

Microrobot-assisted delivery of circular RNA lipid nanoparticles for pulmonary gene therapy

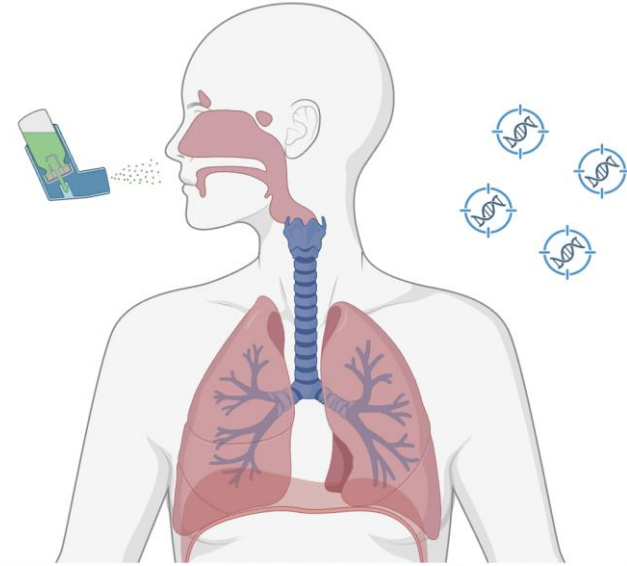
Bianca M. Santana¹, Ritu R. Raj¹, Matthew M. Kwan¹, Katherine A. Trese¹, Taylor R. Ausec¹, Michael A. Evans², C. Wyatt Shields IV¹

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Current limitations in pulmonary mRNA gene therapy

- Mucus entrapment
- Mucociliary clearance
- Accumulation in the distal airways
- Short-lived expression from linear mRNA
- Toxicity



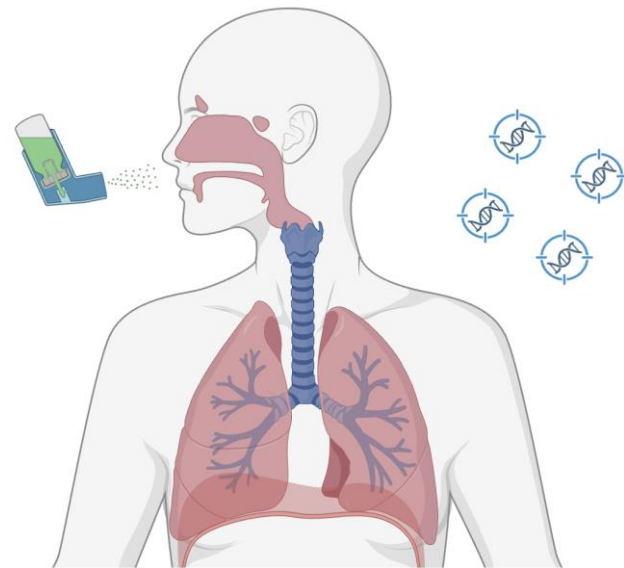
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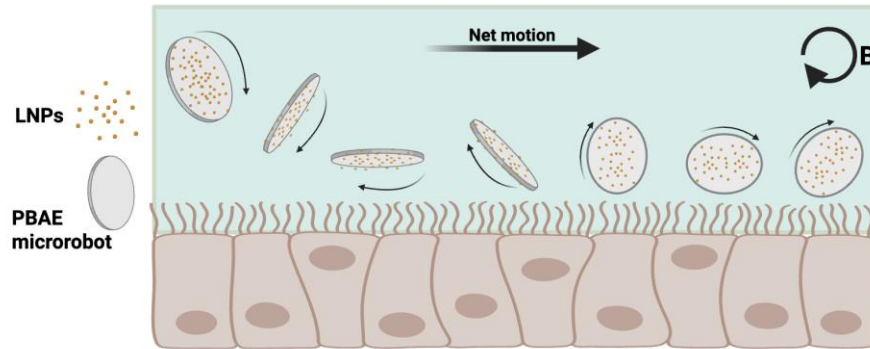
We Need Alternatives

- Actively penetrate lung barriers
 - Nanoparticulated systems rely on passive diffusion
- Sustained gene expression with reduced dosing



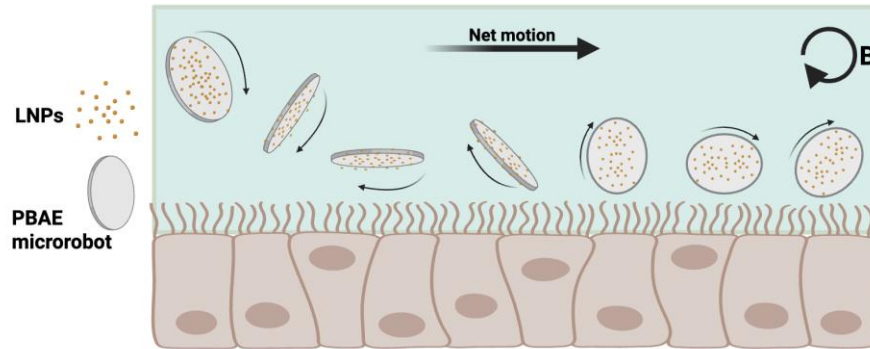
We aim to address two major challenges:

1. Passive nanoparticle transport
2. Short-term expression of therapeutic proteins



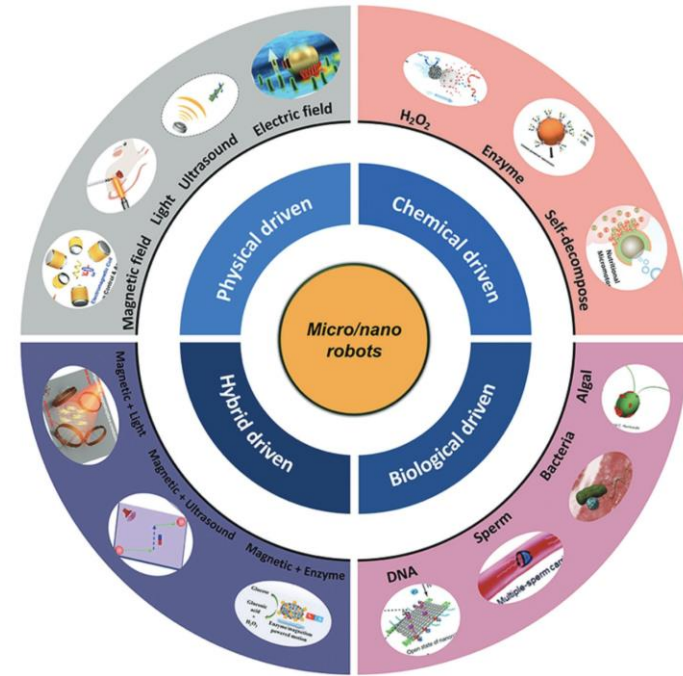
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1. Passive transport: Microrobots can help!

- Nano/micron scale
- Self-propelled or externally actuated
- Navigate through complex environments with precision



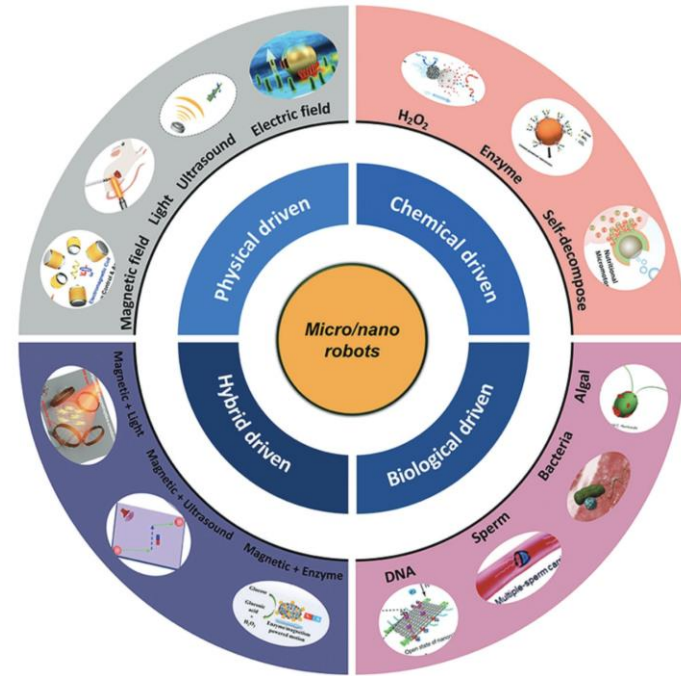
Li et al., 2023 Adv Mat Tech

1. Passive transport: Microrobots can help!

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How?

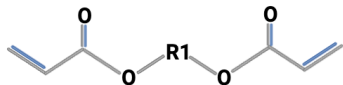
- Can carry gene therapy cargo
- Reach the distal portions of the lungs
- Transverse mucus



Li et al., 2023 Adv Mat Tech

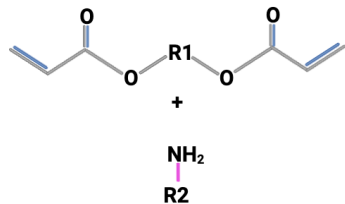
Poly (β -Amino Esters) to fabricate microrobots

- Biodegradable **polymers** synthesized from diacrylates and amines
- Tunable structure enables control over:
 - Degradation rate
 - Charge density
 - Hydrophobicity



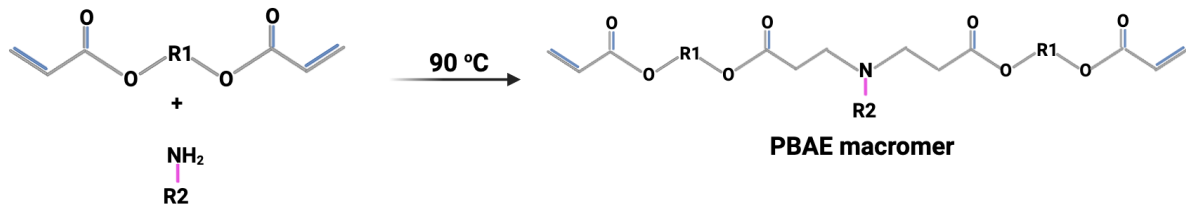
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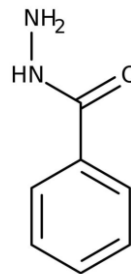
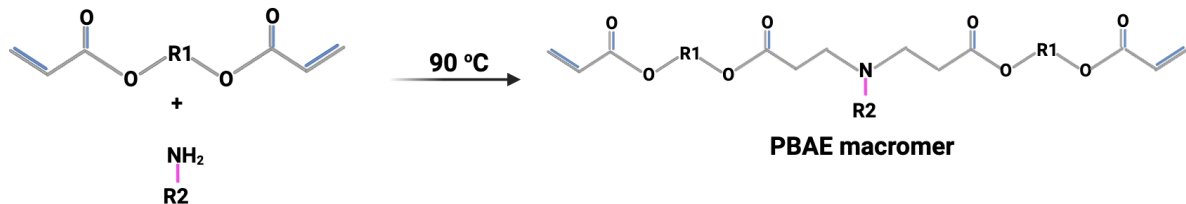
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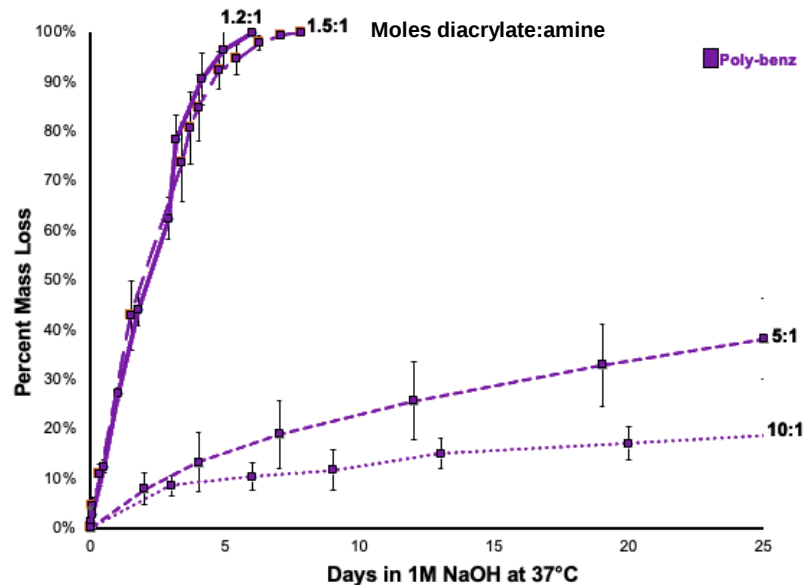
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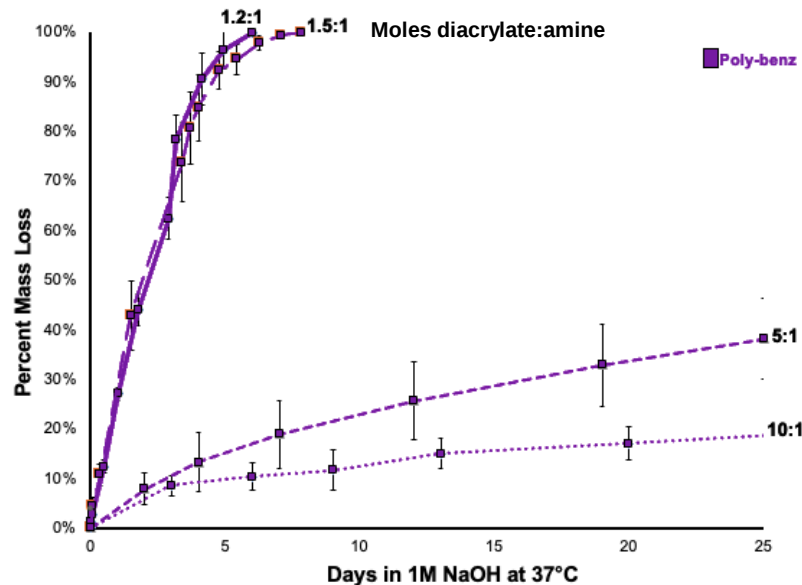


Benzhydrazide

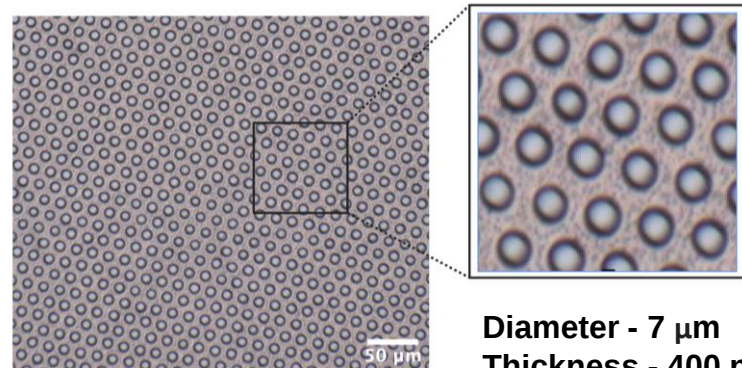
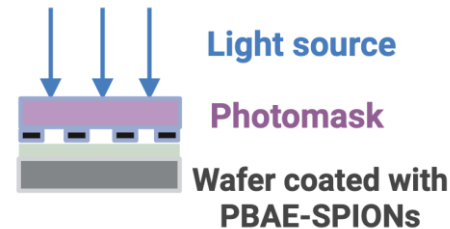
Poly (β -Amino Esters) to fabricate microrobots



Poly (β -Amino Esters) to fabricate microrobots



Contact photolithography



Diameter - 7 μm
Thickness - 400 nm

PBAE microrobots can swim under rotating magnetic fields

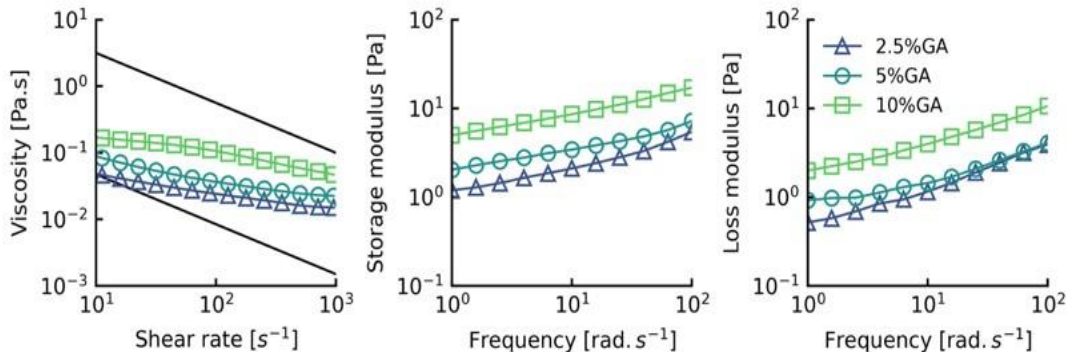


5 mT, 10 Hz – microrobots in water

PBAE microrobots can swim under rotating magnetic fields



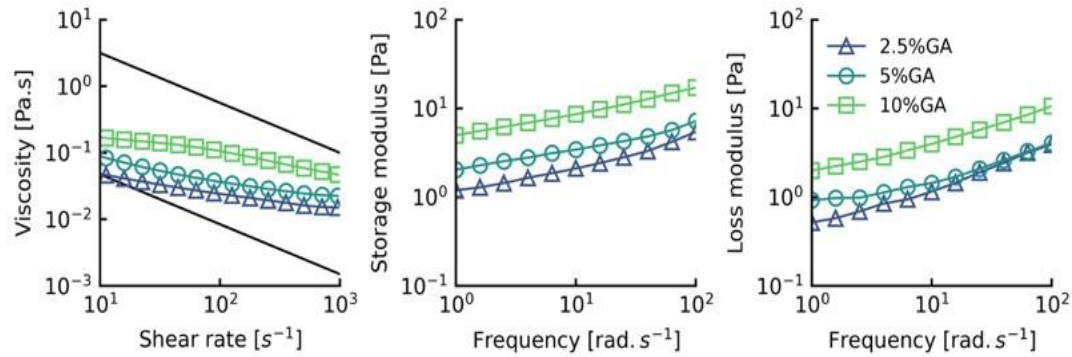
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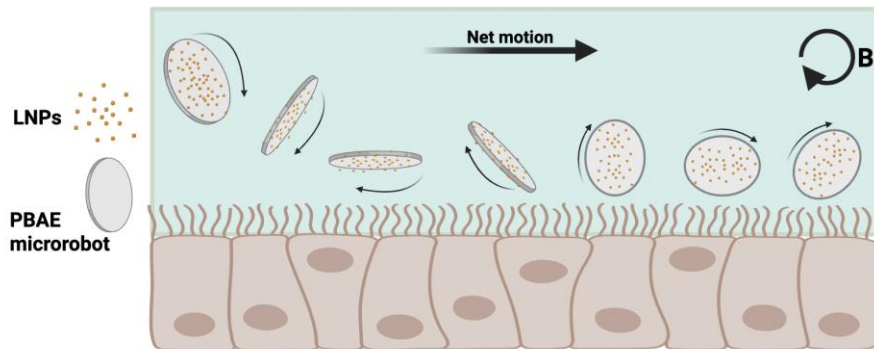
5 mT,10 Hz – microrobots in water



	Water	Mucus
Speed ($\mu\text{m.s}^{-1}$) $\pm$ SD	10.7 ± 4.6	8.4 ± 1.5

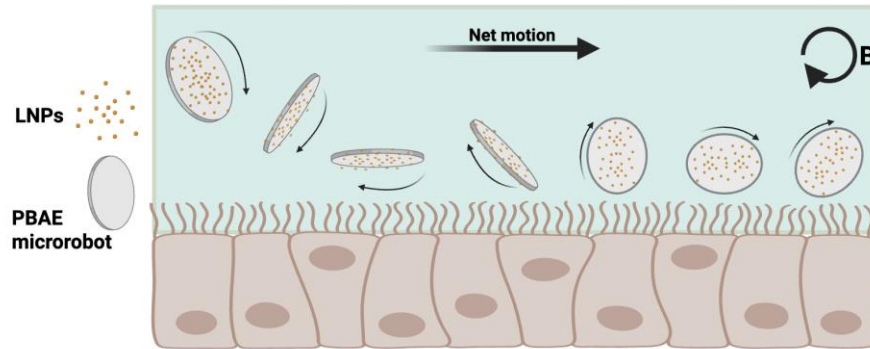
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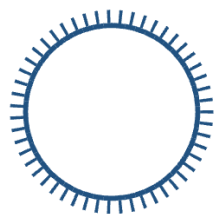


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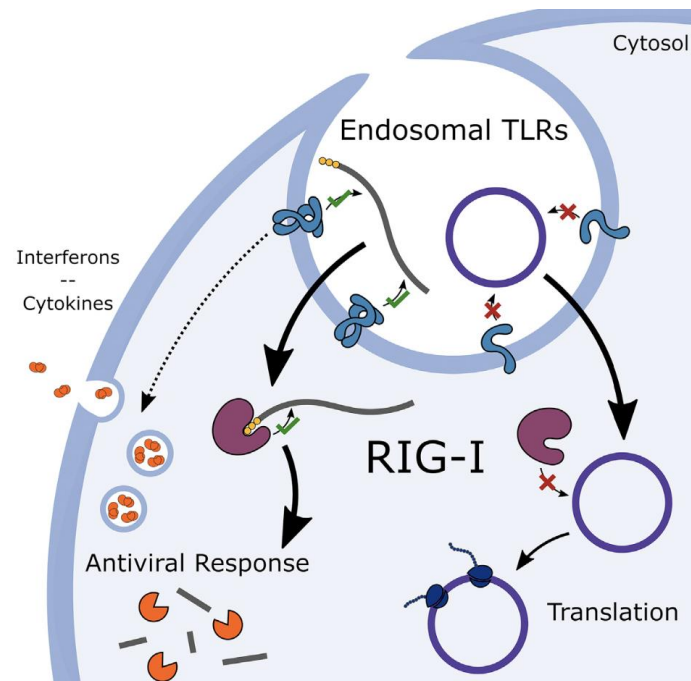
2. Short-term protein expression: circular RNA can help!



circRNA

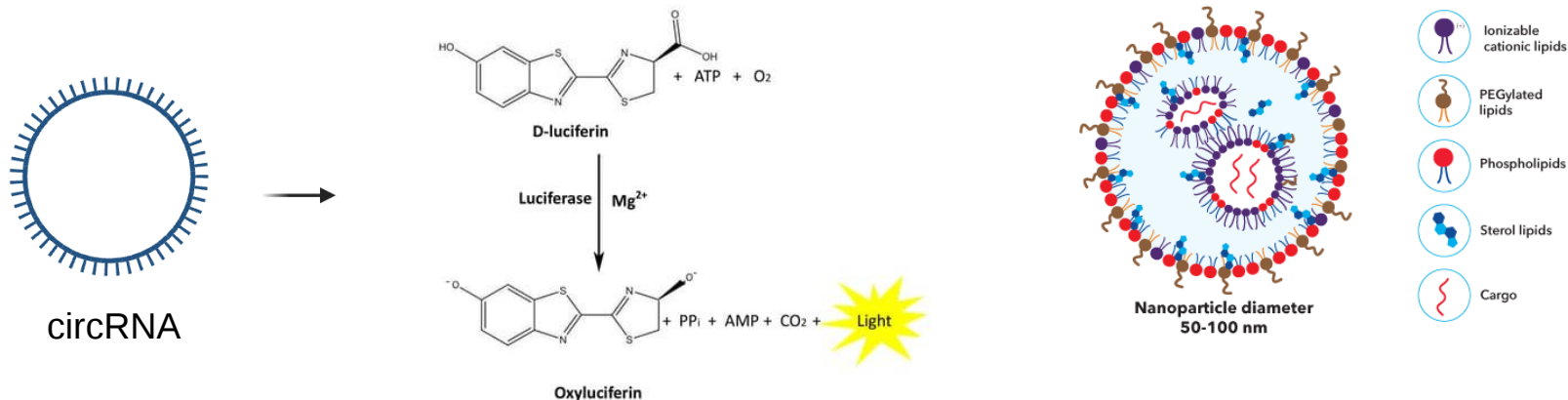


- ✓ Exonuclease resistance
- ✓ RIG and TLRs bypass
- ✓ Stable protein translation

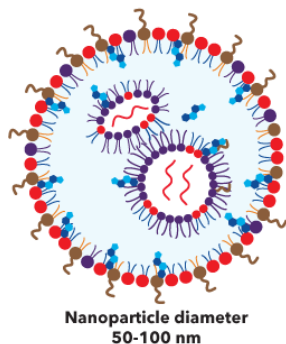


RA Wesselhoeft et al., 2019, Mol Cell

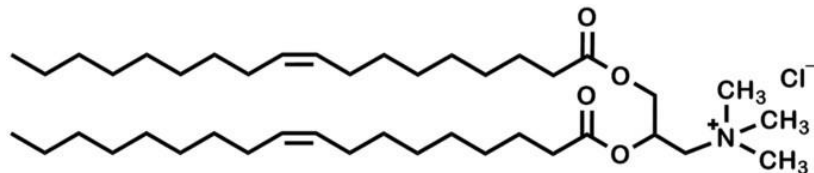
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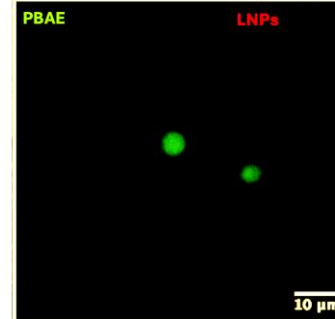
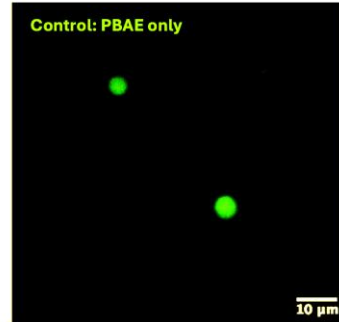
-  Ionizable cationic lipids
-  PEGylated lipids
-  Phospholipids
-  Sterol lipids
-  Cargo



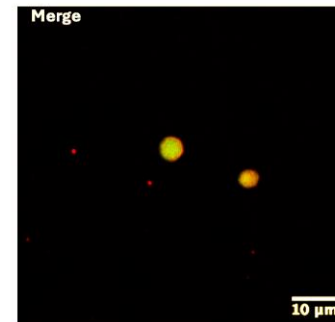
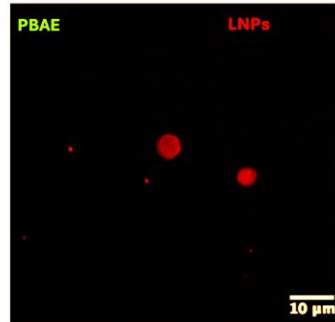
DOTAP

	circRNA 0% DOTAP	circRNA 10% DOTAP	circRNA 20% DOTAP	Linear mRNA 0% DOTAP	Linear mRNA 10% DOTAP	Linear mRNA 20% DOTAP
ζ potential (mv)	0.87	4.77	7.36	-2.62	3.34	7.61

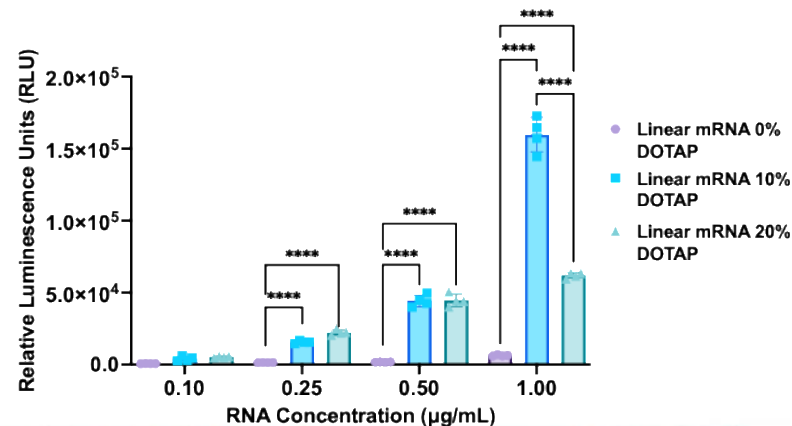
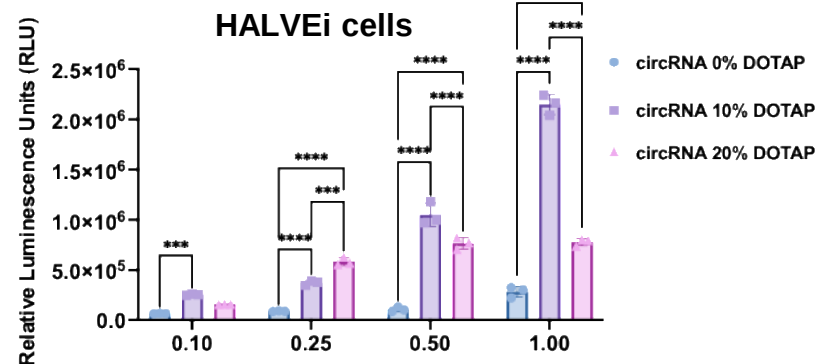
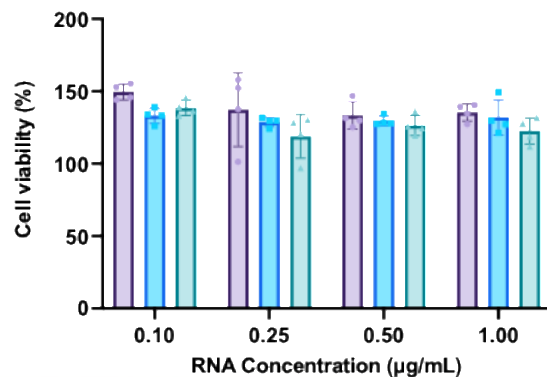
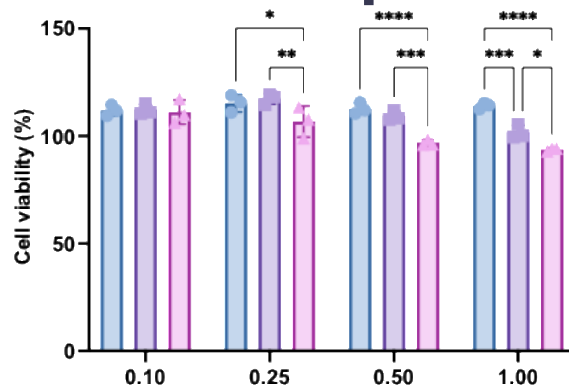
Lipid nanoparticles can be bound to PBAEs robots electrostatically



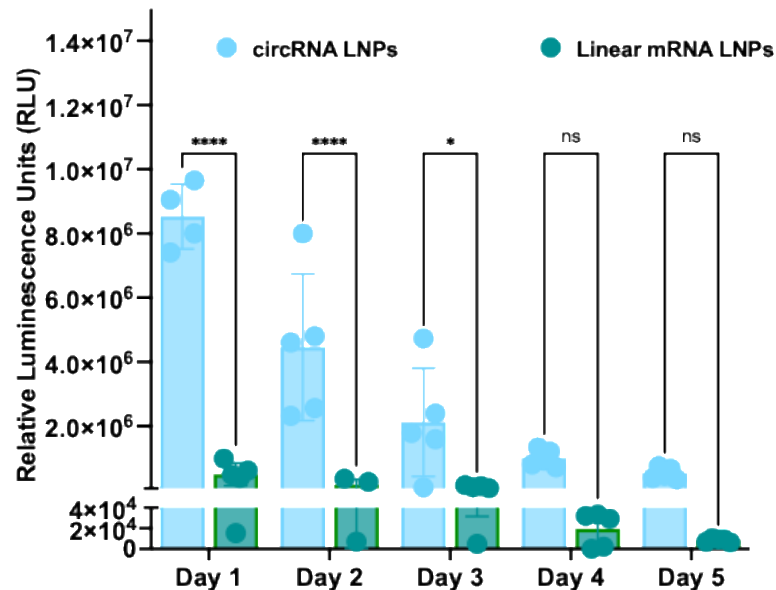
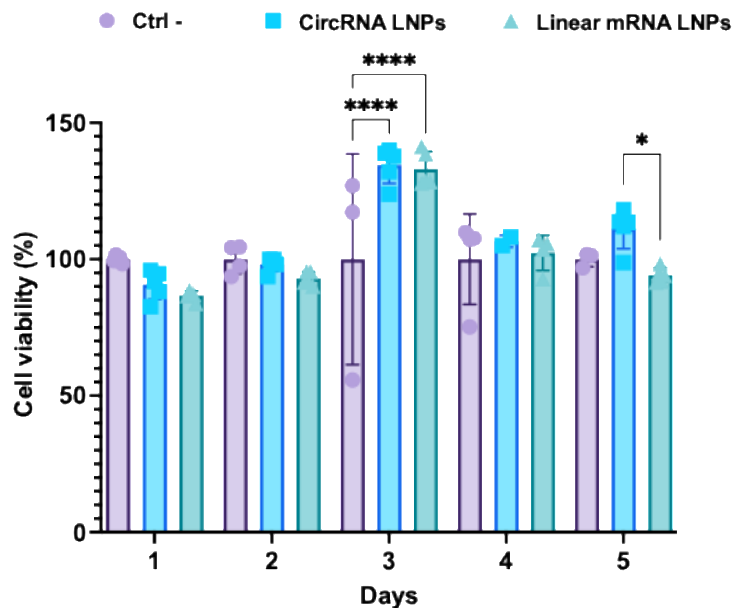
20% DOTAP LNPs



Circular RNA outperform linear mRNA *in vitro*

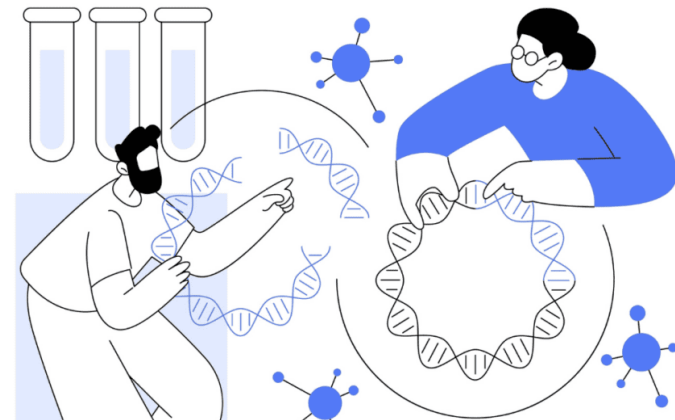


Circular RNA increases protein expression by 20-fold up to 3 days



Future steps

- Evaluate LNP loading and release from PBAE microrobots
- Air-liquid interface cell culture transfection
- *In vivo* studies



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Collin Kemper
Kendra Kreienbrink
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Vivian Nguyen
Caitlin O'Neill
Elizabeth Reichert
Joshua Smith
Matthew Stelzner



the David &
Lucile Packard
FOUNDATION



PHILADELPHIA, PA
JULY 13-18, 2025

**NEXT-GENERATION
DELIVERY INNOVATIONS**

15

JULY

TECH SESSION I:
BIOENGINEERING I



11:38 AM - 11:49 AM

**Poly(β -amino ester) Backpacks for
Adoptive Macrophage Transfers in High-
Grade Serous Carcinoma**

Courtney Bailey

17

JULY

TECH SESSION V: GENE
DELIVERY AND GENE
EDITING III



11:38 AM - 11:49 AM

**Microbot-Assisted Delivery of Circular RNA-
Lipid Nanoparticles to Treat Lung-Associated
Diseases**

Bianca Santana

17

JULY

TECH SESSION V:
NANOMEDICINE AND
NANOSCALE DELIVERY
(FOCUS: NANO)



11:38 AM - 11:49 AM

**Poly (β -amino ester) particles with
programmable shape, stiffness, and
degradation for drug release**

Katie Trese

Poster:
Immuno
Delivery
(Focus Group
- ID)



POSTER # 244

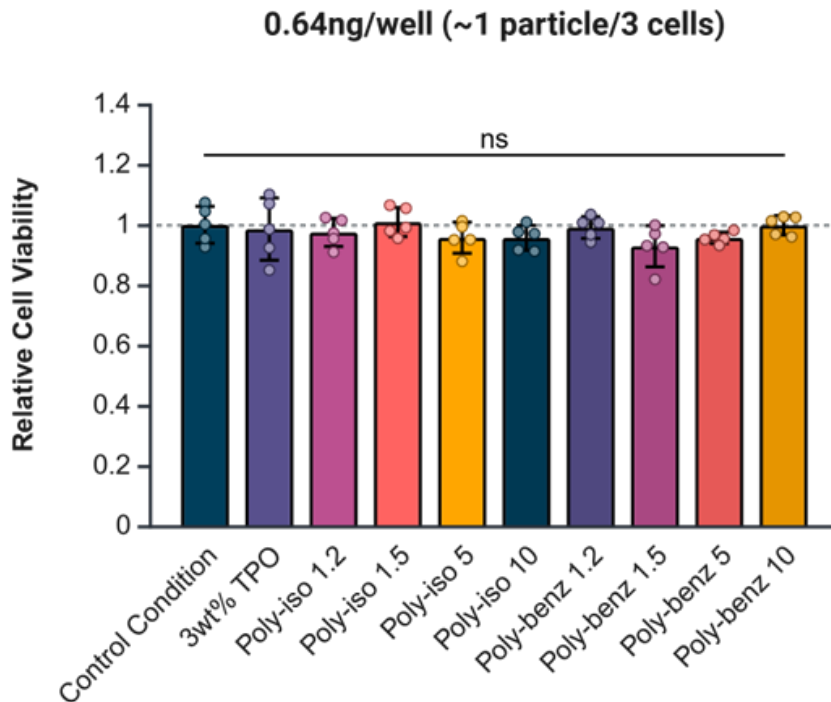
**Particle shape modulates
macrophage phenotype: Insights for
cell-mediated drug delivery systems**

Matt Kwan

Backup slides

LNP	Size (nm)	PDI	Zeta (mv)
0% DOTAP Linear	139	0.24	-2.62
10% DOTAP Linear	164	0.26	3.34
20% DOTAP Linear	254	0.28	7.61
0% DOTAP Circular	91	0.1	0.87
10% DOTAP Circular	133	0.19	4.77
20% DOTAP Circular	139	0.17	7.36

Backup slides



Backup slides

