Lecture: Computational Systems Biology
Universität des Saarlandes, SS 2012

03 Modelling [part 2]

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15.5.2012

Recap

- Some biochemistry basics: chemical reactions, equilibrium, biochemical pathways,
  genes → mRNA → proteins
- Stoichiometry (-ic matrix)
- Kinetic functions: mass action, Michaelis-Menten (enzymatic reactions)
- How to transform a reaction system into a quantitative model, e.g. ordinary differential equation system (ODE)
- Simulation: numerical integration of (stiff) ODEs
- COPASI (exercise)

Equilibrium constant

Example: $S_1 + S_2 \leftrightarrow 2\ P$

$$v = v_f - v_r = k_f \cdot [S_1] \cdot [S_2] - k_r \cdot [P]^2$$

in general:

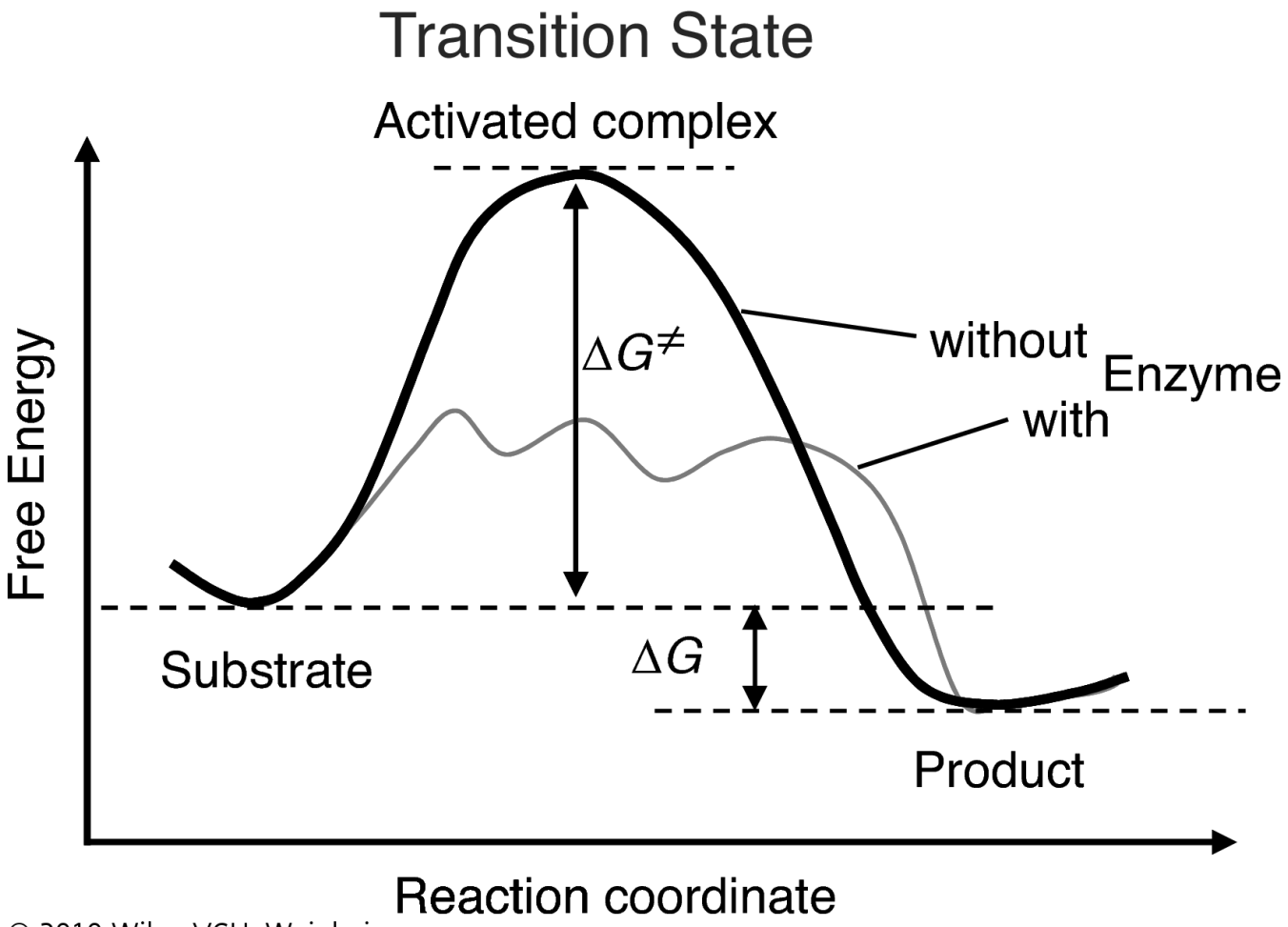
$$v = v_f - v_r = k_f \prod [S_i]^{n_i} - k_r \prod [P_j]^{n_j}$$

Equilibrium constant (determined by respective thermodynamic properties; in equilibrium, the forward rate equals the backward rate):

$$K_{eq} = \frac{k_f}{k_r} = \frac{\prod [P_j]_{eq}^{n_j}}{\prod [S_i]_{eq}^{n_i}}$$

In our example: $K_{eq} = [P]_{eq}^2 / \left([S_1]_{eq} \cdot [S_2]_{eq} \right)$

Enzymatic reactions (transition state theory)



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Enzymes

- **Remember**: enzymes cannot change the free energies of substrates or products, nor their difference
- They only change the way the reaction proceeds microscopically, the so-called reaction path

→ Transition state theory: free energy of (unstable) activated complexes is lowered. This decreases the activation energy and increases the rate(s) of the reaction

Michaelis-Menten mechanism

$$S + E \Leftrightarrow ES \rightarrow P + E$$

Irreversible, one-substrate enzymatic reaction

Important parameters:
$V_{max} = k_{cat} \cdot [E]_{tot}$, $K_M = (k_{off} + k_{cat})/k_{on}$

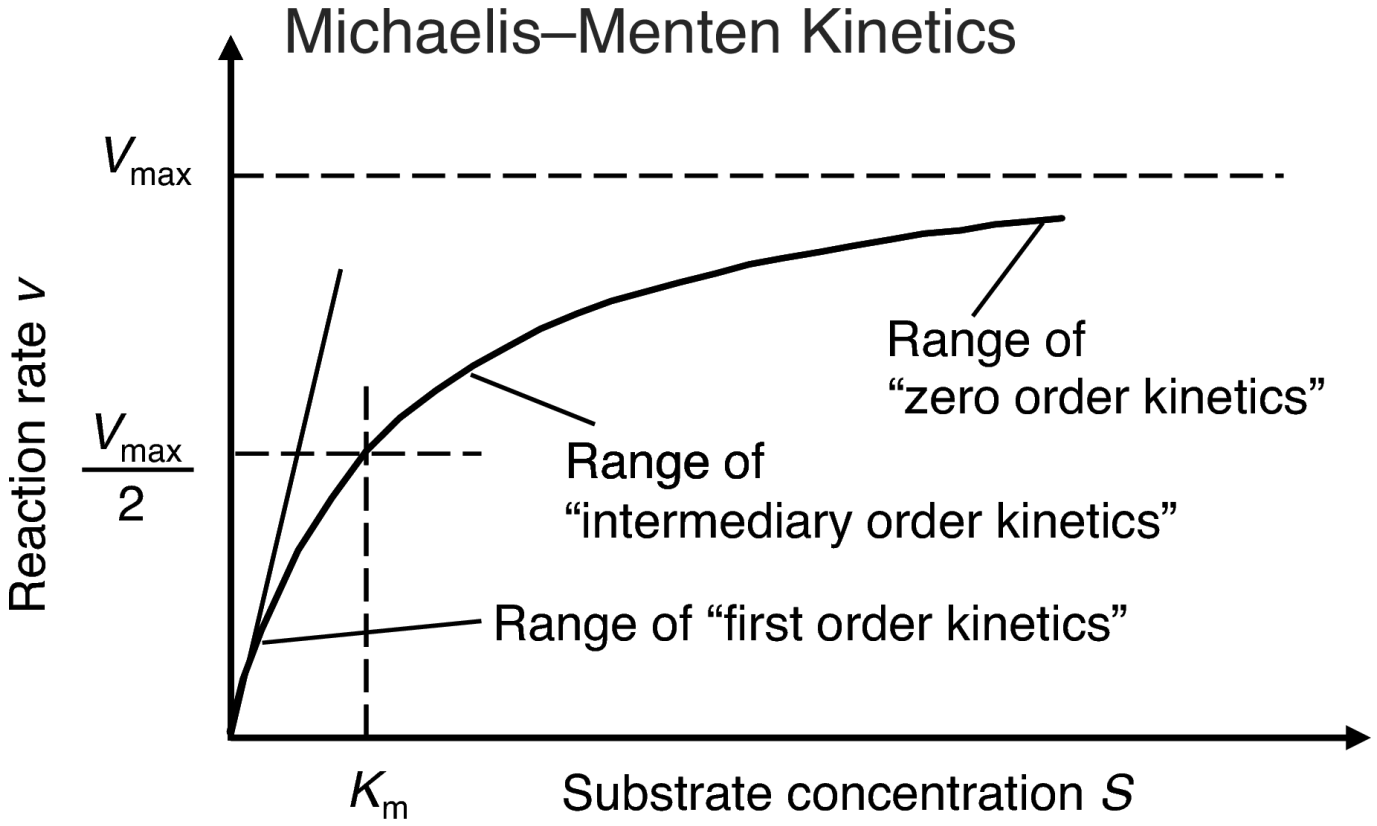
Victor Henri 1903 (bond between enzyme and substrate)

Leonor Michaelis and Maud Menten 1913 (reaction mechanism, quasi-equilibrium assumption, rate law)

G.E. Briggs and J.B.S. Haldane 1925 (quasi-steady-state assumption)

Michaelis-Menten kinetics

$$v = \frac{V_{max} \cdot [S]}{K_M + [S]}$$



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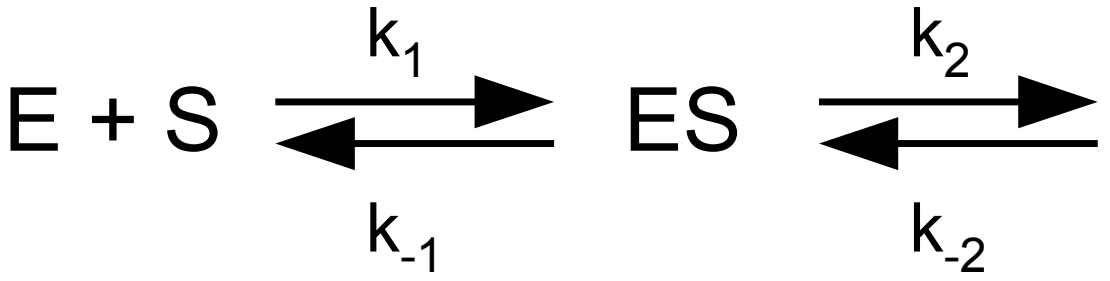
Reversible Michaelis-Menten mechanism

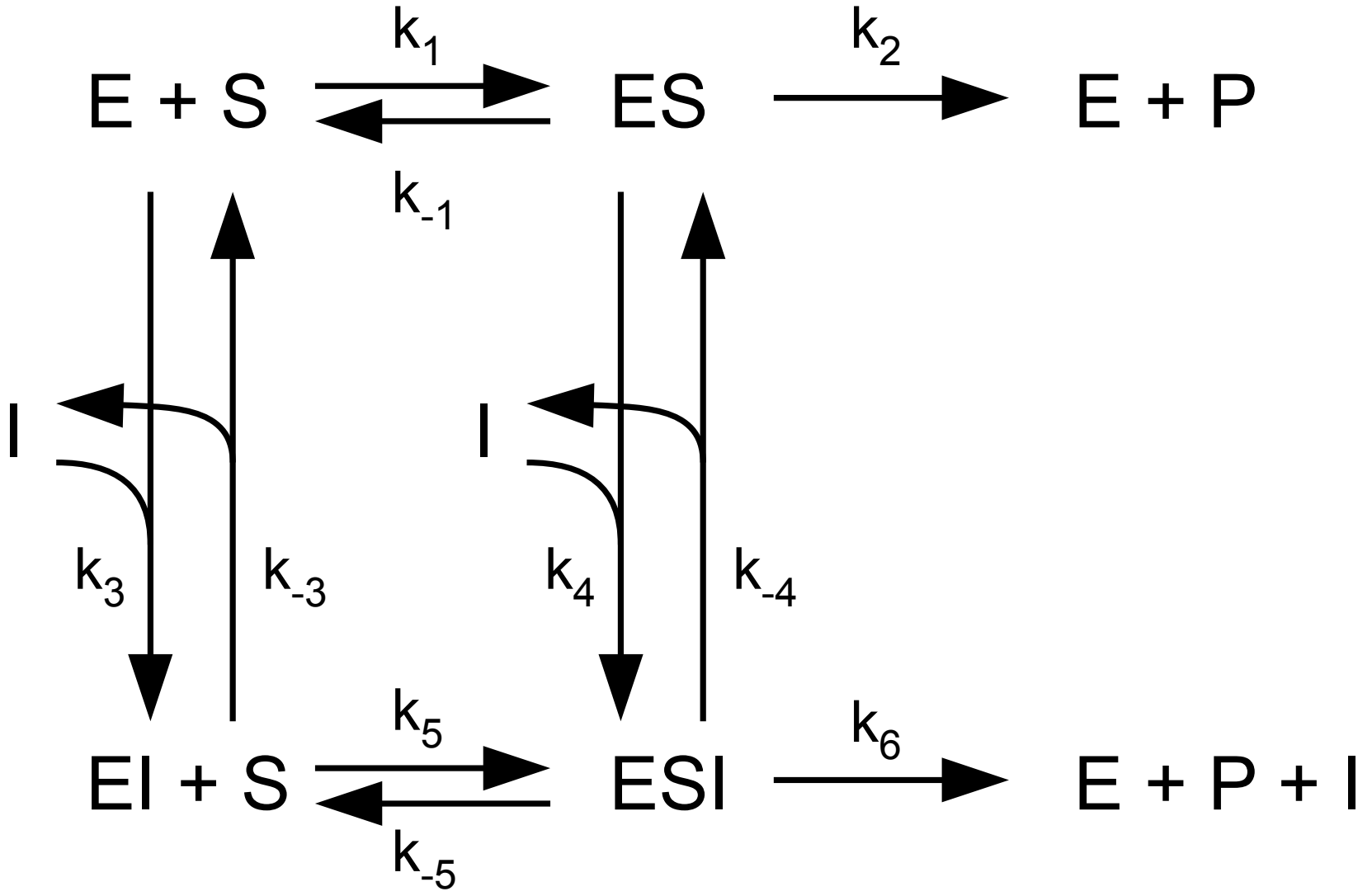


$$v = \frac{d[P]}{dt} = k_2[ES] - k_{-2}[P] = \frac{\left(V^f_{max}/K_{mS}\right)[S] - \left(V^r_{max}/K_{mP}\right)[P]}{1 + [S]/K_{mS} + [P]/K_{mP}}$$

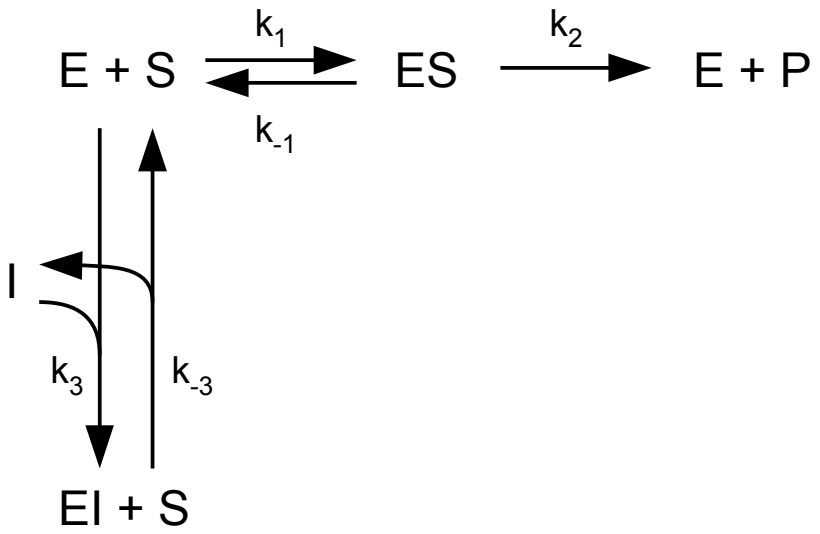
How to derive a rate equation

1) Draw diagram of reaction mechanism (qualitative model)
2) Turn this into a quantitative model with mass action rate laws
3) Sum of all enzyme-containing species is equal to total enzyme concentration $[E]_{tot}$. Therefore, right sides (ODEs) of all enzyme species sum up to zero. This gives one equation
4) Assumption of quasi-steady state for *n-1* enzyme species (set right sides of ODE to zero) together with 3) gives algebraic equations for all *n* enzyme species
5) Reaction rate is equal to product formation. Insert respective concentrations of enzymes species from 4)

Regulation of enzymes by effectors



Competitive inhibition

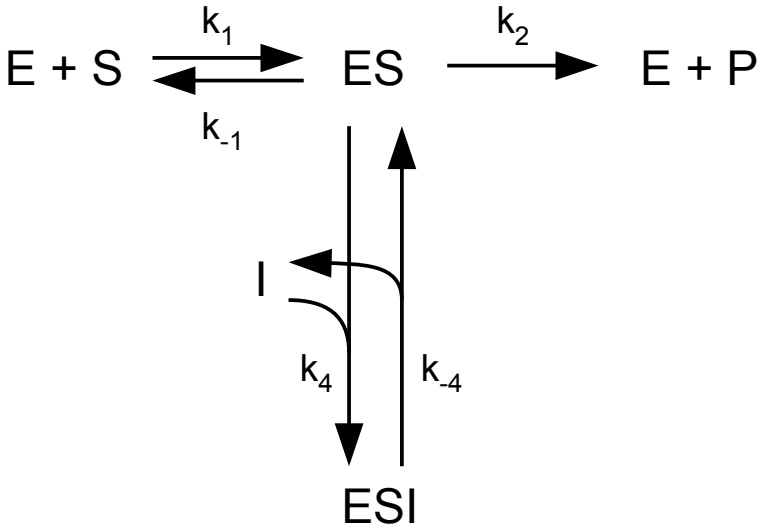


$$v = \frac{V_{max}[S]}{K_m(1+[I]/K_i)+[S]}$$

Note: apparent K_m is increased, V_{max} stays the same.
K_i is dissociation constant of inhibitor.

Reversible version $$v = \frac{V^f_{max}([S]/K_{mS}) - V^r_{max}([P]/K_{mP})}{(1+[I]/K_i)+[S]/K_{mS}+[P]/K_{mP}}$$

Uncompetitive inhibition

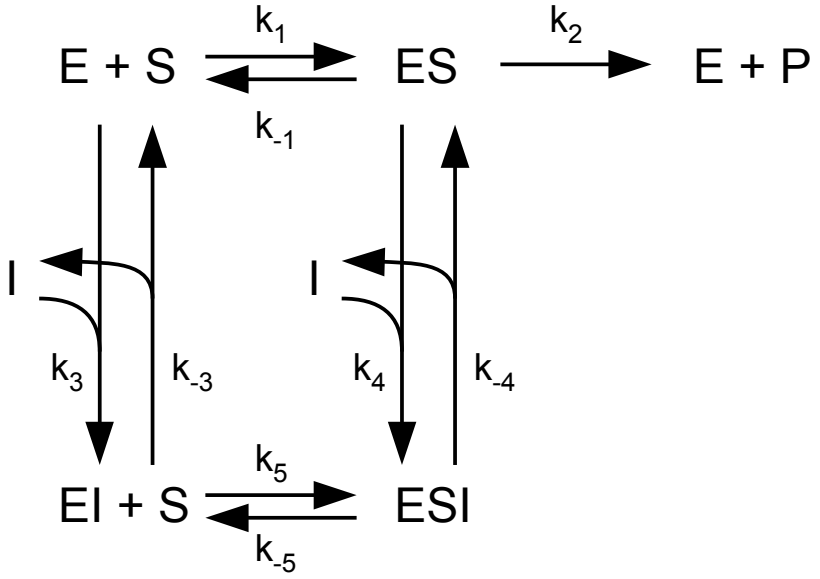


$$v = \frac{V_{max}[S]}{K_m + [S](1 + [I]/K_i)}$$

Note: *S* and *I* do not compete for binding sites.
Increase in *[S]* cannot fully displace the inhibitor
→ original V_{max} cannot be reached. K_m changes also.

Reversible version $$v = \frac{V^f_{max}([S]/K_{mS}) - V^r_{max}([P]/K_{mP})}{1 + ([S]/K_{mS} + [P]/K_{mP})(1 + [I]/K_i)}$$

Noncompetitive inhibition

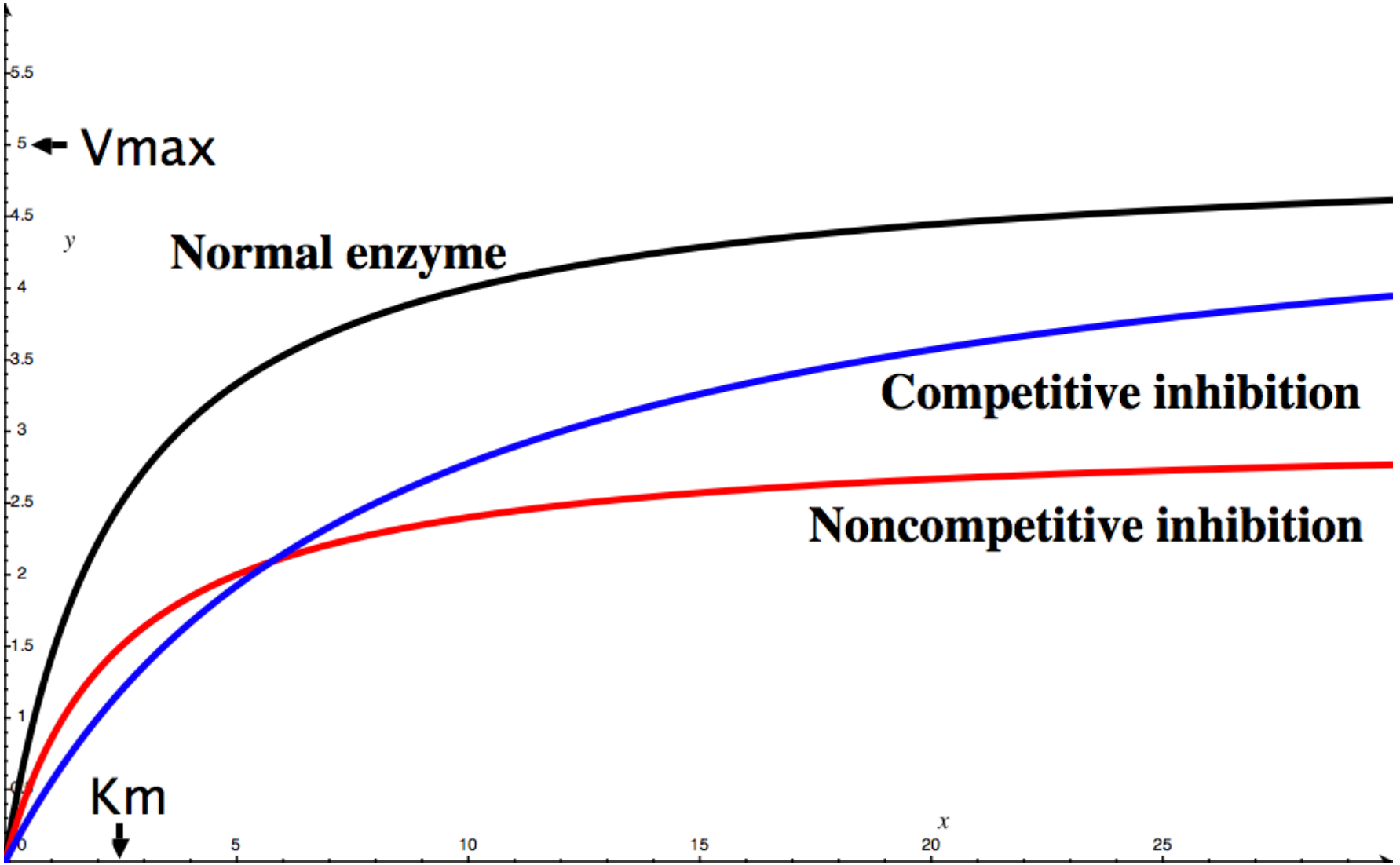


$$v = \frac{V_{max}[S]}{(K_m + [S])(1 + [I]/K_i)}$$

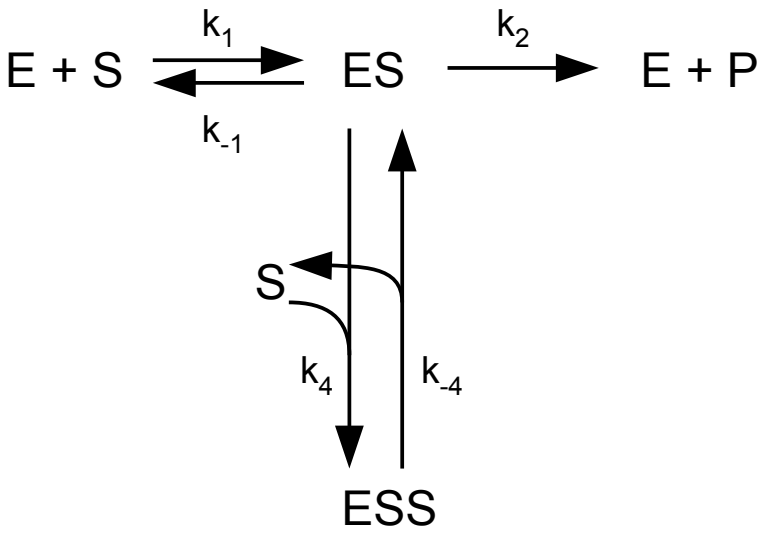
Note: V_{max} changes. K_m stays the same.

Reversible version $$v = \frac{V^f_{max}([S]/K_{mS}) - V^r_{max}([P]/K_{mP})}{(1 + [S]/K_{mS} + [P]/K_{mP})(1 + [I]/K_i)}$$

Rate vs. substrate concentration



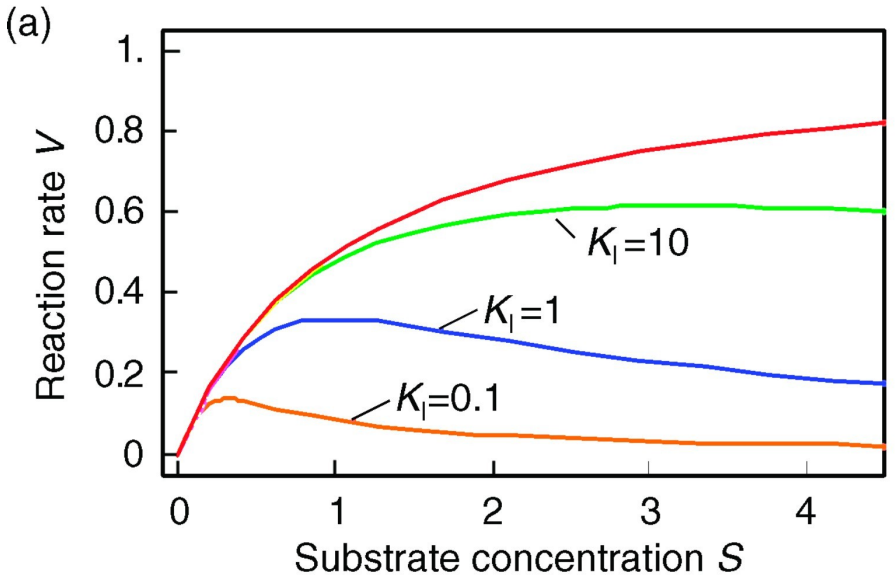
Substrate inhibition

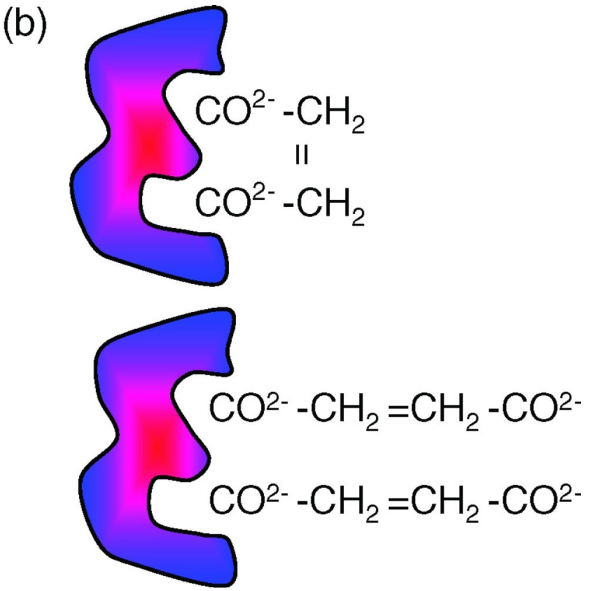


$$v = k_2 \cdot [ES] = \frac{V_{max}[S]}{K_m + [S]\left(1 + \left([S]/K_i\right)\right)}$$

Optimum at $[S]_{opt} = \sqrt{K_m K_i}$

with $v_{opt} = \frac{V_{max}}{1 + 2\sqrt{K_m / K_i}}$





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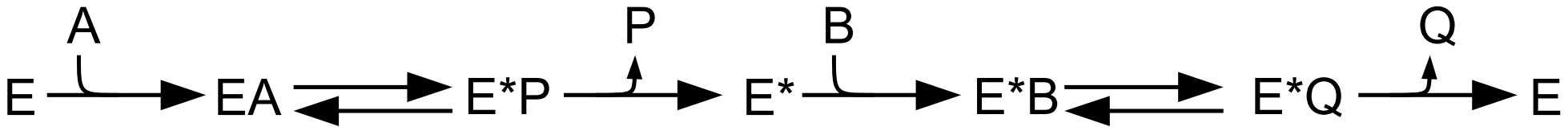
Others

- Mixed inhibition (noncompetitive inhibition with different dissociation constants for *EI* and *ESI*)
- Partial inhibition (product can also be formed from enzyme-substrate-inhibitor complex)
- Multi-substrate reactions:
  - Ternary complex mechanism (random or ordered binding)
  - Ping-pong mechanism (intermediate activated enzyme state *E**)



Hill equation (positive homotropic cooperativity)

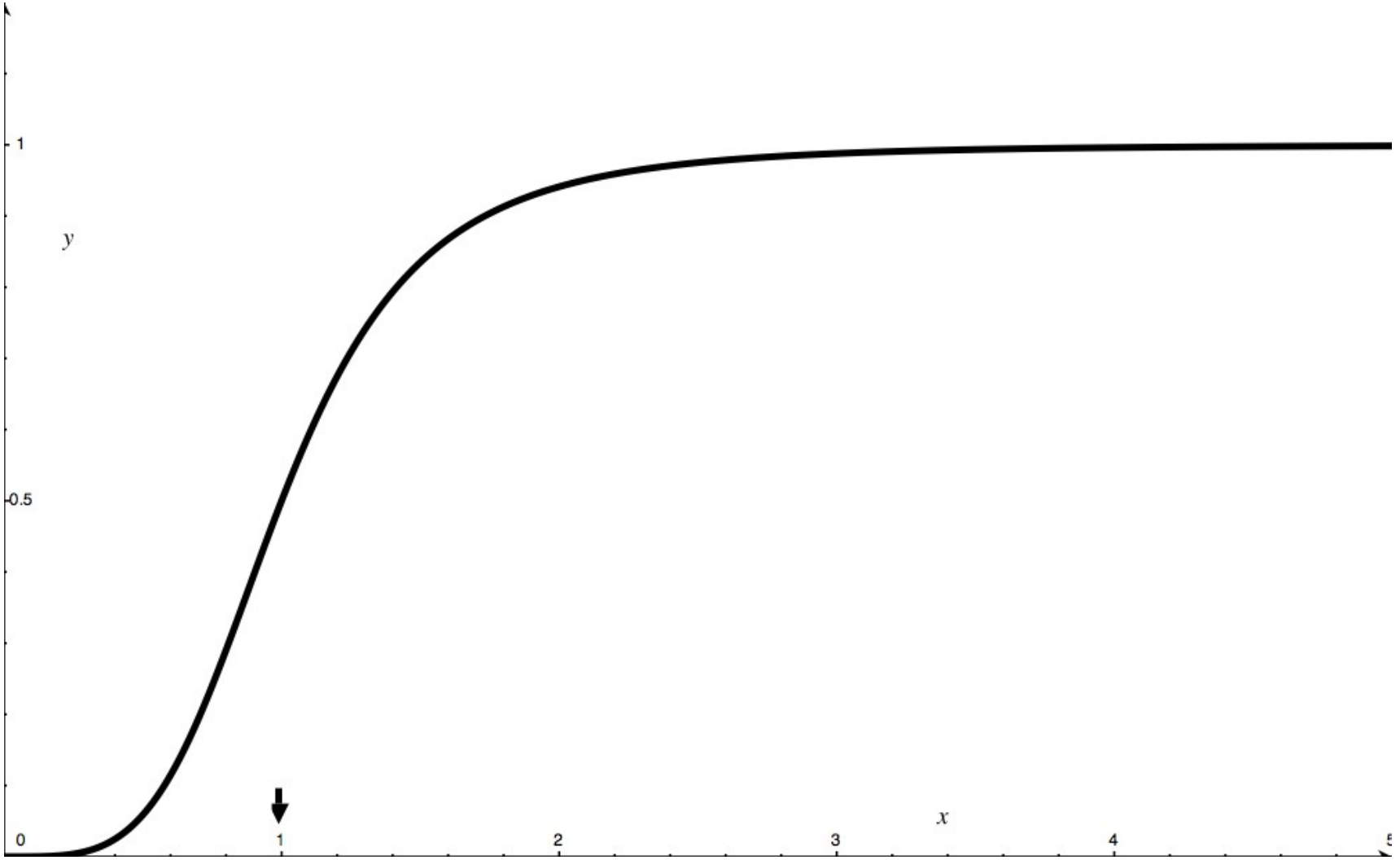
Allosteric regulation: Binding of first substrate ligand facilitates the binding of subsequent substrate ligands.

Complete cooperativity: subsequent ligands bind as soon as first ligand is bound → concentrations of intermediates can be neglected.

For a protein with *h* subunits: $v = \frac{V \cdot [S]^h}{K_{half}^h + [S]^h}$

Hill 1913: binding of oxygen to hemoglobin

Hill curve (Sigmoidal shape of *v* vs. *[S]* curve)



Monod-Wyman-Changeux model

Explains sigmoidal enzyme kinetics. Assumptions:

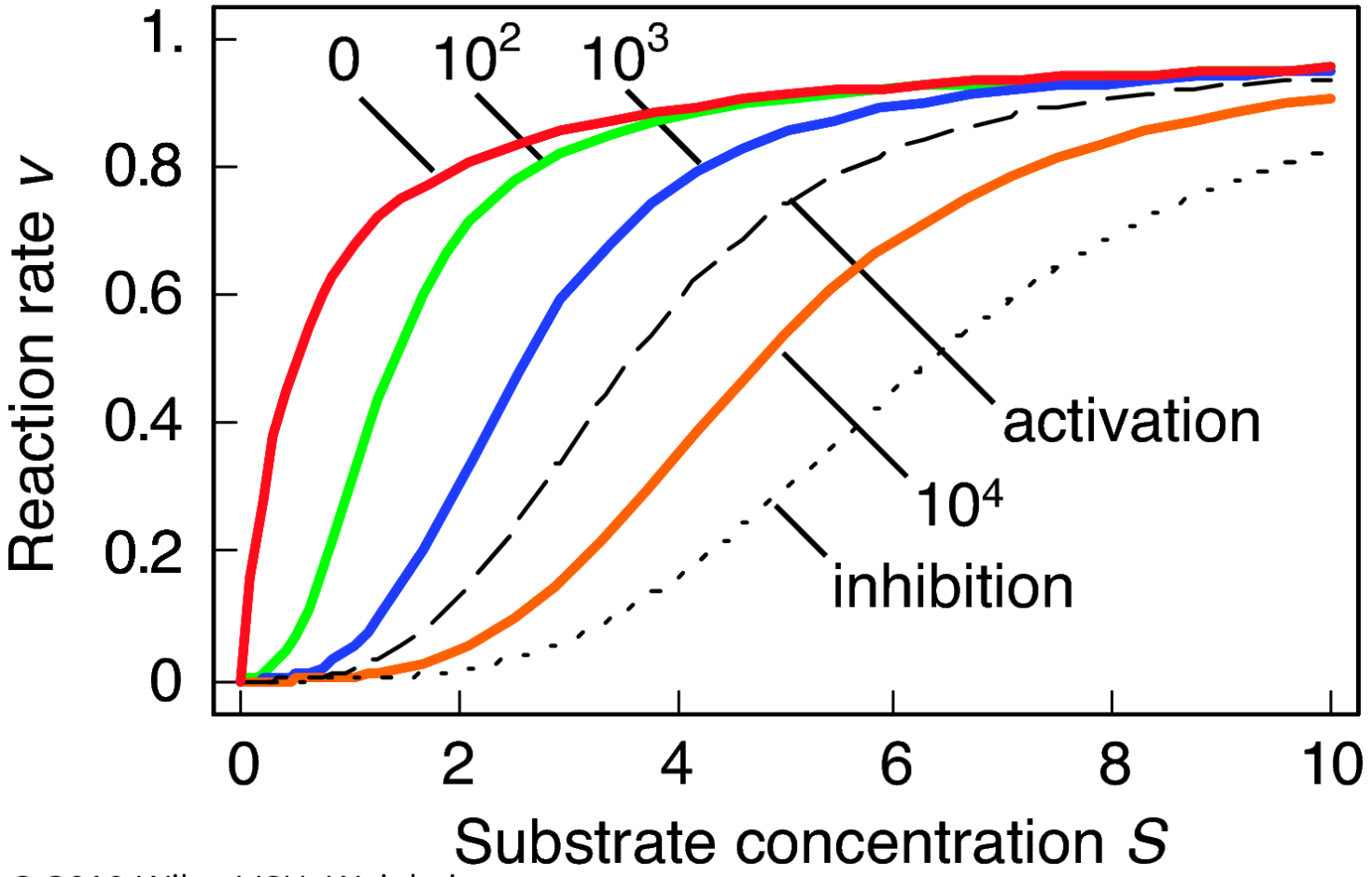
1) Enzyme consists of *n* identical subunits
2) Each subunit can assume an active (R) or inactive (T) confirmation
3) All subunits change conformations at the same time (concerted)
4) Equilibrium between R and T conformation is given by the allosteric constant $L = [T]_0/[R]_0$

Binding constants for R and T conformations are given by K_R and K_T. If binding can only occur to the active form ($K_T = 0$) then

$$v = \frac{V \cdot K_R \cdot [S]}{(1 + K_R \cdot [S])} \cdot \frac{1}{[1 + L/(1 + K_R[S])^n]}$$

Also, heterotropic effects due to the action of positive and negative effectors (inhibitors and activators) can be incorporated (this changes *L*)

Monod-Wyman-Changeux model (cont.)



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Other kinetics frameworks

- Generalised mass action kinetics → **power law kinetics** (nonlinear dependence of rate on concentrations
- Approximate kinetic formats, e.g. **lin-log kinetics** (rate is proportional to enzyme concentration, concentrations normalised to reference state)
- **Convenience kinetics** (generalised form of Michaelis-Menten kinetic with different stoichiometries and enzyme regulation)

Dynamical systems

Biochemical systems described by ODEs

$$\frac{dx_i}{dt} = \dot{x}_i = f_i(x_1, \ldots, x_n, p_1, \ldots, p_l)$$

System state is *n*-dimensional vector of independent variables in **state space**. If initial conditions $x_i(0)$ and parameters p_j are defined we get a particular solution, a path through state space → **trajectory** or time course

What long term behaviours are possible?

Stationary states or steady states

No change over time

Points in state space with $\dot{x} = 0 = f_i(x_{1,}\ldots, x_n, p_{1,}\ldots, p_l)$

The system of *n* differential equations is then described by *n* algebraic equations.

This equation system can have many solutions → multiple steady states

Linearisation

Follow deviation $\hat{\boldsymbol{x}}(t)$ from steady state $\bar{\boldsymbol{x}}$

$$\dot{\boldsymbol{x}} = \boldsymbol{f}\left(\bar{\boldsymbol{x}} + \hat{\boldsymbol{x}}(t)\right) = \frac{d}{dt}\left(\bar{\boldsymbol{x}} + \hat{\boldsymbol{x}}(t)\right) = \frac{d}{dt}\hat{\boldsymbol{x}}(t)$$

Taylor expansion

$$\frac{d}{dt}\hat{x}_i = f_i\left(\bar{x}_1, \ldots, \bar{x}_n\right) + \sum_{j=1}^{n} \frac{\partial f_i}{\partial x_j}\hat{x}_j + \frac{1}{2}\sum_{j=1}^{n}\sum_{k=1}^{n} \frac{\partial^2 f_i}{\partial x_j \partial x_k}\hat{x}_j \hat{x}_k + \ldots$$

Truncate (linearise) (also *f(SS)* vanishes)

$$\frac{d}{dt}\hat{x}_i = \sum_{j=1}^{n} \frac{\partial f_i}{\partial x_j}\hat{x}_j = \sum_{j=1}^{n} \left(a_{ij}\right)\hat{x}_j$$

With a_{ij} elements of the Jacobian matrix $\boldsymbol{J} = \left(a_{ij}\right) = \begin{pmatrix} \frac{\partial f_1}{\partial x_1} & \cdots & \frac{\partial f_1}{\partial x_n} \\ \vdots & \ddots & \vdots \\ \frac{\partial f_n}{\partial x_1} & \cdots & \frac{\partial f_n}{\partial x_n} \end{pmatrix}$

Solving linear ODEs

n = 1

$$\frac{dx_i}{dt} = a_{11} x_1 \qquad x_1(t) = x_1(0)\, e^{a_{11} t}$$

n > 1

$$\dot{\boldsymbol{x}} = \boldsymbol{A}\,\boldsymbol{x} \qquad \boldsymbol{x}(t) = \sum_{i=1}^{n} c_i \boldsymbol{b}^{(i)} e^{\lambda_i t}$$

with eigenvalues λ_i to the corresponding eigenvectors $\boldsymbol{b}^{(i)}$

Inhomogeneous systems can be transformed to homogeneous ones by coordinate transformations.

We get a homogeneous system for the deviations $\hat{\boldsymbol{x}}(t)$

Stability of steady states

- *stable* - the system returns to this state
- *unstable* - the system diverges from this state
- *metastable* - the system behaviour is indifferent

upon a small perturbation of the system from its steady state.

Local stability ↔ Global stability

How to determine stability

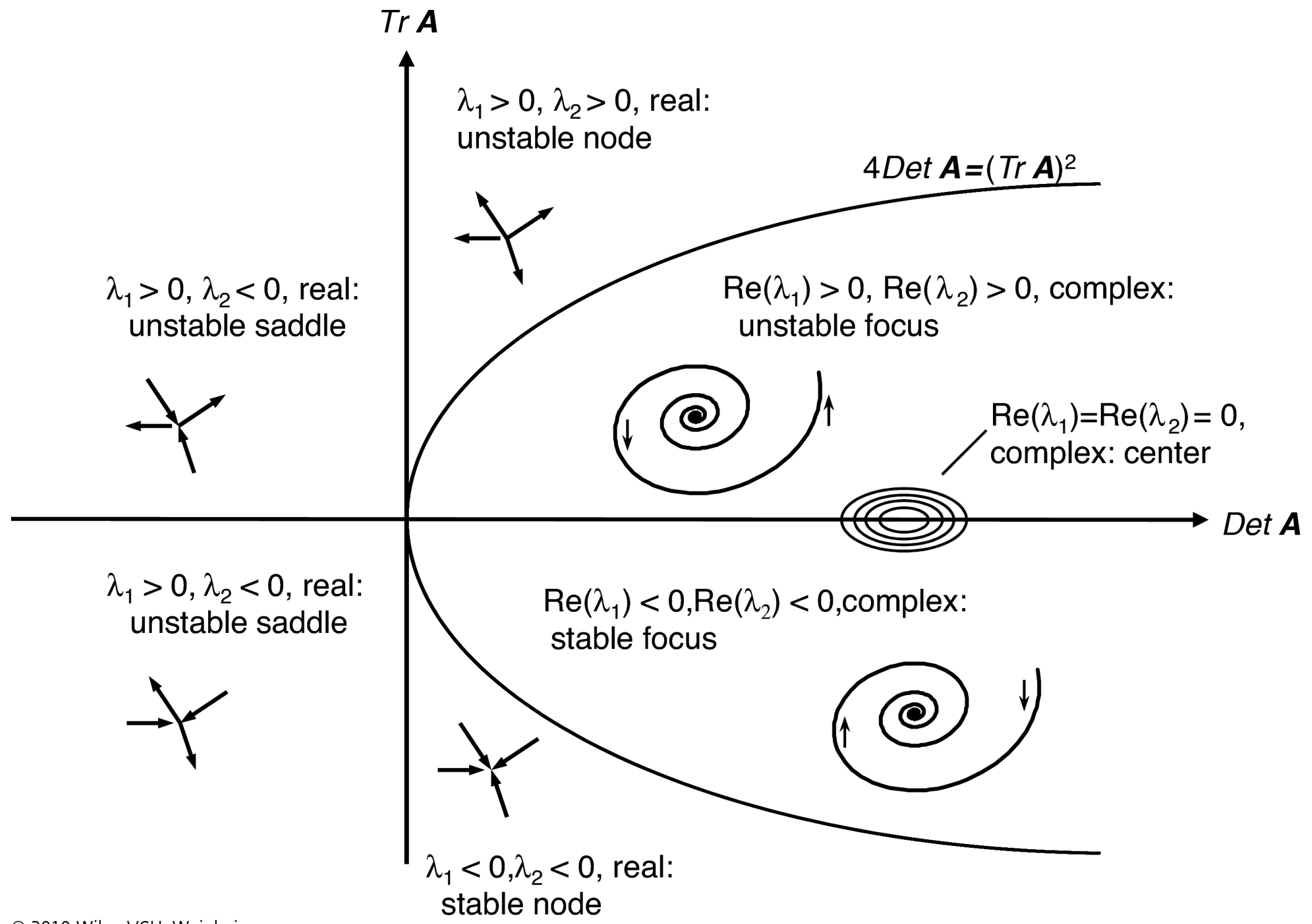
Consider linearised system and investigate the eigenvalues of the Jacobian matrix.

- Asymptotically stable: if all eigenvalues have strictly negative real parts
- Unstable: at least one eigenvalue has a positive real part

1-dim. case: example

2-dim. case: Stability for $Tr\ \boldsymbol{A} < 0$ and $Det\ \boldsymbol{A} \geq 0$

Note: solution contains oscillatory parts in case of complex eigenvalues



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Different asymptotic dynamics

- Steady states (SS)
- Oscillations (periodic behaviour, "limit cycle")
- Chaos (non-periodic, irregular behaviour)

Attractors - sets of states that the system asymptotically converges towards

Changes in asymptotic dynamics or their stability are called ***bifurcations***

Global stability

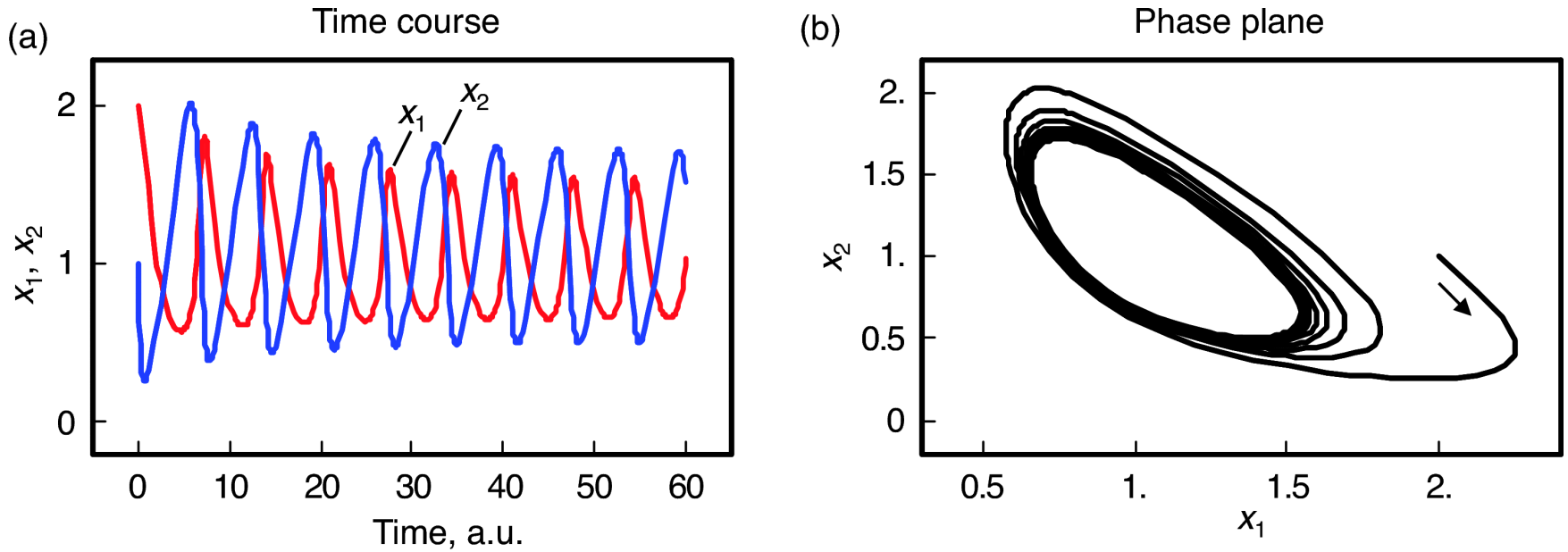
Attractor, e.g. a steady state, is approached for **all** initial conditions

Note: this can be determined for a steady state using a so-called **Lyapunov function** that is decreasing under the system's dynamics and has a minimum at the steady state

Limit cycles

Isolated closed trajectories

All trajectories in its vicinity are periodic solutions winding towards (stable limit cycle) or away from (unstable) the limit cycle



Finding steady states

- Forward integration (→ stable SS)
- Reverse integration (in negative time direction stability properties are reversed also → unstable SS)
- Newton's method

Newton's method

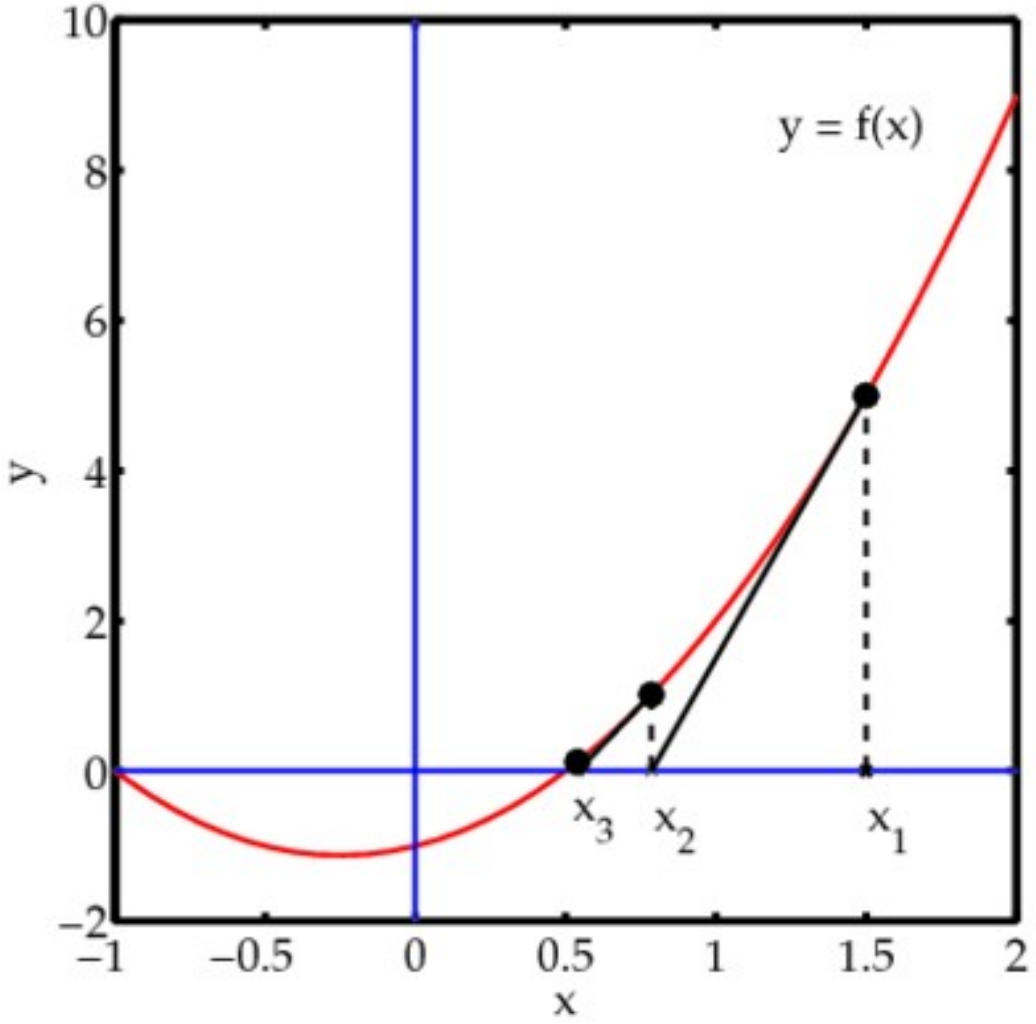
Finding roots (zero values) of general functions

$$x_{n+1} = x_n - \frac{f(x_n)}{f'(x_n)}$$

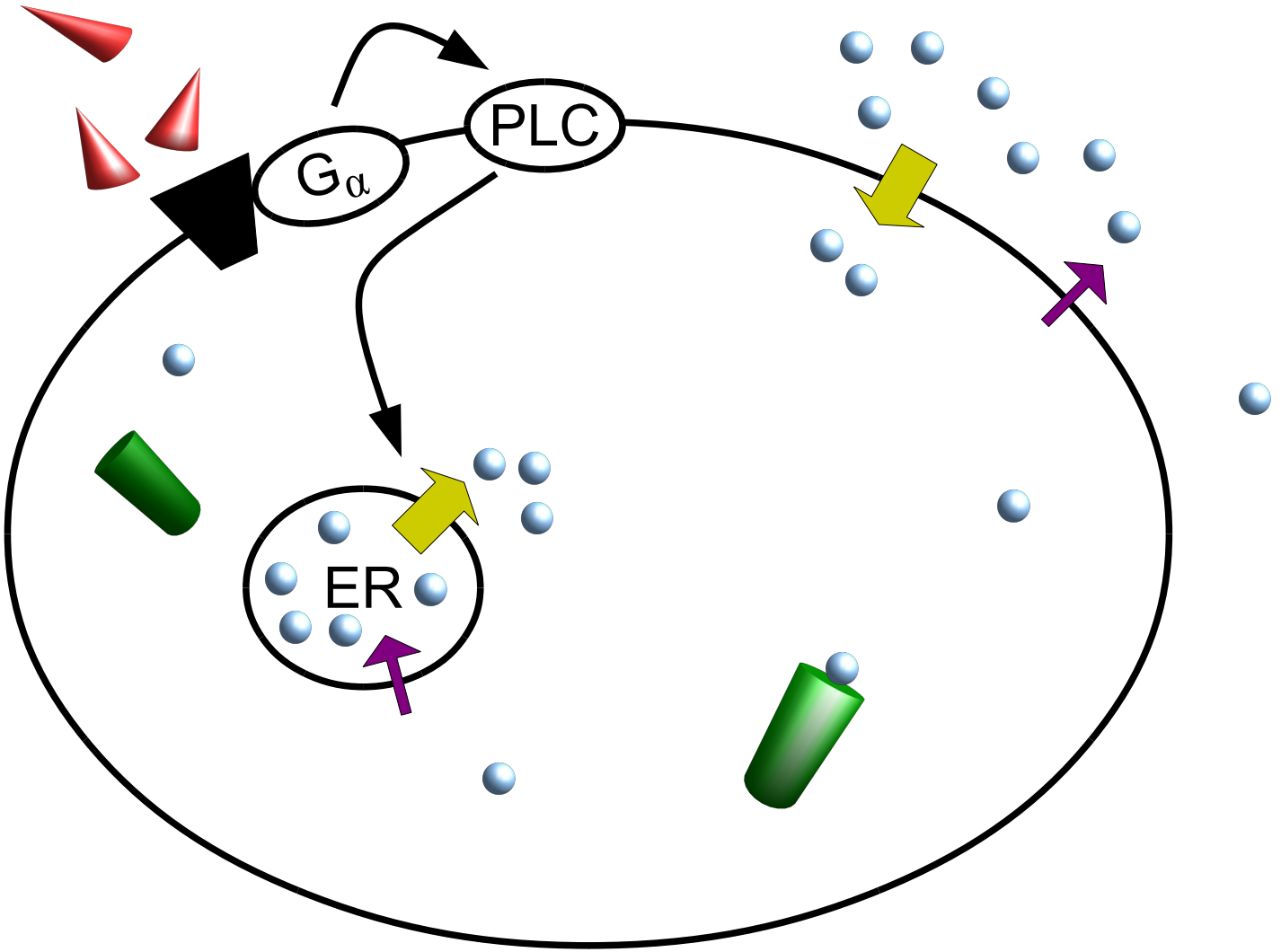
Geometrically: Intersection with the x-axis of the tangent to *f* at the point $(x_n, f(x_n))$

- *f* has to be differentiable
- Convergence is not guaranteed
- If the method converges it does this usually quickly (at least quadratic)

Newton's method (cont.)



Example: Ca^{2+}-signal transduction



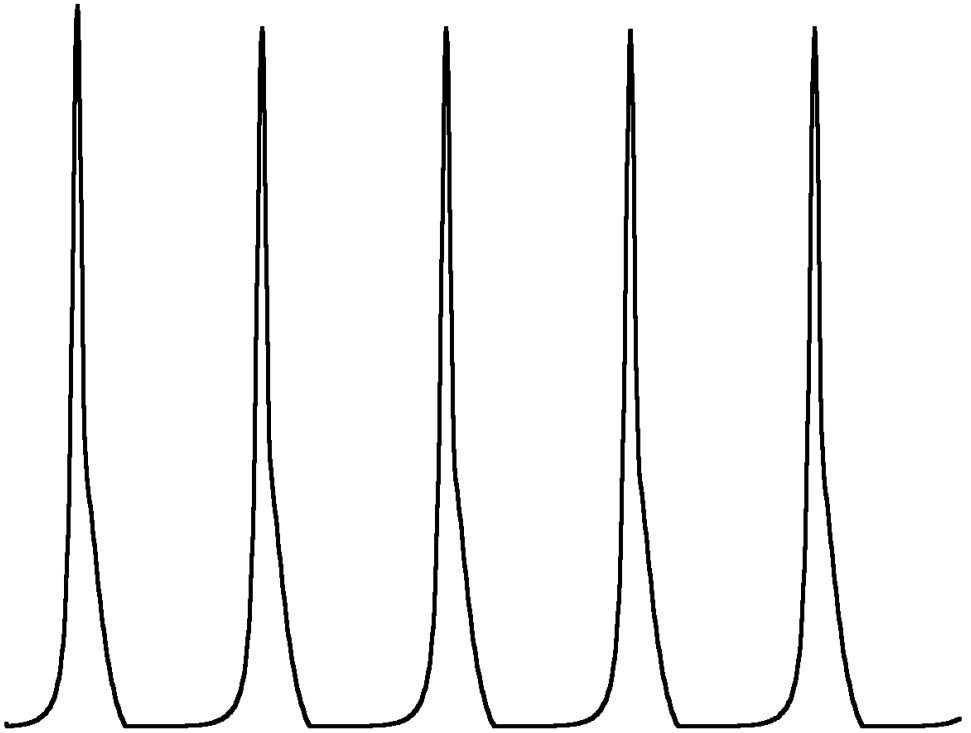
Reaction system (Ca-signal transduction)

R_1	$\rightarrow G_\alpha$	Constant Flux, $k = 0.212$
R_2	$\xrightarrow{G_\alpha} G_\alpha$	Linear Activation, $k = 2.9$
R_3	$G_\alpha \xrightarrow{PLC}$	Irr. Michaelis-Menten-Kinetics, $V_{max} = 1.52, K_m = 0.19$
R_4	$G_\alpha \xrightarrow{Ca^{2+}}$	Irr. Michaelis-Menten-Kinetics, $V_{max} = 4.88, K_m = 1.18$
R_5	$\xrightarrow{G_\alpha} PLC$	Linear activation, $k = 1.24$
R_6	$PLC \rightarrow$	Irr. Michaelis-Menten-Kinetics, $V_{max} = 32.24, K_m = 29.09$
R_7	$\xrightarrow{G_\alpha} Ca^{2+}$	Linear activation, $k = 13.58$
R_8	$Ca^{2+} \rightarrow$	Irr. Michaelis-Menten-Kinetics, $V_{max} = 153, K_m = 0.16$

Kummer et al. (2000), Biophys. J., 79 (3), pp. 1188-1195

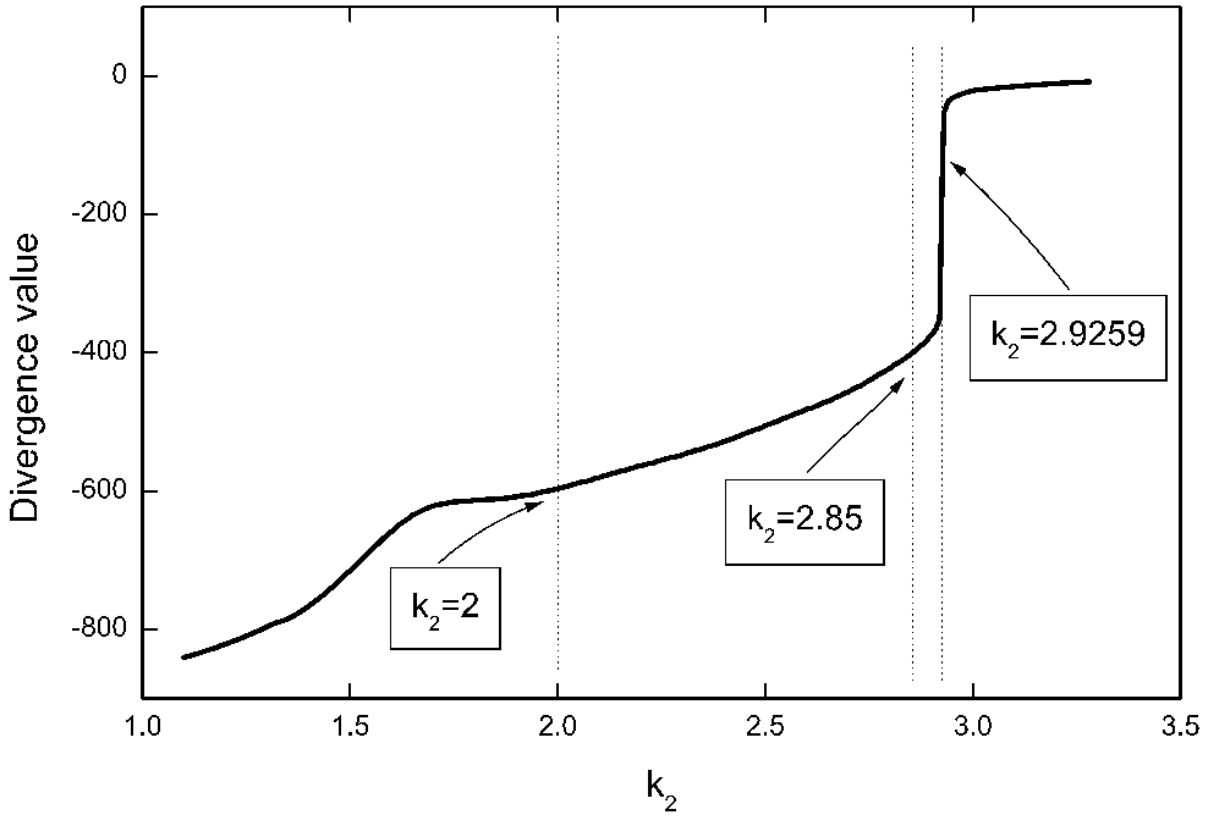
Calcium dynamics (simulated deterministically)

spiking



Dynamics

k_2	**Dynamics**
2	periodic spiking
2.85	periodic bursting
2.9259	chaos
2.99	regular oscillations
3	steady state



Administrative stuff

- 17.5.2012 Christi Himmelfahrt (bank holiday) → **No exercise this week**, worksheet will be discussed next week
- **Registration** for the exam is possible in HISPOS. Final **deadline** for this is two weeks before the exam, e.g. 17.7.2012