



CASSS Mass Spec Symposium 2025

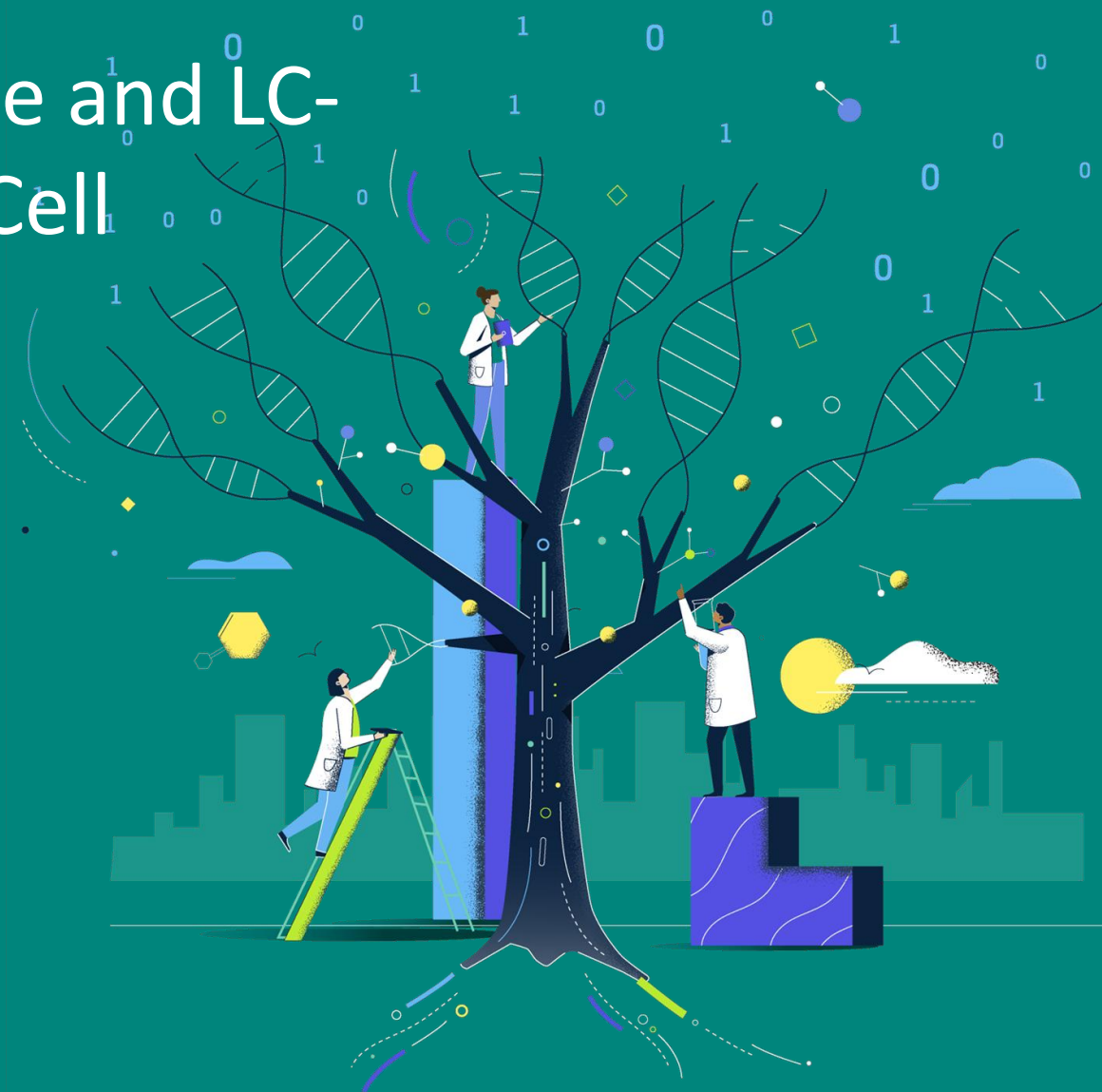
September 23-26, 2025

Combining In-Line Biocapacitance and LC-MS to Monitor and Understand Cell Culture Process in Real Time

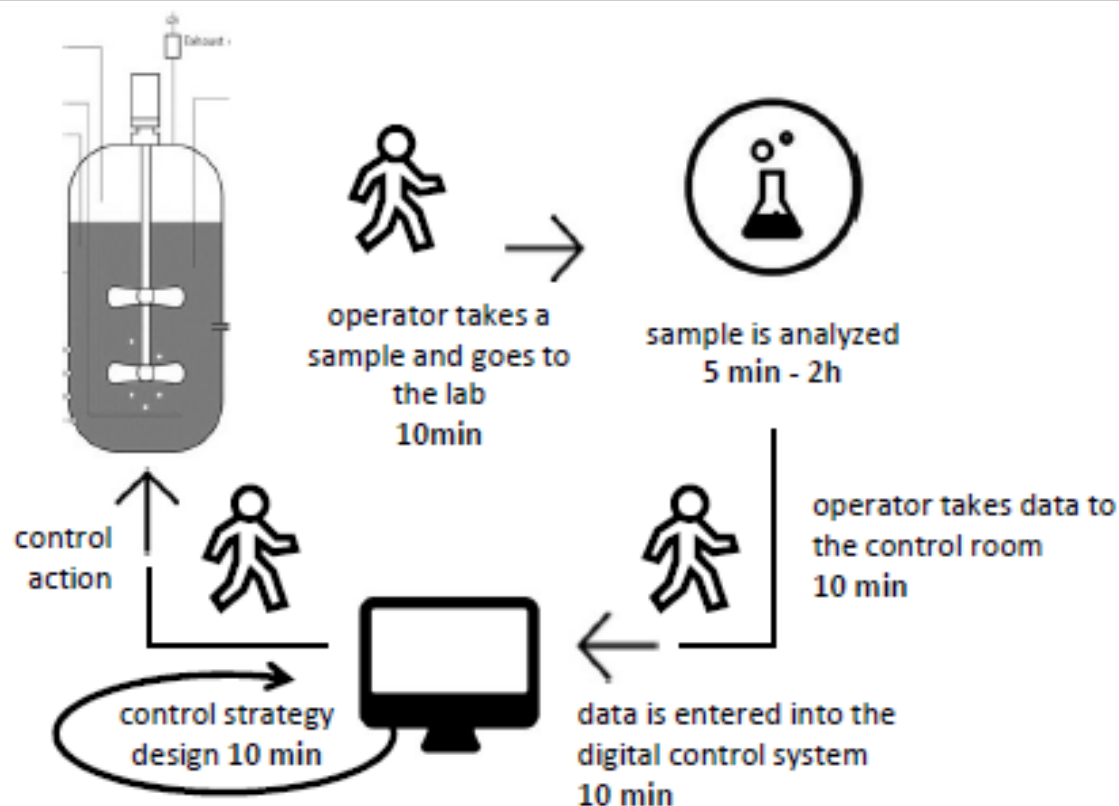
Tingting Jiang

Global Vaccines & Biologics Commercialization

Merck & Co., Inc., Rahway, NJ, USA



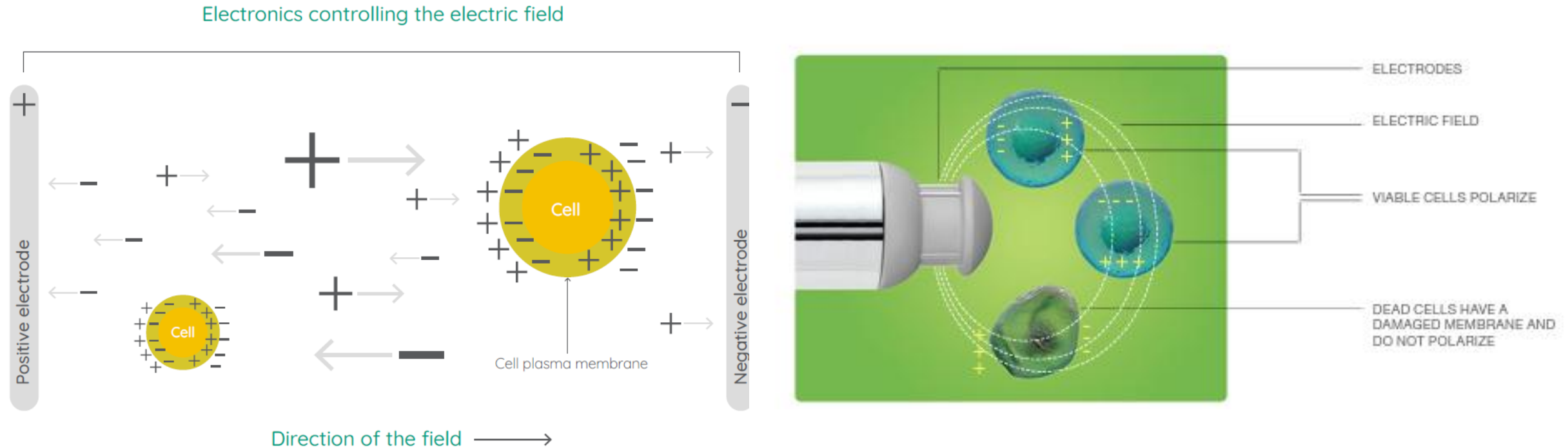
Why use PAT (capacitance spectroscopy) in a bioprocess?



Benefits of PAT

- 24|7 monitoring and control
- Sample is not removed, reactor volume is unaffected
- Reduces operator interaction – lesser risk of contamination, lowers operator error
- Earlier detection of cell physiological changes (apoptosis, viral release)

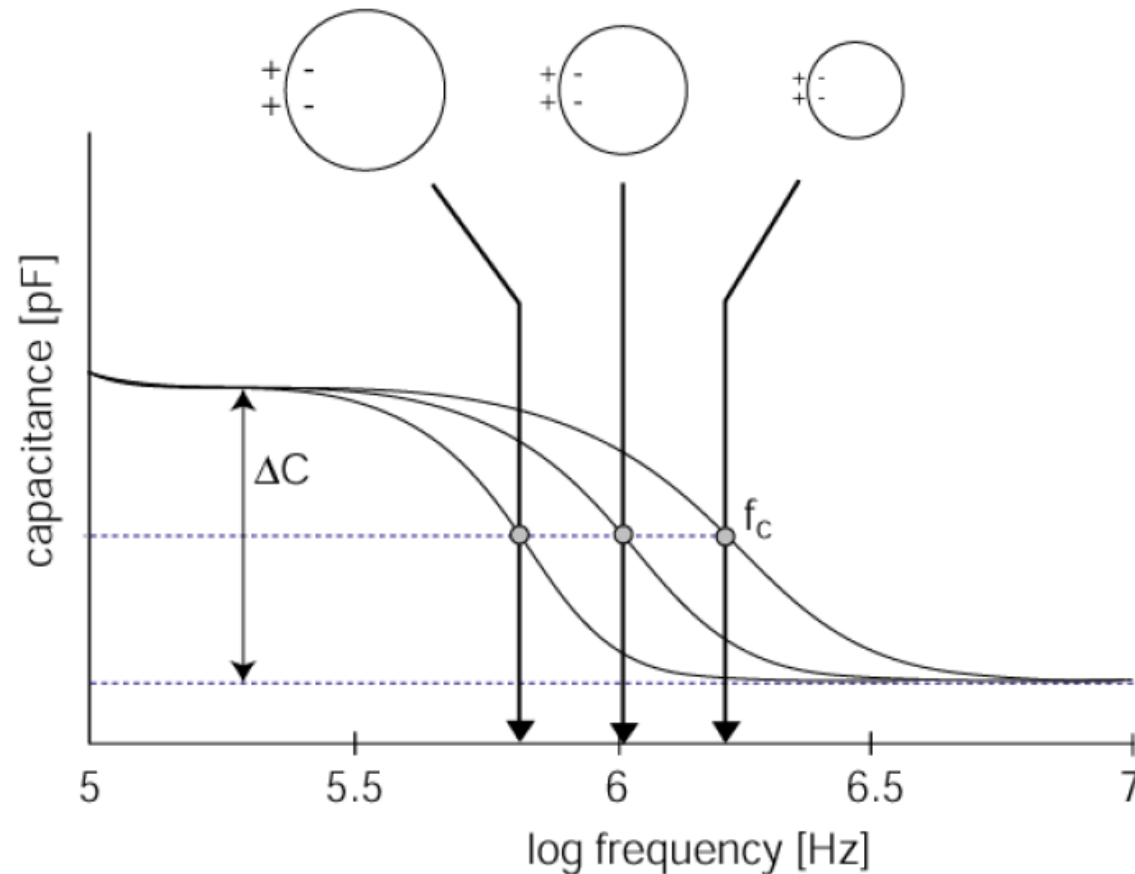
Capacitance - Principle of Measurement



- Measurement of charge separation is capacitance – directly proportional to membrane-bound volume of cells
 - Depends on number and size of cells, electrical properties of cell membrane

Critical Frequency (f_c) provides insight into physiological cell state

As frequency is increased, capacitance decreases, forming a high – low plateau referred as the β -dispersion curve

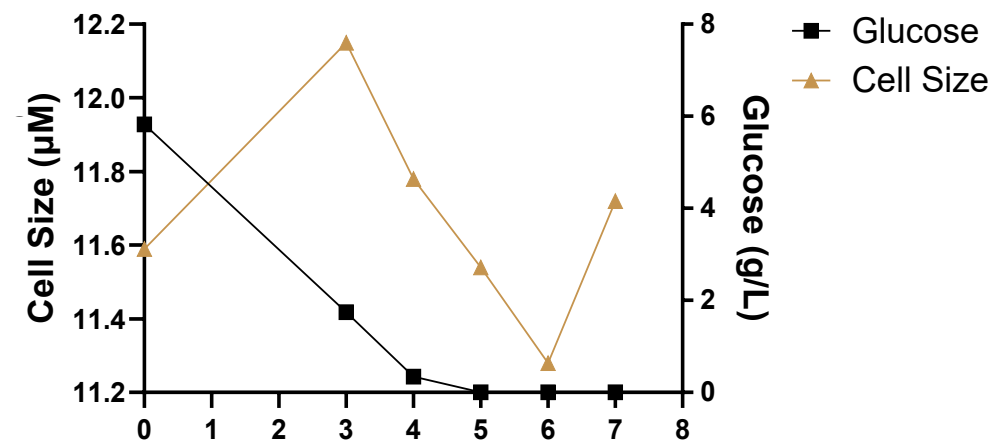
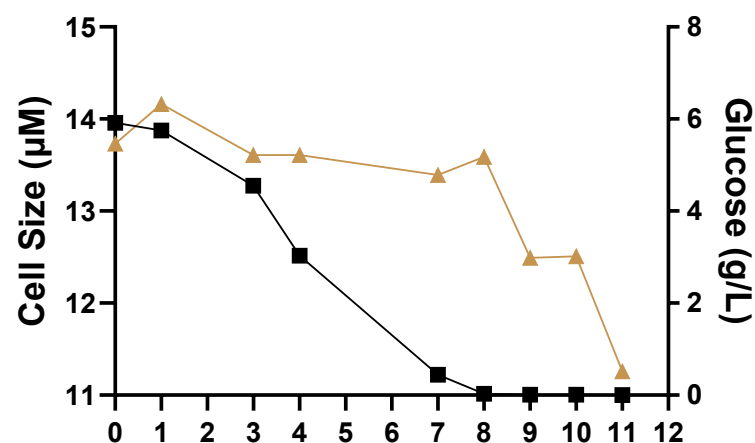
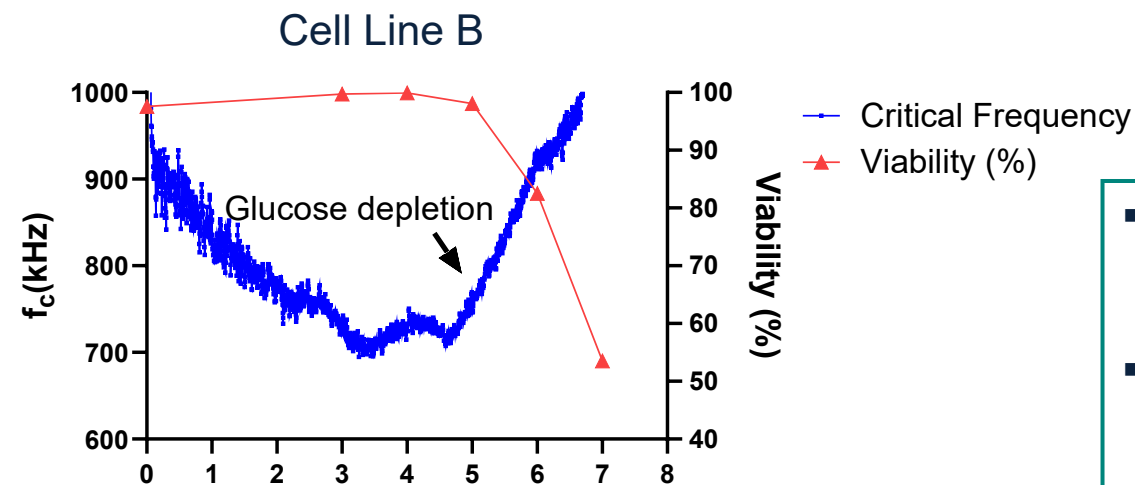
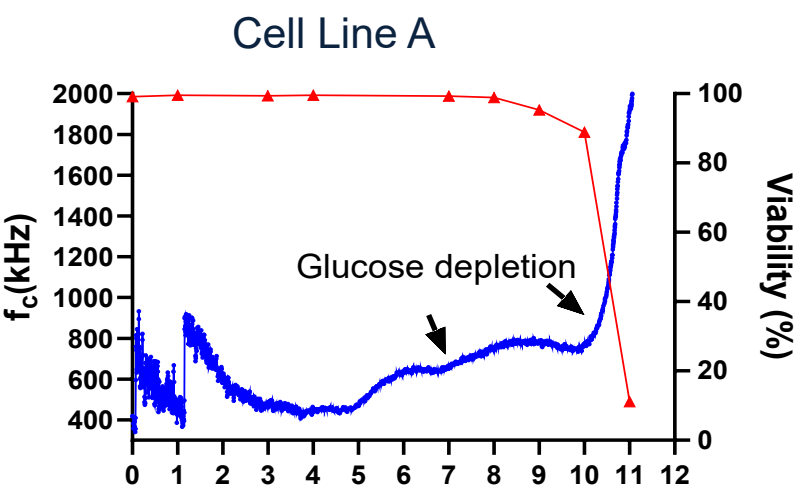


- Critical frequency f_c - midpoint of the β -dispersion curve, **inversely proportional to cell size**

Cell size	Cell Physiological State	f_c Trend Expected
Increase	older non-dividing cells	decrease
Decrease/ shrinkage of cells	early sign of apoptosis	increase

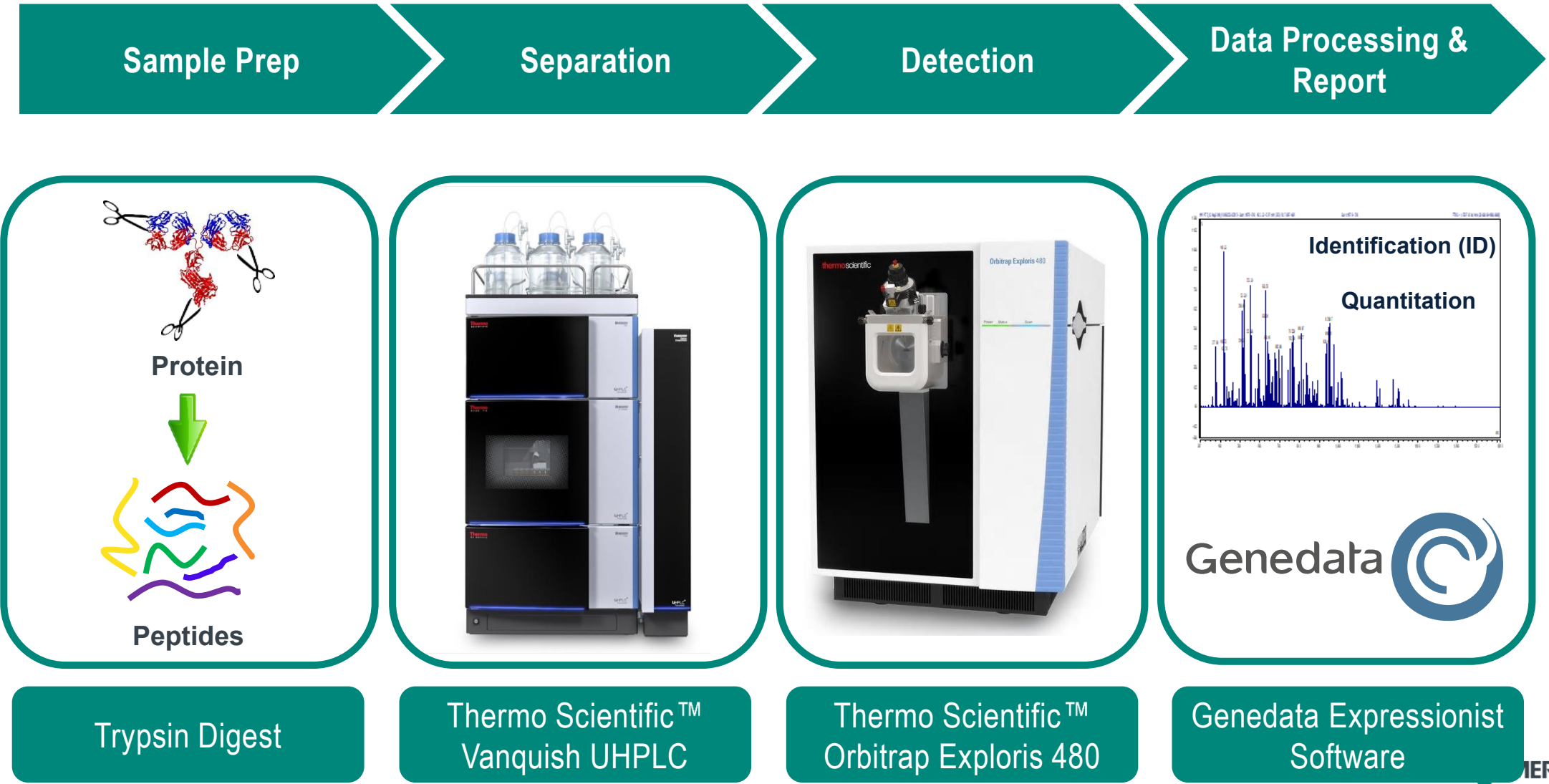
- Real time changes in f_c is seen during nutrient depletion, shear stress, and pH deviations in cell cultures

Fc correlates with nutrient limitation in a batch process



- Cells were not fed to induce nutrient limitation.
- f_c increases with glucose depletion along with decrease in cell size indicating apoptosis.
- Results indicate f_c can correlate with nutrient limitation in real-time.

Application of LC-MS proteomics to detect the changes on the molecular level



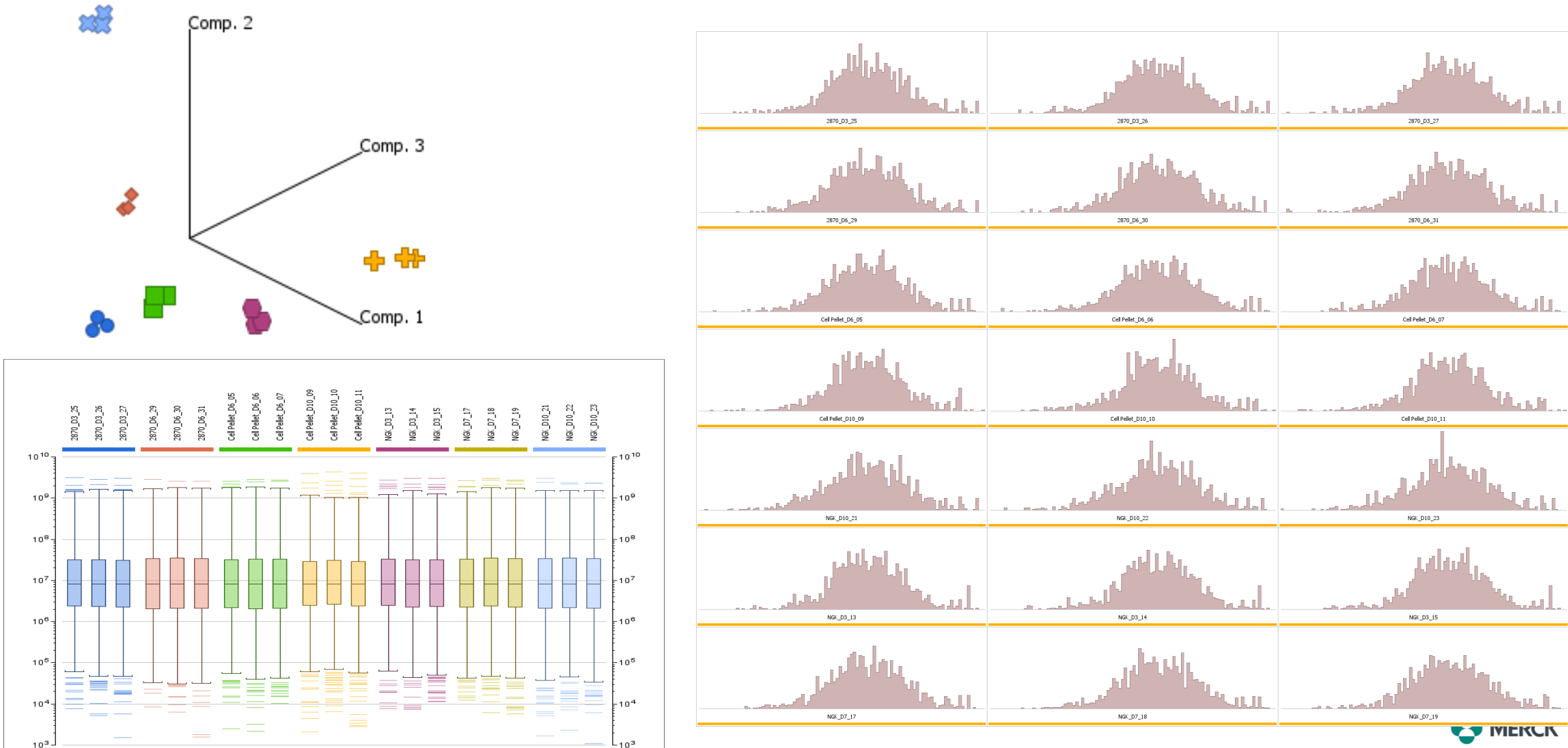
Statistical analysis by Analyst (v.18.5.0)

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 Parallel Plot	 Effect Size	 Divisive Hierarchical
 Tile Display	 2 Groups	 2D Hierarchical
 Scatter Plot	 2 Groups Exact Test	 K-Means
 Bar Charts	 2 Groups Paired	 SOM
 Correlation	 K Groups	 Learning
 Annotation Correlation	 K Ordered Groups	 Parameter Evaluation
 Correlation Circle	 Absent/Present Search	 Classification
 Threshold Scan	 Contrasts	 Cross Validation
 Correlation Network	 Fisher's Exact Test	 Ranking
 Annotation Network	 Proportional Change	 Regression
 Histogram	 Linear Model	 Regression Cross Validation
 Box Plot	 Generalized Linear Model	 Regression Ranking
 Global Distribution	 Logistic Regression	 Gene Ontology Fisher's Exact Test
 Principal Components	 Logistic Regression for Annotations	
 Partial Least Squares	 N-Way ANOVA	
 Orthogonal PLS	 Search by Distance	
 Occupation Display	 Profile Distance Search	
 Annotation Histogram	 Profile Shift Analysis	
 Annotation Scatter Plot	 Periodogram	
 Filter by Valid Value Proportion	 Trend Identification	
 Filter by Average Signal	 Kaplan-Meier Survival Curve	
 Filter by Variance	 Multivariate Cox Regression	
 Filter by Highest Change	 Cox Regression (Per Row)	
 Filter by Threshold Proportions	 Hierarchical	

Analyst offers many data mining, data visualization and statistics capabilities

PCA and Global Distributions



Statistical models

*Clusters - arnd @ Genedata Analyst (ODIN.Genedata.win:8080)

File Annotations Data Analyses Workflows Results Data Settings

File Derivation Annotation

Search Groups

- ☐ All Columns
- ☐ Stress case study (3).gda
- ☐ References
- ☐ Stress case study (3).gda \ References
- ☒ From: Storage Time [C]
 - ☐ 0 Mo
 - ☒ 1.5 Mo
 - ☒ 3 Mo
 - ☒ 6 Mo

Search Analyses

Stress case study

Linear Model

Generalized Linear Model

Generalized Linear Model for Count Data

Logistic Regression

Logistic Regression for Annotations

N-Way ANOVA

Linear Model

Description

Performs a linear (mixed) model analysis on the data matrix. Select one or more factors to be included in the model. The factors can be fixed, random or covariates. Choose either the example model or modify it based on your experimental design.

Settings

a Storage Time [C]

b Temperature

Model

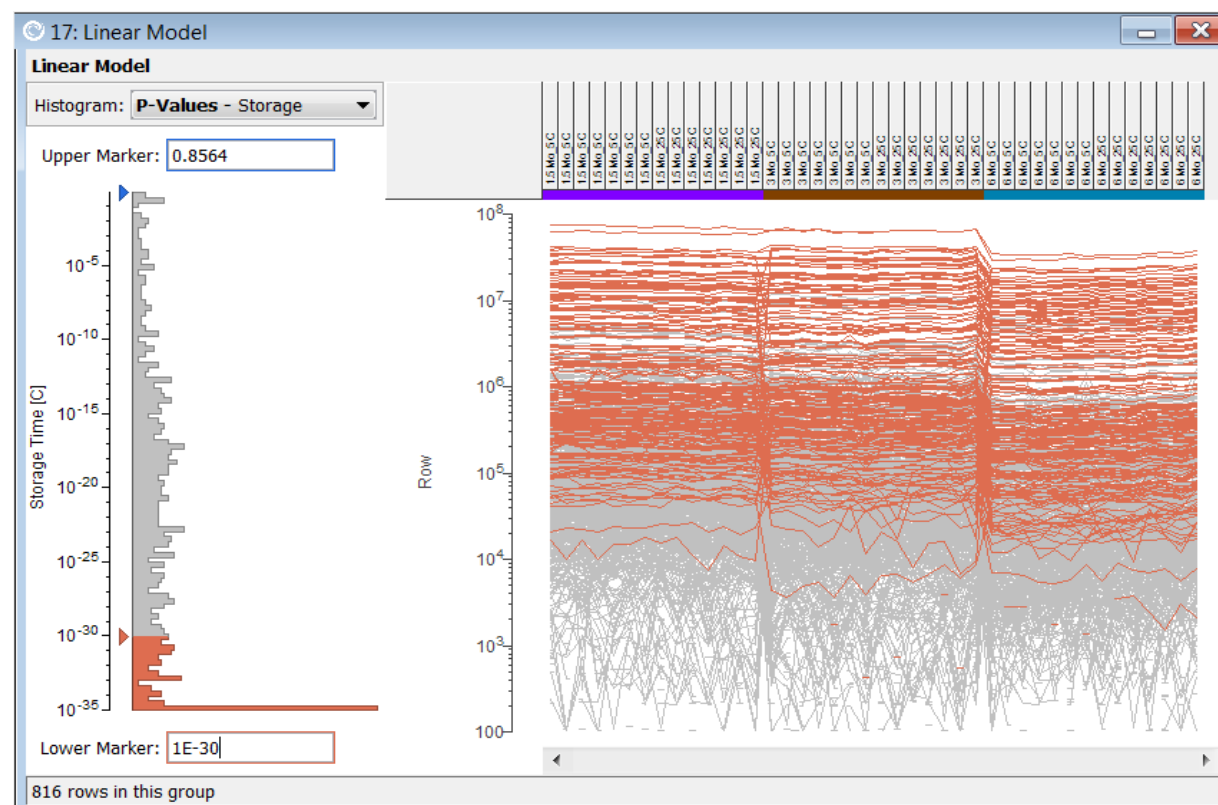
$y \sim a + b + a:b$

Example Model

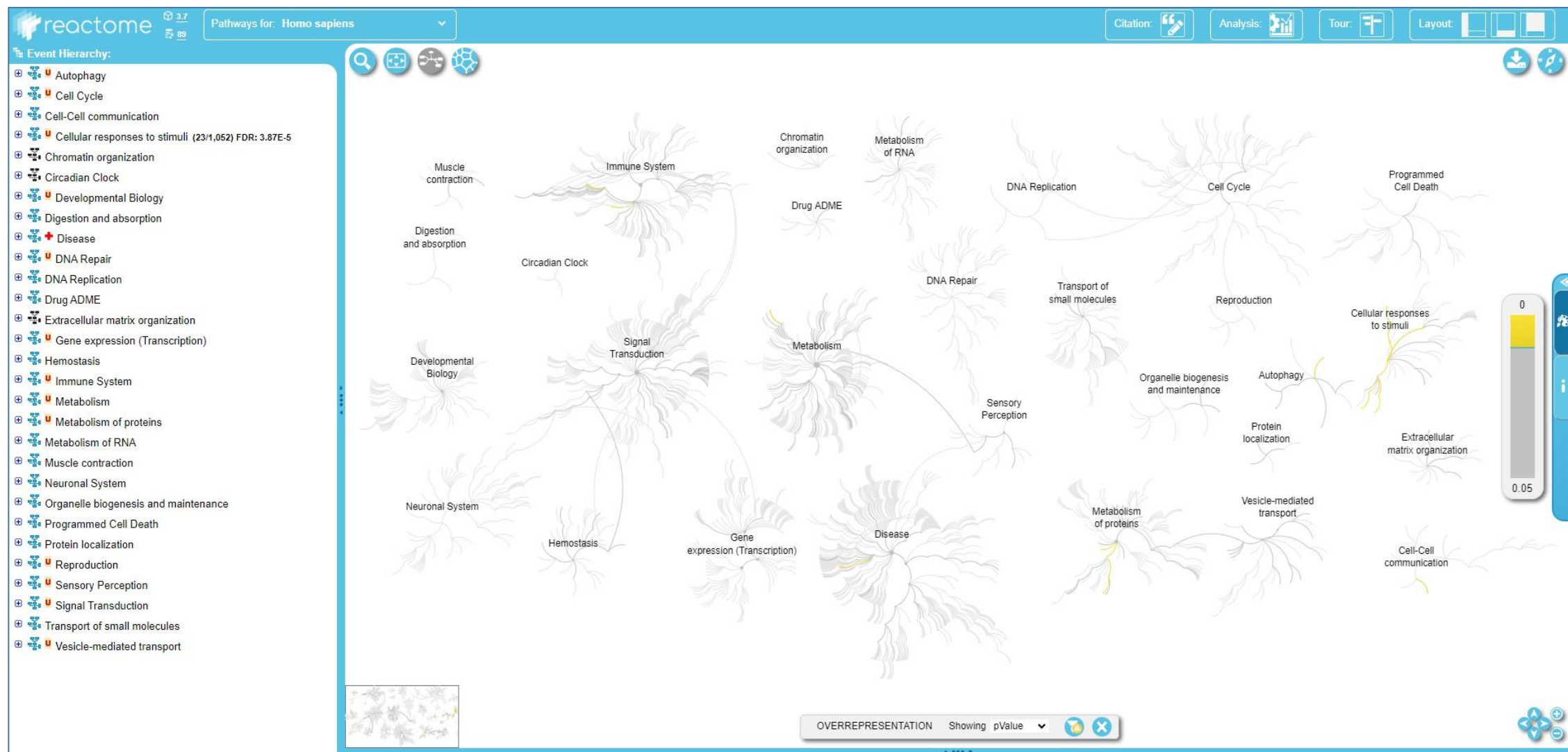
Defaults

Automatically show analysis results

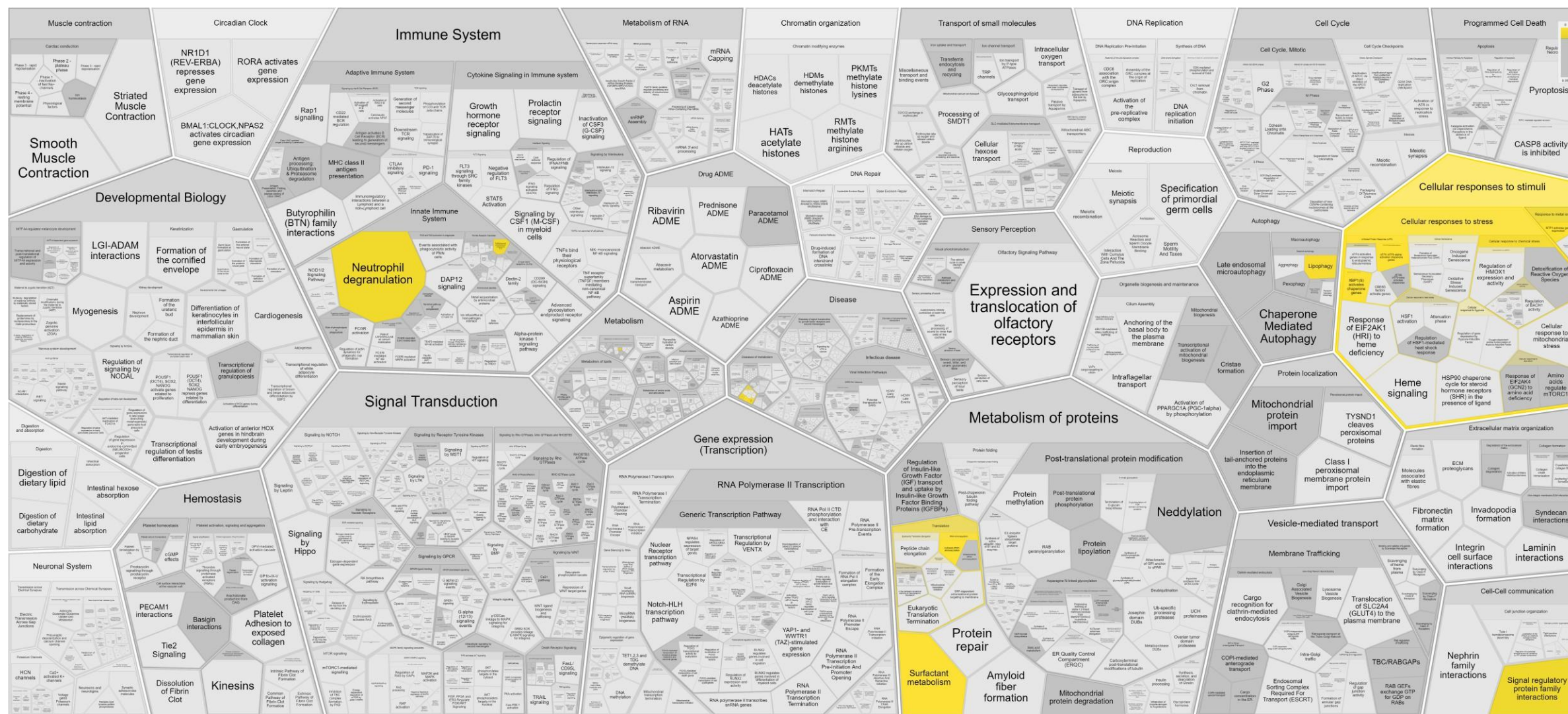
Run



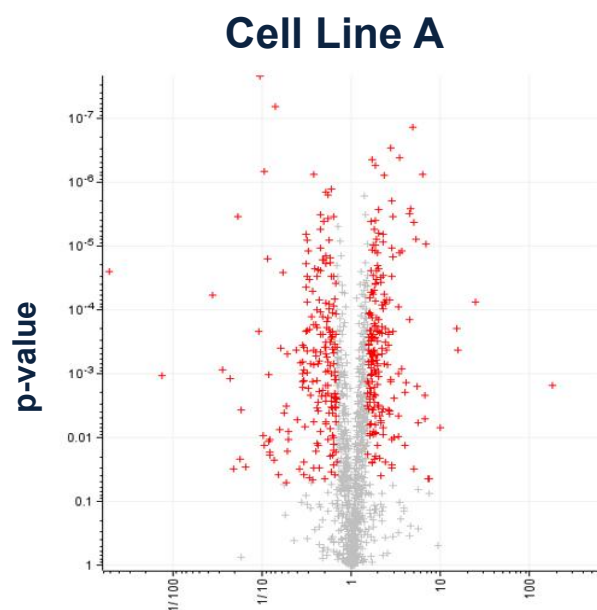
- Overrepresentation Analysis Results (P-value < 0.01)



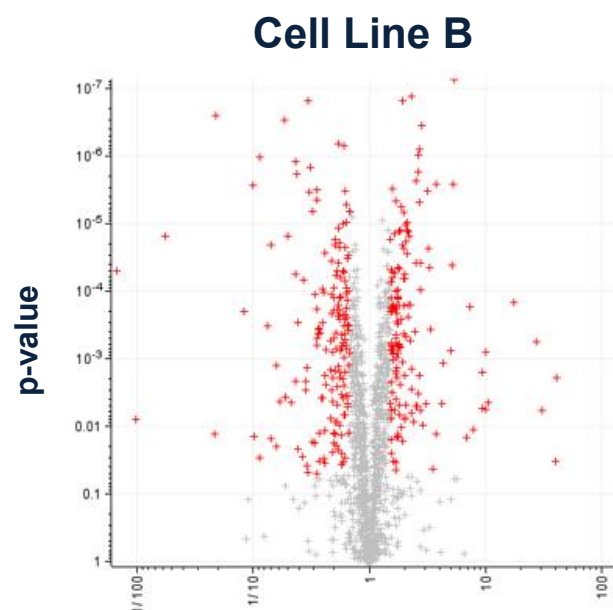
Overrepresentation analysis - Pathway Diagram (P-value < 0.01)



Proteomics analysis reveals increased f_c in batch processes indicate cellular stress



Cell Line A: Ratio (Day 3 vs Day 7)



Cell Line B: Ratio (Day 3 vs Day 6)

Pathway name

Cellular responses to stimuli/ stress

Unfolded Protein Response (UPR)

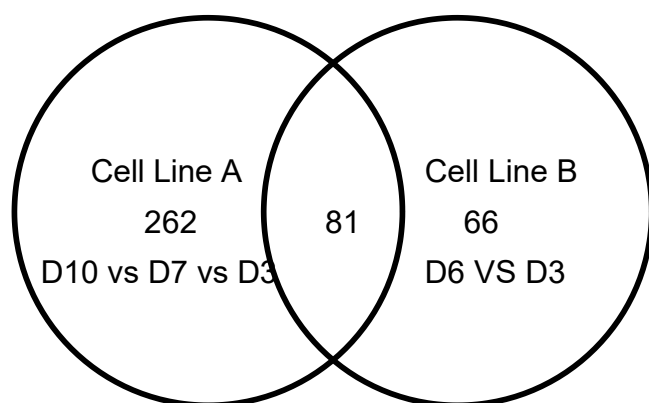
Cytosolic tRNA aminoacylation

ATF6 (ATF6-alpha) activates chaperone genes

IRE1alpha activates chaperones

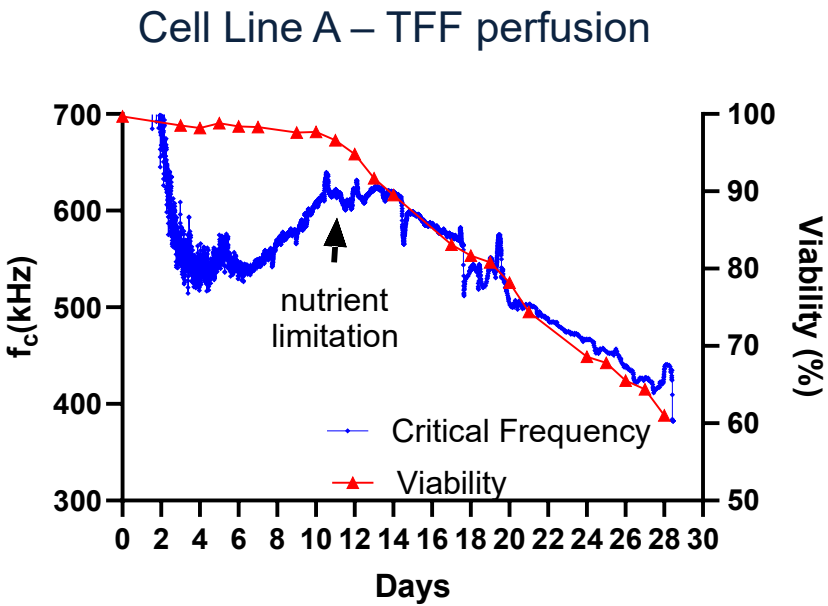
XBP1(S) activates chaperone genes

Mitochondrial unfolded protein response (UPRmt)

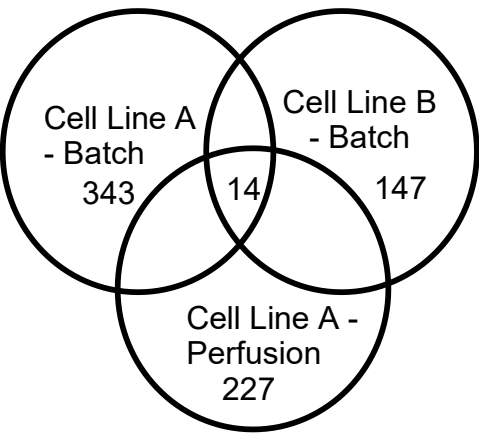


- With f_c increase, 343 genes were upregulated in Cell Line A and 147 genes were upregulated in Cell Line B – **81 genes common** between the 2 cell lines (fold change >1.5 and p-value < 0.05).
- Pathway analysis showed genes were associated with cellular response to stress (nutrient deficiency).

Increased Fc in perfusion and batch processes indicate cellular starvation

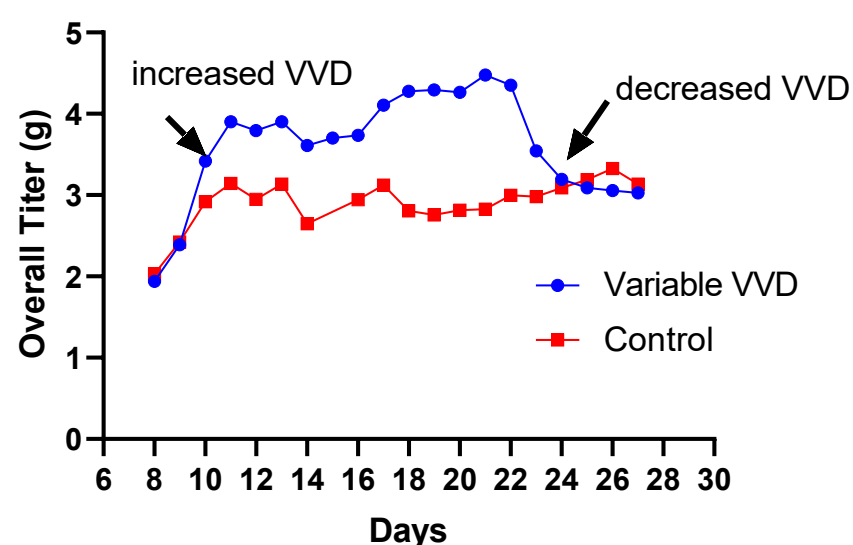
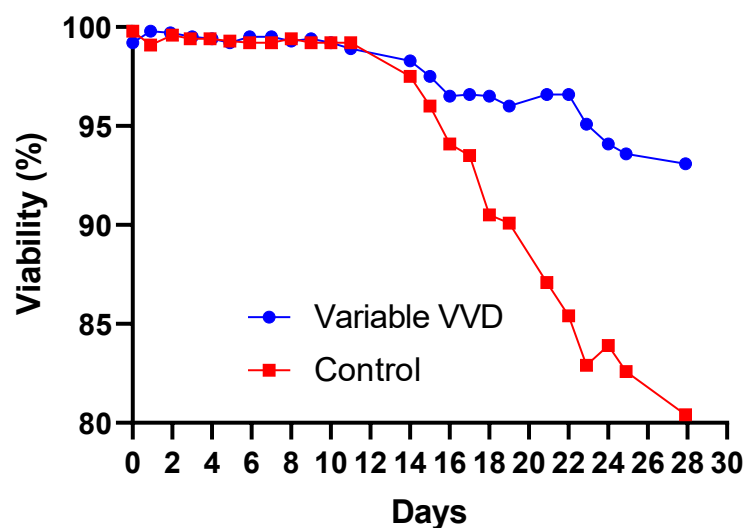
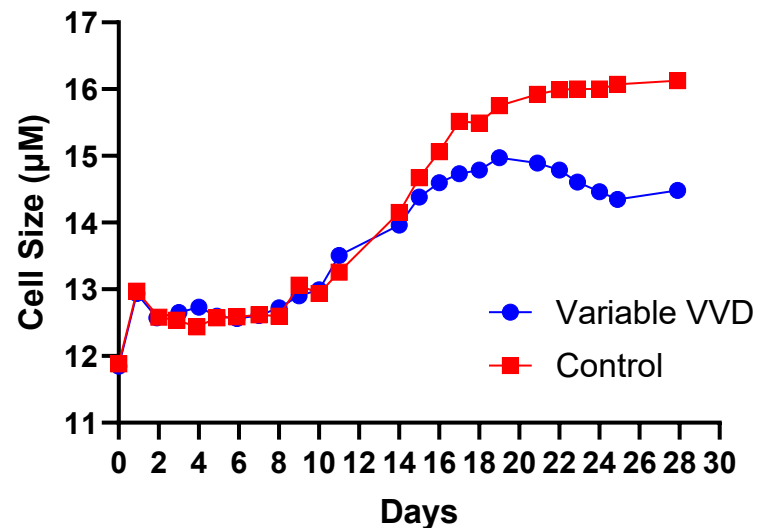
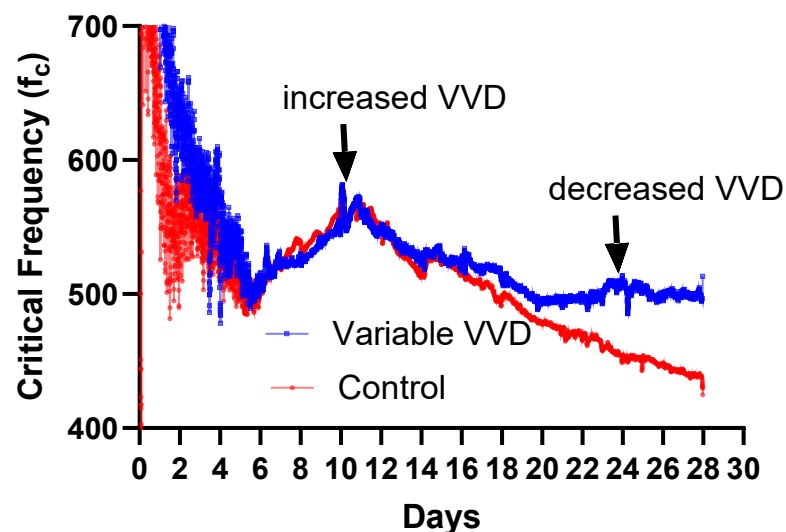


Pathway name
Cellular responses to stimuli/stress
Signal Transduction
Metabolism
MTF1 activates gene expression
Response to metal ions
Regulation of MITF-M-dependent genes involved in lysosome biogenesis and autophagy
Degradation of the extracellular matrix
Cellular response to starvation



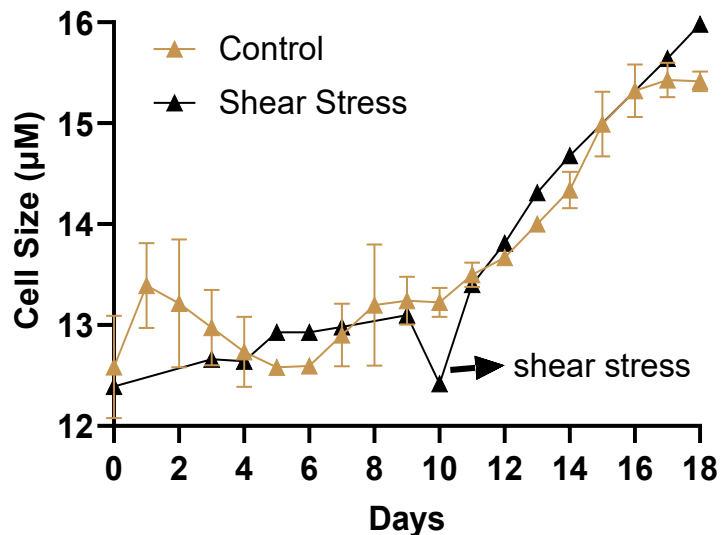
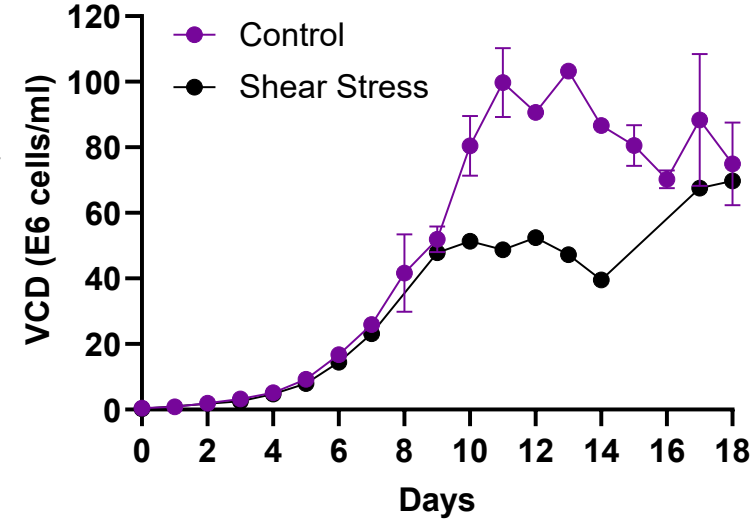
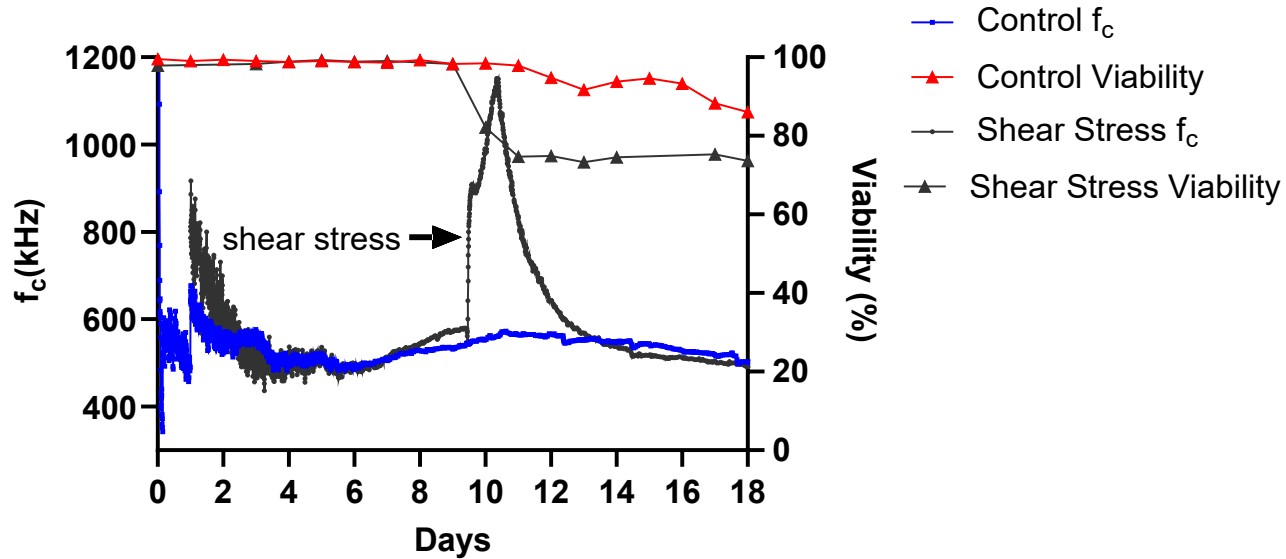
- Fc increases from baseline on D8 to D13 in TFF perfusion process similar to batch process
- **14 genes common** between TFF perfusion and batch processes across 2 cell lines with increased Fc
- Pathway analysis showed genes were associated with cellular response to stress (nutrient deficiency and autophagy).

Feeding strategy based on f_c improves TFF perfusion process performance



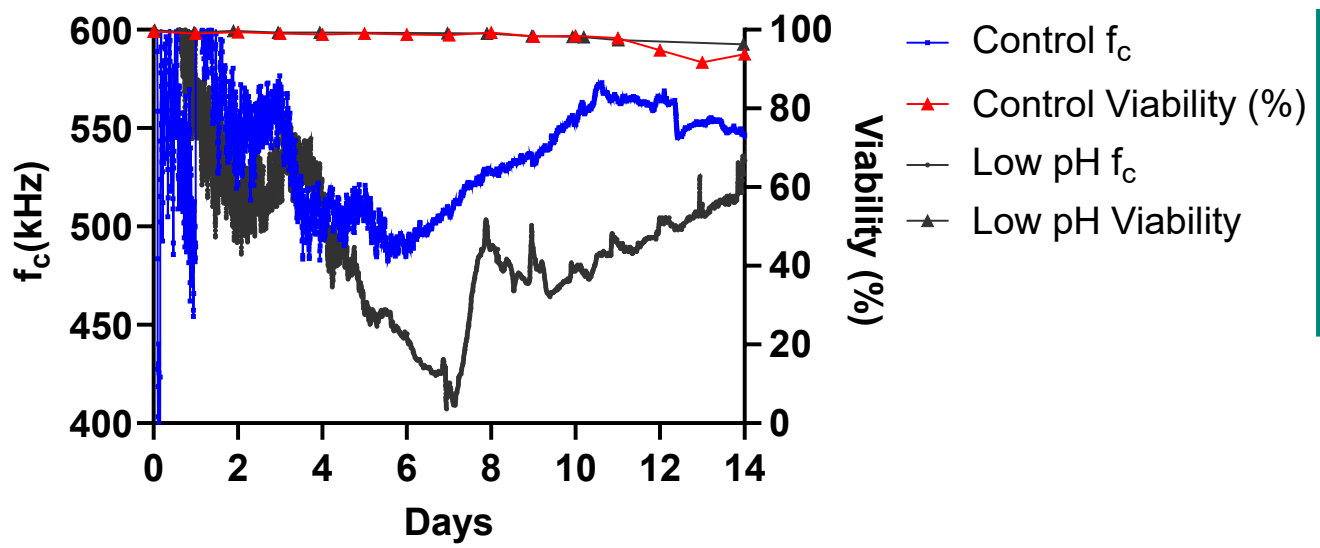
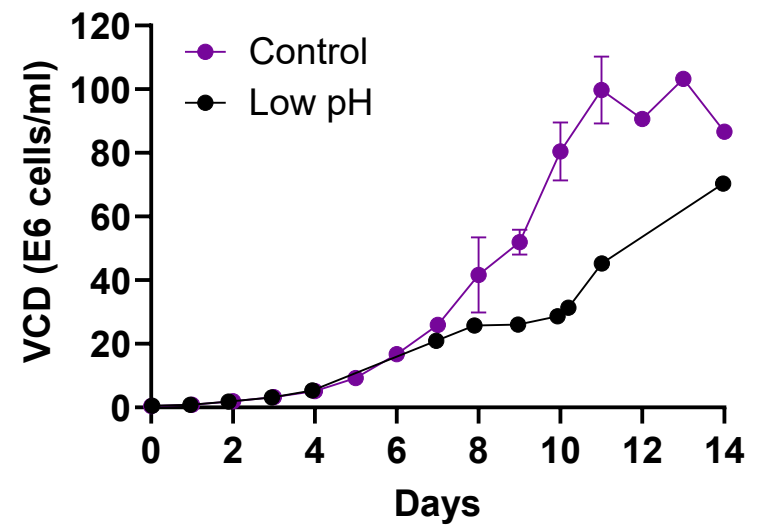
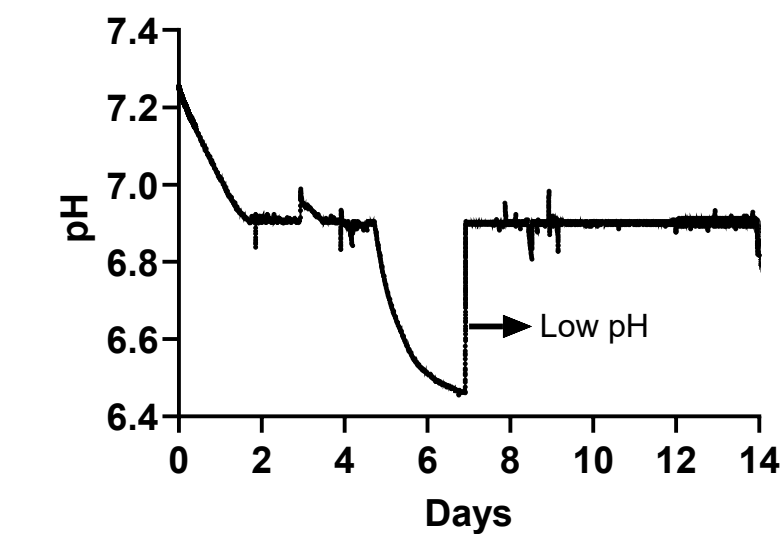
- Maintaining f_c at baseline post day 10 by increasing perfusion flow rate improved overall process performance
- Increased perfusion rate led to younger dividing cells, higher viability, and increased overall titer

Real-time monitoring of shear stress in a perfusion process using f_c



- Shear stress induced by high recirculation rates (day 10) is marked by a sharp increase in f_c – decrease in VCD and viability
- Reduction in cell size on day 10, potentially due to an increase in lysed and apoptotic cells
- The recirculation rate was reduced, resulting in f_c ultimately returning to baseline levels

Real-time monitoring of pH deviation in a perfusion process using f_c



- pH deviation from the normal process – f_c changes in real time
- No significant drop in viability with pH deviation but slower growth compared to control

Conclusions

- Critical Frequency (f_c) is a novel in-line feedback tool to:
 - ✓ Monitor upstream processes in real-time
 - ✓ Identify and act on process deviations quickly in manufacturing environment
 - ✓ Aid in process development and process characterization studies to develop robust upstream processes
- LC-MS proteomics workflow powered by Genedata Expressionist and Analyst is a valuable tool to:
 - ✓ Identify and quantitate the proteins that were upregulated during the process and Fc changes
 - ✓ Confirm that changes in Fc reflected cellular response to stress on the molecular level

Acknowledgements

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