Chiral Quantum Dots Enhanced Small Extracellular Vesicles for Gene Delivery

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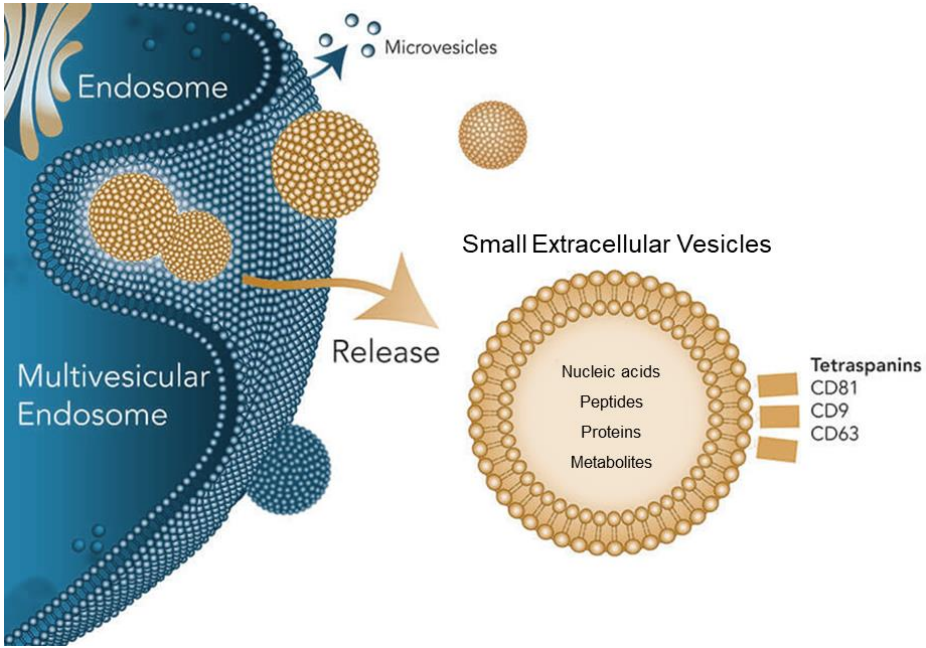
NEXT-GENERATION DELIVERY INNOVATIONS

Small Extracellular Vesicles (sEVs) as Nanocarriers

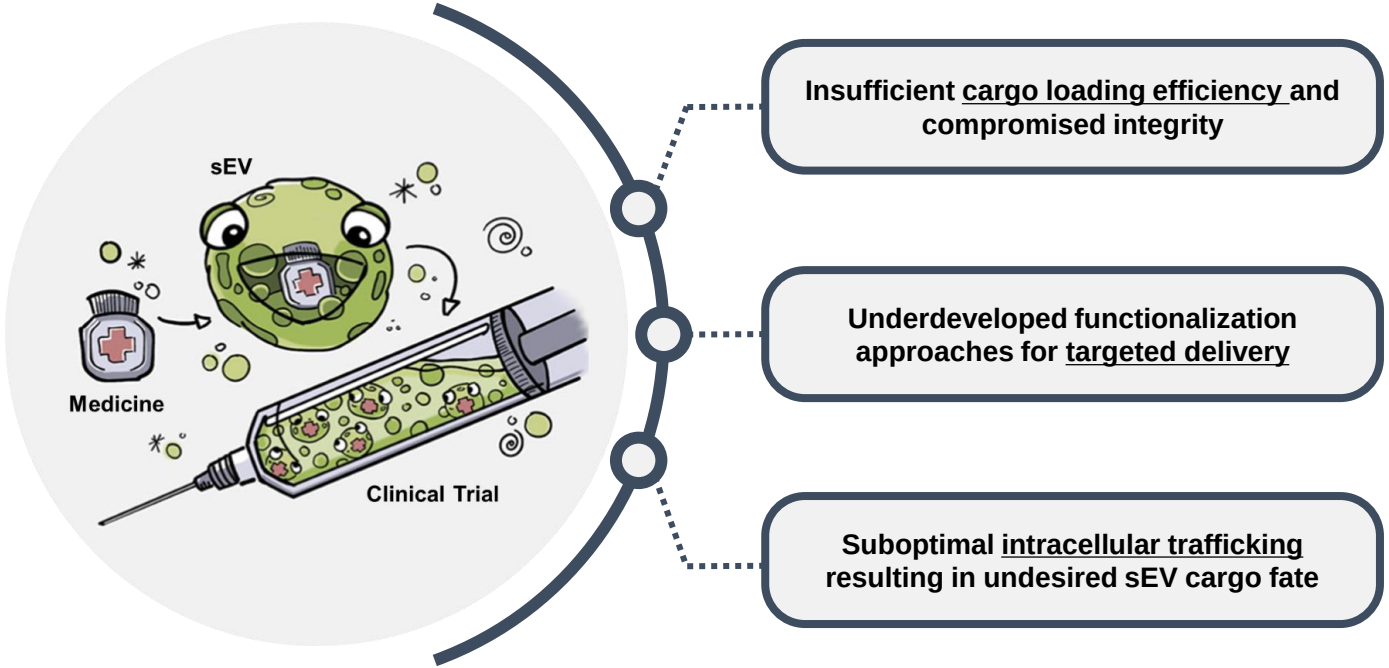


Biological nanoparticles

- One subset of extracellular vesicles
- Size between diameter 50 ~150 nm
- Carry parent cells derived bioactive molecules
- Cell-to-cell communication

sEVs have remarkable potential as one of the candidates for drug delivery platforms.

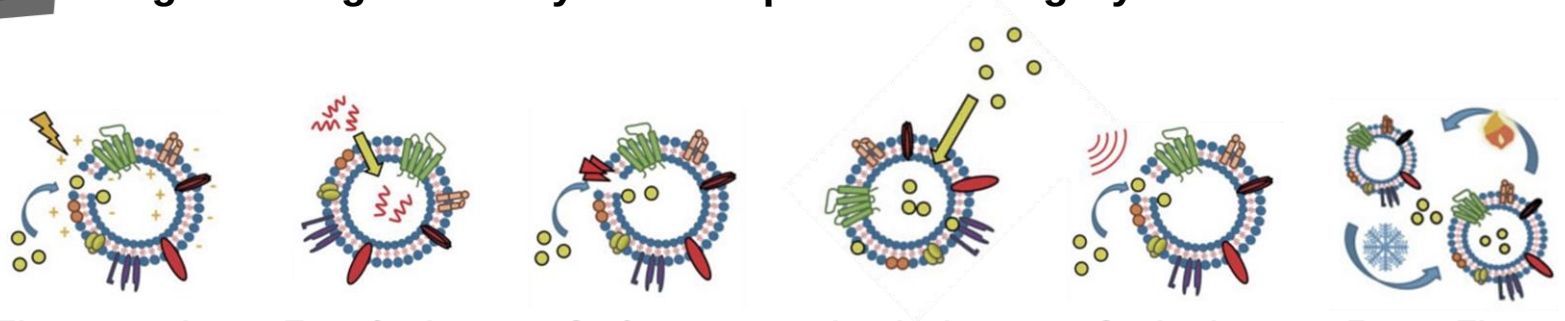
sEVs in Clinical Translation - Unsatisfactory Therapeutic Outcomes



© Annett Mueller. https://www.behance.net/gallery/107041723/Catch-The-Exosomes

CHALLENGE

Cargo loading efficiency and compromised integrity



	Electroporation	Transfection	Surfactant	Incubation	Sonication	Freeze-Thaw
Efficiency	< 20%	N/A	18.5%	1.4~5.1%	8.0~11.0%	4.4~14.7%
Concerns	• Aggregation • Lipid damage • Protein denaturation • Nucleic acid precipitation	• Cytotoxicity • Inefficient packaging • Poor specificity	• Cytotoxicity • Time consuming • Membrane instability	• Time consuming • Inefficient packaging • Small molecules	• Small molecules • Lipid damage • Protein denaturation • Nucleic acid precipitation	• Aggregation • Inefficient packaging

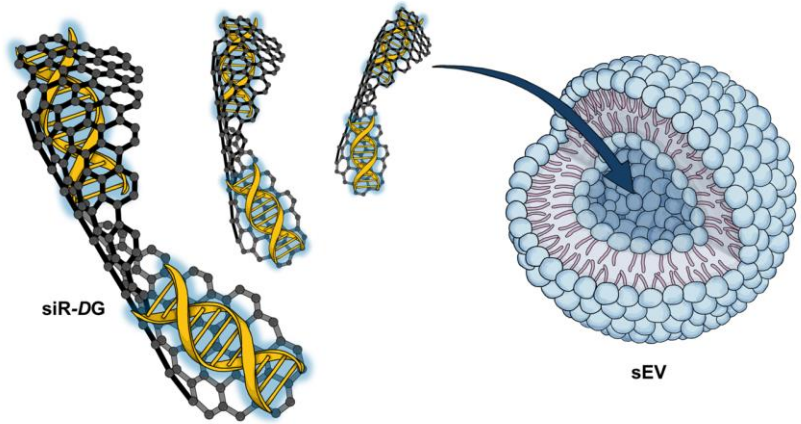
Inefficient cargo loading and compromised sEV integrity demand strategic improvements

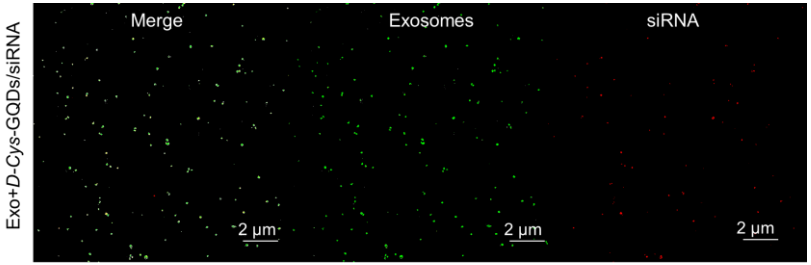
*Fu S, **Wang Y**, Xia X, Zheng JC. NanoImpact. **2020***

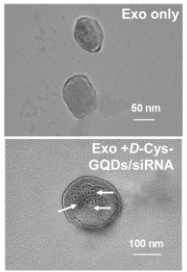
sEV Gene Loading with Chiral Graphene Quantum Dots (GQDs)

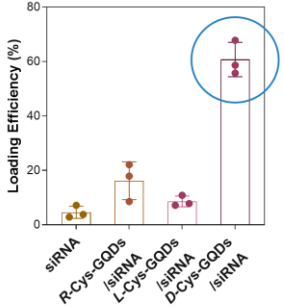
- **Experimental Approach**

Highly efficient siRNA loading







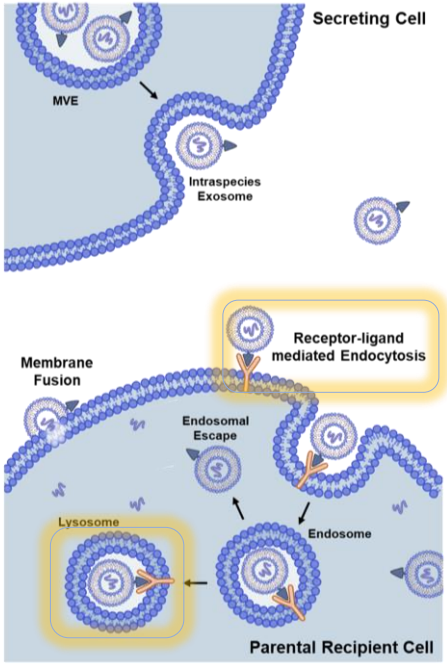


60%

siRNA Loading via Chiral GQDs

Zhang Y., et. al., ***Wang Y.*** *ACS Nano.* ***2023***

CHALLENGE Targeting and intracellular trafficking



*"The **homing effect of sEVs** refers to the ability of sEVs to be specifically transported **back to their cell of origin** or to certain target cells."*

*- Wang et al. JCR. **2024***

*"Intraspecies sEVs preferentially undergo uptake by their parental recipient cells through **receptor−ligand-mediated endocytosis**."*

*- Kim G, et. al., **Wang Y.** ACS Applied Bio Materials. **2024***

*"Only a fraction of the EVs that were endocytosed by cells **exposed their cargo to the cytoplasm**, varying from 10% to **24.5%**."*

*- Joshi et al. ACS nano. **2020***

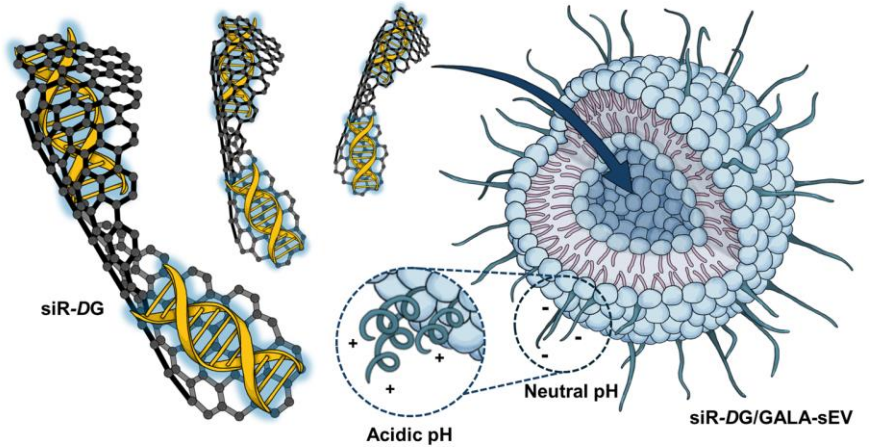
*Kim G, et. al., **Wang Y.** ACS Applied Bio Materials. **2024***

Lysosomal escape of sEVs is key to enabling targeted homing effect and precise intracellular delivery

sEV-Based Gene Delivery System with Maximized Homing Effect

➢ Experimental Approach

Highly efficient siRNA loading and lysosome-escaping functionalization while preserving inherent membrane bioactivity

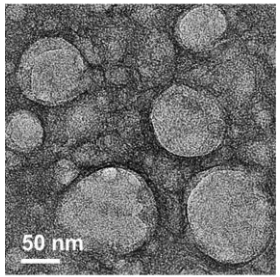


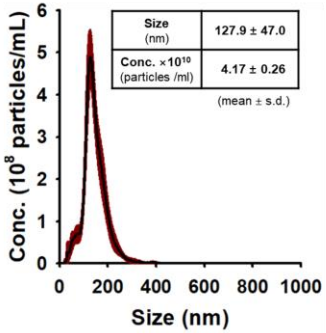
siRNA Loading via Chiral GQDs + sEV Homing Effect + pH-Responsive Lysosomal Escape

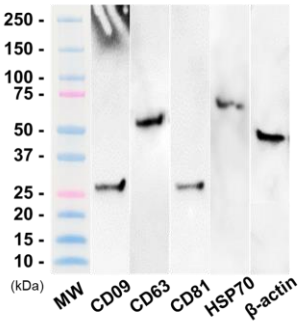
Zhang Y., et. al., ***Wang Y.*** *ACS Nano.* ***2023****; Kim G, et. al.,* ***Wang Y.*** *ACS Appl. Bio Mater.* ***2024****; Kim G, et. al.,* ***Wang Y****. ACS Biomaterials Science & Engineering.* ***2025***

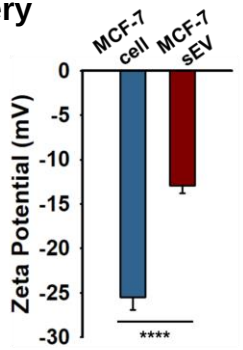
Constituents for multifunctional *DG*/GALA-sEVs

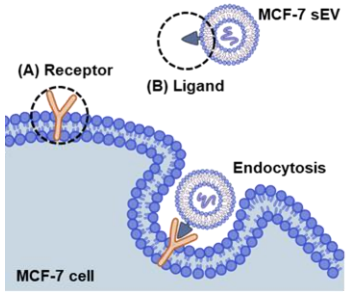
- **Human Breast Cancer (MCF-7) Derived sEVs for Breast Cancer Targeted Delivery**







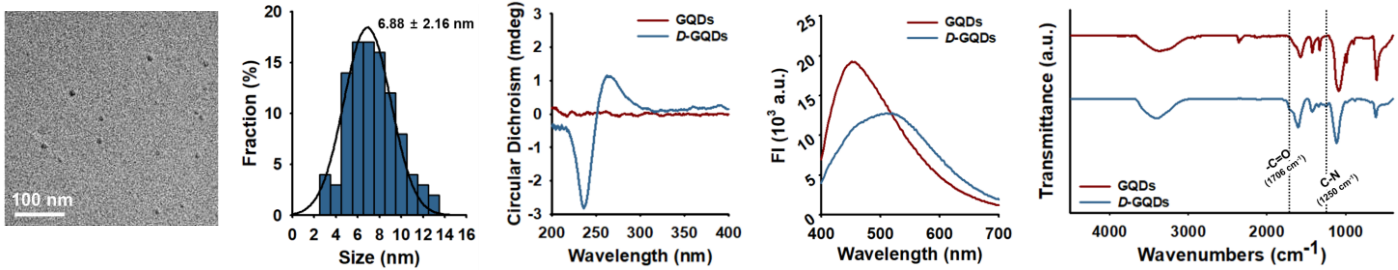




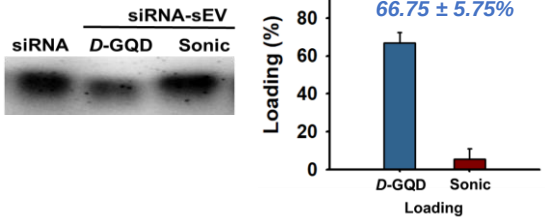
(A) Receptor	EGFR		αvβ3 integrin	TGFBR
(B) Ligand	Nucleolin	Neuropilin-1	Fibrinogen	TGF-β
Accession Number	P19338	O14786	P02671 P02675	P01137 P61812

Constituents for multifunctional *D*G/GALA-sEVs

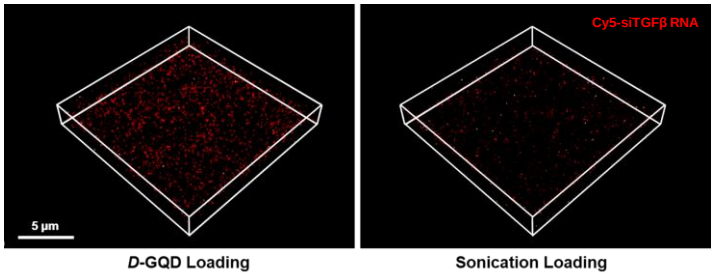
- **Chiral Graphene Quantum Dots Functionalized with *D*-Cysteine (*D*-GQDs)**



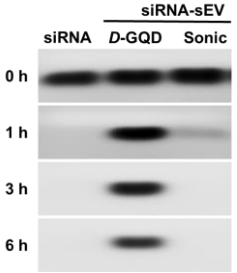
- **siRNA (siTGFβ) Loading Efficiency**



- **siR-*D*G Loaded sEVs**

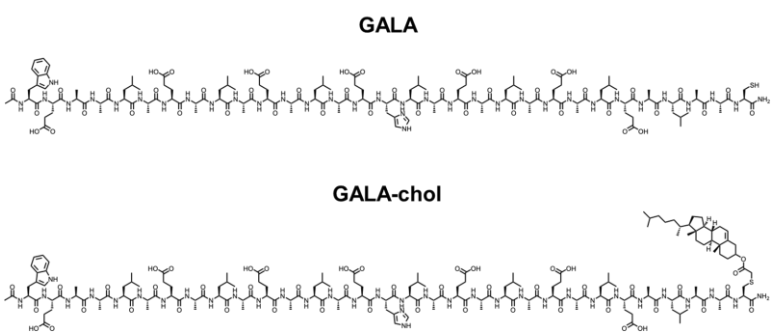


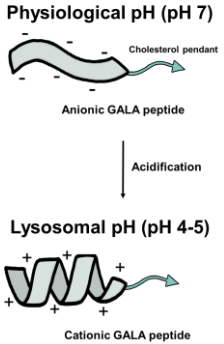
- **siRNA Stability**

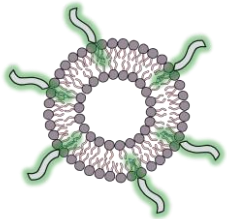


Constituents for multifunctional *DG*/GALA-sEVs

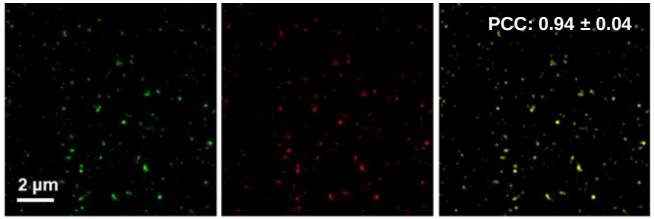
- **pH-Responsive Peptides for Charge-Changeable sEVs in Lysosomal Condition**



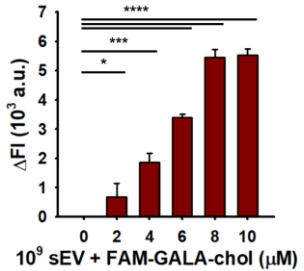




GALA-sEV

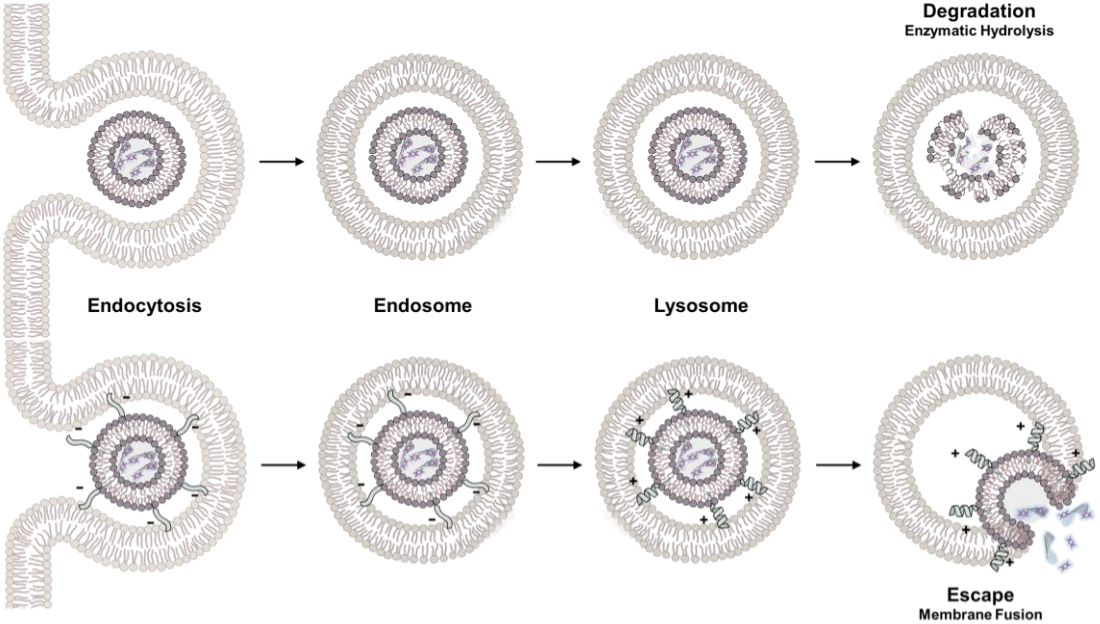


FAM-GALA-chol | sEV membrane | Merged



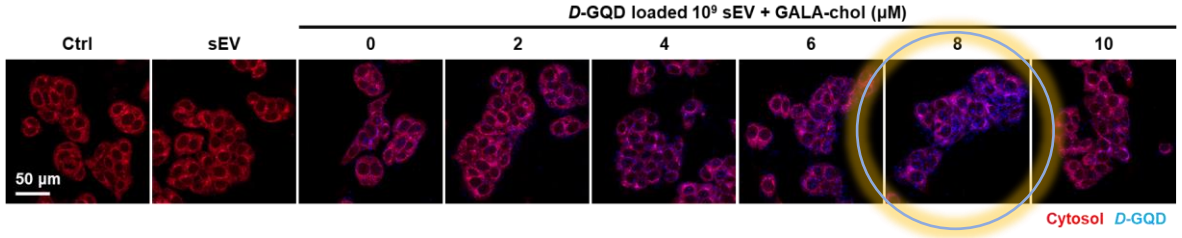
siR-*DG*/GALA-sEVs for Enhanced Cytoplasmic Delivery of siRNA

- **Mechanism of Lysosomal Escape and Subsequent Intracellular siRNA Release**

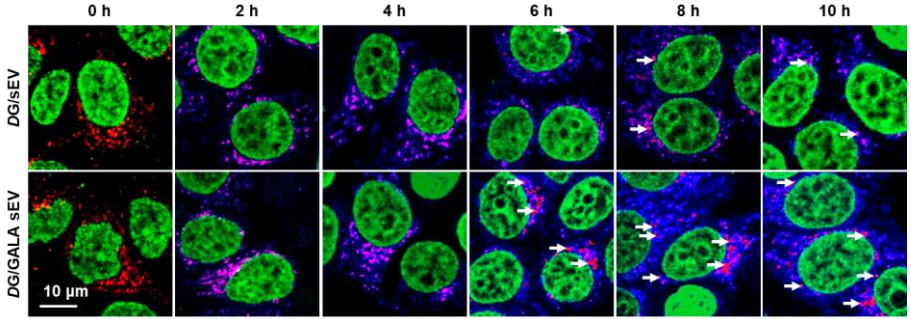


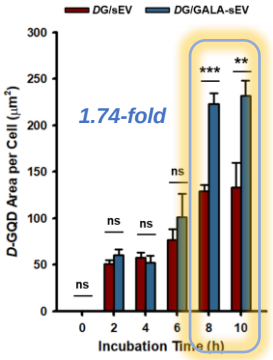
Enhanced Lysosomal Escape of siR-*DG*/GALA-sEVs

➢ **Cytoplasmic Cargo Delivery**

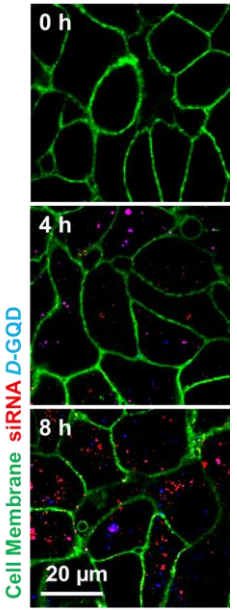


➢ **Time-Dependent Lysosomal Escape**



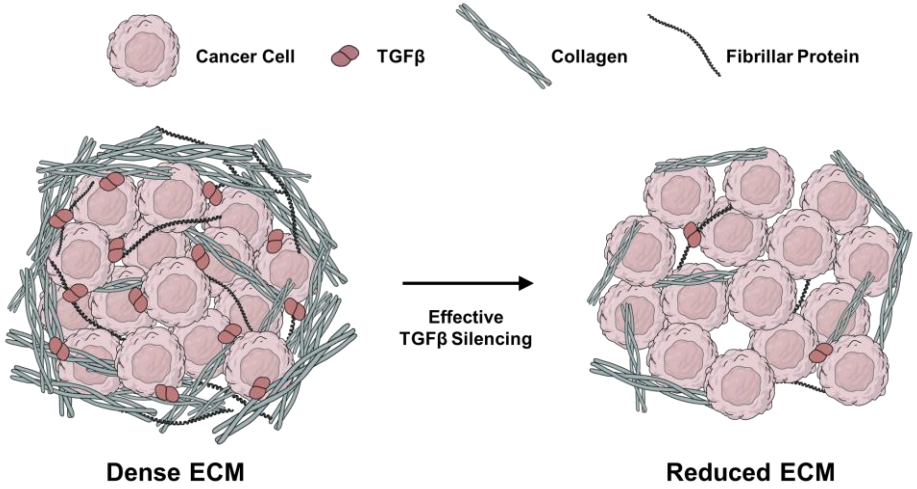


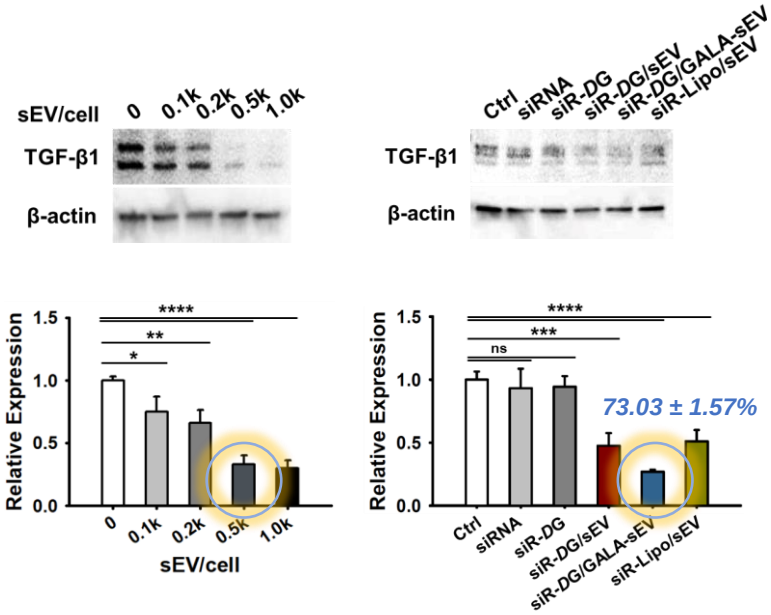
➢ **siRNA Release From *D*-GQDs**



Successful siRNA Transfection via siR-*DG*/GALA-sEVs

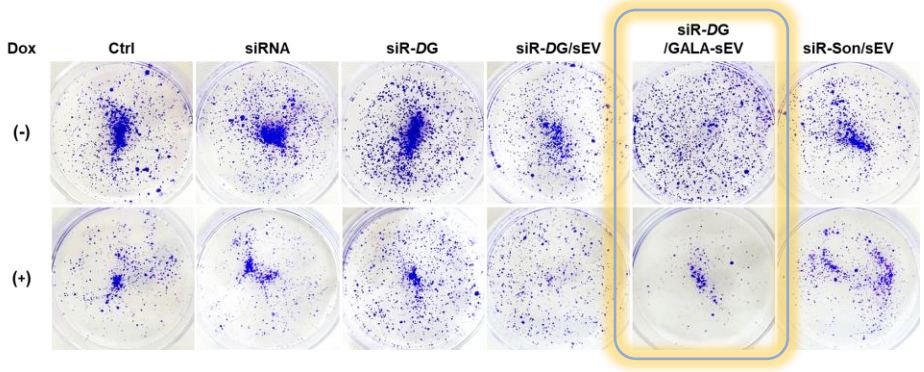
- **TGF-β Silencing Effect**

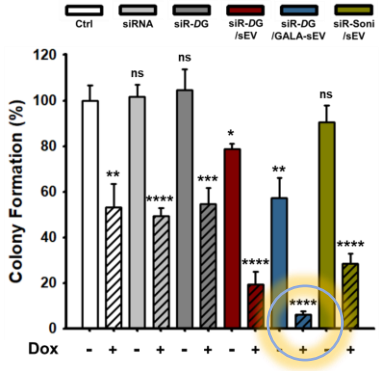




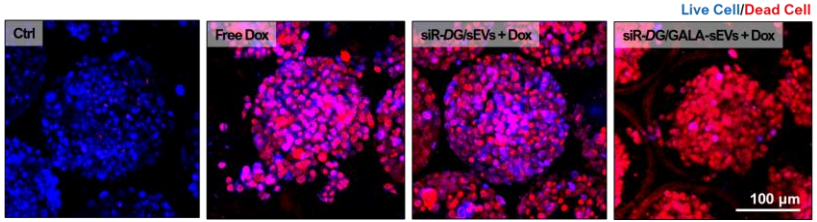
Doxorubicin Combination Treatment with TGF-β Silencing

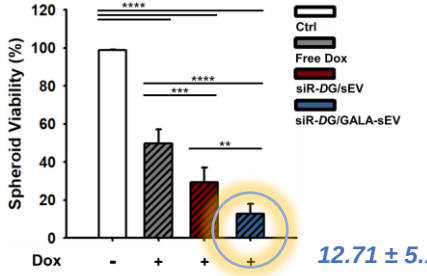
- **Enhanced Dox Sensitivity and Synergistic Therapeutic Effect**





5.97 ± 1.50%





12.71 ± 5.15%

Summary of siR-*DG*/GALA-sEVs

- **67%** of siRNA Loading

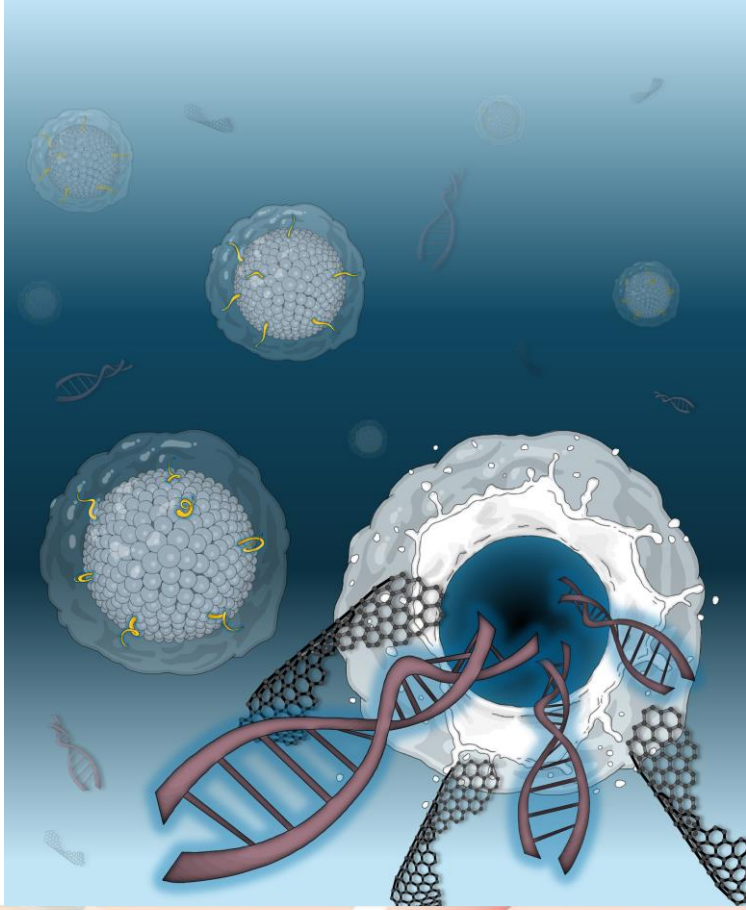
- Inherent **Targeting** Effect (Homing Effect)

- **1.74-fold** Enhanced Cytosolic Cargo Release

- ***73%*** *of TGF-β Knockdown & Enhanced Drug Sensitivity*

By integrating **passive and non-distruptive strategies**, we preserved the **inherent biological advantages** of sEVs.

This approach allows for the full harnessing of their synergistic effects, enhancing targeted gene delivery in **breast cancer to overcome chemoresistance**.



Acknowledgement

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Dr. Sihan Yu

Dr. Bowen Fan

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NDnano Center for Nano Science and Technology
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NIH National Institutes of Health
Harper Cancer Research Institute