Two-Stage Aggregate Formation via Streams in Myxobacteria

M. S. Alber,¹, M. A. Kiskowski,¹, and Y. Jiang²

Mathematics and Physics Departments, University of Notre Dame, Notre Dame, IN 46556.

² Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

(Dated: March 3, 2004)

In response to adverse conditions, myxobacteria form aggregates which develop into fruiting bodies. We model myxobacteria aggregation with a lattice cell model based entirely on short range (non-chemotactic) cell-cell interactions. Local rules result in a two-stage process of aggregation mediated by transient streams. Aggregates resemble those observed in experiment and are stable against even very large perturbations. Noise in individual cell behavior increase the effects of streams and result in larger, more stable aggregates.

PACS numbers: 87.18Ed, 05.65.+b, 87.18.Hf, 05.40.Ca

Introduction.— Fruiting body formation in bacteria occurs in response to adverse conditions [1] and is critical for species survival. When starved, myxobacteria undergo a process of alignment, rippling, streaming and aggregation that culminates in a three-dimensional fruiting body (Fig. 1). This complex, multi-stage morphogenesis must be robust despite internal and external noise.

Canonically, models for bacteria (e.g. E. Coli [3, 4] and Bacillus subtilis [5, 6]) and amoebae (e.g. Dictyostelium discoideum [6, 7]) aggregation have been based on attractive chemotaxis, a long range cell interaction that shares many features of chemical reaction-diffusion dynamics. Initialization of chemotactic signals plays an important role in the initial position of aggregates [3, 8] and subsequent signaling biases cell motion towards developing aggregates [3]. Cells following the maximal chemical gradient navigate towards aggregates which are large and near. In myxobacteria, however, aggregates form without the aid of chemotactic cues [9, 10]. Yet myxobacteria travel large distances to enter an aggregate [11]. Recent computational models based on cell collisions have reproduced the myxobacteria rippling patterns, but did not attempt to model aggregation [12].

During aggregation, myxobacteria cells are elongated with a 7:1 length to width ratio (typically 2 to 12 by 0.7 to 1.2 μ m [13]). They move on surfaces by gliding along their long axis [14]. Fruiting body development is controlled by the C-signal morphogen, which is exchanged by cell-cell contact at cell poles [15]. Different levels of C-signal, encoded by the csgA gene, induce the different stages of fruiting body formation [16, 17]. The expression of csgA is controlled by two feedback loops in the



FIG. 1: Snapshots during the fruiting body formation of Myxococcus xanthus at 0h, 12h, and 61h. From [2] with permission.

signal transduction pathway, one of which is caused by the increased density and alignment in response to Csignal [16, 18]. The second is an intracellular loop via the act operon [19]. Each time a cell receives the Csignal it increases expression of csgA. Aggregates range in size between 10 and 1,000 μ m and are composed of 10^4 to 10^6 cells [13].

Several models have been proposed to explain myxobacteria aggregation [10]. One describes aggregation by cells following the slime trails deposited by other cells, but finds these aggregates unstable without additional chemotaxis [20]. Another suggests that cells form streams by sequential end-to-end contacts due to C-signaling, which coalesce or spiral in on themselves; but these aggregates remain unstable as long as cells are motile [18]. However, experiments show cells move faster within aggregates [21].

We report a new mechanism for aggregate formation in myxobacteria: *two-stage aggregate formation via streams*. This mechanism, based entirely on local cell-cell interactions, accounts for both initialization and formation of large stable aggregates. First, aggregates appear in random positions and cells join aggregates by random walk. Second, the aggregates reorganize as cells redistribute by moving within transient streams connecting aggregates.

Model.— Our model is based on local rules by which cells turn preferentially in directions that increase their level of C-signaling (see [22] for details). Cells move on a hexagonal lattice with periodic boundary conditions. Unit velocities (or channels) are allowed in each of the six directions. Cells are initially randomly distributed with cell density 10, where cell density is the sum of all cell areas divided by total lattice area. We model identical rod-shaped cells as 3×21 rectangles and assume a cell size of $1 \times 7 \ \mu m$. Each cell is represented as follows: (1) a single occupied lattice node corresponds to the position of the cell's center in the xy plane, (2) an occupied channel at this node designates the cell's velocity, and (3) a local neighborhood defines the physical size and shape of the cell. There is an exclusion rule so that there may only be one cell center per channel per node. We also keep track of the C-signal exchange neighborhoods



FIG. 2: Aggregation stages on a 500×500 lattice, which corresponds to an area of 2.8 cm^2 . Local cell density after (a) 200, (b) 900, and (c) 25,000 timesteps. Average cell density is 10. The number of simulated cells is 39,507. The darker shade of gray corresponds to higher cell density. (d) The formation of a stream between two experimental *Myxococcus xanthus* aggregates on the edge of a submerged agar culture after 28 hours after starvation. From [23] with permission.

at the poles of each cell which define the locations of possible C-signaling (end-to-end) interactions between cells. This cell representation is computationally efficient, yet approximates aggregates more closely than using pointlike cells.

Cells first turn stochastically 60 degrees clock-wise or counter-clockwise, or stay in their current direction. The local rule favors directions that maximize the overlap of the C-signal exchange neighborhood at the head of a cell with the C-signal exchange neighborhoods at the tails of neighboring cells. This rule causes cells to align, which is a simplification of the biological hypothesis that alignment and C-signaling reinforce each other (see [16, 18]). Then, all cells move synchronously one node in the direction of their velocity by updating the positions of their centers.

Simulation Results. — Cells aggregate in two distinctive stages in our simulations. During the first stage, cells turn from low density areas towards areas of slightly higher cell density. Initially randomly distributed cells condense into small stationary aggregates (Fig. 2 (a)) which grow and absorb immediately surrounding cells. Next, some adjacent stationary aggregates merge and form long, thin streams which extend and shrink on their own or in response to interactions with other aggregates (Fig. 2 (b)). These streams are transient and eventually disappear, leaving behind a new set of larger, denser stationary aggregates which are stable over time (Fig. 2 (c)). Fig. 2 (d) shows an experimental figure in which two aggregates are interacting via a stream.

Cells in a typical aggregate form an annulus of aligned cells tangent to a hollow center (Fig. 3(a)). Within streams, cells move head to tail with each other in either direction along the stream (see Fig. 3 (b)). Figure 3 (c) shows the details of stream formation from two interacting aggregates. Initial aggregates crowd as they grow. When the distance between aggregates is less than one cell length, they begin exchanging cells, and the cells reorganize into a stream. In contrast to stationary aggregates, cells travel long distances in streams.

Role of noise.— We measured the areas and densities of every stationary aggregate which appeared over the



FIG. 3: Directions of cell centers within (a) a typical annular aggregate on a 30x30 lattice subsection and (b) a typical stream adjacent to a typical aggregate on a 100×100 lattice subsection. (c) Stream formation from two adjacent aggregates at 900, 1000, and 1100 timesteps.



FIG. 4: Area-density phase diagram for (a) 186 stationary aggregates identified within two simulations over 25000 timesteps, (b) an initially small aggregate to which cells are slowly added over 1000 timesteps, and (c) an artificially constructed aggregate (star) over 600 timesteps. Relaxation of perturbation data in (b) and (c) are plotted every 10 timesteps on top of (a).

course of two simulations. These aggregates fall within a narrow region in the area-density phase diagram shown in Fig. 4 (a), which we call an attractor region. As this attractor region covers a continuous rather than disconnected space, the different aggregate structures can continuously transform from one to the other. We now analyze the stability of this attractor region with respect to two kinds of noise: 1) external noise, which includes noise from the initial random distribution of cells and from our perturbations to the system; 2) internal noise, which originates from the stochastic nature of the cell's turning process.

1. External noise. — Simulations for different random initial conditions show that the standard deviation of cell density at each node increases with similar slope and to similar levels (data not shown), indicating that pattern formation is not very sensitive to noise from the initial conditions.

Next we perturb a stable aggregate in two ways. First, we study an adiabatic perturbation by gradually adding cells to an initially small, isolated aggregate. As cells are slowly added, the aggregate increases in area and density while remaining within the attractor region (Fig. 4(b)). The oscillation of the path in the wider region of the attractor corresponds to 'pulsing' of an aggregate [24]. Second, we introduce a non-adiabatic perturbation by placing two duplicate aggregates in close proximity of each other, which creates a new aggregate with double the initial area and the same density. Over 600 timesteps, this aggregate gradually reorganizes so that it has an area and density within the stable region (Fig. 4(c)). Results from applying both kinds of perturbations suggest that the attractor region is stable.

The area-density phase diagram, in addition to prescribing the region of stable aggregates, also helps our understanding of the formation and stability of streams. When two stationary aggregates interact, the newly formed aggregate lies off the attractor region. Large aggregates will fuse and quickly form a new stable aggregate as in Fig. 4 (c) while small aggregates often form streams. As smaller aggregates have a lower cell density, and lower cell C-signaling levels, when small aggregates fuse, they have a longer transient stage and are more likely to form a stream. Cells at the end of streams do not C-signal in the open space, hence cells at these locations will diffuse without any preferred direction. Though some cells diffuse away, most cells randomly turn within a number of timesteps back into the stream. Once cells are re-directed towards the stream, their direction is locked since they are again C-signaling with the stream cells. Cells turning back into the stream over time causes the stream to gradually contract into a stable aggregate.

2. Internal noise. — To evaluate the role of internal noise, we devise a corresponding deterministic model. Instead of using a stochastic process to model cell turning, we use the following function to decide on the cell orientation for the next step:

$$\begin{split} f_i(r,k+1) &= f_{i^{\ominus}}(r-c_{i^{\ominus}},k)\Omega(r-c_{i^{\ominus}},k,c_i) \\ &+ f_{i^{\oplus}}(r-c_{i^{\oplus}},k)\Omega(r-c_{i^{\oplus}},k,c_i) \\ &+ f_i(r-c_i,k)\Omega(r,k,c_i), \end{split}$$

where f is the particle density distribution function over each lattice node r, k is the timestep, and c_i , $c_{i\Theta}$, and $c_{i\oplus}$ represent velocity vectors in the *i*th direction, vectors turning clockwise from the *i*th direction, and vectors turning counter-clockwise respectively. The collision function $\Omega(r, k, i)$ is the probability of a cell at the node rturning towards direction i at the kth timestep. We drop the exclusion principle so that the density of cells may be greater than 1 at a node. This function effectively converts our stochastic model based on cell turning into a deterministic model, analogous to the process of changing a stochastic lattice gas model to a deterministic lattice Boltzmann model [25].

Our simulations show that this deterministic model evolves similarly to the stochastic model, indicating that the aggregation dynamics are not sensitive to internal noise. Namely, many small aggregates appear, then streams form between interacting aggregates. Eventually the streams dissolve and leave behind a larger set of aggregates. One important difference is that streams in the deterministic model are fewer and smaller. Another



FIG. 5: Distribution of stationary aggregate areas for (a) a stochastic simulation after 29000 timesteps and (b) the equivalent deterministic simulation after 3500 timesteps. Both distributions represent the final stable distribution.

difference is that streams are shorter-lived, and the deterministic simulation reaches a steady state much faster. These differences have a critical effect on the way aggregates reorganize. Comparing the size distribution of aggregates in the stochastic model (Fig. 5(a)) with that of the deterministic model (Fig. 5(b)), we see that with the internal noise, aggregates can reach larger sizes. This is not surprising because noise slows the process of stream contraction so that streams persist longer and span a greater area, which enables more aggregates to interact and form larger, more stable aggregates.

Discussion. — In our simulations, streams redistribute cells within fewer, larger aggregates (compare Fig. 2 (a) and (c)). This is a new mechanism for large, stable aggregate formation in which aggregates first form at random locations and then reorganize. The mechanism is robust since streams form when growing aggregates develop too close together. Cells can then span great distances by moving within streams. Streams resettle into stationary aggregates by moving into a pre-existing stationary aggregate or by gradually thickening and contracting.

The aggregates in our simulation reproduce the unique structures of several myxobacteria fruiting bodies. In *Myxococcus xanthus*, the basal region of the fruiting body is a shell of densely packed cells which orbit both clockwise and counter-clockwise around an inner region only one-third as dense [21, 26]. Fig. 3 (a) shows that typical simulation aggregates have this geometry and cell tracking demonstrates that cells orbit clockwise and anticlockwise. Further, aggregates in our simulation often form in clusters of two or three closed orbits while in *Stigmatella erecta*, several fruiting bodies may form in groups and fuse [13].

In experiments, one myxobacteria aggregate has been observed to mysteriously grow as an adjacent aggregate disappears [24]. Our simulations offer a mechanism for this process: a stream may form connecting two adjacent aggregates and cells migrate from the smaller aggregate to the larger aggregate. Experimentally, these streams may not be visible if the density threshold for viewing cells is greater than the density found within the stream. Figure 2 (d) shows a movie snapshot in which a barely visible stream has developed betweens two aggregates. Shortly after the formation of this stream, the two aggregates fuse.

This mechanism suggests several predictions which may be tested experimentally. We predict that the formation of streams and subsequent redistribution of aggregates will be most significant for intermediate initial cell densities. At low density, the initial set of aggregates will form further apart and will not grow as large. At high cell density, very large, dense aggregates form, which fuse immediately into a larger aggregate when they interact rather than forming a stream. The role of external noise can be experimentally tested by reproducing the perturbation experiments we describe in Figure 3(b)and 3(c). Cells may be slowly added to a small aggregate or quickly added to an aggregate by a large amount to observe the cell reorganization over time. Finally, the role of internal noise can be tested experimentally by tuning the amount of C-factor in the cell aggregates. For example, C-signaling can be decreased by diluting a wild-type population with non-C-signaling cells (increasing internal noise) or individual cell C-signaling levels can be increased (decreasing internal noise).

The limited number of directions permitted on a hexagonal lattice results in an overly regular local pattern and limits the size of aggregates in our simulation, since cells capable of turning by 60 degrees at each timestep may follow a circular orbit with a small radius of curvature. Our local rules do not prevent cells from stacking very high, which results in smaller aggregates and thin-

- [1] E. Ben-Jacob *et al.*, Nature (London) **368**, 46 (1994).
- [2] J.M. Kuner and D. Kaiser, J. Bacteriol. 151, 458 (1982).
- [3] L. Tsimring *et al.*, Phys. Rev. Lett. **75**, 1859 (1995).
- [4] M. P. Brenner, L. S. Levitov, and E. O. Budrene, Biophys. J. 74, 1677 (1998).
- [5] M. Matsushita and H. Fujikawa, Physica A 168 498 (1990); I. Golding, Y. Kozlovsky, I. Cohen, and E. Ben-Jacob, Physica A 260, 510 (1998); A. Komoto *et al.*, J. Theor. Biol. 225, 91 (2003).
- [6] E. Ben-Jacob, I. Cohen, and H. Levine, Adv. Phys. 49 4, 395 (2000).
- J. Martiel and A. Goldbeter, Biophys. J. 52, 807 (1987);
 T. H ofer, J.A. Sherratt, and P.K. Maini, Proc. R. Soc. London B 259, 249 (1995).
- [8] J.Y. Wakano et al., Phys. Rev. Lett. 90, 258102 (2003).
- [9] S. Lobedanz and L. Søgaard-Andersen, Genes Dev. 17, 2151 (2003).
- [10] For a review, see: M. Dworkin, Microbiol. Rev. 60, 70 (1996).
- [11] L. Jelsbak and L. Søgaard-Andersen, Curr. Opinion Microbio. 3, 637 (2000).
- [12] O. Igoshin, A. Mogilner, D. Kaiser, and G. Oster, Proc. Natl. Acad. Sci. USA 98, 14913 (2001); U. Borner, A. Deutsch, H. Reichenbach, and M. Bar, Phys. Rev. Lett. 89, 078101 (2002); F. Lutscher and A. Stevens, J. Nonlinear Sci. 12, 619 (2002).

ner streams than there they would be otherwise. E.g., myxobacteria aggregates range in size from 10 to 1000 μ m in diameter, while in our simulations aggregate size is up to 15 μ m. Thus our model only suggests a mechanism qualitatively. Additional rules, such as cell jamming, would be required to reproduce more details of aggregate formation.

Summary.— Our lattice cell model is based on a very simple local rule by which cells align by turning preferentially to make end to end contacts. On average this rule results in cells following the tails of other cells. This mimics C-signaling in myxobacteria, which drives myxobacteria aggregation. In our simulations, distinct aggregate types form which have different behaviors and roles even though they are composed of identical cells following identical rules. Large, stationary aggregates are most stable, but an intermediate motile aggregate (stream) can aid in large aggregate formation. An interesting discovery is that the presence of some internal noise is required for efficient streaming. It is as if the cells must make short-term mistakes for the formation of unstable transients that ultimately results in more efficient aggregation. Our analysis of streams and the role of noise suggest some new experiments.

We thank Drs. Dale Kaiser, Stan Maree and Guy McNamara for fruitful discussions. MSA is partially supported by grant NSF IBN-0083653. MAK and YJ are supported by DOE under contract W-7405-ENG-36. MAK also acknowledges support from CAM and ICSB centers, University of Notre Dame.

- [13] H. Reichenbach, in: M. Dworkin and D. Kaiser (Eds), *Myxobacteria II*, (American Soc. Microbio., Washington DC, 1993).
- [14] R.P. Buchard, Annu. Rev. Microbiol. 35, 497 (1981).
- [15] S.K. Kim and D. Kaiser, Genes Devel. 4, 896 (1990); S.K. Kim and D. Kaiser, Science 249, 926 (1990).
- S.K. Kim and D. Kaiser, J. Bacteriol. **173**, 1722 (1991);
 S. Li, B. Lee and L.J. Shimkets, Genes Dev. **6**, 401 (1992).
- [17] T. Kruse, L. Lobedanz, N.M.S. Berthelsen, and L. Søgaard-Andersen, Mol. Microbiol. 40, 156 (2001).
- [18] L. Jelsbak and L. Søgaard-Andersen, Proc. Natl. Acad. Sci. USA 99, 2032 (2002).
- [19] T.M.A. Gronewold and D. Kaiser, Mol. Microbiol. 40, 744 (2001).
- [20] A. Stevens, SIAM J. Appl. Math. 61, 172 (2000).
- [21] B. Sager and D. Kaiser, Proc. Natl. Acad. Sci. USA 90, 3690 (1993).
- [22] M. S. Alber, Y. Jiang, and M. A. Kiskowski, Physica D (to be published).
- [23] D. Kaiser and R. Welch, J. Bacteriol. 186, 919 (2004).
- [24] D. Kaiser (private communication).
- [25] U. Frisch et al., Complex Systems 1, 648 (1987).
- [26] B. Julien, D. Kaiser and A. Garza, Proc. Natl. Acad. Sci. USA 97, 369 (2000).