Molecular Modeling 2019 -- Lecture 26

Interpretation of a homology model
Review session

Interpreting Homology models

- What is the function of the target? (if known)
- What is the function of the template?
- What is the ligand?
  - If it's an enzyme, the ligand is the substrate.
  - If it's a signaling protein, the ligand may be a kinase, or a peptide, or DNA, or ATP, or another protein.
  - If it's a structural protein, the ligand may be itself, another protein, a carbohydrate, a lipid.
- Where does the ligand bind the template? (if known)
- What residues are involved in binding (and/or activity)?
- Are any of those residues different in the target?

The story of DHFR

Drug design and drug resistance.

Dihydrofolate reductase (DHFR) is a keystone enzyme. Without it, the cell can't synthesize thymidine, which means it can't replicate DNA, which means it can't divide.

Drugs such as methotrexate, that inhibit DHFR, kill fast growing cells, such as aggressive cancers, hair follicles and the cells of the gastrointestinal tract.

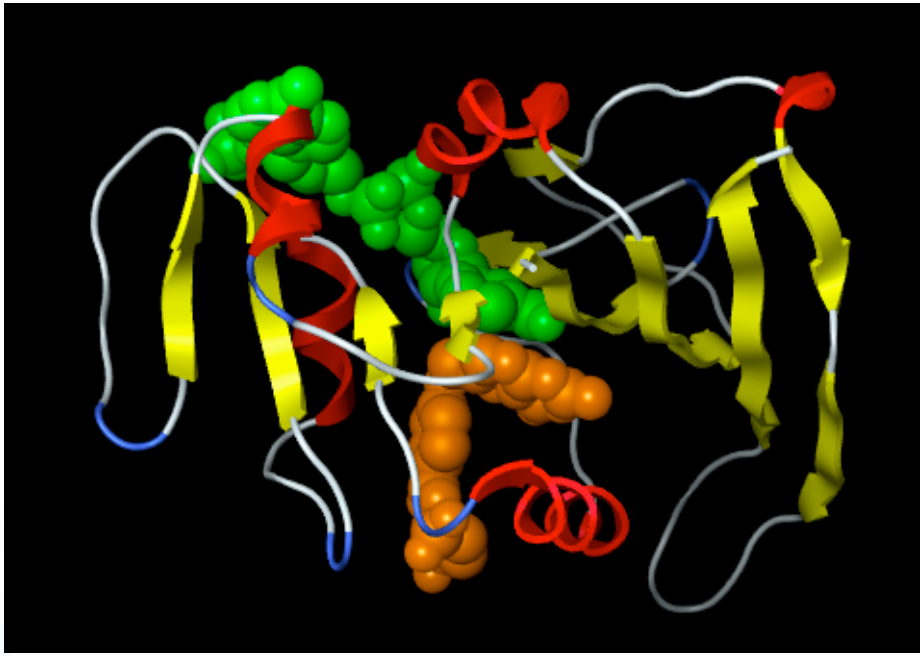
Bacterial DHFR is a drug target for antibiotics such as trimethoprim, which binds much more tightly to bacterial DHFRs than to human DHFR.

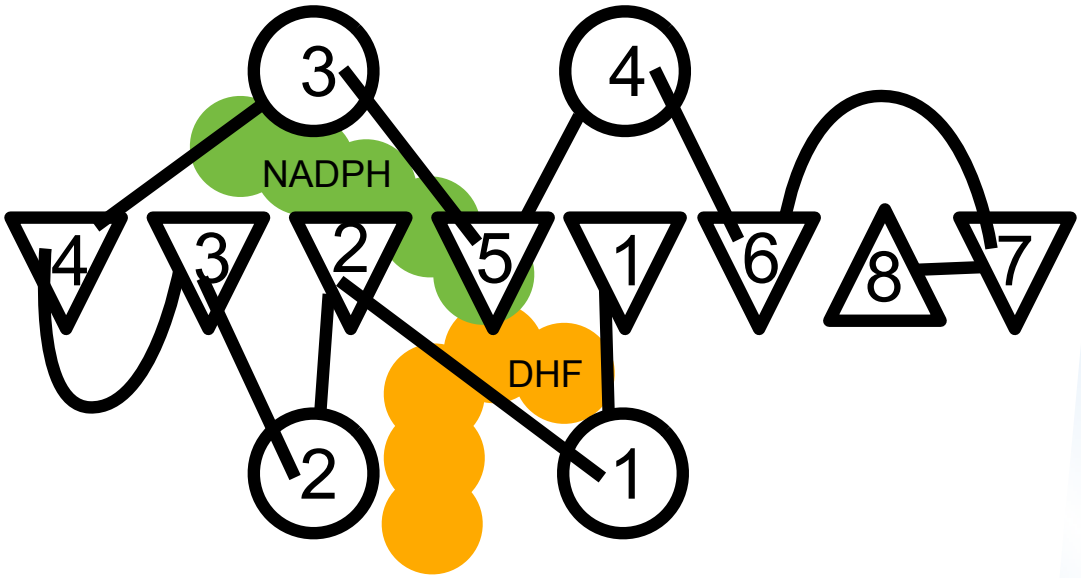
Mutations in bacterial DHFR have produced drug resistance!

Sibley, Carol Hopkins, et al. "Pyrimethamine–sulfadoxine resistance in Plasmodium falciparum: what next?." *Trends in parasitology* 17.12 (2001): 570-571.

Modeling DHFR

dihydrofolate reductase





2 substrates: NADPH (green) and dihydrofolate (DHF, orange)

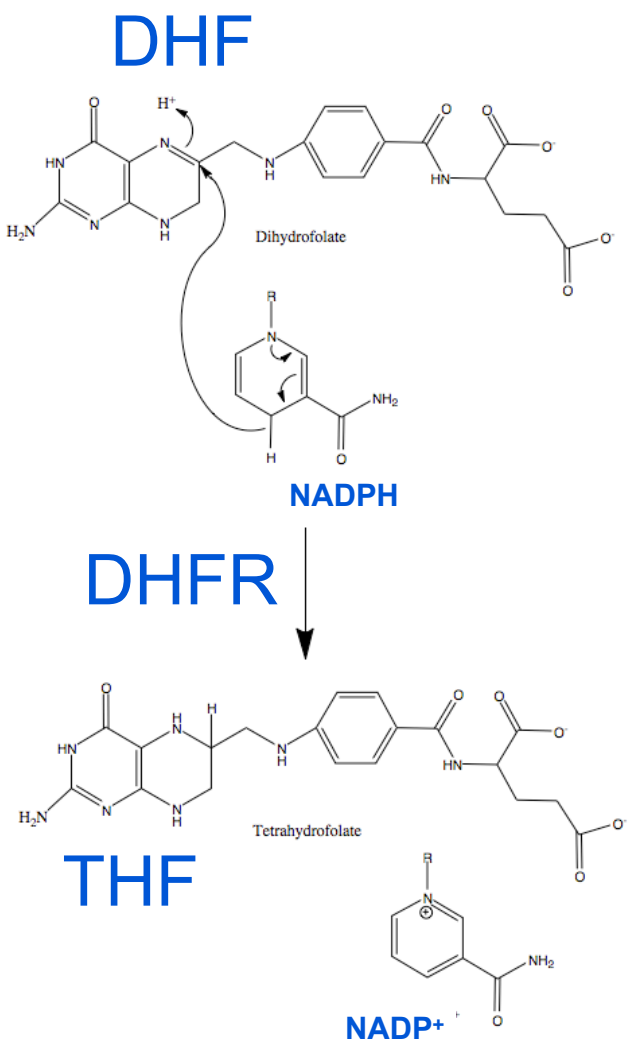
NADPH straddles the beta sheet on the C-terminal side between strands 2and 5. DHF is tucked into the space between helix 1 and the beta sheet.

3-layer, αβα, 2-8-2, mostly parallel beta sheet 43251687 with strand 8 antiparallel to the rest.

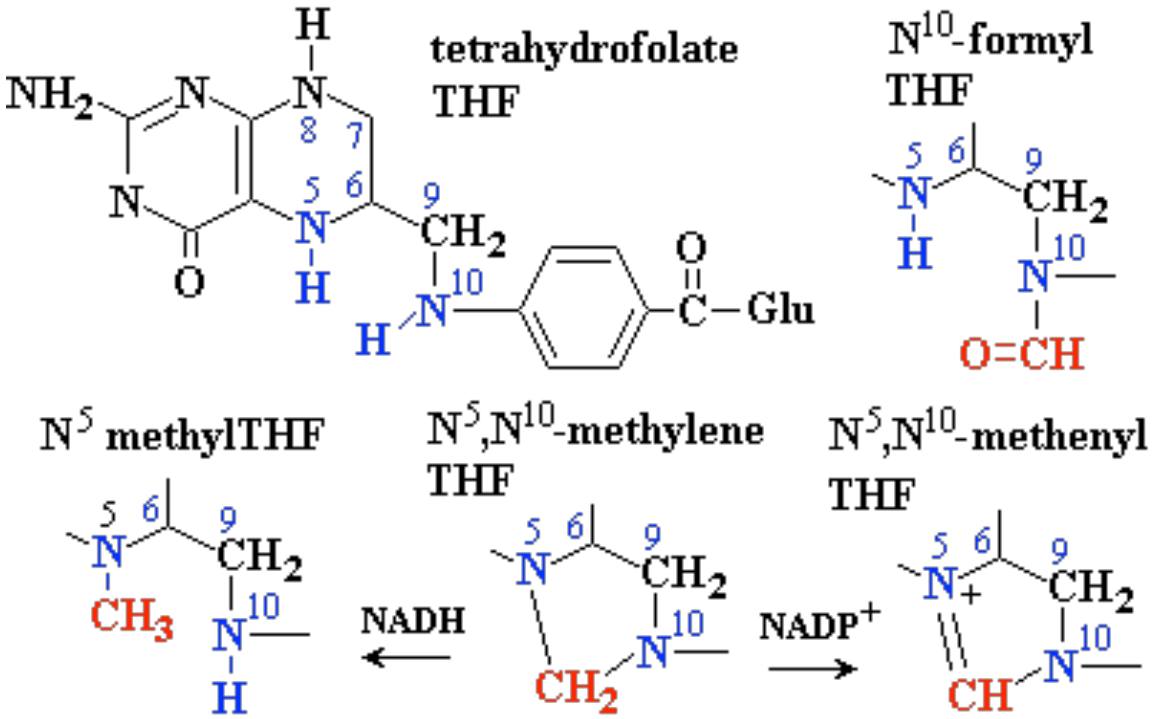
DHFR is required for cell growth

dihydrofolate reductase

- DFHR reduces DHF to THF



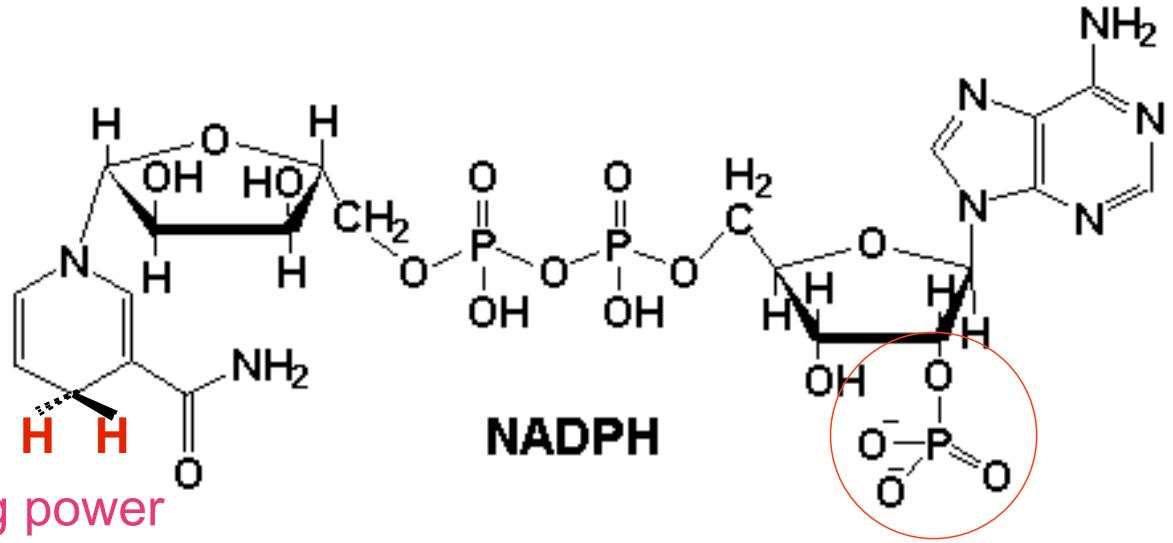
- THF is a 1-carbon carrier



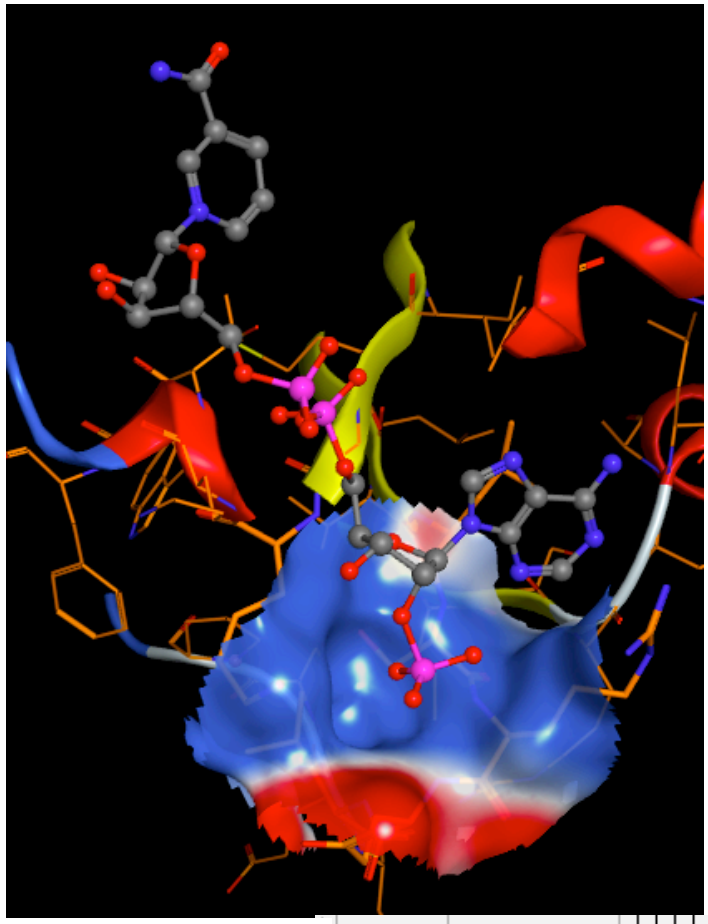
- THF ports a carbon to Uracil, making **Thymidine,** required for cell division.

Substrate specificity in DHFR

- NADH and NADPH both donate H^- (hydride) to reduce carbon.
- Nicotinamide Adenine Dinucleotide (2' Phosphate)
- The only difference is the 2' PO_4 group.
- What residues are responsible for the specificity for NADPH over NADH?



NADPH specificity

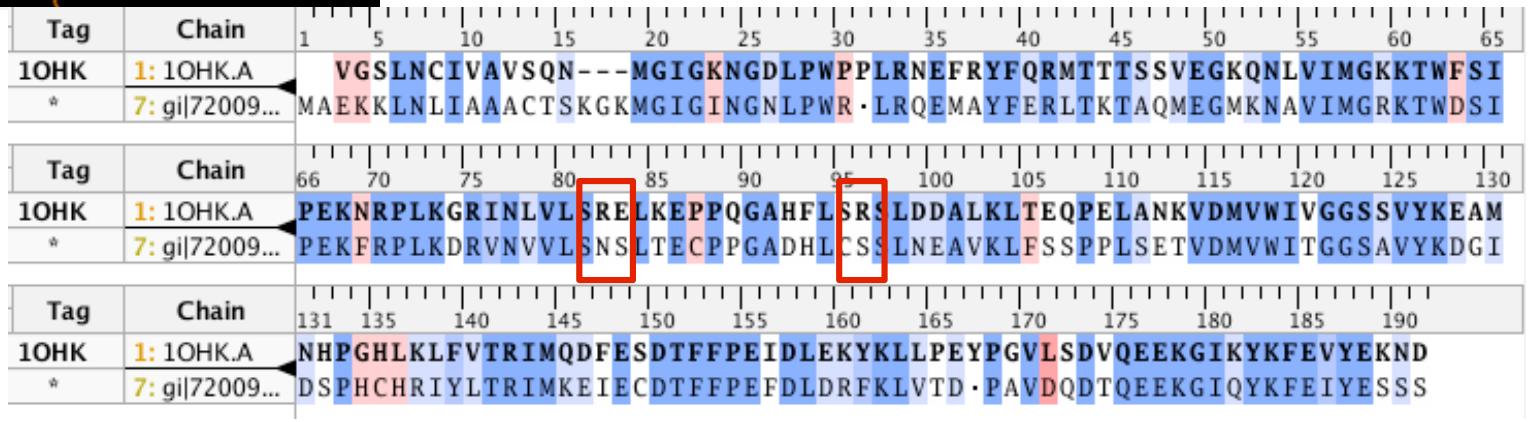


An electrostatic surface shows strong positive charge at the 2‘PO4 site.

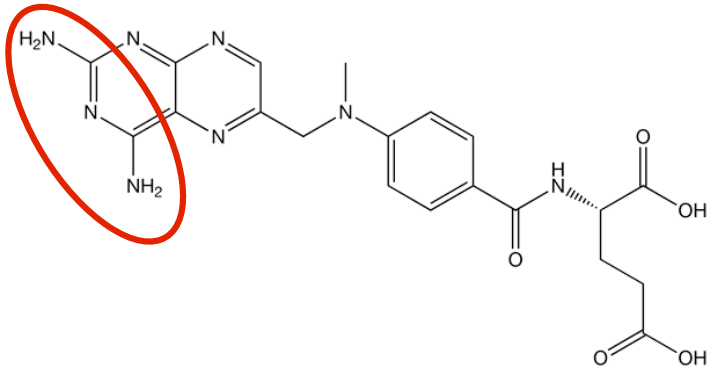
Three positively charged residues are found at the site -- **K55, R77, R92**

If a DHFR homolog is missing one or more of these charged side chains then it may use **NADH** instead of **NADPH** as the cofactor.

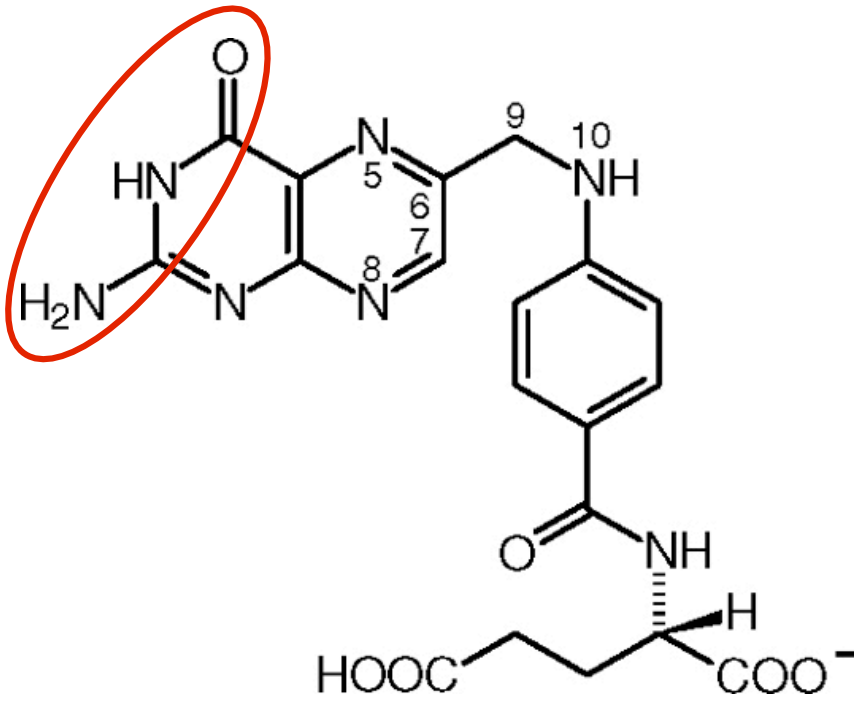
Predicted sea urchin DHFR vs human DHFR (1OHK). Sea urchin lacks charged groups. Could it use NADH? Or is it not a DHFR?



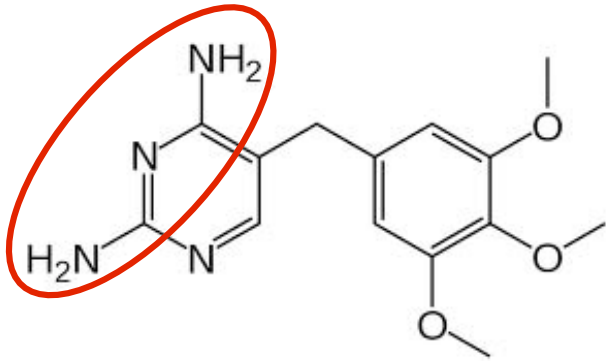
Inhibitors of DHFR



Methotrexate (MTX)



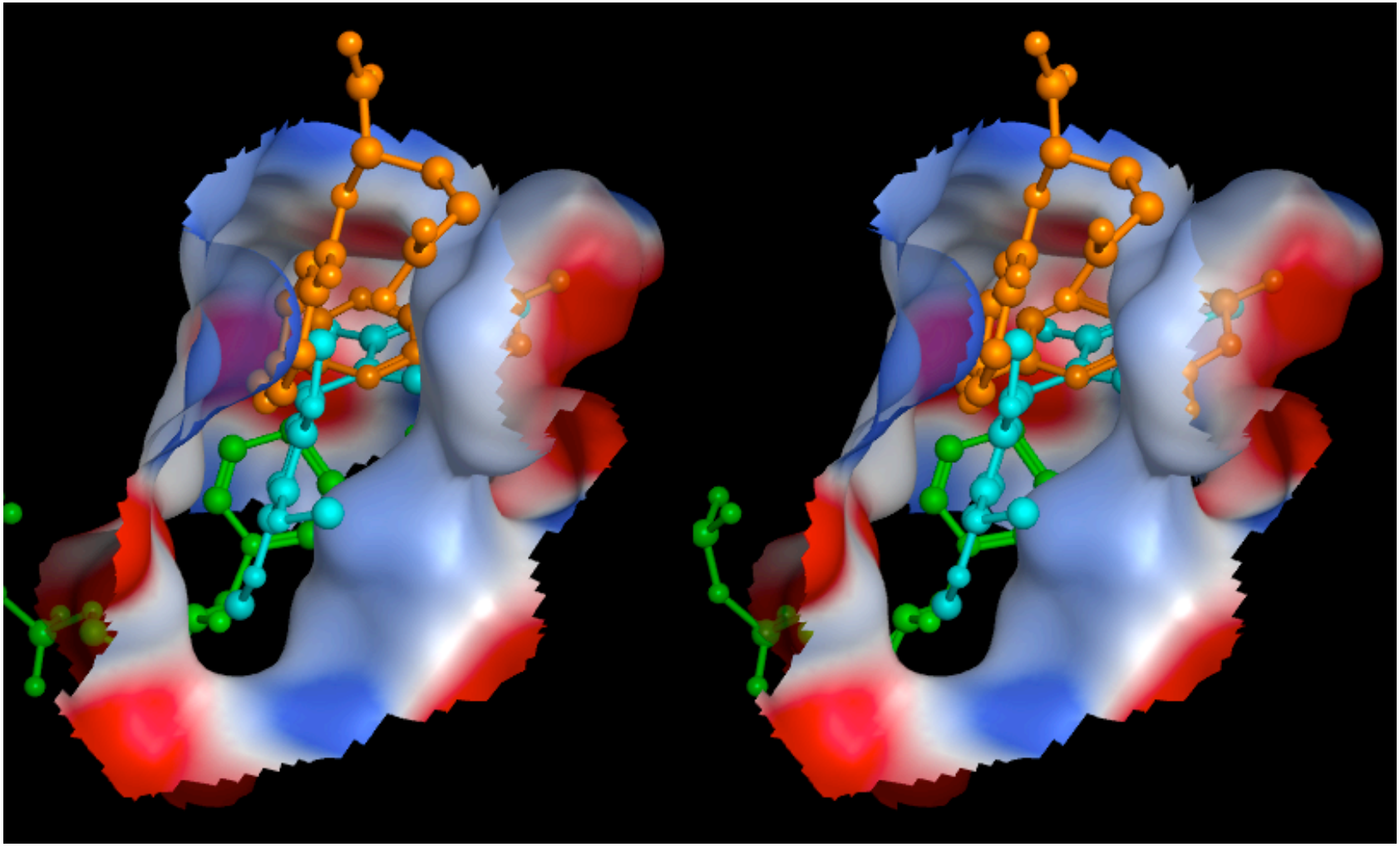
Folate (substrate)



Trimethoprim (TMP)

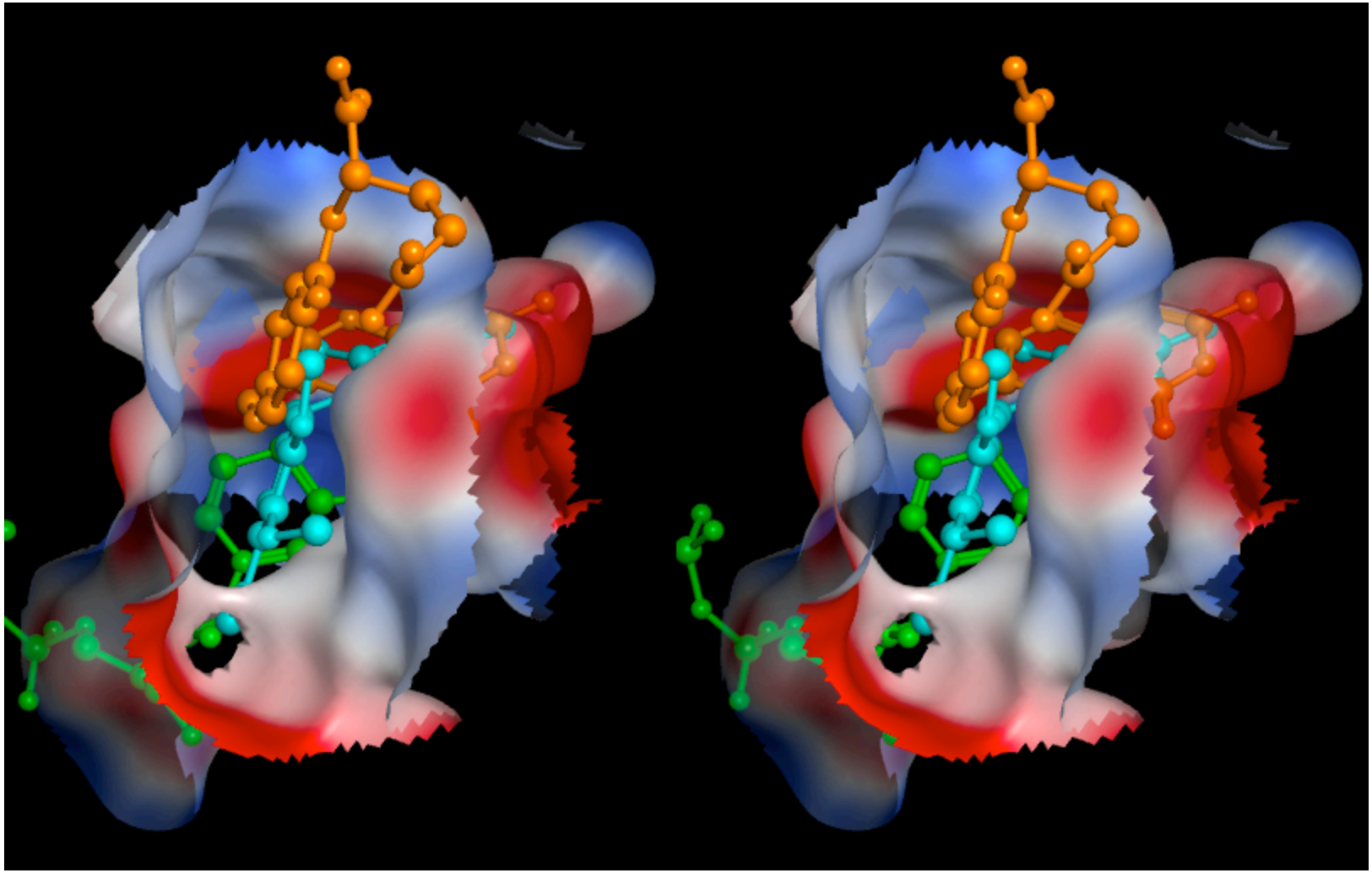
Same binding site. Similar binding mode. Different shape.

E.coli



Green: NADPH, Orange:Folate, Blue: TMP

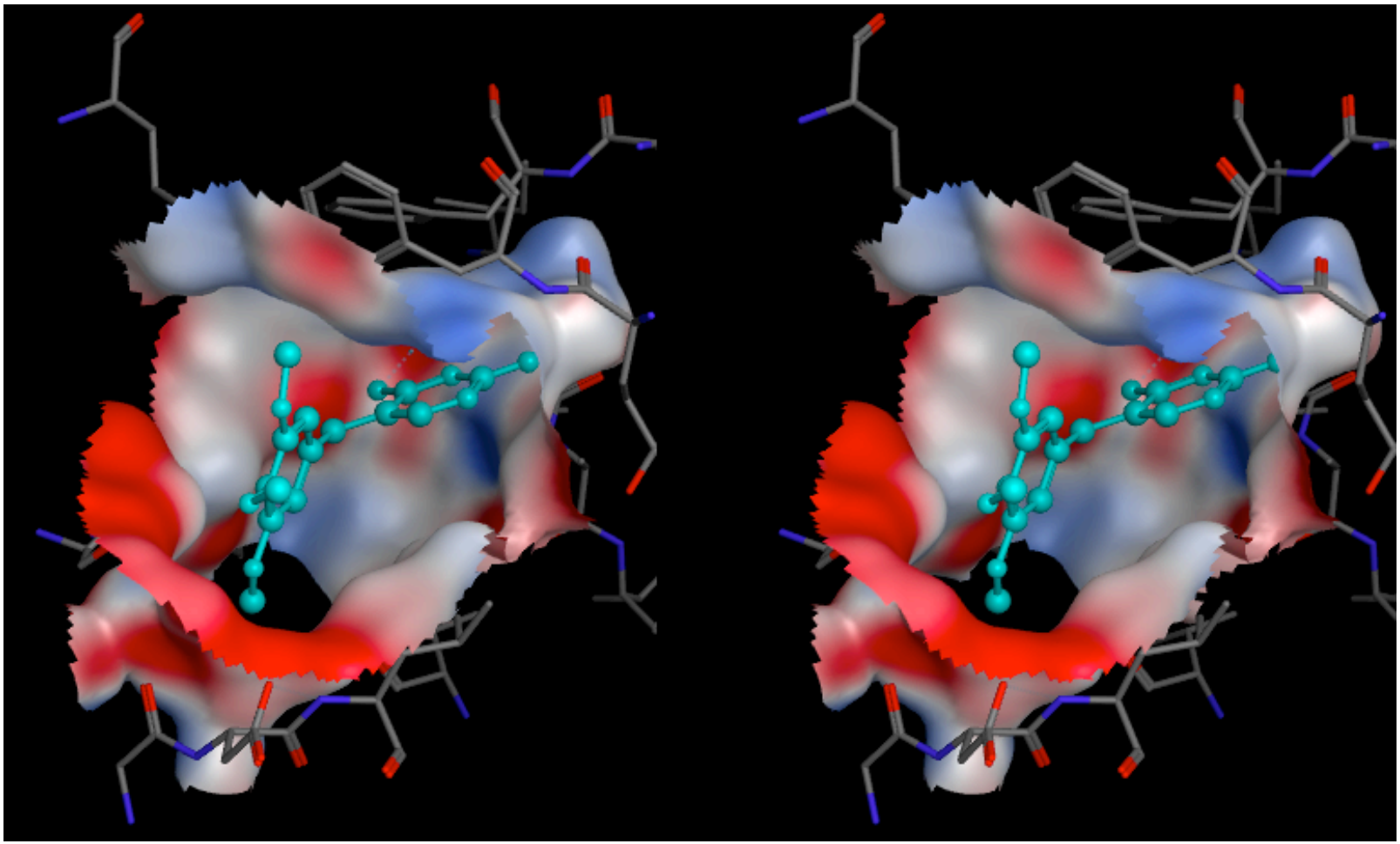
Human



Green: NADPH, Orange:Folate, Blue: TMP

Shape differences

- In the previous two slides you can see that the space occupied by the **TMP fits the bacterial active site** nicely, but in the human enzyme it doesn't fit. It collides with the side of the pocket.
- Can we find the loops responsible for blocking TMP binding in Human?
- Can homology modeling predict TMP resistance based on active site shape differences? (yes!)



Review questions : where do proteins come from?

- What causes Xrays to scatter?
- What causes diffraction?
- What are the results of Xray crystallography?
- What is a temperature factor?
- What wavelength light is used in Xray crystallography?
- What does “resolution” mean in Xray crystallography?
- What is a crystal?
- What wavelength of light is used in NMR?
- What kind of atom resonates with light?
- What is an ensemble in NMR?
- What measure in NMR is the analog of resolution in Xray?
- What type of NMR experiement assigns resonnances to amino acid types?
- What type of NMR experiment provides distances between different parts of the protein chain?

Review questions

- What is a domain?
- What is a “fold” according to SCOP?
- What does “strand order” mean w/respect to SCOP naming?
- What defines a sequence “family”?
- What defines a sequence “superfamily”?
- Draw a beta-alpha-beta unit using TOPS.
- Draw a crude contact maps based on a TOPS diagram.
- Find domain boundaries using a contact map.
- How can we infer domain boundaries using a multiple sequence alignment?

review questions

- What does *sp2* hybridization mean?
- How is energy related to probability?
- What constitutes a “system”?
- Give an example of a state of a system.
- What changes when we minimize the energy?
- Energy can be broken down into what two components?
- Name two stereochemiatry energy functions.
- What is a restraint?
- What is a constraint?
- The hydrophobic effect is an emergent property of what properties of water?
- In what way are H-bonds not properly modeled?
- Is the high energy of a buried unsatisfied H-bond donors an emergent property?

Review questions: local structure

1. What H-bonding pattern defines an alpha helix?
2. What H-bonding pattern defines parallel beta sheet?
3. ... anti-parallel beta sheet?
4. Why does the amphipathic alpha helix have a sequence pattern?
5. How to you apply an augmented matrix to determine the H-bonding partners?
6. What special properties of sequences are predictive of secondary structure? (list a few)
7. How are sequences processed before predicting secondary structure?
8. What is local structure?
9. Give examples of local structures.
10. What is supersecondary structure? Give examples.
11. Why are beta-alpha-beta units right-handed? Give one theory.
12. Same question, alternative theory?

Review: homology modeling

1. What is a loop anchor?
2. What metric is used in the loop database search?
3. What other metric is used?
4. In the sequence editor, how do you move a block of sequence?
5. What is the evolutionary meaning of an insertion?
6. What is the modeling instruction to MOE of an insertion?
7. How can you manually modify an indel for loop search purposes?
8. How likely is a deletion in the middle of a helix?
9. ...deletion in the middle of a sheet?
10. ...insertion in the middle of a helix?
11. ... insertion in the middle of a sheet?

Review questions: refining your model

- Where might you see a **glycine** in the Ramachandran plot?
- Where on the Ramachandran plot does a **proline** always lie?
- How does as a protein modeler **feel** about a buried, unsatisfied, backbone hydrogen bond donor or acceptor?
- What sequence makes a **cis-peptide** acceptable?
- What kind of beta turn has a glycine at the 3rd position?
- What kind of beta turn has a glycine at the fourth position?

Review questions: loops

- Name three ways to create a loop in MOE.
- What is a 4-for-2 loop search?
- How does the PDB mode work for Loop modeler?
- How does the de novo mode work?
-

Review: rotamers

- What is a rotamer?
- What are the letters for chi-1 rotamers?
- Which chi-1 rotamer is preferred in general?
- What is shape complementarity and why is it important?
- Are rotamer preferences depedent on the backbone conformation?
- Why does Nature abhor a void?

Review: validation

- How do you know your model is right?
- How to you know your model is wrong?
- What does confidence mean?
- What is "physics-based" confidence?
- What is "informatics-based" confidence?
- If you find one phi-psi outlier in a protein, does this mean the model is wrong?
- If you find a D-amino acid in a model, does it mean the model is wrong?
- If the model deviates from the template where the sequence is conserved, is it wrong?

Review: interpretation

- Discuss the valid ways of interpreting similarities and differences in a homology model.
- What is rational drug design?
- How can a homology model be used for rational drug design?
- Specifically, what do you look for in a homology model if you want to design a drug that binds one homolog and not the other?
- How is a multiple sequence alignment helpful in deciding whether a target o unknown structure binds a particular ligand when the crystal structure of a homolog with the ligand bound exists?
- What structural characteristic is more powerful in determining whether a ligand (such as a drug) will bind or not bind: shape, charge, or hydrogen bonding? (according to the lecture)