

New and Notable

A Little Engine That Could: ATP-Powered Electrical Battery and Heater Inside Cells

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Statistical equilibrium thermodynamics has always been the theoretical foundation of molecular biophysics, the subdiscipline within biophysics that studies biological macromolecules (1). In addition, it is well understood, from a conceptual level, that living biological processes operate in non-equilibrium settings (2,3). In fact, one of the most important characteristics of a living organism is its ability to convert chemical energy into mechanical work, and in higher-level organisms, even to generate and deliver information. In the past two decades, our understanding of the former in terms of single molecular motors has advanced greatly due to a collaborative endeavor of biophysicists, applied mathematicians, and physical chemists, in terms of both experiments and theoretical models (without exhaustively listing all the references, see a 2009 Themed Issue of PCCP (4)). As for using energy to generate information, the pioneering work of Hodgkin and Huxley (HH) has already shown that it is the interplay between sustained K^+ and Na^+ concentration gradients and the corresponding movements of these two ions that generate the action potential, a prerequisite of neural communication (5). (The story of voltage-gated membrane channels, another key component of the HH system, is a further triumph of molecular

biophysics (6,7).) Still, the HH theory only describes half of the story that connects chemical energy, e.g., ATP, with the neuronal action potential: The resting potentials, i.e., ionic concentration gradients, are sustained (or, more precisely, generated) by the Na^+/K^+ -ATPase pump.

Single-molecule ATPase pumps, e.g., the Na^+/K^+ -ATPase or the sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA), are less understood components of the chemico-mechanico-electrico-info machinery of living organisms. In *Biophysical Journal*, Lervik and colleagues (8) have carried out a *tour de force* thermodynamics analysis of data on ATP hydrolysis and Ca^{2+} transport of SERCA, originally obtained in the 1980s by Caffrey and Feigenson. This study brings their substantial expertise in macroscopic and mesoscopic nonequilibrium thermodynamics, the so-called Dutch school approach, to bear (9–12). Their results show an excellent agreement with the experimentally observed behavior, including the dependence of the pumping flux on the thickness of the supporting lipid bilayer. Thus, an integrative approach to this class of important single-molecule machines seems to be emerging.

Although the successful story of the molecular motor has somewhat paved the way for the development of the study presented here, there are three additional features that are absent in molecular motors. First, statistical dynamics of ions is notoriously difficult, even for the most powerful theoretical physicists. Second, the system is situated in a membrane that is an interface. This could be considered a blessing, as the technology of single-channel recording had been advanced way ahead of single-molecule biophysics in solution; but high-resolution structures for membrane proteins are still a significant challenge to structural biologists (7). And finally, the work described here also reveals a surprising role of temperature gradient! Note that the great success of single-motor-protein bio-

physics is due, to a large extent, to the possibility of sidestepping the nagging issue of temperature gradient in mesoscopic systems, another tough, unyielding subject in theoretical physics.

The electrochemical potential difference is defined as $\Delta G = -e\Delta V + k_B T \ln(c^{in}/c^{out})$, where ΔV is the cross-membrane electrical potential, and the second term is the chemical potential due to ionic concentration difference inside and outside the cell. If $\Delta G = 0$, then $\Delta V = (k_B T/e) \ln(c^{in}/c^{out})$, as is given by the Nernst-Planck equation for electrochemical equilibrium. Therefore, in an isothermal system, the efficiency is simply $\eta = \Delta G \div (\text{ATP hydrolysis free energy}) \times \text{the number of ions pumped per ATP hydrolysis}$. All of this is rather straightforward. However, when thermal energy is involved and a system is nonisothermal, all the above results have to be modified. This is the significance of the nonequilibrium thermodynamics theory (12) in this work.

The thorough thermodynamic analysis has yielded novel insights to electrophysiology. For the first time to our knowledge in biophysics, the authors have estimated that the thermodynamic efficiency of the pump is very low, ~12%, when it is operating under non-electrogenic conditions. This is a very surprising result, especially considering the repeated claims of molecular motors and rotors being highly efficient nanomachines.

The 12% is significantly lower than the previously assumed near-100% efficiency (13–15). The original work of Caffrey and Feigenson has shown, as have later studies (16–18), that the thickness of the lipid membrane modulates the activity of SERCA, with the maximum activity in the bilayer consisting of acyl chains of intermediate length (C_{16} – C_{20} , ~30 Å). One needs to point out that the reconstituted SERCA vesicle system on which the efficiency is estimated is

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different from the native environment of SERCA. Therefore, the SERCA efficiency under native physiological environment requires further investigation, as for any biological study, from in vitro to in vivo. In fact, as already noted by the authors, the Ca^{2+} gradient is another important regulator of pump efficiency, and the experiments of Caffrey and Feigenson were performed under a significantly reduced gradient. It is also conceivable that more ordered membrane bilayer in the reconstituted system causes the pump to be less flexible than in its native environment, thus reducing its efficiency.

Nevertheless, despite the difference in complexity between a model system and the native system, this work provides a strong indication that the assumption thus far that SERCA is able to transport Ca^{2+} with an efficiency close to 100% is likely an overestimate. With a thermodynamic efficiency of 12%, how do we explain the 88% loss? At least some of the loss is accounted for as heat transferred to the surroundings, as previously described by de Meis et al. (19,20), which could mean that SERCA is a thermal regulator. It is not a new idea that SERCA could play a role in nonshivering thermogenesis (e.g., maintaining body temperature); previous studies have shown that the heat loss of the SERCA depends on the tissue, with a greater loss in white muscle and brown adipose tissue than in, e.g., red muscle (20,21).

Thus, the imperfect pump with 12% efficiency is a rather mechanical view of the little engine; maybe as a heater for a biological organism, SERCA does function perfectly well by providing heat energy needed physiologically in a living system. One cannot fully un-

derstand a biological system without reference to its function or, more likely, functions.

Biological thermodynamics is now fully entering a nonequilibrium era in which active biological processes are addressed. This is significant progress in the biophysics of living systems. A complete understanding of the meaning of efficiency, however, requires the functional context of the biological system(s). Thermodynamic energy is responsible not only for mechanical and physiochemical movements, but also for cellular signaling and information processing (22).

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