



Chair: Professor Daniel Bracewell, UCL Department of Biochemical Engineering

Workshop Leaders: Harvey Branton, Dan Bracewell, Karen Polizzi

Agenda

11:00 **Welcome, minutes of the previous meeting, introduction to the agenda**

Oral Presentations

11:15 **Engineering a *Pichia pastoris* cell-free protein synthesis platform**

Rochelle Aw and Karen Polizzi

Department of Chemical Engineering, Imperial College London

A popular expression platform for in vivo protein production is *Pichia pastoris* (syn *Komagataella* spp.); a methylotrophic yeast which is renowned for its high volumetric productivity. We have developed a novel CFPS platform utilising *P. pastoris* as the cell extract. A number of improvements including optimisation of reaction conditions and vector design and overexpression of global regulators of ribosome synthesis were used to increase overall yields.

Aw R, Polizzi KM. Biosensor-assisted engineering of a high-yield *Pichia pastoris* cell-free protein synthesis platform. *Biotechnology and Bioengineering*. 2019;116:656–666.

11:40 **Q&A**

11:45 **Development of a platform for ultra-high-throughput protein evolution focused on protein stability**

Jaime Teneb, Abubaker Mohamed, Noelle Colant, Pierre-Yves Colin and Paul Dalby

Department of Biochemical Engineering, UCL

Directed evolution is a technique that allows improvements on a certain characteristic through cycles of diversification, selection and amplification. This project seeks to establish a platform for protein directed evolution focused on protein stability with the aim of performing early screenings in biopharmaceutical candidates in an ultra-high-throughput fashion. The platform consists of a FACS-like selection using cell-free protein synthesis in droplets to express the different variants of a protein library labelled fluorescently. There are different strategies for selection based on fluorescent markers. This presentation shows the early stages of the project related to the protein labelling through a non-standard amino acid and droplet generation and selection.

12:10 **Q&A**



Future Targeted Healthcare Manufacturing Hub

Cell-Free Synthesis Specialist Working Group

Agenda

Friday 5th April 2019 11.00-15.00

Centre for Process Innovation (CPI) National Biologics Manufacturing Centre
1 Union Square, Central Park, Darlington, DL1 1GL

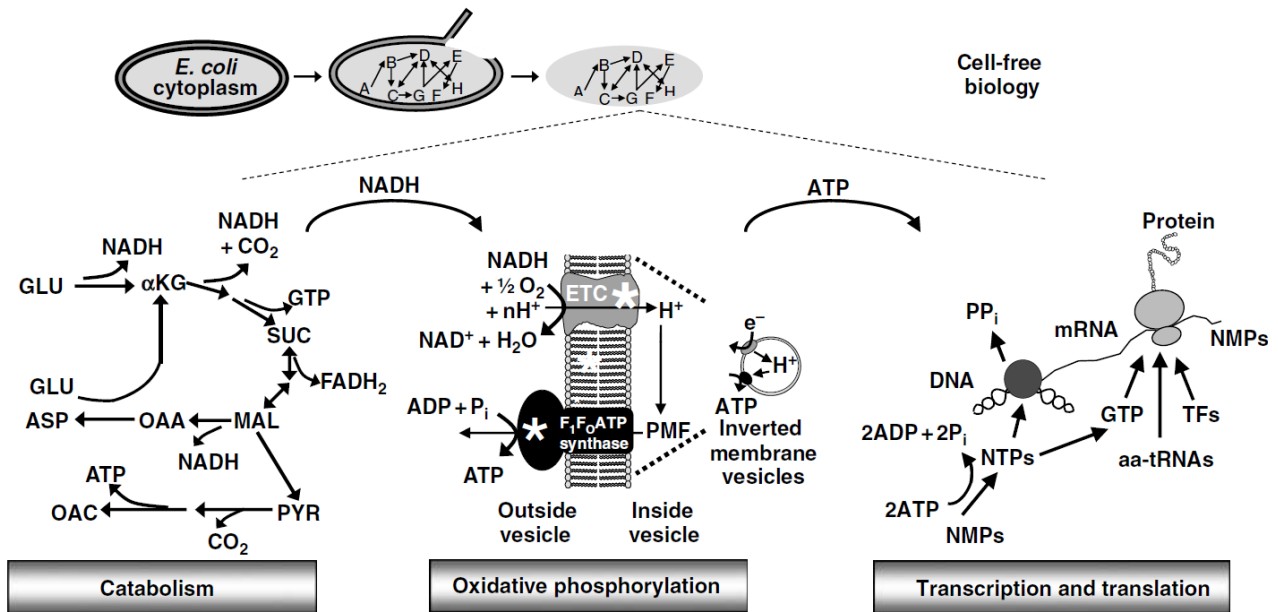
- 12:15** **Development of an In-House Cell Free Extract Process and Robotic Platform for Expression Optimisation Suitable for Large Scale Manufacture**
Jade Tuck and Harvey Branton
CPI Biologics.
- 12:40** **Q&A**
- 12:40** **Introduction to tour of CPI and continuous processing setup in collaboration with Pall Biotech.**
Harvey Branton CPI / John Welsh Pall Biotech

13:00 **Tour and lunch**

Workshops

- 14:00** **Workshop 1: "Measurement of cell extract critical quality attributes for cell free protein synthesis"**
Dan Bracewell (UCL) Karen Polizzi (Imperial College)

If we consider the active elements of metabolism and protein synthesis thought to be active in *E. coli* based cell free protein synthesis (see below), what might we consider critical elements that could form the basis of assays for cell extract CQAs? Conventionally the extract is prepared from rapidly growing cells with the logic that protein synthesis is upregulated under these conditions. Therefore ribosomes would be likely candidates as a CQA, alternatively how critical are the inverted membranes / vesicles supposed to be critical for oxidative phosphorylation, and what is the importance of the cell disruption step in creating these?



Jewett, M.C., Calhoun, K.A., Voloshin, A., Wu, J.J. and Swartz, J.R. (2008) An integrated cell-free metabolic platform for protein production and synthetic biology. *Mol. Syst. Biol.* 4, 220

Workshop 2: “Identify and discuss barriers slowing the commercialization of cell free manufacturing”

Harvey Branton CPI

While progress has been made in the application of cell free technologies within a research environment only limited interest has been shown in its use within commercial manufacture. This workshop will explore the key barriers restricting its commercialisation, once identified they will be ranked and used to help prioritise future projects.

14:45 Feedback and summary from workshop leads

15:00 Close

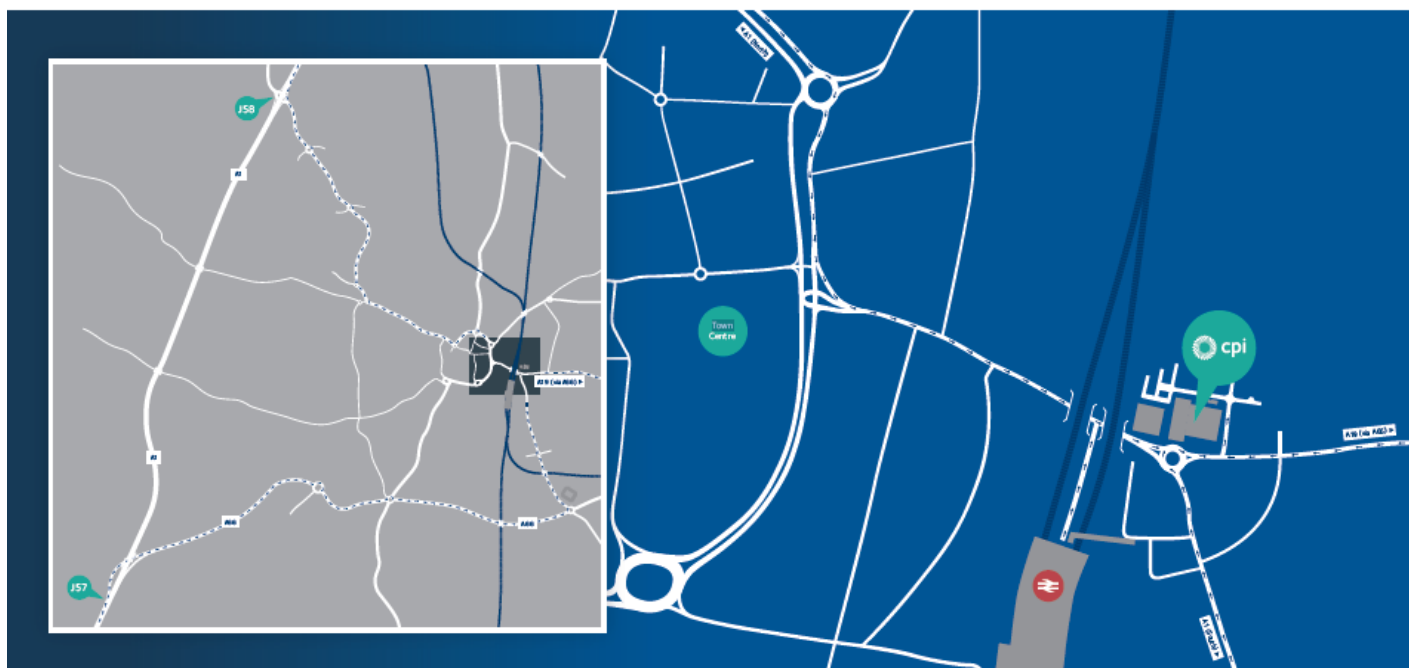
Getting to CPI

National Biologics Manufacturing Centre



CPI, 1 Union Square, Darlington, DL1 1GL, United Kingdom

+44 (0)1325 376 800 www.uk-cpi.com Postcode for sat nav: DL1 1GP



By Road

Please note there is no on-site parking available.

A1 (M) Northbound

1 Exit the A1 (M) at Junction 57 onto the A66 (M) signed Darlington. 2 At the roundabout, take the first exit onto Bridge Rd (A66). 3 At the roundabout, take the third exit onto A66 signed Teesside. 4 At the roundabout, take the first exit onto Neasham Rd signed Town Centre. 5 At the roundabout, take the second exit to stay on Neasham Rd. 6 At the roundabout, take the second exit onto Yarm Rd (B6280) and side left onto John Williams Boulevard South. CPI is on your left.

A1 (M) Southbound

1 Exit the A1 (M) at Junction 58 and take the first exit onto West Auckland Rd. 2 At the roundabout, take the second exit onto West Auckland Rd signed Darlington (A68). 3 At the roundabout, take the second exit onto West Auckland Rd signed Darlington (A68). 4 At the roundabout, take the first exit onto Woodland Rd signed Town Centre (A68). 5 At the roundabout, take the first exit onto Woodland Rd signed Town Centre (A68). 6 At Bondgate Roundabout, bear left onto St Augustines Way signed

A167. 7 At the roundabout, take the second exit onto A167 signed Train Station. 8 At the roundabout, take the second exit onto A167 signed Train Station. 9 Bear left onto Parkgate (B6280) signed Train Station. 10 At the roundabout, take the first exit onto Yarm Rd (B6280) and side left onto John Williams Boulevard South. CPI is on your left.

A19 North/Southbound

1 Exit the A19 onto the A66 signed Darlington and continue on the A66 for 12 miles. 2 At the roundabout, bear left to continue on the A66. 3 Keep left and at the roundabout, continue straight on the A66 signed Train Station. 4 At the roundabout, take the third exit onto Yarm Rd (B6280) signed Train Station. 5 At the roundabout, take the second exit onto Yarm Rd (B6280) signed Train Station. 6 At the roundabout, take the second exit onto Yarm Rd (B6280) signed Train Station. 7 Turn right onto John Williams Boulevard South. CPI is on your left.

P Alternative Parking

With **no on-site parking** available, we recommend visitors arrive by train. However, nearby Pay & Display car parks are available including Park Place, Feethams Multi Storey, and Darlington Train Station. Please visit goo.gl/EpQZbu (Darlington Town Council) for more information and a full list of available car parks.

All parking is at the owner's risk and CPI is not liable for any loss, damage or charges that may occur.

By Train (Recommended)

Darlington Train Station

Head through the ticket gates and exit Darlington Train Station through the main entrance/exit ahead.

Continue down the ramp and turn right to head east on Parkgate.

Head under the bridge and cross over Parkgate road.

Continue east, turning left onto John Williams Boulevard South and follow the footpath west.

By Air

Durham Tees Valley (MME)

12 minute drive

Newcastle International (NCL)

55 minute drive

30 minutes by rail (via Newcastle Central)