nibrt

National Institute for
Bioprocessing Research
and Training

**NIBRT Research Conference E-Proceedings**

# Biopharma Focus on the Future 2023

## Exploring the Future of Biologic Medicines and Advanced Therapies

# Contents



The NIBRT Research Conference Biopharma Focus on the Future 2023 E-Proceedings is a Publication developed by the National Institute for Bioprocessing Research and Training

**Photos:** Chris Bellew/ Fennell Photography and Cristiane Delfina/NIBRT
**Editor:** Cristiane Delfina
**Associate Director of Research:** Jennifer Byrne
**Marketing:** Alison Quinn, Emer Norton

**NIBRT**
Foster Avenue, Mount Merrion, Blackrock, Co. Dublin, A94 X099, Ireland.
Tel: +353 1 215 8100
Email: info@nibrt.ie


# A letter from the CEO

Biopharma and life science has been a jewel the crown of Ireland's FDI and economic successes for numerous decades. **8 of the world's top 10** biopharma companies now have operations in Ireland, and as a sector, biopharma contributes **over €75 billion** in exports annually, with **around 45,000 people** are now directly employed in biopharma facilities nationwide.

It was testament to the immense importance of the sector to Ireland that so many people turned out in person and online, on April 20th, to attend NIBRT's inaugural **'Biopharma Focus on the Future' Conference.**

On the day we welcomed:

- Our partners and clients from across the biopharmaceutical and biotech industry, including senior leaders based in Ireland and from corporate HQs, and representatives from BiopharmaChem Ireland and the Irish Pharmaceutical Healthcare Association.
- NIBRT's close partners from Ireland's leading **higher education institutions** and **research centres,** notably University College Dublin, Trinity College Dublin, University College Cork, University of Galway, Atlantic Technological University and Science Foundation Ireland Research Centre for Pharmaceuticals.
- Our partners from the **NIBRT Global Training Partner Programme** – from K-NIBRT in Korea, from the Canadian Alliance for Skills and Training in Life Sciences in Canada, from Sydney Australia and from various locations in the United States of America (Maryland, Indiana and Texas).

- Members of the **NIBRT Board** and **Scientific Advisory Board.**
- And our funders **Industrial Development Agency - IDA Ireland,** from **Science Foundation Ireland - SFI** and **Enterprise Ireland.**

We were privileged to have the event opened by Simon Coveney TD, Minster for Enterprise, Trade and Employment, and the Minister spoke eloquently on the importance of the industry and the Government's commitment to ongoing investment and support of the sector.

The event marked a big milestone in NIBRT's **IDA-funded Advanced Therapies Expansion Project.** The €21M facility expansion will enable NIBRT take a giant step into the next generation of biopharma manufacturing, the incredibly exciting area of cell and gene therapies and other novel therapies. The facility will also include 'CONCEPT', and new facility with SFI-funded equipment that will allow researchers from academia and the biopharmaceutical industry to access and rapidly generate optimised cell lines for production of recombinant proteins, monoclonal antibodies, cell therapies, gene therapies and RNA therapeutics. On the day, we were able to invite attendees at the conference to be the first non-NIBRT people to tour new Advanced Therapies facility in advance of its opening.

Lastly, we were thrilled to be able to assemble an excellent array of **expert speakers, eminent researchers, academics, clinicians and policy-makers and influencers,** from Ireland and around the world to speak on the day. This 'proceedings report' summarises the important points these speakers made about the future of biopharmaceutical development and manufacturing.

Enjoy the report and the insights!

Darrin Morrissey


## Biopharma Manufacturing Process from Conception to the Shelves

NIBRT is a world leading research institute that undertakes research to grow the fundamental understanding of complex biopharmaceuticals, while delivering impactful solutions that advance the processes for the manufacture of biologic medicines. Inspired by the manufacturing challenges facing the industry, NIBRT Research makes transformative discoveries across multidisciplinary areas such as analytical science, cell and genetic engineering, informatics, and bioprocess engineering. These discoveries are utilized to advance the state-of-the-art in their fields and revolutionize the manufacturing of recombinant proteins, monoclonal antibodies, vaccines and cell and gene therapies.

**www.nibrt.ie**

nibrt
National Institute for Bioprocessing Research and Training

# NIBRT Research Areas

## Cell Engineering

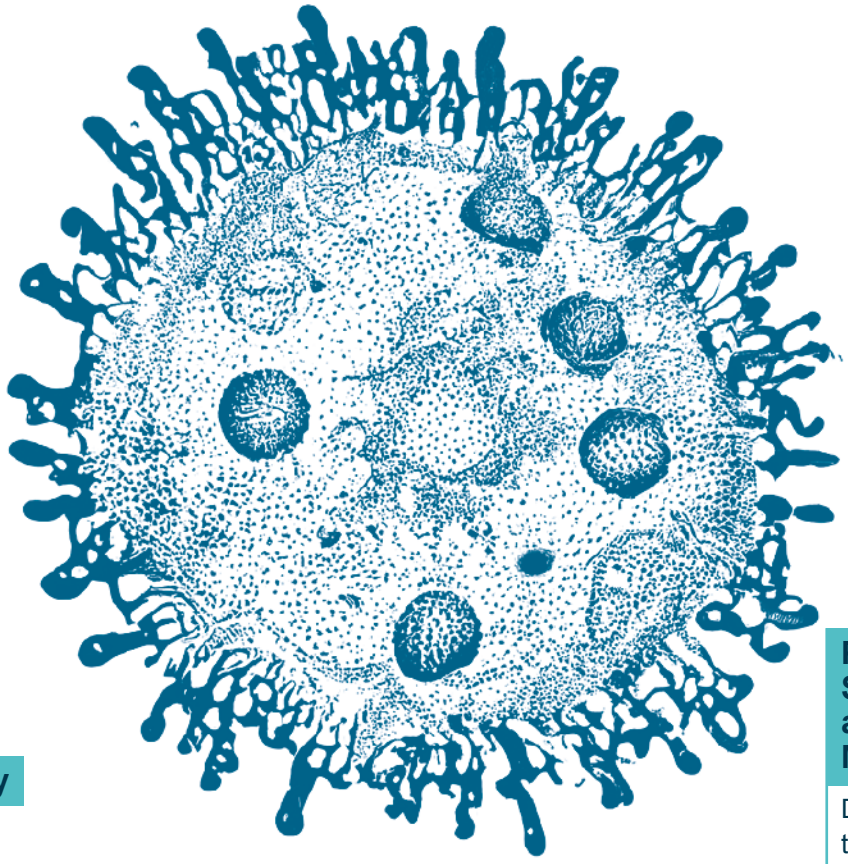
Understanding and improving the cell systems used to create advanced therapies with an emphasis on the cellular and molecular mechanisms involved.

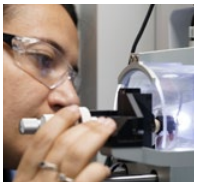
## GlycoScience

Novel, high throughput, robust technologies for the characterisation of protein glycosylation, implicated in all biological processes, and pathologies as well as in biopharmaceutical drugs' efficacy and safety.

## Bioprocess Systems Engineering

Development and manufacture of cell therapies, with a strong emphasis on metabolomics, and their translation towards the clinical research setting.





## Characterization and Comparability

Development and application of liquid phase separations and mass spectrometry for the analysis of complex biological systems.

## Cell Technology

The development and application of analytical solutions for problems associated with the manufacture and characterization of biopharmaceutical, including recombinant proteins, monoclonal antibodies, and advanced therapies.

## Process Engineering, Separations and Advanced Manufacturing

Development of novel technologies in advanced manufacturing, separation and formulation of pharmaceuticals, biopharmaceuticals and ATMPs, with the potential for commercial or societal impact.

## Systems Biology and Data Analytics

Use of next generation sequencing and computational methods to improve biopharmaceutical manufacturing processes.

## Formulation and Stability

The formulation and stability of biopharmaceutical products, especially those containing mRNA and proteins in the amorphous solid state. A fascinating but yet mysterious state of matter in which many protein drugs are stored and/or marketed.

# NIBRT Research Strategy 2024-28

nibrt
National Institute for Bioprocessing Research and Training

## Research Excellence, Reputation and Impact Through Partnership

### Priority

NIBRT will build a strong **international reputation** as an **open research institute** that, in **partnership** with **companies** and **other leading research institutes**, conducts **highest quality research**, delivering **innovation** in biopharma manufacturing science and technology.

### Outcome – end of 2028

- NIBRT conducts the highest quality research and consistently delivers significant impact, with strategic metrics including diversity of funding, scale of funding, number of national and international research collaborations, number of top papers, client impact, technology transfer and wider communication / public engagement.
- NIBRT attracts the best researchers in the field to conduct their research at the Institute, with the NIBRT PI group growing to at least 14 in total number (at least 9 in FTEs) by 2028.
- NIBRT increases its research activity leading to 7-9% annual average growth in research funding.

## Objectives:

1. To conduct research of the highest quality, that is widely recognised as delivering impactful innovation by improving the efficiency, consistency, and safety of manufacturing processes and ultimately improving the product.

2. To develop a diversified portfolio of research in terms of focus and scale:
   - **Focus:** Research that, while building on NIBRT's strengths in advanced analytics research, spans a broad range of biopharma manufacturing processes, from upstream to fill-finish, and biopharmaceutical modalities, from traditional biologics to cell and gene therapies and novel vaccines.
   - **Scale:** Research projects that are funded by research funders, companies, or other sources at varying scales; small, medium, and large. With purposeful targeting of international-scale research initiative(s) in areas of strategic focus for the biopharma industry.

3. To be an open research institute that builds and sustains meaningful research partnerships with and offers open access to relevant research-performing institutes in Ireland, north and south.

4. To increase the frequency and scale of international research partnerships by becoming a consortia member of choice, based on a reputation for excellence and leading infrastructure.

5. To exploit NIBRT's unique position operating at the interface between industry and academia and to create a unique environment in which to conduct research; thereby enabling NIBRT to attract, develop and retain the best for biopharma researchers – from senior PIs to emerging talent.

6. To improve the capacity of NIBRT's research executive support structures – across research office, leadership, communication, hiring, retention and career development – to best support implementation of our research objectives.

7. To opportunistically exploit commercial opportunities arising from IP developed at NIBRT.

# CONCEPT

## National Synthetic Biology and Cell Line Development Facility

**CONCEPT** is a brand-new, state-of-the-art facility constructed at NIBRT and supported through Science Foundation Ireland, with access to emerging technology platforms. We aim to provide a National Core Facility for researchers and the biopharmaceutical industry to access and drive rapid generation of optimised cell lines for production of recombinant proteins, mABs, cell therapies, gene therapies and RNA therapeutics.

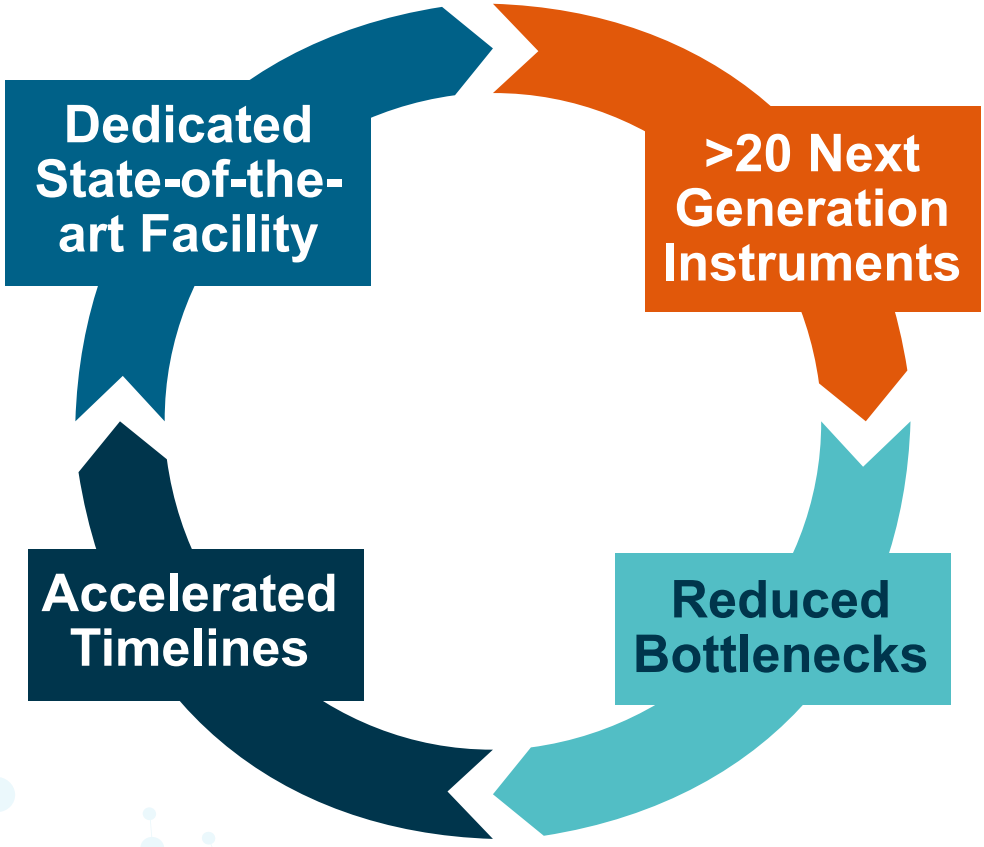
**CONCEPT** will address a gap in the Irish and European research infrastructure ecosystem, by providing a 'one stop shop' for researchers and industry to rapidly generate optimised cell lines and biological material for advanced therapeutics research.

The lead team provides insights, expertise, support and data analytics at every step for Biologics and Advanced Therapy Medicinal Product research and development.

A Not-for-Profit charge model, in line with SFI funding, offers End-to-End workflows or access to individual instruments driven by the customer's unique needs and research goals.

## THE "CONCEPT"

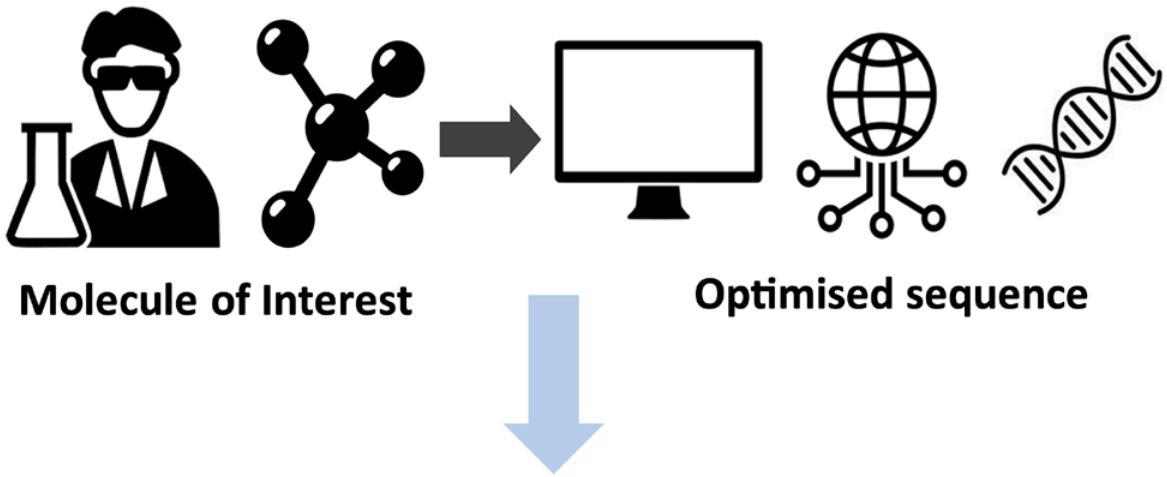
- A researcher presents their CONCEPT (gene identifier, candidate sequence) to our team
- We evaluate the user needs and end-product requirements with our CONCEPT lead team
- We initiate sequence generation and process the idea through our quality-controlled workflows
- To generate a physical Advanced Therapy product (recombinant protein, oligo/ mRNA-LNP, Gene Therapy, Cell Therapy)



## CONCEPT Phase 1: Sequence Generation

A researcher has a molecule of interest (MOI) and needs to generate that MOI or a cell line to express the MOI, to advance their research.

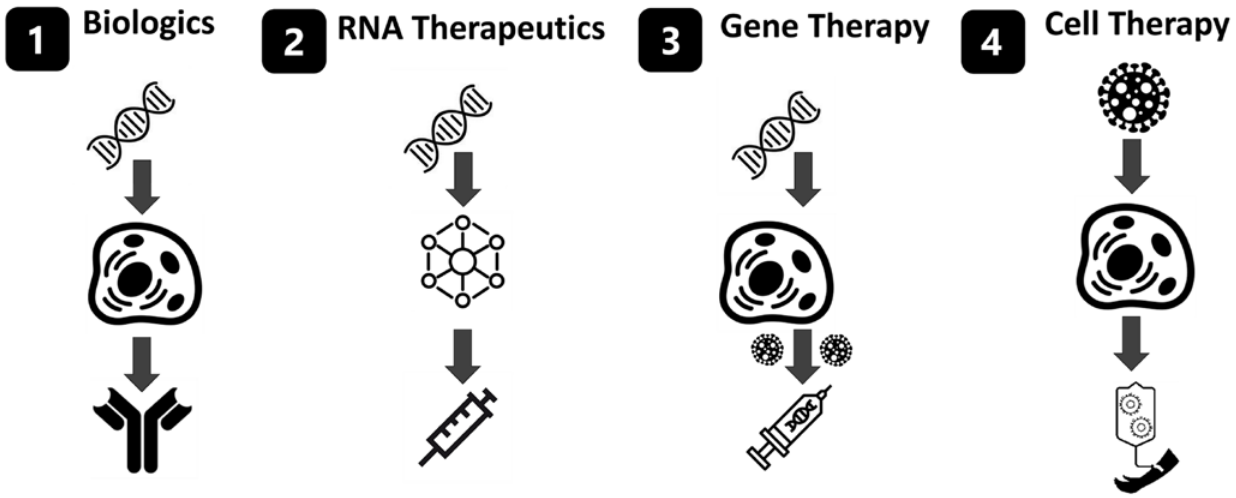
They upload their sequence using online tools, then templates and reagents required for gene creation are shipped to CONCEPT.




## CONCEPT Phase 2: Product Generation

Sequences are generated and processed through one of four workflows, directed by the type of Biologic or Advanced Therapeutic end-product required.

The researcher receives the required physical product; Biologic (mAB or recombinant protein), RNA Therapeutic, Gene Therapy, Cell Therapy or an optimised cell line producing their target molecule.



## Facility

Located in the Cell and Gene Therapy extension at NIBRT, funded by IDA Ireland (Industrial Development Agency).

## Infrastructure

>20 next-generation instruments provide accelerated timelines, quality assurance, increased accuracy and integrity of products generated.

## Flexible Accessible Model

Not-for-Profit charge model.

End-to-End workflows or individual instruments driven by user requirements.

## Website coming soon!

Opening 2023

Contact: jonathan.bones@nibrt.ie, clair.gallagher@nibrt.ie
jennifer.byrne@nibrt.ie or darrin.morrissey@nibrt.ie

IDA Ireland

Science Foundation Ireland SFI For what's next

# Biopharma Focus on the Future 2023

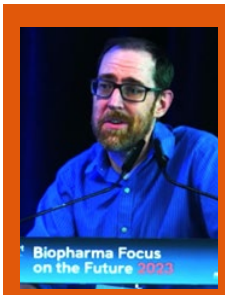
## The word is Collaboration

On the 20th of April, NIBRT hosted the first Biopharma Focus on the Future Research Conference, a landmark event bringing together leading experts in the field of biopharmaceutical manufacturing to explore the latest advances in biotechnology and manufacturing science. The conference was a significant opportunity to showcase Ireland's thriving biopharma research ecosystem and further develop the country's reputation as a hub of innovation and expertise in biopharma manufacturing.

The conference was attended by 100 delegates, as well as live-streamed by over 400 delegates worldwide over the day. Minister Simon Coveney, Minster for Enterprise, Trade and Employment, opened the event and spoke about the importance of the biopharma industry to Ireland's economy and NIBRT's role in supporting its growth.

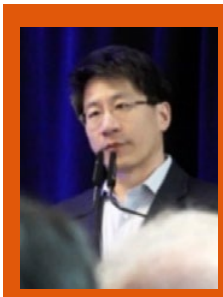
Throughout the day, attendees engaged with internationally recognised academic experts and distinguished speakers present, including

- Professor Kelvin Lee, Director of the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL);
- Professor Bruce Levine, Founding Director of the Clinical Cell and Vaccine Production Facility at the University of Pennsylvania;
- Grainne Power, Ireland's Health Products Regulatory Authority;
- Prof Johan Rockberg, from the KTH Royal Institute of Technology, Stockholm, Sweden;




*"There's a number of challenges outstanding. I think that the biggest one is being able to simplify these complex pharmaceuticals so that they are manufactured in such a way that we can really drive down prices while maintaining quality. But I think that there's an opportunity with a lot of these new computational tools with artificial intelligence to be able to identify where the critical points are, to simplify everything and drive down costs while maintaining quality."*

**Dr Nathan Lewis** - University of California, San Diego, USA



*"When I think about the opportunities for the future, I think about being able to build from the lessons learned from the past. So as the new modalities come to market, and there's a lot of interest in converging manufacturing platforms and creating more robust and resilient platforms, we can take some of the lessons learned in the convergence of maps and try to apply those lessons for the future. And I think that will really help the field move forward and serve patients better.*

*When I think about the challenges, it's really around a workforce, where there is not enough of a manufacturing workforce in place globally let alone, in each individual region to meet the demands and meet the needs for all the facilities that need to be built and that need to be staffed. And that's a challenge that we're going to have to overcome."*

**Prof Kelvin Lee** - National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), Newark, USA

- Prof Dong-Yup Lee, from Sungkyunkwan University, Suwon, Republic of Korea;
- Prof Bjørn Gunnar Voldborg, from the Technical University of Denmark (DTU), Lyngby, Denmark;
- Dr Nathan Lewis, from the University of California, San Diego, USA;
- Dr Piotr Kowalski, who lectures at University College Cork;
- Prof Jane Farrar, from Trinity College Dublin;
- Dr Sandro Matosevic, from Purdue University, West Lafayette, USA.

The event was also live-streamed, with over 400 delegates worldwide registered to view it online over the day.

Topics covered at the conference included advanced analytical characterisation of biologics and advanced therapies, systems biology for biopharmaceutical manufacturing, application of data science for biopharma manufacturing, cell and gene therapy manufacturing science and technology, clinical research, and regulatory science for new modalities.

*"I think we expect to get more and more data. It will be different from what we are familiar with from previously. Data was not consistent, but we expect to get more and more data, which is generated online, in real time. So, a huge amount of big data will be available. The challenge is how to manage this, how to detail them. So, there is an opportunity as well as a challenge."*

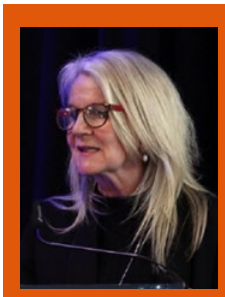
**Prof Dong-Yup Lee** - Sungkyunkwan University, Suwon, Republic of Korea

During the conference, NIBRT introduced attendees to its new Advanced Therapeutics extension, scheduled for completion at the end of July, 2023. This €21 million project, funded by IDA Ireland and the Irish Government, will significantly increase NIBRT's capacity to conduct manufacturing-focused research and training in advanced therapy areas, including cell and gene therapies, mRNA and oligonucleotides and novel vaccines. The 1,800m$^2$ building extension will feature five dedicated laboratories for cutting-edge therapy research and two new training suites. The launch of this state-of-the-art facility further cements Ireland's position as a leader in biopharmaceutical manufacturing research, training, and innovation.

*We are honoured to have hosted the Biopharma Focus on the Future Conference and to have had such a distinguished group of speakers and attendees, including Minister Coveney, said Darrin Morrissey, CEO of NIBRT. This conference is vital to placing Ireland at the centre of innovation in the field. We at NIBRT are very proud to be playing an important role in helping to drive the development of the biopharmaceutical industry in Ireland.*

Minister Coveney spoke at the conference:
*It has been a pleasure to be here today at the Biopharma Focus on the Future 2023 Research Conference and to open the new IDA-funded €21M expansion to the NIBRT facility.*

*With its focus on the future and innovative collaboration, NIBRT is a wonderful example of what Ireland offers to the world. NIBRT provides training and research to thousands, and with valued global partners in the US, Canada, South Korea, China and Australia, this centre reaches far beyond the Irish ecosystem. NIBRT epitomises what makes Ireland a key location on the global pharmaceutical map, and the Irish Government is committed to growing the industry here. This expansion will further embed Ireland in the global biopharma sector."*



*" We are in the era of genomic medicine. We have many tools that allow us to make almost any disease-targeting drug, which is amazing. So we can sequence the whole human genome. We can gene-edit disease targets. Actually, we have the ability now to put the components together to test, novel therapies in precision medicine. But the challenge, really, is that the process of generating therapy for targeting diseases, rare diseases, or even common diseases, it's a really expensive process, and the pricing of novel therapies is very, very high, and it will be tolerated for a few therapies, which is what we have on the market now. But in a few years, there will be a lot more, and that pricing will not be tolerated. So, we've got to find ways of streamlining the process so that we can get these innovative, powerful protein therapies to patients."*

**Prof Jane Farrar** - Trinity College Dublin, Dublin, Ireland

Michael Lohan, CEO of IDA Ireland, said, *The expansion of NIBRT to respond to innovation in the biopharma sector greatly adds to Ireland's reputation as a global location of excellence for next-generation biopharmaceutical products. IDA Ireland continues to partner with and support NIBRT to ensure that Ireland is well-positioned to support companies in advanced therapeutic areas. The completion of NIBRT's Advanced Therapeutics extension in May 2023 will be a significant development for the biopharma industry in Ireland.*


# Summaries of the lectures

**Session 1** **Chair:** Niall Barron

## Opening Remarks: Minister Simon Coveney - Minister for Enterprise, Trade and Employment

### Simon Coveney, Minister for Enterprise, Trade and Employment

*As we look to the future of biopharma, we can glimpse a world in which our children are vaccinated against the worst of seasonal respiratory illnesses.*

*A world in which, at the same time as our cancers are treated, we receive a simple vaccine that future-proofs us against further mutations.*

*And the world in which those of us who experience rare diseases receive bespoke treatments tailored for and created for our bodies and our unique genetic makeup for the first time.*

The Minister for Enterprise, Trade and Employment, Simon Coveney, delivered the conference's opening remarks welcoming all the guests to NIBRT. Onsite attendees included members of the academic community, the Irish government, and funding agencies, as well as representatives of the biopharma Industry from America, Europe, and Asia.

The Minister highlighted NIBRT's historical ground-breaking nature and how it has been reaching its conceptual goals and challenging its partners and potential partners to think further and "out of the box".

*With its focus on the future and innovative collaboration, NIBRT is a fantastic example of what Ireland offers to the world. It's open, it's modern, it's responsive, and most importantly, it's focused on building partnerships for the future.*

NIBRT's focus on the challenges and opportunities surrounding Cell and Gene Therapy research and manufacturing is growing rapidly, with the institute providing state-of-the-art infrastructure and research, and training for over 31,000 learning days to nearly four and a half thousand Irish and international trainees and students last year alone.

Ireland has become a centre of excellence, and Minister Coveney asserted that the Irish Government is deeply committed to growing the biopharma industry here, emphasizing how the pandemic brought home to many of us our vulnerability and, on the other hand, proved how well-directed funding and a clear focus of mind could drive technological innovation at a speed that perhaps surprised many people.

*We're living and working on the very cusp of a new frontier of medicine, and on a day like today, in a place like NIBRT, this transformation in our greater understanding of how we get sick and how we recover feels a bit more tangible than it ever has before.*

Opening Keynote: **Prof. Kelvin Lee**

*National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), Newark, USA*

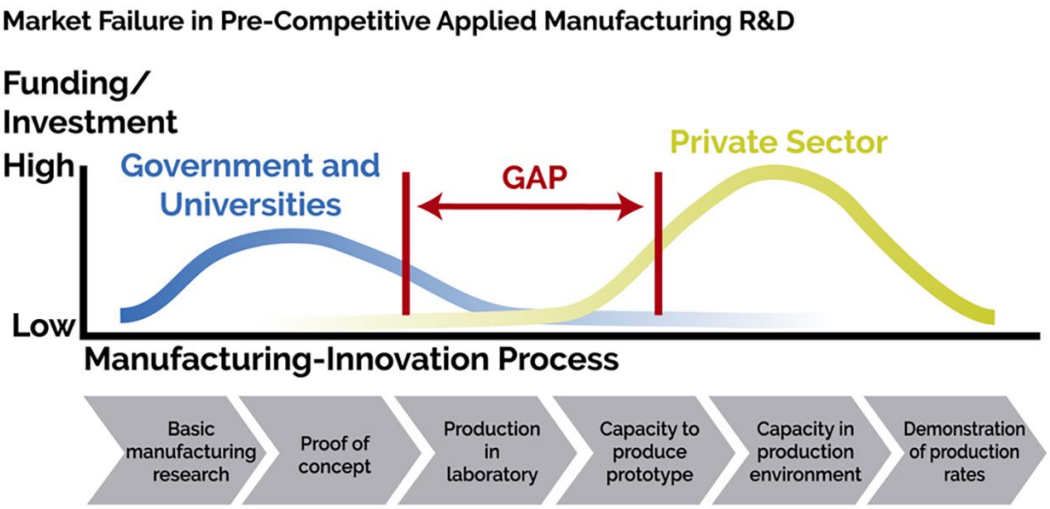
## Biomanufacturing Innovation – the Importance and Impact of Going First Together

Kelvin Lee is the Director of the National Institute for Innovation in Manufacturing Biopharmaceuticals, NIIMBL, a unique public-private partnership with a specific goal: to expedite biopharmaceutical innovation.

By supporting the development of standards that enable more efficient and rapid manufacturing capabilities and being committed to educating and training a world-class biopharmaceutical manufacturing workforce within the United States, NIIMBL aims to make the process of manufacturing biopharmaceuticals more streamlined and effective. The institute is funded by a cooperative agreement with the National Institute of Standards and Technology (NIST) within the U.S. Department of Commerce. In addition, it receives significant additional support from its members, underscoring the importance of collaboration and cooperation.

Kelvins' talk started by raising a historical overview of U.S. Government policies and efforts in keeping the North American advanced manufacturing industry not only strong but leading in crucial fields such as energy, defence, and trade, which was world-leading until about the year 2000, when new dynamics resulted in declines in American manufacturing jobs and advanced technology trade balance.

Investigating what could have caused the drops under the American practices and strategies to grow the manufacturing chain, a gap was detected between the investments in national basic research and the substantial efforts in promoting the market of matured technologies.




*Prof. Kelvin Lee, NIIMBL*

*(…) The philosophy is that as technologies get very mature, they're about to lead to new products, processes and services that the private sector does not hesitate once something is sufficiently derisked to also make investments to really bring those products to market, and where there has been a gap in the United States relative to other countries is in that missing middle, that sort of innovation gap, sometimes called the valley of death.*

*(…) How do you take technologies where a proof of concept has been established but it's not yet ready for full deployment at a commercial scale?*

*So how can we accelerate things and technologies moving through that gap?*

*The identified solution was to create a series of very large-scale public-private partnerships consortia at a scale that hadn't been done before in our country. To bring together universities; to bring together community colleges which do a lot of vocational and worker training, and our national laboratories and marry and partner them with very large global companies, small and medium-sized companies, as well as startup companies, and marry them with government, whether it's the federal government at the national level, state and local governments, as well as an economic development organisations.*

NIIMBL fosters collaboration between diverse industrial and trading actors, exchanging knowledge, expertise, and technology to advance manufacturing in a symbiotic competition to become the number one in their sectors.

The biopharmaceutical industry has its particularities, though. Under market access challenges, fragmented and increasingly complex regulation, high costs and the longest timeframes between research and commercialisation (Deloitte, 2014 - https://www2.deloitte.com/bd/en/pages/life-sciences-and-healthcare/articles/measuring-risk-in-biopharmaceutical-research.html), through a study[1] performed at the suggestion of the U.S. Food and Drug Administration, Lee shared that biopharmaceutical manufacturers tend to prefer to be "fast seconds", to deploy new manufacturing technologies.

They want other companies to go through the regulatory process first before their own company benefits from a shared understanding of the technology.

By understanding the challenges and demands of the sector, NIIMBL set up strategies to increase the gains of this manufacturing sector. Large companies are driven to work together in shaping individual projects, and smaller companies and academic partners are included in the loop. Within a decade, the so-called NIIMBL-Led Process Intensification Program aims to improve facility cost, flexibility, sustainability, and control for each and all, establishing the complex biopharmaceutical sector envisioned ecosystem system of innovation, collaboration, and competition, baptised as Going First Together.[2]

1 Mantle, J. L., & Lee, K. H. (2020). NIIMBL-facilitated Active Listening Meeting between industry and FDA identifies common challenges for adoption of new biopharmaceutical manufacturing technologies. *PDA Journal of Pharmaceutical Science and Technology, 74*(5), 497-508. https://doi.org/10.5731/pdajpst.2019.011049

2 Erickson, J, Baker, J, Barrett, S, et al. (2021). End-to-end collaboration to transform biopharmaceutical development and manufacturing. *Biotechnology Bioengineering*. 118, 3302– 3312. https://doi.org/10.1002/bit.27688


**Prof. Elizabeth Topp**

*National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland*

## Research at NIBRT

The overview of research excellence at the National Institute for Bioprocessing Research and Training, presented by the Chief Scientific Officer, Prof Elizabeth Topp, began by acknowledging the continuous support and engagement from the Irish Industrial Development Agency (IDA) and the Science Foundation Ireland (SFI) agencies.

NIBRT plays a critical role in the provision of research and training solutions in biopharma manufacturing across multidisciplinary areas such as analytical science, cell and genetic engineering, informatics, and bioprocess engineering.

From 2006 to today, it has achieved international relevance in its research fields by gathering next-generation infrastructure and first-class expertise, constantly pushing the current knowledge and practices on recombinant proteins and how to improve their expression, processing, formulation, predictions, and packaging. NIBRT is constantly evolving as the biopharma industry demands innovative processes and solutions, including developing manufacturing-focused research related to the emergence of advanced therapeutics.

2023 will see the launch of an SFI funded disruptive synthetic biology and cell line development facility entitled CONCEPT, which will provide access to unique and leading-edge infrastructure to the community.

Prof. Topp outlined some of the specific areas of biopharmaceutical manufacturing research expertise at NIBRT, including expertise in advanced manufacturing technologies, understanding and optimising the culture conditions; cellular engineering; mass spectrometry based high-resolution analysis; systems biology, data analysis and management; metabolism-driven cellular therapeutics; glycobiology and characterisation of glycosylation; mRNA vaccine stabilisation and engineering.

Partnering with powerful manufacturing pharma industries and organisations, such as Thermo Fisher, NIBRT is physically and influentially expanding.

*What would an institute that involves these things look like? We might draw a little dashed line around the outside, and the dashed line indicates to us that we want to be an open Research Institute. The doors here are always open, and we want to collaborate with those in Ireland and globally.*


**Grainne Power**

*Health Products Regulatory Authority (HPRA), Dublin, Ireland*

## Focus on the Future: A Regulatory Perspective

Grainne Power discussed the role of regulators in the pharmaceutical industry, acknowledging the industry's constant growth in sophistication.

Her talk updated and upgraded the traditional role of a regulator to protect public health by minimising harm associated with the use of medicines or advanced therapies, which can be primarily achieved by focusing on compliance with the law while also ensuring simultaneous focus on systemic risk in any market, and working to manage areas of risk through the development of guidance and regulations. However, modern regulators tend to focus more on the horizon, observing systemic risk and ensuring that regulations are joined up and clear. With the advent of health products and medicines of increasing sophistication comes an increased need for collaboration and understanding the applications of medicinal products as part of a broader health system infrastructure. There is a need to balance minimising risk and maximising gains and innovation, and Power asserted that the regulatory system in Ireland and the European system is working to find the sweet spot of that balance.

The value of external engagement, listening, and learning for regulators to codify regulations that add public value appropriately was highlighted. There is a need for expertise in the regulatory system to effectively interrogate gene therapy technologies and the importance of adapting to the dynamic shift towards precision medicine.

*The regulatory system needs to be flexible and adaptable to innovation, as evidenced by the lessons learned from COVID-19.*

Part of the four pillars of the European Pharmaceutical strategy includes supporting a competitive and innovative European pharmaceutical industry. It must be efficient and effective, with better processes, gatekeeping, analytics, and tools. In that sense, Power addressed initiatives such as early access to scientific advice and regulatory support for innovators and scientific advice to entities before submitting a product for approval.

In these paths of technical improvement and scientific literacy for regulators, as well as responsibility and public commitment on the manufacturing side, a mutual reliance can be established, to maximize those gains and minimize the risk in the protection pursuit of the protection of public health, in the delivery of new convergent technologies.

**Session 2** **Chair:** Jonathan Bones

## Prof. Johan Rockberg

*KTH Royal Institute of Technology, Stockholm, Sweden*

# Systems and Synthetic Biology for improved titre & quality of Non-classical Biologics

Johan Rockberg discussed the use of systems and synthetic biology to improve the quality of non-classical biologics, which has been the primary goal of his group at the Royal Institute of Technology in Sweden.

The group focuses on cellular engineering, working with gene therapies and protein engineering, collaborating with the industry and other centres, such as AdiBioPro and the Genova Centre. The team also have international partners in South Korea, working with CAR-T therapies, monoclonal antibodies, and studies in cancer immunotherapies.

*Cell therapies and gene therapies are coming out into the market. We are now actually curing diseases, which is what we'd like to do. A key problem, however, is that this is very expensive. We can't even afford it in countries like Sweden.*

By addressing the main challenge in the development of biological therapies, namely the costs, Rockberg's group focuses on the application of scalable technologies and different approaches to the production of biologics, which could rely upon the knowledge already acquired to investigate other ways to optimise the steps of the processes, by synthesising or cloning essential and adaptable parts, which could then be used in more patients and treatments.

He provided an example of a scalable version of cell therapies using peptides and antibodies and discussed their challenges, such as product aggregation, and how the group addressed them through gene engineering and autophagy restoration. The research group also investigates the production of intracellular and secreted proteins and the trends observed in high and low-producing clones.

*You may have problems with money, content, purity or stability. But what is clear is that when we try to produce advanced biologics, such as biospecific antibodies, human proteins or even viruses, the cells do not like it. They have mechanisms to avoid producing these things at large scale. They might be toxic. In particular, viruses are. It's not really what cells are designed to produce; on the contrary.*

In conclusion, Rockberg acknowledged the value of the partnerships and highlighted some results already coming from their work.


**Dr. Colin Clarke**

*National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland*

## Annotation of the non-canonical CHO cell translatome

Colin Clarke's talk described his group's analysis of CHO cell non-canonical translation and discovery of cell microproteins.

Clarke raised the challenges of identifying translated regions of the CHO cell genome and the need to move beyond algorithmic predictions.

*Over the last twelve years, we have started to understand that non-canonical translation is much more widespread across mammalian genomes than we realised.*

Using a technique called ribosome footprint profiling, Colin's group identified thousands of new translated regions of the CHO cell genome, including sORFs that were predicted to encode microproteins.

Colin's work impacts on an area of significant interest in the biopharma industry: host cell protein detection. ELISA assay is the most common technique utilised to identify host cell proteins. Mass spectrometry has been recommended as a complementary technique to ELISA for HCP assessment to ELISA capable of sensitive detection of individual HCPs.

The discovery of microproteins extends the mass spectrometry databases and enables improved detection of potential HCPs.

So, what do we know at the moment?

*Non-canonical is widespread across the CHO cell transcriptome.*

*We have extended the annotation, and that increases the coverage of mass-spec-based HCP analysis.*

*Microproteins are a new class of host cell protein, and these proteins have been identified in monoclonal antibody drug products. We're not saying that anybody should be concerned about these, but it's a demonstration that by looking for new areas of translation within the genome, you can extend the database to be able to accurately detect more of the host cell proteins that are in your product. And that could be useful in process development.*

**Dr. Dong-Yup Lee**

*Sungkyunkwan University, Suwon, Republic of Korea*

# The future of bioprocessing: data-driven predictions and model-guided framework for mammalian cell cultures

Prof Dong-Yup Lee spoke passionately about modelling and data driven prediction and how these can be applied to improve processes developments in biomanufacturing, to create information and knowledge out of the heterogeneous, multi-dimensional and unstructured data generated through multi-omics analytical processes.

Data integration is a big challenge. By mechanistically building mathematical models, the researcher and international partners developed and published the first genome scale metabolic model (GEM) of CHO cells[2]. Since then, they have developed omics profiling models, and the framework can be integrated together.

His next step was focusing on processes, as they are more practical and relevant to the industry. *Working with minimum data, how can we get some new insights? Engineering can improve it.*

Professor Dong-Yup Lee then developed what he called digital twins[3], virtual representations that serve as the real-time digital counterpart of physical objects or processes.

*What is the difference between digital twin and just a simulation? The data in the digital twin is a real time data, not previously generated.*

For the manufacturing industry, the physical manufacturing objects are virtualized and represented as avatars seamlessly and closely integrated in both physical and cyber spaces.

Prof. Dong Yup Lee discussed an analysis involving the current mechanistic GEM models available and the efforts in improving its quality to generate reliable data, which can feed into effective data-driven bioprocess models[4].

2 Hefzi H, Ang KS, Hanscho M, Bordbar A, Ruckerbauer D, Lakshmanan M, Orellana CA, Baycin-Hizal D, Huang Y, Ley D, Martinez VS, Kyriakopoulos S, Jiménez NE, Zielinski DC, Quek LE, Wulff T, Arnsdorf J, Li S, Lee JS, Paglia G, Loira N, Spahn PN, Pedersen LE, Gutierrez JM, King ZA, Lund AM, Nagarajan H, Thomas A, Abdel-Haleem AM, Zanghellini J, Kildegaard HF, Voldborg BG, Gerdtzen ZP, Betenbaugh MJ, Palsson BO, Andersen MR, Nielsen LK, Borth N, Lee DY, Lewis NE. A Consensus Genome-scale Reconstruction of Chinese Hamster Ovary Cell Metabolism. Cell Syst. 2016 Nov 23;3(5):434-443.e8. doi: 10.1016/j.cels.2016.10.020. PMID: 27883890; PMCID: PMC5132346.

3 Seo-Young Park, Cheol-Hwan Park, Dong-Hyuk Choi, Jong Kwang Hong, Dong-Yup Lee, Bioprocess digital twins of mammalian cell culture for advanced biomanufacturing, Current Opinion in Chemical Engineering, Volume 33, 2021, 100702, ISSN 2211-3398, https://doi.org/10.1016/j.coche.2021.100702.

4 Park, S.-Y., S.-J. Kim, C.-H. Park, J. Kim and D.-Y. Lee. 2023. Data-driven prediction models for forecasting multi-step ahead profiles of mammalian cell culture towards bioprocess digital twins. Biotechnol. Bioeng., in press. https://onlinelibrary.wiley.com/doi/abs/10.1002/bit.28405

**Session 3** **Chair:** Steven Ferguson / NIBRT


**Prof. Bjørn Gunnar Voldborg**

*Technical University of Denmark (DTU), Lyngby, Denmark*

## The next generation of CHO cells for the production of therapeutic proteins

Bjørn Voldborg provided an overview of the results of the current program in CHO cells at the Technical University of Denmark from the past eleven years. His group aims to produce better CHO cell lines for the industry, improving the product's quantity, quality, and purity.

DTU's international program was built differently than classical research groups, incorporating a flexible research approach, generating considerable data on what CHO cells can or can't do.

In this context, Bjørn's group deepened their understanding of specific phenotypes to develop next-generation CHO cell lines for production of biopharmaceuticals.

One of the outcomes of their research was the pivotal significance of glycans in therapeutic drugs, concluding that comprehending, regulating, and managing glycosylation is indispensable for manipulating antibodies. To assist with understanding, Bjørn's DTU team supports external groups and offers their expertise in glycan characterisation upon request.

*You should consider glyco-efficiency in your cells. You take your gene of interest, make a stable pool, get clones, and go for those which grow well. And in some cases, you can have a very heterogenous selection of glycogens in that pool, and it could be that only one or two of those glycans are active, and the rest is there taking up space.*


**Dr. Nathan Lewis**

*University of California, San Diego, USA*

# Towards Actionable Omics in Cell Factory Design and Manufacturing of Biologics

Nathan Lewis presented his investigation on turning multiple omics tools from observational to actionable omics, to accelerate and optimize the cell line engineering processes by thinking about the cells as little factories.

Lewis starts with a list of fifteen top-selling drugs in 2021, of which more than half are therapeutic proteins produced in CHO cells. Global spending on biologics was over $1 trillion in the past few years, and with that, emerging demands for Antiviral mAbs (monoclonal antibodies), viruses for gene therapy, antivenom and cytokines for cell therapies arrived. The question behind his research is how to optimize these demands driving down the costs, as the ideal scenario would be seeing these number increase due to great accessibility.

In order to achieve these outcomes, Lewis has been pursuing the dynamics of how the cell produce its monoclonal antibodies, proteins, the machinery behind it, and what the cell factory looks like.

One of the problems, Lewis points out, in the research on cell line production processes is the need for more time in the biomanufacturing industry. He organised the process in steps.

1 Transgene transfection (1 week),

2 Amplification /selection of transfected cells (2-4 months),

3 Clone selection from a pool of cells (6 weeks),

4 Clone evaluation (3 months),

5 Media and feed optimization (3 months), scale up testing (2 months)

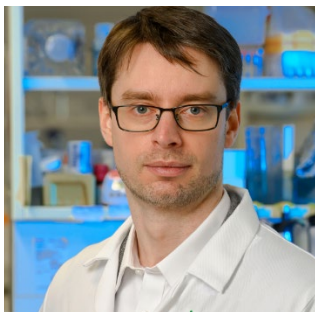
6 Master cell-bank clone selection.

By overviewing the steps, he then asks where, in these procedures we can leverage a lot of the new tools and models emerging over the years, as there are various relevant factors in bioproduction, like media composition, temperature, PH, and feed strategy. Many of these steps ultimately impact the expected results in titre, product quality and production stability.

How do we stack the new technologies to accelerate cell line engineering and development? Genome sequence and computational models are tools that can be used. The generated data can be integrated to make predictions or monitor bioprocesses to find better ways to improve them. Lewis's group is looking to turn the cells into engineerable systems, and the biologics have challenges as cells react in many unwanted forms when forced to do other than what they want. Results can be toxicity, impurity, and quality diversity. To view it as an engineerable system, they indicate the need for appropriate tools, diagrams, and the overview of the whole as a group of smaller parts or subgroups. In doing so, the group teamed with BGI Genomics to obtain the CHO cells' genome sequence and improve its gene annotation. But they want more.

*We want to go beyond, understand the mechanisms, understand the biochemistry and what they are doing, being able to quantify that. What is a cell saying to other cells? How to engineer that? What are all the proteins doing?*

Nathan's goal is not necessarily to create some new tool but to master applying what is available, like using AI, CRISPR, and Omics in cell line development processes, to mitigate the major challenge in biomanufacturing: high costs.



**Dr. Piotr Kowalski**

*University College Cork, Cork, Ireland*

## Engineering circular RNA nanotherapeutics

Circular RNAs (circRNAs) are a new class of non-coding RNAs, characterized by a covalently closed-loop structure and produced from genes through an alternative form of splicing, called back splicing. The lack of free ends necessary for exonuclease-mediated degradation, including 5′ Cap and 3′poly(A) tail, makes circRNAs significantly more stable, extending their half-life intracellularly and in blood as compared to their linear counterparts. They were also shown to possess unique roles and structural features and have been implicated in several human diseases, hinting at their potential for therapeutic applications. Piotr Kowalski's group developed a method for the circularization and purification of large synthetic circRNAs and pioneered the use of these circRNAs for robust and stable protein expression in eukaryotic cells in order to address the short half-life of mRNA in biological systems.

Compared to modified linear messenger RNAs (mRNA), synthetic circRNAs have a longer intracellular half-life, which allows sustained translation driven by the internal ribosome entry site, both in vitro and in vivo after injection of circRNA formulated into lipid nanoparticles. Moreover, in contrast to linear mRNAs, unmodified synthetic circRNA can bypass the cellular RNA sensors thereby avoiding unwanted immune responses in cells and in mice, which is likely attributed to their circular structure. Kowalski's group current work aims to address a significant gap in the knowledge of the utility of circular RNAs for translational research and will develop methods to deliver them inside the cells to help realize their therapeutic potential.

**Session 4** **Chair:** Darrin Morrissey

**Prof. Jane Farrar**

*Trinity College Dublin, Dublin, Ireland*

## Gene Therapy Renaissance: A Snapshot of Preclinical Development to Clinical Applications

Prof. Jane Farrar, who has been working in inherited retinal degenerative disorders for three decades in the Dept of Genetics and Microbiology at Trinity College Dublin, presented the Conference participants with a tour and reflection on the evolution of gene therapy.

A gene therapy initially consists of a therapeutic transgene, a piece of DNA delivered into a cell through a viral or non-viral vector, to express a missing or non-functional protein. This process is also known as somatic cell gene therapy. There are other approaches including gene replacement, to substitute autosomal dominant genes with normal ones, or gene suppression, in which a target gene is switched-off or down-regulated; and the most recent technology of gene editing (e.g. CRISPR) which can also repair DNA.

For Prof.Farrar, the key components for delivering gene therapies are:

- Understanding the molecular basis of diseases to identify the targets.
- Identifying the target patient population for the therapy.
- Creating appropriate model systems such as cells, animals, or organoids (tissues developed from the differentiation to induced pluripotent stem cells and again turned into other tissues with functions equivalent to organs).
- In addition, there is a continuous need to improve vectors for viral and non-viral delivery.

The evolution of gene therapies includes, not just delivering the endogenous gene necessarily, but changing and optimising the nucleotide sequence.

*So, amazingly, we are at the point now in gene therapy where we can, in principle, target any gene. Why is that? Because of gene editing. Because we know the sequence, we know the mutation, we know the target disease, and we can target any gene. Any disease is druggable. It is just costly. That is the problem. And time-consuming as well.*

Farrar provided examples of preclinical, clinical and approved gene therapies. These included examples such as

- Haemoglobinopathies for which Gene Editing therapy has evolved from Basic Research to Clinical Trial through the application of CRISPR (Vertex Inc);
- Preclinical Gene Therapy which included AAV-delivered RP2 gene replacement in a patient-derived retinal organoid model of x-linked RP2 ocular disease (an example of work from her group);
- and the first gene replacement therapy that was FDA and EMA approved, in 2017 and 2019, respectively, which was an Ocular Gene Therapy (Luxturna).

This AAV2 delivered RPE65 gene replacement for an inherited retinal degeneration (IRD), was developed by Profs Jean Bennett, Albert McGuire, Kathy High, Daniel Chung & colleagues, Uni Penn; subsequently, Spark Therapeutics (which was acquired by Roche).

Numerous diseases are under investigation, with the search for targets of rare and complex health conditions like RPE 65, Beta Thalassemia, sickle cell disease, haemophilia and others. Apart from that, Farrar points out a general aim to move gene therapies towards the more common multifactorial disease space and find overarching principles to modulate therapies.

In conclusion, Jane Farrar praised Ireland's achievements and continuous growth in research, biomanufacturing expertise, and infrastructure. She finalised her talk with optimistic and encouraging views on the future of gene therapy.

*We (Ireland) can become a really good clinical trial site if we do enough genomic medicine and if we understand the patient population - the genetic architecture of our patient population. And, of course, we have lots of manufacturing sites now, which is fantastic.*

**Dr. Sandro Matosevic**

*Purdue University, West Lafayette, USA*

# Reprogramming natural killer cells for next-generation immunotherapies of cancer

Cell therapies are a unique class of "drugs" or treatments that utilize cells to treat various conditions. These therapies are referred to as "living drugs" as the cells are "living" when administered to the patient. Sandro Matosevic has a particular focus on cell therapies for cancer treatment. Compared to other drugs, these therapies stand out due to their complex nature and size and the use of proteins and genes as integral components. The process typically begins by drawing the patient's blood, then modifying and expanding the cells in a laboratory setting, frozen and shipped to the hospital where the approved therapy is administered to the patient.

One notable form of engineered cell therapy is CAR-T therapy, which involves modifying immune cells with genes to specifically target tumour cells. This innovative approach enhances the immune system's ability to identify and eliminate cancer cells.

*Cell therapies are based on the function of immune cells and their ability to act as drugs. That ability is often compromised in cancer, and so we've started developing engineered cell therapy. This is where the whole CAR-T field exploded.*

CAR-T therapy has been successful in treating blood cancers, while other cell types like NK (Natural Killer Cell) cells and Dendritic cells (DC) can also be used. NK cells, part of the innate immune system, offer advantages such as rapid response and safety. The mechanism involves recognition of targets, activation of killing signals and elimination of target. Due to its intelligent system, healthy tissues are spared from killing. Matosevic has been exploring this potential and the challenges of controlling and conserving its functions.

In cancer, cell therapies target cells that appear foreign and express activating signals. Different sources, such as peripheral blood, cord blood, and induced pluripotent stem cells, are being explored to overcome limitations in sourcing cells to achieve off-the-shelf therapies. Peripheral blood has advantages due to self-donation, while cord blood allows for the preservation of cell sources. Cell therapy workflows involve selecting a cell source, modifying the cells, and treating the patient.

CAR-T therapies have had more development time and research than CAR-NK therapies. Manufacturing NK Cell therapies face challenges in genetic engineering and viral delivery; they have shorter lifespans, requiring additional boosts such as cytokines. However, they are considered safer than CAR-T cells, and recent discoveries of specific activating signalling domains in NK cells show promise.

Despite the effectiveness of immune cells in eliminating pathogens, there are still challenges in treating cancer with cell therapies.

The response of patients with solid tumours to CAR-T therapies has been limited, raising questions about the reasons for this lack of response. Studies have explored various factors such as drug delivery, persistence, infection, and dosing to identify areas for improvement. Despite manufacturing changes, there is still a challenge in effectively treating difficult cancers that have yet to receive FDA approval. Resistance mechanisms in these aggressive cancers make them highly resistant to therapeutic interventions. Current research efforts in CAR-T and CAR-NK therapies focus on overcoming resistance by engineering cells. Specificity has been critical, ensuring that CAR-T cells target the tumour. However, several factors hinder therapeutic efficacy within the tumour, including metabolic and immunosuppressive conditions, poor immune cell infiltration, drug delivery issues, and checkpoint inhibitors.

Gene expression data analysis in brain cancer patients revealed that inhibitory molecules actively exclude NK cells from the tumour microenvironment. An example of a pathway studied is the CD73-adenosine axis, where adenosine binding to NK cells leads to their functional shutdown. Non-viral gene delivery into NK cells has been explored as an alternative to the use of viruses for the modification of NK cells, using various delivery systems, including nanoparticle-based systems. Functional expression using nanoparticles was achieved and combining a CAR against NKG2D with a monoclonal antibody to block CD73 showed improved efficacy in killing lung cancer cells. As a step further, through engineering, they created a cell therapy that directly targets CD73 without the use of antibodies, showing promising survival responses in lung cancer models.

One of the key challenges to cell-based therapy is to ensure there is sufficient infiltration of immune cells into targeted tumours. Matosevic's group has developed strategies to enhance the infiltration of NK cells into tumors, aided by proteins called chemokines, which attract immune cells to target sites. In addition, cell therapies face challenges such as antigen modulation and heterogeneity in the tumour, potential off-target toxicities, and immune cell dysfunction. To understand these issues, the group examined NK cells from brain cancer patients and found significant differences compared to healthy individuals.

Gene therapy allows researchers to reprogram drugs and develop more potent approaches beyond antigen targeting. Patients with brain cancer lack chemokines that attract immune cells to tumours, causing dysregulation. To address this, they developed triple-engineered, multifunctional NK cells that targeted antigen escape, CD73 inhibition, and therapeutic resistance. In patient-derived brain cancer mice, these engineered cells combined with autophagy inhibitors effectively controlled tumour growth. The therapeutic efficacy of these cells was significantly reduced when placed in a natural tumour environment, emphasizing the need for and potential impact of genetic engineering.

*The future of cell therapy is going to get to a point where we can hopefully make these cells off the shelf, right? We can package them, get them ready, frozen, take a sort of a shelf, and make it sort of as if there is an on-demand therapy.*

Closing Keynote: **Prof. Bruce Levine**

*University of Pennsylvania, Philadelphia, USA*

## Development, Approvals, Access & Prospects of Engineered T Cell Therapies

The immune system is designed to protect one's "self" from non self. The challenge with cancer immunotherapy lies in the immune system's difficulty in recognizing cancer cells derived from one's own cells. Cancer-specific immune cells are scarce, like finding a dull needle in a haystack. To address this, researchers are exploring isolating and delivering rare T-cell receptors designing synthetic receptors that can recognize cancer. Traditional T-cell receptors have limitations, as they require proper antigen presentation and are tissue-specific. Chimeric antigen receptors (CARs) offer an alternative approach, as they recognize surface antigens independently of major histocompatibility complex (MHC) molecules. CAR T-cells engage with tumour cells, activation of the T cell ensues, resulting in the release of perforin and granzyme, which destroys the tumour cells.

CARs emerged in the 1980s when researchers proposed combining antibody and T-cell receptor domains. The first clinical trial of CAR T-cells was presented to the DNA Advisory Committee Meeting in 1994 and conducted in HIV by Cell Genesys. However, the initial results showed temporary cell persistence. During that time, Bruce Levine's postdoctoral project focused on activating T cells using an artificial dendritic cell or artificial antigen-presenting cell. His group used magnetic beads conjugated with antibodies to stimulate and activate T cells more effectively than soluble antibodies and cytokines. This approach was adopted by Cell Genesys, leading to the long-term persistence of their CAR-T cells.

In August 2010, Levine's team initiated their first CAR-T cell trial in cancer, targeting CD19. The process involved isolating T cells from leukapheresis, delivering the CAR gene using a lentiviral vector, and growing the cells in a bioreactor. The results showed remarkable success, with patients experiencing remission and significantly reducing leukaemia cells.

However, challenges, such as severe cytokine release syndrome, were managed using the drug tocilizumab. Despite these obstacles, CAR-T cell therapies have gained approvals for leukaemia, lymphoma, and myeloma. Additional gene-modified cell therapies, including T cell receptor therapy and gene-modified stem cell therapies, are also being developed.

Bruce Levine highlighted the importance of continuous improvement in these therapies to address relapse cases and improve patient outcomes. Two of his patients, Emily and Sophia, were mentioned as examples of both success and challenges in CAR-T cell therapy. Emily achieved long-term remission and is now thriving, while Sophia experienced relapse with CD19-negative disease. CAR T investigators aim to enhance these therapies to benefit a more significant percentage of patients.

In adult patients, two out of the first three patients treated at Penn Medicine survived for a decade[1], showing the durability of T cell persistence. Tracking the CAR T cells in vivo showed two phases of engraftment, with initial persistence of CD8 and gamma delta cells, followed by persistence of CD4 cells. The reasons for long-term persistence are still being studied, including factors like gene disruption, integration in genomic regions associated with better CAR expression, and genetic drift. T cell differentiation is a complex process, with various subsets displaying different functional properties. Higher levels of stem cell memory and central memory T cells have been correlated with more potent CAR-T cell products. However, in people diagnosed with cancer, T cell fitness declines with multiple treatment cycles in cancer, leading to suboptimal raw material for relapsed/refractory patients. Therefore, it is essential to determine what potency level is considered "good enough" given the patient variability.

Many methods have been proposed to enhance CAR-T cell potency, and synthetic biology offers opportunities to create logic gated CARs, checkpoint-resistant CARs, knockouts, switches, and conditional and stealth CARs. Dozens of targets to enhance T cell potency are being explored. CRISPR screens and other studies provide valuable insights into the role of different genes in T cell function. Various models of next-generation CARs are being investigated in preclinical and clinical studies, aiming to improve the potency, efficacy, and safety of CAR-T cell therapies.

CAR-T cell therapy is being enhanced through various strategies. One approach involves using a dominant negative “decoy” receptor to counter immunosuppressive cytokines secreted by tumours. Clinical trials in prostate cancer have showed positive results, although some patients experienced adverse events. Faster manufacturing processes, such as 5-day and 3-day protocols, have been explored, yielding more potent CAR-T cells. Clinical trials using accelerated processes and antitumor cytokines have shown promising responses. Quality control and release testing assess parameters like viability and phenotype, but measuring CAR expression and potency remains challenging. Synthetic biology offers additional opportunities, including engineered cytokine receptors with different signalling domains for improved CAR T cell therapy.

Researchers have collaborated to explore the effects of manipulating cytokine receptors in T cells. Combining different receptors and signaling domains, such as IL-2, IL-7, and IL-9, enhanced the stem cell memory phenotype and improved anti-tumour response in mouse models.

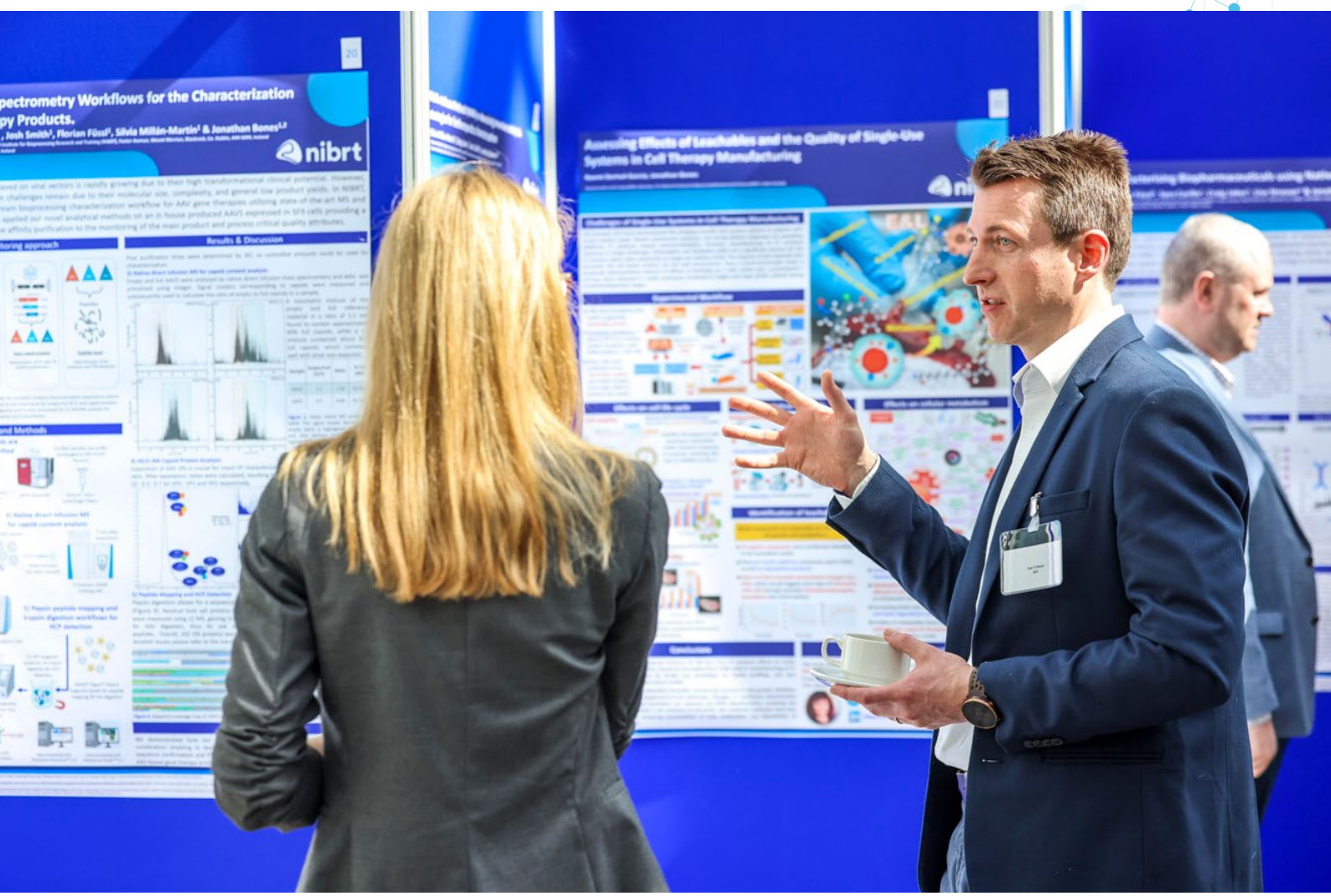
An orthogonal IL-2 receptor with an IL-9 signalling domain was utilized to avoid immune system suppression. Additionally, oncolytic viruses delivered the orthogonal IL-2 receptor, providing an additional therapeutic effect. Gene editing methods, including electroporation and newer techniques like Avectas soluporation method, are being developed to deliver gene-editing and other cargo.

*CARs are moving beyond oncology. Remember the first CAR-T trial? It was in HIV. There are CARs trials in autoimmunity. CAR-T cells and Lupus, where five patients responded. Additional pre-clinical research is ongoing in cardiac fibrosis and perhaps one day CAR- T cells for ageing and other diseases.*

T-cell serial cultivation in mice has shown that these cells can survive and remain functional for many years. Efforts are focused on enhancing potency, shortening manufacturing processes, and providing education and training to improve patient access. Disparities in access to CAR-T cell therapies must be addressed, considering factors like geography and income. Ethical considerations are also crucial, as seen in the November, 2018 case of CRISPR gene-edited babies, where responsible and ethical practices were ignored. It is important to differentiate between scientifically and clinically validated therapies and unproven cellular therapies that make unsupported testimonial claims. The International Society for Cell and Gene Therapy (ICT) has an ethics committee that provides resources[2] to address these issues.

1 Melenhorst, J.J., Chen, G.M., Wang, M. et al. Decade-long leukaemia remissions with persistence of CD4+ CAR T cells. Nature 602, 503–509 (2022). https://doi.org/10.1038/s41586-021-04390-6

2 International Society for Cell & Gene Therapy Committee on the Ethics of Cell and Gene Therapy Patient Resources - https://www.isct-unprovencellulartherapies.org/patient-resources/




# NIBRT Research highlights

The full day of talks and discussions promoted by NIBRT and sponsors during the Research Conference Biopharma Focus on the Future 2023 was wrapped up by posters viewing, and networking, highlighting the ongoing investigations of NIBRT research groups.

Twenty-four posters from NIBRT Research Groups were presented during the Conference.

The following is a selection of nineteen poster abstracts outlining some of the leading-edge research conducted by NIBRT research groups, in collaboration with other research groups, institutions and the industry.

# NIBRT Research highlights

## POSTERS

### The Effect of N6 - methyladenosine Modifications on mRNA Stability and Expression in CHO Cells

**Or Skornik[1], Niall Barron[1]**

*[1] National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland*

Chinese hamster ovary (CHO) cells are the most frequently applied host cell system in the pharmaceutical industry. Current CHO processes can typically yield 3-10 g/L of product, exceeding productivity of many other cell lines. These yields have come about via optimized expression systems as well as genetic engineering advances including overexpression and silencing of specific genes. RNA-based technology is now recognised as a promising approach for cell engineering applications, with the advantages of a fast translation, no size limitation of the transcript delivered into the cell, higher control over the expression of proteins and an easier and cheaper manufacturing process. RNA has not been a popular tool for cell engineering applications until recently due to its immunogenicity. However, recent ground-breaking studies have shown that mRNA post-translational modifications pose a solution to this problem, furthermore, RNA post translational modifications contribute to the transcript stability and expression level. N6 -methyladenosine (m6A) modification is the most abundant mRNA modification and has been proven to improve the mRNA transcript's properties.

In this work, we aimed to transfect CHO cells with modified mRNA transcripts and investigate the modification's influence on the transcript when different concentrations of modified bases were incorporated randomly into the transcript.

Modified mRNA transcripts for green fluorescent protein (GFP) were created using in vitro transcription (IVT) reaction supplemented with 6 different concentrations of m6A bases and adenosine bases (100%, 50%, 25%, 12.5%, 6.25%, 0%). m6A presence in the transcripts was evaluated by Dot Blot. Transcripts were then transfected into CHO cells. We evaluated the GFP expression levels in the transfected CHO cells using flow cytometry.

We show that m6A modified RNA bases, when implemented in an arbitrary manner throughout the mRNA transcript, had a negative effect on the transcript's expression levels. Our results show a negative correlation between the concentration of m6A modified bases used in the IVT reaction and the expression level of the mRNA transcript, as the lowest concentration of modified bases used in the reaction resulted in the highest level of GFP expression, which gradually decreased as mRNA transcripts containing higher concentrations of modified bases were used.

Overall, this study shows the negative effects of randomly incorporated m6A modified bases on mRNA expression levels, suggesting that this modification is closely controlled in vivo and is able to support mRNA stability and expression levels only when incorporated in specific transcript locations.

Or.skornik@nibrt.ie
Niall.barron@nibrt.ie

## Enhancing recombinant protein and viral vector production in mammalian cells by targeting the YTHDF readers of N6–methyladenosine in mRNA

**Nga Lao[1] and Niall Barron[1,2]**

*[1] National Institute for Bioprocessing Research and Training, Blackrock, Dublin 4, Ireland*

*[2] School of Chemical and Bioprocess Engineering, University College Dublin, Belfield, Dublin 4, Ireland*

N6–methyl adenosine (m6A) is the most abundant internal modification on eukaryotic mRNA and has been implicated in a wide range of fundamental cellular processes. This modification is regulated and interpreted by a set of writer, eraser, and reader proteins. To date, there have been no reports on the potential of mRNA epigenetic regulators to influence recombinant protein expression in mammalian cells. In this study we evaluated the potential of manipulating the expression of the m6A YTH domain-containing readers, YTHDF1, 2, and 3 to improve recombinant protein yield based on their role in regulating mRNA stability and promoting translation. Using siRNA-mediated gene depletion, cDNA over-expression and methylation-specific RNA immunoprecipitation, we demonstrate that (i) knock-down of YTHDF2 enhances (~2-fold) the levels of recombinant protein derived from GFP and EPO transgenes in CHO cells; (ii) the effects of YTHDF2 depletion on transgene expression is m6A-mediated and (iii) YTHDF2 depletion or over-expression of YTHDF1 increases viral protein expression and yield of infectious lentiviral particles (~2-3 fold) in HEK293 cells. We conclude that various transgenes can be subjected to regulation by m6A regulators in mammalian cell lines and that these findings demonstrate the utility of epi-transcriptomic-based approaches to host cell line engineering for improved recombinant protein and viral vector production.

## Analysis of mRNA Lipid Nanoparticles (mRNA-LNPs)

**AswathyBalakrishnan[1,2], KaustabhSarkar[3], CaioH.N.Barros[2], BakulBhatnagar[4], RaminDarvari[4], SergueiTschessalov[4], SutapaBarua[4], AgotaCsiki-Fejer[4], JayeshSonje[4], StevenFerguson[1,2], ElizabethTopp[2,3]**

*[1] School of Chemical and Bioprocess Engineering, University College Dublin (UCD), Belfield, Ireland*

*[2] National Institute for Bioprocessing Research and Training (NIBRT), Blackrock, Dublin, Ireland*

*[3] Department of Industrial and Physical Pharmacy, College of Pharmacy, Purdue University, West Lafayette, IN47907, United States*

*[4] Pfizer Inc.,1 Burtt Road, Andover, MA,01810*

mRNA-based therapeutics are commonly delivered in the form of lipid nanoparticles (mRNA-LNPs) which are currently stored and transported as frozen solutions at –20°C or –80°C. Lyophilization is a technique that could potentially be used to improve the long-term stability of mRNA-LNPs. It is a drying method that involves three steps – (i) freezing of the solution (ii) removal of free water by sublimation (primary drying) (iii) removal of bound water by desorption (secondary drying). Here, the effect of lyophilization on the physical stability of RNA (from Saccharomyces cerevisiae) encapsulated in lipid nanoparticles has been studied. Particle size distribution, polydispersity index and encapsulation efficiency have been measured after each stage of the freeze-drying cycle. Images have also been obtained using cryo-electron microscopy which show changes in morphology of the nanoparticles during the freezing and drying processes. The formation of lamellar structures and blebbed compartments can clearly be seen in the images obtained. Apart from these assays, which are typically used to characterize mRNA-LNPs, studies using circular dichroism have also been carried out to analyse the secondary structure of RNA. Understanding the impact of each stage of freeze-drying brings us one step closer to developing an optimum cycle for stable solid-state mRNA-LNP formulations.

## Atomic layer coating on solid myoglobin formulation powder surfaces and its effect on protein stability

**Caio H. N. Barros, Manuel Alfaro, Cormac Costello, Fei Wang, Kedar Sapre, Suneel Rastogi, Shivkumar Chiruvolu, James Connolly, Elizabeth M. Topp**

*National Institute for Bioprocessing Research and Training*

The stability of proteins in solid-state formulations for biopharmaceutical applications is heavily influenced by the excipients. Here, the stability of myoglobin containing powders was assessed following Atomic Layer Coating (ALC), a process traditionally used in semiconductor sciences but seldom used for pharmaceutical solids. Powders containing myoglobin and mannitol (1:1 w/w) were generated by either lyophilization or spray-drying and then subjected to aluminum oxide or silicon oxide ALC coating. These formulations were then stored for 3 months under controlled conditions (53% RH, 40 oC) in open or closed vials. After the storage period, the powders were initially analyzed by X-ray diffraction and Fourier-transform Infrared spectroscopy.

Moisture content determination and reconstitution time were also measured. Native myoglobin recovery and protein aggregate content were measured using Size Exclusion High Performance Liquid Chromatography (SEC-HPLC). Results showed that the recovery of soluble, monomeric myoglobin depended on coating type, drying method and storage conditions. Powders with ALC-coated surfaces had up to 2-fold greater protein recovery than uncoated controls, and some samples showed less soluble aggregate content than the controls, a result attributed in part to the effects of ALC coating on powder moisture content. Overall, ALC coating showed potential to reduce myoglobin aggregation on storage under stressed conditions.

# Detection and quantification of alpha galatosylated n-glycans in porcine notochordal cell matrix and implantable xenogenic commercial products

**Ng Tung Hing M[1,2], Joyce K[3], Gunay B[3], Schmitz T[4], Tryfonidou M[5], Ito K[4], Pandit A[3] and Saldova R[1,2,3]**

*[1]GlycoScience Group, National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland*

*[2]UCD School of Medicine, College of Health and Agricultural Science, University College Dublin, Ireland*

*[3]CÚRAM, SFI Research Centre for Medical Devices, National University of Ireland, Galway, Ireland*

*[4]Orthopaedic Biomechanics, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands*

*[5]Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Porcine notochordal cell-rich NP matrix (NCM) is being developed as a regenerative biomaterial-based treatment for intervertebral disc (IVD) degeneration, a significant cause of low back pain (1). Alpha galactose (α-Gal), a glycosylated epitope present in porcine tissue, is immunogenic for humans because they do not express it due to the inactivation of enzyme α-1,3galactosyltransferase essential for the synthesis of α-Gal. (2). It may be required for biopharmaceuticals to minimise α-Gal content as severe allergic reaction/anaphylactic shock have been reported in response to the epitope. The aim of this project is to determine α-Gal content in porcine NCM, a component of prospective IVD regeneration devices.

N-glycans on glycoproteins from NCM and decellularised NCM (DGAL) were captured in gel-block, reduced and alkylated, digested by Peptide-N-Glycanase F, labelled with 2-aminobenzamide and analysed by hydrophilic interaction ultra-performance liquid chromatography (HILIC-UPLC) and liquid chromatography-mass spectrometry (LC-MS). Exoglycosidase coffee bean α-galactosidase (CBG) was used to identify N-glycans carrying α-gal epitope.

Six replicates of undigested and CBG-digested NCM and DGAL were run on HILIC-UPLC and compared using multivariate statistical analyses with univariate post-hoc analysis. Chromatographic peaks, digested with CBG, were labelled. Digestion patterns of previously identified α-Gal glycans in these peaks were elucidated, and consistently digested glycans were selected. These glycans were cross-compared with LC-MS data. The final list of α-Gal glycans confirmed by CBG-digest and LC-MS was created.

Due to the lack of methodology within the field, there is no current α-gal quantification performed on xenografts. HILIC-UPLC/MS method could be potentially used for this purpose.

This project was funded by the iPSpine project (European Union's Horizon 2020 research and innovation programme under grant agreement No 825925) and Science Foundation Ireland (SFI) and the European Regional Development Fund (Grant Number 13/RC/2073_2).

**References**

1. de Vries S, Doeselaar MV, Meij B, Tryfonidou M, Ito K. Notochordal Cell Matrix As a Therapeutic Agent for Intervertebral Disc Regeneration. Tissue Eng Part A. 2019;25(11-12):830-41.
2. Hilger C, Fischer J, Wolbing F, Biedermann T. Role and Mechanism of Galactose-Alpha-1,3-Galactose in the Elicitation of Delayed Anaphylactic Reactions to Red Meat. Curr Allergy Asthma Rep. 2019;19(1):3.

# High throughput platform for analysis of unconjugated glycans from human breast milk (HMOs) and investigation of their role in microbial transfer

**Matthews E[1], O'Flaherty R[2], Murphy E[3], Cotter PD[4], McAuliffe FM[5], Van Sinderen D and Saldova R[1,7]**

[1]*The National Institute for Bioprocessing, Research, and Training (NIBRT), Dublin, Ireland*

[2]*Department of Chemistry, Human Health Institute Maynooth University, Maynooth, Kildare, Ireland*

[3]*PrecisionBiotics Group Limited, Cork Airport Business Park, Cork, Ireland*

[4]*Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland*

[5]*UCD Perinatal Research Centre, School of Medicine, University College Dublin, National Maternity Hospital, Dublin, Ireland*

[6]*APC Microbiome Ireland, National University of Ireland, Cork, Ireland*

[7]*UCD School of Medicine, College of Health and Agricultural Science and UCD Conway Institute of Biomolecular and Biomedical Research*

The seeding and colonisation events of the infant gut determine an immune and metabolic infrastructure that have a profound, lifelong effect on host health. It is well established that human milk contains an abundance of diverse biomolecules that influence microbial colonization. The Microbe Mom SFI Spoke consortium was set up to investigate the major factors that influence the seeding and proliferation of microbes during mother to infant microbial transfer. A specific aim of this project was to determine the role of HMOs (human milk oligosaccharides) in mother to infant microbial transfer and gut colonisation. The development of a high throughput (HT) method and the subsequent analysis of HMOs in relation to microbial transfer sits as part of this work.

We aimed to develop and validate a high throughput platform to generate reproducible data on the relative abundances of human milk oligosaccharides from 81 breast feeding mothers. To provide phenotypic data for the assignment of Secretor and Lewis genotype status and to analyse this data with respect to the microbial transfer data available for each participant.

Milk was diluted with water (1:1), the lipids were removed via centrifugation and the aqueous layer was recovered and filtered through a 1micron glass fibre plate. Proteins were ethanol precipitated and centrifuged, the upper liquid fraction was collected and dried. All samples were reconstituted in water and subjected to a sequential solid phase C18 and Carbograph microplate extraction protocols. The HMO eluent was labelled with 2 -aminobenzamide and free label and salts were removed using HyperSepDiol plates. The separation of 2AB-derivatized HMOs was carried out by UPLC with fluorescence detection on a Waters ACQUITY UPLC H-Class. The HILIC separation was performed using a Waters Ethylene Bridged Hybrid (BEH) Glycan column using a linear gradient of 88–43% MeCN at 0.56 mL/min over 35 minutes. 20µL sample was injected in 88% v/v MeCN. The system was calibrated using a 2-AB-labeled dextran standard. After the lipid extraction, all samples were processed on a 96 well plate using an 8 channel multi pipette.

The protocol was validated using commercially available HMO standards and the observed Glucose Unit (GU) values obtained were comparable to those in the literature. 50 reproducible glycan peaks were resolved in all samples and integrated. The constituent HMO peaks were assigned. The Secretor status was determined by the presence or near absence of 2'FL and LNFPI and Lewis status was assigned on the relative abundances of 3FL, LDFT, LNFPII, LNFP III and LDFH. Of the 81 individuals 68% are Se+Le+, 5% are Se+Le-, 27% are Se-Le+ and no assignment of Se-Le- has been made. Relative abundances of all HMO peaks are being assessed in relation to microbial transfer.

We report a validated HT method for the analysis of HMOs and its application for investigation of HMOs' role in microbial transfer.

This project has received funding from the Science Foundation Ireland (SFI) under Grant No. 16/SP/3827 (Microbe Mom).

# Spatial Dysregulation of *N*-glycans in Multiple Sclerosis Brain Lesions

**Yagmur Bozkurt[1], Kieran Joyce[1], Jill McMahon[1], Una FitzGerald[1], Radka Saldova[2,3] and Abhay Pandit[1]**

*[1]CÚRAM, SFI Research Centre for Medical Devices, University of Galway, Ireland*

*[2]National Institute for Bioprocessing, Research and Training, Dublin, Ireland*

*[3]School of Medicine, College of Health and Agricultural Science, University College Dublin, Ireland*

Multiple Sclerosis (MS) is a life-long deteriorating disease characterized by inflammation and degeneration of the central nervous system (1). Due to the disease's complex pathophysiology and clinical phenotype, therapeutic options are limited. Oxidative stress and mitochondrial dysfunction are some of the molecular hallmarks of MS (2). Mitochondrial dysfunction results in the accumulation of reactive oxygen and nitrogen species inside neuronal cells, which is further, induces endoplasmic reticulum (ER) stress. This affects post-translational modifications in the ER-Golgi pathway such as N-glycosylation, the addition of glycans to asparagine residues on peptides (3). In this project, we have characterized the glycosignature of MS through lectin microarray and high-throughput N-glycomics analysis on MS and control cases. Isolated glycoproteins from snap-frozen brain samples were labelled and quantified for N- and O-glycan epitopes through lectin microarray assay. For N-glycan analysis, glycans were cleaved via PNGase-F enzyme, labelled with 2AB and analyzed by Hydrophilic Interaction Liquid Chromatography-Ultra performance liquid chromatography (HILIC-UPLC), weak anion exchange chromatograph (WAX), exoglycosidase enzyme digestions and mass spectrometry. Whole glycome analysis (lectin microarray) has shown a significant increase in sialylation, complex antennary structures and GlcNAcylation in MS. Principal component analysis has also shown distinctive clusters of control and MS groups, confirming these differences. N-glycan profiles of MS brain exhibited distinctive features compared to healthy human brain profile, concordant with lectin microarray findings in which sialylated and oligomannose species abundance are notably dysregulated in a spatial dependent manner. These results show that N-glycosylation machinery is dysregulated in MS brain, with specifically conserved patterns across MS lesions and could be further investigated to understand molecular mechanisms underlying disease and identify potential therapeutic targets.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813263. We thank the U.K. MS Society Tissue Bank for the supply of post-mortem tissues.

**References**

1. Walton et al. Rising Prevalence of Multiple Sclerosis Worldwide: Insights from the Atlas of MS, third edition. 2020. Mult. Scler. J. 26, 1816–1821.
2. Simkins et al. Chronic Demyelination and Axonal Degeneration in Multiple Sclerosis: Pathogenesis and Therapeutic Implications. 2021. Curr. Neurol. Neurosci. Rep. 21.
3. Haile et al. Rab32 Connects ER Stress to Mitochondrial Defects in Multiple Sclerosis. 2017. J. Neuroinflammation 14, 1–13.

## Integrated analysis of single cell chromatin accessibility and gene expression in Chinese hamster ovary cells

**Ryan Hagan[1,2], Ioanna Tzani1 and Colin Clarke[1,2]**

*[1].National Institute for Bioprocessing Research and Training, Fosters Avenue, Blackrock, Co. Dublin, Ireland*

*[2].School of Chemical and Bioprocess Engineering, University College Dublin, Belfield, Dublin, Ireland*

The emergence of single cell -omics technologies allows biological data to be captured at a previously unprecedented resolution - from individual cells! Single cell Assay for Transposase Accessible Chromatin (ATAC) sequencing enables profling of the open chromatin landscape at single cell resolution. scATAC-seq data can be integrated with transcriptomic data (e.g. scRNA-seq) to provide further understanding of the relationship between chromatin accessibility and gene expression. In this study we used the 10x Chromium scATAC-seq workfow to analyse chromatin accessibility in four CHO cell populations. Additionally, we performed scRNA-seq analysis using the BD Rhapsody WTA workfow on two of the four CHO populations.

## Understanding the transcriptional response to ER stress in Chinese hamster ovary cells using multiplexed single cell RNA-seq

**Ioanna Tzani[1], Marina Castro-Rivadeneyra[1,2], Stefano Boi[1], Colin Clarke[1,2]**

*[1].National Institute for Bioprocessing Research and Training, Fosters Avenue, Blackrock, Co. Dublin, Ireland*

*[2].School of Chemical and Bioprocess Engineering, University College Dublin, Belfield, Dublin, Ireland*

Single cell RNA-seq (scRNA-seq) has recently been shown to provide a powerful method for the analysis of transcriptional heterogeneity in Chinese hamster ovary (CHO) cells. A potential drawback of current scRNA-seq platforms is that the cost can limit the complexity of experimental design and therefore the utility of the approach. Here, we report the use of oligonucleotide barcoding to perform multiplexed CHO cell scRNA-seq to study the impact of tunicamycin (TM), an inducer of the unfolded protein response (UPR). For this experiment, we treated a CHO-K1 GS cell line with 10µg/ml tunicamycin and acquired samples at 1, 2, 4 and 8 hr post-treatment as well as a non-treated TM-control. We transfected cells with sample-specific polyadenylated ssDNA oligonucleotide barcodes enabling us to pool all cells for scRNA-seq. The sample from which each cell originated was subsequently determined by the oligonucleotide barcode sequence. Visualisation of the transcriptome data in a reduced dimensional space confirmed that cells were not only separable by sample but were also distributed according to time post-treatment. These data were subsequently utilised to perform weighted gene co-expression analysis (WGCNA) and uncovered groups of genes associated with TM treatment. For example, the expression of one group of coexpressed genes was found to increase over the time course and were enriched for biological processes associated with ER stress. The use of multiplexed single cell RNA-seq has the potential to reduce the cost associated with higher sample numbers and avoid batch effects for future studies of CHO cell biology.

# Found in Translation- Microproteins are a new class of potential host cell impurity in mAb drug products

**Marina Castro-Rivadeneyra[1,2], Ioanna Tzani[1], Paul Kelly[1], Lisa Strasser[1], Felipe Guapo[1], Ciara Tierney[1], Lin Zhang[3], Martin Clynes[4], Barry L. Karger[5], Niall Barron[1,2], Jonathan Bones[1,2] and Colin Clarke[1,2]**

[1] *The National Institute for Bioprocessing Research and Training, Dublin*

[2] *School of Chemical and Bioprocess Engineering University College Dublin*

[3] *Bioprocess R&D, Pfzer Inc. Andover, Massachusetts, USA*

[4] *National Institute for Cellular Biotechnology, Dublin City University*

[5] *Barnett Institute, Northeastern University, Boston,Massachusetts, USA*

Mass spectrometry (MS) has emerged as a powerful approach for the detection of Chinese hamster ovary (CHO) cell protein impurities in antibody drug products. The incomplete annotation of the Chinese hamster genome, however, limits the coverage of MSbased host cell protein (HCP) analysis. In this study, we performed ribosome footprint profling (Ribo-seq) to monitor CHO cell translation. The Ribo-seq data was used to discover unannotated proteoforms including 1000s of microproteins (defned as proteins < 100aa).

MS-based HCP analysis was performed for 4 mAb drug products. The resulting data searched against a protein database comprised of previously annotated canonical proteins and novel CHO cell microproteins.

# Ultra-deep next generation mitochondrial genome sequencing reveals widespread heteroplasmy in Chinese hamster ovary cells

**Paul S Kelly[1], Colin Clarke[2], Alan Costello[1], Craig Monger[3], Justine Meiller[1], Heena Dhiman[4], Nicole Borth[4], Michael J Betenbaugh[5], Martin Clynes[1], Niall Barron[6]**

[1] *National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland*

[2] *National Institute for Bioprocessing Research and Training, Fosters Avenue, Blackrock, Co., Dublin, Ireland*

[3] *National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland; National Institute for Bioprocessing Research and Training, Fosters Avenue, Blackrock, Co. Dublin, Ireland*

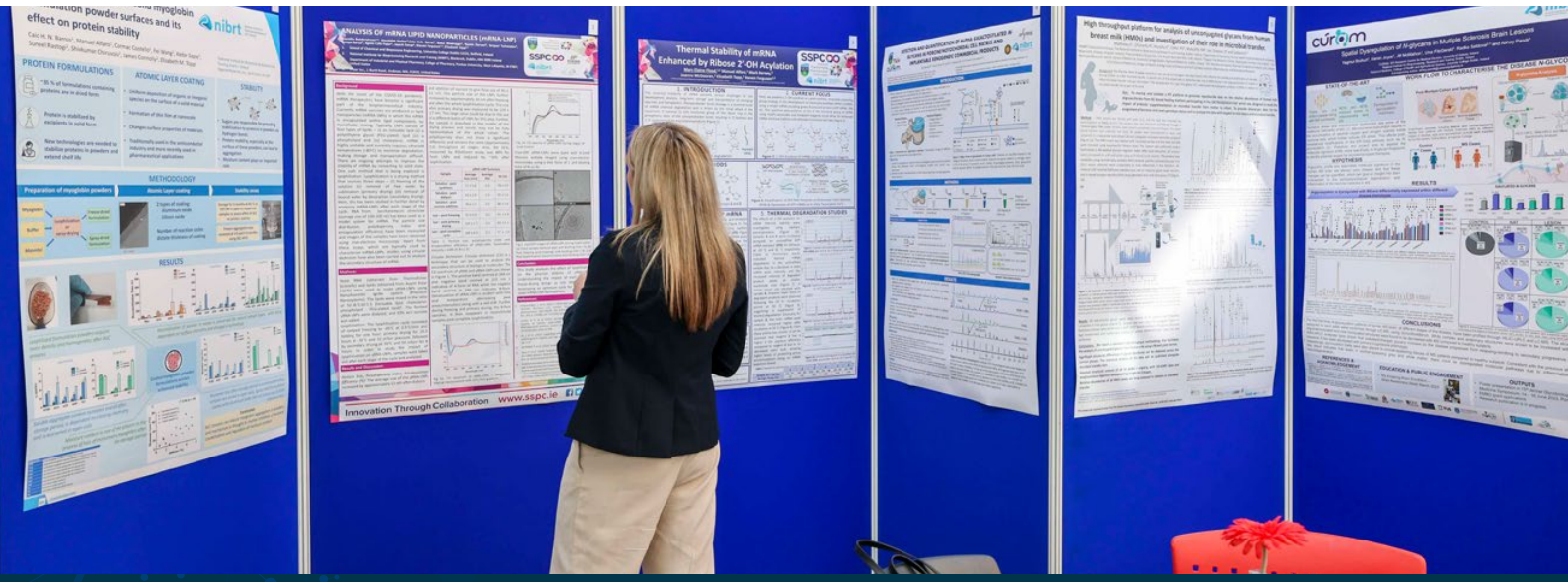
[4] *University of Natural Resources and Life Sciences, Vienna, Muthgasse 18, Vienna 1190, Austria*

[5] *Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA*

[6] *National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland*

*Electronic address: niall.barron@dcu.ie*

Recent sequencing of the Chinese hamster ovary (CHO) cell and Chinese hamster genomes has dramatically advanced our ability to understand the biology of these mammalian cell factories. In this study, we focus on the powerhouse of the CHO cell, the mitochondrion. Utilizing a high-resolution next generation sequencing approach we sequenced the Chinese hamster mitochondrial genome for the first time and surveyed the mutational landscape of CHO cell mitochondrial DNA (mtDNA). Depths of coverage ranging from ~3,319X to 8,056X enabled accurate identification of low frequency mutations (>1%), revealing that mtDNA heteroplasmy is widespread in CHO cells. A total of 197 variants at 130 individual nucleotide positions were identified across a panel of 22 cell lines with 81% of variants occurring at an allele frequency of between 1% and 99%. 89% of the heteroplasmic mutations identified were cell line specific with the majority of shared heteroplasmic SNPs and INDELs detected in clones from 2 cell line development projects originating from the same host cell line. The frequency of common predicted loss of function mutations varied significantly amongst the clones indicating that heteroplasmic mtDNA variation could lead to a continuous range of phenotypes and play a role in cell to cell, production run to production run and indeed clone to clone variation in CHO cell metabolism. Experiments that integrate mtDNA sequencing with metabolic flux analysis and metabolomics have the potential to improve cell line selection and enhance CHO cell metabolic phenotypes for biopharmaceutical manufacturing through rational mitochondrial genome engineering.



## Developing Mass Spectrometry Workflows for the Characterization of AAV Gene Therapy Products

**Felipe Guapo, Lisa Strasser, Josh Smith, Florian Füssl, Silvia Millán-Martín & Jonathan Bones**

*National Institute for Bioprocessing Research and Training*

Investigation into gene therapies based on viral vectors is rapidly growing due to their high transformational clinical potential. However, manufacturing and characterisation challenges remain due to their molecular size, complexity, and general low product yields. In NIBRT, we developed a complete downstream bioprocessing characterization workflow for AAV gene therapies utilizing state-of-the-art MS and LC-MS based approaches. Here we applied our novel analytical methods on an in house produced AAV5 expressed in SF9 cells providing a multi-level analysis ranging from the affinity purification to the monitoring of the main product and process critical quality attributes.

## Assessing Effects of Leachables and the Quality of Single-Use Systems in Cell Therapy Manufacturing

**Noemí Dorival-García & Jonathan Bones**

*National Institute for Bioprocessing Research and Training*

Cell therapies (CT) have demonstrated life-changing benefits and curative options to patients with unmet medical needs. Recent commercial successes and strong clinical responses are propelling additional CT products toward commercialization. However, manufacturing of CT products continues to create challenges. Extractables and leachables (E&L) are a significant concern for the CT industry, which relies exclusively on single-use systems (SUS). The impacts of SUS materials that encounter the cell based product are not well documented. Here, a proof-of-principle study is presented, demonstrating evidence of effects of leachates on T cells. Jurkat cells, a prototypical T cell line, were cultivated in media previously incubated in single-used bags (SUBs) utilized during incubation/expansion stages.

## Multi-attribute Method (MAM) in advancing innovative analytical strategies for biotherapeutics characterization

**Silvia Millán-Martín, Craig Jakes, Sara Carillo & Jonathan Bones**

*National Institute for Bioprocessing Research and Training*

The rapid growth of biotherapeutic industry with more and more complex molecules entering the market forces the need for advanced analytical platforms which can quickly and accurately identify and quantify product quality attributes. Mass spectrometry has the potential to provide more detailed information about the quality attributes of complex products, and MS methods are more sensitive than UV methods for detection of impurities. The multi-attribute method (MAM), a liquid chromatography-mass spectrometry based analytical approach is an emerging platform which supports biotherapeutics characterization and cGMP testing. The main advantage of MAM lies in the ability to monitor multiple quality attributes in a single assay, both at the peptide and the intact level, becoming a more streamlined application for biopharmaceutical production, from research and development to the QC environment. At NIBRT CCL we have developed MAM and iMAM approaches and demonstrated their applicability to monitor critical quality attributes (CQAs) for batch-to-batch comparison, host cell protein (HCP) and sequence variant analysis and to support analytical biosimilarity assessment.

## The Impact of N1-Methylpseudoiridine Bases on mRNA Digest Stability

**Craig Jakes, Maikel Gaitkoski, Felipe Guapo, Silvia Millán-Martín, Sara Carillo & Jonathan Bones**

*National Institute for Bioprocessing Research and Training*

mRNA based vaccines are beneficial due to their specificity, lack of infectivity and their lower cost of manufacturing. Traditionally they suffered from a lack of stability. However, the adaptation of modified bases and the use of lipid nanoparticles as a delivery system has helped overcome this limitation. As a biological product, there is a regulatory requirement to be fully characterised before vaccine administration. LC-MS is ideally suited for the characterisation of mRNA and the identification of its critical quality attributes. In this study we examine the effect incorporation of N1- methylpseudouridine instead of uracil on mRNA digestion using T1 endoribonuclease to generate an oligonucleotide mixture for LC-MS analysis. Close attention was paid on the determination of sequence coverage with respect to digestion time. Additionally, the ability to monitor other mRNA quality attributes, such as polyA tail distribution was examined.

## Characterising Biopharmaceuticals using Native Mass Spectrometry

**Florian Füssl, Sara Carillo, Craig Jakes, Lisa Strasser & Jonathan Bones**

*National Institute for Bioprocessing Research and Training*

Native mass spectrometry (MS) enables the characterization of biopharmaceutical proteins while preserving their native-like structure. Proteins can be analysed using minimal sample preparation and the evaluation of protein higher order structure becomes feasible. At NIBRT CCL, we have developed strategies for coupling native separations directly to native mass spectrometry. Our approaches exploit physicochemical properties such as size, charge or binding affinity of therapeutics of interest to obtain in-depth information on structure and molecular heterogeneity.

## ABSTRACTS

# Loss of Cell Viability is Tracked by Decreased Cytoplasmic Conductivity

**Laura Breen[1], James Flynn[1], Adam Bergin[1], Evangelia Flampouri[1], Elham Salimi[2], Michael Butler[1]**

*[1] National Institute for Bioprocessing Research and Training (NIBRT), Foster Avenue, Dublin, Ireland.*

*[2] Department of Electrical and Computer Engineering, University of Manitoba, Winnipeg, Canada.*

**Presented at Cell Culture Engineering XVIII, Cancun, Mexico, April 2023**

Cell viability has been consistently measured by off-line analysis using the Trypan Blue exclusion assay. Although this has been the gold standard for monitoring cell growth in bioprocesses the limitations are well documented. At the stage that the cells are positive for trypan blue the membrane is damaged, host cell proteins have leaked out and the ability for cell division has long past. The demise of cells can reliably be determined at an earlier time point using biocapacitance. Information on the properties of cells, beyond simple biomass data, can be effectively drawn by modelling the cytoplasmic conductivity($\sigma$i) on the Schwan equation. A recognisable change in cytoplasmic conductivity becomes apparent well before the breakdown of cell membrane integrity. This study comprises three independent bioreactor cultures of the cell line CHO-EG2 grown in Biogro medium in 1L bench-top bioreactors. In-line biocapacitance measurements were recorded for the entire duration and off-line samples were taken daily for trypan blue measurement.

Our results show that capacitance measurements allow for the determination of earlier inflection points in the deterioration of cell health than traditional trypan blue staining. The non-invasive methods described here propose a potential disruptive technology for profiling events associated with the loss of cell viability by modelling the cytoplasmic conductivity.

**References**

(1) Opel, C.F., Li, J. and Amanullah, A. (2010). Biotechnol Progress, 26: 1187-1199

(2) Fazelkhah, A. et al. (2019). Biotechnology and Bioengineering, 116(11), pp. 2896–2905

# Construction of InstantPC-derivatized N-glycan GU database for high-throughput and high sensitivity glycosylation profiling

**Yongjing Xie and Michael Butler**

*National Institute for Bioprocessing Research and Training, Foster Avenue, Mount Merrion, Blackrock, Co. Dublin, Ireland*

**Presented at Bioprocessing Summit Europe, Barcelona, Spain, March 2022**

Glycosylation is well-recognized as a critical quality attribute (CQA) of biotherapeutics being routinely monitored to ensure desired product quality, safety, and efficacy [1]. Additionally, as one of the most prominent post-translational modifications, glycosylation plays a key role in disease manifestation, and changes in glycosylation may serve as a specific and sensitive biomarker for disease diagnostics and prognostics [2]. However, glycans are highly heterogeneous, which has considerably hampered the progress of glycomics. Here, we present an innovative streamlined 96-well-plate-based platform utilizing Agilent AdvanceBio InstantPC label for high-throughput and high-sensitivity glycosylation profiling. This approach offers consistent identification and quantification of diverse N-glycans from various sources, which is rapid, reliable, and ready for automation [3]. However, the limited availability of InstantPC labelled N-glycan standards has significantly hampered the applicability and transferability of this platform for expedited glycan structural profiling. To mitigate this challenge, we have constructed a detailed InstantPC labelled glycan glucose unit (GU) database for hydrophilic interaction liquid chromatography with fluorescence detection (HILIC-FLD) separation and analysis. The complete workflow is presented, which includes innovative 2D liquid chromatography for glycan fraction and separation and Hydrophilic interaction ultra-performance liquid chromatography coupled with electrospray ionization mass spectrometry (HILIC-ESI-MS) for glycan fragmentation and accurate glycan mass confirmation [4]. The constructed InstantPC glycan GU database is accurate and robust. It is believed that this database will enhance the application of the developed platform for high-throughput, high-sensitivity N-glycan profiling, and eventually advance glycan-based biopharmaceutical production and biomedical research. Furthermore, derivatization mechanism, experimental workflow, and how to avoid potential pitfalls to ensure good reproducibility is discussed as well.

## References

(1) Delobel, A. Glycosylation of Therapeutic Proteins: A Critical Quality Attribute. Methods Mol Biol 2021, 2271, 1-21. DOI: 10.1007/978-1-0716-1241-5_1.

(2) Reily, C.; Stewart, T. J.; Renfrow, M. B.; Novak, J. Glycosylation in health and disease. Nat Rev Nephrol 2019, 15 (6), 346-366. DOI: 10.1038/s41581-019-0129-4.

(3) Xie, Y.; Mota, L. M.; Bergin, A.; O'Flaherty, R.; Jones, A.; Morgan, B.; Butler, M. High-throughput and high-sensitivity N-Glycan profiling: A platform for biopharmaceutical development and disease biomarker discovery. Anal Biochem 2021, 623, 114205. DOI: 10.1016/j.ab.2021.114205.

(4) Xie, Y.; Butler, M. Construction of InstantPC derivatized glycan GU database: A foundation work for high-throughput and high-sensitivity glycomic analysis. Glycobiology 2021. DOI: 10.1093/glycob/cwab128.

# Analysis of Plant-Based Protein Hydrolysates for BioPharma by ICP-MS

**Thais McNamara[1], Sandra Roche[1], Michael Butler[1,2]**

*[1]National Institute for Bioprocessing Research & Training, Dublin*

*[2]School of Chemical and Bioprocess Engineering, University College Dublin (UCD), Belfield, Dublin 4, Ireland.*

**Presented at the Irish Mass Spectrometry Society (IMSS), Dublin, May 2023**

Protein hydrolysates (HL) are produced by the controlled chemical or enzymatic breakdown of raw materials. These HLs have been well used over the last decades as media supplements because they are a valuable source of biologically active components and provide optimal cell growth and increased recombinant protein production. The challenge is that HLs are naturally heterogeneous in composition. Therefore, to be used as media supplements for potential therapies there is an increasing interest to develop the HLs as more defined and increase reproducibility. Inductively coupled plasma mass spectrometry (ICP-MS) is a precise analytical technique frequently used for determining trace element concentrations in biological materials. In this work, ICP-MS was used to detect and quantify the trace elements present in a variety of HLs, providing useful information for building bioactivity profiles.

The samples were prepared through a process of acid digestion, dilution, and filtration, making it a quick and cost-effective method. Calibration curves were used in the ICP-MS data analysis to determine the instrument response to the analyte concentration in the sample. ICP-MS revealed the range of trace metals detected in HLs derived from meat, wheat, soy, and cotton. The trace element content of each hydrolysate was clearly different, revealing unique features potentially contributing to unique bioactivity profiles. Principal component analysis (PCA) of the HLs' trace metal profile revealed clustering that indicates a distinct separation based on their origin, suggesting that the trace metal profile is unique to source and consistent throughout each batch of HL.

Overall, the use of ICP-MS for trace element analysis in HLs can contribute to our understanding of the biological roles of trace elements and may have implications for the development of xenogene free media supplements. To further explore the impact of trace metals on CHO cells, we will examine a range of trace metal cocktails, corresponding to the derived HL profiles, to determine the most bio-active mixture. The uptake and accumulation of trace metals in CHO cells will be investigated using ICP-MS to correlate uptake and accumulation with bioactivity, cell growth, and IgG production.

## References

(1) Yao, T.; Asayama, Y. Animal-Cell Culture Media: History, Characteristics, and Current Issues. Reprod. Med. Biol. 2017, 16 (2), 99–117. https://doi.org/10.1002/rmb2.12024.

(2) Nasri, M. Chapter Four - Protein Hydrolysates and Biopeptides: Production, Biological Activities, and Applications in Foods and Health Benefits. A Review; Toldrá, F., Ed.; Advances in Food and Nutrition Research; Academic Press, 2017; Vol. 81, pp 109–159. https://doi.org/10.1016/bs.afnr.2016.10.003.

(3) Butler, M. Serum-Free Media: Standardizing Cell Culture System. Pharm. Bioprocess. 2013, 1, 315–318.

(4) Luo, Y.; Pierce, K. M. Development toward Rapid and Efficient Screening for High Performance Hydrolysate Lots in a Recombinant Monoclonal Antibody Manufacturing Process. Biotechnol. Prog. 2012, 28 (4), 1061–1068. https://doi.org/10.1002/btpr.1568.

(5) Obaidi, I.; Mota, L. M.; Quigley, A.; Butler, M. The Role of Protein Hydrolysates in Prolonging Viability and Enhancing Antibody Production of CHO Cells. Appl. Microbiol. Biotechnol. 2021, 105 (8), 3115–3129. https://doi.org/10.1007/s00253-021-11244-8.

nibrt

National Institute for
Bioprocessing Research
and Training

PM GROUP

BPE BIOPHARMA ENGINEERING

BUILDERS SISK CONTRACTORS Established 1859