Class 11: Structural Bioinformatics pt2

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Background

We saw last day that the PDB has 209,886 entires (Oct/Nov 2025). UniProtKB (i.e. protein sequence database) has 199,579,901 entries.

```
209886/199579901
```

```
[1] 0.001051639
```

So the PDB has 0.1% coverage of the main sequence database.

Enter Alphafold data base (AFDB). < https://alphafold.ebi.ac.uk/ > that attempts to provide computed models for sequences in UniProt

"AlphaFold DB provides open access to over 200 million proteins structure predictions to accelerate scientific research"

AlphaFold

AlphaFold has 3 main outputs

- the predicted coordinates (PDB files)
- A local qualiy score called **pLDDT** (one for each amino acid - per residue measure of local confidence)
- A second quality score **PAE** Predicted Aligned Error for the confidence in the prelative position of each pair of amino acids

We can run Alphafold ourselves if we are not happy with AFDB (i.e. no coverage or poor model).

Interpreting/analyzing AF results in R

```
results_dir <- "HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0"
```

```
# 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] "HIVPR_dimer_23119_0_unrelaxed_rank_001_alphafold2_multimer_v3_model_4_seed_000.pdb"
[2] "HIVPR_dimer_23119_0_unrelaxed_rank_002_alphafold2_multimer_v3_model_1_seed_000.pdb"
[3] "HIVPR_dimer_23119_0_unrelaxed_rank_003_alphafold2_multimer_v3_model_5_seed_000.pdb"
[4] "HIVPR_dimer_23119_0_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.pdb"
[5] "HIVPR_dimer_23119_0_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.pdb"
```

```
library(bio3d)
```

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

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

```
Reading PDB files:
HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelaxed_rank_001_alphafo
HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelaxed_rank_002_alphafo
HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelaxed_rank_003_alphafo
HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelaxed_rank_004_alphafo
HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelaxed_rank_005_alphafo
.....

Extracting sequences

pdb/seq: 1   name: HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelax
pdb/seq: 2   name: HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelax
pdb/seq: 3   name: HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelax
pdb/seq: 4   name: HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelax
pdb/seq: 5   name: HIVPR_dimer_23119_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0_unrelax
```

```r
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
```

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
```

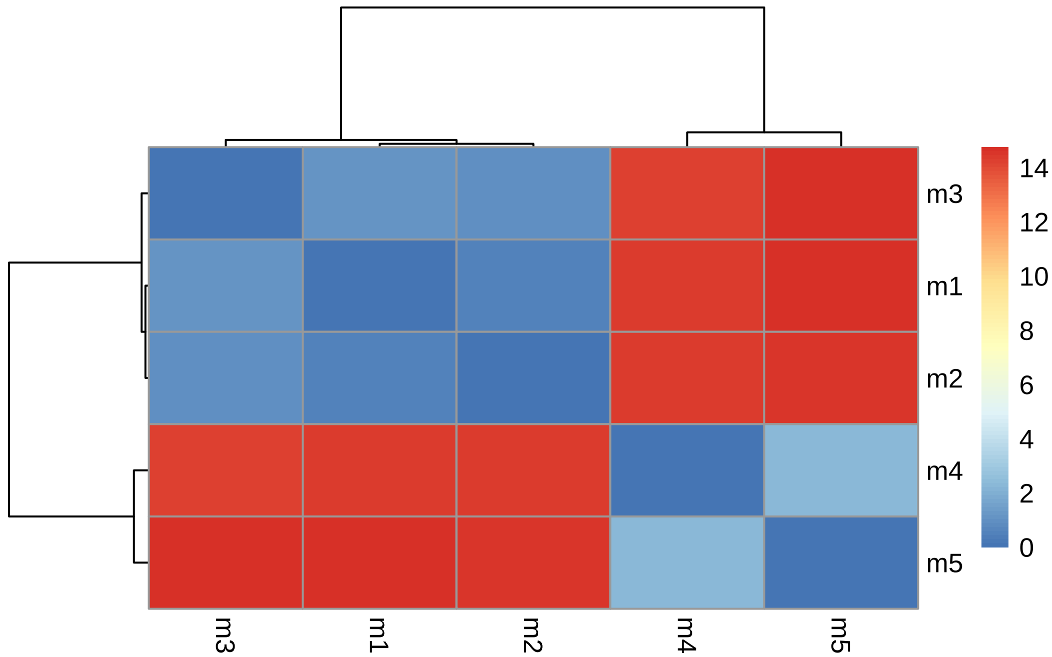
```
range(rd)
```

```
[1]  0.000 14.754
```

Draw a heatmap of these RMSD matrix values

```
library(pheatmap)

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



Here we can see that 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.

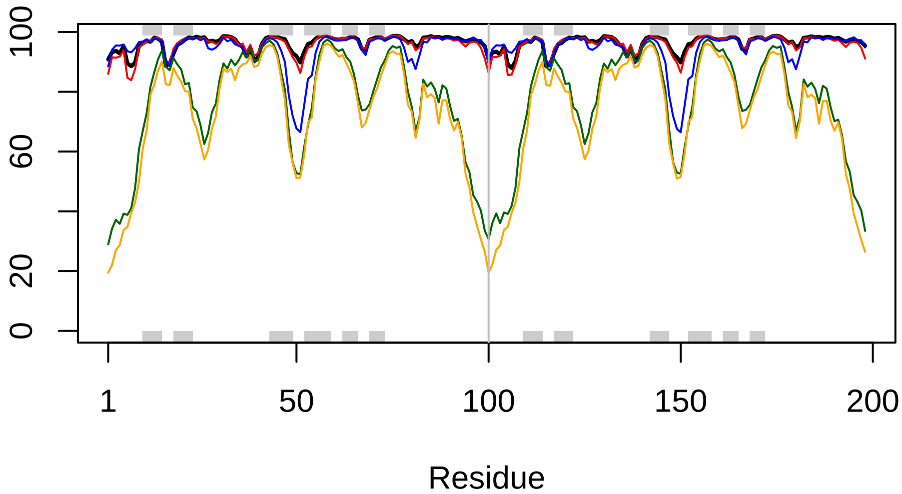
Now lets plot the pLDDT values across all models. Recall that this information is in the B-factor column of each model and that this is stored in our aligned pdbs object as pdbs$b with a row per structure/model.

```
# 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:

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

```
 core size 197 of 198  vol = 9836.196
 core size 196 of 198  vol = 6800.793
 core size 195 of 198  vol = 1322.091
 core size 194 of 198  vol = 1032.249
 core size 193 of 198  vol = 944.133
 core size 192 of 198  vol = 894.048
 core size 191 of 198  vol = 829.724
 core size 190 of 198  vol = 766.628
 core size 189 of 198  vol = 728.681
 core size 188 of 198  vol = 693.317
 core size 187 of 198  vol = 656.166
 core size 186 of 198  vol = 622.063
 core size 185 of 198  vol = 586.601
 core size 184 of 198  vol = 565.358
 core size 183 of 198  vol = 532.747
 core size 182 of 198  vol = 511.421
 core size 181 of 198  vol = 489.077
 core size 180 of 198  vol = 468.599
```

```
core size 179 of 198  vol = 449.222
core size 178 of 198  vol = 433.122
core size 177 of 198  vol = 418.651
core size 176 of 198  vol = 405.069
core size 175 of 198  vol = 391.855
core size 174 of 198  vol = 381.006
core size 173 of 198  vol = 371.54
core size 172 of 198  vol = 355.539
core size 171 of 198  vol = 345.156
core size 170 of 198  vol = 335.982
core size 169 of 198  vol = 325.275
core size 168 of 198  vol = 313.603
core size 167 of 198  vol = 302.908
core size 166 of 198  vol = 293.343
core size 165 of 198  vol = 284.547
core size 164 of 198  vol = 277.932
core size 163 of 198  vol = 269.592
core size 162 of 198  vol = 258.167
core size 161 of 198  vol = 246.856
core size 160 of 198  vol = 239.073
core size 159 of 198  vol = 234.174
core size 158 of 198  vol = 226.309
core size 157 of 198  vol = 221.212
core size 156 of 198  vol = 214.951
core size 155 of 198  vol = 206.027
core size 154 of 198  vol = 197.301
core size 153 of 198  vol = 190.889
core size 152 of 198  vol = 185.403
core size 151 of 198  vol = 179.154
core size 150 of 198  vol = 174.709
core size 149 of 198  vol = 167.269
core size 148 of 198  vol = 160.866
core size 147 of 198  vol = 156.173
core size 146 of 198  vol = 148.395
core size 145 of 198  vol = 143.389
core size 144 of 198  vol = 138.428
core size 143 of 198  vol = 132.99
core size 142 of 198  vol = 126.924
core size 141 of 198  vol = 121.257
core size 140 of 198  vol = 116.407
core size 139 of 198  vol = 112.241
core size 138 of 198  vol = 107.813
core size 137 of 198  vol = 104.807
```

```
core size 136 of 198  vol = 100.968
core size 135 of 198  vol = 97.21
core size 134 of 198  vol = 92.54
core size 133 of 198  vol = 87.964
core size 132 of 198  vol = 85.621
core size 131 of 198  vol = 81.514
core size 130 of 198  vol = 77.627
core size 129 of 198  vol = 74.881
core size 128 of 198  vol = 72.642
core size 127 of 198  vol = 70.352
core size 126 of 198  vol = 68.627
core size 125 of 198  vol = 66.349
core size 124 of 198  vol = 64.019
core size 123 of 198  vol = 61.763
core size 122 of 198  vol = 58.758
core size 121 of 198  vol = 56.354
core size 120 of 198  vol = 54.129
core size 119 of 198  vol = 51.611
core size 118 of 198  vol = 49.502
core size 117 of 198  vol = 48.045
core size 116 of 198  vol = 46.489
core size 115 of 198  vol = 44.599
core size 114 of 198  vol = 43.185
core size 113 of 198  vol = 40.978
core size 112 of 198  vol = 39.029
core size 111 of 198  vol = 36.363
core size 110 of 198  vol = 33.953
core size 109 of 198  vol = 31.343
core size 108 of 198  vol = 29.304
core size 107 of 198  vol = 27.188
core size 106 of 198  vol = 25.655
core size 105 of 198  vol = 24.03
core size 104 of 198  vol = 22.504
core size 103 of 198  vol = 20.906
core size 102 of 198  vol = 19.803
core size 101 of 198  vol = 18.215
core size 100 of 198  vol = 15.652
core size 99 of 198  vol = 14.762
core size 98 of 198  vol = 11.574
core size 97 of 198  vol = 9.391
core size 96 of 198  vol = 7.327
core size 95 of 198  vol = 6.123
core size 94 of 198  vol = 5.592
```

```
 core size 93 of 198  vol = 4.689
 core size 92 of 198  vol = 3.66
 core size 91 of 198  vol = 2.779
 core size 90 of 198  vol = 2.147
 core size 89 of 198  vol = 1.725
 core size 88 of 198  vol = 1.158
 core size 87 of 198  vol = 0.88
 core size 86 of 198  vol = 0.682
 core size 85 of 198  vol = 0.527
 core size 84 of 198  vol = 0.37
 FINISHED: Min vol ( 0.5 ) reached
```

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

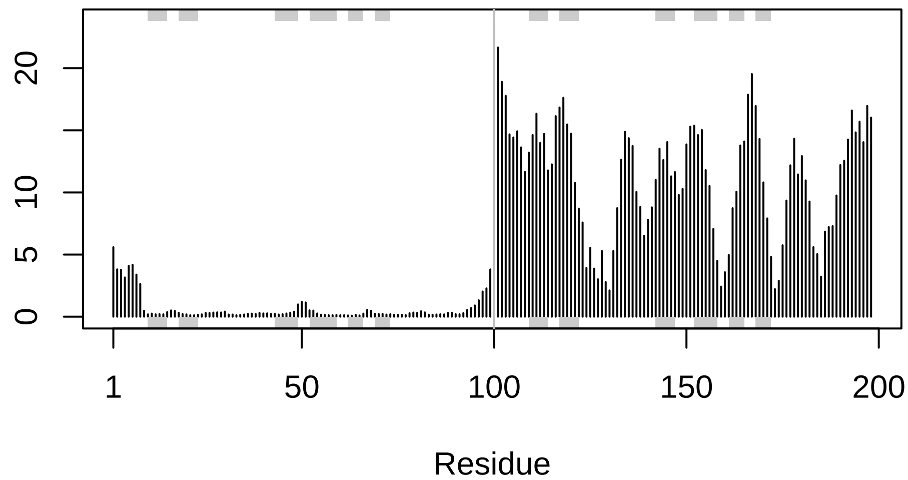
```
# 85 positions (cumulative volume <= 0.5 Angstrom^3)
  start end length
1     9  49     41
2    52  95     44
```

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

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")
```



Here we see that the first chain is largely very similar across the different models. However, the second chain is much more variable - we saw this in Mol* previously

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 4.5.2
```

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

```r
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"
```

```r
# 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
```

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 their max PAE scores?

```r
pae1$max_pae
```

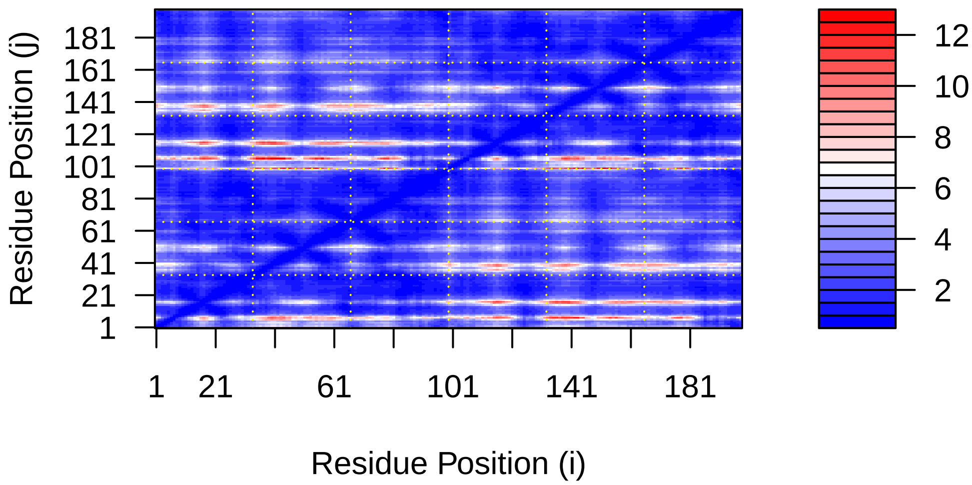
```
[1] 12.84375
```

```r
pae5$max_pae
```

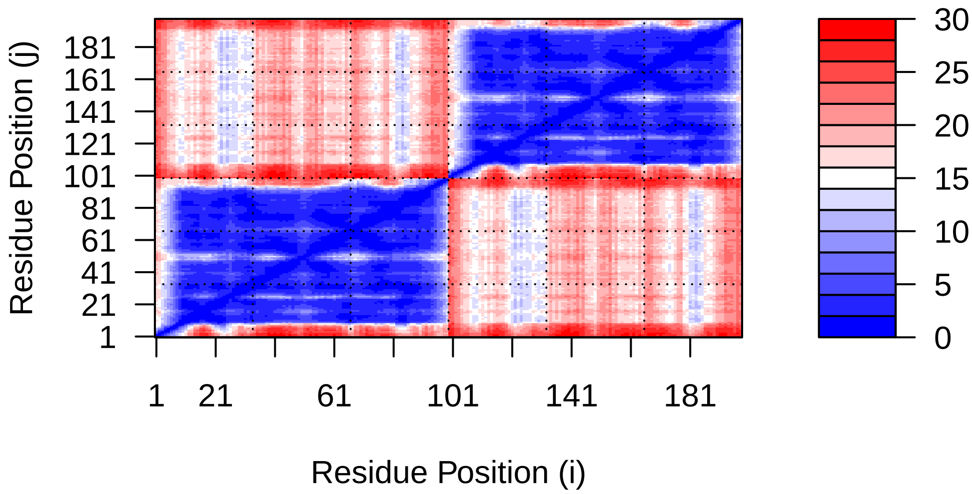
```
[1] 29.59375
```

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:

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

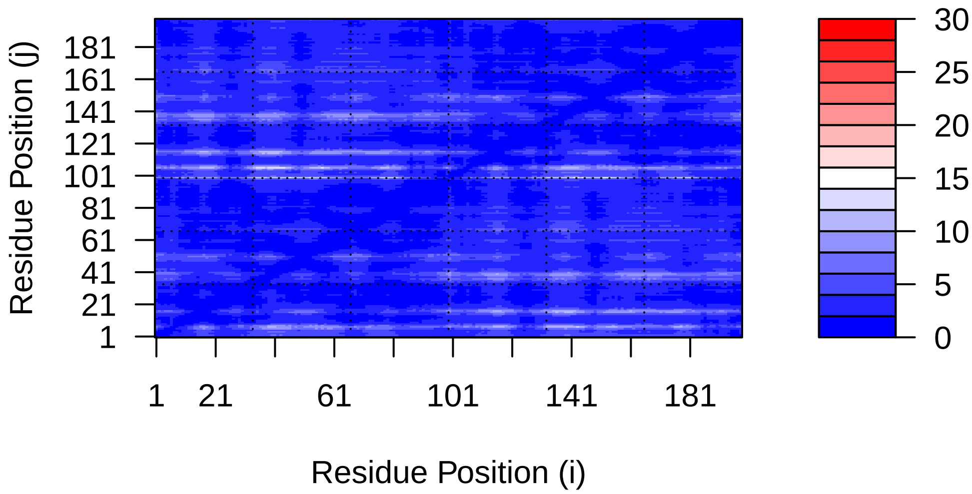



```
plot.dmat(pae5$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)",
          grid.col = "black",
          zlim=c(0,30))
```



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_0.result/HIVPR_dimer_23119_0/HIVPR_dimer_23119_0.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

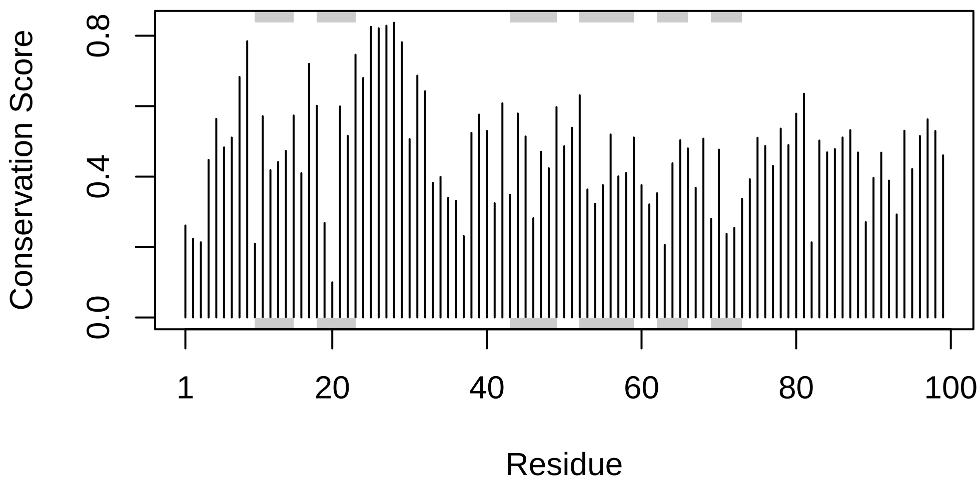
```
dim(aln$ali)
```

```
[1] 5397  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")
```



Note the conserved Active Site residues D25, T26, G27, A28. These positions will stand out if we generate a consensus sequence with a high cutoff value:

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

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

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")
```