Connection between Microscopic Stochastic and Macroscopic Nonlinear Diffusion Models of Reversing Bacteria

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Mariel Zagunis served as US Olympic Flag Bearer For 2012 Opening Ceremony. Former Notre Dame fencer is two-time Olympic gold medalist in sabre, most successful American fencer in Olympic history.
Outline

- Background and Motivation
- Role of Reversals in Swarming of Myxobacteria
- Swarming Model Development and Implementation
- Results on Gliding Bacteria Clustering
- Continuous Limits of Discrete Stochastic Systems
- Conclusions
Self-organisation and Myxobacteria life cycle

Available nutrients - growth, swarming, predation
Starvation - signaling, differentiation, fruiting bodies


Swarming

- **S(ocial)-motility**: pilus retraction PULLS cell forward ONLY when there is a group of cells ahead;

- **A(adventurous)-motility**: slime secretion from the end of a cell PUSHES the cell forward;

- **Reversal**: Individual cell reverses its gliding direction, or polarity, roughly once every 10 minutes;

- **Slime following**: tend to follow the existing slime trails.

- **Mutant types**: cells having only one motility mechanism (A+S-, A-S)

Figures from Dale Kaiser, Nature 2003
A-motility

- 500 nozzles

Images: Kaiser (Curr Bio 2002) and (for WZA), Dong (Nat. 2006)
S-motility

- Type IV Pili: 6-8 nm in diameter
- 1 – 2 cell lengths long
- 4 – 6 pili per cell

AFM Images: Li et al (Microbio 2005) and (for Pil Complex), Ayers et al (J Mol Biol
The collective behavior of Myxobacteria

Collective motion:
- Cells in Layers, clusters, open space
- Driven by motility and signaling
- Gliding Motility (not swimming)
Evidence for a reversal clock

A cytoplasmic His-Asp phospho-relay, like chemotaxis in E.coli

A negative feedback loop, uncovered by simulation. “The frizzilator”.

A guanine nucleotide release protein

A small Ras-like G-protein

De-commmission both types of old engines

Regulator of A and S motilities

Morcos et al., *Pac Symp Biocomput.* 15, 157-165.
Stochastic Model of Bacretial Swarming

- A rectangular section is chosen as simulation domain;
- Cells distributed randomly in space; cell division accounted by a constant cell density in the initial domain;
- Cell flux $\propto$ Swarm expansion rate

Individual cell

Equation of motion:
- Constant speed;
- Variable direction;
- Cellular interactions

Experimental Image  Simulation setup

Radial direction

$$H = \sum_{i=0}^{N-1} K_b (r_i - r_0)^2 + \sum_{i=0}^{N-2} K_b \theta_i^2$$
Hypothesis: Type IV pilus-mediated social interactions have an effect on local alignment of cells during active motion.

(Kearns & Shimkets, Trends in Microbiol. 2001)

Reversal is required for efficient swarming
The reversal frequency is optimized due to natural selection.

Experimental measurements: 8+/−3 minutes
Comparison of experimental data and simulations

<table>
<thead>
<tr>
<th>Strain</th>
<th>Rate of Swarm Expansion (mm/hr)</th>
<th>Gene Deleted</th>
<th>Published Reversal Period (minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ 2</td>
<td>0.155</td>
<td>None</td>
<td>7.21</td>
</tr>
<tr>
<td>DZ 4480</td>
<td>0.028</td>
<td>∆6-393 Frz CD</td>
<td>34.1</td>
</tr>
<tr>
<td>DZ 4481</td>
<td>0.03</td>
<td>Frz E</td>
<td>34.1</td>
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<tr>
<td>DZ 4482</td>
<td>0.123</td>
<td>Frz G</td>
<td>4.15</td>
</tr>
<tr>
<td>DZ 4483</td>
<td>0.033</td>
<td>Frz F</td>
<td>34.1</td>
</tr>
<tr>
<td>DZ 4486</td>
<td>0.055</td>
<td>∆6-132 Frz CD</td>
<td>1.41</td>
</tr>
</tbody>
</table>


How do physical properties of cells impact collective motion?

Two specific questions:
- How adhesion and flexibility impact the clustering of cells.
- How clustering of cells influences the mobility of cells.

To answer these questions, we need:
- Detailed description of cell-cell interactions from experimental movies.
- Computational model that details these physical properties and cell interactions.

Bigger Question:
- Does an optimal adhesion and flexibility combination exist that improves cell mobility in dense single layer populations?
- Do clusters provide an advantage for cells?
Imaging Chamber

- Imaging Chambers were designed by modifying Submerge Agar Chambers (SAC) from (Welch 2001)*.
- Chambers allow for Oil-immersion Microscopy objectives with high-magnification but low working distance.

Welch, PNAS 98:26 14907-14912

- .5 mm Silicon Gasket with Agar Disc
- Glass Slide
- Cover Slip
- Thin layer of water produced from capillarity.
- Bacteria glide on agar disc.
Improved Imaging by using Specialized Chamber

Petri Dishes with dry objective

Agar disc on microscope slide chamber with oil objective
Experimental Clusters
Subcellular Elements (SCE)

- Cells are represented as strings of SCE
- Interaction potentials between elements determine dynamics and model bending and adhesive forces

\[ E_b = \frac{1}{2} k_b (x_{ij} - x_{eq})^2 \]

\[ E_\theta = \frac{1}{2} k_\theta (\theta - \theta_{eq})^2 \]

\[ E_{nb} = \epsilon_{ij} \left( \left( \frac{\sigma_{ij}}{||x_{ij}||} \right)^{12} - \left( \frac{\sigma_{ij}}{||x_{ij}||} \right)^{6} \right) \]

Harvey et al., Phys Bio (2011)
\[ dv_c = M^{-1}f(x)\, dt + \gamma(v_f - v_c)\, dt + \sqrt{2k_B T\gamma M^{-\frac{1}{2}}}\, dW(t). \]

The Langevin equation describes microscopic coupling between the fluid molecules and the SCE meaning that the acceleration of the system particles with mass matrix $M$ is expressed as the sum of a viscous force which is proportional to the particle's velocity $v_c$ (Stokes' law), a noise term $W(t)$ representing the effect of a continuous series of collisions with the atoms of the underlying fluid, and $f(x)$ which is the systematic interaction force derived from the potential energy function. For our model we add the velocity of the fluid $v_f$, from Stokes’ law.
Equations of Motion

Total force found from potentials and slime.

\[
F = F_{slime} + \sum_{E_i=E_b, E_{\theta}, E_{nb}} \nabla_x E_i(x(t))
\]

\[
\frac{d}{dt} x(t) = \frac{1}{\gamma} (R(t) + F)
\]

For bacteria gliding in viscous slime, viscous interactions dominate and inertia can be neglected. Velocity is proportional to applied force with a random noise.

Random perturbations represent imperfections in the substrate that can affect cell direction.
Connecting Simulations to Experiments

Experimental movies are processed to identify cells and find boundaries and center lines of cells.

Cell bending is compared between simulations and experiments.
Total curvature is sum of the curvature measured at each SCE

Harvey et al., Phys Bio (2011)
Graphics Processing Unit (GPU) implementation of SCE Model
Cluster tracking

- Groups are identified and tracked throughout simulation.

Clustering algorithm uses orientation and proximity to determine groups.
Connecting with Experimental Clusters
*LJ = adhesion parameter; larger LJ -> more adhesive cells

1) Higher adhesion increases number of groups.
2) Increases the duration of groups.
Clustering impacts cell mobility

![Graphs showing distance traveled vs number of neighbors for different LJ parameters.](image)

- **LJ 0.0025**
- **LJ 0.01**
- **LJ 0.025**
- **LJ 0.05**
M. xanthus pauses to divide
Pauses for cell division dominate over other cell interactions

…and daughter cells most often move in opposing directions
Summary

- For all observed division events (259)—*M. xanthus* pauses movement.
- These pauses dominate over other potential cell-cell interactions.
- After division occurs, the two new cells often display a polarity in movement.
- The timing and polarity of DK1622, DZ2, and DK1622Δ*pilA* are very similar.
Continuous limits of discrete stochastic systems

\[ E = E_{Adhesion} + E_{Volume} + E_{Flow} \]

**Cellular Potts Model**

**Fig. 2.** The CPM grid showing cells and ECM. The shading denotes the cell type. Different cells (for example, cells 1 and 3) may have the same type. A site S connects up to fourth-neighbor pixels \((N_1, \ldots, N_4)\).
Master equation. $T_l(x, L; x', L')$ and $T_r(x, L; x', L')$ correspond to transitional probabilities for a cell of length $L$ and center of mass at $x$ to change into a cell of length $L$ and center of mass at $x'$. Subscripts $l$ and $r$ correspond to a transition due to the addition removal of a pixel from the left right side of a cell, respectively.
Fokker-Planck Equation and Keller-Segel Model

\[
\partial_t P(x, L, t) = D (\partial_x^2 + 4 \partial_L^2) P + 8D\beta\mu \partial_L (L P) \\
+ D\beta L \partial_x \left[ c'(x) P \right],
\]

\[
\vec{L} = \frac{1}{\lambda} \left[ J_{cm} + \lambda (L - L_T) + \frac{1}{2} \mu c(x) \right], \quad D = \frac{(\Delta x)^2}{8\Delta t}.
\]

\[
p(x, t) = 2\varepsilon \Delta x \sum_{L=(1+\alpha)\varepsilon \Delta x, (3+\alpha)\varepsilon \Delta x, \ldots} P(x, L, t) \\
= \int_{-\infty}^{+\infty} P(x, L, t) dL, \quad \varepsilon \to 0,
\]

\[
E_{\text{min}} = E(L_{\text{min}}), \quad L_{\text{min}} = L_T - \frac{J_{cm}}{\lambda} - \frac{\mu c(x)}{2\lambda},
\]

\[
\Delta E_{\text{length}} = E(L) - E_{\text{min}} = \lambda \vec{L}^2,
\]

\[
P_{Boltz}(x, L) = \frac{1}{Z} \exp(-\beta \Delta E_{\text{length}}),
\]

\[
P(x, L, t) = P_{Boltz}(x, L) p(x, t),
\]

\[
E_{\text{min}} \text{ is a minimum of energy } E(L) \text{ as a function of } L \text{ for a fixed } x.
\]
Comparison between mesoscopic CPM and macroscopic continuous model

(a) Plot of a two-dimensional probability density distributions for a CPM simulation of 12 cells and numerical solution $p(x; y; t)$ of the continuous.

(b) Cross sections of $p_{cpm}(x_0; y; t)$ and $p_{cont}(x_0; y; t)$ at $x_0 = 53.0$ as functions of $y$.

Continuous Limit of the 2D CPM with excluded volume constraint


\[
P(r, L, t + \epsilon^2 \Delta t) = \sum_{j=1}^{2} \left\{ \left[ \frac{1}{2} - \Phi_{j,0}(r - \frac{\epsilon}{2} e_j, L + \epsilon e_j; r, L, t) \right. \right.

\left. - \Phi_{j,r}(r + \frac{\epsilon}{2} e_j, L + \epsilon e_j; r, L, t) - T_l(r + \frac{\epsilon}{2} e_j, L - \epsilon e_j; r, L, t) \right. \right.

\left. - T_r(r - \frac{\epsilon}{2} e_j, L - \epsilon e_j; r, L, t) \right] P(r, L, t) \right. \right.

\left. + \Phi_{j,l}(r, L; r + \frac{\epsilon}{2} e_j, L - \epsilon e_j, t) P(r + \frac{\epsilon}{2} e_j, L - \epsilon e_j, t) \right. \right.

\left. + \Phi_{j,r}(r, L; r - \frac{\epsilon}{2} e_j, L - \epsilon e_j, t) P(r - \frac{\epsilon}{2} e_j, L - \epsilon e_j, t) \right. \right.

\left. + T_l(r, L; r - \frac{\epsilon}{2} e_j, L + \epsilon e_j, t) P(r - \frac{\epsilon}{2} e_j, L + \epsilon e_j, t) \right. \right.

\left. + T_r(r, L; r + \frac{\epsilon}{2} e_j, L + \epsilon e_j, t) P(r + \frac{\epsilon}{2} e_j, L + \epsilon e_j, t) \right} \right. \]
\[ \partial_t p = D_2 \partial^2_r p - \chi_0 \partial_r \cdot [p \partial_r c(r)] \]
\[ + \frac{D_2}{2} (N - 1) \left\{ \partial_x [\psi_x p] + \partial_y [\psi_y p] \right\} \]
\[ \psi_x = \int_{y-L_y^{(min)}}^{y+L_y^{(min)}} \left[ p(x + L_x^{(min)}, y') - p(x - L_x^{(min)}, y') \right] dy' \]
\[ \psi_y = \int_{x-L_x^{(min)}}^{x+L_x^{(min)}} \left[ p(x', y + L_y^{(min)}) - p(x', y - L_y^{(min)}) \right] dx' \]
\[ \chi_0 = -D_2 \mu \beta L_x^{(min)} L_y^{(min)}, (5) \]

where \( D_2 = \frac{(\Delta r)^2}{16 \Delta t}, \partial^2_r = \partial^2_x + \partial^2_y, \chi_0 = -D_2 \mu \beta L_x^{(min)} L_y^{(min)}, L_x^{(min)} = L_{T_x} - \frac{J_{cm}}{\lambda_x}, L_y^{(min)} = L_{T_y} - \frac{J_{cm}}{\lambda_y} \) and \( \int p(r) dr = N \). Lastly, we couple this equation to an equation describing evolution of the external (chemotactic) field \( c \)
\[ \partial_t c = D_c \partial^2_r c - \gamma c + \alpha p \quad (6) \]

where \( D_c, \gamma \) and \( \alpha \) are diffusion, decay and production rates of the field respectively. Note that the chemical is produced by cells.
Equation with nonlinear diffusion term and without blow up in finite time

\[ \partial_t p = D_2 \partial_r \cdot \left[ \frac{1}{(1 - L_x^{(min)} L_y^{(min)} p)^2} \partial_r p \right] - \chi_0 \partial_r \cdot \left[ p \partial_r c(r, t) \right] \]

\[ \partial_t c = D_c \partial_r^2 c - \gamma c + a p \]

Monte Carlo

Chemical Production rate

0.5

1.5

3.0

Continuous Model
\[ u_t = D \left[ \frac{1 + u^2}{(1 - u)^2} u_x \right]_x - \chi(u v_x)_x \]

\[ v_t = D_c v_{xx} - \gamma v + \alpha u \]

on the interval \((0, L), \ L > 0\), satisfying the boundary conditions

\[ u_x(0, t) = v_x(0, t) = u_x(L, t) = v_x(L, t) = 0. \]

1D stochastic model of reversing cells
Nonlinear diffusion equation for a system with reversals

\[ \partial_t p = \partial_x \left[ D(p) \partial_x p \right], \]

where \( p(x) \) is a local cell density (measured in units of volume fraction, i.e. the ratio of volume occupied by cells to the total volume of space), \( x \) is the spatial coordinate and \( D(p) \) is the nonlinear diffusion coefficient determined using Boltzmann-Matano (BM) analysis of stochastic simulations of bacteria motion with different reversal frequencies. Initial cellular distribution for the stochastic model is chosen in the form of a "top-hat" distribution.

Density of bacteria $p_0$ at which there is a transition from motion with collisions to equilibrium motion:

$$p_0 = \left[ T^{-1} \int_0^T (L + vq) dq \right]^{-1} = \frac{L}{L + vT/2}.$$ 

**Nonlinear diffusion coefficient:** 

$$D_{an}(p) = \Theta(p - p_0) \frac{L^2}{2T p^2}.$$
Diffusion Curve for Different Reversal Periods

Diffusion for different reversal periods at $t=128$

- period=2
- period=4
- period=6
- period=8
- period=10
- period=12
- period=14
- period=16
Conclusions

- Existence of optimal reversal frequency maximizing swarming rate has been shown using cell-based off lattice stochastic model.
- Collisions and clusters in experiments have been tracked and used to calibrate and validate SCE model.
- Higher adhesion values lead to increased pair/group duration.
- Excessive adhesion leads to increased cluster size and reduced cell mobility in clusters.
- Cells tend to have more neighbors and do not experience reduced cell mobility.
- Derivation of nonlinear diffusion equations for systems of self-propelled bacteria reversing at different Rates.
Research Group

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- Graduate Students (ND)
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  - Amy Buchmann
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- Undergrad (ND)
  - Fernano Sanjuan

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