

Chapter 2

Accounting for the plasticity and major drivers of minimal cellular metabolism

When model microbes like *E. coli* and *S. cerevisiae* grow fast, they coordinate their growth, metabolic rate, macromolecular makeup, and even morphology with remarkable precision. Such “growth laws” have inspired calibrated theoretical models of physiological adaptation, and also have enabled empirical predictions of cellular attributes. Yet, most of these patterns have been observed during relatively rapid growth or along stereotypical environmental perturbations, such as carbon limitation or sublethal antibiotic dosage. Does comparable coordination exist for cells that are not, or only slowly, growing? What processes set the scale of minimal cellular metabolism, and how plastic are such power requirements across cells and conditions? Towards answering these questions, we formulate simple biophysical estimates that bound the most relevant processes—including protein turnover, ion leakage across membranes (osmoregulation), and signal transduction—that likely dominate the energy budgets of non-growing cells. We give context to these estimates by building a small census of empirical parameters and power densities of cells and organisms in diverse states of activity, unifying diverse data sources.

These data include recent direct laboratory measurements of the power output of non-growing cells. Our analysis stresses the plasticity of basal power requirements across conditions; highlights persistent empirical mismatches between maintenance extrapolated at high growth and measured at low growth; and recommends specific experiments to resolve molecular processes enabled by cellular nonequilibrium. These findings help clarify the minimal and empirical costs of maintaining living matter.

This chapter proceeds in three parts. First, we review both new and classic characteristic scales of minimal microbial metabolism in context of both history and new measurements, contributing to this comparison by inferring new numbers from unusual sources. Second, we discuss where these numbers actually come from and the extremely different conceptual assumptions underlying them. Last, we sweep through an exploratory order of magnitude accounting of what processes struggling cells mainly pay for to keep alive.

2.1 Introduction

2.1.1 Overview

This chapter is motivated by one of the most classic types of experiments in the history of microbiology, namely, those in which the metabolism and growth of bacterial cells is measured in real time in the controlled setting of the lab. Specifically, the cells are provided with some “environment” and the subsequent dynamics is measured. Indeed, Jacques Monod, as one of the greatest modern practitioners of this art, had a beautiful thesis on this topic culminating in the discovery of gene regulation as well as the allosteric concept. Monod waxed eloquently on the importance of these types of experiments: “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....In the present review we shall

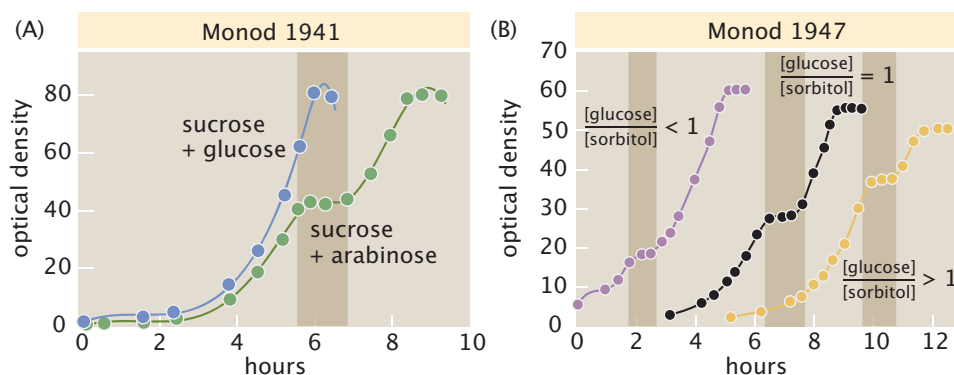


Figure 2.1: Choice of carbon source in the lives of bacterial cells. (A) Growth curves using optical density as a readout of number of cells reveal “enzymatic adaptation,” where cells shift from one carbon source to another as indicated by the vertical brown band. (Specifically, cells were provided fixed initial composition of a mixture of sugars, and the brown bands emphasize when cells switch their physiology to metabolize the second sugar once the first is meaningfully depleted.) (B) Illustration of the shift from glucose to sorbitol as the carbon source for three different experiments involving different ratios of glucose to sorbitol. The shift from one carbon source to the other is indicated by the vertical brown bands. In fact, the lag in growth corresponding to those brown bands reflect the turning on of the genes for the enzymes needed to metabolize the secondary carbon sources. Adapted from Monod (1941) [2] and Monod (1947) [3].

consider bacterial growth as a method for the study of bacterial physiology and biochemistry” [1]. Some of the Monod’s experimental results are shown in Figure 2.1 [2, 3]. In particular, here we see his measurements on bacterial growth where several different carbon sources are offered to the growing bacteria and their growth (as measured by optical density) is monitored over time. These measurements reveal that bacteria have preferences of some carbon sources over others. The shoulders in those curves correspond to the time period in which the cell is turning on the genes associated with the utilization of the less preferred carbon sources.

The work in this chapter was inspired by ongoing experimental efforts in the Newman Laboratory that call for a careful reckoning of bacterial physiology in circumstances that are widely divergent from those used in the famed experiments of Monod described above. In particular, new technologies made it possible to carefully measure the fluxes in electron transfer reactions in *Pseudomonas aeruginosa* in environmental conditions in which the cells

are deprived of oxygen (terminal electron acceptors), resulting in a form of “maintenance metabolism” and we were curious to quantitatively understand what changes to physiology are required to survive in these circumstances.

One of the founders of the modern field of bacterial physiology, Fred Neidhardt, wrote a beautiful article entitled, “Obsession with dN/dt ” that highlights his own lifetime love affair with bacterial growth curves and the allied mathematics [4]. Neidhardt was cognizant that there is more to bacterial physiology than was revealed by the focus on bacterial growth curves: “Did Monod’s views divert attention from the stationary phase and delay serious study of the developmental processes preparing cells for survival during nongrowth? I doubt it. I suggest the opposite is true – that the attention given by Monod to the usefulness of growth analysis in bacterial physiology and biochemistry hastened the day when nongrowth could be meaningfully studied. To recognize the right questions and pursue them required an understanding and an information base gained from growth studies. *The physiology of nongrowth is not the absence of the physiology of growth. Understanding both the phenotypic and genotypic processes involved in stationary-phase physiology involves building on what we understand about growth physiology*” (emphasis added). How ready are we to seize Neidhardt’s gauntlet to build understanding in the nongrowing setting? We direct the remainder of this chapter precisely to begin asking how ideas may need to be generalized or superseded in the case of “maintenance metabolism”—life on the energetic brink.

2.2 Experimental measures and philosophical notions of basal cellular metabolism

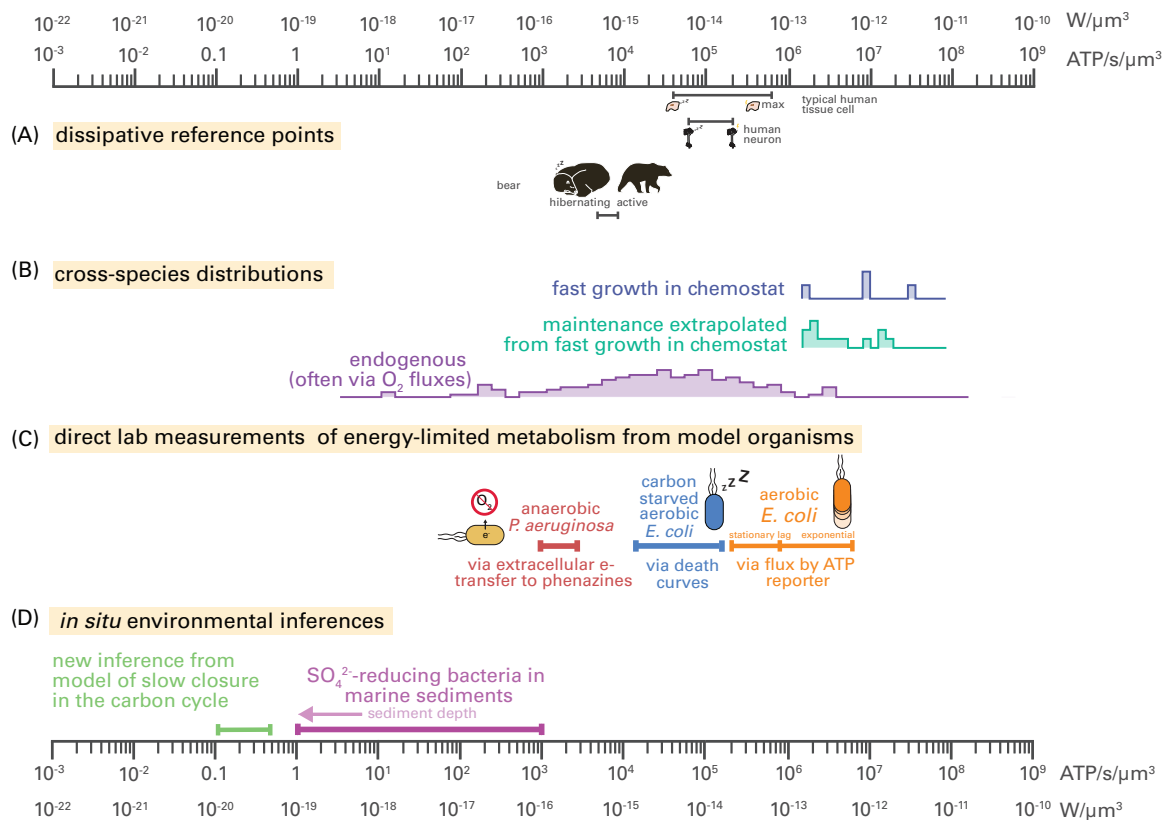


Figure 2.2: Extraordinary metabolic plasticity in microbes, as reported by different experimental means. (A) Metabolic plasticity of broader cell types and famously-hibernating organisms. (We calculated the metabolism of hibernating bears from experimental data in [5].) (B) Traditional laboratory measures of microbial metabolism, including chemostat measurements of species at high growth; extrapolated maintenance values from chemostat measurements; and various microbes persisting using just endogenous energy stores (often measured as oxygen fluxes). Note that maintenance values extrapolated from chemostats are large. (Compiled metabolic data across species were retrieved from [6, 7].) (C) Case studies of direct laboratory measurements of model organisms under nongrowth conditions. (*Pseudomonas* metabolic measurements, in red, were reported by [6]. Metabolic rates of aerobic carbon starved *E. coli*, in blue, were converted from death curve measurements by [8]. [9] reported values of *E. coli* metabolism at different growth stages, measured by ATP reporters, given in orange.) (D) Inferred maintenance values *in situ*. (Metabolic rates of sulfate reducing bacteria, in purple, were converted from values summarized by [10]. In green we give our fresh deduction of metabolism as consequence of the slow closure model in [11] and described in §2.4.)

Just as they amaze us by how rapidly they can devour energy, cells must also astonish us by how slowly they can consume it when conditions demand. While the fastest-growing microbial cells can metabolize almost 100 million ATP equivalents per second per cubic micron, cells deep in ocean sediments have been reported to metabolize at nearly 100 million times slower, as shown by Fig. 2.2(B-D). Cells of the same species (say *E. coli*) regularly slow metabolisms by three orders of magnitude when warranted. This immense metabolic plasticity is extraordinary on broader biological terms. For instance, both bears accomplishing their famous hibernation, or many typical human tissue cells, only reduce their metabolic rates by a factor few (to few times ten) between sustained states of relaxation and maximal exertion (as intimated in Fig. 2.2(A)).

To assemble the numbers reported in Fig. 2.2, we combined both existing compilations of metabolic rates [6, 10] (given as the histogram distributions in Fig. 2.2(B)) and also inferred fresh numbers from distinct sources, next converting them to common units to assess their communion with extant values of different origins.

Where do these highly provocative “basal metabolism” numbers, of the sort visualized in Fig. 2.2, come from? In other words, by what measurements do we arrive at what we purport to know about slow metabolism? Do these measurements cohere, or do they expose numerical and philosophical fractures? We now discuss some dominant means of inferring slow metabolic rates to frame these abiding questions.

Measuring metabolic rates has a distinguished and ingenious history in microbiology. Just thirteen years after he invented the nuclear chain reaction ushering in our nuclear age (and five years after he passionately petitioned the U.S. President and the Manhattan Project to avoid dropping a bomb), physicist Leo Szilard together with biologist Aaron Novick introduced a culturing device known as the chemostat [12, 13, 14], now a foundational technique across biology. As illustrated in Fig. 2.3, the chemostat smoothly controls the abundance of microbes consuming a limiting resource in culture medium.

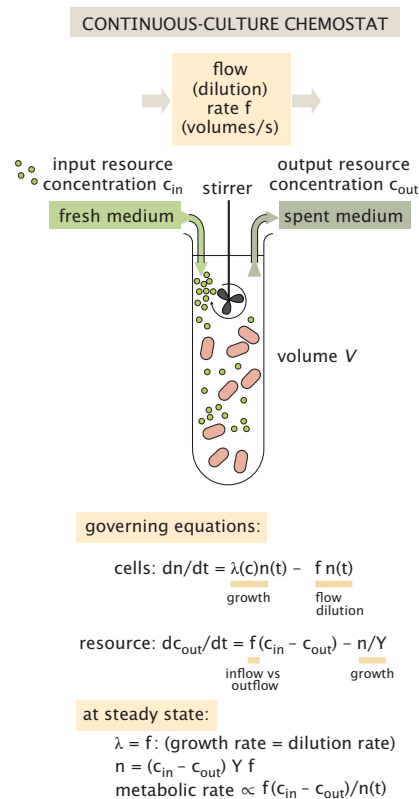


Figure 2.3: Continuous culture chemostats give correlations between bulk carbon consumption rates and fast growth rates. Cells at number density $n(t)$, consuming limiting resource at average concentration $c(t)$ outflowing at the same concentration $c_{out}(t) = c(t)$ at rate f , with a resource yield of Y , manifest a balance between their rate of growth λ and the flow rate. This allows the growth or metabolic rate to be inferred directly from the flow rate and the difference between the resource concentrations of media flowing out and that of the fresh medium flowing in (c_{in}).

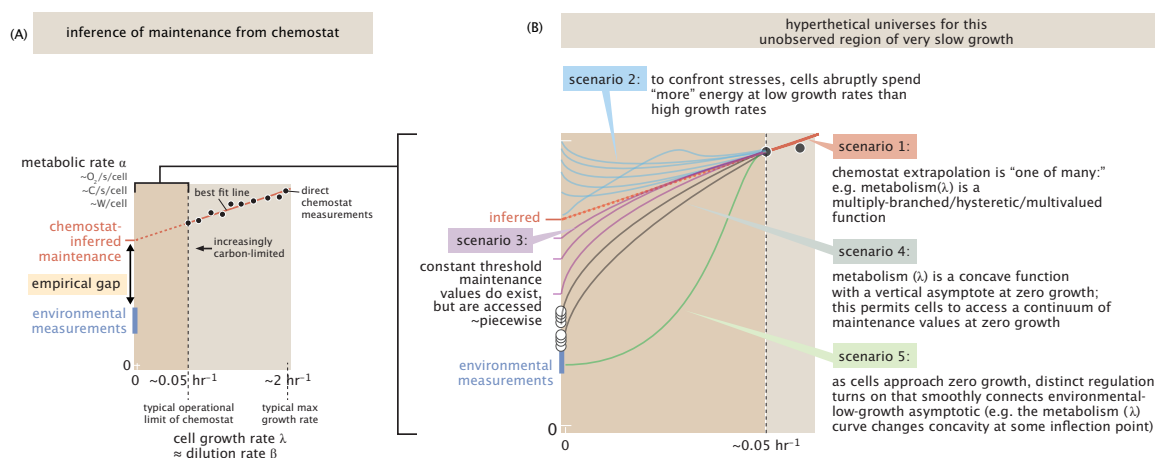


Figure 2.4: Conceptual subtleties of inferring basal metabolism using extrapolation from fast growth measurements in a chemostat.

The operating principle is simple and inspired. With inflow of fresh medium and outflow of cells and spent medium in a fixed volume, cells can impose a steady-state on the abundance of a resource (canonically, the carbon source) required for metabolism and growth. The flow rate of fresh medium sets the steady-state abundance of cells (as hinted at by the equations in Fig. 2.3), and crucially, the difference in resource level between the fresh inflowing medium and the spent outflowing medium reports on the resource consumption rate (and thus, biochemical energy consumption) of the cells.

The ability of the chemostat to rapidly balance and control the context of cells has unlocked a whole industry of deep insights about physiology, especially probing the regimes of rapid, exponential, steady-state bacterial growth and the extraordinary regularities manifested in that biochemical regime. Growth measurements across species soon indicated that when the dilution rate (which entrains the growth rate of the population) of fresh medium is decreased away from the fastest exponential growth attainable by cells, the metabolic rate on a per-cell basis also usually decreases. As illustrated schematically in Fig. 2.4(A), remarkably—for many laboratory strains and medium conditions—this decrease is often linear from fast growth rates to modest growth rates [15]. This line between metabolic rate and growth rate usually has nonzero y -intercept, and foundational studies (including by Pirt and colleagues

[15]) proposed that this extrapolated energy consumption rate at zero growth rate could be interpreted as the basal metabolic rate cells need just to stay alive, not to produce biomass.

This extrapolated intercept from growing cells in chemostats, often denoted β , is perhaps the dominant operationalization of what “basal maintenance metabolism” means when values are reported in the literature [15, 7, 6]. It is invoked in wonderful, deservedly influential, theoretical studies such as Lynch & Marinov’s “The Bioenergetic Costs of a Gene” [16] and beyond. Fixed parameter values inferred from chemostat and culturing studies also enter applied climate models [17]. However, comparing the maintenance values extrapolated from chemostats across species and contexts (the data shown as teal histogram in Fig. 2.2(B)) with the basal metabolisms separately inferred from natural environments (Fig. 2.2(D)) or different laboratory methods (Fig. 2.2(C)) emphasizes that the chemostat estimates of basal metabolism are frequently many orders of magnitude greater than environmental inferences or direct laboratory measures.

This conspicuous empirical gap between chemostat measurements and other routes to basal metabolism values prompts us as a field to introspect about the underlying conceit of these extrapolated measurements. We remark that chemostats have limits on the slowest flow (hence cellular growth) rates they are able to stably operate at, without burgeoning technical tricks and complexity. A characteristic lower limit to the comfortable (common) operation of chemostats is a flow (growth) rate λ of the order of $\lambda_{\min} \gtrsim \text{few} \times 10^{-2} \text{ hr}^{-1}$, corresponding to a generational doubling time of faster than a few tens of hours (as schematized by the dark brown band of a Pirt style plot in Fig. 2.4(A)). By the nature of extrapolation, slower growth and therefore metabolic rates—precisely the regimes of say geobiological interest, anticipating doubling times slower than months—remain directly unobserved.

Accordingly, what nonlinear behaviors linking metabolic rate and growth rate could truly lurk in the limit as growth rate vanishes? Fig. 2.4(B) schematically imagines some

mathematical and physiological behaviors that connect a linear trend of metabolic rate with high growth rate to a spectrum of true underlying basal metabolic rates as bacteria stop growing. Specifically, we speculate microbes could very plausibly change the concavity (in either direction) of their metabolism’s relationship with growth as they become more energy constrained or stressed; this could manifest as the emergence of a burst of energy expenditure or conservation as microbes drop their growth rates relative to the linear extrapolation expectation.

More generally, we offer this cartoon *not* to stray into a pedantically conspiratorial stance of asking, “What if biology is more complicated than a linear extrapolation?”¹ Instead we register these possibilities to ask whether the very act of quantitatively expecting growth rate λ to stay a sufficient, closed “equation of state” for the physiology of a challenged microbial cell, in the bonafide physics sense, may be overly optimistic. Of course, exactly this optimism has been richly rewarded in the case of *fast* growth. As Neidhardt incisively remarked decades ago, “Certain major phenotypic characteristics are coordinated with the absolute growth rate and are completely independent of the chemical nature of the medium. . . . No description of the average bacterial cell of a given species is possible unless one specifies the growth rate” [4]. The near-sufficiency of growth rate alone to specify many aspects of the state of a cell has been more recently ratified in model organisms (growing exponentially in simple environments) to inspirational quantitative precision. This includes the establishment of “growth laws” by the groups of Terence Hwa and many other investigators [18]. We harvest from these examples big assurance that explanatory state variables (like growth rate) do emerge in biology (whose recognition yields thrilling understanding), but *not* that it will be specifically growth rate that is still the “right” state coordinate that best organizes the complexity of microbial metabolism in the limit of energetic challenge. In other words, these ideas require our community be bold

¹Biology always exceeds the complexity of the line; what matters is the conceptual leverage a line does or does not give us.

enough to ask for the new abstractions that replace the growth rate as governing (but measurable) x -coordinate(s) in plots describing microbial cells in the energy-limited setting. Is this a membrane potential? An NAD^+/NADH ratio? Some new organizing variable entirely?

Progress towards new, effective abstractions demands being able to test them out ruthlessly in experiments. Our field has never enjoyed a time like ours when more of the right technological and biological (e.g. genetic) tools are at hand to do this. We now briefly remark on three, newly-direct laboratory measurements contributing values of total basal metabolism. We anticipate these measurements are especially the sort that may help probe physiology in particularly challenging but revealing conditions, each with a corresponding datum shown in Fig. 2.2(C). These are exploiting the quantitative phenomenology of death curves of bacteria to infer cellular energy demands (as shown in Fig. 2.5); direct measurements of ATP flux by reporters over time (as shown in Fig. 2.6(A)); and measurements performed using electrodes on cells reducing terminal oxidants (as shown in Fig. 2.6(B)).

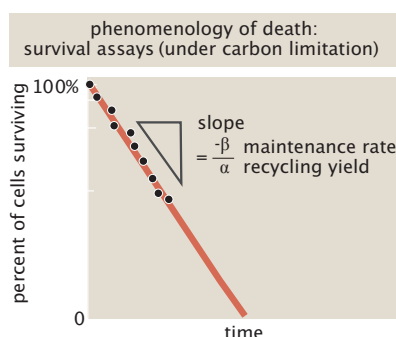


Figure 2.5: Inferring maintenance rate from death rates under carbon limitation. Under suitable conditions, internal population recycling of the energetic content of dying cells implies that the slope of survival curves on a semilog axis reports on the maintenance rate divided by the recycling yield.

How can death report on life? An ascendant tradition has emerged from the work of Schink, Gerland, Basan, and coworkers [19, 8] whose beautiful experiments show how—under stereotypical carbon limitation—*E. coli* cells often die at constant-per-capita rates, as

schematized by Fig. 2.5. This cursorily simple behavior is actually quite a deep and surprising regularity. Why should eight cells become four, four cells become two; two cells become one; and so on, all at a constant kinetic rate? Pulling on this conceptual thread can be a route to measuring valuable metabolic parameters. In the case of carbon limitation, it transpires that a straight line in the decline of (log) cell population emerges from dying cells being recycled by their still-viable neighbors. In the next chapter, we explore the existing math of this scenario much more granularly (when asking how it might be generalized to the richer and entirely physiologically different case of oxygen deprivation). However, for our present discussion, we remark that since (as is intuitive) struggling cells die faster when they harvest energy faster under a limited supply, the rate at which cells die reports quantitatively on the basal energy consumption rate β per cell in these conditions. To enable comparison with the other repertoire of reported values at hand, we translate some of the values of β identified in recent experiments' units [8] to common comparative metabolic units in Fig. 2.2(C) as the blue bar.

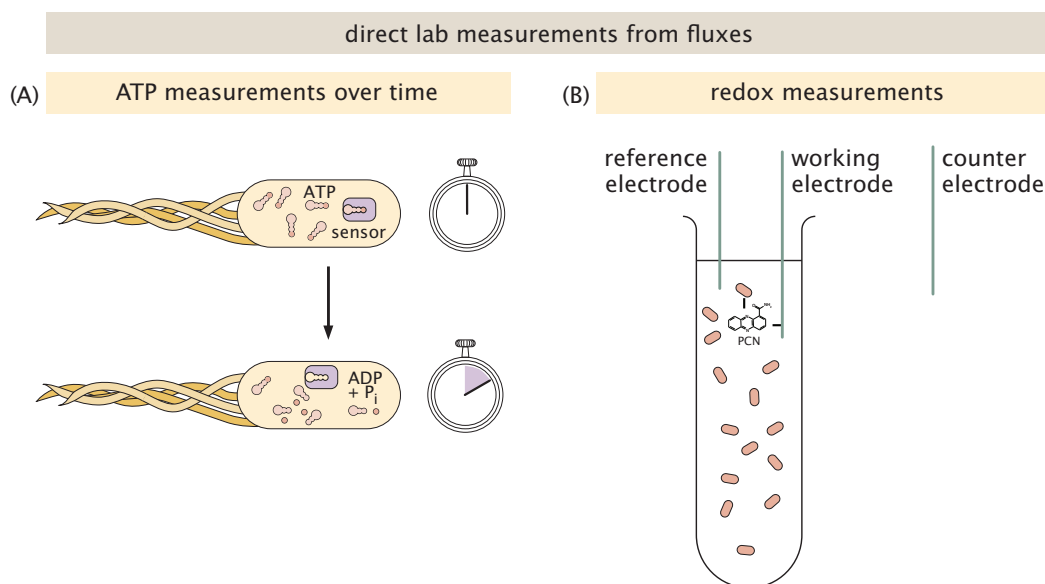


Figure 2.6: Inferring cellular energy consumption by (A) direct measurement of ATP consumption or (B) direct measurement of electron transport by electrode exchanges with extracellular electron carriers such as phenazines (including phenazine-1-carboxamide (PCN)).

Another quite recent, and exciting, glimpse of the real-time metabolism of microbial cells is by directly measuring ATP abundance by fluorescent reporters and watching resulting resolved fluxes [9]. This gives, for instance, the ability to both measure current endogenous ATP levels, and also finely temporally resolve the metabolic status of cells (from changes in this ATP pool) as they exit exponential phase and enter stationary or lag phase. We summarize Deng and coworker's measurements [9] on aerobically-grown *E. coli* in various stages of growth in Fig. 2.2(C) as the orange bars. These measurements show that aerobic *E. coli* slows its metabolic rate from $\sim 6.4 \times 10^6$ ATP/s/cell in exponential phase, to $\sim 8 \times 10^5$ ATP/s/cell in lag phase, to $\sim 2 \times 10^5$ ATP/s/cell in stationary phase. Complementing these changes in metabolic rates, Deng & coworkers also report how the total quasi-steady state abundance of ATP changes across these growth stages; *E. coli* reduces its typical cytosolic ATP pool from ~ 3 mM standing ATP in exponential phase to order ~ 0.6 mM standing ATP in stationary phase [9]. Incidentally, the purview and immediacy of this class of direct measurements of ATP is much wider than whole-cell energetic metabolism. We advance work with members of our laboratory in this direction, using ATP fluorescent reporters to ask how much building microtubule structures costs across space and time, in a later chapter in this thesis.

Most of these methods of experimentally measuring basal metabolisms depend on operating conditions that compel cells to be in particular regimes of physiology. That is, these direct measurements often access the regime of nongrowth by imposing stress along stereotypical axes—overwhelmingly commonly, carbon deprivation (or antibiotics). However, the reasons that cells survive, but do not grow, in natural environments can be much richer and more varied than just lacking carbon sources. Across the biosphere, cells instead often lack terminal electron acceptors like oxygen crucially required to allow them to perform metabolism. Unfortunately, many of the empirical means to assess metabolism we have just discussed do not directly translate effectively to oxygen deprivation. For

instance, fluorescent ATP reporters (like many assays depending on fluorescent proteins) often have completely different and abrogated structure or kinetics in anoxic settings. Similarly, the lovely quantitative picture that so enabled rates of death to report on basal metabolism in the case of carbon limitation is completely upended with terminal electron acceptor deprivation. (How this happens, and whether there may be some quantitative analog to allow death alone to report on metabolic rates, is a question we broach seriously in the next chapter.)

However, compelling work by the laboratory of Dianne Newman and colleagues instead turns the challenges presented by measuring anaerobic metabolism into an advantage. Specifically, some cells who manage to cope with anaerobic environments produce their own extracellular electron acceptors. This is manifested by the opportunistic pathogen *P. aeruginosa*, which produce phenazines, a class of redox active compounds that can mediate extracellular electron transfer. Experimentally, these molecules give a rare handle on measuring metabolic activity, since electrodes can be tuned to the midpoint potential of phenazine and thus selectively resolve the rate at which cells metabolize at high temporal resolution. We depict this scheme schematically in Fig. 2.6(B), and explore fun mathematics animated by these scenarios throughout the next chapter. We indicate the intriguing characteristic scale of metabolic rates measured in these systems in Fig. 2.2(C) as a red bar. Specifically, Ciemniecki *et al.* announce that anaerobic *P. aeruginosa* can metabolize at the striking rate of just $\sim 10^3 - 10^4$ ATP/s/cell.

This small number is tantalizing, in part, because it approaches the amazingly small values of microbial metabolism inferred from natural environments *in situ*, as visualized by Fig. 2.2(D). The tradition of laboratory measurements of microbial metabolism is rich and smart; in this great tradition, however, typical values at the bench are often separated from inferred maintenance rates of microbes in say marine sediments [10] by several orders of magnitude (as seen in the gulf between the lab values of Fig. 2.2(A-C) with the

values of metabolism *in situ* in Fig. 2.2(D)).

To deduce how quiescent microbes in these natural environments metabolize poses daunting technical challenges. Microbes in the ocean floor resist many forms of scrutiny. Mathematics can be one of the only microscopes available to understand these settings. This tradition has a big history. We now offer a very small entry in the spirit of this tradition. Observables like empirical scaling laws in the age of organic carbon in the ocean implicitly report on how slowly microbes metabolize. Recently, Rothman [11] proposed a model of the global carbon cycle that makes a slew of empirically-successful predictions about even the precise values of aged carbon and cellular abundance in the ocean. This model posits that past some age of carbon, microbial metabolism ultimately transitions from accumulating biomass—albeit very slowly—to a constant fundamental basal metabolism independent of growth. We were inspired by Rothman’s elegant model to ask what it predicts for the basal metabolic rate of these bacteria *in situ*. In the appendix of this chapter (§2.4) we derive a simple prediction for this basal metabolic rate and evaluate it numerically using exponents of empirical scaling laws. We arrive at a phenomenally slow metabolic rate of plausibly less than a single ATP consumed per second per microbe, as indicated by the green bar in Fig. 2.2(D). This quantitatively buttresses a view of natural microbes apparently subsisting on extraordinarily slow rates of energy consumption.

By calculating and comparing empirical or inferred numbers such as those in Fig. 2.2 and throughout this chapter, we do not mean to canonize them, or pretend that these quantities are truly well “known” to acceptable precision and rigor. To the contrary. Numbers that matter, as basal metabolisms do both practically (in e.g. climate models) and conceptually (as inputs into nonequilibrium statistical models of living matter), must be measured many times in multiple different ways. Differences between reported basal metabolism numbers are already visible from the subset of settings and separate measurement sources we explored in this chapter. These differences must be reconciled or

attributed to particular features of these biological systems. We view these numbers as urgent provocations for us to account for both true underlying physiological plasticity in metabolism, and also differences in philosophy, measurements, and environmental context.

2.3 Plausible Energy Sinks in the Cell

Having seen just how plastic total basal metabolism appears to be, we now ask, what activities fundamentally set the scale of minimal metabolic rates? In the sections that follow, we strive for a series of increasingly sophisticated attempts to quantitatively reckon with the various dissipative processes within cells. This strategy is illustrated schematically in Figure 2.7 where we show some of the processes that make up the energy budget of microbial cells.

2.3.1 The Cost of Protein Turnover

As seen in Figure 2.7, one of the primary costs incurred by bacterial cells whether in a state of maintenance metabolism or rapid growth is associated with the synthesis of proteins. To that end, in this section, we attempt to make some rough estimates of the power budget associated with the synthesis or resynthesis of proteins.

Cells constantly remake proteins to compensate for their active or spontaneous degradation. The energetic cost to resynthesize proteins can be formidable, as a simple estimate reveals. Specifically—assuming that the cellular abundance of proteins is maintained at a steady-state—the stable copy number N , overall resynthesis rate q , degradation rate k , and typical lifetime τ can be related by Little's Theorem in queuing

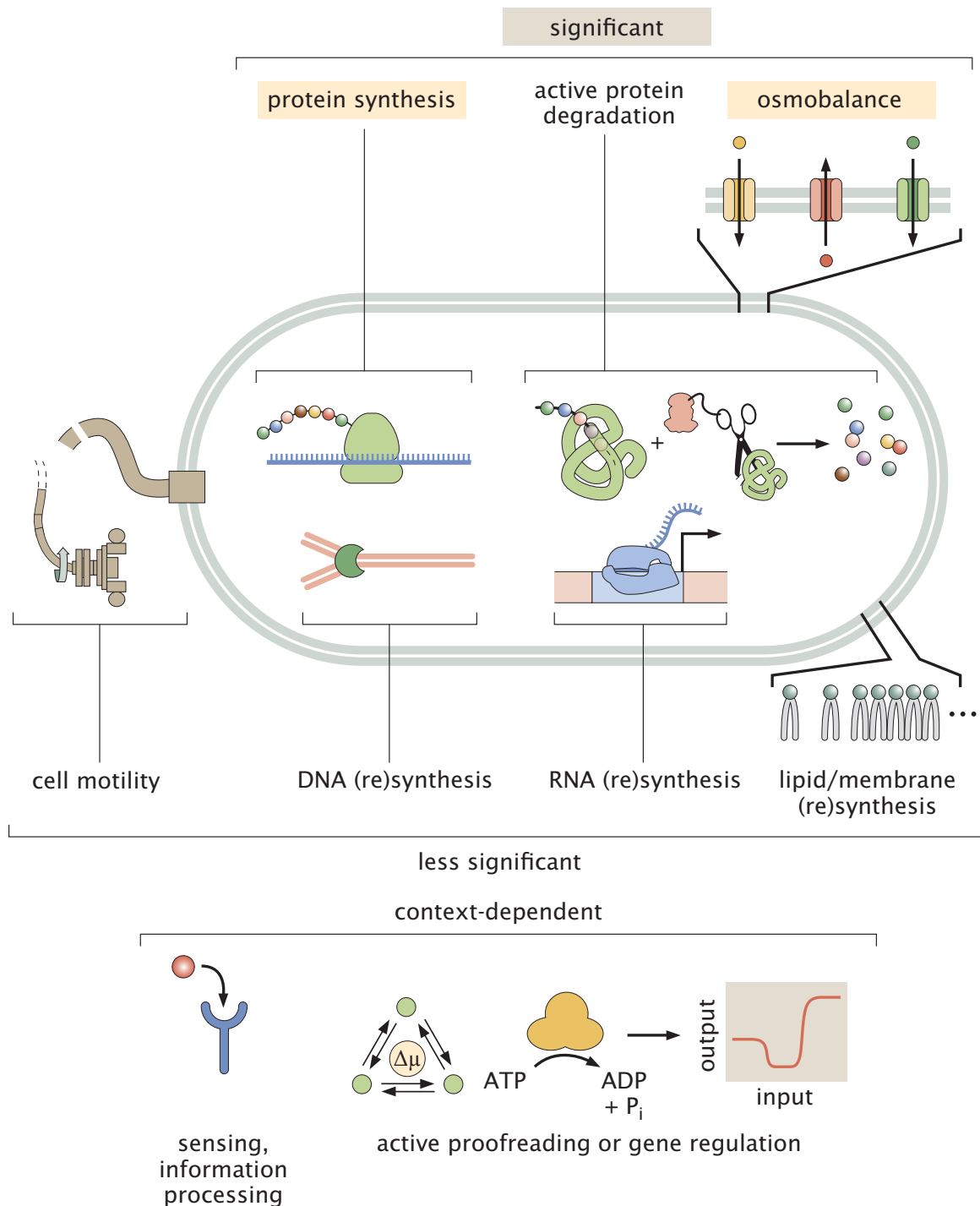


Figure 2.7: Processes that make up microbial energy budgets. Each of the processes shown in the figure can be subjected to quantitative estimates and compared; their balance surely adjusts across conditions of severe energy limitation to active growth. We attempt to categorize these processes by how large a contribution they make to the cell's energy budget, seeking bounds to focusing on the energy-limited case.

theory [20],

$$\frac{N}{\tau} = q = k. \quad (2.1)$$

Typical protein turnover rates in bacteria [21] and eukaryotes [22] are both of order $k \approx f \times 10^{-5} \text{ s}^{-1}$, consistent with measured typical half lives of a few hours (and where remark we adopt the useful convention that $f = \text{few}$ [23]).

We now ask, given these turnover times, how much does it cost to constantly replenish these degraded proteins? Figure 2.8 provides a schematic of our strategy for estimating the power associated with protein replenishment due to turnover. In particular, we imagine that under maintenance conditions, only a fraction (e.g. 10%) of the proteome is replenished. Given the rate of degradation $k \approx f \times 10^{-5} \text{ s}^{-1}$, we can compute the number of new proteins synthesized every second as

$$\text{protein synthesis rate} \approx \frac{1}{10} \times (f \times 10^{-5} \text{ s}^{-1}) \times (f \times 10^6 \text{ proteins}) \approx 10 \frac{\text{proteins}}{\text{s}}, \quad (2.2)$$

where the 1/10 on the right hand side is the fraction of the proteome we imagine is replenished and $f \times 10^6$ proteins is the typical number of proteins per μm^3 (or per bacterial cell). Given that a typical protein is composed of $f \times 10^2$ amino acids and that the cost of each such amino acid incorporation is 4 ATP equivalents, then the cost of maintaining the proteome is

$$\text{power of protein synthesis} \approx \left(10 \frac{\text{proteins}}{\text{s}}\right) \times (f \times 10^2 \text{ aa}) \times \left(4 \frac{\text{ATP}}{\text{aa}}\right) \approx 10^4 \frac{\text{ATP}}{\text{s}}. \quad (2.3)$$

We can formalize the calculations above as follows. Each protein is $\ell \approx$ a few hundred amino acids long and each peptide bond requires $\eta \approx 4$ ATP equivalents to polymerize [25]. Since mammalian and bacterial cells alike typically have concentrations $c \approx$ a few

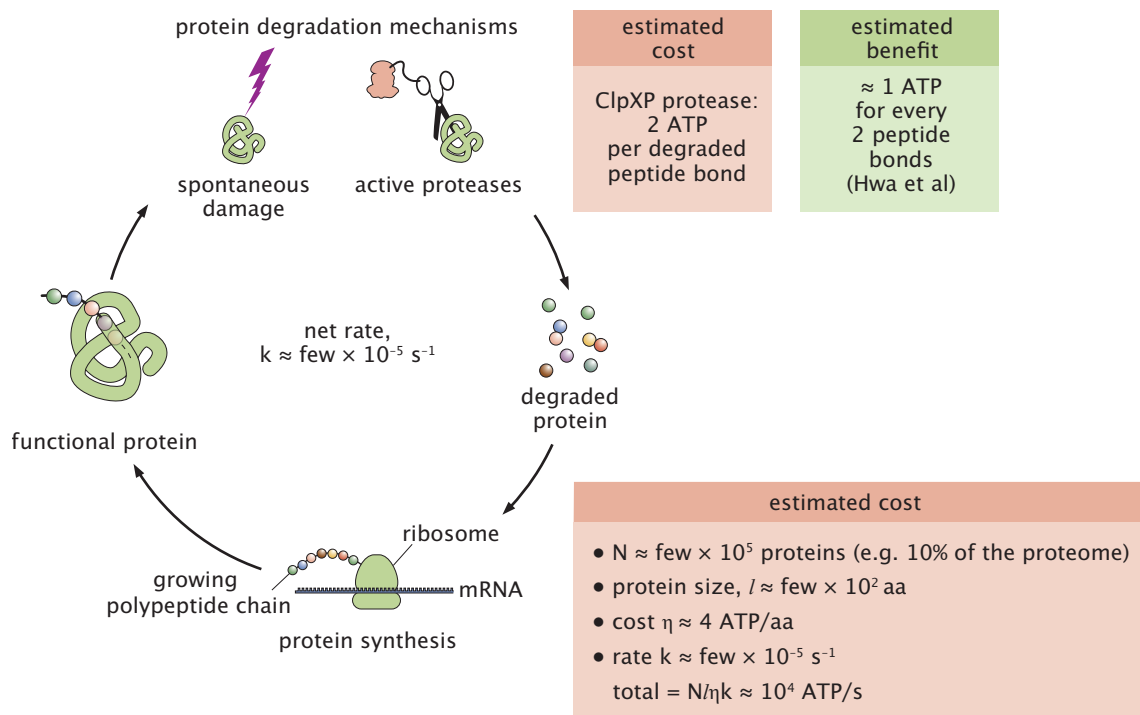


Figure 2.8: Degradation and active resynthesis of proteins enacts a steady-state with characteristic turnover rates and accompanying costs. The cycle on the left shows the life history from newly synthesized protein to degradation. The bottom right recapitulates the key features of the estimates made in the body of the paper. (We note that one enormous part of the picture for which reliable quantitative data are currently in severe deficit is the question of whether protein degradation pathways exist that could, in the net balance, *produce* energy; compelling initial impressions of this possibility are being investigated by Hwa and colleagues [24].)

million proteins per cubic micron [26], protein resynthesis to maintain the standing proteome pool should demand of order $\gamma c k \ell \eta \approx \gamma 10^5 \text{ ATP}/(\mu\text{m}^3 \text{ s})$, where we introduce the parameter γ to represent the fraction of the proteome that is resynthesized during maintenance conditions. It is amusing to speculate on the parameter γ by recalling efforts to create minimal genomes, some of which are ongoing even here at Caltech in the laboratory of Kaihang Wang. Given that the total number of genes in *E. coli* is ≈ 4500 and the minimal genome size is of order 500, one might imagine that under maintenance conditions, a variety of proteins do not undergo replenishment. That said, like with much of the guesswork in this chapter, there is clearly so much more experimental work to be done to pin down the mechanisms of microbial maintenance metabolism.

Cost of protein turnover inferred from cellular data

In a paper now three decades old, Russell and Cook [27] had already thought deeply about these matters, claiming that the empirical rate at which proteins turnover in *E. coli* is not sufficient to account for inferred maintenance rates in this organism. We find it instructive to quote their observations in their entirety. “Radiolabeling experiments indicate that the rate of protein turnover in exponentially growing *E. coli* cells ranged from 0.5 to 2.5 %/h and that RNA turnover paralleled the breakdown of protein. During stationary phase, protein turnover was higher (5% for *E. coli* and 8% for *Bacillus cereus*, but even these rates cannot account for all of the maintenance in *E. coli*. On the basis of a cell composition of 0.5 g of protein per g of cells, 4 ATP equivalents per amino acid polymerized, an average molecular mass for an amino acid of 100 Da, and a maximum ATP production of 24 ATP per glucose, the glucose consumption rate needed to sustain a protein turnover rate of 5%/h would be 0.04 mmol of glucose per g of cells per h. The maintenance rate of *E. coli* is 0.31 mmol of glucose per g of cells per h [as extrapolated from chemostat measurements].”

Let us try to come to terms with what Russell and Cook have said. First, they note that

the cost of protein turnover seems to fall far short of the total power cost of maintenance. However, a protein turnover rate of a few %/hr in stationary phase, assuming a total protein copy number of $N \sim \text{few} \times 10^6$ proteins/cell, corresponds to,

$$\frac{\text{few turned over}}{100 \text{ total}} \times (\text{few} \times 10^6 \text{ total}) \sim 10^5 \frac{\text{proteins turned over}}{\text{hr cell}} \quad (2.4)$$

$$\sim \boxed{\text{few} \times 10 \frac{\text{proteins turned over}}{\text{s cell}}}. \quad (2.5)$$

Since a protein is typically $\ell \sim 300$ aa long and polymerizing each amino acid into a growing peptide chain costs about 4 ATP hydrolyses worth of energy, it takes order 10^3 ATP/protein. This anticipates that protein turnover appears to demand $\boxed{\text{few} \times 10^4 \text{ ATP/s/cell}}$. This numerical estimate is intriguing in the context of recent measurements in *Pseudomonas* which uses phenazine to cycle a median value of a $\text{few} \times 10^3 \text{ e}^-/\text{s/cell}$ [6]. Since each electron transported along relevant metabolic pathways provides about the equivalent of one ATP hydrolysis's worth of energy (up to a factor few), the measurement corresponds to about ten times smaller an ATP consumption rate per *Pseudomonas* cell than demanded by protein turnover in *E. coli* in stationary phase. We consider it plausible that *E. coli* on glucose could easily vary physiologically within a factor ten of the governing properties of *P. aeruginosa* using phenazine. This calculation gives grounds for inferring that a large part of the observed metabolic rate of *Pseudomonas* might indeed be explained by just protein turnover, but explicit turnover data in *Pseudomonas* are obviously warranted.

Specifically, to inform a more useful and representative estimate of the proportion of an anaerobic cell's energy budget destined for protein resynthesis, absolute (not just relative) copy numbers of proteins *and* their turnover rates should be measured. While these values would seem very basic and essential quantities, they appear to be only rarely measured simultaneously in well-defined conditions where other metabolic features are also

measured. We view this deficiency as a direct call to action for our experimental efforts in the community.

The above quick calculation assumes that the total copy number of proteins in stationary growth is still a few million per cell, an abundance informed by growing cells; if the protein count is different in stationary phase, then a few percent turned over per hour corresponds to a different quantitative number turned over per hour than estimated above.

Cost of protein turnover from first principles and chemical rates

One hypothesis advanced for the minimal metabolic energy flux cells need to survive is that slow spontaneous degradation of the proteome must be counteracted by some nonzero rate of protein synthesis, even without growth. Here, we make this claim quantitatively precise using a simple stochastic kinetic model. Next we coarsely estimate whether known mechanisms and kinetics of protein degradation or spontaneous racemization could account for measured maintenance requirements.

Consider a proteome consisting of N proteins, each of which has ℓ amino acids. Each amino acid can fail at rate k (per amino acid per time). Say that m amino-acid failures per protein are required before the whole protein fails (requiring replacement by active synthesis). What is the expected rate at which proteins fail (hence approximately the total rate of new protein synthesis required to maintain a steady pool)?

Our first approximation is to treat the failures of amino acids as statistically independent and following the same Poisson process with failure rate k .² Thus, inside a protein, the net rate at which amino acids fail is the total number of amino acids times this failure rate,

²This treatment of independently-failing-entities is analogous to how the net rate at which rhodopsin photoreceptors show spurious activation with temperature is rare per receptor, but meaningful in aggregate. For instance, one paper writes, “As a toad rod contains about 2×10^9 rhodopsin molecules, and as all these combined rhodopsin molecules give a mean interval between thermal breakdowns of 50 sec, then the mean thermal lifetime per rhodopsin molecule is the product of these, about 10^{11} sec or roughly 3000 years. So, despite the occurrence of the discrete electrical events, the rhodopsin molecule is incredibly stable” [28].

namely

ℓk amino acids failing/second/protein.

The time τ that is needed to wait for m exponentially-distributed events each with failure rate λ is described by a Gamma distribution, $\tau \sim \text{Gamma}(m, \lambda)$, which has mean $\langle \tau \rangle = \frac{m}{\lambda}$. Thus the effective rate at which one protein fails (by reaching ℓ amino acid failures) is $\frac{\ell k}{m}$. Since there are in fact N proteins, each of which can be regarded as failing statistically independently, this predicts that there are

$$k^{\text{tot}} = \boxed{\frac{N \ell k}{m} \text{ proteins failing per second}}. \quad (2.6)$$

Note that this expression is intuitive: the greater the required number m of amino acids per protein failure, the smaller the overall protein failure rate; but the larger the number ℓ of amino acids that could fail per protein, the per-amino-acid failure rate k , or the total number N of proteins, the higher the total protein failure rate.

Now, we hazard a tentative numerical estimate of this total spontaneous protein degradation rate, based on the risk of amino acid “failure” attributed to spontaneous deamidation, isomerization, and racemization of protein residues. Geiger and Clark [29], focusing on asparaginy/aspartyl residues whose associated chemical transformation rates are larger than for other residues, used hexapeptides to measure that L-Asn undergoes spontaneous deamidization with a half-life of $\tau_1 \sim 1.4$ days, followed by further hydrolysis (a reaction with half-life $\tau_2 \sim 2.3$ hr), and racemization (a reaction with half-life $\tau_3 \sim 19.5$ hr). Viewing these transformations as sequential kinetic processes, we

assimilate these values to estimate the overall rate to be

$$k \sim \frac{\ln 2}{\tau_1 + \tau_2 + \tau_3} \quad (2.7)$$

$$\sim \frac{\ln 2}{34 \text{ hr} + 2.3 \text{ hr} + 19.5 \text{ hr}} \quad (2.8)$$

$$\sim 3.5 \times 10^{-6} \text{ amino acids failing/second/protein.} \quad (2.9)$$

Next, recalling the sensitivity of protein function to even single nucleotide polymorphisms in underlying sequence, we imagine that a protein fails when one out of a few amino acids has failed, namely $m/\ell \sim 1/\text{few} = \text{few} \times 10^{-1}$. Last, we take the typical value of a proteome having $N \sim \text{few} \times 10^6$ proteins. These values predict an effective overall rate,

$$k^{\text{tot}} \sim (\text{few} \times 10^6 \text{ proteins}) \left(\frac{\text{few total aa}}{1 \text{ aa failure req'd}} \times \text{failed protein} \right) \left(3.5 \times 10^{-6} \frac{\text{aa failing}}{\text{s protein}} \right) \quad (2.10)$$

$$\sim \boxed{\text{few} \times 10 \text{ proteins "failing"/second/cell}}. \quad (2.11)$$

Note that we must regard this estimate as a (plausibly loose) approximate upper-bound of the spontaneous protein failure rate, given that the rates reported by Greiger and Clark [29] were measured for extremely short polypeptides (reducing steric effects expected to retard these chemical transformations). Further, these rates also describe especially chemically-labile Asp and Asn; other amino acids have reduced analogous spontaneous transformation rates. Last, just reaching a racemized state may not yet render an amino acid functionally deficient, without subsequent chemical and biological transformations not acknowledged here; so the rate of actual protein failure may be slowed by the times these other processes need to complete. Nonetheless, this estimate provides an interesting scale of how quickly spontaneous injurious transformations occur in a cell's proteome. However approximate, we note with surprise and curiosity the remarkable correspondence of the crudely estimated

spontaneous protein “failure” rate, Eq. 2.11, and the empirically-reported rate of protein turnover, Eq. 2.5.

In closing this broad discussion of the cost of protein turnover, we note that we could just as well have asked for the cost of the other processes in the central dogma such as replication and transcription during “maintenance metabolism.” That is, costs of RNA and DNA repair and turnover are also incurred by the cell. Here we simply note that as was already examined in detail by Lynch and Marinov [16]: the cost of transcription is far lower than that of translation, and for now, we hypothesize that that same hierarchy will be true in the context of maintenance metabolism as well. Hence, we view the focus on protein turnover as the key process in the context of the central dogma.

2.3.2 Cost of post-translational protein modifications

Proteins of all types are continuously subjected to modifications by the addition and removal of chemical groups. In a very interesting article (“Post-translational modification: nature’s escape from genetic imprisonment and the basis for dynamic information encoding”), Jeremy Gunawardena asks us to think about post-translational modifications as a way of expanding the natural repertoire of the 20-letter amino acid alphabet. Similarly, Prof. Christopher Walsh wrote a whole book entitled, “Posttranslational Modifications of Proteins: Expanding Nature’s Inventory,” again making the point that by adding chemical groups to proteins we can expand nature’s repertoire, significantly changing their properties. However, we cannot forget, this widespread escape from genetic imprisonment has an energetic cost. In this section, we explore simple estimates of both the ubiquity and the cost of such post-translational modifications, once again trying to establish a baseline of what processes within cells are energetically “costly” and what processes are “cheap.”

To get a feeling for the problem, let’s consider the amendment of proteins by the addition of phosphate groups. The point is that proteins are all made up of the same 20 amino acids. But once those proteins are synthesized, some residues such as threonine, serine and tyrosine, can be modified through the addition of phosphate groups (there are a host of other post-translational modifications such as acetylation, palmitoylation and methylation, to name a few, but for concreteness we focus on phosphorylation). If we consider amino acid usage, we could say that threonine, serine and tyrosine make up roughly 10% of the residues and these are the ones that are available for phosphorylation. Our aim here is to make a simple, but essentially first-principles, estimate of the number of distinct states of phosphorylation there are for a typical protein and then to examine the cost of running the kinases and phosphatases that maintain a steady flux of post-translational modifications.

Figure 2.9 provides a crude representation of proteins that can help us estimate the fraction of surface residues available for post-translational modification. In our toy model we assume

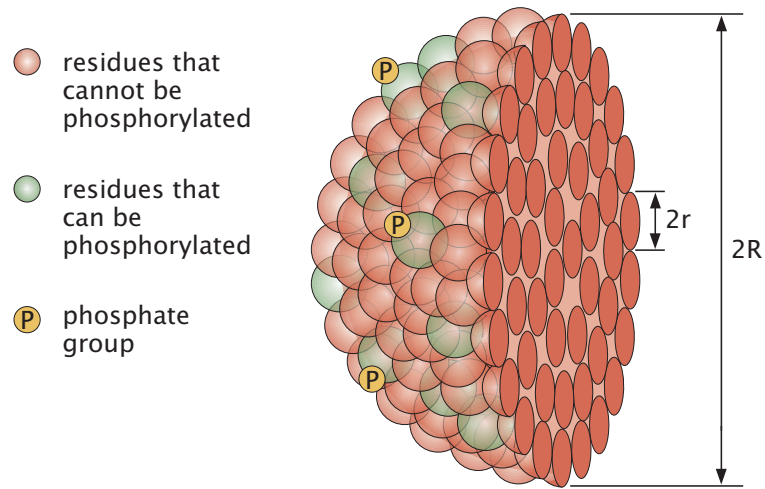


Figure 2.9: Phosphorylation of the amino acids on the surface of a protein. The simple estimate uses the known size of proteins and amino acids to estimate what fraction of the $f \times 100$ amino acids are on the surface of the protein, and then out of those surface exposed amino acids, what fraction are available for phosphorylation, for example.

that each of the N residues making up the protein is itself a sphere. The crude scaling of the radius of our spherical protein will go as $N^{1/3}$. However, we can do a little better. If the amino acids which we imagine as tiny spheres are maximally close packed, then the volume of the protein will be roughly $0.75N \times V_{aa}$. The factor of 0.75 is approximately equal to the volume fraction occupied by close packed spheres. Therefore, the radius of our protein will be given by $R = (0.75N)^{1/3}R_{aa}$. Given that a typical protein has $f \times 10^2$ amino acids and a radius of f nm, the radius of a single amino acid can be estimated as $R_{aa} \approx 0.4$ nm. Therefore, the scaling of the protein radius with the number of amino acids will go approximately as

$$R(N) \approx 0.4 \times (0.75N)^{1/3} \text{ nm.} \quad (2.12)$$

As a very crude estimate for the total number of residues on the surface of the protein, we can take the total surface area and divide it by the 2D projection area of a single residue, that

is

$$S_{\text{array}} = 4\pi R^2, \quad (2.13)$$

$$S_{aa}^{\text{proj}} = \pi R_{aa}^2, \quad (2.14)$$

$$N_{\text{surface}} = \frac{S_{\text{protein}}}{S_{aa}^{\text{proj}}} = 4 \times (0.75N)^{2/3} \approx f \times N^{2/3}. \quad (2.15)$$

Taking $N = f \times 10^2$ for a typical protein, we find that the number of surface residues is approximately

$$N_{\text{surface}} \approx 130. \quad (2.16)$$

A few (f) of 20 amino acids are commonly phosphorylated in bacteria. Making the crude assumption that all amino acids are equally represented on the surface, only 15% of the residues, or $130 \times 0.15 \approx 20$, will on average be phosphorylatable. This means that a total of $2^{20} \approx 10^6$ phosphorylation states are available to a given protein. Given that there are several million proteins in a given *E. coli* cell, this means that there are

$$\text{number of possible proteins in one bacterium} \approx (10^6)^{f \times 10^6}, \quad (2.17)$$

distinct possible proteins in one bacterium! We really do not have any good way to think about these kinds of huge numbers.

To estimate the cost of a phosphorylation-dephosphorylation cycle, we need to now get explicit about the reactions themselves. Here, we follow the development of Hong Qian's excellent 2007 piece entitled, "Phosphorylation energy hypothesis: open chemical systems and their biological functions," which shows how to compute the fluxes in these cycles, and from those fluxes, their cost. We borrow some of these calculations verbatim from *The Restless Cell* by Hueschen and Phillips. Unfortunately, none of these earlier treatments attempted to put in any numbers, which is a shortcoming we try to remedy here. To that

end, Figure 2.10 shows a hypothetical enzyme E that can also exist in a phosphorylated state E^* . We can think of this nonequilibrium steady state as arising from two coupled reactions given by



and



The rate equations describing that process are given by

$$\frac{d[E]}{dt} = -\frac{d[E^*]}{dt} = -k_1[E][ATP] + k_{-1}[E^*][ADP] + k_2[E^*] - k_{-2}[P_i][E], \quad (2.20)$$

where $[E]$ is the concentration of enzyme that is not phosphorylated, $[E^*]$ is the concentration of phosphorylated enzyme, $[ATP]$ is the concentration of ATP, $[ADP]$ is the concentration of ADP and $[P_i]$ is the concentration of phosphate. We have read this equation directly off the two kinetic equations describing the dynamics of interconversion between E and E^* .

We can use eqn. 2.20 to find the steady-state values of $[E]$ and $[E^*]$ by setting the left side equal to zero and using the constraint that $[E] + [E^*] = [E_{tot}]$. Following this strategy, we find

$$[E]^{ss} = \frac{k_{-1}[ADP] + k_2}{k_1[ATP] + k_{-2}[P_i] + k_{-1}[ADP] + k_2} [E_{tot}] \quad (2.21)$$

and

$$[E^*]^{ss} = \frac{k_1[ATP] + k_{-2}[P_i]}{k_1[ATP] + k_{-2}[P_i] + k_{-1}[ADP] + k_2} [E_{tot}], \quad (2.22)$$

where we have introduced the superscript $[E^*]^{ss}$ notation to indicate steady state. Intuitively, the qualitative behavior of these steady-state concentrations make sense since we see that as the excess concentration of ATP is increased, this tilts the balance towards higher levels of

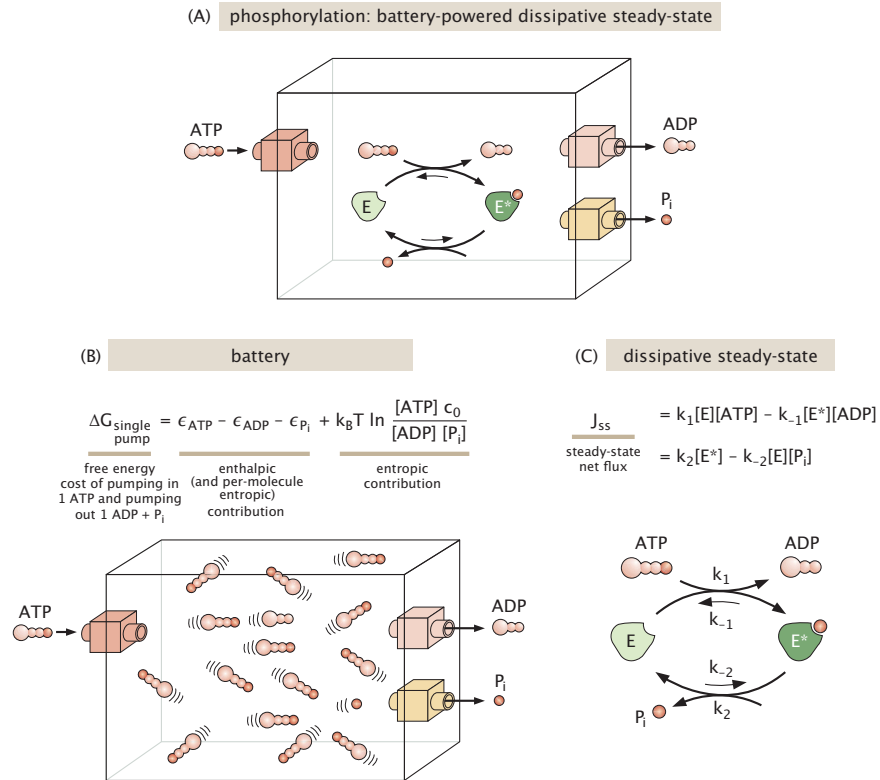


Figure 2.10: Nonequilibrium steady state for phosphorylation-dephosphorylation cycle. (A) Schematic of the chemical reactor system in which the phosphorylation and dephosphorylation of enzyme E takes place. The pumps at the system boundary represent the work that needs to be done to maintain the out of equilibrium concentrations of ATP, ADP and P_i . (B) The free energy cost of active maintenance. For every phosphorylation-dephosphorylation cycle, one ATP needs to be added to the system and one ADP and P_i needs to be removed. (C) Evaluation of the flux in the phosphorylation-dephosphorylation cycle. Adapted from CL Hueschen and R Phillips, *The Restless Cell*, Princeton University Press, 2024.

E^* . We can find the steady-state net flux passing through the reaction given in eqn. 2.18 as

$$J = k_1[ATP][E] - k_{-1}[ADP][E^*]. \quad (2.23)$$

Using the steady-state values of $[E]$ and $[E^*]$ we find the steady-state flux as

$$J^{ss} = \frac{k_1 k_2 [ATP] - k_{-1} k_{-2} [ADP][P_i]}{k_1 [ATP] + k_{-2} [P_i] + k_{-1} [ADP] + k_2}. \quad (2.24)$$

The units of this quantity are $1/s$ and the interpretation is that J^{ss} tells us the net number of reactions per volume per unit time. The flux in reaction given by eqn. 2.19 is the same since the whole reaction network is in steady state (and it follows from the math too). Note that if the reaction were allowed to equilibrate, then $J^{ss} = 0$ implying the allied condition

$$\frac{[ADP]_{eq}[P_i]_{eq}}{[ATP]_{eq}} = \frac{k_1 k_2}{k_{-1} k_{-2}}. \quad (2.25)$$

Given the steady rhythm of phosphorylation of the proteins of the proteome, what can we say about its typical energetic costs? Figure 2.10 gives us one hint as to how to think about the cost of these events. It is convenient to think of the processes of the cell as being powered by a suite of different biological batteries. For photosynthetic cells, that battery is provided by the steady stream of photons impinging on the Earth, each of which is worth roughly $100 k_B T$ of energy. Photosynthetic reactions produce organic matter such as sugars which harbor a huge amount of stored energy ($\approx 1200 k_B T$ per sugar) which can in turn be converted into other kinds of biological batteries such as membrane potentials and ATP. As a reminder, we recall the rule of thumb that $1 \text{ ATP} \approx 20 k_B T$.

To provide more everyday intuition for thinking about these biological batteries, it is helpful to think of one of the most underrated of human technologies, namely, refrigerators. First, remember that a refrigerator doesn't work unless we plug it into an

electric outlet in the wall. In the same way that the dynamics of photosynthesis and carbon fixation does not work if we are not “plugged into” the external energy source of the sun, the refrigerator doesn’t work unless we are plugged into the power grid. In both cases, the energy source is “over there.” That is, we don’t need to concern ourselves with how that energy source works or how much it costs to maintain it, it is just there. For the refrigerator, there is a heat pump that exploits the external energy source to reduce the temperature. The second law of thermodynamics and the corresponding insights about the Carnot cycle tell us how much energy we need to extract from the external power source to achieve that cooling.

Figure 2.10 attempts to make the refrigerator analogy more concrete by considering a system coupled to ATP, ADP and P_i pumps. In the same way that a refrigerator pumps heat, we can think of the reactions within a cell that keep a fixed ratio of ATP to ADP and P_i as mediated by pumps as shown in the figure. Every time one full phosphorylation-dephosphorylation cycle is completed, the pumps will pump in a single ATP and pump out an ADP and P_i with the result that the ratio of these molecules remains constant. Keeping those reservoirs at fixed values is the equivalent of keeping our refrigerator plugged into an outlet. But doing that pumping action comes with a free energy cost of its own. Our goal now is to work out how much energy is consumed for each such pumping event. Once we have that energy per cycle, we can then invoke our usual concept that $\partial F/\partial t = J \Delta\mu$, where $\Delta\mu$ is the cost per cycle and J is the number of such reactions per unit time.

In one of the most inspired and subtle acts of coarse-graining in the history of science, Josiah Willard Gibbs showed how we could capture the nearly infinite degrees of freedom with some reservoir in the simple idea of the chemical potential. The chemical potential is defined as the difference in free energy of the reservoir when it has N and $N - 1$ molecules

of the species of interest. In the present context, the chemical potential of ATP is defined as

$$\mu_{ATP} = \Delta U_{ATP} - T\Delta S_{ATP} \quad (2.26)$$

where we have

$$\Delta U_{ATP} = N\varepsilon_{ATP} - (N - 1)\varepsilon_{ATP} \quad (2.27)$$

and

$$\Delta S_{ATP} = k_B \ln \frac{W(N)}{W(N - 1)}, \quad (2.28)$$

where $W(N)$ is the number of states of the reservoir when there are N ATP molecules present; ε_{ATP} is a phenomenological parameter that captures the energy associated with having a single ATP molecule in solution; k_B is Boltzmann's constant. The ΔS term accounts for the entropy change because during a hydrolysis event, the number of ATPs was reduced by one while the number of ADP and P_i was increased by one. Putting all of this together yields

$$\mu_{ATP} = \varepsilon_{ATP} + k_B T \ln \frac{[ATP]}{c_0}, \quad (2.29)$$

where c_0 is the standard state concentration, usually taken as 1 M. We have similar expressions for ADP and P_i , namely,

$$\mu_{ADP} = \varepsilon_{ADP} + k_B T \ln \frac{[ADP]}{c_0} \quad (2.30)$$

and

$$\mu_P = \varepsilon_P + k_B T \ln \frac{[P_i]}{c_0}. \quad (2.31)$$

These equations are entirely analogous to those we used in the previous section in describing the ions and sugars that were being transported into and out of the cell.

When reactions 1 and 2 both take place, the biochemical system has taken one complete

cycle. The free energy cost associated with one such reaction can therefore be reckoned by appealing to the chemical potentials of the ATP, ADP and P_i in the form

$$\Delta F_{\text{one cycle}} = \mu_{ATP} - \mu_{ADP} - \mu_{P_i}. \quad (2.32)$$

The reason for the different signs is that we have to add an ATP and remove an ADP and P_i , and the definition of the chemical potential therefore mandates these signs. Given the functional form of the chemical potentials derived above, we can rewrite this expression as

$$\Delta F_{\text{one cycle}} = \Delta \varepsilon + k_B T \ln \frac{[ATP]c_0}{[ADP][P_i]}. \quad (2.33)$$

When the system is in equilibrium, as we saw above, the net flux $J^{ss} = 0$ which corresponds to the condition

$$k_1 k_2 [ATP]_{eq} = k_{-1} k_{-2} [ADP]_{eq} [P_i]_{eq} \quad (2.34)$$

which can be rewritten as

$$\frac{[ADP]_{eq} [P_i]_{eq}}{[ATP]_{eq}} = \frac{k_1 k_2}{k_{-1} k_{-2}}. \quad (2.35)$$

Further, at equilibrium, we know that $\Delta F = 0$, permitting us to relate the equilibrium concentrations to $\Delta \varepsilon$. Specifically, in that case we have

$$\Delta \varepsilon = -k_B T \ln \frac{[ADP]_{eq} [P_i]_{eq}}{[ATP]_{eq}} = -k_B T \ln \frac{k_1 k_2}{k_{-1} k_{-2}}, \quad (2.36)$$

permitting us to rewrite the total free energy change in one full cycle as

$$\Delta F_{\text{one cycle}} = k_B T \ln \frac{k_1 k_2 [ATP]}{k_{-1} k_{-2} [ADP][P_i]} = k_B T \ln \frac{J_1 J_2}{J_{-1} J_{-2}}. \quad (2.37)$$

The result worked out here is a special case of a much more general result that can be

written in the form

$$\frac{\partial F}{\partial t} = k_B T \sum_i (J_i - J_{-i}) \ln \frac{J_i}{J_{-i}}, \quad (2.38)$$

which instructs us to sum over all the separate reactions. For our case of interest here, we have two reactions to sum over with the result that

$$\frac{\partial F}{\partial t} = k_B T (J_1 - J_{-1}) \ln \frac{J_1}{J_{-1}} + k_B T (J_2 - J_{-2}) \ln \frac{J_2}{J_{-2}}. \quad (2.39)$$

We can work out the separate contributions by appealing to our analysis above where we worked out the various fluxes, yielding

$$\ln \frac{J_1}{J_{-1}} = \ln \frac{k_1[ATP][E]}{k_{-1}[ADP][E^*]} \quad (2.40)$$

and

$$\ln \frac{J_2}{J_{-2}} = \ln \frac{k_2[E^*]}{k_{-2}[E][P_i]}. \quad (2.41)$$

We note that $J_1 - J_{-1} = J_2 - J_{-2}$, so that we can make the simplification

$$(J_1 - J_{-1}) \ln \frac{J_1}{J_{-1}} + (J_2 - J_{-2}) \ln \frac{J_2}{J_{-2}} = (J_1 - J_{-1}) \ln \frac{J_1 J_2}{J_{-1} J_{-2}}. \quad (2.42)$$

We see that

$$k_B T \ln \frac{J_1 J_2}{J_{-1} J_{-2}} = k_B T \ln \frac{k_1 k_2 [ATP]}{k_{-1} k_{-2} [ADP][P_i]}, \quad (2.43)$$

which is the free energy change in one cycle, while the prefactor $(J_1 - J_{-1})$ gives us the net number of cycles per second. We have found it exceedingly difficult to find measurements of the parameters k_1 , k_{-1} , k_2 and k_{-2} and as a result, we attempt instead to use

$$\frac{\partial F}{\partial t} = k_B T (J_1 - J_{-1}) \ln \frac{J_1 J_2}{J_{-1} J_{-2}}. \quad (2.44)$$

If we take $J_1 = J_2 = 100 \text{ s}^{-1}$ and make the conservative estimate that $J_{-1} = J_{-2} = 1 \text{ s}^{-1}$, this tells us that

$$\frac{\partial F}{\partial t} = k_B T \times \frac{100}{s} \times \ln 10^4 \approx f \times 10 \frac{\text{ATP}}{s}. \quad (2.45)$$

Note that this estimate is for a single protein. Clearly if every protein in the proteome were being phosphorylated at this rate, this would be a huge cost. Measurements and modeling performed in the 1980's on cyclic (de)phosphorylation cascades [30] report that typical operating conditions for such cascades demand at most of order 1 mM ATP/min, corresponding to at most a power per volume of $\text{few} \times 10^5 \text{ ATP}/\mu\text{m}^3 \text{ s}$. Clearly, there is far more to learn about the power associated with escaping genetic imprisonment through post-translational modifications.

2.3.3 Cost of sustaining vital membrane potential and osmalance

Microbial cells build and sustain very different concentrations of ions than their environmental milieu—for instance intracellularly accumulating potassium and reducing sodium significantly relative to typical ambient concentrations [31]. As shown in Figure 2.11, if no active import and export processes were performed by the cells to sustain these concentration differences, ions would leak along their entropic incentive and these gradients would decay to equilibrium uniformity. While a rich array of fascinating enzymatic machinery orchestrates this pumping activity, and the battery of distinct molecular actors and feedbacks are extremely intricate and merit their own individual dissection, here we abstract some of this considerable complexity and formulate some basic fundamental lower bounds and phenomenological estimates on the dissipative costs needed to perform these functions. The concept in this first round of calculations is simply to get a feeling for the relevant orders of magnitude.

As shown in Figure 2.11, despite a very small permeability to ions, there is a leak current

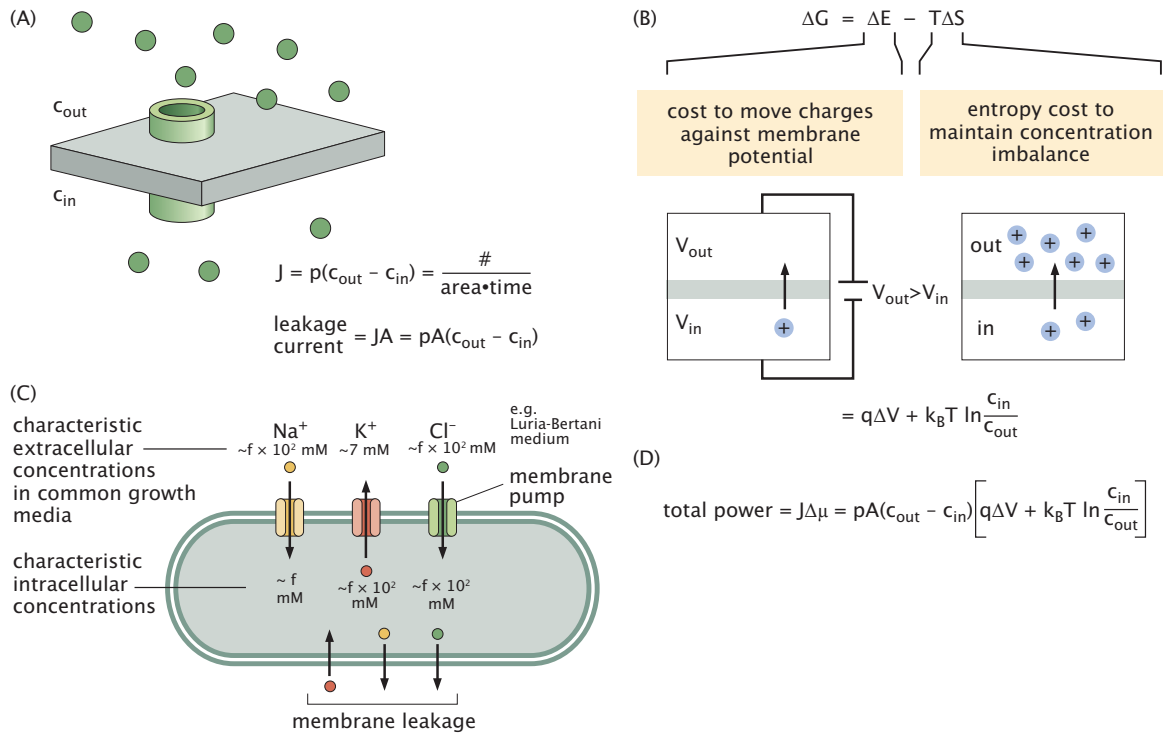


Figure 2.11: Maintaining gradients of ions in a cell requires active energy expenditure lower-bounded by a simple calculation.

of ions across lipid bilayer membranes. In particular, if we assume a typical permeability of $p = 10^{-4} \text{ nm/s} = 10^{-7} \text{ }\mu\text{m/s}$ [32], and a concentration difference across the membrane of order 100 mM, then the flux is given by

$$J_{ion} = p\Delta c \approx 10 \frac{\text{ion}}{\mu\text{m}^2 \text{ s}}. \quad (2.46)$$

For a microbial cell with a surface area of $f \text{ }\mu\text{m}^2$, this corresponds to a leak current of about $f \times 10$ ions/s. As a first sense of scale, the cost to transport one such ion up its gradient is roughly $\Delta G_{ion} = q\Delta V + k_B T \ln(c_{in}/c_{out}) \approx 1 \text{ ATP}$, indicating that the power of this process is about $f \times 10 \text{ ATP/s}$, a tiny fraction of the overall power of the cell in either maintenance or growth stages.

More precisely, as discussed by Kedem and Katchalsky [33] and others [32], for dilute

solutions neglecting the contribution of water fluxes³, the change in chemical potential that accompanies a single solute molecule moving across a membrane from a concentration c_{out} to a concentration c_{in} is

$$\Delta\mu \equiv \mu_s^{\text{out}} - \mu_s^{\text{in}} \approx \frac{\bar{v}}{N_A} \Delta p + k_B T \ln \frac{c_{\text{out}}}{c_{\text{in}}} + q \Delta V, \quad (2.47)$$

where $\bar{v}$ is the partial molar volume; $\Delta p = p_{\text{out}} - p_{\text{in}}$ is the change in pressure; q is the solute's charge; ΔV is the cell membrane potential; and N_A and k_B are Avogadro's constant and Boltzmann's constant, respectively. Under nice conditions, a bacterial cell has a standard membrane potential of $\Delta V \in [70, 200] \text{ mV}$ (BNID 103386; [35]). It is reasonable to take $\Delta V = 0.15 \text{ V}$ as a characteristic value in this range.

At this point, our plan is to examine each of the contributions to $\Delta\mu$ separately, but from the outset, treating the first term involving the pressure as negligible. Thus, we begin by examining the entropic contribution to the chemical potential. This term can be written as

$$\Delta\mu \approx k_B T \ln \frac{c_{\text{out}}}{c_{\text{in}}}. \quad (2.48)$$

The flux j of solute molecules expected to pass a membrane with permeability p (with apologies that the permeability has the same symbol as the pressure) against a concentration difference Δc is well approximated by a linear transport law of the form

$$j = -p \Delta c, \quad (2.49)$$

³In an ideal solution with composition c varying in space as $c(r)$, we can more generally write $\mu(r) = \mu_0 + k_B T \ln c(r)$. See e.g. Atkins & de Paula, *Physical Chemistry*, 9th Ed., pg. 164, eq 5.19 [34] for review of the basic ideal logarithmic variation.

so the total current dN/dt of solvents experienced across a membrane of area J is

$$\frac{dN}{dt} = Aj = -Ap\Delta c. \quad (2.50)$$

Thus, to resist this spontaneous leakage current across the membrane, at least an accompanying free energy dissipation rate dS/dt must be expended by the cell, given by,

$$\frac{dS}{dt} = \frac{dN}{dt} \Delta\mu \quad (2.51)$$

$$= - \boxed{Ap (c_{\text{out}} - c_{\text{in}}) \ln \frac{c_{\text{out}}}{c_{\text{in}}} k_B T}. \quad (2.52)$$

To illustrate the scale of this required dissipation rate to maintain a membrane potential, consider an ion with the following representative features:

- permeability, $p \sim 10^{-7} \mu\text{m/s}$: taken to near be that of potassium, see Reference [32].
- concentration difference: $c_{\text{out}} \sim \text{few mM}$ (in Luria-Bertain medium); $c_{\text{in}} \gtrsim (1-2) \times 10^2 \text{ mM}$ (potassium) (see BNID 115760) Thus, $\Delta c \sim 100 \text{ mM} = 10^8 \mu\text{m}^{-3}$.
- cell membrane area, $A \sim \text{few } \mu\text{m}^2$.

These numbers suggest a characteristic dissipation scale of about,

$$\left| \frac{dS}{dt} \right| \sim \frac{dN}{dt} \Delta\mu = \underbrace{(\text{few } \mu\text{m}^2) (10^{-7} \mu\text{m/s}) (10^8 \mu\text{m}^{-3})}_{\text{few} \times 10 \text{ ions/s}} \underbrace{\ln \frac{150 \text{ mM}}{5 \text{ mM}}}_{\sim \text{few}} k_B T \quad (2.53)$$

$$\sim 100 k_B T/s \quad (2.54)$$

$$\sim \boxed{5 \text{ ATP}/(\text{ion} \cdot \text{s})}. \quad (2.55)$$

With this result for the entropic contribution to the free energy change associated with moving one ion across the membrane in hand, we now turn to the contribution in Eqn. 2.47 due to the membrane potential.

Quantifying the $\frac{dN}{dt} q \Delta V$ term

For an ion of charge $|q| = +1$ (electron/proton equivalents), such as for potassium, $q = 1.6 \times 10^{-19}$ Coulomb. This implies

$$q \Delta V \approx (0.15 \text{ V})(1.6 \times 10^{-19} \text{ C}) \quad (2.56)$$

$$\approx 2.4 \times 10^{-20} \text{ J} \quad (2.57)$$

$$\approx 6 k_B T / \text{ion}. \quad (2.58)$$

This is of the same order as the former $\Delta\mu = k_B T \ln(c_{\text{out}}/c_{\text{in}})$ term. To see that, we note that for a typical ratio $c_{\text{out}}/c_{\text{in}} \approx 100$ and recalling that $\ln 10 \approx 2.3$, we see that in this case $\Delta\mu = k_B T \ln 100 \approx 5 k_B T$, quite in line with the voltage contribution to the chemical potential.

2.3.4 Experimental inference of ion pumping costs

The estimate we just reached, giving an approximate lower bound for the power cost to pump ions and maintain a concentration gradient in a cell, is wholly agnostic to how cells instantiate their balance of these ions using their real and nonideal biochemistry and ion channels. How does the actual power paid by cells to balance ions under environmental challenge relate to this lower bound?

How cells respond to osmotic provocations can be experimentally probed by adjusting the concentration of a salt like sodium chloride in the medium and watching cells metabolize or die differently. Here we turn to a revealing set of such experiments from Schink, Basan & coworkers on *E. coli*.

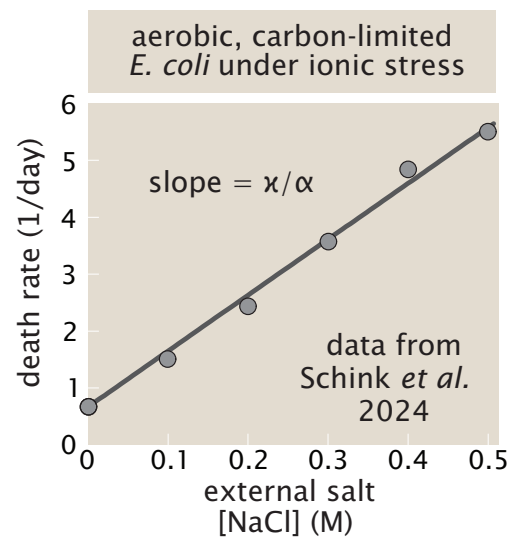


Figure 2.12: Rate at which *E. coli* populations die (aerobically under carbon limitation), as a function of the concentration of sodium chloride in the external medium. (Adapted from Schink *et al.* [8].)

Schink *et al.* report that starving populations of *E. coli* die faster when their medium has more sodium chloride (relative to a typical reference condition). Specifically, as shown by their measurements reproduced in Figure 2.12, the per-capita death rate $\gamma \equiv \frac{1}{N} \frac{dN}{dt}$ of these cells⁴ increases remarkably linearly with the ambient concentration of sodium chloride. Under these (carbon-limited, aerobic) culture conditions, Schink and coworkers also argue that the basal metabolic rate β at which cells consume energy is proportional to this death rate, $\beta = \alpha\gamma$, where α is a fixed proportionality constant specifying the harvestable energetic content released by a dead cell. We alluded to this proportionality in §2.2 and will return to this idea even more forcefully in the next chapter. Accordingly, the linear variation of death rate with external salt level announced by measurements like those in Fig. 2.12 reports that the basal metabolic rate β also varies linearly with salt, namely,

$$\beta = \beta_0 + \kappa[\text{NaCl}], \quad (2.59)$$

where β_0 is the basal metabolic rate at some (negligible) reference level of salt, and κ represents the additional power cost incurred by the cell per increment in sodium chloride concentration past this reference level.

Before theoretically understanding and contrasting this relationship Eq. 2.59's behavior to our earlier theoretical estimate of Eq. 2.52, we take it as a phenomenological fact and immediately probe its numerical implications. Schink *et al.*'s analysis stopped short of calculating or reporting κ , the increase in metabolic activity demanded per additional increment of salt concentration, but the quantities they otherwise report suffice to yield it. Specifically, the slope of the line between death rate and salt in Figure 2.12 gives the value of κ/α , so invoking a physiological value of α can deliver a value of κ . To estimate α , we use a condition where both β and γ under the same conditions were reported or can be

⁴As described further in their methods sections, Schink *et al.* performed these measurements in N-C-minimal medium [8].

estimated, since $\gamma = \beta/\alpha$. Specifically, Schink *et al.* report that cells consume energy at a rate $\beta \sim 0.6$ fmol glucose/cell/day (see [19], Extended Data Figure 10(B)), which we may express as 10^5 ATP/cell/s⁵, in a particular and characteristic medium condition. Under these ambient conditions (43 mM sodium chloride), the trend of Figure 2.12 reports a death rate $\gamma \sim 1 \text{ day}^{-1} = 1.2 \times 10^{-5} \text{ s}^{-1}$. This implies that,

$$\alpha = \frac{\beta}{\gamma} \approx \frac{10^5 \text{ ATP/cell/s}}{1.2 \times 10^{-5} \text{ s}^{-1}} \sim \boxed{8 \times 10^9 \text{ ATP/dead cell}}. \quad (2.62)$$

We now briefly pause to subject this estimated number for α to a smell test. Does this value make sense, if it is to represent the energy effectively harvestable from a dead cell? To ask whether this is plausible, we recall that the energy required to *make* a fresh *E. coli* cell is of order $\text{few} \times 10^{10}$ ATP. This building cost can be called to mind quickly, by remembering that while growing exponentially at a fast doubling time of order $\tau_2 \sim 20\text{--}30 \text{ min} = \text{few} \times 10^3 \text{ s}$, a very good rule of thumb is that cells are spending of order $\rho \sim 10^7 \text{ ATP/s}/\mu\text{m}^3$ [38, 39], immediately giving a total energy expenditure of $\rho\tau_2 = (1\text{--}\text{few}) \times 10^{10}$ ATP to build a cell. Accordingly, the fact that our estimated number suggests a dead cell releases a few times less energy than a fresh one requires to be made is eminently reasonable. Indeed, in other studies, Schink and coworkers measured the relative recycling yield delivered by dead cells compared to the abundance when alive, and also found by distinct and explicit experiments that about a few to ten dead cells give the energy required to build a new cell [19]. This scale—that (under aerobic carbon limitation) dead cells yield a few times less energetic content in recyclable

⁵To understand the inferred metabolic expenditure of *E. coli* in units of ATP/cell/s (permitting comparison to other meaningful dissipative numbers at hand), we recall that the ideal, complete oxidation of 1 molecule of glucose generates the energetic equivalent of 26 ATP molecules (BNID 114969; [36, 37]). This gives,

$$\beta \sim 0.6 \frac{\text{fmol glu}}{\text{CFU day}} \times \frac{10^{-15} \text{ mol}}{\text{fmol}} \frac{26 \text{ mol ATP}}{1 \text{ mol glu}} \frac{6 \times 10^{23} \text{ ATP}}{\text{mol}} \frac{1 \text{ day}}{86400 \text{ s}} \quad (2.60)$$

$$\sim \boxed{10^5 \text{ ATP/s/cell}}. \quad (2.61)$$

necromass than a new cell needs—is an interesting and evocative bioenergetic rule of thumb in its own right.

Armed with this plausible value for the parameter α , we now at last estimate our desired value of κ setting the metabolic burden per an increment in external sodium chloride concentration. Consulting Figure 2.12 reveals that total death rate changes from less than one day^{-1} to close to six day^{-1} over a range of 500 mM external sodium chloride, or a slope of

$$\kappa/\alpha \approx \frac{5 \text{ day}^{-1}}{500 \text{ mM NaCl}} = 10^{-2} \text{ day}^{-1}/(\text{mM NaCl}) \quad (2.63)$$

$$= 1.2 \times 10^{-7} \text{ s}^{-1}/(\text{mM NaCl}). \quad (2.64)$$

Combining our estimates gives

$$\kappa = \frac{\kappa}{\alpha} \times \alpha \quad (2.65)$$

$$\approx (8 \times 10^9 \text{ ATP}) (1.2 \times 10^{-7} \text{ s}^{-1}/(\text{mM NaCl})) \quad (2.66)$$

$$\sim \boxed{10^3 \text{ ATP/s/cell}/(\text{mM NaCl})}. \quad (2.67)$$

This characteristic scale for κ announces, with huge quantitative drama, the metabolic plasticity demanded of microbes like *E. coli* in their variable environments. Consider the scale of fluctuations in ambient salt that cells easily experience. For instance, if one of the human tears of joy shed while writing this thesis fell into one of the 96-well plates hosting cells that inspired us to write it, the unlucky well could experience a $\Delta c = 10 \text{ mM}$ boost in the concentration of sodium ions⁶; our estimated value of κ anticipates *E. coli* would increase its metabolic rate by a considerable $\kappa \Delta c \sim 10^4 \text{ ATP/s/cell}$. For context, such an

⁶Photometry measurements report human tears typically have sodium ion concentrations of $c \sim 150 \text{ mEq/L} = 150 \text{ mM}$ [40], and since teardrops have volumes of a few microliters, eg $V \sim 7 \mu\text{L}$ [41], implying that a typical teardrop offers about 1 micromol of sodium; if added to a well with a typical working volume of $\sim 100 \mu\text{L}$, this anticipates a boost of 10 mM sodium, if e.g. there were negligible sodium to begin with.

increase is about a tenth of the starting reference metabolic rate at 43 mM sodium chloride (see Eq. 2.61), but of the order of the *total* metabolic rate of *P. aeruginosa* directly measured by Ciemniecki and colleagues in the Newman lab [42] using extracellular phenazine cycling. Clearly much more dramatic ionic fluctuations could shape a life of *E. coli* in soils or human guts. Our inferred value for κ predicts that these environmental fluctuations may induce substantial, coupled energetic demands on struggling bacteria as well.

However, intriguingly, the metabolic costs incurred under different conditions or by other species may be substantially different. For instance, just to foreshadow a more rigorous discussion, our analysis of new measurements performed by collaborators in the Newman laboratory at Caltech appear to resolve a much milder variation of *Pseudomonas aeruginosa*'s metabolic rate with ambient sodium chloride than here with *E. coli*. We discuss this evidence in further detail in the succeeding chapter.

Where does the mathematical form and numerical sensitivity of a cell's metabolic response to salt come from? Should Eq. 2.59's linear relationship between total metabolic rate β and external salt concentration be regarded mostly phenomenologically, e.g. as a Taylor expansion to linear order of some more complicated physiological dependence $\beta \equiv \beta([\text{NaCl}])$? Even under this generic interpretation, κ serves as a useful governing effective parameter that concisely summarizes how sensitive metabolism is to the insult of external salt. Indeed, Schink and colleagues motivate this relationship literally by positing the total metabolic rate β is dominated by active pumping against a diffusive flux $P\Delta c$ for fixed permeability P (which subsumes the cell membrane's area over which the leaks can happen) with a concentration difference Δc , where the ion cost is κ . However, we search for more explicit biophysical insight into this question by returning to contrast the empirically linear variation of death (thus metabolic) rate with external salt, Eq. 2.59, with

our earlier coarse biophysical estimate, Eq. 2.52, which predicts that,

$$\text{power cost to pump: } \frac{dS}{dt} = \Delta\mu(pA\Delta c) \quad (2.68)$$

$$= \left(k_B T \ln \frac{c_{\text{out}}}{c_{\text{in}}} \right) pA (c_{\text{out}} - c_{\text{in}}) \quad (2.69)$$

$$= k_B T A p (c_{\text{out}} - c_{\text{in}}) \ln \frac{c_{\text{out}}}{c_{\text{in}}}. \quad (2.70)$$

On its surface, this prediction appears to contradict the simple linear scaling of power with external ion concentration of Eq. 2.59. That is, unlike the literal invocations by Schink and co, the cost κ to pump an ion out of the cytoplasm should manifestly vary with the present balance of inner and outer concentrations, specified by the chemical potential. However, examining two limiting and intentionally extreme cases of ionic regulation gives some clues about how to mathematically reconcile these pictures.

First, imagine that cells maintain their cytoplasmic concentration of ions c_{in} at a fixed level despite any variation in the external levels c_{out} . This evokes a scenario where cytoplasmic functionality is extremely sensitive to the homeostasis of particular ions, compelling cells to exert stringent control to precisely clamp an ionic concentration inside the cell.⁷ For evocative illustration, consider the finding that *E. coli* tightly can maintain cytoplasmic sodium ion concentration to a level within $\sim 5 - 14$ mM Na^+ while subject to external sodium concentrations between 1-85 mM sodium in specific culture conditions [43], illustrating a partial homeostasis. In this simple case, the predicted power cost to pump as the external concentration varies is often only mildly nonlinear over experimentally relevant regimes of external salt concentration; for instance, Figure 2.13 illustrates the relative pumping cost predicted by Eq. 2.70 when the cell fixes its cytoplasmic concentration to 5 mM despite an external salt concentration variation up to

⁷Imagining that cytoplasmic ion concentration c_{in} is constant with respect to even a ten-fold variation in $c_{\text{out}} \gtrsim c_{\text{in}}$ will *not* necessarily be reasonable for all bacterial strains.

500 mM. Note that if the ambient concentration c_{out} is not so different than the cytoplasmic concentration c_{in} , then a Taylor expansion anticipates that the cost varies quadratically in c_{out} . This behavior can be seen by writing the logarithm of the ratio in the pumping cost as

$$\ln \frac{c_{\text{out}}}{c_{\text{in}}} = \ln \frac{c_{\text{in}} + (c_{\text{out}} - c_{\text{in}})}{c_{\text{in}}} = \ln \left(1 + \frac{\Delta c}{c_{\text{in}}} \right) \quad (2.71)$$

$$\approx \frac{\Delta c}{c_{\text{in}}}, \quad (2.72)$$

so $\frac{dS}{dt} \sim (\Delta c)^2/c_{\text{in}}$. Even more usefully, Figure 2.13 shows that over large typical concentration ranges the true nonlinearity of the pumping cost is mild, and for example a line of average slope over a tested domain,

$$\langle \text{slope} \rangle = k_B T p A \ln \left(\frac{c_{\text{out}}^{\text{max}}}{c_{\text{in}}} \right). \quad (2.73)$$

that reproduces the average slope from $c_{\text{out}} = 0$ to a maximum concentration tested ($c_{\text{out}} = c_{\text{out}}^{\text{max}}$) often adequately describes the variation of the predicted pumping cost with external salt concentration. In summary, if the inner concentration of an ion is clamped to some fixed value and the external concentration of ion is varied over a wide range relative to this internal value (while the cell manifests a constant permeability and other properties), the corrections to a linear behavior (with average slope scaling with the permeability of the cell and the area of the cell) should be modest.

Next, we consider a second separate physiological extreme, where the inner concentration of ion is not fixed to a literal constant, but pinned to some multiplicative factor of the external concentration, namely $\frac{c_{\text{in}}}{c_{\text{out}}} := f$ for some fixed multiplicative factor f . (This idea abstractly stylizes some classic observations made on *P. aueriginosa* where sodium concentrations inside cells were reported to be $\sim 90\%$ of the external medium up

to hundreds of millimolar in external salt [44].) In this case, the predicted cost Eq. 2.70 can be written as,

$$\frac{dS}{dt} = k_B T A p (c_{\text{out}} - c_{\text{in}}) \ln \frac{c_{\text{out}}}{c_{\text{in}}} \quad (2.74)$$

$$= k_B T A p c_{\text{out}} (1 - f) \ln f \quad (2.75)$$

$$= \underbrace{(k_B T A p (1 - f) \ln f)}_{\equiv k} c_{\text{out}}. \quad (2.76)$$

Here we see that the metabolic cost is exactly linear in the external salt—without any further approximation required—with the interpretation of the slope k as something proportional to $k_B T A p$ and depending (gently) on the value of the maintained target fraction f .

Thus in either physiological extreme of these two examples, we conclude that the characteristic slope relating pumping cost and external salt concentration should enjoy the interpretation of being nearly linearly proportional to $k_B T A p$. Of course, the real dynamics of ionic regulation in cells is much more complex than acknowledged by these toy scenarios. Specifically, for instance, the inner concentration c_{in} is probably set by a much more intricate web of feedbacks than mere constancy or proportionality to ambient salt.

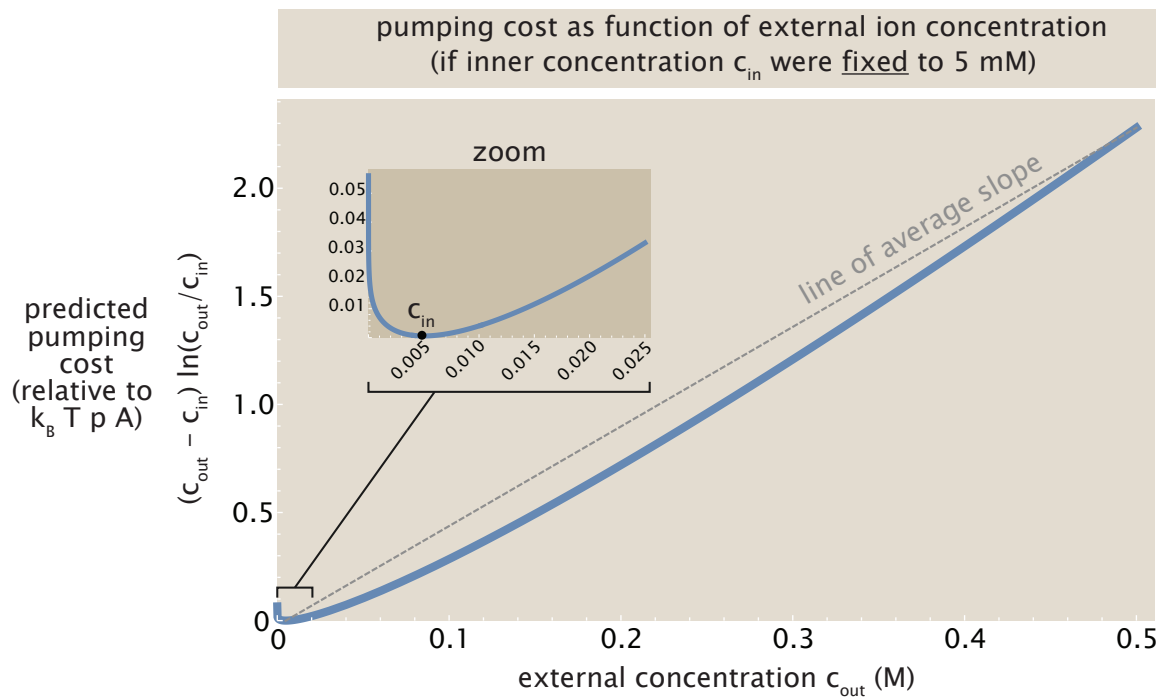


Figure 2.13: Predicted cost of pumping ions as function of the external medium concentration c_{out} up to 500 mM, assuming that cells fix cytosolic concentrations to c_{in} at a value of 5 mM, according to Eq. 2.70. (This power cost is reported in units of $k_B T p A$ on the y -axis.) Notice that the curvature of this function over this range is not dramatic, as judged by only small bowing relative to the average slope over the domain that varies external concentration to $10^3 \times$ the fixed inner concentration. Inset: the cost to pump has the intuitive minimum of zero cost at $c_{in} = c_{out}$, about which the cost function is approximately quadratic.

2.3.5 Cost of cellular motility

Another process highlighted in Figure 2.7 that is shared by many microbes is that of motility. For the purposes of the present estimates, we consider the case of swimming motility powered by the motion of exterior flagella, though there are a number of other flagellum based motilities. In this section, we explore two different ways of assessing the energetic cost and power of cellular motility. First, we consider the fact that swimming organisms must overcome fluid drag. Second, we consider the power consumption of the molecular machines (e.g. the bacterial flagellar motor) that drive motion through viscous fluids.

Before embarking on these estimates, we remark that whether cells move (and so spend accompanying mechanical energy to power their flagella) depends sensitively on their environment. Specifically, many microbes on the severe energetic brink, including in senescent biofilms, do not appear to activate their flagella in a number of natural conditions for a large fraction of their lifetimes. This contextual dependence implies that there is a number of maintenance lifestyles where the mechanical costs of motility are not especially imposed on cells.

The Cost of Overcoming Drag

Whether giant whales migrating across oceans, long distance flying migrations such as that of the bar tailed godwit which travels nonstop between Alaska and New Zealand or a swimming bacterium, in all of these cases, the living organism must overcome the forces of viscous drag. But there the similarity ends. There is roughly a trillion-fold difference in the Reynolds number in these distinct situations, with the whale in a regime of extremely high Reynolds number and the bacterium in the opposite very low Reynolds number regime. As

a result, the drag laws that characterize these two cases are extremely different. To get a feeling for the forces involved in the low Reynolds number setting, we imagine a spherical bacterium of radius a which is met with a drag force known as Stokes drag and described by

$$F_{drag} = 6\pi\eta av, \quad (2.77)$$

where η is the viscosity and v is the velocity of the swimming bacterium. (We note that in general rod-shaped bacteria would experience lesser drags, but such differences in geometry usually matter only within factors of few for typical cellular aspect ratios.) We can make a naive estimate of the power associated with overcoming this mechanical drag by multiplying the drag force and a typical velocity for *E. coli*, namely $v \sim 35 \mu\text{m/s}$ [45], resulting in

$$\text{power of swimming} = 6\pi\eta av^2 \quad (2.78)$$

$$\approx 20 \times (10^{-3} \text{ Pa} \cdot \text{s}) \times (10^{-6} \text{ m}) \times (f \times 10^{-5} \text{ m/s})^2 \quad (2.79)$$

$$\approx f \times 10^2 \text{ ATP/s}. \quad (2.80)$$

This is a very interesting number that shows that the cost of overcoming fluid drag is small in comparison with many of the other much larger powers showcased in Figure 2.7. However, there is more to consider.

The Cost of Running the Flagellar Motors

The swimming motion of a bacterium such as *E. coli* ultimately derives from the rotation of flagella. And each such rotation has a cost, regardless of how much fluid drag the bacterium needs to overcome. In this section, we attempt to estimate this cost for the purposes of comparing it to the cost of overcoming the fluid drag and the many other cellular processes

highlighted in Figure 2.7.

Experiments on swimming in *E. coli* tell us that for every rotation of the flagellar motors that power swimming, the cost is roughly 1200 protons per revolution, and the motors are rotating at a speed of 10 Hz [46]. Further, we will assume that there are multiple such motors. As usual, we turn to our strategy of computing the power as

$$\text{power of flagellar motors} = J_{motors} \times \Delta\mu_{H^+}, \quad (2.81)$$

where J_{motors} is really a statement of how many rotations per second total occur and $\Delta\mu_{H^+}$ is the free energy cost associated with the H^+ ions moving across the membrane to power the motor. To maintain the hydrogen ion biological battery, those H^+ ions needed to be pumped back up their concentration gradient. We recall that the proton motive force is given by

$$\Delta\mu_{H^+} = q\Delta V + k_B T \ln \frac{c_{out}}{c_{in}}, \quad (2.82)$$

where the first term represents the cost of transporting charge against the membrane potential and the second term is associated with the entropy change associated with transporting molecules against their concentration gradient. For characteristic scales in these problems, we estimate $\Delta\mu_{H^+} \approx 10 k_B T$. Hence, for one motor rotating at 10 Hz, this corresponds to a free energy of

$$\text{flagellar motor power} = J_{motors} \times \Delta\mu_{H^+} \quad (2.83)$$

$$\approx 10 \frac{\text{rotations}}{\text{s}^{-1}} \times 10^3 \frac{H^+}{\text{rotation}} \times \frac{10 k_B T}{H^+} \quad (2.84)$$

$$\approx 10^5 \frac{k_B T}{\text{s}} \quad (2.85)$$

$$\approx 5 \times 10^3 \frac{ATP}{\text{s}}. \quad (2.86)$$

Note that this is the power for only a single flagellar motor. In fact, there are often multiple

flagella and hence we can imagine that this power is another five-fold higher than this value, in the range of $f \times 10^4$ ATP/s. As often appears to be the case, the cost of running the machines of the cell often comes out much higher than the dissipative processes that they must act against. In this case, there is a 100-fold difference between the motor power and the mechanical power of swimming.

2.3.6 Cost of membrane synthesis

For one cell to become two, not only must the contents of the cellular interior be doubled, but so too must all of the surface components such as the membrane and the cell wall. If we imagine the bacterial cell membrane as having an area of something like $5 \mu m^2$ and recognizing that there is both an inner and outer membrane, this gives a total area of $20 \mu m^2$. However, given that roughly half of that area is taken up by membrane proteins, we estimate a total lipid area of $10 \mu m^2$. If we now assume that the typical cross sectional area of a lipid molecule is in the range $1/4 - 1/2 nm^2$, this implies that the number of lipids per bacterial cell is something like

$$\text{number of lipids} \approx \frac{10 \times 10^6 nm^2}{1/4 - 1/2 nm^2/\text{lipid}} \approx 2 - 4 \times 10^7 \text{ lipids.} \quad (2.87)$$

Let's now try to estimate the cost of synthesizing all of these lipids in the case of fast growing cells. Roughly, the cost is $f \times 10$ ATP equivalents per phospholipid. Obviously, there is a great diversity of different lipid types and the biochemical pathways for each such lipid are different. But the cost we assume here is a reasonable and conservative guess. Now, given that there are $f \times 10^7$ lipids, we can work out a power

$$\text{power of lipid synthesis} \approx \frac{(f \times 10^7 \text{ lipids}) \times (f \times 10 \text{ ATP/lipid})}{f \times 10^3 s} \approx f \times 10^5 \text{ ATP/s,} \quad (2.88)$$

a significant power investment in comparison with protein synthesis, which is an order of magnitude higher. Note that our calculation, as evidenced by the denominator of $f \times 10^3$ s, reflects the fast growth regime with a division time of less than an hour. To pivot to the case of “maintenance metabolism” would imply knowing something about the rate of turnover of lipids for cells that are not growing at all. Here we are starved of the relevant facts and can only make educated guesses, though we hope that someone might point us to solid experimental facts. If we assume that instead of being replenished on the hour time scale that instead the turnover time is of order a day, this means that the power will be reduced by a factor of between $f \times 10$ and 100, to the much more modest power of between $f \times 10^3$ and 10^4 ATP/s.

A key point of this overall exercise is to take stock of what is already known experimentally and what new experiments should be done in order to refine our understanding of the unusual molecular census of cells in a state of “maintenance metabolism.” As concerns the subject of lipids, we have the strong sense that there is so much more to be known since it is clear that cells “care” about their lipid composition since when that composition is perturbed, cells invoke steps to restore the previous lipid composition, which is a very complex mix. Even the phospholipids have a strange collection of tail lengths whose properties and functional origins remain, for now, unclear: Why are there so many diverse phospholipids? Why their particular lengths? These questions await substantive further inquiry.

2.4 Minimal maintenance power from empirical data in ocean sediment

Next, to complement our more mechanistic and indirect first-principles reasoning about dissipation rates above, we endeavor to estimate how environmental carbon data, interpreted

from the lens of a recent and successful kinetic theory, report on the basal power requirement cells need to survive in real environments.

In a recent tour de force, Prof. Dan Rothman built a beautiful model aiming to account for how quickly microbes degrade carbon in marine and sediment environments [11]. The model is built from assuming that increasingly rare fluctuations in energy available to microbes constrain the degradation of organic carbon. In brief, Rothman's model predicts power laws for the total abundance of carbon $g(t)$; the cell density per volume $N(t)$; and hence the cell-specific carbon degradation rate $b(t)$, all with precise, theoretically-ordained power law exponents in carbon age t . These predictions accord impressively with available data.

Crucially for our interest in quantifying maintenance metabolism, Rothman predicts that the cell-specific carbon oxidation rate $b(t)$ decreases with age until it reaches a minimum viable basal rate $b(t_*)$ at a critical carbon age t_* , after which this rate is constant, $b(t > t_*) \equiv b(t_*)$. Though Rothman's compelling model specifies all the ingredients needed to back-calculate the basal metabolic rate $b(t_*)$ from other numerical features, Rothman's paper stopped short of algebraically or numerically calculating the value demanded of the model given by internal consistency. Here we quickly solve for this basal metabolic rate. Next we evaluate it numerically using Rothman's identified scalings, and so ask what this model predicts as a type of consensus value for the transition to basal metabolism across microbes in the ocean in their natural environments.

As the rate dg/dt at which carbon abundance changes with age is attributable to microbes, specifically

$$\frac{dg}{dt} = -N(t)b(t) \quad (2.89)$$

by definition, this assertion amounts to an unambiguous quantitative prediction for the

value of basal power requirement $b(t_*)$. Rothman's model predicts that carbon ages as

$$g(t) = g_0 t^{-\alpha} \quad (2.90)$$

for a numerical exponent α (a value fixed by theory, and dependent on whether $t < t_*$ or $t > t_*$) and some prefactor g_0 ⁸. This implies that $dg/dt = -\alpha g_0 t^{-\alpha-1}$, or by the definitional equation Eq. 2.89,

$$g(t) = -\frac{t}{\alpha} \frac{dg}{dt} \quad (2.91)$$

$$= \frac{t}{\alpha} N(t) b(t), \quad (2.92)$$

so the basal power requirement rate is given by

$$b(t_*) = \boxed{\frac{\alpha}{t_*} \frac{g(t_*)}{N(t_*)}}. \quad (2.93)$$

⁸More precisely, to enforce continuity at the crossover time t_* , the condition insists that $\lim_{t \rightarrow t_*^+} g(t) = \lim_{t \rightarrow t_*^-} g(t)$, so we have $g(t \leq t_*) = g_1 t^{-\alpha_1}$ and $g(t \geq t_*) = (g_0 t_*^{\alpha_2 - \alpha_1}) t^{-\alpha_2}$. Rothman's model, based on fixed point equations following from extreme value theory, predicts that $\alpha_1 = \exp[-\gamma]/2$ and $\alpha_2 = \exp[-\gamma]$, where $\gamma \approx 0.5772 \dots$ is the Euler-Mascheroni constant.

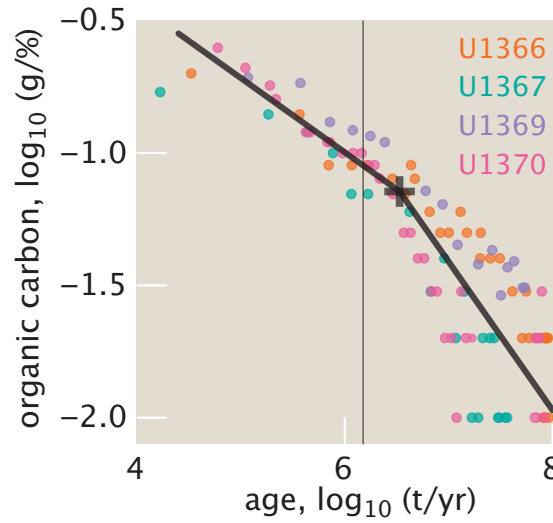


Figure 2.14: Fraction of organic carbon in sediment versus carbon age, as reported by deep marine sediment samples. The gray vertical line indicates the cross-over time $t_* \sim 1.5 \times 10^6$ yr taken by Rothman. Our further simple piecewise fit (indicated by the black line) to these carbon-abundance data $g(t)$ finds the cross-over time $t_*^g = (3.4 \pm 1.0) \times 10^6$ yr, emphasized by the black cross in the plot, within a factor few of Rothman's estimate.

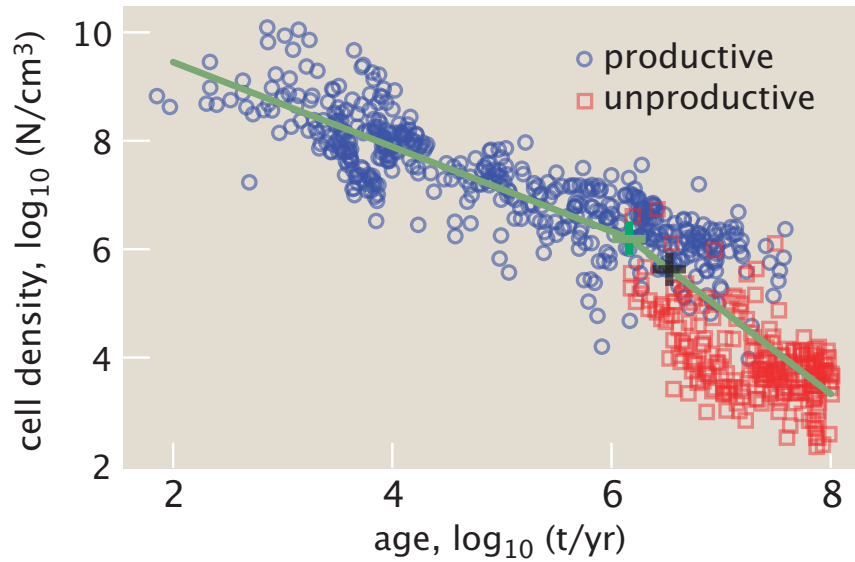


Figure 2.15: Cellular abundance in deep marine sediment versus local carbon age. Measurements from metabolically “productive” and “unproductive” marine sites are colored in blue and red, respectively. A piecewise linear fit to these cell data $n(t)$ alone, indicated by the solid green line, predicts a cross-over time $t_*^n = (1.4 \pm 0.2) \times 10^6$ yr, emphasized by the green cross. For reference, the cross-over time t_*^g inferred earlier from the separate fit to the carbon abundance data $g(t)$ is also displayed, as a black cross.

From organic carbon $g(t)$ and cell abundance $N(t)$ versus age, Rothman approximately takes the cross-over time to be $t_* \sim 1.5 \times 10^6$ yr. We can read off the approximate values of the carbon abundance $g(t_*)$ and the cellular density $N(t_*)$ at the crossover time t_* from plots of data considered by Rothman; Figures 2.14 and 2.15 replot these data, along with our own simple piecewise linear fits to these profiles. With Rothman's original cross-over time $t_* \sim 1.5 \times 10^6$ yr, we observe $g(t_*) \sim 10^{-1}\%$ organic carbon (e.g. a fraction 1/1000th) and $N(t_*) \sim 10^6$ cells/cm³. Finally, we must convert the carbon abundance fraction $g(t_*)$ reported in a mass-percent unit to an actual mass abundance. To do so, we take the density of sediment to be $\rho_s \sim 0.34$ g/cm³, as reported by the original sources of data compiled by Rothman.

Thus,

$$g(t_*) \sim 10^{-3} \frac{\text{g organic carbon}}{\text{g sediment}} \times 0.34 \frac{\text{g sediment}}{\text{cm}^3} \quad (2.94)$$

$$\sim 3.4 \times 10^{-4} \frac{\text{g organic carbon}}{\text{cm}^3}. \quad (2.95)$$

Collectively these values predict,

$$b(t_*) = \frac{\exp[-\gamma]}{1.6 \times 10^6 \text{ yr} \times (\pi \times 10^7 \text{ s/yr})} \frac{10^{-4} \frac{\text{g organic carbon}}{\text{cm}^3}}{10^6 \text{ cells/cm}^3} \quad (2.96)$$

$$\sim 10^{-24} \text{ g organic carbon/cell/s} \quad (2.97)$$

$$\sim 3 \times 10^{-3} \text{ organic carbon molecules/cell/s.} \quad (2.98)$$

Clearly this is a very small uptake rate—it says that a cell waits an average of over five minutes between each uptake of an organic molecule.

Complete oxidation of one gram of glucose yields 1.6×10^4 J of energy; since $k_B T \sim$

$4.1 \times 10^{-21} J$ and $1 \text{ ATP} \sim 20k_B T \sim 8 \times 10^{-20} J$,

$$b(t_*) \sim (10^{-24} \text{ g organic carbon/cell/s}) (1.6 \times 10^4 J/(g \text{ C})) \left(\frac{1 \text{ ATP}}{8 \times 10^{-20} J} \right) \quad (2.99)$$

$$\sim \boxed{0.2 \text{ ATP/cell/s}}. \quad (2.100)$$

This is $\boxed{10^4}$ -fold smaller than the measurement reported by Ciemniecki *et al.* on *Pseudomonas* [6].

We remark that the cross-over age taken above is close to slightly-more-fussily-inferred values. Specifically, we performed separate piecewise linear fits of carbon abundance and cell density to age, where the two allowed slopes are fixed to the theoretically predicted values and the only free parameter is the cross-over age under enforced continuity. On the basis of the carbon abundance data, we found a cross-over time $t_*^g = (3.4 \pm 1.0) \times 10^6 \text{ yr}$, within a factor few of Rothman's cross-over time. On the basis of cell density data, we found $t_*^n = (1.4 \pm 0.2) \times 10^6 \text{ yr}$, essentially the same as Rothman's cross-over time. Thus the cross-over behaviors are indeed likely close to those taken above, within a factor few.

2.5 Outlook

A flurry of foundational discoveries over the past decades have revealed a surprising regularity to how cells act while *growing fast*. Microbial physiology textbooks can now celebrate a set of impressively-general growth laws for the fastest growth rates [47], specific macromolecular compositions [42, 18], and even regulation of size and shape [48] of cells, at least for particular environments and species. This apparent universality in growth invites an optimism for discovering complementary *non-growth* laws that quantitatively account for how cells manage their metabolisms at the precipice of vitality. This setting of cells just surviving in challenging environments has urgent relevance across scales from

single-cell motility [10] or antibiotic persistence, to models of the global atmosphere and carbon cycle [11].

On the other hand, almost from the instant that fixed “maintenance rates” were conceptually and mathematically invoked, microbial physiologists have quarreled about their usefulness or biological interpretability as fixed numbers or mechanistic invariants; see the perspective posed by Tempest & Neijssel [49] forty years ago. In this chapter, to ground such discussion in concrete numbers and learn how they relate to underlying physiological processes, we endeavored an initial tour of how various total basal metabolism values quantitatively and philosophically differ. In many cases, our estimates of underlying physiological processes were separated by orders of magnitude from the purported total metabolic rates of the slowest cells in published data both old and new. These reckonings are approximate and subject to their necessary assumptions, required by a poverty of direct unambiguous measurements on features like membrane permeability, lipid turnover, protein abundance, and beyond specifically in the energy-challenged regimes of interest. Our analysis stress the urgency of more rigorous and cross comparative physiology in the energy-limited regime.

2.6 Acknowledgments

We gratefully acknowledge thoughtful and philosophically generative discussions with Steven Wilbert; Dongyang Li; John Marken; Fang Xiao; and Madhav Mani. We thank members of the Newman and Phillips laboratories for input. We thank the Center for Environmental Microbial Interactions (including a Pilot Grant to GLS) for funding that helped support and catalyze this collaborative work across labs.

Bibliography

- [1] J. Monod. The growth of bacterial cultures. *Annu Rev Microbiol*, 3:371–394, 1949.
- [2] J. Monod. Sur un phénomène nouveau de croissance complexe dans les cultures bactériennes. *Comptes Rendus Des Séances de L'Académie Des Sciences*, 212:934–939, 1941.
- [3] J. Monod. The Phenomenon of Enzymatic Adaptation - and Its Bearings on Problems of Genetics and Cellular Differentiation. *Growth*, 11(4):223–289, 1947.
- [4] Frederick C Neidhardt. Bacterial growth: constant obsession with dn/dt . *Journal of Bacteriology*, 181(24):7405–7408, 1999.
- [5] Øivind Tøien, John Blake, Dale M Edgar, Dennis A Grahn, H Craig Heller, and Brian M Barnes. Hibernation in black bears: independence of metabolic suppression from body temperature. *Science*, 331(6019):906–909, 2011.
- [6] John A Ciemniecki, Chia-Lun Ho, Richard D Horak, Akihiro Okamoto, and Dianne K Newman. Mechanistic study of a low-power bacterial maintenance state using high-throughput electrochemistry. *Cell*, 187(24):6882–6895, 2024.
- [7] Tori M Hoehler, Dylan J Mankel, Peter R Girguis, Thomas M McCollom, Nancy Y Kiang, and Bo Barker Jørgensen. The metabolic rate of the biosphere and its components. *Proceedings of the National Academy of Sciences*, 120(25):e2303764120, 2023.
- [8] Severin Schink, Mark Polk, Edward Athaide, Avik Mukherjee, Constantin Ammar, Xili Liu, Seungeun Oh, Yu-Fang Chang, and Markus Basan. Survival dynamics of starving bacteria are determined by ion homeostasis that maintains plasmolysis. *Nature Physics*, 20(8):1332–1338, 2024.
- [9] Yijie Deng, Douglas Raymond Beahm, Steven Ionov, and Rahul Sarpeshkar. Measuring and modeling energy and power consumption in living microbial cells with a synthetic atp reporter. *BMC biology*, 19(1):101, 2021.
- [10] Tori M Hoehler and Bo Barker Jørgensen. Microbial life under extreme energy limitation. *Nature Reviews Microbiology*, 11(2):83–94, 2013.
- [11] Daniel H Rothman. Slow closure of earth's carbon cycle. *Proceedings of the National Academy of Sciences*, 121(4):e2310998121, 2024.

- [12] Aaron Novick and Leo Szilard. Description of the chemostat. *Science*, 112(2920):715–716, 1950.
- [13] Aaron Novick and Leo Szilard. Experiments with the chemostat on spontaneous mutations of bacteria. *Proceedings of the National Academy of Sciences*, 36(12):708–719, 1950.
- [14] Aaron Novick and Leo Szilard. Experiments on spontaneous and chemically induced mutations of bacteria growing in the chemostat. In *Cold Spring Harbor Symposia on Quantitative Biology*, volume 16, pages 337–343. Cold Spring Harbor Laboratory Press, 1951.
- [15] S. J. Pirt. The maintenance energy of bacteria in growing cultures. *Proc R Soc Lond B Biol Sci*, 163(991):224–31, 1965.
- [16] Michael Lynch and Georgi K Marinov. The bioenergetic costs of a gene. *Proceedings of the National Academy of Sciences*, 112(51):15690–15695, 2015.
- [17] Gangsheng Wang, Melanie A Mayes, Lianhong Gu, and Christopher W Schadt. Representation of dormant and active microbial dynamics for ecosystem modeling. *PloS one*, 9(2):e89252, 2014.
- [18] Matthew Scott, Carl W Gunderson, Eduard M Mateescu, Zhongge Zhang, and Terence Hwa. Interdependence of cell growth and gene expression: origins and consequences. *Science*, 330(6007):1099–1102, 2010.
- [19] Severin J Schink, Elena Biselli, Constantin Ammar, and Ulrich Gerland. Death rate of *E. coli* during starvation is set by maintenance cost and biomass recycling. *Cell systems*, 9(1):64–73, 2019.
- [20] John DC Little et al. A proof for the queuing formula $l = \lambda w$. *Operations research*, 9(3):383–387, 1961.
- [21] Karthik P Jayapal, Siguang Sui, Robin J Philp, Yee-Jiun Kok, Miranda GS Yap, Timothy J Griffin, and Wei-Shou Hu. Multitagging proteomic strategy to estimate protein turnover rates in dynamic systems. *Journal of proteome research*, 9(5):2087–2097, 2010.
- [22] Eran Eden, Naama Geva-Zatorsky, Irina Issaeva, Ariel Cohen, Erez Dekel, Tamar Danon, Lydia Cohen, Avi Mayo, and Uri Alon. Proteome half-life dynamics in living human cells. *Science*, 331(6018):764–768, 2011.
- [23] Sanjoy Mahajan. *Street-Fighting Mathematics: The Art of Educated Guessing and Opportunistic Problem Solving*. MIT Press, Cambridge, Mass., 2010. ISBN 9780262514293 (pbk. alk. paper) 026251429X (pbk. alk. paper).
- [24] Rohan Balakrishnan Miriam Abele Zhongge Zhang Chenhao Wu Lance Freiman Eray Enutson Kevin Corbett Christina Ludwig Matteo Mori Harish Kannan, Gabriel Mullin-Manzanarez and Terence Hwa. Bacteria leverage protein degradation to increase energy supply during starvation. APS March Meeting 2025, 2025. URL <https://summit.aps.org/events/MAR-S71/8>.

- [25] George Stephanopoulos, Aristos A Aristidou, and Jens Nielsen. Metabolic engineering: Principles and methodologies. 1998.
- [26] Ron Milo. What is the total number of protein molecules per cell volume? A call to rethink some published values. *Bioessays*, 35(12):1050–1055, 2013.
- [27] James B Russell and Gregory M Cook. Energetics of bacterial growth: balance of anabolic and catabolic reactions. *Microbiological reviews*, 59(1):48–62, 1995.
- [28] TD Lamb. Sources of noise in photoreceptor transduction. *Journal of the Optical Society of America A*, 4(12):2295–2300, 1987.
- [29] Terrence Geiger and S Clarke. Deamidation, isomerization, and racemization at asparaginy and aspartyl residues in peptides. succinimide-linked reactions that contribute to protein degradation. *Journal of Biological Chemistry*, 262(2):785–794, 1987.
- [30] E Shacter, P Boon Chock, and ER Stadtman. Energy consumption in a cyclic phosphorylation/dephosphorylation cascade. *Journal of Biological Chemistry*, 259(19):12260–12264, 1984.
- [31] Ravish Sharma, Tomohiro Shimada, Vinod K Mishra, Suchitra Upreti, and Abhijit A Sardesai. Growth inhibition by external potassium of escherichia coli lacking ptsn (eiantr) is caused by potassium limitation mediated by ycg. *Journal of bacteriology*, 198(13):1868–1882, 2016.
- [32] Rob Phillips. Membranes by the numbers. *Physics of biological membranes*, pages 73–105, 2018.
- [33] Ora Kedem and Aharon Katchalsky. Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. *Biochimica et biophysica Acta*, 27:229–246, 1958.
- [34] P. Atkins and J. de Paula. *Atkins' Physical Chemistry*. OUP Oxford, 9th edition, 2010. ISBN 9780199543373. URL <https://books.google.com/books?id=BV6cAQAQBAJ>.
- [35] Quang Hon Tran and Gottfried Uden. Changes in the proton potential and the cellular energetics of escherichia coli during growth by aerobic and anaerobic respiration or by fermentation. *European journal of biochemistry*, 251(1-2):538–543, 1998.
- [36] Ron Milo, Paul Jorgensen, Uri Moran, Griffin Weber, and Michael Springer. Bionumbers—the database of key numbers in molecular and cell biology. *Nucleic acids research*, 38(suppl_1):D750–D753, 2010.
- [37] Christoph Kaleta, Sascha Schäuble, Ursula Rinas, and Stefan Schuster. Metabolic costs of amino acid and protein production in escherichia coli. *Biotechnology journal*, 8(9):1105–1114, 2013.
- [38] Ian S Farmer and Colin W Jones. The energetics of *Escherichia coli* during aerobic growth in continuous culture. *European journal of biochemistry*, 67(1):115–122, 1976.
- [39] R. Milo and R. Phillips. *Cell Biology by the Numbers*. Garland Press, New York, NY, 2016.
- [40] Jørn Hess Thaysen and Niels A Thorn. Excretion of urea, sodium, potassium and chloride in human tears. *American Journal of Physiology-Legacy Content*, 178(1):160–164, 1954.

- [41] S Mishima, A Gasset, SD Klyce, and JL Baum. Determination of tear volume and tear flow. *Investigative ophthalmology & visual science*, 5(3):264–276, 1966.
- [42] Griffin Chure and Jonas Cremer. An optimal regulation of fluxes dictates microbial growth in and out of steady state. *Elife*, 12:e84878, 2023.
- [43] Chien-Jung Lo, Mark C Leake, and Richard M Berry. Fluorescence measurement of intracellular sodium concentration in single *Escherichia coli* cells. *Biophysical journal*, 90(1):357–365, 2006.
- [44] Tibor I Matula and Robert A Macleod. Penetration of *Pseudomonas aeruginosa* by sodium chloride and its relation to the mechanism of optical effects. *Journal of Bacteriology*, 100(1):411–416, 1969.
- [45] Graeme Lowe, Markus Meister, and Howard C Berg. Rapid rotation of flagellar bundles in swimming bacteria. *Nature*, 325(6105):637–640, 1987.
- [46] Howard C Berg. The rotary motor of bacterial flagella. *Annual review of biochemistry*, 72(1):19–54, 2003.
- [47] Markus Basan, Sheng Hui, Hiroyuki Okano, Zhongge Zhang, Yang Shen, James R Williamson, and Terence Hwa. Overflow metabolism in *Escherichia coli* results from efficient proteome allocation. *Nature*, 528(7580):99–104, 2015.
- [48] Leigh K Harris and Julie A Theriot. Relative rates of surface and volume synthesis set bacterial cell size. *Cell*, 165(6):1479–1492, 2016.
- [49] David W Tempest and Oense M Neijssel. The status of yatp and maintenance energy as biologically interpretable phenomena. *Annual Review of Microbiology*, 38(1):459–513, 1984.