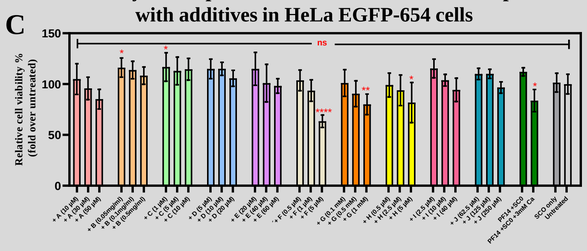
- Silencing of **luciferase** expression was observed by supplementing additives along with PF14 + SCO nanoparticles.
- Concentration-dependent effect with high significance can be seen here.

Cell Viability Profiles of Additives

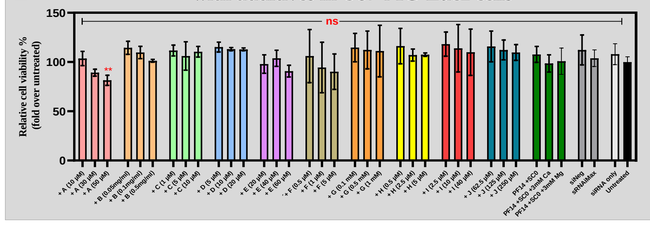
Cell viability of complexes with SCO-705 + PF14 nanoparticles with additives in HeLa pLuc-705 cells



Cell viability of complexes with SCO-654 + PF14 nanoparticles with additives in HeLa EGFP-654 cells



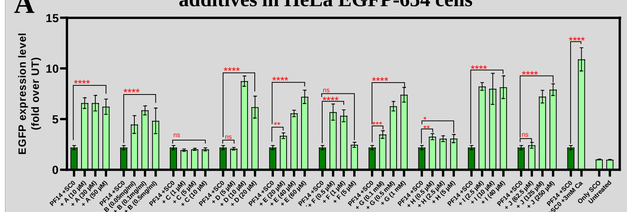
Cell viability of complexes with siRNA + PF14 nanoparticles with additives in U87-MG-Luc2 cells



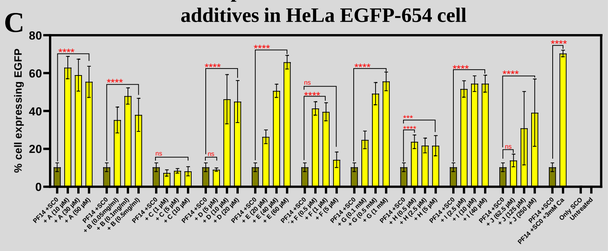
- Additives have different effects depending on the cell line.
- Compounds A and F showed reduced cell viability at higher concentrations in HeLa pLuc-705 cells.
- In the case of U87 MG-Luc2, none of the additives showed reduced viability except compound A at the highest tested concentration.
- Similarly, HeLa EGFP-654 showed reduced viability with some compounds at the highest tested concentration.

Cellular Association and Internalization

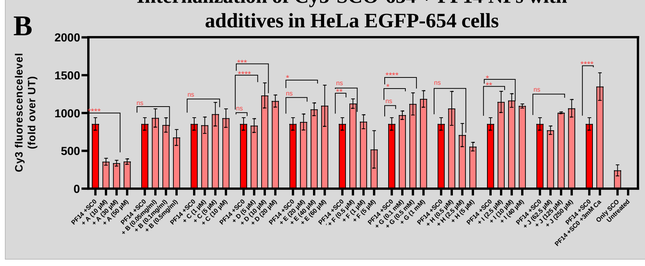
Rescue of EGFP expression with SCO-654 + PF14 NPs with additives in HeLa EGFP-654 cells



Rescue of EGFP expression with SCO-654 + PF14 NPs with additives in HeLa EGFP-654 cell



Internalization of Cy5-SCO-654 + PF14 NPs with additives in HeLa EGFP-654 cells



- Higher levels of cellular association were observed with the addition of additives as shown in figure A.
- With HeLa EGFP, almost all the additives are showing a significant increase in the expression level of EGFP except compound C.
- Figure B shows the association of Cy5 labeled SCO, which is also enhanced by introducing additives.
- Figure C shows the % of cells expressing eGFP protein, which is significantly correlating with Figure A.

Conclusion

- Additives enhance the PF14-mediated transfection of SCO and siRNA into all the used cell lines.
- All the studied additives positively contributed to the accessibility of NAs in cells in a concentration-dependent manner.
- Most of the PF14-NA + additives NPs did not significantly affect the cell viability.

Future Plan

- These additives will be screened with other types of Nas and onther CPPs too.
- Study of mechanism of action of additives-NAs-PF14 will be performed.
- Confocal microscopy imaging will be performed.

Funding

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