Class 9: Structural Bioinformatics 1.

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```
#webshot::install_phantomjs()
```

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.

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).

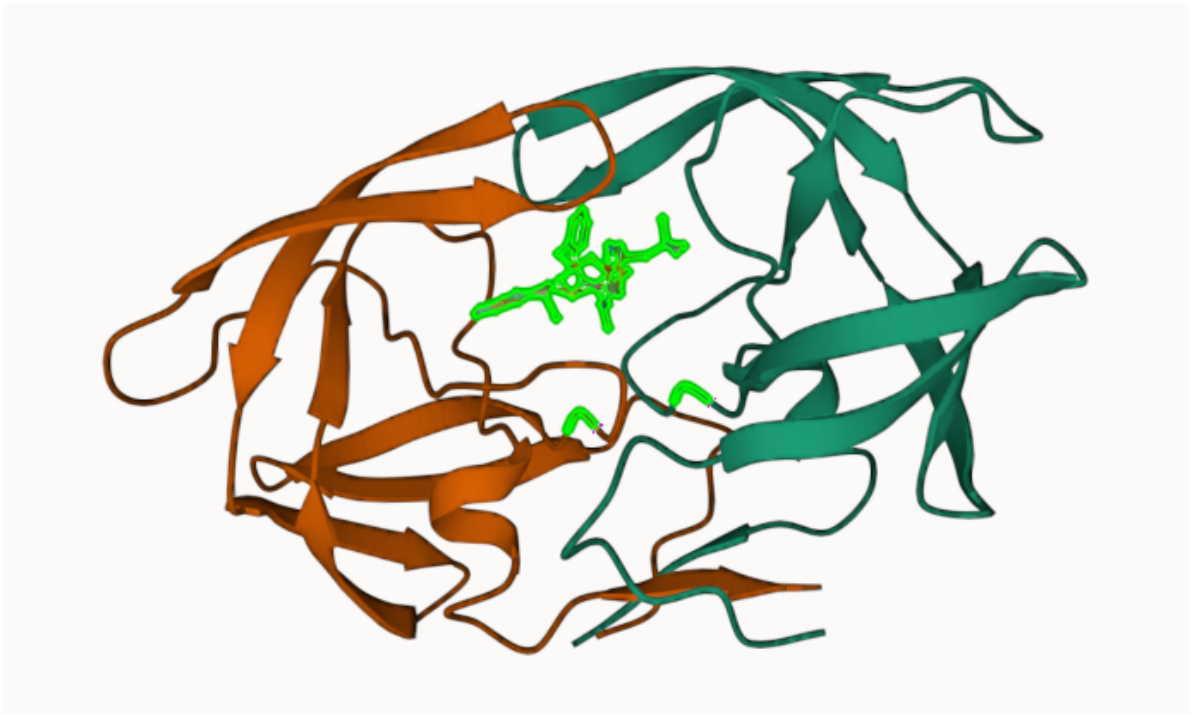


Figure 1: HIV-Pr structure from 1hsg

```
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)
```

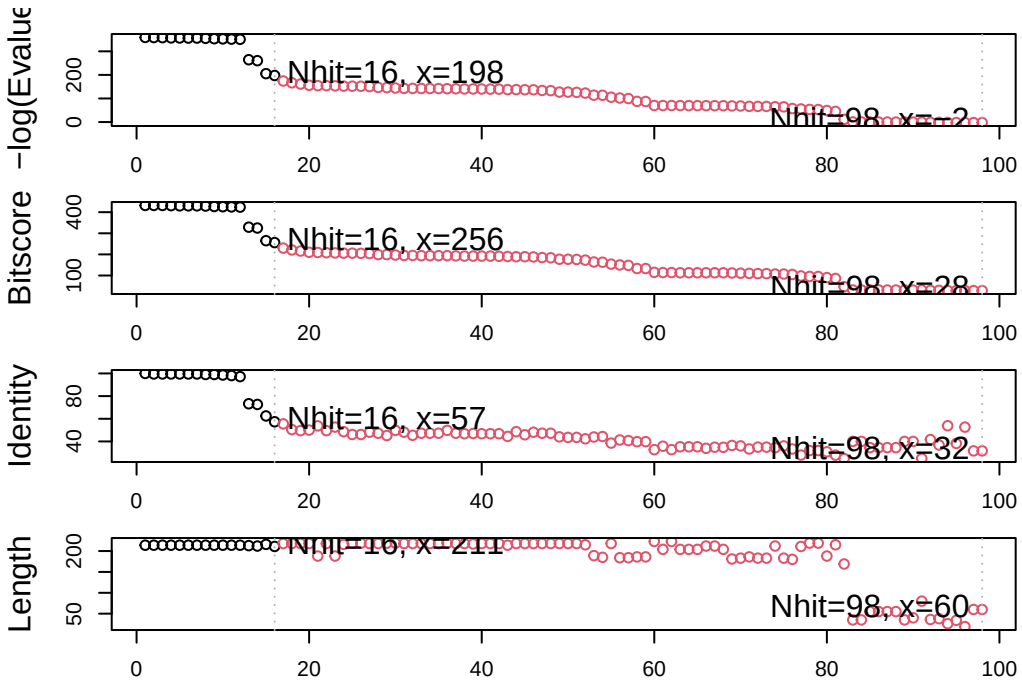
```
Searching ... please wait (updates every 5 seconds) RID = NNM6A3A5016
.........
Reporting 98 hits
```

```
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
```

```
  |
  |                                                                      |   0%
  |
  |=====                                                                 |   8%
  |
  |===========                                                           |  15%
  |
  |================                                                      |  23%
  |
  |======================                                                |  31%
  |
  |============================                                          |  38%
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  |============================================                          |  69%
  |
  |================================================                      |  77%
  |
  |============================================================          |  85%
  |
  |====================================================================  |  92%
  |
  |======================================================================| 100%
```

Align and superose structures

Align all these structures

```
# 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: 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)

dev.off()
```

```
null device
          1
```

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

# adjust plot margins
par(mar = c(1,1,1,1))
```

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
1AKE_A	2.00	Adenylate kinase	Adenylate kinase, active site lid (ADK_lid)
6S36_A	1.60	<NA>	Adenylate kinase, active site lid (ADK_lid)
6RZE_A	1.69	<NA>	Adenylate kinase, active site lid (ADK_lid)
3HPR_A	2.00	<NA>	Adenylate kinase, active site lid (ADK_lid)
1E4V_A	1.85	Adenylate kinase	Adenylate kinase, active site lid (ADK_lid)
5EJE_A	1.90	<NA>	Adenylate kinase, active site lid (ADK_lid)
1E4Y_A	1.85	Adenylate kinase	Adenylate kinase, active site lid (ADK_lid)
3X2S_A	2.80	<NA>	Adenylate kinase, active site lid (ADK_lid)
6HAP_A	2.70	<NA>	Adenylate kinase, active site lid (ADK_lid)
6HAM_A	2.55	<NA>	Adenylate kinase, active site lid (ADK_lid)
4K46_A	2.01	<NA>	Adenylate kinase, active site lid (ADK_lid)
3GMT_A	2.10	<NA>	Adenylate kinase, active site lid (ADK_lid)
4PZL_A	2.10	<NA>	Adenylate kinase, active site lid (ADK_lid)

	ligandId	ligandName
1AKE_A	AP5	BIS(ADENOSINE)-5'-PENTAPHOSPHATE
6S36_A	CL (3),NA,MG (2)	CHLORIDE ION (3),SODIUM ION,MAGNESIUM ION (2)
6RZE_A	NA (3),CL (2)	SODIUM ION (3),CHLORIDE ION (2)
3HPR_A	AP5	BIS(ADENOSINE)-5'-PENTAPHOSPHATE
1E4V_A	AP5	BIS(ADENOSINE)-5'-PENTAPHOSPHATE
5EJE_A	AP5,CO	
1E4Y_A	AP5	
3X2S_A	JPY (2),AP5,MG	
6HAP_A	AP5	
6HAM_A	AP5	
4K46_A	ADP,AMP,PO4	
3GMT_A	SO4 (2)	
4PZL_A	CA,FMT,GOL	

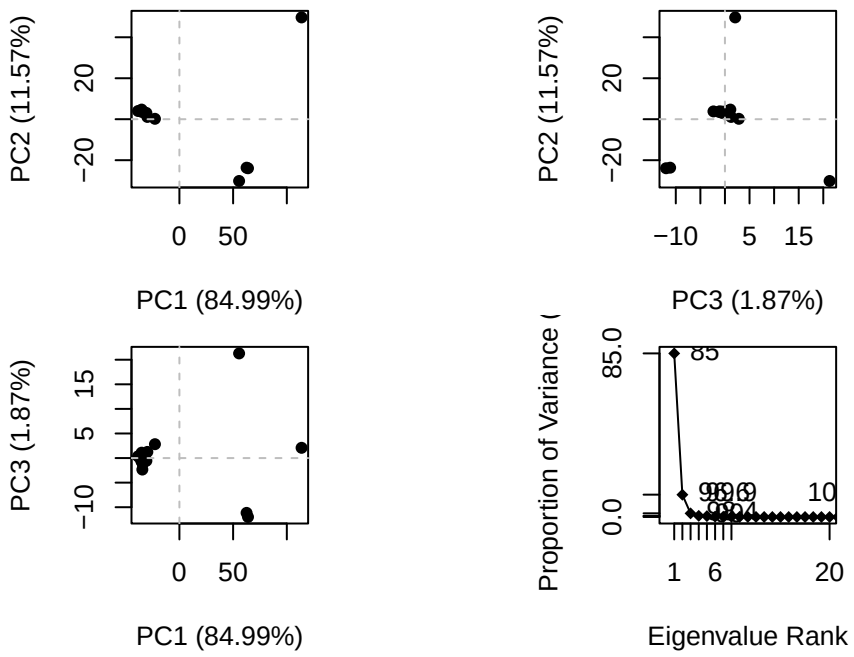
```
5EJE_A                                  BIS(ADENOSINE)-5'-PENTAPHOSPHATE,COBALT (II) ION
1E4Y_A                                                BIS(ADENOSINE)-5'-PENTAPHOSPHATE
3X2S_A N-(pyren-1-ylmethyl)acetamide (2),BIS(ADENOSINE)-5'-PENTAPHOSPHATE,MAGNESIUM ION
6HAP_A                          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

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