Structural Bioinformatics (pt.2)

Raquel Gonzalez (A16207442)

AlphaFold has changed the game for protein structure prediction and allows anyone with sufficient bioinformatics skills to predict the structure of virtually any protein.

We ran AlphaFold via GoogleColab at: https://github.com/sokrypton/ColabFold

In particular we used their AlphaFold2_mmseq2 version that uses mmseq2 rather than HMMMer for sequence search.

The main outputs include a set of **PDB structure files** along with matching **JSON format files** that tell us how good the resulting models might be.

Let's start by loading the PDB structures up in Mol*

```r
# Change this for YOUR results dir name
results_dir <- "hivpr1dimer_23119"

pdb_files <- list.files(path=results_dir,
                        pattern="*.pdb",
                        full.names=TRUE)

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

```
[1] "hivpr1dimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_000.pdb"
[2] "hivpr1dimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_000.pdb"
[3] "hivpr1dimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_000.pdb"
[4] "hivpr1dimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.pdb"
[5] "hivpr1dimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.pdb"
```

```r
library(bio3d)

# Read all data from Models and superpose/fit coordinates
```

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

```
Reading PDB files:
hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_00
hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_00
hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_00
hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_00
hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_00
.....

Extracting sequences

pdb/seq: 1   name: hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_001_alphafold2_multimer
pdb/seq: 2   name: hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_002_alphafold2_multimer
pdb/seq: 3   name: hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_003_alphafold2_multimer
pdb/seq: 4   name: hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_004_alphafold2_multimer
pdb/seq: 5   name: hivpr1dimer_23119/hivpr1dimer_23119_unrelaxed_rank_005_alphafold2_multimer
```

```r
pdbs
```

```
                               1        .         .         .         .         50 
[Truncated_Name:1]hivpr1dime   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:2]hivpr1dime   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:3]hivpr1dime   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:4]hivpr1dime   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:5]hivpr1dime   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
                               ************************************************** 
                               1        .         .         .         .         50 

                              51        .         .         .         .         100 
[Truncated_Name:1]hivpr1dime   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:2]hivpr1dime   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:3]hivpr1dime   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:4]hivpr1dime   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:5]hivpr1dime   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
                               ************************************************** 
                              51        .         .         .         .         100 

                             101        .         .         .         .         150 
[Truncated_Name:1]hivpr1dime   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
```

```
[Truncated_Name:2]hivpr1dime   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:3]hivpr1dime   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:4]hivpr1dime   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:5]hivpr1dime   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
                               **************************************************
                              101       .         .         .         .         150

                              151       .         .         .         .      198
[Truncated_Name:1]hivpr1dime   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:2]hivpr1dime   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:3]hivpr1dime   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:4]hivpr1dime   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:5]hivpr1dime   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
```

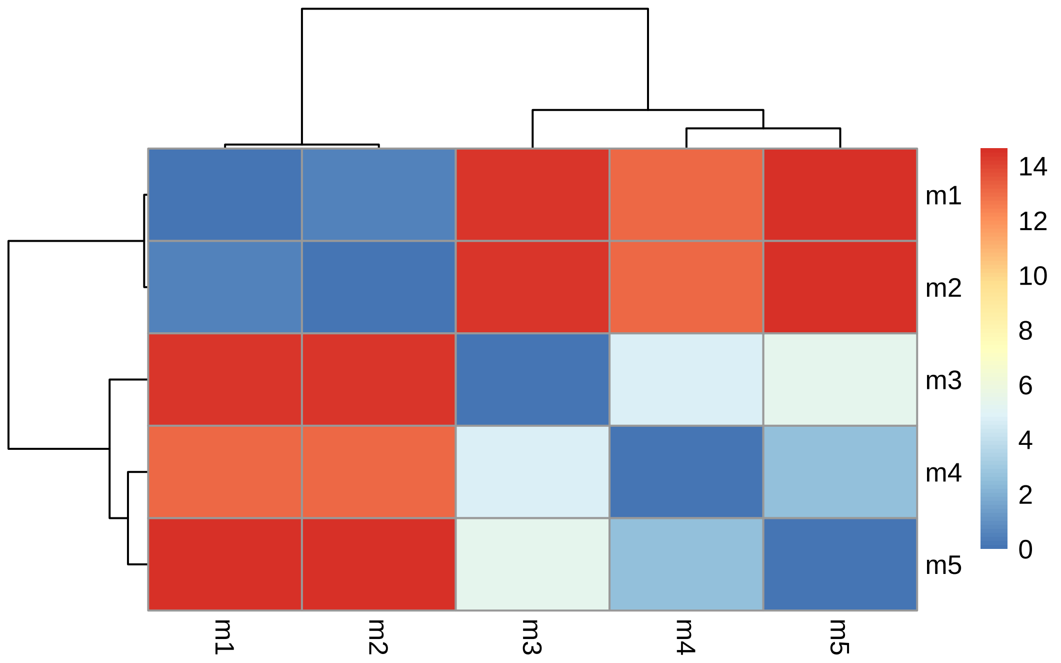
```
range(rd)
```

```
[1]  0.000 14.631
```

Draw a heatmap of these RMSD matrix values.

```r
library(pheatmap)

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



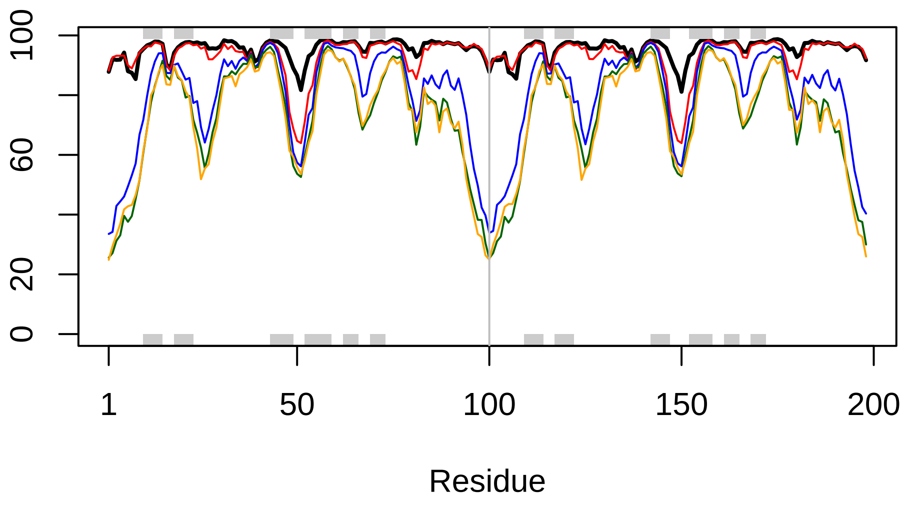
Now lets plot the pLDDT values across all models. Recall that this information is in the B-factor column of each model and that this is stored in our aligned `pdbs` object as `pdbs$b` with a row per structure/model.

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

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

```r
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:

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

```
core size 197 of 198  vol = 4578.336
core size 196 of 198  vol = 3931.103
core size 195 of 198  vol = 3709.727
core size 194 of 198  vol = 3496.014
core size 193 of 198  vol = 3302.428
core size 192 of 198  vol = 3146.468
core size 191 of 198  vol = 3048.959
core size 190 of 198  vol = 2970.348
core size 189 of 198  vol = 2893.007
core size 188 of 198  vol = 2831.818
core size 187 of 198  vol = 2774.499
core size 186 of 198  vol = 2728.035
core size 185 of 198  vol = 2704.937
core size 184 of 198  vol = 2701.97
core size 183 of 198  vol = 2715.897
core size 182 of 198  vol = 2809.84
core size 181 of 198  vol = 2888.937
core size 180 of 198  vol = 2967.269
core size 179 of 198  vol = 3036.243
core size 178 of 198  vol = 3066.274
core size 177 of 198  vol = 3096.82
core size 176 of 198  vol = 3056.401
core size 175 of 198  vol = 3014.755
core size 174 of 198  vol = 2974.999
core size 173 of 198  vol = 2898.037
core size 172 of 198  vol = 2810.159
core size 171 of 198  vol = 2747.518
core size 170 of 198  vol = 2684.42
core size 169 of 198  vol = 2620.339
core size 168 of 198  vol = 2550.863
core size 167 of 198  vol = 2492.567
core size 166 of 198  vol = 2422.963
core size 165 of 198  vol = 2358.901
core size 164 of 198  vol = 2298.277
core size 163 of 198  vol = 2235.903
core size 162 of 198  vol = 2171.006
core size 161 of 198  vol = 2093.544
core size 160 of 198  vol = 2029.129
core size 159 of 198  vol = 1950.943
core size 158 of 198  vol = 1881.001
core size 157 of 198  vol = 1801.491
core size 156 of 198  vol = 1728.877
core size 155 of 198  vol = 1660.022
core size 154 of 198  vol = 1586.134
core size 153 of 198  vol = 1532.702
core size 152 of 198  vol = 1460.171
core size 151 of 198  vol = 1399.236
core size 150 of 198  vol = 1333.893
core size 149 of 198  vol = 1271.731
core size 148 of 198  vol = 1219.48
core size 147 of 198  vol = 1175.987
core size 146 of 198  vol = 1138.462
core size 145 of 198  vol = 1102.108
core size 144 of 198  vol = 1049.627
core size 143 of 198  vol = 1014.047
core size 142 of 198  vol = 970.56
core size 141 of 198  vol = 929.163
core size 140 of 198  vol = 889.089
core size 139 of 198  vol = 846.653
core size 138 of 198  vol = 805.785
core size 137 of 198  vol = 775.019
core size 136 of 198  vol = 743.075
core size 135 of 198  vol = 715.68
core size 134 of 198  vol = 689.773
core size 133 of 198  vol = 660.314
core size 132 of 198  vol = 630.951
core size 131 of 198  vol = 597.191
core size 130 of 198  vol = 566.973
core size 129 of 198  vol = 532.874
core size 128 of 198  vol = 496.192
core size 127 of 198  vol = 463.167
core size 126 of 198  vol = 431.877
core size 125 of 198  vol = 408.848
core size 124 of 198  vol = 376.594
core size 123 of 198  vol = 362.36
core size 122 of 198  vol = 353.633
core size 121 of 198  vol = 331.501
core size 120 of 198  vol = 312.518
core size 119 of 198  vol = 286.715
core size 118 of 198  vol = 262.336
core size 117 of 198  vol = 245.109
core size 116 of 198  vol = 228.342
core size 115 of 198  vol = 210.366
core size 114 of 198  vol = 197.519
core size 113 of 198  vol = 179.392
core size 112 of 198  vol = 161.891
core size 111 of 198  vol = 148.359
core size 110 of 198  vol = 134.477
core size 109 of 198  vol = 121.261
core size 108 of 198  vol = 109.516
core size 107 of 198  vol = 103.031
core size 106 of 198  vol = 96.443
core size 105 of 198  vol = 88.455
core size 104 of 198  vol = 81.816
core size 103 of 198  vol = 74.88
core size 102 of 198  vol = 68.386
core size 101 of 198  vol = 65.937
core size 100 of 198  vol = 62.345
core size 99 of 198  vol = 58.836
core size 98 of 198  vol = 52.868
```

```
 core size 97 of 198  vol = 47.796
 core size 96 of 198  vol = 41.292
 core size 95 of 198  vol = 33.831
 core size 94 of 198  vol = 24.912
 core size 93 of 198  vol = 18.912
 core size 92 of 198  vol = 12.7
 core size 91 of 198  vol = 7.35
 core size 90 of 198  vol = 4.922
 core size 89 of 198  vol = 3.421
 core size 88 of 198  vol = 2.553
 core size 87 of 198  vol = 1.917
 core size 86 of 198  vol = 1.513
 core size 85 of 198  vol = 1.201
 core size 84 of 198  vol = 1.046
 core size 83 of 198  vol = 0.922
 core size 82 of 198  vol = 0.755
 core size 81 of 198  vol = 0.668
 core size 80 of 198  vol = 0.596
 core size 79 of 198  vol = 0.549
 core size 78 of 198  vol = 0.493
 FINISHED: Min vol ( 0.5 ) reached
```

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

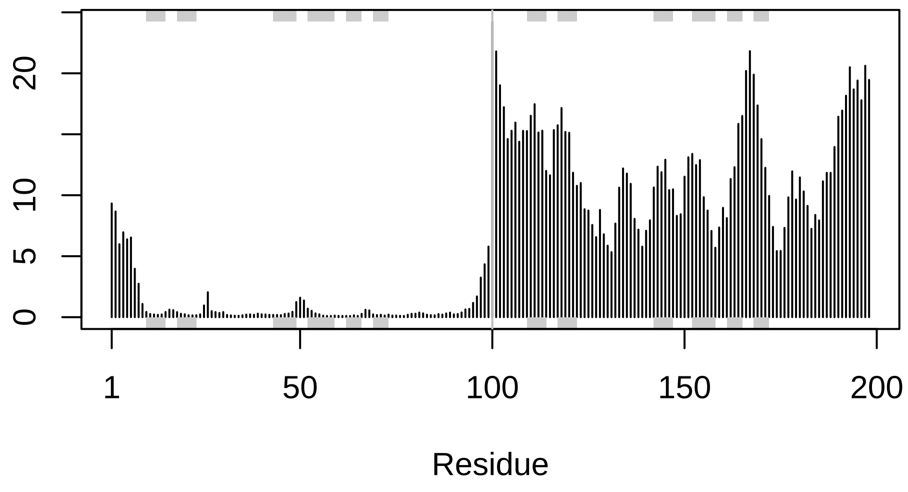
```
# 79 positions (cumulative volume <= 0.5 Angstrom^3)
  start end length
1    10  25     16
2    28  48     21
3    53  94     42
```

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

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

```r
rf <- rmsf(xyz)

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



If the predicted model has more than one domain, each domain may have high confidence, yet the relative positions of the domains may not. The estimated reliability of relative domain positions is in graphs of predicted aligned error (PAE) which are included in the downloadable zip file and analyzed in R above.

Predicted Alignment Error for Domains

```
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 (you can read the others and make similar plots).

```
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] 87.81 92.00 91.81 91.88 94.25 88.00
```

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
```

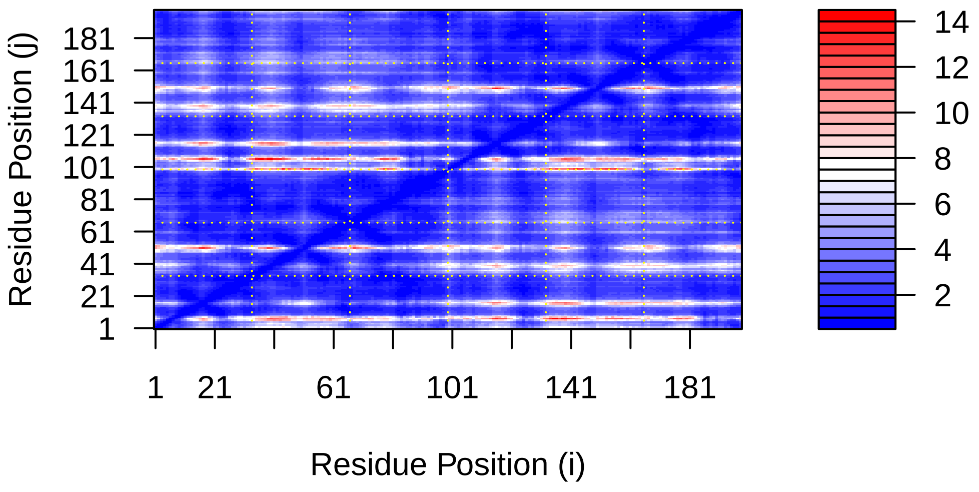
```
[1] 14.09375
```

```
pae5$max_pae
```

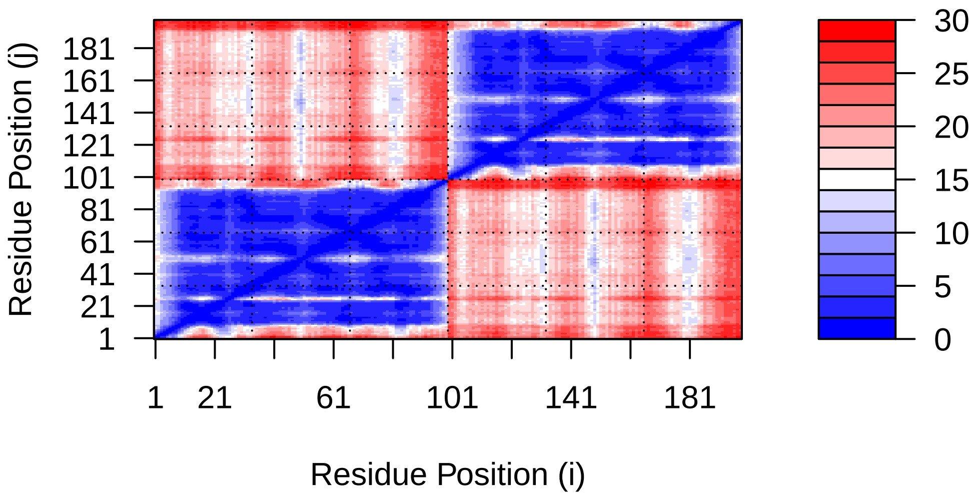
```
[1] 29.29688
```

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:

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

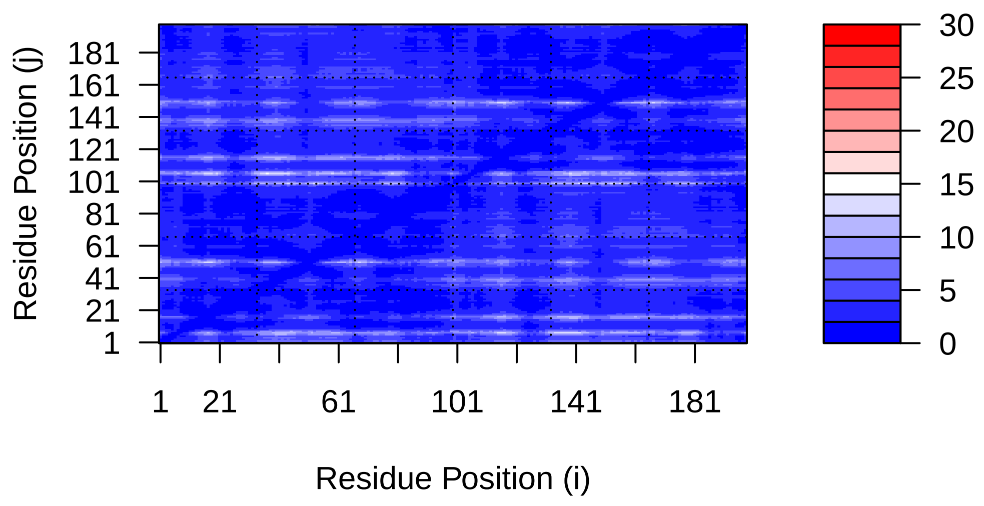


```
plot.dmat(pae5$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)",
          grid.col = "black",
          zlim=c(0,30))
```



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:

```r
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] "hivpr1dimer_23119/hivpr1dimer_23119.a3m"
```

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

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

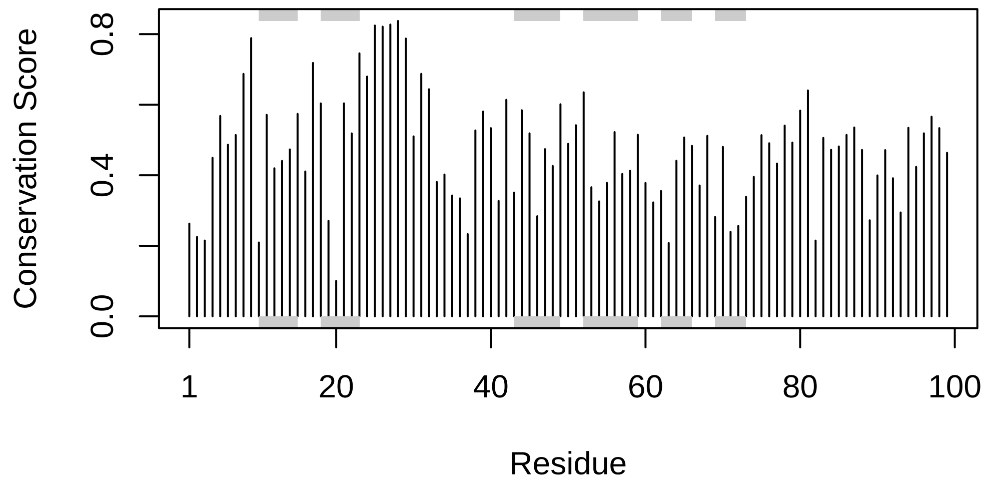
How many sequences are in this alignment

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


Conservation Score
Residue