Supporting Text

S1 Mathematical Analysis of the *C. crescentus* Stalk as a Diffusion Antenna for Nutrient Uptake

The biochemical and proteomic analysis of the *C. crescentus* stalk presented in the main text of this report suggests a direct role in enhancing nutrient uptake. Although increasing receptor surface area is the preferred means for increasing uptake capability in the presence of fluid flow, this is not the case when nutrient uptake is predominantly due to diffusion. In this section, we demonstrate mathematically how growing a stalk represents a beneficial strategy for *C. crescentus* in a diffusion-limited environment by facilitating an optimal arrangement of nutrient receptors. Some of the discussion in the main text of the report is repeated here, so that the present material can be read as a stand-alone document.

S1.1 Summary of Results

In a diffusion-limited environment, simply increasing the number of receptors on the *C. crescentus* cell body may not be the most effective strategy for enhancing nutrient uptake. This can be illustrated by considering the path of a diffusing particle. Microscopically, the random walk trajectory of a diffusing particle explores a given region in space well before wandering away (1). This qualitative picture has important consequences for the rate of uptake (number of particles absorbed per unit time), when particles are dependent on diffusion for contact with their absorbers (i.e., nutrient receptors). For example, given a disk-shaped receptor, adding a second absorber to the cell surface will double the rate of uptake only if it is well separated from the first one, compared to their size. This is because a diffusing particle in the vicinity of the second absorber might instead be absorbed by the first one if the absorbers are placed "too close" to each other, as illustrated in Fig. 7.

The rate of diffusive uptake by N discrete disk-like absorbers on an otherwise nonabsorbing spherical surface was first addressed by Berg and Purcell (2) in their study of the physical limits to the measurement of the concentration of diffusing signaling molecules by

surface receptors in single-celled organisms (for example, in bacterial chemotaxis). They showed that for absorbers of radius, s, distributed uniformly on the surface of a sphere of radius, R, where $R \gg s$, the rate of uptake is initially proportional to N (for $Ns \ll R$) but eventually saturates (for $Ns \sim R$) to the maximum rate that results when the entire surface is covered by absorbers. The individual absorbers are assumed to be perfect in that upon contact with a diffusing molecule, the probability of absorption is equal to one. Treatment of nonperfect absorbers is equivalent to reducing their size, s. In the context of *C. crescentus* stalk formation, we extend this work to show that for the cell to increase its rate of nutrient uptake by diffusion, it is advantageous to arrange additional receptors onto an auxiliary structure such as a stalk. Details of this calculation are given in Section S1.2.

The stalk and cell body can be approximated as prolate spheroidal bodies of dimensions (b, b, ℓ), with $\ell > b$, as indicated in Fig. 7. The maximum rate of uptake $I^{max}_{spheroid}$ is obtained from the solution to the steady state diffusion equation for the substrate concentration in three dimensions in prolate spheroidal coordinates, subject to the boundary conditions of zero concentration, $c(r,t) = 0$, at the absorbing surface, and constant concentration c_0 infinitely far from the surface:

$$I^{max}_{spheroid}(\ell, b) = \frac{4\pi D c_0 \ell \sqrt{1 - b^2/\ell^2}}{\tanh^{-1}\left(\sqrt{1 - b^2/\ell^2}\right)} = 4\pi D c_0 \ell_{\mathit{effective}}\,. \qquad (\mathbf{1})$$

We note that the maximum rate of uptake to the surface is proportional to an effective linear dimension of the body, $\ell_{\mathit{effective}} \equiv \left(e/\tanh^{-1} e\right)\ell$, where $e = \sqrt{1 - b^2/\ell^2}$ is the eccentricity of the spheroid. This is also true for other shapes when uptake is diffusion limited.* In contrast, when the dominant transport of particles to the absorber is not by diffusion, but rather by fluid advection or mixing, then the rate of uptake is no longer proportional to the length of the absorber but rather to its surface area.†

*From dimensional analysis, the rate of diffusive uptake must be proportional to both the diffusion constant and the concentration of substrate molecules, $I \propto Dc_0$, where I has dimensions of number of particles per unit time, D has dimensions of length^2 per unit time and c_0 has dimensions of number of particles per length^3. Hence, I must also depend on the effective *length* of the absorber, $\ell_{\mathit{effective}}$, giving $I \sim Dc_0\ell_{\mathit{effective}}$.

†Again, from dimensional analysis, we must have $I \sim u\, c_0\, A$, where u is the fluid velocity in units of

We address how this maximum rate of uptake is modified with N discrete disk-like absorbers of radius s, by calculating the probability P_{esc} that a diffusing particle that collides with the surface will eventually escape to infinity and not be captured by any of the absorbers. Our analysis extends Berg and Purcell's calculation for the spherical case (2) to the prolate spheroidal geometries that characterize the *C. crescentus* cell body and stalk (see Section S1.2). The modified rate of uptake is given by $I_{spheroid}(N;\ell,b,s) = I^{max}_{spheroid}(\ell,b)(1-P_{esc})$. In the limit of a "cigar-shaped" absorbing surface such as the stalk, where $b \ll \ell$, we have

$$1 - P_{esc} \underset{b\ll\ell}{\approx} \left[1 + \frac{4\ell}{Ns}\,\frac{1}{\ln(2\ell/b) - \frac{4s}{\pi b}}\right]^{-1}, \qquad (\mathbf{2})$$

and in the limit of an absorbing surface such as the cell body, where $b \approx \ell$, we have

$$1 - P_{esc} \underset{b\sim\ell}{\approx} \left[1 + \frac{4b^2}{N\ell s}\,\frac{1}{1 - \frac{2bs}{\ell(b+\ell)}}\right]^{-1}. \qquad (\mathbf{3})$$

We note that the saturation of the modified rate of uptake to the maximum rate with increasing N depends on the ratio of the linear size of the single discrete absorber to an effective linear size of the spheroid.

In the main text (see Fig. 5*A*), we have plotted the rate of uptake by the cell body with N discrete absorbers, $I_{cell}(N) = I_{spheroid}(N;\ell_{cell},b_{cell},s)$ as a function of N in units of the maximum rate of uptake by the cell body, $I_{cell} = I^{max}_{spheroid}(\ell_{cell},b_{cell})$, using typical dimensions for the *C. crescentus* cell body, where $(\ell_{cell},\ b_{cell}) = (0.5,\ 0.25)$ μm and typical sizes of transport porins, $s = 1$ nm (3, 4). Increasing the number of absorbers on the cell body by 100% from $N = 10,000$ to $N = 20,000$ leads to a 5% increase, from 83% to only 88% in the rate of nutrient absorption relative to the maximum rate.

We also plotted (see Fig. 5*B* in the main text) the rate of uptake by the stalk with N discrete absorbers, $I_{stalk}(N) = I_{spheroid}(N;\ell_{stalk},b_{stalk},s)$, as a function of N for typical stalk dimensions, in units of the maximum rate of uptake by the cell body. If the $N = 10,000$ new receptors are placed on a stalk instead of the cell body, for stalks of length $L = 2\ell_{stalk}$

length per unit time, c_0 is the concentration in units of number of particles per length^3, and A is the area of the absorber in units of length^2.

equal to $1, 5$, and 10μm, the rate of uptake is increased by approximately $50\%, 125\%$, and 200%, respectively, of the maximum rate of uptake by the cell body.

For comparison, in Fig. 8*A*, we plot the rate of uptake by an elongated cell with N discrete absorbers, $I_{elongated\ cell}(N) = I_{spheroid}(N; f\ell_{cell}, b_{cell}, s)$, as a function of N in units of the maximum uptake by the cell body for different cell lengths, where $L_{cell} = 2f\ell_{cell}$, and f is a scale factor. In Fig. 8*B*, we plot the rate of uptake for a stalked cell, $I_{stalked\ cell}(N)$, with N discrete absorbers uniformly distributed on the cell body and stalk surfaces. The length of the cell body is held fixed (with $L_{cell} = 1\ \mu$m), while the stalk length is varied to achieve the same total length as the hypothetical elongated cell in Fig. 8*A*.

We note that the corresponding curves in Fig. 8*A* and Fig. 8*B* indicate that the absorption rate for stalked cells is comparable to (though slightly less than) that for elongated cells of the same total length. This is true despite the fact that the surface areas of elongated cells with total lengths of 3, 5, and 10 μm are approximately 2, 2.5, and 3.5 times that of stalked cells of the same length. Strikingly, as shown in Fig. 9, the maximum rates of uptake per unit volume and uptake per unit surface area are significantly greater for stalked cells than for elongated cells. Therefore, growing a stalk would be an efficient strategy for a cell to increase its rate of nutrient uptake while at the same time minimizing the cost of increasing both surface area and volume.

S1.2 Modified Rate of Uptake of an Ellipsoidal Object with N Discrete Absorption Sites

For an ellipsoidal absorber, assumed for simplicity to be a prolate spheroid with dimensions (b, b, ℓ), the total rate of uptake is obtained by solving Laplace's equation in three dimensions in prolate spheroidal coordinates subject to boundary conditions given by $c(r, t) = 0$ at the surface of the absorber and $c(r, t) = c_0$ infinitely far from the absorber

$$I_{spheroid} = \frac{4\pi D c_0 \ell \sqrt{1 - b^2/\ell^2}}{\tanh^{-1}\left(\sqrt{1 - b^2/\ell^2}\right)}\,. \tag{4}$$

With N discrete disk-like absorbers of radius s, to determine how this maximum rate of

uptake is modified we first find the probability that a diffusing particle at a given location in space reaches the surface of the spheroid: this is given by the solution to Laplace's equation in prolate spheroidal coordinates subject to boundary conditions $P = 0$ infinitely far from the surface, and $P = 1$ at the surface of the spheroid

$$P(\xi) = \frac{1}{\tanh^{-1}\sqrt{1-b^2/\ell^2}}\,\tanh^{-1}\sqrt{\frac{1-b^2/\ell^2}{1+\xi/\ell^2}}\,, \qquad (\mathbf{5})$$

where prolate spheroidal coordinates are $\{\xi,\ \zeta,\ \phi\}$

$$\begin{aligned}
\xi &= \frac{1}{2}\left[-(\ell^2+b^2)+\rho^2+z^2+\sqrt{-4\ell^2(b^2-\rho^2)+4b^2z^2+(\ell^2+b^2-\rho^2-z^2)^2}\right], \\
\zeta &= \frac{1}{2}\left[-(\ell^2+b^2)+\rho^2+z^2-\sqrt{-4\ell^2(b^2-\rho^2)+4b^2z^2+(\ell^2+b^2-\rho^2-z^2)^2}\right], \\
\phi &= \tan^{-1}\frac{y}{x}\,,
\end{aligned}$$

with $\xi \geq -\ell^2$, $-\ell^2 \leq \zeta \leq -b^2$, $0 \leq \phi \leq 2\pi$, and $\rho^2 = x^2 + y^2$ (5). The surface of the spheroid in Cartesian coordinates

$$\frac{x^2}{b^2} + \frac{y^2}{b^2} + \frac{z^2}{\ell^2} = 1, \qquad (\mathbf{6})$$

is given in prolate spheroidal coordinates by $\xi = 0$. If the entire surface is absorbing, then the probability that a particle at $\xi(\rho, z)$ will be absorbed is given by Eq. **5**. What if there are N disk shaped absorbers of radius s, while the rest of the surface is non-absorbing? Berg and Purcell (2) addressed this question for the spherical case in their analysis of the physical limits to biochemical signaling in the context of bacterial chemotaxis. Their assumption, which we likewise follow here, is that a particle in the vicinity of the spheroid is destined to make many encounters with the surface before escaping to infinity, if at all. Assuming the absorbers are uniformly distributed on the surface of the spheroid of surface area A, the probability that a given encounter is not with an absorber is $(1 - N\pi s^2/A)P \equiv \beta P$, where the surface area of a prolate spheroid is

$$A = 2\pi b^2 + \frac{2\pi\ell b}{\sqrt{1-b^2/\ell^2}}\sin^{-1}\sqrt{1-b^2/\ell^2}, \qquad (\mathbf{7})$$

with

$$A \underset{b\ll\ell}{\approx} \pi^2\ell b\,, \qquad (\mathbf{8})$$

$$\underset{b\sim\ell}{\approx} 2\pi b(b+\ell)\,. \tag{9}$$

We would like to find the probability that the particle does not encounter an absorber after $n = 0, 1, \ldots\infty$ number of independent hits. For the purpose of an approximate calculation, we define independent hits with the surface as those separated by a distance on the surface approximately equal to s. Following Berg and Purcell, we assert that consecutive hits are independent provided that they are separated by an excursion above the surface by a distance also approximately equal to s. Hence, the probability that a particle in the vicinity of the surface - defined to be a perpendicular distance approximately equal to s above the surface - will escape to infinity is given by

$$P_{esc} = \sum_{n=0}^{\infty}\left[\underbrace{(\beta P_s)^n}_{make\ n\ hits}\cdot\underbrace{(1-P_s)}_{escape\ to\ \infty}\right] = \frac{1-P_s}{1-\beta P_s}\,, \tag{10}$$

where P_s is the probability of encountering the surface from a distance s above it. To detemine what this distance corresponds to in prolate spheroidal coordinates, we note that the element of length in the ξ-direction (perpendicular to the spheroidal suface, $\xi =$ constant) is given by $h_\xi d\xi$, where the scale factor h_ξ is

$$h_\xi = \frac{\sqrt{\xi-\zeta}}{2\sqrt{(\xi+\ell^2)(\zeta+b^2)}}. \tag{11}$$

Hence, for

$$s \sim h_\xi(\xi=0)\delta\xi = \frac{\sqrt{-\zeta}}{2\ell b}\delta\xi, \tag{12}$$

the value of $\delta\xi$ corresponding to a perpendicular distance s above the surface varies with position. Therefore, we consider the average value over the surface of spheroid

$$\langle\delta\xi\rangle = 2\ell b s\left\langle\frac{1}{\sqrt{-\zeta}}\right\rangle, \tag{13}$$

where

$$\left\langle\frac{1}{\sqrt{-\zeta}}\right\rangle = 4\pi b/A\,. \tag{14}$$

Using Eqs. **8** and **9**, we find

$$\langle \delta\xi \rangle \underset{b \ll \ell}{\approx} \frac{8bs}{\pi}\,, \tag{15}$$

$$\underset{b \sim \ell}{\approx} \frac{4b\ell s}{b+\ell}\,. \tag{16}$$

Therefore, we take $P_s = P(\xi = \langle \delta\xi \rangle) = P(8\pi\ell b^2 s/A)$, which for $s/b \ll 1$, becomes

$$P_s \underset{b \ll \ell}{\approx} 1 - \frac{4s}{\pi b}\frac{1}{\ln\left(2\ell/b\right)}\,, \tag{17}$$

$$\underset{b \sim \ell}{\approx} 1 - \frac{2bs}{\ell\left(b+\ell\right)}\,. \tag{18}$$

Finally, with N discrete absorbers the total rate of uptake by the spheroid is modified by the factor $(1 - P_{esc})$

$$I_{spheroid}(N;\ell,b,s)/I^{max}_{spheroid}(\ell,b) = \frac{(1-\beta)\,P_s}{1-\beta P_s}\,, \tag{19}$$

where $\beta = 1 - N\pi s^2/A$ carries the dependence on N, as defined earlier in this section.

S1.3 Discussion

Diffusion in the Periplasmic Space. Although absorption of nutrient molecules from the environment into the cell body cytoplasm takes placed via the two-stage process described in the main text of this report (first, absorption by OM receptors followed by diffusion in the stalk and cell body periplasmic spaces, and then absorption by cell body IM transporters) the role of the stalk is simply to increase the rate of absorption into the cytoplasm where the nutrients will be metabolized. At steady state, the number of nutrient molecules in the perplasm is fixed, and the rate of uptake from the periplasm by the cell body IM is equal to the sum of the rates of uptake by the cell body and stalk OM's, which we have calculated. The time scale τ for reaching steady state depends on the diffusion constant of the nutrient in the periplasm, and is given by $\tau \sim L^2_{stalk}/D_{periplasm}$ for quasi-one dimensional diffusion in the stalk. Diffusion constants of typical proteins in the cytoplasm and in the lipid membrane have been measured in recent experiments, with $D_{membrane} = (1.2 \pm 0.2) \times 10^{-2}$ μm^2/sec (6)

and $D_{cytoplasm} = (2.5 \pm 0.6)\ \mu\text{m}^2/\text{sec}$ (7). Earlier measurements on diffusion of fluorescently labeled maltose-binding protein in the *Escherichia coli* periplasm indicate $D_{periplasm}$ to be comparable to $D_{membrane}$ (8). Using this estimate for $D_{periplasm}$, we find that $\tau \sim 17$ and 42 min for $L_{stalk} = 1$ and 5 μm, respectively. Should $D_{periplasm}$ be larger, for example by an order of magnitude, these characteristic diffusion time scales will be reduced by a factor of 10. Even for long stalks, this time scale is short compared to the duration of the *C. crescentus* cell cycle, which is on the order of hours under nutrient deprived conditions. Hence, the stalk quickly enhances the rate of nutrient uptake into the cytoplasm by an amount equal to the stalk's rate of uptake from the environment.

Alternate Possibilities for Stalk Function. Could the stalk play other roles in addition to that of a "diffusive antenna"? Suppose that, in addition to diffusion, the substrate is also advected by fluid flow. At the stationary surface to which the cells are adhered, the bulk fluid velocity decreases to zero within a boundary layer whose thickness depends on the fluid properties. The rate of uptake in the presence of flow is proportional to uAc_0, where u is the flow velocity, A is the surface area of the absorber, and c_0 is the nutrient concentration. Therefore, the cell body, by virtue of its greater surface area, will have a larger rate of nutrient uptake than the stalk, and a possible additional role of the stalk would then be to lift the cell body out of the boundary layer and into the bulk where the flow velocity is largest. A second possible role for the stalk as a "stem" arises from consideration of the diffusive boundary layer at the surface. As the number of cells attached to a stationary surface becomes appreciable, the concentration of nutrients at the surface decreases from that in the bulk over a thickness proportional to the cell size. Hence, the role of the stalk may additionally be to lift both itself and the cell body into the bulk where the nutrient concentration is less depleted (while the part of the stalk remaining in the cell biomass at the surface could take up nutrients generated by the other cells adhering to the surface). In these ways, prosthecae in the form of "stems" may play additional roles beyond that of

linear appendages that simply project out from anywhere on the cell surface and serve as diffusive antennae.

References

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