

ESC4

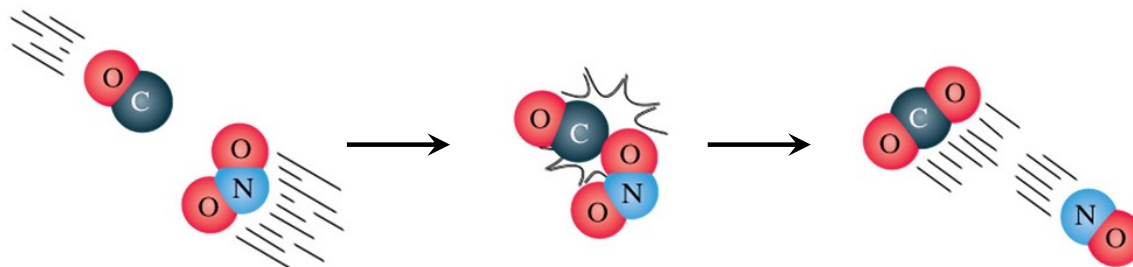
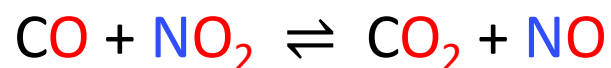
Advanced kinetics approaches to
unravel protein structure and function

Essential kinetics in biochemistry and biophysics

Prof. Francesco Malatesta

Chemical kinetics

While **thermodynamics** deals with the relative **stability** between reactants and products in a chemical reaction, **chemical kinetics** studies the **rate** at which a chemical reaction occurs and its **dependence** on various factors.

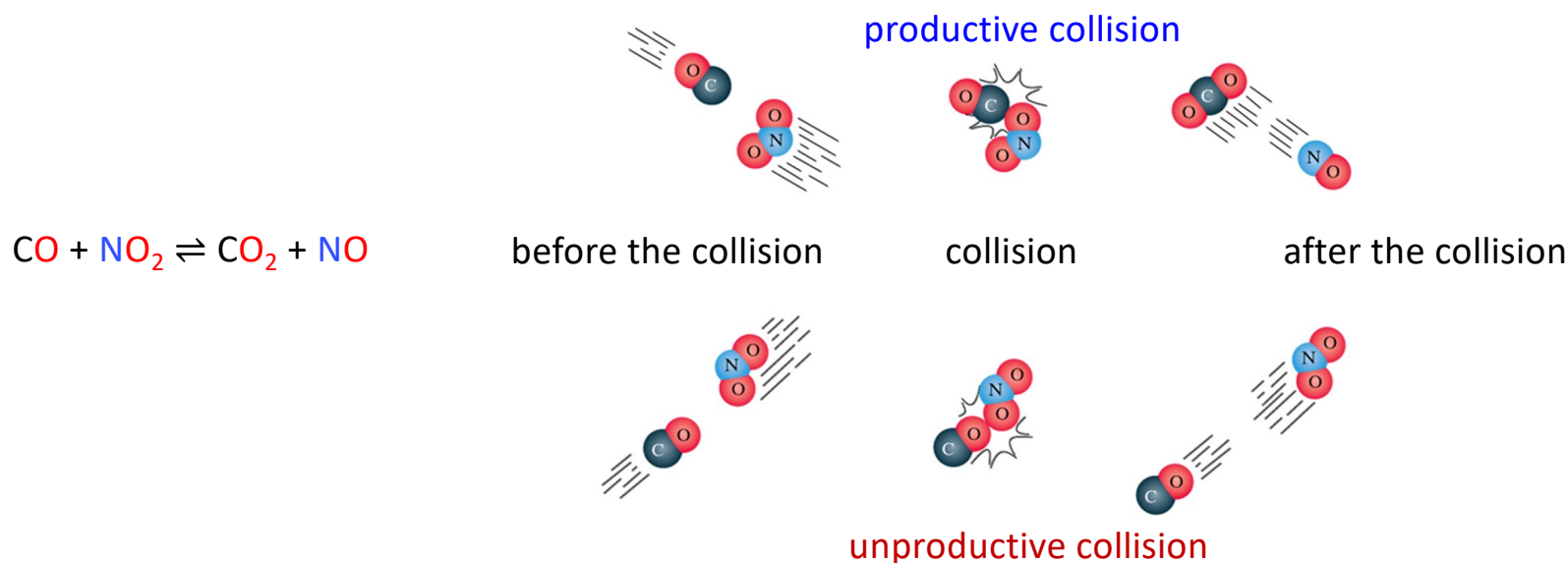


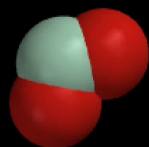
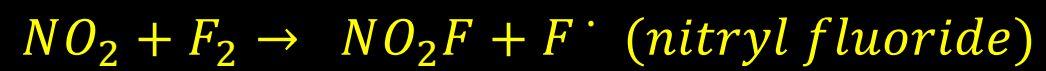
In addition to the description of the dependence of the reaction rate on experimental factors, **chemical kinetics** also examines the **sequence of elementary chemical or physical processes** through which the reactants are converted to products, i.e. the **mechanism**.

The reactions from the microscopic point of view

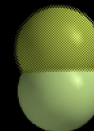
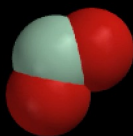
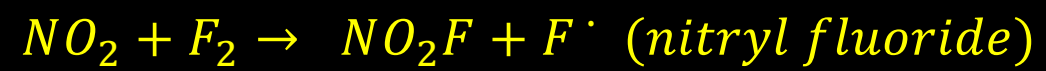
For any chemical reaction to take place, the **collision** and **transition state theories** establish that **3 conditions** must be met:

1. the reagent molecules must **collide** with each other
2. the reagent molecules must collide with **enough energy** to break the bonds involved in the reaction
3. the molecules must collide according to an **orientation** that can allow the rearrangement of the atoms and the formation of the products.





bad orientation



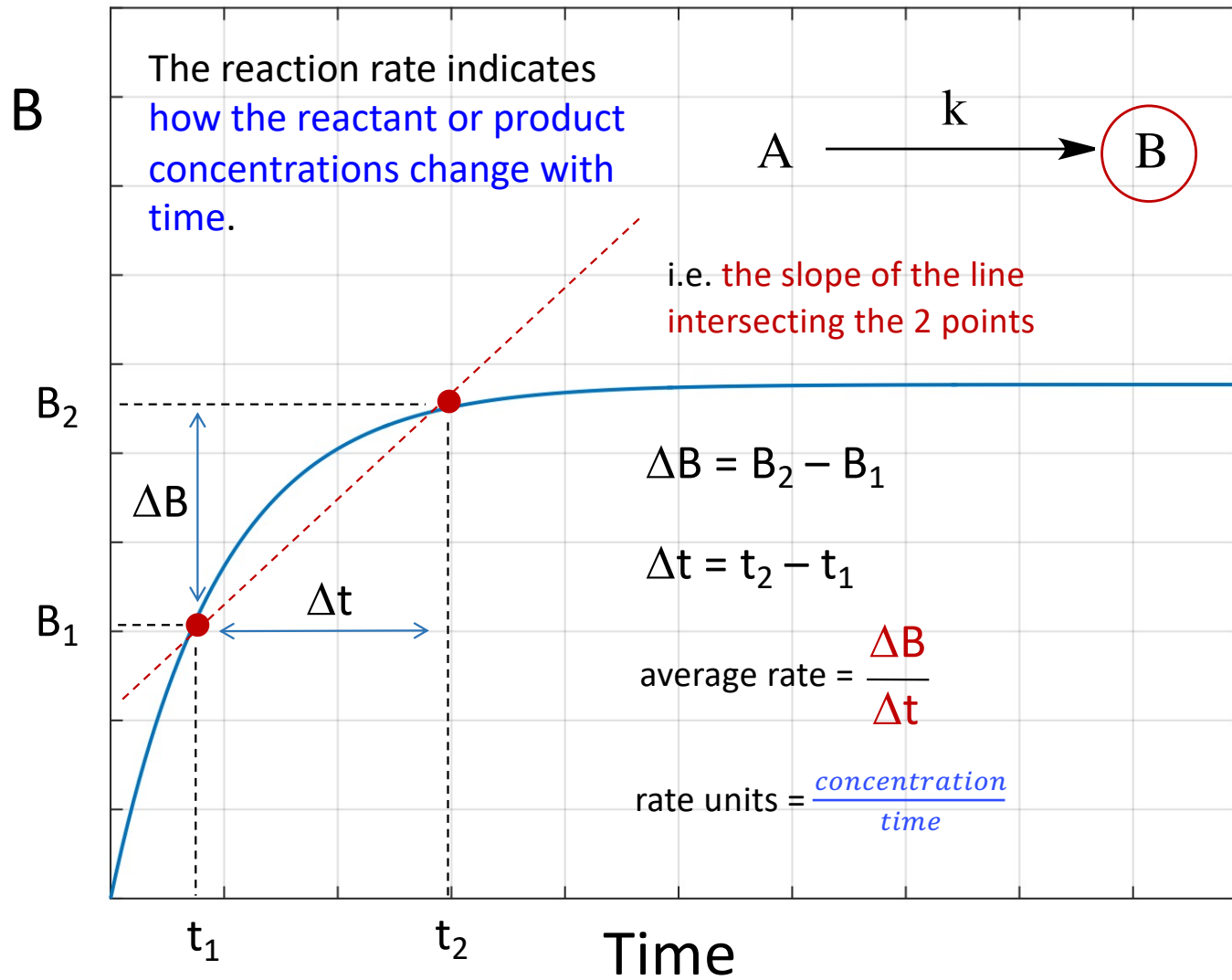
good orientation

Reaction rates depend on several factors

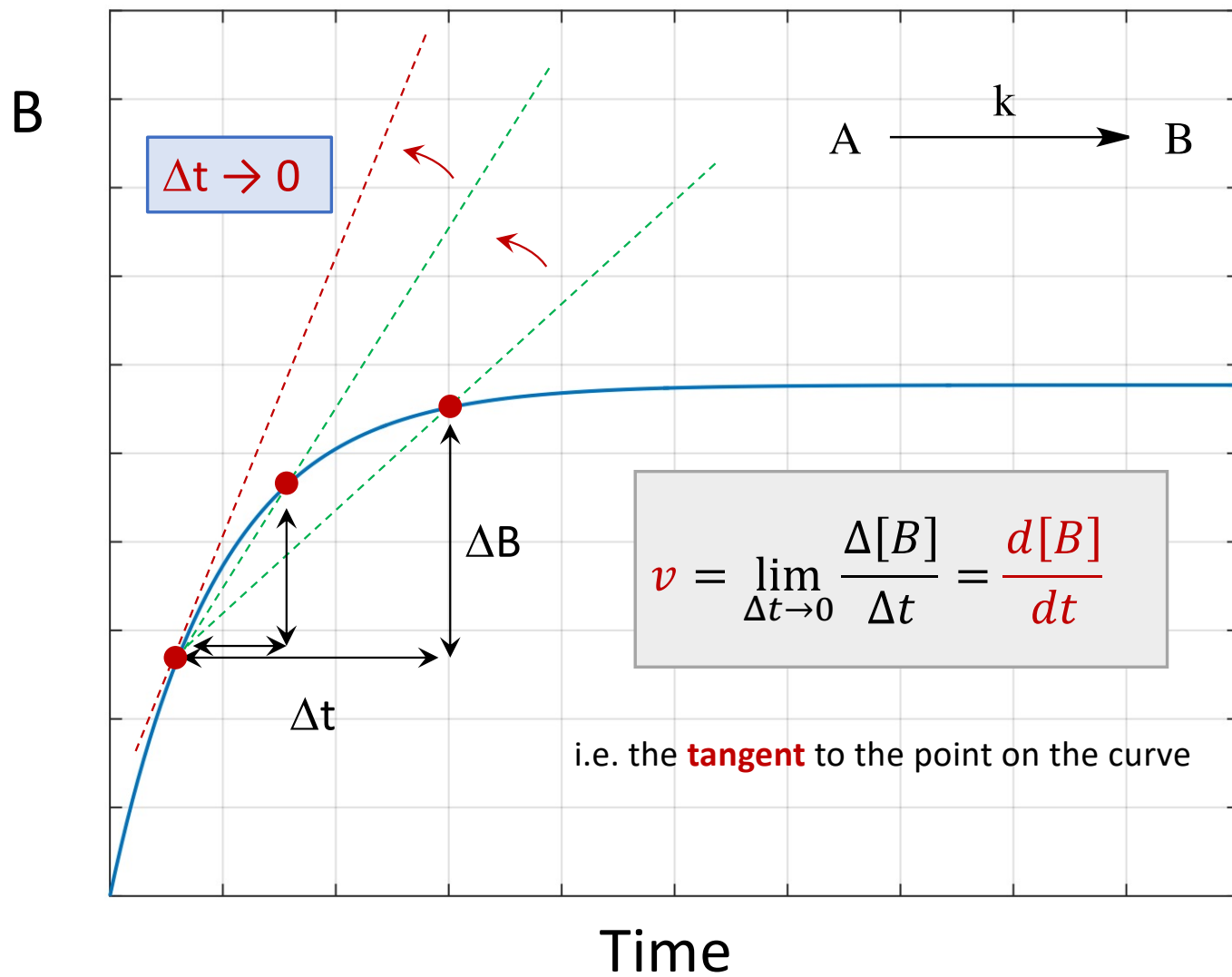
- the **nature** of the reagents
- the **concentration** of reagents
- reaction **temperature** (always)
- the presence of **catalysts** (enzymes, of course!)
- the area of the **interface** (heterogeneous reactions)

But how do we define a reaction rate?

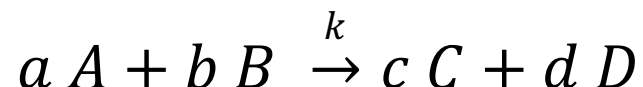
The average rate



The instantaneous rate



Dependence of the reaction rate on concentration



Experimentally (only!) it is found that the **rate of a chemical reaction depends on the concentration of reagents**. The equation which correlates the rate of a reaction with the concentrations of the reagents (products) is known as the **kinetic equation** and has the form:

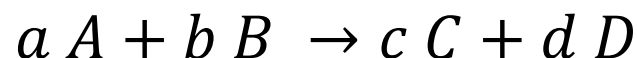
$$v = k [A]^p [B]^q$$

units of k: $M^{-(p+q-1)} s^{-1}$

i.e. the product of concentrations of the reagents raised to suitable coefficients (p, q, ..., *generally different from the stoichiometric coefficients*) multiplied by a constant k. **These coefficients – order – are usually (but not always) integers and must be determined experimentally.**

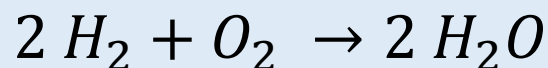
The constant **k** is called the **specific rate constant** and depends on the nature of the reagents and the temperature.

To report the kinetics of a single reaction unambiguously when **different molecules** are formed or consumed at **different rates**, a useful definition is the **reaction velocity scaled by the stoichiometric coefficients**

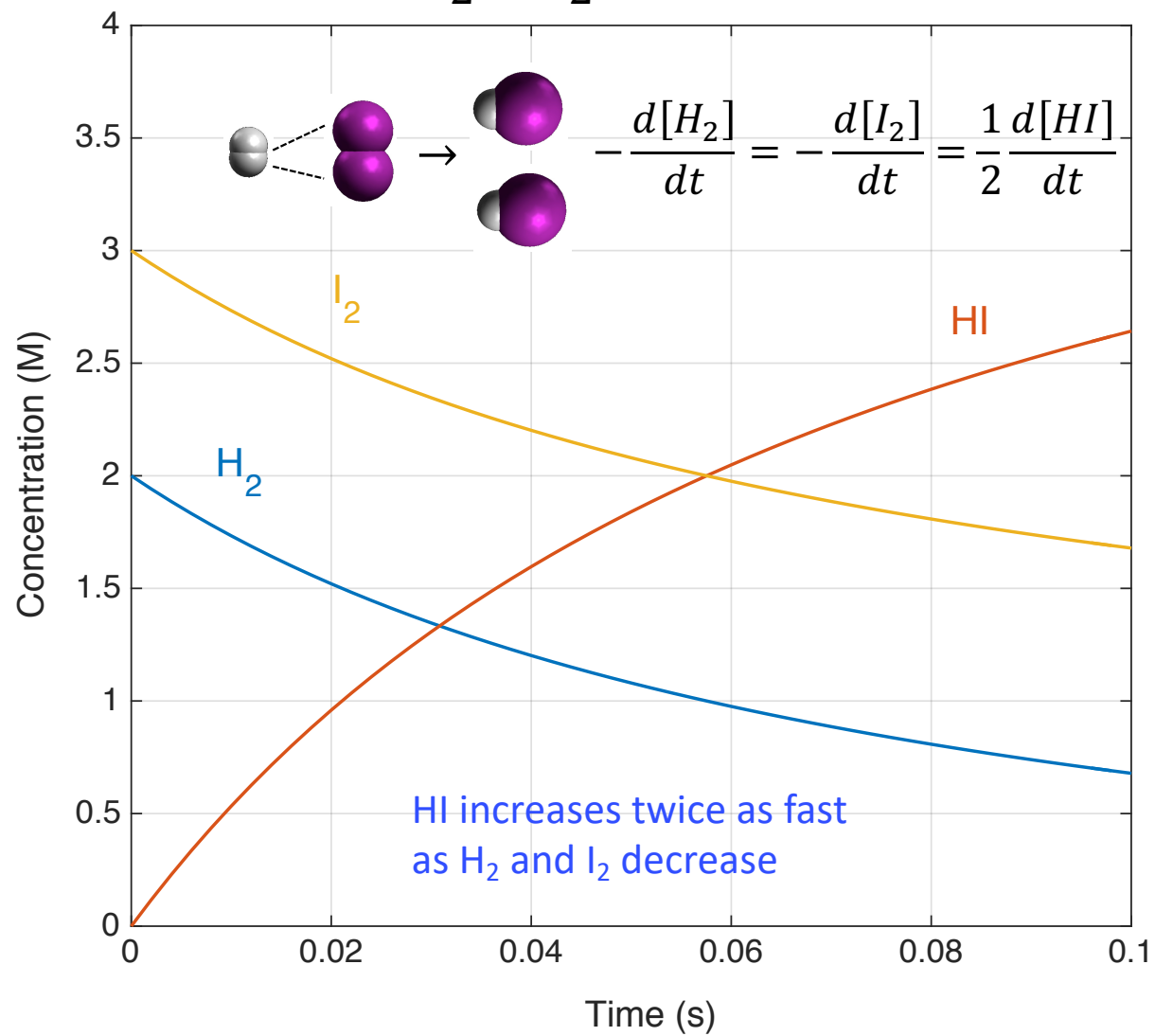
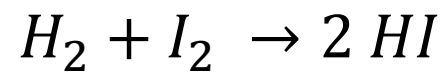


$$v = -\frac{1}{a} \frac{d[A]}{dt} = -\frac{1}{b} \frac{d[B]}{dt} = \frac{1}{c} \frac{d[C]}{dt} = \frac{1}{d} \frac{d[D]}{dt} = k[A]^p[B]^q$$

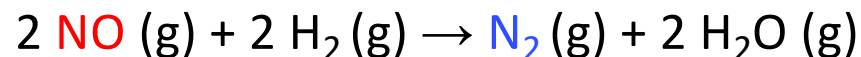
The reason that stoichiometric coefficients appears in the definition is so that the **reaction velocity is equal for species in the reaction that have different stoichiometries**



$$v = -\frac{1}{2} \frac{d[H_2]}{dt} = -\frac{d[O_2]}{dt} = \frac{1}{2} \frac{d[H_2O]}{dt}$$

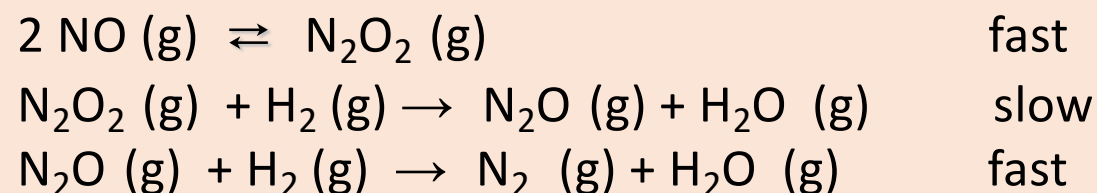


Example on reaction order



kinetic equation $v = k [\text{NO}]^2 [\text{H}_2]$

is **second order with respect to NO**, **first order with respect to H₂** and **third order overall**.



If a reagent does not appear in the kinetic equation for a given reaction, this is of **zero order** with respect to that reagent.

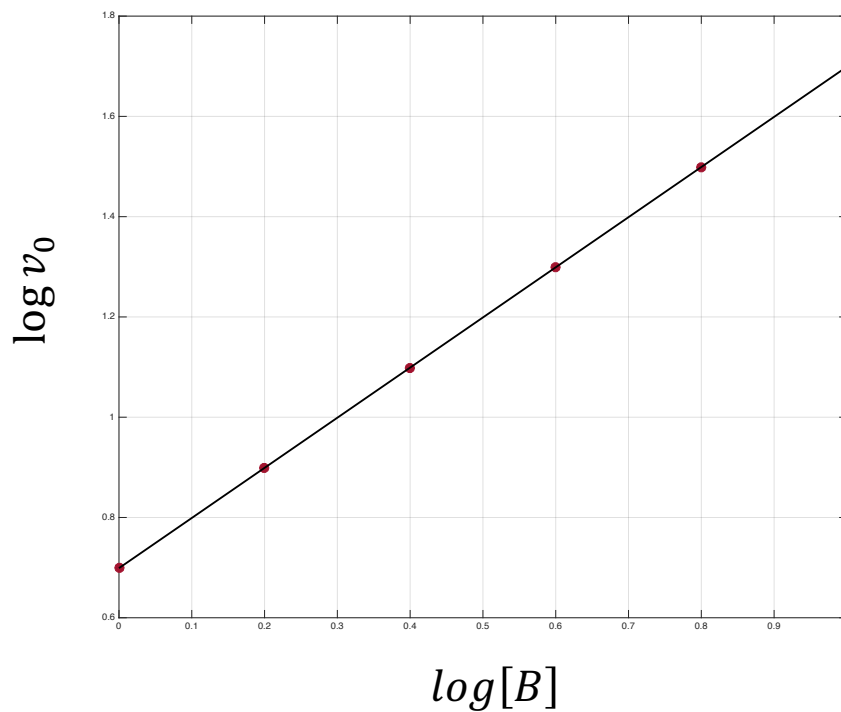
Reaction orders may fractional (i.e. $\frac{1}{2}$, $\frac{3}{2}$...) and even negative

Determination of reaction order

$$v = k_1[A]^p[B]^q - k_2[C]^m$$

when $t \rightarrow 0$ $v_0 \cong k_1[A]^p[B]^q$

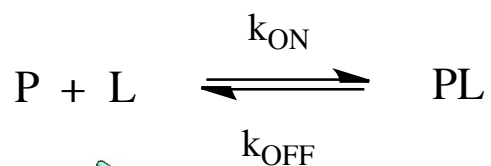
$$\log v_0 = \underbrace{\log k_1 + p \log[A]}_c + q \log[B] = c + q \log[B]$$



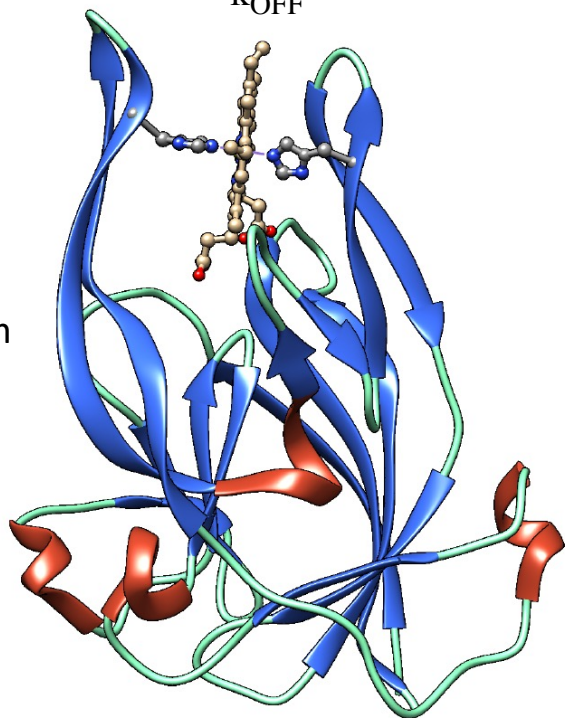
slope = q

yintercept = c

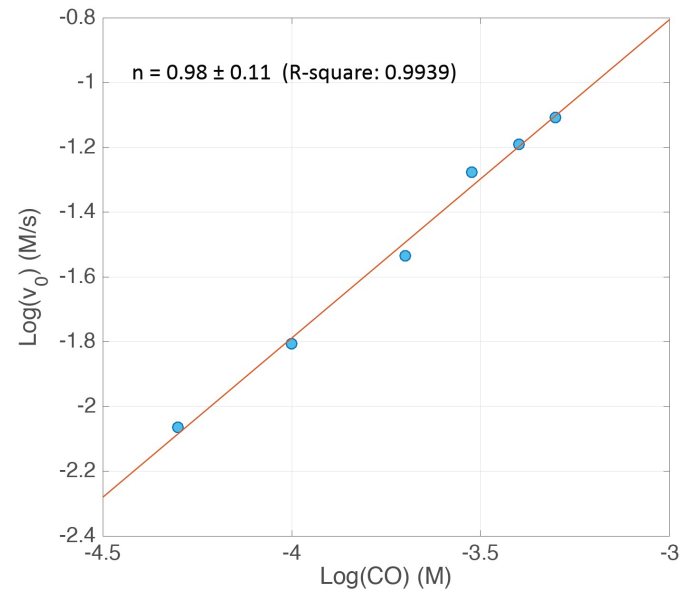
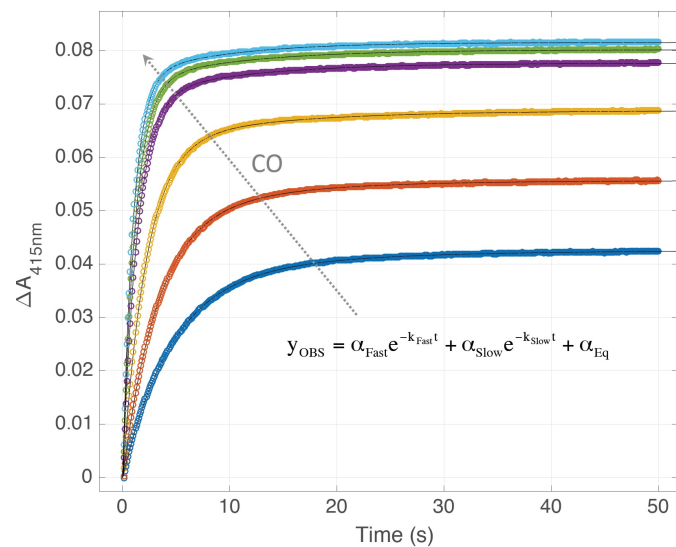
CO binding to HmuY hemophore from *Porphyromonas gingivalis*



pdb: 6ewm

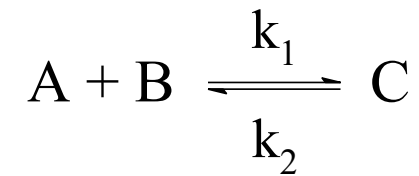


L. Montemiglio, C. Exertier, T. Olczak, F. Malatesta, in preparation

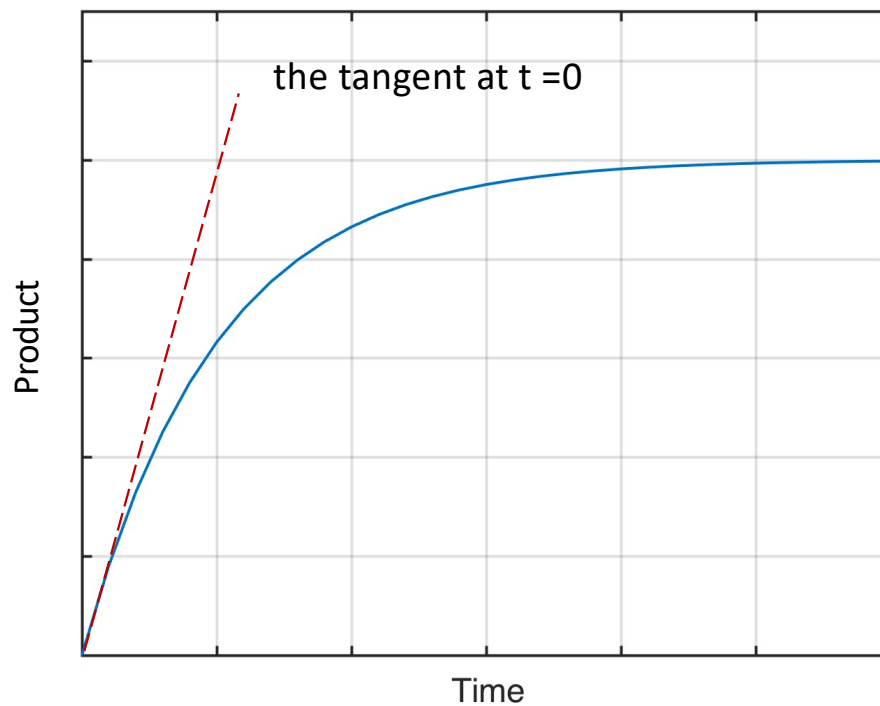


Initial rates

Extremely important in chemistry
(i.e. reaction order determination)



$$v = k_1[A]^p[B]^q - k_2[C]^m \quad \text{when } t \rightarrow 0 \quad v_0 \cong k_1[A]^p[B]^q$$



and in biochemistry:

$$v_0 = \frac{V_{MAX} [S]}{K_M + [S]}$$

The two reaction types found
in all proteins and enzymes

all reaction orders are integer numbers
(i.e. first- & second-order)

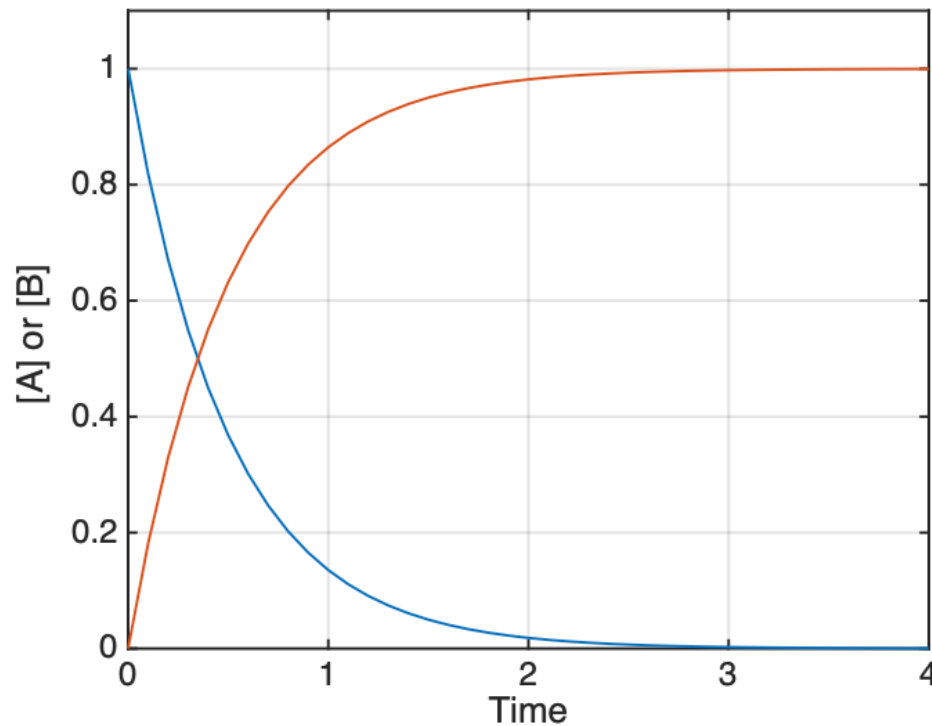
In the reaction $A \xrightarrow{k} B$ the kinetic equation is:

$$v = -\frac{d[A]}{dt} = \frac{d[B]}{dt} = k[A] \quad \text{and is first-order with respect to A.}$$

dimensions of k: time^{-1} (ex. s^{-1})

In enzymes: bond breaking/forming, conformational reactions, ET.

In proteins: folding/unfolding, allosteric changes



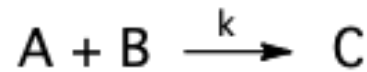
$$-\frac{d[A]}{dt} = k[A]$$

$$\int_{A_0}^0 \frac{d[A]}{[A]} = -k \int_0^t dt$$

$$[A] = A_0 e^{-k t}$$

$$[B] = A_0 (1 - e^{-k t})$$

In the reaction



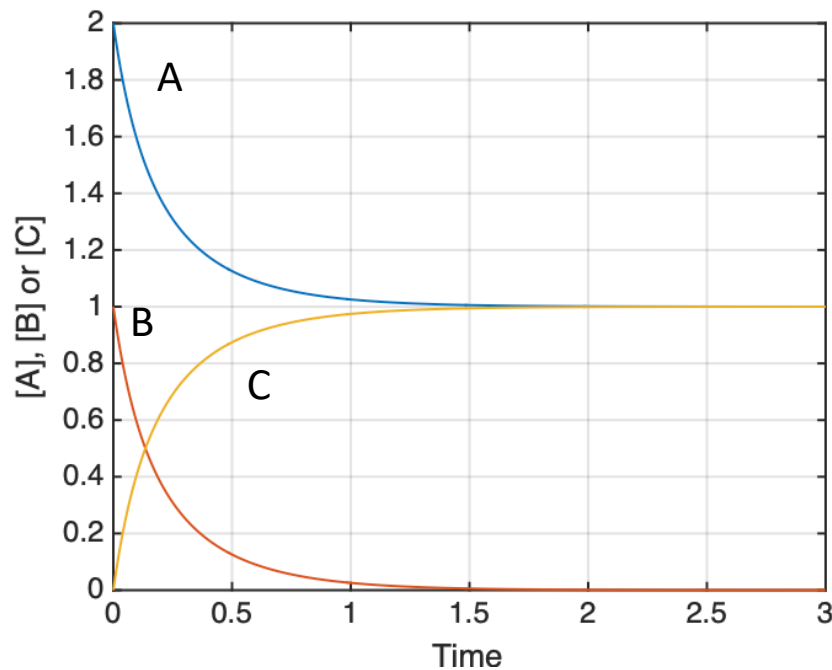
the kinetic equation is:

$$v = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = \frac{d[C]}{dt} = k[A][B]$$

In enzymes and proteins: all binding reactions, ET reactions

and is first-order with respect to A and B and overall **second-order**.

dimensions of k: $M^{-1} \text{time}^{-1}$ (ex. $\mu M^{-1} s^{-1}$)



$$-\frac{d[A]}{dt} = k[A][B]$$

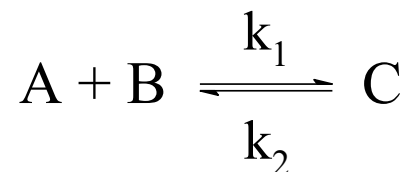
$$[A] = A_0 - x \quad \& \quad [B] = B_0 - x \quad \& \quad [C] = x$$

$$\frac{dx}{dt} = (A_0 - x)(B_0 - x)$$

$$\int_0^x \frac{dx}{(A_0 - x)(B_0 - x)} = -k \int_0^t dt$$

$$x = \frac{A_0 B_0 (1 - e^{-k(B_0 - A_0)t})}{B_0 - A_0 e^{-k(B_0 - A_0)t}}$$

Reactions are usually reversible



rate of the forward
reaction (v_f)

The kinetic equation is:

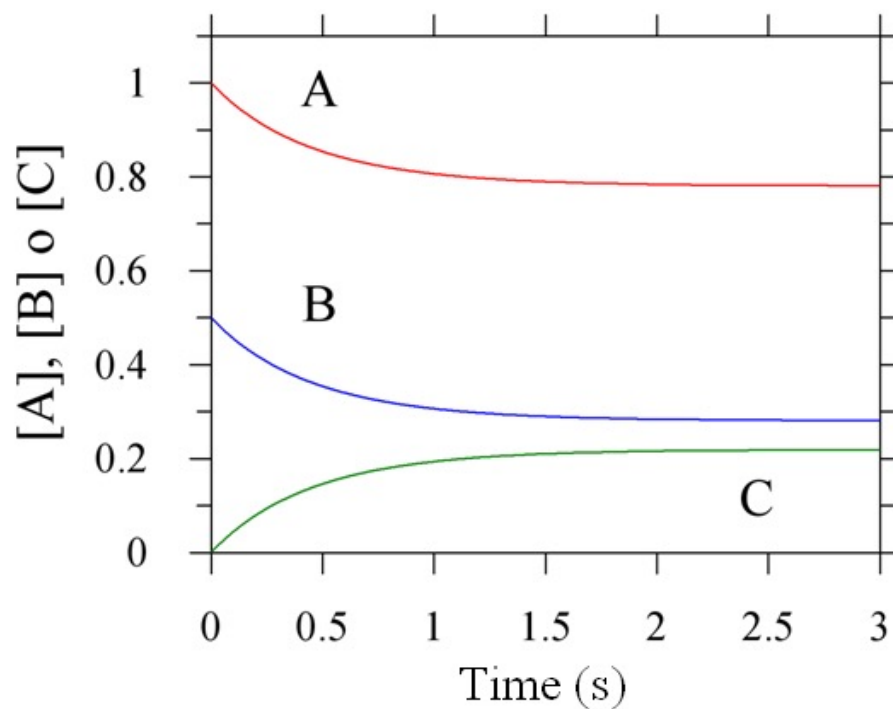
$$v = k_1[A][B] - k_2[C]$$

rate of the reverse
reaction (v_i)

at equilibrium $v_f = v_i$
and

$$K_D = \frac{[A][B]}{[C]} = \frac{k_2}{k_1}$$

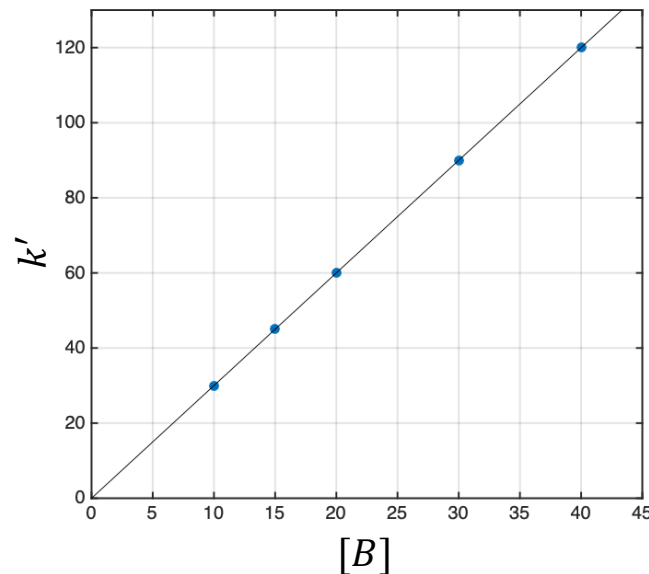
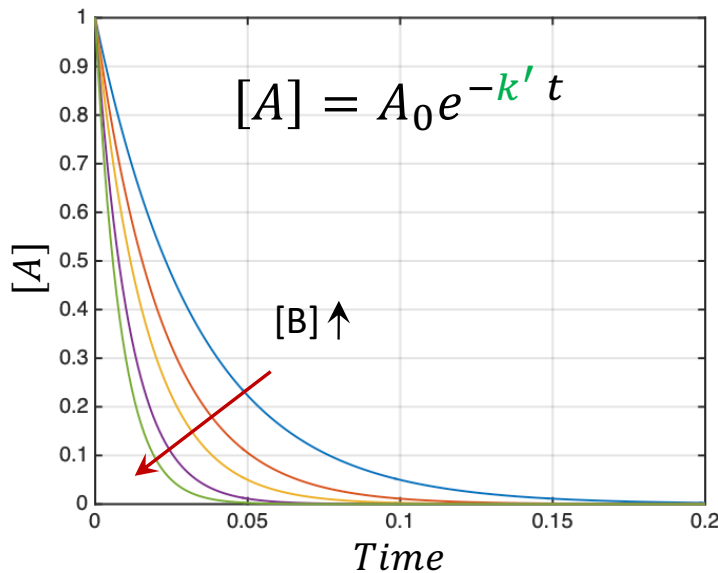
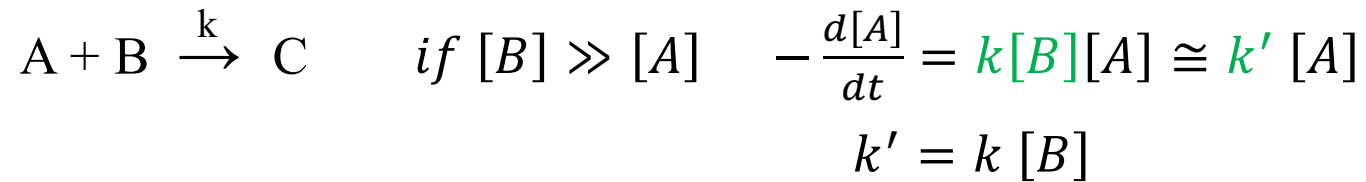
A very useful
complement to
thermodynamics



Pseudo-first order conditions (PFO)

Experimental conditions whereby **one reagent is in large excess** relative to other reagents.

Typically, under these conditions **simple exponential time courses** are found.



PFO conditions are usually used in steady-state enzyme kinetics

Zero-order reactions

In the reaction $A \xrightarrow{k} B$ the kinetic equation is:

$$v = -\frac{d[A]}{dt} = \frac{d[B]}{dt} = k [A]^0$$

$$[A] = A_0 - k t$$

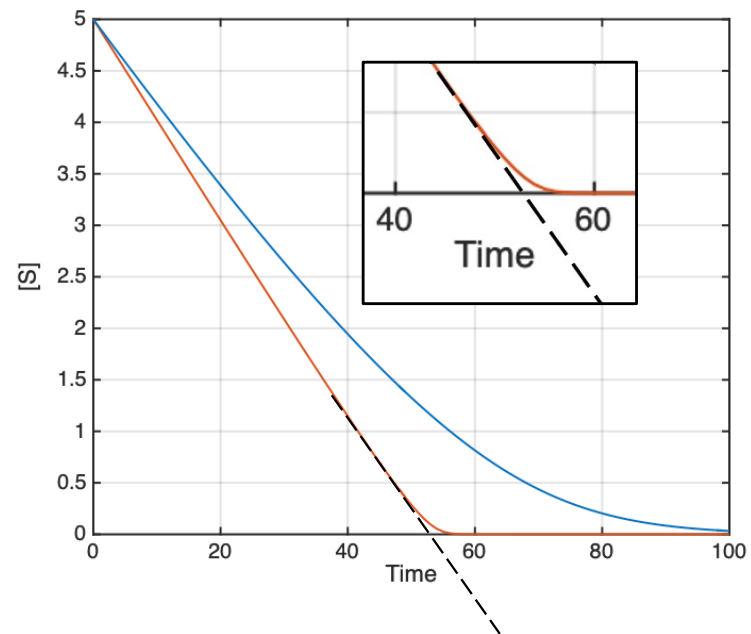
and zero-order with respect to A.

dimensions of k: **concentration time⁻¹**
(ex. M s⁻¹)

Zero-order kinetics are **always an artifact of the conditions** under which the reaction is carried out.

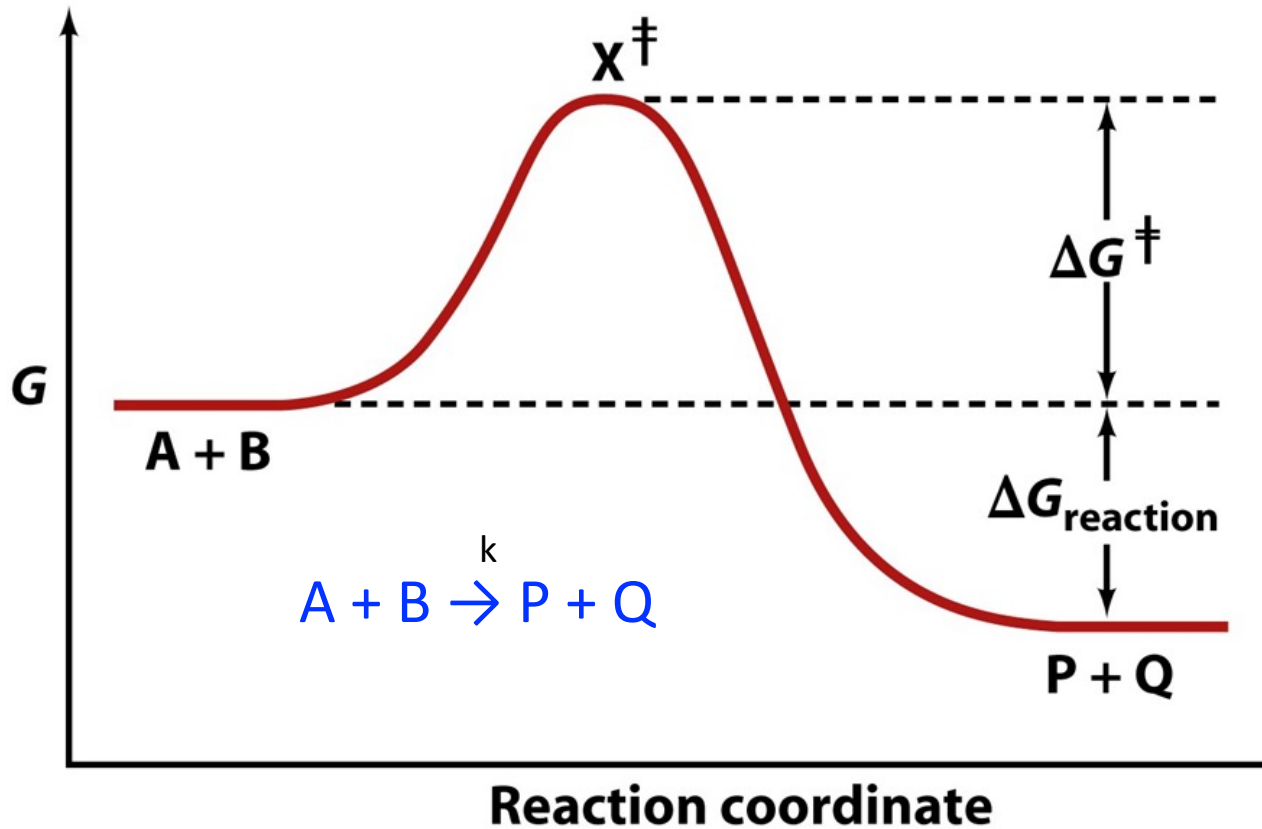
Clearly, a zero-order process **cannot continue after a reactant has been exhausted**. Just before this point is reached, the reaction will revert to another rate law instead of falling directly to zero

The **early stage** of an **enzyme-catalysed reaction under steady-state conditions** is usually **zero-order**



Dependence of the reaction rate on temperature

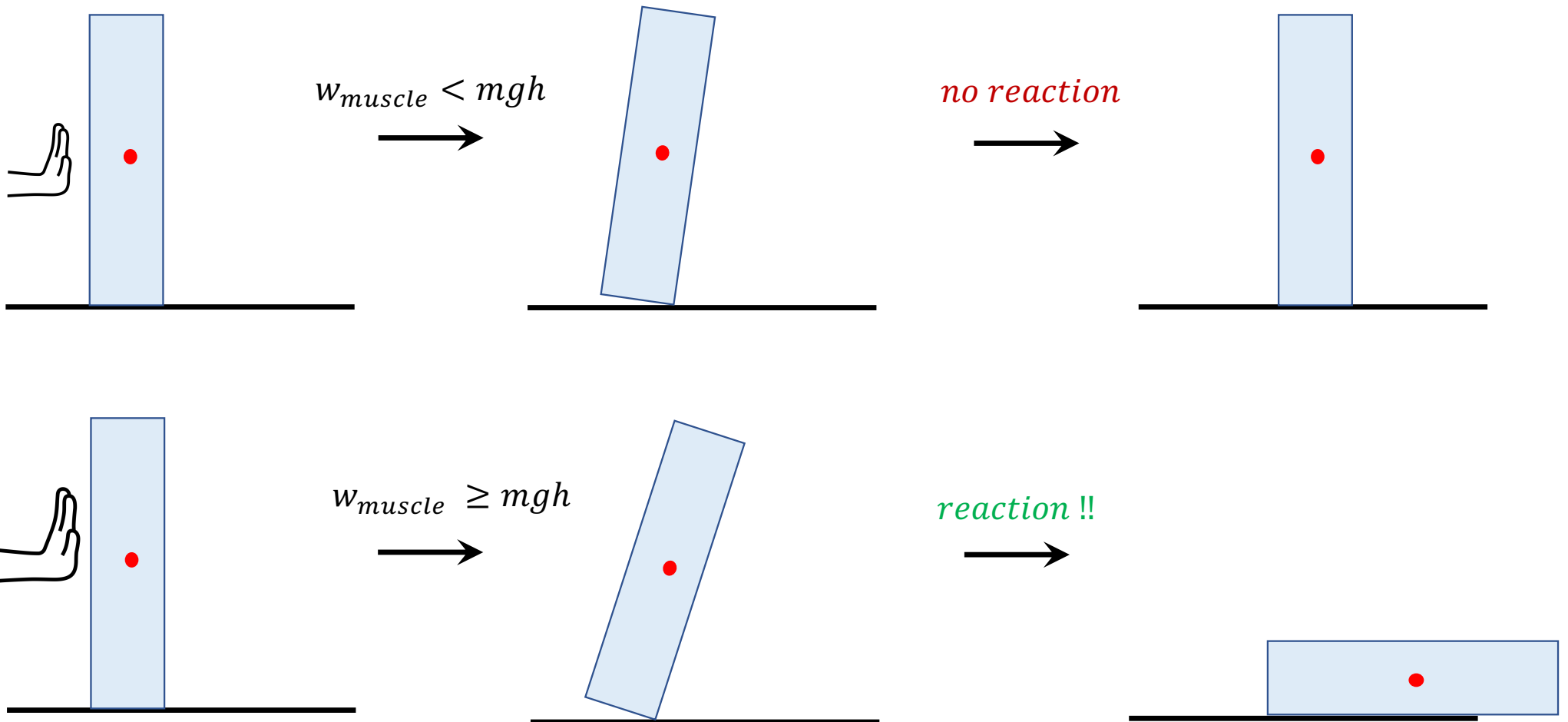
The reaction rate is a function of the **free energy of activation ΔG^\ddagger** (TST)



Eyring–Evans–Polanyi equation

$$k = \frac{\kappa k_B T}{h} e^{-\frac{\Delta G^\ddagger}{RT}}$$
$$= \frac{\kappa k_B T}{h} e^{\frac{\Delta S^\ddagger}{R}} e^{-\frac{\Delta H^\ddagger}{RT}}$$

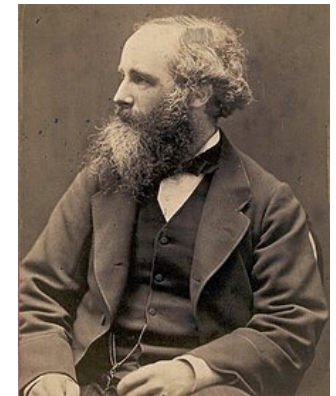
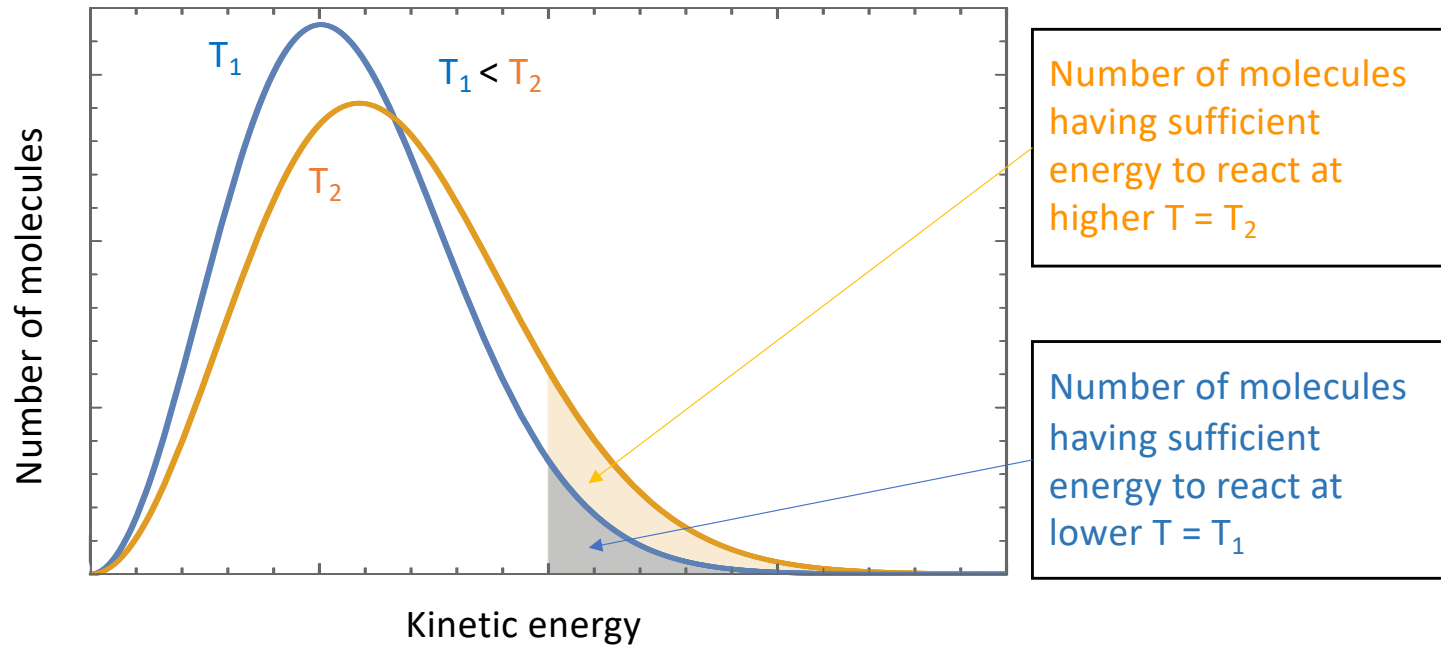
Why do you need energy to start a chemical reaction? **An analogy**



Why does the reaction rate increase with temperature?

$$k = \frac{\kappa k_B T}{h} e^{-\frac{\Delta G^\ddagger}{RT}}$$

Maxwell-Boltzmann distribution



James Clerk Maxwell



Ludwig Boltzmann