

A 3D Multiscale Model of Chemotaxis in Bacteria

Andrew Wu¹, Timothy Davison¹, and Christian Jacob^{1,2}

¹Department of Computer Science, University of Calgary, Calgary, AB, Canada T2N 1N4

²Department of Biochemistry & Molecular Biology, University of Calgary, Calgary, AB, Canada T2N 4N1
cjacob@ucalgary.ca

Abstract

We present an interactive, agent-based, multi-scale 3D model of a colony of *E. coli* bacteria. We simulate chemical diffusion on an agar plate which is inhabited by a colony of bacterial cells. The cells interact with a discrete grid that models diffusion of attractants and repellents, to which the cells react. For each bacterium, we simulate its chemotactic behaviour, making a cell either follow a gradient or tumble. Cell propulsion is determined by the spinning direction of the motors that drive its flagella.

In an agent-based model, we have implemented the molecular elements that comprise the two key chemotactic pathways of excitation and adaptation, which, in turn, regulate the motors and influence a cell's movement through the agar medium. We show four interconnected model layers that capture the biological processes from the colony layer down to the level of interacting molecules.

Introduction

We have implemented a model of a colony of bacterial cells, which we can visualize and interact with at four distinct, yet computationally interconnected levels (Fig. 1). At the “naked eye” level we model the gradient of a diffusing chemical signal, similar to what one observes in a laboratory by looking at an agar plate inhabited by a bacterial colony. Once we zoom closer into the plate, the colony of bacteria becomes visible, which reflects the simulated behaviours of cell clusters. Picking one of the cells transitions to a close-up view of an individual bacterium, with its flagella propelling it through the medium. As the last model level, we can navigate into a bacterium's cytoplasm, where we have implemented the molecular signalling pathways that drive chemotaxis.

E. coli and Chemotaxis

The prokaryotic cell we have modelled is known as *Escherichia coli*. Most strains of *E. coli*, as it is known for short, are harmless. We have billions of these bacteria naturally residing within our intestinal tracts (Zimmer, 2009). *E. coli* has been at the centre of many biological discoveries due

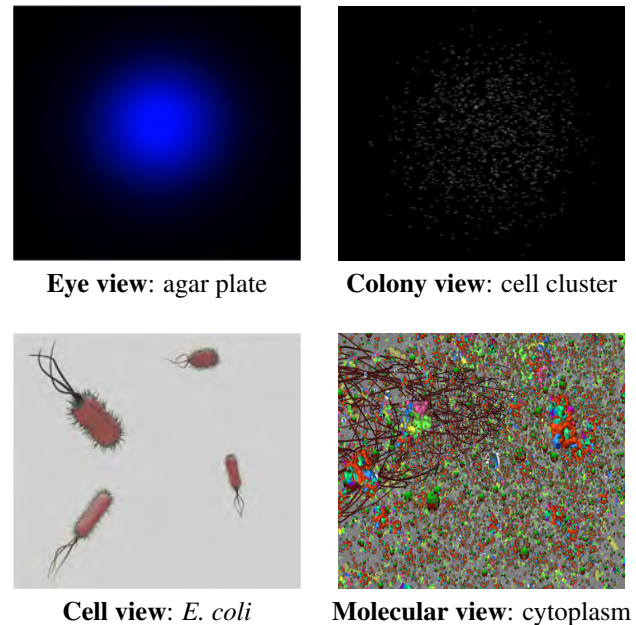


Figure 1: Snapshots of the four layers of resolution within our multi-scale *E. coli* model.

to its ease of growth and adaptability to different conditions and manipulation of its genome (Berg, 2004).

Chemotaxis is a universal attribute of motile cells and organisms. It is the mechanism that dictates their movement in the presence of a stimulus (Wadhams and Armitage, 2004). The stimulus—often chemical (hence “chemo”)—is either an attractant or a repellent (Berg, 2004). A cell like *E. coli* moves in the direction of the higher gradient towards an attractant source (Fig. 8A), thereby exhibiting a positive chemotactic response. Correspondingly, chemo-repellents cause the organism to turn away from the stimulus, thereby exhibiting a negative chemotactic response (Fig. 8B). Starting from a center point, a typical *E. coli* colony expands in an elliptical pattern known as chemotactic rings (Fig. 2).

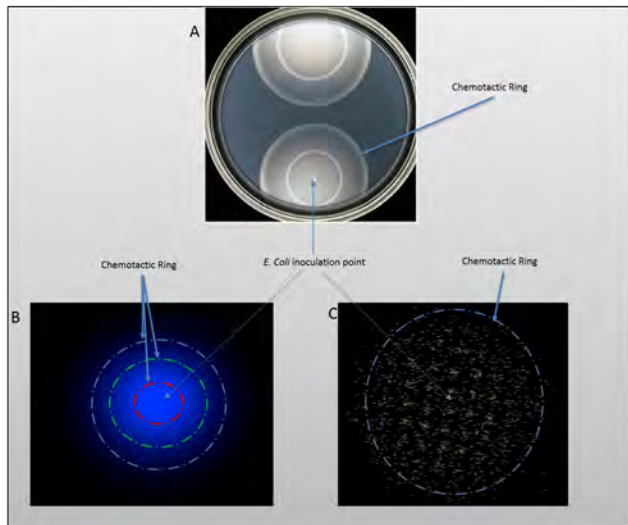


Figure 2: A) A tryptone soft agar plate in which motile cells can swim through water-filled tunnels in the agar. Two chemotactic colonies are shown. As the cells grow, they establish attractant gradients as they consume energy sources saturated in the agar. Printed with permission from Dr. John S. Parkinson Lab. B) Simulated chemotactic gradient in the agar layer. C) Closeup of the chemotactic ring formed by the simulated bacteria colony.

Chemotaxis Receptors

Bacteria use specific receptors to recognize chemical stimulants in their environment (Adler, 1966a,b, 1969, 1973; Adler et al., 1973). Five different receptors known as the methyl-accepting chemotaxis proteins (MCPs) play a key role in the signalling pathways (Berg, 2004): *Tsr*, *Tar*, *Tap*, *Trg*, and *Aer*. Each MCP detects a different chemical. MCPs are usually bundled into clusters at the poles of a bacterium (Adler, 1969; Sourjik, 2004). For simplicity, in our model we include one *generic receptor* which subsumes all MCP properties.

Chemotaxis Pathways

E. coli's chemotactic pathway is comprised of two distinct networks (Hauri and Ross, 1995): (1) the Signal Transduction Cascade, which leads to an *excitation* reaction, and (2) the Methylation Response, which results in *adaptation* (Berg, 2004; Hauri and Ross, 1995; Wadhams and Armitage, 2004). This chemotactic behaviour is a series of runs and tumbles the bacterium performs during its life span.

A bacterium is propelled by flagella filaments (Fig. 3) that extend from its cellular membrane (Alon, 2007). Each flagellum is controlled at its base by a motor inside the cell membrane. The direction in which the motors rotate determines whether the bacterium “runs” or “tumbles”. A *run* is defined by the bacterium swimming in a forward mo-

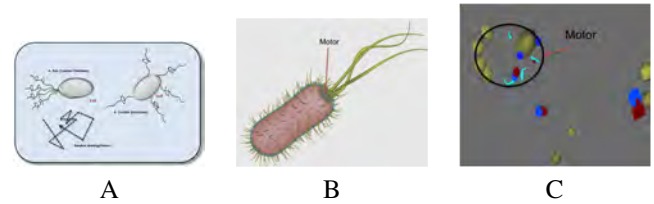


Figure 3: A) When the flagella filaments rotate counter-clockwise, they bundle together and propel the bacterium forward. A clockwise motor rotation makes the bacterium tumble, as the flagella bundles break up. B) Location of the motor unit in our *E. coli* model. C) The motor complex inside the modelled cytoplasm.

Protein	Role
CheA	Phosphorylation protein
CheB	De-methylation protein
CheY	Signal transmission protein
CheZ	De-phosphorylation protein
CheR	Methylation protein
CheW	Binds with CheA to form CheAW complex

Table 1: Proteins and their roles in the chemotaxis pathway

tion. When the motor rotates counter-clockwise, the flagella bundle together to propel the bacterium forward (Berg, 2004). During the *tumble* phase, the motor rotates clockwise (Berg, 2004), which causes the flagella to break from the bundle, resulting in a random change of direction. In the absence of any stimulus, runs and tumbles alternate, which leads to a random walk for the bacterium.

In the presence of a stimulus—whether it be an attractant or repellent—the rotation bias of the flagella is affected by the stimuli. Molecules of a stimulus are picked up by the receptors protruding from the cell membrane. Such a stimulus then triggers the excitation and adaptation response. *E. coli* is constantly comparing the concentration of its current location to its previous location, thus implementing a short-term “memory” that compares present and past information (Segall et al., 1986).

Table 1 summarizes the proteins involved in the chemotaxis signalling pathways in *E. coli*'s cytoplasm. Each protein plays a specific role in one of the two response networks.

Excitation Response: Signal Transduction Cascade

The excitation response directly affects the motion of the bacterium, where a series of signals is transferred downstream from the receptor to a flagellum motor (Fig. 4A). To keep our model simple, we assume that receptors can only be active or inactive. CheAW, a complex formed by CheA and CheW, is bound to the receptor end inside the cell membrane. With an increase in attractant concentration, the receptor becomes inactive, thus increasing CheY concen-

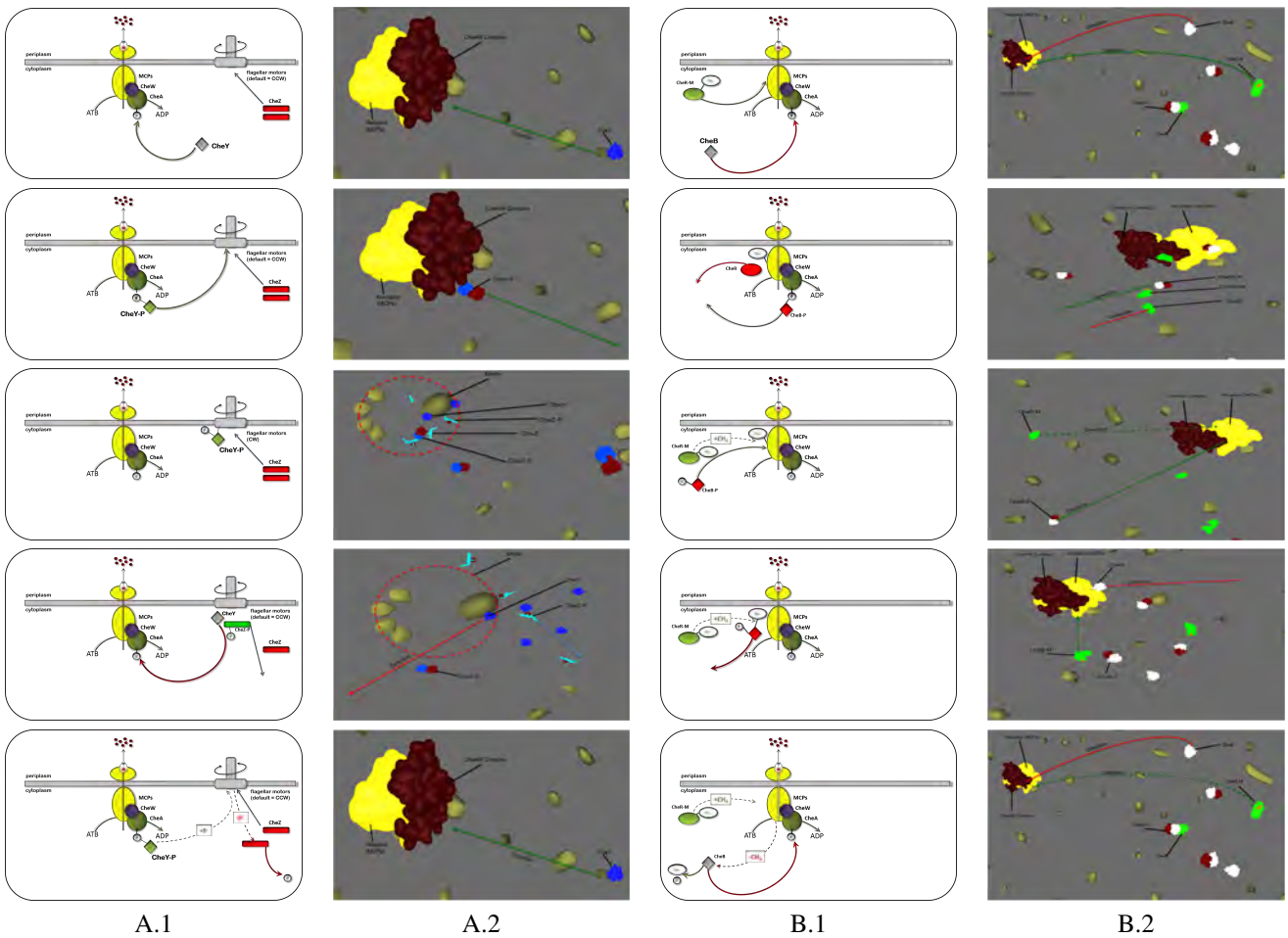


Figure 4: Chemotactic pathways: schematics and translations into our agent-based *E. coli* model. A.1) Excitation; A.2) agent interactions for excitation; B.1) Adaptation; B.2) agent dynamics during adaptation response.

tration, making the motor turn counter-clockwise, and suppressing the bacterium's tumble motion (Miller et al., 2010).

Active CheAW phosphorylates both CheB and CheY. CheB plays a role in adaptation (see below). CheZ's role is to de-phosphorylate CheY-P. The rotation of the flagella is dependent on the concentrations of CheY and CheY-P. A counter-clockwise rotation results from a higher CheY concentration. Likewise, motor rotation occurs in a clockwise fashion when CheY-P is present at a higher concentration than (unphosphorylated) CheY. Increasing attractant or decreasing the repellent concentration will bias the bacterium into swimming in smooth arcs by suppressing CheY phosphorylation, and thereby increasing the concentration of CheY. Similarly, an increase in repellent (or decrease in attractant) results in an increase of CheY-P, which leads to more frequent tumbling.

Adaptation Response: Methylation Adaptation is the process of the cell returning to its normal state of behavior (Fig. 4B). Without methylation, the cell would contin-

uously travel in a straight line regardless of the conditions. Methylation ensures that there is a recovery condition in the cell so that it may continue to query its vicinity for a more favourable location to travel towards.

Methylation involves the CheA-CheW complex again (CheAW), as well as CheR and CheB. CheB is phosphorylated by CheAW into CheB-P, which subsequently removes a methyl group from the receptor (Berg, 2004; Adler et al., 1973; Patnaik, 2007). This process is called de-methylation, after which CheB-P returns to its unphosphorylated state CheB. Regardless of conditions inside or outside of the cell, CheR keeps methylating the receptor, which reactivates it. Consequently, CheB-P and CheR alternate in deactivating and reactivating the receptors.

Models of Bacterial Chemotaxis

There are two key methods for modelling biological systems: mathematical or agent-based approaches. The method of choice depends on the system or process being modelled. In recent years, a new method known as hybrid modelling is

being utilized, which is precisely what we are using to create our chemotaxis multi-scale model. This method combines both mathematical and agent-based models and automatically switches between these two techniques as required.

Following a hybrid approach for our *E. coli* model, we use mathematical equations to track diffusion of attractants and repellents as well as the concentration of cells in an agar plate environment. In order to simulate single-cell behaviours and bio-molecular interactions inside a cell, we use an agent-based model, where we track movements and collisions of elements in 3-dimensional cytoplasmic space. In the following sections, we give a brief overview of related modelling work for *E. coli* chemotaxis.

Mathematical Models of Chemotaxis

Many models of chemotaxis in *E. coli* have been developed over the last few decades, which differ in their comprehensiveness and coverage of pathways (Fernando, 2005). Some models capture general principles, while others focus on particular pathway parts. In fine-tuned models, biochemical parameters are accurately replicated, whereas more robust models replicate a wide range of parameters (Alon, 2007).

In the 1970s, Keller and Segel (1971) proposed a mathematical model that was originally developed to analyze the movement of slime molds. Over many years, their model has served as the foundation for modelling chemotaxis at the population level. Knox et al. (1986) proposed a theoretical model for the adaptation process. This model has formed the basis for later theoretical work on adaptation response and is often replicated in fine-tuned models (Alon, 2007).

Bray et al. developed a computational chemotaxis model (Bray et al., 1993), the Bacterial Chemotaxis Program (BCT), which initially modelled only the excitation response. BCT later incorporated a more biologically accurate representation of the receptor complexes (Bray and Bourret, 1995), binding affinities were optimized by an evolutionary algorithm (Fernando, 2005), and incorporation of receptor clustering and sensitivity (Bray et al., 1998). Bray et al. also created *E. solo* (Bray and Lipkow, 2007), using ordinary differential equations to replicate the signaling reactions in the pathway. *E. solo* provides a graphical display of bacterial movement in a 2D environment.

Barkai and Leibler (1997), presented a model for the adaptation response in chemotaxis, which takes a wide range of possible values for biochemical parameters into account. Their model includes several methylation sites, and reproduces many observations on the dynamical chemotactic behaviour of cells (Barkai and Leibler, 1997). Using a three-component model they showed that the adaptation process is robust rather than fine-tuned (Alon, 2007).

In 1999, the BCT team and Morton-Firth et al. developed *StochSim*, the first stochastic simulation model of bacterial chemotaxis (Morton-Firth et al., 1999). *StochSim* incorporates both excitation and adaptation responses. *Smoldyn*, an

extension of *StochSim*, simulates cell-scale biochemical reactions to capture natural stochasticity data. The program was developed to provide a more realistic way to simulate the diffusion of signaling molecules through the cytoplasm (Andrews and Bray, 2004). *StochSim* was further expanded by Emonet et al. (2005) to develop *AgentCell*, which utilizes agent-based modelling to represent chemotactic responses at the population and single cell level, simulated independently. *AgentCell* accurately reproduces validated results under both stimulated and unstimulated conditions.

A more recent framework to specify and simulate micro-colony growth and molecular signaling for synthetic biology applications was developed by Jang et al. (2012).

Agent Based Models (ABM)

In agent-based approaches one simulates the interactions of elements (“agents”) with other elements and their environment. These interactions often give rise to complex patterns, referred to as emergence (Macal and North, 2005; Bonabeau, 2002). Emergent properties are often not identifiable by looking at the individual agents, but evolve from interactions between agents, as described by Ginovart et al. (2002) for discrete simulations of bacterial cultures .

Most modelers consider any independent component (software, object, model, etc.) with some sort of defined (programmed) behaviour rules to be an agent. The behaviour can range from primitive reactive protocols to adaptive intelligence programs (Mellouli et al., 2003). Berry (1997) proposed that an agent should contain both base-level rules for its behaviour and higher-level protocols to “change the rules” (adaptive intelligence). The base-level rules provide a reaction to the environment, whereas the higher-level protocols provide adaptation to the environment. This is the agent definition we have followed in our *E. coli* model.

Hybrid Chemotaxis Models

The use of hybrid models, which combine mathematical modelling with ABM techniques, is becoming widespread especially with the growth in computational power. This allows for more complex systems to be modelled and simulated such as biological systems and their cellular processes. Hybrid modelling has been applied for tumor growth (Patel et al., 2001) and forest dynamics (Landsberg and Waring, 1997). Hybrid models have been applied for chemotaxis in slime molds (Dallon and Othmer, 1997) and bacteria (Fernando, 2005).

A Hybrid, Multi-scale Model of Chemotaxis

As an extension of *Prokaryo*, a hybrid model of prokaryotic gene regulation (Esmaeili et al., 2015), we have developed a generalized model of *E. coli* chemotaxis. The model captures the key attributes and characteristics of the chemotaxis pathways which control the locomotion of bacteria in a simulated agar. The mathematical model handles all of the

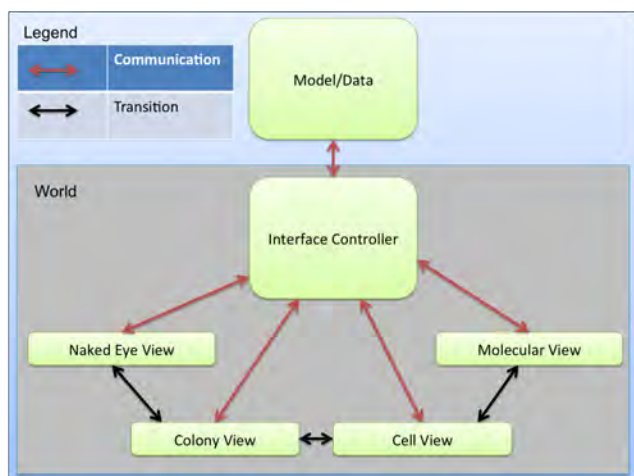


Figure 5: Software architecture and communication hierarchy between the components in the *E. coli* model.

calculations related to intra-cellular and extra-cellular signals as well as concentrations of molecules. The ABM handles interactions among cells (as individual agents on the agar) and among molecules in the cell's cytoplasm. By zooming in and out of the 3D scenario, the scales for the model and its visualization automatically transition between four layers (Fig. 1), which includes switching from ABM calculations to the mathematical model (and back). The mathematical model is always executed in the background; this ensures that information is shared between the layers.

Model Architecture

We have used the latest version of our *LINDSAY Composer 2.0* agent simulation software (Jacob et al., 2012) to implement the multi-scale *E. coli* model. *LINDSAY Composer* provides 3D simulation, including physics and graphics engines, camera navigation, interactive parameter manipulation, scene hierarchies, and an object-oriented programming environment. The architecture of our model consists of the following components (Fig. 5):

Model Data: Simulation data is stored and shared among the layers through this module, which is only accessible through the Interface Controller.

Naked Eye Layer: In this top-level view, concentrations of chemicals (attractants or repellents) as well as colony distribution is visualized by color gradients, which are drawn onto the grid surface that represents the agar environment. Algorithm 1 is used to update the grid.

Colony Layer: In this layer, *E. coli* cells and stimuli are represented through particle systems, which are used to generate the illusion of thousands of cells and molecules in the environment. Movement of the particles is governed by Algorithm 1.

Cell Layer: In order to reach this level, a single cell has been selected on the Colony Layer. An *E. coli* cell is represented as an agent with its own distinct properties. The cell moves based on the other agents and the stimuli in the simulation at each time step.

Molecular Layer: The lowest layer of our simulation captures the molecular details and interactions of the chemotaxis pathways. Molecules and proteins are modelled as individual agents that interact with other agents and the environment.

Simulation Engine: The Interface Controller and model layers are embedded in the simulation environment. Transitions only occur between connected layers.

Interface Controller: The interface controller relays interactions from the user (gestures, mouse clicks and movements, parameter manipulations) to the model layers and the data module.

Model data: The model data is situated in a separate module and shared among the layers by communicating through the Interface Controller.

Algorithm 1 Update of Petri Dish and Colony Layer

```

CREATE:
Initialize 2D grid cells
attractant := 0; repellent := 0; ecoli := 0

ITERATE:
for all gcell ∈ Grid do
  for all egcell ∈ {attractant, repellent, ecoli} do
    if |egcell| ≠ 0 then ▷ Cell contains element(s)
      Apply Gaussian algorithm ▷ Diffusion
      Update gcell
    end if
  end for
  if layer == PetriDish then ▷ Petri dish layer
    isColourCell := true
    Apply colour visuals to gcell
  else ▷ Colony layer
    isParticlesCell := true
    Generate particles for gcell
  end if
end for

```

Molecular Agents

All protein models in our simulation have been extracted from online protein databases. We have listed the 3D shapes and the PDB IDs of our bio-molecular agents in Figure 7. Recall the role of each agent from Table 1. In this paper, we only have space to illustrate two agents and how we have implemented their behaviour rules. More information is available on our project website (LindsayVirtualHuman.org).

CheY protein is phosphorylated by the CheAW complex (Algorithm 2). CheY-P will dock onto the motor unit. Upon

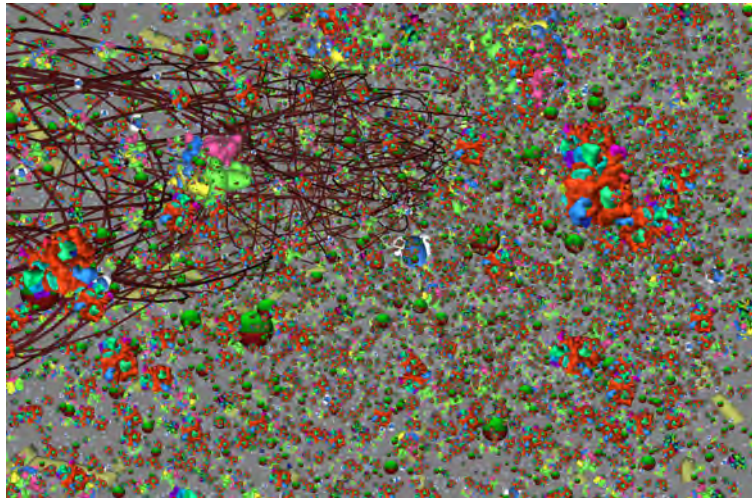


Figure 6: A glimpse of the full-scale simulation inside the *E. coli* cell's cytoplasm.

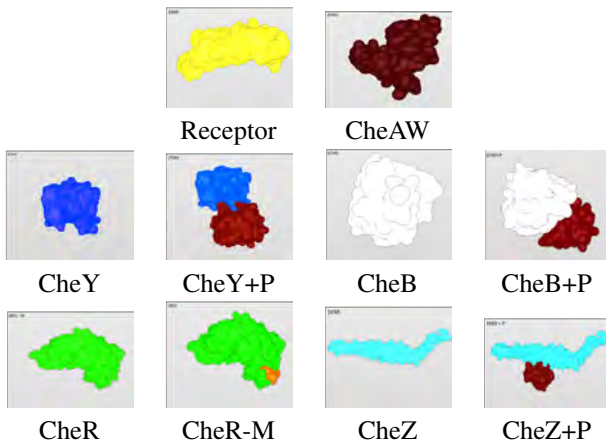


Figure 7: 3D structures of the protein agents in our chemotaxis model as used in the cytoplasm layer (Fig. 6). The meshes were imported using the associated PDB Ids from the Protein Databank (www.wwpdb.org).

collision with CheZ, CheY-P gets de-phosphorilized and follows an attractive force towards the CheAW complex, which will start the interaction loop again.

CheZ is set to be attracted to the motor complex (Algorithm 3). CheZ performs a random walk in the vicinity of the motor complex, checking for collisions with phosphorylated CheY. Upon collision with a CheY-P agent, CheZ removes the phosphate group from CheY, and subsequently enters a short period of inactivity.

An impression of what the simulation looks like—with all molecular agents, including water and lactose, interacting inside the cytoplasmic space—is depicted in Figure 6.

Algorithm 2 CheY

```

1: CREATE:
2: set state to RandomWalk

3: ITERATE:
4: if CheY is Active then
5:   if CheY is not phosphorylated then
6:     if collided with CheAW complex then
7:       set state to phosphorylated
8:       set agent conformation to CheY-P mesh
9:       set attraction to Motor
10:    else
11:      set state to RandomWalk
12:    end if
13:  else ▷ CheY is phosphorylated
14:    if collided with Motor then
15:      set state to BoundToMotor
16:      clear movement velocities
17:      if collided with CheZ then
18:        set state to not phosphorylated
19:        set agent conformation to CheY mesh
20:        set attraction towards CheAW Complex
21:      end if
22:    end if
23:  end if
24: else
25:   set state to RandomWalk
26: end if

```

Simulation Results

We have been using results from our simulations throughout the illustrations in this paper. Starting with our Eye and Colony view, we can see that in an environment such as

Algorithm 3 CheZ

```
1: CREATE:
2: set state to RandomWalk, Active
3: set attraction towards Motor
4: set timer to 100                                ▷ Start timer

5: ITERATE:
6: if CheZ is Active then
7:   if CheZ collided with CheY-P then
8:     remove P from CheY-P
9:     set CheZ to  $\neg$ Active
10:  end if
11: else
12:   timer := timer - 1
13:   if timer == 0 then
14:     set CheZ to Active
15:     timer := 100                                ▷ Restart timer
16:   end if
17: end if
```

an agar plate, with no external stimulus, chemotactic rings are formed (Fig. 2B,C), similar to a wet-lab experiment of *E. coli* growing in a tryptone soft agar plate.

Placing an attractant stimulus in the environment, our simulated *E. coli* cells grow and move towards the origin of attraction (Fig. 8A). Similarly, with a repellent stimulus the colony moves away from the repelling source (Fig. 8B).

In single cell view, we see an *E. coli* bacterium following a gradient or performing a random walk in the absence of a stimulus (Fig. 9).

On the molecular interaction level, we have replicated the different interaction phases of the chemotaxis pathway. Figure 4 illustrates this with a side-by-side comparison of the pathway diagrams and their replication in our agent-based model, where the agent behaviours are driven by short code scripts, such as the examples of Algorithms 2 and 3.

Conclusion

We have introduced a multi-scale, hybrid model that replicates and illustrates chemotaxis of *E. coli* bacteria. We have implemented abstractions of chemotaxis on four levels of detail: from the naked eye and colony level down to single cells and their cytoplasm. Our model system is interactive, provides 3D visuals, and can serve as a tool to learn about and explore behaviours in biological systems arising from the interactions of many constituents across a range of scales of resolution. More information about this system and related simulations can be found on the LINDSAY Virtual Human web site.

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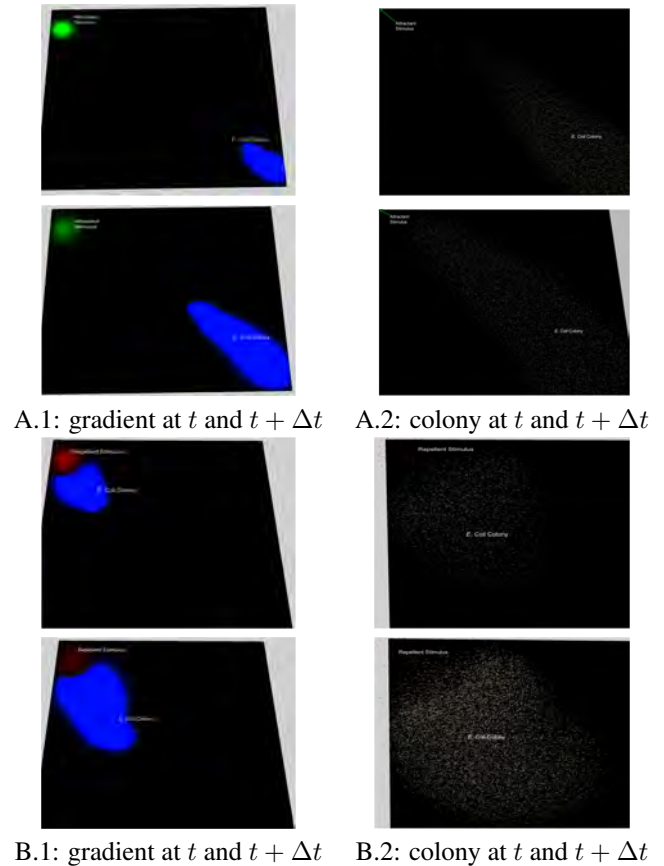


Figure 8: Modeled chemotaxis: Movement toward an attractant (A) and away from a repellent stimulus (B). The left column shows colony movement (blue), repellent (red) and attractant (green); bacterial colony closeups on the right.

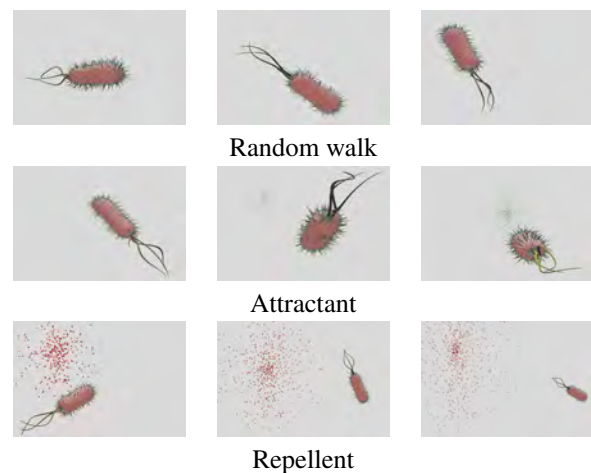


Figure 9: *E. coli* as a single agent in agar environment.

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