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BOOK of ABSTRACTS

ORAL PRESENTATIONS

Lectin-aided flow cytometry reveals a close correlation between cell surface and mAb glycosylation

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Glycosylation is a critical quality attribute (CQA) of monoclonal antibodies (mAbs) because it is crucial for the safety and efficacy of these life-saving biopharmaceuticals [1, 2]. Rapid and robust methods for monitoring mAb glycosylation will facilitate dynamic control strategies that ensure optimal product quality and yield [3]. This work presents a rapid and simple method to quantitatively monitor the critical quality determinants of mAb N-glycosylation (fucosylation and galactosylation) using measurements of cell surface glycosylation with lectin-aided flow cytometry (FC).

A mAb-producing CHO cell line (DP12-GalMax) that has been engineered to achieve >95% mAb β4-galactosylation and >95% α6-fucosylation has been fed with different doses of fluorinated analogues of galactose [4] (2-deoxy-2-fluoro-d-galactose, 2FG) and fucose (2-deoxy-2-fluoro-l-fucose, 2FF). CHO DP12-GalMax cells were fed with 10% 2FG on day 3 and 90% on day 5 of culture at concentrations from 0 to 2mM or with 100% of 2FF at the beginning of culture at concentrations between 0 and 1mM to achieve different levels of each of galactosylation and fucosylation, respectively. Samples for FC and mAb glycoprofiling were taken on day 6 of culture. To analyse cell surface β4-galactosylation for the 2FG experiments, cells were incubated at 37°C for 15 minutes with 33 µg/ml of Texas Red-labelled *Erythrina cristagalli* lectin (EyLabs, CA, USA). For cell surface α6-fucosylation, cells were incubated at room temperature for 30 minutes with 8 µg/ml of the recombinant RPL-Fuc1-Dylight 488 lectin (GlycoSelect, IE). FC on the stained cells was performed on a Beckman Coulter Cytoflex LX. The excitation/emission wavelengths were 561nm/610nm (2FG) and 488nm/525nm (2FF). Glycoprofiling was performed on the mAb product using the LC-MS method developed by Carillo et al., 2020 [5]. Cell density and viability were evaluated using trypan blue dye exclusion.

2FG feeding caused a dose-dependent decrease in both cell surface and mAb β4-galactosylation and presented a strong linear correlation (Pearson's r^2=0.811) between both measurements (Figure1). A strong linear trend (Pearson's r^2=0.995) in concomitant dose-dependent reduction in α6-fucosylation was observed with increasing 2FF feeding (Figure 2). The robustness of the observed correlations suggests that lectin-aided flow cytometry can be used to rapidly monitor the β4-galactosylation and α6-fucosylation of mAbs.

To validate the robustness of the correlation between the cell surface and mAb product galactosylation, another engineered CHO cell line (VRC-GalMax) was fed with the 2FG inhibitor. Based on the cumulative mAb titer and cell density of CHO-VRC-GalMax, cells were fed on days 3, 4, and 5 at a range between 10-80 pg/cell of 2FG. The samples were collected for FC and mAb glycoprofiling on day 6. Once again, 2FG feeding caused a dose-depended decrease in cell surface and mAb β4-galactosylation. We these results, we conclude that our Lectin-FC is an attractive alternative to traditional glycoprofiling because its

associated sample preparation and data acquisition takes 2 hours and costs approximately €10 per sample, which is considerably shorter and less costly than traditional glycoprofiling (~4 hours and ~€50 per sample).

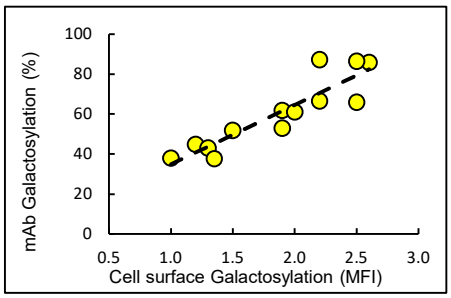

Figure 2. Correlation between cell surface and mAb β4-galactosylation

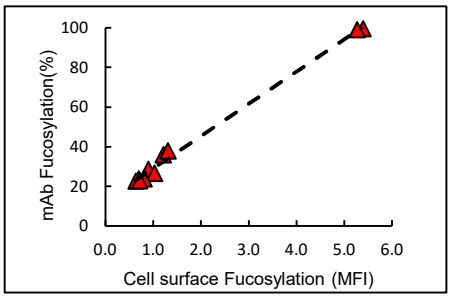

Figure 1. Correlation between cell surface and mAb α6-fucosylation

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Upcycling natural limestone waste for thermochemical energy storage by utilising tailored $CaZrO_3$ nanoadditives

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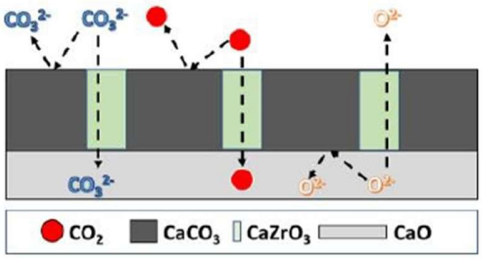
The development of long-term renewable energy storage systems is crucial for decarbonising the energy sector and enabling the transition to a sustainable energy future. Thermochemical energy storage (TCES) systems are well suited for the long-term renewable energy storage as the materials used in these systems have high energy densities, and long storage duration. Among the plethora of TCES materials, calcium carbonate (Limestone) is of particular interest since it exhibits a high enthalpy of reaction, and is Earth-abundant. The main problem with Limestone inhibiting its commercial application for long-term renewable energy storage is its deteriorating cycling performance after several energy charge/discharge cycles [1]. In this study, two $CaZrO_3$ nanoadditives with two different Ca:Zr ratios and tailored oxygen vacancies were synthesised by a precipitation method, and mixed with Limestone waste at three weight concentrations (5,10 and 20 wt%). Their phase, chemical state, and morphology was determined by XRD, XPS and TEM, respectively. The cycling performance of the mixture samples was determined through thermogravimetric analysis. The best-performing sample was the one mixed with 20% $CaZrO_3$ nanoadditives, which contained a large number of oxygen vacancies and thus enhanced ionic conductivity [2], as confirmed by density functional theory (DFT) calculations. This sample exhibited the best effective conversion and the highest energy density values of 0.7 and 2640 kJ/kg, respectively, after 40 cycles.



Graphical abstract: Facilitating the carbonation reaction of Limestone waste by using $CaZrO_3$ nanoadditives.

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Integrated control in pharmaceutical manufacturing

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As the pharmaceutical industry makes a shift towards implementing continuous manufacturing across multiple processes, it is important to have advanced techniques for modelling and control. Moreover, as processes now run continuously for longer periods of time, it is important to investigate the temporal dynamics of process variables to get a better understanding of how the controller needs to learn these dynamics and adapt to them as process time increases. In this study, the modelling capabilities of different machine learning algorithms are compared for temperature data obtained from the static mixer. After stationary modelling, the dataset is converted into a time-shifted time-series dataset to analyse the modelling capabilities of a simple machine learning model for time-dependent data. Future work for this project involves employing a type of recurrent neural network called long short term memory networks to improve the time-series model and also integrate first-principles models into these neural networks.

Structural, dynamical and dielectric properties of water in contact with TiO_2 surfaces via molecular-dynamics simulations.

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Undoubtedly, enhancing our knowledge of the dielectric response, structural, translational-librational and dynamics properties of water either in bulk environment or in its interfacial guise are required to provide detailed understanding of the fundamental properties crucial for the development of more efficient and improved control of photo-energy conversion devices, especially those based on photo-electrochemical (PEC) water splitting. To this end, classical equilibrium molecular-dynamics simulations have been performed to rationalise the intriguing behaviour of bulk water and, in particular, interfacial hydration water layers with both rutile (110) and anatase (101) surfaces of titanium dioxide (TiO_2). The dielectric characteristics of interfacial water in the first-adsorbed water layer evince a markedly reduced value in comparison to the bulk water environment, as well as lower self-diffusivity (owing to surface-confinement effects). In addition, this work presents evidence describing behavioural pattern of adsorbed hydration layers which reflects similarities observed in the underlying surface-identity mechanism of titania slabs used, and on how surface atomistic architecture – indeed, "surface personality" – dictates to a large extent the dielectric "matching" of hydration-water layers adsorbed thereon. Further analyses show evidence of strong-roles underpinning the hydrogen-bond network at the water/titania interfaces.

The exploration of HEK293 cell line engineering strategies to optimize rAAV production for gene therapy application

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Adeno-associated virus (AAV) is a versatile viral vector technology that can be engineered for specific functionality in gene therapy. Genetic diseases are caused by absent or defective genes and gene therapy aims to treat these conditions by delivering a functional copy of the affected gene into a patient's cells (Gonçalves and Paiva, 2017). Gene therapies utilizing AAV as a vector system are emerging as novel therapeutics that have the potential to lead to substantial disease modification of many monogenic disorders (Kuzmin *et al.*, 2021). However, manufacturing these vectors at high quantities and fulfilling current good manufacturing practices (GMP) is still a challenge. High production costs, scalability restrictions, sub-optimal yields and contamination with empty capsids, or mis-packaged DNA are common recurring issues. In this regard, every aspect of the virus, the transgene and the producing cell line must be examined to find potential avenues of improvement to either increase production yield or make it more cost effective. AAV-based treatments often require the administration of very high amounts of virus to deliver sufficient levels of the therapeutic gene to the right cells/organ to achieve a therapeutic effect(Donohue *et al.*, 2021). For example, the delivery of factor VIII gene to the liver for haemophilia treatment. Part of the challenge of systemic delivery is that the immune system will clear a great deal of virus before it gets to the target cells, hence the need for large dosages. This is a major manufacturing challenge and so there is a need for improved production systems or improved AAV tropism to target specific cells and organs more efficiently. The majority of early vector production protocols relied on AAV production in adherent HEK 293 cells in the presence of serum (Hildinger *et al.*, 2007). This technology is challenging to scale-up. The use of a HEK293 suspension system is advantageous as cells grown in suspension are highly scalable and are also usually adapted to grow without the addition of serum or other animal-derived components, making them safer and easier to adapt to cGMP production(Blessing *et al.*, 2018). Extensive research has been devoted to improving the genetic components needed for AAV assembly with little focus placed on the host producer cell itself. This work focuses on the optimization of a HEK293 cell line suspension system in the production of rAAV. A combination of directed evolution approaches and physiochemical parameter optimization were explored to study the impact on rAAV production efficiency in HEK293 production cells, to aid in the development of optimized production systems.

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Development of approaches that enable demand response in wastewater treatment plants

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A high share of renewable energy sources (RES) in electricity calls for flexible options that can balance fluctuating wind and solar power production. Energy and water systems are highly interconnected, but they are often operated independently. Wastewater treatment plants (WWTPs) are among the largest electricity consumers in the municipal sector. Furthermore, they have the capability to stabilize energy grids with their existing aggregates and promote the integration of RES into the energy system. WWTPs are able to handle different energy forms i.e. electricity, heat, biogas, etc., and they are flexible energy prosumers. Therefore, there is an urgent need to investigate the different coupling opportunities for WWTPs and energy systems to benefit from their cooperation. However, due to a sudden increase in the number of innovative wastewater treatment technologies and various integration opportunities with the energy grid, it is impossible to evaluate all scenarios on a real scale. Mathematical models that can holistically evaluate the impact of various integration options on WWTPs and energy systems are required. This study aims to investigate the role of WWTPs on the decarbonization of the energy grid and to assess the coupling opportunities for WWTPs and energy systems for a possible increase in renewables share in the energy grid. To accomplish this, a generic WWTP model will be designed and subsequently integrated into the Spine Toolbox (open source energy system modelling framework), in which the energy systems are modelled. Several integration scenarios such as power to heat (e.g. waste heat from combined heat and power plant units) and power to gas (e.g. direct feed-in of methane into the local gas grid and use of green hydrogen for biological methanization) will be applied to the integrated WWTP and energy systems model in order to investigate the effect of coupling scenarios on the energy grid in terms of promoting the energy transition from to renewables and decreasing the overall energy consumption of the wastewater treatment plants. Furthermore, the overall impact of innovative WWTP technologies on greenhouse gas emissions will be evaluated.

Scalable liquid phase RNA synthesis enhanced by membrane separation

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RNA based gene therapies are on the rise and it is debatable if solid-phase synthesis cannot supply therapeutic oligonucleotides on a ton scale. Liquid phase oligonucleotide synthesis (LPOS) is a possible scalable alternative. The aim of this project is to develop a scalable liquid phase oligonucleotide synthesis (LPOS) platform my organic solvent nanofiltration (OSN). In this work a growing oligonucleotide was tethered to a soluble 4-arm PEG support where reactions are be carried out in liquid phase with intermediate purification performed by a commercially available ceramic OSN membrane. To simplify the process and reduce the number of unit operations a one-pot LPOS (OP-LPOS) method was developed to telescope all reaction steps. Products were analysed by NMR, MALDI-MS and LC-MS. A tetramer phosphorothioate oligonucleotide was produced using this method with stepwise yields of 84-94% and close to quantitative product rejection (99.7-99.9%) by the membrane.

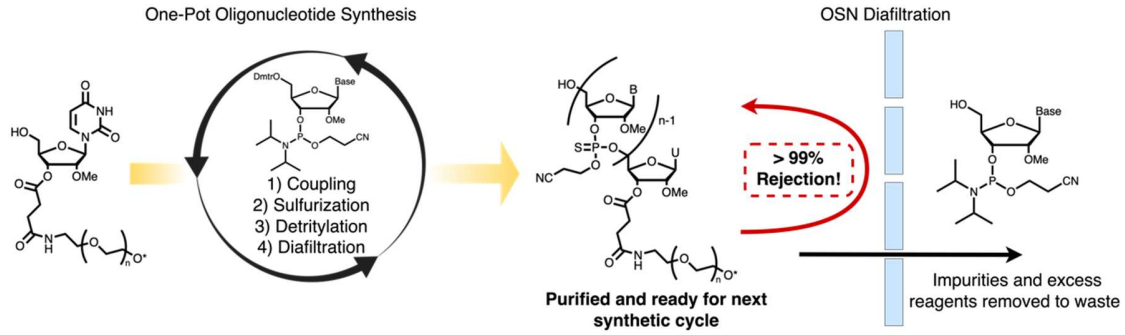

Figure 3: Schematic representation of OP-LPOS with OSN intermediate purification

Development of a platform for the purification of viral vector systems for the manufacture of vaccines and gene therapies

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Cell and gene therapies (CGTs) and novel vaccines are an emerging, rapidly growing area due to their potential to address unmet medical needs. The European Medicines Agency (EMA) refers to these as Advanced therapy medicinal products (ATMPs). [1] The FDA expects 10 CGT products a year to be approved by 2025 and Ireland is well-positioned to expand its manufacturing capabilities into this area.

Adeno-associated viruses (AAV) have become a leading platform for both gene and vaccination delivery to treat and prevent various diseases due to its excellent safety profile and efficient transduction to various target tissues. [2] Since their discovery in 1965, they have become one of the most actively investigated gene therapy vehicles. [3] AAVs are widely used in gene therapy applications with several advantages over other viral vectors including a lack of pathogenicity in humans and the availability of over 150 naturally occurring genotypes and serotypes. Although much work has progressed on the upstream production of these viral vectors, the downstream purification is far less developed.

Downstream purification of viral vectors presents several challenges with the most significant being the limitations in the purification technologies that exist. Although there are methods to purify these, there are drawbacks to existing processes including a lack of scalability or the requirement of many inefficient steps to get the final purified product. Gene therapies frequently require a high dose meaning that scalability is an important factor when developing an appropriate process for commercial supply. Therefore, currently there is significant focus, both within industry and academia on addressing the challenging purification requirements for these biotherapeutics. [4] The lack of a systemic design approach makes efficient and reliable process development difficult to achieve. This presentation will outline the approach to developing a platform approach to the design and scale-up of chromatography operations for the large-scale purification of viral particles for vaccine and gene therapy applications.

Acknowledgements
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Assessment of green hydrogen technology deployment in a long term for large scale power production

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Variable nature of wind energy generation leads to curtailments in electrical grid, especially during low electricity demand and high wind availability periods. Power-to-hydrogen is a promising option to capture the surplus wind energy in the form of green hydrogen produced via water electrolysis. To support the electrical grid at times of low renewable penetration existing gas power plants can be modified to burn blends of hydrogen and natural gas or alternatively hydrogen can be used directly in fuel cells. While modification of existing infrastructure is more competitive in the current market conditions. However, assessment of the technologies is also required, based on the long term approach where the carbon emissions, decline in technology cost, and potential supporting policies are considered. Therefore, the study aims to determine the conditions that will favour fuel cell deployment over the turbine upgrades for blended gas throughout the energy system transition period.

A Large-scale electrical grid and gas network combination is modelled as the overall energy system. Simple cost optimization model is developed considering capital and operational costs, fuel prices, technology features, forecasted electricity demands and emission targets. Gas turbines and fuel cell technology are the options for power production, where wind capacity is the renewable electricity production and grid connected battery storage is also available. Hydrogen supply sources are electrolyzer production and external imports. Electricity demand and renewable electricity sources availability are based on the hourly data for All-Ireland. The model is used to analyse the influences of financial parameters and carbon reduction targets into the optimal infrastructure requirements for 2050 scenarios. The model will be further developed to include various infrastructure locations, potential incentives, and emission reduction strategies. The specific focus of the study will be to analyse the conditions that promote hydrogen usage in fuel cells versus blended gas in turbines.

The conclusions obtained from the study is expected to add value for investment decisions and policies regarding the role of green hydrogen in supporting the energy transition.

The stability of monoclonal antibodies and their high galactose glycoforms

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Monoclonal antibody (mAb) therapeutics are the leading class of biopharmaceutical products, comprising approximately 40% of FDA approvals each year [1]. For FDA approval, demonstration of appropriate fragment crystallisable (Fc) N-glycosylation of mAbs is a critical quality attribute that must be monitored during their production, as glycosylation has been directly linked to the efficacy and safety of mAb based drug products. There is limited available literature surrounding the impact that differing glycan profiles have on the solution stability and aggregation propensity of mAb proteins. By monitoring interprotein interactions, the factors leading to changes in protein self-assembly due to glycans can be better understood, providing insight into the aggregation propensity of these mAbs [2]. Glycans are theorised to affect interprotein interactions through multiple methods, such as masking hydrophobic regions and therefore reducing the hydrophobic effect, impacting electrostatic interactions and the protein isoelectric point, creating additional repulsive interactions through hydration forces, or upsetting orientation specific interactions through steric repulsion [3, 4].

We have produced four different human immunoglobulin G1 (IgG1) proteins, the most common protein used in mAb therapeutics, from Chinese hamster ovary (CHO) cell lines. DP12 parental IgG1 and DP12 GalMax IgG1 contain N-glycans on the Fc region, while VRC01 parental IgG1 and VRC01 GalMax IgG1 contain additional N-glycans on the Fab region of the IgG1 molecules. Solution stability for each protein was determined by dynamic light scattering (DLS) analysis and a polyethylene glycol (PEG) solubility assay. Mass Spectrometry glycan analysis showed a significant increase in galactose containing Fc glycans for IgG1 produced from engineered GalMax cell lines compared to Fc glycans from parental cell lines. k_D values from DLS measurements indicate a minor decrease of between 1 and 2 k_D units for Fab glycosylated protein, suggesting an increase in net attractive protein-protein interactions. A significant shift of between 8°C - 10°C in the PEG solubility assay between Fab and non-Fab glycosylated IgG1 is also reported, also indicating a significant increase in net attractive protein-protein interactions for Fab glycosylated protein. This observed increase in net attractive protein-protein interactions is hypothesised to be due to the presence of the complex, sialylated N-glycans on the Fab region of VRC01 IgG1, resulting in an increase to the aggregation propensity of this protein.

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BOOK of ABSTRACTS

POSTER PRESENTATIONS

Maximizing biopharmaceutical α-2,6 -sialylation through CHO cell glycoengineering

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The Global Biopharmaceutical Market is ever-expanding, estimated to be US$330 bn in 2021, and is expected to reach US$478 bn by 2026 (7.65% CAGR) [1]. Monoclonal antibodies (mAbs) have emerged as a crucial component in the treatment of various illnesses, including cancer and autoimmune disorders, due to their high specificity and therapeutic benefits [2]. These artificially produced glycoproteins possess remarkable abilities to target specific cells and molecules, leading to effective therapeutic outcomes with reduced secondary effects. Glycosylation is the primary source of variability in antibodies [3], with sialylation playing a crucial role in their therapeutic mechanisms. α2,6-linked sialylation can improve the solubility [4], anti-inflammatory activity [4,5], thermal stability, and serum half-life [6] of mAbs. This project aims to maximize α6-sialylation through CHO cell glycoengineering to improve anti-inflammatory and pharmacokinetic properties. The proposed approach builds upon the GalMAX technology that has been previously developed in UCD's ACTG group to enhance galactosylation. The objective is to eliminate α-2,3 sialylation and ectopically express human α-2,6 sialyltransferase to achieve optimal α-2,6 sialylation in CHO cells. Currently, the work in progress involves knocking out the ST3GAL3, ST3GAL4, and ST3GAL6 genes, which encode α2,3 sialyltransferase [7] respectively, by utilizing the CRISPR/Cas 9 genome editing technique. To achieve this, sgRNA design and plasmid construction were executed via USER cloning[8]. Furthermore, we are developing a lectin-assisted flow cytometry method to quantify cell surface sialylation in order to phenotypically characterize the 2,3 K.O. lines and simplify cell line selection. After the lines have been characterized, the next step will be to isolate and purify the product followed by mass spectrometry-based glycan analysis.

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Dial-A-Sugar: Developing actuators for real-time mAb glycosylation control in CHO Cells

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Glycosylation is defined as the attachment of carbohydrates or sugars to the backbone of a protein through an enzymatic reaction and is a form of co-translational or post-translational modification [1]. Due to the numerous variations at the end of glycosylation reactions, the macroheterogeneity (i.e., presence or absence of glycans) and microheterogeneity (i.e., glycosidic linkages and varying degrees of mannosylation, antennarity, core fucosylation, galactosylation, and sialylation) on mAbs heavily influences the safety and efficacy of protein therapeutics [2]. Therefore, Dial-A-Sugar aims to control the biopharmaceutical manufacturing processes by integrating sophisticated computational modelling with cutting-edge synthetic biology tools to produce mAbs containing optimal and consistent glycosylation for safe, efficacious, and cost-effective treatments.

The framework combines all components of Model Predictive Control (MPC): (i) Actuators that enable real-time tuning of mAb glycosylation; (ii) Sensors that analyse cell surface glycosylation; and (iii) a computational model which relates cell culture inputs with mAb synthesis and glycosylation. The focus of this doctorate is on the actuators, where the expression of two glycosylation enzymes, α-1,6 Fucosyltransferase (Fut8) and β1,4-Galactosyltransferase (βGalT1), will be controlled in real-time using lineariser inducible transcriptional circuits.

Dial-A-Sugar will utilise two negatively autoregulated lineariser gene circuits: (i) TetR_Lin; and (ii) PhlF_Lin [3-5]. Both linearisers were chosen as they display highly favourable fold induction and linear dose response [3]. The linearisers will contain a gene of interest (GOI) (either Fut8 or βGalT1) and a repressor protein gene (either TetR or PhlF). Addition of small molecular inducers (SMI) will unbind repressor proteins from the operons to enable transcription of itself and the GOI.

To evaluate the performance of TetR_Lin, the lineariser was stably integrated into the genome of the host cell (CHO-DP12) by random integration, and the performance of the lineariser was assessed. Two regions of linear response between Dox (SMI) concentration and eGFP expression was observed, and results showed a polyclonal population of CHO_TetRLin cells with a compromised basal expression at least one order of magnitude greater than published in literature [3]. In addition, a lower induction range with a linear dose response was observed. We hypothesise that the observed deviations in lineariser performance are due to stoichiometric imbalances between TetR and the GOI, which likely arise due to random integration and polyclonality of the characterised cells.

Therefore, a landing pad system was deemed to be the best solution to aid in controlling the location of gene integration and copy number [6] to ensure stoichiometric equivalence of TetR and the GOI. Two landing pad vectors for safe harbour sites A and T9 have been designed and are currently undergoing construction using a molecular cloning technique known as Gibson Assembly [7]. This path forward was chosen to allow a single copy of the lineariser circuit to be integrated within the genome to, ultimately,

minimise the basal level of GOI expression while maximising fold-induction and linearity of the inducible transcriptional circuit.

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Thermal stability of mRNA enhanced by ribose 2'OH acylation

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The stability limitations of mRNA present serious challenges for the development, analysis, long-term storage and transportation of emerging vaccines and therapeutics. Degradation of this vital biomolecule over time can arise both from spontaneous thermal fragmentation as well as the presence of ubiquitous ribonuclease enzymes. Mechanisms of mRNA degradation are predominantly instigated by the 2'-hydroxyl (2'OH) functional group of the ribose ring unit, which exists in a rapid preequilibrium with an oxyanion state of high nucleophilicity. Hence, designing an inhibitory route to selectively protect the 2'OH functional group of mRNA would allow for enhanced thermal and enzymatic stability with extended functional half-lives (Figure 1).

Here, we present 2'OH acylation as a protecting group strategy in the development of chemically-modified mRNA (cmRNA) using a green fluorescent protein model.[1] The analysis of cmRNA integrity *via* capillary electrophoresis would allow for the structural optimisation of various acyl-adducts as well as a comparative insight into the extent of thermal GFP mRNA degradation.

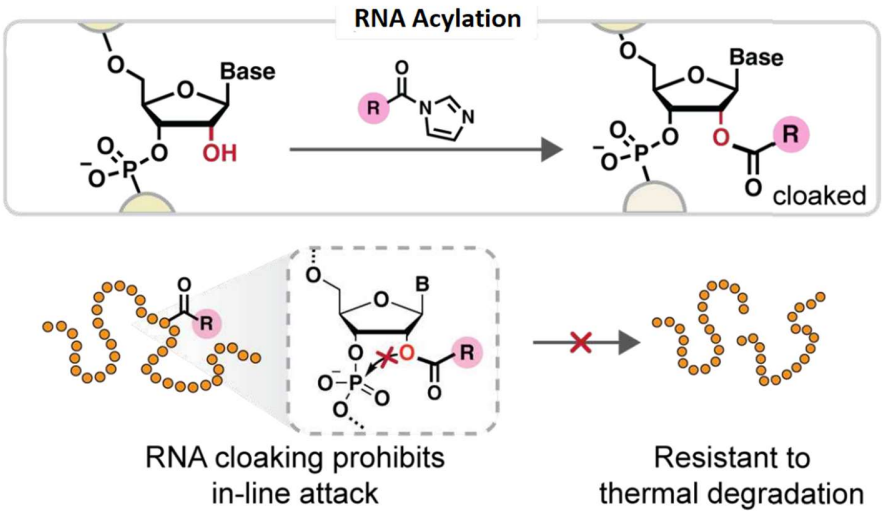

Figure 1: Mechanism of post-synthetic 2'OH acylation to produce chemically-modified mRNA with increased resistance to thermal degradation. Reproduced from Fang et al. (2022) and free to use under a Creative Commons Attribution 4.0 International License.

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Role of calcium in the anaerobic treatment of wastewater

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Anaerobic digestion of organic matter is a process successfully implemented for the treatment of municipal and industrial wastewaters. In this process, microorganisms (bacteria and archaea) break down the organic matter in the absence of oxygen to transform it into new cells and products such as biofertilizers and renewable fuel (biogas) (Kennedy & Lentz, 2000). The state-of-the-art technology for the abatement of high strength wastewaters containing low amounts of particulate matter is the high rate granular sludge anaerobic reactors. The success of these reactors lies in the biomass granulation process, which it can be defined as the aggregation of several microorganisms to form a colony that provides them benefits, i.e. substrate access and protection, over the other microbes (Litser & Ennis, 2004).

The biomass granulation is a complex phenomenon that is governed by several factors. One of the factors that has an extensive role is the concentration of calcium cation (Ca^{2+}). This is because it can enhance the granulation process through ionic bridging and binding of exocellular polymers (Tiwari et al., 2006), and it may play an important function in microbial aggregation (Kosaric & Blaszczyk, 1990). However, high calcium concentration can be detrimental for granulation, and therefore anaerobic treatment. During the methanogenesis phase in the anaerobic digestion process, carbonate ions are produced via inorganic carbon chemistry, which can form precipitates such as calcium carbonate ($CaCO_3$) with calcium (Batstone et al., 2002). Calcium precipitation is undesirable because it can result in negative effects on the process such as the formation of too heavy sludge, inhibition of methanogenic activity, nutrient deficiency, scaling of reactor walls and effluent pipes, and space occupation by inorganic precipitates (Hammes, Seka, De Knijf, & Verstraete, 2003; Van Langerak, 1998; Van Langerak, Hamelers, & Lettinga, 1997).

Research on the presence of calcium in anaerobic wastewater treatment is still in progress. The aim of this research project is to improve the performance of the anaerobic treatment process downstream of a dairy industry by controlling and preventing calcium carbonate precipitation. For this, batch and continuous laboratory scale experimental tests will be carried out, together with the operation of a pilot scale IC reactor to replicate the performance of large scale reactors. In addition, a mathematical model will be developed to obtain a greater understanding of the bioprocess and the model will be used to improve operational efficiency. The model will be validated using data from pilot and full-scale anaerobic reactors.

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The monitoring and optimization of cell culture medium preparation to support bioprocess intensification.

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The aim of this research project is to investigate the application of an image-based process analytical technology (PAT) tool to the real-time monitoring of media prep processes, which can be used to develop a fundamental understanding of the process and inform development and optimization activities. The demands on cell culture medium are ever increasing from process intensification in order to meet current and future pharmaceutical demands. Cell culture medium must supply all nutrients to support cellular growth and production. The greater the cell density and product titre in a process, the greater the nutrient demand and the more challenging it is to achieve and maintain dissolution of the required concentrations of nutrients. Medium preparation is affected by parameters such as mixing time, temperature, pH and addition order of the constituent components. Currently process development relies on trial and error due to the challenges associated with monitoring dissolution endpoint. This project aims to develop the application of image analysis-based process monitoring using Canty's image analysis system as a PAT tool to track the progression and endpoint of dissolution of medium components during the preparation process. The application will support the intensification of cell culture processes requiring media enrichment. The process understanding provided by the Canty technology will be used to develop a process model for media prep which will be used as a digital twin in order to optimize cell culture media processes. The digital twin will be used to perform in silico experiments in order to propose an optimization strategy that will ensure robust, reliable media prep at scale. Technologies such as Malvern and focussed beam reflectance measurement will be used to measure particle size for comparison to the Canty system's output. The information will be used both to develop and validate the Canty method and ensure confidence in its validity and performance. The optimized conditions determined using the model will be experimentally verified. The project will primarily focus on the use of the NISTCHO cell line which produces an IgG monoclonal antibody and its associated basal and feed medias for cell culture. The raw materials i.e. media components will be characterized. The quality of the media prepared will be determined using a combination of analytical methods e.g. HPLC for the determination of amino acid concentration and functional testing. A Design-Of-Experiment approach will be taken to the experimental mapping of the media prep design space in order to maximise the information gained per experiment.

Characterization, modelling and optimization of the cryopreservation and revival of mammalian cells

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The cryopreservation and revival of mammalian cells represent a fundamental step for the manufacturing of biopharmaceutical products and Advanced Therapy Medicinal Products (ATMPs). However, while efforts in intensification of biopharmaceutical processes have been motivated by the need to reduce associated costs, increasing efficiency and the shortening of product cycle-time (i.e., it has been demonstrated that the inoculum production can be reduced by 35% compared to the standard process by increasing the freezing cell density up to 260 x 10^6 cells/ml[1]), the cryopreservation process faces challenges regarding the overall efficiency and capacity. This is due to ice formation and crystallization which can mechanically damage the cells; the usage of cryoprotective agents (CPAs) which can be cytotoxic, and the fluctuations of osmotic balance throughout the phase change which can affect the viability and integrity of cells[2].

Empirical optimization of cryopreservation protocols has been widely discussed in literature for different cell lines and modelling of ice formation inside biological membranes was first reported in the 1980s. However, there is still a lack in fundamental understanding as to the relationship between cryopreservation conditions and the physiological state of the cell upon subsequent thaw.

The aim of this project is to combine computational models with experimental approaches to mechanistically understand the effect of key variables to the process in order to support process intensification and product supply for biopharmaceuticals and advanced therapies respectively. The effect of key variables (i.e., cooling and warming rates, sample volume and freezing cell density) on cell's viability and functionality after the cryopreservation process will be investigated. Initial work will focus on a CHO-K1 cell line as an industrially relevant model. Volume will vary from 1 ml in cryovials to 250 mls in cryobags and the cell density will be varied from 1 x 10^7 cells/ml to greater values. Trypan blue exclusion method based cell counting for out-of-freeze cell viability quantification and flow cytometric analysis for apoptotic and programmed cell death markers, and mitochondrial potential will be used to determine the impact of the cryopreservation step on the CHO cell line.

The experimental data will inform the development of a predictive model to describe the impact of the freezing process on the post-thaw condition of cells by combining deterministic and data-driven methods i.e., hybrid modelling in MatlabR2022a®. While the deterministic approach is built upon Finite Element Modelling (FEM) with differential equations of energy and mass balance over a control volume designed in AutoCAD2023®, the data-driven method establishes the connection between the process conditions and environment to the cell quality attributes.

Ultimately the model developed with be used for the *in silico* optimization of the cryopreservation step, accelerating the development of a robust high-performing freezing process.

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Ammonia removal and recovery from animal manure; State of art and prospects

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The intensification of livestock-based agricultural activities has resulted in the production of large volumes of ammonia-containing manure. Agricultural activities also require ammonia as a fertiliser for crop production. The pumping and spreading of animal manure result in significant atmospheric emissions where ammonia is a major contributor to very fine aerosols ($PM_{2.5}$). Ammonia is also being proposed as a carbon-free fuel alternative to hydrocarbons, and an economic way of transporting green energy. Therefore, there are potential environmental and economic benefits to the recovery of ammonia from animal manures. Several technologies have been studied, such as gas stripping, membrane separation, struvite precipitation, Ion exchange, anammox, and reverse osmosis, each with their own advantages and disadvantages. This presentation will present the current state of the art of ammonia recovery technologies and their commercial development. The long-term goal of this study is to evaluate the process pathways for ammonia recovery that are environmentally and economically advantageous for Irish agriculture. This will require a review of the sources of ammonia regarding quantity and location. It will investigate factors that influence the recovery process including technical parameters, e.g. ammonia concentration, contaminants in the manure, pH, and temperature as well as economic factors, process configuration, the scale of the process, transport, capital, and operational costs. These will be critically evaluated along with future challenges and prospects of ammonia considered.