

## THERMODYNAMICS AND KINETICS OF INTERLINKED MOLECULES

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### EXTENDED ABSTRACT

Molecular systems such as enzymes or synthetic molecular machines carry out their functions in fluctuating environments. The fluctuations arise not only because of the ineluctable presence of thermal noise but also possibly through externally driven fluctuations [1]. Examples of the latter include external fluctuating electric fields that were shown to be able to drive uphill motion of solute molecules across a membrane through a mechanism termed electro-conformational coupling [2]. In the mid 1980's a theory, then known as electro-conformational coupling, and now more generally termed stochastic pumping, was developed to describe the mechanism by which energy from externally driven fluctuations could be harvested by enzymes and used to do work on the environment by forming a non-equilibrium steady state between substrate and product given by [3]

$$\left. \frac{[P]}{[S]} \right|_{ss} = K_{eq} e^{\overline{W_{el}}} \quad (1)$$

where here and elsewhere in this extended abstract energies are given in units of the thermal energy  $k_B T$ . The term  $e^{\overline{W_{el}}}$  is the time-average of the exponential of the work exchanged between the enzyme and the applied electric field in those trajectories in which a molecule of substrate is converted to a molecule of product. This work can be either positive or negative depending on the details of the motion of the charges in the enzyme conformational changes, and hence the term  $e^{\overline{W_{el}}}$  can either favor substrate or product relative to the equilibrium case.

The theory of stochastic pumping is based on the principle of microscopic reversibility (MR) [4] by which, irrespective of whether the system is in equilibrium or not, the ratio of probabilities for forward and microscopic reverse paths between any two states  $i$  and  $j$ , of some molecule of interest is given as the exponent of the basic free-energy difference  $\Delta G_{ij} = G_i - G_j$  plus the work exchanged (excess work) with the environment  $W_S$  in the forward path  $S$  from  $i$  to  $j$

$$\frac{\pi_{(i \xrightarrow{S} j)}}{\pi_{(j \xrightarrow{S^\dagger} i)}} = e^{\Delta G_{ij} + W_S} \quad (2)$$

The term  $\Delta G_{ij}$  is the same for every path  $S$  but away from equilibrium the work exchanged with the environment,  $W_S$ , in the transition  $i \xrightarrow{S} j$  depends on the path  $S$ . We take the sign convention that  $W_S$  is positive when energy is dissipated in the environment in the transition  $i \xrightarrow{S} j$ . Note that the work exchanged in the reverse path is equal and opposite that in the forward path  $W_{S^\dagger} = -W_S$ .

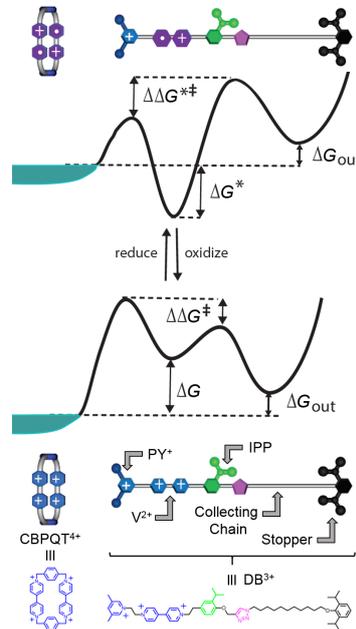
The steady state ratio between concentrations  $[i]$  and  $[j]$  is obtained by summing the probabilities for all paths from  $j$  to  $i$  and dividing by the sum of the probabilities for all paths from  $i$  to  $j$ . We can use MR, Eq. (1), to derive an extension of the Boltzmann expression for the ratio of probabilities to be in two states at a not necessarily equilibrium steady state

$$\left. \frac{[i]}{[j]} \right|_{ss} = \frac{\sum_n \pi_{S_n^\dagger}(j \xrightarrow{S_n^\dagger} i)}{\sum_n \pi_{S_n}(i \xrightarrow{S_n} j)} = e^{-\Delta G_{ij}} \frac{\sum_n \pi_{S_n}(i \xrightarrow{S_n} j) e^{-W_{S_n}}}{\sum_n \pi_{S_n}(i \xrightarrow{S_n} j)} = e^{-\Delta G_{ij}} \langle e^{-W_S} \rangle \quad (3)$$

The second equality arises by application of the identity, Eq. (2), to each term in the numerator of Eq. (3), and the final equality compacts the notation, recognizing the possibly path dependent term as  $e^{-W_{S_n}}$  averaged over all trajectories leading from state  $j$  and  $i$ . The term  $e^{-\Delta G_{ij}}$  is the standard Boltzmann factor familiar from equilibrium statistical mechanics. The apparent simplicity of the second factor,  $\langle e^{-W_S} \rangle$ , belies the fact that, while at equilibrium  $W_S$  is path independent and so the average is just a proportionality constant, away from equilibrium the average over all paths is non-trivial to calculate.

I will discuss how these relationships can be used to understand the mechanism of stochastic pumping to form non-equilibrium steady states of assembled vs. disassembled forms of a mechanically bonded pseudo-rotaxane.

Consider a pseudo-rotaxane comprised of a cyclobis paraquat ring that threads onto a dumbbell shaped rod comprising a viologen ( $V^{2+}$ ) recognition site, a 3,5-dimethylpyridinium ( $PY^+$ ) group on one end to serve as an electrostatically switchable barrier for threading the ring onto the dumbbell, an isopropylphenylene (IPP) group that serves as a steric “speed bump”, separating the recognition site from a long oligomethylene-collecting chain, and a bulky 2,6-diisopropylphenyl stopper [5]. The energy landscape for threading the ring onto the dumbbell depends on whether the ring and the dumbbell are oxidised (lower profile in Fig. 1) or reduced (upper profile in Fig. 1).



**Fig. 1** Schematic illustration of the free energy profile experienced by a  $CBPQT^{4+}$  ring moving along a dumbbell  $DB^{3+}$  when the ring and recognition site are reduced (top profile) and when the ring and recognition site are oxidized (bottom profile). The corresponding structural formulas of  $CBPQT^{4+}$  and  $DB^{3+}$  are reported on the bottom. The level of the blue-green “sea” indicates the chemical potential of the  $CBPQT^{4+}$  rings in the bulk.

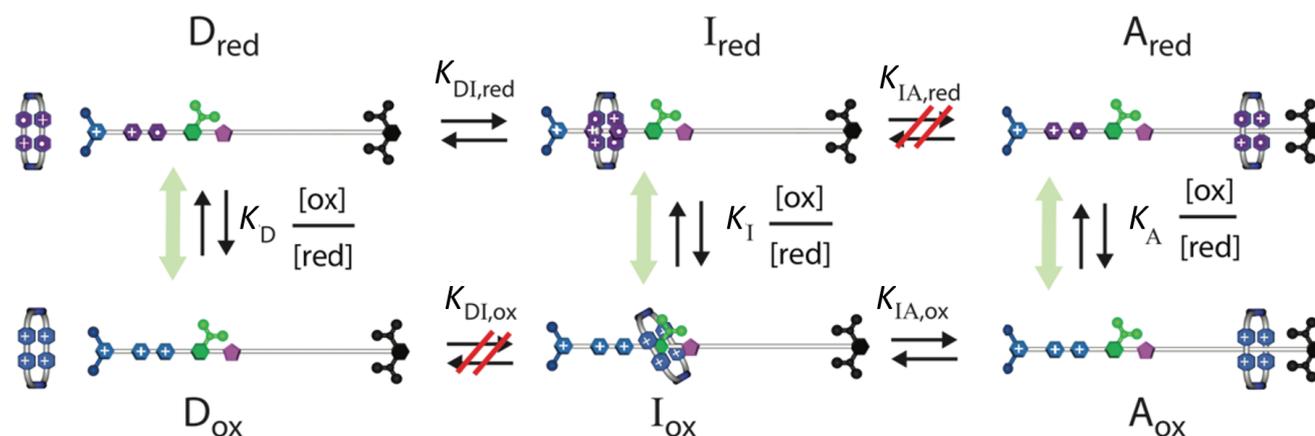
At equilibrium, the ratio between the probability of finding the system assembled with a ring on the collecting chain ( $[A_{ox}] + [A_{red}]$ ) to the probability to be disassembled with no ring on the chain ( $[D_{ox}] + [D_{red}]$ ), is

$$\frac{[A_{ox}] + [A_{red}]}{[D_{ox}] + [D_{red}]} \Bigg|_{eq} = e^{-\Delta G_{out}} \quad (4)$$

The equilibrium occupancy of the collecting chain does not depend on the redox potential of the solution, or on the free-energy of either intermediate state,  $I_{ox}$  or  $I_{red}$ . By externally changing the redox potential back and forth between reducing and oxidising conditions, the energy profile is made to switch back and forth between the upper state and the lower state, giving rise to stochastic pumping<sup>8</sup> by an energy ratchet<sup>31</sup> mechanism. After many cycles the steady-state ratio of the assembled to disassembled molecules reaches<sup>14</sup>

$$\frac{[A_{ox}] + [A_{red}]}{[D_{ox}] + [D_{red}]} \Bigg|_{ss} \approx K_{DI,red} K_{IA,ox} = e^{-\Delta G_{out}} e^{\Delta G - \Delta G^*} \quad (5)$$

where  $\Delta G - \Delta G^* = \ln\left(\frac{K_A}{K_I}\right)$  is the work absorbed when the components are oxidised while in state I (See Fig. 2).



**Fig. 2** Kinetic diagram describing how external oscillation of the redox potential drives dissipative assembly of CBPQT<sup>4+</sup> rings onto a collecting chain. Under reducing conditions the system equilibrates between states  $D_{\text{red}}$  and  $I_{\text{red}}$ , and under oxidizing conditions between  $I_{\text{ox}}$  and  $A_{\text{ox}}$ . Over many cycles reducing and oxidizing conditions the system eventually reaches a steady state in which the net equilibrium between assembled and disassembled molecules is governed by the product  $K_{DI,\text{red}}K_{IA,\text{ox}}$ . Refer to Fig. 1 for the structural formulas.

We can develop intuition about the mechanism of pumping by considering modifications to the design of the pump [6]. For example, if we exchange the  $\text{PY}^+$  group with the IPP steric “speed bump” the transitions  $D_{\text{red}} \rightleftharpoons I_{\text{red}}$  and  $I_{\text{ox}} \rightleftharpoons A_{\text{ox}}$  would become blocked kinetically. This alteration would have the effect of changing the sign of  $\Delta\Delta G^{\ddagger} - \Delta\Delta G^\ddagger$ . The single remaining pathway would then be  $D_{\text{red}} \leftrightarrow D_{\text{ox}} \rightleftharpoons I_{\text{ox}} \leftrightarrow I_{\text{red}} \rightleftharpoons A_{\text{red}} \leftrightarrow A_{\text{ox}}$  and we would find that large amplitude oscillations of the redox potential would lead to the steady state ratio between assembled and disassembled molecules  $\left. \frac{[A_{\text{ox}}] + [A_{\text{red}}]}{[D_{\text{ox}}] + [D_{\text{red}}]} \right|_{\text{ss}} = K_{DI,\text{ox}}K_{IA,\text{red}} = e^{-\Delta G_{\text{out}}}e^{-(\Delta G - \Delta G^*)}$ . The external driving force in this case would favour disassembly, not assembly, relative to the equilibrium case. Non-equilibrium self-assembly requires, in addition to dissipation, intelligent design on the part of the synthetic chemist. We also observe that, in contrast to equilibrium thermodynamics, the free energies of the intermediate states and of the transition states play an important role in determining the steady-state ratio maintained by the external driving force. If  $\Delta G - \Delta G^* = 0$ , the steady state in the presence of redox potential oscillations is the same as the equilibrium ratio, irrespective of the barriers heights. Further, if the difference of the barrier heights between the bulk and the recognition site and between the recognition site and the collecting chain are the same under both oxidising and reducing conditions there is no energy-driven assembly (or disassembly) irrespective of the value of  $\Delta G - \Delta G^*$ . The possibilities are summarised in Table I.

**Table I**  
Effect of thermodynamic and kinetic parameters on externally driven self-assembly

$\Delta G - \Delta G^*$ \ / \ $\Delta\Delta G^{\ddagger} - \Delta\Delta G^\ddagger$	<0	0	>0
<0	Disassembly	No change	Assembly
0	No change	No change	No change
>0	Assembly	No change	Disassembly

This simple synthetic example shows how externally driven fluctuation can drive formation and maintenance of non-equilibrium steady states. Through the rigorous application of fundamental physical principles [7] such as microscopic reversibility the essential back and forth between theory and experimental investigation of synthetically manipulable chemical systems can be carried out to provide insight into the basic mechanisms of self-organization.

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