Class 11 Alphafold

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5. The EBI AlphaFold database

Use the following sequences to search AFDB

> HIV-Pr PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGR-WKPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRN-LLTQIGCTLNF

6. Generating your own structure predictions

First, check the AFDB for the protein of interest. If your structure has already been predicted there, you can download the AFDB PDB file and skip to the Interpreting Results section below.

Otherwise obtain the sequence of your protein of interest, e.g. at UniProt. Click on the FASTA button above the sequence in UniProt. Copy only the sequence, excluding the FASTA header line that begins with ">".

For your first time through this lab I would like you to use the HIV-Pr sequence to generate a single chain model. After your first run you can experiment with generating the biologically relevant homodimer (the monomer is unstable in reality and the dimer is the functional unit): >HIV-Pr PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG-GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF

And for your 2nd run the dimer input. As it is a homodimer this consists of the same sequence twice with a colon between chains:

> HIV-Pr-Dimer PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLP-GRWKPKMIGGIGGFIKVRQYD QILIEICGHKAIGTVLVGPTPVNIIGRN-LLTQIGCTLNF:PQITLWQRPLVTIKIGGQLK EALLDTGADDTVLEEMSLP-GRWKPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPT PVNIIGRN-LLTQIGCTLNF You can also experiment with your "find-a-gene" project sequence as you will need this result for your project work).

Visit AlphaFold2_mmseqs2 Colab notebook (at the time of writing this is currently the preferred AlphaFold version for our current prediction tasks.

Click on "Connect" on top right toolbar to obtain access to computing resources to run AlphaFold on. If successful this will connect you to Google Compute Engine cloud resources with a GPU. A green tick should appear along with RAM and Disk space graphics

In the first main page code cell paste in your query_sequence, making sure to completely replace the default sequence

Enter a descriptive jobname (replacing the default "test" value). Note that the results.zip filename obtained at the end of the full computation will begin with this jobname (but none of its contents include the jobname).

For now leave all other parameters (i.e. the code cells) at their default values. We can explore them later after completing our first successful run.

Back at the very top of the page where we have the "File"/"Edit"/"View" toolbar menu items click "Runtime" > "Run All"

8. Custom analysis of resulting models

Change this for YOUR results dir name

```r
# Change this for YOUR results dir name
results_dir <- "hivprdimer_23119"
```

```r
# 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_4_seed_000.pdb"
[2] "hivprdimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_1_seed_000.pdb"
[3] "hivprdimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_5_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"
```

```r
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_4_seed_000
hivprdimer_23119/hivprdimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_1_seed_000
hivprdimer_23119/hivprdimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_5_seed_000
hivprdimer_23119/hivprdimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000
hivprdimer_23119/hivprdimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000
.....

Extracting sequences

pdb/seq: 1   name: hivprdimer_23119/hivprdimer_23119_unrelaxed_rank_001_alphafold2_multimer_
pdb/seq: 2   name: hivprdimer_23119/hivprdimer_23119_unrelaxed_rank_002_alphafold2_multimer_
pdb/seq: 3   name: hivprdimer_23119/hivprdimer_23119_unrelaxed_rank_003_alphafold2_multimer_
pdb/seq: 4   name: hivprdimer_23119/hivprdimer_23119_unrelaxed_rank_004_alphafold2_multimer_
pdb/seq: 5   name: hivprdimer_23119/hivprdimer_23119_unrelaxed_rank_005_alphafold2_multimer_
```

```r
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
```

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

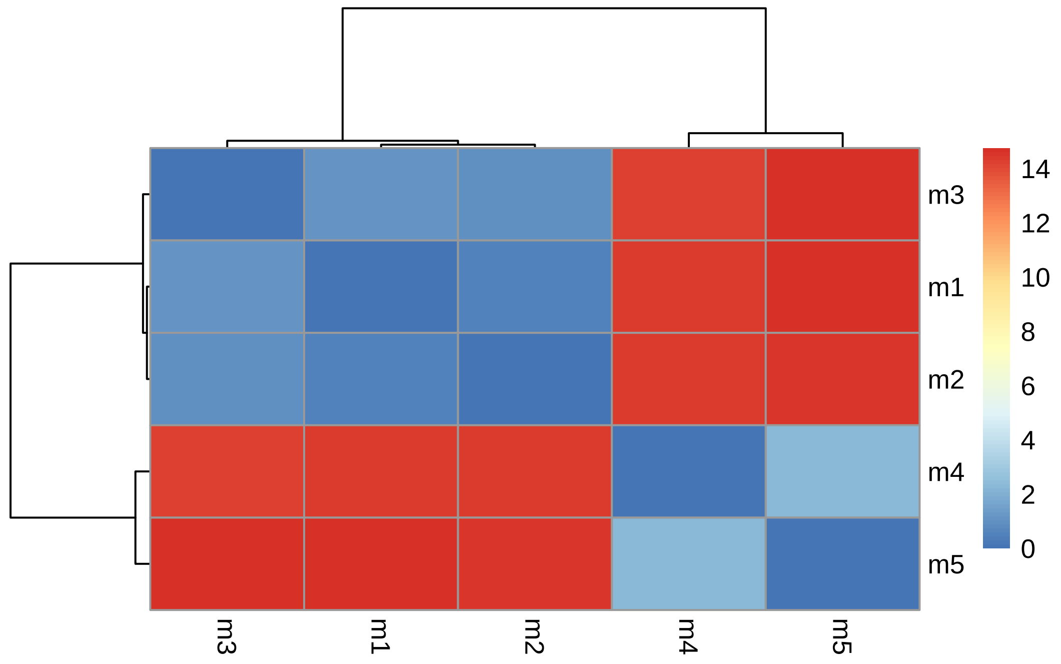
```
Warning in rmsd(pdbs, fit = T): No indices provided, using the 198 non NA positions
```

```r
range(rd)
```

```
[1]  0.000 14.754
```

```r
library(pheatmap)
```

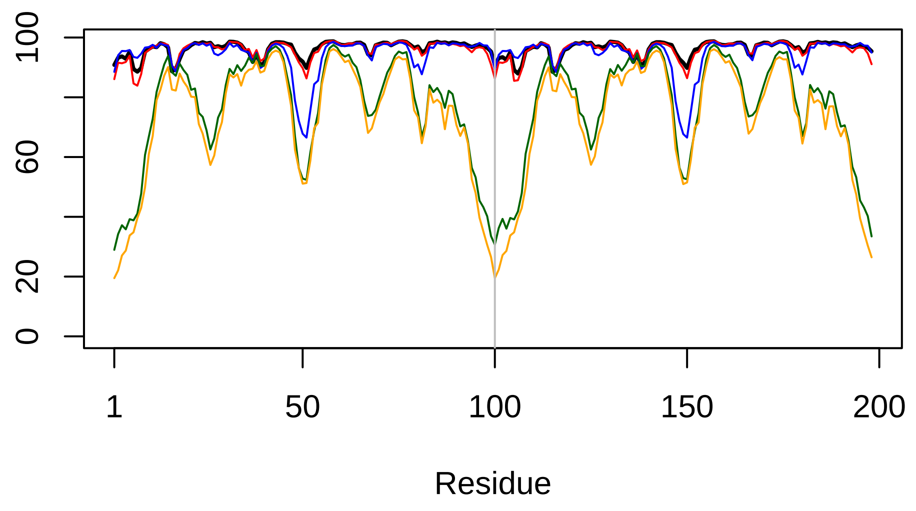
```r
colnames(rd) <- paste0("m",1:5)
rownames(rd) <- paste0("m",1:5)
pheatmap(rd)
```



```r
plotb3(pdbs$b[1,], typ="l", lwd=2, sse=pdbs)
```

```
Warning in plotb3(pdbs$b[1, ], typ = "l", lwd = 2, sse = pdbs): Length of input
'sse' does not equal the length of input 'x'; Ignoring 'sse'
```

```r
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")
```



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

```
core size 197 of 198  vol = 9885.822
core size 196 of 198  vol = 6896.71
core size 195 of 198  vol = 1337.847
core size 194 of 198  vol = 1040.67
core size 193 of 198  vol = 951.857
core size 192 of 198  vol = 899.083
core size 191 of 198  vol = 834.732
core size 190 of 198  vol = 771.338
core size 189 of 198  vol = 733.065
core size 188 of 198  vol = 697.28
core size 187 of 198  vol = 659.742
core size 186 of 198  vol = 625.273
core size 185 of 198  vol = 589.541
core size 184 of 198  vol = 568.253
core size 183 of 198  vol = 545.015
core size 182 of 198  vol = 512.889
core size 181 of 198  vol = 490.723
core size 180 of 198  vol = 470.266
core size 179 of 198  vol = 450.731
core size 178 of 198  vol = 434.735
core size 177 of 198  vol = 420.337
core size 176 of 198  vol = 406.658
core size 175 of 198  vol = 393.334
core size 174 of 198  vol = 382.395
core size 173 of 198  vol = 372.858
core size 172 of 198  vol = 356.994
core size 171 of 198  vol = 346.567
core size 170 of 198  vol = 337.446
core size 169 of 198  vol = 326.659
core size 168 of 198  vol = 314.95
core size 167 of 198  vol = 304.127
core size 166 of 198  vol = 294.552
core size 165 of 198  vol = 285.648
core size 164 of 198  vol = 278.884
core size 163 of 198  vol = 266.765
core size 162 of 198  vol = 258.994
core size 161 of 198  vol = 247.723
core size 160 of 198  vol = 239.84
core size 159 of 198  vol = 234.963
core size 158 of 198  vol = 230.062
core size 157 of 198  vol = 221.985
core size 156 of 198  vol = 215.62
core size 155 of 198  vol = 206.793
core size 154 of 198  vol = 196.984
core size 153 of 198  vol = 188.539
core size 152 of 198  vol = 182.262
core size 151 of 198  vol = 176.954
core size 150 of 198  vol = 170.712
core size 149 of 198  vol = 166.119
core size 148 of 198  vol = 159.796
core size 147 of 198  vol = 153.767
core size 146 of 198  vol = 149.092
core size 145 of 198  vol = 143.657
core size 144 of 198  vol = 137.138
core size 143 of 198  vol = 132.517
core size 142 of 198  vol = 127.231
core size 141 of 198  vol = 121.574
core size 140 of 198  vol = 116.775
core size 139 of 198  vol = 112.57
core size 138 of 198  vol = 108.17
core size 137 of 198  vol = 105.133
core size 136 of 198  vol = 101.249
core size 135 of 198  vol = 97.374
core size 134 of 198  vol = 92.974
core size 133 of 198  vol = 88.184
core size 132 of 198  vol = 84.029
core size 131 of 198  vol = 81.898
core size 130 of 198  vol = 78.019
core size 129 of 198  vol = 75.272
core size 128 of 198  vol = 73.052
core size 127 of 198  vol = 70.695
core size 126 of 198  vol = 68.975
core size 125 of 198  vol = 66.694
core size 124 of 198  vol = 64.394
core size 123 of 198  vol = 62.092
core size 122 of 198  vol = 59.045
core size 121 of 198  vol = 56.629
core size 120 of 198  vol = 54.016
core size 119 of 198  vol = 51.806
core size 118 of 198  vol = 49.652
core size 117 of 198  vol = 48.193
core size 116 of 198  vol = 46.648
core size 115 of 198  vol = 44.752
core size 114 of 198  vol = 43.292
core size 113 of 198  vol = 41.093
core size 112 of 198  vol = 39.147
core size 111 of 198  vol = 36.472
core size 110 of 198  vol = 34.117
core size 109 of 198  vol = 31.47
core size 108 of 198  vol = 29.448
core size 107 of 198  vol = 27.325
core size 106 of 198  vol = 25.822
core size 105 of 198  vol = 24.15
core size 104 of 198  vol = 22.648
core size 103 of 198  vol = 21.069
core size 102 of 198  vol = 19.953
core size 101 of 198  vol = 18.3
core size 100 of 198  vol = 15.723
core size 99 of 198  vol = 14.841
core size 98 of 198  vol = 11.646
core size 97 of 198  vol = 9.434
core size 96 of 198  vol = 7.354
core size 95 of 198  vol = 6.179
core size 94 of 198  vol = 5.666
core size 93 of 198  vol = 4.705
core size 92 of 198  vol = 3.665
```

```
 core size 91 of 198  vol = 2.77
 core size 90 of 198  vol = 2.151
 core size 89 of 198  vol = 1.715
 core size 88 of 198  vol = 1.15
 core size 87 of 198  vol = 0.874
 core size 86 of 198  vol = 0.685
 core size 85 of 198  vol = 0.528
 core size 84 of 198  vol = 0.37
 FINISHED: Min vol ( 0.5 ) reached
```

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

```
# 85 positions (cumulative volume <= 0.5 Angstrom^3)
  start end length
1     9  49     41
2    52  95     44
```

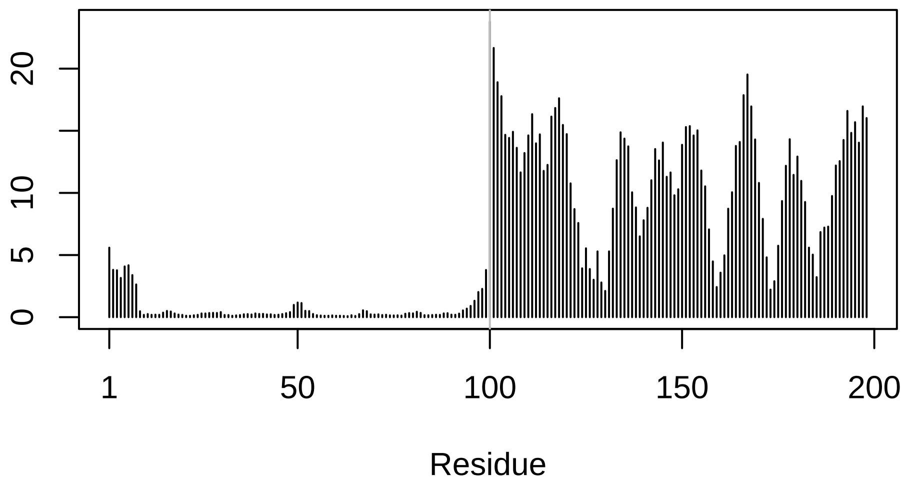
```
xyz <- pdbfit(pdbs, core.inds, outpath="corefit_structures")
```

```
rf <- rmsf(xyz)

plotb3(rf, sse=pdbs)
```

```
Warning in plotb3(rf, sse = pdbs): Length of input 'sse' does not equal the
length of input 'x'; Ignoring 'sse'
```

```
abline(v=100, col="gray", ylab="RMSF")
```



```
library(jsonlite)

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

```
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] 90.81 93.25 93.69 92.88 95.25 89.44
```

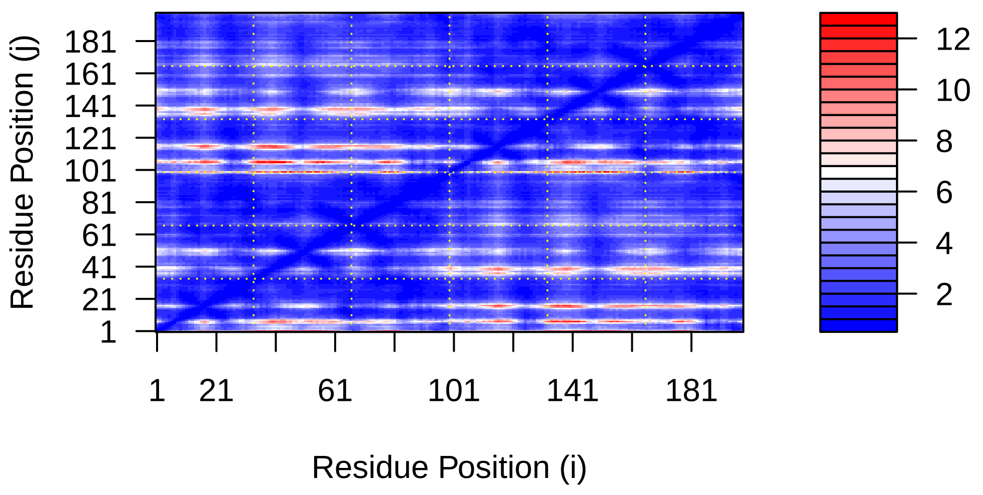
```r
pae1$max_pae
```

```
[1] 12.84375
```

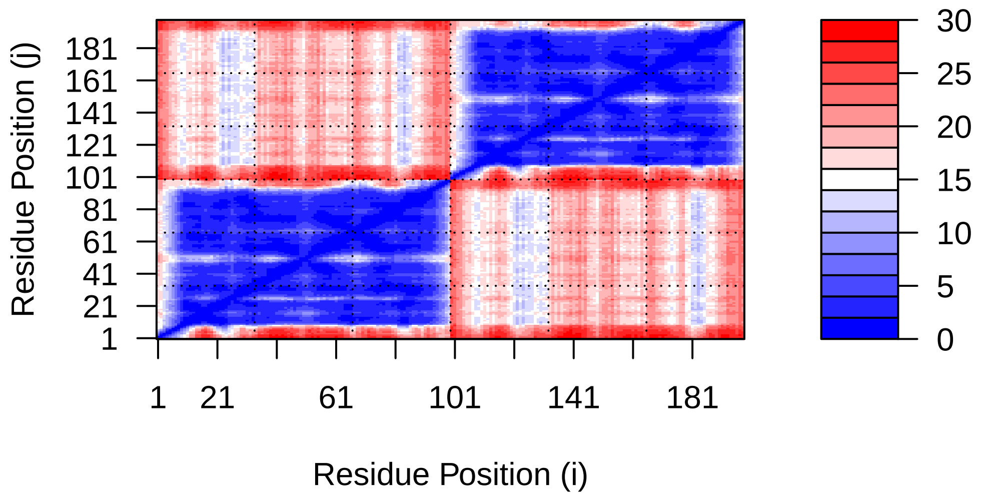
```r
pae5$max_pae
```

```
[1] 29.59375
```

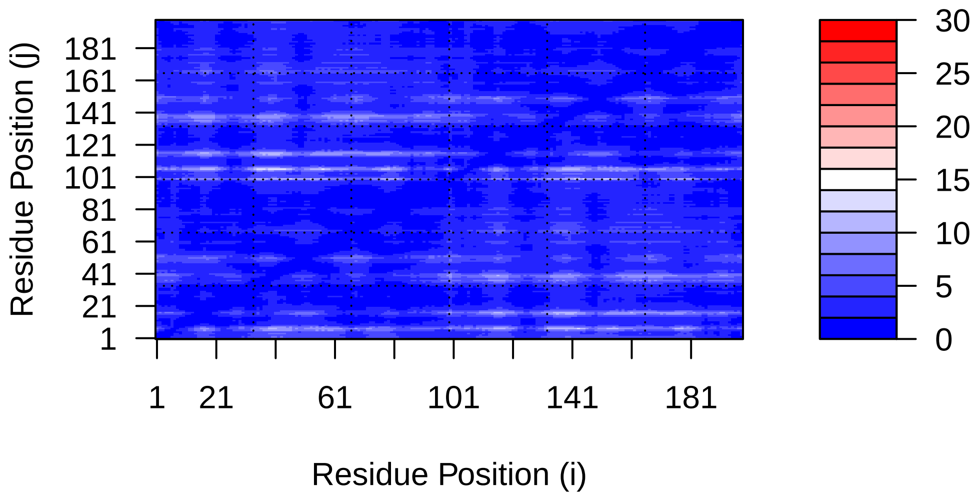
```r
plot.dmat(pae1$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)")
```



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



```
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] "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 **"
```

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

```
[1] 5397  132
```

```r
library(bio3d)

# read one structure (e.g., the first PDB file)
#pdb <- read.pdb(pdb_files[1])

# trim that pdb to chain A (for SSE display)
#sseA <- trim.pdb(pdb, chain = "A")

# now plot
#plotb3(sim[1:99], sse = sseA, ylab = "Conservation Score")
```

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

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

```r
#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")
```