

Class 11: AlphaFold

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Background

We saw last day that the main repository for biomolecular structure (the PDB database) only has ~250,000 entries.

UniProtKB (the main protein sequence database) has over 200 million entries!

AlphaFold

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 EBI AlphaFold database

The EBI AlphaFold database contains lots of computed structure models. It is increasingly likely that the structure you are interested in is already in this database at <https://alphafold.ebi.ac.uk/>

There are 3 major outputs from AlphaFold

1. A model of structure in **PDB** format.
2. a **PLDDT score**: that tells us how confident the model is for a given residue in your protein (High values are good, above 70)
3. a **PAE score** that tells us about protein packing quality.

If you can't find a matching entry for the sequence you are interested in AFDB you can run AlphaFold yourself...

Running AlphaFold

We will use ColabFold to run AlphaFold on our sequence <https://github.com/sokrypton/ColabFold>

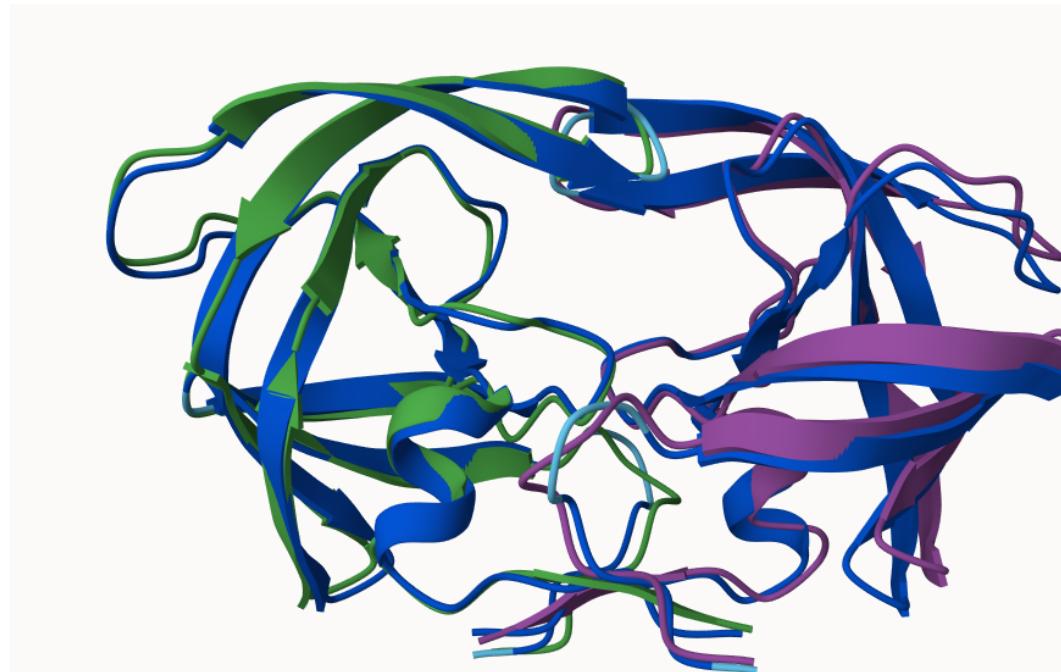


Figure from AlphaFold here!

Interpreting Results

Custom analysis of resulting models

We can read all the AlphaFold results into R and do more quantitative analysis than just viewing the structures in Mol-star:

Read all the PDB models:

```
library(bio3d)
p <- read.pdb("hivpr_23119/hivpr_23119_unrelaxed_rank_001_alphaFold2_multimer_v3_model_4_seed_000.pdb")
```

```
pdb_files <- list.files("hivpr_23119/", pattern = ".pdb", full.names = T)
```

```
pdb <- pdbs(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_seed_000
pdb/seq: 2 name: hivpr_23119//hivpr_23119_unrelaxed_rank_002_alphaFold2_multimer_v3_model_1_seed_000
pdb/seq: 3 name: hivpr_23119//hivpr_23119_unrelaxed_rank_003_alphaFold2_multimer_v3_model_5_seed_000
pdb/seq: 4 name: hivpr_23119//hivpr_23119_unrelaxed_rank_004_alphaFold2_multimer_v3_model_2_seed_000
pdb/seq: 5 name: hivpr_23119//hivpr_23119_unrelaxed_rank_005_alphaFold2_multimer_v3_model_3_seed_000
```

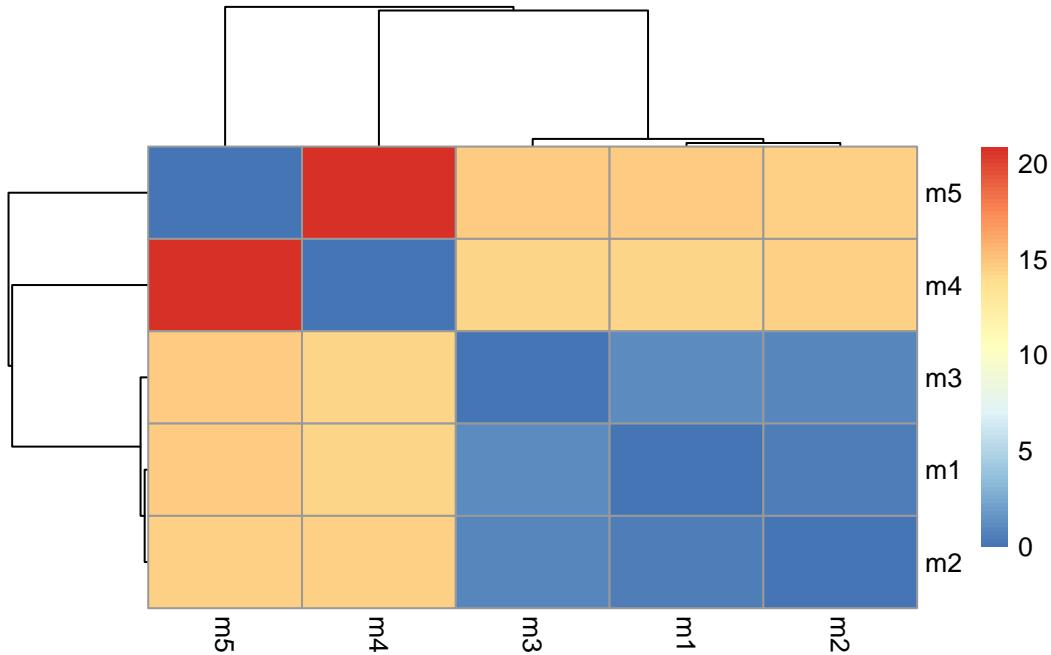
```
#library(bio3dview)
#view.pdb(pdb)
```

How similar or different are my models?

```
rd <- rmsd(pdb)
```

```
Warning in rmsd(pdb): No indices provided, using the 198 non NA positions
```

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



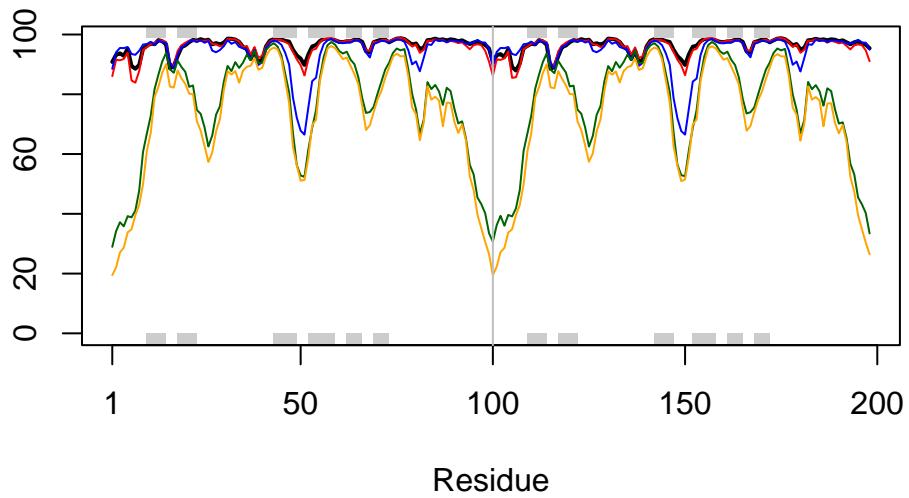
Custom analysis of resulting models

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")
```



```
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)
```

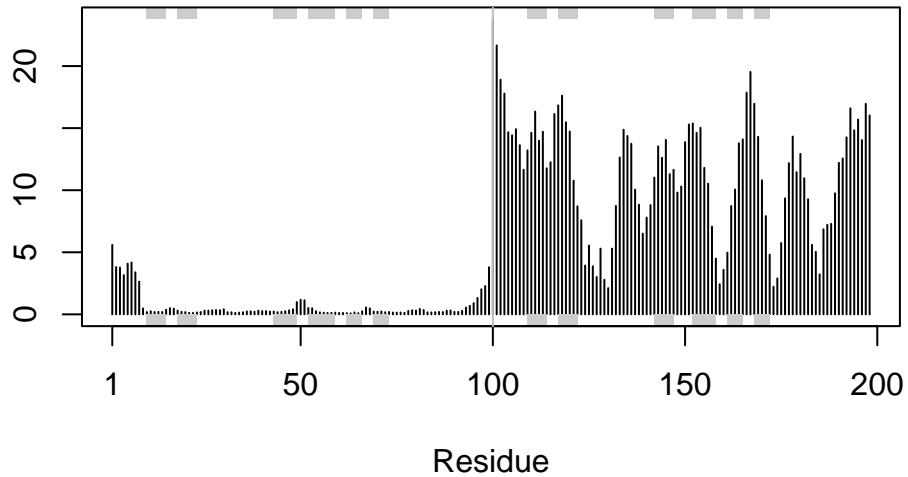
```
# 85 positions (cumulative volume <= 0.5 Angstrom^3)
  start end length
1      9   49     41
2     52   95     44
```

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

Examining the RMSF between positions of the structure of 1hsg.

```
rf <- rmsf(xyz)

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



Predicted Alignment Error for domains

```

library(jsonlite)

# Listing of all PAE JSON files
pae_files <- list.files(path="hivpr_23119/",
                         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
```

Maximum PAE values

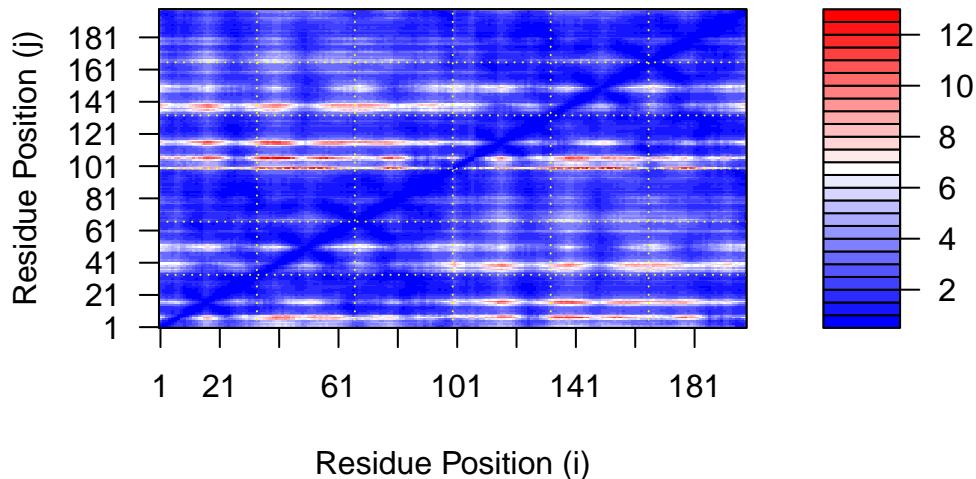
```
pae1$max_pae
```

```
[1] 12.84375
```

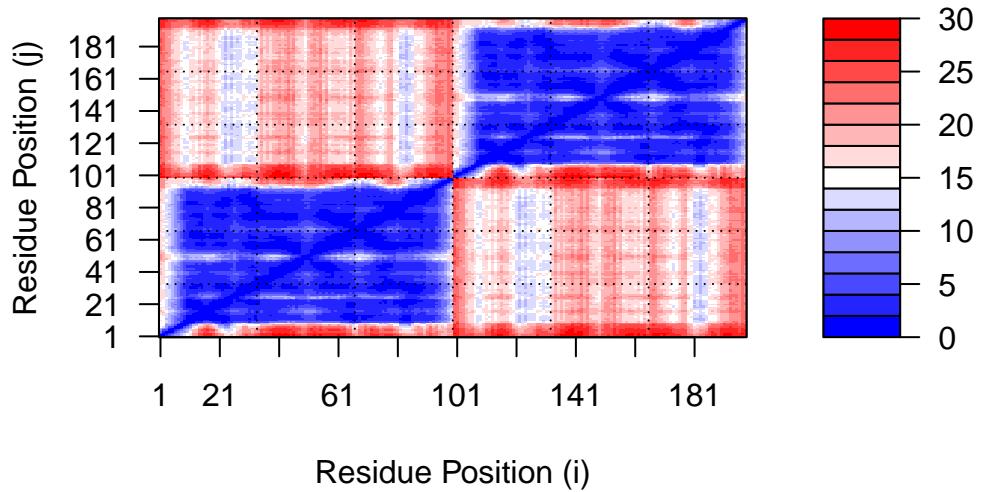
```
pae5$max_pae
```

```
[1] 29.59375
```

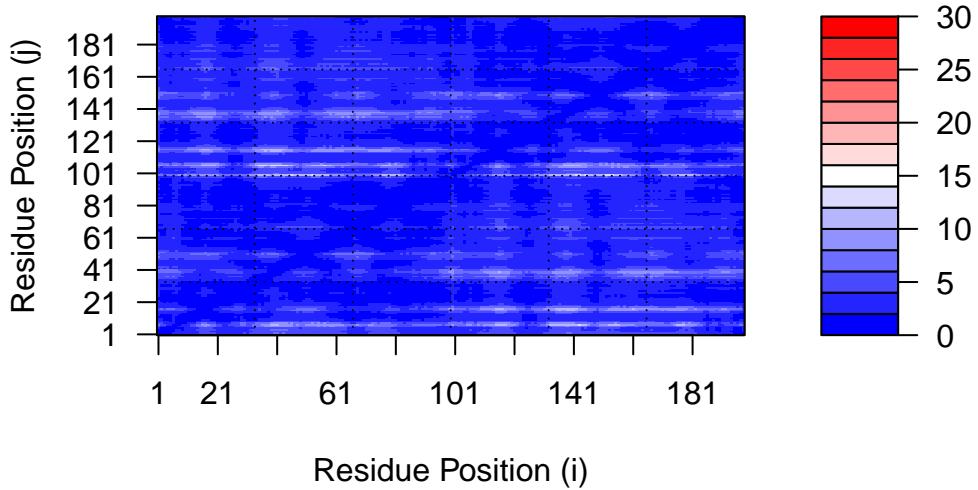
```
plot.dmat(pae1$pae,
           xlab="Residue Position (i)",
           ylab="Residue Position (j)")
```



```
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="hivpr_23119/",
                        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 **"
```

Number of sequences in this alignment

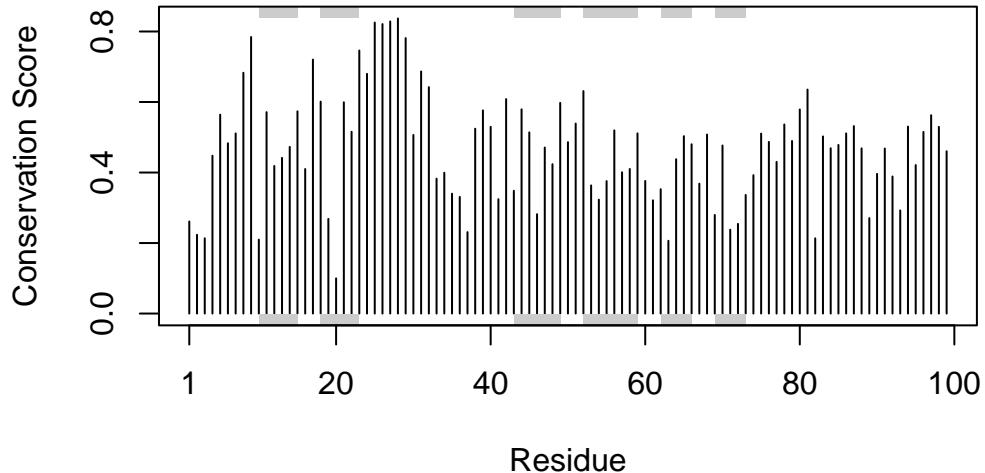
```
dim(aln$ali)
```

```
[1] 5397 132
```

Residue conservation

```
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")
```