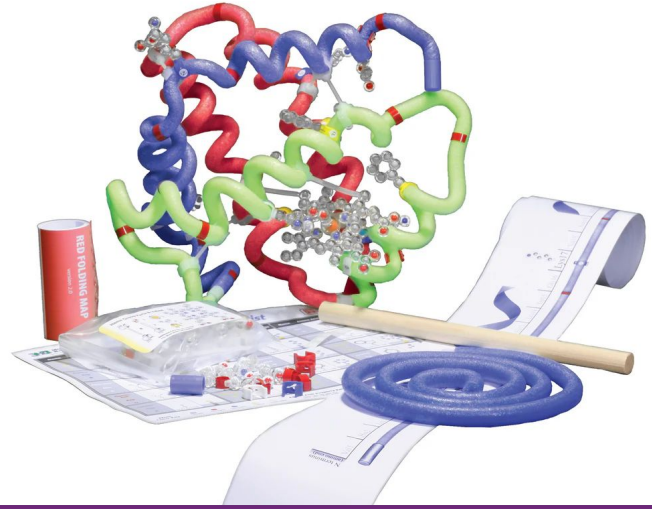
3d molecular designs
Transforming Science Learning

Protein puzzles: Decoding Sickle Cell and CRISPR Solutions

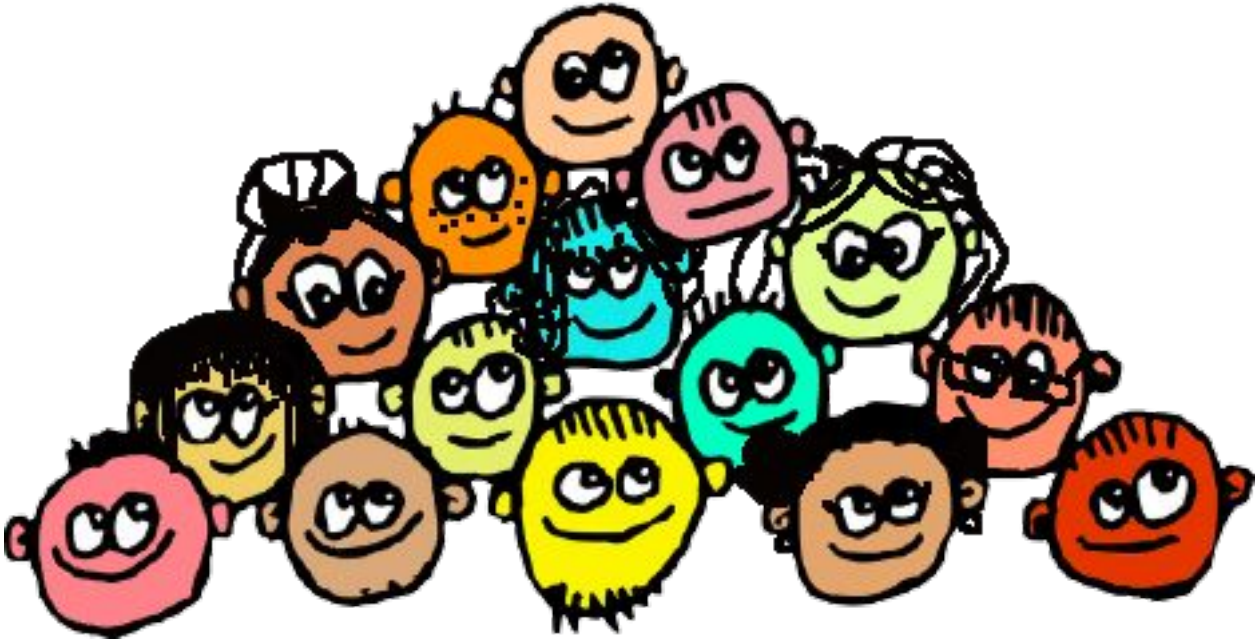
Keri Shingleton, PhD
Holland Hall School
Tulsa, Oklahoma



Anaheim
CALIFORNIA 2024
NABT PROFESSIONAL DEVELOPMENT CONFERENCE
NOVEMBER 14-17, 2024

Who am I?

Who are you?



FDA NEWS RELEASE

FDA Approves First Gene Therapies to Treat Patients with Sickle Cell Disease

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For Immediate Release: December 08, 2023

Today, the U.S. Food and Drug Administration approved two milestone treatments, Casgevy and Lyfgenia, representing the first cell-based gene therapies for the treatment of sickle cell disease (SCD) in patients 12 years and older. Additionally, one of these therapies, Casgevy, is the first FDA-approved treatment to utilize a type of novel genome editing technology, signaling an innovative advancement in the field of gene therapy.

Sickle cell disease is a group of inherited blood disorders affecting approximately 100,000 people in the U.S. It is most common in African Americans and, while less prevalent, also

https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease

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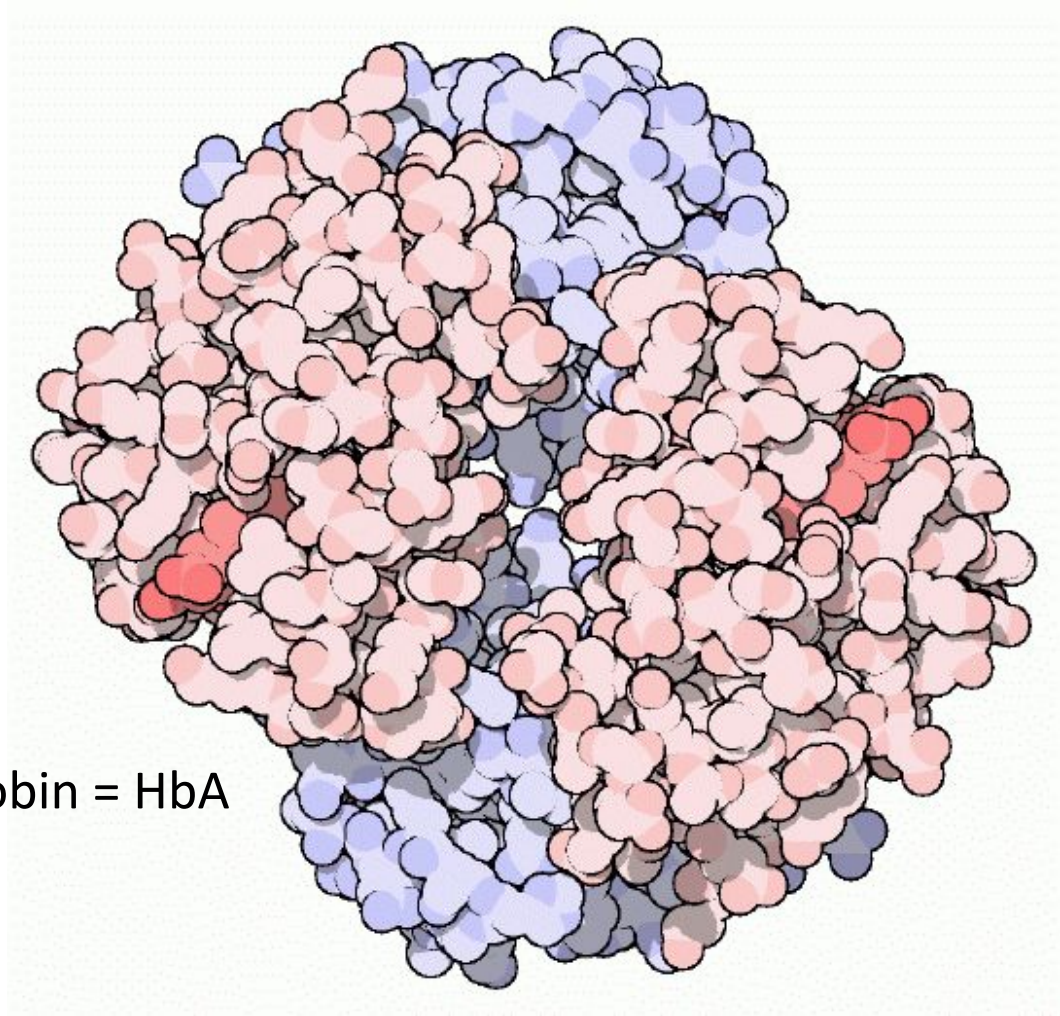
https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease

But how does this work?

And what conceptual ideas are essential for understanding this story?

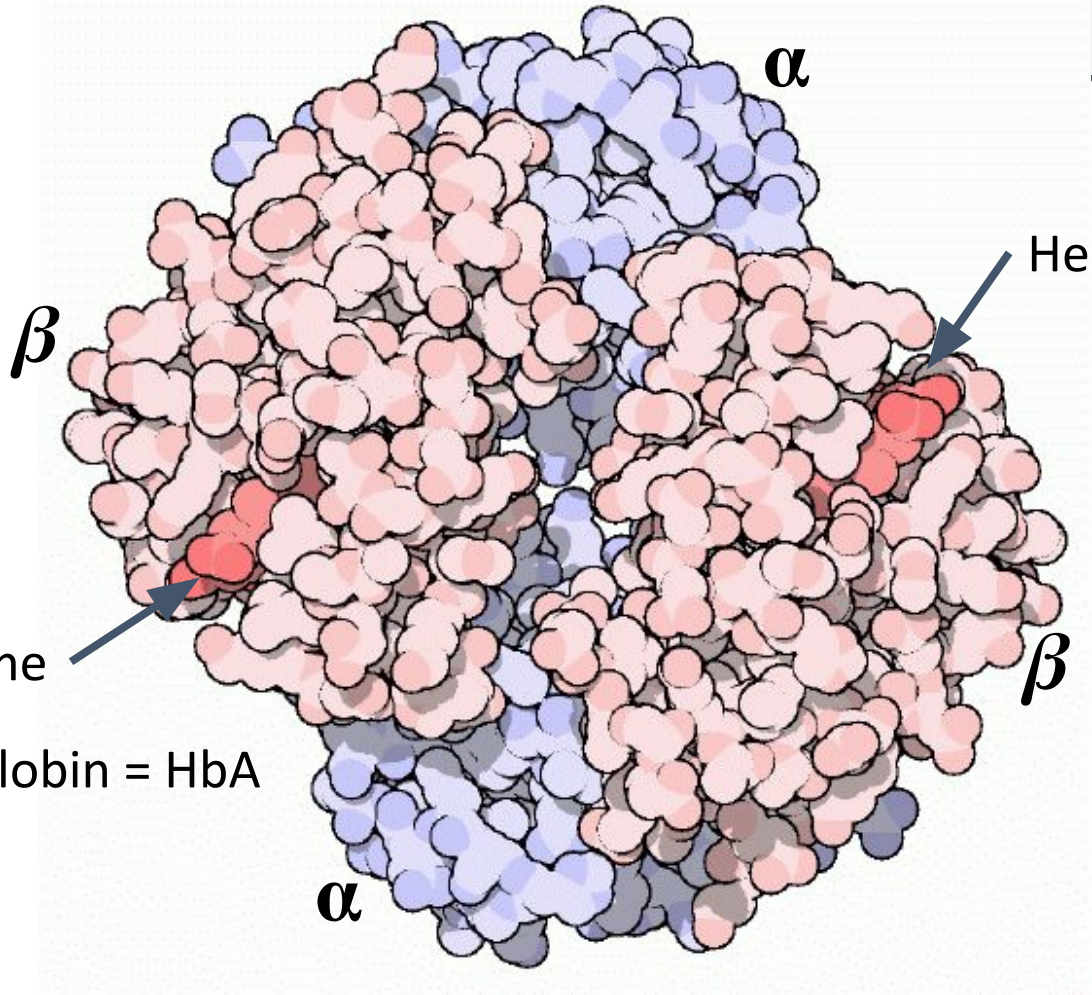
What do you know about sickle cell disease?

What do you wonder?



“Adult” hemoglobin = HbA

https://pdb101.rcsb.org/motm/41



PDB-101 Molecular explorations through biology and medicine
Training and outreach portal of PDB
Molecule of the Month

"Adult" hemoglobin = HbA

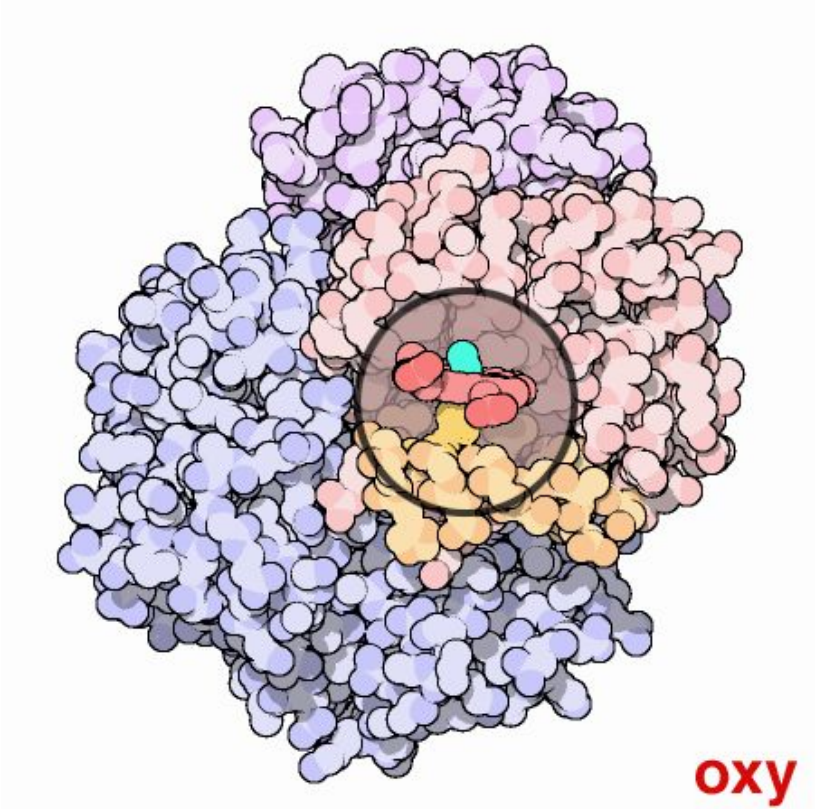
https://pdb101.rcsb.org/motm/41

Hemoglobin in action

One subunit of HbA

One α-chain

One β-chain



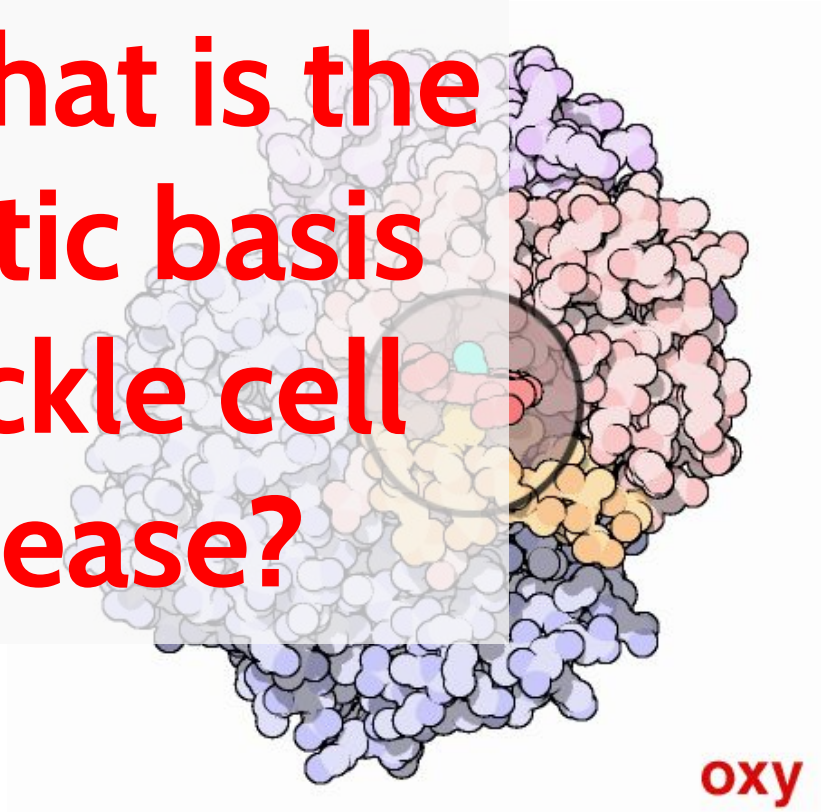
Hemoglobin in action

But what is the genetic basis of sickle cell disease?

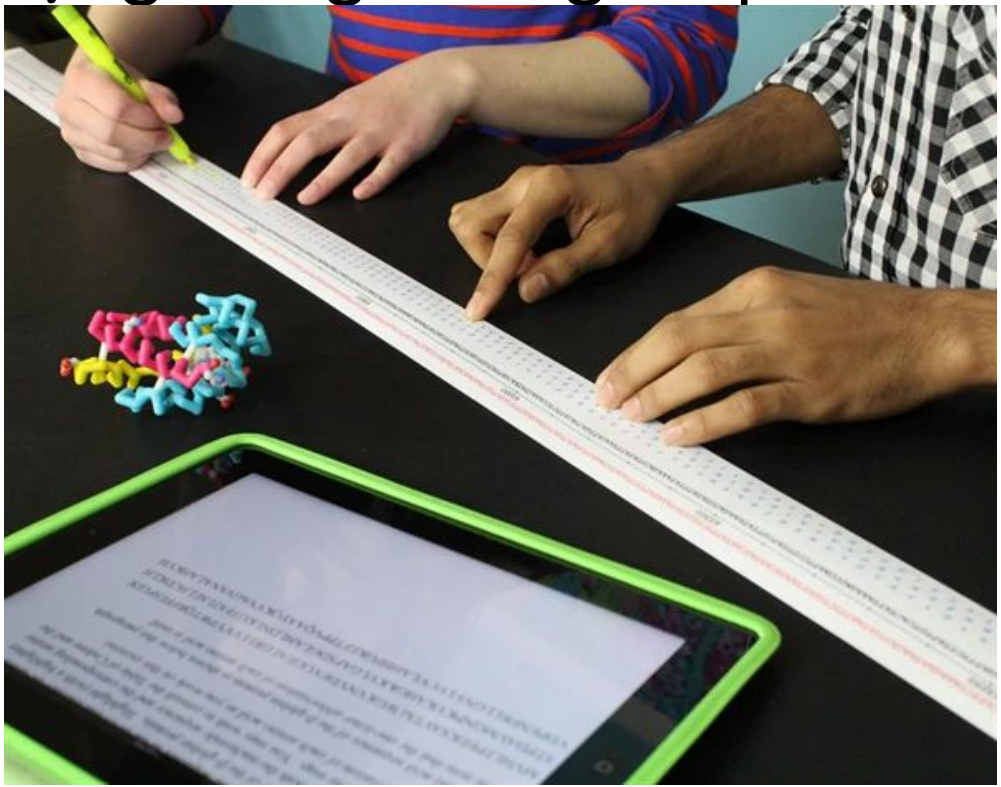
One subunit of HbA

One α-chain

One β-chain

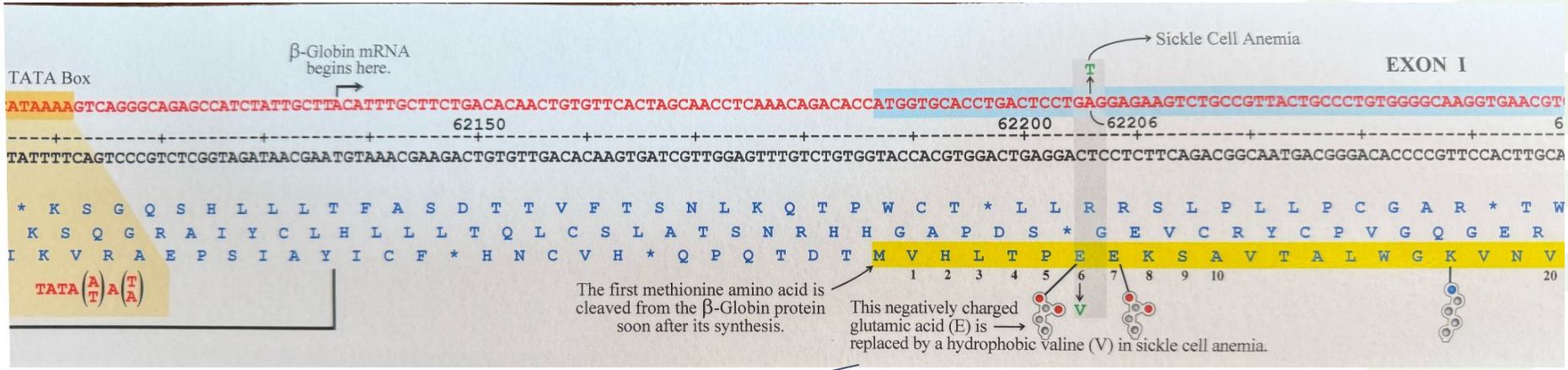


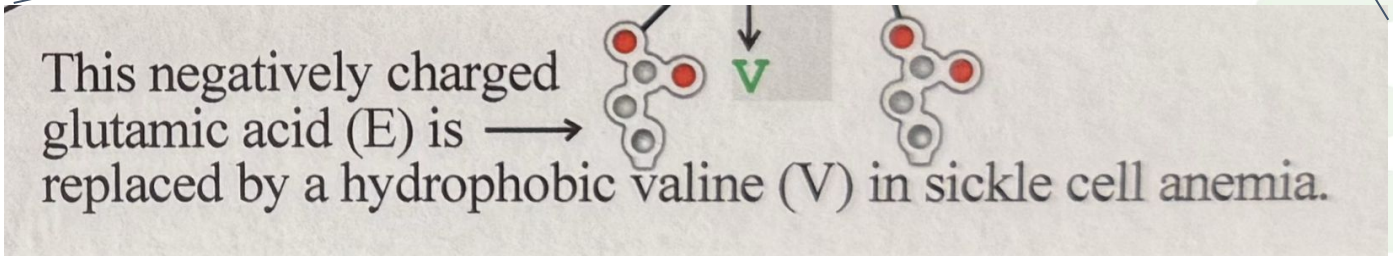
The β-globin gene—a good place to start



What do you notice?

What do you wonder?

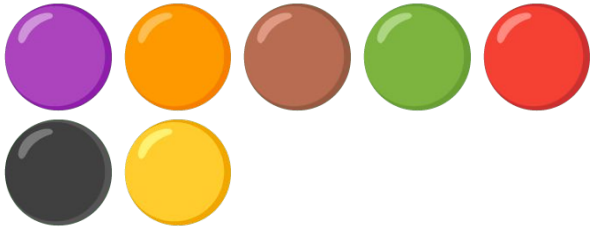

TATA Box
β-Globin mRNA begins here.
Sickle Cell Anemia
EXON I
62150
62200
62206
TATA (A/T) A (T/A)
The first methionine amino acid is cleaved from the β-Globin protein soon after its synthesis.
This negatively charged glutamic acid (E) is replaced by a hydrophobic valine (V) in sickle cell anemia.


This negatively charged glutamic acid (E) is ⟶ V replaced by a hydrophobic valine (V) in sickle cell anemia.

But what is a protein?

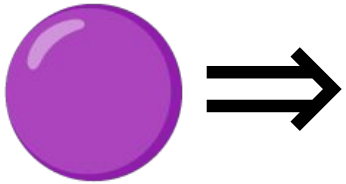
And how does the mutation in the DNA affect the protein?

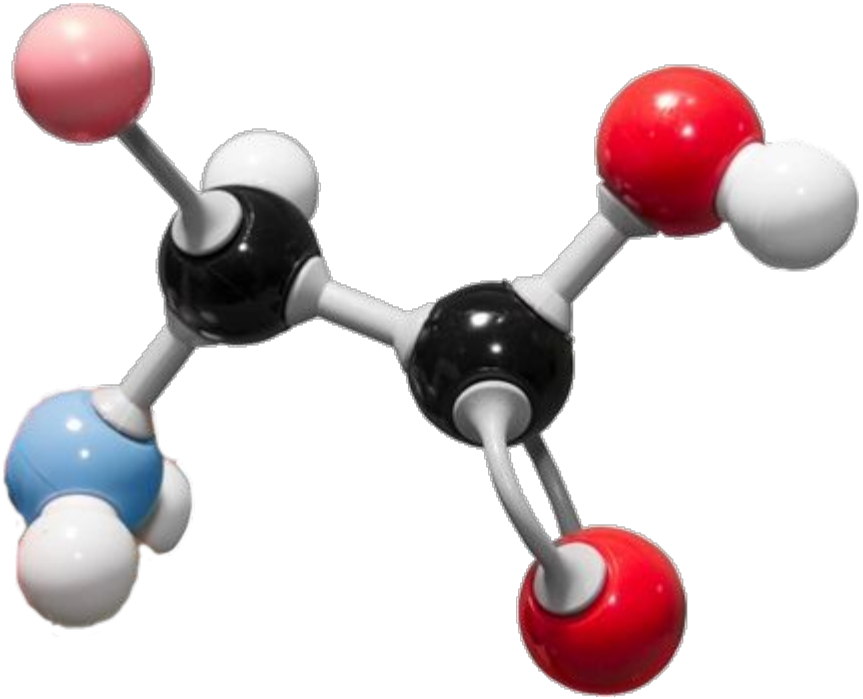
A protein is a polymer, made of monomers.



And the monomer is...

A protein is a polymer of monomers called amino acids.





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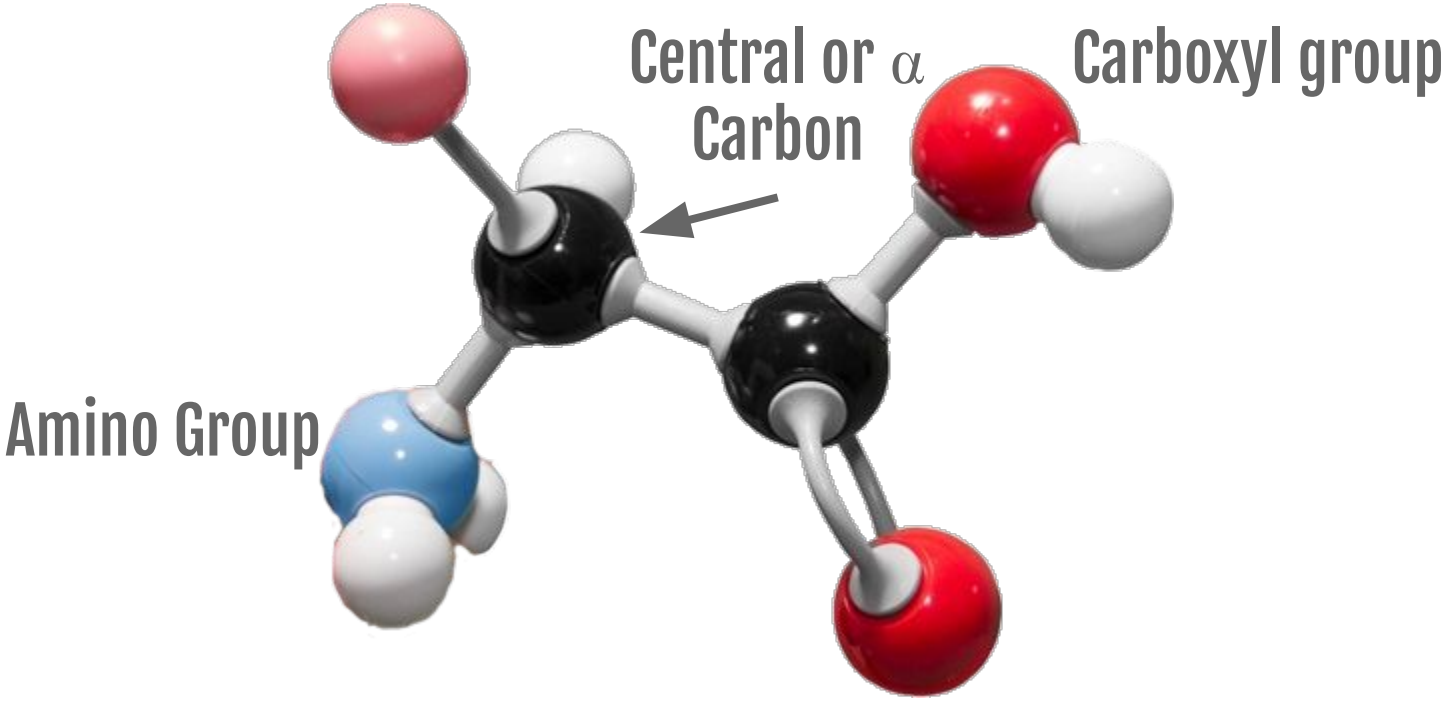
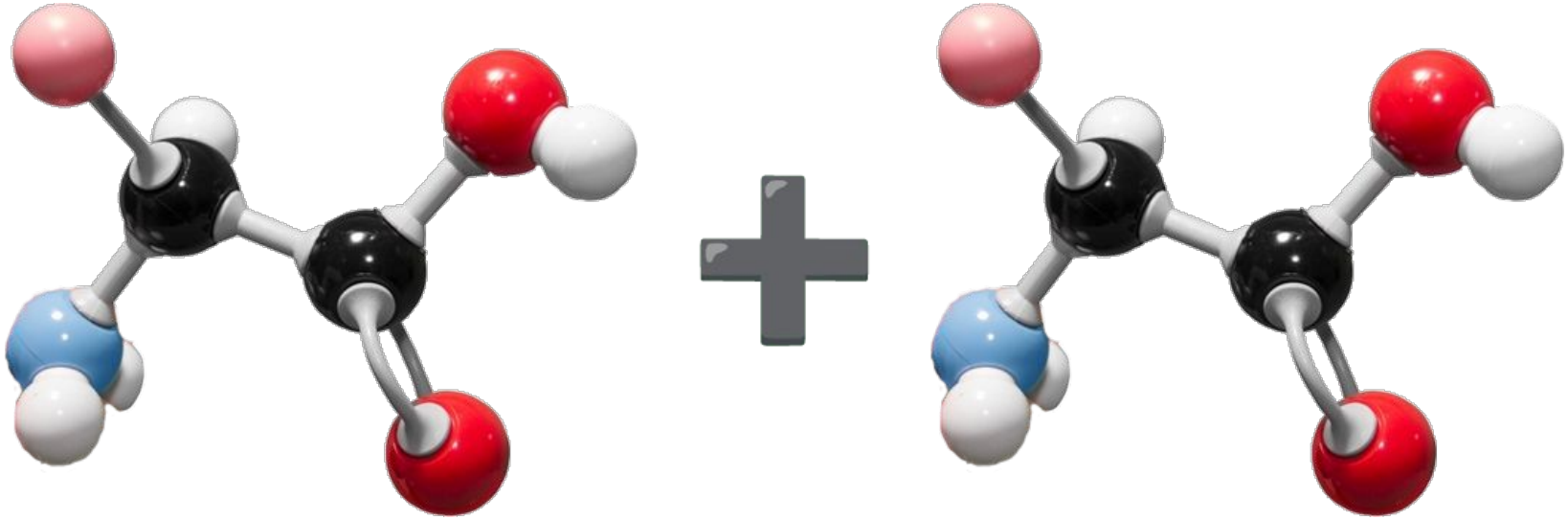
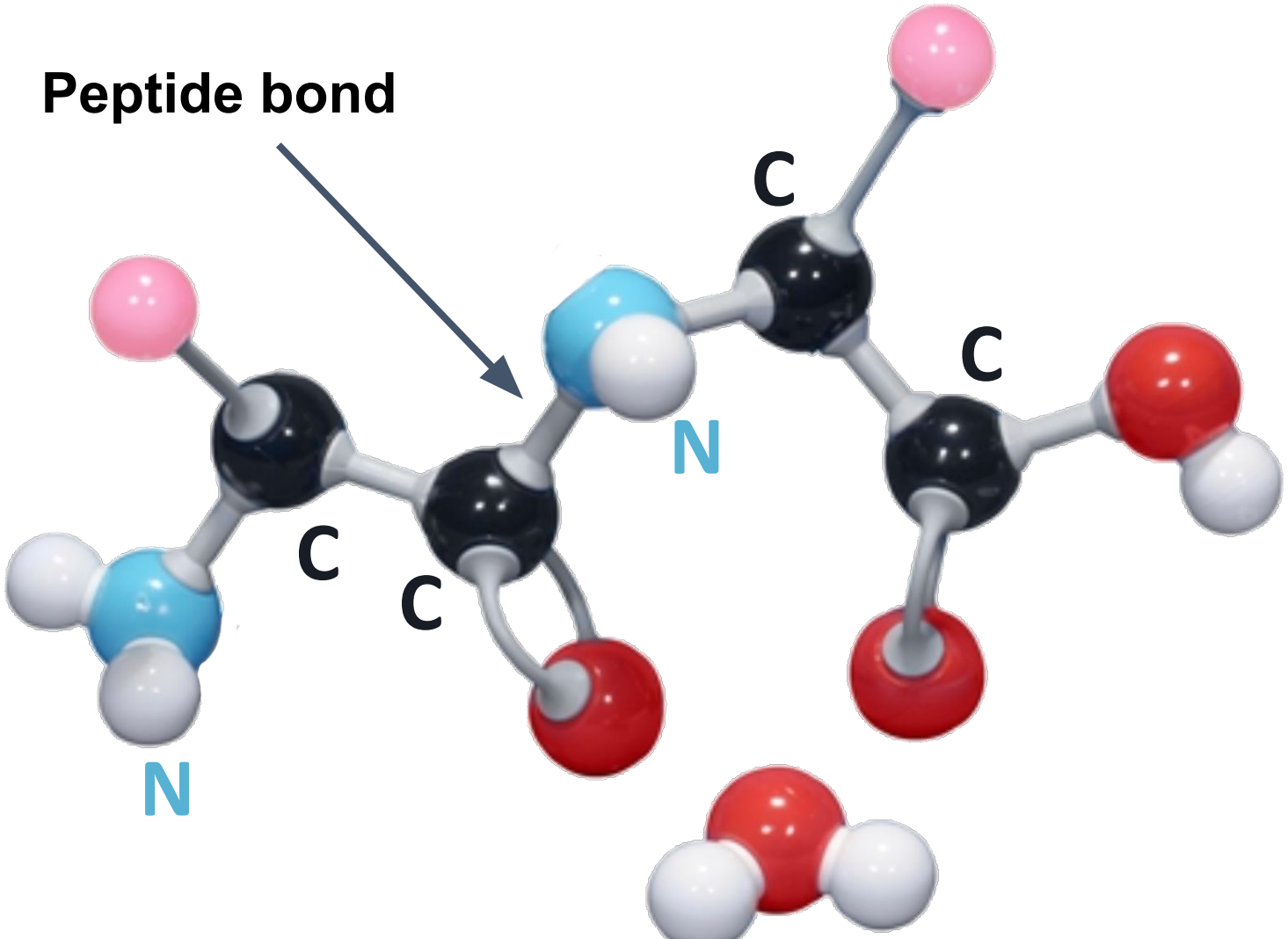


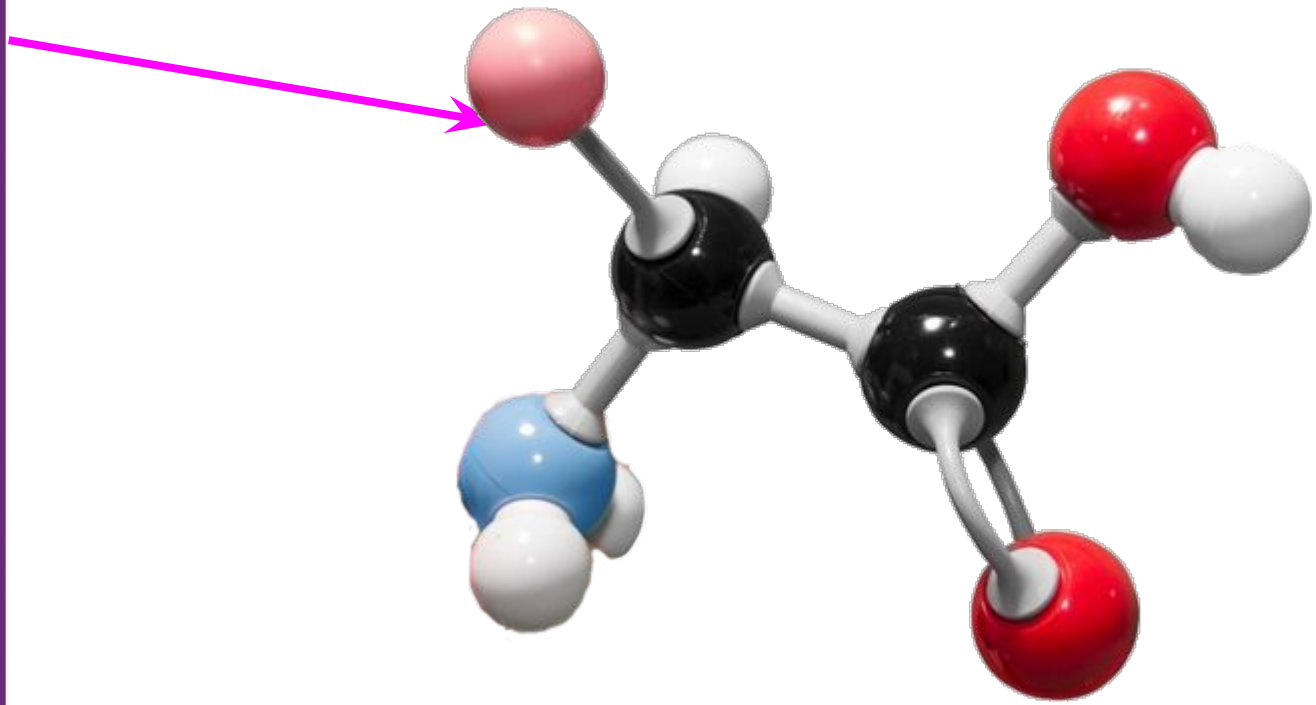
Let’s make a protein… Dehydration Synthesis.

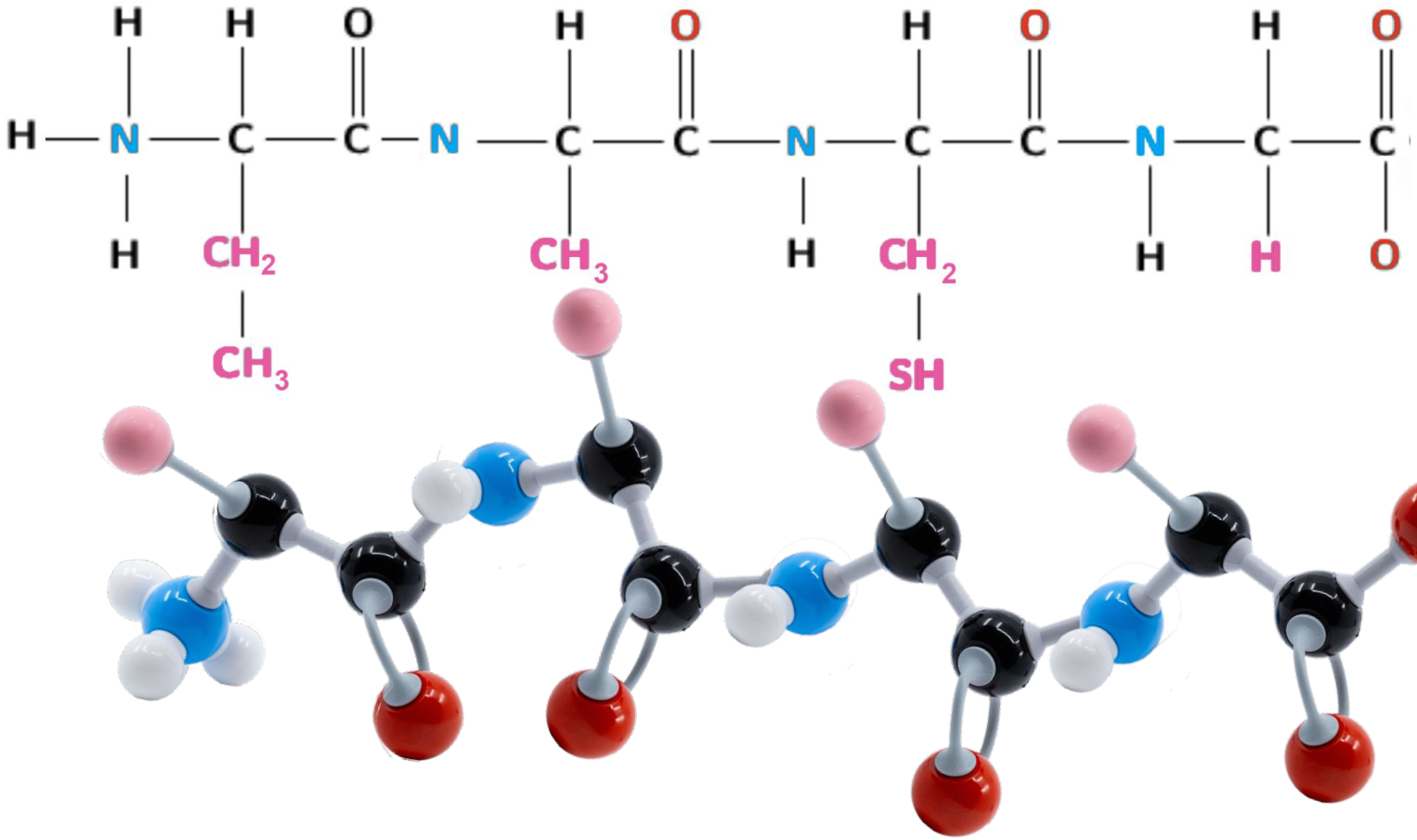



Peptide bond
C
C
C
N
N

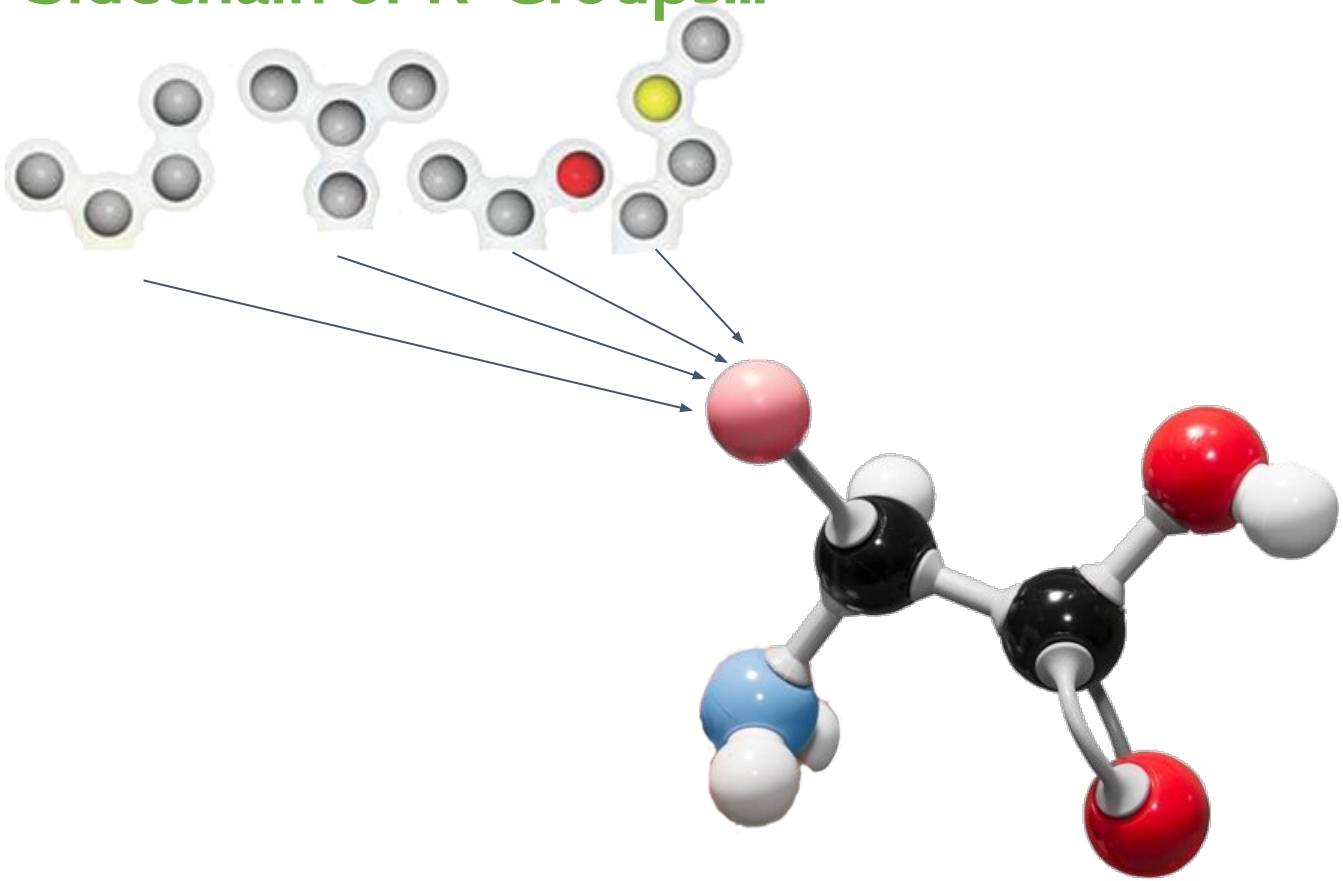
A protein is a polymer of amino acids

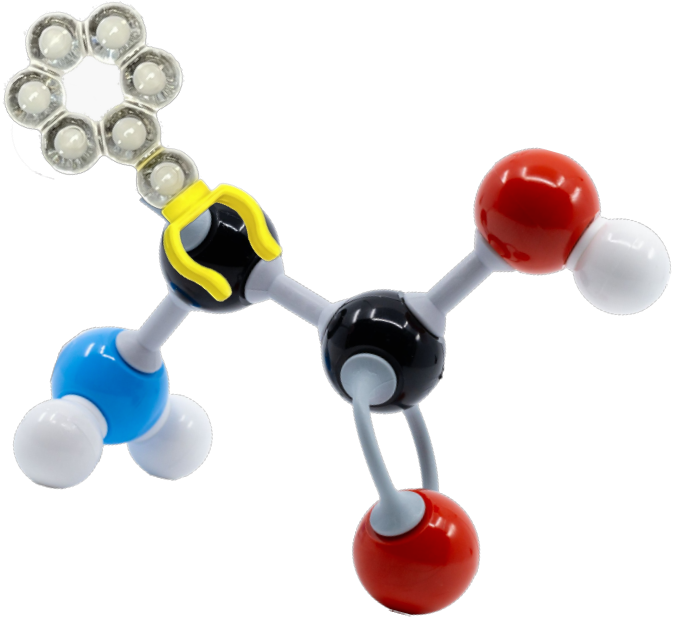
But what is is this???

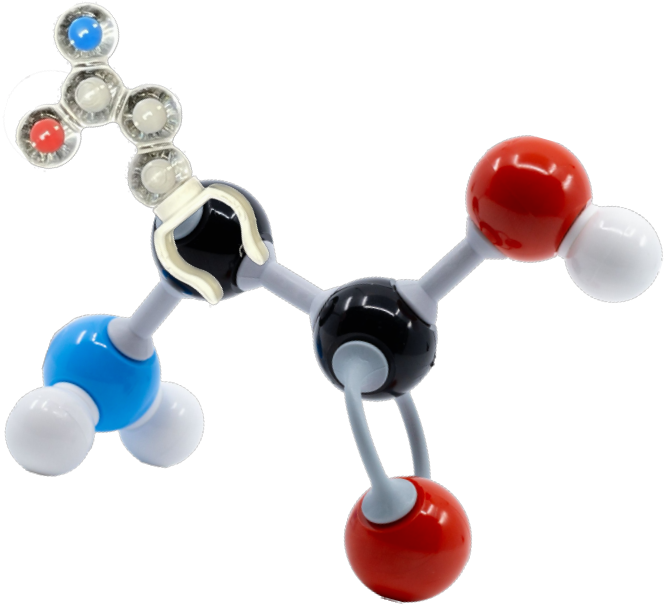



H
H
O
H
O
H
O
H
O
H
N
C
C
N
C
C
N
C
C
N
C
C
H
H
CH2
CH3
H
CH3
H
CH2
H
H
O
CH3
SH

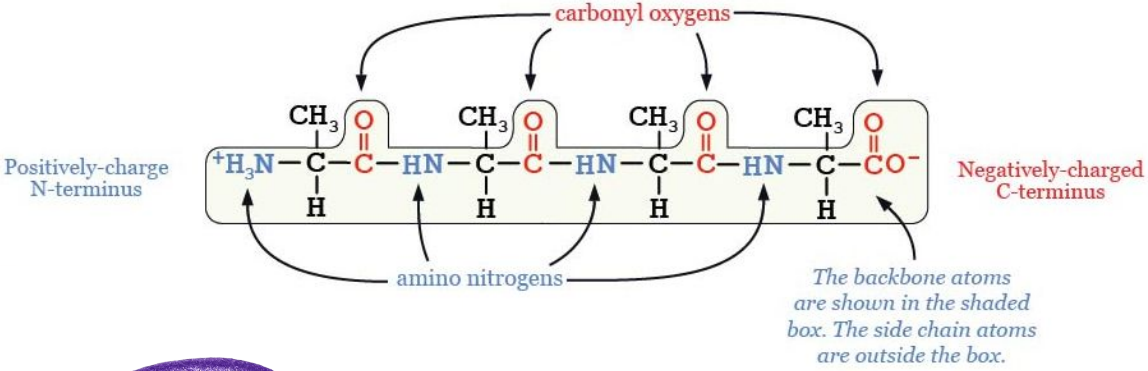
Sidechain or R-Groups...

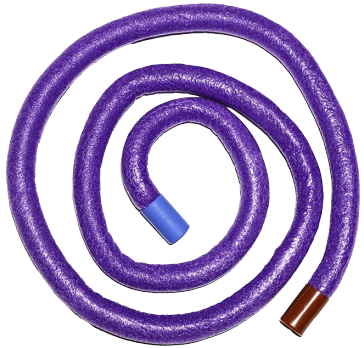




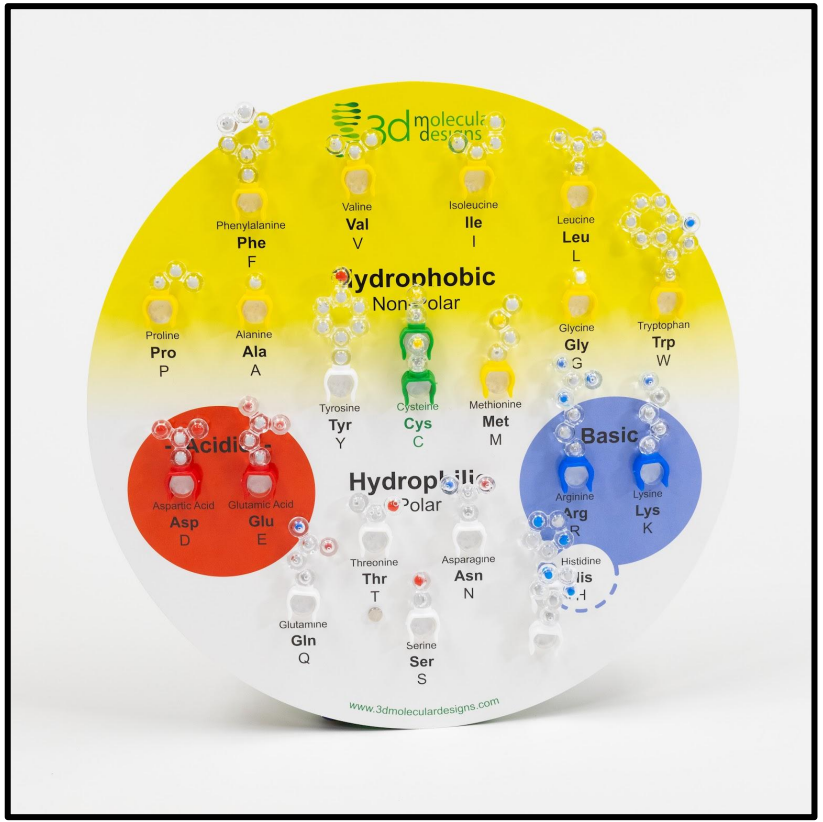


Another Way to Model a Polypeptide





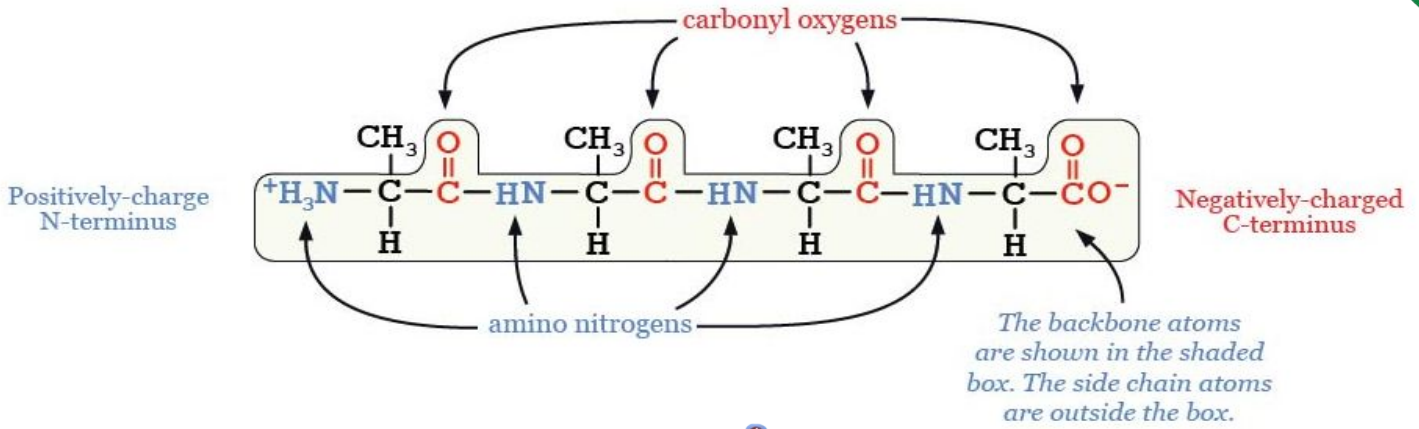
What observations can you make? What do you wonder?



Choose Your Own (Protein) Adventure

- 6 Hydrophobic R-groups (not valine… let's save that one)
- 2 Acidic R-groups
- 2 Basic R-groups
- 2 Cysteine R-groups
- 3 Polar R-groups (white)

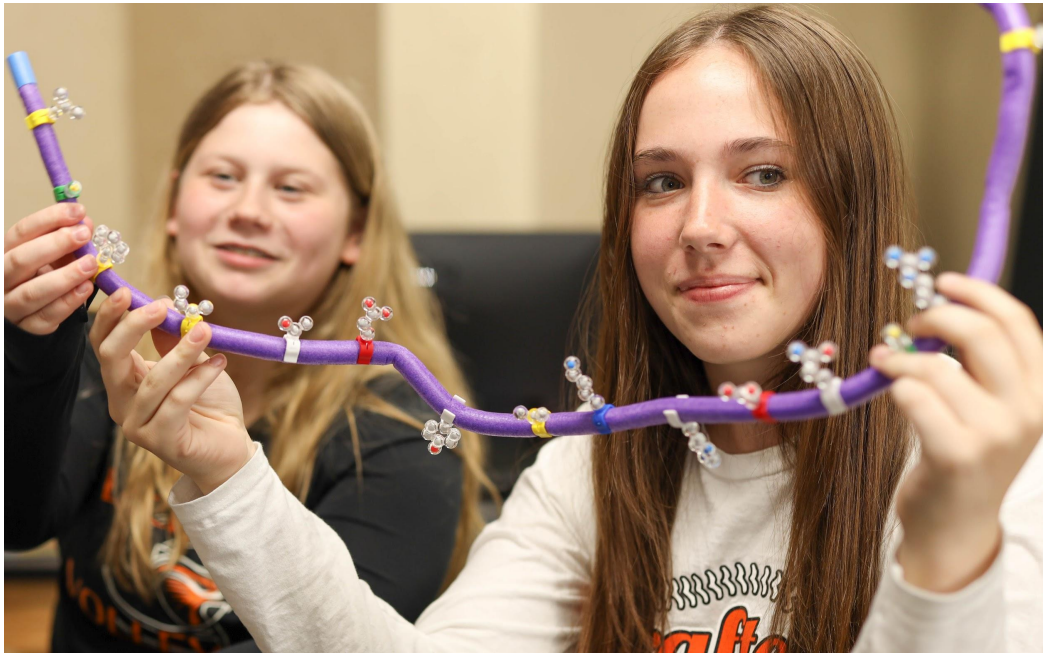
Primary Structure of a Polypeptide



Identifying Various Components of the Model

Primary Structure

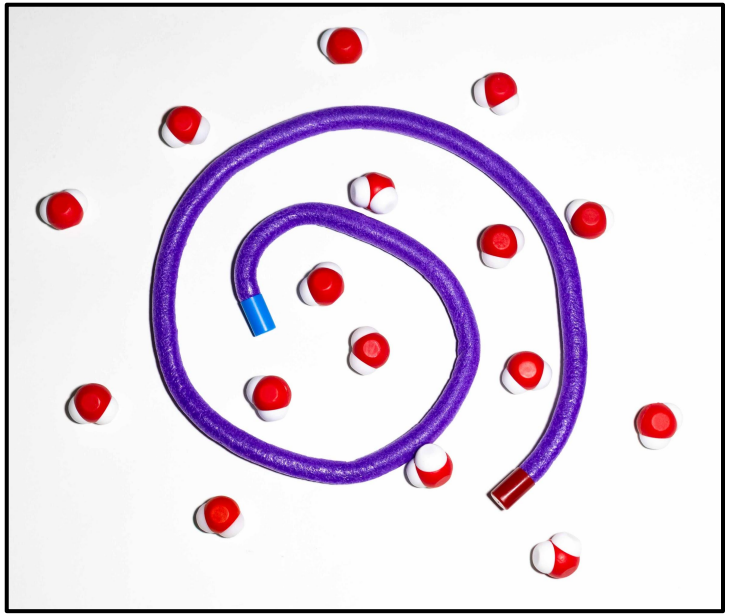
- R-groups
- Peptide bonds
- N-terminus
- C-terminus



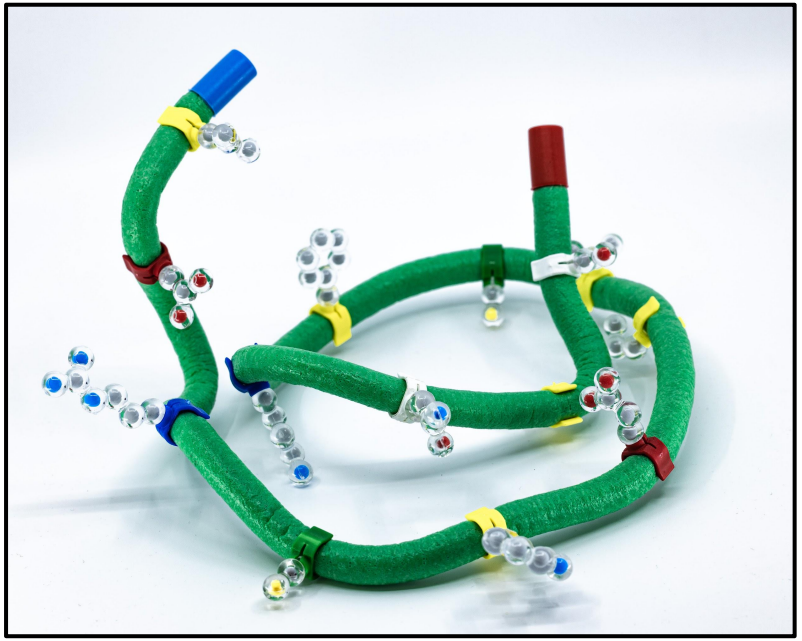
Modeling the behavior of proteins in water

How might different amino acid R groups interact with each other in water (an aqueous environment)?

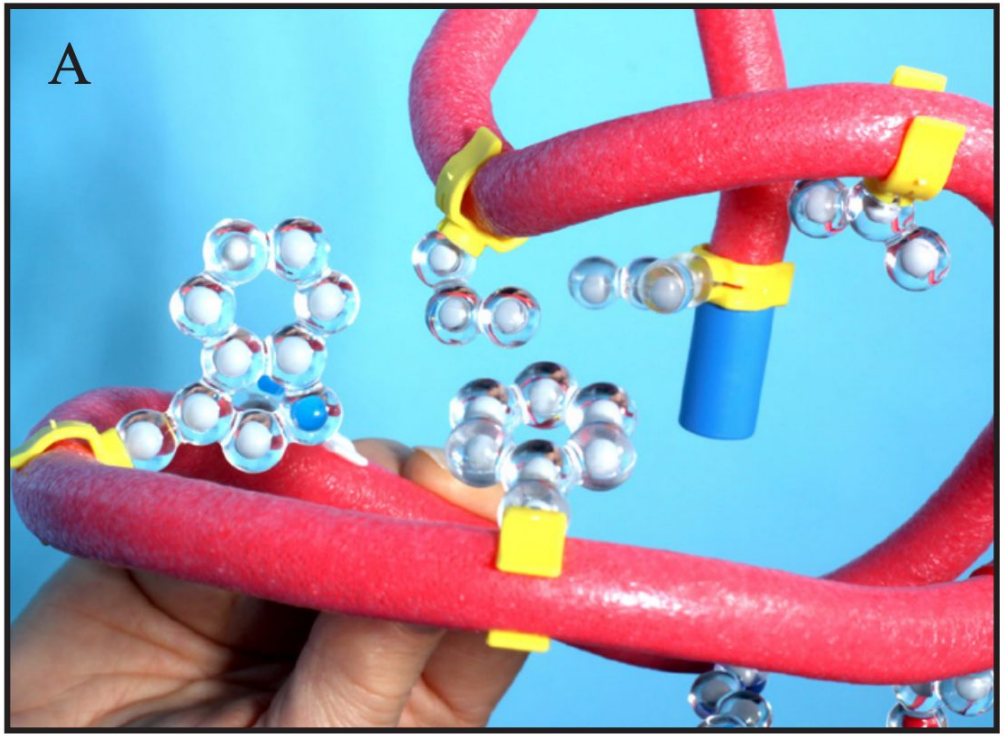
Model these interactions.



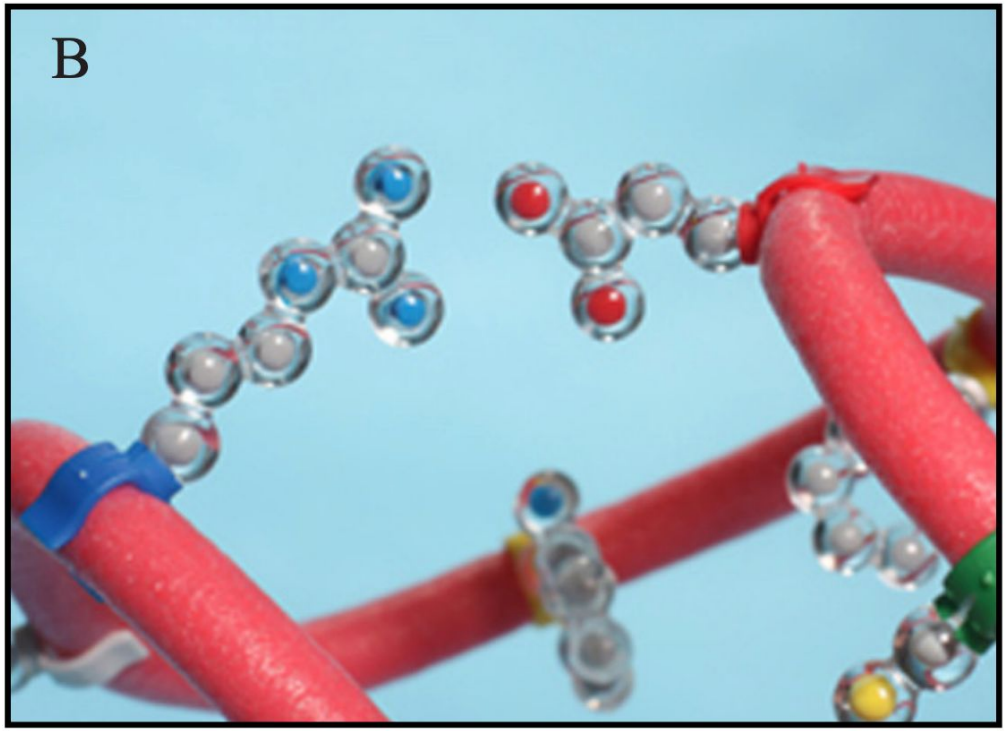
- Hydrogen bonds (hydrophilic R groups)
- Hydrophobic interactions (hydrophobic R groups)
- Ionic bonds (salt bridges)
- Disulfide bridges (cysteine-cysteine)



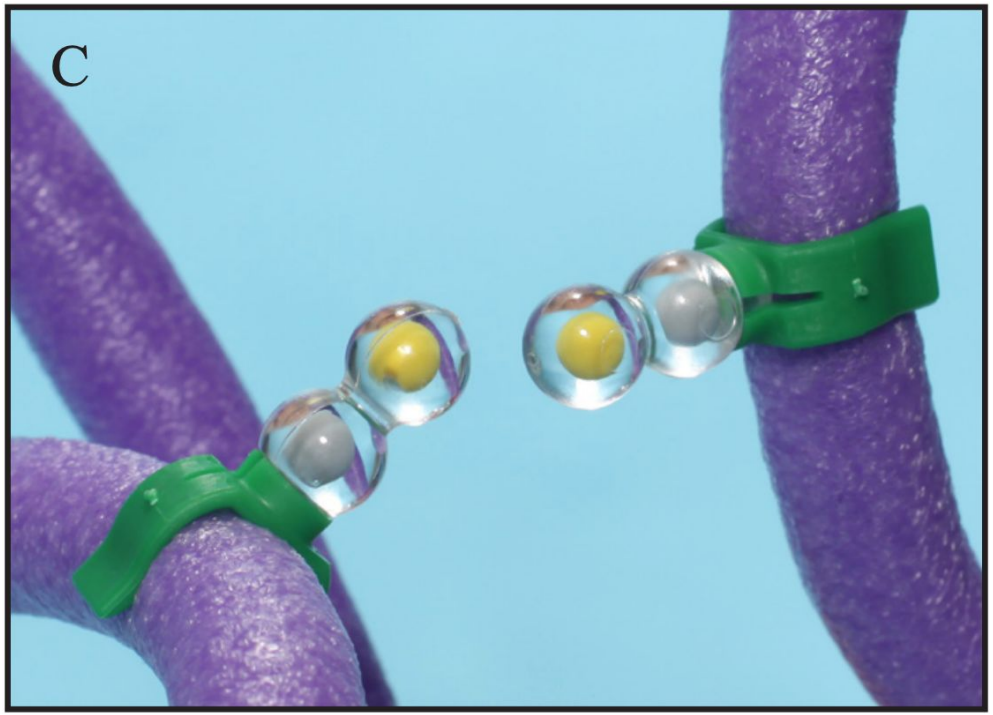
Where will we find hydrophobic side chains?



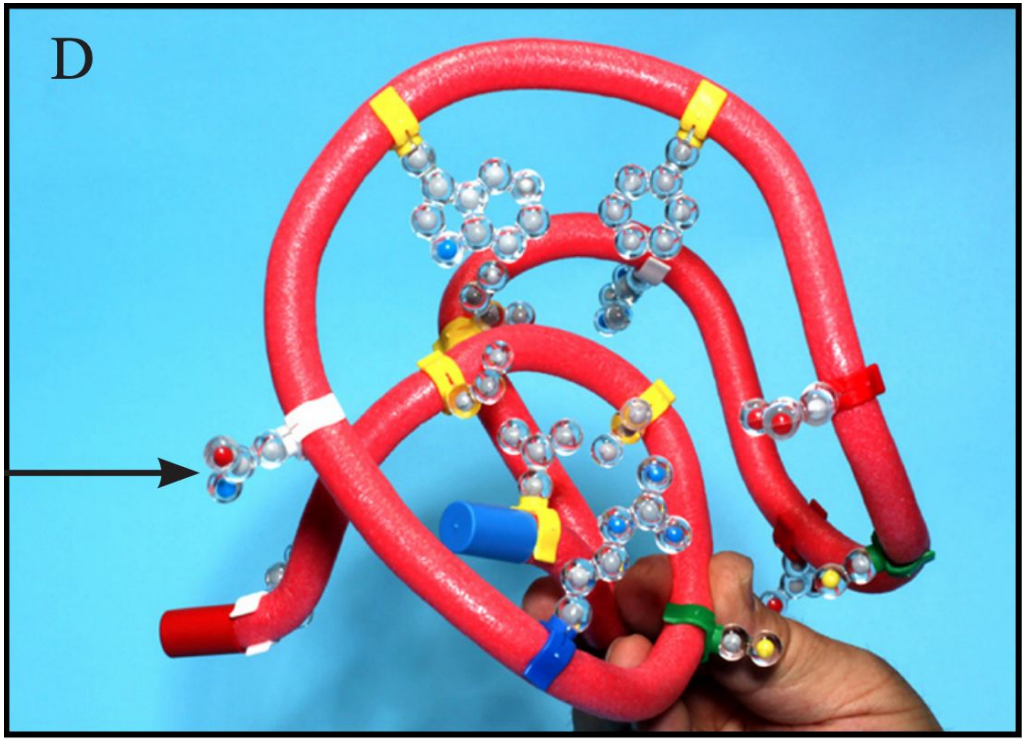
Acidic - Basic associations



Cysteines!



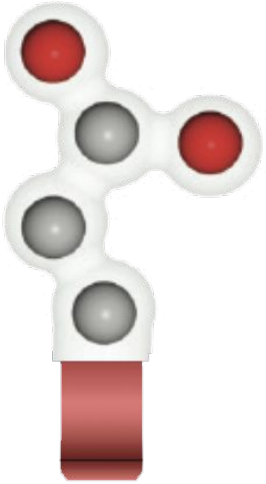
Hydrophilic?



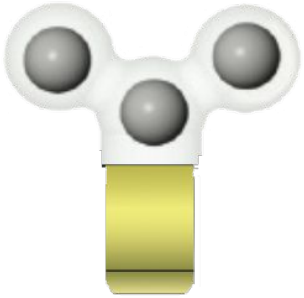
Back to β-hemoglobin

This negatively charged glutamic acid (E) is ⟶  replaced by a hydrophobic valine (V) in sickle cell anemia.

Find this...



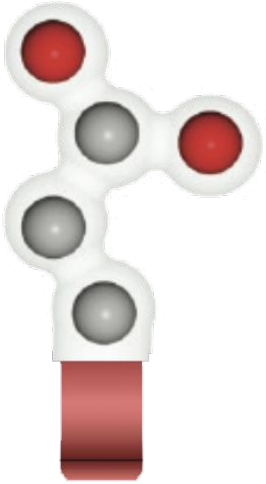
Replace with this



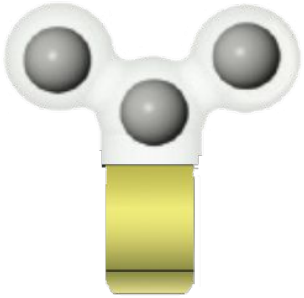
Back to β-hemoglobin

What can we learn from this?

Find this...

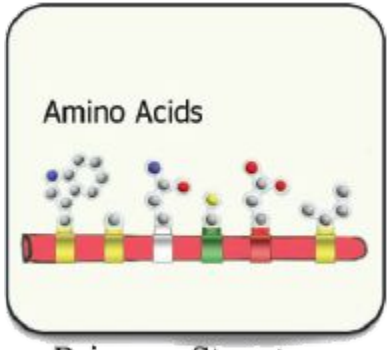


Replace with this

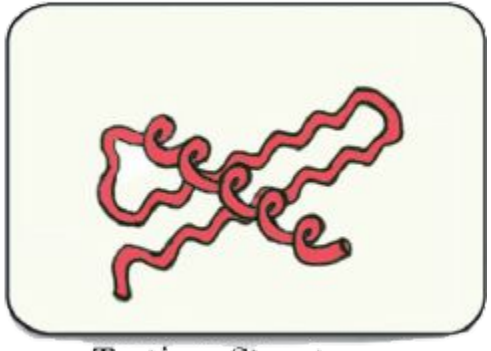


Back to β-hemoglobin

What have we not yet explored?

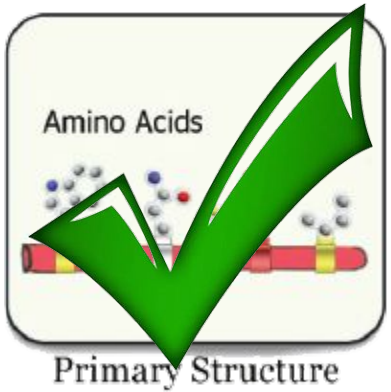


Primary Structure

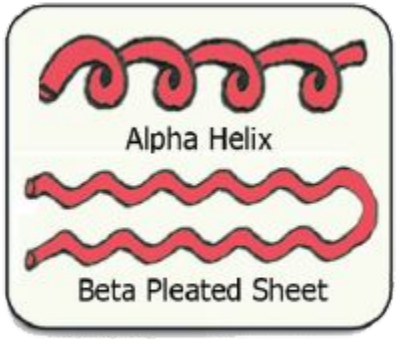

Tertiary Structure

Back to β-hemoglobin

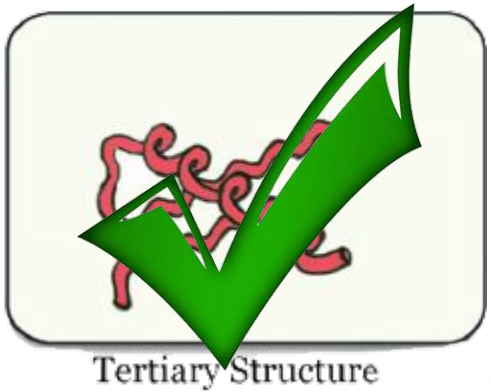
What have we not yet explored?



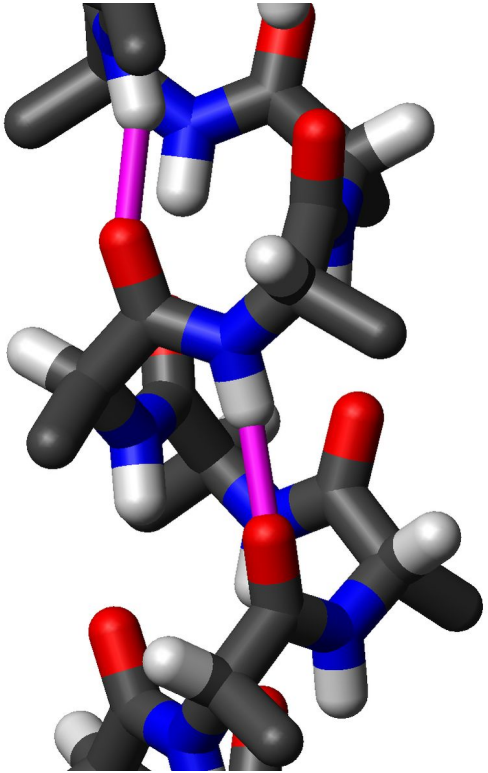
Primary Structure

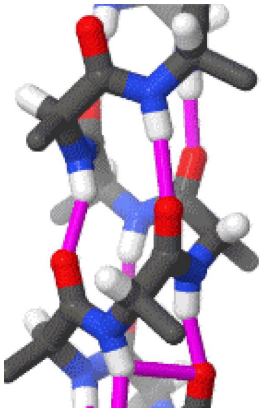


Secondary Structure


Tertiary Structure

What is secondary structure?



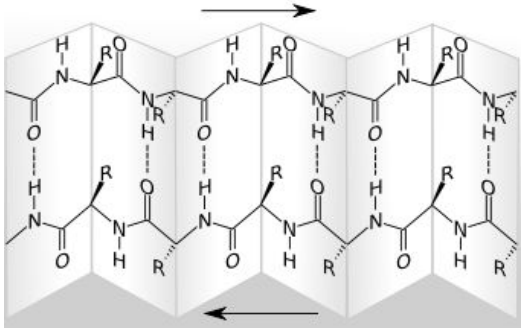


α Helix

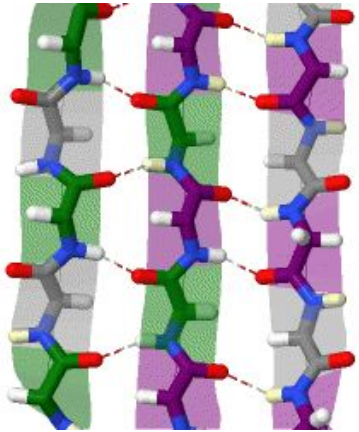
- C=O forms H bond with the N-H of the BACKBONE 4 amino acids ahead in the chain
- Influenced by the sequence of R groups, but R groups are not part of the H-bonds

https://en.wikipedia.org/wiki/Alpha_helix#/media/File:Alpha_helix_neg60_neg45_sideview.png

What is secondary structure?



https://commons.wikimedia.org/wiki/File:Beta-Faltblatt.svg



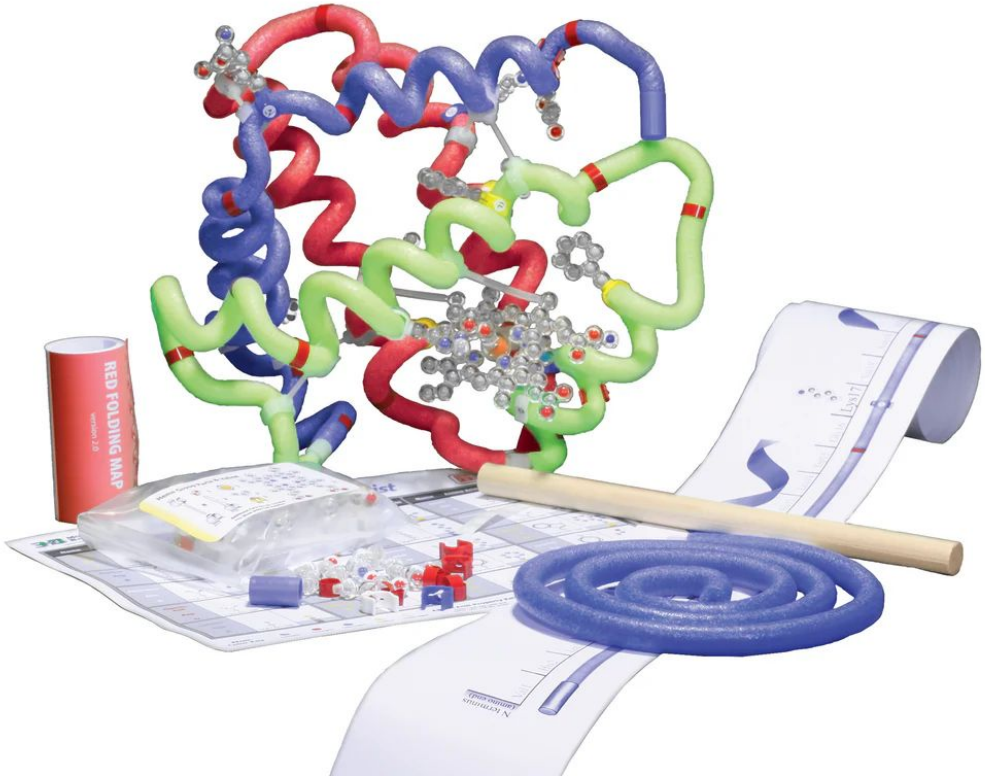
https://upload.wikimedia.org/wikipedia/commons/5/5e/Animated_Beta_sheet.gif

β sheets:

- H bonds between C=O and N-H of the BACKBONE.
- Influenced by the sequence of R groups, but R groups are not part of the H bonds

Secondary structure in β-globin

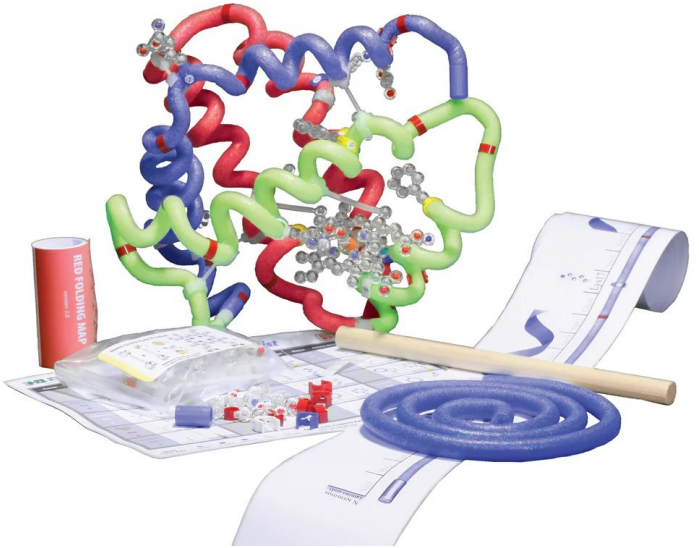
ß-Globin Folding Kit©



Secondary structure in β-globin

ß-Globin Folding Kit©

- 146 amino acids per β-globin chain
- Features secondary structure and a few side chains
- Model splits chain into 3 segments to make it easier to fold...

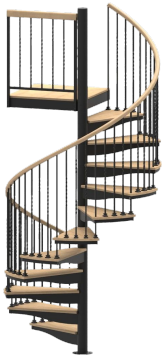


Using your strip as a guide...

- Place the side chains and clips in the correct locations.

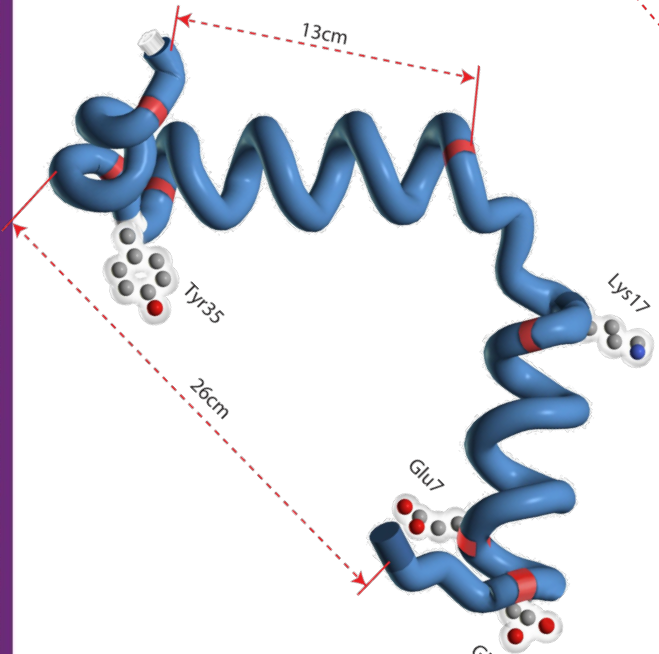
Using your strip as a guide...

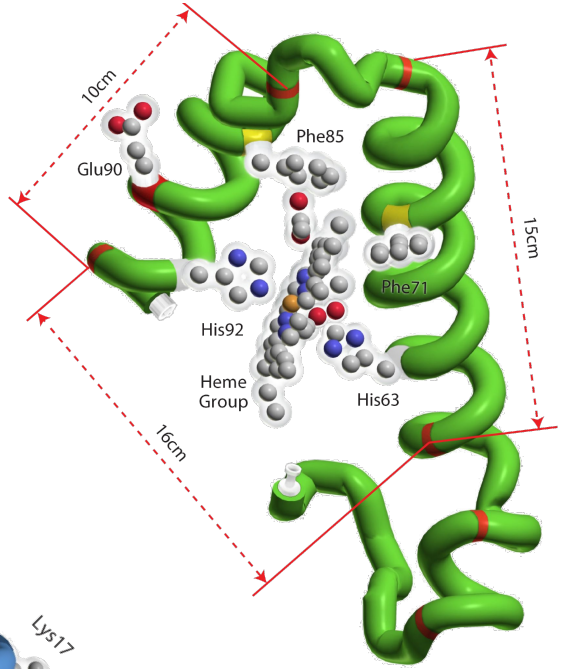
- Place the side chains and clips in the correct location.
- Using the wooden rod as a guide, turn your alpha helix

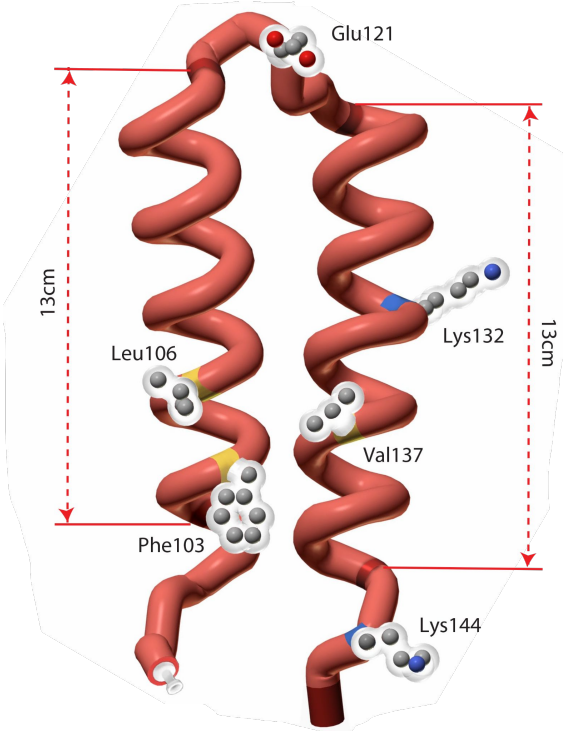


Using your strip as a guide...

- Place the side chains in the correct location.
- Using the wooden rod as a guide, turn your alpha helix
- Green team: place the heme group.

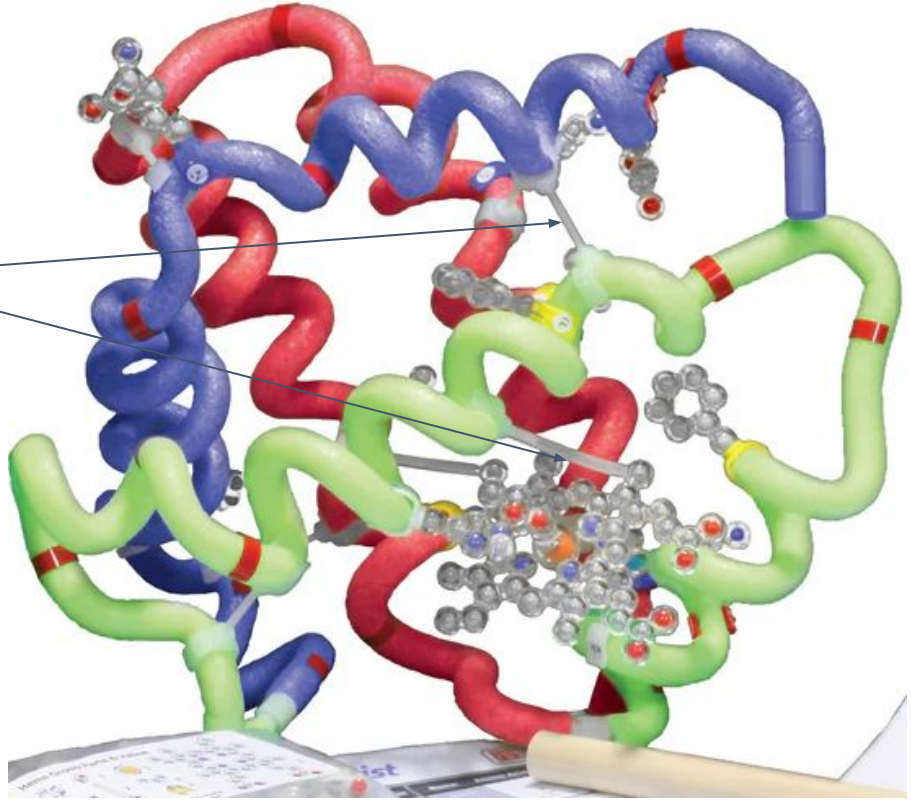

13cm
Tyr35
Lys17
26cm
Glu7


10cm
Glu90
Phe85
15cm
Phe71
His92
Heme
Group
His63
16cm

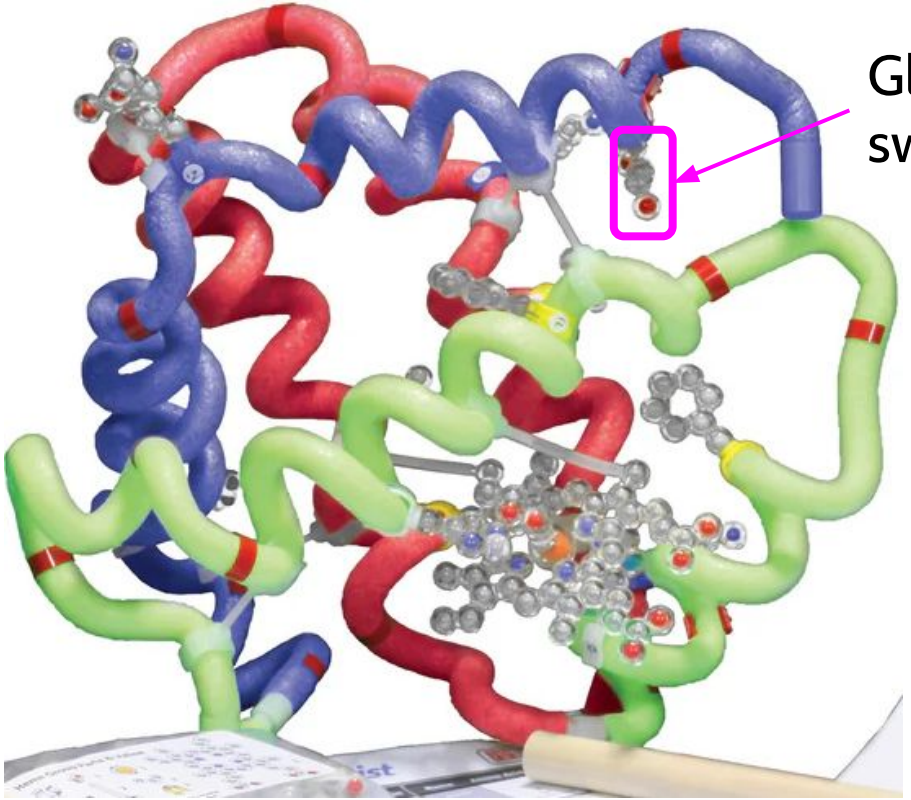

Glu121
13cm
Lys132
13cm
Leu106
Val137
Phe103
Lys144

Come together and put it all together.

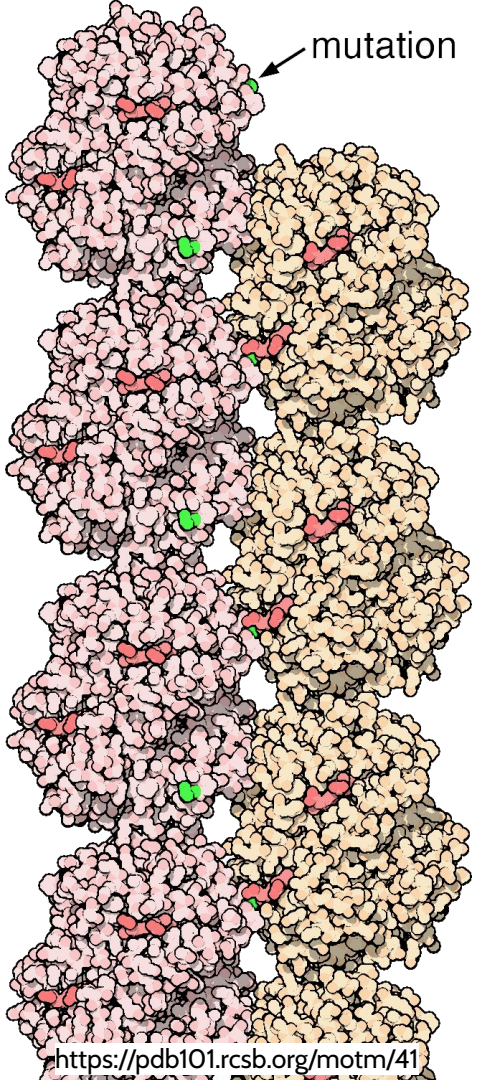
Clips to help stabilize



What would happen now?



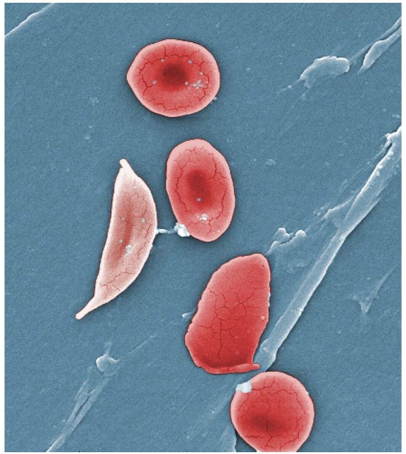
Glutamic Acid 6 switches to valine



https://pdb101.rcsb.org/motm/41

In low oxygen environments:

The hydrophobic valine sticks into a hydrophobic patch on a neighboring molecule.



https://commons.wikimedia.org/wiki/File:1911_Sickle_Cells.jpg

FDA NEWS RELEASE

FDA Approves First Gene Therapies to Treat Patients with Sickle Cell Disease

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For Immediate Release: December 08, 2023

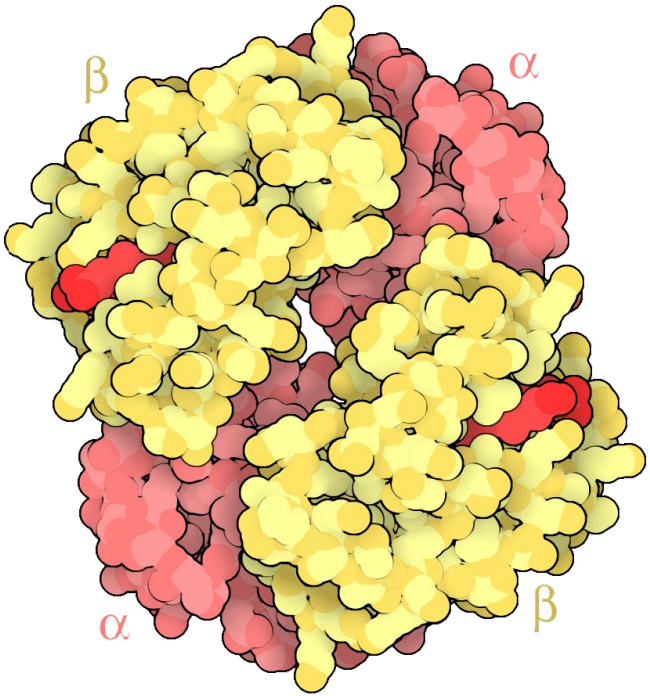
Today, the U.S. Food and Drug Administration approved two milestone treatments, Casgevy and Lyfgenia, representing the first cell-based gene therapies for the treatment of sickle cell disease (SCD) in patients 12 years and older. Additionally, one of these therapies, Casgevy, is the first FDA-approved treatment to utilize a type of novel genome editing technology, signaling an innovative advancement in the field of gene therapy.

Sickle cell disease is a group of inherited blood disorders affecting approximately 100,000 people in the U.S. It is most common in African Americans and, while less prevalent, also

https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease

But how does this work?

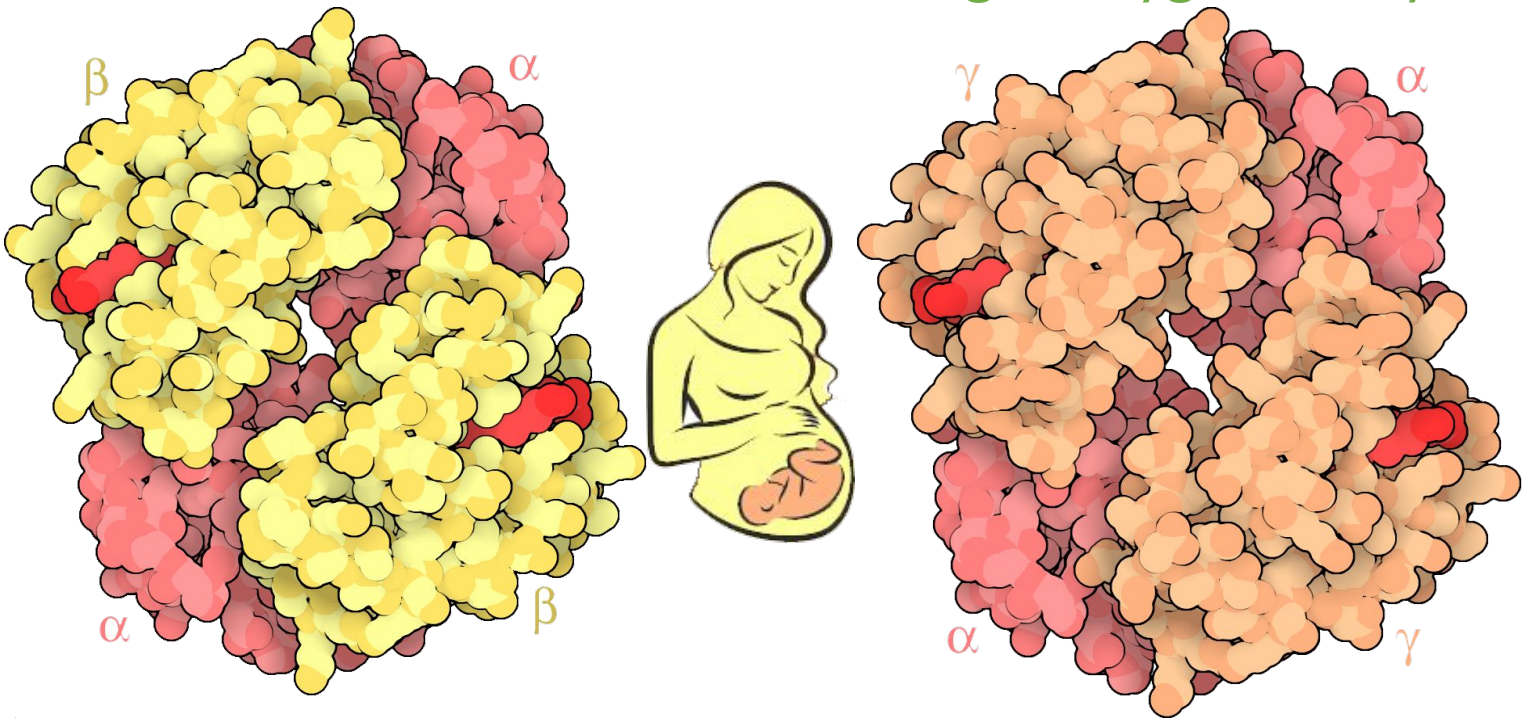
Two forms of human hemoglobin



“Adult” hemoglobin

Two forms of human hemoglobin

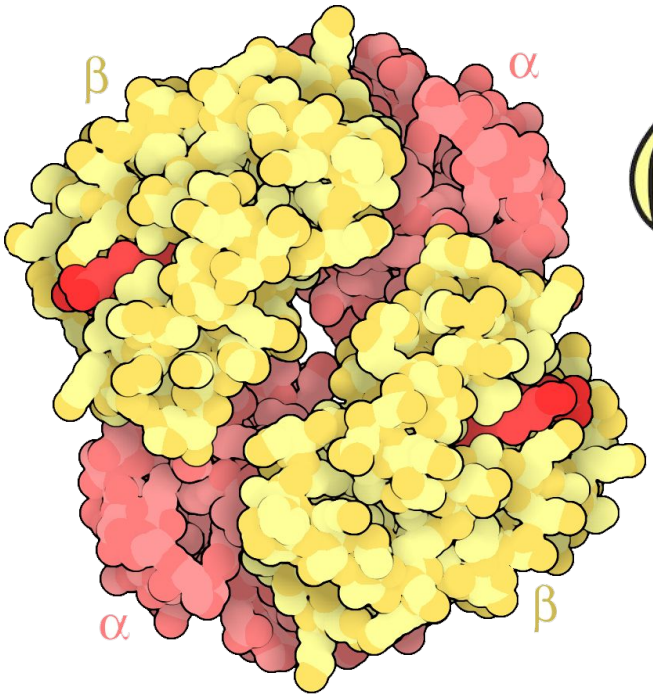
Higher oxygen affinity



"Adult" hemoglobin

Fetal hemoglobin

Two forms of human hemoglobin



"Adult" hemoglobin

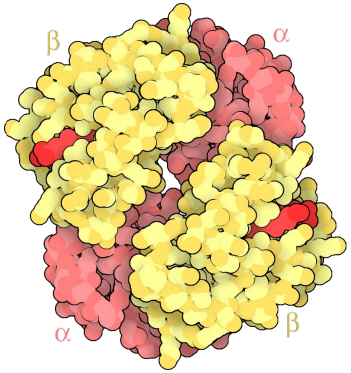
Higher oxygen affinity

BCL11A suppresses production of γ-globin after birth

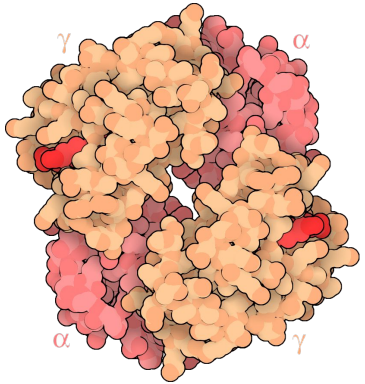
Fetal hemoglobin

Hereditary persistence of HbF (HPBF)

Individuals with mutations in the enhancer region of the erythroid specific BCL11A gene continue to produce γ-globin after birth.



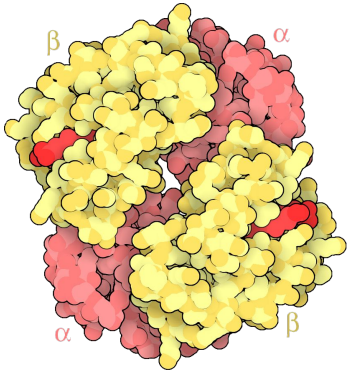
"Adult" hemoglobin



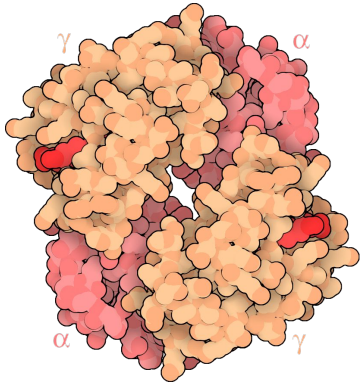
Fetal hemoglobin

Hereditary persistence of HbF (HPBF)

In other words, these mutations suppress the production of the suppressor protein!

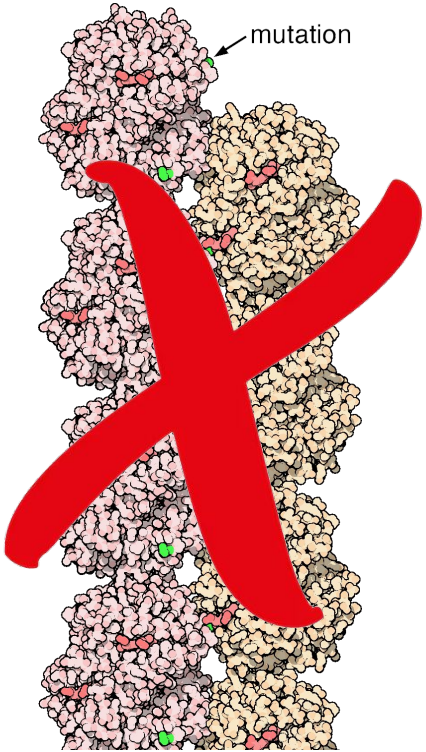


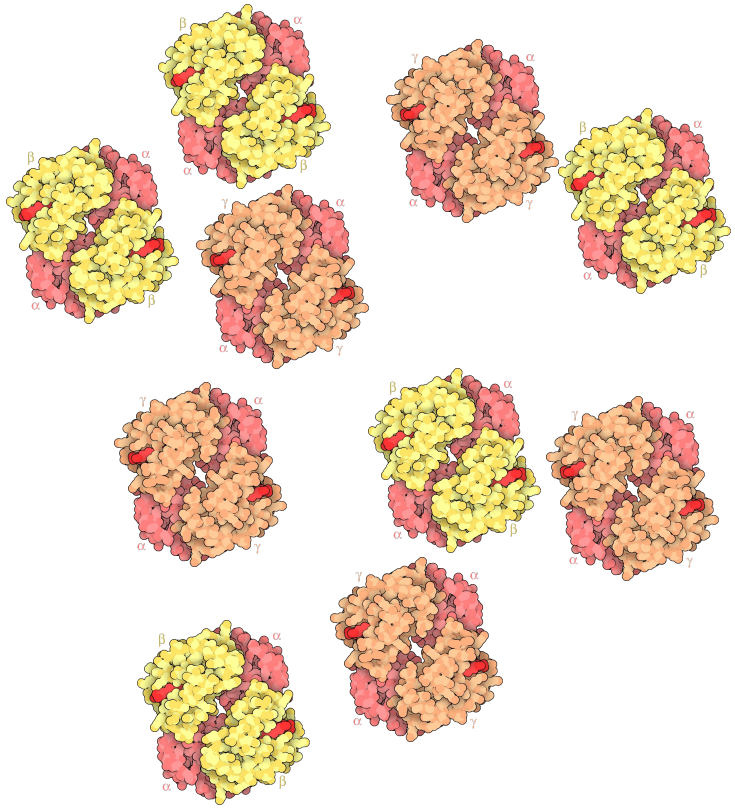
"Adult" hemoglobin



Fetal hemoglobin

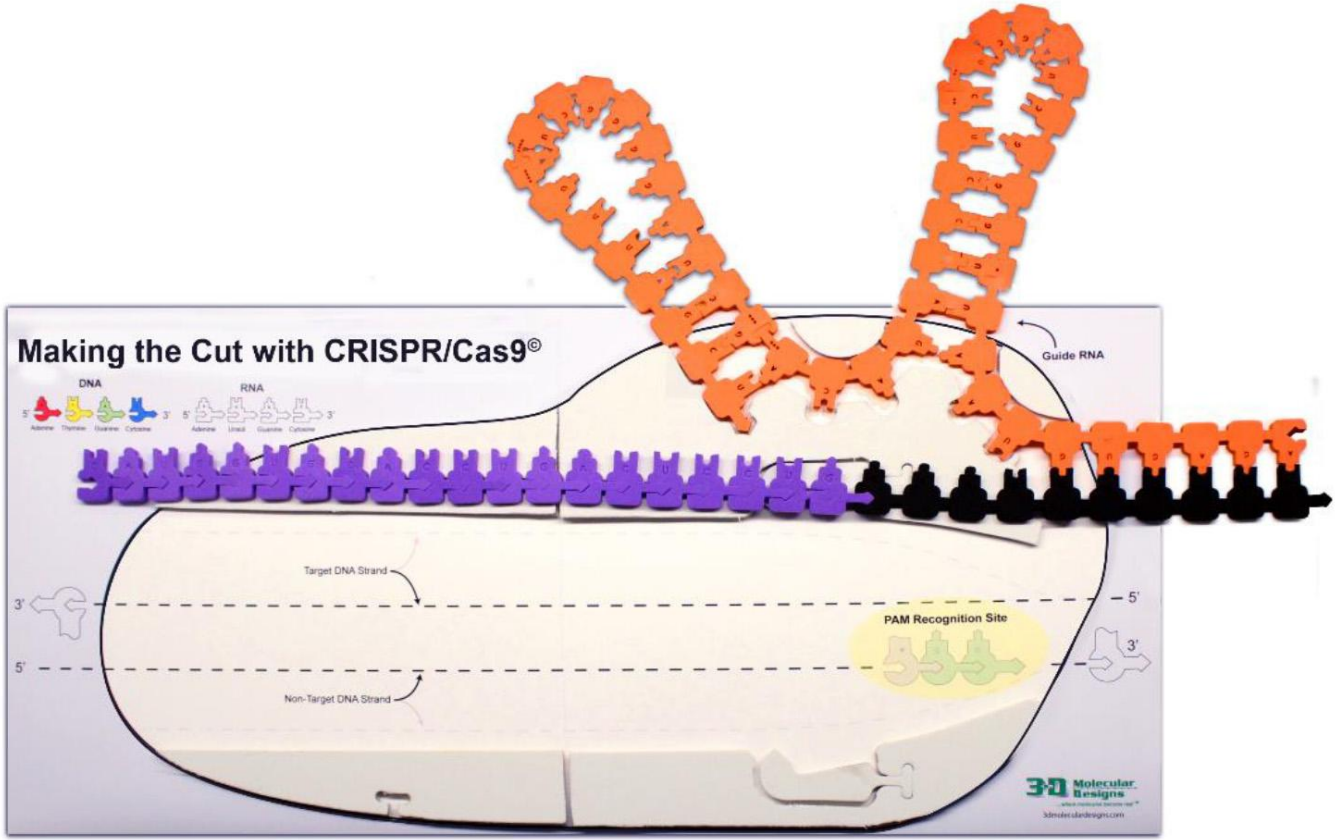
Casgevy–disrupts the enhancer



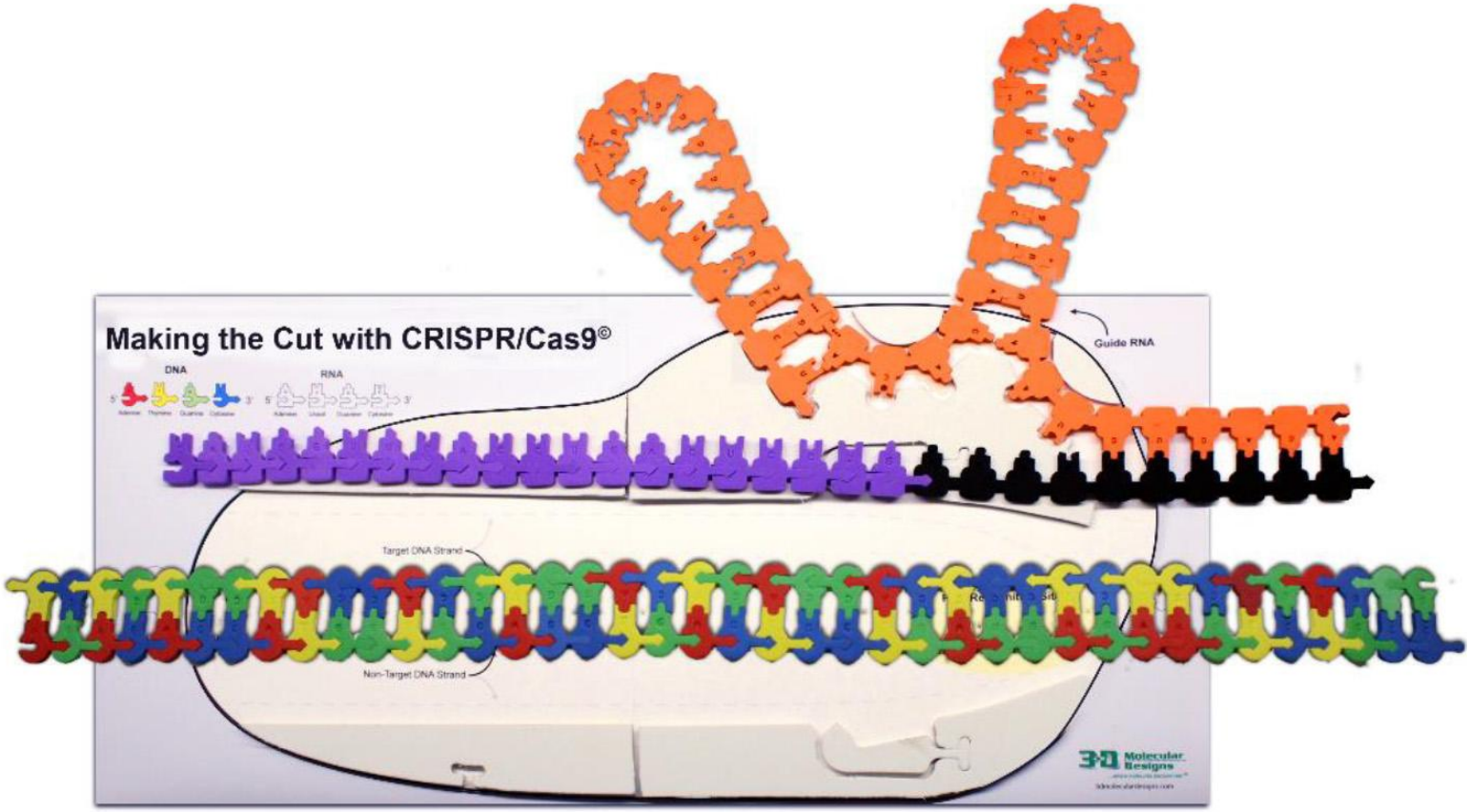


Some Basic Biology

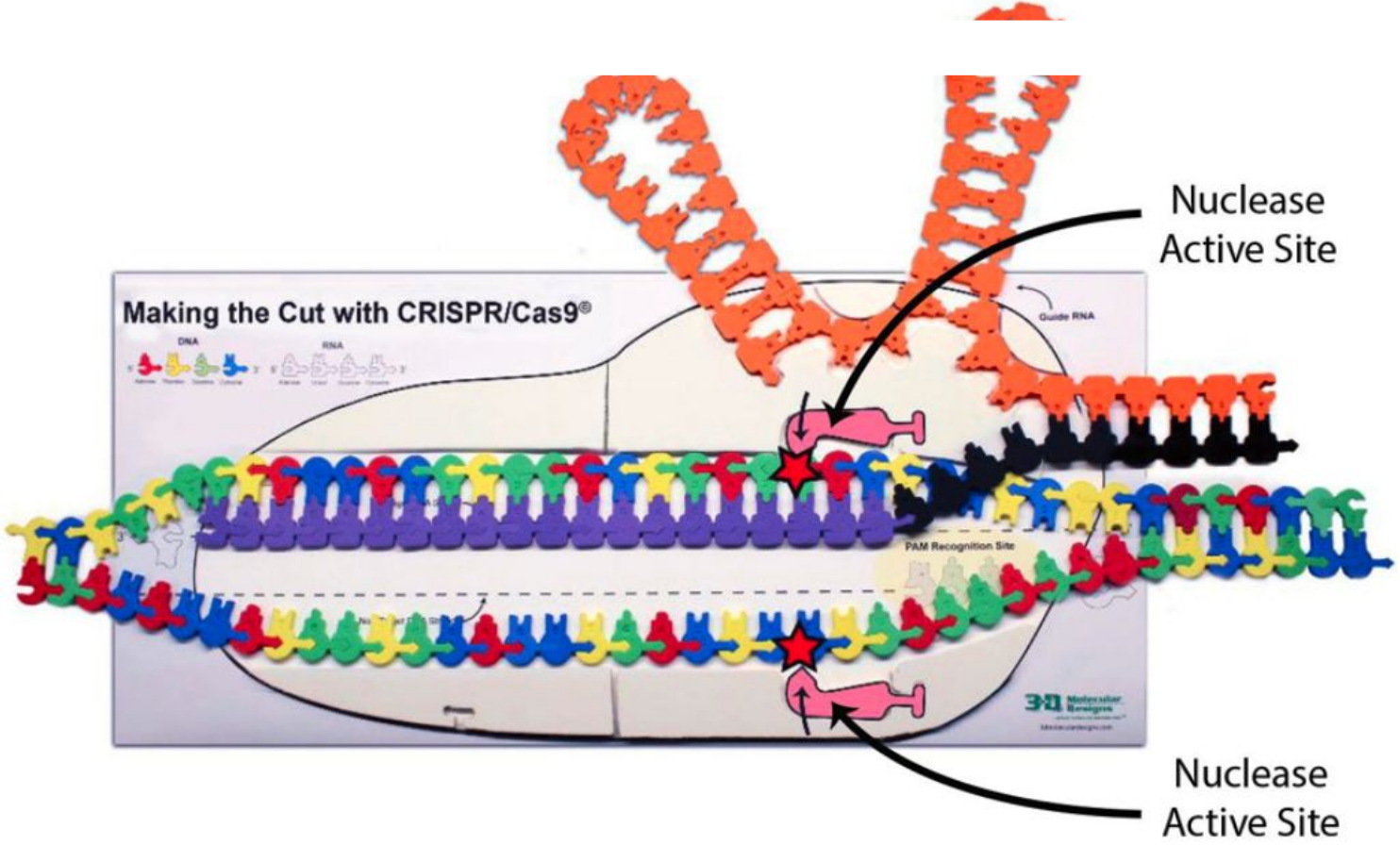
How to CRISPR



How to CRISPR



How to CRISPR



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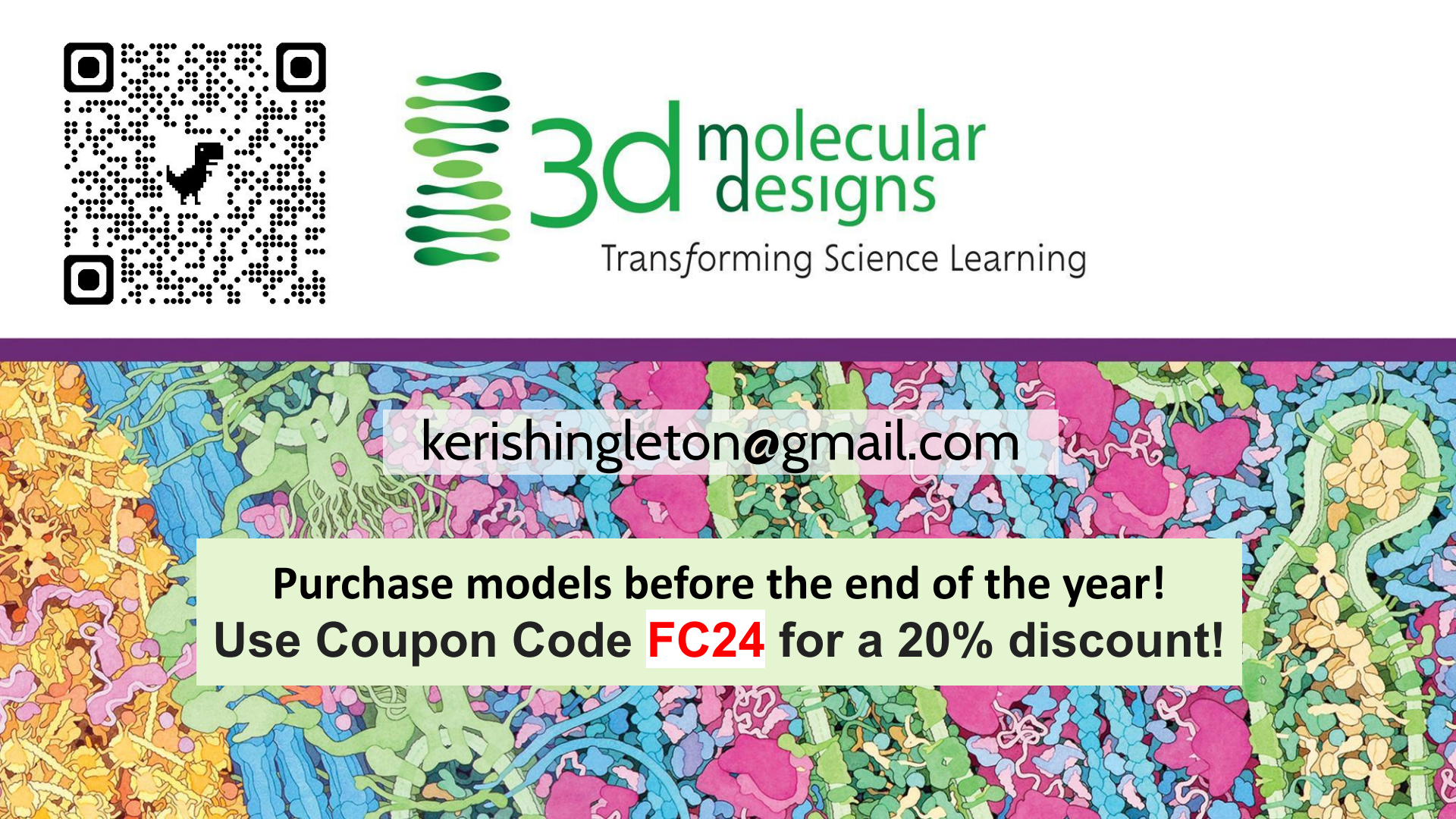
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