Lab 11 - Tue 2.11 - HIV PR Dimer

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Visualization of the Models

Use Mol* to visualize the PDB files regarding the HIV PR Dimer.

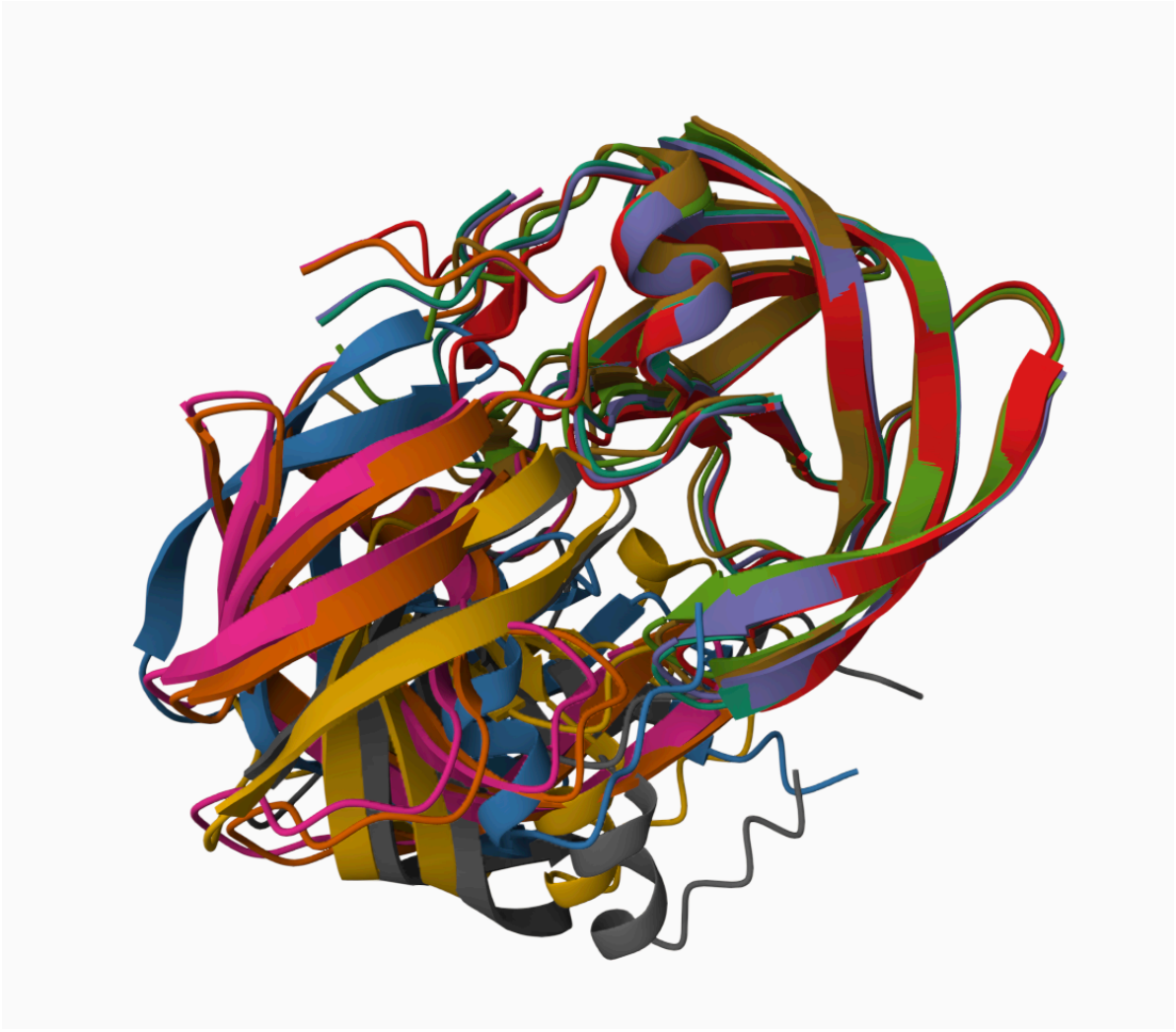


Figure 1: Superposed HIV protein dimer model for chain A

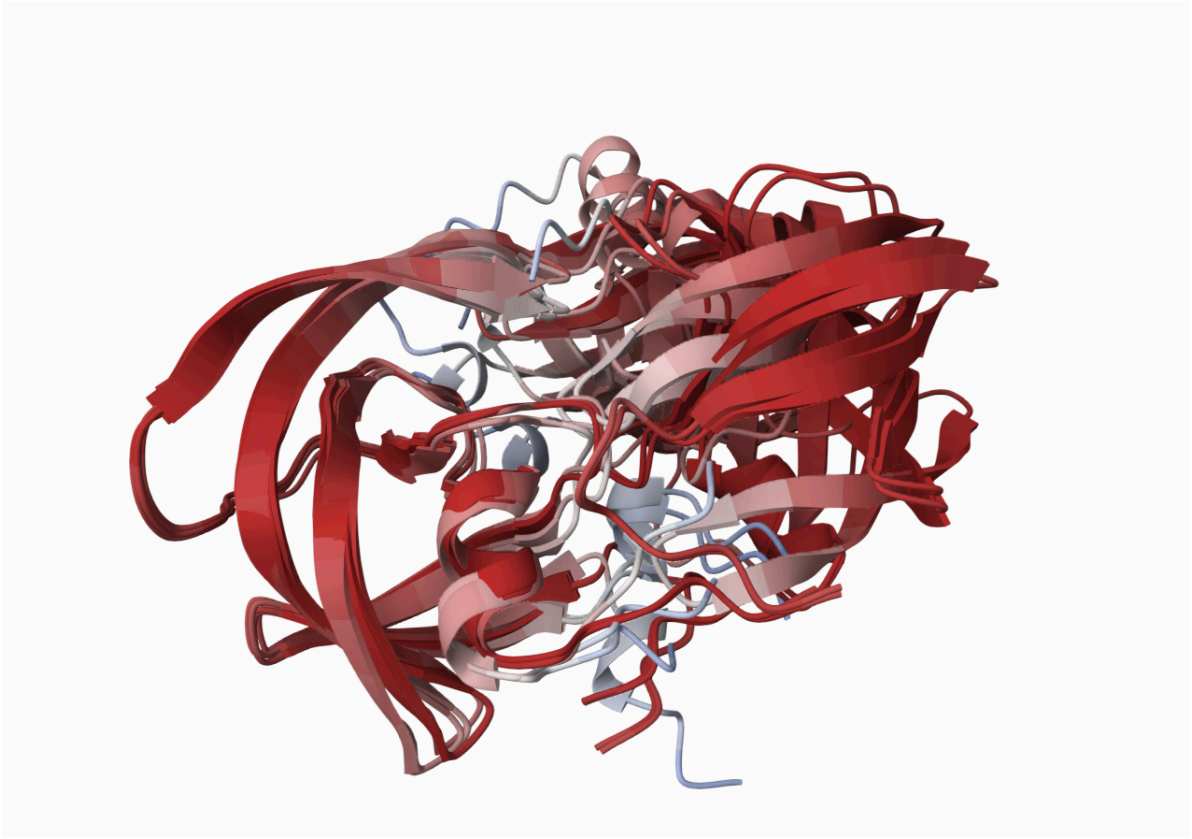


Figure 2: Chain A fitted and pLDDT colored structure model for HIV Protein Dimer

Custom Analysis of Resulting Models

We will read the results of the HIV-Pr dimer AlphaFold2 models into R with the help of the `Bio3D package`.

```
results_dir <- "HIVPrDimer_23119"
```

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

```
[1] "HIVPrDimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_000.pdb"
[2] "HIVPrDimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_000.pdb"
[3] "HIVPrDimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_000.pdb"
```

```
[4] "HIVPrDimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000.pdb"
[5] "HIVPrDimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000.pdb"
```

```
library(bio3d)
# Read all data from Models and superpose/fit coords

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

```
Reading PDB files:
HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_1_seed_000
HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_5_seed_000
HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_4_seed_000
HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_004_alphafold2_multimer_v3_model_2_seed_000
HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_005_alphafold2_multimer_v3_model_3_seed_000
.....

Extracting sequences

pdb/seq: 1   name: HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_001_alphafold2_multimer_v
pdb/seq: 2   name: HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_002_alphafold2_multimer_v
pdb/seq: 3   name: HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_003_alphafold2_multimer_v
pdb/seq: 4   name: HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_004_alphafold2_multimer_v
pdb/seq: 5   name: HIVPrDimer_23119/HIVPrDimer_23119_unrelaxed_rank_005_alphafold2_multimer_v
```

```
pdbs
```

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

                                51        .         .         .         .         100
[Truncated_Name:1]HIVPrDimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:2]HIVPrDimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:3]HIVPrDimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:4]HIVPrDimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
[Truncated_Name:5]HIVPrDimer   GGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFP
```

```
                              **************************************************
                             51        .         .         .         .         100

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

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

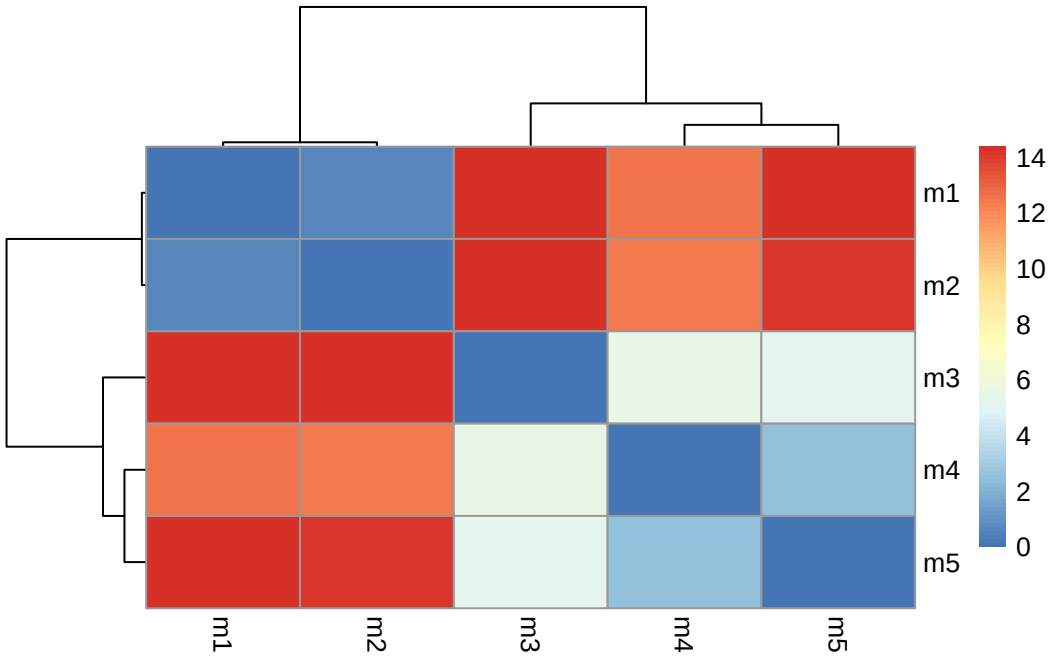
```
Warning in rmsd(pdbs, fit = T): No indices provided, using the 198 non NA positions
```

```
range(rd)
```

```
[1]  0.000 14.376
```

```
#Draw a heatmap of these RMSD matrix values
library(pheatmap)

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



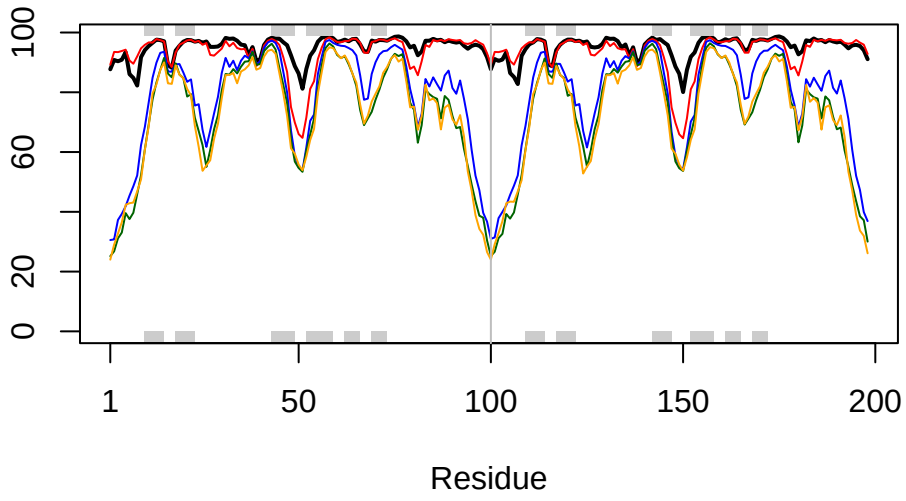
Models 1 and 2 are the most similar to each other. Models 4 and 5 are somewhat similar to each other, and also more similar to model 3 than they are to models 1 and 2.

Let's now plot the pLDDT values across all models.

```
# 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")
```



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 = 4916.702
 core size 196 of 198  vol = 4311.481
 core size 195 of 198  vol = 4101.445
 core size 194 of 198  vol = 3907.124
 core size 193 of 198  vol = 3711.925
 core size 192 of 198  vol = 3546.511
 core size 191 of 198  vol = 3440.437
 core size 190 of 198  vol = 3317.571
 core size 189 of 198  vol = 3220.079
 core size 188 of 198  vol = 3142.057
 core size 187 of 198  vol = 3066.79
 core size 186 of 198  vol = 3015.892
 core size 185 of 198  vol = 2959.969
 core size 184 of 198  vol = 2913.74
 core size 183 of 198  vol = 2880.923
 core size 182 of 198  vol = 2848.081
 core size 181 of 198  vol = 2857.001
 core size 180 of 198  vol = 2871.24
```

```
core size 179 of 198  vol = 2905.696
core size 178 of 198  vol = 2953.776
core size 177 of 198  vol = 3020.847
core size 176 of 198  vol = 3087.22
core size 175 of 198  vol = 3109.99
core size 174 of 198  vol = 3129.601
core size 173 of 198  vol = 3135.085
core size 172 of 198  vol = 3092.283
core size 171 of 198  vol = 3036.012
core size 170 of 198  vol = 2947.995
core size 169 of 198  vol = 2886.897
core size 168 of 198  vol = 2829.355
core size 167 of 198  vol = 2746.377
core size 166 of 198  vol = 2671.189
core size 165 of 198  vol = 2600.848
core size 164 of 198  vol = 2534.651
core size 163 of 198  vol = 2464.3
core size 162 of 198  vol = 2390.171
core size 161 of 198  vol = 2322.47
core size 160 of 198  vol = 2236.698
core size 159 of 198  vol = 2160.475
core size 158 of 198  vol = 2077.281
core size 157 of 198  vol = 2003.596
core size 156 of 198  vol = 1939.94
core size 155 of 198  vol = 1859.188
core size 154 of 198  vol = 1781.083
core size 153 of 198  vol = 1699.1
core size 152 of 198  vol = 1622.558
core size 151 of 198  vol = 1546.319
core size 150 of 198  vol = 1473.01
core size 149 of 198  vol = 1414.087
core size 148 of 198  vol = 1352.547
core size 147 of 198  vol = 1295.278
core size 146 of 198  vol = 1246.999
core size 145 of 198  vol = 1203.962
core size 144 of 198  vol = 1163.009
core size 143 of 198  vol = 1110.955
core size 142 of 198  vol = 1064.672
core size 141 of 198  vol = 1028.458
core size 140 of 198  vol = 986.121
core size 139 of 198  vol = 944.003
core size 138 of 198  vol = 895.914
core size 137 of 198  vol = 853.508
```

```
core size 136 of 198  vol = 827.977
core size 135 of 198  vol = 796.874
core size 134 of 198  vol = 772.763
core size 133 of 198  vol = 743.108
core size 132 of 198  vol = 707.65
core size 131 of 198  vol = 669.172
core size 130 of 198  vol = 634.655
core size 129 of 198  vol = 594.035
core size 128 of 198  vol = 559.154
core size 127 of 198  vol = 525.971
core size 126 of 198  vol = 493.19
core size 125 of 198  vol = 466.473
core size 124 of 198  vol = 438.433
core size 123 of 198  vol = 410.725
core size 122 of 198  vol = 401.38
core size 121 of 198  vol = 391.76
core size 120 of 198  vol = 362.084
core size 119 of 198  vol = 338.183
core size 118 of 198  vol = 312.338
core size 117 of 198  vol = 282.176
core size 116 of 198  vol = 262.215
core size 115 of 198  vol = 241.577
core size 114 of 198  vol = 225.151
core size 113 of 198  vol = 204.137
core size 112 of 198  vol = 185.038
core size 111 of 198  vol = 162.728
core size 110 of 198  vol = 146.181
core size 109 of 198  vol = 133.352
core size 108 of 198  vol = 123.207
core size 107 of 198  vol = 109.228
core size 106 of 198  vol = 98.824
core size 105 of 198  vol = 89.735
core size 104 of 198  vol = 81.206
core size 103 of 198  vol = 74.188
core size 102 of 198  vol = 67.042
core size 101 of 198  vol = 62.043
core size 100 of 198  vol = 58.432
core size 99 of 198  vol = 55.149
core size 98 of 198  vol = 51.114
core size 97 of 198  vol = 45.798
core size 96 of 198  vol = 41.161
core size 95 of 198  vol = 35.619
core size 94 of 198  vol = 29.784
```

```
 core size 93 of 198  vol = 23.233
 core size 92 of 198  vol = 16.669
 core size 91 of 198  vol = 9.459
 core size 90 of 198  vol = 4.595
 core size 89 of 198  vol = 3.161
 core size 88 of 198  vol = 2.678
 core size 87 of 198  vol = 2.293
 core size 86 of 198  vol = 1.935
 core size 85 of 198  vol = 1.619
 core size 84 of 198  vol = 1.367
 core size 83 of 198  vol = 1.09
 core size 82 of 198  vol = 0.906
 core size 81 of 198  vol = 0.764
 core size 80 of 198  vol = 0.649
 core size 79 of 198  vol = 0.596
 core size 78 of 198  vol = 0.53
 core size 77 of 198  vol = 0.486
 FINISHED: Min vol ( 0.5 ) reached
```

Use the identified core atom positions to find more suitable superposition and write out the fitted structures to a directory called `corefit_structures`.

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

```
# 78 positions (cumulative volume <= 0.5 Angstrom^3)
  start end length
1    10  25     16
2    28  48     21
3    53  93     41
```

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

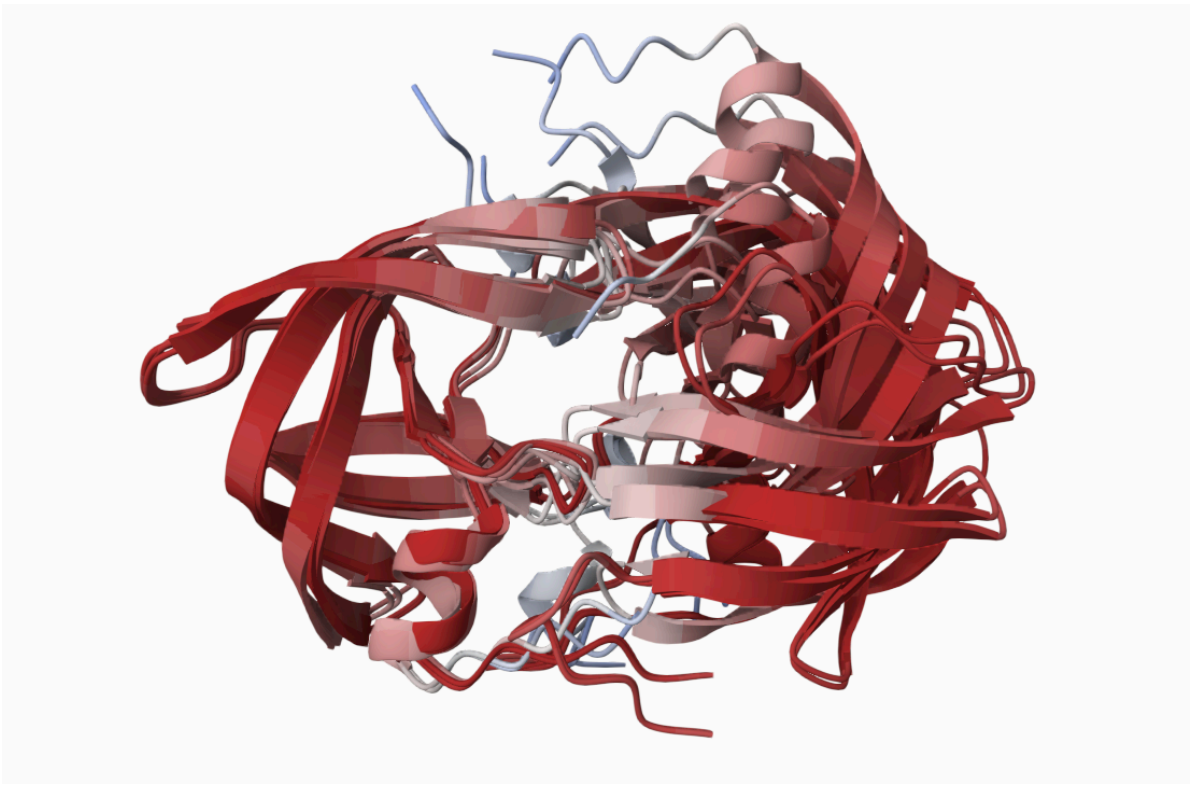
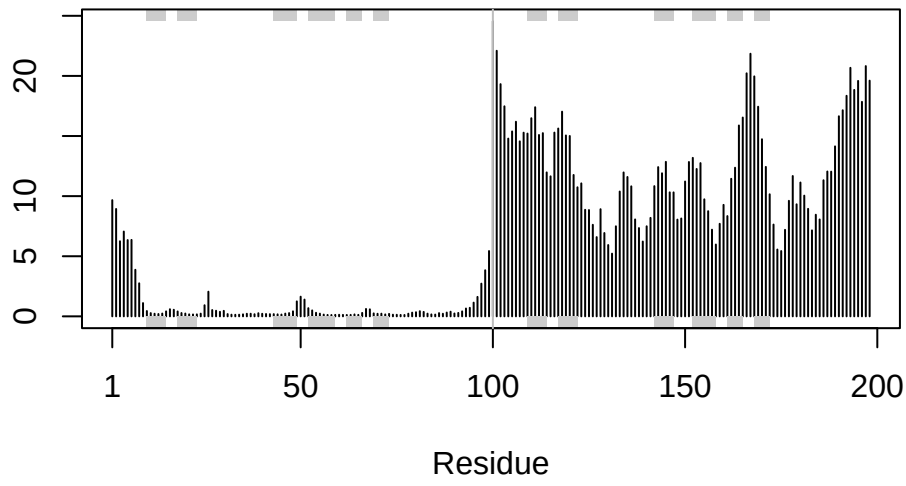


Figure 3: Core superposed structures colored by B-factor i.e pLDDT

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")
```



This plot shows that the first chain is very similar across the different models while the second chain is more variable.

Predicted Alignment Error for Domains

AlphaFold also produces an output called `Predicted Aligned Error (PAE)` that is independent of the 3D structure. This output is detailed in the JSON format result files and contains one for each model structure.

```
library(jsonlite)

# Listing of all PAE JSON files
pae_files <- list.files(path=results_dir,
                        pattern=".*model.*\\.json",
                        full.names = TRUE)
```

We will read the 1st and 5th files to make 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] 87.69 90.81 90.38 90.88 93.44 86.06
```

The maximum PAE values are useful for ranking models.

```
pae1$max_pae
```

```
[1] 15.47656
```

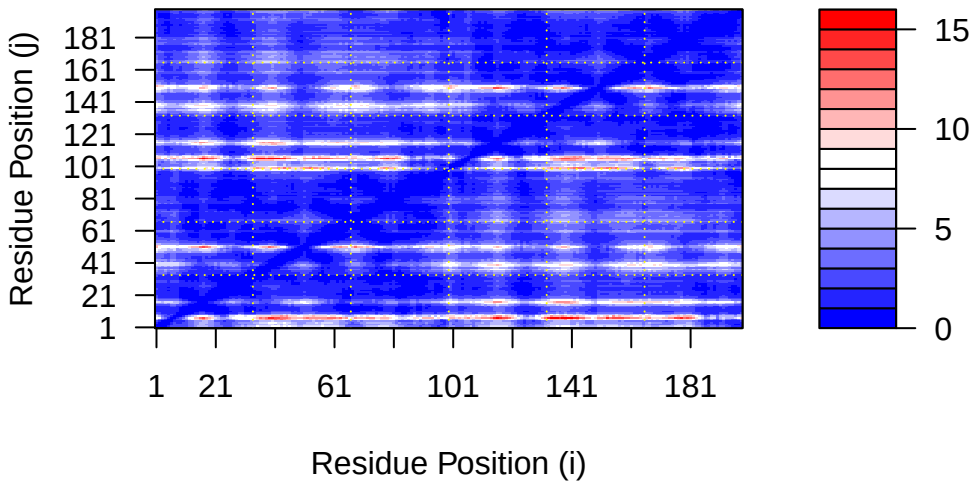
```
pae5$max_pae
```

```
[1] 29.32812
```

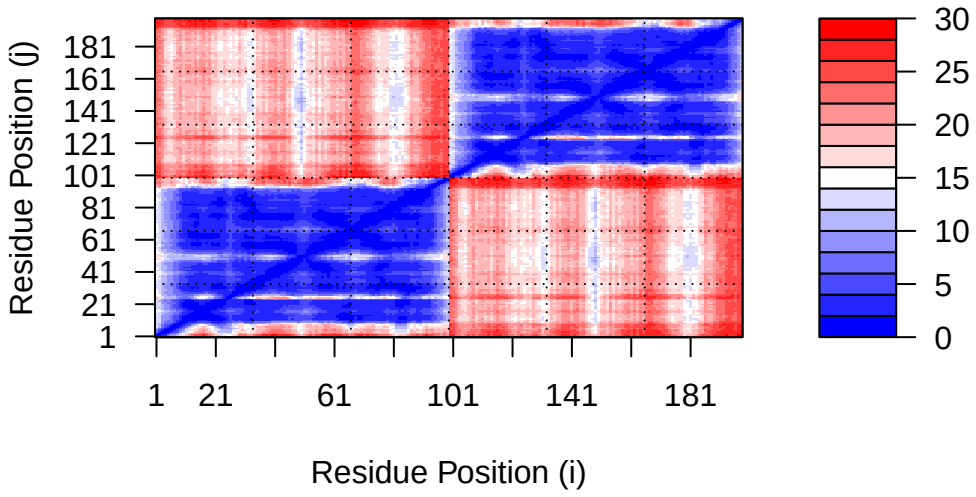
The values here show that model 5 is worse than model 1 since a lower PAE score when comparing between models.

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)")
```

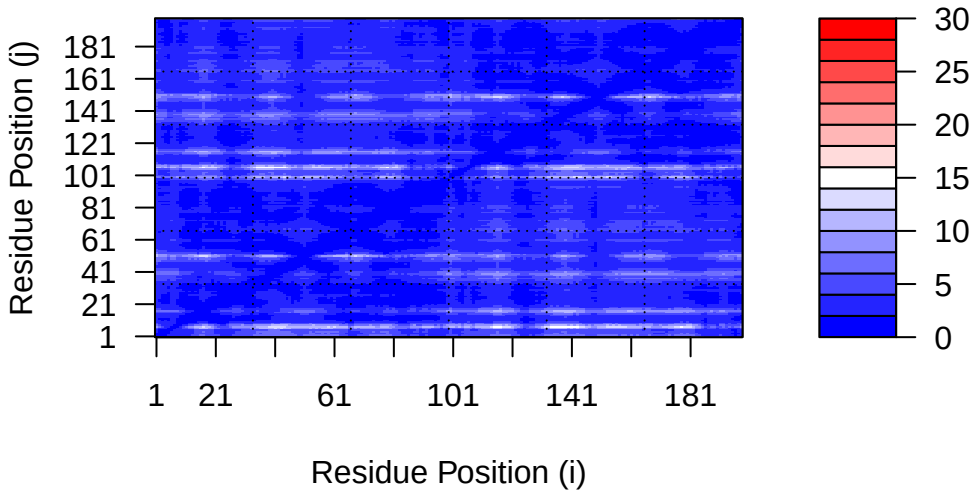



```
plot.dmat(pae5$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)",
          grid.col = "black",
          zlim=c(0,30))
```



It is better to plot using the same z range. Below is the model 1 plot again but with 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

We will now take a look at the alignment file.

```
aln_file <- list.files(path=results_dir,
                       pattern=".a3m$",
                        full.names = TRUE)
aln_file
```

```
[1] "HIVPrDimer_23119/HIVPrDimer_23119.a3m"
```

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

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

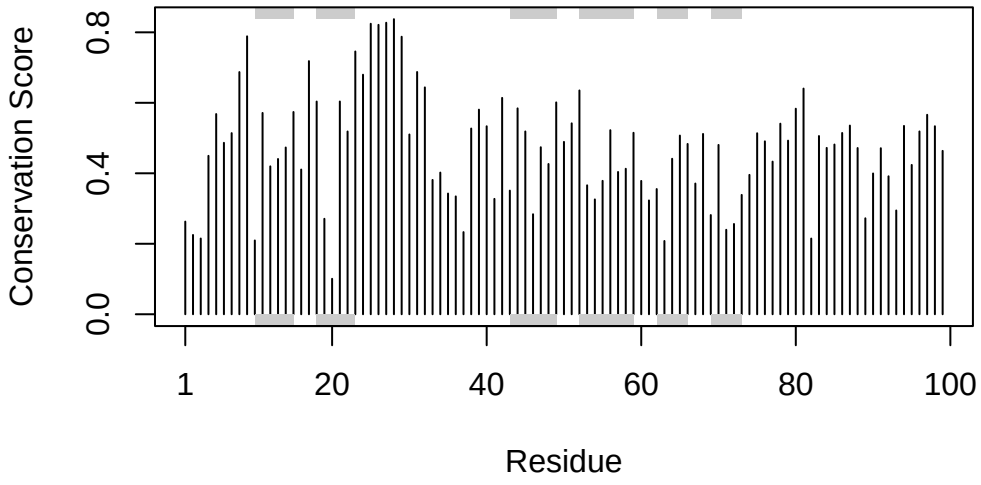
How many sequences are in this alignment?

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

```
[1] 5378  132
```

To score the 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")
```



The plot shows that residues D25, T26, G27, and A28 are highlighy conserved. 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] "-" "-" "-" "-" "-" "-"
```

Let's createa final visualize to show these functionally important sites by mapping the conservation score to the Occupancy column of a PDB file that will be viewed using Mol*.

```
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")
```

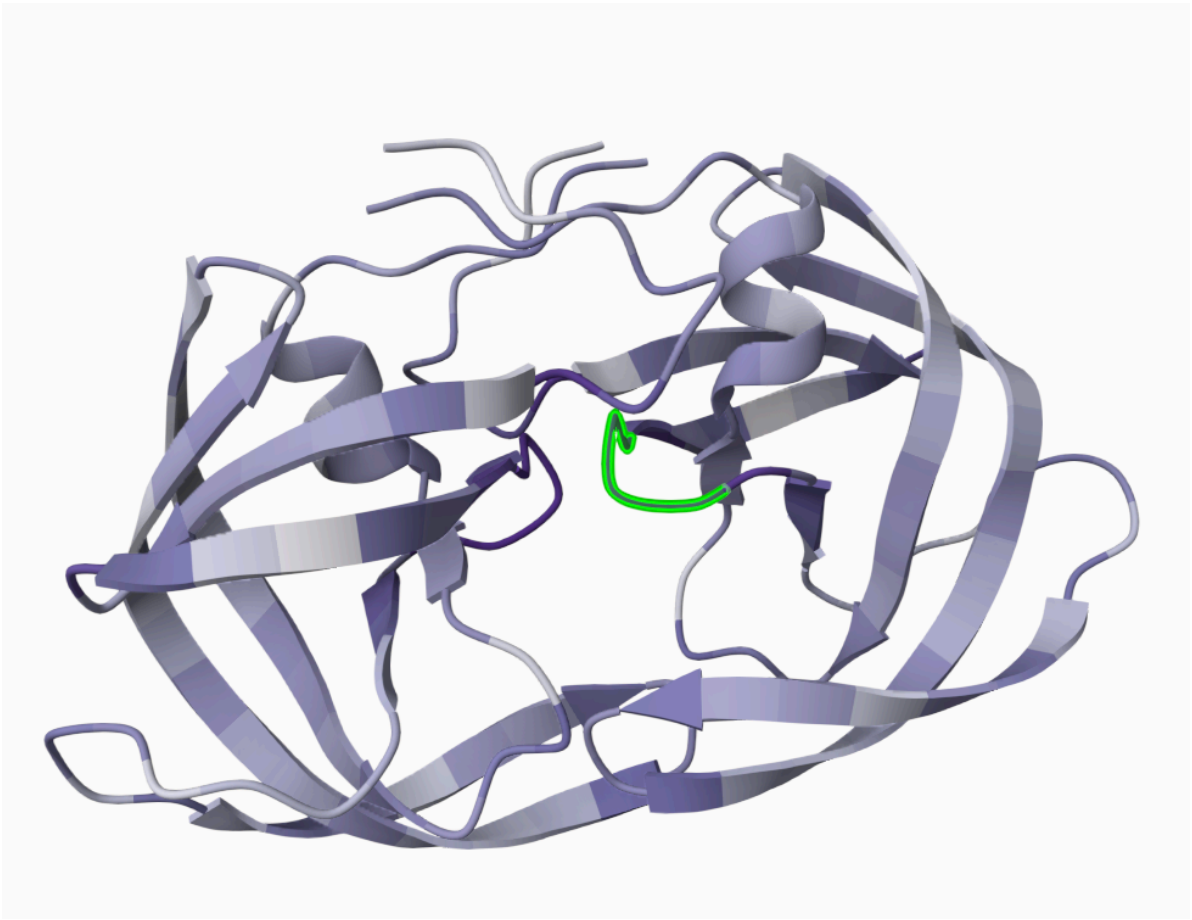


Figure 4: Top ranked dimer model colored by sequence conservation. The dark purple indicates the conserved positions while the DTGA motif of a single chain is highlighted in green.

This figure helps to show the central conserved active site in the model where the natural peptide substrate (and small molecule inhibitors) would bind between domains.