

notes

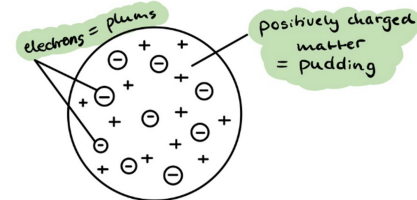
2022. December 11., Sunday PM 2:47

1 The structure of atoms

Thomson's model of the atom. Rutherford's experiment, Rutherford's model of the atom. Bohr's postulates and Bohr's model. Energy levels of the Hydrogen atom according to Bohr's model. Explanation of the line spectra of atoms. The Franck-Hertz experiment and its explanation

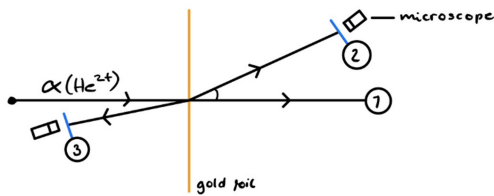
3. THE STRUCTURE OF ATOMS.

Thomson's model of the atom = Plum pudding model



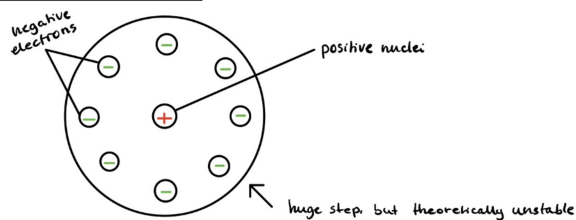
Rutherford's experiment

- Rutherford studied the scattering of α -particles (He^{2+} nuclei) on a thin metal foil



- Results**
 - Most α -particles were not deflected
 - Some α -particles were deflected at low angles
 - Very few α -particles were reflected
- Conclusions**
 - Atoms are made mostly of empty space
 - The positive charge of the atom is concentrated in the nucleus, not evenly distributed in the atom

Rutherford's model of the atom



Bohr's postulates and Bohr's model

1. Electrons in an atom can only have defined orbits
2. When electrons jump from one allowed orbit to another, the energy difference is emitted as a photon

$$hf = E_2 - E_1$$

De Broglie's matter wave hypothesis and its experimental setup

- De Broglie's matter wave hypothesis postulates that any particle that has a linear momentum is also a wave. E.g. a beam of electrons can be diffracted just as a beam of light
- $\lambda = \frac{h}{p}$, where h is Planck's constant, and p is the momentum (mass x velocity)
- The experiment: an electron beam was allowed to fall onto a single crystal of nickel and the diffraction was observed in the reflected beam

Explanation of the line spectra of atoms

- When atoms are excited, they emit light of certain wavelengths which corresponds to different colours
- The emitted light can be observed as a series of coloured lines with dark spaces in between called a line or atomic spectra
- Elements can be identified by their line spectrum

The Frank-Hertz experiment and its explanation

- Frank and Hertz studied the **collision of electrons with mercury**
- **Experiment**
 - The electrons were accelerated by an electric field between the cathode and the grid filled with Hg-vapour
 - After passing the grid, the electrons slowed down and did not reach the anode unless they had gained enough kinetic energy
- **Explanation**
 - The Hg-atoms cannot absorb energy, just well-defined values (4.9 eV)
 - This energy excited the ground state electron to the first excited state, i.e. it equals exactly to the energy difference between the E_1 and E_2 states of the Hg-atom
 - Thus, the experiment gives a proof of the validity of Bohr's theory

2 Dual nature of light and the electrons. Electromagnetic waves

Dual nature of light. Phenomena proving the wave nature of light. Waves, characteristics of waves. The Huygens-Fresnel principle. Diffraction, Interference, the double-slit experiment. Light as a transversal wave, wave polarization. Electromagnetic waves, the electromagnetic spectrum. List of phenomena proving the wave nature of light. **De Broglie's matter wave hypothesis and its experimental proof**

The dual nature of light

→ Has both wave & particle properties.

Wave properties:

- **Diffraction:**
 - The spreading of waves as they pass through a gap.
 - Significant when the wavelength of the wave is similar to the width of gap.
- **Interference:**
 - When two or more sets of waves arrive together at a point.
 - They may either interfere as to cancel out or give a larger amplitude.
- **Polarization:**
 - An unpolarized wave (e.g. light) oscillates in any direction.
 - Polaroid (plastic film) polarizes light waves allowing oscillations in one plane only (often used in sunglasses to eliminate glare).
 - If two Polaroid are crossed, no light will be transmitted.

Particle properties:

- **Photoelectric effect:**
 - Frequency of light does not increase e⁻ emission, only intensity.
 - If light was just a wave, the frequency would also increase e⁻ emission.
- **Electromotive force**
 - The voltage produced by a source of electricity that can drive a current through a circuit.

Waves, characteristics of waves

- **Definition:** a wave is a period disturbance that moves through a medium from one location to another (transport of energy)
- **Types of waves:** longitudinal, transverse, mechanical, electromagnetic
- **Characteristics of waves**
 - Amplitude: the distance from rest to crest
 - Wavelength: the length of one complete wave
 - Frequency: the number of waves that pass a fixed point during one second
 - Period: the time it takes to complete one cycle

Fermat's principle and the Huygens-Fresnel principle

- **Fermat's principle:** also called the principle of the least time – of all the possible paths light can take, it will take the path which requires the shortest time
- **Huygens-Fresnel principle:** every point on the wave front acts as a secondary source which produces wavelets with the same speed as the original wave

Waves, characteristics of waves

- **Definition:** a wave is a period disturbance that moves through a medium from one location to another (transport of energy)
- **Types of waves:** longitudinal, transverse, mechanical, electromagnetic
- **Characteristics of waves**
 - Amplitude: the distance from rest to crest
 - Wavelength: the length of one complete wave
 - Frequency: the number of waves that pass a fixed point during one second
 - Period: the time it takes to complete one cycle

Fermat's principle and the Huygens-Fresnel principle

- **Fermat's principle:** also called the principle of the least time – of all the possible paths light can take, it will take the path which requires the shortest time
- **Huygens-Fresnel principle:** every point on the wave front acts as a secondary source which produce wavelets with the same speed as the original wave

Refraction of light

- **Definition:** refraction of light is the bending of a wave when it enters a medium where its speed will be different

Interference, diffraction, the double-slit experiment

- **Interference:** overlapping of two waves from different sources produce a new wave
 - Constructive or destructive
- **Diffraction:** a phenomenon that occurs when a wave meets an obstacle – defined as the bending of waves around the corners of an obstacle
- **The double slit experiment:** demonstration of the wave-like properties of light;
 - Light from a laser beam is passed through two narrowly spaced slits
 - The light diffracts through the slits and interferes in the space beyond the slits
 - The interference pattern is projected onto a screen

Light as a transverse wave

- Light is a **transverse wave** because its components vibrate perpendicular to the direction of its propagation
- A light wave is an **electromagnetic wave** – it has an electric and a magnetic component

Wave polarization

- **Unpolarized light:** light that is vibrating in more than one plane
- **Polarized light:** light vibrates in a single, well-defined plane
- The process of transforming unpolarized light into polarized light is known as polarization. Only transverse waves can be polarized

Electromagnetic spectrum

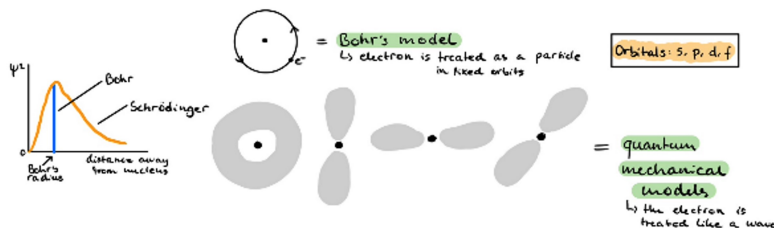
- Gamma rays – X-rays – ultraviolet – visible light – infrared – microwaves – radio waves

3. The quantum-mechanical model of the atom. Orbitals, molecular orbitals

Wave properties of the electron: the wave function and electron states in the atom. Atomic orbitals and their types. Quantum numbers and their physical meaning (orbital angular momentum and spin). The Pauli principle and Hund's rule. The Stern-Gerlach experiment and its interpretation. The Einstein-de Haas experiment. Molecular orbitals: sigma and pi bonds. Energy states of molecules

Wave properties of the electron: the wave function, the electron in the atom, atomic orbitals and their type

- **The wave function – ψ**
 - The square of the wave function ψ^2 , is the probability of finding the electron in a given spatial point (x, y, z)
 - Where ψ^2 is bigger, the more likely it is to find an electron



Quantum numbers and their physical meaning

- **Principal quantum number (n)** → gives the energy, every n value has an energy value
 - $n \rightarrow E_n$
- **The angular quantum number (l)** → gives the magnitude of the angular momentum
- **The magnetic quantum number (m)** → gives the direction of the angular momentum
- Angular momentum (L) → $L = mvr$
 - Momentum = mass in motion
 1. Orbital = rotation of electron around nucleus
 2. Spin = rotation of electron around itself

The Pauli principle and Hund's rule

- **Pauli's exclusion principle** = no two electrons can have the same set of quantum numbers, thus electrons in the same orbital must have opposite spins
- **Hund's rule** = electrons will fill into empty orbitals of the same energy singly, before they begin to pair up into the same orbital

The Stern-Gerlach experiment and its interpretation

- The experiment involves sending a beam of silver atoms through a magnetic field and observing their deflection
- It showed that **space quantization** exists = the electron's magnetic moment can only be in a certain direction

The Stern-Gerlach experiment and its interpretation

- The experiment involves sending a beam of silver atoms through a magnetic field and observing their deflection
- It showed that **space quantization** exists = the electron's magnetic moment can only be in a certain direction

The Einstein-de Haas experiment

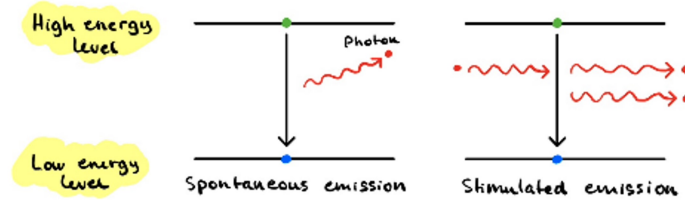
- Proved that the electron's rotation around their own axis (their own angular momentum) and their magnetic moment (spin moment) are tightly connected, so if one is changed, the other will also change

4 The laser

Spontaneous and stimulated emission, population inversion. Energy levels in a laser, metastable state, lifetime of states. The laser oscillator, **resonance condition**. Physical properties of the laser light. Description of one of the laser types. Comparison of continuous wave and pulsed lasers. Medical and other applications of lasers

Spontaneous and stimulated emission, population inversion

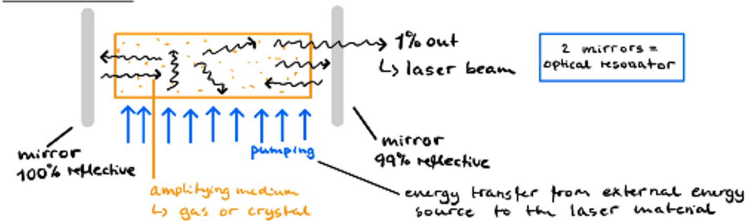
- **Spontaneous emission** takes place without interaction with other photons, and the direction is random
- **Stimulated emission** takes place when the excited electron interacts with another photon
- **Population inversion** = when the majority of the electrons can be found in higher, excited energy states, it is the redistribution of atomic energy levels so that laser action can occur



Energy levels in a laser, metastable state, lifetime of states

- **Two-level lasers** → in the best case we get equal population
- **Three-level lasers** → hitting hard will result in lasing
 - In a three-level laser, the material is first excited to a short-lived high-energy state that spontaneously drops to a somewhat lower energy-state with an unusually long life-time called the **metastable state**
 - The metastable state is important because it traps and holds the excitation energy, building up a population inversion that can be further stimulated to emit radiation
- **Four level lasers** → easy laser operation
 - In a four-level laser, an extra transition state is located between the ground state and the metastable state, which makes them a lot steadier

The laser oscillator



- **Absorption:** the photon is absorbed, so annihilated → its energy is given to the atom, which gets excited
- **Emission:** the atom is de-excited (excited to ground state) → emits a photon
- **Equilibrium:** 3 or more energy levels (lasing cannot occur in a two-level system!), fast and slow transition, pumping = giving energy to electrons → population inversion
- **Optical resonance:** 2 mirrors, 100% and 99% reflective → 1% out (laser beam)

Physical properties of laser light

1. Monochromatic (one colour, one wavelength)
2. Coherent – allow the laser beam to stay narrow over great distances
3. High intensity/power
4. Parallel/small divergence
5. Polarized
6. Short pulses

Description of one laser type

- **He-Ne laser** → mixture of helium and neon gas
 - Low pressure, excited helium atoms collide with neon atoms, four-level laser

Comparison of continuous wave and pulsed lasers

- A continuous wave laser operates with stable or continuous output power
- A pulsed laser is characterized by pulses of energy which occur at a particular pulse repetition frequency

Medical and other applications of laser

- **Surgery:** laser scalpel → coagulation and bleeding free operation
 - Eye surgery → retina detachment, glaucoma, etc.
- **Tattoo removal, hair removal, etc.**

5 Absorption spectroscopy

The concept, types and typical quantities of radiation. Interaction of radiation with matter in general. Energy levels of atoms and molecules, the Jablonski diagram. Main spectroscopic methods, grouping them by the types of interactions and photon energy. Light absorption in general, the Lambert-Beer law. Transmittance, absorbance, absorption coefficient. Absorption spectrum. Structure, operation and applications of absorption photometers.

28. SPECTROPHOTOMETRY.

The concept, types and typical quantities of radiation

- **Radiation** = emission of energy in the form of waves or particles through space or a medium
- Includes:
 - **Electromagnetic radiation**: radio waves, microwaves, infrared, visible light, UV, X-rays and gamma rays
 - **Particle radiation**: alpha radiation (Helium nuclei) and beta radiation (electrons or positrons)
 - **Acoustic radiation**: ultrasound, sound and seismic waves
- Often categorized as **ionizing** or **non-ionizing**
 - Ionizing radiation carries more than 10 eV, enough to ionize atoms or molecules

Interaction of radiation with matter in general

- **Transmission**: radiation can penetrate matter with no interaction
- **Absorption**: radiation can interact with matter and be completely absorbed
- **Reflection**: radiation can interact with matter and be scattered or deflected, losing part of its energy

Energy levels of atoms and molecules, the Jablonski diagram

- If an atom, ion, or molecule is at the lowest possible energy level, it and its electrons are said to be in the ground state
- If it is at a higher energy level, it is said to be excited
- When an atom absorbs energy, it can become excited
- A Jablonski diagram illustrates the electronic states of a molecule and the transitions between them

Main spectroscopic methods, grouping them by the types of interactions and photon energy

- **Spectroscopy** = any measurement in which a property of a substance is examined depending on energy, result from spectroscopy → **spectrum**
- **Classification of spectra based on origin**:
 - **Absorption** and **emission** spectra are distinguished by detecting absorbed and emitted photons
 - **Absorbed photons**: from a lower energy state to a higher energy state)
 - **Emitted photons**: from a higher energy state to a lower energy state
- **Classification of spectra based on their form**:
 - **Line spectrum** → atoms
 - **Band spectrum** → molecules
 - **Continuous spectrum** → glowing substances (based on temperature)

Light absorption in general, the Beer-Lambert law

- **Light absorption**: process by which light is absorbed and converted into energy
- **Beer-Lambert law** → describes light absorption in solutions
 - $I = I_0 10^{-\epsilon c x}$

Transmittance, absorbance, absorption coefficient

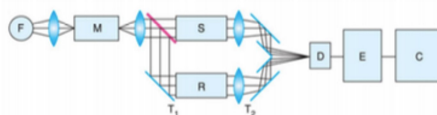
- **Transmittance** = ratio of intensity of incident and transmitted light
- **Absorbance** = ratio between radiation falling upon a solution and radiation through the solution, also called optical density (OD)
- **Absorption coefficient** → determines how far into a material light of a particular wavelength can penetrate before it is absorbed

Absorption spectrum

- **Absorption spectrum** = spectrum of electromagnetic radiation, which passed through a substance and the substance absorbed different wavelength components
- Wide bands → not suitable for qualitative identification
- Spectra of atoms → line
- Spectra of molecules → band

Structure, operation and application of absorption photometers

- **Absorption spectrum can be recorded with a spectrophotometer**
- **Steps**:
 - Light source (F) illuminates in wavelength 200-800 nm
 - Monochromator (M) selects appropriate wavelength
 - Light goes through sample solution (S) and reference solution (R)
 - Photodetector (D) measures light intensity using photoelectric multiplier
 - Data is sent to data processing unit (E) and a computer (C)
 - Mirrors (T₁ and T₂) are used for diverging light



6 Fluorescence spectroscopy

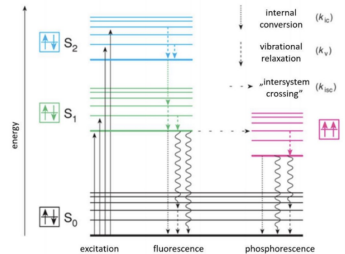
Energy transitions in atoms and in molecules, explained with the Jablonski diagram. Singlet and triplet states. Concept and types of luminescence (fluorescence, phosphorescence). The process of fluorescence, the Kasha rule. Structure and function of the fluorimeter. The concept of excitation and emission spectra, the way they are recorded. The Stokes shift. Fluorescence quantum efficiency and lifetime

Principle

- Some substances can be excited by stimulation and some of their excitation energy is emitted into their environment as the form of electromagnetic waves, light
- This phenomenon is called **luminescence**, the substances that create it are luminophores
 - Light is not generated by high temperatures, so-called "cold emission"

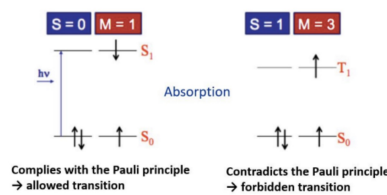
Energy transitions in atoms and in molecules, explained with the Jablonski diagram

- Absorption often results in an excited state → transition from basic vibration level S_0 (ground state) to a vibrational level of an excited state $S_1, S_2, \dots$ (very fast, in femtoseconds)
- Thermal (vibrational) relaxation: the excited electron is set to the lowest vibration level of S_1 energy state
- Excited state is not stable → back to ground state
 - Non-radiative transition** (internal conversion): excitation energy is completely converted into heat
 - Radiative transition (luminescence)**
 - Fluorescence**: electrons go from lowest vibration level of S_1 to ground state in one step within nanoseconds
 - Phosphorescence**: spin of excited electron turns from singlet state S_1 to triplet state T_1 , then from T_1 to ground state (S_0)



Singlet and triplet states

- Multiplicity = number of possible orientation states of magnetic moment assigned to the spin state (S) relative to the directions of the magnetic field
- $M = 2S + 1$
- If $S = 0$, then $M = 1$ → singlet state
- If $S = 1$, then $M = 3$ → triplet state



Types of luminescence

- Chemiluminescence**
 - Light emission that is excited by the energy from chemical reactions
- Bioluminescence**
 - Production and emission of light by a living organism, as a result of a chemical reaction, e.g. fireflies and jelly fish
- Photoluminescence**
 - Light emission that is excited by direct light radiation of certain energy (frequency) and wavelength
 - Luminescent molecules have aromatic rings with conjugated double bonds
 - Two types: fluorescence and phosphorescence

The process of fluorescence, the Kasha rule

- Energy absorption
 - Excitation
 - Release of the accumulated extra energy in the form of light (emission)
- Kasha rule** = in each case, photon emission always starts from the lowest vibrational level of the 1st excited state

Structure and function of the fluorimeter

- Luminescence is measured in a so-called **perpendicular measuring arrangement**
- During the measurement, light from a light source is selected with a **monochromator** to the desired excitation wavelength while the emission wavelength is set to another monochromator
- The light intensity is measured with a **detector** and the data is collected and displayed by a computer

The concept of excitation and emission spectra, the way they are recorded

- Excitation spectrum**: if the excitation wavelength is kept constant in the spectrum as a function of the emission wavelength
 - Represents vibrational levels of the ground state
- Emission spectrum**: if the emission wavelength is kept constant in the spectrum as a function of the excitation wavelength
 - Represents the vibrational levels of excited states
- They are roughly mirror images of each other due to the Stokes shift
- Stokes shift** = the shift to lower energy between the incident light and the emitted light after interaction with a sample

Fluorescence quantum efficiency and lifetime

- Quantum yield** = the ratio between the number of photons emitted and the number of photons absorbed
 - Value between 0 and 1 $\Phi_F = \frac{N_F}{N_A}$
 - $Q = \frac{k_{fluorescence}}{k_{sum}}$
 - k_{sum} is the combined probability of all radiative and non-radiative transitions
- Luminescence lifetime** = the average time between excitation and emission of a photon
 - Fluorescence: short, some nanoseconds
 - Phosphorescence: much longer, few milliseconds to hours

7 Photophysics of molecules. Biomedical applications of fluorescence

Bioluminescence. Green fluorescent protein (GFP). Intrinsic and extrinsic fluorophores, fluorescent labels. Direct and indirect immunofluorescence labeling. Fluorescence sensors (ions, pH, membrane potential). Photobleaching. Photoswitchable fluorophores

Bioluminescence

- Production and emission of light by a living organism
- Form of chemiluminescence
- Luciferin and luciferase
- Enzymes catalyze oxidation of luciferin with cofactors like oxygen

Green fluorescent protein (GFP)

- Versatile biological marker for monitoring physiological processes (fluorescence spectroscopy)
- Exhibits bright green fluorescence when exposed to light in blue to UV range
- Refers to protein first isolated from jellyfish
- Has excitation peak at 395 nm
- Emission peak at 509 nm

Intrinsic and extrinsic fluorophores

- **Intrinsic:** occurs naturally, possess fluorescence
 - tryptophan, tyrosine, phenylalanine, GFP
- **Extrinsic:** added to sample to provide fluorescence when there is no natural fluorescence
 - synthetic dye, GFP, fluorescent antibody

Fluorescent labels

- Molecules that are attached chemically to aid in the detection of a biomolecule
- Selectively binds to specific region on target molecules
- e.g. ethidium bromide, fluorescein, GFP

Direct and indirect immunofluorescence

- Technique used in laboratory to diagnose disease of skin, kidney and other organs
- Application of antibody – fluorophore binds to molecules of sample of patient tissue
 - Direct → 1 antibody, indirect → 2 antibodies
- Fluorophore emits own frequency of light, seen with microscope

Fluorescence sensor (ion, pH, membrane potential)

- Fluorophores are sensitive to environmental parameters → biosensors

Förster resonance energy transfer (FRET) → phenomenon, requirements, application

- Technique to study the distance between two chromophores, called a donor-acceptor pair
- An excited donor transfer energy to an acceptor through a non-radiative process (resonance)
- Highly distance-dependent: process is only effective when separating distance between the donor-acceptor pair is less than 10 nm

Photobleaching

- Photochemical reaction of a dye or fluorophore molecule so that it is permanently unable to make fluorescence → caused by the cleavage of covalent bonds between the fluorophore and surrounding molecules

FRAP (= fluorescence recovery after photobleaching)

- Method for determining kinetics of diffusion through tissue or cells
- Used for the study of cell membrane, diffusion and protein binding

8 Infrared (IR) and Raman spectroscopy

Energy-level system of molecules. Vibrational motion of molecules, natural frequency. Dipole moment for linear and non-linear molecules (e.g. HCH, CO₂), IR-active and IR-inactive vibrations. Vibrational modes of water. Condition of resonance, absorption, the IR spectrum and its interpretation. Applications of IR spectroscopy. Elastic and inelastic light scattering. Recording and interpretation of Raman spectra, Rayleigh peak, Stokes and anti-Stokes shift. Applications of Raman spectroscopy. Advantages and disadvantages of IR and Raman spectroscopy

Energy-level system of molecules

- Rotational levels = kinetic energy is associated with the rotational motion of molecules
- Electronic levels = energy is stored as potential energy in excited electron configurations
- Vibrational levels = the oscillatory motion of atoms or groups within a molecule
- Ground state = lowest possible energy level of a molecule

Vibrational motion of molecules, natural frequency

- Vibrational motion = a periodic motion of the atoms of a molecule relative to each other such that the center of the molecule remain unchanged
- Natural frequency = frequency at which a system tends to oscillate in the absence of any driving force

Dipole moment for linear and non-linear molecules, IR-active and IR-inactive vibrations

- Dipole moments occur when there is a separation of charge
 - If $\mu = 0 \rightarrow$ no dipole moment
- $\text{CO}_2 \rightarrow$ linear molecule
 - Symmetric stretch: $\mu = 0$
 - Asymmetric stretch: $\mu \neq 0$
- $\text{H}_2\text{O} \rightarrow$ non-linear
 - Symmetric stretch: $\mu \neq 0$
 - Asymmetric stretch: $\mu \neq 0$
- Symmetric stretching = two or more bonds vibrate in and out together
- Asymmetric stretching = some bonds are getting shorter as some bonds are getting longer
- **IR-active and IR-inactive vibrations**
 - Symmetric stretch of CO_2 is not present in IR spectroscopy absorption spectrum as the change of the dipole moment is 0
 - Energy of light must be equal a vibrational energy level difference within the molecule

Vibrational modes of water

- Symmetric and asymmetric stretching
- Bend

Condition of resonance, absorption, IR-spectrum and its interpretation

- Resonance = a phenomenon of increased amplitude that occurs when the frequency of a periodically applied force is equal or close to a natural frequency of the system on which it acts; it causes system to oscillate with larger amplitude
- Wavelength found in IR-radiation is a little longer than visible light
 - Useful to find out what kind of bonds are present
- Y-axis: usually labelled transmittance (amount of light that passes through sample)
- X-axis: usually labelled with frequency or wavelength

Applications of IR spectroscopy

- Analyzing samples with covalent bonds
- Used in inorganic and organic chemistry
- FTIR (Fourier transform infrared)

Elastic and inelastic light scattering

- **Elastic scattering** = scattering with no change in photon energy
- **Inelastic scattering** = scattering with change in photon energy

Recording and interpretation of Raman spectra, Rayleigh's peak, Stokes and anti-Stokes shift

- Recording and interpretation of Raman spectra
 - Intense laser is used (785 nm)
 - Relies upon inelastic scattering of photons
 - Steps
 - Sample is illuminated with laser
 - Electromagnetic radiation from the illuminated spot is collected with a lens and sent through a monochromator
 - Elastic scattered radiation (Rayleigh scattering) is filtered out by a notch filter
 - Other radiation is dispersed onto a detector
- Stokes shift = if final state is higher than the initial state, the scattered photon will be shifted to a lower energy (500 nm peak)
- Anti-Stokes shift: photon is shifted to higher energy (-500 nm peak)

Application of Raman spectroscopy

- To identify molecules and study chemical bonding and intermolecular interactions
- To find crystallographic orientation of a sample

Advantages and disadvantages of IR and Raman spectroscopy

- **Advantages**
 - Works on a wide range of concentration
 - Spectra recorded within a couple of seconds
 - Qualitative and quantitative analysis
 - Sample can be liquid, solid, powder, gel
 - Inexpensive
- **Disadvantages**
 - Atoms or monoatomic ions do not have a spectrum
 - Homonuclear diatom molecules do not show IR spectrum
 - Complex mixture and aqueous solutions are difficult to analyse with IR

9 Flow cytometry

Components, operation and applications of a flow cytometer (fluidic and optical system, hydrodynamic focusing). Detected parameters: light scattering and fluorescence emission. Data representation and analysis: list mode, single- and multiparametric representations and their interpretation. Principles of cell sorting

Components, operation and applications of a flow cytometer

- **Flow cytometry** = a system for sensing cells or particles as they move in a liquid stream through a laser beam
- **Components of a flow cytometer**
 - Fluidics → flow and hydrodynamic focusing
 - Optics → light source, filters, mirrors, detectors
 - Electronics, data analysis → conversion of light signal to digital data
 - Sorting → separation of cells
- **Operation of a flow cytometer**
 - Sample of cells or particles (often fluorescently labelled) are put into the instrument
 - Hydrodynamic focusing: sample is focused to flow one cell at a time through a laser beam → the system uses liquid (hydro) to force the cells to travel at the same speed as single cells
 - Sample fluid + sheath fluid → do not mix due to laminar flow
 - Light is scattered → characteristic to cells and their components
 - Forward scattered → information about cell size
 - Side scattered light → information about granularity and structural complexity of the cell
 - Emission of fluorescence → split into defined wavelengths by optical filters and dichroic mirrors
- **Data representation and analysis**
 - List mode → presentation of all measured data corresponding to every single particle; raw data stored in the computer
 - Single parametric representation → histogram
 - Plot of the number of particles characterized by a given parameter
 - e.g. size, intracellular complexity, amount of fluorescence
 - Multiparametric representation → dot plot
 - Correlation between two or more measured parameters
 - Displayed as dots → 1 dot = 1 particle

Principles of cell-sorting

- **Cell separation** = separation of cell populations characterized by a given parameter (e.g. FCS, SSC, FL) for further investigation
- **Principles**
 1. Data collection
 2. Decision: based on gating, the cell population to be sorted and defined
 3. Drop formation: the flow head is shaken by a piezoelectric vibrator to decompose the fluid that leaves the head into droplets (1 droplet = 1 cell)
 4. Electrostatic separation: the droplet to be separated is charged and deflected by an electric field
 5. Separation: 3000-5000 cells per second

Applications

- **Medical biology**: cell signaling, cell-cell interactions, morphological studies, DNA damage and repair, cell cycle, autophagy, cell death, stem cell differentiation
- **Clinical practice**: parasitology, microbiology, oncology, haematology, clinical immunology

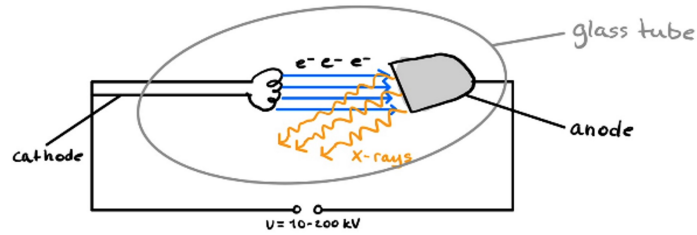
10 X-rays

Physical properties of X-rays and their place within the electromagnetic spectrum (frequency, wavelength, neighboring radiation types). Components and function of an X-ray tube. Formation and mechanism of production of characteristic and braking radiation, comparison of their spectra. Explanation of the cutoff wavelength

Physical properties of X-rays and their place within the EM spectrum

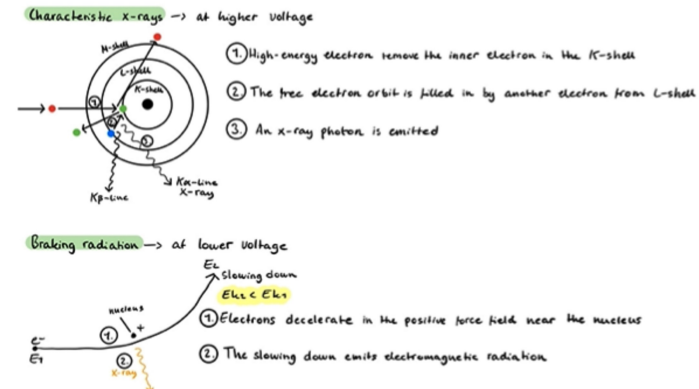
- **What are X-rays?**
 - Electromagnetic wave or photon
 - Wavelength = approx. 10 nm, frequency = $3 \cdot 10^{16} - 3 \cdot 10^{19}$
 - Located between UV-light and gamma rays on the EM spectrum
- **Physical properties:** they can penetrate objects, they have heating effect, fluorescence: when X-rays fall on certain material fluorescence is emitted, ionization: can convert atoms into ions

Components and function of the X-ray tube



- X-rays are produced in high voltage tubes where the cathode is heated at high voltage in vacuum
- The emitted electrons are attracted to the anode and after collision, X-rays are generated

Formation and the mechanism of production of characteristic and braking radiation, comparison of their spectra

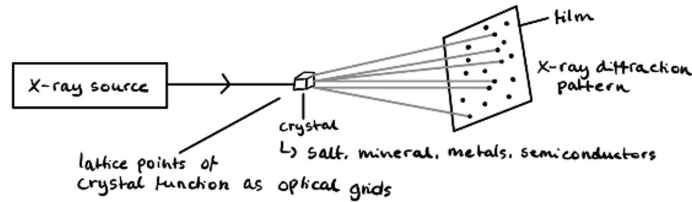


Explanation of cut-off wavelength = the maximum wavelength at which a wave propagates

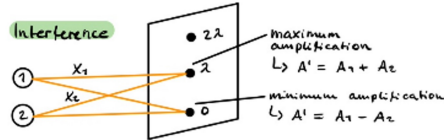
11 X-ray diffraction. The condition of the diffraction, objects that may be studied by X-ray diffraction. The condition for the formation of interference maxima. Laue and Bragg equations. The experimental procedure. The single-crystal and the powder method. Biological applications

The condition of the diffraction, objects that may be studied by X-ray diffraction

- **X-ray diffraction** = a method to investigate crystalline matters involving the diffraction of X-ray radiation on the crystal grid, yielding an interference pattern that can be detected



The condition for the formation of interference maxima



- Interference minima = destructive interference → when the waves cancel each other
- Interference maxima = constructive interference → when you get a diffraction pattern

Laue and Bragg equations

- **Laue equations** → used to calculate the grating constants = the distance of two neighbouring lattice points
 - $a \cdot (\cos \alpha_n - \cos \alpha_0) = k \cdot \lambda$
 - $b \cdot (\cos \beta_n - \cos \beta_0) = l \cdot \lambda$
 - $c \cdot (\cos \gamma_n - \cos \gamma_0) = m \cdot \lambda$
- **Bragg equation** → gives the angles of the scattering
 - $2 \cdot d \cdot \sin \theta = k \cdot \lambda$

The single crystal and the powder method

- **Single crystal method**: one pure, relatively large crystal is produced (tedious and long procedure), then the crystal is irradiated by a wide spectrum of X-rays, and the resulting diffraction pattern is analysed
 - Device: goniometer, rotates the crystal sample to fulfil the Laue rules
- **Powder method**: the sample is ground into very fine grains and placed in a transparent holder; the random orientation of the individual grains ensures that some of them show crystal planes matching the angle of incidence to produce an intensity maximum

Application of x-ray diffraction

- **Single crystal method**: used to determine the structure of an unknown crystal, mainly macromolecules
- **Powder method**: used to get atomic resolution image about substances

12 Diagnostic X-rays, CT

The nature of X-rays, energy and wavelengths. Mathematical description of X-ray absorption (equations, functions, attenuation coefficient, half-value layer). Interactions responsible for absorption. Detection of X-rays. Factors influencing absorption. Contrast materials: principles and examples. Digital subtraction angiography. Dual-energy X-ray absorptiometry (DEXA). Computed tomography (CT): the scheme and operation of the instrument, principles of imaging and computing. Voxels, CT number, Hounsfield units. Windowing.

The nature of x-rays, energy and wavelength

- What are x-rays?
 - Electromagnetic wave or photon
 - Wavelength = approx. 10 nm, frequency = $3 \cdot 10^{16} - 3 \cdot 10^{19}$
 - Located between UV-light and gamma rays on the EM spectrum
- **Physical properties:** they can penetrate objects, they have heating effect, fluorescence: when X-rays fall on certain material fluorescence is emitted, ionization: can convert atoms into ions

Mathematical description of X-ray absorption (equations, functions, attenuation coefficient, half-value layer)

- Exponential absorption law
 - $I = I_0 \cdot e^{-\mu x}$
- Half value layer = amount of absorbing material needed to reduce x-ray intensity by half
 - $x_{\frac{1}{2}} = \frac{\ln 2}{\mu}$
 - μ = attenuation value → describes the imaging beam's reduction in intensity by the tissue which it passes through
- Maximum range = layer of thickness where all radiation is absorbed
 - $R_{max} = 8x_{1/2}$

Interactions responsible for absorption

	Photoeffect	Compton scattering
Interaction with elements on	Inner shell	Outer shell
What happens to the photon?	Disappears	Scattered with low energy
More likely at energies	Less than 25 keV	More than 25 keV
Absorption depends on	Density, type of atom	Density

Detection of x-rays

- Imaging detectors → photographic plates, X-ray film
- Dose measurement devices → ionization chambers, Geiger counters, dosimeters

Factors influencing absorption

- Density
- Type of atom
- 5 times larger absorption in bones than in soft tissue → Calcium

Contrast materials: principles and enhancement

- ^{53}I → in urinary tract, circulatory system
- ^{56}Ba → in digestive tract
- ^{82}Pb → protection
- Provides contrast to the low absorption in soft tissue

Digital subtraction angiography

- Radiographs of vessels of the body after injection of contrast material
- Images produced by subtracting background structures and enhancing the contrast

Dual energy X-ray absorptiometry (DEXA)

- Measurement of bone density
- 2D image

Computed tomography (CT): the scheme and operation of the instrument, principles of imaging and computing

- **Instrument**
 - Gives a cross-sectional image
 - Wide fan-shaped X-ray beam
 - Multiple detectors (detector ring)
 - X-ray source and detectors rotate around the patient
- **Imaging basis** → different absorption properties of the tissues
 - Image taken from different sides creating voxel (volume element)
 - Detectors measure total absorption
 - Computer calculates the different absorption properties of the tissues
 - Goes in slices through the whole body → cross-sections

Voxels, CT numbers, Hounsfield units

- Voxel = volume element, the region in a tissue that corresponds to a pixel in an image
- Hounsfield scale describes radiodensity, its value is the CT number
 - $HU = 1000 \cdot \frac{\mu - \mu_{water}}{\mu_{water} - \mu_{air}}$

13 Gas laws. Phases of water

The concept, types and examples of a thermodynamic system. Properties of the ideal gas as a thermodynamic model system. Gas laws (Boyle's law, Charles's law, Gay-Lussac's law) and their graphic representation. The combined and ideal gas laws. Characteristics of phase diagrams, the phase diagram of water. Thermal expansion of gases and liquids.

The concept, types and examples of thermodynamic systems

- Thermodynamics is the study of heat, work, and energy in a system
- **Open system:** exchange of both energy and matter (e.g. casserole with boiling water, no lid)
- **Closed system:** exchange of energy, but not matter (e.g. casserole with boiling water + lid)
- **Isolated system:** exchange of neither energy nor matter (only theoretical)

Properties of the ideal gas as a thermodynamic model

- The own volume of the gas can be neglected
- There are not attractive/repulsive forces among the molecules
- There is elastic collision between the molecules, no energy is lost during collision

Gas laws

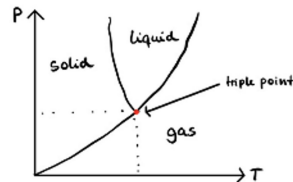
- **Boyle's law** → pressure at constant temperature is inversely proportional to the volume
 - $P_1 \cdot V_1 = P_2 \cdot V_2$
- **Charles' law** → volume at constant pressure is directly proportional to the temperature
 - $\frac{V_1}{T_1} = \frac{V_2}{T_2}$
- **Gay-Lussac's law** → pressure at constant volume is directly proportional to the temperature
 - $\frac{P_1}{T_1} = \frac{P_2}{T_2}$

The combined and ideal gas laws

- **The combined gas law** combines Boyle's, Charles' and Gay-Lussac's laws
 - $\frac{pV}{T} = \text{constant}$
- **The ideal gas law** works for ideal gases and real gases with low pressure and high temp
 - $p \cdot v = n \cdot r \cdot T$

Characteristics of phase diagrams, phase diagram of water

- Major features: phase boundaries and triple point (there the three phases are in equilibrium)
- Phase diagram of water



Thermal expansion of liquids and gases

- Both liquids and gases expand when they get heated
- The atoms themselves do not expand, but the volume they take up does
- A gas that is contained in a fixed volume cannot expand, so the temperature increases

14 Diffusion

Thermal motion of particles, the phenomenon of diffusion, its cause and consequences. Quantitative description of diffusion, Fick's 1st law: matter flow rate, matter flow density, concentration gradient and their relation. Diffusion coefficient, Einstein-Stokes-formula. Relation between the diffusion time and the mean displacement. Classification of transport processes through the cell membrane according to the transport mechanism and energetic requirements

Thermal motion of particles, the phenomenon of diffusion, its cause and consequences

- **Thermal motion of particles**
 - Particles are moving due to the continuous collision with water molecules
 - Elevated temperature results in faster motion, which results in increased frequency of collision
- **Diffusion**
 - Due to the non-uniform distribution of particles, there will be transport of particles from where it is more concentrated to where it is less concentrated, which continues until the distribution of particles is uniform

Quantifying diffusion, Fick's 1st law

- Fick's 1st law = the matter flow density is linearly proportional to the concentration gradient
- Concentration gradient increases → rate of diffusion increases
- $J = -D \frac{\Delta C}{\Delta x}$
where J = matter flow density, D = diffusion coefficient, $\Delta C/\Delta x$ = concentration gradient

Diffusion coefficient, Einstein-Stokes formula

- Do all particles diffuse at the same rate in all media?
- For spherical particles (r = radius) in a viscous medium (η) at T temperature
 - $D = \frac{k}{6\pi} \cdot \frac{T}{\eta r}$
- Temperature (T): the higher temperature, the faster the motion
- Geometry/shape of particle (r): small, round particles diffuse more easily than big, fibrillar particles
- Molecular weight of the particle (M): lighter particles diffuse more easily
- Viscosity of the medium (η): diffusion is faster in low viscosity media

Relation between the diffusion time and the mean displacement

- $Distance \sim \sqrt{time}$
 - Relatively fast (< seconds) over short distances (100 μm)
 - Exceptionally slow (> days) over long distances (1 cm)

Classification of transport processes through the cell membrane according to the transport mechanism and energy requirements

- **Transport mechanism**
 - Without mediator = passive diffusion
 - With mediator = facilitated diffusion (e.g. ion channels, carrier proteins)
- **Energy requirements**
 - Passive transport = no energy requirements
 - Active transport = need energy (e.g. ATP → ADP)

15 Osmosis

Semipermeable membrane. Hydrostatic pressure. The phenomenon of osmosis, its cause and consequences. Osmotic pressure and its interpretation. Van't Hoff's law. Classification of solutions based on their osmotic pressure. Biological relevance of osmosis: red blood cells in different osmotic pressure environment, treatment of edemas and inflamed areas, treatment of constipation, hemodialysis

Semipermeable membrane = allows smaller solvent molecules to pass through, but not the larger solute molecules (e.g. sugar vs water)

- Pore size = selectivity

Hydrostatic pressure = the pressure exerted by a fluid due to gravity

- $p = \rho g h$
Where p = pressure, ρ = density of liquid, g = gravity (9.81), h = height of column

The phenomenon of osmosis, its cause and consequences

- **Osmosis = restricted diffusion**
 - Unidirectional matter flow through a semipermeable membrane which takes place by means of diffusion
 - Smaller molecules reach a uniform distribution while the larger molecules remain in their compartments
- Conditions: semipermeable wall and concentration difference

Osmotic pressure and its interpretation

- Osmotic pressure = pressure that inhibits net solvent flow
- $\pi = icRT$
where i = van't Hoff constant, c = molar concentration, R = universal gas constant, T = temp

Van't Hoff's law: for dilute solutions and perfect semipermeable membranes

- $P_{\text{osmotic}} = cRT$
- The osmotic pressure is linearly proportional to concentration
 - Concentration increases → osmotic pressure increases

Classification of solutions based on their osmotic pressure

- **Hypertonic**
 - Higher concentration: $c > c_x$
 - Higher osmotic pressure: $p > p_x$
 - Shrinking
- **Isotonic**
 - Same concentration
 - Same osmotic pressure
- **Hypotonic**
 - Lower concentration: $c < c_x$
 - Lower osmotic pressure: $p < p_x$
 - Swelling

Biological relevance of osmosis

- Red blood cells in different osmotic pressure environments
 - Isotonic → nothing happens
 - Hypertonic → cells shrink, water OUT
 - Hypotonic → cells swell/burst, water IN
- Treatment of edemas and inflamed areas: $MgSO_4$ solution → hypertonic environment
- Treatment of constipation: laxative salts → hypotonic environment
- Haemodialysis = treatment of patients with severe kidney disease
 - Aim: remove toxins and waste products, retain plasma proteins and cellular elements of the blood

16 Fluid flow

Static, hydrostatic and dynamic pressure. Pascal's law (radial pulse). Shear stress, velocity gradient, the definition of viscosity. Ideal and real fluids. Blood and synovial fluid. Laminar and turbulent flow, stationary flow. Reynolds number. Volumetric flow rate. Continuity equation, Bernoulli's law (aneurysm). Hagen-Poiseuille's law (vasodilation). Stokes's law.

Static, hydrostatic and dynamic pressure

- **Static/hydrostatic pressure** is the pressure that a fluid exerts when it's not moving, and it's caused by the weight of the fluid above it
 - $p = \rho g h$
where p = pressure, ρ = density, g = gravity, h = height of the column
- **Dynamic pressure** is the pressure that a fluid exerts when it's moving
 - $q = \frac{1}{2} \rho v^2$
where q = dynamic pressure, ρ = density, v = velocity of fluid

Hydrostatic pressure: pressure applied to the fluid by the fluid at equilibrium at a given point due to gravity

Pascal's law = pressure in a fluid is equal in all directions

- $P_1 = P_2$

Shear stress, velocity gradient, the definition of viscosity

- **Shear stress** = the force that causes layers to slide upon each other in opposite directions
- **Velocity gradient** = the difference in velocity between layers
- **Definition of viscosity** = viscosity is the measure of a fluid's resistance to flow

Ideal and real fluids

- **Ideal fluids** have no viscosity, e.g. certain liquid He-species
- **Real fluids** have viscosity
 - **Newtonian fluids** (e.g. water) = have constant viscosity, no matter the pressure being applied to the fluid
 - **Non-Newtonian fluids** (e.g. blood and synovial fluid) = viscosity may change if enough force is applied to the fluid

Laminar and turbulent flow, stationary flow

- **Laminar flow** is characterized by low speed, no swirling and on smooth surfaces
- **Turbulent flow** is characterized by high-speed, swirling and on rough surfaces
- They are both unsteady flows, but turbulent flow is an extreme kind
- **Stationary flow**: a flow in which the velocity of the fluid does not change with time

Reynold's number → low number = smooth/constant flow, high number = turbulent flow

- $R = \frac{v \rho r}{\eta}$

Continuity equation, Bernoulli's law

- **Continuity equation** → describes the volumetric flow rate (the flow of fluid which passes a point per unit time)
 - $Q = \frac{v}{t}$
- **Bernoulli's law** = the sum of static, dynamic and hydrostatic pressure is constant
 - $p + p \frac{v^2}{2} + \rho gh = \text{constant}$

Hagen-Poiseuille's law → force because of pressure difference = frictional force

- $I = \frac{\pi r^4}{8\eta} \cdot \frac{\Delta p}{l}$

Stokes' law → describes the frictional force on moving spherical objects

- $F = 6 \cdot \pi \cdot \eta \cdot r \cdot v$

17 Circulation. Work of the heart

Structure of the circulatory system. Blood pressure. The changes of the pressure, cross-section and flow speed along the systemic circulation. Factors affecting the blood flow. Blood viscosity. Vascular resistance. Structure and function of the heart. Pressure and volume changes during the cardiac cycle, pressure-volume curve of the heart. The work of the heart. Frank-Starling law

Structure of the circulatory system

- **Pulmonary circulation:** heart → lung (right ventricle → lung → left atrium)
 - O₂ uptake in the lung, low pressure
- **Systemic circulation:** heart → body (left ventricle → body → right atrium)
 - O₂ transport to the body, high pressure

Blood pressure

- The driving force of the circulation: the pressure gradient created by the heart
- Blood pressure is exerted by the flowing blood on the vessel walls and on the neighbouring fluid. The pressure wave running through the blood puts the vessel walls in motion: expansion and recoil, which is palpable (pulse) and measurable
- Blood pressure = hydrostatic pressure → $p = \rho g h$
 - Depends on: cardiac output (the power of the heart) and vascular resistance
 - Arterial BP: normal value = 120/80 mmHg
 - Venous BP: normal value = 3-6 mmHg

The change of the pressure, cross-section and flow speed along the systemic circulation

- Stenosis (narrowing) – $r \downarrow$ $A \downarrow$ $v \uparrow$ $p \downarrow$
- Aneurism (dilation) – $r \uparrow$ $A \uparrow$ $v \downarrow$ $p \uparrow$

Factors affecting the blood flow

- **Cardiac output:** measure of blood flow from the heart through the ventricles (liters/min)
- **Blood volume:** decrease in blood volume → decrease in blood flow
- **Blood viscosity:** increase in viscosity → decrease in blood flow
- **Length/diameter** of the vessels: decrease in diameter → decrease in blood flow

Blood viscosity

- **Blood is a non-Newtonian fluid** which means that its viscosity is not constant; it may be changed by the pressure exerted on it
- Factors that affect blood viscosity
 - Haematocrit (RBCs/blood ratio)
 - Plasma viscosity: proteins, immunoglobulins
 - Temperature
 - Flow velocity

Vascular resistance

- **Vascular resistance** is the resistance that must be overcome to push blood through the circulatory system
- Vasoconstriction increases vascular resistance, while vasodilation increases it

Structure and function of the heart

- Heart = muscular organ which function is to pump blood
 - 4 chambers → right/left ventricle, right/left atrium
- Cardiac muscle: striated, contains contractile proteins (actin, myosin), sarcomere as functional unit

Frank Starling's law

- An increase in preload (tension of muscle fibers) leads to an increase in stroke volume and the force of contraction, but after reaching the max. tension, stroke volume will decrease

18 The first law of thermodynamics. Heat capacity, enthalpy

The zeroth law of thermodynamics. Internal energy, the equipartition theorem, heat. Equation and graphical interpretation of work. The first law of thermodynamics, perpetual motion machines of the first kind. Enthalpy

The 0th law of thermodynamics

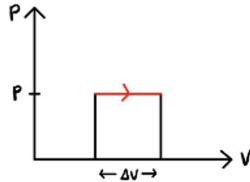
- If body A is in thermodynamic equilibrium state with body B, and body B is in equilibrium state with body C, then A and C are also in equilibrium, so there is no heat transfer between them

Internal energy, the equipartition theorem, heat

- **Internal energy** (= thermal energy) is the energy that an object has which is due to the kinetic and potential energy with the random motions of all the particles that make up the object
- **The equipartition theorem** states that energy is shared equally amongst all energetically accessible degrees of freedom of a system
- **Heat (Q)** is energy transfer to or from a thermodynamic system

Equation and the graphical interpretation of work

- $W = -P\Delta V$
where W = work, P = pressure, V = volume
- Graphical interpretation of work



The 1st law of thermodynamics

- States the conservation energy of a thermodynamic system
 - $\Delta E = Q + W$
 - Energy change = heat + work

Perpetual motion machines of the 1st kind

- A perpetual motion machine of the 1st kind is a machine that can do infinite work without energy input
- This machine is impossible because it violates the 1st law of thermodynamics – energy cannot be created

Enthalpy (H)

- Enthalpy is the total heat content of a system
- ΔH = change in enthalpy, simply energy transfer
 - Positive ΔH = endothermic reaction – system gains heat
 - Negative ΔH = exothermic reaction – system emits heat

19 The second law of thermodynamics. Entropy

Classical and statistical interpretation of entropy (micro- and macrostates, thermodynamic probability, Boltzmann-equation). Different statements of the second law of thermodynamics (involving entropy change, direction of heat transfer, perpetual motion machines of the second kind). Gibbs free energy, its change and the direction of processes

Classical and statistical interpretation of entropy

- **Entropy** = measure of molecular disorder (S)
- **Classical interpretation of entropy**
 - $\Delta S = \frac{Q_{rev}}{T}$
 - The change in entropy is the ratio of the reversibly acquired or given heat and the temperature
- **Statistical interpretation of entropy**
 - $S = k \ln W$
 - W = number of microstates in a given microstate
 - Microstate = the arrangement of each molecule in a system
 - Microstate = macroscopic properties, such as temperature, pressure, volume, etc.

Different statements of the 2nd law of thermodynamics

- **The 2nd law of thermodynamics** states that when energy changes from one form to another, the entropy increases
- **Direction of heat transfer:** heat does not “flow” from a colder body to a hotter body without a change outside of the two bodies, because it is not a spontaneous process
 - The surroundings must do work to make this heat transfer happen
- **Perpetual machines of the 2nd kind**
 - Would be able to absorb all the heat caused by friction between it and the ground and convert it to kinetic energy, and keep moving forever
 - Obeys the 1st law of thermodynamics, but such a machine is impossible because the energy conversion from heat to kinetic energy is not 100%

Gibb's free energy

- $\Delta G = \Delta H - T\Delta S$
- Positive ΔG = endergonic reaction → non-spontaneous
- Negative ΔG = exergonic reaction → spontaneous

20 Protein structure, protein folding, enzymes

Levels of protein structure: primary (peptide bond, properties of amino acids), secondary (types of structures and corresponding bonds), tertiary (bonds providing stability), quaternary. Anfinsen's experiment. Levinthal's paradox. The folding funnel theory and its thermodynamic background (change of free enthalpy). Protein misfolding and its pathological consequences with examples. Gibbs free energy change of enzyme-catalysed reactions, effect of enzymes on the rate of reactions, graphic illustration

19. PROTEIN STRUCTURE, PROTEIN FOLDING. *agree*
An! decide which type of folding will take place.

Levels of protein structure

- Primary structure:** sequence of amino acids, held together by peptide bonds
- Secondary structure:** α -helix, β -sheet, held together by H-bonds
 - H-bond in α -helix: an electrostatic interaction that involves hydrogen $3-6 \text{ \AA}$ in one twist
 - H-bond in β -sheet: two or more **parallel polypeptide chains** stabilized by **H-bonds**
- Tertiary structure:** α -helices and β -sheets are further folded into final functional 3D proteins
 - Chaperones: proteins responsible for the folding
 - Disulphide bonds, H-bonds, hydrophobic interactions
- Quaternary structure:** two or more peptide chains form complex proteins (e.g. hemoglobin)

mark the conformation

Anfinsen's experiment

- Denaturation of the proteins by **urea** *measured by the activity of the enzyme*
- Disruption of disulphide bonds by **mercaptoethanol** *in some cases, there was no enzymatic activity*
- Renaturation by the removal of mercaptoethanol $\rightarrow$ scrambled (inactive) protein, reformed randomly
- Renaturation by the removal of urea $\rightarrow$ native (active) protein, reformed like the original protein
- Conclusion:** **the native form of the protein has the thermodynamically most stable structure**

very fast

Levinthal's paradox

- "How, despite the huge number of conformations accessible to it, a **protein can fold into its one precisely defined native structure so quickly** (sometimes microseconds)?"
- Example: 25 bonds in a protein with 5 conformational states for each bond
 - 1 conformation = 1 ns
 - Total time = $5^{25} \text{ ns} = 95 \text{ years}$
- Conclusion:** **native structure cannot be made randomly, it is under control**

very fast

The folding funnel theory

- protein's native state corresponds to its free energy minimum**
- Smooth funnel** = ultrafast folding, **bumpy funnel** = contains "traps" so proteins would not reach the native state, **avoided by isomerases and chaperones** *if not, get to native state*
- System seeks lowest energy state possible** *The energy gap has higher energy at the native state*

at native state $\Delta G < 0$

Protein misfolding and its pathological consequences

- Misfolding = the folding is not successful (e.g. β -sheets instead of α -helices)
- The misfolded proteins are degraded (lysosomal or ubiquitin-proteasome pathways)
- Or the cell will not remove it
 - Low functionality** $\rightarrow$ **sickle cell anaemia** (abnormal hemoglobin form strands inside red blood cells)
 - Deposits (plaque) within the cells** $\rightarrow$ **Alzheimer's disease, Parkinson's**

Enzymes! *in case of using an enzyme, ΔG won't change just the activation energy decreases so the spontaneity of the reaction is not affected by enzymes.*

20. BIOLOGICAL MEMBRANES, MEMBRANE POTENTIAL

The structure of the cell membrane and its formation, membrane models

21 Sedimentation, electrophoresis

Forces and processes during sedimentation, sedimentation methods. Density-gradient sedimentation. Centrifuge types, preparative and analytical centrifugation. Sedimentation constant. Electrophoretic mobility. Principle of electrophoretic methods, 2D-electrophoresis, isoelectric focusing.

40. SEDIMENTATION, ELECTROPHORESIS.

Forces and processes during sedimentation, sedimentation methods

- Sedimentation** = movement of particles in centrifugal force fields, time-dependent deposition of particles in a suspension
 - Sample: particle suspension (blood, urine, bone marrow, cells, proteins, nucleic acids, etc.)
 - Instrument: laboratory centrifuge → the centrifuge uses the sedimentation principle where the centrifugal acceleration causes denser substances to move outward and less dense substances move toward the center
 - Results: gives information about **size, shape, molecular weight, density**, etc.
- Forces during sedimentation**
 - Centrifugal force, buoyant force, drag force
 - Centrifugal force points out of the centre of rotation
 - The latter two act in the opposite direction of the centrifugal force
- Sedimentation methods**
 - Analytical ultracentrifugation → determination of mass and size of macromolecules
 - Sedimentation velocity method: measures rate at which molecules move in response to centrifugal force
 - Sedimentation equilibrium method: measures weight of molecules
 - Preparative ultracentrifugation → used in pelleting small materials such as membranes, organelles, viruses, DNA/RNA
 - Differential centrifugation: on the basis of mass and size
 - Gradient centrifugation: on the basis of density

Density-gradient centrifugation

- A method where the **components of a sample are separated on the basis of their density**, in a density medium, **according to the centrifugal force they experience**
- Procedure**
 - The centrifuge tube is **filled with a high-density solution** (e.g. sucrose)
 - The **sample is mixed with the high-density solution and centrifuged at high speed**
 - During centrifugation, **a density gradient is built up in the tube and particles of the sample migrate to the layers of the solution with the same density** → *where centrifugal force = buoyant force*
 - More dense components end up further down in the tube**
 - The suspension is collected into fractions and concentration of molecules in the fractions are **determined by using UV-absorption**
 - Proteins → 280 nm, RNA/DNA → 260 nm

Centrifuge types

- Two types of rotors
 - Fixed angle rotors** → tubes are in a constant angle from the axis of rotation
 - Can accommodate more tubes, suitable for pelleting and higher speeds
 - Swing out rotors** → tubes are in vertical position at the beginning, swing out horizontally during rotation
 - Used to separate larger particles in solutions

Sedimentation constant

- A measure of the **rate at which a molecule suspended in a solution sediments in a centrifuge**, i.e. how fast a particle "settles" to the bottom of a solution
- SI-unit → Svedberg, a measure of time, **1 Svedberg = 10^{-13} seconds**
- e.g. ribosomes → 60s and 40s subunits

$$f = \frac{4\pi}{D} = \frac{RT}{ND}$$

$$S = \frac{u}{\omega^2 r} = \frac{1}{f} \left(1 - \frac{\rho}{\rho_0}\right)$$

(sedimentation coefficient)

Sedimentation equilibrium: the velocity is slower so the sedimentation comes w/ diffusion but in opposite directions. In this case we don't need the diffusion constant.

Electrophoretic mobility

e.g. ribosomes → 60s and 40s subunits

$$f = \frac{4\pi}{D} = \frac{RT}{ND}$$

$$S = \frac{u}{\omega^2 r} = \frac{1}{f} \left(1 - \frac{\rho}{\rho_0}\right)$$

(sedimentation coefficient)

Sedimentation equilibrium: the velocity is slower so the sedimentation comes w/ diffusion but in opposite directions. In this case we don't need the diffusion constant.

Electrophoretic mobility

- Physical quantity** describing the electrophoretic properties of particles
- Equals to the electrophoretic velocity unit in a force field**

Principle of electrophoresis, 2D electrophoresis, isoelectric focusing

- Electrophoresis** = movement of particles in an electric field
 - Free electrophoresis** → e.g. capillary electrophoresis
 - Gel electrophoresis**
 - Polycrylamide gel electrophoresis → **proteins**
 - Denaturing: SDS-PAGE → separates according to **size**
 - Non-denaturing: native → separates according to **size, shape and charge**
 - Isoelectric focusing → separation on basis of **isoelectric point**
 - 2D electrophoresis → combination of **SDS-PAGE and isoelectric focusing**
 - Agarose gel electrophoresis → **nucleic acids** (RNA: formaldehyde)
- Principle of electrophoresis**
 - SDS-polycrylamide gel electrophoresis
 - Most used method for protein fractionation**
 - Sample is boiled in the presence of sodium dodecyl sulphate and mercaptoethanol
 - The **mercaptoethanol disrupts the disulphide bonds** and denatures it
 - Sulphate group of the SDS molecule makes the proteins negatively charged**
 - The **negatively charged proteins migrate towards the positive electrode** during the electrophoresis according to their size
 - Smaller molecules migrate faster** and reach the bottom faster
 - Larger molecules remain in the upper region of the gel
 - Results can be viewed with **staining or autoradiography**
 - Staining: **Coomassie blue for proteins, ethidium bromide for nucleic acids**, and **fluorescent dyes for both**
 - Isoelectric focusing**
 - Separation of proteins according to their different **isoelectric points**
 - Isoelectric point = the **pH at which the molecule carries no net electrical charge**, i.e. it is neutral
 - A **pH gradient** is generated in a **polycrylamide gel** in a narrow tube, and the protein mixture is electrophoresed in the gel
 - The proteins migrate **until they reach the pH value** in the tube corresponding to their isoelectric point, **lose their charge and stop moving** → *isoelectric point is when the net charge of the protein will be zero*
 - 2D electrophoresis**
 - 1st step → isoelectric focusing, 2nd step → SDS-PAGE
 - Direction of the migration in the two gels are perpendicular to each other

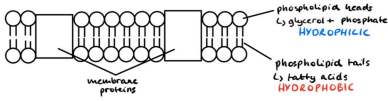
Diagram illustrating 2D electrophoresis:

22 Biological membranes, membrane potential

The structure of the cell membrane and its formation (hydrophobic-hydrophilic interaction), membrane models. Membrane dynamics: lateral and transversal movements. The concept of electric potential. Electrochemical potential, ion channels, ion pumps. Generation, maintenance and measurement of the resting membrane potential. The potassium-hypothesis of Bernstein, Nernst-equation, Donnan potential, Goldman-Hodgkin-Katz equation

20. BIOLOGICAL MEMBRANES, MEMBRANE POTENTIAL

The structure of the cell membrane and its formation, membrane models



Membrane dynamics

- One remarkable feature of all biological membranes is their ability to **change shape without losing their integrity or becoming leaky—they are flexible**.
- Membrane dynamics is the motions that occur in membranes and the structures allowed by these motions: lateral and transverse movements

The concept of electric potential

- Electric potential is the energy of a charged particle in an electric field
 - Moving charged particles = electric current

Electrochemical potential, ion channels, ion pumps

- Chemical potential** is the amount of energy that can be absorbed or released due to a **change in particle number**.
- Electrochemical potential** = the sum of the electric and chemical potential energy, it is the **force controlling the movements of the ions going through a cell membrane**
- ion channels** = **pore-forming membrane proteins that allow ions to pass through**
- ion pumps** = transmembrane proteins that move ions across a membrane against their concentration gradient, through **active transport**

Generation, maintenance and measurement of the resting membrane potential

- Membrane potential** = the electric potential difference across a cell's membrane
 - When positively charged ions (Na^+ , K^+) leave the cell, leaving behind negatively charged ion (Cl^-)
- Maintenance**: keep the ions separated by ion pumps
- Measurement**: a voltmeter can be used to measure the charge difference between the extracellular and intracellular fluids

The potassium-hypothesis of Bernstein

- The cell membrane is **selectively permeable to potassium at rest** and during excitation the membrane permeability to other ions increase
- The intracellular potassium concentration is high**
- The extracellular potassium concentration is low**

Nernst's equation: equilibrium potential = inward and outward flow is balanced

- $E = \frac{RT}{zF} \ln \frac{x_1}{x_2}$ *no active transport should be happening, only passive*
 where z = valence of ion, F = Faraday constant, x_1/x_2 = concentration gradient

Donnan potential

- Describes **the equilibrium that exists between two solutions separated by a membrane**

Handwritten notes:
 ~ -10 to -20 mV
 Goldman Hodgkin's → is the extended version of Nernst's
 concentration of 4 important ions outside and inside

21. CHANGES OF THE MEMBRANE POTENTIAL, ION CHANNELS, ACTION POTENTIAL

23 Changes of the membrane potential, ion channels, action potential

Types of ion channels, function of K⁻ and Na⁺-channels. Types of sensory receptors, modality, adequate stimulus. Conditions of the generation of the action potential. Phases of action potential and the corresponding changes of the ion currents. Refractory phases

concentration of important ions outside and inside

21. CHANGES OF THE MEMBRANE POTENTIAL, ION CHANNELS, ACTION POTENTIAL

Na⁺/K⁺ Pump / ATPase Pump

Types of ion channels, function of the K⁺ and Na⁺ channels

- Types of **potassium channels**
 - Ca²⁺ sensitive – open in presence of Ca²⁺
 - Inwardly rectifying – passes positive charge into the cell
 - Leaking channel – always open
 - Voltage gated – open/close in response to transmembrane voltage
- Types of **sodium channels**
 - Ligand gated – opens when ligand binds
 - Voltage gated – open/close in response to transmembrane voltage
- Function of **K⁺ and Na⁺ channels**
 - K⁺ channels: regulate action potential
 - Na⁺ channels: responsible for the rising phase of the action potential

Types of sensory receptors, modality, adequate stimulus

- Types of sensory receptors
 - Exteroceptors: information from outside of the body (sound, vibration, touch)
 - Interoceptors: information from inside the body
 - Proprioceptors: position of the different parts of the body (muscle, skin, joints)
- Modality = "sense", basic sensory modalities: light, sound, touch, temperature, pressure
- Adequate stimulus = the type of stimulus to which a sensory receptor is especially tuned

Conditions for the generation of action potential

- Action potential generation is the process by which a neuron rapidly depolarizes from a negative resting potential to a more positive potential and is achieved by the influx of cations (Na⁺ or K⁺) through ion channels.

Phases of the action potential

- Resting phase – equilibrium state**
 - Activation gate closed; inactivation gate open
- Depolarization – rising phase**
 - Na⁺ move into the cell, the inner surface of the cell will be positively charged
- Overshoot**
 - Na⁺ movement slows down, K⁺ channels begin to open
- Repolarization – falling phase**
 - K⁺ channels are open; K⁺ move out of the cell
- Undershoot – hyperpolarization**
 - Movement of K⁺ slows down
- Recovery**

Refractory phase = the cell is resistant to stimulus

- Absolute refractory period:** the formation of new action potential is totally blocked
- Relative refractory period:** larger depolarization than threshold is needed to get an action potential

In animals, action potential can only be generated in neurons as well as muscle cells.
diff. to receptor potential

- AP has a threshold, higher intensity gives larger A.P., AP is always uniform and has all or none.
* know the diff. b/w receptor potential and A.P.

22. VISION

24 Molecular mechanisms of biological movement: motor proteins, cytoskeletal polymers

Motor proteins, types of motor proteins. Cytoskeletal polymers, types of cytoskeletal polymers.

Structural polarity and its consequences. Structural and functional characteristics of cytoskeletal polymer based motor proteins. Power stroke, working distance, stroke velocity, cycle time, duty ratio, processivity. Cross-bridge, duty cycle of skeletal muscle myosin II (mechanical and biochemical aspects)

Motor proteins, types of motor proteins

- Microfilament associated: myosin
- Microtubule associated: dynein, kinesin
- Nuclei acid associated: DNA/RNA polymerase, ribosome
- Rotary motor proteins: F-type/V-type ATPases

Cytoskeletal polymers, types of cytoskeletal polymers

- **Cytoskeletal proteins** provide the shape of the cell and mechanical resistance to deformations
- **Microfilaments:** composed of **actins**
 - Function: cytokinesis, provide stability, cell motility
- **Microtubules:** composed of **tubulin**
 - Function: provide structure and shape, cell motility
- **Intermediate filaments:** composed of a variety of proteins (e.g. lamin)
 - Function: mechanical support

Structural polarity and its consequences

- **Structural polarity:** microfilaments, microtubules → **has directionality**
 - Head: N-terminal, motor domain, binding site for cytoskeletal filaments and ATPase
 - Neck: structural and regulatory unit
 - Tail: C-terminal, structural and regulatory unit
- **No structural polarity:** intermediate filaments → **no directionality**

Structural and functional characteristics of cytoskeletal polymer-based proteins

- **Microfilament proteins: myosin**
 - Function: convert chemical energy in the form of ATP to mechanical energy, thus generating force and movement
- **Microtubule proteins: kinesin + dynein**
 - Function: convert chemical energy in the form of ATP to mechanical energy, move cargo (kinesin), beating of flagella (dynein)

Power stroke = when motile forces are generated by a series of structural changes in the actomyosin cross-bridge driven by hydrolysis of ATP

- $ATP \rightarrow ADP + P$
- **Cross-bridge**
 - Myosin binds to actin, releases $ADP + P$
 - Rigor state: myosin remains attached to actin until new ATP binds to the myosin head
 - Myosin + ATP is released from actin

25 Molecular mechanisms of muscle functioning

Structural properties and levels of organization of striated muscles. Sarcomere, filament systems and proteins. What does the force generated by sarcomeres depend on? Sliding filament model. Steric blocking model and regulatory proteins (tropomyosin, troponin system)

Structural properties and levels of organization of striated muscle

- **Structural properties**
 - Attached to bones or skin
 - Contraction: voluntary
 - Striated pattern
- **Levels of organization**
 - Skeletal muscle
 - Muscle fascicle
 - Muscle fiber
 - Myofibril
 - Sarcomere

Sarcomere

- Smallest contractile unit of muscle
- **Thin filament:** actin + regulatory proteins (tropomyosin, troponin, tropomodulin)
- **Thick filament:** myosin II = the motor, energy source: ATP

What does the force generated by sarcomere depend on?

- Storage of glycogen and creatine phosphate → ATP!
- How is ATP used?
 - Myosin binds to actin, releases $ADP + P$
 - Rigor state: myosin remains attached to actin until a new ATP binds to the myosin head
 - Myosin + ATP is released from actin

Steric blocking model

- Muscle function is inhibited when the myosin cannot bind to actin
- Troponin binds to actin
 - Troponin T (tropomyosin binding)
 - Troponin I (inhibitory)
 - Troponin C (Ca^{2+} binding)

26 Mechanical properties of muscles

Stimulus-contraction response of striated muscles: twitch, wave summation, tetanus. Length-dependence of the force developed by the sarcomere (its medical relevance in the functioning of heart muscles). Relation between the force/power and the velocity of contraction. Torque. Equilibrium of rigid bodies. Simple machines, examples. Lever as a simple machine, conditions of equilibrium. Characteristics of mechanically advantageous and disadvantageous levers. Simple machines in the human body, examples of type 1, 2, 3 levers

Stimulus-contraction response of striated muscles

- **Twitch** = a single contraction-relaxation event
- **Wave summation** = two stimuli produce a greater response
- **Tetanus** = incomplete: partial relaxation, complete: continuous contraction with no relaxation (spasm)

Length dependence of the force developed by the sarcomere

- Increase in muscle fiber tension (preload) leads to increase in stroke volume and the contraction force
- Heart muscle relevance: preload = muscle fibers are stretching = ventricle is filled with blood

Torque

- A measure of the force that can cause an object to rotate around an axis
- Muscle torque: the force applied by the muscles through a moment arm of a given length, at a given angle in a joint
- $\tau = rF\sin\alpha$
where r = distance from joint to where the force is applied, F = magnitude of the force, and $\sin\alpha$ = angle

Equilibrium of rigid bodies

- 1st condition: $\vec{F}_{net} = 0$
- 2nd condition: $\vec{\tau}_{net} = 0$

Simple machines, examples

- Simple machines use a single applied force to do work
- **First order**: hammer, claw hammer
- **Second order**: wheel barrow, nut cracker
- **Third order**: human arm, sugar tong

Lever as a simple machine

- $F_{input} \cdot d_{input} = F_{output} \cdot d_{output}$

Characteristics of mechanically advantageous and disadvantageous levers

- **Disadvantageous**: $d_{output} > d_{input} \rightarrow F_{input} > F_{output}$ ☹️
- **Advantageous**: $d_{output} < d_{input} \rightarrow F_{input} < F_{output}$ 😊

Simple machines in the human body, examples of type 1, 2 and 3 levers

- **Type 1 lever**: neck muscle – advantageous
- **Type 2 lever**: leg muscle – advantageous
- **Type 3 lever**: biceps – disadvantageous

27 Mechanical properties of tissues

Spring constant, Hooke's law. Physical model of the perfect elastic body. Mechanical stress, mechanical strain, elastic modulus, elastic energy. Types of mechanical deformations. Stress-strain characteristics of ideal and real elastic bodies. Elastic and plastic region. Ultimate mechanical stress and strain. Properties of viscoelastic materials: creep, stress relaxation, hysteresis. Biomechanical properties of bones: the dependence of its stress-strain characteristics on the type and the rate of deformation. Biomechanical properties of blood vessels: compliance, distensibility

Spring constant, Hooke's law

- **Spring constant** $= k = \frac{F}{\Delta l}$
- **Hooke's law**: the force (F) needed to stretch/compress a spring is linearly proportional to the change in its length (Δl) $= F = k\Delta l$

Physical model of the perfect elastic body = spring

- σ (stress) $= \epsilon$ (strain)
- Stores energy during deformation
- Deformation does not depend on the rate of load being applied
- Upon unloading, the original shape is restored

Mechanical stress, mechanical strain, elastic modulus, elastic energy

- **Mechanical stress**: $\sigma = \frac{\text{force}}{\text{area}}$
- **Mechanical strain**: $\epsilon = \frac{\text{change in length}}{\text{original length}} = \frac{\Delta l}{l_0}$
- **Elastic modulus**: $\frac{\text{stress}}{\text{strain}}$
 - Rigid material: elastic modulus $\uparrow$, stress $\uparrow$, strain $\downarrow$
 - Elastic material: elastic modulus $\downarrow$, stress $\downarrow$, strain $\uparrow$
- **Elastic energy** = the potential energy stored in our bones/joints

Types of mechanical deformations

- Compression, tension = stretch, shear = cut, torsion = twist, bending

Stress/strain characteristics of ideal and real elastic bodies

- **Real elastic bodies**
 - Elastic region: reversible deformation
 - Linear region: reversible deformation $\rightarrow \sigma = E * \epsilon$
 - Plastic region: irreversible damage
- **Ideal elastic bodies** $\rightarrow$ only theoretical
 - No plastic region = no rupture
 - σ (stress) is directly proportional to ϵ (strain) $\rightarrow$ always!

Elastic and plastic region

- **Elastic region**: the region which the material returns to its original state when applied forces are removed
- **Plastic region**: the region in which the material deforms permanently, rupture happens at the ultimate mechanical stress and strain

Properties of viscoelastic materials

- **Creep**: when stress is applied the strain in the medium increases with time
- **Stress relaxation**: when strain is applied the stress in the medium decreases with time
- **Hysteresis**: length of the elastic material will increase if weight is loaded onto it