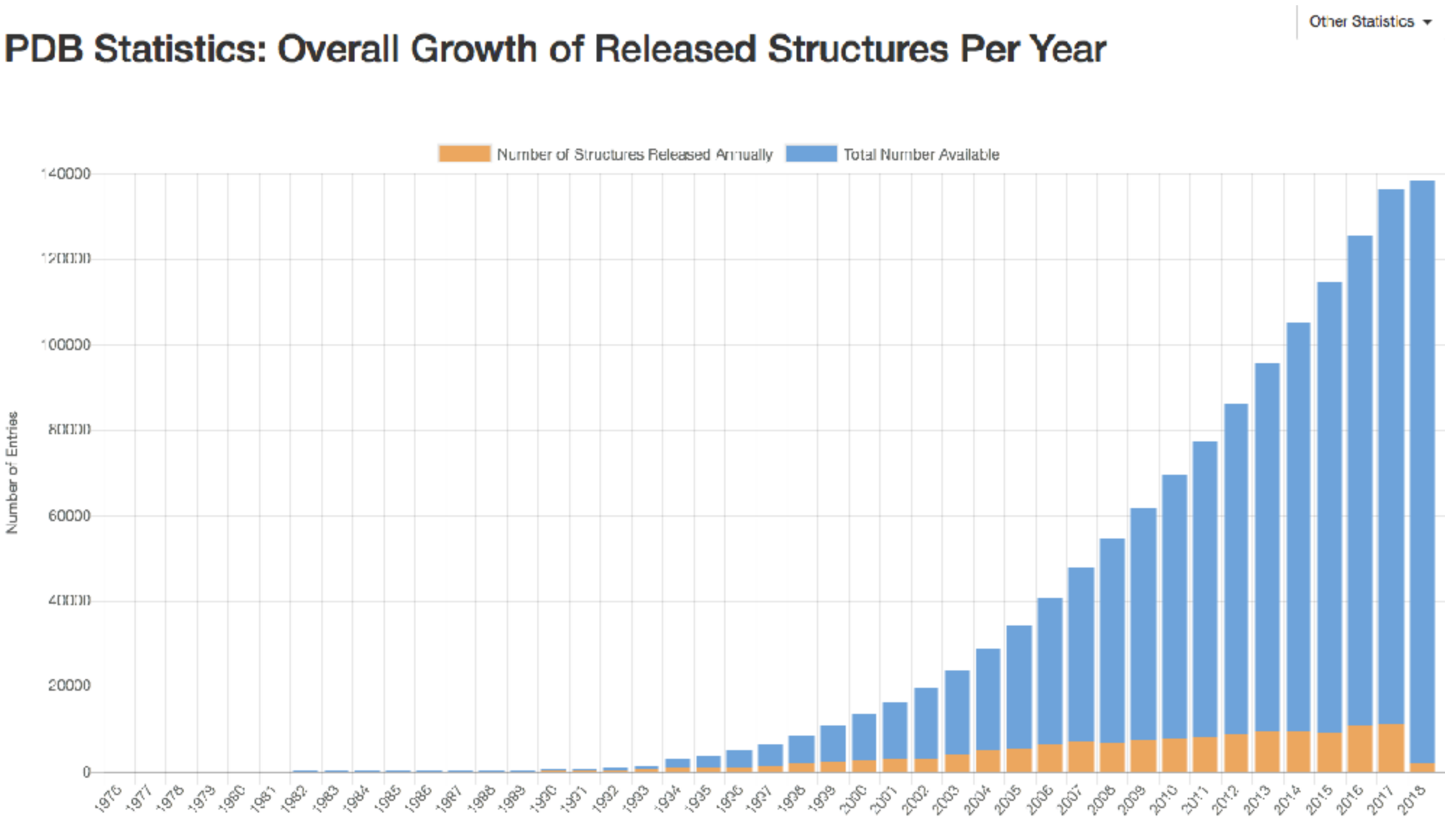
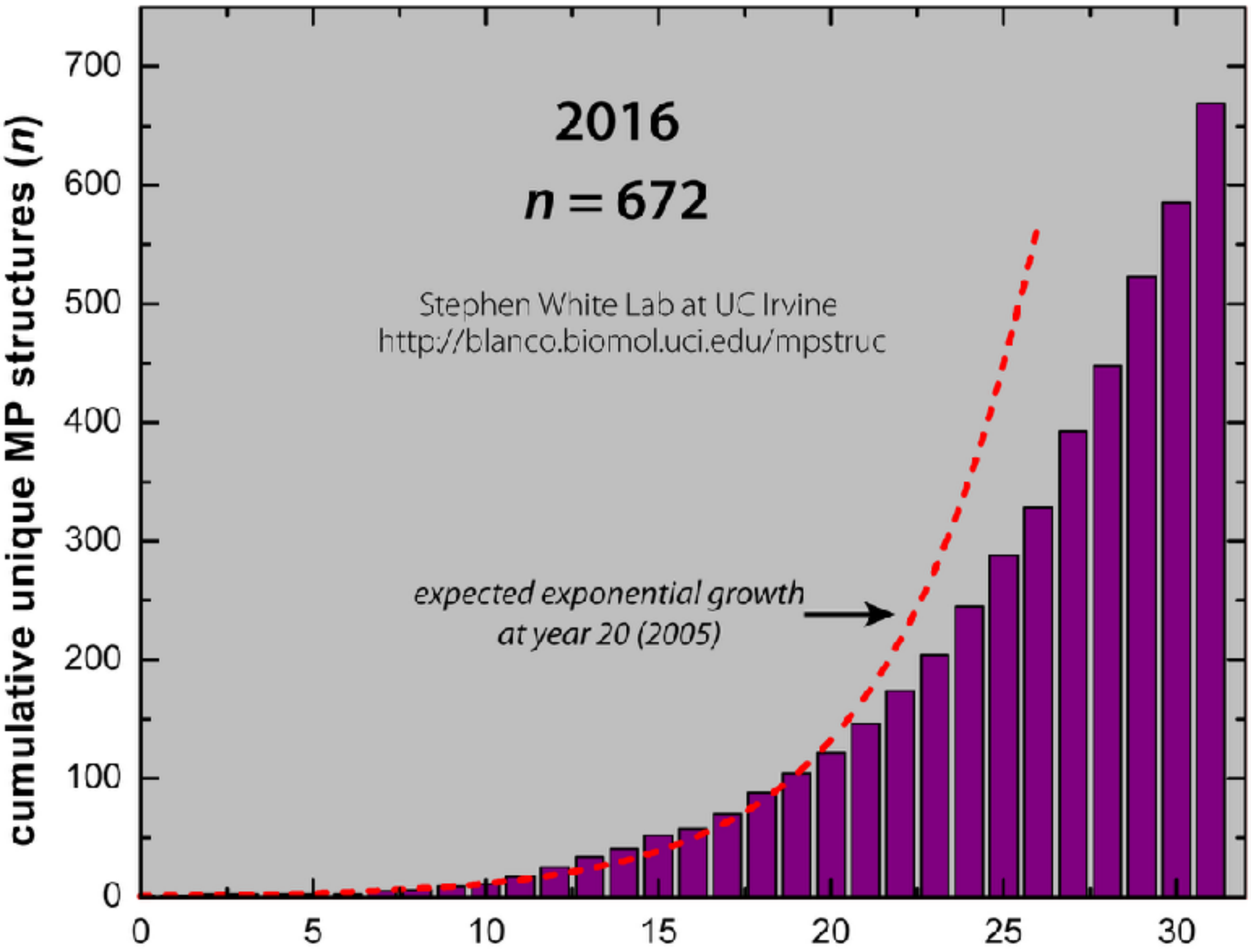
Structure determination has proceeded at a remarkable pace



The Protein Data Bank (PDB) is a repository which stores structures of macromolecules

www.rcsb.org

Membrane protein structures are still extremely rare



Most structures are obtained via crystallography

Other Statistics

PDB Data Distribution by Experimental Method and Molecular Type

Copy CSV

Experimental Method	Proteins	Nucleic Acids	Protein/NA Complex	Other	Total
X-Ray	116115	1916	5922	10	123963
NMR	10660	1236	249	8	12153
Electron Microscopy	1459	31	506	0	1996
Other	210	4	6	13	233
Multi Method	112	4	2	1	119
Total	128556	3191	6685	32	138464

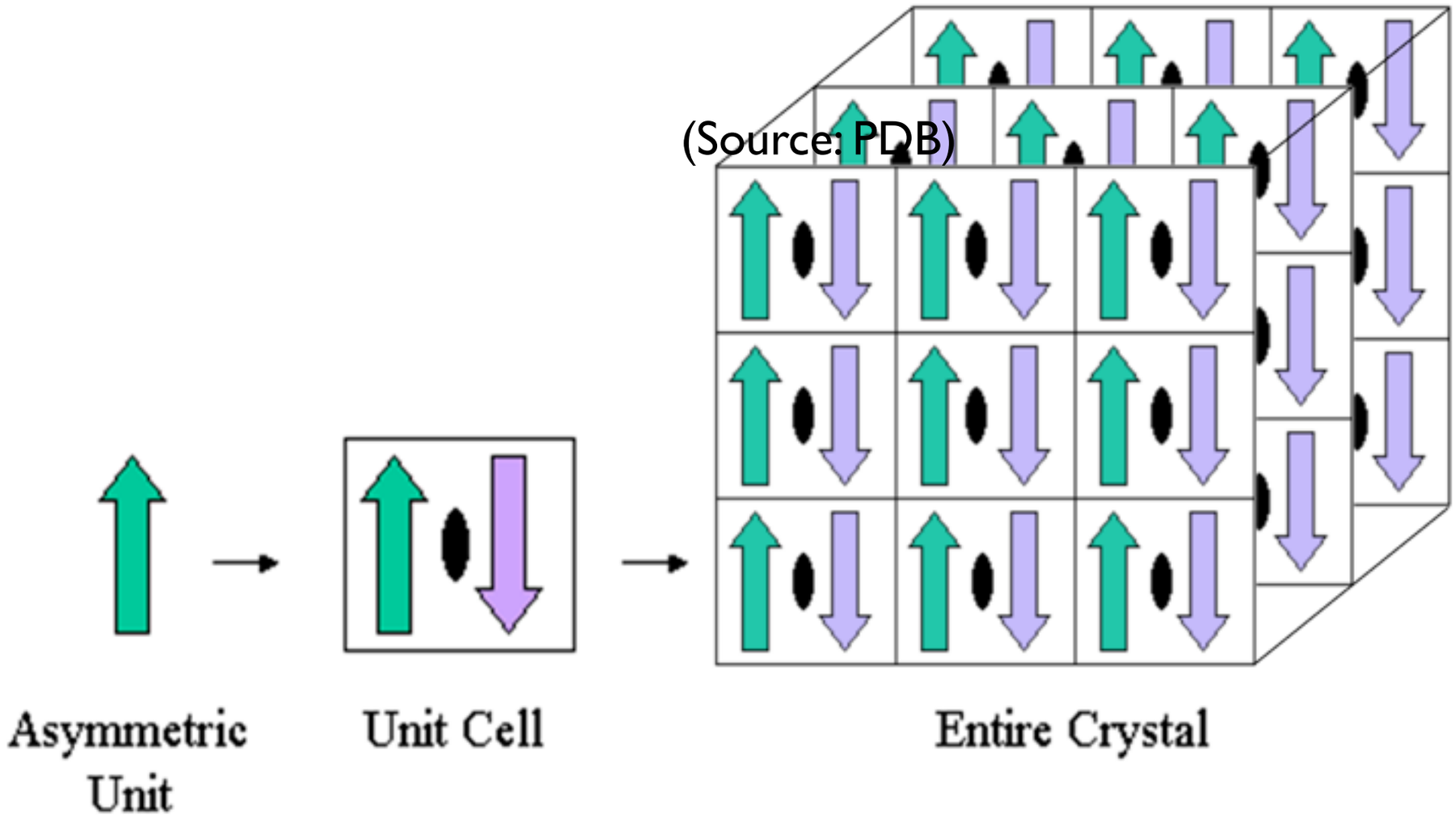
113777 structures in the PDB have a structure factor file.
9490 structures in the PDB have an NMR restraint file.
3242 structures in the PDB have a chemical shifts file.
2022 structures in the PDB have a 3DEM map file.

(RCSB PDB)

What's in a PDB file?

- Coordinates:
  - Atom list
  - 3D position in space
  - "occupancy"
  - Missing information!
- Experimental details
  - What the system is, how the structure was solved, secondary structure information, protein sequence
- Other info:
  - i.e. citations

PDB files for crystals contain just a portion of the whole crystal structure -- the "asymmetric unit"



(Source: PDB)

(Source: PDB)

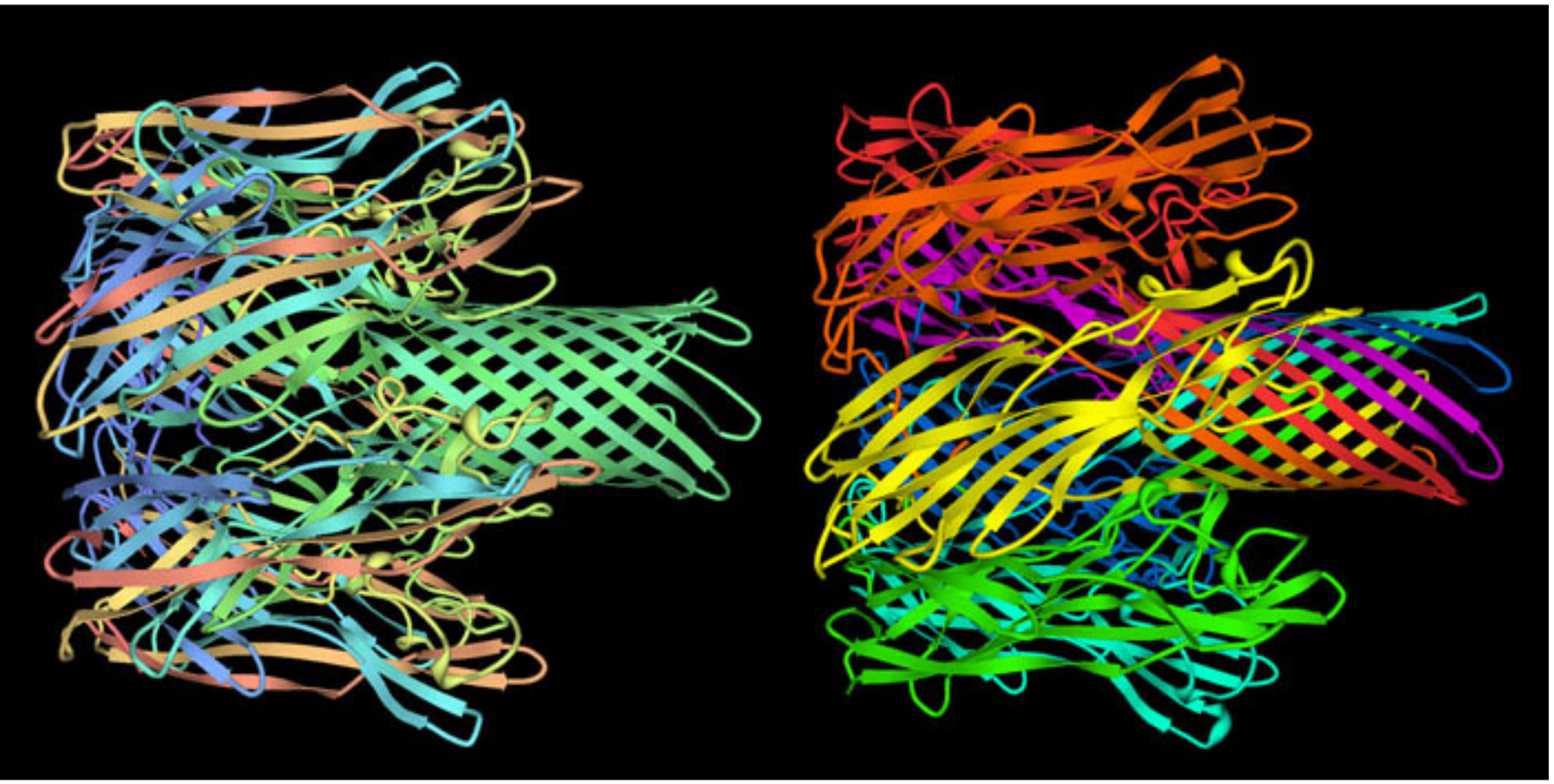
The biological assembly may or may not be the same as the asymmetric unit

- Asymmetric unit can be:
  - A single biological assembly
  - Multiple biological assemblies
  - Part of a biological assembly

(Source: PDB)

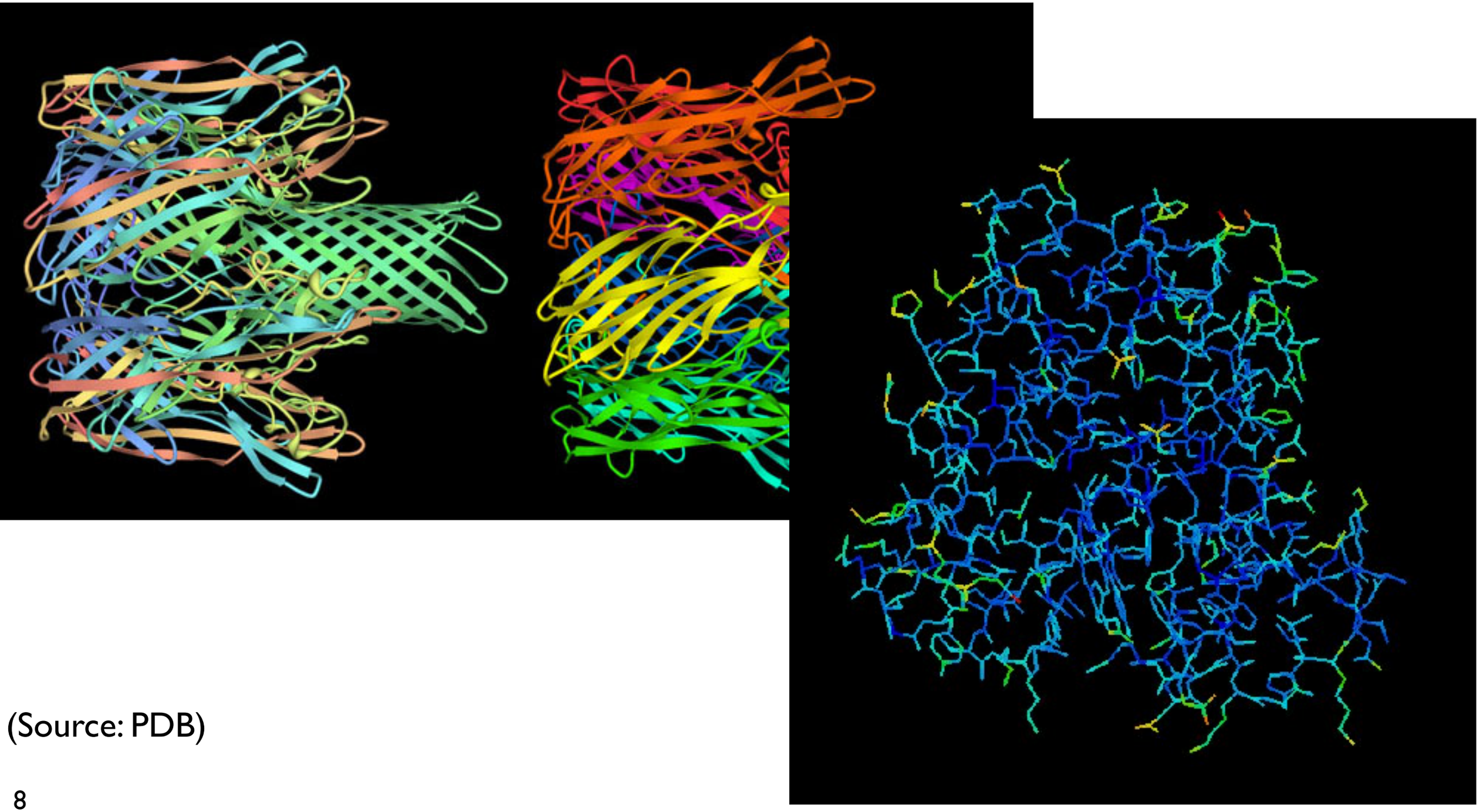
Asymmetric unit with one biological assembly	Asymmetric unit with a portion of a biological assembly	Asymmetric unit with multiple biological assemblies
		
Entry **2hhb** contains **one** hemoglobin molecule (**4 chains**) in the asymmetric unit.	Entry **1hho** contains **half** a hemoglobin molecule (**2 chains**) in the asymmetric unit. A crystallographic two-fold axis generates the other 2 chains of the hemoglobin molecule.	Entry **1hv4** contains **two** hemoglobin molecules (**8 chains**) in the asymmetric unit.

Structures may contain multiple chains and models; temperature factor (B value) information is also deposited



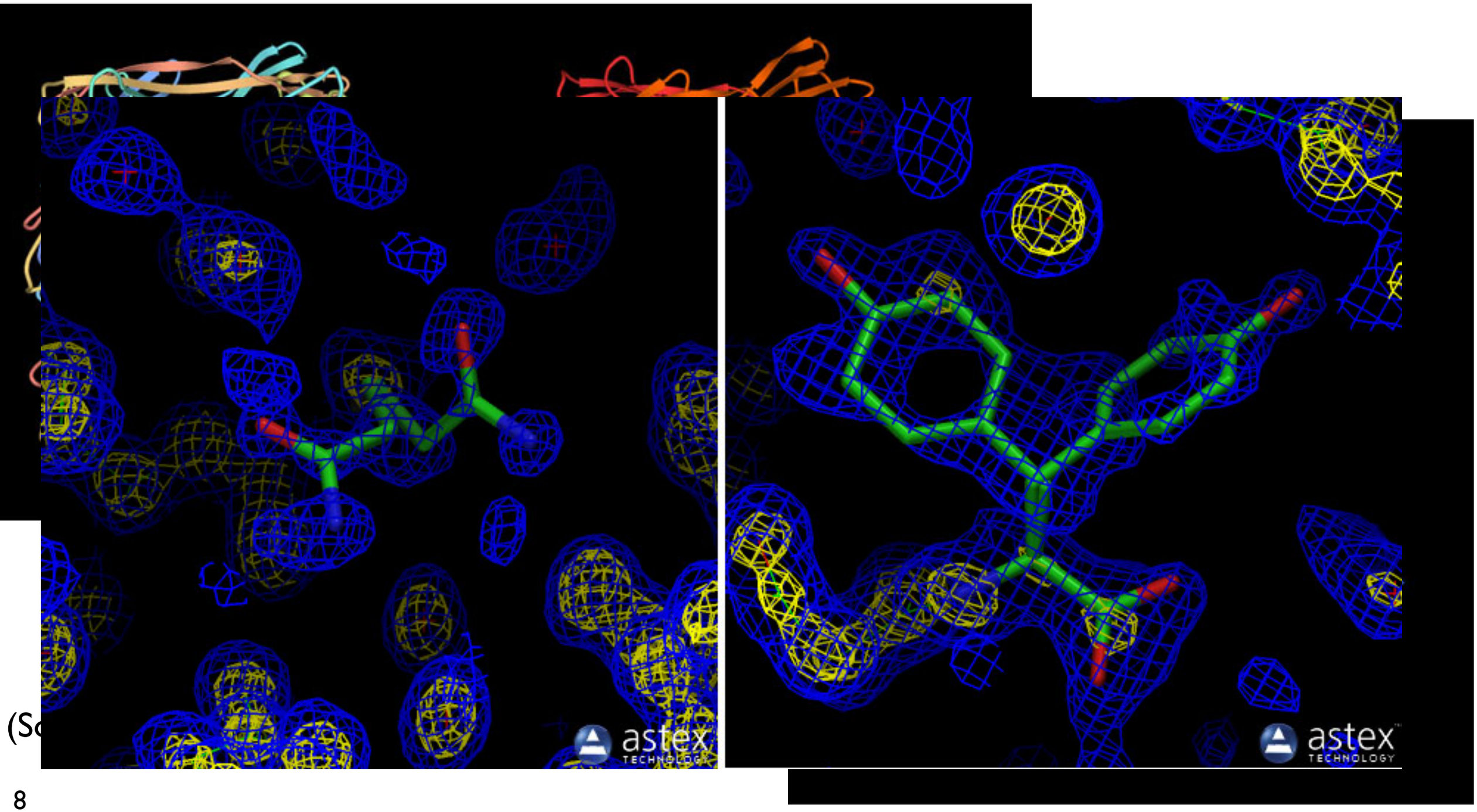
(Source: PDB)

Structures may contain multiple chains and models; temperature factor (B value) information is also deposited



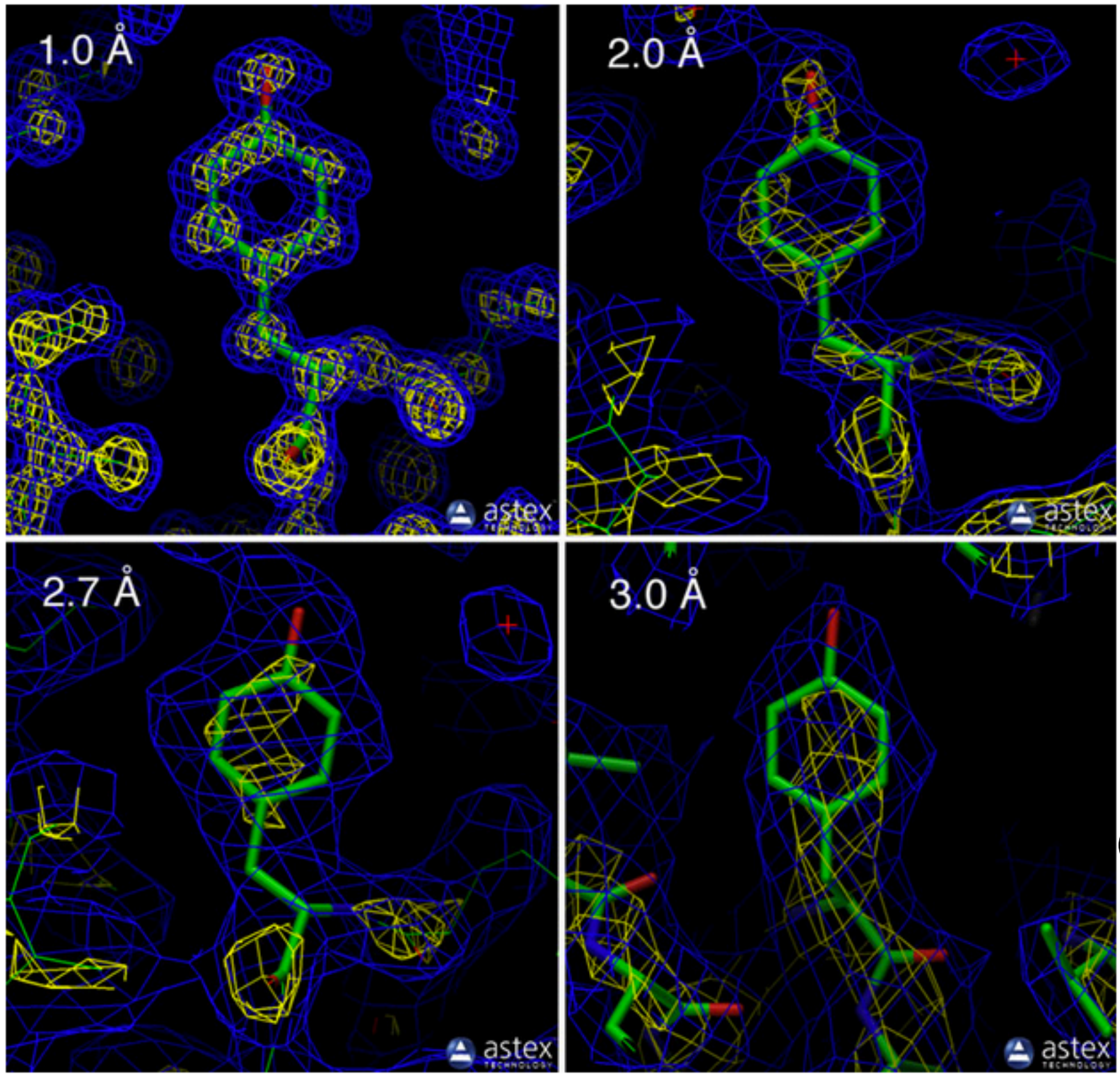
(Source: PDB)

Structures may contain multiple chains and models; temperature factor (B value) information is also deposited



(Sc

Resolution is an important aspect when looking at structures



(Source: PDB)

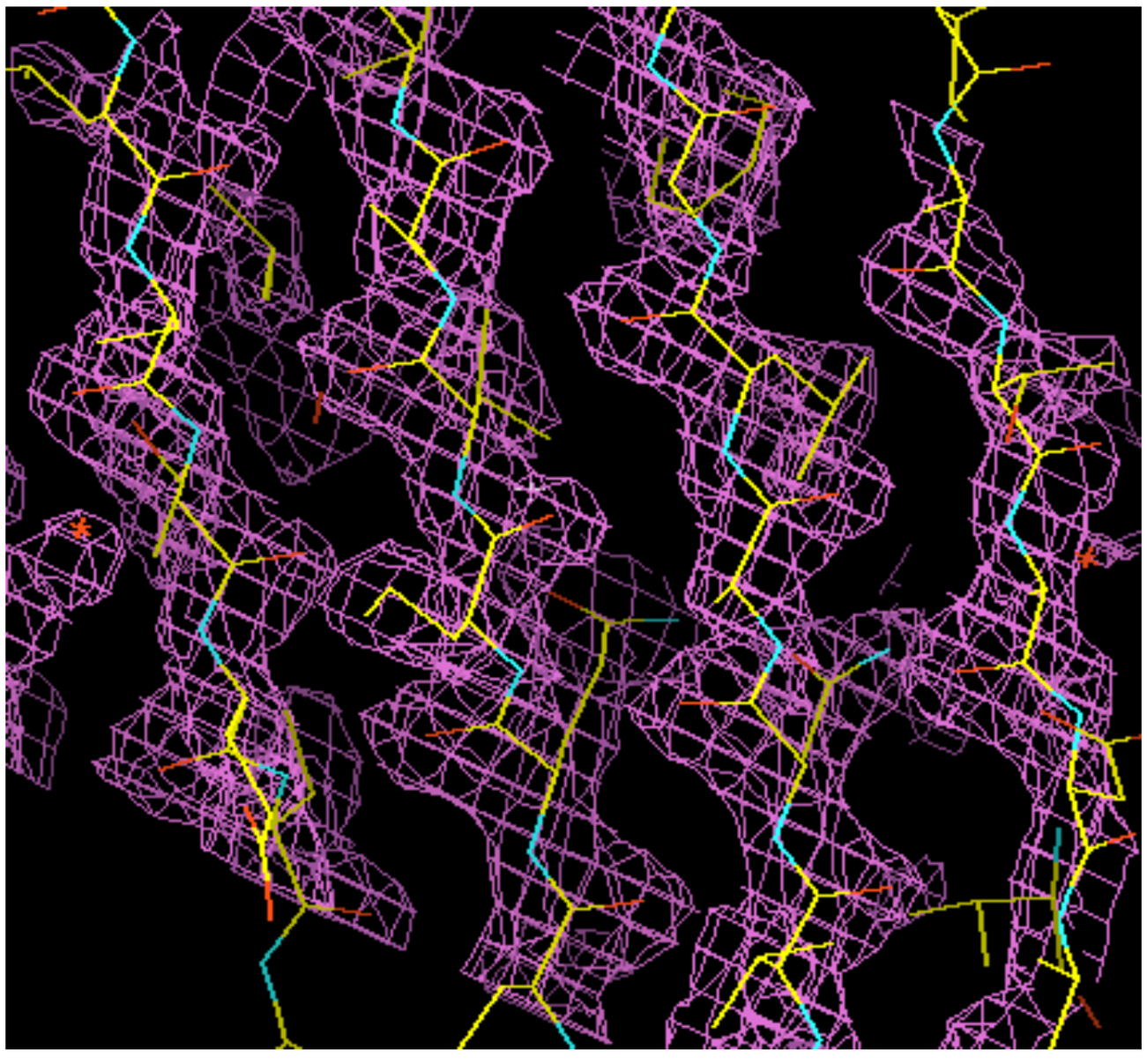
R-value and R-free are important factors in reliability

- R value: Measures how well simulated diffraction pattern matches experiment
  - Ideal: R=0
  - Typical: R~0.2
  - Random: R about 0.63
- But R value is used in building model, which introduces bias

(Source: PDB)

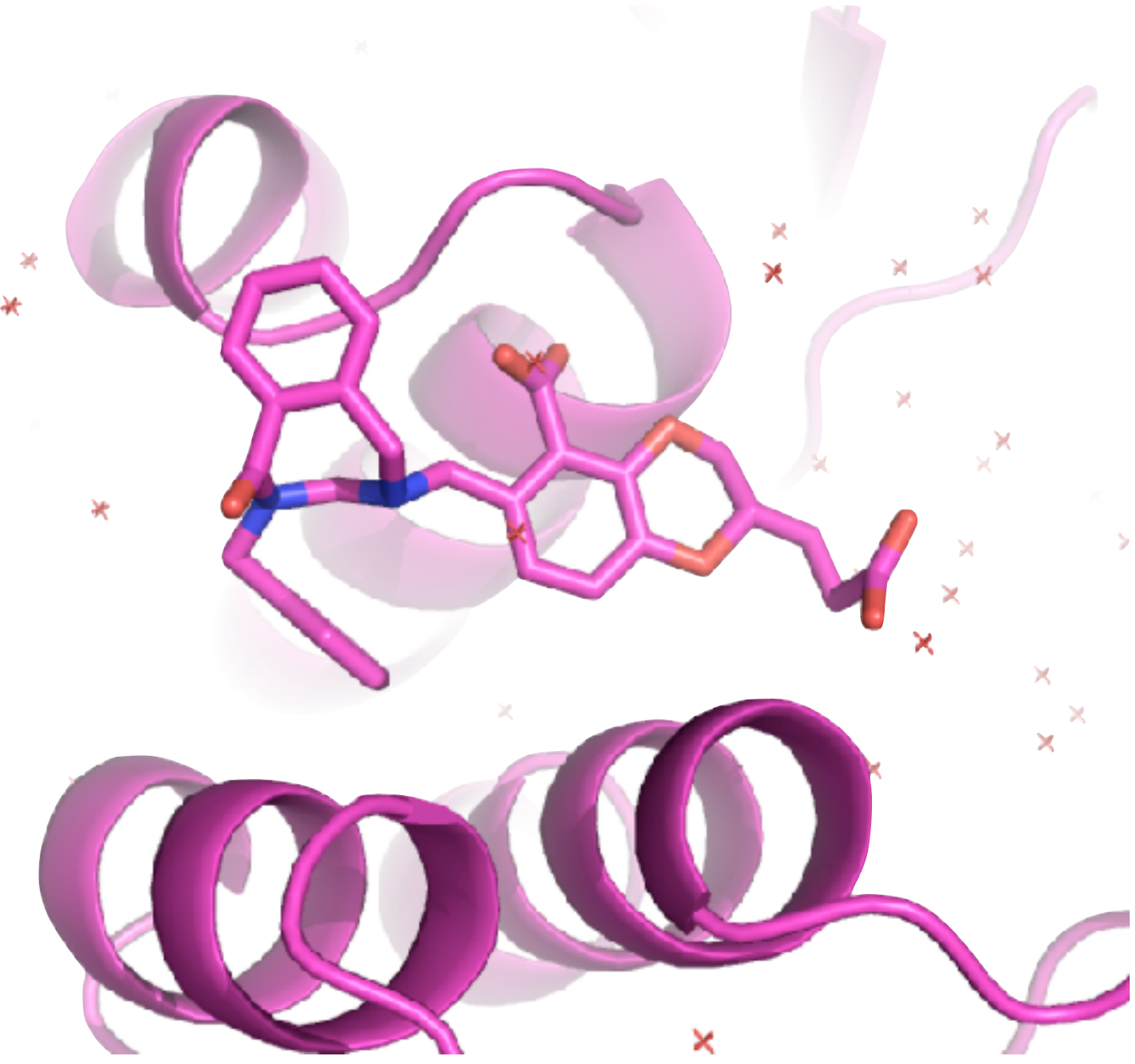
- Better: Use R-free
  - R value on 10% of data which is held back and not used in building model. Typically slightly higher, around 0.26
  - Big concern if it is ever a lot higher than R -- suggests overfitting

At the very least you should visualize the electron density before using a structure for something



The structure is a FIT to the density

A lot of other things can get in the way: How sure are you of what you're looking at?



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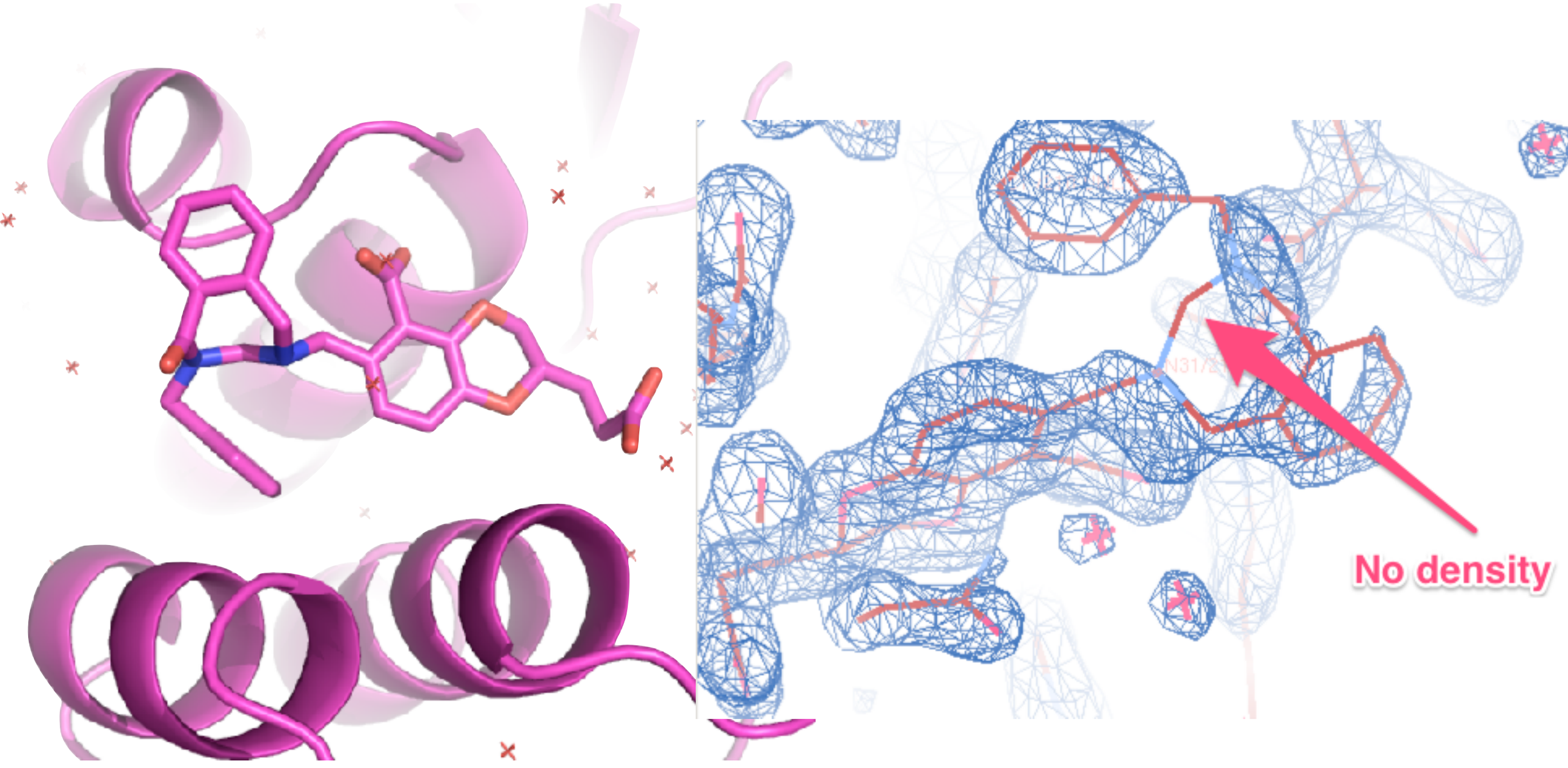
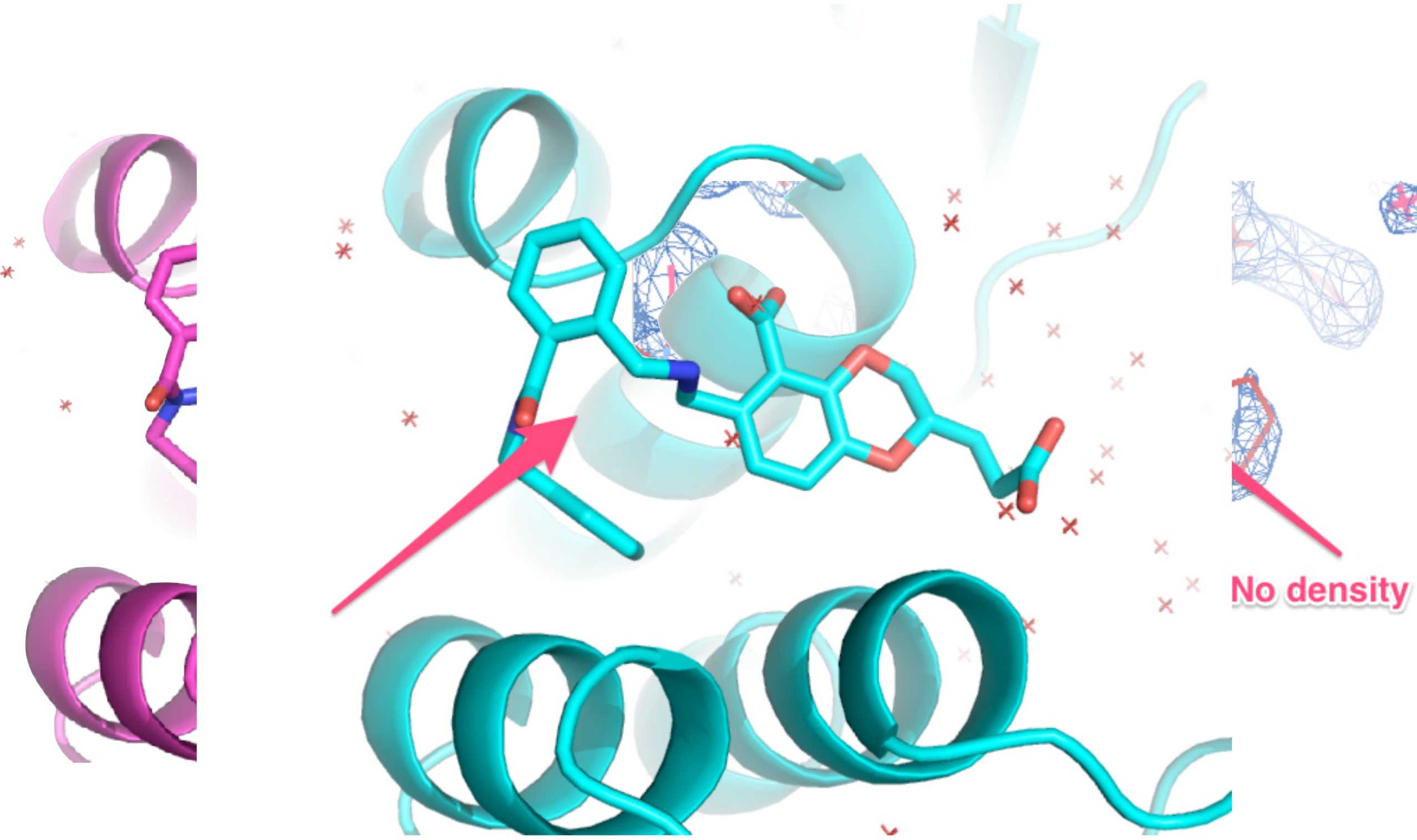
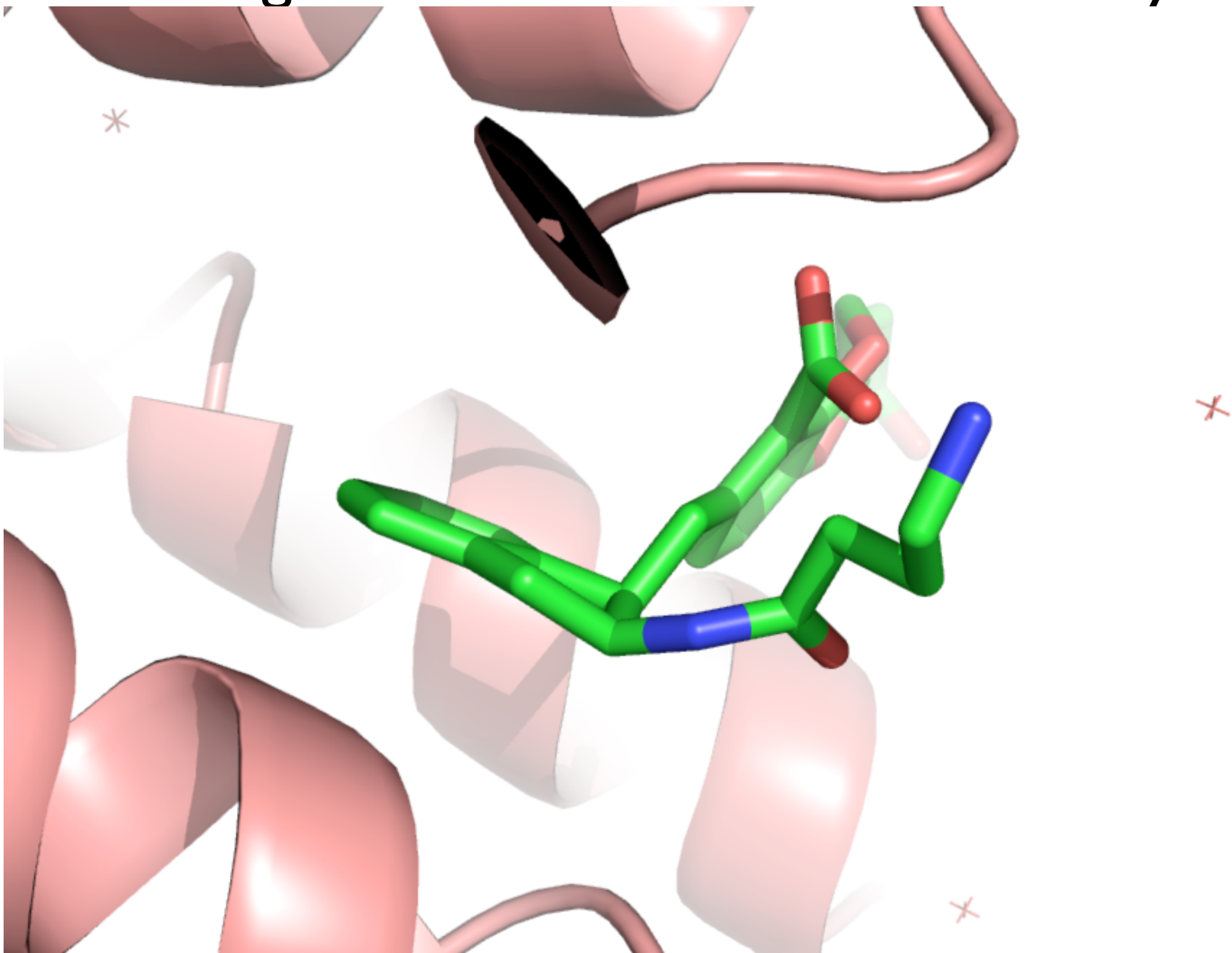


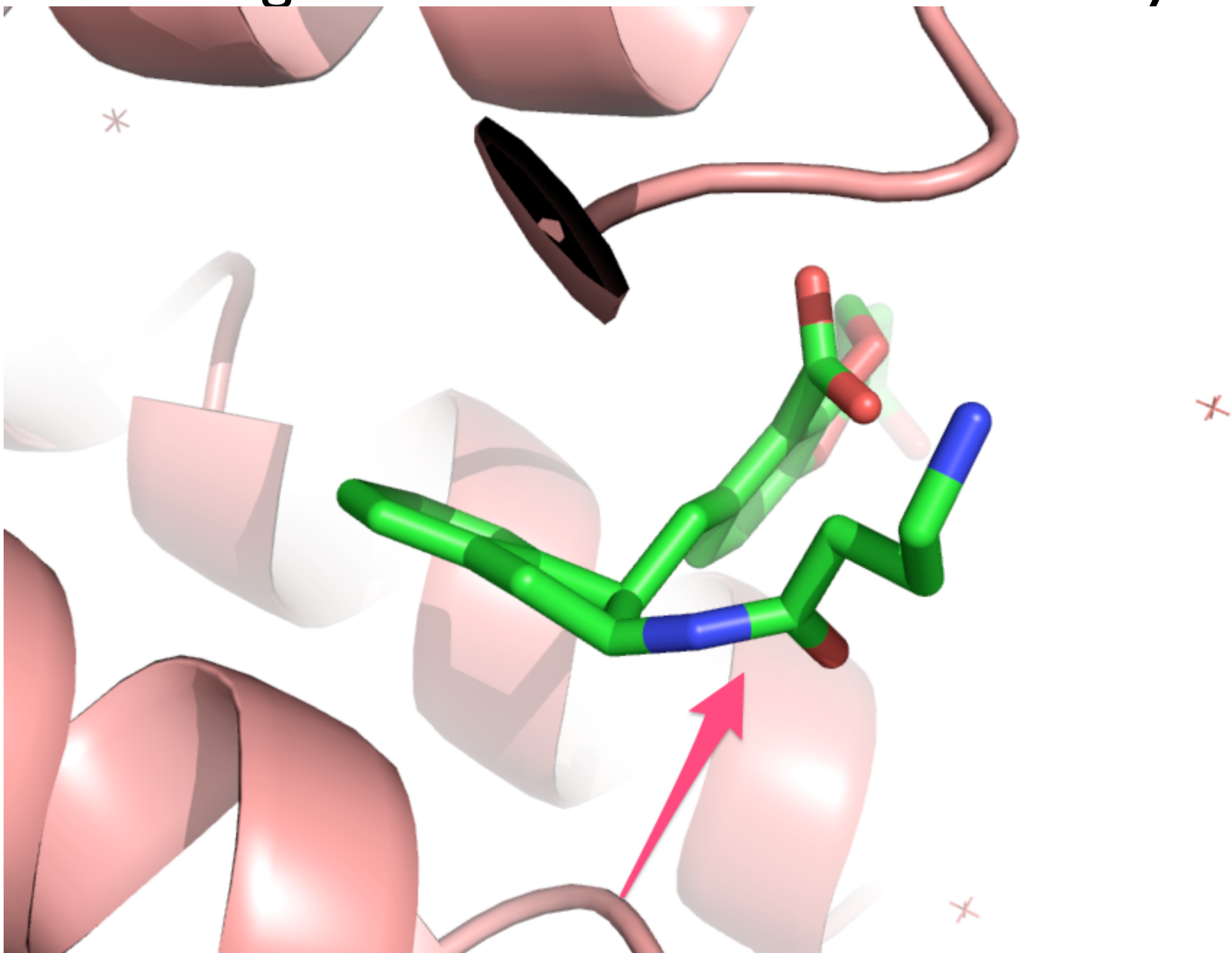
A lot of other things can get in the way: How sure are you of what you're looking at?



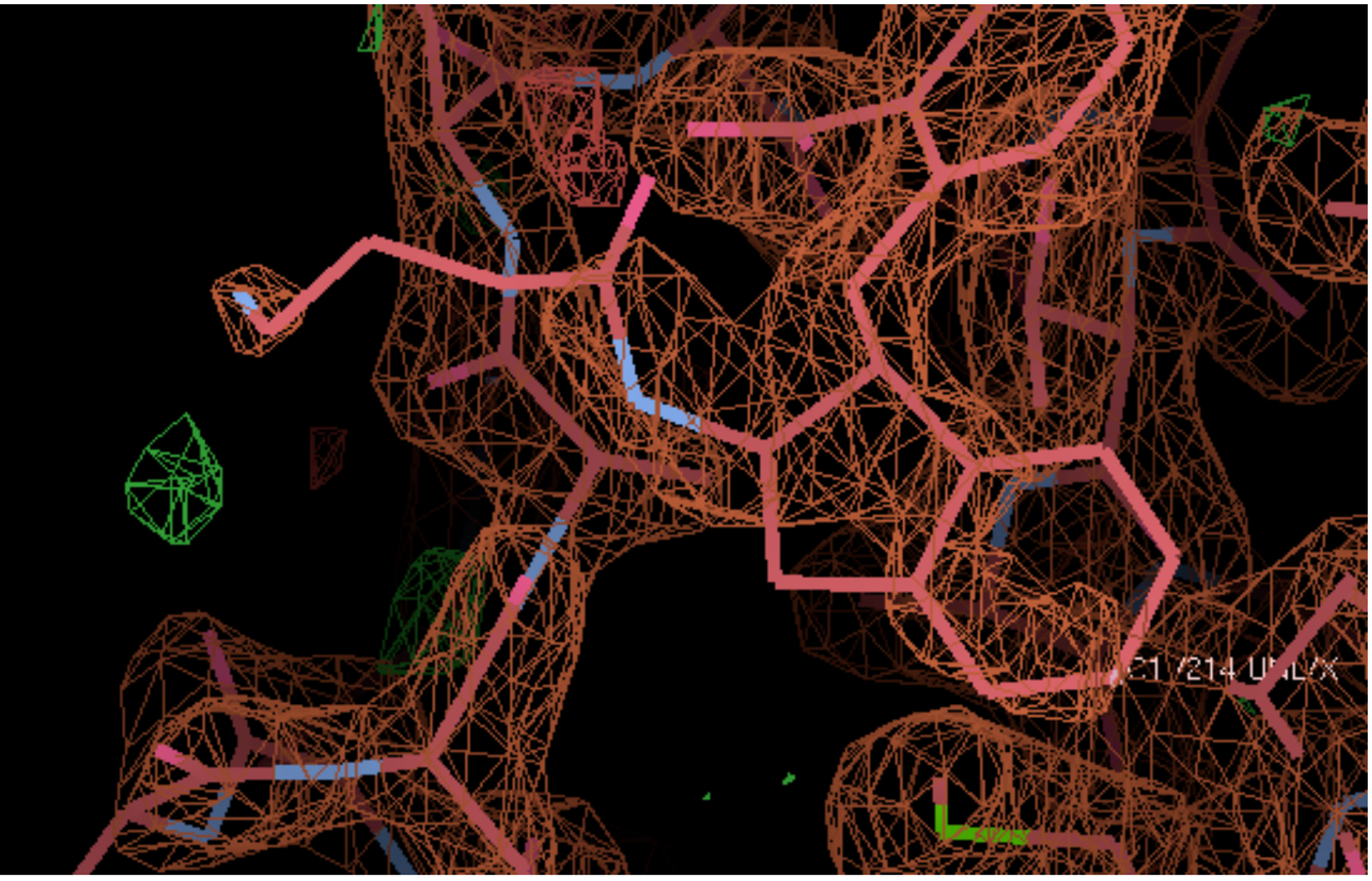
Molecular modeling is used in refinement. Do you have the right balance of forces and density?



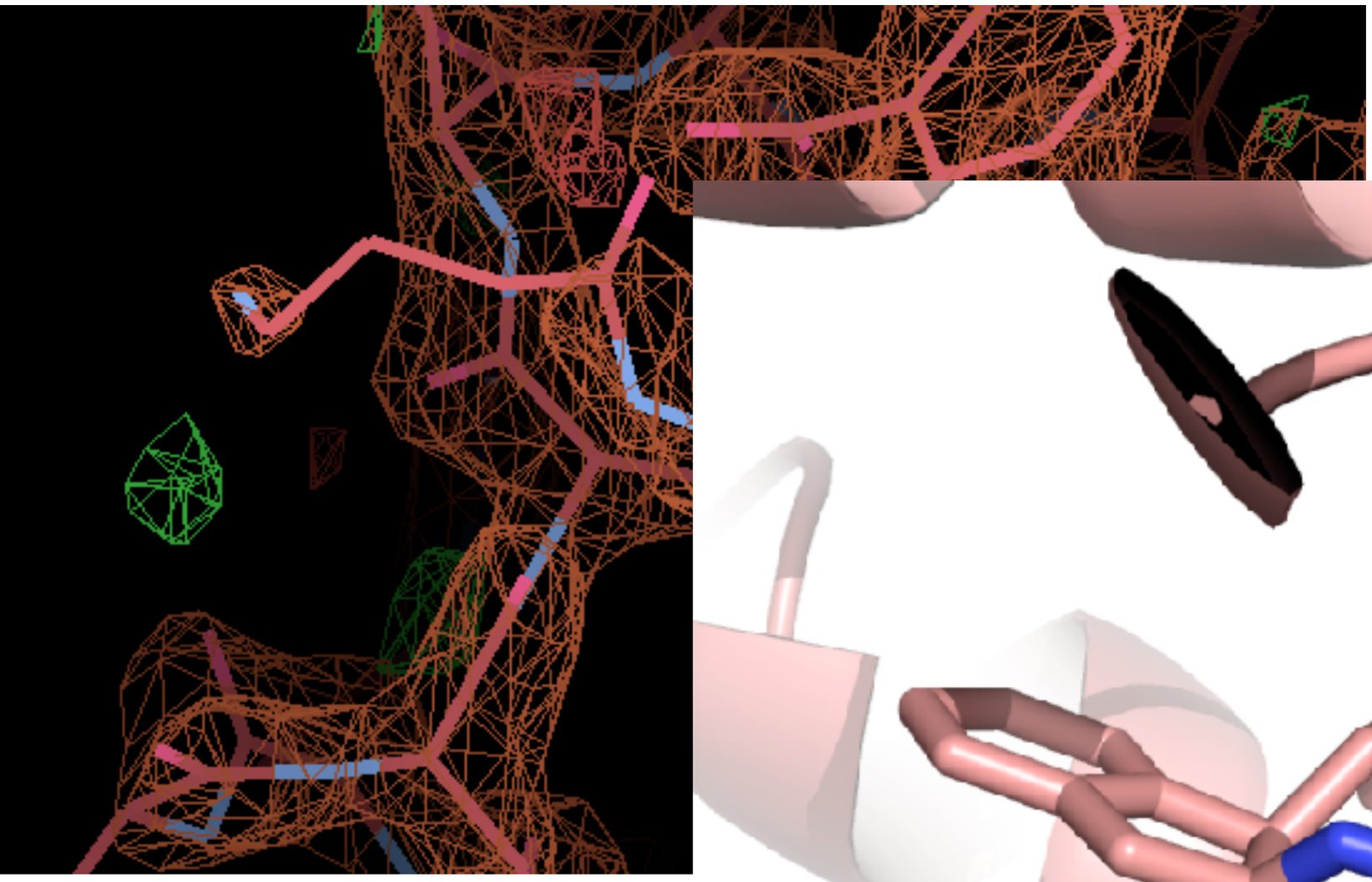
Molecular modeling is used in refinement. Do you have the right balance of forces and density?



Molecular modeling is used in refinement. Do you have the right balance of forces and density?



Molecular modeling is used in refinement. Do you have the right balance of forces and density?



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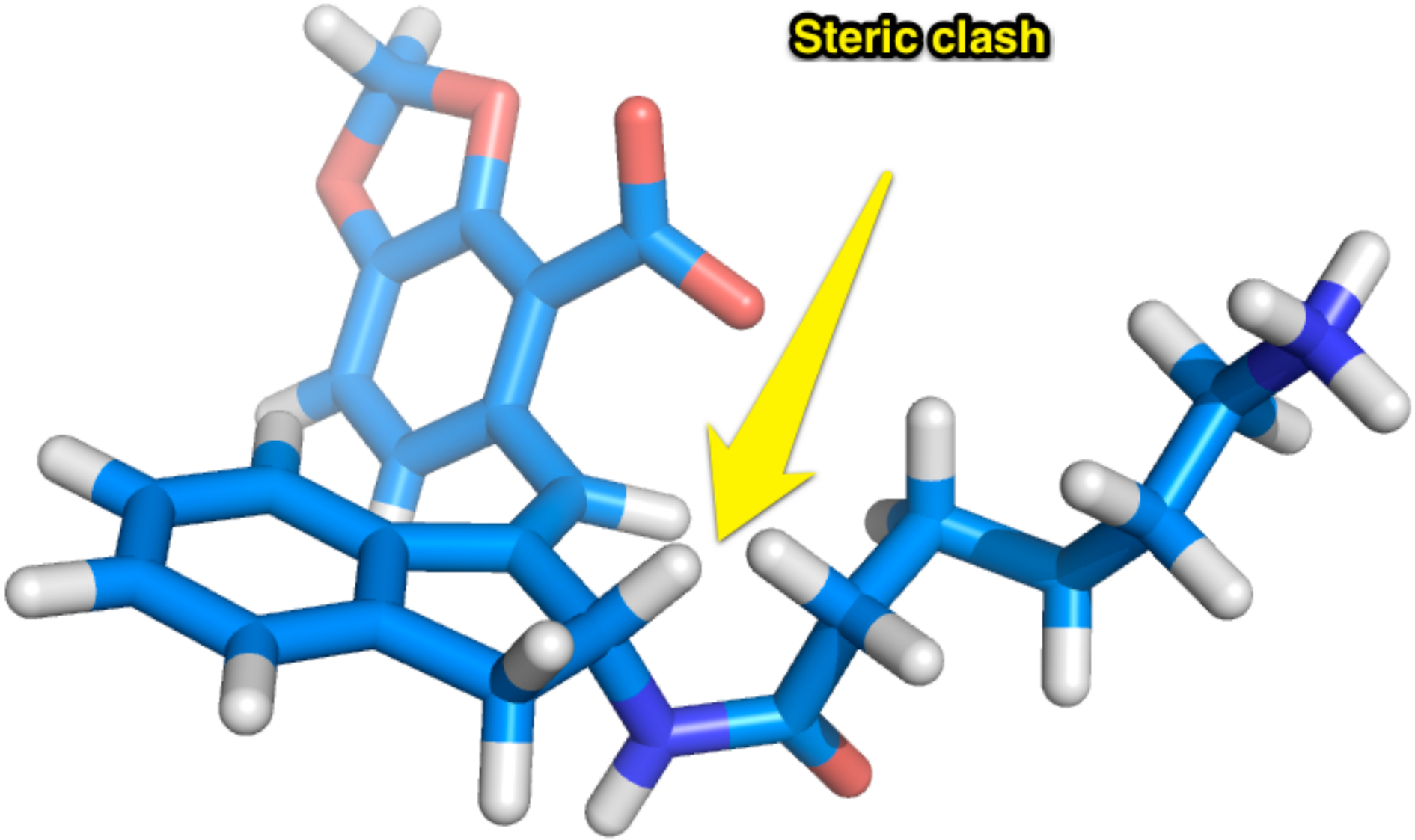
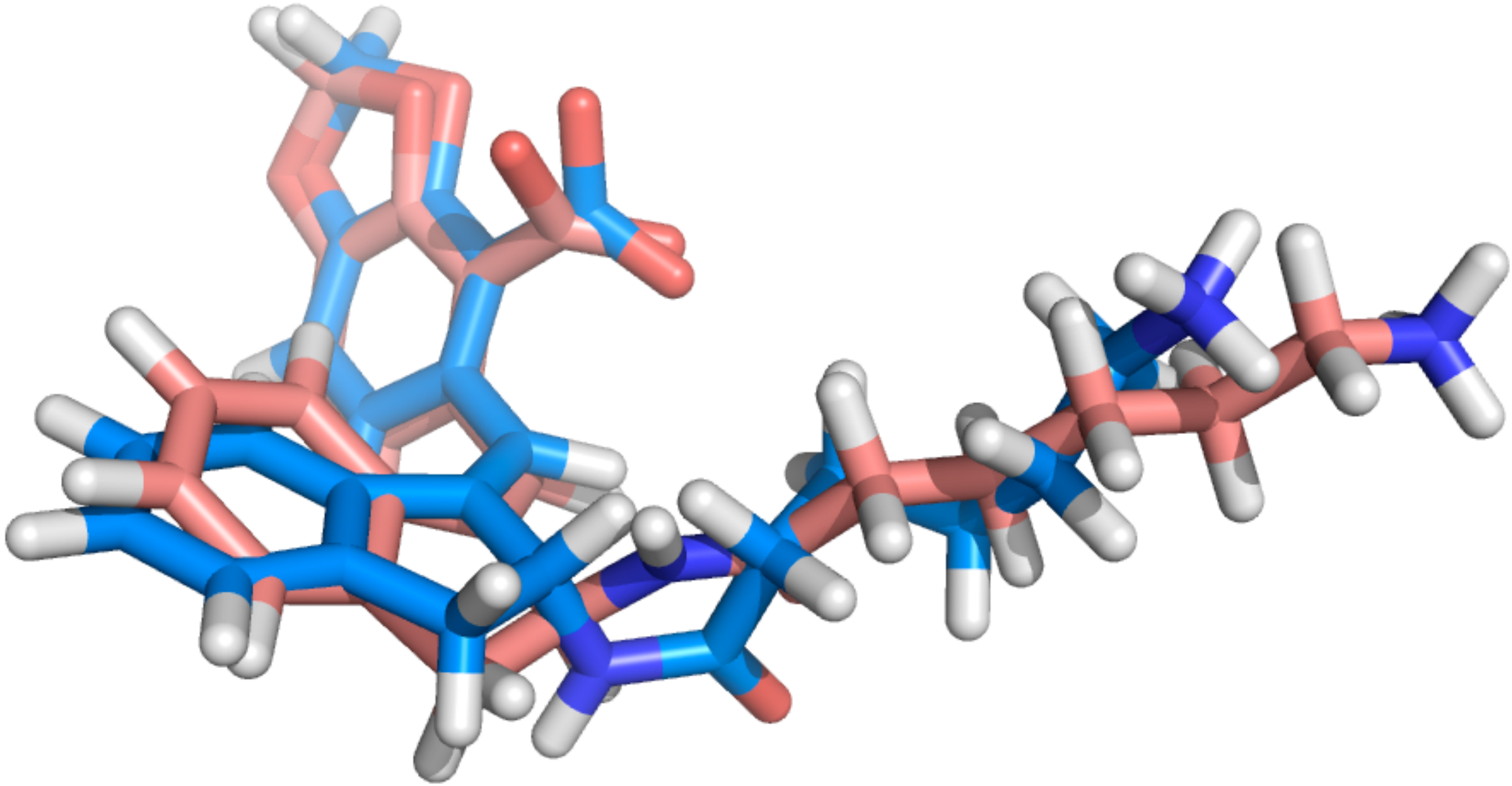
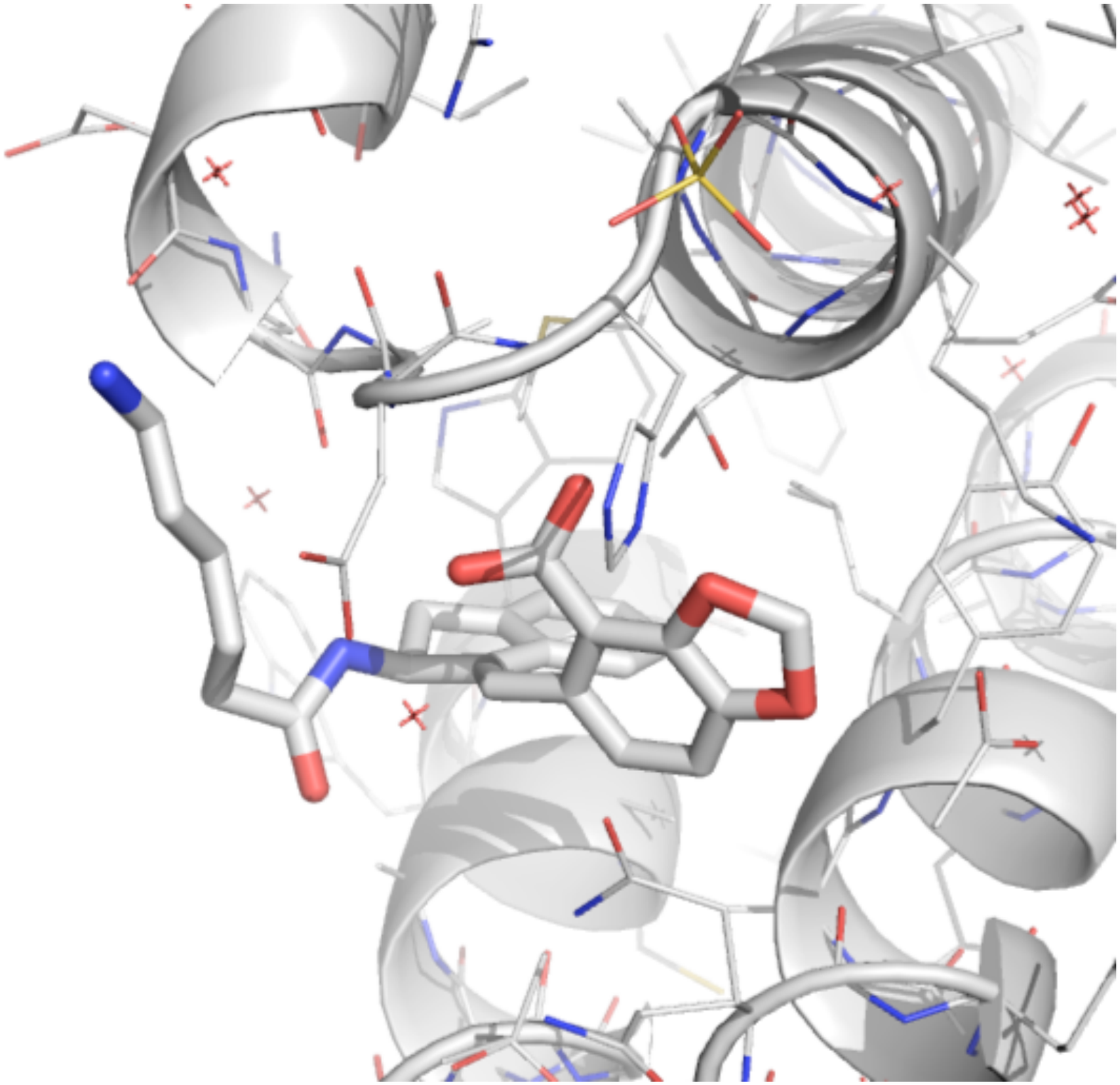


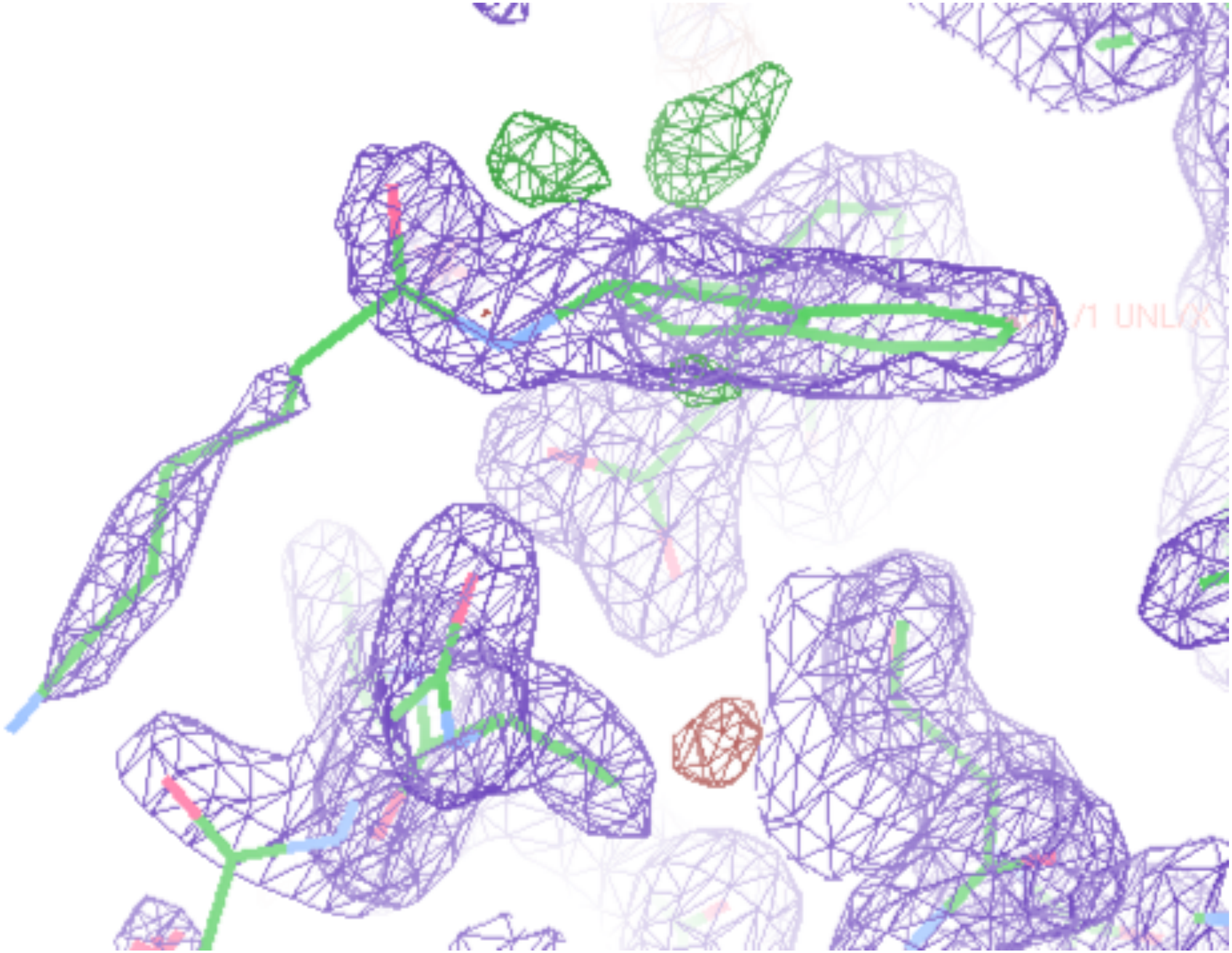
We proposed the opposite stereoisomer, which would eliminate the steric clash



The newly refined model eliminates the steric clash



And it fits the density as well as the original model



PDB information includes sequence info

```
SEQRES   1 B   19   DT  DG  DG  DA  DG  DA  DT  DG  DA  DC  DG  DT  DC
SEQRES   2 B   19   DA  DT  DC  DT  DC  DC
SEQRES   1 A   63  MET ILE VAL PRO GLU SER SER ASP PRO ALA ALA LEU LYS
SEQRES   2 A   63  ARG ALA ARG ASN THR GLU ALA ALA ARG ARG SER ARG ALA
SEQRES   3 A   63  ARG LYS LEU GLN ARG MET LYS GLN LEU GLU ASP LYS VAL
SEQRES   4 A   63  GLU GLU LEU LEU SER LYS ASN TYR HIS LEU GLU ASN GLU
SEQRES   5 A   63  VAL ALA ARG LEU LYS LYS LEU VAL GLY GLU ARG
```

Amino acids	ALA, CYS, ASP, GLU, PHE, GLY, HIS, ILE, LYS, LEU, MET, ASN, PRO, GLN, ARG, SER, THR, VAL, TRP, TYR
Deoxyribonucleotides	DA, DC, DG, DT, DI
Ribonucleotides	A, C, G, U, I

(Source: PDB)

Coordinates are a key section in the PDB file

```
         1         2         3         4         5         6         7         8
12345678901234567890123456789012345678901234567890123456789012345678901234567890
MODEL         1
ATOM      1  N   ALA A   1      11.104   6.134  -6.504  1.00  0.00           N
ATOM      2  CA  ALA A   1      11.639   6.071  -5.147  1.00  0.00           C
...
...
ATOM    293 1HG  GLU A  18     -14.861  -4.847   0.361  1.00  0.00           H
ATOM    294 2HG  GLU A  18     -13.518  -3.769   0.084  1.00  0.00           H
TER     295      GLU A  18
ENDMDL
MODEL        2
ATOM    296  N   ALA  A   1      10.883   6.779  -6.464  1.00  0.00           N
ATOM    297  CA  ALA  A   1      11.451   6.531  -5.142  1.00  0.00           C
...
...
ATOM    588 1HG  GLU A  18     -13.363  -4.163  -2.372  1.00  0.00           H
ATOM    589 2HG  GLU A  18     -12.634  -3.023  -3.475  1.00  0.00           H
TER     590      GLU A  18
```

If you want to work with PDB files, look up the format. Fixed width!

COLUMNS	DATA TYPE	FIELD	DEFINITION
1 - 6	Record name	"ATOM "	
7 - 11	Integer	serial	Atom serial number.
13 - 16	Atom	name	Atom name.
17	Character	altLoc	Alternate location indicator.
18 - 20	Residue name	resName	Residue name.
22	Character	chainID	Chain identifier.
23 - 26	Integer	resSeq	Residue sequence number.
27	AChar	iCode	Code for insertion of residues.
31 - 38	Real(8.3)	x	Orthogonal coordinates for X in Angstroms.
39 - 46	Real(8.3)	y	Orthogonal coordinates for Y in Angstroms.
47 - 54	Real(8.3)	z	Orthogonal coordinates for Z in Angstroms.
55 - 60	Real(6.2)	occupancy	Occupancy.
61 - 66	Real(6.2)	tempFactor	Temperature factor.
77 - 78	LString(2)	element	Element symbol, right-justified.
79 - 80	LString(2)	charge	Charge on the atom.

Crystal structures are limiting in some respects

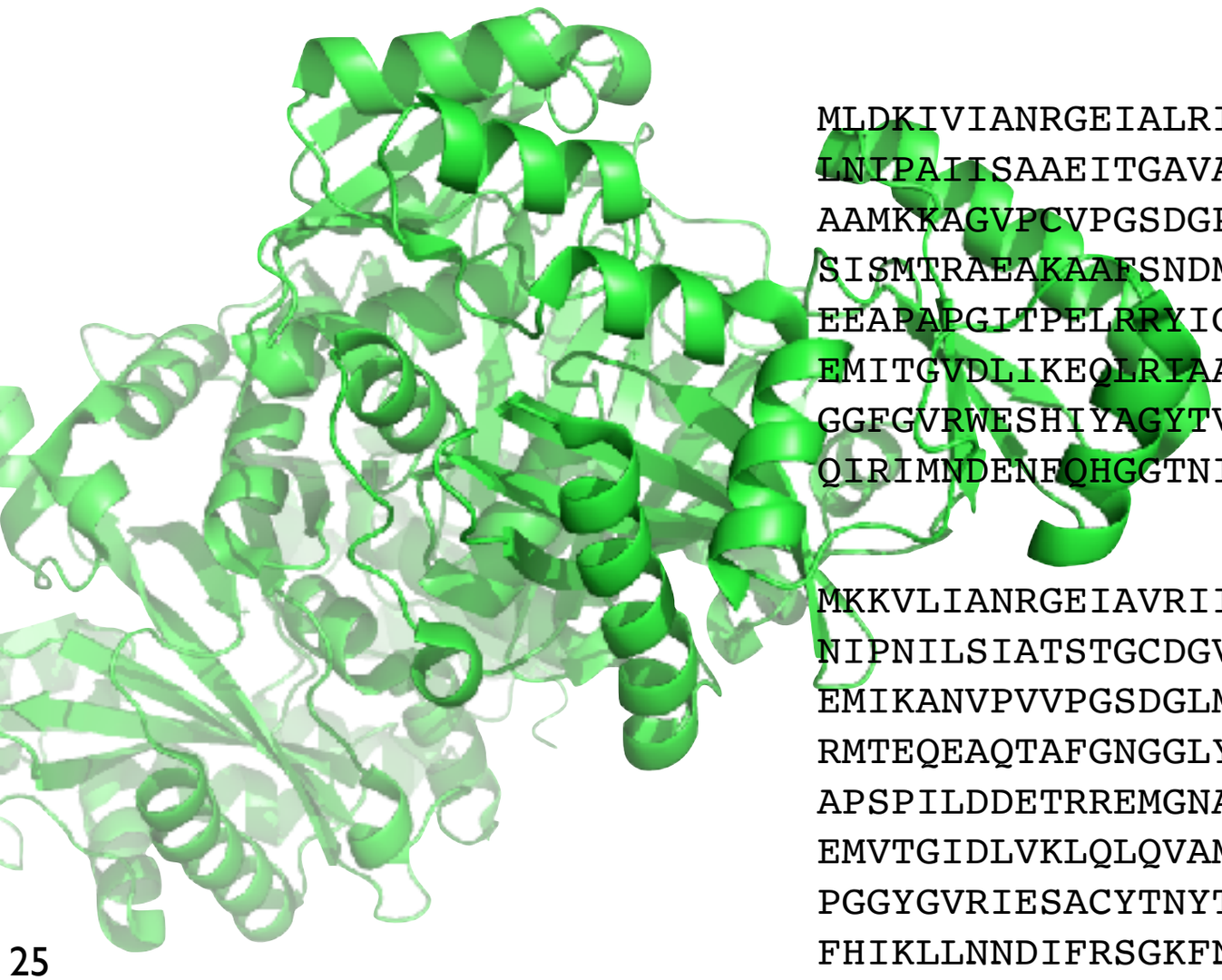
- Proteins are not really static
- They are a *model* fit to the data (the data is the diffraction pattern/electron density)
  - It's easy to confuse them with the data itself
  - Bad modeling yields a bad model!
  - Hetero atoms are particularly problematic (i.e. ligands)
- Crystal conditions, cosolvents, temperature can be important

Homology modeling seeks to solve a common protein structure problem

- We have a protein sequence of interest
  - ex. MET ALA ILE VAL ARG ALA HIS LEU LYS ILE TYR GLY ARG VAL GLN GLY VAL GLY PHE ARG TRP SER MET GLN ARG GLU ...
    - that is, MAIVRAHLKIYGRVQGVGFAWSMNRE...
- We want to predict its structure
  - There are structures of related proteins available
- How do we use the structures of these related proteins to help with our problem?

Homology or comparative models predict new structures based on knowns

- Example: Could we predict the structure of BC in *S. Aureus* based on its structure in *E. Coli?*

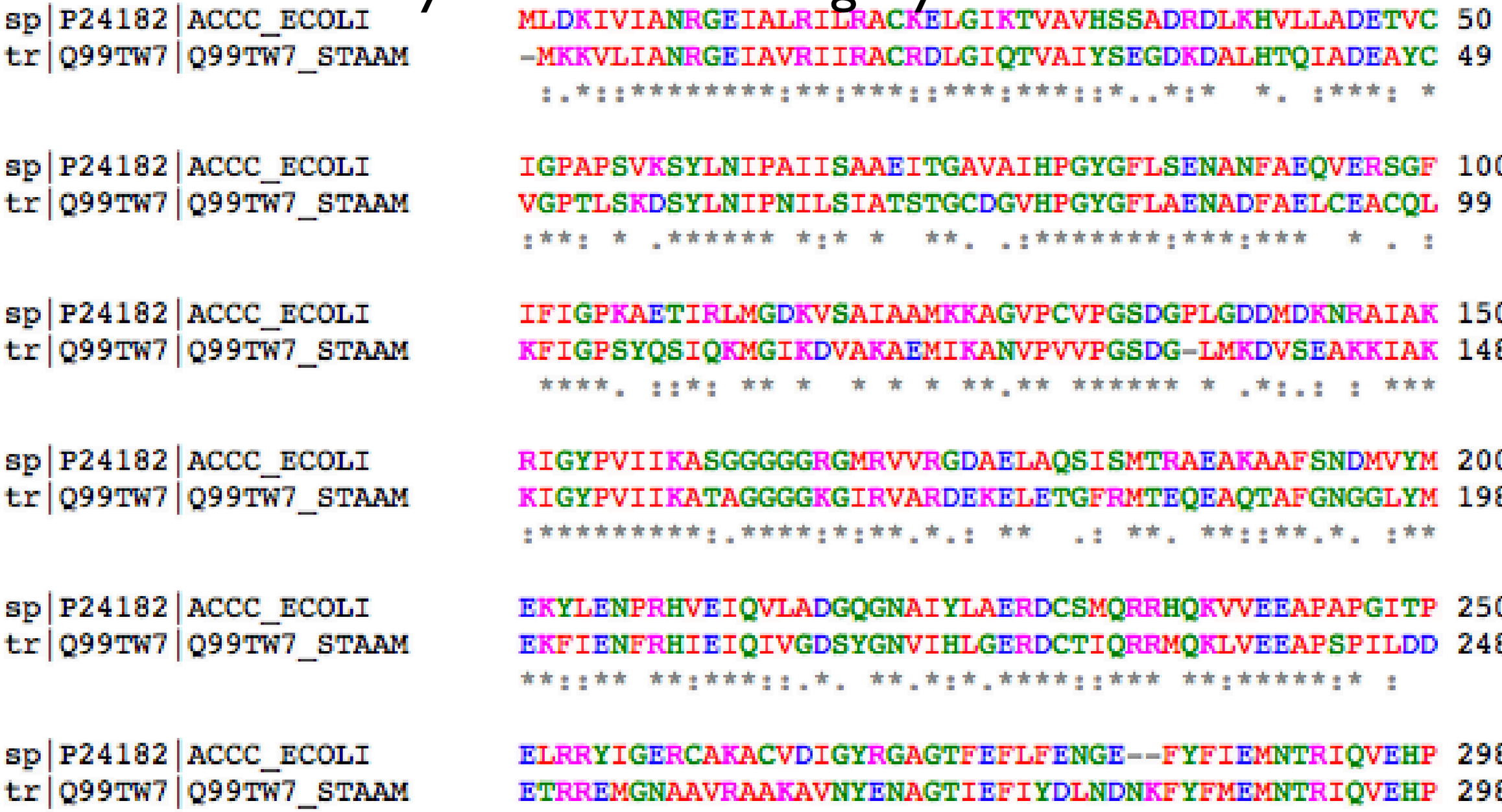


MLDKIVIANRGEIALRILRACKELGIKTVAVHSSADRDLKHVLLADETVCIGPAPSVKSY
LNIPAIISAAEITGAVAIHPGYGFLSENANFAEQVERSGFIFIGPKAETIRLMGDKVSAI
AAMKKAGVPCVPGSDGPLGDDMDKNRAIAKRIGYPVIIKASGGGGGRGMRVVRGDAELAQ
SISMTRAEAKAAFSNDMVYMEKYLENPRHVEIQVLADGQGNAIYLAERDCSMQRRHQKVV
EEAPAPGITPELRRYIGERCAKACVDIGYRGAGTFEFLFENGEFYFIEMNTRIQVEHPVT
EMITGVDLIKEQLRIAAGQPLSIKQEEVHVRGHAVECRINAEDPNTFLPSPGKITRFHAP
GGFGVRWESHIYAGYTVPPYYDSMIGKLICYGENRDVAIARMKNALQELIIDGIKTNVDL
QIRIMNDENFQHGGTNIHYLEKKLGLQEK

MKKVLIANRGEIAVRIIRACRDLGIQTVAIYSEGDKDALHTQIADEAYCVGPTLSKDSYL
NIPNILSIATSTGCDGVHPGYGFLAENADFAELCEACQLKFIGPSYQSIQKMGIKDVAKA
EMIKANVPVVPGSDGLMKDVSEAKKIAKKIGYPVIIKATAGGGGKGIRVARDEKELETGF
RMTEQEAQTAFGNGGLYMEKFIENFRHIEIQIVGDSYGNVIHLGERDCTIQRRMQKLVEE
APSPILDDETRREMGNAAVRAAKAVNYENAGTIEFIYDLNDNKFYFMEMNTRIQVEHPVT
EMVTGIDLVKLQLQVAMGDVLPYKQEDIKLTGHAIEFRINAENPYKNFMPSPGKIEQYLA
PGGYGVRIESACYTNYTIPPYYDSMVAKLIIHEPTRDEAIMAGIRALSEFVVLGIDTTIP
FHIKLLNNDIFRSGKFNTNFLEQNSIMNDEG

At this point, it's hard to say -- how similar is the sequence?

- First, let's try to align the sequences and then color code by amino acid category:



It turns out the structure is already available, and almost identical despite sequence differences

```
sp|P24182|ACCC_ECOLI        MLDKIVIANRGEIALRILRACKELGIKTVAVHSSADRDL
tr|Q99TW7|Q99TW7_STAAM      -MKKVLIANRGEIAVRIIRACRDLGIQTVAIYSEGDKDA
                             :.*::*********:**:*****:***:****::*..*:*

sp|P24182|ACCC_ECOLI        IGPAPSVKSYLNIPAIISAAEITGAVAIHPGYGFLSE
tr|Q99TW7|Q99TW7_STAAM      VGPTLSKDSYLNIPNILSIATSTGCDGVHPGYGF
                             :***: * .****** *:* *  **. .:*****

sp|P24182|ACCC_ECOLI        IFIGPKAETIRLMGDKVSAIAAMKKAGVPCVPGS
tr|Q99TW7|Q99TW7_STAAM      KFIGPSYQSIQKMGIKDVAKAEMIKANVPVVPGS
                             ****. ::*: ** *  * * * **.** **

sp|P24182|ACCC_ECOLI        RIGYPVIIKASGGGGGRGMRVVRGDAELAQSI
tr|Q99TW7|Q99TW7_STAAM      KIGYPVIIKATAGGGGKGIRVARDEKELETGFR
                             :**********:.*****:***.*.: **

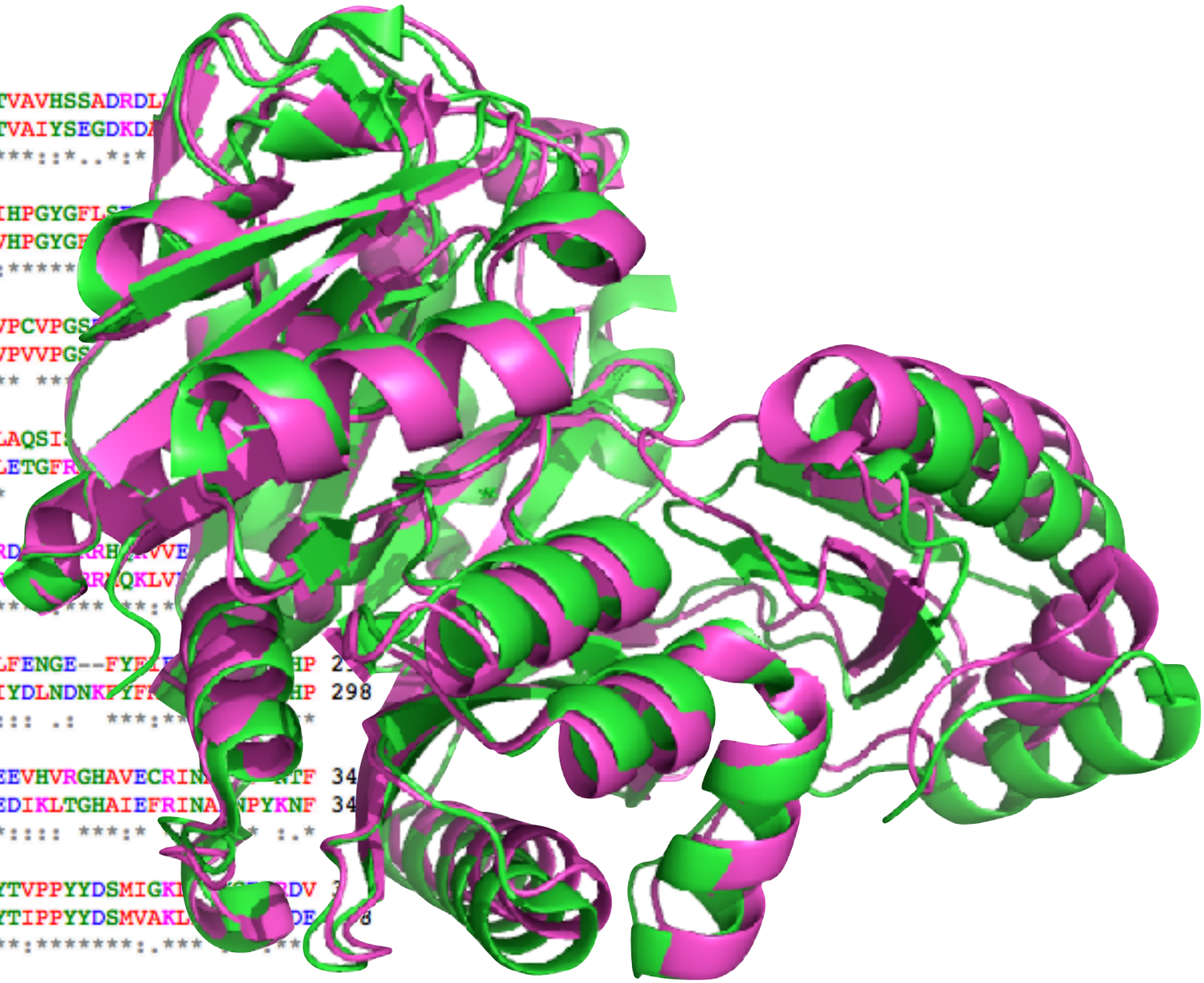
sp|P24182|ACCC_ECOLI        EKYLENPRHVEIQVLADGQGNAIYLAERD
tr|Q99TW7|Q99TW7_STAAM      EKFIENFRHIEIQIVGDSYGNVIHLGER
                             **::** **:******::*. **.*:*.****.**** ***:*

sp|P24182|ACCC_ECOLI        ELRRYIGERCAKACVDIGYRGAGTFEFLFENGE--FYFL
tr|Q99TW7|Q99TW7_STAAM      ETRREMGNAAVRAAKAVNYENAGTIEFIYDLNDNKFYFL
                             * ** :*: ..:*.  :.*..***:*****:: .:  ******

sp|P24182|ACCC_ECOLI        VTEMITGVDLIKEQLRIAAGQPLSIKQEEVHVRGHAVECRIN
tr|Q99TW7|Q99TW7_STAAM      VTEMVTGIDLVKLQLQVAMGDVLPYKQEDIKLTGHAIEFRINA
                             ****:**:**:* ****:* *: *. *******: ****** * :.*

sp|P24182|ACCC_ECOLI        LPSPGKITRFHAPGGFGVRWESHIYAGYTVPPYYDSMIGK
tr|Q99TW7|Q99TW7_STAAM      MPSPGKIEQYLAPGGYGVRIESACYTNYTIPPYYDSMVAK
                             :****** :: ******** **  *:.**:*******:.***

sp|P24182|ACCC_ECOLI        AIARMKNALQELIIDGIKTNVDLQIRIMNDENFQHGGTNIHYLEKKLGLQ 447
tr|Q99TW7|Q99TW7_STAAM      AIMAGIRALSEFVVLGIDTTIPFHIKLLNNDIFRSGKFNTNFLEQNSIMN 448
                             **    .**.*::: **.*.: ::*::::*:: *: *  * ::***::  ::
```



This motivates homology or comparative modeling: Often, proteins have high structural similarity even at low sequence identity

- Many tools available for homology modeling
- In general, high sequence identity is not necessary
  - Above 50% sequence identity, models are generally quite good, with errors mostly in sidechain positioning
  - In 30-50% range, errors can be more severe, but often still tolerable (generally considered acceptable)
  - Below 30%, all bets are off

Sequence identity and sequence similarity are related but different concepts

- A low sequence identity scenario can be OK if sequence similarity is high
- Sequence identity: Fraction of amino acids that are exactly the same
- Sequence similarity: Fraction that have similar properties (i.e. polar, positively charge, negatively charged, hydrophobic)

For high sequence identity models to work, there are two key ingredients

- Good choice of related (template) structure(s)
- Good sequence alignment

```
AAB24882        TYHMCQFHCRYVNNHSGEKLYECNERSKAFSCPSHLQCHKRRQIGEKTHEHNQCGKAFPT 60
AAB24881        --------------------YECNQCGKAFAQHSSLKCHYRTHIGEKPYECNQCGKAFSK 40
                                     ****: .***:  * *:** * :****.:* *******..

AAB24882        PSHLQYHERTHTGEKPYECHQCGQAFKKCSLLQRHKRTHTGEKPYE-CNQCGKAFAQ- 116
AAB24881        HSHLQCHKRTHTGEKPYECNQCGKAFSQHGLLQRHKRTHTGEKPYMNVINMVKPLHNS 98
                 **** *:***********:***:**.: .***************     :  *.: :
```

Graphic from Wikipedia

We will look at Modeller, a useful tool for homology modeling (many other good ones out there)

http://salilab.org/modeller/documentation.html -- has tutorials! (We'll follow one of these)

conda install -c salilab modeller

The process starts with picking our target -- i.e. lactate dehydrogenase from a particular bacteria (Tv)

- Step 1: Get our target sequence and search for related structures

```
>P1;TvLDH
sequence:TvLDH:::::::0.00: 0.00
MSEAAHVLITGAAGQIGYILSHWIASGELYGDRQVYLHLLDIPPAMNRLTALTMELEDCAFPHLAGFVATTDPKA
AFKDIDCAFLVASMPLKPGQVRADLISSNSVIFKNTGEYLSKWAKPSVKVLVIGNPDNTNCEIAMLHAKNLKPEN
FSSLSMLDQNRAYYEVASKLGVDVKDVHDIIVWGNHGESMVADLTQATFTKEGKTQKVVDVLDHDYVFDTFFKKI
GHRAWDILEHRGFTSAASPTKAAIQHMKAWLFGTAPGEVLSMGIPVPEGNPYGIKPGVVFSFPCNVDKEGKIHVV
EGFKVNDWLREKLDFTEKDLFHEKEIALNHLAQGG*
```

- The tutorial provides a Python script which uses Modeller to do this search

- Searches a clustered set of PDB sequences (at 95% similarity) to find those similar to this

Following http://salilab.org/modeller/tutorial/basic.html

This is called building a profile; once we do it, we need to decide what structure to use as a template

```
# Number of sequences:     30
# Length of profile  :    335
# N_PROF_ITERATIONS  :      1
# GAP_PENALTIES_1D   :   -900.0   -50.0
# MATRIX_OFFSET      :    0.0
# RR_FILE            : ${MODINSTALL8v1}/modlib//as1.sim.mat
    1 TvLDH                                S     0   335     1   335     0     0     0    0.    0.0
    2 1a5z                                 X     1   312    75   242    63   229   164   28.   0.83E-08
    3 1b8pA                                X     1   327     7   331     6   325   316   42.    0.0
    4 1bdmA                                X     1   318     1   325     1   310   309   45.    0.0
    5 1t2dA                                X     1   315     5   256     4   250   238   25.   0.66E-04
    6 1civA                                X     1   374     6   334    33   358   325   35.    0.0
    7 2cmd                                 X     1   312     7   320     3   303   289   27.   0.16E-05
    8 1o6zA                                X     1   303     7   320     3   287   278   26.   0.27E-05
    9 1ur5A                                X     1   299    13   191     9   171   158   31.   0.25E-02
   10 1guzA                                X     1   305    13   301     8   280   265   25.   0.28E-08
   11 1gv0A                                X     1   301    13   323     8   289   274   26.   0.28E-04
   12 1hyeA                                X     1   307     7   191     3   183   173   29.   0.14E-07
   13 1i0zA                                X     1   332    85   300    94   304   207   25.   0.66E-05
   14 1i10A                                X     1   331    85   295    93   298   196   26.   0.86E-05
   15 1ldnA                                X     1   316    78   298    73   301   214   26.   0.19E-03
   16 6ldh                                 X     1   329    47   301    56   302   244   23.   0.17E-02
   17 2ldx                                 X     1   331    66   306    67   306   227   26.   0.25E-04
   18 5ldh                                 X     1   333    85   300    94   304   207   26.   0.30E-05
   19 9ldtA                                X     1   331    85   301    93   304   207   26.   0.10E-05
   20 111c                                 X     1   321    64   239    53   234   164   26.   0.20E-03
   21 111dA                                X     1   313    13   242     9   233   216   31.   0.31E-07
   22 5mdhA                                X     1   333     2   332     1   331   328   44.    0.0
   23 7mdhA                                X     1   351     6   334    14   339   325   34.    0.0
   24 1m1dA                                X     1   313     5   198     1   189   183   26.   0.13E-05
   25 1oc4A                                X     1   315     5   191     4   186   174   28.   0.18E-04
   26 1ojuA                                X     1   294    78   320    68   285   218   28.   0.43E-05
   27 1pzgA                                X     1   327    74   191    71   190   114   30.   0.16E-06
   28 1smkA                                X     1   313     7   202     4   198   188   34.    0.0
   29 1sovA                                X     1   316    81   256    76   248   160   27.   0.93E-03
   30 1y6jA                                X     1   289    77   191    58   167   109   33.   0.32E-05
```

This is called building a profile; once we do it, we need to decide what structure to use as a template

```
# Number of sequences:     30
# Length of profile  :    335
# N_PROF_ITERATIONS  :      1
# GAP_PENALTIES_1D   :   -900.0   -50.0
# MATRIX_OFFSET      :    0.0
# RR_FILE            : ${MODINSTALL8v1}/modlib//as1.sim.mat
   1 TvLDH                                S     0   335     1   335     0     0     0    0.    0.0
   2 1a5z                                 X     1   312    75   242    63   229   164   28.   0.83E-08
   3 1b8pA                                X     1   327     7   331     6   325   316   42.    0.0
   4 1bdmA                                X     1   318     1   325     1   310   309   45.    0.0
   5 1t2dA                                X     1   315     5   256     4   250   238   25.   0.66E-04
   6 1civA                                X     1   374     6   334    33   358   325   35.    0.0
   7 2cmd                                 X     1   312     7   320     3   303   289   27.   0.16E-05
   8 1o6zA                                X     1   303     7   320     3   287   278   26.   0.27E-05
   9 1ur5A                                X     1   299    13   191     9   171   158   31.   0.25E-02
  10 1guzA                                X     1   305    13   301     8   280   265   25.   0.28E-08
  11 1gv0A                                X     1   301    13   323     8   289   274   26.   0.28E-04
  12 1hyeA                                X     1   307     7   191     3   183   173   29.   0.14E-07
  13 1i0zA                                X     1   332    85   300    94   304   207   25.   0.66E-05
  14 1i10A                                X     1   331    85   295    93   298   196   26.   0.86E-05
  15 1ldnA                                X     1   316    78   298    73   301   214   26.   0.19E-03
  16 6ldh                                 X     1   329    47   301    56   302   244   23.   0.17E-02
  17 2.ix                                 X     1   331    66   306    67   306   227   26.   0.25E-04
  18 5ldh                                 X     1   333    85   300    94   304   207   26.   0.30E-05
  19 9ldtA                                X     1   331    85   301    93   304   207   26.   0.10E-05
  20 111c                                 X     1   321    64   239    53   234   164   26.   0.20E-03
  21 111dA                                X     1   313    13   242     9   233   216   31.   0.31E-07
  22 5mdhA                                X     1   333     2   332     1   331   328   44.    0.0
  23 7mdhA                                X     1   351     6   334    14   339   325   34.    0.0
  24 1m1dA                                X     1   313     5   198     1   189   183   26.   0.13E-05
  25 1oc4A                                X     1   315     5   191     4   186   174   28.   0.18E-04
  26 1ojuA                                X     1   294    78   320    68   285   218   28.   0.43E-05
  27 1pzgA                                X     1   327    74   191    71   190   114   30.   0.16E-06
  28 1smkA                                X     1   313     7   202     4   198   188   34.    0.0
  29 1sovA                                X     1   316    81   256    76   248   160   27.   0.93E-03
  30 1y6j                                 X     1   289    77   191    58   167   109   33.   0.32E-05
```

PDB code

This is called building a profile; once we do it, we need to decide what structure to use as a template

```
# Number of sequences:     30
# Length of profile  :    335
# N_PROF_ITERATIONS  :      1
# GAP_PENALTIES_1D   :   -900.0   -50.0
# MATRIX_OFFSET      :    0.0
# RR_FILE            : ${MODINSTALL8v1}/modlib//as1.sim.mat
   1 TvLDH                                S     0   335     1   335     0     0     0    0.     0.0
   2 1a5z                                 X     1   312    75   242    63   229   164   28.    0.83E-08
   3 1b8pA                                X     1   327     7   331     6   325   316   42.     0.0
   4 1bdmA                                X     1   318     1   325     1   310   309   45.     0.0
   5 1t2dA                                X     1   315     5   256     4   250   238   25.    0.66E-04
   6 1civA                                X     1   374     6   334    33   358   325   35.     0.0
   7 2cmd                                 X     1   312     7   320     3   303   289   27.    0.16E-05
   8 1o6zA                                X     1   303     7   320     3   287   278   26.    0.27E-05
   9 1ur5A                                X     1   299    13   191     9   171   158   31.    0.25E-02
  10 1guzA                                X     1   305    13   301     8   280   265   25.    0.28E-08
  11 1gv0A                                X     1   301    13   323     8   289   274   26.    0.28E-04
  12 1hyeA                                X     1   307     7   191     3   183   173   29.    0.14E-07
  13 1i0zA                                X     1   332    85   300    94   304   207   25.    0.66E-05
  14 1i10A                                X     1   331    85   295    93   298   196   26.    0.86E-05
  15 1ldnA                                X     1   316    78   298    73   301   211   25.    0.19E-03
  16 6ldh                                 X     1   329    47   301    56   302   244   23.    0.17E-02
  17 2.ix                                 X     1   331    66   306    67   306   227   26.    0.25E-04
  18 5ldh                                 X     1   333    85   300    94   304   207   26.    0.30E-05
  19 9ldtA                                X     1   331    85   301    93   304   207   26.    0.10E-05
  20 111c                                 X     1   321    64   239    53   234   164   26.    0.20E-03
  21 111dA                                X     1   313    13   242     9   233   216   31.    0.31E-07
  22 5mdhA                                X     1   333     2   332     1   331   328   44.     0.0
  23 7mdhA                                X     1   351     6   334    14   339   325   34.     0.0
  24 1m1dA                                X     1   313     5   198     1   199   183   26.    0.13E-05
  25 1oc4A                                X     1   315     5   191     4   186   174   28.    0.18E-04
  26 1ojuA                                X     1   294    78   320    68   285   218   28.    0.43E-05
  27 1pzgA                                X     1   327    74   191    71   190   114   30.    0.16E-06
  28 1smkA                                X     1   313     7   202     4   198   188   34.     0.0
  29 1sovA                                X     1   316    81   256    76   248   160   27.    0.93E-03
  30 1y6jA                                X     1   289    77   191    58   167   109   33.    0.32E-05
```

PDB code

len.

This is called building a profile; once we do it, we need to decide what structure to use as a template

```
# Number of sequences:     30
# Length of profile  :    335
# N_PROF_ITERATIONS  :      1
# GAP_PENALTIES_1D   :   -900.0   -50.0
# MATRIX_OFFSET      :    0.0
# RR_FILE            : ${MODINSTALL8v1}/modlib//as1.sim.mat
   1 TvLDH                                S     0   335     1   335     0     0     0    0.     0.0
   2 1a5z                                 X     1   312    75   242    63   229   164   28.    0.83E-08
   3 1b8pA                                X     1   327     7   331     6   325   316   42.    0.0
   4 1bdmA                                X     1   318     1   325     1   310   309   45.    0.0
   5 1t2dA                                X     1   315     5   256     4   250   238   25.    0.66E-04
   6 1civA                                X     1   374     6   334    33   358   325   35.    0.0
   7 2cmd                                 X     1   312     7   320     3   303   289   27.    0.16E-05
   8 1o6zA                                X     1   303     7   320     3   287   270   26.    0.27E-05
   9 1ur5A                                X     1   299    13   191     9   171   153   31.    0.25E-02
  10 1guzA                                X     1   305    13   301     8   280   265   25.    0.28E-08
  11 1gv0A                                X     1   301    13   323     8   289   274   26.    0.28E-04
  12 1hyeA                                X     1   307     7   191     3   183   173   29.    0.14E-07
  13 1i0zA                                X     1   332    85   300    94   304   207   26.    0.56E-05
  14 1i10A                                X     1   331    85   295    93   298   196   23.    0.36E-05
  15 1ldnA                                X     1   316    78   298    73   301   231   26.    0.19E-03
  16 6ldh                                 X     1   329    47   301    56   302   244   33.    0.17E-02
  17 2.ix                                 X     1   331    66   306    67   306   227   32.    0.25E-04
  18 5ldh                                 X     1   333    85   300    94   304   207   26.    0.30E-05
  19 9ldtA                                X     1   331    85   301    93   304   207   26.    0.10E-05
  20 111c                                 X     1   321    64   239    53   234   164   26.    0.20E-03
  21 111dA                                X     1   313    13   242     9   233   215   31.    0.31E-07
  22 5mdhA                                X     1   333     2   332     1   331   325   44.    0.0
  23 7mdhA                                X     1   351     6   334    14   339   325   34.    0.0
  24 1m1dA                                X     1   313     5   198     1   199   183   26.    0.13E-05
  25 1oc4A                                X     1   315     5   191     4   186   174   28.    0.18E-04
  26 1ojuA                                X     1   294    78   320    68   285   218   28.    0.43E-05
  27 1pzgA                                X     1   327    74   191    71   190   114   30.    0.16E-06
  28 1smkA                                X     1   313     7   202     4   198   188   34.    0.0
  29 1sovA                                X     1   316    81   256    76   248   160   27.    0.93E-03
  30 1y6jA                                X     1   289    77   191    58   167   109   33.    0.32E-05
```

PDB code

len.

% iden.

This is called building a profile; once we do it, we need to decide what structure to use as a template

```
# Number of sequences:     30
# Length of profile  :    335
# N_PROF_ITERATIONS  :      1
# GAP_PENALTIES_1D   :   -900.0   -50.0
# MATRIX_OFFSET      :    0.0
# RR_FILE            : ${MODINSTALL8v1}/modlib//as1.sim.mat
   1 TvLDH                                S     0   335     1   335     0     0     0    0.     0.0
   2 1a5z                                 X     1   312    75   242    63   229   164   28.    .83E-0
   3 1b8pA                                X     1   327     7   331     6   325   316   42.     0.0
   4 1bdmA                                X     1   318     1   325     1   310   309   45.     0.0
   5 1t2dA                                X     1   315     5   256     4   250   238   25.    0.66E-04
   6 1civA                                X     1   374     6   334    33   358   325   35.     0.0
   7 2cmd                                 X     1   312     7   320     3   303   289   27.    0.16E-05
   8 1o6zA                                X     1   303     7   320     3   287   270   26.    0.27E-05
   9 1ur5A                                X     1   299    13   191     9   171   153   31.    0.25E-02
  10 1guzA                                X     1   305    13   301     8   280   265   25.    0.28E-08
  11 1gv0A                                X     1   301    13   323     8   289   274   26.    0.28E-04
  12 1hyeA                                X     1   307     7   191     3   183   173   29.    0.14E-07
  13 1i0zA                                X     1   332    85   300    94   304   207   26.    0.56E-05
  14 1i10A                                X     1   331    85   295    93   298   196   23.    0.86E-05
  15 1ldnA                                X     1   316    78   298    73   301   210   25.    0.19E-03
  16 6ldh                                 X     1   329    47   301    56   302   244   33.    0.11E-02
  17 2ldx                                 X     1   331    66   306    67   306   227   32.    0.25E-04
  18 5ldh                                 X     1   333    85   300    94   304   207   26.    0.80E-05
  19 9ldtA                                X     1   331    85   301    93   304   207   26.    0.10E-05
  20 111c                                 X     1   321    64   239    53   234   164   26.    0.20E-03
  21 111dA                                X     1   313    13   242     9   233   215   31.    0.31E-07
  22 5mdhA                                X     1   333     2   332     1   331   325   44.     0.0
  23 7mdhA                                X     1   351     6   334    14   339   325   34.     0.0
  24 1mldA                                X     1   313     5   198     1   189   183   26.    0.13E-05
  25 1oc4A                                X     1   315     5   191     4   186   174   28.    0.18E-04
  26 1ojuA                                X     1   294    78   320    68   285   218   28.    0.43E-05
  27 1pzgA                                X     1   327    74   191    71   190   114   30.    0.16E-06
  28 1smkA                                X     1   313     7   202     4   198   188   34.     0.0
  29 1sovA                                X     1   316    81   256    76   248   160   27.    0.93E-03
  30 1y6jA                                X     1   289    77   191    58   167   109   33.    0.32E-0
```

PDB code

len.

% iden.

eval.

Next, we zero in on some specific structures which look interesting

```
# Number of sequences:     30
# Length of profile  :    335
# N_PROF_ITERATIONS  :      1
# GAP_PENALTIES_1D   :   -900.0   -50.0
# MATRIX_OFFSET      :    0.0
# RR_FILE            : ${MODINSTALL8v1}/modlib//as1.sim.mat
    1 TvLDH                                S     0   335     1   335     0     0     0    0.     0.0
    2 1a5z                                 X     1   312    75   242    63   229   164   28.   0.83E-08
    3 1b8pA                                X     1   327     7   331     6   325   316   42.    0.0
    4 1bdmA                                X     1   318     1   325     1   310   309   45.    0.0
    5 1t2dA                                X     1   315     5   256     4   250   238   25.   0.66E-04
    6 1civA                                X     1   374     6   334    33   358   325   35     0.0
    7 2cmd                                 X     1   312     7   320     3   303   289   27.   0.16E-05
    8 1o6zA                                X     1   303     7   320     3   287   278   26.   0.27E-05
    9 1ur5A                                X     1   299    13   191     9   171   158   31.   0.25E-02
   10 1guzA                                X     1   305    13   301     8   280   265   25.   0.28E-08
   11 1gv0A                                X     1   301    13   323     8   289   274   26.   0.28E-04
   12 1hyeA                                X     1   307     7   191     3   183   173   29.   0.14E-07
   13 1i0zA                                X     1   332    85   300    94   304   207   25.   0.66E-05
   14 1i10A                                X     1   331    85   295    93   298   196   26.   0.86E-05
   15 1ldnA                                X     1   316    78   298    73   301   214   26.   0.19E-03
   16 6ldh                                 X     1   329    47   301    56   302   244   23.   0.17E-02
   17 2ldx                                 X     1   331    66   306    67   306   227   26.   0.25E-04
   18 5ldh                                 X     1   333    85   300    94   304   207   26.   0.30E-05
   19 9ldtA                                X     1   331    85   301    93   304   207   26.   0.10E-05
   20 111c                                 X     1   321    64   239    53   234   164   26.   0.20E-03
   21 111dA                                X     1   313    13   242     9   233   216   31.   0.31E-07
   22 5mdhA                                X     1   333     2   332     1   331   328   44.    0.0
   23 7mdhA                                X     1   351     6   334    14   339   325   34.    0.0
   24 1mldA                                X     1   313     5   198     1   189   183   26.   0.13E-05
   25 1oc4A                                X     1   315     5   191     4   186   174   28.   0.18E-04
   26 1ojuA                                X     1   294    78   320    68   285   218   28.   0.43E-05
   27 1pzgA                                X     1   327    74   191    71   190   114   30.   0.16E-06
   28 1smkA                                X     1   313     7   202     4   198   188   34     0.0
```

We then run a structural comparison on these to get some additional insight

```
Sequence identity comparison (ID_TABLE):

   Diagonal        ... number of residues;
   Upper triangle ... number of identical residues;
   Lower triangle ... % sequence identity, id/min(length).

          1b8pA @1 1bdmA @1 1civA @2 5mdhA @2 7mdhA @2 1smkA @2
1b8pA @1      327      194      147      151      153       49
1bdmA @1       61      318      152      167      155       56
1civA @2       45       48      374      139      304       53
5mdhA @2       46       53       42      333      139       57
7mdhA @2       47       49       87       42      351       48
1smkA @2       16       18       17       18       15      313

Weighted pair-group average clustering based on a distance matrix:

                                          .----------------------- 1b8pA @1.9    39.0000
                                          |
                                     .----------------------------- 1bdmA @1.8    50.5000
                                     |
                                .------------------------------------ 5mdhA @2.4    55.3750
                                |
                                |                                  .--- 1civA @2.8    13.0000
                                |                                  |
                  .-------------------------------------------------------- 7mdhA @2.4    83.2500
                  |
            .-------------------------------------------------------------- 1smkA @2.5

              +----+----+----+----+----+----+----+----+----+----+----+----+
          86.0600   73.4150   60.7700   48.1250   35.4800   22.8350   10.1900
               79.7375   67.0925   54.4475   41.8025   29.1575   16.5125
```

We then run a structural comparison on these to get some additional insight

```
Sequence identity comparison (ID_TABLE):

   Diagonal        ... number of residues;
   Upper triangle  ... number of identical residues;
   Lower triangle  ... % sequence identity, id/min(length).

          1b8pA @11bdmA @11civA @25mdhA @27mdhA @21smkA @2
1b8pA @1      327     194     147     151     153      49
1bdmA @1       61     318     152     167     155      56
1civA @2       45      48     374     139     304      53
5mdhA @2       46      53                             54
7mdhA @2       47      49                             52
1smkA @2       16      18      17      18      15     313

Weighted pair-group average clustering based on a distance matrix:

                                                  .---------------------- 1b8pA @1.9    39.0000
                                                  |
                                             .----------------------------- 1bdmA @1.8    50.5000
                                             |
                                        .---------------------------------- 5mdhA @2.4    55.3750
                                        |
                                        |                                 .--- 1civA @2.8    13.0000
                                        |                                 |
                                   .-------------------------------------------- 7mdhA @2.4    83.2500
                                   |
                              .-------------------------------------------------- 1smkA @2.5

                              +----+----+----+----+----+----+----+----+----+----+----+----+
                          86.0600   73.4150   60.7700   48.1250   35.4800   22.8350   10.1900
                              79.7375   67.0925   54.4475   41.8025   29.1575   16.5125
```

High sequence similarity

We then run a structural comparison on these to get some additional insight

```
Sequence identity comparison (ID_TABLE):

   Diagonal        ... number of residues;
   Upper triangle  ... number of identical residues;
   Lower triangle  ... % sequence identity, id/min(length).

          1b8pA @11bdmA @11civA @25mdhA @27mdhA @21smkA @2
1b8pA @1      327     194     147     151     153      49
1bdmA @1       61     318     152     167     155      56
1civA @2       45      48     374     139     304      53
5mdhA @2       46      53
7mdhA @2       47      49
1smkA @2       16      18      17      18      15     313

Weighted pair-group average clustering based on a distance matrix:

                                                  .------------------------ 1b8pA @1.9     39.0000
                                                  |
                                              .---------------------------- 1bdmA @1.8     50.5000
                                              |
                                          .-------------------------------- 5mdhA @2.4     55.3750
                                          |                                 |
                                          |                                 .--- 1civA @2.8  13.0000
                                          |                                 |
                                      .-------------------------------------- 7mdhA @2.4     83.2500
                                      |
                                  .------------------------------------------ 1smkA @2.5

                              +----+----+----+----+----+----+----+----+----+----+----+----+
                                                                                    10.1900
                                                                                    .125
```

High sequence similarity

But 7mdh has better resolution

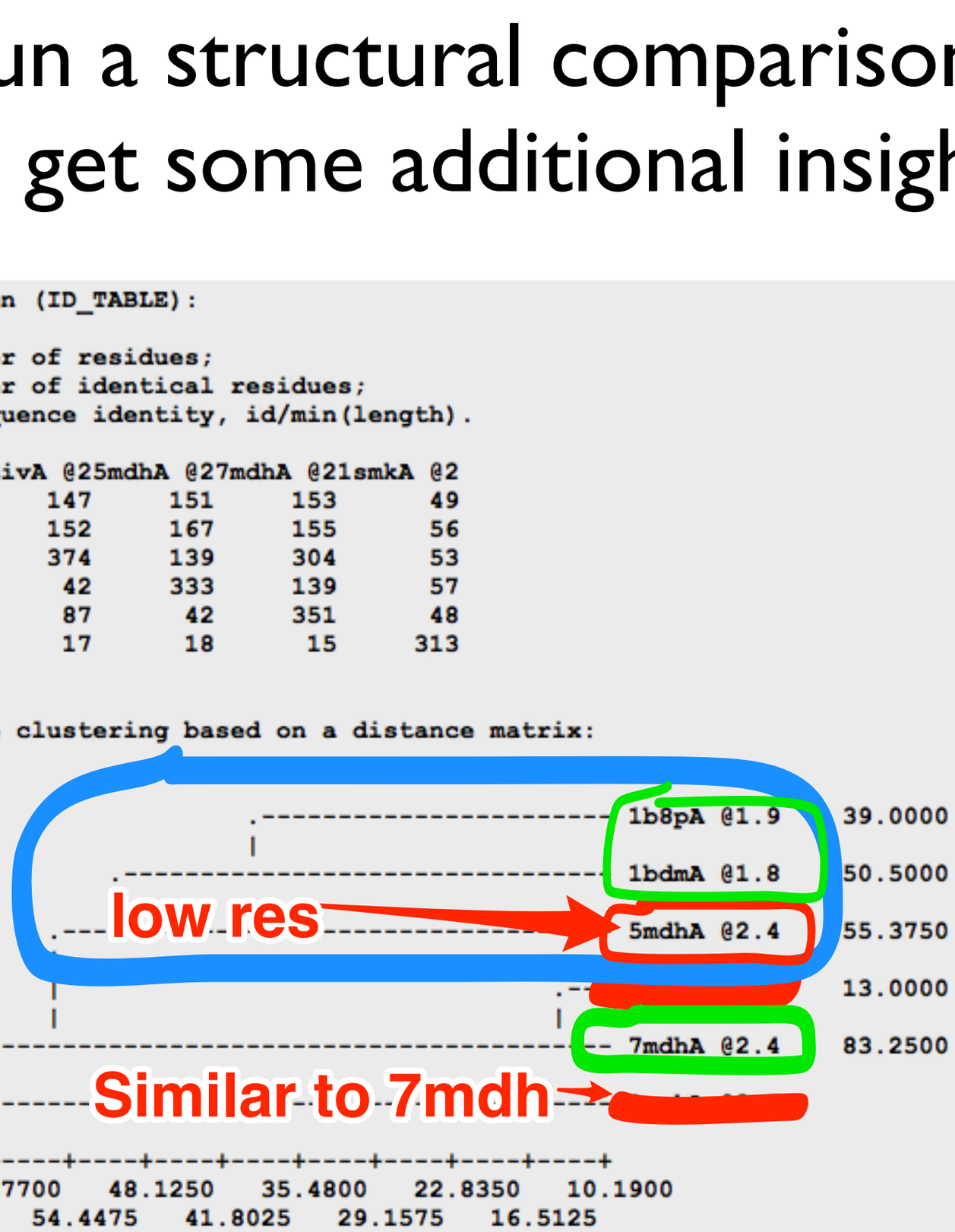
We then run a structural comparison on these to get some additional insight

```
Sequence identity comparison (ID_TABLE):

   Diagonal       ... number of residues;
   Upper triangle ... number of identical residues;
   Lower triangle ... % sequence identity, id/min(length).

         1b8pA @11bdmA @11civA @25mdhA @27mdhA @21smkA @2
1b8pA @1      327     194     147     151     153      49
1bdmA @1       61     318     152     167     155      56
1civA @2       45      48     374     139     304      53
5mdhA @2       46      53      42     333     139      57
7mdhA @2       47      49      87      42     351      48
1smkA @2       16      18      17      18      15     313

Weighted pair-group average clustering based on a distance matrix:
```



For high sequence identity models to work, there are two key ingredients

- Good choice of related (template) structure(s)
- Good sequence alignment

```
AAB24882        TYHMCQFHCRYVNNHSGEKLYECNERSKAFSCPSHLQCHKRRQIGEKTHEHNQCGKAFPT 60
AAB24881        --------------------YECNQCGKAFAQHSSLKCHYRTHIGEKPYECNQCGKAFSK 40
                                     ****: .***:  * *:** * :****.:* *******..

AAB24882        PSHLQYHERTHTGEKPYECHQCGQAFKKCSLLQRHKRTHTGEKPYE-CNQCGKAFAQ- 116
AAB24881        HSHLQCHKRTHTGEKPYECNQCGKAFSQHGLLQRHKRTHTGEKPYMNVINMVKPLHNS 98
                 **** *:***********:***:**.: .*****************    :  *.: :
```

Graphic from Wikipedia

For high sequence identity models to work, there are two key ingredients

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AAB24882        TYHMCQFHCRYVNNHSGEKLYECNERSKAFSCPSHLQCHKRRQIGEKTHEHNQCGKAFPT 60
AAB24881        --------------------YECNQCGKAFAQHSSLKCHYRTHIGEKPYECNQCGKAFSK 40
                                     ****: .***:  * *:** * :****.:* *******..

AAB24882        PSHLQYHERTHTGEKPYECHQCGQAFKKCSLLQRHKRTHTGEKPYE-CNQCGKAFAQ- 116
AAB24881        HSHLQCHKRTHTGEKPYECNQCGKAFSQHGLLQRHKRTHTGEKPYMNVINMVKPLHNS 98
                 **** *:***********:***:**.: .***************     :  *.: :
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Graphic from Wikipedia

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                                    ****: .***:  * *:** * :****.:* *******..

AAB24882        PSHLQYHERTHTGEKPYECHQCGQAFKKCSLLQRHKRTHTGEKPYE-CNQCGKAFAQ- 116
AAB24881        HSHLQCHKRTHTGEKPYECNQCGKAFSQHGLLQRHKRTHTGEKPYMNVINMVKPLHNS 98
                 **** *:***********:***:**.: .*****************    :  *.: :
```

Graphic from Wikipedia

Next, we do a sequence alignment in Modeller

```
 _aln.pos          10         20         30         40         50         60
1bdmA      MKAPVRVAVTGAAGQIGYSLLFRIAAGEMLGKDQPVILQLLEIPQAMKALEGVVMELEDCAFPLLAGL
TvLDH      MSEAAHVLITGAAGQIGYILSHWIASGELYG-DRQVYLHLLDIPPAMNRLTALTMELEDCAFPHLAGF
 _consrvd  *     *  ********* *   ** **  * *  * * ** ** **  *    ********* ***

 _aln.p    70         80         90         100        110        120        130
1bdmA      EATDDPDVAFKDADYALLVGAAPRL---------QVNGKIFTEQGRALAEVAKKDVKVLVVGNPANTN
TvLDH      VATTDPKAAFKDIDCAFLVASMPLKPGQVRADLISSNSVIFKNTGEYLSKWAKPSVKVLVIGNPDNTN
 _consrvd   ** **  **** * * **   *                 *  **   *  *   **  ***** *** ***

 _aln.pos  140        150        160        170        180        190        200
1bdmA      ALIAYKNAPGLNPRNFTAMTRLDHNRAKAQLAKKTGTGVDRIRRMTVWGNHSSIMFPDLFHAEVD---
TvLDH      CEIAMLHAKNLKPENFSSLSMLDQNRAYYEVASKLGVDVKDVHDIIVWGNHGESMVADLTQATFTKEG
 _consrvd    **   *  * * **     ** ***    * * *  *       *****   *  **  *

 _aln.pos    210        220        230        240        250        260        270
1bdmA      -GRPALELVDMEWYEKVFIPTVAQRGAAIIQARGASSAASAANAAIEHIRDWALGTPEGDWVSMAVPS
TvLDH      KTQKVVDVLDHDYVFDTFFKKIGHRAWDILEHRGFTSAASPTKAAIQHMKAWLFGTAPGEVLSMGIPV
 _consrvd          *       *      *   *   **  ****   *** *   *  **  *   **  *

 _aln.pos      280        290        300        310        320        330
1bdmA      Q--GEYGIPEGIVYSFPVTAK-DGAYRVVEGLEINEFARKRMEITAQELLDEMEQVKAL--GLI
TvLDH      PEGNPYGIKPGVVFSFPCNVDKEGKIHVVEGFKVNDWLREKLDFTEKDLFHEKEIALNHLAQGG
 _consrvd     ***  * * ***      *   ****   *   *     *   *  * *
```

The next step is building a model -- or really, multiple models

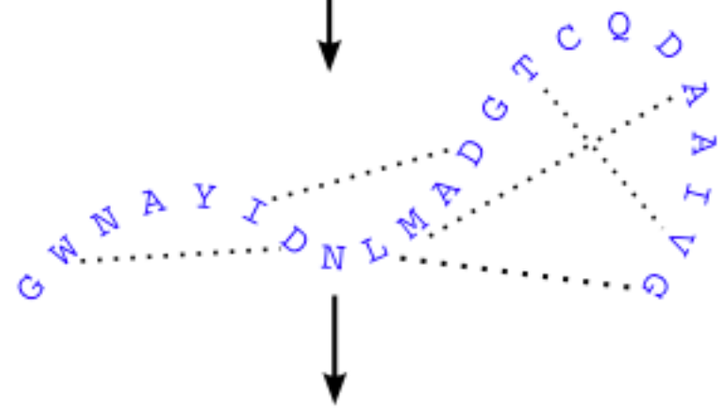
- Model building involves something like:
  - Fit the model backbone trace along the template
  - Build in the sidechains
  - Refine model based on energies
  - Optimize sidechains

Model building works on identifying and optimizing subject to spatial restraints and energy terms

1. Align sequence with structures

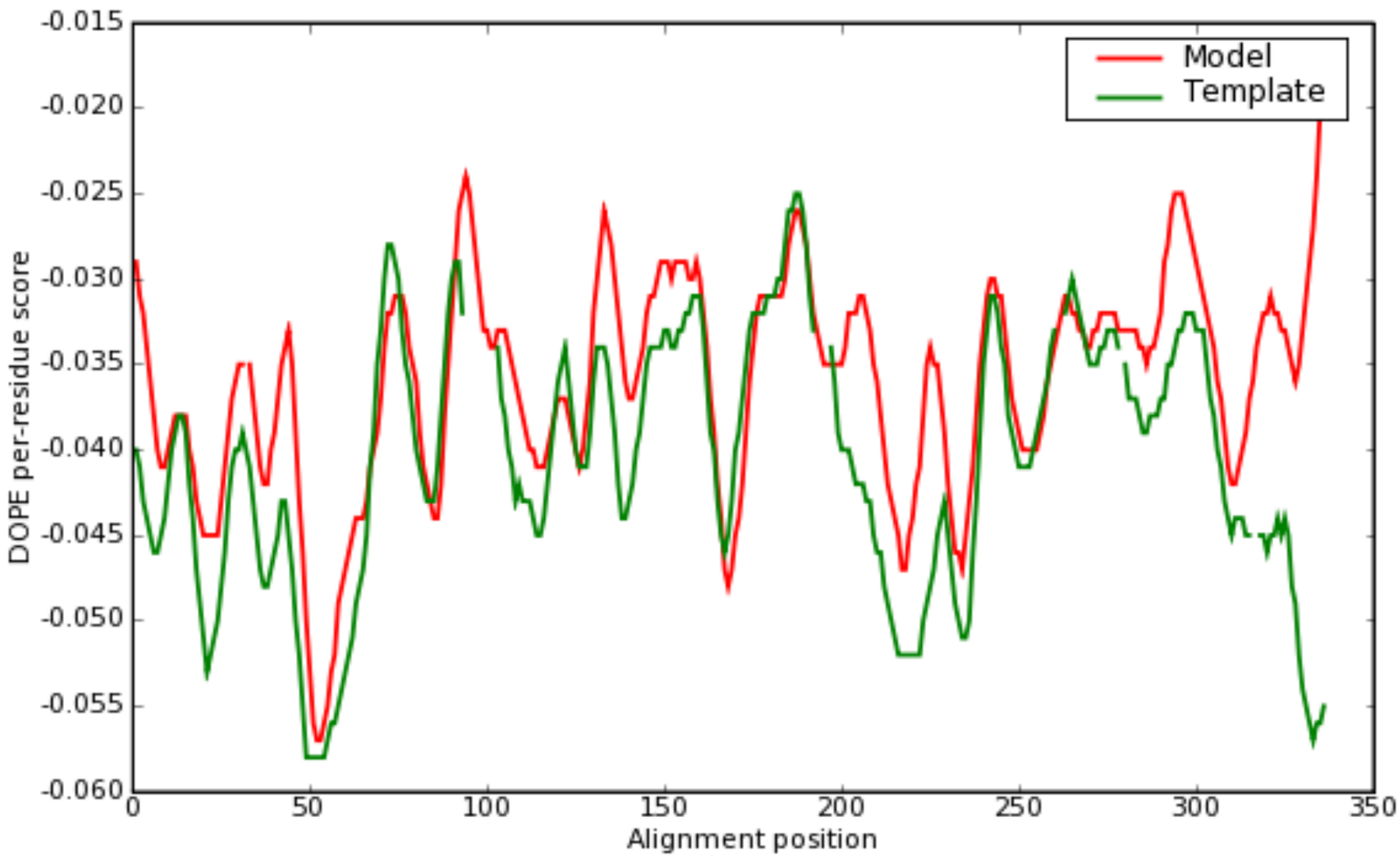
Template structure(s) SWQTYVDTNLVGTGAVTQA--AI
Target sequence -GWNAYIDNLMADGTCQDAAIVG

2. Extract spatial restraints

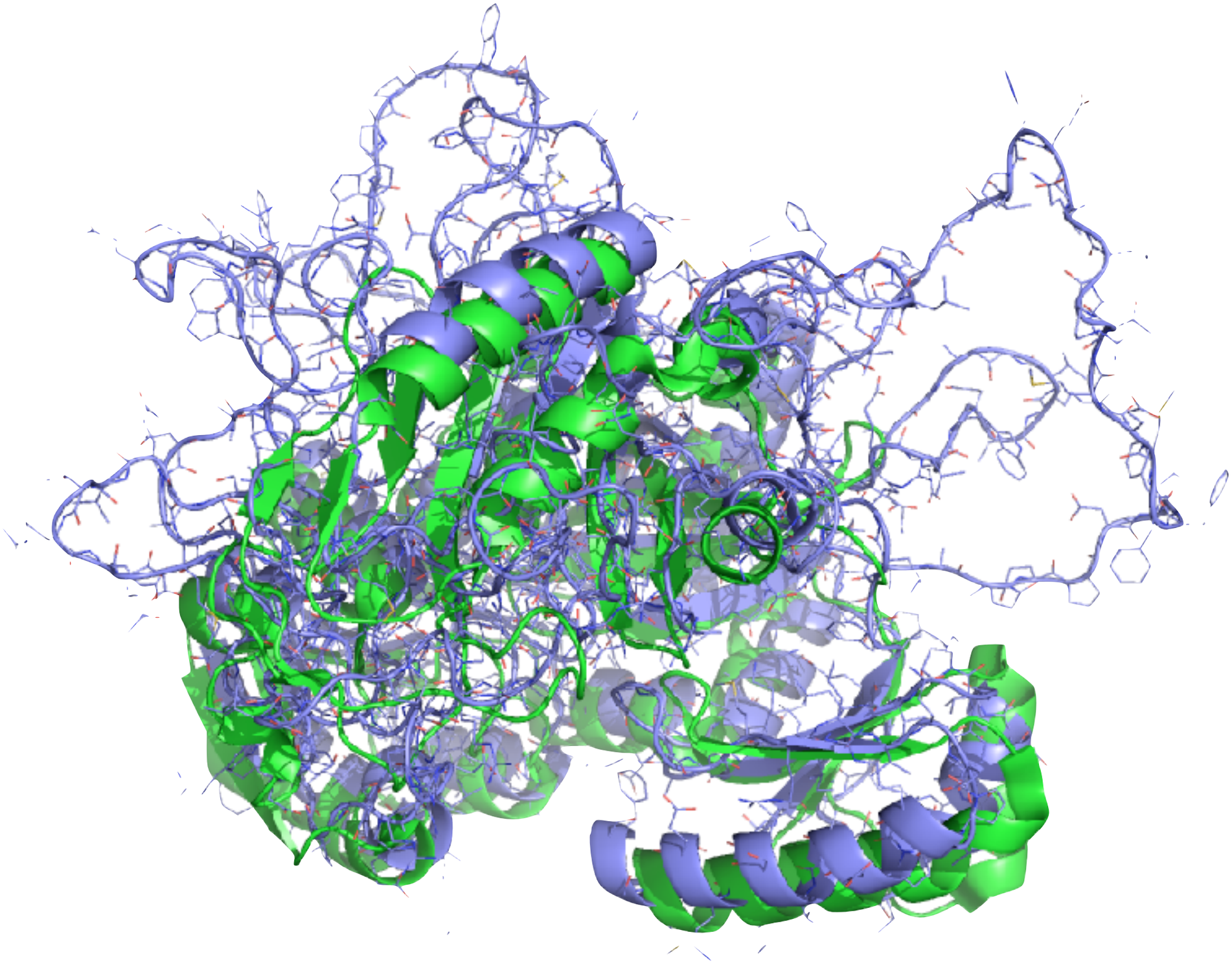


3. Satisfy spatial restraints

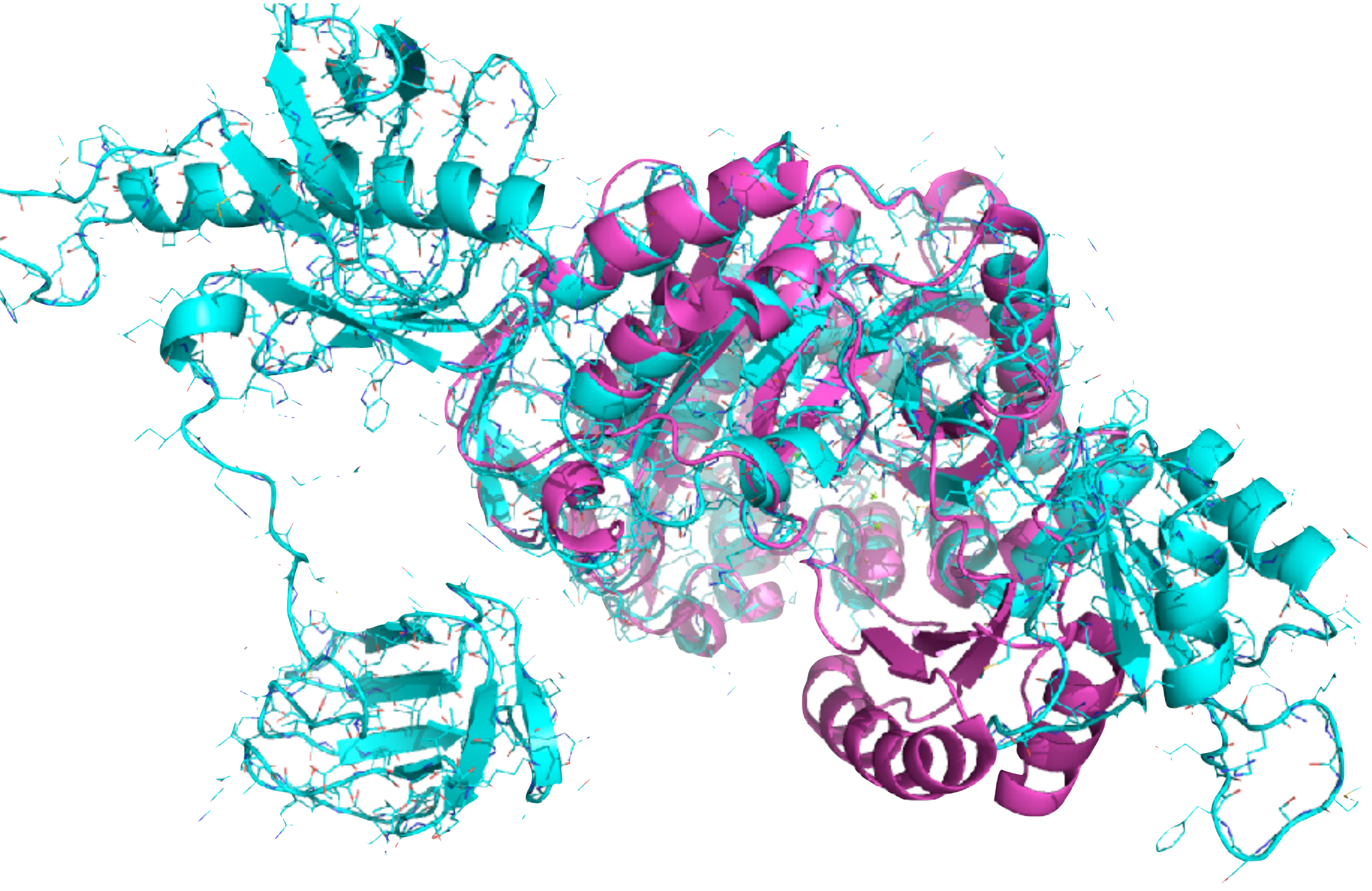
We evaluate the model as a final step with an energy score along the sequence



Looking at the actual model and its overlay onto the template is always helpful



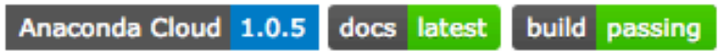
In this case, fixing the alignment solves the problem



More advanced homology modeling can involve additional elements

- Using multiple template structures
  - i.e. one template gives info about one part of the structure, another part about another
- Building in constraints based on experimental data and/or binding partners
- Model refinement, such as with simulations

Ensembler



Software pipeline for automating omics-scale protein modeling and simulation setup.

Online documentation

- Go to the official online documentation.
- Read a preprint of the paper on bioRxiv.
- See the example dataset from modeling all human tyrosine kinases.

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Overview of pipeline

1. Retrieve protein target sequences and template structures.
2. Build models by mapping each target sequence onto every available template structure, using Modeller.
3. Filter out non-unique models (based on a RMSD cutoff).
4. Refine models with implicit solvent molecular dynamics simulation.
5. Refine models with explicit solvent molecular dynamics simulation.
6. (*optional*) Package and/or compress the final models, ready for transfer or for set-up on other platforms such as Folding@Home.

Python can interface easily with the PDB itself: Example, solid state NMR structures

```
import urllib.request
url = 'http://www.rcsb.org/pdb/rest/search'
queryText = """<?xml version="1.0" encoding="UTF-8"?>
<orgPdbQuery>
<version>B0907</version>
<queryType>org.pdb.query.simple.ExpTypeQuery</queryType>
<description>Experimental Method Search : Experimental Method=SOLID-
STATE NMR</description>
<mvStructure.expMethod.value>SOLID-STATE NMR</
mvStructure.expMethod.value>
</orgPdbQuery>"""
print("query:\n", queryText)
print("querying PDB…\n")
req = urllib.request.Request(url, data=queryText.encode())
f = urllib.request.urlopen(req)
result = f.read().decode()

if result:
    print("Found number of PDB entries:", result.count('\n'))
else: print("Failed to retrieve results")
```

Running this produces some simple output; we can also get the referenced structures

```
pdbcodes = result.split()
print(pdbcodes)

url = 'http://www.rcsb.org/pdb/files/%s.pdb' % pdbcodes[0]
file = urllib.request.urlopen(url)
pdbcontents = file.readlines()

['1CEK', '1EQ8', '1M8M', '1MAG', '1MP6', '1MZT', '1NH4', '1NYJ', '1PI7',
'1PI8', '1PJD', '1PJE', '1PJF', '1Q7O', '1RVS', '1XSW', '1ZN5', '1ZY6',
'2C0X', '2CZP', '2E8D', '2H3O', '2H95', '2JSV', '2JU6', '2JZZ', '2K0P',
'2KAD', '2KB7', '2KHT', '2KIB', '2KJ3', '2KLR', '2KQ4', '2KQT', '2KRJ',
'2KSJ', '2KWD', '2KYV', '2L0J', '2L3Z', '2LBU', '2LEG', '2LGI', '2LJ2',
'2LME', '2LMN', '2LMO', '2LMP', '2LMQ', '2LNL', '2LNQ', '2LNY', '2LPZ',
'2LTQ', '2LU5', '2M02', '2M3B', '2M3G', '2M4J', '2M5K', '2M5M', '2M5N',
'2M67', '2MC7', '2MCU', '2MCV', '2MCW', '2MCX', '2MEX', '2MJZ', '2MME',
'2MMU', '2MPX', '2MPZ', '2MS7', '2MSG', '2MTZ', '2MVX', '2MXU', '2N0A',
'2N0R', '2N1E', '2N1F', '2N28', '2N3D', '2N70', '2N7H', '2NNT', '2RLZ',
```

Much more complicated queries of the PDB are straightforward

SEARCH service

This interface exposes the RCSB PDB **advanced search** interface as an XML Web Service.

To use this service, POST a XML representation of an advanced search to **/pdb/rest/search**.

XML representation of advanced search

Every **advanced search** can be represented by XML. To view an example representation, simply execute an advanced search query and then click on the **Result tab**. One of the links on the top of the page is **Query Details**. Clicking on this link will provide the XML representation of the query.

Every query is described by two data items:

- **queryType:** the name of the class that is implementing the query
- **arguments:** depending on the type of query that is being executed one or more differently named arguments need to be provided.

http://www.rcsb.org/pdb/software/rest.do#search

You can easily get example code to help you build your queries

Sample XML Queries:

Author Name

The Query is displayed in the Textbox below

```
<orgPdbQuery>
<queryType>org.pdb.query.simple.AdvancedAuthorQuery</queryType>
<description>Author Search: audit_author.name=Miller, J.S OR
(citation_author.name=Miller, J.S AND
citation_author.citation_id=primary)</description>
<searchType>0</searchType>
<audit_author.name>Miller, J.S</audit_author.name>
</orgPdbQuery>
```

http://www.rcsb.org/pdb/software/rest.do#search

You can even search for a specific sequence without leaving Python

```
<orgPdbQuery>
<queryType>org.pdb.query.simple.SequenceQuery</queryType>
<description>Sequence Search (Structure:Chain = 4HHB:A, Expectation
Value = 10.0, Search Tool = BLAST)</description>
<structureId>4HHB</structureId>
<chainId>A</chainId>
<sequence>VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHGKKV
ADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVST
VLTSKYR</sequence>
<eCutOff>10.0</eCutOff>
<searchTool>blast</searchTool>
</orgPdbQuery>
```

http://www.rcsb.org/pdb/software/rest.do#search

So, finding structures in the PDB is doable, but what if we want to go deeper and DO something with them?

- BioPython (conda install biopython) provides lots of functionality, including some for working with the PDB:

```
from Bio.PDB.PDBParser import PDBParser
from Bio.PDB import PDBList
p = PDBParser(PERMISSIVE = 1) #Ignore many common problems
pdbl = PDBList()
structure_id = '1HXW'
filename = pdbl.retrieve_pdb_file(structure_id, file_format='pdb')
structure = p.get_structure(structure_id, filename)

firstmodel = structure[0]
chain_A = firstmodel['A']
res10 = chain_A[10]
print(res10.get_resname())
```

BioPython has lots of useful functionality for working with the contents of PDBs

```
a.get_name()         # atom name (spaces stripped, e.g. "CA")
a.get_id()           # id (equals atom name)
a.get_coord()        # atomic coordinates
a.get_bfactor()      # B factor
a.get_occupancy()    # occupancy
a.get_altloc()       # alternative location specifie
a.get_sigatm()       # std. dev. of atomic parameters
a.get_siguij()       # std. dev. of anisotropic B factor
a.get_anisou()       # anisotropic B factor
a.get_fullname()     # atom name (with spaces, e.g. ".CA.")
```

For example we can print the coordinates of all CA atoms with B factor greater than 10

```
for model in structure.get_list():
  for chain in model.get_list():
    for residue in chain.get_list():
      if residue.has_id("CA"):
        ca=residue["CA"]
        if ca.get_bfactor()>10.0:
          print(ca.get_coord())
```

Lots of other stuff is also available in BioPython

- Handles common file formats and can extract contents into Python
  - Interfacing with databases, searching, handling formats: BLAST, FASTA, GenBank, PubMed, Medline, SCOP, ...
  - Easy handling of sequences -- comparisons, translation, complementation
  - Working with sequence alignments
  - Clustering/data classification tools
  - Lots of other stuff…
  - BUT if you want to mutate residues in a structure, you need a MODELING tool, e.g. Modeller, Rosetta, etc.

http://biopython.org/DIST/docs/tutorial/Tutorial.pdf

MDTraj

Read, write and analyze MD trajectories with only a few lines of Python code.

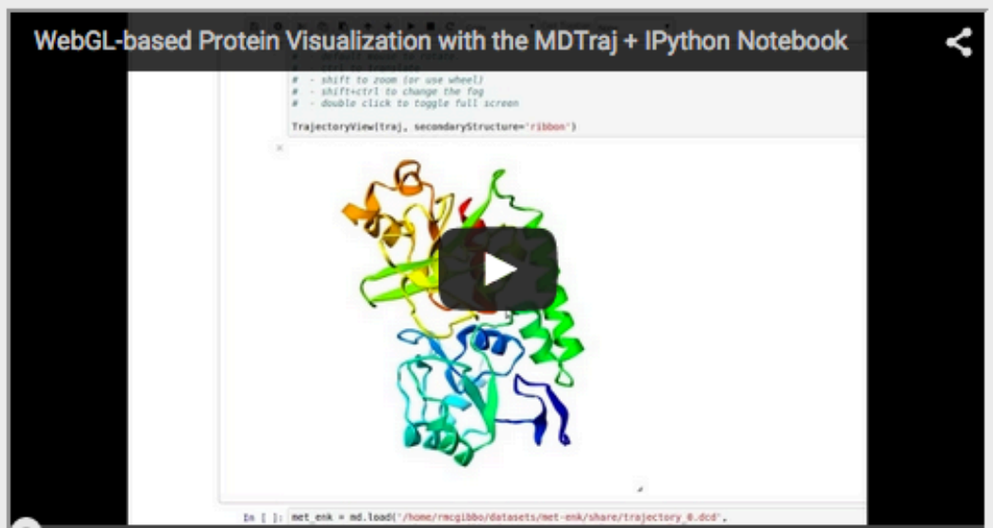
MDTraj is a python library that allows users to manipulate molecular dynamics (MD) trajectories. Extensive *trajectory analysis* routines are implemented. With MDTraj, you can

- Read and write from **every MD format imaginable** (`pdb` , `xtc` , `trr` , `dcd` , `binpos` , `netcdf` , `mdcrd` , `prmtop` , ...)
- Run **blazingly fast** RMSD calculations (4x the speed of the original Theobald QCP).
- Use tons of *analysis* functions like bonds/angles/dihedrals, hydrogen bonding identification, secondary structure assignment, NMR observables.
- **Lightweight API**, with a focus on **speed** and vectorized operations.

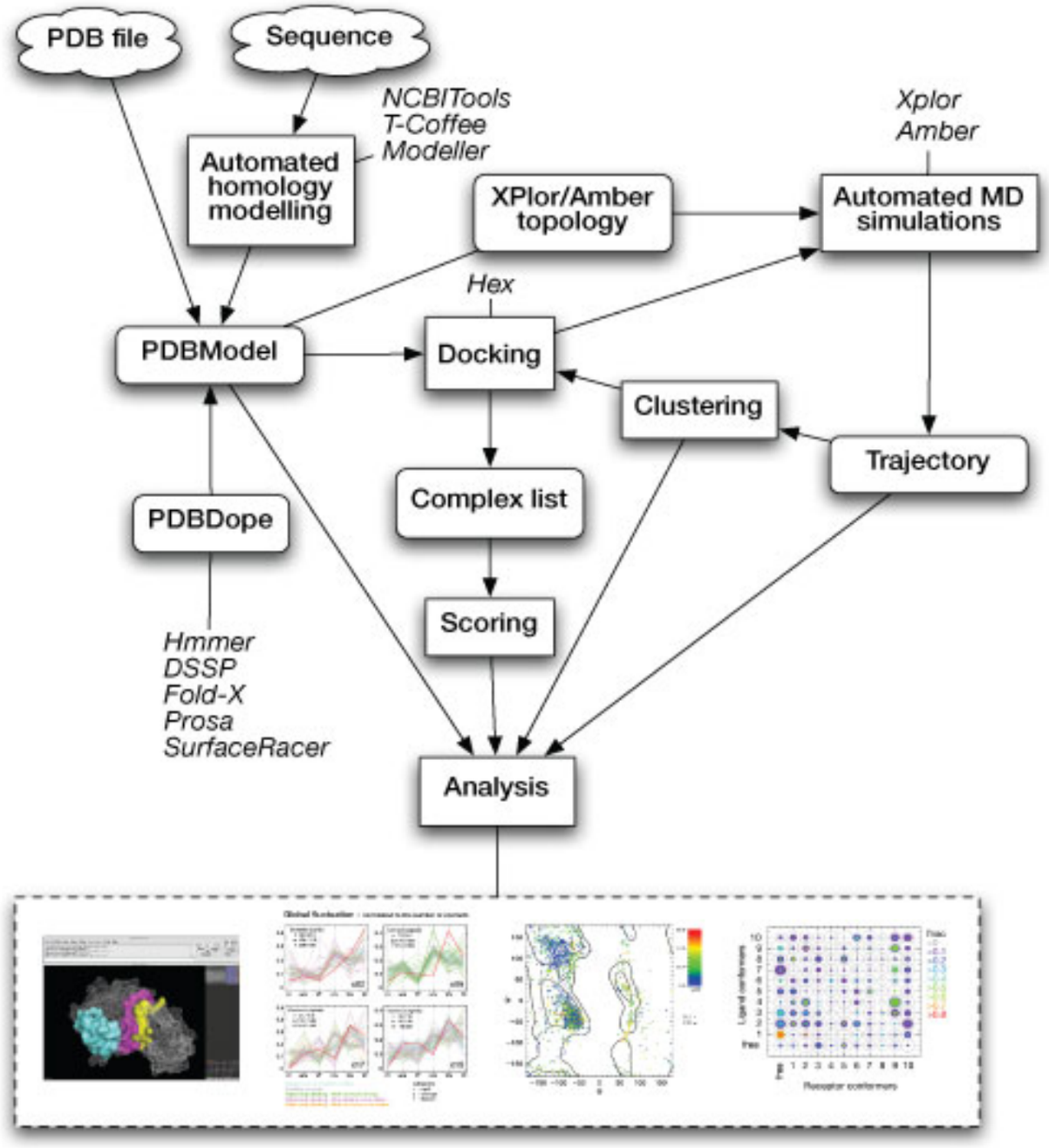
The library also ships with a flexible command-line application for converting trajectories between formats. When you install MDTraj, the script will be installed under the name `mdconvert` .

Download the Code **See it in Action**

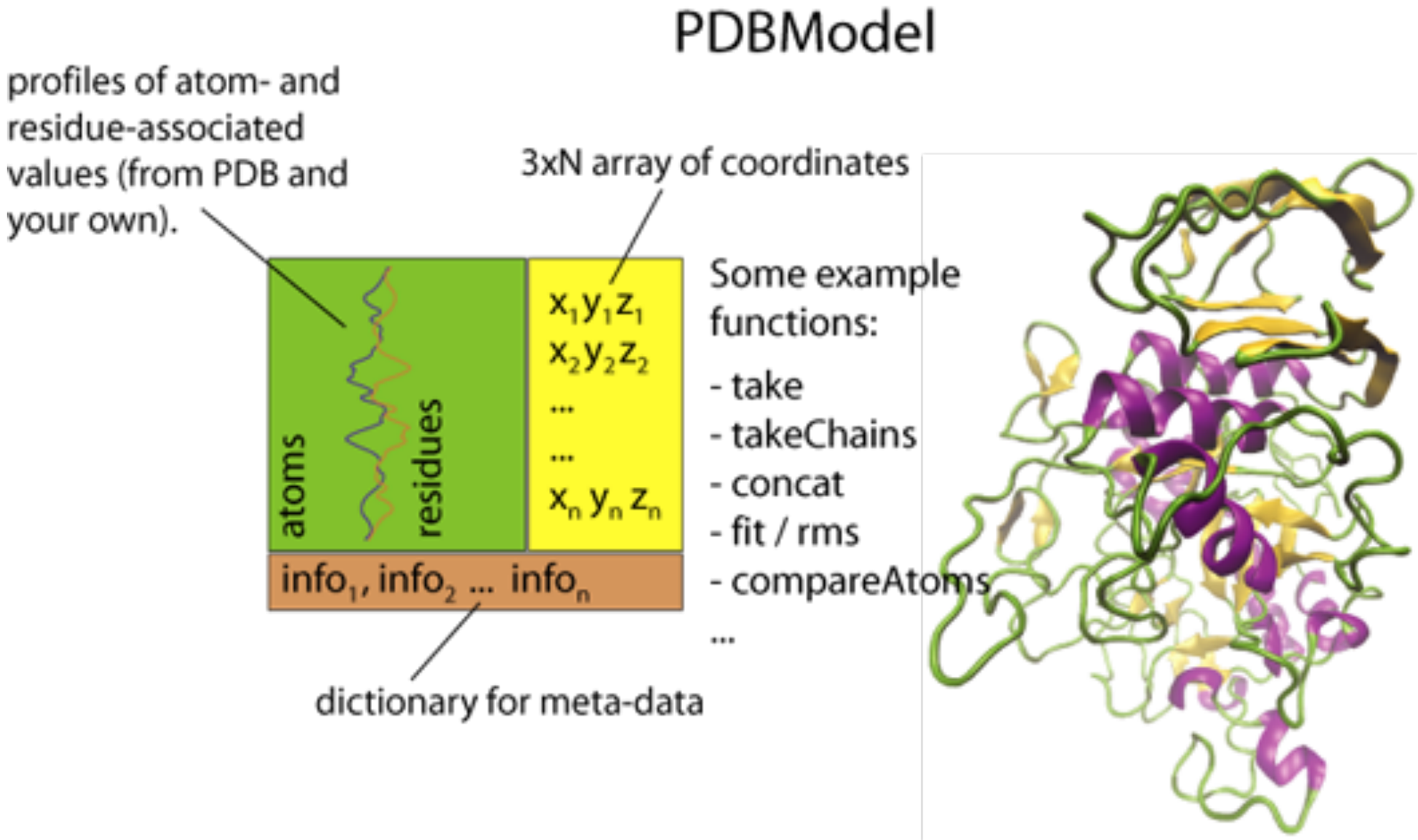
Get Help **Get Involved**



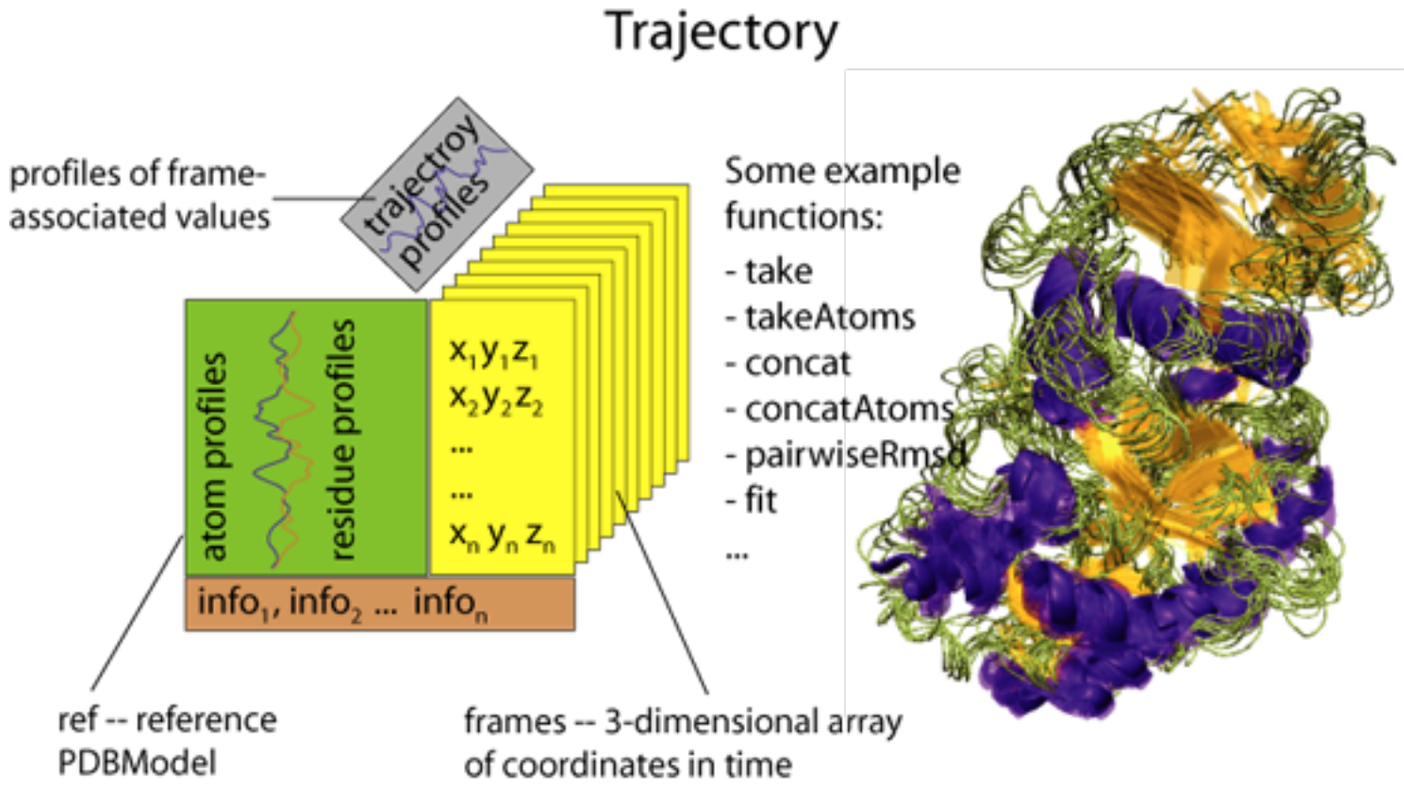
Biskit is a Python library and toolchain with many representations and tools



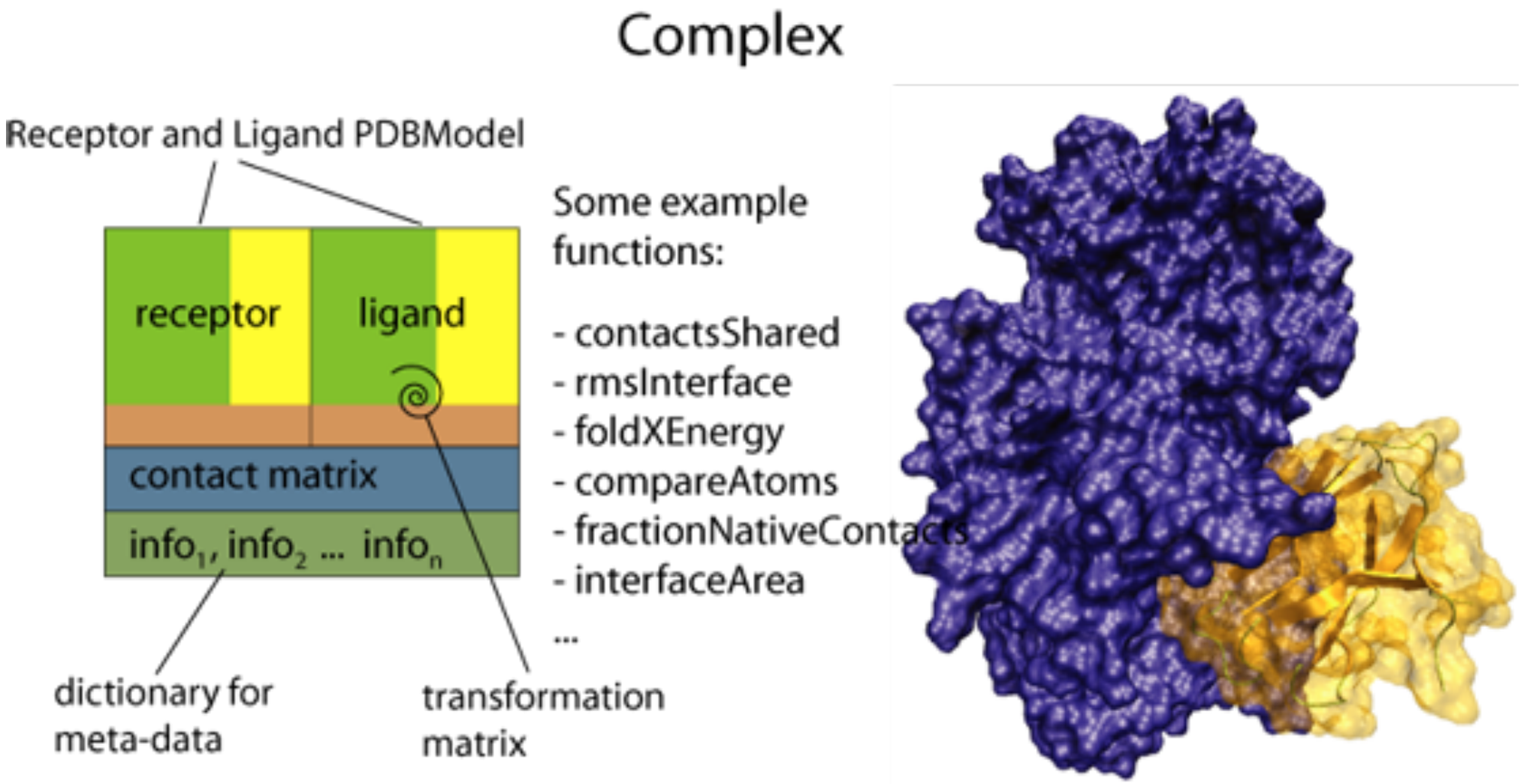
Biskit has an internal representation of PDBs



Biskit also has tools for representing and working with trajectories



Biskit also can handle relative positioning of PDBModels, i.e. for rigid body docking



An example task: Let's align two (related, not identical) proteins and compute their RMSD

```
import Biskit as B

m1 = B.PDBModel( "your/structure1.pdb" )
m2 = B.PDBModel( "your/related/structure.pdb" )

# align both models to the same residue content
# and atom order and content
i1, i2 = m1.compareAtoms( m2 )
m1 = m1.take( i1 ) # take atoms common with m2
m2 = m2.take( i2 ) # take atoms common with m1

## some checking for demonstration
assert len( m1 ) == len( m2 )
assert m1.sequence() == m2.sequence()

## get CA structures
ca1 = m1.compress( m1.maskCA() )
ca2 = m2.compress( m2.maskCA() )

## RMSD
rms = ca1.rms( ca2, fit=1 )
```

Let's calculate the fraction beta sheet content at a protein surface

```
import Biskit as B
import numpy as N

m = B.PDBModel('structure.pdb')

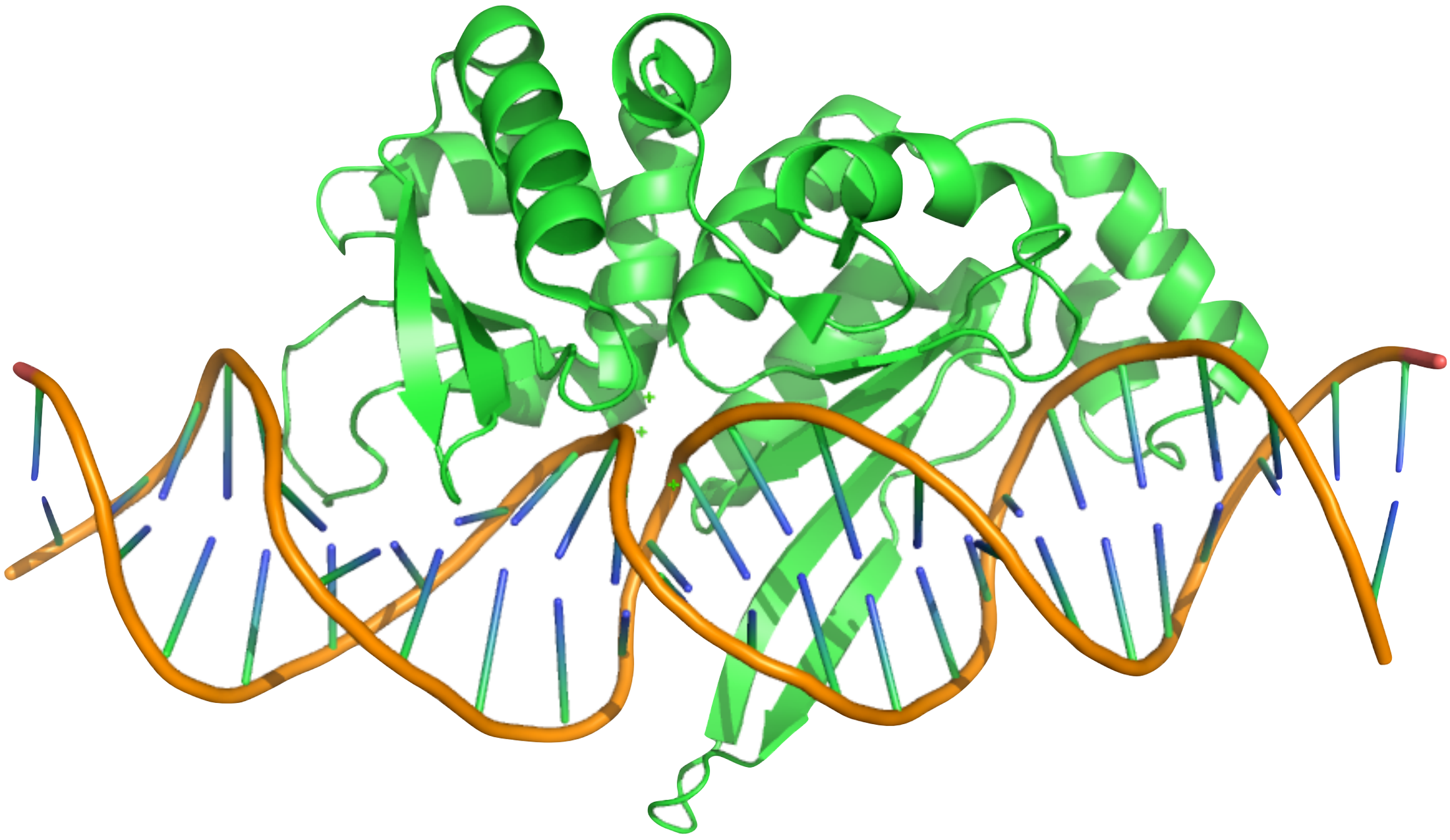
## add information calculated by external programs
d = B.PDBDope(m)
d.addSurfaceRacer()           # MS, AS, relMS, relAS and curvature
d.addSecondaryStructure()     # secondary structure from DSSP

## molecular surface of all atoms
ms_profile = m['MS']

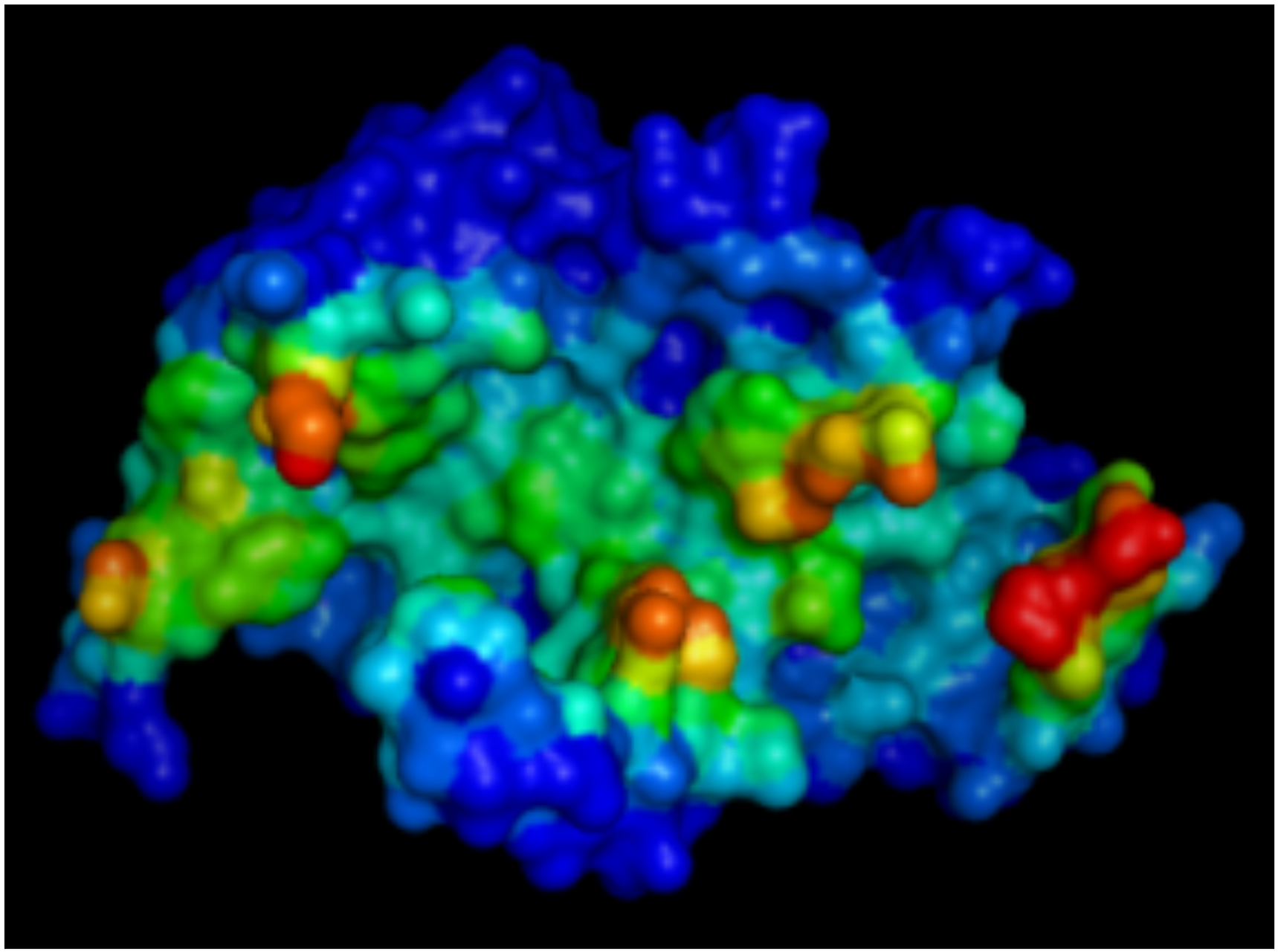
## mask for all atoms of the given secondary structure
ss_mask = N.array( m.res2atomProfile('secondary') ) == 'E'

## fraction of total surface
ss_fraction = N.sum(ms_profile * ss_mask) / N.sum(ms_profile}
```

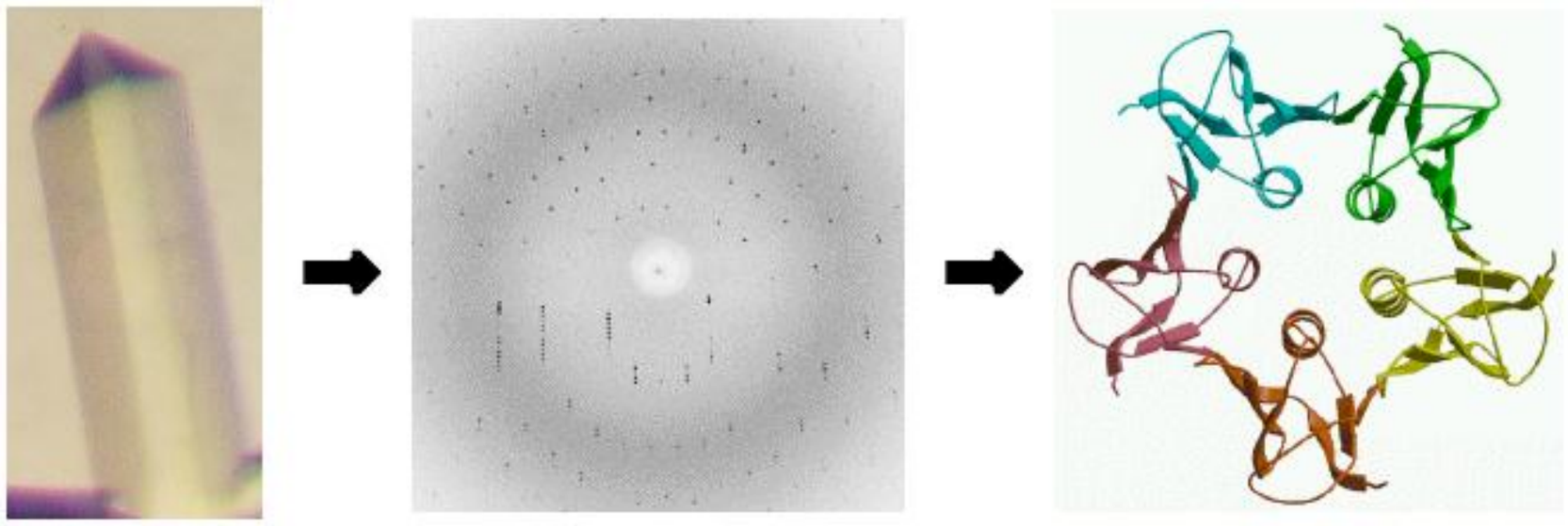
Let's take an example from the Biskit tutorial and look at a protein-DNA complex



Biskit can easily compute the number of protein-DNA contacts and this can be used to color-code in PyMol



Crystallography tries to turn diffraction data into structures



Really, there is not quite enough data -- each atom has about 3-4 parameters, and we have about one observation per parameter

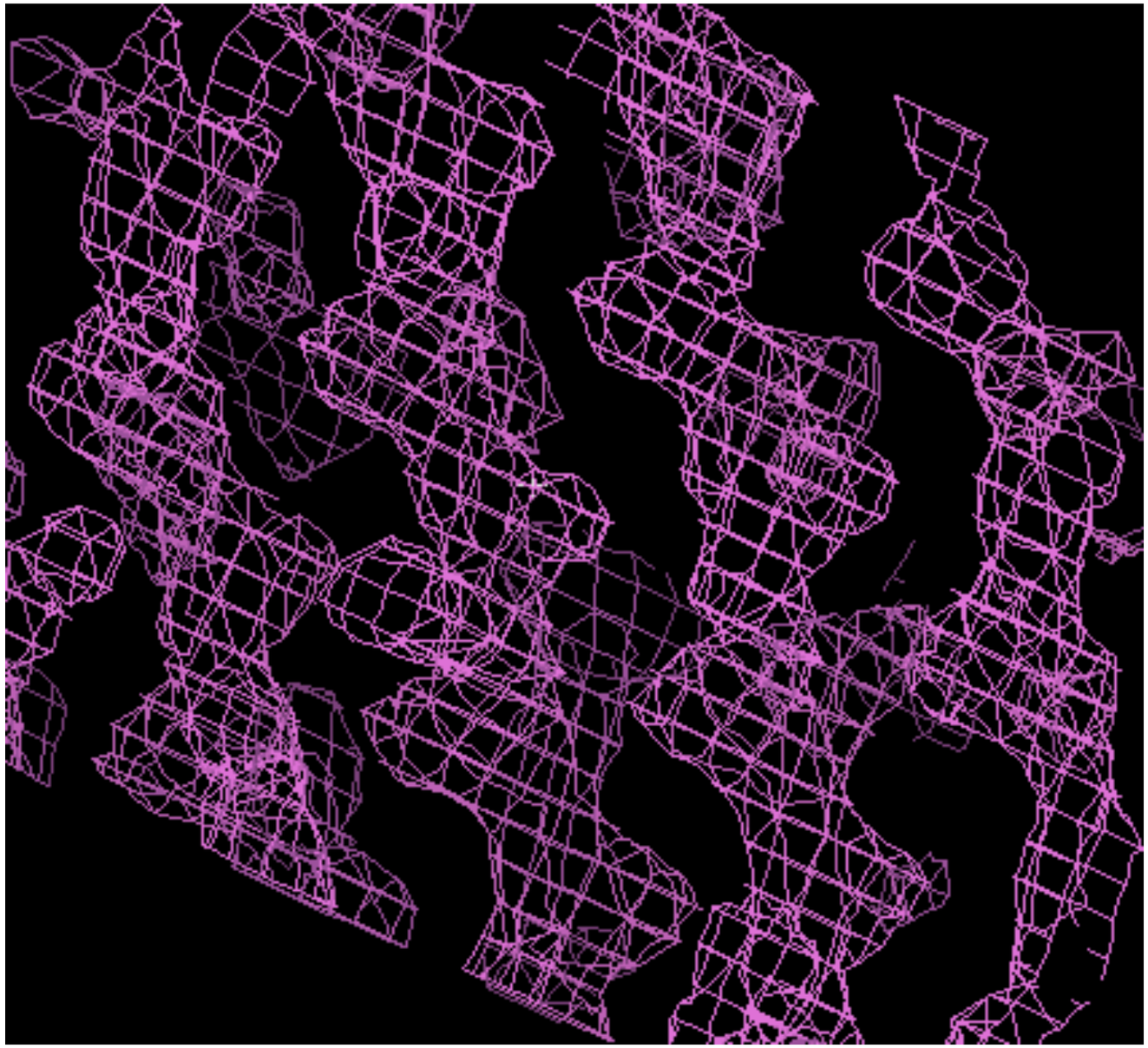
This means we fit a model to the data

http://www-structmed.cimr.cam.ac.uk/Course/Overview/Overview.html

The data actually collected is a diffraction pattern (or set of them) which gives electron scattering amplitudes

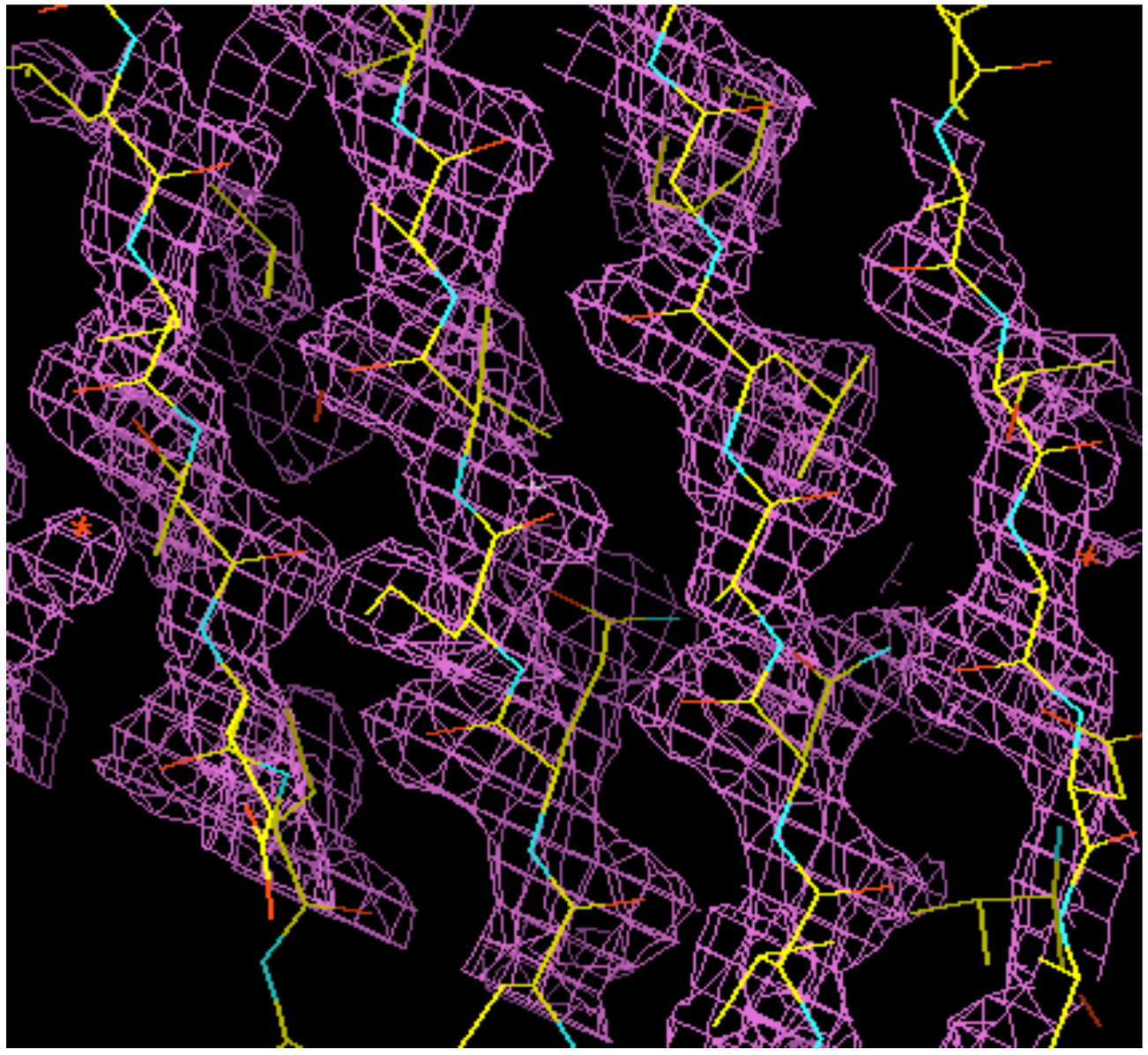
- The refinement process
  - Takes scattering amplitudes
  - Turns it into the electron density everywhere
  - Fits a model to the electron density
- There is a "phase problem" to get from the diffraction pattern to the electron density

But at some point, we have to fit a model to the "data" -- the electron density



It is nonobvious where the atoms should be -- and this is good data

The actual model IS consistent with this data, but the model is not the data



This makes it a lot more obvious what's here

This all results in a model

The model is not the data

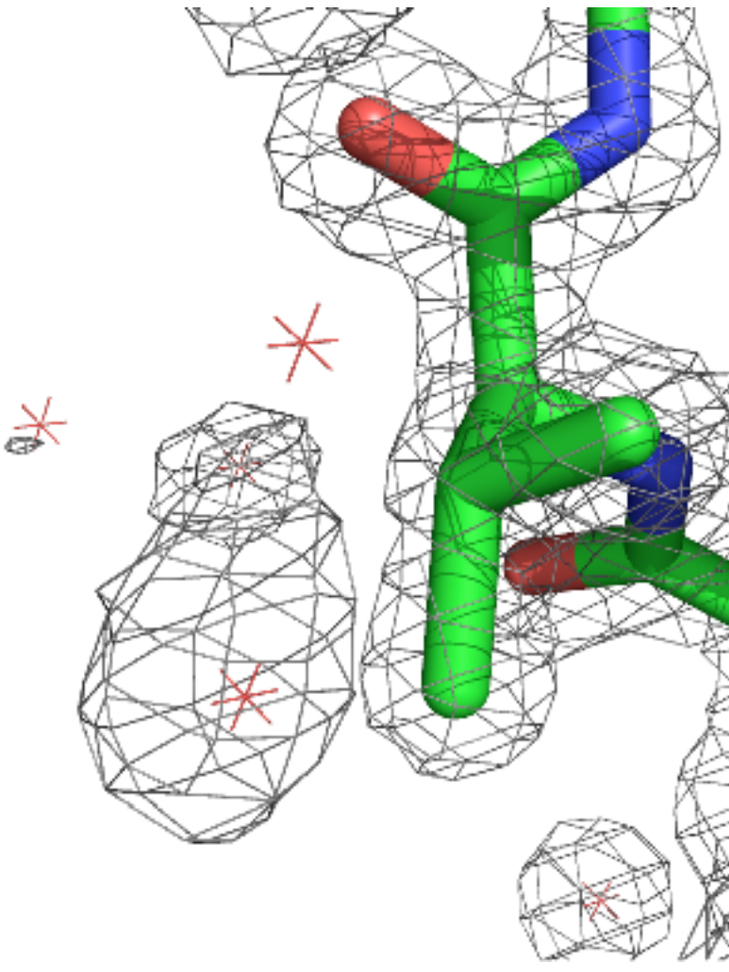
The electron density is (closer to) the data

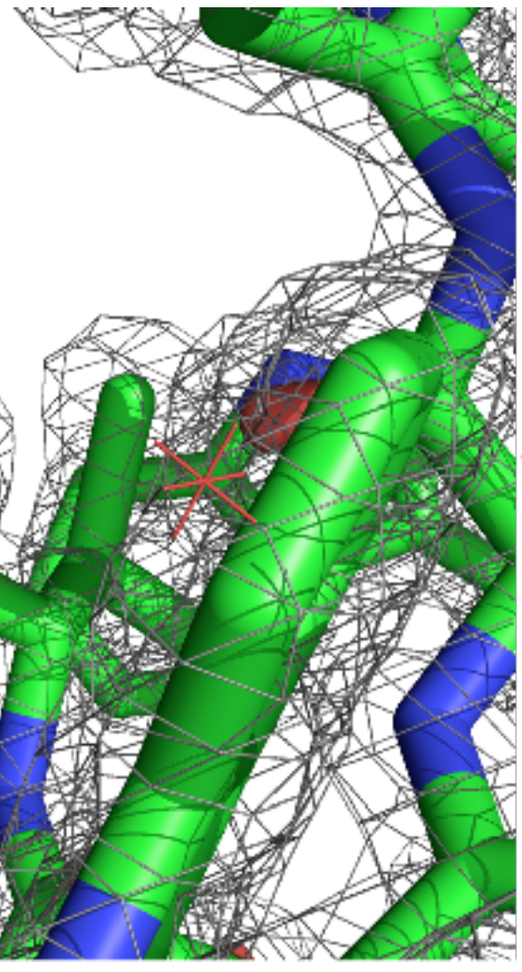
So, one should visualize the electron density to check the model

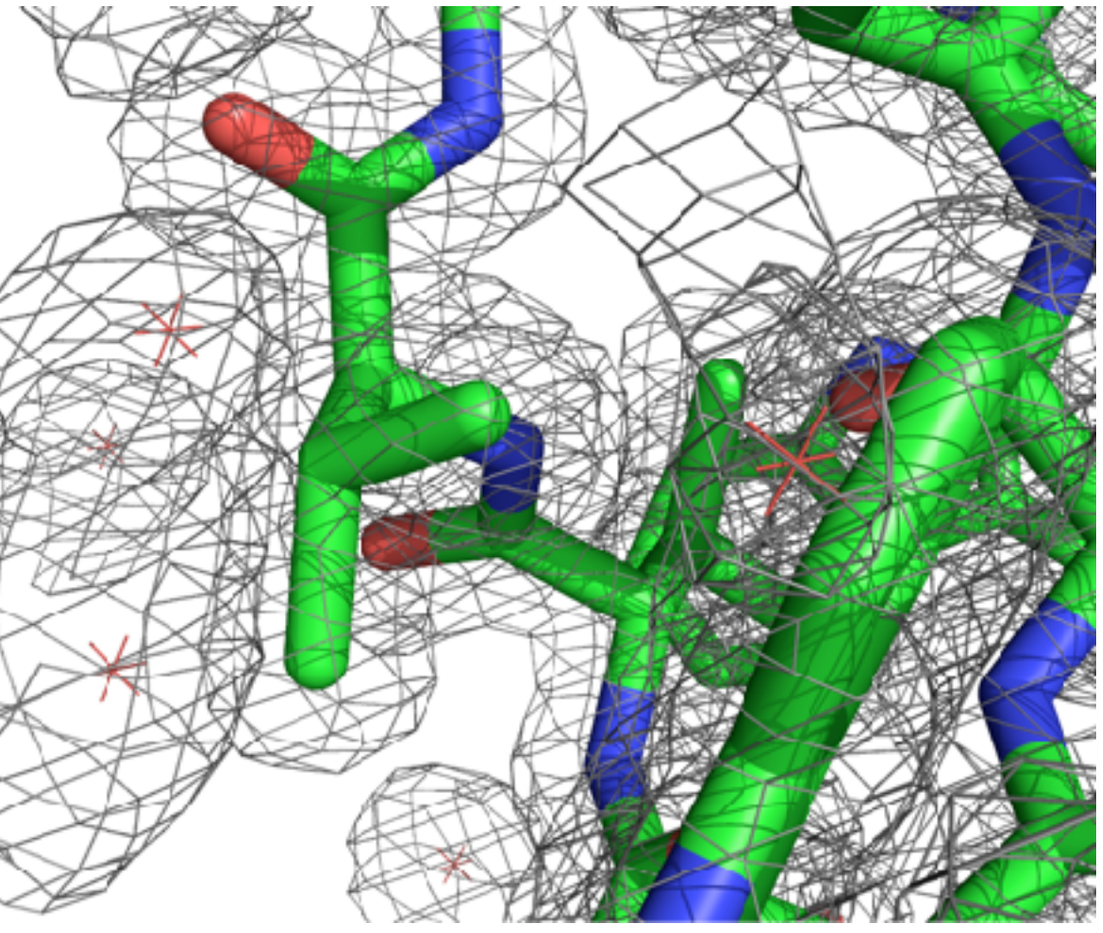
There are two common ways of visualizing the electron density

- "Difference map" $\mathbf{F}_o - \mathbf{F}_c$
  - Shows difference between calculated and observed structure factors
- The map itself, usually as $2\mathbf{F}_o - \mathbf{F}_c = 2\left|\mathbf{F}_o\right| - \left|\mathbf{F_c}\right|$
  - Shows the actual observations, with the difference from the calculated values also appearing to a limited extent

But, there is a value for the electron density everywhere, so we have to select contour levels

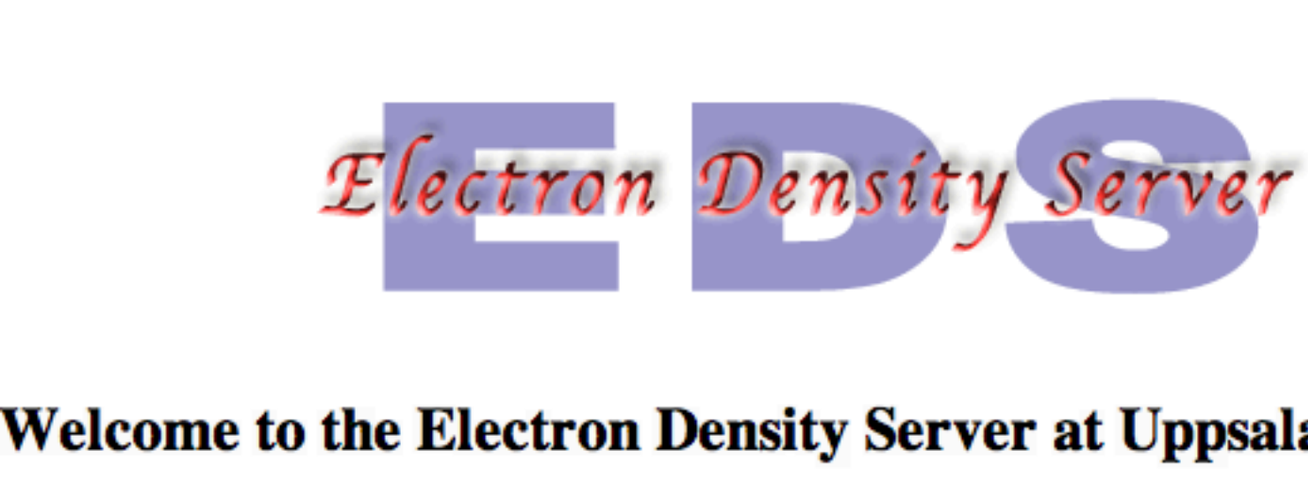






PDB files don't actually contain electron density, though modern ones contain calculated structure factors

- Calculated structure factors yield the electron density
- Usually, see the Electron Density Server to get electron densities



http://eds.bmc.uu.se/eds/

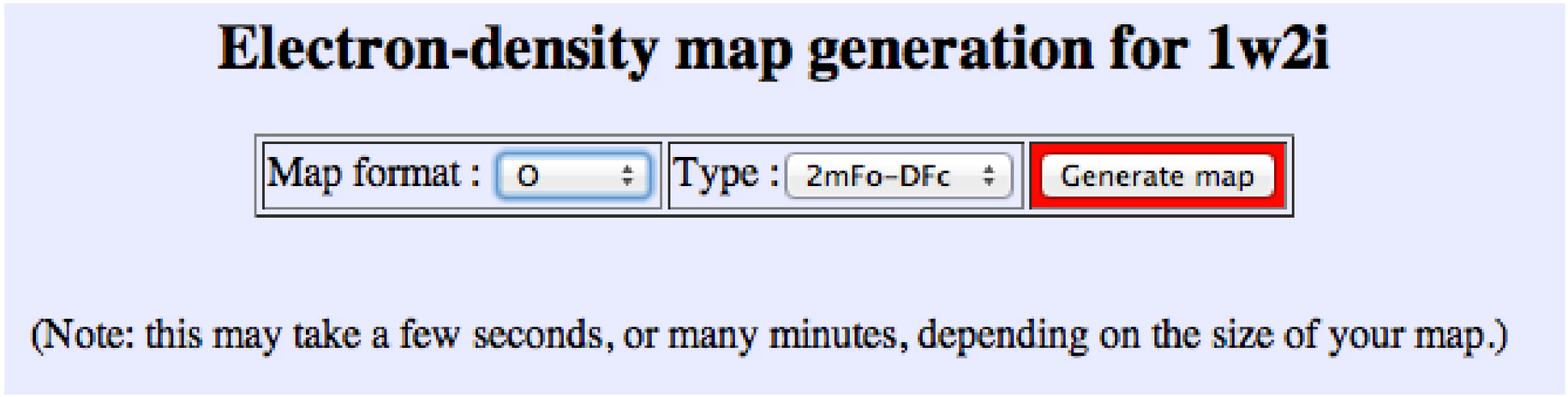
From EDS, you choose a type of map and a format

Electron-density map generation for 1w2i

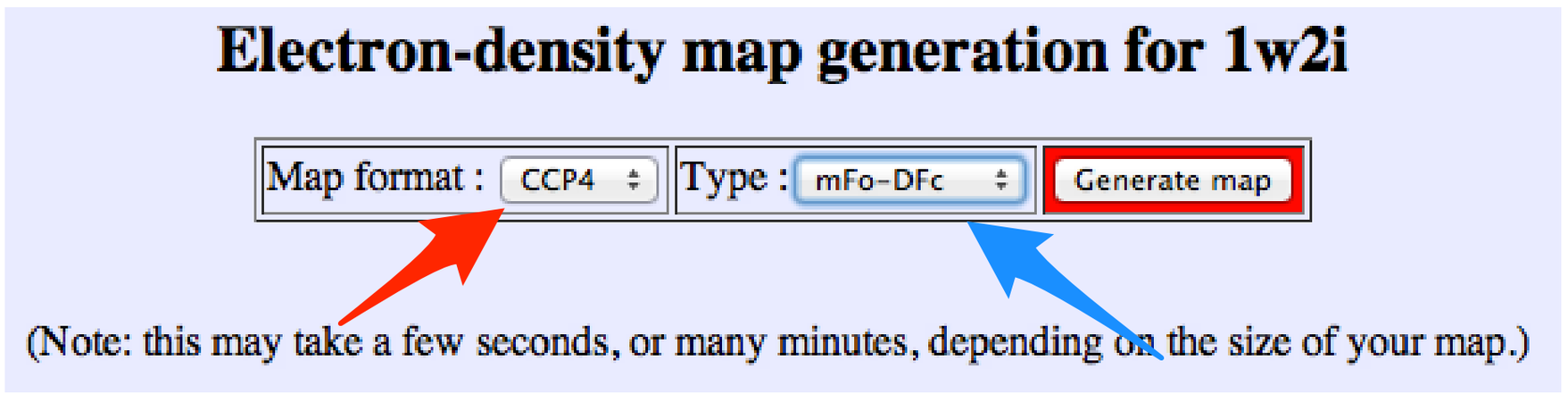
Map format : O | Type : 2mFo-DFc | Generate map

(Note: this may take a few seconds, or many minutes, depending on the size of your map.)

From EDS, you choose a type of map and a format



Let's do a difference map for visualization in PyMol



Next, I download the map to my computer, extract it, and get ready to load into PyMol

Here is your gzipped map : 1w2i_diff.ccp4.gz

- Check out this PyMol tutorial:
  - http://pymolwiki.org/index.php/Display_CCP4_Maps

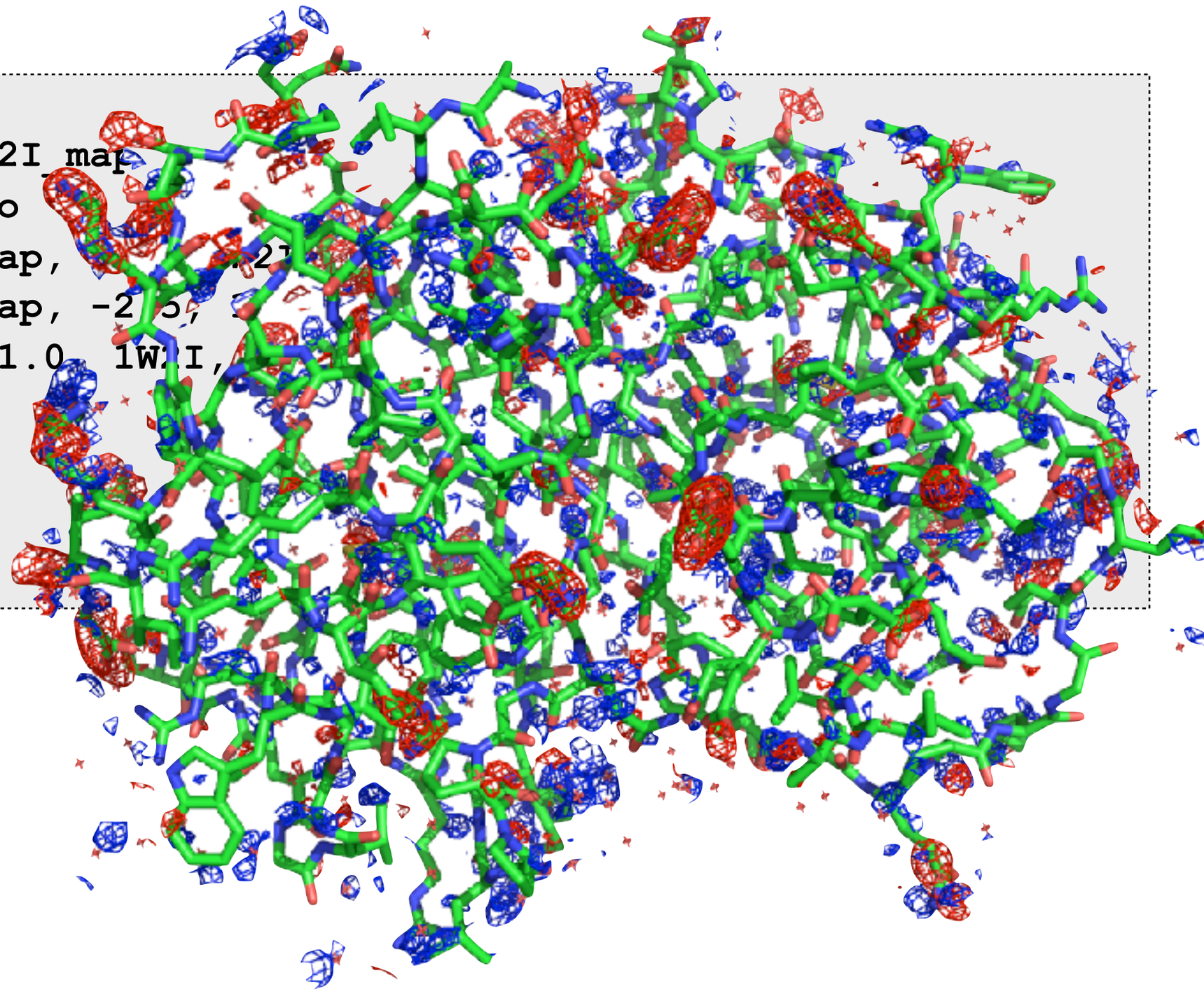
- Note: Difference maps usually contoured at +/- 2.5 sigma

In PyMol:

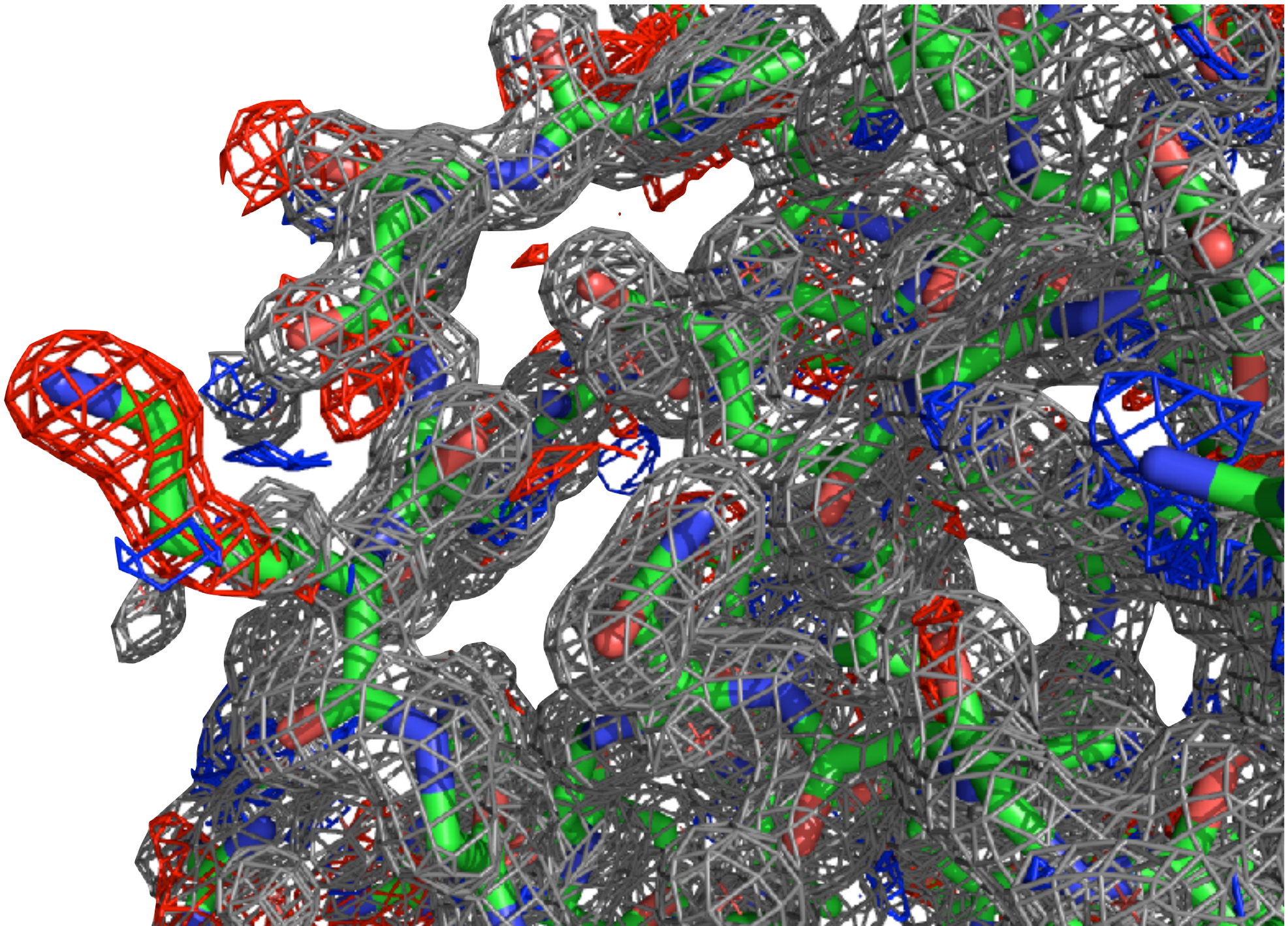
```
load 1W2I.pdb
load 1w2i_diff.ccp4, 1W2I_map
load 1w2i.ccp4, 1W2I_2fo
isomesh map_pos, 1W2I_map, 2.5, 1W2I, carve = 1.6
isomesh map_neg, 1W2I_map, -2.5, 1W2I, carve = 1.6
isomesh map, 1W2I_2fo, 1.0, 1W2I, carve = 1.6
color gray, map
color blue, map_pos
color red, map_neg
show sticks
```

In PyMol:

```
load 1W2I.pdb
load 1w2i_diff.ccp4, 1W2I_map
load 1w2i.ccp4, 1W2I_2fo
isomesh map_pos, 1W2I_map,
isomesh map_neg, 1W2I_map, -2
isomesh map, 1W2I_2fo, 1.0, 1W2I,
color gray, map
color blue, map_pos
color red, map_neg
show sticks
```



Let's focus on an area with large differences and look at the actual density there as well



In some sense, the structure is not the result of crystallography

- The data: The diffraction pattern
- The results: The electron density
- The author's interpretation: The structure

To sum up:

You want to look at the electron density to help assess the model

PyMol and other viewers can help you do so