Molecular Modeling 2020 -- Lecture 21. Fri Apr 10
rotamers and waters

Rotamers

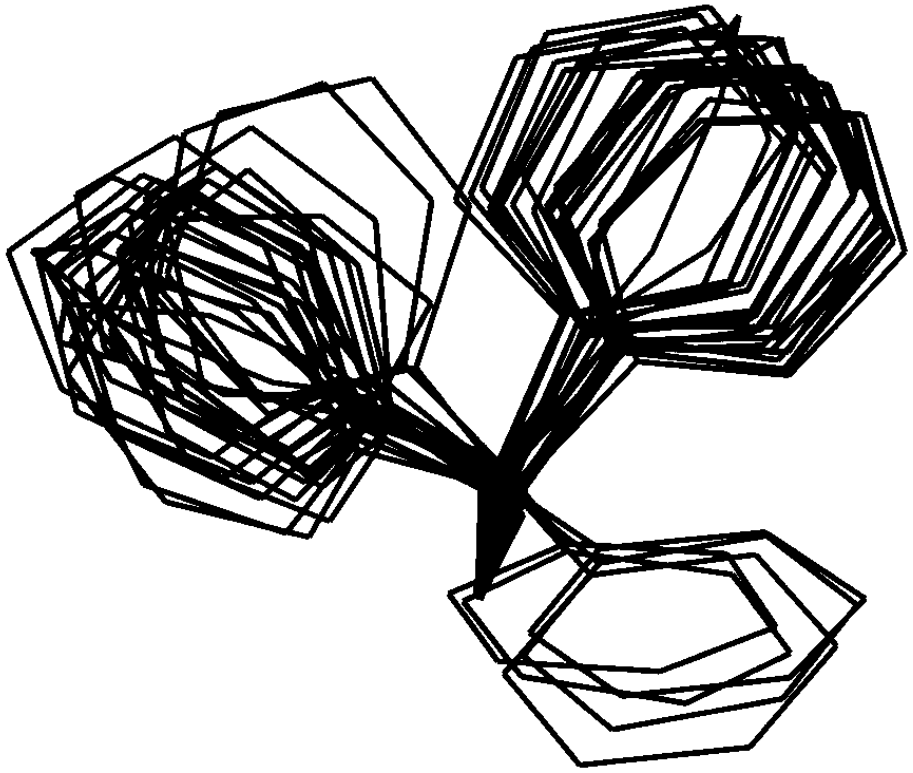
Manual protein design

Packing

Adding waters

Rotamers

Sidechain conformations fall into three classes called rotational isomers, or rotamers.

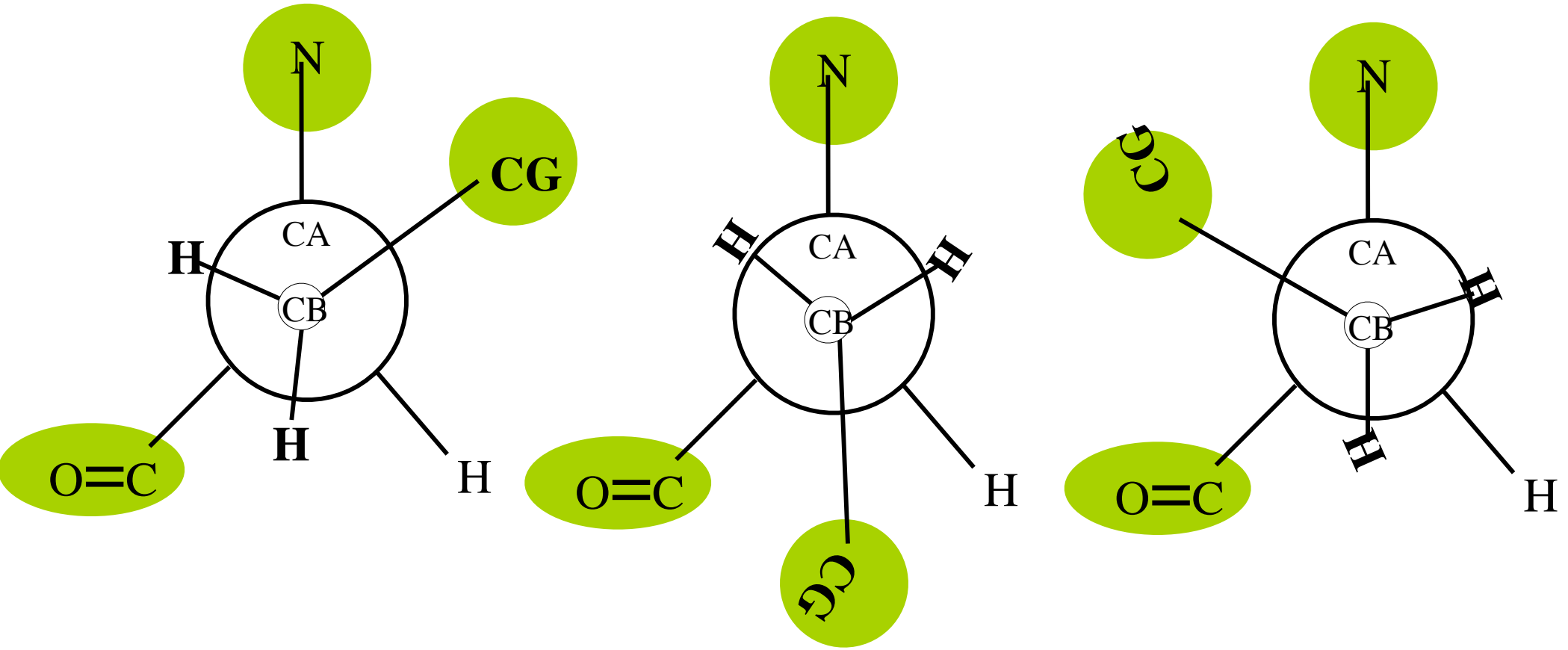


A random sampling of Phenylalanine sidechains, w/ backbone superimposed

Sidechain rotamers

1-4 interactions differ greatly in energy depending on the moieties involved.

Newman projections



"m"

-60° gauche

"t"

180° anti/trans

"p"

+60° gauche

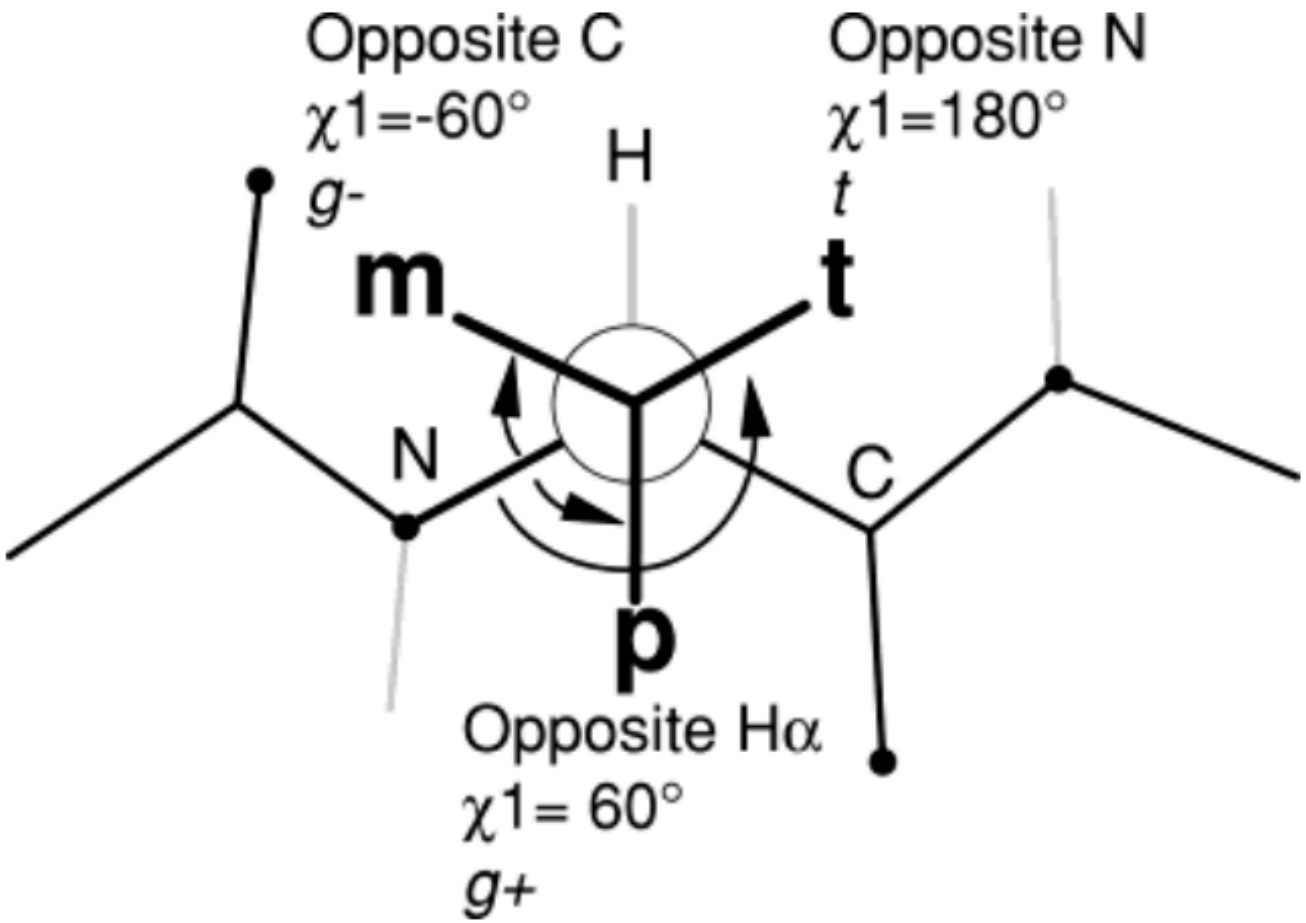


Fig. 1. Illustration of the relationships and nomenclature for the side-chain dihedral angle χ_1. Each of the three staggered conformations is labeled with its χ_1 angle (measured from the backbone N), its officially correct[29] g+ or g− designation, and its **p**, **t**, or **m** nomenclature as used in this work. Note that earlier studies have used opposite g+, g− designations, as discussed in the text (Nomenclature).

SC Lovell, JM Word, JS Richardson and DC Richardson (2000) "The Penultimate Rotamer Library" *Proteins: Structure Function and Genetics* **40:** 389-408.

General generic rotamer preference order for χ_1

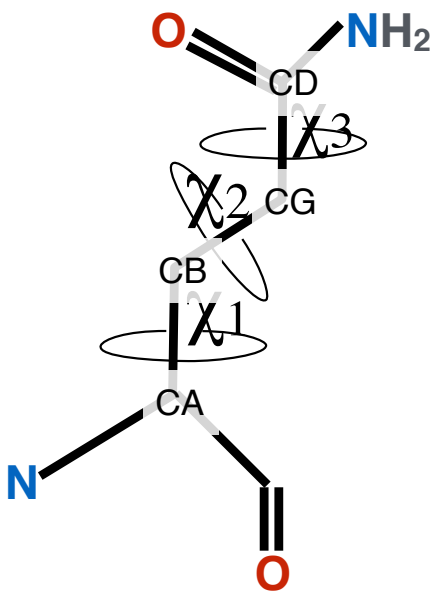
energy of...

$$m < t < p$$

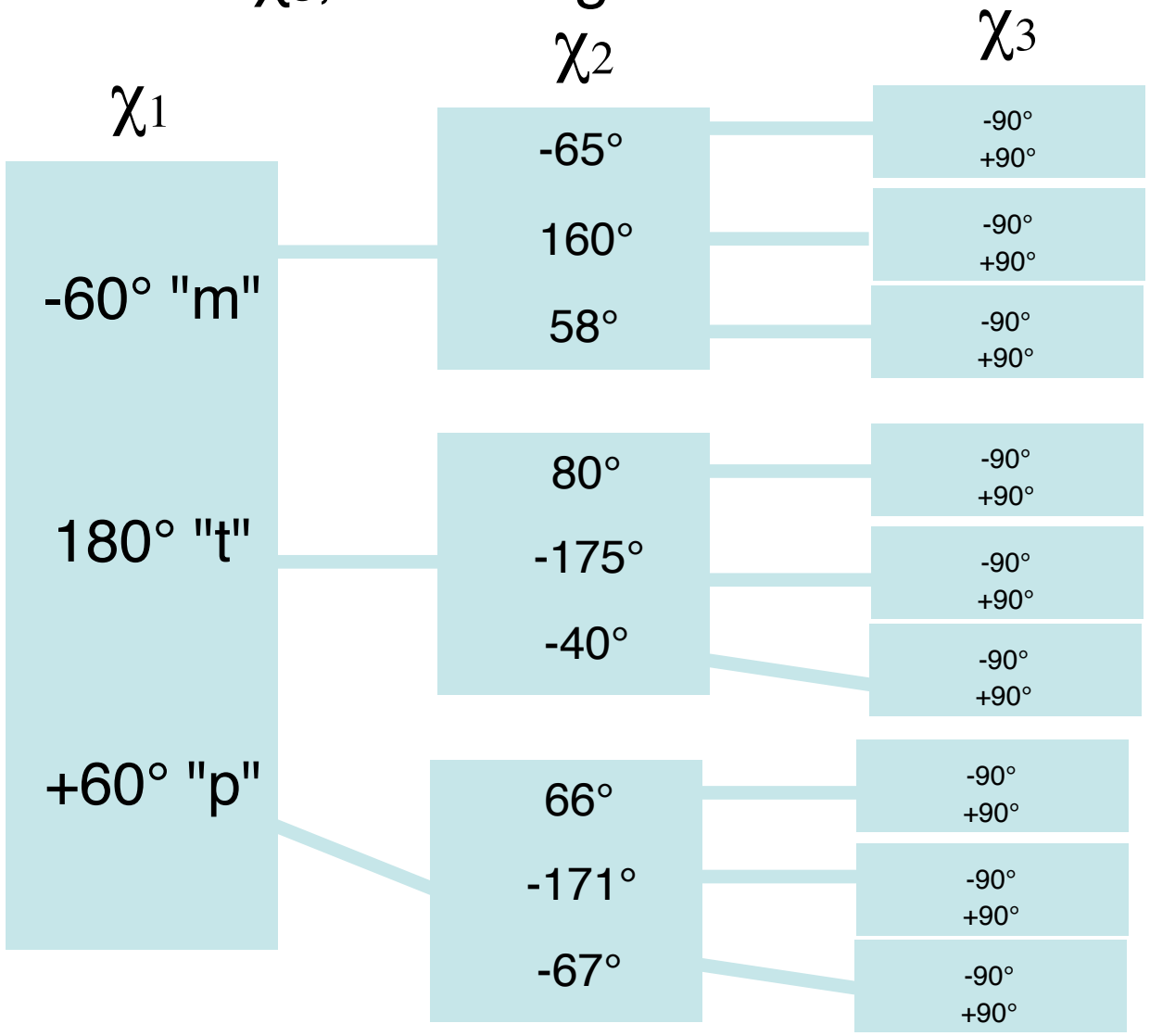
...but, actual rotamer preference depends on
1) the amino acid
2) the backbone conformation
3) packing.

Rotamer tree

χ_1 backbone determines the preference for χ_2 which determines the preference for χ_3, an energetic decision tree of rotamers



Gln

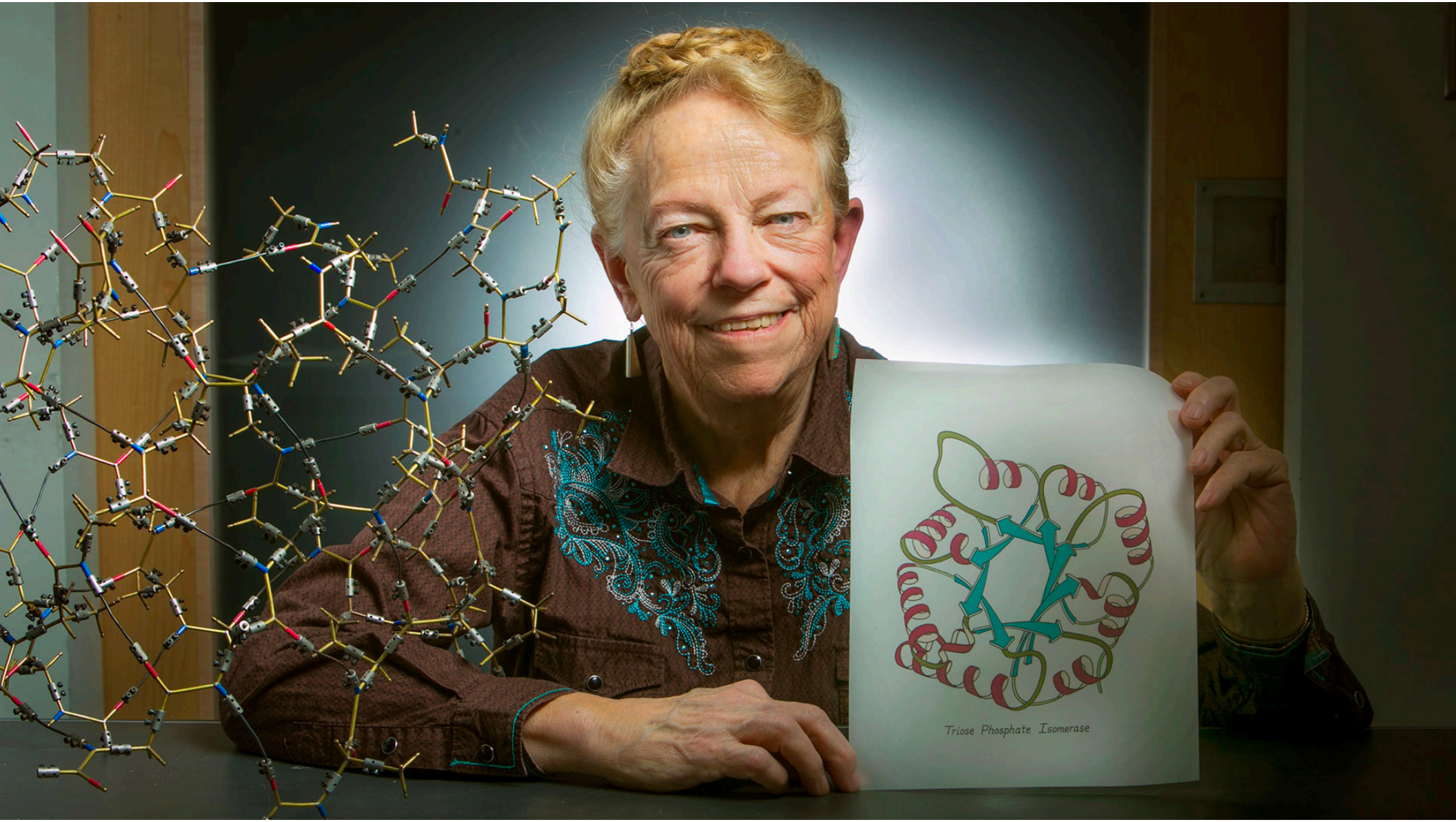


E_{rot}

- The energy of a rotamer can be calculated two ways.
  - Using a force field. (not very accurate)
  - using statistics from the protein data bank. (empirical and accurate)

$$E_{rot} = -RT \log \left(\frac{P(r)}{1-P(r)} \right)$$

where $P(r)$ is the probablity of rotamer r.



Jane Richardson

Rotamer Libraries

Rotamer libraries have been compiled by clustering the sidechains of each amino acid over the whole database. Each cluster is a representative conformation (or rotamer), and is represented in the library by the best sidechain angles (chi angles), the "centroid" angles, for that cluster.

Two commonly used rotamer libraries:

Jane & David Richardson: http://kinemage.biochem.duke.edu/databases/rotamer.php

Roland Dunbrack: http://dunbrack.fccc.edu/bbdep/index.php

Richardson rotamer library

```
G 3 0
 -0.000000         G          -        p:0000     900.     900.     900.     900.     0.000
  0.0000   0.0000   0.0000   N
  1.4700   0.0000   0.0000   CA
  2.0200  -0.7140  -1.2400   HB
-------------------------------------------------------
A 3 0
 -0.000000         A          -        p:0000     900.     900.     900.     900.     13.255
  0.0000   0.0000   0.0000   N
  1.4700   0.0000   0.0000   CA
  2.0200  -0.7140  -1.2400   CB
-------------------------------------------------------
C 4 1
  0.377250         C          -        p:1000      52.     900.     900.     900.     33.851
  0.0000   0.0000   0.0000   N
  1.4700   0.0000   0.0000   CA
  2.0200  -0.7140  -1.2400   CB
  1.2558  -0.0213  -2.7466   SG
  1.6794  -0.5784  -3.7820   HG
  0.377250         C          -        p:2000      62.     900.     900.     900.     34.855
  0.0000   0.0000   0.0000   N
  1.4700   0.0000   0.0000   CA
  2.0200  -0.7140  -1.2400   CB
  1.4900   0.1650  -2.7500   SG
  1.9194  -0.3874  -3.7854   HG
  0.377250         C          -        p:3000      72.     900.     900.     900.     33.924
  0.0000   0.0000   0.0000   N
  1.4700   0.0000   0.0000   CA
  2.0200  -0.7140  -1.2400   CB
  1.7470   0.3158  -2.7228   SG
  2.1829  -0.2329  -3.7575   HG
  0.232074         C          -        t:1000     173.     900.     900.     900.     33.457
  0.0000   0.0000   0.0000   N
```

AA 1-letter code

E_{rot} = log-likelihood

rotamer name, dihedral angle(s). 900 means "n/a"

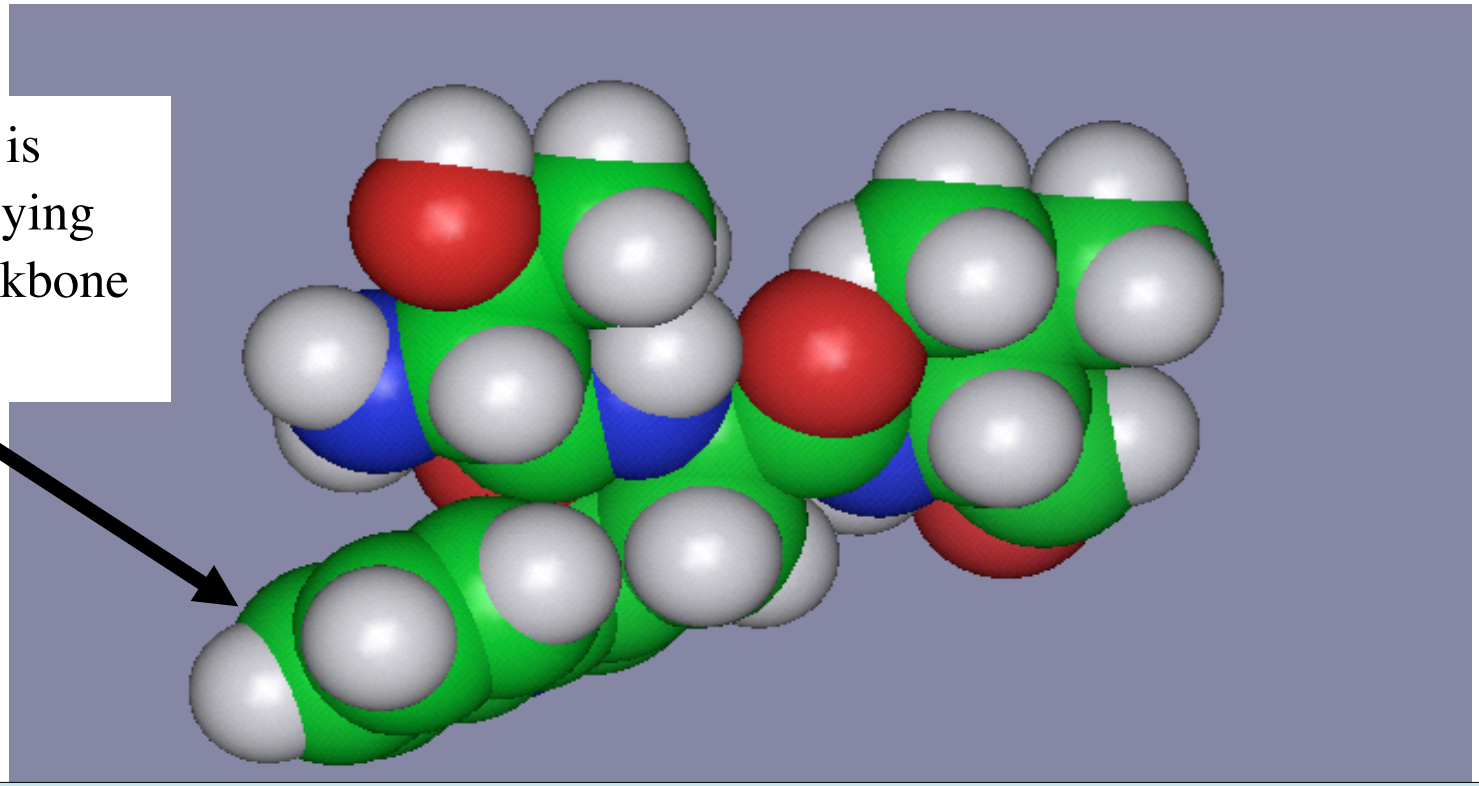
rotamer coordinates

Stats compiled from a set of 240 high resolution PDB structures.

Lovell, S. C., Word, J. M., Richardson, J. S., & Richardson, D. C. (2000). The penultimate rotamer library. *Proteins: Structure, Function, and Bioinformatics*, *40*(3), 389-408.

Rotamer stability depends on $\phi\psi$

W sidechain is shown here lying over Thr backbone

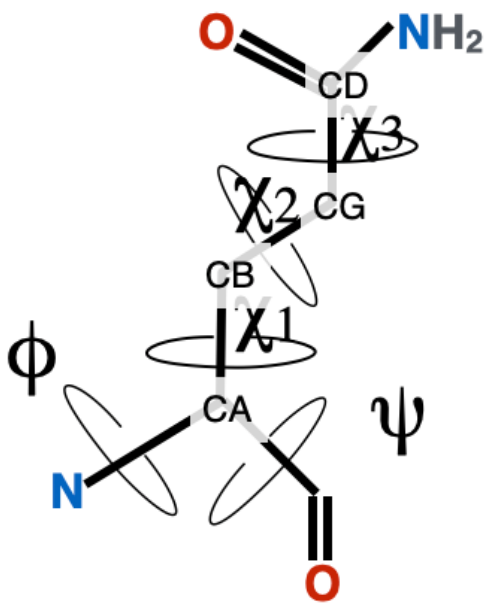


Rotamers of W*:	$\chi1$	$\chi2$	P(ϕ=-140,ψ=160	P(ϕ=-60,ψ=-40
p-90	+60	-90	0.372	0.079
p90	+60	+90	0.238	0.005
t-105	180	-105	0.033	0.251
t90	180	90	0.021	0.268
m0	-65	5	0.038	0.124
m95	-65	95	0.183	0.203

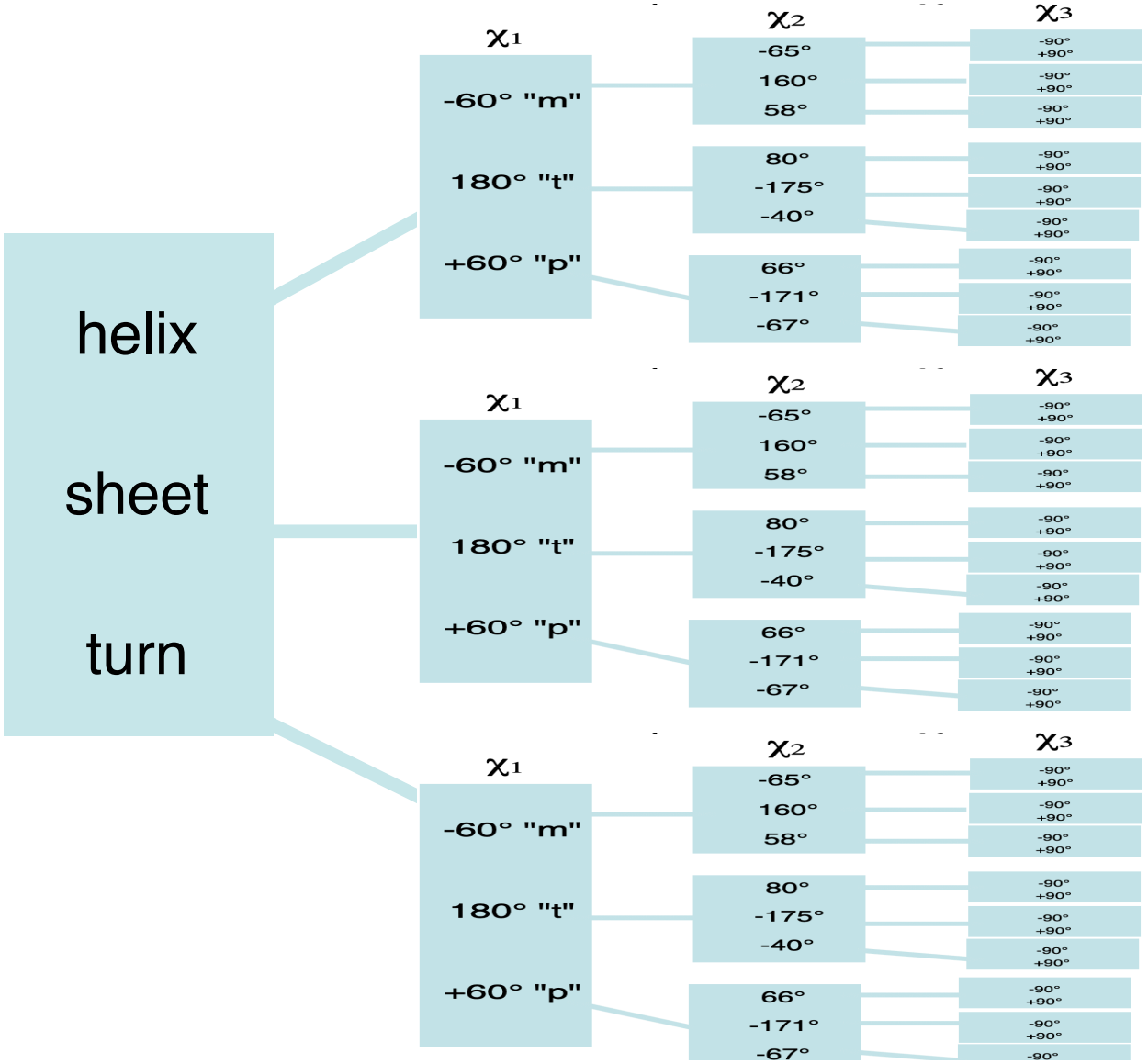
Roland Dunbrack's is a *backbone-dependent* rotamer library

Backbone dependent rotamer tree

$\phi\psi$ determines preference for χ_1 , determines the preference for χ_2 , determines the preference for χ_3,



Gln

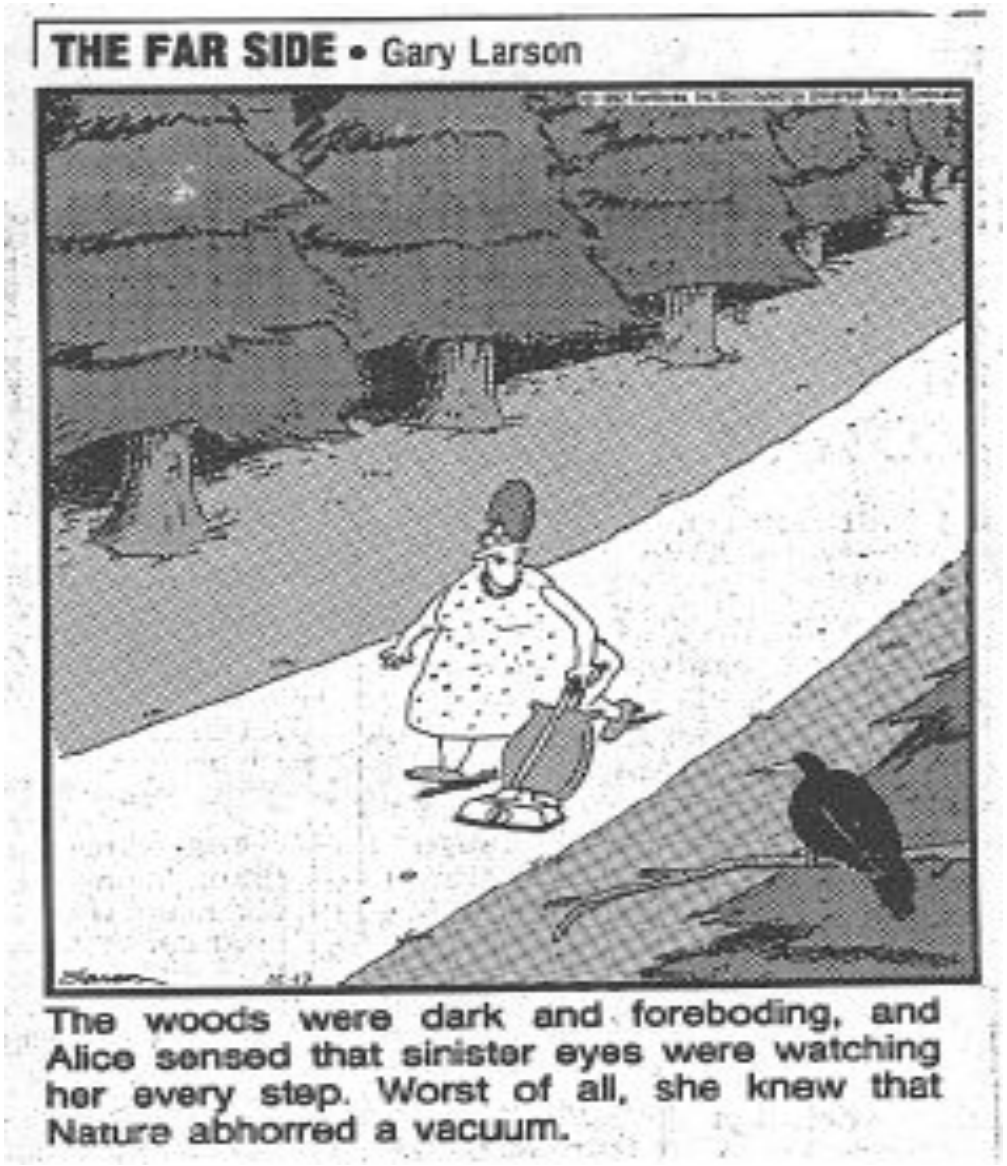


Exercise 21.2: Protein design using Protein | Protein builder

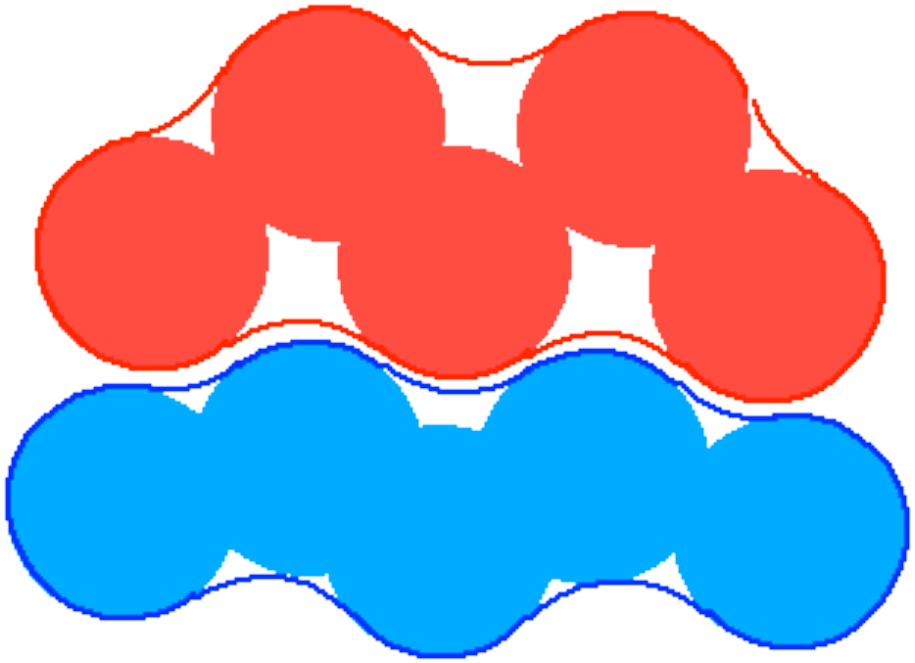
- Open "messedup.moe"
- Using **Protein | Protein builder** , find a better rotamer.
- Select several sidechains that are in mutual contact. Click **REPACK.** wait. What happened?
- Protein design: Select a buried sidechain that is too small, In ***targets*** add large sidechains Trp, Phe. Hit **Rotamers**. Inspect. Select a Trp rotamer. **Keep**.
- Select sidechains near the new Trp side chain. Click **REPACK**. Is the new Trp "happy" where you put it? (Happy means no clashes, no buried H-bond donor/acceptors, no holes, good *shape complimentarity*.)
- Design more residues this way.

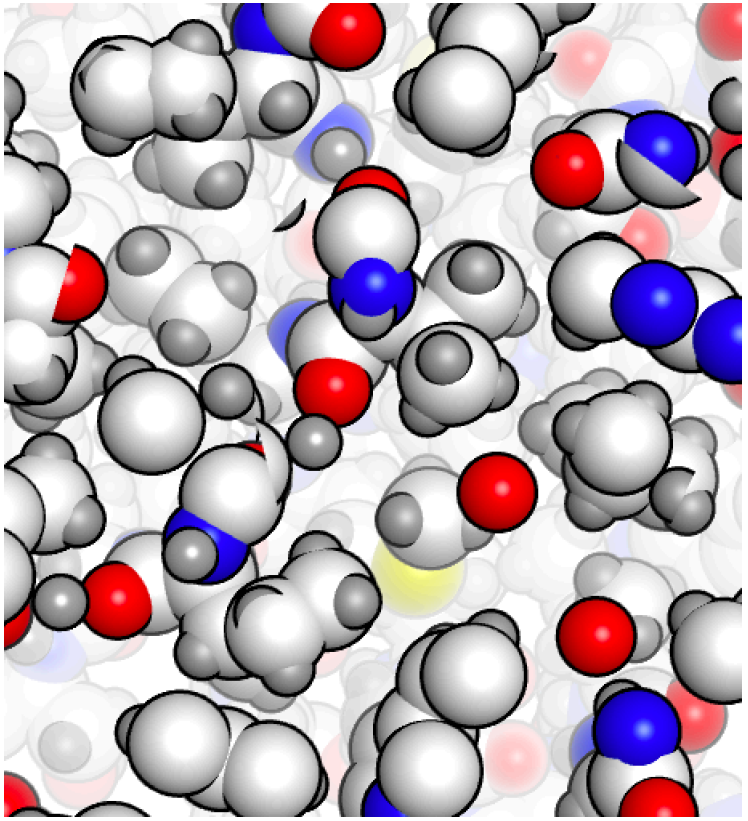
The Protein Core:

Nature abhors a vacuum



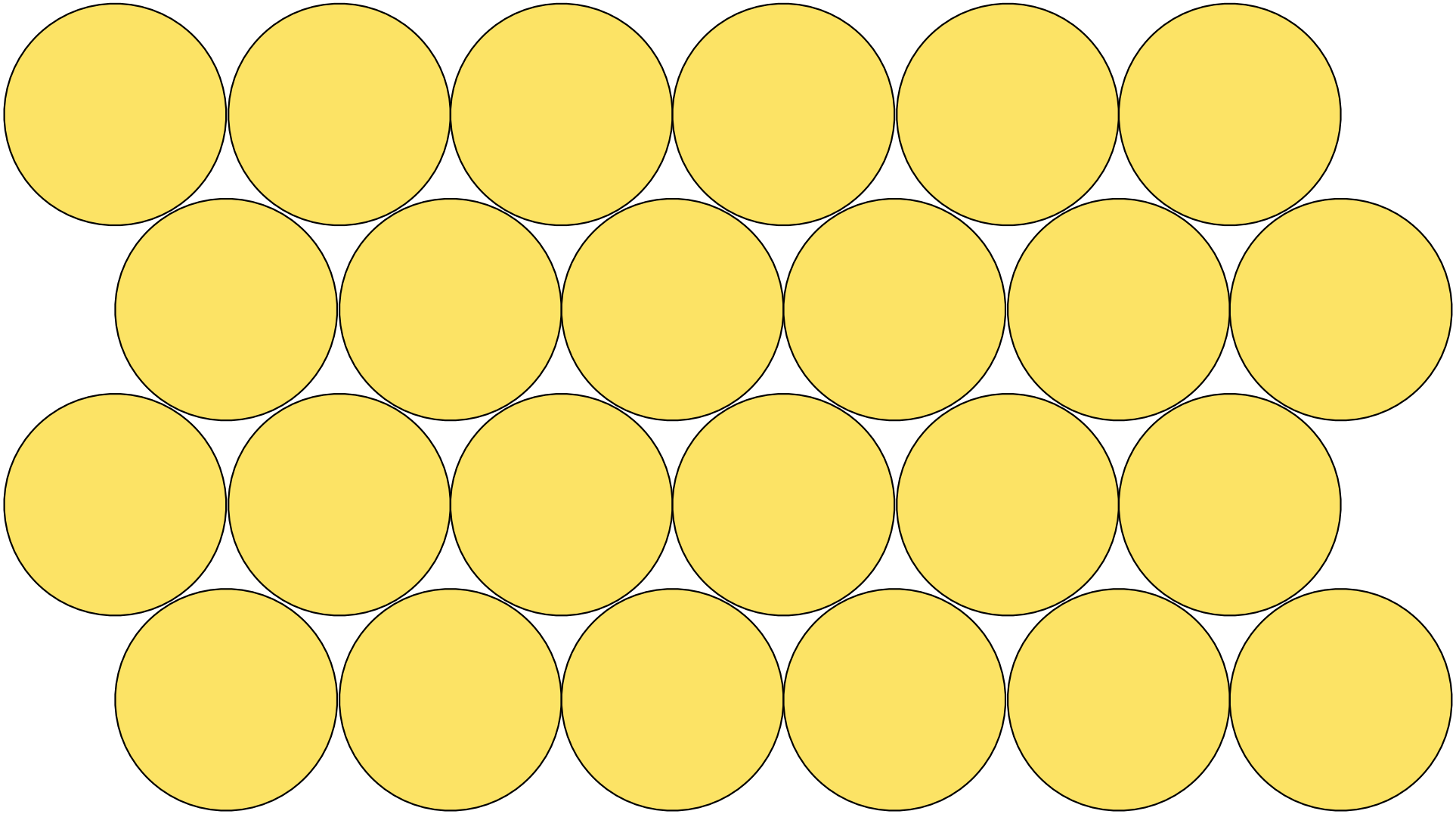
Shape Complementary



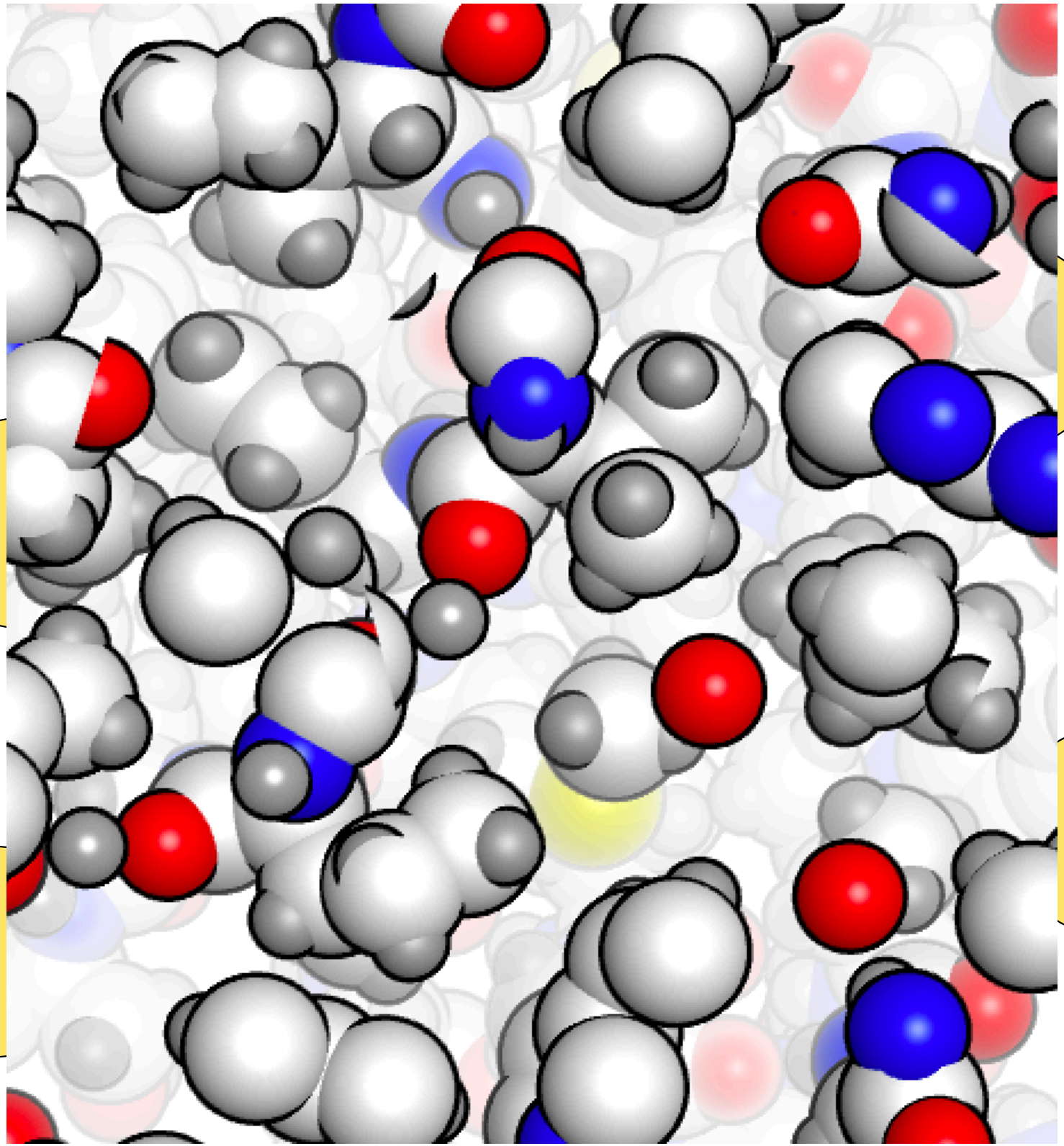


Complementary surfaces leave relatively little unfilled (void) space. Protein cores are well-packed with little empty space.

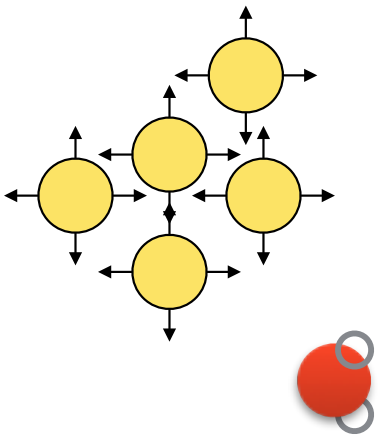
close packing



is **not** the way of proteins.

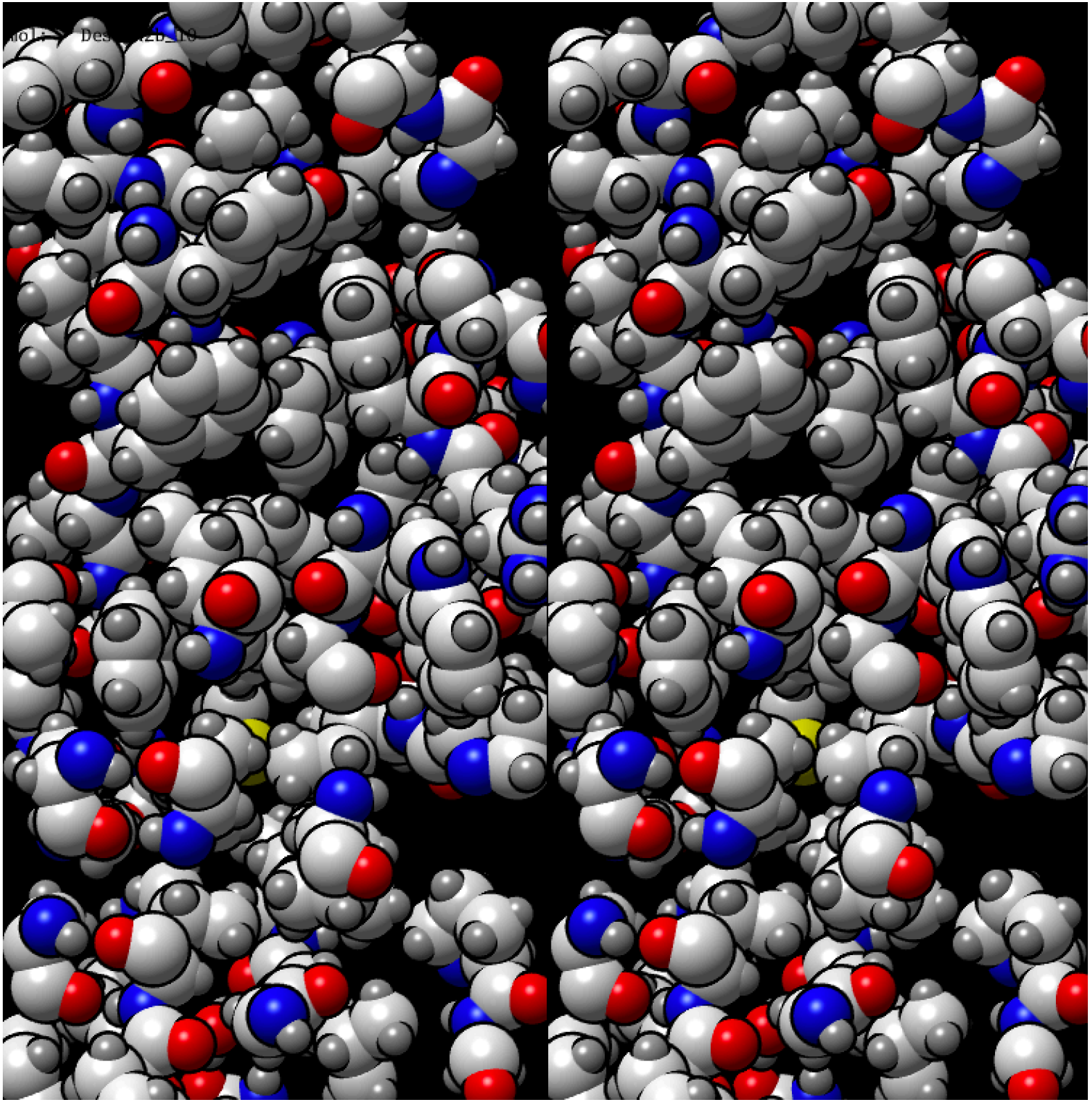


Empty space inside a protein is wide enough for wiggle room, but not wide enough to drive a water molecule through.



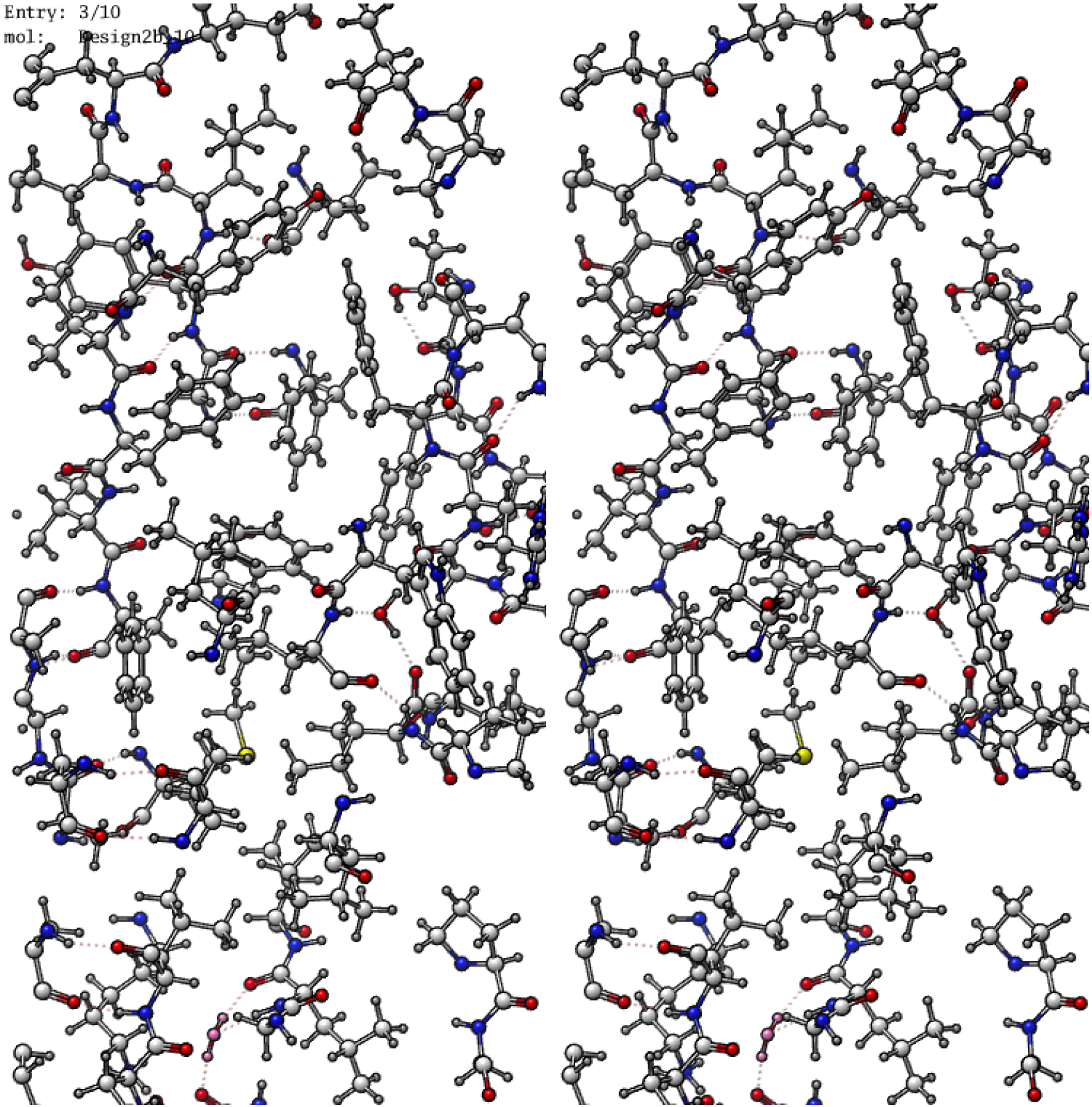
About 1/2 an atom wide.

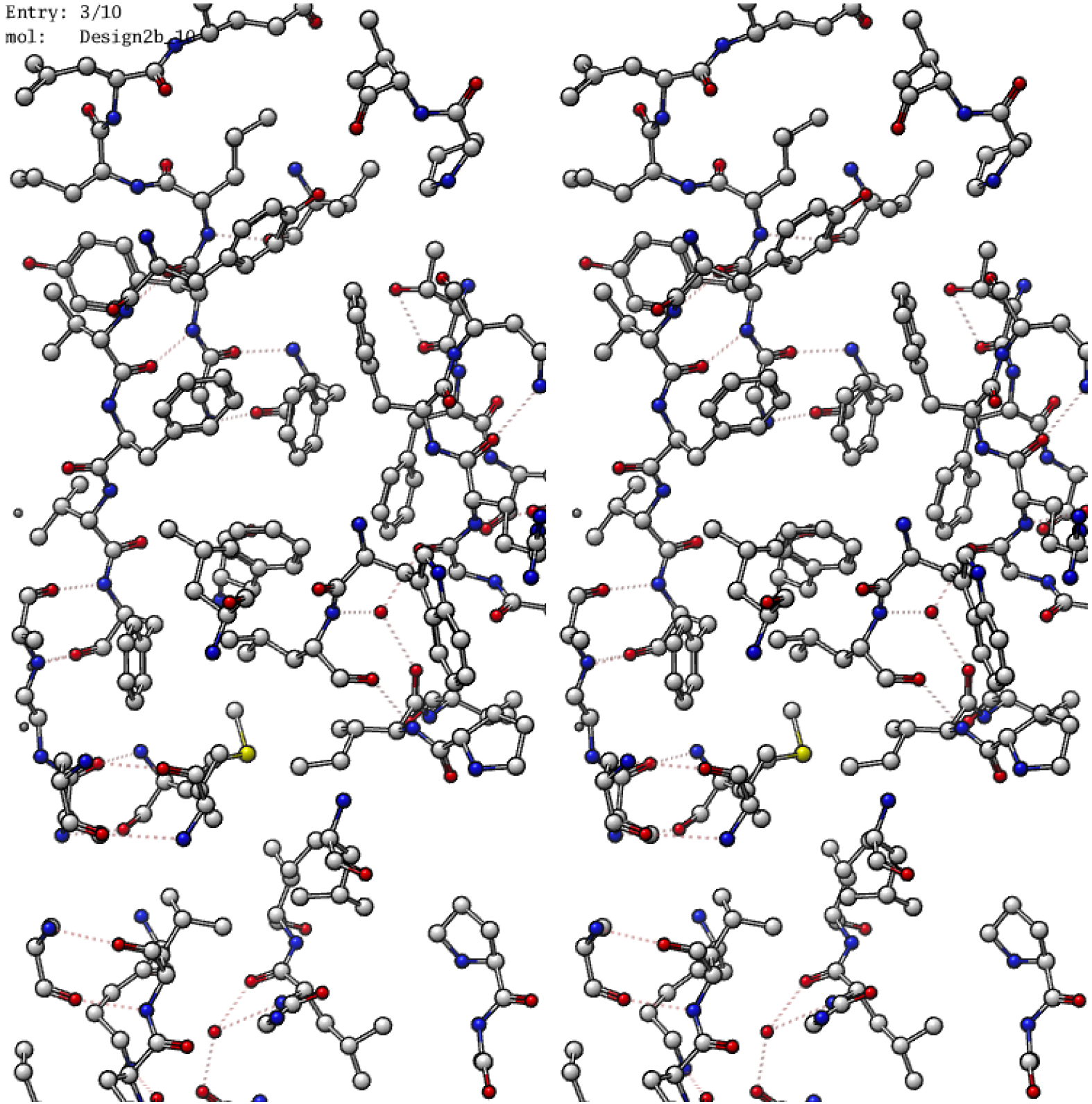
If you add that to the radius of two carbon atoms, you get a typical carbon-carbon non-bonded distance = 1.5 + 1.5 + 0.75 = 3.75Å

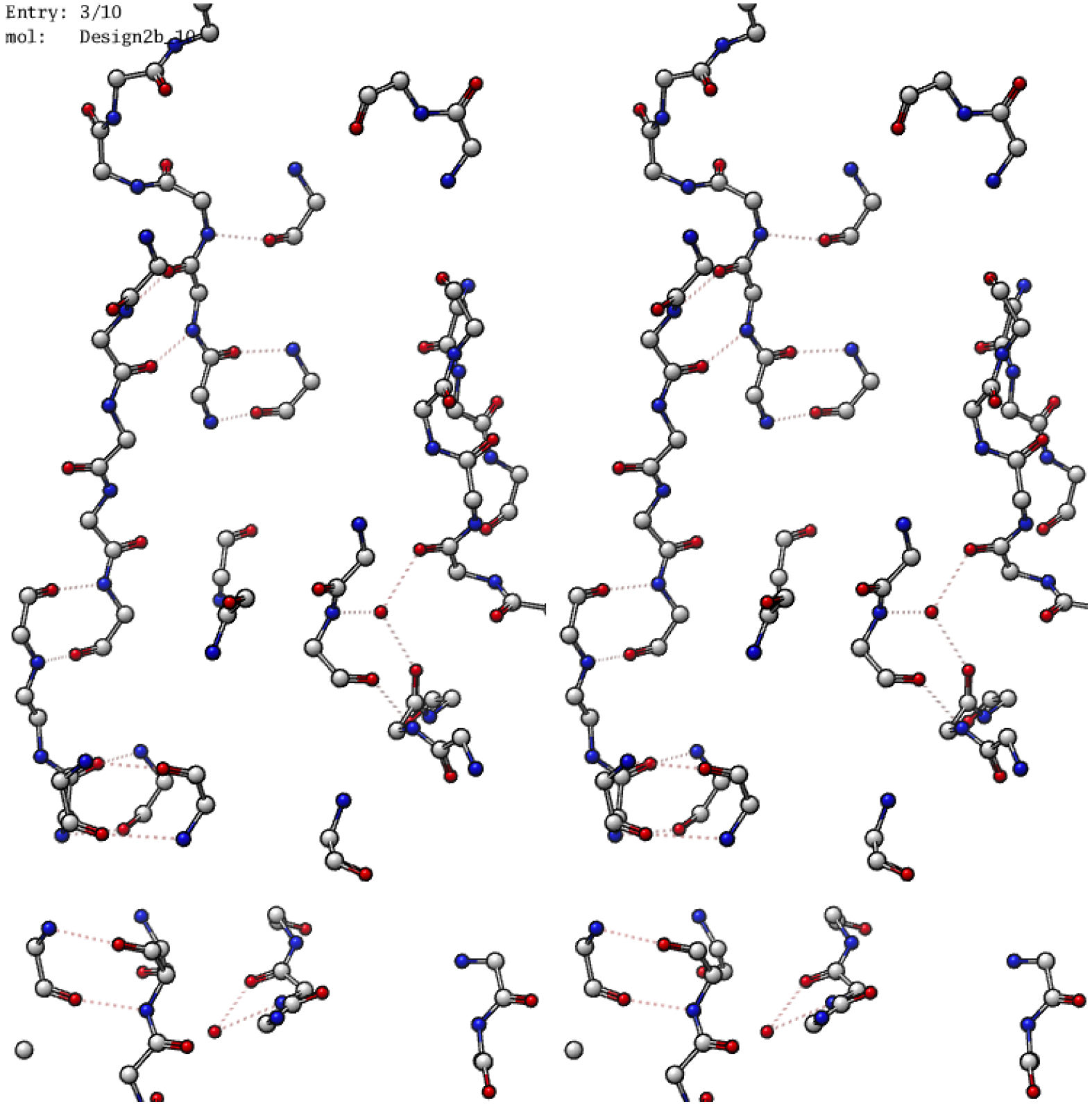


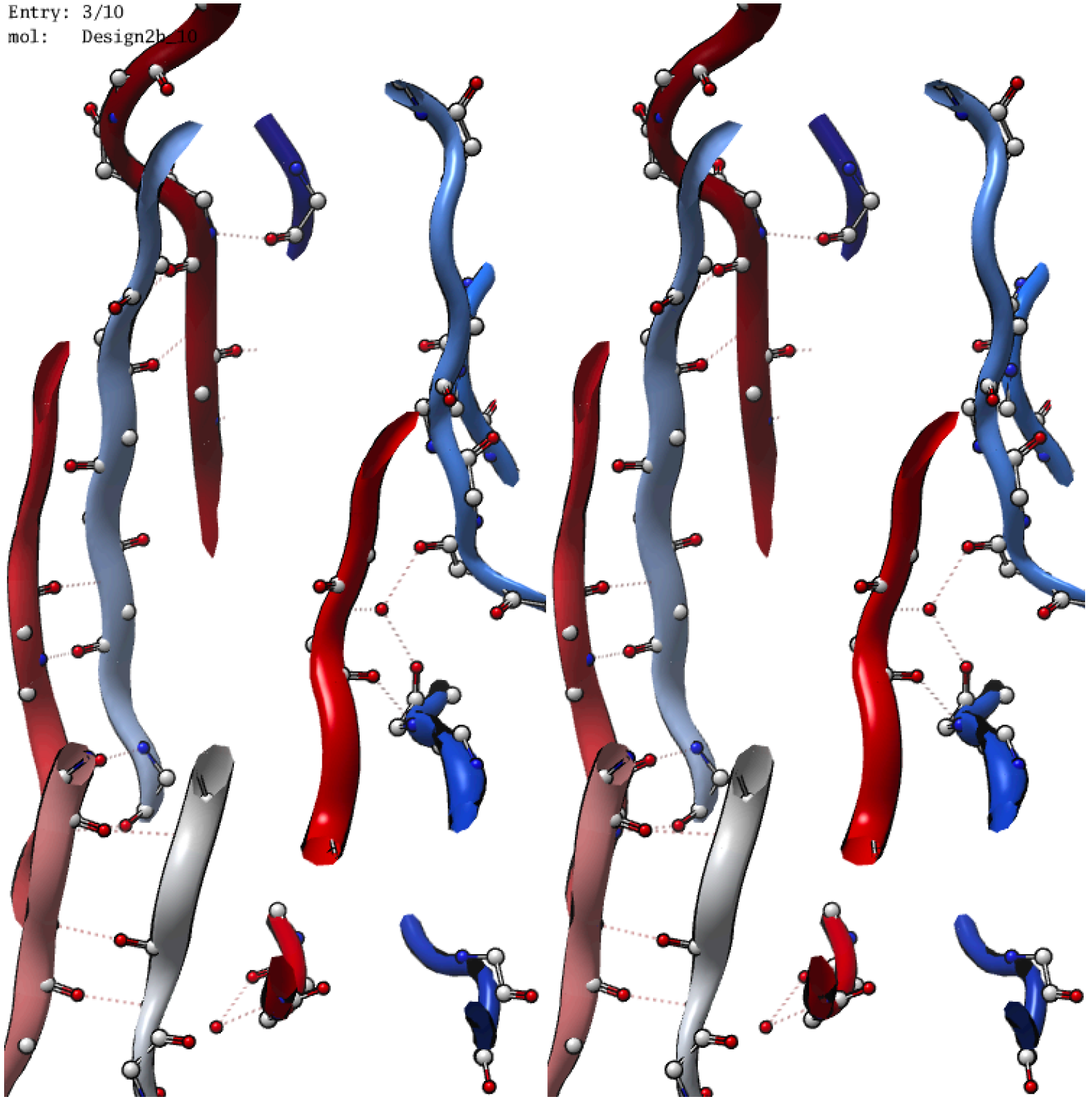
8Å slab

stereo!

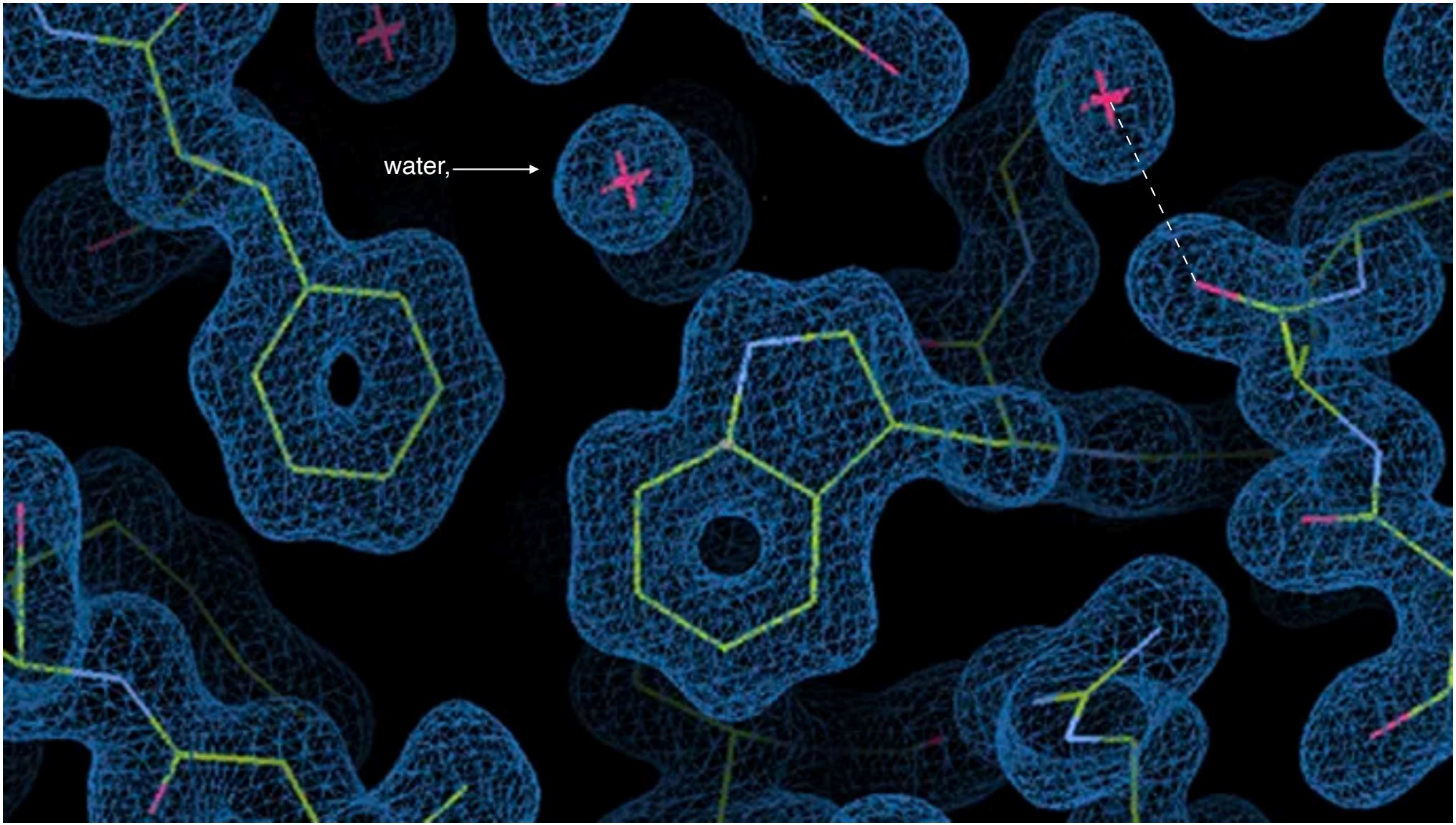

Entry: 3/10
mol: Design2b_10


Entry: 3/10
mol: Design2b_10


Entry: 3/10
mol: Design2b_10


Entry: 3/10
mol: Design2b_10

waters fills polar pockets and cavities



Adding waters one at a time

- Locate an *unsatisfied hydrogen bond donor or acceptor* with enough space to fit a water, and which is in a buried cavity or pocket.
- **Edit | Build | Molecule** (or **Builder**)
- Clear selections.
- Click **O** (oxygen). OK.
- Select the new water molecule. **EPUSIEPF**. **Minimize**.

Exercise 21.3
Adding *freeze dried* waters

Part 1 -- Get started

Make sure Select | Synchronize is checked.

1. Open **1rx5** from **PDB** within **MOE**.
2. **Potential Setup lower corner menu | Load | Amber14EHT**
    1. Select maximum threads.
    2. Fix hydrogens.
    3. Fix charges.
    4. OK.

    You are ready to add waters.

Part 2 -- Hydrate your protein

3. **Compute | Simulations | Dynamics**
    1. Solvent Setup :
    2. Layer, Water, NaCl 0M, 4.0, Delete far, OK.
    3. Cell Setup: No periodicity (don't change it)
    4. Constrain: light bonds
    5. Rigid water
    6. Time step 0.002 ps
    7. NPA algorithm
    8. OK
4. in SEQ window: select all waters and ions.
5. In MOE window: **EPUSIEPF***
6. Minimize.

    Now your protein is hydrated. Go to Part 3.

*EPUSIEPF = Edit | Potential | Unfix, Select | Invert, Edit | Potential | Fix

Freeze dried protein nydrate: remove waters that are exposed to bulk solvent or move too much

Part 3 -- Freeze dry

1. In SEQ window, select water and ions chains.
2. **Select | Selector**, Click UI (user interface)
    1. Check Selected Chains
    2. Operation: or
    3. Connectivity | Accessibility
    4. Probe radius 5.0. <---- critereon for bulk water exposure
    5. Exposed. (Some waters and ions are selected)
    6. Molecule. (Now complete water molecules are selected.)
    7. Note the number of atoms selected. Number of waters is that number divided by 3.
    8. In MOE window, Delete selected.
    9. Repeat 5-8, until...
3. No more exposed waters? Is the number of waters left less than 20? Stop. Go to Part 4.
4. Minimize.
    Now your protein is freeze-dried.

Part 4 -- Molecular dynamics

5. **Select | Solvent**
6. EPUSIEPF
7. **Compute | Simulations | Dynamics**
    1. Change name to water.mdb
    2. Uncheck "rigid water"
    3. Protocol: prod {ps=250 T=500} (You may explore a higher or lower temperature if you do this a second time.)
    4. OK. If the simulation does not finish in time, **Cancel | Dynamics** when told.

Part 5 -- Find stable waters

8. Open water.c.250.mdb (opens in database viewer, DBV)
9. DBV: **File | Browse**
10. Hit the play button. Use the slider to set the speed of playback.
11. In MOE window. remove protein atoms, display ribbon, and
    make waters **spacefill**. Waters sitting in deep energy wells move very
    little. Waters in shallow energy wells move alot and escape to other energy wells.
12. Select the five **least** mobile waters and color them light blue.
13. Stop the animation. Go to the last frame and hit **keep**. (sends frame to MOE window) Close.
14. Display protein as ribbon only. Save the MOE file.
    Upload it to the homework server as Exercise 21.