Modeling Subtilin Production in *Bacillus subtilis* Using Stochastic Hybrid Systems

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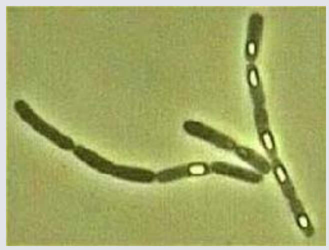
Chess Review
May 10, 2004
Berkeley, CA

Overview

- Goal
  - To study quantitatively the subtilin production in *B. subtilis* cells during different phases of population growth
- Hierarchical Model
  - Microscopic: each cell is modeled as a stochastic hybrid system
  - Macroscopic: ODEs for population level variables
- Simulations
  - SHS method vs. averaging method

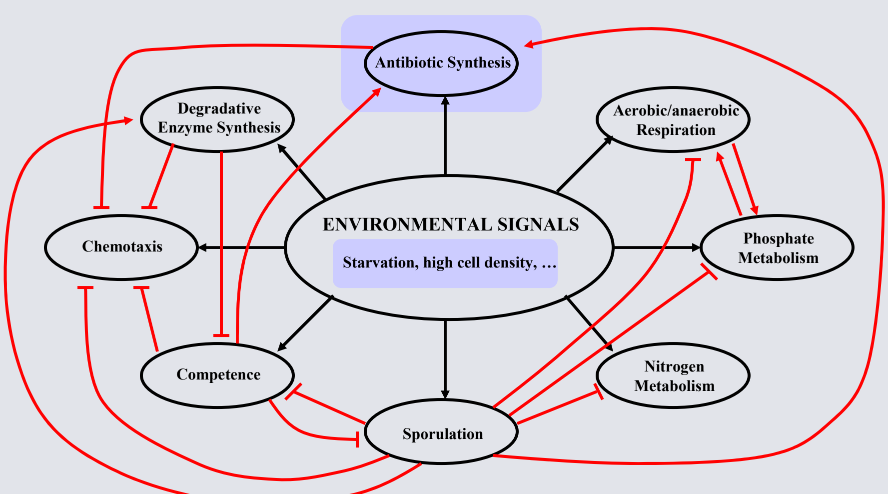
Bacillus subtilis

- *Bacillus subtilis* ATCC 6633
- Survival strategies under environmental stresses
  - Synthesis of antibiotics (*e.g.* subtilin)
  - Sporulation (forming spores)
  - Chemotaxis (moving towards chemical stimulus)



courtesy of Adam Arkin

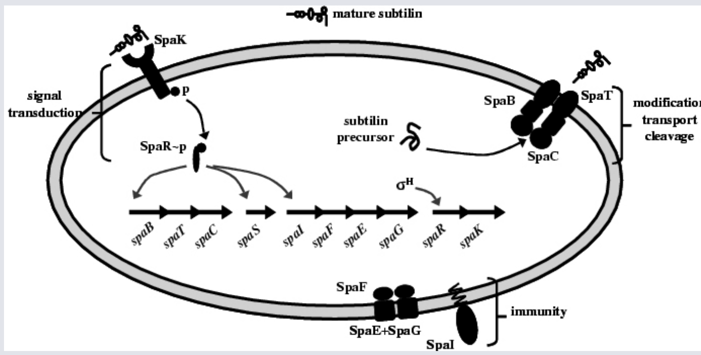
Stress Response Network



Courtesy of Denise Wolf

Subtilin Synthesis Model

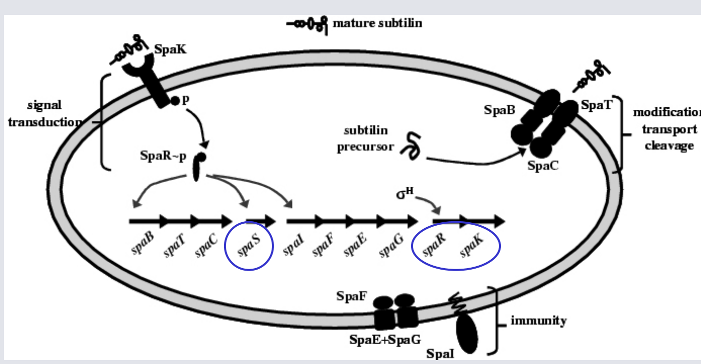
Biosynthesis pathway of subtilin ([Banerjee & Hansen, 1988], [Entian & de Vos, 1996])



Subtilin Synthesis Model

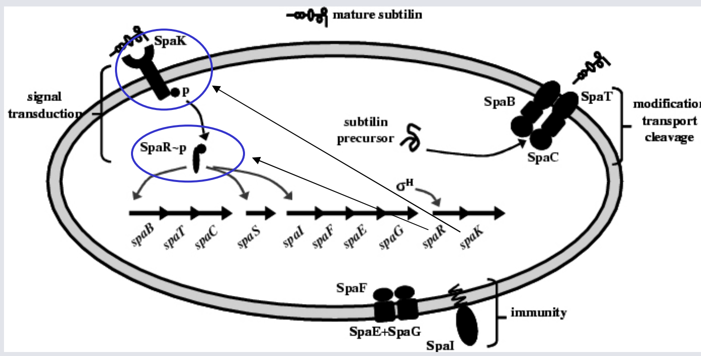
spaRK

spaS



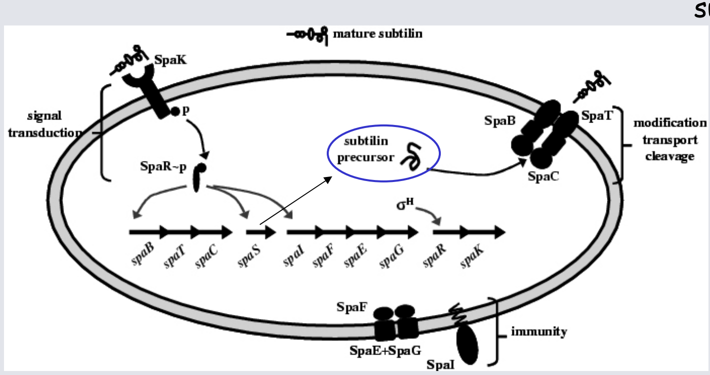
Subtilin Synthesis Model

spaRK → SpaRK   spaS

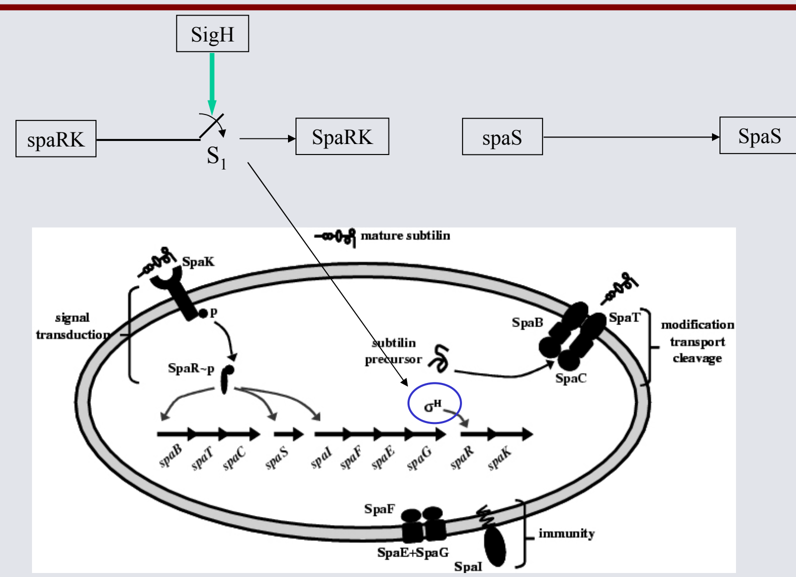


Subtilin Synthesis Model

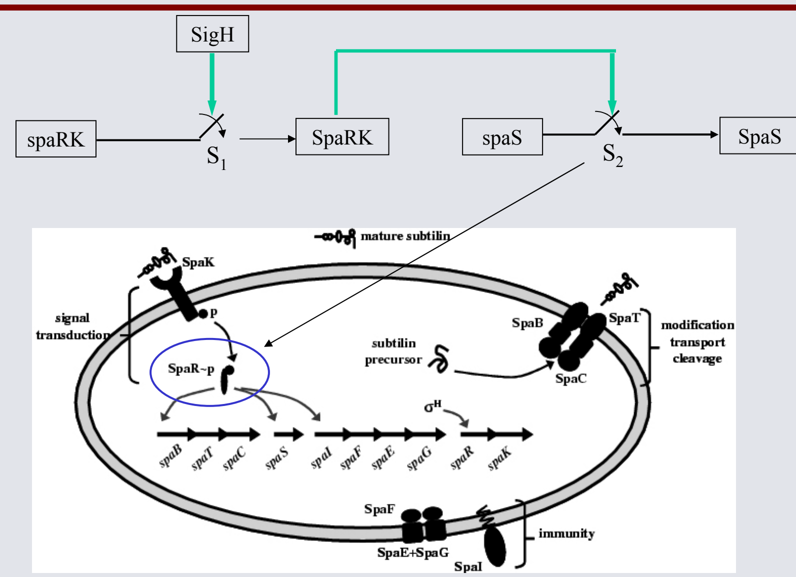
spaRK → SpaRK   spaS → SpaS → subtilin



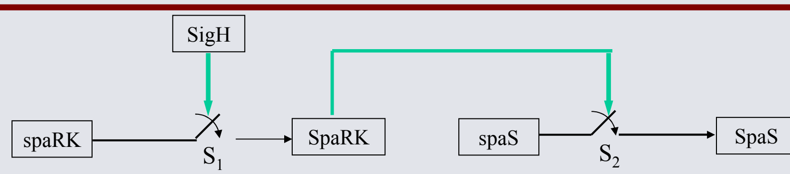
Subtilin Synthesis Model

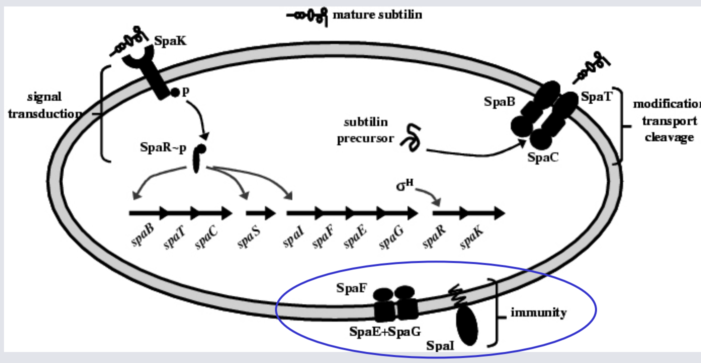


Subtilin Synthesis Model

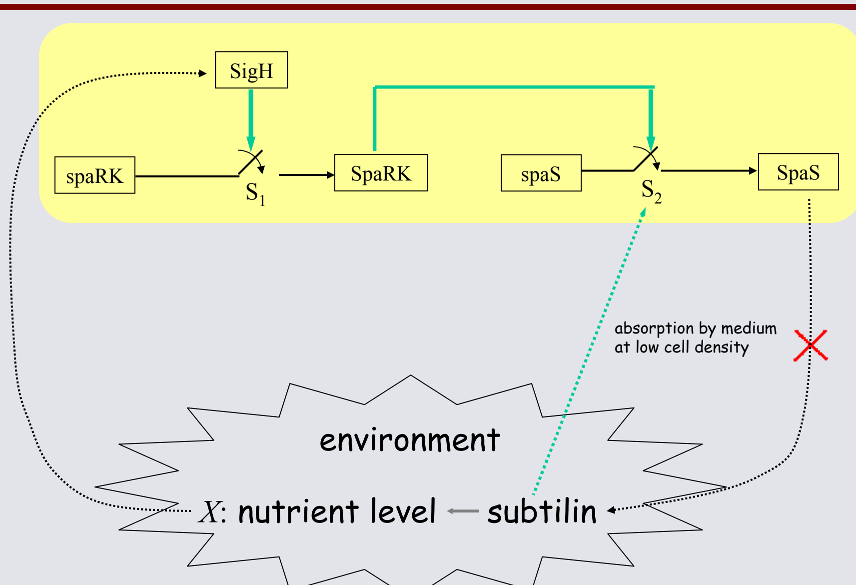


Subtilin Synthesis Model

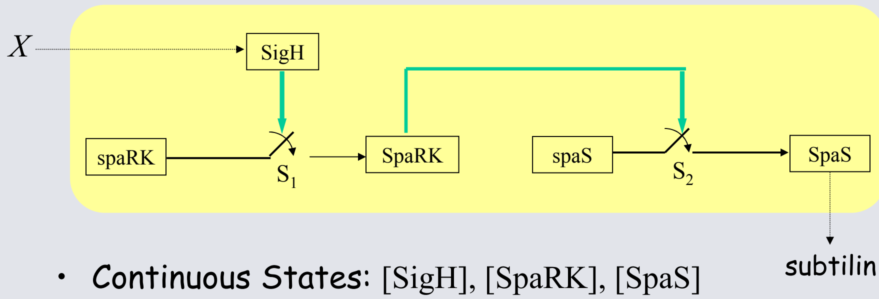




Interaction with Environment

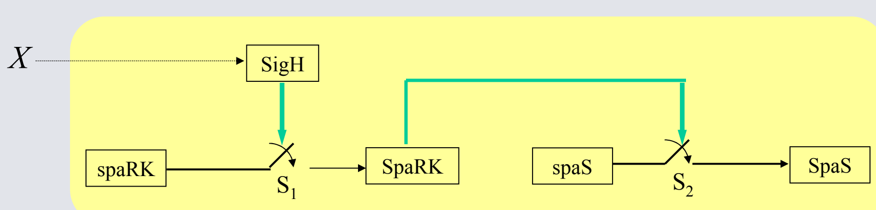


Stochastic Hybrid System Model



- Continuous States: [SigH], [SpaRK], [SpaS]
- Discrete States: S_1 , S_2
- Input: X
- Output: [SpaS]
- Randomness in switching of S_1 , S_2

Synthesis of SigH



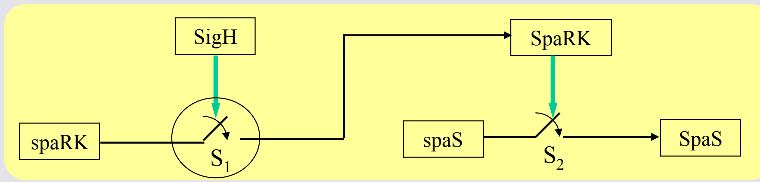
[SigH]: concentration level of SigH

$$\frac{d[\text{SigH}]}{dt} = \begin{cases} -\lambda_1[\text{SigH}], & \text{if } X \geq X_0 \\ -\lambda_1[\text{SigH}] + k_3, & \text{if } X < X_0 \end{cases}$$

natural decaying rate

threshold

Synthesis of SpaRK



- Synthesis of SpaRK is controlled by the random switch S_1

$$\frac{d[\text{SpaRK}]}{dt} = \begin{cases} -\lambda_2[\text{SpaRK}], & \text{if } S_1 \text{ is off } (0) \\ -\lambda_2[\text{SpaRK}] + k_4, & \text{if } S_1 \text{ is on } (1) \end{cases}$$

- S_1 switches every Δ time according to a Markov chain with transition matrix

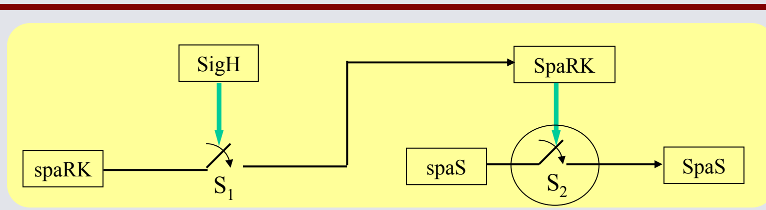
$$\begin{pmatrix} 1-a_0 & a_0 \\ a_1 & 1-a_1 \end{pmatrix}$$, where a_0 and a_1 depend on [SigH].

- Thermodynamics constraint [Shea & Ackers, 1985]:

$$p_{rk} = \frac{e^{-\Delta G_{rk}/RT}[\text{SigH}]}{1 + e^{-\Delta G_{rk}/RT}[\text{SigH}]}$$

ΔG_{rk}: Gibbs free energy when S_1 is on
R: Gas constant
T: Temperature

Synthesis of SpaS



- Synthesis of SpaS is controlled by the random switch S_2

$$\frac{d[\text{SpaS}]}{dt} = \begin{cases} -\lambda_3[\text{SpaS}], & \text{if } S_2 \text{ is off } (0) \\ -\lambda_3[\text{SpaS}] + k_5, & \text{if } S_2 \text{ is on } (1) \end{cases}$$

- S_2 switches every Δ time according to a Markov chain with transition matrix

$$\begin{pmatrix} 1-b_0 & b_0 \\ b_1 & 1-b_1 \end{pmatrix}$$, where b_0 and b_1 depend on [SpaRK].

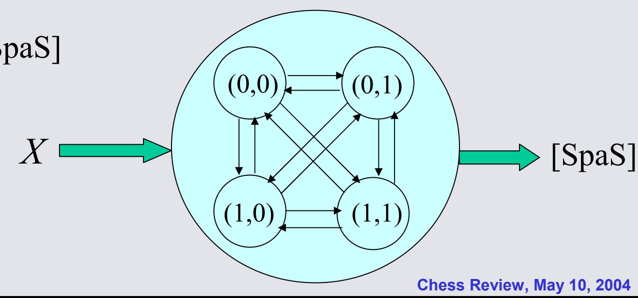
- Thermodynamics constraint:

$$p_s = \frac{e^{-\Delta G_s/RT}[\text{SpaRK}]}{1 + e^{-\Delta G_s/RT}[\text{SpaRK}]}$$

ΔG_s: Gibbs free energy when S_2 is on
R: Gas constant
T: Temperature

Single *B. subtilis* Cell as a SHS

- Continuous state ([SigH], [SpaRK], [SpaS]) $\in R^3$
- Discrete state $(S_1, S_2) \in \{0,1\}\times\{0,1\}$
- Input is nutrient level X
- Output is [SpaS]



Analysis of [SpaRK]

- Fix [SigH], analyze [SpaRK]

$$\frac{d[\mathrm{SpaRK}]}{dt} = \begin{cases} -\lambda_2[\mathrm{SpaRK}], & \text{if } S_1 \text{ is off } (0) \\ -\lambda_2[\mathrm{SpaRK}] + k_4, & \text{if } S_1 \text{ is on } (1) \end{cases}$$

$$\begin{cases} [\mathrm{SpaRK}] \to 0, & \text{if } S_1 \text{ is off } (0) \\ [\mathrm{SpaRK}] \to k_4/\lambda_2, & \text{if } S_1 \text{ is on } (1) \end{cases}$$

Random switching between two stable equilibria

Assume S_1 is in equilibrium distribution

$[\mathrm{SpaRK}]_{n\Delta}$, $n=0, 1, \ldots,$ is a Markov chain

$$\begin{cases} [\mathrm{SpaRK}]_{n\Delta} \to \rho\,[\mathrm{SpaRK}]_{n\Delta} & \text{with probability } 1-p_{rk} \\ [\mathrm{SpaRK}]_{n\Delta} \to \rho\,[\mathrm{SpaRK}]_{n\Delta} + (1-\rho)\, k_4/\lambda_2 & \text{with probability } p_{rk} \end{cases}$$

Equilibrium Distribution of $[\text{SpaRK}]_{n\Delta}$

- Suppose $\rho = 1/m,\ \ k_4/\lambda_2=1$
- Distribution of $[\text{SpaRK}]_{n\Delta}$ as $n\to\infty$ concentrates on $[0,1]$

$$[\text{SpaRK}]_{n\Delta}=0.\underbrace{d_1 \ldots d_l\, xxx \ldots}_{(m\text{-nary})}\ \text{ with probability } (p_{rk})^d\,(1-p_{rk})^{l-d},\ \ d=d_1+\ldots+d_l$$

- Start rational, stay rational

Macroscopic Model

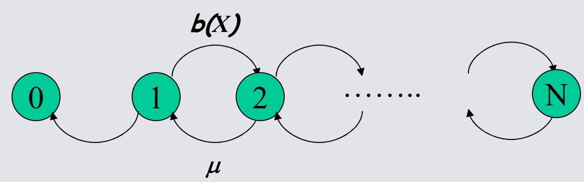
- Population density D governed by the logistic equation

$$\frac{dD}{dt} = rD(1 - D/D_\infty)$$

- Maximal sustainable population size $D_\infty = \min\,(\eta X, D_{\max})$
- Dynamics of nutrient level X

$$\frac{dX}{dt} = \underbrace{-k_1 D}_{\text{consumption}} + \underbrace{k_2 D\,[\text{SpaS}]_{\text{avg}}}_{\text{production}}$$

Birth-and-Death Chain for Population Dynamics



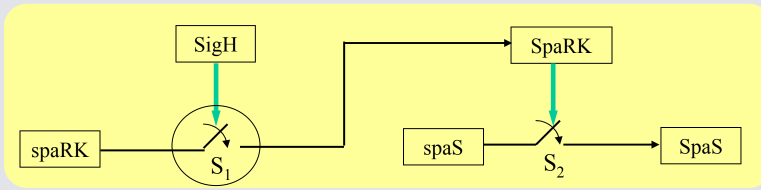
- Population size, n, is governed by a birth-and-death chain
- Birth rate, $b(X)$, depends on the nutrient level X
- Death rate, μ, is fixed
- Dynamics of nutrient level X becomes

$$\frac{dX}{dt} = \underbrace{-k_1 n}_{\text{consumption}} + \underbrace{k_2\, n\, [\mathrm{SpaS}]}_{\text{production}}$$

Some Remarks

- Hierarchical Model
  - Macroscopic model: ODEs
  - Microscopic model: SHS
- A compromise between accuracy and complexity
- More accurate model:
  - Population size as a birth-and-death Markov chain
  - Each cell has an exponentially distributed life span
- Simpler model: averaging method

Averaging Dynamics of [SpaRK]



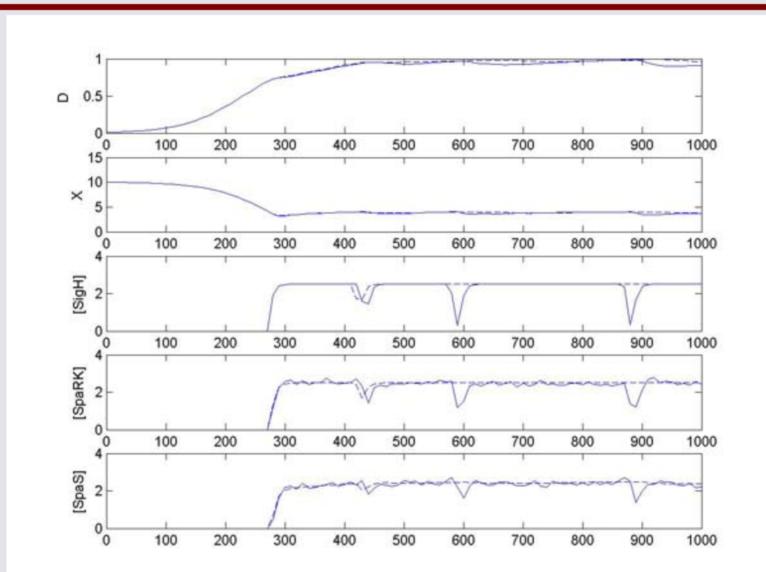
- Synthesis of SpaRK is controlled by the random switch S_1

$$\frac{d[\text{SpaRK}]}{dt} = \begin{cases} -\lambda_2[\text{SpaRK}], & \text{if } S_1 \text{ is off } (0) \\ -\lambda_2[\text{SpaRK}] + k_4, & \text{if } S_1 \text{ is on } (1) \end{cases}$$

- In equilibrium distribution S_1 is on with probability p_{rk}
- The averaged dynamics of [SpaRK] is

$$\frac{d[\text{SpaRK}]}{dt} = -\lambda_2[\text{SpaRK}] + p_{rk}\, k_4$$

Simulations: A Typical System Trajectory



Different Time Scales

- Slower and more deterministic dynamics
  - *D, X*
  - [SigH]
- Faster and more random dynamics
  - [SpaRK]
  - [SpaS]
- Time scale analysis
  - Fixed slower variables, analyze the probabilistic property of the faster variables

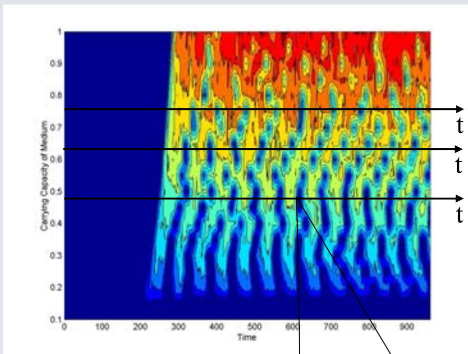
Observations

- Slow variables follow a limit circle
- Fast variables induce random fluctuations around it
- Variance of fluctuations can be controlled by changing the transit probabilities of the switches
  - The less frequent the switches, the larger the variance

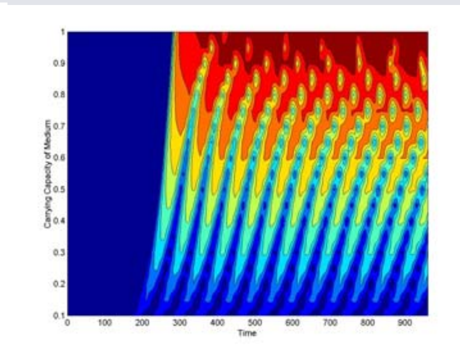
Simulations: Production vs. Max Density

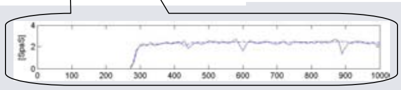
Production level of subtilin as a function of time under different maximal carrying capacity D_{max}

SHS

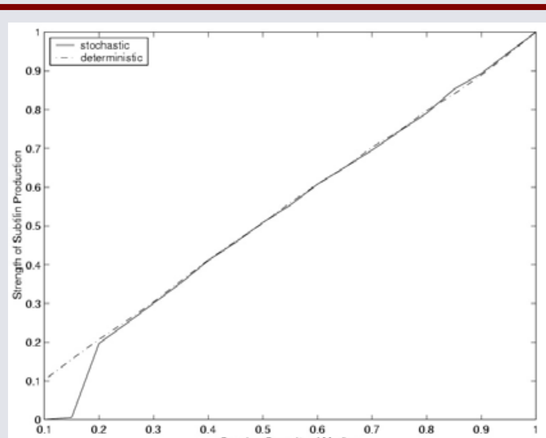


Averaged



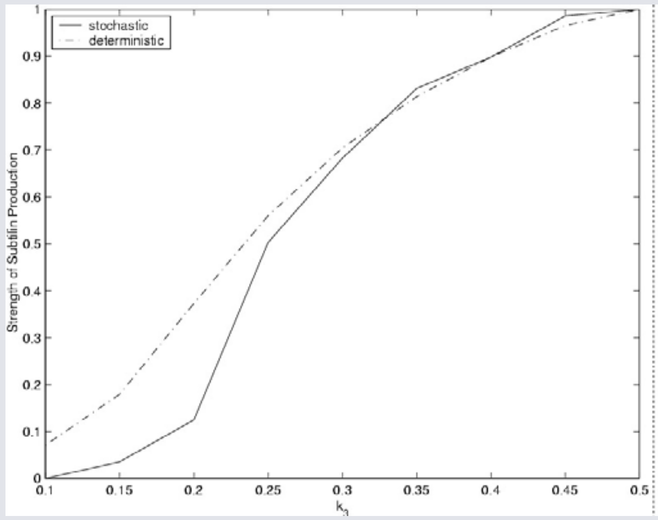


Average Subtilin Production vs. Maximal Carrying Capacity



There is a threshold of D_{max} below which subtilin is not produced consistently, and above which it is produced at linearly increasing rate with D_{max}
[Kleerebezem & Quadri, 2001]

Average Subtilin Production vs. Production Rate of SigH



Conclusions and Future Directions

- A stochastic hybrid system model of subtilin synthesis
- Analysis of fast variable dynamics
- Numerical simulations

- A birth-and-death chain for population growth
- More complete system dynamics
- Model validation

- Acknowledgement:
  - This work is in collaboration with Prof. Adam Arkin (LBNL)