CLASS11 ALPHAFOLD

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Background

In this hands-on session we will utilize AlphaFold to predict protein structure from sequence (Jumper et al. 2021).

Without the aid of such approaches, it can take years of expensive laboratory work to determine the structure of just one protein. With AlphaFold we can now accurately compute a typical protein structure in as little as ten minutes.

The PDB database (the main repository of experinmental structures) only has ~ 250 thousand structures (we saw this in the last lab). The main protein sequence database has over **200 million** sequences! Only 0.125% of known sequences have a known structure ~ this is called the "structure knowledge gap".

```
250000 / 200000000*100
```

```
[1] 0.125
```

- Structures are much harder to determine than sequences
- They are expensive (on avaerage ~ $1million each)
- They take on average 3-5 years to solve!

EBI AlphaFold Database

The EBI has a database of pre-computed AlphaFold(AF) models called AFDB. This is growing all the time and can be useful to check before running AF ourselves.

Running AlphaFold

We can download and run locally (on our own computers) but we need a GPU. Or we can use "cloud" computing to run this on someone elses computers :-)

We will use ColabFold https://github.com/sokrypton/ColabFold

We previously found there was no AFDB entry for our HIV sequence:

```
>HIV-Pr-Dimer
PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQ
```

Here will use AlphaFold2_mmseqs2 (https://colab.research.google.com/github/sokrypton/ColabFold/blob/mai

Interpreting Results

Figure 1: Superposed image HIVPR

Q. Can you identify the most variable regions by eye?

Yes, the most variable regions are the ones with lots of gaps/substitutions and where the sequences do not align well.

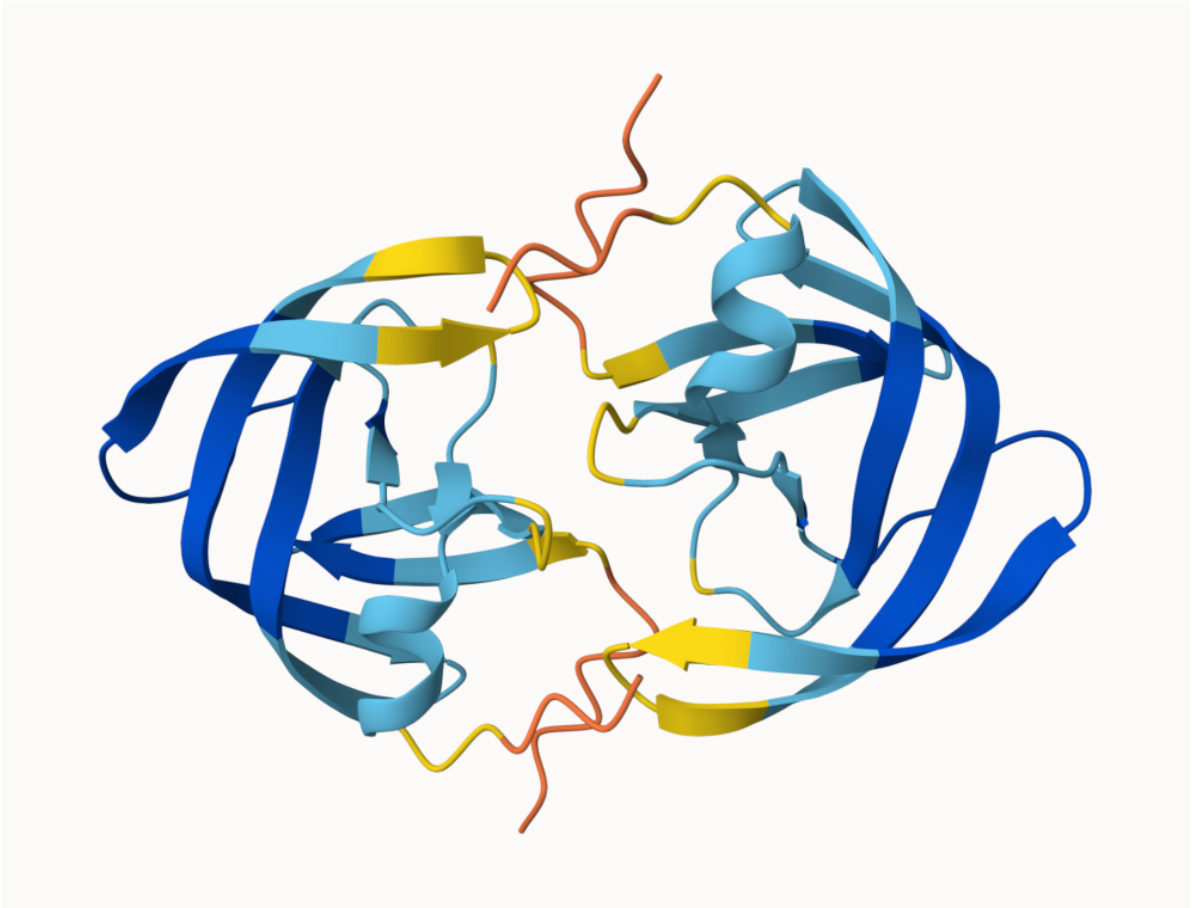


Figure 2: Superposed structures

Figure 3: Coloring by uncertainty disorder

Custom analysis of resulting models

```
results_dir <- "HIVpr_23119"
```

File name for all PDB models

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

basename(pdb_files)
```

```
[1] "HIVpr_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_4_seed_000.pdb"
[2] "HIVpr_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_1_seed_000.pdb"
[3] "HIVpr_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_5_seed_000.pdb"
[4] "HIVpr_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.pdb"
[5] "HIVpr_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.pdb"
```

```
library (bio3d)
```

```
Warning: package 'bio3d' was built under R version 4.4.3
```

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

```
Reading PDB files:
HIVpr_23119/HIVpr_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_4_seed_000.pdb
HIVpr_23119/HIVpr_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_1_seed_000.pdb
HIVpr_23119/HIVpr_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_5_seed_000.pdb
HIVpr_23119/HIVpr_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.pdb
HIVpr_23119/HIVpr_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.pdb
.....

Extracting sequences

pdb/seq: 1   name: HIVpr_23119/HIVpr_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_4
pdb/seq: 2   name: HIVpr_23119/HIVpr_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_1
pdb/seq: 3   name: HIVpr_23119/HIVpr_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_5
pdb/seq: 4   name: HIVpr_23119/HIVpr_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2
pdb/seq: 5   name: HIVpr_23119/HIVpr_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3
```

```
pdbs
```

```
                                1        .        .        .        .         50
[Truncated_Name:1]HIVpr_2311   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:2]HIVpr_2311   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:3]HIVpr_2311   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:4]HIVpr_2311   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:5]HIVpr_2311   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
                               **************************************************
                                1        .        .        .        .         50

                               51        .        .        .        .        100
[Truncated_Name:1]HIVpr_2311   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:2]HIVpr_2311   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:3]HIVpr_2311   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:4]HIVpr_2311   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:5]HIVpr_2311   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
                               **************************************************
                               51        .        .        .        .        100

                              101        .        .        .        .        150
[Truncated_Name:1]HIVpr_2311   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:2]HIVpr_2311   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:3]HIVpr_2311   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:4]HIVpr_2311   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:5]HIVpr_2311   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
                               **************************************************
                              101        .        .        .        .        150

                              151        .        .        .        .      198
[Truncated_Name:1]HIVpr_2311   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:2]HIVpr_2311   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:3]HIVpr_2311   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:4]HIVpr_2311   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:5]HIVpr_2311   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
```

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

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

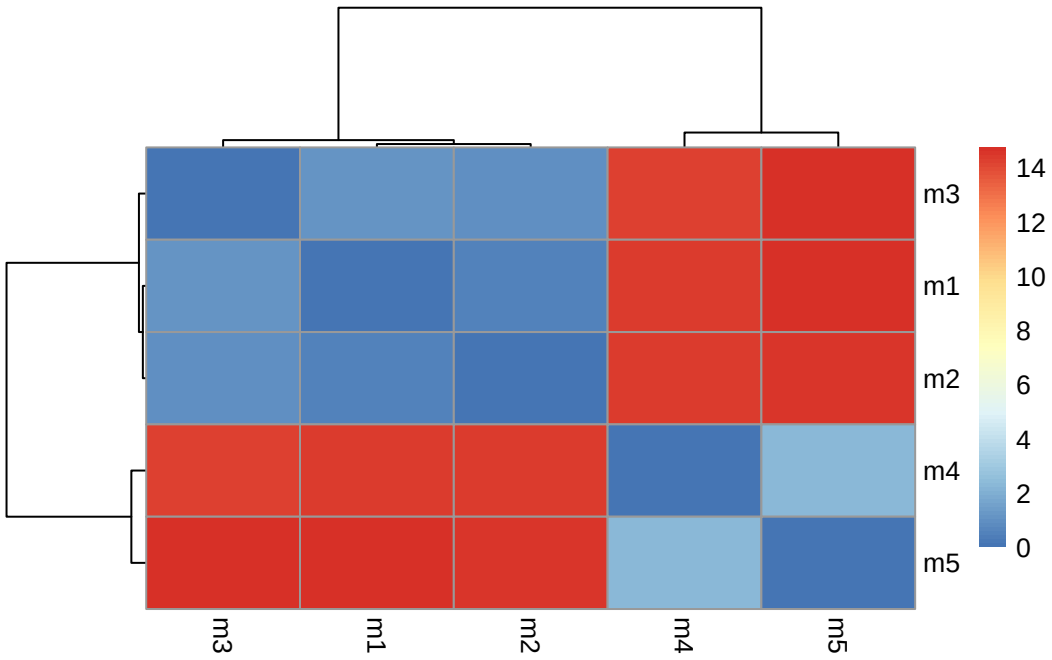
```
range(rd)
```

```
[1]  0.000 14.754
```

```
library(pheatmap)
```

```
Warning: package 'pheatmap' was built under R version 4.4.3
```

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

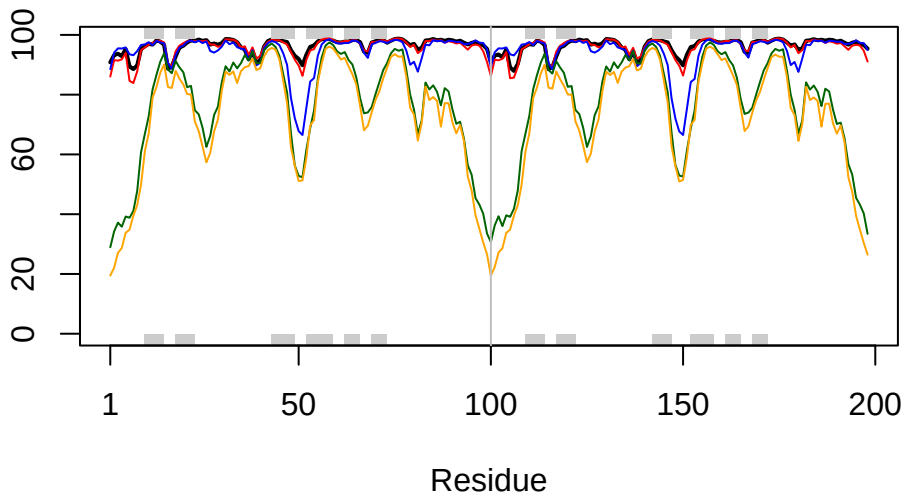


Read a reference PDB structure

```
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")
```




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

```
 core size 197 of 198  vol = 9885.419
 core size 196 of 198  vol = 6898.241
 core size 195 of 198  vol = 1338.035
 core size 194 of 198  vol = 1040.677
 core size 193 of 198  vol = 951.865
```

```
core size 192 of 198  vol = 899.087
core size 191 of 198  vol = 834.733
core size 190 of 198  vol = 771.342
core size 189 of 198  vol = 733.069
core size 188 of 198  vol = 697.285
core size 187 of 198  vol = 659.748
core size 186 of 198  vol = 625.28
core size 185 of 198  vol = 589.548
core size 184 of 198  vol = 568.261
core size 183 of 198  vol = 545.022
core size 182 of 198  vol = 512.897
core size 181 of 198  vol = 490.731
core size 180 of 198  vol = 470.274
core size 179 of 198  vol = 450.738
core size 178 of 198  vol = 434.743
core size 177 of 198  vol = 420.345
core size 176 of 198  vol = 406.666
core size 175 of 198  vol = 393.341
core size 174 of 198  vol = 382.402
core size 173 of 198  vol = 372.866
core size 172 of 198  vol = 357.001
core size 171 of 198  vol = 346.576
core size 170 of 198  vol = 337.454
core size 169 of 198  vol = 326.668
core size 168 of 198  vol = 314.959
core size 167 of 198  vol = 304.136
core size 166 of 198  vol = 294.561
core size 165 of 198  vol = 285.658
core size 164 of 198  vol = 278.893
core size 163 of 198  vol = 266.773
core size 162 of 198  vol = 259.003
core size 161 of 198  vol = 247.731
core size 160 of 198  vol = 239.849
core size 159 of 198  vol = 234.973
core size 158 of 198  vol = 230.071
core size 157 of 198  vol = 221.995
core size 156 of 198  vol = 215.629
core size 155 of 198  vol = 206.8
core size 154 of 198  vol = 196.992
core size 153 of 198  vol = 188.547
core size 152 of 198  vol = 182.27
core size 151 of 198  vol = 176.961
core size 150 of 198  vol = 170.72
```

```
core size 149 of 198  vol = 166.128
core size 148 of 198  vol = 159.805
core size 147 of 198  vol = 153.775
core size 146 of 198  vol = 149.101
core size 145 of 198  vol = 143.664
core size 144 of 198  vol = 137.145
core size 143 of 198  vol = 132.523
core size 142 of 198  vol = 127.237
core size 141 of 198  vol = 121.579
core size 140 of 198  vol = 116.78
core size 139 of 198  vol = 112.575
core size 138 of 198  vol = 108.175
core size 137 of 198  vol = 105.137
core size 136 of 198  vol = 101.254
core size 135 of 198  vol = 97.379
core size 134 of 198  vol = 92.978
core size 133 of 198  vol = 88.188
core size 132 of 198  vol = 84.032
core size 131 of 198  vol = 81.902
core size 130 of 198  vol = 78.023
core size 129 of 198  vol = 75.276
core size 128 of 198  vol = 73.057
core size 127 of 198  vol = 70.699
core size 126 of 198  vol = 68.976
core size 125 of 198  vol = 66.707
core size 124 of 198  vol = 64.376
core size 123 of 198  vol = 61.145
core size 122 of 198  vol = 59.029
core size 121 of 198  vol = 56.625
core size 120 of 198  vol = 54.369
core size 119 of 198  vol = 51.826
core size 118 of 198  vol = 49.651
core size 117 of 198  vol = 48.19
core size 116 of 198  vol = 46.644
core size 115 of 198  vol = 44.748
core size 114 of 198  vol = 43.288
core size 113 of 198  vol = 41.089
core size 112 of 198  vol = 39.143
core size 111 of 198  vol = 36.468
core size 110 of 198  vol = 34.114
core size 109 of 198  vol = 31.467
core size 108 of 198  vol = 29.445
core size 107 of 198  vol = 27.323
```

```
 core size 106 of 198  vol = 25.82
 core size 105 of 198  vol = 24.149
 core size 104 of 198  vol = 22.647
 core size 103 of 198  vol = 21.068
 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 = 9.435
 core size 96 of 198  vol = 7.354
 core size 95 of 198  vol = 6.181
 core size 94 of 198  vol = 5.667
 core size 93 of 198  vol = 4.706
 core size 92 of 198  vol = 3.664
 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")
```

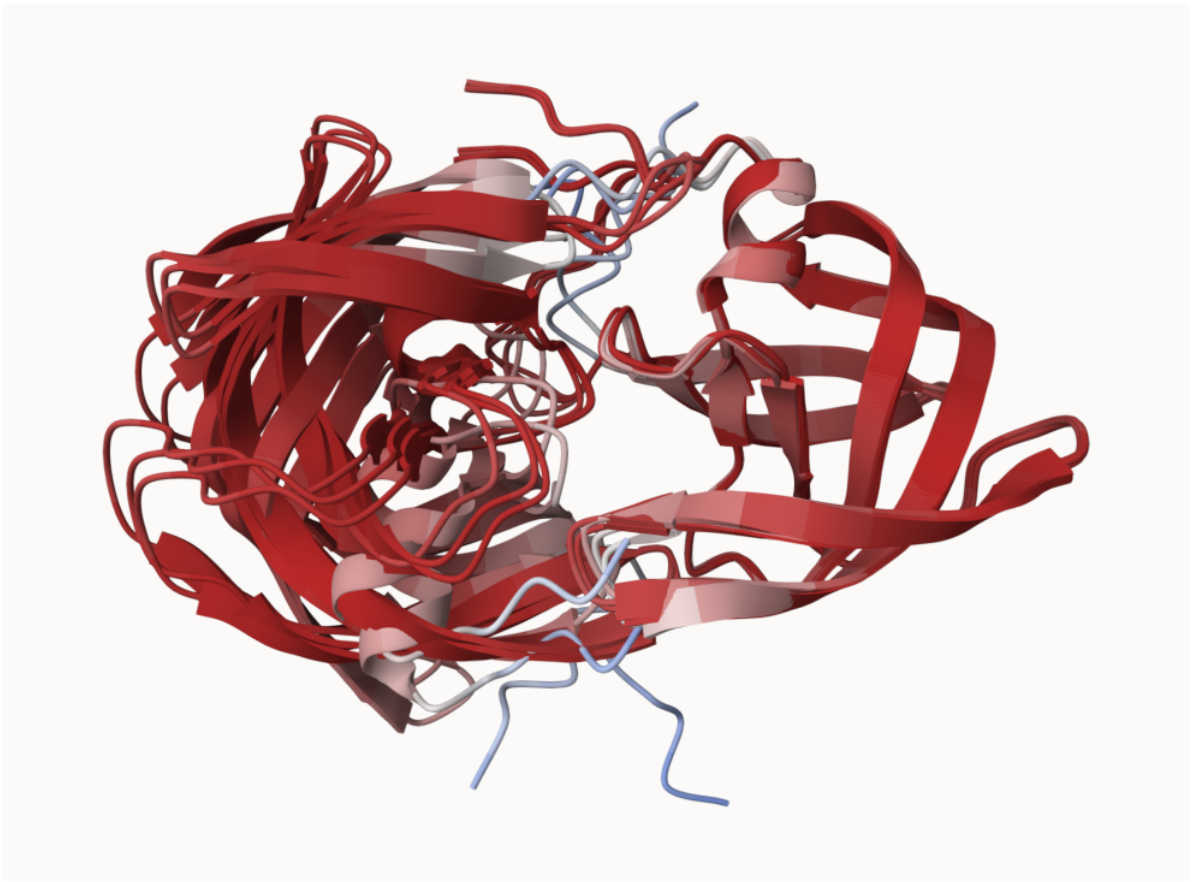
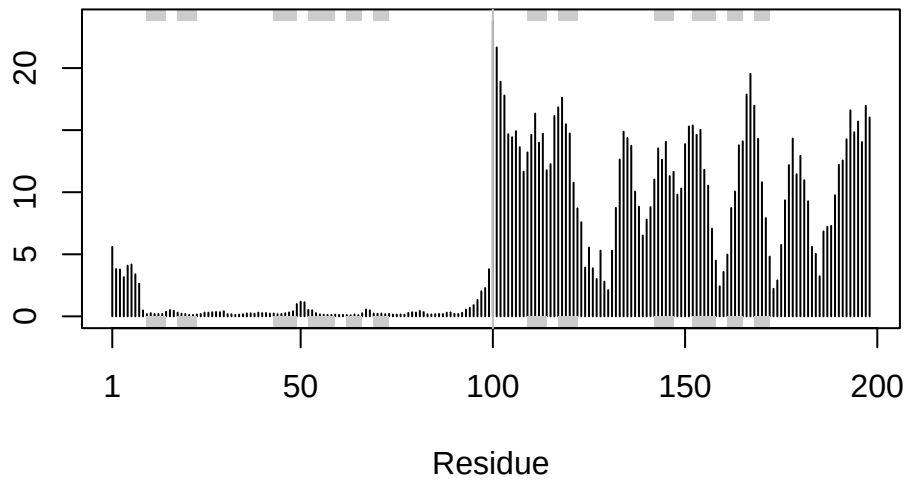


Figure 4: superposed structures colored by B-factor

```
rf <- rmsf(xyz)

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



Predicted Alignment Error for domains

```
library(jsonlite)
```

```
Warning: package 'jsonlite' was built under R version 4.4.3
```

```
# 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
```

```
pae1$max_pae
```

```
[1] 12.84375
```

```
pae5$max_pae
```

```
[1] 29.59375
```

```
pae2 <- read_json(pae_files[2],simplifyVector = TRUE)
pae3 <- read_json(pae_files[3],simplifyVector = TRUE)
pae4 <- read_json(pae_files[4],simplifyVector = TRUE)
```

```
pae2$max_pae
```

```
[1] 20.07812
```

```
pae3$max_pae
```

```
[1] 16.09375
```

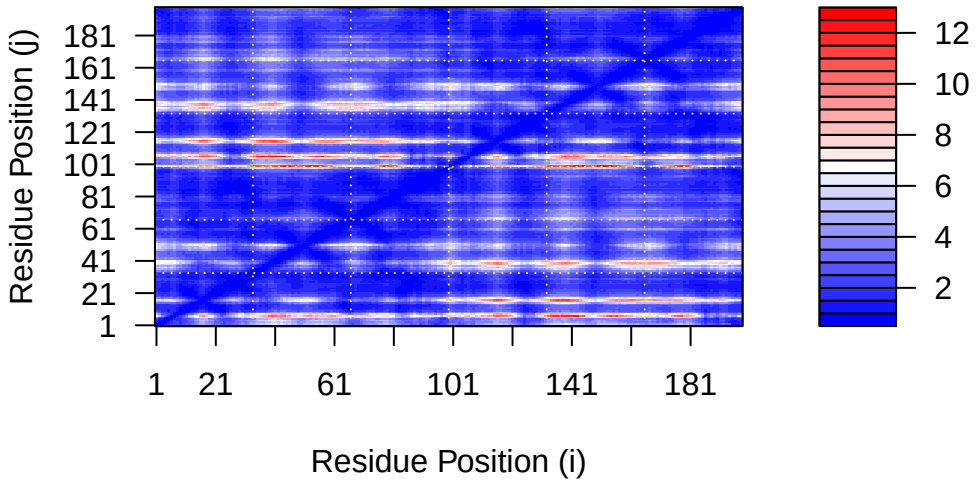
```
pae4$max_pae
```

```
[1] 29.60938
```

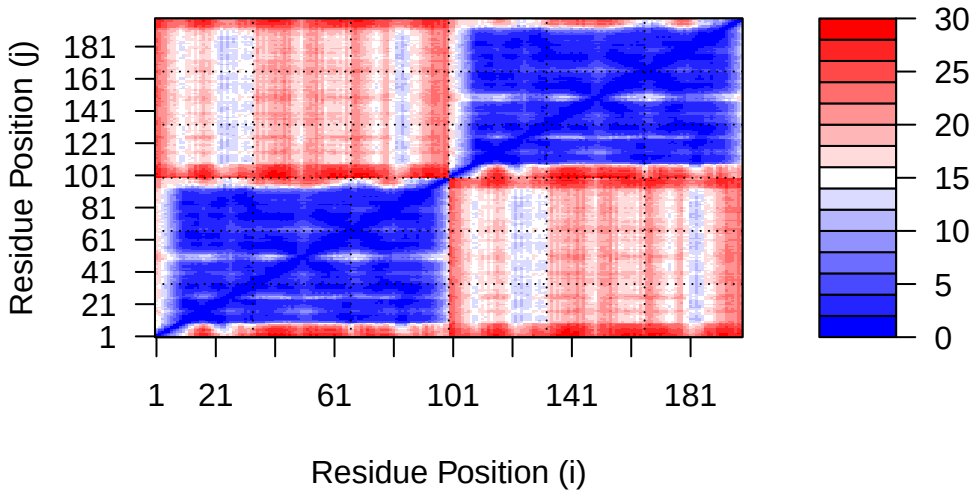
Q. What are the max PAE score for the models?

The max PAE score for model 2 is 20.08. The max PAE score for model 3 is 16.09. The max PAE score for model 4 is 29.61.

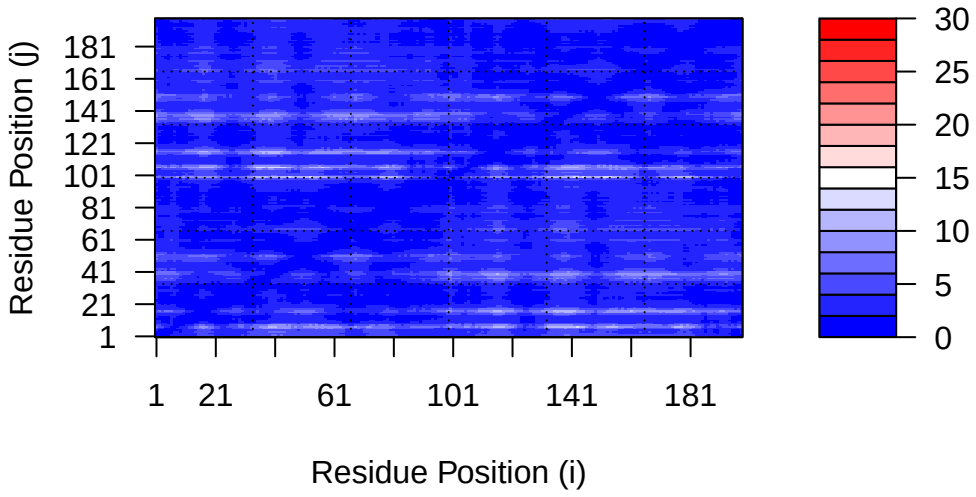
```
plot.dmat(pae1$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)")
```




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




```
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_23119/HIVpr_23119.a3m"
```

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

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

Q. How many sequences are in this alignment?

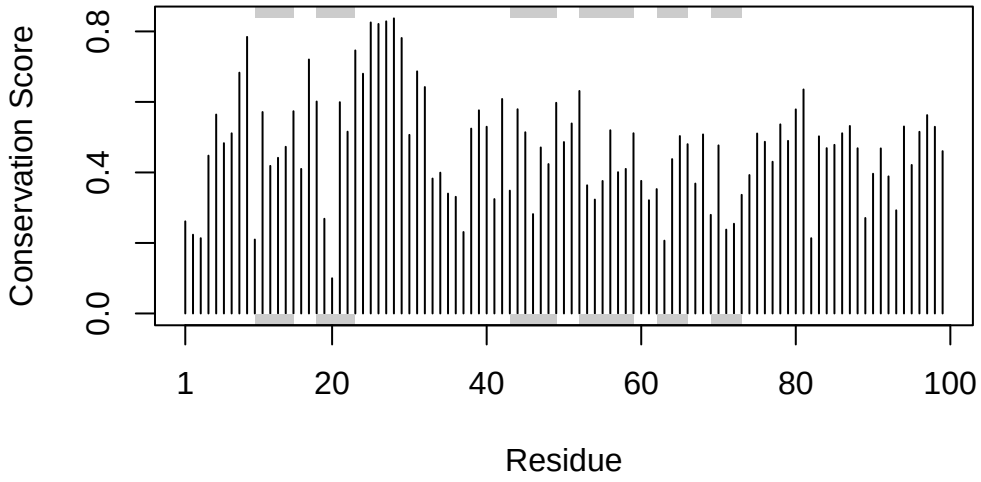
There are 5397 sequences in this alignment.

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

```
[1] 5397  132
```

```
sim <- conserv(aln)
```

```
plotb3(sim[1:99], sse=trim.pdb(pdb, chain="A"),
       ylab="Conservation Score")
```




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

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

```
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")
```