中国药科大学

8th International Symposium on
Phospholipids in Pharmaceutical Research

Researches and development of multifunctional lipopeptides as excipients

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NMPA Key Laboratory for Research and Evaluation of
Pharmaceutical Preparations and Excipient

China Pharmaceutical University

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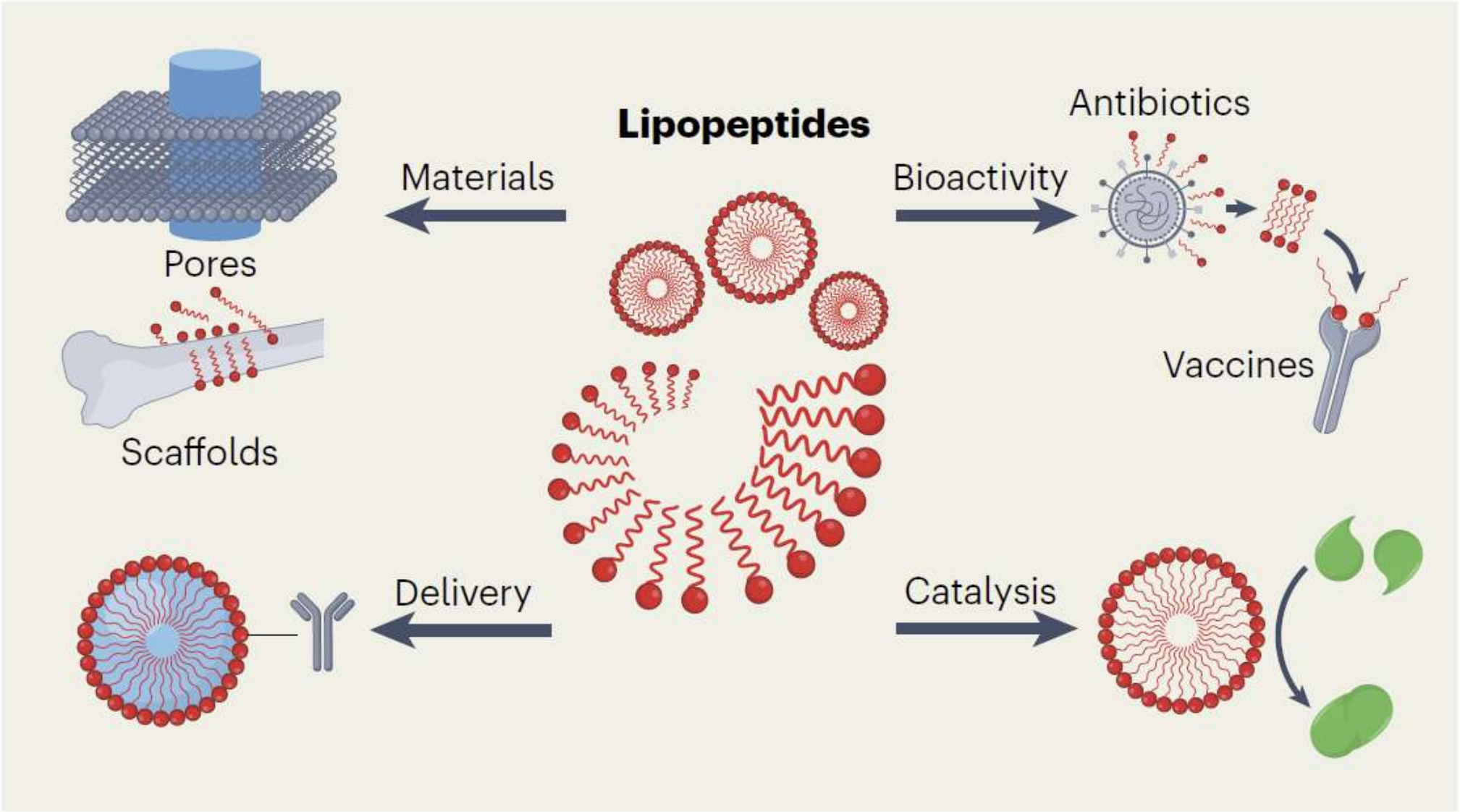
PART ONE

Introduction

Lipopeptides



Nat Rev Chem 2023, 7. 710-731

Lipopeptides developed in Our lab

- 1. Mimicking the viral membrane peptide.
- 1. Dendritic Arginine and/or Lysine rich lipopeptide: stronger enhancing capability than linear membrane-penetrating peptide.
- 2. Cationic lipopeptide for gene delivery:

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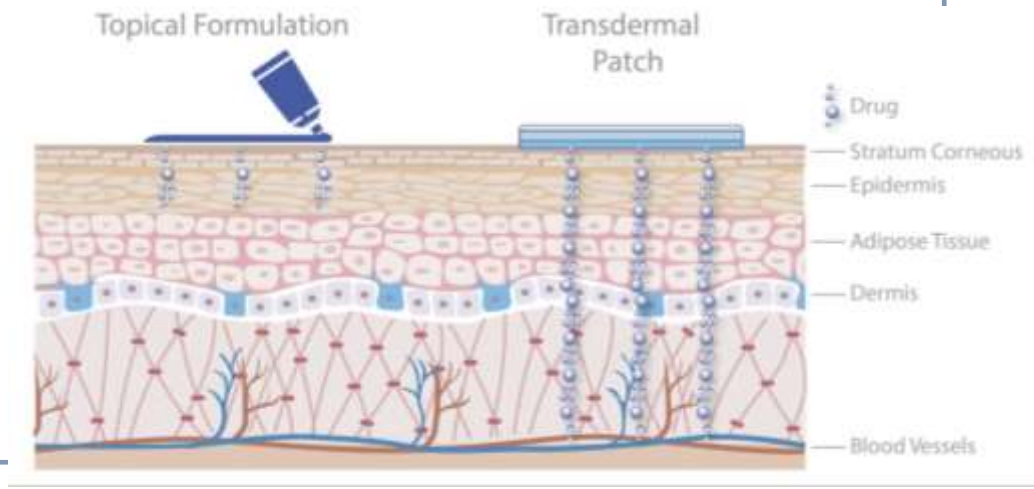
PART TWO

Lipopeptide based transdermal drug delivery system (TDDS)

mimicking the pivotal amino acid sequence in viral protein transduction domains, and virion-like nanostructure by camouflaging viral capsids

Transdermal drug delivery system

Transdermal drug delivery systems are convenient dosage forms both for systemic or local therapy, especially for pain control, geriatric disorders, pediatric disorders, etc.



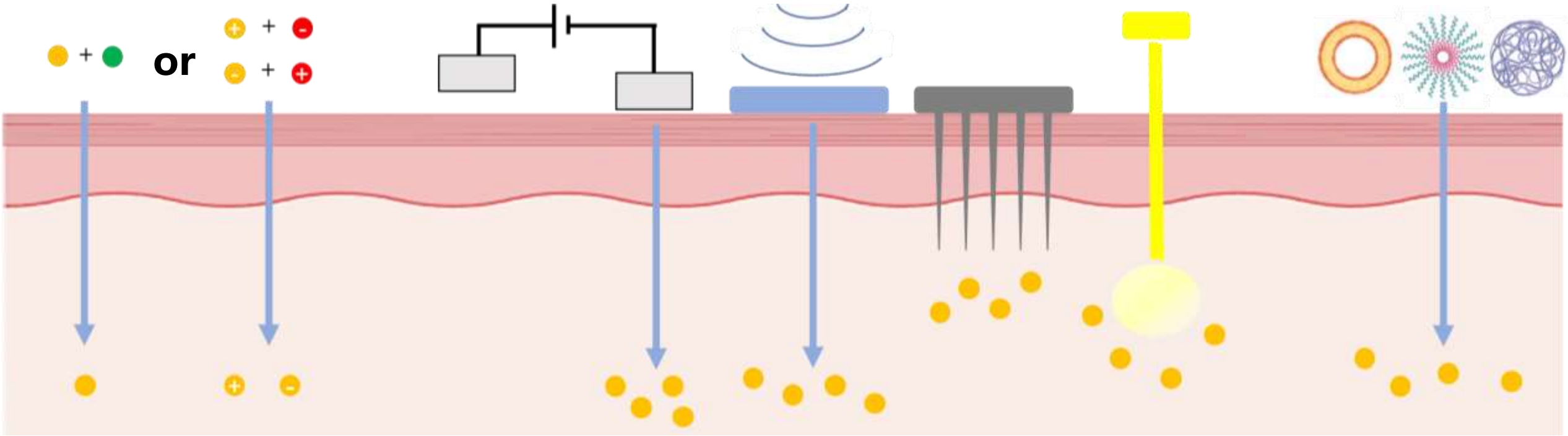
Transdermal drug delivery system

- **Topical drug delivery system** → **Local skin**
- **Transdermal drug delivery system** → **Systemic blood circulation**

Percutaneous absorption of most chemicals is always difficult due to barrier property of skin. Enhancing percutaneous absorption techniques are necessary for most drugs.

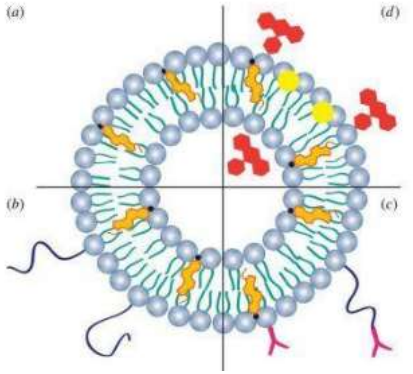
Drug Percutenous Absortion Enhancing Technique

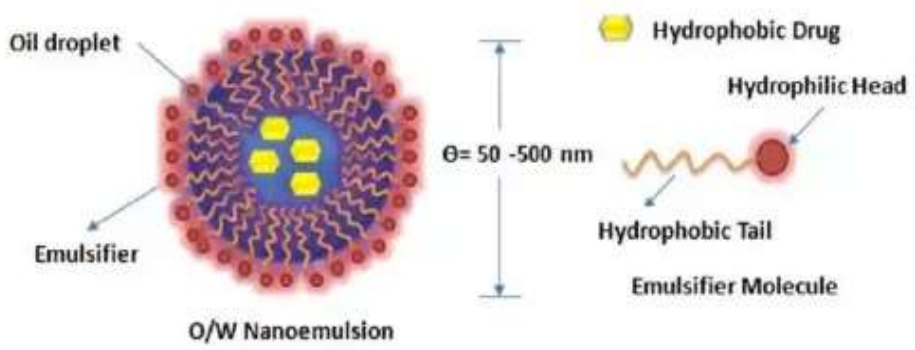
Chemical method **Physical method** **Pharmaceutical method**

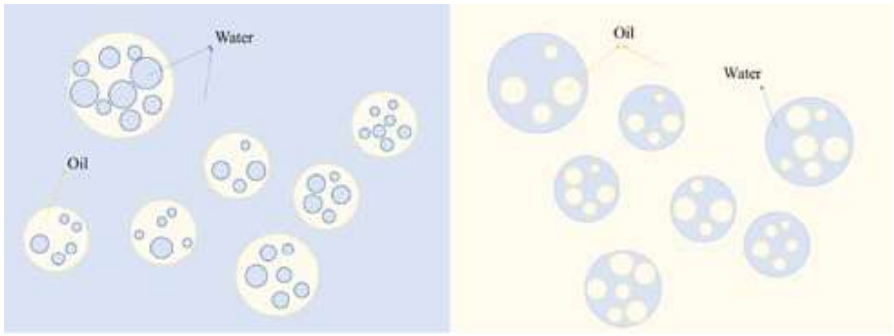


Pharmaceutical Method

- **Liposomes, Niosomes, Ethosomes, Transferosomes (Functionized liposomes)**
- **Emulsion, Microemulsion, Nanoemulsion**
- **Nanospheres, Nanoparticles, Nanotubes, Nanocrystal**

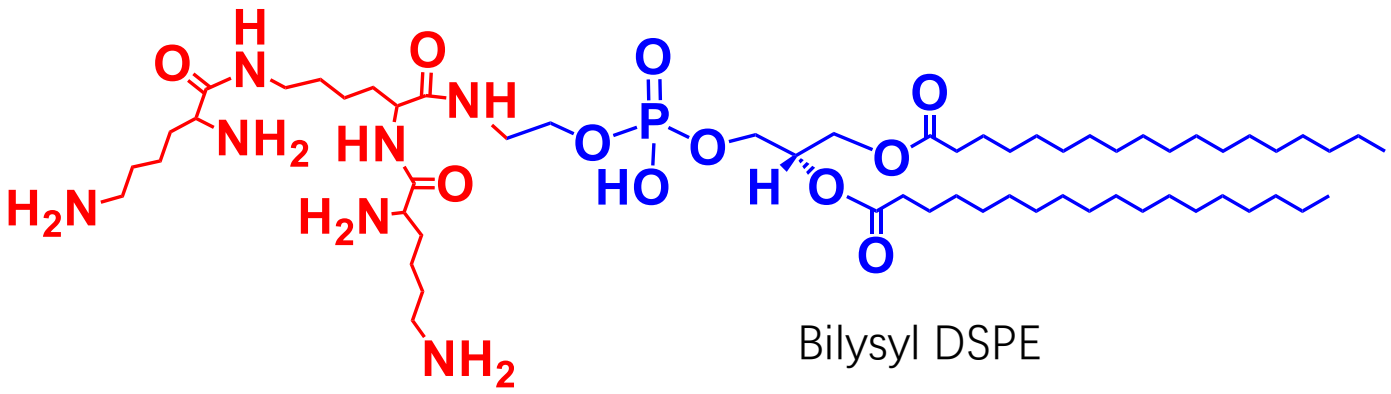




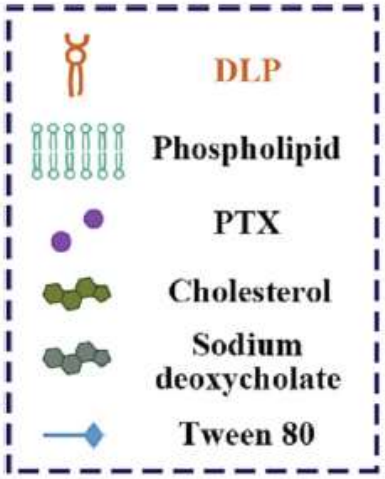


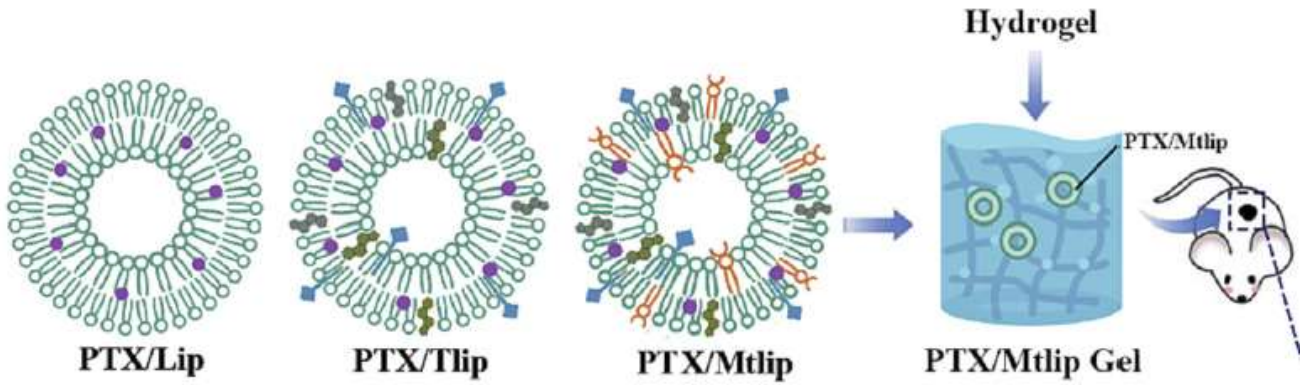
Transferosomes

1. Dendritic lipopeptide (DLP) Based Transferosomes



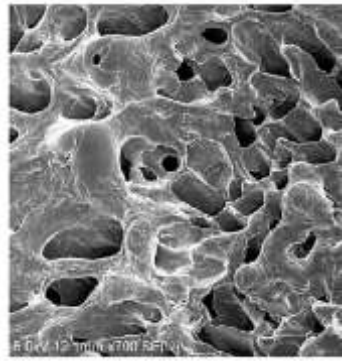
Bilysyl DSPE





Temperature-sensitive gel

SEM



We aimed to melanoma chemotherapy through TDDS.

Journal of Controlled Release 2022,351,245–254

Mtlip：High Skin Tissue Permeability

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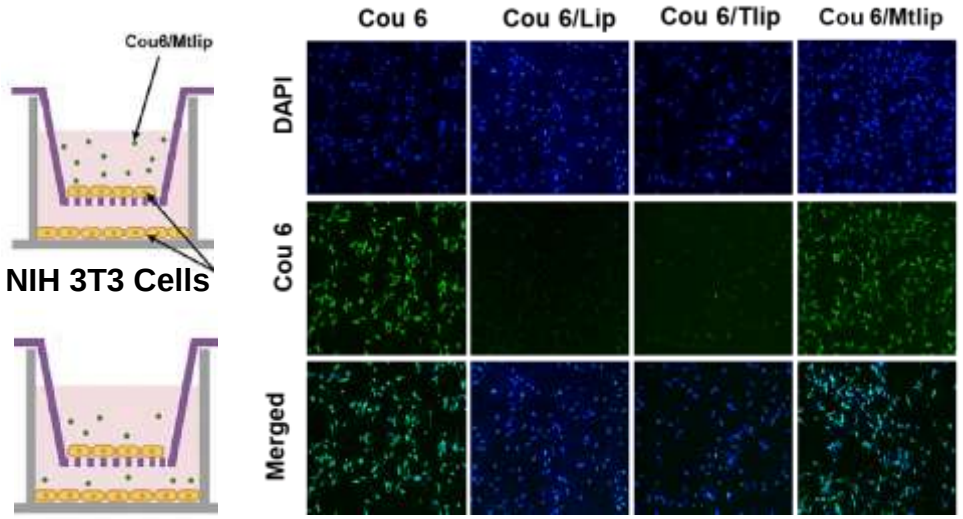
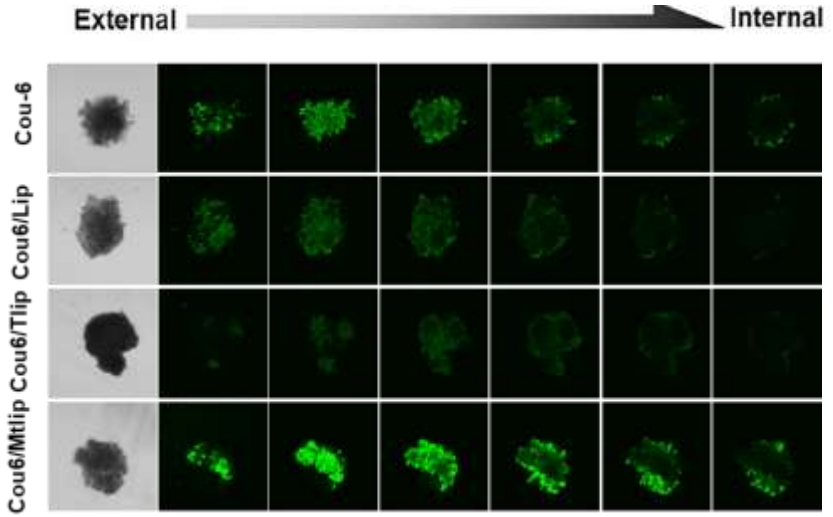
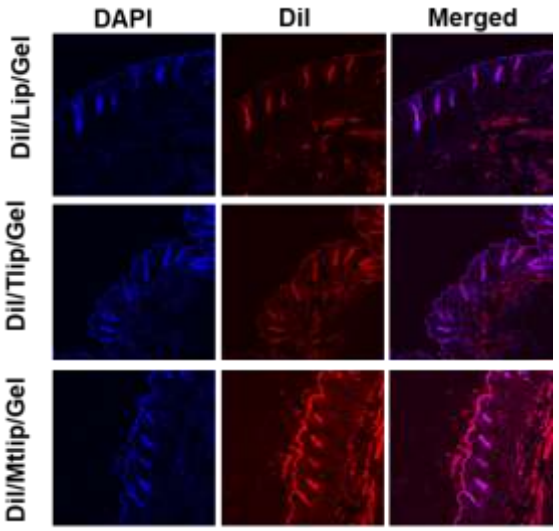
Multilayer cell permeability



Tumor sphere permeability



Transdermal performance



Highly permeable in multi-layered intercellular cells, tumors and skin tissues

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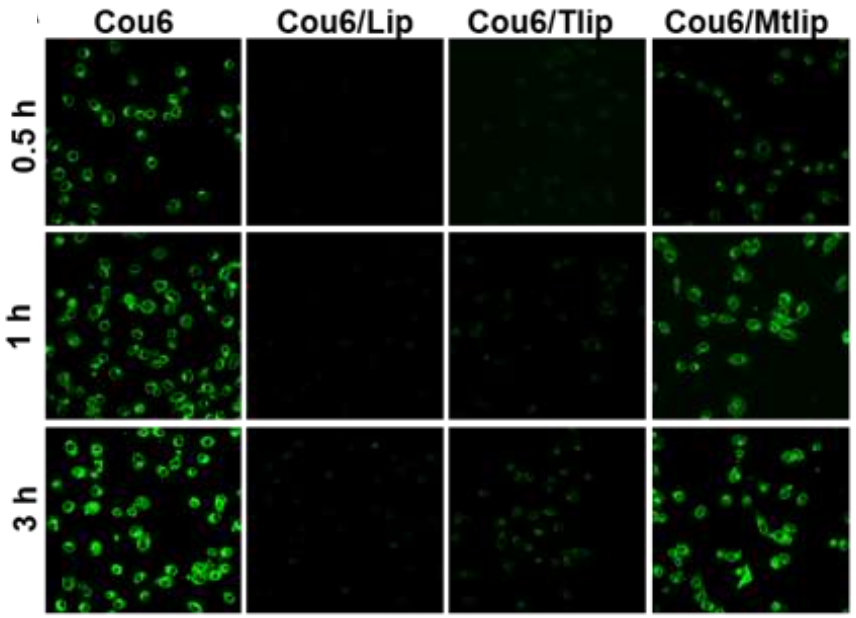
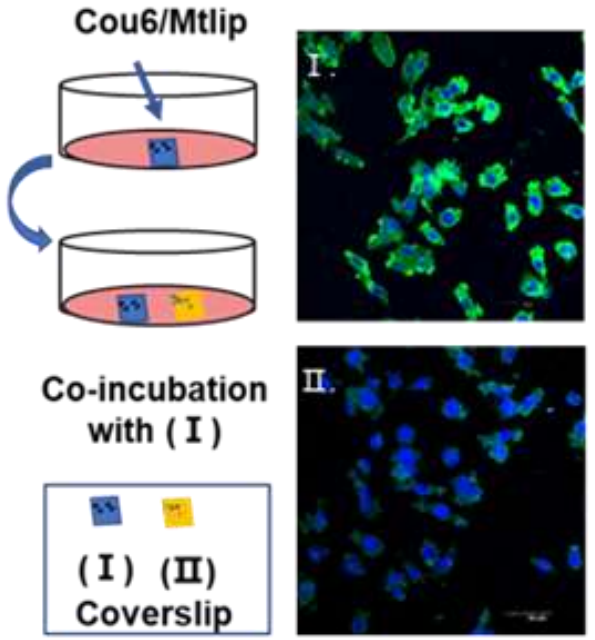
Biological Properties of MTlip

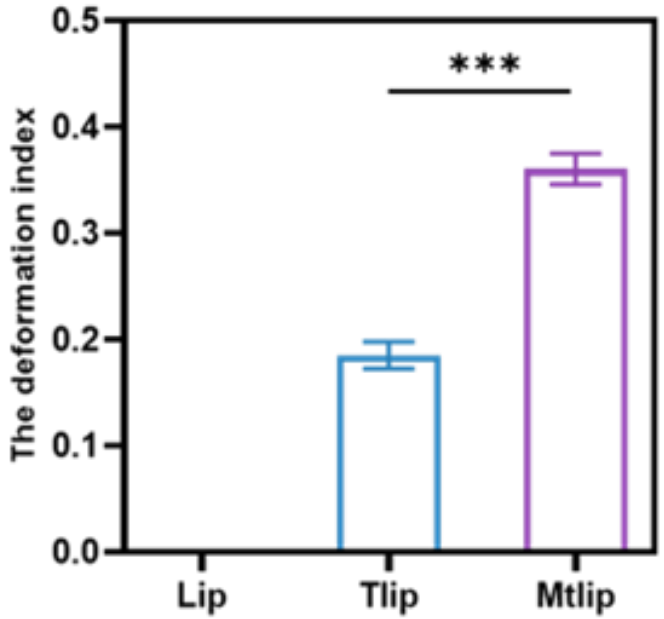
Rapid uptake



Endocytosis and exocytosis effect



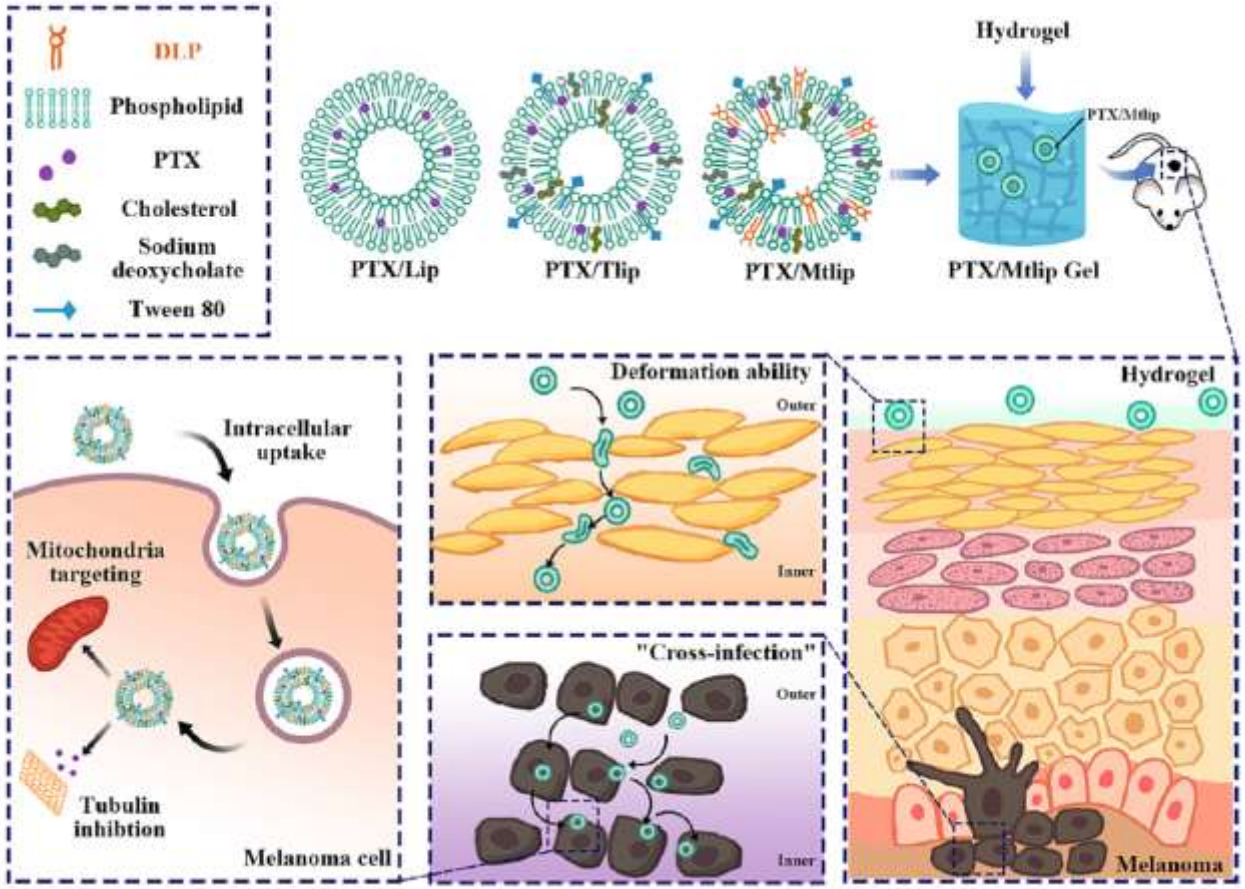
Deformation index



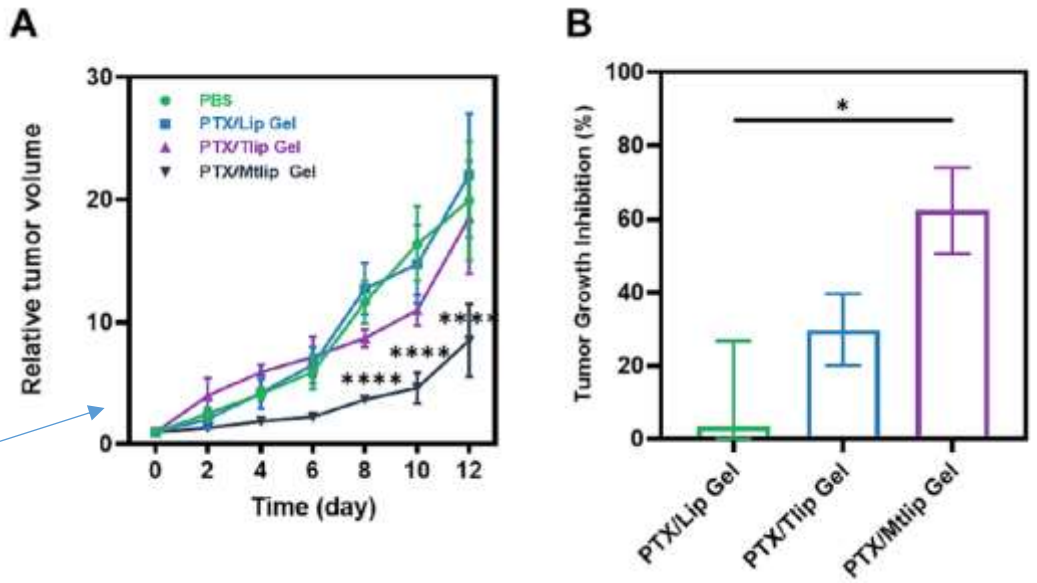
✓ **Rapid uptake, endocytosis, and deformability are the reasons for the increased permeability**

Journal of Controlled Release 2022,351,245–254

Mtlip Hydrogel:Transdermal Enhancer & Organelle Targeting



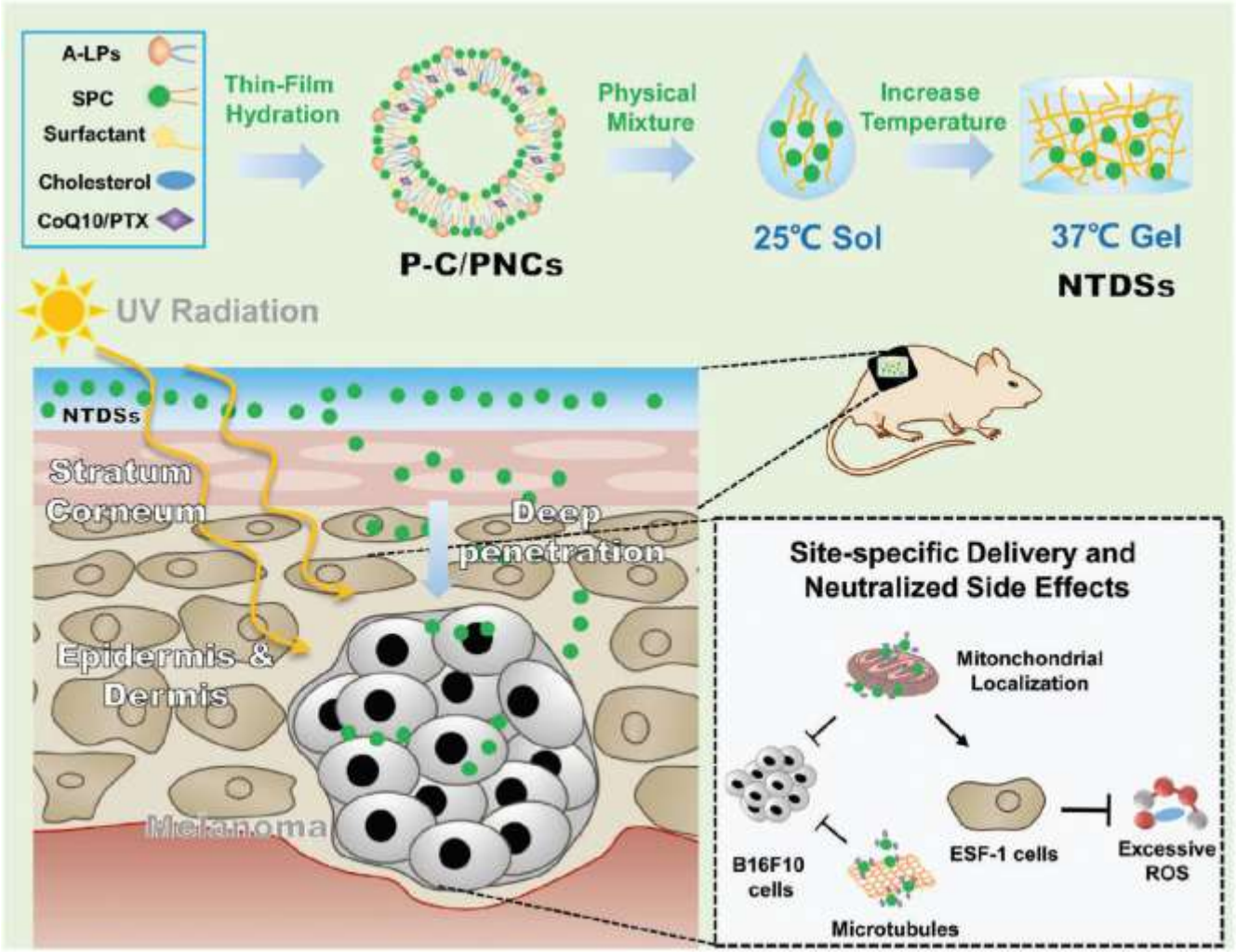
- ✓ Break through the stratum corneum barrier
- ✓ Break through multi-level cell barriers
- ✓ Efficient cellular uptake
- ✓ Mitochondrial localization to clear excess ROS



Antitumor activity. PTX/Lip Gel, PTX/Tlip Gel and PTX/Mtlip Gel were administered to B16F10 tumor-bearing mice via applying hydrogel every day at a PTX dose of 5 mg/kg, PBS was used as a negative control. (A) Relative tumor volume, ****P < 0.0001, statistically significant versus PBS group (two-way ANOVA test). (B) Tumor weight. *P < 0.05 (two-way ANOVA test).

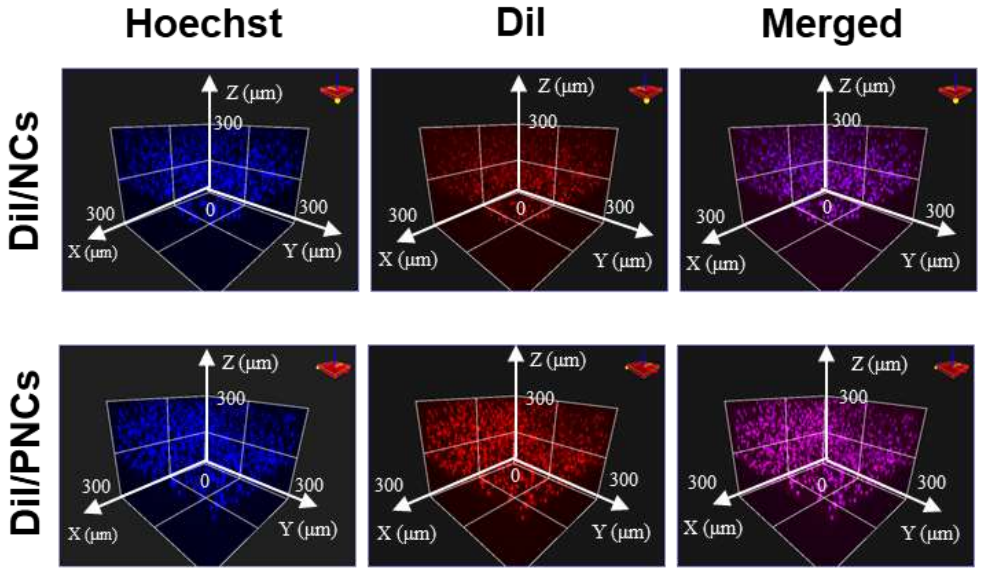
2. **Arginine rich lipopeptides (A-LPs) based Transferosomes**

- **An arginine-rich lipopeptides (A-LPs)** were designed for constructing multifunctional nanocarriers, which attributed to drug encapsulation, deep tissue penetration, high cellular uptake, and organelle targeting.
- **PTX and CoQ10-loaded PNCs (P/C-PNCs):** i) Incorporating antioxidant therapy (CoQ10) into antitumor treatment; ii) Engineering non-invasive penetrating delivery systems, i.e., A-LPs based Transferosomes; iii) Constructing a promising dosing strategy realizes melanoma location for non-invasive transdermal delivery.

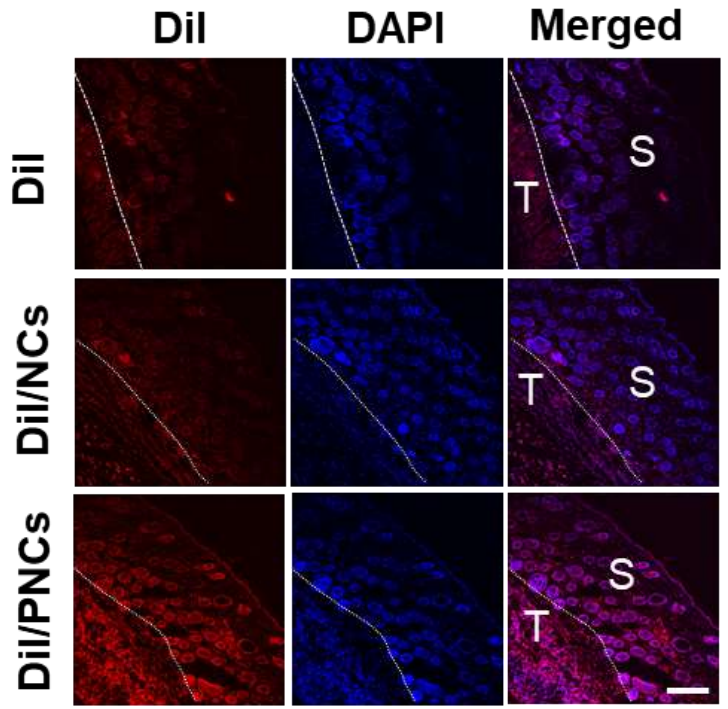


Transdermal Enhancer & Organelle Targeting

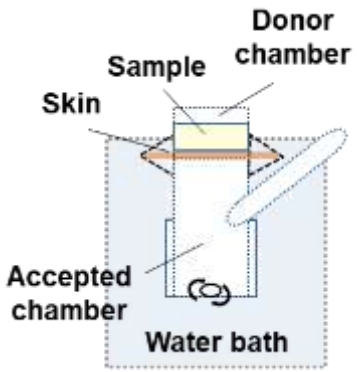
Tissue permeability simulation

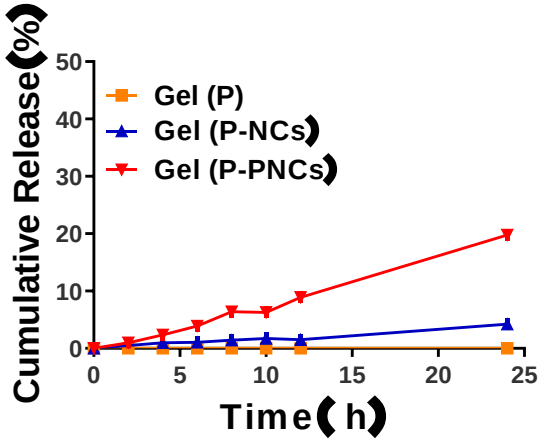


Skin tissue permeability



Transdermal performance of Franz diffusion cells





✓ **Excellent permeability of skin tissues and lesion tissues**

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Transdermal Enhancer & Organelle Targeting

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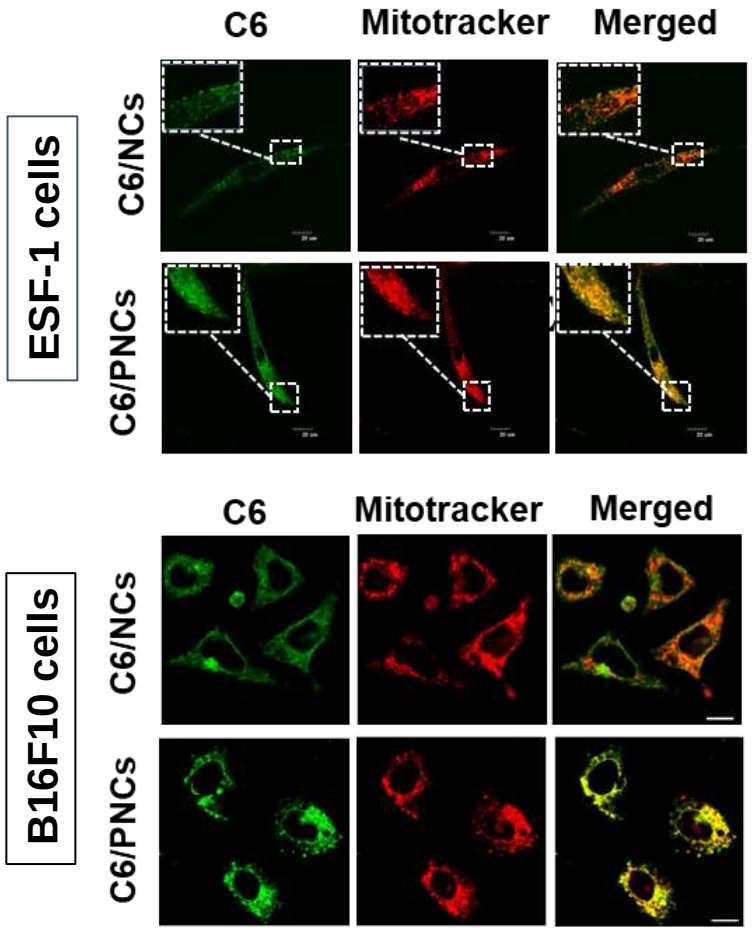
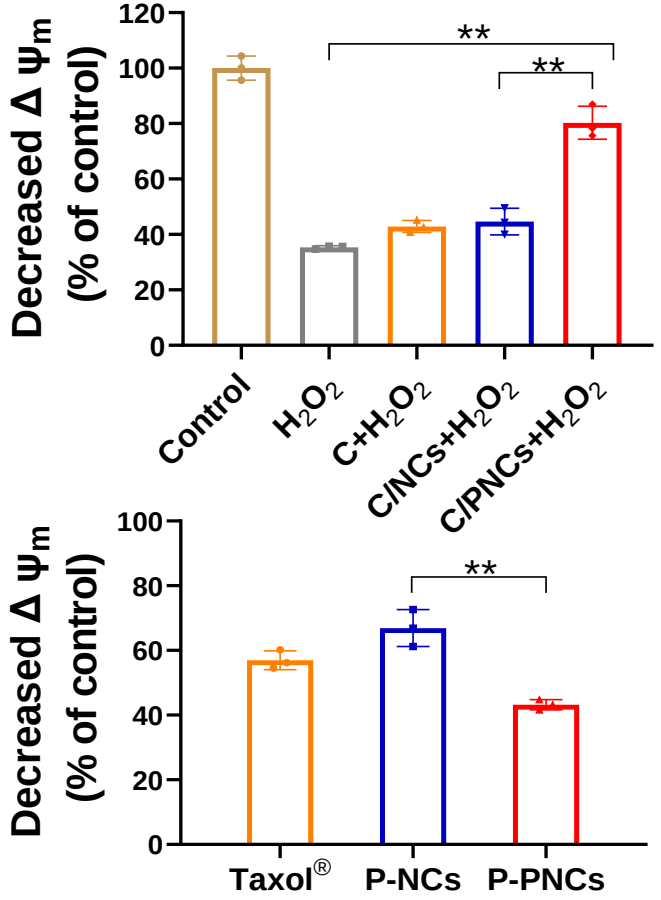
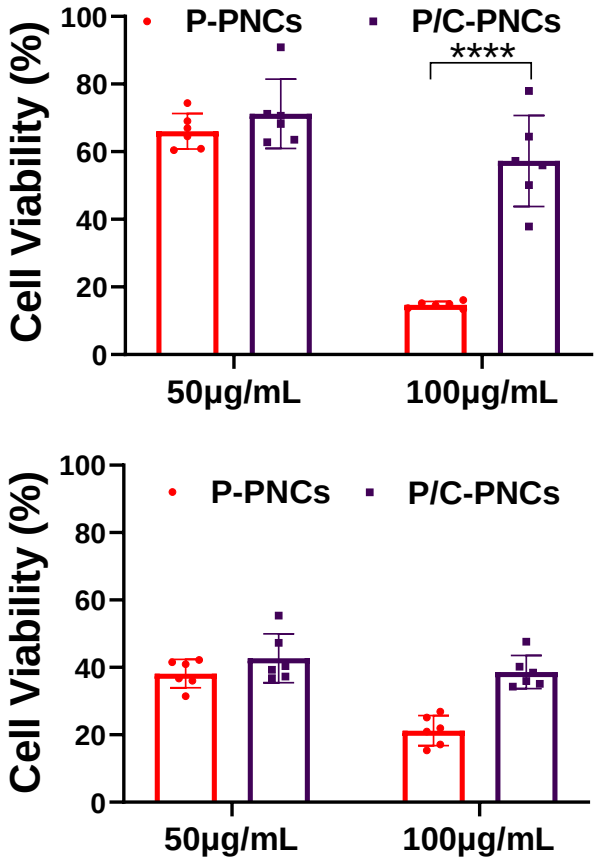
Mitochondrial localization



Mitochondrial membrane potential



Cytotoxicity



Mitochondrial localization leads to attenuation and efficiency

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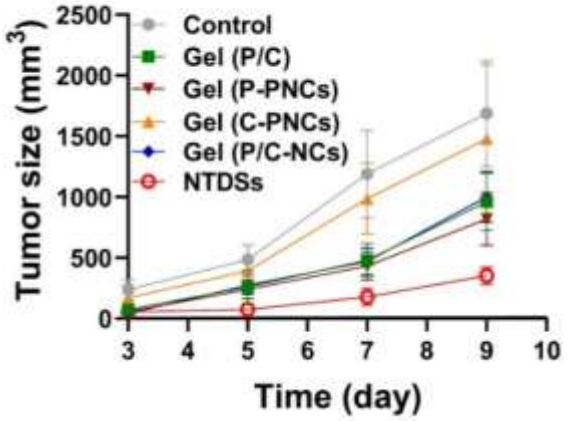
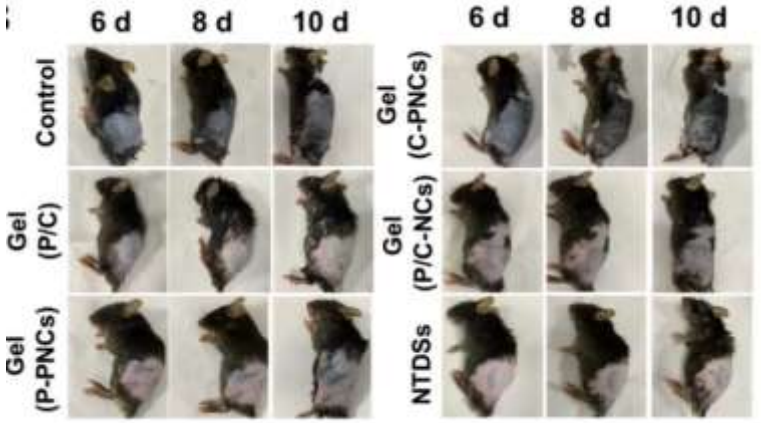
Transdermal Enhancer & Organelle Targeting

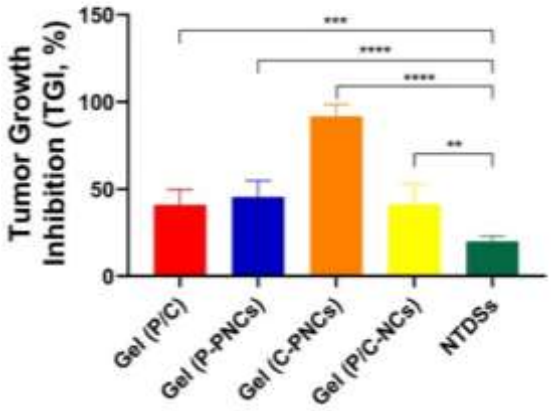
Change of tumor volume



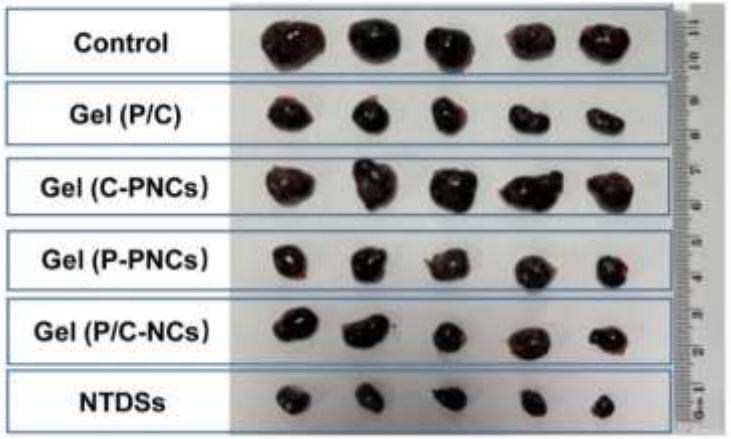
Change in tumor spread area



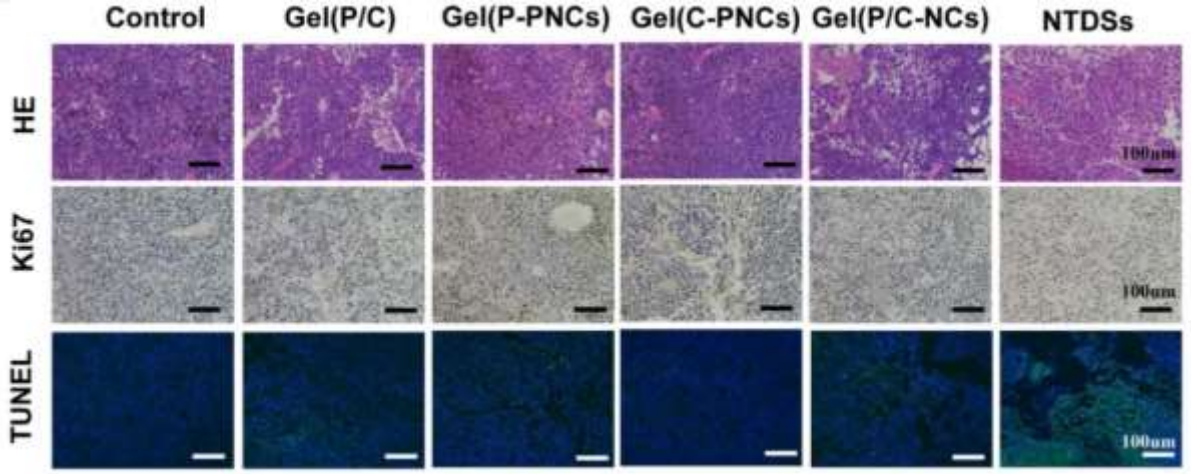
Tumor suppression rate



Tumor morphology after treatment



Immunohistochemistry



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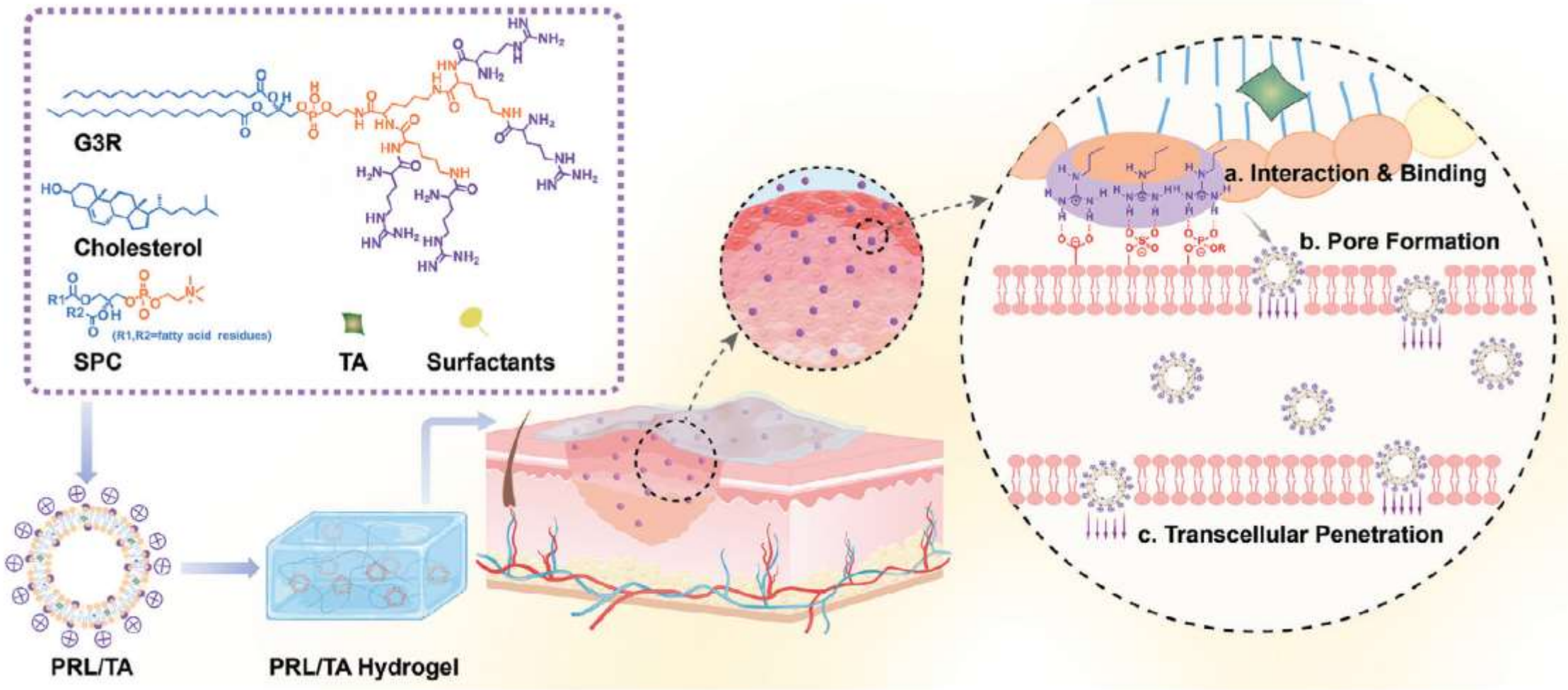
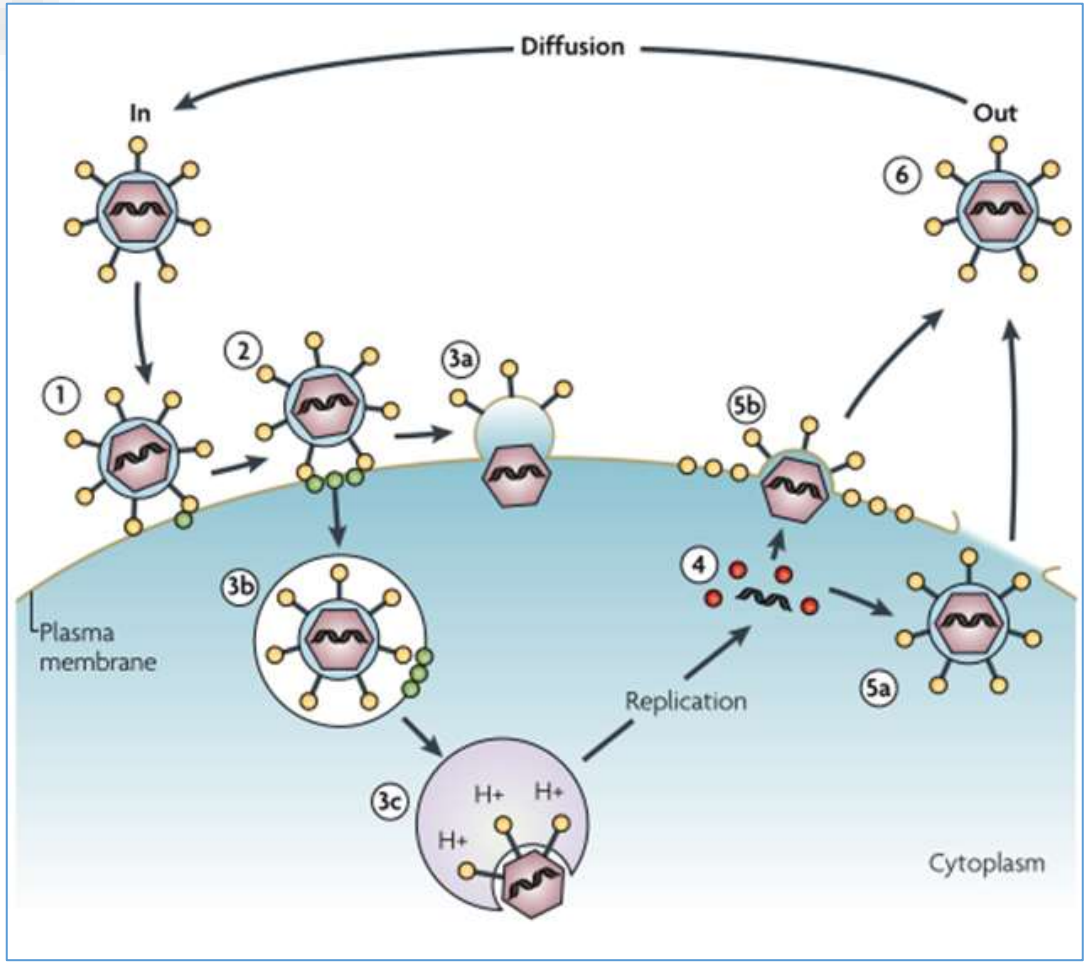
3. Third-generation arginine-containing dendritic lipopeptide (G3R) based Transferosomes

- A tissue and cell dual-penetrating arginine (R)-rich transferosomes (PRL) of triamcinolone acetonide (TA) (PRL/TA) for noninvasive hypertrophic scar treatment.
- TDDS: i) PRLs were incorporated into the hydrogel matrix, which could continuously release and increase transdermal effect via transdermal hydration gradients; ii) The released PRLs could overcome corneum barriers to punch on hypertrophic scar fibroblasts membranes with the help of surfactants Tween 80 and sodium deoxycholate, as did as natural viruses; iii) The deeply delivered TA would promote hypertrophic scar fibroblast apoptosis and collagen fiber remodeling within the thickened dermis and epidermis; iv) Considering the key excipients of PRL/TA, the preparation procedure of G3R is uncomplicated. Thus, PRL/TA exhibits promising possibilities for clinical implementation.

The mechanism for hypertrophic scar treatment by hydrogels encapsulating PRL/TA



Dual Tissue-cell Permeability Performance



- Natural virus: effective tissue infection, rapid entry into target cells
- Key peptide sequences in the protein transduction domain R/KXXR/K
- Ordered nanostructures

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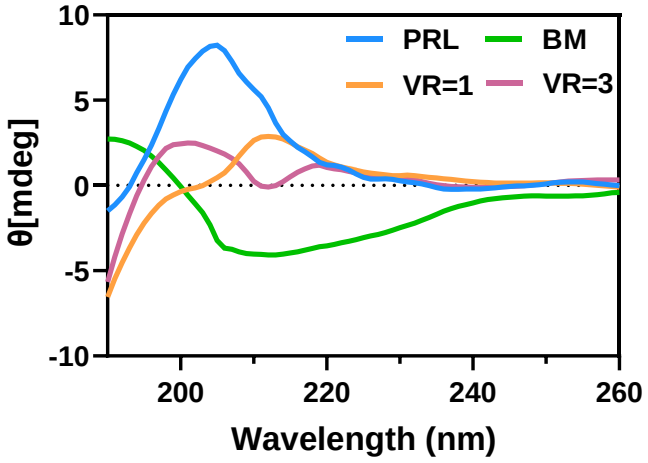
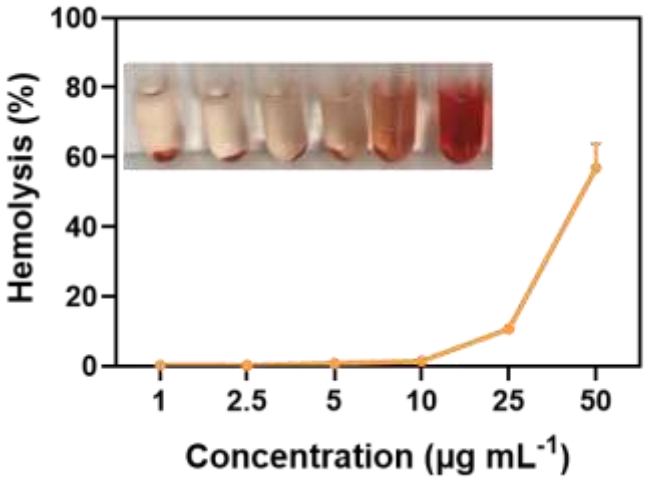
Dual Tissue-cell Permeability Performance

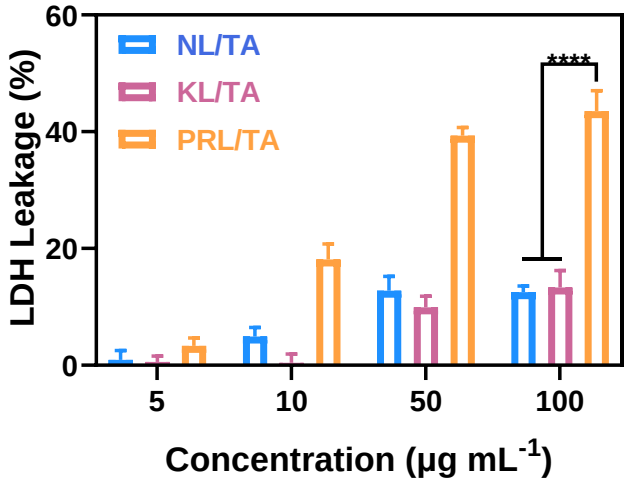
Circular dichroism



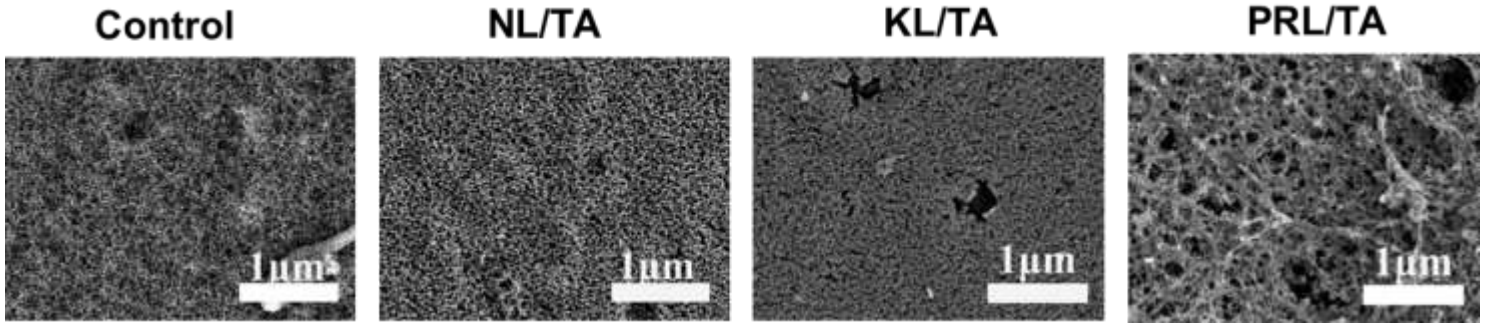
Cell permeabilization



Lactate dehydrogenase release



TEM of Cell membrane



The interaction of cell membranes triggers the permeabilization effect.

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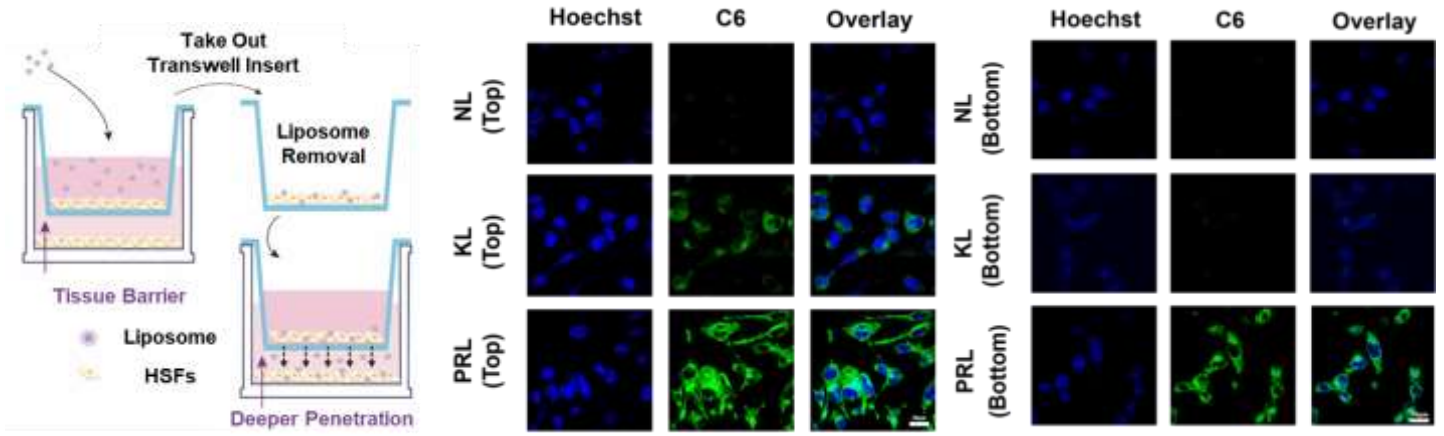
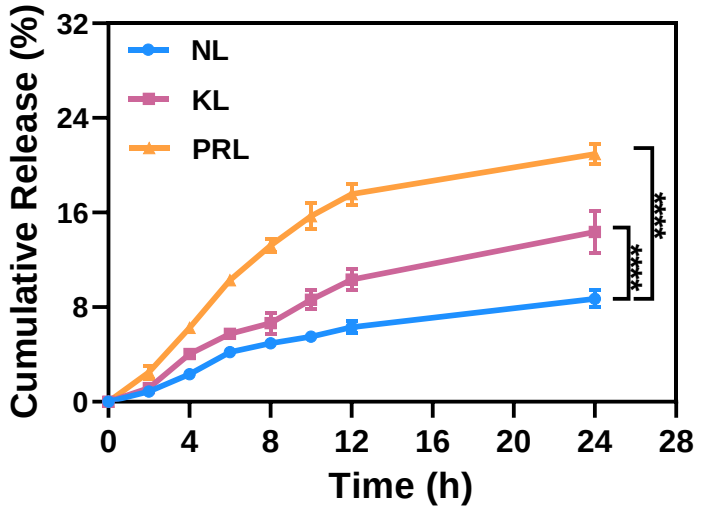
Dual Tissue-cell Permeability Performance

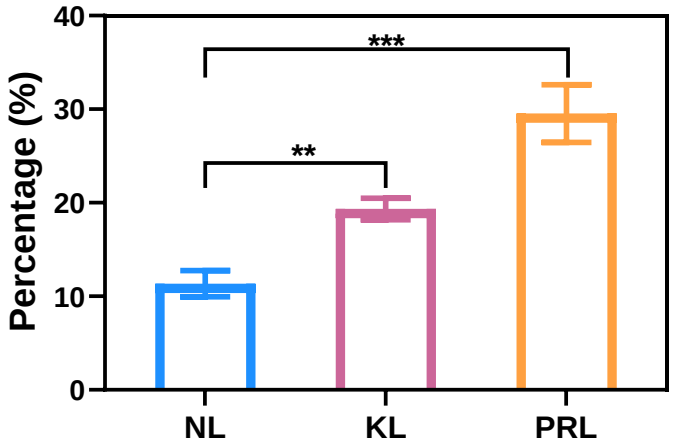
Cell permeability properties



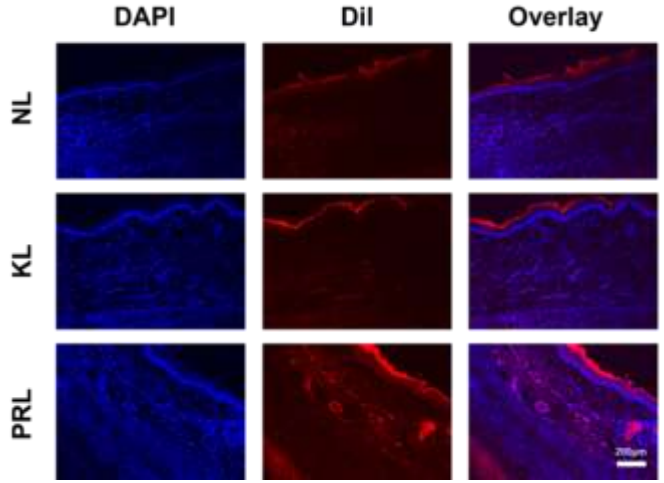
Transdermal performance



Skin tissue retention rate



Skin tissue permeability



Excellent skin tissue and cell-to-cell penetration effect in vitro and vivo

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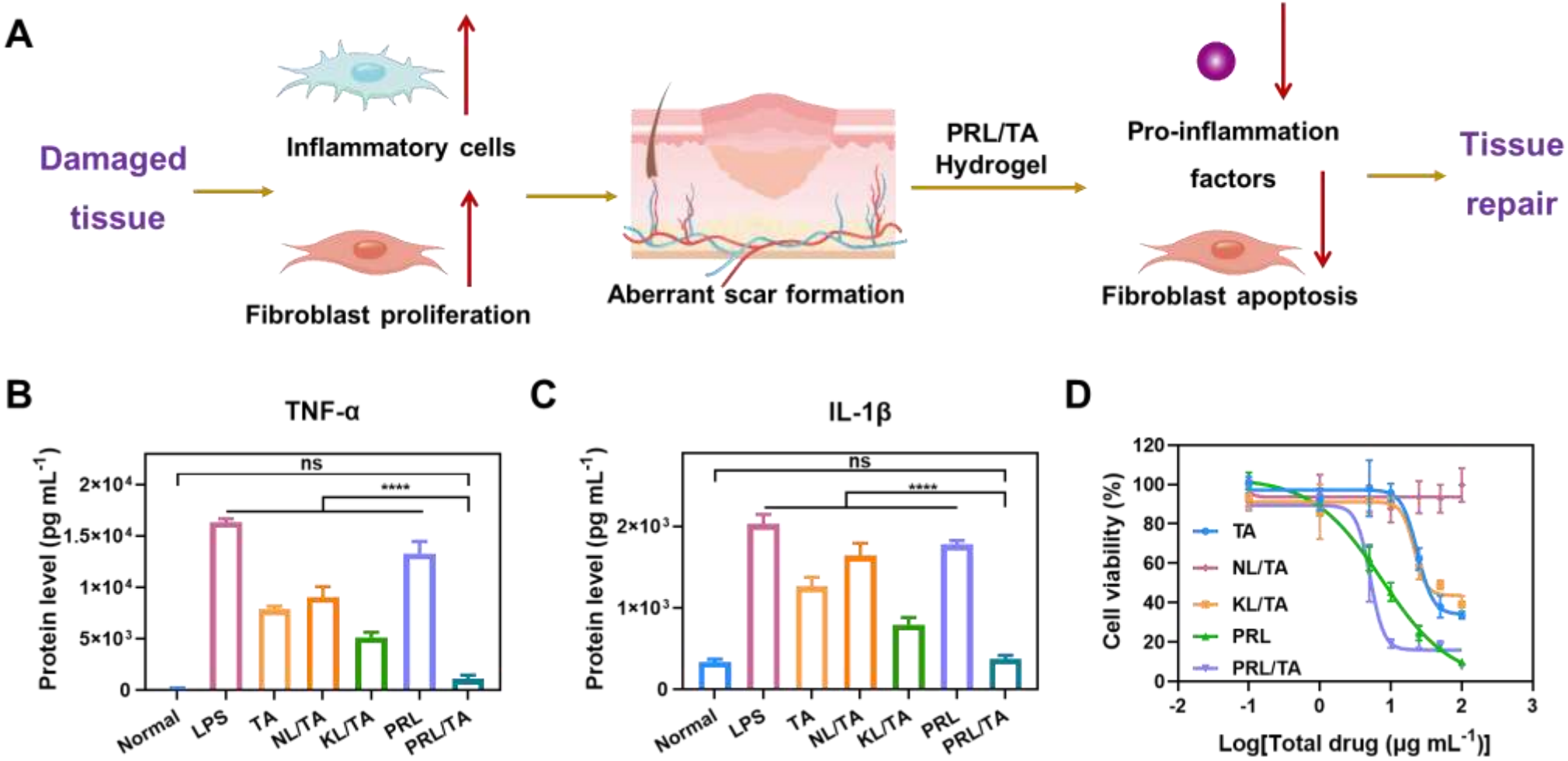
Dual Tissue-cell Permeability Performance



Significant anti-inflammatory and antifibroblast-proliferative effects

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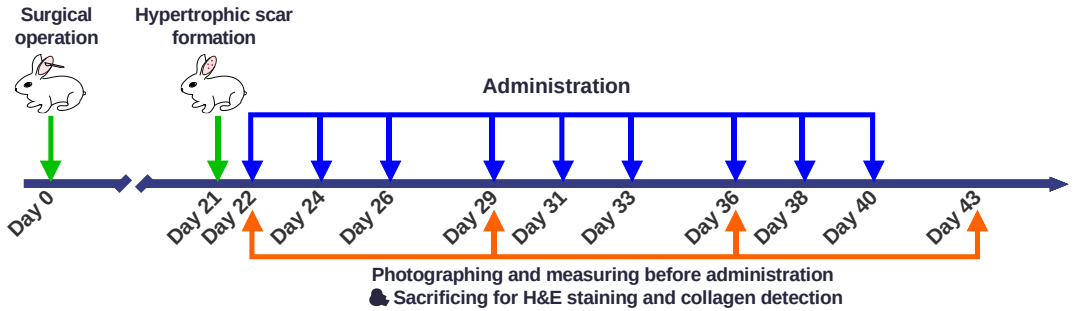
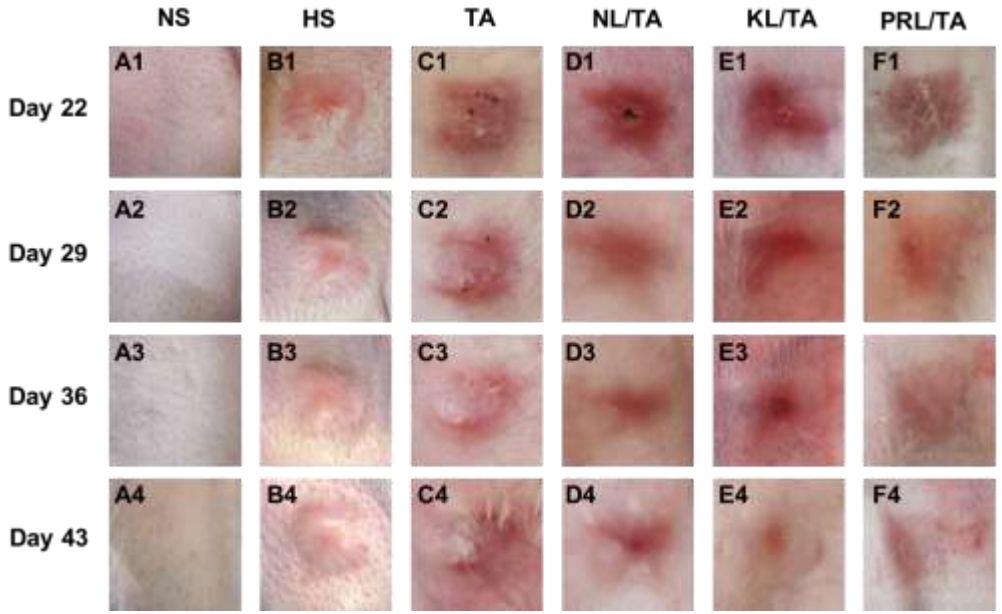
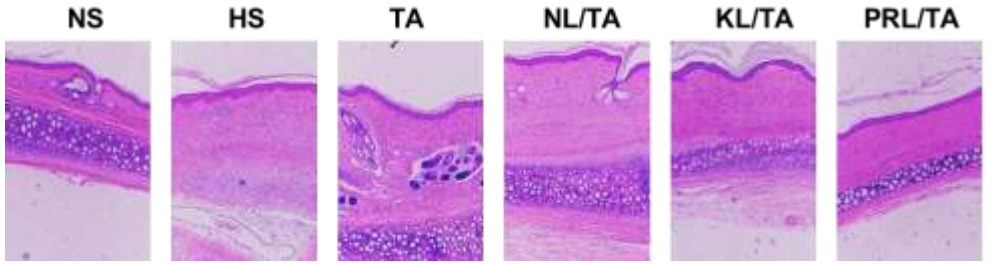
Dual Tissue-cell Permeability Performance

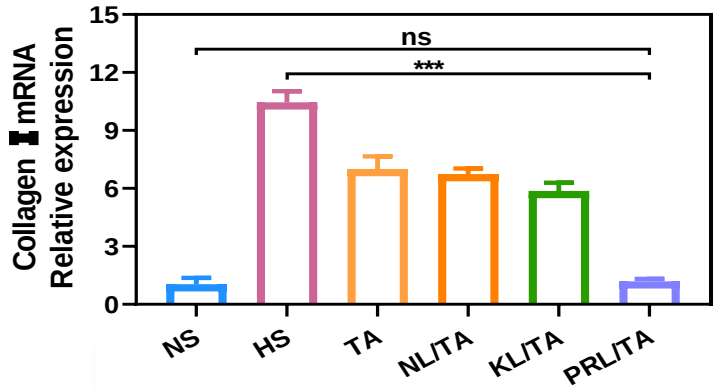




Immunohistochemistry



Collagen expression



Significant antiproliferative scar effect

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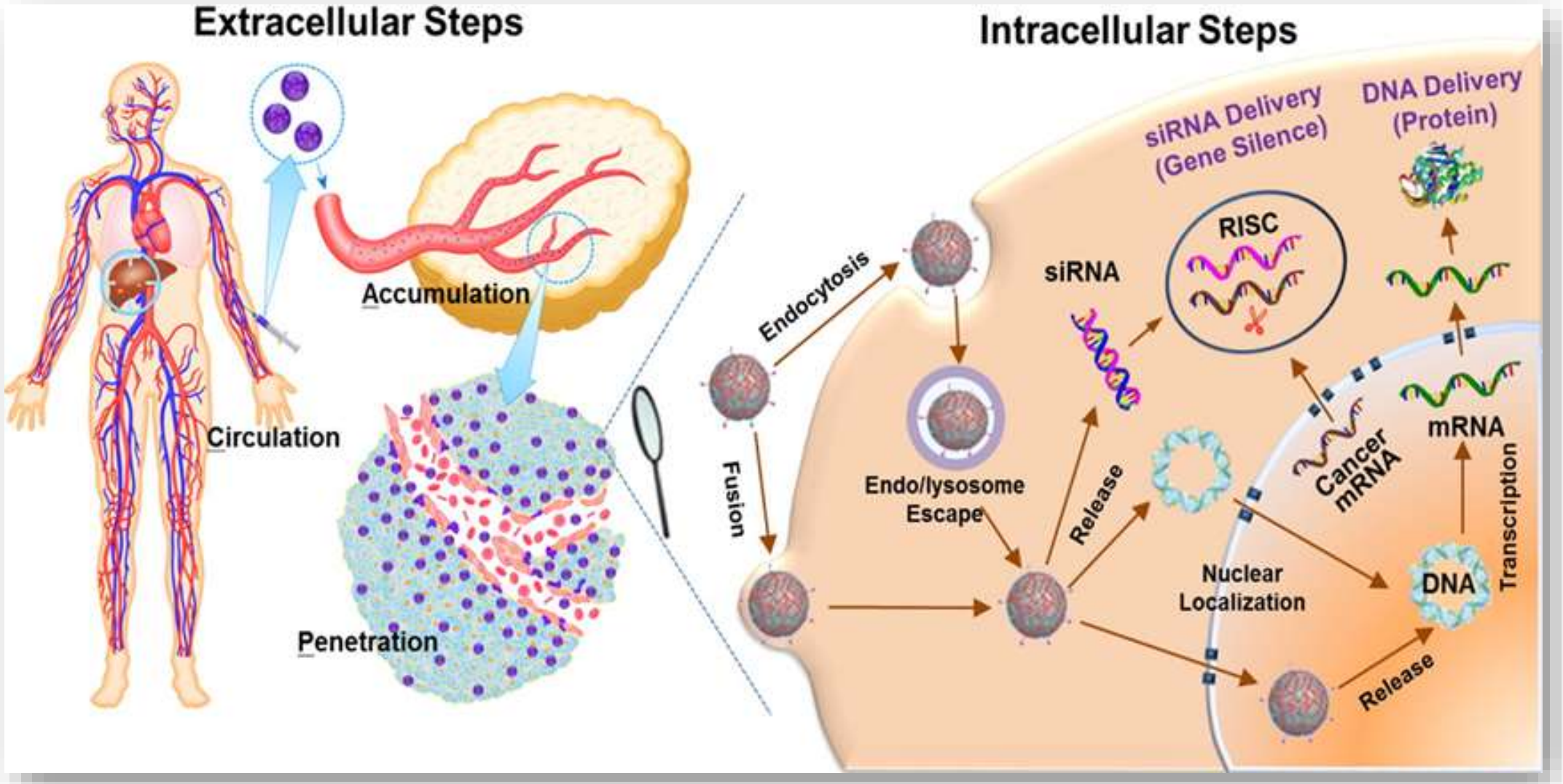
PART THREE

Lipopeptide for Gene Delivery

Gene Delivery



Gene Delivery

Gene delivery solutions

Nucleic Acid Carrier

Viral vectors

Non-viral carriers

High efficiency
Unsafe

Biomembrane penetrating

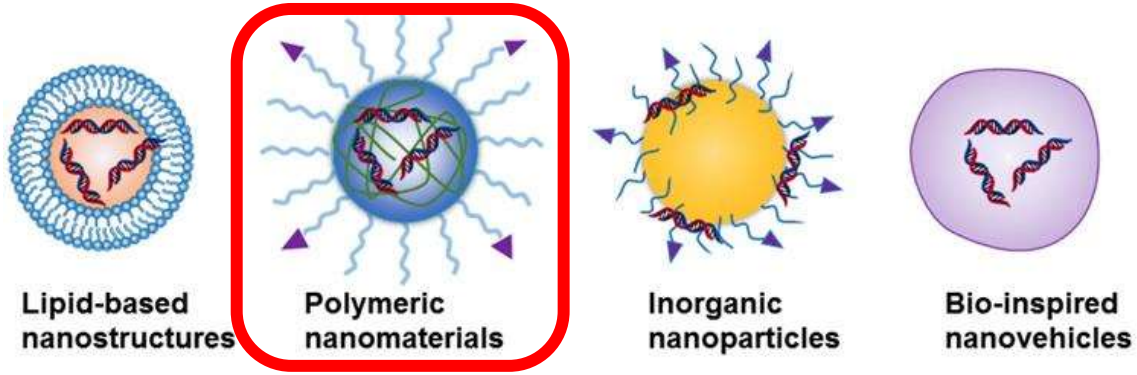
Desianable

Synthesized

Flexible

High-loading

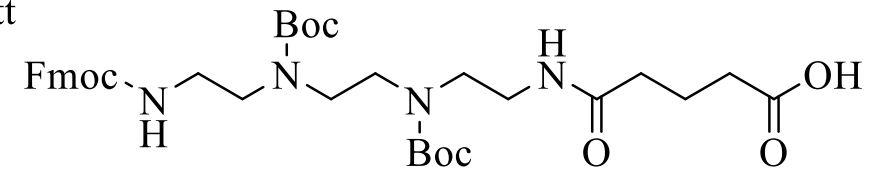
VS



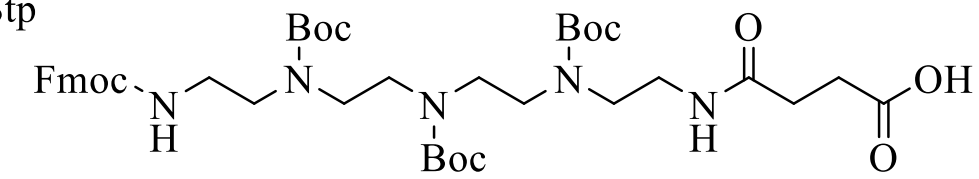
Peptide-like Cationic Oligomers

➢ Protonable artificial amino acid as solid phase synthesis building blocks

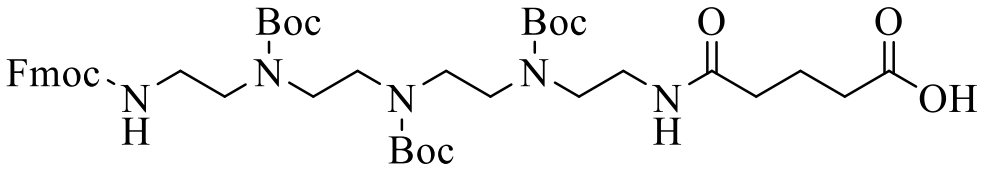
Gtt



Stp



Gtp



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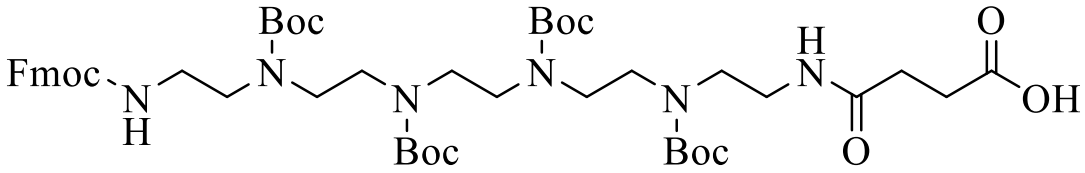
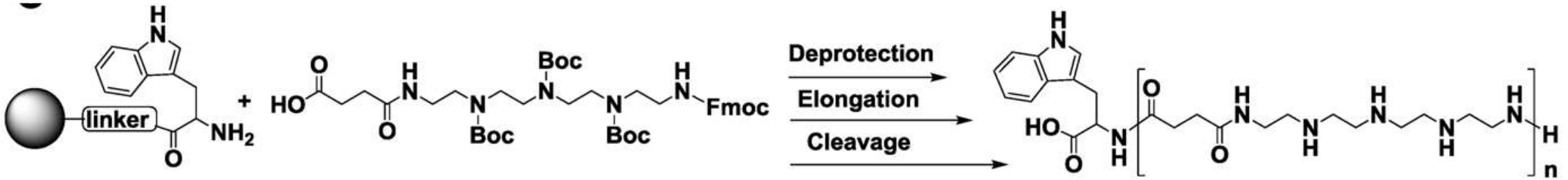
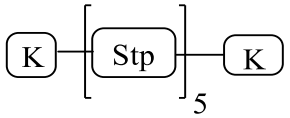


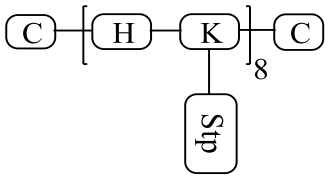
➢ Schematic synthesis process

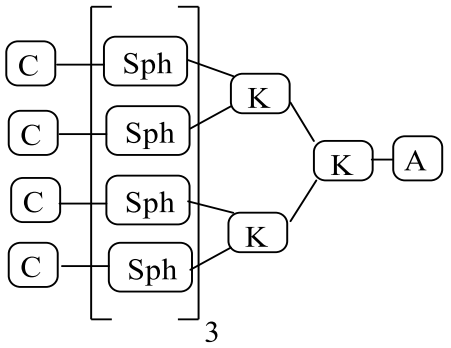


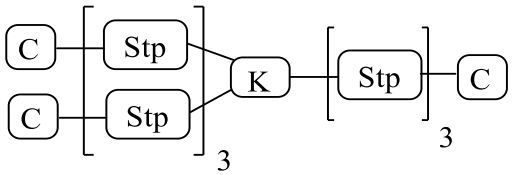
Lipopeptides from Peptide-like Cationic Oligomer

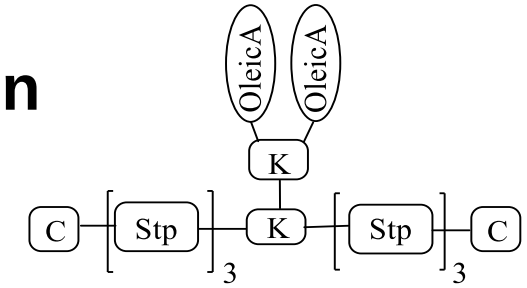
- **Precise Structure**
- **Biodegradable**
- **Multifunctional**
- **Responsive**
- **On-demand Design**

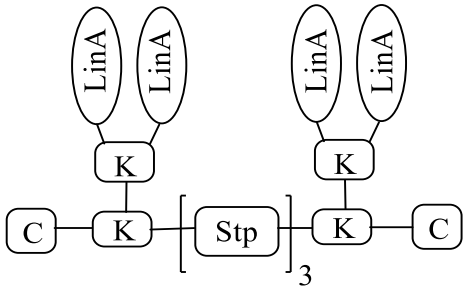


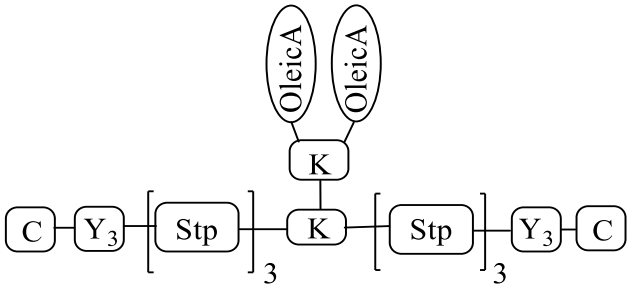


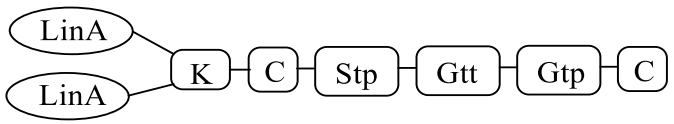


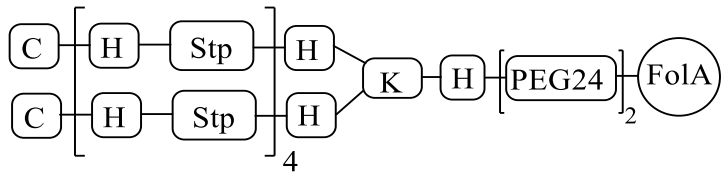












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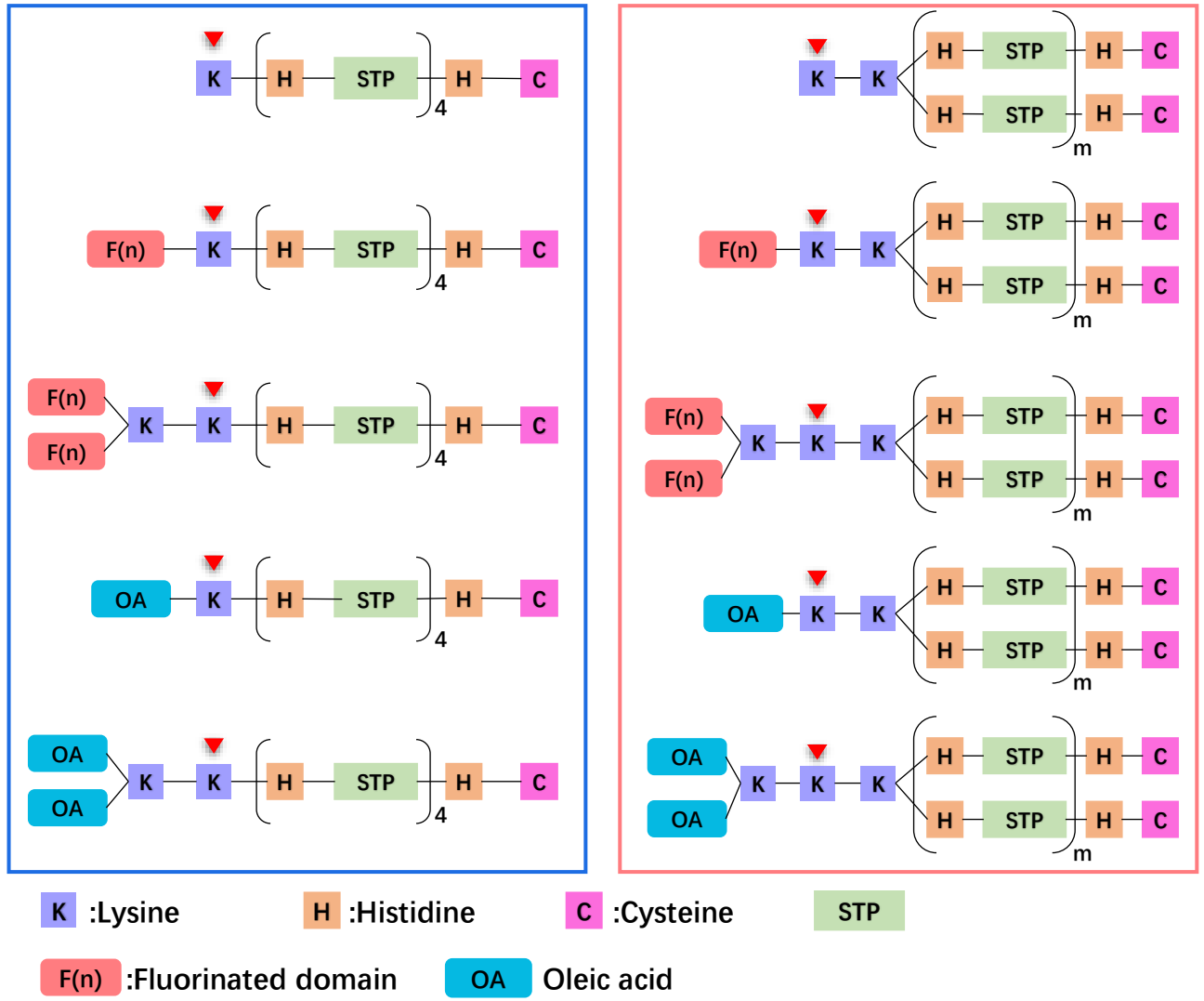
Fluorinated lipopeptides from Peptide-like Cationic Oligomer



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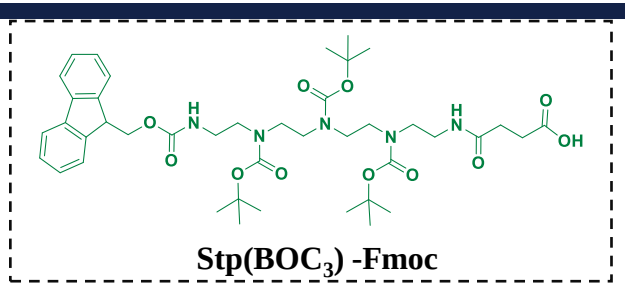

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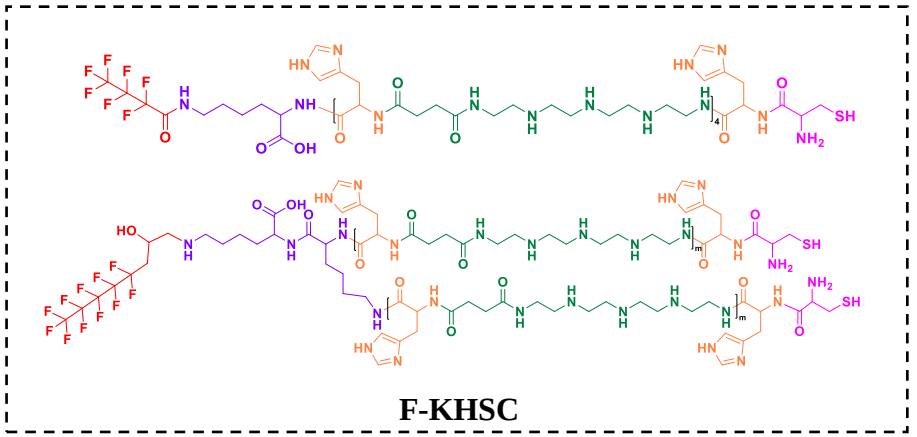

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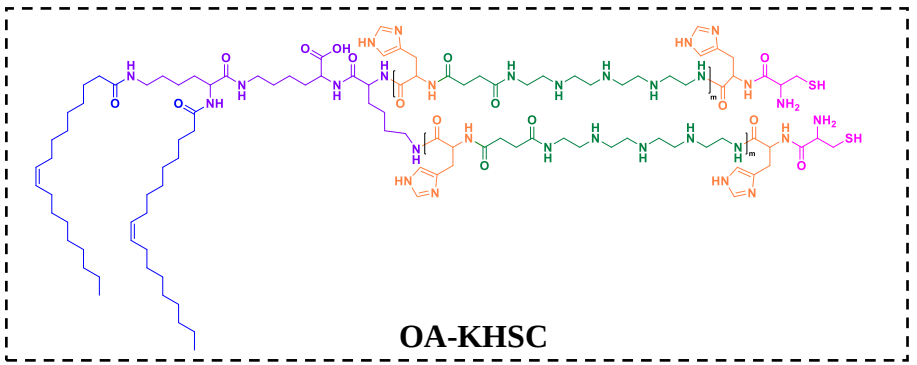
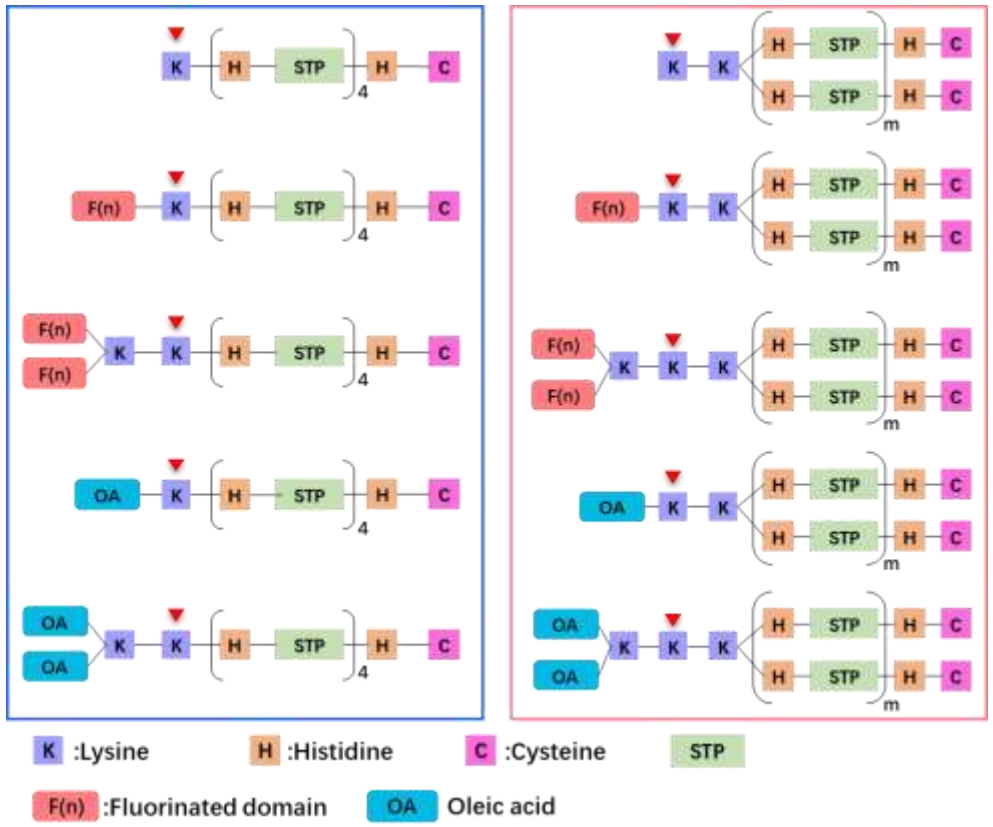
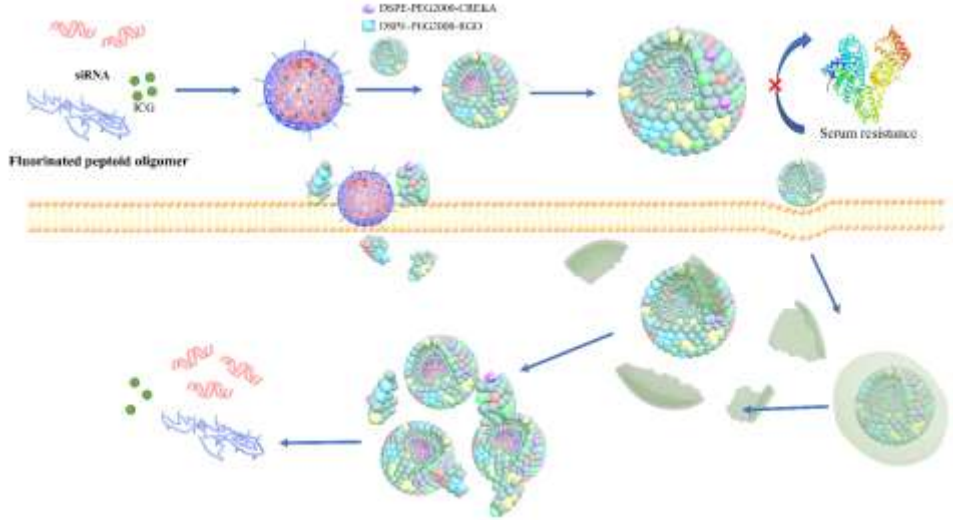


Sequential Tumor Targeted Delivery System

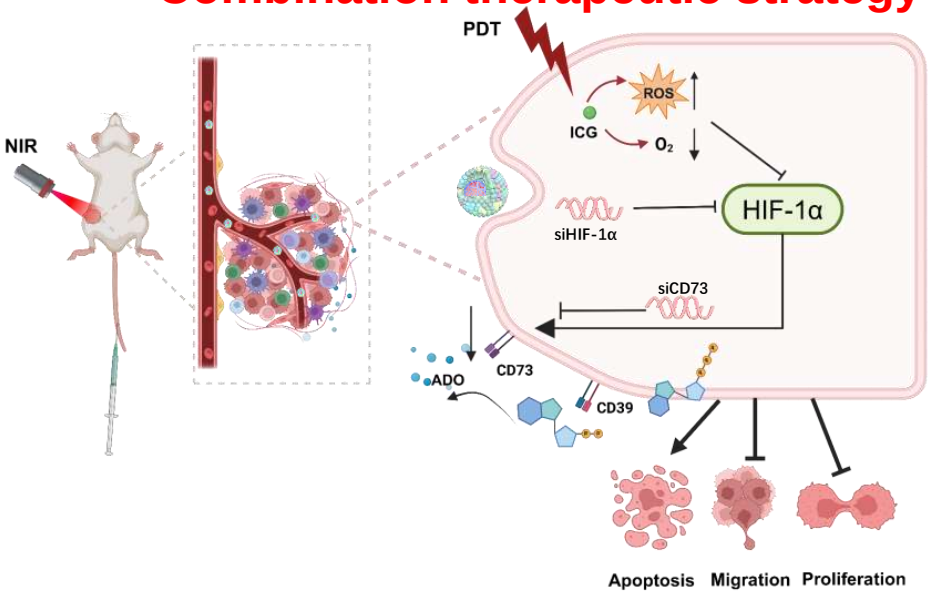


■ Carrier library design and optimization

■ Targeted lipid-polymer hybrid nanoparticles

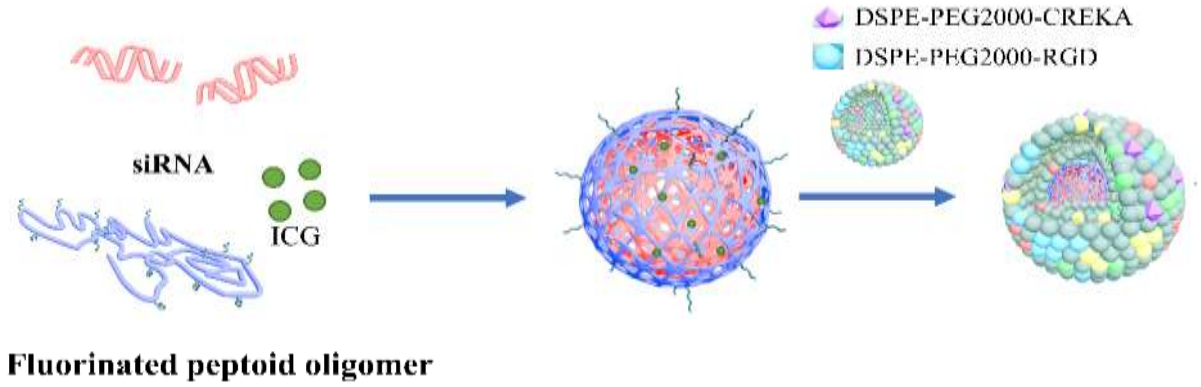


■ Combination therapeutic strategy



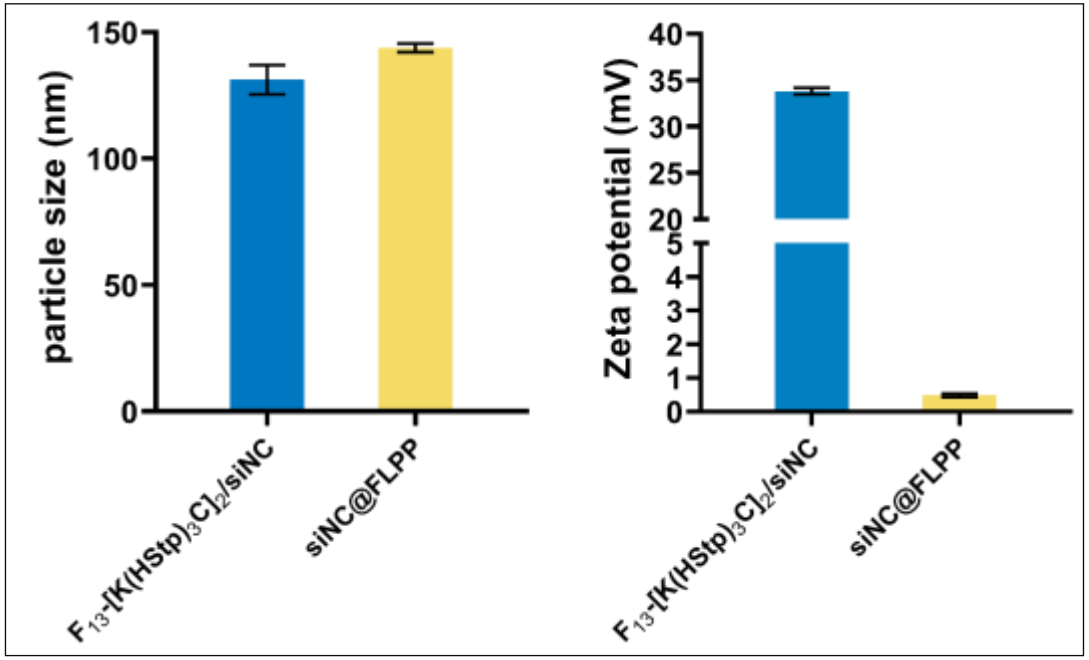
Sequential Tumor Targeted Delivery System

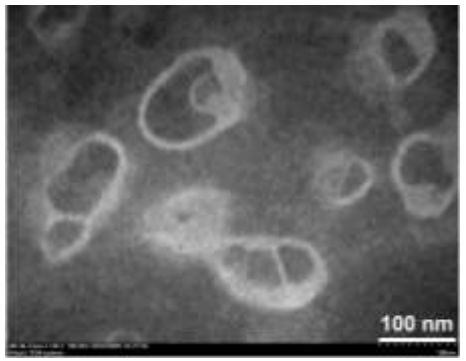
- **Lipid-polymer hybrid nanoparticles**



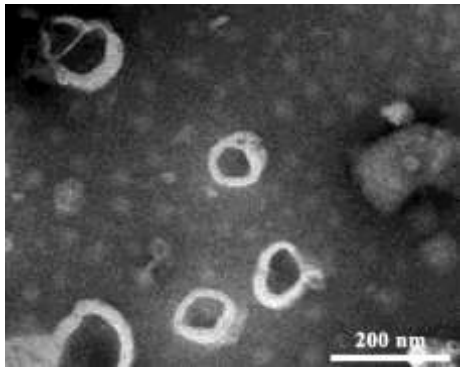
FLPP

Sequential Tumor Targeted Delivery System



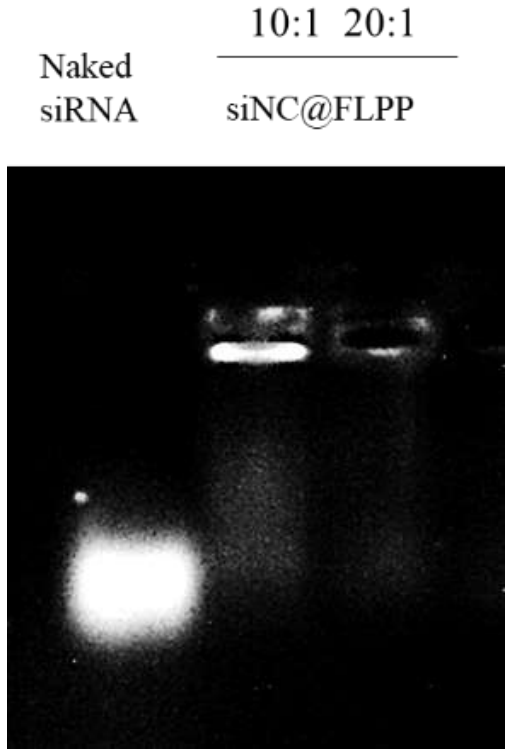


Blank lipid



siNC@FLPP

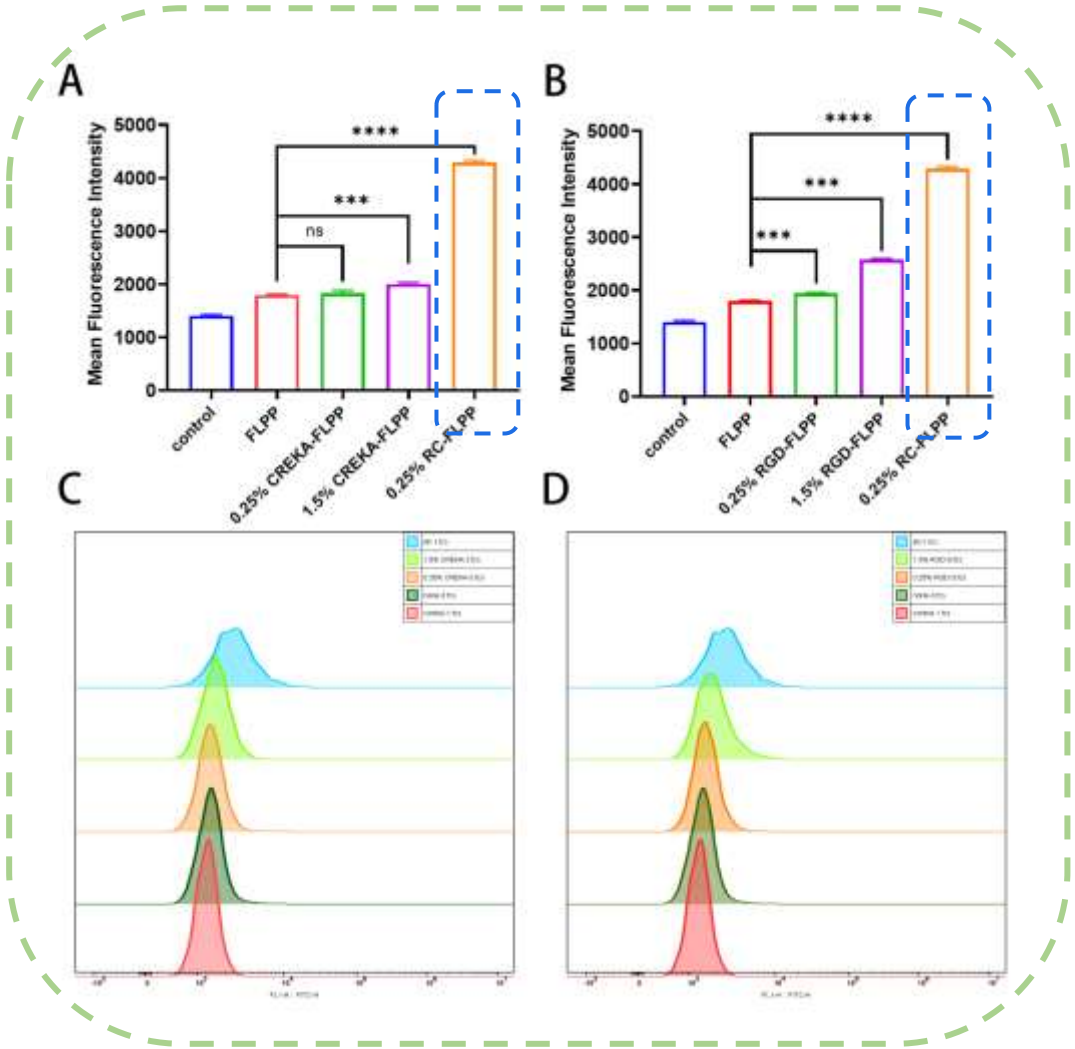
Hollow elliptical or spherical



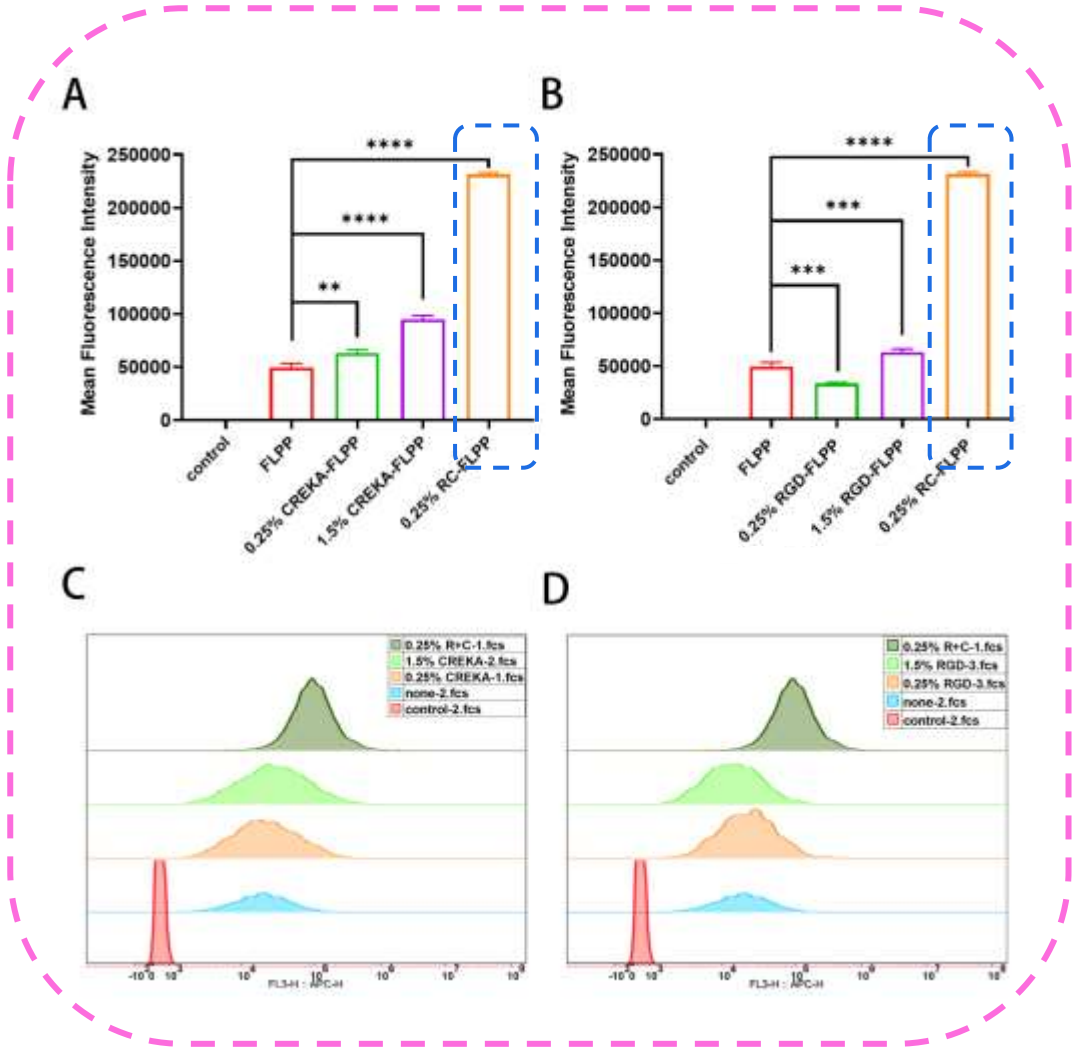
Effective siRNA encapsulation

Sequential Tumor Targeted Delivery System

➢ FLPP demonstrate higher cellular uptake

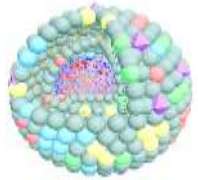


4T1

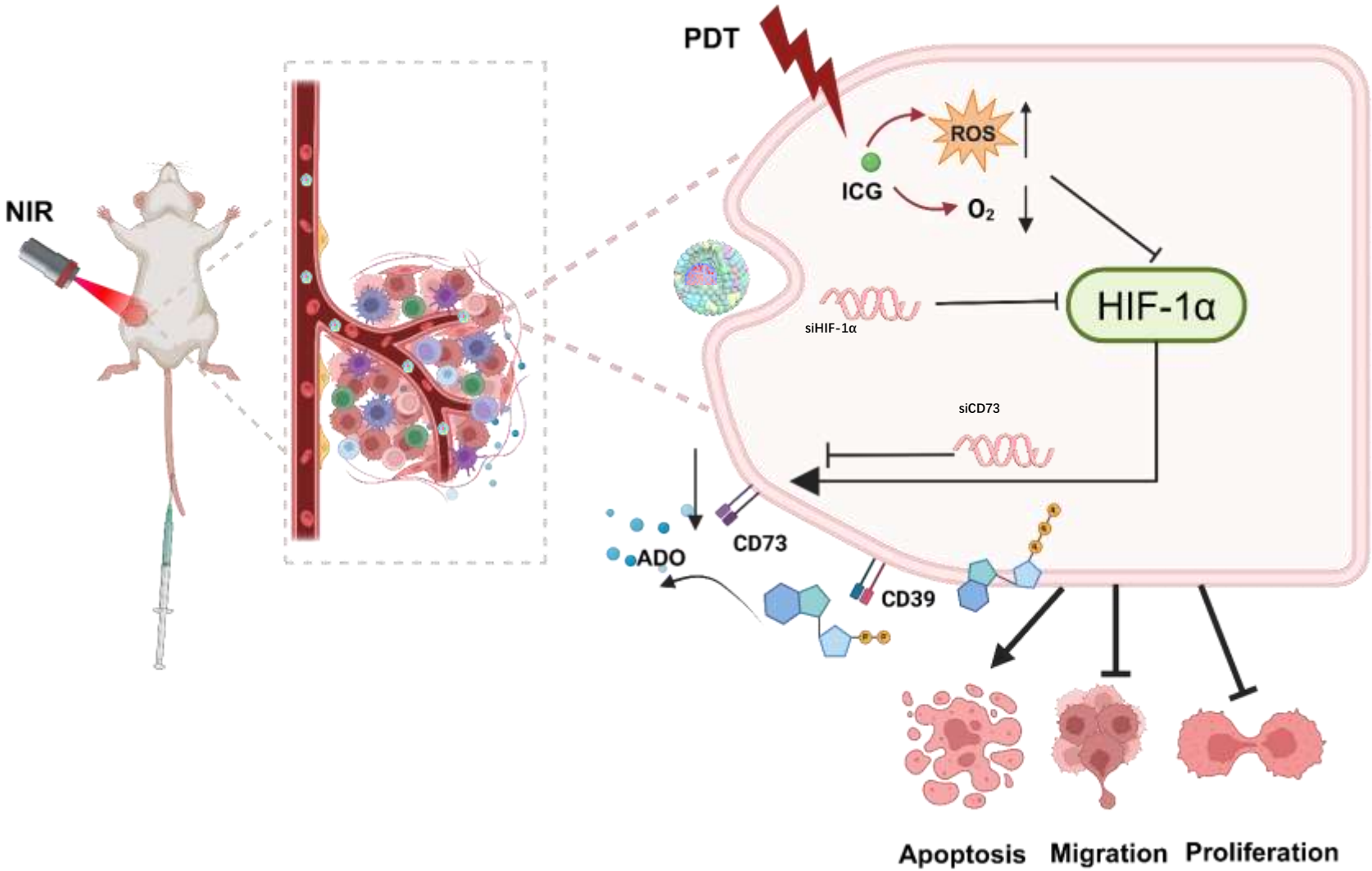


NIH/3T3

Sequential Tumor TDDS for Combination Therapy with PDT

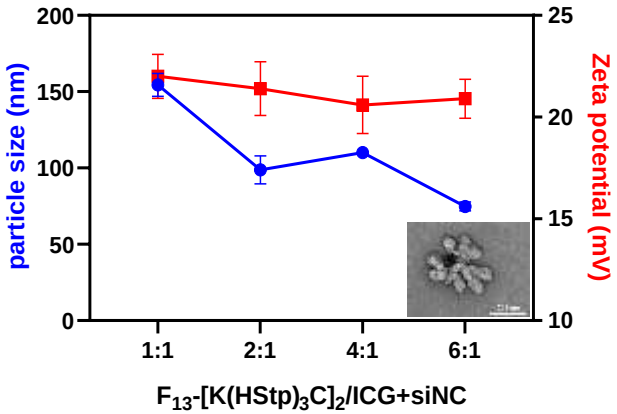


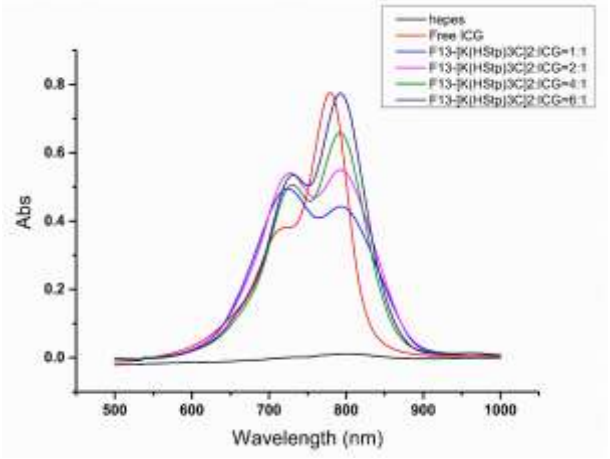
FLPP

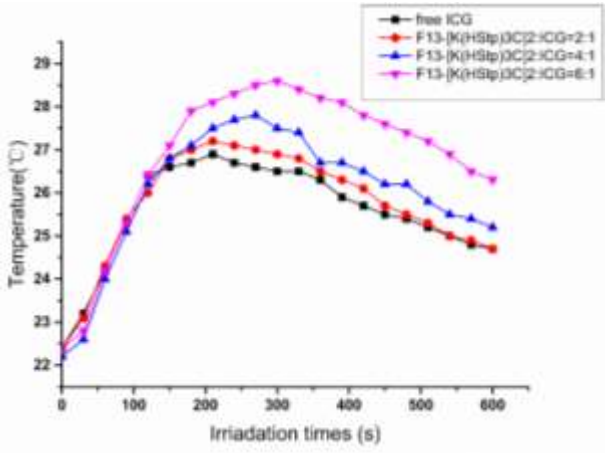


Sequential Tumor TDDS for Combination Therapy with PDT

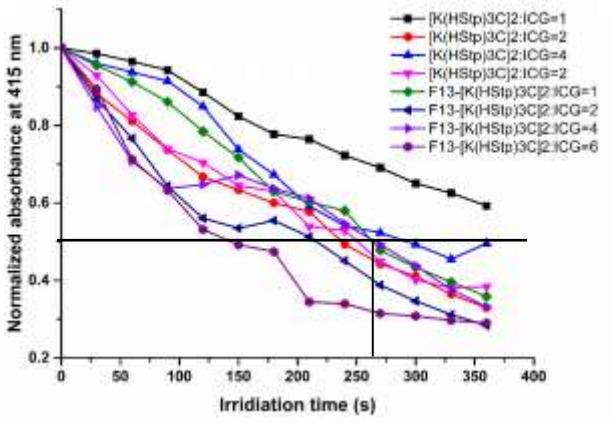
■ Co-loading of siRNA and ICGs with fluorinated carriers



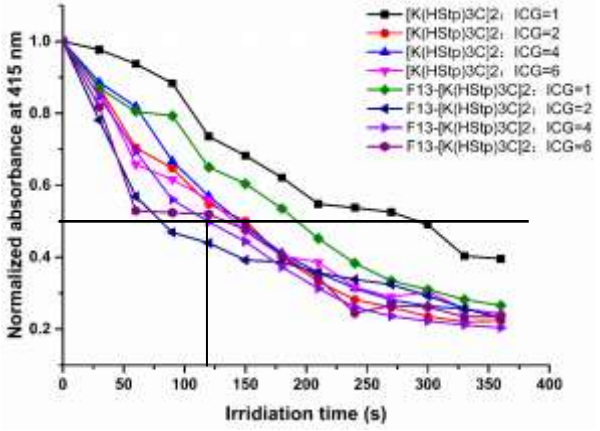




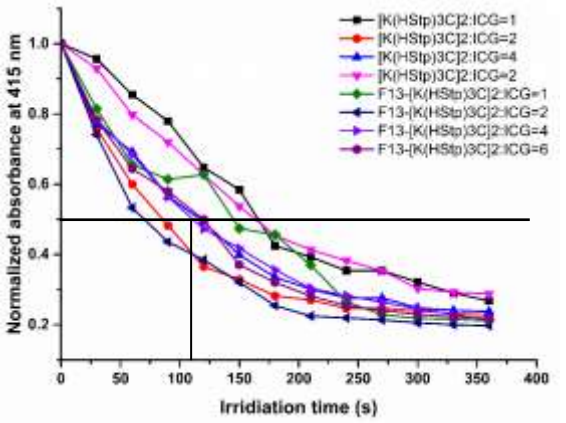
- Fluorinated carriers/ICG polymers improve photothermal conversion efficiency



1 W/cm^2



1.5 W/cm^2



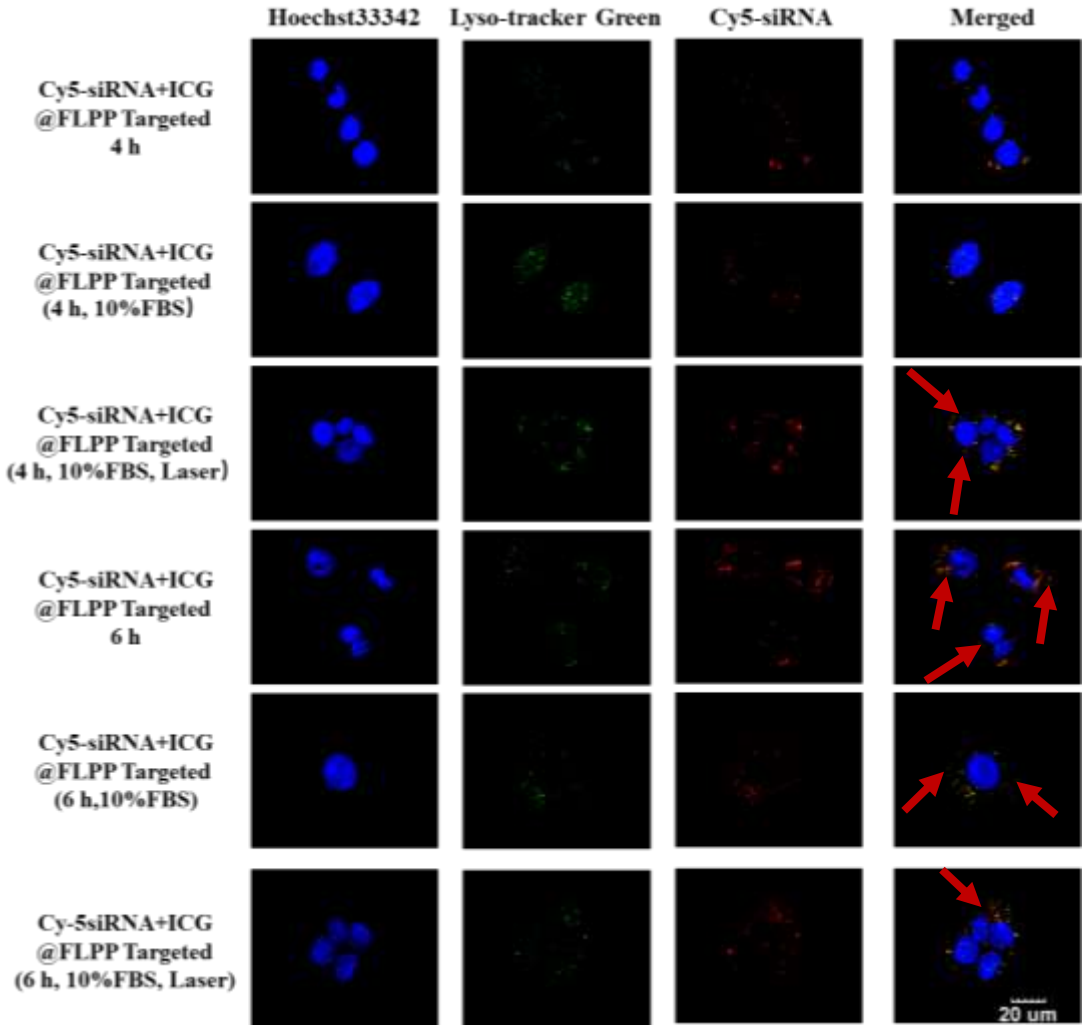
2 W/cm^2

- Increase of laser irradiation power, the faster the photodynamic conversion rate
- Fluorinated carriers higher than unmodified carriers

Sequential Tumor TDDS for Combination Therapy with PDT

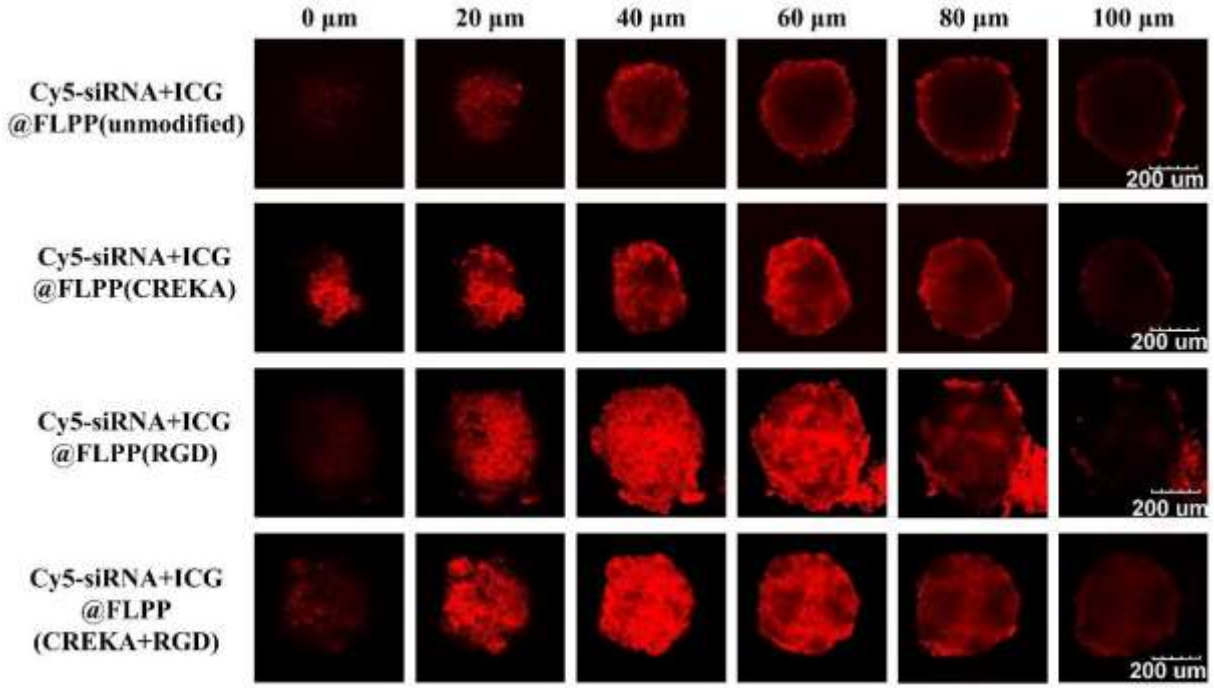
In vitro evaluation of the siRNA+ICG@FLPP delivery system

Endosomal Escape



4T1

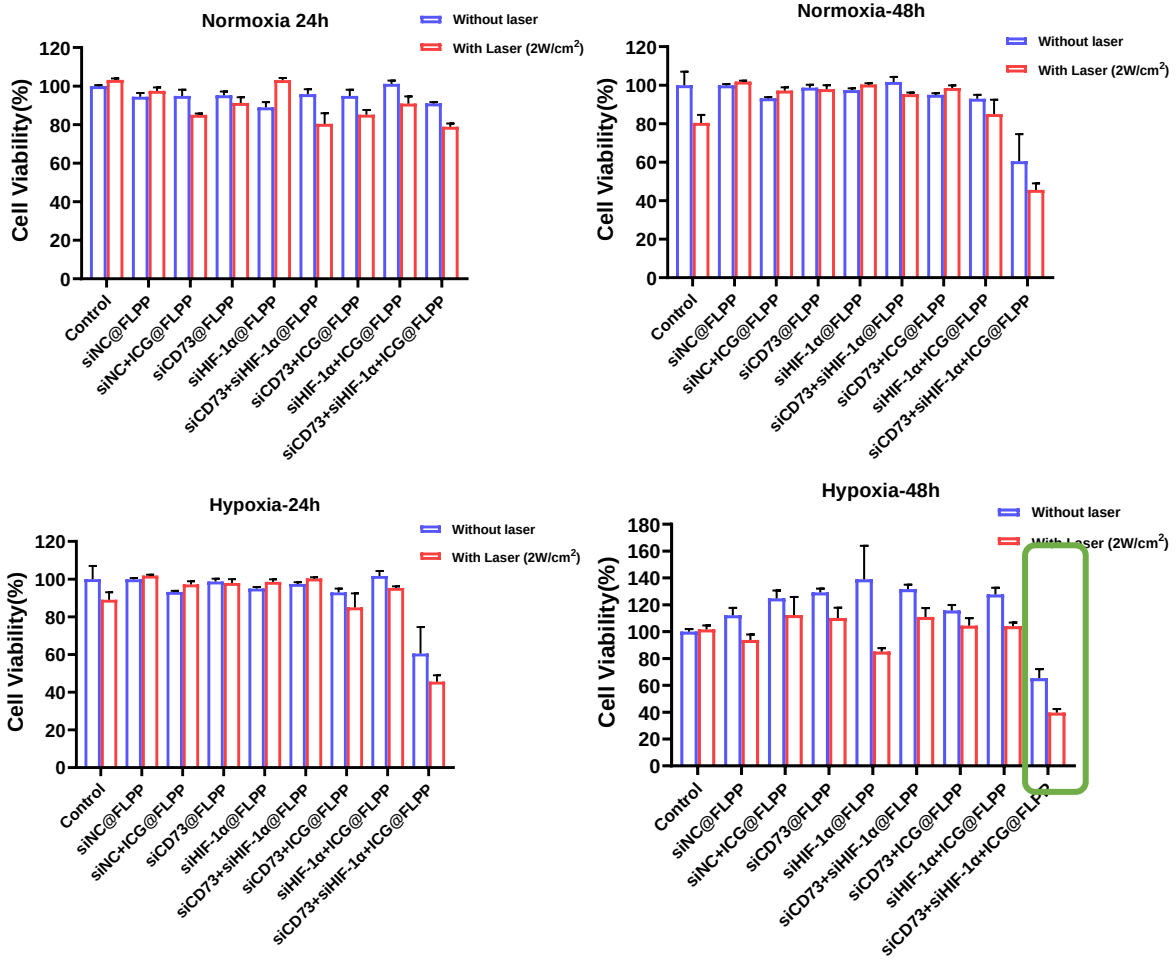
Targeted permeability in mixed tumor cell spheroids



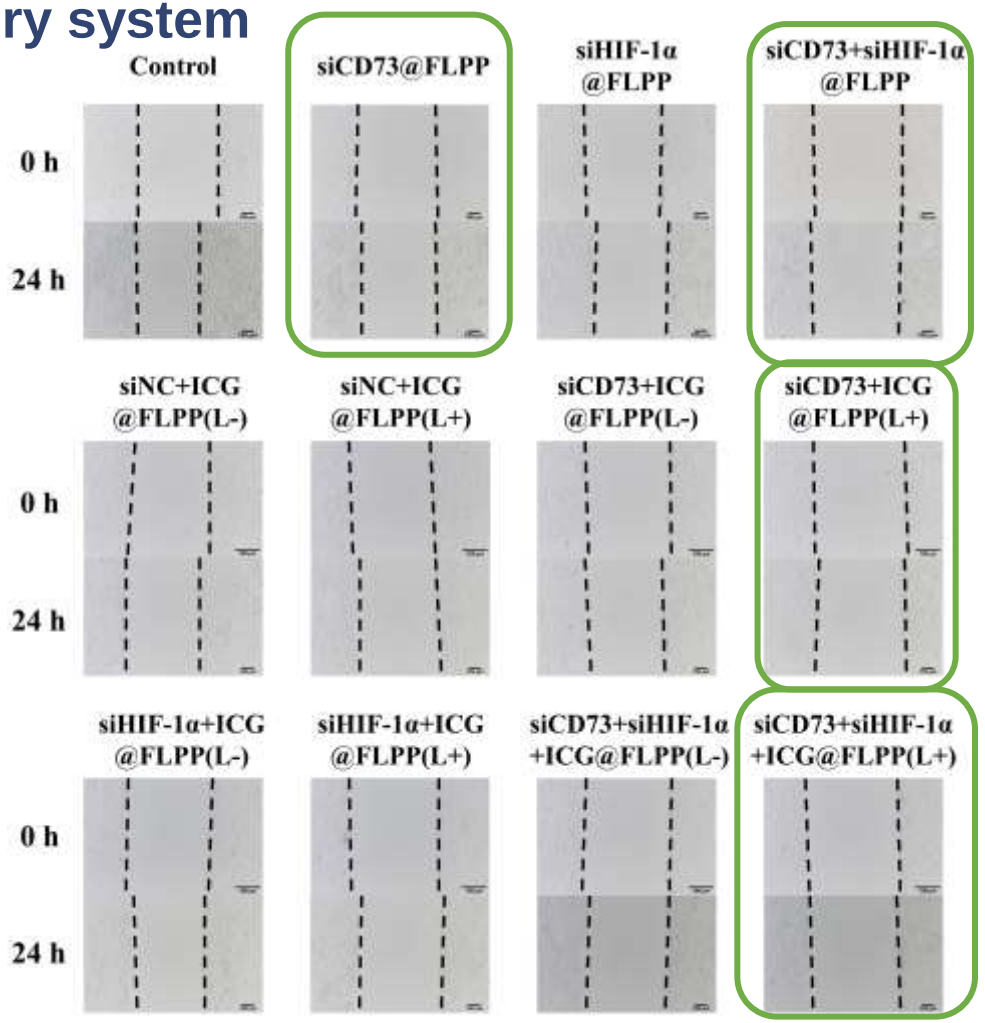
4T1+NIH/3T3

Sequential Tumor TDDS for Combination Therapy with PDT

- In vitro evaluation of the siRNA+ICG@FLPP delivery system



The combination treatment group had a stronger killing effect on cells under hypoxic conditions.

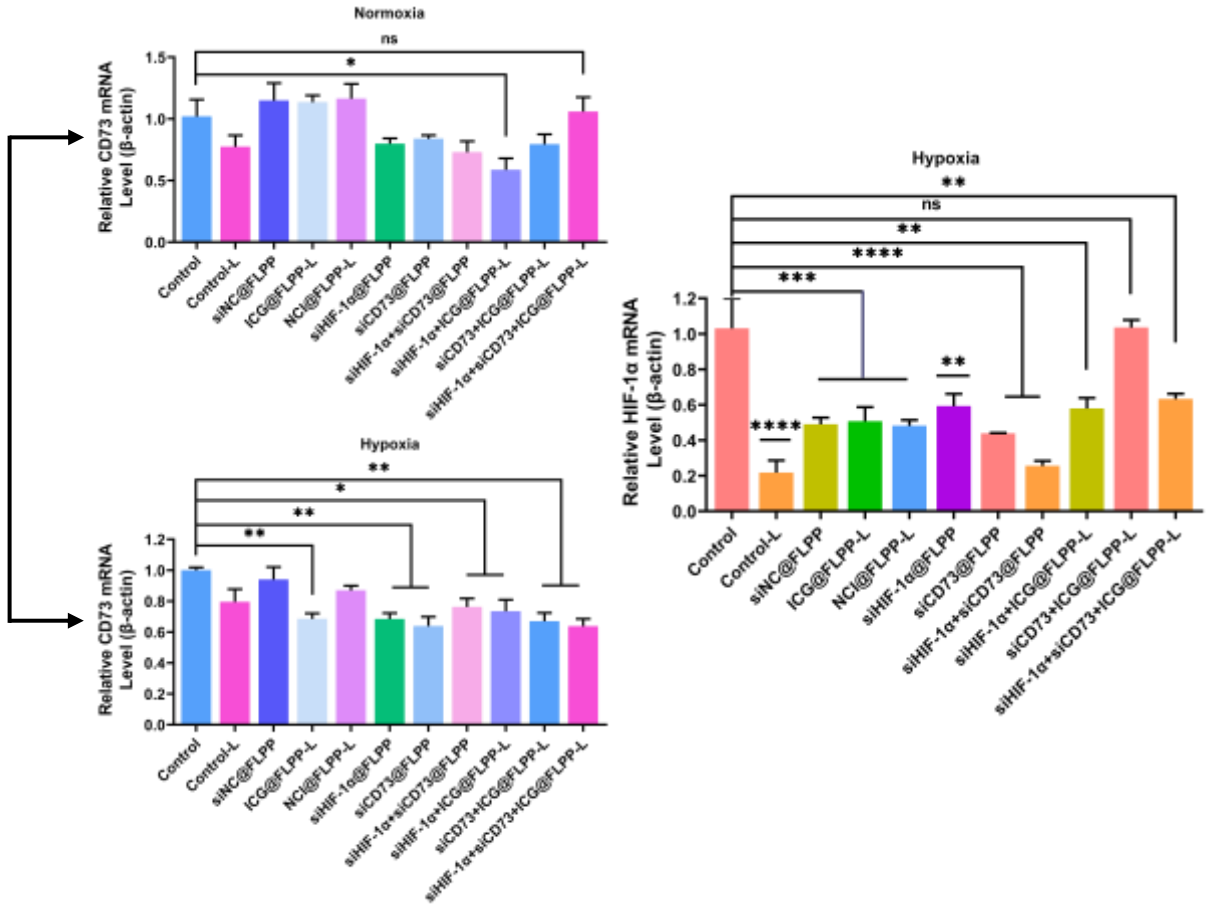


siCD73-containing group had stronger ability to inhibit cell migration.

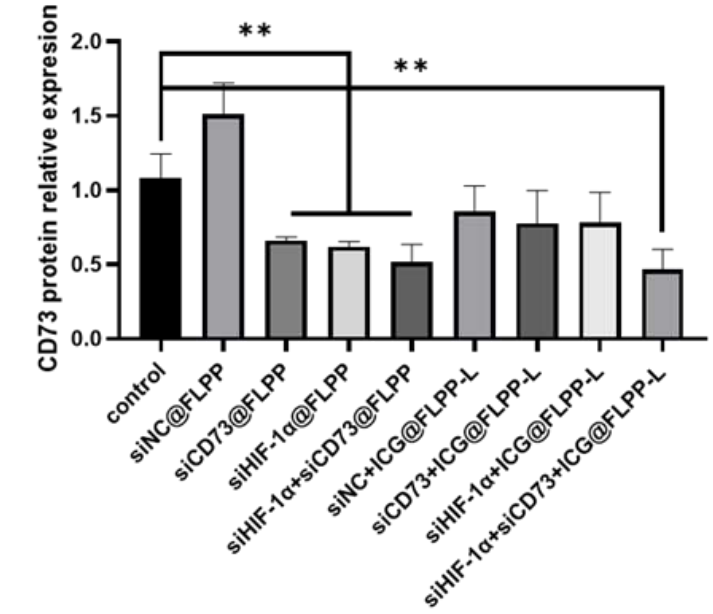
Sequential Tumor TDS for Combination Therapy

- **In vitro evaluation of the siRNA+ICG@FLPP delivery system**

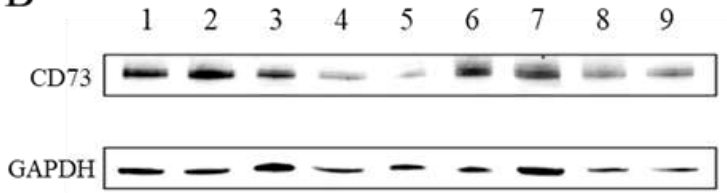
CD73 mRNA level by PCR



A CD73 expression by WB



B



1: Control; 2: siNC@FLPP; 3: siCD73@FLPP; 4: siHIF-1α@FLPP;
5: siHIF-1α+siCD73@FLPP ;6: siNC+ICG@FLPP-L; 7: siCD73+ICG@FLPP-L;
8: siHIF-1α+ICG@FLPP-L; 9: siHIF-1α+siCD73+ICG@FLPP-L

Sequential Tumor TDS for Combination Therapy

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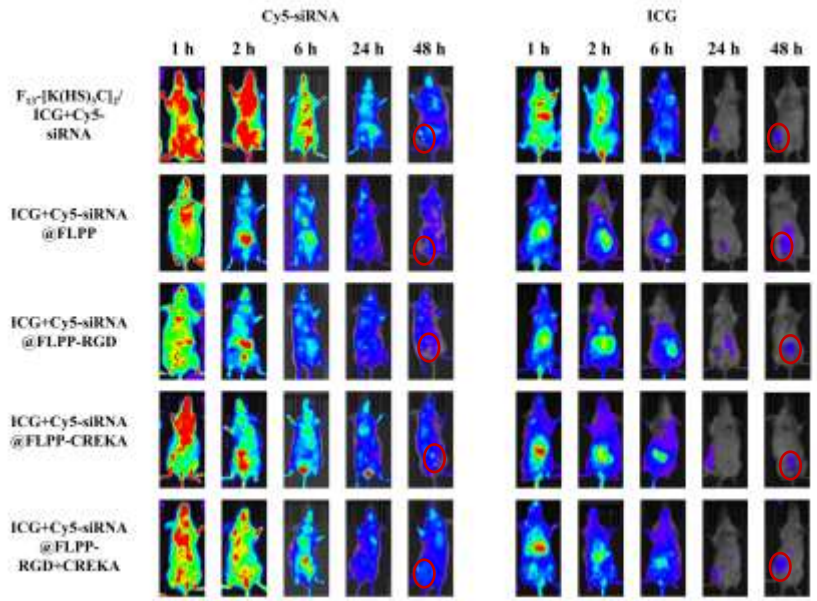
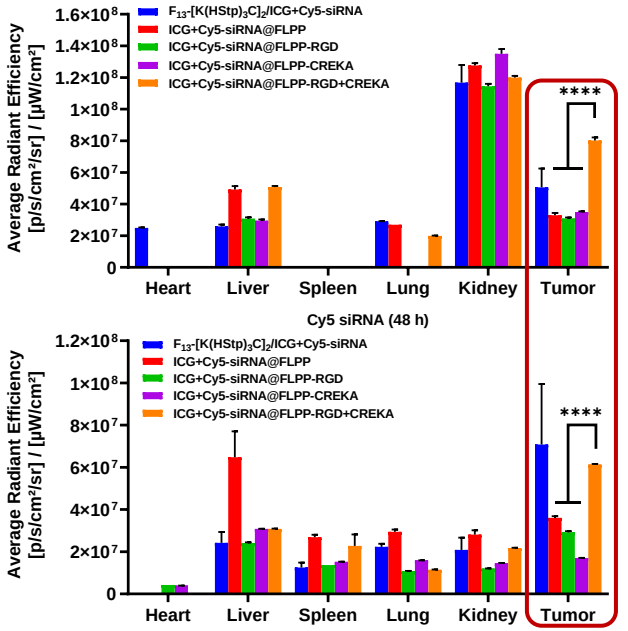
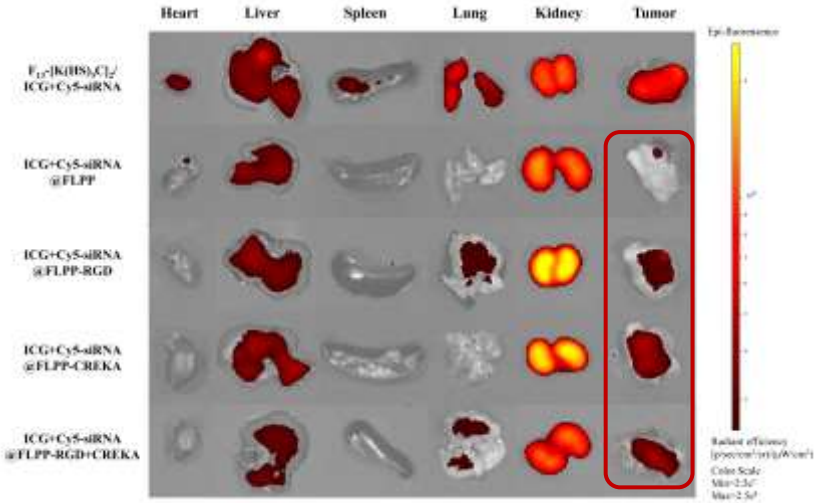
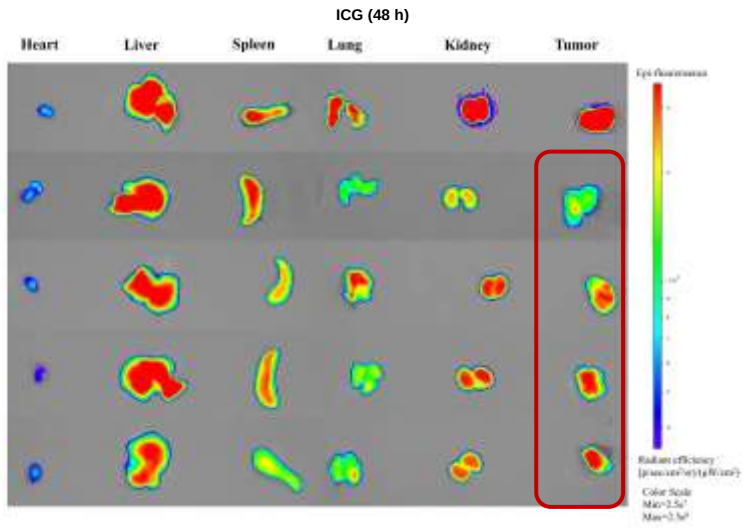
In-vivo distribution

- 48 h after i.v., siRNA and ICG still remain at tumor site.
- targeted FLPPs have stronger tumor tissue accumulation.









Sequential Tumor TDS for Combination Therapy

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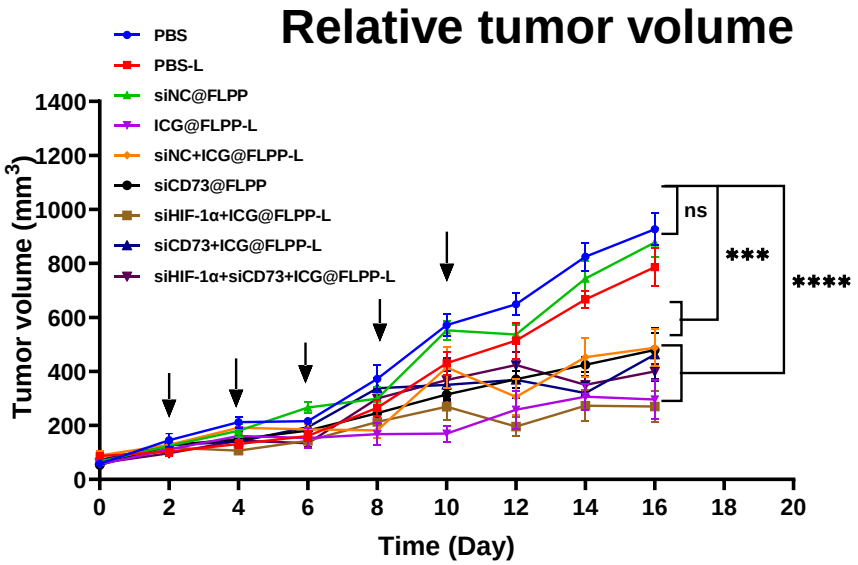
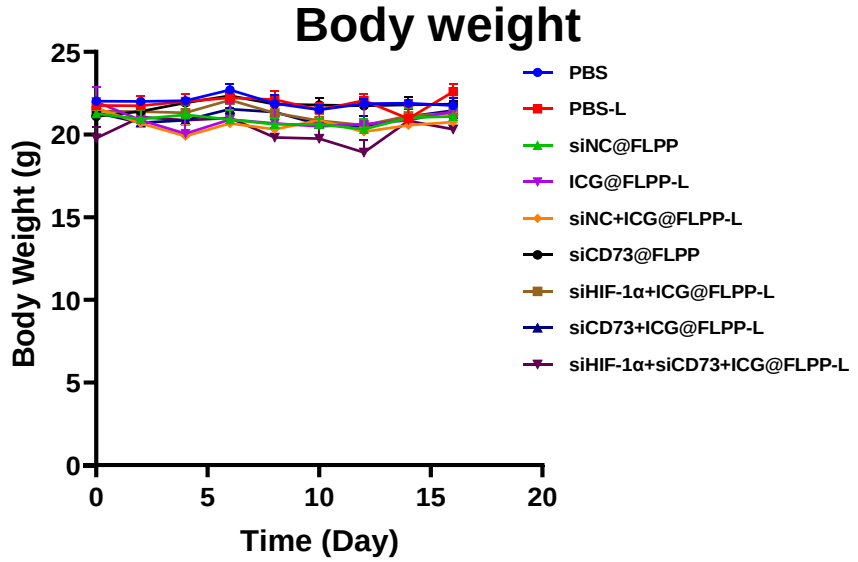
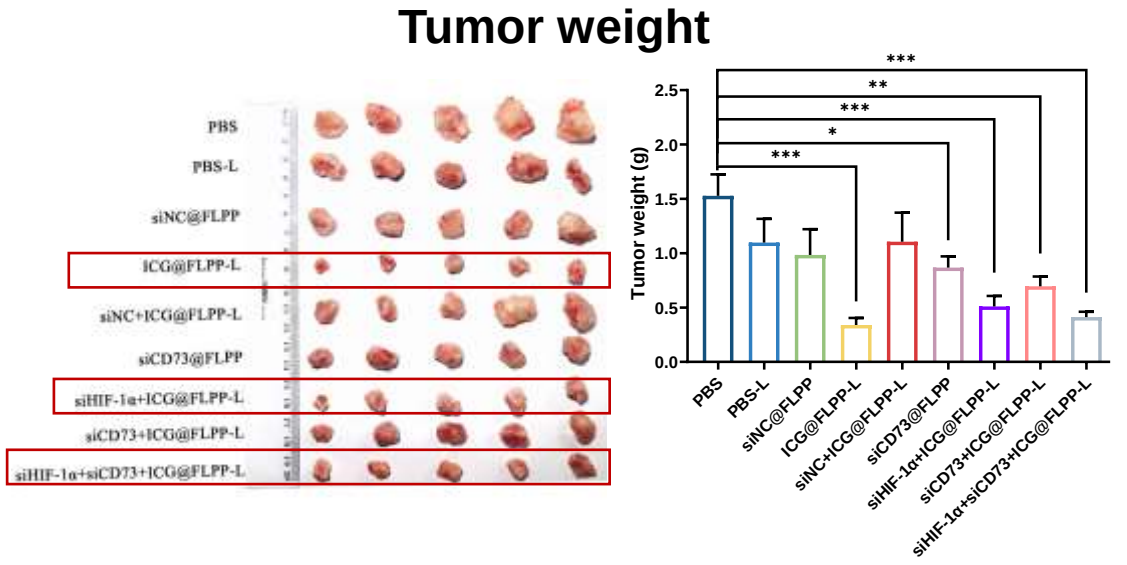
In vivo evaluation of the siRNA+ICG@FLPP delivery system







- siRNA combined with PDT effectively inhibited tumor growth
- Targeting upstream hypoxia on tumor treatment is better than that of downstream adenosine pathway

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PART FOUR

Summary and Perspective

Summary and Perspective

Lipopeptide

- **Advantage**
  - **Multifunction: Versatile for the construction of a wide range of delivery systems; Posses the potential to build delivery platform to meet different delivery needs.**
  - **Precise structure: ease to meet the different functional requirements via structural modification; ease to carry out structure-activity relationship study.**
  - **Biodegradable: Biocompatible and high safety.**
- **Foci in research**
  - **Quality Control as Excipients: structure characterization, assay, impurities, functional related characteristics, stability, etc.**
  - **Scale up Production**
  - **Batch to Batch Consistency**

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Center for Research Development and Evaluation of Pharmaceutical Excipients and Generic Drugs

Key Laboratory of the National Medical Products Administration

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CHINA PHARMACEUTICAL UNIVERSITY

中国药科大学药学院
SCHOOL OF PHARMACY CHINA PHARMACEUTICAL UNIVERSITY

NMPA Key Laboratory for Research and Evaluation of Pharmaceutical Preparations and Excipient

Department of Pharmaceutics, School of Pharmacy, China Pharmaceutical University

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THANKS