# Cell Line qualifying attributes for prediction of good producers

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UMAss Lowell, Biopharmaceutical Summit

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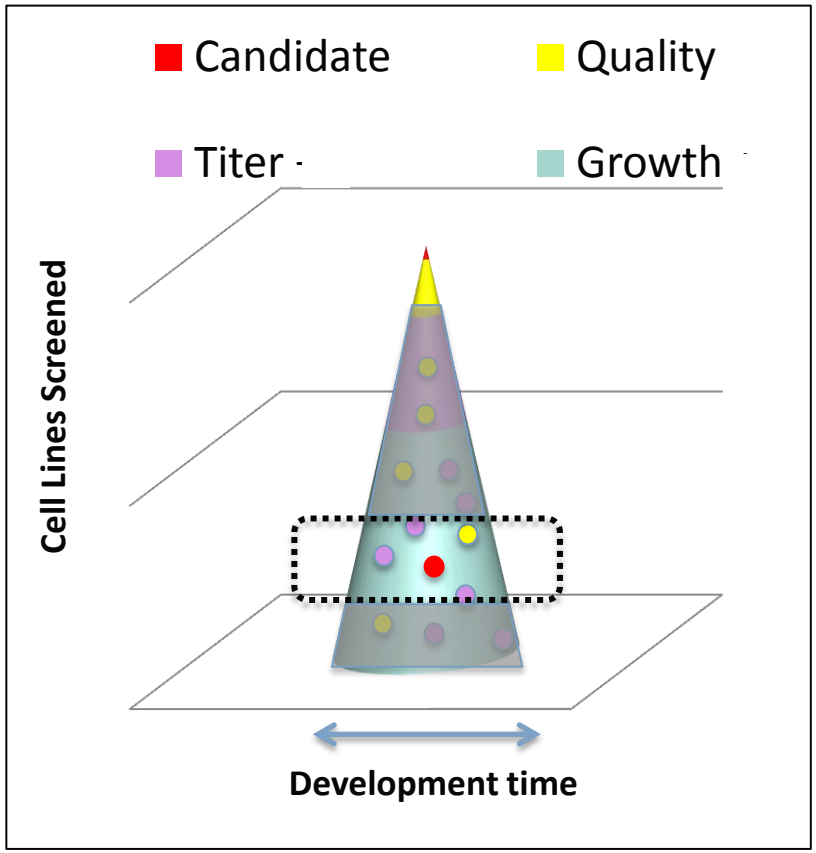
# Cell line development aims to select the “best” candidate

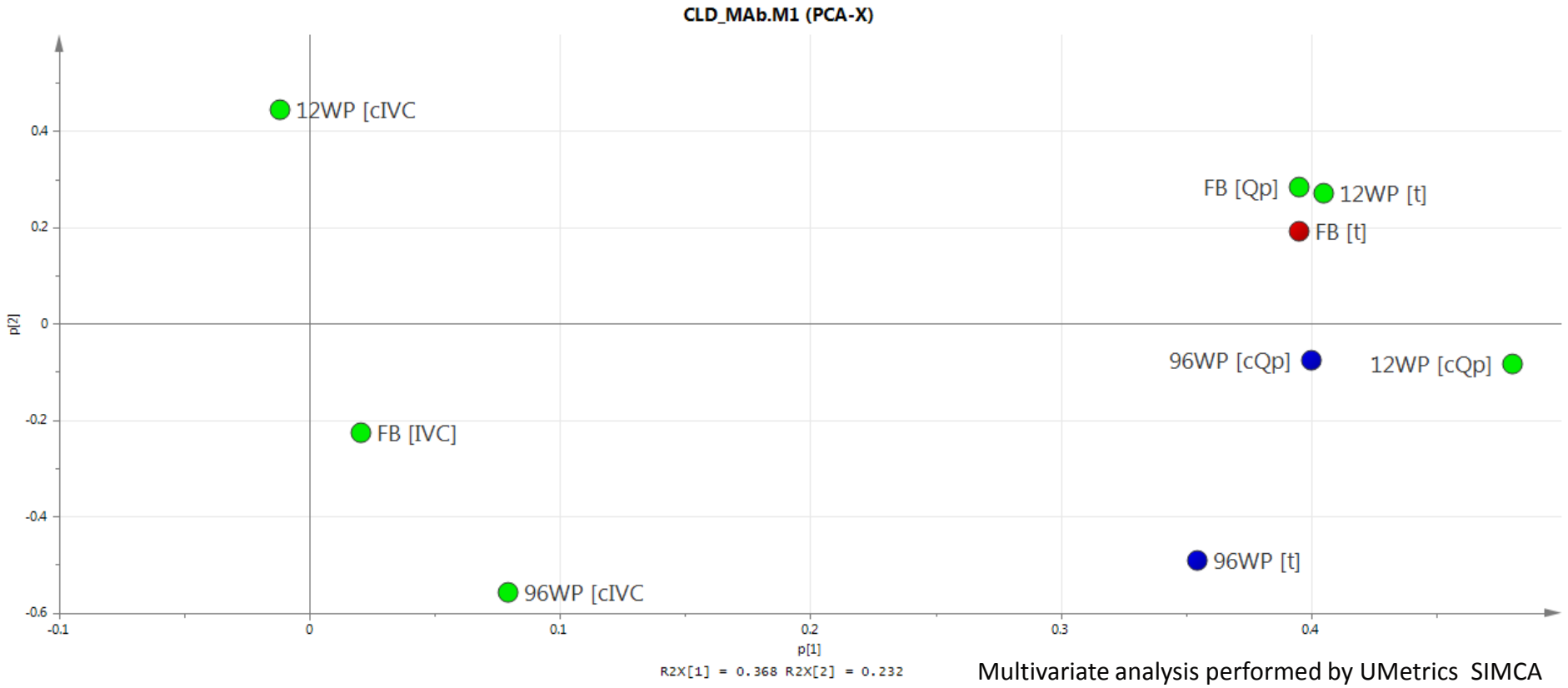



| | Titer [t] | Growth [IVC] | Productivity [Qp] (titer/growth) |
|---|---|---|---|
| 96WP | 96WP[t] | 96WP[IVC] | **96WP[Qp]** |
| 12WP | 12WP[t] | 12WP[IVC] | 12WP[Qp] |
| 96DWP fed-batch [FB] | FB[t] | FB[IVC] | FB[Qp] |

*96WP[IVC] and 12WP[IVC] attributes were generated by corrected confluence analysis*

Three different parental lines originated 320 sub-lines whose 9 attributes were screened throughout the development in order to **explore hypothetical patterns**

# 96WP[cQp] might be a better qualifying attribute than 96WP[t]



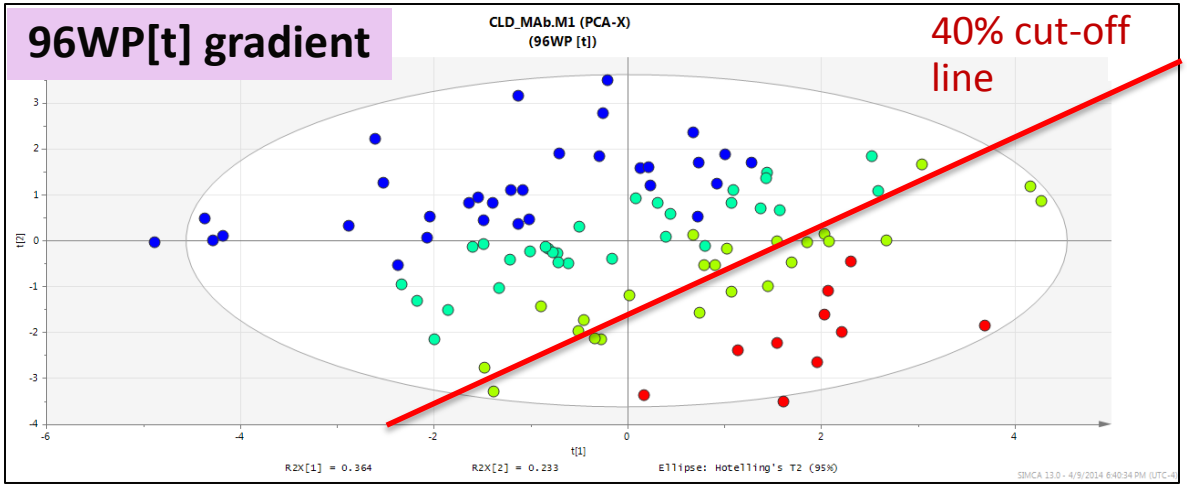

Multivariate analysis performed by UMetrics SIMCA

Multivariate Analysis explains relationships among all the attributes studied.

- Attributes closely located behave similar (positive covariance).
- Attributes far away from the origin **drive the *"best cell line"*** model.

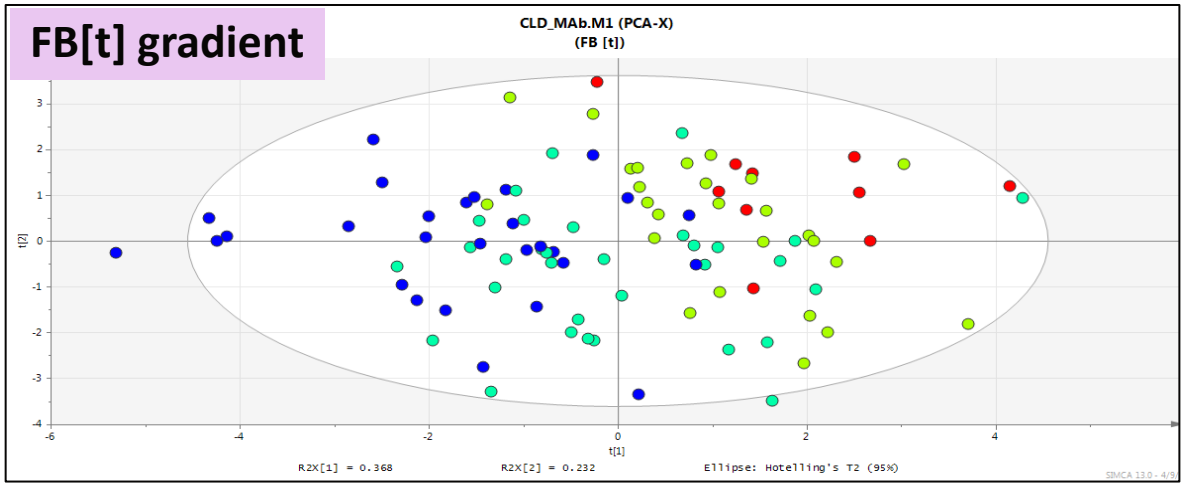
# Using static titer to predict final fed-batch titer

**96WP[t] gradient**




Poor prediction

**FB[t] gradient**




Desired scenario

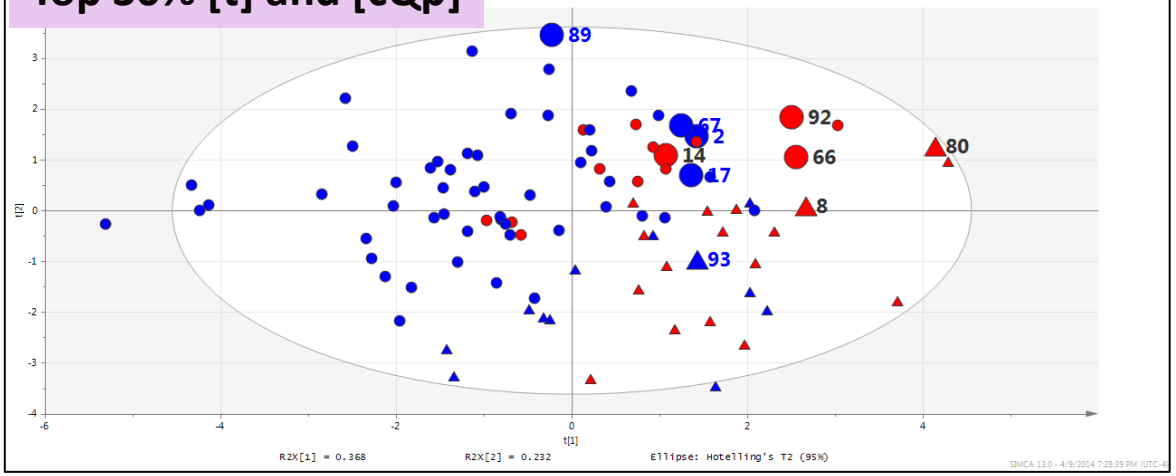
**Dots are cell lines**

- Low titer
- Mid-low
- Mid-high
- High producers

Final top performers in fed-batch are poorly predicted by static titer at 96-well plate. Vessel adaptation, cellular dynamics, mechanics and genetic events contribute to this.

# Top 96WP[cQP] and 96WP[t] performers Vs. Top 10% FB[t]

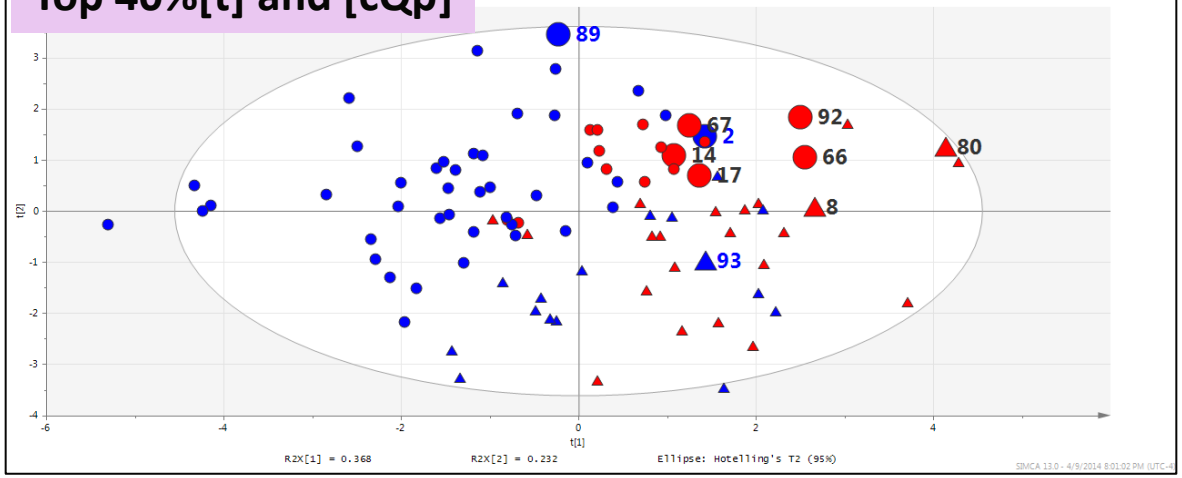
**Top 30% [t] and [cQp]**

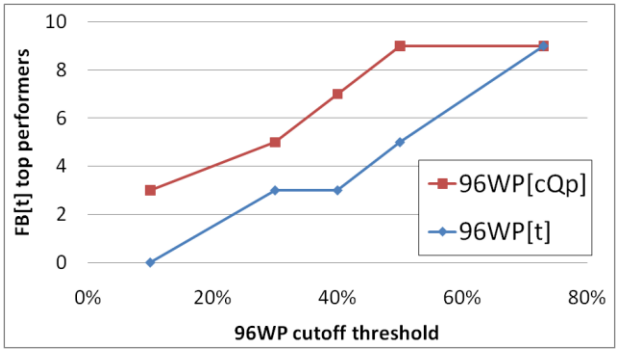



| | [t] high | [t] low |
|---|---|---|
| [cQp] high | ▲ | ● |
| [cQp] low | ▲ | ● |

Magnified shapes are final top performers in fed-batch

**Top 40%[t] and [cQp]**




96WP[cQp] is a powerful tool and captures more final candidates than 96WP[t].

# Conclusions

- Early stage titer in 96WP doesn’t predict top FB producers.
- Productivity in 96WP shows a good predictive power combining existing 96WP[t] with new developed 96WP[IVC] attribute.
- 96WP[cQp] acts like an enhanced prediction tool and it might save about 20-40% initial workload in cell line development.

Biopharmaceutical Summit 2014

# Metabolomics for CHO-Cell Production

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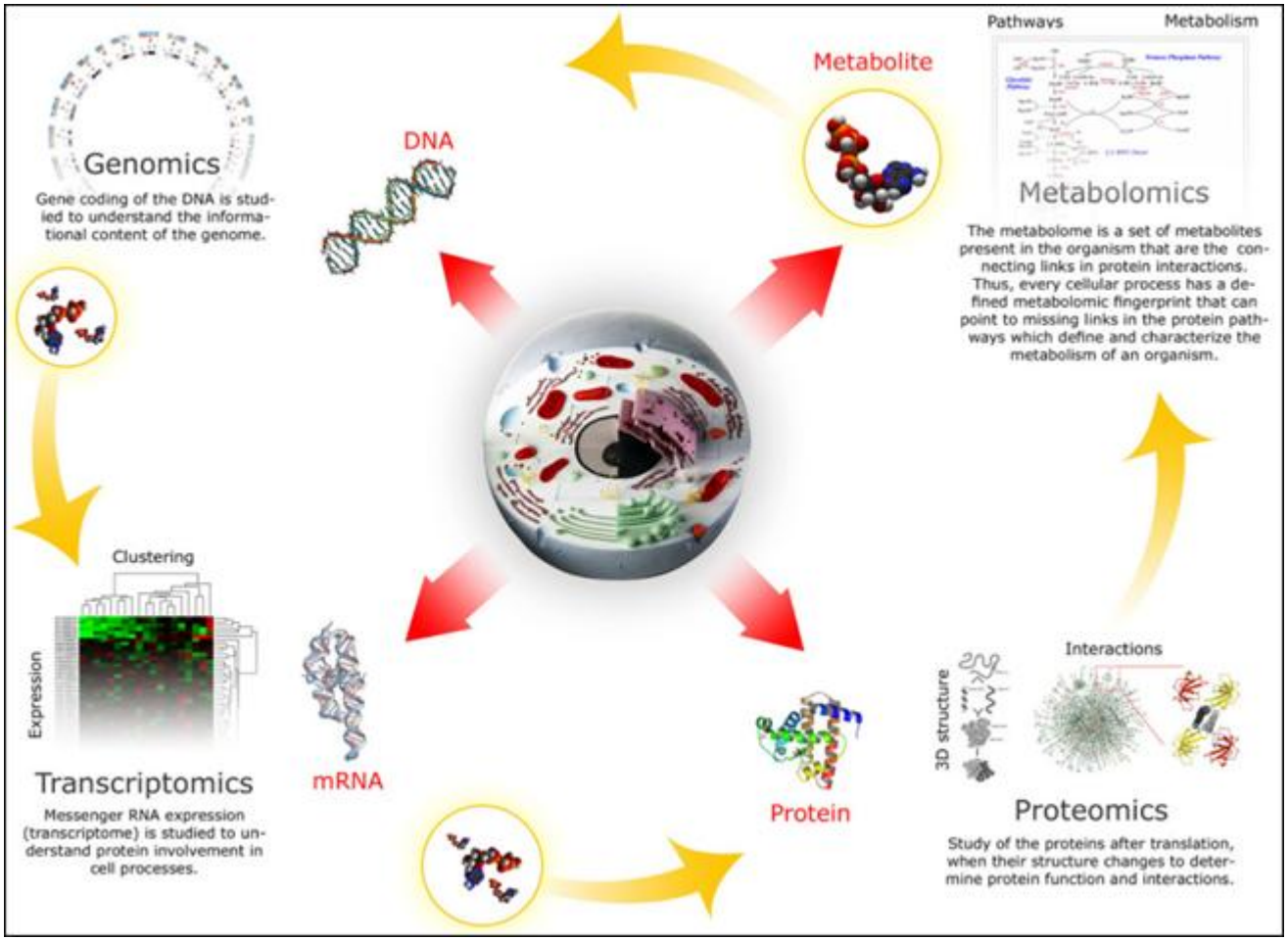
05/30/2014

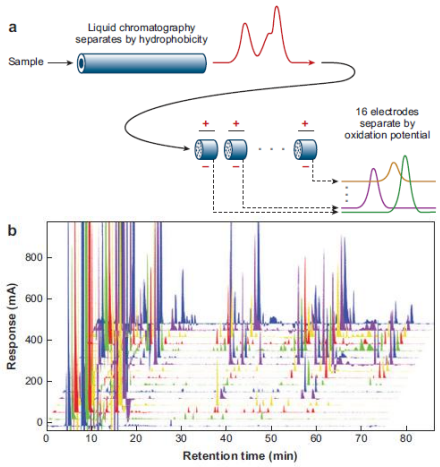
# Overview




**Genomics** and **Proteomics** tell you what **might** happen, but **metabolomics** tells you what actually **did** happen!

# The conceptual approach in metabolomics

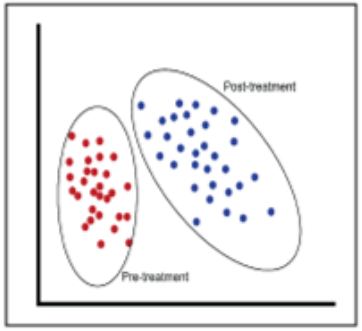
Sample Collection



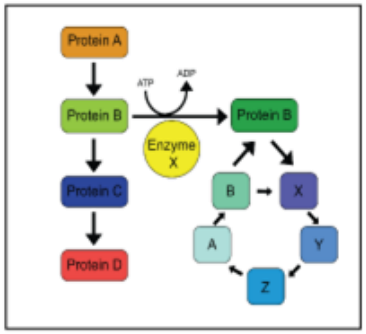

Metabolite identification & quantification
(NMR/GC-MS/LC-MS)

Database Production

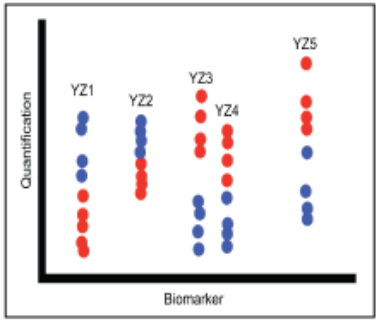
**Computational Analysis**



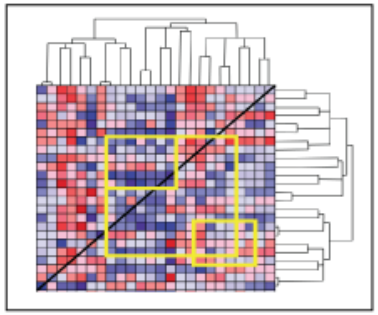

Identify & Compare Classes




Map to Metabolic pathways




Quantify putative biomarkers



Identify metabolite interactions

# Conclusion and Future Directions

- The metabolomics enables the identification of the metabolome while revealing tremendous information about cellular function and the metabolic pathways of a cell. It is increasingly used in investigations of more subtle effects, such as the indication of pharmaceutical efficacy, and the probing of life-style changes, nutrition and the complex interconnection of metabolic processes.

- **Challenges in Metabolomics:**
  (1) metabolites have a wide range of molecular weights and ***large variations*** in concentration,
  (2) the metabolome is much ***more dynamic*** than proteome and genome, which makes the metabolome more ***time sensitive***,
  (3) metabolites can be either polar or nonpolar, as well as organic or inorganic molecules. This makes the ***chemical separation*** a key step in metabolomics,
  (4) metabolites have ***chemical structures***, which makes the ***identification*** using MS an extreme challenge.

- **Future work we will explore:**
  (1) development of metabolite identification and Standardization of culture conditions,
  (2) standardization of sample preparation protocols will ensure reproducibility and reliability,
  (3) development of new sample preparation methods will increase metabolite coverage in future manufacturing cell metabolomics studies.


# Biochemical Reaction Network Modelling for Glycosylation

Sha Sha

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# Cell compartment (Golgi) interpretation

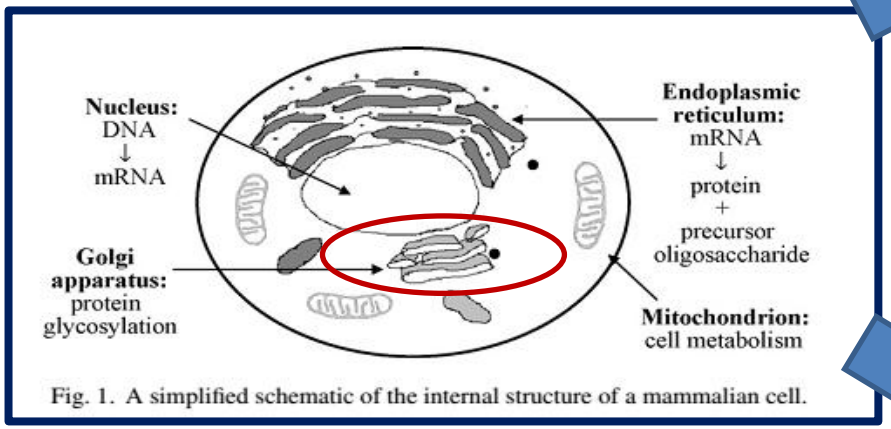
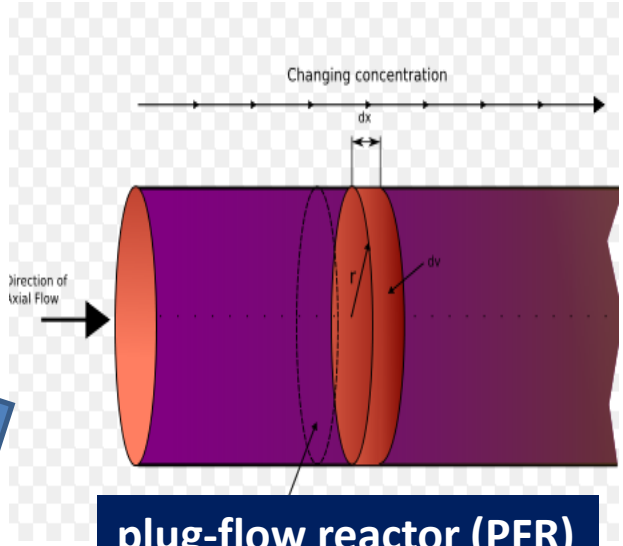
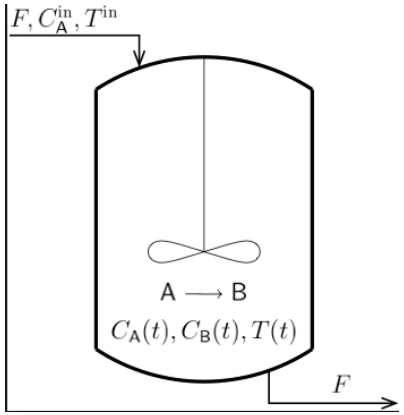


Fig. 1. A simplified schematic of the internal structure of a mammalian cell.

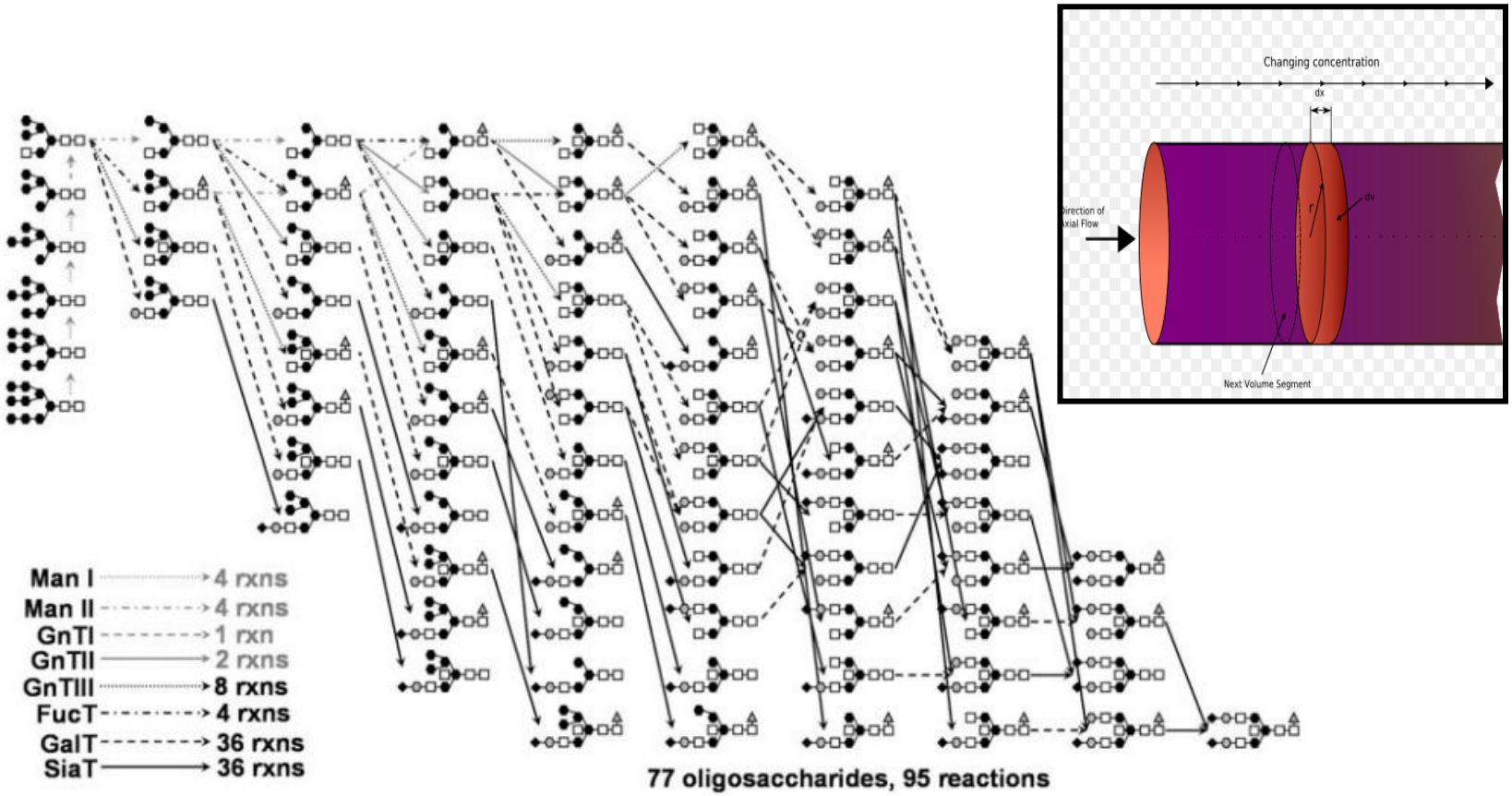



**plug-flow reactor (PFR)**




**countinous well-mixed reactor (CSTR)**

# Chemical reaction for glycosylation



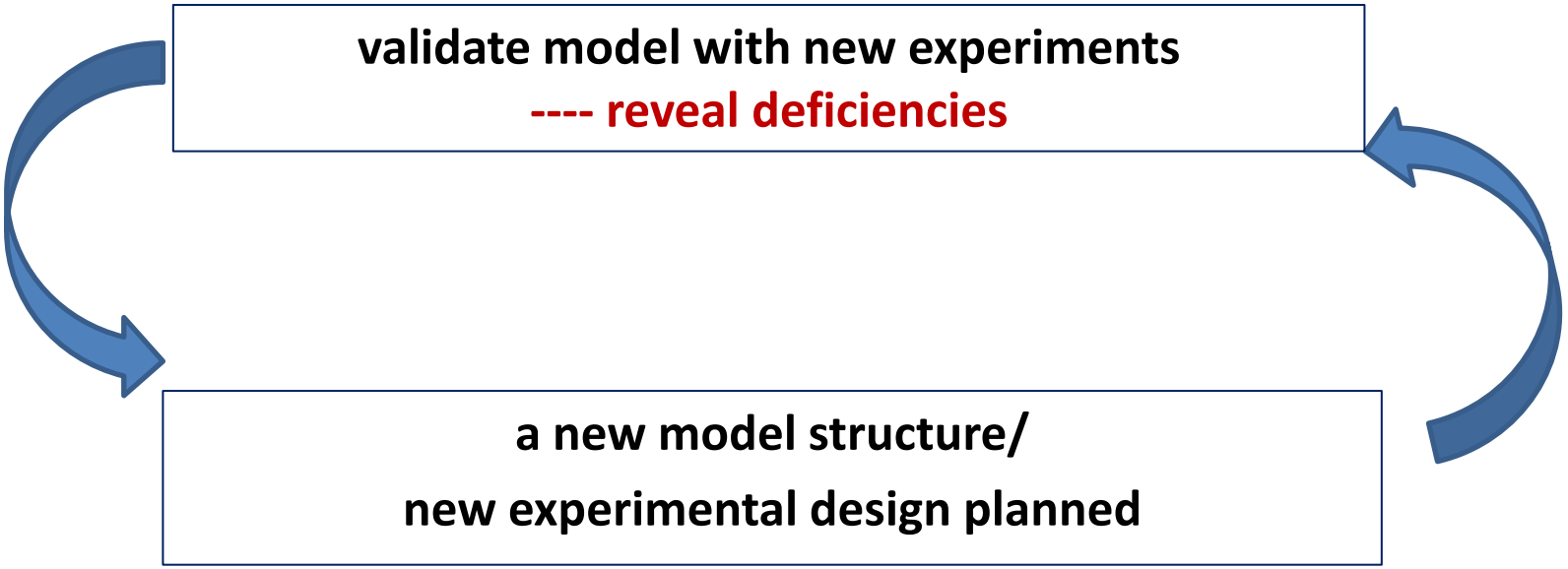

Val et al. *Biotechnol. Prog.*, 2011

# Steps to the first working model

- 1. Define the **frame and structure** of the model based on *a priori* knowledge

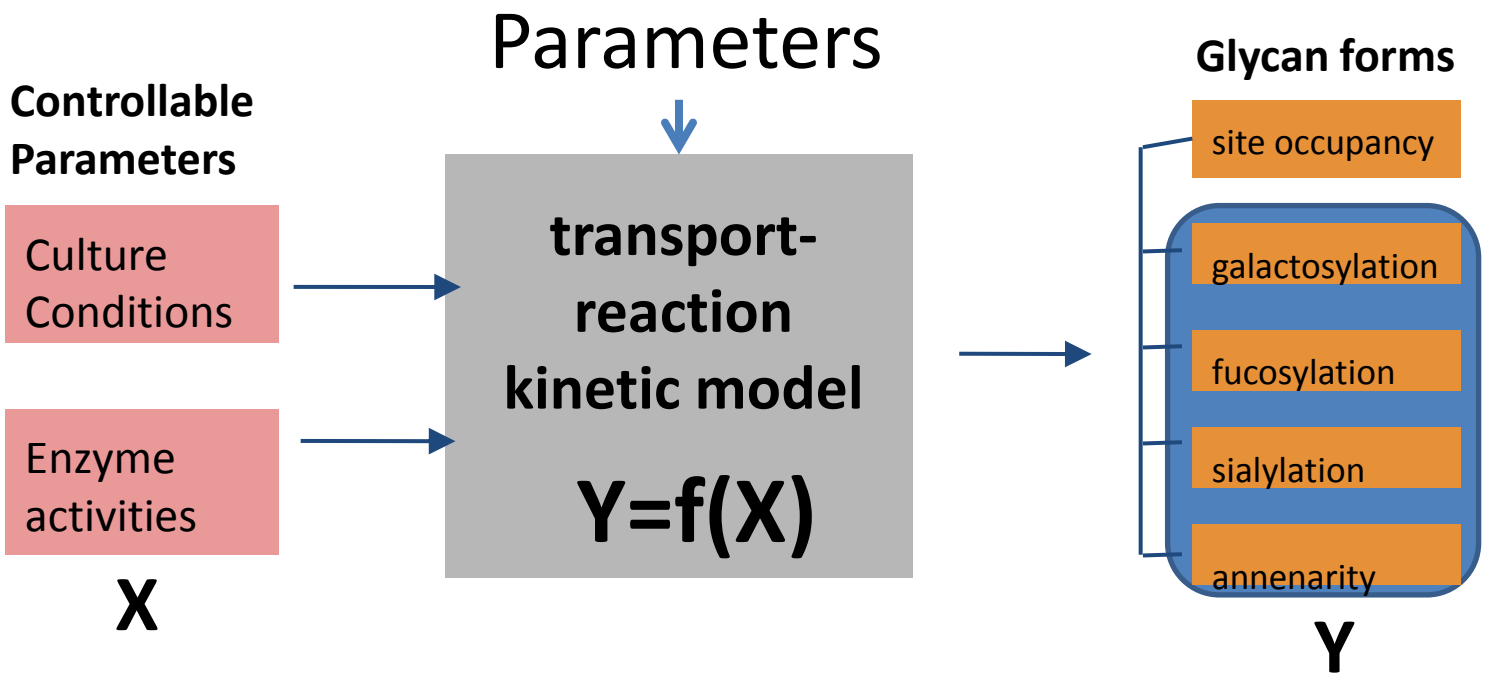
- 2. **Parameter estimation** from available data
  - Golgi size
  - Protein flow rate
  - Donor concentration (carbon feeding, metabolites)
  - Enzyme concentration
  - Enzyme kinetic constants
  - Transport protein concentration
  - Glycan profile
  - …

# Validation and Optimization of model




models and experiments are disigned in tandem ensuring that sets of modelled and measured variables can be matched to each other

# Conclusion




- Simulate, predict and optimise procedures, experiments and therapies
- Disprove hypotheses and to define improved hypotheses (based on comparison of model-predicted and experimentally measured variables)

# Protein A Column: Modeling, Simulation & Multi-Variate Analysis

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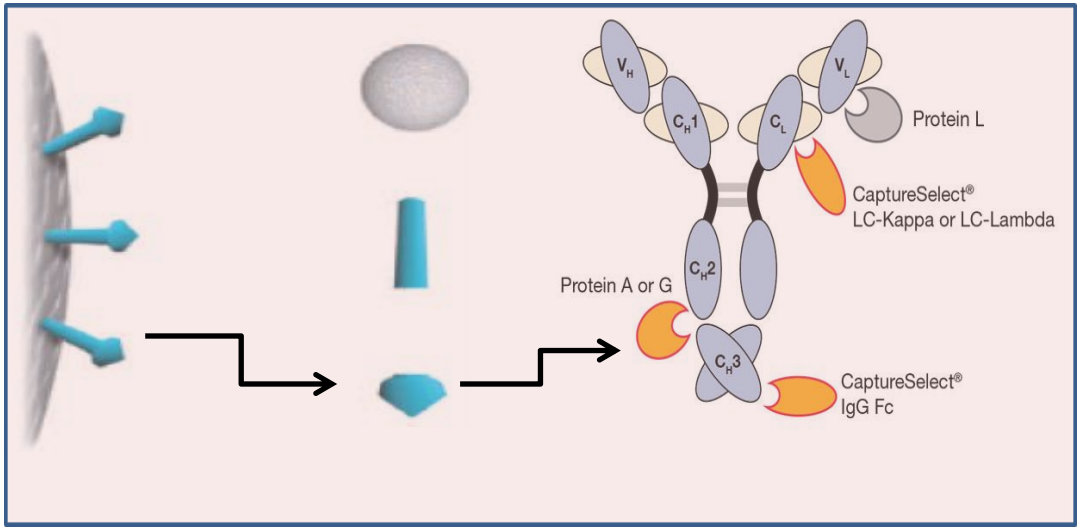
PhD in Chemical Engineering
Umass Lowell, MA

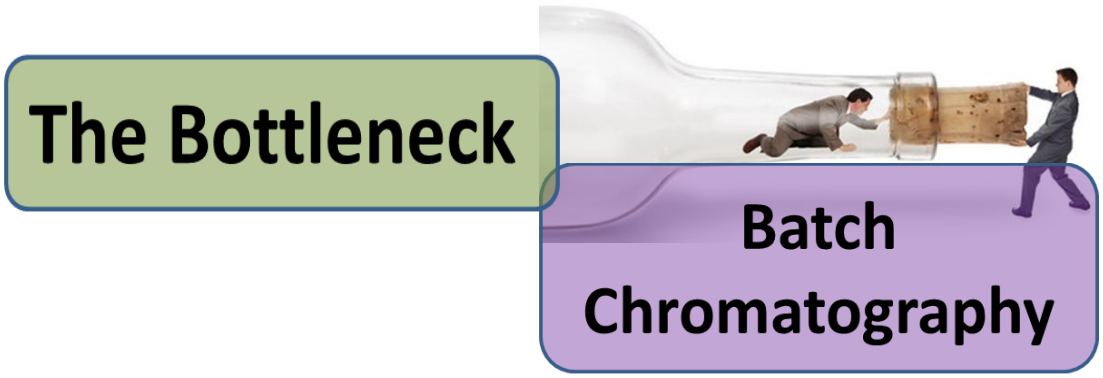
**Advisor: Prof Seongkyu Yoon**

# Why Protein A column?

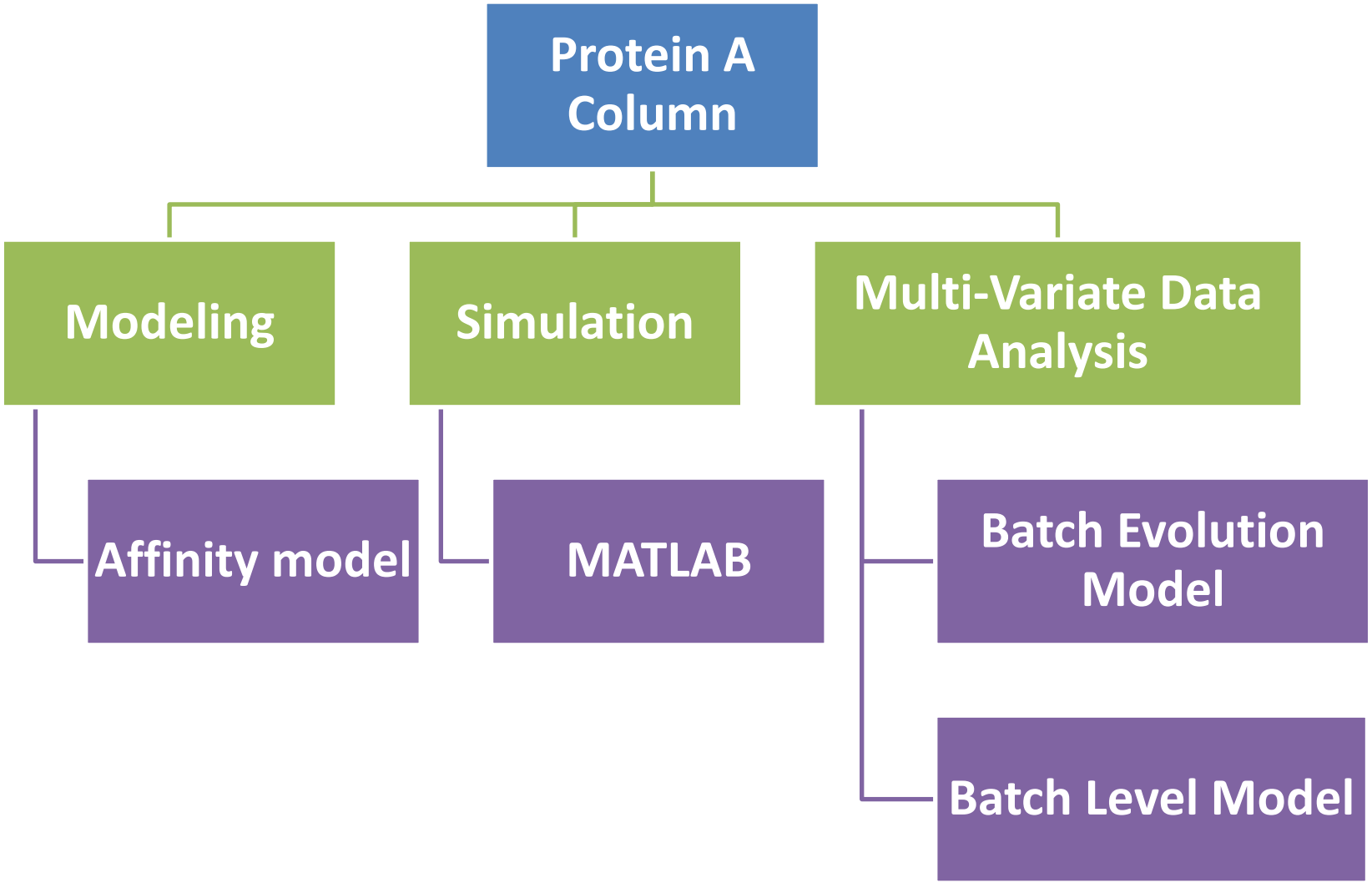
- Protein A: Affinity column used as a capture step




- Affinity binding specific to Monoclonal Antibody provides > 95% purity

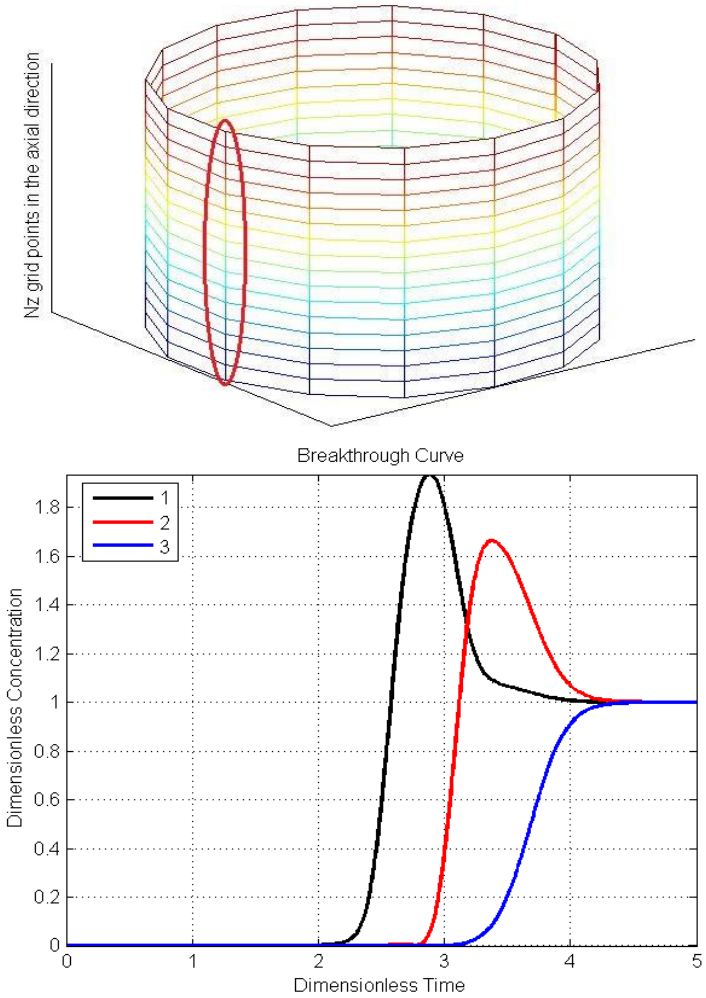


# Research Focus



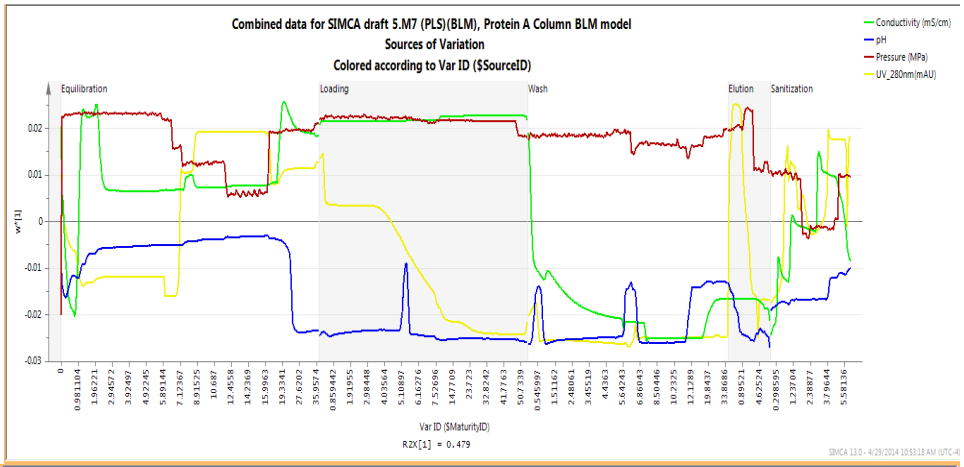
# Modeling & Simulation

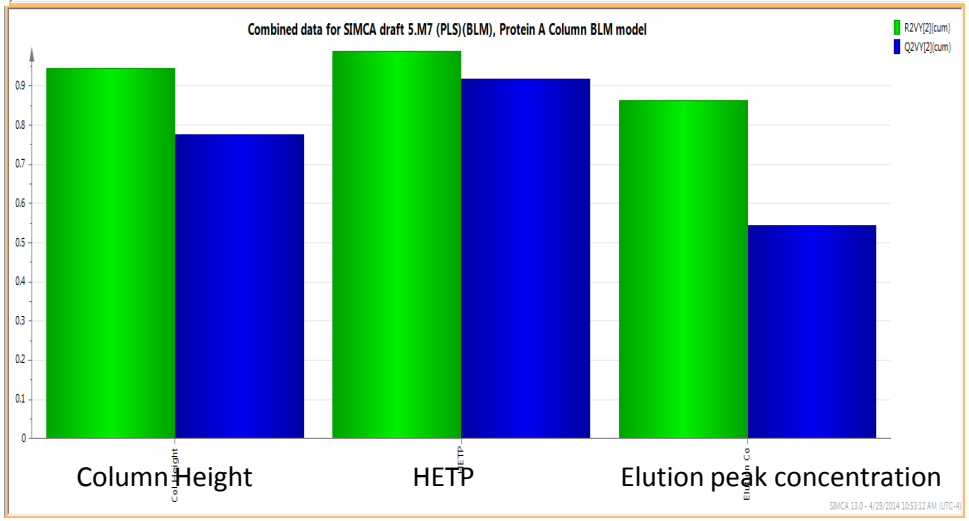
- General Rate Model customized for Affinity column: Protein A
- MATLAB Simulation for Single Column
- Project Single-column simulation to Multi-Column



# Multi-Variate Data Analysis

- Explain the variability in dataset by building a regression model
- Predictability to the user
- Determine the resin shelf-life & online qualification of column
- Used to complement DOE studies in process development



# Conclusion

- Mechanistic model to better explain, simulate, control and analyze the chromatography process.
- The purification process will become robust improving the process economics and increase the productivity.
- A Continuous Downstream Process (CDP) with Model & Simulation will demonstrate the Next Generation Bioprocess.