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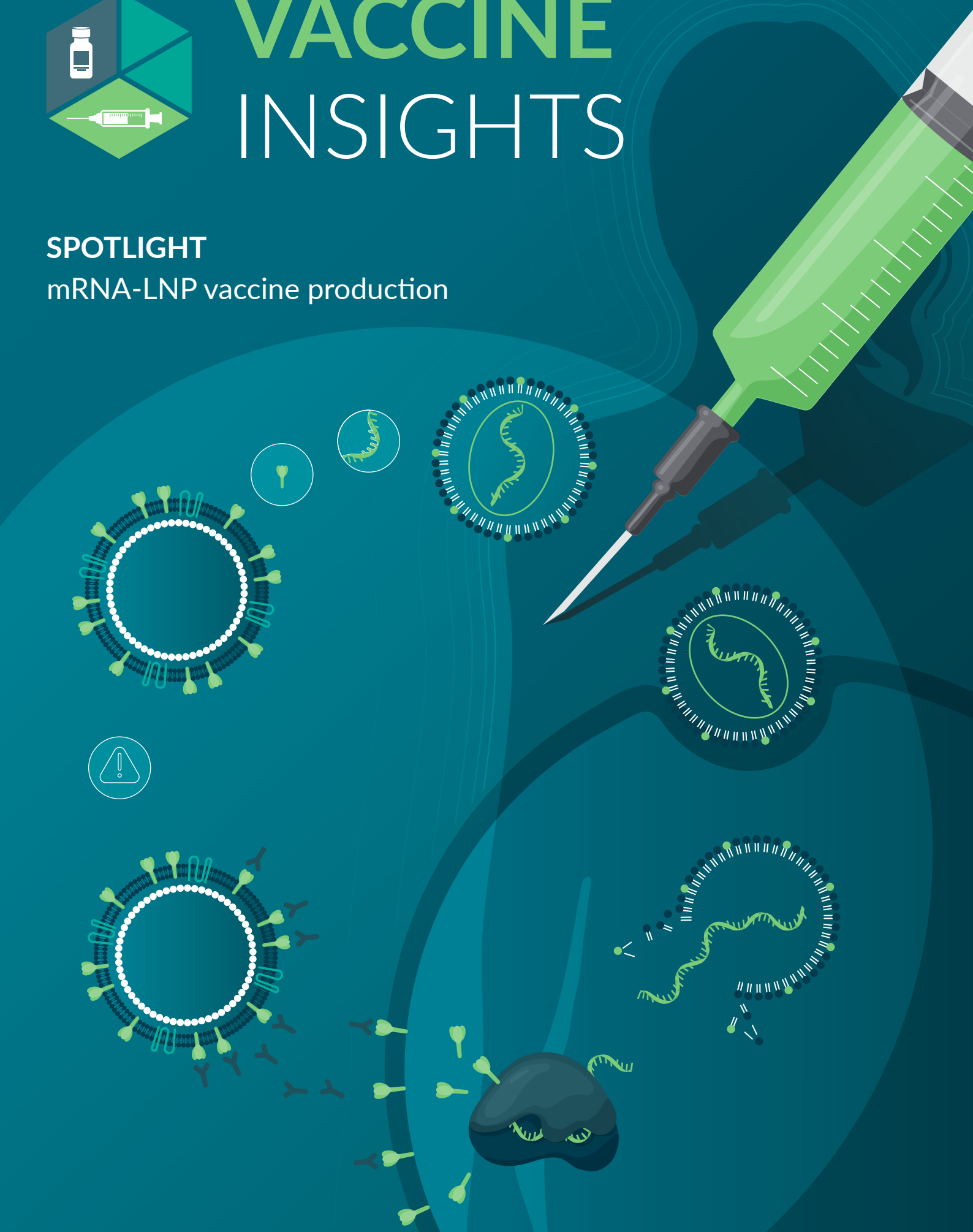
VACCINE INSIGHTS

SPOTLIGHT

mRNA-LNP vaccine production



VACCINE INSIGHTS

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mRNA-LNP vaccine production

VACCINE INSIGHTS

SPOTLIGHT

Advancing mRNA-LNP characterization: innovative chromatographic approaches for next-generation therapeutics

INTERVIEW

"No single analytical technique can comprehensively characterize complex biomolecules such as mRNA-LNP products."

Charlotte Barker (Editor, *Vaccine Insights*) speaks to **Jonathan Maurer** (Postdoctoral Researcher, University of Geneva) about critical quality attributes for mRNA-lipid nanoparticle formulations, novel chromatographic methods for integrity and encapsulation efficiency evaluation, and the technical challenges of characterizing large RNA therapeutics.

Vaccine Insights 2025; 4(8), 269–273 · DOI: 10.18609/vac.2025.040

Q Can you tell us about your background and how you became involved in mRNA-LNP characterization?

JM My background spans multiple analytical disciplines, beginning with forensic science training at the University of Lausanne in Switzerland. This

interdisciplinary training provided early exposure to physics, chemistry, and biology, and taught me how to integrate data across fields.

During my master's program, I specialized in forensic chemistry. From there, I transitioned to toxicology and then clinical chemistry, where I focused on biomarker discovery by quantifying endogenous peptides using liquid chromatography–mass spectrometry.

I am currently a postdoctoral researcher at the University of Geneva, collaborating with Sanofi to improve the characterization of mRNA-lipid nanoparticle therapeutics and vaccines. This field is evolving rapidly, creating significant demand for robust characterization methodologies and demanding continuous adaptation to novel analytical challenges, which makes this research particularly compelling.

Q What critical quality attributes present the greatest analytical challenges for mRNA-LNP formulations?

JM Rather than a single attribute, much of the challenge stems from the diversity of new therapeutics and vaccines entering the market, each presenting unique analytical requirements. However, encapsulation efficiency evaluation emerges as one of the most significant technical challenges.

First, the definition of encapsulation efficiency lacks standardization: does it represent the proportion of mRNA physically within the LNP structure, or the proportion functionally protected by the lipid matrix and capable of reaching ribosomes? This conceptual ambiguity complicates both the development of methods and the interpretation of results.

The challenge is compounded by the inherent instability of LNP. These formulations are extremely sensitive to routine sample handling, including dilution, freeze-thaw cycles, mixing, and standard laboratory manipulations. This instability makes an accurate evaluation exceptionally challenging, as the measurement process itself can alter the parameter being assessed.

These combined challenges position encapsulation efficiency as one of the most complex critical quality attributes to evaluate reliably across different mRNA-LNP products.

Q Can you describe the innovative chromatographic approaches your team published this year and explain how they advance current analytical capabilities?

JM We published different complementary studies. The first investigated ion-pairing reverse-phase chromatography, a method established for small oligonucleotides [1]. The fundamental question was whether this proven approach could be successfully extended to larger mRNA molecules, which exhibit markedly different chromatographic behavior.

This study systematically investigated the impact of ion-pairing agent selection on retention behavior and resolution when analyzing mRNA products. While effective, several limitations emerged. Ion-pairing agents pose environmental and safety concerns, are incompatible with mass spectrometry detection, and samples require extensive

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pretreatment, as LNPs must be disrupted to release encapsulated mRNA and removed to avoid interference with the reverse-phase separation.

To cope with these challenges, the second study investigated hydrophilic interaction liquid chromatography (HILIC) as an alternative to ion-pairing reverse-phase chromatography [2]. While HILIC had been applied to small oligonucleotides, its potential for drug product applications remained largely unexplored.

The key innovation was to demonstrate that drug products can be injected directly, without pretreatment, because the disruption of LNPs was achieved within the column. Indeed, HILIC operates under high-temperature and high-organic solvent conditions, which are highly aggressive for LNPs. As a result, this enables integrity profiling without prior sample manipulation, thereby preserving the native state of mRNA. The method produced excellent results for integrity evaluation. However, current commercial HILIC columns present limitations due to inadequate pore sizes for very large molecular species, suggesting that further development of column technology is required.

Finally, to address the need for innovative methods to evaluate encapsulation efficiency, we recently developed an anion-exchange chromatography approach. In this work, we present a robust method to assess encapsulation while also providing information on mRNA localized at the surface of LNPs [3]. This study deepens the understanding of LNP structure–function relationships and provides an orthogonal alternative to the gold-standard RiboGreen assay for batch release and quality control of mRNA–LNP formulations.

Q Building on these findings, what are your ongoing projects?

JM We are broadening our focus to other critical quality attributes, particularly the structural features at the 5′ cap and poly-A tail. We are developing methods to characterize capping efficiency, poly-A tail length, and heterogeneity — all critical for mRNA stability and translation.

In parallel, we are refining HILIC methodology, as its full potential has not yet been realized. We are evaluating new column types to determine whether HILIC could provide a single platform for both integrity and encapsulation efficiency.

Q How does your academic-industry partnership with Sanofi influence your research approach?

JM Working with Sanofi fosters a uniquely fruitful collaboration posing targeted analytical questions, such as how to evaluate encapsulation or capping efficiency. These industry-driven needs provide clear objectives and realistic timelines for method development, validation, and technology transfer to industrial laboratories.

Access to authentic samples is another major benefit: genuine mRNA, LNP formulations, drug products, vaccines, and intermediates allow us to validate methods under realistic conditions rather than simplified models.

The partnership also enables fundamental research with broader scientific benefits, ensuring outcomes with both immediate industrial relevance and long-term impact.

Q Have you explored these methods with larger RNA molecules like self-amplifying or circular RNA?

JM Not yet, primarily due to fundamental technical limitations. Current column pore sizes are inadequate for such large molecular species, creating separation challenges that cannot be resolved through method optimization alone.

Additionally, minor structural modifications are nearly undetectable in very long RNA molecules. A single nucleotide change in a 30-nucleotide oligonucleotide significantly alters the molecule's physicochemical properties, making detection straightforward. However, the same change in a 30,000-nucleotide molecule becomes essentially insignificant from an analytical perspective.

These challenges often necessitate digestion into smaller fragments, though this compromises analysis of intact structures. Future solutions will require improved separation technologies and novel analytical strategies tailored for large RNA therapeutics.

Q What is your vision for the optimal analytical workflow for mRNA-LNPs over the next 5 years?

JM No single analytical technique can comprehensively characterize complex biomolecules such as mRNA-LNP products. Therefore, adaptive workflows combining multiple tools will be essential.

Multi-dimensional chromatography is expected to become increasingly widespread in the coming years, offering numerous opportunities to streamline the analysis of mRNA–LNPs by integrating information from several orthogonal methods. This approach will help minimize sample manipulation from production through analysis, which is critical for preserving native properties and avoiding artifacts.

Sustainability will also shape the field: greener methods with reduced energy and reagent demands will be essential. The gold standard workflow will combine multiple separation dimensions, minimal sample handling, automated operations, and sustainable practices—supporting both the rapid expansion of the mRNA therapeutics market and the growing complexity of biopharmaceuticals, while addressing diminishing resource availability. The challenge ahead for industries and researchers is clear: to do more with less.

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BIOGRAPHY

Jonathan Maurer is a post-doctoral researcher at the Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, working in the group led by Dr Davy Guillarme. His research focuses on developing advanced chromatographic methods for characterizing mRNA-based drug products in collaboration with Sanofi's mRNA Center of Excellence. He completed his PhD at the University of Lausanne, Switzerland where he specialized in mass spectrometry quantification of endogenous peptides and their clinical relevance. Jonathan has authored over 10 publications in high-impact journals (h-index 6), presented at international conferences, and received over CHF 125,000 in research funding. He is actively involved in scientific communities, serving as a board member of the ccCTA, as the founder and president of its Young Scientists' Club, and as a regular reviewer for leading journals. Passionate about analytical chemistry and innovation, his work bridges academia and industry to advance pharmaceutical science.

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VACCINE INSIGHTS

mRNA-LNP PRODUCTION

SPOTLIGHT

Balancing the tradeoffs in IVT: enzyme engineering, reaction optimization, and dsRNA control for safe, scalable RNA production

Kaixi Zhao and Emily Wen

VIEWPOINT

"The growing demand for RNA-based vaccines and therapeutics has exposed critical trade-offs in current *in vitro* transcription (IVT) workflows. To ensure safe and scalable RNA production, manufacturers must balance enzyme engineering, reaction optimization, and dsRNA control to improve efficiency without compromising quality."

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In vitro transcription (IVT) is a versatile and powerful technique for producing RNA. It supports fundamental RNA research and is essential for the development and manufacture of RNA-based vaccines and therapeutics. IVT began as researchers sought to synthesize RNA using DNA templates in test tubes. Early work in the 1960s and 1970s established that DNA-dependent RNA polymerases (RNAPs) could transcribe DNA *in vitro* when supplied with nucleoside triphosphates and appropriate cofactors.

Bacteriophage RNAPs such as T7, SP6, and T3 recognize a well-defined, short promoter sequence and can initiate, elongate, and terminate transcription without accessory factors. These single-subunit phage RNAPs produce large amounts of full-length RNA from linear DNA or plasmid templates containing the promoter sequences, and they tolerate simple reaction conditions. These properties made them attractive for early biochemical studies.

T7 RNA polymerase was among the first to be cloned and expressed recombinantly in useful amounts [1]. It rapidly became widely used in labs for transcription and expression systems. T7 RNA polymerase is exceptionally fast, precise, and capable of producing large amounts of RNA in a very short reaction time. Later, it became the main workhorse for mass RNA production because it also allows incorporation of various modified nucleotides due to its broad substrate tolerance. SP6 and T3 RNA polymerase were characterized similarly and remain useful alternatives.

Standard IVT with wild-type T7 RNA polymerase often produces immunostimulatory byproducts, notably double-stranded RNA (dsRNA), which triggers innate immune sensing. dsRNAs are highly immunogenic because they are typically only present when cells are infected by viruses; for example, dsRNA is produced during single-stranded RNA virus replication. Therefore, like viral RNA and DNA, dsRNA is considered a virus-associated molecule by cells and can be recognized by host pattern-recognition receptors in innate immune cells [2]. These dsRNAs reduce protein expression and can cause unwanted inflammation; therefore, low levels of dsRNA in drug substances are critical for the safety and efficacy of vaccines and therapeutic products. There are currently no clear guidelines for acceptable dsRNA levels in vaccines or therapeutics, making the removal or avoidance of dsRNA particularly important.

A few purification methods are currently available for dsRNA removal, such as ion-pairing reverse phase high-performance liquid chromatography [3] and cellulose-based purification [4]. Some groups have taken other approaches to reducing dsRNA levels by engineering T7 RNA polymerase to produce less dsRNA under standard IVT conditions [5], or producing T7 RNA polymerase mutants that could tolerate higher temperatures, thus reducing formation of the 3' extension of run-off products [6]. The authors engineered variants of T7 RNA polymerase through structure-guided mutagenesis and directed selection to alter polymerase activity and reduce the formation of immunogenic byproducts during IVT. More companies are developing their versions of T7 mutants that produce minimal or no dsRNA. A third commonly used approach is to optimize IVT reaction conditions so that they generate low levels of dsRNA while preserving all other aspects of IVT performance.

Each approach has its own set of pros and cons. dsRNA removal methods offer versatility and compatibility with varying levels of dsRNA produced from upstream IVT; however, they may not be easily implemented at larger manufacturing scales and often introduce complexity and additional cost to the downstream purification process. Enzyme engineering of T7 polymerase can significantly reduce dsRNA formation and may eliminate the dsRNA-removal step downstream, enabling a

simpler workflow, reduced process-optimization needs, and lower manufacturing costs. However, many engineered enzymes are not readily available for GMP commercial use and are often single-sourced because of IP protection. Single-sourced material faces several operational, regulatory, and quality risks. Interruptions at the supplier can halt the supply chain and delay planned production. Single sourcing reduces resilience and redundancy, complicates risk assessments (e.g., logistical, geopolitical), and makes it harder to reroute supplies when disrupted. The sole supplier also has strong negotiating power, which can lead to limited discounts or the imposition of unfavorable contract terms. Maintaining an additional stockpile or investing more in inventory buffers may be required to mitigate single-source risk.

Optimizing IVT reaction conditions with wild-type T7 facilitates a more straightforward process, as dsRNA levels in the IVT reaction remain within acceptable safety limits, thereby reducing the burden on purification for dsRNA removal. This also provides the flexibility to choose T7 from multiple vendors. IVT reaction optimization can be complex and resource-intensive, sequence-dependent, and often requires high-throughput capability for DOE studies. A platform IVT process can be developed, but it still needs to be validated before production of a new RNA construct. Acceptable safety limits may differ depending on the intended application of the RNA and may need to be established by animal studies and/or clinical trials.

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BIOGRAPHY

Kaixi Zhao is an Associate Principal Scientist at Merck with over 10 years of research experience in the life sciences, focusing on RNA viruses, siRNA, mRNA, and circRNA. Since joining Merck in 2022, her primary responsibilities have been in vaccine bioprocess development, including process optimization, scale-up, technology transfer, and GMP manufacture.

Emily Wen, is currently a Senior Principal Scientist at the Merck Research Laboratory in the department of Vaccines & Advanced Biotechnologies Process Development & Research. Dr Wen has over 30 years of experience and has led process developments for several major

vaccine products from Phase I to Phase III clinical trials, including vaccines against hepatitis B, *Haemophilus influenzae* type B, pneumococcal, meningococcal, and group B streptococcus. Dr Wen has authored a book on vaccine development and production and is an inventor on numerous patents in pneumococcal conjugate vaccine space.

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VACCINE INSIGHTS

mRNA-LNP VACCINE PRODUCTION

SPOTLIGHT

Reengineering membranes for the RNA era: overcoming diffusion, fouling, and scale-up barriers

INTERVIEW

"Ultimately, success will depend on robust standardization of process control and quality assurance across the industry, ensuring reproducibility and regulatory confidence as these systems move toward broader adoption."

Ashling Cannon (Editor, BioInsights) speaks to **Georges Belfort** (Institute Professor of Chemical and Biological Engineering, Rensselaer Polytechnic Institute) about how his work in transport phenomena and membrane science is being applied to the manufacturing and purification of mRNA-LNP vaccines. They discuss how membrane-based systems outperform diffusion-limited chromatography for large biomolecules, strategies to reduce fouling and improve selectivity, and new insights into polymer microstructure and convective flow. Belfort also shares his perspective on designing low-cost, scalable membrane systems for global vaccine access and the need for a standardized RNA platform manufacturing process.

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Q You have had a long and distinguished career in separation science. How did you first become interested in this field, and what ultimately led you to apply your expertise to biotechnology and vaccine-related challenges?

GB My academic career began in chemical engineering at the University of Cape Town. A gifted lecturer, Heinrich Buhr, introduced me to transport phenomena, which unites the movement of heat, mass, and momentum under a single mathematical framework. That elegant connection between theory and application has guided my work ever since.

Since then, I have remained devoted to transport processes, particularly mass and fluid mechanics. In deciding where to focus my research, I have always been guided by societal need. Engineering, to me, should address problems that matter. Early in my career, this meant desalination, developing membranes capable of providing affordable water in arid regions such as California and later Israel. These projects demonstrated the potential of membrane technologies to deliver practical, scalable solutions.

As monoclonal antibodies emerged as leading therapeutics, I saw parallels between water purification and the separation of biological macromolecules. Adapting membrane technologies from environmental to biomedical systems opened a new frontier that combined fundamental engineering with medical impact.

A decisive influence was my wife, RNA biologist Marlene Belfort, whose pioneering research on prokaryotic introns first drew me toward molecular biology. I often say that I entered the field through osmosis; it was impossible not to, surrounded by her journals and discussions at home.

When the COVID-19 pandemic exposed the difficulty of purifying mRNA at scale, it became clear that membrane science could again provide part of the solution. Working with the nearby RNA Institute co-founded by Marlene, and supported by federal pandemic-preparedness funding, our group established a research program focused on scalable, affordable mRNA purification.

Q When it comes to purifying mRNA–LNP vaccines, how do membrane-based strategies compare with more traditional methods?

GB The expansion of RNA technology beyond vaccines into therapeutic applications has created new challenges for purification. In addition to linear RNA, both circular RNA and self-amplifying RNA have demonstrated promising biological activity. However, their manufacturing processes are not yet fully optimized, particularly with respect to large-scale purification.

Traditional chromatographic methods, which perform well for proteins, are poorly suited to these larger RNA molecules. Proteins such as monoclonal antibodies are comparatively small and diffuse easily into the fine pores of chromatography beads. mRNA molecules, and the LNPs that encapsulate them, are approximately an order of magnitude larger. According to Einstein's diffusion relationship, that increase in size translates to at least a tenfold decrease in mobility. The result is inefficient mass transfer, pore blockage, and reduced productivity.

Diffusion-limited resins, the long-standing workhorse of protein purification, are therefore fundamentally incompatible with the scale and structure of RNA molecules. Revisiting published performance data through the lens of transport theory quickly confirms this limitation.

Convective-flow systems, such as membranes and monoliths, provide a more rational solution. In these pressure-driven systems, material is actively transported through a porous medium rather than relying on slow diffusion. This approach supports higher device capacity, shorter residence times, and significantly improved productivity. In practical terms, the question is simple: how much purified material can be produced per unit time and per unit device? For RNA, the answer is unequivocal: membrane systems deliver superior throughput because they harness convective flow instead of diffusion.

Even so-called perfusive chromatography resins, which contain internal channels intended to enhance flow, do not provide a true solution. The majority of fluid still bypasses those channels, resulting in loss of effective surface area. For large RNA molecules, these systems cannot match the efficiency or scalability of well-designed membrane-based approaches.

Q Scaling membrane processes can bring challenges such as fouling and mass-transfer limitations. What design principles have proven most effective for maintaining reliability at larger scales?

GB Fouling has been a central focus of my research for more than three decades, resulting in over 50 publications dedicated to understanding and mitigating this phenomenon. My group has developed high-throughput approaches to evaluate which membrane surfaces are least susceptible to fouling by proteins. Although these methods have not yet been applied to nucleic acids, the same principles can be adapted to assess RNA systems. Screening a range of membrane chemistries at high throughput enables rapid identification of low-fouling materials and process conditions.

The chemistry of the membrane surface is the primary determinant of fouling behavior. Hydrophilic and chemically stable materials tend to resist the adsorption of biomolecules more effectively than hydrophobic ones. Flow modulation through the membrane is the second key factor, as pore structure and flow regime govern both selectivity and fouling kinetics.

There is also a knowledge gap within the biomanufacturing community. Much of the sector's expertise derives from protein chromatography, and comparatively few practitioners are familiar with membrane selection, membrane chemistry, and the associated fluid mechanics. Broader technical understanding is urgently needed so that manufacturing teams can fully exploit the advantages of membrane systems for RNA purification.

Following the *in vitro* transcription step, ultrafiltration and diafiltration are typically employed to remove small reactants and byproducts. Recycling these smaller components back into the reactor can improve yield and cost efficiency.

For the critical capture stage, the team has developed peptide-functionalized membranes that selectively bind the target mRNA; essentially a 'hook' designed for a specific molecule. This approach enables precise discrimination between capped and uncapped RNA and facilitates removal of double-stranded RNA, which is highly immunogenic and can halt production if not eliminated.

By integrating this capture mechanism into membrane systems, the process remains both scalable and operationally straightforward. The result is a high-purity RNA stream with significantly reduced fouling, achieved through deliberate material selection and process design.

Of course, all membranes foul to some degree. The goal is to minimize, not eliminate, fouling. Materials such as regenerated cellulose have proven exceptionally robust in protein separations and are now showing similar promise for nucleic-acid scale-up. These strategies collectively form the basis of a reliable, high-yield membrane-purification process for RNA therapeutics.

Q Your work on interfacial forces and polymer-modified surfaces has been highly influential. How do these insights translate into improved impurity removal during mRNA–LNP purification?

GB Recent investigations revealed a critical structural limitation in many commercial polymer membranes. High-resolution imaging and modeling demonstrated that fluid flow tends to concentrate through narrow, elongated microchannels within the membrane structure, sometimes up to eight times longer than the average pore diameter.

This channeling effect compromises separation efficiency. Instead of diffusing uniformly through a porous medium, solute components travel together through preferential flow paths, reducing surface contact and preventing effective discrimination between species.

Since that publication, the group has made substantial progress in both the analysis, and redesign of membrane architectures. Using *in silico* modeling and experimental validation, new polymer structures are being developed to eliminate channeling entirely and ensure consistent flow distribution across the membrane.

Interfacial-force measurements have also become a valuable tool in this research. By quantifying the adhesive interactions between membrane surfaces and potential foulants, it is possible to identify materials with inherently low-fouling propensity. The same techniques are now applied to study ligand–RNA interactions. For example, by measuring the binding forces of peptide ligands that selectively capture double-stranded RNA, the team can precisely evaluate ligand performance and optimize selectivity.

Together, these investigations link membrane microstructure, interfacial chemistry, and separation performance, providing a foundation for the design of next-generation membranes tailored for RNA purification and other biomanufacturing applications.

Q Continuous purification is gaining attention across biomanufacturing. What are the key hurdles and opportunities for applying continuous membrane systems to mRNA production?

GB In the protein world, one of the most significant rate-limiting steps in continuous processing is viral removal. Viruses are exceptionally small, requiring membranes with tight pore structures. This final filtration stage often becomes a bottleneck, as it restricts overall process throughput.

"Fouling and concentration polarization remain the principal technical barriers to implementing continuous membrane systems for RNA purification."

A similar challenge exists in RNA purification, though for different reasons. mRNA molecules are much larger, but they must still be separated from impurities such as double-stranded RNA, which are nearly identical in size and structure. Achieving that level of selectivity without compromising yield is complex.

Fouling and concentration polarization remain the principal technical barriers to implementing continuous membrane systems for RNA purification. Maintaining process integrity requires careful control of both parameters. The opportunity, however, lies in the superior performance characteristics of membranes. Pressure-driven flow enables high device capacity, low residence times, and compact system design.

Importantly, membranes are inherently modular. Large-scale implementations already exist in other industries; for instance, the largest petroleum plant in the world operates at a lower flow rate than the largest membrane plant. This demonstrates that scaling is not the problem; membranes can be scaled effectively and reliably. The challenge is ensuring stability, reproducibility, and fouling control under continuous operating conditions.

The promise of continuous membrane processing lies in its ability to reduce cost, increase throughput, and integrate multiple purification steps into a single streamlined operation. By combining high throughput with consistent product quality, continuous systems could markedly improve efficiency across RNA manufacturing. Ultimately, success will depend on robust standardization of process control and quality assurance across the industry, ensuring reproducibility and regulatory confidence as these systems move toward broader adoption.

Q Looking ahead, what innovations or hybrid approaches are most likely to enable the next generation of scalable mRNA–LNP purification?

GB The most immediate priority is to design polymer membranes that eliminate channeling entirely. Ensuring uniform flow distribution across the membrane is fundamental to improving efficiency and reproducibility.

Beyond structural optimization, the main challenge is cost. Extending this technology to low- and middle-income countries requires reducing the price of key materials and reagents. The bioreactor used for *in vitro* transcription relies on several expensive components, such as T7 polymerase, capping enzymes, and linear DNA templates. Unless the cost of these inputs is significantly reduced, widespread technology transfer will remain difficult.

My group is actively working to lower production costs through membrane-based approaches. We are exploring the use of lower-cost membranes and innovative capture methods that minimize reagent consumption. Peptide-capture ligands, for instance, have become far more affordable in recent years. They can now be synthesized at scale with high yield, making them a promising option for selective RNA purification at reduced cost.

Ultimately, what made monoclonal antibody manufacturing so successful was the establishment of a platform process; a standardized, transferable production framework that the entire field could adopt. The RNA industry needs the same foundation. Developing a comparable platform process would make RNA manufacturing more efficient, less expensive, and globally accessible, enabling its use across both high-income and resource-limited settings.

BIOGRAPHY

Georges Belfort, Institute Professor at Rensselaer Polytechnic Institute (RPI), USA, is internationally recognized for pioneering work in membrane science and molecular separations for biotechnology applications. A member of the US National Academy of Engineering, his research focuses on continuous bioseparations and purification of nucleic acids, including mRNA vaccines, with support from BioNTech and the Gates Foundation. Dr Belfort has authored over 260 peer-reviewed papers and holds 18 patents in separations science and bioprocessing. His contributions have advanced downstream processing and purification technologies critical to vaccine and therapeutic development.

Georges Belfort PhD, Institute Professor at Rensselaer Polytechnic Institute (RPI), New York, USA

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VACCINE INSIGHTS

IMMUNE RESPONSE UPDATE

Integrating immunopeptidomics and mRNA technology to tackle antimicrobial resistance

INTERVIEW

"Thus, using immunopeptidomics, we are able not only to identify potential vaccine targets but also to prioritize them."

Charlotte Barker (Editor, *Vaccine Insights*) speaks to **Francis Impens** (Group Leader at VIB and Professor at Ghent University) about how advances in proteomics are being applied to antigen discovery for mRNA vaccines against intracellular, antibiotic-resistant bacterial pathogens.

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Q What motivated you to apply proteomics in infection research?

FI I did my PhD in proteomics at Ghent University and became interested in infectious diseases through a collaboration with researchers at the Pasteur Institute in Paris. This experience led me to a postdoctoral position in infection research. I then joined the laboratory I had collaborated with, which specialized in infection by *Listeria monocytogenes*—an intracellular bacterium that causes listeriosis. I became fascinated by how much could still be discovered by applying modern technologies such as

proteomics to such a well-established infection model. When I started my own research group, I decided to combine both areas of expertise—proteomics and infection research.

Q What is the current focus of your group at Ghent University?

FI We use mass spectrometry to investigate the protein interplay between pathogenic bacteria and their host. Most of our current projects focus on the host side.

Our main line of research investigates ubiquitin-like modifications in the immune system, particularly ISG15. A related line of research explores infection-triggered rare diseases. I would describe this as our most fundamental and 'high risk, high gain' area of work.

Additionally, we have a more applied research line focused on antigen discovery for vaccine development. In this area, we use proteomics—and more specifically, immunopeptidomics—to identify bacterial proteins that could serve as potential vaccine candidates.

Q What is immunopeptidomics and how can it be applied for vaccine discovery?

FI Immunopeptidomics is an application of proteomics that has been established for almost 30 years now. This technology allows the identification of peptides that are presented by major histocompatibility complexes (MHCs) on the surface of infected cells, enabling us to determine which bacterial epitopes are displayed on infected cells.

Through this approach, we believe it is possible to perform antigen discovery through the lens of immunology. The underlying idea is that by measuring what the immune system 'sees' from the bacterium, we can obtain a strong proxy or a robust approach for identifying immunodominant antigens that make good vaccine candidates.

Bacterial pathogens are particularly relevant to study because of the rising problem of antimicrobial resistance (AMR). Bacteria are complex pathogens with many expressed proteins—making it difficult to identify suitable antigens as vaccine candidates. This is especially true for intracellular bacteria, for which there are very few licensed vaccines.

One of the most striking findings from our work is that when investigating the epitopes identified for a given bacterium across different screens, cell types, and even organisms, we consistently detect peptides derived from the same bacterial proteins. These recurring peptides are prioritized as vaccine candidates. Thus, using immunopeptidomics, we are able not only to identify potential vaccine targets but also to prioritize them.

Q What are the greatest challenges in this approach?

FI Researchers working in immunopeptidomics use the tools and instruments of proteomics, but every step—from sample preparation to mass spectrometry and data analysis—has unique challenges. For example, most standard proteomics workflows rely on trypsin to digest proteins into smaller peptides, which are then measured by mass spectrometry. MHC peptides are already suitable for mass spectrometry, but they do not share all the features of standard tryptic peptides, which introduces challenges at

"[...] combining immunopeptidomics for antigen discovery with mRNA vaccine formulation for intracellular bacteria creates a powerful approach."

multiple levels. Since these immune peptides are non-tryptic, they have different charge profiles, and the search space is much larger. For example, for MHC Class I, one must consider every possible peptide from 8–13 amino acids in length, which significantly expands the search space compared to regular proteomics applications.

Another key challenge, particularly in bacterial antigen discovery, is maintaining tight control over what we call the false discovery rate. In proteomics, we typically allow for 1% false positive identifications in our data. In the context of infection, bacterial peptides typically constitute less than 1% of all the immunopeptides presented — the vast majority are host-derived peptides. Therefore, we must adapt our process to give extra confidence that the bacterial peptides we identify are not part of the 1% false positives we normally accept.

Although this application has existed for decades, only in the last ten years has mass spectrometry become sensitive enough to detect the few bacterial peptides presented on infected cells. At that point, we realized that this technology was ready to be applied to infectious disease problems, which is why it has become a major axis of our research.

Q How did you come to be involved in developing mRNA vaccines?

FI It happened largely by serendipity. When the COVID-19 pandemic occurred and mRNA vaccines received widespread attention, we had just completed antigen discovery by immunopeptidomics in our model pathogen, *Listeria monocytogenes*.

At that time, there was a research group at the Faculty of Pharmacy at Ghent University that had been working on mRNA vaccines long before the pandemic. It felt natural for me to reach out and say, 'We have these antigens. Can we test them together as an mRNA-LNP formulation and see whether they provide protection in our mouse models?'

Over time, I became more interested in nucleic acid vaccines in general. For intracellular bacteria, which reside inside cells, this approach is particularly relevant because the vaccine allows the host cell to express the bacterial protein. Protective immunity to intracellular bacteria often relies on a strong cellular response. In the case of *Listeria monocytogenes*, our model pathogen, protection is provided exclusively by cytotoxic T cell responses—antibodies do not confer protection. This pattern is common for many intracellular bacteria, and traditional vaccine platforms often struggle to induce robust cellular responses. Therefore, combining immunopeptidomics for antigen discovery with mRNA vaccine formulation for intracellular bacteria creates a powerful approach.

I believe we are now capable of dramatically accelerating vaccine development for this type of pathogen. Based on the proof-of-concept data we generated with *Listeria monocytogenes*, together with our mRNA vaccine colleagues, we launched the EU-funded **BAXERNA consortium**. This consortium brings together 11 institutes across Europe to develop vaccines for *Mycobacterium tuberculosis*, *Mycobacterium ulcerans*, and *Acinetobacter baumannii*—all pathogens associated with AMR.

We believed it was strategic to focus on these pathogens. We have expert partners for each organism and cover the entire vaccine development pipeline. Our group focuses on

antigen discovery, while our partners handle mRNA design and vaccine formulation. We also have access to relevant preclinical models, including non-human primates, and the goal is for the tuberculosis vaccine to reach phase I clinical trials in 2–3 years from now.

This effort illustrates the enormous speed achievable by combining these novel technologies. We nearly have a preclinical candidate now, moving from antigen discovery to vaccine formulation and mouse challenge studies in just 2 years. Even as an academic group, I consider this very fast.

Q How does the greater biological complexity of bacterial pathogens impact mRNA construct design?

FI mRNA construct design remains under-investigated. The current clinical examples are the COVID-19 vaccines, which encode the spike protein of SARS-CoV-2. This protein is naturally meant to be expressed by human cells and displayed on the cell surface as a membrane-anchored protein.

The situation is inherently different for bacterial pathogens, where we are asking eukaryotic human cells to produce a bacterial protein. This is a research area my group intends to investigate in the future, in collaboration with other partners in our network.

Key questions include—should we include signal sequences to target them to the secretory pathway? Should we remove modifications that occur in eukaryotic cells but not in bacteria? Should we add targeting sequences to direct them to specific organelles, such as the lysosome, or to the cell surface? There is still much knowledge to be gained in this area.

Q What key milestones or developments in proteomics tools and technology have enabled this kind of research?

FI One of the most critical historical challenges in immunopeptidomics has been the large amount of input material required because the workflow involves pulling down and purifying the MHC complex, then eluting the peptides. Therefore, scaling down and increasing the throughput of sample preparation protocols is a key step. Over the past few years, we have miniaturized our procedures into a 96-well format, which, together with more sensitive mass spectrometers, enables the application of the technology to a far wider range of biological questions, including antigen discovery.

We now have collaborations with neuroscientists studying neurodegeneration and with colleagues in cancer research. Miniaturization allows us to work with far fewer cells, reducing the requirement from hundreds of millions of cells to just a few million. For infection research, this makes experiments much safer and more feasible.

Applying this technology to biosafety level 3 (BSL-3) or BSL-4 pathogens would otherwise not be possible due to the large amounts of material required. By scaling down, we make such studies possible. For example, we are involved in the EU-funded **COMBINE project**, where we perform antigen discovery for Marburg virus, a filovirus related to Ebola and classified as a BSL-4 pathogen. In collaboration with colleagues at Marburg University, we are working to make the immunopeptidomics workflow compatible with BSL-4 inactivation procedures.

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"In the future, we aim to integrate TCR sequencing alongside immunopeptidomics in our antigen discovery pipeline."

Q How do you apply artificial intelligence (AI) and machine learning (ML) to your work?

FI There are several opportunities to apply AI in computational data analysis. In general, there is a trend in proteomics to use AI-based rescoring of peptide and protein identifications. This means that ML algorithms are used alongside regular search algorithms to analyze the recorded peptide spectral data. Using AI software, we can predict their fragmentation spectrum, ion mobility, and retention time. This information improves the distinction between true positive and negative hits.

While this approach is widely used in proteomics, we have now incorporated it into our immunopeptidomics pipelines. We collaborate on this with computational proteomics scientists in our center, particularly the group led by Dr Lennart Martens.

Another important area is the development of AI-based search engines in proteomics. There is a growing trend in *de novo* search engines, which read peptide sequences directly from fragmentation spectra rather than comparing them against a target database. Historically, these algorithms have not performed well, but the latest AI-driven methods show promising results. We are excited to begin testing these algorithms on our immunopeptidomics data.

In our latest research, we are using T cell receptor (TCR) sequencing to examine the immune response from the other side of the coin. With single-cell RNA sequencing, it is now possible to identify individual TCR sequences and look at clonal expansion. This allows us to gain a more complete immune picture, combining information on the peptides presented—detected by mass spectrometry—with data on the T cells that recognize them. Here again, many AI-based tools are being developed to match immune peptides to TCR sequences.

In the future, we aim to integrate TCR sequencing alongside immunopeptidomics in our antigen discovery pipeline. This will allow us to assess which antigens are truly immunodominant in terms of T cell clonal expansion, potentially further improving antigen prioritization.

Q How do you ensure reproducibility and robustness in large-scale proteomics datasets?

FI For all the infection experiments we perform to discover antigens, we typically use and compare multiple biological replicates of infected and non-infected cells. We also screen different models, including various cell lines and, when feasible, *in vivo* infection models.

Reproducibility in the proteomics pipeline is partly ensured by the core facility we use to analyze our samples. They apply strict QC standards to ensure that the instruments are running to specification. This is a particularly relevant issue in immunopeptidomics because it is a niche application with a lack of standardization.

The Human Proteome Organization (HUPO) has an immunopeptidome project that is now working toward global benchmarking studies involving multiple laboratories. Such initiatives may eventually lead to QC guidelines and more standardized procedures to ensure consistent quality in immunopeptidomics.

Q What is next for your research?

FI aim to develop vaccines for all the pathogens within our scope, which are mainly intracellular bacteria with high levels of AMR. We prioritize targets based on the WHO's AMR priority list and have established collaborations with expert infection research groups for these pathogens. At the same time, we continue to improve our workflows and explore novel technologies, such as TCR sequencing.

I hope that some of the projects we initiate for these bacteria will one day reach the clinic, or that some of the antigens we identify could be adopted in clinical vaccines. We are actively engaging with industrial and nonprofit partners, such as the Gates Foundation and other stakeholders, to support the clinical translation of our vaccines.

In our most speculative discussions, we could even imagine almost custom hospital vaccines that protect against the specific AMR strains circulating in a particular clinic, for the duration of a patient's stay. Such protection for a couple of weeks could be sufficient to prevent infection with dangerous pathogens.

BIOGRAPHY

Francis Impens holds an MSc in Biomedical Sciences and a PhD in the field of mass spectrometry-based proteomics, both obtained at Ghent University, Ghent, Belgium. He then moved to the Pasteur Institute in Paris for a four-year postdoc where he applied his proteomics expertise to study bacterial infection by the foodborne model pathogen *Listeria monocytogenes*. In 2015 Francis moved back to Belgium to lead the Proteomics Core at VIB-UGent and in 2016 he started his own research group, supported by an Odysseus starting grant. His research aims to better understand the fundamental principles of bacteria-host interactions and to develop novel antimicrobial approaches. Francis is a VIB group leader since 2016 and a full professor at Ghent University since 2022. His current projects focus on the development of novel antibacterial nanobodies and mRNA vaccines and in 2023 he was awarded an ERC Consolidator Grant to investigate rare diseases triggered by infection. Since 2023 he co-coordinates the EU-funded BAXERNA project to develop bacterial mRNA vaccines.

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VACCINE INSIGHTS

IMMUNE RESPONSE UPDATE

Rethinking protection: systems immunology, correlates, and vaccine confidence

INTERVIEW

"Improving vaccination coverage starts with improving delivery, reducing logistical hurdles, ensuring supply, and making scheduling more convenient for families."

Ashling Cannon (Editor, BioInsights) speaks with **John Tregoning** (Professor of Vaccine Immunology, Imperial College London) about how high-dimensional analytical tools are transforming the study of vaccine-induced immunity. They discuss the evolution of correlates of protection, the integration of systems immunology and multiomic data, and strategies for capturing immune heterogeneity across populations. Tregoning also reflects on the importance of assay standardization, risk communication, and technology transfer in accelerating vaccine innovation and public confidence in immunization.

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Q Your research has spanned diverse vaccine platforms and respiratory pathogens. How has your understanding of what constitutes a protective immune response evolved with the advent of systems immunology and high-dimensional analytical tools?

JT The idea of correlates of protection has been central to vaccinology for decades. The classic example is influenza, where human challenge studies showed that an antibody titer above a defined threshold reduced the likelihood of infection. This led to the 1 in 40 hemagglutination inhibition (HAI) titer that continues to guide influenza vaccine development.

Correlates of protection allow for smaller, smarter clinical trials. In influenza, for instance, correlates make it possible to adjust vaccine formulations annually without repeating full-scale Phase 3 trials. The same principle applies to pneumococcal vaccines, where adding new serotypes, from seven to 13 and now to 20, was supported by antibody correlates of protection.

Correlates have also guided vaccine design in respiratory syncytial virus (RSV). The monoclonal antibody palivizumab demonstrated that neutralizing antibodies targeting the F protein could prevent severe RSV disease and contributed to antigen design. That success provided the blueprint for antigen-stabilized RSV vaccines.

At a conceptual level, our understanding of immune protection has become more integrated in recent years. The 'Swiss-cheese model', coined by Shane Crotty (La Jolla), illustrates this well: multiple overlapping immune layers, mucosal IgA, serum IgG, T cells, and cell-intrinsic mechanisms, collectively prevent infection. Vaccines likely strengthen many of these layers simultaneously, even though we often measure only a single component of a multifactorial response, i.e., antibodies.

Q Which emerging analytical or immunological tools do you see as most transformative for dissecting vaccine-induced immunity, and what practical barriers still limit their wider adoption?

JT Advances such as single-cell sequencing, spatial transcriptomics, and systems serology have given us unprecedented detail, but there is a danger of 'stamp collecting', subdividing the immune response into ever smaller and potentially transient cell populations. Immune cells are often plastic, and a snapshot at a single time point may not represent their true behavior.

The key is integration. Systems vaccinology, championed by researchers such as Bali Pulendran (Stanford University), links complex datasets to clinical outcomes. For example, if an HAI titer of 1 in 40 defines protection against influenza, can early transcriptional signatures, measured six or 24 hours post-vaccination, predict which individuals will achieve that protective threshold? Such approaches are beginning to reveal gene networks associated with durable immunity.

The challenge lies in combining these data streams into a coherent mechanistic framework. Protection rarely depends on a single gene unless a person has an inborn error of immunity. It is more about how networks of genes and pathways coordinate. Systems approaches will be transformative, but they require sophisticated computational integration and clear hypotheses to translate discovery into understanding.

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Q Immune responses vary widely between individuals and tissues. How should study design and data interpretation account for this biological heterogeneity rather than averaging it away?

JT Vaccines are, by necessity, population medicines. They must be safe, affordable, and effective for the greatest number of people. That means striking a balance between immunogenicity and safety, particularly because vaccines are administered to healthy individuals.

Within any vaccinated population, responses range from non-responders to individuals who experience strong reactions. When billions of doses are given, as during the COVID-19 vaccination campaigns, rare adverse events inevitably appear simply because of the scale of the program. The challenge is to identify and mitigate those risks early without undermining confidence.

Capturing heterogeneity requires both smart trial design and continuous post-marketing surveillance. Early studies should screen for extreme responses, while large-scale rollout demands ongoing monitoring and transparent risk communication. Many apparent cases of 'hesitancy' are in fact access issues: logistical barriers, clinic availability, or scheduling difficulties. Addressing those practical challenges can improve uptake more effectively than debating ideology.

Q As vaccine platforms diversify, how can we meaningfully compare immune readouts across studies or technologies without over-interpreting differences?

JT Standardization is essential. When assays are harmonized, cross-study comparison becomes feasible. The HAI assay for influenza is a good example, widely used, reproducible, and directly comparable across laboratories.

However, as assays become more complex, such as T cell ELISpot or flow cytometry-based analyses, variability increases. Each laboratory introduces subtle differences that complicate interpretation. To compare results meaningfully, either testing must occur in centralized laboratories or protocols must be stringently standardized across sites. The greater the assay complexity, the greater the need for consistency.

Q Public communication often focuses on infection prevention, yet many vaccines confer broader health benefits. How can the scientific community better communicate these additional advantages to strengthen public trust and uptake?

JT A key issue is that most people who are unvaccinated face access barriers rather than ideological resistance. Improving vaccination coverage starts with improving delivery, reducing logistical hurdles, ensuring supply, and making scheduling more convenient for families.

"Vaccinology is a magpie discipline; it borrows and repurposes tools that prove transformative."

Equally important is how we frame vaccine benefits. Beyond preventing infection, vaccines reduce the risk of serious complications such as myocardial infarction. Both influenza and RSV vaccines have been associated with reduced risk of cardiac events. Communicating those broader health impacts resonates more strongly with the public than focusing solely on avoiding mild respiratory illness.

Similarly, the shingles vaccine Shingrix has been linked to a delayed onset of dementia. When people understand that vaccination protects not only against acute infection but also against long-term conditions, the perceived value changes. Viral infections are rarely benign; highlighting their downstream risks helps contextualize why vaccination matters. Clear, evidence-based messaging, rather than endless social-media debate, is the most effective way to sustain trust.

Q What are the biggest gaps in our current toolkit for probing vaccine-elicited immunity, and where do you expect the next breakthroughs to emerge?

JT Vaccine progress is consistently driven by technology. Every major advance, from cell-culture systems for viral vaccines to sugar–protein conjugation for bacterial vaccines, has come from a new enabling technology. Even the success of mRNA vaccines was seeded by innovations in gene therapy and oncology rather than vaccinology itself.

I suspect the next leap will again come from outside the traditional vaccine field, perhaps a technology developed in another biomedical area that can be adapted to immunization. Vaccinology is a magpie discipline; it borrows and repurposes tools that prove transformative.

After two decades in this field, I can say it has never been more dynamic. The integration of new technologies with our growing understanding of immune networks ensures that the coming years will be as exciting as any period in modern vaccine research.

BIOGRAPHY

John Tregoning is currently Professor of Vaccine Immunology at Imperial College London, where he has studied the immune responses to vaccination and respiratory infection for more than 25 years. His group is currently focusing on the immune response to RNA vaccination and RSV infection (exploring how the virus evolves under antibody pressure). John has written more than 90 peer-reviewed scientific articles and over 50 articles on scientific careers for *Nature*, *Science* and *Times Higher Education*. He is also the author of two books ***Live Forever?*** and ***Infectious.***

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