BGGN-213: FOUNDATIONS OF BIOINFORMATICS (Lecture 11)

Structural Bioinformatics (Part 1)
https://bioboot.github.io/bggn213_S18/lectures/#11
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Section 1: Introduction to the RCSB Protein Data Bank (PDB)

The PDB archive is the major repository of information about the 3D structures of large biological molecules, including proteins and nucleic acids. Understanding the shape of these molecules helps to understand how they work. This knowledge can be used to help deduce a structure's role in human health and disease, and in drug development. The structures in the PDB range from tiny proteins and bits of DNA or RNA to complex molecular machines like the ribosome composed of many chains of protein and RNA.

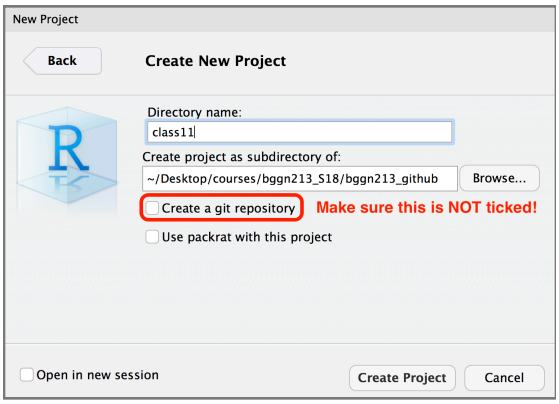
In the first section of this lab we will interact with the main US based PDB website (note there are also sites in Europe and Japan).

Visit: http://www.rcsb.org/ and answer the following questions

NOTE: The "Analyze" -> "PDB Statistics" > "by Experimental Method and Molecular Type" on the PDB home page should allow you to determine most of these answers.

1.1 PDB statistics

Open Open RStudio and begin a new **class11** project **within** your GitHub tacked directory/folder from last day. Make sure "Create a git repository" option is **NOT** ticked. This is because we want to use the same git repository as we used last day and not start a new one **- if you are not sure what this means ask Barry now!**



Next, open a new R Markdown document (**File > New File > R Markdown…**). Chose "**From Template**" and select "**GitHub Document**".

Q1: Download a CSV file from the PDB site (accessible from "**Analyze**" -> "**PDB Statistics**" > "**by Experimental Method and Molecular Type**". Move this CSV file into your RStudio project and determine the percentage of structures solved by X-Ray and Electron Microscopy. From the website what proportion of structures are protein?

Q2: Type **HIV** in the PDB website search box on the home page and determine how many HIV-1 protease structures are in the current PDB?

1.2 The PDB format

Now download the **"PDB File"** for the HIV-1 protease structure with the PDB identifier **1HSG**. On the website you can "*Display*" the contents of this "*PDB format*" file. Alternatively, you can examine the contents of your downloaded file in a suitable text editor.

NOTE: You can also use the **Terminal** tab from within RStudio (or your favorite Terminal/Shell) and try the following command:

```
> more ~/Downloads/1hsg.pdb          ## (use 'q' to quit)
```

NOTE: You can type **1HSG** in the PDB search box to jump to its entry and then click **"Download Files"** to the right of the top display. Selecting "**Display Files**" will allow you to view the PDB file directly in your browser window.

When viewing the file stop when you come the lines beginning with the word "ATOM". We will discuss this ubiquitous PDB file format when you have got this far.

Section 2: Visualizing the HIV-1 protease structure

The HIV-1 protease [1] is an enzyme that is vital for the replication of HIV. It cleaves newly formed polypeptide chains at appropriate locations so that they form functional proteins. Hence, drugs that target this protein could be vital for suppressing viral replication. A handful of drugs - called *HIV-1 protease inhibitors* (saquinavir, ritonavir, indinavir, nelfinavir, etc.) [2] - are currently commercially available that inhibit the function of this protein, by binding in the catalytic site that typically binds the polypeptide.

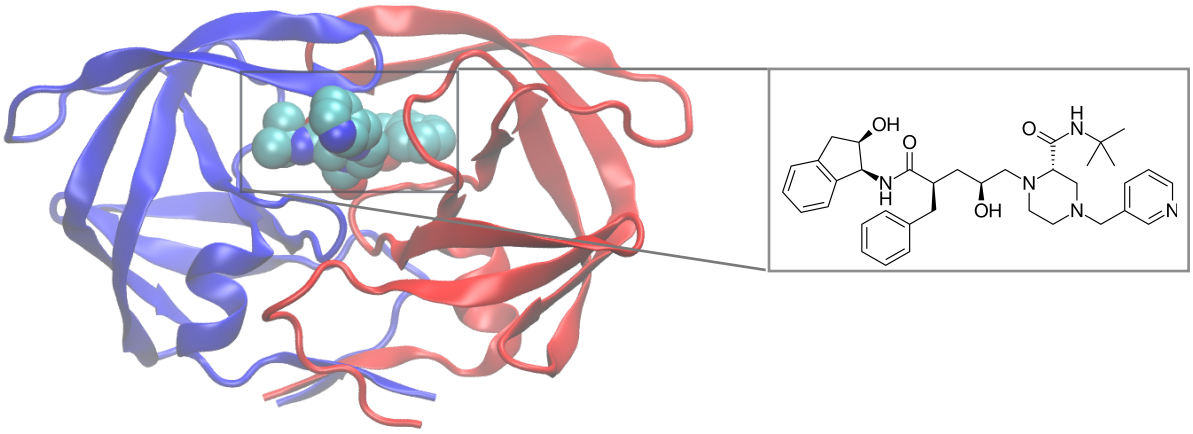


Figure 1. HIV-1 protease structure in complex with the small molecule indinavir.

In this section we will use the 2Å resolution X-ray crystal structure of HIV-1 protease with a bound drug molecule indinavir (PDB ID: 1HSG) [3]. We will use the **VMD molecular viewer** to visually inspect the protein, the binding site and the drug molecule. After exploring features of the complex we will move on to computationally dock a couple of drug molecules into the binding site of HIV-1 protease to see how well computational docking can reproduce the crystallographically observed binding pose. If time permits, we will also explore the conformational dynamics and flexibility of the protein - important for it's function and for considering during drug design.

NOTE: If you have not already done so please download and install VMD from:
http://www.ks.uiuc.edu/Development/Download/download.cgi .

2.1 Getting know VMD

Open VMD and load **1hsg.pdb** by using the **VMD Main** window and going to "***File***" -> "***New Molecule***" and then from the new window that appears click "***Browse***" and select your downloaded **1hsg.pdb** file. Then click "***Load***".

You should now see the protein structure displayed as lines and water molecules as little red dots. Use the mouse to zoom and rotate. Once you have the hang of rotation we will start exploring different "*Graphical Representations*".

VMD can display molecules in various ways by choosing different options in the *Graphical Representations* window shown in **Figure 2**. You can access this window by clicking **Graphics > Representations** from the small **VMD Main** window.

NOTE. Each representation is defined by three main parameters: (**1**) the *drawing method*, (**2**) the *selected atoms* to be included in the representation, and (**3**) the *coloring method* (see **Figure 2** labels 1-3)

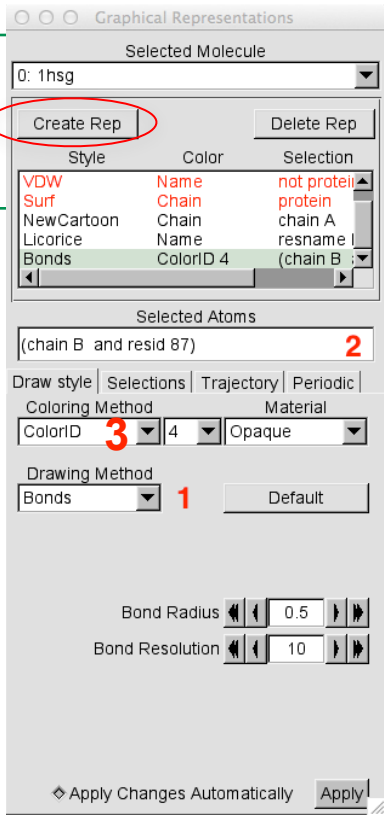
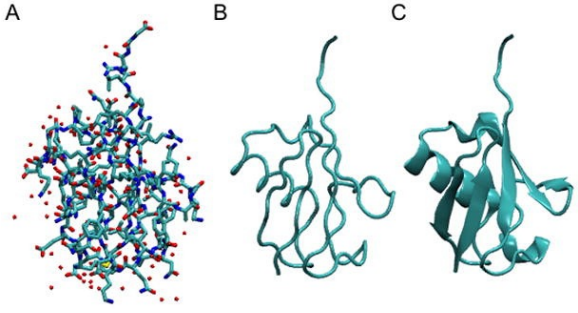


Figure 2. The VMD graphical Representation window. Note that the *Drawing Method* (labeled **1** in the figure) defines which graphical representation is used and (**2**) the *Selected Atoms* determines which part of the molecule is drawn, and (**3**) defines the color it is displayed with. You are encouraged to explore different drawing styles (i.e. *Drawing Methods* - labeled **1**) including *Licorice*, *Tube* and *NewCartoon* (see below for examples A-C).



Also try different selections by entering text in the (*Selected Atoms* box - labeled **2**). Some examples to try include:

```
chain A and backbone
resname ASP
within 5 of resname MK1
```

2.2 Using Atom Selections

Now type "`protein`" in the *Selected Atoms* text box (labeled **2** in Figure 2) and show the protein using the **Cartoon** representation and color by **chain** (see label **3** in Figure 2.)

Lets add a new representation by clicking the **"Create Rep"** (circled in Figure 2) and using the selection text **"not protein and not water"**

Add more representations (by clicking the **"Create Rep"** button) and hiding (by double clicking) or deleting previous ones (with the "**Delete Rep**" button) to explore different representations for both the ligand and the protein.

NOTE: you can use the residue name of the ligand "resname MK1" to select just the ligand.

Water molecules have the residue name HOH. Select and display all water molecules as red spheres. If you think the spheres are too big, how would you reduce their size?

Q3: Water molecules normally have 3 atoms. Why do we see just one atom per water molecule in this structure?

Q4: There is a conserved water molecule in the binding site. Can you identify this water molecule? What residue number does this water molecule have (see note below)?

NOTE: From the **VMD Main** window click **Mouse > Label > Atoms** and then click on the water in question to display its residue number. A short cut is to press the #1 key when your mouse is active in the OpenGL window.

Now you should be able to produce an image similar or even superior to **Figure 1** and save it to an image file on disk with **VMD Main** window, **File > Render > Start Rendering**.

NOTE: You can chose different rendering engines including Tachyon (internal), which is commonly used for publication quality images.

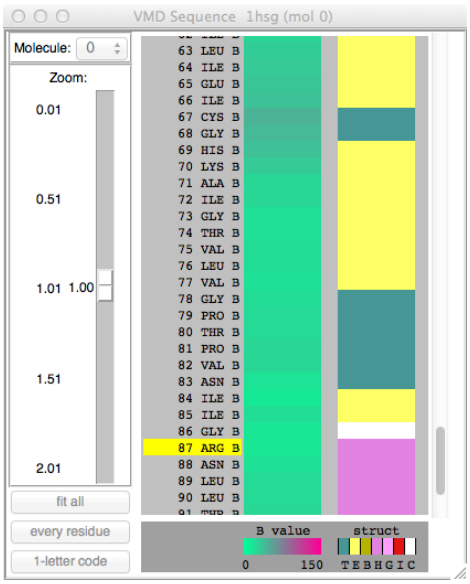
Optional: Generate and save a figure clearly showing the two distinct chains of HIV-protease along with the ligand. You might also consider showing the catalytic residues ASP 25 in each chain (we recommend Licorice for these side-chains). Email this figure to bjgrant@ucsd.edu for grading.

Discussion Topic: Can you think of a way in which indinavir, or even larger ligands and substrates, could enter the binding site?

2.3 Sequence Viewer Extension

When dealing with a protein for the first time, it is very useful to be able to find and display different amino acids quickly. The sequence viewer extension allows viewing of the protein sequence, as well as to easily pick and display one or more residues of interest.

To launch the Sequence Viewer click **VMD Main** window, **Extensions > Analysis > Sequence Viewer**. The different color scales beside the sequence correspond to the B-factor and Secondary structure type (the major ones being Extended (beta) in yellow and Helix in purple).



Q5: As you have hopefully observed HIV protease is a homodimer (i.e. it is composed of two identical chains). With the aid of the graphic display and the sequence viewer extension can you identify secondary structure elements that are likely to only form in the dimer rather than the monomer?

Section3: Introduction to Bio3D in R

Bio3D[1] is an R package for structural bioinformatics[2,3]. Features include the ability to read, write and analyze biomolecular structure, sequence and dynamic trajectory data.

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[1] The latest version of the Bio3D package, full documentation and further vignettes (including detailed installation instructions) can be obtained from the main Bio3D website: thegrantlab.org/bio3d/.

[2] Grant, B.J. et al. (2006) *Bioinformatics* 22:2695--2696.

[3] Skjaerven, L. et al. (2014) *BMC Bioinformatics* 15:399.

3.1 Getting started with Bio3D

In your existing class11 R markdown document load the Bio3D package by typing in a new code chunk:

```
library(bio3d)
```

Side-Note: If you see an error message reported then you will first need to install the package with the command: `install.packages("bio3d")` This is only required once whereas the `library(bio3d)` command is required at the start of every new R session where you want to use Bio3D.

At the R console use the command `lbio3d()` or `help(package=bio3d)` to list the functions within the package and `help(FunctionName)` to obtain more information about an individual function (`help(pca.xyz)`)

Side-note: You can find online documentation at https://www.rdocumentation.org/packages/bio3d/ and at the official Bio3D web-site.

3.2 Bio3D functions and their typical usage

To better understand how a particular function operates it is often helpful to view and execute an example. Every function within the Bio3D package is documented with example code that you can view by issuing the `help()` command.

Running the command `example(function)` will directly execute the example for a given function. In addition, a number of longer worked examples are available as Tutorials on the Bio3D website.

```
example(plot.bio3d)
```

3.3 Working with individual PDB files

Protein Data Bank files (or PDB files) are the most common format for the distribution and storage of high-resolution biomolecular coordinate data. At their most basic, PDB coordinate files contain a list of all the atoms of one or more molecular structures. Each atom position is defined by its x, y, z coordinates in a conventional orthogonal coordinate system. Additional data, including listings of observed secondary structure elements, are also commonly (but not always) detailed in PDB files.

Reading PDB file data into R

To read a single PDB file with Bio3D we can use the `read.pdb()` function. The minimal input required for this function is a specification of the file to be read. This can be either the file name of a local file on disc, or the RCSB PDB identifier of a file to read directly from the on-line PDB repository. For example to read and inspect the on-line file with PDB ID `4q21`:

```
pdb <- read.pdb("1hsg")
```

```
##   Note: Accessing on-line PDB file
```

To get a quick summary of the contents of the `pdb` object you just created you can issue the command `print(pdb)` or simply type `pdb` (which is equivalent in this case):

```
pdb
```

```
##
##  Call:  read.pdb(file = "1hsg")
##
##    Total Models#: 1
##      Total Atoms#: 1686,  XYZs#: 5058  Chains#: 2  (values: A B)
##
##      Protein Atoms#: 1514  (residues/Calpha atoms#: 198)
##      Nucleic acid Atoms#: 0  (residues/phosphate atoms#: 0)
##
##      Non-protein/nucleic Atoms#: 172  (residues: 128)
##      Non-protein/nucleic resid values: [ HOH (127), MK1 (1) ]
##
##    Protein sequence:
##       PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIGGFIKVRQYD
##       QILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFPQITLWQRPLVTIKIGGQLKE
##       ALLDTGADDTVLEEMSLPGRWKPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPTP
##       VNIIGRNLLTQIGCTLNF
##
## + attr: atom, xyz, seqres, helix, sheet,
##         calpha, remark, call
```

Q6. How many amino acid residues are there in this pdb object and what are the two non-protein residues?

Note that the attributes (`+ attr:`) of this object are listed on the last couple of lines. To find the attributes of any such object you can use:

```
attributes(pdb)
```

```
## $names
## [1] "atom"   "xyz"    "seqres" "helix"  "sheet"  "calpha" "remark" "call"
##
## $class
## [1] "pdb" "sse"
```

To access these individual attributes we use the `dollar-attribute` name convention that is common with R list objects. For example, to access the `atom` attribute or component use `pdb$atom`:

```
head(pdb$atom)
```

```
##   type eleno elety  alt resid chain resno insert      x      y      z o
## 1 ATOM     1     N <NA>   PRO     A     1   <NA> 64.080 50.529 32.509 1
## 2 ATOM     2    CA <NA>   PRO     A     1   <NA> 64.044 51.615 33.423 1
## 3 ATOM     3     C <NA>   PRO     A     1   <NA> 63.722 52.849 32.671 1
## 4 ATOM     4     O <NA>   PRO     A     1   <NA> 64.359 53.119 31.662 1
## 5 ATOM     5    CB <NA>   PRO     A     1   <NA> 65.373 51.805 34.158 1
## 6 ATOM     6    CG <NA>   PRO     A     1   <NA> 65.122 52.780 35.269 1
##. <... cut for brevity ...>
```

```
# Print a subset of $atom data for the first two atoms
pdb$atom[1:2, c("eleno", "elety", "x","y","z")]
```

```
##   eleno elety      x      y      z
## 1     1     N 64.080 50.529 32.509
## 2     2    CA 64.044 51.615 33.423
```

```
# Note that individual $atom records can also be accessed like this
pdb$atom$elety[1:2]
```

```
## [1] "N"  "CA"
```

```
# Which allows us to do the following
plot.bio3d(pdb$atom$b[pdb$calpha], sse=pdb, typ="l", ylab="B-factor")
```

Q7. What type of R object is `pdb$atom`? **HINT**: You can always use the str() function to get a useful summery of any R object.

Note that the main `xyz` coordinate attribute is a numeric matrix with 3N columns (each atom has three values x, y and z). The number of rows here correspond to the number of models in the PDB file (typically one for X-ray structures and multiple for NMR structures).

```
# Print a summary of the coordinate data in $xyz
pdb$xyz
```

```
##
##    Total Frames#: 1
##    Total XYZs#:   4341,  (Atoms#:  1447)
##
##     [1]  64.08  50.529  32.509  <...>  74.159  76.923  41.999  [4341]
##
## + attr: Matrix DIM = 1 x 4341
```

```
# Examine the row and column dimensions
dim(pdb$xyz)

## [1]    1 4341

# Print coordinates for the first two atom
pdb$xyz[ 1, atom2xyz(1:2) ]

## [1] 64.080 50.529 32.509 64.044 51.615 33.423
```

Side-Note: The 'pdb' class. Objects created by the `read.pdb()` function are of class "pdb". This is recognized by other so called generic Bio3D functions (for example `atom.select()`, `nma()`, `print()`, `summary()` etc.). A generic function is a function that examines the class of its first argument, and then decides what type of operation to perform (more specifically it decides which specific method to dispatch to). So for example, the generic `atom.select()` function knows that the input is of class "pdb", rather than for example an AMBER parameter and topology file, and will act accordingly.

A careful reader will also of noted that our "pdb" object created above also has a second class, namely "sse" (see the output of `attributes(pdb)` or `class(pdb)`). This stands for *secondary structure elements* and is recognized by the `plot.bio3d()` function to annotate the positions of major secondary structure elements in the marginal regions of these plots (see Figure 1). This is all part of the R S3 object orientation system. This S3 system is used throughout Bio3D to simplify and facilitate our work with these types of objects.

3.5 Atom selection

The Bio3D `atom.select()` function is arguably one of the most challenging for newcomers to master. It is however central to PDB structure manipulation and analysis. At its most basic, this function operates on PDB structure objects (as created by `read.pdb()`) and returns the numeric indices of a selected atom subset. These indices can then be used to access the `$atom` and `$xyz` attributes of PDB structure related objects.

For example to select the indices for all C-alpha atoms we can use the following command:

```
# Select all C-alpha atoms (return their indices)
ca.inds <- atom.select(pdb, "calpha")
ca.inds

##
##  Call:  atom.select.pdb(pdb = pdb, string = "calpha")
##
##    Atom Indices#: 168  ($atom)
##    XYZ  Indices#: 504  ($xyz)
##
## + attr: atom, xyz, call
```

Note that the attributes of the returned `ca.inds` from `atom.select()` include both `atom` and `xyz` components. These are numeric vectors that can be used as indices to access the corresponding atom and xyz components of the input PDB structure object. For example:

```
# Print details of the first few selected atoms
head( pdb$atom[ca.inds$atom, ] )
```

```
##    type eleno elety  alt resid chain resno insert      x      y      z o
## 2  ATOM     2    CA <NA>   MET     A     1   <NA> 64.044 51.615 33.423 1
## 10 ATOM    10    CA <NA>   THR     A     2   <NA> 62.439 54.794 32.359 1
## 17 ATOM    17    CA <NA>   GLU     A     3   <NA> 63.968 58.232 32.801 1
## 26 ATOM    26    CA <NA>   TYR     A     4   <NA> 61.817 61.333 33.161 1
## 38 ATOM    38    CA <NA>   LYS     A     5   <NA> 63.343 64.814 33.163 1
## 47 ATOM    47    CA <NA>   LEU     A     6   <NA> 61.321 67.068 35.557 1
##. <... cut for brevity ...>
```

```
# And selected xyz coordinates
head( pdb$xyz[, ca.inds$xyz] )
```

```
## [1] 64.044 51.615 33.423 62.439 54.794 32.359
```

In addition to the common selection strings (such as 'calpha' 'cbeta' 'backbone' 'protein' 'notprotein' 'ligand' 'water' 'notwater' 'h' and 'noh') various individual atom properties can be used for selection.

```
# Select chain A
a.inds <- atom.select(pdb, chain="A")

# Select C-alphas of chain A
ca.inds <- atom.select(pdb, "calpha", chain="A")

# We can combine multiple selection criteria to return their
intersection
cab.inds <- atom.select(pdb, elety=c("CA","CB"), chain="A",
resno=10:20)
```

We can write new PDB files with the `write.pdb()` function. rom

Q8. Use the Bio3D `write.pdb()` function to write out a protein only PDB file for viewing in VMD. Also write out a second separate PDB file for the ligand with residue name MK1

HINT: In Bio3D you can use the trim.pdb() function together with your atom selections.

Section 4: Working with multiple PDB files

The Bio3D package was designed to specifically facilitate the analysis of multiple structures from both experiment and simulation.

The challenge of working with these structures is that they are usually different in their composition (i.e. contain differing number of atoms, sequences, chains, ligands, structures, conformations etc. even for the same protein as we will see below) and it is these differences that are frequently of most interest.

For this reason Bio3D contains extensive utilities to enable the reading, writing, manipulation and analysis of such heterogenous structure sets. This topic is detailed extensively in the separate **Principal Component Analysis** vignette and **Ensemble Normal Mode Analysis** vignette available from http://thegrantlab.org/bio3d/tutorials.

4.1 Installing the stand-alone muscle alignment program

If you are not on one of the classroom computers you will need to download the appropriate version of the **muscle** multiple alignment program from: https://www.drive5.com/muscle/downloads.htm

Side-Note: For Mac you will most likely want the Intel i86 64 bit version unless you are on a particularly old laptop. Ask Barry if you have questions about this step

On MAC: After downloading MUSCLE, it should be unzipped and renamed to just "**muscle**". If you are on Mac you can move this file to a directory such as "**/usr/local/bin/**"

The easiest way to do this on Mac or Linux is to use the **Terminal**/Shell in your RStudio. At the shell prompt issue the following commands:

```
tar -xvf ~/Downloads/muscle3.8.31_i86darwin32.tar
sudo mv muscle3.8.31_i86darwin32 /usr/local/bin/muscle
```

If you now type **`muscle`** in your terminal you should see the help splash screen for the MUSCLE program. Instead, if you see the response "`bash: muscle: command not found`" something has not worked and you should ask Barry for help!

On WINDOWS: After downloading MUSCLE, it should be moved to your RStudio project directory and renamed to **muscle.exe**. Use your windows file explorer to move the downloaded **muscle3.8.31_i86win32.exe** from your Downloads folder via copy and paste to your Project folder. Then right click to rename to **muscle.exe**.

In your RStudio **Terminal** you should now be able to run the following without error:

```
muscle.exe -version
```

If you get an error message then something has not worked and you should ask Barry for help!

4.2 Aligning multiple structures

Before delving into more advanced analysis (detailed in the next section and additional vignettes) lets examine how we can read multiple PDB structures from the RCSB PDB for a particular protein and perform some basic analysis:

```
# Download some example PDB files
ids <- c("1TND_B","1AGR_A","1TAG_A","1GG2_A","1KJY_A","4G5Q_A")
files <- get.pdb(ids, split = TRUE)
```

The `get.pdb()` function will download the requested files. Argument `split = TRUE` requests further that we want to extract particular chains, i.e. those specified by the `_A` suffix of each PDB ID in the example above. Note that these `ids` could come from the results of a `blast.pdb()` search as described in other vignettes.

The requested chains are then aligned and their structural data stored in a new object `pdbs` that can be used for further analysis. The `pdbaln()` function includes the ability to superimpose, or fit, all structures onto each other using the argument `fit = TRUE`.

Side-Note: You can also provide a vector of PDB IDs, or a list of **pdb** objects as input to `pdbaln()`. Here we use a vector of file names (output from `get.pdb()`).

```
# Extract and align the chains we are interested in
pdbs <- pdbaln(files, fit = TRUE)


# Print to screen a summary of the 'pdbs' object
pdbs
```

Side-Note: If you are on **Windows** and the call to `pdbaln()` yielded an ERROR message it is likely that you need to prove an extra option to the `pdbaln()` function call to tell R where muscle lives on your computer: For example:

```
pdbs <- pdbaln(files, fit = TRUE,
     exefile="C:/Users/barry/Downloads/muscle3.8.31_i86win32.exe")
```

Or if that does not work you can use the online EBI muscle server:

```
pdbs <- pdbaln(files, fit=TRUE, web.args=list(email="your@email.com"))
```

Here we use a vector of file names (output from `get.pdb()`).

Q8: What effect does setting the `fit=TRUE` option have in the related `rmsd()` function? What does RMSD measure and what would the results indicate if you set `fit=FALSE` or removed this option? **HINT**: Bio3D functions have various default options that will be used if the option is not explicitly specified by the user, see `help(rmsd)` for an example and note that the input options with an equals sign (e.g. `fit=FALSE`) have default values.

Here the returned object is of class `pdbs`. Note that it contains a `xyz` numeric matrix of aligned C-alpha coordinates, a `ali` matrix of aligned residues, and a `resno` matrix of aligned residue numbers (see the list of associated attributes (`+ attr`)). These attirbutes can be accessed using the common `$` syntax in R. E.g. use `pdbs$ali` to access the alignment. To access the first few rows of the alignment matrix we use standard subsetting syntax for matrices in R:

```
# Access the first 5 rows, and 8 columns
pdbs$ali[1:5, 1:8]

##                           [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8]
## ./split_chain/1TND_B.pdb "-"  "-"  "-"  "-"  "-"  "-"  "-"  "-"
## ./split_chain/1AGR_A.pdb "L"  "S"  "A"  "E"  "D"  "K"  "A"  "A"
## ./split_chain/1TAG_A.pdb "-"  "-"  "-"  "-"  "-"  "-"  "-"  "-"
## ./split_chain/1GG2_A.pdb "L"  "S"  "A"  "E"  "D"  "K"  "A"  "A"
## ./split_chain/1KJY_A.pdb "-"  "-"  "-"  "-"  "-"  "-"  "-"  "-"

# Associated residues numbers
pdbs$resno[1:5, 1:8]

##                           [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8]
## ./split_chain/1TND_B.pdb   NA   NA   NA   NA   NA   NA   NA   NA
## ./split_chain/1AGR_A.pdb    5    6    7    8    9   10   11   12
## ./split_chain/1TAG_A.pdb   NA   NA   NA   NA   NA   NA   NA   NA
## ./split_chain/1GG2_A.pdb    5    6    7    8    9   10   11   12
## ./split_chain/1KJY_A.pdb   NA   NA   NA   NA   NA   NA   NA   NA
```

Side-Note: The row names of the alignment matrix (`pdbs$ali`) as well as the identifiers component (`pdbs$id`) is set to the file name of the associated PDB file. You can convert these identifiers to their PDB codes using the `basename.pdb()` function (e.g. `basename.pdb(pdbs$id)`).

4.3 Basic structure analysis

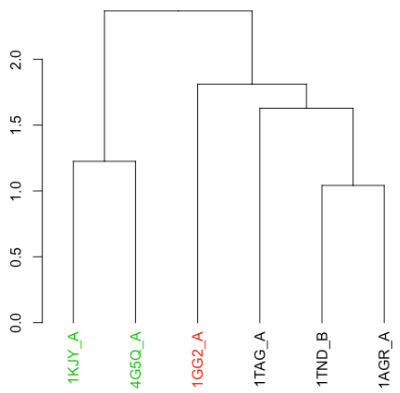
Having the generated `pdbs` object at hand facilitates a range of possibilities for protein structure analysis. This includes sequence identity/similarity, structural deviation, rigid core identification as well as principal component and normal mode analysis. Several Bio3D function are specifically designed to operate on the `pdbs` object, including functions `seqidentity()`, `rmsd()`, `pca()`, `core.find()`, `nma()` and many others.

Below we calculate the pairwise sequence identity between the structures of the `pdbs` ensemble followed by the root mean square deviation (RMSD):

```
# Calculate sequence identity
seqidentity(pdbs)

##         1TND_B 1AGR_A 1TAG_A 1GG2_A 1KJY_A 4G5Q_A
## 1TND_B   1.000  0.693  1.000  0.690  0.696  0.696
## 1AGR_A   0.693  1.000  0.694  0.997  0.994  0.997
## 1TAG_A   1.000  0.694  1.000  0.691  0.697  0.697
## 1GG2_A   0.690  0.997  0.691  1.000  0.991  0.994
## 1KJY_A   0.696  0.994  0.697  0.991  1.000  1.000
## 4G5Q_A   0.696  0.997  0.697  0.994  1.000  1.000

# Calculate RMSD
rmsd(pdbs)

##         1TND_B 1AGR_A 1TAG_A 1GG2_A 1KJY_A 4G5Q_A
## 1TND_B   0.000  1.042  1.281  1.651  2.098  2.367
## 1AGR_A   1.042  0.000  1.628  1.811  1.949  2.244
## 1TAG_A   1.281  1.628  0.000  1.730  1.840  1.885
## 1GG2_A   1.651  1.811  1.730  0.000  1.901  2.032
## 1KJY_A   2.098  1.949  1.840  1.901  0.000  1.225
## 4G5Q_A   2.367  2.244  1.885  2.032  1.225  0.000
```

These pairwise similarity measures facilitate the identification of groups of structures sharing a similar conformation (in case of RMSD) through clustering analysis:

```
# Calculate RMSD
rd <- rmsd(pdbs)

# Clustering
hc <- hclust(as.dist(rd))
grps <- cutree(hc, k=3)

# Plot results as dendrogram
hclustplot(hc, k=3)
```



Section 5: Example Application on Adenylate Kinase (Adk)

In this section we perform PCA on the complete collection of Adenylate kinase structures in the protein data-bank (PDB). Starting from only one PDB identifier (PDB ID 1AKE) we show how to search the PDB for related structures using BLAST, fetch and align the structures, and finally calculate the normal modes of each individual structure in order to probe for potential differences in structural flexibility.

5.1 Search and retrieve Adenylate kinase structures

Below we perform a blast search of the PDB database to identify related structures to our query Adenylate kinase sequence. In this particular example we use function `get.seq()` to fetch the query sequence for chain A of the PDB ID 1AKE and use this as input to `blast.pdb()`. Note that `get.seq()` would also allow the corresponding UniProt identifier.

```
aa <- get.seq("1ake_A")
```

```
## Fetching... Please wait. Done.
```

Next

```
# Blast or hmmer search
b <- blast.pdb(aa)
```

```
##  Searching ... please wait (updates every 5 seconds) RID =
ZM7GP50C014
##  .
##  Reporting 209 hits
```

Function `plot.blast()` facilitates the visualization and filtering of the Blast results. It will attempt to set a seed position to the point of largest drop-off in normalized scores (i.e. the biggest jump in E-values). In this particular case we specify a cutoff (after initial plotting) of 225 to include only the relevant *E.coli* structures:

```
# Plot a summary of search results
hits <- plot(b)
```

```
##    * Possible cutoff values:    198 -3
##              Yielding Nhits:    39 209
##
##    * Chosen cutoff value of:    198
##              Yielding Nhits:    39
```

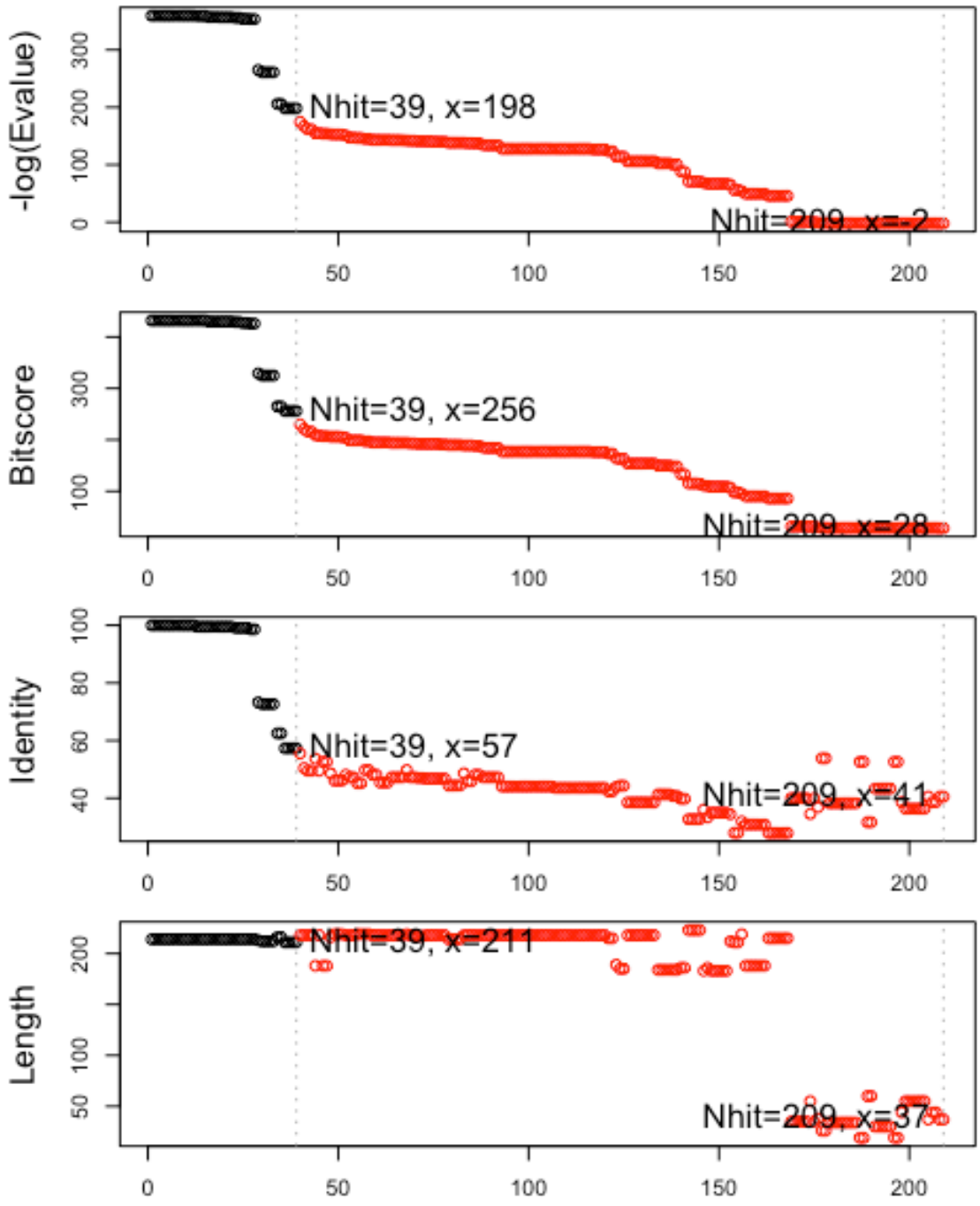


Figure 11: *Blast results. Visualize and filter blast results through function* `plot.blast()`*. Here we proceed with only the top scoring hits (black).*

```
head(hits$pdb.id)
```

```
## [1] "1AKE_A" "1AKE_B" "1ANK_A" "1ANK_B" "4AKE_A" "4AKE_B"
```

The Blast search and subsequent filtering identified a total of 39 related PDB structures to our query sequence. The PDB identifiers of this collection are accessible through the `pdb.id` attribute to the `hits` object (`hits$pdb.id`). Note that adjusting the cutoff argument (to `plot.blast()`) will result in a decrease or increase of hits.

We can now use function `get.pdb()` and `pdbslit()` to fetch and parse the identified structures. Finally, we use `pdbaln()` to align the PDB structures.

```
# Fetch PDBs
files <- get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip =
TRUE)

# Align structures
pdbs <- pdbaln(files)

# Vector containing PDB codes
ids <- basename.pdb(pdbs$id)

# Draw schematic alignment
plot(pdbs, labels=ids)
```

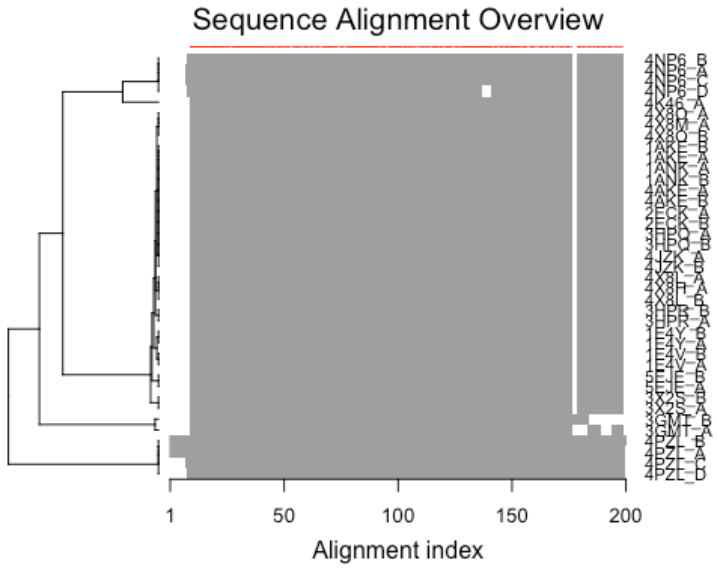


Figure 12: *Schematic representation of alignment. Grey regions depict aligned residues, while white depict gap regions. The red bar at the top depict sequence conservation.*

5.2 Sequence conservation analysis

```
# Calculate sequence conservation
cons <- conserv(pdbs, method="entropy22")


# SSE annotations
sse <- pdbs2sse(pdbs, ind=1, rm.gaps=FALSE)
```

```
# Plot conservation per residue
plotb3(cons, sse=sse, ylab="Sequence entropy")
```

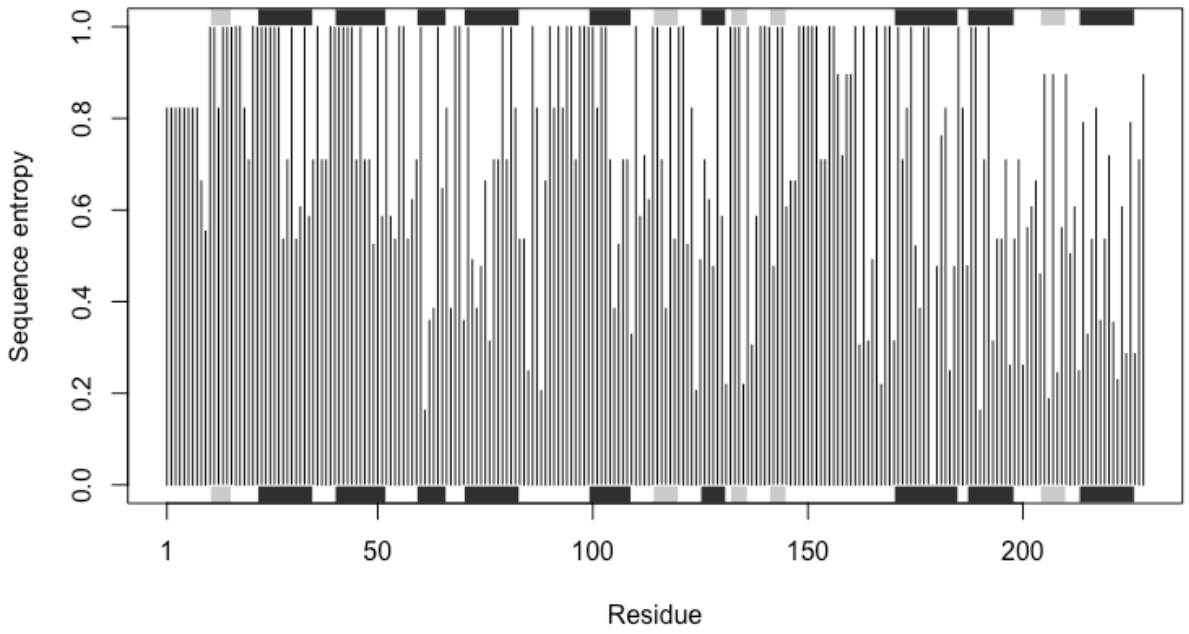


***Figure 13**: Sequence conservation per residue. Here, Shannon's information entropy is used to measure the diversity per alignment column. SSEs are depicted with dark (helices) and light (sheets) grey boxes in marginal regions.*

5.3 Annotate collected PDB structures

Function `pdb.annotate()` provides a convenient way of annotating the PDB files we have collected. Below we use the function to annotate each structure to its source species. This will come in handy when annotating plots later on:

```
anno <- pdb.annotate(ids)
print(unique(anno$source))
```

```
## [1] "Escherichia coli"          "Photobacterium profundum"
## [3] "Vibrio cholerae"           "Burkholderia pseudomallei"
## [5] "Francisella tularensis"
```

5.4 Principal component analysis

Function `pca()` provides *principal component analysis* (PCA) of the structure data. PCA is a statistical approach used to transform a data set down to a few important components that

describe the directions where there is most variance. In terms of protein structures PCA is used to capture major structural variations within an ensemble of structures.

Function `pca()` provides *principal component analysis* (PCA) of the structure data. PCA is a statistical approach used to transform a data set down to a few important components that describe the directions where there is most variance. In terms of protein structures PCA is used to capture major structural variations within an ensemble of structures.

PCA can be performed on the structural ensemble (stored in the *pdbs* object) with function `pca.xyz()`. To obtain meaningful results we first superimpose all structures on the *invariant core* (function `core.find()`).

```
# find invariant core
core <- core.find(pdbs)

# superimpose all structures to core
pdbs$xyz = pdbfit(pdbs, core)

# Perform PCA
pc.xray <- pca(pdbs)
```

Function **rmsd()** will calculate all pairwise RMSD values of the structural ensemble. This facilitates clustering analysis based on the pairwise structural deviation:

```
# Calculate RMSD
rd <- rmsd(pdbs)

# Structure-based clustering
hc.rd <- hclust(dist(rd))
grps.rd <- cutree(hc.rd, k=3)

plot(pc.xray, 1:2, col="grey50", bg=grps.rd, pch=21, cex=1)
```

The plot shows a conformer plot -- a low-dimensional representation of the conformational variability within the ensemble of PDB structures. The plot is obtained by projecting the individual structures onto two selected PCs (e.g. PC-1 and PC-2). These projections display the inter-conformer relationship in terms of the conformational differences described by the selected PCs.

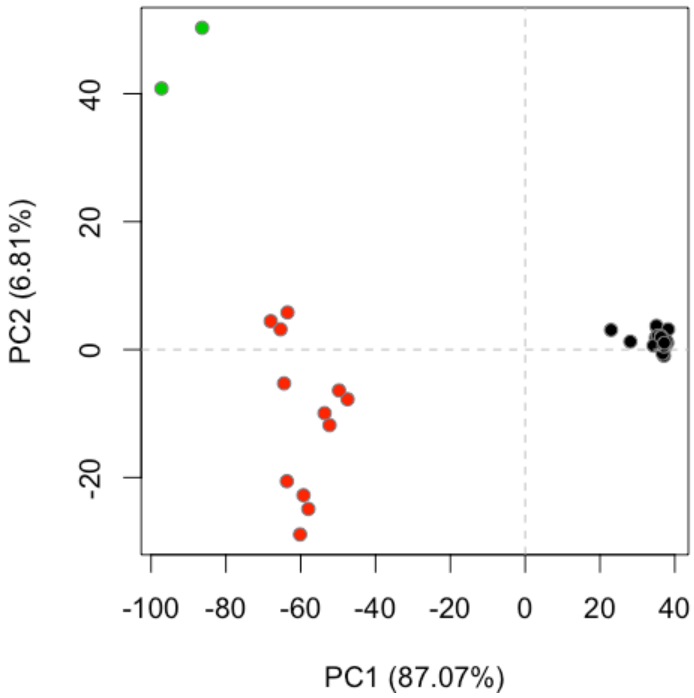


***Figure 14**: Projection of Adenylate kinase X-ray structures. Each dot represents one PDB structure.*

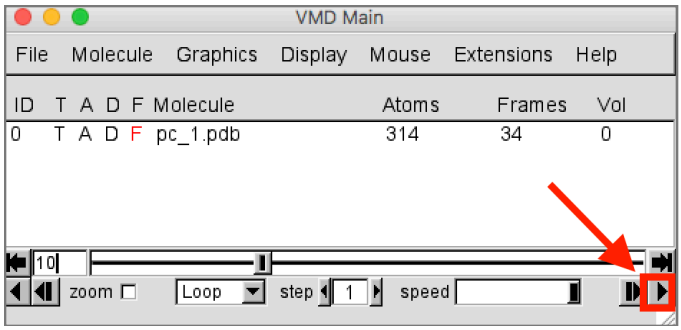
One can then use the `identify()` function to label and individual points.

```
# Left-click on a point to label and right-click to end
identify(pc.xray$z[,1:2], labels=basename.pdb(pdbs$id))
```

To visualize the major structural variations in the ensemble the function **mktrj()** can be used to generate a trajectory PDB file by interpolating along the eigenvector:

```
# Visualize first principal component
mktrj(pc.xray, pc=1, file="pc_1.pdb")
```

You can open this file, `pc_1.pdb`, in VMD, chose the “Drawing Method” **Tube** and “Coloring Method” **Index**. Then click the play button shown below to animate the structure and visualize the major structural variations along PC1.



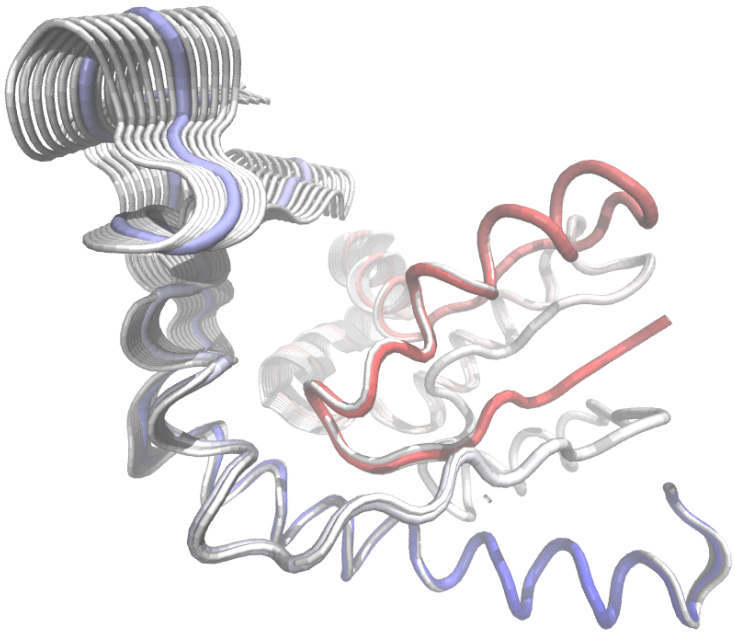


***Figure 15**: Visualization of PC-1 in VMD. Trajectory PDB file is generated using `mktrj()`.*

Side-Note: Remember to save your R markdown script document and Knit to generate a GitHub format .md report. Then stage, commit and push both these documents to GitHub by following the steps outlined in Section 4 - ask Barry if you are unsure of this process.

5.5 Plotting results with ggplot2

```
library(ggplot2)
library(ggrepel)

df <- data.frame(x=pc.xray$z[,1], y=pc.xray$z[,2])
col <- as.factor(grps.rd)

p <- ggplot(df, aes(x, y)) +
            geom_point(aes(col=col), size=2) +
            xlab("PC1") +
            ylab("PC2") +
            scale_color_discrete(name="Clusters") +
        geom_text_repel(aes(label=ids))
p
```

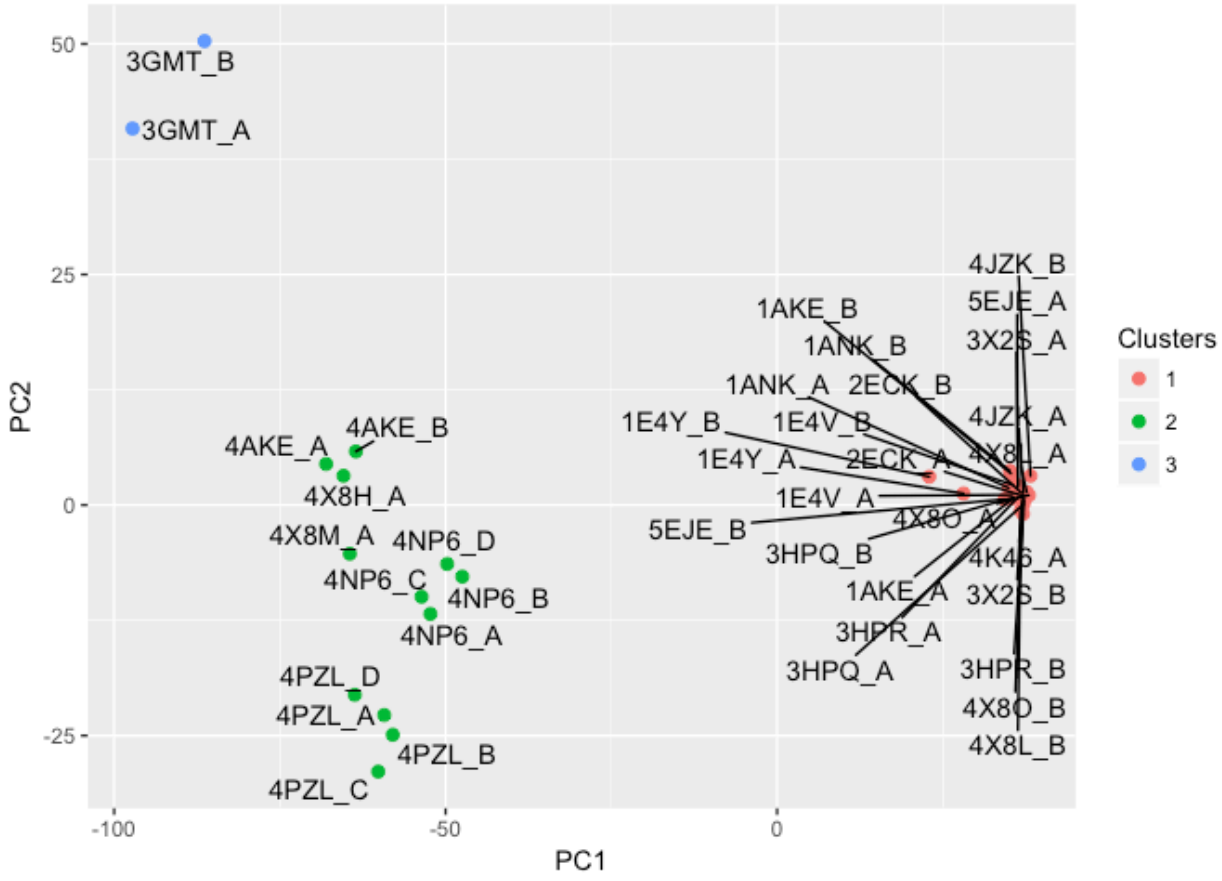


***Figure 16**: Projection of Adenylate kinase X-ray structures using package* **ggplot2**. *Each dot represents one PDB structure.*

Section 6: Normal mode analysis

Function `nma()` provides *normal mode analysis* (NMA) on the complete structure ensemble. This facilitates characterizing and comparing flexibility profiles of related protein structures.

```
# NMA all structures
modes <- nma(pdbs)
```

```
plot(modes, pdbs, col=grps.rd)
```

Side-Note: Again remember to save your R markdown script document and Knit to generate a GitHub format .md report. Then stage, commit and push both these documents to GitHub by following the steps outlined in the last class - ask Barry if you are unsure of this process.

Q9. **Muddy Point Assessment Feeedback**: How Please help us improve this lab session by providing your anonymous opinions here: https://goo.gl/forms/NpFkln0gzxbULi6F3

Section 7: Exploring the conformational dynamics of proteins with Bio3D-web

Visit the new web application Bio3D-web: http://thegrantlab.org/bio3d/webapps watch the introduction video and and click start analysis to begin exploring the conformational dynamics and flexibility of protein structures.

R Session info

```
# Information about the current Bio3D session
sessionInfo()

## R version 3.4.1 (2017-06-30)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Sierra 10.12.6
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/3.4/Resources/lib/libRblas.
0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.4/Resources/lib/
libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] ggrepel_0.7.0    ggplot2_2.2.1    bio3d_2.3-3.9000
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.13     knitr_1.17       magrittr_1.5     munsell_0.4.3
##  [5] colorspace_1.3-2 rlang_0.1.2      stringr_1.2.0    highr_0.6
##  [9] plyr_1.8.4       tools_3.4.1      parallel_3.4.1   grid_3.4.1
## [13] gtable_0.2.0     htmltools_0.3.6  yaml_2.1.14      lazyeval_0.2.0
## [17] rprojroot_1.2    digest_0.6.12    tibble_1.3.4     evaluate_0.10.1
## [21] rmarkdown_1.6    labeling_0.3     stringi_1.1.5    compiler_3.4.1
## [25] scales_0.5.0     backports_1.1.1
```