Supplementary Material (ESI) for Molecular BioSystems

Supplementary information for

Mathematical modeling and Experimental validation of chemotaxis under controlled gradients of methyl-aspartate in *Escherichia coli*

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1 Experimental details

Microorganism

Escherichia coli K-12 (MTCC 1302) lacking Trg receptor was used throughout this study. The strain was obtained from MTCC, IMTECH Chandigarh, India.

Media

The motility buffer (MB) contained (/l of distilled water) K_2HPO_4, 11.2 g; KH_2PO_4, 4.8 g; $(NH_4)_2SO_4$, 2 g; $MgS0_4.\ 7H_20$, 0.25 g; PVP, 1 g; and EDTA, 0.029 g.[1] Luria-Bertani (LB) broth contained LB (/l of distilled water) 25 g. Tryptone medium contained (/l of distilled water) tryptone, 10 g and Nacl, 5 g. Different concentrations of chemotaxis medium was prepared by adding different amounts of MeAsp to the motility buffer under sterilized conditions.

Growth conditions

The growth conditions of the bacteria are critical to the success of the experiments. In the present work, the bacteria were grown as per the following procedure: One loop full of culture from the slant was inoculated into LB media and allowed to grow for 9 hours in the exponential growth phase. The incubation was always carried out at 37^{o}C and 240 rpm. After the culture was grown for 9 hours, 1 ml of the culture broth from LB medium was transferred to tryptone medium and allowed to grow for 6 hours (exponential phase). To ensure that the cells are adapted to tryptone medium, 10 ml of sample was transferred to tryptone medium and grown again for 4 hours (early exponential phase). The biomass was separated by taking 50 ml of the culture into sterilized tubes and centrifuged at 4000 rpm for 10 minutes. The supernatant was decanted and the settled pellet was gently re-suspended in 10 ml motility buffer. The above procedure was repeated three times before introducing the cells into the capillary. High levels of bacterial motility were observed on viewing the cells under an optical microscope. Finally for the chemotaxis measurements, cells were introduced gently by touching the pellet with the mouth of the capillary. The concentration of cells in the capillary was determined using agar plates.

Establishment of 2-NBDG gradients

The micro-capillaries were first sterilized and then were marked with graduations spaced at 0.25 cm along its length. The concentration gradients were first established using different concentrations of 2-(N-(7-nitro-benz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxy-glucose (2-NBDG, a fluorescent glucose) solutions using motility buffer. In order to calibrate the intensity of 2-NBDG solutions, a 5 cm plug of 2-NBDG solution was drawn in the capillary and the ends were sealed with wax. The fluorescence intensity was found to vary linearly with 2-NBDG concentration up to 1000 μM. The calibration chart is shown in Fig. S1(A).

Experiments were performed for establishing steady linear concentration gradients of 2-NBDG. A 4.5 cm liquid plug containing a fixed concentration of 2-NBDG was

drawn in the capillary followed by about 0.5 cm plug of a motility buffer without 2-NBDG. Approximately 10^7 bacteria/ml were taken into the capillary by contacting the pellet (as described earlier) with the mouth of the capillary. Then, the capillary ends were sealed with wax. A schematic diagram of the micro-capillary experimental setup is shown in Fig. S2. Using a microscope with an 4X (numerical aperture = 0.13) objective lens, the fluorescence intensity of 2-NBDG was measured over $0 < x < 1500$ μm as a function of time shown in Fig. S1(B) and the results are shown in Fig. S1(C). It is noticed that within the first 1.5 min, the intensity profiles attains the steady state and there is a negligible change in the concentration profile up to 30 min. These profiles were robust and easily reproducible and were not effected neither by the cell movements nor by the consumption of 2-NBDG by this strain. Further, for the quantification experiments, MeAsp was used instead of 2-NBDG. It is assumed that the gradients obtained with MeAsp is identical to 2-NBDG, since the molecular weight of the two are similar and so the diffusivity in water would also be approximately the same.

Quantification of chemotaxis

Image analysis was used to quantify the movements of *E.coli*. This involves the analysis of moving objects in image sequences. In this section, specifications of optical microscope, image processing and procedure for calculating the cell movements are discussed.

Specifications of optical microscope: IX71 Inverted Microscope (Olympus, Japan) was adopted as the optical microscope. Images were taken using Evolution VF Cooled Monochrome camera (Media Cybernetics, Japan). Bright-filed (BF) illumination with magnification of 40X objective lens (numerical aperture = 0.75).

Image processing procedure: Image-Pro Plus 6.0 image analysis program was used to locate *E.coli* in each frame and follow its motion in subsequent frames. This program uses a single stack TIFF image which contains 500 sequential TIFF images taken at an interval of 0.11 s. In every experiment two stack images were recorded at 500, 1000 and 1500 μm. Note that, the image captured using 40X magnifications encom-

passed a physical area measuring $160 \times 120\ \mu\mathrm{m}^2$.

Procedure for calculating the cell movement: To minimize errors in finding the cell movements, the following two assumptions were made. First, the cells moving close to each other or overlapping cell movements were neglected. Secondly, the cells not in the field of view for the entire time series (minimum 1.1 s) were neglected.

Using Matlab©, each stack image was thresholded so as to clearly distinguish cells from the image background. Then, with the help of the auto tracking option, the movement of cells along the gradient direction (x) was tracked in a sequence of images. The raw data containing spatial location of each cell as a function of time was exported to an Excel file. The procedure for calculating the movement of cell over a period of time was straight forward. Starting from an initial position in the first frame, the displacements between two consequent frames 0.11 s apart were determined. The tracking data with time was collected from six identical experiments for analysis.

2 Model equations for the intracellular pathway

As mentioned in the main text, the modeling of the intracellular pathway, closely follows that of Barkai and Leibler.[2] Let T_i represent the concentration of receptor complexes with i residues methylated and $\alpha_i(L)$ denote the probability that the receptor complex T_i is active when the concentration of chemoattractant is L. The receptor complex can be in one of five methylation states with i = 0, 1, 2, 3 or 4 methyl groups. The total concentration of active receptors is given by,

$$T^A = \sum_0^4 \alpha_i(L) T_i, \tag{S-1}$$

while the total concentration of inactive receptors is given by,

$$T^I = \sum_0^4 (1 - \alpha_i(L)) T_i. \tag{S-2}$$

The binding kinetic equation for active receptor complex is given by,

$$T_F^A + L \rightleftharpoons [T^A L] \tag{S-3}$$

The total active receptor complex concentration T_T^A is given by,

$$T_T^A \rightleftharpoons T_F^A + [T^A L] \tag{S-4}$$

where T_F^A is free (non-ligand bound) active receptor complex concentration and $[T^A L]$ is the ligand bound active receptor complex concentration respectively. The fraction of free active receptor complex concentration from the above equation is given by,

$$\frac{T_F^A}{T_T^A} = \frac{K_L}{K_L + L} \tag{S-5}$$

where K_L is the ligand dissociation constant. Similarly, the fraction of ligand bound receptor complex concentration is given by,

$$\frac{[T^A L]}{T_T^A} = \frac{L}{K_L + L} \tag{S-6}$$

The total probability of the receptor complex being in active state is the sum of the probabilities of the ligand bound and non ligand bound being in active state and is given by,

$$\alpha_i(L) = \frac{\alpha_i^L L}{K_L + L} + \frac{\alpha_i^0 K_L}{K_L + L} \tag{S-7}$$

where the parameters assigned the following numerical values are, α_0^L = 0, α_1^L = 0, α_2^L = 0.1, α_3^L = 0.5, α_4^L = 1, α_0^0 = 0, α_1^0 = 0.1, α_2^0 = 0.5, α_3^0 = 0.75, α_4^0 = 1 and K_L = 1 μM.

These values are taken from Morton-Firth et. al.[3] and are estimated from the free energy states of methylation and the ligand occupancy of receptor complex for MeAsp. The corresponding phosphorylation rate equations with the corresponding rate con-

stants[4] are given by,

$$\frac{dA_p}{dt} = 23.5(T^A)A - 100(A_p)Y - 10(A_p)B \tag{S-8}$$

$$\frac{dY_p}{dt} = 100(A_P)Y - 30(Y_p) \tag{S-9}$$

$$\frac{dB_p}{dt} = 10(A_p)B - (B_p) \tag{S-10}$$

Here, A, A_P, Y, Y_p, B and B_p represent, respectively, the concentrations of CheA, phosphorylated CheA, CheY, phosphorylated CheY, CheB and phosphorylated CheB. Li and Hazelbauer[5] have measured these chemotaxis protein concentrations for wild type and are given by, $A + A_p$ = 5.3 μM, $B + B_p$ = 0.28 μM, $Y + Y_p$ = 9.7 μM and CheR (R) = 0.16 μM. The total receptor concentration (Tar+Tsr), $T_0 + T_1 + T_2 + T_3 + T_4$ = 17 μM and R = 0.16 μM reflecting the reported[6] findings that both Tsr and Tar participate in sensing aspartate.

The Barkai and Leibler[2] model assumes that CheR (R) binds to the inactive receptors (T^I) and the phosphorylated CheB (B_p) binds to the active receptors (T^A). Assuming that, the methylation and demethylation reactions follows Michelis - Menten kinetics, the rate of demethylation and methylation is given by, respectively,

$$r_B = \frac{k_b(B_p)}{K_B + T^A} \tag{S-11}$$

$$r_R = \frac{k_r(R)}{K_R + T^I} \tag{S-12}$$

where, k_b = 0.6 s^{-1} and k_R = 0.75 s^{-1} are the rate constants and K_B = 0.54 μM and K_R = 0.39 μM are the Michaelis constants[4] for receptor demethylation and methylation, respectively.

The rate of methylation is proportional to the concentration of inactive receptors

(1-$\alpha_i(L)$)T_i, and the rate of demethylation is proportional to the concentration of active receptors $\alpha_i(L)\ T_i$. For the receptor T_i, the rate of demethylation is $r_B\ \alpha_i(L)\ T_i$ and the rate of methylation is $r_B\ (1-\alpha_i(L))\ T_i$, the mass balance equations for the corresponding receptor can be given by,

$$\frac{dT_0}{dt} = -r_R(1-\alpha_0(L))T_0 + r_B\alpha_1(L)T_1 \tag{S-13}$$

$$\frac{dT_1}{dt} = -r_R(1-\alpha_1(L))T_1 + r_B\alpha_2(L)T_2 + r_R(1-\alpha_0(L))T_0 - r_B\alpha_1(L)T_1 \tag{S-14}$$

$$\frac{dT_2}{dt} = -r_R(1-\alpha_2(L))T_2 + r_B\alpha_3(L)T_3 + r_R(1-\alpha_1(L))T_1 - r_B\alpha_2(L)T_2 \tag{S-15}$$

$$\frac{dT_3}{dt} = -r_R(1-\alpha_3(L))T_3 + r_B\alpha_4(L)T_4 + r_R(1-\alpha_2(L))T_2 - r_B\alpha_3(L)T_3 \tag{S-16}$$

$$\frac{dT_4}{dt} = r_R(1-\alpha_3(L))T_3 - r_B\alpha_4(L)T_4 \tag{S-17}$$

Using Matlab©, we solved simultaneously the steady state phosphorylation reaction equations and the mass balance equations for the receptors. The optimum parameters of the simulated model are reported in Table S1. The details of ligand concentration gradient obtained from experiments and used in simulation are reported in Table S2.

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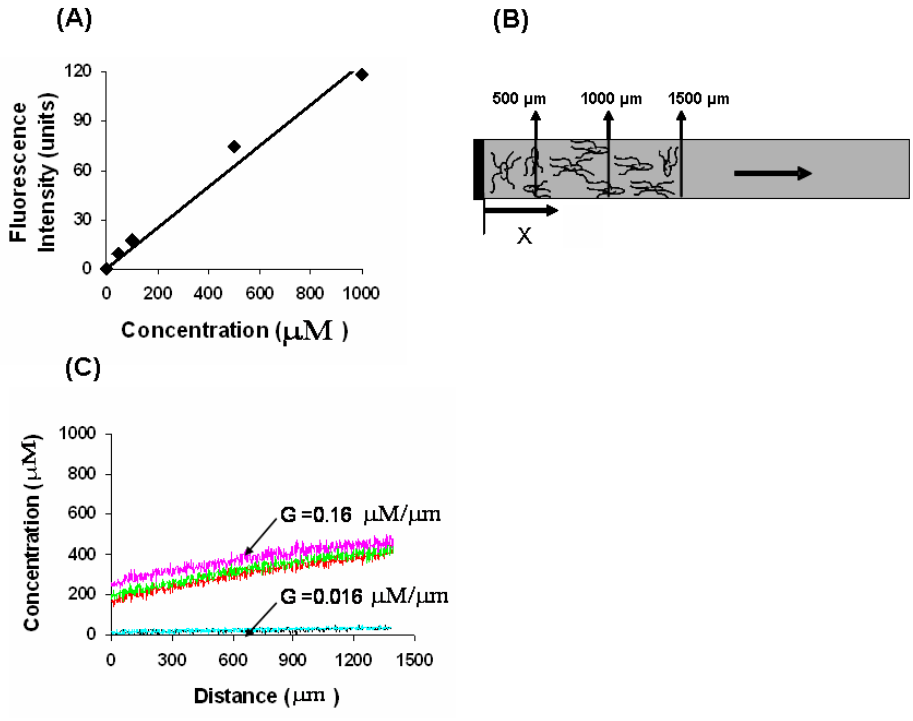

Figure S1: (A) Linear Calibration curve; Fluorescence intensity with varying concentration of 2-NBDG (B) A magnified view of the micro-capillary set-up (C) Stable Linear Gradients; Measured concentration profiles for two gradients: $t = 1.5$ min (black line) and $t = 5$ min (blue line) for $G = 0.016\ \mu$M/μm, t = 1.5 min (red line), $t = 5$ min (green line) and $t = 30$ min (pink line) for $G = 0.16\ \mu$M/μm respectively.

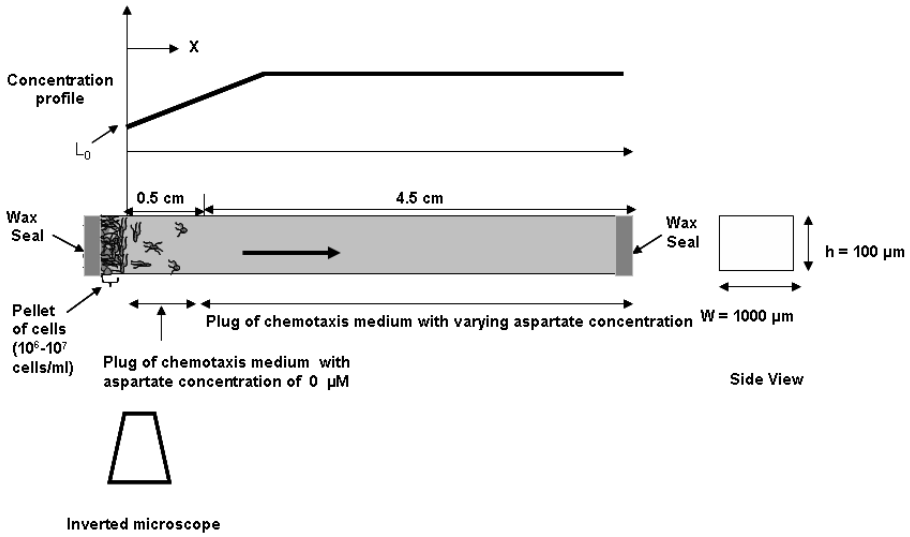

Figure S2: Micro-capillary experimental setup for establishing methyl-aspartate gradients. $x = 0$ is located at the end of the pellet with a ligand concentration, L_0. The experimental values of L_0 and the established gradient (G) is given in Table S2 and these conditions were used in the model simulations

Table S1: Parameters used to simulate the model

Description	Present study	Reported
CheA concentration (A)	5.3 μM	5-5.3 μM ([3,4,7,8])
CheY concentration (Y)	9.7 μM	9.7-18 μM ([3,4,7,8])
CheR concentration (R)	0.16 μM	0.16 - 0.3 μM ([3,4,7,8])
CheB concentration (B)	0.28 μM	0.28 - 2.27 μM ([3,4,7,8])
Receptor concentration (Tar+Tsr)	17 μM	5 - 17 μM ([4,7])
Ligand dissociation constant (K_L)	170 μM	1 - 100000 μM ([2,9,10])
CheR catalytic rate (K_R)	0.39 μM	0.099 - 0.39 μM ([3,4,7,8])
CheB-P catalytic rate (K_B)	0.54 μM	0.54 - 5.5 μM ([3,4,7,8])
CheR Michaelis-Menten (k_r)	0.624 s^{-1}	0.255 - 0.819 s^{-1} ([3,4,7,8])
CheB-P Michaelis-Menten (k_b)	1.2 s^{-1}	0.155 - 6.3 s^{-1} ([3,4,7,8])
CheA autophosphorylation rate	23.5 s^{-1}	15.5 - 50 s^{-1} ([3,4,7,8])
CheY phosphorylation rate	100 μM^{-1} s^{-1}	3 - 100 μM^{-1} s^{-1} ([3,4,7,8])
CheY-P dephosphorylation rate	30 s^{-1}	14.15 - 30.1 s^{-1} ([3,4,7,8])
CheB phosphorylation rate	10 μM^{-1} s^{-1}	3 - 30 μM^{-1} s^{-1} ([3,4,7,8])
CheB-P dephosphorylation rate	1 s^{-1}	0.35 - 1 s^{-1} ([3,4,7,8])
Steady state CheY-P concentration	2.5 μM	2.2 - 4.4 μM ([7,11,12])
Intracellular Hill coefficient (n)	50	10 - 50 ([11,13,14])

Table S2: Gradients and ligand concentration at $x = 0$ obtained through experiments. These conditions were also used in the model simulation.

S. No.	Concentration (L_0, μM) at $x = 0$ cm	Gradient (G, μM/ μm)
1	16	0.016
2	80	0.08
3	160	0.16
4	1600	1.6