ISCI, Volume *12*

Supplemental Information

Dissecting N-Glycosylation Dynamics in Chinese Hamster Ovary Cells Fed-batch Cultures using Time Course Omics Analyses

Madhuresh Sumit, Sepideh Dolatshahi, An-Hsiang Adam Chu, Kaffa Cote, John J. Scarcelli, Jeffrey K. Marshall, Richard J. Cornell, Ron Weiss, Douglas A. Lauffenburger, Bhanu Chandra Mulukutla, and Bruno Figueroa Jr.

Supplemental Information

Supplemental Figures and Legends

Figure S1. Glycoforms attached to monoclonal antibodies produced in CHO fed-batch processes vary with time, and their relative levels could be affected by media components, Related to Figure 2.

A. Time course dynamics of extracellular metabolites and other cell/process variables are shown for CC and HD processes. For both processes, the cells in the culture behaved in similar fashion. B. Time dynamics of specific glycan structures attached to mAbs in cell culture. Various glycosylated species exhibit similar time dynamics despite process variation. C. Differential (instantaneous) time course profiles of major glycan species for the two processes, calculated by taking the differential of the cumulative glycans with respect to the titer produced (see Transparent methods). D. Time course metal ions and trace metal analysis of supernatant samples for both the processes. E. Glycan data from shake experiments under the two media conditions (CC and HD) with swapped manganese levels. Black lines represent data from HiPDOG (HD) process, while grey lines represent data from platform (CC) process.

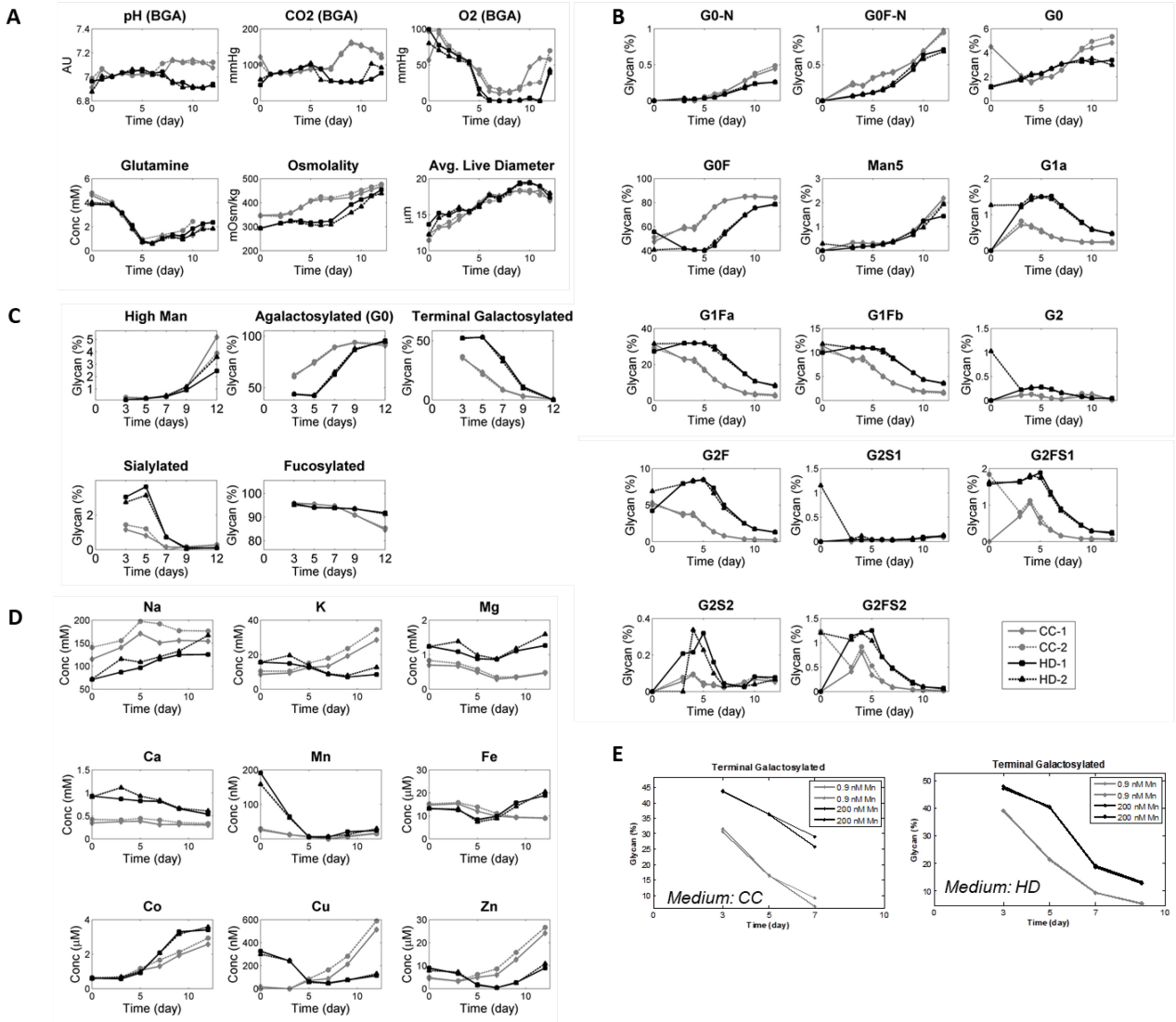


Figure S2. Galactosylation and sialylation steps serve as potential bottlenecks in the N-glycosylation process, Related to Figure 2.

Scatter plots between the fractions of consecutive major glycan species plotted on x-axis (i^{th} species) and y-axis ($i+1^{th}$ species). A. The data plot for G0-N *versus* high mannose species is primarily localized at the intersection of the two axes, suggesting that this step might not be rate limiting. B. The scatter plot of G0 species *versus* G0-N species is primarily localized on the y-axis of the graph, suggesting that GlcNAc addition might also not be rate limiting. C. When the total galactosylated species are plotted against G0 species, a negative correlation is observed, wherein G0 species increase while galactosylated species decrease with increasing time, suggesting that galactosylation might be rate limiting and the rate limitation increases over time. D. Scatter plot of sialylated species *versus* galactosylated species is primarily located near the x-axis of the graph suggesting a significant build-up of the precursors with time i.e. galactosylated species. The trends across days are depicted by lighter to darker shades of grey with increasing time. Arrows indicate time course direction.

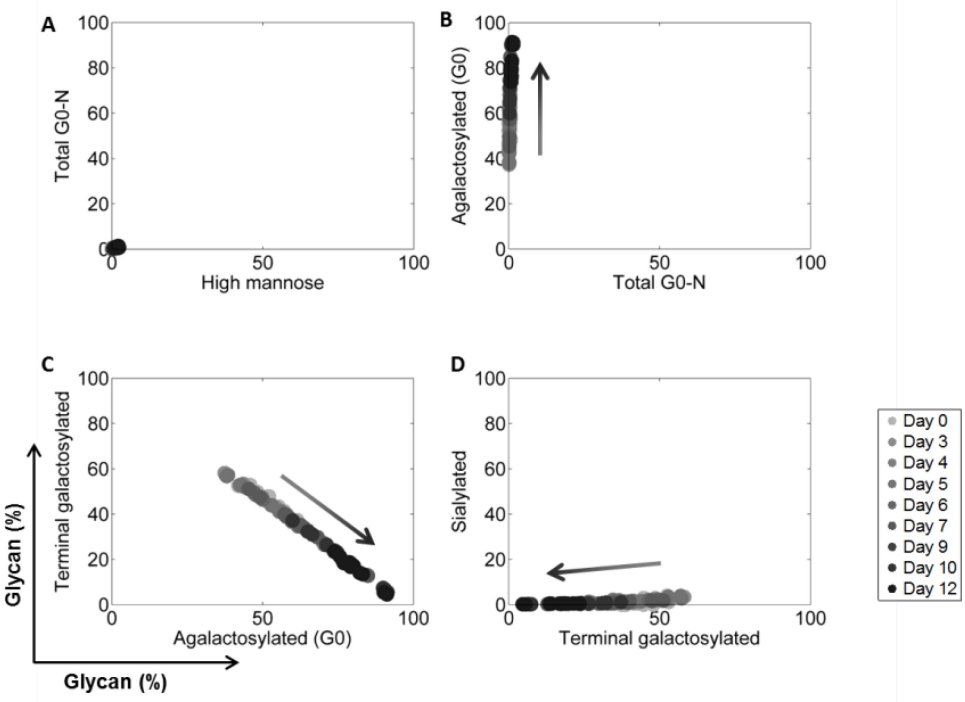


Figure S3. Gene set enrichment analysis of the time course transcriptome data for fed-batch process, Related to Figure 3.

Gene set enrichment analysis on pairwise comparisons of days in the growth phase (days 0, 3, 5) and production phase (days 7, 9 and 12). The bar lengths depict the number of day-by-day comparisons for which the gene set appears to be significantly enriched out of the nine possible day-by-day comparisons. A. A selection of gene sets that are enriched in growth phase. B. A selection of sets that are enriched in the production phase. For complete analysis, see Data S8a and Data S8b.

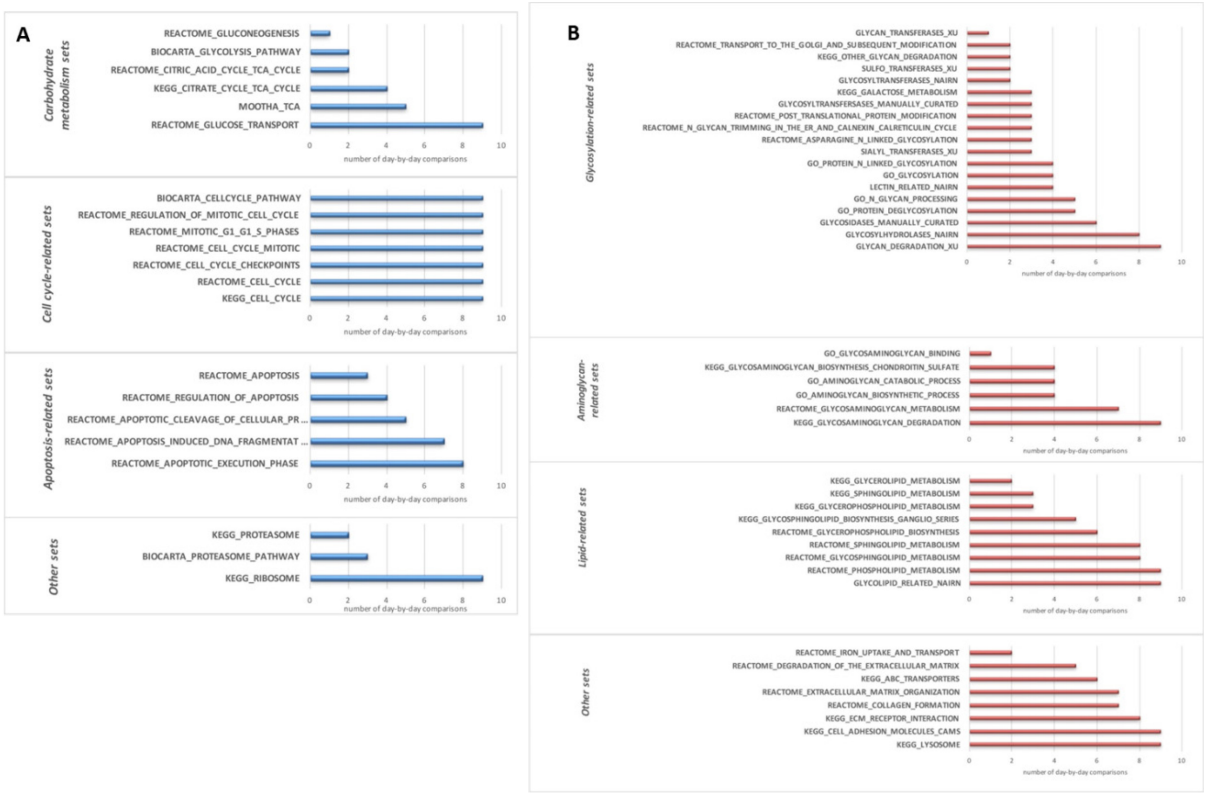


Figure S4. Shift in transcriptional and metabolic profile of CHO cells during cell culture is reflected in the central energy metabolism and related pathways linked to N-glycosylation, Related to Figure 4.

A. Specific glucose consumption and lactate production rates vary significantly over cell culture period for both the processes showing metabolic shift during the culture process. The switching of the ratio qLac/qGlc from positive to negative and back to positive suggests that cells undergo metabolic shifts in both the processes despite one being controlled for lactate (HD) while other not controlled (CD). B. Time dynamics of the metabolites and genes encoding for enzymes involved in central energy metabolism for the two processes. Throughout the cell culture, several enzymes and metabolites involved in central energy metabolism pathways show significant time variation. As these pathways provide precursors for the nucleotide sugar biosynthesis, the temporal variation can potentially affect the dynamics of NSD biosynthesis, thereby affecting in N-glycosylation dynamics.

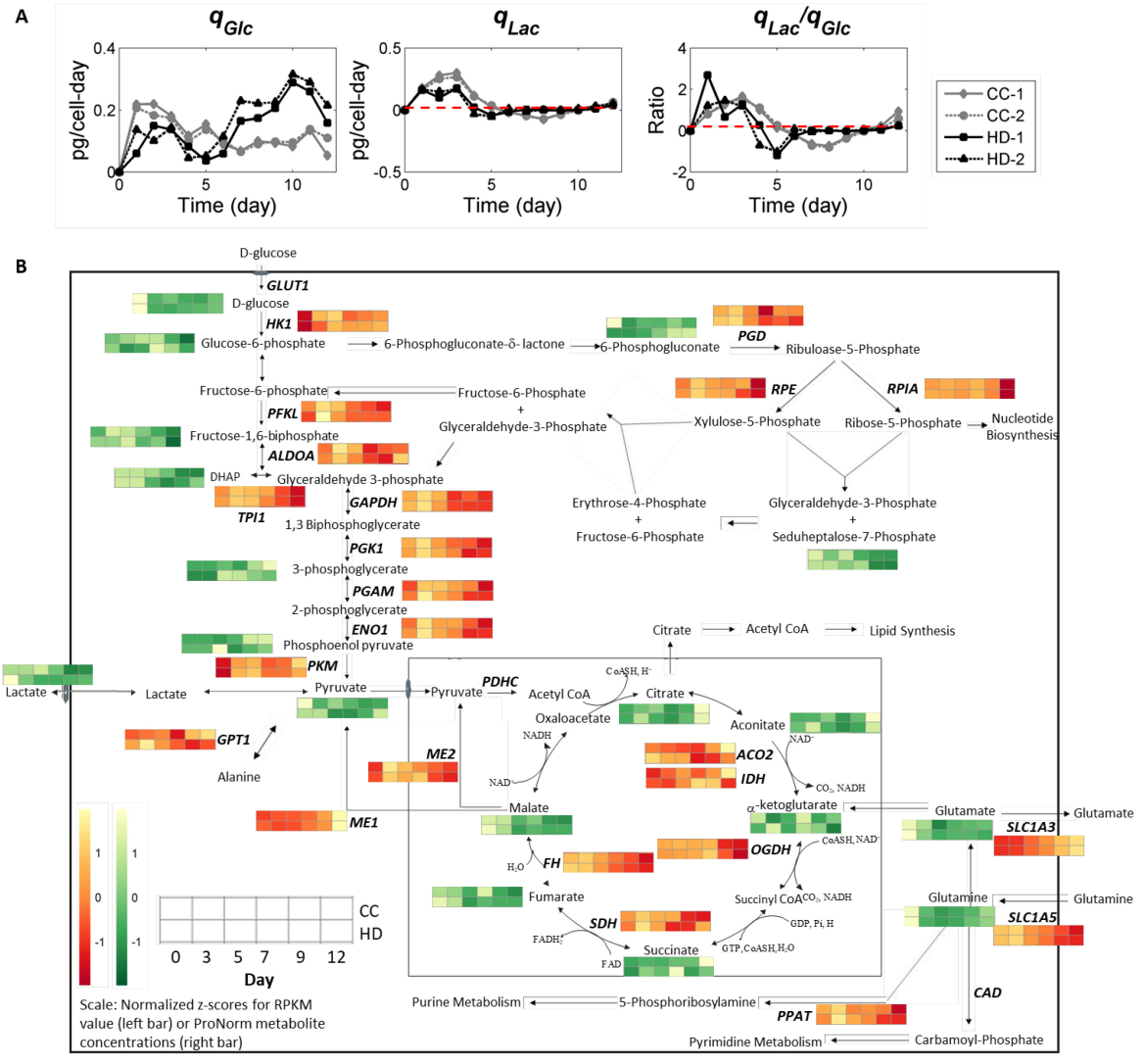


Figure S5. Transcript levels of several glycosylation related enzymes vary significantly with time, Related to Figure 5. Time dynamics of the transcripts of the enzymes directly involved in the N-glycosylation process and expressed in the CHO cell line used. Most of the transcripts wither increase over time or remain relatively constant, except *MGAT2*.

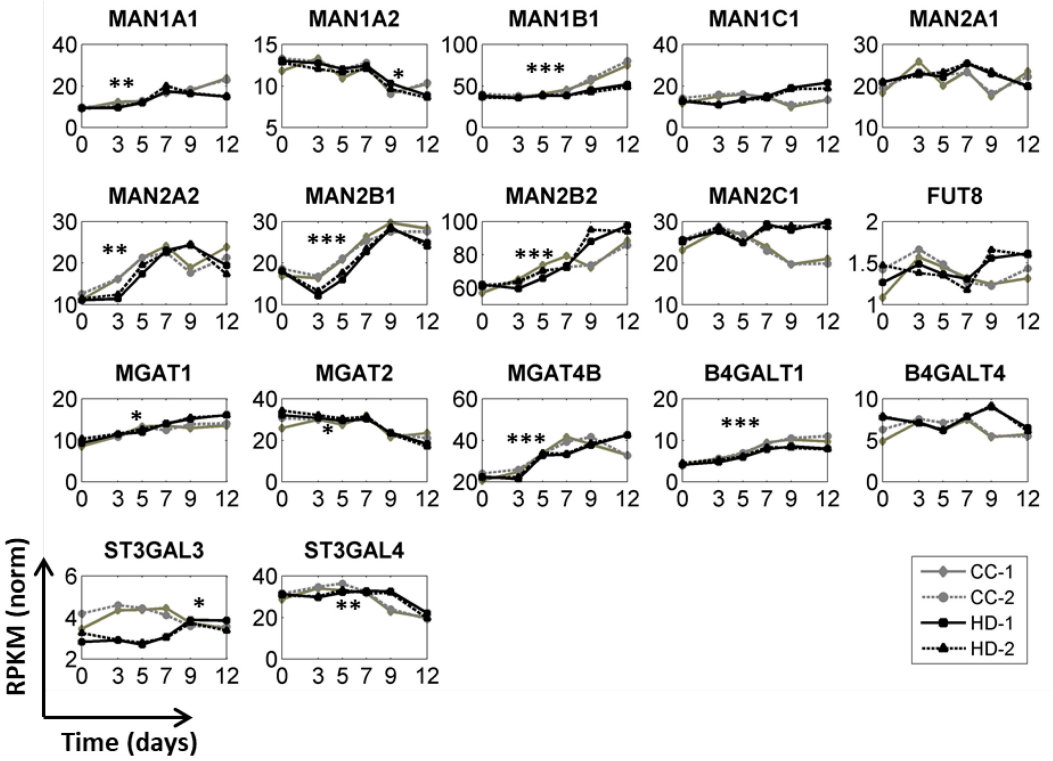


Figure S6. Galactose and not manganese overcomes the temporal heterogeneity in galactosylation, Related to Figure 6.

A. Time dynamics of transcripts of the enzymes involved in the Leloir pathway of UDP-Gal biosynthesis from external galactose supplementation. Both *GALK* and *GALT* remain relatively constant over the course of the fed-batch culture, while GALE increases over time. B. Supplementation of high concentration of manganese (2 uM) along with or without galactose (5g/L) affects cell growth in CC medium, while no significant difference is observed in HD medium. C, D. Glycan profiles of samples collected from cells cultured in fed-batch shake flasks with galactose and manganese supplementation. While manganese supplementation results in dose dependent increase in terminal galactosylated species, it alone is insufficient to sustain high levels of galactosylation in the later days in culture. Galactose supplementation results in sustained levels of galactosylation. The trends remain similar in both the media (CC and HD).

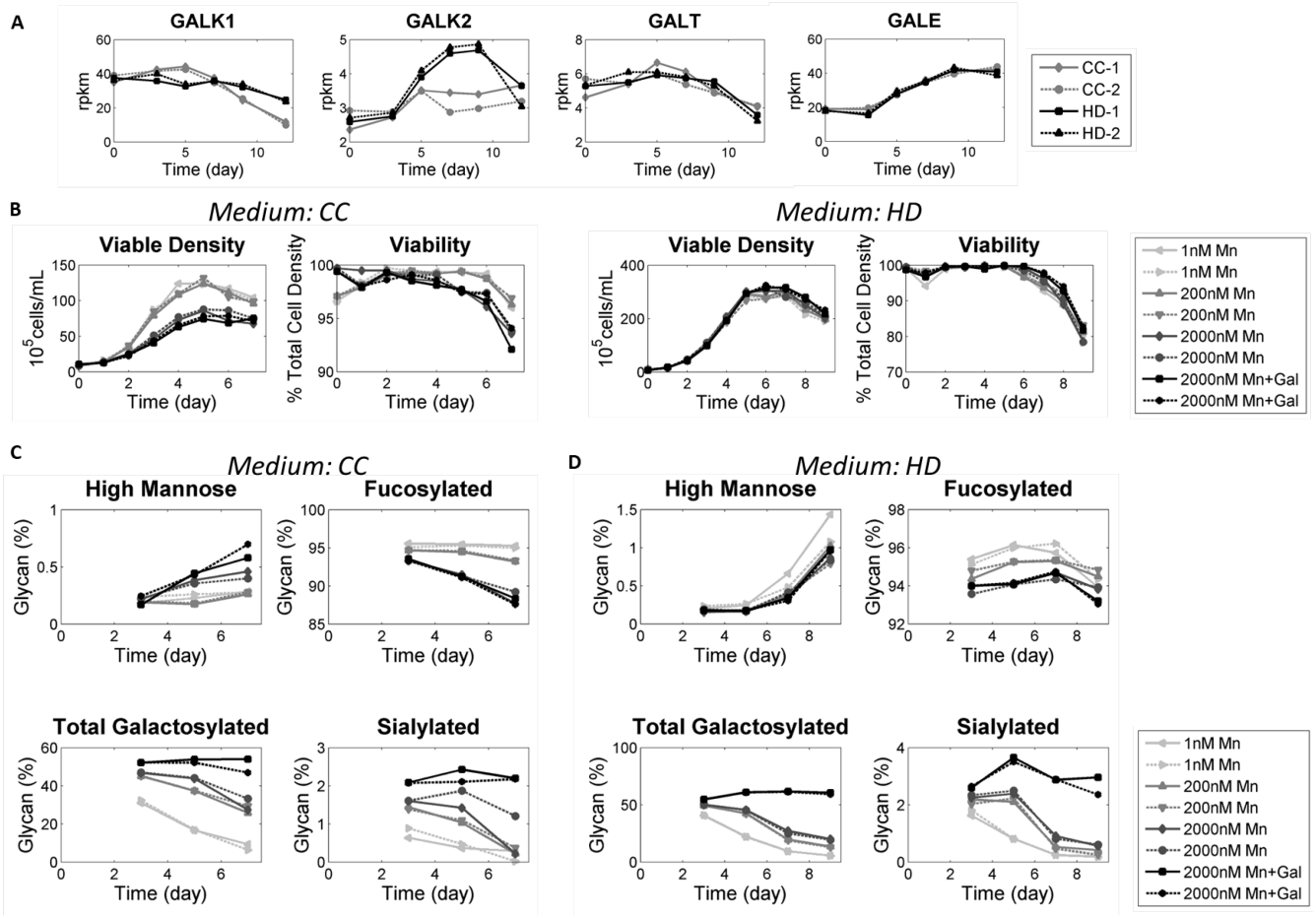
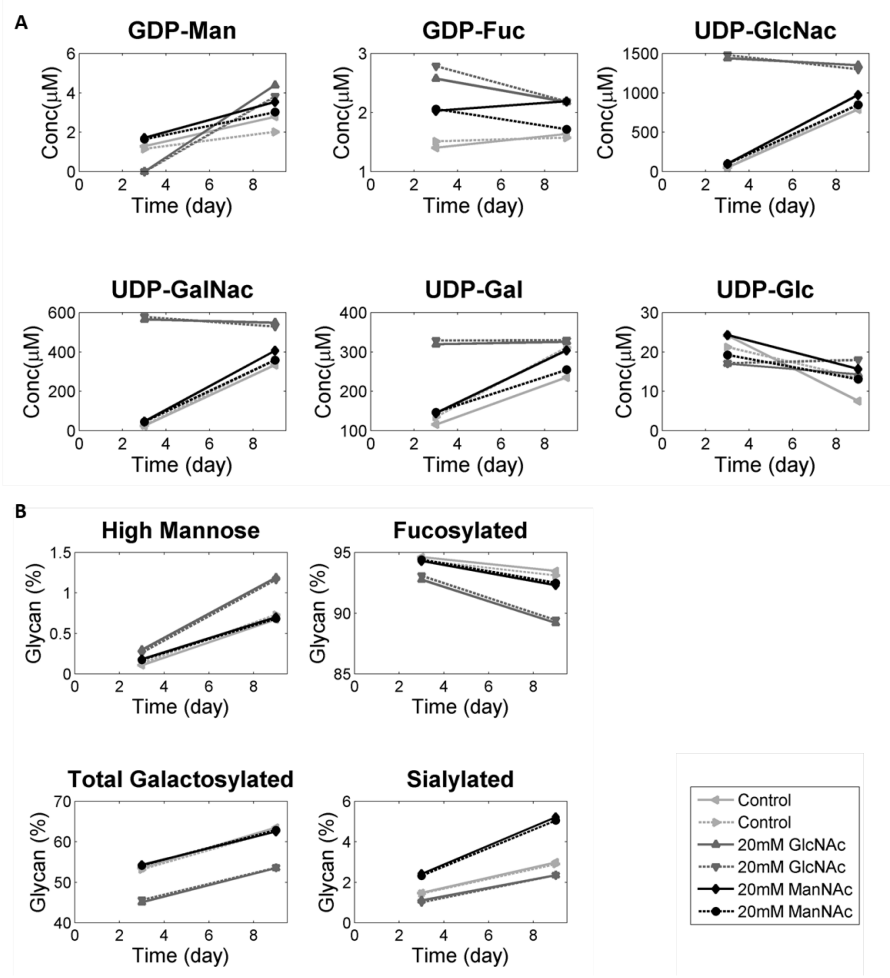


Figure S7. Bypassing CMP-Sia autoregulation by supplementing ManNAc significantly increases intra-cellular pool of CMP-Sia and marginally improves sialylation levels, Related to Figure 6.

A. Intracellular nucleotide sugar donor concentrations for the three conditions (a) control (b) 20 mM GlcNAc supplementation and (c) 20 mM ManNAc supplementation. All the three conditions were supplemented with 2000 nM Mn and 5g/L of Galactose. B. Glycan profiles for samples collected from cells cultured in fed-batch shake flasks with GlcNAc and ManNAc supplementation. Supplementation of ManNAc results around two-fold increase in sialylated species, while GlcNAc results in slightly lower sialylation levels. GlcNAc supplementation also affects the fractions of galactosylated, fucosylated and high mannose species.



TRANSPARENT METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Time course RNA-seq data	This paper	Data S3
Time course intracellular metabolic data	This paper	Data S4
Time course extracellular metabolic data	This paper	Data S5
Experimental Models: Cell Lines		
Hamster: CHO-K1 cell line	ATCC	CCL-61
Software and Algorithms		
edgeR package	(Robinson and Oshlack, 2010)	https://bioconductor.org/packages/release/bioc/html/edgeR.html
Principal Component Analysis	MathWorks Documentations	https://www.mathworks.com/help/stats/pca.html
pheatmap	CRAN-R	https://cran.r-project.org/web/packages/pheatmap/index.html
Gene Set Enrichment Analysis (GSEA)	(Subramanian et al., 2005)	https://software.broadinstitute.org/gsea/index.jsp
Time course gene set analysis (TCGSA)	(Hejblum et al., 2015)	https://cran.r-project.org/web/packages/TcGSA/index.html
maSigPro	(Conesa et al., 2006)	https://www.bioconductor.org/packages/release/bioc/html/maSigPro.html
Other		
Curate gene set list	This paper	Data S7
Curate metabolic set list	This paper	Data S10

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for datasets and/or protocols may be directed to, and will be fulfilled by the Lead Contact Bhanu Chandra Mulukutla (BhanuChandra.Mulukutla@pfizer.com).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell line development

The CHO-K1 cell line (ATCC) was adapted to serum free, suspension growth. Approximately 10 million cells were transfected with a vector expressing both the heavy and light chain genes for the mAb, as well

as a puromycin resistance selectable marker by electroporation. After 24 hours, cells were spun down and re-suspended in media containing 10 µg/mL of puromycin to select for cells with stable vector integration. After addition of selective agent, cells were plated in static 48-well plates at an approximate density of 2000 cells per well. After 3 weeks, wells were screened for mAb titer, and high expressing wells were pooled together, and expanded to shake flasks. After one passage, the cell pool was FACS cloned (Aria II, Becton Dickinson) to one cell per well using previously described methods (Zhang et al., 2015). Subsequent scale up and octet titer screening were performed on clones to select 30 clones for shake flask cultures. The clone with the highest octet titer was chosen to perform the bioreactor and shake flask experiments. Cells were grown in shake flasks with CD-CHO media supplemented with 6mM Glutamine and 10 µg/L of Puromycin (Mirrus, Fisher Scientific).

Fed-batch processes description

Two fed-batch processes were employed as part of this study. These included a platform process (CC-1 and CC-2) that used a modified CD-CHO commercial media as a basal production medium and, a HiPDOG process (HD-1 and HD-2) that used an internally developed high nutrient production medium and employed a HiPDOG (high end pH control of glucose) strategy (Gagnon et al., 2011). The HiPDOG strategy helps restrict lactate production in fed-batch cultures without compromising on the proliferative capability of cells (Gagnon et al., 2011). For both processes, internally developed distinct feed medium and feeding strategies were used. In the CC process, glucose was maintained above 1.5 g/L throughout the process. In the HD process, glucose concentration was kept low by intermittent addition of feed medium containing glucose at the high end of pH dead-band of the process when the HiPDOG strategy was operational (i.e. from day 2-5) and above 1.5 g/L during the post-HiPDOG phase.

Fed-batch bioreactor experiments

Duplicate bioreactor vessels were inoculated at 1E6 viable cells/mL for two different production media viz. platform (CC-1 and CC-2) and HiPDOG (HD-1 and HD-2). The bioreactors were maintained at a pH of 7.05±0.05 in case of CC-1 and CC-2, and at 7.025±0.025 in case of HD-1 and HD-2. Day 6 onwards, the pH for both the conditions was set at 7.05±0.15. Dissolved oxygen was controlled at 40% of air saturation by micro-bubble sparging of a mixture of CO_2/air and O_2. Temperature was controlled at 36.5°C. Several metabolites and biophysical characteristics such as glucose, lactate, K^+, Na^+, Ca^{++}, NH_4^+, glutamate, glutamine, viable cell density, osmolality, and average live cell diameter were determined daily using a NOVA Flex BioProfile Analyzer (Nova Biomedical, Waltham, MA). Offline pH, O_2 and CO_2 measurements were taken daily using a 248 CIBA-Corning blood gas analyzer (Bayer AG, Leverhusen, Germany). The cell culture were sampled on specific days (Day 0, 3, 5, 7, 9 and 12) for cell pellets and supernatant to perform several time course analyses including transcriptomic (RNA-seq) and metabolomics analyses, glycan analysis, nucleotide-sugar analysis as well as for titer measurements. Titers were analyzed using a protein A HPLC (model 1100 HPLC, Agilent Technologies, Inc., Santa Clara, CA, protein A column model 2-1001-00, Applied Biosystems, Foster City, CA).

Fed-batch shake flask experiments

Duplicate shake flasks were inoculated at 1E6 viable cells/mL in either CC medium or in HD medium with added 7 g/L glucose. 6 mM Glutamine was supplemented in the media before inoculation. The flasks were maintained at 36.5°C and 5% CO2 and the shaker speed was set at 140 rpm. pH adjustment to 7.1 was performed daily for each of the flasks using a base solution ($NaHCO_3$ and $KHCO_3$ solution). Appropriate volume of 50% glucose was added so that the glucose in the medium is maintained at a level higher than 1.5 g/L.

Mathematical definition used in the manuscript

a. IVCC based specific productivity (q_P) for *i*th time-point measurement is given by: $q_P = \frac{Titer_i}{IVCC_i}$, where $IVCC_i = IVCC_{i-1} + \frac{(VCD_i + VCD_{i-1})}{2} * (time_i - time_{i-1})$.

b. Specific glucose consumption (q_{Glc}) for *i*th time-point is given by: $q_{Glc} = \frac{rGlc_{consumption}}{0.5*(VCD_i + VCD_{i-1})}$, where, $rGlc_{consumption} = \frac{Glucose_i - Glucose_{i-1} + Glucose\ from\ nutrient\ feed}{time_i - time_{i-1}}$.

c. Specific lactate production (q_{Lac}) for *i*th time-point is given by: $q_{Lac} = \frac{Lactate_i - Lactate_{i-1}}{0.5*(VCD_i + VCD_{i-1})*(time_i - time_{i-1})}$.

METHOD DETAILS

Protein-A purification materials and methods

Protein-A purification materials: 1:1 mixture of Milli-Q water, Acetonitrile (ACN-Omnisolve), trifluoroacetic acid (TFA-Thermo Scientific), PNGaseF (N-glycanase-Prozyme), 5x sodium phosphate buffer (Prozyme), sodium cyanoborohydride (Fluka), DMSO (Fluka), 2-aminobenzamide (Fluka), glacial acetic acid (Sigma), octanal (Sigma-Aldrich), Protein A resin (GE MabSelect), HILIC column: Xbridge Amide 3.5 um, 4.6x150 mm (Waters). Sodium phosphate (J.T. Baker), Acetonitrile (Omnisolve), 1M Tetrabutylammonium acetate (Sigma-Aldrich), Methanol (EMD-Millipore), Phenomenex Aeris PEPTIDE, 1.7 µm, 150 x 2.1 mm, Agilent Zorbax 300SB-CN 5 µm, 4.6 x 250 mm, Amicon 10KMWCO filter (EMD-Millipore). mAb select

Protein-A purification methods: Protein-A resin was washed and added to a 1:1 mixture of sample:water. After incubating at room temperature for 45 minutes in a rocker, the supernatant was removed and the pellets were added to a 96-well plate. The wells of the plate were washed three times with water. To elute the glycans, 6 µL of 0.5 M Tris base pH 10.8 was added to the receiver tray and 250 µL of 50 mM glycine 25 mM sodium chloride pH 3.00 was added to the filter plate. Concentration was determined using the Nano-Drop.

Glycan analysis

Glycan release from mAb: Approximately 100 µg of protein A purified mAb at a volume not exceeding 100 µL was combined with 6 µL of 5x phosphate buffer and 2 µL (4µL if reaction volume >50µL) of PNGaseF and incubated for approximately 16 hours at 37ºC. 10µL of 2-AB derivitization solution (0.35M 2-aminobenzamide/ 1M sodium cyanoborohydride dissolved in a 30/70% acetic acid/DMSO) was added to each sample and incubated for 2 hours at 65ºC. Sample volume was brought to 100µL with water and combined with 600 µL of octanal (Chu et al., 2018). Samples were vortexed and the bottom fraction was filtered through a 0.1 µm spin filter and diluted to 30/70% sample/ACN prior to injection.

Chromatograhy: Mobile phase A (A): 0.05% (v/v) TFA in ACN. Mobile phase B (B): 0.05% (v/v) TFA in Water. HILIC column: Xbridge Amide 3.5 um, 4.6x150 mm (Waters). Temperature: 45ºC. Sample Temperature: 5ºC. System: Waters Alliance. Flow rate: 1mL/min. Fluorescence detector Excitation: 330 nm, Emission: 420 nm. Run time: 60 minutes. Injection volume: 30µL. Gradient: 0-5 min 28% B, 5-42 min 28-42%B,42-43 min 42-90%B, 43-44 min 90%B, 44-45 min 90-28%B, 45-60 min 28%B. For each run sample peaks were identified by retention time comparison to a mass spectrometry characterized reference material.

Intracellular nucleotide sugar analysis

Cellular extraction: 5-10 E6 cells washed in cold PBS and snap frozen were thawed on ice and mixed with 100µL of cold 50:50 ACN:water. Samples were vortexed and centrifuged at 13000g. Supernatant samples were dried in a speedVac, reconstituted in 100 µL of water, and filtered through an Amicon 10kMWCO filter prior to injection.

Chromatography: Mobile Phase A (A): 100 mM Sodium phosphate pH 5.1, 8 mM tetrabutylammonium acetate. Mobile Phase B (B): 30% methanol, 70%A. Two columns were combined in tandem 1 (pump side): ThermoFisher Scientific Accucore Vanquish C18+, 1.5 µm, 100 x 2.1 mm, 2: Agilent Zorbax 300SB-CN 5 µm, 4.6 x 250 mm. Temperature: 15°C. Sample Temperature: 5°C. System: Waters Acquity UPLC H-Class Bio. Flow rate: 0.2 mL/min. UV 260 nm detection. Run Time: 140 minutes. Injection Volume: 5 µL. Gradient: 0-40 min 1%B, 40-90 min 1-50%B, 90-110 min 50%B, 110-111 min 50-1%B, 111-140 min 1%B. Sample peaks were identified by comparison to a reference standard with known amounts of nucleotides and nucleotide sugars bracketed throughout the run.

Differential glycan calculations

The cumulative glycan profile obtained experimentally was transformed into differential glycan data using two-point time derivative with respect to the titer. Mathematically, if Gi,tk is the cumulative value for i^{th} glycan structure on day 'tk', and Ttk is the titer produced until that day, then the differential glycan values can be calculated by using the following relation:

$$g_{j,t_m} = \frac{G_{j,t_m} \times T_{t_m} - G_{j,t_{m-1}} \times T_{m-1}}{\Delta T_{t_m}}$$

Metal ions and trace metal analysis

5 mL of supernatant samples were collected for days 0, 3, 5, 7, 9 and 12 for each of the bioreactors (CC-1, CC2, HD-1 and HD-2). The samples were analyzed using inductively coupled plasma (ICP) mass spectrometry method to determine concentrations of several metal ions known to affect CHO cell metabolism (Figure S1D). For a few of the metal ions, such as sodium, potassium and calcium, the concentrations were also determined using the NovaFlex instrument, confirming that the ICP analysis and NovaFlex measurements were consistent.

RNA-seq analysis

Cell culture from bioreactors were sampled and spun down at 1000 rpm into cells pellets of about 10E6 viable cells and dissolved in Trizol (ThermoFisher Scientific, Waltham, MA) and was flash frozen in dry ice/ethanol bath. The dissolved and frozen cells were sent to Beijing Genomics Institute (BGI, Hong Kong) for RNA isolation, library preparation and sequencing analysis. Samples were run on Illumina HiSeq 2000. RNA-Seq data were mapped to CHO genome using subjunc aligner program from subread-1.4.6 package. The alignment bam files were compared against the gene annotation GFF file, and raw counts for each gene were generated using the featureCounts tool from subread. The raw counts were normalized using TMM method (Robinson and Oshlack, 2010) and the reads per kilobase per million (RPKM) values for each gene was calculated using the edgeR package (https://bioconductor.org/packages/release/bioc/html/edgeR.html).

Intracellular and extracellular metabolomic analyses

Cell pellets (10E6 cells) flash frozen in dry ice/ethanol and supernatant (1 mL) from each bioreactor for each sampling day were sent to Metabolon (Metabolon Inc, Morrisville, NC) for metabolomics analyses. Proteins were removed by methanol precipitation and the metabolites were recovered by vigorous shaking and centrifugation. The extracted samples were run for reverse phase (RP)/UPLC-MS/MS (Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy) with negative ion mode ESI and for

HILIC/UPLC-MS/MS with negative mode ESI. Raw data was extracted, peak-identified and QC processed using Metabolon's hardware and software. The raw ion count data was normalized against the extracted proteins quantified using Bradford assay.

QUANTIFICATION AND STATISTICAL ANALYSIS

Principal Component Analysis (PCA) and Cluster Analysis

After the addition of a value of 1 to RPKM expression values, and performing quantile normalization, principal component analysis was performed in MATLAB on the log2 transformed gene expression data. Each sample was considered an observation and each gene was considered a variable.

Pair-wise cluster analysis was performed for the time point samples for their transcriptome data, intracellular and extracellular metabolite data as well as glycan data. Spearman correlation was used to cluster the samples using the pheatmap package in R. The variance of each gene over time was calculated and the top 10% varying genes were selected for the analysis. This was done to remove the less-varying genes that might not carry the temporal information.

PCA loadings for the transcriptome, extracellular metabolites, intracellular metabolites, as well as for glycosylation related gene set is provided in Data S14. Factors with large loadings indicate that their variations would contribute the most to the particular principal components.

Gene Set Enrichment Analysis (GSEA)

A pair-wise permutation of all the samples within each process (CC or HD) were analyzed using GSEA method to identify pathways that are enriched in either growth phase or in production phase (Data S8b). A score was calculated based on how many times a particular gene-set showed up significant (p-value < 0.05) in CC or HD or both processes for the pairwise permutations.

Preprocessing: To minimize the noise from the low expressing genes, a value of 1 was added to the RPKM gene expression values. Subsequently, quantile normalization was performed so that all samples have a similar expression distribution and to alleviate the inter-sample biases. A manually curated gene set was compiled by combining three curated canonical pathways databases including BioCarta, KEGG, and Reactome, taken from the Broad Institute's Molecular Signatures database v6.0 (MSigDB) (Liberzon et al., 2015). Additional glycan-related gene sets taken from Gene Ontology databases as well as four gene sets comprised of the genes in nucleotide sugar synthesis were added to the combined list. The resulting gene set list is provided as Data S7.

GSEA analysis: Gene set enrichment analysis (GSEA) method was adapted to analyze the time course RNA-seq data from a published source (Subramanian et al., 2005). A two-step analysis was performed: (i) pair-wise comparison of days from distinct phases of cell culture, i.e. growth phase (days 0, 3, 5) and production phase (days 7, 9 and 12), to identify sets that are over-represented in each phase (Data S8a), and (ii) find how many times a gene set was enriched in either of the growth or production phases (Data S8b). For each of these nine comparisons between growth and production phases, GSEA was run using the desktop version with the following settings: Gene sets database: number of permutations: 1000 and permutation type: Gene_set. Since the number of samples in each comparison was 4 (2 per days compared), gene set permutation was performed as recommended by GSEA guidelines as follows. Enrichment statistic: Weighted; Metric for ranking genes: log2_Ratio_of_Classes; Max size: exclude larger sets: 500; Min size: exclude smaller sets: 10 and FDR cut-off of 0.05 was used to select the enriched gene sets that are significant. The outcomes of these set of analyses are the gene sets where its member genes are either enriched or lowered in one phase over another in a coordinated manner (see Data S8b).

Time course gene set analysis (TCGSA)

An extension of gene set analysis for longitudinal data sets (time course data) is available as TCGSA package in R (Hejblum et al., 2015). TCGSA considers a time series data as a single vector measurement and utilizes generalized linear (and/or non-linear) models to compare the two vectors. Thus, in our study, TCGSA was used to analyze gene sets that had time dynamics in the entirety of the cell culture period. This was done by comparing the time series measurement (Day 0 through Day 12) with the basal (Day 0) levels to gain insight into which functional groups were significantly perturbed over the course of cell culture time period. In this method, a gene set is said to be significant if its expression is not stable over time once the variability for genes and biological conditions are accounted for. The genes within a gene sets are modeled with mixed models that includes variables for gene, biological condition, time, intercepts in gene sets as well as random effects and variabilities. The mixed models fit the gene expression profiles to linear polynomials or cubic polynomials or natural cubic splines. For the sake of simplicity, we used the linear polynomial method for our analysis. The null hypothesis that the genes in a gene set are stable over time is compared with alternate hypothesis that the genes may vary significantly over time. A likelihood ratio is calculated for each of the gene sets based on these two hypotheses and a p-value for the significance of the variation of a gene set over time is computed. Multiple testing hypothesis was accounted for by using Bejamini-Yekutieli procedure to control the false discovery rate. A corresponding adjusted p-value or q-value was computed for each of the gene sets under different biological conditions such as media (CC vs HD) and different phases such growth phase (Day 0 – Day 7) vs production phase (Day 7 – Day 12). The RPKM data was pre-processed similarly as described in the previous section for the GSEA analysis.

Time course metabolic set analysis (TCMSA)

An adaptation of TCGSA (described in the previous sub-section), using the same software package was implemented on the time course intracellular metabolomic data set. A curated list of metabolic functional sets was used (see Data S10).

Time course significance analysis of individual transcripts and metabolites using maSigPro

maSigPro is a regression based approach that identifies genes for which there are significant gene expression profile differences between experimental groups in a time course microarray and RNA-seq experiments. An R package available for maSigPro analysis was used to analyze the transcriptomic data (Conesa et al., 2006). maSigPro package was used on quantile normalized rna-seq data and metabolome data to calculate false discovery rate (FDR) normalized p-values for each gene/metabolite that show differential expression over time. Additionally, same approach was implemented on the protein normalized intracellular metabolomic data to identify key metabolites that exhibit significant time profiles during the cell culture.

Statistical analysis and significance

For the statistical tests employed in the manuscript, including two-tailed unpaired t-test, GSEA, TCGSA and maSigPro, statistical significance is defined as $p < 0.05$. A gene or metabolic set (in case of TCGSA/TCMSA) or a gene/enzyme (in case of maSigPro analysis) 'exhibiting significant time dynamics' means that the adjusted p-value is < 0.05 and statistically, they show significant perturbations or changes over the cell culture period.

DATA AND SOFTWARE AVAILABILITY

The time course transcriptomic data, intracellular and extracellular metabolomic data and data generated from the time course omics analyses (functional analyses) are provided in Data S1-S15.

Supplemental References

Chu, A.H.A., Saati, A.E., Scarcelli, J.J., Cornell, R.J., and Porter, T.J. (2018). Reactivity-driven cleanup of 2-Aminobenzamide derivatized oligosaccharides. Anal. Biochem. *546*, 23–27.

Conesa, A., Nueda, M.J., Ferrer, A., and Talón, M. (2006). maSigPro: A method to identify significantly differential expression profiles in time-course microarray experiments. Bioinformatics *22*, 1096–1102.

Gagnon, M., Hiller, G., Luan, Y.T., Kittredge, A., Defelice, J., and Drapeau, D. (2011). High-End pH-controlled delivery of glucose effectively suppresses lactate accumulation in CHO Fed-batch cultures. Biotechnol. Bioeng. *108*, 1328–1337.

Hejblum, B.P., Skinner, J., and Thiébaut, R. (2015). Time-Course Gene Set Analysis for Longitudinal Gene Expression Data. PLoS Comput. Biol. *11*, 1–21.

Liberzon, A., Birger, C., Thorvaldsdóttir, H., Ghandi, M., Mesirov, J.P., and Tamayo, P. (2015). The Molecular Signatures Database Hallmark Gene Set Collection. Cell Syst. *1*, 417–425.

Robinson, M.D., and Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. *11*.

Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., et al. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U. S. A. *102*, 15545–15550.

Zhang, L., Inniss, M.C., Han, S., Moffat, M., Jones, H., Zhang, B., Cox, W.L., Rance, J.R., and Young, R.J. (2015). Recombinase-mediated cassette exchange (RMCE) for monoclonal antibody expression in the commercially relevant CHOK1SV cell line. Biotechnol. Prog. *31*, 1645–1656.