Modelling the chemotactic response of escherichia coli to signalling molecule AI-2

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**MODELLING THE CHEMOTACTIC RESPONSE OF
ESCHERICHIA COLI TO SIGNALLING MOLECULE AI-2**

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Interdisciplinary Graduate School
NEWRI

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TAN PEI YEN

**Interdisciplinary Graduate School
NEWRI**

A thesis submitted to the Nanyang Technological University in partial
fulfillment of the requirement for the degree of
Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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Marcos

Authorship Attribution Statement

This thesis contains material from 1 paper(s) accepted for publication in the following peer-reviewed journal(s) where I was the first and/or corresponding author.

Chapter 3 is accepted for publication to the Journal of Theoretical Biology as P.Y. Tan, Marcos, Y. Liu. Modelling bacterial chemotaxis for indirectly binding attractants.

The contributions of the co-authors are as follows:

- I prepared the manuscript drafts. The manuscript was revised by Asst. Prof Marcos and Prof. Liu.
- I co-designed the study with Asst. Prof Marcos and Prof. Liu. and performed all the simulation work at the School of Mechanical and Aerospace Engineering. I also analyzed the data.

A handwritten signature in black ink, appearing to read 'PY Tan', is positioned above the printed name.

31/07/2019

TAN PEI YEN

Abstract

In this thesis, our focus is the development of indirect binding models to predict the chemotactic response of *E. coli* to the signaling molecule AI-2. Our first model is developed from first-order binding kinetics to represent the interactions between the attractant molecule and the periplasmic binding protein, as well as between the ligand-binding protein pair and the chemoreceptor. The model accounts for the effects of the dissociation constants characterizing each binding interaction, as well as the crucial effect of the inclusion of the periplasmic binding protein concentration. The first-order model is validated through simulations of the capillary assay popularized by Adler and compared with existing experimental results in the literature. The predicted chemotactic response from our model is first compared with experimental reports of maltose chemotactic response as the maltose response is well-studied and there exists a wealth of data in the past literature. Our model achieves good agreement with experimental data for maltose at various levels of periplasmic binding protein concentration as well as extracellular attractant concentration. Moreover, the model is consistent with experimental results of multiple studies for one universal set of kinetic and chemotaxis parameters, lending further support to the accuracy of predictions made.

Our next study involves the parametric analysis of the first-order model to better understand strategies pertaining to the indirect binding mechanism. It appears that the advantage of the indirect binding mechanism can be observed in nutrient-scarce conditions, as a bacterium is predicted to be able to ramp up the magnitude of chemotactic response and shift the sensitivity profile towards dilute attractant concentrations by raising the concentration of periplasmic binding protein moderately. In the third and last study presented in this thesis, we extend the indirect binding model to develop a higher-order model based on specific considerations for the AI-2 system. As the concentration of periplasmic binding protein is much lower than receptor concentration in the case of AI-2, there is a high possibility of ligand depletion, making it necessary to utilize the exact equilibrium balance for the total periplasmic binding protein. The higher-order model exhibits greater agreement with existing experimental data as compared to the earlier first order model. Moving forward, we believe it necessary to develop experimental approaches to aid in model validation, identifying microfluidics as a possible future direction.

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To Yvonne for being my universal constant and always scaling my responses to more physiologically appropriate levels,

To my family for the support and care that you have always shown,

And lastly to Frank for reasons that are difficult to quantify.

Nomenclature

Abbreviations

ABC	ATP-binding cassette
AI-2	Autoinducer-2
ALU	Arbitrary luminescence units
AMC	Activated methyl cycle
ATP	Adenosine triphosphate
BP	Binding protein
P-AI-2	Phosphorylated AI-2

Variables and parameters

$AI2_{in}$	Concentration of intracellular AI-2
B	Bacterial density field
b_{max}	Empirical value for baseline concentration of LsrB
b_{min}	Empirical value for fully induced concentration of LsrB
C	Attractant concentration field
D	diffusion coefficient or diffusivity
J_x	cell flux in 1D
K_d	dissociation constant between attractant (ligand) and chemoreceptor
K_{dp}	dissociation constant between attractant and periplasmic BP
K_{dr}	dissociation constant between attractant-BP complex and chemoreceptor
K_M	Michaelis constant
k_{on}	characteristic association rate
k_{off}	characteristic dissociation rate
n^+	density of cells travelling in 1D towards the right
n^-	density of cells travelling in 1D towards the left
P_b	Fraction of total chemoreceptors bound to ligand
P_t	Total periplasmic binding protein concentration

p^+	probability of cell from n+ population changing to opposite direction
p^-	probability of cell from n- population changing to opposite direction
R_b	Concentration of bound chemoreceptors
R_t	Total chemoreceptor concentration
r_0	translocation rate
r_{max}	maximum reaction rate
T_p	persistence time required for cell flux to approach steady state
V_0	cell swimming velocity
V_C	Chemotactic velocity
β	scaling factor between extracellular and periplasmic attractant concentration
μ	random motility coefficient
σ	chemotactic sensitivity coefficient
τ	mean duration of a straight run
τ_0	mean duration of a straight run in absence of chemoattractant
χ	chemotaxis coefficient

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Chapter 1

Introduction

The ability of simple single-celled organisms such as bacteria to self-organize and engage in coordinated population scale behaviour is surprising, to say the least. Examples of such actions include the formation of regular and intricate patterns on solid and semi-solid agar, such as rings of dots or even a clover-like structure [1]. From an ecological perspective, coordinated bacterial behaviour includes chemotaxis, quorum sensing, self-aggregation, biofilm formation and interspecies signalling. It is not certain if all observed population-scale behaviours are the result of intentional communication amongst members of the population. For example, each cell can perform an independent chemotactic response towards a nutrient source without taking the responses of other nearby cells into account. Thus, the net movement of cells along a chemical concentration gradient may not be the result of a coordinated intercellular response. In this case, the population-scale behaviour, at times resembling a travelling wave [2, 3], may simply represent the average of each individual chemotactic response.

Recent evidence suggests, however, that the existing signalling and communication framework is necessary for *Escherichia coli* to perform at its best in the tasks of biofilm formation [4] and aggregation [5]. Not surprisingly, it was found that the ability to enact a chemotactic response is also related to the optimal expression of population scale behaviours. It would seem that the common thread binding these key phases in the life cycle of *E. coli* is the ability to respond effectively to signalling molecules that are present in the environment.

The ecological niche of *E. coli* varies from the rich human gut to the nutrient-poor aqueous environments existing outside the human body [6]. As the behaviour of the bacterium changes significantly depending on its current environmental conditions, we specify here that our interest lies in the latter case. *E. coli* is more motile and active in nutrient-scarce conditions due to the need to ensure its survival. By understanding the strategies undertaken by this model species to search for nutrients and build up a biofilm “safe-zone”, we place ourselves in a better position to expand our analyses to the larger microbial community.

Going on a tangent, we note that robust modelling and simulation options exist for the prediction of many physical phenomena, including fluid and heat transfer, electromagnetics, chemical reactions, and structural mechanics. Given the predictable nature of microorganisms that do not possess free will and thus must act deterministically, it is curious that modelling is not as well developed in this area. The difficulty in modelling accurate bacterial behaviour appears to stem from stochastic effects; two similar looking cells may react differently to stimuli, depending on fluctuations in intracellular protein concentrations between cells. The presence of randomness in most aspects of bacterial behaviour means that it is nearly impossible to define a standard quantitative response for a given bacterial species to a corresponding stimulus.

However, by modelling cells at the population scale, it becomes possible to average out these relatively small differences in parameters possessed by individual cells. In the formulations of Keller and Segel [7], Rivero et al. [8] and Ford et al. [9], populations of bacteria can be represented as continuous bacterial density fields that undergo diffusion and advection even in the absence of fluid dynamics. Therefore, it appears that theoretical models at the population scale are invaluable for managing the chaos and computational expense that comes from tracking individual cells and the myriad interactions that they must experience. Thus far, the diversity of bacterial and microbial species in real biological samples, such as those found in membrane bioreactors [10], defy modelling and quantification. In time, it may even be possible to develop an equivalent representation for a mixture of dissimilar species as a continuum field of cells that perform a consistent and repeatable function at the population level.

Currently, such a goal remains far off, but has its roots in the accurate modelling of single-species population dynamics for model species such as *E. coli*. In our study, we have identified that the population behaviour of *E. coli* can be strongly influenced by the chemotactic response to signalling molecule AI-2. In the literature review, we discuss the various interactions between *E. coli* and AI-2, which include synthesis and export, uptake as well as chemotaxis. We realize that existing bacterial transport models are developed based solely on considerations that attractant molecules bind directly to the cognate chemoreceptor. In the case of AI-2, however, the attractant first binds to an intermediate periplasmic binding protein called LsrB, before binding as a pair to the receptor Tsr. Current models are thus

incompatible with the increased number of reactions and thus dissociation constants and other parameters necessary to predict the chemotactic response for indirectly binding attractants.

We close the literature review by presenting the research objectives of this thesis. There, we reiterate where gaps in the research topic lie as well as our strategy for navigating the greater topic and the overarching goal. Briefly, we approach this research topic with the future goal of developing predictive techniques for bacterial behaviour that can accurately be applied in engineering design and prototyping, such as medical equipment or wastewater treatment structures. Therefore, we tackle the fundamental component to population scale behaviour in this thesis by seeking to develop models for the accurate representation and understanding of the chemotactic response of *E. coli* to AI-2.

In Chapter 3, we formulate a first-order indirect binding model that allows us to evaluate chemotactic response as a function of the dissociation constants between ligand, binding protein and receptor, as well as the binding protein concentration. The model is first validated on a well-studied indirectly binding attractant, maltose, achieving good agreement with experimental data obtained from numerous studies. We note that the agreement with multiple studies can be achieved without any adjustment of parameter values to fit each individual data set, lending further confidence to the model accuracy. Simulating the AI-2 response, we achieve an equally good agreement with one out of two chemotaxis studies, but not with the second.

In Chapter 4, we conduct further parametric studies on the indirect binding expression developed in the previous chapter, in an attempt to better visualise how chemotactic response varies specifically with changes in attractant concentration. From this study, we believe that the variable level of periplasmic binding protein may assist the bacterium in maximising sensitivity to dilute concentrations of nutrient by raising the binding protein concentration.

In Chapter 5, we develop an advanced higher-order indirect binding model to address potential concerns with ligand depletion in the case of AI-2. Unlike maltose, the AI-2 periplasmic binding protein concentration is small compared to the receptor concentration, leading to a likely scenario of binding protein depletion. In this case, first-order kinetics do not apply. We find that the advanced model gives a visibly better prediction of the same set of experimental data that we initially tested in Chapter 4. We also present an exploratory attempt at bringing AI-2 chemotactic behaviour and uptake kinetics together to evaluate the

effects on predicted chemotactic response in a simulated capillary assay. The scarcity of experimental data in the existing literature prompts us to begin planning for a joint modelling and experimental approach in order to move our research forward, as detailed in the Conclusions and Future work.

Chapter 2

Literature Review

2.1 *Escherichia coli*

Escherichia coli is a Gram-negative, peritrichous bacteria [1] that swims through fluid with the aid of a small number of helical flagella [11-13] that rotate through the action of proton motors embedded in the cell wall [14]. It is a well-known model organism that has been used in the study of microbiology, genetics, virulence [15] and biofilm formation [16], to name just a few. The wealth of knowledge currently amassed regarding so many aspects of this organism makes it the obvious choice for many in both modelling and experimentation. Similarly, we opt to base our studies on *E. coli*, taking it as a general representation of the mechanisms that govern bacterial chemotactic and signalling strategies [17].

E. coli moves in a run-and-tumble fashion that has been characterized extensively by Berg [18] and colleagues [19, 20]. Using a custom-built 3D-tracking microscope, they observed that *E. coli* tends to swim in a nearly straight path for a mean duration of one second in the absence of chemotactic stimuli. Following a straight run, the cell then undergoes a sudden and random reorientation in swimming direction, described as a “tumble”, after which straight-swimming resumes. This behaviour happens in a fashion that is surprisingly consistent and quantifiable, with tumbles occurring on an average of once per second [20] and each time lasting for about a tenth of that duration. At the population scale, these motions are known as random motility and serve to disperse cells homogeneously throughout a region of space in a manner analogous with pure molecular diffusion [7, 9, 21]. The bacterium is able to travel in a straight line during runs by coordinating the direction of rotation of each of its flagella, which rotate counter-clockwise [1] and come together to form a tight bundle [22, 23]. On occasion, one or more of the flagella have been observed to unravel from the bundle and spin in the clockwise direction instead. This action causes an instant reorientation of the cell body as it tumbles on the spot. After a relatively short period of time, the errant flagella resume counter-clockwise rotation, re-joining the bundle and allowing the cell to resume smooth swimming.

At all times, each chemoreceptor phosphorylates a protein called CheY at a certain rate, producing “phospho-CheY” or “P-CheY”. The P-CheY molecule diffuses throughout the cell and binds transiently to any flagellar motor [24]. This binding event causes the motor to switch momentarily from the CCW rotation to CW rotation [1], causing the cell to tumble as the flagellar bundle comes apart. Another enzyme responsible for degradation of P-CheY allows it to be maintained at a steady concentration, resulting in a constant probability for at least one flagellar motor to be bound with P-CheY at any point in time. Thus, the random motility of *E. coli* in the absence of attractant or other stimuli is governed by the constant rate of phosphorylation of CheY by the total number of chemoreceptors present. With this brief introduction to the fundamental behaviour of *E. coli*, we move on to discuss how the pattern of run-and-tumble and random motility is altered by the presence of attractants due to the phenomenon called chemotaxis.

2.2 Chemotaxis

Single-celled organisms such as bacteria navigate their surroundings through the use of simple yet sophisticated sensory systems, responding to various positive and negative stimuli such as heat, light, and chemical attractants or repellents. These are collectively referred to as tactic responses and include chemotaxis [1, 24], thermotaxis [25], phototaxis and rheotaxis [26, 27]. Chemotactic behaviour is governed by the five main receptors in *E. coli*, which are Tar, Tsr, Trg, Tap and Aer [28]. As touched upon in the previous paragraph, the receptors function similarly using a largely conserved series of Che- family proteins that are named for chemotaxis. When a ligand binds to its cognate receptor, it induces a conformational change in the structure of the receptor that then causes the rate of formation of P-CheY to fall. Another protein, CheZ, catalyses the dephosphorylation of P-CheY, meaning that the overall concentration of P-CheY quickly diminishes when the balance with the rate of formation is disturbed. In this manner, tumbling is suppressed when attractant binds to a receptor.

The chemotaxis system of *E. coli* and other bacteria is surprisingly sophisticated in its simplicity. Due to a phenomenon called “signal adaptation” [29], the bacteria are able to ensure that the chemotactic response is only activated in the presence of an attractant concentration gradient, but not in the presence of a homogeneous concentration field. Essentially, adaptation causes the level of activity of the receptor to return to baseline if the

sensed attractant concentration does not change over a period of time. This causes the suppressed rate of tumbling to return to normal frequency as well. When a receptor is bound by an attractant molecule, it undergoes a temporary conformational change in its protein structure, which allows it to become methylated at a number of “adaptation sites”. The effect of this adaptation is opposite from the effect of an attractant molecule binding to the chemoreceptor, resulting in a “de-suppression” of tumbling frequency. This methylation continues to increase over the duration that the receptor remains bound by the ligand, stopping when the level of receptor activity decreases to its base value. The methylation level is not able to fully “catch up” with the effect of attractant binding to the chemoreceptor in the case where the attractant concentration is continually rising, indicating that the cell is swimming in a direction of increasing attractant. In this manner, the bacterial chemotaxis system is able to discriminate between a homogeneous region of high attractant concentration and an attractant concentration gradient, so as to only react to the latter.

In general, the function of the chemotactic apparatus in *E. coli* is well understood after years devoted to its study. Theoretical models are employed to translate individual cell behaviours into predictions regarding the net movement of cell populations. The net result of random reorientations and extended runs in the direction of an attractant source culminates in an advective flux of the cell population as derived in the work of Keller and Segel [7], Ford [9, 30] and Rivero et al. [8], amongst others. This advective term works together with diffusion due to random motility in a manner analogous with molecular and fluid transport.

2.3 Theoretical model for chemotaxis

A large number of mathematical models have been developed in the study of bacterial chemotaxis, from various angles of approach. The models can be broadly classified into either the agent-based approach [31-35], which takes the single-cell perspective [36], or the population-based approach [7-9, 21, 37-40], which views bacterial transport from a field perspective. The bacterial field concept has been used in the development of travelling wave solutions describing the collective behaviour of cells [41, 42]. The most general form of the bacterial transport equation is analogous to the equation for molecular transport, with chemotactic drift represented as an advective term that is attractant concentration-driven rather than flow-driven.

Tindall et al. [39] collated many mathematical models developed prior to 1990, which include the general transport model formulated by Keller and Segel [7, 43], as well as models of greater complexity that were formulated by Alt [21] and Othmer et al. [37]. More recently, Hillen and Painter [40] conducted an extensive study on Partial Differential Equations (PDEs) for bacterial transport, based on the Keller-Segel model, which focused on the various forms of diffusive, advective and source/sink terms that exist in the literature. In particular, multiple expressions for the chemotactic drift velocity term have been proposed, primarily as functions of local attractant concentration and concentration gradient.

The Rivero model of chemotaxis [8] has previously been applied in quantification of experimental data [44] and has been shown to agree well with experimental results. The model links the average population-scale chemotactic drift velocity with a few key parameters including instantaneous chemoattractant concentration and concentration gradient, binding affinity between substrate and chemoreceptor, average individual cell swimming velocity, and a chemotactic sensitivity coefficient. In particular, the chemotactic sensitivity coefficient serves as a measure of the strength of chemotactic response for a bacteria-chemoattractant pair [44, 45].

The model first defines, in one dimension, the densities of cells travelling toward the right and left as n^+ and n^- , respectively. Assuming a constant average swimming velocity V_0 of cells swimming in either direction, the flux expression is obtained as $J_x = V_0(n^+ - n^-)$. This approach favours simplification of the mathematical complexity as well as the fundamental physical picture describing cell flux, at the cost of ease with which the model can be extended into higher dimensions.

Defining conservation equations for the two cell populations n^+ and n^- :

$$\frac{\partial n^+}{\partial t} + \frac{\partial}{\partial x}(V_0 n^+) = p^- n^- - p^+ n^+ \quad (2.1)$$

$$\frac{\partial n^-}{\partial t} - \frac{\partial}{\partial x}(V_0 n^-) = p^+ n^+ - p^- n^-, \quad (2.2)$$

An equation describing the time rate of change of the one-dimensional bacterial flux J_x is obtained by taking the difference between the two equations:

$$\frac{\partial J_x}{\partial t} - V_0^{-1} J_x \frac{\partial V_0}{\partial t} = -J_x(p^+ + p^-) - V_0 \frac{\partial}{\partial x}(V_0 c) - V_0 c(p^+ - p^-) \quad (2.3)$$

By assuming that $\frac{\partial J_x}{\partial t} = 0$ and making J_x the subject, a new flux expression can be obtained:

$$J_x = -\mu \frac{\partial c}{\partial x} + V_c c - \frac{1}{2} \left[\frac{\partial \mu}{\partial x} - \mu \frac{\partial (\ln T_P)}{\partial x} \right] \quad (2.4)$$

where

$$T_P = \left[(p^+ + p^-) - V_0^{-1} \frac{\partial V_0}{\partial t} \right]^{-1} \quad (2.5)$$

is described as a persistence time relating to the time required for cell flux to approach a steady state value,

$$\mu = T_P V_0^2 \quad (2.6)$$

is the random motility coefficient and

$$V_c = T_P V_0 (p^- - p^+) \quad (2.7)$$

is the chemotactic velocity term.

Rivero et al. develop functional forms of the random motility coefficient and chemotactic velocity term for bacterial chemotaxis, by relating the tumbling probabilities p^+ and p^- to changes in chemoreceptor occupancy. Our subsequent formulation of the indirect binding chemotaxis mechanism builds upon the specific form of the Rivero chemotactic velocity term alone while treating the random motility coefficient as an empirical parameter.

The general mechanism behind the bacterial random walk is taken into consideration such that the average cell swimming velocity is independent of the attractant concentration and cells are assumed to have an equal probability of switching to either direction. In doing so, the persistence time is simplified to $T_P = (p^+ + p^-)^{-1}$ and the chemotactic velocity term becomes

$$V_c = V_0 \frac{(p^- - p^+)}{(p^- + p^+)} \quad (2.8)$$

The probability of changing direction is related to the probability of tumbling p_t such that $p^+ = p_t^+ (1 - \Psi)/2$ and $p^- = p_t^- (1 - \Psi)/2$, where Ψ is the directional persistence. The findings of Brown and Berg [19] are applied, relating the cell mean run time to the time rate of change of receptors bound:

$$\ln \tau = \ln \tau_0 + \sigma \frac{dR_b}{dt} \quad (2.9)$$

where τ is the cell mean run time and the reciprocal of the tumbling probability, τ_0 is the mean run time in absence of attractant, σ is the chemotactic sensitivity parameter and R_b is the number of bound receptors expressed in units of molar concentration. Following a series of algebraic manipulations, the final expression for chemotactic velocity is obtained:

$$V_c = V_0 \tanh \left[\sigma V_0 \frac{dR_b}{dC} \frac{dC}{dx} \right] \quad (2.10)$$

Thus far, the bound receptor concentration has been expressed using the first-order kinetic equilibrium as a function of the total receptor concentration R_T , the free ligand concentration, approximated as the total extracellular ligand concentration C , and a characteristic dissociation constant K_d between the receptor and ligand binding pair. However, this simple model does not fully account for a subset of attractants that bind indirectly to the receptor through the activity of a periplasmic binding protein, which may also be involved in uptake of the attractant substrate.

2.4 Direct and Indirect binding mechanisms in the periplasm

The significance of direct versus indirect ligand binding in chemotaxis has previously been discussed [46] but has not been extensively studied. For direct binding, attractant molecules entering the periplasmic space of the cell interact directly with the membrane-spanning chemoreceptor proteins. On the other hand, indirectly-binding attractants interact only with free-roaming binding proteins (BPs) located in the periplasm. The attractant-protein complexes are then able to bind with chemoreceptors and thus trigger the chemotactic response. Indirect binding appears to provide the cell with better control over the chemotactic response by varying the number of available BP molecules [4], with overall response being highly sensitive to expression levels of BPs [46], in exchange for a lowered dynamic range of response. Furthermore, some periplasmic binding proteins appear to serve concurrently in chemosensory and transport roles as components of ATP-binding cassette (ABC) transporters, including the maltose [47] and AI-2 [48] binding proteins. While this clearly allows for coordination in the uptake and chemotactic responses, the specific strategies which result or benefit from this pairing have yet been fully understood.

While fewer attractants require a mediating binding protein in order to be sensed by *E. coli*, one particular molecule of interest does. The signalling molecule AI-2 binds to a periplasmic binding protein LsrB, the complex of which is then sensed by binding to the Tsr receptor. Certain behaviours revolving around AI-2 are conserved in many species of Gram-negative bacteria, lending it the distinction of having many possible roles in the bacterial ecology. These include the role of AI-2 as simply a metabolic by-product, a quorum sensing and intercellular signalling molecule, or a nutrient source, although this last point is debatable [49].

2.5 Autoinducer-2 (AI-2)

The bacterial signalling molecule AI-2 has been widely studied due to its unique role as an inter-species communication mechanism [17, 48-54]. It was first discovered in *Vibrio harveyi*, a bioluminescent marine bacterium [55, 56], for which its synthase LuxS was named. Over time, it was found that LuxS could be identified in diverse species of bacteria [57], including *Escherichia coli* and *Salmonella typhimurium*. Additionally, the role of LuxS in central metabolism as part of the activated methyl cycle (AMC) was also brought to light [58-60]. While the role of AI-2 as a quorum sensing molecule enables coordination of luminescence in *V. harveyi*, the function of AI-2 in other bacteria is less clear. Inhibition of AI-2 synthesis has been implicated in significantly lower levels of surface adhesion of bacteria from an activated sludge sample [61, 62], suggesting a role for AI-2 in surface colonization. The possibility that AI-2 production is simply a by-product of cell metabolism in some species has been considered, highlighting the need to carefully distinguish between metabolic and signalling defects. From the perspective of a microbial community, the level of AI-2 may allow individual cells to assess the health of the colony or the availability of nutrients based on the level of AI-2-producing metabolic activity [63-65].

A number of bacteria have been found to possess an AI-2 uptake mechanism which is modulated by the level of intracellular AI-2 of the bacterium, known as the Lsr system [66], for Lux-S-regulated. This system has been most thoroughly studied in the case of *E. coli* and *S. typhimurium* [52, 53, 67-69], while *lsr* analogs have been identified in a relatively large number of other bacterial species as well [49, 70]. The physiological and ecological roles of AI-2 are rendered more complex by the presence of the Lsr system in certain bacterial species,

suggesting that AI-2 has other functions beyond an existence as a metabolic by-product or a nutrient source. In *E. coli*, the level of AI-2 in a cell culture generally rises in line with cell growth before reaching a threshold and rapidly falling to undetectable levels [71, 72]. The purpose and benefits conferred upon the cells by such behaviour is not well understood, however, the absence of a functional Lsr system has been linked to reduced or atypical biofilm formation in *E. coli* [4, 53, 73]. Chemotaxis to AI-2 has also been shown to influence auto-aggregation in bulk solution, a behaviour which allows *E. coli* to better survive external stressors [5]. Even so, the purpose of a rapid AI-2 uptake mechanism which causes depletion of the chemoattractant in the bulk solution has yet to be fully explained. It has been suggested that the presence of the Lsr system in some bacteria may serve to interfere with signalling in others or allow them to “eavesdrop” [69, 74]. This may remain to be proven until the roles of AI-2 in signalling are better understood and the significance of signal interference can be further investigated.

The Lsr system in *E. coli* comprises of a number of proteins which work together to form a positive feedback loop [32, 52, 75]. Initially, the entire Lsr system is repressed, thus the uptake rate of AI-2 from the extracellular medium is low. AI-2 uptake begins with the periplasmic binding protein LsrB, which then assists in the transfer of the bound molecule into the cell interior via an ATP-binding cassette (ABC) transporter formed by the proteins LsrC, LsrD and LsrA. Here, ATP refers to adenosine triphosphate, a molecule used for energy storage in many kinds of cells. Inside the cell, LsrK phosphorylates AI-2 into P-AI-2, which in turn binds to LsrR. In its base state, LsrR represses induction of the *lsr* operon, thus modulating its own synthesis along with that of all the other Lsr proteins. By binding with P-AI-2, LsrR no longer represses the *lsr* operon, causing the rate of AI-2 uptake and phosphorylation to increase in a positive feedback loop. In time, the Lsr system reaches a fully induced state while simultaneously causing AI-2 to be depleted from the extracellular medium [52]. Numerical simulations of the Lsr system within individual *E. coli* cells have been performed by a number of groups [32, 35], successfully predicting the gradual rise and abrupt fall in extracellular AI-2 concentration. One key challenge in such studies is to identify physiological values for each reaction rate, along with base and maximal concentrations of each system component. Ideally, incorporation of the full range of physiological detail in modelling can allow for accurate characterization of intracellular dynamics. However, inherent fluctuations in the average values for each modeled variable may adversely affect the accuracy of predicted behavior across different cell populations.

E. coli exhibits a chemotactic response to AI-2 which was found to be important in auto-aggregation as well as biofilm formation [5]. AI-2 chemotaxis in *E. coli* is carried out through the periplasmic LsrB protein which is also responsible for AI-2 uptake [48]. However, the other proteins from the Lsr system besides LsrB have been shown to be essentially uninvolved in the chemotactic response [4]. Instead, the LsrB-AI-2 complex has been found to interact with the serine chemoreceptor Tsr [48] and they are hypothesized to have a high binding affinity [4]. This suggests that there may be competition between chemotaxis and uptake behaviours in *E. coli*, in particular where rapid AI-2 uptake could plausibly dampen the chemotactic response. Taking the positive feedback loop of the Lsr system into consideration, it is apparent that the level of periplasmic LsrB protein fluctuates as a function of time and intracellular AI-2 concentration. This may present a unique type of chemotactic behavior in that the availability of periplasmic binding protein varies with local conditions, as opposed to classical chemotaxis models which assume that ligand concentrations vary while receptor concentrations remain constant.

2.6 Significance of AI-2 synthesis, uptake and chemotaxis

Thus far, the chemotactic response of *E. coli* to AI-2 has yet to be directly quantified through measurement of chemotactic sensitivity coefficient. A challenge in AI-2 chemotaxis experiments lies in measurement of the instantaneous concentration profile of AI-2 within the test region. One issue is the need to utilize *E. coli* Lsr mutants deficient in AI-2 uptake but not in sensing, which could significantly differ from wild-type behaviour due to the fact that LsrB remains at basal levels. The other obstacle lies in real time measurement of AI-2 concentration profile. Recently described methods utilize the *V. harveyii* bioassay [56, 76, 77] to quantify AI-2 in units of bioluminescence. The AI-2 concentration is then estimated by comparing against a calibration scale of luminescence against known AI-2 concentrations [72]. The *V. harveyii* bioassay is typically used to determine whether a particular species of bacteria is able to produce AI-2 under a given set of experimental conditions. This is done by the simple observation of bioluminescence generated, which can be measured in terms of Arbitrary Luminescence Units (ALU). The assay is well suited to this task because of the high sensitivity of *V. harveyii* for even very low concentrations of AI-2. On the other hand, the assay is constrained by the fact that it relies on a biological reporter, meaning that the

presence of other chemicals in the AI-2 sample to be tested may influence the result if the additional substances inhibit or enhance the growth of *V. harveyi* in some way. Also, while *V. harveyi* is extremely sensitive to AI-2, each population must be calibrated against a set of pre-defined AI-2 concentrations in order to produce a standard curve before experimental results can be quantified. Thus, the accuracy of the bioassay is limited by potential fluctuations in AI-2 sensitivity amongst *V. harveyi* populations.

Thus far, real-time experimental measurement of an AI-2 concentration field has not been demonstrated. Presently, it is known that *E. coli* exhibits both a chemotactic response and a variable uptake response to AI-2. Additionally, the Lsr system has been shown to be required in some manner for auto-aggregation and healthy biofilm formation. However, it is unclear how chemotaxis and AI-2 uptake specifically contribute to observed population-scale behaviours. Therefore, there is growing interest in defining the link between chemotactic and signalling behaviours in the context of bacterial population dynamics [4, 5, 78].

A recent study by Long et al. [79] found that a signal of some form, secreted by *E. coli* when in the presence of a nutrient source, resulted in an enhancement in the population-scale chemotactic response, including those of cells that did not appear to be near enough to the nutrient source to have initiated a response to it. Although the signal they found was not identified as AI-2, it remains a possibility due to its role as a metabolic by-product that may be formed due to utilization of the nutrient source. This exciting find, coupled with the similarly recent studies of Laganenka [5] and Jani et al. [4] that implicate AI-2 and the chemotaxis phenotype with aggregation and biofilm formation, represent a shift in focus to the ecological role of AI-2 and the chemotactic response.

A number of models of the intracellular dynamics of AI-2 and the Lsr system exist in the literature [32, 75, 80]. These models deal with the balance of the various proteins in single cells using agent-based simulations, or with homogeneous chemical distributions that vary only in time. We note that the combined effects of AI-2 synthesis, uptake and chemotaxis have not been united in a population-scale model using the mathematical approach outlined by Keller and Segel to represent bacteria as a continuous field that undergoes self-driven diffusion and advection. We believe that such a model may be suitable for better understanding the findings detailed in the previous paragraphs which deal with the complex movement of large groups of cells. Consequently, we may be better able to understand how bacterial populations move, not just as a result of the net effect of each individual response to

an external stimuli, but also the potential effects of the presence of the population itself on each individual response.

2.7 Research Objective

Ultimately, the overarching goal of this research topic is to achieve a thorough understanding and quantification of the total response of *E. coli* towards the signalling molecule AI-2, including the interactions amongst signal production, uptake, and chemotactic response, so as to draw insight into the role of AI-2 in the ecology of *E. coli* in the wild. Preliminary forays into this area of research suggest that the extent of knowledge is skewed towards qualitative understanding of the molecular identities and functions of the protein families governing AI-2 synthesis and uptake. Relatively little work has been conducted on the chemotactic response of *E. coli* towards AI-2, in particular the specific effects that may be created by the unusual feature of a dynamically fluctuating periplasmic binding protein concentration.

Quantitative modelling work on the AI-2 uptake kinetics employs a significant amount of guesswork and fitting of kinetic constants to particular sets of experimental data. This stems from the sheer number of parameters that need to be specified for a system of coupled PDEs that tracks most of the proteins related to the AI-2 response. However, such models are difficult to generalize as they rightfully may not apply to other AI-2 uptake scenarios.

In order to bridge the research gaps, it is clear to us that an excellent first step is to tackle the quantification of the variable chemotactic response of *E. coli* towards AI-2 and application to population scale dynamics. Subsequent efforts may be directed at achieving a more generalized uptake model, although it is likely that significant experimental work will be required to quantify the reaction rates involved. The work in this thesis is thus dedicated to the development and validation of chemotaxis models suitable for representation of the response of *E. coli* to AI-2 at the population scale. By laying a strong foundation for the understanding of one key component of the total AI-2 response, we envision that the study of the synthesis and uptake dynamics can gain potential benefits. By being able to isolate the part of response that is due to chemotactic behaviour, we would like to compartmentalize the components of the total response and thus simplify each part of the analyses.

Lastly, I believe that the practical outcome of the completion of such an extensive study is the addition of bacterial population scale dynamics to the existing repertoire of simulation tools that are invaluable in the field of engineering design and prototyping.

Chapter 3

Modelling bacterial chemotaxis for indirectly binding attractants

In this chapter, we develop a novel expression to predict the chemotactic behaviour of *Escherichia coli* towards attractants that bind indirectly through a mediating periplasmic binding protein. Our focus for model validation is the attractant maltose, which has been studied extensively in the literature, thus creating a relative abundance of experimental chemotaxis data for comparison.

The results obtained from the maltose case study can be generalised in the sense that the model can be validated to apply to indirect-binding chemotactic response systems of similar architecture, which is the main aim of this study. On the other hand, the predicted range and magnitude of the chemotactic response is specific to the response of *E. coli* towards maltose and cannot be interpreted in terms of the responses of other bacterial species or the response of *E. coli* towards other chemical attractants. Each attractant has a different binding affinity with its cognate proteins, contributing to the unique range of sensitivity and strength of chemotactic response, which can be seen clearly in past experimental reports. Indeed, one of the focal points of such experimental work has been the quantification of the binding affinity between various attractant-receptor pairs.

In our model, the binding affinity is reflected in two kinetic parameters that must be specified before the equations can be solved, which are the binding affinity between attractant molecule and periplasmic binding protein, followed by the binding affinity between the attractant-BP complex and the chemoreceptor. The two binding affinities must be known for each attractant species that one wishes to model. To do so, we have conducted an extensive search in the related literature to identify experimentally reported values for each binding affinity, represented by dissociation constants.

The uniqueness of the binding affinity values and the experimentally measured chemotactic response for each attractant can be useful in our model validation. By applying experimentally determined values for the dissociation constants, our model is able to predict chemotactic responses that are in close agreement with experimental reports for maltose. The process is repeated for the chemotactic response of *E. coli* towards the AI-2 signalling

molecule, where we note that the values of the dissociation constants differ from those of maltose by several orders of magnitude. In this case, the predicted chemotactic response continues to agree with experimental reports, thus providing stronger evidence for the model validity under different conditions.

As the capillary assay experimental data reports chemotactic response in terms of the relative cell count accumulated in capillaries at the conclusion of the assay, we perform our comparisons by simulating a two-dimensional capillary and reservoir setup and applying the molecular and bacterial transport equations.

3.1 Problem formulation

3.1.1 Preliminaries

Rivero et al. [8] formulated a chemotactic velocity expression in 1D for a bacterium that swims with a run-and-tumble motion, such as *E. coli*. As mentioned previously, the advantage of their chemotactic velocity equation lies in its mathematical simplicity which allows it to be applied in a general bacterial transport model as a self-propelled advection term. The expression, which relates chemotactic drift velocity to the swimming velocity, rate of change of bound chemoreceptors and external attractant concentration gradient, is given by:

$$V_C = V_0 \tanh \left(\sigma V_0 \frac{dR_b}{dC} \frac{dC}{dx} \right) \quad (3.1)$$

V_0 refers to the average swimming velocity of the cell, σ is the chemotactic sensitivity coefficient, R_b refers to the concentration of bound receptors and C is the external attractant concentration. In their formulation, Rivero et al. utilized the finding of Brown and Berg [19] who determined that the logarithm of the average run time τ of a straight swimming cell was related to the time rate of change of the number of bound receptors:

$$\langle \ln \tau \rangle = \ln \tau_0 + \alpha \frac{dP_b}{dt} \quad (3.2)$$

where τ_0 is the mean run time in the absence of attractant, α is a numerical constant, $P_b = R_b/R_T$ and R_T refers to the total number of receptors. Applying the law of mass action, R_b and R_T can also be related by [81]:

$$R_b = R_T \frac{C}{K_d + C} \quad (3.3)$$

which states that at equilibrium, the fraction of bound receptor is a function of the external attractant concentration C and a dissociation constant K_d . Thus, this formulation was derived from the perspective of directly-binding attractants such as amino acids.

This form assumes that the substrate or ligand binds directly with the chemoreceptor, such as serine with the Tsr receptor or aspartate with the Tar receptor [28, 82]. It has been found that other chemoattractants interact with a periplasmic binding protein (BP) to form a complex which then binds to the chemoreceptor [83]. Examples include maltose, which binds indirectly to Tar through the MalE binding protein [47, 84, 85], and AI-2, which binds indirectly to Tsr via the LsrB binding protein [48].

We believe that the binding kinetics for indirectly-binding attractants bear similarity to that described by Kehres [86] for the binding protein-mediated arabinose transport system of *E. coli*. In this work, it is stated that the translocation rate of arabinose into the cell obeys the Michaelis-Menten equation, whereby

$$r_0 = r_{max} \frac{[LP]}{K_M + [LP]} \quad (3.4)$$

with translocation rate R_0 , maximum reaction rate R_{max} , ligand-protein bound complex $[LP]$ and a characteristic Michaelis constant K_M . From the law of mass action, $[LP]$ is expressed as a function of the free ligand concentration $[L]$, total binding protein concentration P_T , and the equilibrium dissociation constant K_d for the ligand-binding protein pair:

$$[LP] = P_T \frac{[L]}{K_d + [L]} \quad (3.5)$$

Substituting (5) back into (4), an expression relating translocation rate to free ligand concentration is obtained:

$$R_0 = R_{max} \frac{P_T [L]}{K_M K_d + K_M [L] + P_T [L]} \quad (3.6)$$

The main feature of this expression is the explicit dependence on the periplasmic binding protein concentration P_T on the arabinose uptake rate. We seek to obtain an expression for

the fraction of bound chemoreceptors that exhibits similar dependence on the periplasmic binding protein concentration.

3.1.2 Formulation of indirect binding expression

The enzyme reactions for indirect binding chemotactic sensing are given by the following equation, based on reversible enzyme kinetics [87]:

[L]: Ligand, [P]: Periplasmic Binding Protein, [R]: Receptor

[LP]: Ligand-BP complex, [LPR]: Ligand-BP-Receptor complex

k_{on} : characteristic association rate, k_{off} : characteristic dissociation rate

Segel formulated a more detailed model of chemotaxis enzyme kinetics [88] by considering the enzyme to exist in two conformational states. Using a simplified approach without consideration of the conformational state of proteins, we formulate a similar but reduced set of equations. By simply considering the mass balance for each chemical species without synthesis or decay, a system of 5 PDEs is obtained:

$$\frac{d[L]}{dt} = -k_{on,1}[L][P] + k_{off,1}[LP] \quad (3.7a)$$

$$\frac{d[P]}{dt} = -k_{on,1}[L][P] + k_{off,1}[LP] \quad (3.7b)$$

$$\frac{d[LP]}{dt} = k_{on,1}[L][P] - k_{off,1}[LP] - k_{on,2}[LP][R] + k_{off,2}[LPR] \quad (3.7c)$$

$$\frac{d[R]}{dt} = -k_{on,2}[LP][R] + k_{off,2}[LPR] \quad (3.7d)$$

$$\frac{d[LPR]}{dt} = k_{on,2}[LP][R] - k_{off,2}[LPR] \quad (3.7e)$$

In enzyme kinetics, intermediate chemical species such as [LP] and [LPR] typically begin at zero concentration, rapidly increase at the start of the reaction, and remain approximately constant until ligand concentration begins to fall significantly near the end of the reaction. The steady-state assumption is applied such that the concentration of ligand-protein complex

can be expressed as a function of free ligand concentration and a characteristic binding or dissociation constant [87].

In bacterial chemotaxis, it is not necessary for attractant molecules to be transported into the cell [2]. As such, we assume in our model that the ligand concentration is perfectly conserved, under the condition that the characteristic timescale for observation of chemotactic response is shorter than the timescale for decay.

At steady state, $\frac{d[LPR]}{dt} = 0$ and from Equation 7e,

$$K_{dr}[LPR] = [LP][R], \text{ where } K_{dr} = k_{off,2}/k_{on,2}$$

Since the total receptor density R_T is the sum of bound ($[LPR]$) and unbound receptors $[R]$,

$$K_{dr}[LPR] = [LP](R_T - [LPR]) \text{ for } [R] = R_T - [LPR]$$

and thus $[LPR]$ can be expressed as:

$$[LPR] = R_T \frac{[LP]}{K_{dr} + [LP]}. \quad (3.8)$$

Similarly, for $\frac{d[LP]}{dt} = 0$ at steady state, and defining $[P] = P_T - [LP]$, we obtain

$$[LP] = P_T \frac{[L]}{K_{dp} + [L]} \quad (3.9)$$

where P_T is the total concentration of periplasmic BP molecules, while K_{dr} is the dissociation constant for the Receptor-binding protein pair and K_{dp} is the dissociation constant for the binding Protein-attractant pair.

Substituting (9) into (8), we obtain:

$$[LPR] = \frac{R_T P_T [L]}{K_{dp} K_{dr} + K_{dr} [L] + P_T [L]} = \frac{R_T P_T C}{K_{dp} K_{dr} + K_{dr} C + P_T C} = N_b \quad (3.10)$$

From this point, free ligand concentration $[L]$ will be expressed as the external attractant concentration C , and $[LPR]$ expressed as the concentration of bound receptors R_b .

$$(R_b)_{indirect} = \frac{R_T P_T C}{K_{dr} K_{dp} + K_{dr} C + P_T C} \quad (3.11)$$

We assume that the receptor and binding protein concentrations R_T and P_T do not vary as functions of the external attractant concentration C . Taking derivative of R_b with respect to C , we obtain:

$$\left(\frac{dR_b}{dC}\right)_{indirect} = \frac{R_T P_T K_{dr} K_{dp}}{(K_{dr} K_{dp} + K_{dr} C + P_T C)^2} \quad (3.12)$$

Rearranging Eq. 12,

$$\left(\frac{dR_b}{dC}\right)_{indirect} = \alpha \left(\frac{K_{d,eq}}{(K_{d,eq} + C)^2}\right) \quad (3.13)$$

where

$$\alpha = \frac{R_T P_T}{K_{dr} + P_T} \quad (3.14a)$$

$$K_{d,eq} = \frac{K_{dr} K_{dp}}{K_{dr} + P_T} \quad (3.14b)$$

In the case of attractants that bind to a periplasmic binding protein before binding as a complex to the chemoreceptor, each of the two binding events is characterized by a dissociation constant. In our notation, K_{dp} refers to the dissociation constant K_d of the binding between the attractant and the periplasmic binding protein. Similarly, K_{dr} refers to the dissociation constant of the binding between the periplasmic binding protein and the chemoreceptor.

The parameters of the indirect binding expression can be regrouped (Eq. 13) to achieve the same form as the original expression for chemotactic velocity derived by Rivero et al. [8], with a single dissociation constant $K_{d,eq}$ (Eq. 14b) as a function of K_{dp} and K_{dr} . However, the effects of the relative binding sensitivity between attractant, periplasmic binding protein and chemoreceptor on the overall chemotactic response are less apparent in this form.

Substituting Equation 12b back into Equation 1, and grouping $\sigma V_0 R_T = X$, we obtain in 1D:

$$V_C = V_0 \tanh \left(X \frac{P_T K_{dr} K_{dp}}{(K_{dr} K_{dp} + K_{dr} C + P_T C)^2} \frac{dC}{dx} \right) \quad (3.15)$$

Brosilow et al. [89] found that the 1D Rivero chemotaxis equation (Eq. 1) could be applied in two dimensions. This approximation was found to agree well with predictions made by the three-dimensional model of Alt et al. [21]. We therefore obtain:

$$V_{C,x} = V_0 \tanh \left(X \frac{P_T K_{dr} K_{dp}}{(K_{dr} K_{dp} + K_{dr} C + P_T C)^2} \frac{\partial C}{\partial x} \right) \quad (3.16a)$$

$$V_{C,y} = V_0 \tanh \left(X \frac{P_T K_{dr} K_{dp}}{(K_{dr} K_{dp} + K_{dr} C + P_T C)^2} \frac{\partial C}{\partial y} \right) \quad (3.16b)$$

The chemotaxis coefficient X is formed from the chosen group $\sigma V_0 R_T$ to eliminate swimming velocity V from within the hyperbolic tangent function, in contrast with previous studies which define $X_0 = \sigma V_{1D}^2 N_T$ [8, 44], sharing the same units as the diffusion coefficient.

While the periplasmic BP for maltose chemotaxis is assumed constant at approximately 1mM concentration [90], the periplasmic BP for AI-2 chemotaxis is known to be highly variable [4]. As mentioned earlier, the LsrB concentration is dependent on the level of induction of the *lsr* operon, which in turn is modulated by the intracellular AI-2 concentration [52]. Ultimately, the LsrB concentration is related to the extracellular AI-2 concentration through a series of reactions, we assume that it remains constant in our current study.

3.1.3 Capillary assay simulation

Capillary assay numerical simulations were conducted using COMSOL Multiphysics® software to model bacterial transport within the 2D geometry of a capillary tube and an external reservoir. Dimensions are shown in Figure 1. The capillary tube is positioned such that the tube entrance is situated at the centre of the reservoir, with no-flux boundary conditions applying to all other interfaces. The free triangular meshing scheme is applied across the whole geometry, with maximum element size of 1.5×10^{-4} m and minimum element size of 3.0×10^{-7} m. Initially, the bacterial concentration in the reservoir is set at $B = B_0$, while $B = 0$ inside the capillary region. Conversely, we set $C = 0$ in the reservoir and $C = C_0$ inside the capillary. We assume a constant unit depth (direction into the paper) for both the capillary and the reservoir, with the capillary volume approximated as a cuboid rather than a true cylinder. The relative chemotactic response is reported as the ratio of the number of cells N_C predicted to enter the capillary against the initial total cell count N_T , with cell counts per unit length obtained from the integral of B within each region of interest.

Figure 3-1: Geometry for simulated capillary assay in 2D (Not drawn to scale). Chemotactic drift velocity is measured along centreline AB along which attractant gradients are steepest. Only the first 10mm from the entrance of the capillary tube is included according to the reported loading conditions [3].

The predicted cell density within the capillary region is computed by solving the system of two PDEs as given below [7]:

$$\frac{\partial C}{\partial t} - D \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) = 0 \quad (3.17a)$$

$$\frac{\partial B}{\partial t} - \mu \left(\frac{\partial^2 B}{\partial x^2} + \frac{\partial^2 B}{\partial y^2} \right) + \frac{\partial}{\partial x} (V_{C,x} B) + \frac{\partial}{\partial y} (V_{C,y} B) = 0 \quad (3.17b)$$

Here, C represents the attractant concentration and B represents the bacterial concentration, while the total cell count, defined as the integral of B within a specified region, is denoted by the variable N . The values used for diffusion and random motility coefficients are $D = 1 \times 10^{-9} m^2 s^{-1}$ and $\mu = 1 \times 10^{-10} m^2 s^{-1}$ [44] throughout the study. The attractant concentration is assumed to undergo only pure diffusion with zero advection, which is the case for the stationary fluid medium within the capillary and reservoir regions. Effects of decay or depletion of attractant due to bacterial uptake are not considered so as to simplify the analysis and better understand the chemotactic response in isolation. The bacterial concentration is assumed to be subject to diffusion due to random motility as well as advection due to chemotaxis behaviour only. Cell growth and death rates are not considered as they are assumed negligible within the timescale of the capillary assay. The specified

tolerance used for the time-dependent study is 1×10^{-5} , with the result appearing to converge for a tolerance of up to 1×10^{-2} .

3.1.4 Parameters for indirect binding chemotaxis of *E. coli* towards maltose

The widely accepted concentration of maltose binding protein in the periplasm of *E. coli* is approximately 1mM based on osmotic shock experimental data and derived from the average number of molecules per cell. Dietzel et al. [91] reported a count of 20000 molecules per cell, while Kellerman and Szmelcman [92] reported 30000 molecules per cell and Koman et al. [93] reported 45000 molecules per cell. These studies were conducted between 1974 and 1979 and there do not appear to be more recent reports on the periplasmic binding molecule concentration to date.

Zhang et al. [90, 94] conducted studies using *E. coli* strains YZ9, YZ11 and YZ12, corresponding to maltose binding protein concentrations of 100%, 23% and 11% respectively, relative to the wild-type *E. coli* RP437 specimen. Conversely, Manson and Boos [85] utilized a *malE*⁺ phenotype with reported 13mg of maltose binding protein per 10^{12} cells. For a molecular weight of 40661 daltons [85], this is equivalent to roughly 3.20×10^{-19} mol/cell. *E. coli* has a typical cell volume of $0.6\mu\text{m}^3$ and the periplasmic space has been estimated to be 30% [95] of the total cell volume or smaller than 10% [96]. Ultimately, this works out to a periplasmic maltose binding protein concentration of between 1.77mM to 10.66mM for the *malE*⁺ phenotype.

The K_{dr} value of 0.25mM is given in Manson and Boos [85] for binding between maltose binding protein and the Tar chemoreceptor. The dissociation constant was estimated by analysing the change in chemotactic response of cells in a capillary assay possessing different known levels of maltose binding protein concentration. Subsequent studies generally refer directly to this first reported value of the dissociation constant and there do not appear to be differing reports of the K_{dr} value in more recent literature.

The value of K_{dp} has been reported to be between 1 μ M to 3.5 μ M. Szmelcman and Schwartz [97] found the dissociation constant between maltose and maltose binding protein to be 1 μ M. Using an osmotic shock technique, Duplay et al. [98, 99] report a K_{dp} value of 3.5 μ M. Finally, Manson and Boos [85] employed an intermediate value of $K_{dp} = 3\mu\text{M}$ in their

calculations based on an overall range between 1 μ M and 5 μ M discussed in the work of Schwartz et al. [100].

The chemotaxis coefficient X_0 introduced by Ford et al. [30] and Rivero et al. [8] comprises the chemotactic sensitivity coefficient and is defined as $X_0 = \sigma V^2 R_T$, where V is the cell swimming velocity in 1D, R_T is the total concentration of chemoreceptors and $\sigma = \frac{\alpha}{R_T}$ (See Equation 2). The average chemotaxis coefficient in the study of Ford et al. was reported as $8 \times 10^{-5} \text{cm}^2 \text{s}^{-1}$ for response of *E. coli* to fucose, which is a non-metabolizable analog of galactose that binds to the Trg receptor. More recently, Ahmed et al. reported values of X_0 between $4.1 \times 10^{-4} \text{cm}^2 \text{s}^{-1}$ and $5.0 \times 10^{-4} \text{cm}^2 \text{s}^{-1}$ in microfluidic experiments involving *E. coli* and α -methylaspartate [101]. An earlier work [44] involving the same bacteria and attractant reported a slightly higher value of $X_0 = 12.5 \times 10^{-4} \text{cm}^2 \text{s}^{-1}$. Finally, Lewus and Ford [102] collated values of X_0 reported in the literature for responses to fucose, methylaspartate and serine. Fucose responses were consistently in the order of $10^{-5} \text{cm}^2 \text{s}^{-1}$, while methylaspartate responses were between $2.4 \times 10^{-4} \text{cm}^2 \text{s}^{-1}$ and $7.5 \times 10^{-4} \text{cm}^2 \text{s}^{-1}$. Interestingly, the response of *Salmonella typhimurium* towards serine was also reported as $7.5 \times 10^{-4} \text{cm}^2 \text{s}^{-1}$. We note that methylaspartate and maltose chemotactic responses are mediated by the Tar receptor while those of serine and AI-2 are mediated by the Tsr receptor.

In our study, the chemotaxis coefficient X is defined as $X = \sigma V R_T$ and therefore $X = \frac{X_0}{V}$. For an average cell swimming velocity of $30 \mu\text{m s}^{-1}$, the largest value of X based on $X_0 = 12.5 \times 10^{-4} \text{cm}^2 \text{s}^{-1}$ is $4.16 \times 10^{-3} \text{m}$ while the smallest based on $X_0 = 8 \times 10^{-5} \text{cm}^2 \text{s}^{-1}$ is $2.67 \times 10^{-4} \text{m}$. The simulations presented in the following section are repeated for $X = 1 \times 10^{-4} \text{m}$, $X = 1 \times 10^{-3} \text{m}$ and $X = 1 \times 10^{-2} \text{m}$. Comparisons of predicted cell count within capillaries against existing experimental data, along with evaluation of the maximum predicted chemotactic velocity, clearly identify $X = 1 \times 10^{-4} \text{m}$ as the most suitable value of the chemotaxis coefficient to be employed in the study. This finding is further elaborated in the results where the effect of varying X is discussed in particular, whereas all other results presented are based on $X = 1 \times 10^{-4} \text{m}$ as the default value for the chemotaxis coefficient.

3.1.5 Parameters for indirect binding chemotaxis towards AI-2

The dissociation constant K_{dp} for binding between AI-2 and its periplasmic binding protein LsrB has been reported as 0.16mM by Zhu and Pei [103] and more recently as $2 \times 10^{-4} \text{mM}$

by Torcato et al. [104]. One possible reason given for the large difference in measurements is the presence of boron in the study of Zhu and Pei, which binds with the AI-2 molecule and forms a molecule that is not sensed by *E. coli*, thus explaining the lowered sensitivity.

The periplasmic binding protein concentration P_T has been quantified by one study thus far [4], which reports a value of 240 ± 75 molecules of LsrB per cell when chemotaxis is most active. This corresponds to a concentration of approximately 8.3×10^{-4} mM per cell, or somewhat larger if the volume of the periplasm is taken into account as well. The same study also quantified levels of periplasmic binding protein for *lsr* mutants, which varies between 0.2 to 7.5 times of the value given above.

The dissociation constant K_{dr} for binding between the LsrB-AI-2 complex and the Tsr chemoreceptor has not been quantified in the existing literature. Using the method described by Manson and Boos [85] for quantifying the binding affinity of loaded maltose binding protein with the Tar receptor, we first attempt to estimate the K_{dr} of loaded LsrB with the Tsr receptor. The data used was adapted from the work of Jani et al. [4] who tested chemotactic response of various *lsr* mutants of *E. coli* to AI-2 using the capillary assay. In their report, they quantified LsrB levels of each mutant, ranging from 20% of wild-type LsrB concentration in the $\Delta lsrK$ mutant to approximately 750% in the case for the $\Delta lsrR$ mutant. A total of seven data points were extrapolated from their findings, corresponding to mutants with different LsrB levels, and curve fitting was conducted to visualize change in cell count with varying LsrB concentration (Fig. 2). We estimate the half-maximal response to occur at $P/P_T \approx 0.1$, rounding to $K_{dr} \approx 0.1 \mu\text{M}$ for $P_T = 0.83 \mu\text{M}$.

Figure 3-2: Capillary assay data points extracted from the study of Jani et al. [4]. Capillary cell count is plotted against periplasmic binding protein concentration ratio and K_{dr} is estimated from the half maximal response. The maximal response is defined here as the largest value of each plotted curve, corresponding to the rightmost edge. The K_{dr} value is then found by finding the value of P that matches the half-maximal response from the equation of the fitted curve. Each line type corresponds to an attractant concentration and set of experimental data points, while each data point within the same series represents an *E. coli* mutant with a different LsrB concentration.

Finally, we assume that the chemotaxis coefficient X should be of the same order of magnitude in the case of AI-2, such that $X = 1 \times 10^{-4} \text{m}$. X is formed by grouping the chemotactic sensitivity coefficient σ , the swimming velocity V and the total number of receptors R_T . *E. coli* possesses roughly twice as many Tar as Tsr receptors [4], implying that the value of X is twice as large for maltose as it is for AI-2 if σ and V can be assumed constant. The average swimming speed of the bacterium typically lies between $20 \mu\text{ms}^{-1}$ to $30 \mu\text{ms}^{-1}$ and can be assumed constant within this range. However, the chemotactic sensitivity describes the change in mean run time per unit time rate of change of receptor occupancy, which condenses the mechanisms of receptor binding, flagellar motor signalling, tumbling inhibition and adaptation into a single empirical coefficient. To the best of our knowledge, the value of σ has only been experimentally determined in the original study of Brown and Berg [19]. Ford developed an analytical expression to estimate the X_0 based on the initial attractant concentration C_0 in a capillary assay, thus highlighting the fact that X_0 , and by extension σ , is likely to vary across the chemotactic response range. By evaluating the

accumulated cell fraction and maximum chemotactic velocity at different levels of X , we find the rationale for choosing $X = 1 \times 10^{-4}\text{m}$ in the case of maltose to apply to AI-2 as well.

3.2 Results and Discussion

3.2.1 Prediction of chemotactic response to maltose

3.2.1.1 Scaling of dissociation constant K_{dp}

We first conducted a parametric sweep for the dissociation constant K_{dp} ranging from sub-micromolar to molar attractant concentrations. For each attractant concentration C_0 , we ran the capillary assay simulation as described in methods (Fig. 1) and computed the number of cells within the capillary region after a period of one hour. Comparing our results against the experimental work in the literature, we observe that the peak chemotactic response predicted by our model for the physiological K_{dp} value of $3\mu\text{M}$ occurs at significantly lower attractant concentrations than previous experimental reports.

We find that $K_{dp} = 100\mu\text{M}$ (Fig. 3A) provides a much better fit for the chemotaxis simulation as compared to the experimental reported value of $K_{dp} = 3\mu\text{M}$ over a wide range of maltose source concentration (Fig. 3B), based on comparisons with the experimental results presented by Hazelbauer [84], Manson and Boos [85], and Zhang et al. [90]. Notably, this includes *E. coli* mutants with altered levels of maltose binding protein, ranging from 23% (strain YZ11) to 1000% (estimated value for the *malE*⁺ phenotype). In the case of the capillary assay study reported by Hazelbauer [84], we additionally found good quantitative agreement in the predicted cell count for the attractant concentration eliciting peak chemotactic response. In short, the total initial cell count in their study was estimated to lie between 3×10^5 and 1×10^6 , while approximately 2.6×10^4 cells were recorded in the capillary at peak accumulation. Using the higher estimation of total cell count, the ratio of cells found within the capillary is thus 0.026, which is matched near identically by our predicted value for $P_T = 1\text{mM}$ (Fig. 3A, dashed line). Comparisons with other chemotaxis assay studies yielded qualitative agreement, owing to uncertainty in estimating cell accumulation in capillaries as a fraction of the total initial cell count. We note, in particular, that our model predicts a distinct shift in peak response from $C_0 = 1\text{mM}$ to $C_0 = 0.1\text{mM}$ as

the periplasmic binding protein concentration is raised to $P_T = 5\text{mM}$ (Fig. 3A, dotted line) and beyond, which is in strong agreement with the results obtained by Manson and Boos [85] for *malE*⁺ cells. Conversely, when P_T is lowered to 0.25mM , corresponding to strain YZ11 [90], the peak response remains at the same initial attractant concentration. Again, this is in agreement with the experimental report, although we are not able to determine if the decrease in magnitude of response from wild-type is accurately represented.

When the experimentally reported value of $K_{dp} = 3\mu\text{M}$ is used in the capillary assay simulation (Fig. 3B), we observe that the predicted response differs significantly from existing experimental data. Considering specifically the case where $P_T = 1\text{mM}$ (Figs. 3A & 3B, dashed lines), the peak predicted chemotactic response occurs at two orders of magnitude of attractant concentration C_0 lower than the experimental profile. Furthermore, the predicted response appears highly saturated at $C_0 = 1\text{mM}$ such that only a minimal number of cells are able to enter the capillary. This contrasts with numerous experimental reports [84, 105] which indicate maximum response to an initial attractant concentration of $C_0 = 1\text{mM}$.

Figure 3-3: Sensitivity profiles based on predicted cell count from capillary assay simulations. The ratio of the number of cells within the capillary region N_C over the total initial cell count N_T is plotted against initial attractant concentration. Parameter values used are (A): $X = 1 \times 10^{-4}\text{m}$, $K_{dr} = 0.25\text{mM}$ and $K_{dp} = 0.1\text{mM}$ and (B): $X = 1 \times 10^{-4}\text{m}$, $K_{dr} = 0.25\text{mM}$ and $K_{dp} = 3\mu\text{M}$. The K_{dp} value reported in the literature is $3\mu\text{M}$ or $3.5\mu\text{M}$, thus the value of K_{dp} used in set (A) is scaled by a factor of approximately 30. $P_T = 1\text{mM}$ corresponds to a typical wild-type *E. coli* specimen. $P_T = 0.25\text{mM}$ corresponds to strain YZ11 from Zhang et al. [90]. $P_T = 5\text{mM}$ to $P_T = 10\text{mM}$ are estimates of the heightened maltose binding protein concentration in the *malE*⁺ strain from the work of Manson and Boos [85].

We believe that the observed difference in effective K_{dp} value from experimental reports could be attributed to a difference in the concentration of maltose between the periplasmic space and extracellular medium. Equation 15 relates the extracellular attractant concentration directly to the chemotactic response, making the implicit assumption that the extracellular attractant concentration is equal to the concentration of attractant that has diffused into the periplasm through porins. In general, the extracellular attractant concentration is not the same as the concentration within the periplasm unless the extracellular concentration does not change with time. Even so, active maltose transport from the periplasm into the cell suggests that attractant concentrations within the periplasm remain lower than extracellular levels. Detailed single-cell models of substrate transport [80] track the periplasmic attractant concentration separately from both intra- and extracellular attractant concentrations, while relating them through the rate of diffusion via porins and uptake through membrane-spanning transporters. It suffices in our current study to assume that the difference in attractant concentration across the outer membrane can be satisfactorily addressed by scaling the parameter K_{dp} . For the experimentally reported value $K_{dp} = 3\mu\text{M}$, the scaling factor thus appears to be approximately 30.

Based on this finding, we introduce the scaling factor β to Equation 15 such that:

$$V_C = V_0 \tanh \left(X \frac{P_T K_{dr} K_{dp} \beta}{(K_{dr} K_{dp} \beta + K_{dr} C + P_T C)^2} \frac{dC}{dx} \right) \quad (3.18a)$$

The change in chemotactic response with increasing values of β are plotted in Figure 4. The response profile translates by one order of magnitude to the right for each tenfold increase in the value of β , showing that β scales proportionally with the extracellular attractant concentration C_0 . Indeed, the above equation can be rearranged to express that the extracellular attractant concentration scales inversely with β :

$$V_C = V_0 \tanh \left(X \frac{P_T K_{dr} K_{dp}}{\beta (K_{dr} K_{dp} + K_{dr} \frac{C}{\beta} + P_T \frac{C}{\beta})^2} \frac{dC}{dx} \right). \quad (3.18b)$$

This form of the expression relates more directly to the discussion in the preceding paragraph as it states that the extracellular attractant concentration is effectively reduced by a factor of β in the periplasm. This simplification of the actual dynamic fluctuation of attractant

concentration in the periplasm may be justified in conditions where the time rate of change of periplasmic attractant concentration can be assumed constant.

Figure 3-4: Change in predicted chemotactic response with varying β . The sensitivity profile undergoes pure translation towards the right for each tenfold increase in the value of β . Peak chemotactic response shifts rapidly from $C_0 = 0.1\text{mM}$ to $C_0 = 1\text{mM}$ between $\beta = 10$ and $\beta = 20$ and reaches a maximum value of approximately 0.025 (ratio of cells accumulated in capillary against total number of cells) by $\beta = 30$. Subsequent increase in β has a more pronounced effect on the chemotactic response to the next highest attractant concentration level, in this case where $C_0 = 10\text{mM}$.

The curve of $\beta = 1$ (Fig. 4) corresponds to Figure 3B where all parameters are unchanged from their experimentally reported values, while the curve of $\beta = 30$ corresponds most closely to the estimated best-fitting case in Figure 3A with $K_{dp} = 100\mu\text{M}$. The chemotactic response to $C_0 = 1\text{mM}$ predicted by $\beta = 30$ is near maximal and further increases in β result in more pronounced response toward the initial attractant concentration of $C_0 = 10\text{mM}$.

When β exceeds 50, the response to $C_0 = 10\text{mM}$ becomes greater than the response to $C_0 = 0.1\text{mM}$. Since the peak chemotactic response remains at $C_0 = 1\text{mM}$ between $\beta = 20$ to $\beta = 100$, the relative strength of predicted responses to the adjacent attractant concentrations of C_0 may be useful in refining the estimation of β . Presently, the value of $\beta = 30$ has been chosen to best represent the existing experimental data [84]. As shown in Figure 3A with $P_T = 1\text{mM}$, $\beta \approx 30$ predicts a relatively larger response at $C_0 = 0.1\text{mM}$ than $C_0 = 10\text{mM}$, matching the earlier experimental report of Hazelbauer.

3.2.1.2 Effect of other parameters on predicted chemotactic response

We considered the possibility that the difference between predicted and actual chemotactic sensitivity curves (Fig. 3B) could be attributed to errors in the value of the other parameters K_{dr} and X . First, we note a key difference in the methods in which K_{dr} and K_{dp} were experimentally quantified. The dissociation constant K_{dp} , for maltose with maltose binding protein, reported in previous literature was measured through experiments carried out using maltose binding protein that was released from the *E. coli* cell periplasm via osmotic shock. As per the earlier discussion, this implies that the experimentally determined value of K_{dp} must be applied together with the actual attractant concentration of the space within which the binding occurs, i.e. the periplasmic attractant concentration. On the other hand, K_{dr} was quantified by Manson and Boos [85] based on chemotaxis capillary assay data using strains of *E. coli* possessing varying levels of maltose binding protein. By plotting chemotactic response against binding protein concentration, they were able to estimate the concentration of binding protein which corresponded to the half maximal response defining the dissociation constant K_d . Thus, we believe that the reported value of K_{dr} is unaffected by differences in extracellular and periplasmic attractant concentrations as it was quantified based on the cellular response at the population scale.

To test this, we vary K_{dr} from 0.01mM to 100mM while keeping all other parameters at their default levels. We find that the change in response differs (Fig. 5A) depending on whether K_{dr} is raised or lowered from the baseline value of $K_{dr} = 0.25\text{mM}$ reported in experimental studies. Lowering K_{dr} results in a shift in the sensitivity profile towards smaller values of C_0 within the range of C_0 tested, which is a similar effect to that achieved by lowering K_{dp} . Conversely, raising K_{dr} does not cause the sensitivity profile to shift toward higher attractant concentrations any further. Instead, we observe that the overall chemotactic response diminishes as K_{dr} is increased past 0.25mM, indicating that it is not possible to achieve good agreement with the experimental sensitivity profile by manipulating K_{dr} alone. Furthermore, the chemotactic response at $C_0 = 1\text{mM}$ and above is almost completely insensitive to changes in the value of K_{dr} .

Figure 3-5: (A) Change in predicted chemotactic response with varying K_{dr} . The physiological or baseline level of K_{dr} is 0.25mM (dotted line). Lowering K_{dr} causes the sensitivity profile to shift towards more dilute attractant concentrations (solid and dashed lines), while increasing K_{dr} above the baseline value results in a net decrease in the total chemotactic response to all levels of C_0 . Changes in the level of K_{dr} do not appear to affect the upper limit to the sensitivity range ($C_0 = 1\text{mM}$ and above). (B) Change in chemotactic response with varying X . The originally estimated value of X as applied to all other simulations is $X = 1 \times 10^{-4}\text{m}$. While increases in the value of X result in a shift in the peak response to higher levels of attractant concentration as well as a greater range of response, this is accompanied by a large increase in the fraction of cells accumulating in the capillary beyond physiologically observed levels. There is negligible response for values of X smaller than $1 \times 10^{-4}\text{m}$.

By quantifying K_{dr} from the total concentration of periplasmic binding protein rather than the fraction that is bound to maltose, it is possible that the actual sensitivity of binding between receptor and maltose-bound binding protein is underestimated. Thus, we expect the value of K_{dr} to be smaller if it were to be quantified using the osmotic shock or similar method. As this would cause the discrepancy between the predicted and experimental sensitivity profiles to become larger (Fig. 5A, solid and dashed lines), it appears highly unlikely that an error in the value of K_{dr} is responsible for the difference between experimental and predicted chemotactic responses.

Increasing the chemotaxis coefficient X by orders of magnitude causes the sensitivity profile to shift to the right and the range of response to widen, while also greatly increasing the ratio of cells accumulated in the capillary (Fig. 5B). Conversely, the fraction of cells entering the capillary becomes negligible when X is lowered to $1 \times 10^{-5}\text{m}$ and below. In the case of $X = 1 \times 10^{-2}\text{m}$, the peak chemotactic response is shifted to $C_0 = 1\text{mM}$, thus agreeing with

previous experimental results. However, the predicted fraction of cells is large in comparison with experimental data which report the cells accumulated in the capillary to be 10% of the estimated total number of cells based on initial cell density or less. Moreover, the predicted chemotactic velocity is extremely high ($\frac{V_C}{V} \geq 0.95$) for responses to all tested attractant concentrations, representing the unlikely scenario where nearly all cells are swimming deterministically in the direction of the steepest attractant concentration gradient. It is apparent that scaling the chemotaxis coefficient has a simultaneous effect on the fraction of cell accumulation, average chemotactic velocity and peak chemotactic response, making it impossible to address the difference between experimental and simulated chemotactic response simply by adjusting X alone.

We also compare the maximum predicted chemotactic velocity to $C_0 = 1\text{mM}$ for each value of X (Fig. 6) across the entire simulation time range of one hour. The chemotactic velocity is represented as the maximum value of V_C along line AB (Fig. 1), which runs along the capillary axis across the full length of the capillary and reservoir combined. In general, the maximum chemotactic velocity occurs at the entrance of the capillary where attractant gradients are steepest. As mentioned in the previous paragraph, the predicted chemotactic velocity for $X = 1 \times 10^{-2}\text{m}$ is improbably large (Fig. 6, solid line), while falling far below 10% of swimming speed at $X = 1 \times 10^{-5}\text{m}$ and below. The maximum velocity predicted in the case of $X = 1 \times 10^{-3}\text{m}$ (Fig. 6, dashed line) remains large ($\frac{V_C}{V} \geq 0.5$) for the first twenty minutes or so. Noting that the peak chemotactic response occurs at $C_0 = 0.1\text{mM}$ instead of 1mM (Fig. 5B, dashed line), we expect the maximum predicted chemotactic velocity to be somewhat higher at the attractant concentration eliciting the peak response. Consequently, it appears that simulations run using $X = 1 \times 10^{-3}\text{m}$ may predict an excessively large chemotactic velocity for a certain range of the attractant concentration C_0 .

Figure 3-6: Ratio of maximum chemotactic velocity V_c against swimming velocity V evaluated along line AB (See Fig. 1) for different levels X over a simulation time of 1 hour. The typical ratio of chemotactic velocity to swimming speed is approximately 10% [1], although higher values such as 40% have been reported as well [44]. The chemotactic velocity ratio predicted by $X = 1 \times 10^{-2}\text{m}$ is thus excessively high and does not match observations based on run-and-tumble swimming behaviour. The predicted chemotactic velocity always begins at the maximum value due to the discontinuous jump in attractant concentration across the mouth of the capillary at the beginning of simulation. Disregarding early time-steps, the chemotactic velocity ratio predicted by $X = 1 \times 10^{-3}\text{m}$ persists above 40% for approximately 20 minutes. Conversely, the ratio rapidly falls below 10% when $X = 1 \times 10^{-4}\text{m}$ and remains at roughly 2% at the end of simulation time.

3.2.2 Prediction of chemotactic response to AI-2

The two values of K_{dp} reported in the literature for binding of AI-2 to the LsrB periplasmic binding protein are $K_{dp} = 0.16\text{mM}$ and $K_{dp} = 0.2\mu\text{M}$. We first study the chemotactic response based on the product βK_{dp} in lieu of evaluating the suitability of each possible K_{dp} value. The change in chemotactic response for different values of βK_{dp} ranging between 0.01mM to 50mM are plotted in Figure 7. As before, the chemotactic response profile undergoes pure translation to the right for each order of magnitude increase in βK_{dp} (Fig. 7A). Narrowing the range of observation (Fig. 7B), we find that setting $\beta K_{dp} = 30\text{mM}$ gives a simulated response that matches the experimental observations of Hegde et al. most closely. In their study, the cell accumulation was reported in terms of the estimated actual cell count, which we approximated as a ratio of the total cell number using their reported initial cell concentration of $5 \times 10^7\text{cells/ml}$ and assuming a reservoir volume of 0.3ml. This volume is consistent with the dimensions described by Adler in the original capillary assay study [3].

The value of $\beta K_{dp} = 30\text{mM}$ corresponds to $\beta = 187.5$ for the higher value of $K_{dp} = 0.16\text{mM}$ and $\beta = 1.5 \times 10^5$ for $K_{dp} = 0.2\mu\text{M}$. We believe that the latter value of β is less likely as it implies that the periplasmic concentration of AI-2 may be as small as $10^{-3}\mu\text{M}$ while the extracellular concentration is in the order of 1mM . The passive rate of diffusion of AI-2 into the cell periplasm via porins is unlikely to differ greatly from that of maltose molecules. Thus, the main factor governing periplasmic concentrations of attractants appears to be the specific rate of uptake via transport apparatus. Present information is insufficient to conclude that $\beta = 1.5 \times 10^5$ is indeed overly large, and by extension that one value of K_{dp} is more suitable for characterising AI-2 chemotaxis than the other.

Figure 3-7: Predicted chemotactic response at different values of βK_{dp} : (A) $\beta K_{dp} = 0.01\text{mM}$ to 50mM . The sensitivity profile undergoes pure translation in the direction of increasing C_0 for each order-of-magnitude increase in βK_{dp} . (B) $\beta K_{dp} = 1\text{mM}$ to 50mM . The curve predicted by setting $\beta K_{dp} = 30\text{mM}$ corresponds most closely to existing experimental reports, which indicate that the chemotactic response to AI-2 increases exponentially from $C_0 = 1\mu\text{M}$ to $C_0 \approx 3162\mu\text{M}$ ($\text{log}_{10} C_0 = 3.5$). The experimental data curve is estimated from the capillary assay data in Hegde et al. [48] using their reported cell density of 5×10^7 cells/ml and assuming the volume of the Adler capillary assay to be 0.3ml .

The concentration of periplasmic binding protein in the AI-2 system of *E. coli* is not constant with time. The experimental report of Jani et al. [4] suggests that the level of binding protein LsrB varies between 0.2 to 8 times of the characteristic concentration of approximately $0.83\mu\text{M}$. The low level of LsrB corresponds to the *lsrK* mutant which only expresses basal levels of the Lsr proteins and cannot be further induced due to the deficiency in production of

phospho-AI-2. The high level of LsrB corresponds to the *lsrR* mutant which is assumed to express maximal levels of the Lsr proteins due to the lack of inhibition of the *lsr* operon.

We examine how the level of periplasmic binding protein affects chemotactic response of *E. coli* to AI-2 across the range of LsrB discussed above. Our model predictions do not agree very closely with the experimental results of Jani et al. [4] for the chemotactic response of *lsr* mutants (Fig. 8). Using $\beta K_{dp} = 30\text{mM}$ to represent the dissociation constant of AI-2 and LsrB binding, we compare the chemotactic response at three different levels of binding protein concentration P_T . Their experimental findings convey a trend that the relative increase in cell count per level of attractant concentration C_0 decreases steadily and gradually approaches a maximum cell count over the range $C_0 = 1\mu\text{M}$ to $C_0 = 1000\mu\text{M}$. Our model instead predicts an exponentially growing rate of cell accumulation per level of C_0 for the same range of C_0 tested, which is in closer agreement with the earlier study of Hegde et al. (Fig. 7B).

Figure 3-8: Predicted chemotactic response to three different levels of LsrB periplasmic binding protein concentration with $X = 1 \times 10^{-4}\text{m}$, $K_{dp} = 1 \times 10^{-4}\text{mM}$ and $\beta K_{dp} = 30\text{mM}$. $P = 0.25P_T$ corresponds to the *lsrK* mutant which remains at baseline levels of *lsr* induction due to its inability to produce phospho-AI-2. $P = 10P_T$ approximates the *lsrR* mutant which is in a fully induced state as the *lsr* operon is never repressed. The chemotactic response at $P = 10P_T$ is relatively large at $C_0 = 1\text{mM}$ as compared to the other curves. The cell fraction is estimated from data of Jani et al. [4] based on an initial cell density of $2.5 \times 10^8\text{cells/ml}$.

3.3 Chapter Summary

In this study, we have formulated an indirect binding expression which builds upon the original chemotaxis model of Rivero et al. The indirect binding expression takes the binding of ligand to periplasmic binding protein into account, followed by binding of the ligand-periplasmic binding protein complex to the chemoreceptor itself. The kinetic equilibrium state of the indirect binding thus involves two dissociation constants and is dependent on the periplasmic binding protein concentration, such that the predicted chemotactic response is non-existent in the limit that the periplasmic binding protein concentration is zero. We additionally find that a clear distinction must be made between extracellular and periplasmic attractant concentration in our model. By assuming that attractant concentrations in the two spaces are equal, our model appears to significantly over-estimate the sensitivity of the chemotactic response such that the predicted peak response occurs at much smaller attractant concentrations than the experimental data. We address this matter by introducing a scaling factor to the dissociation constant for binding between the attractant and the periplasmic binding protein, representing an effective lowering of the overall sensitivity. Alternatively, the scaling factor can be expressed to “scale down” the extracellular attractant concentration when viewed from the perspective of the periplasmic space.

The formulated expression is tested by conducting capillary assay simulations for the chemotactic responses of the *E. coli* bacterium to maltose and AI-2. Using values for the dissociation constants and periplasmic binding protein concentration reported in the previous literature, we find that the maltose response, in particular, is in close agreement with the model predictions using a scaling factor of approximately 30. On the other hand, our model is consistent with one experimental study on AI-2 chemotaxis but differs significantly from another study involving *lsr* mutants with varying levels of periplasmic binding protein concentration. One reason for the model limitation is the greater complexity of the AI-2 response as compared to chemotaxis towards maltose. The AI-2 system features variable levels of periplasmic binding protein as well as uptake rate, both of which are functions of the intracellular AI-2 concentration. Furthermore, AI-2 is a chemoattractant that is produced, sensed and taken in by *E. coli*, making the extracellular concentration profile difficult to predict. Nevertheless, we believe that the formulated indirect binding expression has provided valuable insight into simplified modelling of chemotactic responses to attractants that are mediated by a periplasmic binding protein.

Chapter 4

Further parametric studies on the chemotactic velocity expression

In the previous chapter, we formulated an expression for the chemotactic response of *Escherichia coli* towards attractants that bind indirectly with the chemoreceptor via a mediating periplasmic binding protein (BP):

$$V_C = V \tanh \left(X \frac{P_T K_{dr} K_{dp}}{(K_{dr} K_{dp} + K_{dr} C + P_T C)^2} \frac{dC}{dx} \right) \quad (4.1)$$

where V_C is the chemotactic velocity, V is the average swimming speed, X is the chemotaxis coefficient, P_T is the concentration of periplasmic binding protein, K_{dr} and K_{dp} are dissociation constants, and C represents the extracellular attractant concentration.

Two binding coefficients are necessary due to the intermediate binding protein. K_{dp} characterizes binding between the attractant or ligand molecule and the binding protein, forming a complex, while K_{dr} characterizes the subsequent binding step between the complex and the chemoreceptor. We utilize the simplest description which assumes that the periplasmic binding protein can only bind with a receptor when it is in complex with the target ligand, excluding the possibility that the binding protein molecule may bind with the receptor in the absence of attractant. The formulated expression (Eq. 1) predicts the magnitude of chemotactic velocity at a point characterized by attractant concentration C and concentration gradient $\left(\frac{dC}{dx}, \frac{dC}{dy}\right)$ in 2D. While C can represent an arbitrary attractant chemical, the predicted response is unique for each bacteria-attractant pair due to the unique values of dissociation constants between attractant molecule, binding protein and chemoreceptor. Thus, using Equation 1 to predict chemotactic response requires prior knowledge of the dissociation constants, which must be obtained experimentally or from existing literature.

We performed validation of our formulated expression through the use of two-dimensional capillary assay simulations, modelling the experimental setup described by Adler [3] for quantification of chemotactic response strength. The bacterial population within the reservoir and capillary regions is represented as a bacterial density or concentration field that is initially homogenous within the reservoir region. The bacterial distribution is governed by the

bacterial transport equation in the absence of external flow, while the attractant concentration is assumed to diffuse freely out of the capillary mouth. Similarly, the bacterial distribution is subject to diffusion via random motility, representing the net effect of run-and-tumble activity of individual cells on the population spread [7]. In the absence of external flow, bulk movement of bacteria in a preferred direction is brought about by advection due solely to the chemotactic velocity (Eq. 1).

By simulating the capillary assay experiment and computing the approximate number of cells that are located within the capillary after a certain duration, we were able to make direct comparisons with previous experimental studies in the literature, ultimately achieving close agreement for the maltose chemotactic response across multiple studies. On the other hand, we were able to achieve good agreement in our predictions with the experimental data of Hegde et al. [48] but only marginally so with the data of Jani et al. [4].

Here, we express the extracellular attractant concentration C in terms of the dissociation constant K_{dp} , and the periplasmic BP concentration P_T in terms of the dissociation constant K_{dr} , allowing us to analyse the formulated expression in terms of the concentration profile alone. We thus conduct a parametric study of the modified expression in 1D, allowing us to go into further detail and better understand the significance of the indirect binding mechanism as compared to the simpler direct binding mode seen in amino acids [2].

4.1 Problem formulation

4.1.1 Non-dimensional indirect binding expression

In general, the attractant concentration profile varies spatially and temporally from the initial source concentration C_0 by a function $f(x, t)$:

$$C = C_0 f(x, t) \quad (4.2)$$

where $f(x, t)$ can be any function describing the attractant concentration profile, such as linear, Gaussian or step. It follows that the gradient of C is expressed as

$$\frac{dC}{dx} = C_0 \frac{df(x, t)}{dx} \quad (4.3)$$

By prescribing C_0 as a multiple of K_{dp} such that $C = f(x, t)AK_{dp}$ and $\frac{dC}{dx} = \frac{df(x, t)}{dx}AK_{dp}$ where A is an arbitrary constant, we find that Equation 1 becomes independent of K_{dp} :

$$V_C = V \tanh \left(X \frac{AP_T K_{dr}}{(K_{dr} + K_{dr}Af + P_T Af)^2} \frac{df}{dx} \right) \quad (4.4)$$

Similarly, taking P_T as a multiple of K_{dr} such that $P_T = BK_{dr}$, we eliminate both parameters from the equation, leaving

$$\bar{V} = \tanh \left(X \frac{AB}{(1 + Af + ABf)^2} \frac{df}{dx} \right) \quad (4.5)$$

where $\bar{V} = \frac{V_C}{V}$ is defined as the ratio of chemotactic velocity over the average cell swimming velocity. The expression can be fully nondimensionalized by introducing $\bar{X} = \frac{X}{L}$ and $\bar{x} = \frac{x}{L}$ where L is the characteristic length scale over which the concentration profile develops, thus obtaining

$$\bar{V} = \tanh \left(\bar{X} \frac{AB}{(1 + Af + ABf)^2} \frac{df}{d\bar{x}} \right) \quad (4.6)$$

By specifying a function $f(x, t)$ that describes the spatial and temporal variation of the attractant concentration profile, we can make use of Equation 6 to better understand the indirect binding chemotactic response in terms of the relative concentrations of attractant and periplasmic binding protein. In the following section, we discuss the choice of $f(x, t)$ based on free diffusion from a semi-infinite source.

In the previous study/Chapter, we found that the periplasmic attractant concentration seems to differ from the extracellular attractant concentration by a factor of β , with the concentration in the periplasm being smaller than the extracellular concentration. By fitting the model with experimentally reported values of dissociation constants and comparing with experimentally reported sensitivity curves, we have estimated the value of β to be approximately 30 in the case of maltose chemotactic response. In the case of AI-2, the value of β remains uncertain because two different values have been reported for the value of dissociation constant K_{dp} , prompting us to fit the model using the product βK_{dp} as a single variable. In this chapter, we are concerned with the effect of varying parameter A , representing the ratio of extracellular attractant source concentration C_0 to the dissociation constant K_{dp} . While β is an important variable for quantification of chemotactic response, it

is less vital for the parametric study. Therefore, we assume that β is represented implicitly by the K_{dp} parameter in this study.

For this parametric study, our focus is on the effect of varying the periplasmic binding protein concentration at different orders of magnitude relative to the dissociation constant K_{dr} characterizing the half-maximal response concentration. Specifically, the value of K_{dr} represents the periplasmic binding protein concentration at which half of the total number of target chemoreceptors will be bound. We first present the parametric sweep of ratios A and B , followed by discussing the difference between direct and indirect binding modes of chemotactic response, as well as the possible effects of a dynamically varying level of P_T .

4.1.2 Attractant concentration profile

We choose to model the attractant concentration profile for the 1D parametric study of the indirect binding expression using the case of free diffusion from a semi-infinite source of strength C_0 . This bears similarity to the diffusion of ligand from the mouth of a capillary tube into a large surrounding reservoir. The boundary conditions for this scenario are $C(-\infty, t) = 0$ and $C(L, t) = C_0$, while our region of interest is $0 \leq x \leq L$. The initial condition is $C(x, 0) = 0$ and at the steady state, $C(x, t)$ approaches C_0 as t approaches infinity.

The attractant concentration profile is governed by the 1D molecular transport equation. Assuming pure diffusion and applying the boundary conditions, we obtain the expressions for the attractant concentration profile and concentration gradient profile:

$$C = C_0 \left(1 + \operatorname{erf} \left(\frac{x-L}{\sqrt{4Dt}} \right) \right) \quad (4.7)$$

$$\frac{dC}{dx} = \frac{C_0}{\sqrt{\pi Dt}} e^{\left(\frac{x-L}{\sqrt{4Dt}} \right)^2} \quad (4.8)$$

To simplify the problem, we further choose a fixed time point t_0 representing an intermediate state between the initial and steady state conditions. t_0 is arbitrarily chosen to provide a reasonable physiological context for the kind of attractant concentration gradients that can plausibly be encountered by bacteria (*E. coli*) in the wild (Fig. 1). To that effect, we avoided the earliest stages of the development of the concentration profile due to extremely large

concentration gradients, while still maintaining $C(0, t_0) \approx 0$ to observe the full range of response from $C = 0$ to $C = C_0$.

Figure 4-1: (A) Attractant concentration profile based on the function $f(x, t) = 1 + \text{erf}\left(\frac{x-L}{\sqrt{4Dt}}\right)$ and (B) Concentration gradient from $\frac{df}{dx} = \frac{1}{\sqrt{\pi Dt}} \exp\left(\frac{x-L}{\sqrt{4Dt}}\right)^2$. Parameter values chosen are: Characteristic length $L = 0.01\text{m}$, molecular diffusion coefficient $D = 1 \times 10^{-9}\text{m}^2\text{s}^{-1}$, fixed time point $t = t_0 = 5000\text{s}$.

Aside from selection of t_0 , we estimated the diffusion coefficient D to be $1 \times 10^{-9}\text{m}^2\text{s}^{-1}$ which is typical for small organic molecules and the characteristic length scale L to be 0.01m , based on the largest dimension involved in concentration profile development within the capillary assay experimental setup of Adler [3]. All fixed parameters applied in this study are reported in Table 1.

4.1.3 Estimation of chemotaxis coefficient X

Using the defined function $f(x, t)$ and its derivative, we proceed to evaluate the chemotactic velocity expression (Eq. 5) along the length $0 \leq x \leq L$ through a sweep of parameters A and B . Our previous study allows us to better understand the effect of varying chemotaxis coefficient X as well as the estimated range of values that X can likely take. An X value of $1 \times 10^{-3}\text{m}$ results in an average predicted chemotactic velocity ratio of approximately $\frac{v_c}{v} = 0.4$ during the peak chemotactic response, which is significantly higher than the typical value of 0.1 estimated by Berg [1]. Higher values of X predict that the chemotactic velocity ratio approaches the limit of swimming velocity, implying that cells swim deterministically towards the attractant source and thus contradicting the run-and-tumble behaviour that has

been described in experimental observations. We find that the predicted chemotactic response for $X = 1 \times 10^{-4}\text{m}$ lies within expected levels not exceeding $\frac{V_c}{V} = 0.1$ for a wide range of attractant concentration source values, making this the reasonable choice for estimation of X value in our current study. Furthermore, smaller orders of magnitude of X result in maximum chemotactic velocity ratios below $\frac{V_c}{V} = 0.01$, corresponding to an actual drift velocity of less than one cell length per second.

Table 4-1: List of parameters used in this study.

Parameter (symbol)	Value (unit)
Characteristic length (L)	0.01m
Diffusion coefficient (D)	$1 \times 10^{-9}\text{m}^2\text{s}^{-1}$
Time constant (t_0)	5000s
Chemotaxis coefficient (X)	$1 \times 10^{-4}\text{m}$

4.2 Results and Discussion

We have defined two parametric ratios, $A = \frac{C}{K_{dp}}$ and $B = \frac{P_T}{K_{dr}}$ and used them to nondimensionalize the indirect binding equation formulated in our previous study. The parameter A expresses the extracellular attractant concentration as a function of the dissociation constant K_{dp} , while B expresses the periplasmic binding protein concentration P_T as a function of the dissociation constant K_{dr} . We now carry out a series of parametric studies in 1D by evaluating the indirect binding expression (Eq. 5) along the concentration profile defined previously (Fig. 1). Based on the parametric study, we discuss how the indirect binding mechanism may function to benefit the cell in its chemotactic response. We analytically compare the direct and indirect binding modes to better understand the advantages and disadvantages that are posed by either binding mode. Finally, we briefly discuss the unique features of the *E. coli* AI-2 response in the context of our understanding of the indirect binding mechanism thus far.

4.2.1 Range of response at fixed levels of periplasmic binding protein ratio B

We first examine the indirect binding mechanism by holding B constant and varying A across a wide range to understand how the range of response is affected by the periplasmic binding protein concentration (Fig. 2). The maximum and minimum values of both A and B used in this study are 1×10^4 and 1×10^{-2} , respectively. For each level of B , only the corresponding range of A which elicits a significant response is shown.

The strength of chemotactic response is relatively low when the periplasmic binding protein concentration P_T is much smaller than the dissociation constant K_{dr} , $P_T \ll K_{dr}$ and hence $B \leq 0.1$ (Fig. 2A). At this low concentration of binding protein, it appears that higher attractant concentrations must be applied to achieve significant chemotactic velocities. Only a negligible chemotactic response is predicted at concentration levels smaller than $A = 1$ and higher than $A = 10000$, demonstrating a reduced range of response as compared to higher values of B (Fig. 2B-F).

Increasing the level of B by one order of magnitude (Fig. 2B), we see that the range of significant chemotactic response rises to include a range of six orders of magnitude of attractant concentration represented by parameter A . Furthermore, we note that the average strength of the chemotactic response appears to be higher as a result of increasing B via comparison with Figure 2A. The response to the prescribed non-linear attractant concentration profile appears nearly symmetrical about the point where peak response occurs, reflecting the relative effects of the gradually increasing values of $f(x, t)$ and $\frac{df}{dx}$ over the length L . It is apparent from the indirect binding equation (Eq. 5) that the chemotactic response grows with $\frac{df}{dx}$ but is inversely proportional to the square of the attractant concentration $f(x, t)$. Thus, at values of x larger than that of the peak response, it appears that the effect of attractant saturation outweighs the additional extension to mean run time per unit increase in the time rate of change of the attractant.

Figure 4-2: Variation of A with B held constant. B takes a different value in each sub-figure: (A) $B = 0.1$, (B) $B = 1$, (C) $B = 10$, (D) $B = 100$, (E) $B = 1000$, (F) $B = 10000$. In each case, A is varied between 1×10^{-2} and 1×10^4 , however only curves which predict a significant response are retained in the plot. Parameters A and B

become inversely proportional from approximately $B \geq 10$. This means that the plots from (D to F) will appear similar for the extended range beyond $A \leq 0.01$, but will be scaled in magnitude in response to increasing B .

At higher levels of B (Fig. 2C-F), we note that predicted chemotactic responses hit a maximum value of approximately 2.5% of the swimming velocity. This is attributed to our choice for the function of the attractant concentration profile as well as the region of observation. By extending the graph towards $x = -\infty$, one would observe that the chemotactic response begins from zero, as expected. In theory, the maximum chemotactic response is achieved when $C = 0$ where Equation 5 reduces to:

$$\bar{V} = \tanh\left(XAB \frac{df}{dx}\right) \quad (4.9)$$

Clearly, there is limited physical context in this extreme case as it is necessary for the concentration gradient profile to be zero as well in the absence of attractant. This is a main cause for unrealistically large chemotactic responses predicted at the beginning of numerical simulations, where the attractant concentration profile is discretized to give $C = 0$ and $\frac{dc}{dx}$ is extremely large across a single element.

As the periplasmic binding protein concentration is further increased above the value of K_{dr} , such that $B \gg 1$, the response curves for each level of A exhibit a gradual shift in position in the negative x -direction. This implies that the specific concentration of P_T that a bacterium possesses has a pronounced effect on the attractant concentration range to which it will respond optimally, and furthermore that the response can be actively fine-tuned through modulation of the periplasmic binding protein concentration. Dynamic levels of P_T are observed in the chemotactic response system of *E. coli* towards signalling molecule AI-2, where the concentration of P_T may potentially vary up to fifty times between the basal and fully-induced states [4].

4.2.2 Changes in response to fixed stimulus at different levels of B

The effect of the periplasmic binding protein concentration on the ability to respond to a particular attractant concentration can be seen by varying parameter B while keeping A constant (Fig. 3). As was done in Figure 3, B is varied between 1×10^{-2} and 1×10^4 and only the levels of B that predict a significant chemotactic response are displayed in each sub-plot. An “optimal response” can be described in Figures 4A-C where the response curve

peaks at approximately $\frac{x}{L} = 0.5$, with a different corresponding level of B in each case. At low levels of A where $A \ll 1$, relatively high levels of periplasmic binding protein ($B \geq 1$) concentration are required to achieve significant chemotactic response (Fig. 3A-B).

As the initial attractant concentration is further increased, the effect of varying the periplasmic binding protein concentration becomes less pronounced due to the highly saturating levels of attractant present (Fig 3E-F). At such high attractant concentrations, it appears that the ratio $B = 1$ provides a marginally more persistent response within the range of x considered. The responses predicted by both higher and lower levels of B diminish more rapidly in the direction of positive x , clearly demonstrating that the predicted chemotactic response becomes insensitive to changes in periplasmic binding protein concentration at saturating attractant concentrations. In other words, the evolutionary strategy of adjusting P_T for optimal adaptation to current stimuli is potentially valuable for magnifying the chemotactic response at dilute attractant concentrations (Fig. 3A), while remaining limited by the saturating effect of large attractant concentrations (Fig. 3F).

Figure 4-3: Variation of B with A held constant. A takes a different value in each sub-figure: (A) $A = 0.01$, (B) $A = 0.1$, (C) $A = 1$, (D) $A = 10$, (E) $A = 100$, (F) $A = 1000$. The procedure is similar to that described in Figure 2, where only levels of B predicting significant chemotactic responses are retained in plots. Chemotactic responses become extremely small (within the observation range) for all levels of B when $A \geq 1000$.

4.2.3 Effective use of the indirect binding mechanism

We believe that the existence of the indirect binding mode of chemotactic response may confer some benefits for *E. coli* and other bacteria, which can include greater control over the range of response as well as sensitivity to a particular attractant concentration [46]. As noted in our previous study/Chapter, the significance of indirectly binding attractants in chemotaxis has primarily been addressed by Neumann et al. [46], while later works have mainly built upon or made reference to their findings. Ecologically, being able to control the periplasmic binding protein concentration may serve as a way for bacteria to boost their response to dilute levels of attractant when faced with nutrient scarcity. In this section, we examine how observations gleaned from the parametric variation in the previous sections may be used to better understand possible strategies that arise from the indirect binding mechanism.

In Figures 2E and 2F, the attractant response range depicted is incomplete due to the limit set on the overall range as mentioned previously. In fact, by extending the range of attractant concentrations tested to include more dilute concentrations, the response curves of Figures 2E and 2F appear near-identical to Figure 2D. The only observable difference is that for each order of magnitude increase in B , and hence P_T , the corresponding attractant concentration range similarly decreases by one order of magnitude, but preserves the relative shape and position of the concentration profiles. For the given attractant concentration profile, it appears that the attractant concentration range and the value of parameter B scale infinitely in this manner for the range $100 \leq B \leq \infty$.

It is reasonable to believe that there is a trade-off between the metabolic cost of synthesizing the periplasmic binding protein and the benefit gained in terms of heightened chemotactic response towards the target attractant. In general, the increase in chemotactic response per increase in binding protein concentration is dependent on the typical abundance of the attractant chemical species that is encountered in the environment. We expect that this may serve to set an upper limit to the viable periplasmic binding protein concentration that a cell can maintain under its usual environmental conditions. *E. coli* has been observed to undergo an increase in concentration of some species of periplasmic binding proteins in nutrient limited conditions, presumably to maximise substrate uptake and avoid a starvation scenario [106]. As some periplasmic binding proteins play a dual role in intracellular transport and chemotaxis, a heightened level of P_T at dilute attractant concentrations may simultaneously aid the cell in rapidly locating scarce substrate molecules.

In general, it appears disadvantageous for the cell to maintain levels of P_T that are far below the dissociation constant K_{dr} , as the overall chemotactic response is diminished for all attractant concentrations (Fig. 2A). Thus, it is plausible that the basal levels of P_T are such that $B \geq 1$ at the minimum, giving predicted peak chemotactic responses of at least 1% of the swimming speed. The magnitude of chemotactic response continues to rise as the value of B is increased (Fig. 2C-D), but subsequently reaches a peak within our window of observation, $0 \leq x \leq L$. For example, comparing Figures 2D and 2F, the improvement in sensitivity to a dilute attractant concentration, $A = 0.01$ (solid line), only improves by roughly twofold when the value of B is increased hundredfold.

On the other hand, it seems counterproductive for the bacterium to maintain a high concentration of P_T when attractant concentrations are much larger than the dissociation constant K_{dp} such that $A \gg 1$. This can be seen from Figures 3D-F where the chemotactic response becomes diminished at a shorter distance away from $x = 0$ for larger values of B . If periplasmic binding protein levels are moderately raised when the cell is faced with nutrient-limited conditions, it then stands to reason that the binding protein levels fall back to basal levels when the cell subsequently experiences a persistent nutrient-rich environment. With regard to our chosen attractant concentration profile, $B = 1$ is a good candidate to represent the basal level of P_T that maximises chemotactic response within the range of observation. We note that the selective pressure for the chemotaxis phenotype may also be diminished in nutrient-rich conditions [107], meaning that the maximum chemotactic response is no longer prioritized by the cell. In such a situation, the basal level of P_T may even be far lower.

The parametric sweep and existing evidence in the literature suggest that it is most efficient for bacteria to maintain a level of P_T such that $1 \leq B \leq 100$. By modulating the periplasmic binding protein concentration within this range, our model predicts that the cells are able to achieve a significant chemotactic response range of up to six orders of magnitude of attractant source concentration (between $1 \times 10^{-3} \leq A \leq 1 \times 10^3$). Next, we would like to understand how the indirect binding mechanics compare against the simpler direct binding mode of chemotactic response.

4.2.4 A comparison between direct and indirect binding chemotaxis modes

Chemotaxis with direct binding involves attractant molecules directly interacting with the chemoreceptor and is the more commonly observed mode of binding (Chemotactic response

is mediated by a specific periplasmic binding protein for only five or six known attractants in *E. coli* [1]). The Tar and Tsr receptors are so named for the main substrates that bind directly with them, which are the amino acids aspartate and serine respectively. Since the receptor concentrations are assumed to remain constant, this implies that the magnitude and range of chemotactic response to any given attractant concentration is static.

The time rate of change of bound receptors for direct binding is given by the equation [8]

$$\frac{dR_B}{dt} = \frac{dR_b}{dC} \frac{dC}{dt} \quad (4.10)$$

$$\frac{dR_b}{dC} = R_T \frac{K_d}{(K_d + C)^2} \quad (4.11)$$

where R_T is the total concentration of chemoreceptors, K_d is the dissociation constant between the attractant ligand and the receptor, and C is the extracellular attractant concentration.

We find that the indirect binding expression (Eq. 1) can be rearranged to give a similar form as Equation 11, with an additional coefficient that scales the total receptor concentration.

From our earlier work, the term $\frac{dR_b}{dC}$ for indirect binding is given by

$$\frac{dR_b}{dC} = \frac{R_T P_T K_{dr} K_{dp}}{(K_{dr} K_{dp} + K_{dr} C + P_T C)^2} \quad (4.12)$$

Factoring out the attractant concentration C , we obtain

$$\frac{dR_b}{dC} = \frac{R_T P_T K_{dr} K_{dp}}{(K_{dr} K_{dp} + (K_{dr} + P_T) C)^2} \quad (4.13)$$

Defining $(K_{dr} + P_T)$ as a common factor,

$$\frac{dR_b}{dC} = \frac{R_T P_T K_{dr} K_{dp}}{(K_{dr} + P_T)^2 \left(\frac{K_{dr} K_{dp}}{(K_{dr} + P_T)} + C \right)^2} \quad (4.14)$$

$$\frac{dR_b}{dC} = R_{T,eq} \frac{K_{d,eq}}{(K_{d,eq} + C)^2} \quad (4.15)$$

where $R_{T,eq} = \frac{R_T P_T}{K_{dr} + P_T}$ and $K_{d,eq} = \frac{K_{dr} K_{dp}}{K_{dr} + P_T}$.

In this manner, an indirectly binding attractant can technically be quantified without knowledge of the periplasmic binding protein concentration P_T by simply fitting experimental data to the equivalent dissociation constant $K_{d,eq}$, with the limitation that P_T should remain constant over the course of the experiments to preserve accuracy. Likewise, by fitting the chemotaxis coefficient X (Eq. 1) to experimental data on a case-by-case basis, the factor $\frac{P_T}{K_{dr}+P_T}$ that scales the total receptor concentration R_T can be absorbed into the estimated value of X without explicit knowledge of P_T .

When expressed in this form, we can see that one function of the periplasmic binding protein is to allow the cell to adjust the equivalent dissociation constant in response to the external stimulus C . By increasing the concentration of P_T , the product $K_{d,eq} = \frac{K_{dr}K_{dp}}{K_{dr}+P_T}$ becomes smaller in magnitude, which serves to increase sensitivity to dilute attractant concentrations and trading off the ability to maintain a significant chemotactic response to larger attractant concentrations. Presumably, the bacterium is able to reduce levels of periplasmic binding protein by relaxing synthesis in favourable conditions, although we are not aware of any examples that demonstrate this specific case.

The total receptor availability is gated by the concentration of P_T since the factor $\frac{P_T}{K_{dr}+P_T}$ is always smaller than one. This implies that the indirect binding mode of chemotaxis is not able to make use of all available receptors except when $P_T \gg K_{dr}$ making the difference negligible. Consequently, the magnitude of the overall chemotactic response is reduced by some extent. Comparing the direct and indirect binding modes for $K_d = K_{d,eq}$, it appears that the indirect binding mechanism allows flexibility in the range in exchange for strength of the chemotactic response at similar levels of attractant.

Finally, we note the key dependence of the indirect binding mechanism on the dissociation constant K_{dr} characterizing binding between the loaded binding protein and the chemoreceptor. The output of the indirect binding chemotactic system is relatively poor in the case of either extreme; $P_T \gg K_{dr}$ and $P_T \ll K_{dr}$. In the former case, the available receptors are fully utilized but the system may become over-sensitive to the attractant, as the equivalent dissociation constant becomes a small fraction of K_{dp} which characterizes binding between the attractant and the periplasmic binding protein. This can be advantageous in nutrient-limited environments but not for response optimization to make full use of a rich nutrient

source. In the latter case, it is straightforward to assume that extremely low levels of utilization of available receptors will result in negligible chemotactic response to any concentration of attractants. Based on the significance of the parameter K_{dr} , it is evident that the bacterium is best able to make use of the features of the indirect binding mechanism when $B = 1$ or within the range $0.1 \leq B \leq 10$.

4.2.5 Unique conditions regarding the AI-2 response system of *E. coli*

Certain features of the AI-2 chemotactic response defy the conventional paradigm that has been established for chemotactic response towards both direct- and indirectly binding attractants. The multi-faceted identity of AI-2 includes a metabolic by-product, an interspecies signalling molecule, a quorum sensing regulator, but essentially not a nutrient on its own as far as *E. coli* is concerned [49]. The full repertoire of responses that *E. coli* exhibits towards AI-2 is challenging to model as it involves AI-2 synthesis based on cell density and nutrient availability, variable chemotactic response dependent on the current level of periplasmic binding protein concentration [49], variable rate of AI-2 uptake dependent on the same periplasmic binding protein, and modulation of periplasmic binding protein concentration through the current level of intracellular AI-2 [70].

Presently, the indirect binding model developed in our previous work is moderately successful in predicting the chemotactic response of *E. coli* to AI-2. We have applied the model to greater effect in the prediction of maltose chemotaxis and we believe that the main difference lies in the fact that periplasmic binding protein concentrations are variable for AI-2 but constant for maltose. Additionally, while the binding protein concentration of MalE, for maltose, is much higher than the total receptor density, the opposite is true for the AI-2 binding protein LsrB. MalE is present in the periplasm of *E. coli* at a concentration of 1mM [85], while LsrB is present at the much smaller concentration of approximately 1 μ M based on an average of 240 molecules per cell [4]. The total receptor count of Tar is rounded off to about 5000 copies per cell, with Tsr having roughly half that amount, corresponding to receptor densities in the order of 10^{-2} mM [4].

In ligand binding kinetics, the first order approximation of the steady-state balance of the ligand-enzyme pair can only be applied when the ligand concentration is at least ten times higher than the enzyme concentration [108]. This is because it assumes that the fraction of total ligand bound in ligand-enzyme complex is negligible compared to the free ligand

concentration. For the indirect binding mechanism, we are interested in the relative concentrations of the periplasmic binding protein complexes that are free versus bound to receptors. The total periplasmic binding protein concentration is sufficiently high relative to the receptor density for nutrient-based attractant such as maltose. On the other hand, the concentration of periplasmic binding protein LsrB is only a fraction of the receptor density in the case of signalling molecule AI-2. This highlights the need for implementing the exact equilibrium balance expression [108] for all binding interactions between ligand, periplasmic binding protein and receptor, and is the topic of our subsequent work (See Chapter 6).

Before ending off, we would like to frame the discussions presented in the previous sections in the context of the unique AI-2 chemotactic response exhibited by *E. coli*. From Section 3.3, the use of a mediating periplasmic binding protein may be different in the case of AI-2 as compared to nutrient-based attractants. AI-2 is typically present in the environment at relatively small concentrations and peaks at approximately 30 μ M in *E. coli* growth culture due to rapid uptake of the substrate in the late exponential phase. However, due to its role as a quorum-sensing signal for coordination of population-scale behaviour [53], it is imperative that a cell only responds when the signal concentration passes a minimum threshold. This may explain why intracellular LsrB begins at a low level and only ramps up as AI-2 is sequestered in the cell. Essentially, it implies that the cell is able to selectively respond when it is “worthwhile to do so”. Assuming that the AI-2 concentration is homogeneous within the cell’s local area, this also serves a purpose of coordinating LsrB levels amongst the nearby population and hence the population chemotactic response. To find out more about this exciting possibility, we are aware of the need for a more robust chemotaxis model that addresses both the scarce ligand problem and the variable level of periplasmic binding protein.

4.3 Chapter Summary

We have conducted a simple parametric study for the indirect binding expression formulated in our previous study. Possible bacterial strategies that can make use of the flexibility afforded by the variable level of periplasmic binding protein are suggested by the predicted chemotactic responses at different levels of extracellular concentration and periplasmic binding protein concentration. In particular, the indirect binding mode appears to favour

heightened sensitivity to dilute attractant concentrations when the periplasmic binding protein concentration is relatively high with respect to the dissociation constant. It comes to our attention that the unusual low levels of periplasmic binding protein concentration in the AI-2 system, coupled with dilute levels of extracellular AI-2 expected to be found in the environment, demands a more robust form of the indirect binding expression. In our subsequent work (Chapter 6), we further extend the model to account for tight-binding mechanics, also known as the ligand-depletion regime, by treating the attractant-binding protein complex as the depleting ligand.

Chapter 5

Modelling the variable chemotactic response of *Escherichia coli* to AI-2

The signalling molecule AI-2 is a metabolic by-product of the Activated Methyl Cycle (AMC) found in many gram-negative bacteria including *Escherichia coli* [66]. The synthesis of AI-2 is regulated by a protein called LuxS, so named for its first observed role in bioluminescence in *Vibrio harveyi* [55]. While other bacteria do not exhibit bioluminescence, they do possess the LuxS homolog [57] which retains its main role in central metabolism [58].

E. coli interacts with AI-2 in the environment in a number of ways. Firstly, it exhibits a chemotactic response to AI-2 through the indirect binding mechanism [48] where, unlike maltose, the periplasmic binding protein concentration can vary significantly over a period of exposure to extracellular AI-2. In our previous study (Chapter 4), we satisfactorily modelled the chemotactic response of *E. coli* to maltose through the development of an indirect binding expression for predicting the chemotactic velocity. The model allows us to factor in the effects of the periplasmic binding protein concentration as well as the binding kinetics between the attractant and the periplasmic binding protein, and the receptor and the attractant-binding protein pair.

In contrast with the maltose response, the predictions made by our model are not in such close agreement with experimental data for the AI-2 chemotactic response from the literature [4]. Nevertheless, it is clear from the study that the AI-2 response is indeed consistent with indirect binding mechanics. Upon further consideration, we believe that the AI-2 chemotactic response can be better predicted with a more robust model for the chemotactic velocity that allows for variable periplasmic binding protein concentration. Moreover, we expect that the best representation can be obtained by specifying the binding protein concentration as a function of intracellular AI-2.

5.1 Problem formulation

5.1.1 General binding model

Ligand-receptor kinetic models often simplify the problem by assuming that the free ligand concentration, being in excess, can be approximated as the total ligand concentration [81], which is one of the main assumptions of Michaelis-Menten reaction models. As this assumption only applies in the case where the total enzyme concentration is much smaller than the ligand concentration, ligand depletion must be taken into account when the two are of comparable levels or when the ligand is dilute relative to the receptor concentration [108]. Such is the case in the response of *E. coli* towards AI-2, in which the periplasmic binding protein LsrB concentration is much smaller than the concentration of cognate Tsr receptors [4]. In our earlier model, we essentially treat the binding kinetics of the attractant and binding protein separately from the binding kinetics between the attractant-binding protein pair and the chemoreceptor. By doing so, we implicitly assume that the concentration of the final binding product is negligible when compared with the concentrations of free attractant and free binding protein. The effect of assuming that the free ligand concentration is equal to the total ligand concentration is less pronounced in the predictive chemotactic model, as discussed briefly by Rivero et al. [8]. At dilute attractant concentrations, where the error due to overestimation of free ligand can be large, predicted chemotactic responses are typically negligible in magnitude due to the shallow concentration gradient, thus minimizing the effect of said error.

On the other hand, we believe that the potential error has a greater impact on the predicted response when it applies to the periplasmic binding protein concentration. As a simple example, for a periplasmic binding protein concentration P that is equal in value to dissociation constant K_{dr} , the first order binding kinetics state that the fraction of bound receptors is equal to $\frac{P}{K_{dr}+P} = 0.5$. If the total receptor concentration R_T greatly exceeds the total binding protein concentration P_T , $R_T \gg P_T$, then it is impossible for half of R_T to be bound with P due to a shortage in the actual number of molecules of binding protein present. As this nuance is not reflected in the first order binding kinetics, it appears likely for the receptor occupancy to be over-predicted in the case of chemotactic response of *E. coli* to AI-2 where the concentration of P_T is indeed about ten times smaller than that of R_T .

An exact algebraic expression can be formulated for the fraction of bound receptors in terms of the extracellular attractant concentration and total binding protein and receptor concentrations. As before (Chapter 4), we write the kinetic equations governing the time rate of change of each molecular species involved in the indirect binding mechanism:

$$\frac{d[L]}{dt} = -k_{on,1}[L][P] + k_{off,1}[LP] \quad (5.1a)$$

$$\frac{d[P]}{dt} = -k_{on,1}[L][P] + k_{off,1}[LP] \quad (5.1b)$$

$$\frac{d[LP]}{dt} = k_{on,1}[L][P] - k_{off,1}[LP] - k_{on,2}[LP][R] + k_{off,2}[LPR] \quad (5.1c)$$

$$\frac{d[R]}{dt} = -k_{on,2}[LP][R] + k_{off,2}[LPR] \quad (5.1d)$$

$$\frac{d[LPR]}{dt} = k_{on,2}[LP][R] - k_{off,2}[LPR] \quad (5.1e)$$

Our focus is on Equations **1c** and **1e** which respectively describe the time rate of change of the ligand-periplasmic binding protein complex $[LP]$ and the final binding product $[LPR]$ between ligand, periplasmic binding protein and receptor. The free concentrations of ligand, binding protein and receptor are denoted as $[L]$, $[P]$ and $[R]$ respectively. At steady state,

$$\frac{d[LP]}{dt} = 0 \quad (5.2a)$$

and

$$\frac{d[LPR]}{dt} = 0. \quad (5.2b)$$

From Equation 2a, we obtain

$$K_{dp}[LP] = [L][P] \quad (5.3)$$

where $K_{dp} = \frac{k_{off,1}}{k_{on,1}}$ is the dissociation constant characterizing binding between the ligand and the periplasmic binding protein. The balance of each molecular species requires that

$$L_T = [L] + [LP] + [LPR] \quad (5.4a)$$

$$P_T = [P] + [LP] + [LPR] \quad (5.4b)$$

where L_T is the total ligand concentration and is equal to the extracellular attractant concentration C that we have used elsewhere in this work, and P_T is the total periplasmic binding protein concentration. Substituting into Equation 3,

$$K_{dp}[LP] = (L_T - [LP] - [LPR])(P_T - [LP] - [LPR]) \quad (5.5)$$

Following algebraic manipulation, a solution for $[LP]$ can be found by taking the subtractive root of the quadratic equation:

$$[LP] = \frac{C+P_T+K_{dp}}{2} - [LPR] - \sqrt{\left(\frac{C+P_T+K_{dp}}{2}\right)^2 - CP_T - K_{dp}[LPR]} \quad (5.6)$$

This is similar to the formulation of the Morrison equation for tightly binding inhibitors [109], an extension of the well-known Michaelis-Menten kinetics that also results in a quadratic equation. In the Morrison equation, only the smaller root is applicable because it allows the predicted response to become saturated. From Equation 2b, the expression for $[LPR]$ is

$$K_{dr}[LPR] = [LP][R] \quad (5.7)$$

where $K_{dr} = \frac{k_{off,2}}{k_{on,2}}$ is the dissociation constant characterizing binding between the ligand-binding protein pair and the chemoreceptor. Unlike Equation 3, we already have an expression for $[LP]$ based on Equation 6. However, choosing

$$R_T = [R] + [LPR], \quad (5.8)$$

where R_T is the total number of receptors, brings us eventually to an implicit expression for the solution of $[LPR]$. Substituting Equation 8 into Equation 7, we obtain

$$K_{dr}[LPR] = [LP](R_T - [LPR]), \quad (5.9)$$

hence

$$[LPR] = R_T \frac{[LP]}{K_{dr} - [LP]}. \quad (5.10)$$

The appearance of $[LP]$ in both numerator and denominator, coupled with the square root of $[LPR]$ in the expression for $[LP]$ (Eq. 6), result in an implicit solution for $[LPR]$ that is best solved iteratively. As the chemotactic velocity expression $V_C = V \tanh\left(X \frac{d[LPR]}{dC} \frac{\partial C}{\partial x}\right)$ depends

on the derivative of the bound receptor concentration with respect to extracellular attractant concentration C (Chapter 4), it is necessary to apply implicit differentiation on Equation 10. However, returning to Equation 7, we consider the fact that $R_T \gg P_T$ for the AI-2 chemotactic response, with $R_T \approx 2500$ copies per cell and $P_T \approx 240$ copies per cell at maximum chemotactic response [4]. We note that this is a conservative estimate of R_T in particular, which may be higher as reported in other studies [110, 111] and based on the expected 2:1 ratio of Tar to Tsr receptors. With this in mind, we assume that the free receptor concentration $[R]$ is approximately equal to the total receptor concentration R_T . From Equation 7, we then obtain

$$[LPR] = [LP] \frac{R_T}{K_{dr}} \quad (5.11a)$$

$$[LPR] = \frac{R_T}{K_{dr}} \left(\frac{C + P_T + K_{dp}}{2} - [LPR] - \sqrt{\left(\frac{C + P_T + K_{dp}}{2} \right)^2 - C P_T - K_{dp} [LPR]} \right) \quad (5.11b)$$

which simply involves rearranging and making $[LPR]$ the subject. The final result of these series of steps is

$$[LPR] = R_B = N_1 - \sqrt{N_1^2 - N_2} \quad (5.12)$$

where

$$N_1 = \frac{R_T(C + P_T + K_{dp})}{2(K_{dr} + R_T)} - \frac{R_T^2 K_{dp}}{2(K_{dr} + R_T)^2} \quad (5.13a)$$

$$N_2 = \frac{R_T^2 C P_T}{(K_{dr} + R_T)^2} \quad (5.13b)$$

and R_B is the concentration of bound receptors. In this case, finding the derivative of R_B with respect to C is a straightforward task, giving us:

$$\frac{dR_b}{dC} = \frac{dN_1}{dC} - \frac{2N_1 \frac{dN_1}{dC} - \frac{dN_2}{dC}}{2\sqrt{N_1^2 - N_2}} \quad (5.14)$$

Substituting into the 2D chemotactic velocity equations [89] (Chapter 4):

$$V_{C,x} = V_0 \tanh \left(\frac{x}{R_T} \frac{\partial C}{\partial x} \left\{ \frac{dN_1}{dC} - \frac{2N_1 \frac{dN_1}{dC} - \frac{dN_2}{dC}}{2\sqrt{N_1^2 - N_2}} \right\} \right) \quad (5.15a)$$

$$V_{c,y} = V_0 \tanh \left(\frac{X}{R_T} \frac{\partial C}{\partial y} \left\{ \frac{dN_1}{dC} - \frac{2N_1 \frac{dN_1}{dC} - \frac{dN_2}{dC}}{2\sqrt{N_1^2 - N_2}} \right\} \right) \quad (5.15b)$$

In Chapter 4, the chemotaxis coefficient X is defined as the product of the “chemotactic sensitivity coefficient” σ [8], the cell swimming velocity V and the total receptor concentration R_T , $X = \sigma V R_T$. The chemotactic velocity equation is then expressed as $V_C = V \tanh \left(\sigma V \frac{dR_b}{dC} \frac{dC}{dx} \right) = V \tanh \left(X \frac{dP_b}{dC} \frac{dC}{dx} \right)$, where $P_b = \frac{R_b}{R_T}$ is the fraction of receptors bound. The current expression for $\frac{dR_b}{dC}$ does not permit for R_T to be easily factored out, making it necessary to specify the chemotaxis coefficient as $\frac{X}{R_T}$.

In the next section, we test the newly formulated indirect binding model (Eq. 14) for AI-2 chemotaxis by conducting capillary assay simulations as well as a simple parametric study for the sake of validation and comparison against our past results (Chapters 4 & 5) that are obtained using the simpler model. Equation 10 gives the exact representation of the $[LPR]$ or R_B concentration at equilibrium, meaning that it should be equally applicable to all indirect binding chemotactic response systems including maltose and AI-2. However, it involves the solution of an implicit expression, thus slightly increasing the complexity required to compute the predicted chemotactic velocity. By simplifying the expression, we essentially constrain ourselves to the scenario in which the receptor concentration is significantly larger than the periplasmic binding protein concentration, $R_T \gg P_T$, making the model described by Equations 12 and 14 an indirect binding model specifically for the *E. coli* AI-2 chemotactic response. Before proceeding to the results, we also present the formulation of a minimalistic set of equations that allow for modelling of the variable level of periplasmic binding protein P_T as a function of the intracellular AI-2 concentration.

5.1.2 Controlling the variation of periplasmic binding protein (LsrB)

As mentioned at various points in this work, the level of LsrB, which plays a role in both AI-2 chemotaxis as well as AI-2 uptake, varies over time as its rate of synthesis is governed by the intracellular AI-2 concentration. The variation of periplasmic binding protein LsrB was represented in a homogeneous uptake model by Zargar et al. [80], who observed from past experimental data that the LsrB concentration varied proportionally with the rate of cell growth. This method, however, implies that the rate of production of LsrB is independent of

the extracellular AI-2 concentration. From their approach, we introduce a modification to express the periplasmic binding protein concentration as a function of the intracellular AI-2 concentration that we believe to be applicable to shorter timescales of one hour or less. Thus, as our focus is on the initial chemotactic response and the subsequent “ramp-up” due to increased periplasmic binding protein, we do not consider long-term effects on the periplasmic binding protein concentration such as gradual decay in the absence of AI-2 in the environment.

A quick summary of the AI-2 system dynamics: The presence of LsrB in the cell periplasm of wild-type *E. coli* is also representative of the level of AI-2 transport and uptake activity that is currently experienced by the cell [48]. LsrB forms part of an ABC transporter apparatus for taking up extracellular AI-2, known collectively as LsrACDB [71]. Two other proteins, LsrK and LsrR, serve to regulate the overall levels of *lsr* family proteins in the cell [52], including themselves and LsrACDB. LsrK phosphorylates intracellular AI-2, forming AI-2P which then binds to LsrR [75]. On its own, LsrR represses the *lsr* operon, keeping levels of Lsr proteins low. AI-2P relieves this repression effect when binding to LsrR, allowing transcription of Lsr proteins. This arrangement forms a forward feedback loops that results in full induction of the Lsr system following prolonged uptake of extracellular AI-2 of a certain threshold concentration.

It is thus partially correct to say that the level of LsrB is a function of the level of intracellular AI-2. While the intracellular AI-2 concentration rises due to uptake of extracellular AI-2, it is also significantly depleted by the phosphorylating action of LsrK, making there essentially two separate populations of AI-2 and AI-2P molecules existing in the cell. However, in the normal progression of the positive feedback loop of the AI-2 system, it is evident that the total concentration of sequestered AI-2 in either form rises gradually, as do the various proteins of the Lsr system. In this case, we assume that first order kinetics is sufficient to describe the concentration of LsrB in relation to the total intracellular AI-2 concentration. Thus, for

$$AI2_{ex} = C_0 - (AI2_{in} + AI2P_{in}) \quad (5.16)$$

we represent the LsrB concentration as a function of AI-2 concentration:

$$LsrB = B_{min} + (B_{max} - B_{min}) \frac{AI2_{in} + AI2P_{in}}{K_{mid} + AI2_{in} + AI2P_{in}} \quad (5.17)$$

or simply

$$LsrB = b_{min} + (b_{max} - b_{min}) \frac{AI2_{in}}{K_{mid} + AI2_{in}} \quad (5.18)$$

if we simply consider the sum of all intracellular AI-2 that has been imported into the cell from the extracellular source in the bulk media. b_{min} and b_{max} are the theoretical minimum and maximum LsrB concentrations, $AI2_{ex}$ and $AI2_{in}$ are the extra- and intracellular AI-2 concentrations, and $AI2P_{in}$ is the concentration of phosphorylated AI-2 within the cell cytoplasm.

The values of b_{min} and b_{max} are assumed to be consistent with LsrB concentrations pertaining to the fully repressed and fully induced states of the *lsr* operon respectively [4], thus $b_{min} = 0.25 \times 10^{-4} \text{mM}$ and $b_{max} = 8 \times 10^{-4} \text{mM}$. In *E. coli* growth assays, the extracellular AI-2 concentration peaks at approximately $30 \mu\text{M}$ and subsequently becomes rapidly depleted [72]. The midpoint value of AI-2 concentration that allows the LsrB concentration to be maximal at peak extracellular AI-2 concentration is thus estimated to lie around $K_{mid} = 10 \mu\text{M}$, as a rough estimate. In the study of Zargar et al., the LsrB concentration was varied as a function of the cell optical density:

$$\frac{dLsrB}{dt} = \mu OD_{600} - decay \quad (5.19)$$

where μ is the constant of growth of the bacterial population and *decay* is proportional to *LsrB*, estimated to be 2% per minute. The rate of extracellular AI-2 uptake was given as:

$$\frac{dAI2_{ex}}{dt} = -k_{in}(LsrB)(AI2_{ex}) + k_{out}AI2_{in} \quad (5.20)$$

Because the LsrB concentration in their study differed greatly from ours, we find it necessary to adjust the k_{in} value to scale the rate of uptake. By doing so, we achieved good agreement (Fig. 1A) in the AI-2 uptake dynamics between our functional form (Eq. 18) and theirs (Eq. 19). Over the course of 270 minutes, the predicted concentration of LsrB (Fig. 1B) is seen to rise by approximately twenty times from the base level representing the fully repressed *lsr* operon.

Figure 5-1: (A) Comparison of uptake rates of extracellular AI-2 based on our model and the model of Zargar et al. (B) Change in periplasmic binding protein concentration over time in the simulated uptake model. The binding protein concentration is assumed to be average over a perfectly homogeneous solution of molecular and bacterial species since the system of coupled PDEs only varies with time.

Based on our modified value of k_{in} obtained in our scaling adjustment, we choose to adopt the functional form of AI-2 uptake that is used by Zargar et al. [80]. For the uptake of AI-2 at longer timescales leading to AI-2 depletion from the bulk medium, it may be necessary to use the functional form of at least first-order kinetics to represent the gradually decreasing rate of uptake as the extracellular AI-2 concentration approaches zero. This can be seen from the batch culture experiments conducted by Taga and Xavier [72], who quantified the rise and fall of AI-2 for *E. coli* in growth media over a period of twelve hours. In the case of chemotaxis experiments lasting for about one hour in the case of the capillary assay, there is little risk of depletion or even of significant uptake of the substrate in many cases, thus the simple linear functional form suffices.

Finally, we present the minimal set of equations required to model variable periplasmic binding protein concentration over the course of one hour, which is the typical timescale for capillary assay experiments:

$$\frac{\partial C}{\partial t} - D \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) = -\text{uptake} \quad (5.21a)$$

$$\frac{\partial B}{\partial t} - \mu \left(\frac{\partial^2 B}{\partial x^2} + \frac{\partial^2 B}{\partial y^2} \right) + \frac{\partial}{\partial x} (V_{Cx} B) + \frac{\partial}{\partial y} (V_{Cy} B) = 0 \quad (5.21b)$$

$$\frac{\partial AI2_{in}}{\partial t} - \mu \left(\frac{\partial^2 AI2_{in}}{\partial x^2} + \frac{\partial^2 AI2_{in}}{\partial y^2} \right) + \frac{\partial}{\partial x} (V_{Cx} AI2_{in}) + \frac{\partial}{\partial y} (V_{Cy} AI2_{in}) = uptake \quad (5.21c)$$

$$uptake = k_{in} P_{T,ex} C \quad (5.21d)$$

$$P_{T,ex} = P_T Q_{cell} B \quad (5.21e)$$

$$P_T = b_{min} + (b_{max} - b_{min}) \frac{AI2_{in}}{K_{mid} + AI2_{in}} \quad (5.21f)$$

In this system of equations, C is the extracellular AI-2 concentration, with diffusion coefficient D . B is the bacterial density, defined as the number of cells per unit volume, with random motility coefficient μ and chemotactic velocities V_{Cx} and V_{Cy} in the respective directions.

The intracellular AI-2 concentration $AI2_{in}$ is quantified from the fixed external reference frame, as opposed to the reference frame of the individual cell of volume $0.6 \times 10^{-18} \text{m}^3$. It is to be understood as the moles of AI-2 per cell multiplied by the cell density as seen from the perspective of the reference frame containing the simulated capillary and reservoir. Likewise, the variable $P_{T,ex}$ is quantified from the external reference frame and is defined as the concentration of P_T per cell multiplied by the cellular volume Q_{cell} and the cell density (Eq. 21e). In contrast, the chemotactic velocity (Eq. 15) and its constituents (Eq. 13 & 14) are quantified from the perspective of the individual cell. The variable P_T (Eq. 21f) is thus defined as the moles of periplasmic binding protein per cell divided by the cellular volume Q_{cell} . This is because each cell represents a separate, enclosed space in which the apparent concentration of AI-2 and periplasmic binding protein is heightened.

In this Chapter, the model developed (Eq. 15) is designed specifically for prediction of the *E. coli* AI-2 chemotactic response, which is unique because the concentration of periplasmic binding protein P_T is much lower than the chemoreceptor concentration R_T . In contrast, Jani and colleagues [4] noted that periplasmic binding protein is typically in relative abundance as compared to chemoreceptor. Therefore, our model here formalises the assumption that $P_T \ll R_T$, with the main implication being that the concentration of bound periplasmic binding protein represents a significant fraction of the total binding protein concentration. By taking this into consideration, the equations for chemical balance at equilibrium are significantly altered from our previous model in Chapter 3. In theory, we expect that the present model can be applied to other indirect-binding attractants that are similarly characterized by the

condition $P_T \ll R_T$. Thus far, we have not identified such an attractant from the literature which would serve as a useful further source of model validation.

The second assumption made is that the concentration of bound receptor is negligible since the total periplasmic binding protein concentration is much smaller, such that $R \approx R_T$. While this assumption is appropriate for AI-2, it is unsuitable for attractants such as maltose where a large fraction of chemoreceptors may be bound due to the abundance of binding protein complexes. As such, we note that the higher-order model developed in this Chapter is less general than the previously developed model in Chapter 3, being specialised to better predict the chemotactic response of *E. coli* towards AI-2.

Finally, we note that the representation of AI-2 uptake mechanics and change in LsrB concentration over time has been significantly simplified in comparison to the full series of interactions that are known to occur. The system of PDEs (Eq. 21) is thus only applicable for short durations of time such as the typical one hour taken for a capillary assay experiment. As the AI-2 uptake is modelled as a function of periplasmic binding protein alone, the accuracy will be adversely affected when the AI-2 concentration approaches zero and the model will fail due to AI-2 concentration becoming negative. For the AI-2 uptake rate calibrated according to Zargar et al. (Fig. 5-1), AI-2 depletion occurs after approximately 4 hours [80]. In our study, we simulate capillary assay experiments not exceeding one hour in duration, thus ensuring that the accuracy of the uptake expression is minimally affected. Another limitation may be presented in the empirical expression relating P_T to the intracellular AI-2 concentration. In the work of Zargar et al. [80], the concentration of P_T was instead related to the cell density, which also represents a significant simplification of the underlying mechanics. Noting that P_T increases over time as long as the intracellular AI-2 concentration rises, we chose an expression that better reflects the physical scenario while ensuring that the change in P_T over time remained within estimated physiological limits. Nevertheless, it remains to be seen if the actual time rate of change of P_T is indeed consistent with our model predictions. Thus far, we have not encountered experimental studies measuring the change in periplasmic binding protein concentration in real time, while the only quantification of LsrB has been conducted by Jani et al. [4].

5.2 Results and Discussion

5.2.1 Capillary assay simulations

We first test the AI-2 indirect binding expression (Eq. 14) by conducting 2D capillary assay simulations according to the methods specified in Chapter 4. Here, our focus is on comparing the predictions made by the current model against our earlier model which employs only first-order binding kinetics. In our earlier work, we discovered that either the extracellular attractant concentration C or the dissociation constant K_{dp} must be scaled by a factor of β to account for the difference in attractant concentrations between the extracellular space and the cell periplasm. Here, we vary the product of βK_{dp} by several orders of magnitude and record the chemotactic response to a range of extracellular AI-2 concentrations (Fig. 2). The broad sweep of βK_{dp} identifies that the best agreement with past experimental data can be obtained using a value of $10 \leq \beta K_{dp} \leq 100$ (Fig. 2A). In the study of Hegde et al., the chemotactic response profile is seen to rise steadily between $C_0 = 1 \times 10^{-3} \text{ mM}$ and $C_0 = 1 \times 10^{0.5} \text{ mM}$. This is first observed in Figure 2A for the curve of $\beta K_{dp} = 10 \text{ mM}$, as predicted chemotactic responses peak at smaller attractant concentrations for lower values of βK_{dp} .

Figure 5-2: (A) Comparison of predicted chemotactic responses for the higher-order model over a range of attractant concentrations C_0 , based on increasing values of the product βK_{dp} . (B) Same as (A), with the range of βK_{dp} narrowed down to $10 \text{ mM} \leq \beta K_{dp} \leq 30 \text{ mM}$.

Thereafter, a closer inspection of βK_{dp} within the initial range identified above reveals that the chemotactic response for $\beta K_{dp} = 10\text{mM}$ begins to plateau when $C_0 > 1\text{mM}$ (Fig. 2B). Gradually increasing βK_{dp} , we observe that the predicted response resumes its steady increase at $C_0 = 1 \times 10^{0.5}\text{mM}$ for $\beta K_{dp} = 15\text{mM}$ and above, shown in Figure 2 up to a value of $\beta K_{dp} = 30\text{mM}$. In our previous study, we find that the best-fitted value of βK_{dp} is approximately 30mM , thus agreeing well with our current finding.

Interestingly, with all other parameter values kept constant from the previous study, including $X = 1 \times 10^{-4}\text{m}$, $K_{dr} = 1 \times 10^{-4}\text{mM}$ and $P_T = 1 \times 10^{-3}\text{mM}$, we find that the predicted chemotactic response for the current model is significantly smaller than the predicted response of the earlier model (Fig. 3A)., as well as from the experimental data to which the earlier response was fitted. Varying the value of X by a small extent between the range of $X = 1 \times 10^{-4}\text{m}$ and $X = 3 \times 10^{-4}\text{m}$, we find that the experimental data can be well approximated by $X = 2 \times 10^{-4}\text{m}$ (Fig. 3B). Notably, it appears that the fit obtained agrees more closely with the existing experimental data as compared with our earlier model.

Figure 5-3: (A) Comparison of predicted chemotactic response between different models and the experimental data of Hegde et al. [48]. The magnitude of response predicted by the higher-order model is roughly half that of the first-order model and the experimental data. (B) Chemotactic responses predicted by the higher-order model at different level of the chemotaxis coefficient X . A close fit to experimental data is achieved for $X = 2 \times 10^{-4}\text{m}$.

Finally, we vary the periplasmic binding protein concentration P_T between the maximum and minimum levels defined by the fully induced and fully repressed states of the Lsr system. Jani et al. [4] reported that the LsrB concentration of $\Delta lsrK$ mutants, which are unable to initiate induction of the Lsr system, is about 25% of the wild-type LsrB concentration. Conversely, the LsrB concentration is about 800% of WT levels in the $\Delta lsrR$ which is assumed to express maximal levels of Lsr proteins at all times due to an inability to repress the *lsr* operon. Comparing against our earlier predicted chemotactic response (Fig. 4A), we find that the effect of raising P_T past the wild-type level of approximately 240 copies per cell is significantly more pronounced in our current model (Fig. 4B). While levels of chemotactic response are similar at the low attractant concentrations, $C_0 \leq 1 \times 10^{-2}$ mM, responses predicted by the higher order model (Fig. 4B) are at least twice as high as those predicted by the first order model (Fig. 4A) for attractant concentrations of $C_0 = 1 \times 10^{-1}$ mM and above.

Figure 5-4: (A) Chemotactic responses predicted by the first-order model for varying levels of the periplasmic binding protein concentration P_T . (B) Chemotactic responses predicted by the higher-order model for varying levels of the periplasmic binding protein concentration P_T .

The key difference in predictions made by our current model and the previous study is the relative change in chemotactic response when P_T is raised from WT levels by one order of magnitude such that $P_T \approx 0.01$ mM. Previously, the effect of raising the level in P_T is essentially a shift in the sensitivity profile by one order of magnitude of attractant concentration towards the left (Fig. 4A). Conversely, the higher-order model predicts that the accumulated cell count remains relatively low at all tested attractant concentration levels for P_T at WT level and below (Fig. 4B). Therefore, by raising P_T by one order of magnitude, the

model predicts a many-fold increase in the expected chemotactic response, especially at the attractant concentration $C_0 = 10\text{mM}$.

The results obtained from capillary assay simulations provide us with two main observations. Firstly, it appears that the specific sensitivity profile predicted by the higher-order model provides a somewhat better fit with experimental data as compared with our earlier model (Fig. 3), although both models can certainly be said to represent the data with satisfactory accuracy. Secondly, the high model predicts a significant amplification effect to the chemotactic response when the periplasmic binding protein concentration is raised above the WT baseline of $P_T = 8.3 \times 10^{-4}\text{mM} \approx 0.001\text{mM}$.

5.2.2 Parametric analysis

To get a more complete picture of this amplifying response, we conduct a short parametric study, using the method described in Chapter 5 and based on the same Gaussian attractant concentration profile, to compare the chemotactic response predicted by the first-order and higher-order models in 1D (Fig. 5). We vary the attractant source concentration C_0 from $1 \times 10^{-3}\text{mM}$ to 1mM , followed by from 1mM to $1 \times 10^3\text{mM}$, for the three levels of periplasmic binding protein concentration P_T discussed earlier.

Somewhat unexpectedly, we find that the responses predicted by the higher-order model are generally relatively small as compared to the first order model (Fig. 5A & 5B). This observation applies for all attractant concentrations below $C_0 = 1\text{mM}$ regardless of the level of P_T applied. The amplifying effects of the raised level of P_T manifest at higher attractant concentrations of $C_0 \geq 10\text{mM}$ (Fig. 5C & 5D), seen as a spike in the chemotactic response (red curves) at various points along the x -axis. When comparing the relative width of the chemotactic responses for the higher-order model, we note that narrower curves do not represent a smaller response range, as they actually correspond to larger concentrations of C_0 and thus a commensurate increased attractant concentration range across the length L . It appears that the higher-order model predicts that the maximum chemotactic response grows gradually with attractant source concentration within the regime marked by $C_0 \geq 10\text{mM}$, while retaining a similar range of response.

Figure 5-5: (A & C) $P_t = 8.3 \times 10^{-4} \text{ mM}$. (B & D) $P_t = 8.3 \times 10^{-3} \text{ mM}$. Note that the plot of $C_0 = 1 \text{ mM}$ is the same curve in each top-bottom pair; corresponding to the dash-dotted line in the upper sub-figures and to the solid line in the lower sub-figures. Curves plotted in blue represent chemotactic response predicted by the first order model from our previous study. Curves plotted in red represent chemotactic response predicted by the higher-order model developed in the current Chapter. (A & B) At low attractant concentrations, the response predicted by the higher-order model is much lower than that predicted by the first order model. (C & D) Sharp spikes in the predicted response for the higher order model (red curves) suggest a narrow response range within which the cells appear to be highly sensitive to changes in the attractant concentration.

The one-dimensional parametric analysis reveals that the amplification of chemotactic response in the higher-order model is actually dependent upon two factors. We have already seen that a modest increase in response is observed when the attractant concentration is raised beyond a certain threshold, even at WT levels of periplasmic binding protein P_T (Fig. 5C). Increasing the concentration of P_T results in an additional amplification step of approximately

equal proportions, as the peak response climbs from $\frac{V_C}{V} \approx 0.0075$ to $\frac{V_C}{V} \approx 0.065$ for a tenfold increase in P_T (Figs. 5C & 5D, dash-dotted lines). Moreover, we observe the response range to grow significantly, essentially doubling for an order of magnitude increase in P_T . We note that under these conditions, the maximum chemotactic response predicted by the higher-order model far outstrips the prediction of the first-order model.

While the prediction of a two-part amplification effect in the higher-order indirect binding chemotaxis model introduces interesting possibilities for bacterial strategies, we note that the static models have limited applicability in accurately predicting the actual dynamic chemotactic response of *E. coli* towards the AI-2 signalling molecule. Our earlier model fitting (Fig. 1B) clearly shows that the level of periplasmic binding protein LsrB can rise significantly in the first hour of exposure to extracellular AI-2, during which the chemotactic response may potentially rise drastically as seen from comparing Figures 5C and 5D. To better understand this, we have formulated a rudimentary model to predict chemotactic response that is mediated by a dynamically varying periplasmic binding concentration.

5.2.3 Variable periplasmic binding protein concentration

The system of equations (Eq. 21) are used to conduct a capillary assay simulation and compare against predictions made by the higher-order model (Eq. 15) with static values of periplasmic binding protein concentration P_T . Keeping all other parameters the same as utilized in Figure 4B, the effect of allowing P_T to vary dynamically is, predictably, a moderate increase in the magnitude of chemotactic response between the WT level (Fig. 6, dotted line) and the fully induced level that is ten times higher than WT (Fig. 6, dash-dotted line).

Figure 5-6: Comparison of predicted chemotactic response for the variable periplasmic binding protein model (solid curve) and responses predicted by the higher-order indirect binding chemotaxis model featuring static levels of P_T .

One interesting feature of the variable P_T chemotaxis model is that the slope of the predicted sensitivity curve becomes shallower over two orders of magnitude of the extracellular attractant concentration, rather than culminating in a sharp peak that is observed in the maltose chemotactic response (Chapter 4) as well as in the experimental data for AI-2 chemotaxis in the study of Hegde et al. [48]. We expect that the reason for this can jointly be attributed to the change in magnitude of chemotactic response as the P_T concentration increases, as well as the dilution of attractant due to uptake. Evidently, the effects of diminishing attractant source strength are more pronounced with higher initial attractant concentration levels as the rate of synthesis of LsrB increases correspondingly.

The predictions made by the variable P_T model are not easily tested against experimental data, making it challenging to proceed with the full analysis of the total AI-2 response that can be exhibited by *E. coli*. On one hand, there is currently scarce data in the literature pertaining to detailed, thorough and quantitative AI-2 chemotaxis experiments. On the other, the use of empirical constants and uptake rates fitted to singular data sets become a hindrance as the model complexity increases, as they can cause the model to lose generality by only describing a specific set of results under environmental conditions that may not be fully understood. It is clear that further steps in this research are best to be taken with a joint experimental and modelling approach, with the existing models serving to inform us of the kind of experiments to be conducted and the data to be obtained.

5.3 Chapter Summary

In this study, we have formulated higher-order indirect binding models to specifically address the conditions pertaining to the chemotactic response of *E. coli* towards AI-2. Unlike nutrient-based attractants, the concentration of periplasmic binding protein LsrB is significantly lower than the total receptor concentration, leading to the unusual situation of potential ligand depletion in both the substrate and binding protein species. Compared to the first-order model, the higher-order model predicts overall lower chemotactic responses when the extracellular attractant concentration is dilute. Interestingly, the predicted response can then be amplified by two factors. Sharp spikes in the chemotactic response can be observed when the attractant concentration is raised above a certain threshold. Thereafter, the magnitude of chemotactic response of the spikes can grow by several orders of magnitude in response to increasing levels of periplasmic binding protein concentration. In contrast, chemotactic responses predicted by the first order model involve relatively small changes in response magnitude and the sensitivity profile can be observed to shift along the attractant concentration range with respect to changes in binding protein concentration.

As an exploratory study, we developed a model for predicting chemotactic response mediated by dynamically varying periplasmic binding protein concentration as a function of AI-2 uptake. As expected, the model appears to predict a chemotactic response that lies between the low and high levels of response, although we note that the peak response remains significantly lower than the case of maximum LsrB expression. It is challenging to probe further into the relationship between LsrB concentration and chemotactic response dynamics at this stage, using either modelling or experimental approaches alone. While it is not clear at this point whether the predictions made by the higher-order model developed in this study are indeed a better representation of the AI-2 chemotactic behaviour, we note that chemotactic responses based on static values of periplasmic binding protein concentration P_T may not present a complete picture of the response dynamics. Thus far, we have conducted verification of the interesting details uncovered in the 1D parametric analyses through corroboration of bacterial accumulation in capillary simulations with experimental data in the literature. This approach has proven fruitful and generally has achieved excellent agreement between the experimental chemotaxis data, the independently reported values for chemotactic and binding kinetic parameters, and the predicted outcome of both the first-order and higher-order models.

Nevertheless, the choice of bacterial cell count as the key metric of comparison is not without its hazards as it relies on the accuracy of plate count techniques as well as a significant amount of guesswork involved in the average cell density at the start of chemotaxis experiments. Moving forward, this extended exercise in bacterial chemotaxis modelling through Chapters 4 to 6 serves as a valuable foundation in developing a systematic approach to obtaining specific and useful experimental verification.

Chapter 6

Conclusion and Future work

6.1 Conclusion

To conclude, we initiated the work presented in this thesis to develop a model for representing the chemotactic response of *E. coli* towards signalling molecule AI-2. The first model developed is based on only first-order kinetics, which makes several limiting assumptions regarding the relative concentrations of ligand, binding protein and receptor. We find that the model is well validated by simulating the chemotactic response to the well-studied indirectly-binding attractant maltose and comparing against experimental results in the literature. Our model is able to correctly predict that the sensitivity profile shifts by one order of magnitude towards more dilute attractant concentration when the periplasmic binding protein concentration is raised by a commensurate amount. Furthermore, the sensitivity profile decreases in magnitude but does not translate horizontally for a decrease in periplasmic binding protein concentration, which agrees with experimental data as well. The model is able to achieve good agreement for predicting chemotactic response towards AI-2 when compared with the experimental data of one capillary assay study. However, predicted responses at various other levels of the periplasmic binding protein deviates significantly from experimental data that is presented in an alternative work.

Our parametric analysis suggests possible strategies for bacterial chemotaxis that can make use of the indirect binding mechanism. Observing that the predicted response for the first-order model grows in magnitude and shifts towards dilute attractant concentrations when the periplasmic binding protein concentration is raised, we believe that this allows bacteria to greatly increase their sensitivity to dilute nutrient gradients at the expense of maintaining a higher concentration of binding protein. This may be a cost-benefit analysis for the cell to avoid starvation by channelling resources to maximise chemotactic sensitivity and response. We note that the observation may not apply to the case of AI-2 as it is not known to be a nutrient for *E. coli* and thus there may not be a reason to respond strongly to dilute AI-2.

The unusual characteristics of the variable binding protein concentration and the low base levels of binding protein relative to receptors in the AI-2 system of *E. coli* prompt us to develop a higher-order model that properly addresses the ligand depletion scenario. In this model, we assume that the receptor concentration is larger than the periplasmic binding protein concentration by a factor of ten, simplifying the free receptor concentration to be approximately the same as the total receptor concentration. We find that the higher-order model gives a closer fit with the experimental results as compared to the first-order model with all kinetic parameters kept the same, while raising the chemotaxis coefficient by a small amount to scale up the chemotactic response in the higher-order case. Finally, we formulate a basic model for the AI-2 uptake and chemotactic response to allow us to observe the effect of varying the periplasmic binding protein concentration dynamically as a function of intracellular AI-2. Without a suitable point of experimental comparison, however, we are unable to fully validate our observations regarding the predicted sensitivity profile.

6.2 Future work

The ultimate goal of this research topic is to develop a better understanding of the population-scale dynamics of *E. coli* behaviour and their implications on its ecology and life-cycle. By doing so, we envision that it will be possible to develop methods for accurately predicting bacterial transport and colonisation events in engineering structures that can come into contact with microbiological contaminants.

Undoubtedly, the modelling efforts can be redoubled to include more aspects of bacterial behaviour, such as a full synthesis-uptake-chemotaxis model. However, we believe that the reliance on estimation and fitting of empirical constants results in limited applicability of a developed model to general scenarios. Thus, we are interested in developing the experimental arm of this research topic before proceeding with advanced modelling methods. The work completed thus far is suitable for providing a better understanding on the type of experiments that need to be conducted to allow rigorous testing of existing models.

We believe that a more thorough set of capillary assay simulations will be invaluable in testing the limits of the developed models. To obtain more data points, it will be necessary to quantify bacterial accumulation at more time intervals as well as for a greater range of attractant concentrations. It may be more economical and enlightening to carry out this task using microfluidic capabilities. We are looking into the use of stopped-flow microfluidic

chambers for observation of bacterial transport, where attractant concentration gradients can be applied to the chamber through porous membranes that are impervious to particles at the size of the cells. By recording cell chemotactic behaviour through real-time microscopy instead of through plating techniques, the method of quantification of bacterial chemotaxis changes dramatically to a more mechanistic context based on analysis of directional persistence of individual cells.

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