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Why Cells are Microscopic: A Transport-Time Perspective

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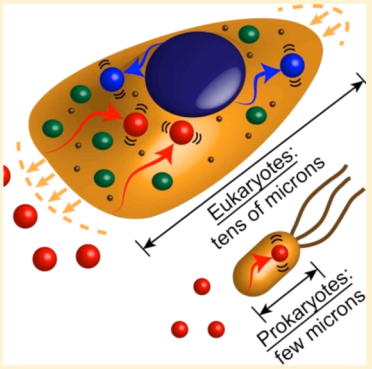
ABSTRACT: Physical−chemical reasoning is used to demonstrate that the sizes of both prokaryotic and eukaryotic cells are such that they minimize the times needed for the macromolecules to migrate throughout the cells and interact/react with one another. This conclusion does not depend on a particular form of the crowded-medium diffusion model, as thus points toward a potential optimization principle of cellular organisms. In eukaryotes, size optimality renders the diffusive transport as efficient as active transport − in this way, the cells can conserve energetic resources that would otherwise be expended in active transport.



SECTION: Kinetics and Dynamics

A striking property of cells − one that still awaits an explanation from "first principles" − is that although cells of different types have different compositions[1,2] and perform markedly different functions, their sizes in the vast majority of cases fall within a relatively narrow range, ∼1 μm for prokaryotes and in tens of micrometers for eukaryotes.[3,4] Numerous models, ranging from the maximization of resource/nutrient uptake[5] to the rates of protein synthesis[6] to the synchronization of calcium oscillations,[7] have been proposed to explain this regularity, but their predictions are largely qualitative and limited in scope. Similarly, "heuristic" arguments invoking the maximization of surface-to-volume ratio correctly conclude that cells should be small but provide no quantitative insights as to how small exactly (in fact, in the absence of any other considerations, the surface-to-volume ratio would be the largest if cells were infinitesimally small). Here we approach the question of cell size from the perspective of optimally timed intracellular transport. Using scaling analysis and accounting for the effects of molecular crowding, we show that the actual sizes of both prokaryotes and eukaryotes are such as to ensure the fastest possible intracellular diffusive transport or signaling. Although scaling arguments are an obvious simplification overlooking the structural and functional details of the cell interior, our results hold irrespective of which particular model is used to describe diffusion through a crowded medium; this regularity suggests that optimization of cell size with respect to transport times is not a model-specific numerical coincidence but rather an architectural principle (likely, one of many) nature has used in designing the cellular units of animate matter. Our analyses also help rationalize when it is preferable for eukaryotic cells to use diffusive versus active transport and save energetic resources, and how the sizes of such cells are tuned with the properties of cytoskeletal fibers underlying cell motility.

The starting point for our analysis is the observation that certain types and numbers, n, of (macro)molecules (proteins, nucleic acids, etc.) are necessary for cells' proper functioning. It has been estimated experimentally[8] that prokaryotes contain on the order of $n \approx 3 \times 10^6$ macromolecules, whereas in the more complex eukaryotes this number is $n \approx 8 \times 10^9$. If the cells were very large, then the average distances between these components would also be large and any interactions/reactions between them would occur on very long time scales, τ. Conversely, if the components were all "crammed" into a very small cell, then the crowding would hinder molecular transport,[9,10] leading, again, to long times required for the molecules to find appropriate targets with which to interact/react. We argue that the actual cell sizes are not only somewhere in between these two regimes (which is obvious) but also are such that they minimize/"optimize" the characteristic transport times (Figure 1).

In our analysis, we focus on the diffusive/passive intracellular transport operative in all prokaryotes and in eukaryotes, where it regulates such important processes as the signaling cascades, organization of mitotic spindle, frequency entrainment via chemical waves, or the assembly of cytoskeletal components involved in cell motility (for review, see ref 1). Unlike active/motor transport in eukaryotic cells whose rate depends predominantly on the molecular details of the motor/cargo interactions,[11,12] diffusion times are known to scale strongly

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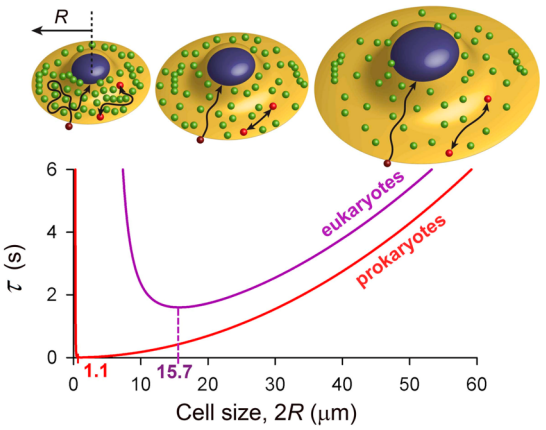

Figure 1. Characteristic times of diffusive transport in differently sized cells. (top) If cells were very small (left), then the crowding of their (macro)molecular contents would slow down diffusion markedly. This slowness is indicated schematically by the long, "wiggly" trajectories of molecules "finding one another" (two red dots) or migrating toward the nucleus (brown dot), as in signaling cascades.[1] Conversely, if cells were very large (right), then diffusion over large distances would, again, make the characteristic diffusion times, τ, very long. The middle picture illustrates the hypothesized "optimal" cell size for which τ is expected to be minimal. If our hypothesis is correct and cells are really optimized for the fastest possible diffusive transport, then the τ versus cell-size dependence should exhibit a minimum. This is, indeed, confirmed by the $\tau(2R)$ dependencies calculated as in the main text and plotted in the (bottom) graph. The minimal τ corresponds to the 15.7 μm size of eukaryotes (violet line) and 1.1 μm size of prokaryotes (red line).

with the dimensions to be traveled and the crowding of the medium in which diffusion takes place. It is, therefore, the diffusive transport that could be "optimized" most readily by varying cell size. Such an optimization could also translate into energetic savings for the cell because diffusion is powered by thermal noise and does not require expenditure of chemical energy (see later in the text).

Scaling Approach. Given that a model of intracellular transport accounting for all structural details is unrealistic, we approach the problem using scaling analysis, in which one uses characteristic ("order of magnitude") parameters describing the system under study. Despite its apparent simplicity, scaling arguments have been used widely in engineering and physics and can yield valuable predictions regarding fluid flows, extrapolate specifications of real-size ships or planes from the corresponding small-scale models,[13] generalize characteristics of phase transitions,[14] rationalize metabolic and circulatory trends across various organisms,[15] and so on. In this spirit, we consider a characteristic time for a diffusive process to transport a macromolecule over a given distance; in three dimensions, this time is $\tau = L^2/6D$. Here the characteristic length scale of the problem can be taken as the cell radius, $L = R$, and D is the diffusion coefficient, which depends on the size of the migrating macromolecules (of typical radii denoted as r) and — importantly — on the "crowding" within the cell. Mathematically, the question we pose is this: Given the number of "necessary components", n, what is the size of the cell that minimizes τ?

Diffusion in a Crowded Cell Interior. To answer this question, it is necessary to relate D to n, r, and R. Diffusivity of particles through a viscous and crowded medium is, in general, governed by three major effects: (i) friction of the solvent, quantified by the viscosity coefficient, (ii) solvent-mediated (known as hydrodynamic) interactions, and (iii) direct contacts between the particles, also referred to as crowding or obstruction effects. Because the relative contributions of these effects vary depending on the particle concentration, it is difficult to develop a "universal" model that would describe diffusivity over the entire possible range of (volumetric) packing fractions, φ. At very low values of φ (i.e., in a very dilute solution), the particles do not "feel" one another, and their diffusivity is well-approximated by the familiar Einstein–Stokes equation: $D_0 = k_BT/6\pi\mu r$. As crowding, φ, increases, and interactions between particles become important. Cukier has shown[16,17] that these interactions suppress diffusivity approximately exponentially, $D = D_0\exp(-\kappa r)$, where κ is the screening constant whose dependence on φ can be expressed as $\kappa = k_C\varphi^\gamma$, where k_C is a system-dependent constant, and γ can be 1/2, 3/4, or 1, depending on the case considered. Specifically, in the so-called semidilute regime (a.k.a. homogeneous regime), $\gamma = 3/4$ and $D = D_0\exp(-k_C r\varphi^{3/4})$. This scaling, however, is *not* applicable to more crowded media, such as a cell, when physical obstructions can become dominant. In this so-called heterogeneous regime, $\gamma = 1/2$ (see refs 16 and 17) such that $D = D_0\exp(-k_0\varphi^{1/2})$; this expression agrees with the experimental data reported by Boyer and Hsu.[18]

Remarkably, identical scaling has been derived by a fundamentally different reasoning by Ogston[19] and is known as the obstruction theory. In this formulation, the infinite-dilution Einstein–Stokes diffusivity, $D_0 = (k_BT/6\pi\mu r)$, is corrected by an exponential factor accounting for the presence of the "obstacles", $D = (k_BT/6\pi\mu r)\exp(-k_0\varphi^{1/2})$, where $k_0 = (r_f + r)/r_f$, r is a typical radius of a diffusing macromolecule, r_f is the typical size of obstacles (e.g., other macromolecules), and both r and r_f are assumed to be of similar sizes (e.g., ~3 to 4 nm, a typical radius of a protein). Furthermore, $\varphi \approx n(r/R)^3$ is the volume fraction of macromolecules in the cell, k_B is Boltzmann's constant, T is the temperature, and $\mu = 3.0$ mPa·s is the experimentally determined "effective" viscosity of the cytoplasm (about three times that of water[20]).

With these preliminaries, the characteristic times can be rewritten as a function of cell radius as $\tau(R) = (R^2/6D) = (\pi\mu r/k_BT)(R^2/(\exp(-k_0n^{1/2}r^{3/2}/R^{3/2})))$ or, alternatively, as a function of the volume fraction $\tau(\varphi) = (R^2/6D) = (\pi\mu r^3/k_BT)n^{2/3}(\varphi^{-2/3}/(\exp(-k_0\varphi^{1/2})))$. The minima of these functions correspond to the values of $R_{min\_\tau} = (3/2)^{2/3}n^{1/3}r$ (nm) and $\varphi_{min\_\tau} \approx 0.4$. If our original hypotheses were correct, then these predicted cell sizes and packing fractions should match those observed in nature. They do. Specifically, the size of a prokaryotic cell (having $n \approx 3 \times 10^6$ macromolecular components) corresponding to the optimal/minimal τ is calculated (Figure 1, bottom) at $2R_{min\_\tau} = 1.1$ μm, whereas the size of an eukaryotic cell ($n \approx 8 \times 10^9$) minimizing transport times is predicted to be $2R_{min\_\tau} = 15.7$ μm; these values are indeed very close to the typical sizes of prokaryotes and eukaryotes. Also, the predicted packing fraction $\varphi_{min\_\tau} \approx 0.4$ is close to the experimental estimates,[21,22] as is the typical diffusion coefficient (calculated $D \approx 0.65 \times 10^{-7}$ cm^2/s vs $O(10^{-7}$ cm^2/s) from experiments).[20,23]

An important point to make is that the above results are not an artifact of a specific diffusion model chosen. For instance, when a conceptually different model of hard-sphere (HS) diffusivity (perhaps, the simplest treatment of diffusion in crowded media) is used, the reasoning analogous to that described above still predicts cell sizes to be $O(10$ μm) for

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eukaryotes, $O(1\ \mu m)$ for prokaryotes, and optimal packing fractions between 0.2 and 0.3. Specifically, in the HS model,[24] the diffusivity in collections of incompressible spheres is given by $D_{HS} = \tilde{D}_0(1 - \rho^*/1.09)(1 + \rho^{*2}(0.4 - 0.83\rho^{*2}))$, where $\tilde{D}_0 = (3r(k_BT/\pi m)^{1/2}/8\rho^*)$ is the HS diffusivity at low density (from Enskog's theory, see[25]), with m being the HS mass and ρ^* the reduced number density of hard spheres (which is related to the volume packing fraction by $\varphi = \pi\rho^*/6$). Also, $\rho^* = 1.09$ corresponds to the glass transition ($D = 0$), and $\rho^* = 1.216$ is Bernal's random close-packed density. (See ref 24 and note that the system freezes before it reaches the close packed density.) Rewriting in terms of the packing fraction (note that we correct for the absence of solvent in the HS model by replacing the Enskog's infinite dilution diffusivity with that of the diffusivity in the Einstein−Stokes equation), we have $D = D_0(1 - (6/\pi)\varphi/1.09)(1 + (6^2/\pi^2)\varphi^2(0.4 - 0.83(6^2/\pi^2)\varphi^2))$. Substituting into the expression for characteristic diffusive time yields:

$$\tau = \frac{R^2}{6D} = \frac{\pi\mu r^3}{k_BT} n^{2/3} \frac{\varphi^{-2/3}}{\left(1 - \frac{6}{\pi}\varphi/1.09\right)\left(1 + \frac{6^2}{\pi^2}\varphi^2\left(0.4 - 0.83\frac{6^2}{\pi^2}\varphi^2\right)\right)}$$

Following the same reasoning as for the Cukier−Ogston model above, the optimal packing fraction is calculated at $\varphi_{min\_\tau} \approx 0.23$, and the corresponding cell sizes are $2R_{min\_\tau} = 1.4\ \mu m$ for prokaryotes and 19.6 μm for eukaryotes.

We make two further comments regarding the parameters of our model and the very use of diffusion formalism to describe passive intracellular transport. First, we note that the general predictions of the model remain valid when a finite spread in the sizes r of the diffusing macromolecules is taken into account. For instance, Figure 2a shows the actual distribution of the radii of proteins found in *E. coli* prokaryotes; for 95% of these proteins ($1.3 < r < 5.3$ nm), the optimal cell size, $2R_{min\_\tau}$, is predicted to be between 0.5 and 2 μm. Similarly, for 86% of proteins found in *Saccharomyces cerevisiae* yeasts/eukaryotes ($0.95 < r < 3.8$ nm, Figure 2b), the predicted optimal size is within 5−20 μm. The second remark is that although some authors have argued that at least some passive intracellular transport is not diffusive but rather anomalous/subdiffusive, the degree of this anomaly has been found to be small, with characteristic exponents between 0.7 and 0.9 (vs 1 in classical diffusion).[26] In addition, subdiffusion only occurs over the time scales of milliseconds, beyond which the trajectory of a macromolecule becomes diffusive.[27,28]

Further Biological Consequences. The optimization of cell size for the fastest diffusive transport appears to have further consequences for cell functioning and properties. A case in point here is the relationship between the size and "energy management" in eukaryotic cells. Unlike prokaryotes, which move their internal components predominantly by diffusion, eukaryotes can also employ active transport along cytoskeletal fibers (microtubules (MTs) or microfilaments[8]). Whereas this mode of transportation costs chemical energy (in the form of high-energy molecules like ATP, GTP, or NADH), it proceeds with constant speed (e.g., ~3 μm/s for vesicles[8]), and the times of transport scale linearly with the distance to be traveled, $\tau_{active}(L) \propto L$. Thus, if the distances are large enough, then one would expect active transport to be faster than diffusion for which $\tau_{diff}(L) \propto L^2$. Yet, for the optimally sized eukaryotes, the opposite is true. To show this, consider Figure 3, which plots and compares the characteristic times required for active transport and for the passive/diffusive transport of "cargos" of different radii r over a given distance L through a medium characterized by the "optimal" packing fraction φ_{min_τ} (as determined in equations above). As seen, for relatively small molecular cargos $r < \sim 4$ nm, $\tau_{diff} < \tau_{active}$ provided that $L < \sim 8$ μm; remarkably, this value of L corresponds to the optimal radius of eukaryotic cells R_{min_τ} (see Figure 1). In other words, the sizes of the eukaryotes are such that to move small molecules or typically sized proteins (refs 31−33 and also Figure 2), these cells do not need to pay the "chemical price" of active transport because simple diffusion is more rapid. Of course, if the loads to carry are larger, then diffusion would be

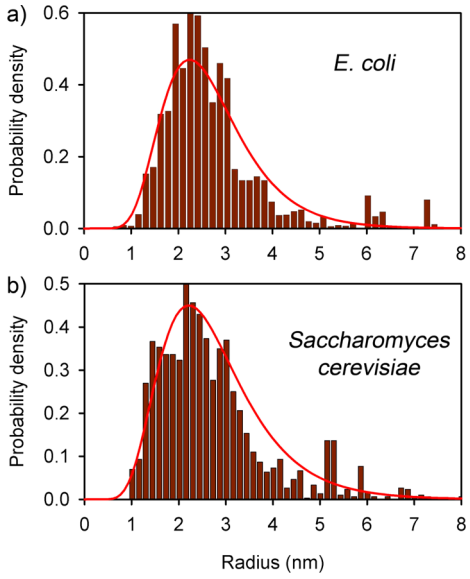

Figure 2. Distributions of the radii of proteins found in (a) *E. coli* and (b) *Saccharomyces cerevisiae*. The distributions were derived from the Protein Data Bank (www.rcsb.org/pdb/) by converting molecular weights to protein radii, r. In this conversion, proteins were approximated as spherical such that $r = (3MW/4\pi \times 1000\rho N_A)^{1/3}$, where MW is the molecular weight in g/mol, ρ is density in kg/m^3, and N_A is the Avogadro constant. Density of proteins is found to be related to their molecular weights by[29] ρ (kg/m^3) = 0.00141 + 0.000145 exp(−MW (Da)/13 000). The distributions thus obtained fit well to log-normal distributions (red lines), as previously suggested in ref 30.

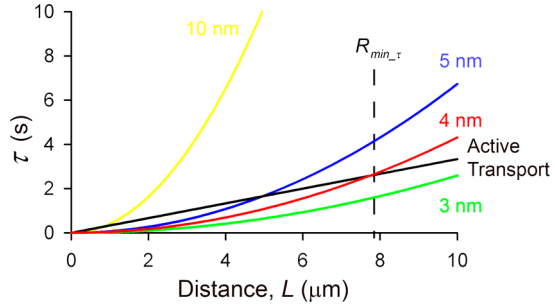

Figure 3. Calculated times, τ, needed to transport a "cargo" of a given radius, r = 3−10 nm, either by diffusion or by active transport over a distance L. Provided that $L < \sim 8$ μm, diffusion transports small molecules and typical macromolecules (of size up to 3 to 4 nm) faster than active transport. Remarkably, this value of L corresponds to the optimal radius R_{min_τ} of an eukaryotic cell calculated in the main text.

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very slow and the cell should and does use active transport. For example, to move a typical vesicle (loaded with proteins, hormones, neurotransmitters, or digestive enzymes[3] packaged in the Golgi apparatus) of size[34] $r \approx 50$ nm from trans-Golgi network to the cell periphery, active transport requires a time of ~2.67 s. If the same vesicle were to diffuse over the same distance, then the time would be a staggering $\sim 10^6$ s.

Whereas the examples above indicate that cell sizes are optimal for diffusive transport speeds, it would be somewhat naive to expect that this criterion is the sole one that dictates cell size. Even in everyday engineering practice, complex systems are typically optimized for several properties/functions simultaneously; for instance, a size of a jet plane is optimized for a combination of speed, desired range, passenger capacity, and fuel/operational costs. It should only be expected that an engineer as skilled as nature itself would strive to perfect cell sizes for multiple properties at once — and it certainly does. To illustrate this point further, we take another look at the network of MT "rails" discussed above as well as other cytoskeletal fibers (intermediate filaments (IFs) and actin filaments (AFs)) that are together important for cell micromechanics[35] and motility.[36,37] The rigidity of these fibers can be quantified by the persistence length, l_P; high l_P means that a fiber is straight and rigid, whereas a low persistence length represents a structure that is wavy and flexible. Importantly, the ratios of the experimentally determined persistence lengths[38] and the optimal cell size we calculated above are on the order of 0.1 for IFs, 1 for AFs, and 10^2 for MTs. This ordering makes perfect biological sense. Specifically, on the scale of the cell size, the IFs are flexible and can thus resist tensile stresses imposed on the cell by straightening up;[39,40] the AFs are "semi-flexible" by themselves but upon polymerizing into actin networks can increase their rigidity by orders of magnitude[41] to form lamellipodial protrusions that push the cell forward; the MTs are rigid and thus can efficiently play their role of "struts" sustaining compressive loads within the cell and maintaining cell shape[42] (and also providing straight tracks for active transport). If cells were significantly smaller than they really are, then all fibers would appear rigid on the scale of the cell size, and the overall structure would be incapable of deformation necessary for cell-shape changes during cell migration or division, stretching of alveolar epithelial cells during breathing,[43] or proper functioning of vascular smooth muscle cells.[44] Conversely, if the cells were larger, then all cytoskeletal fibers would be too flexible, and the "soft" cells would not be mechanically sturdy or capable of efficient locomotion via the actin cytoskeleton.

To summarize, given a specific number n of macromolecular components required for proper functioning, evolution appears to have selected cell sizes such as to minimize the characteristic times of the diffusive transport of these components across the cell. This optimality of transport times is also beneficial to other functions of the cell. Although our analysis and conclusions apply to the majority of cell types, there are notable exceptions — for example, oocytes and some motor and sensory neurons — where requirements other than transport efficiency might affect cell size. For instance, it has been suggested that oocytes are large because they need to store material required for the development of an entire new organism[3] and also because they optimize the likelihood for fertilization (larger targets for sperm cells[45]). In the nervous system, signal transmission through one long neuron/axon may be needed to ensure direct and rapid propagation of electrical signals. (Instead, if the same signal was transmitted by several shorter axons, then transmission would be slowed down by signaling at synapses.[46]) Not surprisingly, because diffusion across these large cells would be very slow, both oocytes and neurons rely heavily on bidirectional active transport along MTs.

From a chemistry point of view, our analysis is relevant to the recent effort to synthesize cell mimics ("protocells"[47−49]). If such artificial systems were ever to resemble real cells, then their sizes should be scrutinized for transport speeds, packing fractions, and other pertinent parameters using the scaling rules/arguments similar to those we applied in the present work.

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Notes

The authors declare no competing financial interest.

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