BIOQUANTS

1. Introduction & Growth laws in biology

Multidisciplinary approaches. – in vitro → in vivo → therapeutic protocols → clinical trials → clinical practice
– observation → ind e dip variable → param → math model → qualitative analysis → coding+simulation → validation

How to find equilibrium points? – **Scalar** variable: •$f'(u_{eq})<0$ stable •$f'(u_{eq})=0$ increase •$f'(u_{eq})>0$ unstable
– **Vector** variable: •all eigen $J_f(u_{eq})<0$ stable •at least one =0 increase •at least one >0 unstable
n=2 **Hurwitz Criterion**: u_{eq} stable if **$tr(J_f(u_{eq}))<0$ & $det(J_f(u_{eq}))>0$**

Growth laws. – Time evolution of variations of N(t) used to •test hypothesis •estimate future
I. **Linear** •**$N'(t)=a$** •**$N(t)=at+N_0$**
II. **Exponential** •**$N'(t)=aN(t)$** •**$N(t)= N_0e^{at}$**
III. **Logistic** •**$N'(t)=aN(t)-aN^2(t)/k$** •**$N(t)=kN_0/N_0+(k-N_0)e^{-at}$**→ k:=**carrying capacity** (the max n of individual in a domain)
IV. **Richard's curve** •Generalized **$N'(t)=aN(t)(1-(N(t)/k)^v)$** •Gompertz **$N'(t)=aN(t)ln(k/N(t))$** •Sigmoid **$N'(t)=aN(t)((N(t)/k)^b-1)$**

2. Math models for Epidemiology

Small-pox vaccine. – Small-pox is caused by an airborne virus
– In **1759 Daniel Bernoulli** developed a mathematical model with the aim of demonstrating the benefits of the vaccinations
– Bernoulli proposed to compare 2 states of humanity
I. Population model in which small-pox does not exist thanks to vaccination $N(t) = N_o e^{-mt}$
•population does not proliferate •people vaccinated at birth •individual died only for natural reason (m)
II. Population model in which small-pox exists $N(t) = N_0 e^{-mt}[1 - M(1 - e^{-ct})]$
•population does not proliferate •population is divided into: •susceptible •recovered
– The extinction of the population is more rapid in the second scenario → vaccination had clear benefits

Behavioural Epidemiology. – A world-wide recent trend has shown a **reduction in the n of vaccinated individuals** → pseudo rational behaviour
I. Economic approach: based on the evaluation of the ratio between costs and benefits of vaccination → **imitation game**
We complete the SIR system with the percentage of people that decided to be vaccinated at time t: $\boldsymbol{p'(t) = k\Delta E(t)p(t)(1 - p(t))}$, where $\boldsymbol{\Delta E(t) = h(I(t)) - \alpha(p(t))}$ is the **benefit/cost of vaccination**
II. Theory of information: based on the **past/present information** about the severity of the disease
The fraction of vaccinated is modelled with $\boldsymbol{p(t) = p_0 + p_1 M(t)}$, •$p_0$ is fraction of newborns vaccinated
• $p_1 M(t)$ is time dependent fraction of newborn whose vaccination depends on the perceived social alert
$\boldsymbol{M(t) = \int_{-\infty}^{t} g(S(\tau), I(\tau))\tau e^{-a(t-\tau)} d\tau}$ is composed of 2 factors: social alert based on •the present state •the past epidemic event

Compartmental model in epidemiology. – **Assumptions**: •population divided into compartments(=label) •individual progress between compartments
•based on systems of differential equations

I. SIR. – Compartments: •**susceptible**, •**infectious**, •**removed**
– Hp: •proliferation is negligible •homogeneous and isolated population $S(t) + I(t) + R(t) = k$ •no incubation •no time-decaying power infection
– $\begin{cases} \boldsymbol{S'(t) = -\beta S(t)I(t)} \\ \boldsymbol{I'(t) = \beta S(t)I(t) - \gamma I(t)} \\ \boldsymbol{R'(t) = \gamma I(t)} \end{cases}$
–The **basic reproduction number** $\boldsymbol{R_0 = \frac{\beta}{\gamma} N = \frac{S(0)}{S^*}}$ is a measure of the expected n of new infections from a single infection where all subjects are susceptible and S^* is the **epidemic threshold**
– We have 2 possibilities: •$\boldsymbol{R_0 > 1}$ the epidemic will **invade** the population
•$\boldsymbol{R_0 < 1}$ the disease will naturally **extinguish**
– **Herd immunity**. The individuals who are immune to the disease at the beginning of the epidemic act as barrier that protect the population
– At the beginning it is possible to predict the development: •S(t) decrease •R(t) increase •I(t) depend on R_0: $\boldsymbol{I_{max} = I_0 + S_0 - \frac{\gamma}{\beta} - \frac{\gamma}{\beta} \ln\left(\frac{\beta}{\gamma} S_0\right)}$

II. SIR with demographic process. – SIR predicts the decay of the disease on every occasion → **it cannot predict endemicity of the pandemic**
– To overcome this limitation, we must include the **demographic process**: •the population is not close •newborns go to susceptible •born&deth rate are equal and constant (m)
– $\begin{cases} \boldsymbol{S'(t) = mN - \beta S(t)I(t) - mS(t)} \\ \boldsymbol{I'(t) = \beta S(t)I(t) - \gamma I(t) - mI(t)} \\ \boldsymbol{R'(t) = \gamma I(t) - mR(t)} \end{cases}$
–The **basic reproduction rate** is $\boldsymbol{R_0 = \frac{\beta N}{m+\gamma}}$ → •$\boldsymbol{R_0 > 1}$ disease remain **pandemic**
•$\boldsymbol{R_0 < 1}$ disease **decay**

III. SIRV. – Compartments: •**susceptible**, •**infectious**, •**removed**, •**vaccinated**
– Hp **vaccination**: •vaccinated individuals have a life-lasting immunity •belong to V-compartment
•p:=fraction of vaccinated newborns •N is assumed constant
– $\begin{cases} \boldsymbol{S'(t) = mN(1 - p) - \beta S(t)I(t) - mS(t)} \\ \boldsymbol{I'(t) = \beta S(t)I(t) - \gamma I(t) - mI(t)} \\ \boldsymbol{R'(t) = \gamma I(t) - mR(t)} \\ \boldsymbol{V'(t) = mpN - mV(t)} \end{cases}$
– The **critical reproduction rate** is $\boldsymbol{R_V = R_0(1 - p) = \frac{\beta N}{m+\gamma}(1 - p)}$→ •$\boldsymbol{R_0 > 1}$ disease remain **pandemic**
•$\boldsymbol{R_0 < 1}$ disease **decay**
– The **threshold of the herd immunity** $\boldsymbol{p_c = 1 - \frac{1}{R_0}}$ is the minimal fraction of vaccinated newborns above which the population is protected by a dramatic spread of the disease

IV. SIS. – Some infections do **not** confer **long-lasting immunity**
– $\begin{cases} \boldsymbol{S'(t) = -\beta S(t)I(t) + \gamma I(t)} \\ \boldsymbol{I'(t) = \beta S(t)I(t) - \gamma I(t)} \end{cases}$
– The dynamics of the infectious subpopulation are ruled by a logistic function $\boldsymbol{I(t) = \frac{(N - \gamma/\beta)I_0}{I_0 + (N - \gamma/\beta - I_0)e^{-(\beta N - \gamma)t}}}$: • $\boldsymbol{\beta N - \gamma < 0}$ the disease **extinct**
• $\boldsymbol{\beta N - \gamma > 0}$ the disease **diffuses**

V. SIRD. – This model distinguishes •**recovered** •**deceased** as consequence of the disease

$$- \begin{cases} S'(t) = -\beta S(t) I(t) \\ I'(t) = \beta S(t) I(t) - \gamma I(t) - \mu I(t) \\ R'(t) = \gamma I(t) \\ D'(t) = \mu I(t) \end{cases}$$

VI. MSIR. – Newborns are immune to many diseases for few months due to protection given by maternal antibodies → **passive immunity**

$$- \begin{cases} S'(t) = -\beta S(t) I(t) + \mu M(t) \\ I'(t) = \beta S(t) I(t) - \gamma I(t) \\ R'(t) = \gamma I(t) \\ M'(t) = \lambda - \mu M(t) \end{cases}$$

3. Math models for chemical Reactions and Pathways

– The **law of mass action** describes the rate at which chemicals (A, B) interact to form a product (C): $A + B \longrightarrow^{k} C$

– The **rate of the reaction** k is the rate of accumulation of the product, depending on •n of collision •collision sufficiently energetic (temperature)

– Most reaction can proceed in both directions $A + B \rightleftharpoons_{k_-}^{k_+} C$ with the **forward and reverse rate constant**

• The concentration varying as $\frac{d[A]}{dt} = k_-[C] - k_+[A][B]$, $\frac{d[B]}{dt} = k_-[C] - k_+[A][B]$, $\frac{d[C]}{dt} = -k_-[C] + k_+[A][B]$

• At the **equilibrium** the concentration are such as $k_{eq} = \frac{k_-}{k_+} = \frac{[A]_{eq}[B]_{eq}}{[C]_{eq}}$, where k_{eq} is the **equilibrium constant**: •$k_{eq} \ll 1, [C]_{eq}$ is high

•$k_{eq} \gg 1, [C]_{eq}$ is low

Enzyme Kinetics. – **Enzyme** •are **proteins** that help to convert **substrates** into products •speed up the reaction •high specific

– A reaction catalysed by an enzyme is $S + E \rightarrow P + E$. **Theoretically**, the velocity must be increased linearly, but in practice increase only at a given extent. To explain, separate the reaction $S + E \rightleftharpoons_{k_{-1}}^{k_1} C \rightleftharpoons_{k_{-2}}^{k_2} P + E$. Assuming $k_{-2} \to 0$, the law of mass action is $\frac{ds}{dt} = k_{-1}c - k_1 se, \frac{de}{dt} = k_{-1}c - k_1 se + k_2 c, \frac{dc}{dt} = -k_{-1}c + k_1 se - k_2 c, v = \frac{dp}{dt} = k_2 c$

We observe $e' + c' = 0 \to e(t) + c(t) = e(0) \forall t$ and $p(t) = \int k_2 c(\tau) d\tau$

Hence, we have 2 possible hp to explain the studied behaviour:

1. **Equilibrium approximation:** $k_1, k_{-1} \gg k_2$ •s, c are in rapid equilibrium •**Michaelis-Menten constant** $K_1 = \frac{k_{-1}}{k_1}$

•the **velocity is the rate** at which the final product is formed $v = \frac{v_{max} s}{K_1 + s} \to v_{max}$ •**saturation** behaviour

2. **Quasi steady-state approximation:** $\varepsilon = \frac{e_0}{s_0} \ll 1$ •defining non-dimensional variable $v = \frac{v_{max} s}{K_m + s}, K_m = \frac{k_{-1} + k_2}{k_1}$ •**saturation** behaviour

– **Parameter estimation**. It is easier to estimate via empirical data interpreting them as unknowns of linear laws (stochastic data analysis is required)

Enzyme inhibition. – Inhibitors disrupt the catalytic action of an enzyme •**irreversible** •**allosteric**=bind a non-active site •**competitive**=bind active site

– **Competitive inhibitors** prevents the association of the enzyme with substrate $S + E \rightleftharpoons_{k_{-1}}^{k_1} C_1 \to^{k_2} P + E, E + I \rightleftharpoons_{k_{-3}}^{k_3} C_2$. Using law of mass action and assuming **2.**, the **velocity** of the reaction is $v = \frac{v_{max} s}{K_m(1 + {}^{i}/_{K_i}) + s}, K_m = \frac{k_{-1} + k_2}{k_1}, K_i = \frac{k_{-3}}{k_3}$

•as i increases, v decreases •wrt **2.** v is reduced by the presence of the inhibitors

Enzyme cooperation. – **Cooperativity**: binding of a ligand to one site on a multi-site protein changes the affinity of the other site for that ligand

– Assuming that an enzyme can bind 2 substrate, law of mass action, **2.**: $S + E \rightleftharpoons_{k_{-1}}^{k_1} C_1 \to^{k_2} P + E$, $S + C_1 \rightleftharpoons_{k_{-3}}^{k_3} C_2 \to^{k_4} P + C_1$

•**Positive cooperativity**: first binding increases affinity at remaining sites → $v = \frac{k_4 s^2 e_0}{K_p{}^2 + s^2}, K_p = k_1 k_2$ sigmoidal binding curve

•**Negative cooperativity**: first binding decreases affinity at remaining sites → shallower-than-hyperbolic curve

•**No cooperativity**: independent sites → $v = \frac{2 v_{max} s}{K_n + s}, K_n = \frac{k_{-1} + k_2}{k_1}$ → hyperbolic behaviour

Feedback loops. – **Positive feedback loop**. A change in a variable amplifies itself, reinforcing the direction of the change

$$\begin{cases} \frac{dx}{dt} = p_x(y) - \delta_x x \\ \frac{dy}{dt} = p_y(x) - \delta_y y \end{cases} \to \begin{cases} x_{eq} = \frac{p_x(y_{eq})}{\delta_x} \\ y_{eq} = \frac{p_y(x_{eq})}{\delta_y} \end{cases}$$

•**Michaelis-Menten form:** $p_y(x) = \frac{\alpha_y x}{K_y + x}, p_x(y) = \frac{\alpha_x y}{K_x + y}$

•**Hill-type laws:** $p_y(x) = \frac{\alpha_y x^m}{K_y{}^m + x^m}, p_x(y) = \frac{\alpha_x y^n}{K_x{}^n + y^n}$

– **Negative feedback loop**. A change in a variable counteracts itself, driving the system back toward stability

$$\begin{cases} \frac{dx}{dt} = \alpha_x(X - x) - I_x(y) x \\ \frac{dy}{dt} = \alpha_y(Y - y) - I_y(x) y \end{cases} \to \begin{cases} x_{eq} = \frac{\alpha_x X}{\alpha_x + I_x(y_{eq})} \\ y_{eq} = \frac{\alpha_y Y}{\alpha_y + I_y(x_{eq})} \end{cases}$$

•**Hill-type laws:** $I_y(x) = \frac{\beta_y x^2}{K_y{}^2 + x^2}, I_x(y) = \frac{\beta_x y^2}{K_x{}^2 + y^2}$

Chemical reaction network. – A **chemical reaction network** is a dynamic system, composed of a finite set of reactions among a finite set of chemicals

– The CRN have a graphical representation in terms of weighted directed graph

→ •**Petri Net**

•**C-graph**: ▪reversible ▪weakly reversible •connected

4. Pharmacokinetics & Pharmacodynamics

The importance of modelling in drug development. – The pipeline is •healthy young people •target patient •prove positive effect •new trial

PK. – **Modelling how the drug passes through the body**, modelling time-evolving concentration: •absorption •distribution •metabolism •elimination

–The **concentration of the drug** is calculated as $\boldsymbol{C(t) = \frac{A(t)}{V}}$, $A(t)$ = amount of the drug, V = volume of distribution

– The **elimination rate** is $\boldsymbol{e_r = kA}$, where $\boldsymbol{k = \frac{CL}{V}}$ is the **elimination rate constant** and CL is the **clearance** that measures the volume of blood which is cleared from the drug per unit of time

– The body can be divided into interconnected parts, called **compartments**

I. One-compartmental models → drug distributed rapidly to all parts of the body

– **IV administration**: drug administrated directly into the bloodstream $\boldsymbol{\frac{dA_{bolus}(t)}{dt} = -kA_{bolus}(t)} \rightarrow A_{bolus}(t) = A_{bolus}(0)e^{-kt}$ exponential decay for $A(t)$

The **max concentration** of a drug in the blood is $t_{max} = 0, \boldsymbol{C_{max} = \frac{A_{bolus}(0)}{V}}, \quad t_{0.5} = \frac{\ln(2)}{k}, A(t_{0.5}) = \frac{A(0)}{2}$

– **EV administration**: add a technical absorption compartment (**gut** compartment): the rate of change in the gut is $\boldsymbol{\frac{dA_{gut}(t)}{dt} = -k_aA_{gut}(t)}$

The **rate of change** in the central is $\boldsymbol{\frac{dA_{oral}(t)}{dt} = Fk_aA_{gut}(t) - kA_{oral}(t)}$, where F is the **bioavailability**=fraction of drug that reach the CC

$A_{oral}(t) = \frac{k_aFA_{gut}(0)(e^{-kt} - e^{-k_at})}{k_a - k}$

The **max concentration** of a drug in the blood is $t_{max} = \frac{1}{k_a - k} ln\left(\frac{k_a}{k}\right), \boldsymbol{C_{max} = \frac{k_aFA_{bolus}(0)(e^{-kt_{max}} - e^{-k_at_{max}})}{V(k_a - k)}}$

– Concentration profiles of drugs are shown on a semi log-scale → we can read the elimination rate constant on the graph

– How to prolong the effect of a drug? •**Constant rate infusion** $\boldsymbol{A_{cc}(t) = \frac{R_{in}}{k}(1 - e^{-kt})}$, where R_{in} is the constant infusion rate

•**Multiple dosing**: administration of pills at constant time $R_{in} = \frac{FA_{pill}}{\tau}$, $\boldsymbol{A_{MD,oral}(t) = \sum_{n=0}^{N-1} A_{CC,oral}(t - n\tau)}$. The *ss concentration* is $\frac{FA_{pill}}{k\tau}$

II. Two-compartmental models → central compartment **CC** + one peripherical compartment **PC**

– The drug is distributed from CC to PC and simultaneously eliminated from CC → an equilibrium is established between the drug in CC and PC

– **IV administration** $\begin{cases} \frac{dA_{CC,bolus}(t)}{dt} = K_{21}A_{PC,bolus} - K_{12}A_{CC,bolus} - K_{10}A_{CC,bolus} \\ \frac{dA_{PC,bolus}(t)}{dt} = -K_{21}A_{PC,bolus} + K_{12}A_{CC,bolus} \end{cases}$, $K_{21} = \frac{Q}{V_{PC}}, K_{12} = \frac{Q}{V_{CC}}, K_{10} = \frac{CL}{V_{CC}}, A_{CC,bolus}(t) = {A_{CC,bolus}}^d + {A_{CC,bolus}}^e$

– **EV administration** $\begin{cases} \frac{dA_{CC,oral}(t)}{dt} = FK_aA_{gut} + K_{21}A_{PC,oral} - K_{12}A_{CC,oral} - K_{10}A_{CC,oral} \\ \frac{dA_{PC,oral}(t)}{dt} = -K_{21}A_{PC,oral} + K_{12}A_{CC,oral} \\ \frac{dA_{gut}(t)}{dt} = -K_aA_{gut} \end{cases}$, $C_{CC,oral}(t) = {C_{CC,oral}}^d + {C_{CC,oral}}^e + {C_{CC,oral}}^a$

III. Three-compartmental models → after drug administration to CC, the drug is: •distributed slowly to a shallow **peripherical compartment SPC**
•distributed more slowly to a **deep peripherical compartment DPC**
•eliminated by **CC**

$$\begin{cases} \frac{dA_{CC,bolus}(t)}{dt} = K_{21}A_{SPC,bolus} - K_{12}A_{CC,bolus} + K_{31}A_{DPC,bolus} - K_{10}A_{CC,bolus} \\ \frac{dA_{SPC,bolus}(t)}{dt} = -K_{21}A_{SPC,bolus} + K_{12}A_{CC,bolus} \\ \frac{dA_{DPC,bolus}(t)}{dt} = -K_{31}A_{DPC,bolus} + K_{13}A_{CC,bolus} \end{cases}$$

PD. – **Modelling the effect of the drug on the body**

1. Receptor theory. – The plasma membrane cell **PM** is covered by different receptors. Drugs are divided into •**agonist**: structural change receptor
•**antagonist**: bind the receptor

– Chemical reaction is $[D] + [R] \rightleftharpoons_{k_-}^{k_+} [DR]$: the **binding property** determines the fraction of bound drug at eq → $A_{ffinitty} = k_{eq}^{-1}$

– To increment the result of the drug: •increment drug administrated •increment the affinity between drug and receptor

– To have an effect E, a drug must be able to both **bind** and **activate** its receptor → $E_{max} = \alpha([R] + [RD])$

– By using law of mass kinetics and steady state conditions → $\frac{[RD]}{[RD]+[R]} = \frac{[D]}{[D]+K_{eq}}, \boldsymbol{K_{eq} = \frac{[R][D]}{[RD]}}, \boldsymbol{E = \frac{E_{max}[D]}{[D]+K_{eq}}}$

– The **equilibrium constant** K_{eq} determines the **concentration of the drug necessary to obtain half of** $\boldsymbol{E_{max}}$

2. Exam Model. – It is used to describe the relation between the concentration and the effect of a drug

•**Sigmoid Model**: $E(C) = E_0 + \frac{E_{max}C^n}{C^n + EC_{50}^n}$ •**Linear Model**: $E(C) = E_0 + SC$ •**Log-linear Model**:$E(C) = mlog(C + C_0)$

– It is possible to add an additional technical compartment: **effect compartment EC** → •negligible volume •no mass transfer between EC and CC

5. Dynamics with space variable

– Different elements are characterized by different mathematical representation: •**genes**: boolean •**chemicals**: density/concentration
•**cell**: •**continuous model** describe populations as a set of equations, are easy to analyse, capture average behaviour of many cells, but do not include individual cell morphology or variability
•**individual cell-based models IBM** represent each cell separately, allowing heterogeneous behaviours and morphology to be included, but are harder to analyse and computationally expensive

Discrete particle models. – Each cell is a **material point** with •**mass** •**position** •**type**

– Assuming •cell move in an extremely viscous environment •the extension of cell-cell contacts is lower wrt the extension of cell-substrate contacts

–The **cell dynamics** is described by a phenomenological postulation of velocity contributions: $\boldsymbol{\frac{dx_i(t)}{dt} = \frac{F_i(t)}{\lambda_i^{c-s}} = v_i = v_i^{rand} + v_i^{env} + v_i^{cell-cell} + v_i^{other}}$

– $v_i^{cell-cell}$ results from the superposition of contributions of pairwise interactions that depends on the relative distance. We account for:

•**repulsive** interactions •**adhesive** interactions → $\boldsymbol{v_i^{cell-cell} = -\sum_{j=1, j\neq i}^{n(t)} K(x_i(t) - x_j(t)) \frac{x_i(t) - x_j(t)}{|x_i(t) - x_j(t)|}}$ •$K < 0$: cell **repulsive** behaviour
•$K > 0$: cell **adhesive** behaviour

– The **intercellular interaction kernel K** can be derived from a scalar interaction potential: $\boldsymbol{u'(r) = K(r)}, \lim_{r\to\infty} u(r) = 0,$ where $r = |x_i(t) - x_j(t)|$

Teo. •If u is **H stable**, at the equilibrium the minimal interparticle distance is bounded below by a positive real value
•If u is **not H stable**, at the equilibrium the maximal interparticle distance is bounded from above

Criterion for the stability property. If $\int_0^\infty u(r)rdr > 0$ the potential u, and the kernel K, are H stable

Cell sorting. – **Pattering** of different cell lines according to the specific adhesiveness

– v_i^{env} implements the movement of a cell in response to environmental signals → 2 possibilities: •**local term** $v_i^{env}(t) = v_i^{env} \frac{\nabla c(x_i(t),t)}{|\nabla c(x_i(t),t)|}$

•**non-local integral term** $v_i^{env}(t) = v_i^{env} \int_{A(t)} w_i(y,t)c(y,y)\big(y - x_i(t)\big)dy$

▪If c is diffusion chemical → v_i^{env} is **chemotaxis**

▪If c is non-diffusion chemical → v_i^{env} is **hypotaxis**

▪If c is rigidity → v_i^{env} **durotaxis**

NC-PC system. – Describes the interaction between **neuronal cells (NC)** and **placodal cells (PC)**, NC migration is guided by chemotactic cues secreted by PCs. The dynamics are modelled with ODEs where each term represents cell–cell forces, NC–PC interactions, extracellular matrix effects, and random movement

Cellular POTTS model. – The cellular Potts model CPM is a lattice-based **Montecarlo** technique which follows an **iterative and stochastic energy-minimization algorithm**

– Individuals move and behave to iteratively and stochastically reduce system energy H according to a **Metropolis** algorithm that ends when a global minimum is reached

I. Chose a lattice site x and attempt to copy its spin into a random chosen neighbouring lattice site

II. Compute the difference in the system energy ΔH

III. The attempt is accepted with a **Boltzmann-like probability**

– The system energy is defined by a **Hamiltonian** H:

•**Adhesions terms** between individual or between individuals or compartments

•**Biophysical** attributes of individual sub compartments (e.g., target volume or perimeter)

•**Effective** and generalized forces (e.g., chemotaxis, external fields)

6. Reaction Network RN

– A **RN** is a tuple (X, C, R) → X:=species (A, B, C), C:=complexes (non-negative linear combination of X: $A + B, C$), R:=reactions e.g., $A + B \to C$

– The **state of a deterministic RN** is a vector x whose components are the current concentrations of the available chemical species

Kinetics. – In mass action kinetics the **reaction rate** $\lambda_{y \to y'}$ is proportional to the production of the concentrations of the participating molecules

– The deterministic model of a RN is the **ODE system** $\frac{dx}{dt} = \sum_{y \to y'} (\boldsymbol{y' - y}) \boldsymbol{\lambda_{y \to y'}}(\boldsymbol{x})$

In matrix form → $\dot{x} = \Gamma\Lambda(x)$, where Γ is the **stochiometric matrix** and Λ contains the reaction rate, i.e. the speed of the reactions

– The **reaction rate function is fulfil** meaning that non-negative concentrations are mapped to non-negative rates → $\lambda_j(x) = 0 \leftrightarrow x_i = 0$

– A dynamic RN is a collection of reactions between species with ≥ 0 concentrations and a kinetic

Reaction Graph. – The (strongly) connected components are called (strong) **linkage classes** LC

– If a strong LC is not connected to other LC it is called **terminal** → there must be at least one terminal strong LC within each LC

– A RG is said to be **weakly reversible** if all LC are terminal strong LC

Conservation law. – Given an ODE system any vector in the left kernel of Γ represents a **conservation law** → $w\Gamma = 0$

– There are $d = n - s$ independent conservation law, with n the n of species and $s = rank(\Gamma)$

– If there is a conservation law with all coefficients being positive, then the RN is said to be **conservative**

– The **deficiency** of a RN is $\boldsymbol{d = m - l - s} \to m$ is the n of complexes, l is the n of LC, $s = rank(\Gamma)$

– A state x of a RN is **complex balanced** if, at x, for any complex y the sum of the fluxes of all reactions pointing to y equals the sum of all fluxes of the reactions outgoing from $y \Rightarrow \forall \boldsymbol{y} \in \boldsymbol{C}\ \sum_{y' \in C} \boldsymbol{\lambda_{y \to y'}}(\boldsymbol{x}) = \sum_{y \in C} \boldsymbol{\lambda_{y' \to y}}(\boldsymbol{x})$

Teo. If a deterministic RN is complex balanced, the reaction graph is weakly reversible. Under mass-action kinetics, all equilibria are CB and exists exactly a positive equilibrium in each stochiometric compatibility class (= translation of the stoichiometric space), which is locally asymptotically stable

Deficiency Zero Teo – local version. If a network of reactions has zero deficiency and the reaction graph is weakly reversible, then in every class of positive stoichiometric compatibility there exists exactly one positive equilibrium, which is locally asymptotically stable. There cannot be multiple positive equilibria or positive limit cycles

Deficiency Zero Theorem – global version. Let RN be a network with $d = 0$. For each class of stoichiometric compatibility containing a positive state: if the reaction graph is weakly reversible, then there exists exactly one positive equilibrium in that class, which is globally asymptotically stable with respect to that class

7. Stochastic Reaction Network

– The main difference wrt the deterministic RN is that we do not count the density, but the **population** through a continuous time Markov chain

Stochastic mass-action Kinetics. – The **stochastic model** is CTMC$(X_t)_{t \geq 0}$ → the reaction take place $X_{t^+} = X_{t^+}(y' - y)$ confined to a certain stoichiometric compatibility class

– The **stochastic mass-action reaction rates** are $\boldsymbol{\lambda_{y \to y'}}(\boldsymbol{x}) = \boldsymbol{k_{y \to y'}} \frac{x!}{(x-y)!} \mathbf{1}_{\{x \geq y\}}$

– The **transition probability function** is a time-varying probability

$$\boldsymbol{P_t(y.x) = P(X_t = x | X_0 = y) = \sum_{k \neq x} P_t(y,k) q(k,x) - \lambda(x) P_t(x,x)}$$

Stationary distribution. – π is the stationary distribution of the stochastic reaction system if $P(X_0 = x) = \pi(x) \Rightarrow P(X_t = x) = \pi(x)$

– π if exists **fulfils the master equation** $\boldsymbol{\pi(x)} \sum_{y \to y'} \boldsymbol{\lambda_{y \to y'}}(\boldsymbol{x}) = \sum_{y \to y'} \boldsymbol{\pi(x - y' + y) \lambda_{y \to y'}}(\boldsymbol{x - y' + y})$ (**Kolmogorov forward**)

•This is equivalent to solve $\boldsymbol{\pi Q = 0}$, where Q is the **rate matrix**

Explosive. – The chain is **not-explosive** if $P_k(T_\infty = \infty) = 1$, $T_\infty = \lim_{t \to \infty} T_i$

– **Teo**. If the chain is not-explosive, there is at most one stationary distribution

If the chain is explosive, there can be arbitrary many

Teo. Assume that the state space is irreducible. A chain is **positive recurrent** ⇔ it is not explosive and exists a stationary distribution. If so π is unique and $\lim_{t \to \infty} P(X_i = x) = \pi(x)$

Complex balanced for deterministic RN – Teo. If a deterministic RN has a complex balanced equilibrium, it has exactly one positive equation on every stoichiometric compatibility class, which is complex balanced. Each component of the graph is weakly reversible

Complex balanced for stochastic RN – Teo. If C is a complex balanced equation of a stochastic RN, then the stationary distribution on any irreducible component Δ for the stochastic system is $\boldsymbol{\pi_\Delta(x) = M_\Delta^C \prod_{i=1}^k \frac{c_i^{x_i}}{x_i!}}$

Teo. Consider a zero-deficiency RN •If it is weakly reversible, the state space is union of irreducible components
•Otherwise, does not exists positive irreducible component
•Moreover, the stationary distribution on every irreducible component is complex balanced

Density dependent families of MC. – A family of MC is density dependent •In each configuration only a finite n of states changes is possible

Kurtz's Teo. •The initial condition is $X^V(0) = Vx_0$
•For every possible state change l exists a continuous positive function $f_l: \lambda^V_{y\to y'}(k) = Vf_{y'\to y}\left(\frac{k}{V}\right)$

– The **density process** $\boldsymbol{Z^V(t)} = \frac{X^V(t)}{V}$ converges almost surely to the deterministic solution $X(t)$ of the d-dimensional ODE system $\dot{\boldsymbol{X}}(\boldsymbol{t}) = \sum_{l\in C} \boldsymbol{l f_l(X(t))}$

8. Mechanistic Models in biology and Medicine

– **Pharmacokinetics** is a part of drug development that studies how a drug is absorbed, distributed, metabolized and excreted from our bodies
– The typical experiment tracks the concentration of a drug over time from its administration. The experimental points can be denoted by $\{Y_{ij}\}$ and are the concentration measurements relative to the i-th patient at time t_{ij} after injecting the pill. A mathematical translation of this kind of model describes the amount of drug in the digestive system A(t) and the concentration of the drug in the blood compartment C(t)

$$\begin{cases} \dot{A}(t) = -k_a A(t) \\ \dot{C}(t) = \frac{k_a}{V} A(t) - k_c C(t) \\ A(0) = 0, C(0) = 0 \end{cases}$$

For the statistical analysis of the data it might be reasonable to assume a model of the kind $Y_{ij} = C(t_{ij}, V_i, k_{ia}, k_{ic}) + \epsilon_{ij}, \quad \epsilon_{ij} \backsim F, \quad (V_i, k_{ia}, k_{ic}) \backsim G$

9. Clinical trials

Clinical research phase of drug development. – It is a study **aimed at comparing** the effects and the value of a set of interventions on humans compared to a control
– A clinical trial •has **control group** •is conducted on humans •is prospective •studies effects-values

– The **perfect experiment** must be •comparative •replicative •unbiased •randomized •blocked or stratifies •optimal •ethical
– Randomization and stratification are method to minimize the risk of introducing **systematic biases** into the data → could invalidate the trial
– **Endpoints** are the specific outcomes measured in a clinical trial to assess the treatment's efficacy and safety
→ •binary variable •continuous measurement •duration variable

The 4 phases of clinical trials. I. First experimentation on humans → •max tolerate dose •bioavailability •PK/PD •repeated dose
II. Demonstration of activity → •min effective dose •scale to evaluate the effectiveness of the drug
III. Comparative evaluation of a final dose → •search for adverse events •placebo-controlled or active-controlled •comparative and confirmatory trials
IV. Monitoring of drug already on the market → •long term study (side effects) •specific effects, subpopulation

10. Statistics for standard clinical trials

– A **standard superiority clinical trial** compares the average response of two independent groups of patients: a **treatment** group T and a **control** group C. Each patient yields a numeric outcome (e.g. blood pressure). We model outcomes as independent samples from Gaussian distributions
→ •$\left(Y_{T_1}, \cdots, Y_{T_{n_t}}\right)$: $Y_{T_i} \sim^{iid} \mathcal{N}(\mu_T, \vartheta_T^2)$ •$\left(Y_{C_1}, \cdots, Y_{C_{n_c}}\right)$: $Y_{C_i} \sim^{iid} \mathcal{N}(\mu_C, \vartheta_C^2)$

– A **binary endpoint** is a binary random variable that measures the effect of T
– The practical question is: **is the treatment better than the control?** Formally we test whether the treatment mean is larger $\boldsymbol{\mu_T > \mu_C}$

Hypothesis testing problem. – **Null hypothesis:** no superiority $\boldsymbol{\vartheta = \mu_T - \mu_C \leq 0}$
– **Alternative hypothesis:** treatment is superior $\boldsymbol{\vartheta > 0}$
– **Interpretation of results:** rejecting H_0 means the observed data are unlikely under H_0 at the chosen significance level
– **Rejection region**:

Error type. – **Type I error** $\boldsymbol{\alpha}$: probability of incorrectly rejecting H_0 when it is true (false positive). Common choices: 0.05 or 0.01
– **Type II error** $\boldsymbol{\beta}$: probability of failing to reject H_0 when the treatment truly is better (false negative)
– **Power** $\boldsymbol{1-\beta}$: probability that the test correctly detects a real effect of the chosen size
– **Power function**:

– Definition of the **sample size** to get a specific power, considering $n_t = n_c = n$, $\boldsymbol{n \geq \frac{(\phi^{-1}(\alpha)+\phi^{-1}(\beta))^2(\vartheta_T^2+\vartheta_C^2)}{\theta_A^2}}$

Teo. – Assuming ϑ_T, ϑ_C **known**:
– Assuming ϑ_T, ϑ_C **not known**: •n_t, n_c large
•n_t, n_c small, $\vartheta_T^2 = \vartheta_C^2$
•Otherwise

A confidence interval approach. – **Two side** confidential interval
– **One side** confidential interval

Kind of trials. – **Non-inferiority trials test** whether a treatment is **not worse** than control by more than a pre-set margin Δ
– **Equivalence trials** check whether the difference between treatment and control lies **within a margin**
– For **binary endpoints**, responses follow a Bernoulli/Binomial model and the treatment effect is typically measured as the **difference (or ratio) of proportions** between groups

Superiority trials with binary endpoints. – The goal is to find a statistical procedure that is able to check the superiority of treatment over control
– The setting considers superiority trials with binary endpoints, where treatment and control responses follow **Bernoulli distributions**. The hypothesis test is based on standardized statistics for the difference in proportions, and one-sided confidence intervals can be constructed to decide whether to reject H_0
– The **delta method** is a technique to approximate the distribution of a function of an estimator by applying a first-order Taylor expansion, allowing us to derive asymptotic variances for nonlinear transformations (e.g., log or logit of an estimator)
– Instead of the Wald interval, the log-likelihood ratio or the log odds ratio (via delta method) provides more reliable inference, especially under worst-case scenarios

Superiority trials nonparametric. – The **Wilcoxon Rank-Sum test** is a non-parametric alternative to the two-sample t-test, used when normality cannot be assumed. It works by ranking all observations and comparing the sum of ranks between treatment and control. Under the null, the test statistic has a known mean and variance, and for large samples it can be approximated by a normal distribution, making the test both robust and practical

Time to event data. – Time-to-event data are analysed through the survival function $S(t) = P(T > t)$, which measures the probability of surviving beyond time t

– The **hazard rate** quantifies the probability of experiencing the event at time t, given survival up to that point. Because censoring often occurs, likelihood methods are used to estimate parameters

I. Parametric approach

II. Non-parametric approach

III. In the non-parametric case, the **Kaplan–Meier estimator** provides an empirical estimate of survival, where each death decreases survival by a factor depending on the ratio of observed deaths to the number of subjects at risk

11. Single arm adaptive trials → **goal**: comparison treatment T and control C

– Different design: •**Fixed** sample design •**Sequential** sample design •**Adaptive** design •**Bayesian** method

– Sequential and two-stage designs improve efficiency by allowing early stopping for futility or success, while controlling type I error and ensuring sufficient power

I. A simple fixed-sample design → **ex**. phase I of a trial of a new cancer drug

– Each patient is followed for a certain time, and the **endpoint** is whether the patient succeeds

– We need a rule to decide whether to continue or abandon the trial → can be expressed as **hypothesis test** $\boldsymbol{H_0: p = p_0}$, $\boldsymbol{H_1: p = p_1}$

- •If the proportion of successes is too low, the trial is stopped (reject H_1)
- •If it is high enough, the treatment is declared promising (reject H_0)

– Using a **normal approximation** of the binomial, the rejection region is chosen so that type I error $\leq \alpha$ and power $= 1 - \beta$, this leads to the **sample size** formula: $n = \left(\frac{z_{1-\alpha}\sqrt{np_0(1-p_0)}+z_{1-\beta}\sqrt{np_1(1-p_1)}}{p_1-p_0}\right)^2$

II. A simple sequential design → this setting is more appropriate when it is more important to discard a bad treatment that progress a new one

- Decisions are taken as patients are enrolled, possibly stopping earlier

Single stage design	Sequential design	Two stage design

12. Two stage designs for normally distributed data → trial continues to a second stage only if results after the first stage are inconclusive

I. A typical fixed-sample design → **goal**: test whether the experimental treatment is better than control by comparing the mean response

– Enrol n_1 patients and compute the test statistic; if it is too low, the trial stops (treatment not effective), if it is very high, efficacy is declared immediately, otherwise the trial continues to **Stage II**

– **Hypothesis test.** $\boldsymbol{H_0: \mu = \mu_0, H_1: \mu = \mu_1 > \mu_0}$

– **Test statistic.** $\boldsymbol{Z = \frac{(Y_t - Y_c)\sqrt{n}}{\sqrt{2\vartheta^2}}}$

– **Sample size.** $\boldsymbol{n = \left(\frac{z_{1-\beta}+z_{1-\alpha}}{\vartheta_R}\right)^2 4\vartheta^2}$

II. A two-stage design → **goal**: improve efficiency by allowing an *interim analysis* after the first n_1 patients before proceeding to the full sample size

– Include an additional n_2 patients, combine the evidence from both stages into a global test statistic, and apply the final decision threshold to accept or reject the treatment

– The two-stage design allows an **interim analysis** after $n_1$patients, with the option to **stop early** for efficacy or futility. This increases efficiency and can reduce sample size, while still controlling the Type I error through appropriate critical values.

13. Phylogenetics

– **DNA sequences** are represented as successions of letters: **Adenine**, **Cytosine**, **Guanine**, **Thymine**

– **Phylogenetic** studies evolutionary relationships among biological entities → it understands the **evolutionary distance** between DNA sequences and builds a phylogenetic tree representing how molecular evolution happened for the DNA sequences

– To find the right tree topology, one must first **align** the sequences to find the highest n of matches. Once they are aligned, **the tree can be built** in different manners:

I. Parsimony: the best model is the one with less n of changes to explain the data

II. Distance Method: uses probabilistic models to compute pairwise distances

– Distances are the estimates of the **branch length** separating a pair of sequences/species

– The optimal tree is generated estimating and updating evolutionary distances and the reconstructing a phylogenetic tree from the distance matrix

– Neighbour-joining method: clustering method that start with a starting tree and iteratively joints couples of nodes and computes the distance

Algo. •For all tip compute $\boldsymbol{\mu_i = \frac{1}{n-2}\sum_{j=1}^{n} D_{ij}}$ •Chose ij to minimize $\boldsymbol{D_{ij} - \mu_i - \mu_j}$ •Join ij and compute branch lengths $\boldsymbol{v_i = 0.5D_{ij} + 0.5(\mu_i - \mu_j)}$ and $\boldsymbol{v_j = 0.5D_{ij} + 0.5(\mu_j - \mu_i)}$ •Compute the distance between ij and all the tips •Delete tips i and j with ij •Continue till end

– Nucleotide substitution models: the simplest way to compute distances between sequences is to use the n of nucleotides substitution between each pair of sequences.

Algo. •$X(t)$ is the state of a given site •The transition prob matrix $\boldsymbol{P_{ij}(t) = P(X(t) = j|X(0) = i)}$ •The substitution rate matrix Q is the instantaneous rate of change $\boldsymbol{P = e^{Qt}}$

Hp •nucleotide substitution occurs as single independent events •sites evaluate independently →the process of substitution is modelled by CTMC Each model has a **stationary distribution** of the nucleotide frequencies and **transition rate matrix Q** →

Name	Transition rate matrix Q	Info
JC69		•nucleotide frequences are equally distributed $\pi_i = 0.25$ •$P(t) = e^{Qt} \rightarrow \boldsymbol{p_o(t) = 0.25 + 0.75e^{-4\alpha t}}$ is the prob of staying in the same state $\rightarrow \boldsymbol{p_1(t) = 0.25 - 0.25e^{-4\alpha t}}$ is the prob of transitioning •$\boldsymbol{p(t) = 0.75 - 0.75e^{-0.75t}}$ is the prob that, at time t, the descendant nucleotide is different from the ancestral one
K80		•distribution between **transitions** (A,G) or (T,C) and **transversion** are different •distance between sequence $d = (\alpha + 2\beta)t$ •at the equilibrium $\pi = 0.25$
HKY		•it allows for stationary frequencies π to be inequal and maintains different distribution rates between transitions and transversions
GTR		•**most general model** allowing all exchangeability rates between nucleotides to differ

– Gamma distributions across site: to represent variants in substitutions rates across sites one can apply
•site specific rate multipliers •derived the rate multipliers from a discrete mean-one gamma distribution

– The goal is to **estimate the parameters** of a phylogenetic tree from aligned DNA sequences, where sequence evolution is modelled as a CTMC with substitution rate matrix Q, using two main approaches:

I. Max likelihood: best tree st $\boldsymbol{\max P(D|M)}$ → useful to evaluate the tree that you have to decide which is the most descriptive of the data

– Objective to **find the parameters that maximise the probability of the data given the model and tree topology** → $\boldsymbol{\hat{\theta} = arg\max_{\theta} P(D \mid T, \theta)}$

Algo. •Compute likelihood for all the site •Compute the total likelihood $\boldsymbol{L = P(D|T) = \prod_{i=1}^{m} P(D^{(i)}|T)}$ •Compute it recursively, to simplify, for each node $\boldsymbol{L_k^{(i)}(s) = \sum_x P(x|s,t_l)L_l^{(i)}(x)\sum_y P(y|s,t_m)L_m^{(i)}(y)}$ •Results at the root node $\boldsymbol{L^{(i)} = \sum_x \pi_x L_0^{(i)}(x)}$

II. Bayesian inferences: best tree st $\boldsymbol{\max P(M|D)}$

– Objective **estimate the posterior distribution on trees** → explore the tree space using a MCMC technique to select trees with high posteriori probabilities, given a prior distribution and likelihood function

Algo. The **Metropolis-Hastings algorithm** is used to explore the tree space •Initial tree T_i •New tree is proposed T_j •The acceptance ratio is $\boldsymbol{R = \frac{P(T_j)P(D|T_j)}{P(T_i)P(D|T_i)}}$ •If $R \geq 1$ accept T_j as current tree

•Otherwise, $R \leq 1$, draw a uniform random number and if it is less then R reject T_j •Repeat