Lipid nanoparticle-mediated metabolic reprogramming of dendritic cells for mRNA vaccines

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CRS 2025 ANNUAL MEETING & EXPOSITION
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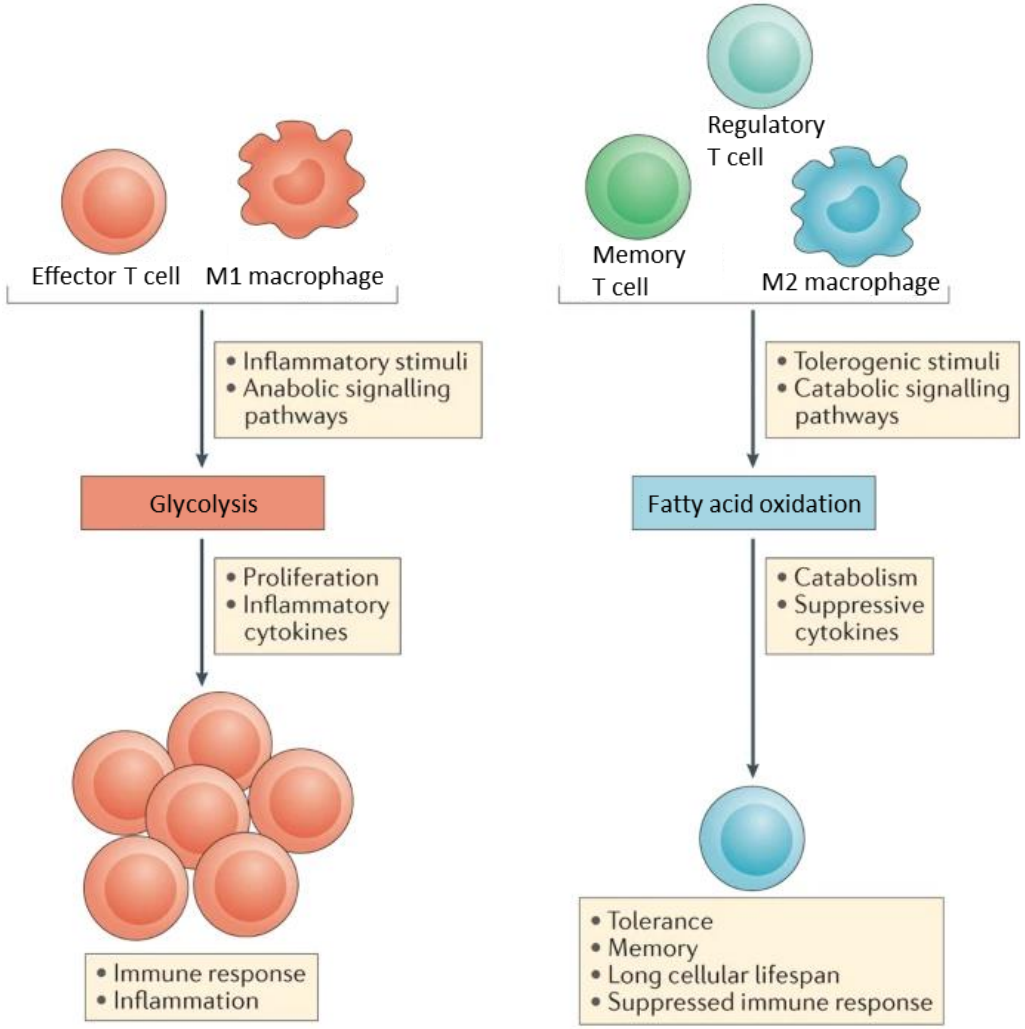
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Metabolism as a key target for immune modulation

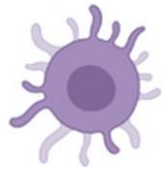
Cell type-specific metabolic traits



Nat Rev Immunol. 2016 Sep;16(9):553-65

Coordination between function and metabolism is essential for the immune responses

Effector function

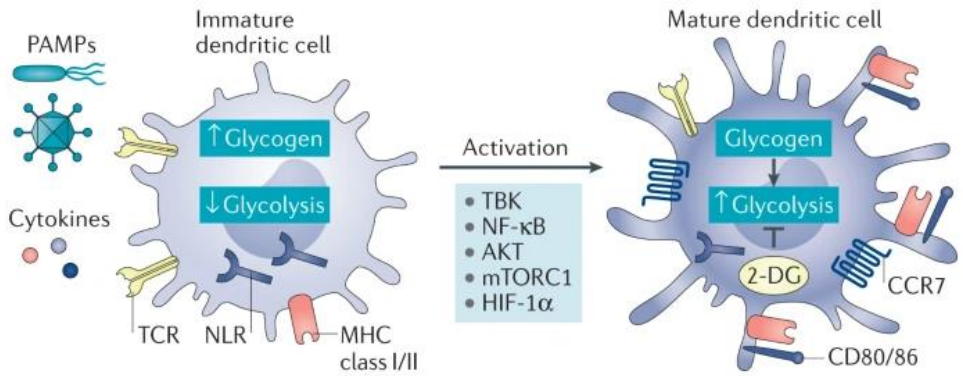


Regulatory/Memory function

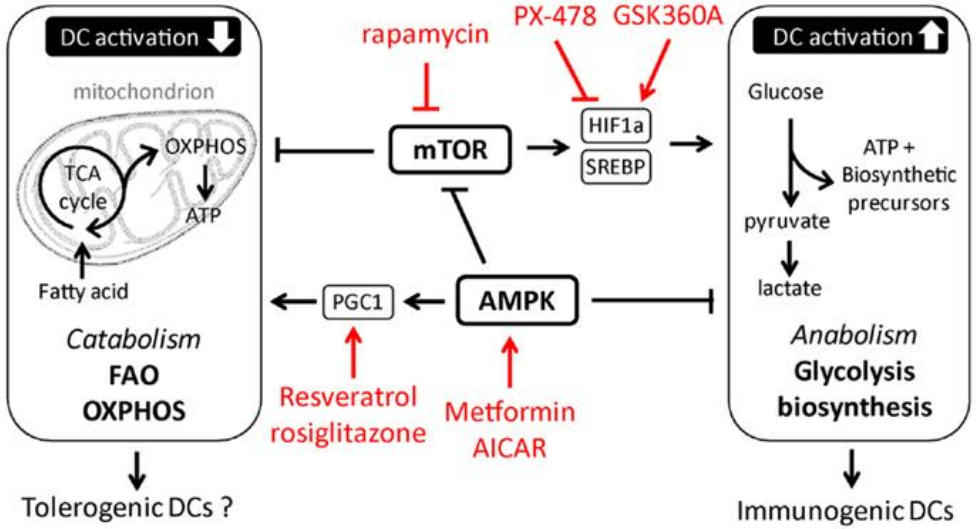
Glycolysis	Oxidative phosphorylation
Pentose phosphate pathway	Fatty acid oxidation
Fatty acid synthesis	TCA

Modulating metabolism in immune cells is an effective approach to induce desired immune responses

Metabolic modulation in dendritic cells



Nat Rev Nephrol. 2021 Jul;17(7):465-480



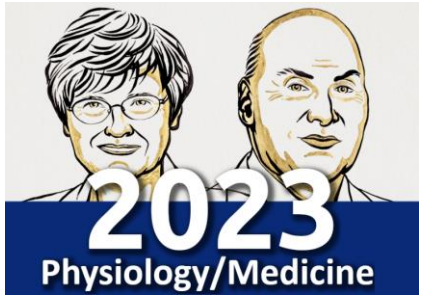
Front Immunol. 2014 May 8;5:203

- The link between cell function and metabolism is also crucial in dendritic cells
- During maturation, antigen presentation, migration, and cytokine expression primarily rely on glycolysis
- Efficient regulation of glucose metabolism and energy supply can enhance vaccine efficacy

Can we modulate dendritic cell metabolism using LNPs for mRNA vaccine?

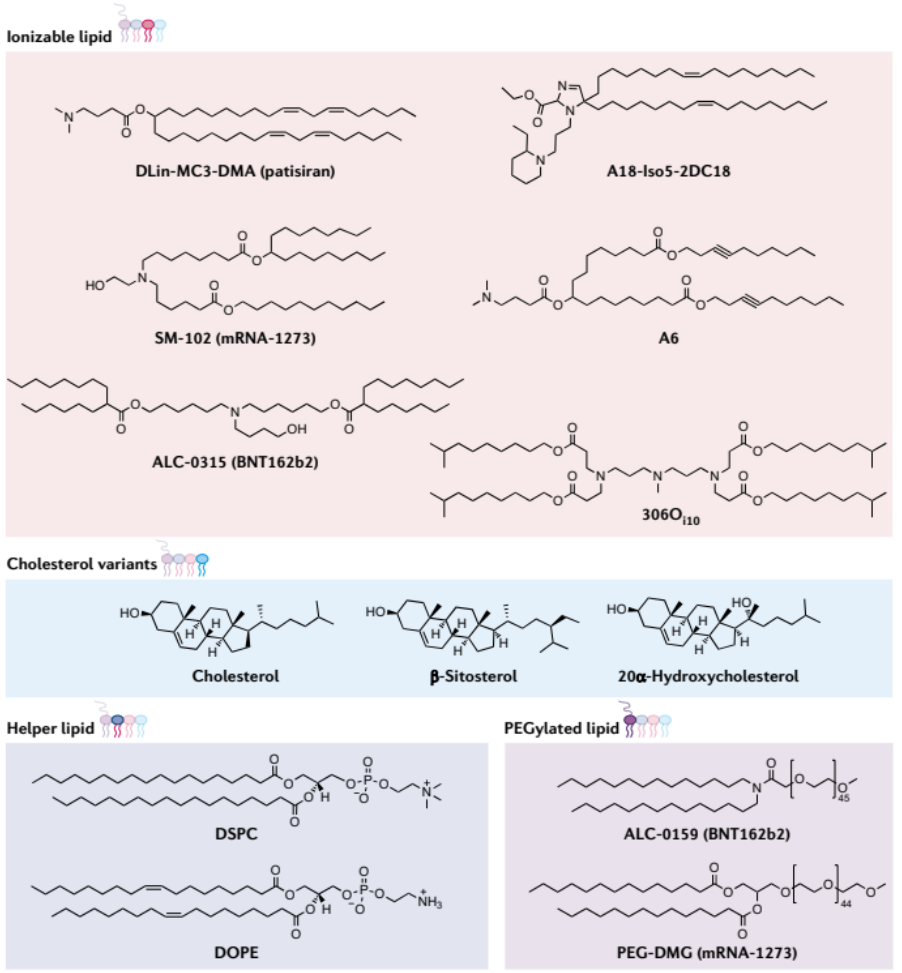
Lipid nanoparticles (LNPs) for mRNA vaccine delivery

mRNA chemistry for vaccine
(The Nobel prize, 2023)



FDA approved mRNA vaccines

FDA approval	Brand	Product	Indication	Sponsor
2020	COMIRNATY	mRNA/LNP	COVID-19	Pfizer /BioNTech
2020	SPIKEVAX	mRNA/LNP	COVID-19	Moderna
2024	mRESVIA	mRNA/LNP	Respiratory syncytial virus	Moderna



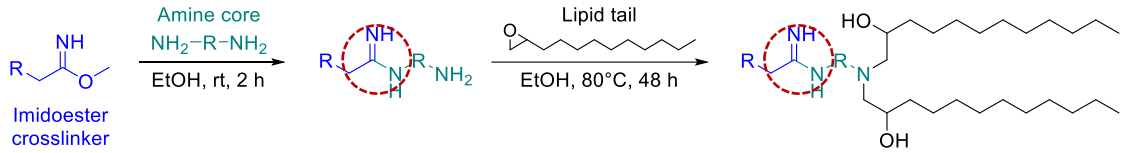
Nat Rev Drug Discov. 2021 Nov;20(11):817-838

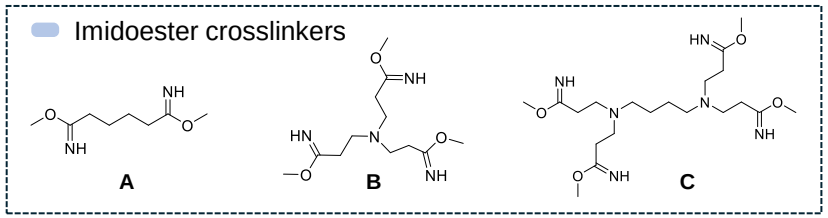
LNPs are the most clinically advanced delivery vehicle for vaccines

The transfection potency of LNP is critically dependent on the ionizable cationic lipid component

Crosslinked ionizable lipids for metabolic regulation

Ionizable lipid library design

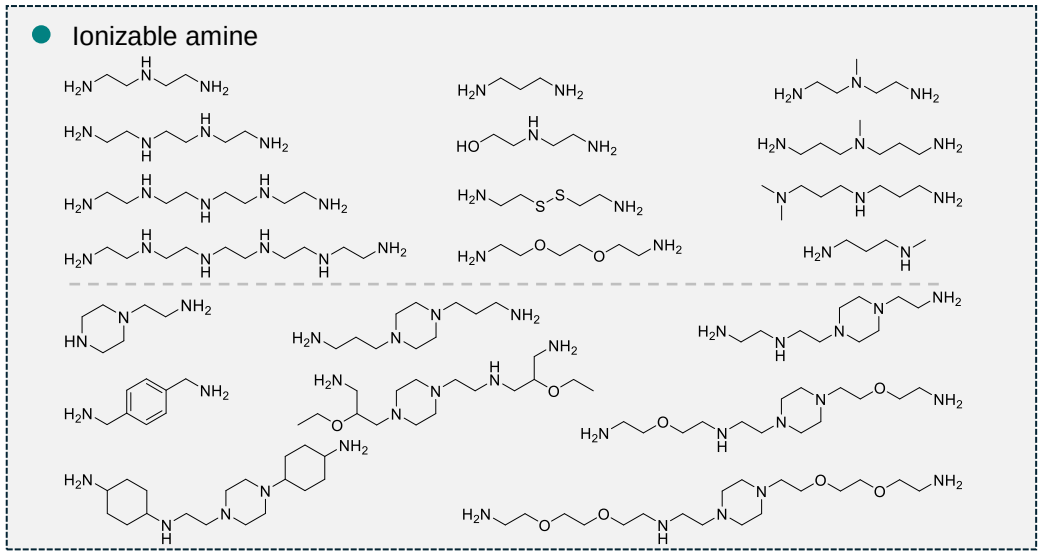




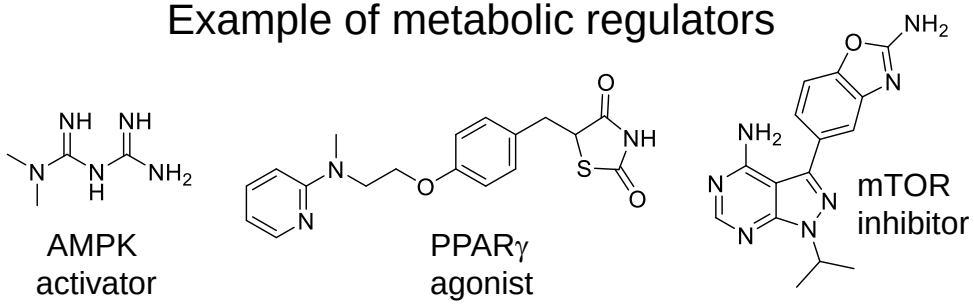
A: 2-arm imidoester (2a)

B: 3-arm imidoester (3a)

C: 4-arm imidoester (4a)



Example of metabolic regulators



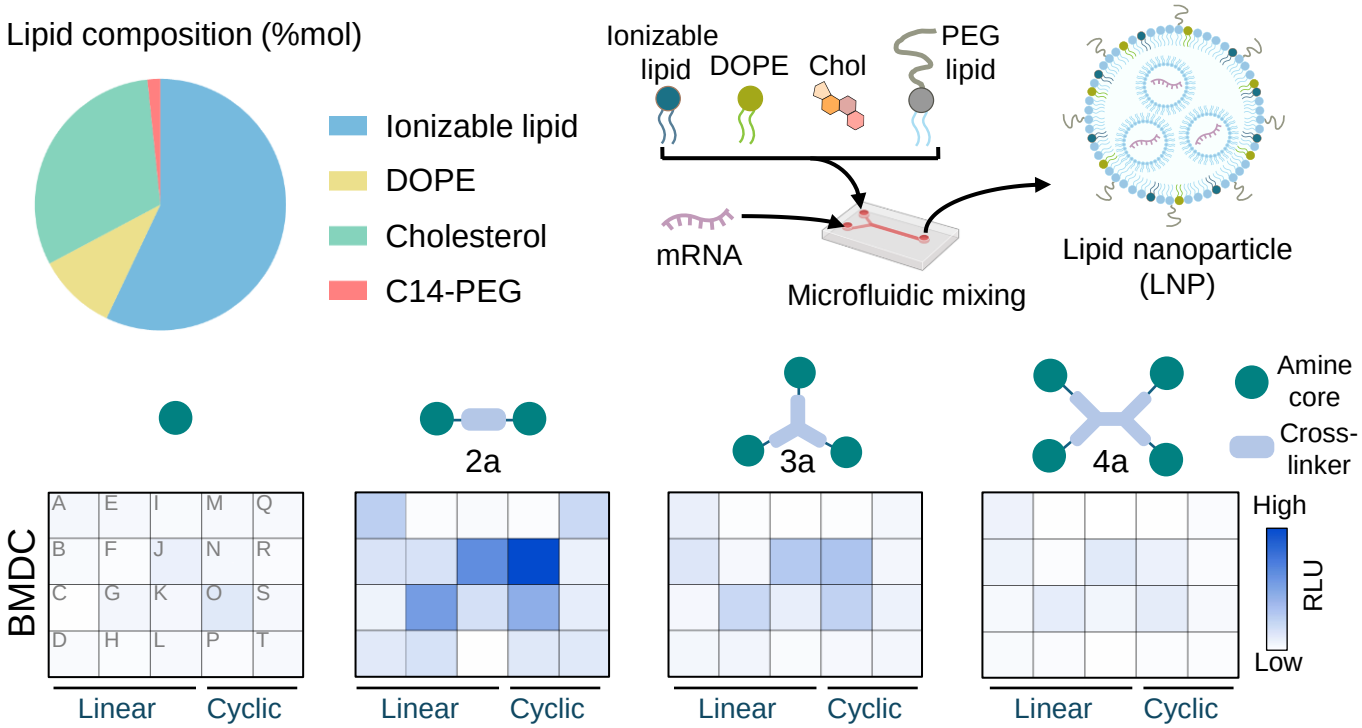
- Reaction between imidoester crosslinkers and amine compounds followed by C12 lipid conjugation created **amidine** incorporated ionizable lipids

- The rapid and straightforward reaction generated a library of novel ionizable lipids in a high throughput manner.

Kim et al., in revision

Crosslinked ionizable lipids for metabolic regulation

In vitro LNP screening in BMDCs



- *In vitro* mLuc transfection to bone marrow-derived dendritic cells (BMDCs)
- The 2a-based ionizable lipids improved mRNA transfection
- C12-2aN was derived as a top-performing ionizable lipid

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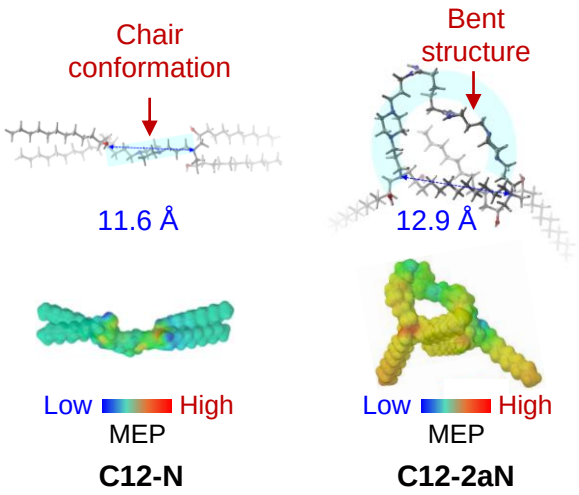
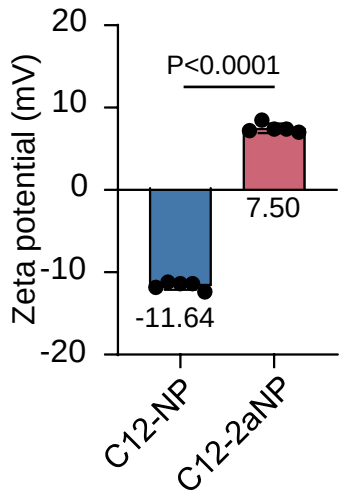
Impact of crosslinkers on lipid structure and mRNA delivery

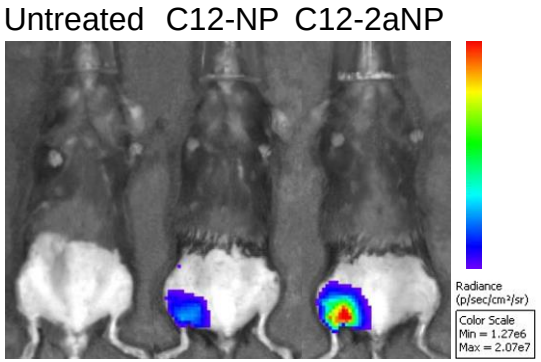
Lipid characterization

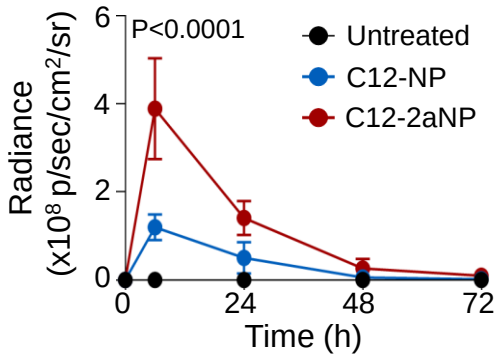




- C12-2aN has a bent structure, adopting conical shape, which may promote the hexagonal phase transition of the lipid bilayer

Intramuscular injection of LNP





- The insertion of amidines resulted in a positive surface charge of the C12-2aN based LNP (C12-2aNP)
- The 2a-based ionizable lipids improved *in vivo* mRNA transfection

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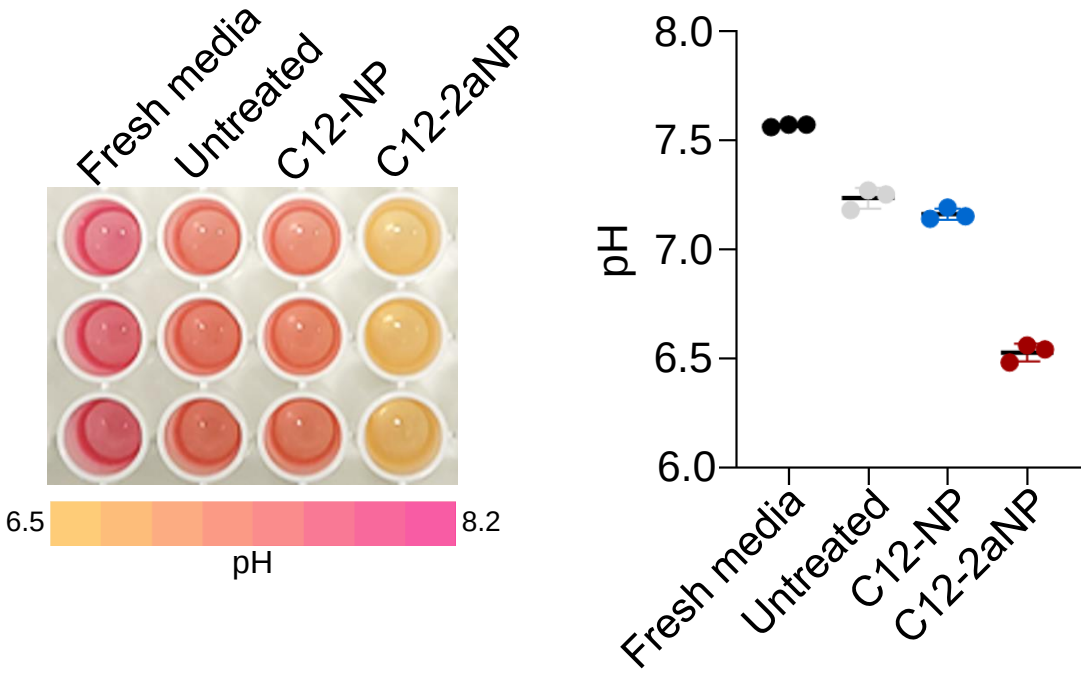
Crosslinked LNP induces a metabolic switch to glycolysis in dendritic cells



- A significant decrease in cell media pH was observed after 24 h of LNP incubation with BMDCs
- Acidification of the medium is indicative of proton and lactate secretion resulting from an elevated cellular metabolic rate

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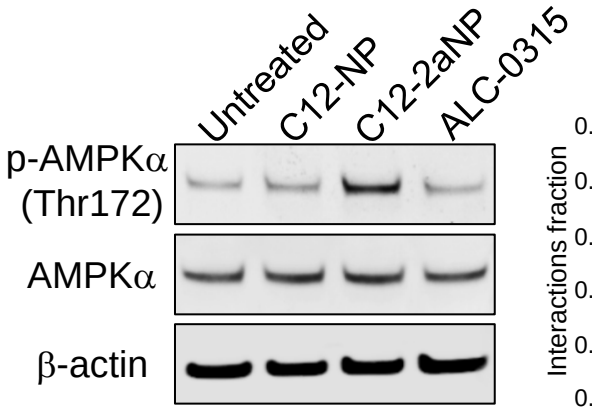
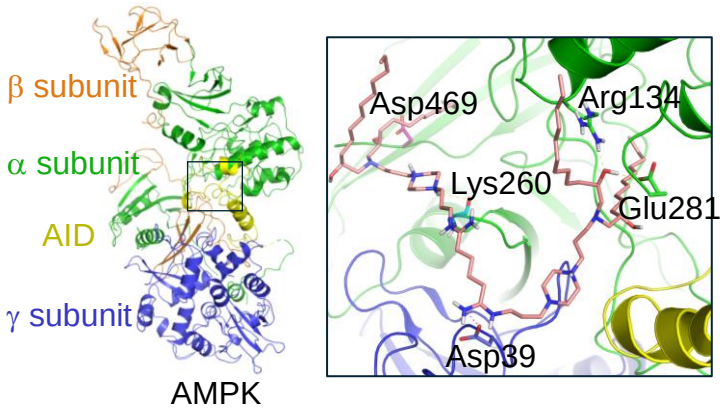
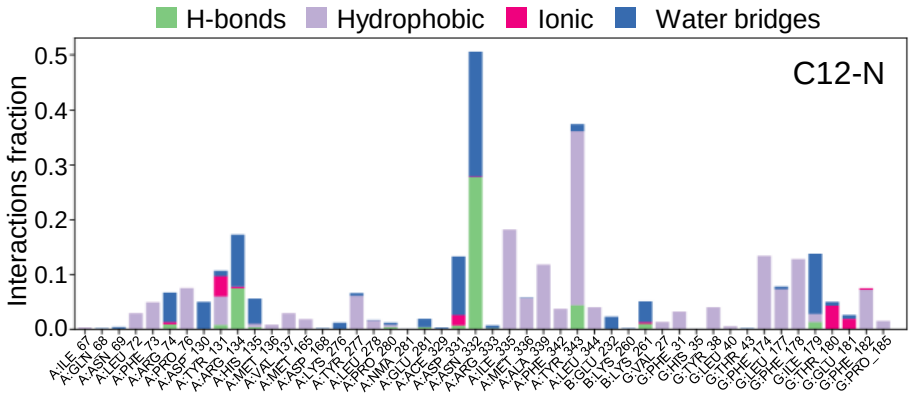
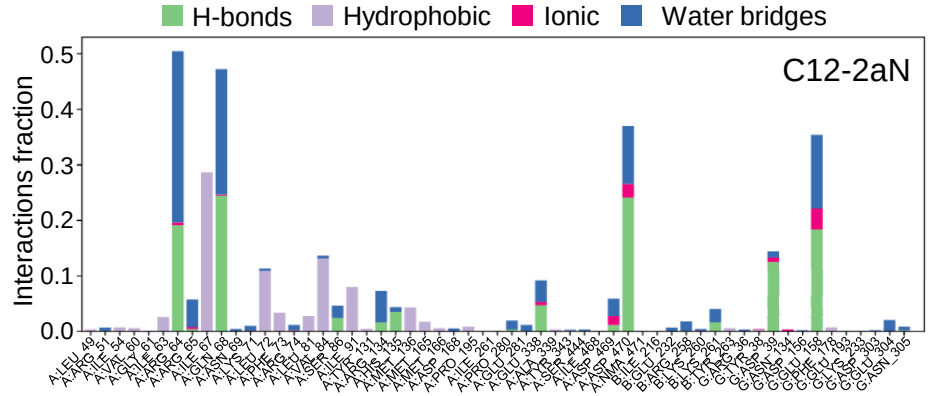
Crosslinked LNP induces a metabolic switch to glycolysis in dendritic cells









- Western blot analysis showed a significant increase in the phosphorylation of AMPKα, a key regulator of cellular metabolism
- As the linker functions as a stronger proton donor and provides additional charge for interactions, C12-2aN exhibited an increased hydrophilic interactions fraction compared to C12-N

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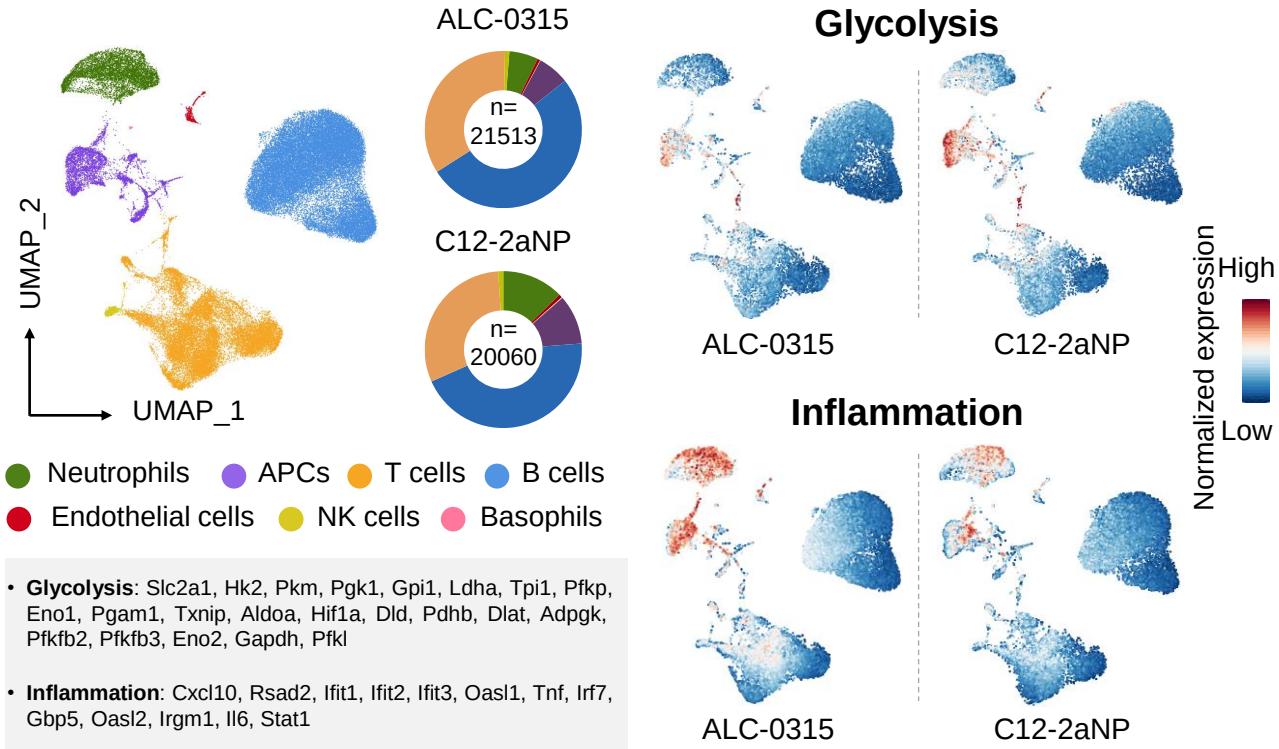
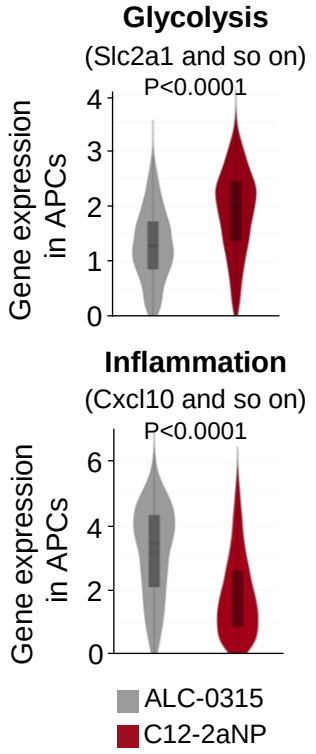
Selective stimulation of glycolysis in dendritic cells by crosslinked LNP

Single-cell RNA sequencing of CD45$^+$ immune cells



- **Glycolysis**: Slc2a1, Hk2, Pkm, Pgk1, Gpi1, Ldha, Tpi1, Pfkp, Eno1, Pgam1, Txnip, Aldoa, Hif1a, Dld, Pdhb, Dlat, Adpgk, Pfkfb2, Pfkfb3, Eno2, Gapdh, Pfkl
- **Inflammation**: Cxcl10, Rsad2, Ifit1, Ifit2, Ifit3, Oasl1, Tnf, Irf7, Gbp5, Oasl2, Irgm1, Il6, Stat1



- In the APC clusters, cells from the C12-2aNP group exhibited significantly higher expression levels of glycolysis-associated genes compared to those from the ALC-0315 group
- On the other hand, the expression of acute inflammation-related genes was notably elevated in both APC and neutrophil clusters in the ALC-0315 group, whereas it was significantly reduced in the C12-2aNP group

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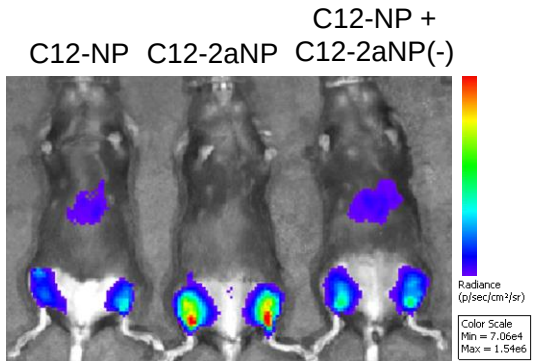
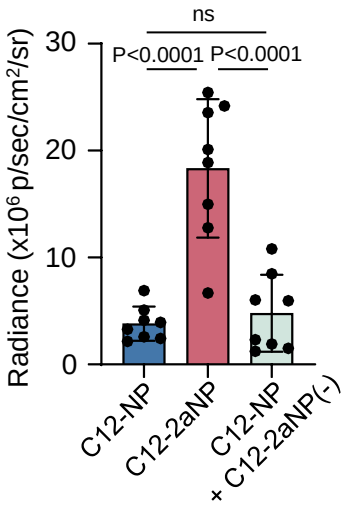
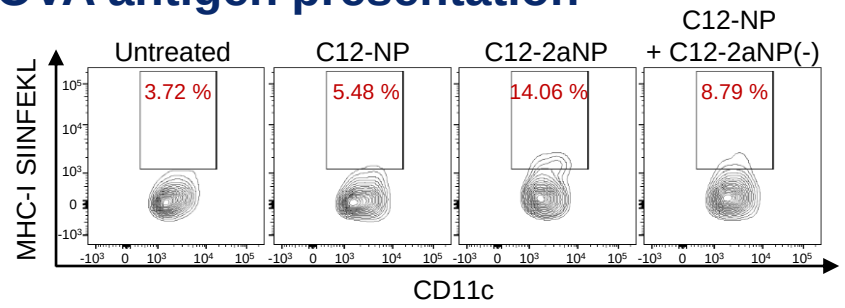
Increased glycolysis induced by crosslinked LNPs leads to enhanced antigen presentation in dendritic cells

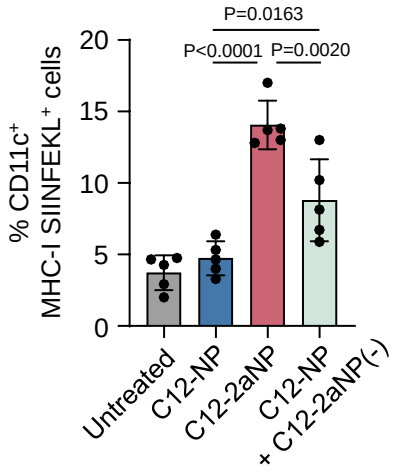
mRNA expression





OVA antigen presentation





C12-2aNP(-) : C12-2aNP with non-functional mRNA

- While co-treatment with C12-NP and C12-2aNP(-) resulted in comparable mRNA transfection efficiency compared to C12-NP alone, it significantly enhanced OVA antigen presentation, indicating that glycolysis stimulation is critical for effective antigen presentation
- This effect was further amplified by C12-2aNP treatment, suggesting that both enhanced glycolysis and efficient mRNA transfection are essential for optimal antigen presentation in dendritic cells.

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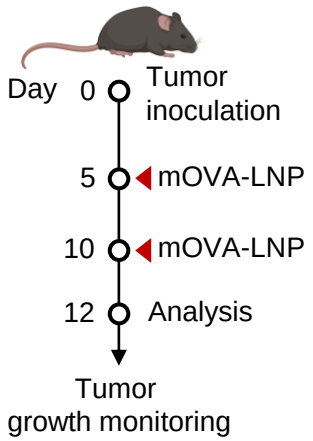
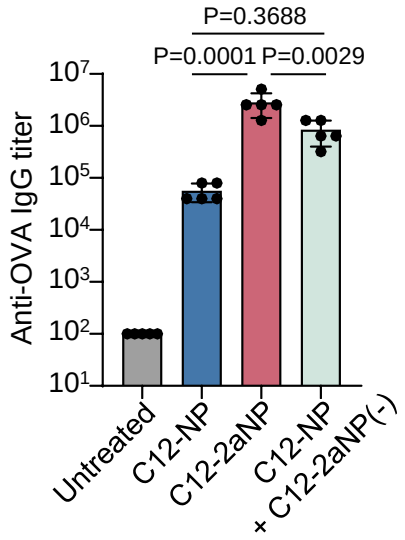
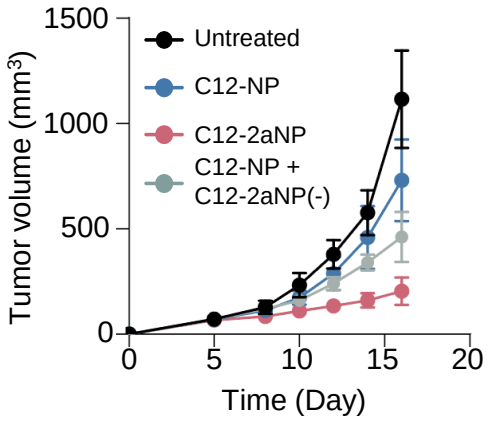
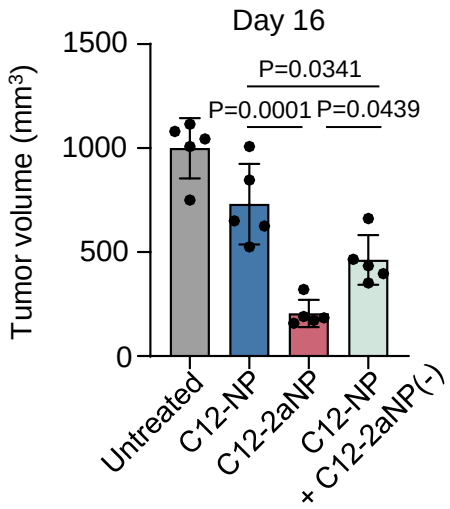
Increased glycolysis induced by crosslinked LNPs improves vaccine efficacy in a cancer vaccine model









- Co-administration of C12-NP with C12-2aNP(-)—thereby selectively promoting glycolysis—enhanced both humoral and cellular immune responses in OVA cancer vaccine model
- Increased glycolysis led to a substantial reduction in tumor size
- As observed with C12-2aNP treatment, achieving optimal vaccine efficacy requires the simultaneous induction of efficient mRNA delivery and metabolic reprogramming.

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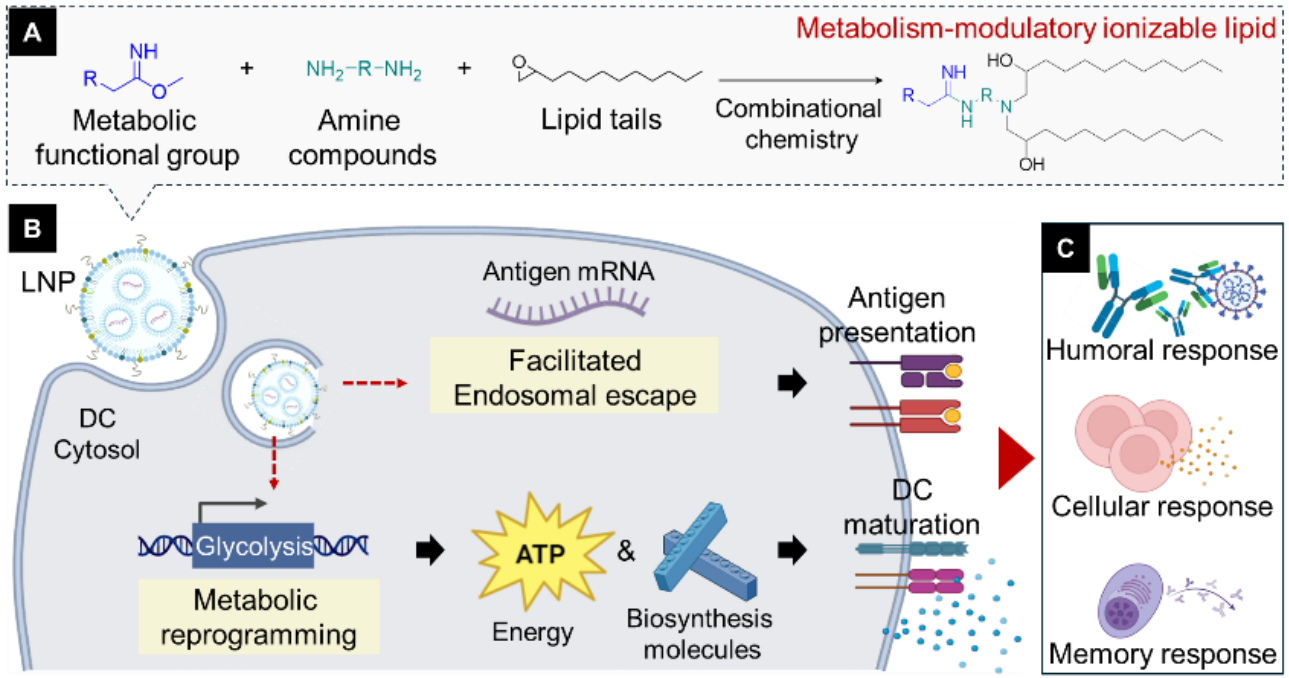
Summary & Future direction



- High-throughput synthesis of ionizable lipids with an imidoester crosslinker enabled the discovery of a multifunctional ionizable lipid, C12-2aN
- C12-2aNP-mediated stimulation of glycolysis enhanced antigen presentation, leading to improved vaccine efficacy
- Overall, the integration of mRNA delivery and metabolic reprogramming presents significant potential for next-generation mRNA LNP vaccines

- The rational design of ionizable lipid chemistry to modulate immune cell metabolism may open new avenues in immune engineering beyond vaccines.

Acknowledgement

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