class09

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```
library(dplyr)
```

```
##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

1: Introduction to the RCSB Protein Data Bank (PDB)

PDB Statistics

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

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

92.99%

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

0.8681 - Protein(only) 0.9782 - Protein included

> 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?

4,926 Structures

2. Visualizing the HIV-1 Protease Structure

Using Mol*

Mol* homepage at: https://molstar.org/viewer/ To load a structure from the PDB we can enter the PDB code and click "Apply" in the "Download Structure" menu.

Getting to Know HIV-Pr

Let's temporally toggle OFF/ON the display of water molecules and change the display representation of the Ligand to Spacefill. Three dots for "Ligand" > Add Representation > Spacefill

Let's also change the protein "Polymer" > "Set Coloring" > "Residue Property" > "Secondary Structure".

Saving an Image

Save high-resolution image to computer: find "iris-like" screenshot icon on right side of display region and select resolution and click download.

```
# insert an image
knitr::include_graphics("1HSG(1).png.png")
```

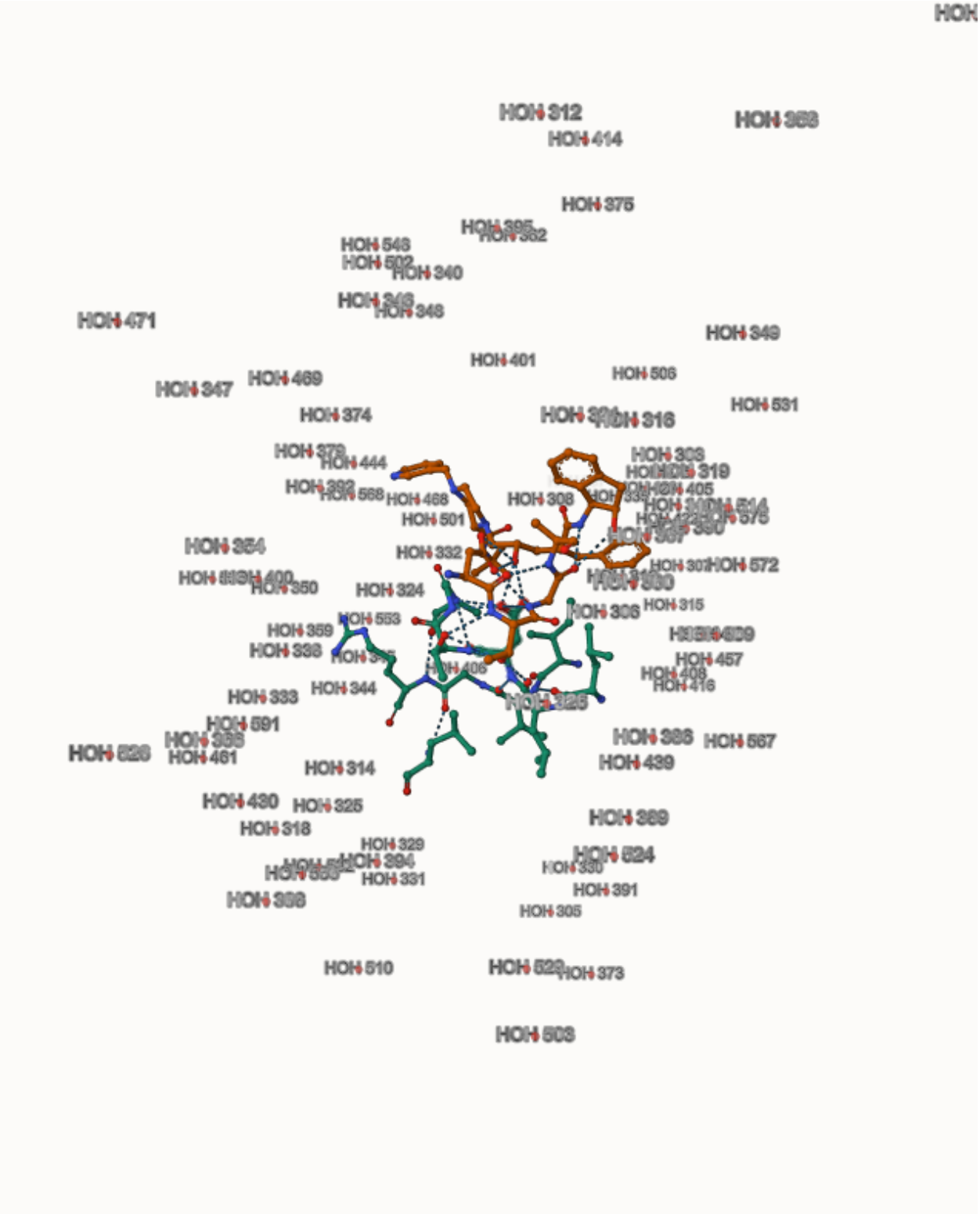
The Important Role of Water

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

When label representation is on, we can see that the one atom per water molecule is named HOH. The red spheres is O.

> 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

```
knitr::include_graphics("1HSGWater.png")
```

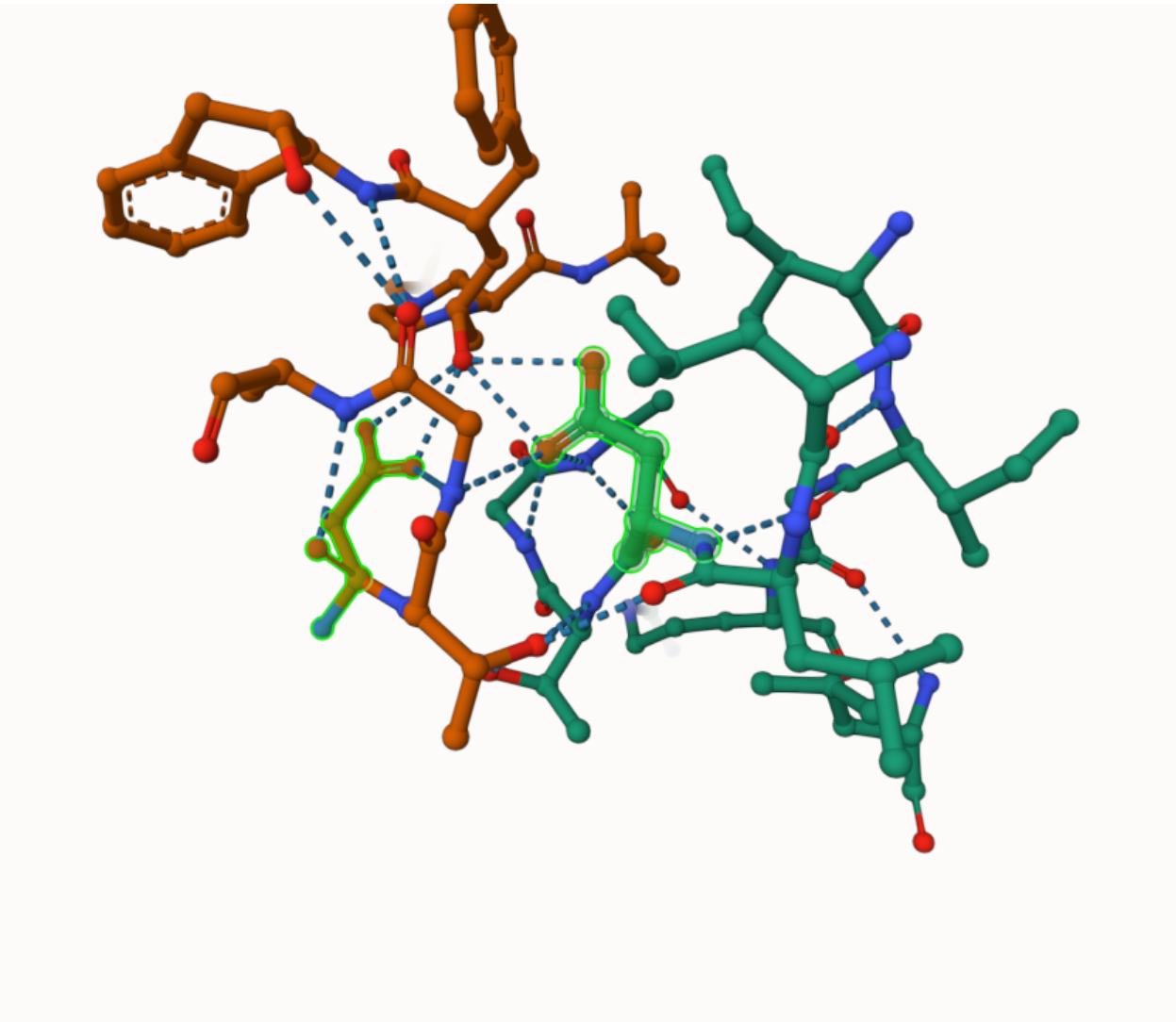


HOH 308

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.

```
knitr::include_graphics("1HSG.png")
```



The image above shows two Asp 25 positions (B and A) in the 3D structure of 1HSG.

3.Introduction to Bio3D in R

```
# load Bio3D package
library(bio3d)
```

Reading PDB File Data into R

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

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

```
# quick summary of contents of pdb object
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

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

HOH (127), MK1 (1)

Q9: How many protein chains are in this structure?

2

Note that the attributes (+ attr:) of this object are listed on the last couple of lines. To find the attributes of any such object you can use:

```
attributes(pdb)
```

```
## $names
## [1] "atom"   "xyz"    "seqres" "helix"  "sheet"  "calpha" "remark" "call"
##
## $class
## [1] "pdb" "sse"
```

To access these individual attributes we use the dollar-attribute name convention that is common with R list objects. For example, to access the atom attribute or component use 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>
```

Predicting functional motions of a single structure

Let's read a new PDB structure of Adenylate Kinase and perform Normal mode analysis.

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

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

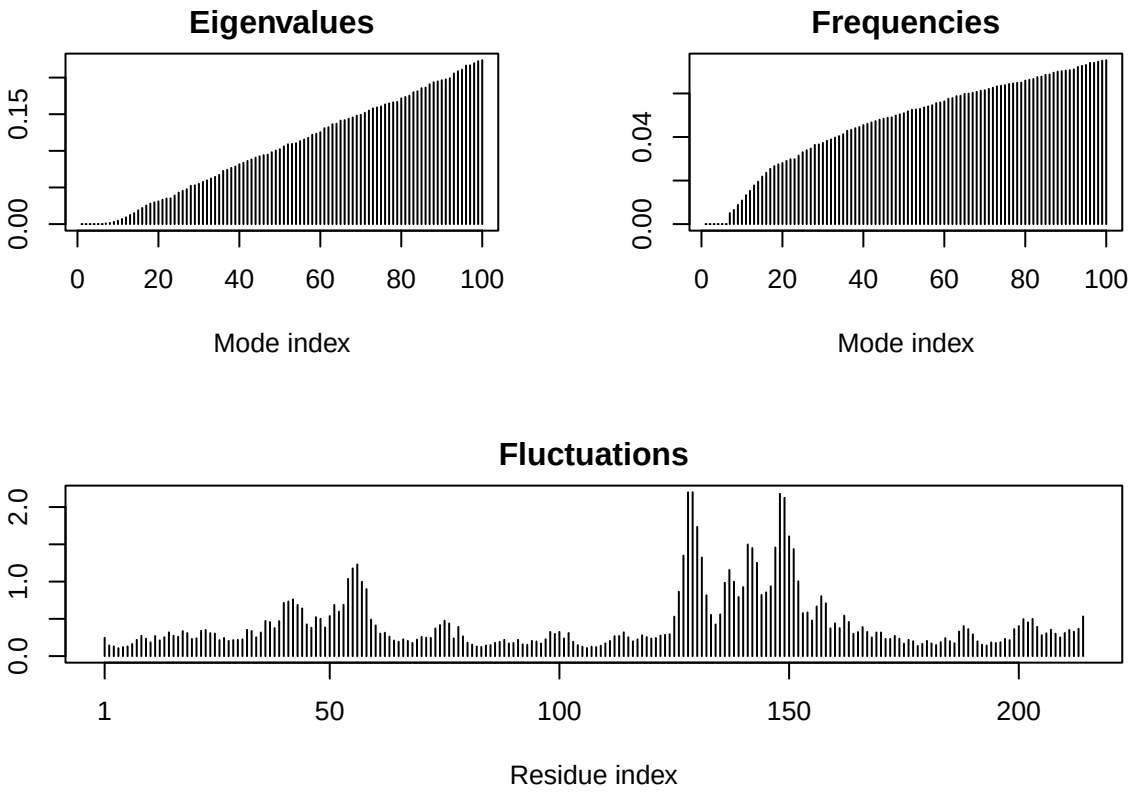
```
adk
```

```
##
##  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
```

```
# Perform flexiblity prediction
m <- nma(adk)
```

```
##  Building Hessian...     Done in 0.03 seconds.
##  Diagonalizing Hessian...    Done in 0.2 seconds.
```

```
plot(m)
```



4. Comparative structure analysis of Adenylate Kinase

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?:

devtools::install_bitbucket("Grantlab/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, i.e. how long is this sequence?

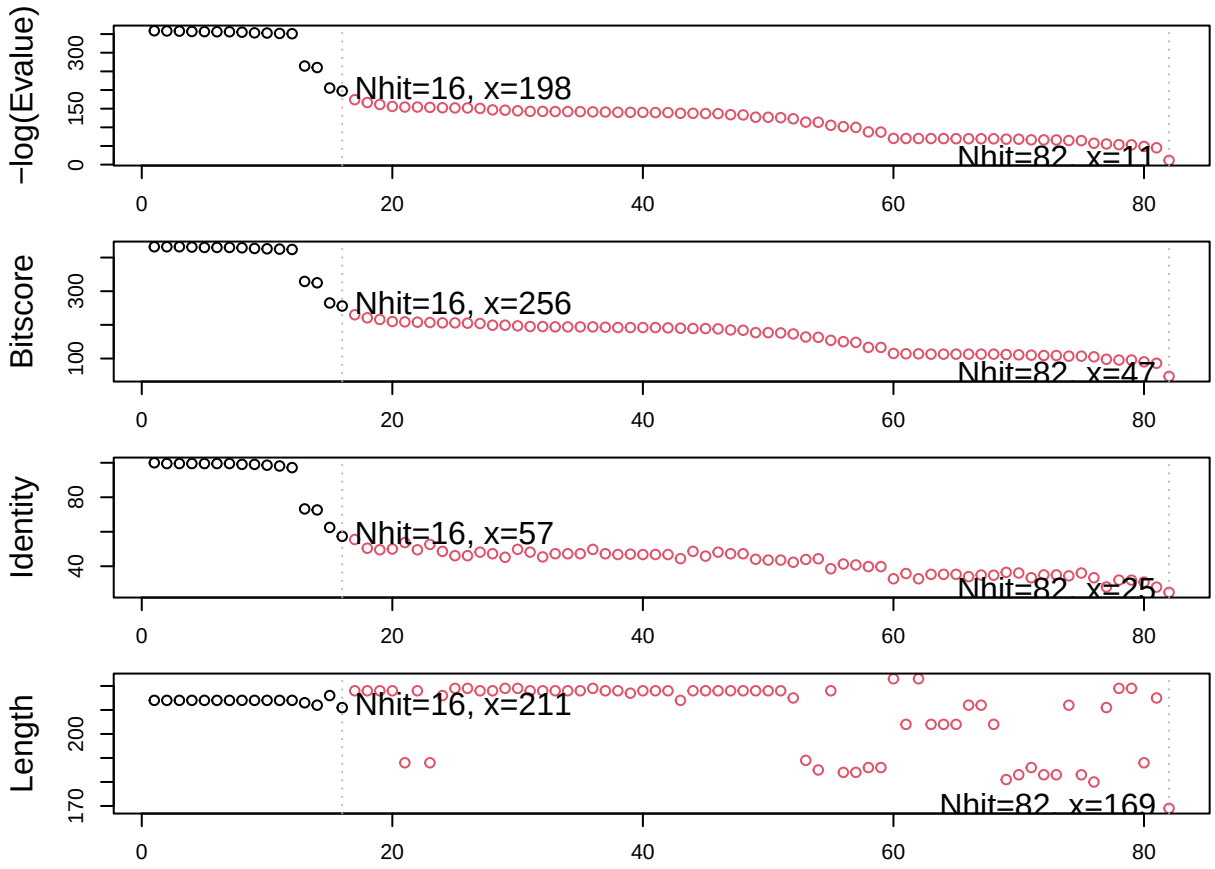
214

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

```
##  Searching ... please wait (updates every 5 seconds) RID = 5JHB69E901N
##  .
##  Reporting 82 hits
```

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

```
##    * Possible cutoff values:    197 11
##              Yielding Nhits:    16 82
##
##    * 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"
```

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

```
# Download releated 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/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/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/3GMT.pdb exists. Skipping download

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

##   |                                                                      |
```

Align and superpose structures

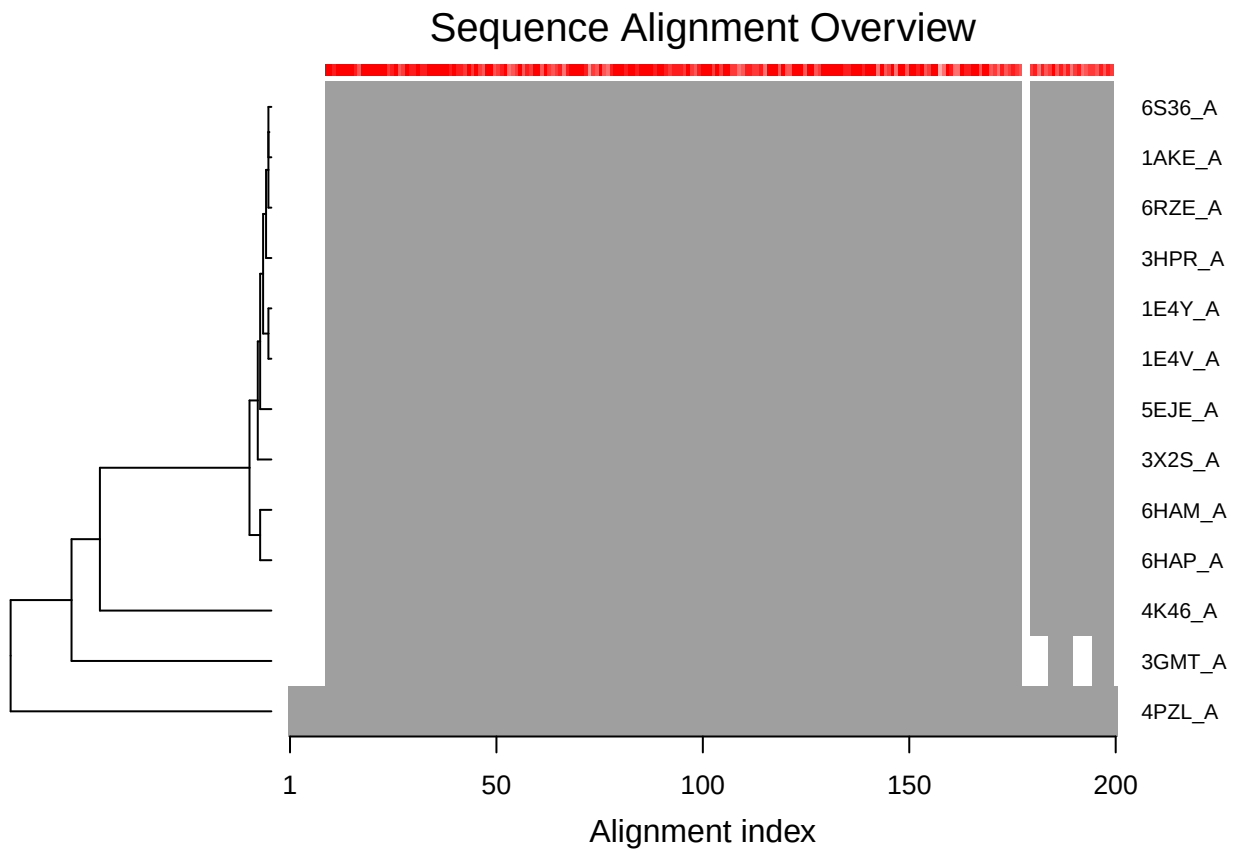
```
# Align releated PDBs
pdbs <- pdbaln(files, fit = TRUE, exefile="msa")
```

```
## 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)

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



Annotate collected PDB structures

The function pdb.annotate() provides a convenient way of annotating the PDB files we have collected.

```
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"
```

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
##
## 1AKE_A STRUCTURE OF THE COMPLEX BETWEEN ADENYLATE KINASE FROM ESCHERICHIA COLI AND THE INHIBITOR AP5A
## 6S36_A
```

```
## 6RZE_A
## 3HPR_A
## 1E4V_A
## 5EJE_A                                                                    Crystal struc
## 1E4Y_A
## 3X2S_A
## 6HAP_A
## 6HAM_A
## 4K46_A
## 3GMT_A
## 4PZL_A                                                                 The crystal struc
##                                                      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
```

```
sessionInfo()
```

```
## R version 4.2.3 (2023-03-15 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19044)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.utf8
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.utf8
```

```
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] bio3d_2.4-4 dplyr_1.1.1
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.10            GenomeInfoDb_1.34.9    pillar_1.9.0
##  [4] compiler_4.2.3         highr_0.10             XVector_0.38.0
##  [7] bitops_1.0-7           tools_4.2.3            zlibbioc_1.44.0
## [10] digest_0.6.31          jsonlite_1.8.4         evaluate_0.20
## [13] lifecycle_1.0.3        tibble_3.2.1           pkgconfig_2.0.3
## [16] rlang_1.1.0            cli_3.6.1              rstudioapi_0.14
## [19] curl_5.0.0             yaml_2.3.7             parallel_4.2.3
## [22] xfun_0.38              fastmap_1.1.1          GenomeInfoDbData_1.2.9
## [25] httr_1.4.6             knitr_1.42             generics_0.1.3
## [28] Biostrings_2.66.0      vctrs_0.6.1            S4Vectors_0.36.2
## [31] IRanges_2.32.0         stats4_4.2.3           grid_4.2.3
## [34] tidyselect_1.2.0       glue_1.6.2             R6_2.5.1
## [37] fansi_1.0.4            rmarkdown_2.21         msa_1.30.1
## [40] magrittr_2.0.3         htmltools_0.5.5        BiocGenerics_0.44.0
## [43] utf8_1.2.3             RCurl_1.98-1.12        crayon_1.5.2
```