Class 11: Comparative Analysis of Structures

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We need some packages for todays class. These include `bio3d` and `msa`.

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

Install `install.packages("BiocManager")` and then `BiocManager::install("msa")` all entered in the R "brain" console.

```
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
```

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

Q12. True or False? Functions from the devtools package can be used to install packages from GitHub and BitBucket?

TRUE

Q13. How many amino acids are in this sequence, i.e. how long is this sequence?

214

Now I can search the PDB database for related sequences:

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

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

These are the related structures in the PDB database that we found via a BLAST search…

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

```
 [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"
```

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

For this we can use the `pdb.annotate()` function

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

```
#attributes(anno)
#head(anno)
```

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

```
# 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.gz exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/6S36.pdb.gz exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/6RZE.pdb.gz exists. Skipping download
```

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

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/1E4V.pdb.gz exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/5EJE.pdb.gz exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/1E4Y.pdb.gz exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/3X2S.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/6HAP.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/6HAM.pdb.gz exists. Skipping download

Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):
pdbs/4K46.pdb.gz exists. Skipping download

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

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


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```

```
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  |
  |====================================================================== | 100%
```

Now we have all these related structures we can Align and Supperpose...

```
# 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
```

```
pdbs
```

```
                                 1        .         .         .         40
[Truncated_Name:1]1AKE_A.pdb     ----------MRIILLGAPGAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:2]6S36_A.pdb     ----------MRIILLGAPGAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:3]6RZE_A.pdb     ----------MRIILLGAPGAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:4]3HPR_A.pdb     ----------MRIILLGAPGAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:5]1E4V_A.pdb     ----------MRIILLGAPVAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:6]5EJE_A.pdb     ----------MRIILLGAPGAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:7]1E4Y_A.pdb     ----------MRIILLGALVAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:8]3X2S_A.pdb     ----------MRIILLGAPGAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:9]6HAP_A.pdb     ----------MRIILLGAPGAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:10]6HAM_A.pdb    ----------MRIILLGAPGAGKGTQAQFIMEKYGIPQIS
[Truncated_Name:11]4K46_A.pdb    ----------MRIILLGAPGAGKGTQAQFIMAKFGIPQIS
[Truncated_Name:12]3GMT_A.pdb    ----------MRLILLGAPGAGKGTQANFIKEKFGIPQIS
[Truncated_Name:13]4PZL_A.pdb    TENLYFQSNAMRIILLGAPGAGKGTQAKIIEQKYNIAHIS
                                           **^*****  *******  *  *^ *  **
                                 1        .         .         .         40

                                41        .         .         .         80
[Truncated_Name:1]1AKE_A.pdb     TGDMLRAAVKSGSELGKQAKDIMDAGKLVTDELVIALVKE
[Truncated_Name:2]6S36_A.pdb     TGDMLRAAVKSGSELGKQAKDIMDAGKLVTDELVIALVKE
[Truncated_Name:3]6RZE_A.pdb     TGDMLRAAVKSGSELGKQAKDIMDAGKLVTDELVIALVKE
[Truncated_Name:4]3HPR_A.pdb     TGDMLRAAVKSGSELGKQAKDIMDAGKLVTDELVIALVKE
[Truncated_Name:5]1E4V_A.pdb     TGDMLRAAVKSGSELGKQAKDIMDAGKLVTDELVIALVKE
```

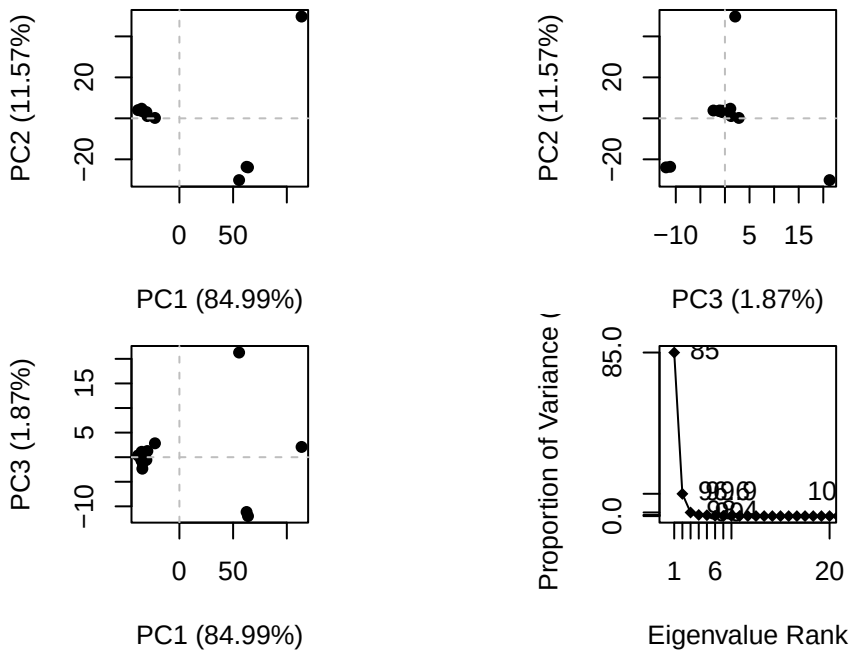
```
[Truncated_Name:6]5EJE_A.pdb    TGDMLRAAVKSGSELGKQAKDIMDACKLVTDELVIALVKE
[Truncated_Name:7]1E4Y_A.pdb    TGDMLRAAVKSGSELGKQAKDIMDAGKLVTDELVIALVKE
[Truncated_Name:8]3X2S_A.pdb    TGDMLRAAVKSGSELGKQAKDIMDCGKLVTDELVIALVKE
[Truncated_Name:9]6HAP_A.pdb    TGDMLRAAVKSGSELGKQAKDIMDAGKLVTDELVIALVRE
[Truncated_Name:10]6HAM_A.pdb   TGDMLRAAIKSGSELGKQAKDIMDAGKLVTDEIIIALVKE
[Truncated_Name:11]4K46_A.pdb   TGDMLRAAIKAGTELGKQAKSVIDAGQLVSDDIILGLVKE
[Truncated_Name:12]3GMT_A.pdb   TGDMLRAAVKAGTPLGVEAKTYMDEGKLVPDSLIIGLVKE
[Truncated_Name:13]4PZL_A.pdb   TGDMIRETIKSGSALGQELKKVLDAGELVSDEFIIKIVKD
                                ****^*  ^* *^ **   *  ^*   ** *  ^^ ^*^^
                               41        .         .         .         80

                               81        .         .         .         120
[Truncated_Name:1]1AKE_A.pdb    RIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:2]6S36_A.pdb    RIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:3]6RZE_A.pdb    RIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:4]3HPR_A.pdb    RIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:5]1E4V_A.pdb    RIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:6]5EJE_A.pdb    RIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:7]1E4Y_A.pdb    RIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:8]3X2S_A.pdb    RIAQEDSRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:9]6HAP_A.pdb    RICQEDSRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:10]6HAM_A.pdb   RICQEDSRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFD
[Truncated_Name:11]4K46_A.pdb   RIAQDDCAKGFLLDGFPRTIPQADGLKEVGVVVDYVIEFD
[Truncated_Name:12]3GMT_A.pdb   RLKEADCANGYLFDGFPRTIAQADAMKEAGVAIDYVLEID
[Truncated_Name:13]4PZL_A.pdb   RISKNDCNNGFLLDGVPRTIPQAQELDKLGVNIDYIVEVD
                                *^   *   *^* ** **** **  ^   *^ ^**^^* *
                               81        .         .         .         120

                              121        .         .         .         160
[Truncated_Name:1]1AKE_A.pdb    VPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTG
[Truncated_Name:2]6S36_A.pdb    VPDELIVDKIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTG
[Truncated_Name:3]6RZE_A.pdb    VPDELIVDAIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTG
[Truncated_Name:4]3HPR_A.pdb    VPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDGTG
[Truncated_Name:5]1E4V_A.pdb    VPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTG
[Truncated_Name:6]5EJE_A.pdb    VPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTG
[Truncated_Name:7]1E4Y_A.pdb    VPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTG
[Truncated_Name:8]3X2S_A.pdb    VPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTG
[Truncated_Name:9]6HAP_A.pdb    VPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTG
[Truncated_Name:10]6HAM_A.pdb   VPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTG
[Truncated_Name:11]4K46_A.pdb   VADSVIVERMAGRRAHLASGRTYHNVYNPPKVEGKDDVTG
[Truncated_Name:12]3GMT_A.pdb   VPFSEIIERMSGRRTHPASGRTYHVKFNPPKVEGKDDVTG
[Truncated_Name:13]4PZL_A.pdb   VADNLLIERITGRRIHPASGRTYHTKFNPPKVADKDDVTG
                                *    ^^^ ^ *** *  *** **  ^*****  *** **
```

```
                                121        .         .         .         160

                                161        .         .         .         200
[Truncated_Name:1]1AKE_A.pdb      EELTTRKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:2]6S36_A.pdb      EELTTRKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:3]6RZE_A.pdb      EELTTRKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:4]3HPR_A.pdb      EELTTRKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:5]1E4V_A.pdb      EELTTRKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:6]5EJE_A.pdb      EELTTRKDDQEECVRKRLVEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:7]1E4Y_A.pdb      EELTTRKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:8]3X2S_A.pdb      EELTTRKDDQEETVRKRLCEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:9]6HAP_A.pdb      EELTTRKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:10]6HAM_A.pdb     EELTTRKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGN
[Truncated_Name:11]4K46_A.pdb     EDLVIREDDKEETVLARLGVYHNQTAPLIAYYGKEAEAGN
[Truncated_Name:12]3GMT_A.pdb     EPLVQRDDDKEETVKKRLDVYEAQTKPLITYYGDWARRGA
[Truncated_Name:13]4PZL_A.pdb     EPLITRTDDNEDTVKQRLSVYHAQTAKLIDFYRNFSSTNT
                                  * *  * ** *^ *  **  *   *  ** ^*
                                161        .         .         .         200

                                201        .         .      227
[Truncated_Name:1]1AKE_A.pdb      T--KYAKVDGTKPVAEVRADLEKILG-
[Truncated_Name:2]6S36_A.pdb      T--KYAKVDGTKPVAEVRADLEKILG-
[Truncated_Name:3]6RZE_A.pdb      T--KYAKVDGTKPVAEVRADLEKILG-
[Truncated_Name:4]3HPR_A.pdb      T--KYAKVDGTKPVAEVRADLEKILG-
[Truncated_Name:5]1E4V_A.pdb      T--KYAKVDGTKPVAEVRADLEKILG-
[Truncated_Name:6]5EJE_A.pdb      T--KYAKVDGTKPVAEVRADLEKILG-
[Truncated_Name:7]1E4Y_A.pdb      T--KYAKVDGTKPVAEVRADLEKILG-
[Truncated_Name:8]3X2S_A.pdb      T--KYAKVDGTKPVAEVRADLEKILG-
[Truncated_Name:9]6HAP_A.pdb      T--KYAKVDGTKPVCEVRADLEKILG-
[Truncated_Name:10]6HAM_A.pdb     T--KYAKVDGTKPVCEVRADLEKILG-
[Truncated_Name:11]4K46_A.pdb     T--QYLKFDGTKAVAEVSAELEKALA-
[Truncated_Name:12]3GMT_A.pdb     E-------NGLKAPA-----YRKISG-
[Truncated_Name:13]4PZL_A.pdb     KIPKYIKINGDQAVEKVSQDIFDQLNK
                                           *
                                201        .         .      227

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

Class:
  pdbs, fasta

Alignment dimensions:
```

```
  13 sequence rows; 227 position columns (204 non-gap, 23 gap)

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

Principal Component Analysis

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




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

Protein Structure Prediction with AlphaFold

Custom Analysis of Resulting Models

For tidiness we can move our AlphaFold results directory into our RStudio project directory. In this example my results are in the director `results_dir`

Figure 1: HIV monomer

```
results_dir <- "hivpr_dimer_23119/"
```

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

```
[1] "hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_s
[2] "hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_s
[3] "hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_s
[4] "hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_s
[5] "hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_s
```

```
library(bio3d)

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

```
Reading PDB files:
hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_0
hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_0
hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_0
hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_0
hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_0
.....

Extracting sequences

pdb/seq: 1   name: hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_001_alphafold2_multime
pdb/seq: 2   name: hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_002_alphafold2_multime
pdb/seq: 3   name: hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_003_alphafold2_multime
pdb/seq: 4   name: hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_004_alphafold2_multime
pdb/seq: 5   name: hivpr_dimer_23119//hivpr_dimer_23119_unrelaxed_rank_005_alphafold2_multime
```

```
pdbs
```

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

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

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

                              151        .        .        .        .      198
[Truncated_Name:1]hivpr_dime    GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:2]hivpr_dime    GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:3]hivpr_dime    GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:4]hivpr_dime    GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:5]hivpr_dime    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
```

Calculate the RMSD between all models.

```
rd <- rmsd(pdbs)
```

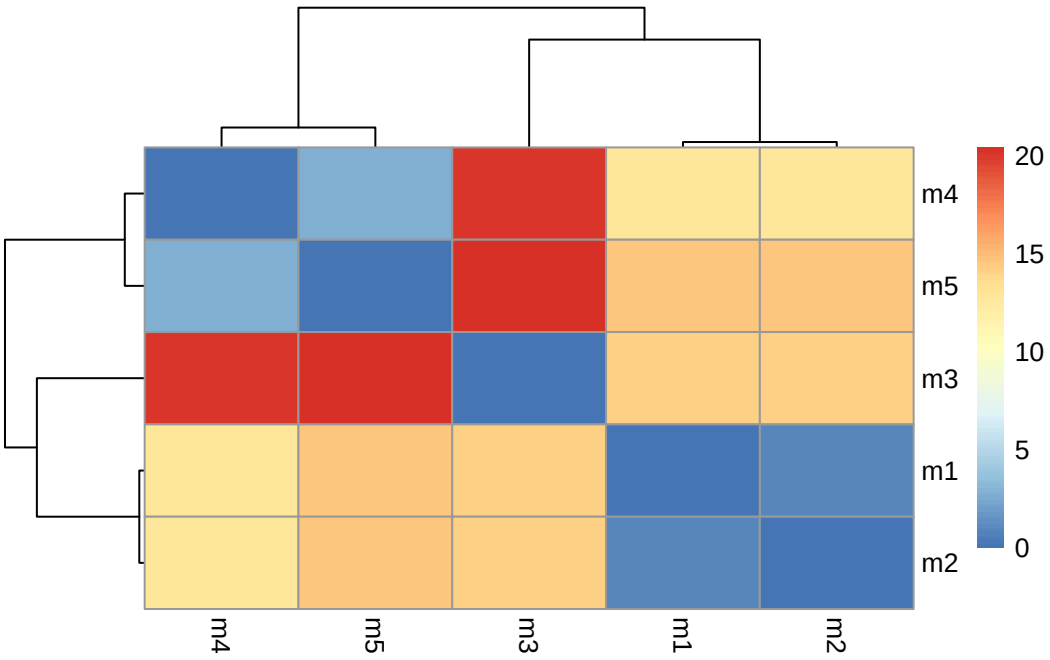
```
Warning in rmsd(pdbs): No indices provided, using the 198 non NA positions
```

```
range(rd)
```

```
[1]  0.000 20.431
```

Draw a heatmap of RMSD matrix values.

```
library(pheatmap)

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



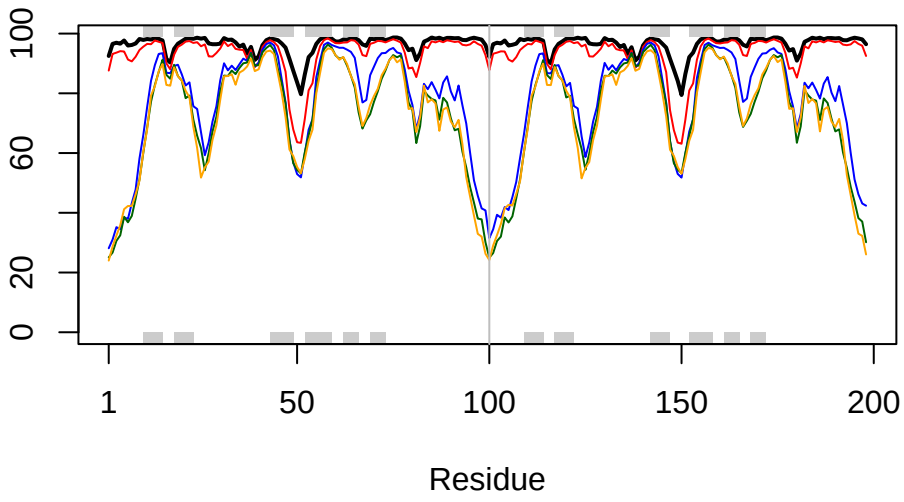
And a plot pLDDT values across all models.

```
# 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$b, typ="l", lwd=2, sse=pdb)
points(pdbs$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:

```
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
```

We can use the identified core atom positions as a basis for a more suitable superposition:

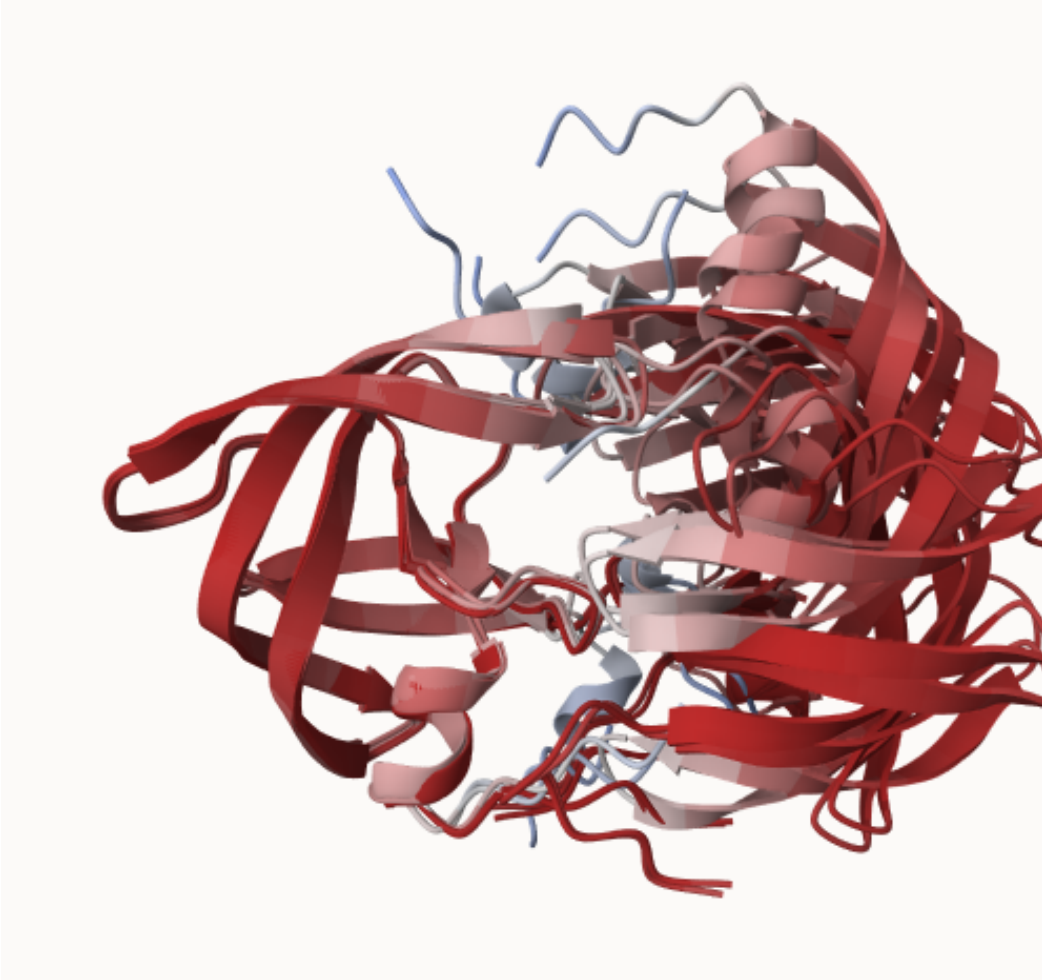
```
core.inds <- print(core, vol=0.5)
```

```
# 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 Uncertenty/Disorder (i.e. the B-factor column that contains the pLDDT scores):

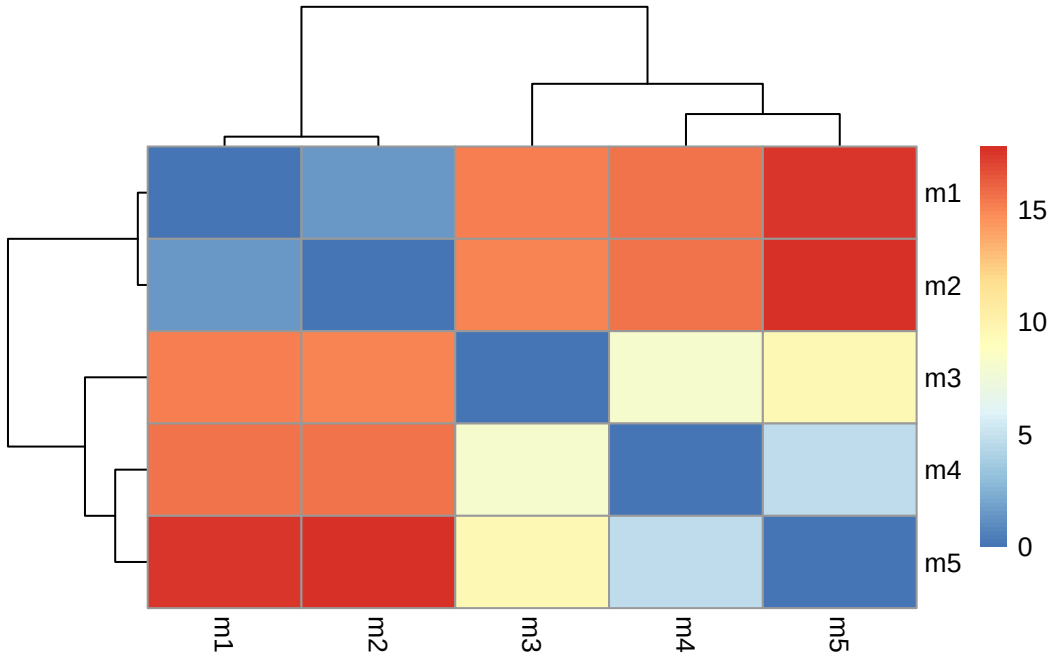


Now we can update our RMSD analysis and examine the RMSF between positions of the structure:

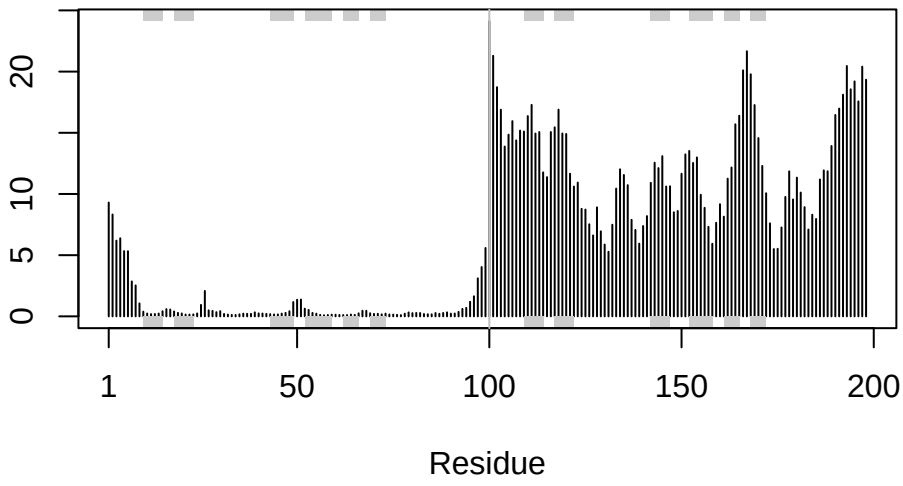
```
rd <- rmsd(xyz)
```

```
Warning in rmsd(xyz): No indices provided, using the 198 non NA positions
```

```
# Change the names for easy reference
colnames(rd) <- paste0("m",1:5)
rownames(rd) <- paste0("m",1:5)
pheatmap(rd)
```




```
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 files, one for each model structure.

Below we read these files and see that AlphaFold produces a useful inter-domain prediction for model 1 but not for model 5:

```
library(jsonlite)

# 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"
```

```
# Per-residue pLDDT scores
#  same as B-factor of PDB..
head(pae1$plddt)
```

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

The maximum PAE values - we can see that model 5 is much worse than model 1. The lower the better.

```
pae1$max_pae
```

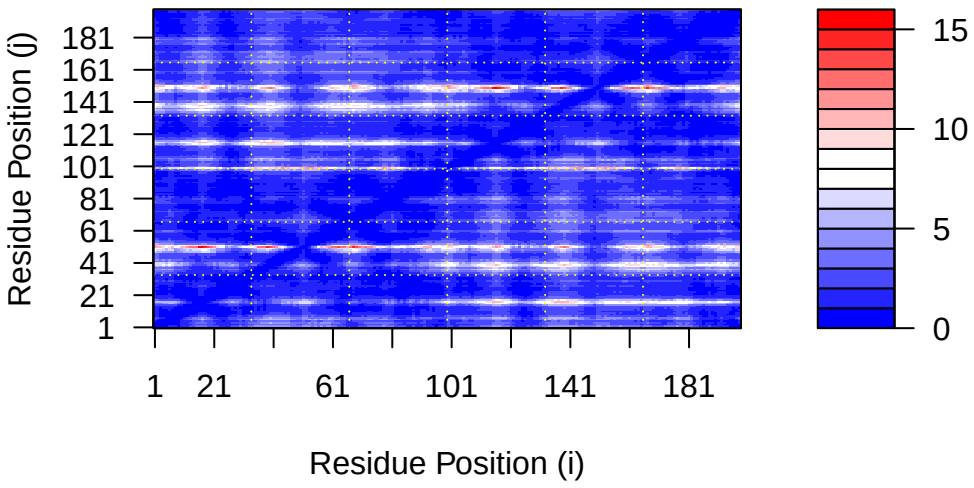
```
[1] 15.54688
```

```
pae5$max_pae
```

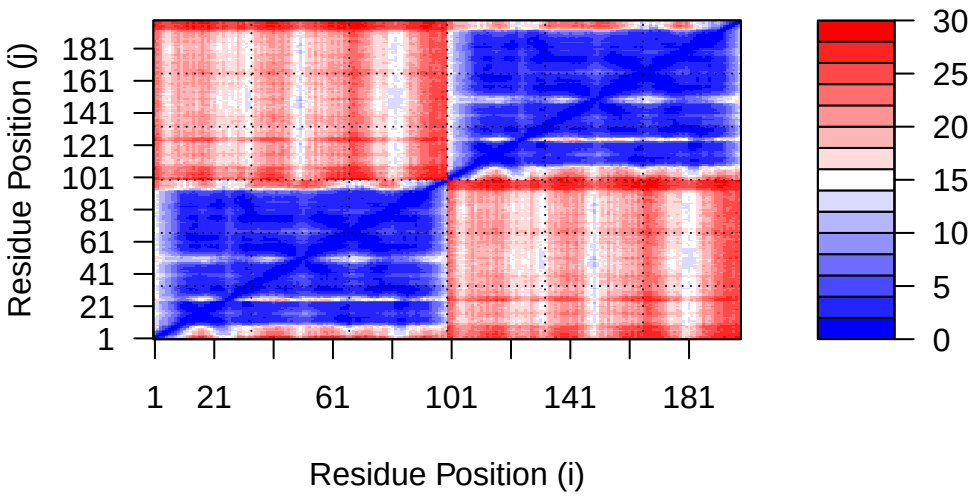
```
[1] 29.29688
```

We can plot these with ggplot or with functions from the Bio3D package:

```
library(bio3d)

plot.dmat(pae1$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)")
```

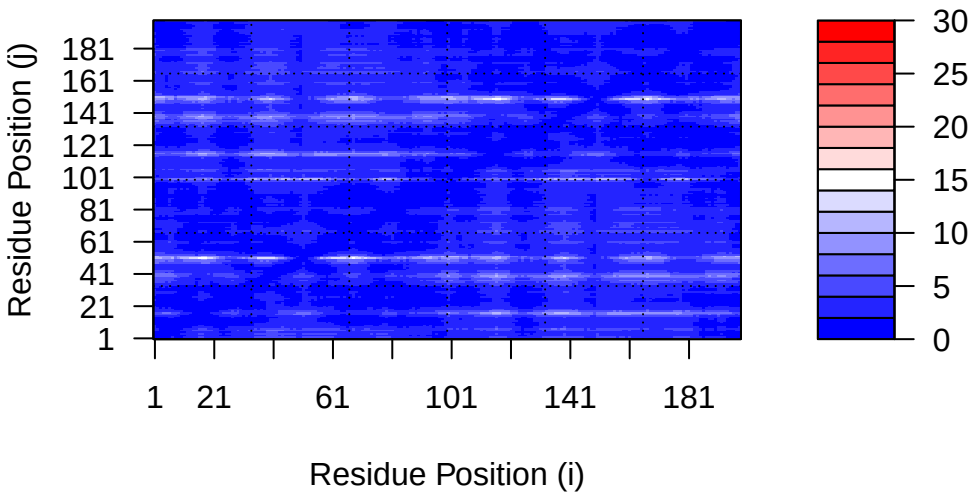



```
plot.dmat(pae5$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)",
          grid.col = "black",
          zlim=c(0,30))
```



We should really plot all of these using the same z range. Here is the model 1 plot again but this time using the same data range as the plot for model 5:

```
plot.dmat(pae1$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] "hivpr_dimer_23119//hivpr_dimer_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 in this alignment?

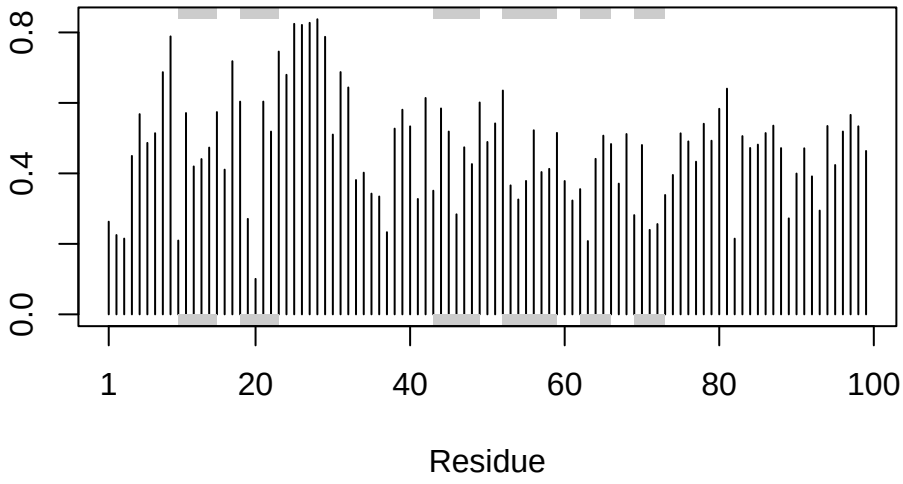
```
dim(aln$ali)
```

```
[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"))
```



Note the conserved Active Site residues D25, T26, G27, A28.

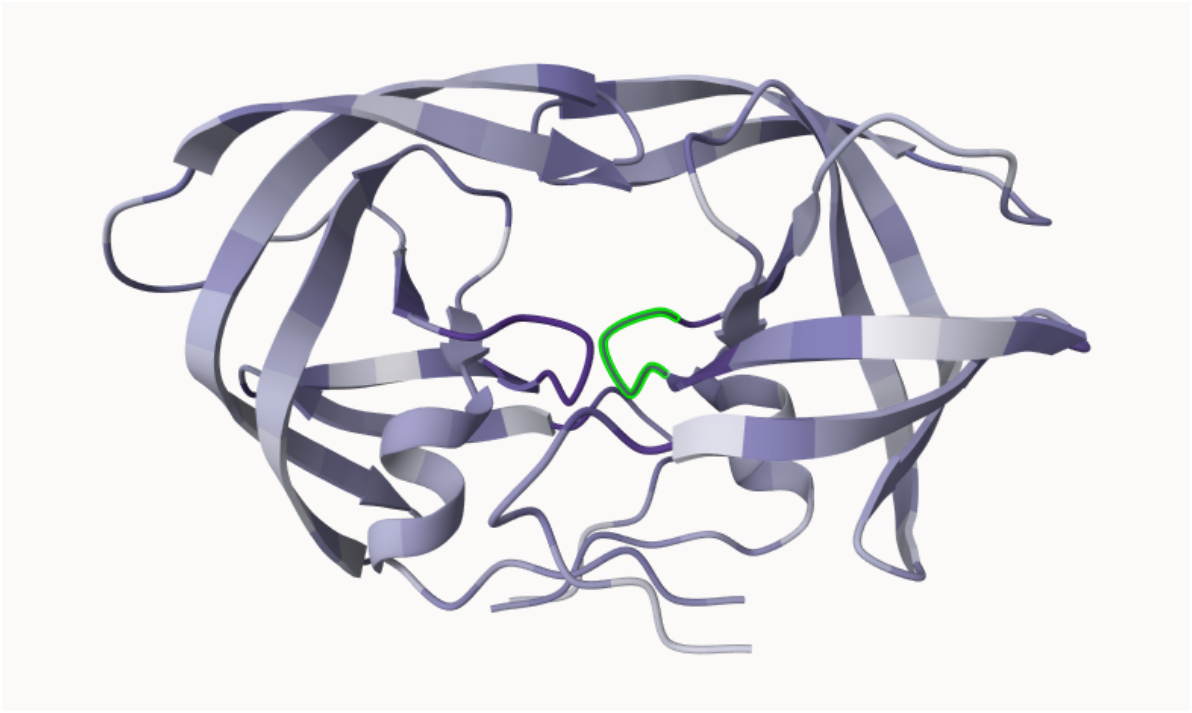
```
con <- consensus(aln, cutoff = 0.9)
con$seq
```

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

For a final visualization 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")
```



Note that 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.