Class 10: Structural Bioinformatics (pt1)

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1. The PDB database

The main repository of biomolecular data is called the PDB (Protein Data Bank) and can be found at: https://www.rcsb.org/

Let's see what it contains in terms of type of molecule and method of strucutre determination (Analyze > PDB Stats > By Mol Type and Method)

```
pdbstats <- read.csv("Data Export Summary.csv")
pdbstats
```

```
           Molecular.Type   X.ray     EM    NMR Multiple.methods Neutron Other
1          Protein (only) 169,563 16,774 12,578              208      81    32
2 Protein/Oligosaccharide   9,939  2,839     34                8       2     0
3              Protein/NA   8,801  5,062    286                7       0     0
4     Nucleic acid (only)   2,890    151  1,521               14       3     1
5                   Other     170     10     33                0       0     0
6  Oligosaccharide (only)      11      0      6                1       0     4
    Total
1 199,236
2  12,822
3  14,156
```

```
4   4,580
5     213
6      22
```

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

```
nocomma <- sub(",","", pdbstats$X.ray)
sum(as.numeric(nocomma))
```

```
[1] 191374
```

Let's try the **readr** package and its newer `read_csv()` function.

```
library(readr)

pdbstats <- read_csv("Data Export Summary.csv")
```

```
Rows: 6 Columns: 8
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Molecular Type
dbl (3): Multiple methods, Neutron, Other
num (4): X-ray, EM, NMR, Total

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
pdbstats
```

```
# A tibble: 6 x 8
  `Molecular Type`   `X-ray`    EM   NMR `Multiple methods` Neutron Other  Total
  <chr>                <dbl> <dbl> <dbl>              <dbl>   <dbl> <dbl>  <dbl>
1 Protein (only)      169563 16774 12578                208      81    32 199236
2 Protein/Oligosacc~    9939  2839    34                  8       2     0  12822
3 Protein/NA            8801  5062   286                  7       0     0  14156
4 Nucleic acid (onl~    2890   151  1521                 14       3     1   4580
5 Other                  170    10    33                  0       0     0    213
6 Oligosaccharide (~      11     0     6                  1       0     4     22
```

The resulting column names are "untidy" with the spaces and mix of upper and lower case letters that will make working with the columns a pain. We'll use the **janitor** package and it's `clean_names()` function to clean up the untidy columns.

```
colnames(pdbstats)
```

```
[1] "Molecular Type"   "X-ray"            "EM"               "NMR"
[5] "Multiple methods" "Neutron"          "Other"            "Total"
```

```
library(janitor)
```

```
Attaching package: 'janitor'
```

```
The following objects are masked from 'package:stats':

    chisq.test, fisher.test
```

```
pdbstats <- clean_names(pdbstats)
```

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

```
n.total <- sum(pdbstats$total)
n.xray <- sum(pdbstats$x_ray)

n.xray
```

```
[1] 191374
```

```
n.total
```

```
[1] 231029
```

In UniProt, there are 253,206,171 sequences, and there are only 231,029 known structures in the PDB. This is a tiny fraction!!!!

```
n.total / 253206171 * 100
```

```
[1] 0.09124146
```

Next clss we will see how bioinformatics methods can help predict structure from sequence with accuracy approaching x-ray methods.

```
n.xray/n.total * 100
```

```
[1] 82.83549
```

82.8% are solved by x ray.

```
n.em <- sum(pdbstats$em)

n.em
```

```
[1] 24836
```

```
n.total
```

```
[1] 231029
```

```
n.em/n.total * 100
```

```
[1] 10.75017
```

10.8% are solved by em.

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

```
round(pdbstats$total[1] / n.total * 100, digits = 2)
```

```
[1] 86.24
```

86.2% of structures in PDB are protein.

> 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?

```
There are experimental (27,589)
```

2. Molecular visualization with Mol*

Mol-star is a new online structure viewer that is taking over the wold of biomolecular visualization. Let's see how to use it from https://molstar.org/viewer/

My first mage from Mol* of HIV-Pr

Figure 1: Fig.1 A first view of HIV-PR dimer PDB: 1HSG

I want an image that shows the binding cleft for the MK1 inhibitor, an image of the most valuable water in human history, and an image showing the catalytic ASP amino-acids.

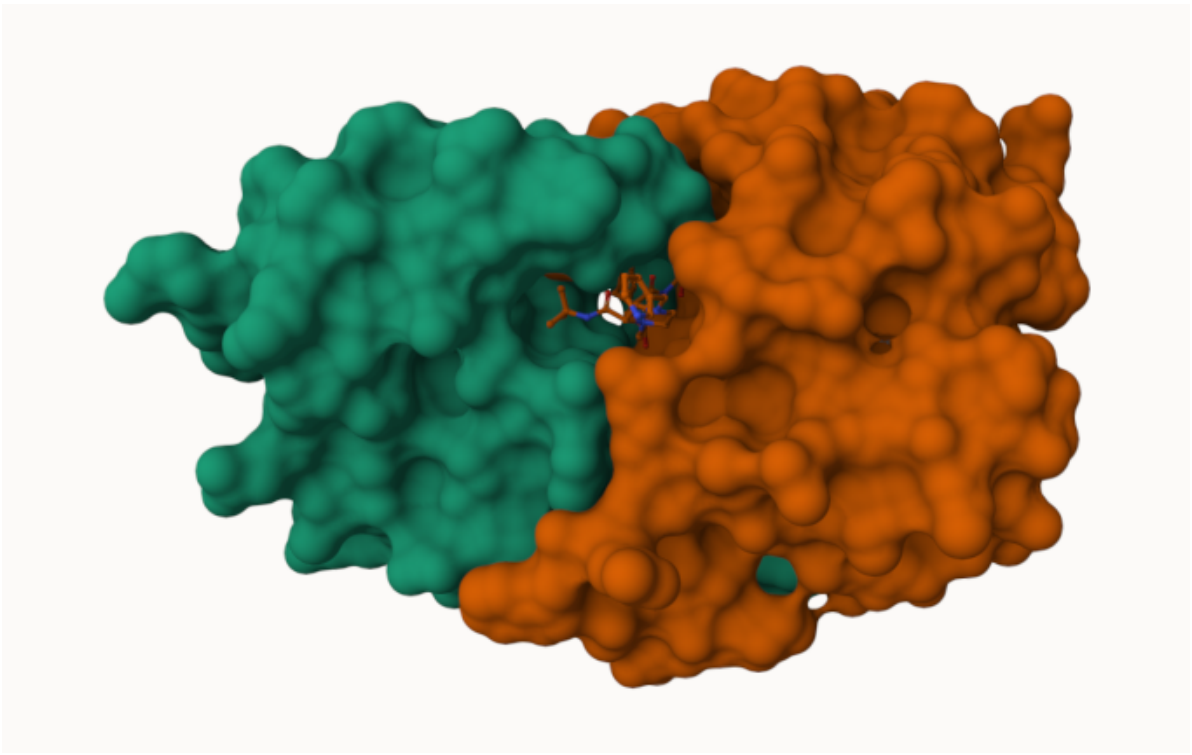


Figure 2: Fig.2 1HSG surface

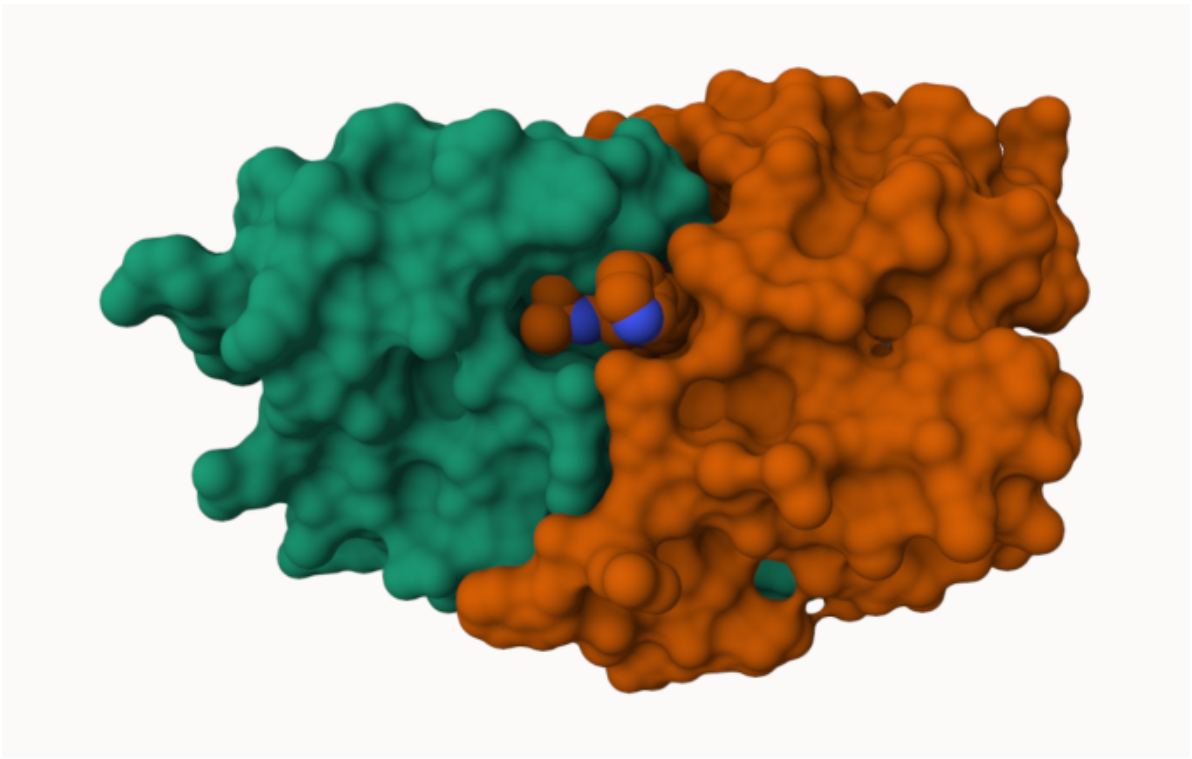


Figure 3: Fig.3 1HSG surface with ligand as spacefill

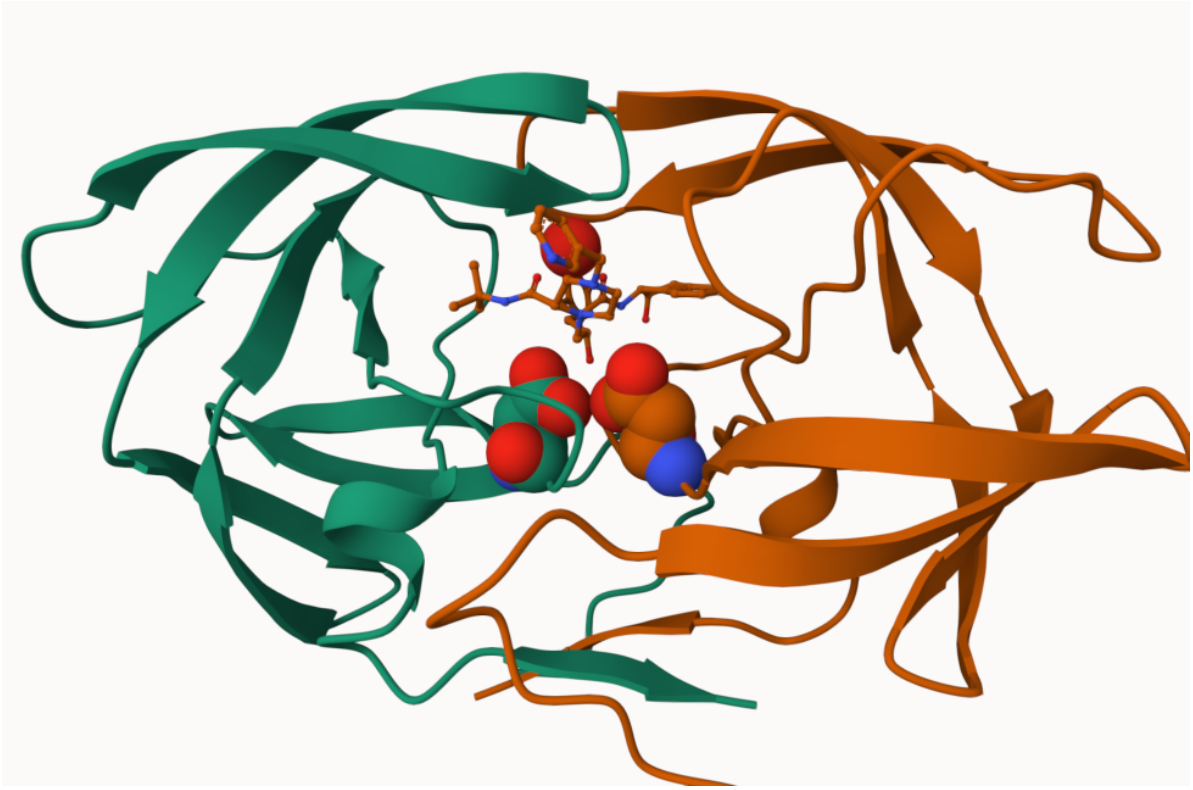


Figure 4: Fig.4 Closer look with H2O 308 and catalytic asp residue

3. Using the Bio3D package

This package has tons of tools and utilities for structural bioinformatics.

```
library(bio3d)

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

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

```
hiv
```

```

 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
```

```
head(hiv$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>
```

```
s <- pdbseq(hiv)
head(s)
```

```
  1   2   3   4   5   6
"P" "Q" "I" "T" "L" "W"
```

Q.How long is this sequence / how many amino acids are in the structure?

```
length(s)
```

```
[1] 198
```

Predict the functional motions

Let's read a new structure "6s36"

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

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

```
pdb
```

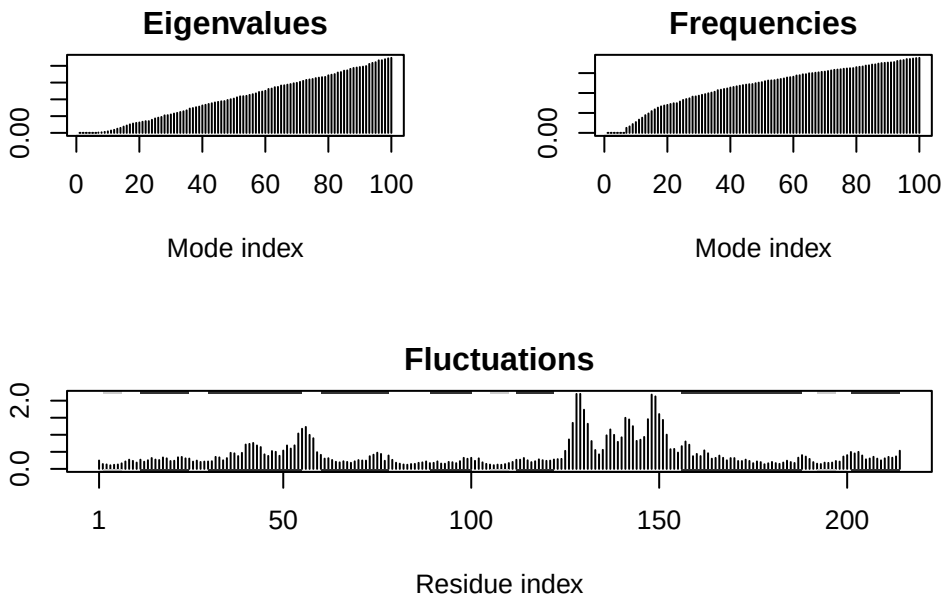
```
 Call:  read.pdb(file = "6s36")

   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
```

We can run a NMA calculation on this structure:

```
m <- nma(pdb)
```

```
 Building Hessian...        Done in 0.06 seconds.
 Diagonalizing Hessian...   Done in 0.3 seconds.
```

```
plot(m, sse=pdb)
```



We can write out a trajectory of the predicted dynamics using the `mktrj()` function

```
mktrj(m, file="results.pdb")
```

4. Comparative structure analysis of Adenylate Kinase

```
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
```

Search the PDB database for related sequences

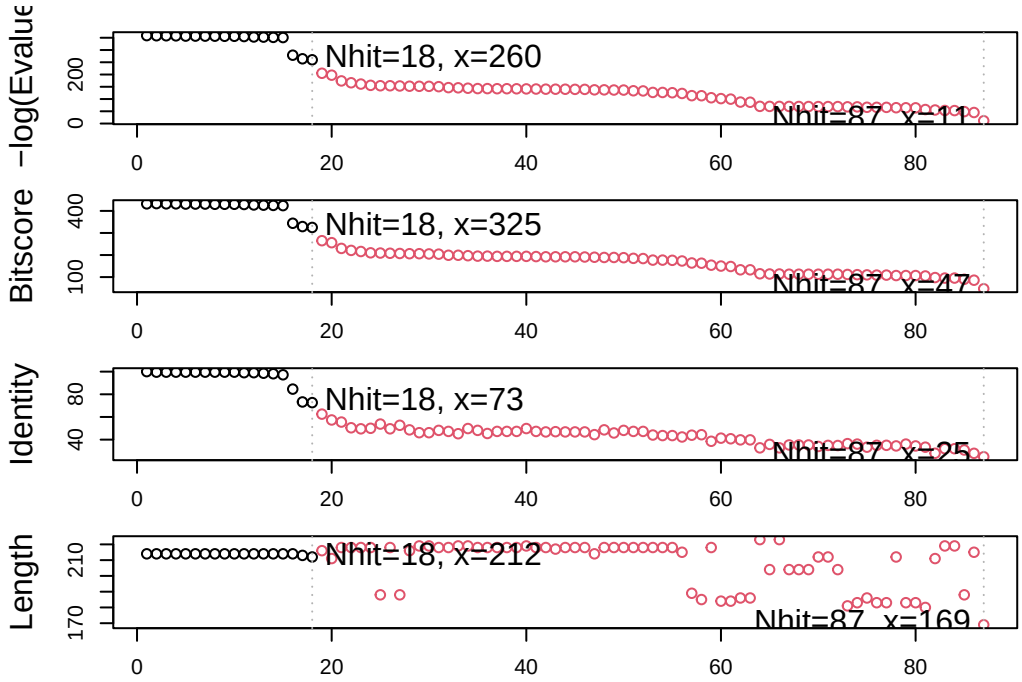
```
blast <- blast.pdb(aa)
```

```
 Searching ... please wait (updates every 5 seconds) RID = UDGCFC3F016
 ..................................................................
 Reporting 87 hits
```

```
hits <- plot(blast)
```

```
  * Possible cutoff values:    260 11
```

```
          Yielding Nhits:    18 87

 * Chosen cutoff value of:    260
          Yielding Nhits:    18
```




```
head(blast$raw)
```

```
        queryid subjectids identity alignmentlength mismatches gapopens q.start
1 Query_3456637     1AKE_A  100.000             214          0        0       1
2 Query_3456637     8BQF_A   99.533             214          1        0       1
3 Query_3456637     4X8M_A   99.533             214          1        0       1
4 Query_3456637     6S36_A   99.533             214          1        0       1
5 Query_3456637     8Q2B_A   99.533             214          1        0       1
6 Query_3456637     8RJ9_A   99.533             214          1        0       1
  q.end s.start s.end    evalue bitscore positives
1   214       1   214 1.61e-156      432    100.00
2   214      21   234 2.64e-156      433    100.00
3   214       1   214 2.89e-156      432    100.00
4   214       1   214 4.24e-156      432    100.00
5   214       1   214 1.13e-155      431     99.53
6   214       1   214 1.13e-155      431     99.53
```

```
hits$pdb.id
```

```
 [1] "1AKE_A" "8BQF_A" "4X8M_A" "6S36_A" "8Q2B_A" "8RJ9_A" "6RZE_A" "4X8H_A"
 [9] "3HPR_A" "1E4V_A" "5EJE_A" "1E4Y_A" "3X2S_A" "6HAP_A" "6HAM_A" "8PVW_A"
[17] "4K46_A" "4NP6_A"
```

Download all these structures to our project directory.

```
#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 exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/8BQF.pdb exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/4X8M.pdb exists. Skipping download

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

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/8Q2B.pdb exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/8RJ9.pdb exists. Skipping download

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

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/4X8H.pdb exists. Skipping download

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

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

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

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

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

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

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

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/8PVW.pdb exists. Skipping download

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

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/4NP6.pdb exists. Skipping download


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```
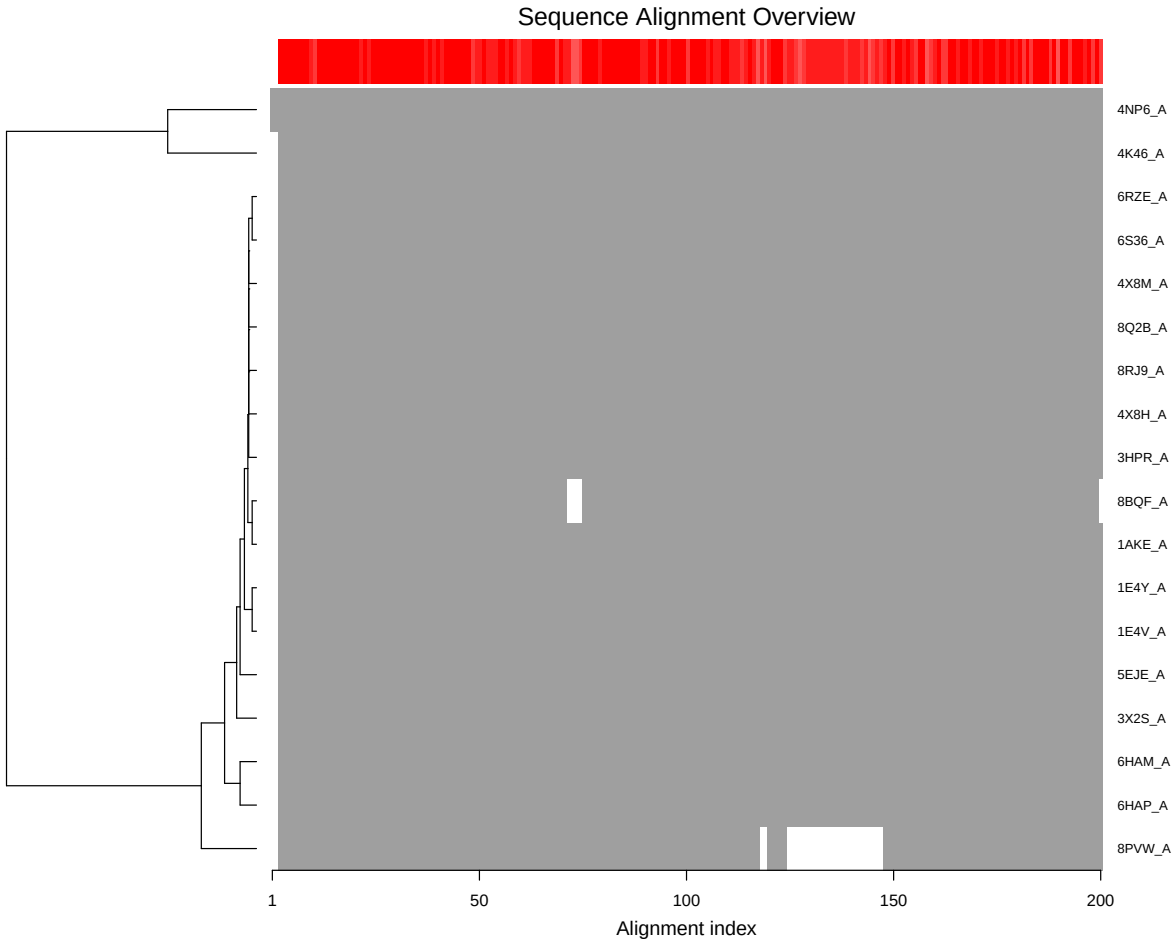

```
# Align releated PDBs
pdbs <- pdbaln(files, fit = TRUE, exefile="msa")
```

```
Reading PDB files:
pdbs/split_chain/1AKE_A.pdb
pdbs/split_chain/8BQF_A.pdb
pdbs/split_chain/4X8M_A.pdb
pdbs/split_chain/6S36_A.pdb
pdbs/split_chain/8Q2B_A.pdb
pdbs/split_chain/8RJ9_A.pdb
pdbs/split_chain/6RZE_A.pdb
pdbs/split_chain/4X8H_A.pdb
pdbs/split_chain/3HPR_A.pdb
pdbs/split_chain/1E4V_A.pdb
pdbs/split_chain/5EJE_A.pdb
```

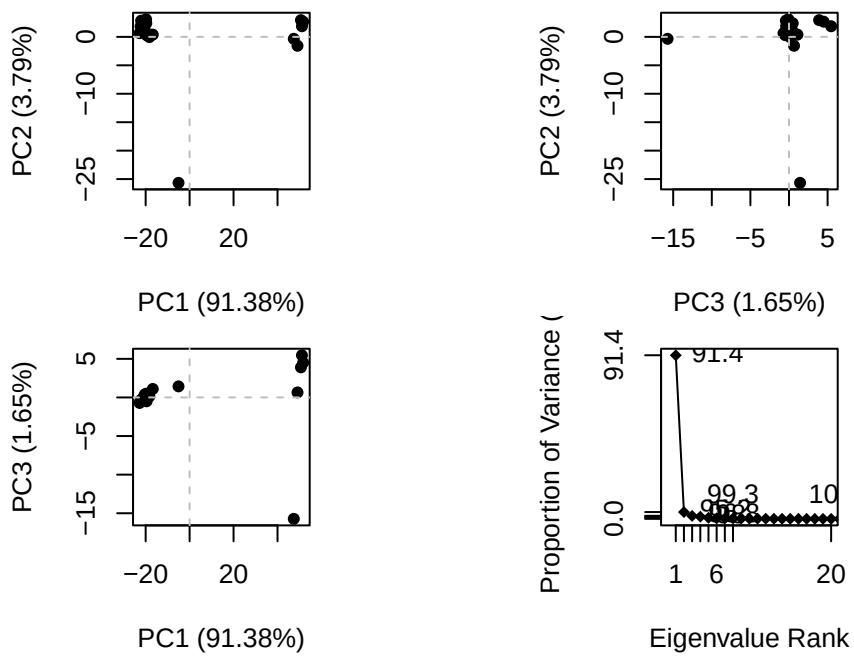
```
pdbs/split_chain/1E4Y_A.pdb
pdbs/split_chain/3X2S_A.pdb
pdbs/split_chain/6HAP_A.pdb
pdbs/split_chain/6HAM_A.pdb
pdbs/split_chain/8PVW_A.pdb
pdbs/split_chain/4K46_A.pdb
pdbs/split_chain/4NP6_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
.   PDB has ALT records, taking A only, rm.alt=TRUE
..   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
..   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/8BQF_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 3   name: pdbs/split_chain/4X8M_A.pdb
pdb/seq: 4   name: pdbs/split_chain/6S36_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 5   name: pdbs/split_chain/8Q2B_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 6   name: pdbs/split_chain/8RJ9_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 7   name: pdbs/split_chain/6RZE_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 8   name: pdbs/split_chain/4X8H_A.pdb
pdb/seq: 9   name: pdbs/split_chain/3HPR_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 10   name: pdbs/split_chain/1E4V_A.pdb
pdb/seq: 11   name: pdbs/split_chain/5EJE_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 12   name: pdbs/split_chain/1E4Y_A.pdb
pdb/seq: 13   name: pdbs/split_chain/3X2S_A.pdb
```

```
pdb/seq: 14   name: pdbs/split_chain/6HAP_A.pdb
pdb/seq: 15   name: pdbs/split_chain/6HAM_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 16   name: pdbs/split_chain/8PVW_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 17   name: pdbs/split_chain/4K46_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 18   name: pdbs/split_chain/4NP6_A.pdb
```

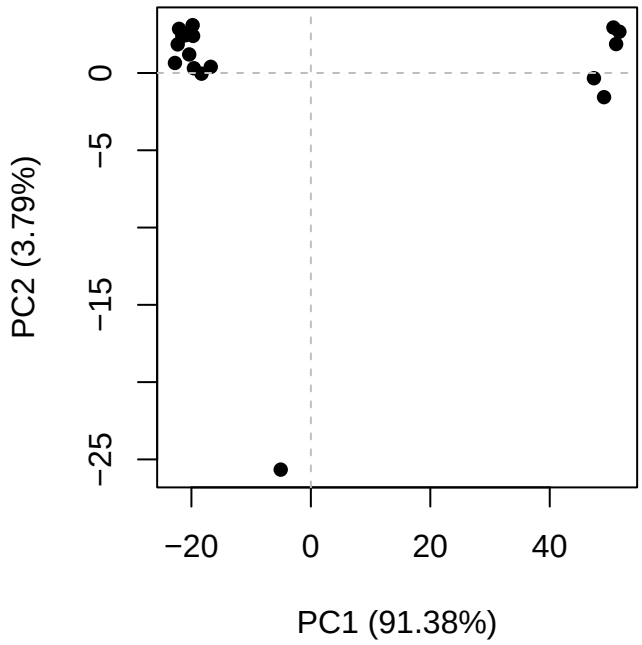
```r
# Vector containing PDB codes for figure axis
ids <- basename.pdb(pdbs$id)
# Draw schematic alignment
plot(pdbs, labels=ids)
```



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



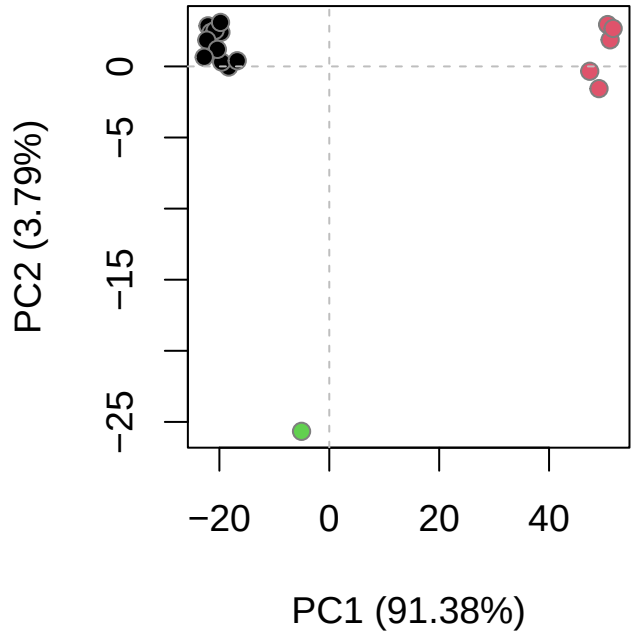

```
plot(pc.xray, pc.axes = c(1,2))
```




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

```
Warning in rmsd(pdbs): No indices provided, using the 182 non NA positions
```

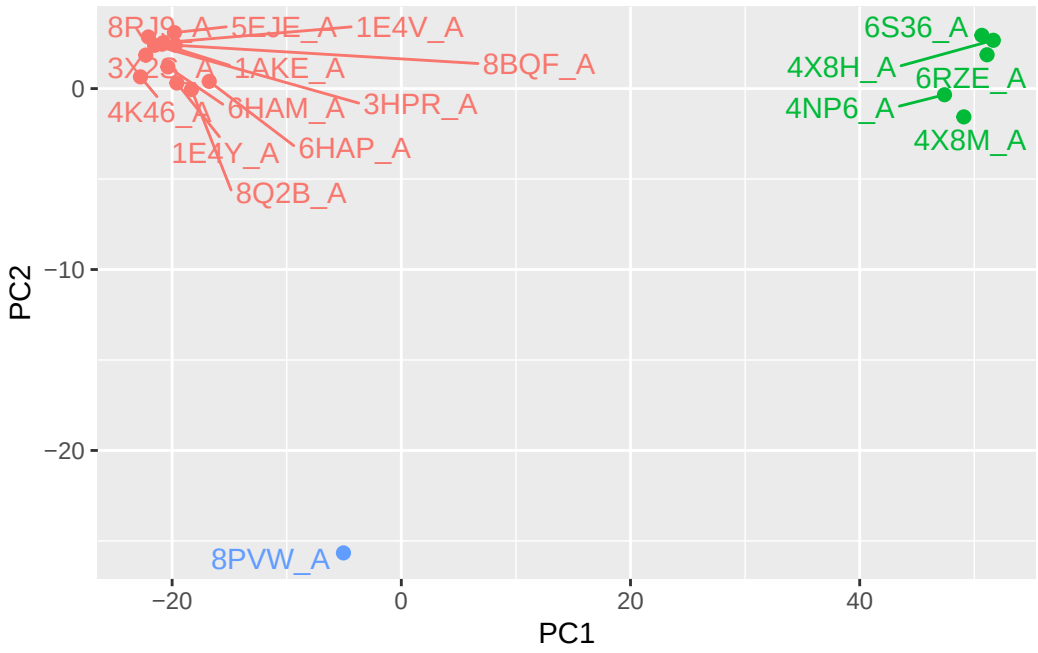
```
# Structure-based clustering
hc.rd <- hclust(dist(rd))
grps.rd <- cutree(hc.rd, k=3)

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



We can view the main PC1 captured displacements with the mktrj function again:

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

```
#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
```


8RJ9_A
5EJE_A
1E4V_A
8BQF_A
3X2S_A
1AKE_A
4K46_A
6HAM_A
3HPR_A
1E4Y_A
6HAP_A
8Q2B_A
6S36_A
4X8H_A
6RZE_A
4NP6_A
4X8M_A
8PVW_A
0
−10
−20
PC2
−20
0
20
40
PC1