FCB unit V

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Molecular Switch

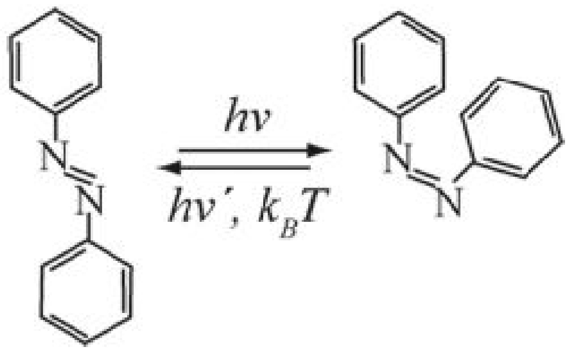
Common examples of switches around us.

A molecular switch is a molecule that can be reversibly shifted between two or more stable states with the help of stimulus.

Factors stimulating switching process: Ph, Light, Temperature, presence of ions, electricity, other heavy metal ions.

Examples of molecular switch

- Acidochromic molecular switches: Ph Indicators and plants like rose, cornflowers.
- Photochromic molecular switches: works with specific wavelength of light. Example Biotin or Vitamin B.

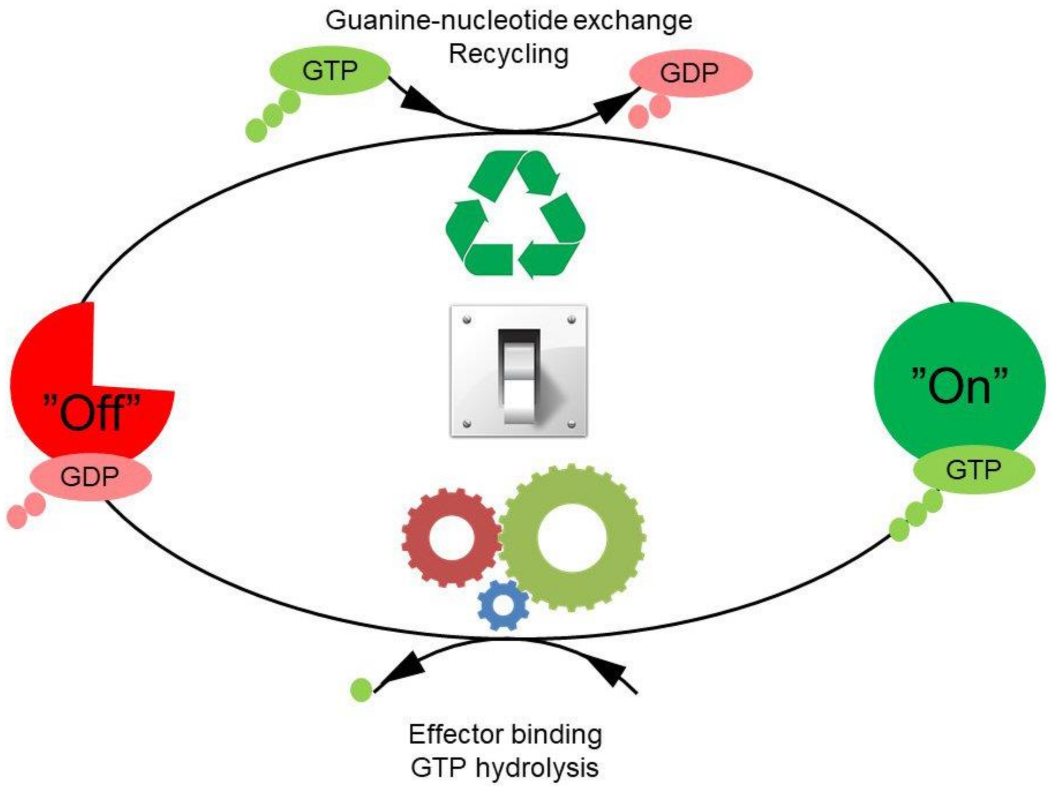


Cis-trans isomerization of azobenzene exposed to heating and light.

- Nanoparticle Switches: Au, Ag, Cu, Ni, Cr all nanoparticle posses this particular switch

All these are gold nano particles under Different wavelength of light.

Mechanism of molecular switch



Example of GTPase mediated molecular switch.
In bacterial protein translation, GTPase enzyme
Cycles between on and off state post binding with
GTP molecule.

Flux balance analysis

- Mathematical analysis of flux associated with biochemical reactions inside a cell.

- A quick revision of the law of mass action?
- How to represent metabolic networks:
    - Stoichiometric coefficients
    - The stoichiometric matrix
    - System equations

The Law of Mass Action

- The reaction rate is proportional to the probability of a collision of the reactants.
- This probability is in turn proportional to the concentration of reactants, to the power of the molecularity: e.g. the number in which they enter the specific reaction.

$$S_1 + S_2 \rightleftarrows 2P$$

$$v = v_+ - v_- = k_+ S_1 \cdot S_2 - k_- P^2$$

- A more general formula for substrate concentrations Si, and product concentrations Pj is:

$$v = v_+ - v_- = k_+ \prod_i S_i^{mi} - k_- \prod_j P_j^{mj}$$

Equilibrium constrains

The equilibrium constant Keq characterizes the ratio of substrate and product concentrations in equilibrium (Seq and Peq), that is, the state with equal forward and backward rates.

$$Keq = \frac{k_+}{k_-} = \frac{\prod P_{eq}}{\prod S_{eq}}$$

he dynamics of the concentrations can be described by Ordinary Differential Equations (ODE), e.g. for the S1+S2→ 2P reaction:

$$1.\quad \frac{d}{dt} S_1 = \frac{d}{dt} S_2 = -v$$

$$2.\quad \frac{d}{dt} P = 2v$$

Laws of mass action for substrate decay

- The kinetics of a simple decay (molecular destruction) such as:

$$S \longrightarrow$$

$$\boxed{\begin{array}{l} 1.\ v = kS \\ 2.\ \dfrac{d}{dt}S = -kS \end{array}}$$

- Integration of this ODE from time t = 0 with the initial concentration S0 to an arbitrary time t with concentration S(t) yields the temporal expression:

$$\boxed{\int_{S_0}^{S} \frac{dS}{S} = \int_{t=0}^{t} k\,dt \quad \text{or} \quad S(t) = S_0 e^{-kt}}$$

Stoichiometric coefficients

- Stoichiometric coefficients denote the proportion or substrates and products involved in a reaction.

$$S_1 + S_2 \rightleftarrows 2P$$

- The stoichiometric coefficients of S1 S2 and P are -1, -1, and 2.
- ODE equation will be.

$$\frac{d}{dt}S_1 = \frac{d}{dt}S_2 = -v \text{ and } \frac{d}{dt}P = 2v$$

- For a metabolic network consisting of m substances and r reactions, the systems dynamics is described by systems equations

$$\frac{dS_i}{dt} = \sum_{j=1}^{r} n_{ij} v_j \ \text{ for } i = 1, \ldots, m$$

Stoichiometric Matrix

Example 1

A → B

A + B → C

B + C → 2 A

$$\begin{array}{cc} \begin{matrix} j_1 & \quad j_2 & \quad j_4 \end{matrix} & \\ \begin{bmatrix} -1 & -1 & 2 \\ 1 & -1 & -1 \\ 0 & 1 & -1 \end{bmatrix} & \begin{matrix} A \\ B \\ C \end{matrix} \end{array}$$

- **Example 2**

A → B

A + E → 2 C

B + C → D + E

2 E + C → 2 A + B

$$\begin{array}{cc} \begin{matrix} j_1 & \quad j_2 & \quad j_3 & \quad j_4 \end{matrix} & \\ \begin{bmatrix} -1 & -1 & 0 & 2 \\ 1 & 0 & -1 & 1 \\ 0 & 2 & -1 & -1 \\ 0 & 0 & 1 & 0 \\ 0 & -1 & 1 & -2 \end{bmatrix} & \begin{matrix} A \\ B \\ C \\ D \\ E \end{matrix} \end{array}$$

Stoichiometric Matrix

- Example of a network

$$
\begin{array}{ccccc}
\xleftrightarrow{v_1} & S_1 & \xleftrightarrow{v_2} & 2S_2 & \xleftrightarrow{v_3} \\
 & \updownarrow v_4 & & & \\
 & S_3 & & &
\end{array}
$$

$$
\begin{array}{lcccccc}
\text{reaction :} & & v_1 & v_2 & v_3 & v_4 & \\
 & N = & \left(\begin{matrix} 1 \\ 0 \\ 0 \end{matrix} \right. & \begin{matrix} -1 \\ 2 \\ 0 \end{matrix} & \begin{matrix} 0 \\ -1 \\ 0 \end{matrix} & \left. \begin{matrix} 1 \\ 0 \\ 1 \end{matrix} \right) & \begin{matrix} S_1 \\ S_2 \\ S_3 \end{matrix}
\end{array}
$$

Flux analysis

- Metabolic network consist of three elements:
- S vector or Stoichiometric matrix
- V vector or reaction velocities
- P vector or parameter vector or known metabolites.

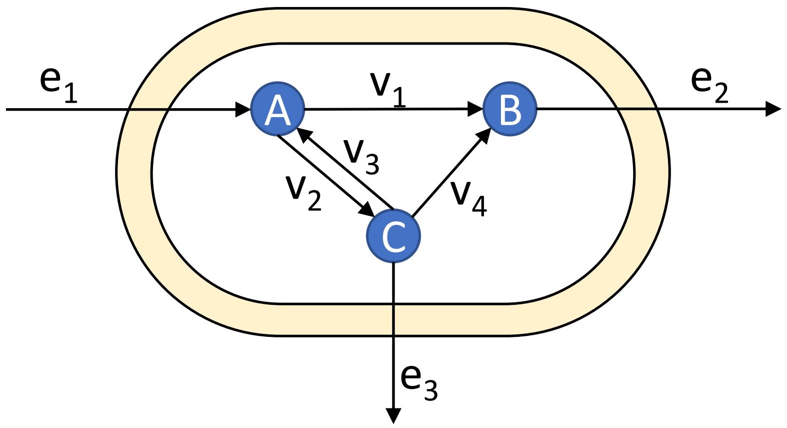
- For a metabolic network that contains m metabolites and n metabolic fluxes, all the transient material balances can be represented by a single matrix equation:

$$\frac{d\mathbf{X}}{dt} = \mathbf{S} \cdot \mathbf{v} - \mathbf{b}$$

where X is an m dimensional vector of metabolite amounts per cell, v is the vector of n metabolic fluxes, S is the stoichiometric m × n matrix, and b is the vector of known metabolic demands.

A Vector Example

A simple network



Linear Transformation

$$\frac{dx}{dt} = S * v \qquad \begin{bmatrix} \frac{dA}{dt} \\ \frac{dB}{dt} \\ \frac{dC}{dt} \end{bmatrix} = \begin{bmatrix} -1 & -1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ e_1 \\ e_2 \\ e_3 \end{bmatrix}$$

Stoichiometric Matrix

Linear Differential Equations

$$\frac{dA}{dt} = -v_1 - v_2 + v_3 + e_1$$

$$\frac{dB}{dt} = v_1 + v_4 - e_2$$

$$\frac{dA}{dt} = v_2 - v_3 - v_4 - e_3$$

Dynamic Mass Balance (Steady State)

$$0 = S * v \qquad \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix} = \begin{bmatrix} -1 & -1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ e_1 \\ e_2 \\ e_3 \end{bmatrix}$$

Note: More unknown variables than equations, thus no unique solutions! Need constraints!

Detailed biological example

$$\frac{d}{dt} Gluc6P = v_1 - v_2 - v_3$$

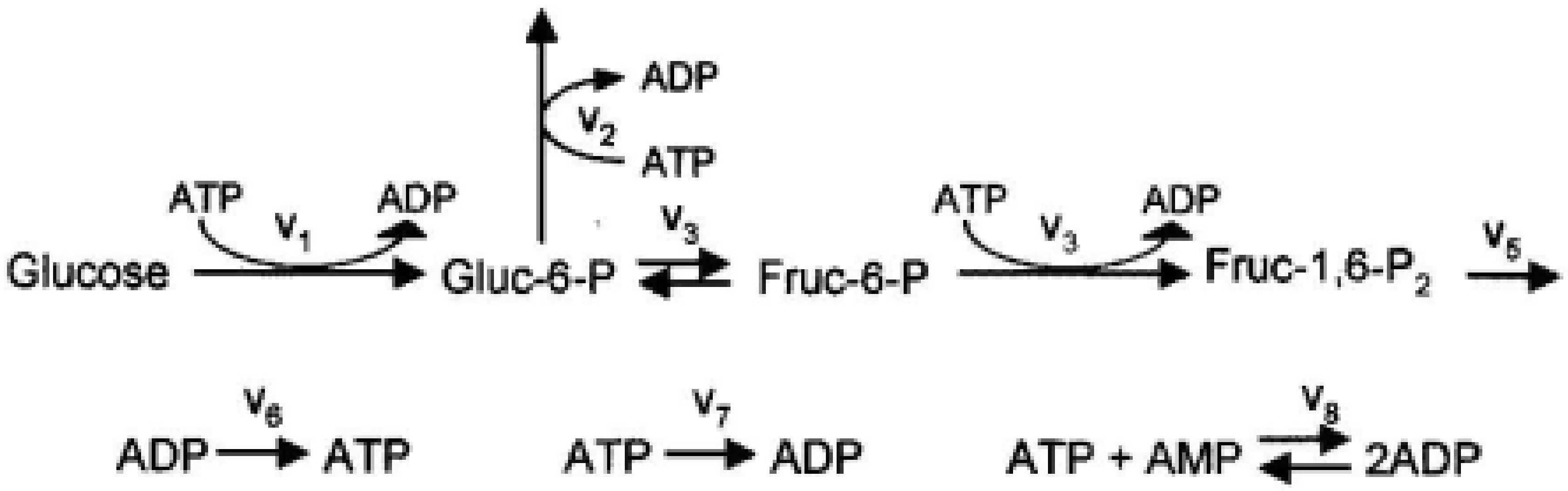
$$\frac{d}{dt} Fruc6P = v_3 - v_4$$

$$\frac{d}{dt} Fruc1{,}6P_2 = v_4 - v_5$$

$$\frac{d}{dt} ATP = -v_1 - v_2 - v_4 + v_6 - v_7 - v_8$$

$$\frac{d}{dt} ADP = v_1 + v_2 + v_4 - v_6 + v_7 + 2\,v_8$$

$$\frac{d}{dt} AMP = -v_8 \,.$$



Glycolysis FBA example (just for knowledge)

$$v_1 = \frac{V_{max,1}\,ATP(t)\cdot Glucose}{1+\frac{ATP(t)}{K_{ATP,1}}+\frac{Glucose}{K_{Glucose,1}}+\frac{ATP(t)}{K_{ATP,1}}\cdot\frac{Glucose}{K_{Glucose,1}}} \text{ or } v_1 = \frac{V_{max,1}\,ATP(t)}{K_{ATP,1}+ATP(t)}$$

$$v_2 = k_2\,ATP(t)\cdot Gluc6P(t)$$

$$v_3 = \frac{\frac{V^f_{max,3}}{K_{Gluc6P,3}}Gluc6P(t)-\frac{V^r_{max,3}}{K_{Fruc6P,3}}Fruc6P(t)}{1+\frac{Gluc6P(t)}{K_{Gluc6P,3}}+\frac{Fruc6P(t)}{K_{Fruc6P,3}}}$$

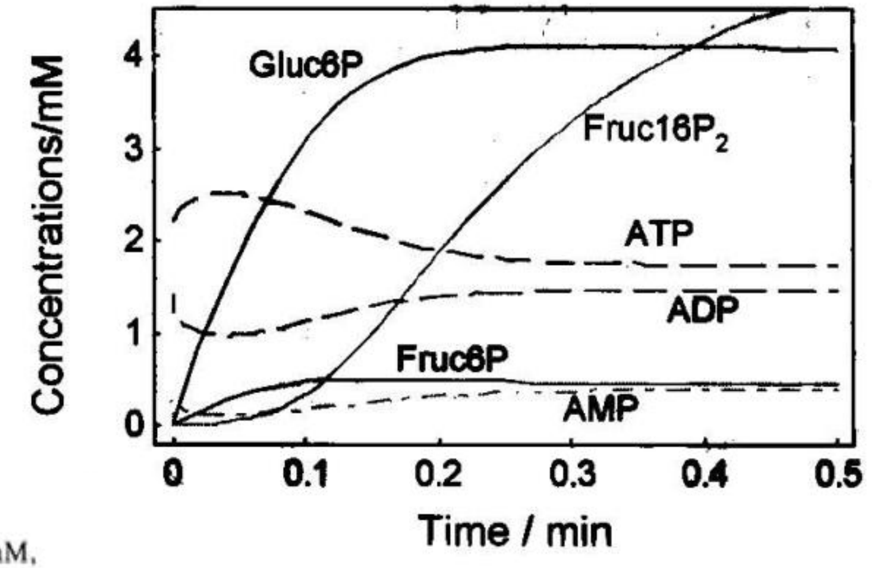
$$v_4 = \frac{V_{max,4}\,(Fruc6P(t))^2}{K_{Fruc6P,4}\left(1+\kappa\left(\frac{ATP(t)}{AMP(t)}\right)^2\right)+(Fruc6P(t))^2}$$

$$v_5 = k_5\,Fruc1{,}6P_2(t)$$

$$v_6 = k_6\,ADP(t)$$

$$v_7 = k_7\,ATP(t)$$

$$v_8 = k_{8f}\,ATP(t)\cdot AMP(t) - k_{8r}\,(ADP(t))^2,$$



with the following parameters:

$Glucose = 12.8174\,\mathrm{mM}$, $V_{max,1} = 1398.00\,\mathrm{mM\cdot min^{-1}}$, $K_{ATP,1} = 0.10\,\mathrm{mM}$,
$K_{Glucose,1} = 0.37\,\mathrm{mM}$, $V_{max,1} = 50.2747\,\mathrm{mM\cdot min^{-1}}$

$k_2 = 2.26\,\mathrm{mM^{-1}\cdot min^{-1}}$

$V^f_{max,3} = 140.282\,\mathrm{mM\cdot min^{-1}}$, $V^r_{max,3} = 140.282\,\mathrm{mM\cdot min^{-1}}$, $K_{Gluc6P,3} = 0.80\,\mathrm{mM}$,
$K_{Fruc6P,3} = 0.15\,\mathrm{mM}$

$V_{max,4} = 44.7287\,\mathrm{mM\cdot min^{-1}}$, $K_{Fruc6P,4} = 0.021\,\mathrm{mM^2}$, $\kappa = 0.15$

$k_5 = 6.04662\,\mathrm{min^{-1}}$

$k_6 = 68.48\,\mathrm{min^{-1}}$

$k_7 = 3.21\,\mathrm{min^{-1}}$

$k_{8f} = 432.9\,\mathrm{mM^{-1}\cdot min^{-1}}$, $k_{8r} = 133.33\,\mathrm{mM^{-1}\cdot min^{-1}}$

Mutational studies in a population

What Are Mutations?

Changes in the nucleotide sequence of DNA

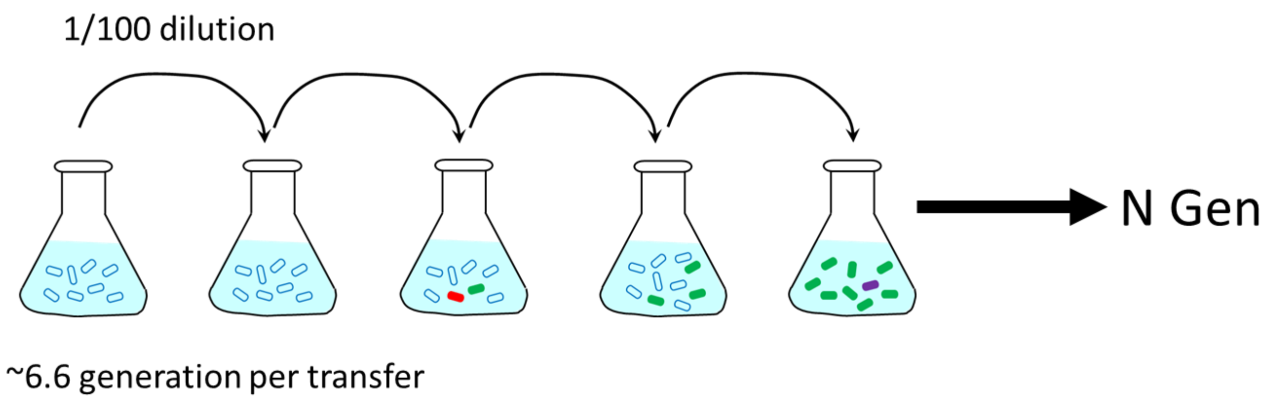
- Mutations happen regularly
- Almost all mutations are neutral
- Chemicals & UV radiation cause mutations
- Many mutations are repaired by enzymes present in cells

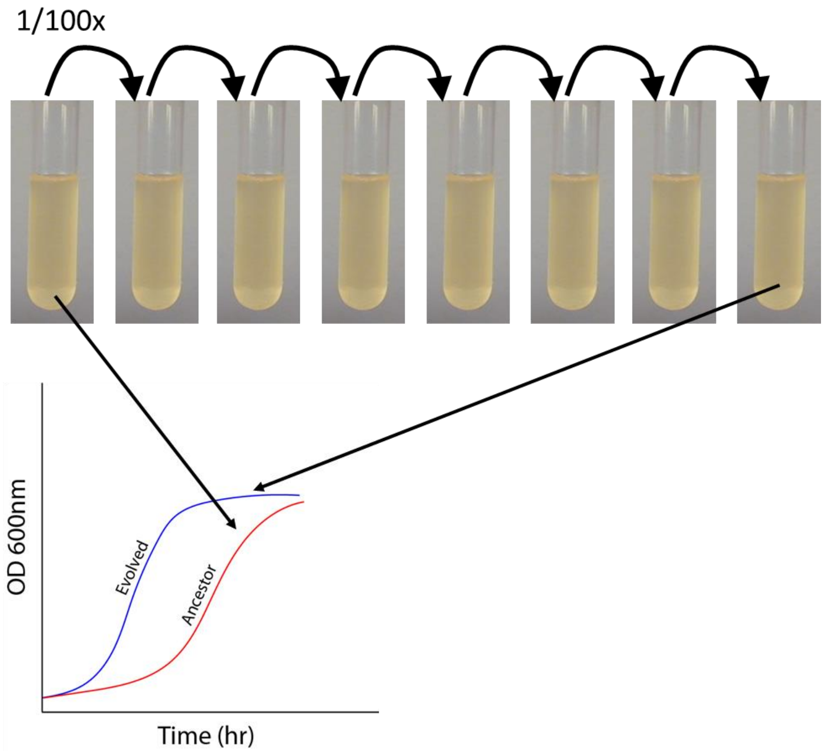
Sickle cell anaemia

Thalassemia

Cystic fibrosis

Natural mutation





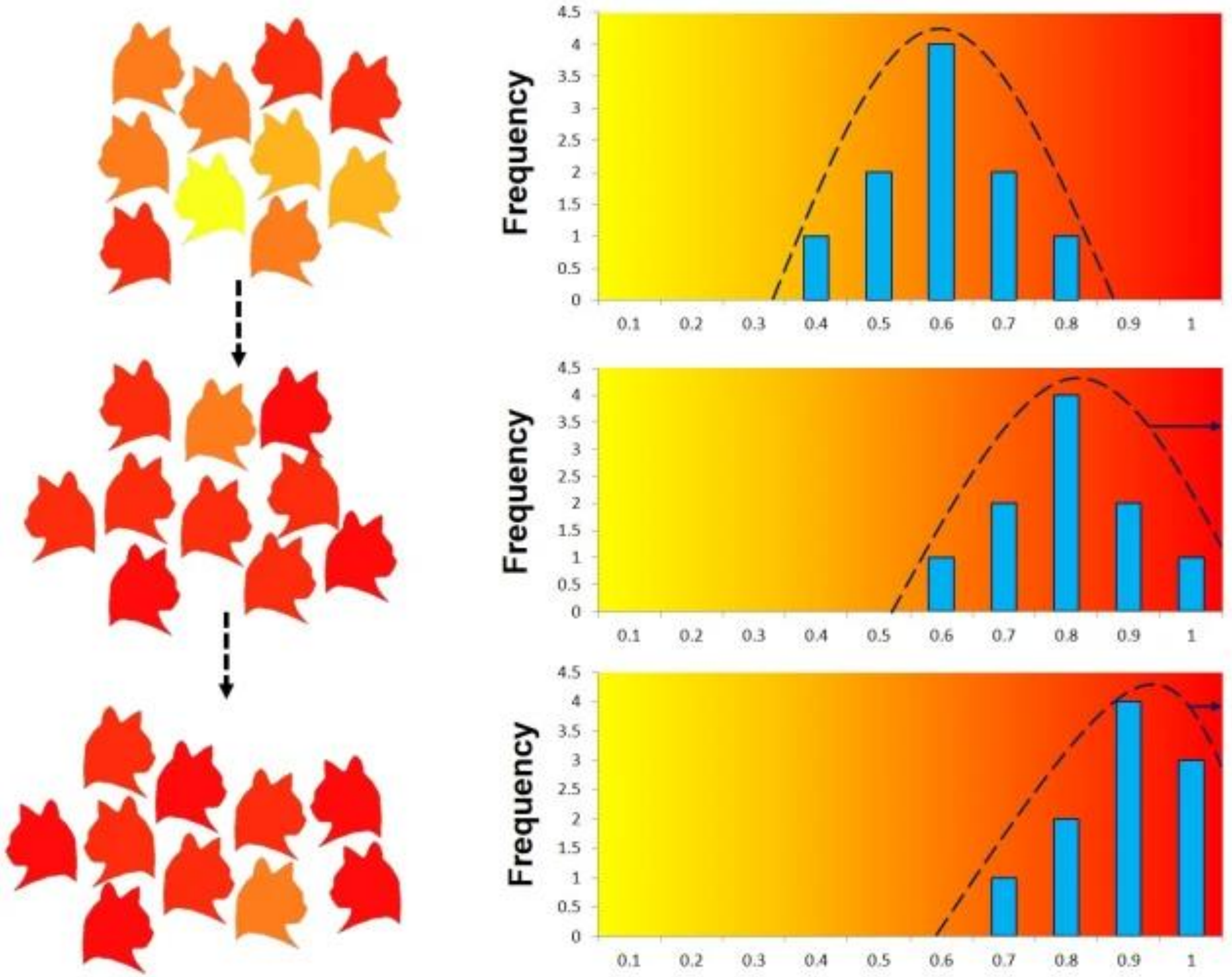
Can we observe mutations in lab?

Rechard Lenski: Michigan S.U.

Long term evolution experiment on *E. coli.*

Image source: Lenski's twitter account

Wave model for mutation



Frequency of mutant and WT changes with time. With time mutant frequency increases when the mutation is beneficial mutation. Frequency of WT decreases with time. The overall pattern follow the wave function.