Why microbes secrete molecules to modify their environment: the case of iron-chelating siderophores

Supplementary Information

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1 Variational formulation of the reaction-diffusion problem

We numerically integrate the reaction-diffusion equations for the two cell case using FEniCS [1]. In order to solve the time dependent problem, we use a finite differences approximation for the time dimension, and solve the variational formulation at each time step using the finite element method. For completeness, we here briefly reiterate the formulation of the time-dependent variational formulation.

Variational formulation for the stationary problem. The finite element method uses the weak variational formulation of the system of partial differential equations of the reaction-diffusion system. Briefly, a PDE for the function $u(r, z)$ with cylindrical symmetry defined on $(r, z) \in \Omega$ of the form,

$$-\frac{\partial}{\partial r}\left(rD\frac{\partial u}{\partial r}\right) + rD\frac{\partial^2 u}{\partial z^2} = rf(u),$$

is multiplied by a test function $v(r, z)$ and integrated over Ω,

$$-\int_\Omega \nabla \cdot (rD\,\nabla u)\, v\, dx = \int_\Omega rfv\, dx.$$

The left-hand side can be integrated by parts, and by forcing $v = 0$ on the boundaries where u is known. The cylindrically symmetrical problem stated in the weak variational formalism is thus,

$$\int_\Omega rD\,\nabla u \cdot \nabla v\, dx = \int_\Omega rfv\, dx.$$

Equivalently, for a spherically symmetric problem,

$$\int_\Omega r^2 D\,\nabla u \cdot \nabla v\, dx = \int_\Omega r^2 fv\, dx.$$

Variational formulation time-dependent problem. Here we use the standard finite difference discretization of the time derivative, such that $\partial_t u \approx (u^{(k)} - u^{(k-1)})/\Delta t$. The time dependent PDE equation becomes,

$$\frac{\partial u}{\partial t} \approx \frac{u^{(k)} - u^{(k-1)}}{\Delta t} = rf^{(k)} + \nabla \cdot (rD\,\nabla u^{(k)})$$

We then iterate along the finite differences by successively solving the following equation using finite elements for a known $u^{(k-1)}$,

$$\int_\Omega \left\{u^{(k)}v^{(k)} + \Delta t\, rD\,\nabla u^{(k)} \cdot \nabla v^{(k)}\right\} dx = \int_\Omega \left\{u^{(k-1)} + \Delta t\, rf^{(k)}v^{(k)}\, dx\right\} dx.$$

2 The spatio-temporal effects of siderophore secretion on the distribution of iron in space

Strong temporal dynamics become visible when quantifying the radial distributions of iron, siderophores and bound iron over time for aggregates of different sizes in the presence of secreted siderophores (Fig. S4). As soon as the cell begins to secrete siderophores, the iron close to the cell is

quickly bound and depleted, and the iron-siderophore complexes are subsequently taken up by the cell with a high probability. At the minimal size of iron sources ($k = 1$) the diffusion speed is maximal and the bound free iron close to the cell is quickly replenished by new iron diffusing towards the cell from a distance. Therefore, the iron concentration close to the cell is roughly the same as the background level (Fig. S4a). New siderophores thus quickly encounter free iron to bind, which can then be taken up by the cell, resulting in a high concentration of bound iron-siderophore complexes close to the cell (see Methods, Eq. 17). The cell is thus transport- and not diffusion-limited. In this fast diffusion limit the background concentration of iron is almost constant, and the iron uptake rate is primarily determined by the secretion rate of siderophores (see Methods and Völker & Wolf-Gladrow [2]).

At higher aggregation levels, though, fresh iron diffuses towards the cell more slowly, eventually resulting in a total depletion of free iron close to the cell (Fig. S4b,c). At the same time, free siderophores diffuse away from the cell and a 'hot-spot' for the formation of bound siderophores builds up at a certain distance from the cell, resulting in a traveling peak in the distribution of bound iron over time (Fig. S4b,c). As bound iron is either taken up by the cell or diffuses away, this peak eventually flattens out over time and moves away from the cell. For large k, it can reach up to 1 mm, i.e. secreting cells create a large region where no unchelated, free iron is available. The physicochemical properties of iron thus play an important role in determining the degree to which bacteria influence and modify their environment when secreting siderophores. In such regions where only siderophore-bound iron is present, other bacteria that are not able to access chelated iron could have competitive disadvantages [3].

3 Iron uptake competition

Once secreted siderophores encounter and bind iron, the chelates must successfully return to cell via diffusion. For a single cell, this process can be modelled by diffusion around a spherical sink. We generally know the probability that a chelate arrives at the cell, i.e. the hitting probability of a small diffusing particle starting at a distance r on a sphere with radius R. In one or two dimensions, $p_1 = 1$, but in three dimensions $p_1 \approx R/r$. We are interested in the probability that a chelate is 'stolen' from a cell A by a cell B, i.e. the fraction of trajectories from the initial position O to A that pass through B. We can decompose the probability that the chelate arrives at A into direct paths, that do not pass through B, and indirect paths, that pass through B,

$$\Pr(0 \to \mathrm{A}) = \Pr(0 \to \mathrm{A}\backslash\mathrm{B}) + \Pr(0 \to \mathrm{B}\backslash\mathrm{A}) \Pr(\mathrm{B} \to \mathrm{A}).$$

Let $p_1 = R/r \approx \Pr(0 \to \mathrm{A})$ be the hitting probability for a particle starting at a large distance r and $b = \Pr(0 \to \mathrm{A}\backslash\mathrm{B})$ be the probability of arriving at A by avoiding a cell B at a distance d (which is approximately the same as arriving at B by avoiding A for $r \gg d$). Let $c = R/d \approx \Pr(\mathrm{B} \to \mathrm{A})$ be the hitting probability of A when starting at B (or of B when starting at A). Thus, $p_1 = b(1 + c)$, and hence $b = p_1/(1 + c)$. The fraction of chelates that would arrive at A by first passing through B is thus,

$$f_2 = \frac{\Pr(0 \to \mathrm{B}\backslash\mathrm{A}) \Pr(\mathrm{B} \to \mathrm{A})}{\Pr(0 \to \mathrm{A})} = \frac{c}{1 + c}. \tag{1}$$

In three dimensions, for two cells at a distance d, $c \approx R_B/d$. Thus, for an immediately neighbouring cell, $d \to R_B$, $c \to 1$ and $f_2 = 1/2$, such that half of the chelates are stolen by B. However for cells that are sufficiently far apart, $d \gg R_B$ and $c \ll 1$, such that $f_2 \approx c$ and the cell *de facto* no longer

feels the competition of the other cell. In one or two dimensions, however, $c = 1$ for all distances and hence the competition is always $1/2$.

4 Influence of siderophore production rate on uptake of sufficient iron to initiate cell division

Siderophore secretion can transiently speed up iron acquisition. To investigate how these transient effects scale with bacterial growth, we define the 'uptake time' as the required time until a cell has acquired enough iron to produce sufficient biomass to initiate cell division (assuming an iron content of 10^6 Fe atoms = 1.66 amol per cell [4]; Fig. S6). Note that, if cells are limited by iron only, the uptake time is equal to the inter-division time.

The increased iron uptake rate achieved by secretion at low iron solubility, although only transient, can significantly shorten the uptake time (Fig. S6a). A non-motile cell relying on direct physical contact would have an uptake time of over a year for parameters close to the marine environment (iron concentration of ρ_0 = 0.1 nM; particle sizes around 200 nm; dissolution rate $\kappa_L = 10^{-6}$). When a cell starts to secrete siderophores, the uptake time at these conditions is significantly reduced to a few weeks, depending on the production rate. Note, that secretion strongly buffers the impact of increasing particle size on the uptake time.

For small particles, a secreter cell has a longer uptake time than a cell using direct uptake. Here, particles diffuse away from proximity of the cell much more quickly than they are dissolved, such that there is net loss of adsorbed siderophores close to the cell, before they are released from the aggregate as siderophore-iron chelates.

The uptake time is strongly influenced by the production rate of siderophores. Overall, the higher the siderophore production rate, the wider the range of environments in which siderophore secretion speeds up iron acquisition compared to direct uptake. Also, at a fixed particle size, higher production rates will decrease the uptake time. However, the incremental reduction in uptake time decreases as the production rate increases until the uptake time levels off and further increasing the production rate no longer has an effect (Fig. S6b). At these high production rates, aggregates quickly become saturated with adsorbed siderophores and are thus dissolved at the maximum possible rate. Hence, for each environment, there is a maximum production rate above which the uptake time can no longer be decreased further.

References

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5 Supplementary Figures

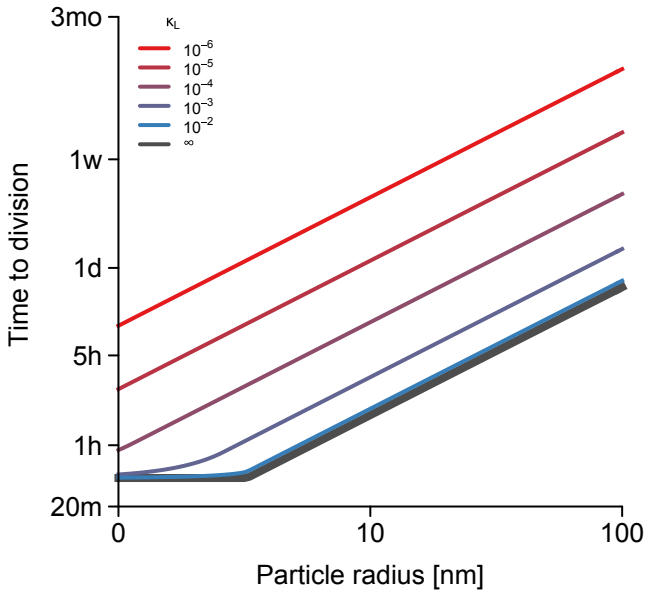

Figure S1: Time required to directly take up sufficient iron for division. The necessary time to reach enough iron for division (10^6 ions) increases as a power law with the aggregation level, k, and the dissolution rate, κ_L. The thick black lines show the required time at maximum uptake rate, i.e. when whole aggregates are taken up instantaneously ($\kappa_L \to \infty$). The background iron concentration is $\rho = 0.1\,\mathrm{nM}$.

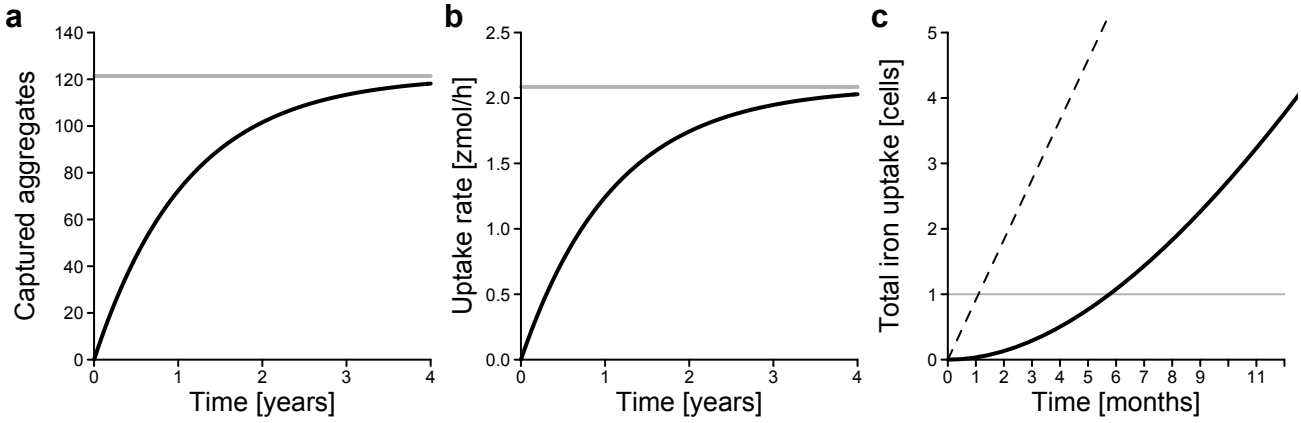

Figure S2: Time dynamics for direct uptake for $k = 10^5$ (particle radius around 5 nm). Both the number of captured aggregates that a cell is actively dissolving (a) as well as the resulting uptake rate of iron (b) only slowly reach their maximum over a period of years. **c.** The maximum possible uptake rate (slope of dashed line) is determined by the diffusion of iron particles towards the cell. However, because of the slow approach to the maximal values in panels a and b, the cells generally only take up iron far below the maximum possible rate. One zetamole (zmol) = 10^{-21} mol.

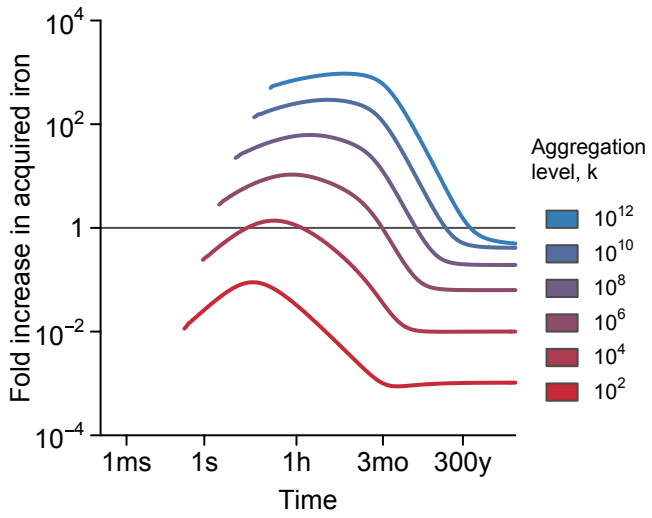

Figure S3: Relative difference in acquired iron over time for secretion compared to direct uptake. The transient increased uptake rate under siderophore secretion results in a large increase in the amount of acquired iron over time. Even at moderate aggregation levels, $k = 10^6, r_k = 10\mu m$, this advantage can last for weeks.

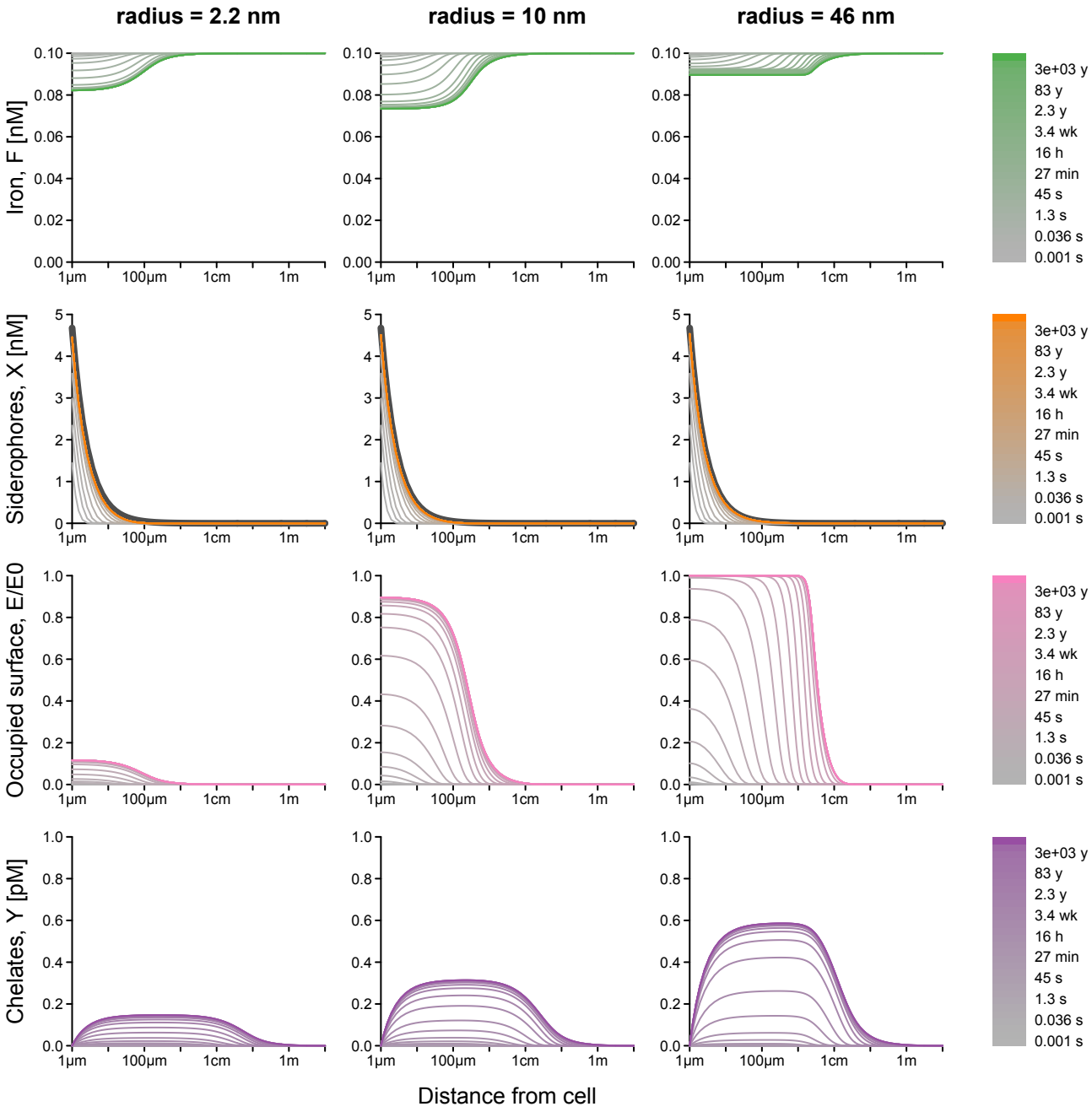

Figure S4: Radial distribution of free iron, free siderophores, ligated surface fraction and iron-siderophore complexes over time. The figure columns correspond to different values of iron aggregation, with each aggregate containing k iron ions. The intensity of the color indicates the time. See also Fig. S5 for $k = 10^6, 10^8, 10^{10}$.

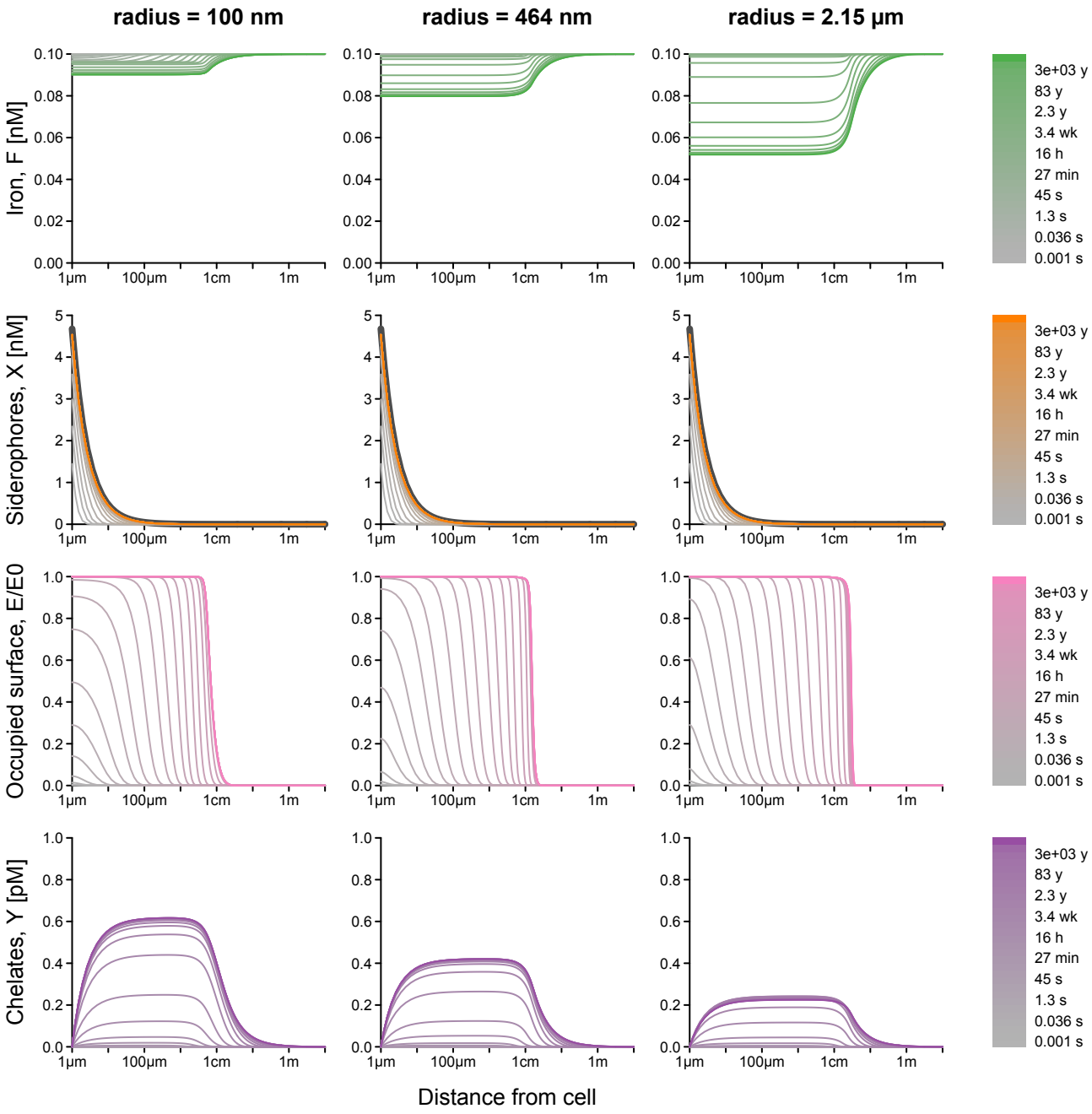

Figure S5: Radial distribution of free iron, free siderophores and iron-siderophore complexes over time (continued).

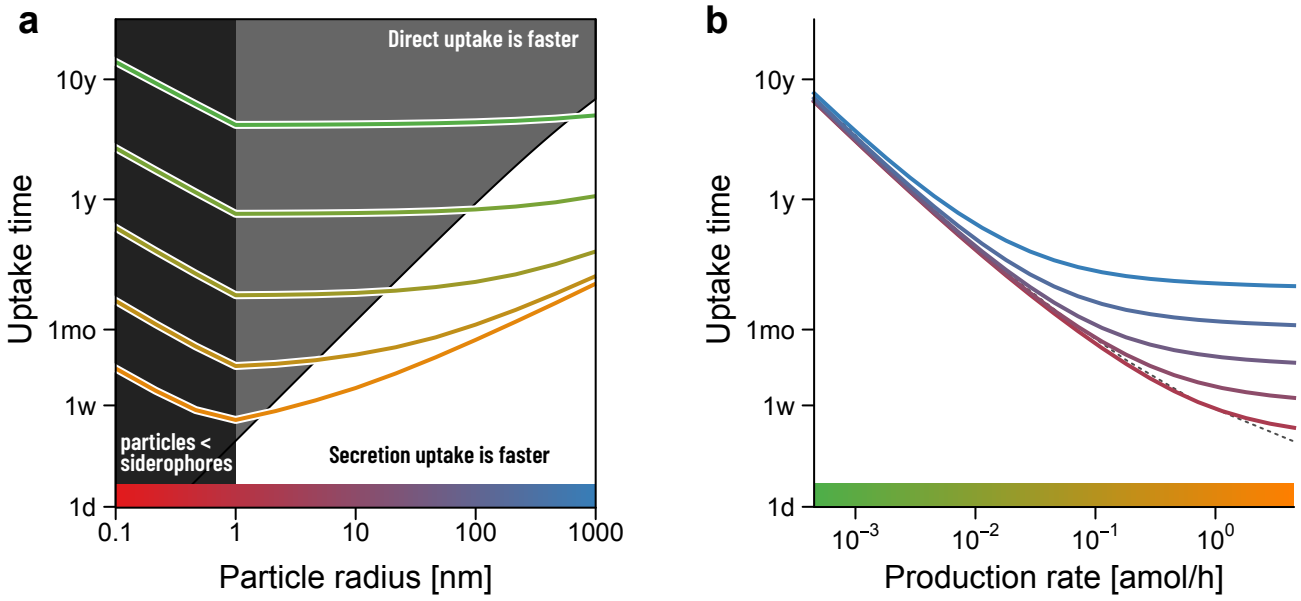

Figure S6: Uptake time for secreter cells. a. The time required to take up enough iron for division ('uptake time') depends on the particle size and the siderophore production rate. The black line dividing the grey and white areas indicates the uptake time for a cell relying on direct uptake. Particle sizes and production rate combinations that fall to the left of this line are values where secretion-based uptake is slower than direct uptake (grey area). Particles sizes and production rate combinations that fall to the right are conditions where secretion-mediated uptake is faster (white area). For small particles, a secreter has higher uptake times than a non-secreter. For large particles, a secreter shortens its uptake time compared to a non-secreter. The full color scale for the production rate is shown in Panel b on the x-axis. **b.** Uptake time is influenced by the siderophore production rate. As the production rate increases from low levels ($< 10^{-3}\,\mathrm{amol/h}$), the uptake rate decreases with slope close to -1, indicating a linear relationship between production rate and uptake time. At higher production levels, the relationship between production rate and uptake time flattens out, indicating that a further increase in production rate only has a small effect on reducing the uptake time. The full color scale for the aggregation level is shown in Panel a on the x-axis.

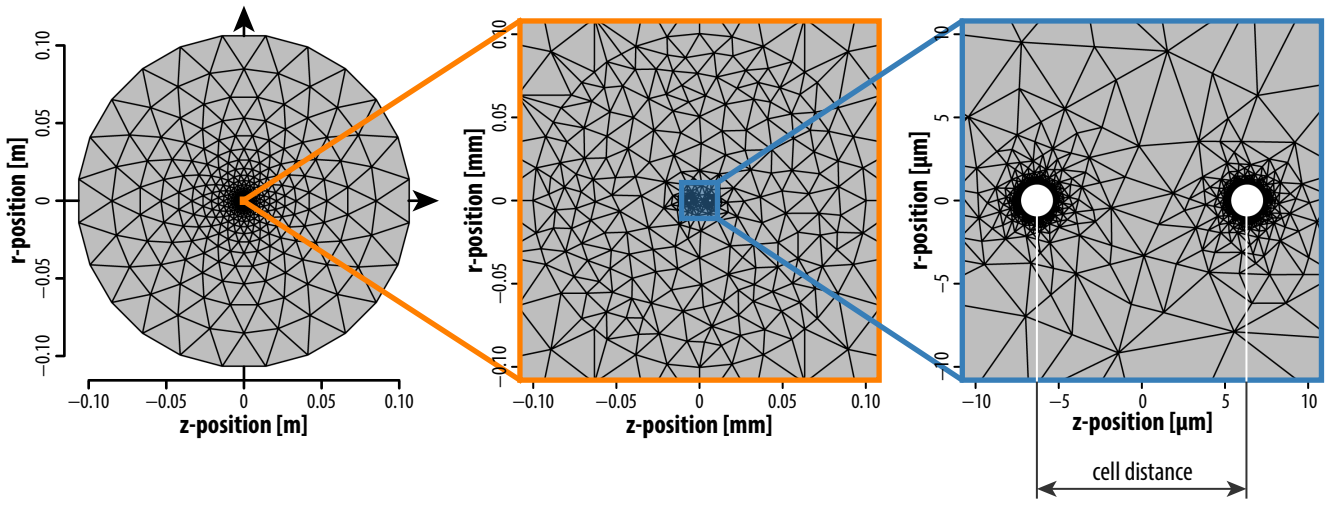

Figure S7: Example mesh used in the 2D numerical integration scheme. The cylindrical symmetry of two cells can be exploited to only solve the reaction-diffusion equations in two dimensions. The large length scale differences between cell spacing and siderophore diffusion require the use of an expanding mesh. First, for $r < 0.1$ mm a mesh was created and refined using the mesh generation routines in FEniCS [1]. Then, for $r > 0.1$ mm a regular circular expanding mesh was added to the local mesh.

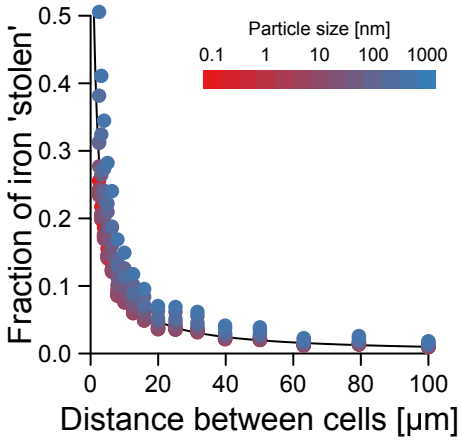

Figure S8: The fraction of iron that is stolen by a neighboring cell depends on the distance between cells. The approximation for the fraction of iron that is stolen by a neighbor at a distance d (black line) is in excellent agreement with the numerical solutions at different aggregation levels (colored points). The color scale is the same as in Fig. 3.