Supplementary Information to:

Quantitative chemical biosensing by bacterial chemotaxis in microfluidic chips

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This supplementary information contains:

- Supplementary Figures S1-S6
- Supplementary Tables S1, S2
- Metropolis script

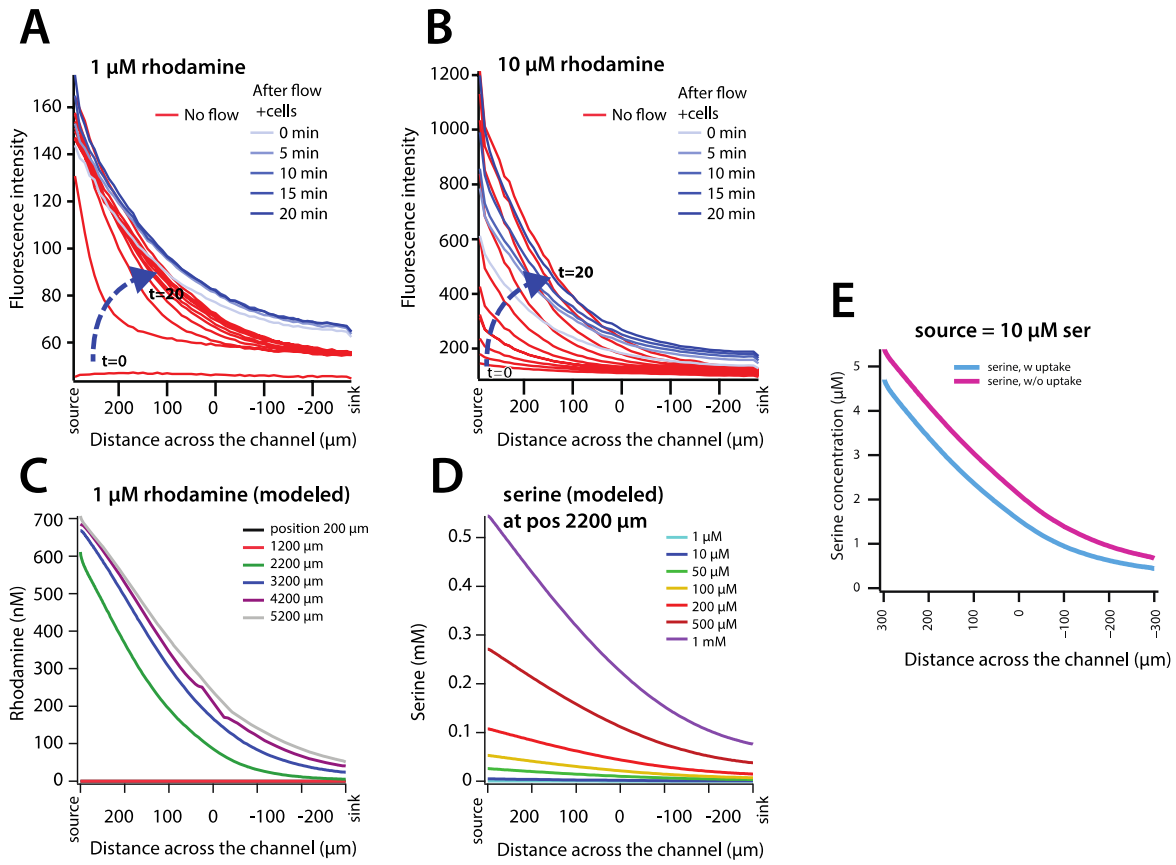

Figure S1. Gradient formation across the observation (middle) channel of the chip. Fluorescence profile formation across the channel without flow with (A) 1 µM or (B) 10 µM rhodamine in the source channel during 20 min (*red* lines, every 2 min), and after starting *E. coli* cell flow in the observation channel for another 20 min (*blue* lines, every 5 min). (C) Modeled rhodamine steady-state gradients at different transects along the observation channel. (D) Modeled serine gradients across the observation cell channel at position 2200 µm for seven source concentrations, as indicated, at the flow conditions computed as in Figure 1C. (E) Modeled effect of serine metabolism of *E. coli* on the established gradient at 10 µM serine source concentration and position 2200 µm in the observation channel. Note that for ease of comparison to the chip design, the distance across the channel is represented from 300 to –300 µm.

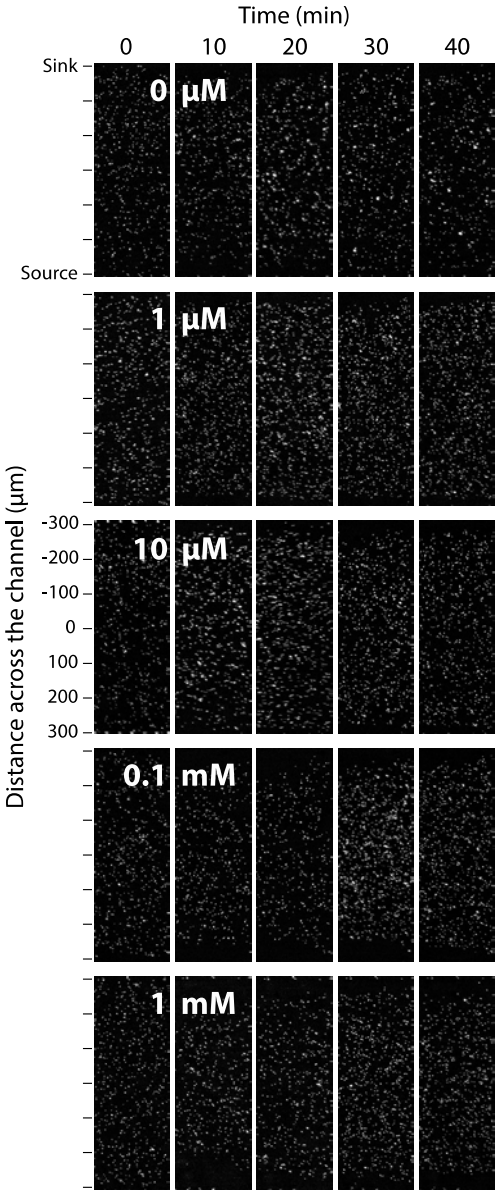

Figure S2: Non-chemotactic cell distribution of *E. coli* Δ*fliC*-mcherry toward serine. Images showing the distribution of Δ*fliC*-mcherry at the 2100-2200 µm location on the chip over time (0 - 40 min) as a function of the indicated serine concentration. Top is sink channel, bottom is source channel.

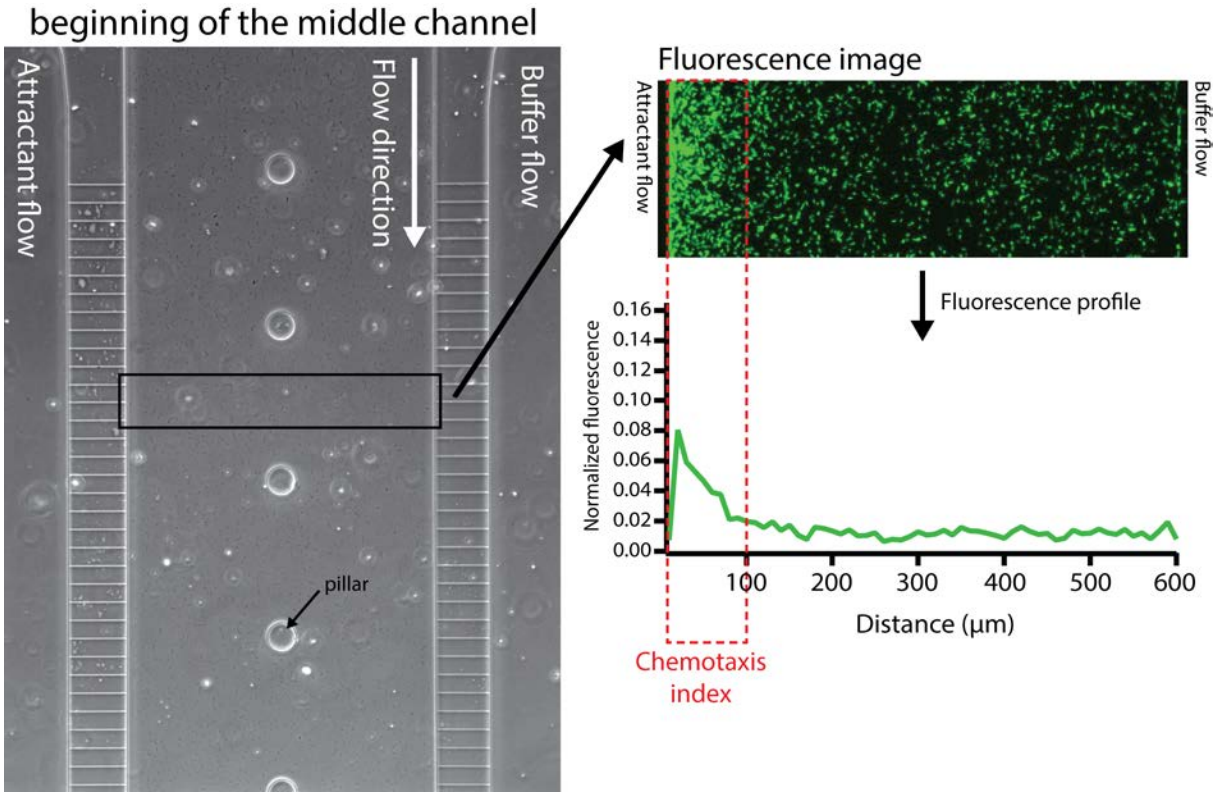

Figure S3: Chemotaxis index measurement setup. Chemotaxis response was quantified in a zone of 600 x 100 microns at a distance of 400 microns from the beginning of the filters. Fluorescence intensity profiles were extracted from the fluorescence images using ImageJ and normalized by the total fluorescence in the zone of measurement. The chemotaxis index was calculated as the proportion of fluorescence in the 100 µm segment closest to the source of attractant compared to the total fluorescence across the channel.

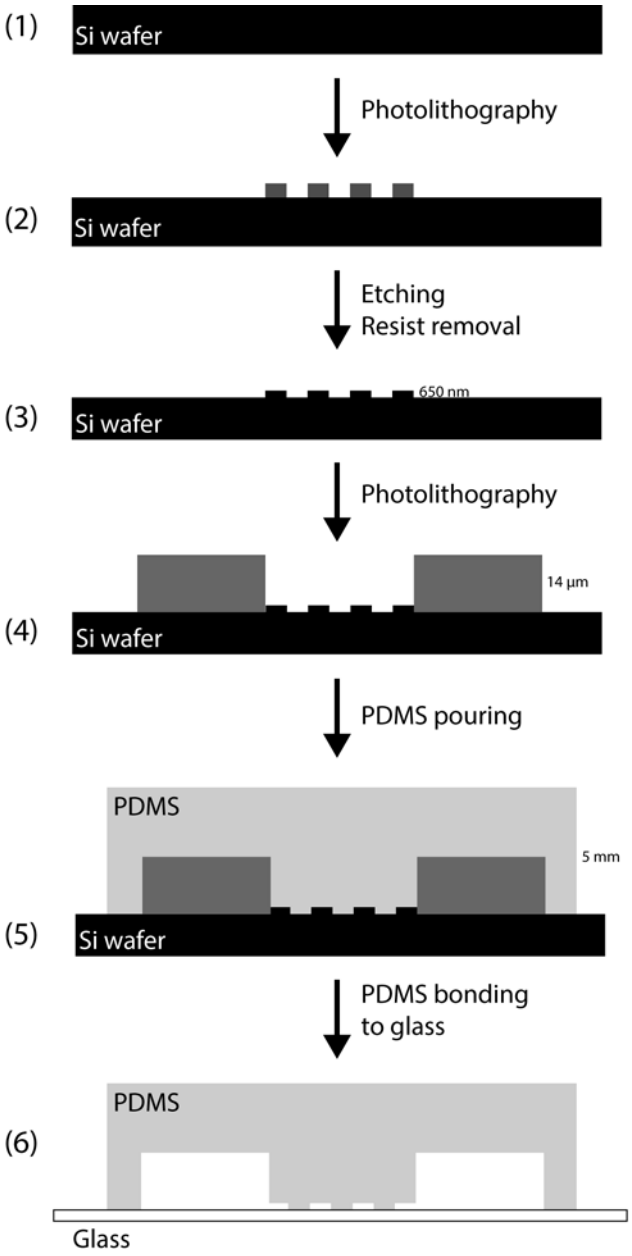

Figure S4: Microfluidic chip fabrication procedure. The fabrication procedure starts with a silicon wafer (1). A photolithography process produces a layer of resist at the filter position that protects this zone during the etching step (2). The etching results in the formation of the negative of the 650 nm high channels of the filters (3). A second step of photolithography produces the mold of thechannels with a resist layer of 14 microns high (4). This inverted mold is used multiple times to produce the PDMS chips, by pouring PDMS on it and let polymerize (5). Once polymerized, the PDMS is peeled off the inverted mold and, after punching holes for the inlets, is bonded to the glass slide by a plasma treatment (6).

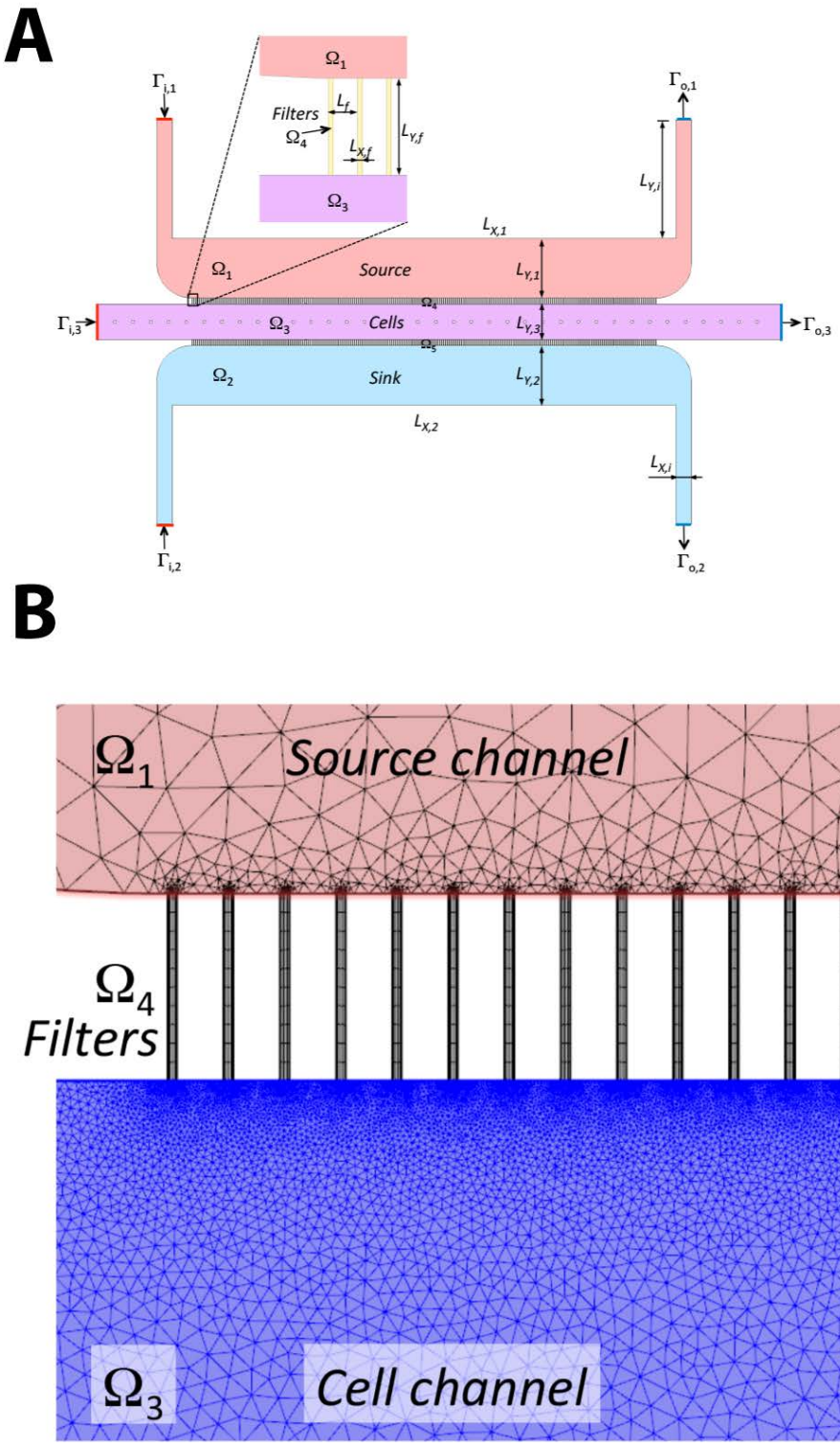

Figure S5. **(A)** Model geometry, dimensions, domains and boundaries. Ω_1: Source domain (fed with chemoattractant solution), Ω_2: Sink domain (fed with water), Ω_3: Cells domain (fed with a suspension of cells), Ω_4 and Ω_5: Filter domains (separate the cells from source and sink channels). $\Gamma_{i,1}$, $\Gamma_{i,2}$, $\Gamma_{i,3}$: Inflows, $\Gamma_{o,1}$, $\Gamma_{o,2}$, $\Gamma_{o,3}$: Outflows. The geometry dimensions are listed in Table S1. **(B)** Finite element mesh detail in the neighborhood of the filter region.

Table S1. Parameters of the continuum steady-state model

Description	Symbol	Value	Units	Source
Geometry				
Height (*z*-direction)				
- channel	h	14	μm	experimental
- filter		0.25	μm	
Cell channel				
- width	$L_{Y,3}$	0.6	mm	experimental
- length	$L_{X,3}$	11.5	mm	
Source and sink channels				experimental
- width	$L_{Y,1}$, $L_{Y,2}$	1	mm	
- length	$L_{X,1}$, $L_{X,2}$	9	mm	
Inlet/outlet channels				experimental
- width	$L_{X,i}$	0.26	mm	
- length	$L_{Y,i}$	2	mm	
Filters				experimental
- width	$L_{X,f}$	5	μm	
- length	$L_{Y,f}$	100	μm	
Filter spacing	L_f	30	μm	experimental
Flow				
Water viscosity (at 20°C)	μ	0.001	Pa s	-
Water density	ρ	1000	kg m^{-3}	-
Flow rate source/sink channels inlet	$F_{in,l}$	0.25	μL min^{-1}	experimental
Flow rate cell channel inlet	$F_{in,m}$	0.003	μL min^{-1}	experimental
Solutes				
Diffusion coefficient[a)]				
- rhodamine B	D_S	3.6×10^{-10}	m^2 s^{-1}	(Culbertson *et al.*, 2002) (Ma *et al.*, 2005)
- serine		8.9×10^{-10}		
Serine concentration in inflow	$c_{S,i}$	1, 10, 20, 50, 100, 200, 500, 1000	μmol L^{-1}	experimental
Maximum serine uptake rate	v_{max}	338	nmol $mmol^{-1}$ min^{-1}	(Kayahara *et al.*, 1992)
Michaelis-Menten half-saturation coefficient	K_m	6	μmol L^{-1}	(Kayahara *et al.*, 1992)
Cells				
Basic motility coefficient	D_X	3×10^{-9}	m^2 s^{-1}	estimated
Chemotaxis sensitivity coefficient	χ_0	8×10^{-4}	cm^2 s^{-1}	estimated after (Chen *et al.*, 1998)
Receptor-ligand dissociation constant	K_C	25–30	μM	estimated after (Kalinin *et al.*, 2009)
Cell concentration in inflow	$c_{X,i}$	10	mmol L^{-1}	experimental
Maximum cell density	$c_{X,max}$	$10\times c_{X,i}$	mmol L^{-1}	[b)]

a) corrected for 20 °C

b) To calculate the biomass in mM we use a typical elemental formula of *E. coli* cells of $CH_{1.8}O_{0.5}N_{0.2}$ that corresponds to a molecular dry weight of 24.6 g/C-mol biomass. At an estimated individual *E. coli* cell weight of 300 fg and a starting suspension of $8 \cdot 10^8$ cells per mL, this corresponds to 10 C-mmol biomass L^{-1}.

Table S2. Parameters, variables and functions used in the Metropolis model

Description	**Symbol in script**	**Symbol in main text**	**Value**	**Units**	**Source**
Counter for simulation steps		*s*	0, 1, 2, ..., 1200	steps	1200 corresponds to 60 min
Distance per time step of simulation	d		20	µm	Chosen
Grid width	h	L_y	600	µm	Channel width
Grid length	l	L_x	2500	µm	Channel length
Cell simulation window position			2000–2500	µm	
Random angle	r	θ	rand(0:360)	degrees	
Chemotactic sensitivity	ksi_0	ξ	$1 \cdot 10^5$	-	(Chen *et al.*, 1998)
Receptor-ligand dissociation constant	c_dis	K_C	0.03	mM	(Kalinin *et al.*, 2009)
Free parameter Gibbs-Boltzmann equation	beta	β	-0.000013	-	Fitting
Correction factor	alpha	α	0.4	-	Fitting
Gradient					
Local serine concentration in y-direction for inlet concentration	g(y)	$c_S(p)$		mM	
0 mM ser			0	mM	Definition
0.001 mM ser			-0.0006/(0.715*exp(-0.0056*y)+0.684)+0.00093	mM	Fitting
0.01 mM ser			-0.009/(1.027*exp(-0.005*y)+1)+0.0095	mM	Fitting
0.1 mM ser			-0.09/(1.34*exp(-0.005*y)+1.04)+0.092	mM	Fitting
1 mM ser			-0.9/(1.5*exp(-0.0054*y)+1.03)+0.9	mM	Fitting
Mean gradient					
Local serine starting concentration for inlet concentration	c	$c_{S,0}$		mM	
0 mM			1	mM	Arbitrary
0.001 mM ser			0.00028	mM	Fitting
0.01 mM ser			0.00236	mM	Fitting
0.1 mM ser			0.0268	mM	Fitting

1 mM ser			0.279	mM	Fitting
Adaptation function		*a(t)*	$1/(1+e^{-k*(\frac{s}{100}-s_0)})$		Logistic function
steepness	k	*k*			
mid-sigmoidal inflection point on *simulation*-axis	s_0	s_0			
0 mM ser			1		Definition
0.001 mM ser			k=2.5; s_0=0.01	-	Fitting
0.01 mM ser			k=2; s_0=0.1	-	Fitting
0.1 mM ser			k=1.5; s_0=2	-	Fitting
1 mM ser			k=1, s_0=3	-	Fitting

Metropolis script

```
function metropolis(x,y,g,d,c_dis,ksi_0,beta,alpha,a,c)

#x,y; starting position on the (x,y) grid within cell accumulation window position
#Number of simulation steps: 1200 (corresponding in the experiments to 60 min; so ~3 s equivalent to each simulation step). This function is called at each time step.
#Number of cells: 5000
#Number of simulations: 5
#sind(r): sinus in degrees of random angle
#cosd(r): cosinus in degrees of random angle
#rand(): random number between 0 and 1 from a uniform distribution
#h=600 (grid width)

## function H ##
   r = rand(0:360);

   Hx = ksi_0*(c_dis*c/(c+c_dis)^2)^alpha*(g(y)/c);
   Hy = ksi_0*(c_dis*c/(c+c_dis)^2)^alpha*(g(y+d*sind(r))/c);

## metropolis algorithm ##

   if sind(r)<=0
      if y <= -d*sind(r)
         y = 0
         x = x + d*cosd(r)
      else
         y = y + d*sind(r)
         x = x + d*cosd(r)
      end

   else
      if exp(-beta*a*(Hy-Hx)) >= rand()
         if y >= h - d*sind(r)
            y = h
            x = x + d*cosd(r)
         else
            y = y + d*sind(r)
            x = x + d*cosd(r)
         end

   end

   return [x,y]
end
```

Supplementary references

Chen, K.C., Ford, R.M., and Cummings, P.T. (1998) Perturbation expansion of Alt's cell balance equations reduces to Segel's one-dimensional equations for shallow chemoattractant gradients. *SIAM J Appl Math* **59**: 35-57.

Culbertson, C.T., Jacobson, S.C., and Ramsey, J.M. (2002) Diffusion coefficient measurements in microfluidic devices. *Talanta* **56**: 365-373.

Kalinin, Y.V., Jiang, L., Tu, Y., and Wu, M. (2009) Logarithmic sensing in *Escherichia coli* bacterial chemotaxis. *Biophys J* **96**: 2439-2448.

Kayahara, T., Thelen, P., Ogawa, W., Inaba, K., Tsuda, M., Goldberg, E.B., and Tsuchiya, T. (1992) Properties of recombinant cells capable of growing on serine without NhaB Na+/H+ antiporter in *Escherichia coli*. *J Bacteriol* **174**: 7482-7485.

Ma, Y., Zhu, C., Ma, P., and Yu, K.T. (2005) Studies on the diffusion coefficients of amino acids in aqueous solutions. *J Chem Eng Data* **50**: 1192-1196.