Class 9: Structural Bioinformatics 1.

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The RCSB Protein Data Bank (PDB)

Protein structures by X-ray crystallography dominate this database. We are skipping Q1-2 as the website was too slow.

Visualizing the HIV-1 protease structure

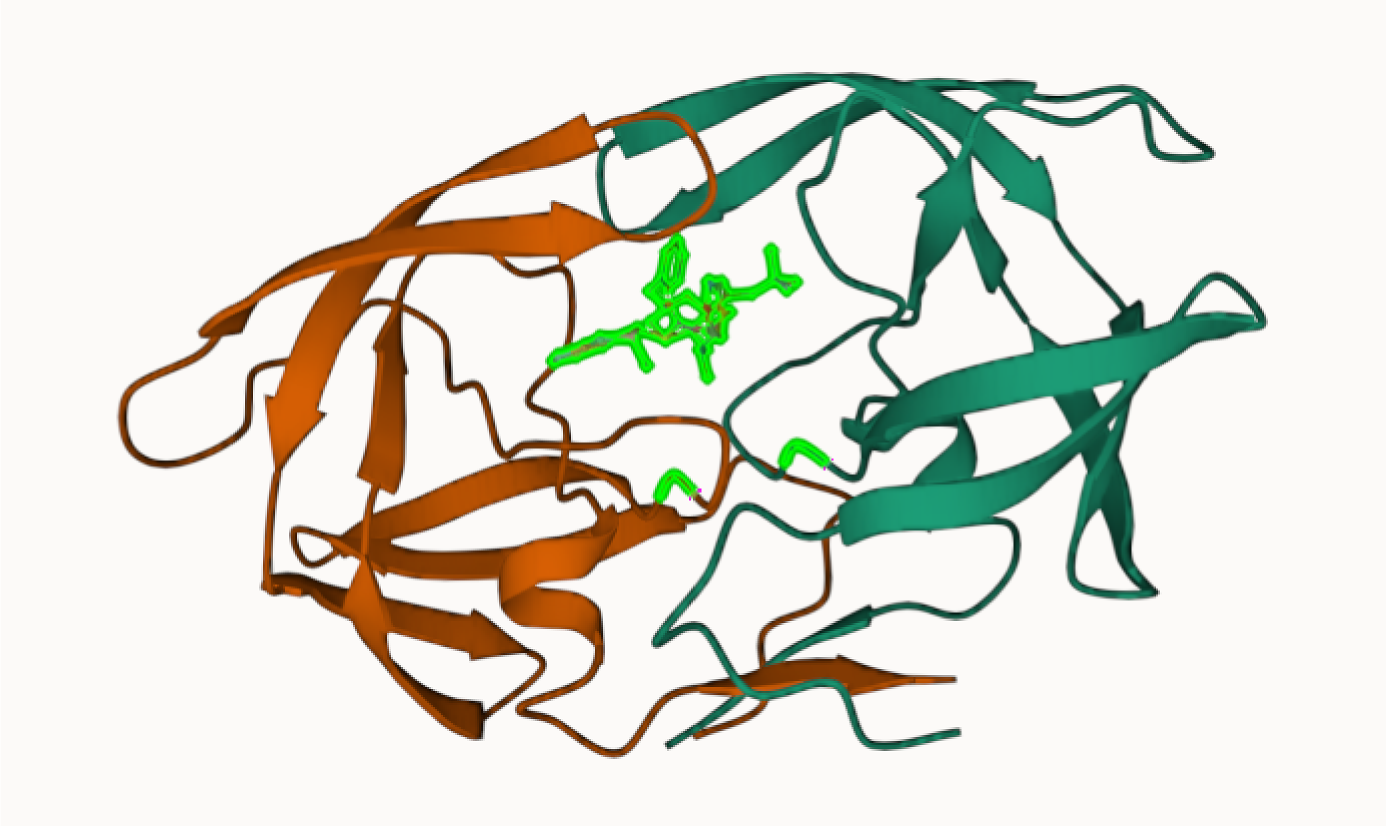
> Question 4: Water molecules normally have 3 atoms. Why do we see just one atom per water molecule in this structure?

We only see one atom per water molecule because the hydrogens are too small to be seen, so only the oxygens are visible at this resolution.

> Question 5: 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?

The critical conserved water molecule is near the ligand at residue number 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 (we recommend "Ball & Stick" for these side-chains). Add this figure to your Quarto document.



HIV-Pr structure from 1hsg

3. Introduction to Bio3D in R

Bio3D is an R package for structural bioinformatics. To use it we need to call it with `library()` function (just like any package).

```
library('bio3d')
```

TO read a PDB file we can use `read.pdb()`

```
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 acids

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

One of the two non-protein residues is MK1, the drug ligand.

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

Threre are two chains in this protein structure.

```
attributes(pdb)
```

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

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

THe ATOM records of a PDB file are stored in `pdb$atom`

```
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>
```

4. Comparative structure analysis of Adenylate Kinase (ADK)

Installed packages in console.

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

msa is found only on BioConductor and not CRAN.

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

bio3d-view is not found on BioConductor or CRAN.

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

TRUE.

We will start our analysis with a single PDB id (code from the PDB database): 1AKE

First we get it’s primary sequence:

```
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, i.e. how long is this sequence?

There are 214 amino acids in this sequence.

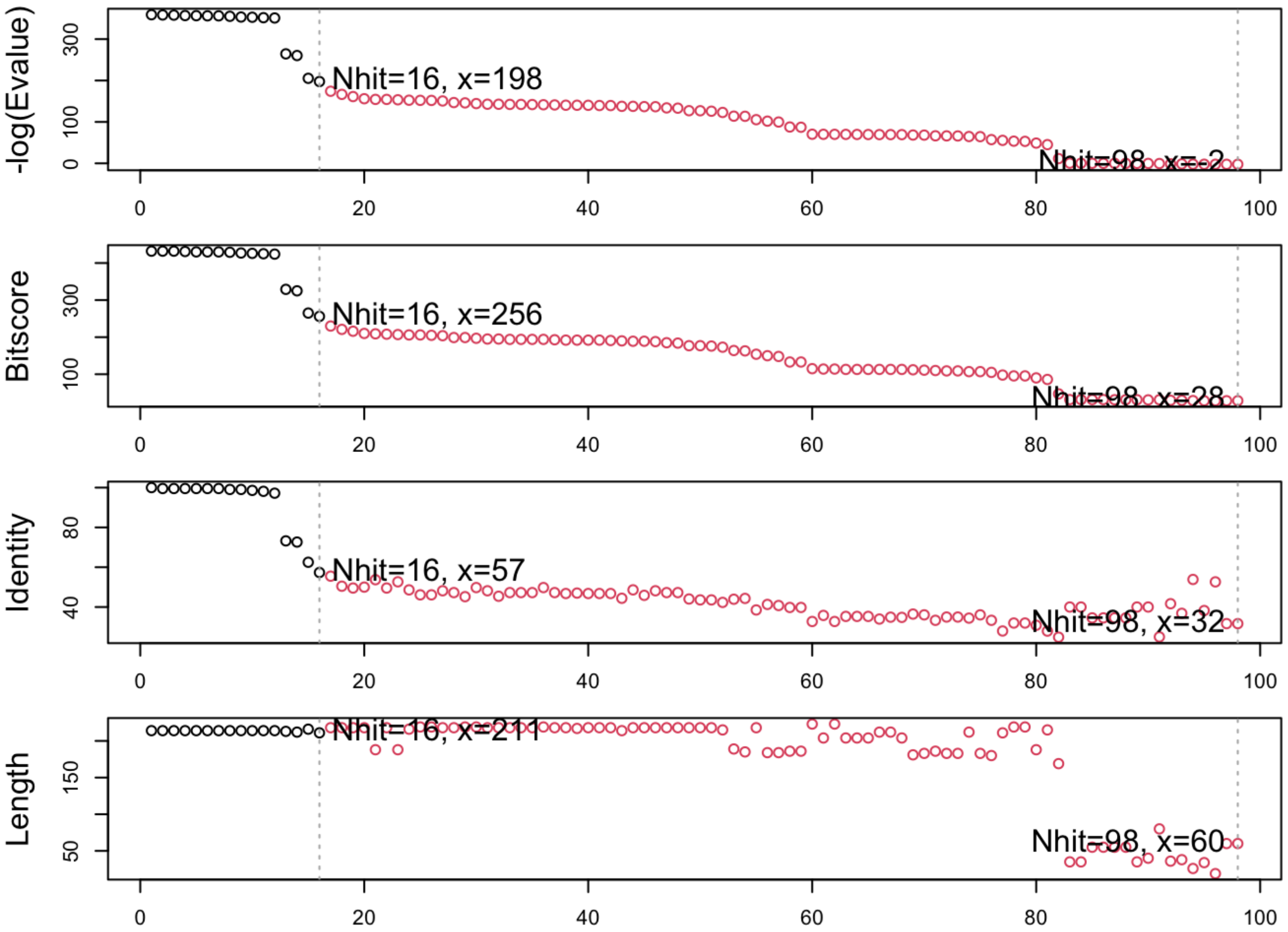
```
# Blast or hmmer search
b <- blast.pdb(aa)
```

```
 Searching ... please wait (updates every 5 seconds) RID = NGC8HYNT013 
 .........................
 Reporting 98 hits
```

```
hits <-  plot(b)
```

```
  * Possible cutoff values:    197 -3 
            Yielding Nhits:    16 98 

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



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

Use these ADK structures for analysis

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

Download all these PDB files from the online database

```
# 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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```

Align and superose structures

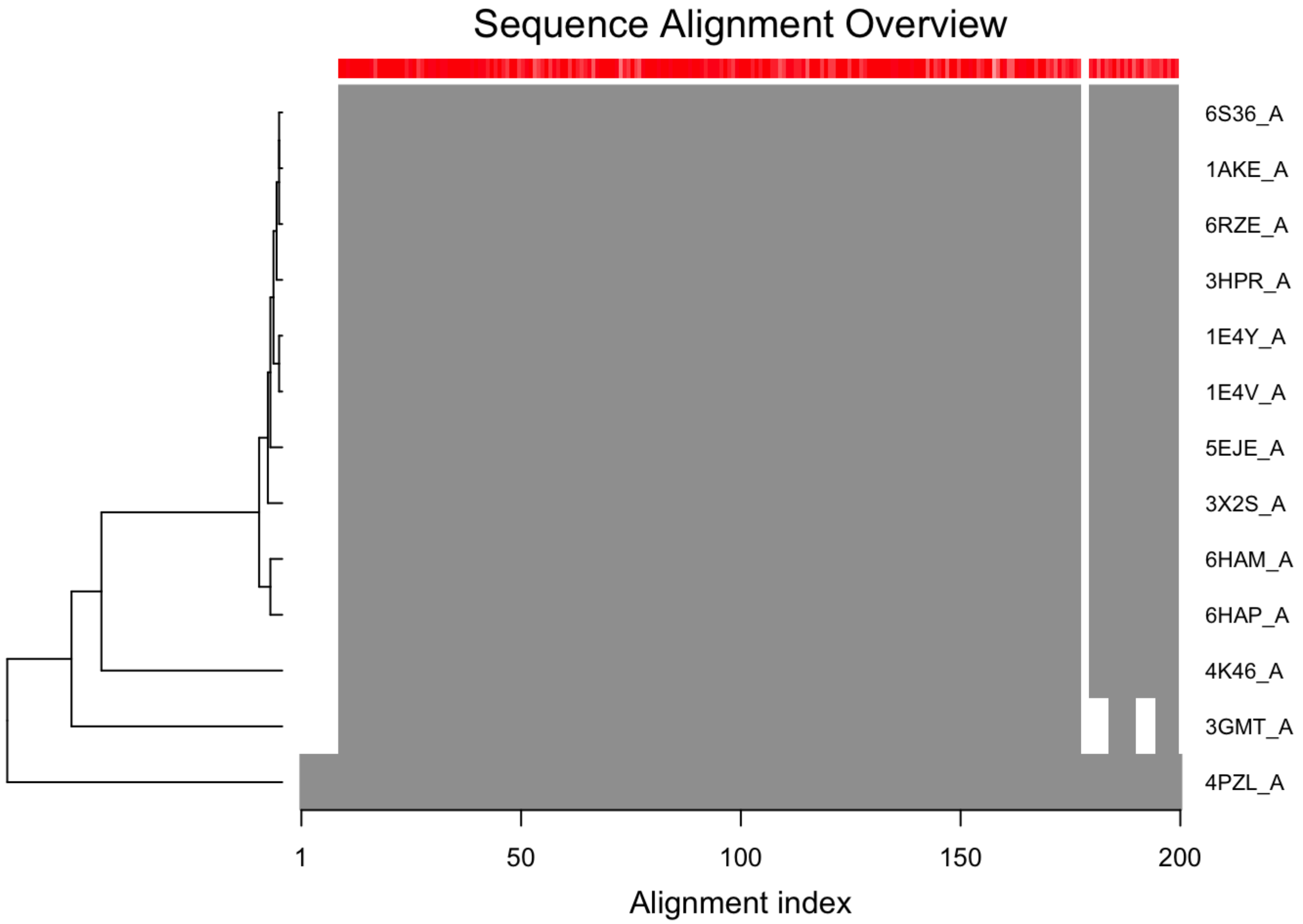
Align all these structures

```r
# Align releated 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: 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
```

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

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



Annotate collected PDB structures

Annotating 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"
```

Viewing 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
```

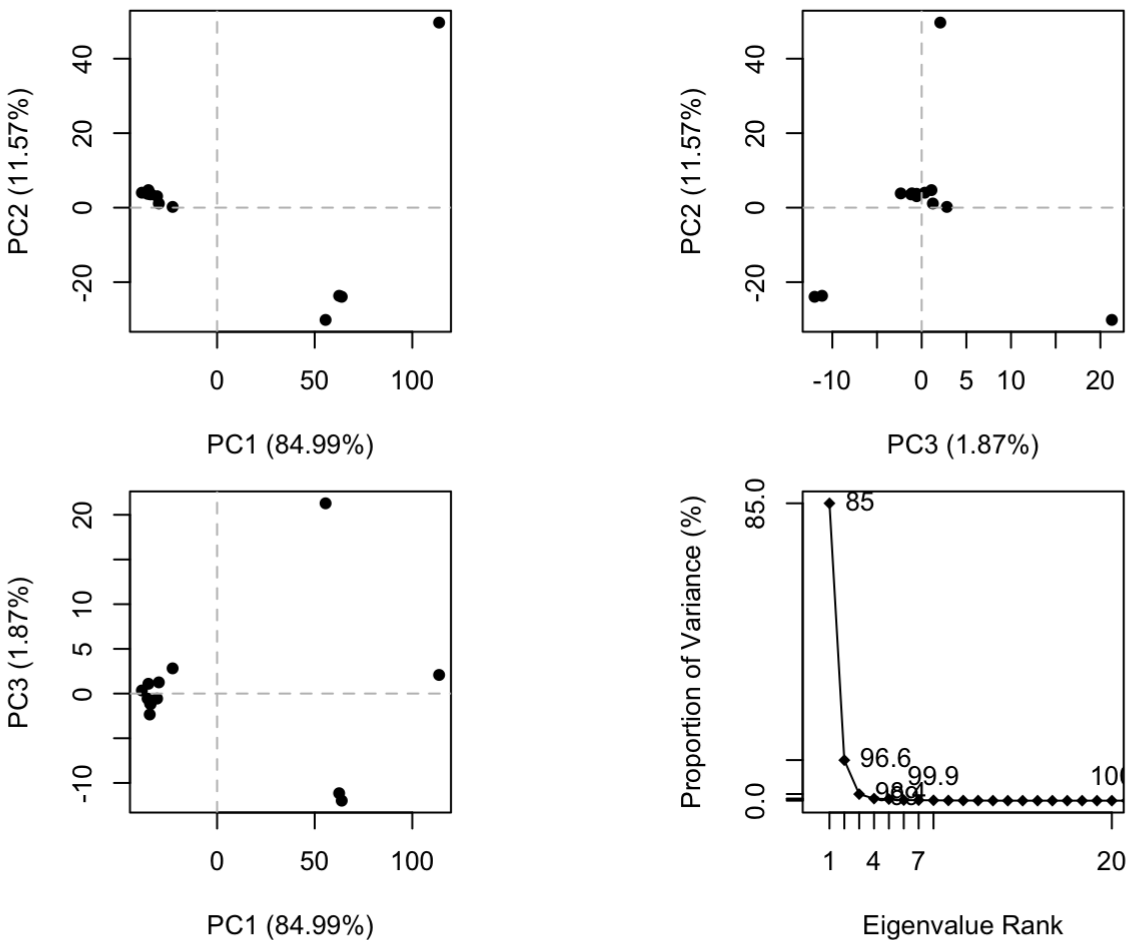
```
5EJE_A                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE,COBALT (II) ION
1E4Y_A                                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE
3X2S_A N-(pyren-1-ylmethyl)acetamide (2),BIS(ADENOSINE)-5'-PENTAPHOSPHATE,MAGNESIUM ION
6HAP_A                                                 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

Performing PCA

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

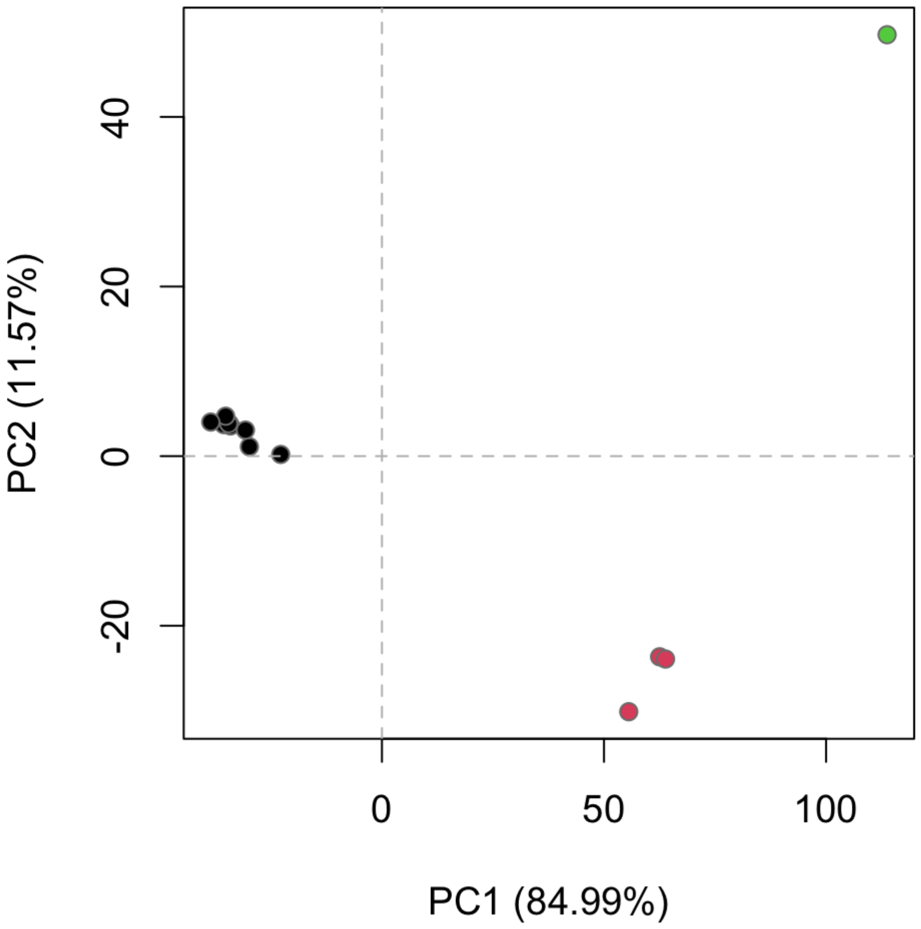


Calculating pairwise RMSD values

```
# 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)
```

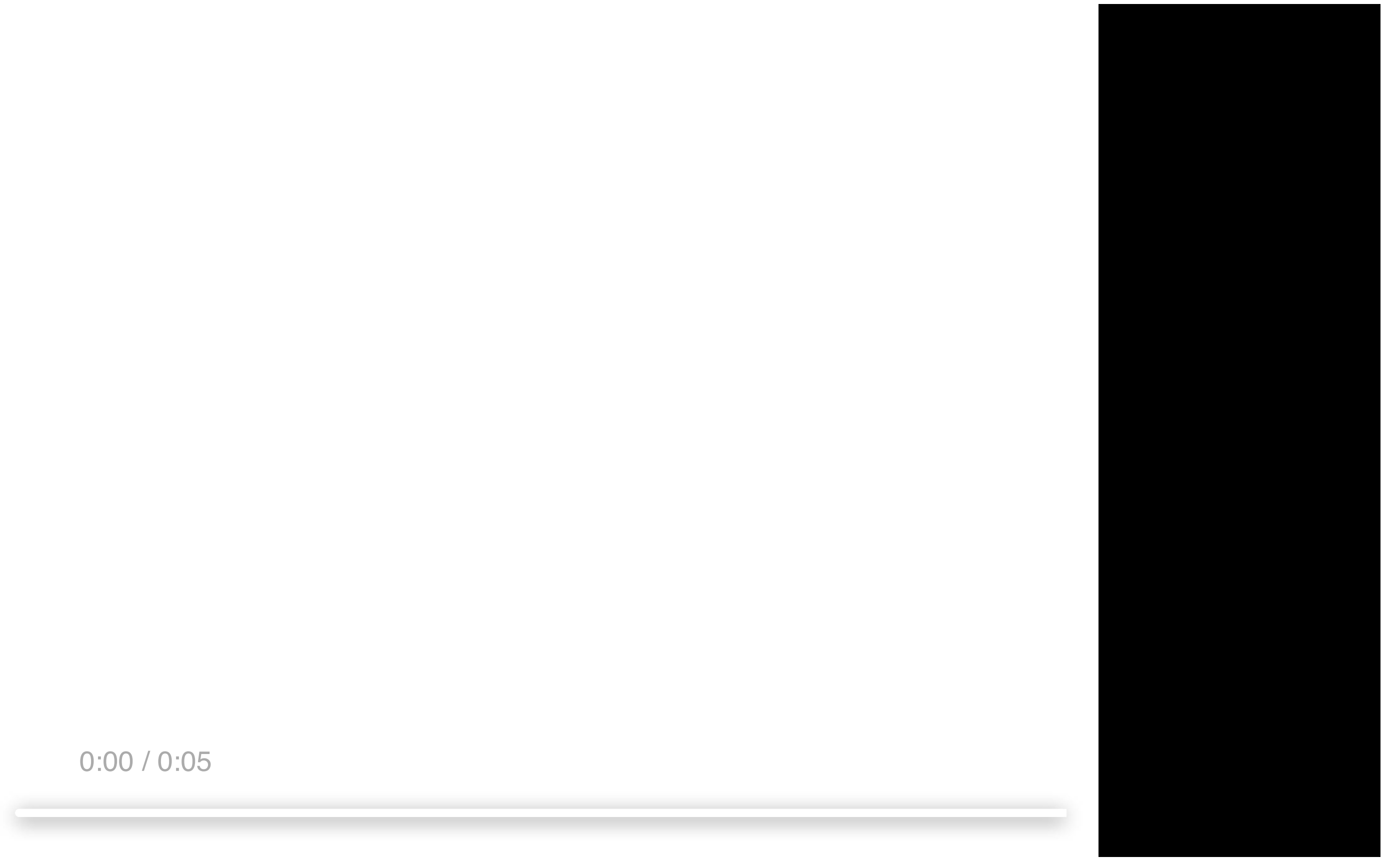


5. Optional Further Visualization

Trying to visualize major structural variation

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

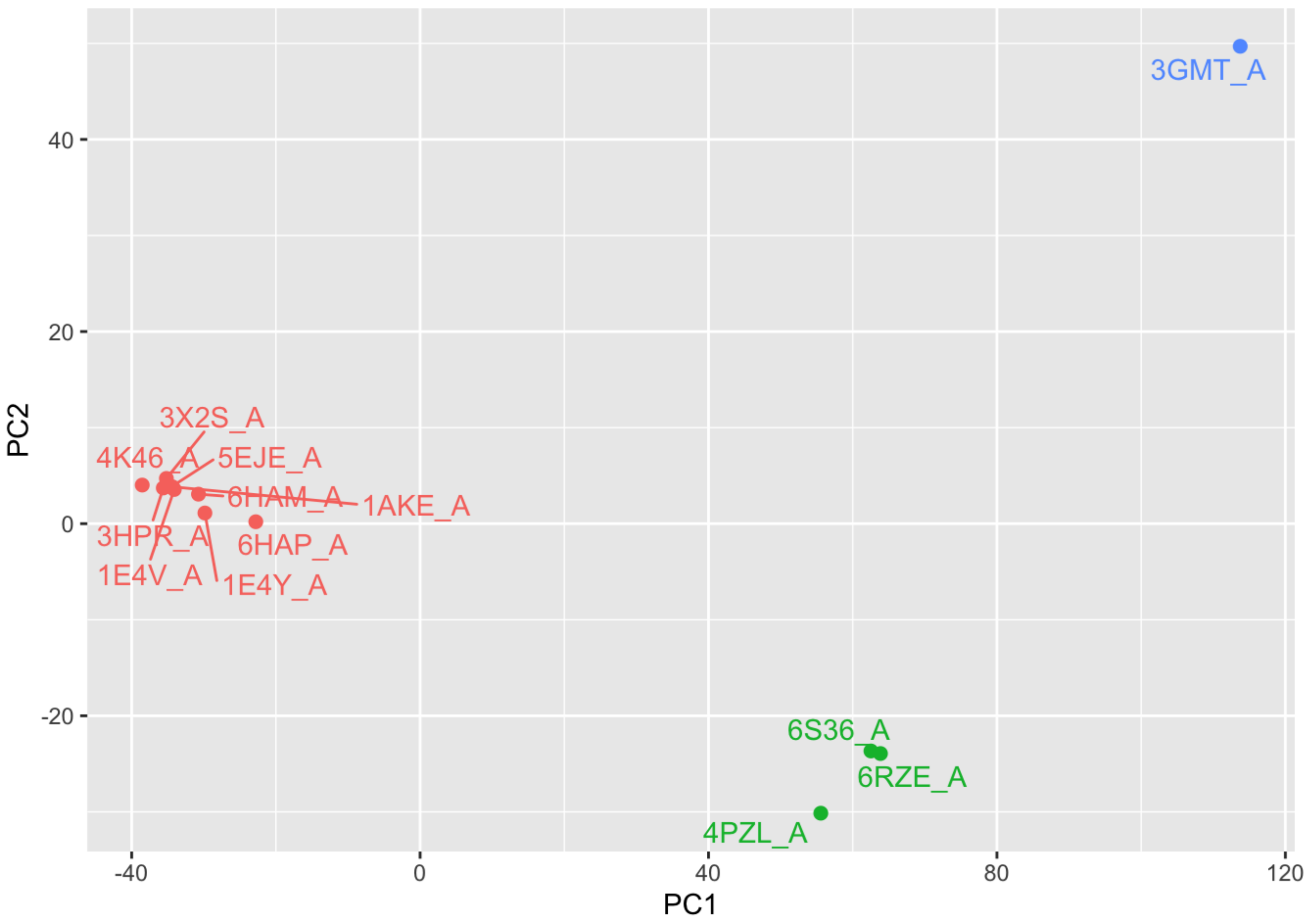
Animated visualizations



Animated PC Visualization

Plotting main results with ggplot

```
#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),
                 ids=ids)

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
```



6. Normal mode analysis [optional]

Doing NMA on pdbs

```
# 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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```
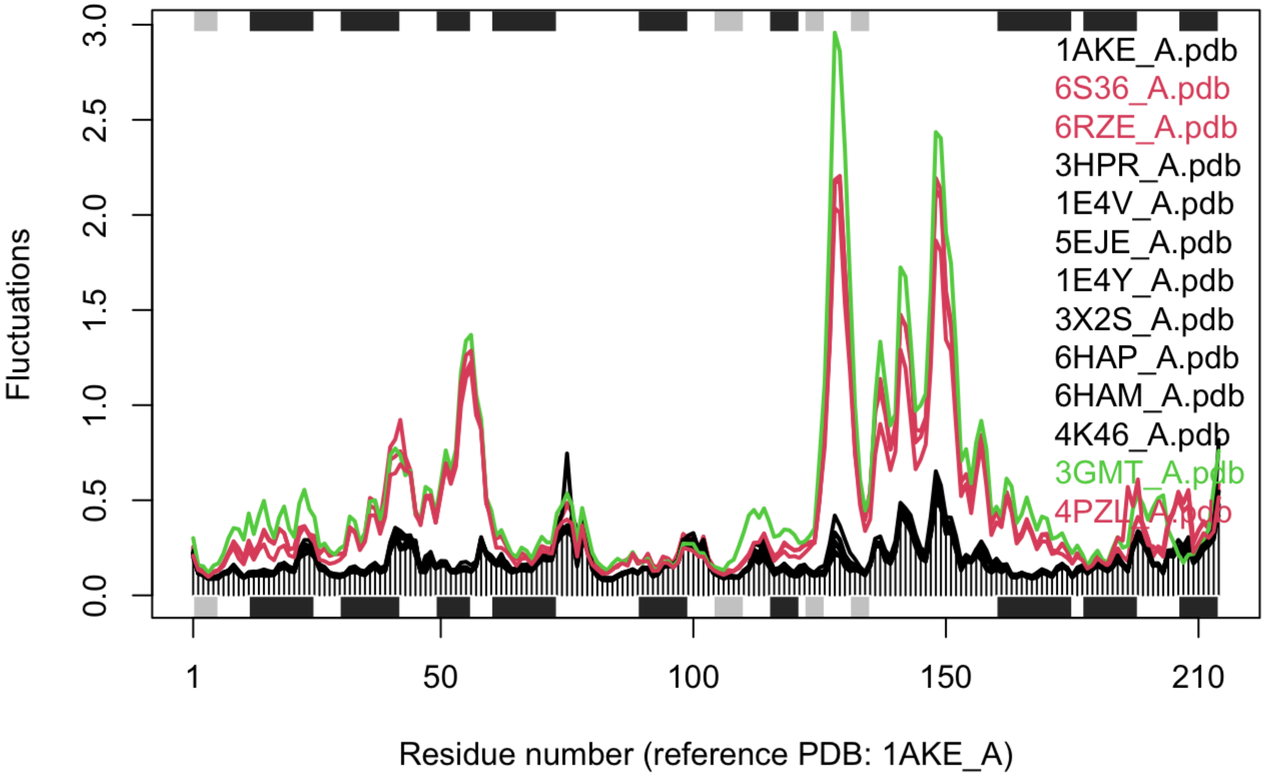

Plotting results

```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 quite different. They seem to differ most around residue number 40-50 and from 130-150. This is probably because these are regions that change with the two major conformational states for Adk. That is, they are the flexible binding-site regions that would change their structure upon binding of a ligand. Therefore, those regions exhibit a lot of fluctuation.