# Ensemble Modeling of Yeast Metabolic Oscillations

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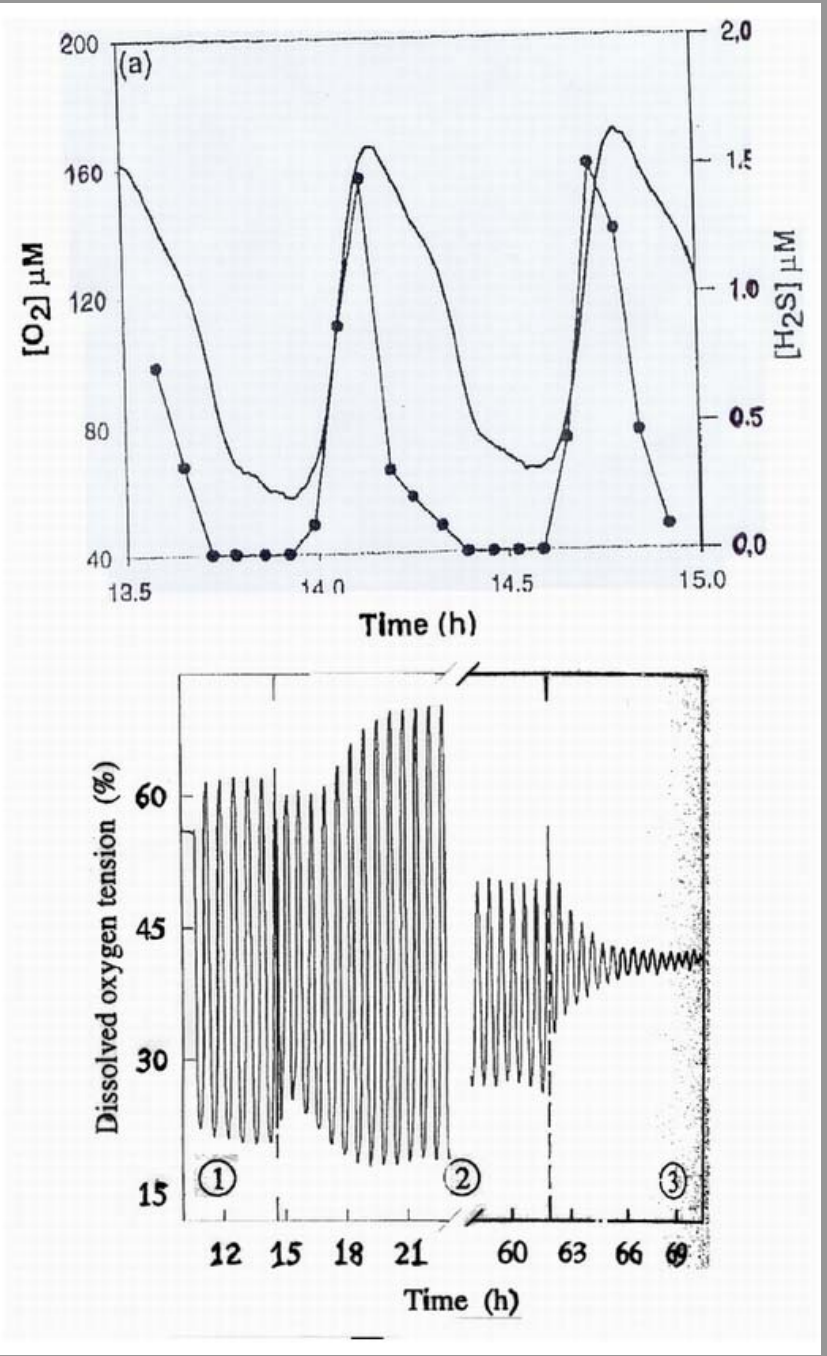
# Autonomous Yeast Oscillations

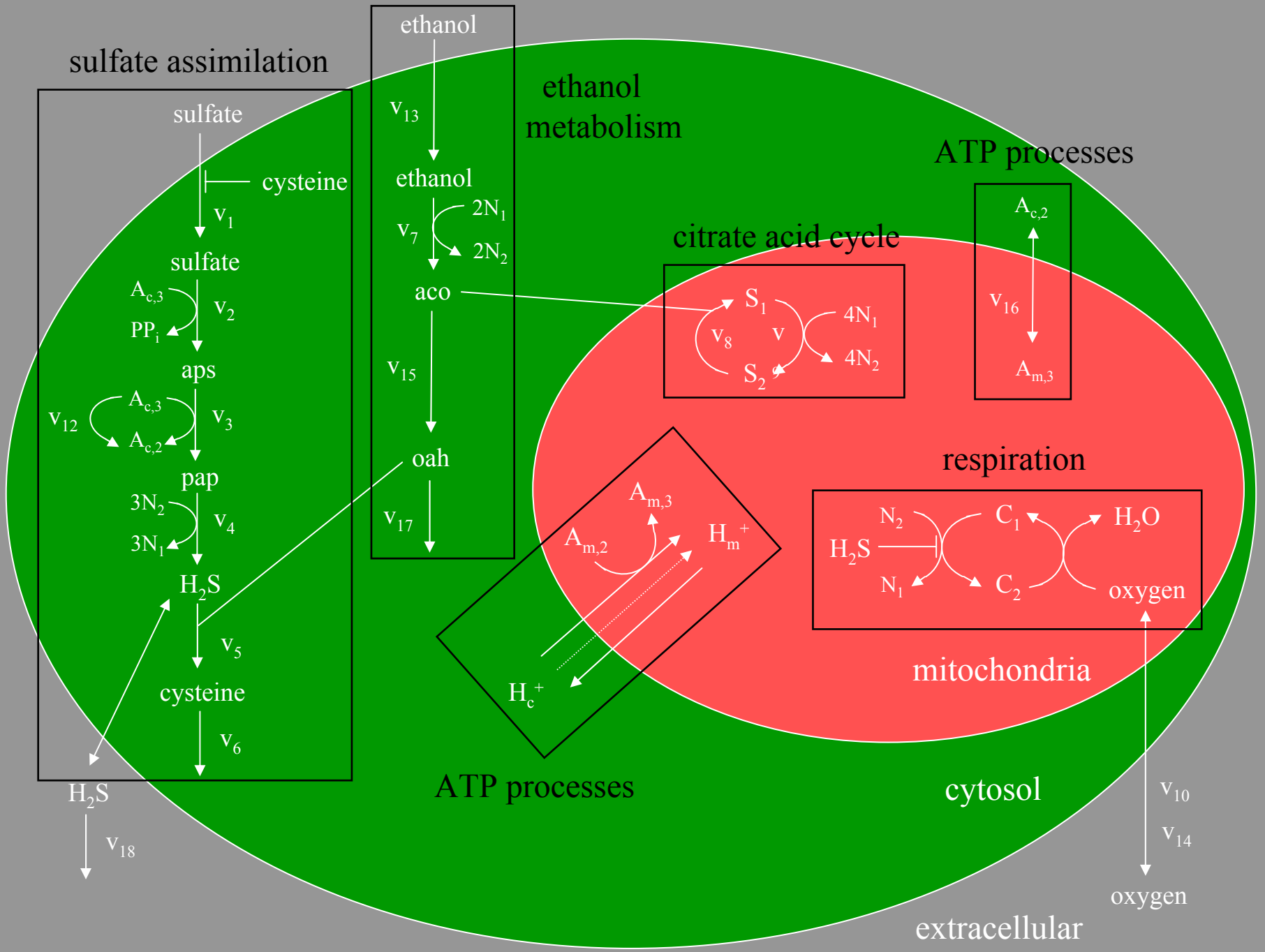
- *Saccharomyces cerevisae*
  - » Ordinary baker's yeast
  - » Essential for brewing & baking
  - » Genetic engineering applications
  - » Model eukaryotic organism (Hartwell, 2001)
- Autonomous oscillations in continuous culture
  - » Glycolytic oscillations (~ 1 min period)
  - » Short period metabolic oscillations (~ 40 min period)
  - » Cell cycle related oscillations (~ 2 h period)
- Requirements
  - » Oscillations at single cell level
  - » Synchronization of cellular oscillations

# Metabolic Oscillations

- Conditions
  - » Continuous fermentor
  - » Various media
  - » Aerobic growth conditions
- Single cell oscillations
  - » Involves sulfate assimilation pathway
  - » Inhibition of sulfate uptake by cysteine (Sohn & Kuriyama, 2001)
- Synchronization
  - » Mediated by diffusable volatile species
  - » Hydrogen sulfide (Sohn et al., 2000)



# Structured Cell Model (Wolf et al., 2001)



# Individual Cell Model

- Intracellular balances

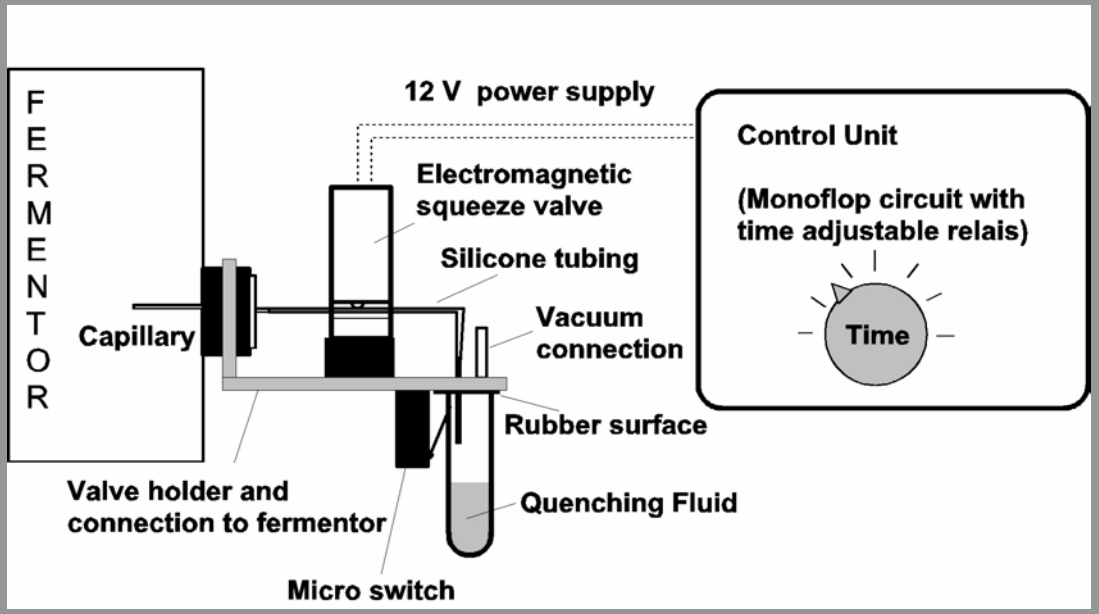
$$\frac{dx}{dt} = f(x, v_0, v_{10}, v_{13}) \quad x \in R^{13}$$

  - » Minimal description of oxidation phosphorylation
  - » Lumped respiratory processes

- Key regulatory effects
  - » Inhibition of sulfate uptake by cysteine ⇨ individual cell oscillations
  - » Inhibition of respiratory chain by $H_2S$

- Reaction kinetics
  - » Mass action kinetics
  - » Kinetic parameters chosen heuristically

# Modeling of Intracellular Kinetics




- Glucose pulse experiments
  - » Extract & inactivate samples on second time scale
  - » Perform assays to determine concentration profiles
  - » Fit kinetic expressions
- Available results
  - » Glucose transport (Rizzi et al., 1996)
  - » Glycolytic pathway (Theobald et al., 1997)
  - » Pentose phosphate pathway (Vaseghi et al., 1999)
  - » PKA/PKF signal transduction pathway (Vaseghi et al., 2001)

# Structured PBE Modeling

- Unstructured PBE models
  - » Single variable used to describe cellular state
  - » Difficult to extract PBE functions from data
  - » Limited predictive capability over range of conditions
- Structured PBE models (Nielsen & Villadsen, 1992)

$$\underbrace{\frac{\partial \Psi(x,t)}{\partial t}}_{\text{accumulation}} + \underbrace{\nabla_x[r(x,t)\Psi(x,t)]}_{\text{intracellular reactions}} = \underbrace{[\mu(x,t)-\mu(t)]\Psi(x,t)}_{\text{differential biomass production}}$$

  - » Intracellular species concentrations: $x_1(t)\ldots, x_n(t)$
  - » Mass fraction distribution: $\Psi(x,t)$
  - » All functions computable from intracellular kinetics
- Discretization of the intracellular state
  - » N node points ⇨ $N^n$ ODEs
  - » n = 13, N = 10 ⇨ ~$1x10^{11}$ ODEs
  - » Computationally intractable

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# Cell Ensemble Modeling

- Basic approach
  - » Develop individual cell model
  - » Determine primary sources of heterogeneity
  - » Construct ensemble of individual cell models
  - » Simulate cell population dynamics
  - » Compute distribution properties from ensemble data
- Previous studies
  - » *E. coli* aerobic growth (Domach & Shuler, 1984)
  - » *E. coli* anaerobic growth (Ataai & Shuler, 1985)
  - » *E. coli p*lasmid instability (Kim & Shuler, 1990)
  - » Yeast glycolytic oscillations (Henson et al., 2002)

# Cell Ensemble Model

- Objectives
  - » Investigate synchronization mechanism involving $H_2S$
  - » Explore methods for introducing heterogeneity
  - » Numerical solution of large cell ensembles
- Extracellular $H_2S$ balance
  - » Neglect other balances
  - » Model removal of $H_2S$ as degradation reaction

$$\frac{dC_{H2S,ex}}{dt} = \frac{\varphi}{M}\sum_{i=1}^{M}\kappa(C_{H2S,i} - C_{H2S,ex}) - k_{18}C_{H2S,ex}$$

- Computational aspects
  - » M = 10000 cells ⇨ ~130,000 ODEs
  - » Utilize highly banded approximation of Jacobian matrix
  - » 500 min simulation ⇨ 50 min CPU time

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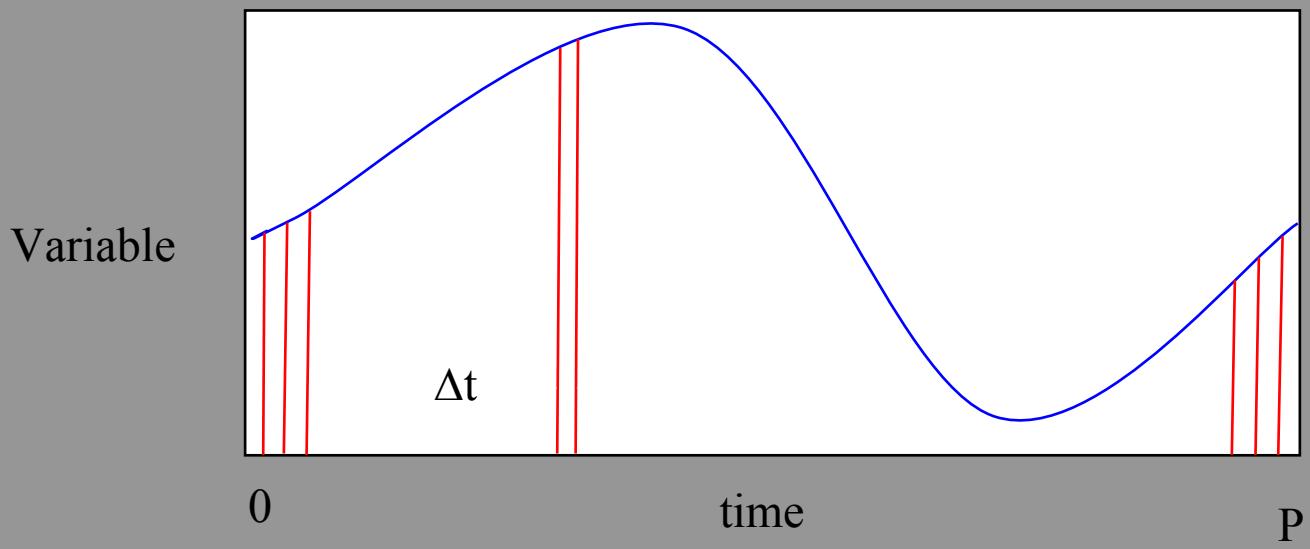
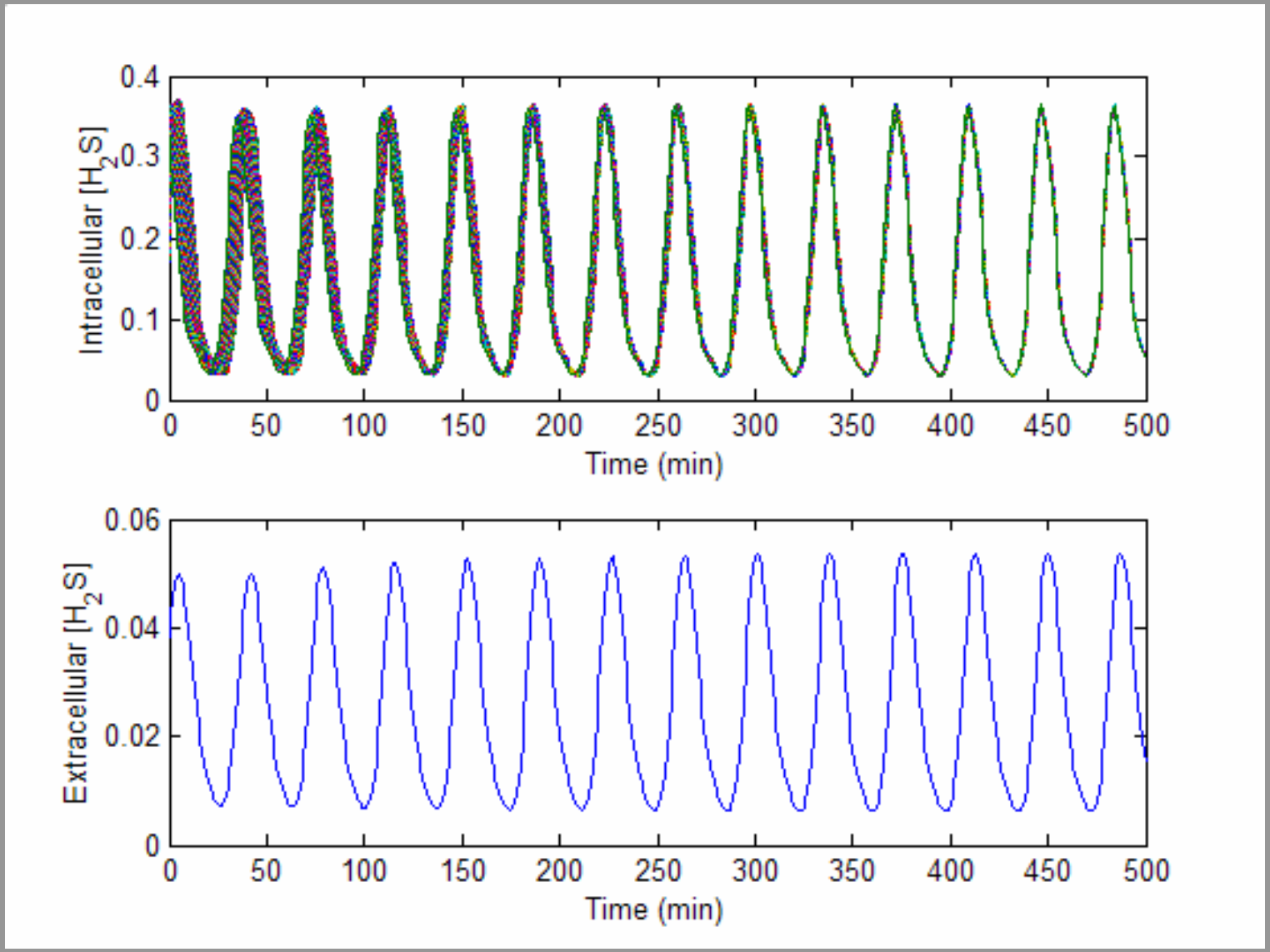
# Introducing Heterogeneity

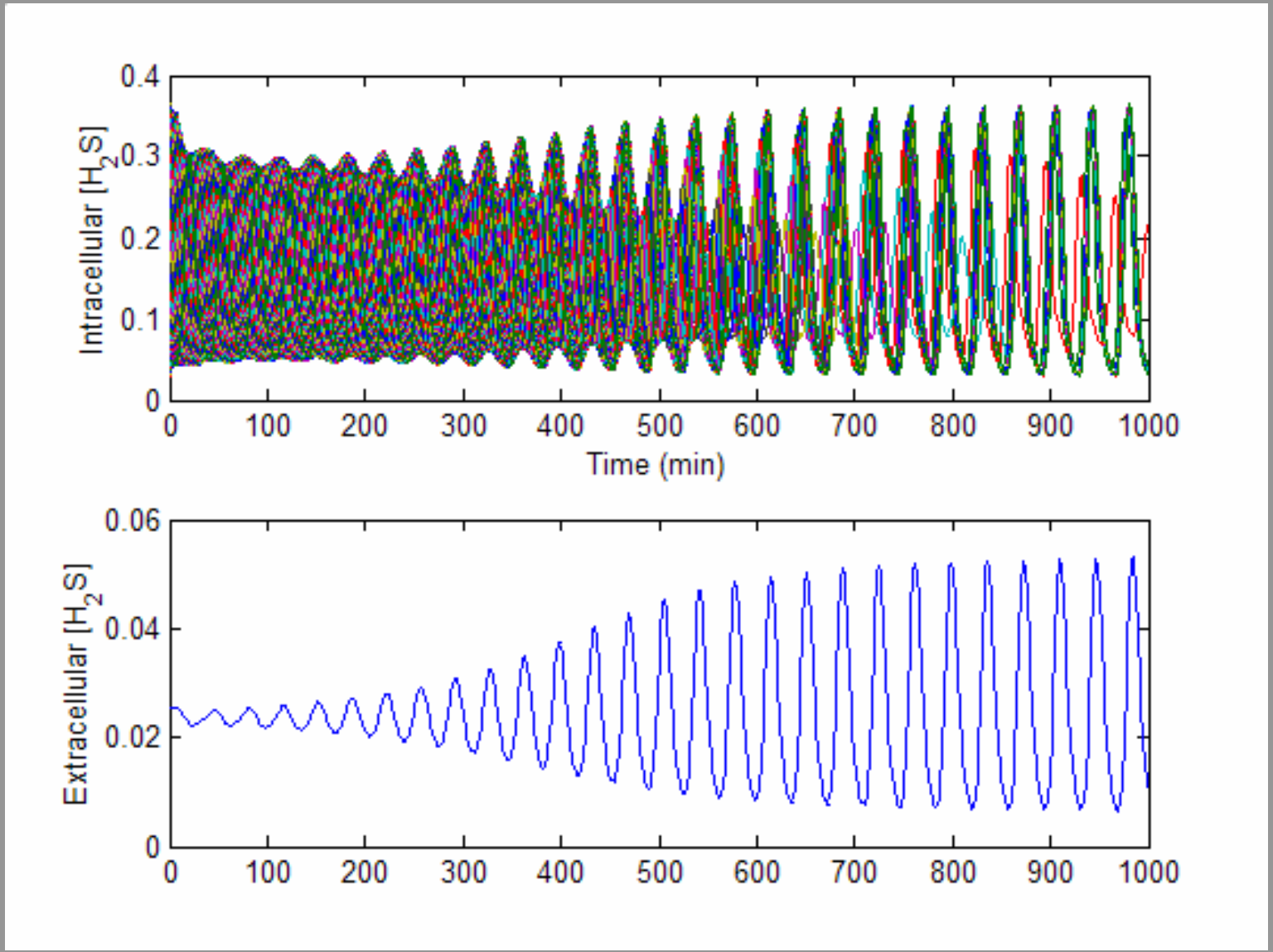
- Randomization of initial state (Henson et al., 2002)
  - » Generates heterogeneous cell population
  - » Leads to unrealistic combinations of intracellular concentrations
- Alternative approach
  - » Simulate synchronous oscillation over one period
  - » Store intracellular state at equally spaced time points
  - » Utilize data set to initialize cell ensemble



# Moderately Heterogeneous Population



# Completely Heterogeneous Population



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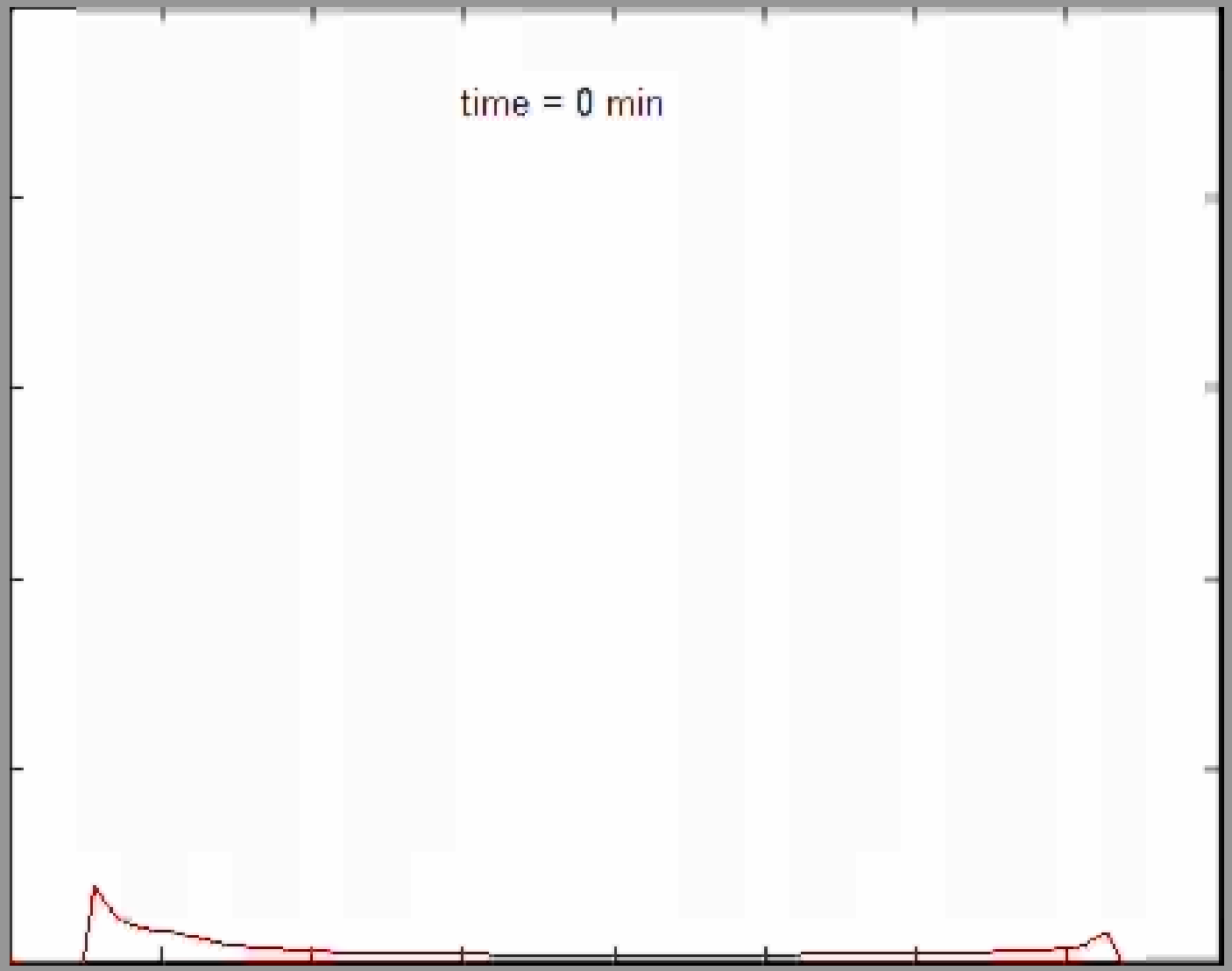


Number Distribution

$[H_2S]$

# Conclusions

- Autonomous yeast oscillations
  - » Oscillations at single cell level
  - » Synchronization of cellular oscillations
- Population balance equation (PBE) models
  - » Unstructured models lack mechanistic description of intracellular events
  - » Structured models difficult to formulate & solve
- Cell ensemble models
  - » Single cell models can be incorporated directly
  - » Computationally tractable
  - » Successful application to yeast metabolic oscillations
  - » Application to cell cycle related oscillations forthcoming