Supplementary Material for: Diffusiophoresis in Cells: a General Non-Equilibrium, Non-Motor Mechanism for the Metabolism-Dependent Transport of Particles in Cells

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<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacteria cell size</td>
<td>1 µm</td>
</tr>
<tr>
<td>total protein concentration</td>
<td>$c_p \simeq 3 \times 10^6 / \mu m^3$</td>
</tr>
<tr>
<td>in cytoplasm</td>
<td>$c_p \simeq 3 \times 10^6 / cell$</td>
</tr>
<tr>
<td>total metabolite concentration</td>
<td>$c_M \simeq 10^8 / \mu m^3$</td>
</tr>
<tr>
<td>in cytoplasm</td>
<td>$c_M \simeq 200 \ mM$</td>
</tr>
<tr>
<td>ATP concentration</td>
<td>$c_{ATP} \sim 10^7 / \mu m^3$</td>
</tr>
<tr>
<td>in cytoplasm</td>
<td>$10^7 / cell$</td>
</tr>
<tr>
<td>ATP diffusion constant</td>
<td>$D_{ATP} \sim 5 \times 10^5 \mu m^2 / s$</td>
</tr>
<tr>
<td>(in solution &amp; in vivo)</td>
<td></td>
</tr>
<tr>
<td>ATP hydrodynamic diameter</td>
<td>1.4 nm</td>
</tr>
<tr>
<td>ATP molecular weight</td>
<td>507 g/mol</td>
</tr>
<tr>
<td>Debye length $\kappa^{-1}$ in 200 mM KCl</td>
<td>0.7 nm</td>
</tr>
<tr>
<td>viscosity of water</td>
<td>$\eta_W \sim 10^{-3} Pa s$</td>
</tr>
<tr>
<td>Power consumption</td>
<td>$P \sim 10^{-12} W$</td>
</tr>
<tr>
<td>of bacterial cell [1, 5]</td>
<td>$P \sim 10^7 ATP/s$</td>
</tr>
</tbody>
</table>

TABLE I. Table of the values I use for properties of the cytoplasm. The value for the concentration of ATP is from Bennett et al. [2], who give 10 mM as the ATP concentration in their rapidly growing E. coli. Other measurements [1] for E. coli have given similar but typically a little lower values, and Traut [6] gives 3 mM $\sim 10^6 / \mu m^3$ for example mammalian cells. The concentration of small cations (eg K$^+$ and Na$^+$) and anions (eg Cl$^-$) in E. coli will vary with external growth conditions, but 200 mM is a typical value [1]. N.B., 1 mM $\simeq 6 \times 10^6 / \mu m^3$. Most of the other numbers are from Cell Biology by the Numbers [1, 7].

NUMBERS FOR PROPERTIES OF A BACTERIAL CELL

To estimate diffusiophoretic velocities in a bacterial cell, we need estimates for a number of properties of a typical bacterial cell, by which I mean E. coli. These are collected in Table I.

Many of these numbers are from the excellent reference, Cell Biology by the Numbers by Milo and Phillips. This is available as both a book [1], and an online resource [7].

ESTIMATION OF THE SIZE OF GRADIENTS IN THE TEMPERATURE

The relative rates of heat and molecular diffusion, is characterised by the Lewis number: $Le = \alpha / D$. Here $\alpha$ and $D$ are the thermal diffusivity, and the diffusion constant of the molecule, respectively. Even for fast diffusing species, such as molecules like ATP, the diffusion constant $\sim 100 \mu m^2 / s$ [1, 4], while for water (and hence the cytoplasm which is mainly water) $\alpha \sim 10^7 \mu m^2 / s$ [8]. Thus for small molecules in the cytoplasm, $Le \sim 10^{3}$, and so temperature gradients relax about a thousand times faster than gradients in ATP.

Momentum diffuses with a diffusion constant of the kinematic viscosity, about $\nu \sim 10^8 \mu m^2 / s$ for water. Thus in cells pressure gradients relax even faster than temperature gradients, and we therefore expect pressure gradients to be negligible.

We can estimate the size of temperature gradients as follows. A growing bacterial cell of volume $1 \mu m^3$ has a power consumption of order $10^{-12} W$, see Table I. If we naively assume that the metabolism is concentrated in say, the left-half of the cell, then crossing the mid-point of the cell we have of order $10^{-12} W$ of heat, or $\sim 1 \ W / \mu m^2$, for a cell cross-section of $1 \mu m^2$. The thermal conductivity of water is of order $1 \ W / K / m$ [8], so a flux of $1 \ W / m^2$ is driven by a gradient of order $1 \ K / m$.

Thus we conclude that active bacterial cells have temperature gradients across of them that are of order $1 \ K / m$, or that the temperature differences across the cells are no more than $1 \mu K$. This is a general observation, cells are made of matter with high thermal conductivity, and so cannot support significant temperature gradients. So, presumably, the recent claim [9] that mitochondria are $10 \mu K$ hotter than the surrounding cytoplasm is incorrect.

GRADIENT IN ATP ACROSS THE CYTOPLASM

To obtain a simple estimate of the size of ATP gradients I assume a one-dimensional geometry in which ATP synthases are along two parallel flat cell walls at $z = \pm w/2$. I assume that the system is at steady state. As the time taken for ATP to diffuse across the cell is only $0.01 \ s$, steady state will be achieved in much less than a second. The cell width $w = 1 \mu m$. To get a simple one-dimensional model I then ignore gradients parallel to the wall, and assume the concentration of ATP depends only on the distance $z$ from the wall. If the proteins consuming ATP (= the ATP sinks) are uniformly distributed,
then the concentration of ATP in the cytoplasm obeys

\[ D_{\text{ATP}} \left( \frac{d^2 c_{\text{ATP}}(z)}{dz^2} \right) - k_{\text{ATP}} c_{\text{ATP}}(z) = 0 \]  

(1)

Here \( D_{\text{ATP}} \) is the diffusion constant for ATP, and \( k_{\text{ATP}} \) is the rate constant for ATP consumption, assumed uniform in the cytoplasm. If the ATP synthases along the cell wall maintain the ATP concentration at a fixed value \( c_{\text{ATP}}(z = \pm w/2) \), this provides the boundary conditions needed to solve this differential equation. The solution is then

\[ c_{\text{ATP}}(z) = c_{\text{ATP}}(z = \pm w/2) \frac{\cosh(-z/l_G)}{\cosh(w/(2l_G))} \]  

(2)

with the lengthscale of the gradient \( l_G = (D_{\text{ATP}}/k_{\text{ATP}})^{1/2} \). The gradients are then of order \( c_{\text{ATP}}(z = \pm w/2)/l_G \). For \( D_{\text{ATP}} = 100 \mu m^2/s \) and \( k_{\text{ATP}} = 1/s, l_G = 10 \mu m. \) Using \( c_{\text{ATP}}(z = \pm w/2) = 10^8/\mu m^3 \), the gradients \( |\nabla c_{\text{ATP}}| \sim 10^6/\mu m^4 \). This one-dimensional model neglects both the discrete nature of the ATP source (ATP synthase at the membrane), and fluctuations.

**GRADIENTS OF METABOLITES NEAR A METABOLON**

In the main part of this paper we considered one metabolite: ATP. Here we consider a metabolite produced/consumed by a large protein complex — these large complexes are sometimes called metabolons [10]. Metabolons are physical assemblies of many proteins, including copies of multiple species of enzyme in the same pathway, i.e. if a synthetic pathway requires enzymes A, B and C, with B catalysing a reaction on a product of enzyme A, etc, then many of the copies of A, B and C may be together in an physical assembly of perhaps hundreds or thousands of molecules. This may enhance the efficiency of this pathway [10]. I will show that near these metabolons, we should also expect large gradients in the concentrations of metabolites.

For simplicity, I approximate a metabolon by a sphere of radius \( R_M \), producing fluxes of order \( k = 10^5/s \), of a single molecule with diffusion constant \( D_M \). A single urease can catalyse the hydrolysis of urea at a rate of \( 10^8 \) umoles/min [13], so this flux could be produced by a hundred copies of a high turnover enzyme. I assume that just the reactants interact with the particle, including products just complicates the expressions a little.

Our model is essentially that studied in detail by Reigh et al. [12]. Following Reigh et al., we estimate the steady-state gradient. At steady state, the concentration \( c_M \) obeys Laplace’s equation \( \nabla^2 c_M = 0 \). I use spherical coordinates centred on the metabolon, with \( r \) the distance from the centre of the metabolon. Then the flux is

\[ \nabla c_M(r) = -\frac{k}{4\pi D_M r^2} \hat{r} \]  

(3)

as this gives the required total flux \( k \) over a surface enclosing the metabolon. For a metabolite diffusion coefficient \( D_M \sim 100 \mu m^2/s \), and \( k = 10^5/s \), the flux is

\[ \nabla c_M(r) \sim \frac{10^{14}}{\mu m^4} \hat{r} \]  

(4)

where we approximated \( 4\pi \) by 10, as the expression is approximate. At a distance of order 100 nm from the centre of the metabolon, the gradient is of order \( 10^{28}/\mu m^4 \) or \( 10^4/\mu m^4 \).

Reigh et al. [12] tested the simple theory above by essentially exact computer simulations of a simple model. There was semiquantitative agreement between the theory and computer simulations.

**GRADIENTS OF SMALL IONS SUCH AS POTASSIUM AND CHLORIDE**

Small ions such as potassium, sodium and chloride are abundant in cells, \( 10^8/\mu m^3 \sim 100 \) mM [1], but we expect the gradients in their concentration to be very small. So we do not expect significant phoretic effects due to gradients in the concentration of small ions, in cells growing in an environment where the osmotic pressure is constant.

The timescale for potassium turnover in *E. coli* has been measured at of order \( 10^3/s \), [13]. Potassium is the most abundant cation in cytoplasm, while chloride is the most abundant anion [1]. Presumably, due to electroneutrality, the flux of anions and cations has to be the same.

The diffusion constant of potassium chloride in water is of order \( 10^5 \) umoles/min [14], so a potassium ion will diffuse across a bacterial cell in about 1 ms. This is a factor of \( 10^6 \) times smaller than the timescale for potassium uptake, and so we expect the gradients in cells, of the concentration of potassium, and chloride, to be very small. As diffusiophoresis is driven by gradients, this implies that diffusiophoresis driven by small ions should typically be irrelevant. An exception may be pollen tubes, a very specialised and large type of cell where large gradients are found, see the work of Lipchinsky [15].

**ALTERNATIVE METABOLISM-DEPENDENT MECHANISMS OF TRANSPORT IN CELLS**

Diffusiophoresis is unlikely to be the only non-motor-driven metabolism-dependent transport mechanism in cells. In this section, I briefly consider two other possible mechanisms for transport in cells, that rely on the cell’s metabolism. These are advection of a particle due to flow in the cytoplasm, and metabolism-dependent processes
accelerating thermal diffusion by making the cytoplasm less sticky. In eukaryote cells, there is also transport of particles as the cargos of motor proteins.

**Transport by flow**

**Cytoplasmic streaming**

It is clear that in a number of large cells (≥ 100 µm), there is significant flow of the cytoplasm. This corresponds to a large $v_{adv}$ term in Eq. (1) in the main text, with a $v_{adv}$ that is relatively uniform over large regions of the space, and relatively constant in time. This is sometimes called cytoplasmic streaming [16, 17]. Cytoplasmic streaming is driven by motors and the cytoskeleton, and it clearly contributes to transport in a number of very large cells [16, 17]. These large cells include 100 µm Drosophila oocytes [17], and plant cells that can be centimetres long [16]. Speeds of tens of nanometres per second were measured in the oocytes, while much faster speeds are found in larger cells. I am not aware of studies of cytoplasmic streaming in eukaryote cells of more typical size, ~ 20 µm across, or in prokaryote cells.

**Random stirring of the cytoplasm**

Mikhailov and Kapral [18, 19] have considered stirring of the cytoplasm by energy-consuming but non-motor proteins. Here by stir, I mean generate transient flow in more-or-less random directions in the cytoplasm, as opposed to the fast directed flow seen in cytoplasmic streaming. So, here $v_{adv}$ varies rapidly in space and time. They considered proteins that consume ATP and generate force dipoles, which stir the surrounding cytoplasm, thus accelerating diffusion in this cytoplasm. Note that proteins free in the cytoplasm generate force dipoles not forces, due to Newton’s Third Law.

They studied active proteins at a concentration $c_{FD}$, that generate force dipoles of root-mean-strength strength $F\delta$ with a characteristic correlation time $\tau_{FD}$. Mikhailov and Kapral found that these force dipoles increase diffusion by an amount (Eq. (10) of Mikhailov and Kapral [18]) $\Delta = 0.01c_{FD}(F\delta)^2\tau_{FD}/\eta l_c$, for $l_c$ a small lengthscale cutoff, approximately equal to the distance of closest approach between the active protein, and the protein whose diffusion is being accelerated. This follows from the fact that a force dipole induces flow at speed $v \sim F\delta/\eta l_c^2$, a distance $r$ away.

Subsequent computer simulations of a simple model system, by Dennison *et al.* [20] found a relatively small effect on diffusion, of order 10% or less. However, the size of the increase in diffusion is very sensitive to a number of parameters so it is hard to estimate how large an affect it could have in cells, without better data on the cytoplasm.

**Metabolism-dependent viscosity**

The speed of diffusion is reduced by drag on the diffusing particle. This drag will increase if the particle sticks to the proteins in the cytoplasm. Thus any energy-consuming process, such as those involving chaperones, which unsticks proteins, will cause a metabolism-dependent increase in diffusion. We do not know if such a process contributes to the results of Parry et al. [21], or is a general source of metabolism-dependent diffusion.

It is also worth noting that the metabolism is typically inhibited by depleting the ATP in a cell. ATP at physiological concentrations, is known [22] to strongly interact with proteins. Recent work of Patel et al. [22] showed that ATP inhibits proteins undergoing liquid/liquid phase separation. The liquid/liquid separation is into coexisting phases with high and low concentrations of protein. Note that this effect of ATP is due to physical interactions between ATP (a relatively large and amphiphilic ion) and proteins, the ATP is not consumed, it is a purely equilibrium effect. Thus when depleting the ATP in a cell, some interactions of the particle may change, in addition to the suppression of the metabolism removing ATP gradients.