Physical bioenergetics: Energy fluxes, budgets, and constraints in cells

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Cells are the basic units of all living matter which harness the flow of energy to drive the processes of life. While the biochemical networks involved in energy transduction are well-characterized, the energetic costs and constraints for specific cellular processes remain largely unknown. In particular, what are the energy budgets of cells? What are the constraints and limits energy flows impose on cellular processes? Do cells operate near these limits, and if so how do energetic constraints impact cellular functions? Physics has provided many tools to study nonequilibrium systems and to define physical limits, but applying these tools to cell biology remains a challenge. Physical bioenergetics, which resides at the interface of nonequilibrium physics, energy metabolism, and cell biology, seeks to understand how much energy cells are using, how they partition this energy between different cellular processes, and the associated energetic constraints. Here we review recent advances and discuss open questions and challenges in physical bioenergetics.

physical bioenergetics | energy fluxes | energetic costs | energetic constraints

Cells function out of thermodynamic equilibrium: They use metabolic pathways to transform matter and energy into the building blocks of cellular components and consume Gibbs energy to power cellular processes. Despite detailed knowledge about the biochemistry and cell biology of biosynthesis and energy metabolism (1–3), much less is known about the energetic costs and constraints of cellular processes.

The flow of energy through cells arises from the conversion of Gibbs energy derived from the environment. Rates of energy flows are characterized by energy fluxes, which can be used to quantify energetic costs. These energy fluxes can also impose constraints on cellular processes ranging from growth (4) to activities of molecular motors (5–7) to cellular information processing (8–10). Physical bioenergetics sits at the interface between nonequilibrium physics, energy metabolism, and cell biology. It seeks to understand how much energy cells are using, how they partition this energy into different cellular processes, and the associated energetic

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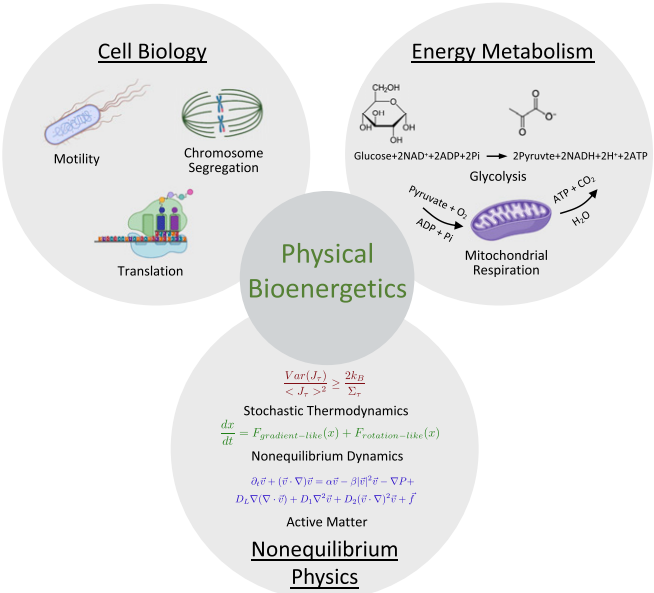

Fig. 1. Physical bioenergetics resides at the interface of cell biology, energy metabolism, and nonequilibrium physics and seeks to reveal the energetic costs and constraints in cells. It combines insights from each field to understand how much energy cells use, how they partition this energy into different cellular processes, and the associated energetic constraints.

constraints (Fig. 1). In contrast to traditional bioenergetic research (11), physical bioenergetics studies energetic costs and constraints in cells by leveraging tools from nonequilibrium physics. Applying these tools to cell biology is associated with many challenges. Energy fluxes, such as the flux of adenosine 5′-triphosphate (ATP), couple many cellular processes, making it difficult to account for all the biochemical reactions involved in ATP production and consumption. While this difficulty could potentially be addressed with a coarse-grained model of ATP turnover, it is unclear how to perform coarse graining of complex biochemical networks in a systematic way. Active-matter physics has provided insights into the emergent dynamics of cellular structures, but energy fluxes that power these dynamics are rarely considered explicitly (12–16). Stochastic thermodynamics has provided lower bounds on entropy production and energy dissipation of cellular processes, but it is unknown how close to these limits cells operate (17–21). Thus, physical bioenergetics presents a challenge to extend theories and approaches developed in nonequilibrium physics to cell biology.

In this paper, we will review and discuss three important open questions in physical bioenergetics and demonstrate the recent progress and emerging challenges. First, what are the energy fluxes in cells, and how can they be measured? Second, what are the energetic costs of key cellular processes? Finally, to what extent do energy fluxes constrain cellular processes? We will address these questions with illustrative examples and highlight how the addition of physics and physical approaches could help answer these questions (Fig. 1).

Open Question: What Are the Energy Fluxes in Cells?

One of the defining features of life is the exchange of energy with the environment, characterized by a net flow of energy. In cells, these energy flows arise from the conversion of Gibbs energy derived from the environment. This energy is transformed into an intermediate form (e.g., ATP) before being converted to biomass, heat, or wastes (Fig. 2). The energetic costs of cellular processes can be quantified by the rates of energy flows, or energy fluxes, through these processes (22). Energy fluxes can also impose constraints on cellular processes, for example by limiting the growth rate of the cell (4) or inducing speed–accuracy trade-offs on cellular information processing (8–10). To reveal energetic costs and constraints in cells, it is crucial to measure energy fluxes and their variations. In this section, we will review techniques to measure energy fluxes both at the global level and through specific metabolic pathways and will discuss the strengths, limitations, and associated challenges of these techniques.

The global energy flux can be quantified by measuring net fluxes through the cell, e.g. through measurements of the rate of oxygen consumption or heat production. Intracellular energy fluxes through specific pathways, including those that produce and consume ATP, can be quantified by measuring the fluxes of metabolites. Energy fluxes can be dynamic, e.g. for cells progressing through the cell cycle (23, 24) or during development (25–29), or constant, e.g. for cells at steady state such as oocytes arrested at meiosis II (30). Spatial effects, such as the diffusion and transport of metabolites or energy-producing organelles, also shape energy fluxes through the cell (31–34). Because the dynamics of energy fluxes are sensitive to changes in cellular energy usage, they provide an important readout of the energy expenditures of the cell (23, 24, 27, 29).

Decades of research have provided a remarkable body of detailed information about the metabolic pathways which convert energy from the environment to forms usable for cellular processes (1, 2). Although the enzymology of these pathways has been well characterized, it is often unclear how much energy cells use and how this energy is partitioned into different cellular processes. One component of the global energy flux is the heat flux. A significant number of studies have measured the heat flux through cells, namely the rate at which cells dissipate energy in the form of heat generated through all cellular activities. Heat generation, which can be expressed in units of power in watts or joules per second, contains contributions from all of the biochemical reactions happening in the cell. This global heat flux represents the enthalpic part of the global energy flux and can be measured using calorimetry (23, 24, 27, 35–40). For aerobic organisms, another component of the global energy flux is the

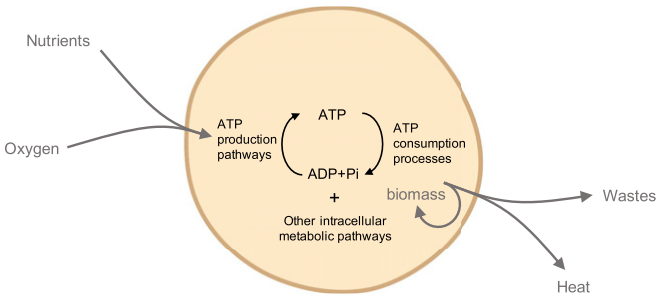

Fig. 2. Schematic of typical cellular chemical and energy fluxes. Energy fluxes represent the conversion rate of Gibbs energy derived from the environment, e.g. from nutrients, through cellular activities to waste products, biomass, or heat. Gray arrows represent the global energy flux through the cell. The enthalpic part of the global energy flux can be measured as the heat production rate of the cell. For aerobic organisms, measurements of the OCR can be related to the rate of heat production through Thornton's rule (46). Intracellular energy fluxes are represented by the fluxes through specific metabolic pathways, such as those that produce and consume ATP.

oxygen consumption rate (OCR), measured using respirometry (41–43). OCR measurements can be interpreted as the global heat flux under conditions where all energy is derived through respiration, and when nonrespiration-related oxygen consumption is negligible (44, 45). Because the net chemical reaction for respiration is equivalent to combustion, when the above conditions are met the OCR is proportional to the amount of heat released, known as Thornton's rule (46). For this reason, OCR measurements are sometimes referred to as indirect calorimetry.

A limitation of both calorimetry and OCR is that they are both global measurements with contributions from many metabolic pathways, making it challenging to measure the energy flux through a particular cellular process. These global measurements provide only an indirect readout of intracellular energy fluxes. To understand intracellular energy fluxes, it is crucial to measure the metabolic fluxes through specific pathways. Biochemical techniques such as radioactive isotope ^{14}C labeling have been applied to measure such fluxes, for example the carbon flux during photosynthesis (47). ^{13}C metabolic flux analysis using mass spectrometry is the predominant technique used to measure intracellular fluxes (48). However, these techniques involve destructive sampling, limiting live-cell measurements. It therefore remains a challenge to measure intracellular fluxes in living cells.

Additionally, almost all measurements of metabolic fluxes are done on populations of cells, providing a measure of the average metabolic fluxes, obscuring the inherent variations at the individual cell level. Microscopy-based single-cell measurements have led to insights into the mechanisms of stochastic cellular growth (49, 50) and revealed oscillatory metabolic dynamics (51), demonstrating the importance of single-cell measurements in revealing metabolic heterogeneity hidden on a population level.

Spatial effects may also play a role in shaping intracellular energy fluxes. In developing embryos, gradients of glycolytic activity have been linked to cell differentiation (52, 53). Mitochondria, key energy-generating organelles, have been observed to be associated with the cytoskeleton (31) and endoplasmic reticulum (32) and display intracellular heterogeneities in membrane potential (54) and mitochondrial DNA sequence (55), implying the potential existence of complex intracellular patterning of energy flows. Glycolytic enzymes also associate with the actin cytoskeleton, allowing cells to couple glycolytic flux with changes in the mechanical environment of the cell (56). While the subcellular distributions of metabolite concentrations including ATP (57), reduced nicotinamide adenine dinucleotide (NADH) (58), and glucose (59) have been characterized with fluorescence microscopy (57–64), it remains a challenge to measure the fluxes of these metabolites with subcellular resolution. Consequently, the spatial patterning of energy fluxes in cells is largely unknown. Mammalian oocytes provide model systems where subcellular spatial patterning of energy fluxes may be present (30, 33, 34). Due to their relatively large sizes, a substantial intracellular gradient of adenosine 5′-diphosphate (ADP) could develop due to localized ATP consumption, for example by the spindle (Fig. 3*A*). Mitochondria have been observed to surround the meiotic spindle, suggesting ATP production could be spatially distributed (33, 34). ATP (57) and ATP:ADP (60) biosensors provide powerful tools that can be used to measure the spatial gradients of these metabolites. The relative rates of localized ATP production and consumption could be inferred by combining such measurements with theoretical reaction–diffusion modeling of ATP and ADP. Such an approach could also potentially be used to estimate how much energy the localized process is using.

Developing new techniques capable of measuring metabolic fluxes of specific pathways with single-cell or even subcellular resolution would help reveal the dynamics of energy fluxes in cells. Substantial recent progress has been made in this direction. An assay based on the coarsening of emulsion droplets has been developed to monitor the glucose uptake rate of single yeast cells (65). Raman spectroscopy is a potential candidate that can allow for spatiotemporal inhomogeneities in nutrient uptake rate to be measured with single-cell resolution (66). This can be done, for example, using glucose analogs displaying spectral peaks in the silent region of the cell (67). Empirical or model-based inference approaches are potential methods to obtain metabolic fluxes with subcellular resolution from fluorescence imaging of metabolite concentrations (30, 64, 68). A model based on NADH redox reactions has been used to infer mitochondrial metabolic fluxes from fluorescence lifetime imaging of NADH with subcellular resolution (30).

Taken together, while powerful techniques have been developed to measure energy fluxes, limitations exist for measuring the dynamic changes and spatial heterogeneity of energy fluxes. As a result, the spatiotemporal dynamics of energy fluxes, both between and within cells, remain largely unknown. To bridge this gap, techniques to measure energy fluxes with high spatiotemporal resolution are needed.

Open Question: What Are the Energetic Costs of Key Cellular Processes?

A myriad of cellular processes require energy, frequently in the form of ATP. While we have a reasonably good understanding of how cells generate energy through central metabolism (1, 2), the energetic costs of specific cellular processes are less known (69–71). Specific cellular processes carry associated energetic costs. These costs can be quantified by the rate of Gibbs energy change. One useful way to quantify Gibbs energy change is to count the ATP equivalents, i.e., the Gibbs energy change per ATP hydrolysis, consumed per unit time by the cellular process. Alternatively, since the energy fluxes through all cellular processes sum up to the global energy flux of the cell, energic cost of a specific cellular process can be quantified by the fraction of the global energy flux associated with that process. Cellular energetic costs include biosynthesis (72), signaling (23, 24, 73, 74), maintaining chemical gradients (75), error correction (8, 9), motility (75), gene regulation (76, 77), and building of cellular structures, such as the cytoskeleton (22). Fig. 3 provides three examples of cellular processes that contribute to the energy budget of cells: spindle self-organization and chromosome segregation in a mammalian oocyte, protein synthesis in growing cells, and sensory adaptation in chemotactic *Escherichia coli*. An open challenge is to measure the energetic costs of such cellular processes. An accounting of cellular energy tells us not only about the known processes but also about the processes that we could be overlooking. For example, the KaiABC system serves as a cyanobacteria circadian oscillator, which keeps time through a sequence of phosphorylation reactions on hexameric KaiC. While each phosphorylation–dephosphorylation cycle is known to take 2 ATP per monomer, the experimentally measured ATP consumption rate is significantly higher at 16 ATP per monomer. A recent theoretical study suggests that this excess energy is not simply wasted—it may be responsible for synchronizing the state between KaiABC complexes, allowing for a global synchronization between the clocks (78).

The energetic costs of cellular processes can be estimated through counting of ATP equivalents consumed per unit time by

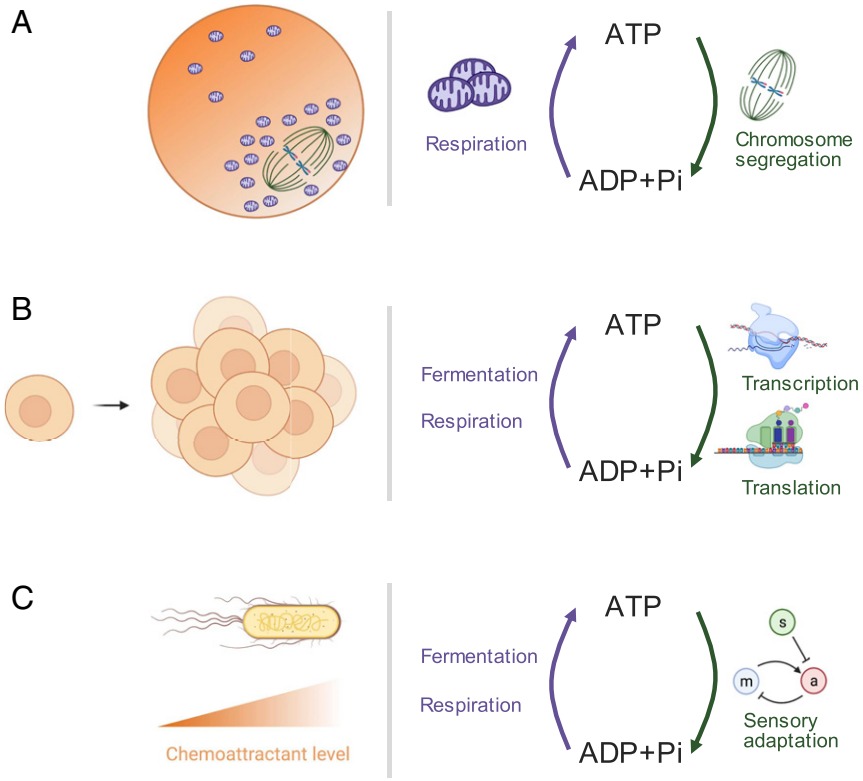

Fig. 3. Examples of cellular processes that contribute to the cellular energy budget. (*A*) Spindle self-organization and chromosome segregation in a mammalian oocyte. (*B*) Protein synthesis with transcription and translation in growing cells. (*C*) Sensory adaptation in chemotactic *E. coli*. s, a, and m represent input, output, and controller, respectively, in the adaptive feedback network (10).

each of the intermediate steps associated with the process. This approach has been used for estimating building costs by analyzing biosynthetic pathways, summing up the number of ATP equivalents required for each intermediate step (27, 69, 70). The counting of ATP equivalents for each step can then be combined with measurements of the step's rate to estimate the overall rate of energy consumption for building the component. As an example, counting of ATP equivalents has been applied to estimate the energetic cost of protein synthesis in growing cells by summing up the number of ATP equivalents required for each intermediate step, including amino acid synthesis, transcription, and translation (69–72, 79) (Fig. 3*B*). This counting of ATP equivalents can then be combined with measurements of the rate of protein synthesis to estimate the rate of energy consumption associated with protein synthesis. This rate can further be compared with the global energy flux of the cell to infer the relative energetic cost of protein synthesis. These estimates heavily rely on knowing all the steps involved in the process, their rates, and the corresponding number of ATP equivalents required. For some less-characterized cellular processes it can be challenging to know all of the coupled steps. For example, a full determination of the energetic costs of chromosome segregation in eukaryotic cells would need to include the energetic costs of assembling the spindle, moving chromosomes, signaling, and error correction, among others (Fig. 3*A*). In the absence of detailed knowledge about all of the coupled processes underlying the process of interest, it is difficult to estimate the energetic costs of the process. Therefore, to test if estimates from ATP counting fully account for the energetic costs, direct measurements are needed.

One approach to directly measure the energetic cost of a process is through process inhibition. Assuming that inhibiting one target process does not affect the activities of other cellular processes, the energetic cost of the target process can be measured as the change of the global energy flux using respirometry or calorimetry when the targeted process is inhibited (22). However, inhibition experiments always carry the potential for off-target effects and can change the activity of other processes coupled to the targeted one (80). As a change in the global energy flux reflects changes in all the processes affected, a coupling between the targeted process and other cellular processes could potentially complicate the interpretation of such measurements. For example, inhibiting a process that represents a significant fraction of the cell's ATP consumption rate could change the ATP concentration, which could in turn change the activities of other ATP-consuming pathways. In this case, the measured change in the global energy flux could include additional contributions from these downstream effects in addition to the contribution from the inhibited process. Moreover, global energy flux may not be uniquely determined by the energetic demand imposed by ATP users but also depends on the rates of ATP synthesis and proton leak in mitochondria (81). Hence, understanding how ATP synthesis and proton leak are modulated by the inhibition is required to estimate energetic costs from such experiments. Models of metabolic control are required to understand the coupling of ATP synthesis, proton leak, and ATPases (45). To determine the true energetic cost associated with a given cellular process, a combined approach is required, where the energetic cost is measured using both the counting of ATP equivalents and measuring change of global energy flux following specific process inhibition (80).

Measuring how global energy fluxes change during development also provides information on the energetic costs of cellular

processes. For example, calorimetry has been used to observe that heat production by embryos contains a component which oscillates in synchrony with the cell cycle as a result of cell-cycle signaling during the development of zebrafish (24) and *Xenopus* embryos (23). It remains a challenge to understand what physiological changes during embryo development contribute to the dynamics of energy fluxes (27, 29, 82–84).

The energetic costs considered above represent direct cost, defined as the number of ATP equivalents used by a given cellular process. In some situations, e.g. for processes that generate biomass, indirect opportunity costs may be relevant. Opportunity costs represent the ATP that could have been generated from metabolic precursors or reducing intermediates had they been used to generate ATP instead of biomass (70). While opportunity costs are not direct energetic costs they could have consequences for evolutionary fitness, particularly in situations where carbon is limiting, and thus the consequences for diverting carbon to biomass are more acute. Understanding how to relate energetic costs with fitness costs is an important open question connecting physical bioenergetics with evolutionary cell biology (69, 70, 85–88).

Overall, while there is promising work in estimating the energetic costs of cellular processes (22, 69, 70, 78), the energy budget of cells remains largely unexplored. There are challenges associated with estimating energetic costs from ATP counting and process inhibition. Overcoming these challenges requires understanding the intermediate steps associated with the process of interest, and the coupling between ATP production and ATP consumption.

Open Question: To What Extent Do Energy Fluxes Constrain Cellular Processes?

Cellular processes are powered by the dissipation of Gibbs energy, and the rate of energy dissipation can impose constraints on cellular processes. These constraints manifest in the limit of cell growth rate (4) (Fig. 4*A*), the efficiency of molecular motors (5–7) (Fig. 4*B*), and the trade-off between speed and accuracy in cellular information processing (8–10) (Fig. 4*C*). In this section, we will discuss these examples in detail and demonstrate how tools from nonequilibrium physics have been applied to understand energetic constraints.

Intrinsic limits on the Gibbs energy dissipation rate could impose constraints on cell growth rate. It has recently been suggested that the transition from respiration to fermentation at high glucose uptake rates is caused by an upper limit in the cellular Gibbs energy dissipation rate and that the maximal cellular growth rate is determined by this limit (4) (Fig. 4*A*). Alternative explanations for this phenomenon include electron-transport-chain enzymes reaching saturating concentrations in the cell membrane (89, 90) and efficient proteome allocation (91). It remains a challenge to reconcile these different explanations.

Stochastic thermodynamics theories have been used to predict limits on the thermodynamic efficiency of molecular motors. Thermodynamic efficiency is defined as the useful energy dissipation rate, such as mechanical power, divided by the total energy dissipation rate, such as that from ATP hydrolysis. Thermodynamic uncertainty relations reveal that energy dissipations constrain current fluctuations at steady state for nonequilibrium systems (19, 92–94). Applying this relation to molecular motors working against an external force or torque, such as moving cargo in a viscous environment, results in the prediction of a universal upper bound to thermodynamic efficiency (95) (Fig. 4*B*). The Harada–Sasa equality, which relates the energy dissipation rate to the extent of the violation of the fluctuation–response relation, has been used to estimate the efficiency of single kinesin motors as ~20% (5). In contrast, the rotary motor F1-ATPase has been suggested to operate near 100% efficiency (6, 7). A candidate explanation for the stark contrast between the efficiencies of these two motors invokes reversibility of their kinetics (5). While both motors consume ATP when taking a forward step, F1-ATPase can synthesize ATP during backward rotations. As such, and in contrast to kinesin, the energy for these backward steps can be conserved. Understanding the evolutionary pressures on motor efficiencies is an active area of research (96).

Nonequilibrium physics theories have also predicted trade-offs in biochemical circuits constrained by the energy dissipation rate. In the context of processes such as DNA replication, the amount of Gibbs energy provided by nucleotides sets a trade-off between replication speed, accuracy of copies, and energy dissipation (9). Kinetic proofreading is a mechanism for error correction in biochemical reactions at the cost of energy expenditure and is important for quality control and accuracy in many cellular processes (8, 97). Keeping track of energy expenditure and understanding how energy dissipation contributes to enhance sensitivity and increase speed and/or accuracy of information processing in cells is both an experimental and a theoretical challenge (8, 10, 74, 76, 77, 98–103). New insights on the energy–performance trade-off in biochemical networks have enabled identification of useful design principles for biological networks to achieve their targeted functions efficiently (78, 104, 105). For example, Gibbs energy dissipation is necessary during chemosensory adaptation in *E. coli*,

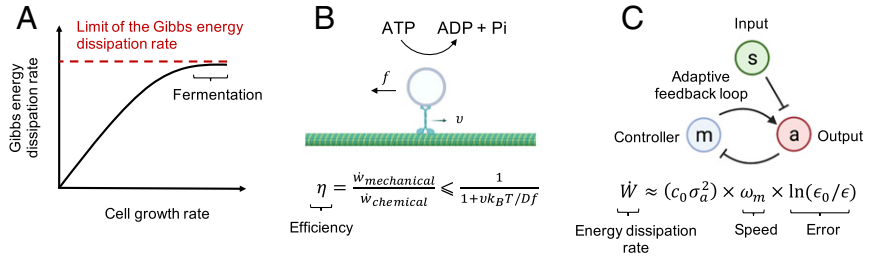

Fig. 4. Examples of limits and constraints imposed on cellular processes by energy fluxes. (*A*) A limit of Gibbs energy dissipation rate has been proposed for growing *E. coli* and yeast. In this model, cells switch from respiration to fermentation when the rate of Gibbs energy dissipation nears this limit and the maximal cell growth rate is determined by this limit (4). (*B*) Thermodynamic uncertainty relations predict an upper limit for the energetic efficiency of molecular motors (95). *v*, *f*, *D*, k_B, and *T* represent velocity, force, diffusion constant, Boltzmann constant, and temperature, respectively. (*C*), A coarse-grained adaptive feedback model predicts an energy–speed–accuracy trade-off during sensory adaptation, including during bacterial chemotaxis, yeast osmosensing, olfactory adaptation, and rhodopsin adaptation (10). c_0 and ε_0 are system-dependent constants, σ_a^2 is the variance of the activity "a" fluctuation, ω_m is the adaptation speed, and ε is the adaptation error.

where energy must be spent in order to adapt to changing concentrations of chemoattractants while maintaining sensitivity of the chemoreceptors (Fig. 3*C*). Coarse-grained models of the feedback network predict a general energy–speed–accuracy trade-off relation in sensory adaptation. This model predicts a decrease of adaptation speed without compromising adaptation accuracy in starving *E. coli*, which has been confirmed experimentally (10) (Fig. 4*C*).

These examples highlight the role energetic constraints can play in understanding the limits of cellular growth rates, the efficiency of molecular motors, and the speed–accuracy trade-off in cellular information processing while opening further questions. To what extent do energetic constraints exist for other cellular processes? If an energetic constraint exists for a given process, do cells operate near this limit? Addressing these questions will require the pairing of quantitative measurements and the development of new theories based on nonequilibrium physics. Recent advances in nonequilibrium dynamics (106–109) and stochastic thermodynamics (17–21) have provided tools to study nonequilibrium processes and define physical limits. Such tools have helped quantify entropy production (110–115), energy dissipation (18, 19, 92, 116, 117), work (118, 119), and free energy transduction in nonequilibrium systems (120). Applying thermodynamic principles to metabolic networks has resulted in the discovery of energetic constraints on metabolic fluxes (4, 121–123). New experimental and theoretical approaches have been developed to identify nonequilibrium dynamics in biological systems by quantifying the breaking of detailed balance (124–126) and irreversibility (127, 128). The violation of the fluctuation–dissipation relation has also been used to quantify energy dissipation at the single-molecular level (5). It remains a challenge to apply these theories to cell biology to understand the reciprocal relationship between energy dissipation and cellular functions (129–136).

Conclusions and Outlook: How Nonequilibrium Physics Can Shed Light on These Questions

Physical bioenergetics aims to reveal energetic costs and constraints in cells, leveraging tools from nonequilibrium physics and applying them to open questions in cell biology and energy metabolism. The central themes of physical bioenergetics are to understand how much energy cells use, how they partition this energy into different cellular processes, and the associated energetic constraints. New technologies are being developed to study energy fluxes with single-cell and subcellular resolution (30, 57, 59–64, 67, 137), which make it possible to study the correlation between energy fluxes and subcellular processes. Nonequilibrium physics provides new tools to interpret these measurements in terms of dynamics (106–108), entropy production (21, 111, 112, 115), energy dissipation (18, 19, 92, 116, 117) and their correlation with emergent dynamics of cellular structures (13, 14, 124, 126, 128).

Despite these recent advances, challenges and opportunities remain in physical bioenergetics. Experimentalists and theorists face the challenge to understand how cells use energy to build cellular structures and drive cellular processes. How do we most usefully define and measure spatiotemporal energy fluxes and connect it to physical quantities such as efficiency, enthalpy, entropy, and Gibbs energy dissipation on the cellular and subcellular scale? Do we have sufficient quantitative knowledge about the biochemistry and rates of cellular processes in order to make calculations about their energy usage and their magnitude within a cellular energy budget? What are the constraints and trade-offs imposed by the energy dissipation rate on cellular processes and what are the resulting consequences on cellular functions?

Nonequilibrium physics, in combination with approaches from biochemistry and cell biology, provides additional tools that hold the promise of addressing these questions. Applying these tools to cellular systems is an open challenge for both experimentalists and theorists which will require the extension of existing physical theories and development of new experimental approaches. Physical bioenergetics provides a testing ground for these theories and will inform the development of nonequilibrium physics. For example, applying stochastic thermodynamics to molecular motors has revealed constraints on their efficiencies (5–7) (Fig. 4*B*), but it is unclear if motors operate near this predicted limit in cells. Active-matter physics has helped provide a quantitative framework for the rich spatiotemporal dynamics of cellular structures which arise from energy fluxes, but the energy fluxes themselves are rarely studied directly. Since energy fluxes in cells are not always constant, it is hence important to consider the interplay between the dynamics of energy fluxes and the dynamics of cellular structures. (Fig. 3*A*) (12, 138–140).

Another fundamental challenge to modeling cellular systems lies in systematically developing phenomenological and coarse-grained models for complex metabolic networks. Computational models are frequently constructed to study metabolic networks by incorporating all known relevant metabolic pathways (141–143). While these models have provided many important insights into metabolism, they are usually dependent on a large number of parameters that can be highly context-dependent and are difficult to measure in vivo. Coarse-grained and phenomenological models are potentially useful tools to model metabolic networks with few effective parameters. This approach has proven useful in many fields. For example, in active-matter physics, cytoskeletal dynamics can be well-described by field theories using few phenomenological parameters and without detailed knowledge of the system's microscopic properties (12, 13, 15, 16, 144, 145). Techniques have also been developed to coarse-grain detailed microscopic models to obtain hydrodynamic models for these systems (146–149). This coarse-grained modeling approach has started seeing applications in modeling cellular energy flows (30, 89, 91, 150–154). Such coarse-grained, phenomenological laws can potentially be used to interpret the changes of global energy fluxes as measured by OCR (28, 43) and heat production rate (23, 24) and provide insights into the energy usage of cells.

How do we know if we have satisfactorily answered the open questions proposed in this perspective? It boils down to making quantitative predictions using theoretical models and testing them experimentally in cells. For example, can we predict the energetic cost of specific cellular processes? Can we predict the dynamical behaviors of cellular structures? Can we predict the relevant energetic limits of cellular processes? Can we integrate this information to make testable predictions about the evolution of cellular features? Overall, physical bioenergetics provides an opportunity for scientists across disciplines to bring together recent experimental and theoretical advances and address new questions and challenges arising from this new perspective.

Glossary

- Active-matter physics: Subfield of physics studying systems of interacting entities that are out of equilibrium due to the dissipation of energy at the scale of the system's constituents. Active-matter systems frequently display emergent phenomena, including collective motion and self-organization. In cell biology, one well-studied example of active matter is the cytoskeleton.

- Coarse-grained models: Theoretical or computational models that simulate the behaviors of complex systems using a simplified representation by decreasing the degrees of freedom of the system.
- Efficiency: A fraction between 0 and 1 that describes how much of a total quantity is spent usefully for a given process. Depending on the context, efficiencies can have numerous definitions (5, 6, 42, 99, 120, 155–158). For example, in the context of energy efficiency, one may define an efficiency as the fraction of Gibbs energy per glucose that is used to generate ATP from glycolysis, which is ~50% (155).
- Energetic cost: The amount of energy required by a process. In the cellular context, energetic costs are typically considered in the equivalent number of ATP hydrolyses required to release the same amount of Gibbs energy per unit time or measured as the fraction of global energy flux associated with a certain cellular process.
- Energetic constraints: Limits and trade-offs on the performances of cellular processes induced by the Gibbs energy dissipation rate or other energetic factors. Examples include the limitation on the cell growth rate, the efficiencies of molecular motors, and the energy–speed–accuracy trade-off of cellular information processing.
- Energy dissipation: A process in which energy is transformed from an initial form to a final form, and the capacity of the final form to do work is less than that of the initial form.
- Energy flow: The conversion of energy from one form to another. In cells, the global energy flow frequently represents the conversion of Gibbs energy from the nutrients through cellular activities to biomass or heat, through intermediate metabolic steps such as the production and consumption of ATP.
- Energy flux: The rate of energy flow. For cells, measurements of energy fluxes include global fluxes such as the rates of oxygen consumption and heat production and fluxes through specific metabolic pathways.
- Energy metabolism: The sum of the processes used by cells to convert energy from one form to another, such as ATP, that is more available for cellular machinery to use. Examples of such processes include glycolysis, respiration, and photosynthesis.
- Enthalpy: Thermodynamic state function characterized by the sum of a system's internal energy and the product of pressure and volume. For a system at constant pressure that is not exchanging mass, changes in the system's enthalpy correspond with the quantity of heat released ($\Delta H > 0$) or absorbed ($\Delta H < 0$) by the system.
- Entropy: Thermodynamic quantity associated with the irreversibility of a process. For an isothermal system, the entropy production is related to the decrease of free energy of the system.
- Gibbs energy: Thermodynamic potential that constrains the maximum amount of reversible work that can be extracted from a process at a constant temperature and pressure and characterizes how far away a system is from equilibrium. Gibbs energy is also referred to as Gibbs free energy. Changes in Gibbs energy can be related to changes in enthalpy (H) and entropy (S) through the relation $\Delta G = \Delta H - T\Delta S$. In the context of chemical reactions, the difference in Gibbs energy between states characterizes if the reaction is favorable or spontaneous to proceed.
- Global energy flux: The rate at which cells convert energy through all cellular activities.
- Intracellular metabolic fluxes: The rates of turnover of molecules through specific metabolic pathways inside the cell.
- Nonequilibrium physics: Subfield of physics studying systems that are not in thermodynamic equilibrium. At thermodynamic equilibrium, there are no net macroscopic flows of energy and matter within the system or between systems.
- Stochastic thermodynamics: Subfield of physics studying the fluctuations and distributions of thermodynamic quantities, including work, entropy production, and energy dissipation, in small systems such as biopolymers, enzymes, and molecular motors. One of its applications in physical bioenergetics is to study the dynamics of molecular motors.

Data Availability. There are no data underlying this work.

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