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Towards model-based bioprocess characterization: A mathematical model of cell cycle, metabolism and apoptosis of mAb-producing mammalian cells

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A predictive model of cell-cycle, metabolism, and apoptosis of mAb-producing GS-NSO cells

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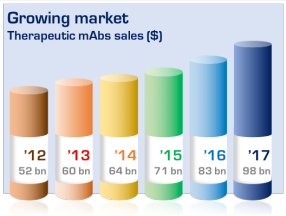
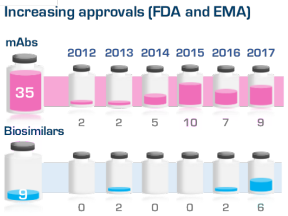
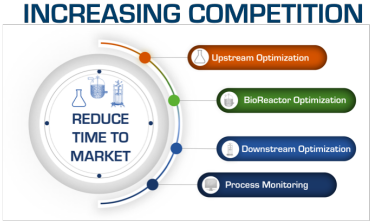
Motivation and Objectives





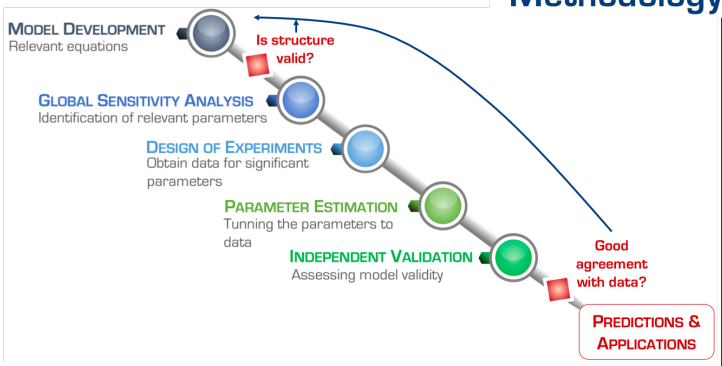
PROCESS DEVELOPMENT NEEDS TO COPE WITH INCREASING COMPETITION



SAVE TIME & MONEY

DO IT MODEL BASED!

Methodology



Optimal Operation
- Medium Design
- Feeding Strategy

BioReactor Optimization
- Integrate design with kinetics
- CFD
- Model-based design

Downstream Optimization
- Optimize strategies
- Optimize operations
- Integrate with upstream

Results

1. Model Development

Integrated, first-principles, upstream model

CELL CYCLE — Population Balance Equations; 3 Lumped phases considered; Dependence on metabolism

PBM Based on cyclins and DNA

Track cell-cycle & understand differential mAb production [1]

$$\frac{\partial N_{i=G1,S,G2}(x_i,t)}{\partial t} + \frac{\partial}{\partial x_k}[r_i(x_k,S)N_i(x_i,t)] = -\Gamma_i(x,S)N_i(x_i,t) - [\mu_A(g)+\mu_D(g)]N_i(x_i,t) + h\int_{x_{imin}}^{x_{imax}} p.\Gamma_j(x_j,S)N_j(x_j,t)\,dy$$

APOPTOSIS — ODEs; 4 key genes considered; Relationship with metabolism

Caspases 3 and 8, bcl-2 and bax expression vs. nutritional stresses

$$\frac{dmRNA_{cB}}{dt} = \beta_{cB}\sum_{i=Asp,Glu,Arg}\frac{km_{i,cB}}{[i]+km_{i,cB}} - \alpha_{cB}.mRNA_{cB}$$

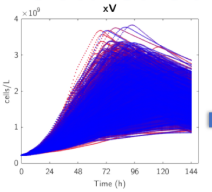
METABOLISM — ODEs; 19 Amino Acids, Glucose; ATP production and usage; mAb production

Predict nutrient requirements, metabolic shifts, ATP consumption and mAb production [2]

$$\frac{d(V.[AA_i])}{dt} = xV.V.\left(Q_{prod,j} - Q_{x,j} - Q_{mAB,j} - Q_{tca,j}\right)$$

Final Structure:
970 algebraic eqs, 455 differential eqs, 62 pars. Running time: 2 seconds

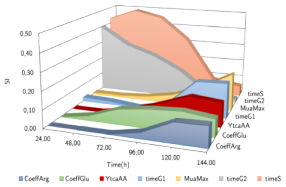
2. Global Sensitivity Analysis



5000 simulations

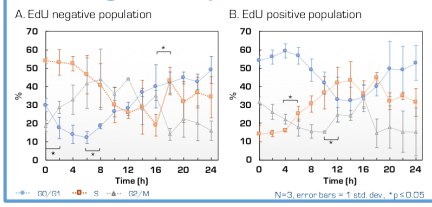
First and Second order parameter interactions

30 significant parameters



Biological Significance
1. Until 72h, the viable cells (xV) profile is largely dominated by the cycling times;
2. Between 72 and 120h the usage of Glutamate and Arginine dominate the profile;
3. On the later stages, the apoptosis rate dominates the profile.

3. Design of Experiments: Determining Cycling times



30μM EdU added during exponential phase

Populations tracked every 2h for 24h – times most important at early times according to GSA

Results:
TimeG1 = 10h
TimeS = 6h
TimeG2/M = 6h

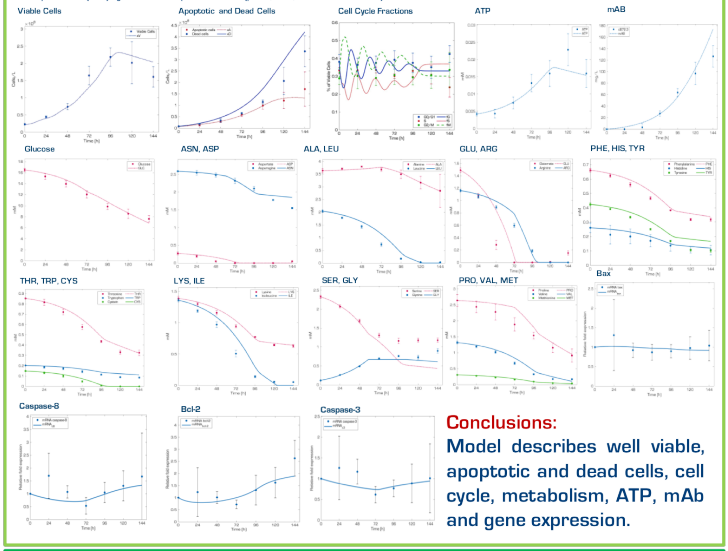
Total time = 22h

N=3, error bars = 1 std. dev., *p≤0.05

4. Parameter Estimation

Re-estimated 30 significant parameters grouped by biological meaning using Maximum Likelihood Estimation

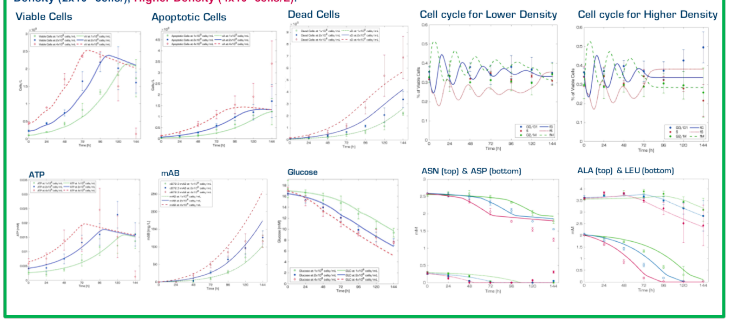
Model predictions (lines) agree well with experimental data (points, N=3, bars = 1 std deviation)



Conclusions:
Model describes well viable, apoptotic and dead cells, cell cycle, metabolism, ATP, mAb and gene expression.

5. Model Validation

Two independent sets of experimental data prove model predictive capabilities: Lower Density (1x10⁸ cells/L), Nominal Density (2x10⁸ cells/), Higher Density (4x10⁸ cells/L).



Conclusions

A validated, predictive mathematical model of cell-cycle, metabolism, mAb production and apoptosis has been obtained and may be used to improve bioprocessing operating conditions or other biological systems.

Acknowledgements

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References

[1]. Garcia-Munzer et al. ***Plos Comput. Biol.*** 2015, *11 (2)*
[2]. Quiroga-Campano et al. ***Met. Eng.*** 2018, 47