

Overview of Research

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- **Microtubules**

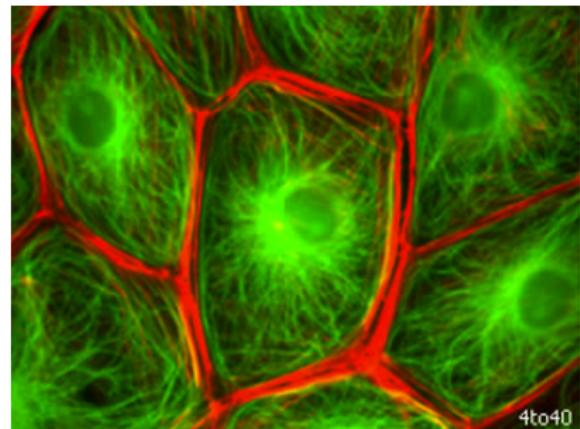
- *M. xanthus*

Microtubules

- **Background**
- **Current Model**
- **Future Work**

Background

- Microtubules (MTs) are components of the cytoskeleton, that give cells shape, the ability to move, and maintain internal organization.
- MTs act as train tracks for the molecular motors that catalyze the movement of various cargos, and pull the chromosomes apart during cell division.



¹"Microtubule dynamic instability: the role of cracks between protofilaments" Li, Li, Goodson, and Alber; 2014 *Soft Matter*

Microtubules

Background

- MTs are dynamic: they grow and shrink in a stochastic process called dynamic instability (DI) to explore space within the cell and respond quickly to changes in the environment.
- DI is regulated by a multitude of proteins that bind to MTs and alter various aspects of DI.

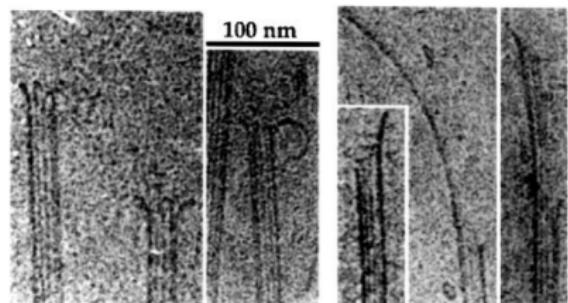


Figure: From "Microtubule Polymerization Dynamics" by Desai and Mitchison

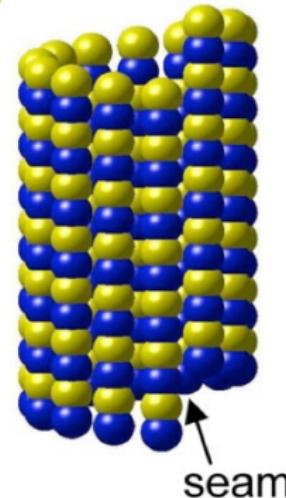
¹"Microtubule dynamic instability: the role of cracks between protofilaments" Li, Li, Goodson, and Alber; 2014 *Soft Matter*

Microtubules

Background

- Each protofilament is formed of dimer comprised of an $\alpha - \beta$ ordered chain of tubulin monomers
- Dimer sub-units are arranged in a lattice as 13 parallel protofilaments, held together by horizontal lateral bonds, *except* at the seam where there is a 3-monomer shift, forming a helical shell around the MT.

α tubulin
 β tubulin

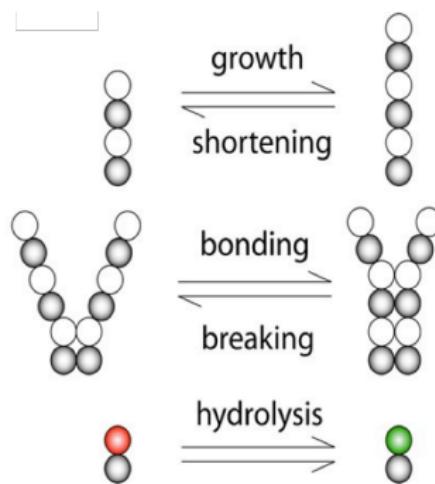


²"The mechanisms of microtubule catastrophe and rescue: implications from analysis of a dimer-scale computational model" Margolin, Gregoretti, Cickovski, Li, Shi, Alber, and Goodson; 2011 *Molecular Biology of the Cell*

Microtubules

Background

- Dimers can be in a phosphorylated state GTP tubulin, or through hydrolysis become GDP tubulin
- Lateral bonds can be created to form a continuous shell around the MT, or break separating protofilament tips, which curl back like “ram horns”.
- Dimers can polymerize and cause the protofilaments to grow, or contrarily, depolymerize and shorten



²“The mechanisms of microtubule catastrophe and rescue: implications from analysis of a dimer-scale computational model” Margolin, Gregoretti, Cickovski, Li, Shi, Alber, and Goodson; 2011 *Molecular Biology of the Cell*

Microtubules

Background

- GTP tubulin-rich region at the MT tip, or “GTP cap”, predisposes the MT to growth due to the strong lateral bonds within the GTP subunits.
- Loss of the GTP cap could trigger catastrophe, and the rapid depolymerization of the MT.

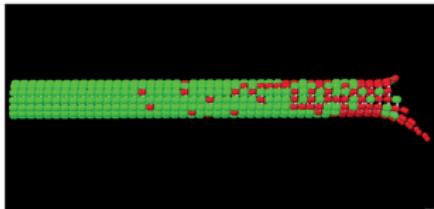


Fig. 1 Three dimensional representation of a typical microtubule tip structure that occurs in the simulated microtubules during the growth phase ($10 \mu\text{M}$ tubulin). The left end is the minus end and the right end is the plus end. Green and red subunits represent GDP and GTP-bound tubulin subunits respectively, while the white bars represent lateral bonds.

¹“Microtubule dynamic instability: the role of cracks between protofilaments” Li, Li, Goodson, and Alber; 2014 *Soft Matter*

Microtubules

Course Grid Model

Microtubules

Detailed Grid Model

- Rates constants for possible events:

Polymerization (GTP only) - k_{poly}^{GTP}

Depolymerization - $k_{depoly}^{GTP}, k_{depoly}^{GDP}$

Lateral bond formation - $k_{bond}^{TT}, k_{bond}^{DT}, k_{bond}^{TD}, k_{bond}^{DD}$

Lateral bond breakage - $k_{break}^{TT}, k_{break}^{DT}, k_{break}^{TD}, k_{break}^{DD}$

Hydrolysis - k_h

- The events are drawn from the parameters provided, and are models as a Poisson processes, with arrival times drawn from its respective rate:

$$t_i = \frac{\ln(r)}{k_i}$$

- The event with the shortest time is implemented.

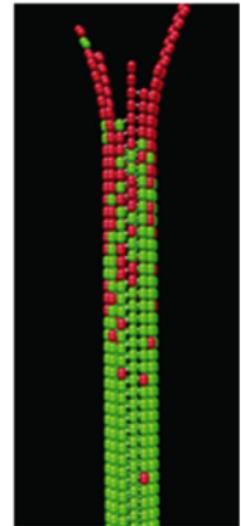
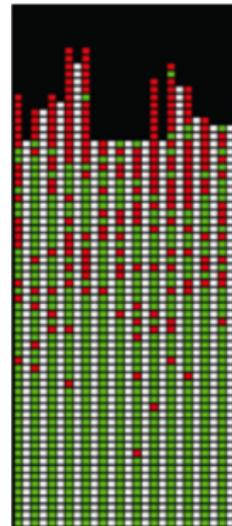
Microtubules

Detailed Grid Model

Microtubules

Detailed Grid Model

- Successfully captures dynamics for MT with 13 protofilaments
- Establishes role of “cracks” and the importance of dimer state at the bottom of the crack for successful growth of the MT.



¹“Microtubule dynamic instability: the role of cracks between protofilaments” Li, Li, Goodson, and Alber; 2014 *Soft Matter*

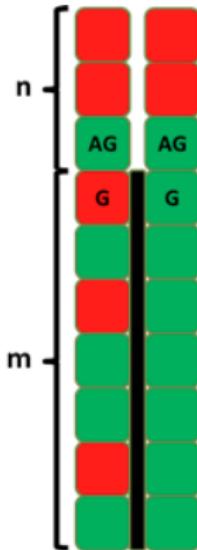
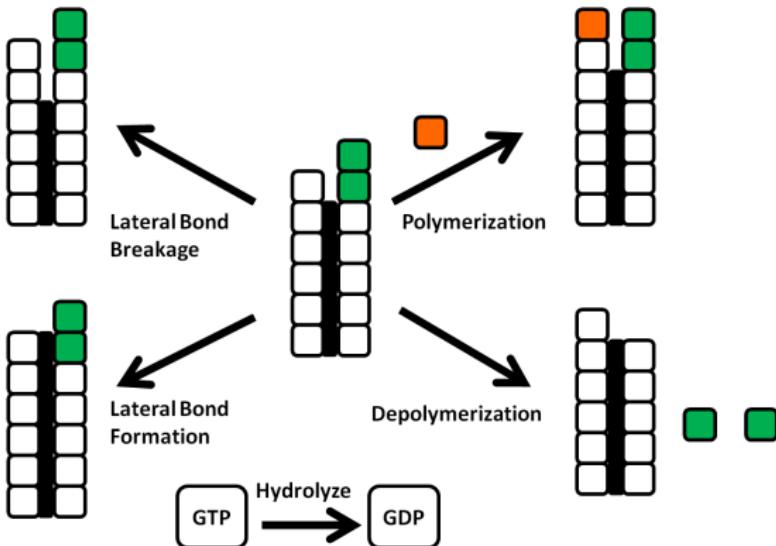
Microtubules

Current Model

- Focus on 2 protofilaments.
- Minimal consideration needed to model a lateral bond.

Microtubules

Current Model



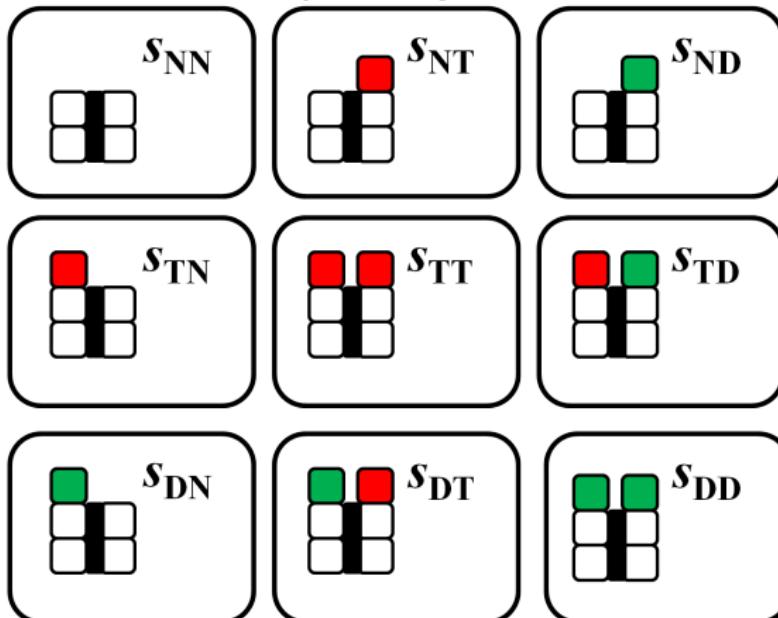
$$\alpha = P(\text{G subunit is GTP-bound})$$

Microtubules

Current Model

Simplified Case: Maximum tip length allowed $n = 1$.

MT Tip Configurations



Microtubules

Current Model

Simplified Case: Maximum tip length allowed $n = 1$.

- Example: Master Equation for configuration s_{NN}

$$\begin{aligned} \frac{dp(s_{NN})}{dt} = & p(s_{NT})k_{\text{depoly}} + p(s_{ND})k_{\text{depoly}} + p(s_{TN})k_{\text{depoly}} + p(s_{DN})k_{\text{depoly}} + \\ & + p(s_{TT})k_{\text{bond}}^{\text{TT}} + p(s_{TD})k_{\text{bond}}^{\text{TD}} + p(s_{DT})k_{\text{bond}}^{\text{DT}} + p(s_{DD})k_{\text{bond}}^{\text{DD}} - \\ & - p(s_{NN}) \left(\alpha^2 k_{\text{break}}^{\text{TT}} + \alpha(1-\alpha)k_{\text{break}}^{\text{TD}} + (1-\alpha)\alpha k_{\text{break}}^{\text{DT}} + (1-\alpha)^2 k_{\text{break}}^{\text{DD}} + \right. \\ & \left. + k_{\text{poly}}^{\text{GTP}} \left(\frac{c}{c_{1/2}+c} \right) + k_{\text{poly}}^{\text{GTP}} \left(\frac{c}{c_{1/2}+c} \right) \right) \end{aligned}$$

Microtubules

Current Model

General Case: Maximum tip length allowed = n .
($n \leq 10$ in practice).

- Master Equation for arbitrary configuration x

$$\begin{aligned} \frac{d}{dt} p(x) = & \sum_y p(y) k_{\text{poly}}^{\text{GTP}} \left(\frac{c}{c_{1/2} + c} \right) + \sum_y p(y) k_{\text{depoly}}^{\text{GTP}} + \sum_y p(y) k_{\text{depoly}}^{\text{GDP}} + \sum_y p(y) k_h \\ & + \sum_y p(y) k_{\text{bond}}^{\text{TT}} + \sum_y p(y) k_{\text{bond}}^{\text{TD}} + \sum_y p(y) k_{\text{bond}}^{\text{DT}} + \sum_y p(y) k_{\text{bond}}^{\text{DD}} \\ & + \sum_y p(y) k_{\text{break}}^{\text{TT}} + \sum_y p(y) k_{\text{break}}^{\text{TD}} + \sum_y p(y) k_{\text{break}}^{\text{DT}} + \sum_y p(y) k_{\text{break}}^{\text{DD}} - p(x) \sum k_* \end{aligned}$$

Upcoming Work

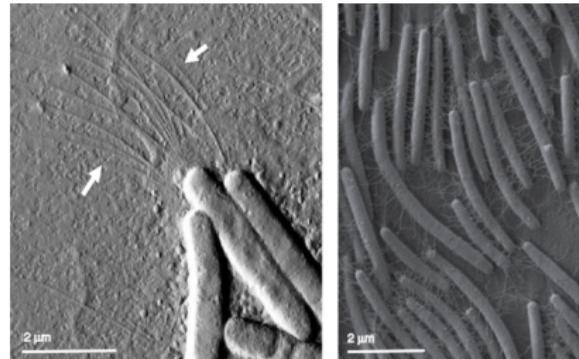
- Use steady-state of master equation to solve for the probability to be in a particular MT tip configuration, which are needed to calculate the net polymerization velocities.
- Depending only the kinetic rate constants for MT dynamics, identify critical concentrations for (i) Elongation and (ii) Unbounded Growth using the 2-PF model.
- Extend the detailed grid model to include effects of MT binding proteins on growth dynamics.

Myxobacteria

- **Background**
- **RomR and Cell Polarity**
- **Future Work**

Background

- *M. xanthus* are a gram-negative bacteria commonly found in soil, typically near decomposing plantlife
- Motility gained from coordinated movement to help the population “swarm”. The unique property of periodic reversals in direction have shown to benefit swarming.



³“Gliding Motility Revisited: How Do the Myxobacteria Move without Flagella?” Mauriello, Mignot, Yang, and Zusman; 2010 *Microbiology and Molecular Biology Reviews* Vol 74 No 2

⁴“Periodic reversal of direction allows Myxobacteria to swarm” Wu, Kaiser, Jiang, and Alber, 2009 *PNAS* Vol. 106, No. 4

Background

- Primary types of non-vegetative motion: Adventurous (A) Motility, and Social (S) Motility.
- Display periodic reversals in direction of motion.
- Characteristic pausing mechanism during division events.

³“Gliding Motility Revisited: How Do the Myxobacteria Move without Flagella?” Mauriello, Mignot, Yang, and Zusman; 2010 *Microbiology and Molecular Biology Reviews* Vol 74 No 2

Myxobacteria

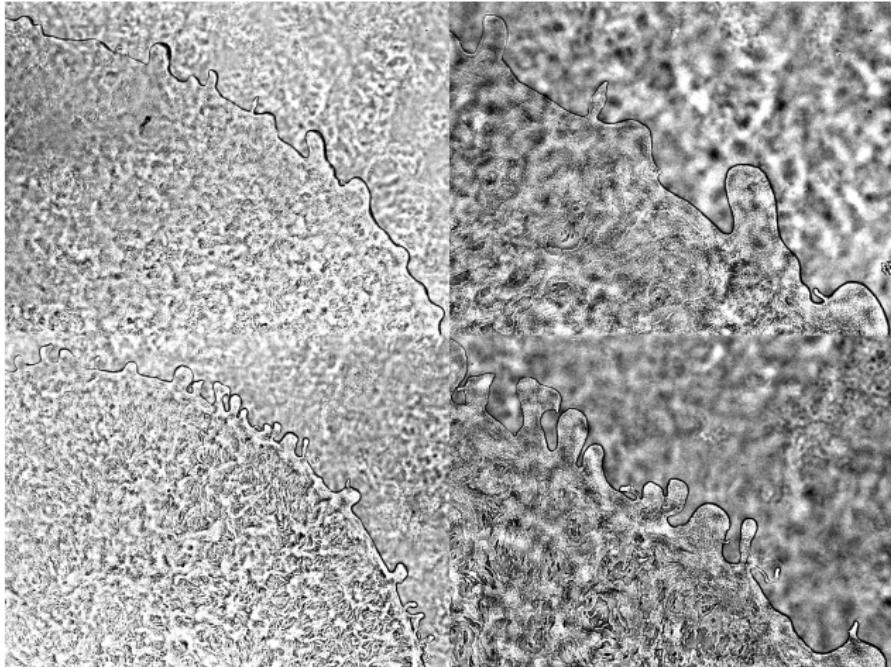
Wild Type

4x

10x

DK1622

DZ2



Myxobacteria

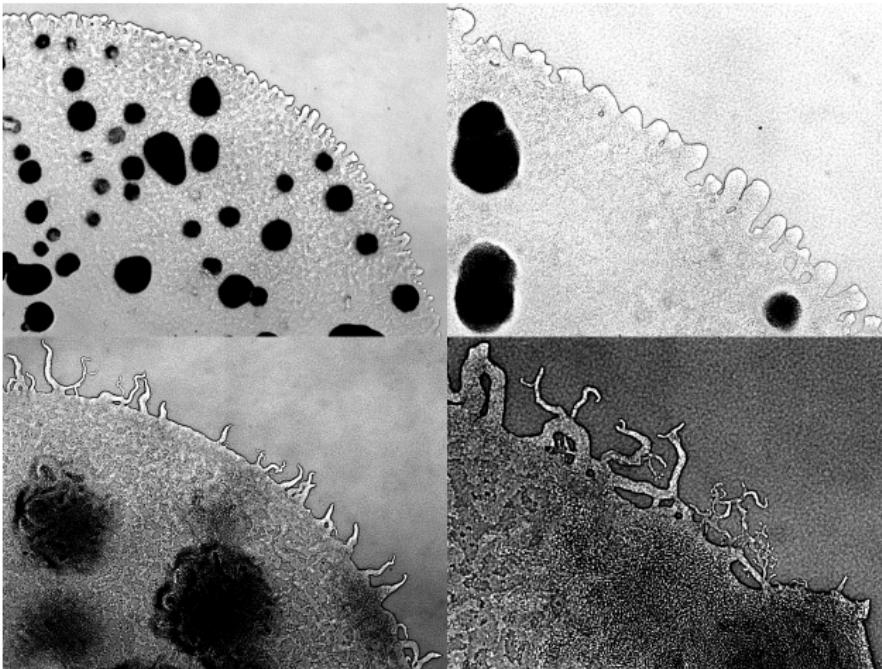
AS-Mutants

DK1240
 $(A^- S^+)$

DK8615
 $(A^+ S^-)$

4x

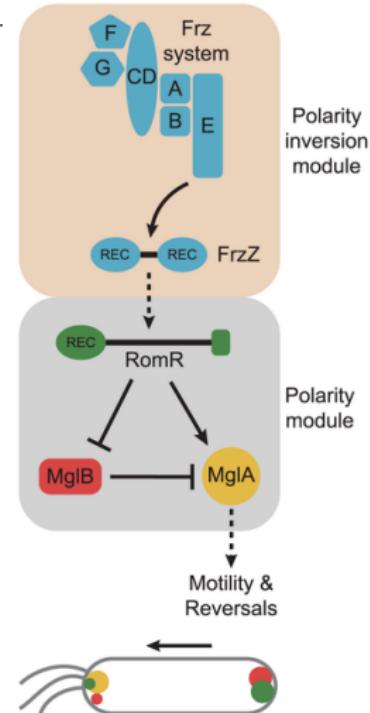
10x



Myxobacteria

RomR and Cell Polarity

- The Frz pathway regulates reversal periods of 8-15 minutes.
- MgIA and MgIB accumulation levels dictate leading and lagging poles selection.

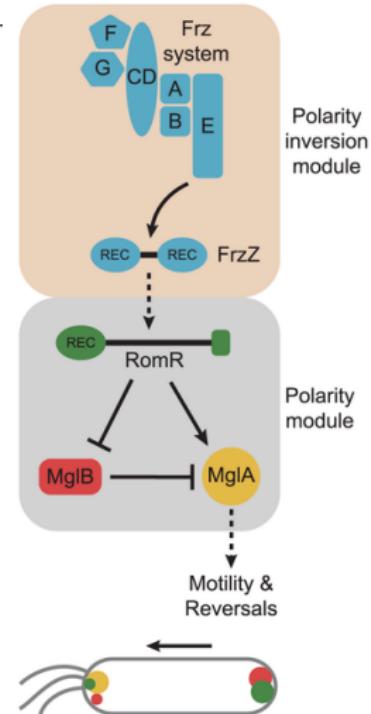


⁵"A Response Regulator Interfaces between the Frz Chemosensory System and the MgIA/MgIB GTPase/GAP Module to Regulate Polarity in *Myxococcus xanthus*" Keilberg, Wuichet, Drescher, Sogaard-Andersen; 2012
PLOS Genetics 8(9): e1002951

Myxobacteria

RomR and Cell Polarity

- RomR accumulates in the pole regions of the cell body by binding to MgIB and MgIA.
- RomR tends to have higher expression in the lagging pole.
- The role of RomR is unclear, though mutants that down-regulate RomR lose motility functions.



⁵"A Response Regulator Interfaces between the Frz Chemosensory System and the MgIA/MgIB GTPase/GAP Module to Regulate Polarity in *Myxococcus xanthus*" Keilberg, Wuichet, Drescher, Sogaard-Andersen; 2012 PLOS Genetics 8(9): e1002951

Myxobacteria

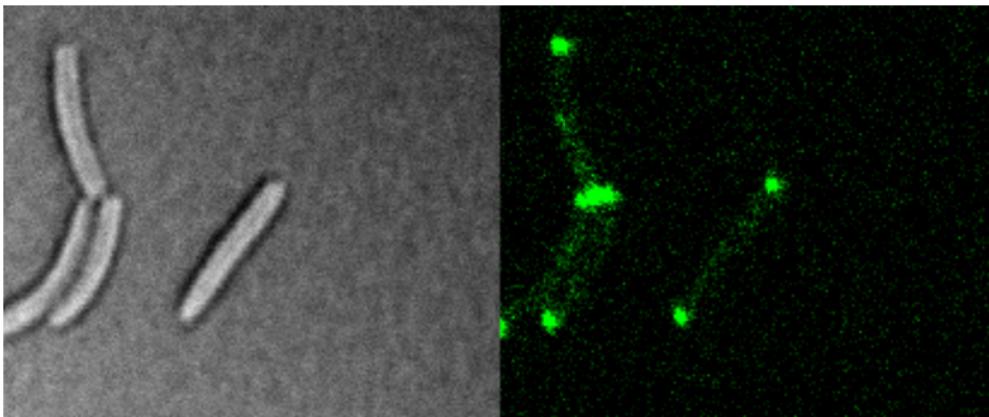
RomR and Cell Polarity

Sample of a WT-RomR Mutant

Myxobacteria

RomR and Cell Polarity

Sample of a WT-RomR Mutant



RomR and Cell Polarity

- Issues with 60x Experimental Setup:
 - Timesteps are too long to capture RomR dynamics.
 - Prolonged laser exposure affects bacteria behavior.
 - Need isolated cells for reliable tracking.
- Solution: 100x objective lens
 - 0.5 sec frametime.
 - Avoiding laser exposure side-effects.
 - Long duration video.
 - Spacial resolution degraded - tradeoff.

Myxobacteria

RomR and Cell Polarity

Sample video of 100x objective experiment

Myxobacteria

RomR and Cell Polarity

Sample of Isolated Reversal Event

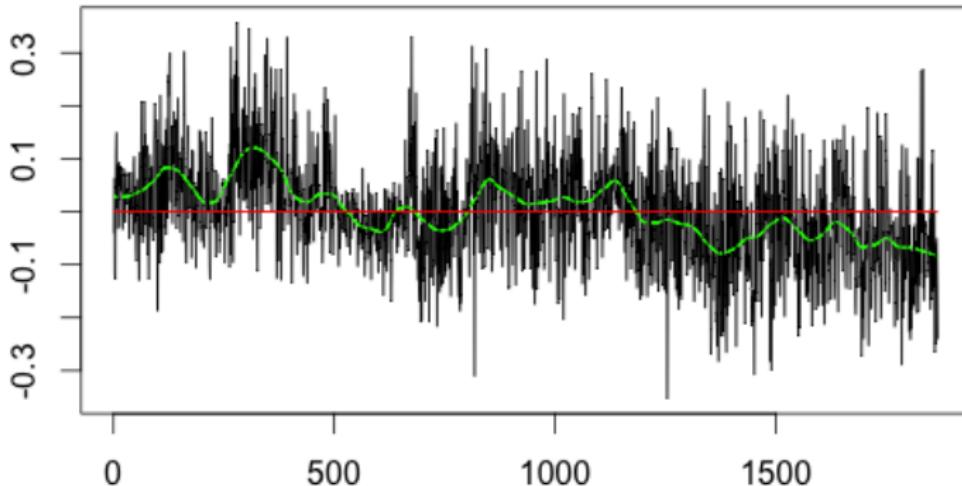
Myxobacteria

RomR and Cell Polarity

Sample of Segmentation of Region of Interest

Myxobacteria

RomR and Cell Polarity



- x-axis = time

- y-axis =
$$\frac{(\text{Pole 1 Intensity}) - (\text{Pole 2 Intensity})}{\text{Total Intensity}}$$

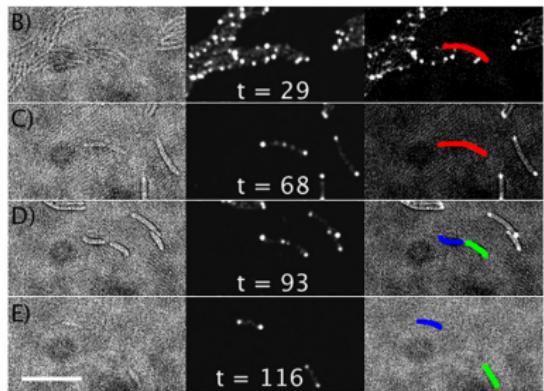
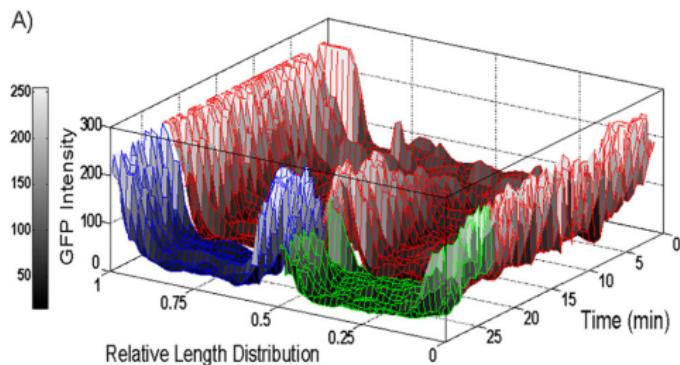
→ Relative difference in RomR expression between poles

Myxobacteria

RomR and Cell Polarity

Myxobacteria

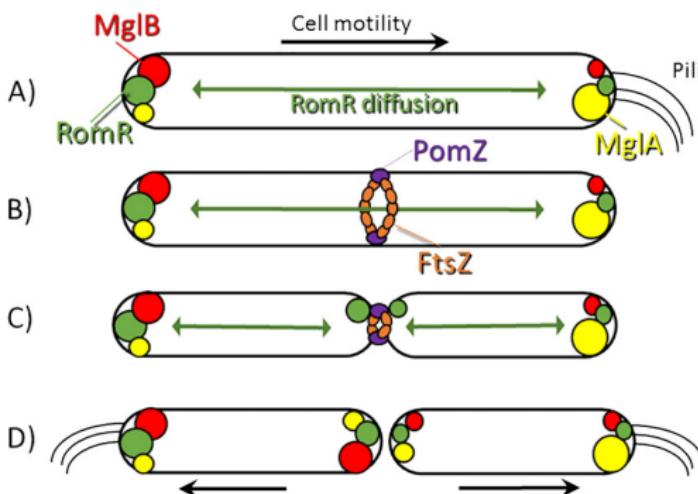
RomR and Cell Polarity



⁶“Cell Division Resets Polarity and Motility for the Bacterium *Myxococcus xanthus*” Harvey, Madukoma, Mahserejian, Alber, Shrout; 2014 J. Bacteriology Vol. 196 #22

Myxobacteria

RomR and Cell Polarity



⁶“Cell Division Resets Polarity and Motility for the Bacterium *Myxococcus xanthus” Harvey, Madukoma, Mahserejian, Alber, Shrout; 2014 J. Bacteriology Vol. 196 #22*

Future Work

- Improve experimental technique to reduce noise during microscopy.
- Complete mathematical modeling for dynamic RomR distribution during reversal and division events.
- Extend experimental protocol to track multiple proteins at time (ie MglA and MglB).

Thank
You