

# A three-dimensional model of myxobacterial fruiting-body formation

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**Myxobacterial cells are social; they swarm by gliding on surfaces as they feed cooperatively. When they sense starvation, tens of thousands of cells change their movement pattern from outward spreading to inward concentration and form aggregates that become fruiting bodies. Cells inside fruiting bodies differentiate into round, nonmotile, environmentally resistant spores. Traditionally, cell aggregation has been considered to imply chemotaxis, a long-range cell interaction that shares many features of chemical reaction–diffusion dynamics. The biological evidence, however, suggests that *Myxococcus xanthus* aggregation is the consequence of direct cell-contact interactions that are different from chemotaxis. To test whether local interactions suffice to explain the formation of fruiting bodies and the differentiation of spores within them, we have simulated the process. In this article, we present a unified 3D model that reproduces in one continuous simulation all the stages of fruiting-body formation that have been experimentally observed: nonsymmetric initial aggregates (traffic jams), streams, formation of toroidal aggregates, hemispherical 3D mounds, and finally sporulation within the fruiting body.**

aggregation | lattice gas | myxobacteria | pattern formation

One fundamental problem in cell biology is how cells assemble organs and tissues that consist of properly positioned differentiated cells. The decisions to differentiate often depend on local interactions among those cells (1). Although the complex interactions among cells in animal organogenesis have received the most attention, genetic programs for differentiation also are found in bacteria. One of the most accessible systems for the study of development is the delta proteobacterium *Myxococcus xanthus*. Myxobacteria are found in cultivated soils all over the earth. Their biological success is due to social behavior that resembles that of the cellular slime molds and, to some extent, the development of animals and plants (2).

Two types of mathematical models can be applied to biological problems: continuous models, which use families of differential or integro-differential equations to describe “fields” of interaction, and discrete models, in which space, time, or state may be discrete. Either type may be deterministic or stochastic. Discrete models can describe the behavior of individual cells in a population, and stochastic Universal Lattice Gas Cellular Automaton (LGCA) models are versatile, readily designed, and rapidly evaluated. Biological LGCA employ a regular, finite lattice and include a finite set of cell states, an interaction neighborhood, and probabilistic local rules that determine the cells’ movements and transitions between states (for a review, see ref. 3).

Our earlier LGCA model, based on simplified point representations for cells and incorporating local cell–cell contact signaling, succeeded in reproducing the streaming and early aggregation phases of myxobacterial development (4, 5). Here, we present a unified 3D stochastic biological LGCA model of fruiting-body formation with extended cell representation. This model adds important components to our previous model (6), such as spores and slime. These new features include C-signal-level-dependent sporulation, passive spore transport, and the

effects of slime on gliding motility. The model reproduces in one continuous simulation all the experimentally observed stages of fruiting-body formation: nonsymmetric initial aggregates (traffic jams), streams, formation of toroidal aggregates, hemispherical 3D mounds, and finally sporulation within the fruiting body.

Myxobacteria move by gliding across the surface of soil particles or of agar in the laboratory. In soil they feed on colonies of other bacteria like packs of microbial wolves; myxobacterial cells surround a colony and secrete hydrolytic enzymes that digest the prey. Because myxobacteria compete with many other micropredators in soil, their food supply is often depleted. As they approach nutrient depletion, *M. xanthus* stop growing and use a quorum sensor to evaluate the local cell density. Given a quorum, tens of thousands of cells undertake an organized, collective effort that culminates in the formation of a spore-filled fruiting body. How is the morphogenesis of a fruiting body programmed? Experiments have suggested several important elements.

**Motility.** Myxobacterial cells are elongated, flexible rods with a 7:1 length-to-width ratio: typically  $2\text{--}12 \times 0.7\text{--}1.2 \mu\text{m}$  (7). They have no flagella and are unable to swim. Instead, cells move on surfaces by gliding along their long axis (8). They move in one direction for a while before smoothly reversing their direction of movement (9). Reversals can have a constant period with little variation (10). Two molecular motors, retractile type IV pili at their leading end (S-motility) (11) and jets for secreting a slime gel at the trailing end (A-motility) (12), provide gliding thrust. The slime-secretion engine can be considered a model; additional evidence can be found in ref. 14 (also R. Yu and D.K., unpublished data). Although reversals are not included in the model described here, they are needed for the traveling waves that preceded aggregation. Recently, we have shown that the model produces aggregates and spores even if reversals are included. Reversals of gliding direction requires, in addition to the two motors, the small G protein *mglA* (13, 14). *MglA* mutants have been said to hyper-reverse (15), but this is misleading. In fact, they secrete slime from both poles and oscillate rapidly back and forth at very low amplitude. The oscillations are due to statistical fluctuations in secretion, not to changes in polarity (16).

**Signaling.** Two modes of cell–cell communication for myxobacterial fruiting-body development are unique. First, they employ diffusible amino acids for quorum-sensing (the A-signal) to certify the presence of enough cells to complete a fruiting body. Quorum sensors in most bacteria are homoserine lactones like

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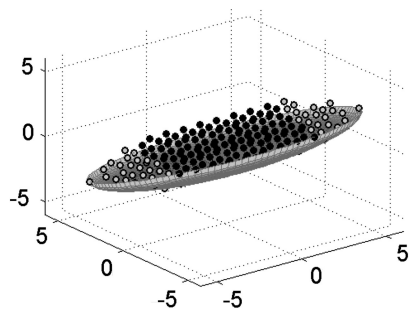
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**Fig. 7.** A single myxobacterial cell body (dark dots) with its signaling poles (light dots) as arrays of pixels on a 3D hexagonal lattice contained in an ellipsoid of particular size. The surface of the cell is indicated by gray coloration.

cal microscopy. In particular, the internal organization of a simulated fruiting body corresponded to the observation of two distinctive domains: a low-density inner domain and a motile, densely packed shell-like outer domain. We also showed that proposed passive spore transport mechanism, provided by circling rod-like cells, results in the delivery of spores to an aggregate's inner area, where they accumulate and form a mature fruiting body.

Because it captures all stages of fruiting body formation, our 3D computational model might serve as a tool for analyzing proposed mechanisms for building multicellular structures dependent on cell contact signaling without chemotaxis. Also, this model should be capable of simulating the competition between normal and cheating mutant cells by varying the cell's motility and ability to accumulate C-signal.

**Computational Model.** Our 3D biological LGCA model is based on local rules that have been suggested by the experiments with *M. xanthus* described above. The model deals with cell movement, C-signaling, slime-trail following, and sporulation. The cell body is represented by an array of pixels on a 3D hexagonal lattice that is generated automatically when the geometric shape, orientation, and size of the cell are given. For this study we approximate rod cells as ellipsoids (see Fig. 7) and spores as spheres of the same volume.

Each cell has two poles: a head and a tail, whose sizes, as well as cell size itself, can be varied. For the simulations reported here, cells that are 11.0  $\mu\text{m}$  in length and 2.4  $\mu\text{m}$  in diameter were used. On a 3D hexagonal lattice each node has 12 nearest neighbors.

The long axis of each cell has 12 possible orientations (or channels). By an exclusion rule of the model, there can be only one cell center of mass per node per channel. Thus, a maximum 12 cell centers, each occupying a different channel, can occupy a single node on the lattice. Apart from the centers, nodes occupied by the extended cell bodies can overlap. As mentioned above, real myxobacteria are flexible and can turn by bending at small angles. The hexagonal lattice constrains this small turning angle to 60°. Cells are allowed to climb over one another and to glide down the other side to embody the fact that myxobacteria move only by gliding on a surface, as described in *Motility*. A cell moves to an upper layer only

if all neighboring nodes in the current layer are occupied, and it moves preferentially in the direction that maximizes the overlap of its pole area with the slime density in the immediate neighborhood. We do not model slime explicitly but instead use a mathematical measure of the slime density field. The field describes the amount of slime deposited by other cells at each location. A sequence of adjacent lattice sites with large amounts of slime indicates a slime trail. An individual cell responds to the slime field by adjusting its orientation to align with the slime trails.

Cells align with one another as they follow slime trails laid down by other cells. At each time step, we first calculate the probabilities of all possible reorientations of a particular cell as a function of the amount of overlap of its head pole with the slime density field. The field records the total number of slime trails that crossed the node at the previous time step. A cell can turn by 60° in the 3D hexagonal lattice or preserve its current orientation. The probability of choosing orientation ( $i$ ) is

$$P_i = \frac{\exp(\beta C^{(i)})}{Z},$$

where  $\beta$  is an alignment parameter,  $Z$  is the normalization factor ( $Z = \sum_i P_i$ ), and  $C^{(i)}$  describes the overlap between the leading pole of the current cell oriented along the  $i$ -th direction and slime trails of cells in the neighborhood. Cells are allowed to climb over one another and to glide down the other side. A cell moves up one layer only if all neighboring nodes in the current layer are occupied. This condition embodies the fact that *M. xanthus* moves only when it is in contact with a surface, as described above.

Also, the poles of a cell are the only C-signaling-sensitive areas. In the model, C-signaling occurs when the end of one cell overlaps with the end of another cell. After each cell-cell interaction, the C-signal level in both cells increases by 1 unit. The threshold level of C-signal serves as a developmental clock. Above a threshold value of accumulated C-signal, a rod cell differentiates into a nonmotile round spore. To preserve the cell volume, we represent a spore as a sphere with the radius of  $\approx 2.3$  units (31). We also differentiate between outer-domain spores (or outer spores) and inner-domain spores (or inner spores). An outer spore is a newly differentiated cell born in the outer domain of an aggregate, which is incapable of active movement. They can be transported passively by motile rod-like cells. An inner spore, by contrast, is a completely motionless spore that has been delivered to the aggregate's inner domain. The passive transport of outer spores is modeled as the result of simple mechanical collisions. If the spore center lies on the line corresponding to the long axis of a rod-like cell, the spore is moved in the direction of motion of the rod-like cell. The spore can be also pushed at some angle to the incoming rod-like cell's long axis, depending on the mutual locations of the rod-like cell and spore centers.

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