lab9

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RCSB Protein Data Bank

This database has mostly x-ray crystalography. Class skipped Q1-3 because the site was too slow.

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

```
169794/196779*100
```

```
[1] 86.28665
```

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

```
(171221+10444+10876)/196779
```

```
[1] 0.9784631
```

> 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?

4703 structures

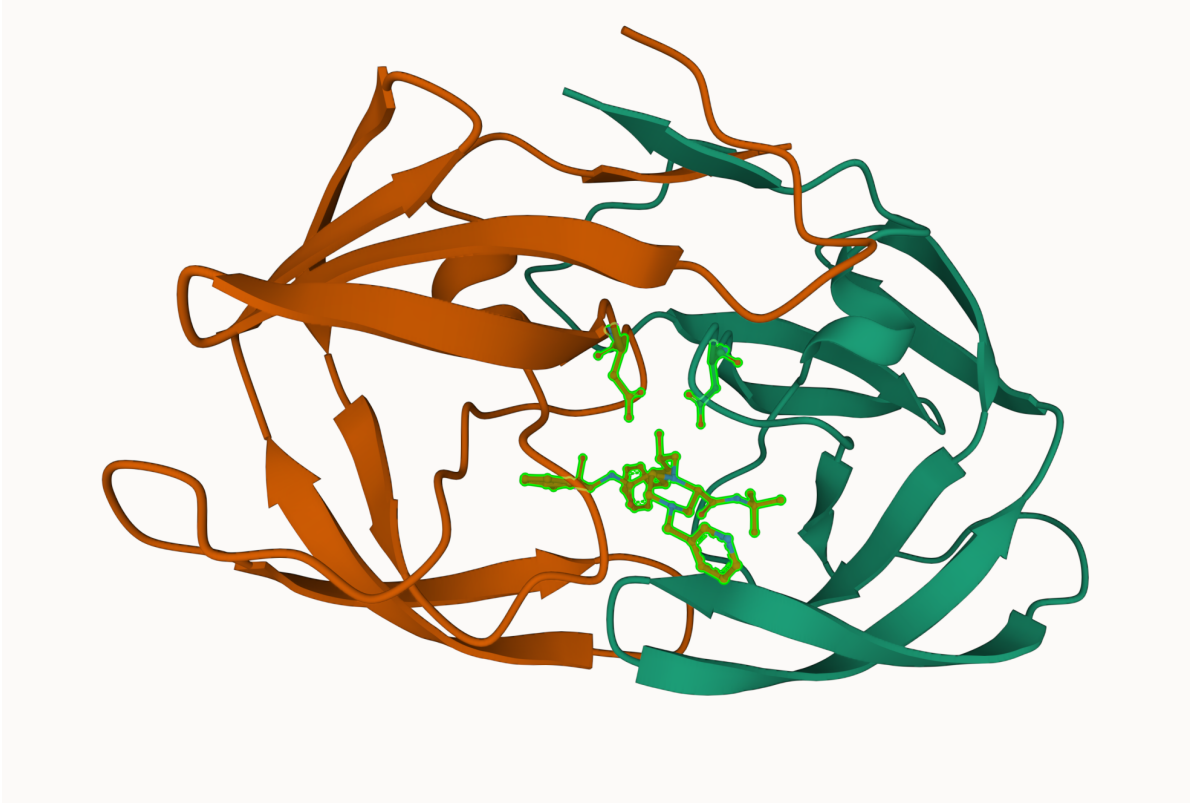
> Q4: Water molecules normally have 3 atoms. Why do we see just one atom per water molecule in this structure?

We only see one atom because it only displays the oxygen atom because the hydrogen atoms are too small to be imaged.

> 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?

The water molecule has a residue number of 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. Discussion Topic: Can you think of a way in which indinavir, or even larger ligands and substrates, could enter the binding site?



A conformational change can allow for a larger ligand and substrates to enter the binding site.

Intro to Bio3D in R

Bio3D is an R package for structural bioinformatics. Bring in bio3d package.

```
library(bio3d)
```

Read PDB file from online repository.

```
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?

198 amino acid residues

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

HOH, also MK1

Q9: How many protein chains are in this structure?

2 chains

Look at attributes and head of atom

```
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>
```

Comparative Structure Analysis of Adenylate Kinase

Install necessary packages

```
#install.packages("ggrepel")
#install.packages("devtools")
#install.packages("BiocManager")
#BiocManager::install("msa")
#devtools::install_bitbucket("Grantlab/bio3d-view")
```

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

msa

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

Retrieve ADK data. We will start with PDB id 1AKE.

```
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?

214

We can use this sequence to BLAST the PDB and find similar sequences and structures.

```
b <- blast.pdb(aa) #takes a very long time to search
```

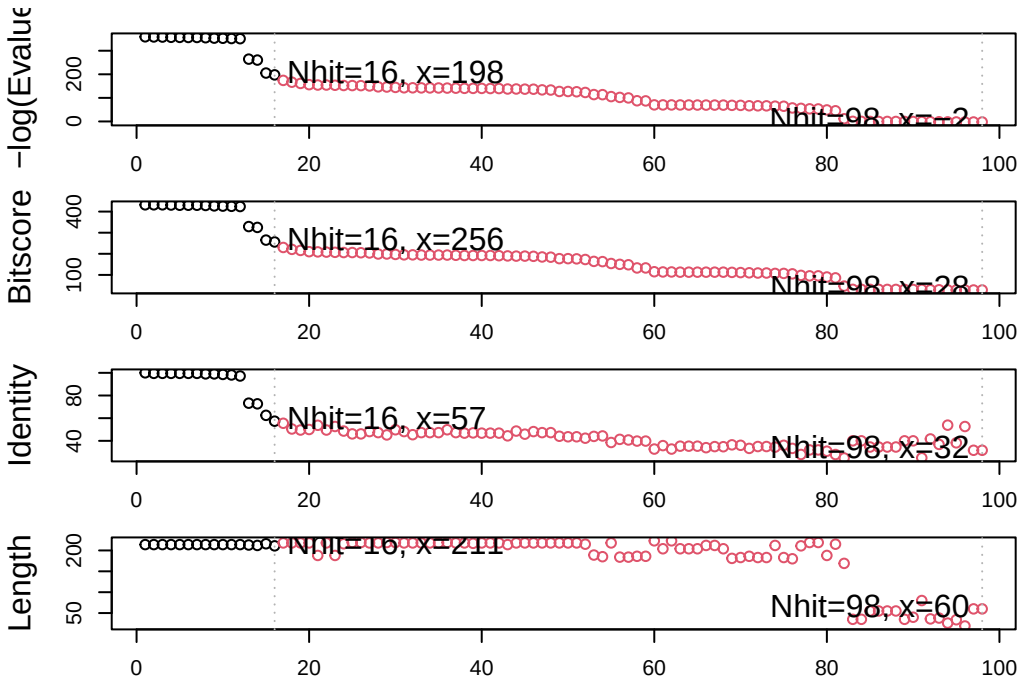
```
Searching ... please wait (updates every 5 seconds) RID = NH1E3XWB013
.
Reporting 98 hits
```

We can plot a summary of search results or list out top hits.

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

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

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



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

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

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

Download related PDB files from online database.

```
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/
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/
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/
4K46.pdb exists. Skipping download

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

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

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

Align related PDBs

```
pdbs <- pdbaln(files, fit = TRUE, exefile="msa")
```

```
Reading PDB files:
pdbs/split_chain/1AKE_A.pdb
pdbs/split_chain/4X8M_A.pdb
pdbs/split_chain/6S36_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/4K46_A.pdb
pdbs/split_chain/4NP6_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/4X8M_A.pdb
pdb/seq: 3   name: pdbs/split_chain/6S36_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 4   name: pdbs/split_chain/6RZE_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 5   name: pdbs/split_chain/4X8H_A.pdb
pdb/seq: 6   name: pdbs/split_chain/3HPR_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 7   name: pdbs/split_chain/1E4V_A.pdb
pdb/seq: 8   name: pdbs/split_chain/5EJE_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 9   name: pdbs/split_chain/1E4Y_A.pdb
pdb/seq: 10   name: pdbs/split_chain/3X2S_A.pdb
pdb/seq: 11   name: pdbs/split_chain/6HAP_A.pdb
pdb/seq: 12   name: pdbs/split_chain/6HAM_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 13   name: pdbs/split_chain/4K46_A.pdb
   PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 14   name: pdbs/split_chain/4NP6_A.pdb
pdb/seq: 15   name: pdbs/split_chain/3GMT_A.pdb
pdb/seq: 16   name: pdbs/split_chain/4PZL_A.pdb
```

Plot schematic alignment.

```
ids <- basename.pdb(pdbs$id) #create vector of PDB codes for axis titles
#plot(pdbs, labels=ids) #error related to figure margins too large
```

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] "Vibrio cholerae O1 biovar El Tor str. N16961"
[7] "Burkholderia pseudomallei 1710b"
[8] "Francisella tularensis subsp. tularensis SCHU S4"
```

```
anno
```

```
       structureId chainId macromoleculeType chainLength experimentalTechnique
1AKE_A        1AKE       A           Protein         214                 X-ray
4X8M_A        4X8M       A           Protein         214                 X-ray
6S36_A        6S36       A           Protein         214                 X-ray
6RZE_A        6RZE       A           Protein         214                 X-ray
4X8H_A        4X8H       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
4NP6_A        4NP6       A           Protein         217                 X-ray
3GMT_A        3GMT       A           Protein         230                 X-ray
4PZL_A        4PZL       A           Protein         242                 X-ray
       resolution       scopDomain                                      pfam
1AKE_A      2.000 Adenylate kinase Adenylate kinase, active site lid (ADK_lid)
4X8M_A      2.600             <NA> Adenylate kinase, active site lid (ADK_lid)
6S36_A      1.600             <NA> Adenylate kinase, active site lid (ADK_lid)
6RZE_A      1.690             <NA> Adenylate kinase, active site lid (ADK_lid)
4X8H_A      2.500             <NA> Adenylate kinase, active site lid (ADK_lid)
3HPR_A      2.000             <NA> Adenylate kinase, active site lid (ADK_lid)
1E4V_A      1.850 Adenylate kinase Adenylate kinase, active site lid (ADK_lid)
5EJE_A      1.900             <NA> Adenylate kinase, active site lid (ADK_lid)
1E4Y_A      1.850 Adenylate kinase Adenylate kinase, active site lid (ADK_lid)
3X2S_A      2.800             <NA> Adenylate kinase, active site lid (ADK_lid)
6HAP_A      2.700             <NA> Adenylate kinase, active site lid (ADK_lid)
6HAM_A      2.550             <NA> Adenylate kinase, active site lid (ADK_lid)
4K46_A      2.010             <NA> Adenylate kinase, active site lid (ADK_lid)
4NP6_A      2.004             <NA> Adenylate kinase, active site lid (ADK_lid)
```

```
3GMT_A      2.100            <NA> Adenylate kinase, active site lid (ADK_lid)
4PZL_A      2.100            <NA> Adenylate kinase, active site lid (ADK_lid)
             ligandId
1AKE_A             AP5
4X8M_A            <NA>
6S36_A CL (3),NA,MG (2)
6RZE_A    NA (3),CL (2)
4X8H_A            <NA>
3HPR_A             AP5
1E4V_A             AP5
5EJE_A          AP5,CO
1E4Y_A             AP5
3X2S_A   JPY (2),AP5,MG
6HAP_A             AP5
6HAM_A             AP5
4K46_A      ADP,AMP,PO4
4NP6_A            <NA>
3GMT_A         SO4 (2)
4PZL_A       CA,FMT,GOL
                                                                        ligandName
1AKE_A                                                 BIS(ADENOSINE)-5'-PENTAPHOSPHATE
4X8M_A                                                                          <NA>
6S36_A                                  CHLORIDE ION (3),SODIUM ION,MAGNESIUM ION (2)
6RZE_A                                                   SODIUM ION (3),CHLORIDE ION (2)
4X8H_A                                                                          <NA>
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
4NP6_A                                                                          <NA>
3GMT_A                                                                   SULFATE ION (2)
4PZL_A                                                 CALCIUM ION,FORMIC ACID,GLYCEROL
                                          source
1AKE_A                          Escherichia coli
4X8M_A                          Escherichia coli
6S36_A                          Escherichia coli
6RZE_A                          Escherichia coli
4X8H_A                          Escherichia coli
3HPR_A                   Escherichia coli K-12
```

1E4V_A					
5EJE_A					
1E4Y_A					
3X2S_A					
6HAP_A					
6HAM_A					
4K46_A					
4NP6_A					
3GMT_A					
4PZL_A					

1AKE_A	STRUCTURE OF THE COMPLEX BETWEEN ADENYLATE KINASE FROM ESCHERICHIA COLI AND THE INHIBI				
4X8M_A					
6S36_A					
6RZE_A					
4X8H_A					
3HPR_A					
1E4V_A					
5EJE_A					Cryst
1E4Y_A					
3X2S_A					
6HAP_A					
6HAM_A					
4K46_A					
4NP6_A					
3GMT_A					
4PZL_A					The cryst

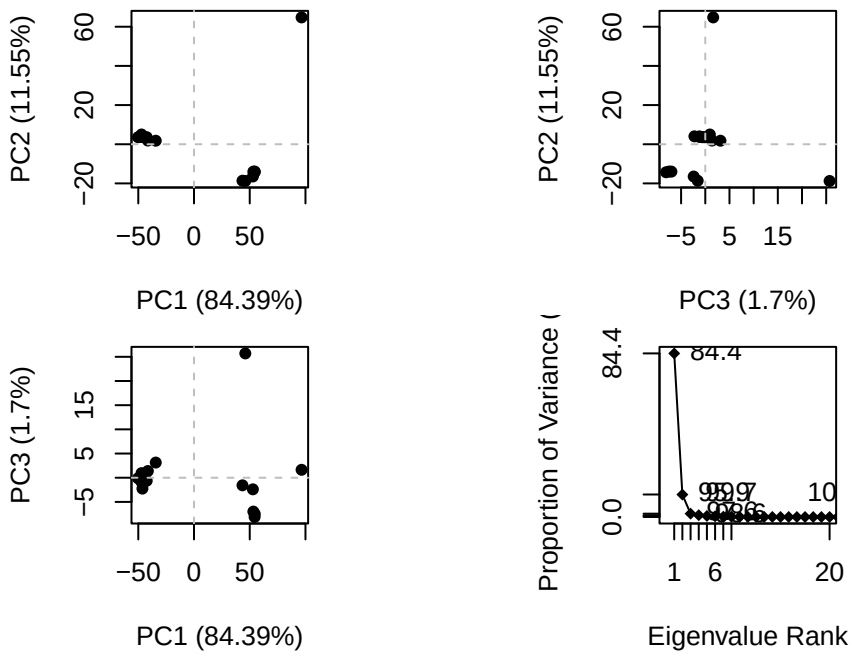
	citation	rObserved	rFree
1AKE_A	Muller, C.W., et al. J Mol Biol (1992)	0.19600	NA
4X8M_A	Kovermann, M., et al. Nat Commun (2015)	0.24910	0.30890
6S36_A	Rogne, P., et al. Biochemistry (2019)	0.16320	0.23560
6RZE_A	Rogne, P., et al. Biochemistry (2019)	0.18650	0.23500
4X8H_A	Kovermann, M., et al. Nat Commun (2015)	0.19610	0.28950
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
4NP6_A	Kim, Y., et al. To be published	0.18800	0.22200
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
4X8M_A 0.24630    C 1 2 1
6S36_A 0.15940    C 1 2 1
6RZE_A 0.18190    C 1 2 1
4X8H_A 0.19140    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
4NP6_A 0.18600       P 43
3GMT_A 0.23500   P 1 21 1
4PZL_A 0.19130       P 32
```

PCA

Perform PCA and plot.

```
pc.xray <- pca(pdbs)
plot(pc.xray)
```



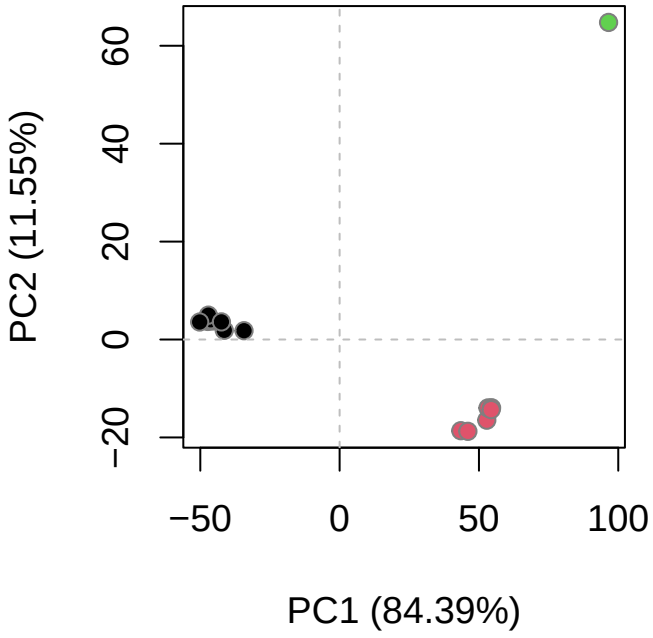
`rmsd()` will calculate all pairwise RMSD values of the structural ensemble for cluster analysis based on pairwise structural deviation.

```
# 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 visualization

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

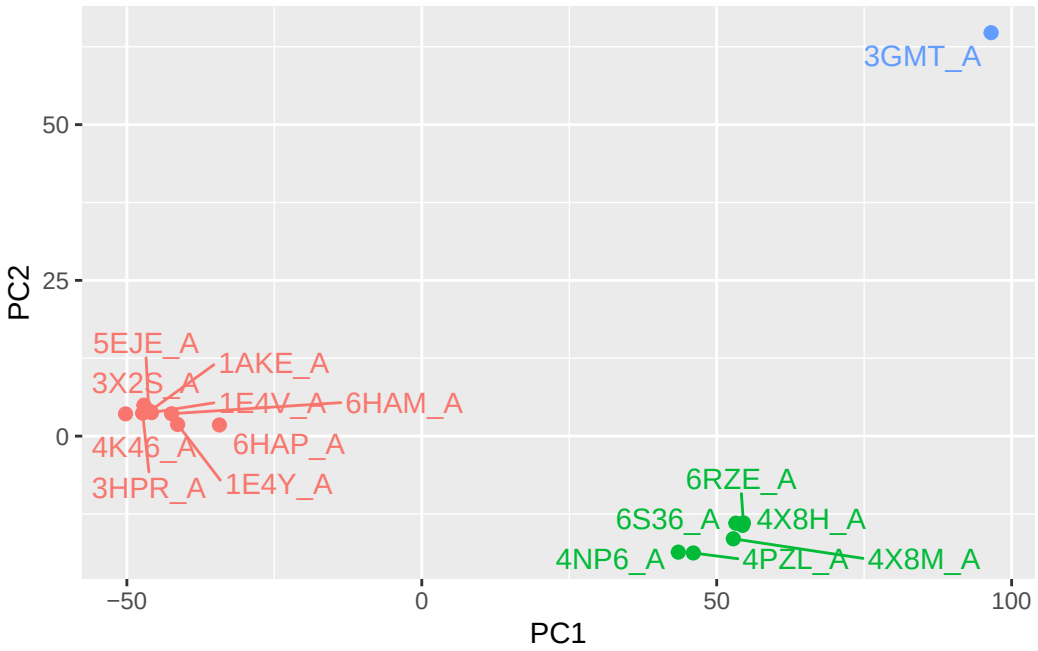
Upload this file into molstar for 3D animation.

```
#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
```



Normal Mode Analysis

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

```
Details of Scheduled Calculation:
  ... 16 input structures
  ... storing 606 eigenvectors for each structure
  ... dimension of x$U.subspace: ( 612x606x16 )
  ... coordinate superposition prior to NM calculation
  ... aligned eigenvectors (gap containing positions removed)
  ... estimated memory usage of final 'eNMA' object: 45.4 Mb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```

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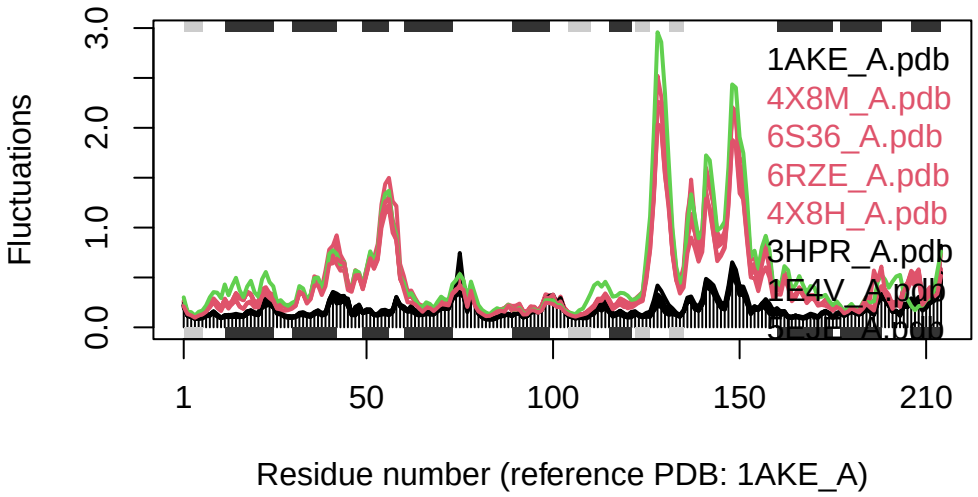
```

```
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?

This plot groups the different proteins by which are most similar to each other, showing 3 different groups: red, green, and black. The colored lines are more similar to each other than they are to the black lines. They differ most in the 2 nucleotide-binding site regions where there are the highest peaks because they are flexible based on nucleotide binding.