Class 11: Structural Bioinoformatics pt2

Jiayi Zhou (PID:A17856751

Table of contents

AlphaFold Data Base (AFDB)

The EBI maintains the largest database of AlphaFold structure prediction models at: https://alphafold.ebi.ac.uk

From last class (before Halloween) we saw that PDB has 244,290 (Oct 2025)

The total number of protein sequences in UniProtKB is 199,579,901.

> Key Point: This is a tiny fraction of sequence space that has structural coverage (0.12%)

```
244290/199579901*100
```

```
[1] 0.1224021
```

AFDB is attempting to address this gap...

there are two "Quality Scores" from Alphafold, one for residues (i.e. each amino acid) called **pLDDT** score. The other **PAE** score measures the confidence in the relative position of two residues (i.e. a score for every pair of residues).

Generating your own structure predictions

Figure of 5 generated HIV_PR models

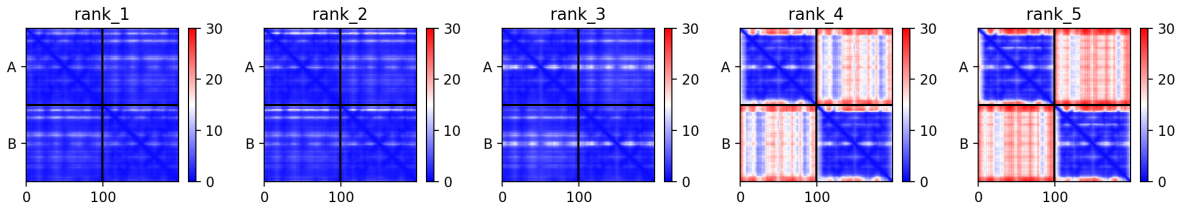


Figure 1: Figure of 5 generated HIV_PR models

Figure of sequence coverage

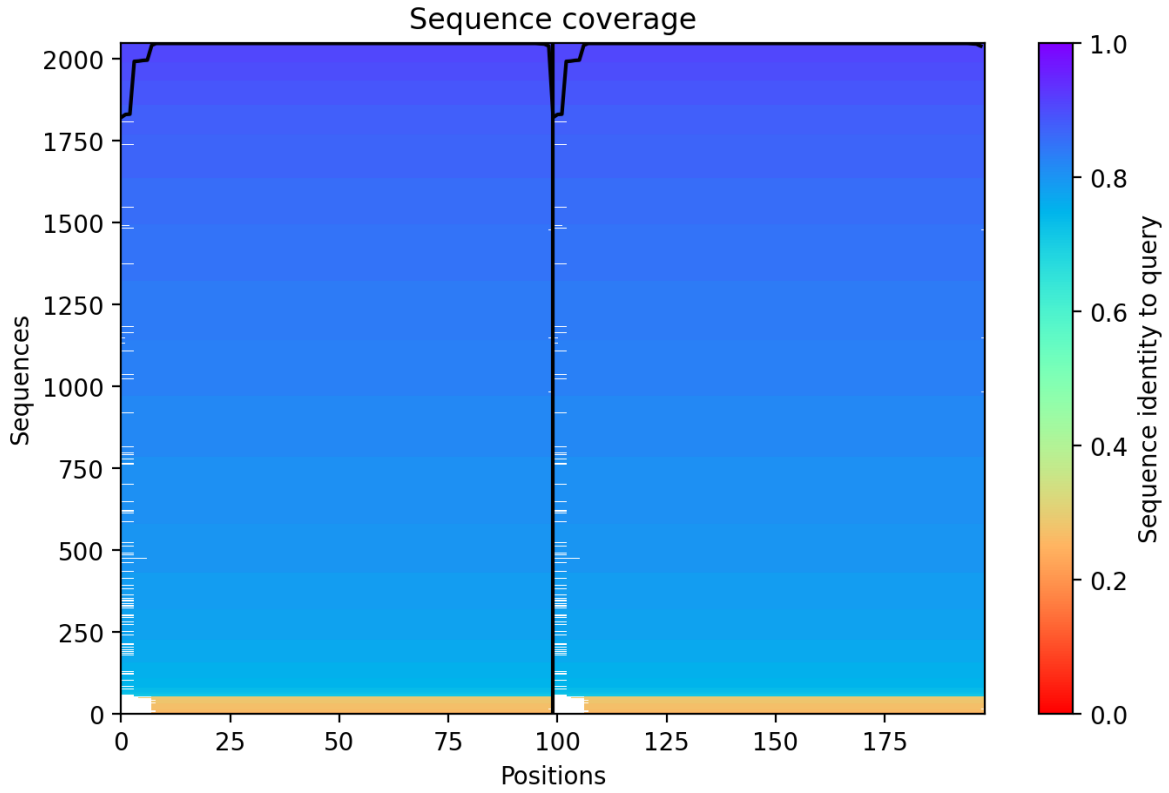
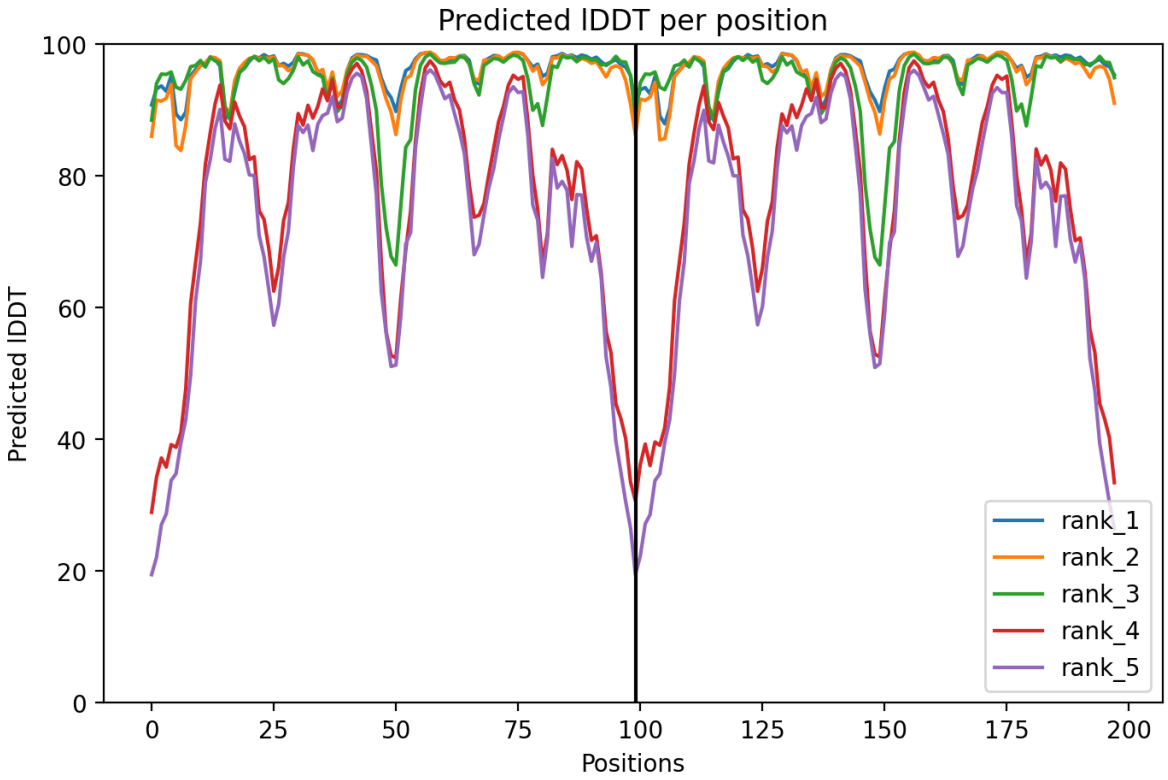


Figure 2: Figure of sequence coverage

Figure of Predicted IDDT per position



Visualization of the models and their estimated reliability

Fitted and pLDDT colored Nras structure models.

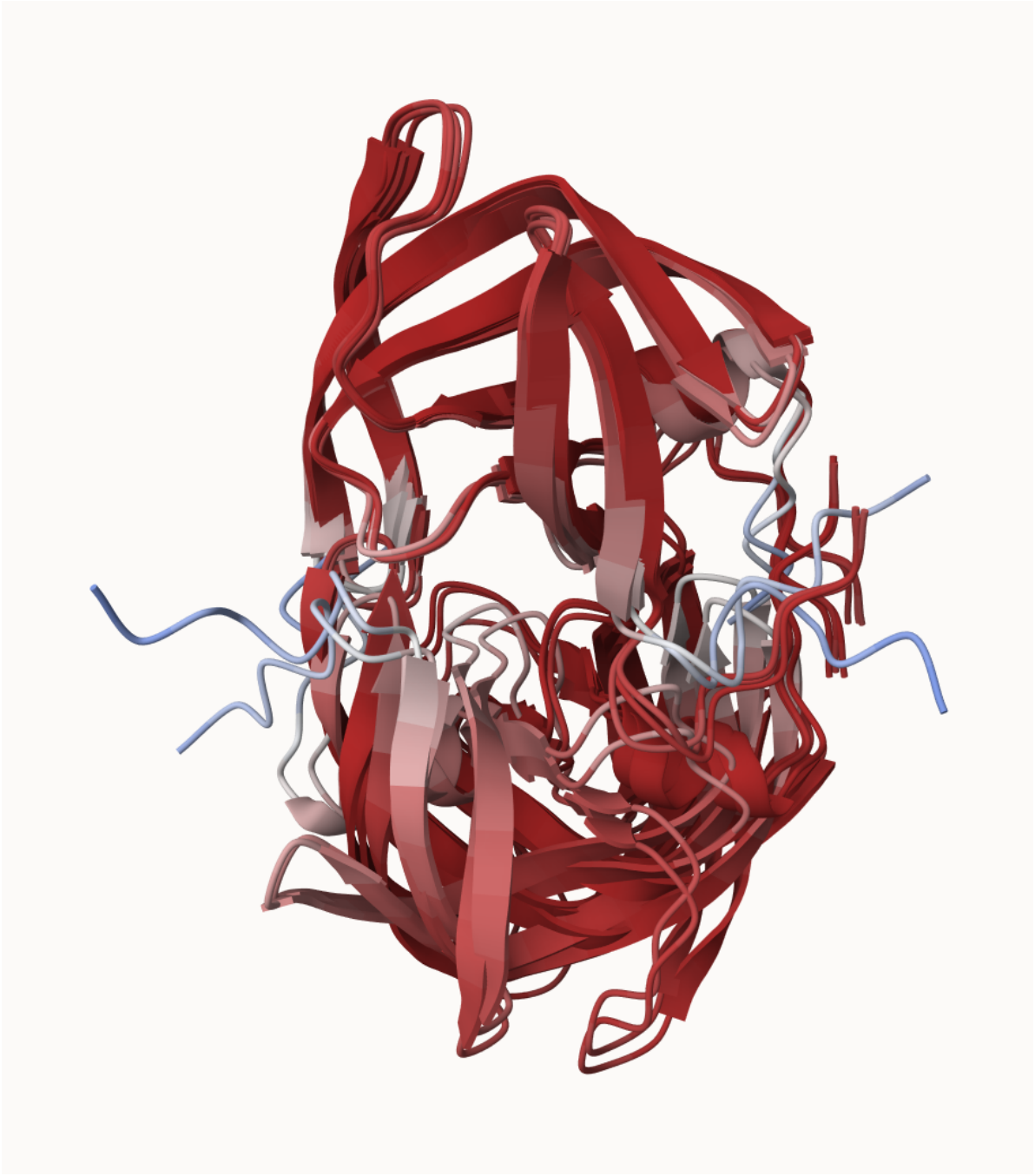


Figure 3: Fitted and pLDDT colored Nras structure models

Coloring the Residue property of Secondary Structure

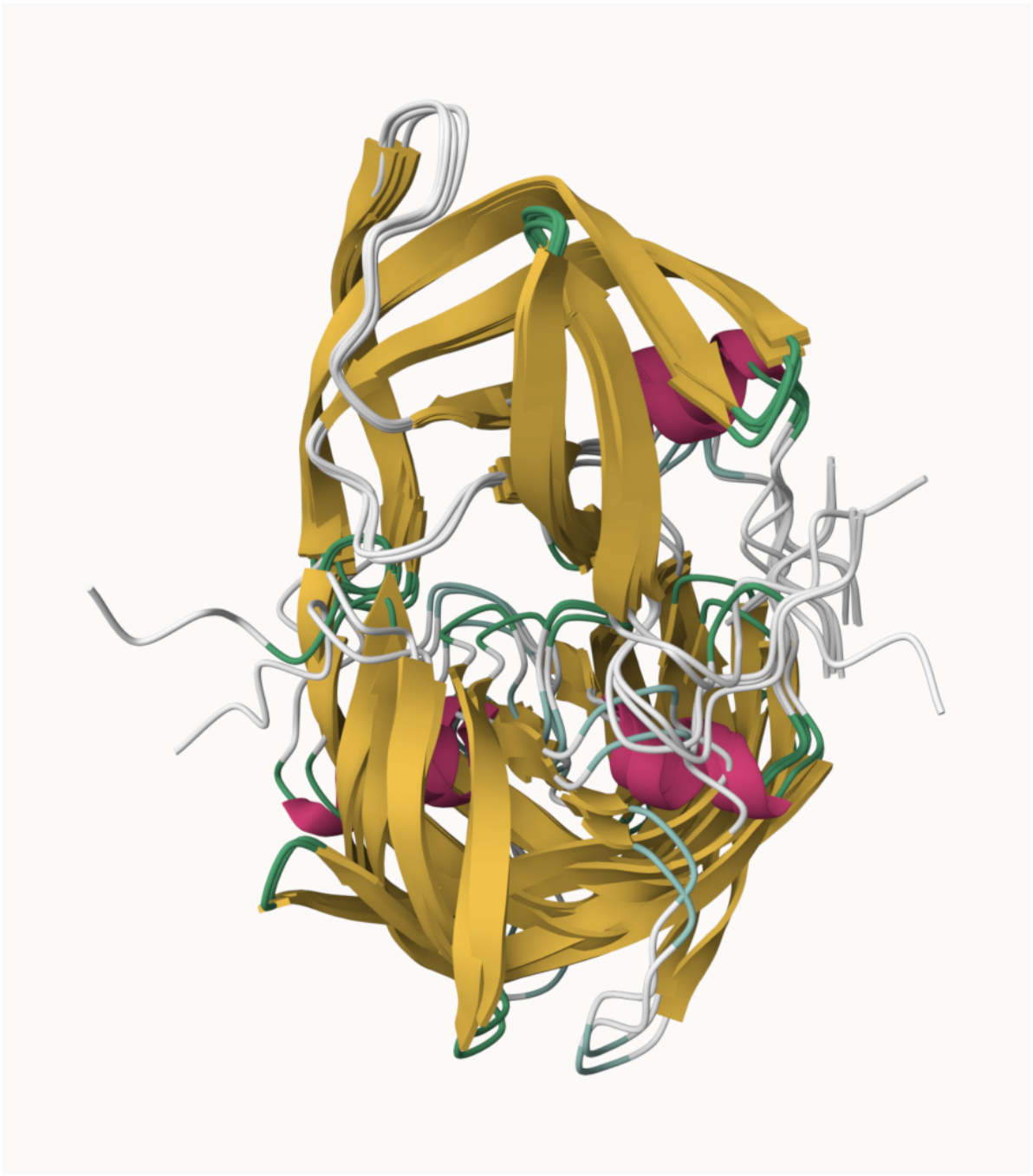
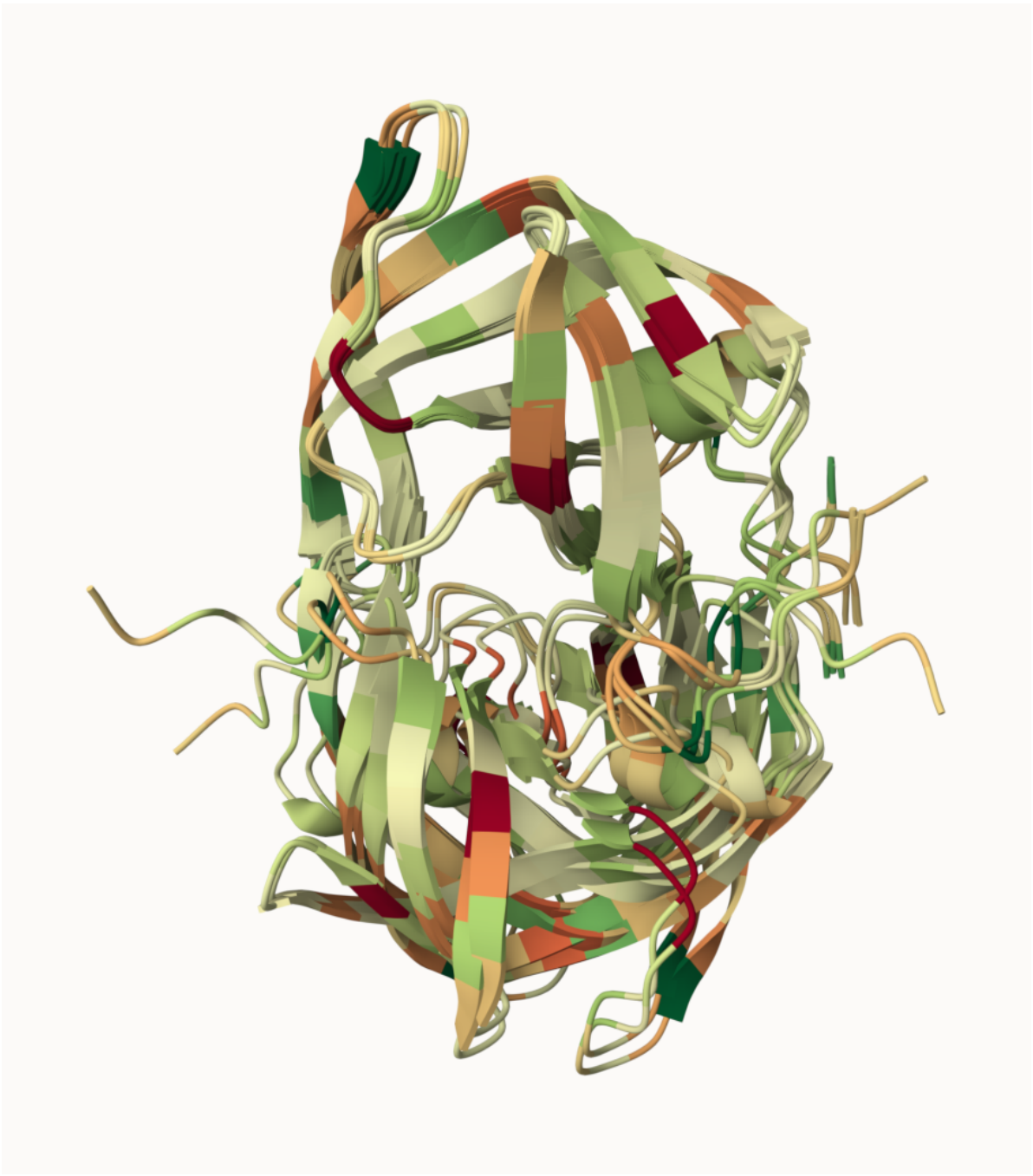


Figure 4: Secondary Structure

Coloring the Residue property of Hydrophobicity



Coloring the Residue property of Accessible surface Area

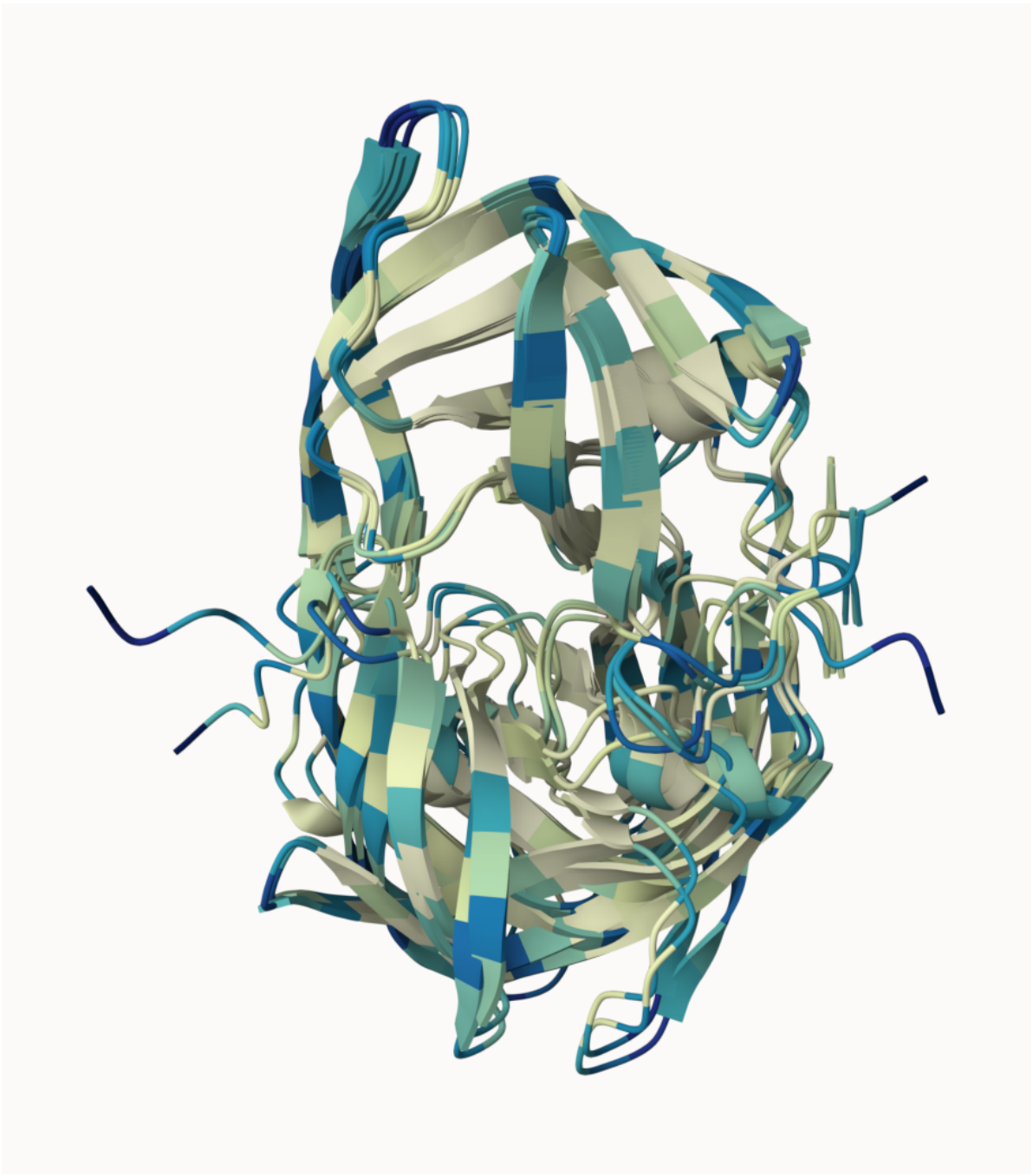


Figure 5: Accessible surface Area

Custom analysis of resulting models in R

Now we read the results of the more complicated HIV-Pr dimer AlphaFold2 models into R using `Bio3D package`.

To move our AlphaFold results directory into our RStudio project directory...

```
results_dir <- "hivprdimer_23119/"

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

Print out our PDB file names

```
basename(pdb_files)
```

```
[1] "HIVPRDimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_4_seed_000.pdb"
[2] "HIVPRDimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_1_seed_000.pdb"
[3] "HIVPRDimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_5_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)

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

```
Reading PDB files:
hivprdimer_23119//HIVPRDimer_23119_unrelaxed_rank_001_alphafold2_multimer_v3_model_4_seed_000
hivprdimer_23119//HIVPRDimer_23119_unrelaxed_rank_002_alphafold2_multimer_v3_model_1_seed_000
hivprdimer_23119//HIVPRDimer_23119_unrelaxed_rank_003_alphafold2_multimer_v3_model_5_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_
pdb/seq: 2   name: hivprdimer_23119//HIVPRDimer_23119_unrelaxed_rank_002_alphafold2_multimer_
pdb/seq: 3   name: hivprdimer_23119//HIVPRDimer_23119_unrelaxed_rank_003_alphafold2_multimer_
pdb/seq: 4   name: hivprdimer_23119//HIVPRDimer_23119_unrelaxed_rank_004_alphafold2_multimer_
pdb/seq: 5   name: hivprdimer_23119//HIVPRDimer_23119_unrelaxed_rank_005_alphafold2_multimer_
```

To 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
```

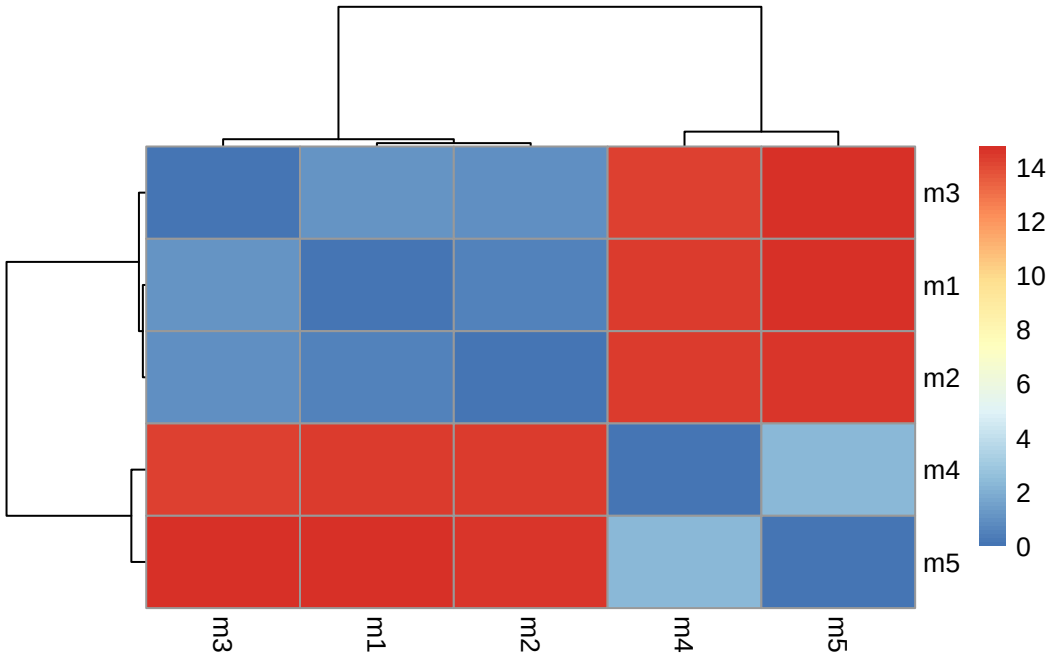
```
range(rd)
```

```
[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)
```

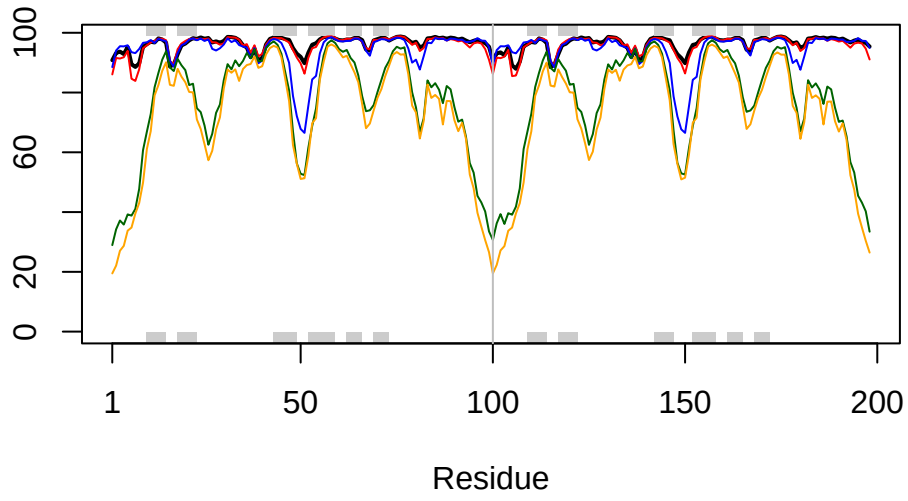


Models 1 and 2 are most similar to each other, while 4 and 5 resemble each other and are closer to 3 than to 1 or 2.

```
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 = 9885.822
 core size 196 of 198  vol = 6896.71
 core size 195 of 198  vol = 1337.847
 core size 194 of 198  vol = 1040.67
 core size 193 of 198  vol = 951.857
 core size 192 of 198  vol = 899.083
 core size 191 of 198  vol = 834.732
 core size 190 of 198  vol = 771.338
```

```
core size 189 of 198  vol = 733.065
core size 188 of 198  vol = 697.28
core size 187 of 198  vol = 659.742
core size 186 of 198  vol = 625.273
core size 185 of 198  vol = 589.541
core size 184 of 198  vol = 568.253
core size 183 of 198  vol = 545.015
core size 182 of 198  vol = 512.889
core size 181 of 198  vol = 490.723
core size 180 of 198  vol = 470.266
core size 179 of 198  vol = 450.731
core size 178 of 198  vol = 434.735
core size 177 of 198  vol = 420.337
core size 176 of 198  vol = 406.658
core size 175 of 198  vol = 393.334
core size 174 of 198  vol = 382.395
core size 173 of 198  vol = 372.858
core size 172 of 198  vol = 356.994
core size 171 of 198  vol = 346.567
core size 170 of 198  vol = 337.446
core size 169 of 198  vol = 326.659
core size 168 of 198  vol = 314.95
core size 167 of 198  vol = 304.127
core size 166 of 198  vol = 294.552
core size 165 of 198  vol = 285.648
core size 164 of 198  vol = 278.884
core size 163 of 198  vol = 266.765
core size 162 of 198  vol = 258.994
core size 161 of 198  vol = 247.723
core size 160 of 198  vol = 239.84
core size 159 of 198  vol = 234.963
core size 158 of 198  vol = 230.062
core size 157 of 198  vol = 221.985
core size 156 of 198  vol = 215.62
core size 155 of 198  vol = 206.793
core size 154 of 198  vol = 196.984
core size 153 of 198  vol = 188.539
core size 152 of 198  vol = 182.262
core size 151 of 198  vol = 176.954
core size 150 of 198  vol = 170.712
core size 149 of 198  vol = 166.119
core size 148 of 198  vol = 159.796
core size 147 of 198  vol = 153.767
```

```
core size 146 of 198  vol = 149.092
core size 145 of 198  vol = 143.657
core size 144 of 198  vol = 137.138
core size 143 of 198  vol = 132.517
core size 142 of 198  vol = 127.231
core size 141 of 198  vol = 121.574
core size 140 of 198  vol = 116.775
core size 139 of 198  vol = 112.57
core size 138 of 198  vol = 108.17
core size 137 of 198  vol = 105.133
core size 136 of 198  vol = 101.249
core size 135 of 198  vol = 97.374
core size 134 of 198  vol = 92.974
core size 133 of 198  vol = 88.184
core size 132 of 198  vol = 84.029
core size 131 of 198  vol = 81.898
core size 130 of 198  vol = 78.019
core size 129 of 198  vol = 75.272
core size 128 of 198  vol = 73.052
core size 127 of 198  vol = 70.695
core size 126 of 198  vol = 68.975
core size 125 of 198  vol = 66.694
core size 124 of 198  vol = 64.394
core size 123 of 198  vol = 62.092
core size 122 of 198  vol = 59.045
core size 121 of 198  vol = 56.629
core size 120 of 198  vol = 54.016
core size 119 of 198  vol = 51.806
core size 118 of 198  vol = 49.652
core size 117 of 198  vol = 48.193
core size 116 of 198  vol = 46.648
core size 115 of 198  vol = 44.752
core size 114 of 198  vol = 43.292
core size 113 of 198  vol = 41.093
core size 112 of 198  vol = 39.147
core size 111 of 198  vol = 36.472
core size 110 of 198  vol = 34.117
core size 109 of 198  vol = 31.47
core size 108 of 198  vol = 29.448
core size 107 of 198  vol = 27.325
core size 106 of 198  vol = 25.822
core size 105 of 198  vol = 24.15
core size 104 of 198  vol = 22.648
```

```
 core size 103 of 198  vol = 21.069
 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 = 9.434
 core size 96 of 198  vol = 7.354
 core size 95 of 198  vol = 6.179
 core size 94 of 198  vol = 5.666
 core size 93 of 198  vol = 4.705
 core size 92 of 198  vol = 3.665
 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")
```

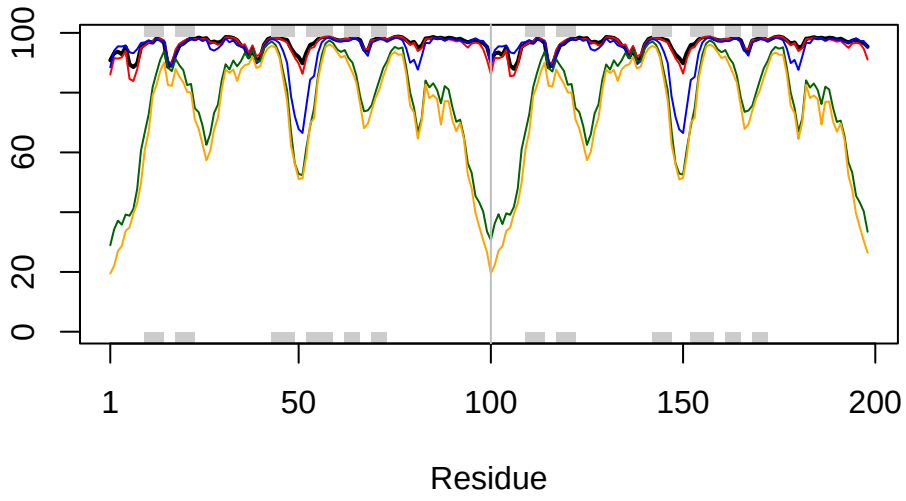
We can also plot the pLDDT values across all models

```
pdb <- read.pdb("1hsg")
```

```
  Note: Accessing on-line PDB file

Warning in get.pdb(file, path = tempdir(), verbose = FALSE):
/var/folders/hw/mb7lcvrd0v17c4nnmhd27vr40000gn/T//Rtmp07QOJb/1hsg.pdb exists.
Skipping download
```

```
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")
```



To enhance the alignment of our models, we can identify the most stable "rigid core" shared among them using the `core.find()` function:

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

```
 core size 197 of 198  vol = 9885.822
 core size 196 of 198  vol = 6896.71
 core size 195 of 198  vol = 1337.847
 core size 194 of 198  vol = 1040.67
 core size 193 of 198  vol = 951.857
 core size 192 of 198  vol = 899.083
 core size 191 of 198  vol = 834.732
 core size 190 of 198  vol = 771.338
 core size 189 of 198  vol = 733.065
 core size 188 of 198  vol = 697.28
```

```
core size 187 of 198  vol = 659.742
core size 186 of 198  vol = 625.273
core size 185 of 198  vol = 589.541
core size 184 of 198  vol = 568.253
core size 183 of 198  vol = 545.015
core size 182 of 198  vol = 512.889
core size 181 of 198  vol = 490.723
core size 180 of 198  vol = 470.266
core size 179 of 198  vol = 450.731
core size 178 of 198  vol = 434.735
core size 177 of 198  vol = 420.337
core size 176 of 198  vol = 406.658
core size 175 of 198  vol = 393.334
core size 174 of 198  vol = 382.395
core size 173 of 198  vol = 372.858
core size 172 of 198  vol = 356.994
core size 171 of 198  vol = 346.567
core size 170 of 198  vol = 337.446
core size 169 of 198  vol = 326.659
core size 168 of 198  vol = 314.95
core size 167 of 198  vol = 304.127
core size 166 of 198  vol = 294.552
core size 165 of 198  vol = 285.648
core size 164 of 198  vol = 278.884
core size 163 of 198  vol = 266.765
core size 162 of 198  vol = 258.994
core size 161 of 198  vol = 247.723
core size 160 of 198  vol = 239.84
core size 159 of 198  vol = 234.963
core size 158 of 198  vol = 230.062
core size 157 of 198  vol = 221.985
core size 156 of 198  vol = 215.62
core size 155 of 198  vol = 206.793
core size 154 of 198  vol = 196.984
core size 153 of 198  vol = 188.539
core size 152 of 198  vol = 182.262
core size 151 of 198  vol = 176.954
core size 150 of 198  vol = 170.712
core size 149 of 198  vol = 166.119
core size 148 of 198  vol = 159.796
core size 147 of 198  vol = 153.767
core size 146 of 198  vol = 149.092
core size 145 of 198  vol = 143.657
```

```
core size 144 of 198  vol = 137.138
core size 143 of 198  vol = 132.517
core size 142 of 198  vol = 127.231
core size 141 of 198  vol = 121.574
core size 140 of 198  vol = 116.775
core size 139 of 198  vol = 112.57
core size 138 of 198  vol = 108.17
core size 137 of 198  vol = 105.133
core size 136 of 198  vol = 101.249
core size 135 of 198  vol = 97.374
core size 134 of 198  vol = 92.974
core size 133 of 198  vol = 88.184
core size 132 of 198  vol = 84.029
core size 131 of 198  vol = 81.898
core size 130 of 198  vol = 78.019
core size 129 of 198  vol = 75.272
core size 128 of 198  vol = 73.052
core size 127 of 198  vol = 70.695
core size 126 of 198  vol = 68.975
core size 125 of 198  vol = 66.694
core size 124 of 198  vol = 64.394
core size 123 of 198  vol = 62.092
core size 122 of 198  vol = 59.045
core size 121 of 198  vol = 56.629
core size 120 of 198  vol = 54.016
core size 119 of 198  vol = 51.806
core size 118 of 198  vol = 49.652
core size 117 of 198  vol = 48.193
core size 116 of 198  vol = 46.648
core size 115 of 198  vol = 44.752
core size 114 of 198  vol = 43.292
core size 113 of 198  vol = 41.093
core size 112 of 198  vol = 39.147
core size 111 of 198  vol = 36.472
core size 110 of 198  vol = 34.117
core size 109 of 198  vol = 31.47
core size 108 of 198  vol = 29.448
core size 107 of 198  vol = 27.325
core size 106 of 198  vol = 25.822
core size 105 of 198  vol = 24.15
core size 104 of 198  vol = 22.648
core size 103 of 198  vol = 21.069
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 = 9.434
 core size 96 of 198  vol = 7.354
 core size 95 of 198  vol = 6.179
 core size 94 of 198  vol = 5.666
 core size 93 of 198  vol = 4.705
 core size 92 of 198  vol = 3.665
 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
```

The resulting core atom positions then serve as a reference for a refined superposition, after which we can save the fitted structures to `corefit_structures`:

```
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")
```

We can now visualize these structures in Mol* and apply coloring based on the Atom Property **Uncertainty/Disorder**, corresponding to the **B-factor** column that stores the **pLDDT** scores.

/Users/jennyzhou/Downloads/M1_CONSERV.PDB-2.png

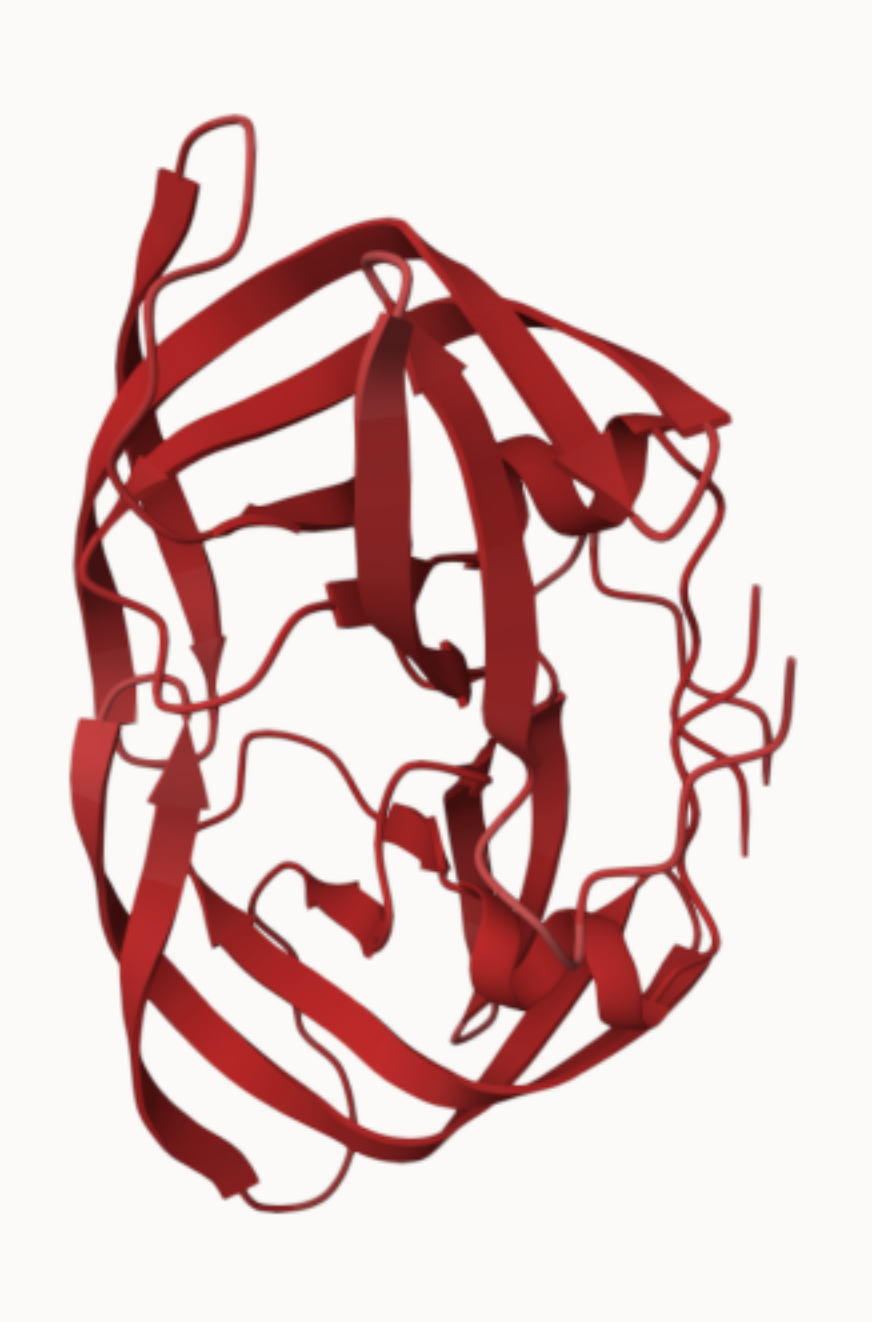
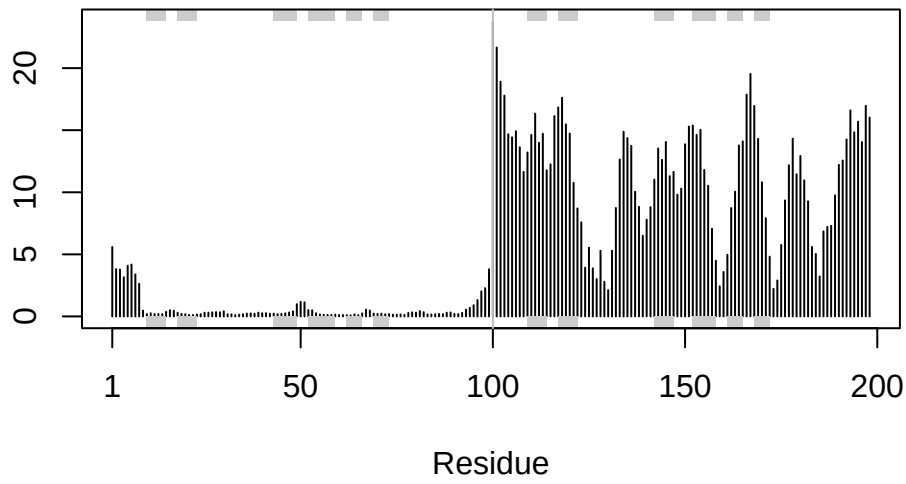


Figure 6: Figure of m1_conserv Uncertainty

Examine the RMSF between positions of the structure.

```
rf <- rmsf(xyz)

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




```
library(bio3d)

m1 <- read.pdb(pdb_files[1])
m1
```

```
 Call:  read.pdb(file = pdb_files[1])

   Total Models#: 1
     Total Atoms#: 1514,  XYZs#: 4542  Chains#: 2  (values: A B)

     Protein Atoms#: 1514  (residues/Calpha atoms#: 198)
     Nucleic acid Atoms#: 0  (residues/phosphate atoms#: 0)

     Non-protein/nucleic Atoms#: 0  (residues: 0)
     Non-protein/nucleic resid values: [ none ]
```

```
   Protein sequence:
      PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIGGFIKVRQYD
      QILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFPQITLWQRPLVTIKIGGQLKE
      ALLDTGADDTVLEEMSLPGRWKPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPTP
      VNIIGRNLLTQIGCTLNF

+ attr: atom, xyz, calpha, call
```

```
head(m1$atom)
```

```
  type eleno elety  alt resid chain resno insert      x      y      z o     b
1 ATOM     1     N <NA>   PRO     A     1   <NA> 16.922 -3.898 -6.254 1 90.81
2 ATOM     2    CA <NA>   PRO     A     1   <NA> 16.891 -2.467 -6.562 1 90.81
3 ATOM     3     C <NA>   PRO     A     1   <NA> 16.406 -1.617 -5.395 1 90.81
4 ATOM     4    CB <NA>   PRO     A     1   <NA> 15.930 -2.373 -7.746 1 90.81
5 ATOM     5     O <NA>   PRO     A     1   <NA> 15.820 -2.146 -4.445 1 90.81
6 ATOM     6    CG <NA>   PRO     A     1   <NA> 15.031 -3.559 -7.598 1 90.81
  segid elesy charge
1  <NA>     N   <NA>
2  <NA>     C   <NA>
3  <NA>     C   <NA>
4  <NA>     C   <NA>
5  <NA>     O   <NA>
6  <NA>     C   <NA>
```

```
library(bio3d)

m1 <- read.pdb(pdb_files[1])
m1
```

```
 Call:  read.pdb(file = pdb_files[1])

   Total Models#: 1
     Total Atoms#: 1514,  XYZs#: 4542  Chains#: 2  (values: A B)

     Protein Atoms#: 1514  (residues/Calpha atoms#: 198)
     Nucleic acid Atoms#: 0  (residues/phosphate atoms#: 0)

     Non-protein/nucleic Atoms#: 0  (residues: 0)
     Non-protein/nucleic resid values: [ none ]
```

```
   Protein sequence:
      PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIGGFIKVRQYD
      QILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFPQITLWQRPLVTIKIGGQLKE
      ALLDTGADDTVLEEMSLPGRWKPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPTP
      VNIIGRNLLTQIGCTLNF

+ attr: atom, xyz, calpha, call
```

```
m1$atom$b
```

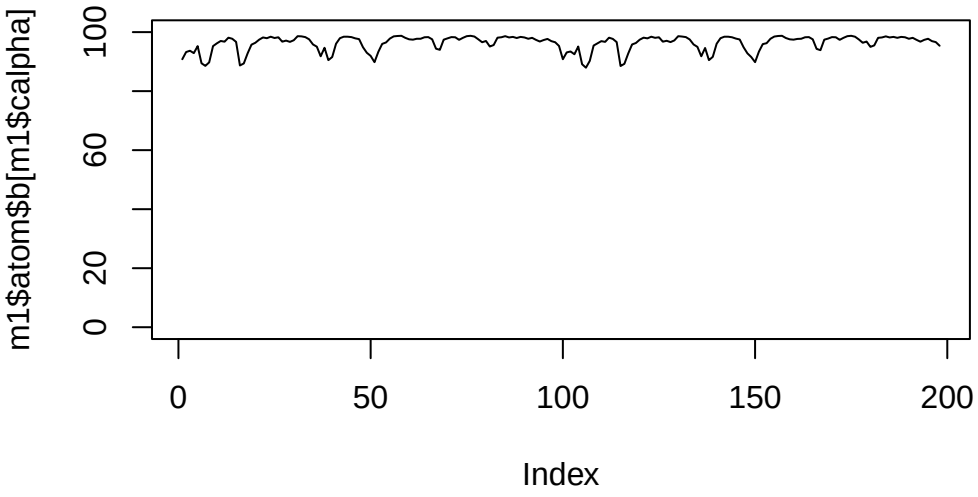
```
  [1] 90.81 90.81 90.81 90.81 90.81 90.81 90.81 93.25 93.25 93.25 93.25 93.25
 [13] 93.25 93.25 93.25 93.25 93.69 93.69 93.69 93.69 93.69 93.69 93.69 93.69
 [25] 92.88 92.88 92.88 92.88 92.88 92.88 92.88 95.25 95.25 95.25 95.25 95.25
 [37] 95.25 95.25 95.25 89.44 89.44 89.44 89.44 89.44 89.44 89.44 89.44 89.44
 [49] 89.44 89.44 89.44 89.44 89.44 88.56 88.56 88.56 88.56 88.56 88.56 88.56
 [61] 88.56 88.56 89.75 89.75 89.75 89.75 89.75 89.75 89.75 89.75 89.75 89.75
 [73] 89.75 95.25 95.25 95.25 95.25 95.25 95.25 95.25 96.19 96.19 96.19 96.19
 [85] 96.19 96.19 96.19 96.19 97.00 97.00 97.00 97.00 97.00 97.00 97.00 96.75
 [97] 96.75 96.75 96.75 96.75 96.75 96.75 98.12 98.12 98.12 98.12 98.12 98.12
[109] 98.12 98.12 97.75 97.75 97.75 97.75 97.75 97.75 97.75 97.75 97.75 96.69
[121] 96.69 96.69 96.69 96.69 96.69 96.69 96.69 88.69 88.69 88.69 88.69 89.38
[133] 89.38 89.38 89.38 92.81 92.81 92.81 92.81 92.81 92.81 92.81 92.81 92.81
[145] 95.75 95.75 95.75 95.75 95.75 95.75 95.75 95.75 96.38 96.38 96.38 96.38
[157] 96.38 96.38 96.38 96.38 96.38 97.44 97.44 97.44 97.44 97.44 97.44 97.44
[169] 97.44 97.44 98.19 98.19 98.19 98.19 98.19 97.94 97.94 97.94 97.94 97.94
[181] 97.94 97.94 97.94 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.00
[193] 98.00 98.00 98.00 98.00 98.00 98.00 98.00 98.25 98.25 98.25 98.25 98.25
[205] 98.25 98.25 96.75 96.75 96.75 96.75 97.12 97.12 97.12 97.12 97.12 96.69
[217] 96.69 96.69 96.69 96.69 96.69 96.69 96.69 97.25 97.25 97.25 97.25 97.25
[229] 97.25 97.25 97.25 98.62 98.62 98.62 98.62 98.62 98.62 98.62 98.56 98.56
[241] 98.56 98.56 98.56 98.56 98.56 98.31 98.31 98.31 98.31 98.31 98.31 98.31
[253] 98.31 97.56 97.56 97.56 97.56 97.56 97.56 97.56 97.56 97.56 95.81 95.81
[265] 95.81 95.81 95.81 95.81 95.81 95.81 95.81 95.06 95.06 95.06 95.06 95.06
[277] 95.06 95.06 95.06 91.81 91.81 91.81 91.81 91.81 91.81 94.69 94.69 94.69
[289] 94.69 94.69 94.69 94.69 94.69 90.50 90.50 90.50 90.50 90.50 90.50 90.50
[301] 91.56 91.56 91.56 91.56 96.06 96.06 96.06 96.06 96.06 96.06 96.06 96.06
[313] 96.06 96.06 96.06 97.94 97.94 97.94 97.94 97.94 97.94 97.94 97.94 97.94
[325] 97.94 97.94 97.94 97.94 97.94 98.44 98.44 98.44 98.44 98.44 98.44 98.44
[337] 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.31 98.31 98.31
[349] 98.31 98.31 98.31 98.31 98.31 98.31 97.88 97.88 97.88 97.88 97.88 97.88
[361] 97.88 97.88 97.62 97.62 97.62 97.62 97.62 97.62 97.62 97.62 94.88 94.88
```

```
[373] 94.88 94.88 93.00 93.00 93.00 93.00 91.94 91.94 91.94 91.94 91.94 91.94
[385] 91.94 91.94 89.81 89.81 89.81 89.81 93.31 93.31 93.31 93.31 96.00 96.00
[397] 96.00 96.00 96.00 96.00 96.00 96.00 96.00 96.00 96.00 96.50 96.50 96.50
[409] 96.50 96.50 96.50 96.50 96.50 97.81 97.81 97.81 97.81 97.81 97.81 97.81
[421] 97.81 97.81 98.56 98.56 98.56 98.56 98.56 98.56 98.56 98.69 98.69 98.69
[433] 98.69 98.69 98.69 98.69 98.69 98.69 98.69 98.69 98.75 98.75 98.75 98.75
[445] 98.75 98.75 98.75 98.75 98.75 98.06 98.06 98.06 98.06 98.06 98.06 98.06
[457] 98.06 98.06 98.06 98.06 98.06 97.56 97.56 97.56 97.56 97.56 97.56 97.56
[469] 97.56 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.75 97.75
[481] 97.75 97.75 97.75 97.75 97.75 97.75 97.75 97.75 97.75 97.75 97.75 97.75
[493] 97.75 97.75 98.25 98.25 98.25 98.25 98.25 98.25 98.25 98.25 98.31 98.31
[505] 98.31 98.31 98.31 98.31 98.31 98.31 98.31 97.56 97.56 97.56 97.56 97.56
[517] 97.56 97.56 97.56 94.38 94.38 94.38 94.38 94.38 94.38 94.00 94.00 94.00
[529] 94.00 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.88
[541] 97.88 97.88 97.88 97.88 97.88 97.88 97.88 97.88 98.31 98.31 98.31 98.31
[553] 98.31 98.25 98.25 98.25 98.25 98.25 98.25 98.25 98.25 97.38 97.38 97.38
[565] 97.38 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.62 98.62 98.62 98.62
[577] 98.62 98.62 98.62 98.75 98.75 98.75 98.75 98.75 98.75 98.75 98.75 98.50
[589] 98.50 98.50 98.50 98.50 98.50 98.50 97.56 97.56 97.56 97.56 96.56 96.56
[601] 96.56 96.56 96.56 96.56 96.56 97.00 97.00 97.00 97.00 97.00 97.00 97.00
[613] 95.12 95.12 95.12 95.12 95.12 95.12 95.12 95.62 95.62 95.62 95.62 95.62
[625] 95.62 95.62 98.12 98.12 98.12 98.12 98.12 98.12 98.12 98.12 98.25 98.25
[637] 98.25 98.25 98.25 98.25 98.25 98.25 98.62 98.62 98.62 98.62 98.62 98.62
[649] 98.62 98.62 98.19 98.19 98.19 98.19 98.38 98.38 98.38 98.38 98.38 98.38
[661] 98.38 98.38 98.38 98.38 98.38 98.00 98.00 98.00 98.00 98.00 98.00 98.00
[673] 98.00 98.38 98.38 98.38 98.38 98.38 98.38 98.38 98.38 98.19 98.19 98.19
[685] 98.19 98.19 98.19 98.19 98.19 97.75 97.75 97.75 97.75 97.75 97.75 97.75
[697] 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.06 97.38 97.38 97.38
[709] 97.38 97.38 97.38 97.38 97.38 96.81 96.81 96.81 96.81 97.31 97.31 97.31
[721] 97.31 97.31 97.31 97.69 97.69 97.69 97.69 97.69 97.69 97.69 96.94 96.94
[733] 96.94 96.94 96.94 96.94 96.94 96.94 96.56 96.56 96.56 96.56 96.56 96.56
[745] 96.56 96.56 95.31 95.31 95.31 95.31 95.31 95.31 95.31 95.31 95.31 95.31
[757] 95.31 90.81 90.81 90.81 90.81 90.81 90.81 90.81 93.12 93.12 93.12 93.12
[769] 93.12 93.12 93.12 93.12 93.12 93.44 93.44 93.44 93.44 93.44 93.44 93.44
[781] 93.44 92.50 92.50 92.50 92.50 92.50 92.50 92.50 95.12 95.12 95.12 95.12
[793] 95.12 95.12 95.12 95.12 89.12 89.12 89.12 89.12 89.12 89.12 89.12 89.12
[805] 89.12 89.12 89.12 89.12 89.12 89.12 87.94 87.94 87.94 87.94 87.94 87.94
[817] 87.94 87.94 87.94 90.25 90.25 90.25 90.25 90.25 90.25 90.25 90.25 90.25
[829] 90.25 90.25 95.44 95.44 95.44 95.44 95.44 95.44 95.44 96.19 96.19 96.19
[841] 96.19 96.19 96.19 96.19 96.19 96.94 96.94 96.94 96.94 96.94 96.94 96.94
[853] 96.69 96.69 96.69 96.69 96.69 96.69 96.69 98.12 98.12 98.12 98.12 98.12
[865] 98.12 98.12 98.12 97.69 97.69 97.69 97.69 97.69 97.69 97.69 97.69 97.69
[877] 96.62 96.62 96.62 96.62 96.62 96.62 96.62 96.62 88.50 88.50 88.50 88.50
```

```
 [889] 89.25 89.25 89.25 89.25 92.75 92.75 92.75 92.75 92.75 92.75 92.75 92.75
 [901] 92.75 95.81 95.81 95.81 95.81 95.81 95.81 95.81 95.81 96.31 96.31 96.31
 [913] 96.31 96.31 96.31 96.31 96.31 96.31 97.44 97.44 97.44 97.44 97.44 97.44
 [925] 97.44 97.44 97.44 98.12 98.12 98.12 98.12 98.12 97.88 97.88 97.88 97.88
 [937] 97.88 97.88 97.88 97.88 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.44
 [949] 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.25 98.25 98.25 98.25
 [961] 98.25 98.25 98.25 96.75 96.75 96.75 96.75 97.06 97.06 97.06 97.06 97.06
 [973] 96.62 96.62 96.62 96.62 96.62 96.62 96.62 96.62 97.19 97.19 97.19 97.19
 [985] 97.19 97.19 97.19 97.19 98.62 98.62 98.62 98.62 98.62 98.62 98.62 98.50
 [997] 98.50 98.50 98.50 98.50 98.50 98.50 98.31 98.31 98.31 98.31 98.31 98.31
[1009] 98.31 98.31 97.50 97.50 97.50 97.50 97.50 97.50 97.50 97.50 97.50 95.81
[1021] 95.81 95.81 95.81 95.81 95.81 95.81 95.81 95.81 95.00 95.00 95.00 95.00
[1033] 95.00 95.00 95.00 95.00 91.81 91.81 91.81 91.81 91.81 91.81 94.62 94.62
[1045] 94.62 94.62 94.62 94.62 94.62 94.62 90.50 90.50 90.50 90.50 90.50 90.50
[1057] 90.50 91.62 91.62 91.62 91.62 96.06 96.06 96.06 96.06 96.06 96.06 96.06
[1069] 96.06 96.06 96.06 96.06 97.94 97.94 97.94 97.94 97.94 97.94 97.94 97.94
[1081] 97.94 97.94 97.94 97.94 97.94 97.94 98.44 98.44 98.44 98.44 98.44 98.44
[1093] 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.44 98.25 98.25
[1105] 98.25 98.25 98.25 98.25 98.25 98.25 98.25 97.81 97.81 97.81 97.81 97.81
[1117] 97.81 97.81 97.81 97.50 97.50 97.50 97.50 97.50 97.50 97.50 97.50 94.88
[1129] 94.88 94.88 94.88 92.81 92.81 92.81 92.81 91.56 91.56 91.56 91.56 91.56
[1141] 91.56 91.56 91.56 89.81 89.81 89.81 89.81 93.38 93.38 93.38 93.38 95.94
[1153] 95.94 95.94 95.94 95.94 95.94 95.94 95.94 95.94 95.94 95.94 96.25 96.25
[1165] 96.25 96.25 96.25 96.25 96.25 96.25 97.75 97.75 97.75 97.75 97.75 97.75
[1177] 97.75 97.75 97.75 98.50 98.50 98.50 98.50 98.50 98.50 98.50 98.69 98.69
[1189] 98.69 98.69 98.69 98.69 98.69 98.69 98.69 98.69 98.69 98.75 98.75 98.75
[1201] 98.75 98.75 98.75 98.75 98.75 98.75 98.00 98.00 98.00 98.00 98.00 98.00
[1213] 98.00 98.00 98.00 98.00 98.00 98.00 97.56 97.56 97.56 97.56 97.56 97.56
[1225] 97.56 97.56 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.69
[1237] 97.69 97.69 97.69 97.69 97.69 97.69 97.69 97.75 97.75 97.75 97.75 97.75
[1249] 97.75 97.75 97.75 98.25 98.25 98.25 98.25 98.25 98.25 98.25 98.25 98.31
[1261] 98.31 98.31 98.31 98.31 98.31 98.31 98.31 98.31 97.56 97.56 97.56 97.56
[1273] 97.56 97.56 97.56 97.56 94.31 94.31 94.31 94.31 94.31 94.31 93.88 93.88
[1285] 93.88 93.88 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44 97.44
[1297] 97.81 97.81 97.81 97.81 97.81 97.81 97.81 97.81 97.81 98.31 98.31 98.31
[1309] 98.31 98.31 98.25 98.25 98.25 98.25 98.25 98.25 98.25 98.25 97.38 97.38
[1321] 97.38 97.38 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.62 98.62 98.62
[1333] 98.62 98.62 98.62 98.62 98.75 98.75 98.75 98.75 98.75 98.75 98.75 98.75
[1345] 98.44 98.44 98.44 98.44 98.44 98.44 98.44 97.50 97.50 97.50 97.50 96.38
[1357] 96.38 96.38 96.38 96.38 96.38 96.38 96.81 96.81 96.81 96.81 96.81 96.81
[1369] 96.81 95.00 95.00 95.00 95.00 95.00 95.00 95.00 95.50 95.50 95.50 95.50
[1381] 95.50 95.50 95.50 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.19
[1393] 98.19 98.19 98.19 98.19 98.19 98.19 98.19 98.56 98.56 98.56 98.56 98.56
```

```
[1405] 98.56 98.56 98.56 98.19 98.19 98.19 98.19 98.38 98.38 98.38 98.38 98.38
[1417] 98.38 98.38 98.38 98.38 98.38 98.38 98.06 98.06 98.06 98.06 98.06 98.06
[1429] 98.06 98.06 98.38 98.38 98.38 98.38 98.38 98.38 98.38 98.38 98.25 98.25
[1441] 98.25 98.25 98.25 98.25 98.25 98.25 97.75 97.75 97.75 97.75 97.75 97.75
[1453] 97.75 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.06 98.06 97.38 97.38
[1465] 97.38 97.38 97.38 97.38 97.38 97.38 96.75 96.75 96.75 96.75 97.38 97.38
[1477] 97.38 97.38 97.38 97.38 97.75 97.75 97.75 97.75 97.75 97.75 97.75 96.94
[1489] 96.94 96.94 96.94 96.94 96.94 96.94 96.94 96.62 96.62 96.62 96.62 96.62
[1501] 96.62 96.62 96.62 95.38 95.38 95.38 95.38 95.38 95.38 95.38 95.38 95.38
[1513] 95.38 95.38
```

```
plot( m1$atom$b[m1$calpha],typ="l", ylim=c(0,100))
```



Predicted Alignment Error for domains

AlphaFold also provides a *Predicted Aligned Error (PAE)* output, stored in per-model JSON files. PAE measures the expected positional error between residues, independent of the 3D structure.

In R, we can read these files with jsonlite

```
library(jsonlite)

pae_files <- list.files(path=results_dir,
                        pattern=".*model.*\\.json",
                        full.names = TRUE)
```

for example

```
pae1 <- jsonlite::read_json(pae_files[1], simplifyVector = TRUE)
pae5 <- jsonlite::read_json(pae_files[5], simplifyVector = TRUE)
attributes(pae1)
```

```
$names
[1] "plddt"   "max_pae" "pae"     "ptm"     "iptm"
```

```
head(pae1$plddt)
```

```
[1] 90.81 93.25 93.69 92.88 95.25 89.44
```

Each file contains per-residue pLDDT, max_pae, and full pae matrices. Lower PAE values indicate higher confidence. For example:

```
pae1$max_pae  #(better)
```

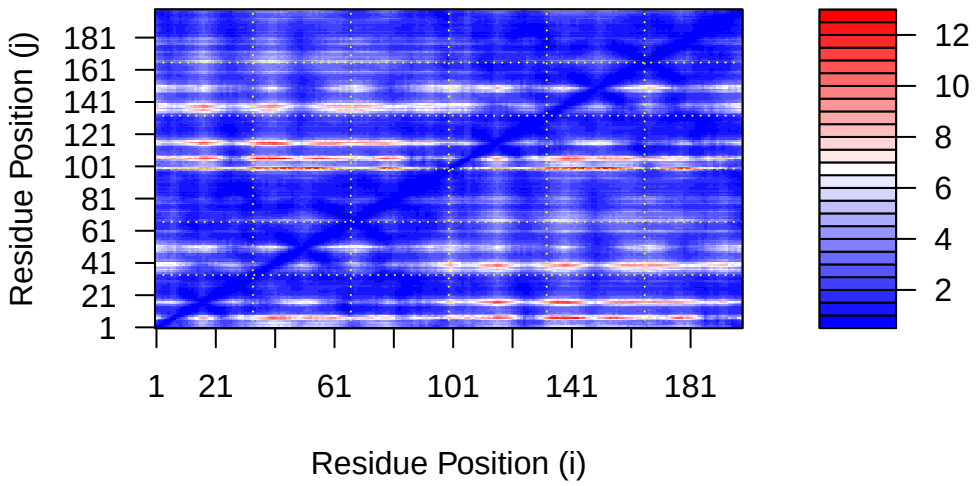
```
[1] 12.84375
```

```
pae5$max_pae  # (worse)
```

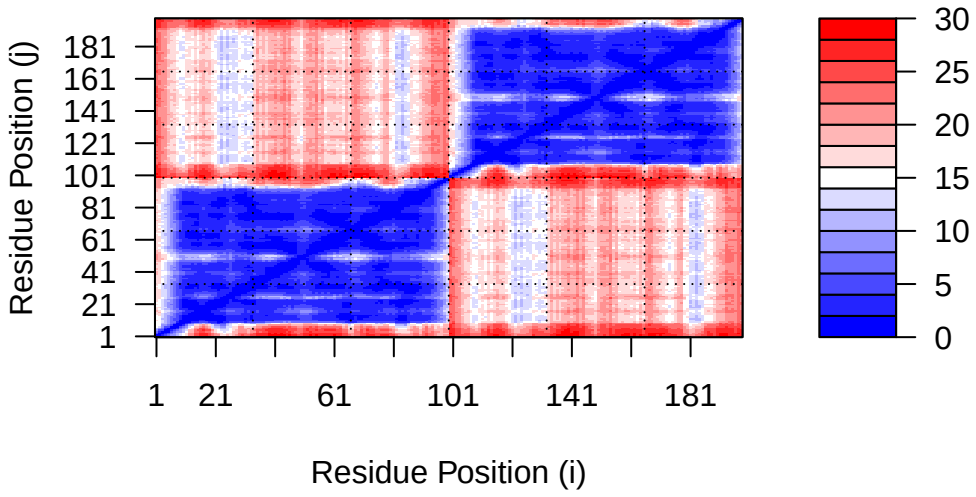
```
[1] 29.59375
```

We can visualize the residue-to-residue PAE matrices with Bio3D:

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

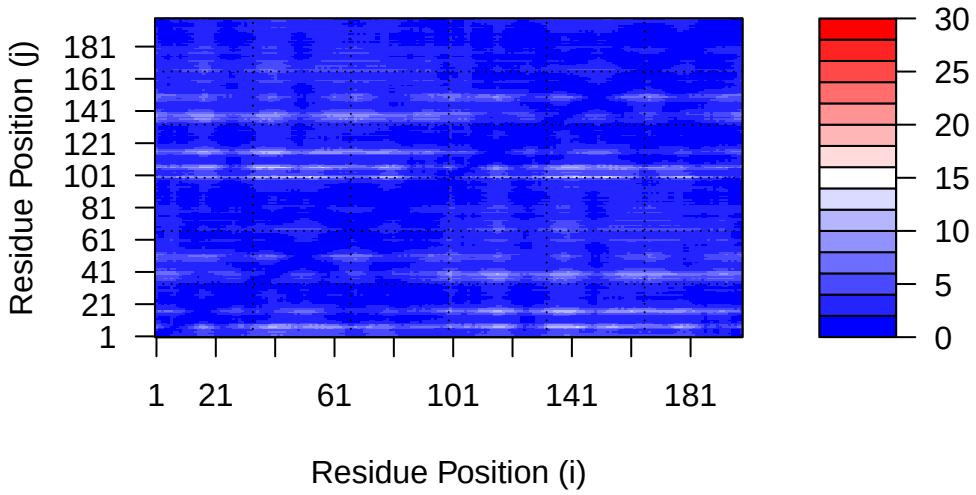



```
plot.dmat(pae5$pae,
          xlab="Residue Position (i)",
          ylab="Residue Position (j)",
          grid.col = "black",
          zlim=c(0,30))
```



Using the same z-range across models allows direct comparison

```
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] "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)
```

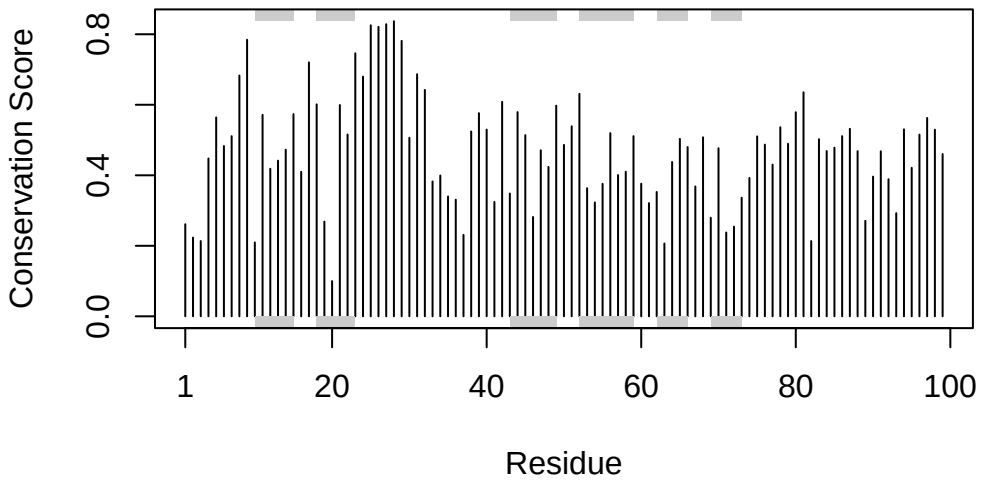
```
[1] 5397  132
```

The alignment contains 5,397 sequences and 132 positions.

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")
```



Highly conserved active-site residues (D25–A28) are evident. A consensus sequence at a 0.9 cutoff confirms these conserved motifs:

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

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

To visualize conservation, we mapped these scores to the **Occupancy** column of a PDB file:

```
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")
```

```
getwd()
```

```
[1] "/Users/jennyzhou/Desktop/BIMM 143/github/Class 11"
```

```
list.files()
```

```
 [1] "Accessible_Surface_Area.png"
 [2] "aln.fa"
 [3] "Class 11_files"
 [4] "Class 11.html"
 [5] "Class 11.qmd"
 [6] "Class 11.rmarkdown"
 [7] "Class 11.Rproj"
 [8] "Class-11_files"
 [9] "Class-11.rmarkdown"
[10] "corefit_structures"
[11] "Fitted_and_pLDDT_colored_Nras_structure models.png"
[12] "HIVPRDimer_23119"
[13] "HIVPRDimer_23119_coverage.png"
[14] "HIVPRDimer_23119_pae.png"
[15] "HIVPRDimer_23119_plddt.png"
[16] "Hydrophobicity.png"
[17] "m1_conserv.pdb"
[18] "M1_CONSERV.PDB-2.png"
[19] "M1_CONSERV.PDB.png"
[20] "Secondary_structure.png"
```

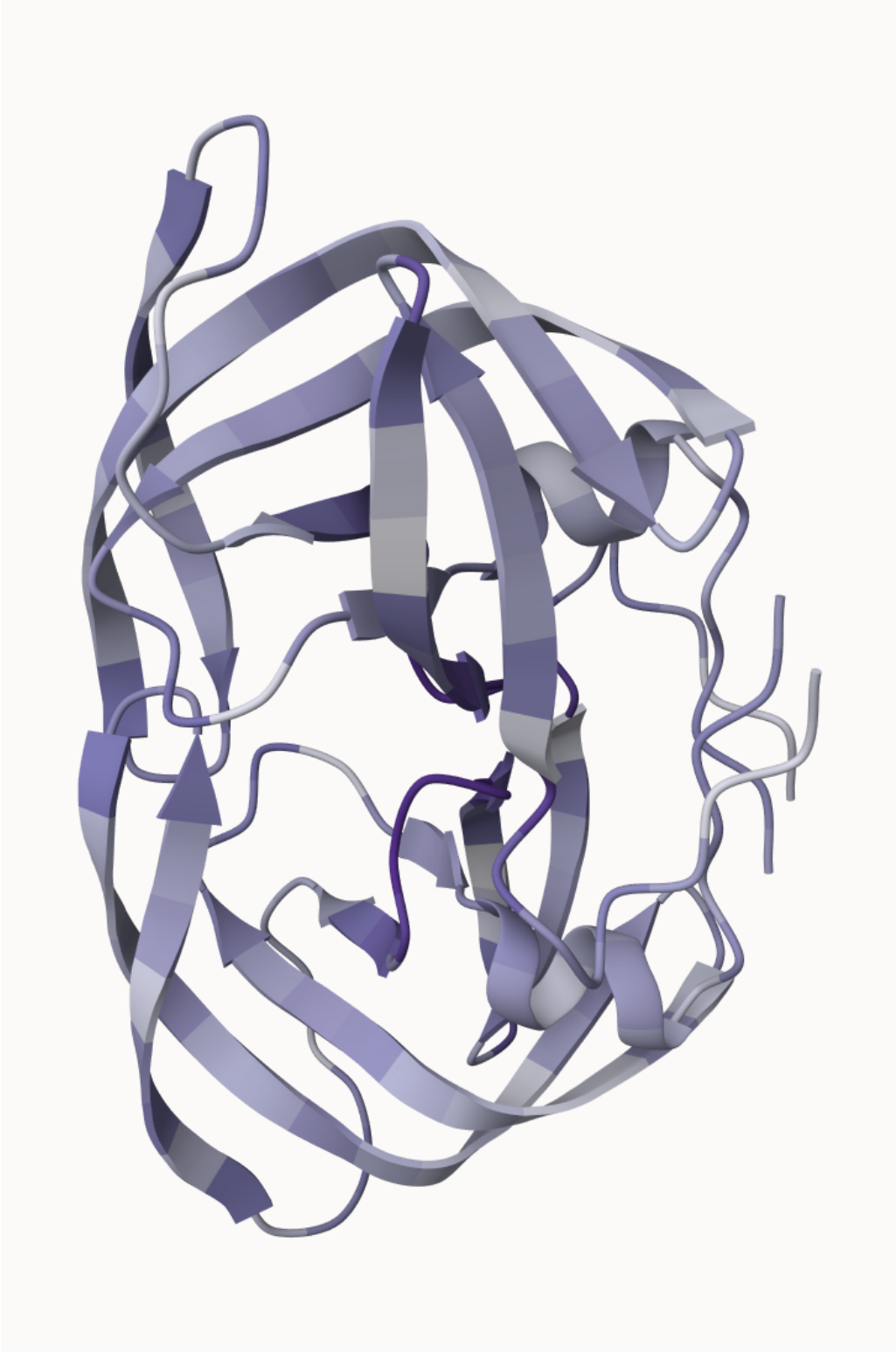


Figure 7: Figure of m1_conserv Occupancy