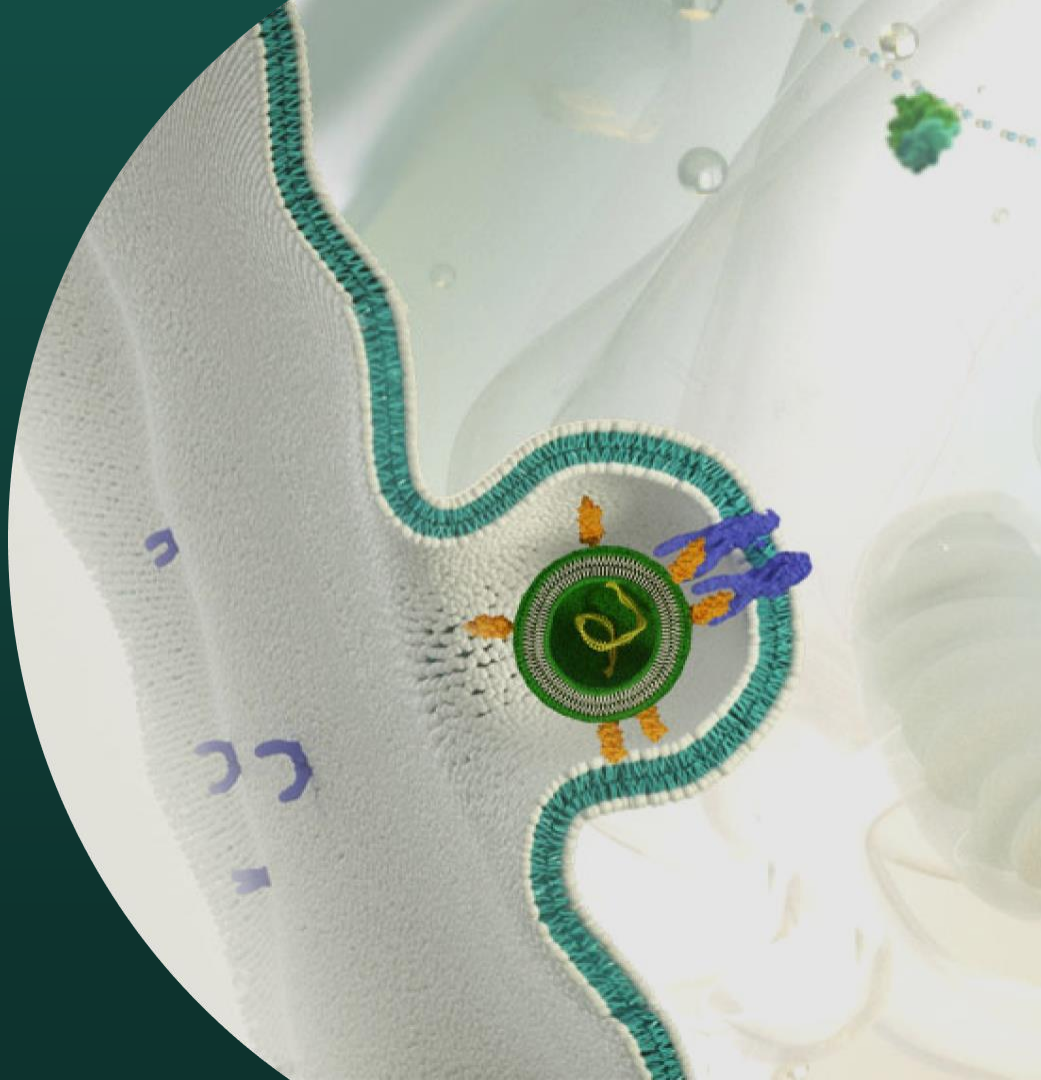
ACUITAS THERAPEUTICS

Optimization of ALC-315® yields superior ionizable lipids for LNP mediated nucleic acid delivery

Polina Blagojevic, PhD



The Rise of Nucleic Acid Based Therapeutics

… not just a prophylactic infectious disease vaccine platform

.

Gene Editing
Expression of a **genome editing** protein to modify gene expression.

Gene Modulation
Expression of an **epigenic editor** to modify gene expression **without changing the genetic code.**

Vaccines
Intracellular expression of viral or bacterial proteins generating protective **immune response.**

Expression of **tumour antigens** (personalized vaccines).

Antibodies
Expression of **prophylactic or therapeutic antibodies** to treat current and emerging diseases.

Protein Replacement & Therapeutic Protein Delivery
Expression of a **human protein** to address genetic disorders (i.e., hemophilia, cystic fibrosis, etc.).

Acuitas technology enabled life saving treatment

The NEW ENGLAND JOURNAL of MEDICINE

CURRENT ISSUE SPECIALTIES TOPICS

ORIGINAL ARTICLE | BRIEF REPORT

Patient-Specific In Vivo Gene Editing to Treat a Rare Genetic Disease

Authors: Kiran Musunuru, M.D., Ph.D. , Sarah A. Grandinette, B.S., Xiao Wang, Ph.D., Taylor R. Hudson, M.S., Kevin Briseno, B.S., Anne Marie Berry, M.S., Julia L. Hacker, M.S., Alvin Hsu, B.S., Rachel A. Silverstein, B.S., Logan T. Hille, Ph.D., Aysel N. Ogul, Nancy A. Robinson-Garvin, Ph.D., Juliana C. Small, Ph.D., Sarah McCague, M.S., Samantha M. Burke, B.S.N., Christina M. Wright, M.D., Ph.D., Sarah Bick, M.D., Venkata Indurthi, Ph.D., Shweta Sharma, M.S., Michael Jepperson, M.S., Christopher A. Vakulskas, Ph.D. , Michael Collingwood, B.S., Katie Keogh, Ph.D., Ashley Jacobi, B.S., Morgan Sturgeon, Ph.D., Christian Brommel, M.S., Ellen Schmaljohn, Ph.D., Gavin Kurgan, Ph.D., Thomas Osborne, B.S., He Zhang, Ph.D., Kyle Kinney, Ph.D. , Garrett Rettig, Ph.D., Christopher J. Barbosa, Ph.D., Sean C. Semple, Ph.D., Ying K. Tam, Ph.D., Cathleen Lutz, Ph.D., Lindsey A. George, M.D., Benjamin P. Kleinstiver, Ph.D., David R. Liu, Ph.D., Kim Ng, M.D., Sadik H. Kassim, Ph.D., Petros Giannikopoulos, M.D., Mohamad-Gabriel Alameh, Ph.D., Fyodor D. Urnov, Ph.D. , and Rebecca C. Ahrens-Nicklas, M.D., Ph.D. -37 Author Info & Affiliations

Published May 15, 2025 | N Engl J Med 2025;392:2235-2243 | DOI: 10.1056/NEJMoa2504747 | VOL. 392 NO. 22

Desperately ill baby healed with personalized gene therapy, doctors say

Baby one of 350 million people worldwide with rare diseases

The Associated Press · Posted: May 15, 2025 1:43 PM PDT | Last Updated: May 15, 2025

Listen to this article
Estimated 6 minutes

KJ Muldoon after a follow up dose of an experimental gene editing treatment at the hospital in April 2025. (Chloe Dawson/Children's Hospital of Philadelphia/The Associated Press)

ACUITAS THERAPEUTICS

Why LNP are Important

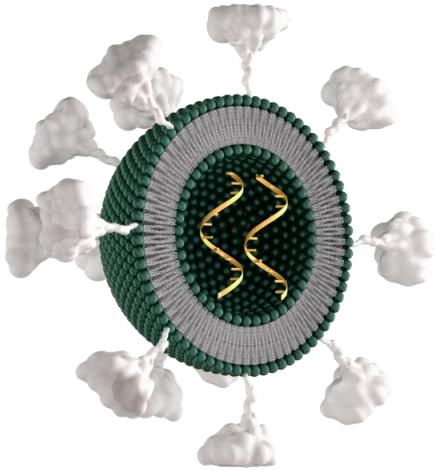
LNP protect the nucleic acid, enabling its delivery into the patient's cells

THE CURRENT CHALLENGE

New therapeutic drugs and vaccines based on nucleic acids are only effective if delivered into cells successfully.

Nucleic acids / mRNA are poor drug substances on their own.

An enabling delivery technology that efficiently & safely delivers these drugs is required.



THE SOLUTION

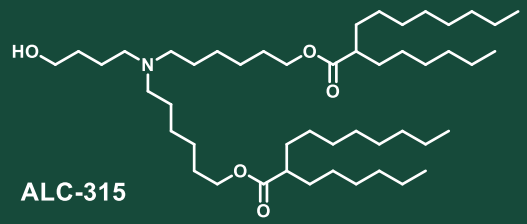
Utilizing LNPs, the nucleic acid is protected and can be successfully delivered into cells.

Composition of Contemporary LNP

Typical Characterization:

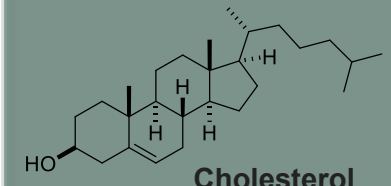
Apparent pKa	RNA content
Encapsulation	Lipid content
Particle size (PDS)	Nucleic acid integrity/purity
Polydispersity index (PDI)	Lipid adducts

Ionizable lipid



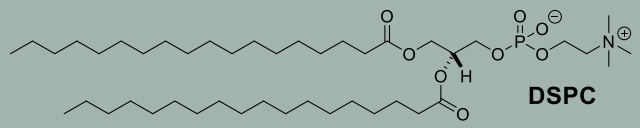
ALC-315

Sterol



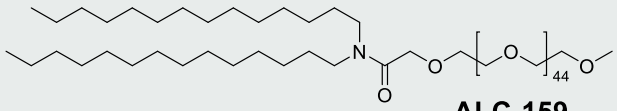
Cholesterol

Phospholipid



DSPC

PEG lipid



ALC-159

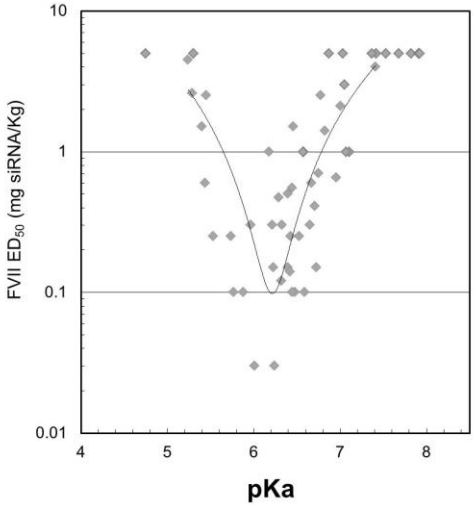
ACUITAS THERAPEUTICS

Specific pKa Required for LNP Activity

Both LNP formation and performance are strongly pH dependent.

Intrinsic pKa of an ionizable lipid vs. apparent pKa of an LNP

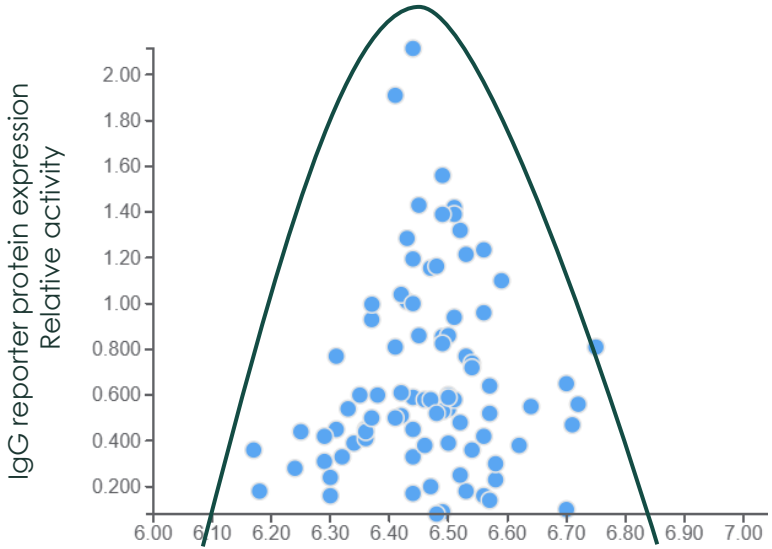
The narrow ideal apparent pKa range represents balance between requirement for:



- Minimum surface charge in the blood compartment (safety)
- Maximum surface charge in acidified endosomes (potency)

Angew. Chem. Int. Ed. 2012, 51, 8529 –8533: Maximum gene silencing associated with a narrow pKa range (siRNA)

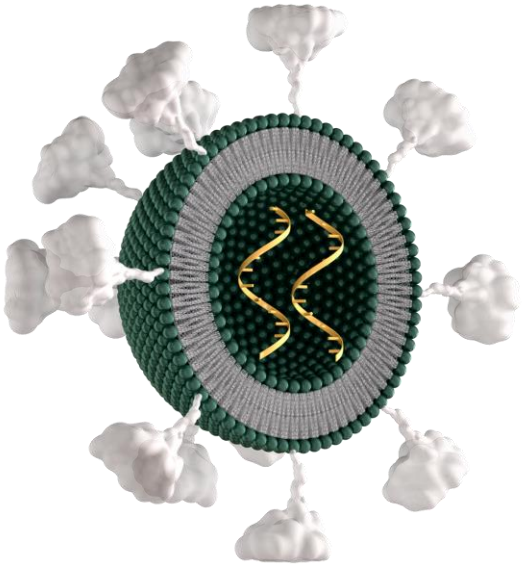
In vivo relative activity vs. pKa
mRNA-LNP



Apparent pKa: necessary but not sufficient requirement for high activity

ACUITAS THERAPEUTICS

Ionizable lipids are key components of LNPs



Ionizable lipid structure drives **apparent pKa** and other LNP properties, including encapsulation, particle size, polydispersity index, activity, and RNA-adduct formation

RESEARCH ARTICLE | MEDICAL SCIENCES

Rational design and modular synthesis of biodegradable ionizable lipids via the Passerini reaction for mRNA delivery

Yue Xu, Fanglin Gong, Alex Golubovic, +5, and Bowen Li Authors Info & Affiliations

Edited by Samir Mitragotri, Harvard University, Boston, MA; received May 13, 2024; accepted January 2, 2025 by Editorial Board Member Rakesh K. Jain

January 30, 2025 | 122 (5) e2409572122 | https://doi.org/10.1073/pnas.2409572122

Article | Open access | Published: 30 December 2024

Artificial intelligence-driven rational design of ionizable lipids for mRNA delivery

Wei Wang, Kepan Chen, Ting Jiang, Yiyang Wu, Zheng Wu, Hang Ying, Hang Yu, Jing Lu, Jinzhong Lin & Defang Ouyang

Nature Communications **15**, Article number: 10804 (2024) | Cite this article

RESEARCH ARTICLE | MEDICAL SCIENCES

Combinatorial design of ionizable lipid nanoparticles for muscle-selective mRNA delivery with minimized off-target effects

Jingan Chen, Yue Xu, Muye Zhou, +7, and Bowen Li Authors Info & Affiliations

Edited by Craig Hawker, University of California Santa Barbara, Santa Barbara, CA; received June 5, 2023; accepted October 30, 2023

December 7, 2023 | 120 (50) e2309472120 | https://doi.org/10.1073/pnas.2309472120

ACUITAS THERAPEUTICS

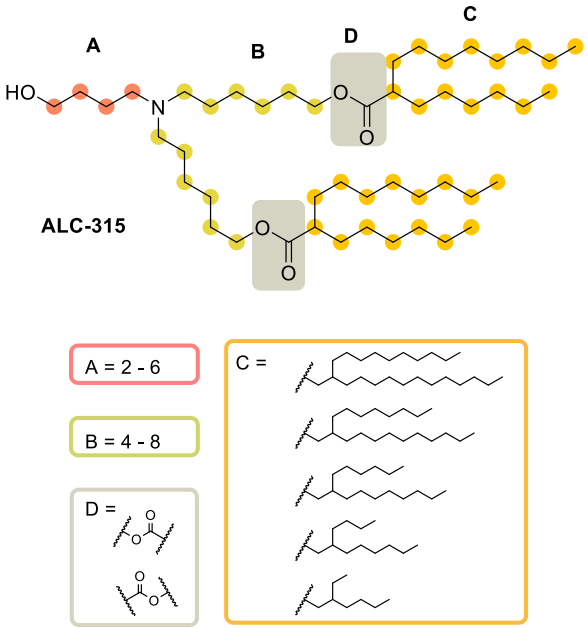
Structural space of ionizable lipids is complex

Even minimal structural changes can significantly affect LNP properties and performance.

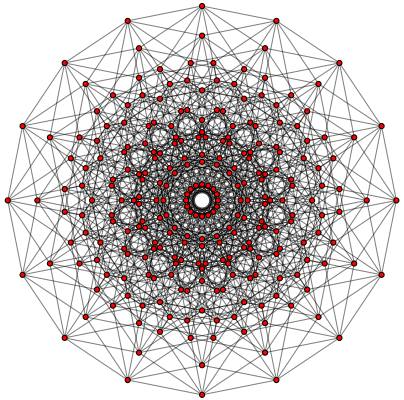
Blind combinatorial exploration of vast chemical space risks missing optimal ionizable lipids.

How do we explore such a broad structural space?

Rational design with structural iterations around proven chemical matter.

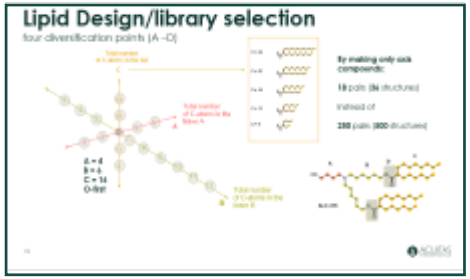


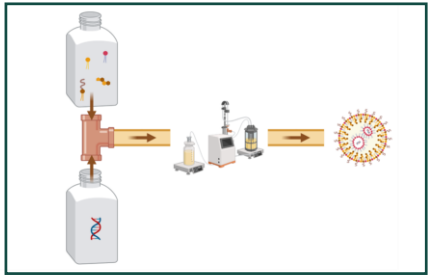
If A, B and C can have 5 values each and D has 2 possible values, there are already 250 theoretical unique structures.

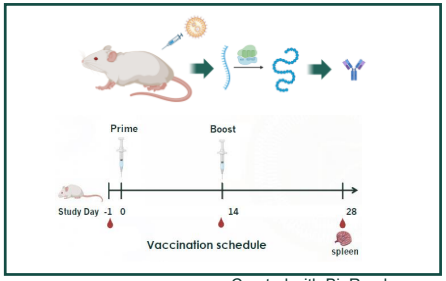


ACUITAS THERAPEUTICS

The search for active ALC-315 analogues







Created with BioRender.com

Lipid Design/Synthesis

- ALC-315 analogues
- Rational design
- Iterative approach

Pilot Formulation

- pKa **5.8-6.8**
- Encapsulation **> 80%**
- Size < **95nm**
- PDI < **0.2**

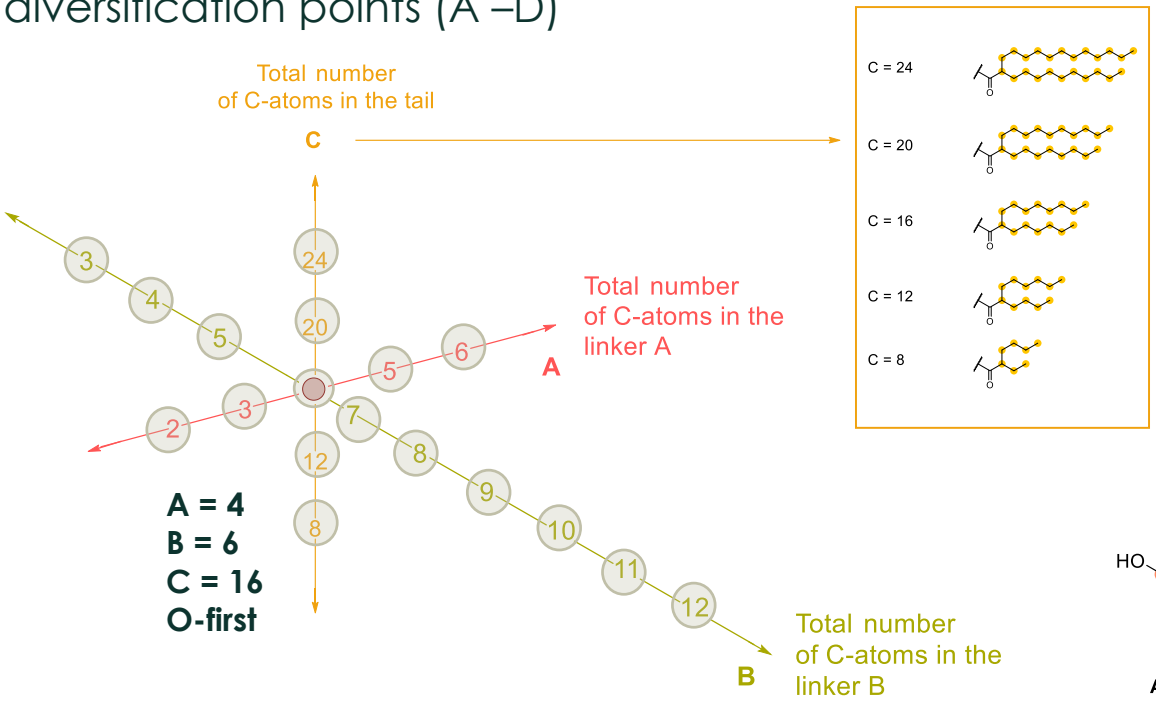
***In vivo* screening**

- IgG reporter protein expression
- Influenza A virus (A/Puerto Rico/8/1934 (H1N1)) mRNA immunization

ACUITAS THERAPEUTICS

Lipid Design/library selection

four diversification points (A –D)

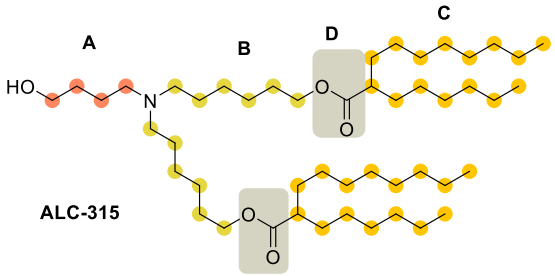


By making only axis compounds:

18 pairs (**36** structures)

instead of

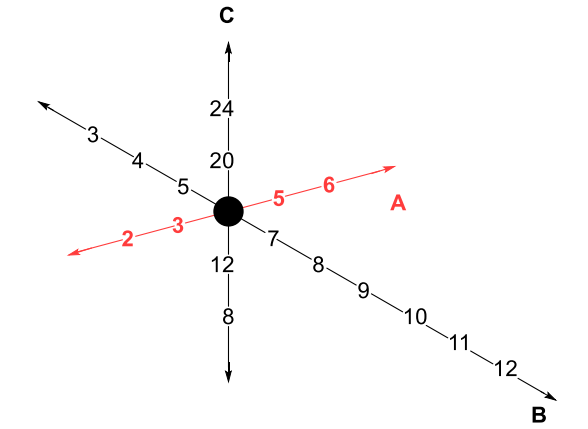
250 pairs (**500** structures)

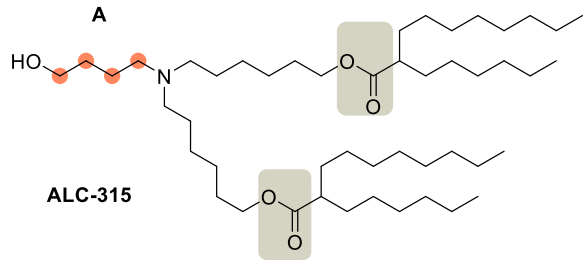


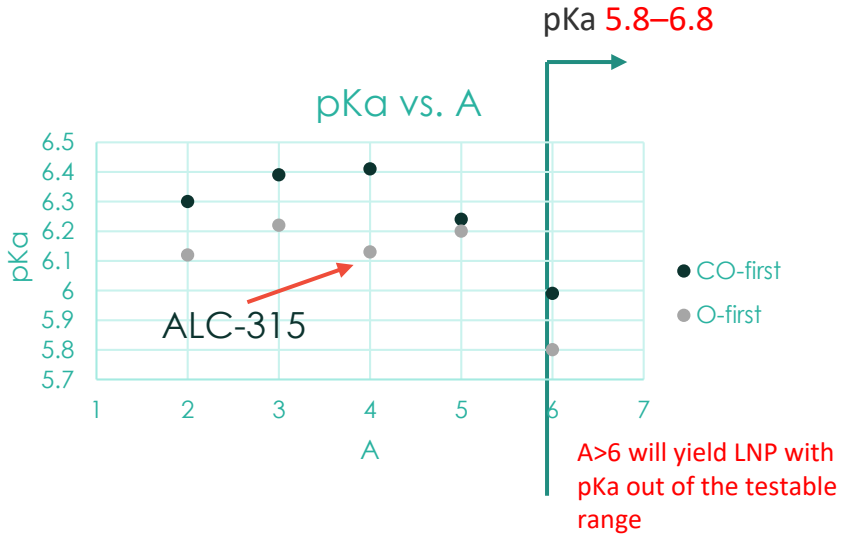
ACUITAS THERAPEUTICS

ALC-315 analogues: variations in linker A

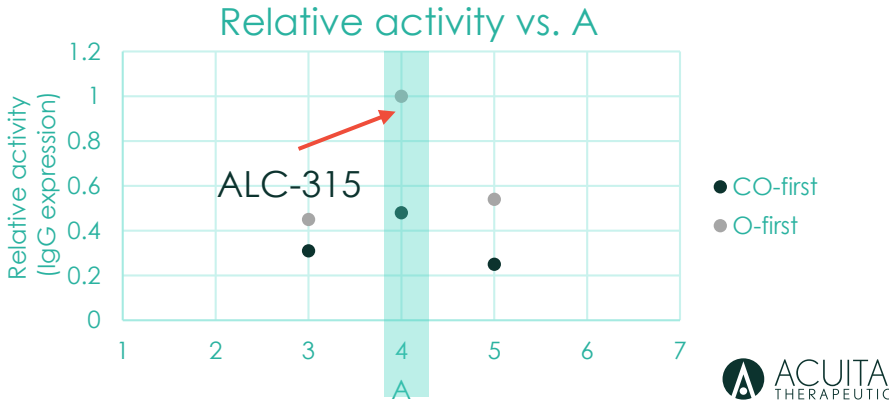
A = 2 – 6, **B** fixed at 6, **C** fixed at 16







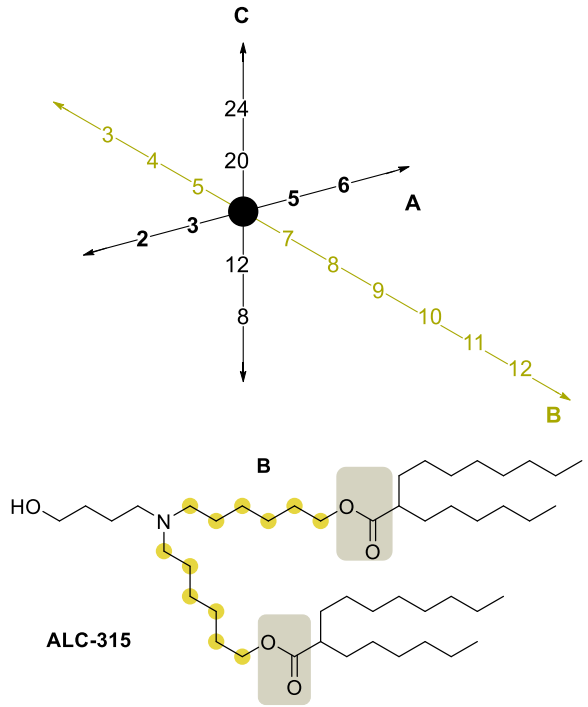
A>6 will yield LNP with pKa out of the testable range

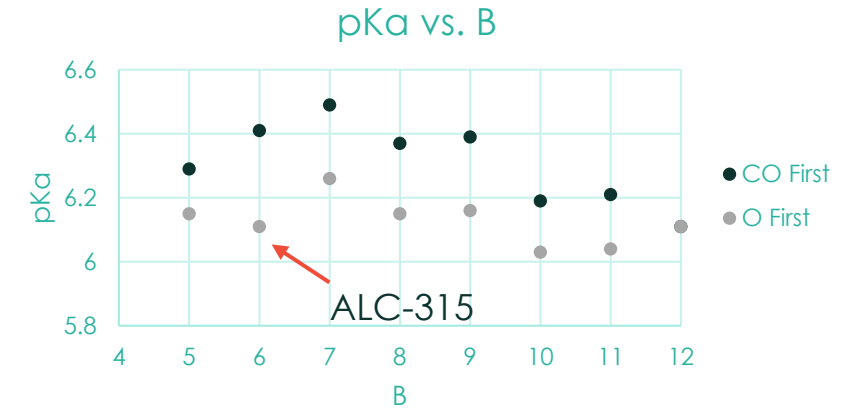


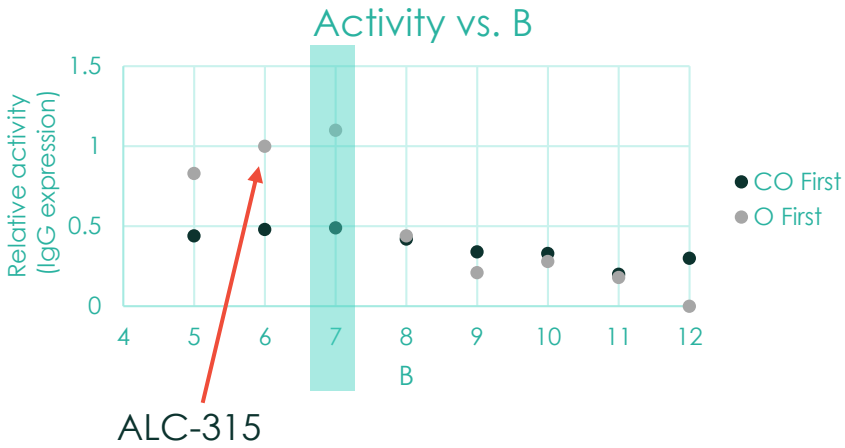
ACUITAS THERAPEUTICS

ALC-315 analogues: variations in spacer B

A fixed at 4, **B** 4-12, **C** fixed at 16



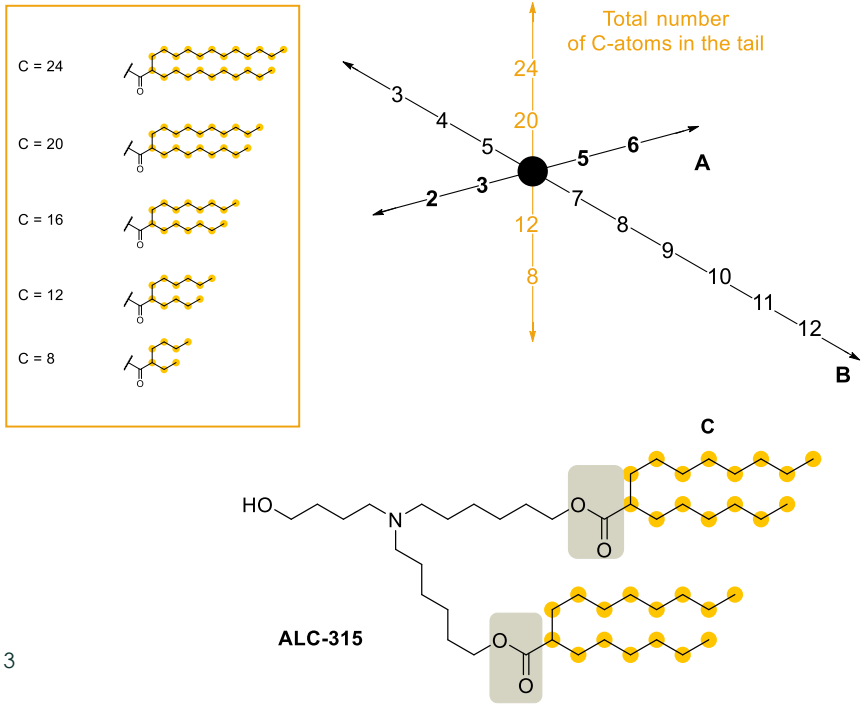


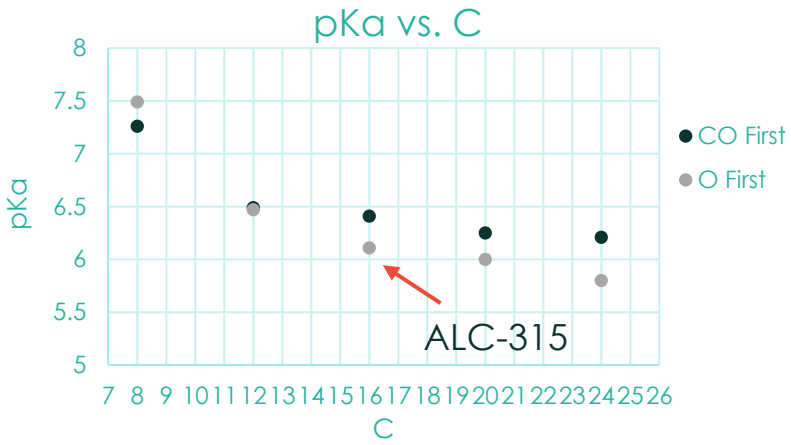


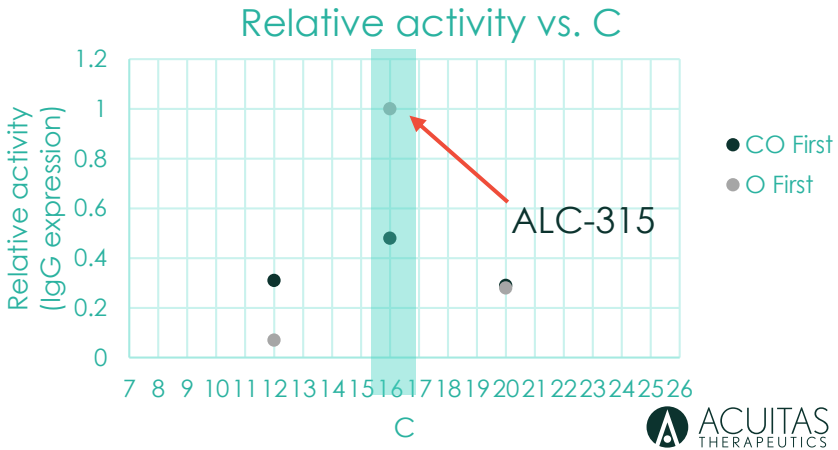
ACUITAS THERAPEUTICS

ALC-315 analogues: variations in tail C

A fixed at 4, **B** fixed at 6 , **C** = 8-24



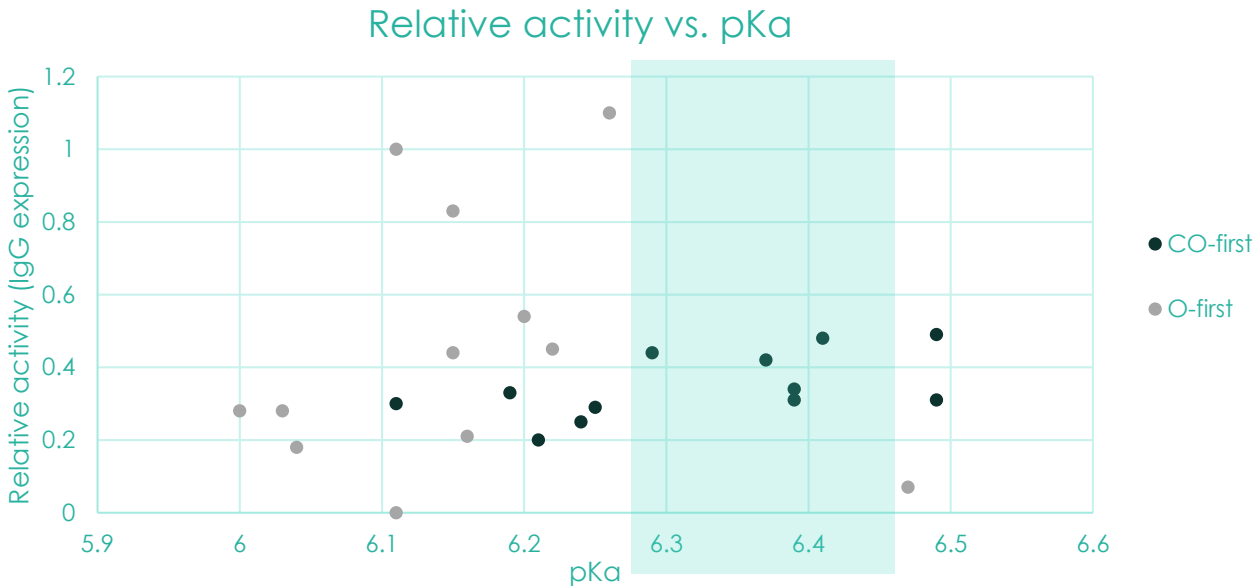




ACUITAS THERAPEUTICS

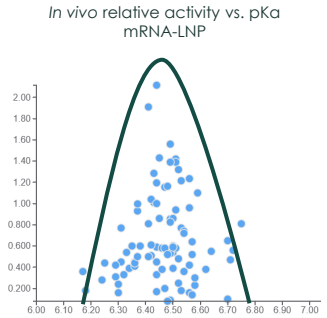
Will maximizing pKa improve activity?

Should we make off-axis lipids?



In CO-first series increase in activity seems to generally corelate with the increase in pKa values (6-6.5 pKa range)

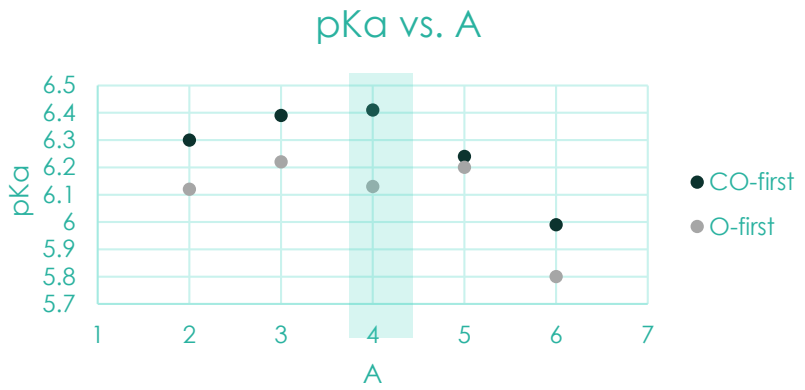
In O-first series activity seems to peak between 6.1 and 6.30, but no examples in 6.30 to 6.45 range.

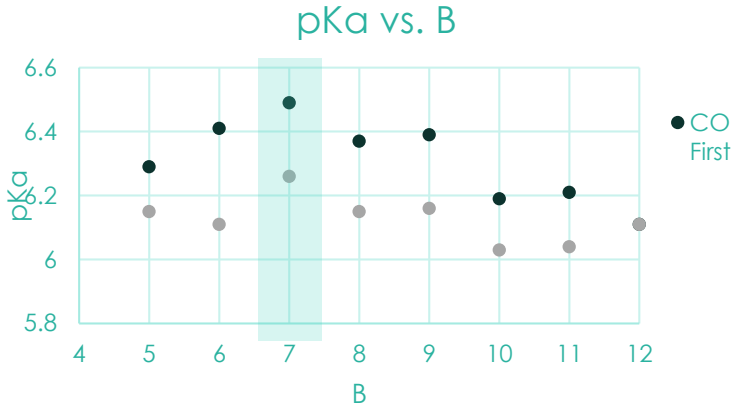


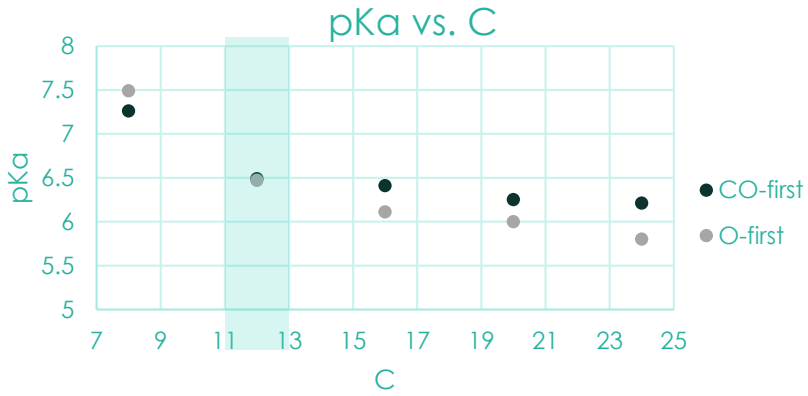
ACUITAS THERAPEUTICS

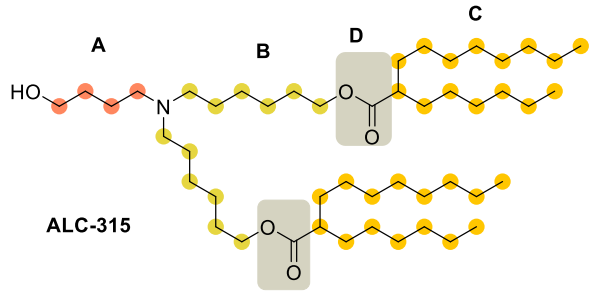
Adjusting pKa

Designing off-axis lipids





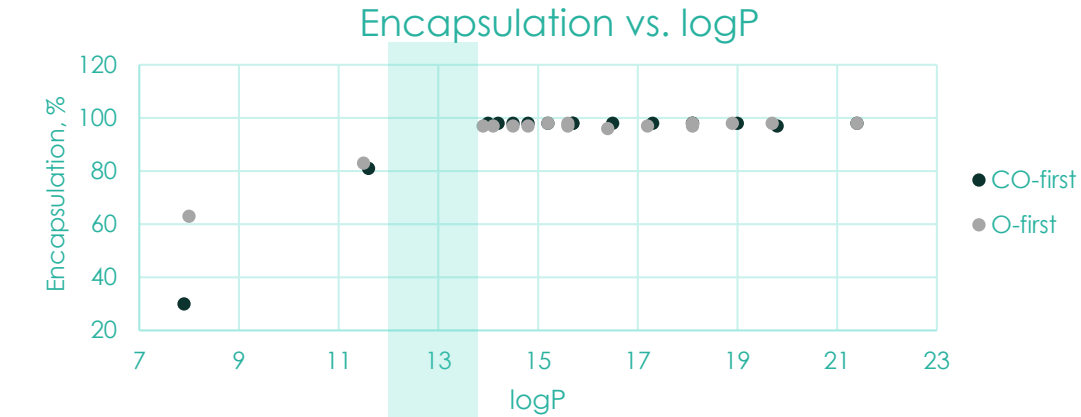


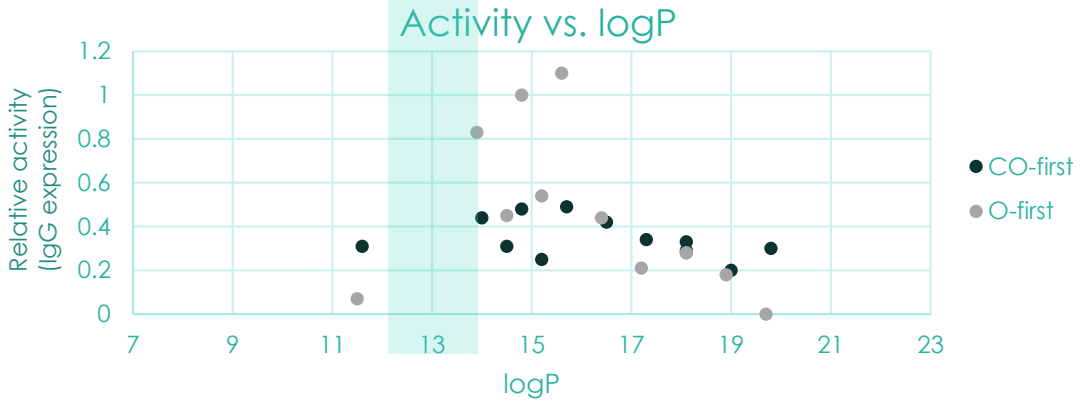


ACUITAS THERAPEUTICS

Adjusting logP

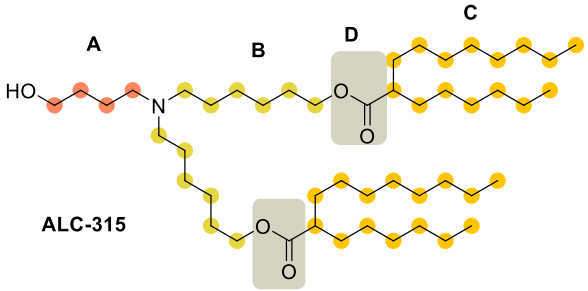
Designing off-axis lipids





LNP encapsulation efficiency, particle size and stability depend on logP.

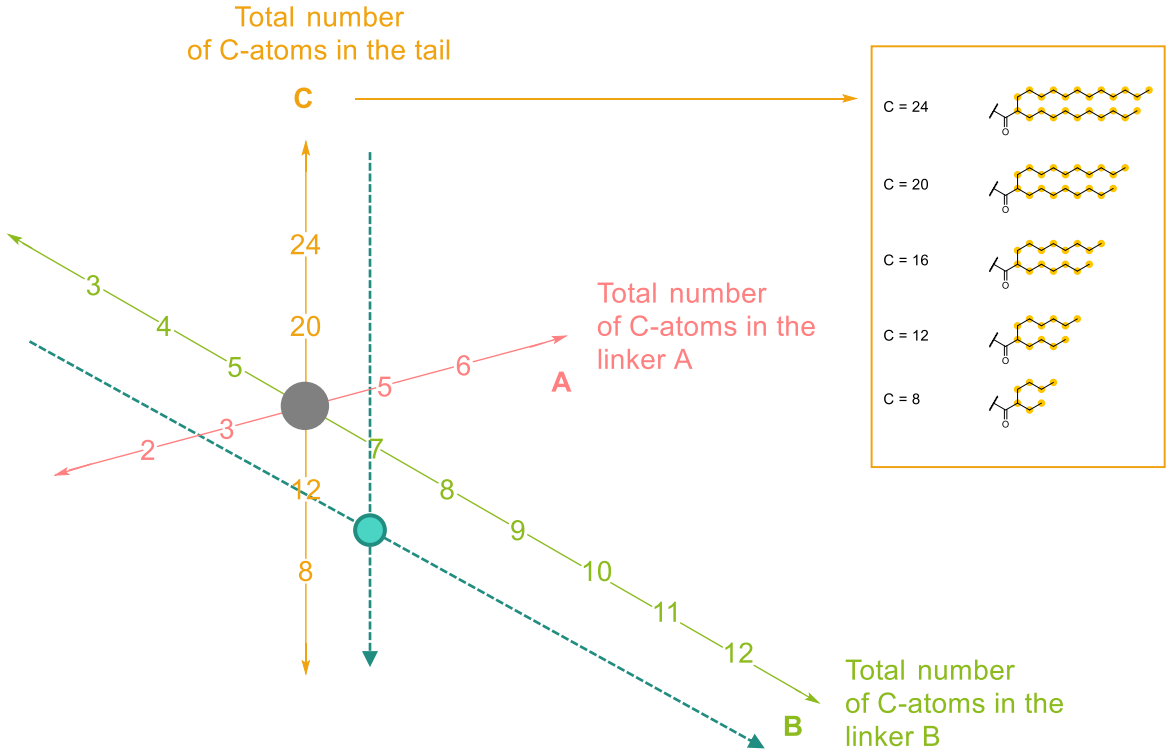
LNP activity can be influenced by logP.

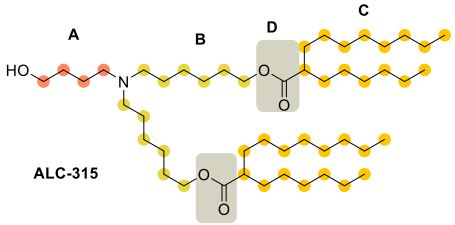


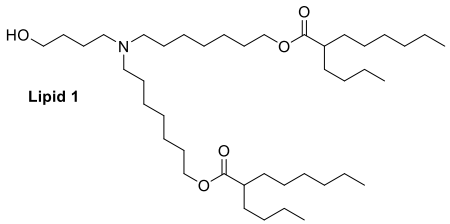
ACUITAS THERAPEUTICS

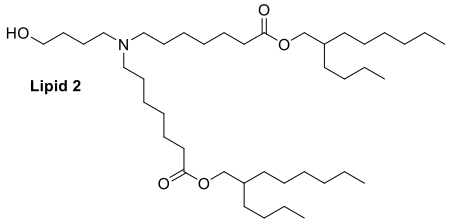
Adjusting pKa and logP

Designing off-axis lipids



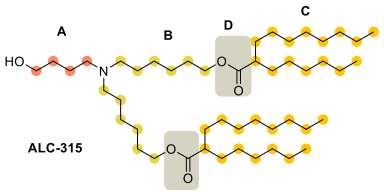


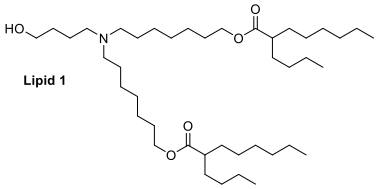


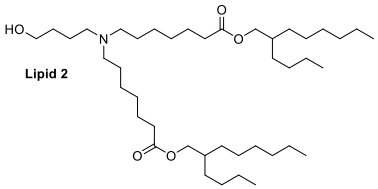


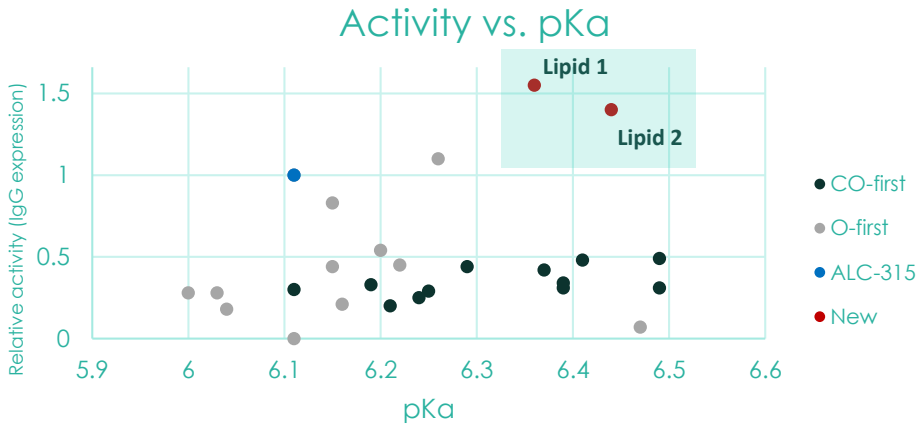
ACUITAS THERAPEUTICS

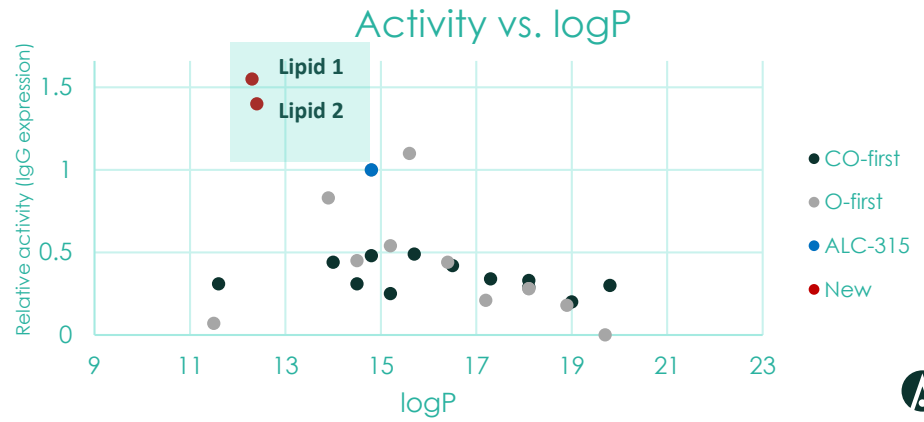
New lipids outperformed ALC-315 in IgG assay





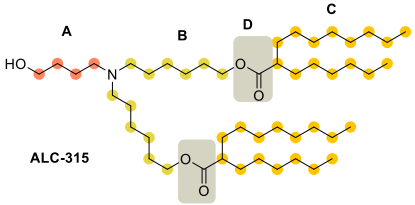


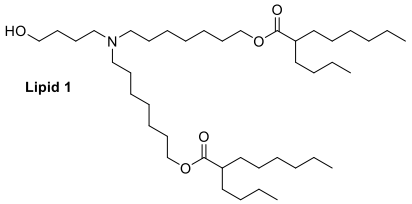


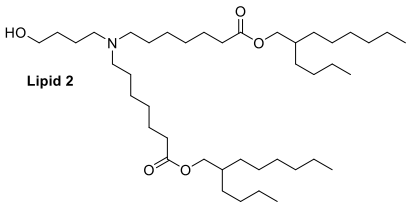


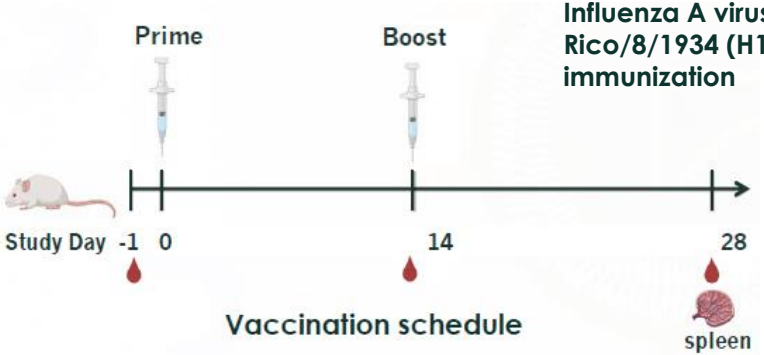
ACUITAS THERAPEUTICS

New lipids outperformed ALC-315 in immunization efficiency



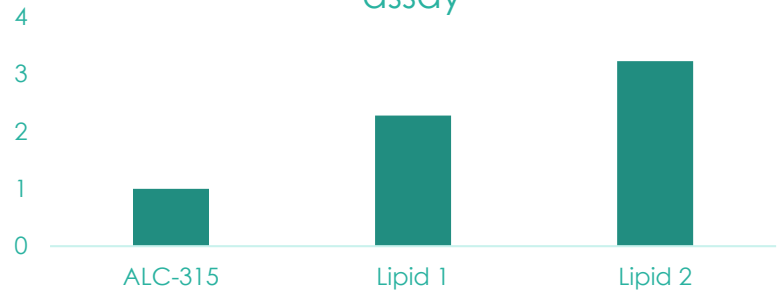






Influenza A virus (A/Puerto Rico/8/1934 (H1N1)) mRNA immunization

Relative activity, PR8 immunization assay



ACUITAS THERAPEUTICS

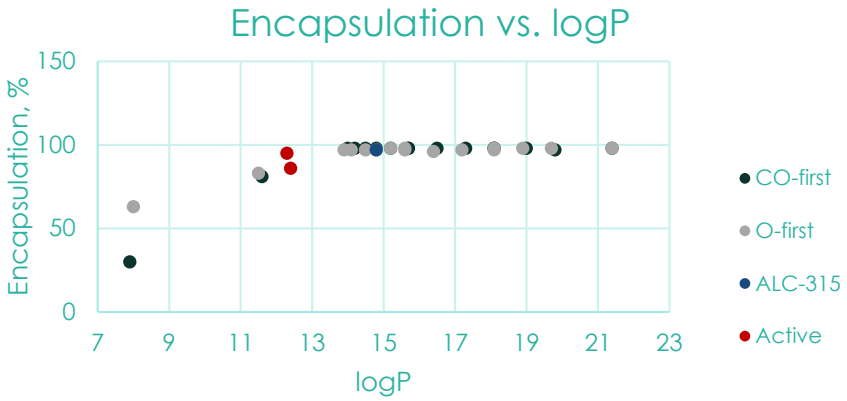
Conclusions

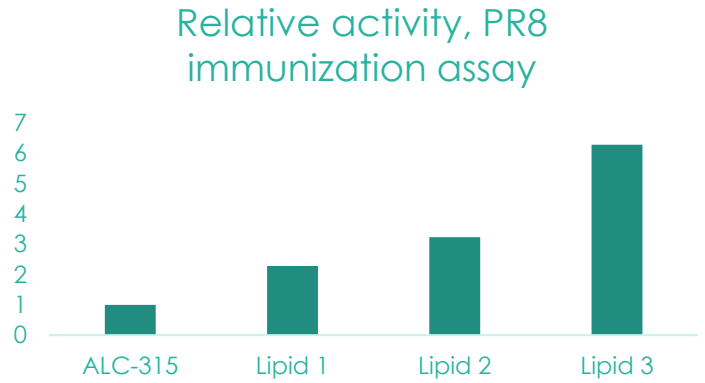
Targeted structural iterations enabled faster exploration of broad chemical space.

Compounds with better activity than ALC-315 successfully identified:

- IgG expression assay: 50% increase in activity
- Influenza A virus immunization: 2.3-3.3-fold higher antibody neutralization titers
- No observable adverse clinical signs.

ALC-315 outperformed in encapsulation efficiency and size stability: it is a premier benchmark lipid.





ACUITAS THERAPEUTICS

Thank you!

ACUITAS THERAPEUTICS