# Longer ORF PDB search and results

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The code in this document is made to be useful with either the long or the short ORF, but in the set-up below the sequence to use is set to the long ORF.

```
# Set-up
library("bio3d")
library("ggplot2")
library("ggrepel")
library("msa")
library("bio3d.view")

# Load sequence of POI
seqL <- read.fasta("long.ORF.fa")
seqS <- read.fasta("short.ORF.fa")

#Choose which sequence to use
seq <- seqS
```

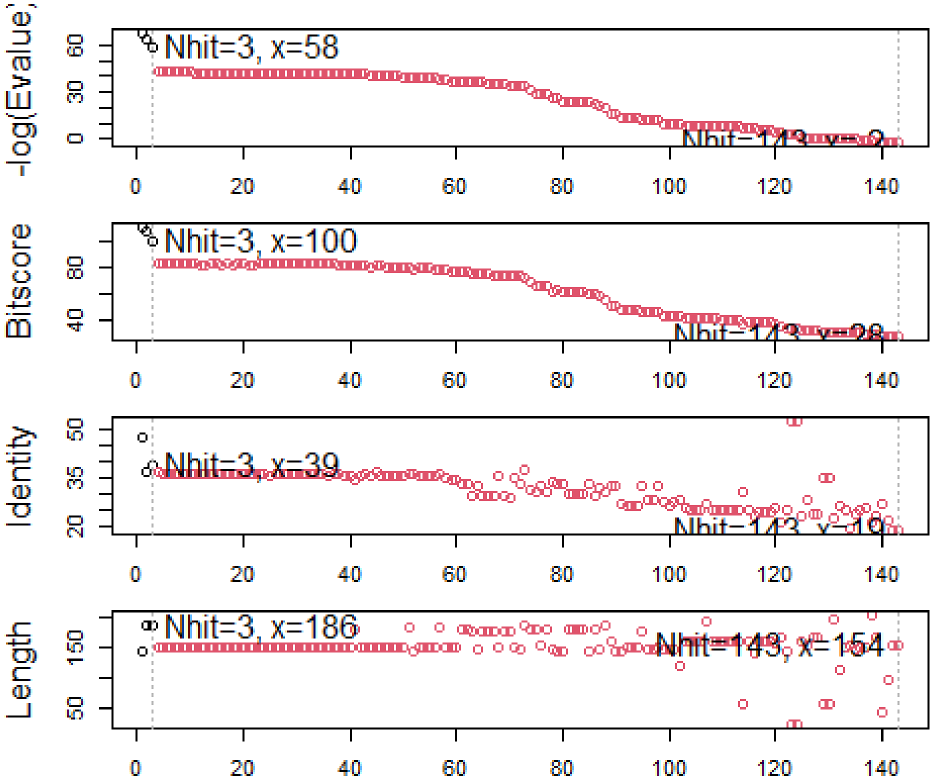
After set up the blast search can be completed and the summary statistics of this search can be plotted.

```
# Blast search
blast <- blast.pdb(seq, database = "pdb")

##  Searching ... please wait (updates every 5 seconds) RID = 1ZR0NWEN013
##  .
##  Reporting 143 hits

# Plot summary statistics of results
hits <- plot(blast)

##   * Possible cutoff values:    58 -3
##             Yielding Nhits:    3 143
##
##   * Chosen cutoff value of:    58
##             Yielding Nhits:    3
```



```
# Print the IDs of the hits above the threshold
hit.IDs <- hits$pdb.id
hit.IDs

## [1] "6GYH_A" "7BMH_A" "5AWZ_A"
```

There are 3 hits that pass the statistical threshold, namely: 6GYH_A, 7BMH_A, 5AWZ_A. More information can be found on these by interrogating the blast results.

```
# Show the hit table for the top hits which pass the threshold
head(blast$hit.tbl, n = length(hit.IDs))

##       queryid subjectids identity alignmentlength mismatches gapopens
q.start
## 1 Query_40791     6GYH_A   47.222             144         74        2
35
## 2 Query_40791     7BMH_A   36.898             187        112        2
1
## 3 Query_40791     5AWZ_A   38.710             186         85        5
2
##   q.end s.start s.end   evalue bitscore positives mlog.evalue pdb.id
acc
## 1   177      77   219 7.74e-30      110     61.81    67.03115 6GYH_A
6GYH_A
## 2   181      92   278 2.13e-28      108     52.94    63.71626 7BMH_A
7BMH_A
```

```
## 3   176      61   228 4.29e-26      100     51.08    58.41093 5AWZ_A
5AWZ_A
```

We can also download these PDB files, annotate them for more information and align them with our sequence to get an overview of sequence alignment.

```
# Download related PDB files
files <- get.pdb(hits$pdb.id, path="pdbs", split=TRUE, gzip=TRUE)
```

```
##   |
|                                                                      |   0%
|
|=======================                                               |  33%
|
|===============================================                       |  67%
|
|======================================================================| 100%
```

```
# Align related PDBs
pdbs <- pdbaln(files, fit = TRUE, exefile="msa")
```

```
## Reading PDB files:
## pdbs/split_chain/6GYH_A.pdb
## pdbs/split_chain/7BMH_A.pdb
## pdbs/split_chain/5AWZ_A.pdb
##    PDB has ALT records, taking A only, rm.alt=TRUE
## .   PDB has ALT records, taking A only, rm.alt=TRUE
## .   PDB has ALT records, taking A only, rm.alt=TRUE
## .
##
## Extracting sequences
##
## pdb/seq: 1   name: pdbs/split_chain/6GYH_A.pdb
##    PDB has ALT records, taking A only, rm.alt=TRUE
## pdb/seq: 2   name: pdbs/split_chain/7BMH_A.pdb
##    PDB has ALT records, taking A only, rm.alt=TRUE
## pdb/seq: 3   name: pdbs/split_chain/5AWZ_A.pdb
##    PDB has ALT records, taking A only, rm.alt=TRUE
```

```
# Vector containing PDB codes for figure axis
ids <- basename.pdb(pdbs$id)

# Annotate hits for more information on the hits
anno <- pdb.annotate(ids)

# Find the organisms these PDB hits come from
unique(anno$source)
```

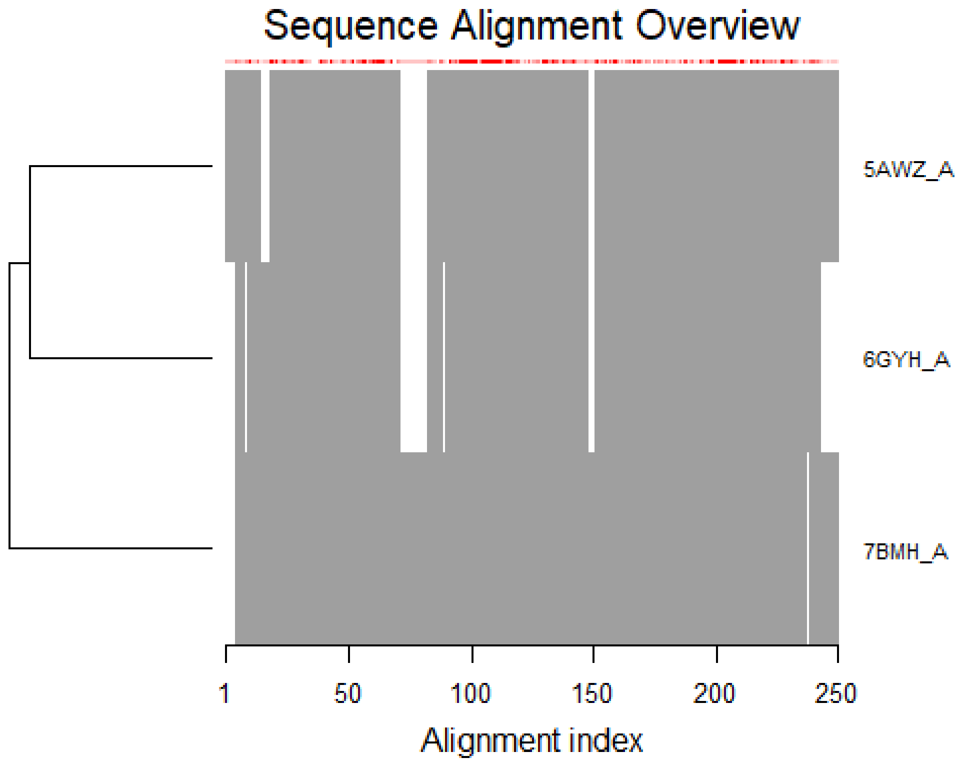
```
## [1] "Coccomyxa subellipsoidea C-169" "Leptosphaeria maculans"
## [3] "Acetabularia acetabulum"
```

```
# View more information on the hits
anno

##        structureId chainId macromoleculeType chainLength
experimentalTechnique
## 6GYH_A        6GYH       A           Protein         236
X-ray
## 7BMH_A        7BMH       A           Protein         324
X-ray
## 5AWZ_A        5AWZ       A           Protein         244
X-ray
##        resolution scopDomain
pfam
## 6GYH_A       2.00       <NA> Bacteriorhodopsin-like protein
(Bac_rhodopsin)
## 7BMH_A       2.20       <NA> Bacteriorhodopsin-like protein
(Bac_rhodopsin)
## 5AWZ_A       1.57       <NA> Bacteriorhodopsin-like protein
(Bac_rhodopsin)
##                                   ligandId
## 6GYH_A                     RET,CLR,OLB (4)
## 7BMH_A                    LFA (22),OLA (3)
## 5AWZ_A OCT (2),C14,RET,OLB,D12 (2),D10 (3)
##
ligandName
## 6GYH_A                                  RETINAL,CHOLESTEROL,(2S)-2,3-
dihydroxypropyl (9Z)-octadec-9-enoate (4)
## 7BMH_A
EICOSANE (22),OLEIC ACID (3)
## 5AWZ_A N-OCTANE (2),TETRADECANE,RETINAL,(2S)-2,3-dihydroxypropyl (9Z)-
octadec-9-enoate,DODECANE (2),DECANE (3)
##                                source
## 6GYH_A Coccomyxa subellipsoidea C-169
## 7BMH_A         Leptosphaeria maculans
## 5AWZ_A        Acetabularia acetabulum
##
structureTitle
## 6GYH_A                    Crystal structure of the light-driven proton
pump Coccomyxa subellipsoidea Rhodopsin CsR
## 7BMH_A                        Crystal structure of a light-driven proton
pump LR (Mac) from Leptosphaeria maculans
## 5AWZ_A Crystal Structure of the Cell-Free Synthesized Membrane Protein,
Acetabularia Rhodopsin I, at 1.57 angstrom
##                                                              citation
rObserved
## 6GYH_A                           Fudim, R., et al. Sci Signal (2019)
0.19398
## 7BMH_A                      Zabelskii, D., et al. Commun Biol (2021)
0.23840
## 5AWZ_A Furuse, M., et al. Acta Crystallogr D Biol Crystallogr (2015)
```

```
0.17760
##            rFree   rWork  spaceGroup
## 6GYH_A 0.22493 0.19231         H 3
## 7BMH_A 0.28470 0.23610 P 21 21 21
## 5AWZ_A 0.19410 0.17690    C 1 2 1

# Draw schematic alignment
plot(pdbs, labels=ids)
```

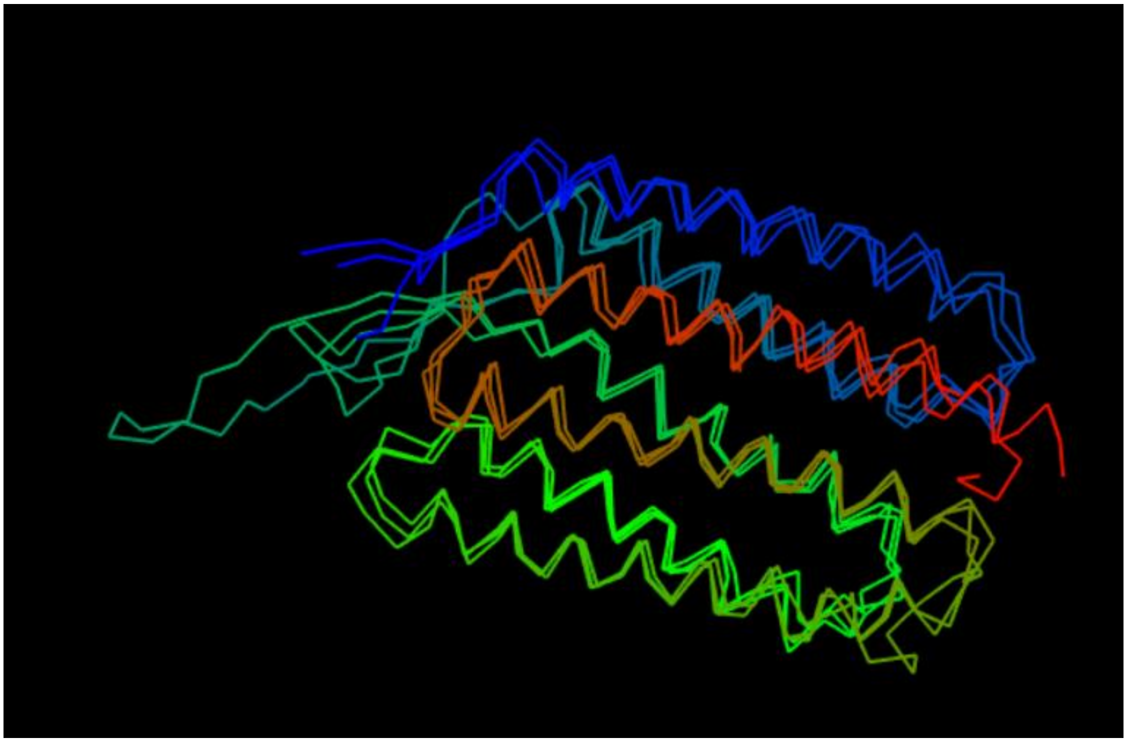


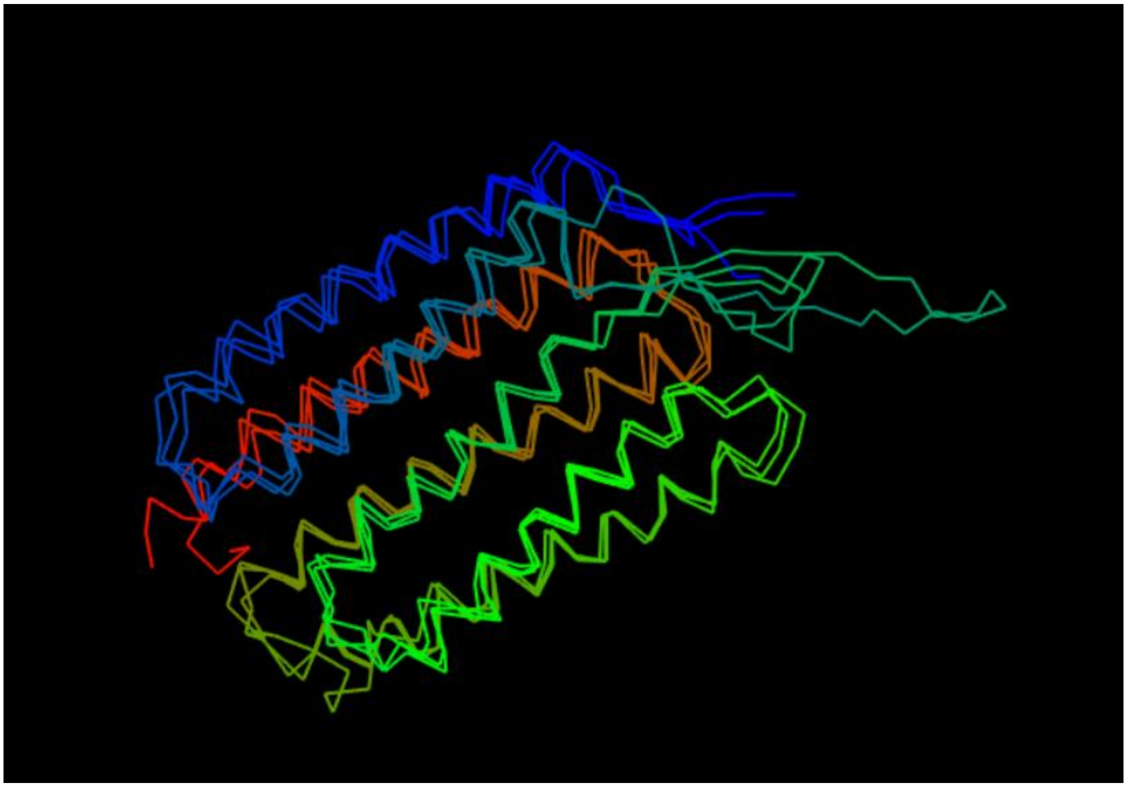
We can also plot the three structures we have found as follows:

```
# Set up
library(bio3d.view)
library(rgl)

# Plot
#view.pdbs(pdbs)
```

The `View.pdbs()` function brings up an interactive viewer, which cannot be directly viewed in the markdown document, so instead two screen-shots of this have been inserted.





With more proteins it could be interesting to plot variability, or even do PCA using the amino acid position data, but with only three proteins this is not useful.

## Session Information

```
sessionInfo()
```

```
## R version 4.1.2 (2021-11-01)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 22000)
## 
## Matrix products: default
## 
## locale:
## [1] LC_COLLATE=English_United Kingdom.1252 
## [2] LC_CTYPE=English_United Kingdom.1252   
## [3] LC_MONETARY=English_United Kingdom.1252
## [4] LC_NUMERIC=C                           
## [5] LC_TIME=English_United Kingdom.1252    
## 
## attached base packages:
## [1] stats4    stats     graphics  grDevices utils     datasets  methods  
## [8] base     
## 
## other attached packages:
##  [1] rgl_0.108.3           bio3d.view_0.1.0.9000 msa_1.26.0           
##  [4] Biostrings_2.62.0     GenomeInfoDb_1.30.1   XVector_0.34.0       
##  [7] IRanges_2.28.0        S4Vectors_0.32.3      BiocGenerics_0.40.0  
## [10] ggrepel_0.9.1         ggplot2_3.3.5         bio3d_2.4-3.9000     
## 
## loaded via a namespace (and not attached):
##  [1] tidyselect_1.1.2       xfun_0.29              purrr_0.3.4           
##  [4] colorspace_2.0-2       vctrs_0.3.8            generics_0.1.2        
##  [7] htmltools_0.5.2        yaml_2.2.2             utf8_1.2.2            
## [10] rlang_1.0.1            pillar_1.7.0           glue_1.6.1            
## [13] withr_2.4.3            GenomeInfoDbData_1.2.7 lifecycle_1.0.1       
## [16] stringr_1.4.0          zlibbioc_1.40.0        munsell_0.5.0         
## [19] gtable_0.3.0           htmlwidgets_1.5.4      evaluate_0.15         
## [22] knitr_1.37             extrafont_0.17         fastmap_1.1.0         
## [25] curl_4.3.2             parallel_4.1.2         fansi_1.0.2           
## [28] Rttf2pt1_1.3.10        highr_0.9              Rcpp_1.0.8            
## [31] scales_1.1.1           jsonlite_1.8.0         digest_0.6.29         
## [34] stringi_1.7.6          dplyr_1.0.8            grid_4.1.2            
## [37] cli_3.2.0              tools_4.1.2            bitops_1.0-7          
## [40] magrittr_2.0.2         RCurl_1.98-1.6         tibble_3.1.6          
## [43] extrafontdb_1.0        crayon_1.5.0           pkgconfig_2.0.3       
## [46] ellipsis_0.3.2         httr_1.4.2             rmarkdown_2.11        
## [49] rstudioapi_0.13        R6_2.5.1               compiler_4.1.2
```