

class09

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Class 9: Structural Bioinformatics (Pt. 1)

1: Introduction to the RCSB Protein Data Bank (PDB)

The PDB database

The main repo for biomolecular structure data is the Protein Data Bank (PDB):
<https://www.rcsb.org/>

Analyze > PDB Statistics > by Experimental Method and Molecular Type

```
# import with basic function  
  
data <- read.csv("Data Export Summary.csv")  
head(data)
```

	Molecular.Type	X.ray	EM	NMR	Integrative	Multiple.methods
1	Protein (only)	176,378	20,438	12,709	342	221
2	Protein/Oligosaccharide	10,284	3,396	34	8	11
3	Protein/NA	9,007	5,931	287	24	7
4	Nucleic acid (only)	3,077	200	1,554	2	15
5	Other	174	13	33	3	0
6	Oligosaccharide (only)	11	0	6	0	1
	Neutron	Other	Total			
1	83	32	210,203			
2	1	0	13,734			
3	0	0	15,256			
4	3	1	4,852			
5	0	0	223			
6	0	4	22			

This reads in data as ‘chr’ type because of commas. below we will use readr package instead

```
# import data with readr package
library(readr)
stats <- read_csv("Data Export Summary.csv")
```

Rows: 6 Columns: 9
-- Column specification -----
Delimiter: ","
chr (1): Molecular Type
dbl (4): Integrative, Multiple methods, Neutron, Other
num (4): X-ray, EM, NMR, Total

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.

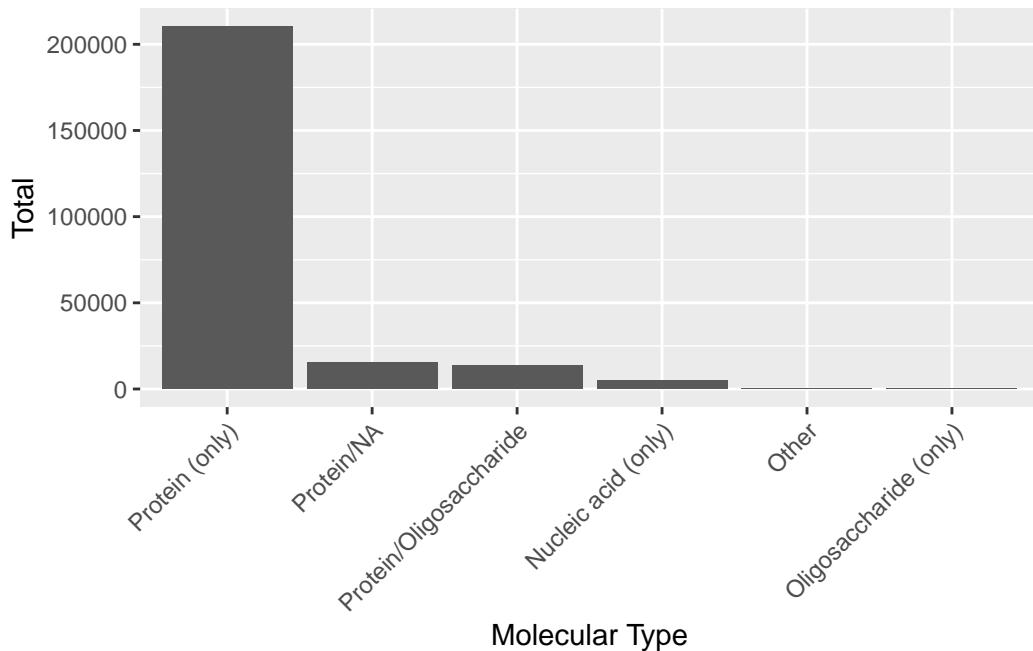
```
head(stats)
```

```
# A tibble: 6 x 9
`Molecular Type`    `X-ray`     EM     NMR Integrative `Multiple methods`  Neutron
<chr>                <dbl>    <dbl>   <dbl>      <dbl>            <dbl>    <dbl>
1 Protein (only)    176378  20438  12709      342           221     83
2 Protein/Oligosacch~ 10284   3396    34        8            11      1
3 Protein/NA         9007    5931   287       24            7      0
4 Nucleic acid (only) 3077    200    1554      2            15      3
5 Other                 174    13     33        3            0      0
6 Oligosaccharide (o~  11      0      6         0            1      0
# i 2 more variables: Other <dbl>, Total <dbl>
```

Q1: What percentage of structures in the PDB are solved by X-Ray and Electron Microscopy.

EM: 12%
X-ray: 81%

```
# plot barchart of molecular types
library(ggplot2)
ggplot(stats) +
  aes(reorder(`Molecular Type`, -Total), Total) +
  geom_col() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1)) +
  xlab("Molecular Type")
```



Q2: What proportion of structures in the PDB are protein?

```
stats[stats$`Molecular Type`=="Protein (only)", "Total"] / sum(stats$Total)
```

```
      Total
1 0.860465
```

Protein (only): 0.860465

Q3: Type HIV in the PDB website search box on the home page and determine how many HIV-1 protease structures are in the current PDB?

4,866 structures

2. Visualizing the HIV-1 protease structure

The *Mol viewer is embeded in many bioinformatics sites. The homepage is: <https://molstar.org/>
Use markdown format to insert image



Figure 1: The HIV-Pr dimer with bound inhibition

Q4: Water molecules normally have 3 atoms. Why do we see just one atom per water molecule in this structure?

This view is only displaying the “backbone”

Q5: There is a critical “conserved” water molecule in the binding site. Can you identify this water molecule? What residue number does this water molecule have

Residue number: 308

Q6: Generate and save a figure clearly showing the two distinct chains of HIV-protease along with the ligand. You might also consider showing the catalytic residues ASP 25 in each chain and the critical water (we recommend “Ball & Stick” for these side-chains). Add this figure to your Quarto document.



Figure 2: The HIV-Pr dimer with bound inhibition. ASP 25 and critical water molecule are highlighted

Q7: [Optional] As you have hopefully observed HIV protease is a homodimer (i.e. it is composed of two identical chains). With the aid of the graphic display can you identify secondary structure elements that are likely to only form in the dimer rather than the monomer?

FOOBAR

3. Introduction to Bio3D in R

```
library(bio3d)
hiv <- read.pdb("1hsg")
```

Note: Accessing on-line PDB file

```
hiv
```

Call: read.pdb(file = "1hsg")

Total Models#: 1

Total Atoms#: 1686, XYZs#: 5058 Chains#: 2 (values: A B)

Protein Atoms#: 1514 (residues/Calpha atoms#: 198)

Nucleic acid Atoms#: 0 (residues/phosphate atoms#: 0)

Non-protein/nucleic Atoms#: 172 (residues: 128)

Non-protein/nucleic resid values: [HOH (127), MK1 (1)]

Protein sequence:

```
PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWPKMIGGIGGFIKVRQYD
QILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFPQITLWQRPLVTIKIGGQLKE
ALLDTGADDTVLEEMSLPGRWPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPTP
VNIIGRNLLTQIGCTLNF
```

+ attr: atom, xyz, seqres, helix, sheet,
calpha, remark, call

```
# check out data
head(hiv$atom)
```

	type	eleno	elety	alt	resid	chain	resno	insert	x	y	z	o	b
1	ATOM	1	N	<NA>	PRO	A	1	<NA>	29.361	39.686	5.862	1	38.10
2	ATOM	2	CA	<NA>	PRO	A	1	<NA>	30.307	38.663	5.319	1	40.62
3	ATOM	3	C	<NA>	PRO	A	1	<NA>	29.760	38.071	4.022	1	42.64
4	ATOM	4	O	<NA>	PRO	A	1	<NA>	28.600	38.302	3.676	1	43.40
5	ATOM	5	CB	<NA>	PRO	A	1	<NA>	30.508	37.541	6.342	1	37.87
6	ATOM	6	CG	<NA>	PRO	A	1	<NA>	29.296	37.591	7.162	1	38.40

```
segid elesy charge
1 <NA>      N   <NA>
2 <NA>      C   <NA>
3 <NA>      C   <NA>
4 <NA>      O   <NA>
5 <NA>      C   <NA>
6 <NA>      C   <NA>
```

Q7: How many amino acid residues are there in this pdb object?

198 amino acid residues

Q8: Name one of the two non-protein residues?

HOH

Q9: How many protein chains are in this structure?

2

Blast

```
# run Blast on chain A sequence
# chainA <- trim.pdb(hiv, chain="A")
# chainA.seq <- pdbseq(chainA)
# blast <- blast.pdb(chainA.seq)
```

Re-run, this time caching the results

```
chainA <- trim.pdb(hiv, chain="A")
chainA.seq <- pdbseq(chainA)
blast <- blast.pdb(chainA.seq)
```

Searching ... please wait (updates every 5 seconds) RID = GD4YASA0016

.....

Reporting 249 hits

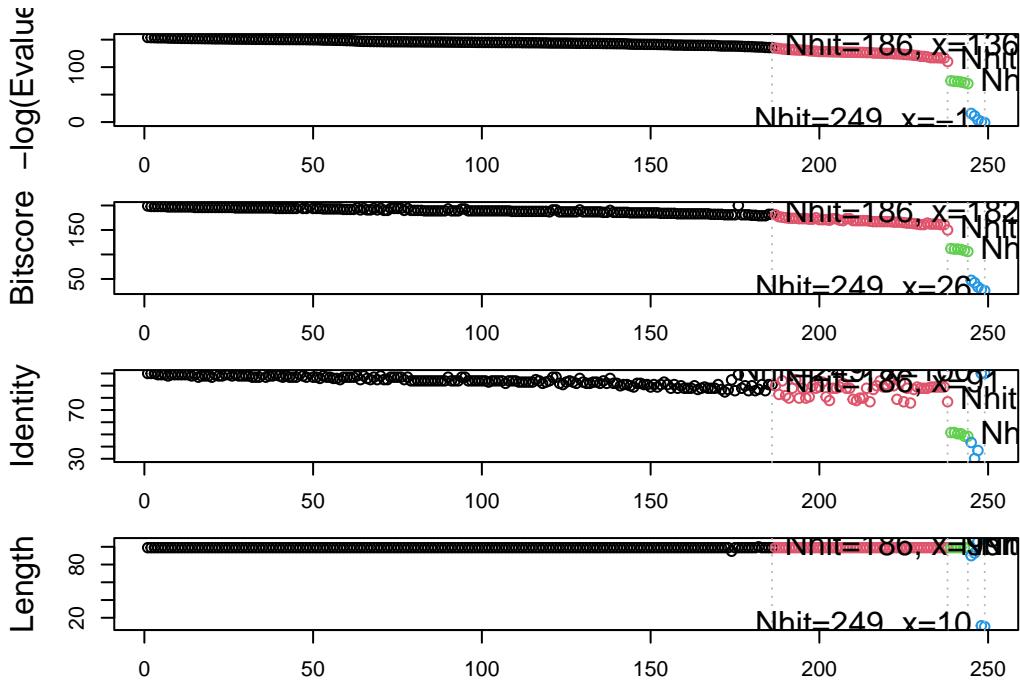
```
head(blast$hit.tbl)
```

	queryid	subjectids	identity	alignmentlength	mismatches	gapopens	q.start	q.end	s.start	s.end	evalue	bitscore	positives	mlog.evalue	pdb.id	acc	
1	Query_5653435	1W5V_A	100.00		99	0	0		99	12	110	1.38e-67	199	100	153.9511	1W5V_A	1W5V_A
2	Query_5653435	2FDE_A	100.00		99	0	0		99	2	100	1.70e-67	198	100	153.7426	2FDE_A	2FDE_A
3	Query_5653435	1AJV_A	100.00		99	0	0		99	1	99	1.99e-67	198	100	153.5851	1AJV_A	1AJV_A
4	Query_5653435	2R38_A	98.99		99	1	0		99	1	99	2.50e-67	198	100	153.3569	2R38_A	2R38_A
5	Query_5653435	2R3T_A	98.99		99	1	0		99	1	99	2.50e-67	198	100	153.3569	2R3T_A	2R3T_A
6	Query_5653435	1HXB_A	98.99		99	1	0		99	1	99	2.50e-67	198	100	153.3569	1HXB_A	1HXB_A

```
# plot Blast results
hits <- plot(blast)
```

* Possible cutoff values: 135 110 69 -2
Yielding Nhits: 186 238 244 249

* Chosen cutoff value of: 69
Yielding Nhits: 244



```
head(hits$pdb.id)
```

```
[1] "1W5V_A" "2FDE_A" "1AJV_A" "2R38_A" "2R3T_A" "1HXB_A"
```

Prediction of functional motion

Normal Mode Analysis (NMA): predict large-scale motion/flexibility/dynamics of a molecule

```
adk <- read.pdb("1ake")
```

Note: Accessing on-line PDB file
PDB has ALT records, taking A only, rm.alt=TRUE

```
adk_A <- trim.pdb(adk, chain='A')  
adk_A
```

```
Call: trim.pdb(pdb = adk, chain = "A")

Total Models#: 1
Total Atoms#: 1954, XYZs#: 5862 Chains#: 1 (values: A)

Protein Atoms#: 1656 (residues/Calpha atoms#: 214)
Nucleic acid Atoms#: 0 (residues/phosphate atoms#: 0)

Non-protein/nucleic Atoms#: 298 (residues: 242)
Non-protein/nucleic resid values: [ AP5 (1), HOH (241) ]

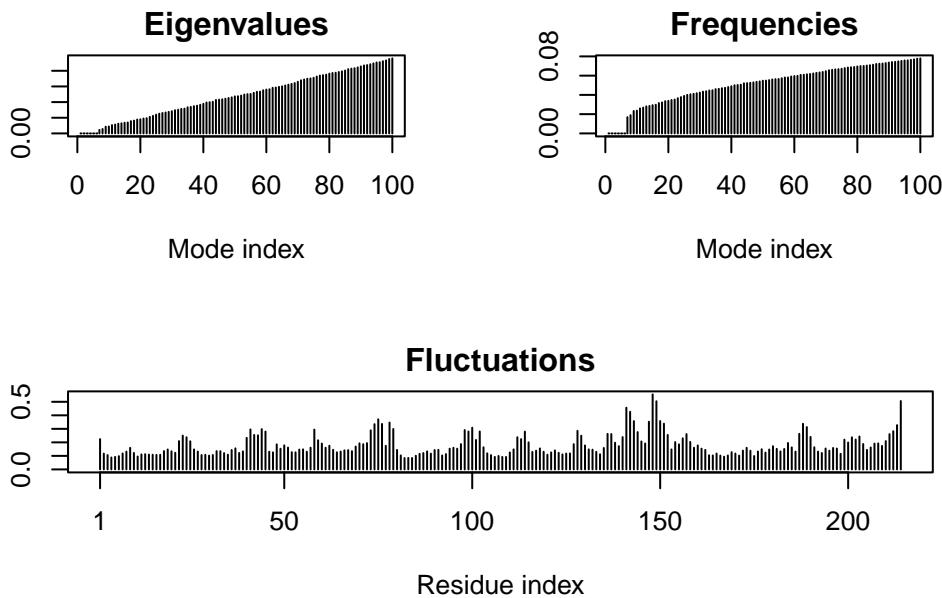
Protein sequence:
MRIILLGAPGAGKGTQAQFIMEKYGIPQISTGDMRLRAAVKSGSELGKQAKDIMDAGKLVT
DELVIALVKERIAQEDCRNGFLLDGFPRТИPQADAMKEAGINVVDYVLEFDVPDELIVDRI
VGRRVHAPSGRVYHVKFNPPKVEGKDDVTGEELTRKDDQEETVRKRLVEYHQMTAPLIG
YYSKAEAGNTKYAKVDGTPVAEVRADLEKILG

+ attr: atom, helix, sheet, seqres, xyz,
calpha, call
```

```
m <- nma(adk_A)
```

```
Building Hessian...           Done in 0.03 seconds.  
Diagonalizing Hessian...     Done in 0.33 seconds.
```

```
plot(m)
```



Let's write out a “trajectory” of predicted motion. We'll load this into molstar

```
mktrj(m, file="adk_nma.pdb")
```

Play with 3D viewing in R

We can use the new “**bio3dview**” package, which is not yet on CRAN, to render integrative 3D views in R and html quarto output reports.

To install from GitHub, we can use the **pak** package.

```
# library(pak)  
# pak::pak("bioboot/bio3dview")  
# install.packages("NGLVieweR")
```

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

This will allow us to programmatically toggle visualization settings.

4. Comparative structure analysis of Adenylate Kinase

Install the following necessary packages in the R console:

```
install.packages("bio3d") install.packages("devtools") install.packages("BiocManager")
BiocManager::install("msa") devtools::install_bitbucket("Grantlab/bio3d-view")
```

Q10. Which of the packages above is found only on BioConductor and not CRAN?

msa

Q11. Which of the above packages is not found on BioConductor or CRAN?:

bio3d-view

Q12. True or False? Functions from the devtools package can be used to install packages from GitHub and BitBucket?

True

Search and retrieve ADK structures

Use function get.seq() to fetch the query sequence for chain A of the PDB ID 1AKE

```
library(bio3d)
aa <- get.seq("1ake_A")
```

Warning in get.seq("1ake_A"): Removing existing file: seqs.fasta

Fetching... Please wait. Done.

```
aa
```

1 60
pdb|1AKE|A MRIILLGAPGAGKGTQAQFIMEKYGIPQISTGMLRAAVKSGSELGKQAKDIDAGKLVT
1 60

61 120
pdb|1AKE|A DELVIALVKERIAQEDCRNGFLLDGFPRTIPQADAMKEAGINVDYVLEFDVPDELIVDRI
61 120

121 180
pdb|1AKE|A VGRRVHAPSGRVYHVKFNNPPKVEGKDDVTGEELTTRKDDQEETVRKRLVEYHQMTAPLIG
121 180

181 214
pdb|1AKE|A YYSKAEAGNTKYAKVDGTPVAEVRADLEKILG
181 214

Call:

```
read.fasta(file = outfile)
```

Class:

fasta

Alignment dimensions:

1 sequence rows; 214 position columns (214 non-gap, 0 gap)

+ attr: id, ali, call

Q13. How many amino acids are in this sequence, i.e. how long is this sequence?

214

Blast search the sequence

```
# Blast search  
#b <- blast.pdb(aa)  
  
# Plot a summary of  
#hits <- plot(b)  
  
# List out some 'to'  
#head(hits$pdb.id)
```

Shortcut above by manually setting blast results

```
hits <- NULL  
hits$pdb.id <- c('1AKE_A', '6S36_A', '6RZE_A', '3HPR_A', '1E4V_A', '5EJE_A', '1E4Y_A', '3X2S_A', '6HA
```

Use function get.pdb() and pdbslit() to fetch and parse the identified structures.

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

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/1AKE.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/6S36.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/6RZE.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/3HPR.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/1E4V.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/5EJE.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/1E4Y.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/3X2S.pdb exists. Skipping download
```

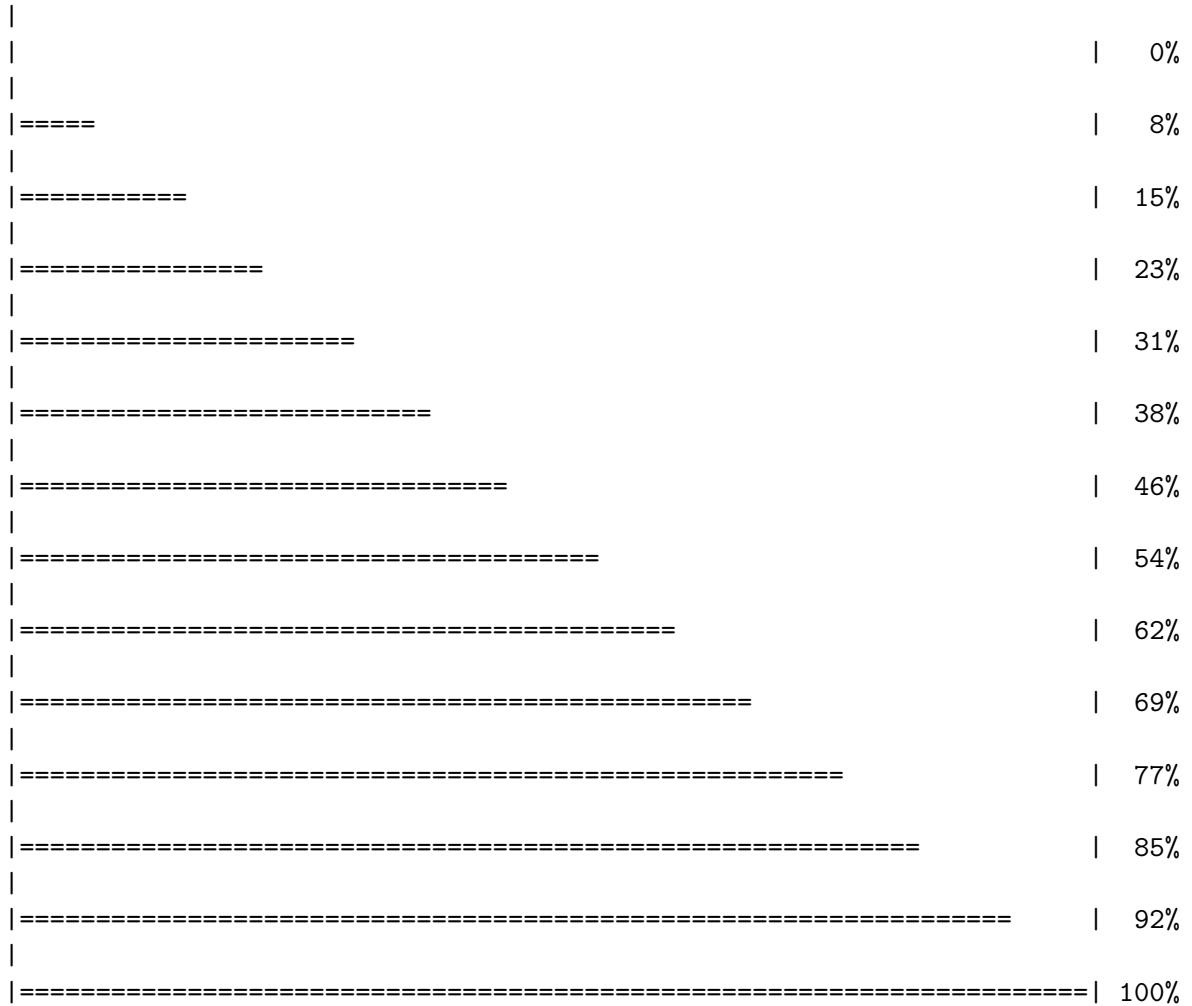
```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/6HAP.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/6HAM.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/4K46.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/3GMT.pdb exists. Skipping download
```

```
Warning in get.pdb(hits$pdb.id, path = "pdbs", split = TRUE, gzip = TRUE):  
pdbs/4PZL.pdb exists. Skipping download
```



Align and superpose structures

Use the `pdbaln()` function to align and also optionally fit (i.e. superpose) the identified PDB structures.

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

```
Reading PDB files:
pdbs/split_chain/1AKE_A.pdb
pdbs/split_chain/6S36_A.pdb
pdbs/split_chain/6RZE_A.pdb
pdbs/split_chain/3HPR_A.pdb
pdbs/split_chain/1E4V_A.pdb
pdbs/split_chain/5EJE_A.pdb
pdbs/split_chain/1E4Y_A.pdb
pdbs/split_chain/3X2S_A.pdb
pdbs/split_chain/6HAP_A.pdb
pdbs/split_chain/6HAM_A.pdb
pdbs/split_chain/4K46_A.pdb
pdbs/split_chain/3GMT_A.pdb
pdbs/split_chain/4PZL_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
.    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
.    PDB has ALT records, taking A only, rm.alt=TRUE
...
...
```

Extracting sequences

```
pdb/seq: 1  name: pdbs/split_chain/1AKE_A.pdb
    PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 2  name: pdbs/split_chain/6S36_A.pdb
    PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 3  name: pdbs/split_chain/6RZE_A.pdb
    PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 4  name: pdbs/split_chain/3HPR_A.pdb
    PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 5  name: pdbs/split_chain/1E4V_A.pdb
```

```

pdb/seq: 6    name: pdbs/split_chain/5EJE_A.pdb
    PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 7    name: pdbs/split_chain/1E4Y_A.pdb
pdb/seq: 8    name: pdbs/split_chain/3X2S_A.pdb
pdb/seq: 9    name: pdbs/split_chain/6HAP_A.pdb
pdb/seq: 10   name: pdbs/split_chain/6HAM_A.pdb
    PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 11   name: pdbs/split_chain/4K46_A.pdb
    PDB has ALT records, taking A only, rm.alt=TRUE
pdb/seq: 12   name: pdbs/split_chain/3GMT_A.pdb
pdb/seq: 13   name: pdbs/split_chain/4PZL_A.pdb

```

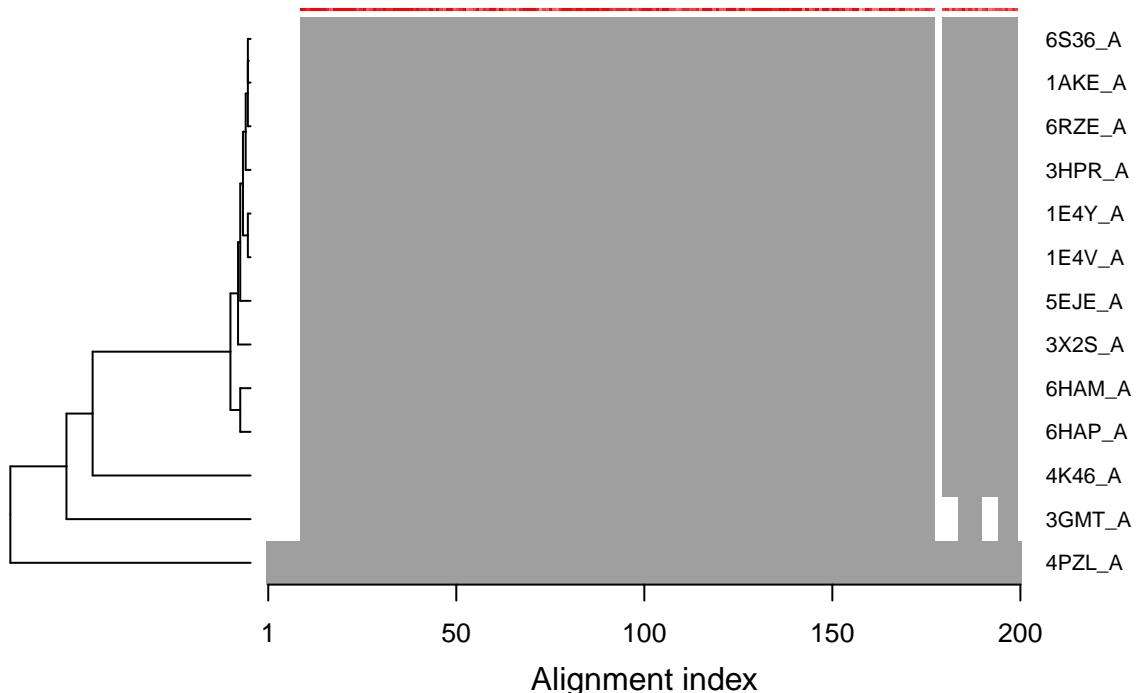
```

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

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

```

Sequence Alignment Overview



Annotate collected PDB structures

```
anno <- pdb.annotate(ids)
unique(anno$source)
```

```
[1] "Escherichia coli"
[2] "Escherichia coli K-12"
[3] "Escherichia coli O139:H28 str. E24377A"
[4] "Escherichia coli str. K-12 substr. MDS42"
[5] "Photobacterium profundum"
[6] "Burkholderia pseudomallei 1710b"
[7] "Francisella tularensis subsp. tularensis SCHU S4"
```

```
head(anno)
```

	structureId	chainId	macromoleculeType	chainLength	experimentalTechnique
1AKE_A	1AKE	A	Protein	214	X-ray
6S36_A	6S36	A	Protein	214	X-ray
6RZE_A	6RZE	A	Protein	214	X-ray
3HPR_A	3HPR	A	Protein	214	X-ray
1E4V_A	1E4V	A	Protein	214	X-ray
5EJE_A	5EJE	A	Protein	214	X-ray
	resolution	scopDomain			pfam
1AKE_A	2.00	Adenylate kinase		Adenylate kinase	(ADK)
6S36_A	1.60	<NA>	Adenylate kinase, active site lid	Adenylate kinase	(ADK_lid)
6RZE_A	1.69	<NA>		Adenylate kinase	(ADK)
3HPR_A	2.00	<NA>		Adenylate kinase	(ADK)
1E4V_A	1.85	Adenylate kinase		Adenylate kinase	(ADK)
5EJE_A	1.90	<NA>		Adenylate kinase	(ADK)
	ligandId			ligandName	
1AKE_A	AP5		BIS(ADENOSINE)-5'-PENTAPHOSPHATE		
6S36_A	CL (3),NA,MG (2)		CHLORIDE ION (3),SODIUM ION,MAGNESIUM ION (2)		
6RZE_A	NA (3),CL (2)		SODIUM ION (3),CHLORIDE ION (2)		
3HPR_A	AP5		BIS(ADENOSINE)-5'-PENTAPHOSPHATE		
1E4V_A	AP5		BIS(ADENOSINE)-5'-PENTAPHOSPHATE		
5EJE_A	AP5,CO	BIS(ADENOSINE)-5'-PENTAPHOSPHATE,COBALT (II) ION	source		
1AKE_A		Escherichia coli			
6S36_A		Escherichia coli			
6RZE_A		Escherichia coli			

3HPR_A Escherichia coli K-12
 1E4V_A Escherichia coli
 5EJE_A Escherichia coli O139:H28 str. E24377A

1AKE_A STRUCTURE OF THE COMPLEX BETWEEN ADENYLYATE KINASE FROM ESCHERICHIA COLI AND THE INHIBITOR 6S36_A

6RZE_A
 3HPR_A
 1E4V_A
 5EJE_A

Crys

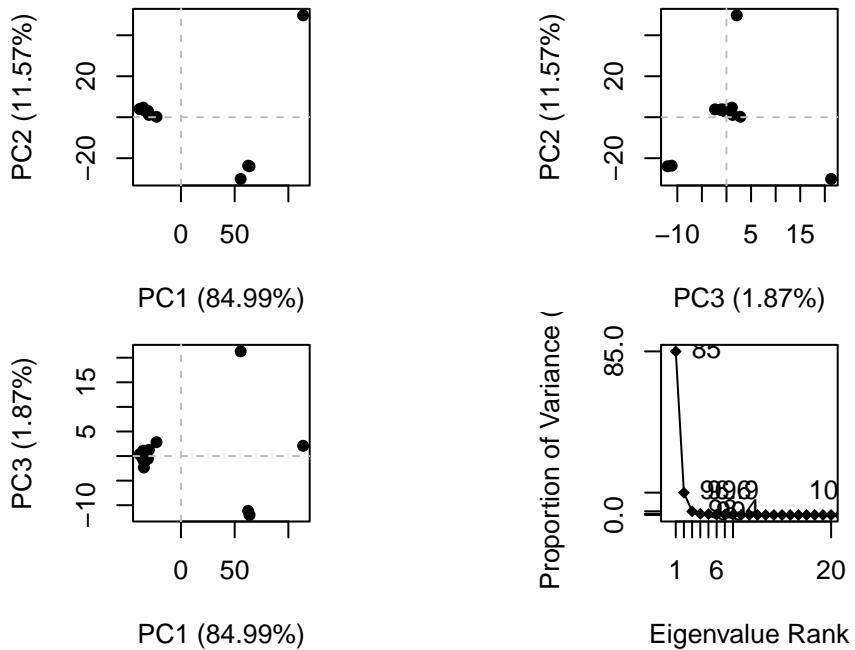
		citation	rObserved	rFree
1AKE_A	Muller, C.W., et al. J Mol Biology (1992)	0.1960	NA	
6S36_A	Rogne, P., et al. Biochemistry (2019)	0.1632	0.2356	
6RZE_A	Rogne, P., et al. Biochemistry (2019)	0.1865	0.2350	
3HPR_A	Schrank, T.P., et al. Proc Natl Acad Sci U S A (2009)	0.2100	0.2432	
1E4V_A	Muller, C.W., et al. Proteins (1993)	0.1960	NA	
5EJE_A	Kovermann, M., et al. Proc Natl Acad Sci U S A (2017)	0.1889	0.2358	
rWork spaceGroup				
1AKE_A	0.1960 P 21 2 21			
6S36_A	0.1594 C 1 2 1			
6RZE_A	0.1819 C 1 2 1			
3HPR_A	0.2062 P 21 21 2			
1E4V_A	0.1960 P 21 2 21			
5EJE_A	0.1863 P 21 2 21			

Principal component analysis

```

# Perform PCA
pc.xray <- pca(pdbs)
plot(pc.xray)

```

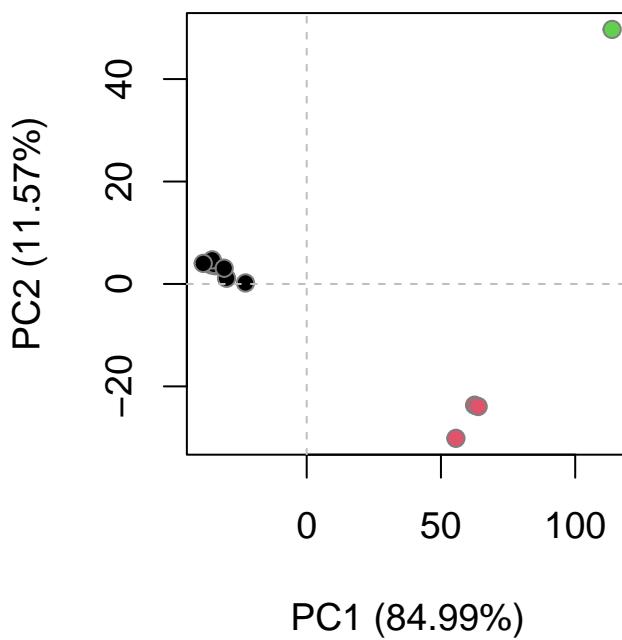


```
# Calculate RMSD
rd <- rmsd(pdfs)
```

Warning in rmsd(pdfs): No indices provided, using the 204 non NA positions

```
# Structure-based clustering
hc.rd <- hclust(dist(rd))
grps.rd <- cutree(hc.rd, k=3)

plot(pc.xray, 1:2, col="grey50", bg=grps.rd, pch=21, cex=1)
```



6. Normal mode analysis [optional]

Function `nma()` provides normal mode analysis (NMA) on both single structures (if given a single PDB input object) or the complete structure ensemble (if provided with a PDBe input object). This facilitates characterizing and comparing flexibility profiles of related protein structures.

```
# NMA of all structures
modes <- nma(pdbs)
```

Details of Scheduled Calculation:

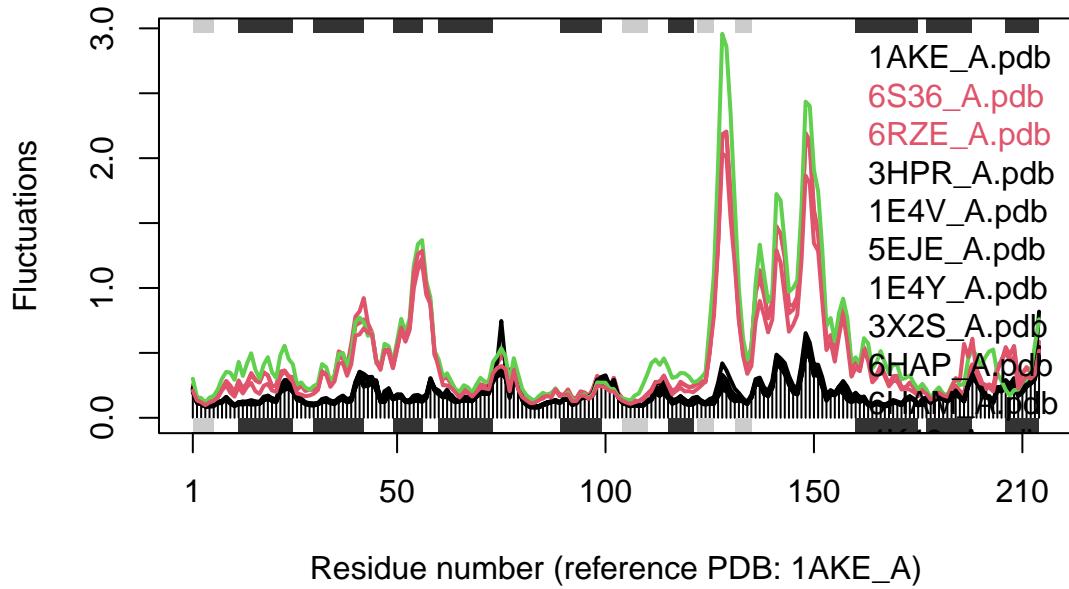
- ... 13 input structures
- ... storing 606 eigenvectors for each structure
- ... dimension of x\$U.subspace: (612x606x13)
- ... coordinate superposition prior to NM calculation
- ... aligned eigenvectors (gap containing positions removed)
- ... estimated memory usage of final 'eNMA' object: 36.9 Mb

|
| 0%

```
|  
|=====| 8%  
|  
|=====| 15%  
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|=====| 23%  
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|=====| 31%  
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|=====| 62%  
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|=====| 69%  
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|=====| 77%  
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|=====| 85%  
|  
|=====| 92%  
|  
|=====| 100%
```

```
plot(modes, pdbs, col=grps.rd)
```

Extracting SSE from pdbs\$sse attribute



Q14. What do you note about this plot? Are the black and colored lines similar or different? Where do you think they differ most and why?

Collectively these results indicate the existence of two major distinct conformational states for Adk. These differ by a collective low frequency displacement of two nucleotide-binding site regions that display distinct flexibilities upon nucleotide binding.