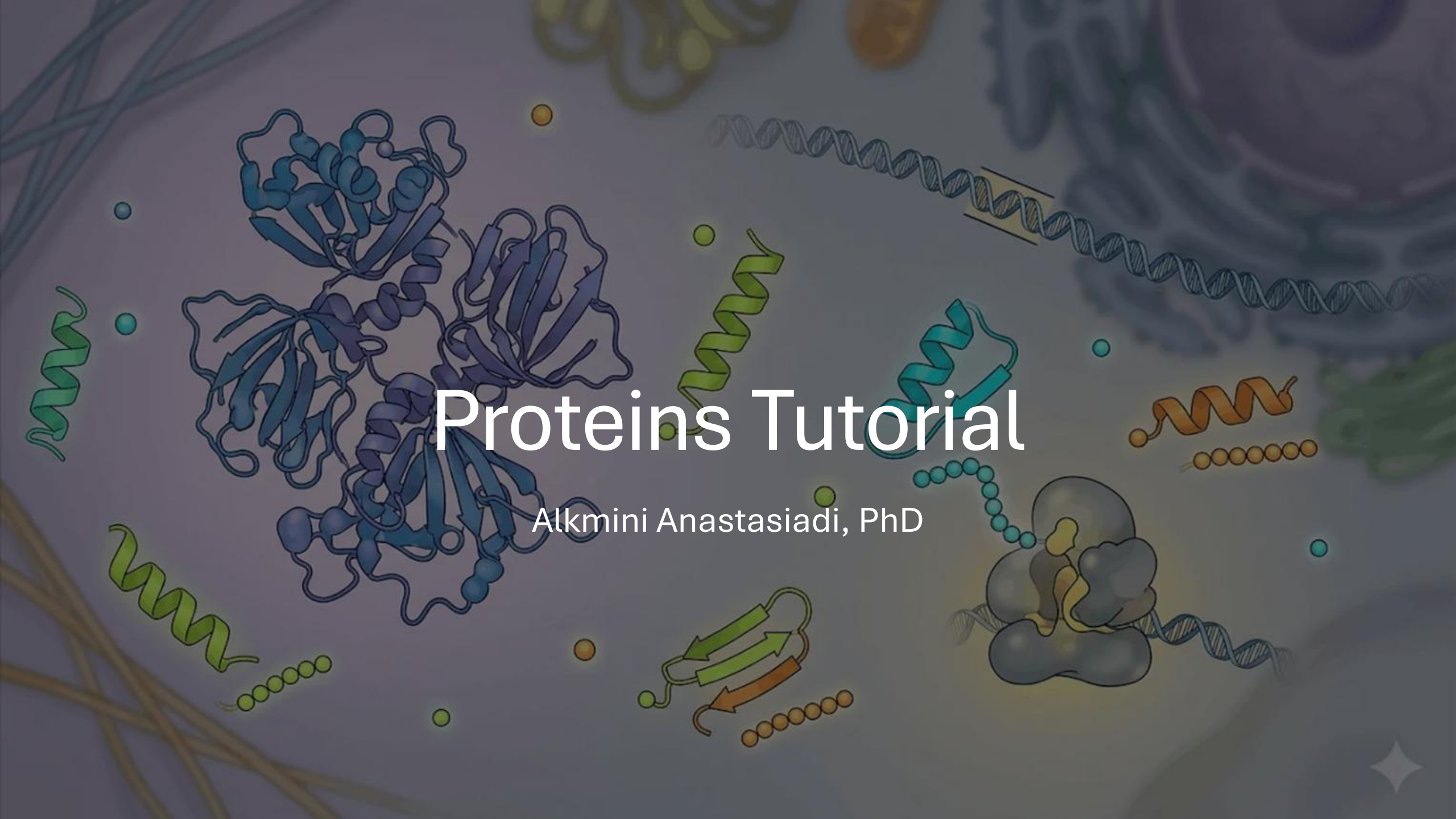
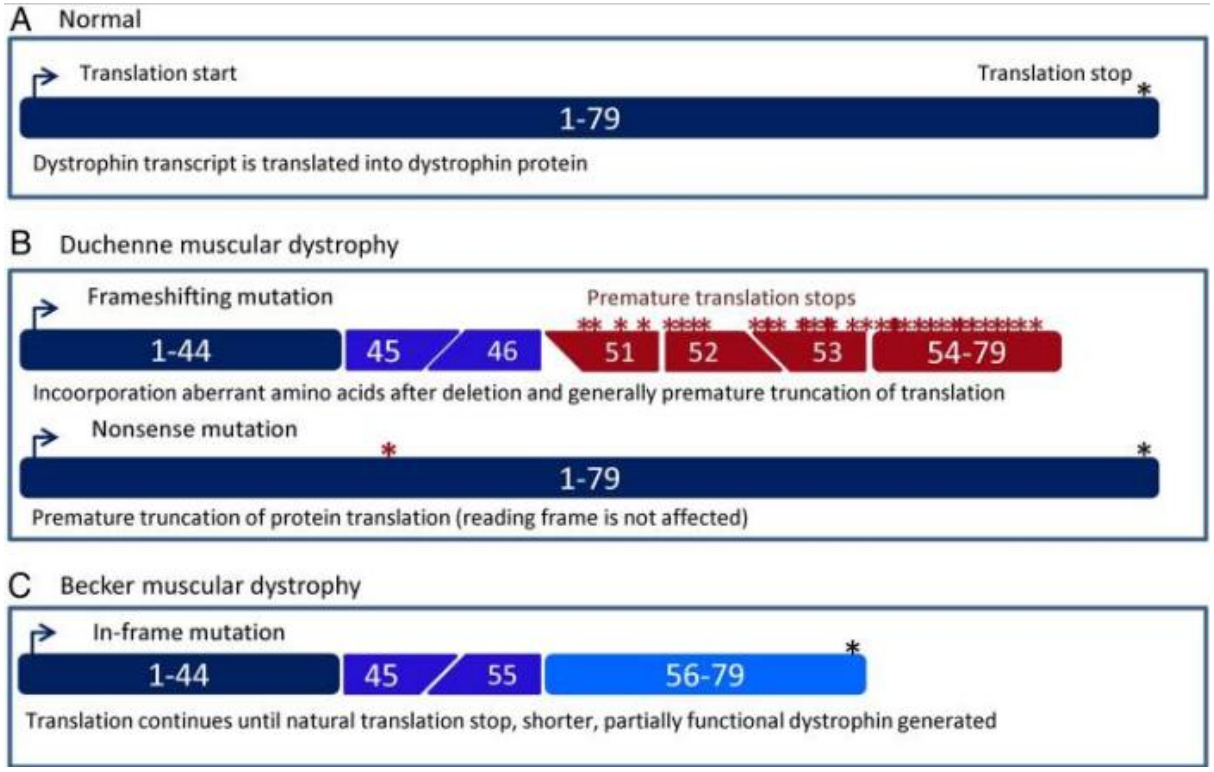


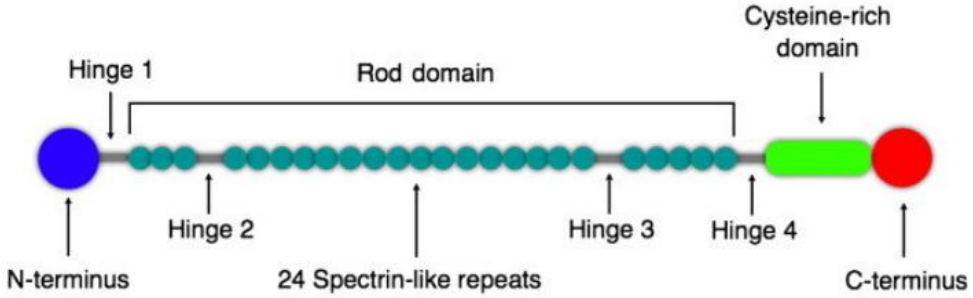
Proteins Tutorial

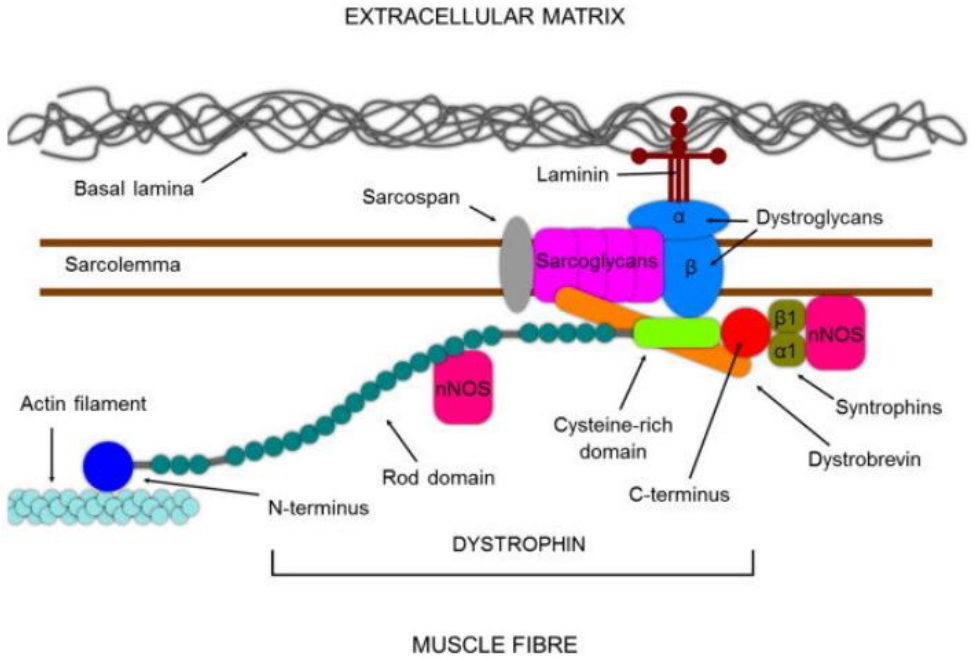
Alkmini Anastasiadi, PhD

Domains – Are they relevant to disease states?

- Dystrophin links the muscle cytoskeleton to the membrane
  - **actin-binding domain**
  - **long rod of 24 repeats**
  - **membrane-anchoring domains**







Why Becker muscular dystrophy is milder than Duchenne muscular dystrophy?

In-frame mutation → shorter dystrophin but with both terminal domains → links actin to membrane

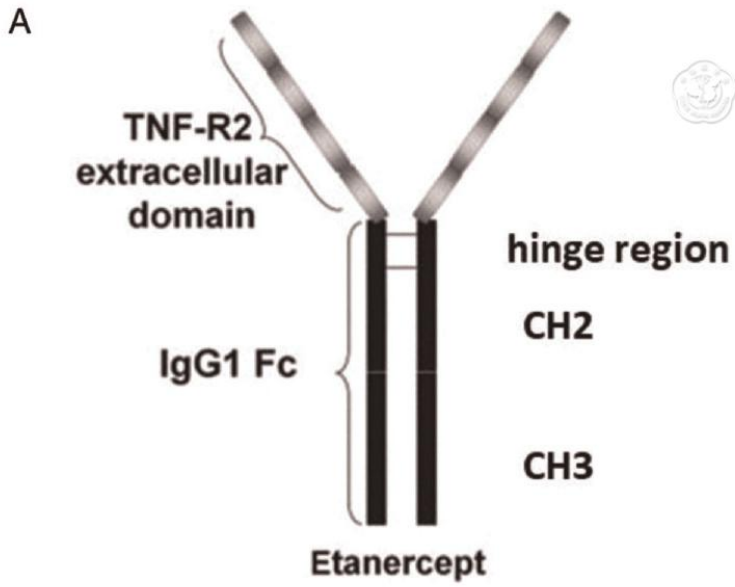
Frameshifting mutation → anchoring end is lost

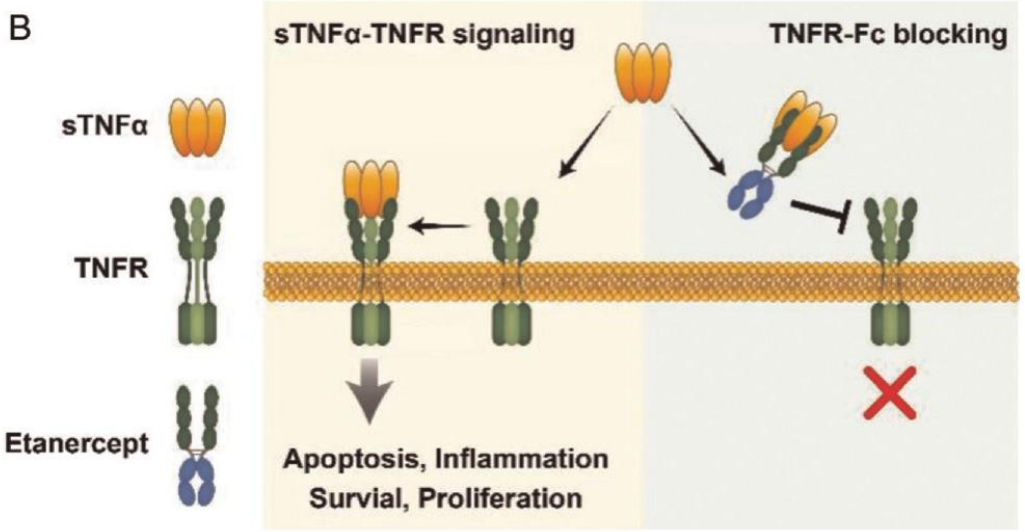
Domains – Are they relevant to therapy?

- TNF (tumor necrosis factor): regulates inflammation, apoptosis, and defense during infection.
- Target-cells can “listen to” TNF’s message through a receptor they have on their surface (it has a domain that can bind TNF).
- In rheumatoid arthritis it is overproduced within the joint lining → inflammation and tissue destruction

What drug can we design?

- We can have the TNF-catching domain of the receptor as a drug ➔ it “grabs” TNF in the blood before it reaches cells
- On its own it would be cleared within hours. We can add the tail domain of an antibody ➔ now it is recognized as “one of ours”, so it stays in the blood for days.





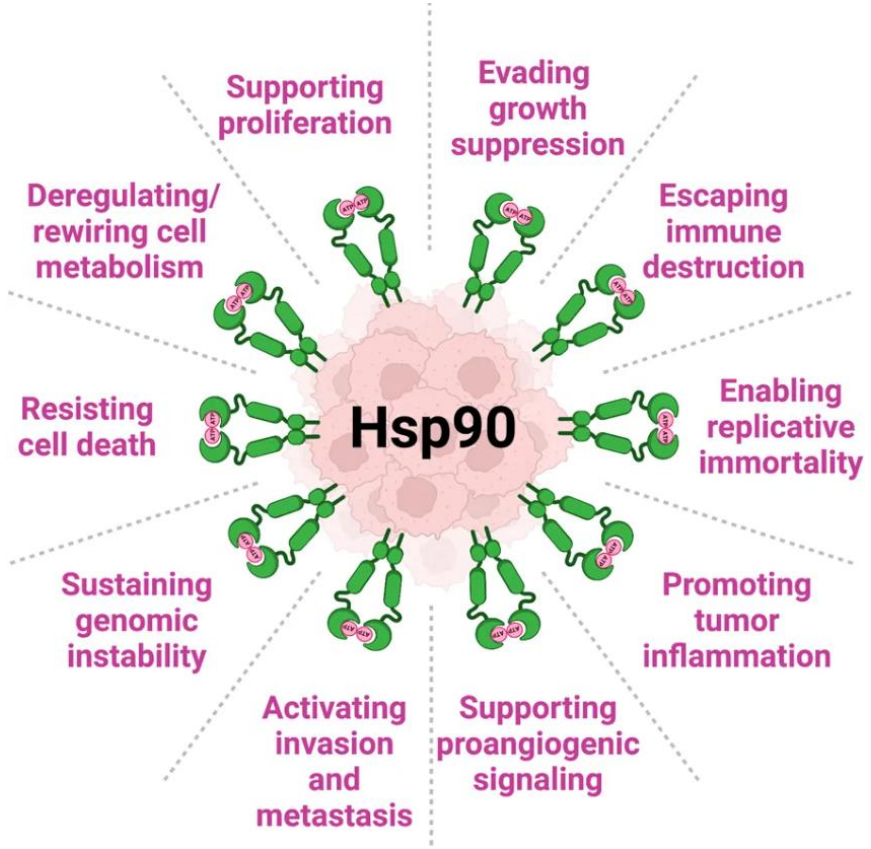
Chaperones

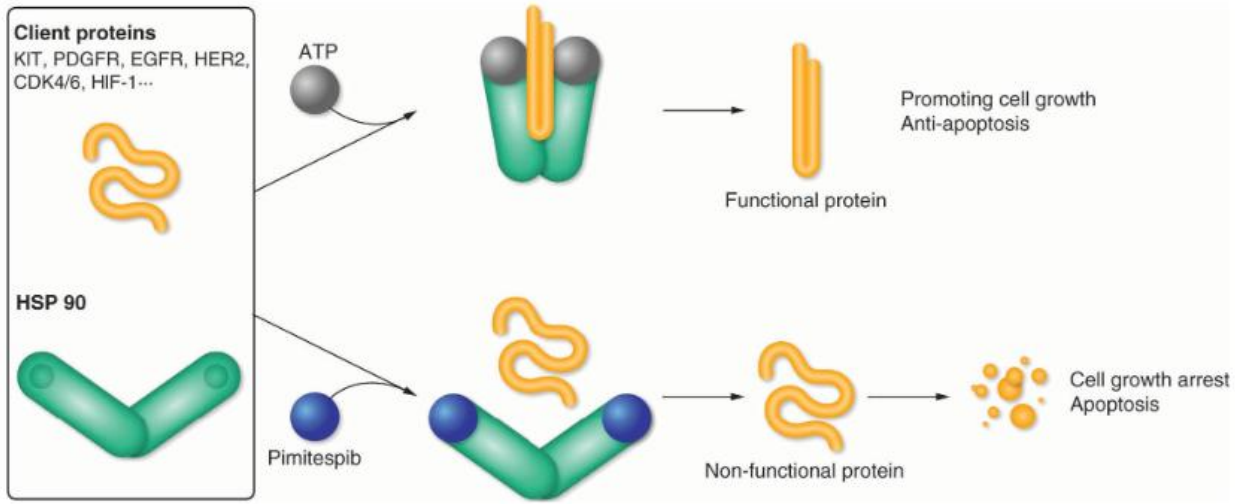
- Cancer cells are characterized by genetic instability and the production of mutated or overexpressed proteins.
- HSP90: molecular chaperones essential for folding, unfolding, degradation and activity of a wide range of proteins

➔ HSP90 binds to the unstable proteins of cancer cells (known as *client proteins*) and shields them from degradation.

How can we "take advantage" of this information?

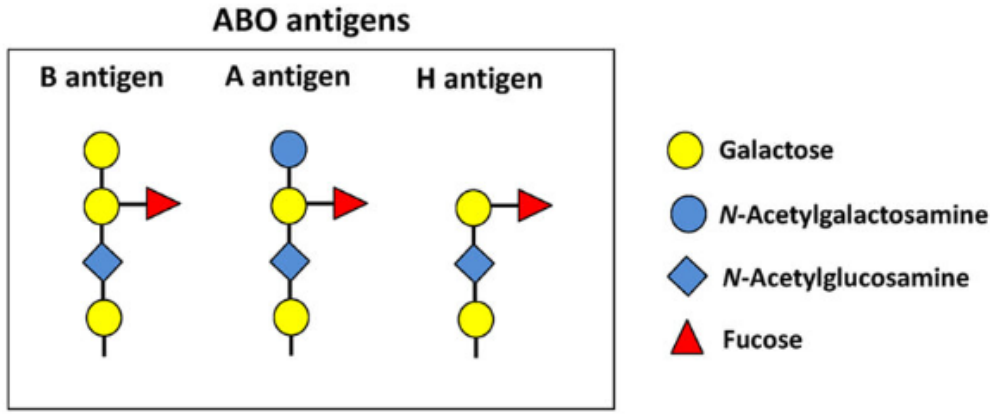
➔ HSP90 inhibitors (Pimitespib; gastrointestinal stromal tumors)

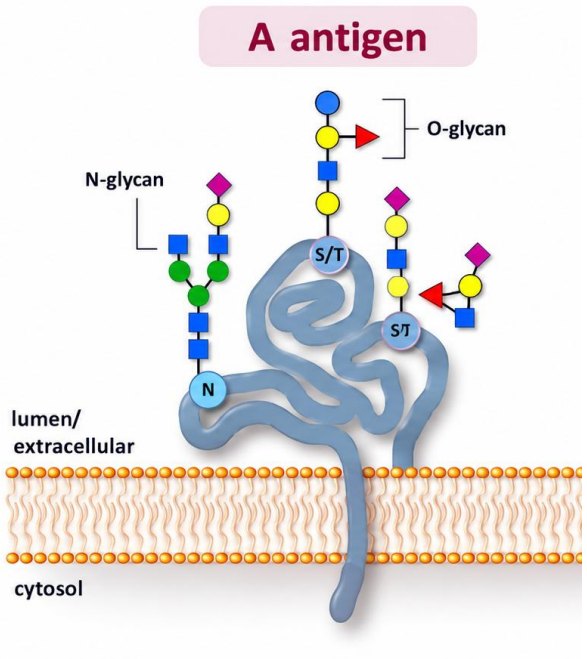


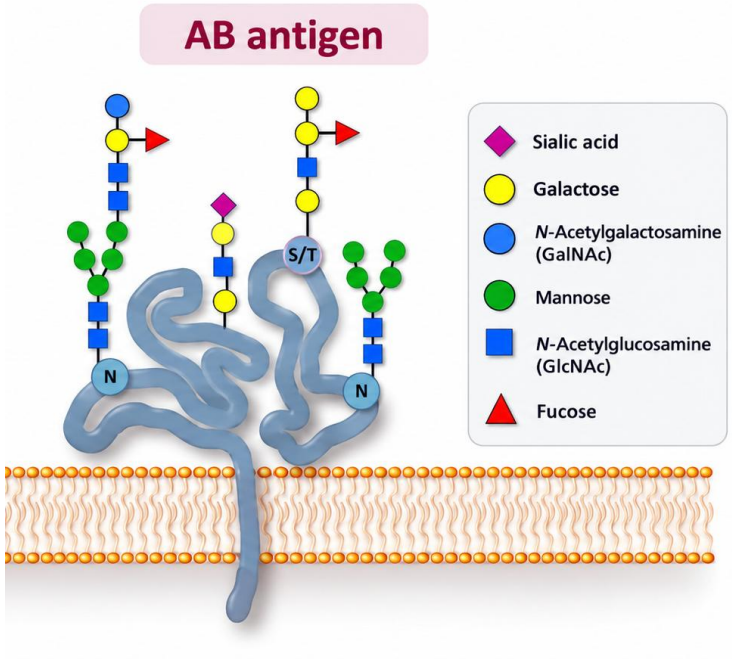


Post-translational Modifications

- Red cell membrane proteins carry sugar chains ➔ ABO blood group







We make antibodies against the sugar we lack: group A people have anti-B, group B people have anti-A.

- **A group A patient is given group B blood by mistake. Within minutes: fever, back pain, dark urine. Why?**

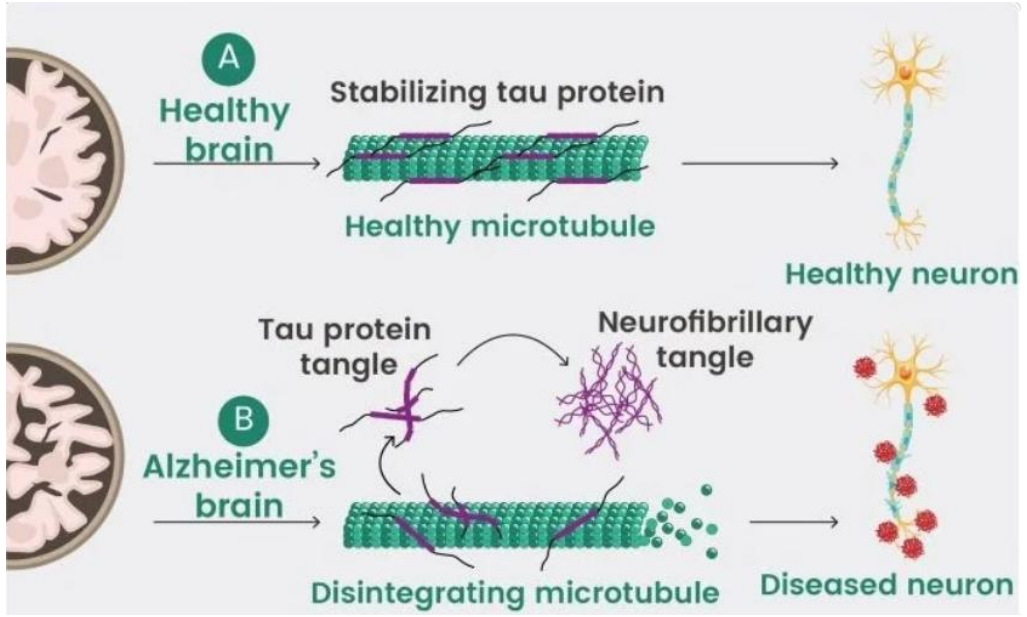
➔ They have antibodies against B group → immune reaction → hemolysis

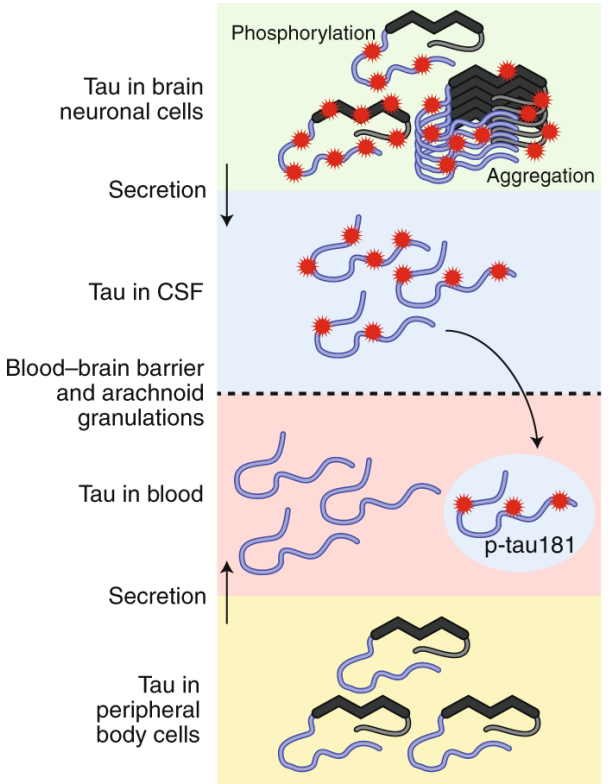
- **Why can group O red cells be given to almost anyone?**

➔ They carry neither A nor B sugar → nothing on their cells can be recognized by anti-A or anti-B antibodies

Post-translational Modifications

- Tau stabilizes microtubules in neurons; phosphorylation regulates its binding.
- In Alzheimer's disease tau is hyperphosphorylated, detaches and forms tangles. **Why would too many phosphate tags make tau fall off the microtubules? Can we use this modification in diagnosis?**
  - Each phosphate adds negative charge → tau is attracted less and less until it is repelled and falls off → microtubules become unstable, and loose tau sticks to itself and forms tangles inside the neuron.
  - Today a blood test that measures tau with a phosphate at one specific position helps diagnose Alzheimer’s disease.



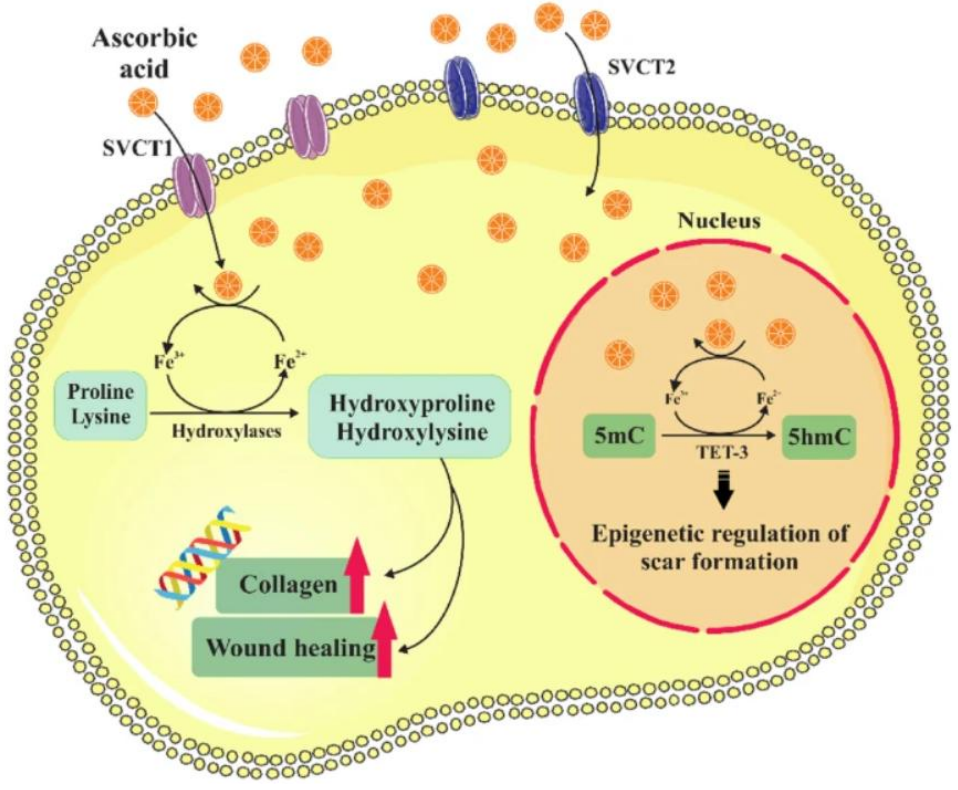




Post-translational Modifications

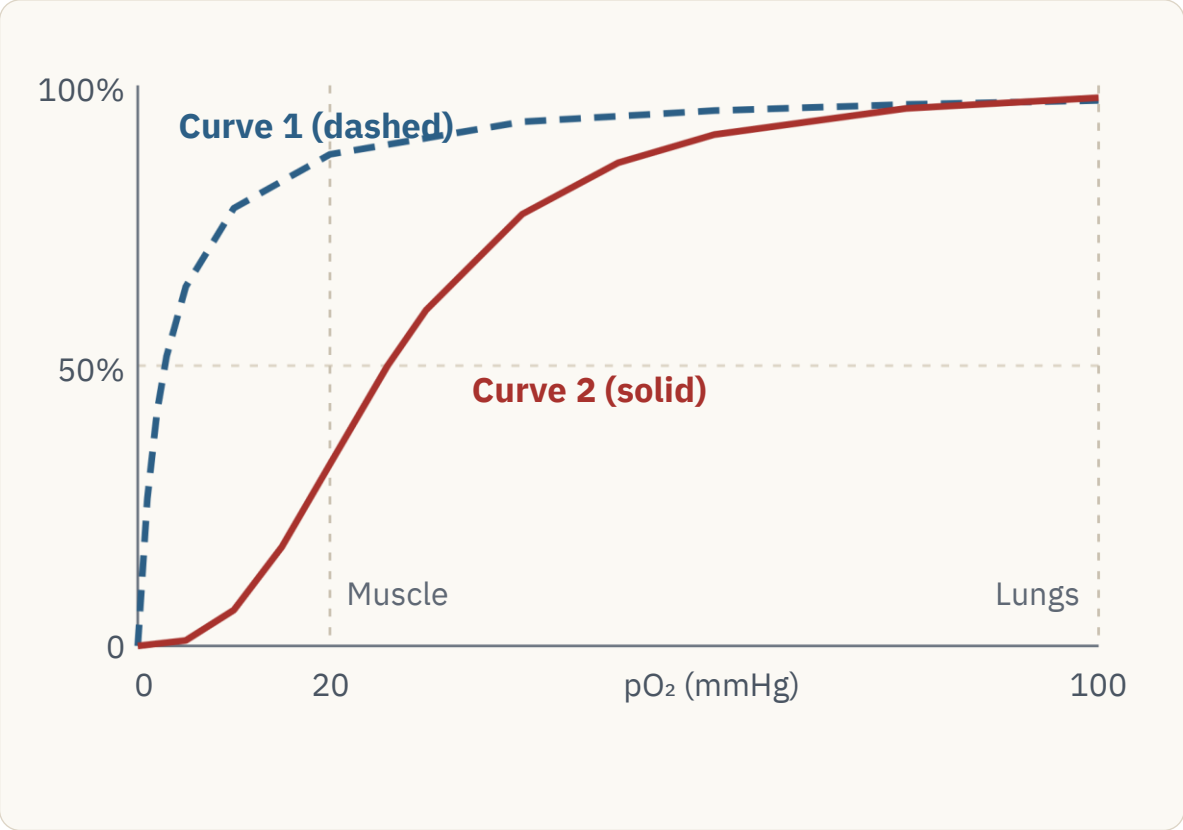
On long voyages without fresh fruit, sailors bled from their gums, bruised easily, and old healed wounds broke open again. **Why does a lack of vitamin C weaken collagen?**

➔ Without vitamin C (ascorbic acid) → proline is not hydroxylated → collagen that needs hydroxyproline becomes unstable



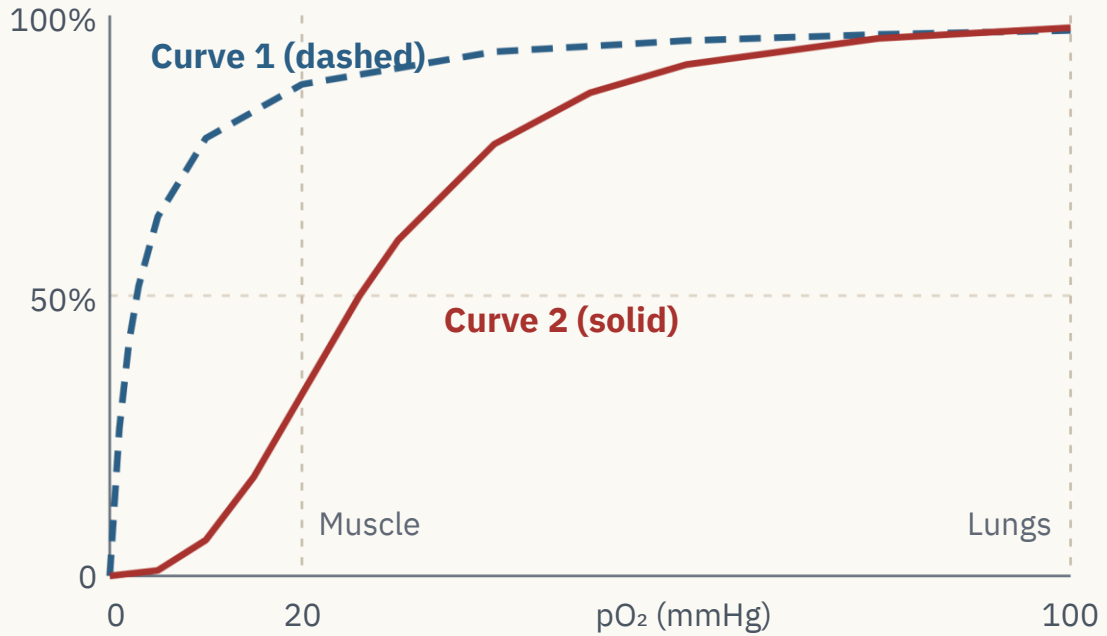


Hemoglobin Regulation



1. Which curve is myoglobin and which is hemoglobin?
   - Myoglobin and hemoglobin
2. pO_2 is ~100 mmHg in the lungs and ~20 mmHg in working muscle. Which protein releases more O_2 between the two?
   - Myoglobin has one chain and is ~90% saturated at 20 mmHg, so it stores O_2 in muscle. Hemoglobin has four chains and falls from ~98% to ~30% between lungs and muscle ➔ it delivers about two-thirds of its O_2.
3. What structural feature makes the S-shaped curve possible?
   - Cooperativity between subunits

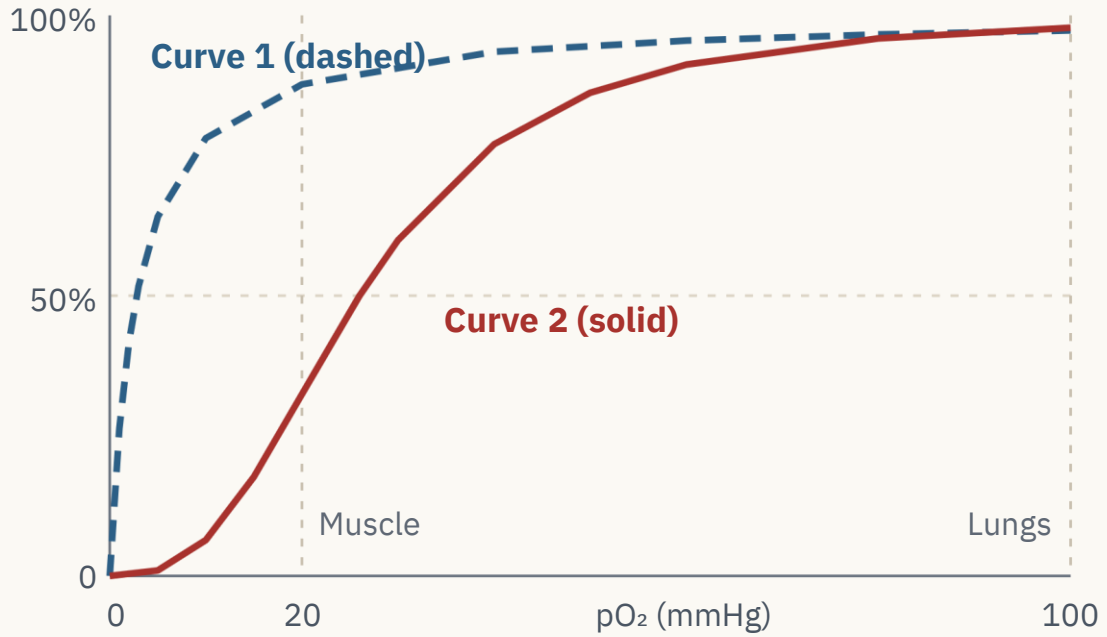
Hemoglobin Regulation



A runner sprints for the last 400 meters. Her thigh muscles are warm, acidic and full of CO_2. Her red cells also contain 2,3-bisphosphoglycerate (2,3-BPG), a by-product of glycolysis.

1. For H^+, CO_2, 2,3-BPG and heat: does each shift the curve right or left?
2. 2,3-BPG binds only deoxy-Hb. What does that do to O_2 release?
3. HbF ($\alpha_2\gamma_2$) binds 2,3-BPG weakly. Why does that help the fetus?

Hemoglobin Regulation

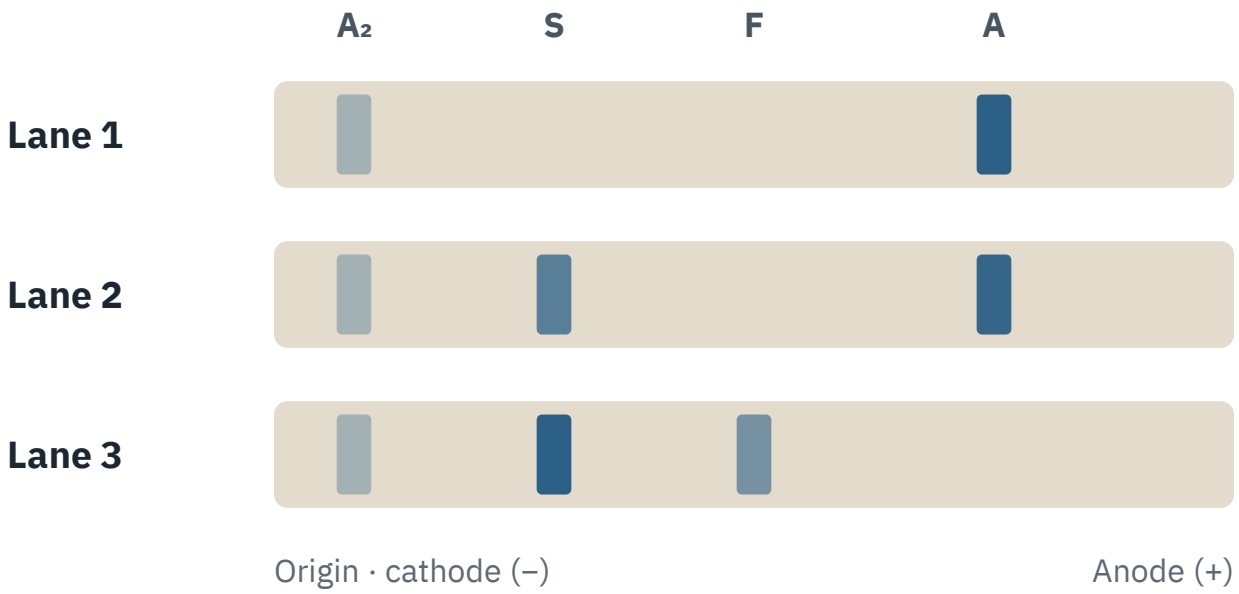


A runner sprints for the last 400 meters. Her thigh muscles are warm, acidic and full of CO_2. Her red cells also contain 2,3-bisphosphoglycerate (2,3-BPG), a by-product of glycolysis.

Factor	How it acts on the protein	Curve shifts
H^+ (Bohr effect)	Protonates histidines (e.g. His146 of β); new salt bridges stabilise deoxy-Hb	Right
CO_2	Binds the N-terminal amino groups as carbamate, and lowers pH	Right
2,3-BPG	Binds positive His and Lys in the central cavity between the β chains of deoxy-Hb	Right
Heat	Weakens O_2 binding	Right
Fetal Hb	γ chains carry Ser at 143 instead of His: weaker 2,3-BPG binding, higher affinity	Left

Hemoglobinopathies

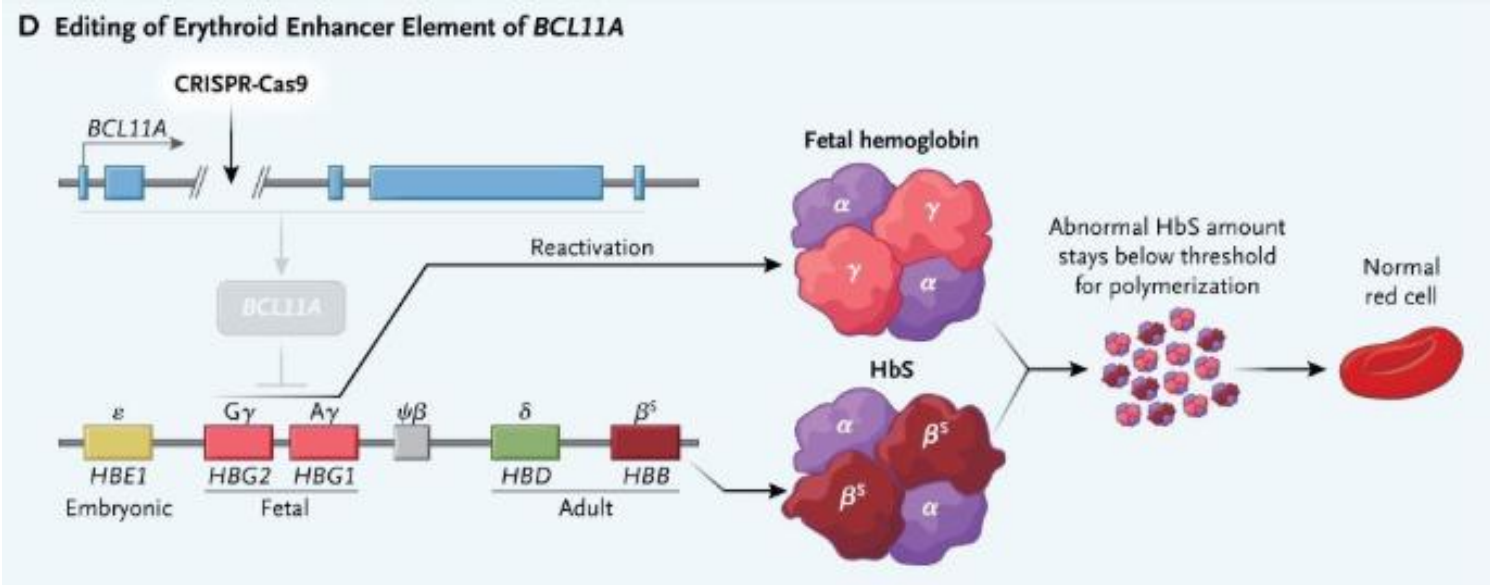
Eleni is an 18-month-old toddler who presented symptoms of anemia. They visited the hospital with her mother and the medical professionals performed Hb electrophoresis. Lane 1 is a healthy adult. Lanes 2 and 3 are Eleni and her mother. **Which is which? At pH 8.6 every hemoglobin is negative. Why does HbS lag behind HbA? Why is there so much HbF in lane 3?**



- Lane 2 is the mother: sickle cell trait (HbAS), more HbA than HbS, and healthy. Lane 3 is Eleni (HbSS): no HbA at all.
- The mutation (glutamic acid to valine) removes one negative charge per β chain, two per tetramer. Less negative, HbS moves more slowly towards the anode.
- At 18 months the switch from γ to β chains is still underway, and HbF tends to stay higher in sickle cell disease.

Hemoglobinopathies

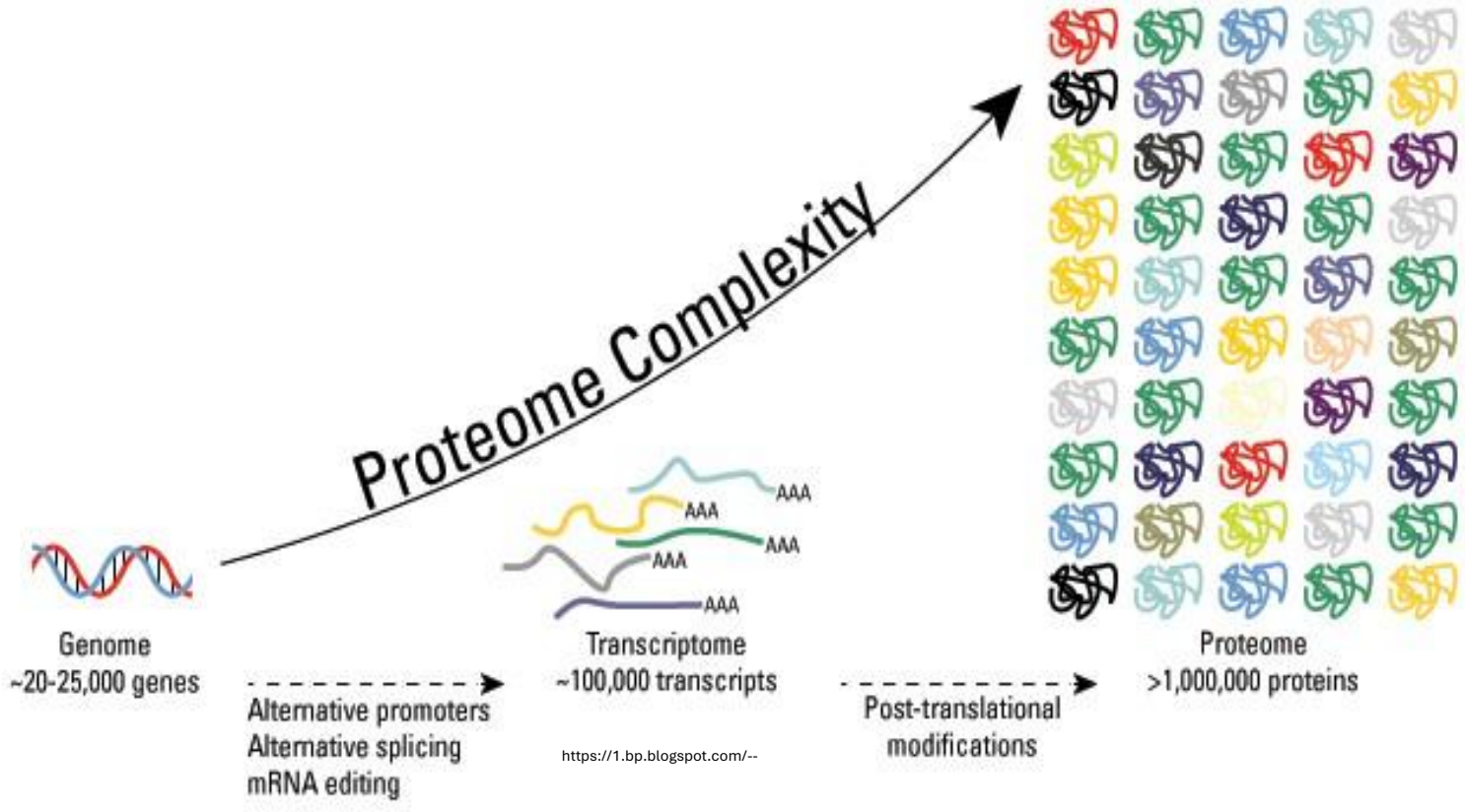
Eleni starts hydroxyurea, which raises HbF. When she is older, a gene therapy such as exa-cel could edit her own stem cells so that they switch γ-globin back on. **Both treatments raise HbF ($\alpha_2\gamma_2$). Why does that help? Exa-cel disables a repressor of γ-globin in red cell precursors. Which level of protein structure does it finally change?**



- More HbF → HbF cannot join the fibers and dilutes HbS → fewer, slower fibers, fewer crises.
- Her red cells assemble $\alpha_2\gamma_2$ (HbF) in place of $\alpha_2\beta^s_2$ (HbS) → quaternary structure is affected

Studying the Proteome

- Proteome: the totality of expressed proteins in a given cell type or organism, at a given time, under specific conditions. Proteomics is the study of the proteome.
- We can isolate proteins to study them:
  - Electrophoresis
  - Chromatography
- We can study proteins in a targeted way:
  - Use of antibodies
- We can holistically study proteins:
  - Mass spectrometry
- We can examine 3D structures:
  - X-ray crystallography
  - Nuclear magnetic resonance (NMR)



Studying the Proteome

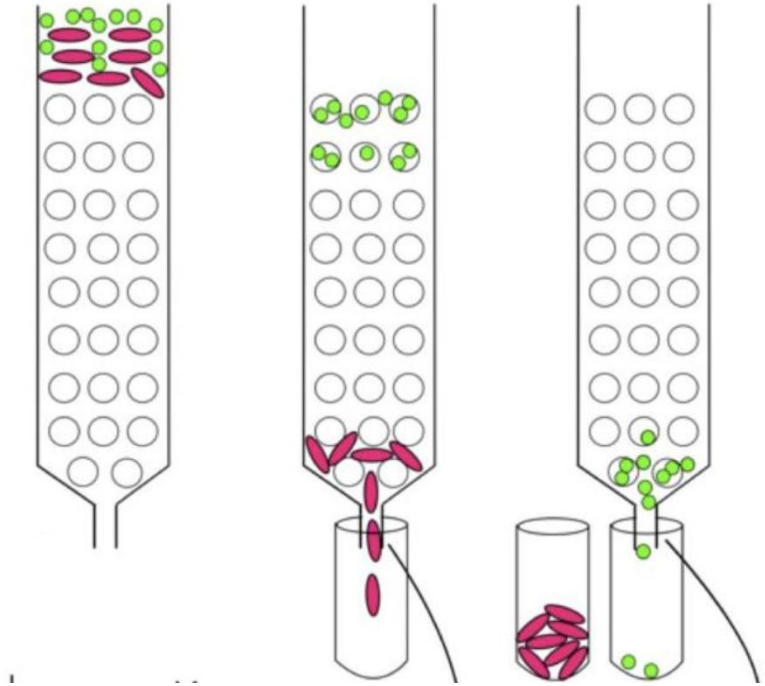
Column Chromatography

- Proteins can be separated based on:
  - **Size** (Gel filtration or Size exclusion chromatography)
  - **Chemical affinity** (Affinity chromatography)
  - **Charge** (Ion exchange chromatography)
- In all cases the basic principles are:
  - **Stationary Phase**: A solid or semi-solid material packed tightly inside a vertical glass or plastic tube
  - **Mobile Phase**: A liquid solvent that continuously flows down through the stationary phase.
  - Components of the mixture **interact differently** with the stationary and mobile phases.
  - **Fraction Collection**: The liquid exiting the bottom of the column is gathered in sequential fractions and analyzed to isolate pure substances.

Studying the Proteome

Column Chromatography

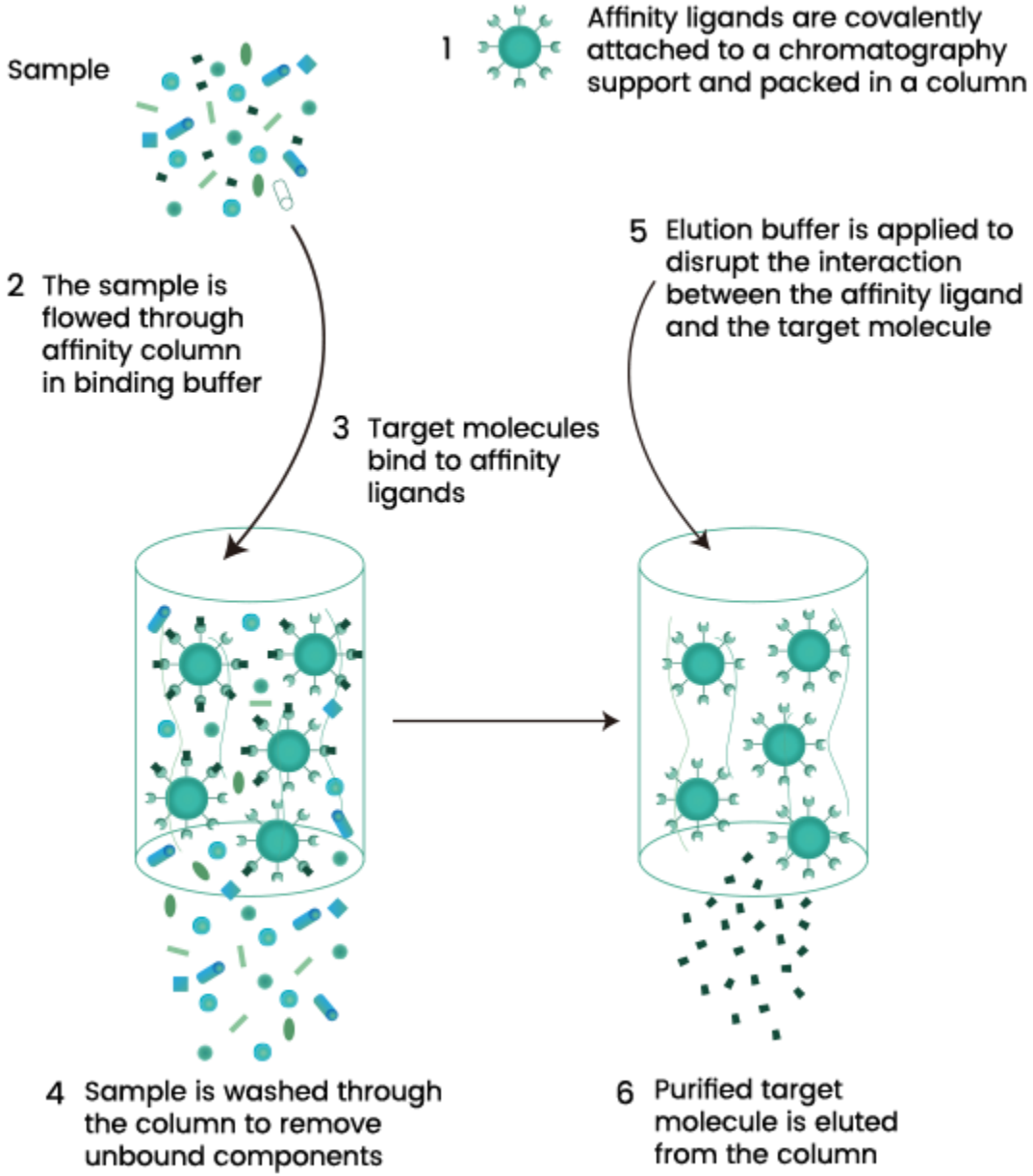
- Gel filtration or Size exclusion chromatography
  - The stationary phase contains **porous beads**
  - The mobile phase is a **buffer** that continuously carries the sample through the porous column.
  - Smaller proteins can enter the pores of the beads → their flow is slowed down → **larger proteins arrive first at the bottom of the column**



Studying the Proteome

Column Chromatography

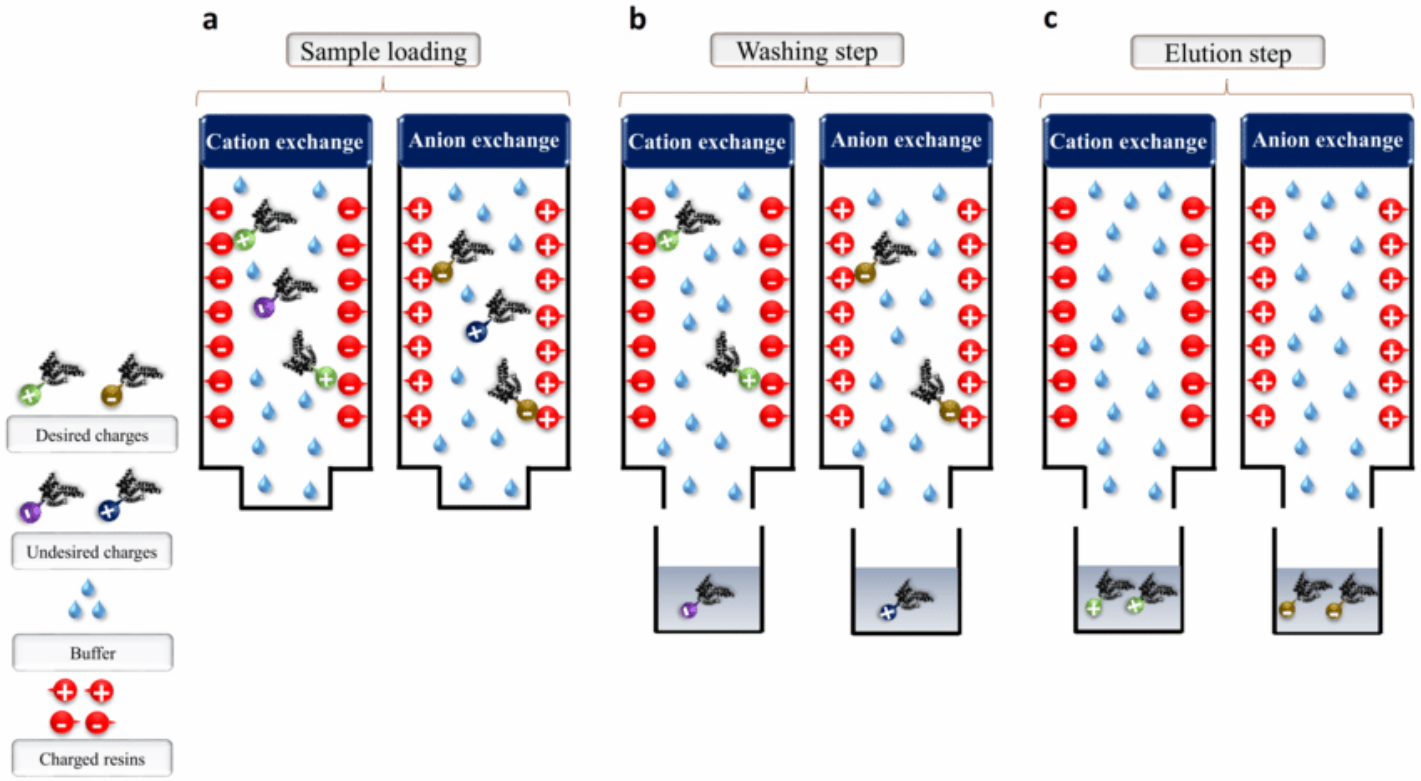
- Affinity chromatography
  - The stationary phase contains **beads with affinity ligands** that can specifically bind the protein of interest
  - The mobile phase is a **buffer** that continuously carries the sample through the column.
  - **Unbound** (unwanted) proteins are **washed** through the column
  - To release the bound proteins, we use another buffer (**different pH**) → **protein of interest is unbound and collected**



Studying the Proteome

Column Chromatography

- Ion exchange chromatography
  - Stationary phase: **beads with specific charge** (cation or anion exchange)
  - Mobile phase: a **buffer** that carries the sample through the column.
  - Proteins of the **opposite charge** of the one in the column → **bind** to the charged beads
  - **Unbound** (unwanted) proteins are **washed** out
  - Buffer with **increased salt levels** → ions compete for the binding place of charged proteins → **protein of interest is displaced and collected**



Studying the Proteome

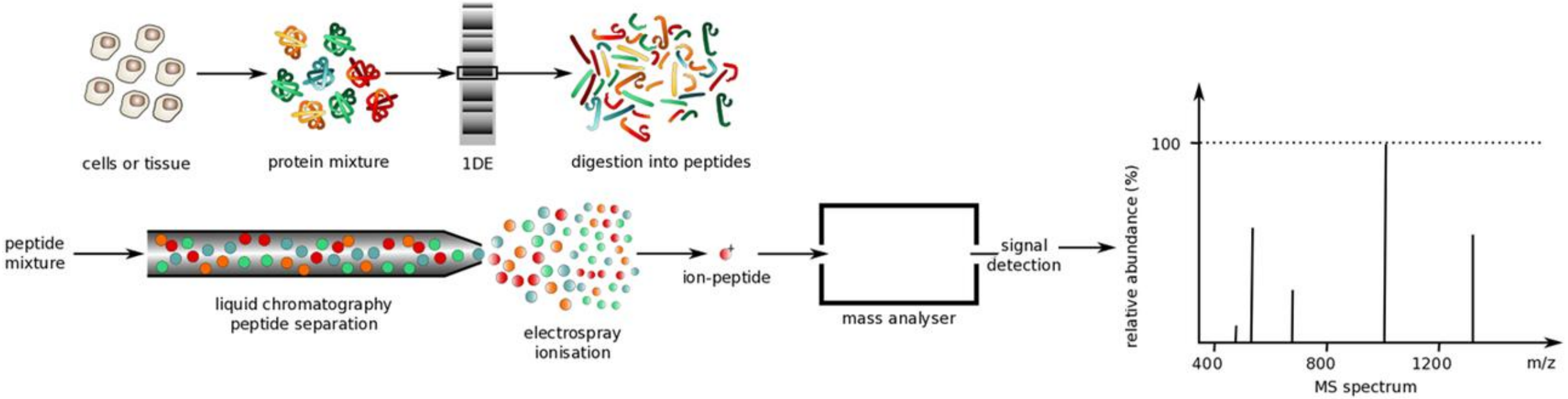
High Performance Liquid Chromatography-HPLC

	Classic column	**HPLC**
Particles	Large beads	Tiny beads (3–5 μm)
Flow	Gravity	Pump, high pressure
Time	Hours	Minutes
Detection	Collect fractions, measure later	Detector reads continuously
Result	Fractions	Chromatogram: time = identity, area = amount

- The column separates; the detector identifies.
- For known molecules (e.g., hemoglobins) a light detector is enough.
- For unknown molecules, such as thousands of peptides in proteomics, the column is coupled to a mass spectrometer (LC-MS).

Studying the Proteome

Mass spectrometry

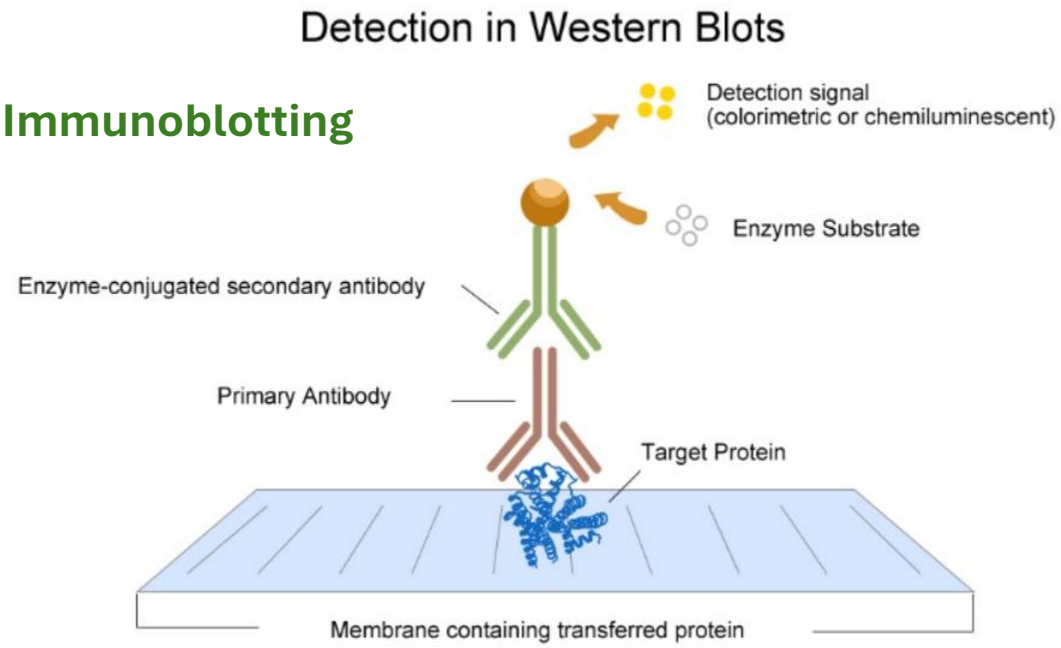


Peptides separated by chromatography are ionized → they are analyzed based on their mass and charge → **every molecule has a characteristic spectral "fingerprint"**

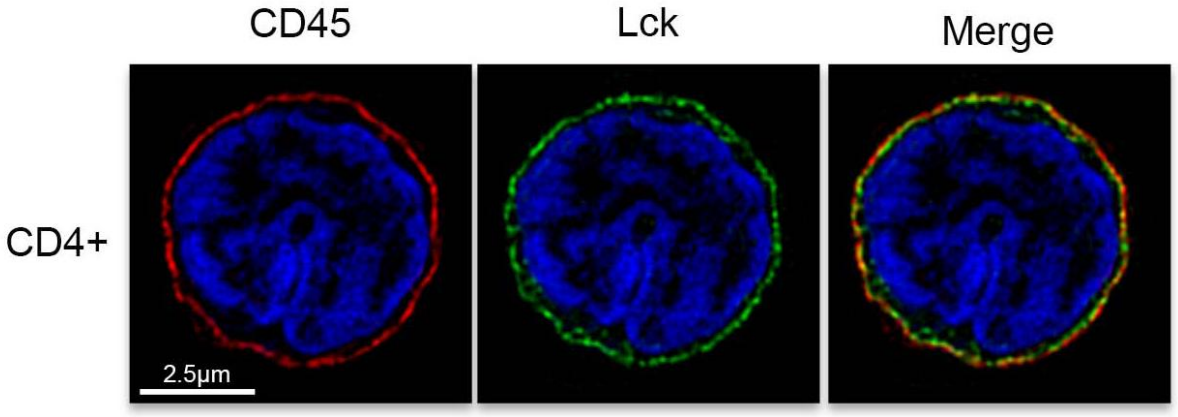
Studying the Proteome

Antibody-based protein analysis

Immunoblotting



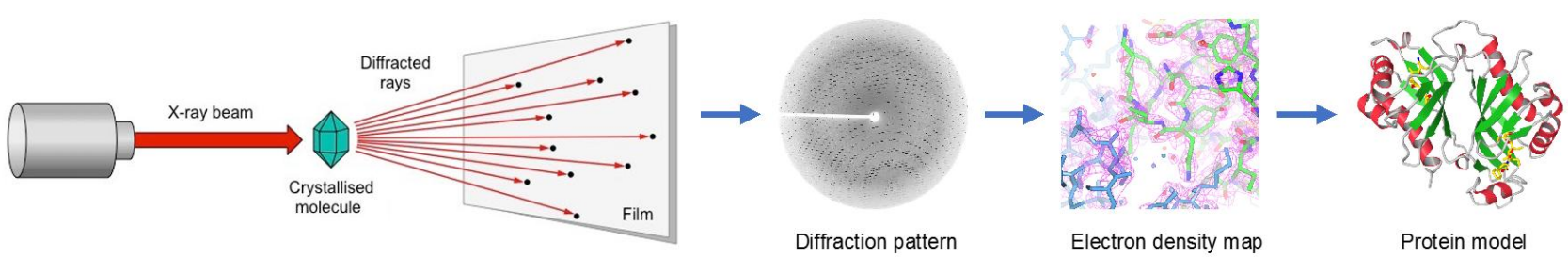
Immunofluorescence Microscopy



Studying the Proteome

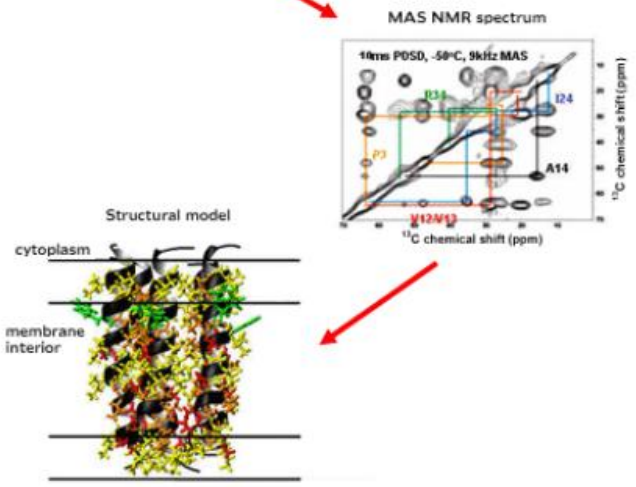
Finding out the 3D structure of a protein

X-ray crystallography





Nuclear Magnetic Resonance



Why do we care about the 3D structure of proteins in medicine?

➔ Many drugs specifically bind on proteins (inhibitors, enhancers etc.) → we need to know the target's 3D structure to design a complementary specific drug.