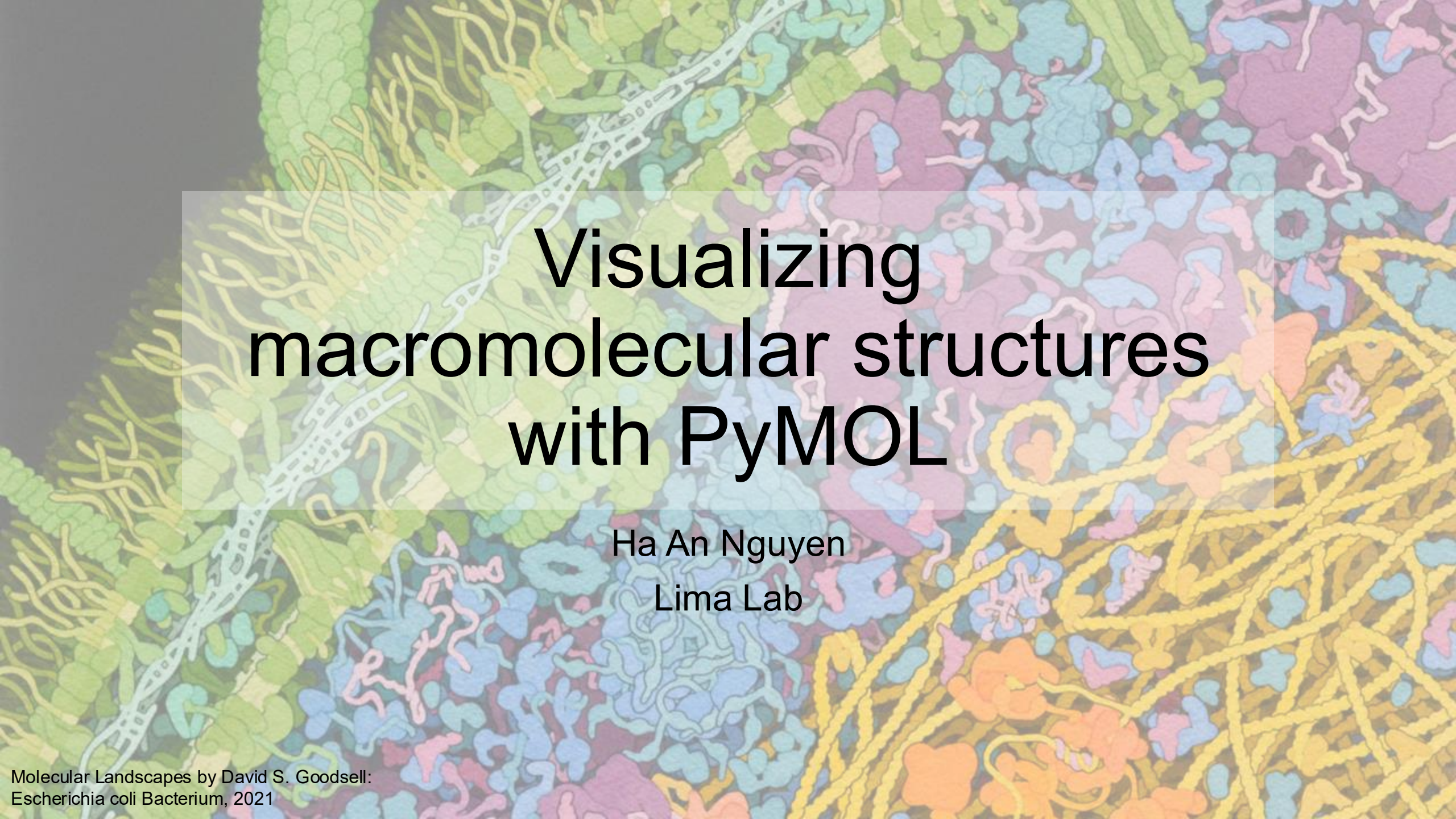


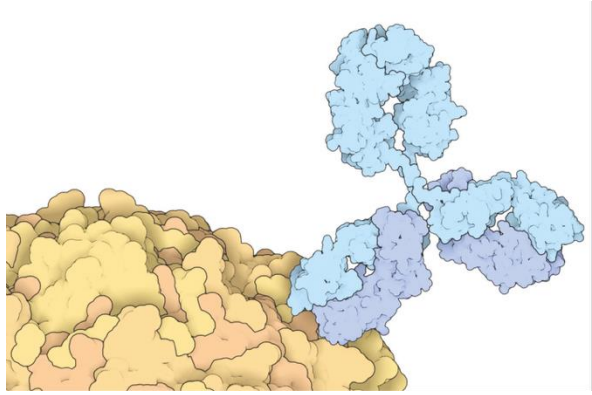
Visualizing macromolecular structures with PyMOL

Ha An Nguyen

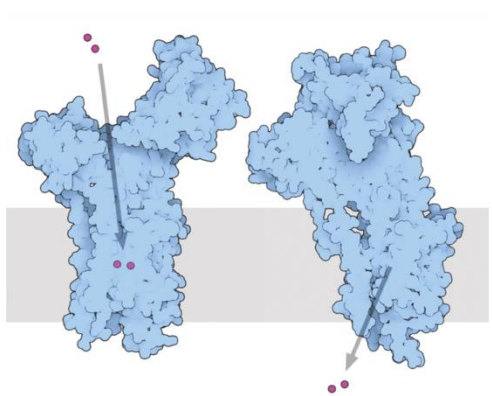
Lima Lab

Molecular Landscapes by David S. Goodsell:
Escherichia coli Bacterium, 2021

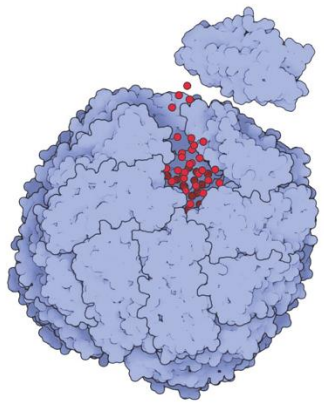
Structure informs function of proteins



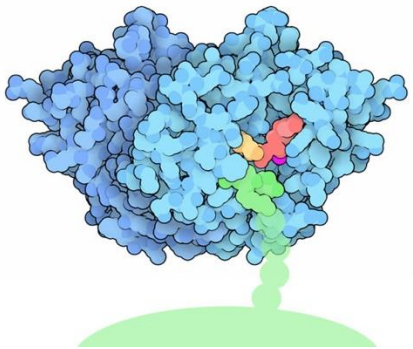
IgG



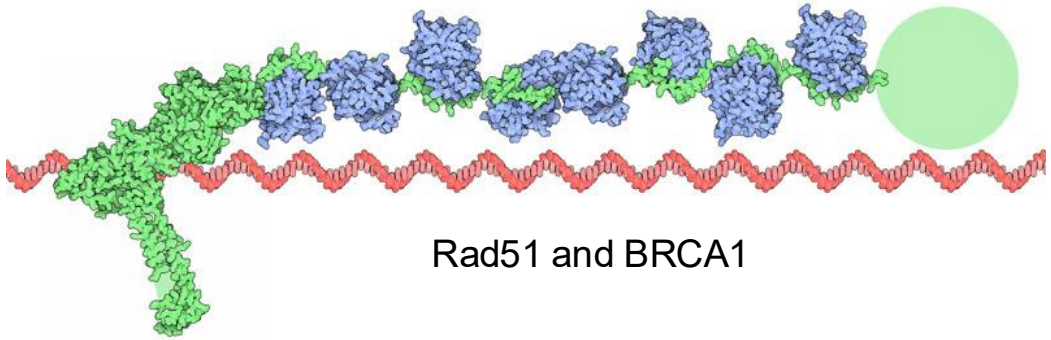
Ca pumps



Ferritin

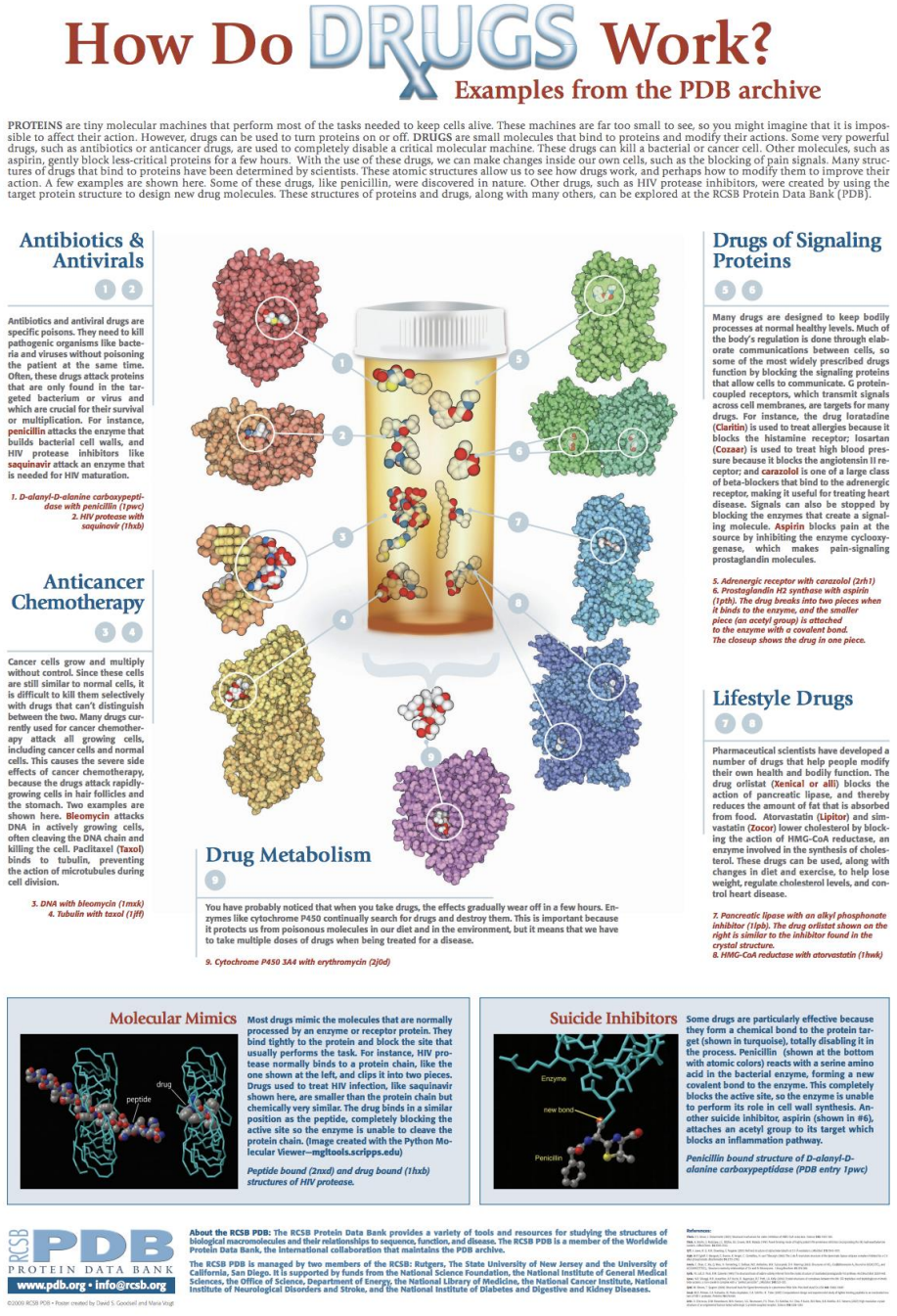


ABO Blood Type Glycosyltransferases



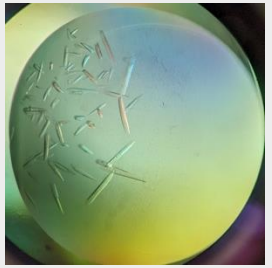
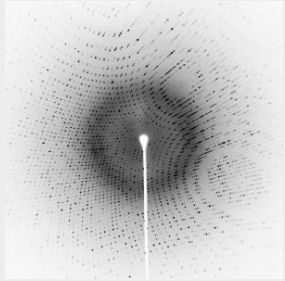
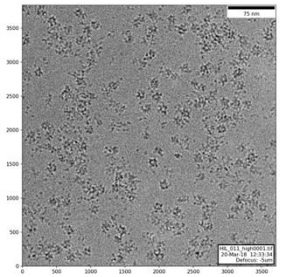
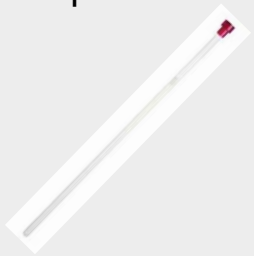
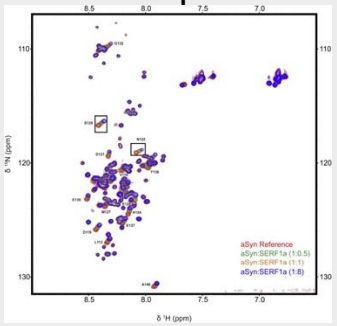
Rad51 and BRCA1

- Amino acid sequence to full functional complex
  - Mutations
  - Drug design

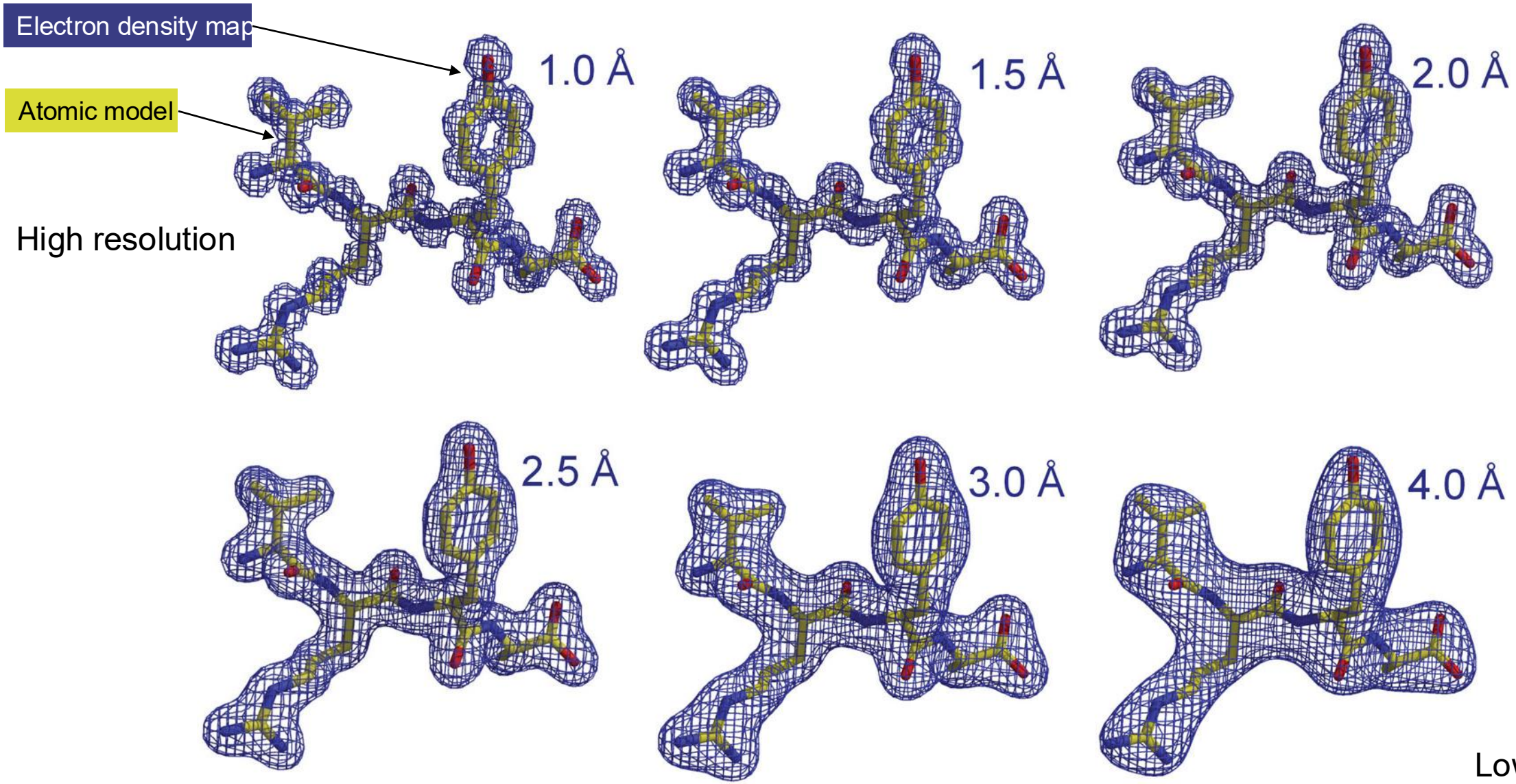


Source: PDB, www.rcsb.org

How do we determine protein structures?

Method	Sample	Instrument	Data	Advantages	Limitations
X-ray crystallography	Crystals 	Synchrotron 	Diffraction pattern 	• High-res data • Very common • No size restriction	• Dependent on crystallization • Limited conformations
Cryo-electron microscopy	Sample vitrified on grids 	Electron microscope 	Micrographs 	• Can capture states • Small amount of sample required • Fast sample prep	• Size limit (smaller = harder) • Preferred orientation • Computationally intensive
Nuclear Magnetic Resonance Spectroscopy	Sample in solvent 	NMR Spectrometer 	NMR spectra 	Only method for real-time, in-solution motion detection	• Size limit (larger = harder) • Need a lot of very pure sample

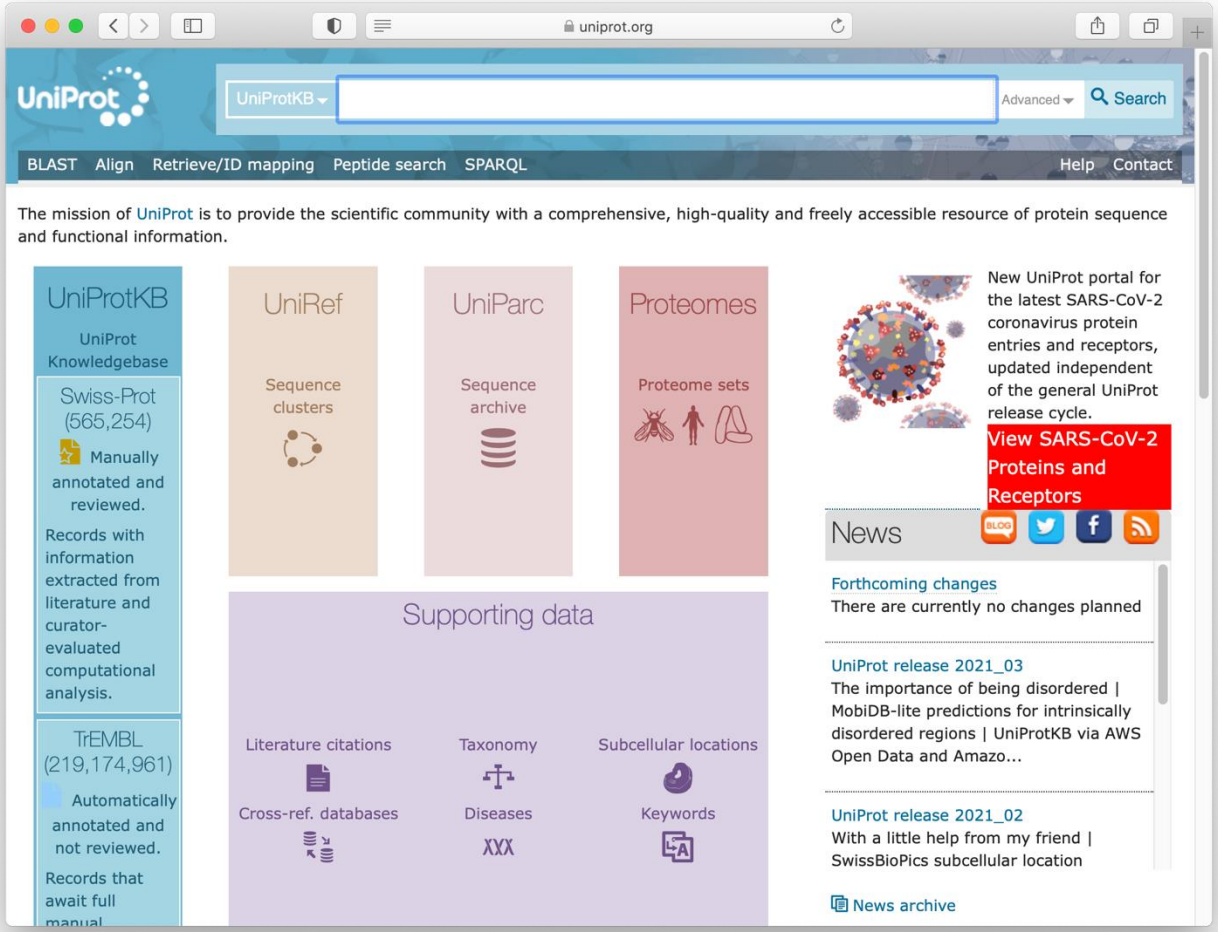
Resolution and information level



Source: Biomolecular Crystallography: Principles, Practice, and Application to Structural Biology

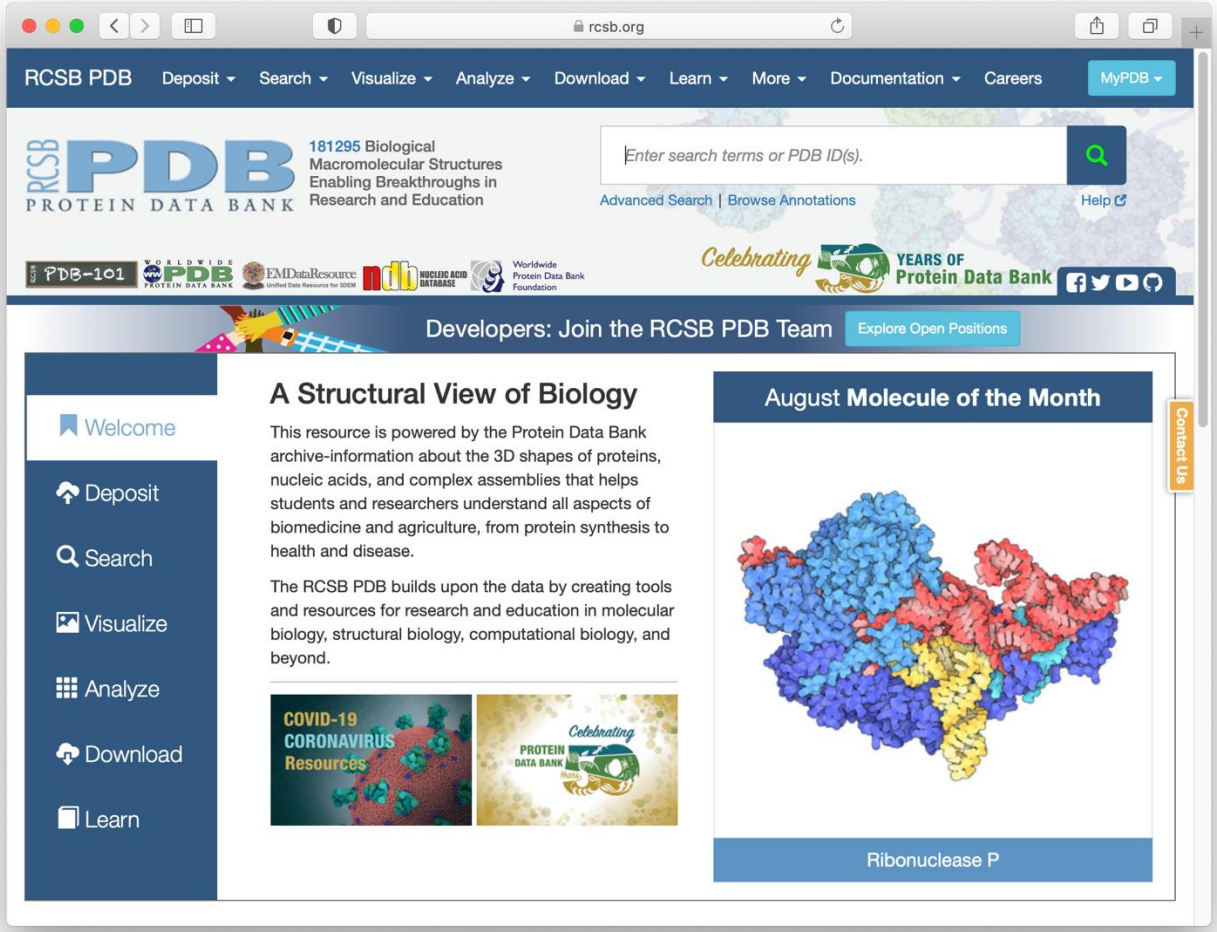
Where to find protein information

UniProt: **Uni**versal **Prot**ein Resource
www.uniprot.org



protein sequence and functional information

RCSB PDB: **P**rotein **D**ata**B**ank
www.rscb.org



central repository of biomolecular structures

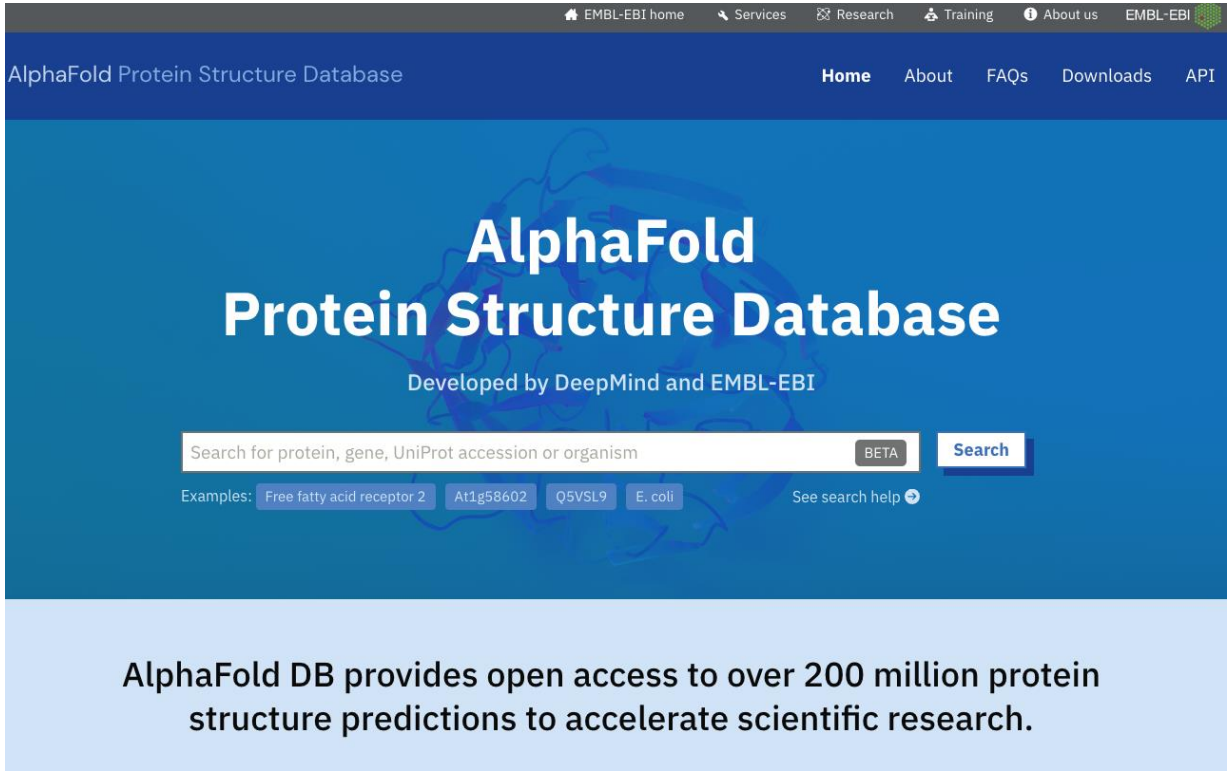
AlphaFold

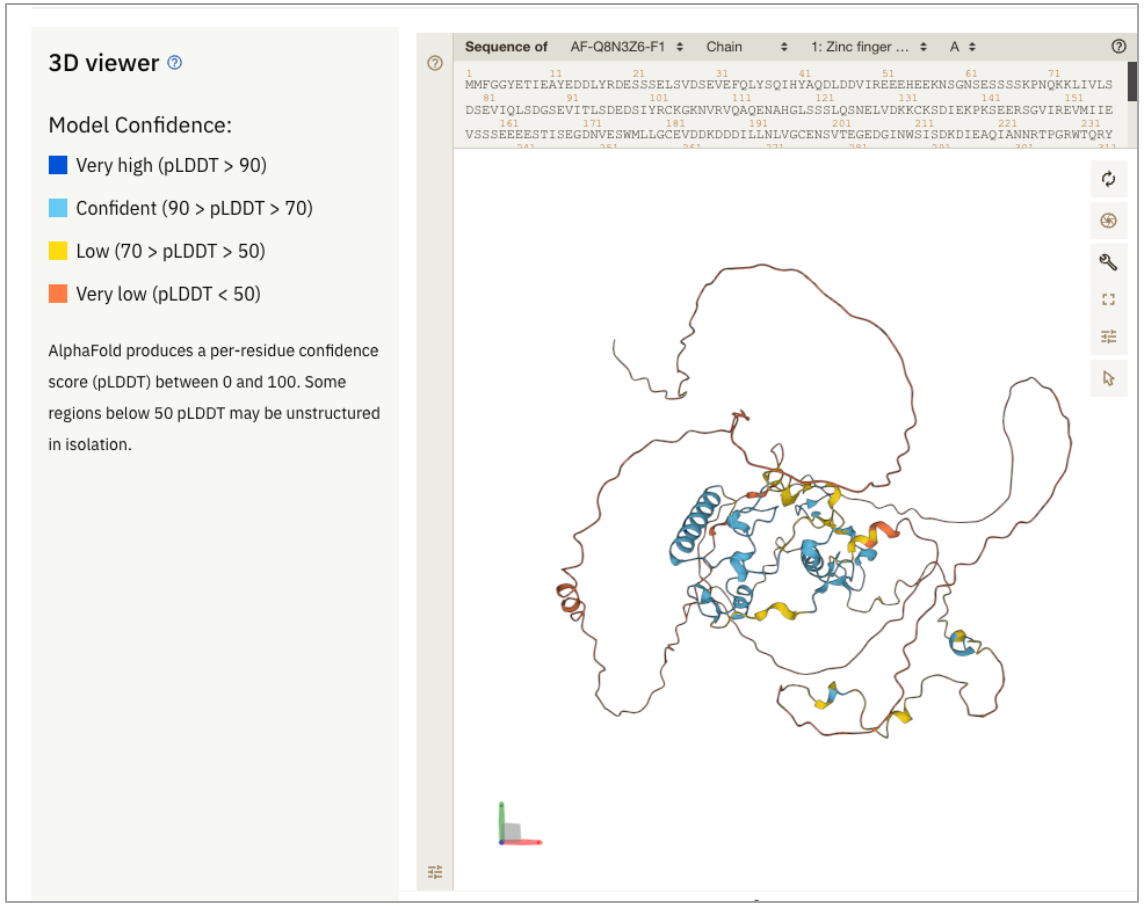
Database: https://alphafold.ebi.ac.uk/

models for almost every protein in Uniprot

Revolutionary tool for structural biology, but proteins are chaotic

post-translational modifications (PTMs), environment, ligands, oligomerization etc…





https://alphafold.ebi.ac.uk/entry/Q8N3Z6

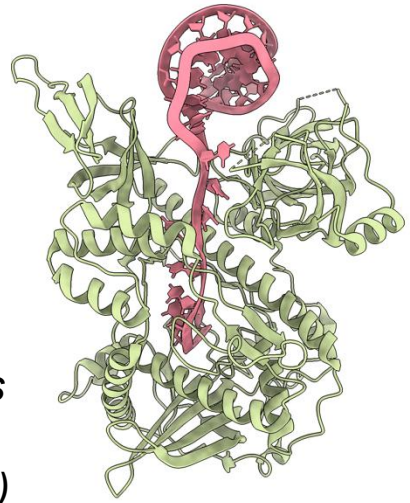
Useful resources

Name	Link	Purpose
UniProt	https://www.uniprot.org/	Protein information database
Protein Data Bank (PDB)	https://www.rcsb.org/	Protein structure repository
PyMOL	https://pymol.org/	Download PyMOL
PyMOL wiki	www.pymolwiki.org	Information on PyMOL such as examples of settings
ConSurf	http://consurf.tau.ac.il	Evolutionary conservation of amino/nucleic acid sequences
PISA	www.ebi.ac.uk/pdbe/pisa/	Protein interface and assemblies
Dali	http://ekhidna2.biocenter.helsinki.fi/dali/	Search PDB for similar structures
EMDB	https://www.ebi.ac.uk/emdb/	Repository for EM/ET data
AlphaFold database	https://alphafold.ebi.ac.uk/	Database of AlphaFold predictions
ChimeraX	https://www.cgl.ucsf.edu/chimerax/	Another visualization software, works well with EM maps

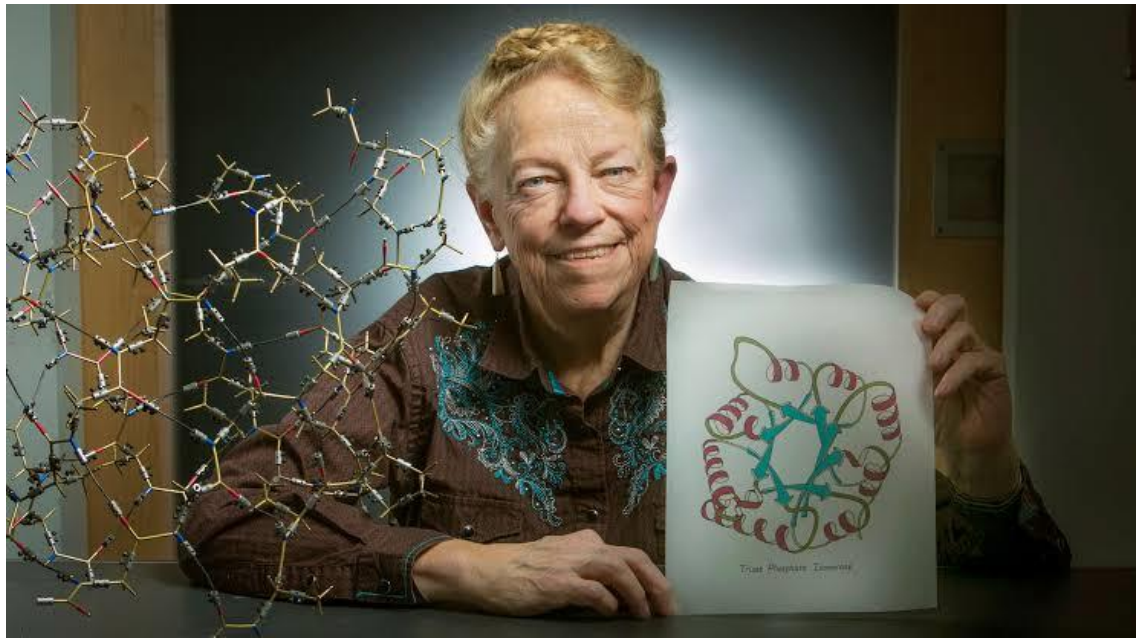
overview of structure files

model

- the coordinates of the atoms
- file types: .pdb, .cif



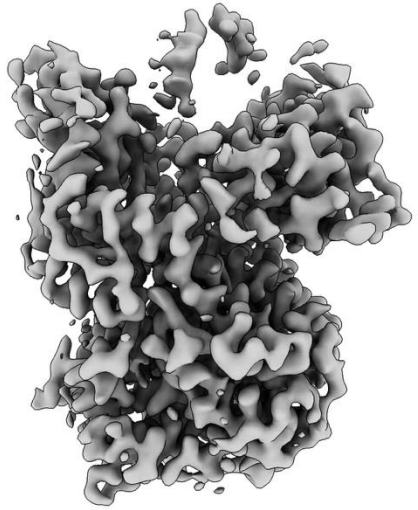
Note: Cartoon (ribbon) representation is an artistic interpretation of structures invented by Dr. Jane Richardson (Duke)



https://stories.duke.edu/sciences-mother-of-ribbon-diagrams-celebrates-50-years-at-duke

map

- the experimental data with which you can place atoms into position (x-ray or EM)
- can also be called structure factors or map coefficients
- file types: .map, .mrc, .ccp4, .mtz, .cif



emd_27829.map

```
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2  0000 0000 0000 0000 0000 0000 2c01 0000
3  2c01 0000 2c01 0000 0000 4943 0000 4943
4  0000 4943 0000 b442 0000 b442 0000 b442
5  0100 0000 0200 0000 0300 0000 9a89 00bf
6  ecc7 3c3f 34c1 c1b8 0100 0000 0000 0000
7  0000 0000 0000 0000 0000 0000 0000 0000
8  0000 0000 0000 0000 0000 0000 0000 0000
9  0000 0000 0000 0000 0000 0000 0000 0000
10 0000 0000 0000 0000 0000 0000 0000 0000
11 0000 0000 0000 0000 0000 0000 0000 0000
12 0000 0000 0000 0000 0000 0000 0000 0000
13 0000 0000 0000 0000 0000 0000 0000 0000
14 4d41 5020 4441 0000 e0eb 913c 0100 0000
15 3a3a 3a3a 454d 4441 5441 4241 4e4b 2e6f
16 7267 3a3a 3a3a 454d 442d 3237 3832 393a
17 3a3a 3a20 2020 2020 2020 2020 2020 2020
18 2020 2020 2020 2020 2020 2020 2020 2020
19 2020 2020 2020 2020 2020 2020 2020 2020
20 2020 2020 2020 2020 2020 2020 2020 2020
21 2020 2020 2020 2020 2020 2020 2020 2020
22 2020 2020 2020 2020 2020 2020 2020 2020
23 2020 2020 2020 2020 2020 2020 2020 2020
```

The primary information stored in the PDB archive consists of coordinate files that list of atoms and their 3D location in space stored as plain text (ASCII) files Either as .pdb or mmcif (.cif) format

1f88.pdb

```
HEADER    SIGNALING PROTEIN                       29-JUN-00   1F88
TITLE     CRYSTAL STRUCTURE OF BOVINE RHODOPSIN
CAVEAT     1F88    MAN E 3 HAS WRONG CHIRALITY AT ATOM C1
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: RHODOPSIN;
COMPND   3 CHAIN: A, B
SOURCE    MOL_ID: 1;
SOURCE   2 ORGANISM_SCIENTIFIC: BOS TAURUS;
SOURCE   3 ORGANISM_COMMON: CATTLE;
SOURCE   4 ORGANISM_TAXID: 9913;
SOURCE   5 ORGAN: RETINA;
SOURCE   6 OTHER_DETAILS: BOVINE RETINA, ROD CELL OUTER SEGMENTS
KEYWDS    PHOTORECEPTOR, G PROTEIN-COUPLED RECEPTOR, MEMBRANE PROTEIN, RETINAL
KEYWDS   2 PROTEIN, VISUAL PIGMENT, SIGNALING PROTEIN
EXPDTA    X-RAY DIFFRACTION
AUTHOR    T.OKADA,K.PALCZEWSKI,R.E.STENKAMP,M.MIYANO
REVDAT   5   29-JUL-20 1F88    1       CAVEAT COMPND REMARK HETNAM
REVDAT   5 2                   1       LINK   SITE   ATOM
REVDAT   4   13-JUL-11 1F88    1       VERSN
REVDAT   3   24-FEB-09 1F88    1       VERSN
REVDAT   2   19-AUG-08 1F88    1       REMARK
REVDAT   1   04-AUG-00 1F88    0
ATOM     16  ND2 ASN A   2      46.634  -7.296 -23.187  1.00 59.21           N
ATOM     17  N   GLY A   3      44.982  -2.466 -23.598  1.00 49.38           N
ATOM     18  CA  GLY A   3      45.060  -1.050 -23.332  1.00 48.15           C
ATOM     19  C   GLY A   3      44.975  -0.193 -24.576  1.00 47.25           C
ATOM     20  O   GLY A   3      45.481  -0.545 -25.645  1.00 48.00           O
ATOM     21  N   THR A   4      44.321   0.946 -24.419  1.00 45.08           N
ATOM     22  CA  THR A   4      44.165   1.892 -25.494  1.00 44.41           C
ATOM     23  C   THR A   4      45.409   2.775 -25.508  1.00 44.61           C
ATOM     24  O   THR A   4      45.662   3.492 -24.545  1.00 44.85           O
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ATOM     26  OG1 THR A   4      41.781   1.901 -25.083  1.00 44.84           O
ATOM     27  CG2 THR A   4      42.649   3.650 -26.435  1.00 43.69           C
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ATOM     33  CG  GLU A   5      49.822   3.538 -27.693  1.00 49.26           C
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ATOM     35  OE1 GLU A   5      51.016   1.511 -28.240  1.00 55.51           O
ATOM     36  OE2 GLU A   5      52.047   3.411 -28.638  1.00 51.62           O
ATOM     37  N   GLY A   6      47.387   5.855 -27.102  1.00 48.23           N
```

Header

Atom coordinates

what's in a pdb file

atom #, atom name, residue name, chain id, residue number, x/y/z coordinates of the atom, occupancy, bfactor, atom element

8e29.pdb

```
HEADER    RNA BINDING PROTEIN/RNA                 14-AUG-22   8E29
TITLE     HUMAN DIS3L2 IN COMPLEX WITH HAIRPIN C-U12
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: DIS3-LIKE EXONUCLEASE 2;
COMPND   3 CHAIN: A;
COMPND   4 SYNONYM: HDIS3L2;
COMPND   5 EC: 3.1.13.-;
COMPND   6 ENGINEERED: YES;
COMPND   7 MOL_ID: 2;
COMPND   8 MOLECULE: RNA HAIRPIN C-U12;
COMPND   9 CHAIN: B;
COMPND  10 ENGINEERED: YES
SOURCE    MOL_ID: 1;
SOURCE   2 ORGANISM_SCIENTIFIC: HOMO SAPIENS;
SOURCE   3 ORGANISM_COMMON: HUMAN;
SOURCE   4 ORGANISM_TAXID: 9606;
SOURCE   5 GENE: DIS3L2, FAM6A;
SOURCE   6 EXPRESSION_SYSTEM: SPODOPTERA FRUGIPERDA;
SOURCE   7 EXPRESSION_SYSTEM_TAXID: 7108;
SOURCE   8 MOL_ID: 2;
SOURCE   9 SYNTHETIC: YES;
SOURCE  10 ORGANISM_SCIENTIFIC: SYNTHETIC CONSTRUCT;
SOURCE  11 ORGANISM_TAXID: 32630
KEYWDS    3'-5' EXONUCLEASE, DS-RNA BOUND EXONUCLEASE, HUMAN EXONUCLEASE, RNA
KEYWDS   2 BINDING PROTEIN-RNA COMPLEX
EXPDTA    ELECTRON MICROSCOPY
```

8e29.pdb

```
SEQRES  61 A  885  ALA PHE ASP VAL LEU VAL LEU ARG TYR GLY VAL GLN LYS
SEQRES  62 A  885  ARG ILE TYR CYS ASN ALA LEU ALA LEU ARG SER HIS HIS
SEQRES  63 A  885  PHE GLN LYS VAL GLY LYS LYS PRO GLU LEU THR LEU VAL
SEQRES  64 A  885  TRP GLU PRO GLU ASP MET GLU GLN GLU PRO ALA GLN GLN
SEQRES  65 A  885  VAL ILE THR ILE PHE SER LEU VAL GLU VAL VAL LEU GLN
SEQRES  66 A  885  ALA GLU SER THR ALA LEU LYS TYR SER ALA ILE LEU LYS
SEQRES  67 A  885  ARG PRO GLY THR GLN GLY HIS LEU GLY PRO GLU LYS GLU
SEQRES  68 A  885  GLU GLU GLU SER ASP GLY GLU PRO GLU ASP SER SER THR
SEQRES  69 A  885  SER
SEQRES   1 B   33    U   C   G   G   C   G   C   C   U   U   U   C   G
SEQRES   2 B   33    A   G   G   C   G   C   C   G   U   U   U   U   U
SEQRES   3 B   33    U   U   U   U   U   U   U
HELIX    1 AA1 SER A   55  GLY A   66  1                                  12
HELIX    2 AA2 ILE A  329  GLY A  341  1                                  13
HELIX    3 AA3 SER A  348  GLU A  353  1                                   6
HELIX    4 AA4 PRO A  364  PHE A  368  5                                   5
HELIX    5 AA5 SER A  421  ARG A  430  1                                  10
HELIX    6 AA6 PRO A  445  GLU A  451  1                                   7
HELIX    7 AA7 GLU A  495  GLU A  502  1                                   8
HELIX    8 AA8 PRO A  509  LEU A  513  5                                   5
HELIX    9 AA9 SER A  521  GLY A  547  1                                  27
HELIX   10 AB1 GLU A  577  PHE A  601  1                                  25
HELIX   11 AB2 GLN A  614  GLN A  627  1                                  14
HELIX   12 AB3 ALA A  637  GLY A  649  1                                  13
HELIX   13 AB4 ASP A  651  SER A  666  1                                  16
HELIX   14 AB5 PHE A  706  LEU A  719  1                                  14
HELIX   15 AB6 ALA A  728  SER A  765  1                                  38
SHEET    1 AA1 6 ILE A  69  VAL A  72  0
SHEET    2 AA1 6 LEU A 110  LEU A 115 -1  O  VAL A 113   N  ILE A  69
SHEET    3 AA1 6 ARG A 231  GLU A 240 -1  O  TYR A 237   N  VAL A 112
SHEET    4 AA1 6 ILE A  94  ASP A  97  1  N  PHE A  95   O  ARG A 231
SHEET    5 AA1 6 GLU A  82  ILE A  85 -1  N  ILE A  85   O  ILE A  94
SHEET    6 AA1 6 ARG A  74  ILE A  75 -1  N  ARG A  74   O  PHE A  84
SHEET    1 AA2 6 ALA A 246  LYS A 252  0
SHEET    2 AA2 6 TYR A 265  PRO A 270 -1  O  LEU A 267   N  LYS A 252
SHEET    3 AA2 6 ILE A 278  PRO A 281 -1  O  VAL A 280   N  ALA A 266
SHEET    4 AA2 6 ALA A 315  GLY A 324  1  O  ALA A 315   N  TYR A 279
SHEET    5 AA2 6 LEU A 300  ASP A 307 -1  N  ILE A 302   O  ALA A 320
SHEET    6 AA2 6 ALA A 246  LYS A 252 -1  N  ALA A 247   O  CYS A 303
```

```
ATOM     20  N   GLU A  51      87.388  80.367 121.779  1.00 85.42           N
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ATOM     23  O   GLU A  51      87.163  79.215 119.271  1.00 85.42           O
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ATOM     25  CG  GLU A  51      83.734  79.732 121.773  1.00 85.42           C
ATOM     26  CD  GLU A  51      82.477  80.461 121.378  1.00 85.42           C
ATOM     27  OE1 GLU A  51      82.293  80.702 120.169  1.00 85.42           O
ATOM     28  OE2 GLU A  51      81.670  80.783 122.274  1.00 85.42           O
ATOM     29  N   THR A  52      85.998  77.507 120.177  1.00 86.60           N
ATOM     30  CA  THR A  52      86.234  76.597 119.068  1.00 86.60           C
ATOM     31  C   THR A  52      85.477  77.029 117.819  1.00 86.60           C
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ATOM     34  OG1 THR A  52      84.375  75.100 119.320  1.00 86.60           O
ATOM     35  CG2 THR A  52      86.189  74.865 120.861  1.00 86.60           C
ATOM     36  N   TYR A  53      85.988  76.602 116.670  1.00 86.91           N
ATOM     37  CA  TYR A  53      85.354  76.896 115.395  1.00 86.91           C
ATOM     38  C   TYR A  53      84.110  76.027 115.212  1.00 86.91           C
ATOM     39  O   TYR A  53      83.850  75.102 115.982  1.00 86.91           O
ATOM     40  CB  TYR A  53      86.329  76.677 114.245  1.00 86.91           C
ATOM     41  CG  TYR A  53      87.400  77.727 114.118  1.00 86.91           C
ATOM     42  CD1 TYR A  53      88.429  77.574 113.214  1.00 86.91           C
ATOM     43  CD2 TYR A  53      87.373  78.876 114.883  1.00 86.91           C
ATOM     44  CE1 TYR A  53      89.401  78.522 113.088  1.00 86.91           C
ATOM     45  CE2 TYR A  53      88.344  79.831 114.759  1.00 86.91           C
ATOM     46  CZ  TYR A  53      89.350  79.648 113.862  1.00 86.91           C
ATOM     47  OH  TYR A  53      90.316  80.599 113.736  1.00 86.91           O
ATOM     48  N   MET A  54      83.333  76.325 114.178  1.00 98.72           N
ATOM     49  CA  MET A  54      82.160  75.527 113.852  1.00 98.72           C
ATOM     50  C   MET A  54      82.382  74.709 112.587  1.00 98.72           C
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ATOM     53  CG  MET A  54      80.567  77.235 114.879  1.00 98.72           C
ATOM     54  SD  MET A  54      78.941  77.984 114.674  1.00 98.72           S
ATOM     55  CE  MET A  54      79.049  78.572 112.987  1.00 98.72           C
ATOM     56  N   SER A  55      81.452  73.786 112.252  1.00105.56           N
```
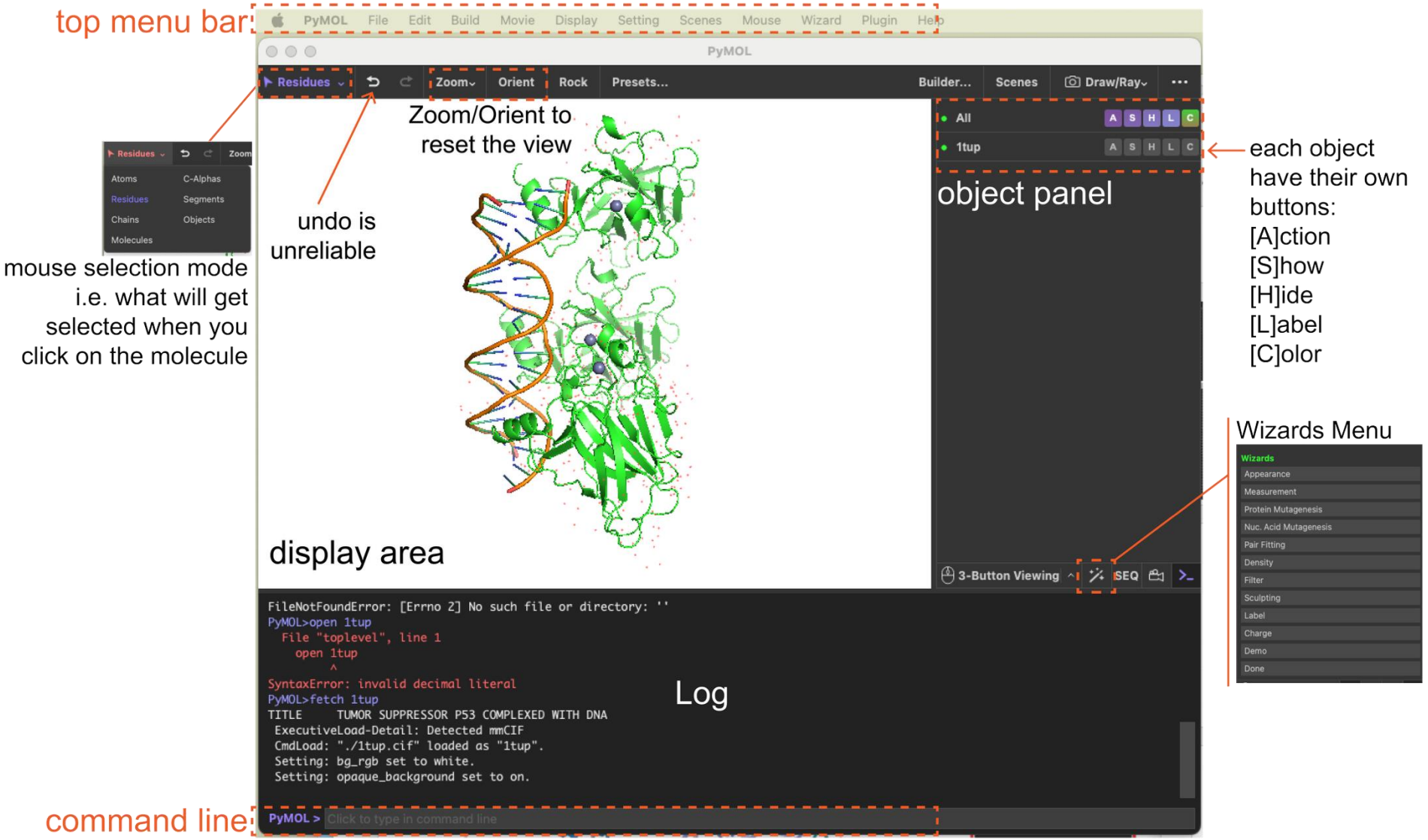

Why and how to visualize protein structures?

- To observe and analyze a structure of interest.
- To make images or movies to illustrate certain properties of the structure of interest.
- There are numerous programs and websites to visualize macromolecular structures. PyMOL and UCSF Chimera / ChimeraX are most used.

PyMOL tutorial

PyMOL interface



In this ppt, [square brackets] indicates these buttons

1. Loading structures

File > Get PDB > 1tup

OR

1. Go to PDB website: www.rscb.org
2. Find molecule of interest using search bar

Search for: **p53 complexed with dna**

3. Download Files > PDB Format > 1tup.pdb file and open it in PyMOL

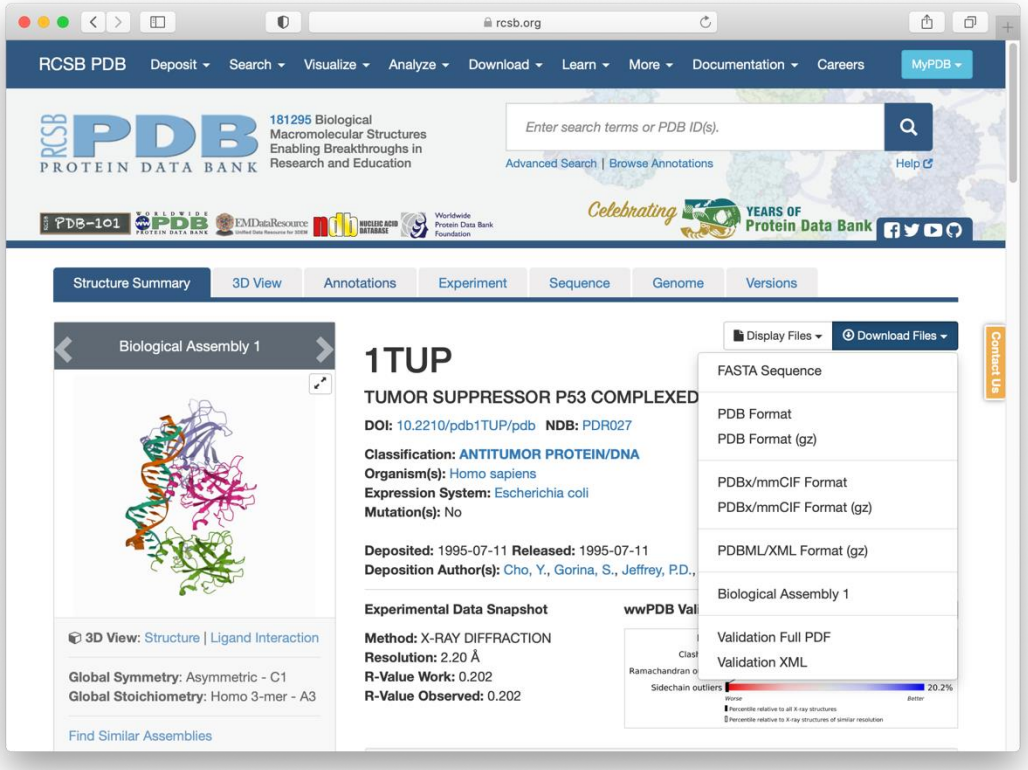
using File>Open

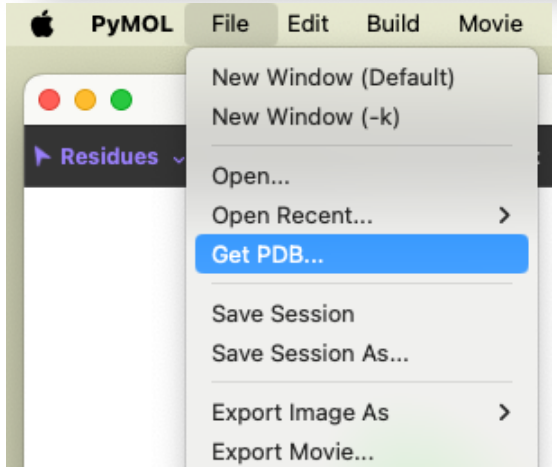
OR

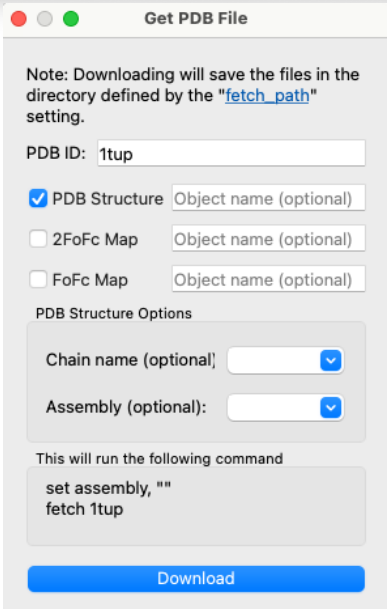
Fetch .pdb file directly to PyMOL using File>Get PDB… and providing 4 letter code from PDB entry: **1TUP**

OR

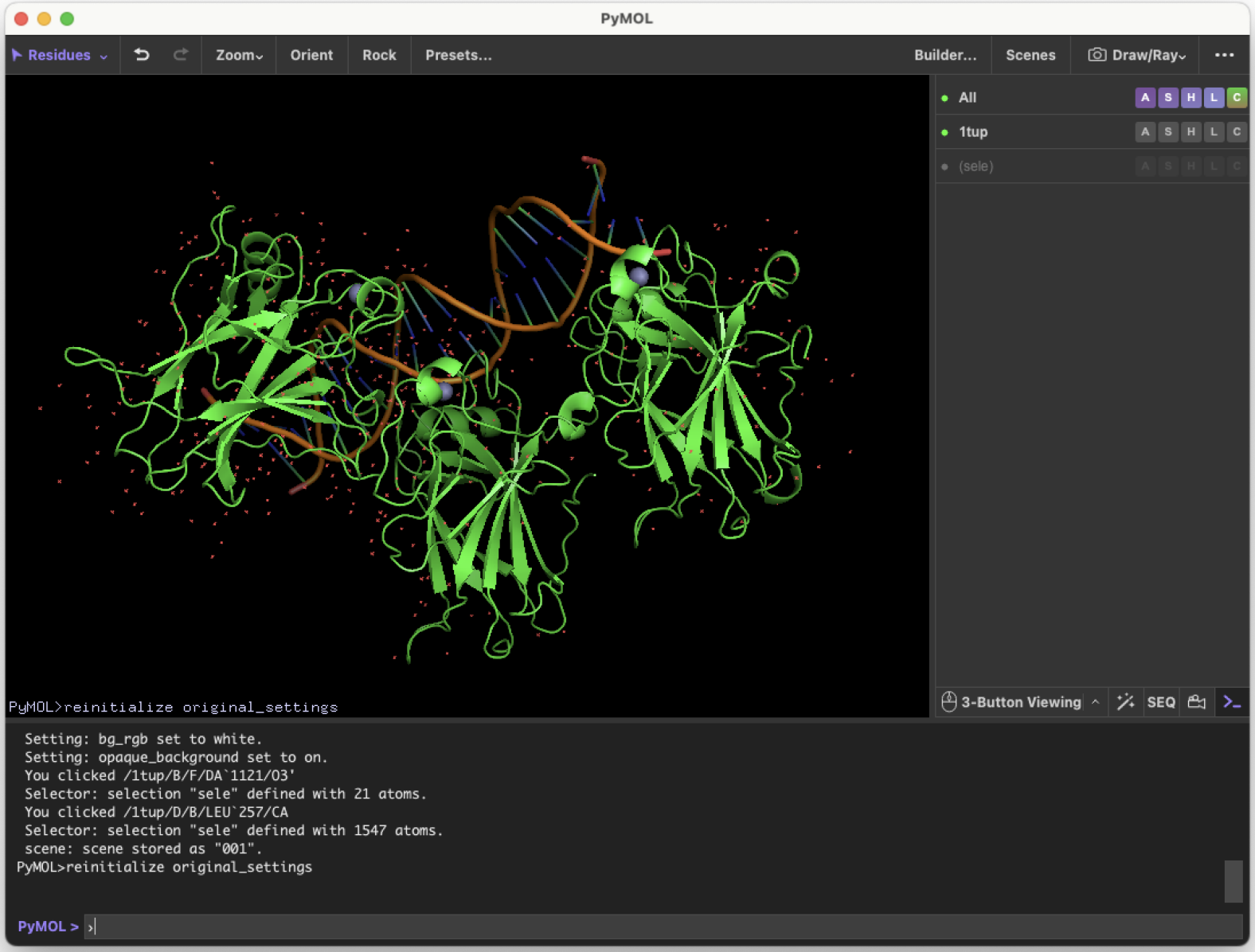
Command line: `fetch 1tup`





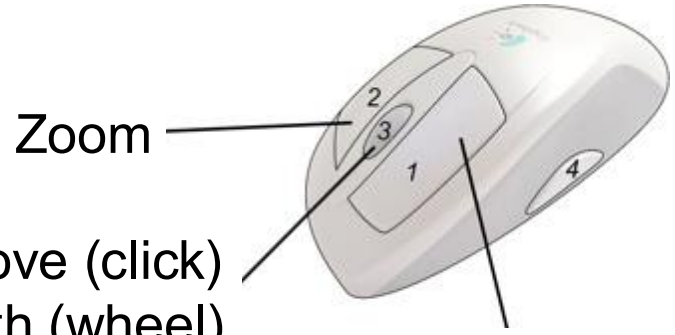


1tup.pdb loaded in PyMOL

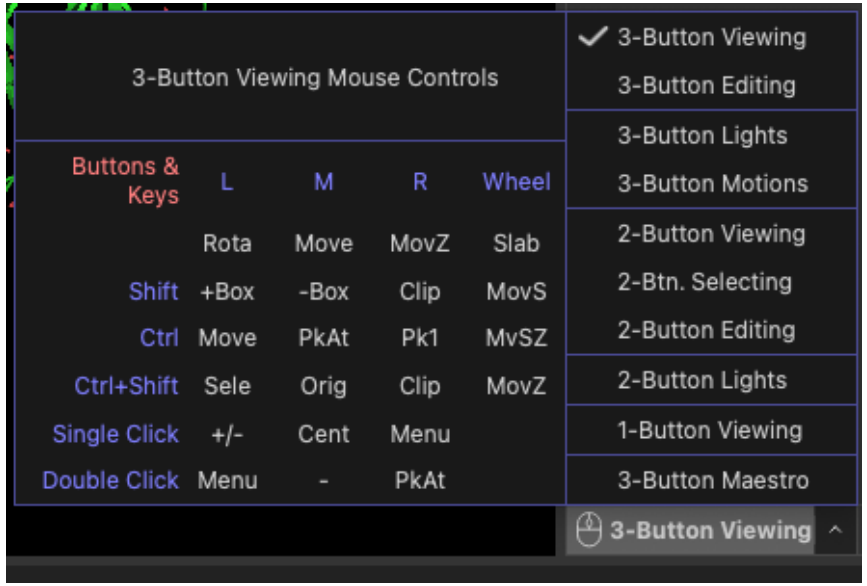


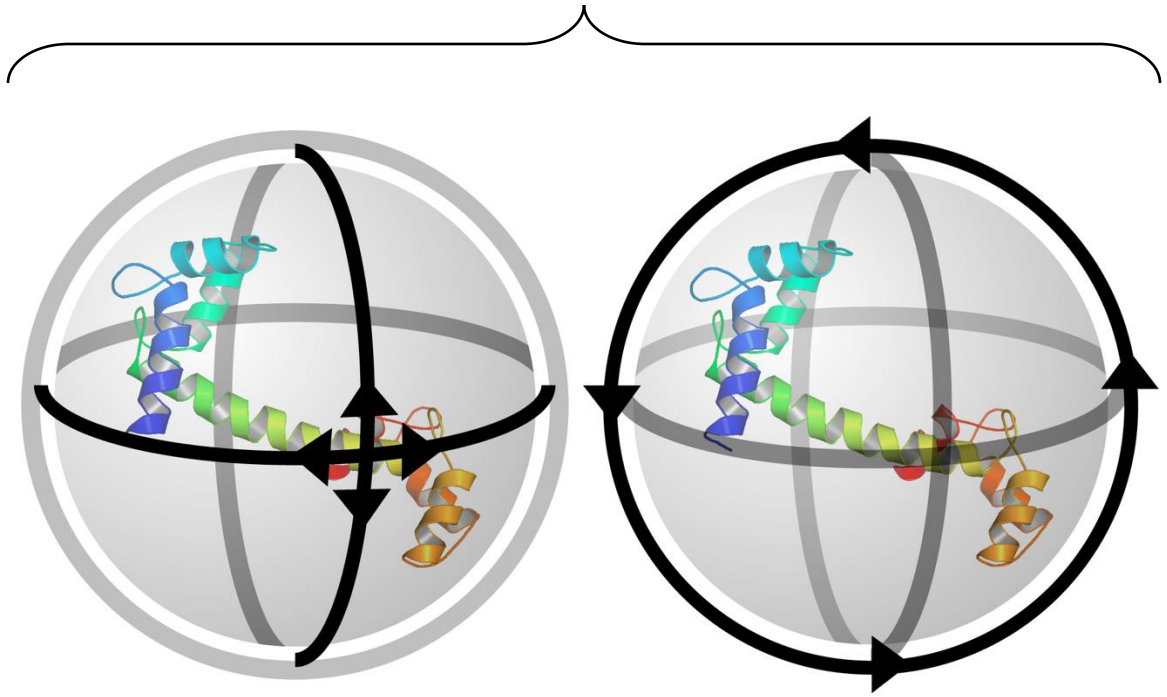
2. Mouse controls

If you get lost:
Zoom>All or Orient



Mouse functions + modifier keys:





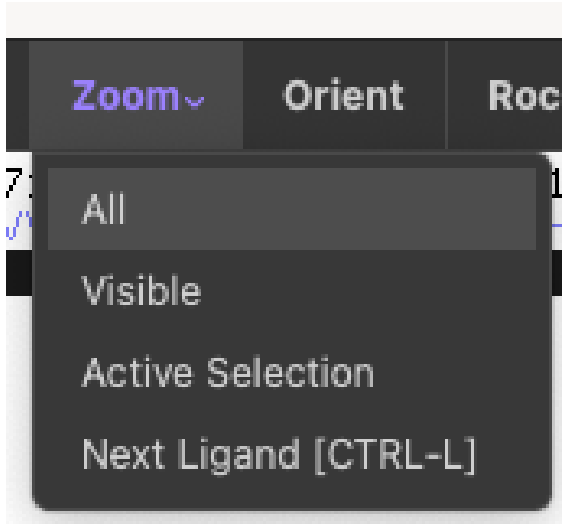
Mouse modes

Viewing Mode: view, rotate, translate, and change the representations of objects (you will use this mostly)

Editing Mode: rotate bonds, replace atoms, physically move atoms and residues, etc.

General notes on PyMOL

- Save sessions **(File->Save session…)** from time to time
- Use the **Zoom** or **Orient** buttons on the top panel if you get lost
- To change display quality, select Display > Quality > Maximum quality (or other)



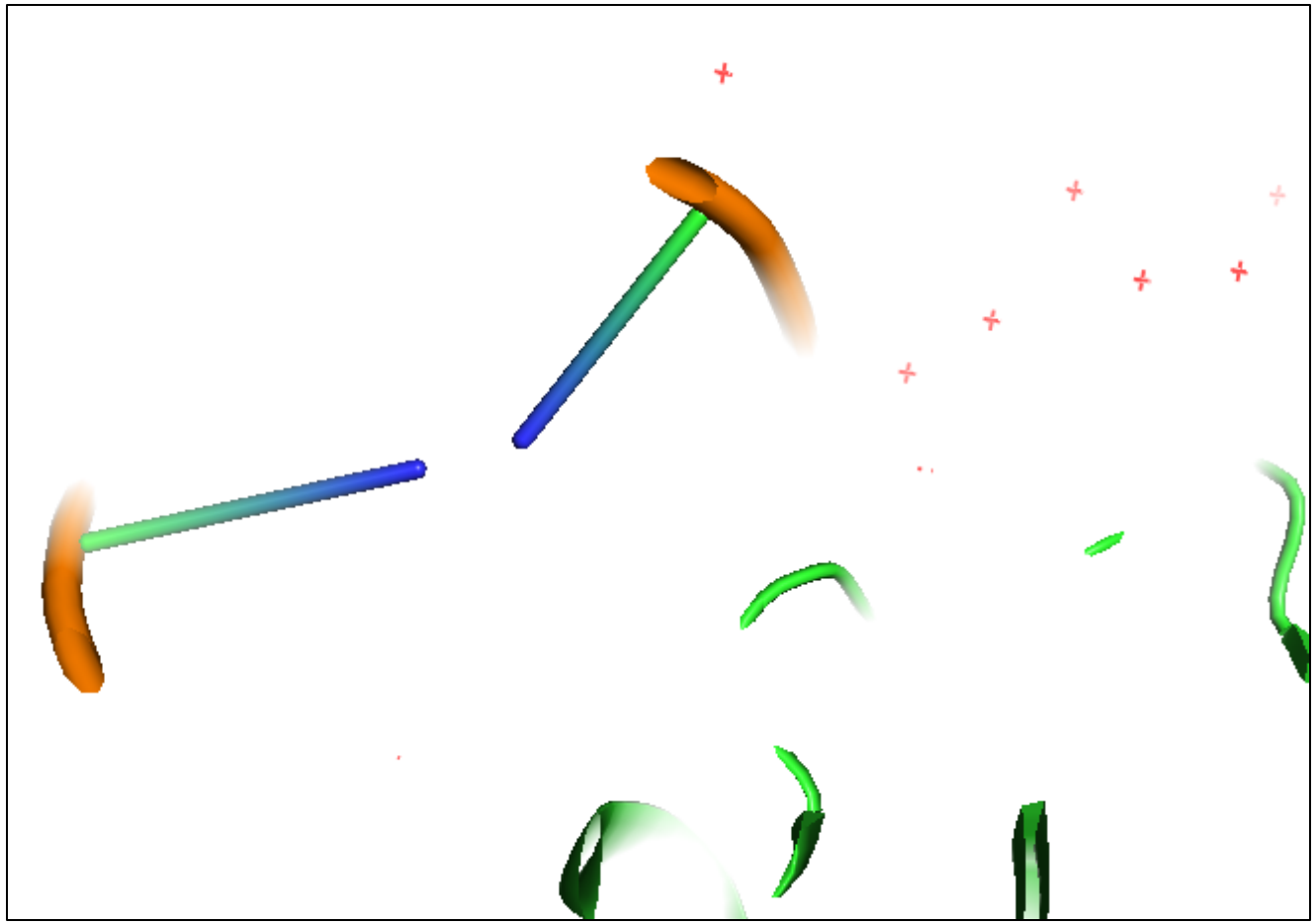
Exercise 1: Adjusting view and slab

1. Reset the view.

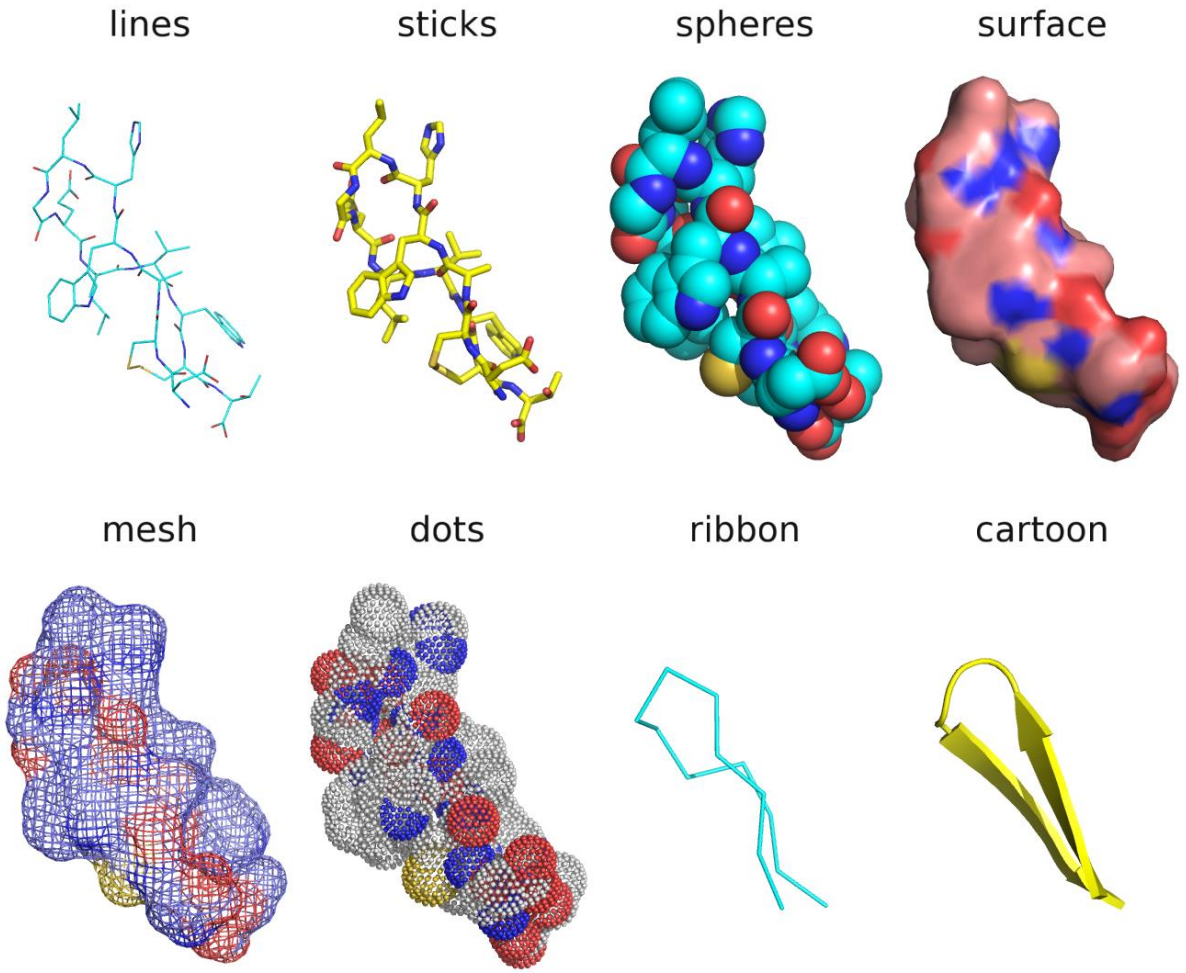
- Locate DNA in the complex, adjust view and slab so you can see single base pair and move the slab so you can see single base-pair as you move the slab along Z-axis.

2. Reset, look around

- How many protein chains are bound to this dsDNA?
- Do all the protein chains bind DNA similarly?



3. Representations



Different representations convey different information

Object and Selections panel – used to change representations and much more

Selections and objects play slightly different roles in PyMOL.

- An **Object** can be created from selections
  - Can be moved and aligned!

- A **Selection** belongs to a specific object
  - Selections are used to modify representation of parts of an object
  - **You cannot display a selection without displaying its original object source**

4. Selections – how to make them

There are multiple ways to perform selections:

By clicking on the molecule:

- Single left mouse click be default selects single residue

(can also be used to select atom/chain/molecule etc. depending by setting in Mouse->Selection Mode menu)

- Shift + Left Mouse button selects a box

By clicking on the sequence:

- Select Sequence On in the Display Menu to show sequence; click on the residues

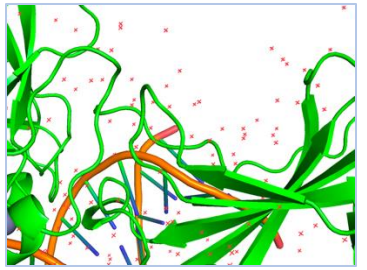
OR

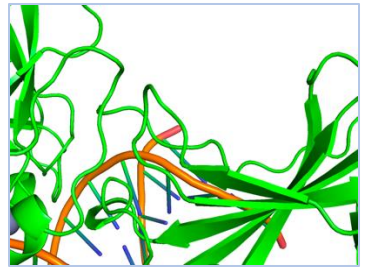
command line: https://pymolwiki.org/index.php/Selection_Algebra

Exercise 2: Changing representations

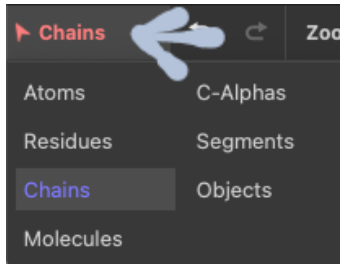
1. Reset the view.
2. Hide waters:
   [H] > waters
3. Select individual protein chains and change their colors:
   a. Change the mouse selection mode to chains.
   b. Click on a chain. In the Object Panel, click on [C] button of <sele>, choose a color.
4. Set the DNA representation to licorice sticks and change color by element:
   Make sure your mouse selection mode is still chains.
   a. Click the 2 DNA strands. [S] > as > licorice – sticks.
   b. [C] > by element > choose a color palette.

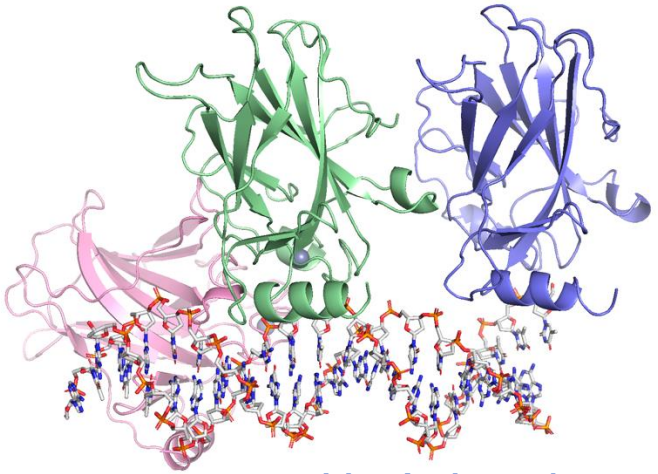
2



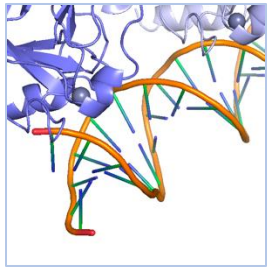


3a

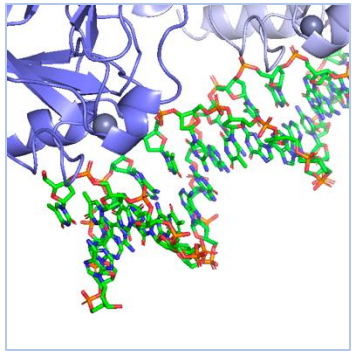




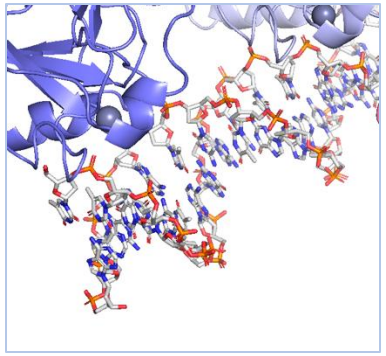
Ha An's colors:
pink, palegreen, slate



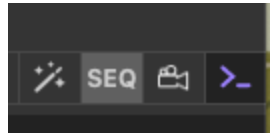
4a



4b



Making Selections using Sequence Display



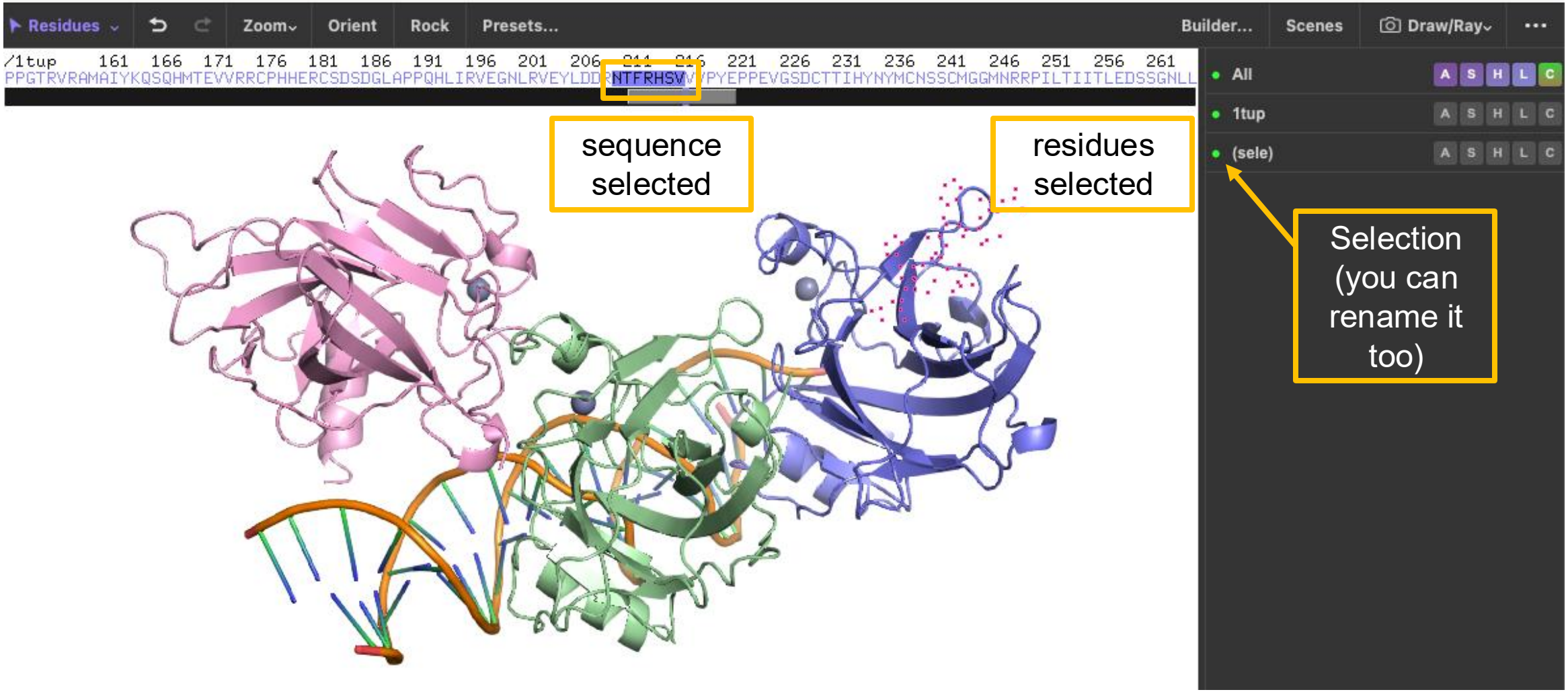
-Select Display->Sequence On to show sequence; click on the residues

-Colors of sequence corresponds to colors in structure
-0 = water
-Residue number corresponds to the residue directly below the first digit

-Display->Sequence Mode->Residue Names changes Sequence display to three-letter amino acid code

if you don’t see the numbering, might need to change the background color to white (Display > Background > White)

How selections are indicated



Selections – command line:

Command line offers powerful way of making selections
Hierarchy: model → chain → resn (residue name) → resi (residue number) → name (atom name)

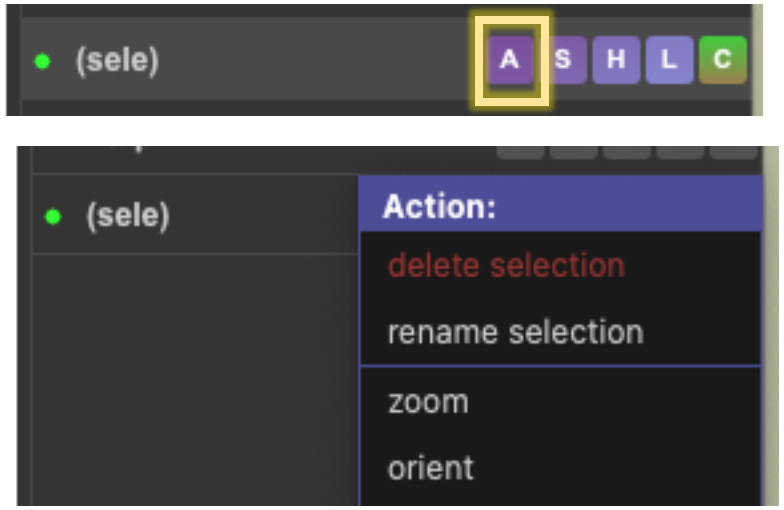
Examples:
- `select chain A` **– select chain A**
- `select resn lys` **– select all lysines**
- `select resi 175` **- select residue 175 in all chains**
- `select (resi 175 or resi 245) and chain B`
  - select residues 175 and 245 in chain B
- `select interface, (chain B within 4 of chain E:F) or (chain E:F within 4 of chain B)` **– make selection named interface with all atoms located less than 4A apart between chain B and chain E:F**

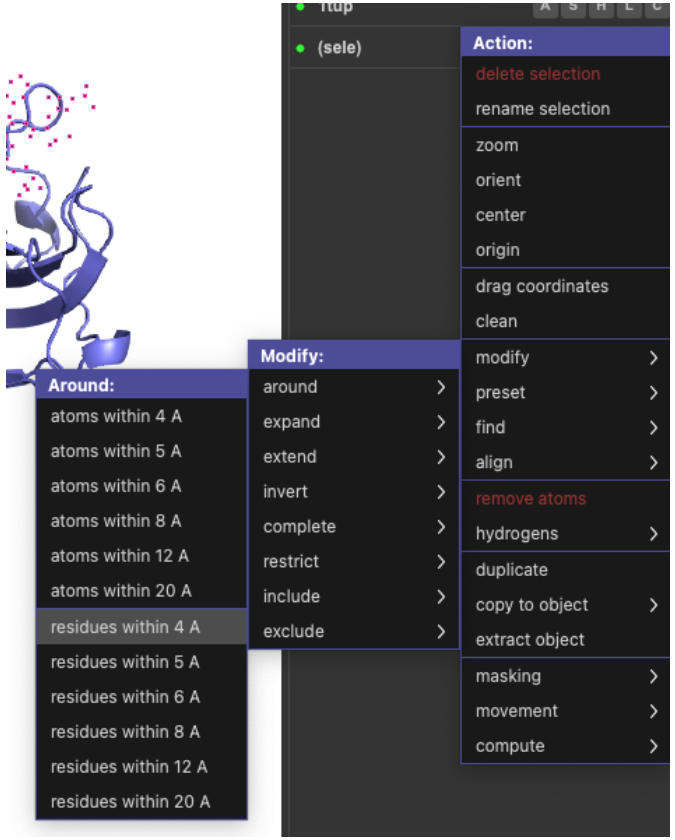
Type: help select **to see help**

Full explanation: https://pymolwiki.org/index.php/Selection_Algebra

Selection and Objects

- rename selection: <sele> [A] > rename selection
- copy selection to new object: <sele> [A] > copy to object
- select all residues within 4Å of selection: <sele> [A] > modify > around > residues within 4 A
  (this selects the atoms; you can show sidechains by going to [S] > sidechain > stick)





Exercise 3: Working with sequences

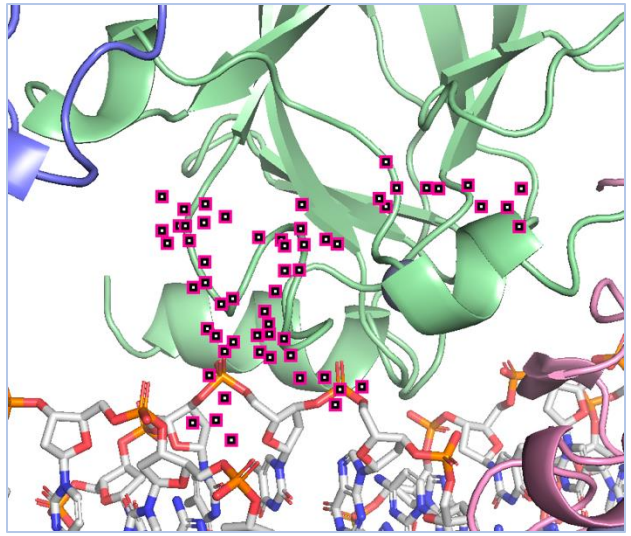
Certain residues are most frequently mutated in cancer:

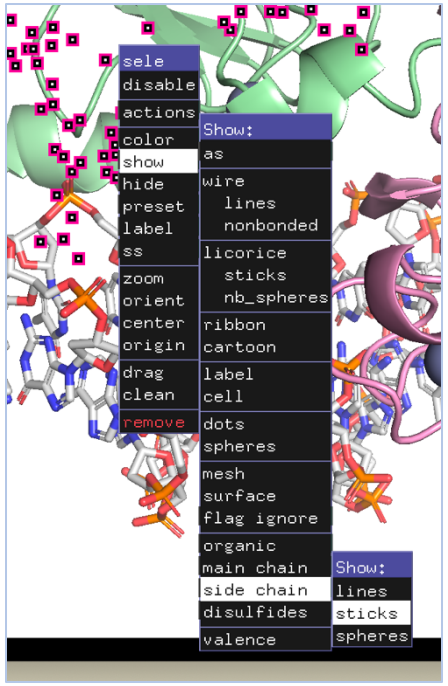
R175, G245, R248, R249, R273, R282.

Select and show the side chains for these residues

in chain B (middle protein molecule) and look at them.

- Display > Sequence
- Make sure your selection mode is ‘Residues”

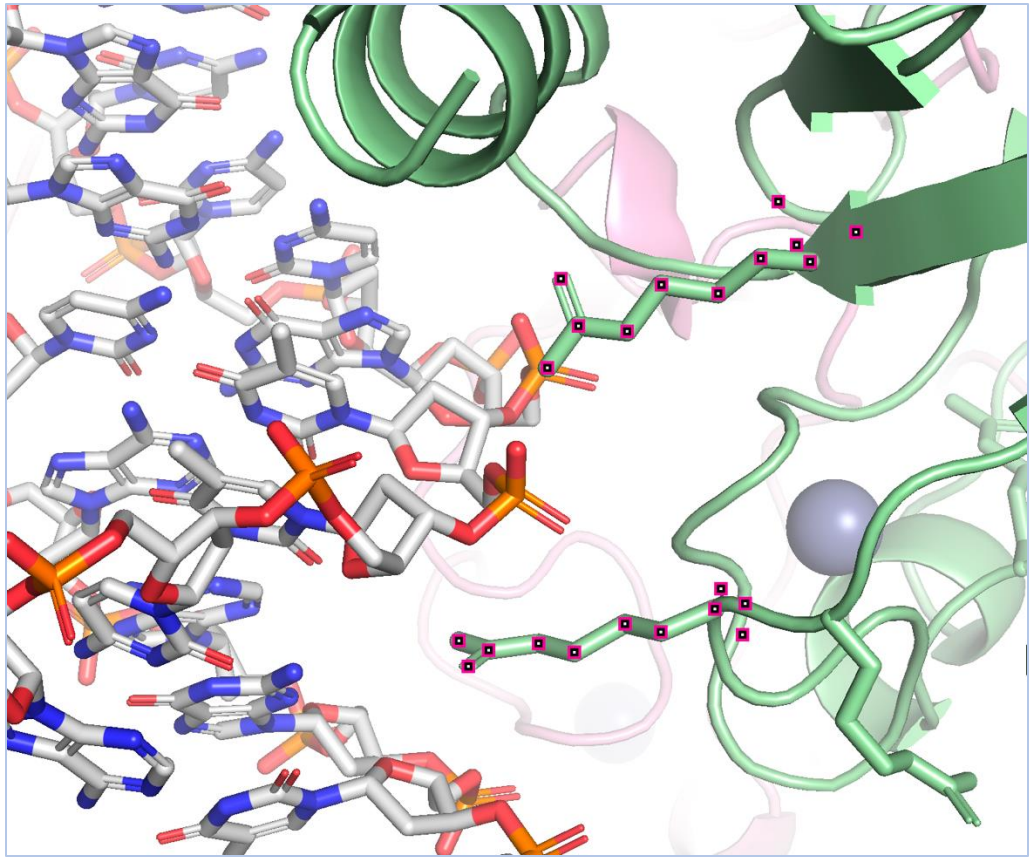




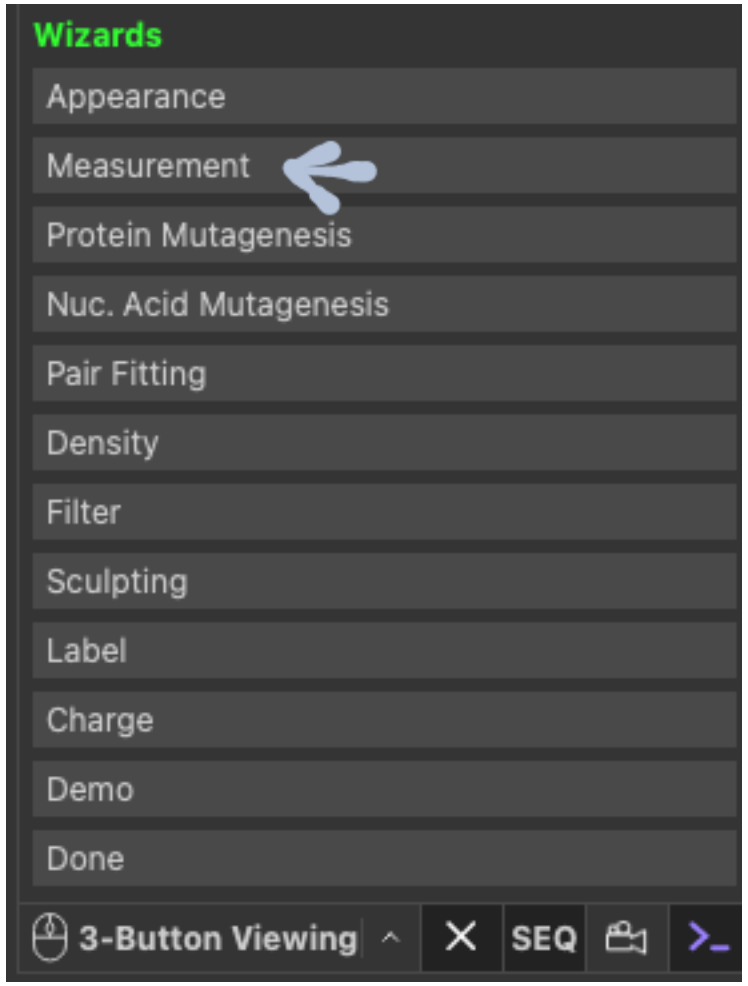
Exercise 3: Working with sequences

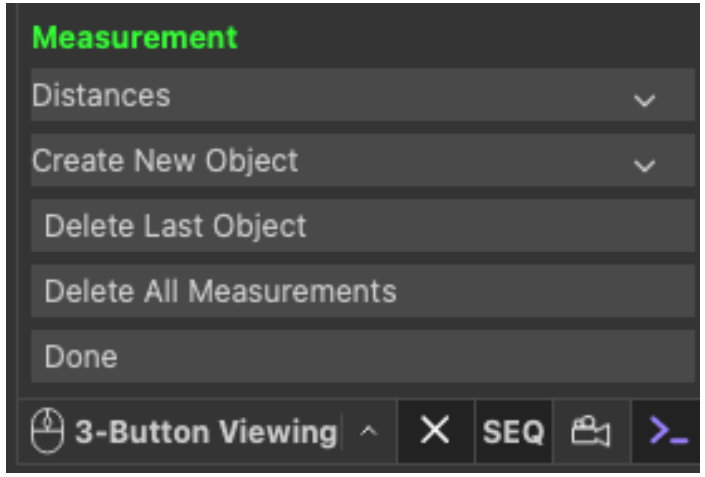
Q: Where are these residues located?

Q: What could be the role of these residues?

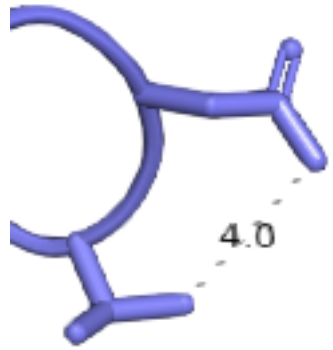


5. Making measurements



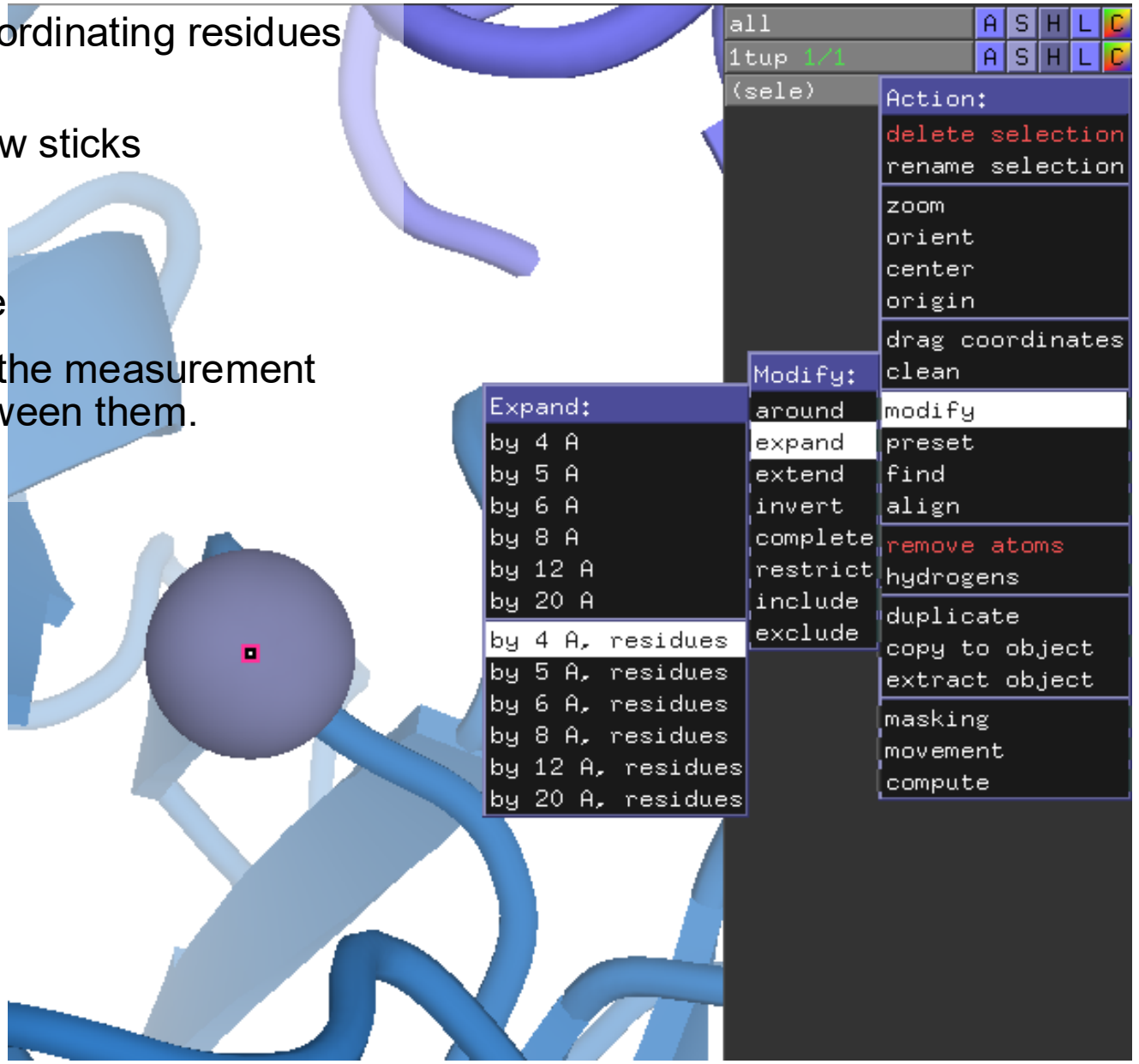


click the first atom, then
click the second atom



Exercise 4: Measure the coordinating distances of the Zn atom

- Select a Zn atom, expand the selection to select coordinating residues
  <sele> [A] > modify > expand > by 4 A, residues
- change the representation of the side chains to show sticks
  <sele> [S] > sidechain > sticks
- you can also change the color of this selection:
  <sele> [C] > by element > choose your color palette
- In the menu bar, Wizard > Measurement to turn on the measurement option click two atoms to measure the distance between them.



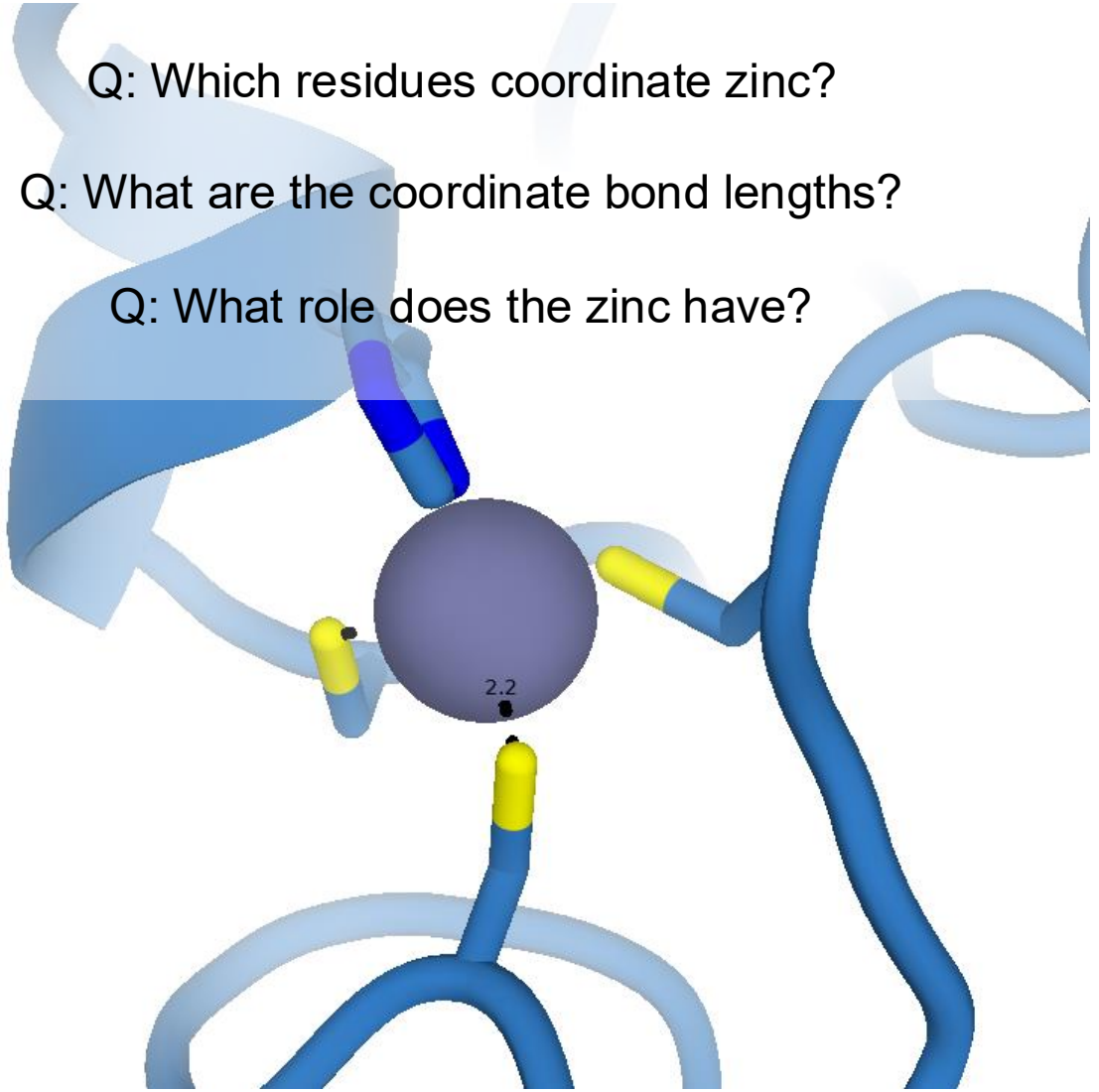
Exercise 4: Measure the coordinating distances of the Zn atom

The zinc ion has been shown to be important in DNA binding.

Q: Which residues coordinate zinc?

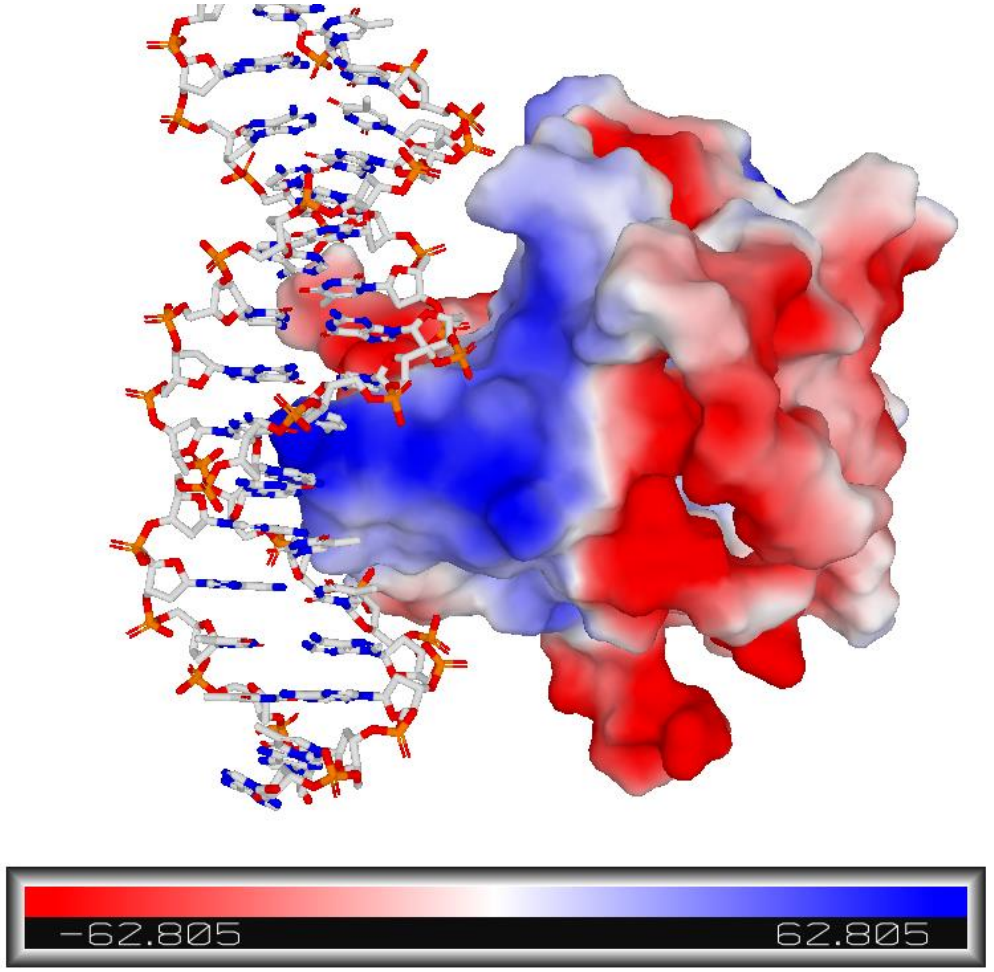
Q: What are the coordinate bond lengths?

Q: What role does the zinc have?



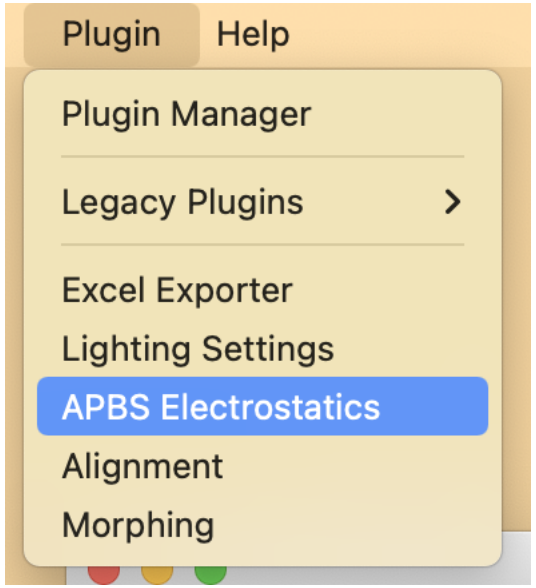
Exercise 5: Showing electrostatics

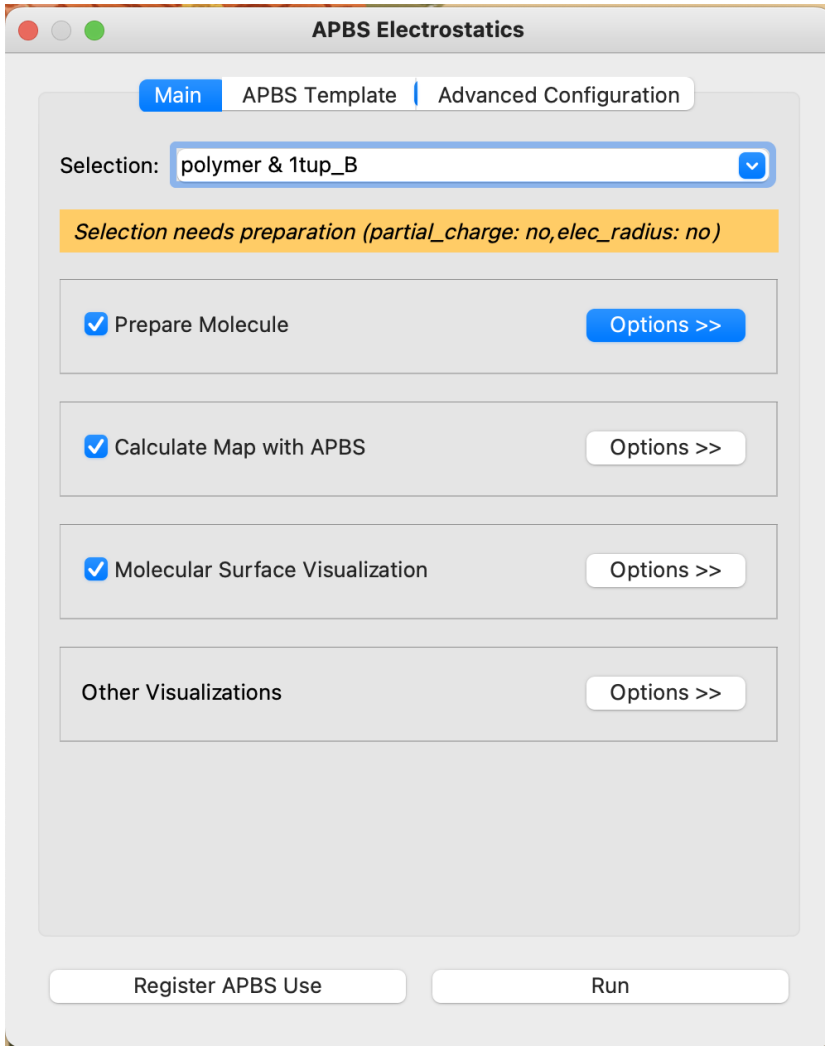
- Create new individual objects that contain the protein chains A, B and C individually
  (use the command `split_chains 1tup`)
- Generate electrostatic potential surfaces quickly:
  [A] > generate > vacuum electrostatics > protein contact potential
- Do you see any electronegative or electropositive patches? Where are they located?

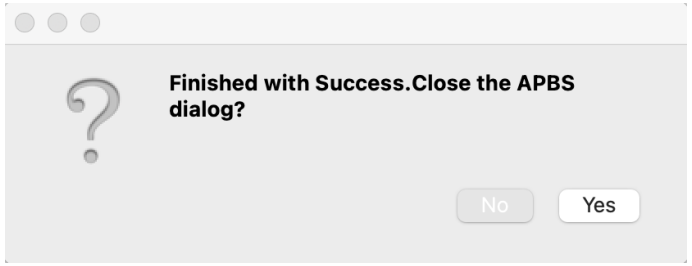


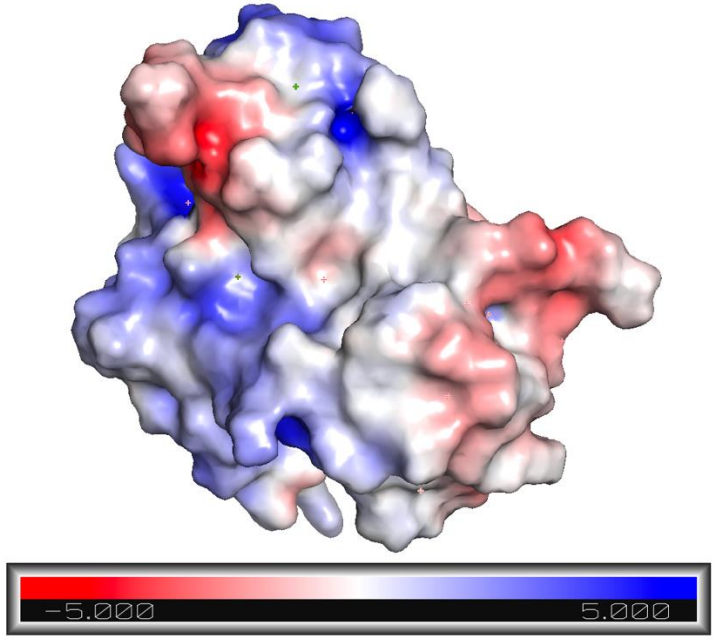
Better (more proper) way to generate electrostatics by APBS:

- Plugin > APBS electrostatics > choose your selection > Run (takes some time)
  wait till “Finished with Success” popup









If this errors out for you, try going into Prepare Molecule > Options > Method: change to “use formal_charge and vdw”

Exercise 6: Align the three protein chains

- you should have the 3 protein chains in separate objects
- align chains A and C to chain B

  [A] > align > to molecule > chain B
- Are the proteins adopting different conformations?

You can align all proteins to a currently selected one
A→Action→Align→All to this

or align proteins one by one
A→Action→Align→To Molecule

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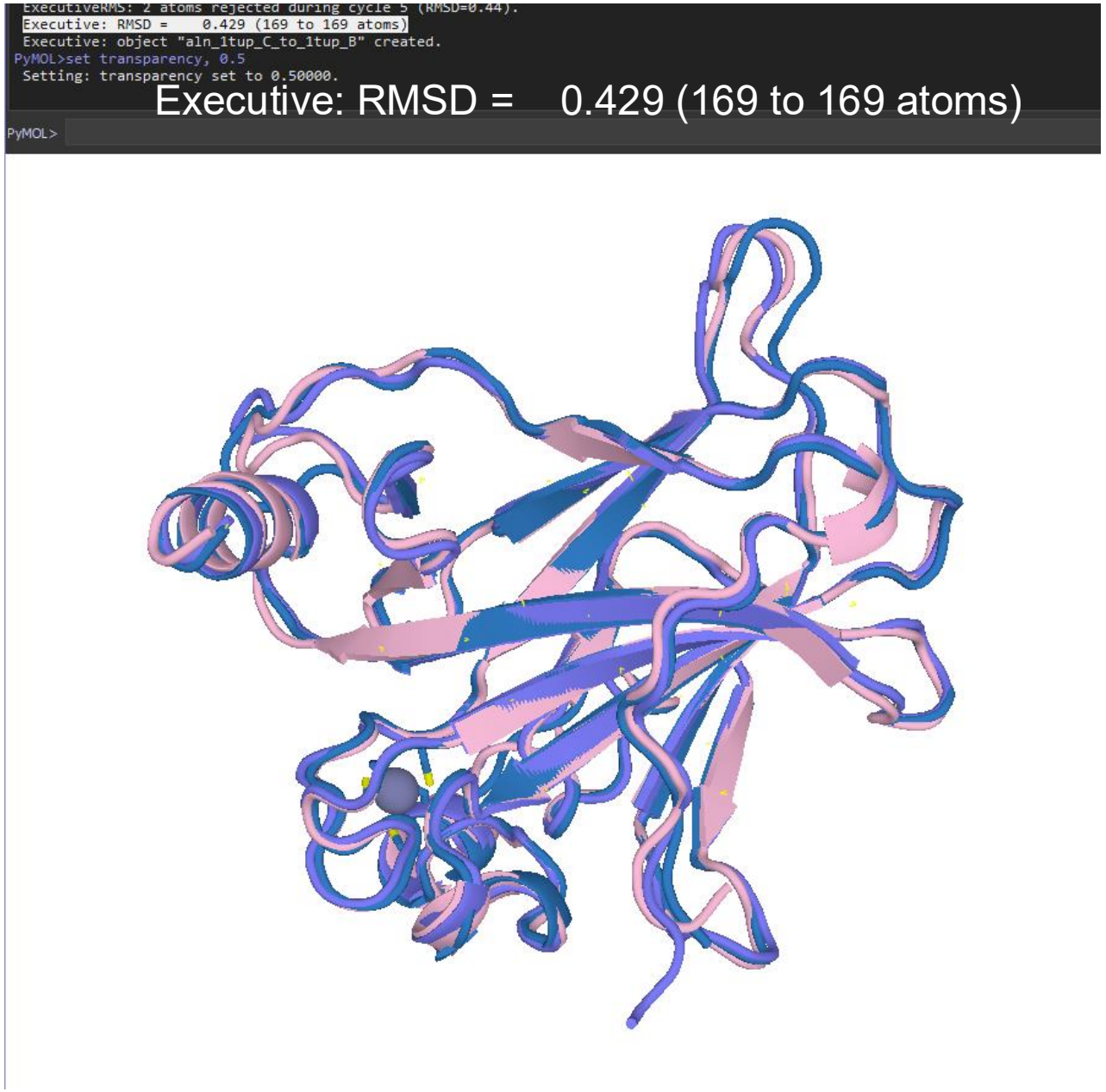
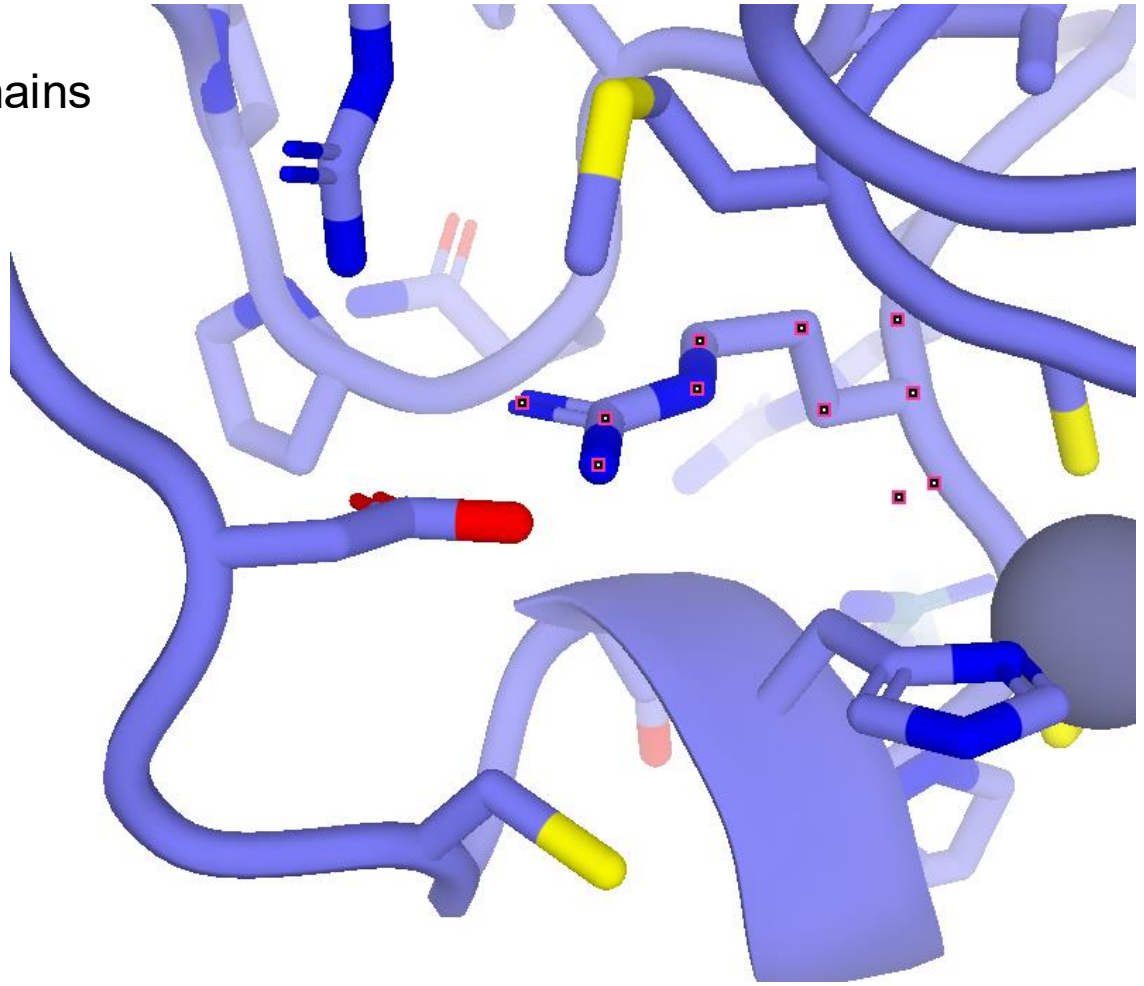
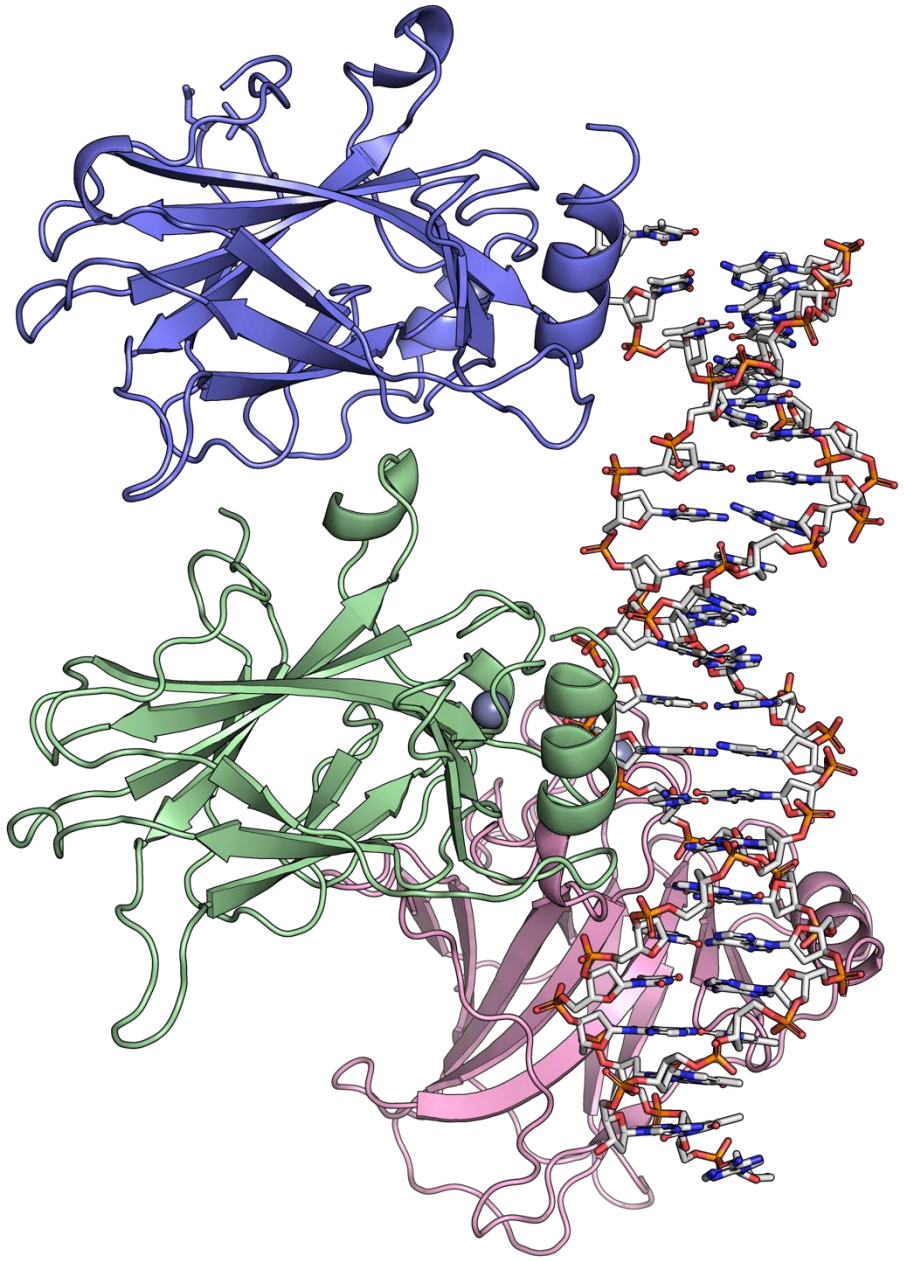


Exercise 7: perform an in silico alanine mutation

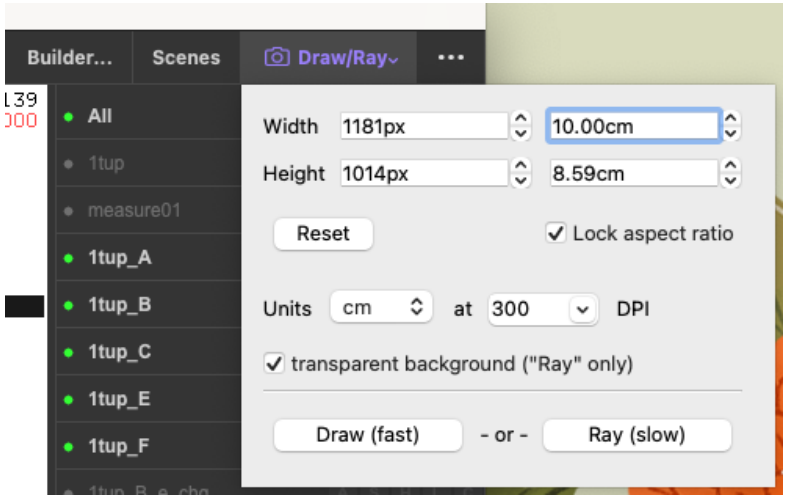
- Select R175 in chain A, show and color its sidechain.
- expand the selection to residues within 5 Å and show sidechains
- Go to menu bar Wizard > Mutagenesis
- In the panel on the right, click on ‘No Mutation’, select ALA.
- Click on the R175 that you want to mutate
- Then click Apply
- What could be an effect of this mutation?





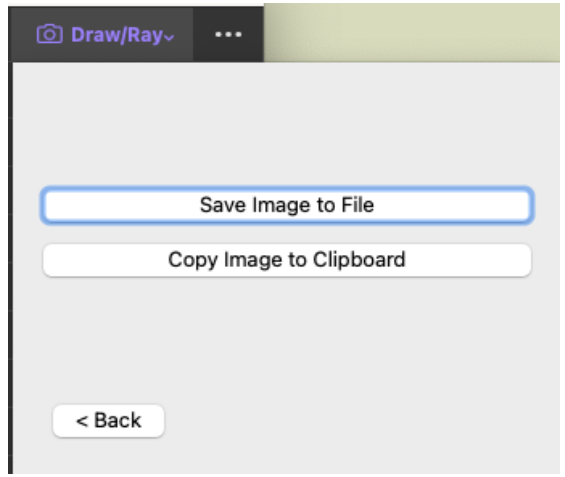
10. Make a figure

Reset everything (can just open a new window)
1tup
Recolor each protein chain a different color
Set DNA chains to be sticks, color by element
Draw/Ray: Ray(slow) > save image as > png to somewhere




```
Ha An's settings to
make it look pretty:
set spec_count, 0
set spec_reflect, 1

set ray_shadows, off
set ray_trace_mode, 1
```



https://pymolwiki.org/index.php/Ray

Bonus: look at electron density of an inhibitor

Explore electron density in the crystal structure of COVID-19 main protease in complex with an inhibitor N3 (pdb: 6lu7)

Get pdb… **6lu7:** PDB and 2FoFc map

- Hide waters
- Create a mesh representing the map:
  - Action > mesh > @level 1.0
  - `isomesh map, 6lu7_2fofc, 1, 3bep, carve = 2.5`
- Color the mesh (e.g. blue > density)
- Color protein chain and inhibitor ([C]->color by chain, [S]->as licorice (sticks)

- Explore electron density for the inhibitor