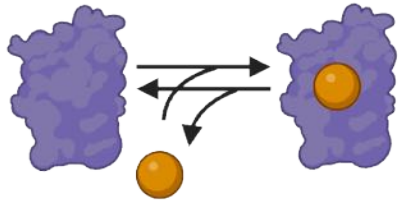
Models of state-transition diagrams, dissociation constants, and diffusion-limited rate constant

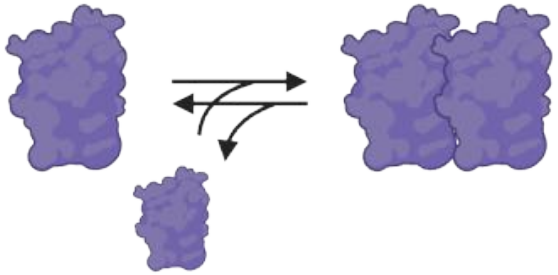
basic modeling of biological networks

Two new models

model 1: small molecule binding



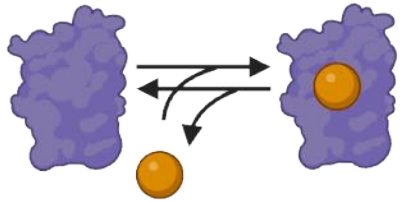
model 2: complex formation (dimerisation)



Exercise:

1. Give the differential equations for the concentrations, and the mass-action kinetics.
2. Reduce the number of differential equations, by using the conservation of the total number of receptors. (Assume that the small molecule is in great excess relative to the concentration of receptors. Such that the free concentration of small molecule remains approximately constant, despite the small molecule binding to the receptor.)
3. Show/proof that the conservation equations for the total concentrations that you used are correct, using the differential equations.

Answers



model:

$$R + S \rightleftharpoons RS$$

$$v = k^+ \cdot r \cdot s - k^- \cdot rs$$

$$\frac{dr}{dt} = -v, \quad \frac{ds}{dt} = -v, \quad \frac{drs}{dt} = v$$

$$r_{tot} = r + rs$$

$$s_{tot} = s + rs, \quad \text{assume excess of s: } s_{tot} \gg r_{tot} \;\Rightarrow\; s_{tot} \approx s$$

simplify using the conservation equation for total receptors:

$$r_{tot} = r + rs, \quad \Rightarrow \quad r = r_{tot} - rs$$

$$\frac{drs}{dt} = v = k^+ \cdot r \cdot s - k^- \cdot rs = k^+ \cdot (r_{tot} - rs) \cdot s - k^- \cdot rs$$

$$\text{Use excess condition: } \frac{drs}{dt} = k^+ \cdot (r_{tot} - rs) \cdot s_{tot} - k^- \cdot rs$$

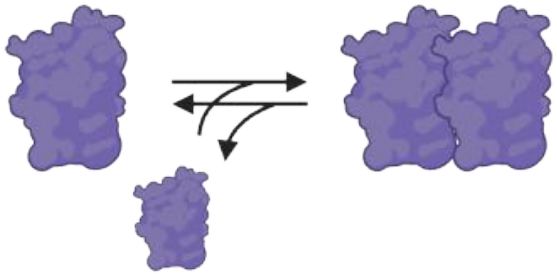
we started with 3 differential equations, now only 1 left!

Proof that $r_{tot} = r + rs$

$$r_{tot} = r + rs \;\Rightarrow\; \frac{dr_{tot}}{dt} = 0 = \frac{dr}{dt} + \frac{drs}{dt} = -v + v = 0!$$

- because r_{tot} is fixed its time derivative is zero
- because r_{tot} is conserved $\frac{dr}{dt} = -\frac{drs}{dt}$, any change in either r or rs is mirrored by an opposite change of the other

Answers



model:

$$r + r \rightleftharpoons r_2$$

$$v = k^+ \cdot r^2 - k^- \cdot r_2$$

$$\frac{dr}{dt} = -2v, \quad \frac{dr_2}{dt} = v$$

$$r_{tot} = r + 2r_2$$

simplify using the conservation equation for total receptors:

$$r_{tot} = r + 2r_2 \;\Rightarrow\; r = r_{tot} - 2r_2$$

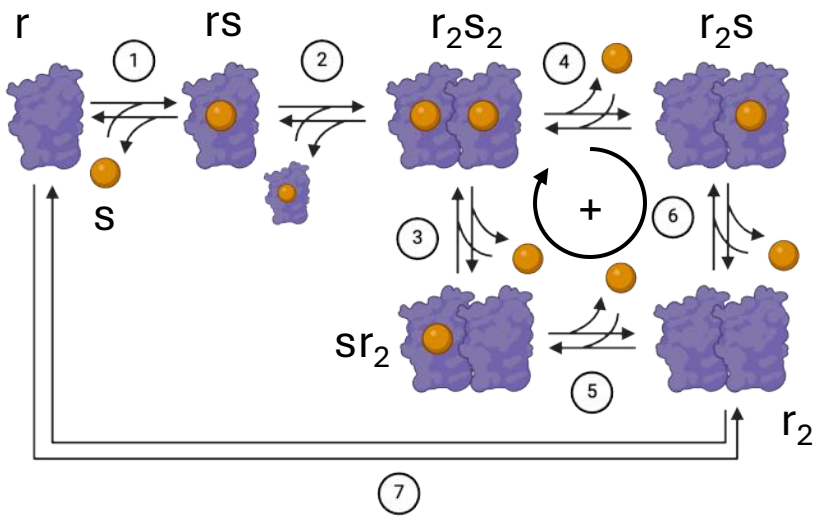
$$\frac{dr_2}{dt} = v = k^+ \cdot r^2 - k^- \cdot r_2 = k^+ \cdot (r_{tot} - 2r_2)^2 - k^- \cdot r_2$$

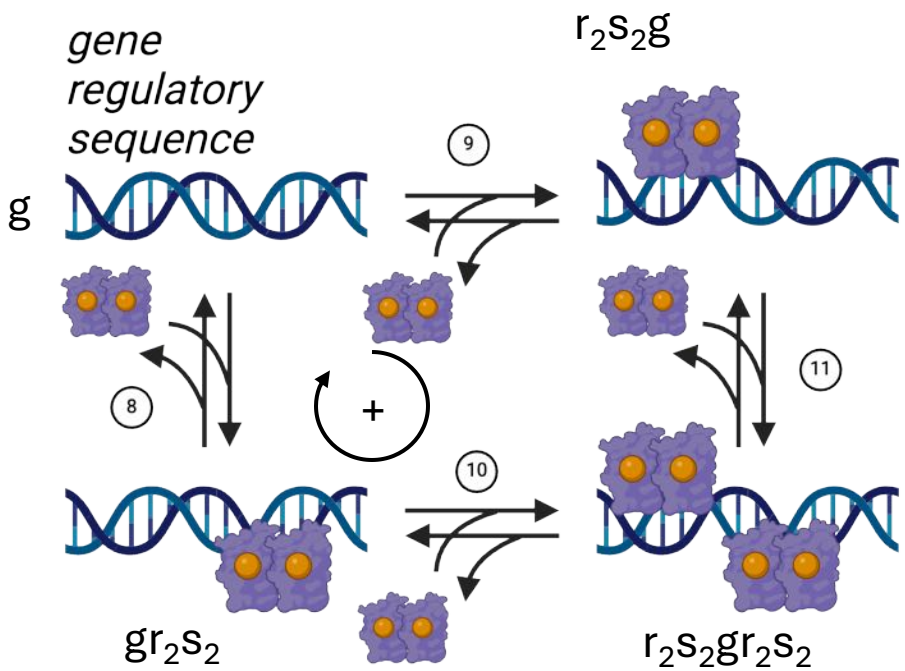
we started with 2 differential equations, now only 1 left!

proof that convervation equation is correct:

$$r_{tot} = r + 2r_2 \;\Rightarrow\; \frac{dr_{tot}}{dt} = 0 = \frac{dr}{dt} + 2\frac{dr_2}{dt} = -2v + v = 0!$$

A bigger model

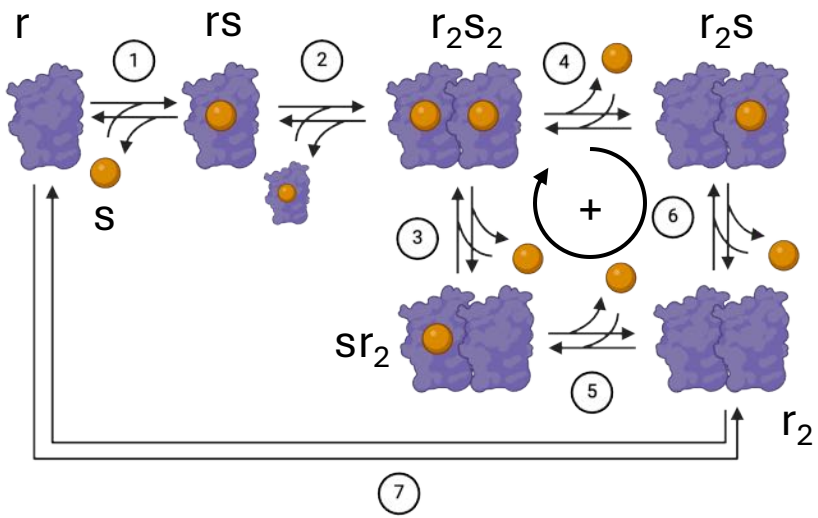


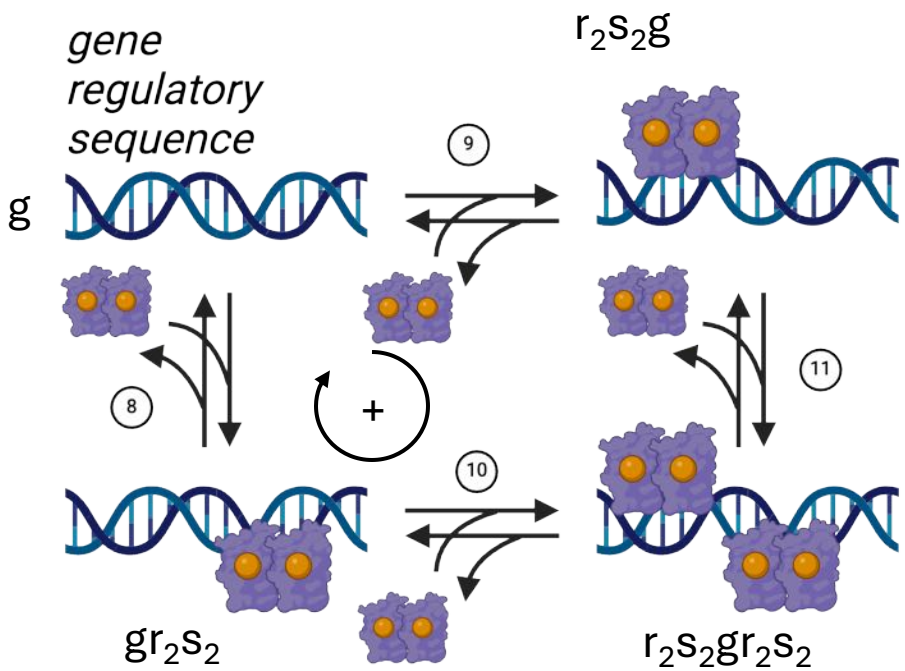


1. How many conservation relationships exist? (Same question, but phrased differently: how many relations exist that relate concentrations to a total concentration value?)

2. Show/proof that you are right, using the differential equations of the concentrations.

conservation relations of S, R and G





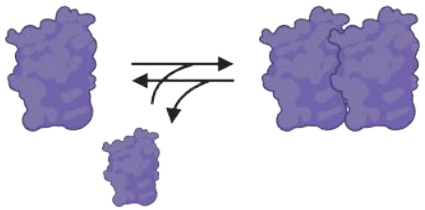
$$s_{tot} = s + rs + sr_2 + r_2s + 2r_2s_2 + 2gr_2s_2 + 2r_2s_2g + 4r_2s_2gr_2s_2$$

$$r_{tot} = r + 2r_2 + rs + 2sr_2 + 2r_2s + 2r_2s_2 + 2gr_2s_2 + 2r_2s_2g + 4r_2s_2gr_2s_2$$

$$g_{tot} = g + gr_2s_2 + r_2s_2g + r_2s_2gr_2s_2$$

Numerical solution of differential equations

Consider the model:



$$r + r \rightleftharpoons r_2$$

$$v = k^+ \cdot r^2 - k^- \cdot r_2$$

$$\frac{dr}{dt} = -2v, \ \frac{dr_2}{dt} = v$$

$$r_{tot} = r + 2r_2$$

You would add to the computer

$$v = k^+ \cdot r^2 - k^- \cdot r_2$$

$$\frac{dr}{dt} = -2v, \ \frac{dr_2}{dt} = v$$

$$k^+ = 3\mu M^{-1}s^{-1}, \ k^- = 2\ s^{-1}, \ r(0) = 1\ \mu M, \ r_2(0) = 0\mu M$$

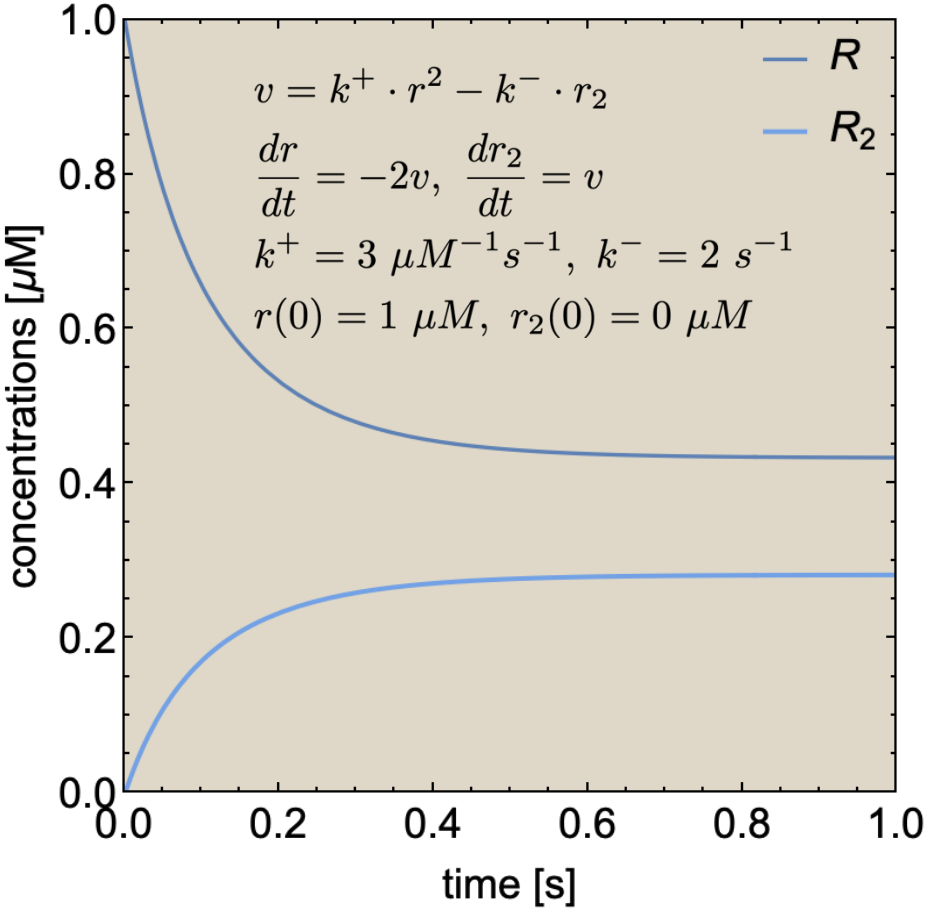
The computer then computes the concentrations of r and r_2 as function of time, by

$$r(t + \Delta t) \approx r(t) + \frac{dr(t)}{dt}\Delta t = r(t) - 2v\Delta t$$

$$r_2(t + \Delta t) \approx r_2(t) + \frac{dr_2(t)}{dt}\Delta t = r_2(t) + v\Delta t$$

following by the next time $t + 2\Delta t$ given the numbers at $t + \Delta t$

For instance, we would then obtain the following result



- the system moves to a stationary state
- in this stationary state the concentrations are constant
- the rate of the reaction must now be zero
- when reaction rates are zero in a stationary state, this stationary state is called a state of (thermodynamic) equilibrium or, equivalently, and equilibrium state

Exercise: calculate the concentrations of R and R_2 in the equilibrium state.

$$v = k^+ \cdot r_e^2 - k^- \cdot r_{2,e} = 0 \ \Rightarrow \ r_{2,e} = \frac{k^-}{k^+} r_e^2$$

$$r_{tot} = r + 2r_2 = r_e + 2\frac{k^-}{k^+} r_e^2 \ \Rightarrow \ 2\frac{k^-}{k^+} r_e^2 + r_e - r_{tot} = 0$$

$$r_e = \frac{1}{4}\left(-4 - \frac{k^-}{k^+} + \frac{\sqrt{k^-(k^- + 8k^+)}}{k^+} + 4r_{tot}\right)$$

Equilibrium constants

Consider: $2r \rightleftharpoons r_2$

$$v = k^+ \cdot r^2 - k^- r_2$$

Each reaction has an equilibrium constant K_{eq}.

A reaction is in equilibrium when its rate is 0:

$$v = k^+ \cdot r_e^2 - k^- r_{2,e} = 0 \;\Rightarrow\; \frac{r_e^2}{r_{2,e}} = \frac{k^-}{k^+} = K_{eq}$$

An equilibrium constant relates the concentrations of the reactants.

Note:

$$v = k^+ r^2 - k^- r_2 = k^+ r^2 \left(1 - \frac{k^- r_2}{k^+ r^2}\right) = k^+ r^2 \left(1 - \frac{r_2}{r^2 K_{eq}}\right)$$

Dissociation constants for complex formation reactions

Consider:

$$A + B \rightleftharpoons AB$$

$$v = k^{+} \cdot a \cdot b - k^{-} ab$$

$$a_{tot} = a + ab,\ b_{tot} = b + ab$$

$$\text{equilibrium: } K_{eq} = \frac{a \cdot b}{ab} = \frac{k^{-}}{k^{+}} = K_D \text{ dissociation constant}$$

$$\text{Association constant } K_A \text{ equals } K_D^{-1}$$

Dissociation constants for complex formation reactions

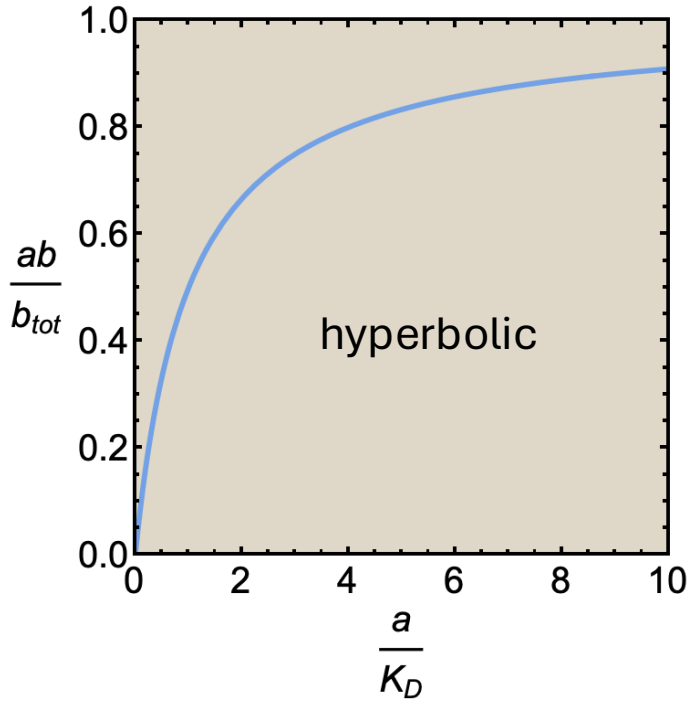
Now consider A in great excess wrt B.

Then $a_{tot} \gg b_{tot}$ and hence ab limited by b_{tot} and $ab \ll a_{tot}$ and $a \approx a_{tot}$.

$$b_{tot} = b + ab = b + \frac{a \cdot b}{K_D} = b\left(1 + \frac{a}{K_D}\right) = b\left(1 + \frac{a_{tot}}{K_D}\right)$$

$$b = b_{tot}\frac{1}{1 + \frac{a_{tot}}{K_D}}, \quad \boxed{ab = \frac{a \cdot b}{K_D} = b_{tot}\frac{\frac{a_{tot}}{K_D}}{1 + \frac{a_{tot}}{K_D}}}$$

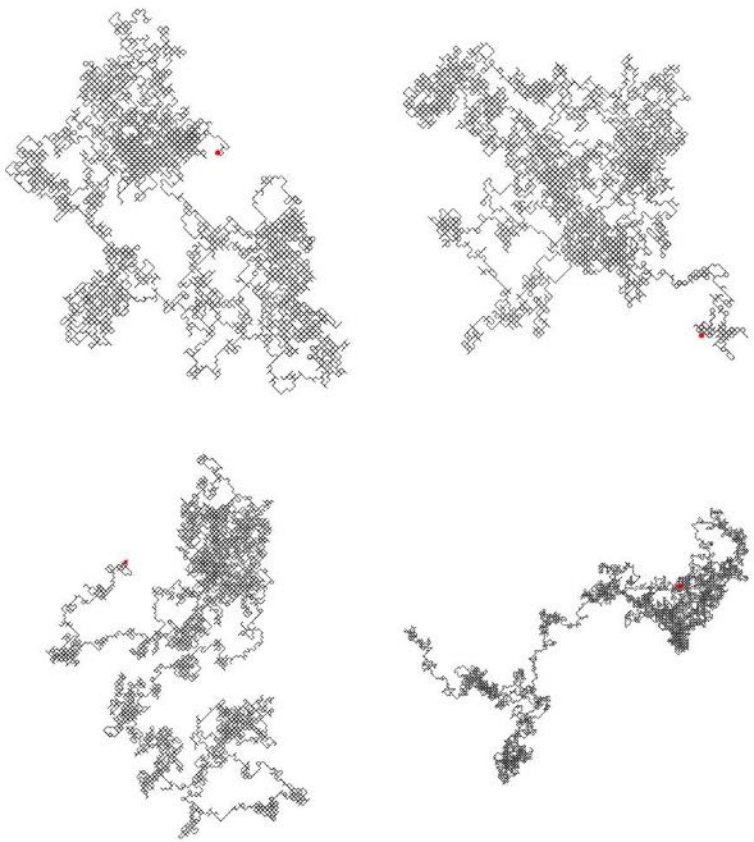
binding curve or binding polynomial

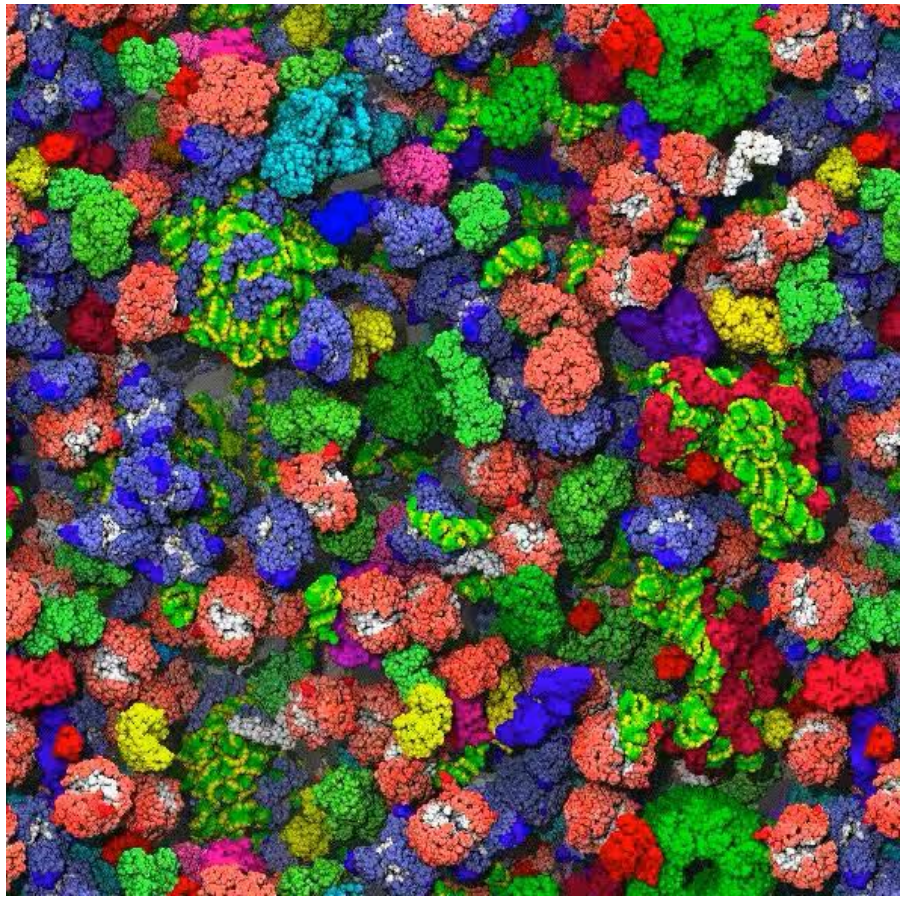


$$\text{when: } a = K_D \text{ then } ab = \frac{b_{tot}}{2}$$

Operational meaning of K_D “half-saturation constant”

Diffusion-limited rate constants for complex formation: diffusion





Diffusion-limited rate constants for complex formation: collision

$$A + B \rightleftharpoons AB \quad \text{(reaction)}$$

One can think of this reaction as a collision followed by an intramolecular process, provided that the molecules collided in the correct orientation. Hence,

$$A + B \rightleftharpoons AB^{\ddagger} \rightleftharpoons AB \quad \text{(collision followed by intramolecular process)}$$

collision complex

The reaction time is the sum of the collision time + intramolecular time.
Reaction time ≥ colission time. The collision time is given by:

$$\tau = \frac{V}{4\pi(D_A + D_B)(r_A + r_B)}$$

Calculate collision time for:
V = 1~fl, D=diffusion coefficient=5 $\mu m^2\ s^{-1}$
r=protein radius = 5 nm.

Due to the fact that a reaction time cannot be shorter than the collision time, a complex formation rate constant is always smaller then:

$$A + B \rightleftharpoons AB$$

$$0 \leq k^{+} \leq 10^{9}\ M^{-1} s^{-1}$$

Realistic value about to 50-100 fold smaller than limit.

We will use this later.

Belousov Zhabotinsky reaction