Structural Bioinformatics (pt2)

Irene Hsieh (A16197563)

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

In particular we used their AlphaFold2_mmseqs2 version that uses mmseq2 rather than HMMMer for sequence search

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

Lets start by loading these structures up in Mol*

```
library(bio3d)
# Change this for YOUR results dir name
results_dir <- "hivpr1dimer_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] "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"
```

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

```
Reading PDB files:
hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_0
hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_0
hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_0
hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_0
hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_0
.....

Extracting sequences

pdb/seq: 1   name: hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_001_alphafold2_multime
pdb/seq: 2   name: hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_002_alphafold2_multime
pdb/seq: 3   name: hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_003_alphafold2_multime
pdb/seq: 4   name: hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_004_alphafold2_multime
pdb/seq: 5   name: hivpr1dimer_23119//hivpr1dimer_23119_unrelaxed_rank_005_alphafold2_multime
```

```
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
```

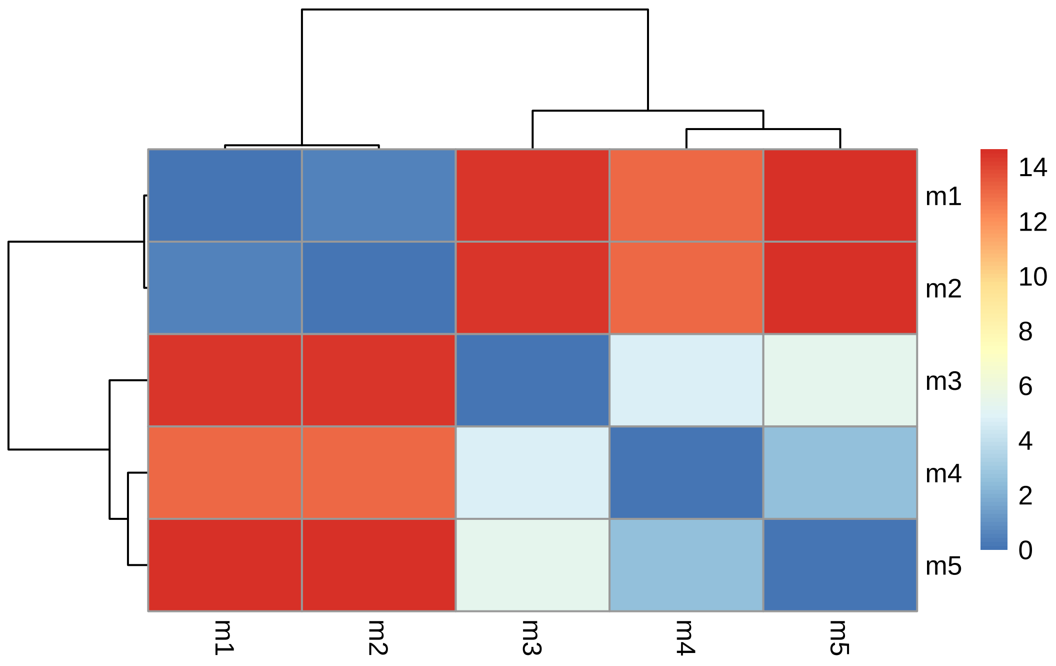
```r
rd <- rmsd(pdbs, fit=T)
```

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

```r
range(rd)
```

```
[1]  0.000 14.631
```

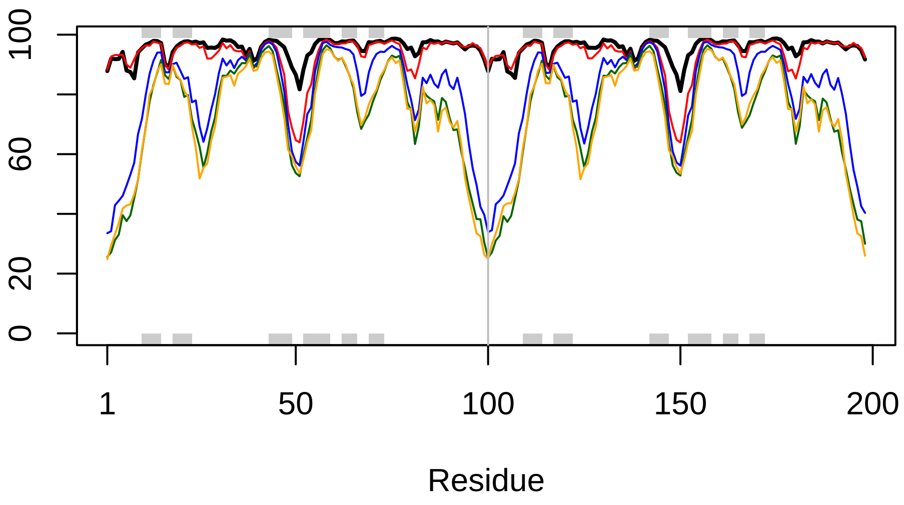
```r
library(pheatmap)

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



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

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

```
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")
```



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

```
core size 197 of 198  vol = 4578.352
core size 196 of 198  vol = 3931.113
core size 195 of 198  vol = 3709.737
core size 194 of 198  vol = 3496.023
core size 193 of 198  vol = 3302.436
core size 192 of 198  vol = 3146.478
core size 191 of 198  vol = 3048.969
core size 190 of 198  vol = 2970.358
core size 189 of 198  vol = 2893.016
core size 188 of 198  vol = 2831.829
core size 187 of 198  vol = 2774.51
core size 186 of 198  vol = 2728.049
core size 185 of 198  vol = 2704.953
core size 184 of 198  vol = 2701.988
core size 183 of 198  vol = 2715.915
core size 182 of 198  vol = 2809.862
core size 181 of 198  vol = 2888.961
core size 180 of 198  vol = 2967.296
core size 179 of 198  vol = 3036.25
core size 178 of 198  vol = 3066.28
core size 177 of 198  vol = 3096.829
core size 176 of 198  vol = 3056.412
core size 175 of 198  vol = 3014.767
core size 174 of 198  vol = 2975.012
core size 173 of 198  vol = 2898.05
core size 172 of 198  vol = 2810.173
core size 171 of 198  vol = 2747.532
core size 170 of 198  vol = 2684.434
core size 169 of 198  vol = 2620.353
core size 168 of 198  vol = 2550.877
core size 167 of 198  vol = 2492.582
core size 166 of 198  vol = 2422.978
core size 165 of 198  vol = 2358.916
core size 164 of 198  vol = 2298.292
core size 163 of 198  vol = 2235.918
core size 162 of 198  vol = 2171.02
core size 161 of 198  vol = 2093.559
core size 160 of 198  vol = 2029.144
core size 159 of 198  vol = 1950.957
core size 158 of 198  vol = 1881.015
core size 157 of 198  vol = 1801.506
core size 156 of 198  vol = 1728.892
core size 155 of 198  vol = 1660.037
core size 154 of 198  vol = 1586.149
core size 153 of 198  vol = 1532.718
core size 152 of 198  vol = 1460.186
core size 151 of 198  vol = 1399.251
core size 150 of 198  vol = 1333.908
core size 149 of 198  vol = 1271.747
core size 148 of 198  vol = 1219.496
core size 147 of 198  vol = 1176.003
core size 146 of 198  vol = 1138.478
core size 145 of 198  vol = 1102.124
core size 144 of 198  vol = 1049.642
core size 143 of 198  vol = 1014.063
core size 142 of 198  vol = 970.575
core size 141 of 198  vol = 929.178
core size 140 of 198  vol = 889.104
core size 139 of 198  vol = 846.668
core size 138 of 198  vol = 805.8
core size 137 of 198  vol = 775.034
core size 136 of 198  vol = 743.09
core size 135 of 198  vol = 715.695
core size 134 of 198  vol = 689.788
core size 133 of 198  vol = 660.329
core size 132 of 198  vol = 630.966
core size 131 of 198  vol = 597.207
core size 130 of 198  vol = 566.989
core size 129 of 198  vol = 532.89
core size 128 of 198  vol = 496.208
core size 127 of 198  vol = 463.183
core size 126 of 198  vol = 431.893
core size 125 of 198  vol = 408.864
core size 124 of 198  vol = 376.61
core size 123 of 198  vol = 362.377
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
```

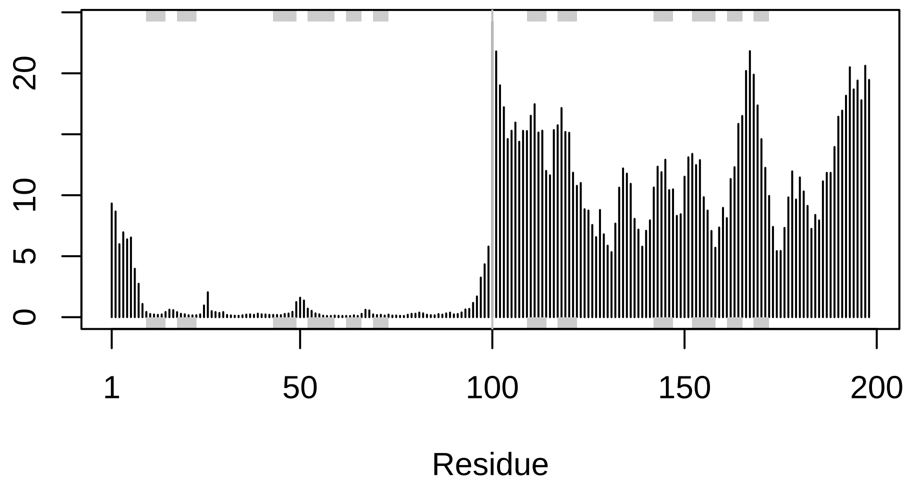
```
 core size 91 of 198  vol = 7.35
 core size 90 of 198  vol = 4.922
 core size 89 of 198  vol = 3.421
 core size 88 of 198  vol = 2.553
 core size 87 of 198  vol = 1.917
 core size 86 of 198  vol = 1.513
 core size 85 of 198  vol = 1.201
 core size 84 of 198  vol = 1.046
 core size 83 of 198  vol = 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")
```

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

```r
pae1$max_pae
```

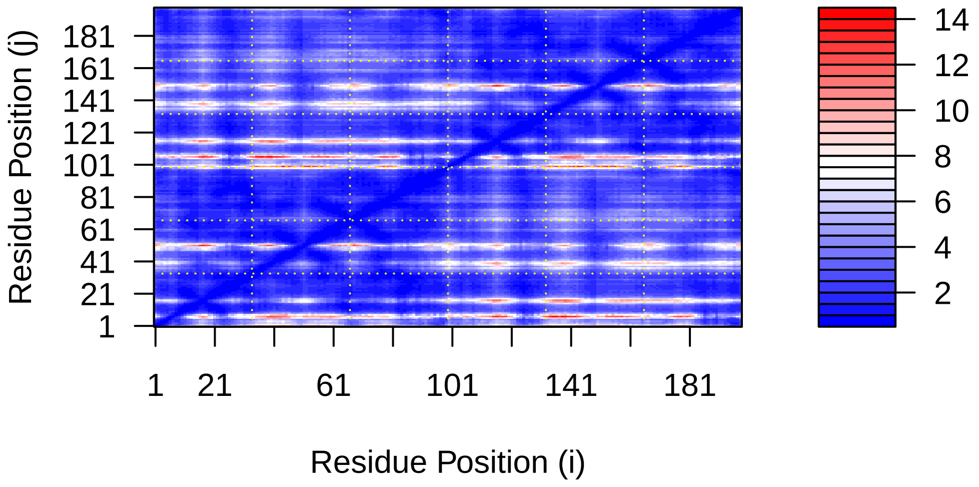
```
[1] 14.09375
```

```r
pae5$max_pae
```

```
[1] 29.29688
```

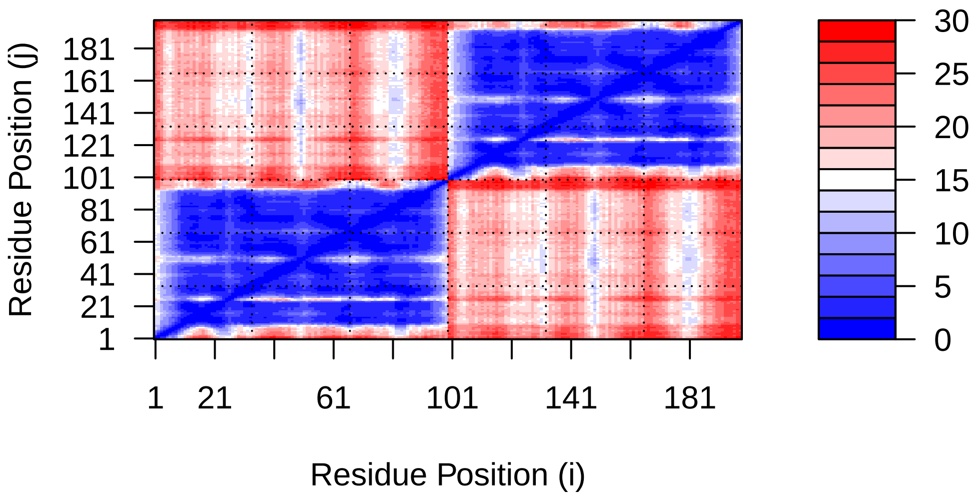
For model 1:

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



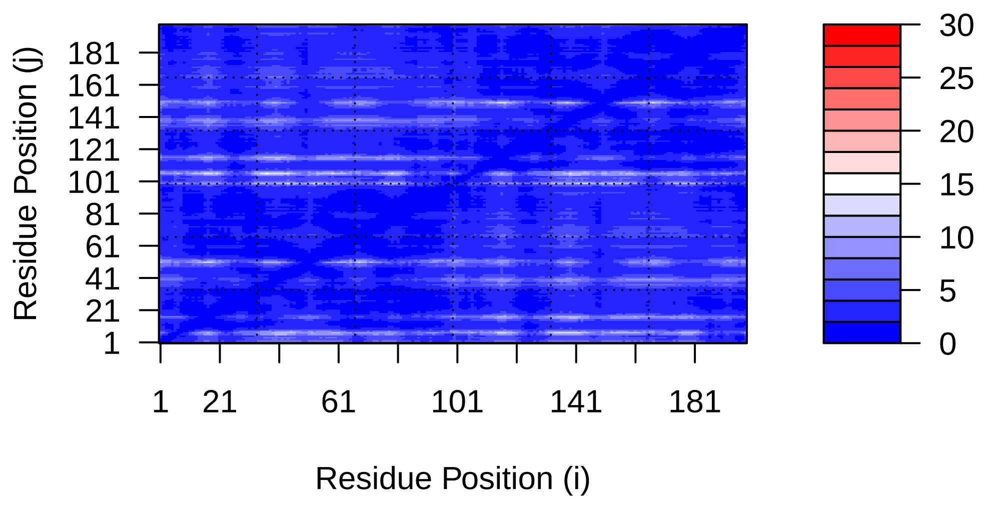
For model 5:

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



Make model 1 With the same z range of 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] "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 **"
```

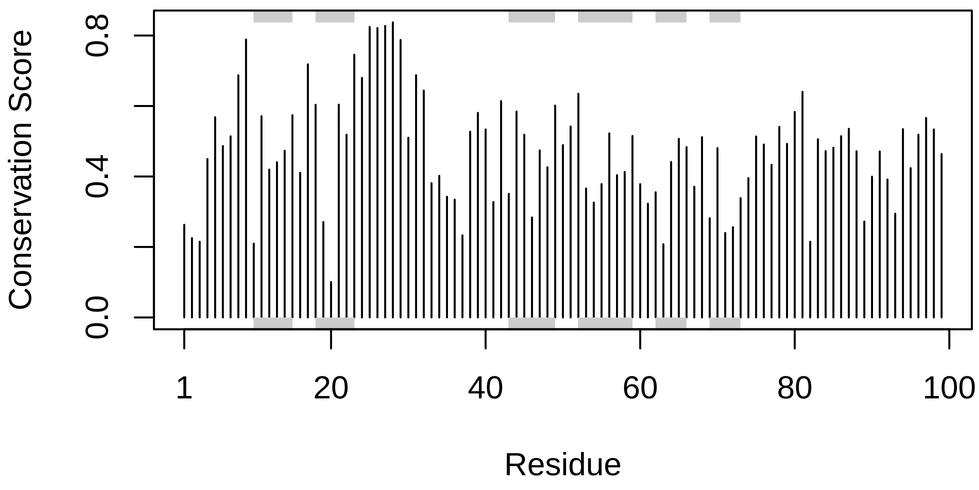
How many sequences are in this alignment

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

```
[1] 5378  132
```

Score residue conservation in the alignment

```r
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

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

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

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")
```