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NUCLEIC ACID INSIGHTS

CONTENT PILLARS

mRNA

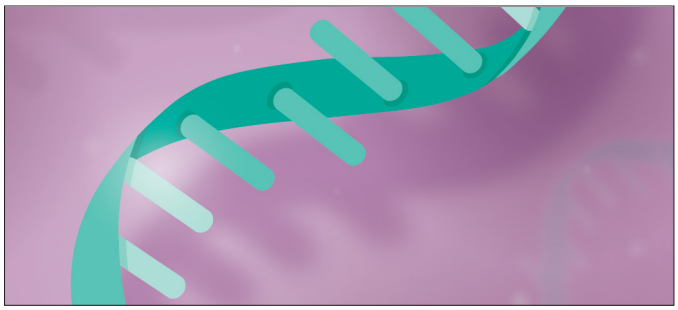
Manufacturing: upstream/synthesis

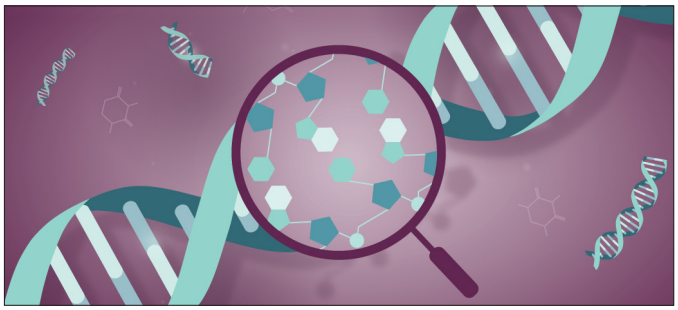
Oligonucleotides

Analytics

NUCLEIC ACID INSIGHTS

CONTENTS VOLUME 2 · ISSUE 3





mRNA
MANUFACTURING: UPSTREAM/SYNTHESIS

SPOTLIGHT

Enhancing mRNA results through strategic RNA design and quality control

INTERVIEW

"There are several design elements known to improve stability and longevity of mRNA in cells while also reducing innate immunogenicity..."

In the last few years, mRNA has moved to the fore in the vaccine and cell and gene therapy fields, and as a result, its successful production for these downstream applications has never been more crucial. In this podcast episode, **Róisin McGuigan**, Editor, *Nucleic Acid Insights*, speaks to **Ryan Lahr**, Senior R&D Scientist, CELLSCRIPT, about design, production, and quality control of synthesized mRNA for translation in cells.

Nucleic Acid Insights 2025; 2(3), 33–38 · DOI: 10.18609/nuc.2024.007

Q Can you tell me a bit about yourself and the current work that you do?

RL I work as part of the product development team at CELLSCRIPT™. Our company develops high-quality, easy-to-use technologies for downstream applications where mRNA is translated in cells, which includes the areas of cell and gene therapy

research and mRNA vaccine development. We provide technologies for all aspects of mRNA synthesis, including the use of modified nucleosides in mRNA. Additionally, we have recently launched benchtop PAGE-based quality control kits for assessing mRNA capping efficiency and tail length.

Q Can you frame the key challenges and considerations when optimizing mRNA design for different research applications in the vaccine and cell and gene therapy fields?

RL Robust protein expression of mRNA is essential for successful applications in vaccine development and cell and gene therapy research. The structure of an mRNA molecule consists of a 5′ cap, an RNA transcript, and a 3′ poly(A) tail. The transcript contains the coding region to be translated into a protein by ribosomes in cells. The 5′ cap interacts with cellular translation machinery for recruitment of the mRNA to ribosomes for translation, whereas the 3′ poly(A) tail is involved in protecting the transcript from exonuclease digestion in cells. All three regions must be carefully considered when designing your transcript for successful translation in cells.

There are several design elements known to improve stability and longevity of mRNA in cells while also reducing innate immunogenicity, which is the body's first line of defense against foreign substances. Researchers should consider incorporating these design elements into their mRNA to address their specific application needs. One critically important consideration is minimization of immunogenic response by cells. A well-documented method for reducing immunogenicity is incorporation of modified nucleosides like pseudouridine and N1-methylpseudouridine into mRNA transcripts.

Another important consideration concerns the 5′ cap structure. Complete capping of the 5′ end of all mRNAs in your sample, as well as modifications to the 5′ cap structure, are important for both the recruitment of cellular machinery for translation and *in vivo* stability of your mRNA. Additionally, 3′ poly(A) tail length should be taken into account to ensure the mRNA transcript survives exonuclease digestion in cells. Synthesized mRNA with poly(A) tails shorter than 150 to 200 A's—the typical tail length found in nature—may experience digestion that negatively affects stability of the transcript.

Q Can you expand on the impact design decisions around nucleoside composition, cap structure, and length of poly(A) tail have on both the quality and functionality of mRNA?

RL Methylation of the 5′ cap (Cap-0) results in a structure called Cap-1 that helps pattern recognition sensors in the cell to mark the mRNA as 'self' versus 'non-self'. As mentioned, the poly(A) tail protects mRNA from exonuclease degradation on the 3′ end. However, it is also required for mRNA expression, as the binding of proteins to the poly(A) tail stimulate ribosome recruitment for translation.

Poly(A) tail length shortens over time in the cell through a process known as deadenylation. Once a poly(A) tail reaches a minimum length, the mRNA is decapped and then degraded from the 5′ end. In nature, the rate of deadenylation plays an important role in

> "...confirming that your mRNA is of sufficient quality for your application is a step that shouldn't be overlooked."

gene regulation as the length of the poly(A) tail determines the half-life of the mRNA. Having longer tails will increase the half-life of your mRNA transcript and therefore increase the total quantity of protein expressed. With all this in mind, it is recommended to start with tails of at least 150–200 A's.

Q How can the risk of innate immunity development best be addressed?

RL Cells have evolved mechanisms to quickly recognize and respond to foreign or 'non-self' RNA as a defense against viral infection. These mechanisms are part of what is referred to as innate immunity and include specialized protein receptors called pattern recognition sensors that constantly search RNA for landmarks that denote 'self' versus 'non-self' RNA. The design challenge for researchers is to introduce foreign RNA for protein expression in cells that also has incorporated features that pattern recognition sensors will confirm as 'self'.

Incorporation of modified nucleosides, like pseudouridine and N1-methylpseudouridine, has been shown to reduce cellular innate immune responses. Use of modified nucleosides in mRNA has been particularly beneficial for therapeutic applications.

Another modification that also helps to mark the mRNA as 'self' is the presence of a Cap-1, which is a cap methylation modification only found in higher eukaryotes. 'Non-self' RNAs are either quickly degraded in the cell or elicit apoptosis. Because of this, Cap-1 capped mRNA is expressed at higher levels in cells when compared to Cap-0 capped or even uncapped RNA.

Because double-stranded RNA (dsRNA) is present during the replication cycles of most eukaryotic viral infections, cells have evolved pattern recognition sensors to detect the presence of dsRNA in the cell and then elicit an innate immune response. Unfortunately, dsRNA is also produced as an unwanted byproduct during *in vitro* transcription and, thus, all mRNA preps contain some percentage of dsRNA. Removal of this byproduct is essential for reducing immunogenicity and thus ensuring increased expression of the mRNA.

Q What advice would you share with developers looking to optimize mRNA design and synthesis for their own specific application?

RL There are many resources that discuss optimization of mRNA design. I'd recommend checking out CELLSCRIPT's website, where you can find answers to frequently asked questions and additional tips and tricks for design. You can also find a variety of kits, enzymes, and assays for both mRNA synthesis and QC, as well as can connect directly with an mRNA expert to discuss your research needs in a one-on-one format.

Finally, don't forget quality control testing of your mRNA—confirming that your mRNA is of sufficient quality for your application is a step that shouldn't be overlooked.

Q What are the key considerations—and the common pitfalls—when developing a quality control strategy for synthesized transcripts? What analytical tools and techniques are available to assess quality?

RL The current USP guidelines recommend that both capping and tailing analysis be performed via LC-MS or HPLC. However, there is a high capital cost associated with this equipment both from the initial investment for the equipment to specialized training and ongoing maintenance that these systems require. The high-cost barrier for this instrumentation often means that researchers will use a third party to perform their testing.

Outsourcing quality control testing can have a lot of downsides, including large sample amount requirements, the possibility that samples can be lost or damaged during shipment, long lead times (often weeks) to get results, and the extensive communication needed with the testing facility. Additionally, third-party outsourcing can prove to be very expensive.

As a new entry into the mRNA QC market, CELLSCRIPT has recently introduced our EZ-QC™ mRNA QC Assay Kits. The EZ-QC kits are fluorescence-based PAGE assays that analyze 5′ capping efficiency and 3′ poly(A) tail length. Assays are performed on the benchtop, using common lab equipment, and can be completed in a single day.

Q Finally, what advice would you give to new researchers interested in using mRNA for translation in cells?

RL Take your time when designing your mRNA and focus on the downstream application. For example, how concerned are you about immunogenicity? Would using modified nucleosides be beneficial for your application? How much dsRNA contamination can your application tolerate? Also, just like with any area of science, expect that there might be a lot of trial and error for new scientists.

BIOGRAPHY

Ryan Lahr received his BsC from the University of Northern Iowa, Cedar Falls, IA, USA and has since built a career of over 15 years in the biotech industry, where he has developed products for Hologic, Illumina, and now CELLSCRIPT as a Senior R&D Scientist.

Ryan Lahr, Senior R&D Scientist, CELLSCRIPT, Madison, WI, USA

AUTHORSHIP & CONFLICT OF INTEREST

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CELLSCRIPT™
RNA for Translation in Cells

This is a transcript of a podcast interview.
You can also listen to the recorded podcast here:

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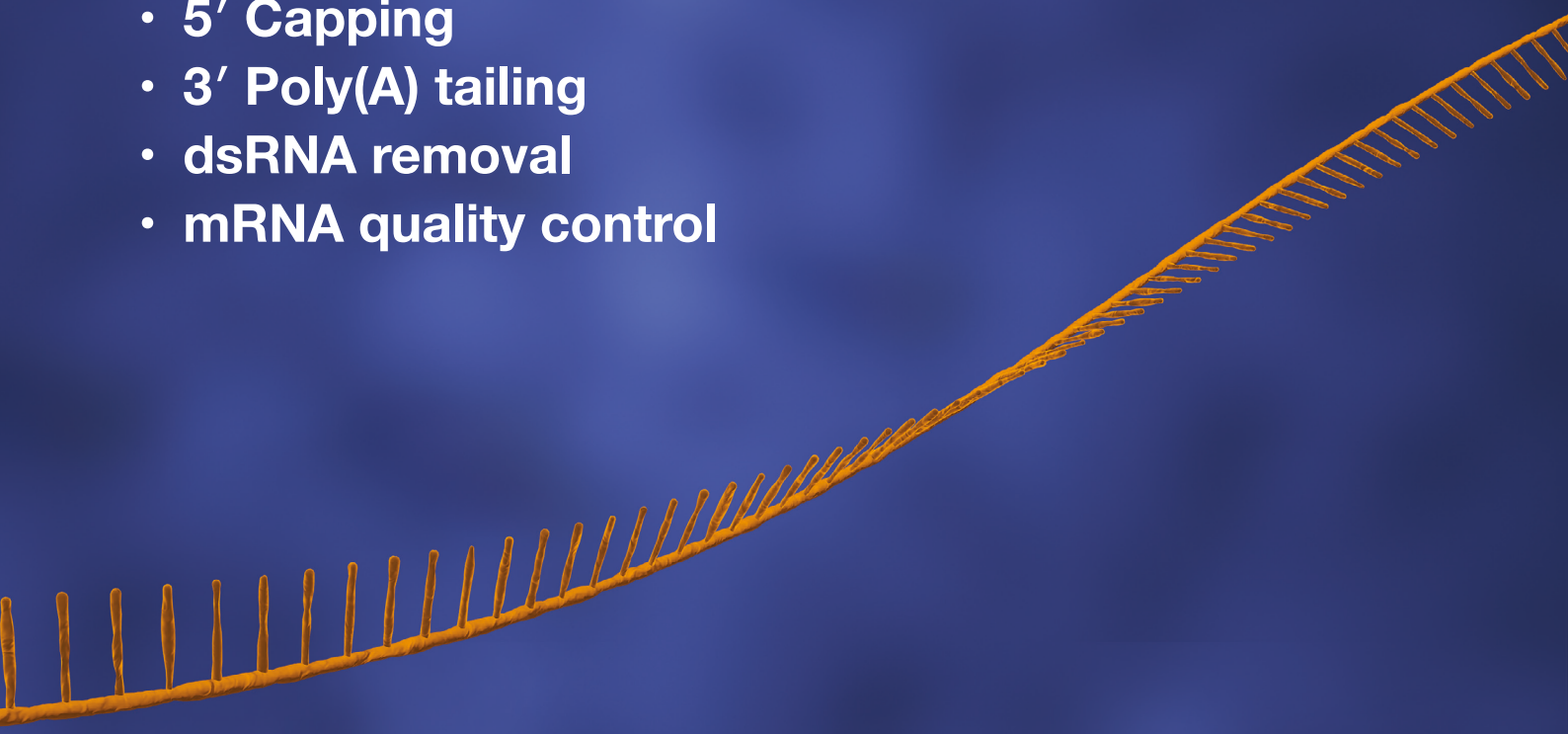
RNA for Translation in Cells

Create mRNAs that translate into discoveries Technologies for:

- IVT with canonical/modified nucleosides
- 5′ Capping
- 3′ Poly(A) tailing
- dsRNA removal
- mRNA quality control



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NUCLEIC ACID INSIGHTS

Meet the

Nucleic Acids Insights Editorial Board

An integral part of the team that brings you *Nucleic Acid Insights* is our fantastic Editorial Advisory Board. This article is part of our 'Meet the EAB' series, created to showcase the leaders in the field who provide their time and expertise to help to steer the scope and focus of the journal.

INTERVIEW

Naim Nazef, Vice President of Oligonucleotide Chemistry, Denali Therapeutics

Nucleic Acid Insights 2025; 2(3), 89–91 · DOI: 10.18609/nuc.2025.014

Q Can you tell us a bit about your current role and the areas you are active in?

I am currently leveraging antibody-based Tfr binders to deliver oligonucleotides (siRNA and ASOs) across the blood-brain barrier (BBB) to address various neurodegenerative disorders of the CNS. Historically, the nucleic acid field has faced significant challenges in achieving extrahepatic delivery of oligonucleotides. However, recent advancements—by Denali and others—have demonstrated the ability to systemically deliver oligonucleotides into the deep regions of the CNS. This represents a game-changing milestone for the field, enabling the development of safer drugs and significantly enhancing the patient experience by moving away from invasive local intrathecal injections.

Additionally, I am deeply interested in discovering new cell-selective receptors to enable targeted delivery of oligonucleotides to specific cell types and tissues. I am also focused on improving the safety and efficiency of delivery by advancing endosomal escape mechanisms in oligonucleotide conjugates.

Q How did your education and career path lead you to the nucleic acids space?

My path to nucleic acids research has been both serendipitous and transformative, shaped by diverse experiences in biochemistry, analytical chemistry, and synthetic chemistry. Born and educated in the UK, I earned a BSc in Biochemistry from the University of Manchester in 1998. Early in my career, I gained expertise in mass spectrometry at Micromass, which led me to NeoGenesis in the US, where I developed a SMol HT affinity-selection mass spectrometry platform. This was my first exposure to a fast-paced drug discovery research environment and really sparked my love for drug discovery. At Merck Research Laboratories in Boston, I expanded my knowledge in drug metabolism and pharmacokinetics, exploring small molecule absorption, distribution, metabolism, and elimination, and bioanalysis. My curiosity about synthetic chemistry led me to pursue an MS in Chemistry at Northeastern University, followed by a PhD in Natural Product Synthetic Chemistry at the University of Edinburgh. I firmly believed that to excel as a chemist, one must have a solid foundation in both organic synthesis and analytical chemistry. In 2011, I joined Dicerna Pharmaceuticals, marking my entry into the nucleic acids field as a PhD scientist. Dicerna's mission to develop oligonucleotide-based therapeutics captivated me, and I embraced the opportunity to apply my skills to this emerging modality. Initially, I focused on developing innovative bioanalytical methods to characterize dicer-substrates encapsulated in LNPs. My role rapidly expanded to include managing all oligonucleotide synthesis activities and leading chemistry efforts to develop the successful GalXC® GalNAc-siRNA delivery platform.

Later, at Takeda Pharmaceuticals, I led an oligonucleotide team, advancing programs across liver and CNS diseases. I now lead the discovery of antibody-oligonucleotide conjugate (AOC) therapeutics at Denali. My broad interdisciplinary training has enabled me to take a holistic view of the end-to-end drug discovery process and effectively contribute to solving complex scientific problems.

Q What are your top predictions for the next five years in the nucleic acids field, and what developments do you most hope to see?

In the next five years, the field will make significant strides in overcoming delivery challenges. Extrahepatic delivery technologies such as antibodies targeting the transferrin receptor will expand, enabling efficient internalization and gene target engagement. I foresee the clinical validation of BBB-crossing Tfr-based AOCs as a transformative approach to treating CNS neurodegenerative disorders, offering less invasive alternatives to intrathecal delivery. I anticipate advancements in identifying cell-surface receptors that can be leveraged to selectively deliver therapeutics to additional cell types, such as immune cells, through in vivo screening approaches. This progress could expand the use of oligonucleotides into immunology, a field where they are currently underutilized but hold significant therapeutic potential.

LNP delivery technologies will continue to improve, driven by AI/ML advancements that refine tissue- and cell-specific delivery. The addition of protein-based targeting ligands to LNPs will enhance their precision, safety, and tolerability. Conversely, AAV-based gene

therapies will face challenges, including pre-existing immunity, high manufacturing costs, limited payload capacity, and steep treatment expenses, hindering patient access. The one-time treatment model will also restrict long-term revenue opportunities. These limitations will open doors for LNP-CRISPR and mRNA-based technologies, which promise to address many of AAV's drawbacks.

I hope to see advancements in oligonucleotide manufacturing beyond traditional solid-phase synthesis, which is less scalable compared to other modalities. This limitation hinders its potential to address larger disease indications where cost is a critical factor for payers. Progress has been made in liquid-phase and enzymatic-based synthesis approaches, and I expect large pharmaceutical companies, with their greater resources and commercial focus, to lead efforts in improving scalability.

Q What was your motivation for joining the board of *Nucleic Acid Insights*, and what do you most hope to see the journal achieve as we enter our second year?

My motivation for joining the board stems from a desire to advance the field of nucleic acid drug discovery and development. I am excited to collaborate with fellow industry leaders to shape discussions on the most pressing topics and foster innovative research within this rapidly evolving space. By contributing to the journal, I hope to help curate content that addresses the most critical and current challenges our readers face, ensuring it remains a valuable resource for the field.

I envision sharing my experiences and insights as a key aspect of my involvement, as I believe knowledge exchange is crucial for a new field to advance as rapidly as possible. Additionally, I am committed to upholding the highest standards of quality and integrity in the journal's content while influencing its direction through feedback on manuscript submissions. This allows us to not only highlight groundbreaking research but also set the journal's scope and standards to reflect the needs and aspirations of the nucleic acids field.

As *Nucleic Acid Insights* enters its second year, I look forward to seeing it become a trusted platform for disseminating impactful research, sparking meaningful dialogue, and shaping the future of nucleic acid therapeutics. My goal is to ensure that it continues to inspire and inform researchers while enhancing its visibility within the field of nucleic acid research.

Q What was your song of 2024?

Good Luck, Babe! by Chappell Roan. It reminds me of the type of 80s music that I used to listen to growing up in the UK.

BIOCONJUGATION INSIGHTS

We are delighted to announce the launch of *Bioconjugation Insights*, a brand new peer-reviewed journal from BioInsights covering one of the most exciting and fast-moving frontiers in therapeutic development.

Bioconjugation represents an intersection of biology and chemistry that continues to redefine drug discovery, targeted delivery, and diagnostics. *Bioconjugation Insights* will provide dedicated coverage of the field via a fully digital, open access publication.

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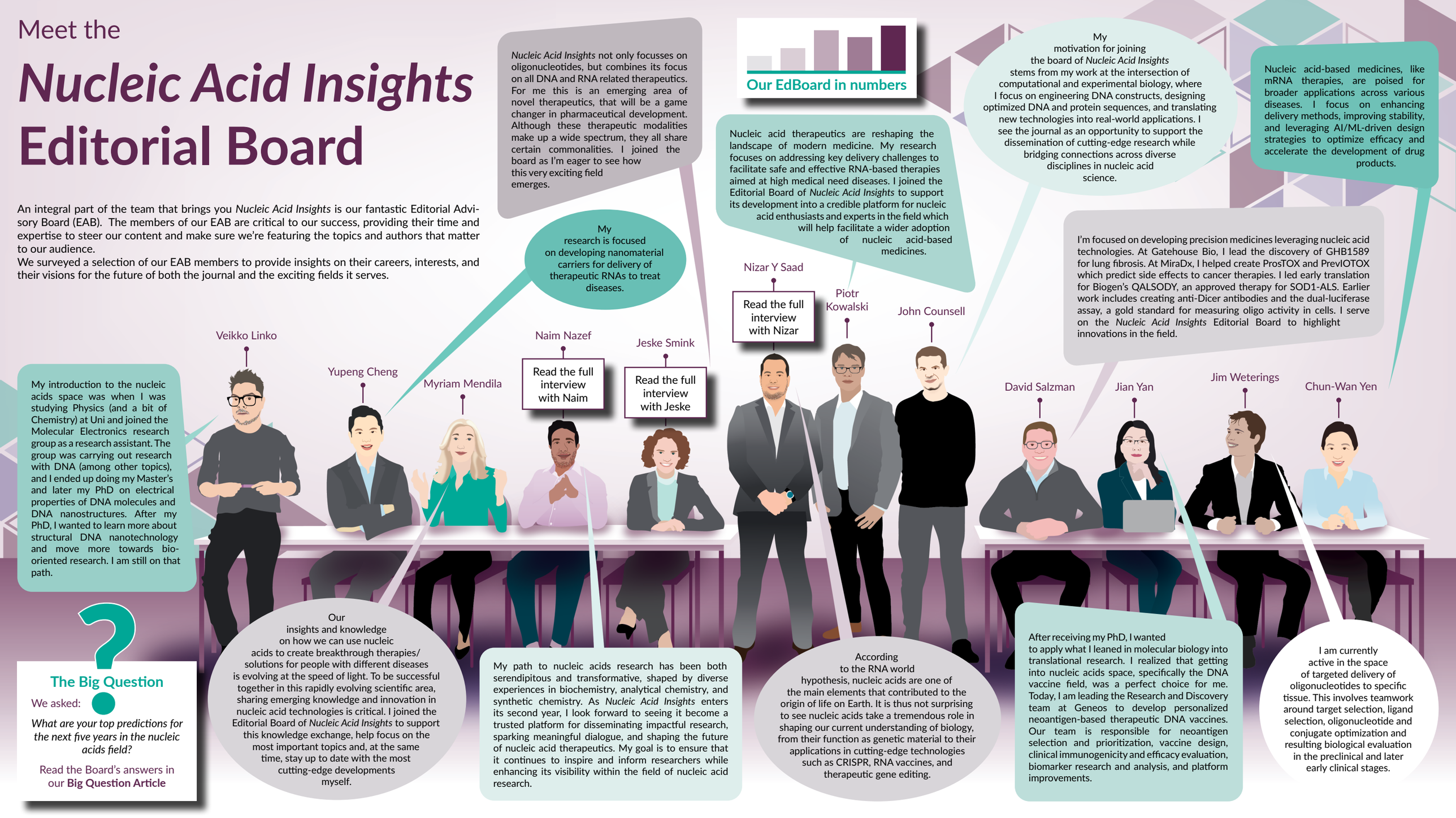
Meet the

Nucleic Acid Insights Editorial Board

An integral part of the team that brings you *Nucleic Acid Insights* is our fantastic Editorial Advisory Board (EAB). The members of our EAB are critical to our success, providing their time and expertise to steer our content and make sure we're featuring the topics and authors that matter to our audience.
We surveyed a selection of our EAB members to provide insights on their careers, interests, and their visions for the future of both the journal and the exciting fields it serves.

Our EdBoard in numbers



Veikko Linko

My introduction to the nucleic acids space was when I was studying Physics (and a bit of Chemistry) at Uni and joined the Molecular Electronics research group as a research assistant. The group was carrying out research with DNA (among other topics), and I ended up doing my Master's and later my PhD on electrical properties of DNA molecules and DNA nanostructures. After my PhD, I wanted to learn more about structural DNA nanotechnology and move more towards bio-oriented research. I am still on that path.

Yupeng Cheng

My research is focused on developing nanomaterial carriers for delivery of therapeutic RNAs to treat diseases.

Myriam Mendila

Our insights and knowledge on how we can use nucleic acids to create breakthrough therapies/solutions for people with different diseases is evolving at the speed of light. To be successful together in this rapidly evolving scientific area, sharing emerging knowledge and innovation in nucleic acid technologies is critical. I joined the Editorial Board of *Nucleic Acid Insights* to support this knowledge exchange, help focus on the most important topics and, at the same time, stay up to date with the most cutting-edge developments myself.

Naim Nazef

Read the full interview with Naim

Nucleic Acid Insights not only focusses on oligonucleotides, but combines its focus on all DNA and RNA related therapeutics. For me this is an emerging area of novel therapeutics, that will be a game changer in pharmaceutical development. Although these therapeutic modalities make up a wide spectrum, they all share certain commonalities. I joined the board as I'm eager to see how this very exciting field emerges.

Jeske Smink

Read the full interview with Jeske

My path to nucleic acids research has been both serendipitous and transformative, shaped by diverse experiences in biochemistry, analytical chemistry, and synthetic chemistry. As *Nucleic Acid Insights* enters its second year, I look forward to seeing it become a trusted platform for disseminating impactful research, sparking meaningful dialogue, and shaping the future of nucleic acid therapeutics. My goal is to ensure that it continues to inspire and inform researchers while enhancing its visibility within the field of nucleic acid research.

Nizar Y Saad

Read the full interview with Nizar

Nucleic acid therapeutics are reshaping the landscape of modern medicine. My research focuses on addressing key delivery challenges to facilitate safe and effective RNA-based therapies aimed at high medical need diseases. I joined the Editorial Board of *Nucleic Acid Insights* to support its development into a credible platform for nucleic acid enthusiasts and experts in the field which will help facilitate a wider adoption of nucleic acid-based medicines.

Piotr Kowalski

According to the RNA world hypothesis, nucleic acids are one of the main elements that contributed to the origin of life on Earth. It is thus not surprising to see nucleic acids take a tremendous role in shaping our current understanding of biology, from their function as genetic material to their applications in cutting-edge technologies such as CRISPR, RNA vaccines, and therapeutic gene editing.

John Counsell

My motivation for joining the board of *Nucleic Acid Insights* stems from my work at the intersection of computational and experimental biology, where I focus on engineering DNA constructs, designing optimized DNA and protein sequences, and translating new technologies into real-world applications. I see the journal as an opportunity to support the dissemination of cutting-edge research while bridging connections across diverse disciplines in nucleic acid science.

David Salzman

I'm focused on developing precision medicines leveraging nucleic acid technologies. At Gatehouse Bio, I lead the discovery of GHB1589 for lung fibrosis. At MiraDx, I helped create ProsTOX and PrevIOTOX which predict side effects to cancer therapies. I led early translation for Biogen's QALSODY, an approved therapy for SOD1-ALS. Earlier work includes creating anti-Dicer antibodies and the dual-luciferase assay, a gold standard for measuring oligo activity in cells. I serve on the *Nucleic Acid Insights* Editorial Board to highlight innovations in the field.

Jian Yan

After receiving my PhD, I wanted to apply what I leaned in molecular biology into translational research. I realized that getting into nucleic acids space, specifically the DNA vaccine field, was a perfect choice for me. Today, I am leading the Research and Discovery team at Geneos to develop personalized neoantigen-based therapeutic DNA vaccines. Our team is responsible for neoantigen selection and prioritization, vaccine design, clinical immunogenicity and efficacy evaluation, biomarker research and analysis, and platform improvements.

Jim Weterings

Nucleic acid-based medicines, like mRNA therapies, are poised for broader applications across various diseases. I focus on enhancing delivery methods, improving stability, and leveraging AI/ML-driven design strategies to optimize efficacy and accelerate the development of drug products.

Chun-Wan Yen

I am currently active in the space of targeted delivery of oligonucleotides to specific tissue. This involves teamwork around target selection, ligand selection, oligonucleotide and conjugate optimization and resulting biological evaluation in the preclinical and later early clinical stages.

The Big Question

We asked:

What are your top predictions for the next five years in the nucleic acids field?

Read the Board's answers in our **Big Question Article**

Meet the *Nucleic Acid Insights* Editorial Board

How do you get to work?

What language(s) do you speak?

How many years have you been active in the nucleic acids field?

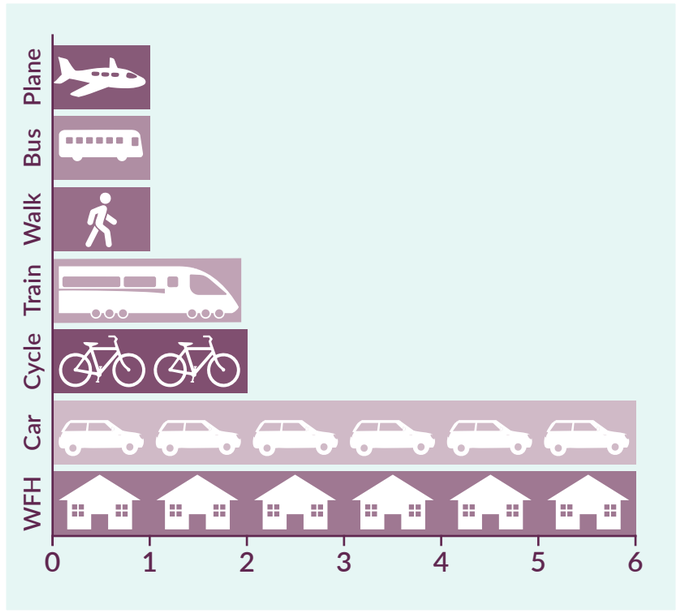
What type of organization(s) do you work for?

Favorite cheese?

What area(s) of the nucleic acids space are you primarily active in?

What is your location?

What other countries have you lived in?



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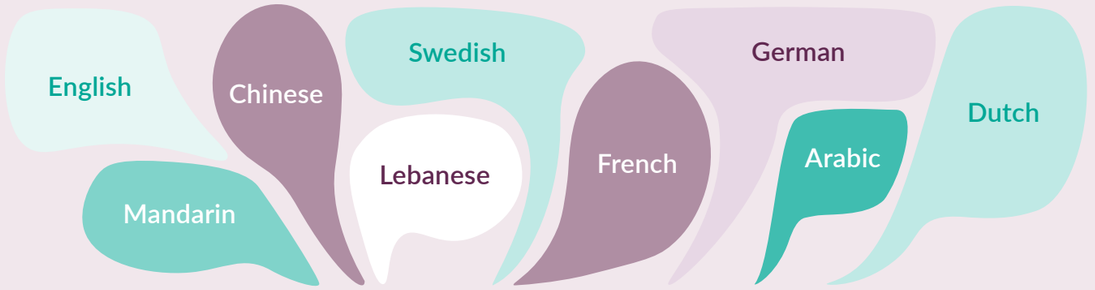

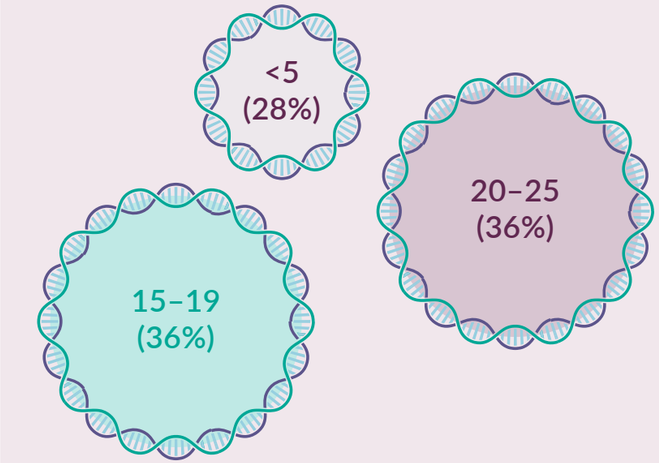

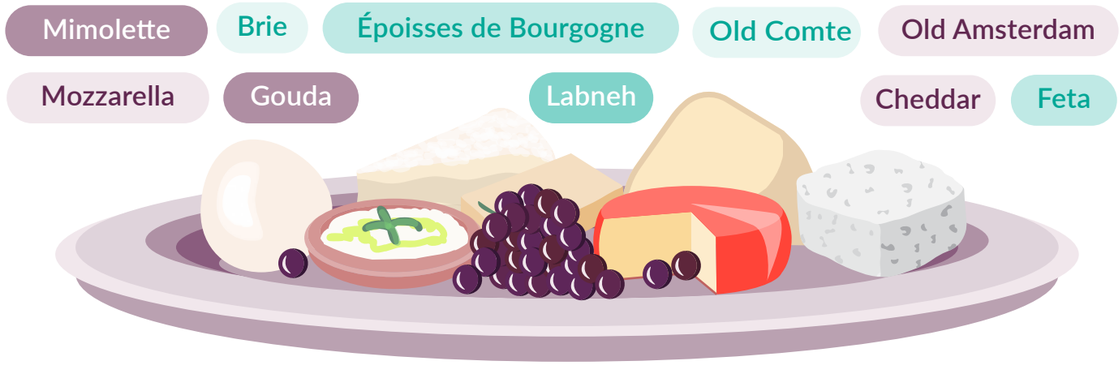

42%

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- Nanoparticles
- Oligonucleotides
- RNA
- mRNA

17%

- DNA