Cardiolipin-containing liposomes for intracellular drug delivery

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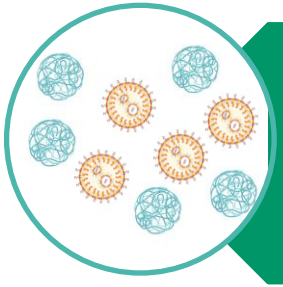
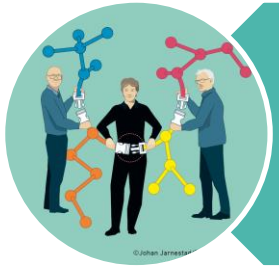
8th International Symposium on Phospholipids in Pharmaceutical Research

Phospholipid Research Center

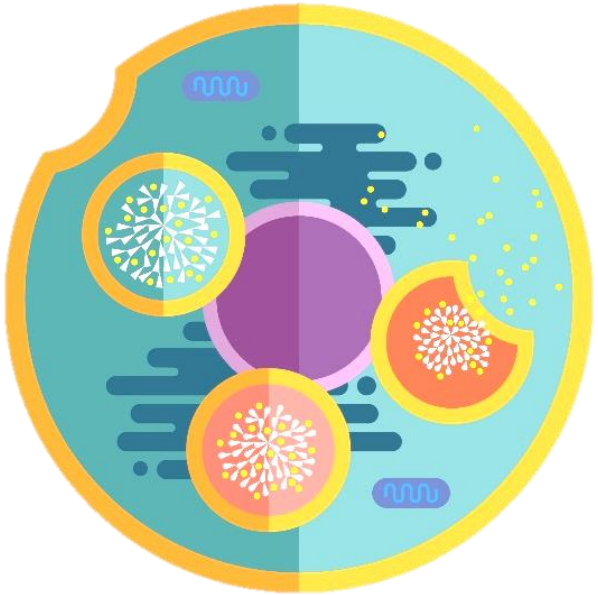
Intracellular delivery: understanding and development



1. Polymer and lipid nanoparticles for intracellular delivery: design and applications



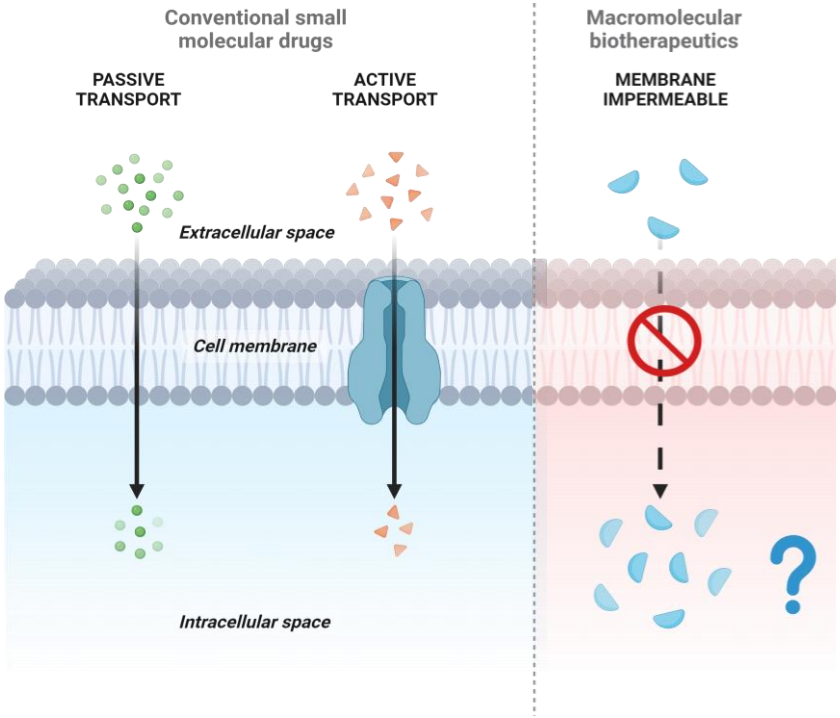
2. Intracellular delivery quantification by bioorthogonal reactions

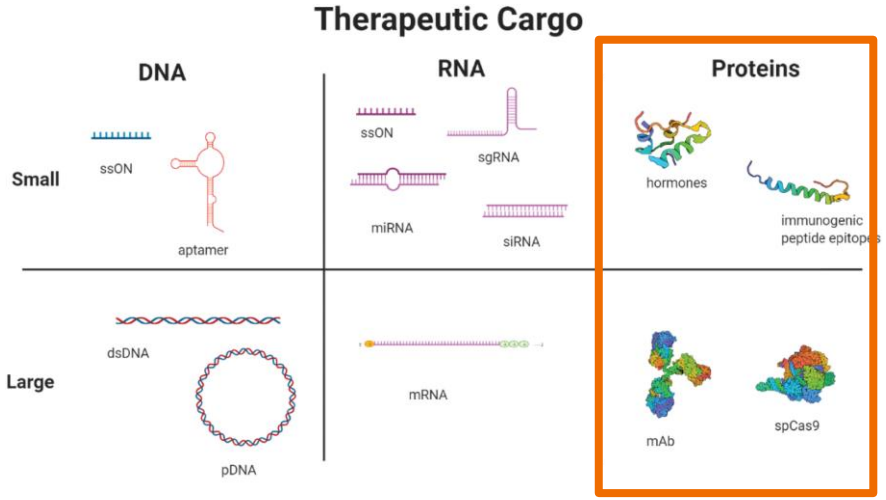


https://www.helsinki.fi/en/researchgroups/intracellular-drug-delivery

Cover Image. Chemistry of Materials 29.14 (2017)

Intracellular delivery of biotherapeutics

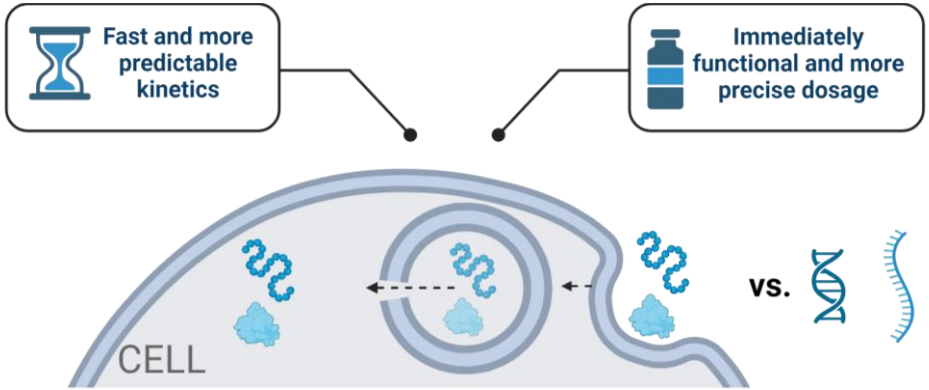


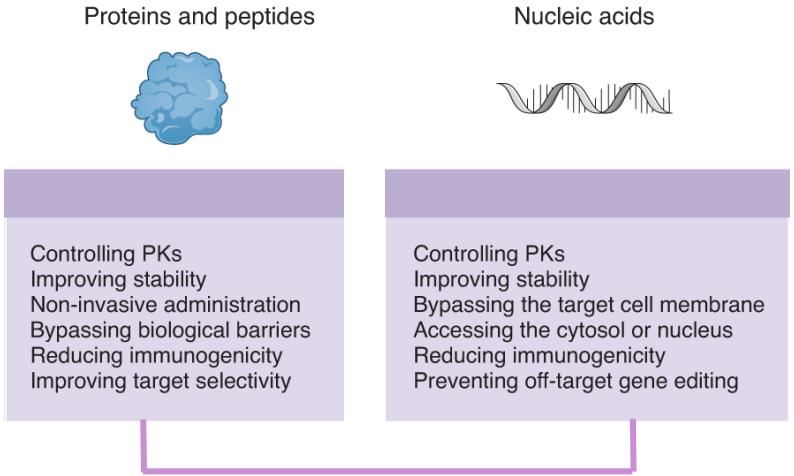


Journal of Controlled Release 336 (2021): 583-597.

Intracellular delivery of therapeutic proteins: why

Protein vs. Gene delivery





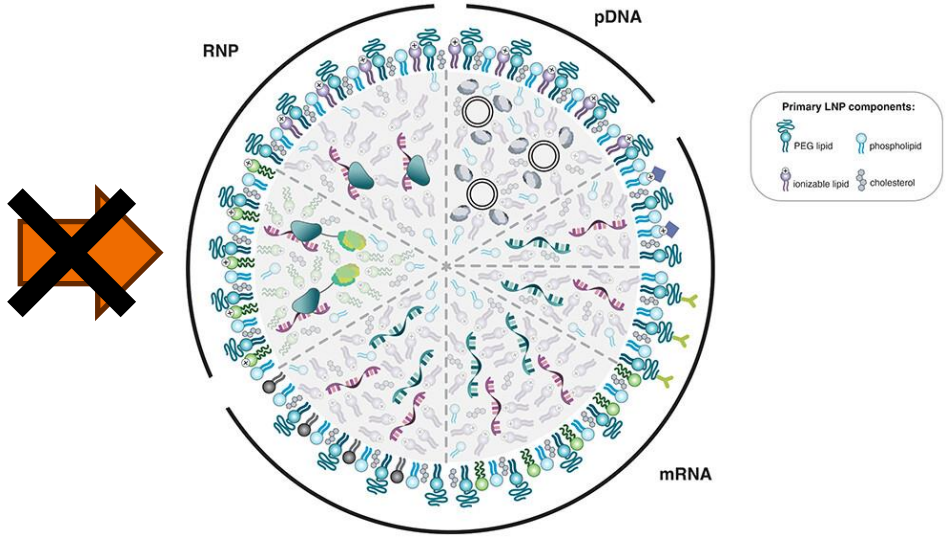
Similar challenges in formulation and delivery

Nature Biomedical Engineering, 5, 951–967 (2021).

Intracellular delivery of therapeutic proteins: how

Unique challenges in protein formulation and delivery

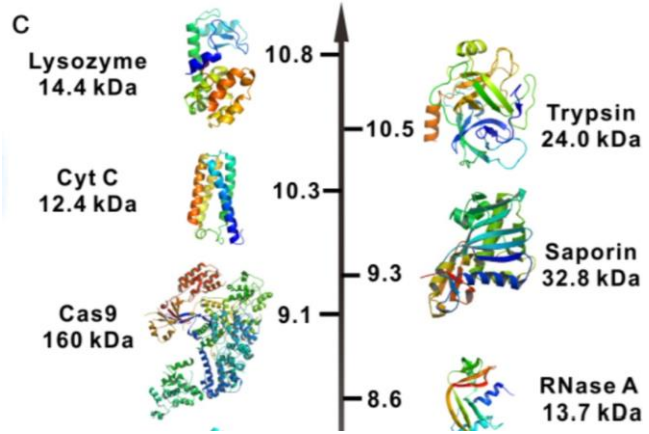
- Maintaining protein **folding and function**
- Achieving sufficient **loading/encapsulation efficiency**
- Larger size and more difficult to **bypass the membrane barrier**
- **Greater structural variations** and thus the results of model proteins hardly transfer to actual payloads



Mol. Pharmaceutics 2022, 19, 6, 1669-1686

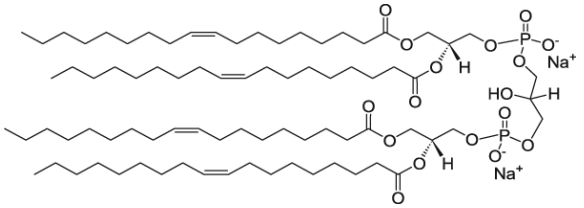
Intracellular delivery of apoptotic/cytotoxic proteins

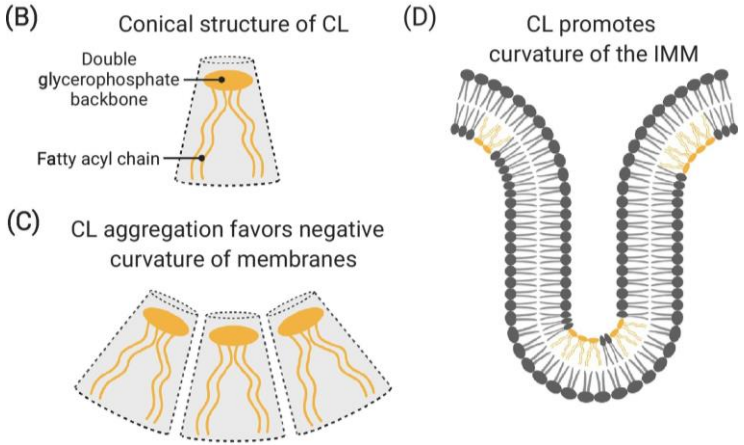
Positively charged at physiological pH



Protein Isoelectric point

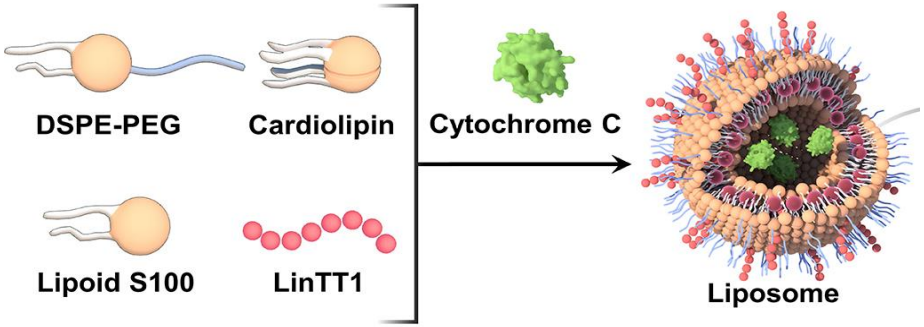
Anionic lipid - Cardiolipin





Science Advances, 5 (2019), Article eaaw8922
Trends in Endocrinology & Metabolism, 2021, 32, 224–237

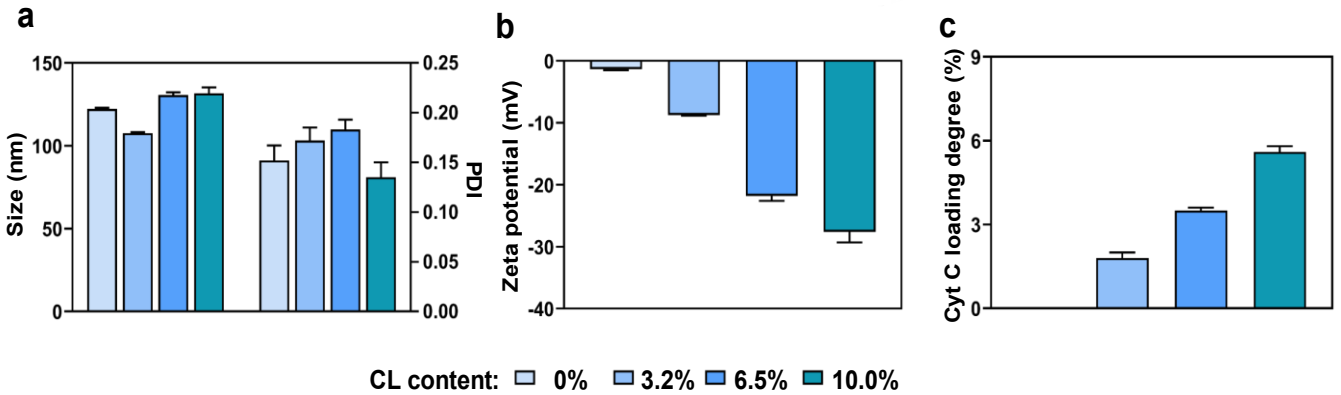
Cardiolipin-containing liposomes for Cyt C delivery



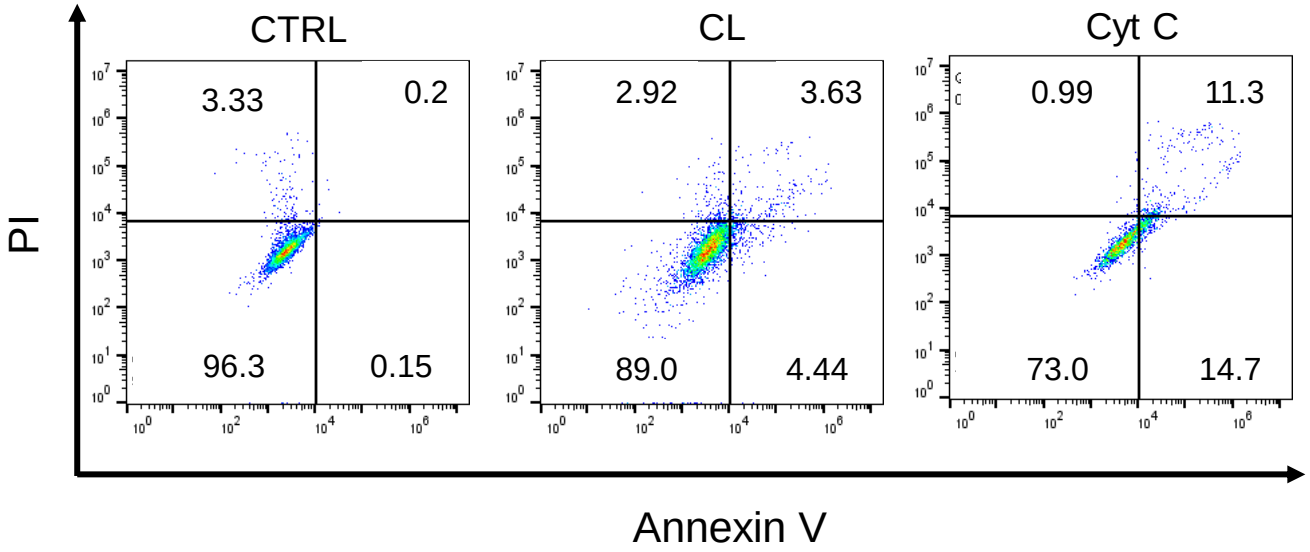
Cardiolipin → **Anionic fusogenic lipids**

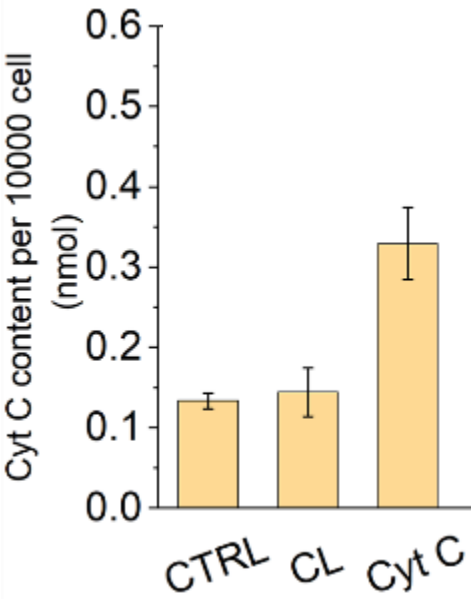
DSPE-PEG, Lipoid S100 → **Helper lipids**

LinTT1 → **Tumor targeting peptides**



Intracellular Cyt C delivery and tumor apoptosis





The delivery of cytochrome c (Cyt c) only moderately increase tumor apoptosis

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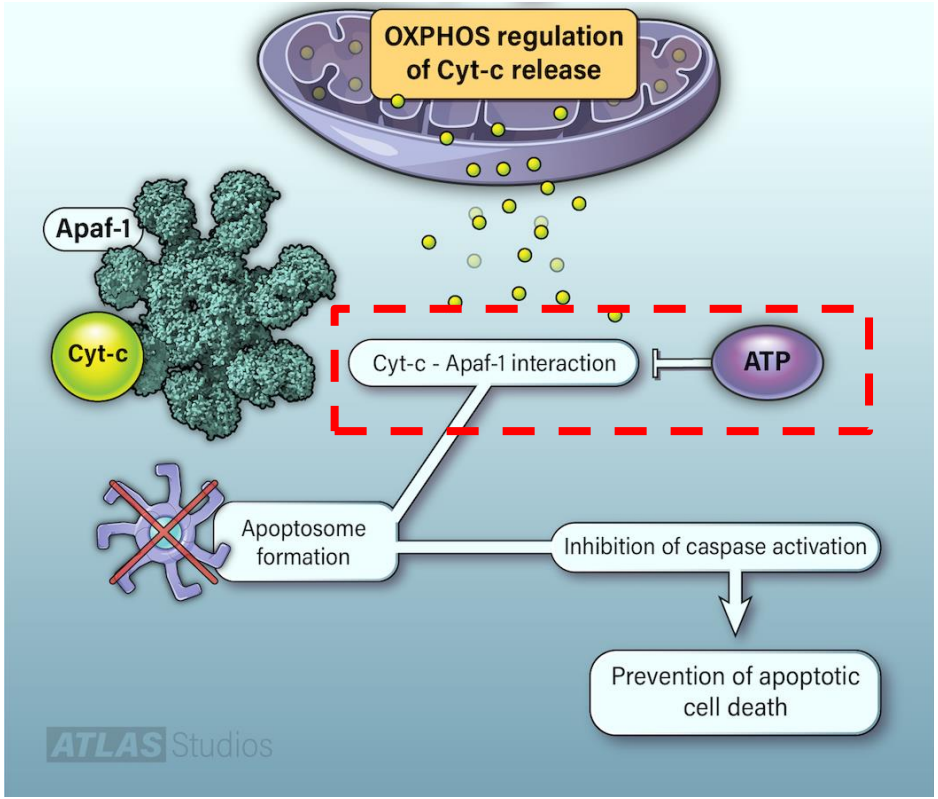
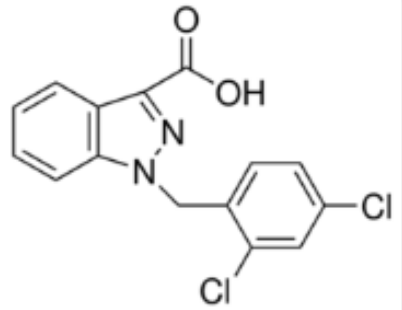
Advanced Functional Materials, 2023, 2305316

Intracellular Cytc delivery and tumor apoptosis





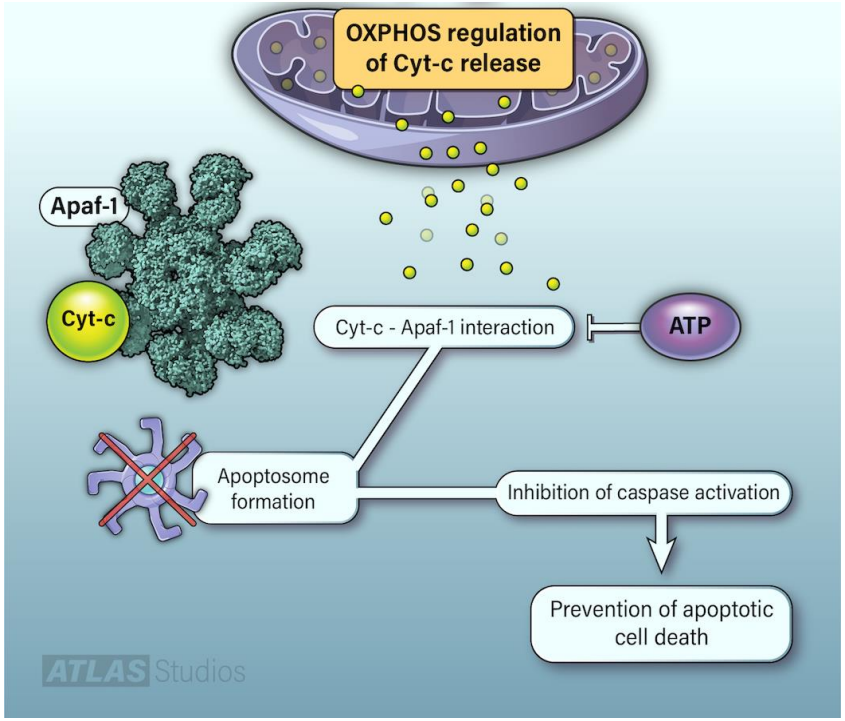
Lonidamine (LND)

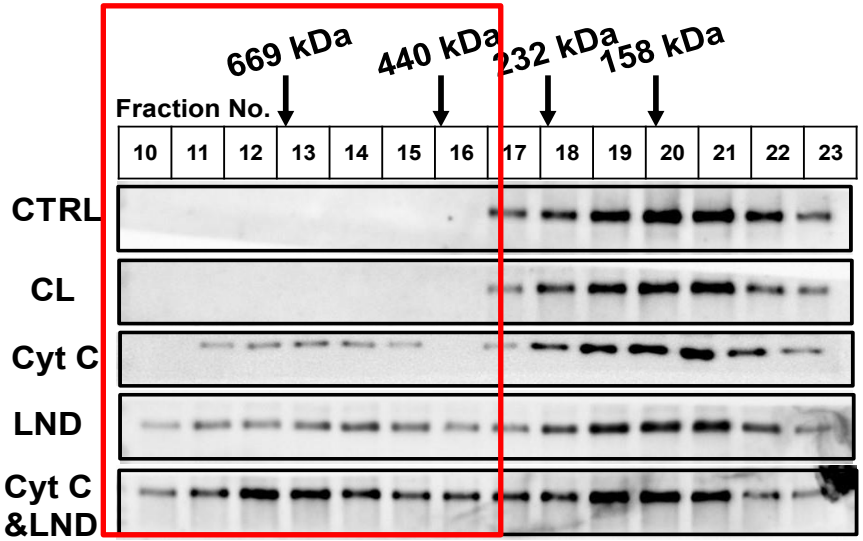
Inhibit tumor aerobic glycolysis

ATP inhibits cytochrome c (Cyt c) and Apaf-1 assembly into apoptosomes

https://www.roswellpark.org/research/labs/chandra-lab/apoptosome-dynamics

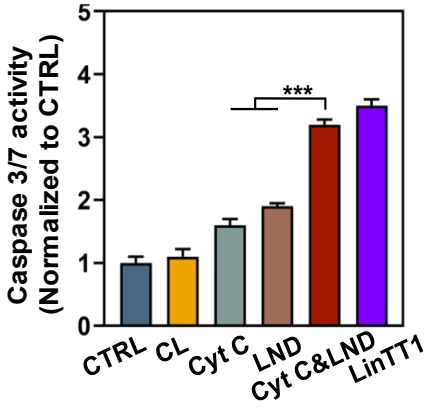
Co-delivery of Cytc and LND: pro-apoptotic mechanisms

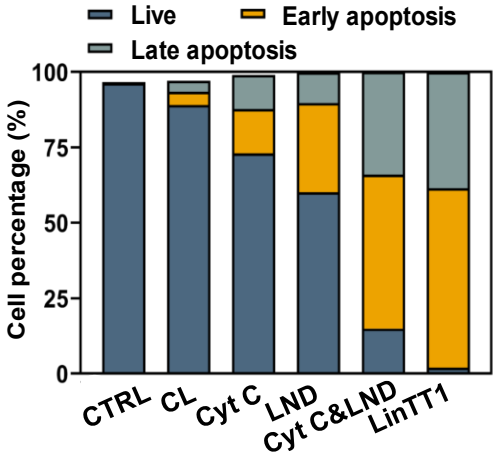


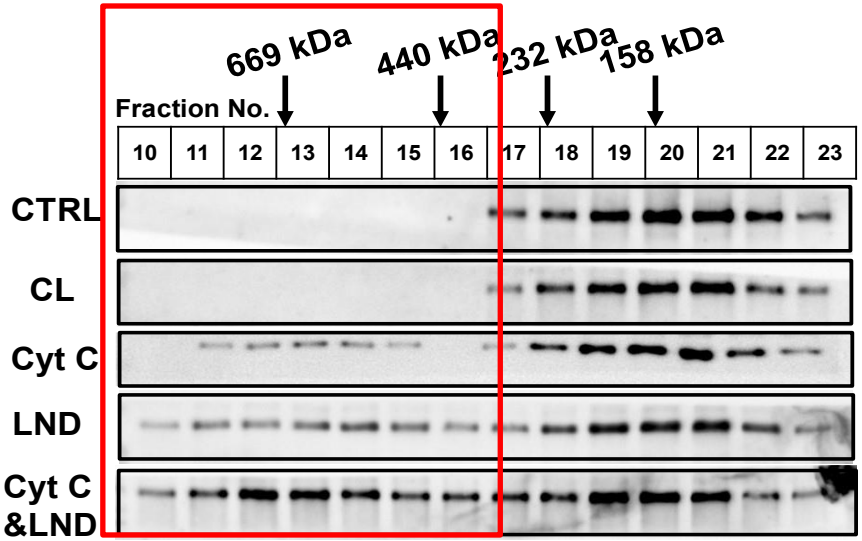


Apoptosome level significantly increased in the Cyt C and LND co-delivery formulation

Co-delivery of Cytc and LND: pro-apoptotic mechanisms

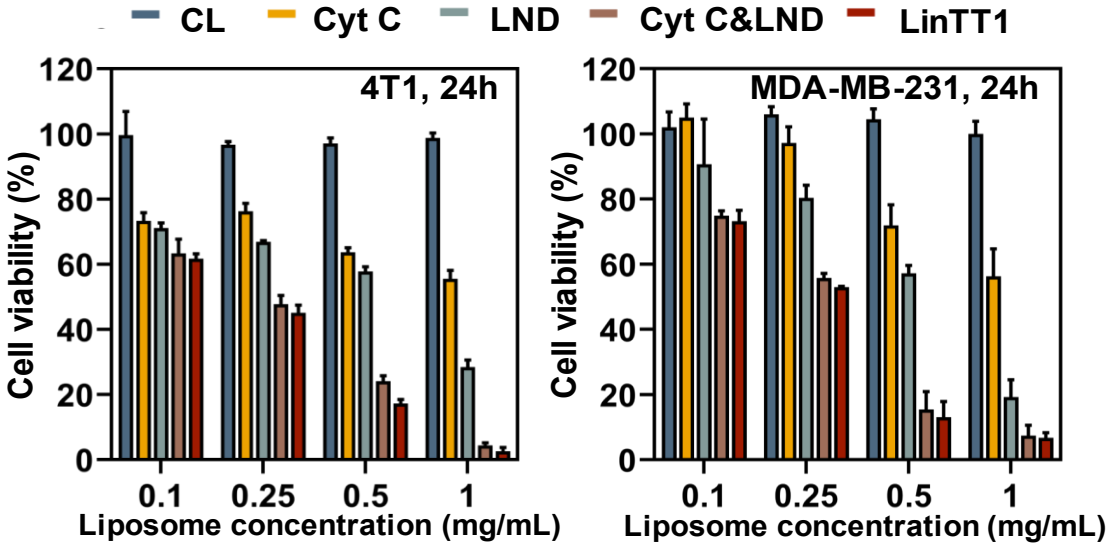


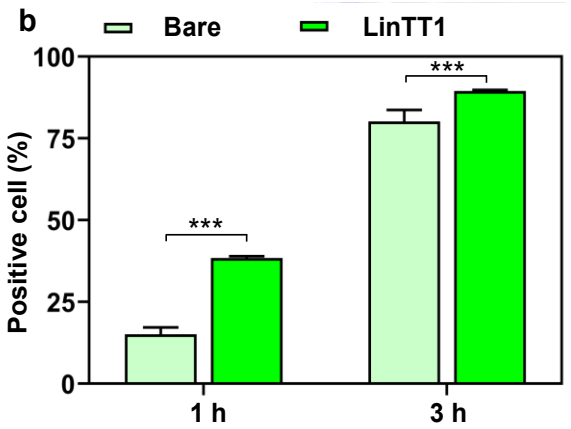




The co-delivery of cytochrome c (Cyt c) and lonidamine (LND) by anionic liposomal carriers could **promote apoptosome formation and the subsequent cancer apoptosis**

Co-delivery of Cytc and LND: anti-tumor effects





The LinTT1 peptide facilitate cellular uptake of the nanoparticles.

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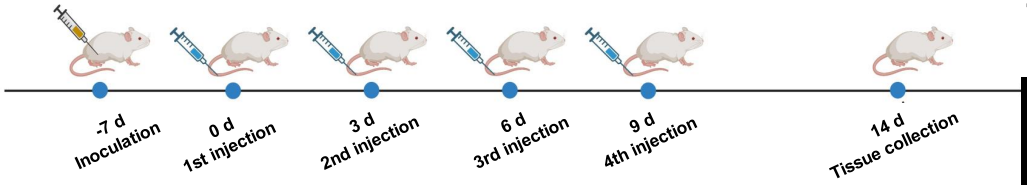
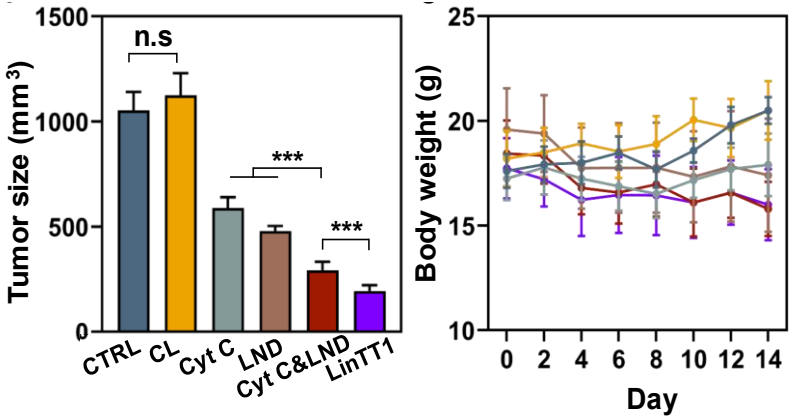
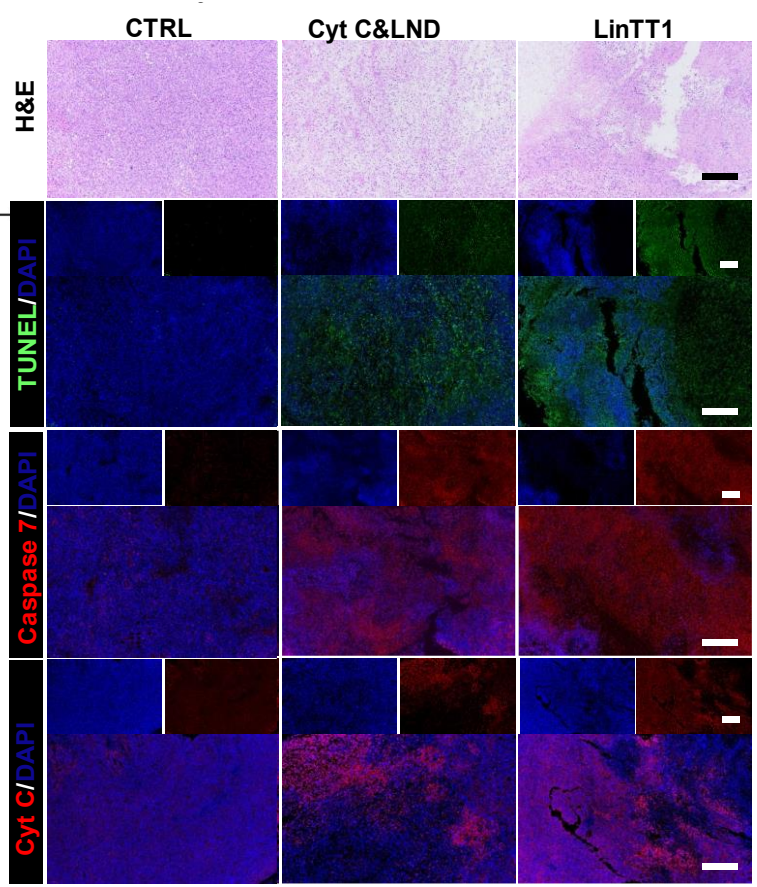
In vivo anti-tumor effects



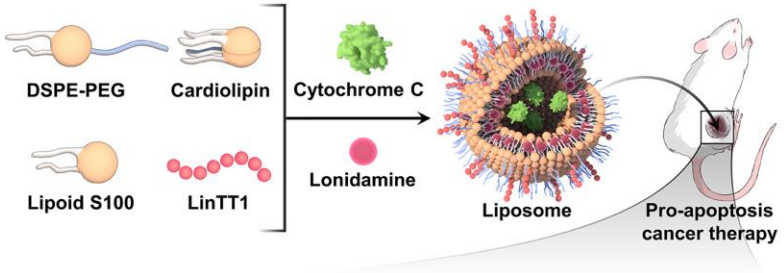


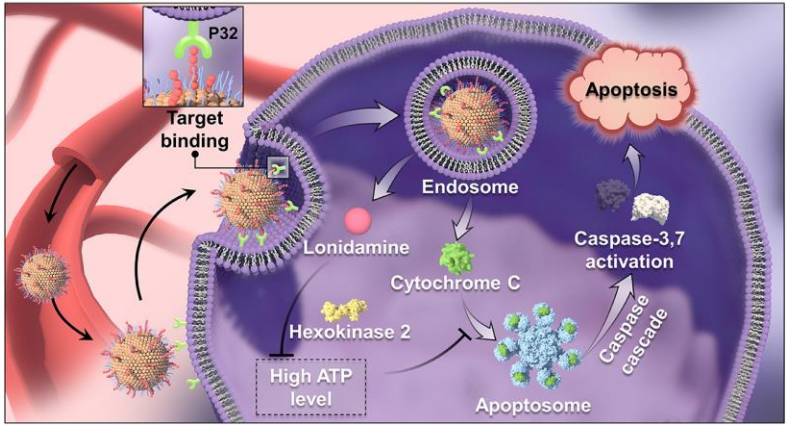


Advanced Functional Materials, 2023, 2305316

Summary

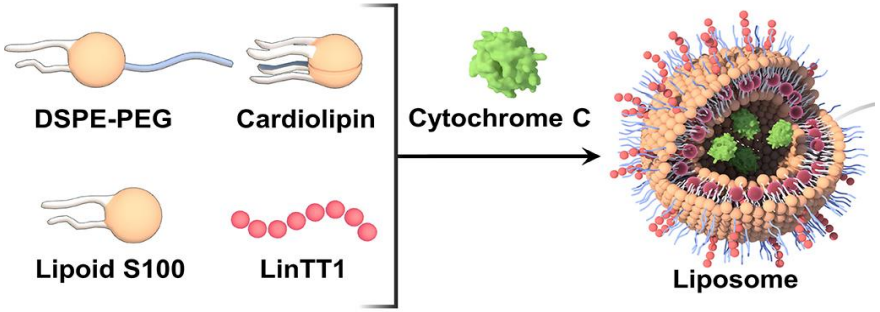
- We developed a cardiolipin containing liposomal formulation for the co-delivery of apoptotic protein, Cyt C, and a metabolic inhibitor, Lonidamine.
- The delivery of Cyt C alone is not enough to trigger sufficient apoptosis, due to inhibited apoptosome assembly.
- Further studies would focus on detailing the endosomal escape properties of cardiolipin containing liposomes, and their potential for other cytotoxic protein intracellular delivery.

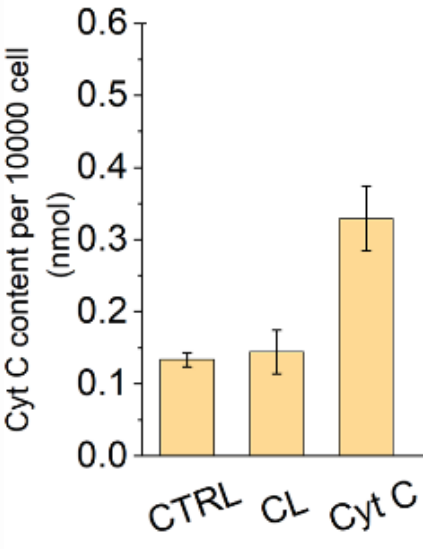




Intracellular delivery quantification by bioorthogonal reactions

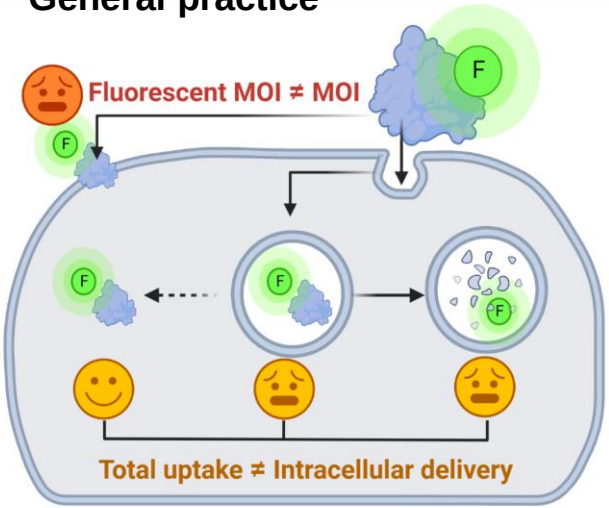
It is challenging to **quantitatively analyze intracellular delivery in live cells.**





Intracellular delivery quantification

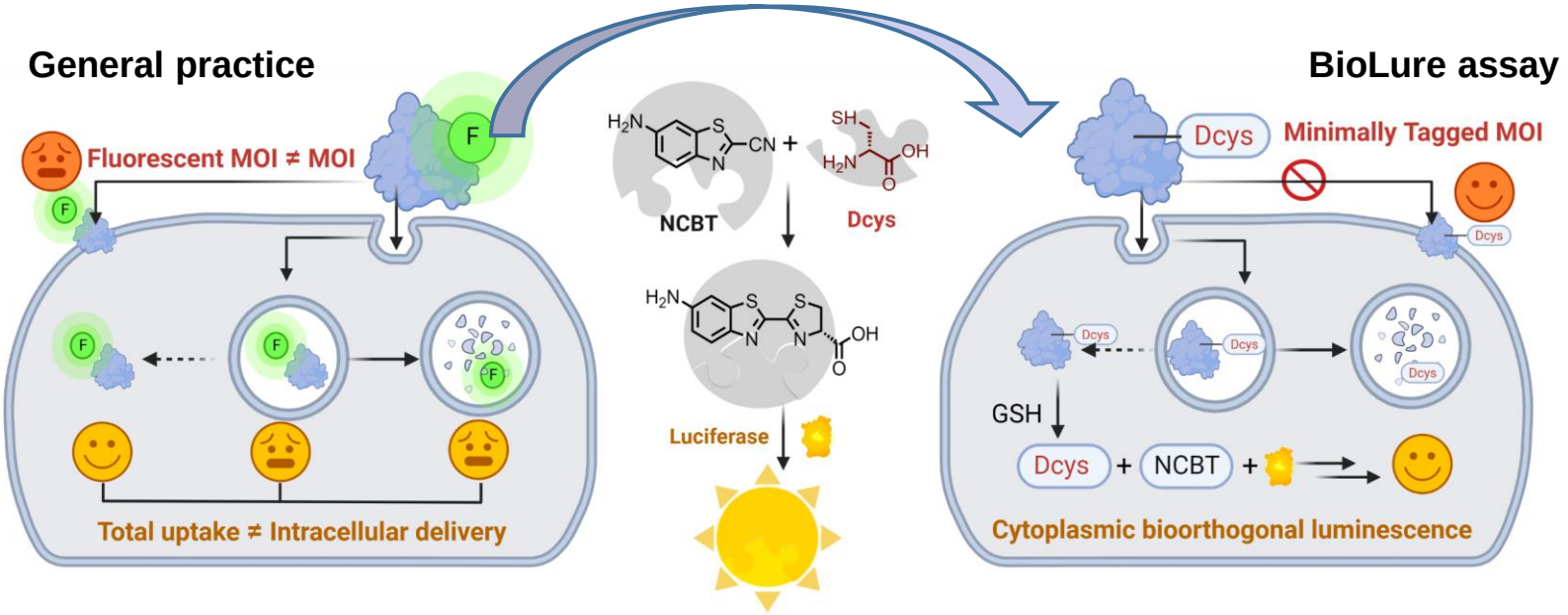
General practice



Challenges:

- Labeling
- Location
- Sensitivity

Intracellular delivery quantification by bioorthogonal reactions

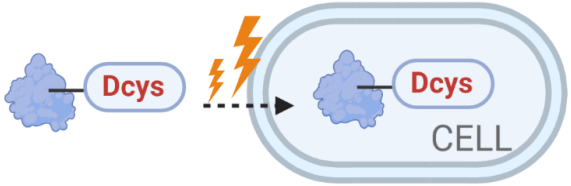


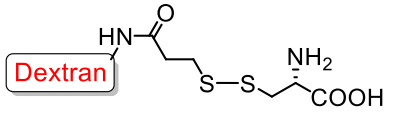
Challenges:

- Labeling
- Location
- Sensitivity

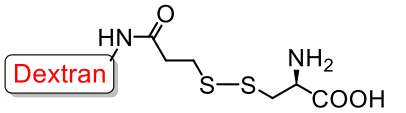
- ✓ Fast
- ✓ Selective
- ✓ Biocompatible
- ✓ Sensitive

Intracellular delivery quantification-model drug

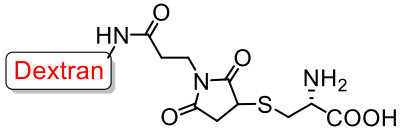




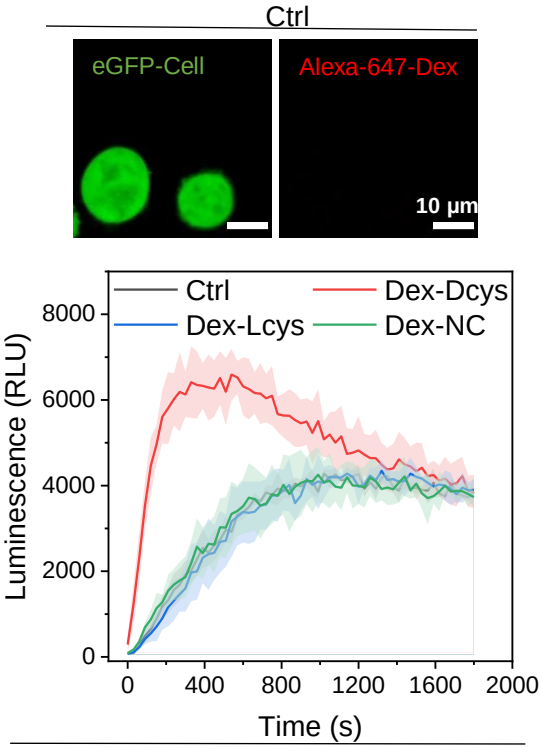
Dex-Dcys



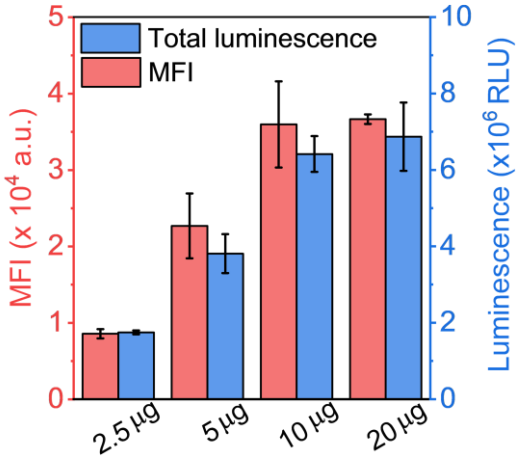
Dex-Lcys



Dex-NC



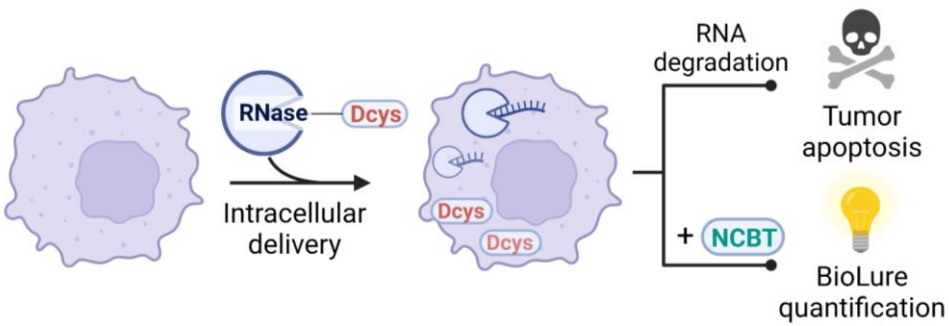
Detectable and specific

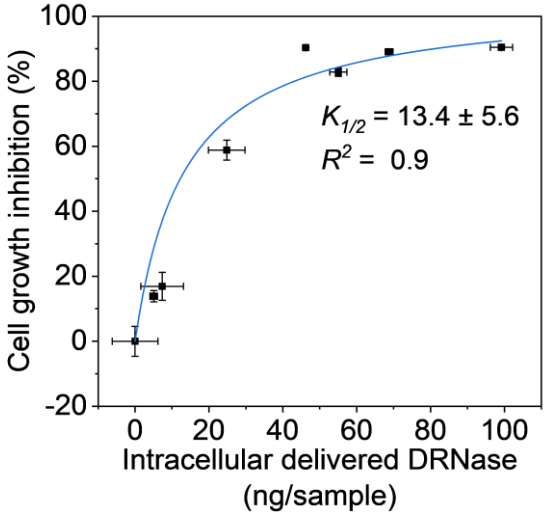


Consistent with complementary method

Communications Chemistry, 2024, 7(1), 181.

Intracellular delivery quantification-cytotoxic protein





BioLure can be used to **estimate the amount of intracellularly delivered molecules**, and thus establishes the link between the cytosolic concentration of intracellularly-delivered biotherapeutics and their therapeutic efficacy.

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Acknowledgments

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- Prof. Wenguo Cui (Shanghai Jiao Tong University)

https://www.helsinki.fi/en/researchgroups/intracellular-drug-delivery