

BIOMANUFACTURING SOLUTIONS
TO TRANSFORM HEALTHCARE
PRECISION IN THE
BIOTHERAPEUTICS SECTOR



Rapid iterative design of tandem-core virus-like particles using *Escherichia coli*-based cell-free protein synthesis

Wednesday 6 April 2022, 09:15-09:45

Dr Beatrice Melinek EngD CEng MChemE

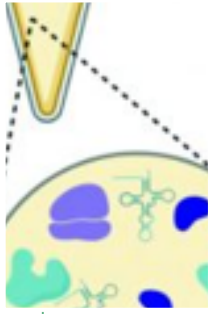
N. Colant, J. Teneb, S. Frank, S. Goldrick, W. Rosenberg, D. Bracewell



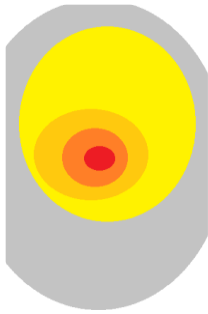
www.ucl.ac.uk/biochemeng/hub

Twitter: @FutureHealthHub

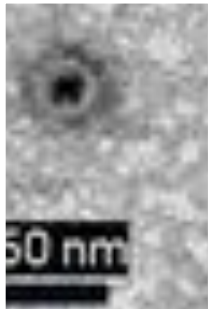
Content: CFS as a platform screening process



Cell-free synthesis and the FTHM Hub



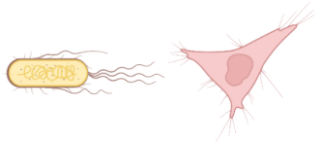
Rational strategies for improving reaction performance



Use as a screening platform: a case study

- ❖ Why is CFS special as a process?
- ❖ The Hub Vision
- ❖ How to start using CFS quickly
- ❖ How to get the most from your CFS reaction
- ❖ How to have enough protein for analysis
- ❖ Application of CFS as a screening tool to improve construct design

Cell-free synthesis: cell-based versus cell-free production 1

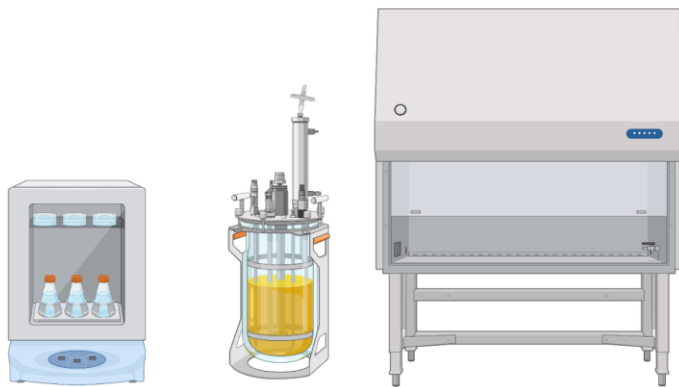


Long product production timelines

~3 weeks for bacterial cells

6-10 weeks for mammalian cells

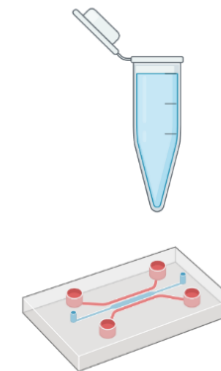
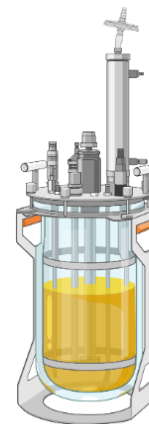
Multiple, complex machinery



Short product production timelines

2-4 days

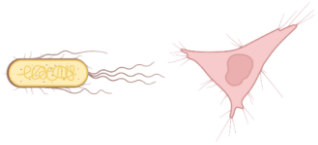
Single, simple machine if scale fixed



(Swartz, 2012)

Created in biorender

Cell-free synthesis: cell-based versus cell-free production 2



High degree of variability

Complicated to model and control



Improved reproducibility

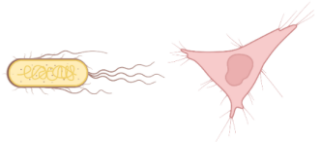
Reduced set of reactions still active

Increased predictability based on
Critical Process Parameters (CPPs)

Ease of containment and/or reduced
intervention

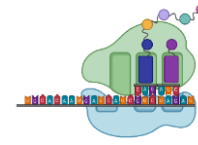
Improved control of product quality

Cell-free synthesis: cell-based versus cell-free production 3



High up-front investment, but lower ongoing costs.

Well understood and characterised.



Low up-front investment, but high ongoing costs (fresh reagents needed).

In development from a 'black-box'.

Cell-free synthesis: The Hub Vision

CFPS as a production platform for rapid & distributed manufacture of proteins.

Distributed manufacturing of drug for increased drug stratification, can be enabled by CFPS.



Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in
**Chemical
Engineering**

Cell free protein synthesis: a viable option for stratified medicines manufacturing?

Olotu W .Ogonah¹, Karen M Polizzi² and Daniel G Bracewell¹



BIOPROCESS TECHNICAL

Toward a Roadmap for Cell-Free Synthesis in Bioprocessing

Beatrice Melinek, Noelle Colant, Christos Stamatis, Christopher Lennon, Suzanne S. Farid, Karen Polizzi, Mark Carver, and Daniel G. Bracewell



Runner Up: Upstream

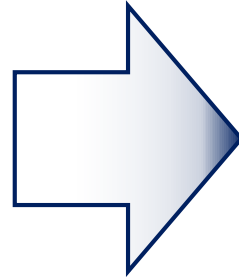


Cell-free synthesis: Features of a great screening platform

Rapid screening of protein candidate constructs, can be enabled by CFPS

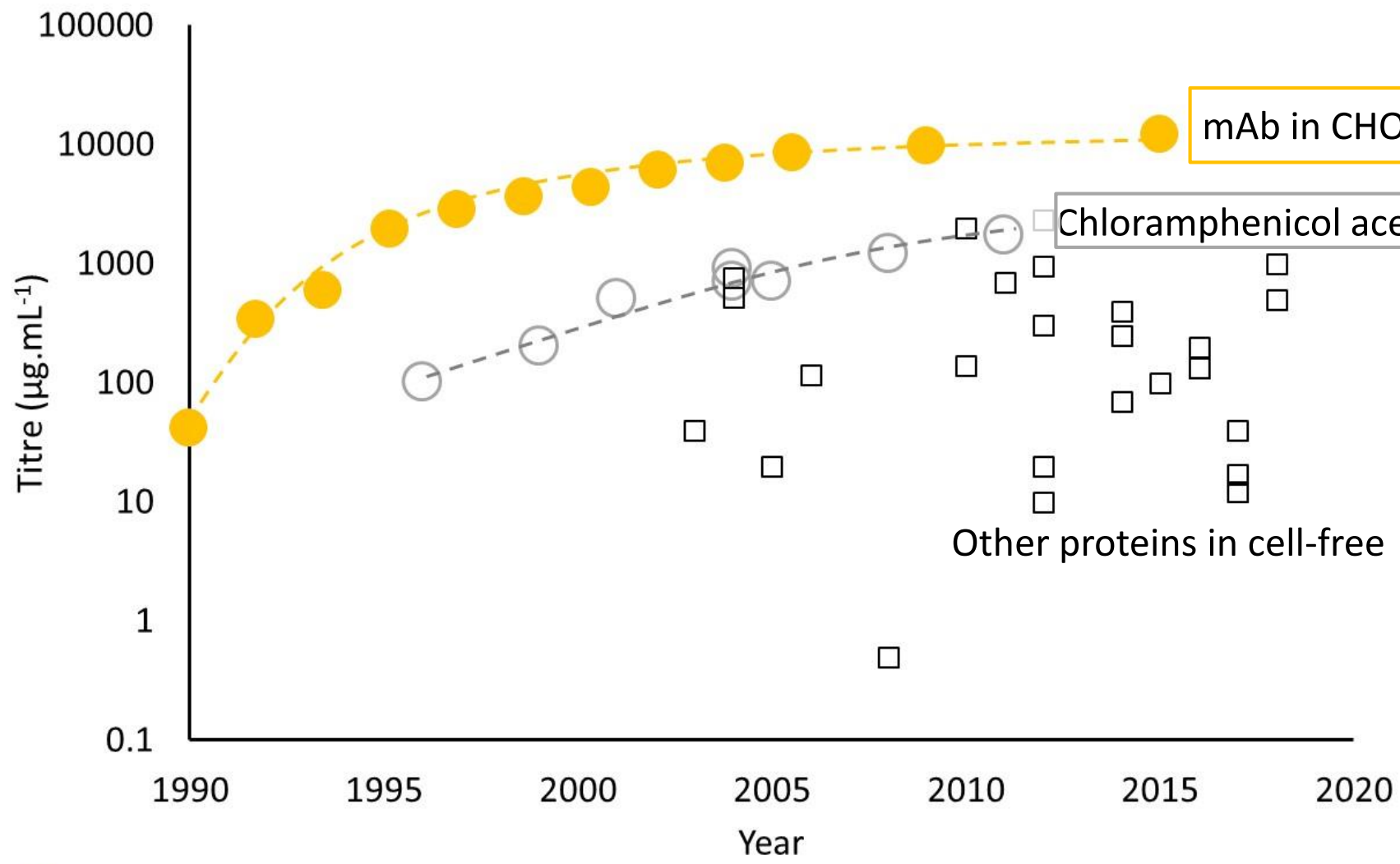
Cell based work is:

- ❖ Time consuming
- ❖ Complex
- ❖ Variable



- ❖ A simple set-up can be used, which is:
 - ❖ Easy to automate allowing high-throughput;
 - ❖ Simple to operate by people with a range of expertise
- ❖ Increased predictability based on critical process parameters (CPPs), so you can quickly establish a workable yield;
- ❖ Improved reproducibility, so you can be confident the differences come from you construct design changes;
- ❖ Rapid – reactions complete within hours
- ❖ Components can be made in bulk and stored frozen or lyophilised

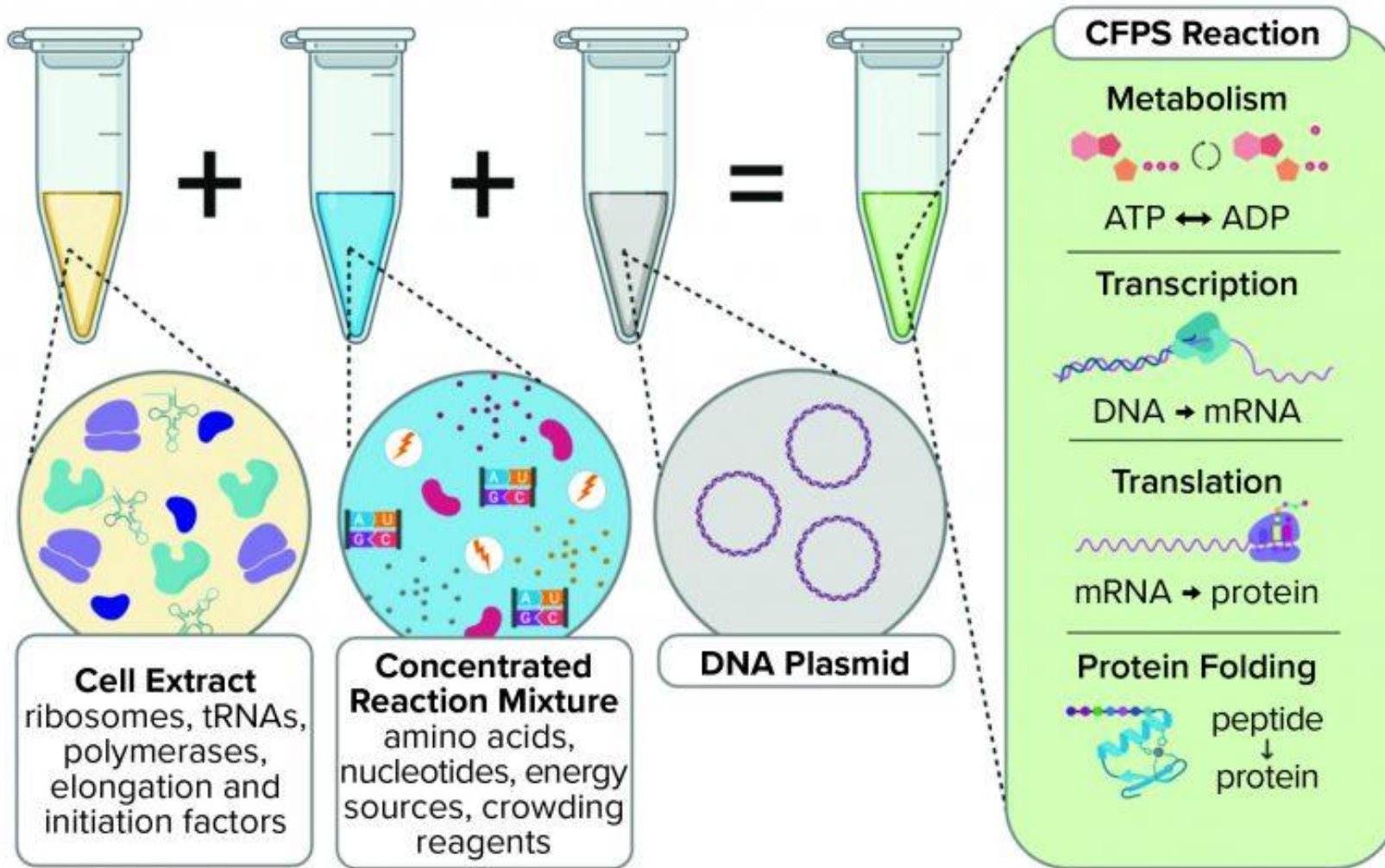
Cell-free synthesis: Step 1 - Improving titres



● Cell-based GS-CHO (mAb) ○ Cell-Free (Chloramphenicol acetyltransferase) □ Cell-Free (other proteins)



Experimental Method: Cell-free Protein Synthesis



E.coli whole cell lysate (shake flask)

+

E.coli grown plasmid (shake flask), purified using Qiagen midi-prep

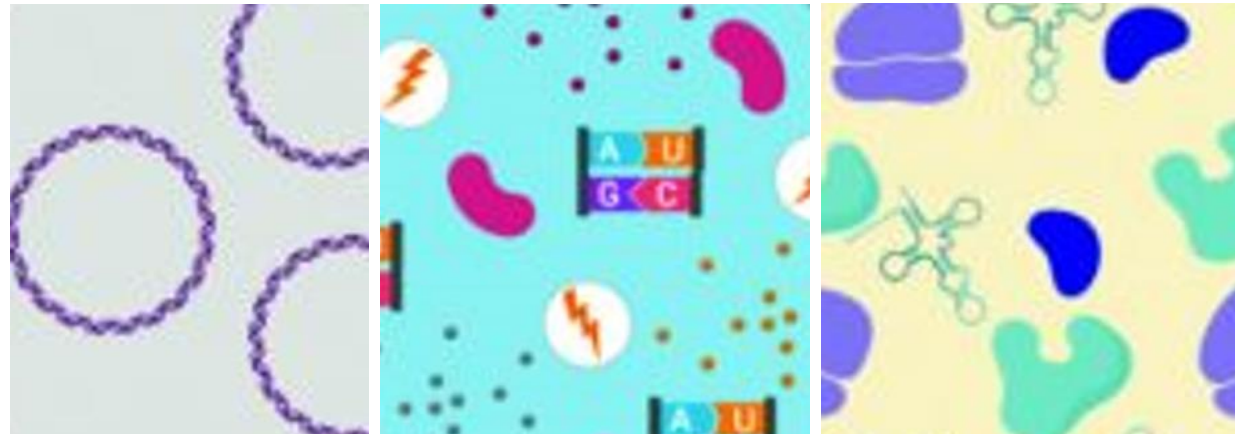
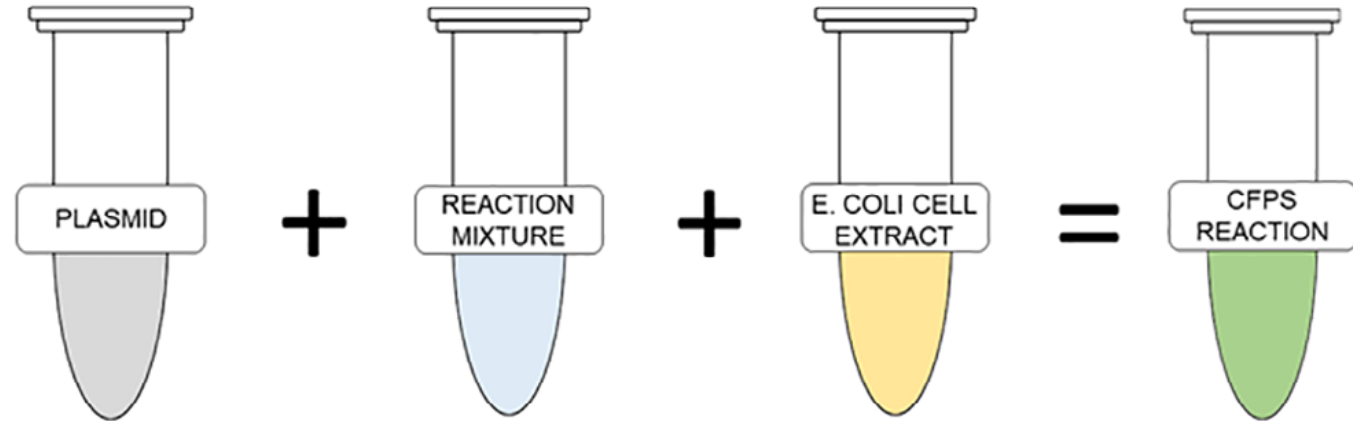
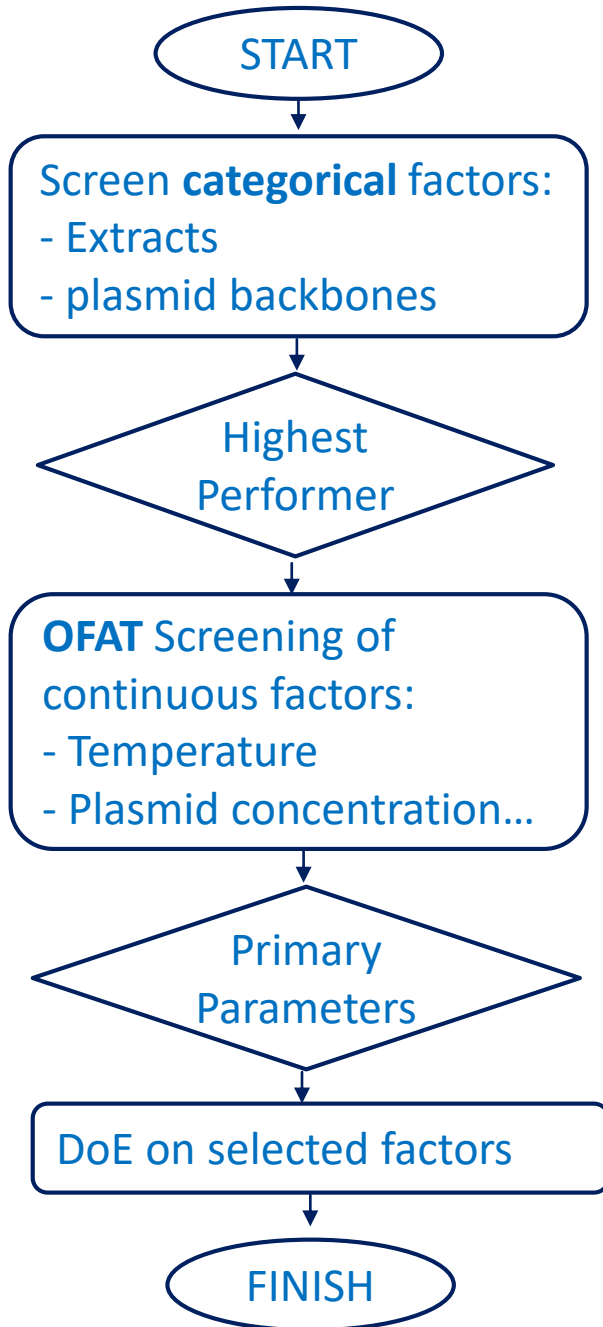
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Kwon reaction mix

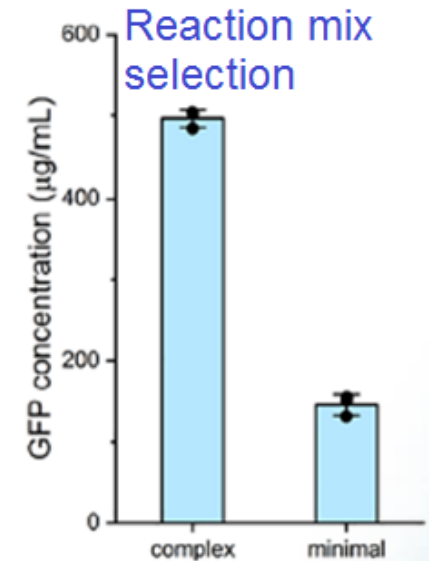
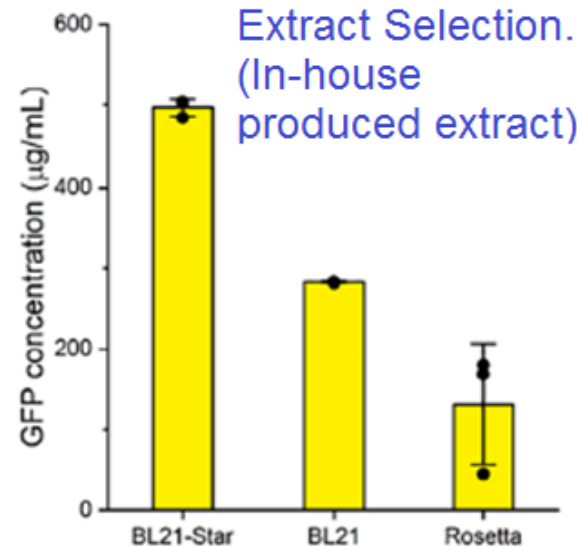
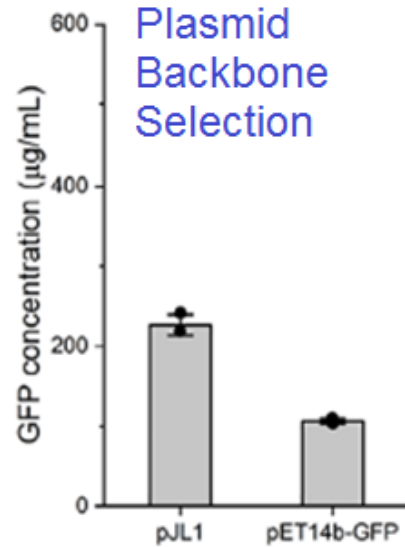
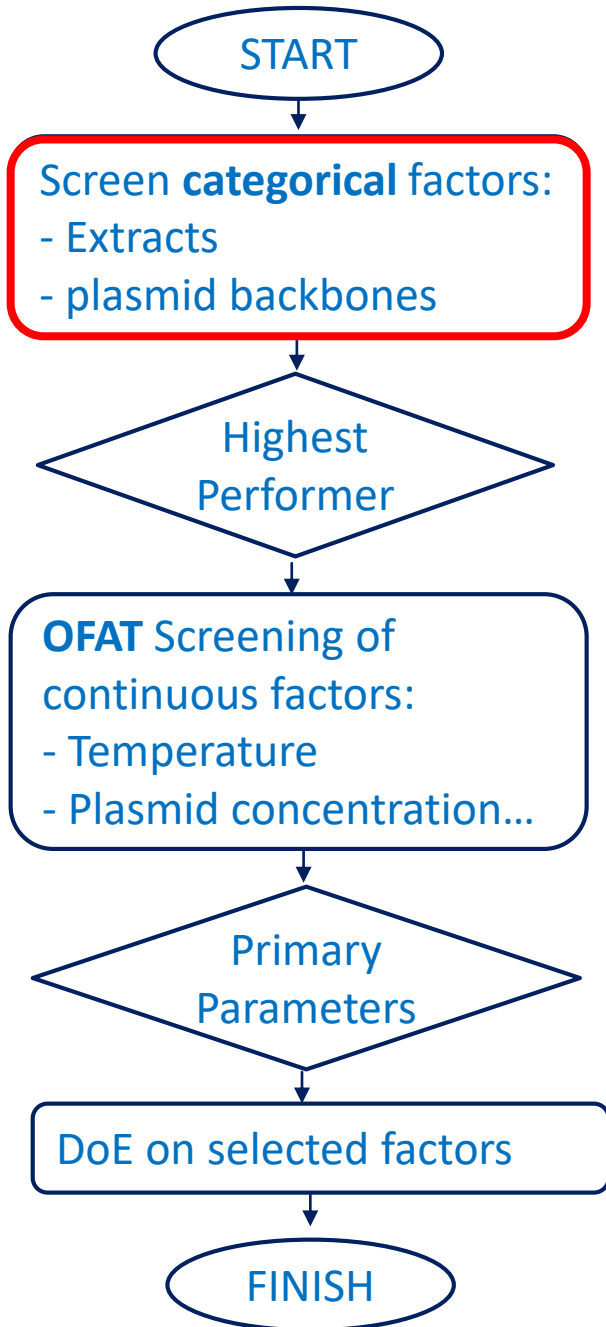
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Amino Acids

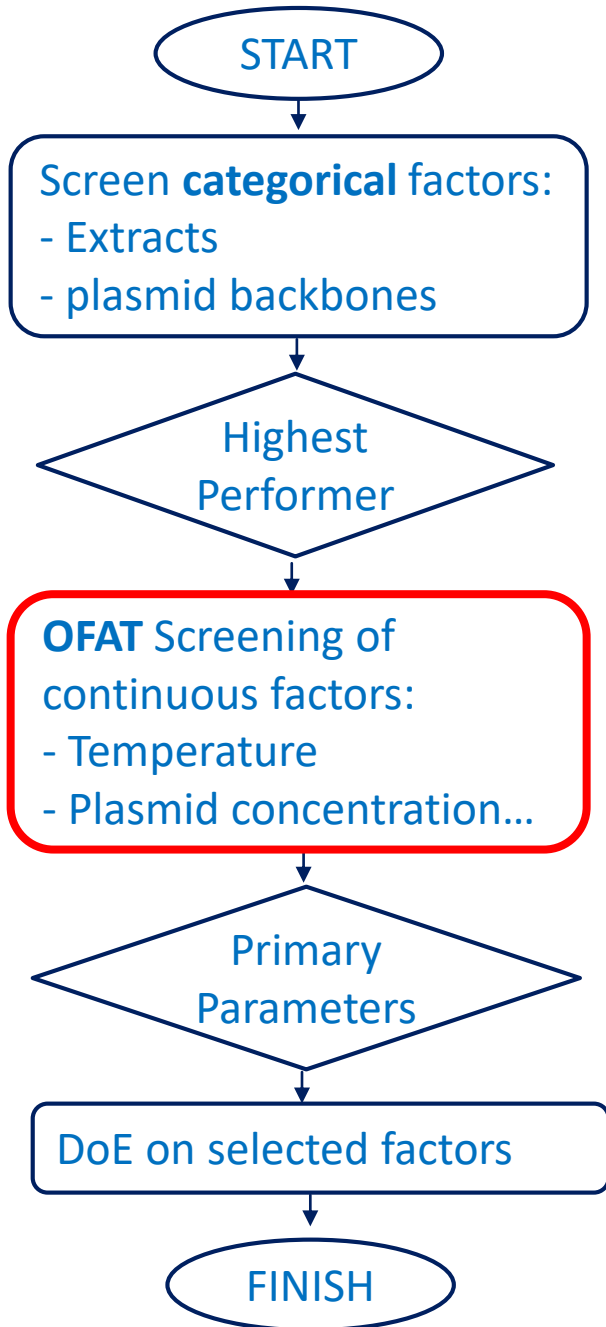
Setup Strategy: Factors to consider



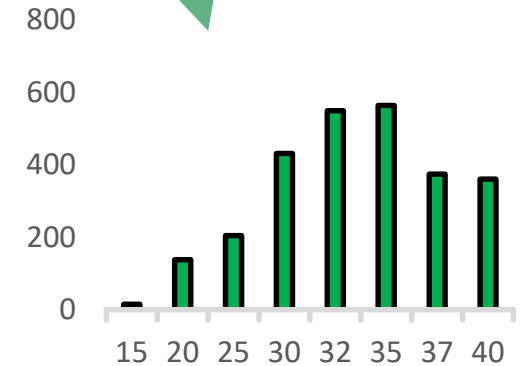
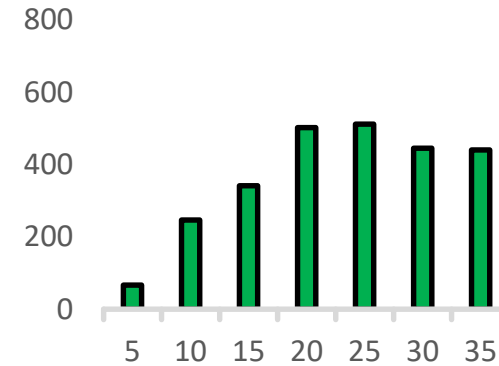
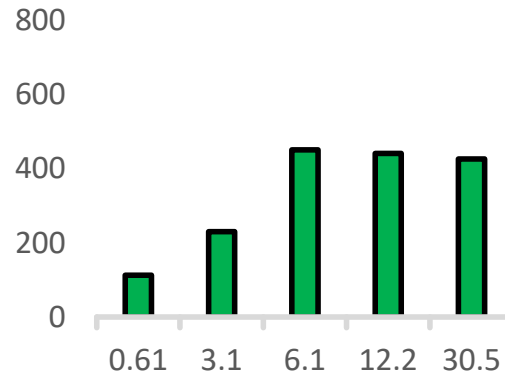
Setup Strategy: Factors to consider



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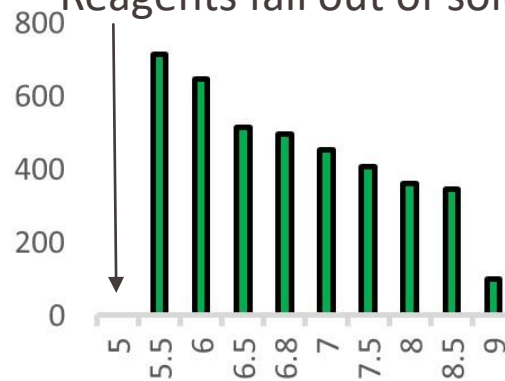


Graphs of Product Concentration ($\mu\text{g/mL}$)

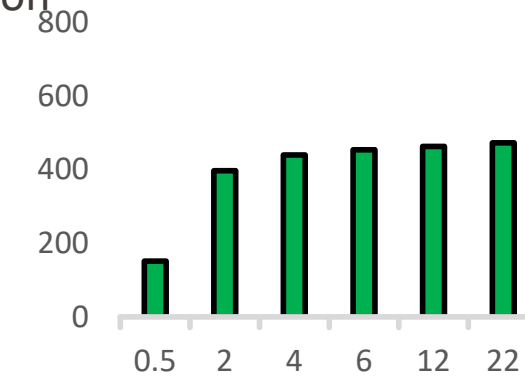


Plasmid Concentration (mM) Amount of extract (%v/v) Temperature ($^{\circ}\text{C}$)

Reagents fall out of solution

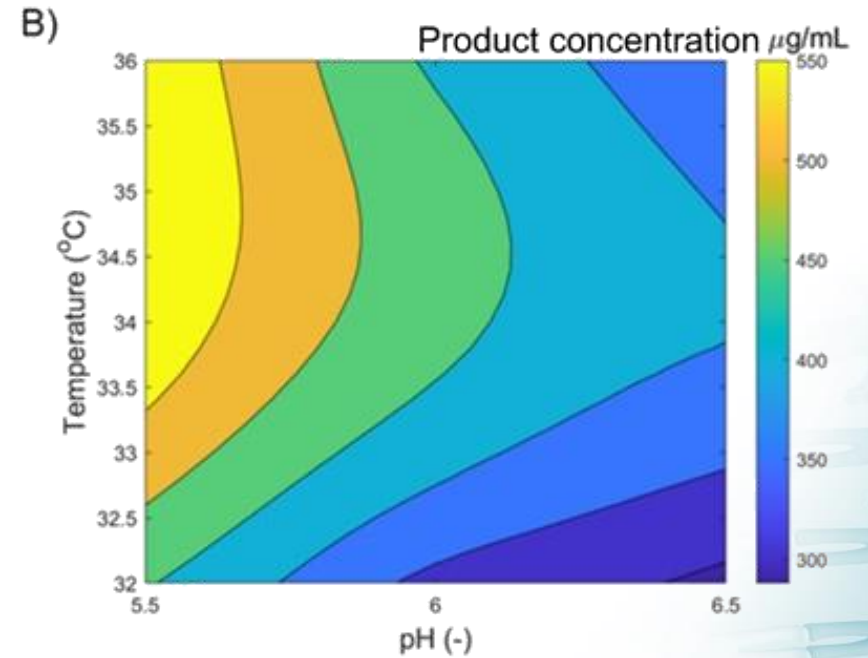
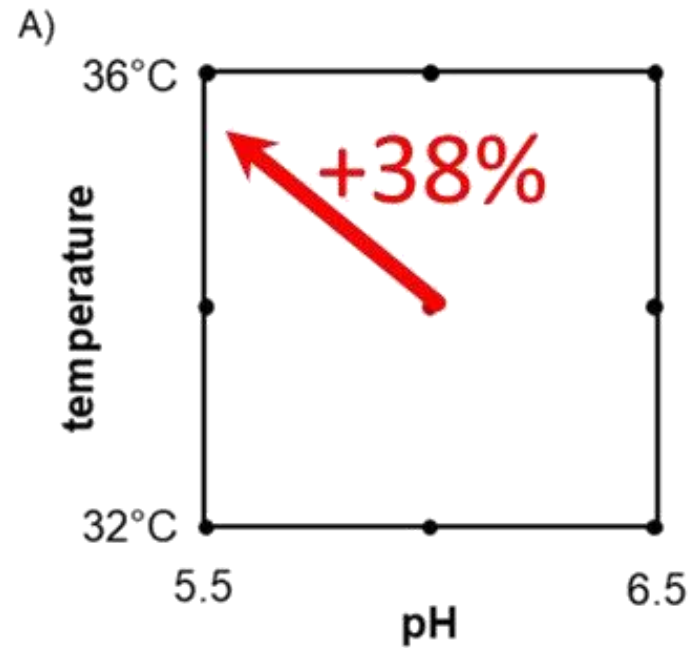
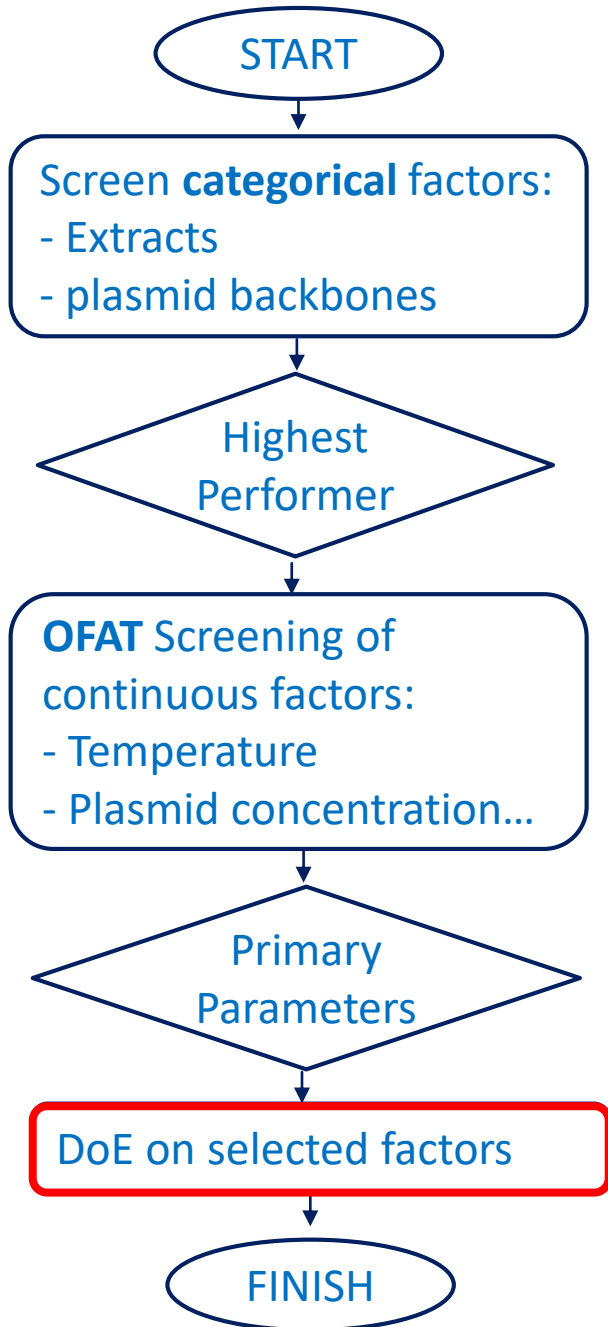


pH of reaction mixture



Reaction Length (hrs)

Setup Strategy: Factors to consider

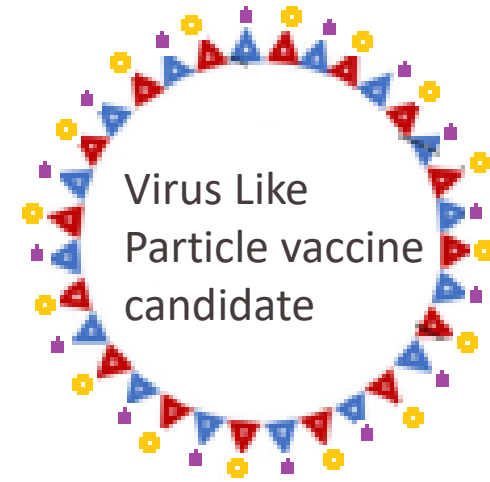




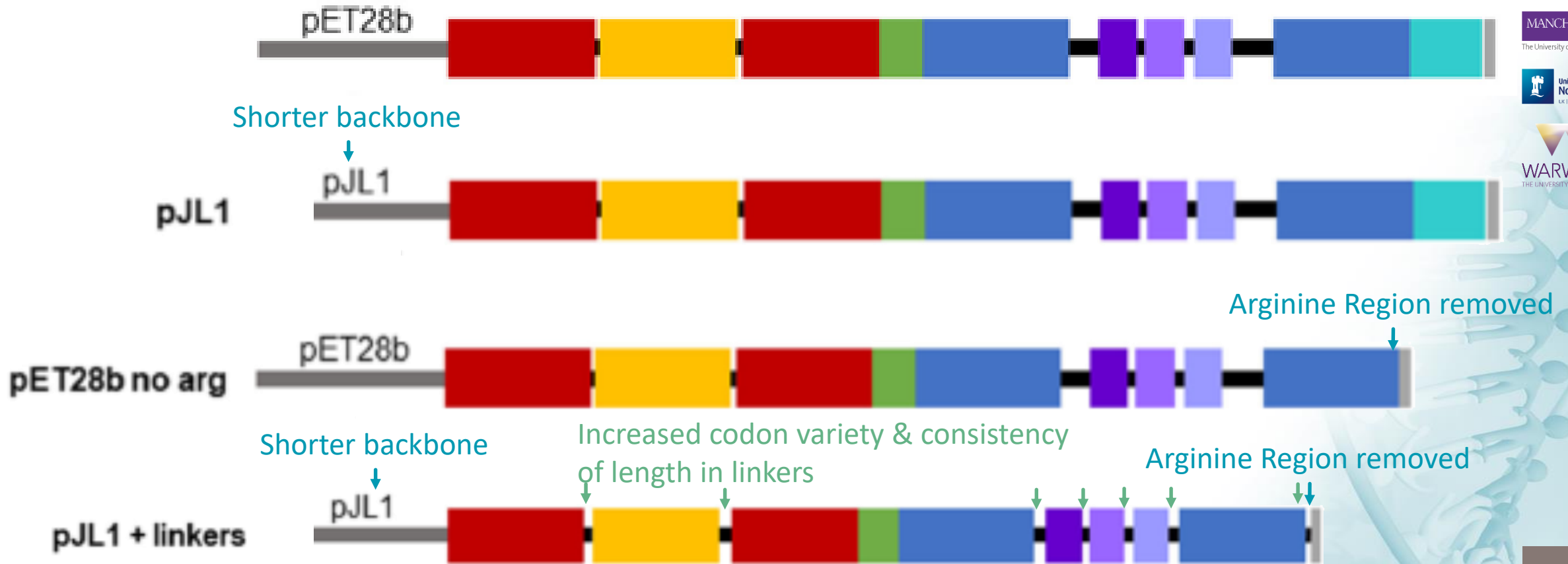
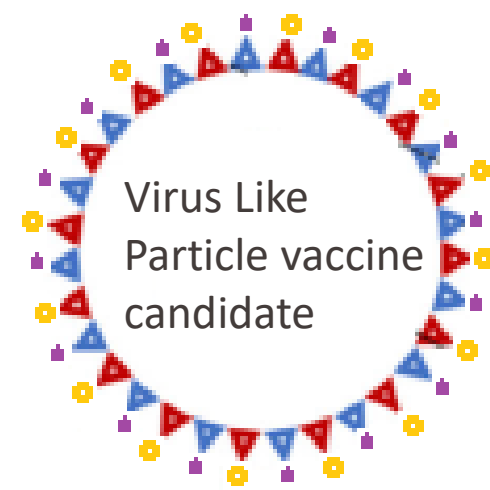
Setup Strategy: Conclusions

- ▶ **Cell-free titres** low relative to cell-based, so improvements needed
- ▶ **'Standard conditions'** are not universal
- ▶ **Simple 3-step Algorithm** for titre improvement
- ▶ Can be completed in as little as 48 hours
- ▶ Requires no expertise in cell-free
- ▶ Comparable results to in-depth titre optimisation studies

Experimental Method: VLP Case Study

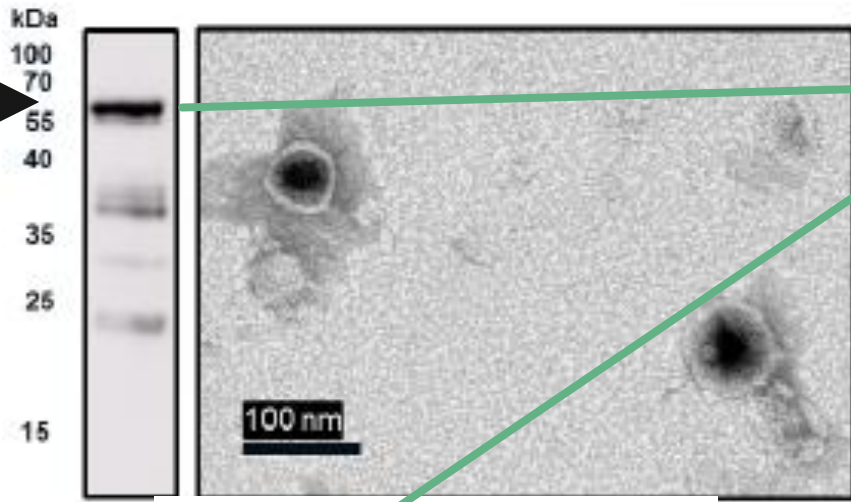


Case Study: Application of CFPS to screening

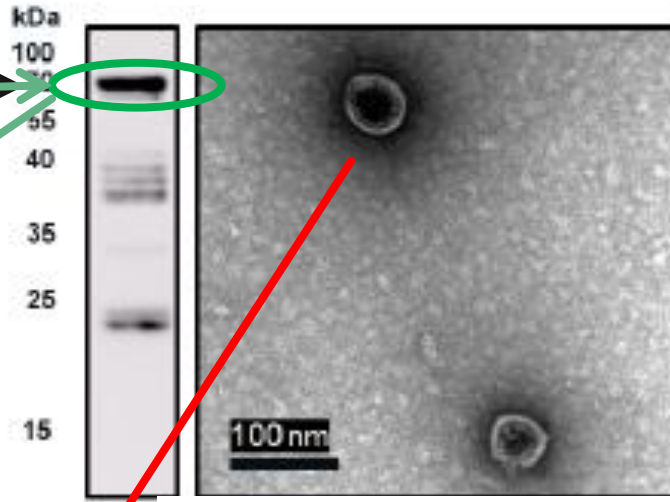


Case Study: Application of CFPS to screening

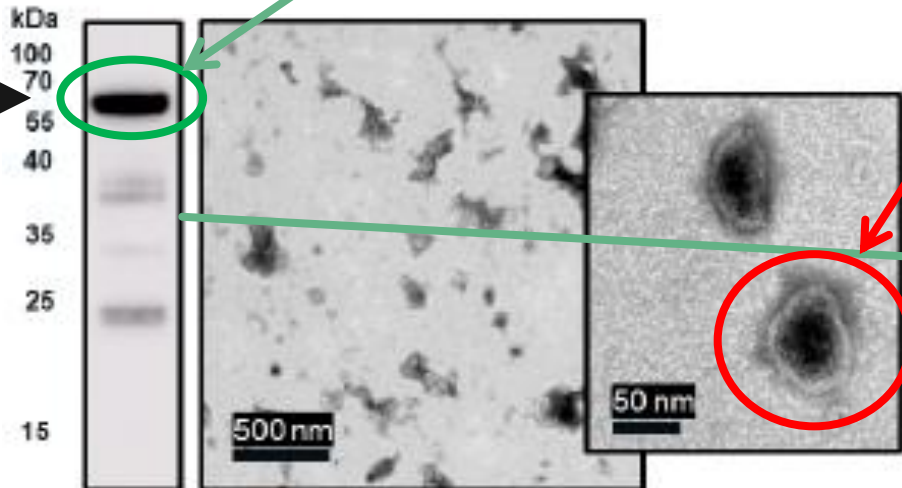
pET28b backbone



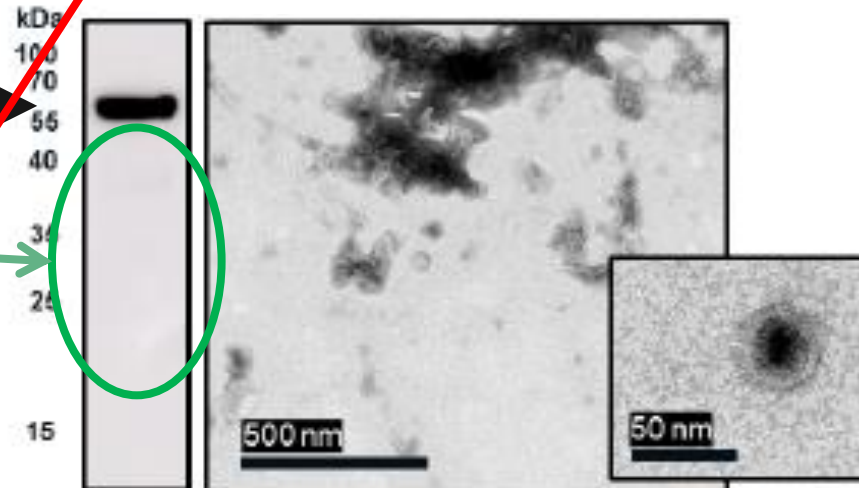
PJL1 backbone



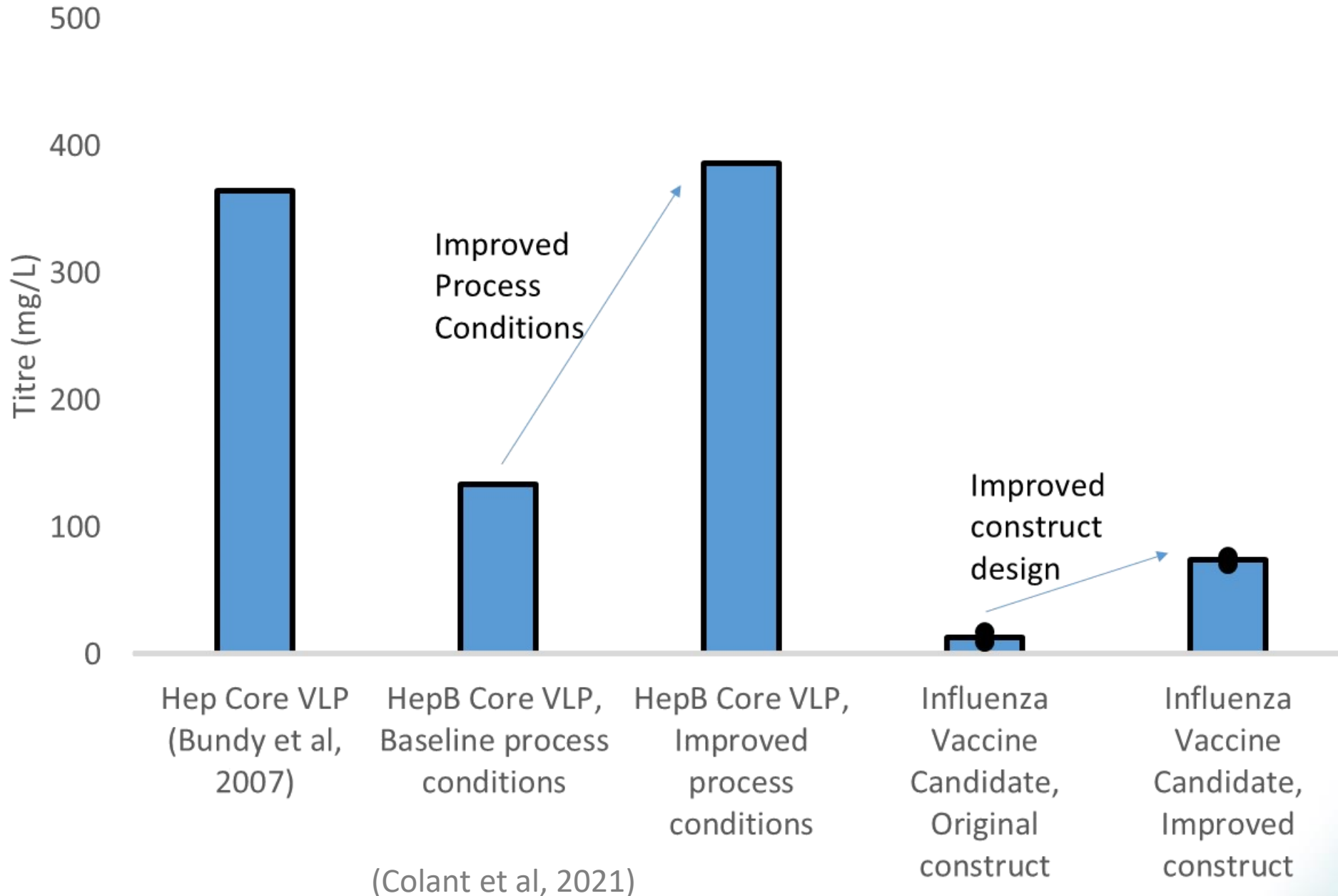
No Arginine Region



No Arginine Region + Linkers (+PJL1)



Case Study: Impact on titres in context



Cell-Free Synthesis: Conclusions Titre Improvement



- ▶ Applying a simple 3-step algorithm the titre for:
 - ▶ GFP was improved by 38%
 - ▶ and for HepB Core VLP by 190%
- ▶ Minimum cell-free expertise is required
- ▶ The process is rapid (48 hrs), but is an essential first step to use of cell-free protein synthesis for construct screening



Cell-Free Synthesis: Conclusions Construct Screening

- ▶ We used the 3-step algorithm to optimise HepB Core VLP titres
- ▶ The same conditions were applied to a derivative of the HepB Core VLP, with influenza antigens
- ▶ **Achievements:**
 - ▶ **Demonstrate large and complex protein produced in cell-free**
 - ▶ **Substantial improvement in rate (1 week for 8 constructs -> 1 day for 100s of constructs)**

Cell-Free Synthesis: Screening Future Directions



- ▶ **Quality by Design:** consistent extract production and/or reactions
- ▶ **Analytics:** automating high-throughput preparation and analysis
- ▶ **Cell-Free to Cell-based:** demonstrating consistency of results from this screening and results in cell-based, to allow for subsequent cell-based manufacture.
- ▶ **Cell-Free pDNA production:** Further increase in production & prototyping rate



Imperial College
London



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RESEARCH ARTICLE BIOTECHNOLOGY PROGRESS

A rational approach to improving titer in *Escherichia coli*-based cell-free protein synthesis reactions

Noelle Colant¹ | Beatrice Melinek¹ | Jaime Teneb¹ | Stephen Goldrick¹ | William Rosenberg² | Stefanie Frank¹ | Daniel G. Bracewell¹

vaccines MDPI

Article
***Escherichia Coli*-Based Cell-Free Protein Synthesis for Iterative Design of Tandem-Core Virus-Like Particles**

Noelle Colant¹, Beatrice Melinek¹, Stefanie Frank¹, William Rosenberg² and Daniel G. Bracewell^{1*}

Thank you!