Adiabatic operation of a molecular machine

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Operation of a molecular machine is often thought of as a “far from equilibrium” process in which energy released by some high free energy fuel molecule or by light is used to drive a nonequilibrium “power stroke” to do work on the environment. Here we discuss how a molecular machine can be operated arbitrarily close to chemical equilibrium and still perform significant work at an appreciable rate: micrometer per second velocities against piconewton loads. As a specific example, we focus on a motor based on a three-ring catenane similar to that discussed by Leigh [Leigh DA, Wong JKY, Dehez F, Zerbetto F (2003) Nature 424:174–179]. The machine moves through its working cycle under the influence of external modulation of the energies of the states, where the modulation is carried out slowly enough that the state probabilities obey a Boltzmann equilibrium distribution at every instant. The mechanism can be understood in terms of the geometric phase [Berry MV (1990) Phys Today 43(12):34–40] in which the system moves adiabatically around a closed loop in parameter space, completing, on average, nearly one-half mechanical cycle each time it does so. Because the system is very close to equilibrium at every instant, the efficiency can approach 100%.

There has been great recent progress in constructing molecular machines from complexes held together by mechanical rather than chemical bonds (1–3). Structures with fascinating topologies have been created (4) and combined to assemble intricate devices, including molecular “elevators” (5) and nanoscale switchable valves (6). Perhaps the greatest interest in these mechanically linked molecules lies in the fact that they can be engineered to have large-scale motions controllable through external changes in the environment. This feature facilitates the design of molecular motors (7, 8) and rotors (9), devices that can be caused to undergo directional motion by an external source of chemical (10), optical (11), or electrical (12) energy. Control is achieved by incorporating two or more recognition stations (sites where the components of the complex interact strongly) to define several intracomplex binding sites (13, 14) and, hence, several configurational states of the molecule. The relative thermodynamic stability of the different sites (and hence states) is controlled by, e.g., protonation/deprotonation, reduction/oxidation, or absorption of light. By changing the pH, redox potential, or light intensity, the molecule can be switched from one state to another externally.

Recently catenanes—structures with two or more interlocked rings—have been designed that allow external control not only of the relative stabilities of the binding sites but also of the relative abilities of the pathways linking the sites (10). By carrying out a sequence of external cyclical changes to theabilities and stabilities of the sites, the rings could be squeezed to undergo directional rotation by a mechanism similar to peristalsis (15), except that the transitions occur by thermal noise so the system operates as a Brownian motor (16).

Here I will describe how a molecular machine can be operated arbitrarily close to chemical equilibrium at every instant and still do significant work at an appreciable rate. Consider the three-ring catenane shown in Fig. 1. The larger gray ring has three distinct recognition stations, labeled 1, 2, and 3, for the two identical yellow rings. The yellow rings cannot pass one another, nor can they occupy the same station, because they make thermally activated transitions from one station to another. Thus, there are a total of three states, labeled A, B, and C. The interaction between a yellow ring with a station is characterized by an interaction energy, \( E_1, E_2, E_3 \leq 0 \). So the total energy of each state is some constant \( K \), which depends on the three rings independent of one another, plus the two interaction energies; i.e., \( E_A = K + E_1 + E_3, E_B = K + E_2 + E_3, \) and \( E_C = K + E_2 + E_3 \). The diagram is identical to that used by Onsager in describing the connection between chemical “detailed balance” and his reciprocal relations for coupled transport (17). Unlike Leigh’s catenane (18), which has all of the same characteristics as those listed above, it is interesting to consider the behavior of the system if two orthogonally addressable stations are controlled by reversible chemical reactions that use chemical reagents rather than light. Working with units in which the product of the Kelvin temperature and the Boltzmann constant is one (\( k_B T = 1 \)) the equilibrium probabilities for the three states are given by a Boltzmann distribution

\[
P_i = \frac{e^{-E_i}}{Z}; \quad i = A, B, C, \tag{1}
\]

where the equilibrium probability to be in state \( i \) is the average number of molecules in state \( i \) at equilibrium divided by the total number of molecules \( \bar{P}_i = \pi_i / N, i = A, B, C \) and

\[
Z = e^{-E_A} + e^{-E_B} + e^{-E_C} \tag{2}
\]

where \( E_A, E_B, E_C \) are the interaction energies between the yellow rings at the three sites:

\[
E_A = \frac{1}{2} k_B T \left( \ln \left( \frac{N_A}{N_B} \right) + \ln \left( \frac{N_C}{N_A} \right) \right)
\]

\[
E_B = \frac{1}{2} k_B T \left( \ln \left( \frac{N_B}{N_C} \right) + \ln \left( \frac{N_A}{N_B} \right) \right)
\]

\[
E_C = \frac{1}{2} k_B T \left( \ln \left( \frac{N_C}{N_A} \right) + \ln \left( \frac{N_B}{N_C} \right) \right)
\]

\[
N_A = N_B = N_C = N/3
\]

\[
\pi_i = \frac{N_i}{N}
\]

\[
\bar{P}_i = \frac{N_i}{N}
\]

\[
Z = e^{-E_A} + e^{-E_B} + e^{-E_C}
\]

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Fig. 1. Schematic illustration of the possible transitions for a catenane comprising one large ring with three distinct recognition stations for two identical small rings linked to the large ring but not to each other. Energetic conditions strongly favoring each state are indicated.
is the partition function. The equilibrium constants are
\[ K_{ij} = \frac{\tilde{P}_j}{\tilde{P}_i} = \frac{k_{ji}}{k_{ij}} = e^{E_{ji}}, \quad i = A, B, C; \quad j = A, B, C; \quad j \neq i \]

from which we easily derive
\[ k_{ji}\tilde{P}_j - k_{ij}\tilde{P}_i = 0. \]

The relation
\[ k_{AB}k_{BC}k_{CA} = k_{AC}k_{CB}k_{BA}, \]
a corollary of detailed balance (17), holds, irrespective of the values of the energies. Eq. 5 is a necessary but not sufficient condition for detailed balance (19).

Periodic modulation of the energies \( E_i, i = 1, 2, 3 \), even at modulation carried out so slowly that Eq. 1 holds at every instant, can drive directional rotation of the small rings about the larger ring. Consider the sequence of steps beginning with \( E_1, E_3 \ll E_2 \), where the system is almost certainly in state A. First, we slowly increase \( E_1 \) and simultaneously decrease \( E_2 \) to reach the condition \( E_2, E_3 \ll E_1 \). During this process, the yellow ring originally on base 1 will have been transferred by an equilibrium process to base 2 to reach state B. Because the energy \( E_3 \) remains very low, the ring on base 3 will not have moved, so the transfer can only have occurred in the clockwise direction. We then slowly increase \( E_1 \) and simultaneously decrease \( E_3 \), reaching the condition \( E_1, E_2 \ll E_3 \), to transfer the ring on base 3 to base 1, also in the clockwise direction, to arrive at state C. Finally, we slowly increase \( E_2 \) and simultaneously decrease \( E_3 \), reaching the original condition \( E_1, E_3 \ll E_2 \), and transferring the ring on base 2 to base 3 in the clockwise direction, regaining the original state A. The cycling \( A \rightarrow B \rightarrow C \rightarrow A \) is accompanied by clockwise cycling of each yellow ring through the bases in the order 1 \( \rightarrow \) 2 \( \rightarrow \) 3 \( \rightarrow \) 1, with two cycles through the states necessary to give each ring one clockwise turn. Note that the slow, continuous changes in the interaction energies of individual molecules described in this theoretical discussion is almost impossible to accomplish experimentally with chemical systems. Such modulation can, however, be accomplished at the ensemble level by, e.g., very slow titrations of pH and redox potential, as discussed below.

Directional cycling due to a process in which the state probabilities are given by the Boltzmann distribution at every instant may be surprising given that the instantaneous steady state flux \( J_{ij} = k_{ij}\tilde{P}_i - k_{ji}\tilde{P}_j = 0 \) is zero (Eq. 4). However, when the external conditions are caused to change, even very slowly, we must account for the changing state probabilities by adding that fraction, \( F_{ij} \), of the “transient” change in the probability \( d\tilde{P}_i/dt \) coming directly from (to) state \( j \), to the steady state flux (20, 21) to get \( J_{ij} = J_{ij}^0 + F_{ij}d\tilde{P}_j/dt \).

Without loss of generality we focus on the transition \( A \rightleftharpoons B \). For very slow cyclical modulation, the transient term and, hence, the total flux because \( J_{ij}^0 = 0 \), is
\[ F_{AB}^{ad} = \omega \int F_{AB}^{ad}d\tilde{P}_A, \]
where \( \omega \) indicates integration over a cycle of the external modulation and \( \omega \) is the frequency at which the external modulation is carried out. Under adiabatic conditions, the fraction \( F_{AB}^{ad} \) of the change in \( P_A \) direct from (to) B is independent of direction,
\[ F_{AB}^{ad} = \frac{k_{AB}}{k_{AB} + k_{AC}} = \frac{k_{BA}\tilde{P}_B}{k_{BA}\tilde{P}_B + k_{CA}P_C}. \]

The adiabatic flux per cycle \( \int F_{AB}^{ad} d\tilde{P}_A \) is purely geometric and does not depend on frequency. Furthermore, because both \( F_{AB}^{ad} \) and \( \tilde{P}_A \) can vary at most between 0 and 1, the limit is one cycle through the states per cycle of modulation, and one turn for each ring per two cycles of the modulation. This geometric effect is what Michael Berry (22) has termed the geometric phase. A similar picture emerges for a wide variety of physical phenomena, including dissipationless pumping of electrons (23, 24), a mechanism for biomolecular ion pumps (21, 25), phase control in oscillating chemical reactions (26), and swimming at low Reynolds number (27, 28). The argument of the integral in Eq. 6 is the product of a kinetic factor \( F_{AB}^{ad} \) that parametrizes the lability of the transition \( A \rightleftharpoons B \) relative to the lability of the transition \( A \rightleftharpoons C \), and a thermodynamic factor \( d\tilde{P}_A \) that parameterizes the stability of state A relative to states B and C. If \( F_{AB}^{ad} \) is relatively large when \( \tilde{P}_A \) is decreasing and \( F_{AB}^{ad} \) is relatively small when \( \tilde{P}_A \) is increasing the system cycles in the clockwise direction. In the rising phase, molecules are recruited into state A predominately through the B \( \rightleftharpoons C \) A pathway, and, in the decreasing phase, molecules are dismissed from state A predominately through the direct transition \( A \rightleftharpoons B \).

Because each transition involves breaking the interaction between only one station and one ring, the rate constants for the situation shown in Fig. 1 are
\[ k_{AB} = k_{CB} = \omega e^{E_{1}^{3}} \]
\[ k_{BC} = k_{AC} = \omega e^{E_{1}^{3}} \]
\[ k_{CA} = k_{BA} = \omega e^{E_{2}^{3}}, \]
where \( \omega \) is a frequency factor and \( E^3 \) is the height of the barrier relative to zero energy. With these rate constants we have
\[ F_{AB}^{ad} = \frac{1}{1 + e^{E^3 - E_1}}, \]
and the probability to be in state A can be rewritten from Eq. 1 as \( P_A = e^{E_1^3}/(e^{E_1^3} + e^{E_2^3} + e^{E_3^3}) \). In Fig. 2 we see a plot of \( F_{AB}^{ad} \) vs. \( \tilde{P}_A \) for a specific case in which the interaction energy of station 1 is controlled by protonation/deprotonation and the interaction energy of station 2 is controlled by reduction/oxidation. The pH and redox potential are changed by slow titration. The energies are calculated as the averages
\[ E_1 = f_1 E_1^{prot} + (1 - f_1) E_1^{deprot} \]
\[ E_2 = f_2 E_2^{ox} + (1 - f_2) E_2^{red}, \]
where \( f_i = n_i/N \), \( i = 1, 2 \) is the fraction of molecules with station 1 protonated \((i = 1)\) or with station 2 oxidized \((i = 2)\). The
The interaction energy of station 3 is taken to be independent of the external conditions.

We start with strongly reducing \( f_2 \approx 0 \) strongly acidic conditions \( (f_1 \approx 1) \). Each catenane is almost certainly in state A \( (P_A^0 = 1) \) because \( E_1 = -10, E_3 = -5 \ll E_2 = 0 \). The fraction \( P_{B}^{10} \approx 0 \) because it is far more likely to go from A to B via C by overcoming two small barriers, each \( 5 + E^3 \) high, rather than directly by overcoming one large barrier of \( 10 + E^3 \) high. We slowly titrate with base to reach a strongly reducing \( f_2 \approx 0 \) and strongly basic condition \( (f_1 \approx 0) \), where \( E_1 = -5 \ll E_2 = 0, E_1 = 0 \) and so the \( P_A \approx P_B \approx 0.5 \). Of those catenanes now in state B, about half will have come clockwise directly from A and about half counterclockwise through C. We next slowly titrate with oxidant to reach a strongly oxidizing \( f_2 \approx 1 \) and strongly basic condition \( (f_1 \approx 0) \) where \( E_2 = -10, E_3 = -5 \ll E_1 = 0 \) and so \( P_B \approx 1 \). Of the new recruits into state B, almost all with have come directly from A for a total of 75% clockwise directly from A and 25% counterclockwise through C. We now titrate with acid to reach a strongly oxidizing condition \( f_2 \approx 1 \) and a strongly acidic condition \( (f_1 \approx 0) \) where \( E_2 = -10 \) and \( E_1 = -10 \ll E_3 = -5 \) and so \( P_C \approx 1 \). Almost all transitions from C to A will have occurred via the direct, clockwise route because \( E_2 \) is very low during the entire process. Finally, we titrate with redundant to return to the state in which we started, with a strongly reducing condition \( f_2 \approx 0 \) and a strongly acidic condition \( (f_1 \approx 0) \) where \( E_1 = -10 \) and \( E_3 = -5 \ll E_2 = 0 \) and so \( P_A \approx 1 \). Almost all transitions from C to A will have occurred via the direct, clockwise route because \( E_2 \) is very low during the entire process. Through two cycles of modulation acid \( \rightarrow \) base, reducing \( \rightarrow \) oxidizing, base \( \rightarrow \) acid, and oxidizing \( \rightarrow \) reducing, there is an \( \approx 75\% \) chance that the two yellow rings on any one catenane will have each made a clockwise turn, a 25% chance that the two yellow rings on any one catenane will have simply shuffled back and forth, and almost no chance that the two yellow rings on any one catenane will have executed a counterclockwise turn. The average net clockwise turn per two cycles of the modulation is therefore 0.75, the area enclosed by the loop shown in Fig. 2 and the value of the integral in Eq. 6. If we reverse the external modulation to go around the loop in Fig 2 clockwise, the direction of the rotation of the yellow rings is reversed.

We have shown that slow external modulation can break microscopic reversibility - each yellow ring sees the stations, on average, in the order 1, 2, 3, which is microscopically distinguishable from 3, 2, 1. In solution, the catenanes would be disordered, so the broken microscopic reversibility does not imply net rotation relative to a fixed laboratory frame of reference. To accomplish net rotation relative to the laboratory frame, we can immobilize the catenane at a surface, but immobilization presents new difficulties: If the large ring is fixed, there is no topology in which the yellow rings can make a full turn. If both yellow rings are immobilized, the catenane can move through the three states only by rotation of the large ring relative to the two yellow rings. In this case there is only one degree of freedom and the activation energy for a transition is governed by the states energies because interaction between the bases and both yellow rings must by broken to move from one state to the next. For this situation the rate constants are

\[
\begin{align*}
  k_{AB} &= k_{AC} = \beta e^{E_3-E_1} \\
  k_{BC} &= k_{BA} = \beta e^{E_3-E_2} \\
  k_{CA} &= k_{CB} = \beta e^{E_2-E_3}
\end{align*}
\]  

[11]

Inserting these expressions into Eq. 7, we see that \( F_0 \) is constant, independent of the values of the base interaction energies \( E_1, E_2, \) and \( E_3 \). Thus, the integral in Eq. 6 is zero: Slow modulation of the interaction energies does not cause directional rotation. For this reason, modulation of the interaction energies of three stations for a catenane with only two rings did not lead to directional flux, even though the system was caused to move through the states A then B then C again with almost perfect positional integrity (18). Because the fractional flux is independent of the modulation in moving from A to B, the path A \( \rightarrow \) C \( \rightarrow \) B is used as frequently as the path A \( \rightarrow \) B, etc. The independence of the motion of any two rings of the three-ring catenane is crucial to the function of this molecule as an adiabatic directional machine.

There are other, more creative architectures and modulation protocols by which spatially directed rotation can be achieved relative to a fixed frame of reference. One is shown in Fig. 3. Superficially, the mechanism by which the molecular machine depicted in Fig. 3 undergoes directional rotation is very similar to how a ratcheting screwdriver is used to drive a screw. With a macroscopic screwdriver, we turn the screwdriver (and the screw) a one-third turn clockwise (processes a, c, and e), then the screwdriver is reset by turning back one-third turn (processes b, d, f). Resetting doesn’t undo the forward turn of the screw because the ratchet releases in the counterclockwise direction but holds in the clockwise direction. This turning and resetting is continued until the job is completed.

Similarly, in the molecular machine, destabilizing the immobilized yellow ring with its current station when the free yellow ring is stable on the station immediately clockwise induces the large ring to make one-third turn clockwise (processes a, c, and e). The machine is reset by destabilizing the interaction energy of the free yellow ring with its current station, thereby inducing the yellow ring to move back (counterclockwise) one-third turn without the large ring moving (processes b, d, and f). Cyclical modulation of the relative energies \( E_1, E_2, \) and \( E_3 \) in the order shown leads to continued clockwise rotation of the gray ring. At a deeper level, however, there is an enormous and very important difference between the macroscopic ratcheting screwdriver and the molecular machine. With the screwdriver, the torque is applied externally: We force the motion in the desired direction. In the molecular machine, however, chemical or light energy is used to change the relative stability of the interactions, but the movement (transition from an unstable to a stable state) is caused by thermal noise and a priori occurs with equal likelihood forward and backward. In processes a, c, and e, the gray ring turns clockwise not because of a clockwise torque but
because the free yellow ring prevents counterclockwise motion. The molecular machine functions as a Brownian motor (16, 20), in which advantage is taken of thermal noise that causes motion in all directions and input chemical energy is used to prevent motion that is unwanted, leaving behind the motion that is wanted (29).

The motor shown in Fig. 3 can be used to do work at an appreciable rate against a significant load, even when operated adiabatically. Any cyclic chemical process (including all enzymes, molecular motors, and molecular pumps) can equilibrate among all states, even if one transition is kinetically blocked; e.g., in the cycle A ↔ B ↔ C ↔ A the states A, B, and C can rapidly equilibrate if the transitions B ↔ C ↔ A are very fast, even if the direct transition A ↔ B is very slow. Because of the kinetic block, however, a constant load or torque applied to the machine in Fig. 3 would not cause fast continuous cycling, $\approx A \mid \mid \mid | B \rightarrow C \rightarrow A \mid \mid \approx \infty$. The position of the kinetic block in the cycle is indicated by the double vertical lines. Roughly speaking, the loading that can be supported and the rate for continuous cycling, $k_{\text{cycle}}$, is governed by the exponential of the highest kinetic barrier of the system, whereas the rate of chemical equilibration is governed by the exponential of the highest kinetic barrier of the system, whereas the rate of chemical equilibration $k_{\text{chem}}$ is governed by the exponential of the next highest barrier. If these two rates are well separated, there is a significant dynamic range in which the machine can be modulated slowly enough that the system is in chemical equilibrium at every instant in time but rapidly enough that the rate of modulation-induced cycling is large compared with the downhill slip due to a load.

Operation of a molecular motor or pump to do work can be thought of as sequentially moving the kinetic block [processes termed ratcheting and linkage by Leigh and colleagues (7)], then changing the state energies (shifting the thermodynamic balance), followed by chemical equilibration (escapement). In terms of the kinetic and thermodynamic parameters $F_i$ and $P_i$, ratcheting and linkage are those processes in which the $F_i$ values (the labilities) are changed, and balance and escapement are those processes in which the equilibrium constants and, hence, the $P_i$ are changed, respectively. In adiabatic operation, escapement occurs simultaneously with shifting balance. Repeating the external cycle frequently in comparison to $k_{\text{cycle}}$ but infrequently in comparison to $k_{\text{chem}}$ causes the system to do work (e.g., lift a weight in the environment), even while in chemical equilibrium at every instant. For catenanes, the chemical relaxation time can be on the 100-μs time scale, so operation at several hundred cycles per second is certainly possible. For a reasonably sized catenane, say 3 nm in diameter ($\approx\pi \times 10$ nm in circumference), this frequency corresponds to a velocity of several micrometers per second around the ring against a load $F \approx 2–3$ pN for a 10 $k_BT \approx 40$ pN nm modulation of the energies. Larger modulation of the energies would allow for doing work against a larger load.

The energy input comes in shifting the thermodynamic balance (i.e., from the modulation of the interaction energies of the stations), and, of course, the input power must be greater than the output power. However, for an adiabatically driven motor, the system is close to chemical equilibrium at every instant; therefore, the efficiency can be very close to unity. Light is a natural candidate as an energy source to drive a molecular machine. Lehn (30) has proposed a very simple imine-based molecule that should display broken microscopic reversibility in the presence of light and broken macroscopic reversibility when immobilized on a surface. Balzani et al. (11) have demonstrated a rotaxane that can be autonomously driven by light. One intriguing possibility for fueling a chemically driven motor is to use an oscillating chemical reaction (26). Autonomous operation of a chemically driven pump also is possible if a catalytic function is incorporated (31), as in the case of biomolecular motors, where binding of substrate influences the energies of the stations differently than does binding of the product through allosteric interactions.

Biomolecular motors are often described in rather violent terms, with mechanisms involving judo throws (32), power strokes (33), and steam engines (34). In light of the fact that inertia is negligible for molecules in solution and that molecular motors are at every instant in mechanical equilibrium (35) perhaps a “kinder, gentler” description in which the motion occurs by thermally activated transitions between locally equilibrated states (36) is more appropriate. Here, I have gone a step further and have shown how a molecular motor can operate at significant velocity (micrometers per second) against nonnegligible loads ($\approx\pi \times 5$ pN) arbitrarily close to both chemical and mechanical equilibrium at every instant.