Class 10

AUTHOR
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We need some packages for today's class, including `bio3d` and `msa`.

The `msa` package is from BioConductor. These packages focus on genomics type work and are managed by the `BiocManager` package

install BiocManager by `install.packages()`, then `BiocManager::install("msa")` in thr R console.

```
library(bio3d)
```

```
Warning: package 'bio3d' was built under R version 4.3.1
```

```
# Human TRIM46 protein isoform3, refseq accession NP_001393174
aa <- get.seq("NP_001393174")
```

```
Warning in get.seq("NP_001393174"): Removing existing file: seqs.fasta

Fetching... Please wait. Done.
```

```
aa
```

```
               1        .        .        .        .        .        60
NP_001393174.1   MAEGEDMQTFTSIMDALVRISLCSGEREARDRGLGRSVNQPKAGALEKLQTSMKNMEKEL
               1        .        .        .        .        .        60

              61        .        .        .        .        .       120
NP_001393174.1   LCPVCQEMYKQPLVLPCTHNVCQACAREVLGQQGYIGHGGDPSSEPTSPASTPSTRSPRL
              61        .        .        .        .        .       120

             121        .        .        .        .        .       180
NP_001393174.1   SRRTLPKPDRLDRLLKSGFGTYPGRKRGALHPQVIMFPCPACQGDVELGERGLAGLFRNL
             121        .        .        .        .        .       180

             181        .        .        .        .        .       240
NP_001393174.1   TLERVVERYRQSVSVGGAILCQLCKPPPLEATKGCTECRATFCNECFKLFHPWGTQKAQH
             181        .        .        .        .        .       240

             241        .        .        .        .        .       300
NP_001393174.1   EPTLPTLSFRPKGLMCPDHKEEVTHYCKTCQRLVCQLCRVRRTHSGHKITPVLSAYQALK
             241        .        .        .        .        .       300

             301        .        .        .        .        .       360
NP_001393174.1   DKLTKSLTYILGNQDTVQTQICELEEAVRHTEVSGQQAKEEVSQLVRGLGAVLEEKRASL
             301        .        .        .        .        .       360

             361        .        .        .        .        .       420
NP_001393174.1   LQAIEECQQERLARLSAQIQEHRSLLDGSGLVGYAQEVLKETDQPCFVQAAKQLHNRIAR
             361        .        .        .        .        .       420
```

```
               421       .         .         .         .         .         480
NP_001393174.1   ATEALQTFRPAASSSFRHCQLDVGREMKLLTELNFLRVPEAPVIDTQRTFAYDQIFLCWR
               421       .         .         .         .         .         480

               481       .         .         .         .         .         540
NP_001393174.1   LPPHSPPAWHYTVEFRRTDVPAQPGPTRWQRREEVRGTSALLENPDTGSVYVLRVRGCNK
               481       .         .         .         .         .         540

               541       .         .         .         .         .         600
NP_001393174.1   AGYGEYSEDVHLHTPPAPVLHFFLDSRWGASRERLAISKDQRAVRSVPGLPLLLAADRLL
               541       .         .         .         .         .         600

               601       .         .         .         .         .         660
NP_001393174.1   TGCHLSVDVVLGDVAVTQGRSYWACAVDPASYLVKVGVGLESKLQESFQGAPDVISPRYD
               601       .         .         .         .         .         660

               661       .         .         .         .         .         720
NP_001393174.1   PDSGHDSGAEDATVEASPPFAFLTIGMGKILLGSGASSNAGLTGRDGPTAGCTVPLPPRL
               661       .         .         .         .         .         720

               721       .         .         .         .         .         780
NP_001393174.1   GICLDYERGRVSFLDAVSFRGLLECPLDCSGPVCPAFCFIGGGAVQLQEPVGTKPERKVT
               721       .         .         .         .         .         780

               781      788
NP_001393174.1   IGGFAKLD
               781      788

Call:
  read.fasta(file = outfile)

Class:
  fasta

Alignment dimensions:
  1 sequence rows; 788 position columns (788 non-gap, 0 gap)

+ attr: id, ali, call
```

Now I can seqrch the PDB database for related sequences

```
#b <- blast.pdb(aa)
#head(b)
```

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

List out some top hits

```
#hits
```

This protein only has one hit in PDB... Proceed with class example for practice.

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

Side-note: Let's annotate these structures (in other words, find out what they arem what species they are from, stuff about the experiment they were solved in etc.)

We can use `pdb.annotate()` for this.

```
anno <- pdb.annotate(hits$pdb.id)

attributes(anno)
```

```
$names
 [1] "structureId"           "chainId"               "macromoleculeType"
 [4] "chainLength"           "experimentalTechnique" "resolution"
 [7] "scopDomain"            "pfam"                  "ligandId"
[10] "ligandName"            "source"                "structureTitle"
[13] "citation"              "rObserved"             "rFree"
[16] "rWork"                 "spaceGroup"

$class
[1] "data.frame"

$row.names
 [1] "1AKE_A" "6S36_A" "6RZE_A" "3HPR_A" "1E4V_A" "5EJE_A" "1E4Y_A" "3X2S_A"
 [9] "6HAP_A" "6HAM_A" "4K46_A" "3GMT_A" "4PZL_A"
```

```
head(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
       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)
              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 BIS(ADENOSINE)-5'-PENTAPHOSPHATE,COBALT (II) ION
                                    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

structureTitle
1AKE_A STRUCTURE OF THE COMPLEX BETWEEN ADENYLATE KINASE FROM ESCHERICHIA COLI AND THE
INHIBITOR AP5A REFINED AT 1.9 ANGSTROMS RESOLUTION: A MODEL FOR A CATALYTIC TRANSITION STATE
6S36_A
Crystal structure of E. coli Adenylate kinase R119K mutant
6RZE_A
Crystal structure of E. coli Adenylate kinase R119A mutant
3HPR_A
Crystal structure of V148G adenylate kinase from E. coli, in complex with Ap5A
1E4V_A
Mutant G10V of adenylate kinase from E. coli, modified in the Gly-loop
5EJE_A
Crystal structure of E. coli Adenylate kinase G56C/T163C double mutant in complex with Ap5a
                                                    citation rObserved  rFree
1AKE_A                Muller, C.W., et al. J Mol Biol (1992)    0.1960     NA
6S36_A                 Rogne, P., et al. Biochemistry (2019)    0.1632 0.2356
6RZE_A                 Rogne, P., et al. Biochemistry (2019)    0.1865 0.2350
3HPR_A Schrank, T.P., et al. Proc Natl Acad Sci U S A (2009)    0.2100 0.2432
1E4V_A                  Muller, C.W., et al. Proteins (1993)    0.1960     NA
5EJE_A Kovermann, M., et al. Proc Natl Acad Sci U S A (2017)    0.1889 0.2358
        rWork spaceGroup
1AKE_A 0.1960  P 21 2 21
6S36_A 0.1594    C 1 2 1
6RZE_A 0.1819    C 1 2 1
3HPR_A 0.2062  P 21 21 2
1E4V_A 0.1960  P 21 2 21
5EJE_A 0.1863  P 21 2 21
```

Now we can download all these structures for analysis with the `get.pdb()` function.

```
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


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```

(Mol* viewer – open file – select all structures from pdbs folder… Hard to interpret)

Now we have all these related structures...

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

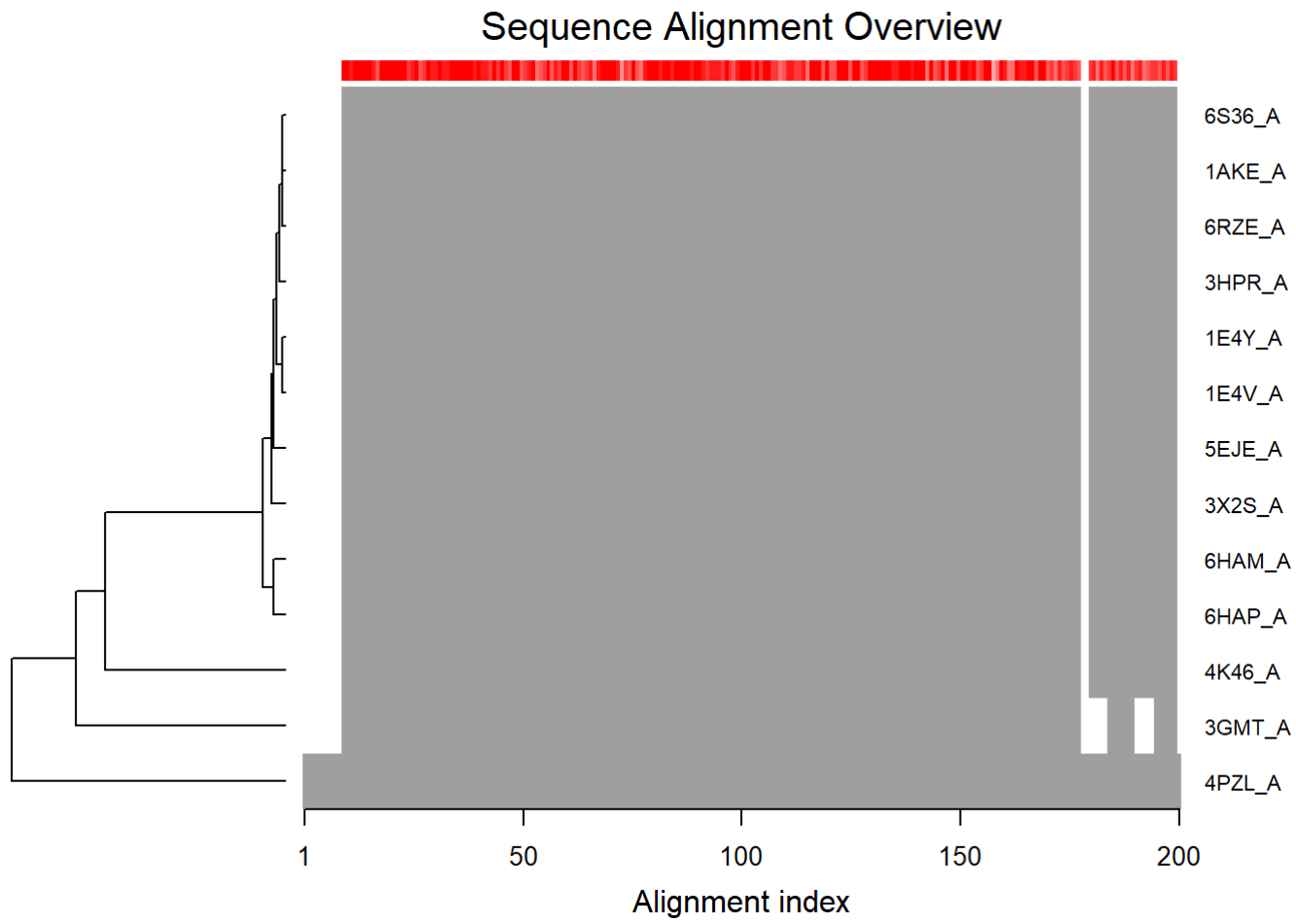
```
Reading PDB files:
pdbs/split_chain/1AKE_A.pdb
pdbs/split_chain/6S36_A.pdb
pdbs/split_chain/6RZE_A.pdb
pdbs/split_chain/3HPR_A.pdb
pdbs/split_chain/1E4V_A.pdb
pdbs/split_chain/5EJE_A.pdb
pdbs/split_chain/1E4Y_A.pdb
pdbs/split_chain/3X2S_A.pdb
pdbs/split_chain/6HAP_A.pdb
pdbs/split_chain/6HAM_A.pdb
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)
```



We can annotate the 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] "Burkholderia pseudomallei 1710b"
[7] "Francisella tularensis subsp. tularensis SCHU S4"
```

```
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	source
1AKE_A	AP5	BIS(ADENOSINE)-5'-PENTAPHOSPHATE	Escherichia coli
6S36_A	CL (3),NA,MG (2)	CHLORIDE ION (3),SODIUM ION,MAGNESIUM ION (2)	Escherichia coli
6RZE_A	NA (3),CL (2)	SODIUM ION (3),CHLORIDE ION (2)	Escherichia coli
3HPR_A	AP5	BIS(ADENOSINE)-5'-PENTAPHOSPHATE	Escherichia coli K-12
1E4V_A	AP5	BIS(ADENOSINE)-5'-PENTAPHOSPHATE	Escherichia coli
5EJE_A	AP5,CO	BIS(ADENOSINE)-5'-PENTAPHOSPHATE,COBALT (II) ION	Escherichia coli O139:H28 str. E24377A
1E4Y_A	AP5	BIS(ADENOSINE)-5'-PENTAPHOSPHATE	
3X2S_A	JPY (2),AP5,MG	N-(pyren-1-ylmethyl)acetamide (2),BIS(ADENOSINE)-5'-PENTAPHOSPHATE,MAGNESIUM ION	
6HAP_A	AP5	BIS(ADENOSINE)-5'-PENTAPHOSPHATE	
6HAM_A	AP5	BIS(ADENOSINE)-5'-PENTAPHOSPHATE	
4K46_A	ADP,AMP,PO4	ADENOSINE-5'-DIPHOSPHATE,ADENOSINE MONOPHOSPHATE,PHOSPHATE ION	
3GMT_A	SO4 (2)	SULFATE ION (2)	
4PZL_A	CA,FMT,GOL	CALCIUM ION,FORMIC ACID,GLYCEROL	

```
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

structureTitle
1AKE_A STRUCTURE OF THE COMPLEX BETWEEN ADENYLATE KINASE FROM ESCHERICHIA COLI AND THE
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6S36_A
Crystal structure of E. coli Adenylate kinase R119K mutant
6RZE_A
Crystal structure of E. coli Adenylate kinase R119A mutant
3HPR_A
Crystal structure of V148G adenylate kinase from E. coli, in complex with Ap5A
1E4V_A
Mutant G10V of adenylate kinase from E. coli, modified in the Gly-loop
5EJE_A
Crystal structure of E. coli Adenylate kinase G56C/T163C double mutant in complex with Ap5a
1E4Y_A
Mutant P9L of adenylate kinase from E. coli, modified in the Gly-loop
3X2S_A
Crystal structure of pyrene-conjugated adenylate kinase
6HAP_A
Adenylate kinase
6HAM_A
Adenylate kinase
4K46_A
Crystal Structure of Adenylate Kinase from Photobacterium profundum
3GMT_A
Crystal structure of adenylate kinase from burkholderia pseudomallei
4PZL_A                                                                                  The
crystal structure of adenylate kinase from Francisella tularensis subsp. tularensis SCHU S4
                                                    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
```
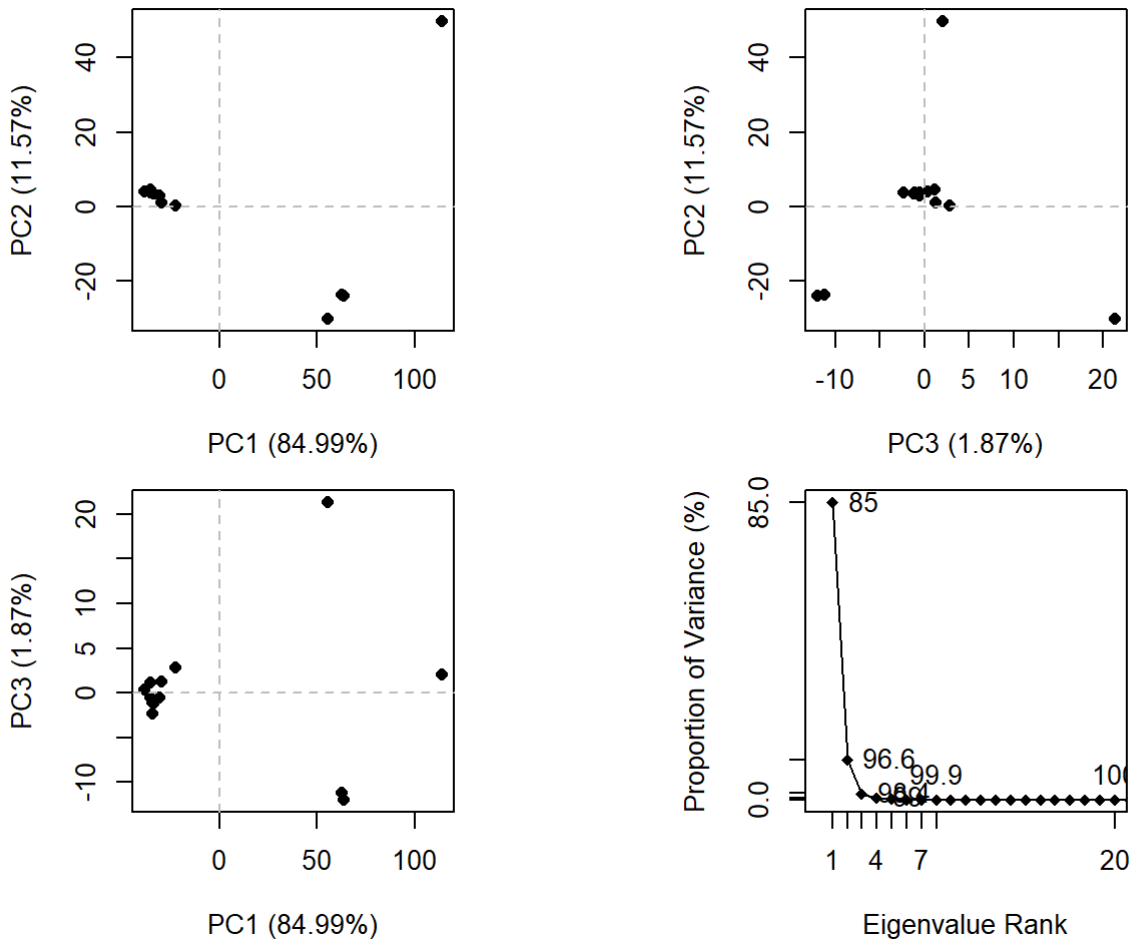

```
1E4V_A 0.19600  P 21 2 21
5EJE_A 0.18630  P 21 2 21
1E4Y_A 0.17800   P 1 21 1
3X2S_A 0.20700 P 21 21 21
6HAP_A 0.22370    I 2 2 2
6HAM_A 0.20311       P 43
4K46_A 0.16730 P 21 21 21
3GMT_A 0.23500   P 1 21 1
4PZL_A 0.19130       P 32
```

We can conduct PCA

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



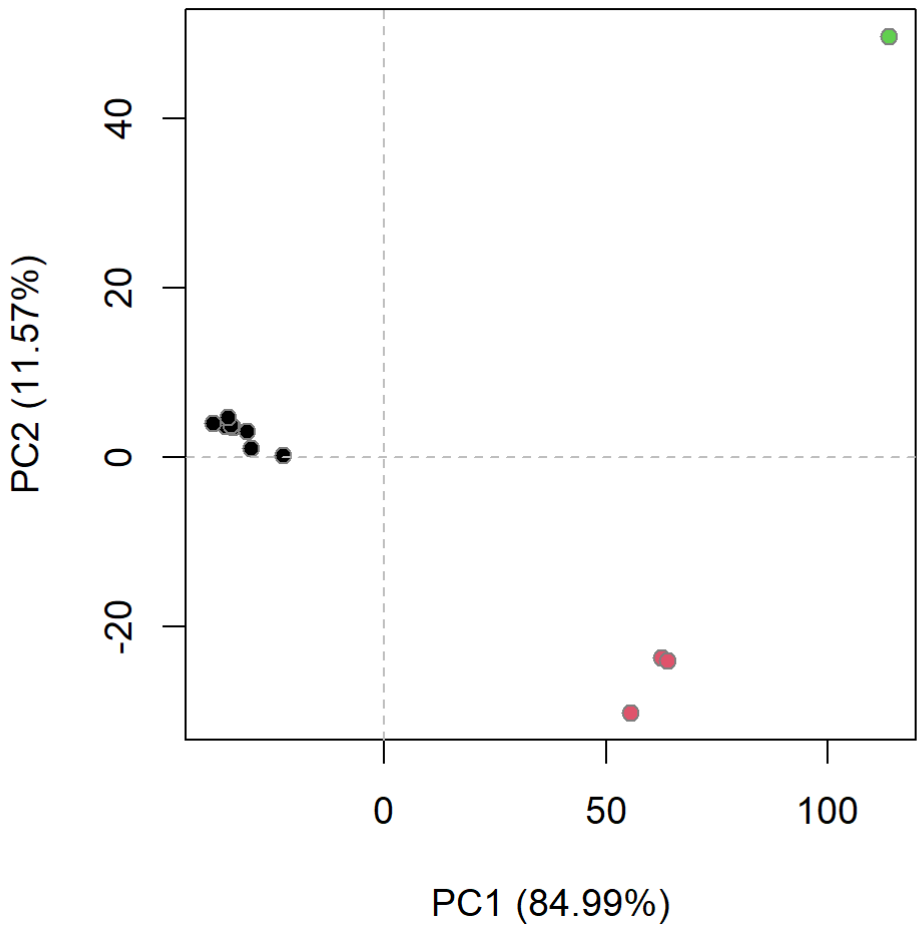
Function `rmsd()` will calculate all pairwise RMSD values of the structural ensemble. This facilitates clustering analysis based on the pairwise structural deviation:

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

```
Warning in rmsd(pdbs): No indices provided, using the 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)
```



Further visualization

```
pc1 <- mktrj(pc.xray, pc=1, file="pc_1.pdb")
```

Open file in Mol* to visualize the major structural variations along PC1.

Alphafold Prediction of protein structure

paste protein seq, for multiple chains, separate by ":".

Download result into project folder, unzip. Open the pdb files in Mol*.

Superposition – chains – select chains– superpose Components – polymer … – set coloring – atom property – uncertainty / disorder

snapshot, download.

Gene of interest: NOTAMACROPUS EUGENII TRIM46 monomer model png:

Custom analysis of dimer model

We will read the results of the more complicated HIV protein dimer AlphaFold2 models into R with the help of the Bio3D package.

First load the sequence information into R.

```
results_dir <- "hivprdimer_23119/"
```

```
# File names for all PDB models
pdb_files <- list.files(path=results_dir,
                        pattern="*.pdb",
                        full.names = TRUE)

# Print our PDB file names
basename(pdb_files)
```

```
[1] "HIVPrdimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_000.pdb"
[2] "HIVPrdimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_000.pdb"
[3] "HIVPrdimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_000.pdb"
[4] "HIVPrdimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.pdb"
[5] "HIVPrdimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.pdb"
```

```
library(bio3d)

# Read all data from Models
#  and superpose/fit coords
pdbs <- pdbaln(pdb_files, fit=TRUE, exefile="msa")
```

```
Reading PDB files:
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_000.p
db
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_000.p
db
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_000.p
```

```
db
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.p
db
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.p
db
.....

Extracting sequences

pdb/seq: 1   name:
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_000.p
db
pdb/seq: 2   name:
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_000.p
db
pdb/seq: 3   name:
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_000.p
db
pdb/seq: 4   name:
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.p
db
pdb/seq: 5   name:
hivprdimer_23119/HIVPrdimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.p
db
```

Quick overview of the sequences

```
pdbs
```

```
                             1        .         .         .         .         50
[Truncated_Name:1]HIVPrdimer   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:2]HIVPrdimer   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:3]HIVPrdimer   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:4]HIVPrdimer   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:5]HIVPrdimer   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
                             **************************************************
                             1        .         .         .         .         50

                            51        .         .         .         .         100
[Truncated_Name:1]HIVPrdimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:2]HIVPrdimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:3]HIVPrdimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:4]HIVPrdimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:5]HIVPrdimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
                             **************************************************
                            51        .         .         .         .         100

                           101        .         .         .         .         150
[Truncated_Name:1]HIVPrdimer   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:2]HIVPrdimer   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:3]HIVPrdimer   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:4]HIVPrdimer   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:5]HIVPrdimer   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
                             **************************************************
```

```
                           101        .         .         .         .         150

                           151        .         .         .         .       198
[Truncated_Name:1]HIVPrdimer   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:2]HIVPrdimer   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:3]HIVPrdimer   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:4]HIVPrdimer   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:5]HIVPrdimer   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
                               ***************************************************
                           151        .         .         .         .       198

Call:
  pdbaln(files = pdb_files, fit = TRUE, exefile = "msa")

Class:
  pdbs, fasta

Alignment dimensions:
  5 sequence rows; 198 position columns (198 non-gap, 0 gap)

+ attr: xyz, resno, b, chain, id, ali, resid, sse, call
```

RMSD is a standard measure of structural distance between coordinate sets. We can use the `rmsd()` function to calculate the RMSD between all pairs models.

```
rd <- rmsd(pdbs, fit=T)
```

```
Warning in rmsd(pdbs, fit = T): No indices provided, using the 198 non NA positions
```

```
range(rd)
```

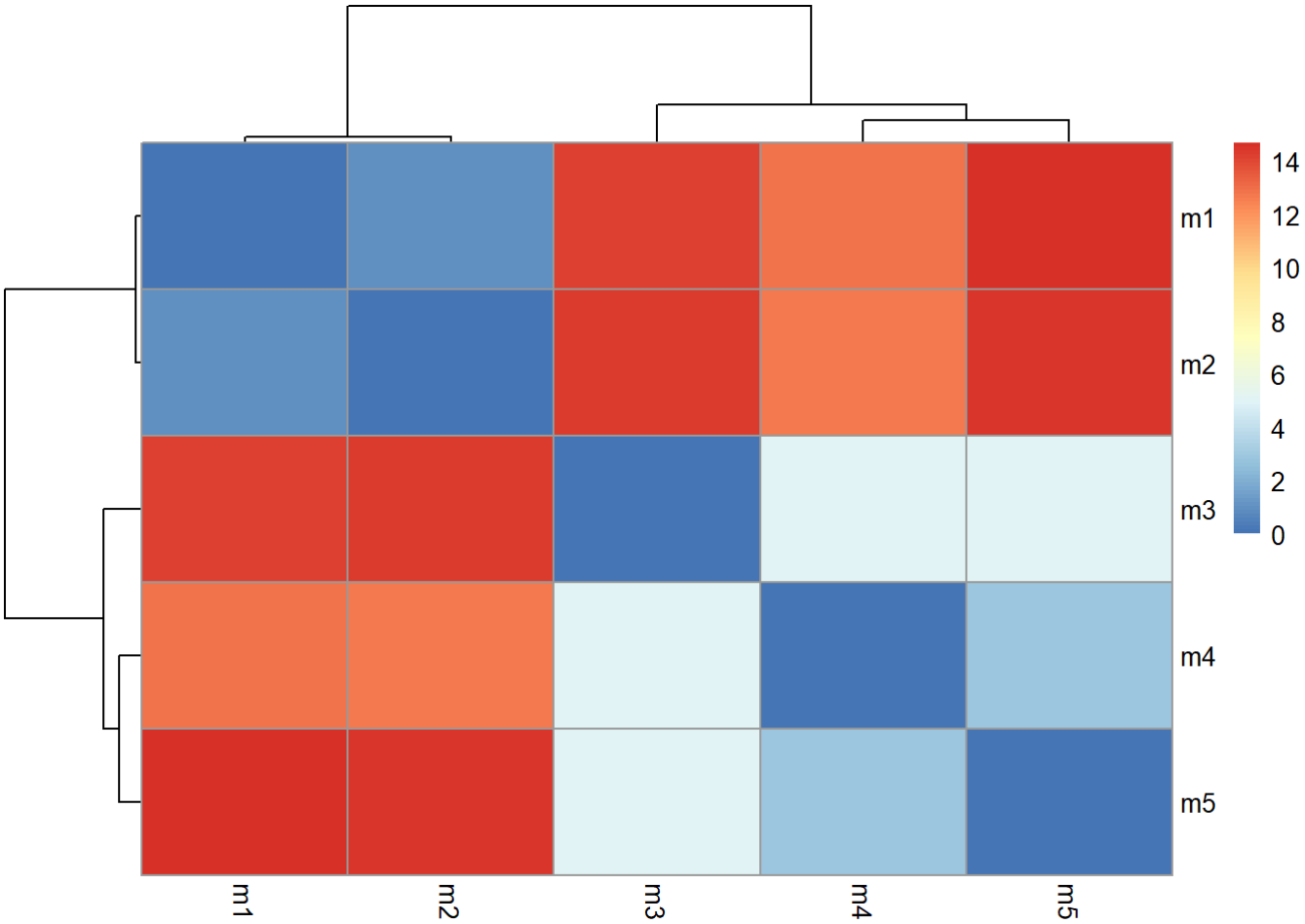
```
[1]  0.000 14.689
```

Draw a heatmap of these RMSD matrix values

```
library(pheatmap)
```

```
Warning: package 'pheatmap' was built under R version 4.3.2
```

```
colnames(rd) <- paste0("m",1:5)
rownames(rd) <- paste0("m",1:5)
pheatmap(rd)
```



Models 1 and 2 are more similar to each other than they are to any other model. Models 4 and 5 are quite similar to each other and in turn more similar to model 3 than to models 1 and 2. We will see this trend again in the pLDDT and PAE plots further below.

Now lets plot the pLDDT values across all models. Recall that this information is in the B-factor column of each model and that this is stored in our aligned pdbs object as pdbs$b with a row per structure/model.

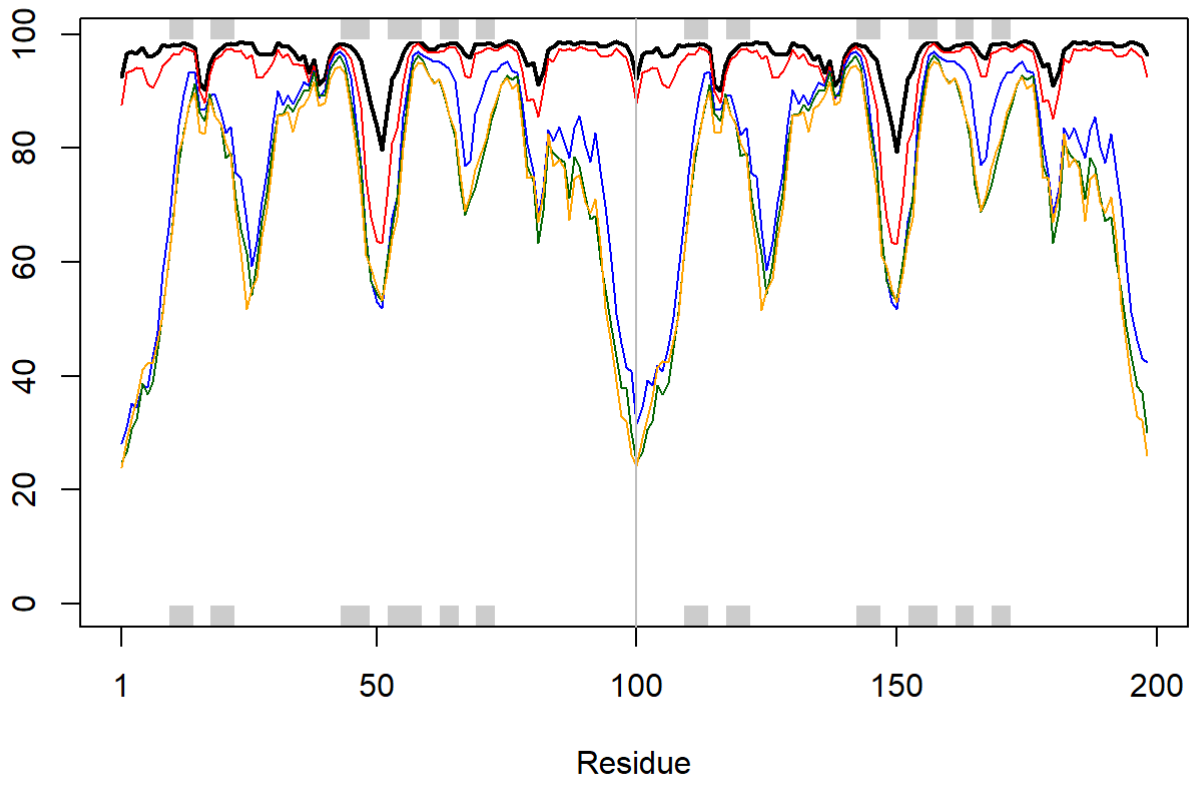
As no TRIM46 sequence is identified in PDB database, I will use human TRIM46 alphafold prediction as a reference for the following practices

```
# Read a reference PDB structure
pdb <- read.pdb("1hsg")
```

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

You could optionally obtain secondary structure from a call to `stride()` or `dssp()` on any of the model structures.

```
plotb3(pdbs$b[1,], typ="l", lwd=2, sse=pdb)
points(pdbs$b[2,], typ="l", col="red")
points(pdbs$b[3,], typ="l", col="blue")
points(pdbs$b[4,], typ="l", col="darkgreen")
points(pdbs$b[5,], typ="l", col="orange")
abline(v=100, col="gray")
```



We can improve the superposition/fitting of our models by finding the most consistent "rigid core" common across all the models. For this we will use the `core.find()` function, and then use the identified core atom positions as a basis for a more suitable superposition and write out the fitted structures to a directory called corefit_structures:

```
core <- core.find(pdbs)
```

```
core size 197 of 198  vol = 6154.839
core size 196 of 198  vol = 5399.676
core size 195 of 198  vol = 5074.795
core size 194 of 198  vol = 4802.518
core size 193 of 198  vol = 4520.256
core size 192 of 198  vol = 4305.362
core size 191 of 198  vol = 4089.792
core size 190 of 198  vol = 3886.145
core size 189 of 198  vol = 3758.321
core size 188 of 198  vol = 3620.18
core size 187 of 198  vol = 3496.698
core size 186 of 198  vol = 3389.985
core size 185 of 198  vol = 3320.114
core size 184 of 198  vol = 3258.683
core size 183 of 198  vol = 3208.591
core size 182 of 198  vol = 3156.736
core size 181 of 198  vol = 3141.668
core size 180 of 198  vol = 3136.574
core size 179 of 198  vol = 3155.52
core size 178 of 198  vol = 3185.362
```

```
core size 177 of 198  vol = 3204.487
core size 176 of 198  vol = 3211.978
core size 175 of 198  vol = 3234.993
core size 174 of 198  vol = 3244.062
core size 173 of 198  vol = 3237.845
core size 172 of 198  vol = 3218.77
core size 171 of 198  vol = 3180.743
core size 170 of 198  vol = 3130.369
core size 169 of 198  vol = 3067.881
core size 168 of 198  vol = 2989.546
core size 167 of 198  vol = 2928.272
core size 166 of 198  vol = 2851.193
core size 165 of 198  vol = 2780.877
core size 164 of 198  vol = 2708.433
core size 163 of 198  vol = 2636.516
core size 162 of 198  vol = 2563.25
core size 161 of 198  vol = 2478.024
core size 160 of 198  vol = 2404.793
core size 159 of 198  vol = 2330.997
core size 158 of 198  vol = 2250.477
core size 157 of 198  vol = 2159.432
core size 156 of 198  vol = 2070.759
core size 155 of 198  vol = 1983.579
core size 154 of 198  vol = 1917.913
core size 153 of 198  vol = 1842.556
core size 152 of 198  vol = 1775.398
core size 151 of 198  vol = 1695.133
core size 150 of 198  vol = 1632.173
core size 149 of 198  vol = 1570.391
core size 148 of 198  vol = 1497.238
core size 147 of 198  vol = 1434.802
core size 146 of 198  vol = 1367.706
core size 145 of 198  vol = 1302.596
core size 144 of 198  vol = 1251.985
core size 143 of 198  vol = 1207.976
core size 142 of 198  vol = 1167.112
core size 141 of 198  vol = 1118.27
core size 140 of 198  vol = 1081.664
core size 139 of 198  vol = 1029.75
core size 138 of 198  vol = 981.766
core size 137 of 198  vol = 944.446
core size 136 of 198  vol = 899.224
core size 135 of 198  vol = 859.402
core size 134 of 198  vol = 814.694
core size 133 of 198  vol = 771.862
core size 132 of 198  vol = 733.807
core size 131 of 198  vol = 702.053
core size 130 of 198  vol = 658.757
core size 129 of 198  vol = 622.574
core size 128 of 198  vol = 578.29
core size 127 of 198  vol = 543.07
core size 126 of 198  vol = 510.934
core size 125 of 198  vol = 481.595
core size 124 of 198  vol = 464.672
```

```
 core size 123 of 198  vol = 451.721
 core size 122 of 198  vol = 430.417
 core size 121 of 198  vol = 409.141
 core size 120 of 198  vol = 378.942
 core size 119 of 198  vol = 348.325
 core size 118 of 198  vol = 324.738
 core size 117 of 198  vol = 312.394
 core size 116 of 198  vol = 300.89
 core size 115 of 198  vol = 279.976
 core size 114 of 198  vol = 263.434
 core size 113 of 198  vol = 250.263
 core size 112 of 198  vol = 229.592
 core size 111 of 198  vol = 209.929
 core size 110 of 198  vol = 196.379
 core size 109 of 198  vol = 180.628
 core size 108 of 198  vol = 167.088
 core size 107 of 198  vol = 155.875
 core size 106 of 198  vol = 142.595
 core size 105 of 198  vol = 128.924
 core size 104 of 198  vol = 114.054
 core size 103 of 198  vol = 100.936
 core size 102 of 198  vol = 90.431
 core size 101 of 198  vol = 81.972
 core size 100 of 198  vol = 74.017
 core size 99 of 198  vol = 66.855
 core size 98 of 198  vol = 59.525
 core size 97 of 198  vol = 52.263
 core size 96 of 198  vol = 43.699
 core size 95 of 198  vol = 35.813
 core size 94 of 198  vol = 28.888
 core size 93 of 198  vol = 20.692
 core size 92 of 198  vol = 14.975
 core size 91 of 198  vol = 9.146
 core size 90 of 198  vol = 5.232
 core size 89 of 198  vol = 3.53
 core size 88 of 198  vol = 2.657
 core size 87 of 198  vol = 1.998
 core size 86 of 198  vol = 1.333
 core size 85 of 198  vol = 1.141
 core size 84 of 198  vol = 1.012
 core size 83 of 198  vol = 0.891
 core size 82 of 198  vol = 0.749
 core size 81 of 198  vol = 0.618
 core size 80 of 198  vol = 0.538
 core size 79 of 198  vol = 0.479
 FINISHED: Min vol ( 0.5 ) reached
```

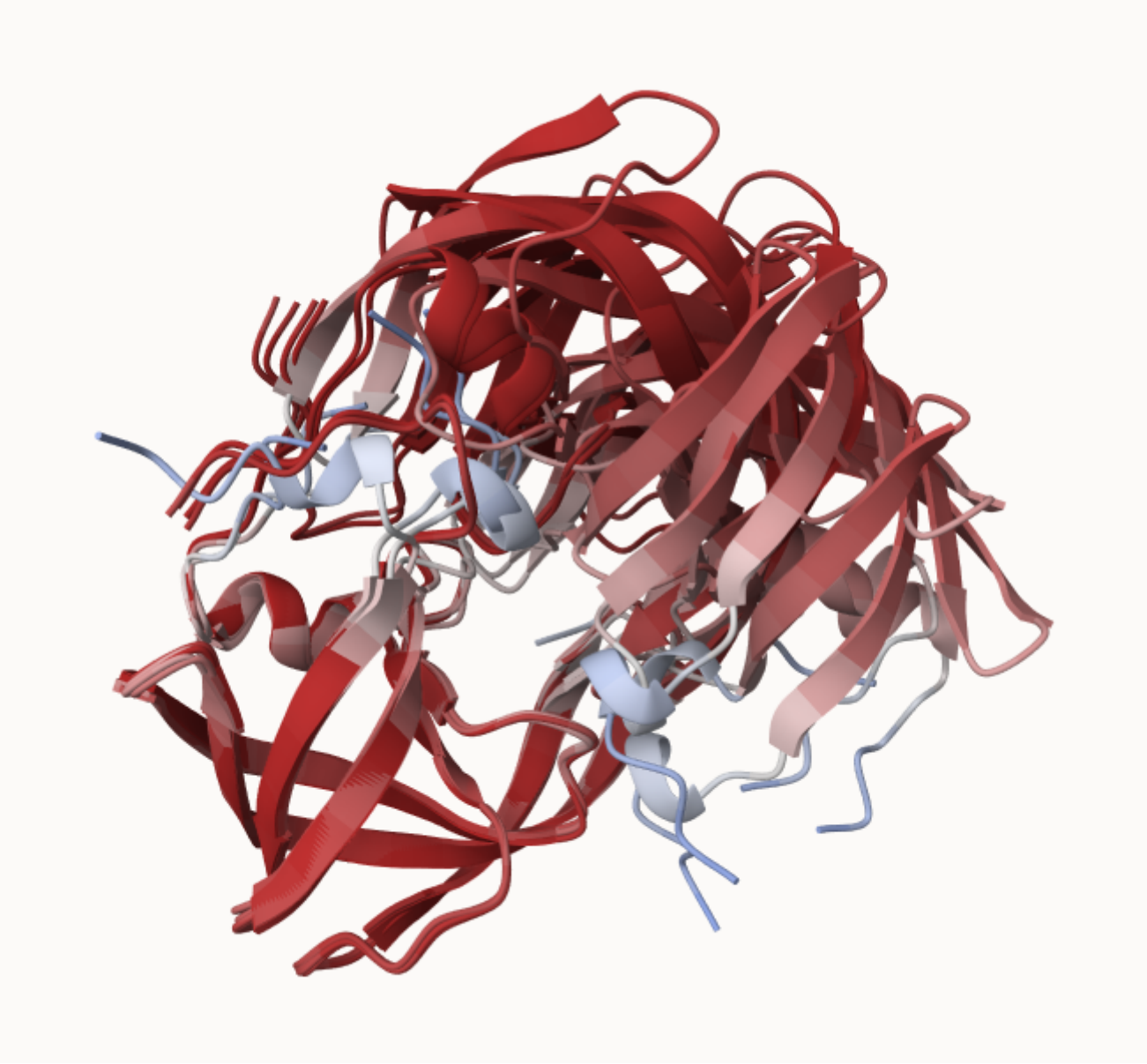
```
core.inds <- print(core, vol=0.5)
```

```
# 80 positions (cumulative volume <= 0.5 Angstrom^3)
  start end length
1    10  25     16
```

```
2     27  48      22
3     53  94      42
```

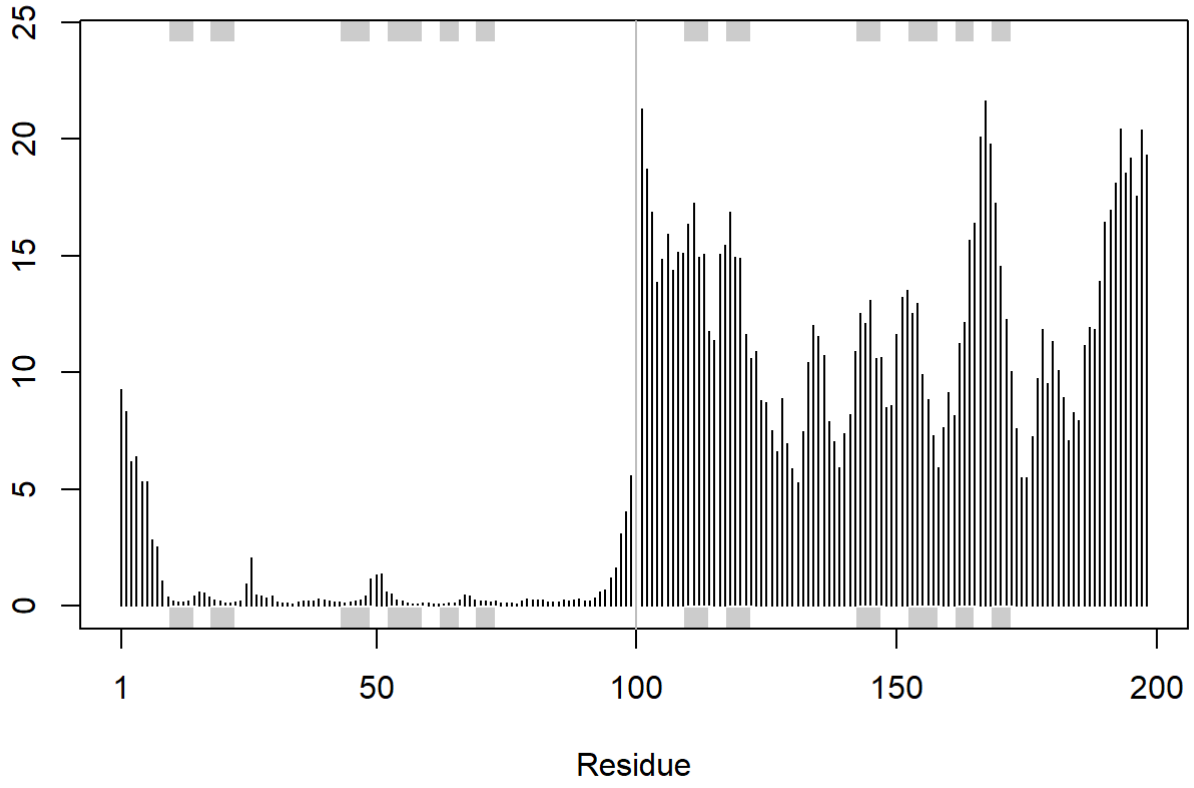
```
xyz <- pdbfit(pdbs, core.inds, outpath="corefit_structures")
```

The resulting superposed coordinates are written to a new director called corefit_structures/. We can now open these in Mol* and color by the Atom Property of Uncertainty/Disorder (i.e. the B-factor column that contains the pLDDT scores):



Now we can examine the RMSF between positions of the structure. RMSF is an often used measure of conformational variance along the structure:

```
rf <- rmsf(xyz)

plotb3(rf, sse=pdb)
abline(v=100, col="gray", ylab="RMSF")
```



Predicted Alignment Error for Domains

Independent of the 3D structure, AlphaFold produces an output called Predicted Aligned Error (PAE). This is detailed in the JSON format result files, one for each model structure.

Below we read these files and see that AlphaFold produces a useful inter-domain prediction for model 1 (and 2) but not for model 5 (or indeed models 3, 4, and 5):

```
library(jsonlite)
```

```
Warning: package 'jsonlite' was built under R version 4.3.2
```

```
# Listing of all PAE JSON files
pae_files <- list.files(path=results_dir,
                        pattern=".*model.*\\.json",
                        full.names = TRUE)
```

For example purposes lets read the 1st and 5th files:

```
pae1 <- read_json(pae_files[1],simplifyVector = TRUE)
pae5 <- read_json(pae_files[5],simplifyVector = TRUE)

attributes(pae1)
```

```
$names
[1] "plddt"    "max_pae" "pae"     "ptm"     "iptm"
```

```
head(pae1$plddt)
```

```
[1] 92.50 96.56 96.94 96.62 97.69 96.00
```

The maximum PAE values are useful for ranking models. Here we can see that model 5 is much worse than model 1. The lower the PAE score the better. How about the other models, what are thir max PAE scores?

```
pae1$max_pae
```

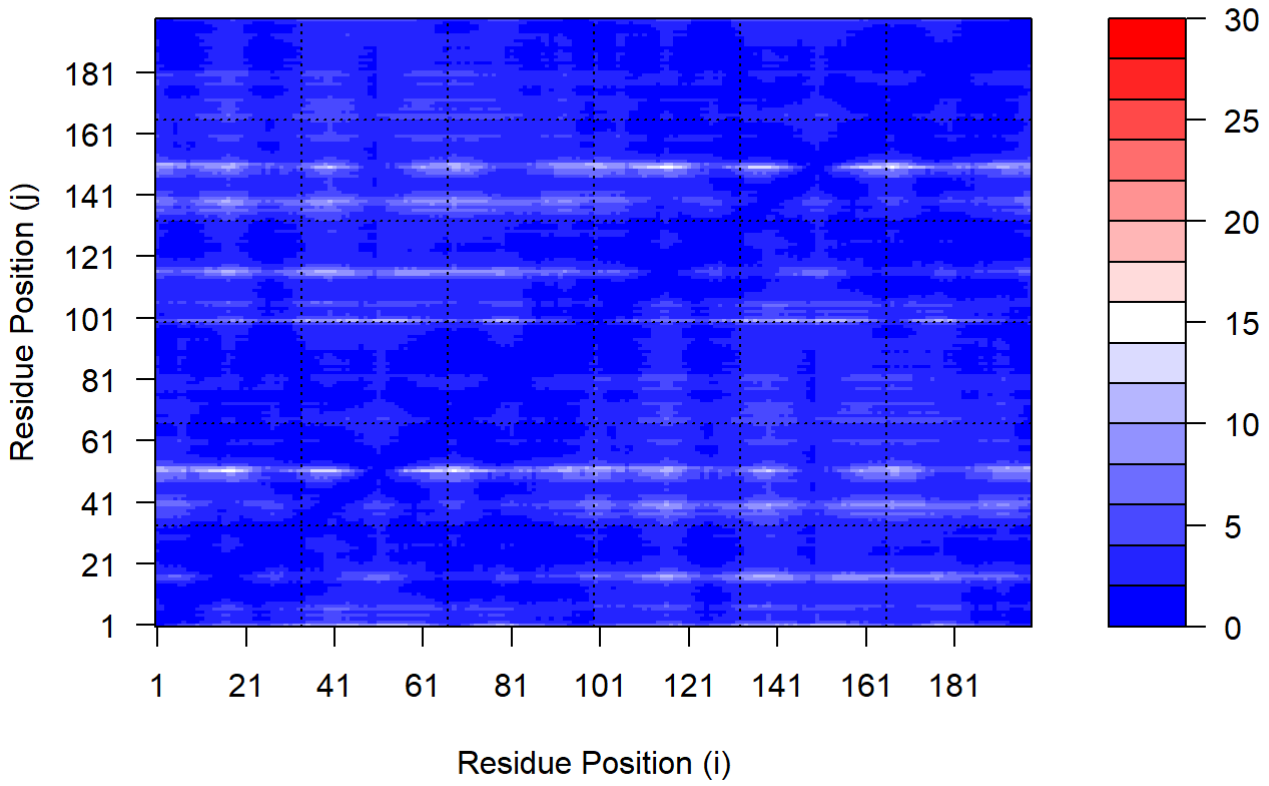
```
[1] 15.54688
```

```
pae5$max_pae
```

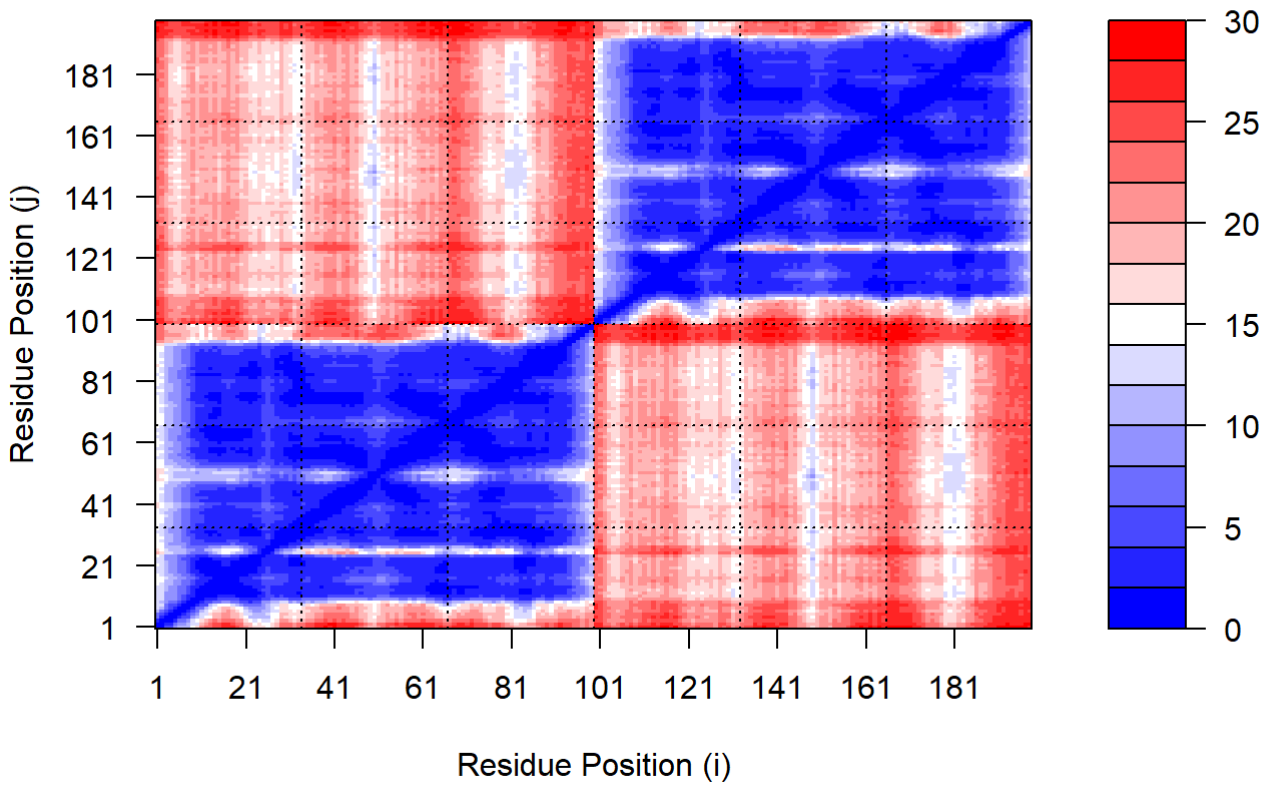
```
[1] 29.29688
```

We can plot the N by N (where N is the number of residues) PAE scores with ggplot or with functions from the Bio3D package:

```
#m1
plot.dmat(pae1$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)",
          grid.col = "black",
          zlim=c(0,30))
```




```
#m5
plot.dmat(pae5$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)",
          grid.col = "black",
          zlim=c(0,30))
```



Residue conservation from alignment file

```
aln_file <- list.files(path=results_dir,
                       pattern=".a3m$",
                        full.names = TRUE)
aln_file
```

```
[1] "hivprdimer_23119/HIVPrdimer_23119.a3m"
```

```
aln <- read.fasta(aln_file[1], to.upper = TRUE)
```

```
[1] " ** Duplicated sequence id's: 101 **"
[2] " ** Duplicated sequence id's: 101 **"
```

How many sequences are there in this alignment

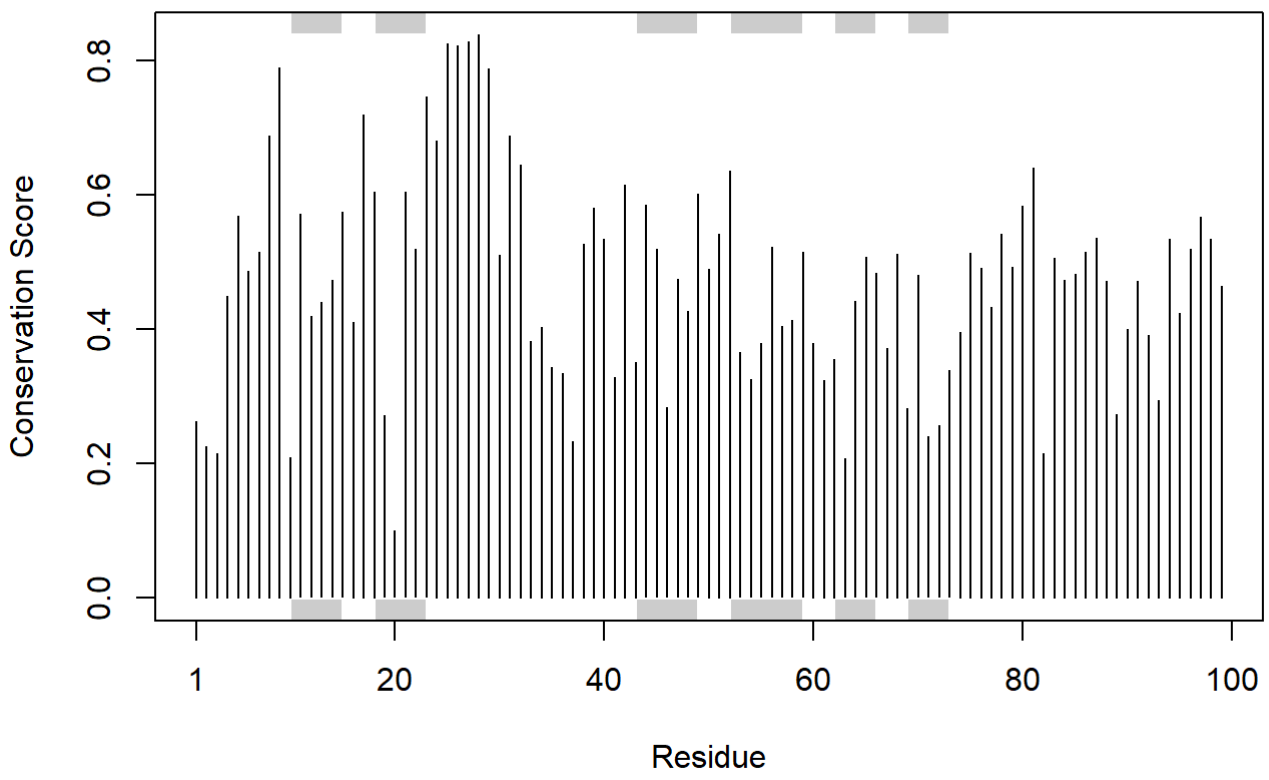
```
dim(aln$ali)
```

```
[1] 5378  132
```

We can score residue conservation in the alignment with the conserv() function.

```
sim <- conserv(aln)
plotb3(sim[1:99], sse=trim.pdb(pdb, chain="A"),
```

```
      ylab="Conservation Score")
```




```
con <- consensus(aln, cutoff = 0.9)
con$seq
```

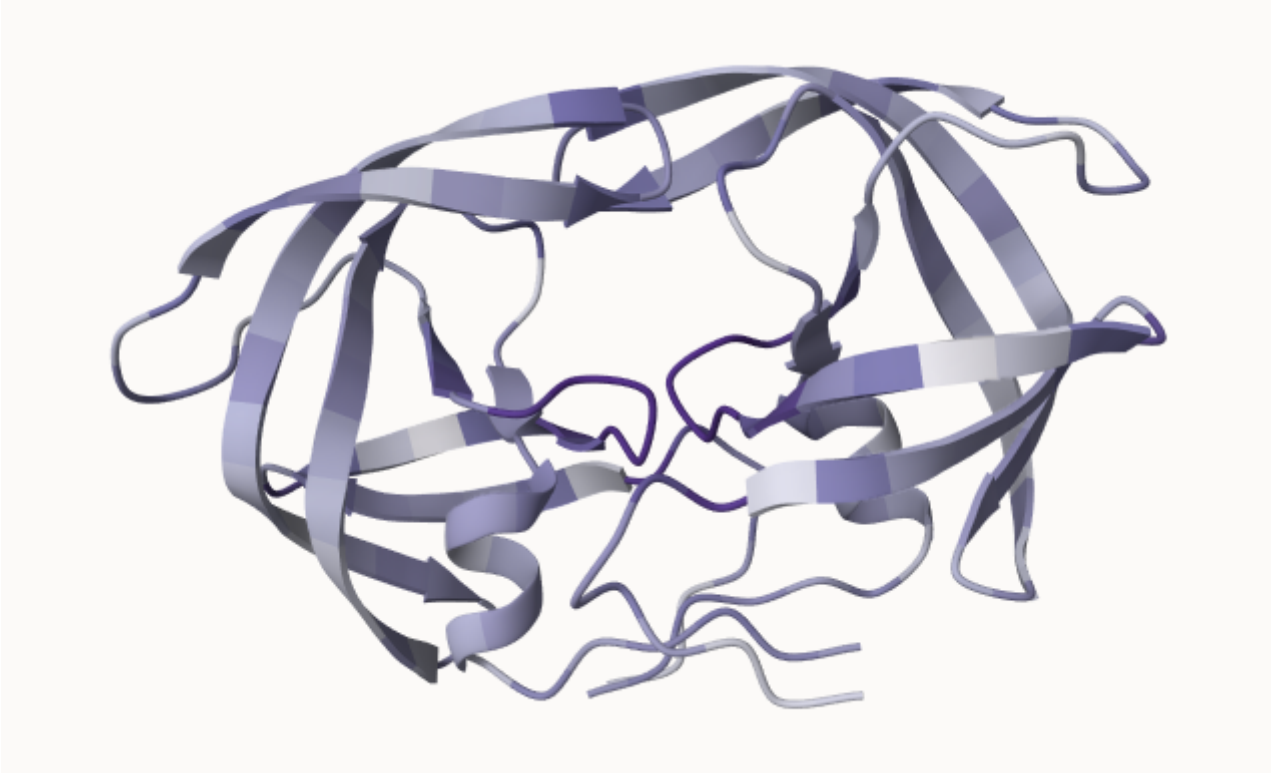
```
  [1] "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-"
 [19] "-" "-" "-" "-" "-" "-" "D" "T" "G" "A" "-" "-" "-" "-" "-" "-" "-" "-"
 [37] "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-"
 [55] "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-"
 [73] "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-"
 [91] "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-"
[109] "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-" "-"
[127] "-" "-" "-" "-" "-" "-"
```

The conserved DTGA is appearant.

For a final visualization of these functionally important sites we can map this conservation score to the Occupancy column of a PDB file for viewing in molecular viewer programs such as Mol*, PyMol, VMD, chimera etc.

```
m1.pdb <- read.pdb(pdb_files[1])
occ <- vec2resno(c(sim[1:99], sim[1:99]), m1.pdb$atom$resno)
write.pdb(m1.pdb, o=occ, file="m1_conserv.pdb")
```

Mol* visualization: coloring by Occupancy



We can now clearly see the central conserved active site in this model where the natural peptide substrate (and small molecule inhibitors) would bind between domains.