

# Innovations Enabling the Development of Intensified Processes for RNA-LNPs

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Technology Lead - Biologics



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**We help companies to  
develop, prove, scale-up  
and commercialise new  
products and processes**



# CPI Pharma - Biologics

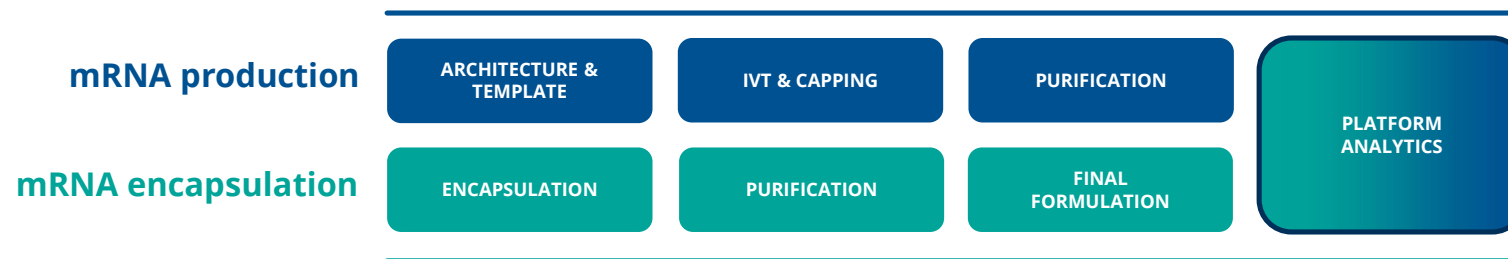
## National Biologics Manufacturing Centre

1. £38m **state-of-the art**, 5,000m<sup>2</sup> facility in Darlington, UK.
2. **13 flexible laboratories** designed to handle BSL1 and BSL2 organisms
3. **ISO 9001 certified scale-up**, with **approved GMP facility** for RNA-LNPs
4. Comprehensive expertise in **cell-free**, **microbial** and **mammalian** expression systems for the production of a wide range of products including:
  - Monoclonal antibodies
  - Viral vectors
  - mRNA
  - pDNA
  - Recombinant proteins
  - Microbial therapeutics
  - Antibody-drug conjugates
  - Nanomedicines

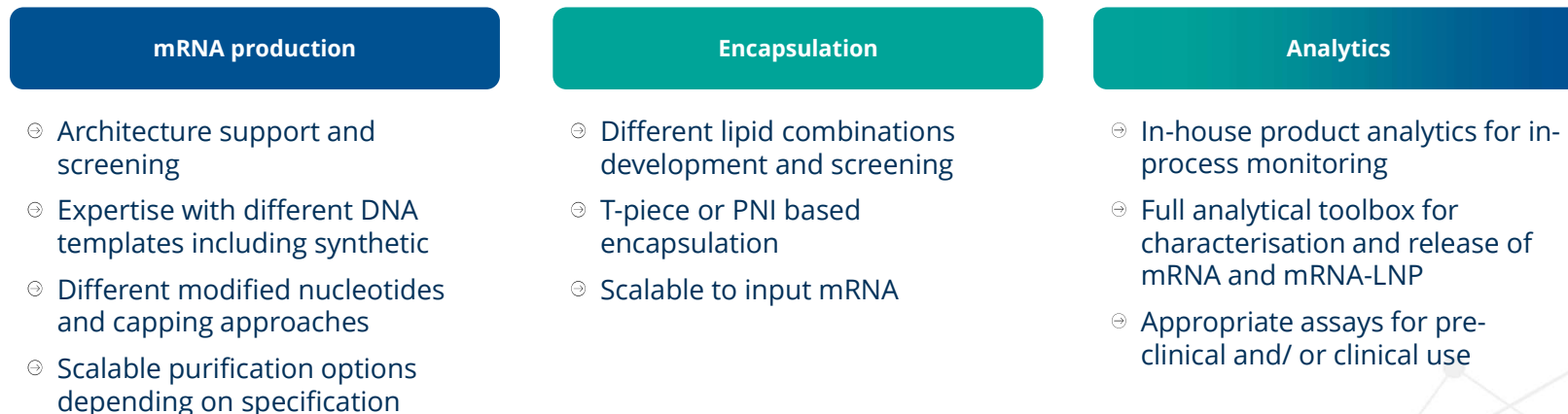


# mRNA offering at CPI's RNA Centre of Excellence

⊖ Scalable platform for  $\mu\text{g}$   $\rightarrow$  g manufacture of mRNA and saRNA LNPs



⊖ Material production for pre-clinical or clinical applications



# Intracellular Drug Delivery Centre (IDDC)

- Centre of Excellence for companies/academics to access state of the art capabilities and expert support
- Design, formulation, characterisation and manufacture of nano-delivery systems (NDS) for multiple payloads and targets
- R&D programmes to solve challenges & flexible industry engagement model

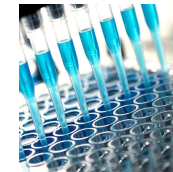
## CPI are leading this multi-partner programme



## We are addressing critical scientific and commercial challenges

in vitro models
Thermostability and alternative delivery routes
Payload transport and release in the body
Delivery beyond the liver
Prediction of immunogenicity, safety and adverse reactions
Unclear pathways to product registration
Supply chains and manufacturing
Complex IP landscape and high licensing costs

## R&D and business support work packages



LNP formulation screening and characterisation

Enhanced methods to enable in vitro -in vivo correlation

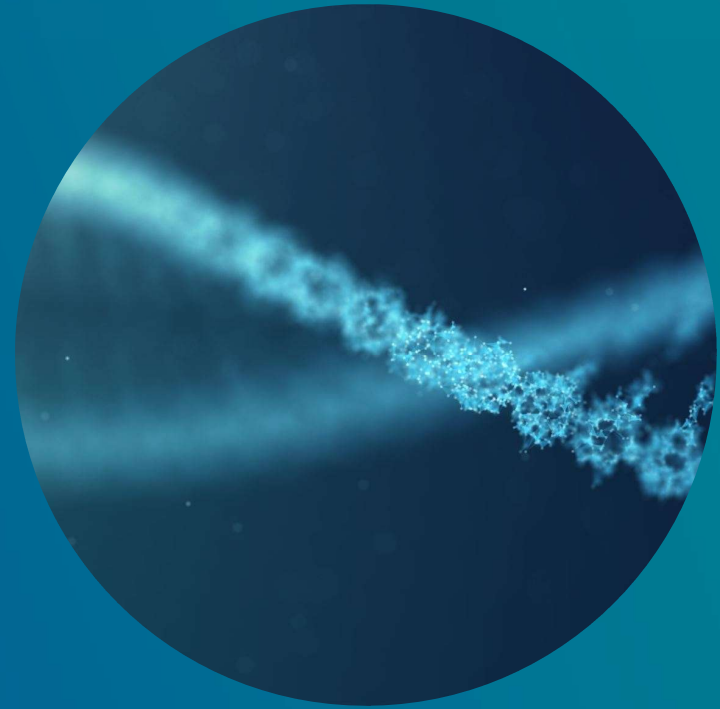


LNP digital manufacturing

Supporting the ecosystem



# mRNA technology and manufacture

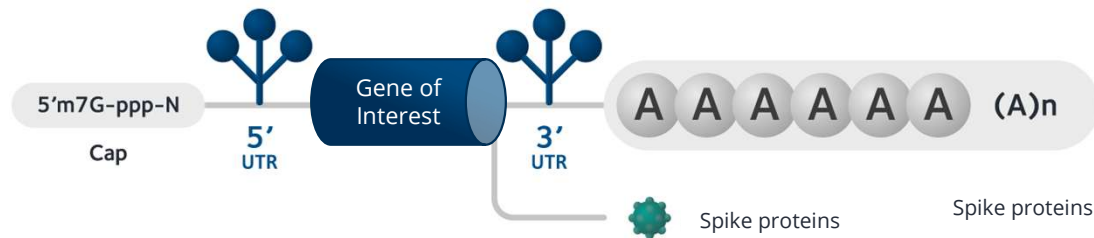


Let's innovate together  
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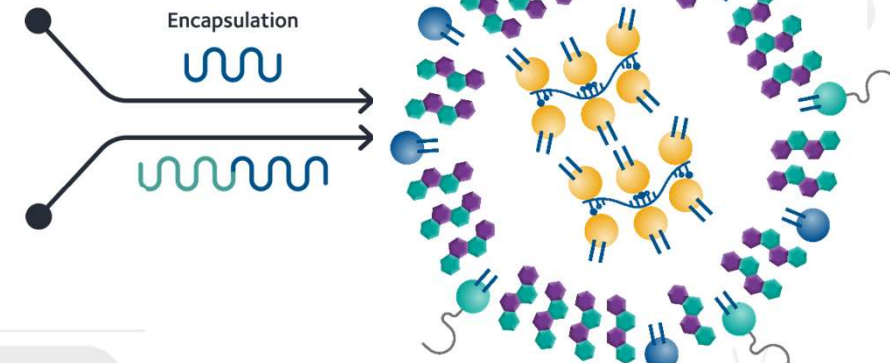


# RNA technology

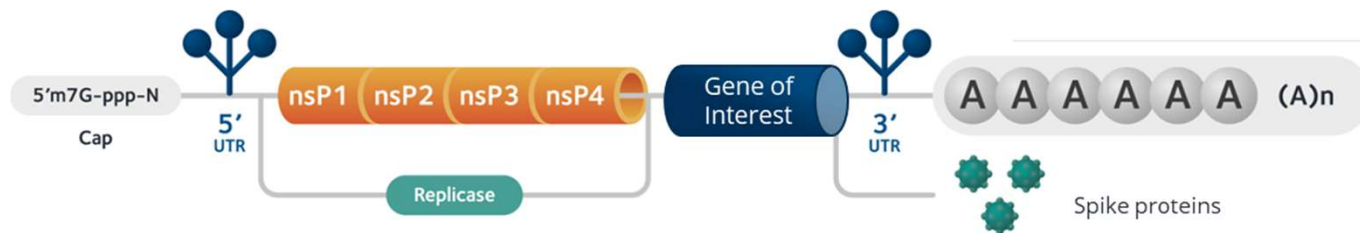
## Conventional non-amplifying mRNA



## mRNA vaccine nanoparticle



## Self amplifying mRNA (Replicon)



# Synthetic production – Enzymes, DNA template + LNPs = mRNA-LNPs



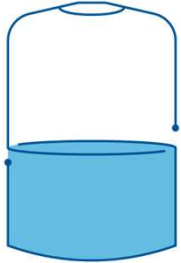
DNA template



T7 RNA polymerase



Additional enzymes



Nucleotide triphosphates (NTPs)



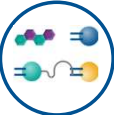
Capping agent



Buffer components



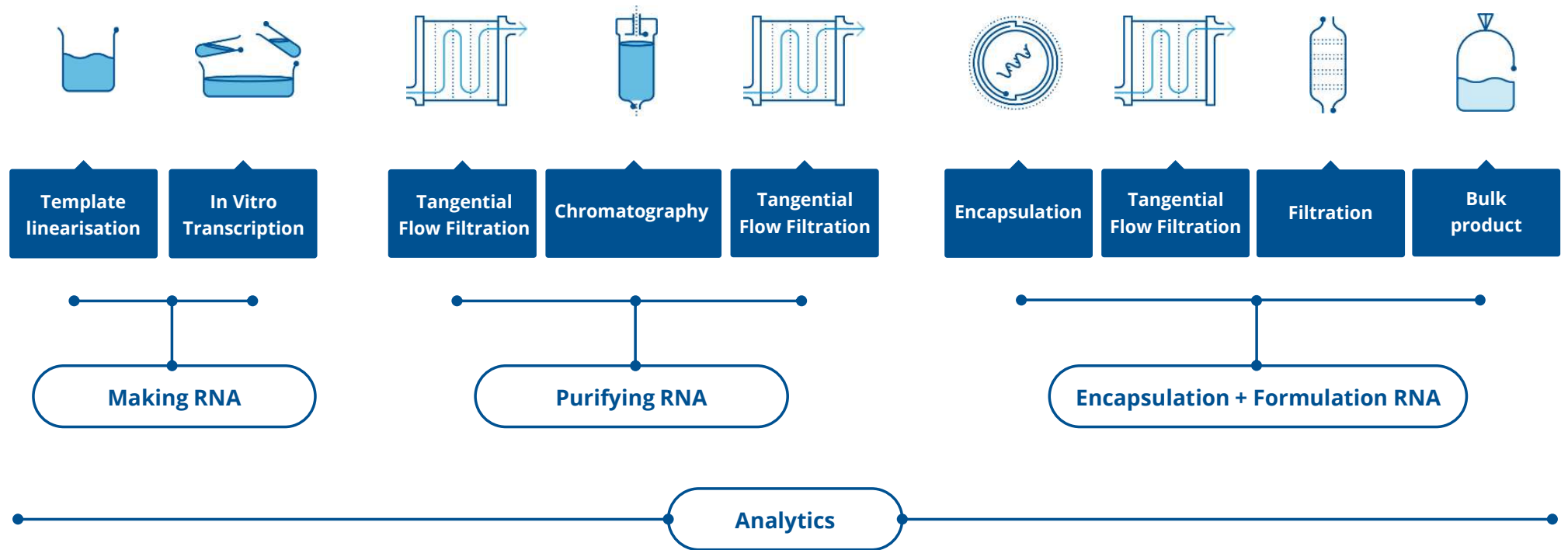
Lipids



25,000 doses/ L at 100µg  
Production scale <100L

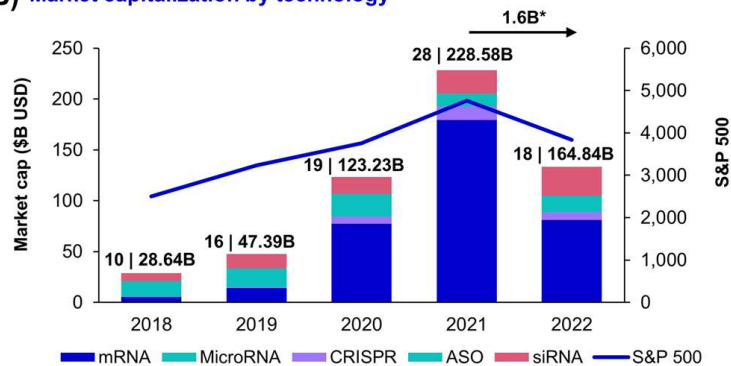


# mRNA-LNP Platform Overview

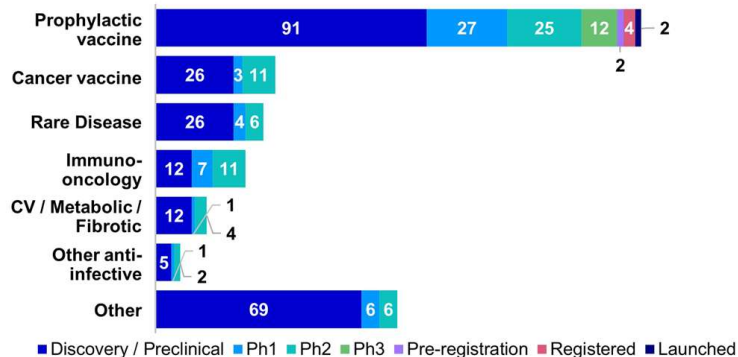


# Intensification driver - Increasing demand, & CoGs

## B) Market capitalization by technology



## D) 2023 mRNA pipeline activity by therapeutic area



Jones, C.H., Androsavich, J.R., So, N. et al. Breaking the mold with RNA—a “RNAissance” of life science. npj Genom. Med. 9, 2 (2024). <https://doi.org/10.1038/s41525-023-00387-4>

**Table 1.** Estimated total annual cost needed to start up mRNA vaccine manufacturing in injectable medicines facilities and produce 100 million doses of mRNA vaccines

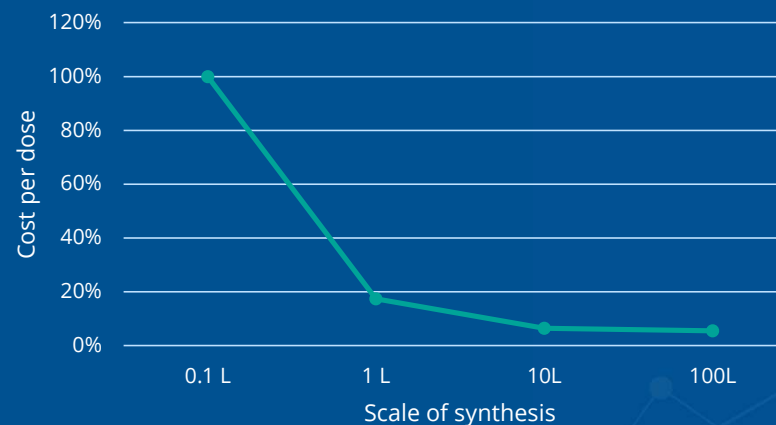
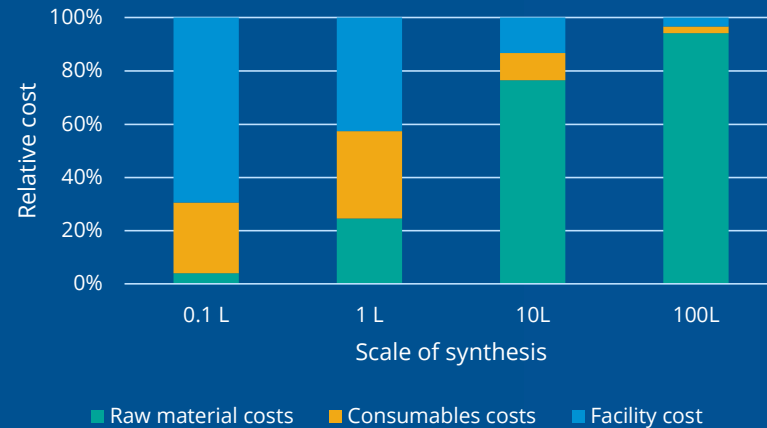
Cost category	Costs for starting up and producing 100 million mRNA-1273 doses	Costs for starting up and producing 100 million BNT162b2 doses
Total operating costs for drug substance <sup>a</sup>	\$241.6 million	\$88.1 million
Facility-related operating costs <sup>b</sup>	\$4.3 million	\$2.5 million
Consumables and single-use equipment costs	\$59.5 million	\$17.5 million
<b>Total costs of raw materials</b>	<b>\$162.9 million</b>	<b>\$54.2 million</b>
Total costs of labour	\$12.7 million	\$11.9 million
Total operating fill and finish costs for producing 100 million doses <sup>c</sup>	\$28.4 million	\$39 million
<b>TOTAL</b>	<b>\$270 million</b>	<b>\$127.1 million</b>

Process-cost modelling for producing 100 million COVID19 mRNA vaccine doses per year at injectable medicines manufacturing sites, MSF Access 2021

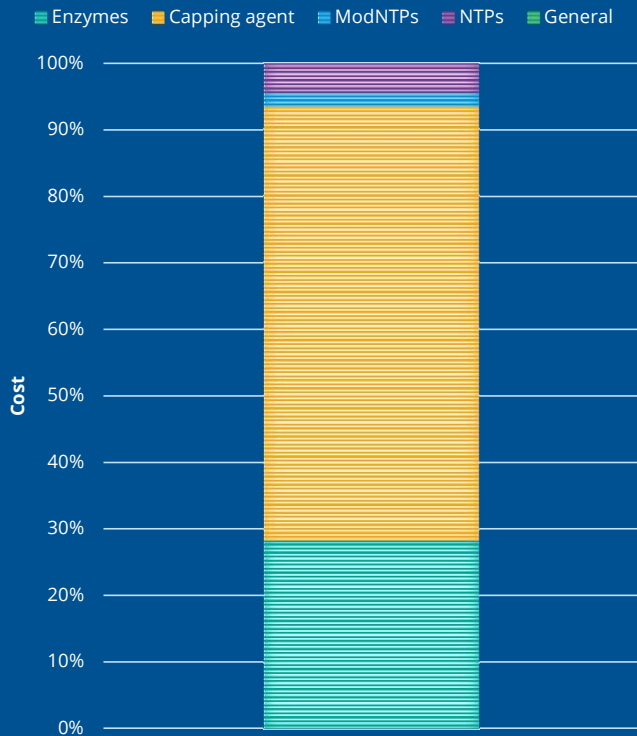
- To date limited products on market worldwide, and all for treatment of infectious diseases
- Increasing numbers of products entering trial covering broader application, therapeutic vaccines & therapeutic products
- Cost of raw materials a key driver

## Intensification driver – dose size

- Infectious disease vaccines <100ug
- Therapeutic vaccines <10mg
- Enzyme replacement & *in-vivo* expressed therapeutics >10mg (potentially over 100mg in some cases)
- All driving increased demand for mRNA-LNPs

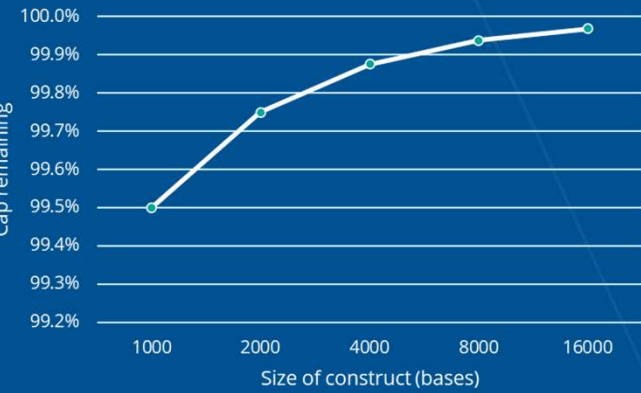
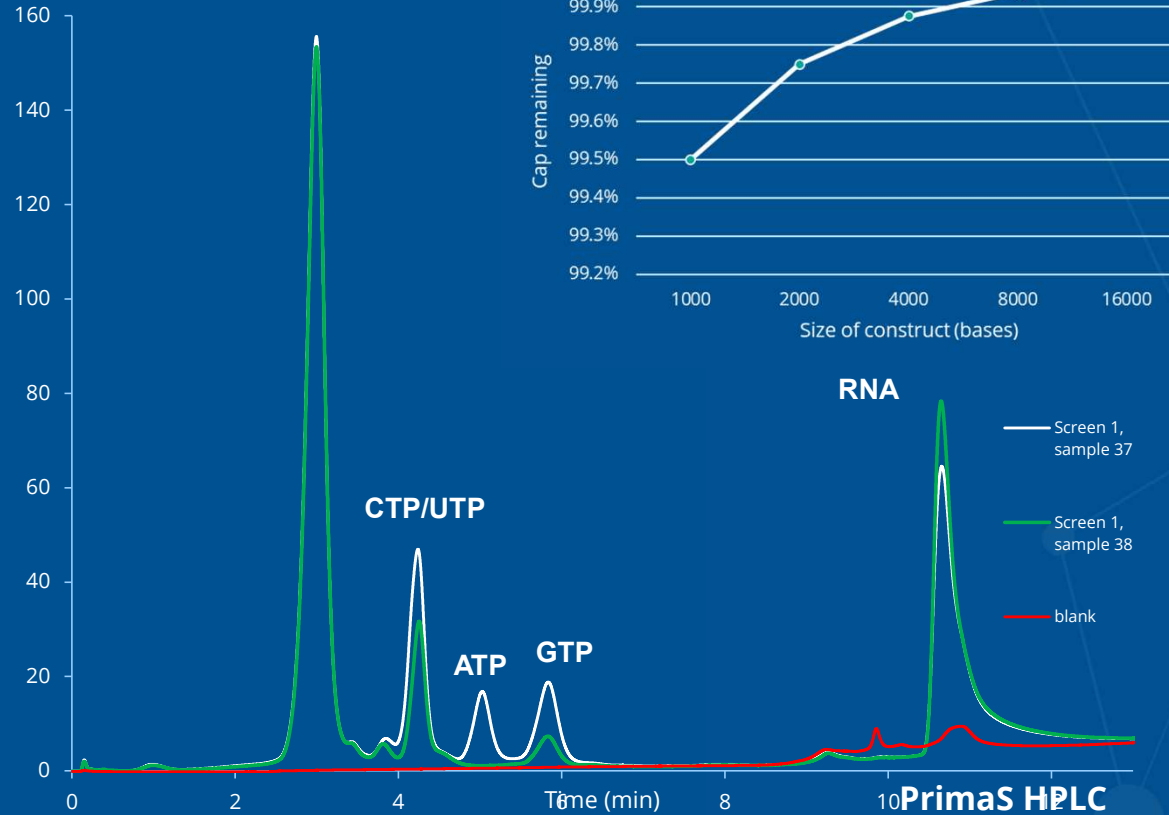


### IVT COST BREAKDOWN



### Cleancap

Absorbance (mAu)



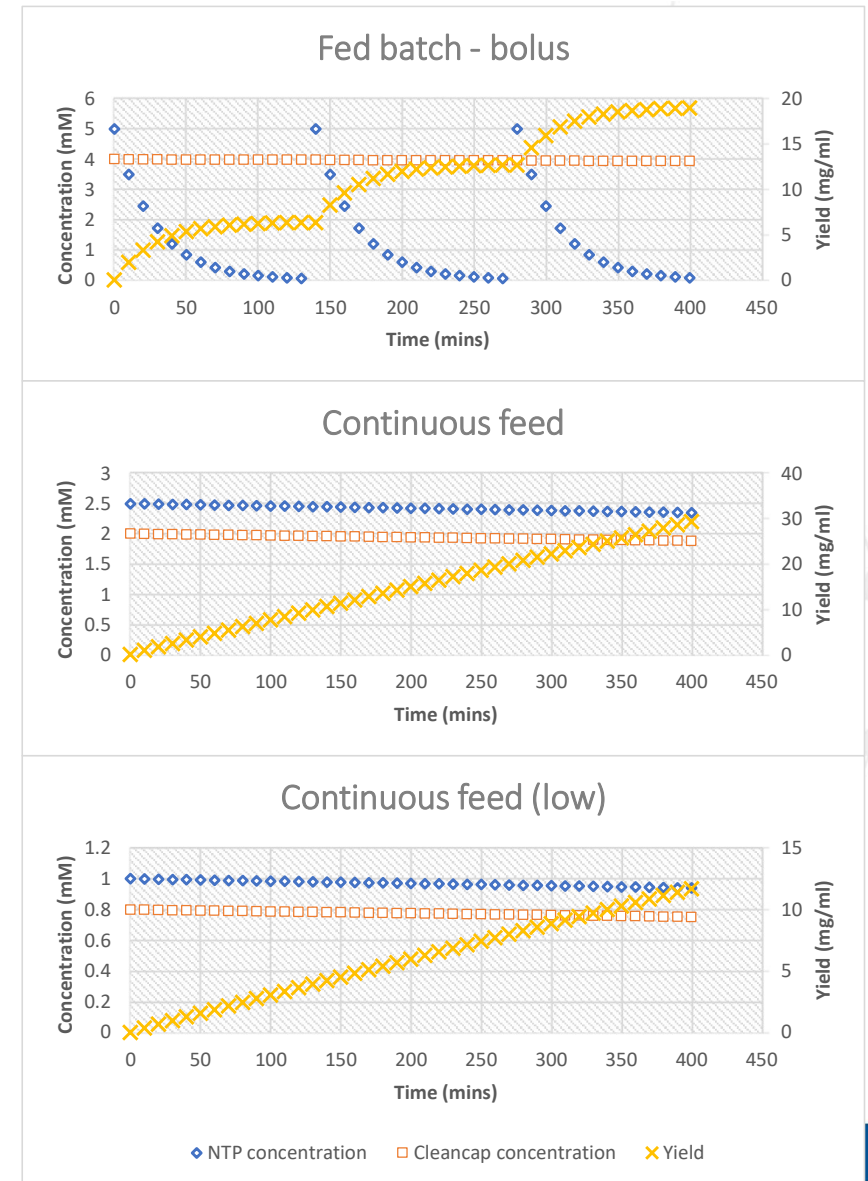
## Material (in)efficiency

- Core cost driver in reactions currently co-transcriptional cap, of which the majority is unused
- T7 polymerase & DNA template cost generally next most expensive items – key opportunities to improve cost (& sustainability) of products

# Reaction feeding – Beyond a supra-batch

- High performance batch reactions can be achieved through use of greater concentration of NTPs (+Mg<sup>2+</sup>), however need to keep ratio NTP : Cap means reduced efficiency of cap usage.
  - At higher NTP concentration, need to adjust other raw materials with additional cost implications
- More efficient to control reaction feed at lower overall concentration, through without removal of orthophosphate reaction will plateau

Starting NTP concentration (mM)	Theoretical yield (mg/ml)	Cleancap utilised
5	6.3	1.99%
10	12.6	0.99%
20	25.2	0.49%

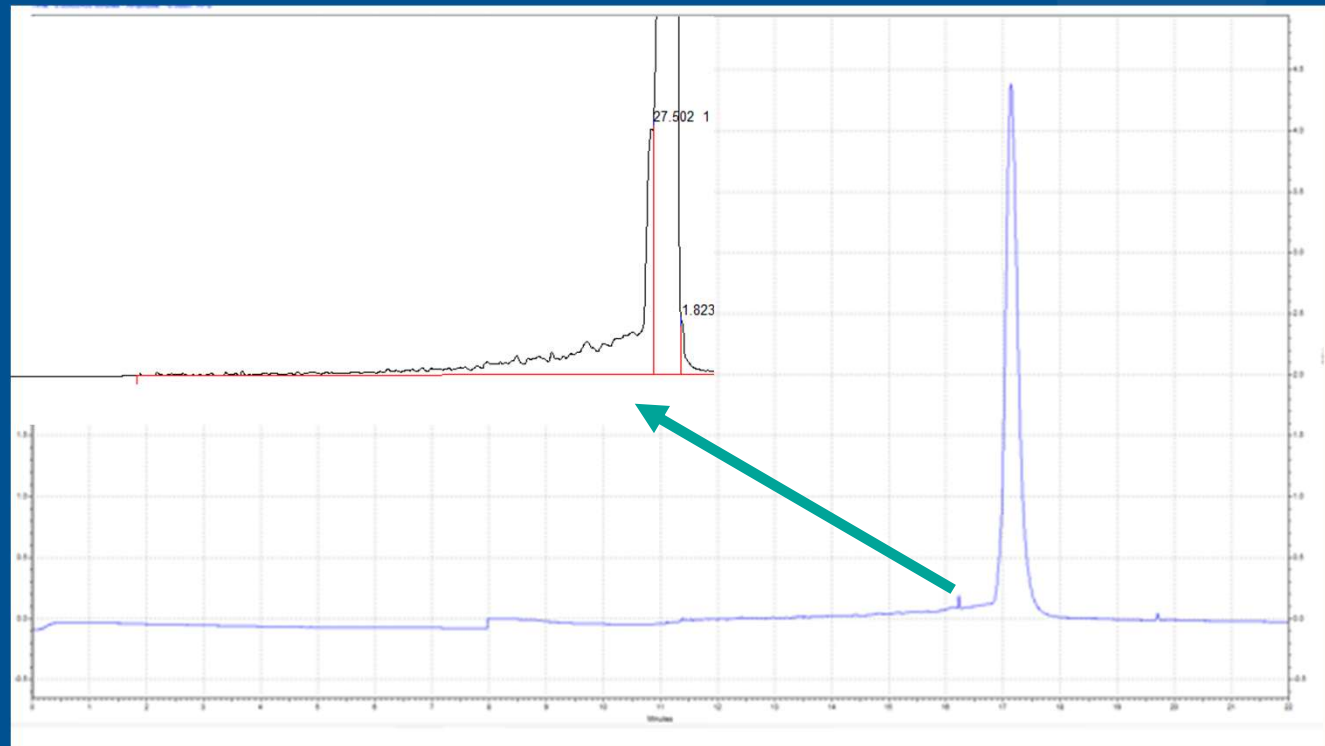


# Product quality

mRNA highly labile from chemical and RNase catalysed degradation

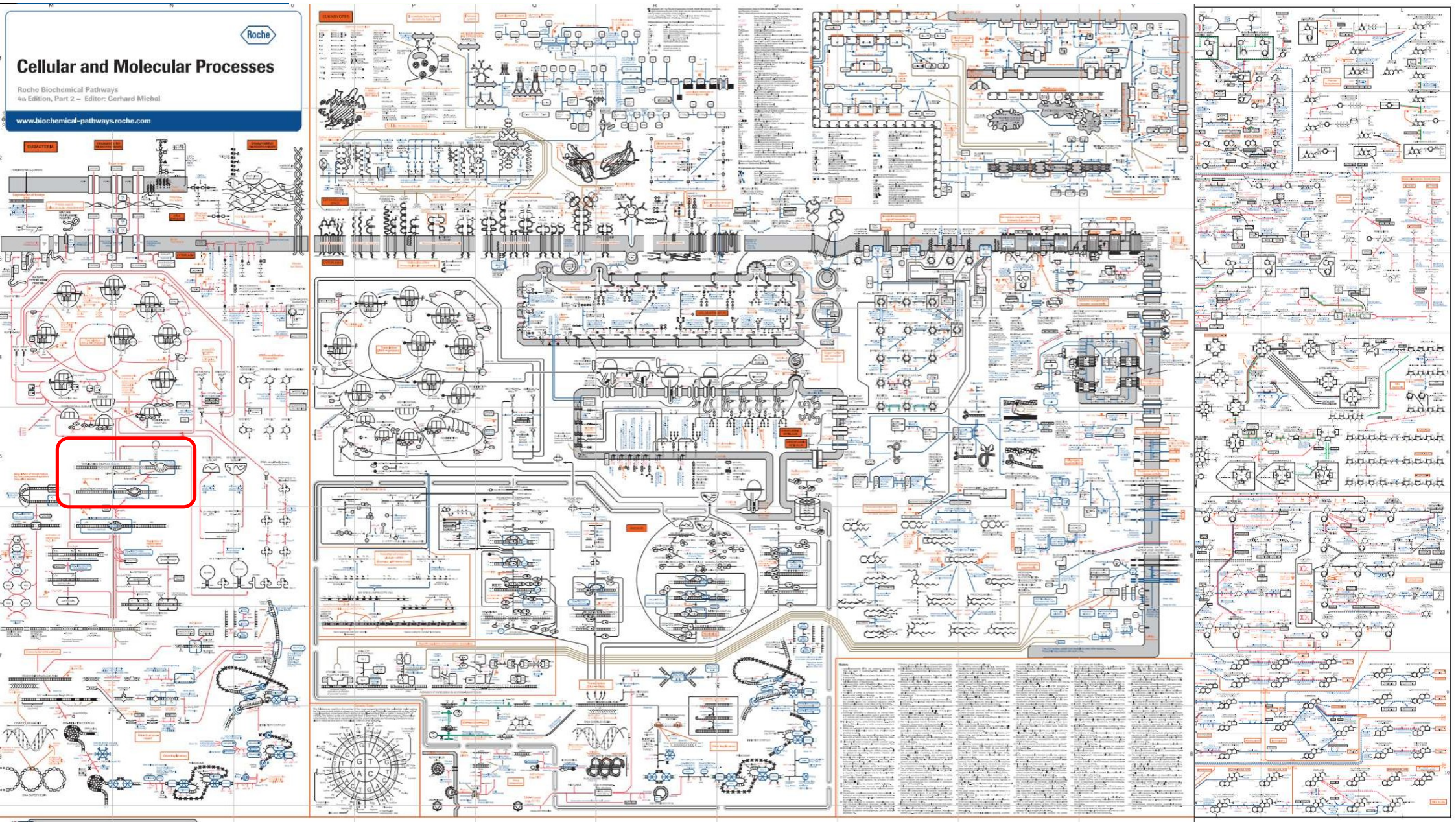
Though no significant degradation encountered in standard process, for therapeutic mRNA products efficacy critical and integrity a key determinant

IVT reactions relatively short, with longer reactions driving reductions leading to some reduction in quality



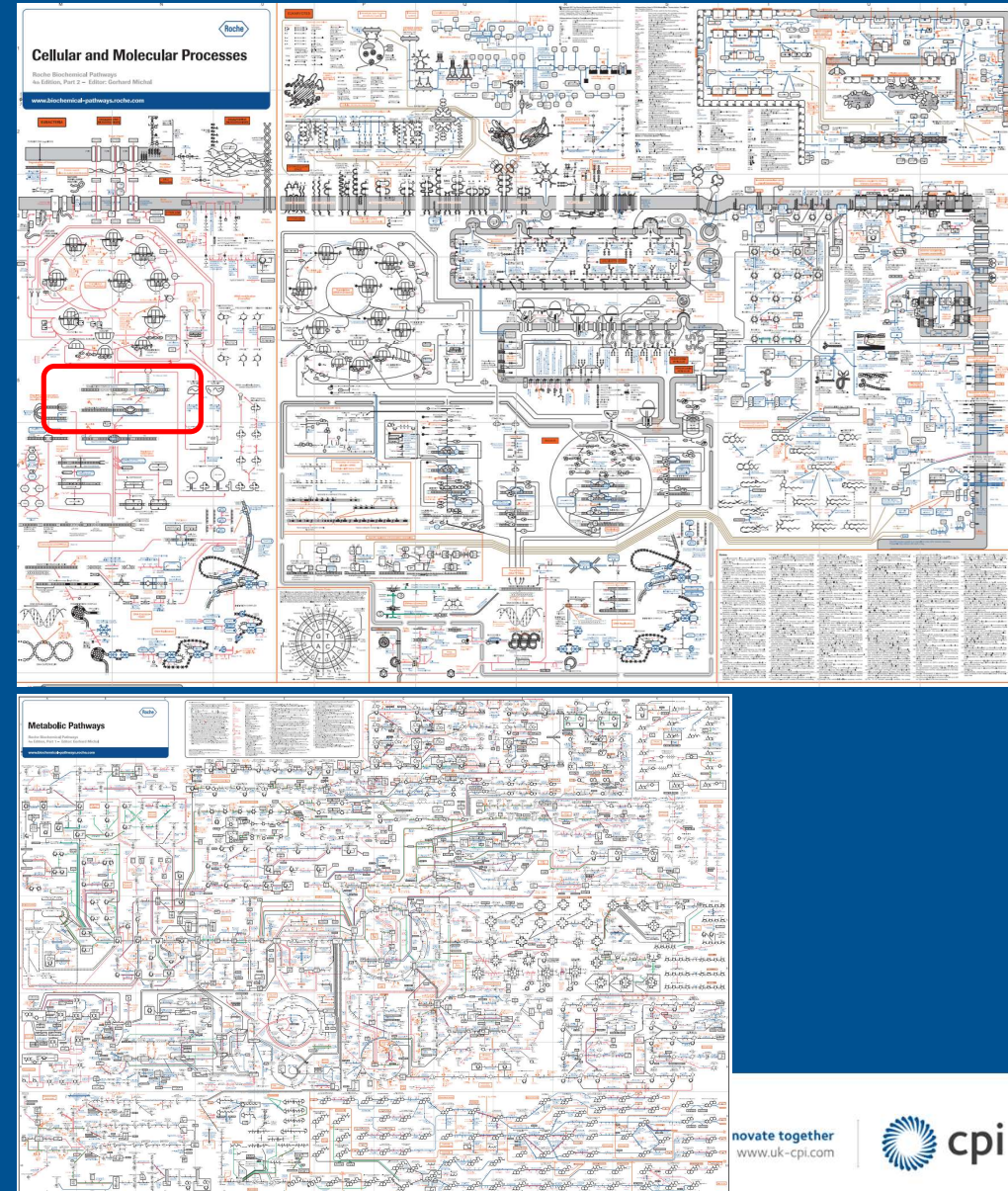
# Cellular and Molecular Processes

Roche Biochemical Pathways  
4th Edition, Part 2 – Editor: Gerhard Michal  
[www.biochemical-pathways.roche.com](http://www.biochemical-pathways.roche.com)



# Opportunity - Complexity

- Intensification aided by process understanding & models
- Upstream component significantly less complex than cell systems, with single enzymatic reaction responsible for synthesis
- No post translational modifications



# Opportunity

## Cost

- Maximising use of high value raw materials, particularly at *in-vitro* transcription stage, to enable significant reduction in cost per dose, particularly at scales >100ml

## Quality

- Faster processing of mRNA and LNP – reduced opportunity for degradation

## Risk

- Scale down of reaction – reduced risk owing to high costs in larger vessels
- High volumetric dose and fast reaction kinetics mean limited need for long continuous processes, thereby reducing risk

## Complexity

- Ability to model reactions owing to relatively reduced levels of complexity vs cell based systems

# Biologics process intensification – Continuous E2E mAb production

## PROJECT AIM



Develop a continuous biologics manufacturing testbed with integrated advanced process analytics and control to allow predictive real-time analysis of critical process parameters and critical quality attributes



### Progress



From Apr 2021  
To Jan 2024

### Funding

**£3.1m** Innovate UK

£2.1m  
Grant

£1.0 m  
Industrial Match

# Project UK Continuous 2

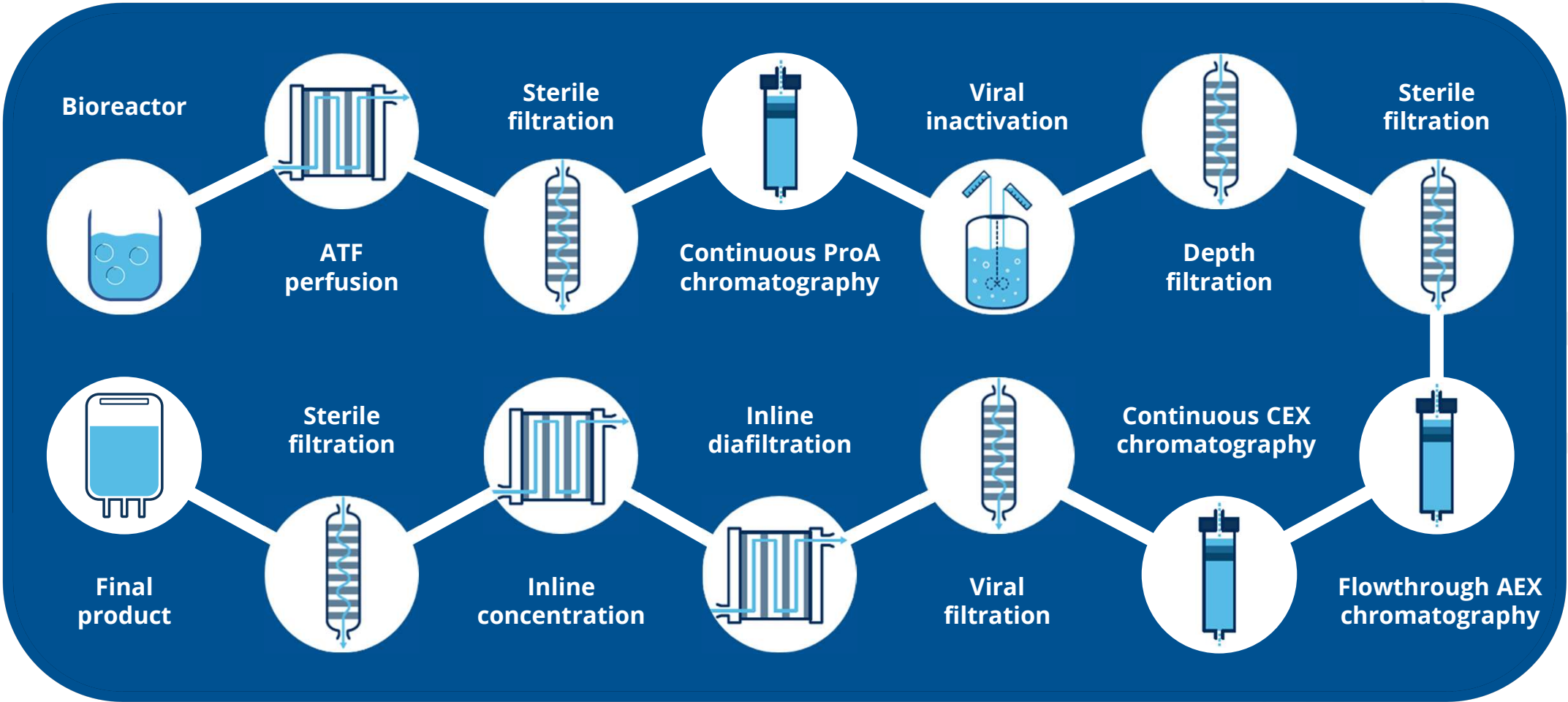
## Integrating Continuous Technologies For Rapid Delivery of Cost Effective Biotherapeutics to Patients

- Create a prototype, fully integrated continuous bioprocessing system and demonstrate operational functionality by performing continuous process runs for monoclonal antibody production.
- Use integrated PAT to measure and enable control over critical process parameters.
- Demonstrate automated control and predictive modelling on the continuous production system by producing models based on collected data.
- Compare facility outputs, cost breakdown, sustainability metrics, facility footprint and investment risk to existing batch processes.



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# CPI's Continuous Process Development Platform for mAbs

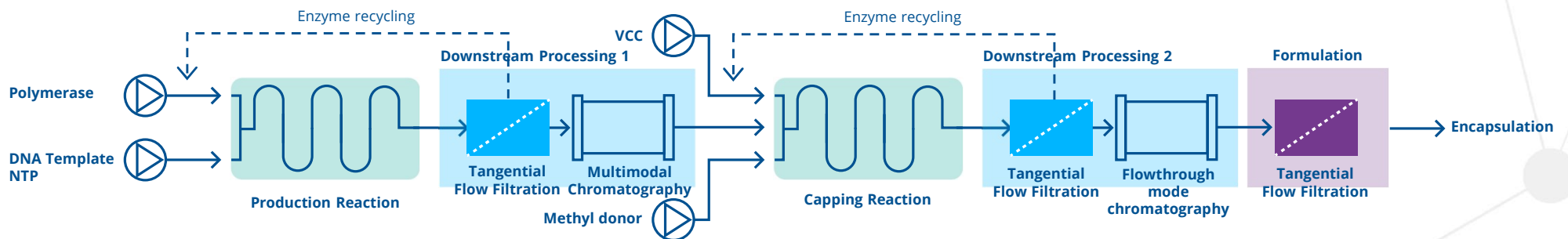


- Enabled by smart surge tanks, robust modelling & control, vendor collaboration & novel technology (e.g. SPTFF, MIR)

# mRNA-LNP process intensification

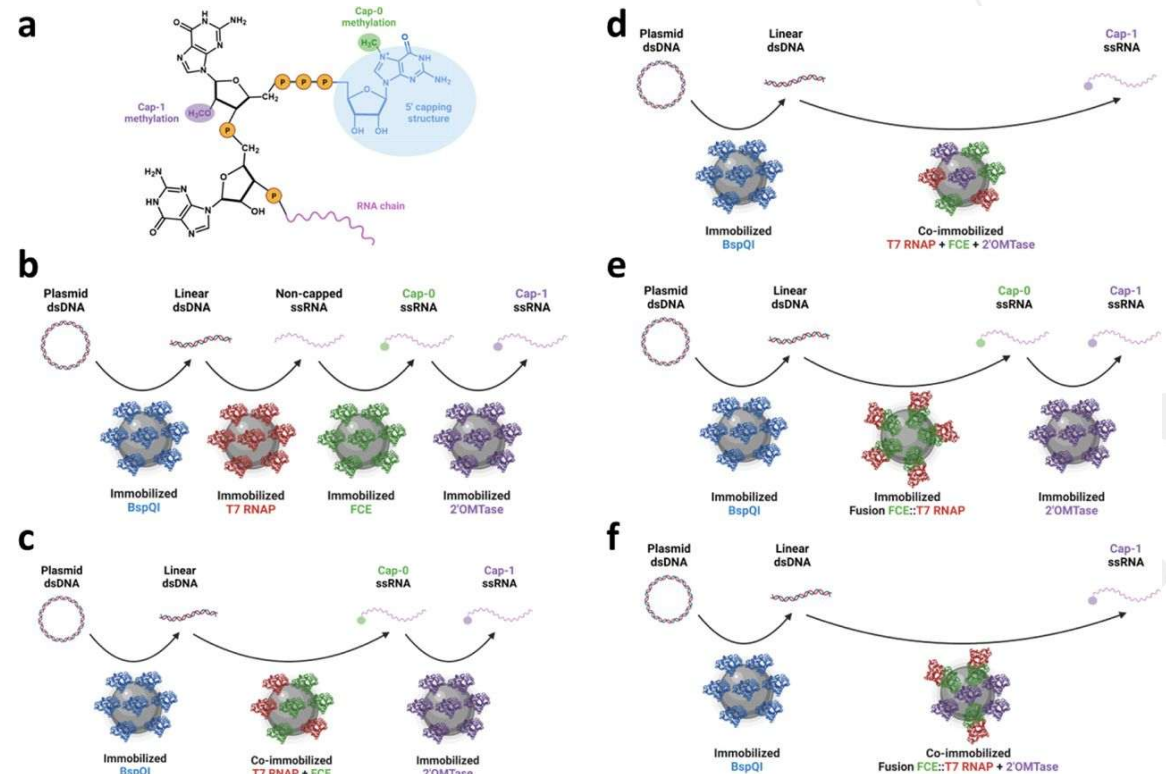
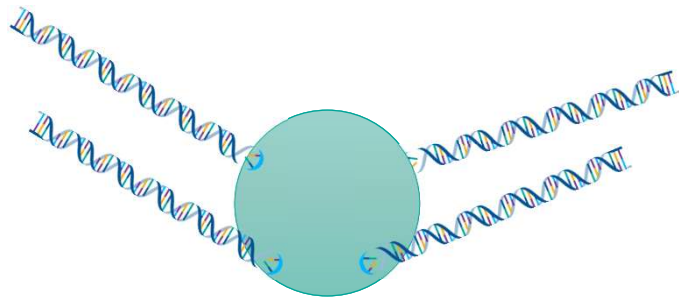
# Intensification approaches – Continuous?

- Continuous processing applies well to mRNA based processes owing to innovations and expertise built in Biologics processing to date
- Example below - composed of a two-step enzymatic reaction, followed by enzyme recycling using tangential flow filtration (TFF), and two multimodal chromatography steps
- The first chromatography step would act in bind and elute mode for the intermediate purification and the second in flowthrough mode as a polishing step
- Limitation through **DNA template loss**, and enzymatic capping necessitates additional unit operations



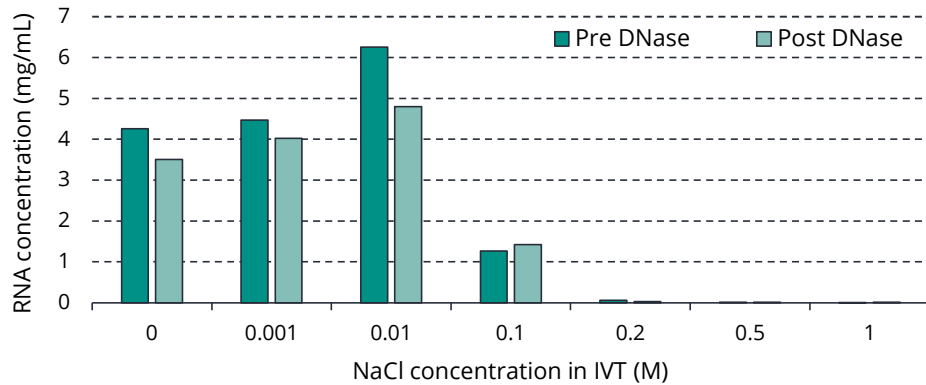
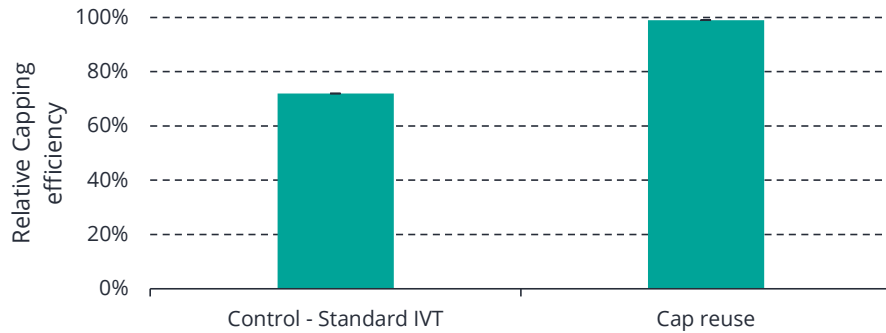
# Immobilisation

- Ability to immobilise nucleic acids and proteins to a solid scaffold (typically beads) well understood and demonstrated
- Gives greater flexibility in system design, and ability to separate RNA & DNA in reaction



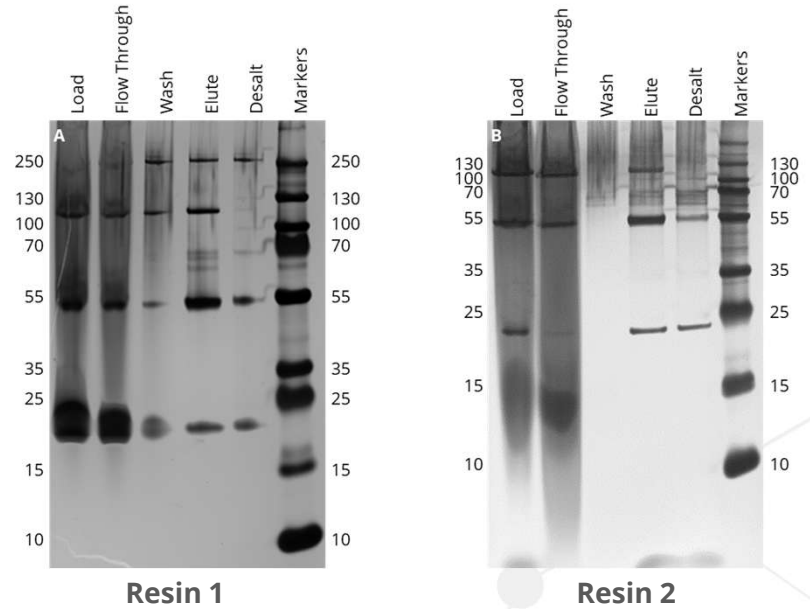
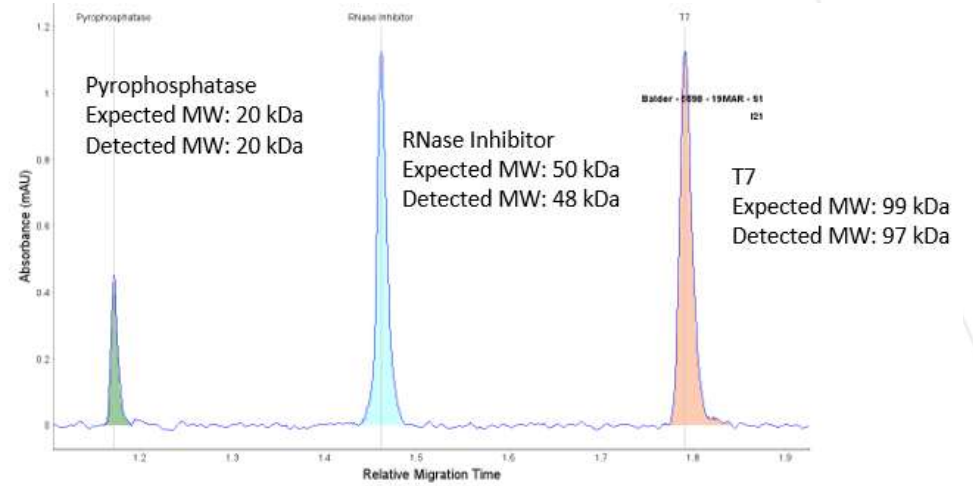
Streamlined DNA template preparation and co-transcriptional 5' capped RNA synthesis enabled by solid-phase catalysis, *bioRxiv*, 2024  
 Guillermo García-Marquina, Aihua Zhang, Michael Sproviero, Yi Fang, Andrew F. Gardner, G. Brett Robb, S. Hong Chan and Ming-Qun Xu

# Raw material re-use

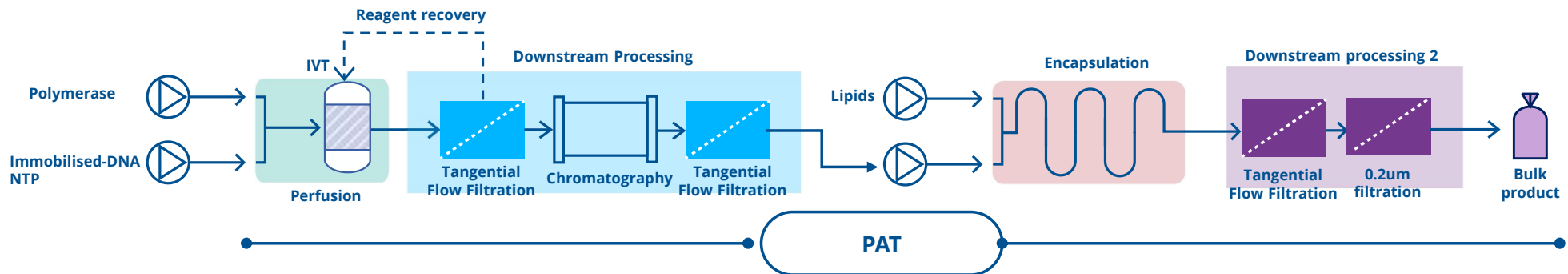
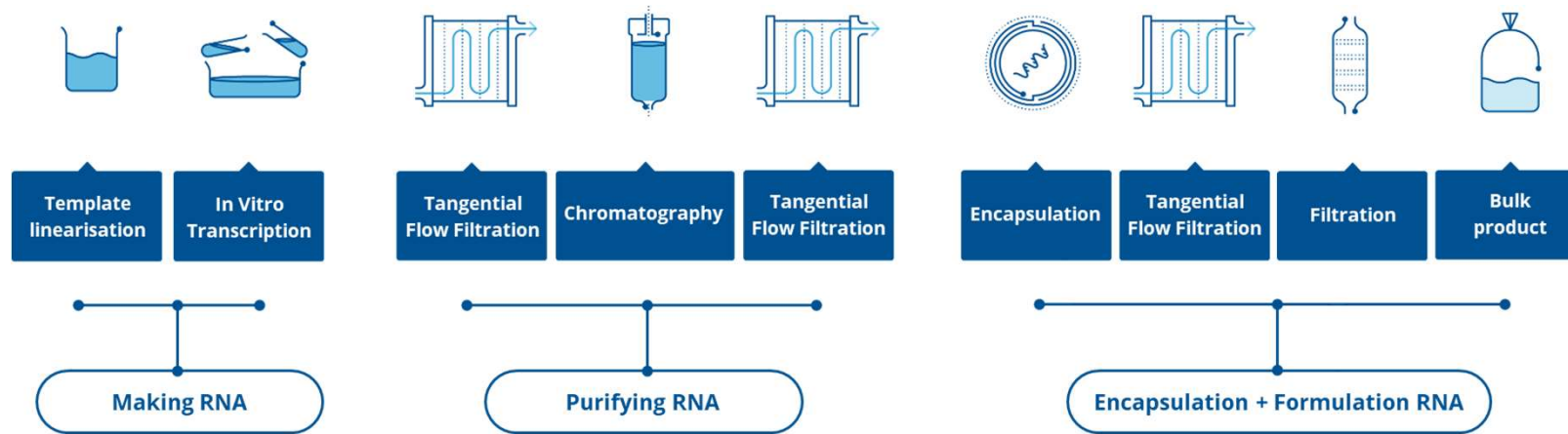


Project Balder : Manufacturing high purity mRNA at significantly reduced cost

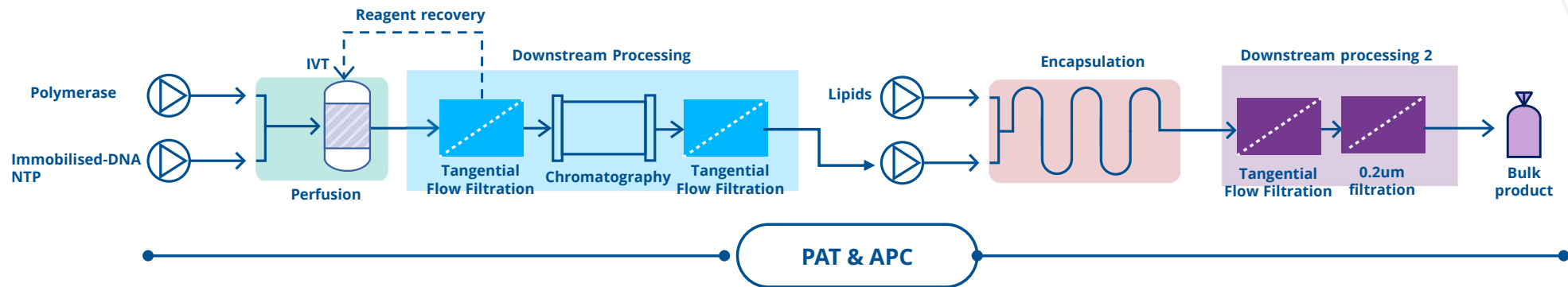
Biotoolumics, 4Basebio & CPI. Funded by IUK



# Integrated mRNA-LNP process example



# Integrated mRNA-LNP process example



## Optimisations:

- Immobilisation of DNA template to enable retention in reaction and perfusion approach
  - Potential for plug flow reactor or continuous counter current alternative
- Modified T7 polymerase to reduce dsRNA content & improve capping. To reduce downstream challenge and enable flow through approach
  - Potential to adopt OligodT simulated moving bed/ PCC for purification
- Application of single pass-TFF expertise and utilisation of smart surge tanks to enable semi-continuous operation

# mRNA/ LNP platform analytics

## mRNA

PARAMETER	METHOD
Concentration	A260 absorbance
Identity	Sequencing*
Integrity	CE, Labchip, RP-HPLC, ddPCR
Potency	Cell based assay
Impurities	Endotoxin, residual protein, residual template DNA, dsRNA
<i>Structural characterisation</i>	<i>CD, DLS, SEC-MALS, MFI</i>
In process monitoring	AGE, Qubit
High resolution characterisation	LC-MS
5' cap & 3' polyA	RL-HPLC

## mRNA-LNP

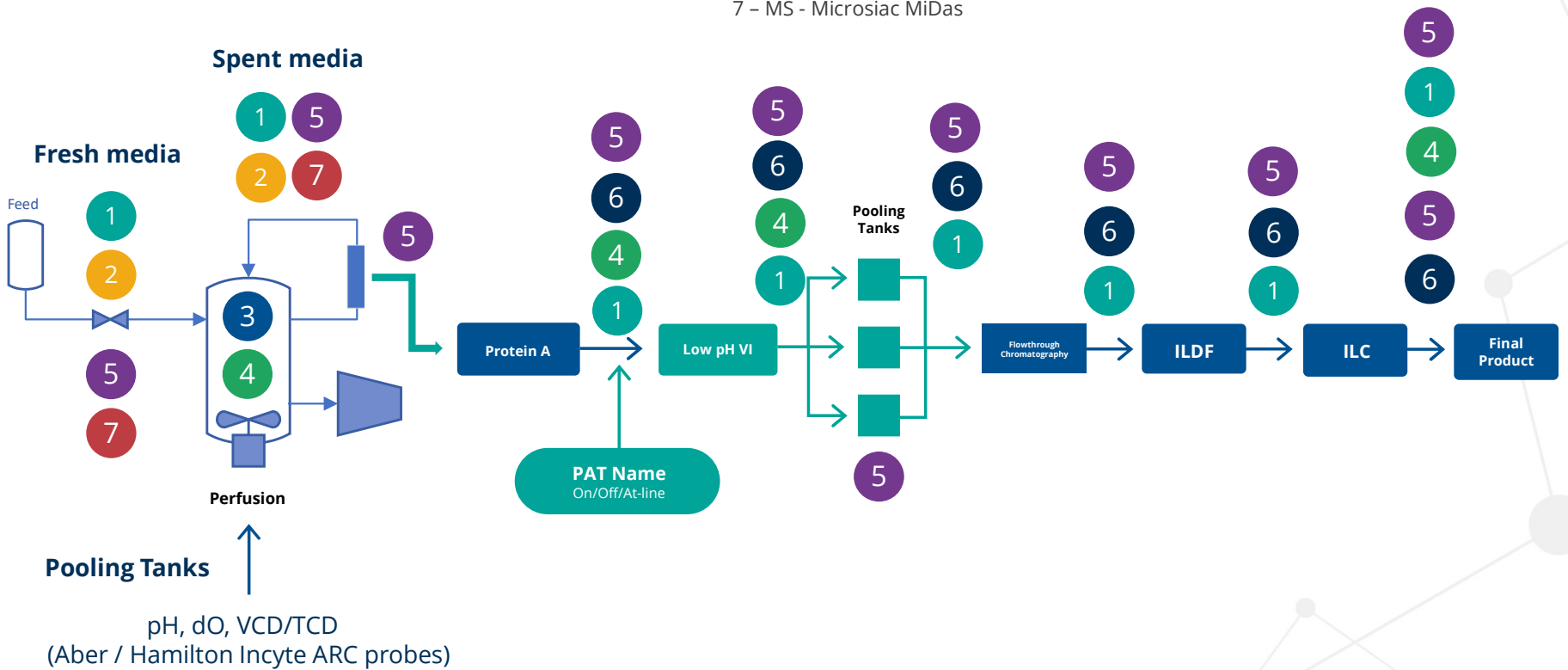
PARAMETER	METHOD
mRNA concentration	A260 absorbance, Ribogreen
mRNA integrity	AGE, CE, RP-HPLC
% mRNA encapsulation	Ribogreen
LNP size and PDI	DLS
Lipid content & identity	RP-HPLC
Residual solvent	GC
Impurities	Endotoxin*
Potency	Cell-based assay

\*Outsourced

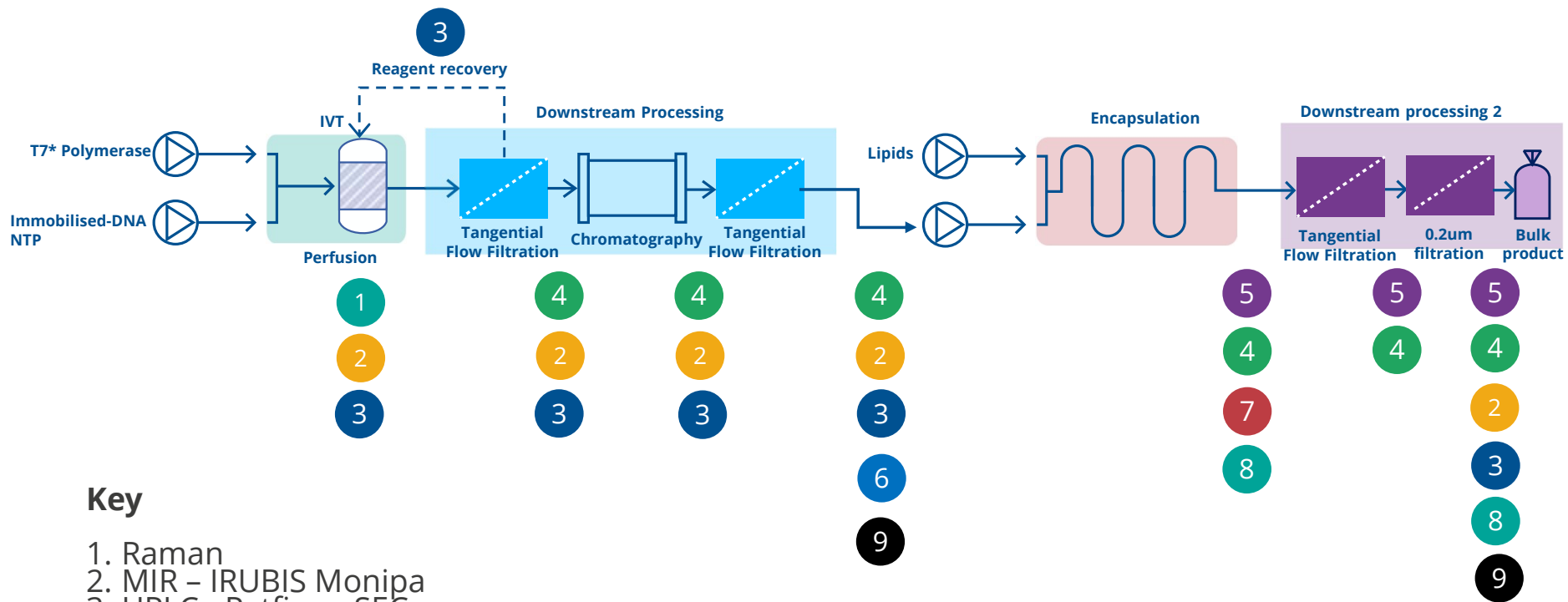
# Overall Process Flow PAT mAb continuous

## Key

- 1 - IOR - PALL mPATH
- 2 - ATEEM - Horiba Aqualog + sipper
- 3 - Raman - Thermo DXR3 + probe
- 4 - MIR - IRUBIS Monipa and/or RedshiftBio AQS3
- 5 - MS - Sciex X500B
- 6 - HPLC-SEC + autosampler
- 7 - MS - Microsiac MiDas

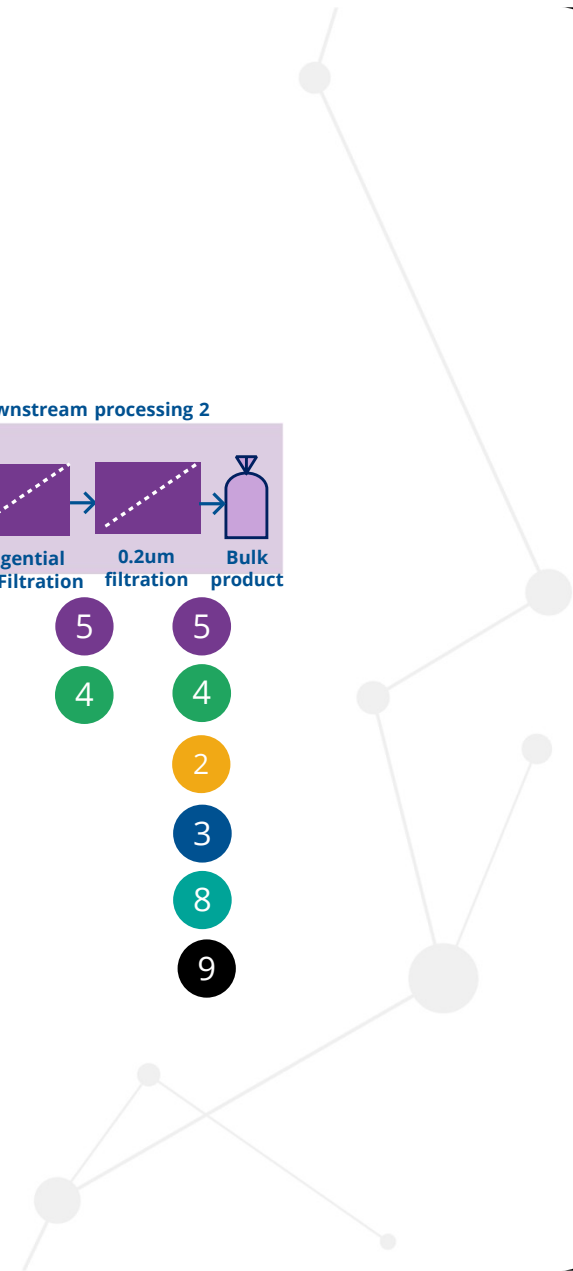


# Integrated mRNA-LNP PAT



## Key

1. Raman
2. MIR – IRUBIS Monipa
3. HPLC - Patfix or SEC
4. UV
5. RT-MALS – UltraDAWN
6. NMR – Bruker
7. Mass photometry - Refyn
8. Zetasizer - Malvern
9. Mass spec - Bioaccord



# Challenges to deployment

## Bespoke solution

- Specific application and scale render conventional bioprocessing equipment unsuitable, necessitating development of novel hardware

## Flexibility

- RNA-LNP technology developing at pace, need modular construction to enable modification to capture new developments

## Limits of intensification

- Evidence showing significant yields achieved through intensification of reactions, but limited data on product quality impacts & “Goldilocks zone” of yield(+CoGs) v quality

# Summary

- **Experience built intensifying and automating antibody-based processes is applicable to intensification of RNA-LNP products**
- **RNA based products offer opportunities for intensification owing to relative simplicity of production versus cell-based systems**
- **Intensification of RNA process will enable significant reductions in cost of goods and improve sustainability of manufacturing**

# Thank you



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