Mathematical modelling of protein oscillations in bacteria

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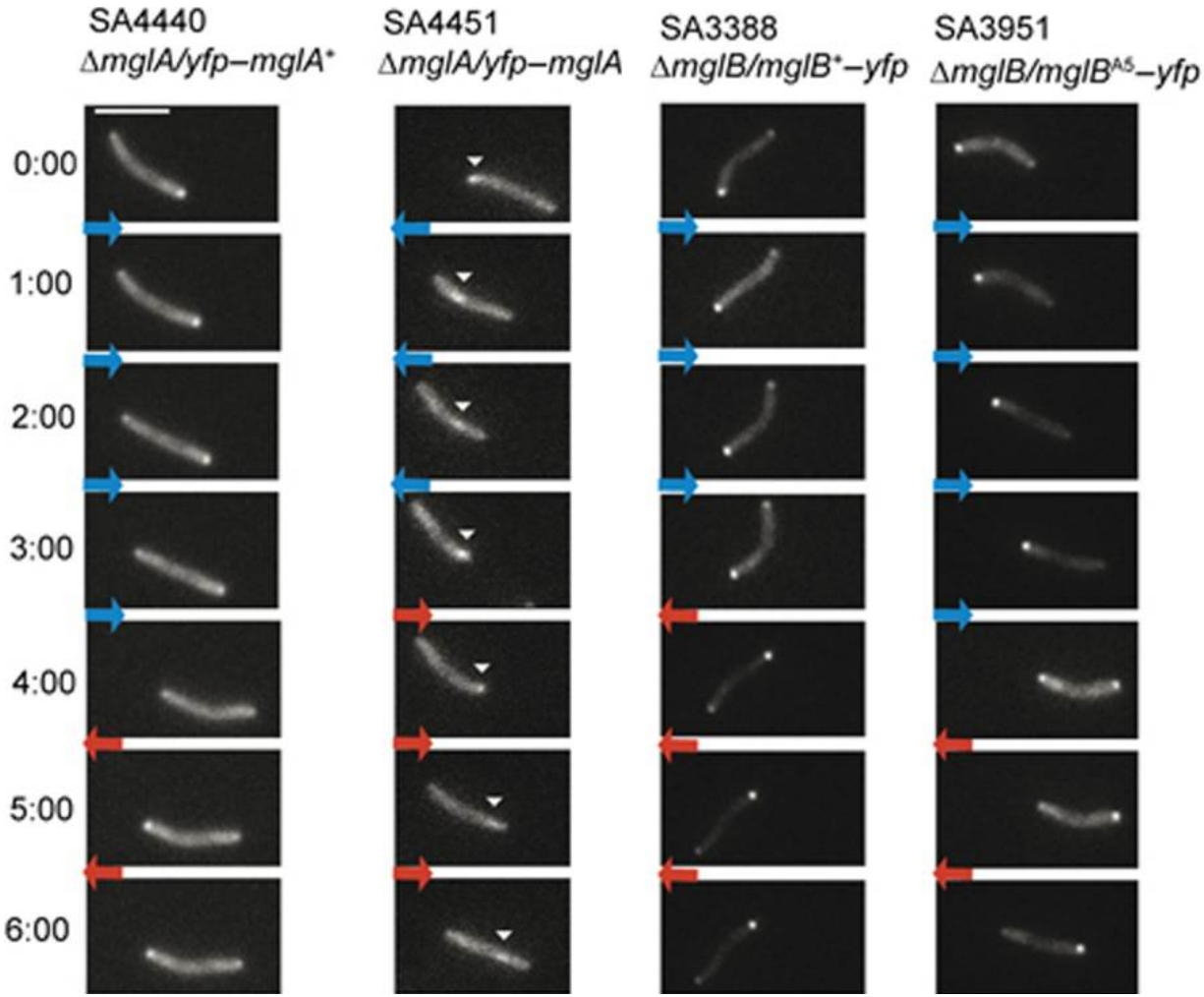
Oscillations in Cells

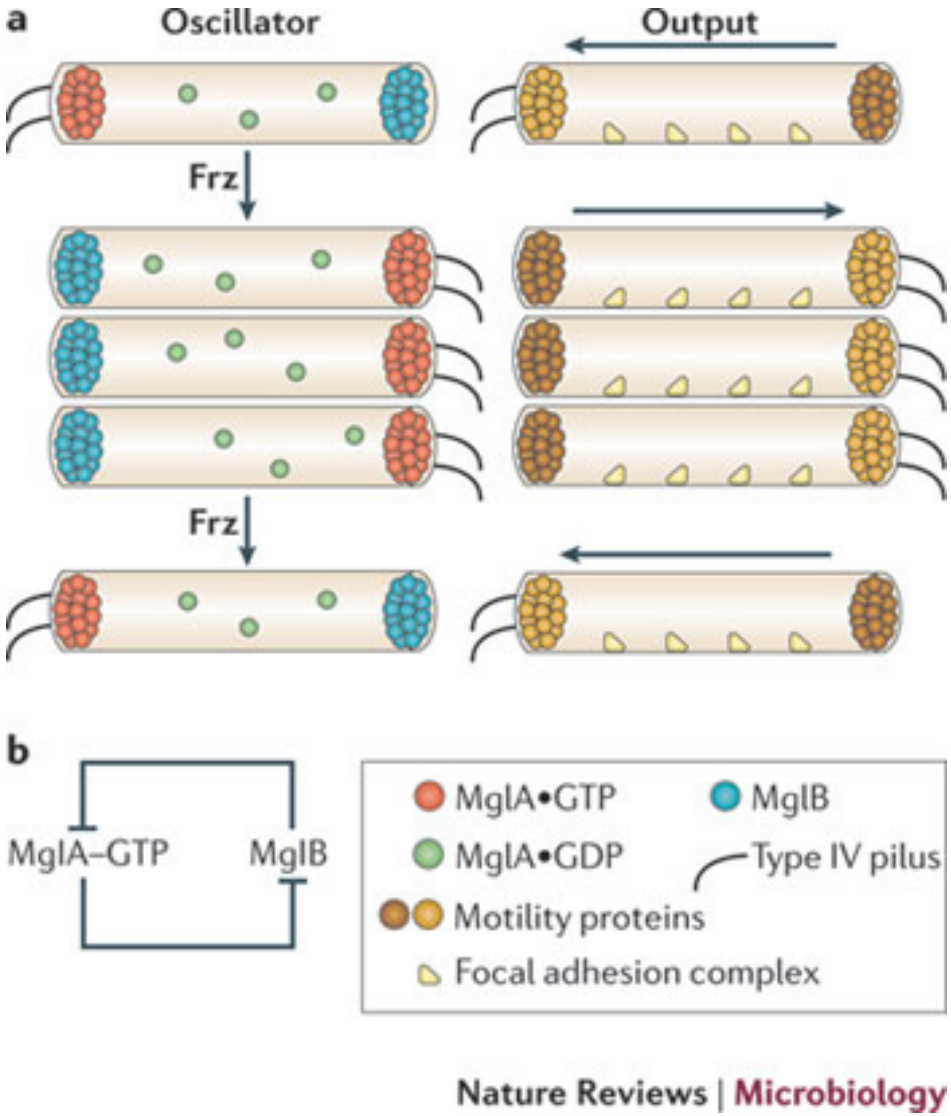
- Min-System in *Escherichia coli* controlling cell division site (Meinhardt and de Boer, 2001; Kruse, 2002; Loose, Kruse, Schwille, 2011)
- Cell orientation, polarity, direction of cell motion
  - Rhythmic movement of plasmodia (Tero, Kobayashi, Nakagaki, 2005; Miyaji and Ohnishi, 2007)
  - Mgl/Frz oscillator in *Myxococcus xanthus* regulating the localisation of motility proteins at the cell poles (Rashkov et al, 2012, 2013, 2014)
  - Dynamics of Cdc42 oscillation in fission yeast (Xu and Jilkine, 2018)

Microscopic time-lapse movies of *M. xanthus*



Miertzschke et al. *EMBO J.* (2011)

Regulatory Network for Cell Polarity in *M. xanthus*



Lenz and Søgaard-Andersen (2011)

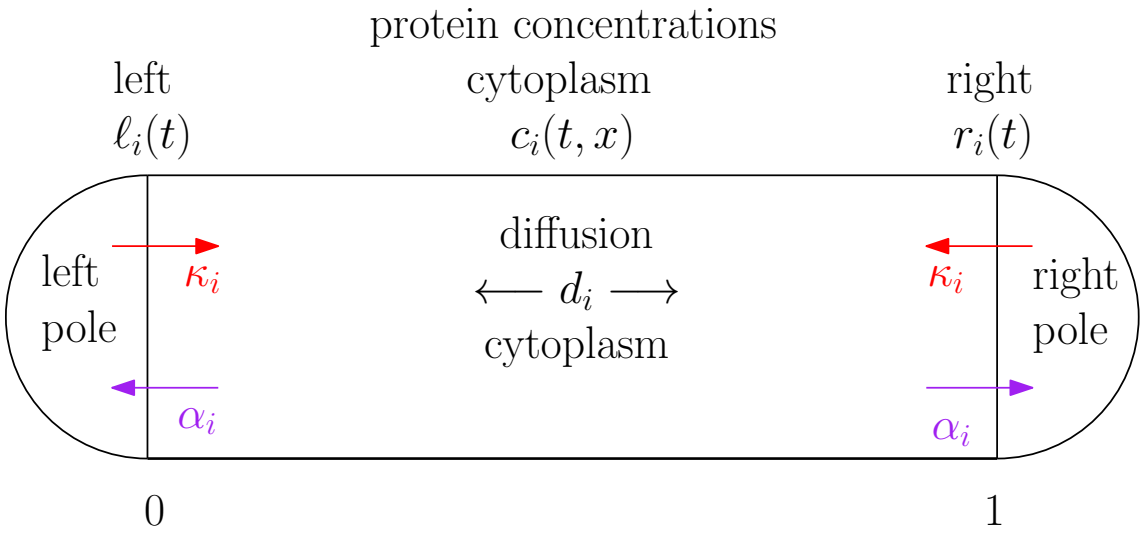
Experimental Observations

- polarity *fixed*: MglA-GTP, MglB stay bound at opposite poles
- signaling of Frz chemosensory system: polarity *inverted*
  - MglA-GTP, MglB released from the poles, transported via cytoplasm and rebind at the opposite poles
  - re-organisation of motility apparatus
- wild type cell: occasional inversion of the cell polarity
- no Frz: no inversion
- mutant cell: highly regular, periodic inversion of cell polarity

Mathematical Objectives

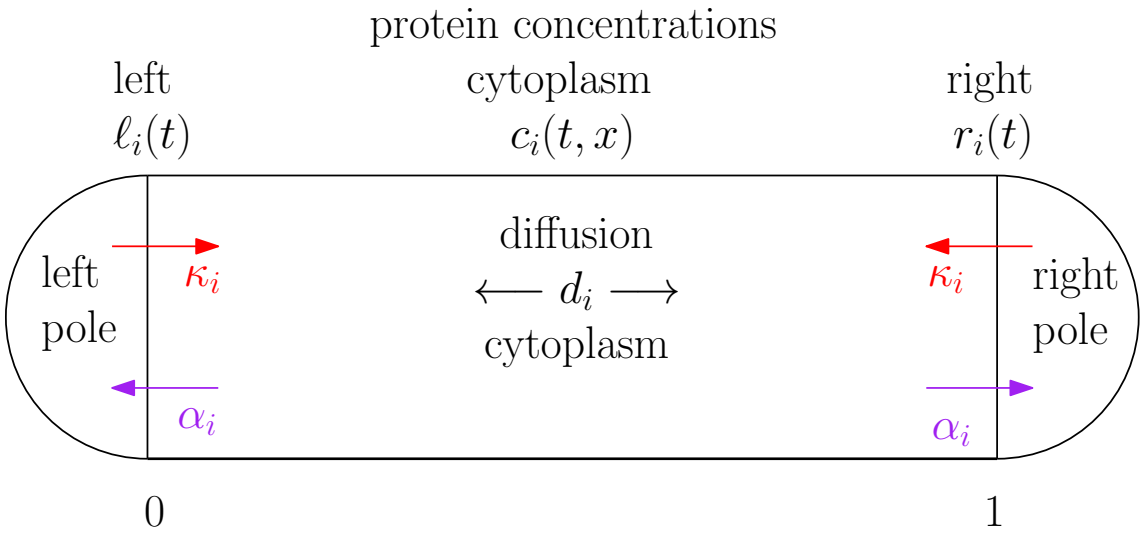
- minimal model
  - as few assumptions as possible because the complexity of signalling/regulatory networks can increase exponentially
- questions
  - polarity set-up
  - pole-to-pole relocation of the regulatory proteins
  - mutant: oscillations governed only by endogenous laws?
  - wild type: response to external triggers?
- parameters not known
- robustness against parameter variation

Model Outline



- *diffusive* transport through cytoplasm $[0,1]$
- binding sites at *poles* at 0 and 1
- i is protein: Mgl**A**-G**T**P, Mgl**A**-G**D**P, Mgl**B**
- effective rates
  - binding/on-rate $\alpha_i = \alpha_i(\ell_i|r_i)$
  - unbinding/off-rate $\kappa_i = \kappa_i(\ell_i|r_i)$

Simpler Model



- identical laws for both poles $\rightarrow$ no directional bias
- vector notation for dependent variables
  $c(t,x) := (c_i)(t,x), \quad \ell(t) := (\ell_i)(t), \quad r(t) := (r_i)(t), \quad i = 1, \ldots n$

Reaction-diffusion System

$$
\begin{aligned}
\frac{\partial c}{\partial t} &= D\Delta c \\
D^{-1}\frac{d\ell}{dt} &= (\underbrace{A(\ell)c(0)}_{\text{binding}} - \underbrace{K(\ell)\ell}_{\text{unbinding}}) \\
D^{-1}\frac{dr}{dt} &= (\underbrace{A(r)c(1)}_{\text{binding}} - \underbrace{K(r)r}_{\text{unbinding}})
\end{aligned}
$$

- $D = \operatorname{diag}(d_i) > 0$ - diffusion matrix
- $A(\cdot) = \operatorname{diag}(\alpha_i(\cdot)) \geq 0$ - matrix of on-rates
- $K(\cdot) = \operatorname{diag}(\kappa_i(\cdot)) \geq 0$ - matrix of off-rates

Boundary Conditions Total Mass Conservation

Lemma (R. et al., *Bull. Math. Biol.* 2012)

Let α_i, κ_i be continuous functions. With boundary conditions

$$\begin{aligned} \partial_x c(t,0) &= A(\ell)c(0) - K(\ell)\ell, \\ \partial_x c(t,1) &= -A(r)c(1) + K(r)r, \end{aligned}$$

the total mass of each protein

$$m_i(t) := \ell_i(t) + \int_0^1 c_i(t,x)\,dx + r_i(t), \quad i = 1, \dots n$$

is constant for all $t \geq 0$.

Recap from Dynamical Systems

- Periodic solutions in time/space
- Where to start?
- Construct a locally asymptotically unstable steady state $(\hat{\ell}, \hat{c}(x), \hat{r})$.
- Limit cycle arising due to a Hopf bifurcation
- Perturbation of a heteroclinic orbit $\rightarrow$ swinging between two saddle points

Steady States

- Steady state $(\hat{\ell}, \hat{c}(x), \hat{r}), x \in (0,1)$.
- Boundary conditions $\rightarrow$ the steady state $\hat{c}$ is constant in x.
- Symmetry of the equations for the poles $\rightarrow$ steady states are symmetric at the poles: $(\hat{r}, \hat{c}, \hat{\ell})$ is also a steady state.
- Start with a biologically relevant steady state and do a linear stability analysis.

Analysis of the Linear System

For small perturbations of the steady state $\tilde{\ell} = \ell - \hat{\ell}, \tilde{r} = r - \hat{r}, \tilde{c} = c - \hat{c}$:

$$\begin{aligned}
\frac{\partial \tilde{c}}{\partial t} &\doteq D\Delta\tilde{c} \\
\frac{d\tilde{\ell}}{dt} &\doteq DA_{\hat{\ell}}\tilde{c}(0) + DV_{\hat{\ell}}\tilde{\ell} \\
\frac{d\tilde{r}}{dt} &\doteq DA_{\hat{r}}\tilde{c}(1) + DV_{\hat{r}}\tilde{r},
\end{aligned}$$

with matrices

$$\begin{aligned}
(A_{\hat{\ell}}) &= \operatorname{diag}(\alpha_i(\hat{\ell})), \quad (A_{\hat{r}}) = \operatorname{diag}(\alpha_i(\hat{r})) \\
(V_{\hat{\ell}})_{ij} &= \partial_j(\alpha_i c_i - \kappa_i \ell_i)|_{(\hat{\ell},\hat{c})} \\
(V_{\hat{r}})_{ij} &= \partial_j(\alpha_i c_i - \kappa_i r_i)|_{(\hat{c},\hat{r})}.
\end{aligned}$$

Separation-of-Variables *Ansatz*

$$\big(\tilde{\ell}(t), \tilde{c}(t,x), \tilde{r}(t)\big) := e^{\lambda t}\big(\boldsymbol{l}, \boldsymbol{c}(x), \boldsymbol{r}\big).$$

Solve for eigenvalue λ, and vectors $\boldsymbol{l}, \boldsymbol{c}(x), \boldsymbol{r}$

$$\begin{aligned} \lambda \boldsymbol{c} &= D\Delta \boldsymbol{c} \\ \lambda \boldsymbol{l} &= DA_{\hat{\ell}}\boldsymbol{c}(0) + DV_{\hat{\ell}}\boldsymbol{l} \\ \lambda \boldsymbol{r} &= DA_{\hat{r}}\boldsymbol{c}(1) + DV_{\hat{r}}\boldsymbol{r} \end{aligned}$$

under the boundary conditions:

$$\begin{aligned} \partial_x \boldsymbol{c}(0) &= \lambda D^{-1}\boldsymbol{l} \\ \partial_x \boldsymbol{c}(1) &= -\lambda D^{-1}\boldsymbol{r}. \end{aligned}$$

Auxiliary Eigen-Boundary Problem

Problem (R. et al. *Int. J. Biomath. Biostat.*, 2013)

Find $\lambda, \mathbf{c}(x)$*:*

$$\lambda \boldsymbol{c} = D\Delta \boldsymbol{c}$$

subject to Robin boundary conditions

$$\begin{aligned} (I - \lambda^{-1} V_{\hat{\ell}} D)\partial_x \boldsymbol{c}(0) &= A_{\hat{\ell}} \boldsymbol{c}(0) \\ (I - \lambda^{-1} V_{\hat{r}} D)\partial_x \boldsymbol{c}(1) &= -A_{\hat{r}} \boldsymbol{c}(1) \end{aligned}$$

For Hopf bifurcation: solutions λ with $\operatorname{Re}\lambda > 0$!

Transcendental Problem

Lemma (R. et al., 2013)

$\lambda \neq 0$ is a solution of the auxiliary problem if the determinant

$$\begin{vmatrix} A_{\hat{\ell}} & -Q_{\hat{\ell}} \\ Q_{\hat{r}}\lambda D^{-1} f(\lambda D^{-1}) + A_{\hat{r}} g(\lambda D^{-1}) & Q_{\hat{r}} g(\lambda D^{-1}) + A_{\hat{r}} f(\lambda D^{-1}) \end{vmatrix}$$

vanishes. Here

$$Q_{\hat{\ell}} = I - \lambda^{-1} V_{\hat{\ell}} D, \quad Q_{\hat{r}} = I - \lambda^{-1} V_{\hat{r}} D,$$

$$f(z) = \frac{\sinh \sqrt{z}}{\sqrt{z}}, \quad g(z) = \cosh \sqrt{z} \quad \textit{component-wise.}$$

Infinitely many solutions: pick dominant λ.

Possible Scenarios

- Dynamics dependent on rates α_i, κ_i
- Biologically relevant on-/off-rates devised according to mathematical analysis
- *Two* proteins
- "*stalker*" scenario (R. et al, 2012):
  - 1 always binds to the poles
  - 2 (the "*stalker*") follows 1 and repels it from the poles
- "*antagonist*" scenario (R. et al, 2013):
  - 1, 2 are off-phase and occupy exactly one pole over an extended time period
  - configuration switches fast

Steady State: 'Stalker' Scenario

- Assume $\alpha_i > 0$, $\hat{\ell}_i, \hat{c}_i(x), \hat{r}_i \neq 0, \hat{\ell} = \hat{r}$
- Identical on-/off-matrices at the poles

$$A_{\hat{\ell}} = A_{\hat{r}} := \hat{A}, \quad V_{\hat{\ell}} = V_{\hat{r}} := \hat{V}$$

- For $D \equiv I$ and $\hat{A} = \alpha I$, the eigen-boundary problem for λ reduces to an eigenvalue problem for a 2×2-matrix

Eigenvalue analysis

Let ϱ be an eigenvalue $\alpha^{-1}\hat{V}$.
Solve $F(\lambda) = \varrho$ where

$$F(\lambda) = \frac{\lambda}{\alpha} + \sqrt{\lambda}\tanh\frac{\sqrt{\lambda}}{2}.$$

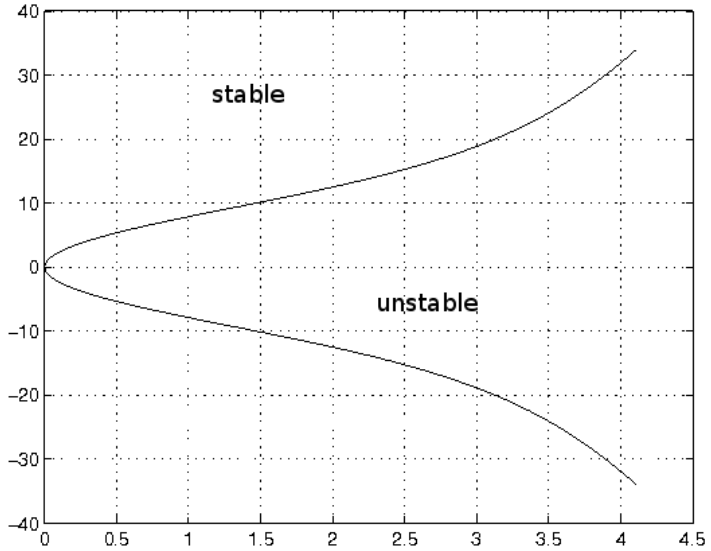


Figure: Location of eigenvalue ϱ of $\alpha^{-1}\hat{V}$ in $\mathbb{C}$ determines the sign of $\mathrm{Re}\,\lambda$ and the local stability of the steady state (R. et al. 2012).

'Stalker' Scenario (R. et al, 2012)

Analysis of eigenvalue conditions for the matrix $\hat{V}$ implies possible rates of the form

$$\begin{aligned}
\text{Binding rates} \quad & \alpha_1(q_1, q_2) = (1 - a_1) + a_1 q_1^2, \\
& \alpha_2(q_1, q_2) = (1 - a_2) + a_2 q_1, \\
\text{Unbinding rates} \quad & \kappa_1(q_1, q_2) = q_2, \\
& \kappa_2(q_1, q_2) = \frac{a_3}{1 + (a_3 - 1) q_2}, \\
\text{Diffusion constants} \quad & d_1 = d_2 = 1
\end{aligned}$$

‘Stalker’ Scenario: Numerical simulation

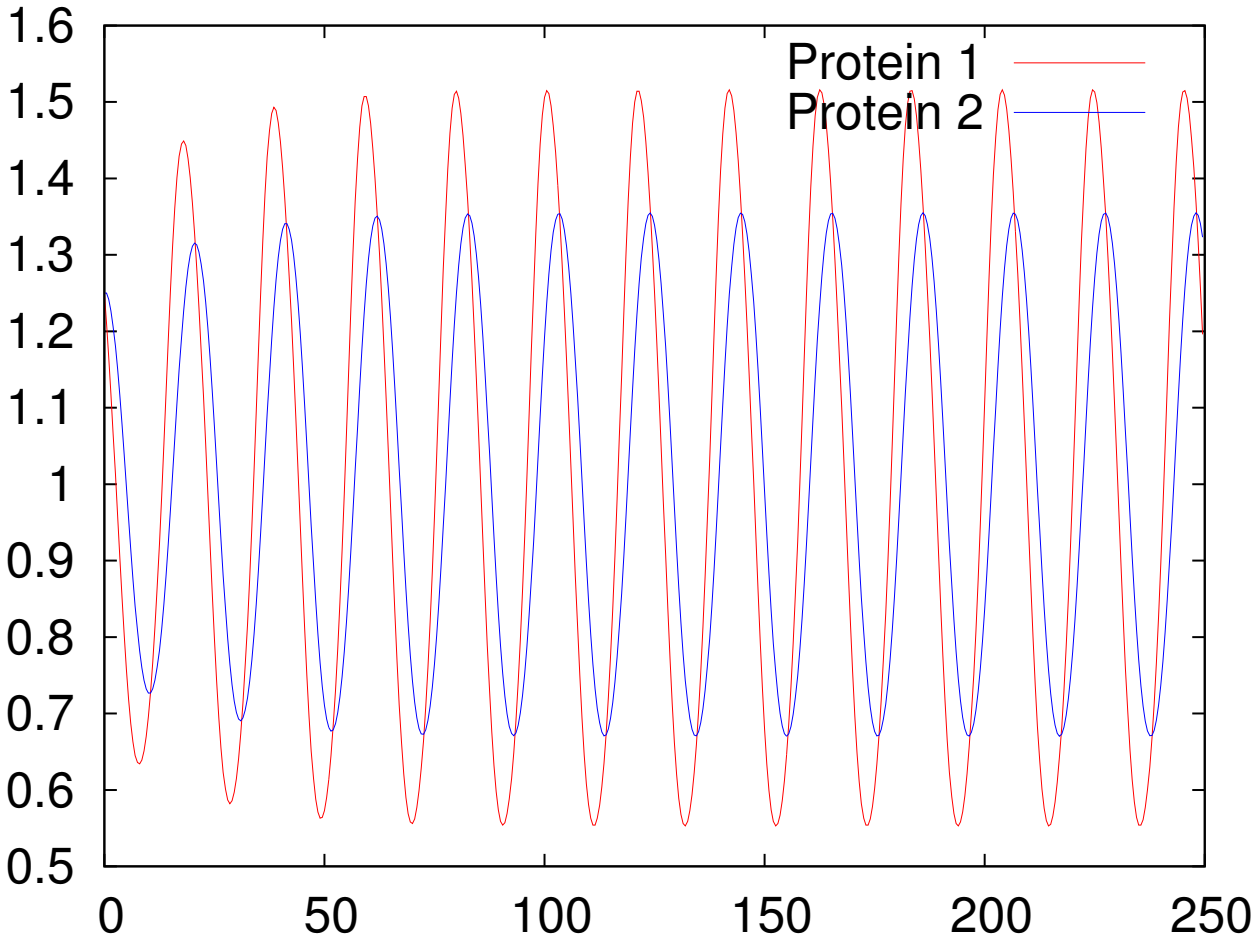


Figure: Oscillations have sinusoidal shape.

R. et al. (2012)

Robustness

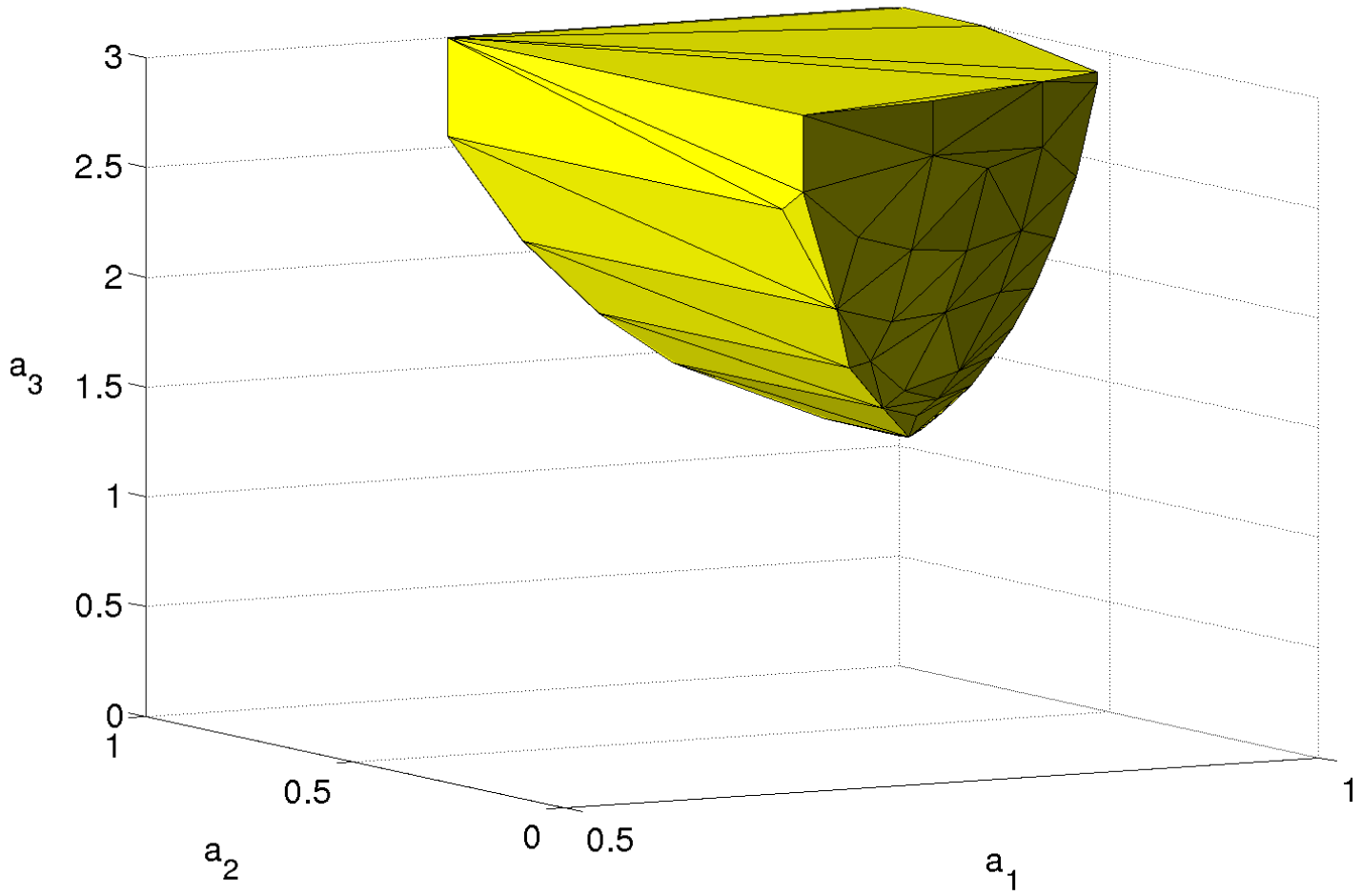
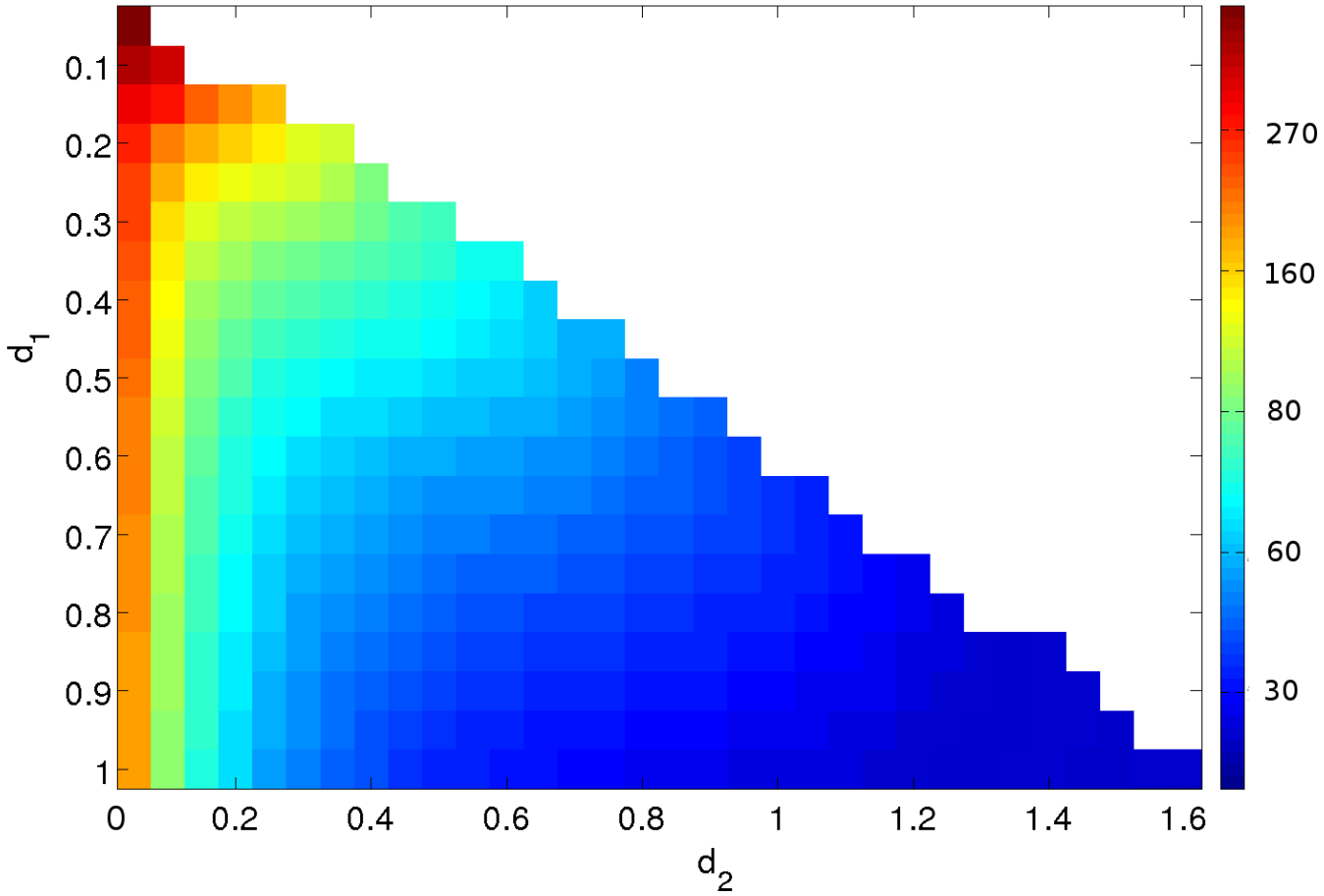


Figure: Surface in parameter space where the Hopf bifurcation occurs for the on-/off-rates used in R. et al. 2012.

R. et al. (2012)

Varying the Diffusion Constants



Period of oscillation depends on the diffusion constants.

R. et al. (2012)

Steady States: 'Antagonist' Scenario

- Asymmetric distribution in steady state ('antagonists'):
  - $\hat{\ell}_1 = m_1, \hat{c}_1 = \hat{r}_1 = 0.$
  - $\hat{\ell}_2 = 0, 0 < \hat{c}_2 < \hat{r}_2.$
- Perturbed heteroclinic orbit
- Restrictions on on-/off-rates in steady state are met when
  - $\kappa_1(q_1, q_2) = k_1(q_2)q_2.$
  - $\alpha_2(q_1, q_2) = a_2(q_1)q_2.$
- Auxiliary problem for λ: different matrices at the poles - must solve the full transcendental problem

'Antagonist' Scenario (R. et al. 2013)

Possible rates:

$$\begin{aligned}
\text{Binding rates} \quad & \alpha_1(q_1, q_2) = 1 - a_1 + a_1 q_1^2, \\
& \alpha_2(q_1, q_2) = \left(\frac{a_2 + 1}{a_2 + 2} + \frac{q_1}{a_2 + 2} \right) q_2 \\
\text{Unbinding rates} \quad & \kappa_1(q_1, q_2) = \frac{(1 + a_3) q_2}{a_3 + q_2} \\
& \kappa_2(q_1, q_2) = \frac{1 + a_2}{a_2 + q_2} \\
\text{Diffusion constants} \quad & d_1 = d_2 = 1
\end{aligned}$$

‘Antagonist’ Scenario

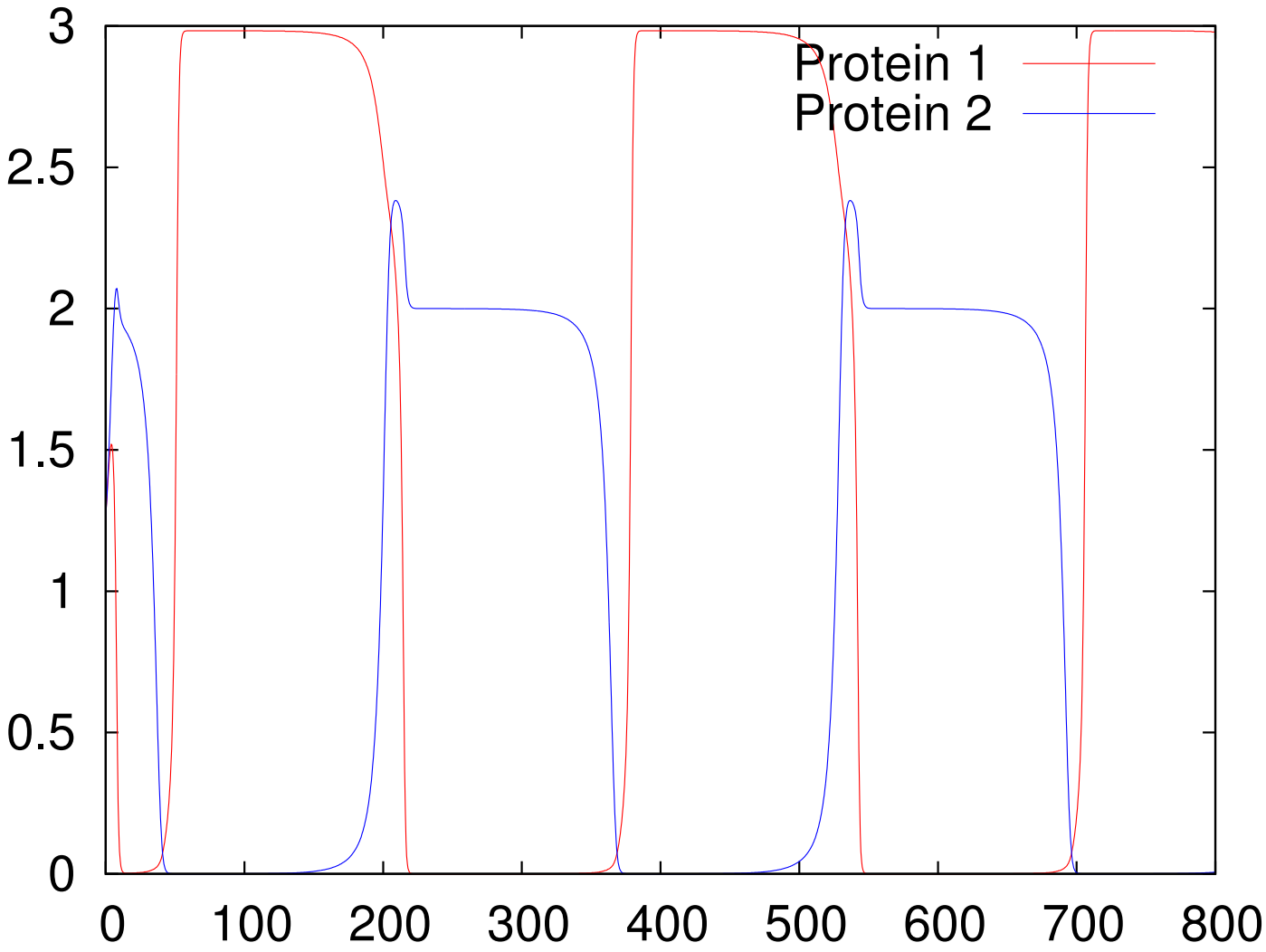
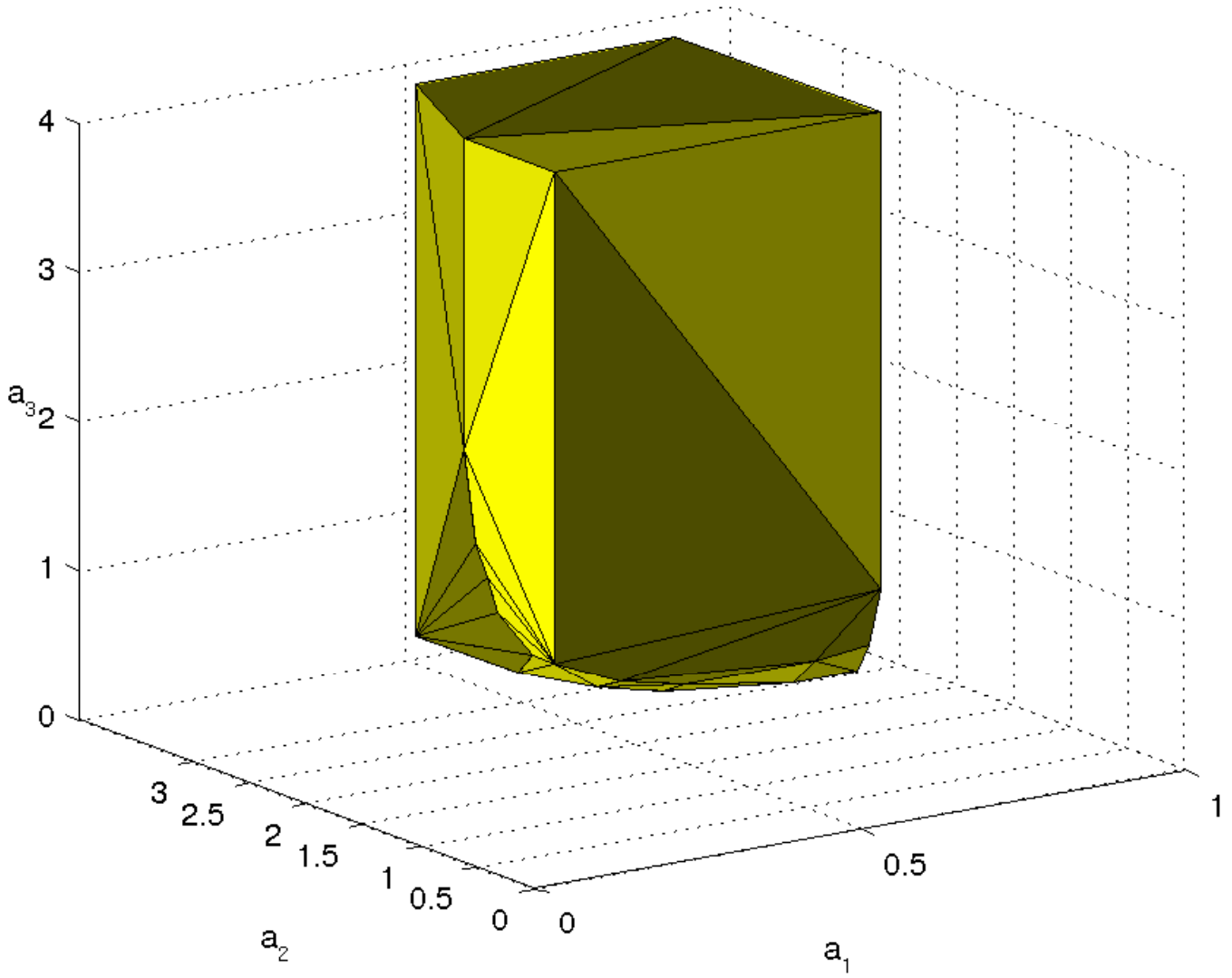


Figure: Concentrations at the pole are nearly perfectly off-phase.

Robustness



R. et al (2013)

Back to *M. xanthus*

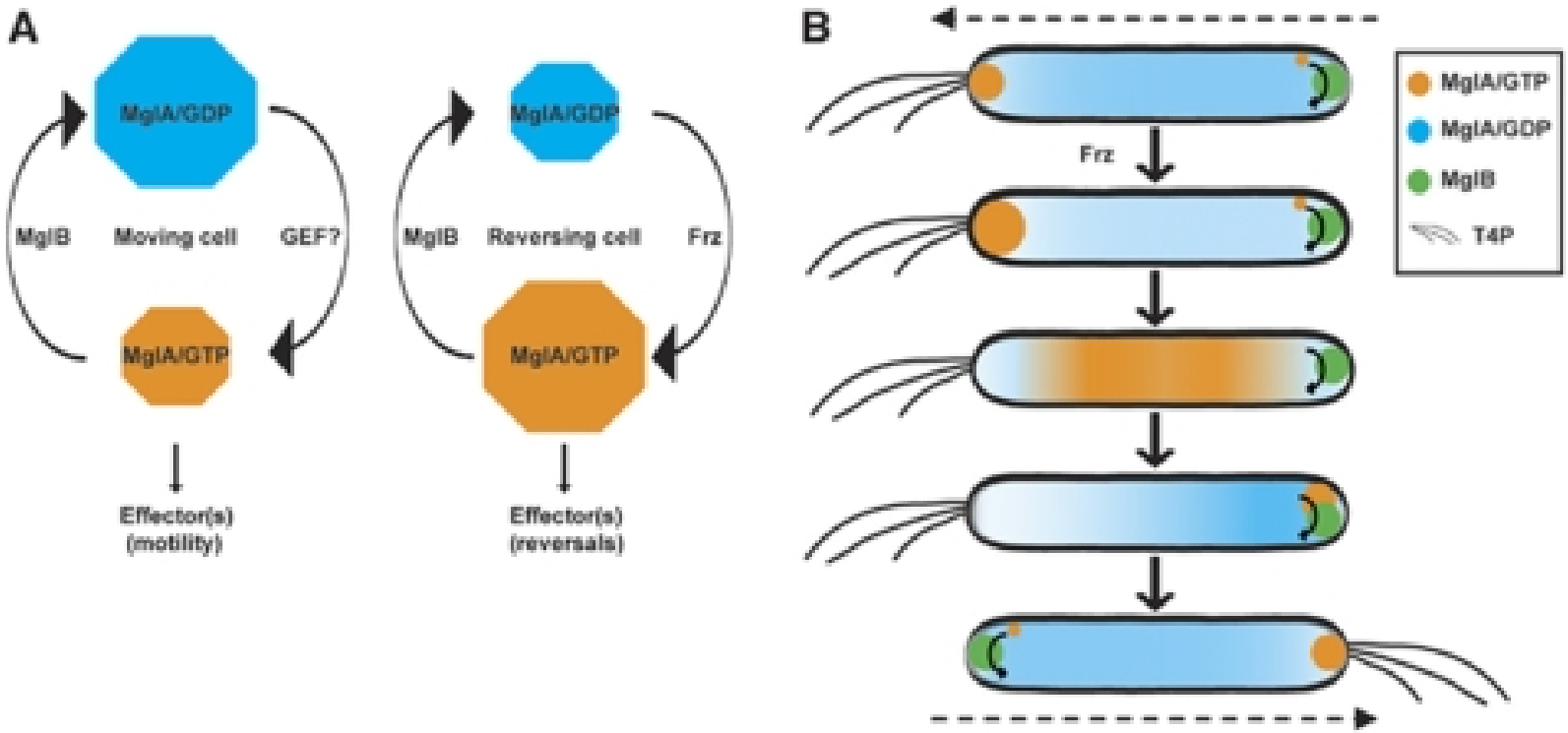


Figure: Biochemical Interactions. MglB (GAP) converts MglA-GTP to MglA-GDP but only MglA-GTP can bind to the poles.

Leonardy et al. (2010)

Biochemical Interactions

- conversion of MglA-GTP to MglA-GDP stimulated by MglB
- *not known* how Frz signalling causes the release of MglA-GTP, MglB from the poles
- Frz signalling *modelled* as a pulse $\beta(t)$ that stimulates the conversion of MglA-GDP to MglA-GTP
- net rate of transition between MglA-GTP and MglA-GDP:

$$\phi(c_{\mathrm{AT}}, c_{\mathrm{B}}, c_{\mathrm{AD}}) = \underbrace{\beta(t)c_{\mathrm{AD}}}_{\text{activation}} - \underbrace{\gamma(c_{\mathrm{B}})c_{\mathrm{AT}}}_{\text{deactivation}}$$

Model Equations

cytoplasm: transport & net transition

$$
\begin{aligned}
\partial_t c_{\mathrm{AT}} &= \Delta c_{\mathrm{AT}} + \phi(c_{\mathrm{AT}}, c_{\mathrm{B}}, c_{\mathrm{AD}}) && \text{MglA-GTP} \\
\partial_t c_{\mathrm{B}} &= \Delta c_{\mathrm{B}} && \text{MglB} \\
\partial_t c_{\mathrm{AD}} &= \Delta c_{\mathrm{AD}} - \phi(c_{\mathrm{AT}}, c_{\mathrm{B}}, c_{\mathrm{AD}}) && \text{MglA-GDP}
\end{aligned}
$$

poles: binding/unbinding

$$
\begin{aligned}
\ell'_{\mathrm{AT}} &= \alpha_{\mathrm{AT}}(\ell_{\mathrm{AT}}, \ell_{\mathrm{B}}) c_{\mathrm{AT}}(0) - \kappa_{\mathrm{AT}}(\ell_{\mathrm{AT}}, \ell_{\mathrm{B}}) \ell_{\mathrm{AT}} && \text{MglA-GTP} \\
r'_{\mathrm{AT}} &= \alpha_{\mathrm{AT}}(r_{\mathrm{AT}}, r_{\mathrm{B}}) c_{\mathrm{AT}}(1) - \kappa_{\mathrm{AT}}(r_{\mathrm{AT}}, r_{\mathrm{B}}) r_{\mathrm{AT}} \\
\ell'_{\mathrm{B}} &= \alpha_{\mathrm{B}}(\ell_{\mathrm{AT}}, \ell_{\mathrm{B}}) c_{\mathrm{B}}(0) - \kappa_{\mathrm{B}}(\ell_{\mathrm{AT}}, \ell_{\mathrm{B}}) \ell_{\mathrm{B}} && \text{MglB} \\
r'_{\mathrm{B}} &= \alpha_{\mathrm{B}}(r_{\mathrm{AT}}, r_{\mathrm{B}}) c_{\mathrm{B}}(1) - \kappa_{\mathrm{B}}(r_{\mathrm{AT}}, r_{\mathrm{B}}) r_{\mathrm{B}}
\end{aligned}
$$

Model Equations

cytoplasm: transport & net transition

$$\begin{aligned} \partial_t c_{\mathrm{AT}} &= \Delta c_{\mathrm{AT}} + \phi(c_{\mathrm{AT}}, c_{\mathrm{B}}, c_{\mathrm{AD}}) && \text{MglA-GTP} \\ \partial_t c_{\mathrm{B}} &= \Delta c_{\mathrm{B}} && \text{MglB} \\ \partial_t c_{\mathrm{AD}} &= \Delta c_{\mathrm{AD}} - \phi(c_{\mathrm{AT}}, c_{\mathrm{B}}, c_{\mathrm{AD}}) && \text{MglA-GDP} \end{aligned}$$

poles: binding/unbinding

$$\begin{aligned} \ell'_{\mathrm{AT}} &= \alpha_{\mathrm{AT}}(\ell_{\mathrm{AT}}, \ell_{\mathrm{B}}) c_{\mathrm{AT}}(0) - \kappa_{\mathrm{AT}}(\ell_{\mathrm{AT}}, \ell_{\mathrm{B}}) \ell_{\mathrm{AT}} && \text{MglA-GTP} \\ r'_{\mathrm{AT}} &= \alpha_{\mathrm{AT}}(r_{\mathrm{AT}}, r_{\mathrm{B}}) c_{\mathrm{AT}}(1) - \kappa_{\mathrm{AT}}(r_{\mathrm{AT}}, r_{\mathrm{B}}) r_{\mathrm{AT}} \\ \ell'_{\mathrm{B}} &= \alpha_{\mathrm{B}}(\ell_{\mathrm{AT}}, \ell_{\mathrm{B}}) c_{\mathrm{B}}(0) - \kappa_{\mathrm{B}}(\ell_{\mathrm{AT}}, \ell_{\mathrm{B}}) \ell_{\mathrm{B}} && \text{MglB} \\ r'_{\mathrm{B}} &= \alpha_{\mathrm{B}}(r_{\mathrm{AT}}, r_{\mathrm{B}}) c_{\mathrm{B}}(1) - \kappa_{\mathrm{B}}(r_{\mathrm{AT}}, r_{\mathrm{B}}) r_{\mathrm{B}} \end{aligned}$$

boundary conditions: total mass conservation of MglA, MglB

Signalling Regimes

Frz signalling

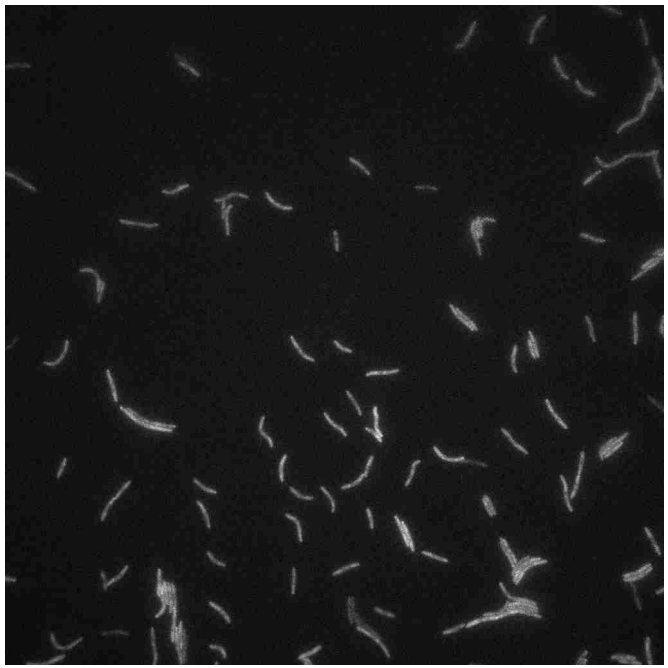
- absent: $\beta(t) \equiv 0$
- continuous: $\beta(t) = \epsilon > 0$
- stochastic:

$$\beta(t) = \begin{cases} \epsilon > 0 & \text{for short intervals } \delta t \approx 0 \\ 0 & \text{else} \end{cases}$$

The sequence of inter-arrival times for the pulse follows a Poisson process with parameter ν over a fixed time interval

Model Validation

time-lapse movie



experiment vs. simulation

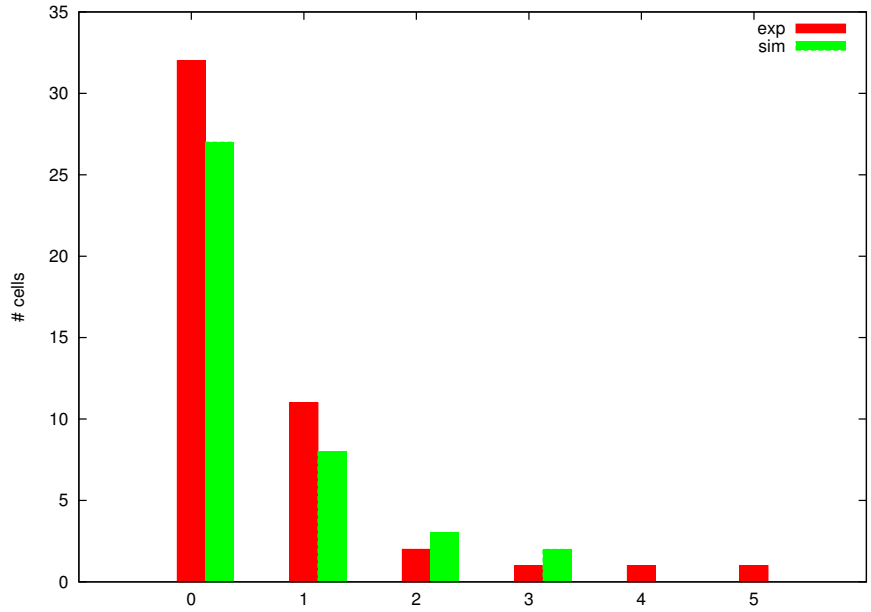


Figure: Reversal counts from an experimental sample (red) vs. reversal counts from a simulation using a Poisson process for the pulse with parameter ν (green).

Summary

- model captures biologically relevant regimes in the network
- spatio-temporal oscillations not of "delay-ODE type"
- oscillations – consequence of Hopf bifurcation (equal diffusion constants!)
- importance of boundary conditions

Literature

- Rashkov P, Schmitt BA, Søgaard-Andersen L, Lenz P, Dahlke S (2012) *Bull. Math. Biol.* 74: 2183-2203
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- Rashkov P, Schmitt BA, Keilberg D, Beck K, Søgaard-Andersen L, Dahlke S (2014) *Math. Biosci.* 258: 189-200

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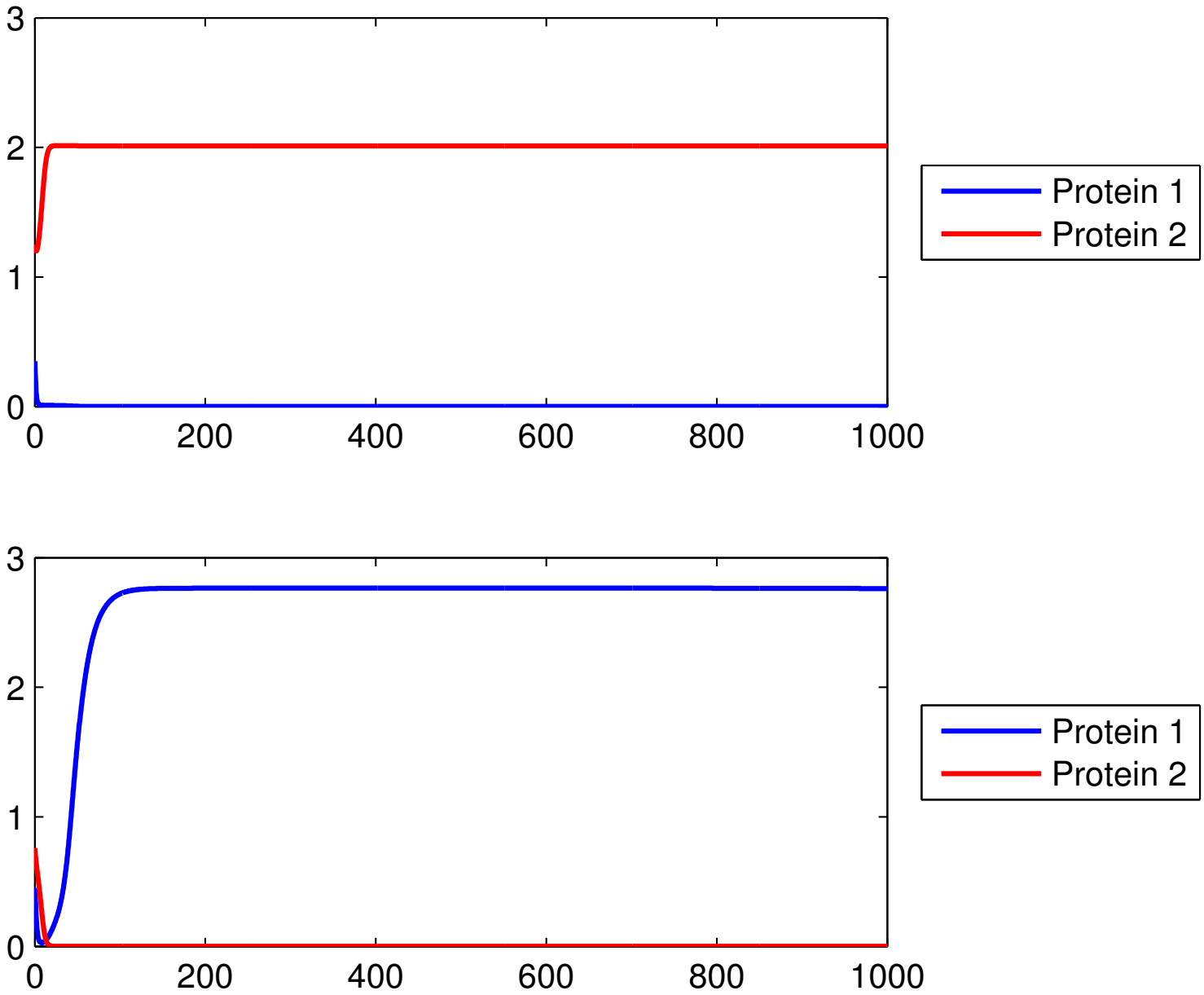
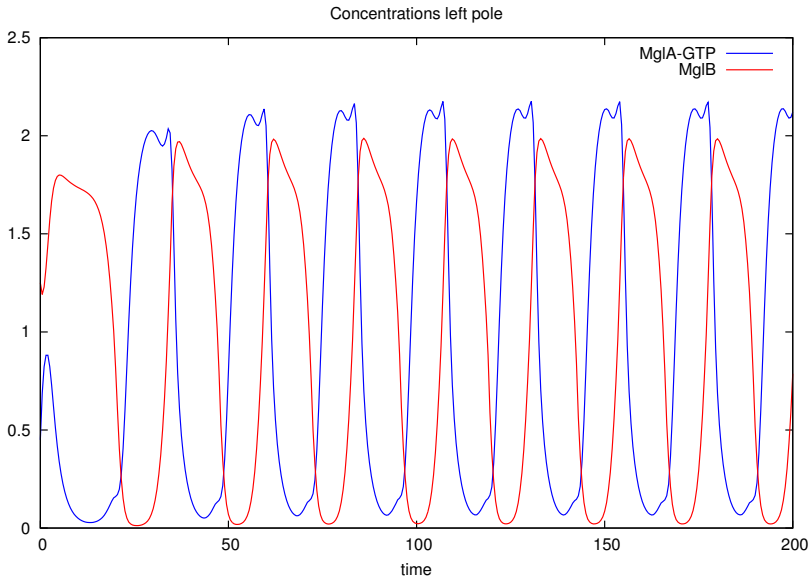
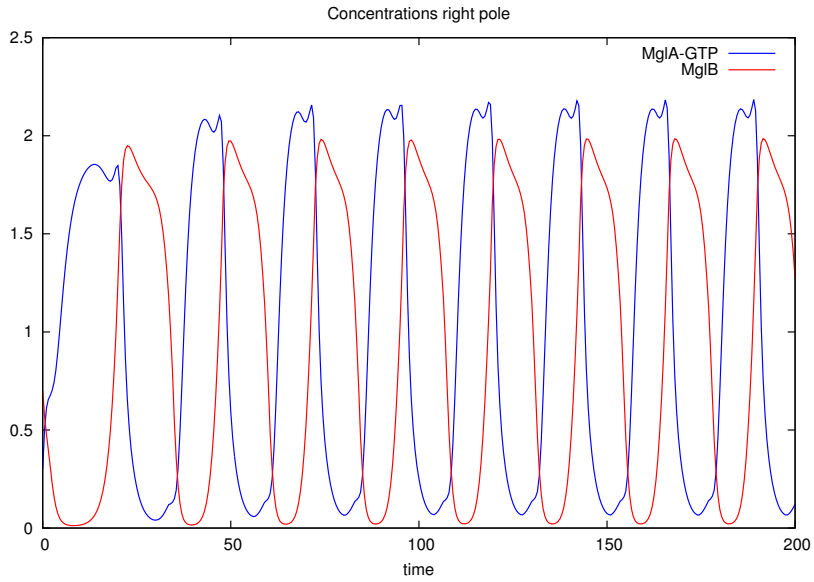
Thank you very much!

Frz signalling Absent



Continuous Frz Signalling





Stochastic Frz Signalling, pulse β by Poisson law