

## Research



**Cite this article:** Briegel HJ, Popescu S. 2013  
Intra-molecular refrigeration in enzymes. *Proc R Soc A* 469: 20110290.  
<http://dx.doi.org/10.1098/rspa.2011.0290>

Received: 5 March 2013

Accepted: 28 June 2013

### Subject Areas:

biophysics, thermodynamics

### Keywords:

novel biological effects, enzymes, thermodynamics

### Authors for correspondence:

Hans J. Briegel

e-mail: [hans.briegel@uibk.ac.at](mailto:hans.briegel@uibk.ac.at)

Sandu Popescu

e-mail: [s.popescu@bristol.ac.uk](mailto:s.popescu@bristol.ac.uk)

# Intra-molecular refrigeration in enzymes

Hans J. Briegel<sup>1,2</sup> and Sandu Popescu<sup>3</sup>

<sup>1</sup>Institut für Theoretische Physik, Universität Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria

<sup>2</sup>Institut für Quantenoptik und Quanteninformation der Österreichischen Akademie der Wissenschaften, Innsbruck, Austria

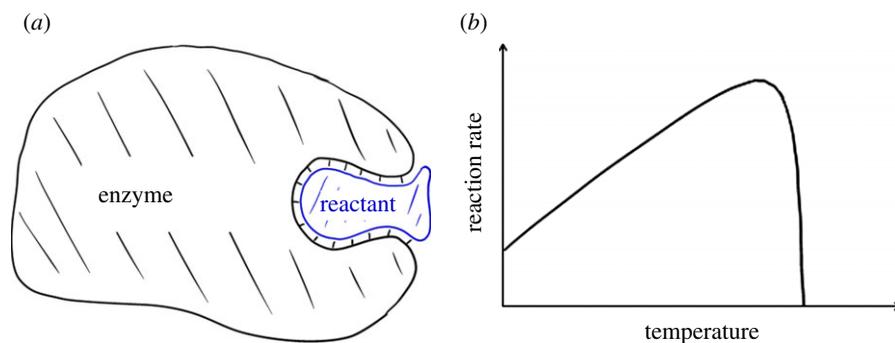
<sup>3</sup>H.H. Wills Physics Laboratory, University of Bristol, Tyndall Avenue, Bristol BS8 1TL, UK

We present a simple mechanism for intra-molecular refrigeration, where parts of a molecule are actively cooled below the environmental temperature. We discuss the potential role and applications of such a mechanism in biology, in particular in enzymatic reactions.

## 1. Introduction

In this paper, we would like to suggest the possibility that enzymes can cool their active sites below the environmental temperature and thereby increase their functionality. A similar cooling mechanism might be used by molecular machines to cool parts of the machine and to reduce the deteriorating effects of thermal noise. The very concept of intra-molecular refrigeration was proposed by us in a recent paper [1]. Here, we discuss it in more detail and present, for the first time, a possible molecular mechanism.

Let us first discuss why we believe that molecular cooling is important and what functionality it could have in biological systems. To start with, we note that for many species, including mammals and birds, the ability to cool parts of their body is essential to survive. Their body temperature is kept largely stable at a specific temperature even when they live in an environment with varying temperatures. This is only possible because they have a built-in cooling system. Such a cooling mechanism works however at a large scale—essentially at the scale of the whole multicellular organism and generally involves the interaction of many organs. The type of cooling we are interested in here is, however, completely different and could, in principle,



**Figure 1.** (a) Molecular model of catalysis: binding of a reactant molecule to the active site of the enzyme. (b) Temperature dependence of a chemical reaction which is catalyzed by an enzyme. (Online version in colour.)

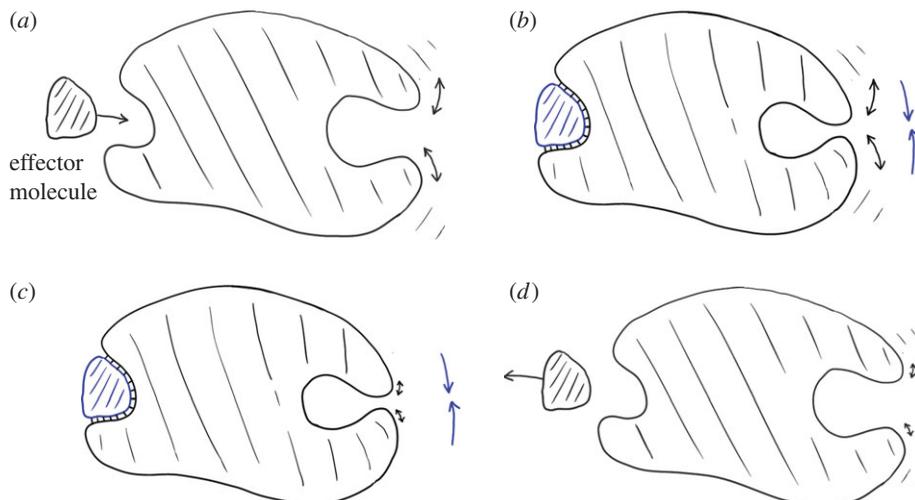
exist in all three domains of life—bacteria, archaea and eukarya. We suggest the possibility that cooling below environmental temperature may occur at molecular level.

If cooling could be achieved at molecular level, this would have obvious benefits. An example is an increased efficiency of catalysis: many proteins act as catalyzers (enzymes) with very high specificity [2]. They have active sites in the shape of cavities, which bind only molecules that fit precisely into the cavity, like a hand in a glove (figure 1a). When a reactant molecule (substrate) binds to the active site, its activation energy to react with some other molecule is lowered, and the reaction is thus speeded-up owing to the presence of the enzyme. After the reaction has taken place, the reactant molecule (product) leaves the cavity. The temperature dependence of the reaction rate in the presence of an enzyme typically looks like the curve displayed in figure 1b. At moderate temperatures, the reaction rate increases with the temperature through an increased thermal energy of the reactant molecules. At higher temperature, however, vibrations in the protein may lead to a deformation of the cavity, which leads in turn to a reduced efficiency to bind reactant molecules (and eventually to de-naturation) and hence to a decreased rate of reaction.<sup>1</sup> From this description [2], it is clear that, if an enzyme would succeed in cooling its active site below the environmental temperature by any mechanism (provided it has no detrimental side effects) then the enzymatic reaction will become more resistant and functionality will be maintained at higher temperatures. Cooling could, in principle, be achieved by different methods. The model we describe here is based on conformational changes, which is a common feature in molecular dynamics.

In the larger context of this study, there is enormous interest in the possibility of controlling enzymes for improved catalysis and, more generally, in manipulating bio-molecular activity. Recent works have discussed the control of enzymatic catalysis by microwave irradiation [4] and, in particular, by conformation modulation using photo-activated molecular switches [5]. Even though related with these studies, our paper is however concerned with an *intrinsic* phenomenon of cooling, where entropy is continuously removed from the active site during normal biological activity, without any external active control.

We want to emphasize from the beginning that we do not have at present any evidence for the existence of such a cooling phenomenon in nature. Indeed, with our paper, the very concept of enzymes cooling one of their active sites is presented for the first time. As far as we know, nobody ever searched for the presence of such behaviour, presumably for the simple reason that it was not known what to search for. Nevertheless, the rough estimation that can be done based on very limited present knowledge of the values of relevant parameters suggests that the existence of such a phenomenon is indeed possible. The main aim of the paper is to present the idea and hopefully this way to initiate the experimental search.

<sup>1</sup>Note that, up to a certain point, vibrations in the protein may in fact be beneficial for the reaction rate, via 'conformational sampling' [3]; a too high temperature will however result in too strong vibrations and eventually lead to a decrease of the reaction rate.



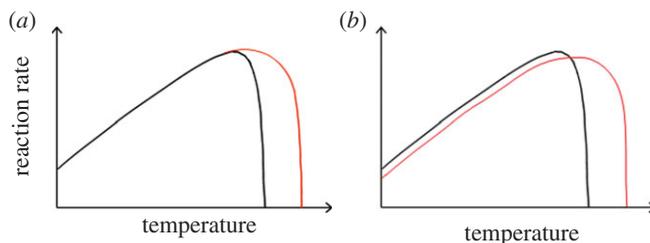
**Figure 2.** Illustration of refrigeration via allosteric conformational change. (a) At very high temperatures, deformation and vibrations of the cavity at the active site will prevent reactant molecules from binding to the enzyme. (b,c) Cooling of the active site can be achieved through a conformational change that contracts the cavity temporarily. Since in the ‘closed’ position the cavity is stiffer, at thermal equilibrium the amplitude of vibrations is smaller than in the ‘open’ position. Heat is pumped out of the molecule during stages (b,c) of the process: when the active site of interest closes (b), its temperature increases. While still in the closed configuration (c), the molecule re-thermalizes by dissipating energy into the environment. Free energy needs to be supplied, for example, by ATP or a similar effector molecule, docking to another active (so-called allosteric) site. (d) Upon re-opening of the cavity, the amplitude of vibration remains reduced, until re-thermalization.

## 2. Molecular mechanism

Let us now describe the molecular model, on which we base our arguments, in a little more detail. This is the hand-in-glove model as illustrated in figure 1*a*. Clearly, there are more advanced models of enzyme activity, but our arguments will apply to all of these model (see comments below). As we have mentioned, the basic mechanism that is responsible for catalysis is the binding of the substrate to the active site of the enzyme, which has the effect of lowering the activation energy for the chemical reaction.

The shape of the cavity at the active site is defined by a specific arrangement of functional groups, which matches with the molecular structure of the substrate. When the ambient temperature increases, there will be more frequent (and higher energetic) collisions of reactant molecules with the enzyme which increases the reaction rate. However, when the temperature becomes too high, it will lead to increased thermal vibrations of the functional groups near the active site as indicated in figure 2. This is valid both in the naive hand-in-glove model, as described above, and in more sophisticated models in which a certain flexibility and mobility of the active site is required. In all these cases, too much fluctuation will kill the efficiency. As a consequence, the substrate will no longer be able to bind to the enzyme and the reaction stops.

The simple refrigeration mechanism we propose is based on a conformational change of the enzyme, which contracts the cavity at the active site and thereby stabilizes its vibrating parts. The reason is that, if the cavity contracts, its walls will interact with each other, making the whole cavity ‘stiffer’. Suppose the cavity stays closed until it reaches thermal equilibrium. At equilibrium, owing to their mutual interaction, the amplitude of the movement of the walls relative to each other is reduced when compared with what it was when the cavity was open (figure 2*b*). Indeed, we can see this in a different way: if the amplitude of the oscillation in the stiffer configuration would be the same as in the open configuration, it would cost more energy, that is, above the average energy corresponding to the ambient temperature. Suppose



**Figure 3.** Proposal of an experiment. (a) Predicted temperature dependence of the rate of an enzymatic reaction, without and with (red) supply of effector molecules. (b) For lower temperatures, the cooling may have a detrimental effect on the enzyme efficiency.

now the cavity re-opens at a speed fast relative to the thermalization rate but slow compared with the period of vibration. In this case, the opening does not itself amplify the amplitude of vibration. Hence, the cavity ends up in the open position with reduced vibrations, that is, at a lower temperature, as is indicated in figure 2c. The cavity is now cold and ready to accept new reactants.

As in any refrigerator, in order for cooling to work, there must be a supply of free energy. We suggest as a possible mechanism that the conformational change is induced by an allosteric process, in which the effector molecule supplies the free energy, as illustrated in figure 2. However, we emphasize that the essential part of the proposal is the refrigeration via conformational change. How exactly the conformational change is produced, and how the free energy is delivered, is irrelevant.

### 3. Experiment

An experimental demonstration of molecular cooling could be straightforward. Suppose one finds a catalyzing enzyme with an active site as described. Several measurement rounds are then performed, observing the enzyme activity (reaction rate) as a function of temperature. In each round, the same concentrations of enzyme and substrate are used, but the concentration of the effector molecules is varied. The predicted temperature dependence of the chemical reaction rate would then be as described in figure 3a (red curve). The enzyme will retain its function up to higher temperatures owing to the molecular cooling effect when effector molecules are present, compared with the situation when there are no effectors. It is also possible that for lower temperatures, the cooling may have a detrimental effect on the enzyme efficiency, since during the time when the active site is closed, it cannot accept any substrate (figure 3b).

### 4. Physical model

Although we talk about cooling at a molecular level, the refrigeration mechanism is essentially classical, that is, not quantum mechanical. For our purposes, the different parts of the enzyme constituting the active site can be viewed as mechanical systems of different masses, which can oscillate around their equilibrium configuration. At increasing temperatures, the different parts of the system start vibrating with increasing amplitudes, which diminishes the functionality of the enzyme.

The active site of the enzyme can vibrate in many complicated ways. Technically, there are many vibration modes. For simplicity, here we analyse the case of just two oscillators, which stresses the essential features of the model. The two oscillating parts could represent the two ‘lips’ of the cavity in figure 2a. The vibrations indicated in the figure then correspond to excitations of the oscillators around their respective equilibrium positions. Contracting the cavity brings the parts together; when the two lips are closer, additional forces will act on them. Whatever the sign

of these forces (attractive or repulsive) will be, the net effect is to make the entire system tighter and increase the frequency of the vibrations.

This process can be modelled by an extra interaction potential which is switched on in the contracted configuration. While the exact nature of these interactions is not essential, its main effect is to increase the frequency of the oscillators which will be energetically more difficult to excite.

The Hamilton function for this system is given by

$$H(t) = \frac{P_1^2}{2m_1} + \frac{P_2^2}{2m_2} + \frac{\kappa_1}{2} X_1^2 + \frac{\kappa_2}{2} X_2^2 + \frac{\kappa(t)}{2} (X_2 - X_1)^2, \quad (4.1)$$

where  $X_i$  is the displacement of mass  $m_i$  from its equilibrium position,  $P_i$  the corresponding canonical momentum and  $\kappa_i$  the spring constant associated with the  $i$ th oscillator. The last term describes the variable interaction potential, which is switched on in the contracted configuration, accounted for by the variable spring constant  $\kappa(t)$ . As we are only interested in the system near the equilibrium, we can choose quadratic oscillator potentials for all the involved potentials. For simplicity, we shall assume equal masses and spring constants for both oscillators, i.e.  $m_1 = m_2 = m$  and  $\kappa_1 = \kappa_2 = \kappa$ . It is convenient to introduce new variables,  $X_{\text{cm}} = (X_1 + X_2)/2$ ,  $P_{\text{cm}} = P_1 + P_2$  for the centre of mass, and  $x = X_1 - X_2$ ,  $p = (P_2 - P_1)/2$  for the relative displacement coordinates and momenta. The transformed Hamilton function then reads

$$H(t) = \frac{P_{\text{cm}}^2}{2M} + \frac{\kappa_{\text{cm}}}{2} X_{\text{cm}}^2 + \frac{p^2}{2\mu} + \frac{\kappa_{\text{rel}} + \kappa(t)}{2} x^2 \quad (4.2)$$

with  $\kappa_{\text{cm}} = 2\kappa$ ,  $\kappa_{\text{rel}} = \kappa/2$ ,  $M = 2m$ ,  $\mu = m/2$ . The motions of the centre of mass and the relative displacement coordinates are decoupled and describe harmonic oscillations. While the first does not lead to a deformation of the cavity, the latter does. We will thus concentrate on the motion of the relative displacements in the following, described by

$$H(t) = \frac{p^2}{2\mu} + \frac{\kappa_{\text{rel}} + \kappa(t)}{2} x^2. \quad (4.3)$$

When the cavity is open,  $\kappa(t) \simeq 0$ , and, due to the influence of the environment, the relative mode of oscillation will be thermally excited. Upon contracting the cavity, the coupling function  $\kappa(t)$  will effectively be switched on and tighten the potential. This corresponds to an increase of the spring constant, which makes this oscillator stiffer. To understand how this can lead to cooling, it is convenient to use a quantum mechanical description of the oscillator, where the energies are quantized. We would like to emphasize however that this quantum mechanical description holds both in the deep quantum regime near the ground state and in the classical regime when we are far away from the ground state.

The Hamilton operator corresponding to (4.3) can be written in the form

$$H(t) = \hbar\omega(t)(a^\dagger a + \frac{1}{2}) \quad (4.4)$$

with a time-dependent frequency

$$\omega(t) = \sqrt{\frac{\kappa_{\text{rel}} + \kappa(t)}{\mu}}, \quad (4.5)$$

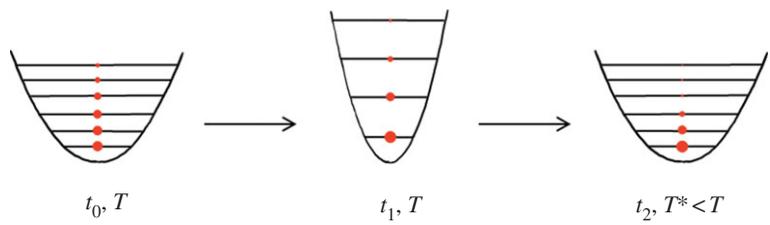
and ladder operators

$$a = a_t = \frac{x}{\sqrt{2\hbar/\mu\omega(t)}} + i\frac{p}{\sqrt{2\hbar\omega(t)\mu}} \quad (4.6)$$

and

$$a^\dagger = a_t^\dagger = \frac{x}{\sqrt{2\hbar/\mu\omega(t)}} - i\frac{p}{\sqrt{2\hbar\omega(t)\mu}}. \quad (4.7)$$

For a fixed frequency,  $\omega(t) = \omega = \text{const.}$ , the possible energies of this oscillator are given by the formula  $E_n = \hbar\omega(n + 1/2)$ ,  $n = 1, 2, \dots$ . In a thermal environment at temperature  $T$ , the different energies  $E_n$  will be found with probabilities  $p_n = Z^{-1} \exp(-n\hbar\omega/k_B T)$ , with  $k_B$  and  $\hbar$  denoting the



**Figure 4.** Refrigeration of the molecular vibrations through a conformational change (see text). (Online version in colour.)

Boltzmann and Planck constant, respectively, and  $Z$  a normalization factor (called the partition function) which ensures  $\sum_n p_n = 1$ .

In the open and contracted configuration, the oscillator will have two different frequencies,  $\omega_0$  and  $\omega_1 > \omega_0$ , respectively. Suppose that the system starts at time  $t_0$  in the open configuration, with a spacing of the allowed energies  $\Delta E_n^0 = \hbar\omega_0$  and with a thermal-state distribution  $p_n(t_0) = Z^{-1} \exp(-n\hbar\omega_0/k_B T)$ . Upon contraction, the spacing between the allowed energies will increase to  $\Delta E_n^1 = \hbar\omega_1 > \hbar\omega_0$  (figure 4). The thermalization of the oscillator is a process with a characteristic time scale that depends on the interaction strength between the system and the environment. Ideally we assume that, after contraction, the system stays long enough in this configuration, such that it has time to thermalize. The resulting thermal distribution at time  $t_1$  is then  $p_n(t_1) = Z^{-1} \exp(-n\hbar\omega_1/k_B T)$ . Note that, as the spacing between the energy levels has increased, a larger fraction of the population will move to lower energies, as indicated in figure 4.

Upon re-opening, the system will move back to the open configuration (with frequency  $\omega_0$ ). Let the time required for opening be  $\tau_{\text{open}}$ . Whether or not cooling takes place depends on two time scales. The first is the thermalization time  $\tau_{\text{therm}}$ , and the second the period of oscillation  $\tau_{\text{osc}} = 2\pi\omega^{-1}$ . The ideal regime is when the transition is fast compared with thermalization but slow compared with the oscillation period, i.e.  $\tau_{\text{osc}} \ll \tau_{\text{open}} \ll \tau_{\text{therm}}$ . In that case, the system will cool down. Indeed, in this regime, the opening is adiabatic and the relative population of the different energy levels does not change during the transition. In this ideal case, right after returning to the open configuration, i.e. at time  $t_2 = t_1 + \tau_{\text{open}}$ , the population of the energy levels will still be the same as at time  $t_1$ , that is  $p_n(t_2) = Z^{-1} \exp(-n\hbar\omega_1/k_B T) = Z^{-1} \exp(-n\hbar\omega_0/k_B T^*)$ . With respect to the new energy spectrum (spacing  $\Delta E_n^0 = \hbar\omega_0$ ), this corresponds however to a thermal state with reduced temperature  $T^* = (\omega_0/\omega_1)T$ . After such a cooling cycle, the temperature of the active site of the enzyme has thus been reduced by the amount  $\Delta T/T = 1 - \omega_0/\omega_1$ . We note however that, as shown later, the cooling effect takes place, albeit with reduced efficiency, also outside the ideal regime described above.

In the remainder of this paper, we illustrate the essential dynamics of the refrigeration process for a time-dependent oscillator frequency  $\omega(t)$ . For the present purpose, it suffices to describe the thermalizing effect of the environment by a master equation of the form

$$\frac{\partial}{\partial t} \rho(t) = -\frac{i}{\hbar} [H(t), \rho(t)] + L_t \rho(t) \tag{4.8}$$

with  $H(t)$  as in (4.4) and with a dissipative term

$$L_t \rho(t) = -\frac{\gamma}{2} (v + 1) \{ a^\dagger a \rho + \rho a^\dagger a - 2a \rho a^\dagger \} - \frac{\gamma}{2} v \{ a a^\dagger \rho + \rho a a^\dagger - 2a^\dagger \rho a \}. \tag{4.9}$$

Here,  $\rho(t)$  is the state (density matrix) of the system at time  $t$ . The parameter  $\gamma$  is the thermal relaxation rate that describes how fast the system would approach, for any fixed configuration ( $\omega = \text{const.}$ ), its thermal equilibrium state

$$\rho_{\text{therm}}(\omega) = \rho(t \rightarrow \infty)|_{\omega=\text{const.}} = Z^{-1} \exp\left(-\frac{\hbar\omega}{k_B T} a^\dagger a\right), \tag{4.10}$$

and  $\nu$  is the mean number of thermal excitations in that equilibrium state,  $\nu = \nu(\omega) = 1/[\exp(\hbar\omega/k_B T) - 1]$ . For a time-dependent oscillator frequency,  $\omega = \omega(t)$ , this ‘instantaneous’ thermal state thus changes with time, as does  $\nu = \nu[\omega(t)]$ . Unless the motion of the oscillator is very slow compared with the relaxation time, it will be driven out of equilibrium, which gives rise to the described refrigeration effect. In the regime where the oscillation period is short compared with all other timescales (adiabatic regime),  $\tau_{\text{osc}} \ll \tau_{\text{open}}, \tau_{\text{therm}}$ , the solution can be written in the form [6]

$$\rho(t) = \frac{1}{\eta(t)} \left[ 1 - \frac{1}{\eta(t)} \right]^{a^\dagger a}, \quad (4.11)$$

where  $\eta(t)$  is a solution of the differential equation

$$\frac{d}{dt} \eta(t) = -\gamma \eta(t) + \gamma \{ \nu[\omega(t)] + 1 \} \quad (4.12)$$

which can be readily integrated to give

$$\eta(t) = e^{-\gamma(t-t_1)} \eta(t_1) + \gamma \int_{t_1}^t ds \frac{e^{-\gamma(t-s)}}{1 - e^{-\hbar\omega(s)/k_B T}}. \quad (4.13)$$

The function  $\eta(t)$  has a simple interpretation, it is equal to the mean number of excitations (plus unity) in the oscillator at time  $t$ , i.e.  $\eta(t) = \langle a^\dagger a \rangle + 1$ . The state (4.11) describes a Boltzmann distribution corresponding to a thermal state, albeit with a temperature  $T(t)$  reduced below the temperature  $T$  of the environment,

$$\frac{T(t)}{T} = \frac{\log[\nu(t)/(\nu(t) + 1)]}{\log[1 - 1/\eta(t)]} = \frac{\log[\nu(t)/(\nu(t) + 1)]}{\log[\langle a^\dagger a \rangle / (\langle a^\dagger a \rangle + 1)]}. \quad (4.14)$$

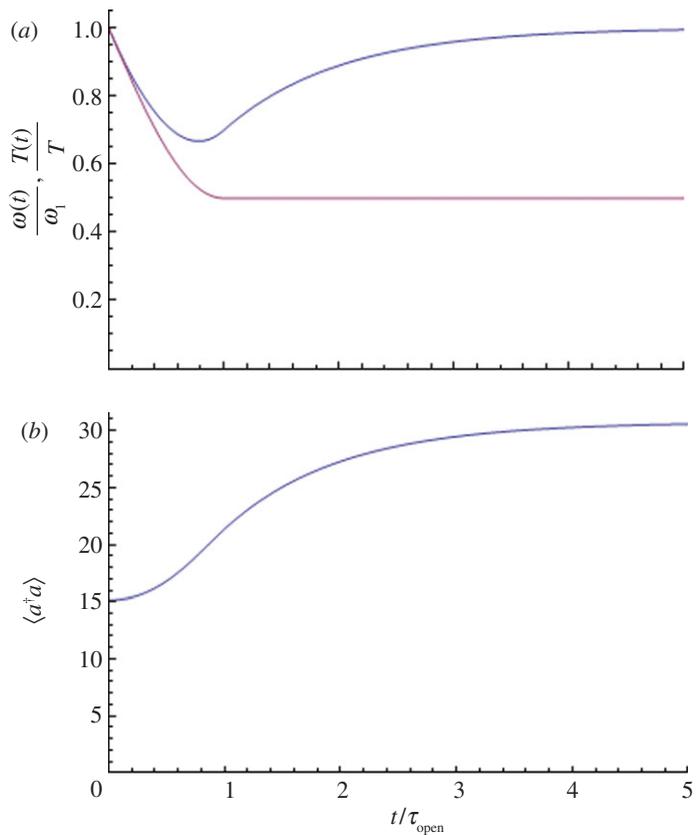
Let us now have a closer look at the essential part of the process, that is, when the molecule moves from the closed configuration (after thermalization) at time  $t_1$  back to the open configuration, which it reaches at time  $t_2 = t_1 + \tau_{\text{open}}$ . In figure 5, we plot the temperature of the oscillator—representing the active site—as a function of time. As an example, we choose the specific time profile  $\omega(t) = \omega_1 + (\omega_0 - \omega_1) \sin((\pi/2)(t/\tau_{\text{open}}))$ , for  $0 = t_1 \leq t \leq t_2 = \tau_{\text{open}}$  and  $\omega(t) = \omega_0$  for  $t > \tau_{\text{open}}$ , but other profiles give similar results. The function  $\eta(t)$  in (4.13), like  $\omega(t)$ , can be expressed in terms of the dimensionless quantities  $\omega_1/\omega_0$ ,  $\hbar\omega_0/k_B T$  and  $t/\tau_{\text{open}}$ .

A crucial issue is, of course, to determine the ranges of values of the parameters (opening time, oscillation frequencies and thermalization time) relevant to our system. This is however not so straightforward, since these parameters vary over considerably large ranges [7]. They depend on the type of molecule we consider, on its size, on the size of the active site, on which parts of the active site contract (i.e. the whole site or only subparts involving some of the functional groups) and on the degree of interaction with the environment. Furthermore, not all such parameters are typically available for the same molecule, but some parameters are known for some molecules, other parameters for other molecules.

For the figure, we have chosen the following values:  $\hbar\omega_0/k_B T = 0.032$ ,  $\omega_1/\omega_0 = 2$ ,  $\gamma \tau_{\text{open}} = 1$ . At room temperature,  $T = 300$  K, this corresponds to molecular oscillation times  $\tau_{\text{osc}} = 2\pi/\omega_0 = 5$  ps,  $\tau'_{\text{osc}} = 2\pi/\omega_1 = 2.5$  ps, which is a typical time scale of elastic vibrations. Possible values for the opening time  $\tau_{\text{open}}$ , which is the time for (part of) a conformational transition, may range from fractions of nanoseconds up to microseconds and beyond, depending on the size of the protein and the specific type of transition. Similarly, the thermalization time  $\tau_{\text{therm}} \equiv \gamma^{-1}$  may vary significantly, depending on coupling of the oscillators to the environment. For fast configurational processes, an exemplary choice would be  $\tau_{\text{open}} = 100$  ps as the time scale for re-opening the cavity and  $\gamma^{-1} = 100$  ps as the thermalization time for the vibrations.

As we have mentioned, these values will depend on the specific molecular realization, and all of these numbers are subject to considerable variation.<sup>2</sup> But it seems reasonable to assume that the time scales of the conformational transition and of the cavity vibrations are separated by

<sup>2</sup>For recent reviews, where one can find a discussion of some of the timescales, see e.g. [3,8,9] and references cited therein.



**Figure 5.** Refrigeration of the active site as a function of time. (a) Temperature  $T(t)/T$  of the active site (blue) and frequency of vibrations  $\omega(t)/\omega_1$  (red). (b) Average number of quanta  $\langle a^\dagger a \rangle(t)$  in the vibration mode. (Parameters:  $\omega_1/\omega_0 = 2$ ,  $\gamma \tau_{\text{open}} = 1$ ,  $\hbar\omega_0/k_B T = 0.032$ . At  $T = 300$  K, this corresponds to  $\tau_{\text{osc}} = 2\pi/\omega_0 = 5$  ps as the time scale of elastic vibrations.)

at least one order of magnitude, i.e.  $\tau_{\text{osc}}, \tau'_{\text{osc}} \ll \tau_{\text{open}}$  (which is the so-called adiabatic regime, with  $\dot{\omega}/\omega \ll \omega$ ). The timescale for  $\tau_{\text{open}}$  may also be much larger; likewise, the timescale for thermalization may be different. Whether or not the refrigeration effect can be observed depends on the timescale of the thermalization relative to the opening time, i.e. on  $\tau_{\text{open}}/\tau_{\text{therm}} = \gamma \tau_{\text{open}}$ . As long as the thermalization time is larger or at least comparable with the opening time, i.e.  $\gamma \tau_{\text{open}} \lesssim 1$ , the effect can clearly be observed, as demonstrated in figure 5. One can see how the effective temperature of the cavity  $T(t)$  drops during the opening transition to about 65% of the environmental temperature  $T$ . After the cavity has reached the open position, its temperature relaxes to the environmental temperature. It should however be noted that a useful effect of refrigeration may persist up to several multiples of  $\tau_{\text{open}}$ : for example, a refrigeration by  $1^\circ$  K or more, corresponding to a relative value  $T(t)/T = 299/300 = 0.997$  or less, is maintained for up to about  $6 \times \tau_{\text{open}}$ . Of course, the effect is more persistent for longer thermalization times  $\tau_{\text{therm}}$ .

To explain the refrigeration mechanism, we have described the enzyme as a system of coupled harmonic oscillators. We want to emphasize however that the main result does not change if we assume anharmonic couplings in the vibrations or the conformational changes. Indeed, the main ingredients of the mechanism we presented are (i) the system cyclically goes between two stages—an open one, where the spacing between the energy levels is smaller and a closed one where the spacing between the energy levels is larger; (ii) the system stays in the closed stage for long enough time to thermalize to the environmental temperature, and (iii) the system re-opens adiabatically slowly, so that the population of the different energy states is not changed considerably. Whenever a system undergoes the above transformation, the end result is that

for a given period of time immediately after the opening, the system is in the open stage with reduced fluctuations. The exact distribution of the energy levels (equal spacing for a harmonic oscillator versus unequal spaces of an anharmonic oscillator) is irrelevant. While, of course, the exact quantitative results will depend on all the parameters, the qualitative result, i.e. the mere existence of cooling, will not.

## 5. Conclusions

The mechanism for intra-molecular refrigeration that we have discussed in this paper is simple. It is based on conformational changes that induce time-dependent forces between different parts of a molecule, reducing their relative vibrations. It works as long as the configurational changes, which may involve only small parts of a protein, happen faster or at least on a comparable timescale as the damping of the relevant vibrations. The changes in local temperature, which can be generated by this mechanism, are, even for moderate variations of the binding potential, quite significant: it should be remembered that, for many biological processes, a refrigeration by only a few degree Kelvin can make all the difference.

Many variations of this scheme are conceivable. For example, it is possible that cooling owing to conformational variations may take place at other sites, near the active site, but without changing the latter's shape. Through heat transfer to the cooling centres, effective refrigeration of the active site would be possible without interrupting the catalyzer process.

The improvement of enzyme functionality is but one example of the potential applications of intra-molecular refrigeration, but there should be many others. Molecular refrigeration might also play a role, for example, in molecular machines, to stabilize their operation against the accumulation of noise and errors or in more complex processes such as those involved in protein synthesis.

Last, but not least, the possibility of maintaining temperature gradients on a molecular scale is by itself of considerable interest for the investigation of thermodynamical processes at the bio-molecular level.

**Acknowledgements.** We would like to thank Judith Klinman for interesting and helpful comments on the manuscript.

**Funding statement.** H.J.B. acknowledges support from the Austrian Science Fund (FWF) through projects F04011 and F04012. S.P. acknowledges support from ERC Advanced grant NLST and Templeton Foundation.

## References

1. Briegel HJ, Popescu S. 2009 Entanglement and intra-molecular cooling in biological systems? A quantum thermodynamic perspective. See <http://arxiv.org/abs/0806.4552>.
2. Alberts B *et al.* 2008 *Molecular biology of the cell*. New York, NY: Garland Science.
3. Klinman JP. 2009 An integrated model for enzyme catalysis emerges from studies of hydrogen tunneling. *Chem. Phys. Lett.* **471**, 179–193. (doi:10.1016/j.cplett.2009.01.038)
4. Young DD, Nichols J, Kelly RM, Deiters A. 2008 Microwave activation of enzyme catalysis. *J. Am. Chem. Soc.* **130**, 10 048–10 049. (doi:10.1021/ja802404g)
5. Agarwal PK, Schultz Ch, Kalivretenos A, Ghosh B, Sheldon B. 2012 Engineering a hypercatalytic enzyme by photoactivated conformation modulation. *J. Phys. Chem. Lett.* **3**, 1142–1146. (doi:10.1021/jz201675m)
6. Englert BG, Naraschewski M, Schenzle A. 1994 Quantum-optical master equations: an interaction picture. *Phys. Rev. A.* **50**, 2667–2679. (doi:10.1103/PhysRevA.50.2667)
7. Frauenfelder H, Sligar SG, Wolynes PG. 1991 The energy landscapes and motions of proteins. *Science* **254**, 1598–1603. (doi:10.1126/science.1749933)
8. Agarwal PK. 2006 Enzymes: an integrated view of structure, dynamics and function. *Microb. Cell Fact.* **5**, 2. (doi:10.1186/1475-2859-5-2)
9. Gilmore J, McKenzie RH. 2008 Quantum dynamics of electronic excitations in biomolecular chromophores: role of the protein environment and solvent. *J. Phys. Chem. A* **112**, 2162–2176. (doi:10.1021/jp710243t)