Class 9: Structural Bioinformatics (Pt. 1)

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Introduction to the RCSB Protein Data Bank (PDB)

What is in the PDB anyway?

The main database of biomolecular structures is called the PDB and is available at www.rcsb.org.

Let's begin by seeing what is in this database:

PDB Statistics

Download a CSV file from the PDB site (accessible from "Analyze" > "PDB Statistics" > "by Experimental Method and Molecular Type").

> Q1. What percentage of structures in the PDB are solved by X-Ray and Electron Microscopy?

```
pdbstats <- read.csv("Data Export Summary.csv", row.names = 1)
head(pdbstats)
```

```
                          X.ray    EM    NMR Multiple.methods Neutron Other
Protein (only)          152,809 9,421 12,117              191      72    32
Protein/Oligosaccharide   9,008 1,654     32                7       1     0
Protein/NA                8,061 2,944    281                6       0     0
Nucleic acid (only)       2,602    77  1,433               12       2     1
Other                       163     9     31                0       0     0
Oligosaccharide (only)       11     0      6                1       0     4
                          Total
Protein (only)          174,642
Protein/Oligosaccharide  10,702
Protein/NA               11,292
Nucleic acid (only)       4,127
Other                       203
Oligosaccharide (only)       22
```

```
n.xray <- sum(as.numeric(gsub(",", "", pdbstats$X.ray)))
n.em <- sum(as.numeric(gsub(",", "", pdbstats$EM)))
n.total <- sum(as.numeric(gsub(",", "", pdbstats$Total)))
p.xray <- (n.xray / n.total) * 100
```

```
p.em <- (n.em / n.total) * 100
round(p.xray, 2)
```

```
[1] 85.9
```

```
round(p.em, 2)
```

```
[1] 7.02
```

There are 172654 (85.9%) protein structures in the X.ray and 14105 (7.02%) protein structures in the Electron Microscopy in the current PDB database.

> Q2. What proportion of structures in the PDB are protein?

```
as.numeric(gsub(",", "", pdbstats$Total)) / n.total
```

```
[1] 0.8689175473 0.0532469600 0.0561824587 0.0205335642 0.0010100105
[6] 0.0001094593
```

It looks like about 86.9% are protein structures.

> Q3. 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?

It is not straight-forward to find all HIV-1 protease structures using plain text searching on the database.

Visualizing the HIV-1 protease structure

> Q4. Water molecules normally have 3 atoms. Why do we see just one atom per molecule in this structure?

Depending on the xray quality, it is hard to see the hydrogen atoms because they're so small.

> Q5. There is a critical “conserved” water molecule in the binding site. Can you identify this water molecule? What residue number does this water molecule have?

HOH 308

> Q6: 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 and the critical water (we recommend “Ball & Stick” for these side-chains). Add this figure to your Quarto document.

HIV figure

Introduction to Bio3D in R

We will use the `bio3d` package for this:

```
library(bio3d)
```

Reading PDB file data into R

```
# accessing online PDB file
pdb <- read.pdb("1hsg")
```

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

```
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
```

> Q7. How many amino acid residues are there in this pdb object?

There are 198 amino acid residues.

> Q8. Name one of the two non-protein residues?

Water (HOH)

> Q9. How many protein chains are in this structure?

There are 2 protein chains in this structure.

```
attributes(pdb)
```

```
$names
[1] "atom"   "xyz"    "seqres" "helix"  "sheet"  "calpha" "remark" "call"  

$class
[1] "pdb" "sse"
```

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

```
  type eleno elety  alt resid chain resno insert      x      y     z o     b
1 ATOM     1     N <NA>   PRO     A     1   <NA> 29.361 39.686 5.862 1 38.10
2 ATOM     2    CA <NA>   PRO     A     1   <NA> 30.307 38.663 5.319 1 40.62
3 ATOM     3     C <NA>   PRO     A     1   <NA> 29.760 38.071 4.022 1 42.64
4 ATOM     4     O <NA>   PRO     A     1   <NA> 28.600 38.302 3.676 1 43.40
5 ATOM     5    CB <NA>   PRO     A     1   <NA> 30.508 37.541 6.342 1 37.87
6 ATOM     6    CG <NA>   PRO     A     1   <NA> 29.296 37.591 7.162 1 38.40
  segid elesy charge
1  <NA>     N   <NA>
2  <NA>     C   <NA>
3  <NA>     C   <NA>
4  <NA>     O   <NA>
5  <NA>     C   <NA>
6  <NA>     C   <NA>
```

What is the first residue 3 letter code?

```
pdb$atom$resid[1]
```

```
[1] "PRO"
```

```
aa321(pdb$atom$resid[1])
```

```
[1] "P"
```

Predicting functional motions of a single structure

```
adk <- read.pdb("6s36")
```

```
  Note: Accessing on-line PDB file
   PDB has ALT records, taking A only, rm.alt=TRUE
```

```
adk
```

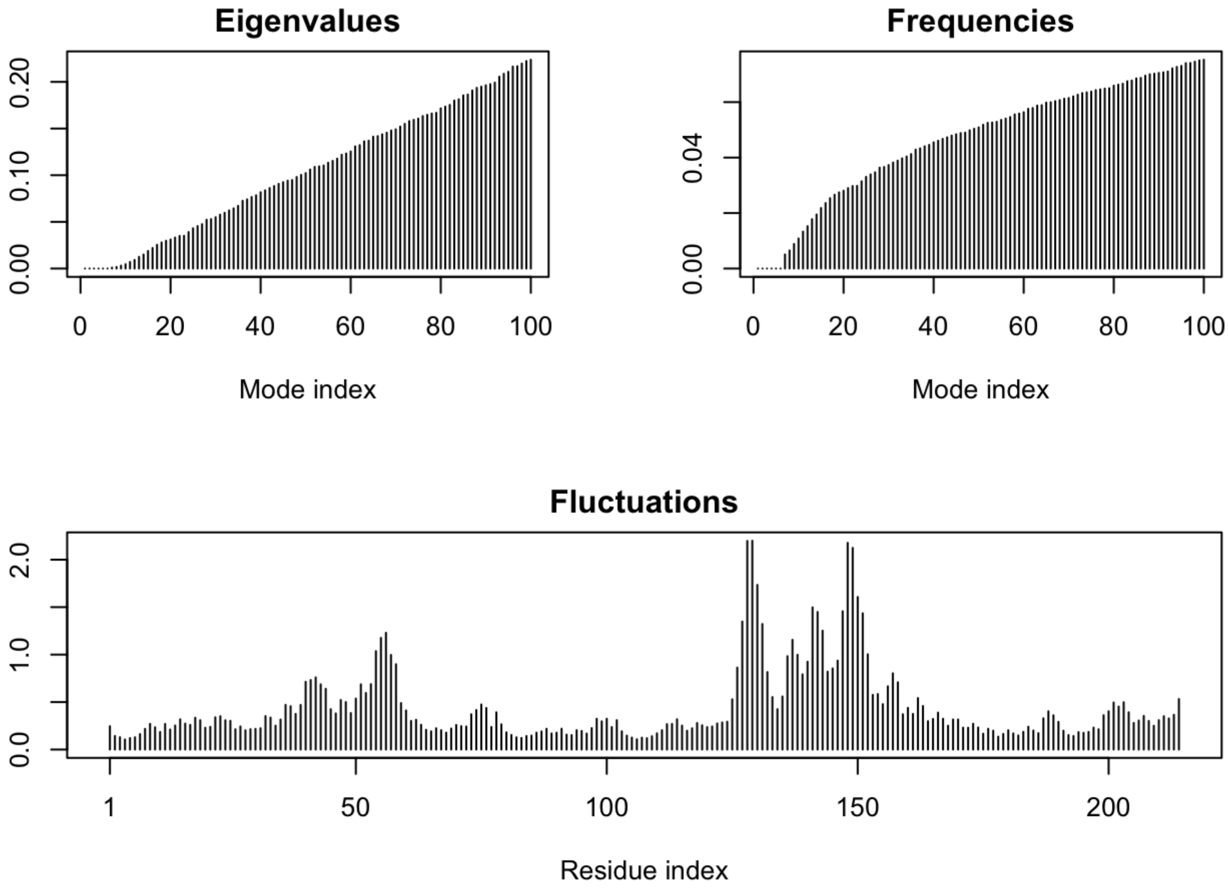
```

 Call:  read.pdb(file = "6s36")

   Total Models#: 1
     Total Atoms#: 1898,  XYZs#: 5694  Chains#: 1  (values: A)

     Protein Atoms#: 1654  (residues/Calpha atoms#: 214)
     Nucleic acid Atoms#: 0  (residues/phosphate atoms#: 0)

     Non-protein/nucleic Atoms#: 244  (residues: 244)
     Non-protein/nucleic resid values: [ CL (3), HOH (238), MG (2), NA (1) ]

   Protein sequence:
      MRIILLGAPGAGKGTQAQFIMEKYGIPQISTGDMLRAAVKSGSELGKQAKDIMDAGKLVT
      DELVIALVKERIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFDVPDELIVDKI
      VGRRVHAPSGRVYHVKFNPPKVEGKDDVTGEELTTRKDDQEETVRKRLVEYHQMTAPLIG
      YYSKEAEAGNTKYAKVDGTKPVAEVRADLEKILG

+ attr: atom, xyz, seqres, helix, sheet,
        calpha, remark, call
```

Normal mode analysis (NMA) is a structural bioinformatics method to predict protein flexibility and potential functional motions (aka conformational changes).

```
# perform flexibility prediction
m <- nma(adk)
```

```
 Building Hessian...        Done in 0.031 seconds.
 Diagonalizing Hessian...   Done in 0.308 seconds.
```

```
plot(m)
```



```r
mktrj(m, file="adk_m7.pdb")
```

Comparative structure analysis of Adenylate Kinase

Today we are continuing where we left off last day building towards completing the loop from biomolecular structural data to our new analysis methods like PCA and clustering.

Install bio3d, devtools, and BiocManager (msa).

> Q10. Which of the packages above is found only on BioConductor and not CRAN?

msa is found only on BioConductor.

> Q11. Which of the above packages is not found on BioConductor or CRAN?

bio3d-view

> Q12. True or False? Functions from the devtools package can be used to install packages from GitHub and BitBucket?

True

Search and retrieve ADK structures

```
library(bio3d)
aa <- get.seq("1ake_A")
```

```
Warning in get.seq("1ake_A"): Removing existing file: seqs.fasta

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

```
aa
```

```
             1        .         .         .         .         .         60 
pdb|1AKE|A   MRIILLGAPGAGKGTQAQFIMEKYGIPQISTGDMLRAAVKSGSELGKQAKDIMDAGKLVT
             1        .         .         .         .         .         60 

            61        .         .         .         .         .         120 
pdb|1AKE|A   DELVIALVKERIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFDVPDELIVDRI
            61        .         .         .         .         .         120 

           121        .         .         .         .         .         180 
pdb|1AKE|A   VGRRVHAPSGRVYHVKFNPPKVEGKDDVTGEELTTRKDDQEETVRKRLVEYHQMTAPLIG
           121        .         .         .         .         .         180 

           181        .         .         .   214 
pdb|1AKE|A   YYSKEAEAGNTKYAKVDGTKPVAEVRADLEKILG
           181        .         .         .   214 

Call:
  read.fasta(file = outfile)

Class:
  fasta

Alignment dimensions:
  1 sequence rows; 214 position columns (214 non-gap, 0 gap)

+ attr: id, ali, call
```

> Q13. How many amino acids are in this sequence?

There are 214 amino acids.

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

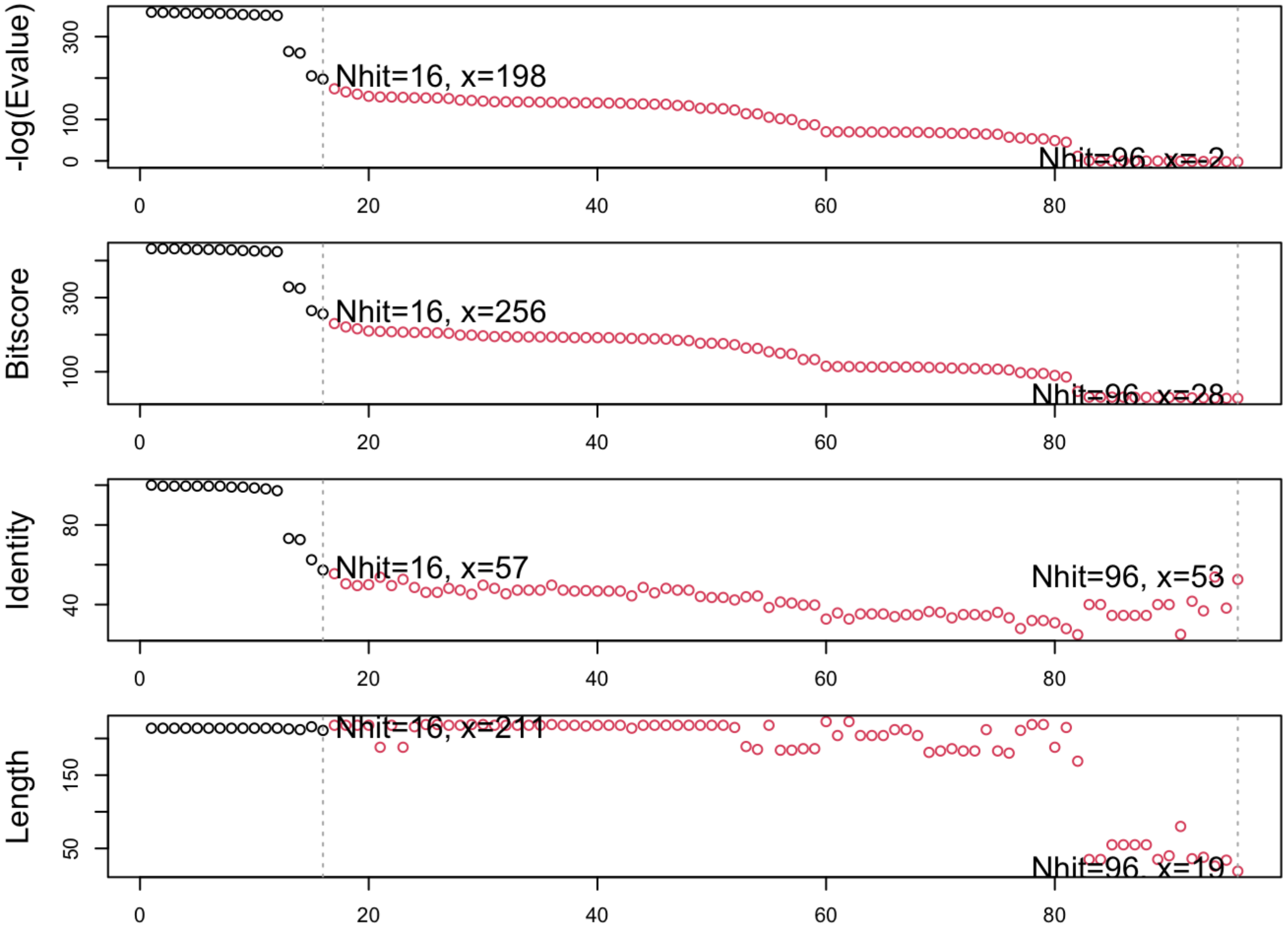
I could save and load my blast results next time so I don’t need to run the search every time.

```
# saveRDS(b, file = "blast_results.RDS")
```

```
b <- readRDS(file = "blast_results.RDS")
```

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

```
  * Possible cutoff values:    197 -3
            Yielding Nhits:    16 96

  * Chosen cutoff value of:    197
            Yielding Nhits:    16
```



```
# list out some 'top hits'
head(hits$pdb.id)
```

```
[1] "1AKE_A" "4X8M_A" "6S36_A" "6RZE_A" "4X8H_A" "3HPR_A"
```

```r
hits <- NULL
hits$pdb.id <- c('1AKE_A','6S36_A','6RZE_A','3HPR_A','1E4V_A','5EJE_A','1E4Y_A','3X2S_A',
```

```r
# download related PDB files
files <- get.pdb(hits$pdb.id, path="pdbs", split=TRUE, gzip=TRUE)
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/1AKE.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/6S36.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/6RZE.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/3HPR.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/1E4V.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/5EJE.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/1E4Y.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/3X2S.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/6HAP.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/6HAM.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/4K46.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/3GMT.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/4PZL.pdb.gz exists. Skipping download
```

```
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  |
  |===========================================================           |  85%
  |
  |=================================================================     |  92%
  |
  |======================================================================| 100%
```
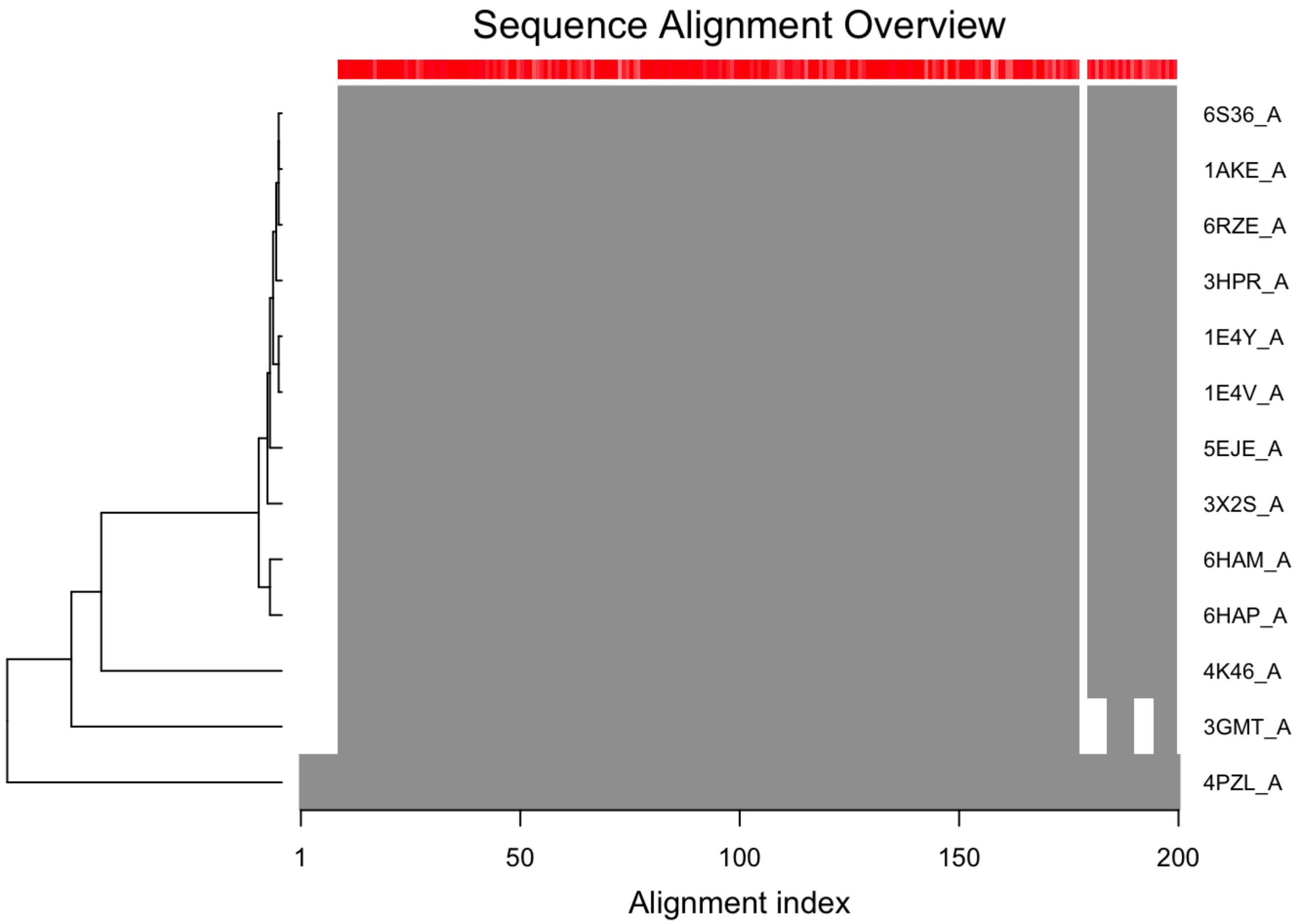

Align and superpose structures

```r
# align related PDBs
pdbs <- pdbaln(files, fit = TRUE, exefile = "msa")
```

```
Reading PDB files:
pdbs/split_chain/1AKE_A.pdb
pdbs/split_chain/6S36_A.pdb
pdbs/split_chain/6RZE_A.pdb
pdbs/split_chain/3HPR_A.pdb
pdbs/split_chain/1E4V_A.pdb
pdbs/split_chain/5EJE_A.pdb
pdbs/split_chain/1E4Y_A.pdb
pdbs/split_chain/3X2S_A.pdb
pdbs/split_chain/6HAP_A.pdb
pdbs/split_chain/6HAM_A.pdb
pdbs/split_chain/4K46_A.pdb
pdbs/split_chain/3GMT_A.pdb
pdbs/split_chain/4PZL_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
.   PDB has ALT records, taking A only, rm.alt=TRUE
.   PDB has ALT records, taking A only, rm.alt=TRUE
.   PDB has ALT records, taking A only, rm.alt=TRUE
..   PDB has ALT records, taking A only, rm.alt=TRUE
....   PDB has ALT records, taking A only, rm.alt=TRUE
.   PDB has ALT records, taking A only, rm.alt=TRUE
...

Extracting sequences

pdb/seq: 1   name: pdbs/split_chain/1AKE_A.pdb 
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 2   name: pdbs/split_chain/6S36_A.pdb 
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 3   name: pdbs/split_chain/6RZE_A.pdb 
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 4   name: pdbs/split_chain/3HPR_A.pdb 
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 5   name: pdbs/split_chain/1E4V_A.pdb 
pdb/seq: 6   name: pdbs/split_chain/5EJE_A.pdb 
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 7   name: pdbs/split_chain/1E4Y_A.pdb 
pdb/seq: 8   name: pdbs/split_chain/3X2S_A.pdb 
pdb/seq: 9   name: pdbs/split_chain/6HAP_A.pdb 
pdb/seq: 10   name: pdbs/split_chain/6HAM_A.pdb 
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 11   name: pdbs/split_chain/4K46_A.pdb 
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 12   name: pdbs/split_chain/3GMT_A.pdb 
pdb/seq: 13   name: pdbs/split_chain/4PZL_A.pdb 
```

```r
# vector containing PDB codes for figure axis
ids <- basename.pdb(pdbs$id)

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



Grey regions = aligned residues White regions = gap regions Red bar = sequence conservation

Annotate collected PDB structures

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

```
[1] "Escherichia coli"
[2] "Escherichia coli K-12"
[3] "Escherichia coli O139:H28 str. E24377A"
[4] "Escherichia coli str. K-12 substr. MDS42"
[5] "Photobacterium profundum"
[6] "Burkholderia pseudomallei 1710b"
[7] "Francisella tularensis subsp. tularensis SCHU S4"
```

We can view all available annotation data:

```
anno
```

```
       structureId chainId macromoleculeType chainLength experimentalTechnique
1AKE_A        1AKE       A           Protein         214                 X-ray
6S36_A        6S36       A           Protein         214                 X-ray
```

```
6RZE_A        6RZE       A           Protein         214                 X-ray
3HPR_A        3HPR       A           Protein         214                 X-ray
1E4V_A        1E4V       A           Protein         214                 X-ray
5EJE_A        5EJE       A           Protein         214                 X-ray
1E4Y_A        1E4Y       A           Protein         214                 X-ray
3X2S_A        3X2S       A           Protein         214                 X-ray
6HAP_A        6HAP       A           Protein         214                 X-ray
6HAM_A        6HAM       A           Protein         214                 X-ray
4K46_A        4K46       A           Protein         214                 X-ray
3GMT_A        3GMT       A           Protein         230                 X-ray
4PZL_A        4PZL       A           Protein         242                 X-ray
       resolution       scopDomain                   pfam         ligandId
1AKE_A       2.00 Adenylate kinase Adenylate kinase (ADK)              AP5
6S36_A       1.60             <NA> Adenylate kinase (ADK) CL (3),NA,MG (2)
6RZE_A       1.69             <NA> Adenylate kinase (ADK)    NA (3),CL (2)
3HPR_A       2.00             <NA> Adenylate kinase (ADK)              AP5
1E4V_A       1.85 Adenylate kinase Adenylate kinase (ADK)              AP5
5EJE_A       1.90             <NA> Adenylate kinase (ADK)           AP5,CO
1E4Y_A       1.85 Adenylate kinase Adenylate kinase (ADK)              AP5
3X2S_A       2.80             <NA> Adenylate kinase (ADK)   JPY (2),AP5,MG
6HAP_A       2.70             <NA> Adenylate kinase (ADK)              AP5
6HAM_A       2.55             <NA> Adenylate kinase (ADK)              AP5
4K46_A       2.01             <NA> Adenylate kinase (ADK)      ADP,AMP,PO4
3GMT_A       2.10             <NA> Adenylate kinase (ADK)          SO4 (2)
4PZL_A       2.10             <NA> Adenylate kinase (ADK)       CA,FMT,GOL
                                                                             ligandName
1AKE_A                                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE
6S36_A                                    CHLORIDE ION (3),SODIUM ION,MAGNESIUM ION (2)
6RZE_A                                                  SODIUM ION (3),CHLORIDE ION (2)
3HPR_A                                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE
1E4V_A                                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE
5EJE_A                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE,COBALT (II) ION
1E4Y_A                                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE
3X2S_A N-(pyren-1-ylmethyl)acetamide (2),BIS(ADENOSINE)-5'-PENTAPHOSPHATE,MAGNESIUM ION
6HAP_A                                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE
6HAM_A                                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE
4K46_A                   ADENOSINE-5'-DIPHOSPHATE,ADENOSINE MONOPHOSPHATE,PHOSPHATE ION
3GMT_A                                                                  SULFATE ION (2)
4PZL_A                                                 CALCIUM ION,FORMIC ACID,GLYCEROL
                                                 source
1AKE_A                                 Escherichia coli
6S36_A                                 Escherichia coli
6RZE_A                                 Escherichia coli
3HPR_A                            Escherichia coli K-12
1E4V_A                                 Escherichia coli
5EJE_A           Escherichia coli O139:H28 str. E24377A
1E4Y_A                                 Escherichia coli
3X2S_A         Escherichia coli str. K-12 substr. MDS42
6HAP_A           Escherichia coli O139:H28 str. E24377A
6HAM_A                            Escherichia coli K-12
4K46_A                         Photobacterium profundum
```

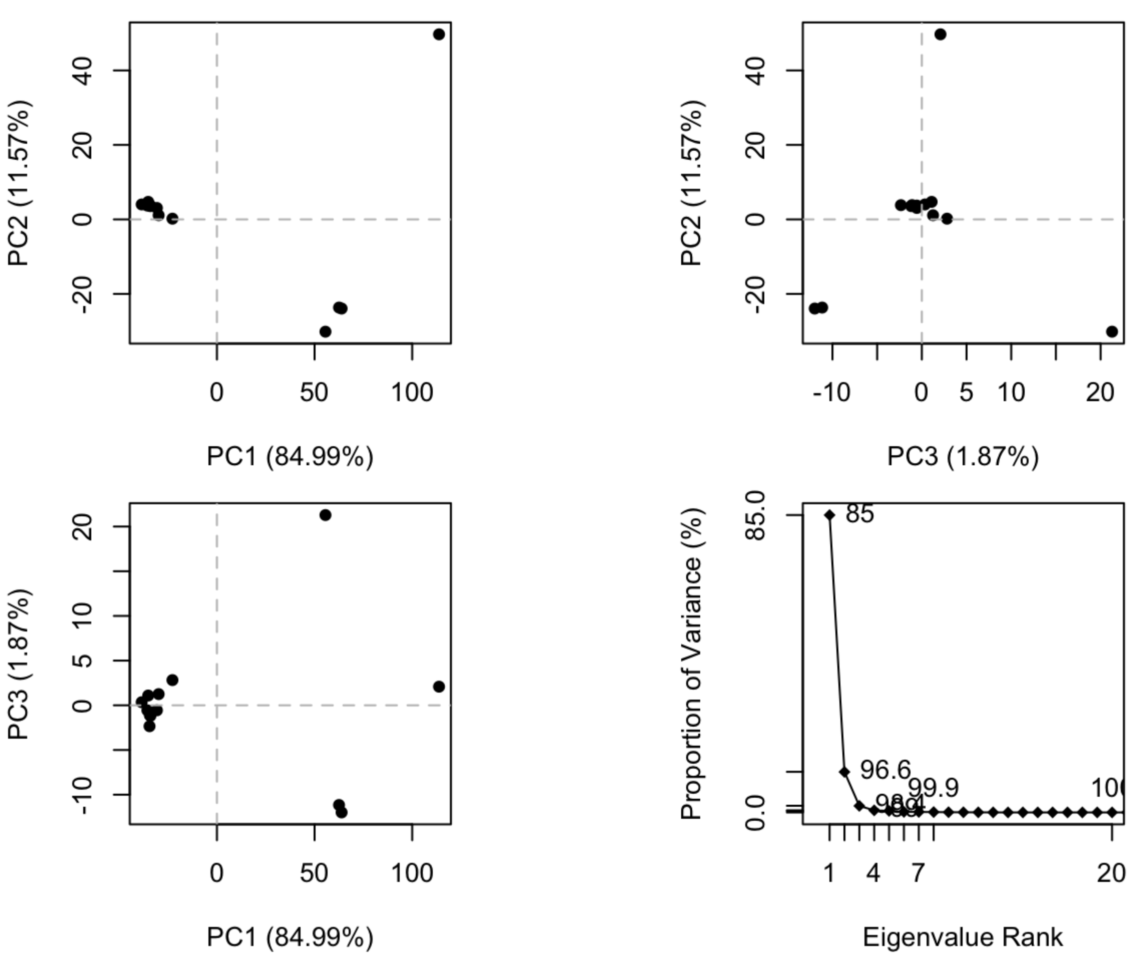
```
3GMT_A                  Burkholderia pseudomallei 1710b
4PZL_A Francisella tularensis subsp. tularensis SCHU S4

structureTitle
1AKE_A STRUCTURE OF THE COMPLEX BETWEEN ADENYLATE KINASE FROM ESCHERICHIA COLI AND THE
INHIBITOR AP5A REFINED AT 1.9 ANGSTROMS RESOLUTION: A MODEL FOR A CATALYTIC TRANSITION
STATE
6S36_A
Crystal structure of E. coli Adenylate kinase R119K mutant
6RZE_A
Crystal structure of E. coli Adenylate kinase R119A mutant
3HPR_A
Crystal structure of V148G adenylate kinase from E. coli, in complex with Ap5A
1E4V_A
Mutant G10V of adenylate kinase from E. coli, modified in the Gly-loop
5EJE_A
Crystal structure of E. coli Adenylate kinase G56C/T163C double mutant in complex with
Ap5a
1E4Y_A
Mutant P9L of adenylate kinase from E. coli, modified in the Gly-loop
3X2S_A
Crystal structure of pyrene-conjugated adenylate kinase
6HAP_A
Adenylate kinase
6HAM_A
Adenylate kinase
4K46_A
Crystal Structure of Adenylate Kinase from Photobacterium profundum
3GMT_A
Crystal structure of adenylate kinase from burkholderia pseudomallei
4PZL_A                                                                              The
crystal structure of adenylate kinase from Francisella tularensis subsp. tularensis SCHU
S4
                                                     citation rObserved   rFree
1AKE_A                 Muller, C.W., et al. J Mol Biol (1992)   0.19600      NA
6S36_A                  Rogne, P., et al. Biochemistry (2019)   0.16320 0.23560
6RZE_A                  Rogne, P., et al. Biochemistry (2019)   0.18650 0.23500
3HPR_A  Schrank, T.P., et al. Proc Natl Acad Sci U S A (2009)   0.21000 0.24320
1E4V_A                   Muller, C.W., et al. Proteins (1993)   0.19600      NA
5EJE_A  Kovermann, M., et al. Proc Natl Acad Sci U S A (2017)   0.18890 0.23580
1E4Y_A                   Muller, C.W., et al. Proteins (1993)   0.17800      NA
3X2S_A                Fujii, A., et al. Bioconjug Chem (2015)   0.20700 0.25600
6HAP_A               Kantaev, R., et al. J Phys Chem B (2018)   0.22630 0.27760
6HAM_A               Kantaev, R., et al. J Phys Chem B (2018)   0.20511 0.24325
4K46_A                    Cho, Y.-J., et al. To be published    0.17000 0.22290
3GMT_A Buchko, G.W., et al. Biochem Biophys Res Commun (2010)   0.23800 0.29500
4PZL_A                       Tan, K., et al. To be published    0.19360 0.23680
         rWork spaceGroup
1AKE_A 0.19600  P 21 2 21
6S36_A 0.15940    C 1 2 1
6RZE_A 0.18190    C 1 2 1
```

```
3HPR_A 0.20620   P 21 21 2
1E4V_A 0.19600   P 21 2 21
5EJE_A 0.18630   P 21 2 21
1E4Y_A 0.17800    P 1 21 1
3X2S_A 0.20700 P 21 21 21
6HAP_A 0.22370     I 2 2 2
6HAM_A 0.20311        P 43
4K46_A 0.16730 P 21 21 21
3GMT_A 0.23500    P 1 21 1
4PZL_A 0.19130        P 32
```

Principal component analysis

We will use the `pca()` function from the `bio3d` package as this one is designed to work nicely with biomolecular data.

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



These are the results of PCA on Adenylate kinase X-ray structures. Each dot represents one PDB structure.

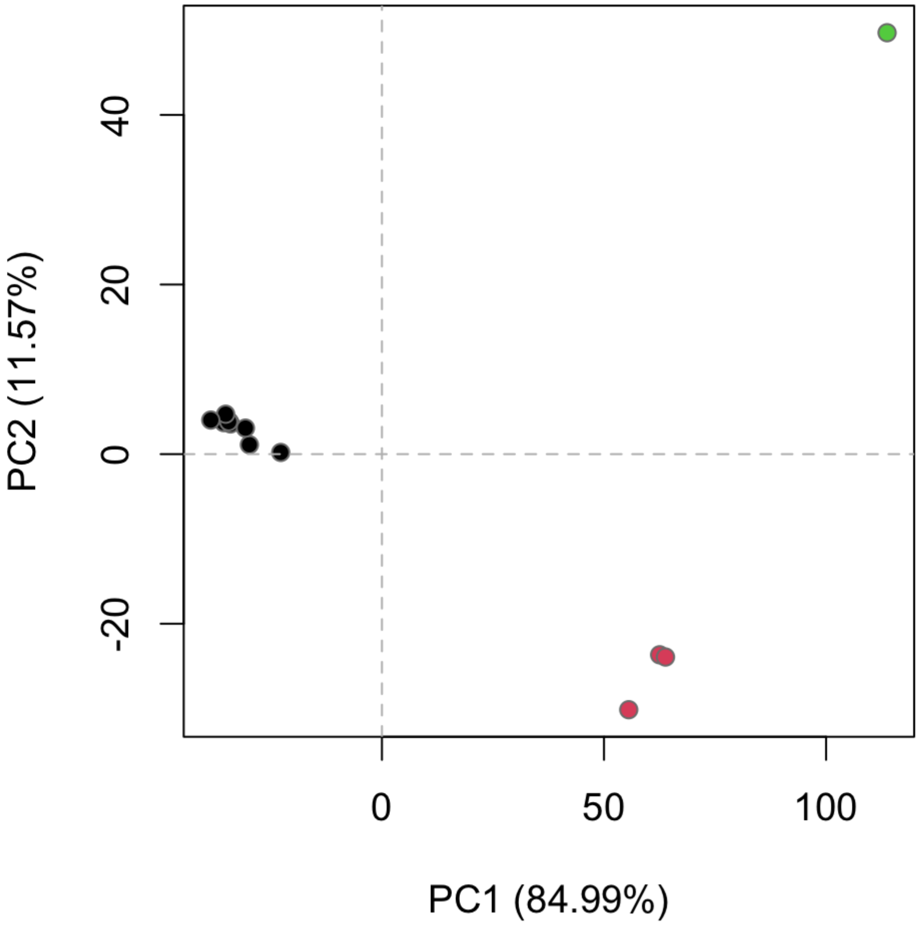
We can focus in on PC1 and PC2.

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)
```

```
Warning in rmsd(pdbs): No indices provided, using the 204 non NA positions
```

```
# 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)
```

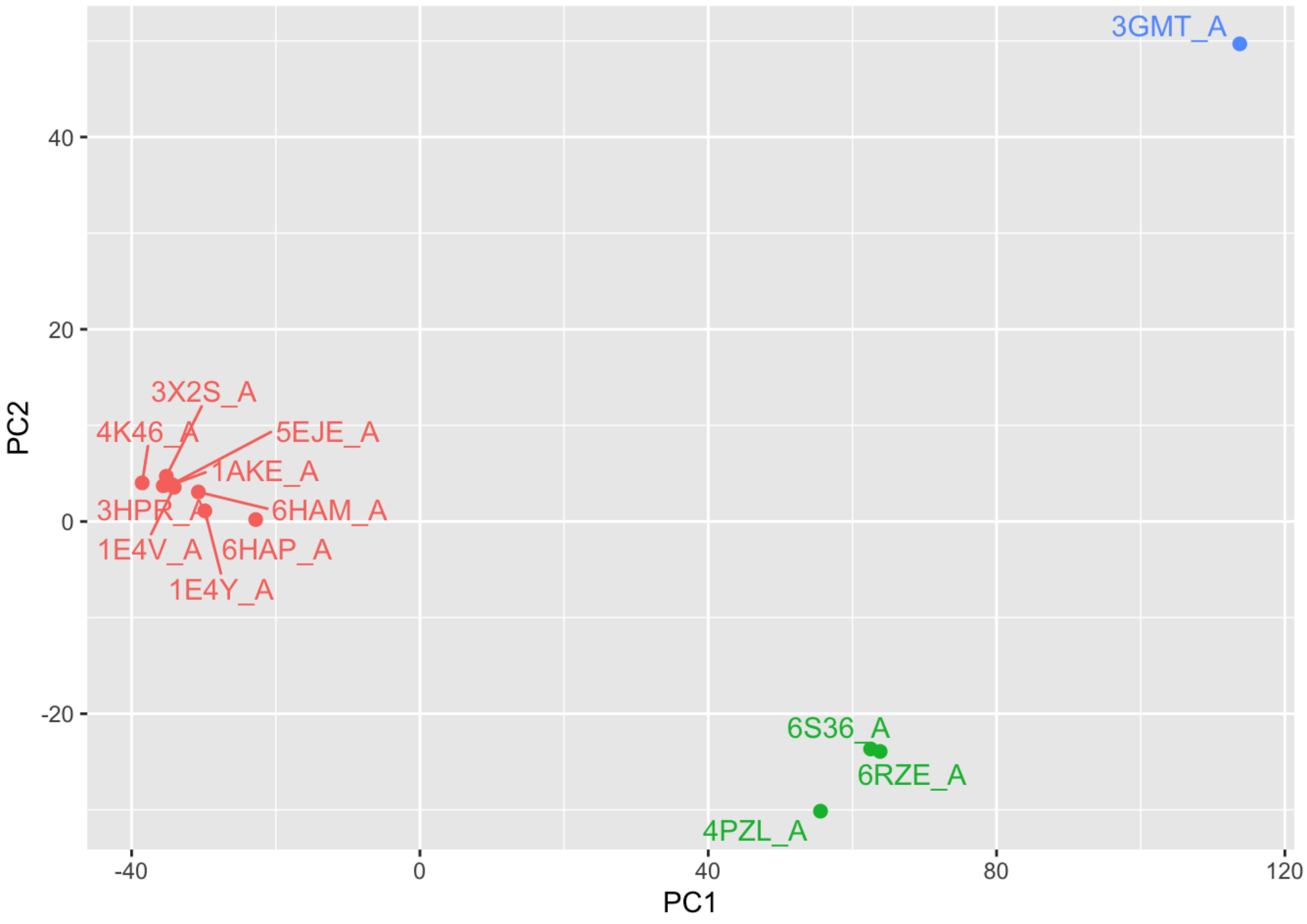


Optional further visualization

```
# visualize first principal component
pc1 <- mktrj(pc.xray, pc = 1, file = "pc_1.pdb")
```

You can view this in Molstar by opening the “pc_1.pdb” file. You can also look at the animations.

```
# plotting results with ggplot2
library(ggplot2)
library(ggrepel)

df <- data.frame(PC1 = pc.xray$z[, 1], PC2 = pc.xray$z[, 2], col = as.factor(grps.rd), id

p <- ggplot(df) +
  aes(PC1, PC2, col = col, label = ids) +
  geom_point(size = 2) +
  geom_text_repel(max.overlaps = 20) +
  theme(legend.position = "none")
p
```



Normal mode analysis

Function `nma()` provides normal mode analysis (NMA) on both single structures (if given a single PDB input object) or the complete structure ensemble (if provided with a PDBS input object). This facilitates characterizing and comparing flexibility profiles of related protein structures.

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

```

Details of Scheduled Calculation:
  ... 13 input structures 
  ... storing 606 eigenvectors for each structure 
  ... dimension of x$U.subspace: ( 612x606x13 )
  ... coordinate superposition prior to NM calculation 
  ... aligned eigenvectors (gap containing positions removed)  
  ... estimated memory usage of final 'eNMA' object: 36.9 Mb 


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```
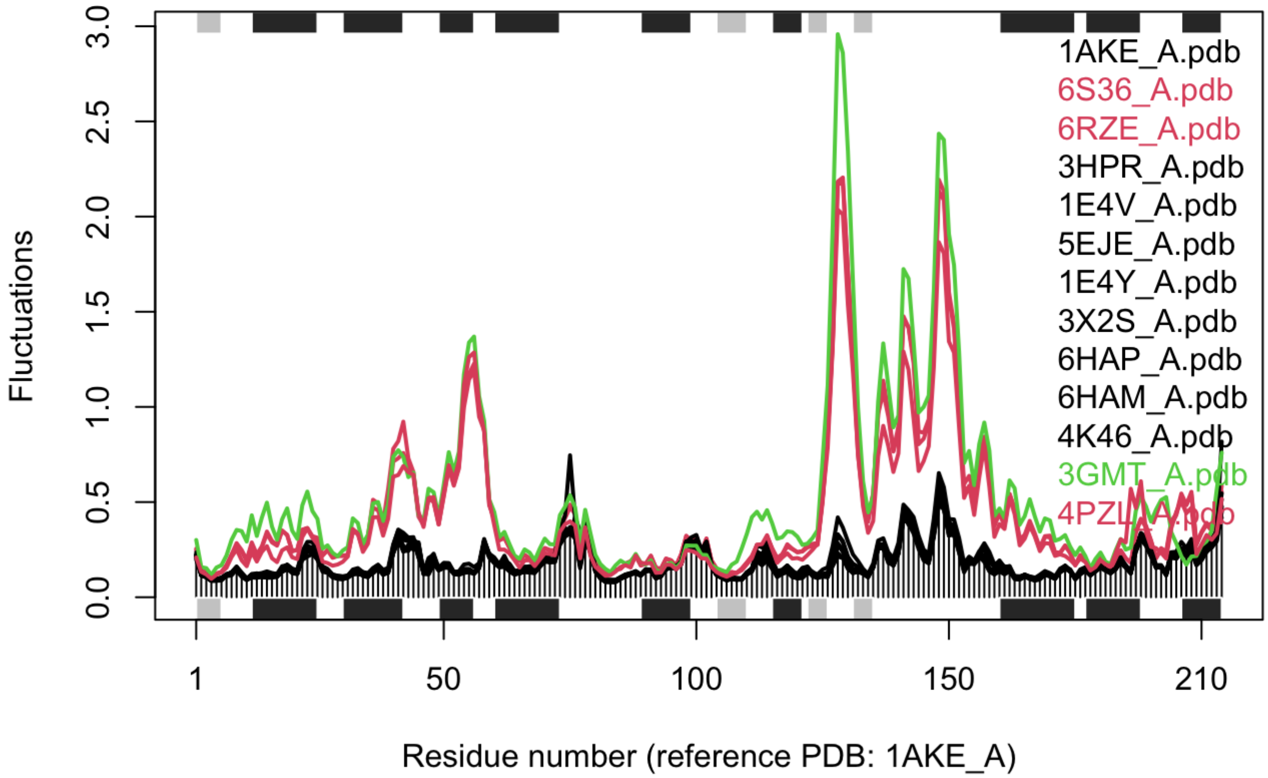

```r
plot(modes, pdbs, col = grps.rd)
```

```
Extracting SSE from pdbs$sse attribute
```



> Q14. What do you note about this plot? Are the black and colored lines similar or different? Where do you think they differ most and why?

The black and colored lines are different at many points. They differ around residues 50 and in between 100 and 150, or basically around where there are higher fluctuations.