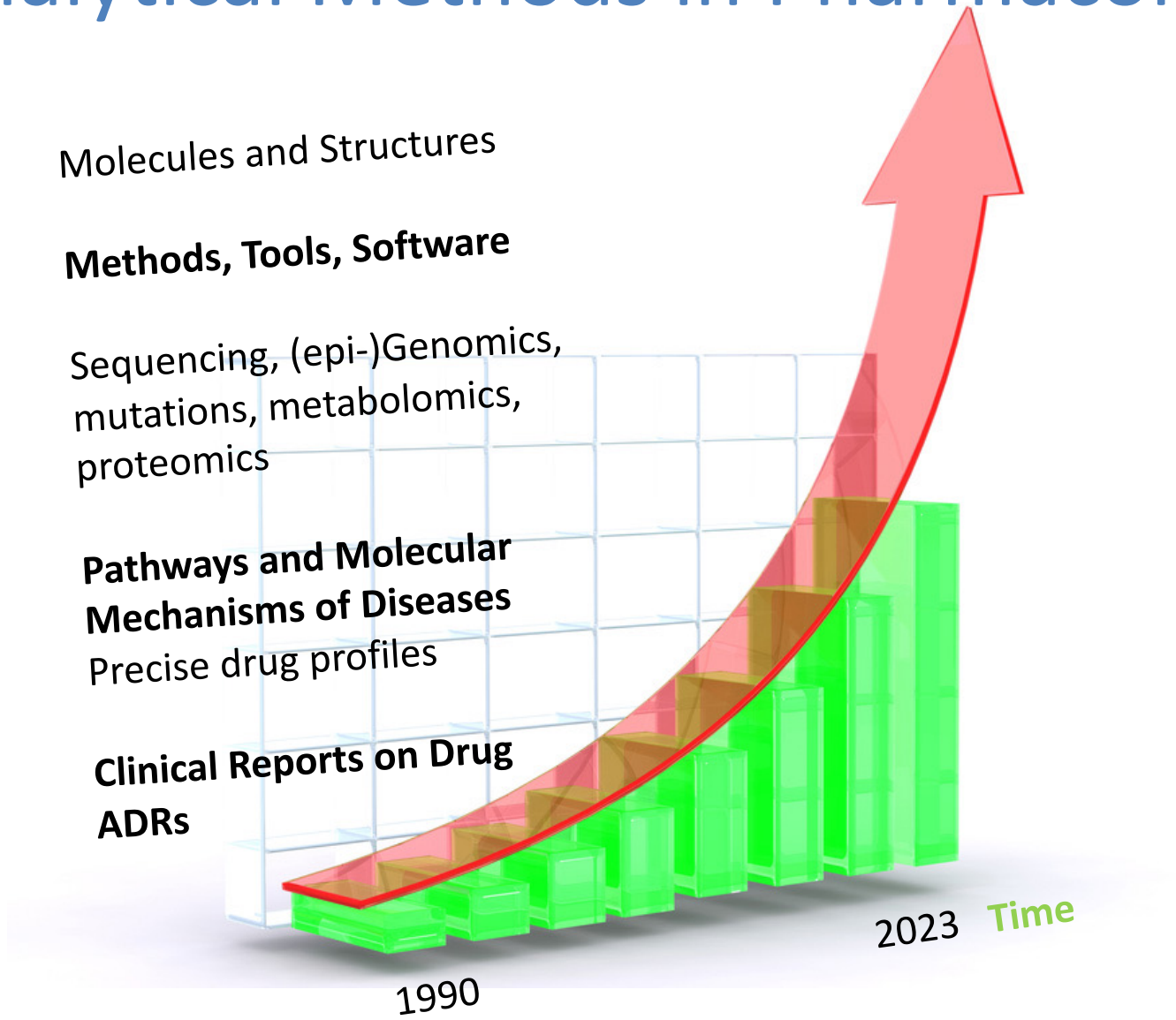
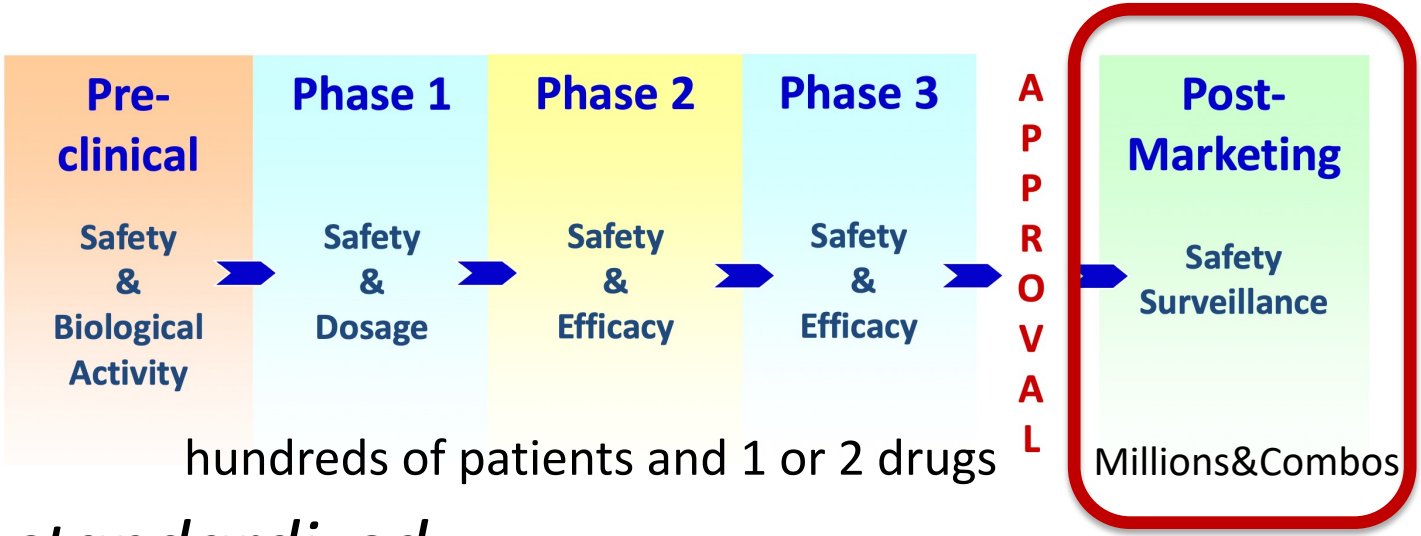
Data and Physical Foundation of Analytical Methods in Pharmacology



Drugs have a diverse range of side effects

FDA Adverse Event Reporting System

- **Post-marking safety surveillance**: 19 M reports



- AE *terms standardized*
- Drugs from the same target or same class (e.g. NSAIDs) have different adverse effects, and sometimes beneficial effects!

Some severe Adverse Effects of Drugs

- Death
- Fatigue
- Nausea
- Diarrhoea
- Headache
- Dyspnoea
- Pain
- Dizziness
- Vomiting
- Rash, pruritus
- Pyrexia
- Acute kidney injury, renal failure
- Constipation
- Anxiety, depression

FDA categories
- Death
- Life-threatening
- Hospitalization (initial or prolonged)
- Disability - significant, persistent, or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities or quality of life.
- Congenital abnormality
- Requires intervention to prevent permanent impairment or damage

The **FDA Adverse Event Reporting System** (**FAERS** or AERS) database of 18.75 million reports designed to support the post marketing safety surveillance program for all approved drug and therapeutic biologic products.

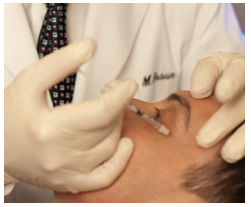
- … (total number of ADRs in FAERS: N=17,870, frequent: n=11,500)

FAERS/ADRs research projects at SSPS

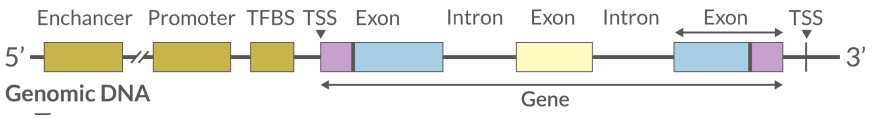
- Makunts T, Burkhart K, Abagyan R, Lee P. **Retrospective analysis of clinical trial safety data for pembrolizumab reveals the effect of co-occurring infections on immune-related adverse events.** *PLoS One*. 2022 Feb
- Wollmer MA, Makunts T, Krüger THC, Abagyan R. **Postmarketing safety surveillance data reveals protective effects of botulinum toxin injections against incident anxiety.** *Sci Rep*. 2021 Dec 21;11(1):24173. doi: 10.1038/s41598-021-03713-x. PMID: 3493409
- Keshishi D, Makunts T, Abagyan R. **Common osteoporosis drug associated with increased rates of depression and anxiety.** *Sci Rep*. 2021 Dec 14;11(1):23956. doi: 10.1038/s41598-021-03214-x. PMID: 34907232; PMCID: PMC8671447
- Nguyen VN, Abagyan R, Tsunoda SM **mTOR Inhibitors Associated with Higher Cardiovascular Adverse Events - A Large Population Database Analysis.** *Clin Transplant*, 2021 Jan 21
- Cohen IV, Makunts T, Moumedjian T, Issa MA, Abagyan R. **Cardiac adverse events associated with chloroquine and hydroxychloroquine exposure in 20 years of drug safety surveillance reports.** *Sci Rep*. 2020 Nov 5;10(1)
- Makunts T, Wollmer MA, Abagyan R, **Postmarketing safety surveillance data reveals antidepressant effects of botulinum toxin across various indications and injection sites.** *Sci Rep*. 2020 Jul 30;10(1):12851
- Makunts T, Alpatty S, Lee KC, Atayee RS, Abagyan R
  Proton-pump inhibitor use is associated with a broad spectrum of neurological adverse events including impaired hearing, vision, and memory.
  Sci Rep, 2019 Nov 21, 9, 17280
- Makunts T, Cohen IV, Awdishu L, Abagyan R
  Analysis of postmarketing safety data for proton-pump inhibitors reveals increased propensity for renal injury, electrolyte abnormalities, and nephrolithiasis.
  Sci Rep, 2019 Feb 19, 9, 2282
- Makunts T, U A, Atayee RS, Abagyan R
  Retrospective analysis reveals significant association of hypoglycemia with tramadol and methadone in contrast to other opioids.
  Sci Rep, 2019 Aug 28, 9, 12490
- Makunts T, Cohen IV, Lee KC, Abagyan R
  Population scale retrospective analysis reveals distinctive antidepressant and anxiolytic effects of diclofenac, ketoprofen and naproxen in patients with pain.
  PLoS One, 2018, 13, e0195521
- Cohen IV, Makunts T, Atayee R, Abagyan R
  Population scale data reveals the antidepressant effects of ketamine and other therapeutics approved for non-psychiatric indications.
  Sci Rep, 2017 May 3, 7, 1450

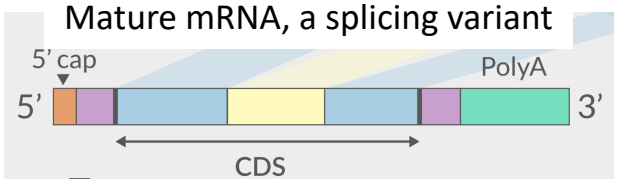
- Your publication here

Analyzing 18.75 million ADR reports for 10,000 drugs and 17,800 adverse effects submitted to the FDA by pharmacists and physicians

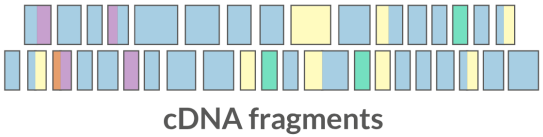


(sc)RNA-**sequencing**, gene Expression, Mutations, Disease, Drug Treatment





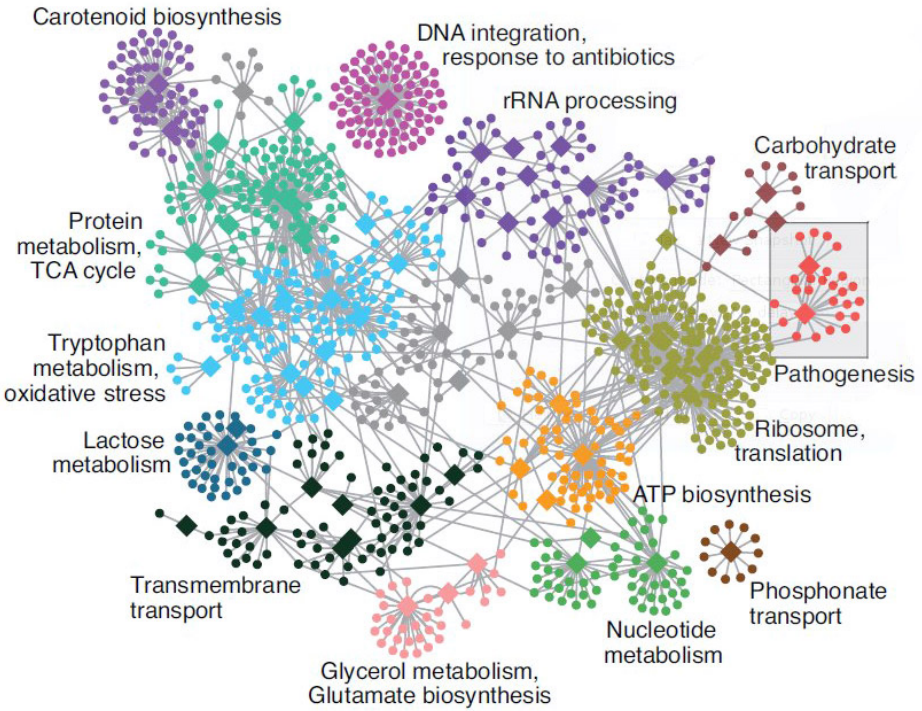
cDNA ds-cDNA is more stable



Sequencer (e.g., Illumina) generates "reads"
The reads are aligned to genomic DNA

Reveal: expression; mutations; alternative splicing, variants

- Patient's tissue samples can be sequenced to find levels of expression, mutations, splicing variants of > 20,000 genes.
- Subtypes of disease, or individual metabolic differences, may need different treatments
- Genes can be mapped on functional gene networks (Gene Ontology, a.k.a. GO)

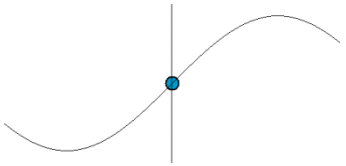


Crystals of Drugs and their Targets

X-ray crystallography and high-res-EM is used to determine three-dimensional structure of drugs, their targets of drug-target complexes

Waves

An X-ray picture (radiograph), taken by Wilhelm Röntgen in 1896, of Albert von Kölliker's hand.



Wilhelm Röntgen

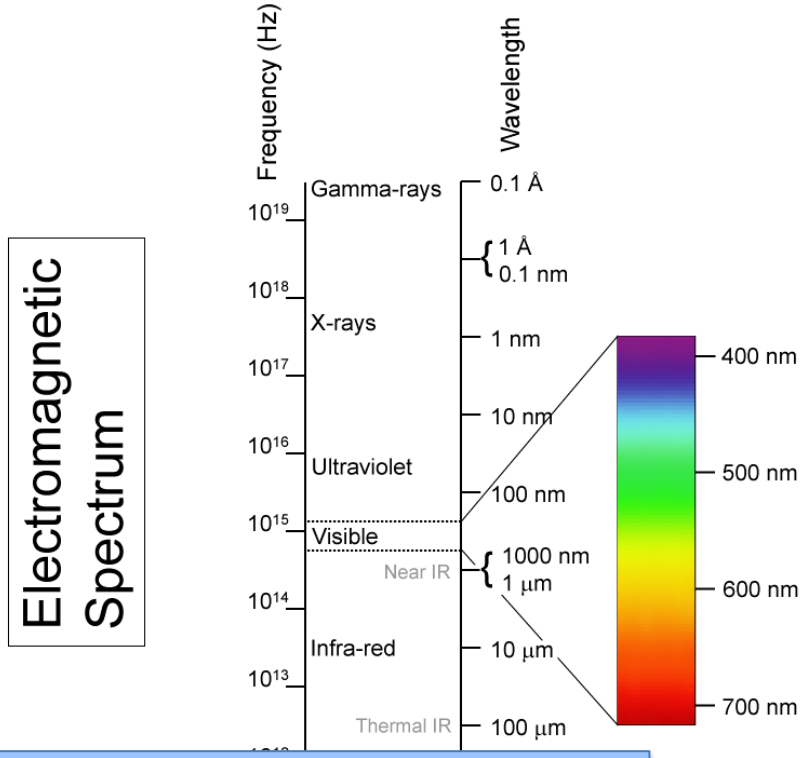
$$\lambda = c / f$$

$$\omega = 2\pi f = 2\pi / T$$

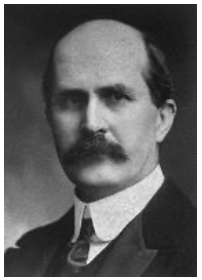
$$E = hf = \hbar\omega$$

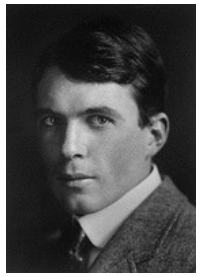
$$E_{mole} = N_A \hbar\omega$$

$$E_{X-ray} \sim 6 \bullet 10^{23} 10^{-34} 10^{18} \sim 6 \bullet 10^{7}\, J / mol!$$



Diffraction: Bragg's Law



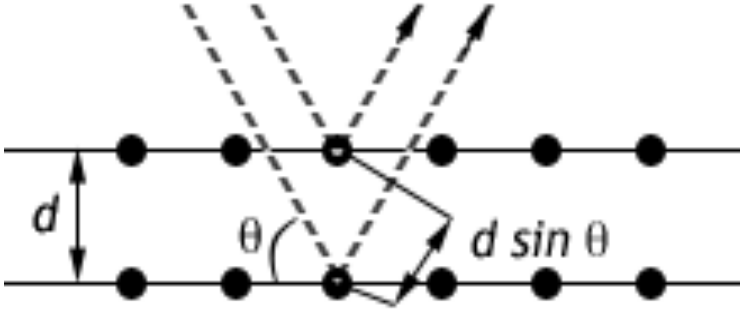


In 1915, William Henry Bragg and William Lawrence Bragg were awarded the Nobel Prize for their contributions to crystal structure analysis. They were the first and (so far) the only father-son team to have jointly won the prize.

- The path difference is $2d\sin\theta$
- The diffraction condition for a crystal

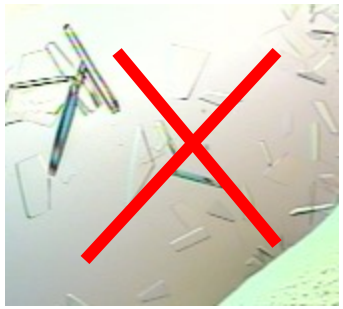
$$2d \cdot \sin\theta = n\lambda$$

  - λ is the wavelength of gamma-rays (~ 1.5A)
  - *d* is the distance between planes,
  - θ is the angle of diffraction
  - and *n* is an integer known as the *order* of the diffracted beam

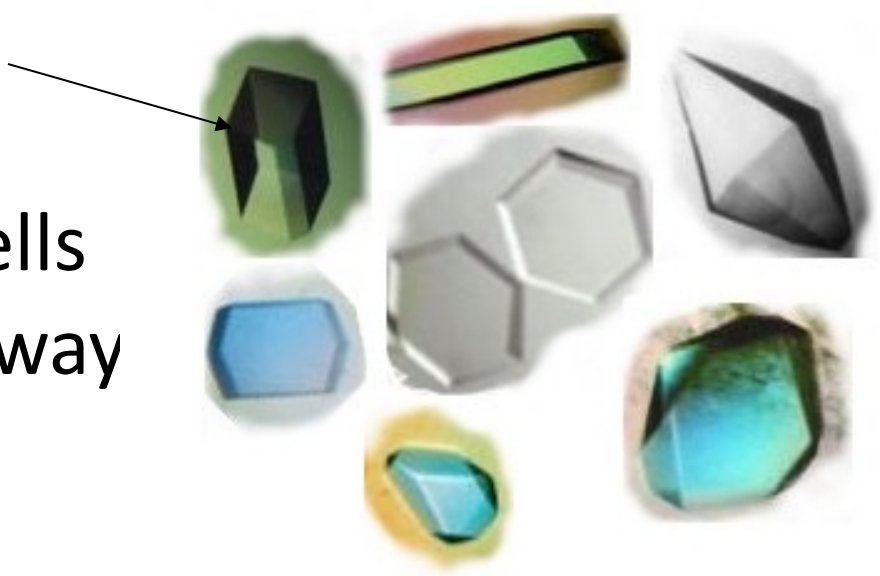


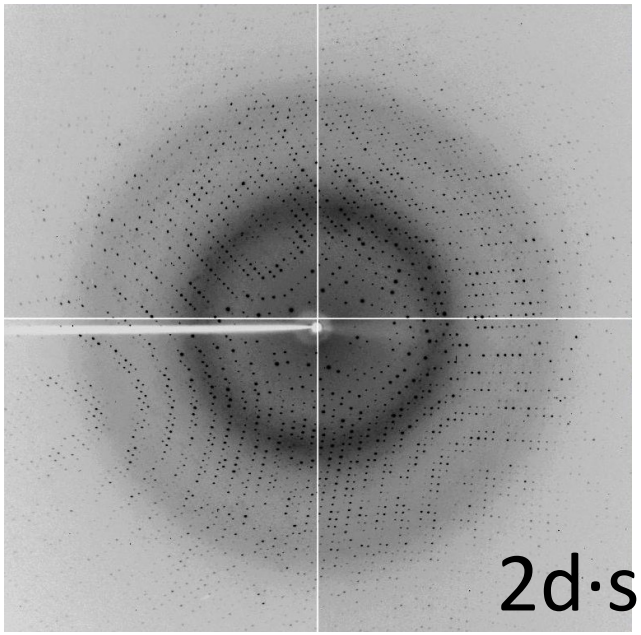
- Coherence will only be achieved for certain values of θ for each set of planes

Single Crystal

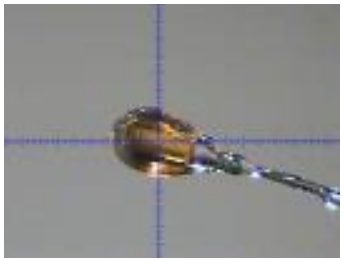


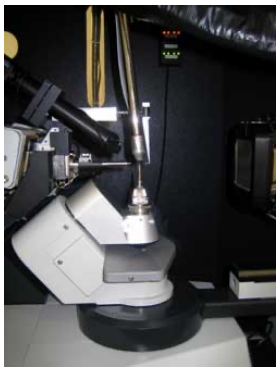
- Only one crystal is mounted for X-ray crystallography
- In this crystal all unit cells are oriented the same way





$$2d \cdot \sin\theta = n\lambda$$



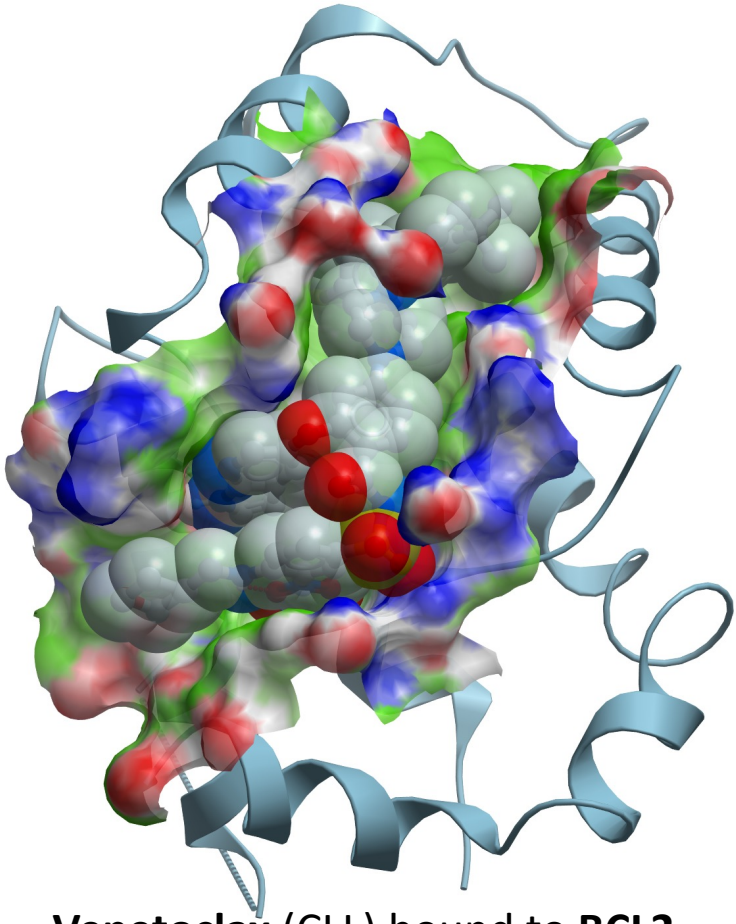


Drug Targets in Protein Data Bank (PDB)

- Most of drug targets have been crystallized and 3D atomic structure determined by X-ray crystallography, EM (hi-res), NMR and other methods

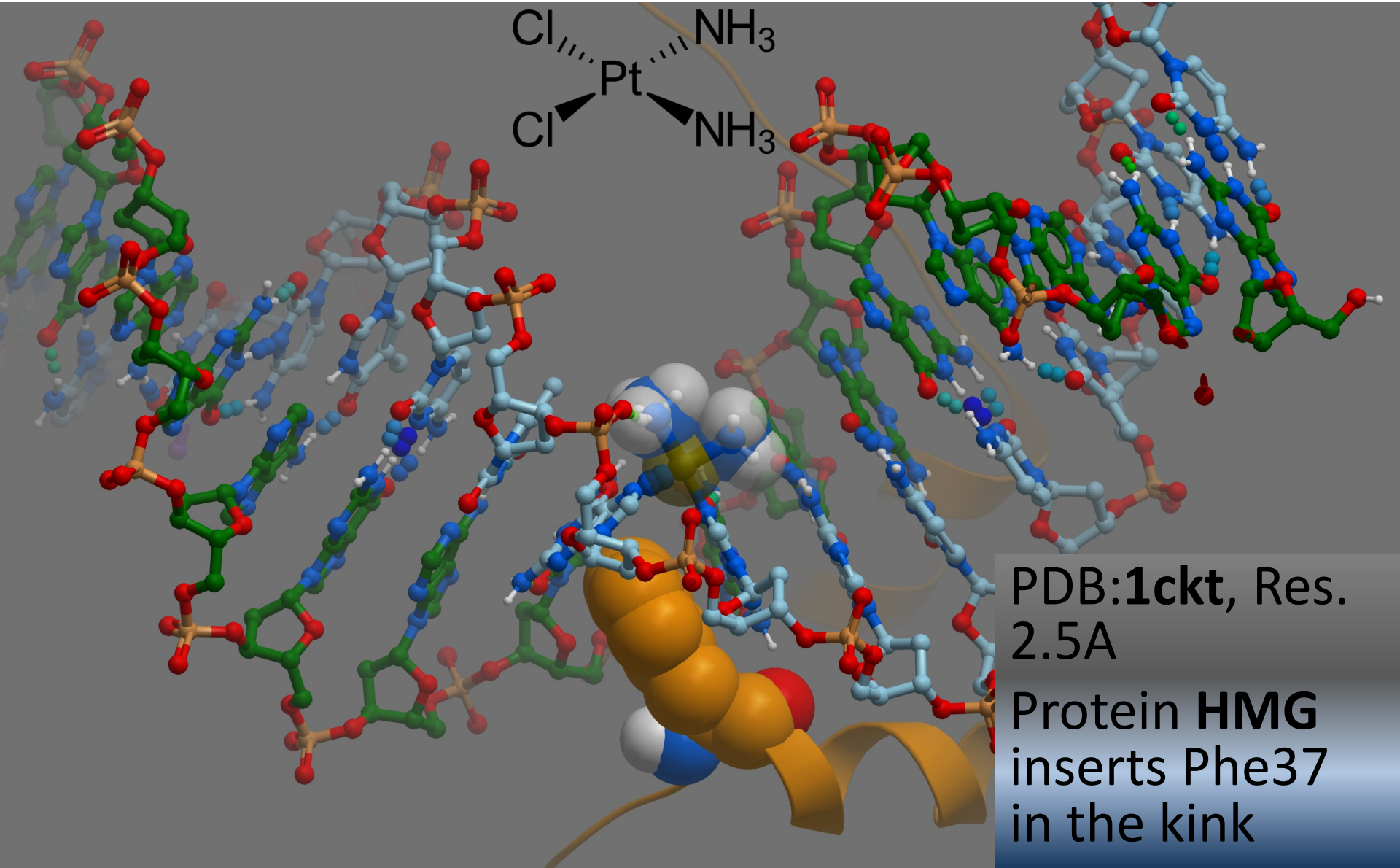
- Many drugs can be found in the PDB bound to their targets in 3D

- PDB contains >200,000 experimental structures and > 1M low quality models predicted by Google AlphaFold(2).



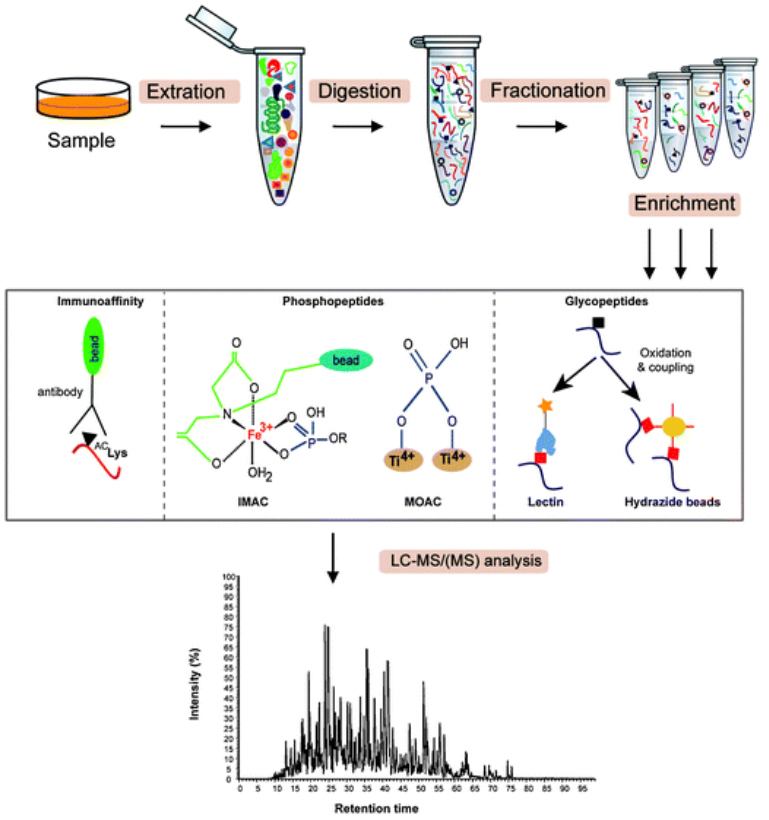
Venetoclax (CLL) bound to **BCL2**

Cisplatin bends DNA by make bonds with two Guanines

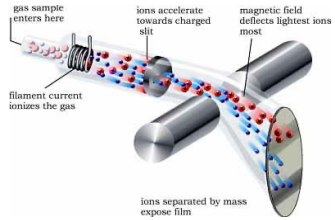


Analytical Methods in Pharmacology

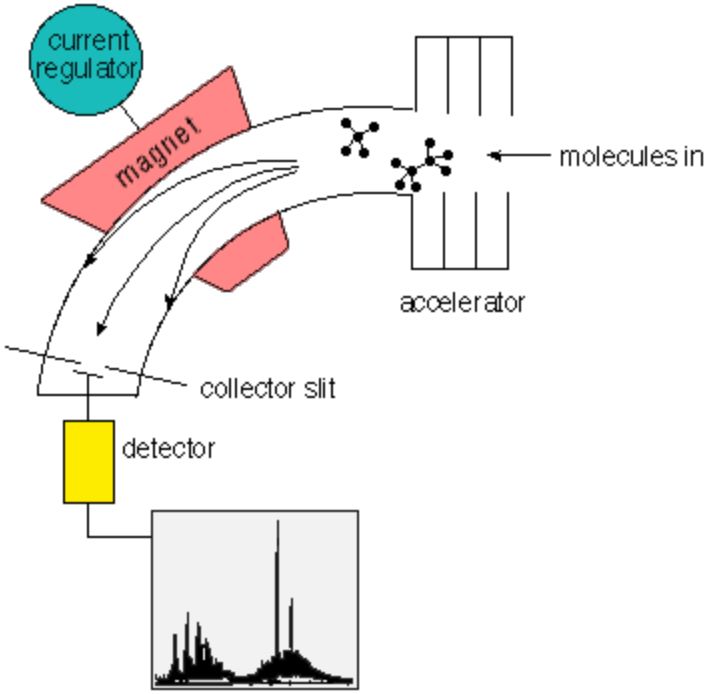
- Crystallography
- Gene sequencing
- Spectroscopy, -metry
- Mass Spectrometry
- Chromatography (e.g. LC)
- Nuclear Magnetic Resonance
- Binding assays
- PCR, .. *more* …



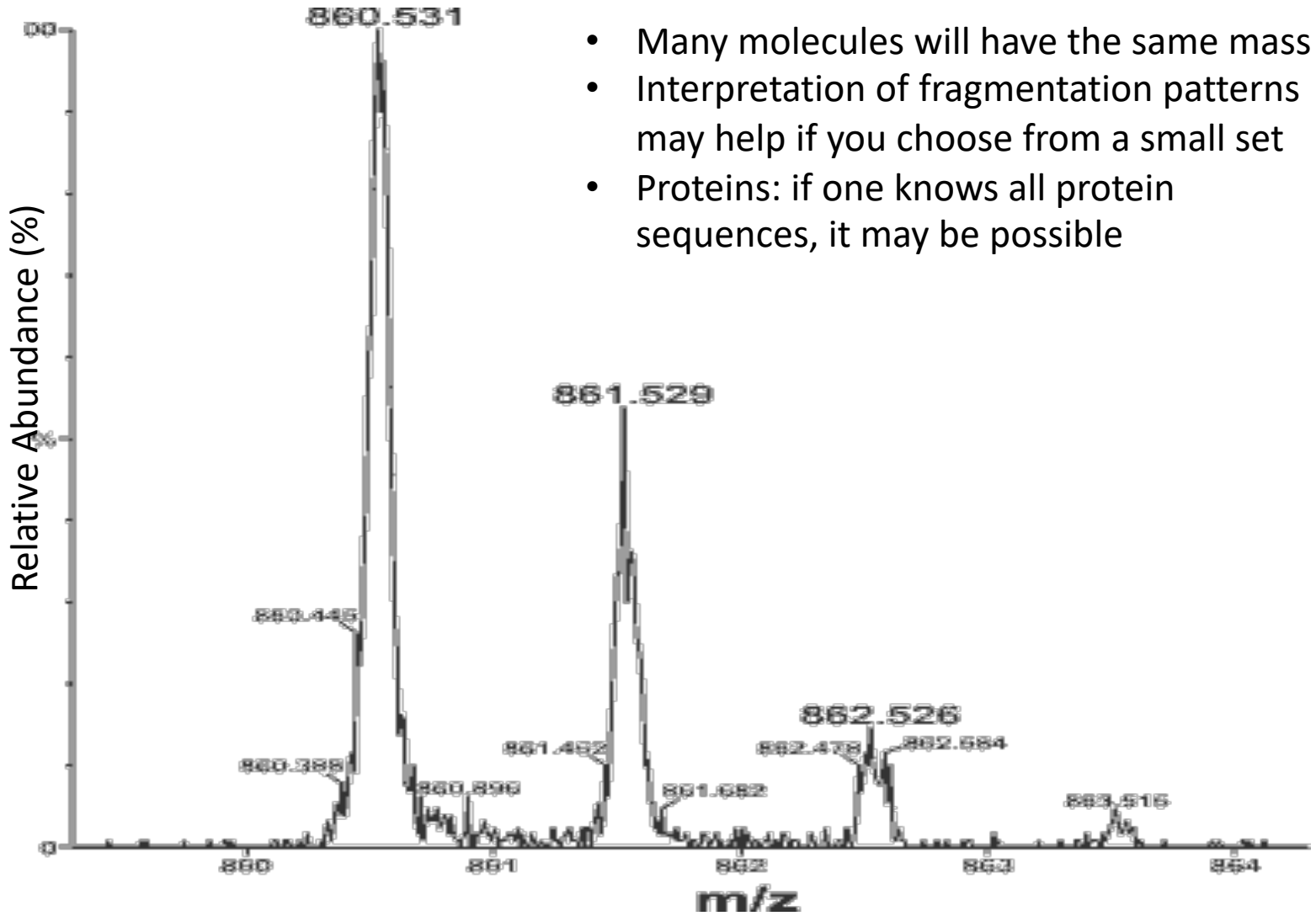
Mass Spectrometry



- Analytical tool measuring molecular weight of molecules in chemistry, biology and pharmacology
- Only picomolar concentrations required
- Within 5 ppm for small organic molecules
- For a 40 kDa protein, there is a 4Da error
- MS can detect amino acid substitutions, post-translational modifications



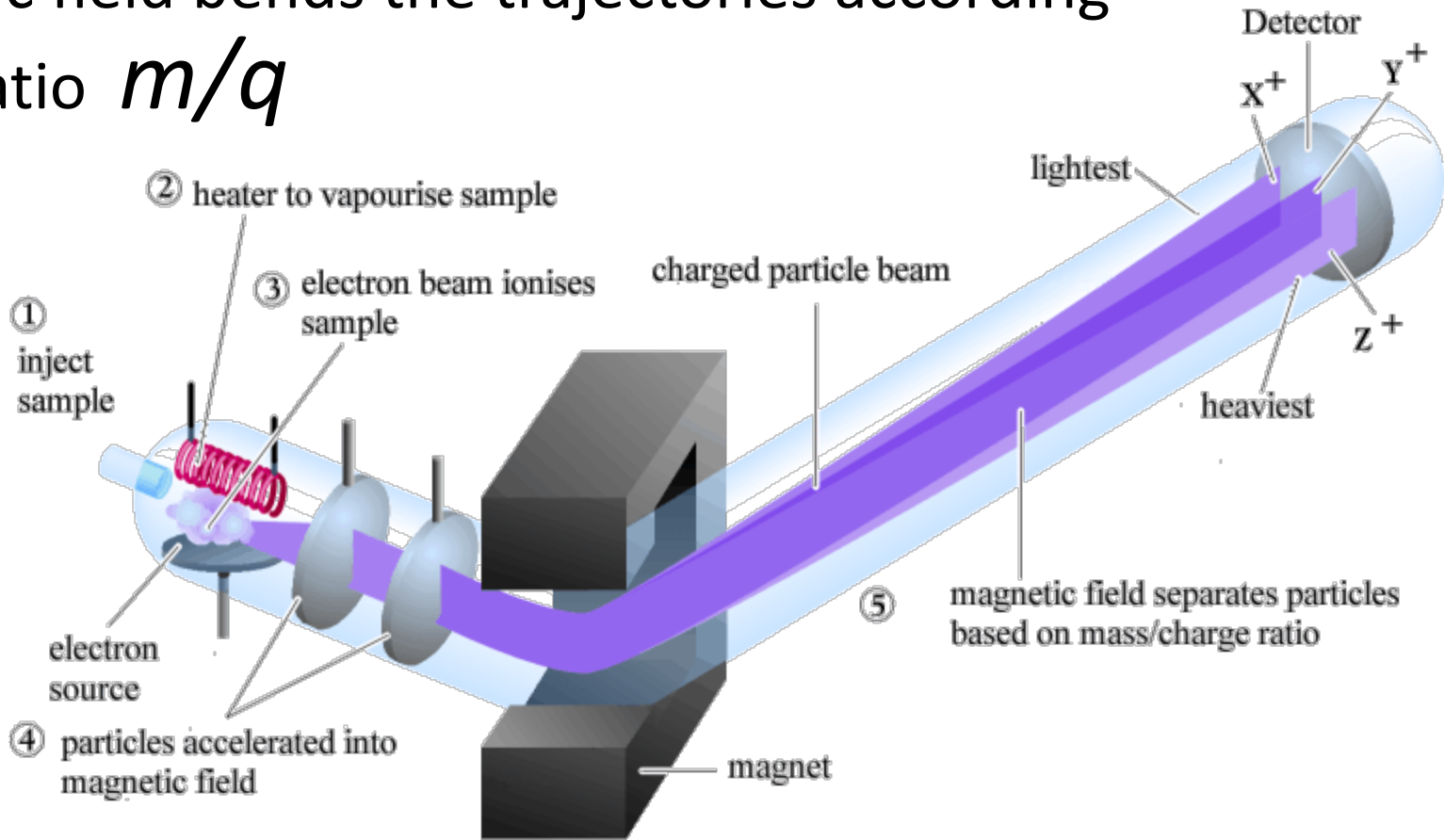
Mass (divided by charge) Spectrum



- Many molecules will have the same mass
- Interpretation of fragmentation patterns may help if you choose from a small set
- Proteins: if one knows all protein sequences, it may be possible

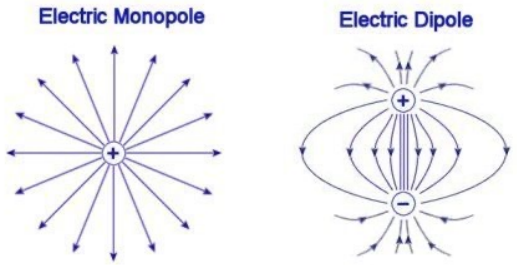
Physics behind MS

- An ion moves in a magnetic field
- A neutral molecule CAN NOT BE measured with MS
- Magnetic field bends the trajectories according to the ratio m/q

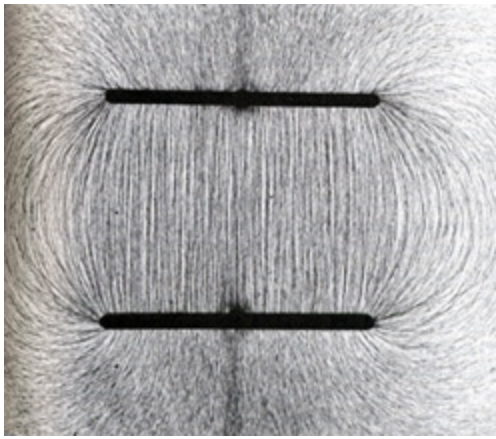


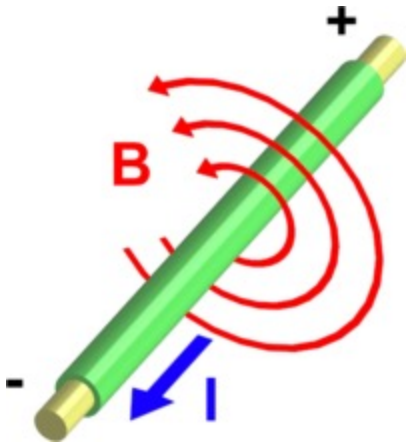
Electric (E) and Magnetic (B) Fields

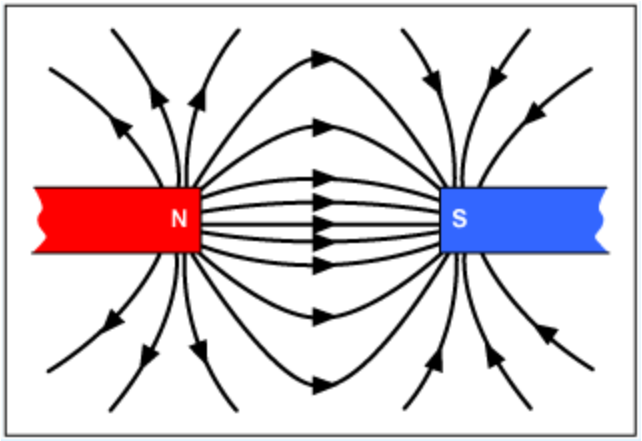
- Field = Force on a prob charge q=1
- Current (*I*) flowing through a wire produces a magnetic field , B [tesla]

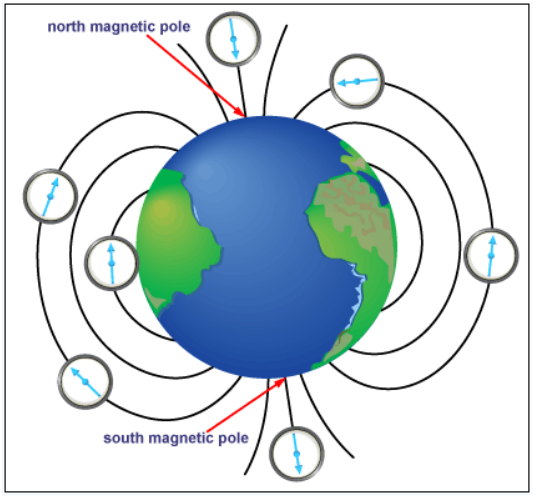


$$E_{Coulomb} = C\frac{q_{source}}{r^2}$$



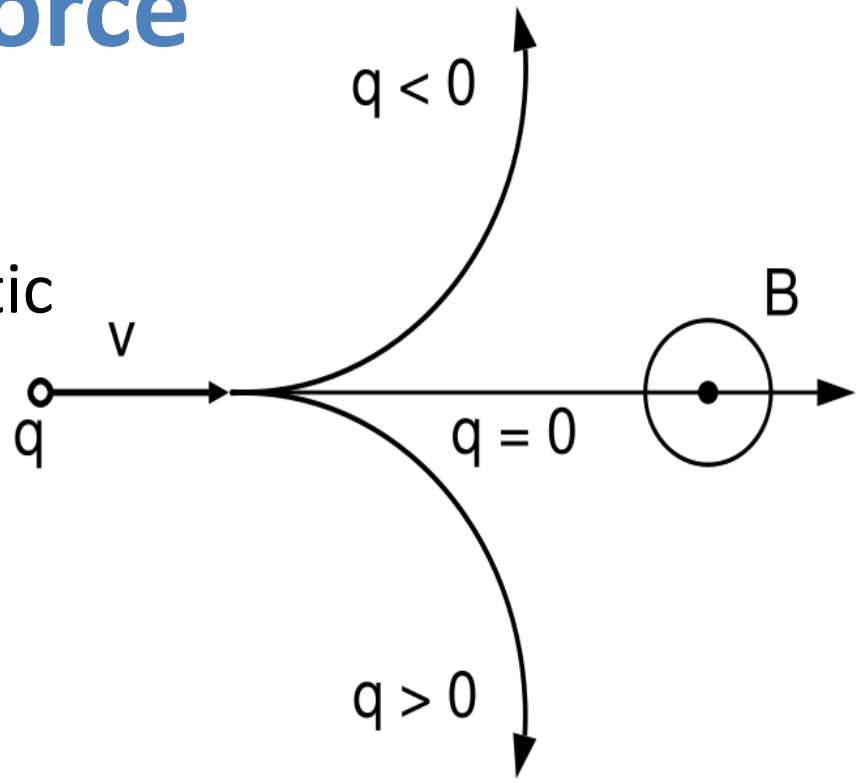


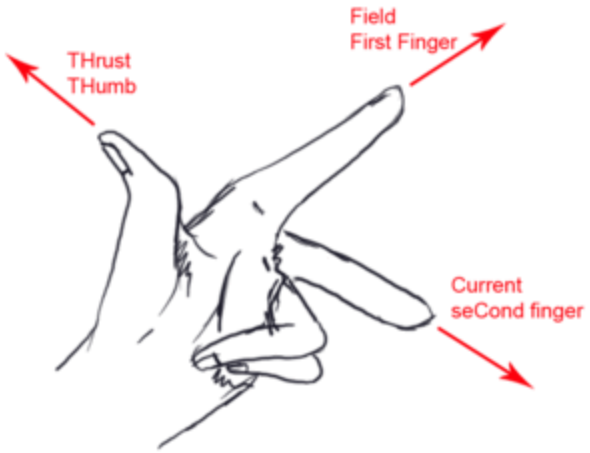




Lorenz Force

- When charged molecules move in electric and magnetic fields
- In electric fields they accelerate. $\mathbf{F}_{el} = q\mathbf{E}$
- In magnetic fields their trajectories bend $\mathbf{F}_{mg} = q\boldsymbol{v}\mathbf{B}$





$$\mathbf{F} = q(\mathbf{E} + \mathbf{v} \times \mathbf{B})$$

$$(m/q)\mathbf{a} = \mathbf{E} + \mathbf{v} \times \mathbf{B}$$

× is the vector cross product

Radius of ion trajectory

- The magnetic force is perpendicular to **V** – it causes circular motion with acceleration v^2/R by providing the centripetal force, R – is the radius
- If magnetic field **B** is perpendicular to V, then radius R can be calculated

$$m\mathbf{a} = q\mathbf{v} \times \mathbf{B}$$

$$\frac{mv^2}{R} = qvB$$

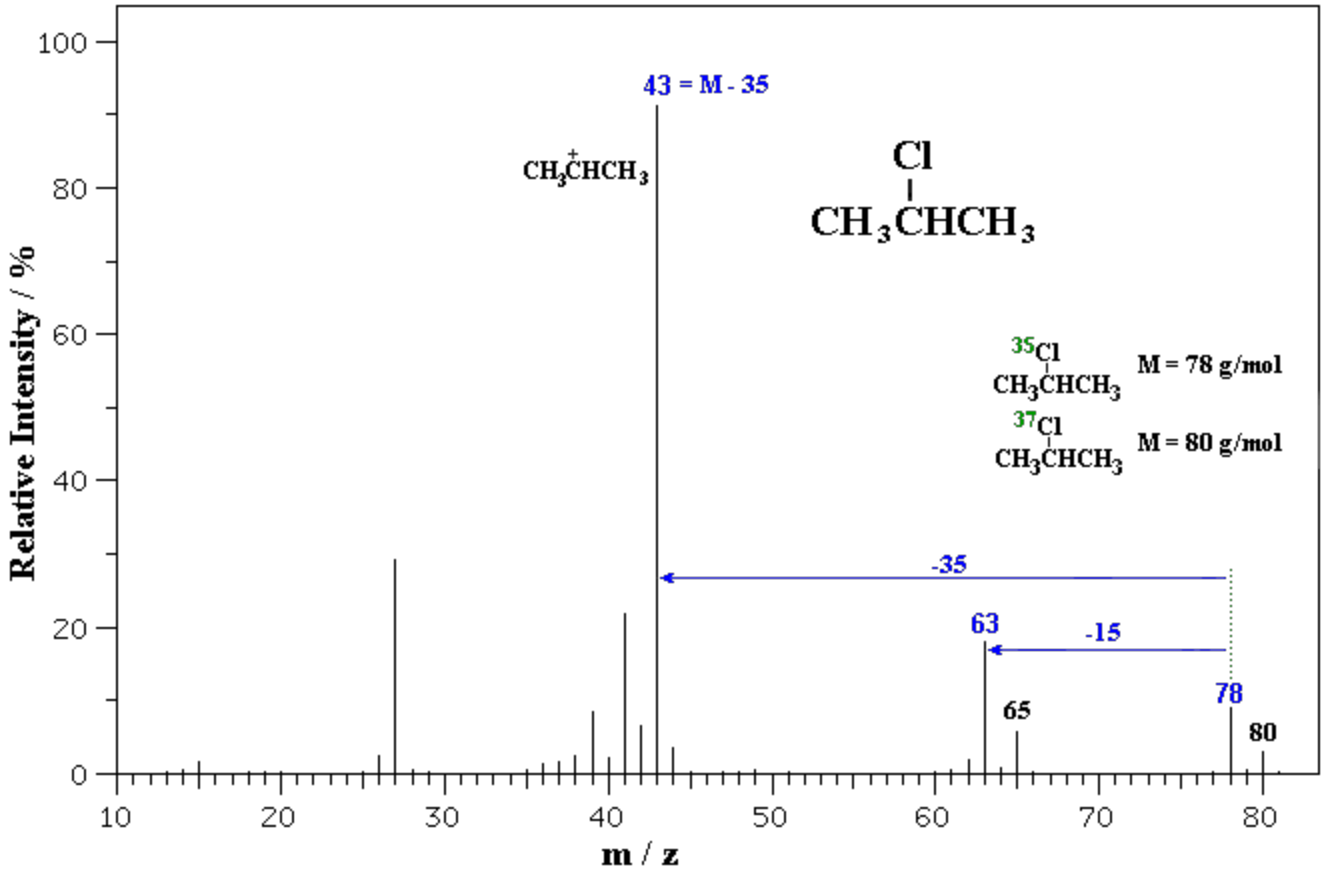
$$R = \frac{m}{q}\frac{v}{B}$$

Mass-to-charge ratio

- *m/q*, or *m/e, or m/z*, z is the electronic charge of a molecular ion or fragment. If z=+1,m = m/z
- Mass: isotopes are detected. E.g. ^{12}C, ^{13}C, ^{79}Br : ^{81}Br, intensity 1:1 and ^{35}Cl : ^{37}Cl, intensity 3:1
- Charge: in an **electron impact** mass spectrometer, a high energy beam of electrons displaces an electron from the organic molecule to form a **radical cation** known as the **molecular ion.**
- If the molecular ion is too unstable then it can **fragment** to give other smaller ions.
- Useful links: https://www.instructables.com/id/How-to-Read-a-Simple-Mass-Spectrum/

Spectrum of 2-chloropropane

- Loss of Cl gives the main peak.



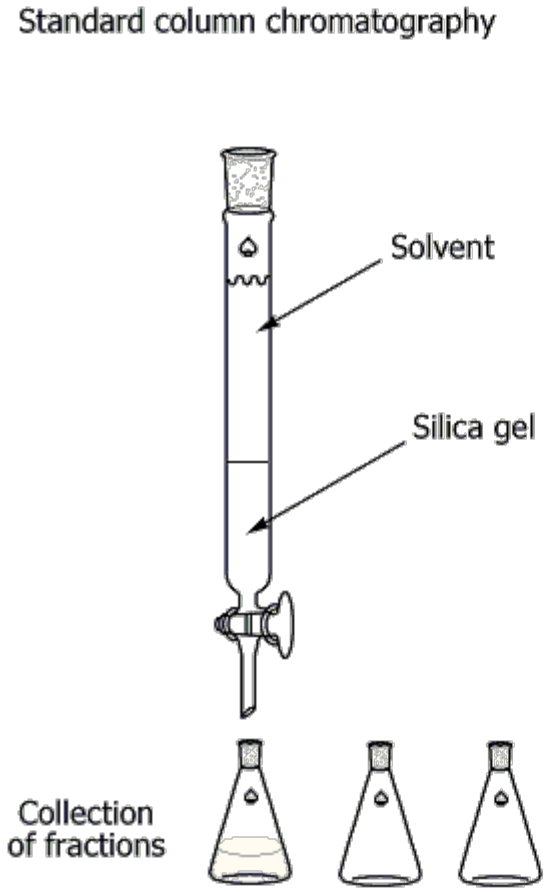
Separation of mixtures

- Chromatography, (greek, color- writing)
- Chromatography exploits partition between a mobile phase and a stationary phase to separate the components in a mixture.
- Molecules interact with the stationary phase based on charge, relative solubility or adsorption
- Gas, thin layer, paper,
- Column (liquid)
  - ion-exchange (charge)
  - size-exclusion (size)
  - Affinity
  - FPLC (proteins) and HPLC (pressure)

Mikhail Semenovich Tsvet, 1872 – 1919, a Russian botanist. He used liquid - adsorption column chromatography

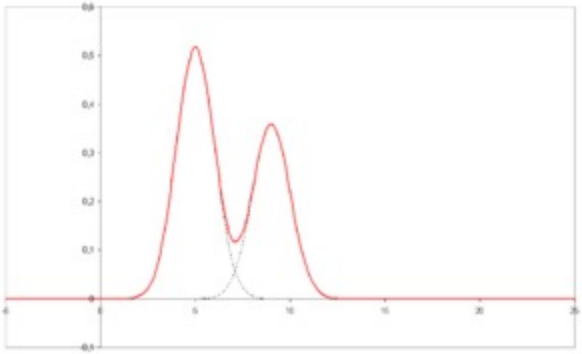
Thin layer chromatography is used to separate components of chlorophyll

Chromatography terms



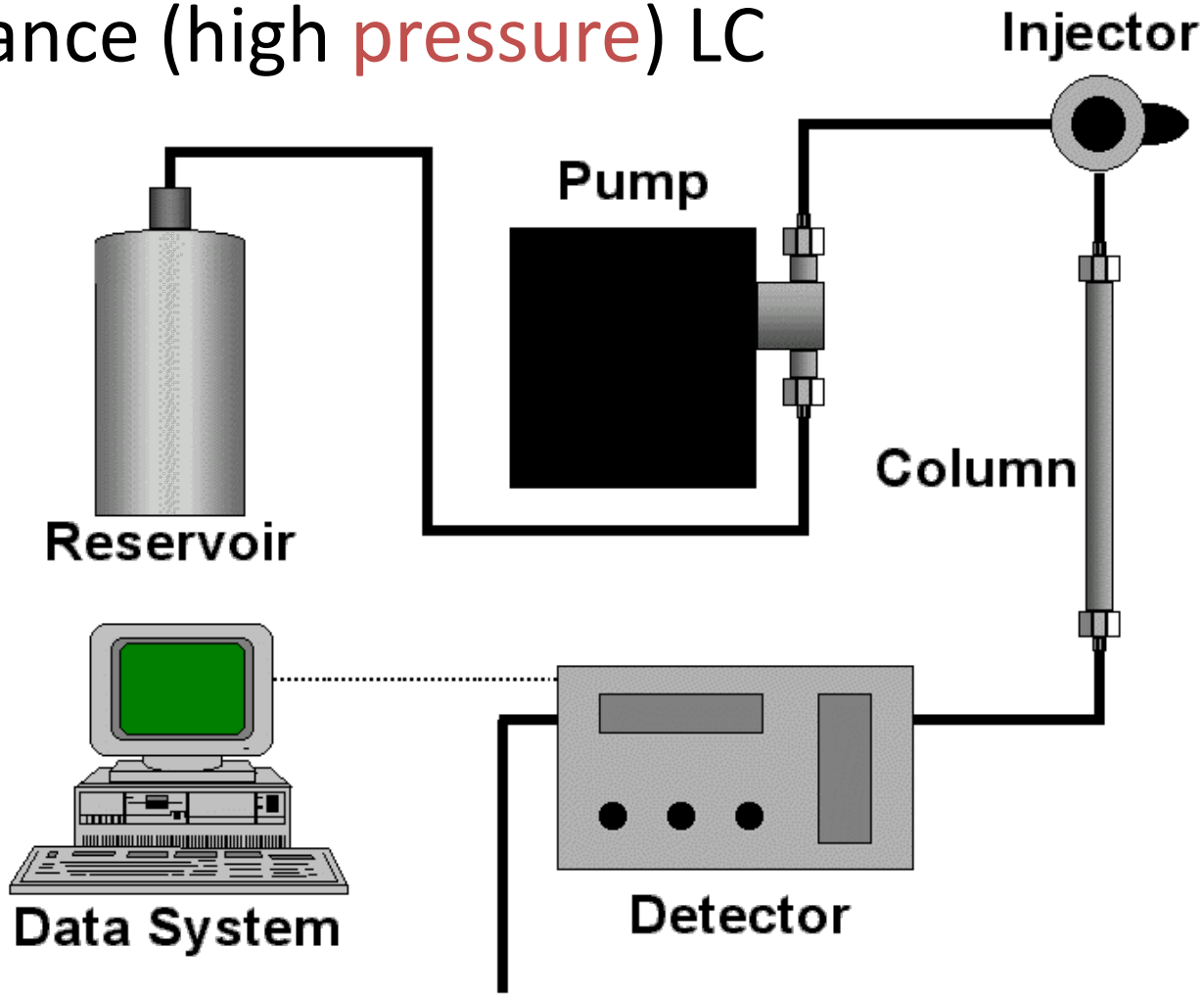
- **Preparative chromatography** is used to nondestructively purify sufficient quantities of a substance for further use, rather than analysis. *Large HPLC columns.*
- **Analytical chromatography** is used to determine the identity and concentration of molecules in a mixture. Quantitative. *Mid-size HPLC columns*
- LC of LCMS. *Small HPLC columns*.

- The **analyte** is the substance which is to be purified
- A **chromatogram** is the visual output of the chromatograph. Different peaks -> different molecules
- The **mobile phase** is the analyte and solvent mixture which travels through the stationary phase
- The **retention time** is the characteristic time it takes for a particular molecule to pass through the system
- **Stationary phase**: Examples include the silica layer, or columns

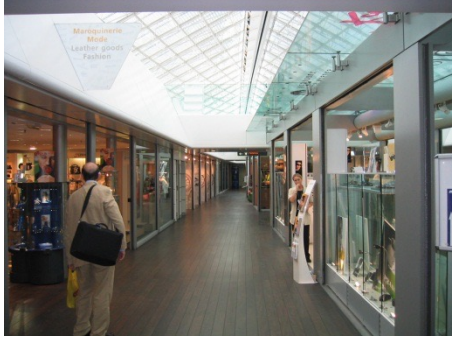


HPLC

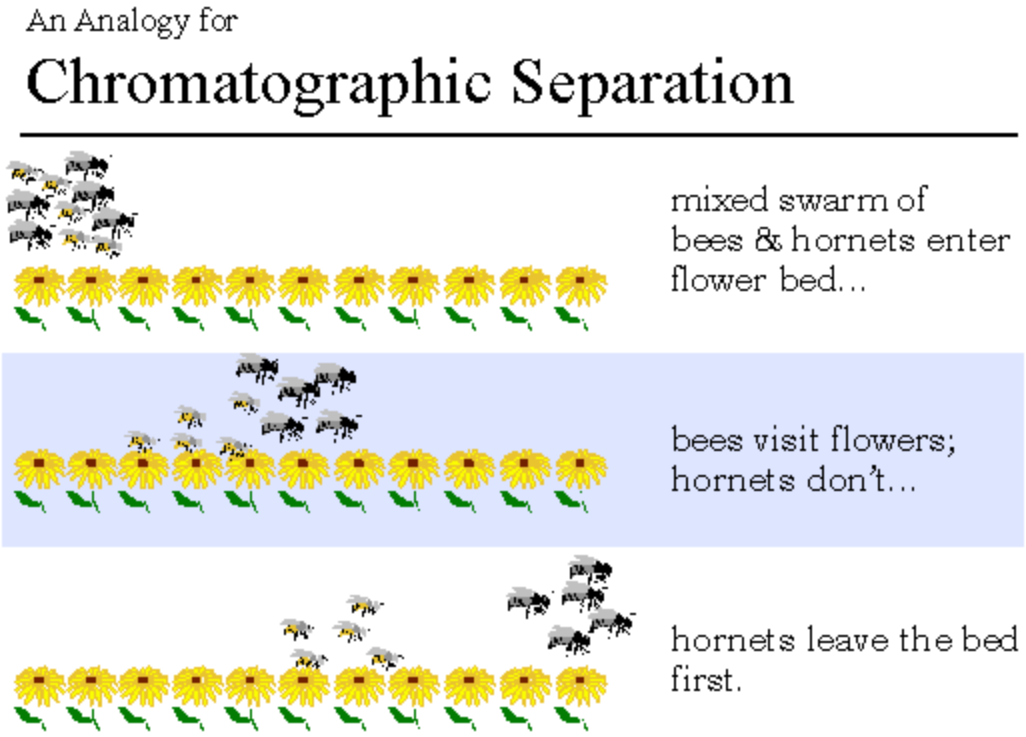
- High-performance (high pressure) LC



Shopping in Factory outlets



- The *elution time* depends on the number and duration of stops



The Final

Don’t worry, it’s not tennis – you always win

Laptop, Tablet, iPad of other mobile device is fine for 3 things:

Calculator

Access to the lecture notes (! A big win)

Entering the answers usual way

Time limit of 2 hours is strict. The page will be closed at 12pm sharp.