ECHELON BIOSCIENCES™
A VION BIOSCIENCES COMPANY

1 LIPIDS
2 RNA
3 SYNTHESIS
4 FORMULATION

LNP CATALOG

+ ACCELERATE YOUR DISCOVERY

ECHELON'S CAPABILITIES

01/ **LIPID SYNTHESIS**
02/ **IN VITRO TRANSCRIPTION**
03/ **LNP FORMULATION**
04/ **LNP CHARACTERIZATION**
05/ **EXPRESSION SCREENING**

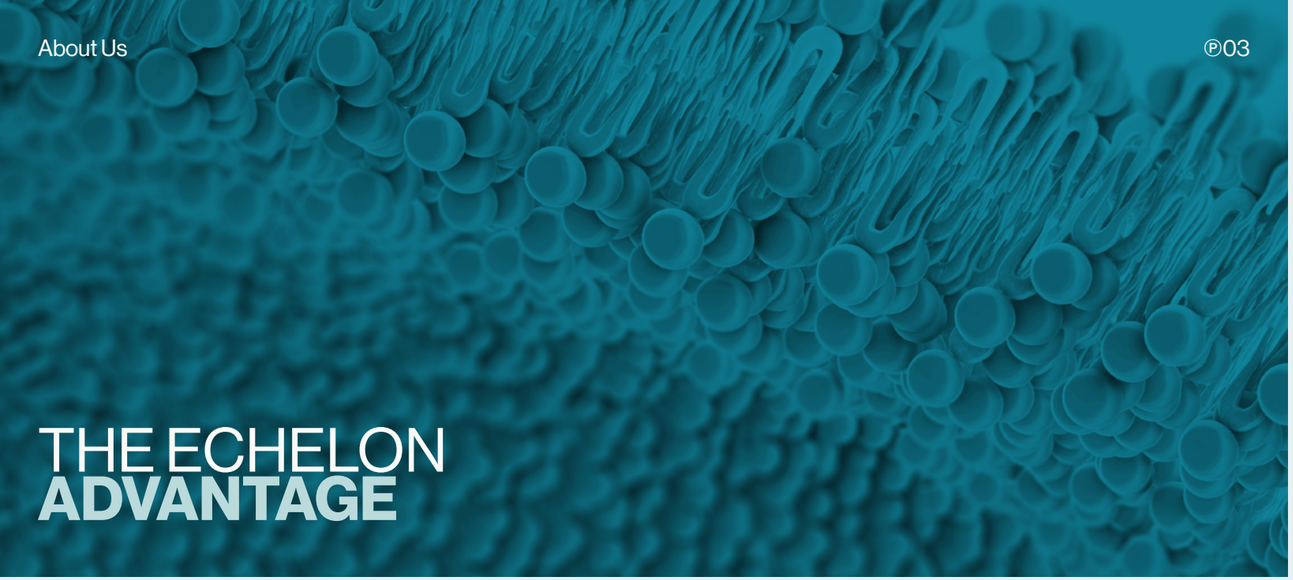
01 Echelon's chemists have decades of experience in synthesizing complex lipids and are continually adding to our ionizable lipid catalog. Their expertise can support building and screening novel ionizable lipids and other custom synthesis solutions.

02 Our scientists will assist with the design of any IVT template prior to RNA synthesis. We have experience with incorporation of modified nucleotides and synthetic cap structures. In-house templates for mRNA IVT are available.

03 Echelon's LNP platform utilizes gold-standard microfluidic mixing for LNP development and screening. Our devices are cartidge-free, saving time and cost associated with formulation.

04 Downstream analysis meets all current standards for LNP characterization and includes encapsultion efficiency, zeta potential, particle size, molecular weight and polydispersity.

05 Expression screening is a standard part of our process whether testing a novel RNA or using a reporter to screen lipid formulations. Multiple cell lines are available in cases where cell specificity may be a concern.



THE ECHELON ADVANTAGE

01

/ WHO WE ARE

We are a lipid company with decades of hard-won experience. We take great pride in our accomplishments but never settle for what's been done before. We are resolute in our mission to advance science for the betterment of all humankind and continue moving science forward to enable discovery of new ideas and benefits. We understand science's power and consistently seek to use our knowledge and experience to benefit our customers, all people and the planet.

02

/ EXPERTISE

Echelon has been built on the foundation of our expertise in lipids, lipid signaling, and molecular biology. This means we're not only skilled at chemically synthesizing lipids, but we understand how they function in a biological context. We're not just experts at manufacturing products, we're experts in the underlying biology of how and why they work.

03

/ QUALITY CONTROL

We adhere to stringent quality control manufacturing procedures including triplicate QA/QC checkpoints. For many years, loyal customers have trusted the reliability of Echelon products.

04

/ SUPPORT

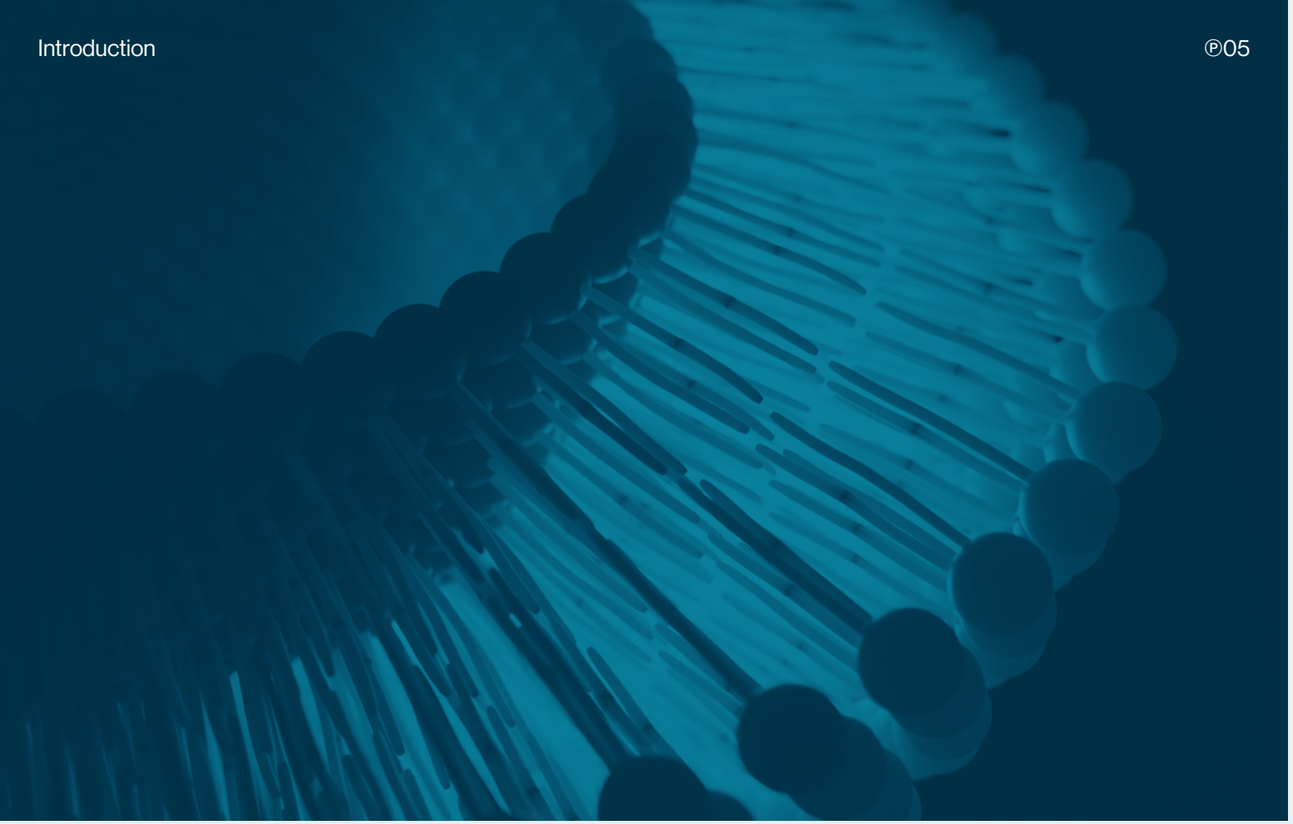
The questions and concerns of our clients and customers are our highest priority. We pride ourselves on our responsiveness, providing solutions as quickly as possible. When needed, our team will perform troubleshooting experiments with your scientists to keep your project moving forward.

PRIMER/LIPID NANOPARTICLES

Genetic therapies have now been pursued for some time, and several DNA-based gene therapies are now approved. However, DNA gene therapies remain limited as they commonly use viral vectors that can cause unwanted immune activation. Alternative therapeutics based on RNA could hypothetically bypass some of the issues associated with DNA gene therapies and have been the focus of intense research and development. The approval and effectiveness of the first RNA medication and mRNA-based vaccines within the last 5 years has only heightened the interest in these types of therapies. The main drawback to using RNA as a therapy is that naked RNA cannot simply be injected because it is immunogenic, easily susceptible to enzymatic degradation, and is not taken up by cells. Therefore, alternative delivery systems are required for delivering therapeutic RNA.

The advent of the vaccines for SARS-COV-2 / COVID19 has generated an explosion in interest in creating new medicines based on their core technology: lipid nanoparticles (LNPs). LNPs package RNA in a lipid coat that protects it from degradation. This allows the RNA cargo to enter cells where it can then be released into the cytoplasm for synthesis of putatively therapeutic proteins.

While schematically simple, care must be taken in the design of both RNA cargo and the lipid formulation of the LNP in order to ensure sufficient delivery of potentially therapeutic cargos.



CONCERNS+ CONSIDERATIONS

As with any new class of drugs or therapeutics, there are a set of basic considerations for designing LNPs. For mRNA LNPs, the RNA and the charged lipids they interact with can be immunogenic. This may be desirable for applications such as vaccines, but other modalities that require repeat dosing such as genetic editing therapies may encounter negative effects. Therefore, when screening ionizable lipids, it is advisable to take into account the level of immune activation and balance this against the extent of cargo delivery and protein translation.

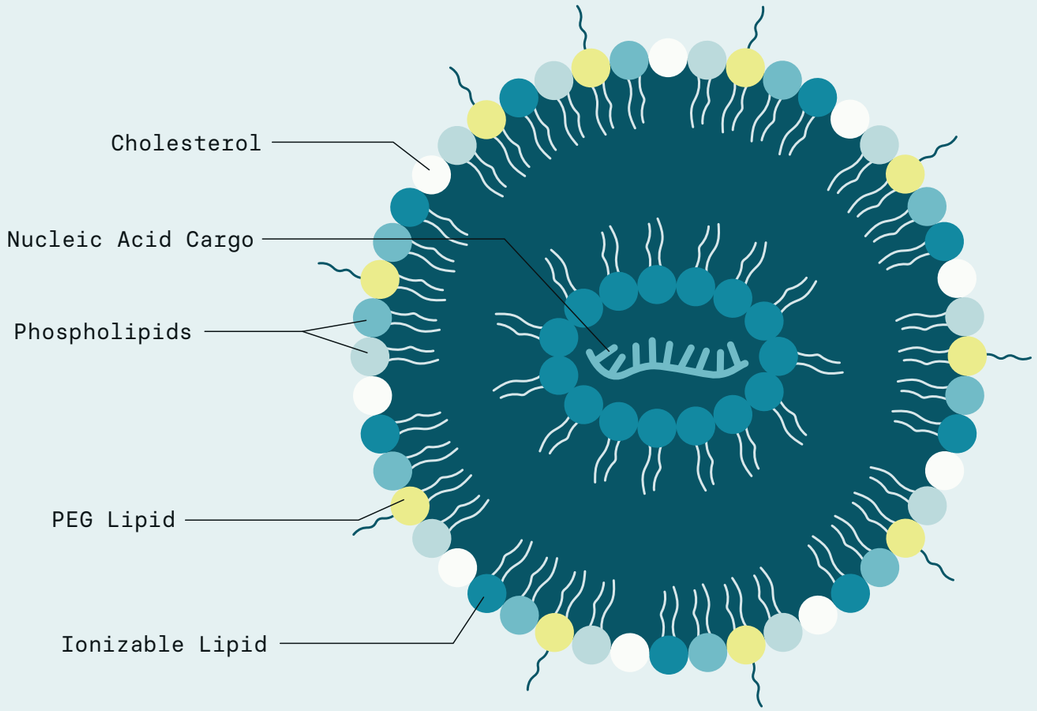
PEGylated lipids are incorporated in LNPs to increase LNP half-life and help prevent elimination. However, they also stimulate production of anti-PEG antibodies which can actually accelerate clearance from the circulation. Considering this, there is an interest in PEG alternatives such as polysarcosine that can reduce immunogenicity without loss of transfection efficiency.

Another ongoing challenge in the LNP field is targeting for cell-specific delivery. LNP based vaccines benefit from a systemic response, but other therapies require delivery of LNP cargos to specific tissues or cells to be effective. Popular strategies to counter accumulation of LNPs in off-target tissues have been altering the biodistribution of LNPs via changes in surface charge or the addition of a targeting peptide or antibody.

LNP COMPONENTS

The building blocks of LNPs are remarkably simple. A typical LNP includes an ionizable or cationic lipid, cholesterol, at least one phospholipid, and a polyethylene glycol (PEG) modified lipid. These lipid components are used to shield or encapsulate a nucleic acid cargo that encodes a therapeutic protein.

The type of cargo ranges from mRNA or siRNA to plasmid DNA. The choice of lipids in the formulation is influenced by the cargo type.



TARGETING MODIFICATIONS

The biggest current obstacle to development of successful LNP therapeutics is target selectivity. Due to their size and other physiochemical properties, unmodified LNPs bioaccumulate in specific organs. This can drastically reduce their therapeutic potential.

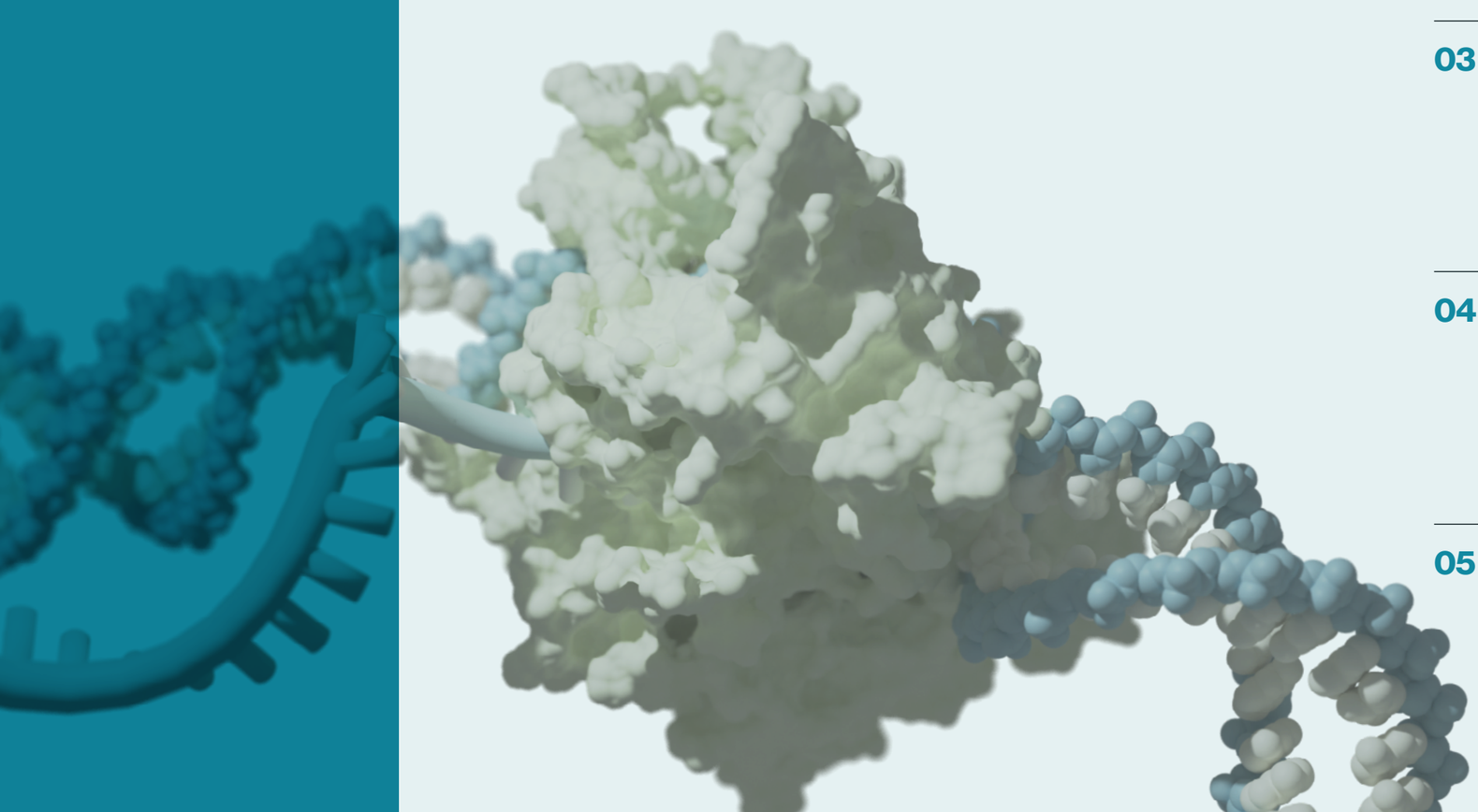
Several strategies have emerged to address this issue. In some cases, simply altering the lipid composition and therefore the LNP surface charge is sufficient to alter biodistribution. More precise targeting may be achieved by the addition of small molecules or proteins to the surface of the LNP. This may take the form of a lipid conjugated ligand or attaching antibodies or peptides via established methods such as biotin-streptavidin interactions or Click Chemistry.

These approaches add a layer of complexity to LNP formulation, but they offer a vast pool of possible ligand-receptor or antibody antigen combinations that could be used to tailor unique LNPs.

RNA SYNTHESIS

The majority of RNA species produced for LNPs utilize in vitro transcription (IVT) as the method for synthesis. This method can be utilized with different RNA polymerases making it extremely flexible. The **TYPE** of RNA as well as specific sequence requirements can influence the polymerase used for synthesis. Specific **COMPONENTS** of the RNA sequence, such as UTRs, coding region, ribosome entry sites, and poly(A) tails are incorporated based on the type of RNA being produced, which is in turn be informed by therapeutic goal of the LNP.

While some RNA species may need little additional processing, IVT mRNA requires a **CAPPING** step at some point during synthesis. The extent of capping, poly(A) addition and sequence integrity can all be assessed during the **SYNTHESIS + ANALYSIS** steps from initial IVT to downstream purification. Expression screening is also recommended to ensure that any novel RNA is translation competent (has the intended functional effect on a target cell).



01

The **TYPE** of RNA to be used in an LNP formulation is the first consideration when designing a therapeutic cargo. siRNA, mRNA, and circular RNAs, for instance, have different lengths, 5’ and 3’ elements and sequence requirements to be effective. Even when the minimally required sequence elements or components are present, there may be additional editing and optimization required due to the cell type or tissue targeted.

02

The **COMPONENTS** of an RNA are largely dictated by the type of RNA. mRNA, for example, requires untranslated regions (UTRs) at the 5’ and 3’ end of the coding region as well as a poly(A) tail. These extra sequence components stabilize the mRNA transcript and allow it to engage with the translation machinery once delivered to the cytoplasm of the cell. Some of these sequences may be generalizable, but specific 5’ or 3’ UTRs may boost expression in certain cells. Codon optimization of the coding region may also be beneficial.

03

mRNA cargos have an additional **CAPPING** step for complete synthesis. Eukaryotic transcripts have a 5’ cap made of a nucleotide analog that conveys added stability and is necessary for proper translation. Synthetic cap analogs can be incorporated during IVT or added with a secondary reaction step following synthesis of the transcript. Various cap analogs are available for incorporation. The choice of cap analogs hinges on a delicate balance of immunogenicity and translation efficiency.

04

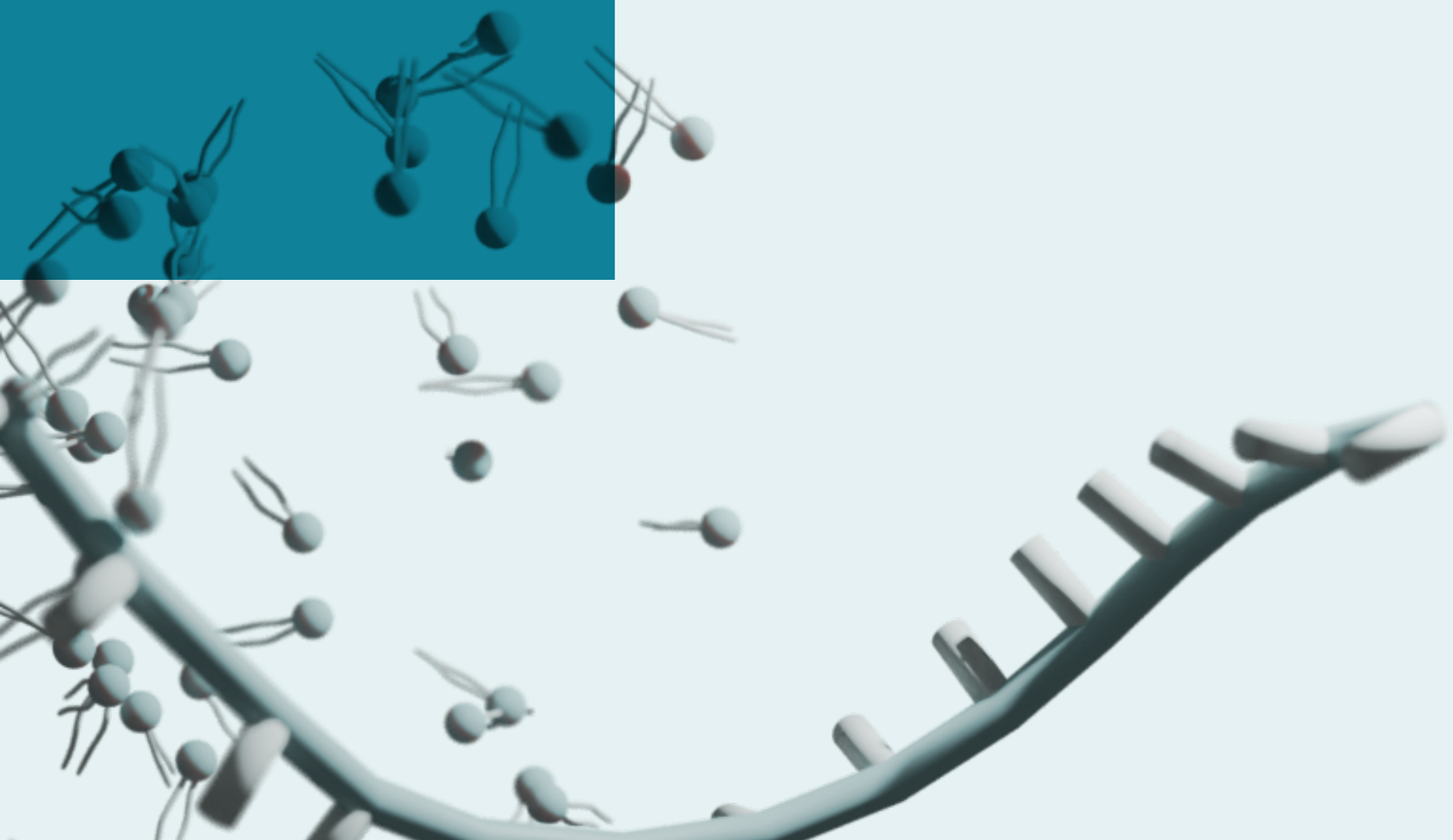
RNA **SYNTHESIS** is usually done using In Vitro Transcription (IVT). This system requires a linear DNA template and specific sequence elements in the promoter that will permit transcription with recombinant RNA polymerase enzymes such as T7. IVT is flexible in that modified nucleotides are easily substituted, and the reaction can be scaled easily once initial reaction conditions are optimized. The reaction templates may be produced by several methods, including restriction digestion, PCR, or oligonucleotide complementation.

05

ANALYSIS of RNA transcripts involves a series of steps both before and after LNP encapsulation. Initially, the transcript is purified via chromatography which removes excess nucleotides and allows for an assessment of the relative purity of the reaction. The correct length and sequence of the transcript must then be verified. Once the initial quality of the RNA transcript has been confirmed, it is ready for LNP formulation. The integrity of the transcript is rechecked following encapsulation to ensure that no fragmentation or degradation occurred during LNP formulation.

LNP FORMULATION

01/ **DESIGN**
02/ **CARGO SYNTHESIS**
03/ **FORMULATION**
04/ **ANALYSIS**
05/ **EXPRESSION SCREENING**



Successful LNP formulation requires multiple points of consideration. The **DESIGN** of the LNP must take into account the target cell or tissue, as well as how effectively a given ionizable lipid encapsulates and then releases a cargo molecule. **CARGO SYNTHESIS** must include elements that are amenable to stability and expression of nucleic acid elements during initial cloning. Subsequent amplification or transcription of DNA or RNA may also require incorporation of modified nucleotides to decrease immunogenicity.

Initial screening for LNP **FORMULATION** can be conducted once adequate cargo material has been obtained. The goal is to assess appropriate assembly conditions between various ionizable lipids and the cargo and to assess different lipid compositions. Downstream **ANALYSIS** of LNPs provides additional information beyond simple encapsulation, which is critical for determining if a formulation is successful. Further testing via **EXPRESSION SCREENING** is also highly advisable as not all formulations that appear to pass quality checks during analysis will lead to functional LNPs.

01

The primary concern in **DESIGN** of LNPs is the ionizable lipid and how well it encapsulates and releases a given cargo. The choice of ionizable lipid is also influenced by the type of nucleic acid cargo. dsDNA and mRNA or siRNA may preferentially interact with structurally different classes of ionizable lipids. Other factors such as the surface charge and size of the LNP may be tuned by careful selection of the type and molar percentages of the phospholipids and PEG lipids in the formulation.

02

CARGO SYNTHESIS of RNA for LNPs is generally performed by in vitro transcription from a DNA template. For mRNA cargos, the selection of non-coding elements such as the UTRs that stabilize the transcript and enhance translation should be made on the basis of the tissue or cell type that the LNP will be delivered to. Other elements, such as the type of cap structure that will be incorporated, should be made ahead of time as these may require specific sequences at the beginning of the transcript.

03

Once lipids have been selected and cargo prepared, LNP **FORMULATION** can move forward. The current standard for this step is the use of automated microfluidic mixers to ensure reproducibility. These mixers allow for precise, individual control of the rapid mixing of organic phase lipids with the aqueous phase cargo. Mixers also allow for a great deal of flexibility in terms of the ratios at which the two components are mixed, which can be critical when dealing with unique RNAs.

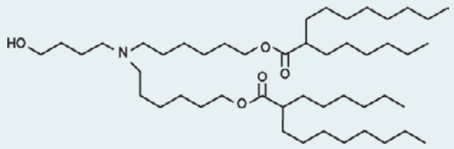
04

Initial **ANALYSIS** of LNPs is performed by examining the level of encapsulation of the cargo molecule. Downstream characterization examines the particle molecular weight, size, surface charge, and polydispersity of the LNP sample. Formulation and analysis can be seen as paired steps as they can be very iterative during screening. Deciding when a specific formulation is sufficient is largely dependent on one or more of these analytical parameters.

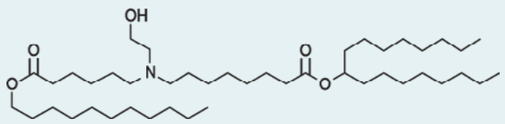
05

In vitro **EXPRESSION SCREENING** is often carried out as a supplemental step following formulation and analysis. Although in vitro and in vivo expression do not always correlate, expression screening can provide early validation of an LNP formulation. This may help accelerate subsequent formulation screens or in selecting which candidates to test in vivo. Adding benchmark LNPs that have high expression in similar contexts can also make this method more reliable.

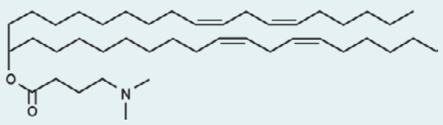
01 **ALC-0315**
[Catalog Number / N-1020]



02 **SM-102**
[Catalog Number / N-1102]



03 **DLIN-MC3-DMA**
[Catalog Number / N-1282]



IONIZABLE LIPIDS

Ionizable lipids are the key component to building a fully formed lipid nanoparticle. These specialized lipids obtain a positive charge in acidic pH, allowing them to interact with and encapsulate nucleic acids which contain negative charges via their phosphate groups.

Screening and selection of ionizable lipids is based on the type of nucleic acid cargo, the target tissue and therapeutic application.

CUSTOM **IONIZABLE LIPIDS**

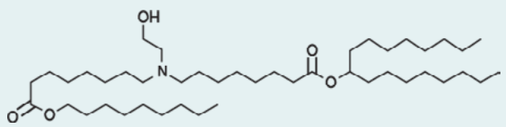
The need for new ionizable lipids is growing as the types of cells targeted for therapy and varities of RNA continue to expand. Echelon's chemists have years of expertise in lipid synthesis and are actively adding new ionizable lipids to our product catalog.

If you have a novel ionizable or cationic lipid structure you would like to screen or if an item is not listed in our catalog, we can synthesize it. Our LNP group can then pre-screen any new ionizable lipids with benchmark reporter mRNAs for encapsulation and transfection efficiency.

IONIZABLE LIPIDS	CATALOG NUMBER
AA3-Dlin	N-1003
LP-01	N-1007
306Oi10	N-1010
DOG-IM4	N-1017
6,6'-trehalose dioleate	N-1025
YSK05	N-1050
Lipid 10	N-1110
306-O12B	N-1128
C12-200	N-1220
306-N16B	N-1316
Lipid 319	N-1319
4A3-SC8	N-1438

See full product listing on page 14

04 **LIPID 5**
[Catalog Number / N-1005]



05 **CKK-E12**
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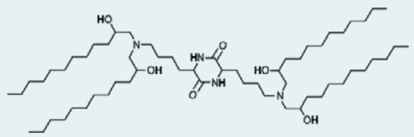

06 **9A1P9**
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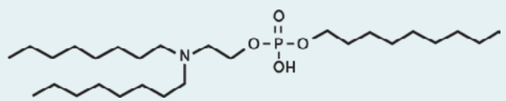
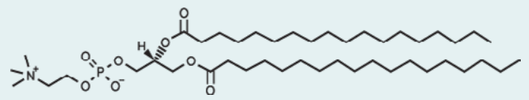
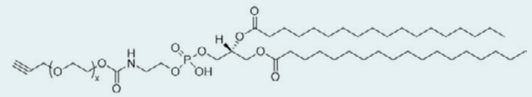


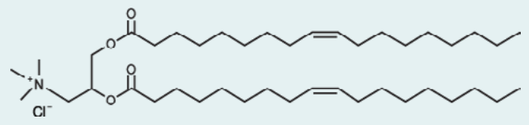
01 **DSPC (18:0/18:0 PC)**
[Catalog Number / L-1118]



02 **DSPE-PEG2000-Alkyne**
[Catalog Number / L-2812]



03 **18:1 TAP (DOTAP)**
[Catalog Number / N-1380]

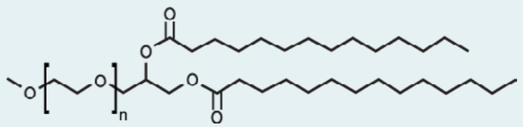


HELPER LIPIDS

Helper lipids refer to the remaining lipid components of LNPs outside of the ionizable lipid. These typically include a combination of cholesterol, a phospholipid, and a PEGylated lipid to build the outer membrane structure that fully encloses nucleic acids captured by the ionizable lipids.

Stability and other physiochemical properties of LNPs can be tuned and refined by adjusting the type and ratio of these lipids across LNP formulations.

04 **DMG-PEG 2000**
[Catalog Number / N-2014]



05 **mPEG-DSPE 2000**
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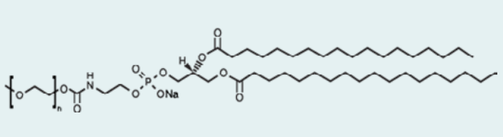

06 **ALC-0159**
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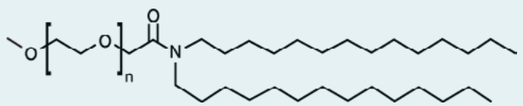

LIPID CONJUGATION

An emerging and growing need in the LNP field is targeting for site-specific delivery. The current, most straightforward means to address this is by modifying phospholipids or PEGylated lipids to conjugate a peptide, antibody, or other ligand.

A variety of solutions are available including lipids modified with biotin, streptavidin, or azide to facilitate attachment of targeting moeities.

HELPER LIPIDS	CATALOG NUMBER
9(10)-Nitrooleic Acid	L-0112
PLPC (16:0/18:2 PC)	L-1122
DOPC (18:1/18:1 PC)	L-1182
POPC (16:0/18:1 PC)	L-1618
DPPE (16:0/16:0 PE)	L-2116
DSPE (18:0/18:0 PE)	L-2118
DOPE (18:1/18:1 PE)	L-2182
DSPE-PEG2000-Maleimide	L-2810
DSPE-PEG2000-NH2	L-2811
DSPE-PEG2000-COOH	L-2814
7-Dehydrocholesterol (7-DHC)	L-6004
Ergosterol	L-6005
Cholesterol	L-6012

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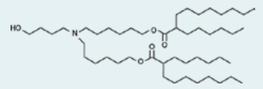
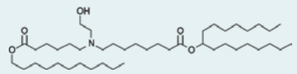
01 **LNP TRAILBLAZER KIT**
[Catalog Number / K-6300L]

02 **LNP TRAILBLAZER KIT / PRE-MIXED**
[Catalog Number / K-6300M]

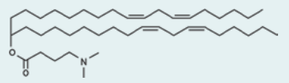
ALC-0315



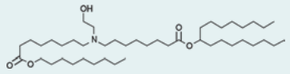
SM-102



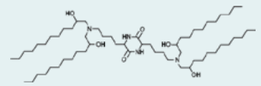
DLin-MC3-DMA



Lipid 5



cKK-E12



TRAILBLAZER LNP KITS

Echelon's Trailblazer LNP Kits are designed to simplify early stage exploration of LNPs. Each kit provides a buffer for diluting nucleic acids and the lipids necessary to form LNPs either lyophilized and pre-mixed.

The pre-mixed LNP kit utilizes a standardized molar percentage for each lipid so that accurate comparisons can be made. The lyophilized LNP kit allows the experimenter to adjust the ratio of the lipid components as needed.

CUSTOM **KITS**

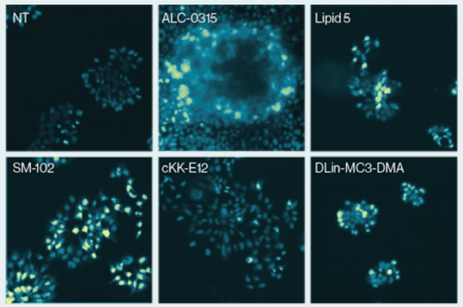
The ionizable lipids provided in the Trailblazer Kits are among the most common benchmark lipids found in the literature. Three of these have been previously utilized in FDA-approved formulations.

Additional ionizable lipids can be added into the kit format upon request. The kit reagents can also be scaled if there is a need to perform a large screen beyond the listed volumes of the stock kit.

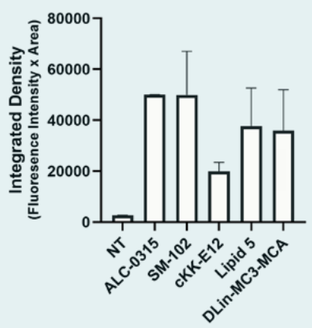
SCALING UP

The Trailblazer Kits are compatible with microfluidic devices, however we recommend utilizing custom services for intermediate to large scale LNP formulation. Production of mRNA, lipid mixtures, and LNPs at scale in a quality controlled environment reduces variability and can help control for experimental artifacts.

Echelon has custom IVT and LNP services available to meet your needs.



HEK293



IONIZABLE LIPIDS	CATALOG NUMBER
1-A-N	N-1001
Lipid OA2	N-1002
AA3-Dlin	N-1003
Lipid 5	N-1005
Lipid A6	N-1006
LP-01	N-1007
YHS-12	N-1009
306Oi10	N-1010
cKK-E12	N-1012
NT1-014B	N-1014
CL4H6	N-1016
DOG-IM4	N-1017
ALC-0315	N-1020
2000i10	N-1021
OF-02	N-1022
244cis	N-1023
Lipid C24	N-1024
6,6'-trehalose dioleate	N-1025
6-trehalose monooleate	N-1026
C12-113	N-1027
304013	N-1029
3-A2-7b	N-1030
Lipid 15	N-1033
N2-3L	N-1034
Lipid 35	N-1035
Lipid 8-014B	N-1036

IONIZABLE LIPIDS	CATALOG NUMBER
YSK05	N-1050
ATX-100	N-1100
Lipid U-101	N-1101
SM-102	N-1102
Lipid M	N-1103
Lipid 10	N-1110
11-A-M	N-1111
TT3	N-1113
4-010b1	N-1114
FTT5	N-1115
80-018	N-1118
306-012B	N-1128
93-0170	N-1170
93-017S	N-1175
OC2-K3-E10	N-1210
C12-200	N-1220
DLin-MC3-DMA	N-1282
306-N16B	N-1316
Lipid 319	N-1319
DOTMA	N-1381
DODMA	N-1382
PPZ-A10	N-1410
4A3-SC8	N-1438
CAP2	N-1912
9A1P9	N-1919

HELPER LIPIDS	CATALOG NUMBER
9(10)-Nitrooleic Acid	L-0112
DSPC (18:0/18:0 PC)	L-1118
DSPC-d9 (18:0/18:0 PC-d9)	L-1118D
PLPC (16:0/18:2 PC)	L-1122
DOPC (18:1/18:1 PC)	L-1182
POPC (16:0/18:1 PC)	L-1618
DPPE (16:0/16:0 PE)	L-2116
DSPE (18:0/18:0 PE)	L-2118
DOPE (18:1/18:1 PE)	L-2182
DOPE-Lipoic Acid (S-DOPE)	L-2310
DSPE-PEG2000-Maleimide	L-2810
DSPE-PEG2000-NH2	L-2811
DSPE-PEG2000-Alkyne	L-2812
DSPE-PEG2000-Biotin	L-2813
DSPE-PEG2000-COOH	L-2814
DSPE-PEG2000-DBCO	L-2815
DSPE-PEG2000-Mannose	L-2816
DSPE-PEG2000-Azide	L-2817
mPEG-DSPE 2000	L-2820
DOTAP	N-1380
ALC-0159	N-2010
PEG2000-C-DMG	N-2012
DMG-PEG 2000	N-2014
Beta-Sitosterol	L-6001
Stigmasterol	L-6002
20a-Hydroxycholesterol	L-6003
7-Dehydrocholesterol (7-DHC)	L-6004
Ergosterol	L-6005
Cholesterol	L-6012

LNP KITS	CATALOG NUMBER
TRAILBLAZER	K-6300L
TRAILBLAZER PRE-MIXED	K-6300M

AVAILABLE KIT IONIZABLE LIPIDS:

SM-102
CKK-E12
ALC-0315
DLIN-MC3-DMA
LIPID 5

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