Class 9: Structural Bioinformatics pt 1

Seona Patel (PID: A69035519)

The PDB Database

The main repository for biomolecular structure data is the Protein Data Bank (PDB) https://www.rcsb.org

Let's have a quick look at the composition of this database:

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

```
           Molecular.Type   X.ray     EM    NMR Integrative Multiple.methods
1          Protein (only) 176,378 20,438 12,709         342              221
2 Protein/Oligosaccharide  10,284  3,396     34           8               11
3              Protein/NA   9,007  5,931    287          24                7
4     Nucleic acid (only)   3,077    200  1,554           2               15
5                   Other     174     13     33           3                0
6  Oligosaccharide (only)      11      0      6           0                1
  Neutron Other   Total
1      83    32 210,203
2       1     0  13,734
3       0     0  15,256
4       3     1   4,852
5       0     0     223
6       0     4      22
```

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

```
stats$X.ray <- gsub(",", "", stats$X.ray)
sum(as.numeric(stats$X.ray))
```

```
[1] 198931
```

This is annoying, lets try a different import function from the **readr** package.

```
library(readr)
```

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

```
Rows: 6 Columns: 9
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Molecular Type
dbl (4): Integrative, 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.
```

```
stats
```

```
# A tibble: 6 x 9
  `Molecular Type`    `X-ray`    EM   NMR Integrative `Multiple methods` Neutron
  <chr>                 <dbl> <dbl> <dbl>       <dbl>              <dbl>   <dbl>
1 Protein (only)       176378 20438 12709         342                221      83
2 Protein/Oligosacch~   10284  3396    34           8                 11       1
3 Protein/NA             9007  5931   287          24                  7       0
4 Nucleic acid (only)    3077   200  1554           2                 15       3
5 Other                   174    13    33           3                  0       0
6 Oligosaccharide (o~      11     0     6           0                  1       0
# i 2 more variables: Other <dbl>, Total <dbl>
```

```
round(sum(stats$EM)/sum(stats$Total)*100,2)
```

```
[1] 12.27
```

```
round(sum(stats$`X-ray`)/sum(stats$Total)*100,2)
```

```
[1] 81.43
```

So 81.43% of the structures in the PDB are solved by X-ray and 12.27% of the structures are solved by EM.

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

```r
round(stats[1,9]/sum(stats$Total)*100,2)
```

```
  Total
1 86.05
```

So 86.05% are protein.

> Q3: Make a bar plot overview of Molecular type composition using ggplot

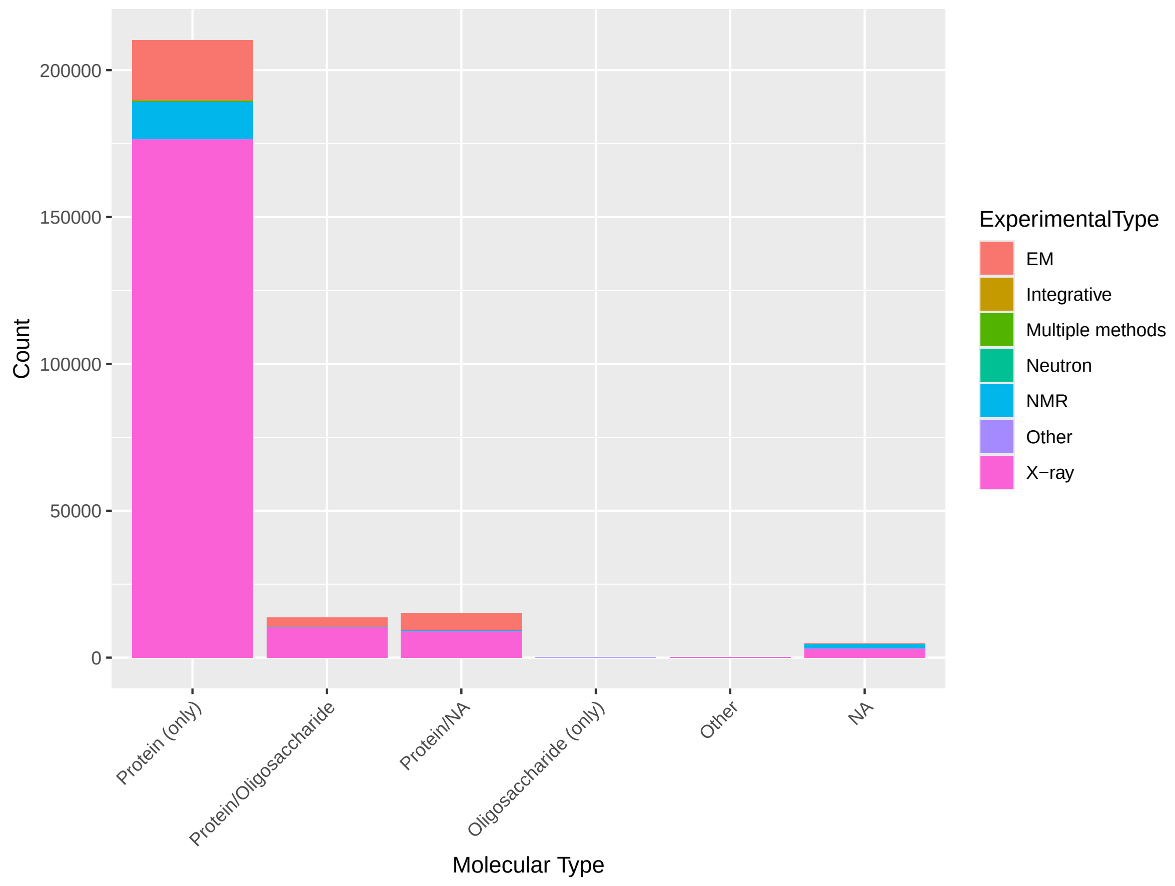
```r
library(ggplot2)
library(tidyr)
```

```r
stats$`Molecular Type` <- factor(stats$`Molecular Type`, levels = c("Protein (only)", "Prote
```

```r
stats_long <- pivot_longer(
  stats,
  cols = -c(Total, `Molecular Type`),
  names_to = "ExperimentalType",
  values_to = "Count"
)
head(stats_long)
```

```
# A tibble: 6 x 4
  `Molecular Type`  Total ExperimentalType  Count
  <fct>             <dbl> <chr>             <dbl>
1 Protein (only)   210203 X-ray            176378
2 Protein (only)   210203 EM                20438
3 Protein (only)   210203 NMR               12709
4 Protein (only)   210203 Integrative         342
5 Protein (only)   210203 Multiple methods    221
6 Protein (only)   210203 Neutron              83
```

```r
ggplot(stats_long) +
  aes(x=`Molecular Type`, y = Count, fill = ExperimentalType) +
  geom_col() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```

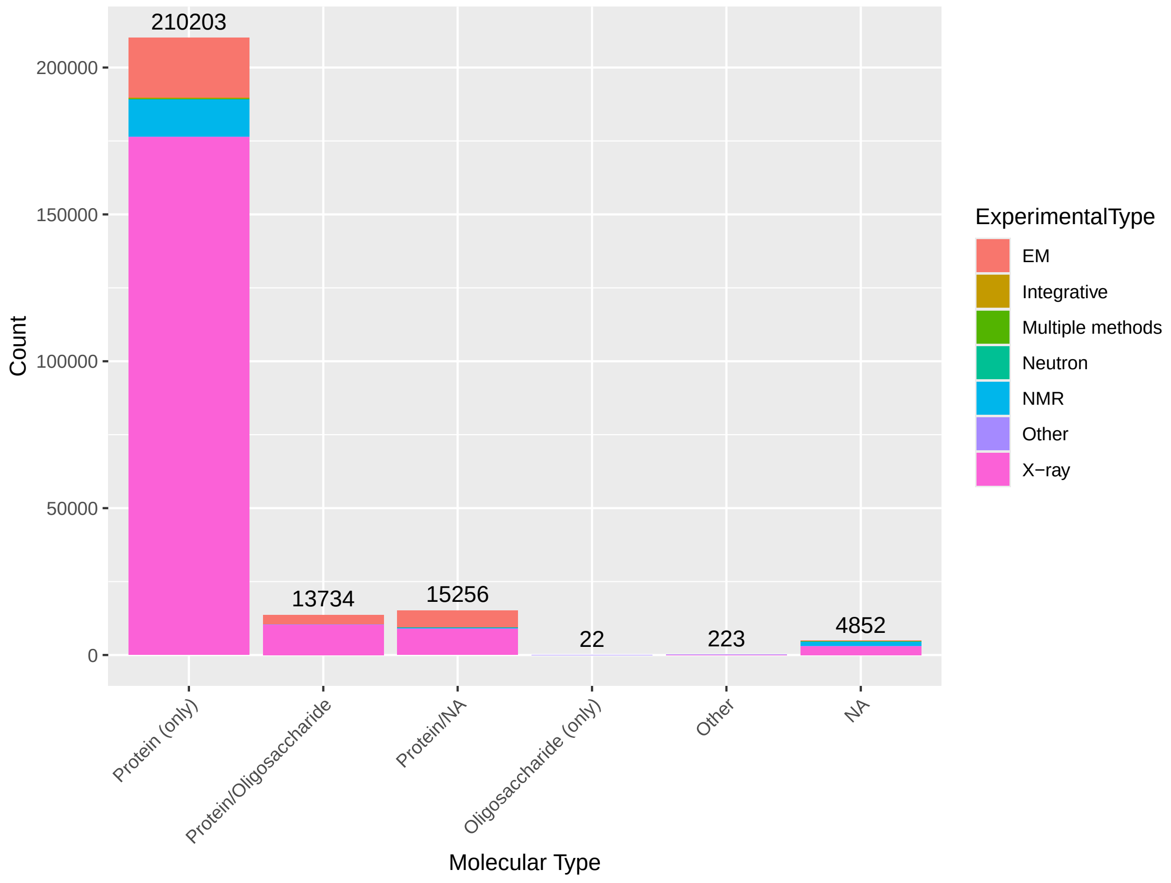


```
library(dplyr)
```

```

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
```

```
totals_df <- stats_long |>
  group_by(`Molecular Type`) |>
  summarise(Total = sum(Count))
```

```r
ggplot(stats_long) +
  aes(x = `Molecular Type`, y = Count, fill = ExperimentalType) +
  geom_col() +
  geom_text(
    data = totals_df,
    aes(x = `Molecular Type`, y = Total, label = Total),
    vjust = -0.5,
    inherit.aes = FALSE
  )+
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```



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

We only see one oxygen per water molecule because hydrogen atoms are hard to detect due to their low electron density in x-ray crystallography.

> 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

This water molecule is HOH 308.

Visualizing structure data

The Mol* viewer is embedded in many bioinformatics websites.The homepage is https://molstar.org/

I can insert any figure or image file using markdown format.

> 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.

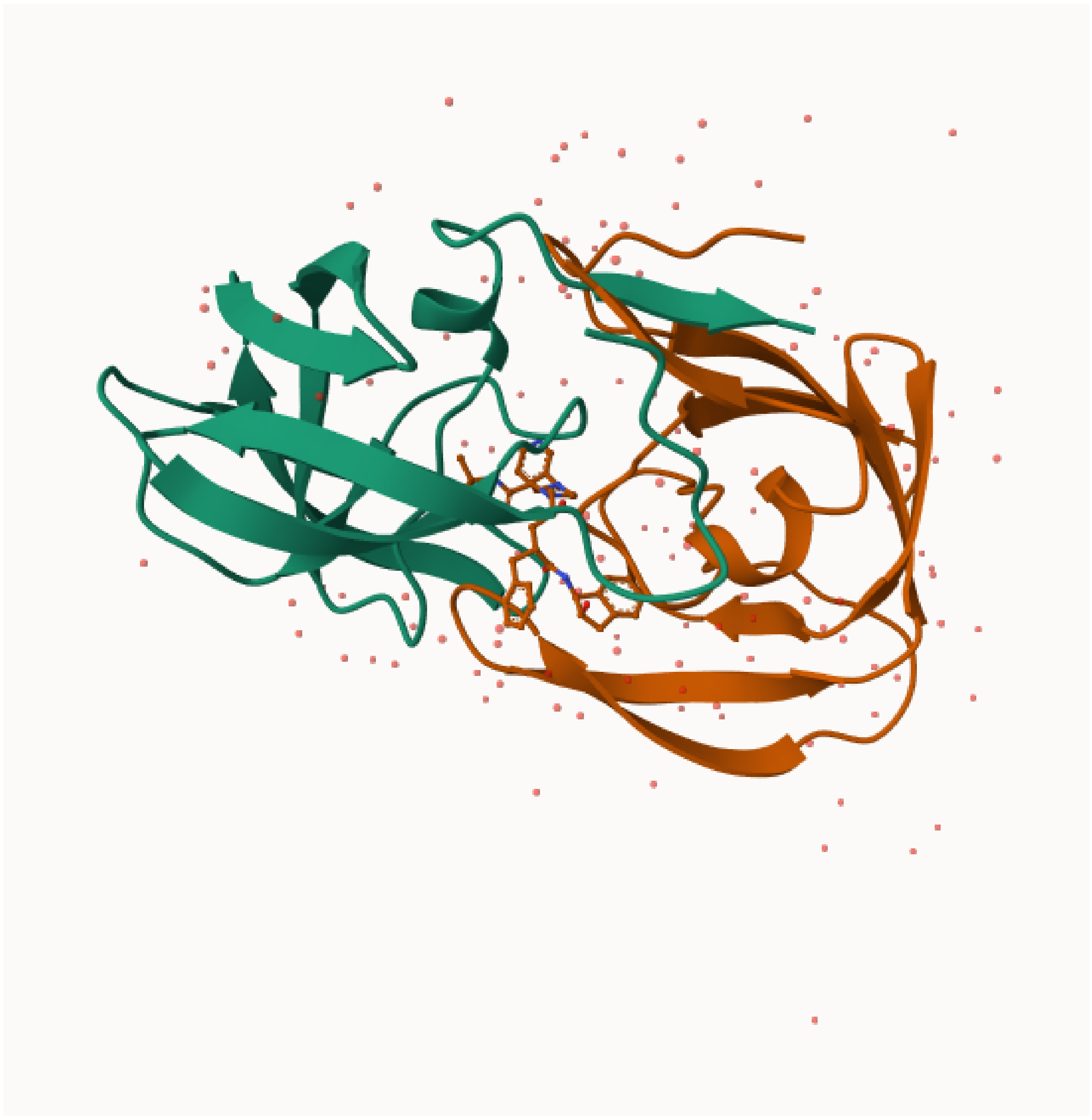


Figure 1: The HIV-Pr dimer with bound inhibitor

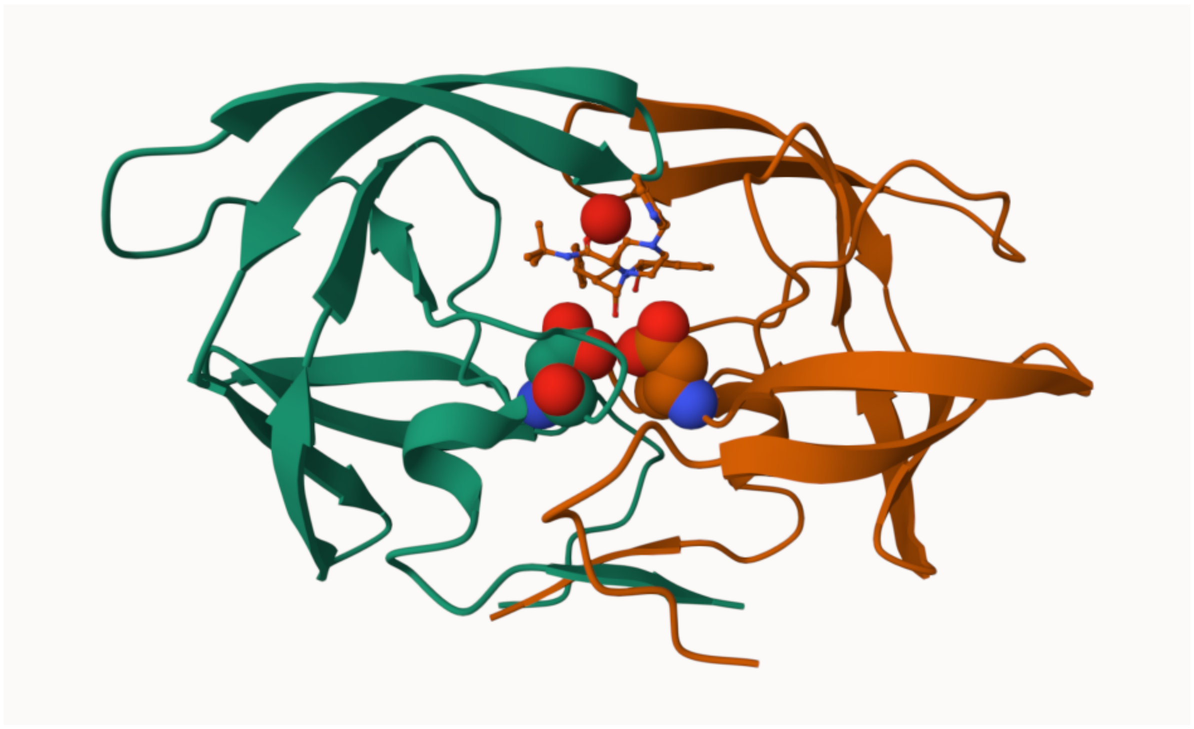


Figure 2: The catalytic ASP25 and active-site water

##Bio3D package for structural bioinformatics

We can use the bio3d package to read and analyze biomolecular data in R:

```
library(bio3d)
```

```
Warning: package 'bio3d' was built under R version 4.5.2
```

```
hiv <- read.pdb(file = "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
```

> Q7: How many amino acid residues are there in this pdb object?

198 amino acids

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

MK1

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

There are 2 protein chains in this structure

```r
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>
```

```r
pdbseq(hiv)
```

```
  1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19  20 
"P" "Q" "I" "T" "L" "W" "Q" "R" "P" "L" "V" "T" "I" "K" "I" "G" "G" "Q" "L" "K" 
 21  22  23  24  25  26  27  28  29  30  31  32  33  34  35  36  37  38  39  40 
"E" "A" "L" "L" "D" "T" "G" "A" "D" "D" "T" "V" "L" "E" "E" "M" "S" "L" "P" "G" 
 41  42  43  44  45  46  47  48  49  50  51  52  53  54  55  56  57  58  59  60 
"R" "W" "K" "P" "K" "M" "I" "G" "G" "I" "G" "G" "F" "I" "K" "V" "R" "Q" "Y" "D" 
 61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80 
"Q" "I" "L" "I" "E" "I" "C" "G" "H" "K" "A" "I" "G" "T" "V" "L" "V" "G" "P" "T" 
 81  82  83  84  85  86  87  88  89  90  91  92  93  94  95  96  97  98  99   1 
"P" "V" "N" "I" "I" "G" "R" "N" "L" "L" "T" "Q" "I" "G" "C" "T" "L" "N" "F" "P" 
  2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19  20  21 
"Q" "I" "T" "L" "W" "Q" "R" "P" "L" "V" "T" "I" "K" "I" "G" "G" "Q" "L" "K" "E" 
 22  23  24  25  26  27  28  29  30  31  32  33  34  35  36  37  38  39  40  41 
"A" "L" "L" "D" "T" "G" "A" "D" "D" "T" "V" "L" "E" "E" "M" "S" "L" "P" "G" "R" 
 42  43  44  45  46  47  48  49  50  51  52  53  54  55  56  57  58  59  60  61 
"W" "K" "P" "K" "M" "I" "G" "G" "I" "G" "G" "F" "I" "K" "V" "R" "Q" "Y" "D" "Q" 
 62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80  81 
"I" "L" "I" "E" "I" "C" "G" "H" "K" "A" "I" "G" "T" "V" "L" "V" "G" "P" "T" "P" 
 82  83  84  85  86  87  88  89  90  91  92  93  94  95  96  97  98  99 
"V" "N" "I" "I" "G" "R" "N" "L" "L" "T" "Q" "I" "G" "C" "T" "L" "N" "F" 
```

Let’s trim to chain A and get just it’s sequence

```r
chainA <- trim.pdb(hiv, chain="A")
chainA.seq <- pdbseq(chainA)
chainA.seq
```

```
  1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19  20 
"P" "Q" "I" "T" "L" "W" "Q" "R" "P" "L" "V" "T" "I" "K" "I" "G" "G" "Q" "L" "K" 
 21  22  23  24  25  26  27  28  29  30  31  32  33  34  35  36  37  38  39  40 
"E" "A" "L" "L" "D" "T" "G" "A" "D" "D" "T" "V" "L" "E" "E" "M" "S" "L" "P" "G" 
 41  42  43  44  45  46  47  48  49  50  51  52  53  54  55  56  57  58  59  60 
"R" "W" "K" "P" "K" "M" "I" "G" "G" "I" "G" "G" "F" "I" "K" "V" "R" "Q" "Y" "D" 
 61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80 
"Q" "I" "L" "I" "E" "I" "C" "G" "H" "K" "A" "I" "G" "T" "V" "L" "V" "G" "P" "T" 
 81  82  83  84  85  86  87  88  89  90  91  92  93  94  95  96  97  98  99 
"P" "V" "N" "I" "I" "G" "R" "N" "L" "L" "T" "Q" "I" "G" "C" "T" "L" "N" "F" 
```

Let's blast

Cache the blast so rendering doesn't take forever

```
blast <- blast.pdb(chainA.seq)
```

```
 Searching ... please wait (updates every 5 seconds) RID = GGVK33A7016
 ................................
 Reporting 249 hits
```

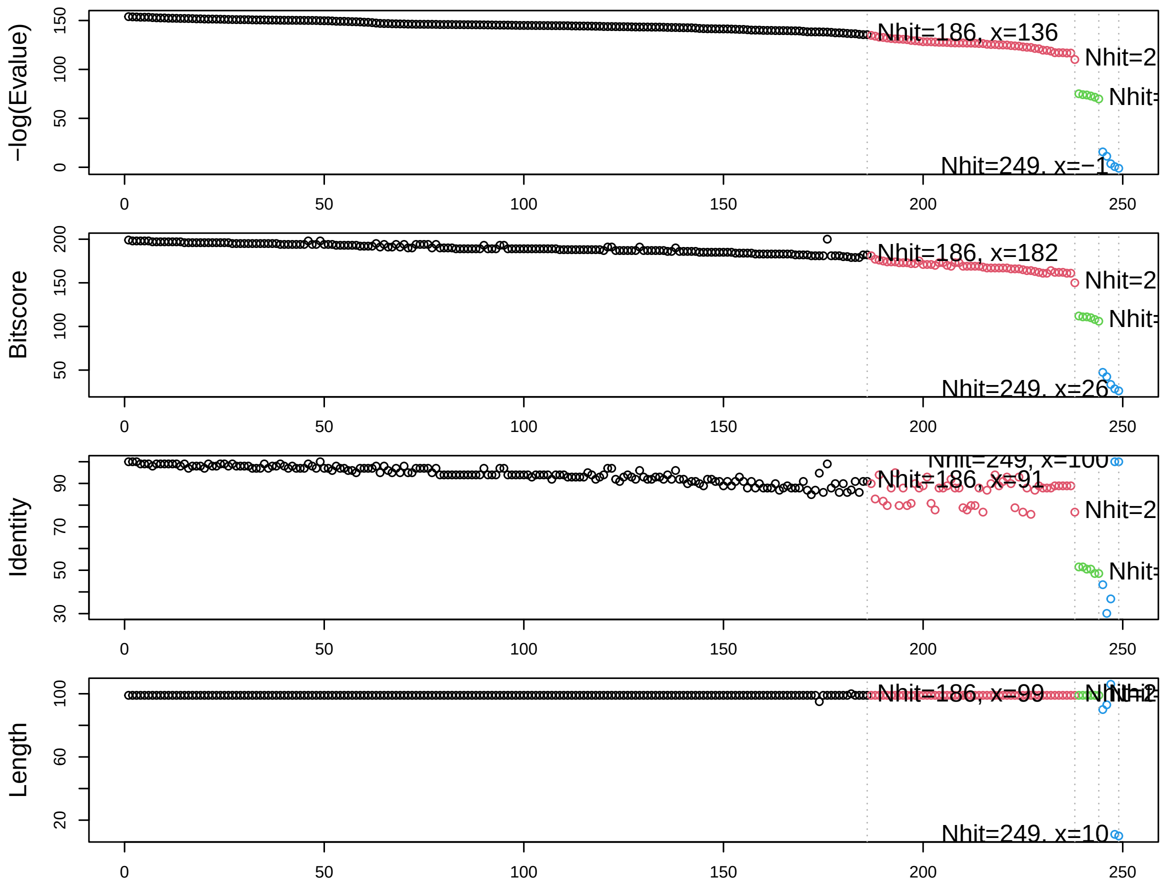
```
head(blast$hit.tbl)
```

```
        queryid subjectids identity alignmentlength mismatches gapopens q.start
1 Query_3882723     1W5V_A   100.00              99          0        0       1
2 Query_3882723     2FDE_A   100.00              99          0        0       1
3 Query_3882723     1AJV_A   100.00              99          0        0       1
4 Query_3882723     2R38_A    98.99              99          1        0       1
5 Query_3882723     2R3T_A    98.99              99          1        0       1
6 Query_3882723     1HXB_A    98.99              99          1        0       1
  q.end s.start s.end   evalue bitscore positives mlog.evalue pdb.id    acc
1    99      12   110 1.38e-67      199       100    153.9511 1W5V_A 1W5V_A
2    99       2   100 1.70e-67      198       100    153.7426 2FDE_A 2FDE_A
3    99       1    99 1.99e-67      198       100    153.5851 1AJV_A 1AJV_A
4    99       1    99 2.50e-67      198       100    153.3569 2R38_A 2R38_A
5    99       1    99 2.50e-67      198       100    153.3569 2R3T_A 2R3T_A
6    99       1    99 2.50e-67      198       100    153.3569 1HXB_A 1HXB_A
```

Plot a quick overview of blast results

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

```
  * Possible cutoff values:    135 110 69 -2
            Yielding Nhits:    186 238 244 249

  * Chosen cutoff value of:    69
            Yielding Nhits:    244
```



Every dot is a hit on this.

Get the accession number of all of the hits:

```
hits$pdb.id
```

```
 [1] "1W5V_A" "2FDE_A" "1AJV_A" "2R38_A" "2R3T_A" "1HXB_A" "1BV9_A" "1AAQ_A"
 [9] "1AXA_A" "1HVS_A" "1ZP8_A" "2QHC_A" "1A8G_A" "2O4L_A" "5COK_A" "1TCX_A"
[17] "2Z54_A" "1D4S_A" "1BV7_A" "1BWA_A" "1A9M_A" "2FLE_A" "1ODY_A" "1GNN_A"
[25] "1GNM_A" "5YRS_B" "1HEF_E" "1ODX_A" "4QGI_A" "1BVE_A" "2AZ8_A" "1A30_A"
[33] "6DH6_A" "6DH0_A" "2I4D_A" "6OOS_A" "1RL8_A" "5YRS_A" "1ZSF_A" "2Q64_A"
[41] "6DH3_A" "2NPH_A" "2Q63_A" "1LZQ_A" "1FB7_A" "1G6L_A" "1HIV_A" "6OOU_A"
[49] "1HVC_A" "2I4V_A" "2AZ9_A" "6OOT_A" "2P3B_B" "5KAO_A" "2WLO_A" "6OPT_A"
[57] "1IZI_A" "1MRX_A" "2PYM_A" "2PYN_A" "1DMP_A" "4K4P_A" "1LV1_A" "1AID_A"
[65] "1LV1_A" "1ZBG_A" "3TKG_A" "1HVC_A" "5YOK_A" "1G6L_A" "1FGC_C" "3K4V_A"
[73] "3KT5_A" "3KT5_A" "4QLH_A" "4QLH_A" "2F3K_A" "4Q5M_A" "2AOC_A" "3B80_A"
[81] "3VF5_A" "2AVQ_A" "1DW6_C" "1KZK_A" "2HS1_A" "1K6C_A" "1MTB_A" "4Q1X_A"
```

```
 [89] "4Q1W_A" "4Q5M_A" "3D1X_A" "2AVM_A" "3PWM_A" "3KT2_A" "3KT2_A" "1SDV_A"
 [97] "3JVW_A" "3OY4_A" "1A94_A" "2HS2_A" "4EJ8_A" "2FGU_A" "2AVV_A" "3JW2_A"
[105] "3BVA_A" "1FFF_C" "3S43_B" "2NXD_A" "1FG6_C" "1EBK_C" "4Q1Y_A" "3EL4_A"
[113] "1F7A_A" "1K2B_A" "2FGV_A" "1Z8C_A" "2G69_A" "3EL9_A" "3OXV_A" "1BDR_A"
[121] "3N3I_A" "3N3I_A" "3OXW_A" "3S43_A" "3EM3_A" "3CYW_A" "5KQX_A" "2B60_A"
[129] "7DOZ_A" "1K2C_A" "1MT7_A" "3EM4_A" "4QJ9_A" "1BDL_A" "3LZS_A" "5T84_A"
[137] "4DQB_A" "7DOZ_A" "4QJ2_A" "3LZV_A" "1SGU_A" "2FXE_A" "1BDQ_A" "3U71_A"
[145] "2R5P_A" "4OBD_A" "7MAS_A" "3IXO_A" "3D3T_A" "5YOJ_A" "3LZU_A" "4NJS_A"
[153] "3EKP_A" "1B6J_A" "3EKQ_A" "2RKF_A" "1C6X_A" "7MAR_A" "4DQF_A" "1RPI_A"
[161] "3OU1_B" "3PJ6_A" "2P3A_A" "6OGQ_A" "3OQ7_A" "5KR1_A" "3OQD_A" "4RVI_A"
[169] "3OQA_A" "1B6K_A" "3OUD_B" "6MK9_A" "3SO9_A" "1Q9P_A" "6I45_A" "7SEP_A"
[177] "4NJT_A" "3BXR_A" "4YOA_A" "4DQC_A" "2FDD_A" "2RKG_A" "4DQH_A" "2P3C_A"
[185] "4EP2_A" "4EP2_A" "4EQ0_A" "4NPT_A" "6OPU_A" "4NPU_A" "3U7S_A" "3HAW_A"
[193] "2AZB_A" "3TTP_A" "3HBO_A" "3GGU_A" "7N6T_A" "6OPV_A" "4EQ0_A" "6OPX_A"
[201] "2O4N_A" "5T2E_A" "3UCB_A" "3KA2_A" "3FSM_A" "6OPW_A" "2AZC_A" "3FSM_A"
[209] "3HLO_A" "2P3D_A" "3T3C_A" "7MYP_A" "6O54_X" "6OPY_A" "4Z4X_A" "6OPZ_A"
[217] "2JE4_A" "1DAZ_C" "7MAP_A" "7MAQ_A" "1K1U_A" "2B7Z_A" "3MWS_A" "1K1T_A"
[225] "8DCH_A" "3I2L_A" "6P9A_A" "2FXD_A" "2J9J_A" "3DCK_A" "2J9J_B" "3NXE_A"
[233] "2O40_A" "2O40_A" "3NXE_A" "3KA2_A" "3HLO_A" "5B18_A" "1SIP_A" "2SAM_A"
[241] "1AZ5_A" "1SIV_A" "1HII_A" "1IVP_A"
```

Prediction of Functional motions

We can run an Normal Mode Analysis (NMA) to predict large scale motions/flexibility/dynamics of any biomolecule that we can read into R.

Let's look at ADK and chain A only!

```r
adk <- read.pdb("1ake")
```

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

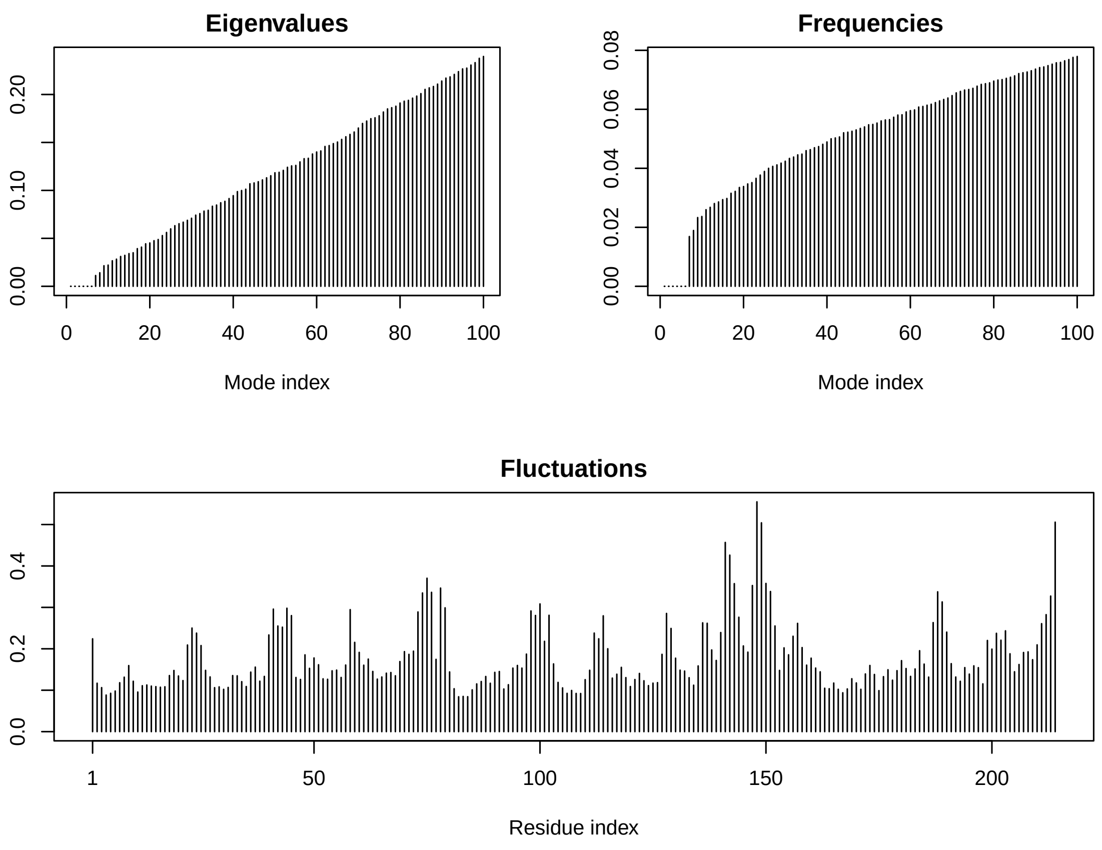
```r
adk_A <- trim.pdb(adk, chain="A")
adk_A
```

```

 Call:  trim.pdb(pdb = adk, chain = "A")

   Total Models#: 1
     Total Atoms#: 1954,  XYZs#: 5862  Chains#: 1  (values: A)
     Protein Atoms#: 1656  (residues/Calpha atoms#: 214)
     Nucleic acid Atoms#: 0  (residues/phosphate atoms#: 0)

     Non-protein/nucleic Atoms#: 298  (residues: 242)
     Non-protein/nucleic resid values: [ AP5 (1), HOH (241) ]

   Protein sequence:
      MRIILLGAPGAGKGTQAQFIMEKYGIPQISTGDMLRAAVKSGSELGKQAKDIMDAGKLVT
      DELVIALVKERIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFDVPDELIVDRI
      VGRRVHAPSGRVYHVKFNPPKVEGKDDVTGEELTTRKDDQEETVRKRLVEYHQMTAPLIG
      YYSKEAEAGNTKYAKVDGTKPVAEVRADLEKILG

+ attr: atom, helix, sheet, seqres, xyz,
        calpha, call
```

```
m <- nma(adk_A)
```

```
 Building Hessian...        Done in 0.02 seconds.
 Diagonalizing Hessian...   Done in 0.25 seconds.
```

```
plot(m)
```



This tells me about the flexibility of the protein.

Let's write out a "trajectory" of predicted motion

```r
mktrj(m, file="adk_nma.pdb")
```

Play with 3D viewing in R

We can use the new **bio3dview** package, which is not yet on CRAN, to render interactvie 3D views in R and HTML quarto output reports.

To install from GitHub we can use the **pak** package.

```r
pak::pak("bioboot/bio3dview")
```

```
! Using bundled GitHub PAT. Please add your own PAT using `gitcreds::gitcreds_set()`.
```

```
Loading metadata database

Loading metadata database ... done
```

```
No downloads are needed

1 pkg + 40 deps: kept 40 [6.5s]
```

```r
library(bio3dview)
#view.pdb(adk)
```

Comparative structure analysis of Adenylate Kinase

The goal of this section is to perform principal component analysis (PCA) on the complete collection of Adenylate kinase structures in the protein data-bank (PDB).

Adenylate kinase (often called simply Adk) is a ubiquitous enzyme that functions to maintain the equilibrium between cytoplasmic nucleotides essential for many cellular processes. Adk operates by catalyzing the reversible transfer of a phosphoryl group from ATP to AMP. This reaction requires a rate limiting conformational transition (i.e. change in shape). Here we analyze all currently available Adk structures in the PDB to reveal detailed features and mechanistic principles of these essential shape changing transitions.

The bio3d package pca() function provides a convenient interface for performing PCA of biomolecular structure data. As we have discussed in previous classes, PCA is a statistical approach used to transform large data-sets down to a few important components that usefully describe the directions where there is most variance. In terms of protein structures PCA can be used to capture major structural variations within a set of structures (a.k.a. structure ensemble). This can make interpreting major conformational states (such as 'active' and 'inactive' or 'ligand bound' and 'un-bound' states) and structural mechanisms for activation or regulation more clear.

Overview

Starting from only one Adk PDB identifier (PDB ID: 1AKE) we will search the entire PDB for related structures using BLAST, fetch, align and superpose the identified structures, perform PCA and finally calculate the normal modes of each individual structure in order to probe for potential differences in structural flexibility.

> 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

Search and retrieve ADK structures

Below we perform a blast search of the PDB database to identify related structures to our query Adenylate kinase(ADK) sequence. In this particular example we use function get.seq() to fetch the query sequence for chain A of the PDB ID 1AKE and use this as input to blast.pdb(). Note that get.seq() would also allow the corresponding UniProt identifier.

```
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

Now we can use this sequence as a query to BLAST search the PDB to find similar sequences and structures.

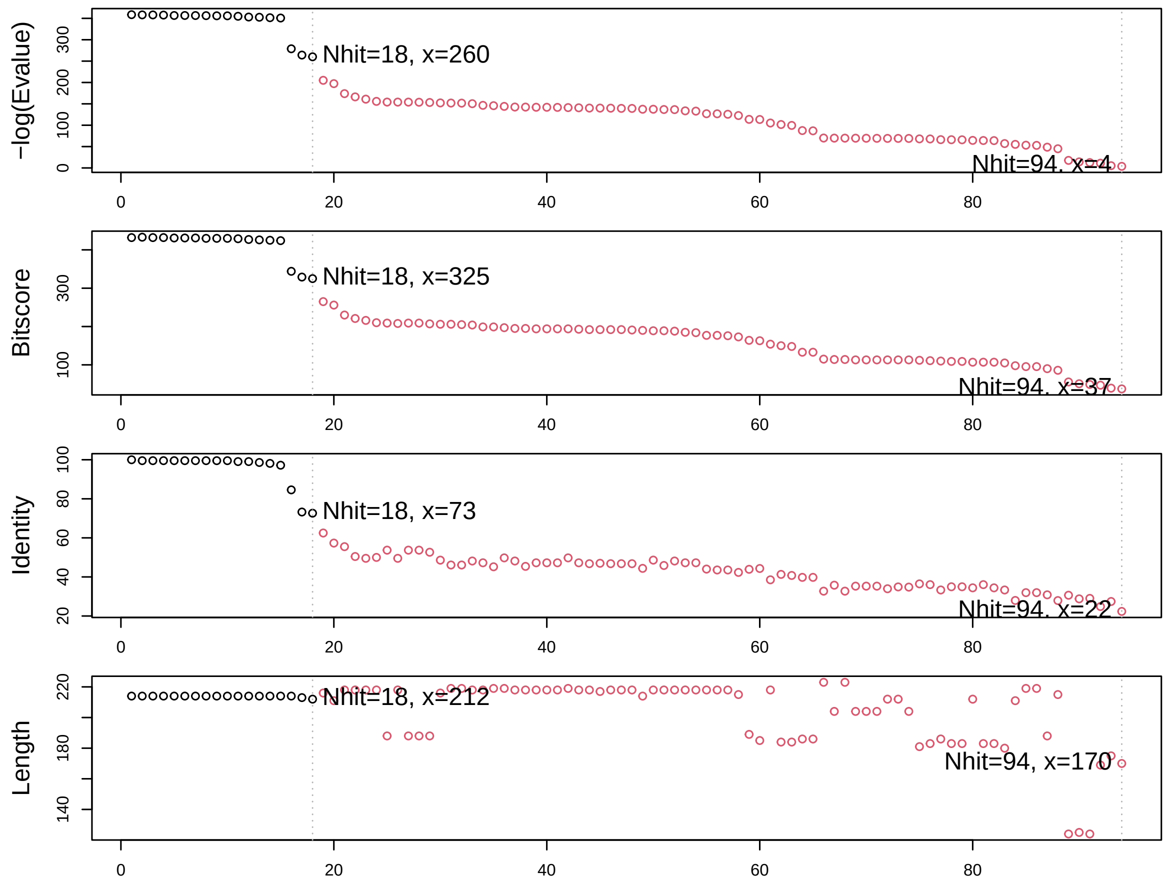
```r
b <- blast.pdb(aa)
```

```
 Searching ... please wait (updates every 5 seconds) RID = GGVT366W014
 ....................
 Reporting 94 hits
```

The function plot.blast() facilitates the visualization and filtering of the Blast results. It will attempt to set a seed position to the point of largest drop-off in normalized scores (i.e. the biggest jump in E-values). In this particular case we specify a cutoff (after initial plotting) of to include only the relevant E.coli structures:

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

```
 * Possible cutoff values:    260 3
            Yielding Nhits:    18 94

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



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

```
[1] "1AKE_A" "8BQF_A" "4X8M_A" "6S36_A" "8Q2B_A" "8RJ9_A"
```

The Blast search and subsequent filtering identified a total of 13 related PDB structures to our query sequence. The PDB identifiers of this collection are accessible through the `$pdb.id` attribute to the hits object (i.e. `hits$pdb.id`). Note that adjusting the cutoff argument (to `plot.blast()`) will result in a decrease or increase of hits.

We can now use function `get.pdb()` and `pdbslit()` to fetch and parse the identified structures.

```r
# 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/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 and superpose structures

Next we will use the `pdbaln()` function to align and also optionally fit (i.e. superpose) the identified PDB structures.

```r
# Align related 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
```

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

# Draw schematic alignment

plot(pdbs, labels=ids)
```

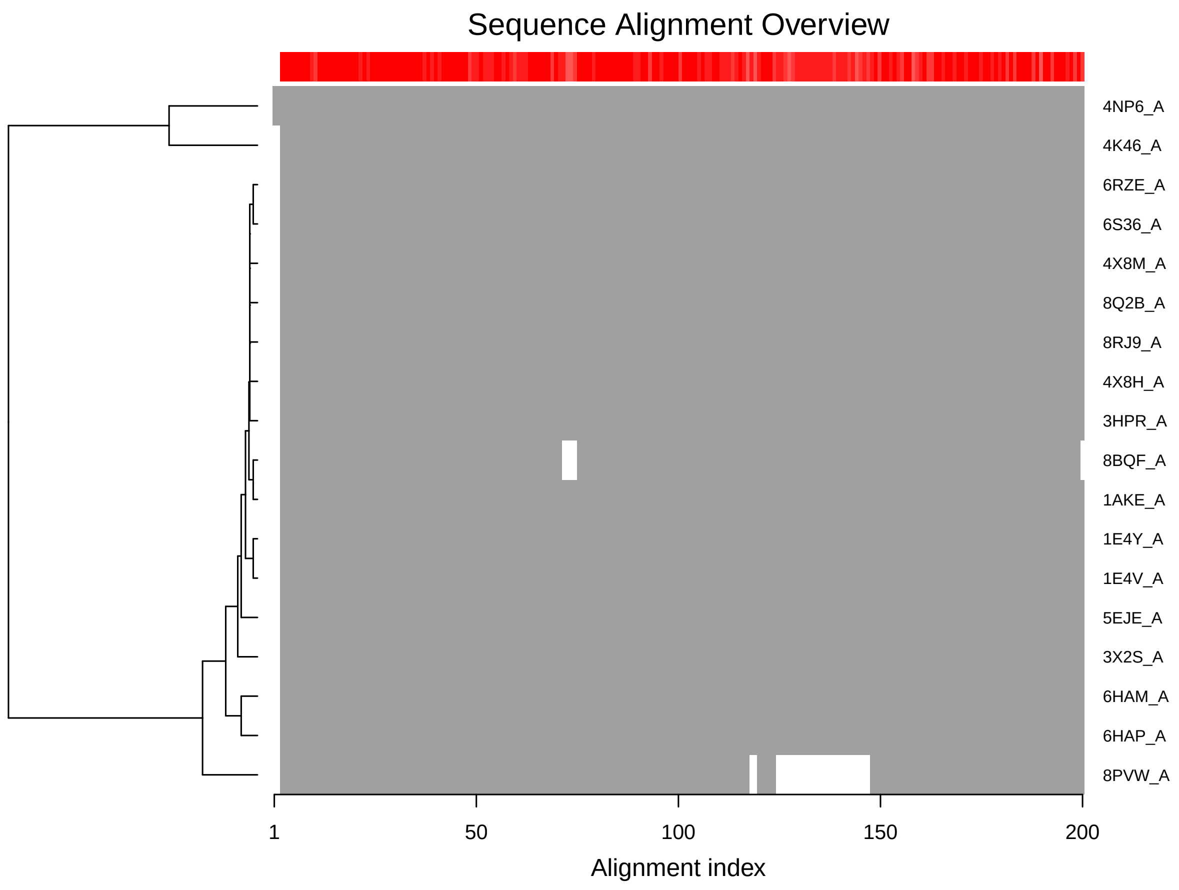


Figure 7: Schematic representation of alignment. Grey regions depict aligned residues, while

white depict gap regions. The red bar at the top depict sequence conservation

Annotate collected PDB structures

The function pdb.annotate() provides a convenient way of annotating the PDB files we have collected. Below we use the function to annotate each structure to its source species. This will come in handy when annotating plots later on:

```r
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"
```

We can view all available annotation data:

```r
anno
```

```
       structureId chainId macromoleculeType chainLength experimentalTechnique
1AKE_A        1AKE       A           Protein         214                 X-ray
8BQF_A        8BQF       A           Protein         234                 X-ray
4X8M_A        4X8M       A           Protein         214                 X-ray
6S36_A        6S36       A           Protein         214                 X-ray
8Q2B_A        8Q2B       A           Protein         214                 X-ray
8RJ9_A        8RJ9       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
8PVW_A        8PVW       A           Protein         187                 X-ray
4K46_A        4K46       A           Protein         214                 X-ray
4NP6_A        4NP6       A           Protein         217                 X-ray
```

```
       resolution       scopDomain                                        pfam
1AKE_A      2.000 Adenylate kinase                      Adenylate kinase (ADK)
8BQF_A      2.050             <NA>                      Adenylate kinase (ADK)
4X8M_A      2.600             <NA>                      Adenylate kinase (ADK)
6S36_A      1.600             <NA> Adenylate kinase, active site lid (ADK_lid)
8Q2B_A      1.760             <NA> Adenylate kinase, active site lid (ADK_lid)
8RJ9_A      1.590             <NA> Adenylate kinase, active site lid (ADK_lid)
6RZE_A      1.690             <NA>                      Adenylate kinase (ADK)
4X8H_A      2.500             <NA>                      Adenylate kinase (ADK)
3HPR_A      2.000             <NA>                      Adenylate kinase (ADK)
1E4V_A      1.850 Adenylate kinase                      Adenylate kinase (ADK)
5EJE_A      1.900             <NA>                      Adenylate kinase (ADK)
1E4Y_A      1.850 Adenylate kinase                      Adenylate kinase (ADK)
3X2S_A      2.800             <NA>                      Adenylate kinase (ADK)
6HAP_A      2.700             <NA> Adenylate kinase, active site lid (ADK_lid)
6HAM_A      2.550             <NA>                      Adenylate kinase (ADK)
8PVW_A      2.000             <NA> Adenylate kinase, active site lid (ADK_lid)
4K46_A      2.010             <NA>                      Adenylate kinase (ADK)
4NP6_A      2.004             <NA>                      Adenylate kinase (ADK)
                ligandId
1AKE_A               AP5
8BQF_A               AP5
4X8M_A              <NA>
6S36_A CL (3),NA,MG (2)
8Q2B_A      AP5,SO4,MPO
8RJ9_A          ADP (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
8PVW_A               AP5
4K46_A       ADP,AMP,PO4
4NP6_A              <NA>
                                                                  ligandName
1AKE_A                                   BIS(ADENOSINE)-5'-PENTAPHOSPHATE
8BQF_A                                   BIS(ADENOSINE)-5'-PENTAPHOSPHATE
4X8M_A                                                               <NA>
6S36_A                 CHLORIDE ION (3),SODIUM ION,MAGNESIUM ION (2)
```

```
8Q2B_A BIS(ADENOSINE)-5'-PENTAPHOSPHATE,SULFATE ION,3[N-MORPHOLINO]PROPANE SULFONIC ACID
8RJ9_A                                                      ADENOSINE-5'-DIPHOSPHATE (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
8PVW_A                                                  BIS(ADENOSINE)-5'-PENTAPHOSPHATE
4K46_A                    ADENOSINE-5'-DIPHOSPHATE,ADENOSINE MONOPHOSPHATE,PHOSPHATE ION
4NP6_A                                                                              <NA>
                                             source
1AKE_A                             Escherichia coli
8BQF_A                             Escherichia coli
4X8M_A                             Escherichia coli
6S36_A                             Escherichia coli
8Q2B_A                             Escherichia coli
8RJ9_A                             Escherichia coli
6RZE_A                             Escherichia coli
4X8H_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
8PVW_A                        Escherichia coli K-12
4K46_A                     Photobacterium profundum
4NP6_A Vibrio cholerae O1 biovar El Tor str. N16961

1AKE_A STRUCTURE OF THE COMPLEX BETWEEN ADENYLATE KINASE FROM ESCHERICHIA COLI AND THE INHIB
8BQF_A
4X8M_A
6S36_A
8Q2B_A                                              E. coli Adenylate Kinase variant D158A (A
8RJ9_A                                                        E. coli adenylate kinase Asp84A
6RZE_A
4X8H_A
3HPR_A
```

```
1E4V_A
5EJE_A                                                                                                   Crys
1E4Y_A
3X2S_A
6HAP_A
6HAM_A
8PVW_A
4K46_A
4NP6_A
                                                       citation rObserved   rFree
1AKE_A            Muller, C.W., et al. J Mol Biology (1992)   0.19600      NA
8BQF_A  Scheerer, D., et al. Proc Natl Acad Sci U S A (2023)   0.22073 0.25789
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
8Q2B_A              Nam, K., et al. J Chem Inf Model (2024)   0.18320 0.22440
8RJ9_A                       Nam, K., et al. Sci Adv (2024)   0.15190 0.20290
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
8PVW_A             Rodriguez, J.A., et al. To be published    0.18590 0.23440
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
         rWork spaceGroup
1AKE_A 0.19600  P 21 2 21
8BQF_A 0.21882  P 2 21 21
4X8M_A 0.24630    C 1 2 1
6S36_A 0.15940    C 1 2 1
8Q2B_A 0.18100   P 1 21 1
8RJ9_A 0.15010  P 21 21 2
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
8PVW_A 0.18340  P 2 21 21
4K46_A 0.16730 P 21 21 21
4NP6_A 0.18600       P 43
```

Principle Component Analysis

Function pca() provides principal component analysis (PCA) of the structure data. PCA is a statistical approach used to transform a data set down to a few important components that describe the directions where there is most variance. In terms of protein structures PCA is used to capture major structural variations within an ensemble of structures.

PCA can be performed on the structural ensemble (stored in the pdbs object) with the function pca.xyz(), or more simply pca().

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

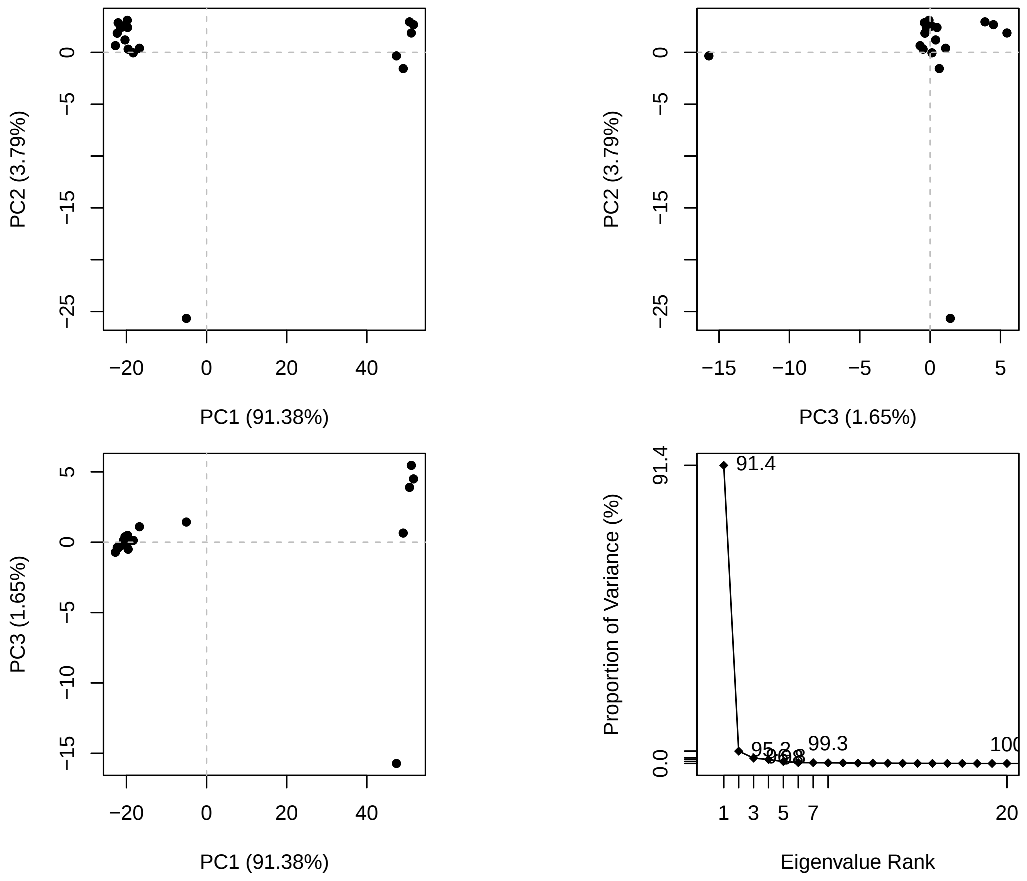


Figure 9: Results of PCA on Adenylate kinase X-ray structures. Each dot represents one PDB structure.

Function rmsd() will calculate all pairwise RMSD values of the structural ensemble. This facilitates clustering analysis based on the pairwise structural deviation:

```
# 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)
```

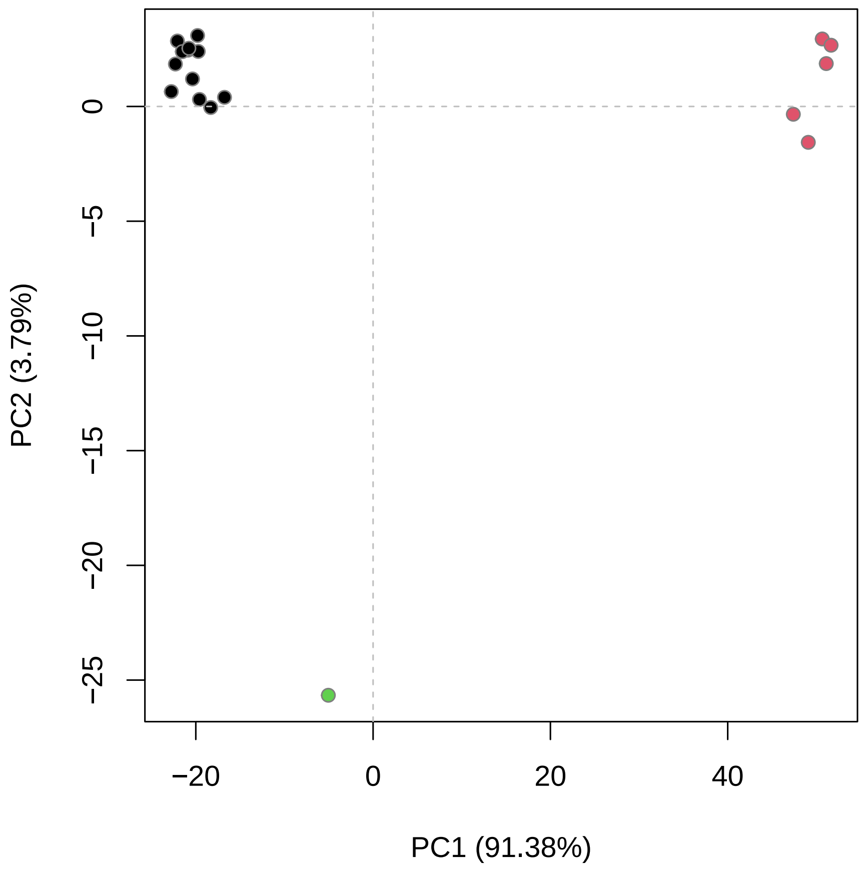


Figure 10: Projection of Adenylate kinase X-ray structures. Each dot represents one PDB structure.

The plot shows a conformer plot – a low-dimensional representation of the conformational variability within the ensemble of PDB structures. The plot is obtained by projecting the individual structures onto two selected PCs (e.g. PC-1 and PC-2). These projections display the inter-conformer relationship in terms of the conformational differences described by the selected PCs.

5. Optional further visualization

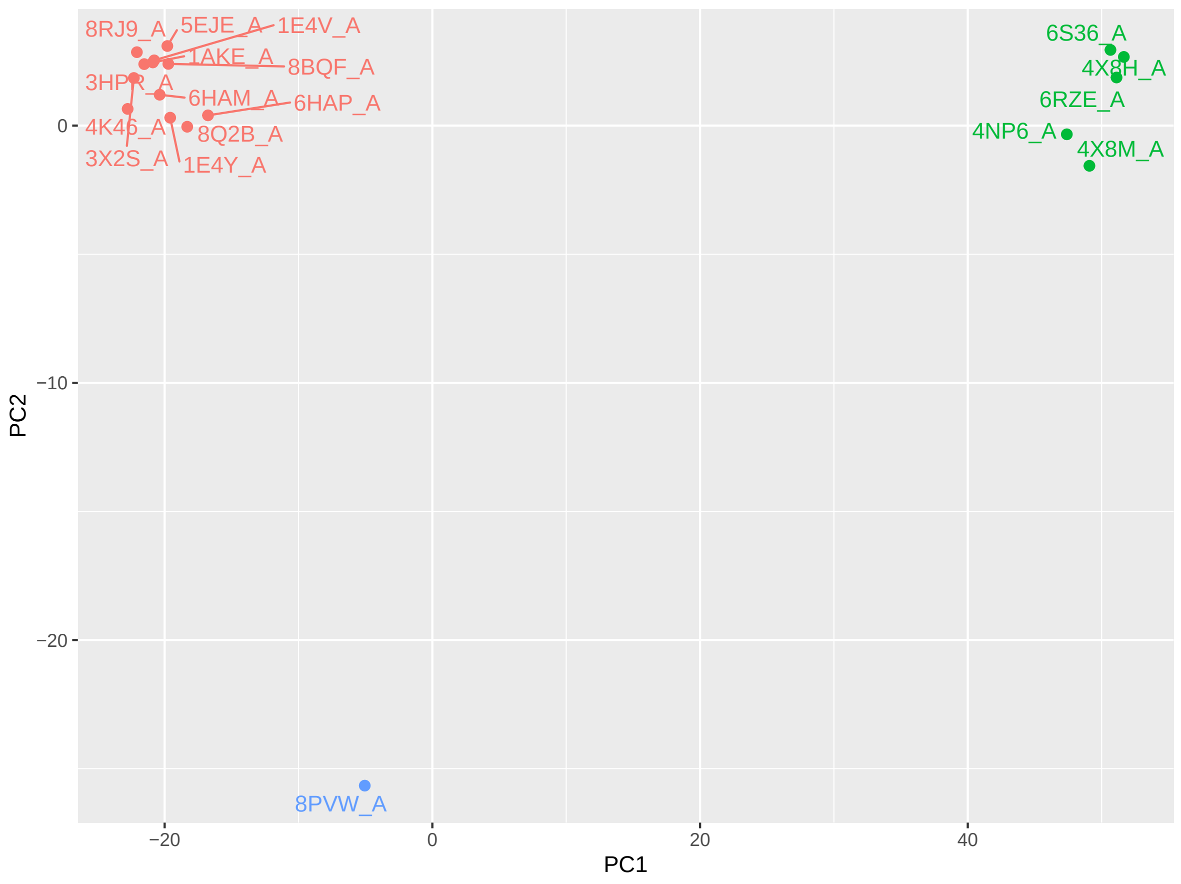
To visualize the major structural variations in the ensemble the function mktrj() can be used to generate a trajectory PDB file by interpolating along a give PC (eigenvector):

```
# 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
1AKE_A
8BQF_A
3HPR_A
6HAM_A
6HAP_A
4K46_A
8Q2B_A
3X2S_A
1E4Y_A
6S36_A
4X8H_A
6RZE_A
4NP6_A
4X8M_A
8PVW_A
PC2
PC1