# Fundamental limits on the rate of bacterial cell division

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**Abstract** This will be written next

## Uptake of Nutrients

In order to build new cellular mass, the molecular and elemental building blocks must be scavenged from the environment in different forms. Carbon, for example, is acquired via the transport of carbohydrates and sugar alcohols with some carbon sources receiving preferential treatment in their consumption (**?**). Phosphorus, sulfur, and nitrogen, on the other hand, are harvested primarily in the forms of inorganic salts, namely phosphate, sulfate, and ammonia (**??????**). All of these compounds have different permeabilities across the cell membrane and most require some energetic investment either via ATP hydrolysis or through the proton electrochemical gradient to bring the material across the hydrophobic cell membrane. Given the diversity of biological transport mechanisms and the vast number of inputs needed to build a cell, we begin by considering transport of some of the mot important cellular ingredients: carbon, nitrogen, oxygen, hydrogen, phosphorus, and sulfur.

The elemental composition of *E. coli* has received much quantitative attention over the past half century (**????**), providing us with a starting point for estimating the copy numbers of various transporters. While there is some variability in the exact elemental percentages (with different uncertainties), we can estimate that the dry mass of a typical *E. coli* cell is ≈ 45% carbon (BNID: 100649, **?**), ≈ 15% nitrogen (BNID: 106666, **?**), ≈ 3% phosphorus (BNID: 100653, **?**), and 1% sulfur (BNID: 100655, **?**). In the coming paragraphs, we will engage in a dialogue between back-of-the-envelope estimates for the numbers of transporters needed to facilitate these chemical stoichiometries and the experimental proteomic measurements of the biological reality. Such an approach provides the opportunity to test if our biological knowledge is sufficient to understand the scale at which these complexes are produced. Specifically, we will make these estimates considering a modest doubling time of 5000 s, a growth rate of ≈ 0.5 $hr^{-1}$, the range in which the majority of the experimental measurements reside.

## Nitrogen Transport

Before we begin our back-of-the-envelope estimations, we must address which elemental sources must require proteinaceous transport, meaning that the cell cannot acquire appreciable amounts simply via diffusion from the membrane. The permeability of the lipid membrane to a large number of solutes has been extensively characterized over the past century. Large, polar molecular species (such as various sugar molecules, sulfate, and phosphate) have low permeabilities while small, non-polar compounds (such as oxygen, carbon dioxide, and ammonia) can readily diffuse across the membrane. Ammonia, a primary source of nitrogen in typical laboratory conditions, has a permeability on par with water ($\approx 10^5$ nm/s, BNID:110824 **?**). In particularly nitrogen-poor conditions, *E. coli* expresses a transporter (AmtB) which appears to aid in nitrogen assimilation, though the mechanism and kinetic details of transport is still a matter of debate (**??**). Beyond ammonia, another plentiful source of nitrogen come in the form of glutamate, which has it's own complex metabolism and scavenging pathways. However, nitrogen is plentiful in the growth conditions examined in this work, permitting us to neglect nitrogen transport as a potential rate limiting process in cell division in typical experimental conditions. We direct the reader to the supplemental information for a more in-depth discussion of permeabilities and a series of calculations revealing that active nitrogen transport can be neglected for the purposes of this article.

## Carbon Transport

We begin our estimations with the most abundant element in *E. coli* by mass, carbon. Using $\approx 0.3$ pg as the typical *E. coli* dry mass (BNID: 103904, **?**), we estimate that $\approx 10^{10}$ carbon atoms must be brought into the cell in order to double all of the carbon-containing molecules (***Figure 1***(A, top))). Typical laboratory growth conditions, such as those explored in the aforementioned proteomic data sets, provide carbon as a single class of sugar such as glucose, galactose, or xylose to name a few. *E. coli* has evolved myriad mechanisms by which these sugars can be transported across the cell membrane. One such mechanism of transport is via the PTS system which is a highly modular system capable of transporting a diverse range of sugars (**?**). The glucose-specific component of this system transports $\approx 200$ glucose molecules per second per channel (BNID: 114686, **?**). Making the assumption that this is a typical sugar transport rate, coupled with the need to transport $10^{10}$ carbon atoms, we arrive at the conclusion that on the order of 1,000 transporters must be expressed in order to bring in enough carbon atoms to divide in 5000 s, diagrammed in the top panel of ***Figure 1***(A). This estimate, along with the observed average number of the PTS system carbohydrate transporters present in the proteomic data sets (**????**), is shown in ***Figure 1***(A). While we estimate 1500 transporters are needed with a 5000 s division time, we can abstract this calculation to consider any particular growth rate given knowledge of the cell density and volume as a function of growth rate and direct the reader to the SI for more information. As revealed in ***Figure 1***(A), experimental measurements exceed the estimate by several fold, illustrating that transport of carbon in to the cell is not rate limiting for cell division.

The estimate presented in ***Figure 1***(A) neglects any specifics of the regulation of carbon transport system and presents a data-averaged view of how many carbohydrate transporters are present on average. Using the diverse array of growth conditions explored in the proteomic data sets, we can explore how individual carbon transport systems depend on the population growth rate. In ***Figure 1***(B), we show the total number of carbohydrate transporters specific to different carbon sources. A striking observation, shown in the top-left plot of ***Figure 1***(B), is the constancy in the expression of the glucose-specific transport systems. Additionally, we note that the total number of glucose-specific transporters is tightly distributed $\approx 10^4$ per cell, the approximate number of transporters needed to sustain rapid growth of several divisions per hour, as indicated by the grey shaded line. This illustrates that *E. coli* maintains a substantial number of complexes present for transporting glucose which is known to be the preferential carbon source (**???**).

It is now understood that a large number of metabolic operons are regulated with dual-input logic gates that are only expressed when glucose concentrations are low (mediated by cyclic-AMP

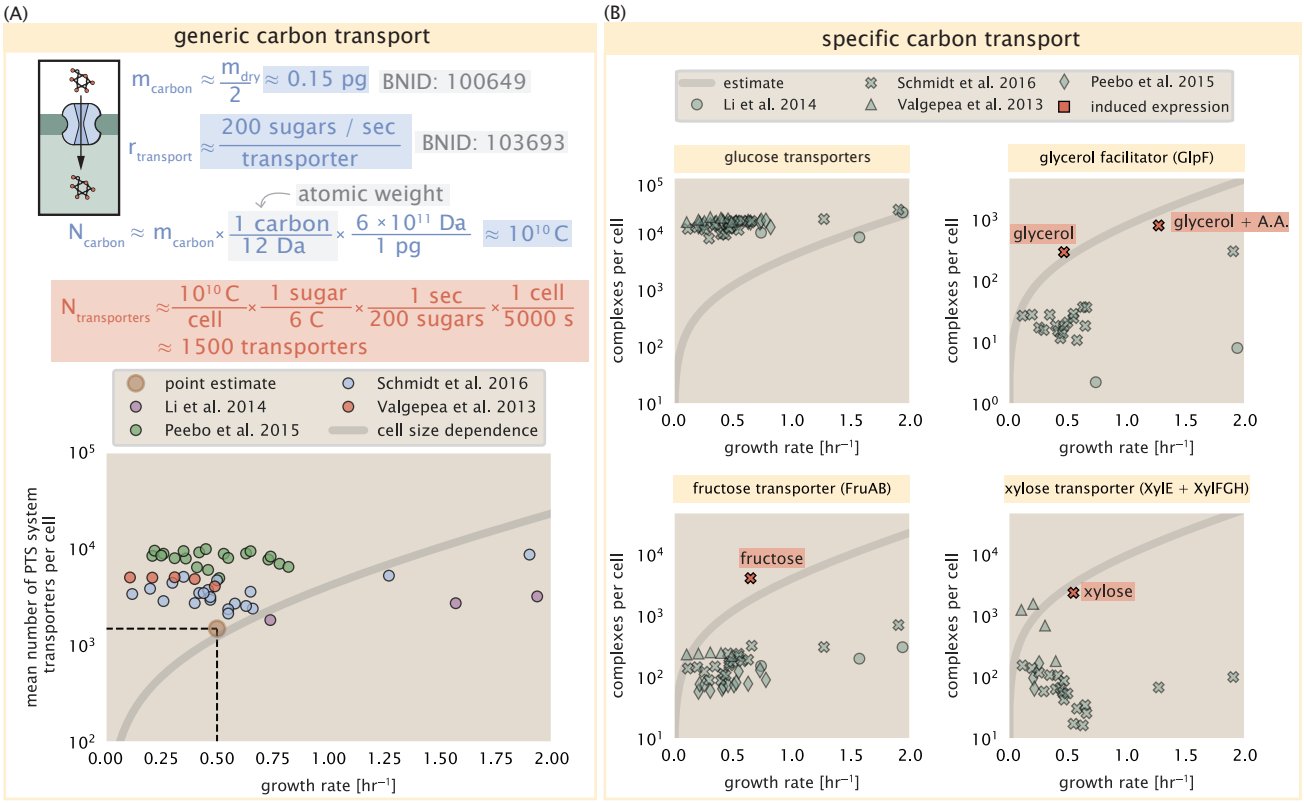


**Figure 1. The abundance of carbon transport systems across growth rates.** (A) A simple estimate for the minimum number of generic carbohydrate transport systems (top) assumes $\approx 10^{10}$ C are needed to complete division, each transported sugar contains $\approx 6$ C, and each transporter conducts sugar molecules at a rate of $\approx 200$ per second. Bottom plot shows the estimated number of transporters needed at a growth rate of $\approx 0.5$ per hr (light-brown point and dashed lines). Colored points correspond to the mean number of PTS system sugar transporters (complexes annotated with the Gene Ontology term GO:0009401) for different growth conditions across different published datasets. (B) The abundance of various specific carbon transport systems plotted as a function of the population growth rate. Red points and red-highlighted text indicate conditions in which the only source of carbon in the growth medium induces expression of the transport system. Grey line in (A) and (B) represents the estimated number of transporters per cell at a continuum of growth rates.

receptor protein CRP) and the concentration of other carbon sources are elevated (**??**). A famed example of such dual-input regulatory logic is in the regulation of the *lac* operon which is only natively activated in the absence of glucose and the presence of allolactose, an intermediate in lactose metabolism (**?**), though we now know of many other such examples (**???**). This illustrates that once glucose is depleted from the environment, cells have a means to dramatically increase the abundance of the specific transporter needed to digest the next sugar that is present. Several examples of induced expression of specific carbon-source transporters are shown in ***Figure 1***(B). Points colored in red (labeled by red text-boxes) correspond to growth conditions in which the specific carbon source (glycerol, xylose, or fructose) is present. These plots show that, in the absence of the particular carbon source, expression of the transporters is maintained on the order of $\sim 10^2$ per cell. However, when induced, the transporters become highly-expressed and fall close to the predicted number of transporters needed to facilitate growth on that substrate alone, shown as a transparent grey line. Together, this generic estimation and the specific examples of induced expression suggest that transport of carbon across the cell membrane, while critical for growth, is not the rate-limiting step of cell division.

## Phosphorus and Sulfur Transport

We now turn our attention towards other essential elements, namely phosphorus and sulfur. Phosphorus is critical to the cellular energy economy in the form of high-energy phosphodiester bonds making up DNA, RNA, and the NTP energy pool as well as playing a critical role in the post-translational modification of proteins and defining the polar-heads of lipids. In total, phosphorus makes up ≈3% of the cellular dry mass which in typical experimental conditions is in the form of inorganic phosphate. The cell membrane has remarkably low permeability to this highly-charged and critical molecule, therefore requiring the expression of active transport systems. In *E. coli*, the proton electrochemical gradient across the inner membrane is leveraged to transport inorganic phosphate into the cell (**?**). Proton-solute symporters are widespread in *E. coli* (**??**) and can have rapid transport rates of 50 to 100 molecules per second for sugars and other solutes (BNID: 103159; 111777, **?**). As a more extreme example, the proton transporters in the $F_1$-$F_0$ ATP synthase, which leverage the proton electrochemical gradient for rotational motion, can shuttle protons across the membrane at a rate of ≈ 1000 per second (BNID: 104890; 103390, (**?**)). In *E. coli* the PitA phosphate transport system has been shown to very tightly coupled with the proton electrochemical gradient with a 1:1 proton:phosphate stoichiometric ratio (**??**). Taking the geometric mean of the aforementioned estimates gives a plausible rate of phosphate transport on the order of 300 per second. Illustrated in ***Figure 2***(A), we can estimate that ≈ 150 phosphate transporters are necessary to maintain an ≈ 3% dry mass with a 5000 s division time. This estimate is again satisfied when we examine the observed copy numbers of PitA in proteomic data sets (plot in ***Figure 2***(A)). While our estimate is very much in line with the observed numbers, we emphasize that this is likely a slight over estimate of the number of transporters needed as there are other phosphorous scavenging systems, such as the ATP-dependent phosphate transporter Pst system which we have neglected.

Satisfied that there are a sufficient number of phosphate transporters present in the cell, we now turn to sulfur transport as another potentially rate limiting process. Similar to phosphate, sulfate is highly-charged and not particularly membrane permeable, requiring active transport. While there exists a H+/sulfate symporter in *E. coli*, it is in relatively low abundance and is not well characterized (**?**). Sulfate is predominantly acquired via the ATP-dependent ABC transporter CysUWA system which also plays an important role in selenium transport (**??**). While specific kinetic details of this transport system are not readily available, generic ATP transport systems in prokaryotes transport on the order of 1 to 10 molecules per second (BNID: 109035, **?**). Combining this generic transport rate, measurement of sulfur comprising 1% of dry mass, and a 5000 second division time yields an estimate of ≈ 1000 CysUWA complexes per cell (***Figure 2***(B)). Once again, this estimate is in notable agreement with proteomic data sets, suggesting that there are sufficient transporters present to acquire the necessary sulfur. In a similar spirit of our estimate of phosphorus transport,

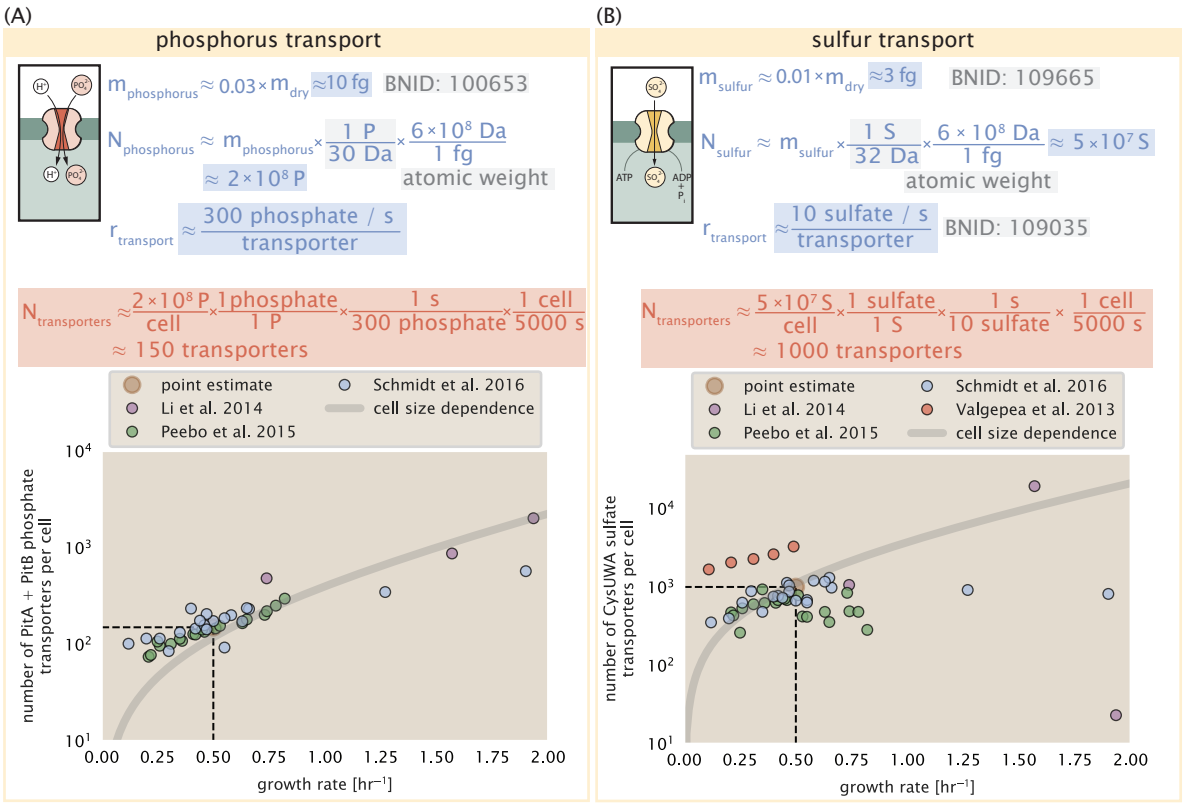


**Figure 2. Estimates and measurements of phosphate and sulfate transport systems as a function of growth rate.** (A) Estimate for the number of PitA phosphate transport systems needed to maintain a 3% phosphorus *E. coli* dry mass. Points in plot correspond to the the total number of PitA transporters per cell. (B) Estimate of the number of CysUWA complexes necessary to maintain a 1% sulfur *E. coli* dry mass. Points in plot correspond to average number of CysUWA transporter complexes that can be formed given the transporter stoichiometry $[CysA]_2[CysU][CysW][Sbp/CysP]$. Grey line in (A) and (B) represents the estimated number of transporters per cell at a continuum of growth rates.

we emphasize that this is likely an overestimate of the number of necessary transporters as we have neglected other sulfur scavenging systems that are in lower abundance.

## Limits on Transporter Expression

So which, if any, of these processes may be rate limiting for growth? As suggested by **??** (B), induced expression can lead to an order-of-magnitude (or more) increase in the amount of transporters needed to facilitate transport. Thus, if acquisition of nutrients was the limiting state in cell division, could expression simply be increased to accommodate faster growth? A way to approach this question is to compute the amount of space in the bacterial membrane that could be occupied by nutrient transporters. Considering a rule-of-thumb for the surface area of *E. coli* of about 6 $\mu m^2$ (BNID: 101792, **?**), we expect an areal density for 1000 transporters to be approximately 200 transporters/$\mu m^2$. For a typical transporter occupying about 50 nm$^2$/dimer, this amounts to about only 1 percent of the total inner membrane (**?**). In addition, bacterial cell membranes typically have densities of $10^5$ proteins//$\mu m^2$ (**?**), implying that the cell could accommodate more transporters of a variety of species if it were rate limiting. As we will see in the next section, however, occupancy of the membrane can impose other limits on the rate of energy production.

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