The PDB and Molecular Visualization

Biochemistry Boot Camp 2019
Session #8
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Properties of PDB Files

- Experimental methodology:
  - X-Ray: Typically more precise
  - NMR: Need lots of "restraints;" sometimes hard to assess quality
- Most PDB files are solved using X-ray crystallography
  - 141,000 structures total
  - 126,000 are crystal structures

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Building an X-Ray Structure

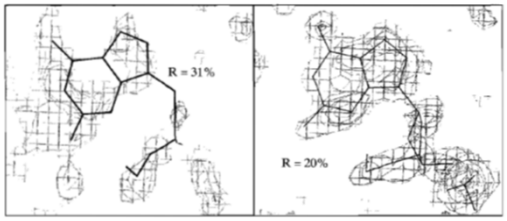

Figure 6.31 Effect of refinement on structure. The guanine nucleotide of a DNA fragment is shown with its electron density map prior to refinement and after refinement. Prior to refinement, the *R* factor is 31%. The structure is refined against the data to an *R* factor of 20%, which is one criterion of a good fit of the model to the data.

- **At first:** look for gross structural features (helix, backbone), then add side chains
- Molecular mechanics are used to help refine positions

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Guidelines for X-Ray Quality

- **R-factor:** Less than 25% (ideally, less than 20%)
- **R-Free:** Bigger than R, but smaller than 25%
- **Resolution:** Less than 2.5 Å, but think about how much you need (1.5 Å usually very good)
  - At ~1 Å hydrogens become visible
- **Validation:** No clashes, good torsions, etc.
- **Water**: 2-5 molecules / kDa

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Protein Data Bank Revisited

- http://rcsb.org/
- **Input:** Protein name, PDB ID, authors, etc.
- **Output:** 3D coordinates of protein structures
  - Author information on methods
  - Cofactors and other information

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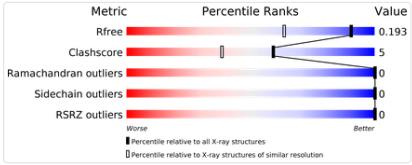
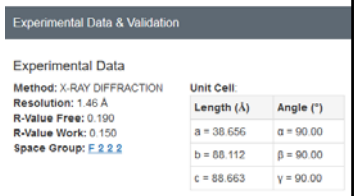
Assessing a Crystal Structure: 3TJW

Structure Validation

View Full Validation Report or Ramachandran Plots



- PDB contains a lot of useful information for determining how good a crystal structure is
- **Things to look at:** R, R_{free}, resolution, structure validation



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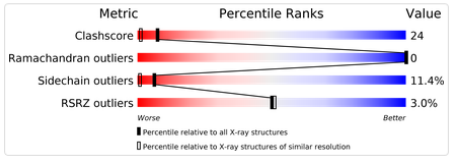
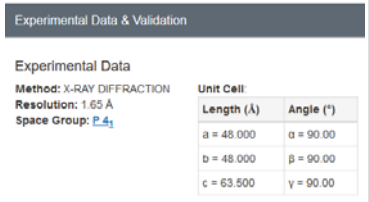
Assessing a Crystal Structure: 1SNC

Structure Validation

View Full Validation Report or Ramachandran Plots



- Not every protein will have R_{free}



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PDB Files: A Closer Look

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Obtaining PyMOL

- Recent versions of educational PyMOL (EduPyMOL) are great, although time-limited.
- Older educational builds can be obtained by registering at: http://www.pymol.org/educational/
- **Mac or PC:** Fill in form and follow instructions for downloading; follow standard procedure
- **Linux:** Open source (full version) can be obtained through most distributions (e.g, apt, yum)

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Obtaining PyMOL

- PyMOL Website: http://pymol.org/ep
  Username: dec2018
  Password: betasheet
- Older versions for Windows XP, etc. (version 1.3r1) are available at:
  Windows: http://goo.gl/0mRH2O
  Mac: http://goo.gl/U68Hoc
  Linux: http://goo.gl/HMZvPU

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Obtaining PyMOL on Linux/Debian

- PyMOL is open source, and an alternative is downloading compiled versions on Linux
- Linux versions (i.e., versions compiled independently from Schrodinger) are not crippled, even the latest version. They do lack some Schrodinger-specific features.
- On Debian/Ubuntu (easy, but you need admin access):

```
apt-get install pymol
```

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Obtaining PyMOL on CentOS

- On Redhat/Centos (harder, and you still need admin access):

```
1. sudo yum update
2. sudo yum install epel-release
3. sudo yum install subversion gcc gcc-c++
   kernel-devel python-devel tkinter python-pmw
   glew-devel freeglut-devel libpng-devel
   freetype-devel libxml2-devel apbs
```

- The commands above install the necessary libraries for compiling PyMOL
- The final command should be typed entirely on one line.

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Obtaining PyMOL: CentOS (cont.)

- Obtain the latest tar.bz2 file for PyMOL from http://sourceforge.net/projects/pymol/?source=typ_redirect
- Extract the source code using the following command:
  `tar xfj <source file name>`
- This will extract the source code to a directory with a similar name as the tar.bz2 archive (e.g. `pymol-v2.1.0.tar.bz2` extracts to `pymol-v2.1.0`)

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Obtaining PyMOL: CentOS (cont.)

- Finally, compile and install python. Change to the python source directory and type (will take some time):

```
1. python setup.py build
2. sudo python setup.py install
```

- You can then run PyMOL from the setup directory (you can also create a link):

```
1. cd setup      (still from within the PyMOL directory)
2. ./pymol
```

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Alternatives to PyMOL

- UCSF Chimera (https://www.cgl.ucsf.edu/chimera/)
  - Absolutely free, although tricky to learn. A large and growing user base.
- VMD (http://www.ks.uiuc.edu/Research/vmd/)
  - Optimized for looking at MD simulations
- More information about history at http://www.umass.edu/microbio/rasmol/history.htm

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Quick Intro to PyMOL

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PyMOL Tools: Protein Alignment

- Download two files from the web page: 1F8A and 1PIN

```
fetch 1f8a
fetch 1pin
```

- Open both PDB files in PyMOL
- Align both structures using the following command (uses similar residues as cues):

```
align 1f8a and resi 80-160, 1pin and resi 80-160
```

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PyMOL Tools: Measurement

- Measure distances and dihedral angles using PyMOL (Wizard → Measurement)
- Recall that phi (ϕ) is defined as the dihedral angle defined by:

$$C'_{i-1} - N_i - C_{\alpha,i} - C'_i$$

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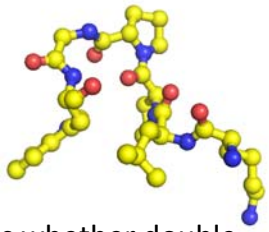
PyMOL Tools: Ball-and Stick

- Combining small spheres with stick model can produce a pleasing result

```
show sticks
show spheres
set sphere_scale=0.3
set valence=0
```



- The final command toggles whether double bonds are shown

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PyMOL Tools: Transparent Surfaces

- Show surfaces using

```
show surface, selection
```

- Transparency can be applied to different renderings (e.g. cartoon vs. spheres)

```
set sphere_transparency=0.65
```

- Surface transparency is a global property, so a separate object must be created if mixed results are desired (see next slide)

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PyMOL Tools: Transparent Surfaces

- **Example:** Cartoons under a semi-transparent surface
  1. First, create a duplicate object by clicking 1f8a (A) → Copy to Object → New
  2. Then show surface for the new object (obj01)
     ```
     show surface, obj01
     color grey, obj01
     ```
  3. Set transparency for the new object
     ```
     set transparency=0.5, obj01
     ```
  4. Show cartoons for the original object
     ```
     hide everything, 1f8a
     show cartoon, 1f8a
     color yellow, 1f8a
     ```

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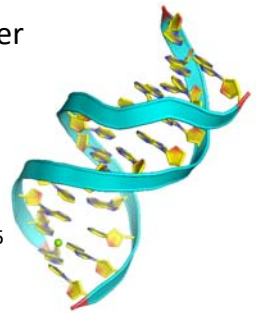
PyMOL Tools: Awesome DNA

- Grab the Dickerson dodecamer
  ```
  fetch 4c64
  ```
- Reasonable first attempt
  ```
  set cartoon_ring_mode=3
  cartoon dumbbell
  set cartoon_dumbbell_radius=0.25
  color cyan, elem P
  ```



- My go-to pages for DNA:
  https://kpwu.wordpress.com/2006/10/22/pymol-fancy-dna-helix-and-filled-rings/
  https://pymolwiki.org/index.php/Examples_of_nucleic_acid_cartoons

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PyMOL Tools: Ray Tracing Tips

- **Ray Tracing:** Simulation of photon light paths, reflection, and scattering to give a photorealistic image
- New Draw/Ray Panel
  - Fine control over resolution/image size
  - Automatic removal of background (a bit buggy); for best results, maximize slab so no fading
  - Display → Background: Can set color
- Save image: Use File → Export Image As… (or click Draw/Ray again)

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Summary

- PDB files are complex models, derived from experimental data
  - Need to assess these models, too
- Atom names and properties are stored in the PDB, can be used to investigate structures
- PyMOL can make interesting pictures, but it is also a very powerful analytical tool

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