Class 11: Alpha Fold

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#Alpha Fold

AlphaFold is a new bioinformatics tool developed by DeepMind that uses AI to predict protein structure - with high reliability!

We can run AlphaFold on our own computers by installing it or we can run on GoogleColab (without needing to install anything) via: https://github.com/sokrypton/ColabFold. To run locally, it is simply a matter of copying the code from above and putting it directly into R.

If you run the it on Google Colab, you will get a zip folder. Make sure to move it into your project folder to make it accessible.

Since it is a folder, we need to be able to read it in. Use below:

```
#Read in name of folder
pth <- "HIV1dimer_23119/"

list.files(path=pth)
```

```
 [1] "cite.bibtex"
 [2] "config.json"
 [3] "HIV1dimer_23119_coverage.png"
 [4] "HIV1dimer_23119_env"
 [5] "HIV1dimer_23119_pae.png"
 [6] "HIV1dimer_23119_plddt.png"
 [7] "HIV1dimer_23119_predicted_aligned_error_v1.json"
 [8] "HIV1dimer_23119_scores_rank_001_alphafold2_multimer_v3_model_1_seed_000.json"
 [9] "HIV1dimer_23119_scores_rank_002_alphafold2_multimer_v3_model_5_seed_000.json"
[10] "HIV1dimer_23119_scores_rank_003_alphafold2_multimer_v3_model_4_seed_000.json"
[11] "HIV1dimer_23119_scores_rank_004_alphafold2_multimer_v3_model_2_seed_000.json"
[12] "HIV1dimer_23119_scores_rank_005_alphafold2_multimer_v3_model_3_seed_000.json"
[13] "HIV1dimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_000.pdb"
[14] "HIV1dimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_000.pdb"
```

```
[15] "HIV1dimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_000.pdb"
[16] "HIV1dimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.pdb"
[17] "HIV1dimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.pdb"
[18] "HIV1dimer_23119.a3m"
[19] "HIV1dimer_23119.csv"
[20] "HIV1dimer_23119.done.txt"
[21] "log.txt"
```

The .a3m file is your multiple sequence alignment (MSA) file in the AlphaFold output.

Now we want to return a single file or file of interest. The `list.files()` function has an argument `pattern =` that we can use to do so. To get the full path and be able to actually open file, you need to call or find the path. Use another argument `full.names =` and set as `TRUE`.

```r
aln.file <- list.files(path=pth, pattern = ".a3m", full.names = TRUE)
```

```r
library(bio3d)
# to.upper arguments converts all the letters to uppercase.
aln <- read.fasta(aln.file, to.upper = TRUE)
```

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

```r
attributes(aln)
```

```
$names
[1] "id"   "ali"  "call"

$class
[1] "fasta"
```

This is a big alignment / large FASTA file. Not worth printing. So use dim to get overall picture.

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

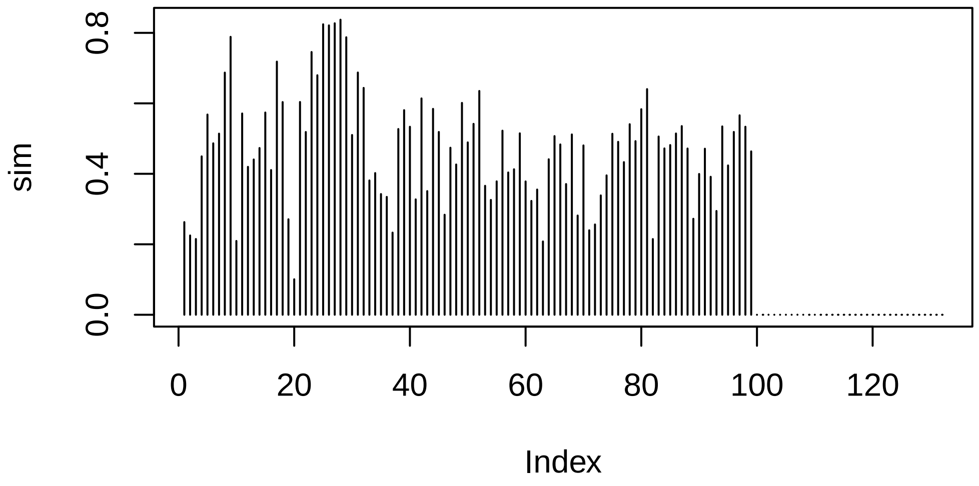
```
[1] 5378  132
```

Lets calculate sum summary info such as conservation scores.

```r
#conserv is a function within the Bio3D package.
sim <- conserv(aln)
```

Highest values (1) shows the location of conservation of amino acids.

```r
plot(sim, typ = "h")
```



We can summarize the conserved column (the ones with high scores above) via a consensus sequence.

```r
#default cutoff is 0.6, which might be too low. There are multiple outputs, so call just t
consensus(aln, cutoff = 0.9)$seq
```

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

Read all out structure files into R

Read the PAE (predicted alignment error files) into R to make sense of the different multichain models. These are stored in the .json files.

The PAE from AlphaFold is a metric for the reliability of protein structure predictions.

```r
library(jsonlite)
```

Now we need to find out json files. There are multiple json files so make sure you are reading into the right ones. We will cheat below but you can use this syntax to modify and call files `.*model.*\\.json` where the `*` means anything before.

```r
pae.files<- list.files(path=pth, pattern = "000.json", full.names = TRUE)
```

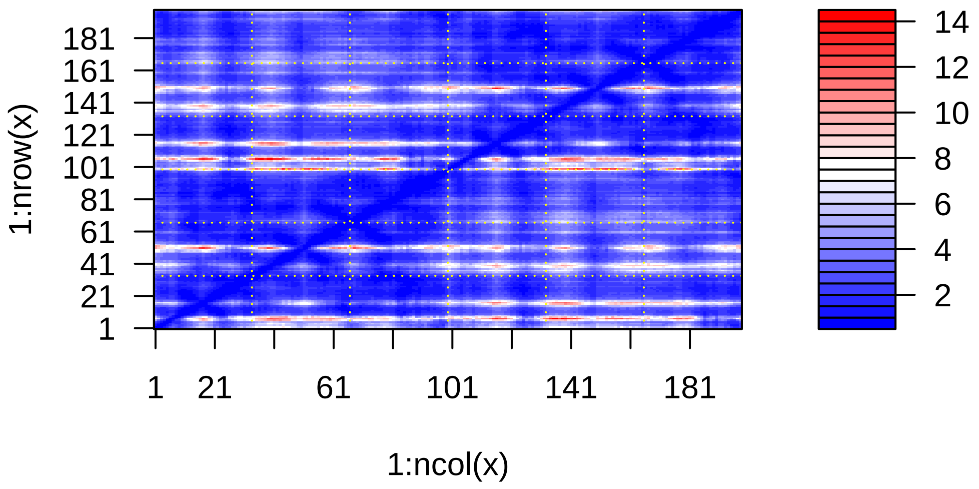
```r
pae5 <- read_json(pae.files[5], simplifyVector = TRUE)
pae1 <- read_json(pae.files[1], simplifyVector = TRUE)
```

```r
dim(pae1$pae)
```

```
[1] 198 198
```

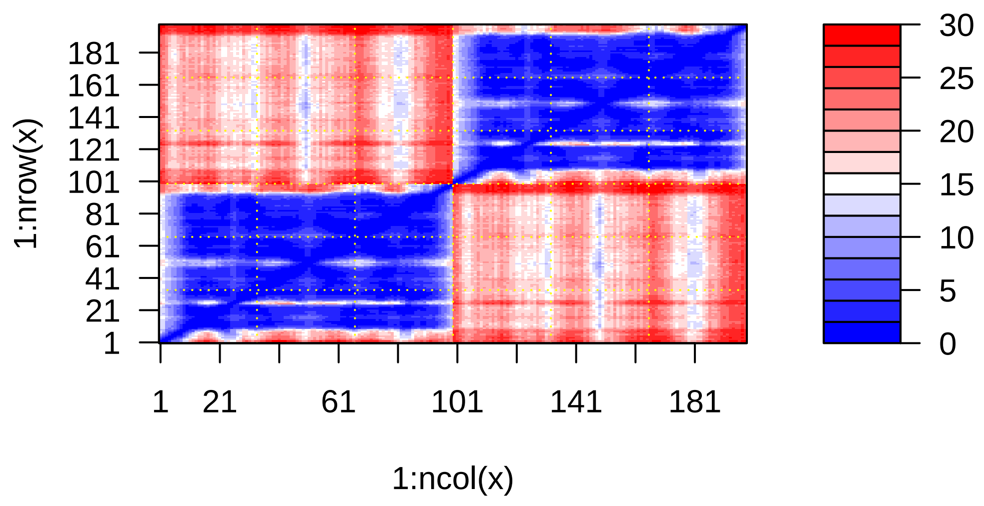
This is a good predicted alignment. Low PAE score.

```r
plot.dmat(pae1$pae)
```

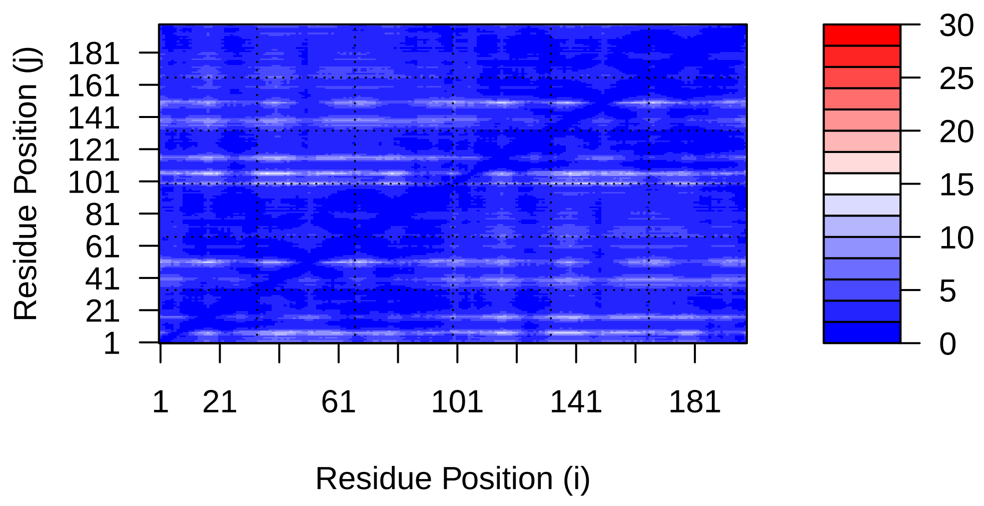


This is a bad PAE score as shown with red and blue portions in graph.

```
plot.dmat(pae5$pae)
```



```
plot.dmat(pae1$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)",
          grid.col = "black",
          zlim=c(0,30))
```



To visualize the predicted structures like you would in Molstar, we can customize the parameters here like we would there.

In this section we will read the results of the more complicated HIV-Pr dimer AlphaFold2 models into R with the help of the `Bio3D` package. You can do the same thing for the monomer models if you wish but again they will be less interesting as the monomer is not physiologically relevant.

For tidiness we can move our AlphaFold results directory into our RStudio project directory. In this example my results are in the directory `pth` (set above). You should change this to match your directory/folder name.

```
# Change this for YOUR results dir name
# results_dir <- "hivprdimer_23119/" this was already written above

pth
```

```
[1] "HIV1dimer_23119/"
```

Our visualized predicted structures are stored in the PDB files within the output. So select them as we did above.

```r
# File names for all PDB models
pdb_files <- list.files(path=pth,
                        pattern="*.pdb",
                        full.names = TRUE)

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

```
[1] "HIV1dimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_000.pdb"
[2] "HIV1dimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_000.pdb"
[3] "HIV1dimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_000.pdb"
[4] "HIV1dimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.pdb"
[5] "HIV1dimer_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:
HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_000.
HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_000.
HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_000.
HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.
HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.
.....

Extracting sequences

pdb/seq: 1   name: HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_001_alphafold2_multimer_v
pdb/seq: 2   name: HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_002_alphafold2_multimer_v
pdb/seq: 3   name: HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_003_alphafold2_multimer_v
pdb/seq: 4   name: HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_004_alphafold2_multimer_v
pdb/seq: 5   name: HIV1dimer_23119//HIV1dimer_23119_unrelaxed_rank_005_alphafold2_multimer_v
```

If your `pdbaln()` function gives an error message then you likely do not have the msa package from BioConductor installed correctly. You will need to run `install.packages("BiocManager")` and then `BiocManager::install("msa")` in your console.

A quick view of model sequences - this should be a boring alignment in the sense that all sequences are the same.

```
pdbs
```

```
                                 1        .         .         .         .         50 
[Truncated_Name:1]HIV1dimer_   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:2]HIV1dimer_   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:3]HIV1dimer_   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:4]HIV1dimer_   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
[Truncated_Name:5]HIV1dimer_   PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGI
                               ************************************************** 
                                 1        .         .         .         .         50 

                                51        .         .         .         .         100 
[Truncated_Name:1]HIV1dimer_   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:2]HIV1dimer_   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:3]HIV1dimer_   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:4]HIV1dimer_   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:5]HIV1dimer_   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
                               ************************************************** 
                                51        .         .         .         .         100 

                               101        .         .         .         .         150 
[Truncated_Name:1]HIV1dimer_   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:2]HIV1dimer_   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:3]HIV1dimer_   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:4]HIV1dimer_   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
[Truncated_Name:5]HIV1dimer_   QITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIG
                               ************************************************** 
                               101        .         .         .         .         150 

                               151        .         .         .         .       198 
[Truncated_Name:1]HIV1dimer_   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:2]HIV1dimer_   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:3]HIV1dimer_   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:4]HIV1dimer_   GFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
[Truncated_Name:5]HIV1dimer_   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.

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

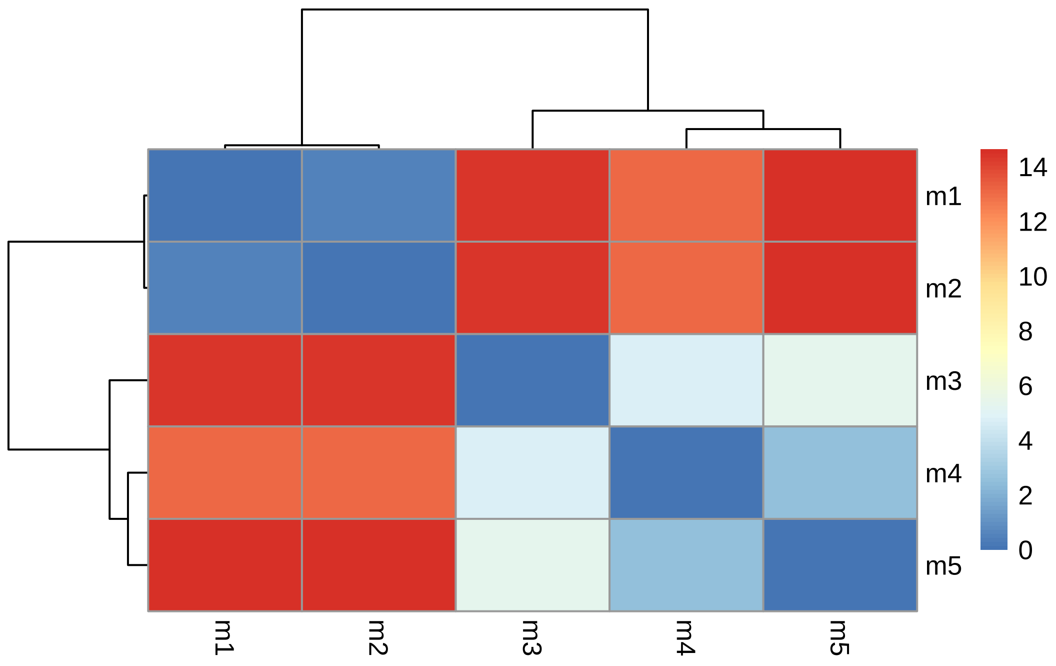
```
Warning in rmsd(pdbs, fit = T): No indices provided, using the 198 non NA positions
```

```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)
```



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.

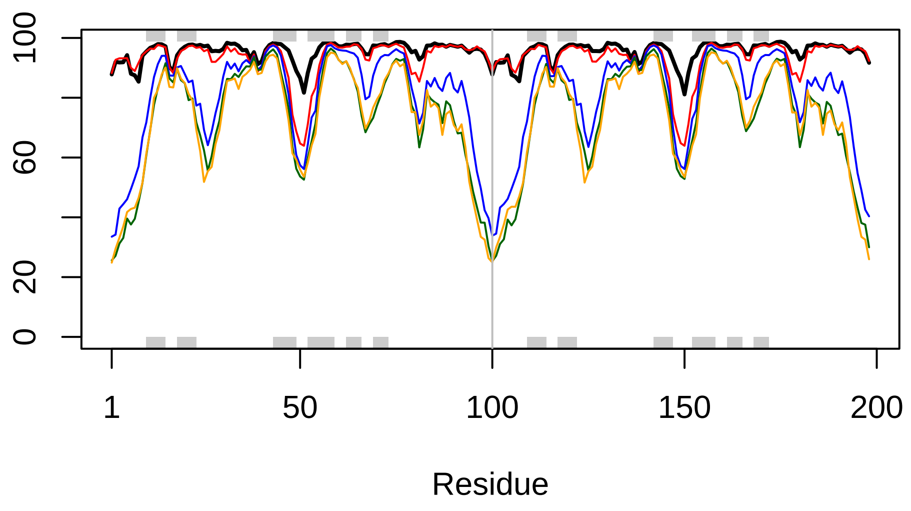
```
# Read a reference PDB structure
reference_pdb <- read.pdb("1hsg")
```

```
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$b[1,], typ="l", lwd=2, sse=reference_pdb)
points(pdbs$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 = 4578.346
core size 196 of 198  vol = 3931.108
core size 195 of 198  vol = 3709.733
core size 194 of 198  vol = 3496.019
core size 193 of 198  vol = 3302.432
core size 192 of 198  vol = 3146.474
core size 191 of 198  vol = 3048.964
core size 190 of 198  vol = 2970.354
core size 189 of 198  vol = 2893.012
core size 188 of 198  vol = 2831.825
core size 187 of 198  vol = 2774.506
core size 186 of 198  vol = 2728.043
core size 185 of 198  vol = 2704.946
core size 184 of 198  vol = 2701.981
core size 183 of 198  vol = 2715.909
core size 182 of 198  vol = 2809.853
core size 181 of 198  vol = 2888.95
core size 180 of 198  vol = 2967.282
core size 179 of 198  vol = 3036.256
core size 178 of 198  vol = 3066.287
core size 177 of 198  vol = 3096.833
core size 176 of 198  vol = 3056.414
core size 175 of 198  vol = 3014.768
core size 174 of 198  vol = 2975.013
core size 173 of 198  vol = 2898.051
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
```

We can now use the identified core atom positions as a basis for a more suitable superposition and write out the fitted structures to a directory called `corefit_structures`:

```
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
```

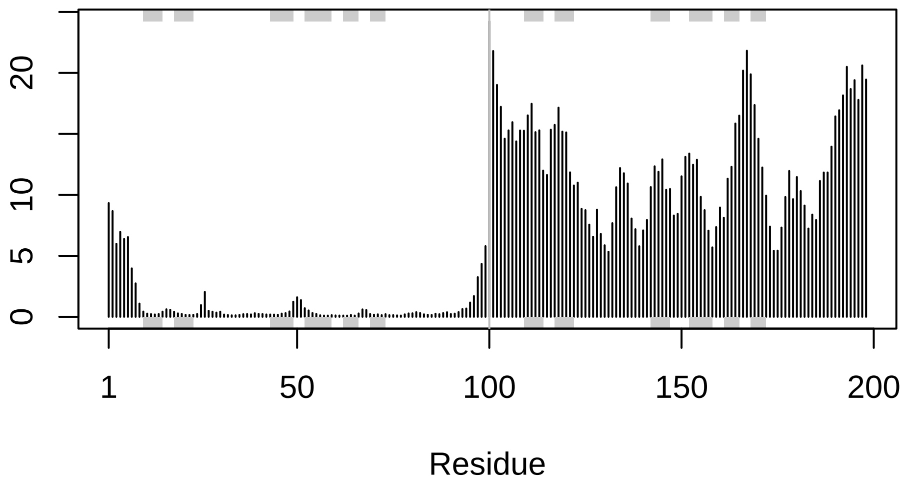
```
xyz <- pdbfit(pdbs, core.inds, outpath="corefit_structures")
```

The resulting superposed coordinates are written to a new director called `corefit_structures/`. We can now open these in Mol* and color by the Atom Property of Uncertainty/Disorder (i.e. the B-factor column that contains the pLDDT scores Figure 19):

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=reference_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 (Figure 19).

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.

```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")
```

Here is an image of this data generated from and Mol* using coloring by Occupancy. This is done in a similar manor to the pLDDT coloring procedure detailed above (Figure 20).