

Fundamental limits on the rate of bacterial cell division

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Abstract This will be written next (promise).

Introduction

The observed range of bacterial growth rates is enormously diverse. In natural environments, some microbial organisms might double only once per year (*Mikucki et al., 2009*) while in comfortable laboratory conditions, growth can be rapid with several divisions per hour (*Schaechter et al., 1958*). This six order-of-magnitude difference in time scales encompasses different microbial species and lifestyles, yet even for a single species such as *E. coli*, the growth rate can be modulated over a similar scale by tuning the type and amount of nutrients in the growth medium. This remarkable flexibility in growth rate illustrates the intimate relationship between environmental conditions and the rates at which cells convert nutrients into new cellular material – a relationship that has remained a major topic of inquiry in bacterial physiology for over a century (*Jun et al., 2018*).

As was noted by Jacques Monod, “the study of the growth of bacterial cultures does not constitute a specialized subject or branch of research, it is the basic method of Microbiology.” Those words ring as true today as they did when they were written 70 years ago (*Monod, 1949*). Indeed, the study of bacterial growth has undergone a renaissance. Many of the key questions addressed by the pioneering efforts in the middle of the last century can be revisited by examining them through the lens of the increasingly refined molecular census that is available for bacteria such as the microbial workhorse *Escherichia coli*. In this work, we explore an amalgamation of recent proteomic data sets to explore fundamental limits of bacterial growth.

Several of the evergreen questions about bacterial growth that were originally raised by microbiologists in the middle of the 20th century can now be reframed in light of this newly available data. For example, what biological processes set the absolute speed limit for how fast bacterial cells can grow and reproduce? How do cells alter the absolute numbers and relative ratios of their molecular constituents as a function of changes in growth rate or nutrient availability? In this paper, we address these two questions from two distinct angles. First, as a result of an array

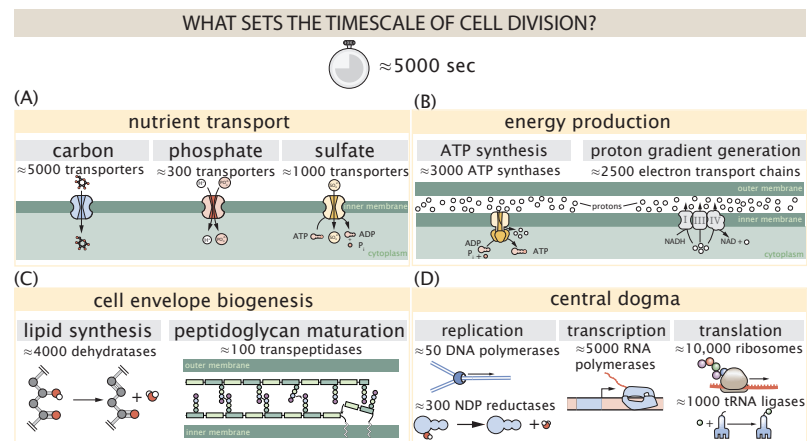


Figure 1. Transport and synthesis processes necessary for cell division. We consider an array of processes necessary for a cell to double its molecular components, broadly grouped into four classes. These categories are (A) nutrient transport across the cell membrane, (B) energy production (namely, ATP synthesis), (C) cell envelope biogenesis, and (D) processes associated with the central dogma. Numbers shown are the approximate number of complexes of each type observed at a growth rate of 0.5 hr^{-1} , or a cell doubling time of $\approx 5000 \text{ s}$.

of high-quality proteome-wide measurements of the *E. coli* proteome under myriad growth conditions, we have a census that allows us to explore how the number of key molecular players change as a function of growth rate. This census provides a window into the question of whether the rates of central processes such as energy generation or DNA synthesis are regulated systematically as a function of cell growth rate by altering protein copy number in individual cells. Second, by compiling molecular turnover rate measurements for many of the fundamental processes associated with bacterial growth, we can make quantitative estimates to determine whether the observed protein copy numbers under varying conditions appear to be in excess of what would be minimally required to support cell growth at the observed rates.

In this paper, we make a series of order-of-magnitude estimates for the copy numbers and growth rate dependent expression of a variety of different processes, schematized in **Figure 1**, informed by the collection of proteomic data sets. We use these estimates to explore which, if any, of the hypothesis illustrated in **Figure 1** may act as molecular bottlenecks that limit bacterial growth. Specifically, we leverage a combination of *E. coli* proteomic data sets collected over the past decade using either mass spectrometry (Schmidt et al., 2016; Peebo et al., 2015; Valgepea et al., 2013) or ribosomal profiling (Li et al., 2014) across 31 unique growth conditions. Throughout, our estimates we consider a modest growth rate of $\approx 0.5 \text{ hr}^{-1}$ corresponding to a doubling time of ≈ 5000 seconds, as the data sets heavily sample this regime. While we formulate point estimates for the complex abundances at this division time, we consider how these values will vary at other growth rates due to changes in cell size, surface area, and chromosome copy number (Taheri-Araghi et al., 2015).

Broadly, we find that for the majority of these estimates the protein copy numbers appear well-tuned for the task of cell doubling at a given growth rate. From our analysis, it emerges that translation, particularly of ribosomal proteins, is the most plausible candidate for a molecular bottleneck. We reach this conclusion by considering that translation is 1) a rate limiting step for the fastest bacterial division, and 2) a major determinant of bacterial growth across the nutrient conditions we have considered under steady state, exponential growth. This enables us to suggest that the long-observed correlation between growth rate and cell size (Schaechter et al., 1958; Si et al., 2017) can be simply attributed to the increased absolute number of ribosomes per cell under conditions supporting extremely rapid growth, a hypothesis which we formally mathematize and explore.

Box 1. The Rules of Engagement for Order-Of-Magnitude Estimates

This work relies heavily on using so-called "back-of-the-envelope" estimates to understand the abundances and growth-rate dependences of a variety of molecular complexes. This moniker arises from the limitation that any estimate should be able to fit on the back of a postage envelope. As such, we must draw a set of rules governing our precision and sources of key values.

The rule of "one, few, and ten". The philosophy behind order-of-magnitude estimates is to provide a estimate of the appropriate scale and a prediction with infinite accuracy. As such, we define three different scales of precision in making the estimates. The scale of "one" is reserved for values that range between 1 and 2. For example, If a particular process has been experimentally measured to transport 1.87 protons per second, we approximate this process to require 2 protons per event. The scale of "few" is reserved for values ranging between 3 and 5. For example, we will often use Avogadro's number to compute the number of molecules in a cell given a concentration and a volume. Rather than using Avogadro's number as 6.02214×10^{23} , we will approximate it as 5×10^{23} . Finally, the scale of "ten" is reserved for values which we know within an order of magnitude. If a particular protein complex is present at 883 copies per cell, we say that it is present in approximately 10^3 copies per cell. These different scales will be used in combination to arrive at simple estimates that report the expected scale of the observed data. Therefore, the estimates presented here should not be viewed as hard-and-fast predictions of precise copy numbers, but as approximate lower (or upper) bounds for the number of complexes that may be needed to satisfy some cellular requirement.

Furthermore, we use equality symbols ($=$) sparingly and frequently defer to approximation (\approx) or scaling (\sim) symbols when reporting an estimate. When \approx is used aside a value, we are implicitly stating that we are confident in this estimate within a factor of a few. When a scaling symbol \sim is used, we are stating that we are confident in our estimate to within an order of magnitude.

The BioNumbers Database as a source for values. In making our point estimates, we often require approximate values for key cellular properties, such as the elemental composition of the cell, the average dry mass, or approximate rates of synthesis an polymerization. We rely heavily on the BioNumbers Database (*Milo et al., 2010*) as a repository for such information. Every value we draw from the BioNumbers Database as an associated BioNumbers ID number, abbreviated as BNID. When we have used a value from the data base in making our estimate, we provide this ID number (or numbers, depending on the quantity) in parentheses on in grey-boxes in the figures. For some processes, values needed to complete the estimate were not present in the data base. In these cases, we turned to the original literature and have provided citations to the appropriate references from where the value was obtained.

Uncertainty in the data sets and the accuracy of an estimate. The data sets presented analyzed in this work are the products of extremely careful experimentation with the aim to report, to the best of their ability, the absolute copy numbers of proteins in the cell. These data, collected over the span of a few years, come from different labs and use different internal standards, controls, and even techniques. As a result, there is notable disagreement in the observed copy numbers for some complexes between the different data sets. In assessing whether our estimates could explain the observed scales and growth-rate dependencies, we considered the degree of variation between the different data sets. For example, say a particular estimate undercuts the observed data by an order of magnitude. If all data sets agree within a factor of a few of each other, we revisit our estimate and consider what we may have missed. However, if the data sets themselves disagree by an order of magnitude between them, we determine that our estimate is appropriate given the variation in the data.

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 (Monod, 1947). Phosphorus, sulfur, and nitrogen, on the other hand, are harvested primarily in the forms of inorganic salts, namely phosphate, sulfate, and ammonia (Jun et al., 2018; Assentoft et al., 2016; Stasi et al., 2019; Antonenko et al., 1997; Rosenberg et al., 1977; Will-sky et al., 1973). All of these compounds have different permeabilities across the cell membrane (Phillips (2018) 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 most 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 (Neidhardt et al., 1991; Taymaz-Nikerel et al., 2010; Heldal et al., 1985; Bauer and Ziv, 1976), 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 $\approx 45\%$ carbon (BNID: 100649, Milo et al. (2010)), $\approx 15\%$ nitrogen (BNID: 106666, Milo et al. (2010)), $\approx 3\%$ phosphorus (BNID: 100653, Milo et al. (2010)), and 1% sulfur (BNID: 100655, Milo et al. (2010)). 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 $\approx 0.5 \text{ hr}^{-1}$, 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 across 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 ($\approx 10^5 \text{ nm/s}$, BNID:110824 Milo et al. (2010)). 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 are still a matter of debate (van Heeswijk et al., 2013; Khademi et al., 2004). Beyond ammonia, another plentiful source of nitrogen come in the form of glutamate, which has its 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 $\approx 0.3 \text{ pg}$ as the typical *E. coli* dry mass (BNID: 103904, Milo et al. (2010)), 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 2(A, top)). Typical laboratory growth conditions, such as those explored in the aforementioned

171 proteomic data sets, provide carbon as a single class of sugar such as glucose, galactose, or xylose
 172 to name a few. *E. coli* has evolved myriad mechanisms by which these sugars can be transported
 173 across the cell membrane. One such mechanism of transport is via the PTS system which is a
 174 highly modular system capable of transporting a diverse range of sugars (Escalante et al., 2012).
 175 The glucose-specific component of this system transports ≈ 200 glucose molecules per second per
 176 transporter (BNID: 114686, Milo et al. (2010)). Making the assumption that this is a typical sugar
 177 transport rate, coupled with the need to transport 10^{10} carbon atoms, we arrive at the conclusion
 178 that on the order of 1,000 transporters must be expressed in order to bring in enough carbon
 179 atoms to divide in 5000 s, diagrammed in the top panel of Figure 2(A). This estimate, along with
 180 the observed average number of the PTS system carbohydrate transporters present in the pro-
 181 teomic data sets (Schmidt et al., 2016; Peebo et al., 2015; Valgepea et al., 2013; Li et al., 2014), is
 182 shown in Figure 2(A). While we estimate 1500 transporters are needed with a 5000 s division time,
 183 we can abstract this calculation to consider any particular growth rate given knowledge of the cell
 184 density and volume as a function of growth rate and direct the reader to the SI for more informa-
 185 tion. As revealed in Figure 2(A), experimental measurements exceed the estimate by several fold,
 186 illustrating that transport of carbon into the cell is not rate limiting for cell division. Abstracting this
 187 point estimate at 5000 s to a continuum of growth rates (grey line in Figure 2(A)) reveals an excess
 188 of transporters at other growth rates, though in rapid growth regimes, the abundance is below our
 189 simple estimate.

190 The estimate presented in Figure 2(A) neglects any specifics of the regulation of the carbon
 191 transport system and presents a view of how many carbohydrate transporters are present on
 192 average. Using the diverse array of growth conditions explored in the proteomic data sets, we
 193 can explore how individual carbon transport systems depend on the population growth rate. In
 194 Figure 2(B), we show the total number of carbohydrate transporters specific to different carbon
 195 sources. A striking observation, shown in the top-left plot of Figure 2(B), is the constancy in the
 196 expression of the glucose-specific transport systems. Additionally, we note that the total number
 197 of glucose-specific transporters is tightly distributed at $\approx 10^4$ per cell, the approximate number of
 198 transporters needed to sustain rapid growth of several divisions per hour. This illustrates that *E.*
 199 *coli* maintains a substantial number of complexes present for transporting glucose regardless of
 200 growth rate, which is known to be the preferential carbon source (Monod, 1947; Liu et al., 2005;
 201 Aidelberg et al., 2014).

202 It is now understood that a large number of metabolic operons are regulated with dual-input
 203 logic gates that are only expressed when glucose concentrations are low (mediated by cyclic-AMP
 204 receptor protein CRP) and the concentration of other carbon sources are elevated (Gama-Castro
 205 et al., 2016; Zhang et al., 2014b). A famed example of such dual-input regulatory logic is in the regu-
 206 lation of the *lac* operon which is only natively activated in the absence of glucose and the presence
 207 of allolactose, an intermediate in lactose metabolism (Jacob and Monod, 1961), though we now
 208 know of many other such examples (Ireland et al., 2020; Gama-Castro et al., 2016; Belliveau et al.,
 209 2018). This illustrates that once glucose is depleted from the environment, cells have a means to
 210 dramatically increase the abundance of the specific transporter needed to digest the next sugar
 211 that is present. Several examples of induced expression of specific carbon-source transporters
 212 are shown in Figure 2(B). Points colored in red (labeled by red text-boxes) correspond to growth
 213 conditions in which the specific carbon source (glycerol, xylose, or fructose) is present. These plots
 214 show that, in the absence of the particular carbon source, expression of the transporters is main-
 215 tained on the order of $\sim 10^2$ per cell. However, when the transport substrate is present, expression
 216 is induced and the transporters become highly-expressed. The grey lines in Figure 2(B) show the
 217 estimated number of transporters needed at each growth rate to satisfy the cellular carbon re-
 218 quirement. It is notable that in all cases, the magnitude of induced expression (shown in red) falls
 219 close to the estimate, illustrating the ability of the cell to tune expression in response to changing
 220 environments. Together, this generic estimation and the specific examples of induced expression
 221 suggest that transport of carbon across the cell membrane, while critical for growth, is not the

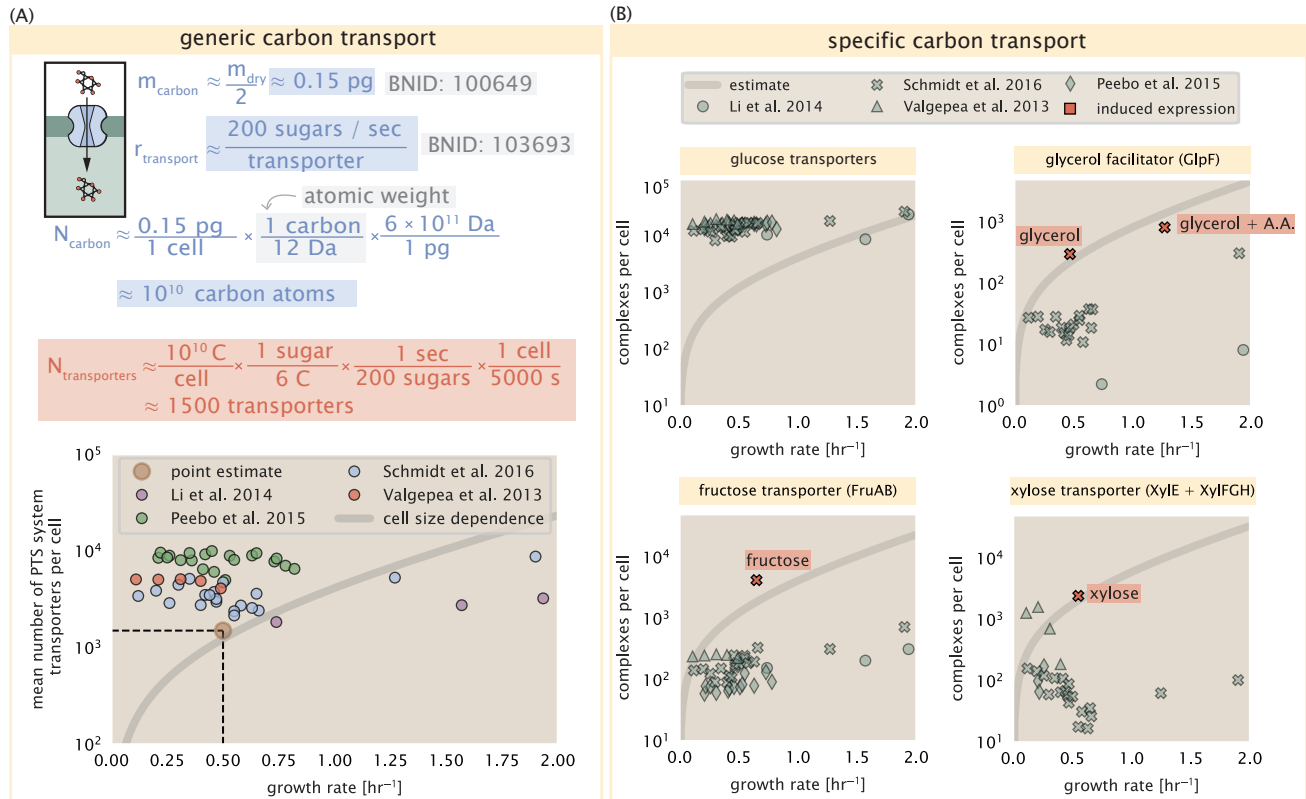


Figure 2. The abundance of carbon transport systems across growth rates. (A) A simple estimate for the minimum number of generic carbohydrate transport systems (top) assumes $\approx 10^{10}$ C are needed to complete division, each transported sugar contains ≈ 6 C, and each transporter conducts sugar molecules at a rate of ≈ 200 per second. Bottom plot shows the estimated number of transporters needed at a growth rate of ≈ 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. The rates of substrate transport to compute the continuum growth rate estimate (grey line) were 200 glucose $\cdot \text{s}^{-1}$ (BNID: 103693, *Milo et al. (2010)*), 2000 glycerol $\cdot \text{s}^{-1}$ (*Lu et al., 2003*), 200 fructose $\cdot \text{s}^{-1}$ (assumed to be similar to PtsI, BNID: 103693, *Milo et al. (2010)*), and 50 xylose $\cdot \text{s}^{-1}$ (assumed to be comparable to LacY, BNID:103159, *Milo et al. (2010)*). Red points and 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.

222 rate-limiting step of cell division.

223 **Phosphorus and Sulfur Transport**

224 We now turn our attention towards other essential elements, namely phosphorus and sulfur. Phos-
 225 phorus is critical to the cellular energy economy in the form of high-energy phosphodiester bonds
 226 making up DNA, RNA, and the NTP energy pool as well as playing a critical role in the post-translational
 227 modification of proteins and defining the polar-heads of lipids. In total, phosphorus makes up
 228 $\approx 3\%$ of the cellular dry mass which in typical experimental conditions is in the form of inorganic
 229 phosphate. The cell membrane has remarkably low permeability to this highly-charged and critical
 230 molecule, therefore requiring the expression of active transport systems. In *E. coli*, the proton elec-
 231 trochemical gradient across the inner membrane is leveraged to transport inorganic phosphate
 232 into the cell (*Rosenberg et al., 1977*). Proton-solute symporters are widespread in *E. coli* (*Ramos*
 233 *and Kaback, 1977; Booth et al., 1979*) and can have rapid transport rates of 50 to 100 molecules per
 234 second for sugars and other solutes (BNID: 103159; 111777, *Milo et al. (2010)*). As a more extreme
 235 example, the proton transporters in the F_1F_0 ATP synthase, which use the proton electrochemical
 236 gradient for rotational motion, can shuttle protons across the membrane at a rate of ≈ 1000 per
 237 second (BNID: 104890; 103390, (*Milo et al., 2010*)). In *E. coli* the PitA phosphate transport system
 238 has been shown to be very tightly coupled with the proton electrochemical gradient with a 1:1
 239 proton:phosphate stoichiometric ratio (*Harris et al., 2001; Feist et al., 2007*). Taking the geometric
 240 mean of the aforementioned estimates gives a plausible rate of phosphate transport on the order
 241 of 300 per second. Illustrated in *Figure 3(A)*, we can estimate that ≈ 150 phosphate transporters
 242 are necessary to maintain an $\approx 3\%$ dry mass with a 5000 s division time. This estimate is consistent
 243 with observation when we examine the observed copy numbers of PitA in proteomic data sets (plot
 244 in *Figure 3(A)*). While our estimate is very much in line with the observed numbers, we emphasize
 245 that this is likely a slight overestimate of the number of transporters needed as there are other
 246 phosphorous scavenging systems, such as the ATP-dependent phosphate transporter Pst system
 247 which we have neglected.

248 Satisfied that there are a sufficient number of phosphate transporters present in the cell, we
 249 now turn to sulfur transport as another potentially rate limiting process. Similar to phosphate,
 250 sulfate is highly-charged and not particularly membrane permeable, requiring active transport.
 251 While there exists a H^+ /sulfate symporter in *E. coli*, it is in relatively low abundance and is not well
 252 characterized (*Zhang et al., 2014a*). Sulfate is predominantly acquired via the ATP-dependent ABC
 253 transporter CysUWA system which also plays an important role in selenium transport (*Sekowska*
 254 *et al., 2000; Sirko et al., 1995*). While specific kinetic details of this transport system are not readily
 255 available, generic ATP transport systems in prokaryotes transport on the order of 1 to 10 molecules
 256 per second (BNID: 109035, *Milo et al. (2010)*). Combining this generic transport rate, measurement
 257 of sulfur comprising 1% of dry mass, and a 5000 second division time yields an estimate of ≈ 1000
 258 CysUWA complexes per cell (*Figure 3(B)*). Once again, this estimate is in notable agreement with
 259 proteomic data sets, suggesting that there are sufficient transporters present to acquire the nec-
 260 essary sulfur. In a similar spirit of our estimate of phosphorus transport, we emphasize that this is
 261 likely an overestimate of the number of necessary transporters as we have neglected other sulfur
 262 scavenging systems that are in lower abundance.

263 **Limits on Transporter Expression**

264 So which, if any, of these processes may be rate limiting for growth? As suggested by *Figure 2*
 265 (B), induced expression can lead to an order-of-magnitude (or more) increase in the amount of
 266 transporters needed to facilitate transport. Thus, if acquisition of nutrients was the limiting state
 267 in cell division, could expression simply be increased to accommodate faster growth? A way to
 268 approach this question is to compute the amount of space in the bacterial membrane that could
 269 be occupied by nutrient transporters. Considering a rule-of-thumb for the surface area of *E. coli* of
 270 about $6 \mu m^2$ (BNID: 101792, *Milo et al. (2010)*), we expect an areal density for 1000 transporters to

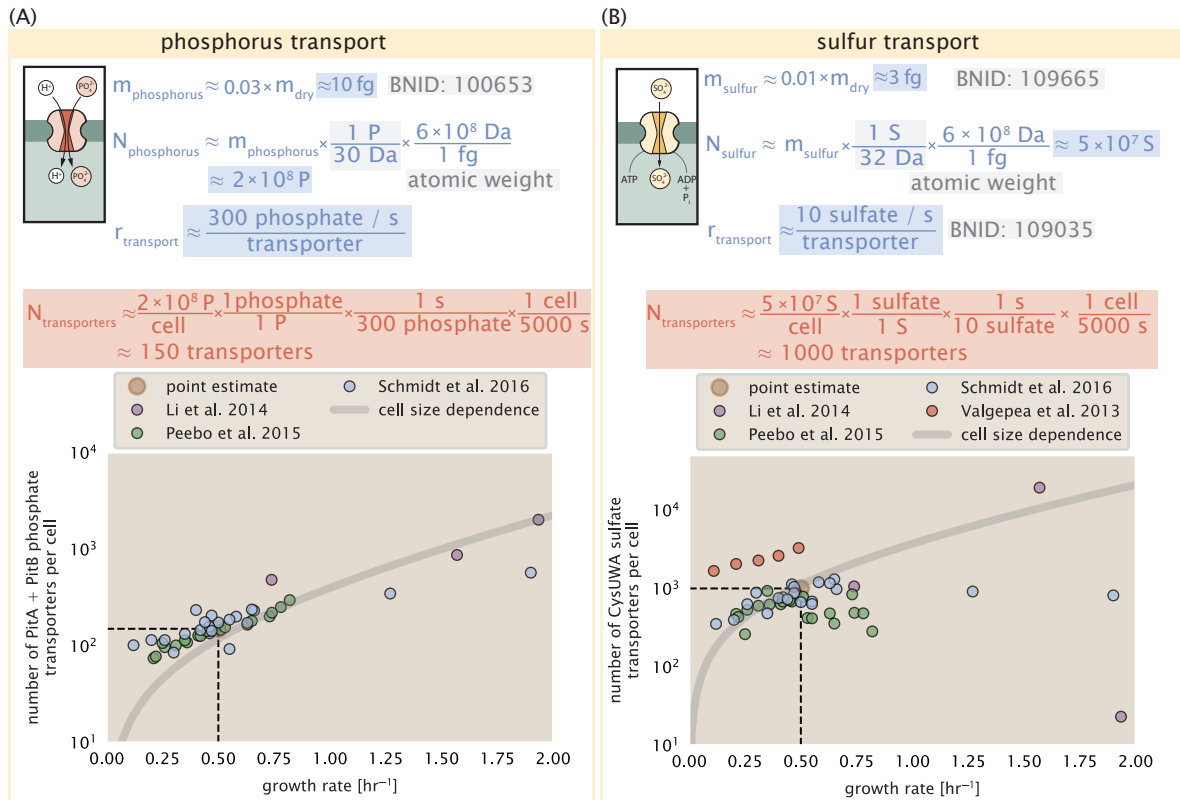


Figure 3. Estimates and measurements of phosphate and sulfate transport systems as a function of growth rate. (A) Estimate for the number of PitA phosphate transport systems needed to maintain a 3% phosphorus *E. coli* dry mass. Points in plot correspond to the the total number of PitA transporters per 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.

271 be approximately 200 transporters/ μm^2 . For a typical transporter occupying about 50 nm^2 /dimer,
272 this amounts to about only 1 percent of the total inner membrane (*Szenk et al., 2017*). In addition,
273 bacterial cell membranes typically have densities of 10^5 proteins/ μm^2 (*Phillips, 2018*), implying that
274 the cell could accommodate more transporters of a variety of species if it were rate limiting. As we
275 will see in the next section, however, occupancy of the membrane can impose other limits on the
276 rate of energy production.

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