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9AM - 5PM

ROOM L024 MASON HAYES THEATRE
SUTHERLAND SCHOOL OF LAW,
BELFIELD, UCD

BOOK OF ABSTRACTS

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

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Background and Novelty

Single cell RNA-seq (scRNA-seq) has recently been shown to be a powerful method for understanding of transcriptional heterogeneity in monoclonal antibody (mAb) producing Chinese hamster ovary (CHO) cell lines[1]. However more complex experimental designs can be prohibitively expensive with some commercial platforms. In this study sample specific oligonucleotide labelling[2] was used to monitor the transcriptome following the induction of endoplasmic reticulum (ER) stress using tunicamycin.

Experimental approach

A non-mAb producing CHOK1GS cell line was treated with 10µg/ml tunicamycin, and cells were captured at selected timepoints post treatment. To prepare for multiplexed scRNA-seq each sample was transfected with a distinct short barcode oligonucleotide (SBO) and pooled samples were loaded onto two lanes of the 10X Genomics Chromium platform. Following cell isolation and RNA/barcode oligo capture, 4 libraries (2 x cellular RNA and 2 x barcode oligos) were prepared and sequenced on an Illumina NovaSeq configured to yield 28 x 91 bp reads.

Results and Discussion

For the cellular RNA, Kallisto | bustools[3] was used to generate a cell-gene matrix and CITE-seq count[4] was used for the SBO libraries to yield > 4,000 individual cells and the corresponding sample barcodes from the pooled samples. The individual samples were demultiplexed in Seurat[5] by classifying the identity of each sample using the nucleotide barcodes. Following pre-processing and dimensionality reduction the samples were clearly distributed according to sample identity and timepoint post tunicamycin treatment. Through single cell weighted gene coexpression network analysis (WGCNA) groups (modules) of genes were resolved that were correlated across the sample set. Of the identified gene modules, 3 were associated with progression of the cells over the time course and were enriched for categories such as ER stress, the unfolded protein response and sterol metabolism. This work demonstrates the utility of multiplexed scRNA-seq for improving the understanding of CHO cell biology.

Acknowledgements and Funding

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Advanced Formulation of Co-processed Ionic Liquid Drugs via Spray Drying for Incorporation into Solid Dosage Forms

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Transforming active pharmaceutical ingredients (APIs) into analogous ionic liquid forms (API-ILs) holds the potential to concurrently solve the increasingly commonplace problems of poor aqueous solubility and solid state instability of APIs. However, as this class of compounds mostly exist as viscous oils or waxes, handling, processing, and formulation are extremely challenging. Therefore, before these promising forms of active pharmaceutical ingredients (APIs) can be routinely incorporated into oral solid dosage forms (OSDs), questions remain as to how best to overcome their particularly poor material properties.

To address this, spray drying API-ILs with other excipients was found to be an excellent solidification method. This can be achieved in one of two ways by the appropriate selection of carrier material to form single or two-phase solid forms and was applicable to a range of API-ILs. Selecting an API-IL insoluble polymer (ethyl cellulose) to form two-phase systems allows for up to 75% w/w API-IL to be loaded into the polymer and recovered as a fine powder, whilst up to 90% w/w API-IL content can be recovered as a solidified paste. Alternatively, an exceptionally high glass transition temperature (Tg) polysaccharide (maltodextrin) can be used to form a single-phase solid solution with the API-IL. Using this approach, however, only allowed for 50% w/w loading of the API-IL in the polysaccharide to be successfully produced. This was due to Tg suppression becoming too substantial at higher loadings for a viable product to be recovered. Perhaps counterintuitively, this points towards the immiscible approach being more desirable for solidification of API-ILs.

As the intended use for the spray dried materials is in OSDs, dissolution studies were performed in representative dissolution media and showed that solidification of the API-IL in this manner had no detrimental effect on release characteristics, even when encapsulated in the immiscible and water insoluble polymer. These results demonstrate that API-ILs spray dried at high loadings in immiscible or high T_g materials preserve solubility and solid state stability enhancements of the API-IL form. At the same time, these approaches provide solid powders for processing and further formulation unit operations, and so represent an exciting new platform that allows API-ILs to fulfil their potential for formulating poorly soluble compounds as OSDs

Sequencing of mitochondrial DNA from single CHO cells reveals widespread heteroplasmy

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Background and novelty: Chinese Hamster Ovary (CHO) cells have a long history in the biopharmaceutical industry, producing some of the most effective medicines available today. To encourage homogeneity in drug product, biopharmaceuticals must be manufactured using a producer cell line derived from a single-cell clone. However, it's recognised that genetic and phenotypic heterogeneity between individual cells in a clonal CHO population tends to arise over time. Bulk analysis of CHO cell populations has shown that considerable variation can arise within the mtDNA sequence (heteroplasmy) which could have implications for the performance of the cell line in a bioprocess. By analysing the mtDNA differences of single cells within the same population, this heterogeneity can be mapped with greater resolution. This may help understand the impact of this variation in recombinant protein production and perhaps reveal genetic engineering targets for improved bioreactor performance. Here, we present a full workflow from cell culture to bioinformatic analysis and provide preliminary evidence of significant mtDNA heteroplasmy across a small panel of individual cells.

Results and Discussion: CHO single-cells were isolated by FACS into lysis buffer with emphasis on simple and reproducible gating. After optimisation of the lysis buffer, PCR kit and purification system, long-range PCR (LRPCR) cycle number was kept lower than any other published method. Subsequent Illumina library prep and iSeq sequencing provided input data to a bespoke bioinformatics pipeline. Preprocessing was performed in Linux and data analysis in R. We analysed 4 single cells to demonstrate the functionality of our protocol. Variant calling against the CHO-K1 reference sequence identified heteroplasmy within single cells. For example, a frameshift variant at nt11,431 was found to be shared between 2 single cells but not found in a mixed population, suggesting that some heteroplasmic variants may only be sporadically found in single cells above 2% frequency, but not evenly across a population. Finally we discuss the possibility for future investigations at a single-mitochondrial level.

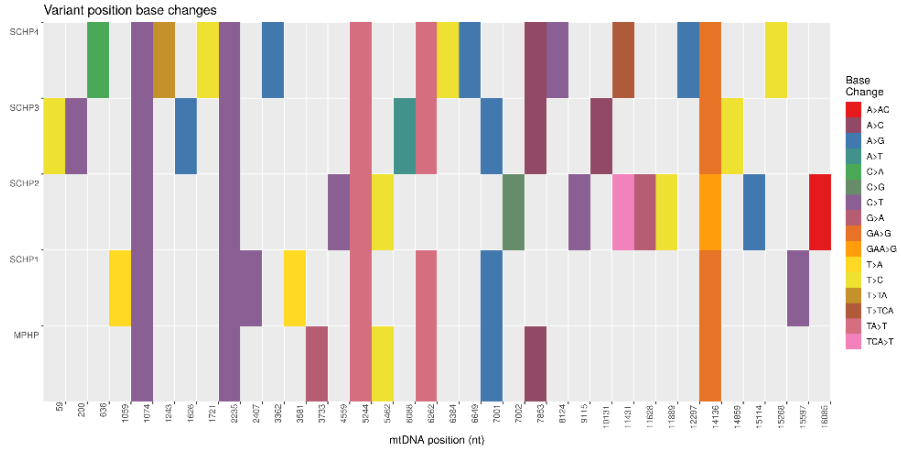

Figure: Variant position base changes in each sample. SCHP = single cell. MPHP = 4000 cells.

Smart Management of Combined Sewer Systems together with End-of-pipe Treatment Plants

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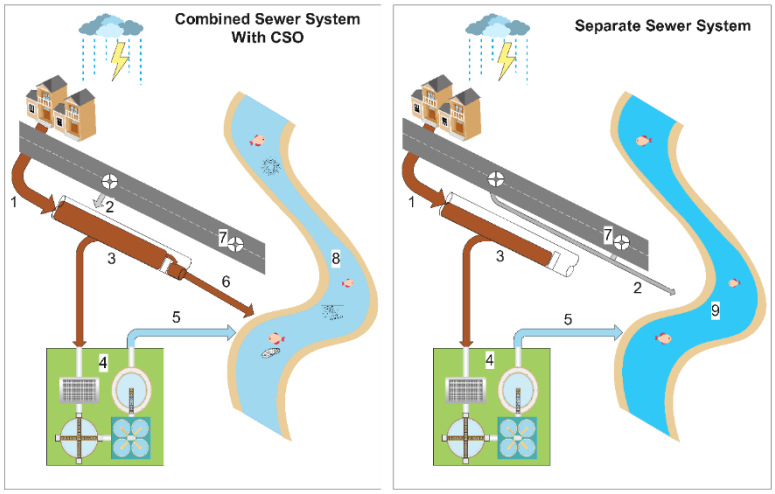
Sewer systems (Figure 1) are an essential part of sanitation infrastructure, but they have been under the pressure of climate change and increasing urbanization. In developing nations, the majority of sewer networks are combined sewer systems (CSS), and during wet weather conditions, intrusion of runoff into the pipes remarkably increase the flow which can exceed the capacity of the end-of-pipe wastewater treatment plant (WWTP). The surplus water, known as combined sewer overflow (CSO), contributes significantly to water contamination and has negative impacts on human health and receiving water bodies. Consequently, when CSSs overflow during heavy rains, untreated wastewater is released into nearby receiving waters via a bypass line within the WWTP. If there is no method to relieve the pressure, the sewer system will surcharge, and sewage will back up into buildings, clog storm drains, and flood streets. Extending WWTP capacity and restructuring sewage networks by segregating storm water conduits and wastewater pipes are two solutions for dealing with the overflows. However, due to changes in land use over the pipes, land restrictions in heavily urbanized areas, and simply economic reasons, expanding WWTP capacities and renewing the sewer system may not always be possible.



1. Wastewater
2. Stormwater
3. CSS
4. WWTP
5. WWTP Discharge
6. WW Bypass
7. Manhole
8. Polluted Receiving Waterbody
9. Receiving Waterbody

Figure 1: Combined and separate sewer system.

The broader scope of this PhD research is to investigate how modeling and artificial intelligence (AI) may be used to construct and operate resilient urban water infrastructures in a changing climate, as well as how AI can be utilized to mitigate CSO and stormwater. The main objectives of this project are

- To mathematically model the sewer network of a selected investigation site by using Stormwater Modeling Software (SWMM).
- To develop smart management options to mitigate CSOs by using AI and machine learning techniques.

Several scenarios will be simulated by varying rain intensity, duration and return periods, location of low impact development (LID) and grey-green infrastructures and the results will be evaluated by using statistical indicators. This will help to identify the optimum management strategy to mitigate the CSOs in the investigated region and to relive the WWTP from untreated wastewater by-passes.

Green Hydrogen in Gas Pipelines & the End-Users Limits

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In recent years, the development of renewables in energy systems has been targeted in many countries to support decarbonisation objectives. The European Green Deal frames Europe's response to climate change challenges and commits to delivering net-zero greenhouse gas emissions at the EU level by 2050 and an EU-wide GHG emissions reduction target of at least 55% for 2030. In line with EU ambition, the Irish Government's Programme commits to achieving a 51% reduction in Ireland's overall GHG emissions from 2021 to 2030, and to achieving net-zero emissions no later than 2050. These are challenging targets, in particular as Ireland has already missed targets for 2020. Gas networks are a key component of the global energy infrastructure, and Ireland's gas network provides almost one-third of all primary energy 40% of heating and more than half of Ireland's electricity generation. Ireland's gas transmission system is among the most developed gas networks in EU countries and it can make a significant contribution to decreasing emissions by providing a more sustainable and secure transition to a clean energy future. Natural gas is a cleaner alternative to conventional hydrocarbons such as oil and coal and can replace these fuels in heat and power generation. The gas network can also transport renewable gas such as green hydrogen and biomethane for long-term energy sustainability and decarbonisation of the gas supply.

The decarbonisation of gas networks incorporating hydrogen generated from excess renewable electricity is an exciting prospect, especially so in Ireland with an abundance of wind resources. However, the management of a gas network incorporating a new component that has different physical and chemical characteristics, coupled with varying production poses significant challenges for gas network operators. The main objective of this research is to ensure a reliable and safe supply of adequate energy to residential end-users while decarbonising the gas network. Dealing with varying hydrogen blends poses significant challenges for gas network operators to ensure hydrogen concentration meets the limits of end-user devices. At the first phase of the project, for the first time in Ireland, an experimental research investigates the effects of various hydrogen concentrations on common existing end-user devices found in the Irish gas network. The project will examine the potential range of hydrogen blends that may be incorporated into the Irish gas network from 2-20% Hydrogen. This research work will provide a detailed account of the tests and experiments that will be carried out as part of this project in the UCD Integrated energy Lab and the Gas Innovation Centre of Gas Network Ireland. This includes modelling of pressure drop in an example of distribution pipe rig, combustion and ignition temperature, and comparing the accuracy of meters before and after blending hydrogen through the pipelines. The second phase of the project aims to develop strategies to manage the incorporation of hydrogen production from renewable resources while at the same time ensuring the quality and quantity of energy supply for all gas end-users. Using the Irish gas network as a case study end-user and network parameters will be incorporated into the model to identify the locations and capacity to incorporate renewable hydrogen into the gas network. Further investigations evaluating green hydrogen storage and its capacity to maximise the decarbonisation potential will also be conducted.

Membrane bioreactors to produce polyhydroxyalkaonate (PHA) from CO_2

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Polyhydroxyalkanoate (PHA) is a promising alternative to petroleum-derived plastics due to their comparable physical and chemical properties and biodegradability. Many microorganisms can produce PHA as an intracellular energy and carbon storage material. Microorganisms such as *Cupriavidus necator* can metabolize CO_2 as a carbon source and produce PHA when a mixture of H_2, CO_2 and O_2 gas is supplied. Thus, it is possible to produce PHA directly from CO_2 which would reduce greenhouse gas emissions. However, the optimum gas composition ratio for cell growth is 7:2:1 for H_2:O_2:CO_2 which is within the gas-explosion range [1]. To eliminate the explosion risk, the oxygen concentration should be maintained below the lower explosion limit however this limits the growth and productivity due to oxygen limitation. Furthermore, gas fermentation faces substrate limitation due to the low solubility of gases in the culture medium. Membranes have the potential to help achieve high gas transfer efficiencies at low gas supply rates due to the high specific surface area available for transfer. Thus, membrane bioreactors are promising reactor systems for gas fermentation process. This work demonstrates the applicability of membrane bioreactors to deliver gaseous substrates for the production of PHA. To accomplish this, reusable systems were constructed using plastic centrifugal tubes and gas-permeable polydimethylsiloxane (PDMS) membrane fibres. Preliminary results indicate that comparable cell concentrations can be reached when membranes are used for provision of gaseous substrates. Further research will include investigating the effect of operational parameters such as the specific surface area of the fibers, composition of gas and recirculation flowrate on the production of PHA in a membrane bioreactor in order to develop a scale-up strategy.

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Degradation of an Organic Demon in Water Using Visible Light Photocatalysis

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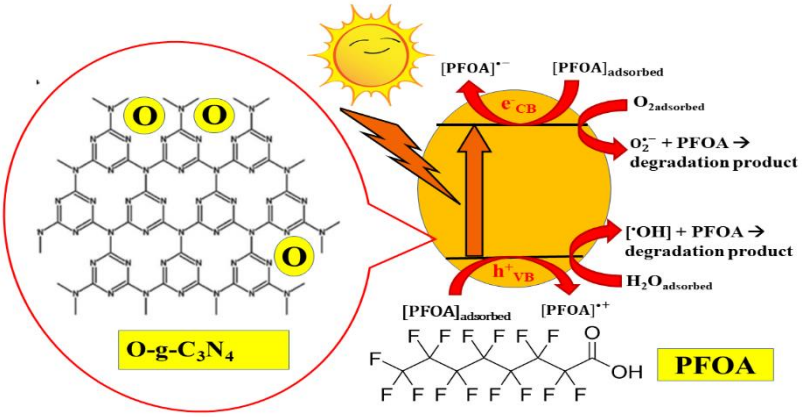
The scarcity of the world's freshwater supplies is a result of increasing level of water pollution, inadequate treatment of wastewater and removal of wetlands. Per and poly-fluoroalkyl substances (PFA), which are frequently used industrially as a fire retardant, surfactant and coating for metals remain un-remediated following traditional biological treatments of wastewater thus escape to pollute surface and ground waters [1]. Because of its persistency in the environment, several restrictive regulations and health advisories have been established by European Union Stockholm convention and the World Health Organization. Sunlight-driven photocatalysis is an emerging sustainable advanced oxidation process which can eliminate PFA from water at low costs. Metal-free graphitic carbon nitride (g-C_3N_4) has attracted intensive interest as a promising photocatalyst because of its narrow band gap, nontoxicity, high stability and low cost. However, in order to compensate low separation rate of photogenerated excitons and low solar utilization of pristine g-C_3N_4, a doping strategy using non-metal atoms is required. The degradation behaviour of perfluoroalkyls over g-C_3N_4 and modified g-C_3N_4 is not yet reported in the literature and therefore, this study reveals the possibility of using this catalyst for the photocatalytic degradation of perfluoroalkyl compounds in water. A model compound, perfluorooctanoic acid (PFOA), has been used here.

In this study, metal free oxygen doped graphitic carbon nitride (O-g-C_3N_4) photocatalysts were synthesized following pyrolysis of melamine and oxalic acid. The catalysts showed absorption in the visible range of solar spectrum. Compared to g-C_3N_4, the absorption edge of O-g-C_3N_4 showed a red shift, indicating that the visible-light absorption ability of O-g-C_3N_4 was significantly enhanced with increase in the oxygen content in g-C_3N_4. The planar structure g-C_3N_4 was destroyed in O-g-C_3N_4 which led to an n-$\pi*$ electron transition and hence, response range was expanded to the visible region. The morphology of g-C_3N_4 and O-g-C_3N_4 was characterized by XRD spectroscopy. The photocatalytic performance of 30%-O-doped g-C_3N_4 after 4 h of irradiation using a 700 W solar simulator showed 20% degradation of the 30 ppm PFOA present in water compared to negligible degradation in the presence of the pristine g-C_3N_4.



A cartoon of photocatalytic degradation of PFOA in water using solar light

Reference

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Designing nano-additive for Limestone based thermochemical energy storage to improve cycling performance

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For the replacement of fossil fuels to renewable energy sources, long-term and large-scale energy storage is required. Thermal energy storage has been identified as a commercially viable option for such purpose. In detail, thermochemical energy storage systems are considered the most promising among others, such as sensible and latent heat storage systems. Their uniqueness lies in their ability to store heat in the form of reversible chemical reactions. The reversible reaction of Limestone, as shown in Eq (1), to lime (CaO) and CO_2 at high temperatures (890 °C) is one of the most potential and effective thermochemical reaction due to its high enthalpy which is linked to high heat storage capacity. Unfortunately, this reaction suffers from incomplete reversibility (Limestone cycling). There are two steps involved in one complete cycle: 1. Desorption of CO_2 and 2. Absorption of CO_2. During the cycling process, Limestone encapsulates the unreacted lime, and therefore CO_2 diffusion is restricted due this formed product layer hindering the direct contact of CaO and CO_2[1]. In addition, the layer formation of Limestone on the lime grains, inhibits the free movement of necessary ions (such as, O^{2-}-, CO_3^{2-} and Ca^{2+}) which are crucial for the reaction completion[2]. My research focus is to use bi-metallic oxides in the form of nano-additives to: (i) achieve the complete reversible reaction of Limestone, and (ii) enhance the ion mobility of necessary ions.

$$CaCO_3(s) \leftrightarrow CaO(s) + CO_2(g)\ \Delta H_{890\,^\circ C} = 165.7\ kJ;\ \Delta S_{890\,^\circ C} = 143.0\ J\ K^{-1};\ \Delta G_{890\,^\circ C} \approx 0\ kJ \quad (1)$$

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Molecular-dynamics simulation of proteins in external electric fields

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The interaction between a protein and external electric field (EF) can alter its structure and dynamical behaviour, which has a potential impact on the biological function of proteins. With increasing attention focused on the potential damage of microwave-frequency radiation exposure to human health, as well as the development of nanotechnology industry, the effect of extraneous EFs on biomolecules is becoming a particularly pertinent issue. In this presentation, the application of EF in a wide variety of proteins' dynamical behaviour shall be discussed, including folding, dipolar response, absorption and agglomeration, where clear non-thermal field effects were observed by non-equilibrium molecular-dynamics simulations.

The prototype of the field-effect study on protein folding is a small artificial protein – chignolin, which only consists of 10 amino acids and can achieve fast-folding and conformational transitions. Compared to zero-field condition, the solvated chignolin undergoes dramatically different folding pathways and forms a new, quasi-stable structure in the presence of externally-applied EFs (both static and alternating). For larger proteins like prion, it is difficult to obtain complete folding information by fully deterministic sampling, since they need much longer folding periods and have more complex pathways. Biased sampling, such as ratchet-and-pawl molecular simulation, is a useful tool to find protein intermediates.

As a regulating agent between protein and the environment in *vivo*, water plays a fundamental part in the field response of proteins. By applying EF with a serial of intensity and frequency, the hydration dynamic of hen egg-white lysozyme (HEWL) and its hydration layer to EF was investigated, focusing on their different dipolar response.

Finally, future work, including the field effect on characteristics of insulin's absorption on gold surface and protein agglomeration will be discussed, which are motived by their potential application in bioengineering, and understanding microscopic mechanisms of field effects will help to improve current technology.

Characterization, Modelling and Optimization of the Cryopreservation and Revival of Mammalian Cells

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According to the European Medicines Agency (EMA): *"Advanced Therapy Medicinal Products (ATMPs) are medicines for human use that are based on genes, tissues or cells"*. They represent almost 300 products in development within the EU and their continuous use in upstream manufacturing is highly susceptible to change their genetic and epigenetic aspects over time. Therefore, the cryopreservation, which consists in the preservation of tissues and cells at cryogenic temperature, is used to mantain the aspects of the original cell line (WATSON & CADWEL, 2019; HUNT, 2019).

This study is going to focus on the modelling and optimization of cryopreservation and revival of mammalian cells. Many factors can influence the cryopreservation of cell lines, such as the protocol for temperature increase/decrease, the routine for cryoprotective agent (CPA) elution and the freezing cointainer system (MERYMAN, 2007; HUNT, 2019). Hence, in order to evaluate the process, we are going to apply mathematical modelling and parameters estimation routines to experimental data to comprehend how those variables can affect the solution vitrification or ice formation phenomena and to eventually damage the cell membrane due to osmotic stress or ice crystal growth (MERYMAN, 2007; PEGG, 2002).

The results from this study and its final optimization protocol could impact on the market lauching of new ATMPs, since they depend on continuous scientific developments and compliance to regulation (YURK, ESHUIS & WILBIK, 2021). It represents also an alternative to process intensification in a growing market: according to Visiongain (2021), the ATMPs are expected to reach an amount of U$ 59.91 billion in the global market by 2031.

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Next-gen glycoengineering: combining cellular and metabolic engineering to fine-tune mAb β1,4 galactosylation

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Key Words: Antibody glycoengineering, Metabolic engineering, CHO cells, Flow cytometry, LC-MS.

Background and novelty: N-linked galactosylation is a major source of heterogeneity in commercial monoclonal antibody (mAb) products [1]. In addition, higher levels of galactosylation are reported to enhance mAb effector functions (i.e., CDC and ADCC) [2]. Despite the clear need to tightly control mAb galactosylation, strategies to do so remain limited: standard cell engineering strategies are not amenable for real-time control applications, advanced cell engineering strategies require multiple time-consuming genetic engineering events [3, 4], and metabolic glycoengineering strategies achieve only a narrow range of control at the expense of cell growth and product yield [5, 6]. Here, we present a novel approach that enables real-time galactosylation control across a broad range by feeding a decoy substrate, 2-deoxy-2-fluoro-d-galactose (2FG), to CHO cells that have been engineered to produce hypergalactosylated mAb product.
Experimental approach: CHO-DP12 cells producing a 93% galactosylated mAb were cultured in fed-batch mode, where 2FG was fed (10% on day 3 and 90% on day 5) to achieve concentrations ranging from 0.1mM to 2mM. Daily samples were collected to monitor cell growth and metabolic behaviour, while product titre was quantified with Protein A HPLC. On day 6 of the culture, lectin-aided flow cytometry was used to monitor cell surface galactosylation and samples were taken for mAb LC-MS glycoprofiling [7].
Results and discussion: Our glycoengineering strategy achieved dose-dependent control of mAb galactosylation ranging from 45% to 93% (Figure 1). No detrimental effect of 2FG feeding on cell growth and mAb titre was observed with up to 2mM of 2FG. With increasing 2FG concentrations, we observed accumulation of undesired aglycosylated mAb product. Upon further analysis, the lowest achieved level of mAb galactosylation (45% galactosylated species) was observed to occur because half of all mAb (at 93% galactosylation) is produced before the first 2FG feed and not due to limited 2FG activity

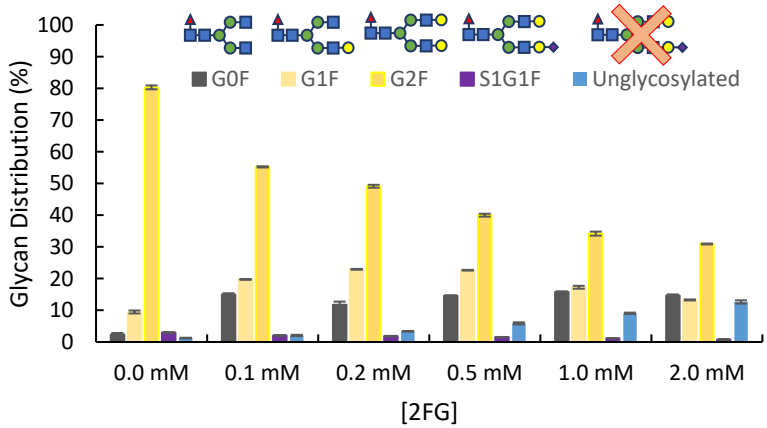

Figure 1. Glycan distribution of the produced mAb with increased concentrations of 2FG.

With further development, our novel mAb glycoengineering strategy can be deployed for real-time control of mAb glycosylation and contribute to the assurance of a critical quality attribute in biopharmaceutical manufacturing.

Acknowledgments
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