Molecular Modeling 2020-- Lecture 20, loops and linkers

Homology modeling

Grafting

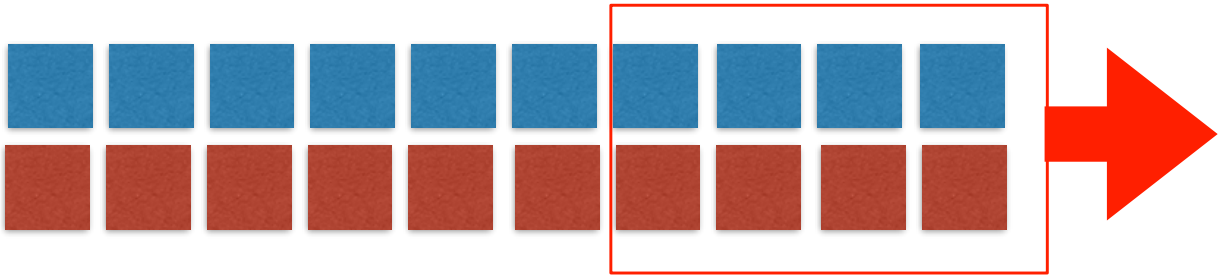
linker design

De novo loop building

Loop modeling by manual alignment

Current alignment (all matches)

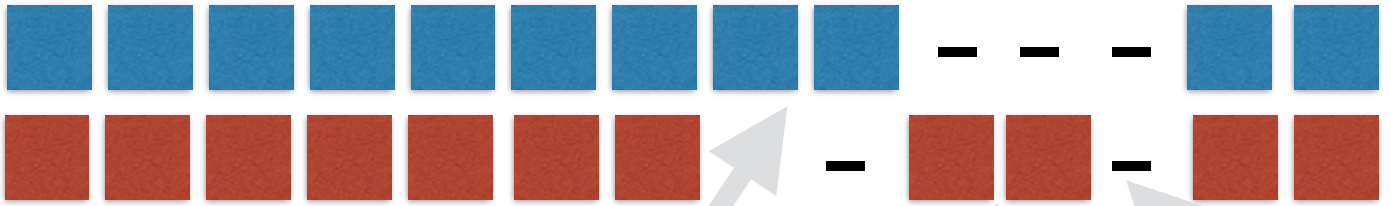
target
template



select block, **option/alt-middlemouse** drag right (creates space)

Select, **left-mouse** drag residues you want to model into the space, unaligned.

target
template

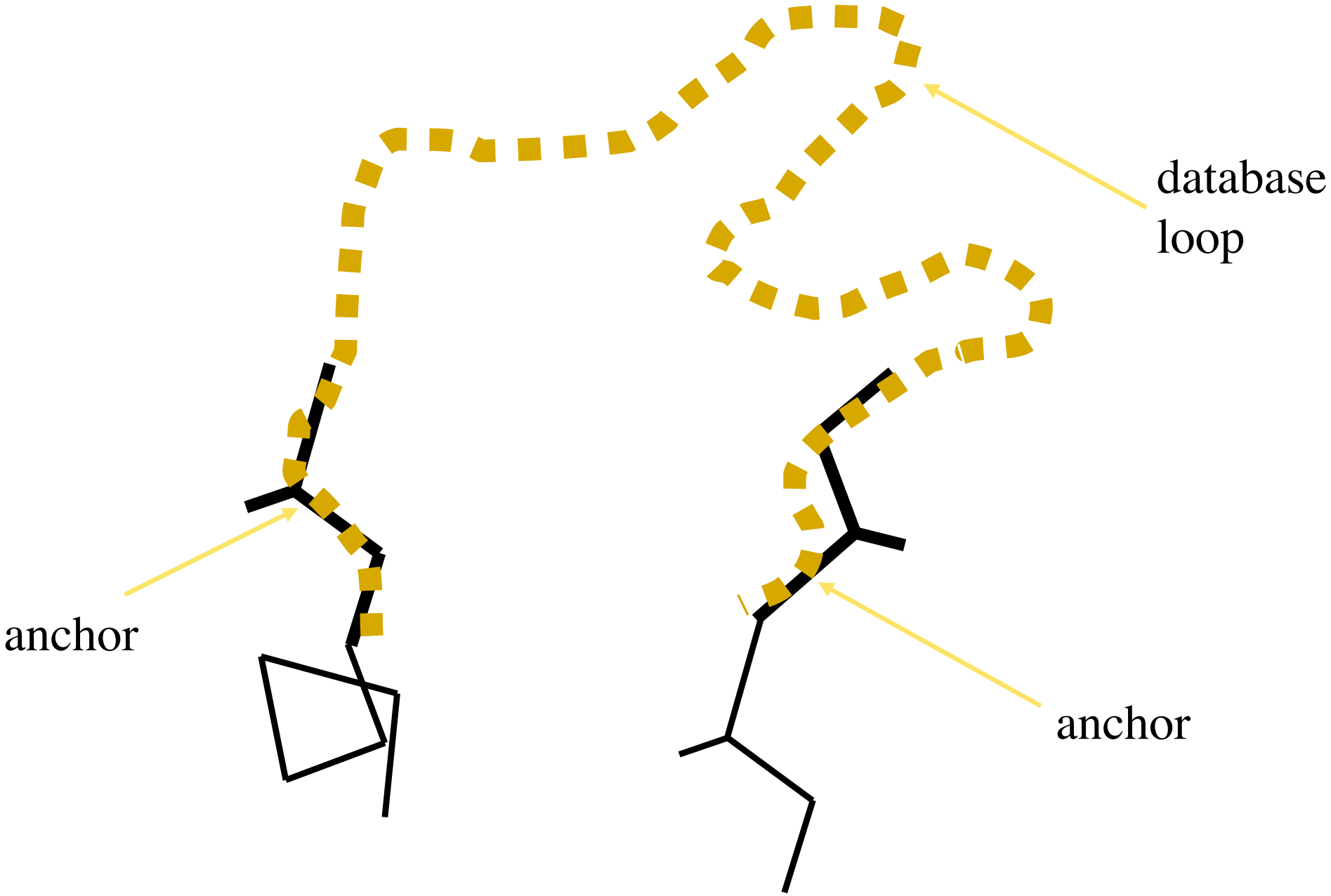


residues to be added by loop search

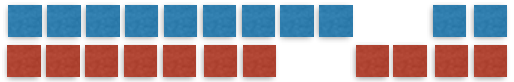
residues to be deleted

ignored by MOE

Automated Loop Search

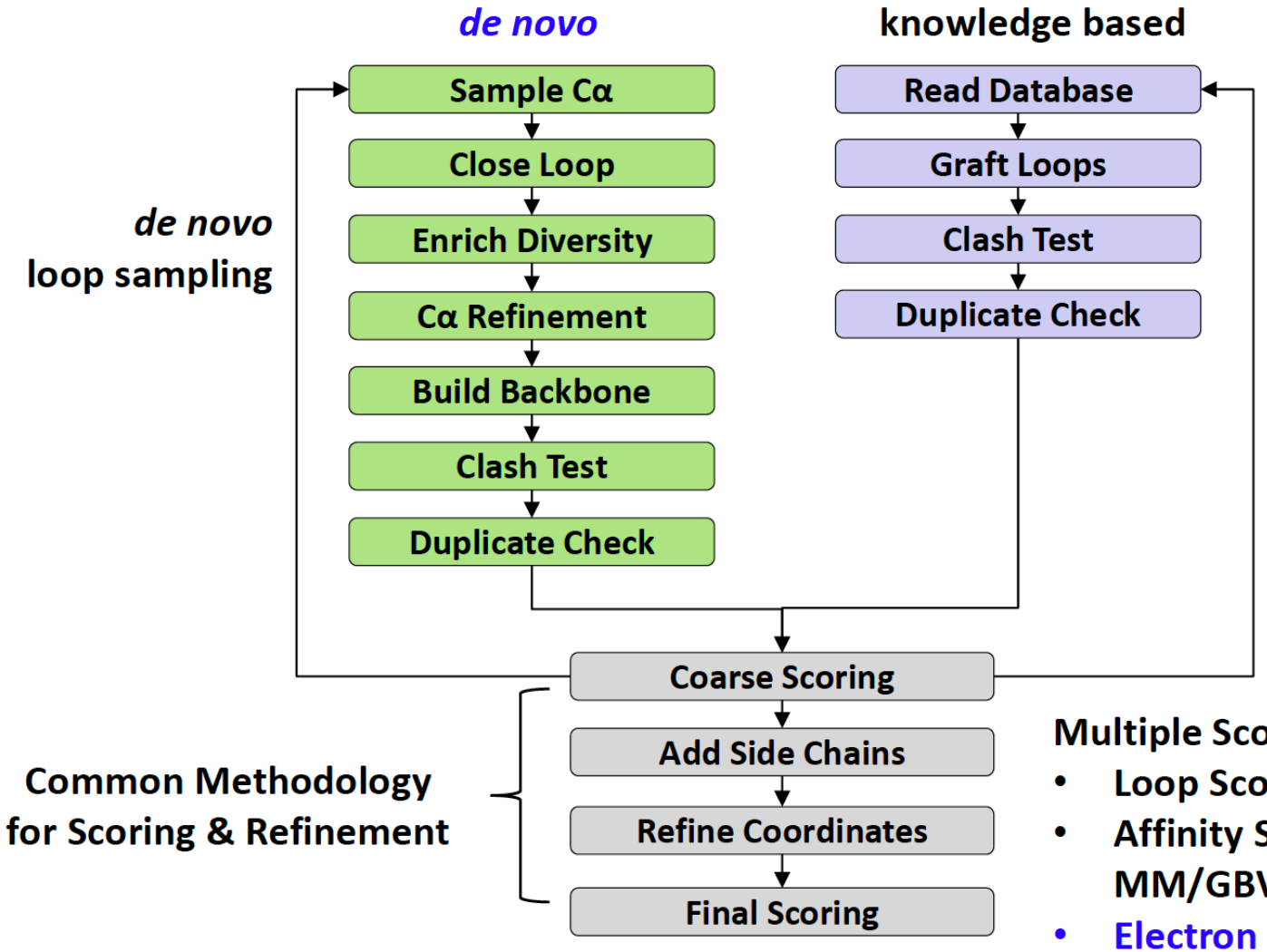


Exercise 20.1 Loop search by Homology Modeling

- Open messedup.moe (course website or LMS)
- Delete 2gb1 and target sequence, leaving only the model.
- Find the bad part of the model. Mark it.
- Select model chain. **Edit | copy as... fasta**. **Edit | paste**.
- Unalign segment to be modeled 
- Run **Homology Model** (Open Database Viewer.)
- Browse the loops (Hide everything in MOE window, then **DBV: File | Browse...**)
  - Hit start button. MOE window cycles through models.
  - **MOE**: **Protein | Geometry | phi-psi,** Resides: Browser
  - Mark the ones with the fewest outliers.
  - Choose one. Send to MOE.

Exploring the MOE loop modeling function

- **Interactive protein loop modeling procedure in MOE**



Custom databases

Variable length loop

(Faster) Knowledge-based loop grafting algorithm

Multiple Scoring Functions

- Loop Scoring with AMBER/GBVI
- Affinity Scoring with GBVI/WSA or MM/GBVI
- Electron Density Scoring

- **Search for multiple loops simultaneously**

Demo/Exercise 20.2: De novo loop search

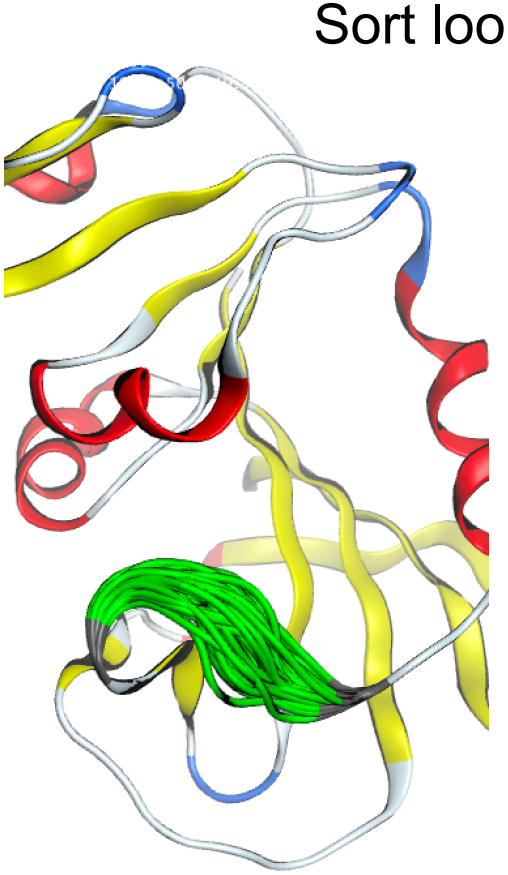
SEQ: Select a loop to be modeled.

Protein | Loop Modeler.

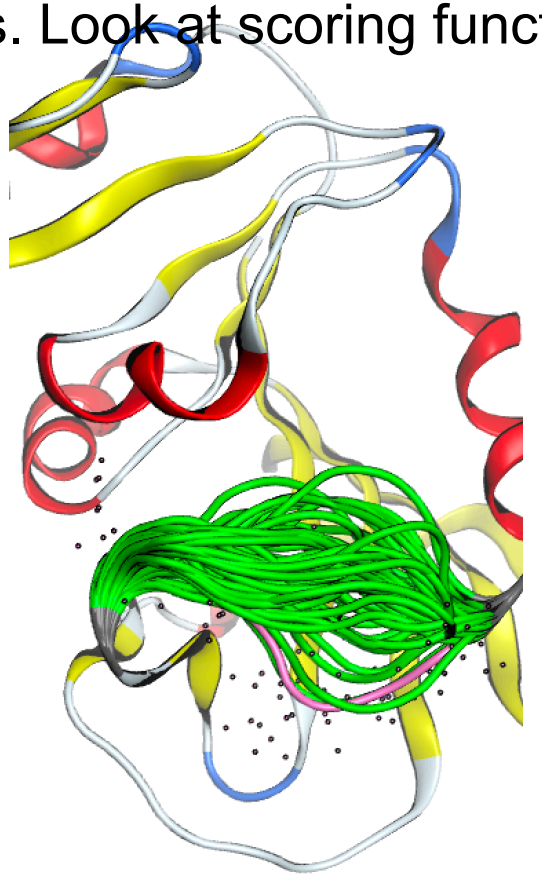
Select "**de novo**".

Set Loop Limit=100. Run. Wait and watch.

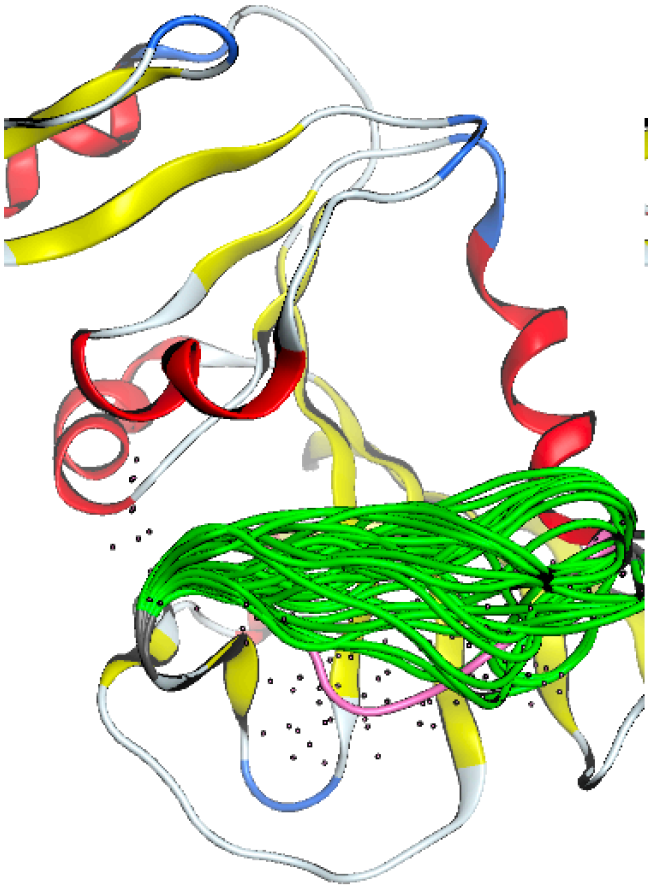
Sort loops. Look at scoring function.



length=4



length=6



length=8

Demo/Exercise 20.3: PDB loop search

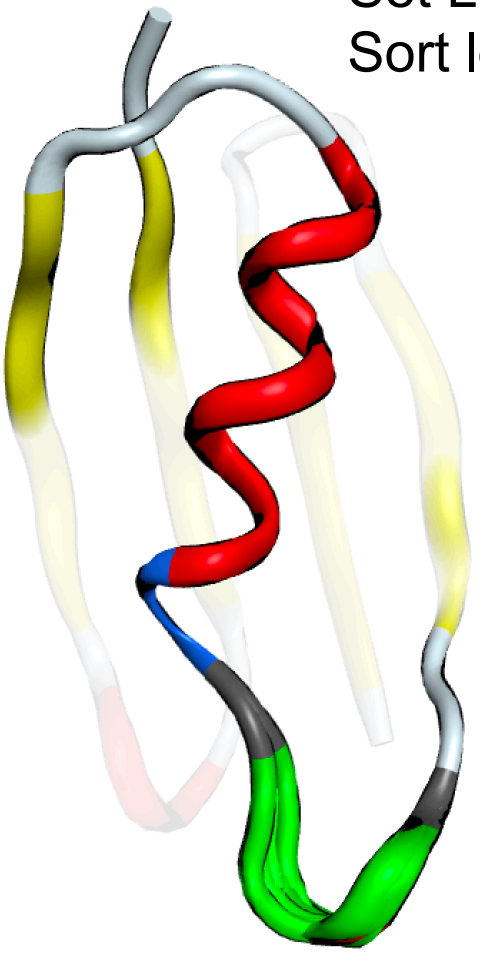
SEQ: Select a loop to be modeled.

Protein | Loop Modeler.

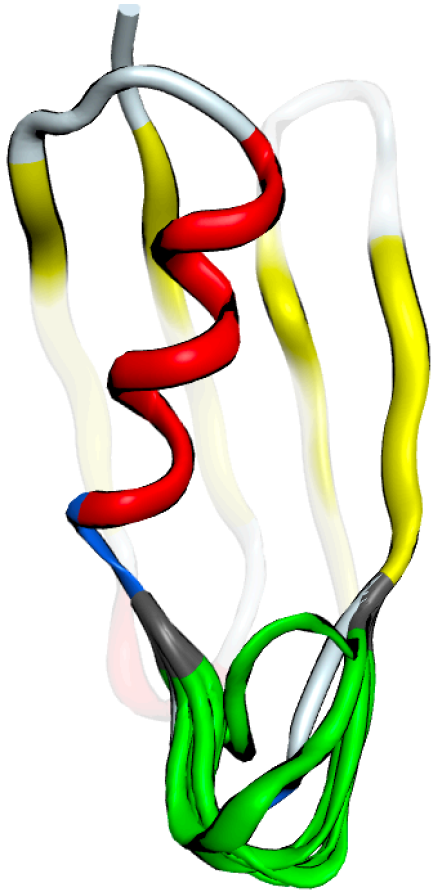
Select "**PDB**".

Set Loop Limit=100. Run. Wait and watch.

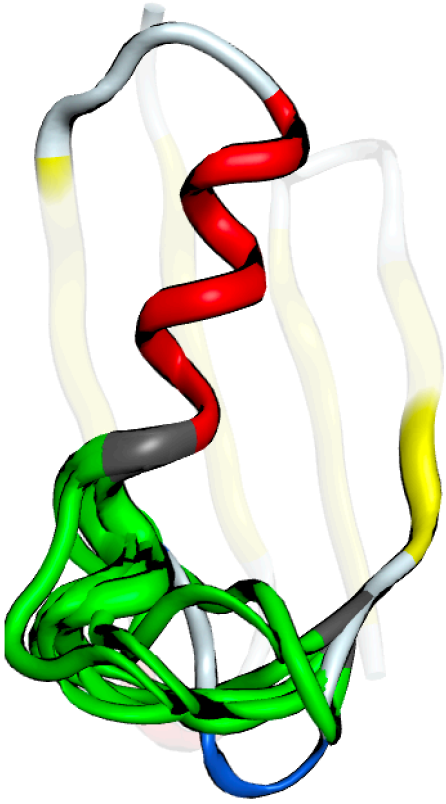
Sort loops. Look at scoring function.



length=4

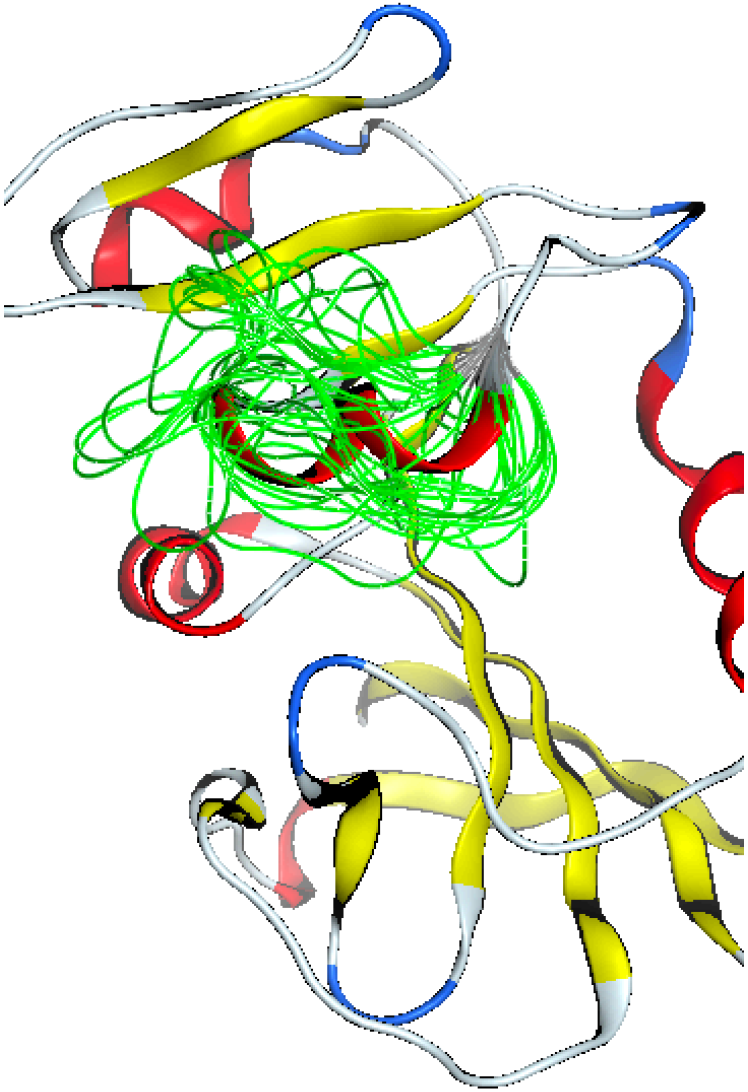


length=6



length=8

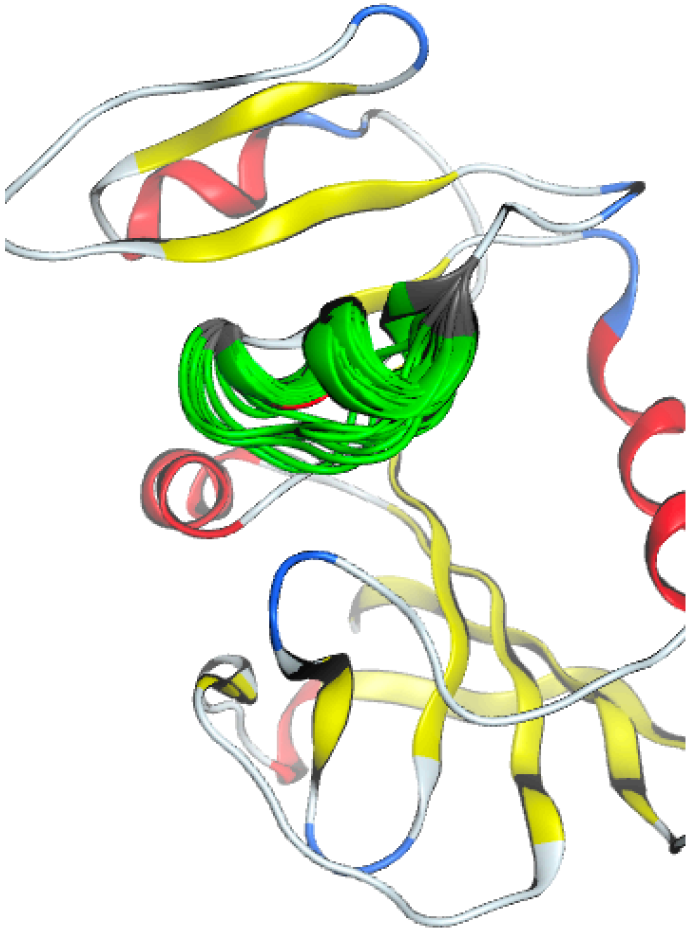
Loop modeler in "de novo" mode can't make a helix



...in 100 tries.

Protein | Loop modeler
"de novo" mode is not ready for primetime.

Loop Modeler in "PDB" mode finds mostly helix, for a helical segment.



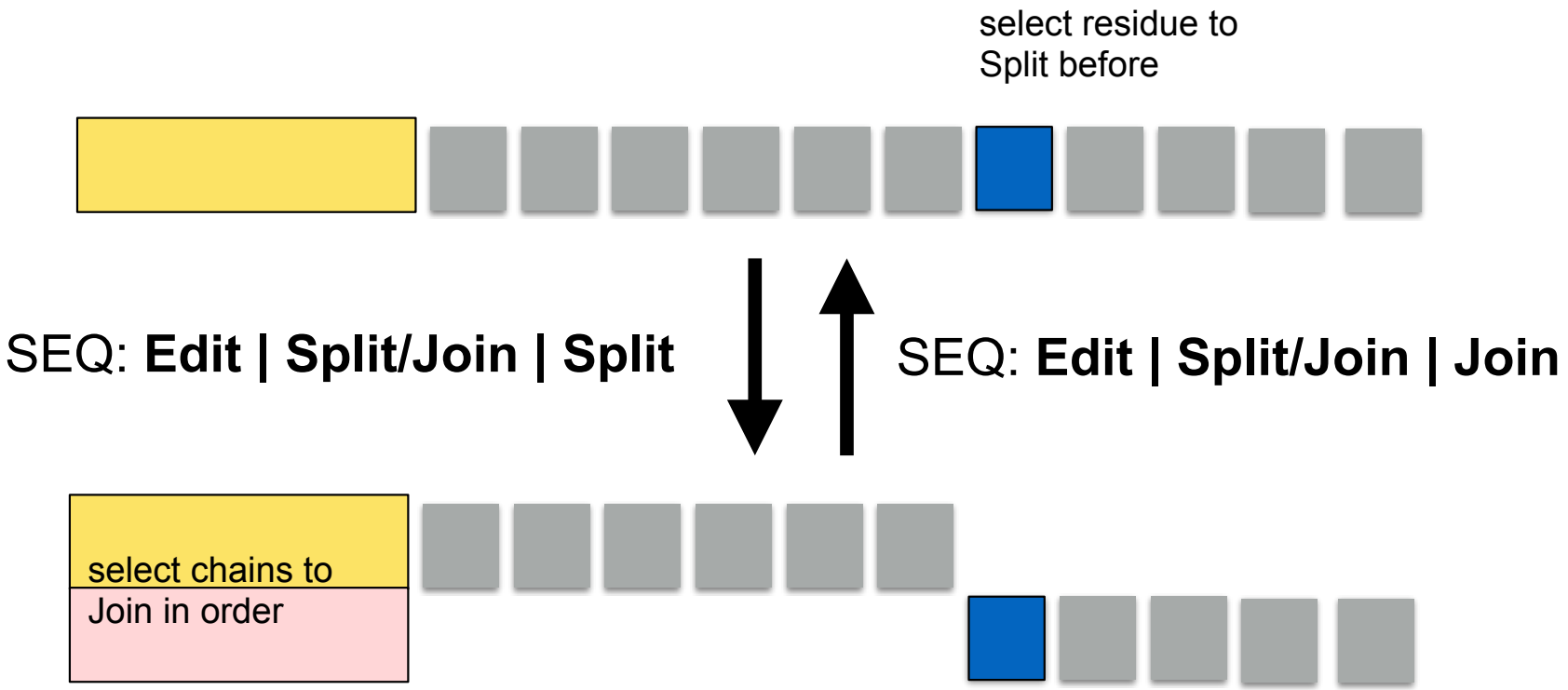
Use **Protein | Loop modeler** in "**PDB**" mode , or use **Homology Model**

Why?

de novo vs PDB

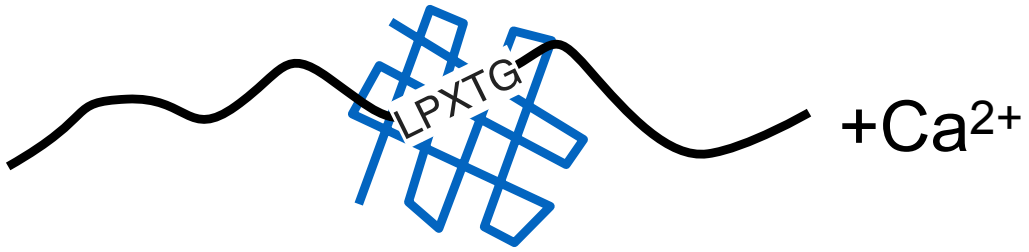
- As the loop length increases, the conformational space possible increases *exponentially*
- Random likelihood of canonical secondary structure is low. Random search doesn't find it.
- In PDB loops, frequency of secondary structure in proportional to its frequency in real proteins.

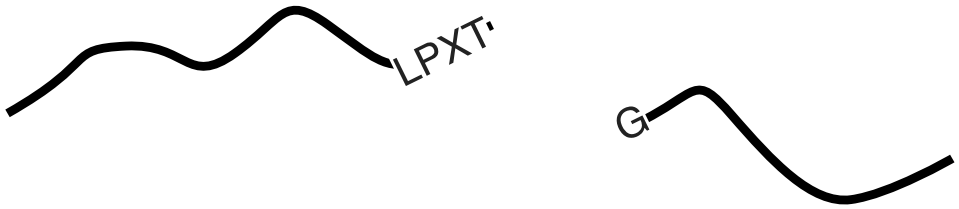
Splitting/joining



Linker design -- Sortase A

Sortase A is a bacterial protease that cuts at the sequence c. Cutting is triggered by calcium ions.

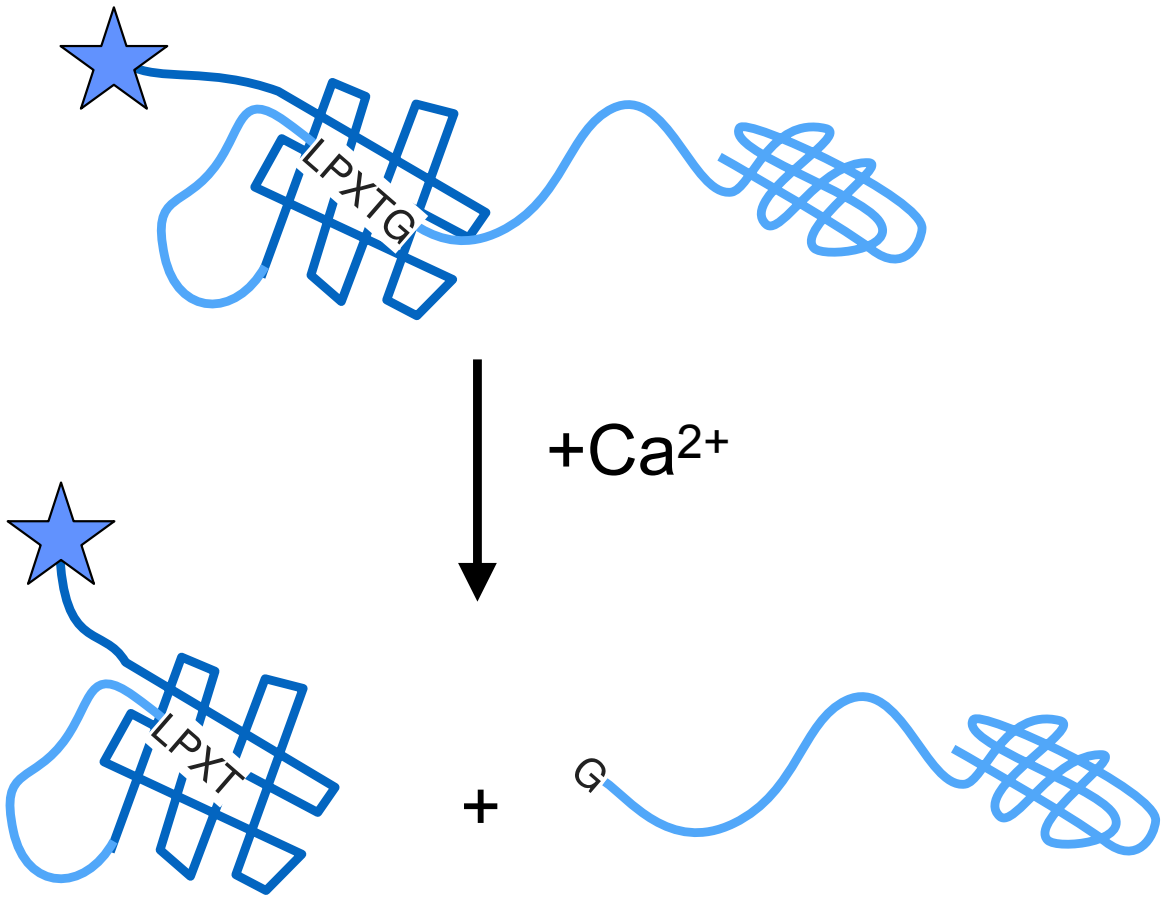




in-line Sortase A construct

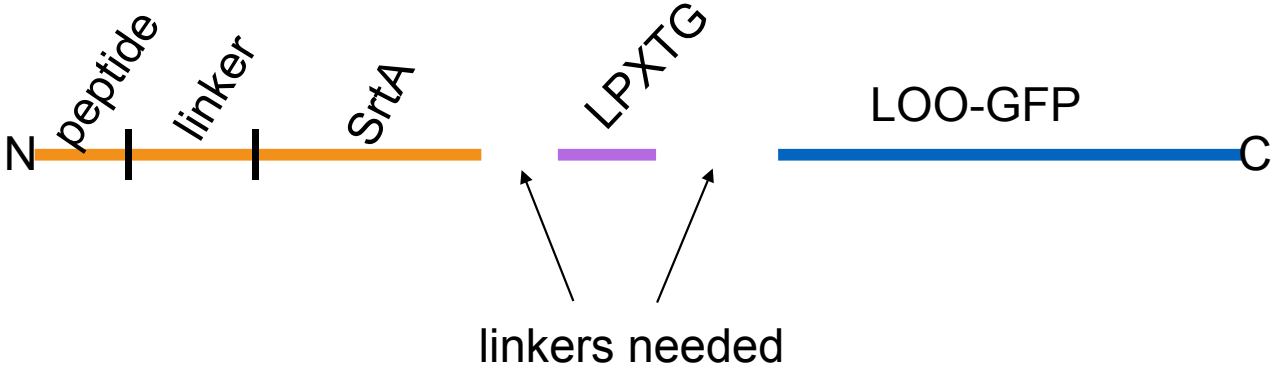
SrtA may be cloned inbetween two protein segments, then cleaved, leaving two separate chains which may then be separated by chromotography.

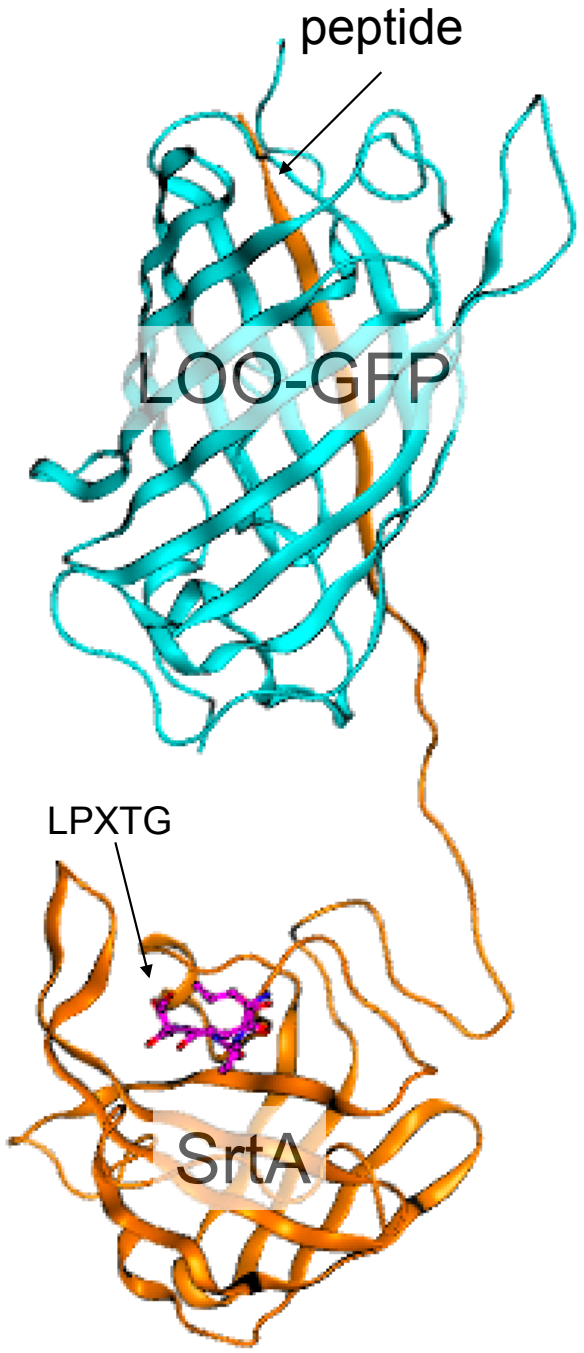
The N-terminus may be linked to an affinity reagent ★ which is then removed by adding Ca^{2+}



Self-priming biosensor

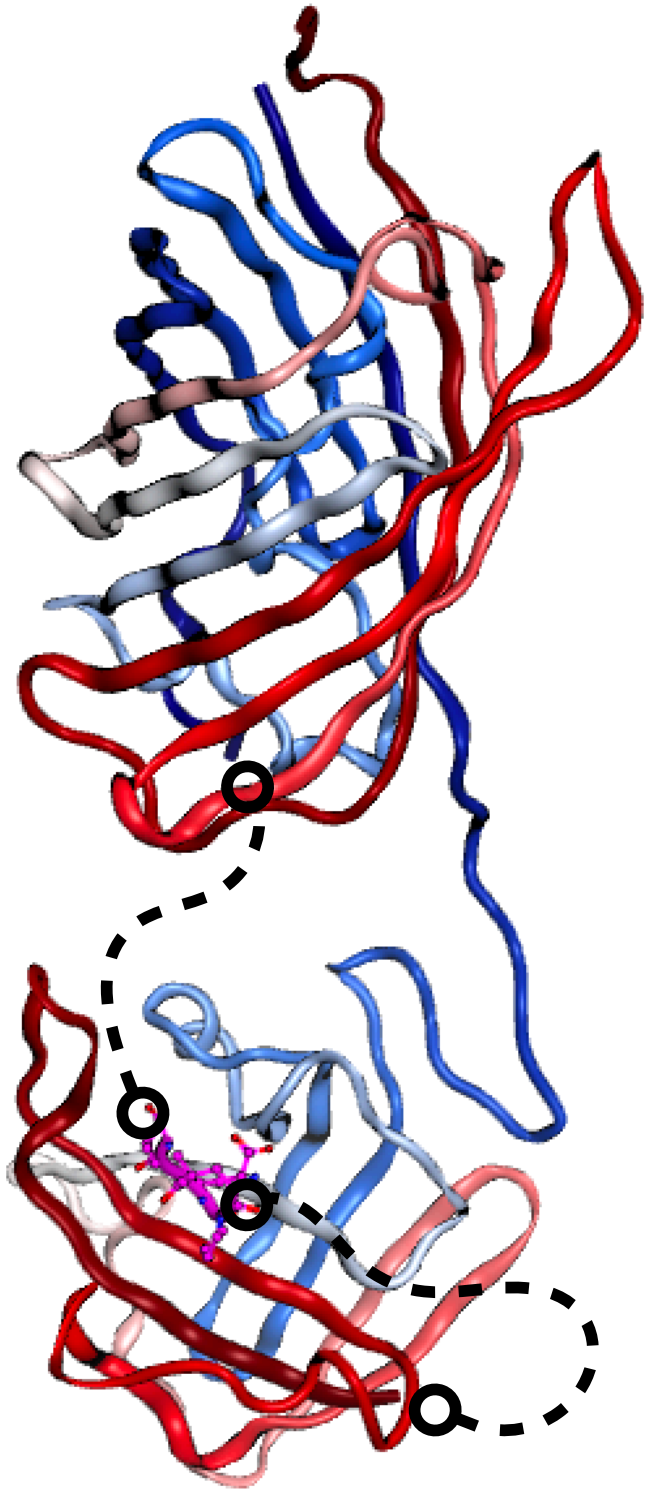
Leave-one-out green fluorescent protein -- a peptide biosensor that glows when peptide is bound. It needs to have a peptide bound in order to mature. Then the peptide must be removed. We can use SrtA and its target LPXTG inline as a self-cleaving, covalently linked peptide, removed by adding calcium!





1. Link the **C-terminus of SrtA** to the **N-terminus of the LPETG peptide**.

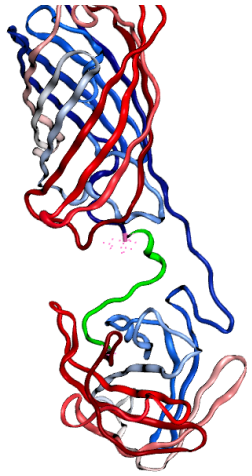
2. Link the **C-terminus of LPETG** to the **N-terminus of GFP**



Exercise 20.3: Design a linker to span from C-terminus of SrtA to the active site.

Open **SrtA.moe** from course web site (or LMS).
Select one C-terminus and one N-terminus to link and run Protein | Linker Modeler.

Protein | Linker Modeler
Select C-terminus of peptide-SrtA and the L of LPETG
Click "**Anchors in Selection**"
Make the link 15 glycines, **G(15). Apply**
Check **PDB** ~~or de novo~~. (Dont run de novo. De novo is very slow.)
RMSD limit: 0.75
Maximum anchor error: 0.8
Maximum anchor RMSD: 0.4
Run.



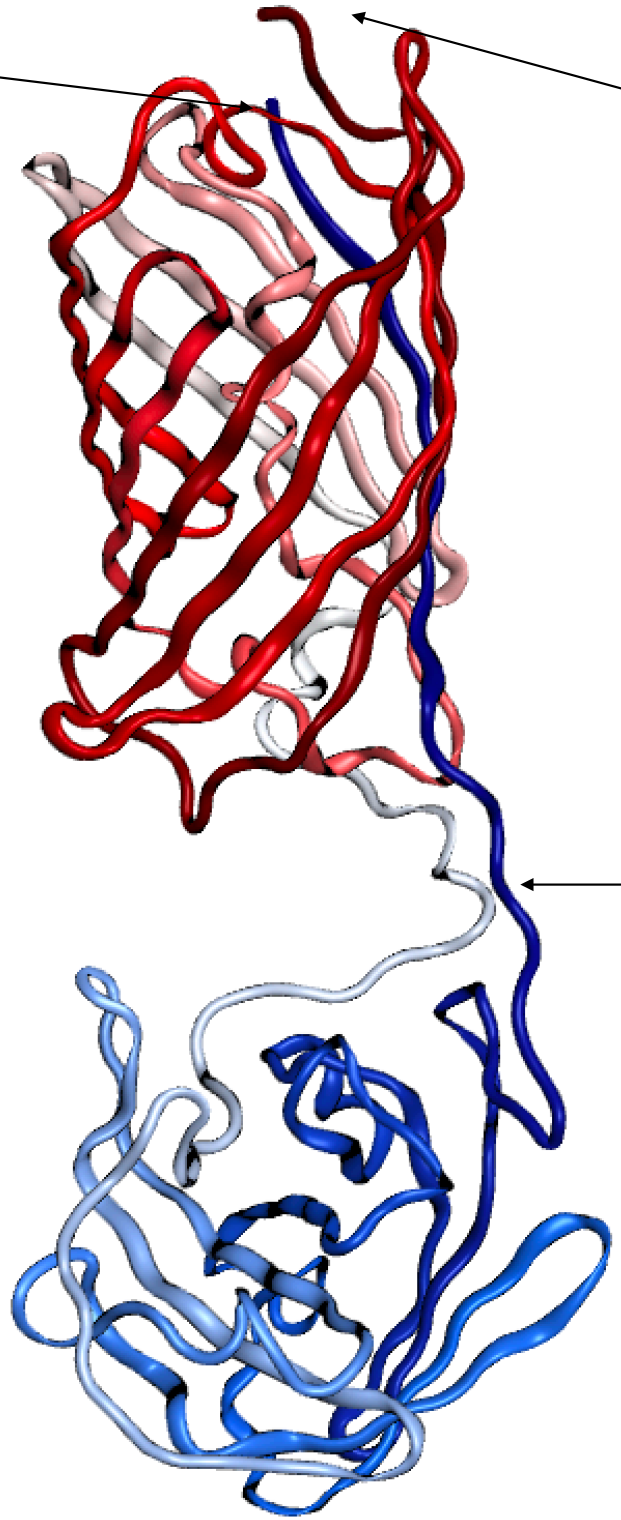
Linker designs appear in Loop Browser. Select to visualize.

Select a linker, **Build.**
Send to MOE. Display ribbon only. Color ribbon by Terminus.

N-terminus

C-terminus

Finished product



new linkers
all glycines

Review questions

- 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?
- Why do protein modelers need to make linkers?
- What are the considerations when designing a linker?