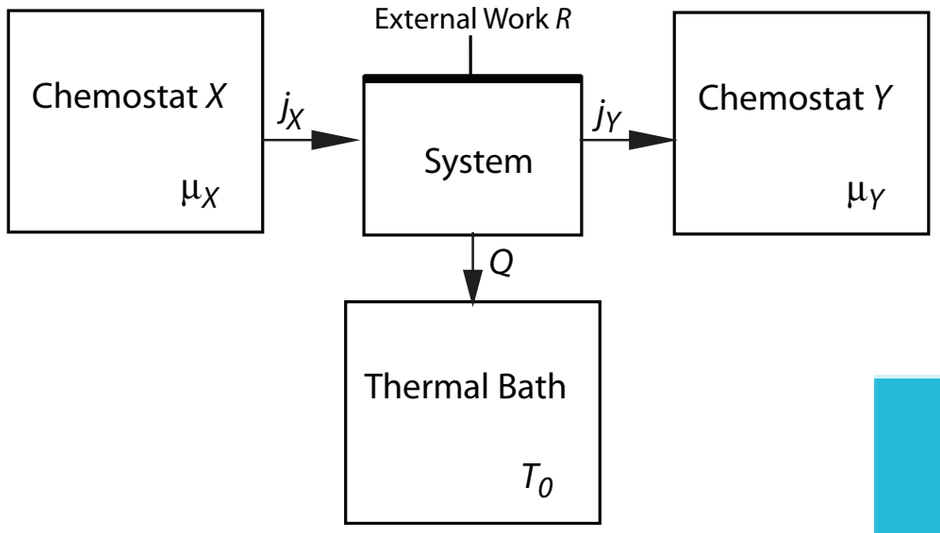
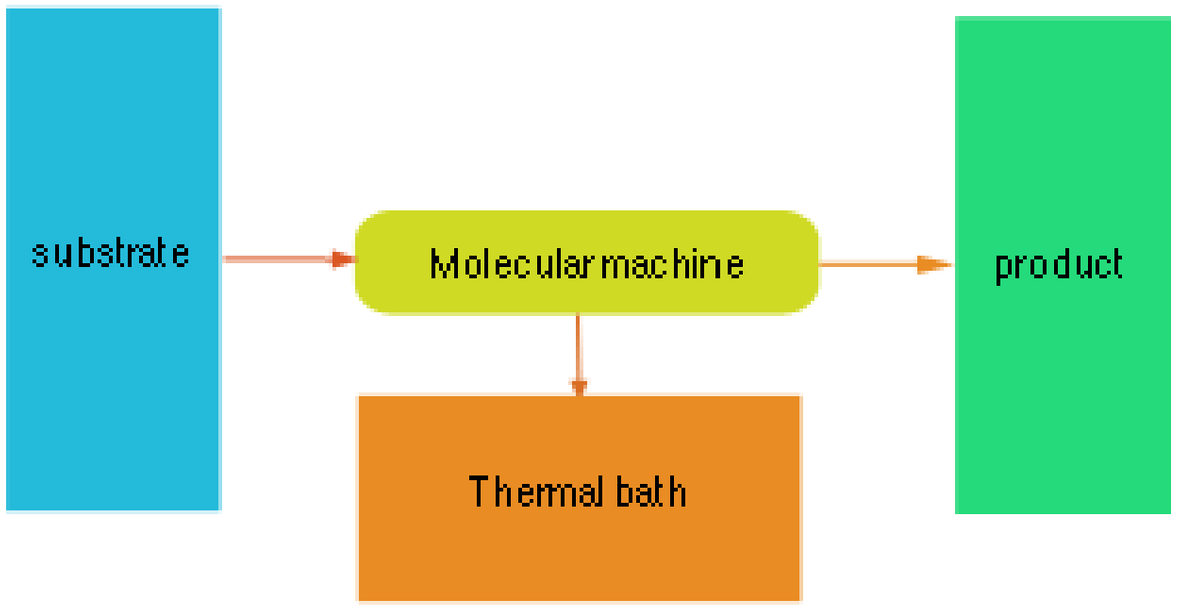
金沢大学
KANAZAWA UNIVERSITY

Introduction into the Theory of Biological Nanomachines

Alexander S. Mikhailov/Holger Flechsig

Lectures 3 & 4

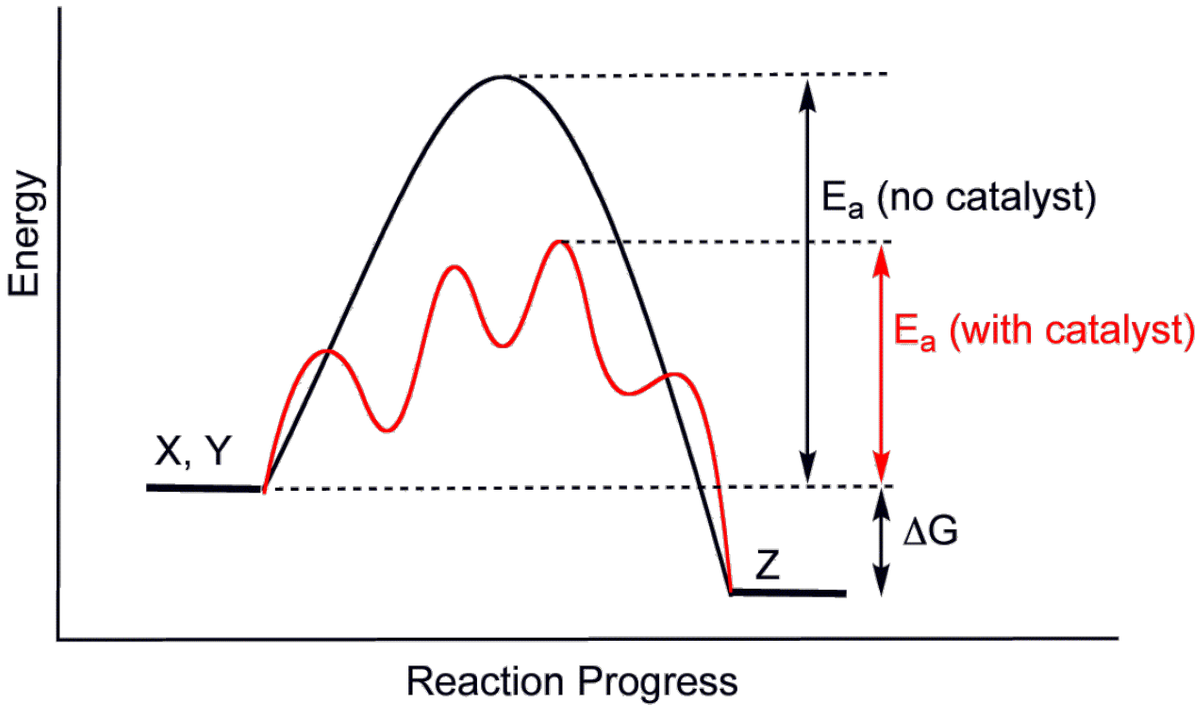




Molecular machines are single-molecule chemical engines!
All of them represent various *enzymes.*

Therefore, we have to consider single-molecule kinetics and thermodynamics of enzymes.

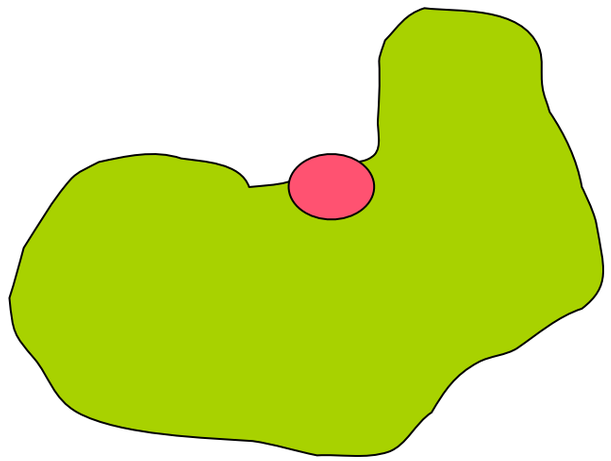
Catalytic reactions



Many reactions proceed only very slowly in their pure form. They can be however greatly accelerated when catalysts are added. The role of a catalyst is to lower the energy barrier for a reaction, thus strongly increasing its rate.

There are many inorganic catalysts. Any modern car is using the reaction of CO oxidation into CO_2 on a catalytic platinum surface.

Enzymes are single-molecule protein catalysts



Enzyme protein

The active center typically contains a metal ion. In its simplest version, the enzyme protein just provides mechanical support for the active ion and ensures an optimal local environment for the catalytic reaction event.

A catalytic reaction typically proceeds through formation of substrate-enzyme and product-enzyme complexes. There may be also several intermediate products, two different substrates, two different products etc. Protein conformations may also change depending on the ligand state.

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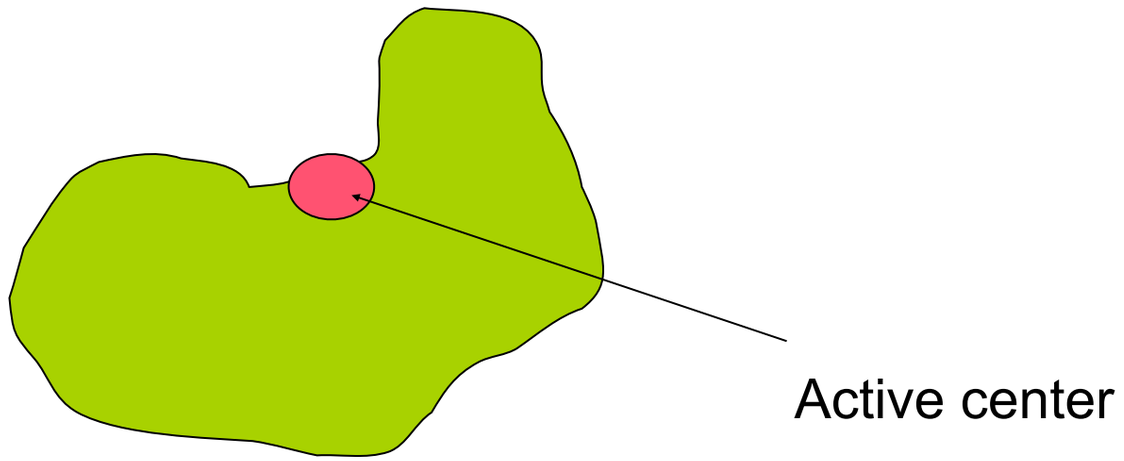

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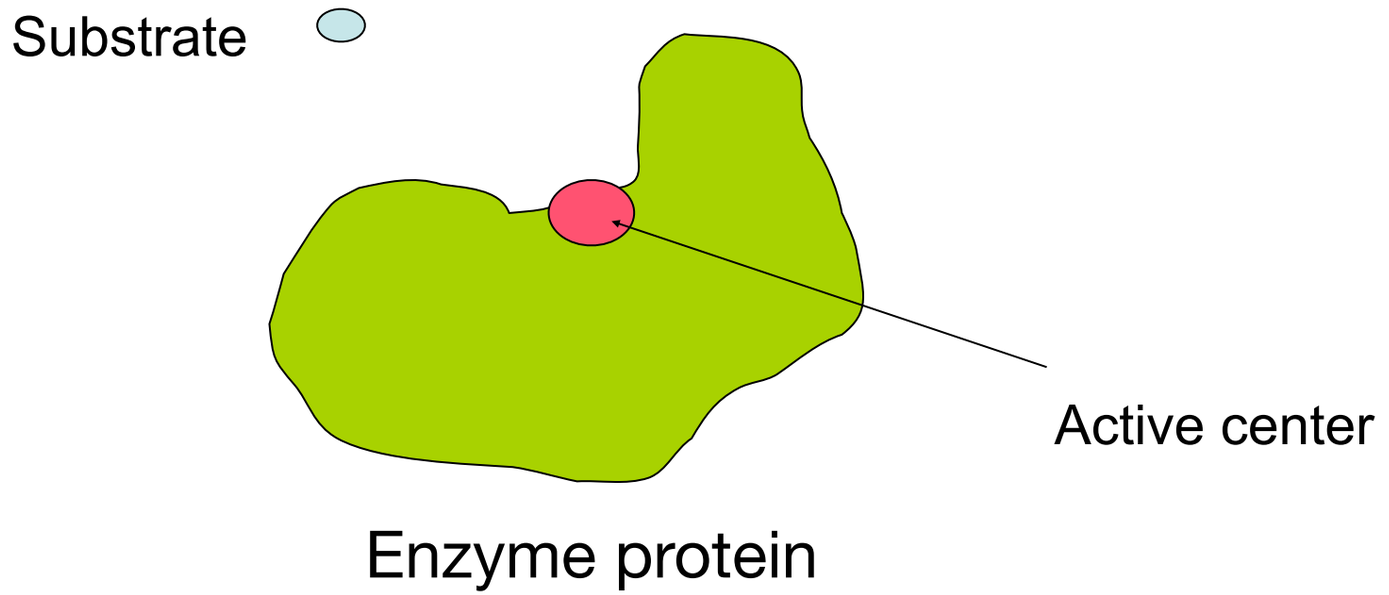

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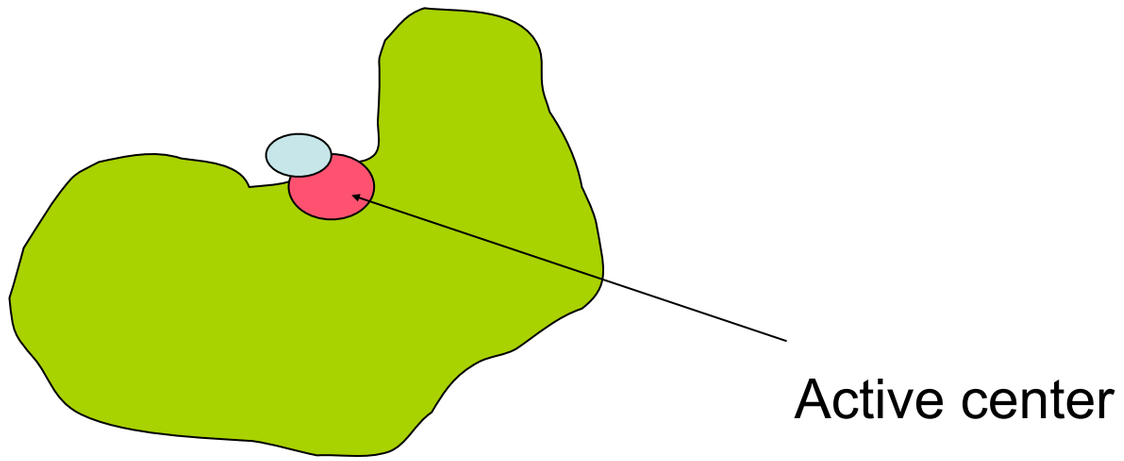

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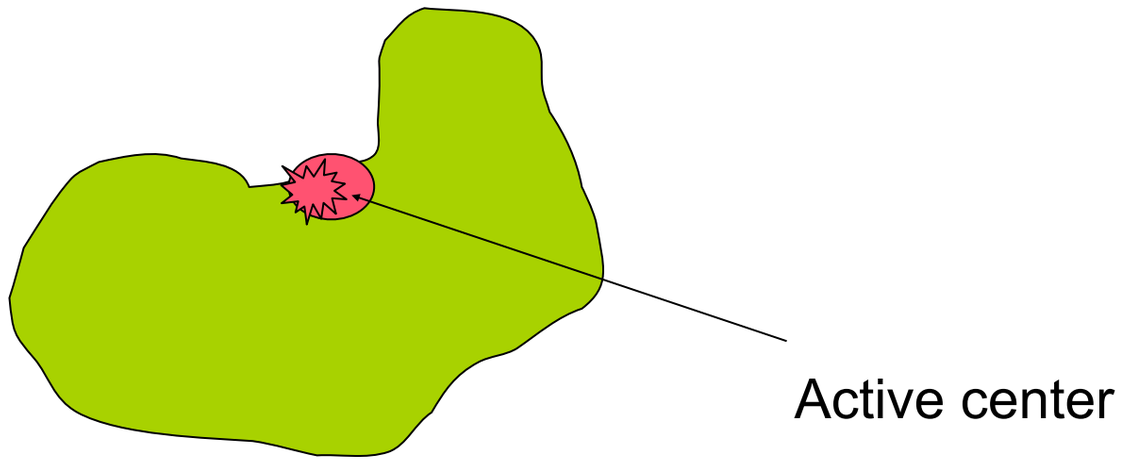

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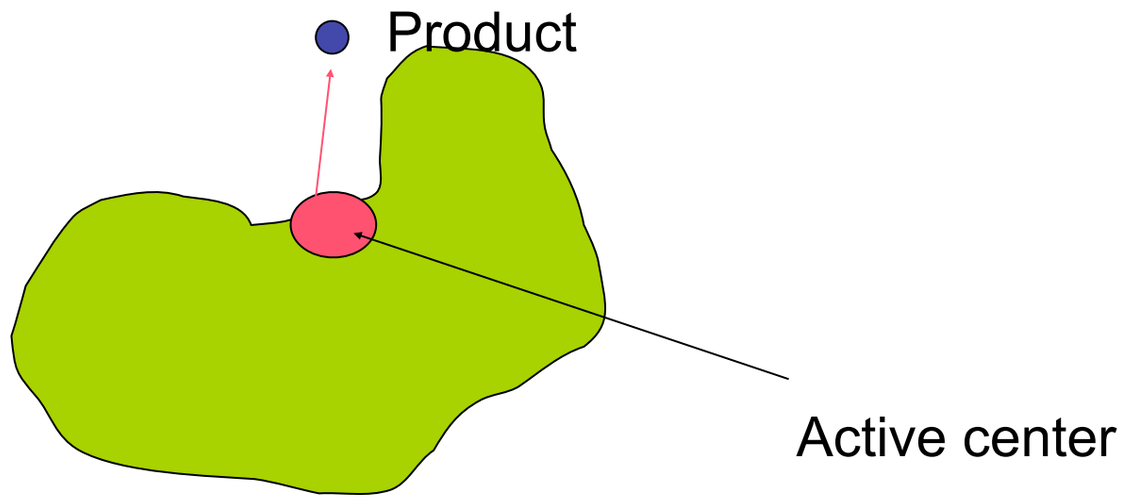

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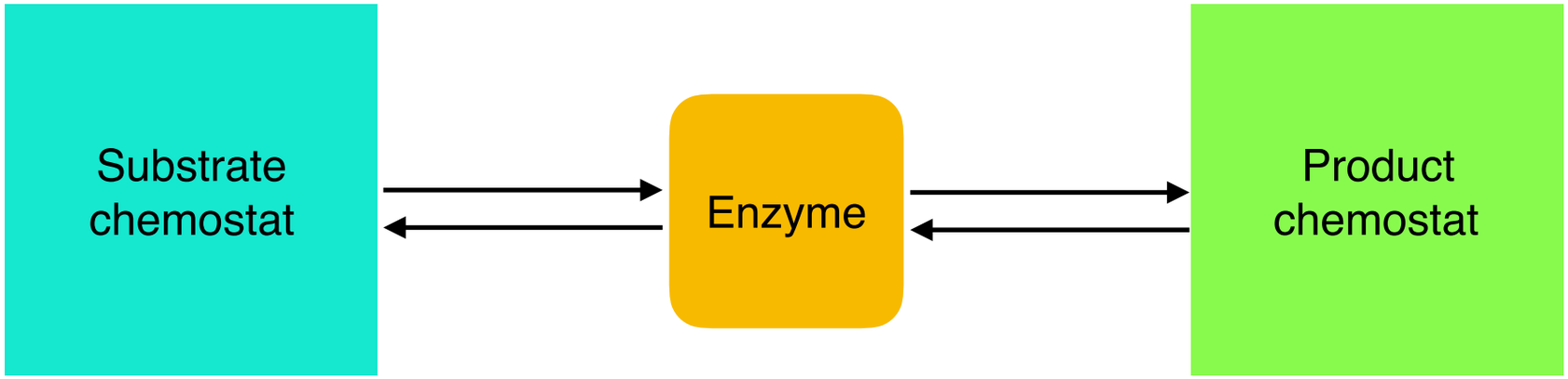
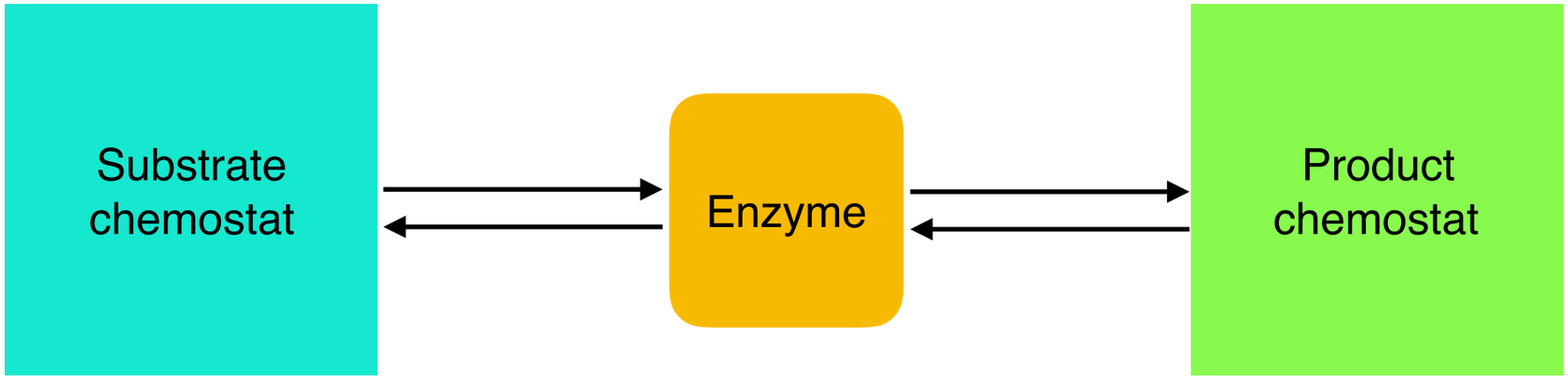
Markov network models of enzymes

First we will consider a simple reaction scheme. A substrate binds to a free enzyme and forms a substrate-enzyme complex. Then, the substrate is converted into a product and thus a product-enzyme complex is formed. Finally, the product is released and the enzyme returns to its free form. All these reaction steps are generally reversible. Each transition is characterised by some probability rate.

$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

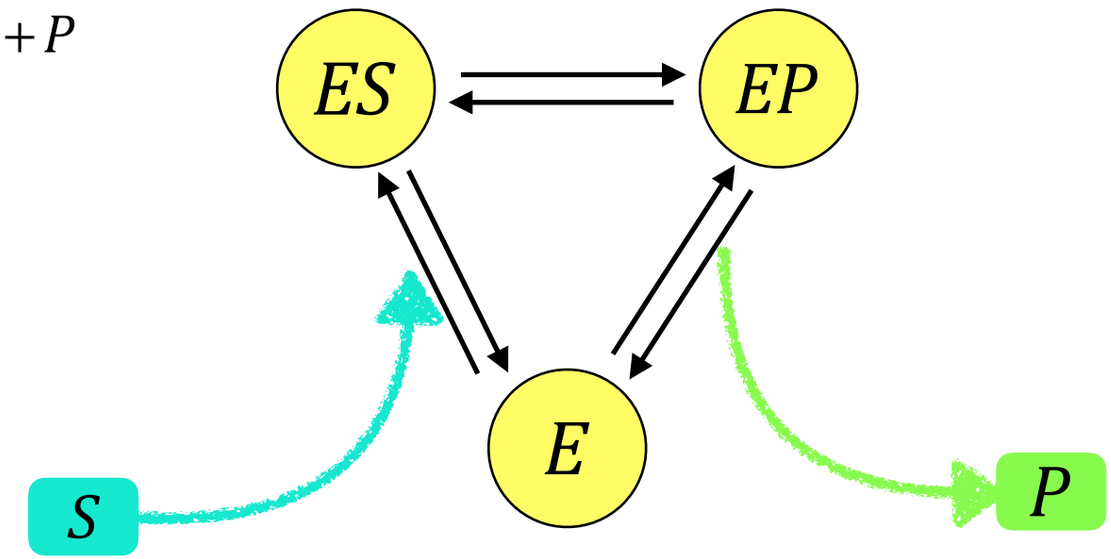
There is only one enzyme molecule. There are many available substrate and product molecules and their number practically does not change when the enzyme operates.



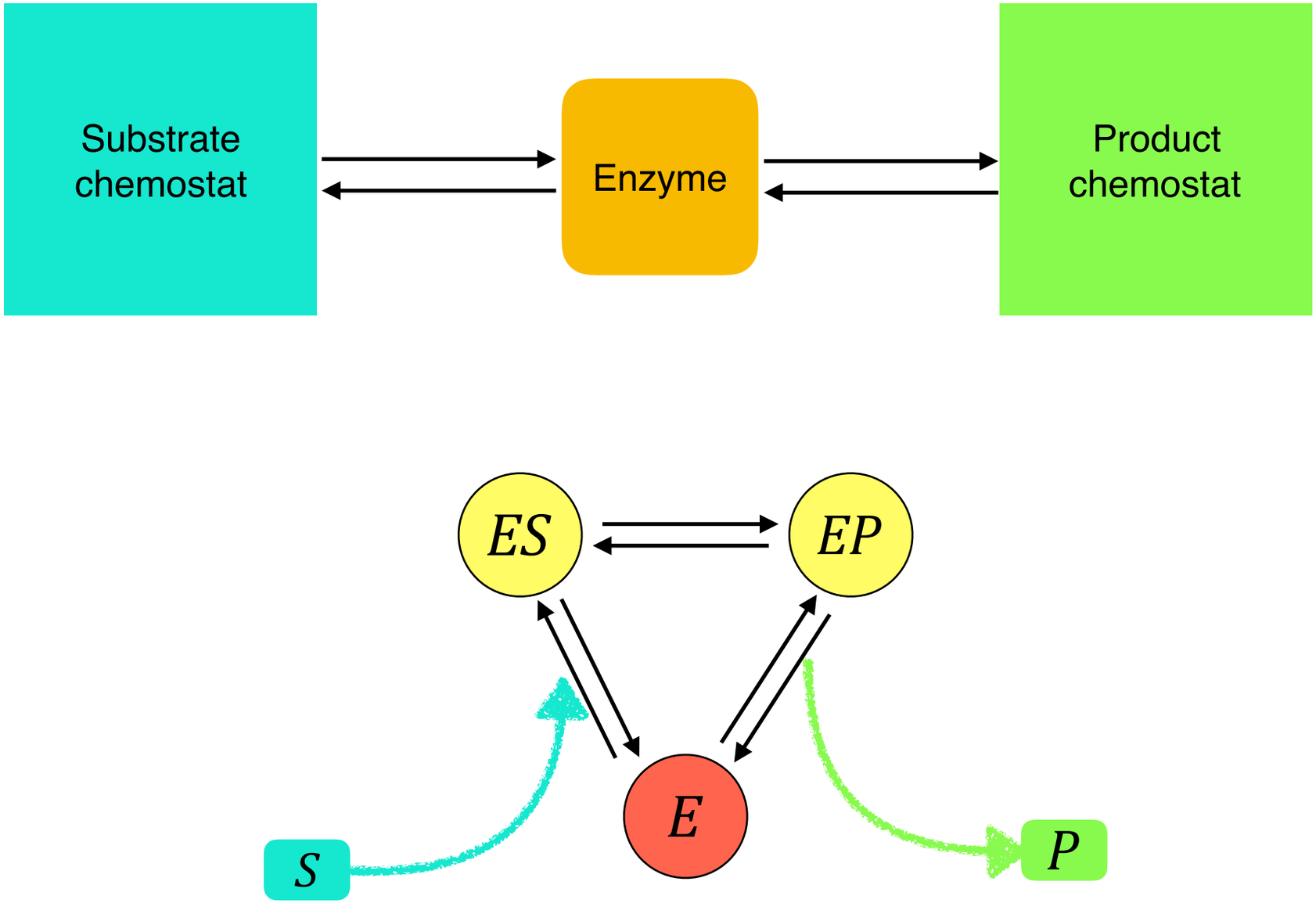


The reaction scheme

$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$



At each time moment, only one state can be occupied. Sudden jumps between the states take place, with the jump probability per unit time determined by the respective reaction rate. There is no memory of the previous states.



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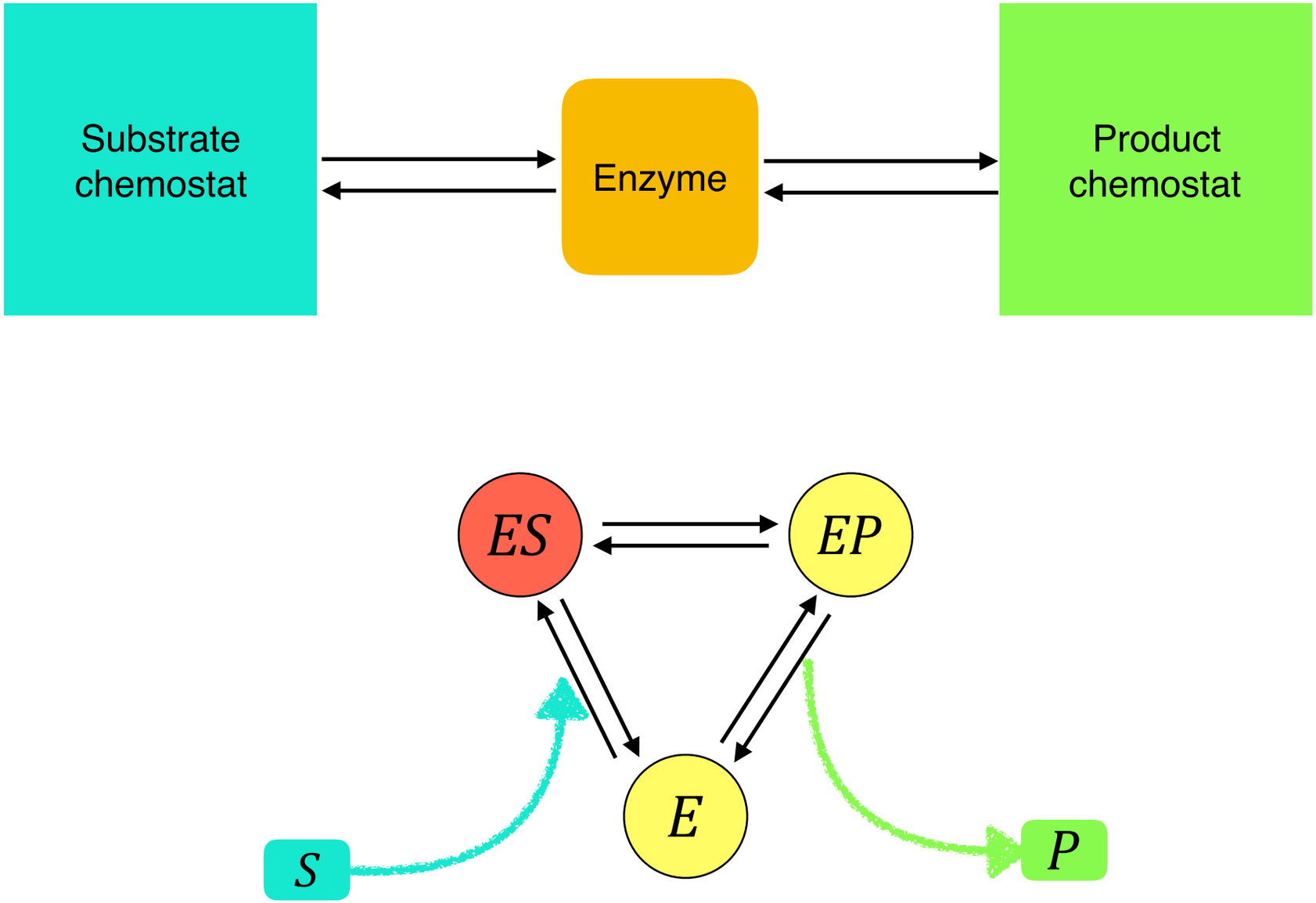

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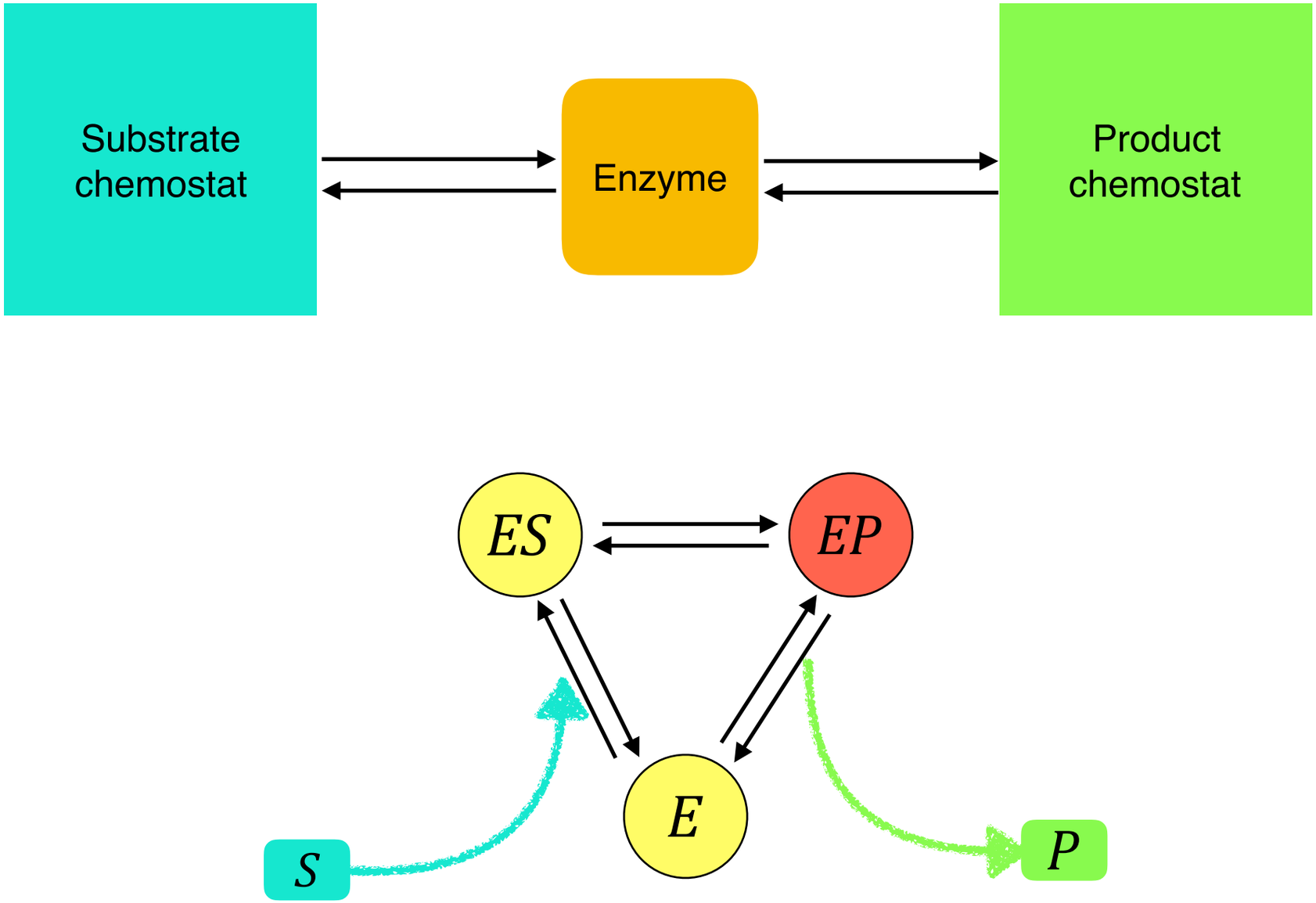

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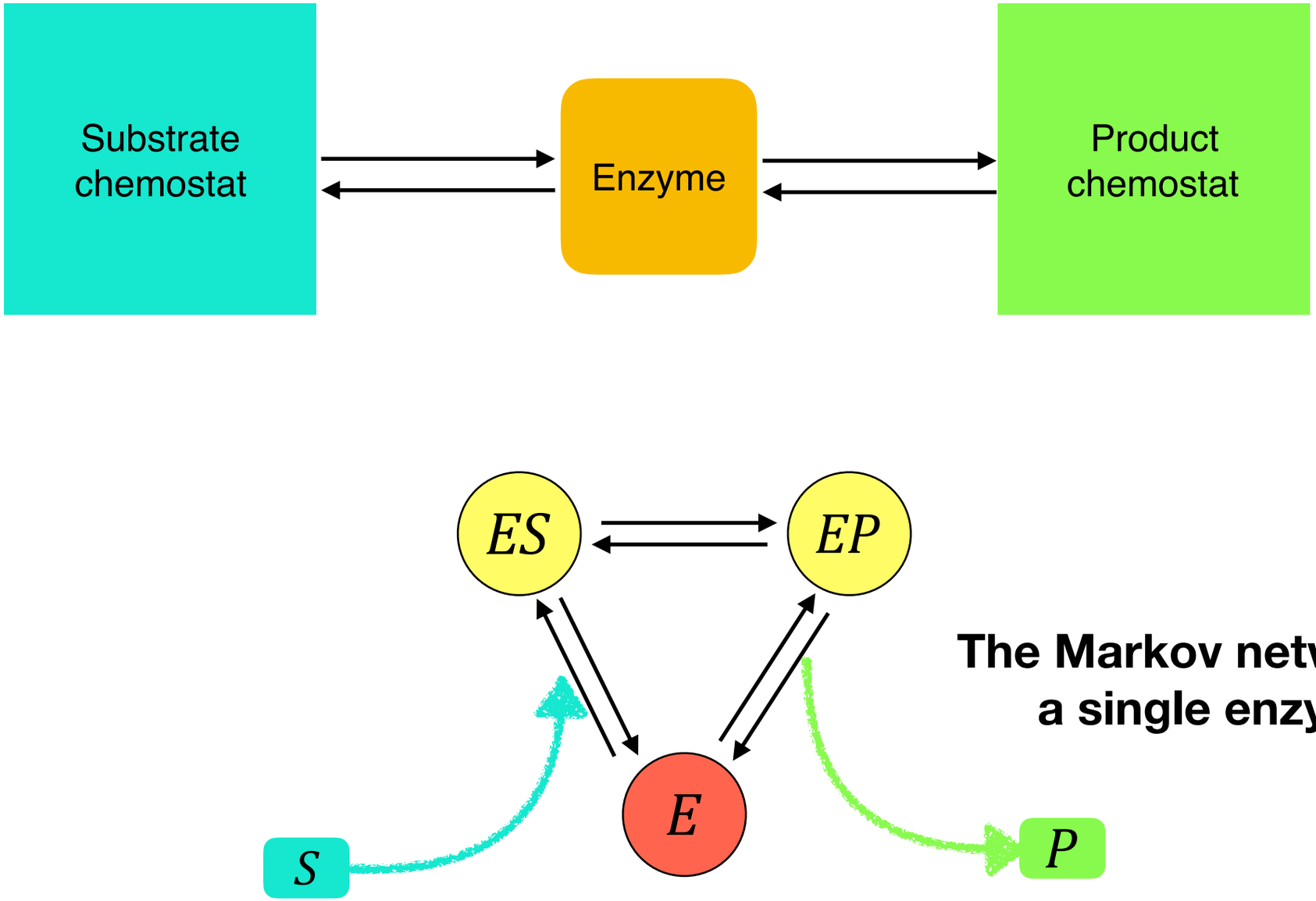
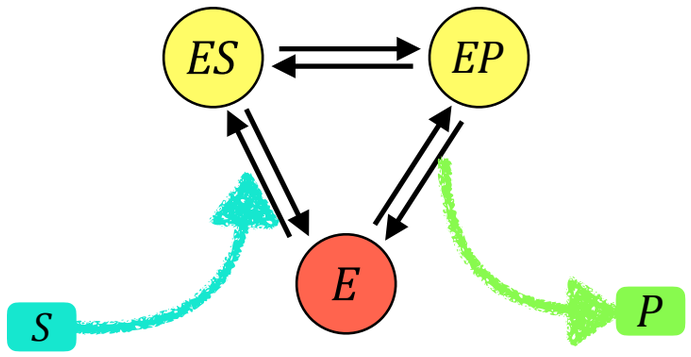


The Markov network of a single enzyme

At each time moment, only one state can be occupied. Sudden jumps between the states take place, with the jump probability per unit time determined by the respective reaction rate. There is no memory of the previous states.



Occupation probabilities of the states

$$p_E(t),\ \ p_{ES}(t),\ p_{EP}(t),\quad p_E(t)+p_{ES}(t)+p_{EP}(t)=1$$

Transition rates

$$w_{E\to ES},\ w_{ES\to EP},\ w_{EP\to E}$$

$$w_{E\leftarrow ES},\ w_{ES\leftarrow EP},\ w_{EP\leftarrow E}$$

$w_{E\to ES}=c_S v_{E\to ES},\quad w_{E\to EP}=c_P v_{E\to EP}$
where c_S and c_P are (constant) substrate and product concentrations.

How the occupation probability of the substrate-enzyme state will change within a short time because of transtions between the states E and ES?

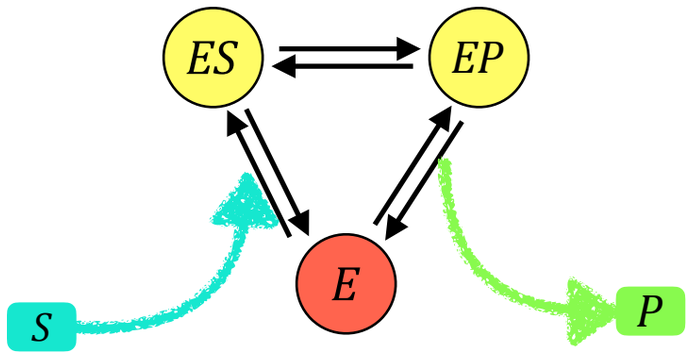
$$\Delta p_{ES}=\left(w_{E\to ES}\Delta t\right)p_E-\left(w_{E\leftarrow ES}\Delta t\right)p_{ES}\quad\Rightarrow\quad\frac{\Delta p_{ES}}{\Delta t}=w_{E\to ES}p_E-w_{E\leftarrow ES}p_{ES}$$

$$\frac{dp_{ES}}{dt}=w_{E\to ES}p_E-w_{E\leftarrow ES}p_{ES}+w_{ES\leftarrow EP}p_{EP}-w_{ES\to EP}p_{ES}$$

$$\frac{dp_{EP}}{dt}=w_{ES\to EP}p_{ES}-w_{ES\leftarrow EP}p_{EP}+w_{E\to EP}p_E-w_{EP\to E}p_{EP}$$

$$p_E(t)=1-p_{ES}(t)-p_{EP}(t)$$

This system represents the **master equation** for the considered Markov network.

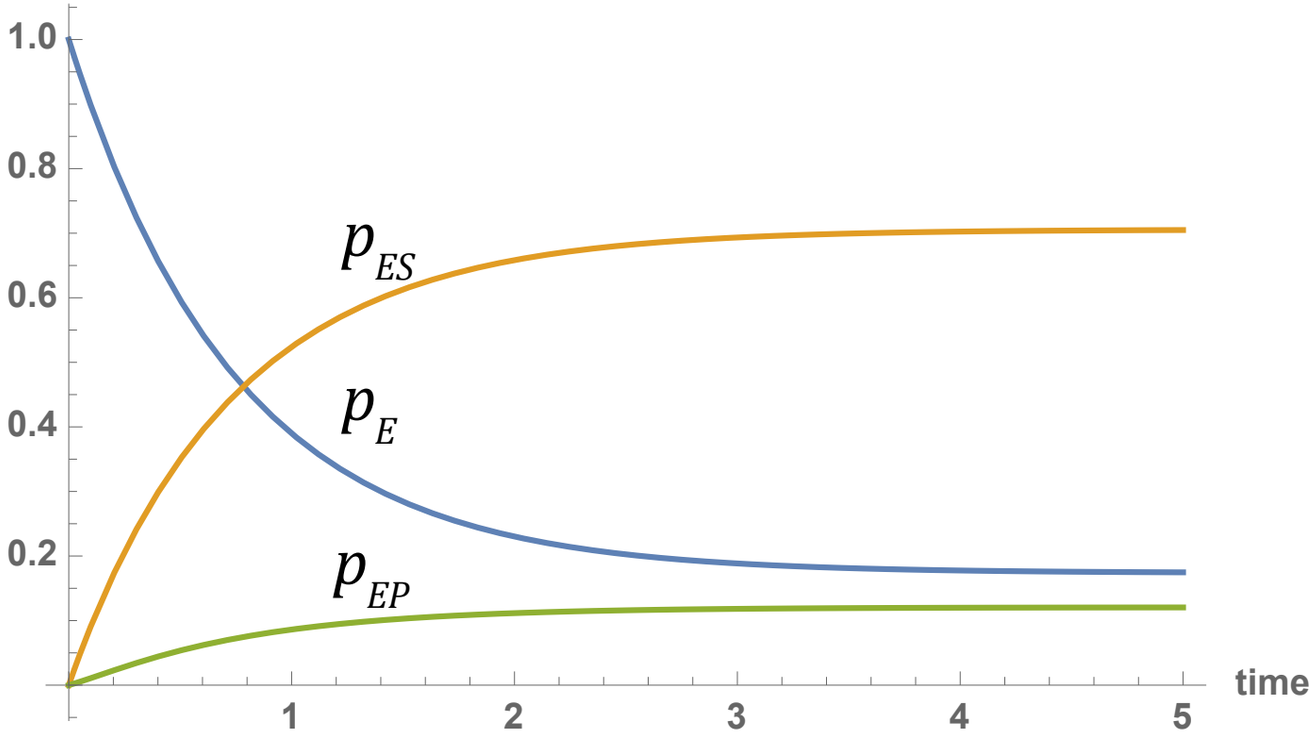


$$\frac{dp_{ES}}{dt} = w_{E\to ES}p_E - w_{E\leftarrow ES}p_{ES} + w_{ES\leftarrow EP}p_{EP} - w_{ES\to EP}p_E$$

$$\frac{dp_{EP}}{dt} = w_{ES\to EP}p_{ES} - w_{ES\leftarrow EP}p_{EP} + w_{EP\leftarrow E}p_E - w_{EP\to E}p_{EP}$$

$$p_E(t) = 1 - p_{ES}(t) - p_{EP}(t)$$

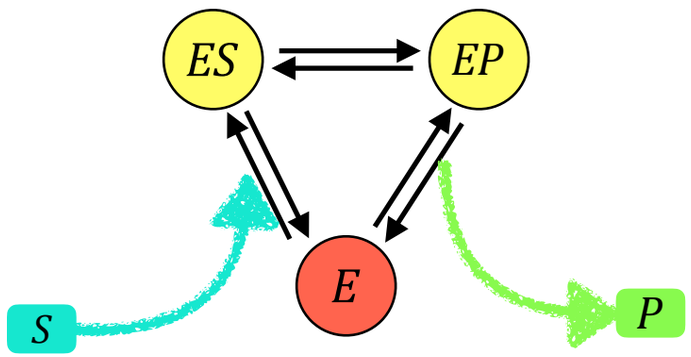
Suppose $w_{E\to ES} = 1\ \text{ms}^{-1}$, $w_{E\leftarrow ES} = 0.1\ \text{ms}^{-1}$, $w_{ES\to EP} = 1\ \text{ms}^{-1}$, $w_{ES\leftarrow EP} = 5\ \text{ms}^{-1}$, $w_{EP\to E} = 1\ \text{ms}^{-1}$, $w_{EP\leftarrow E} = 0.1\ \text{ms}^{-1}$ and that at the initial time moment $t = 0$ the enzyme is in the state E, that is $p_E(0) = 1$, $p_{ES}(0) = p_{EP}(0) = 0$.



The system evolves to a steady state.

$$\bar{p}_E = 0.17,\ \ \bar{p}_{ES} = 0.71,\ \ \bar{p}_{EP} = 0.12$$

Occupation probabilities in the steady state give relative times that the enzyme stay in different chemical states.

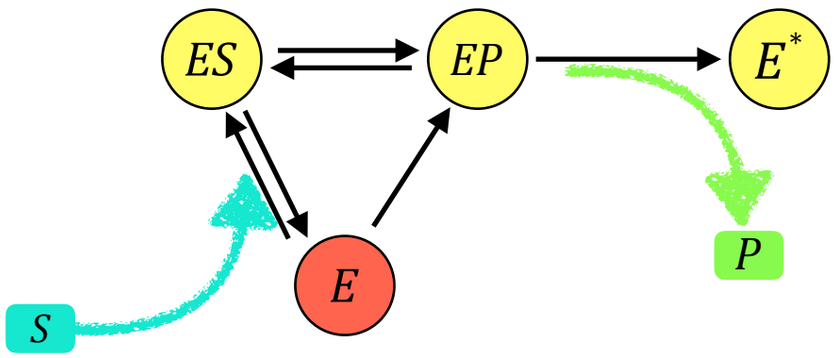


But we can also determine other statistical properties of the enzyme.

For example, what is the probability distribution for its cycle times?

To determine this distribution, we assume that, after product release, the enzyme goes into a non-reactive state E^*.

$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightarrow E^* + P$$



$$\frac{dp_{ES}}{dt} = w_{E\to ES}p_E - w_{E\leftarrow ES}p_{ES} + w_{ES\leftarrow EP}p_{EP} - w_{ES\to EP}p_{ES}$$

$$\frac{dp_{EP}}{dt} = w_{ES\to EP}p_{ES} - w_{ES\leftarrow EP}p_{EP} - w_{EP\to E}p_{EP}$$

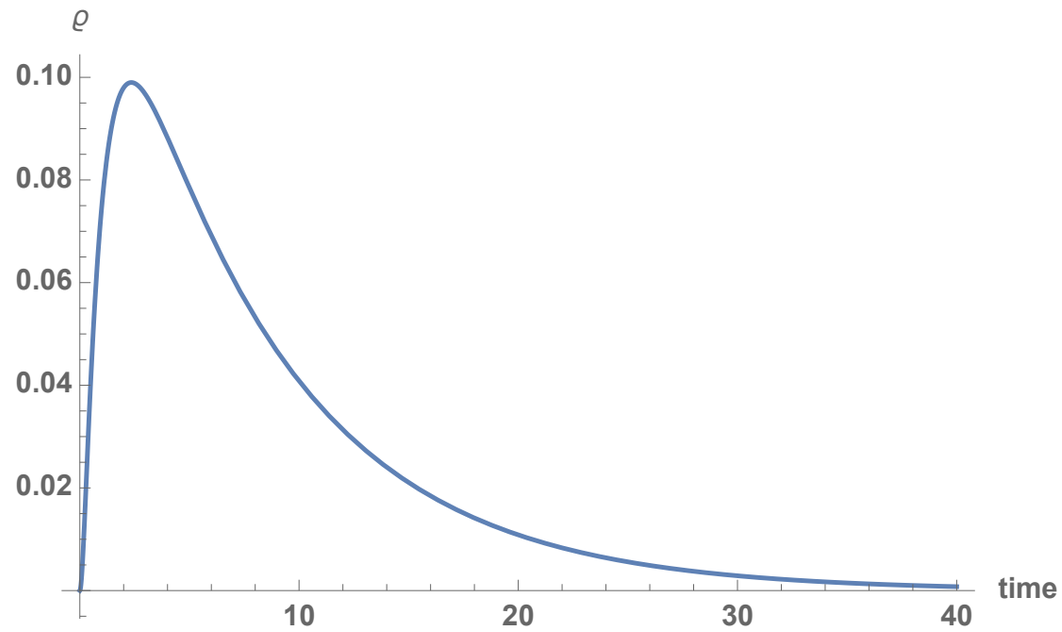
$$\frac{dp_E}{dt} = w_{E\leftarrow ES}p_{ES} - w_{E\to ES}p_E$$

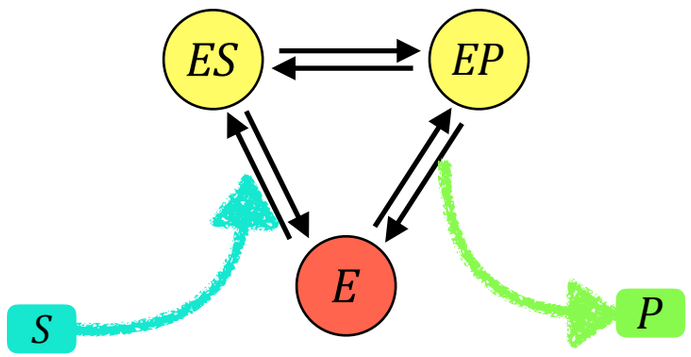
$$p_{E^*}(t) = 1 - p_{ES}(t) - p_{EP}(t) - p_E(t)$$

With the initial conditions $p_E(0)=1,\ p_{ES}(0)=p_{EP}(0)=p_{E^*}(0)=0,$ the solution $p_{E^*}(T)$ gives us the probability that the enzyme has finished its cycle by time T. Then, the probability that it will finish it turnover cycle within time ΔT will be $\rho(T)\Delta T$, where

$$\rho(T) = \frac{dp_{E^*}}{dT}$$

is the distribution of turnover times.





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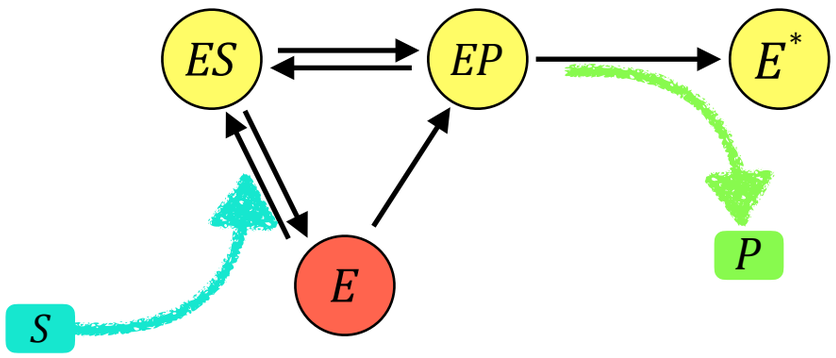

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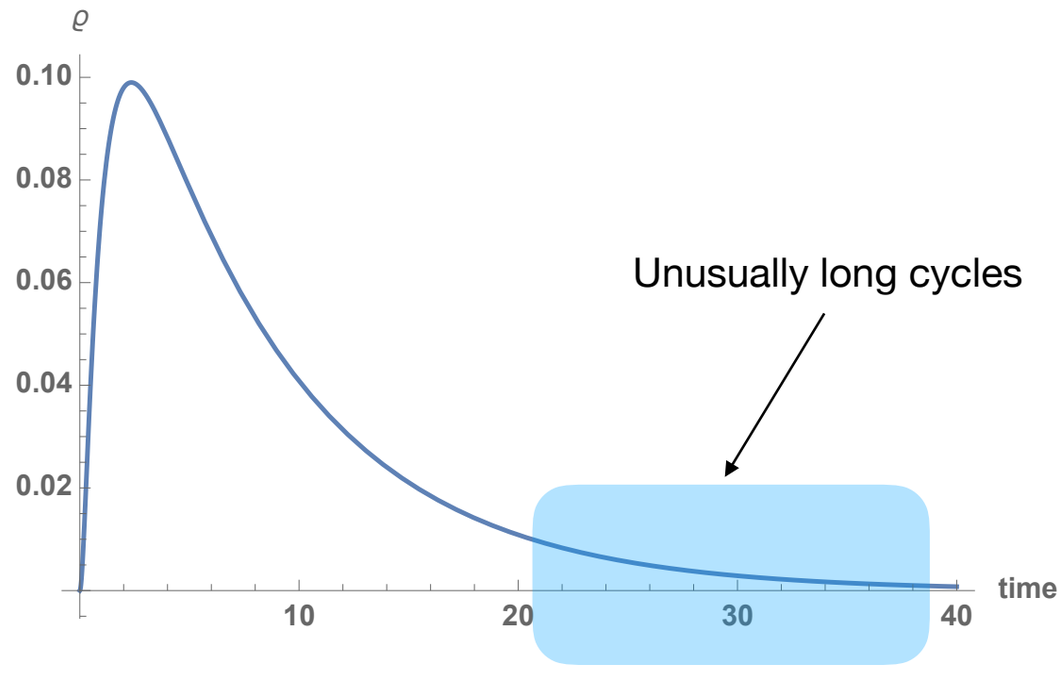
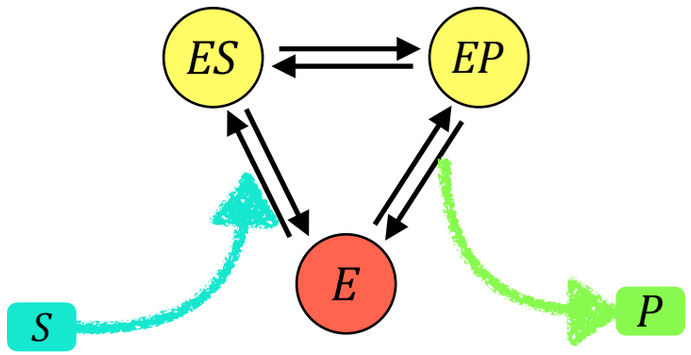
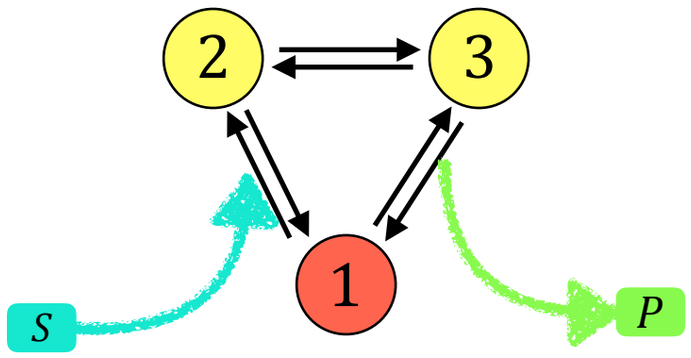






Now we will change the notations. We enumerate the states

$$"E"=1,\ "ES"=2,\ "EP"=3$$

and denote the transition rates as

$$w_{E\to ES}=k_{21},\ w_{E\leftarrow ES}=k_{12},w_{ES\to EP}=k_{32},\ w_{ES\leftarrow EP}=k_{23},w_{EP\to E}=k_{13},\ w_{EP\leftarrow E}=k_{31}$$

We arrive at state 2 from state 1

$$\frac{dp_1}{dt}=k_{12}p_2-k_{21}p_1+k_{13}p_3-k_{31}p_1$$
$$\frac{dp_2}{dt}=k_{21}p_1-k_{12}p_2+k_{23}p_3-k_{32}p_2$$
$$\frac{dp_3}{dt}=k_{32}p_2-k_{23}p_3+k_{31}p_1-k_{13}p_3$$

$$\Longleftrightarrow\quad \boxed{\frac{dp_i}{dt}=\sum_{j=1}^{3}\left(k_{ij}p_j-k_{ji}p_i\right)}$$

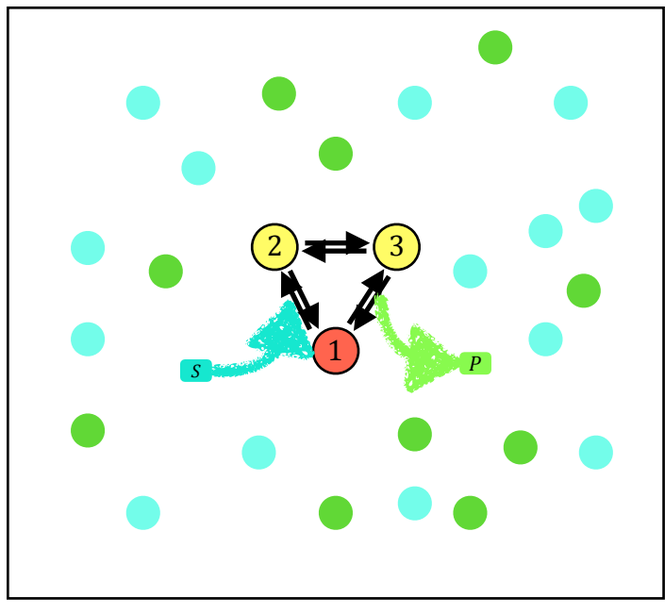
This is the general form of the master equation for Markov networks.

Detailed Balance conditions

The master equation can be also written as

$$\frac{dp_i}{dt} = \sum_j \left(k_{ij} p_j - k_{ji} p_i \right) \quad \Rightarrow \quad \frac{dp_i}{dt} = \sum_j J_{ij}$$

where $J_{ij} = k_{ij} p_j - k_{ji} p_i$ is the probability flux between the states i and j



$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

Suppose that the enzyme is in a small closed compartment. Then, the numbers of substrate and product molecules will change until the **thermal equilibrium** is reached. In this thermal equilibrium, the following conditions will be satisfied:

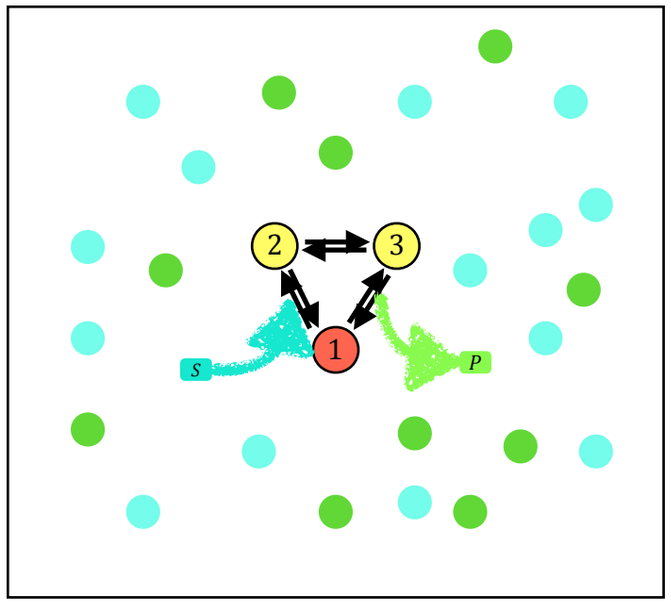
1. All probability fluxes vanish, $\bar{J}_{ij} = 0.$

2. The occupation probability of each state i is determined only by its Gibbs free energy g_i and the Boltzmann distribution holds,

$$\bar{p}_i = \frac{1}{Z} \exp\left(-\frac{g_i}{k_B T} \right)$$

What are the consequences of these conditions?

Detailed Balance conditions



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$$\frac{w_{ES\to EP}}{w_{ES\leftarrow EP}} = \exp\left(-\frac{g_{EP} - g_{ES}}{k_B T}\right)$$

Let us first consider a transition that does not involve binding or release of ligands (substrate or product). The detailed balance then implies that

$$\bar{J}_{ij} = k_{ij}\bar{p}_j - k_{ji}\bar{p}_i = 0 \quad \Rightarrow \quad \frac{k_{ij}}{k_{ji}} = \frac{\bar{p}_i}{\bar{p}_j} = \exp\left(-\frac{g_i - g_j}{k_B T}\right)$$

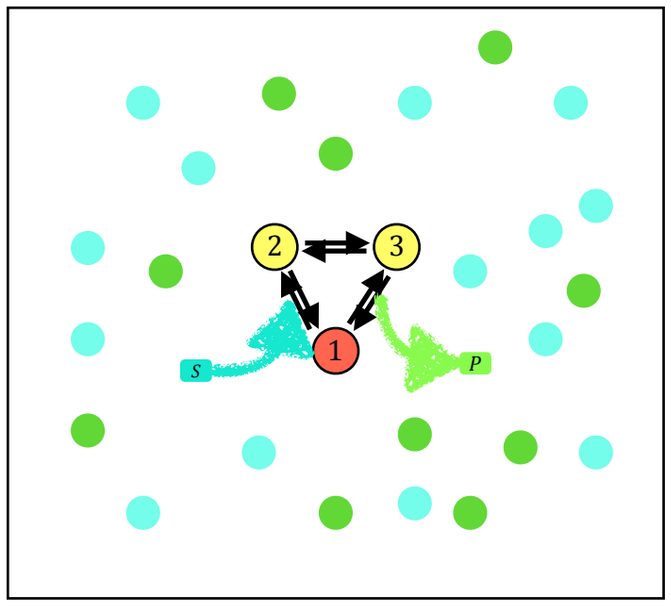
and therefore

$$\boxed{\frac{k_{ij}}{k_{ji}} = \exp\left(-\frac{g_i - g_j}{k_B T}\right)}$$

where g_i and g_j are Gibbs free energies of internal molecular states i and j

This condition is **general**, it holds not only at thermal equilibrium. The rates of forward and backward transitions between different molecular states **are not independent!**

Detailed Balance conditions



$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

Now we consider the transitions that involve binding or release of a substrate or product,

$$E + S \rightleftarrows ES$$

Because one free substrate molecule disappears after binding, the detailed balance condition in this case is

$$\frac{w_{E\to ES}}{w_{E\leftarrow ES}} = \exp\left(-\frac{g_{ES} - g_E - \mu_s}{k_B T}\right)$$

where μ_s is the chemical potential of free substrate molecules.

In a similar way, we obtain

$$\frac{w_{EP\to E}}{w_{EP\leftarrow E}} = \exp\left(-\frac{g_E + \mu_p - g_{EP}}{k_B T}\right)$$

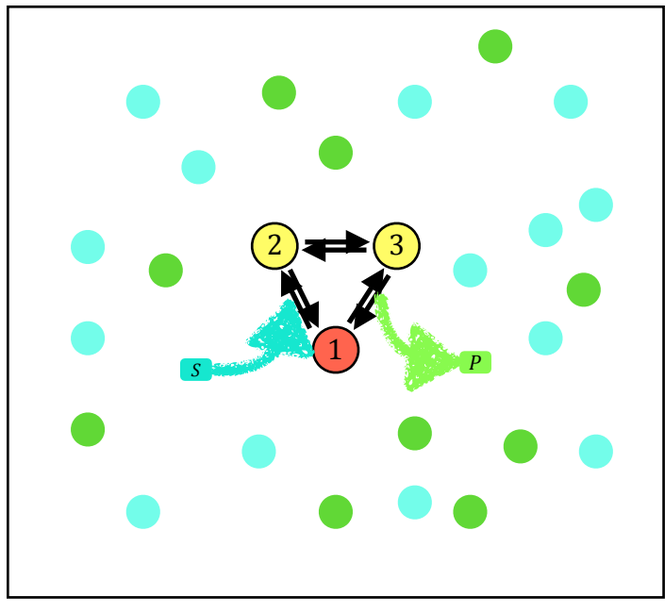
where μ_p is the chemical potential of free product molecules

Detailed Balance conditions

$$g_E + \mu_p - g_{EP} = -k_B T \ln\left(\frac{w_{EP\to E}}{w_{EP\leftarrow E}}\right)$$

$$g_{EP} - g_{ES} = -k_B T \ln\left(\frac{w_{ES\to EP}}{w_{ES\leftarrow EP}}\right)$$

$$g_{ES} - g_E - \mu_s = -k_B T \ln\left(\frac{w_{E\to ES}}{w_{E\leftarrow ES}}\right)$$



$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

Summing these three equations, we get a thermodynamic identity:

$$\mu_s - \mu_p = k_B T \ln\left(\frac{w_{EP\to E} w_{ES\to EP} w_{E\to ES}}{w_{EP\leftarrow E} w_{ES\leftarrow EP} w_{E\leftarrow ES}}\right)$$

This is the special case of the general **Schnakenberg identity** for chemical Markov networks.

The energy landscape

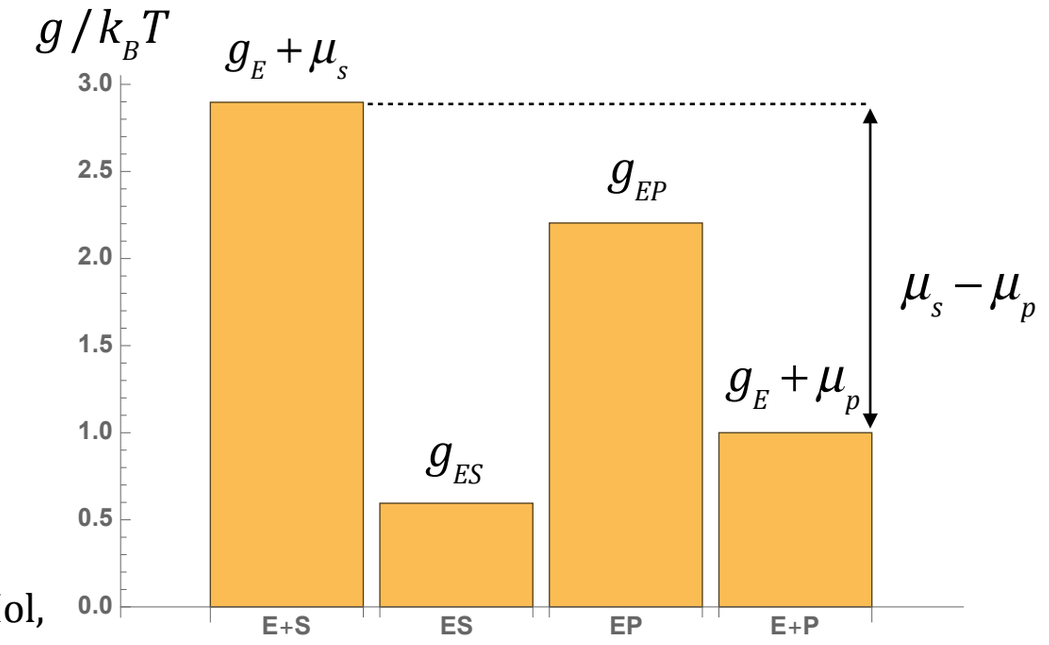
If all reaction rates are known, we can use detailed balance conditions to reconstruct the energy landscape of the enzyme. After simple calculation, we find

$$g_{EP} = (g_E + \mu_p) + k_B T \ln\left(\frac{w_{EP\to E}}{w_{EP\leftarrow E}}\right)$$

$$g_{ES} = (g_E + \mu_p) + k_B T \ln\left(\frac{w_{EP\to E}}{w_{EP\leftarrow E}}\right) + k_B T \ln\left(\frac{w_{ES\to EP}}{w_{ES\leftarrow EP}}\right)$$

$$\left(g_E + \mu_s\right) = (g_E + \mu_p) + k_B T \ln\left(\frac{w_{EP\to E}}{w_{EP\leftarrow E}}\right) + k_B T \ln\left(\frac{w_{ES\to EP}}{w_{ES\leftarrow EP}}\right) + k_B T \ln\left(\frac{w_{E\to ES}}{w_{ES\leftarrow E}}\right)$$

$$\mu_s - \mu_p = k_B T \ln\left(\frac{w_{EP\to E}}{w_{EP\leftarrow E}}\right) + k_B T \ln\left(\frac{w_{ES\to EP}}{w_{ES\leftarrow EP}}\right) + k_B T \ln\left(\frac{w_{E\to ES}}{w_{ES\leftarrow E}}\right)$$



$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

Suppose $v_{E\to ES} = 0.1\ \mathrm{ms^{-1}mMol^{-1}}$, $w_{E\leftarrow ES} = 0.1\ \mathrm{ms^{-1}}$, $w_{ES\to EP} = 1\ \mathrm{ms^{-1}}$,
$w_{ES\leftarrow EP} = 5\ \mathrm{ms^{-1}}$, $w_{EP\to E} = 1\ \mathrm{ms^{-1}}$, $v_{EP\leftarrow E} = 0.1\ \mathrm{ms^{-1}mMol^{-1}}$, $c_s = 10\ \mathrm{mMol}$, $c_p = 3\ \mathrm{mMol}$,
$w_{E\to ES} = c_s v_{E\to ES} = 1\ \mathrm{ms^{-1}}$, $w_{EP\leftarrow E} = c_p v_{EP\leftarrow E} = 3\ \mathrm{ms^{-1}}$

$$g_{EP} = (g_E + \mu_p) + 1.204\ k_B T$$
$$g_{ES} = (g_E + \mu_p) - 0.405\ k_B T$$
$$\left(g_E + \mu_s\right) = (g_E + \mu_p) + 1.897\ k_B T$$

$$\mu_s - \mu_p = 1.897\ k_B T$$

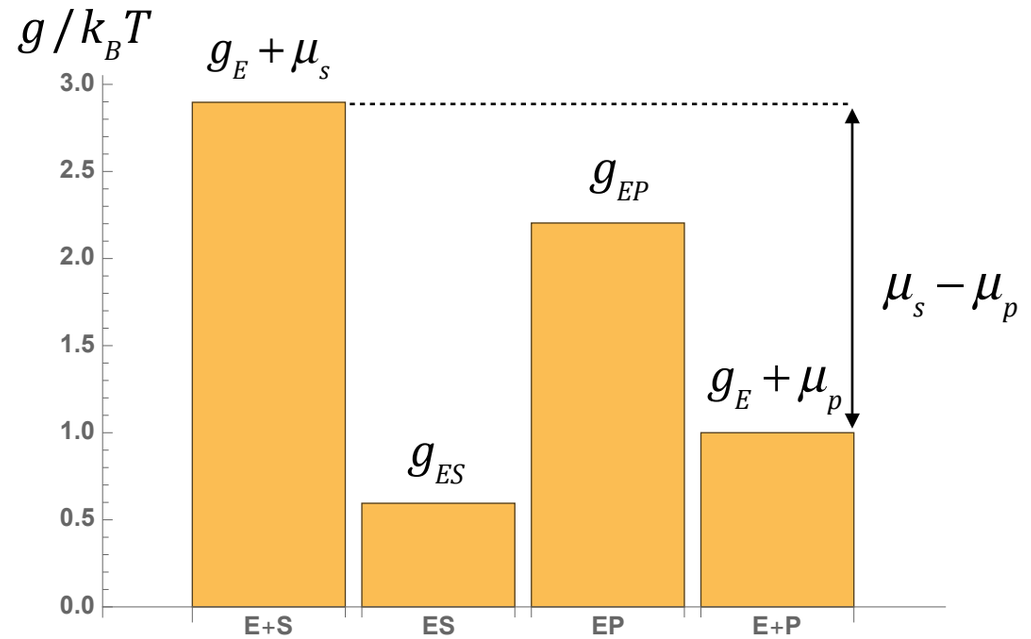
The energy landscape

Suppose $v_{E\to ES}=0.1\ \mathrm{ms^{-1}mMol^{-1}}$, $w_{E\leftarrow ES}=0.1\ \mathrm{ms^{-1}}$, $w_{ES\to EP}=1\ \mathrm{ms^{-1}}$,
$w_{ES\leftarrow EP}=5\ \mathrm{ms^{-1}}$, $w_{EP\to E}=1\ \mathrm{ms^{-1}}$, $v_{EP\leftarrow E}=0.1\ \mathrm{ms^{-1}mMol^{-1}}$, $c_s=10\ \mathrm{mMol}$, $c_p=3\ \mathrm{mMol}$,
$w_{E\to ES}=c_s v_{E\to ES}=1\ \mathrm{ms^{-1}}$, $w_{EP\leftarrow E}=c_p v_{EP\leftarrow E}=3\ \mathrm{ms^{-1}}$

$$g_{ES}-(g_E+\mu_p)=0.405\ k_BT$$

$$g_{EP}-(g_E+\mu_p)=1.204\ k_BT$$

$$\mu_s-\mu_p=1.897\ k_BT$$



$$S+E\rightleftharpoons ES\rightleftharpoons EP\rightleftharpoons E+P$$

Chemical potential of a weak solutions of substrate and product molecules are

$$\mu_s=\mu_s^0+k_BT\ln c_s$$
$$\mu_p=\mu_p^0+k_BT\ln c_p$$

where μ_s^0 and μ_p^0 are the chemical potentials at unit concentrations.

$$\mu_s^0-\mu_p^0=\mu_s-\mu_p-k_BT\ln\frac{c_s}{c_p}=0.693\ k_BT$$

Entropy at nanoscales

Entropy is microscopically defined as (it is then also known as Shannon or information entropy)

$$\boxed{S = -\sum_i p_i \ln p_i}$$

Microscopic entropy is measured in bits, 1 bit = $\ln 2$

What is the rate of change of the entropy?

$$\frac{dS}{dt} = -\sum_i \frac{dp_i}{dt}\ln p_i - \sum_i p_i \frac{1}{p_i}\frac{dp_i}{dt} = -\sum_i \frac{dp_i}{dt}\ln p_i == -\sum_{ij} J_{ij}\ln p_i = -\frac{1}{2}\left(\sum_{ij} J_{ij}\ln p_i + \sum_{ij} J_{ji}\ln p_j\right) = \frac{1}{2}\sum_{ij} J_{ij}\left(\ln p_j - \ln p_i\right)$$

$\frac{d}{dt}\sum_i p_i = 0$

$\frac{dp_i}{dt} = \sum_j J_{ij}$

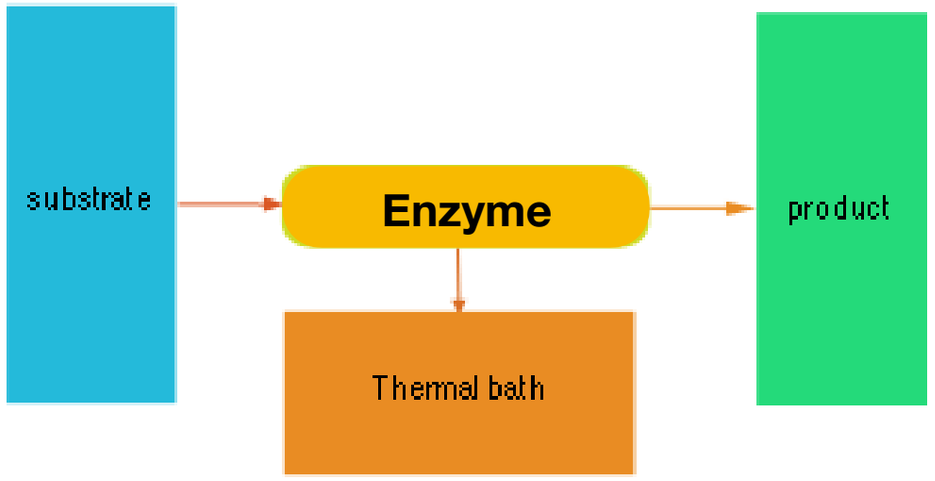
$J_{ji} = -J_{ij}$

$J_{ij} = k_{ij}p_j - k_{ji}p_i$

Second Law of Thermodynamics for microscopic open systems

$$\frac{dS}{dt} = \frac{1}{2}\sum_{ij} J_{ij} \ln \frac{p_j}{p_i} = \frac{1}{2}\sum_{ij}\left[J_{ij} \ln\left(\frac{k_{ij} p_j}{k_{ji} p_i}\right) - J_{ij} \ln\left(\frac{k_{ij}}{k_{ji}}\right)\right] \equiv \sigma - h$$

σ h



$$\frac{dS}{dt} = \sigma - h$$

Entropy production

Entropy export

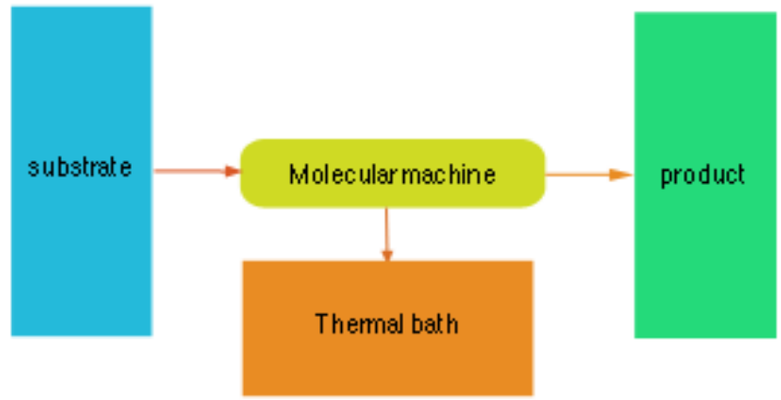
Entropy production and flow in a Markov network

$$\frac{dS}{dt} = \sigma - h$$

Entropy production

Entropy export

The Second Law of thermodynamics for a microscopic system



$$\sigma = \frac{1}{2}\sum_{ij}\sigma_{ij}, \quad h = \frac{1}{2}\sum_{ij}h_{ij}$$

$$\sigma_{ij} = J_{ij}\ln\left(\frac{k_{ij}p_j}{k_{ji}p_i}\right) \qquad \sigma_{ij} \geq 0$$

$$h_{ij} = J_{ij}\ln\left(\frac{k_{ij}}{k_{ji}}\right)$$

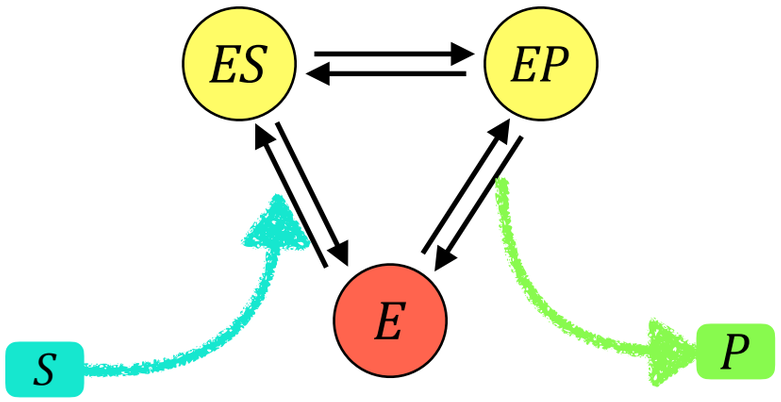
Entropy production in transition $i \rightleftarrows j$

Entropy export (or import) in transition $i \rightleftarrows j$

In a non-equilibrium state, each transition is accompanied by entropy production and by export or import of entropy. At thermal equilibrium, all fluxes vanish - therefore entropy is not produced, imported or exported in this state.

In the steady state $\frac{dS}{dt} = 0$ and therefore $\sigma = h$. However, $\sigma_{ij} \neq h_{ij}$!

Entropy production and flow in an enzyme



$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

We can determine entropy production and its export in the steady state.

It can be shown that the flux is constant for all transitions in the steady state of this system.

$$J_{E,ES} = J_{ES,EP} = J_{EP,E} = J \qquad J = w_{E\to ES}\bar{p}_E - w_{E\leftarrow ES}\bar{p}_{ES}$$

The flux yields the mean turnover rate of the enzyme.

$$\frac{dS}{dt} = \sigma - h = 0$$

$$\sigma = \sigma_{E,ES} + \sigma_{ES,EP} + \sigma_{EP,E}$$

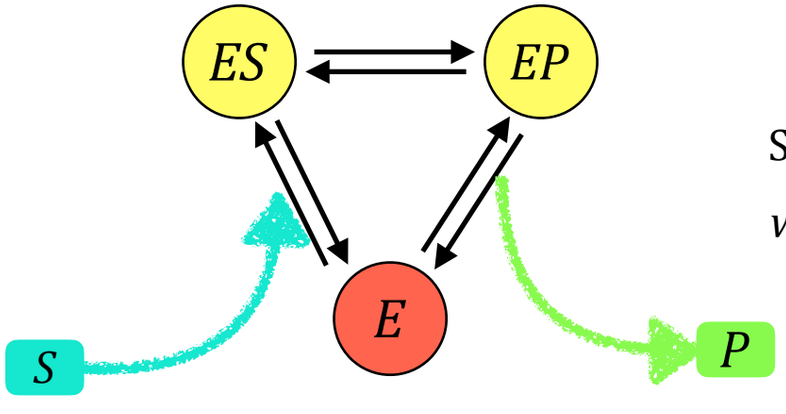
$$h = h_{E,ES} + h_{ES,EP} + h_{EP,E}$$

$$\sigma = h$$

$$\sigma_{E,ES} = J\ln\left(\frac{w_{E\to ES}\bar{p}_E}{w_{E\leftarrow ES}\bar{p}_{ES}}\right),\quad \sigma_{ES,EP} = J\ln\left(\frac{w_{E\to ES}\bar{p}_{ES}}{w_{ES\leftarrow EP}\bar{p}_{EP}}\right),\quad \sigma_{EP,E} = J\ln\left(\frac{w_{EP\to E}\bar{p}_{EP}}{w_{EP\leftarrow E}\bar{p}_E}\right)$$

$$h_{E,ES} = J\ln\left(\frac{w_{E\to ES}}{w_{E\leftarrow ES}}\right),\quad h_{ES,EP} = J\ln\left(\frac{w_{ES\to EP}}{w_{E\leftarrow ES}}\right),\quad h_{EP,E} = J\ln\left(\frac{w_{EP\to E}}{w_{EP\leftarrow E}}\right)$$

Entropy production and flow in an enzyme

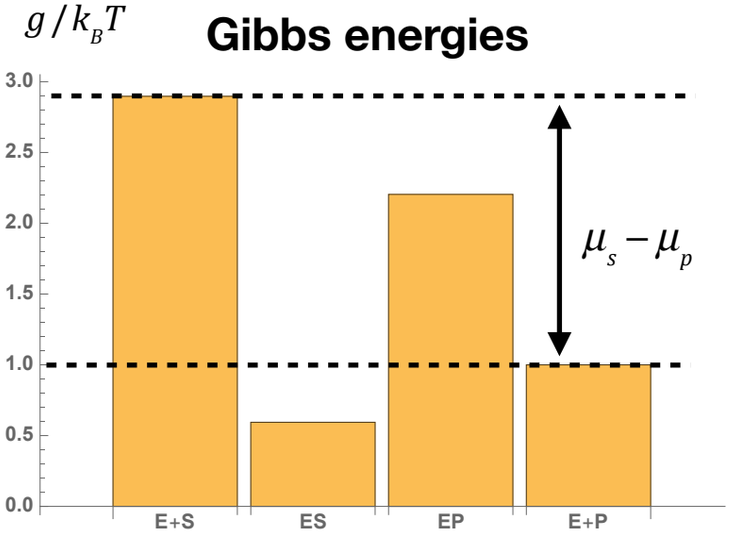


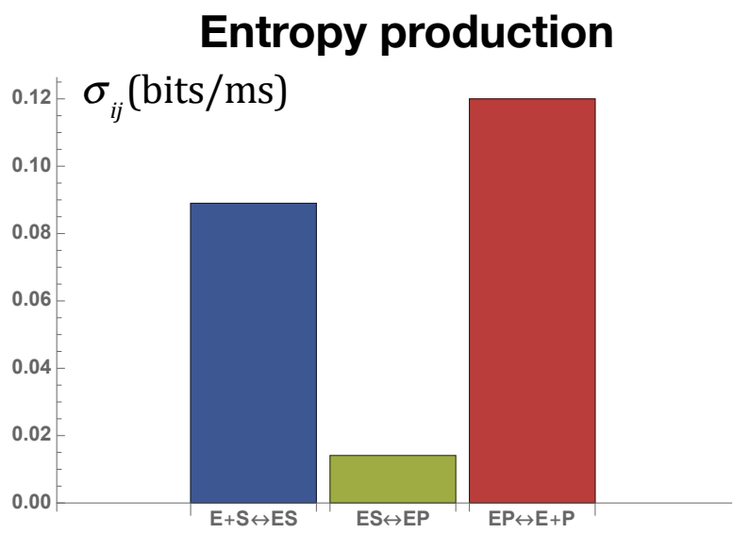
$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

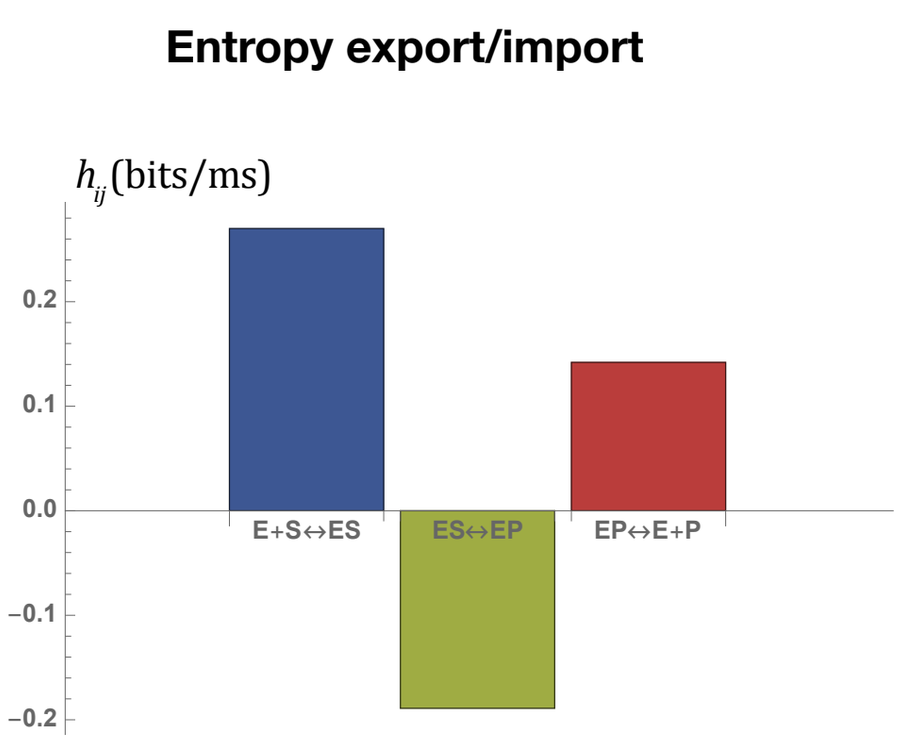
Suppose $v_{E\to ES} = 0.1\ \text{ms}^{-1}\text{mMol}^{-1}$, $w_{E\leftarrow ES} = 0.1\ \text{ms}^{-1}$, $w_{ES\to EP} = 1\ \text{ms}^{-1}$,

$w_{ES\leftarrow EP} = 5\ \text{ms}^{-1}$, $w_{EP\to E} = 1\ \text{ms}^{-1}$, $v_{EP\leftarrow E} = 0.1\ \text{ms}^{-1}$, $c_s = 10\ \text{mMol}$, $c_p = 3\ \text{mMol}$

Turnover rate $J = 0.081\ \text{ms}^{-1}$, turnover time $T = 1/J = 12.3\ \text{ms}$





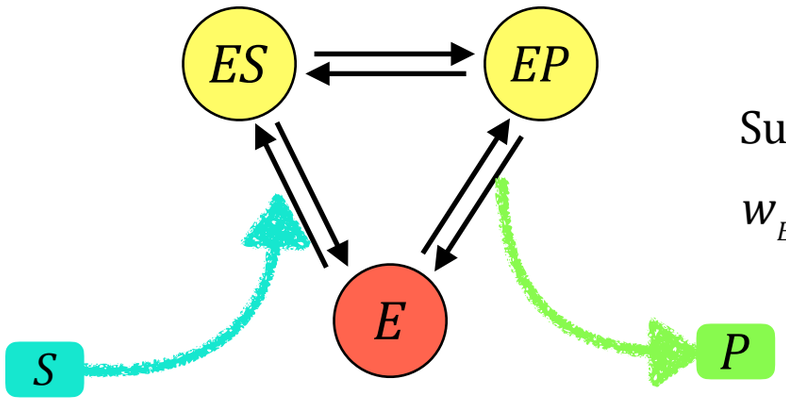


This enzyme generates (and exports) entropy

at the rate $\sigma = 0.223$ bits/ms,

or $\sigma / J = 2.73$ bits per its turnover time.

Entropy production and flow in an enzyme



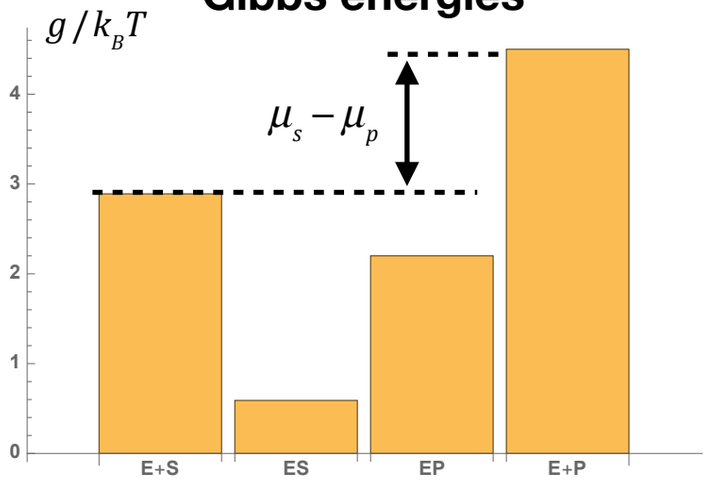
$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

Suppose $v_{E\to ES} = 0.1\ \mathrm{ms}^{-1}\mathrm{mMol}^{-1}$, $w_{E\leftarrow ES} = 0.1\ \mathrm{ms}^{-1}$, $w_{ES\to EP} = 1\ \mathrm{ms}^{-1}$,
$w_{ES\leftarrow EP} = 5\ \mathrm{ms}^{-1}$, $w_{EP\to E} = 1\ \mathrm{ms}^{-1}$, $v_{EP\leftarrow E} = 0.1\ \mathrm{ms}^{-1}$, $c_s = 10$ mMol, $c_p = 100$ mMol

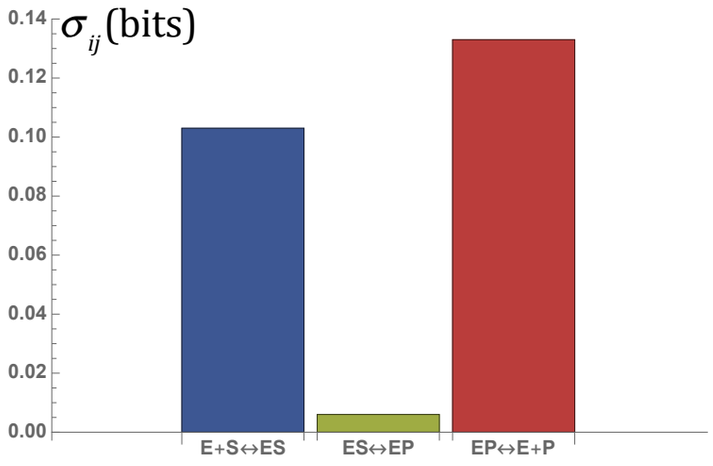
Under such conditions, the enzyme operates,
on the average, in the opposite direction (converts product into substrate)

Flux $J = -0.057\ \mathrm{ms}^{-1}$, turnover time $T = 1/|J| = 17.5$ ms

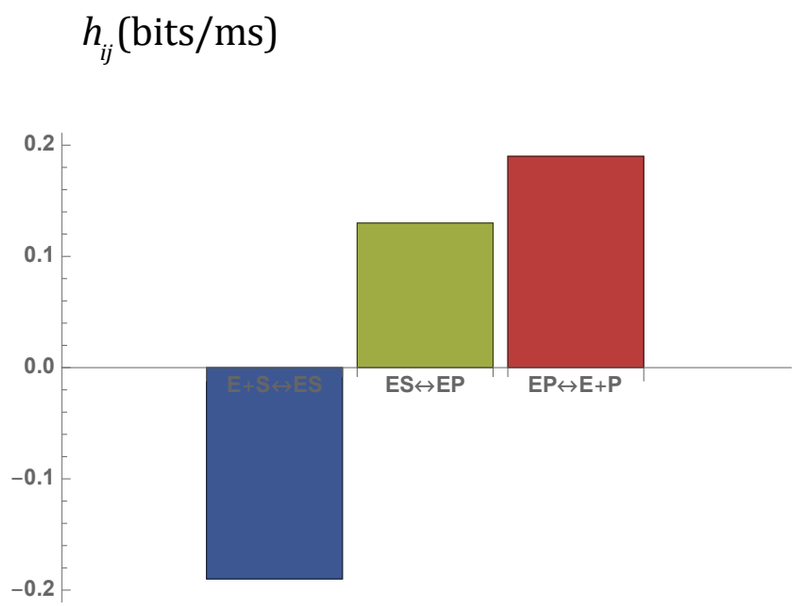
Gibbs energies



Entropy production



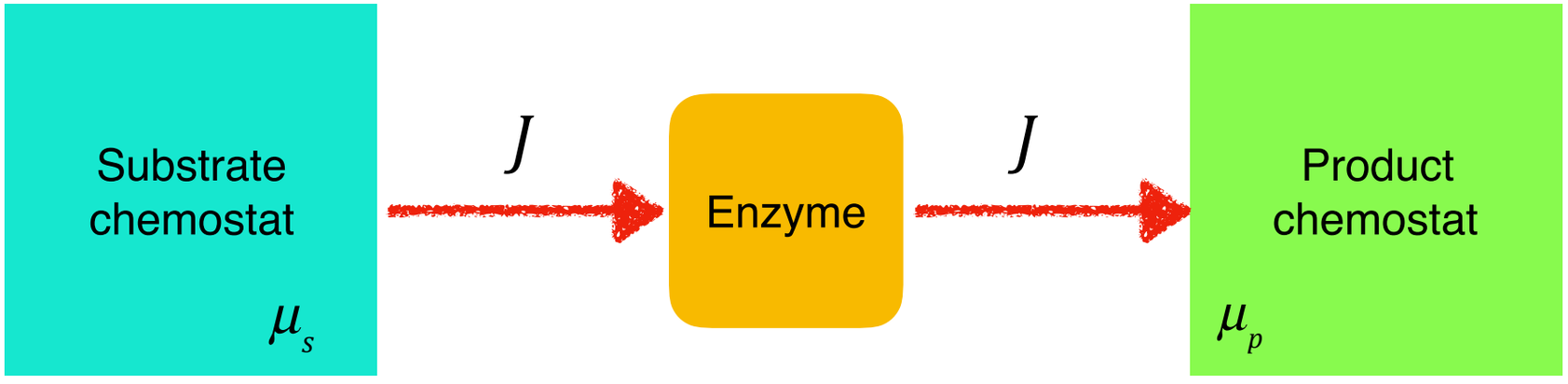
Entropy export/import



This enzyme generates (and exports) entropy
at the rate $\sigma = 0.133$ bits/ms,
or $\sigma / J = 2.32$ bits per its turnover time.

Direction of enzymic reaction

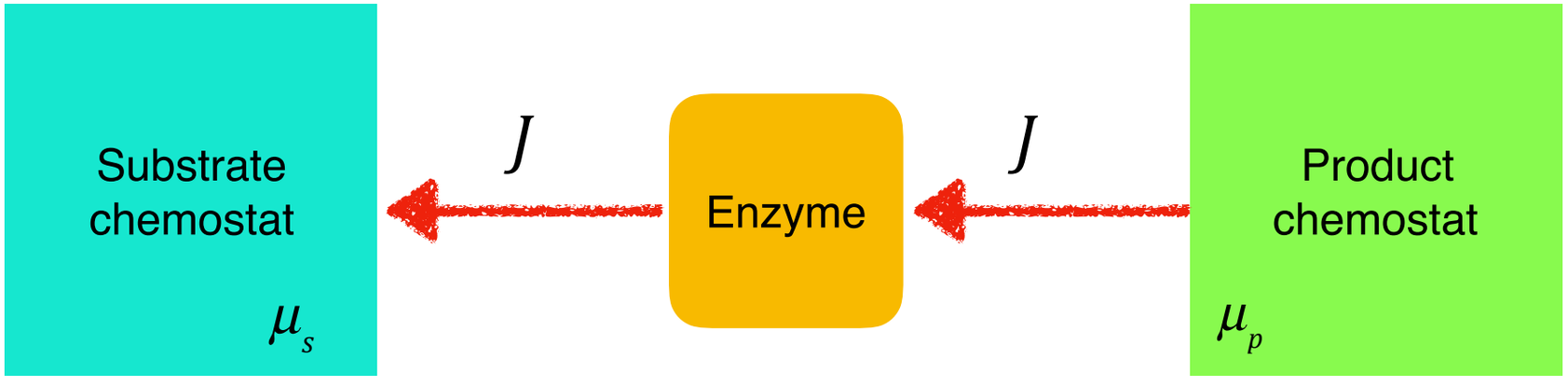
$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$



$$\mu_s > \mu_p$$

Direction of enzymic reaction

$$S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$



$$\mu_s < \mu_p$$

$$\mu_s = \mu_s^0 + k_B T \ln c_s$$
$$\mu_p = \mu_p^0 + k_B T \ln c_p$$

Reaction direction can be controlled by changing substrate and/or product concentrations.

The direction is reversed (the flux vanishes, *J* = 0) when

$$\mu_s = \mu_p \quad \Rightarrow \quad \mu_s^0 + k_B T \ln c_s = \mu_p^0 + k_B T \ln c_p$$

$$\ln \frac{c_s}{c_p} = \frac{\mu_p^0 - \mu_s^0}{k_B T}$$

The energy landscape

Suppose $\nu_{E\to ES}=0.1\ \mathrm{ms^{-1}mMol^{-1}}$, $w_{E\leftarrow ES}=0.1\ \mathrm{ms^{-1}}$, $w_{ES\to EP}=1\ \mathrm{ms^{-1}}$,

$w_{ES\leftarrow EP}=5\ \mathrm{ms^{-1}}$, $w_{EP\to E}=1\ \mathrm{ms^{-1}}$, $\nu_{EP\leftarrow E}=0.1\ \mathrm{ms^{-1}mMol^{-1}}$, $c_s=10\ \mathrm{mMol}$, $c_p=3\ \mathrm{mMol}$,

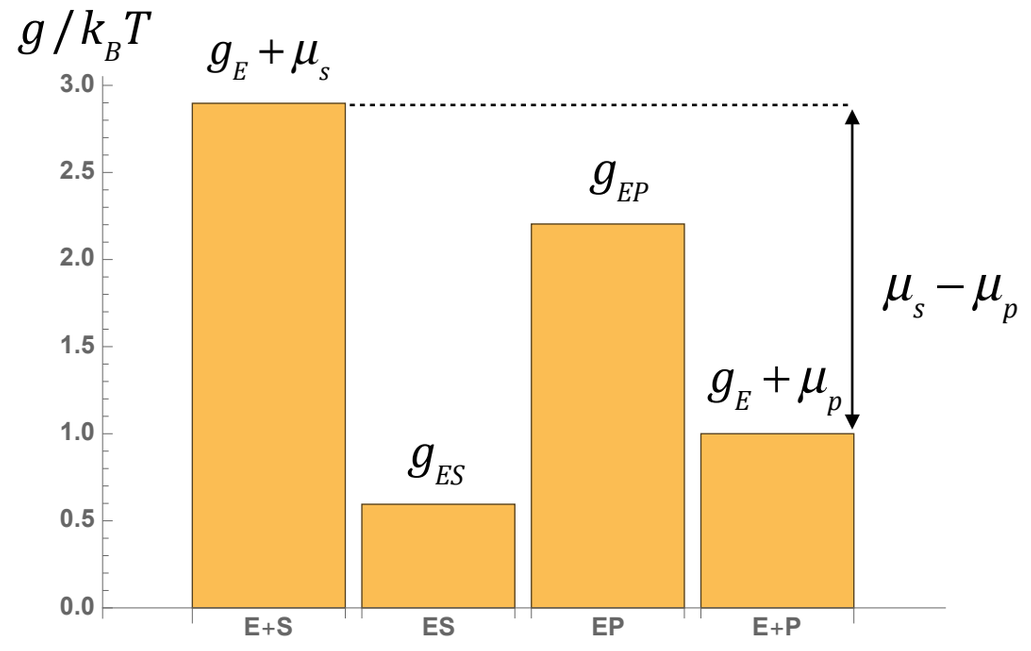
$w_{E\to ES}=c_s\nu_{E\to ES}=1\ \mathrm{ms^{-1}}$, $w_{EP\leftarrow E}=c_p\nu_{EP\leftarrow E}=3\ \mathrm{ms^{-1}}$

$$g_{ES}-(g_E+\mu_p)=0.405\ k_BT$$

$$g_{EP}-(g_E+\mu_p)=1.204\ k_BT$$

$$\mu_s^0-\mu_p^0==0.693\ k_BT$$

$$S+E\rightleftharpoons ES\rightleftharpoons EP\rightleftharpoons E+P$$



The reaction direction becomes reversed at the concentration ratio

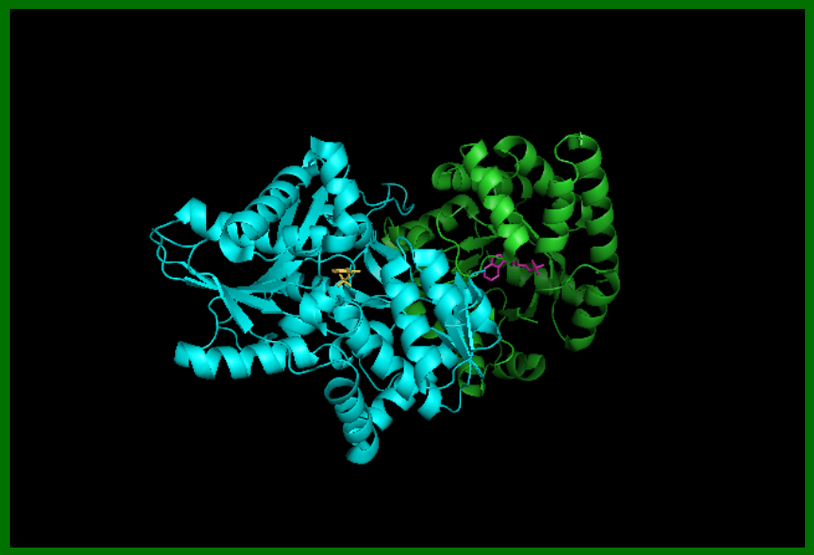
$$\frac{\tilde{c}_p}{\tilde{c}_s}=\exp\left(\frac{\mu_s^0-\mu_p^0}{k_BT}\right)=e^{0.693}=1.99$$

But suppose that the difference of chemical potentials of products and substrates (at unit concentrations) is large. Then the required reversion ratio becomes extremely large.

$$\mu_s^0-\mu_p^0==20\ k_BT\ \Rightarrow\ \frac{\tilde{c}_p}{\tilde{c}_s}=\exp\left(\frac{\mu_s^0-\mu_p^0}{k_BT}\right)=e^{20}=4.85\times10^8$$

Such reactions are practically irreversible.

Channeling enzyme Tryptophan Synthase



This enzyme synthesises an essential amino acid tryptophan from serine and indole glycerol phosphate (IGP). It is employed by all bacteria, plants, yeast and molds, but not by the higher organisms (they get this amino acid with food). Its substrate IGP is present only in small amounts in a cell, and high efficiency is required. Its intermediate product indole is hydrophobic and can easily escape through the cell membrane. Therefore, its release into cytoplasm must be avoided.

Nature has found an elegant solution for these constraints. The entire synthesis with 13 elementary reaction steps is performed within a single enzyme molecule with two catalytic active centers, and the intermediate indole is channeled within the protein from one center to another.

This enzyme has been extensively experimentally investigated. Several reviews are available, with the authors describing tryptophan synthase as **„an allosteric molecular factory“** and **„a channeling nanomachine“.** Recently, we have constructed a complete microscopic kinetic theory and investigated stochastic thermodynamics of such chemical nanomachine.

D. Loutchko, D. Gonze and A. Mikhailov „Single-molecule stochastic analysis of channeling enzyme tryptophan synthase“ J. Phys. Chem. B 120, 2179 (2016)
D. Loutchko, M. Eisbach and A. Mikhailov „Stochastic thermodynamics of a chemical nanomachine: the channeling enzyme tryptophan synthase“ J. Chem. Phys. 146, 025101 (2017)

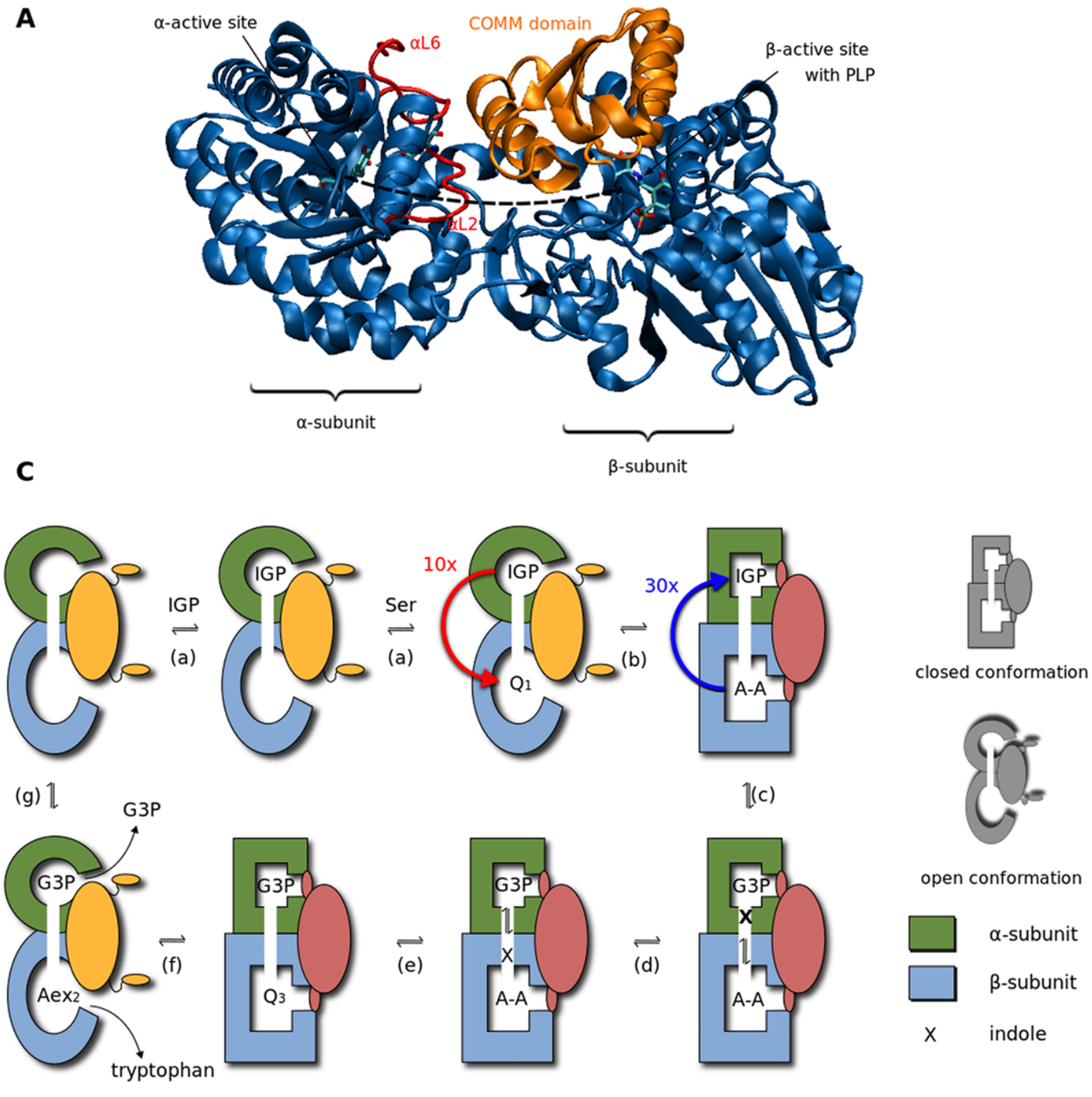
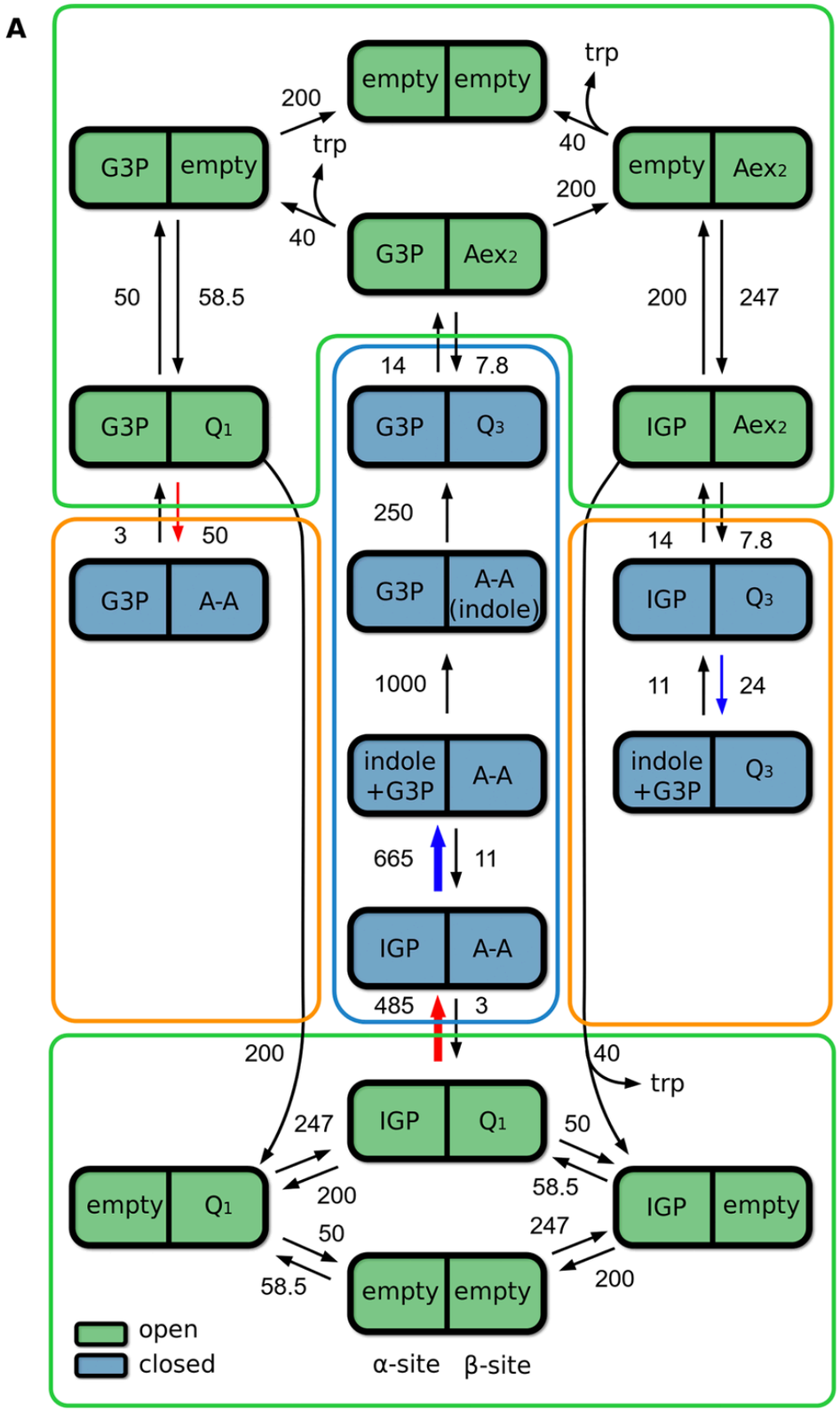
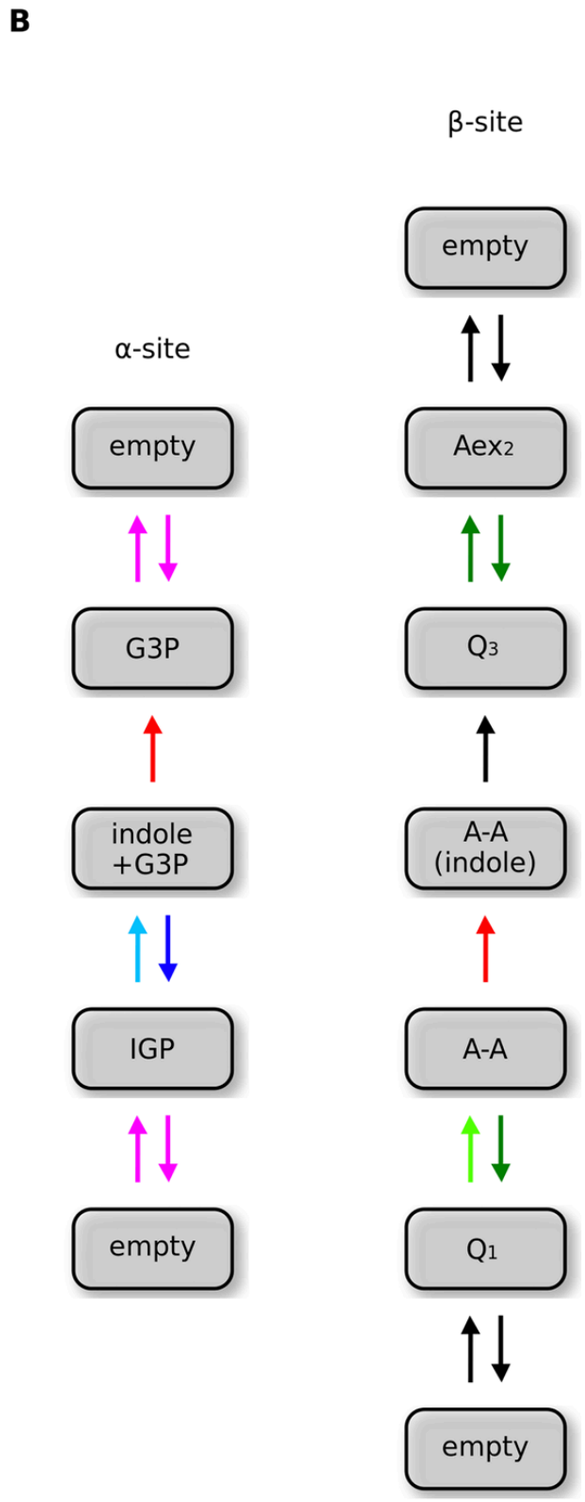


Figure 1. Tryptophan synthase as a chemical nanomachine. (A) Structure of the enzyme from *Salmonella typhimurium* (PDB code: 2J9X[22]). (B) Reduced reaction scheme. The internal aldimine E(Ain) corresponds to the PLP cofactor without any bound ligand. It is therefore referred to as *empty* throughout the text. (C) Operation of the machine: Once substrates are bound (a) at both catalytic sites, IGP activates (b) the formation of A-A and the enzyme adopts the closed conformation. A-A activates (c) the cleavage of IGP and indole is channeled (d) to the β-site where it reacts (e) with A-A to give Q_3. Q_3 undergoes (f) further transformations that return of the enzyme to the open conformation where tryptophan and G3P are released (g).

Markov network of tryptophan synthase

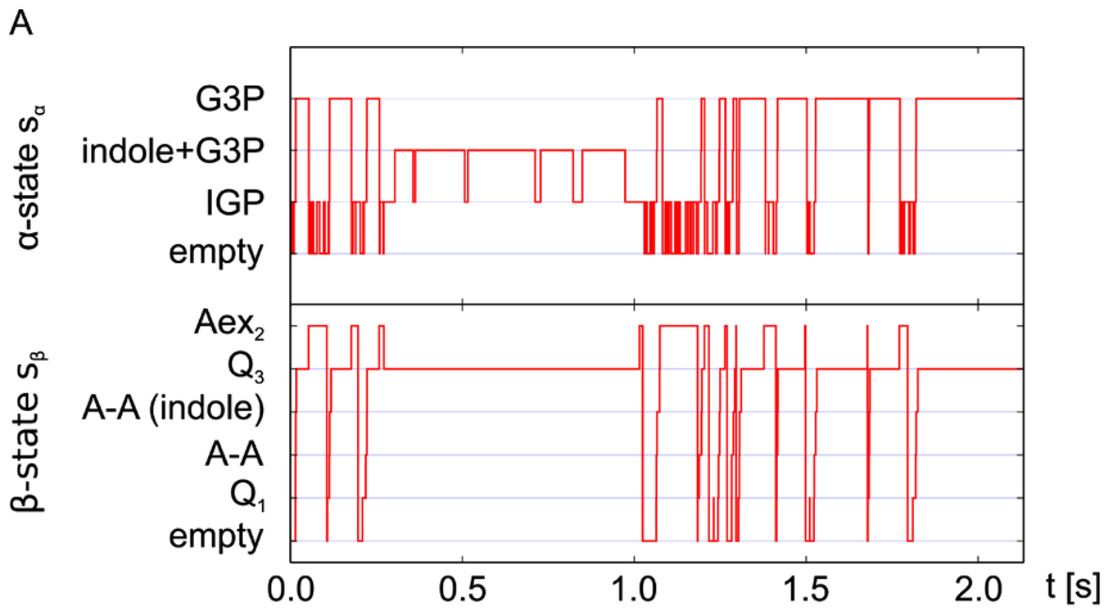




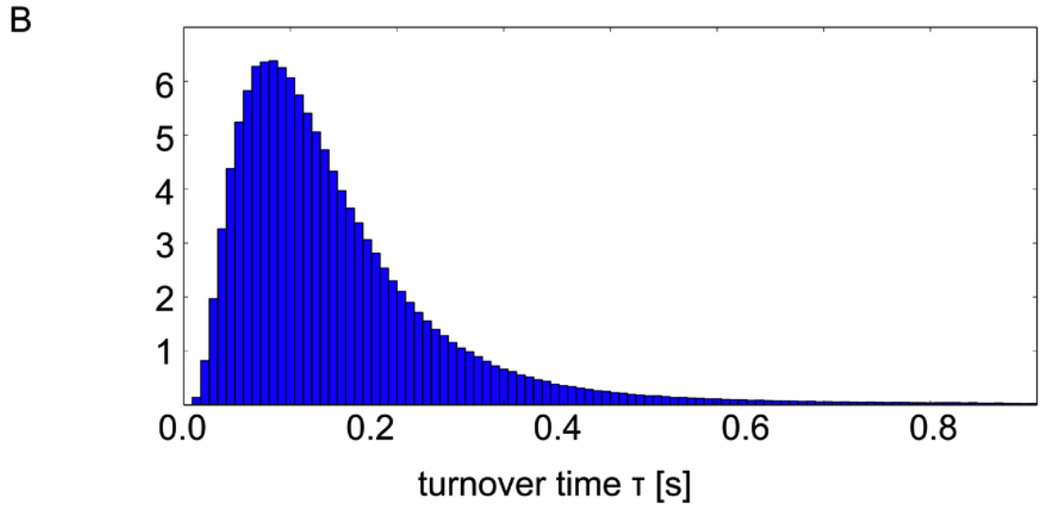
Joint chemical states of two subunits

Reaction networks in individual subunits

Numerical stochastic simulations



Tryptophan synthase is a very slow enzyme. Its mean turnover time is 0.15 s. The cycles of duration of almost a second are also occasionally taking place.



Synchronization of intramolecular processes tryptophan synthase

How to characterise correlations in the states of two subunits?

For each state i or j of two subunits, we introduce binary variables $X_i(t)$ and $Y_j(t)$ such that

$X_i(t)=1$ if α-subunit is in state i at time t, $X_i(t)=0$ otherwise

$Y_j(t)=1$ if β-subunit is in state j at time t, $Y_j(t)=0$ otherwise

The Pearson correlation coefficients for the states of the two subunits are determied as

$$c_{ij}=\frac{\langle X_i(t)Y_j(t)\rangle-\langle X_i(t)\rangle\langle Y_j(t)\rangle}{\sqrt{\langle X_i^2(t)\rangle-\langle X_i(t)\rangle^2}\sqrt{\langle Y_j^2(t)\rangle-\langle Y_j(t)\rangle^2}}$$

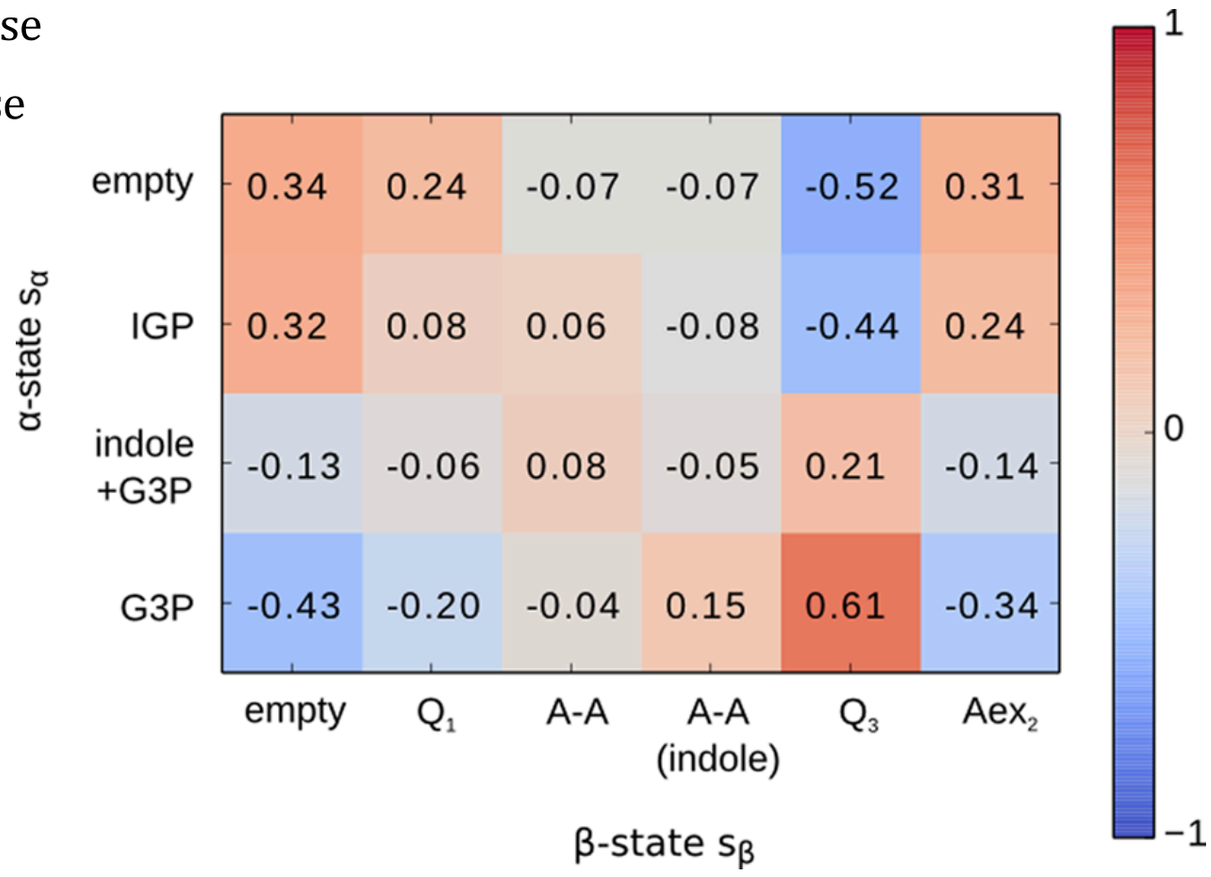
These coefficients take the maximum value

$c_{ij}=+1$ if $X_i(t)\equiv Y_j(t)$ (complete correlation)

and the minimum value

$c_{ij}=-1$ if $X_i(t)\equiv 1-Y_j(t)$ (complete anti-correlation)

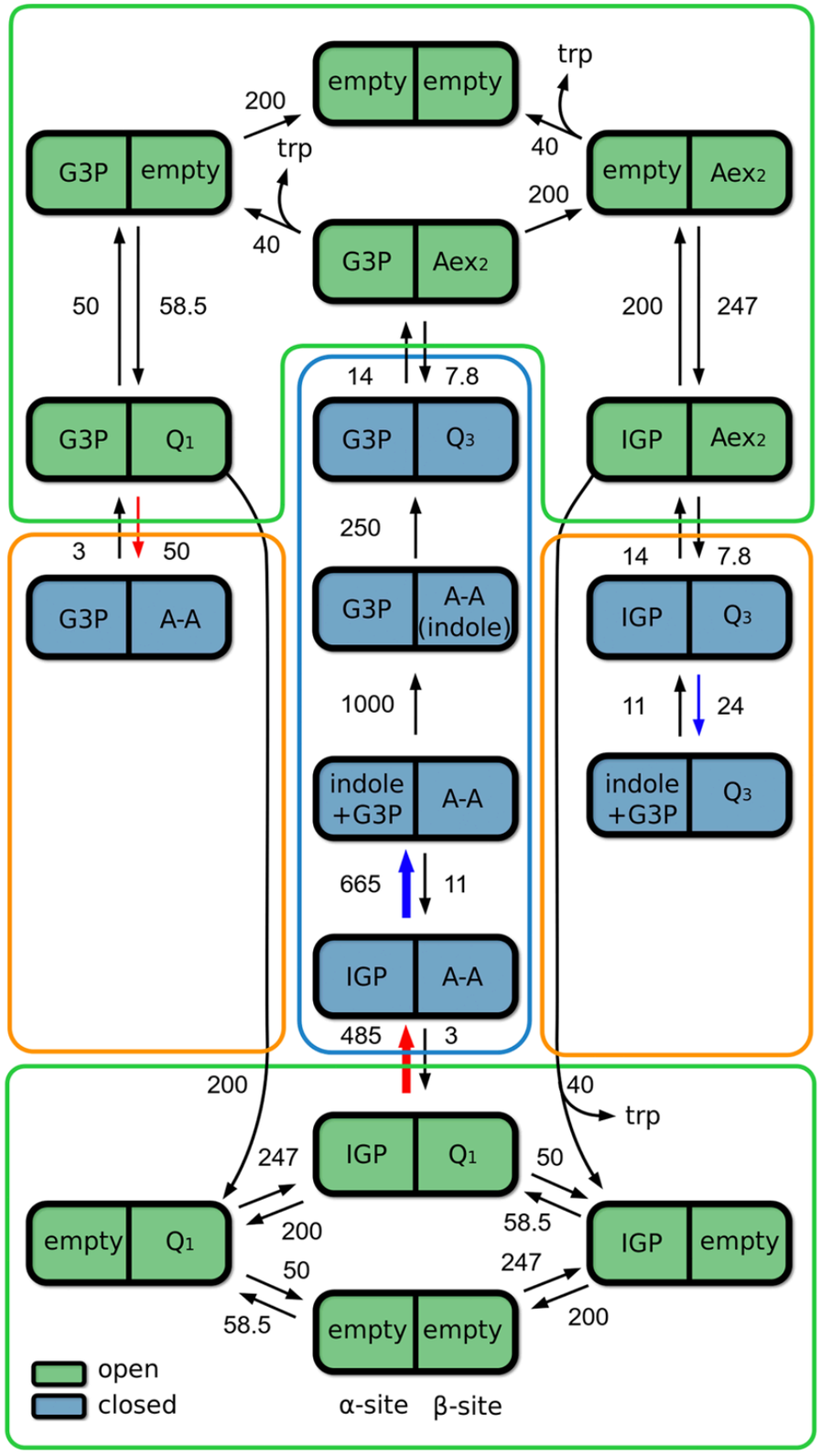
If there is no correlation, $c_{ij}=0$.



Pearson correlation coefficients between the states of two subunits

Strong intramolecular correlations develop, processes in two subunits become synchronised.

Synchronization of intramolecular processes tryptophan synthase



There is also another way to characterise synchronisation.

Suppose that both subunits are initiall (at $t=0$) in the empty states. We can run a stochastic simulation and determine times t_{IGP} and t_{Q_1} when substrates arrive into the subunits. By running many simulations, the mean square-root difference of these times

$$\sigma_{IGP,Q_1}=\sqrt{\left\langle\left(t_{IGP}-t_{Q_1}\right)^2\right\rangle}$$

can be found.

In a similar way, we can find the mean square-root difference

$$\sigma_{\text{indole+G3P,A-A}}=\sqrt{\left\langle\left(t_{\text{indole+G3P}}-t_{\text{A-A}}\right)^2\right\rangle}$$

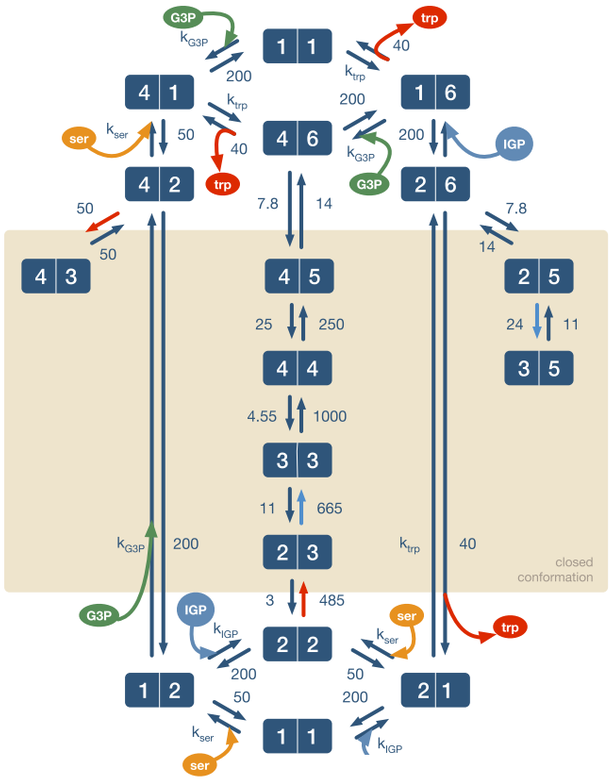
of the times when both subunits are in the pre-channeling state.

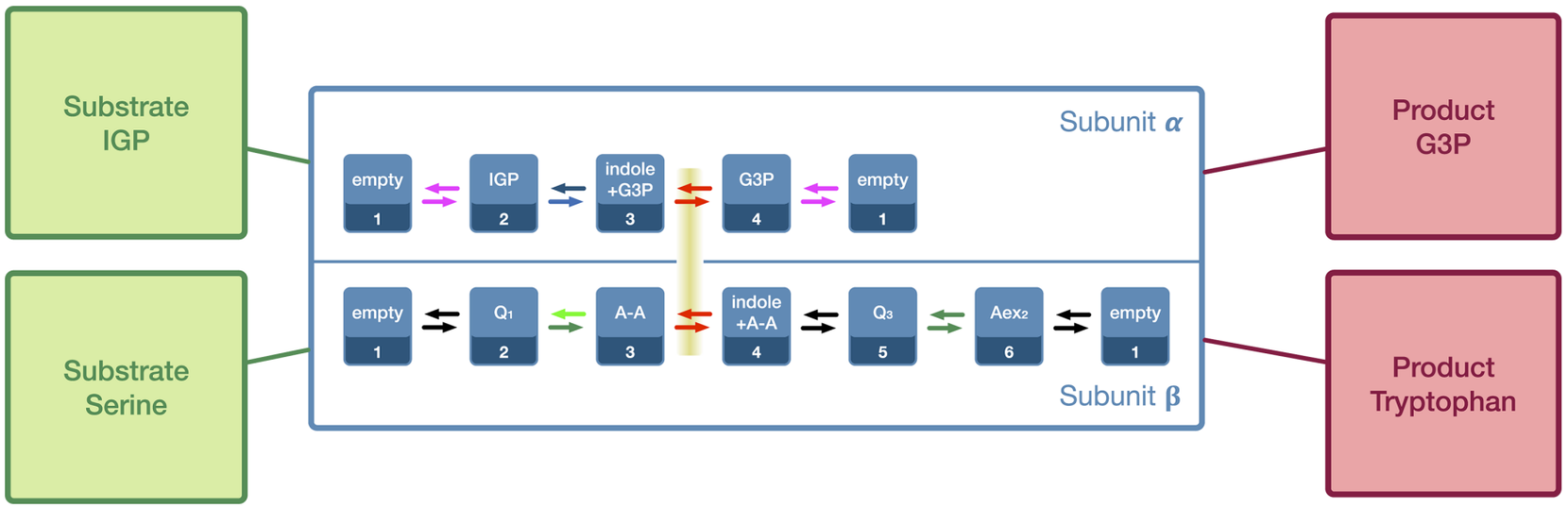
Statistical analysis of simulation data yields

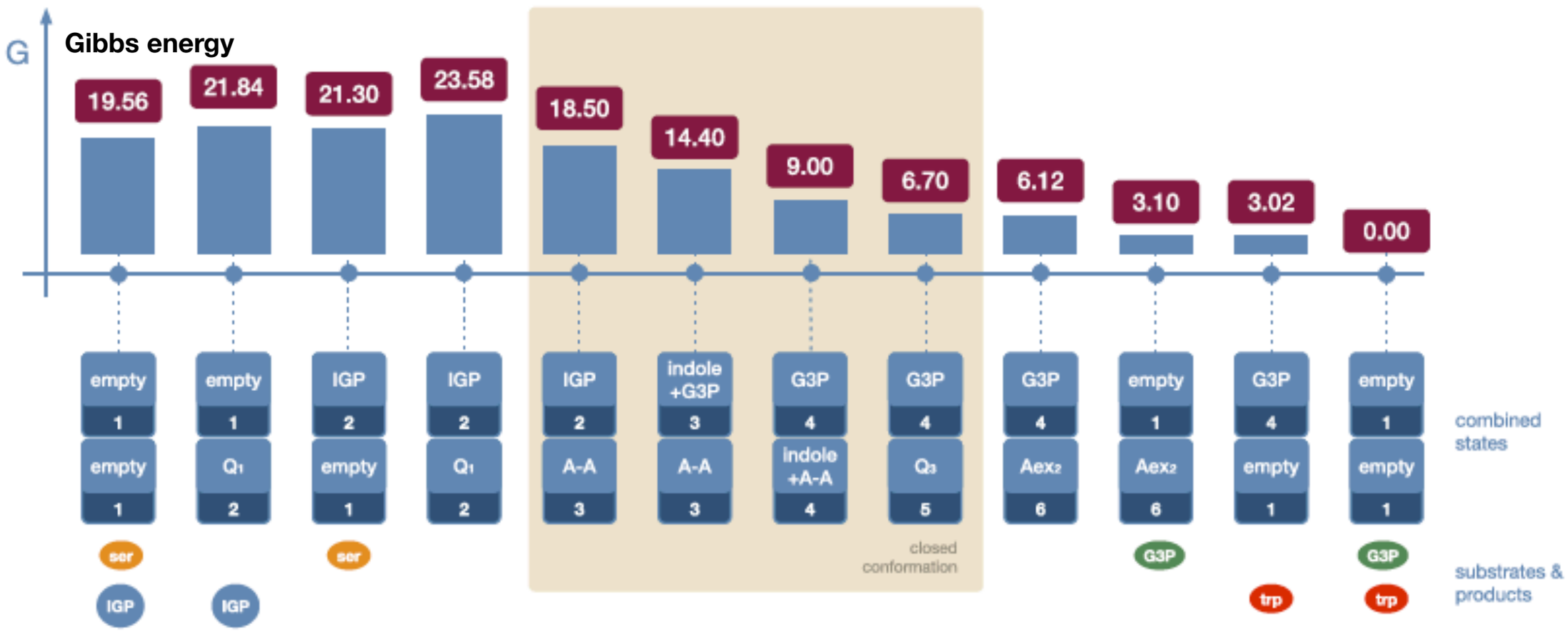
$$\sigma_{IGP,Q_1}=22\text{ ms},\quad \sigma_{\text{indole+G3P,A-A}}=2.4\text{ ms}$$

While binding of substrates occurs at largely different rimes, both subunits almost simultaneously arrive at the pre-channeling states.

Energy landscape of tryptophan synthase



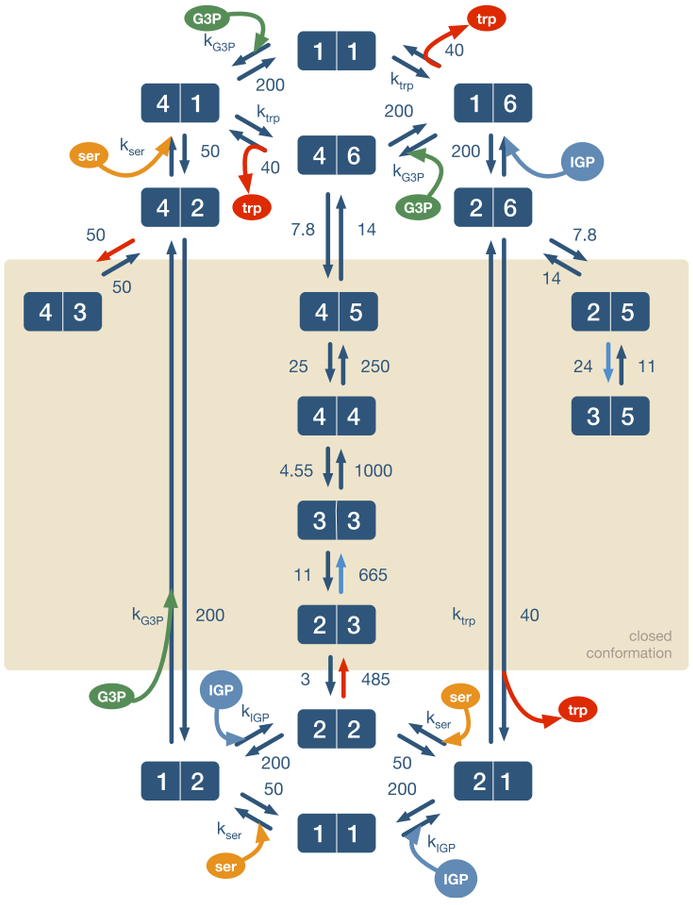




$$\left(\mu^0_{serine} + \mu^0_{IGP}\right) - \left(\mu^0_{G3P} + \mu^0_{tryptophan}\right) = 20.46\ k_B T$$

The reaction is practically irreversible !

Entropy production



Tryptophan synthase produces 27.79 bits of entropy per turnover cycle.

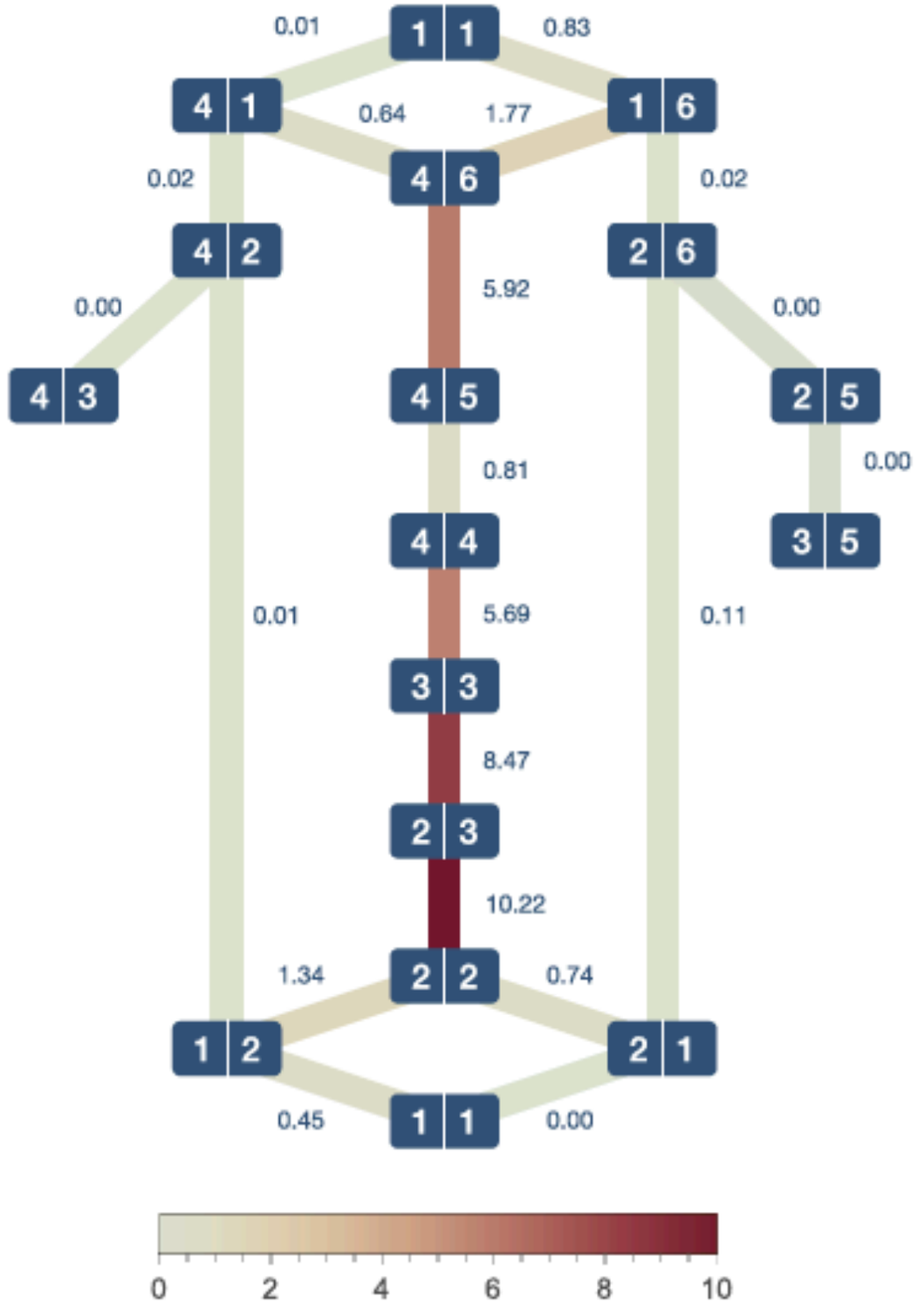
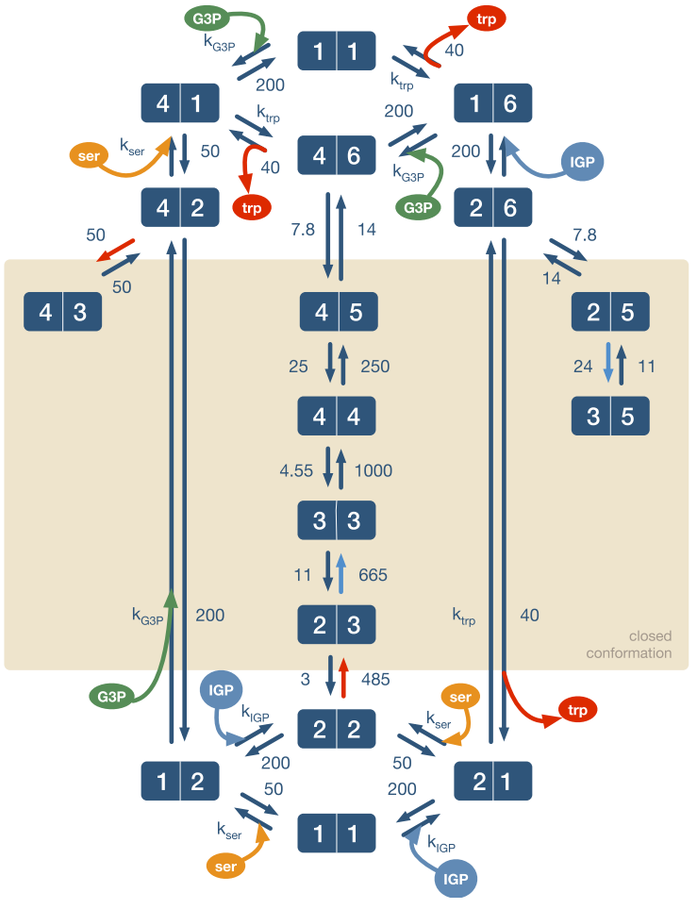


FIG. 5. Entropy production in different transitions in the nonequilibrium steady-state. The values of entropy production are given in units of bit s^{-1} next to the links between the states. Additionally, color coding of the links according to the corresponding entropy production is used.

Entropy export/import



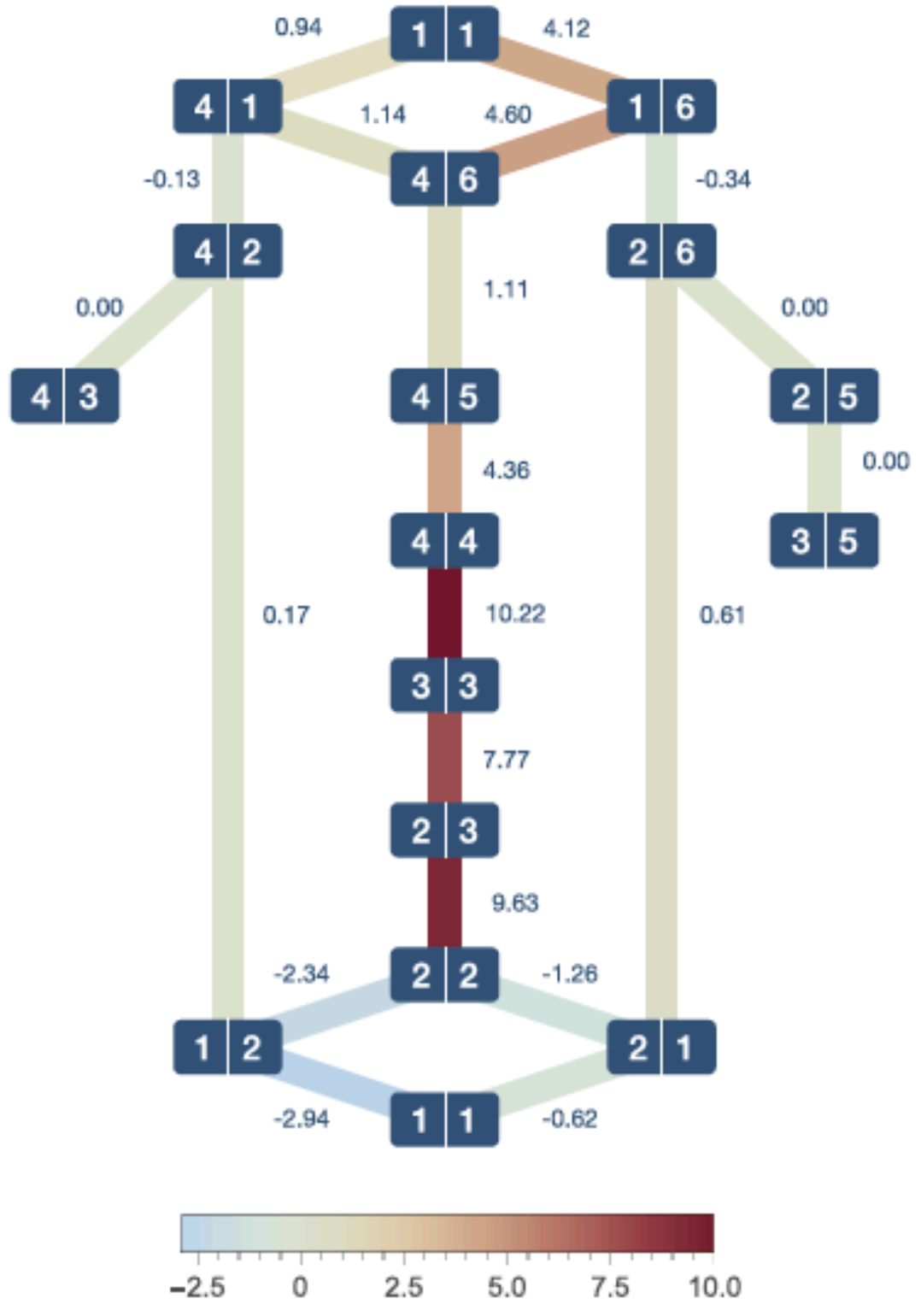


FIG. 6. Rates of entropy export in individual transitions in tryptophan synthase. The same notations as in Fig. 5 are used.