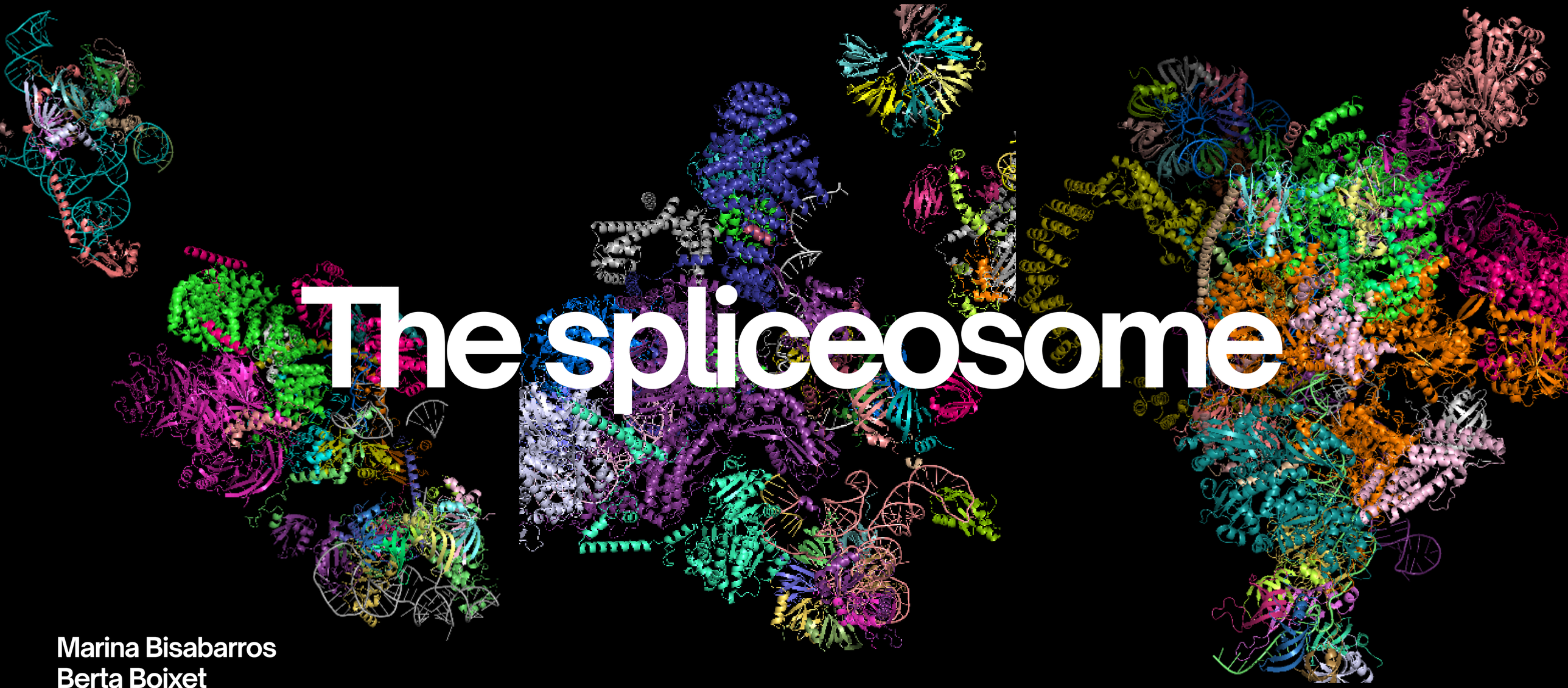
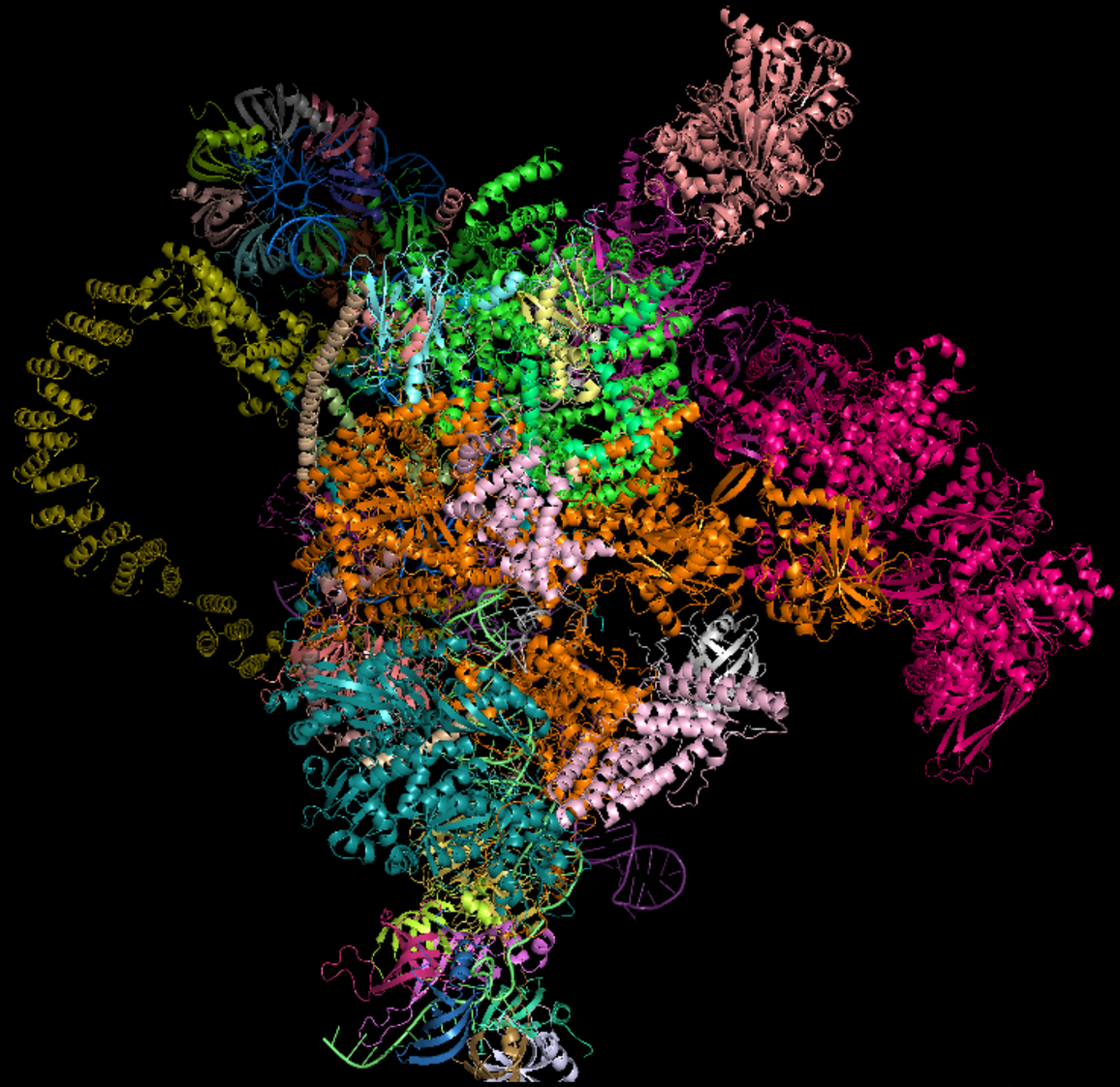


The spliceosome

Marina Bisabarros
Berta Boixet
Núria Càlix
Alexia Garcia

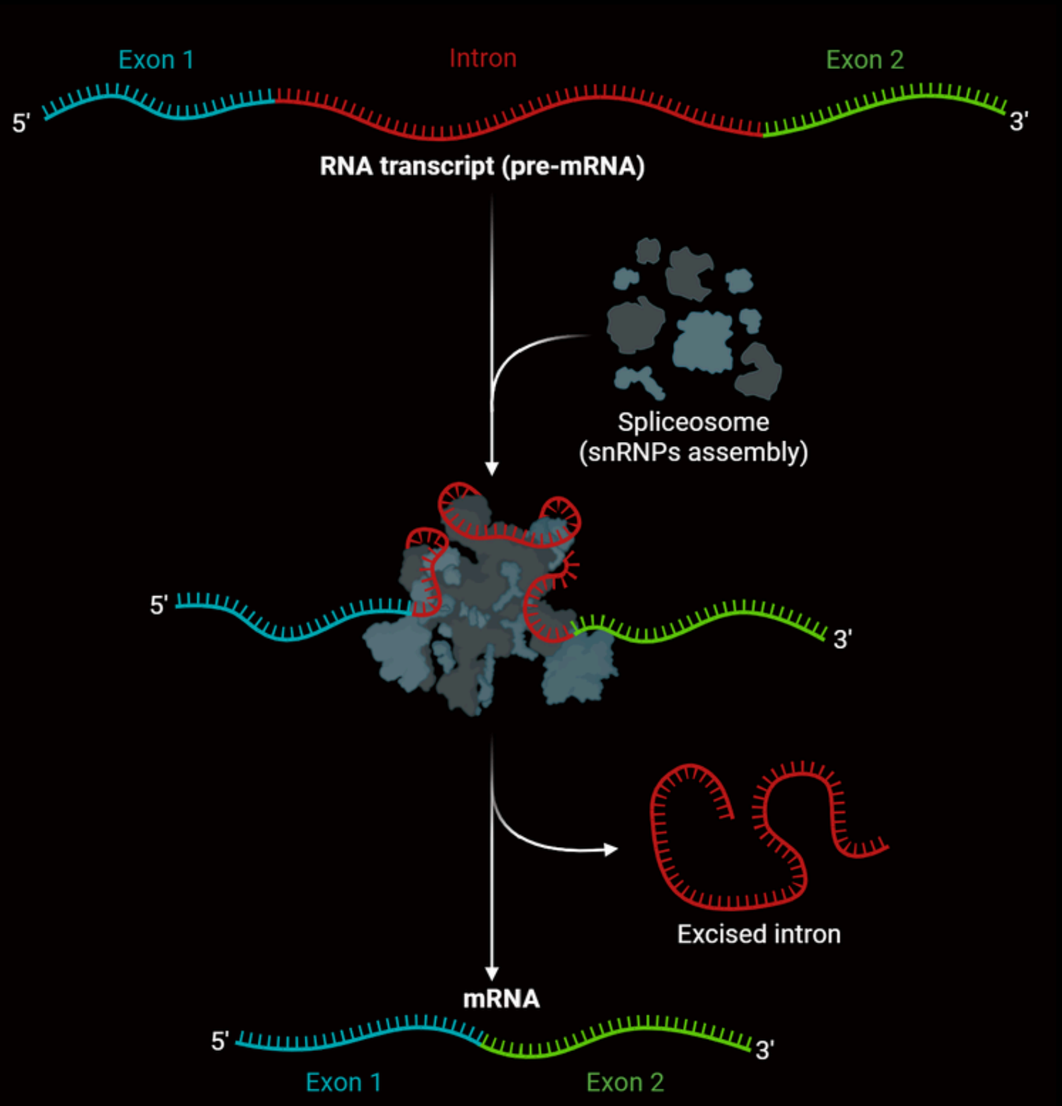
Index

1. **Splicing mechanism and complex**
2. **Brr2**
3. **N cassette and C cassette comparison**
4. **Ski2 like motifs**
5. **Interactions with ATP, Mg2+**
6. **Interactions between NC-CC**
7. **Inhibition of Brr2**
8. **Evolution**
9. **Retinitis pigmentosa type 33**



Cryo-EM structure of a human activated spliceosome (early Bact) (5Z58) Homo Sapiens - 4.9Å

Splicing



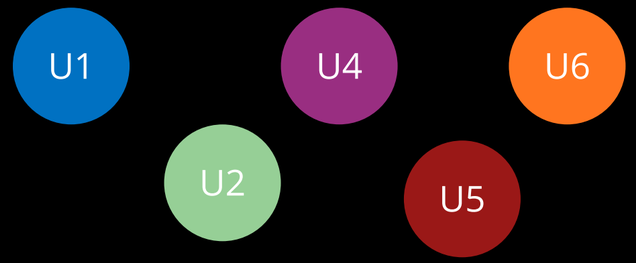
Intron parts



Spliceosome subunits

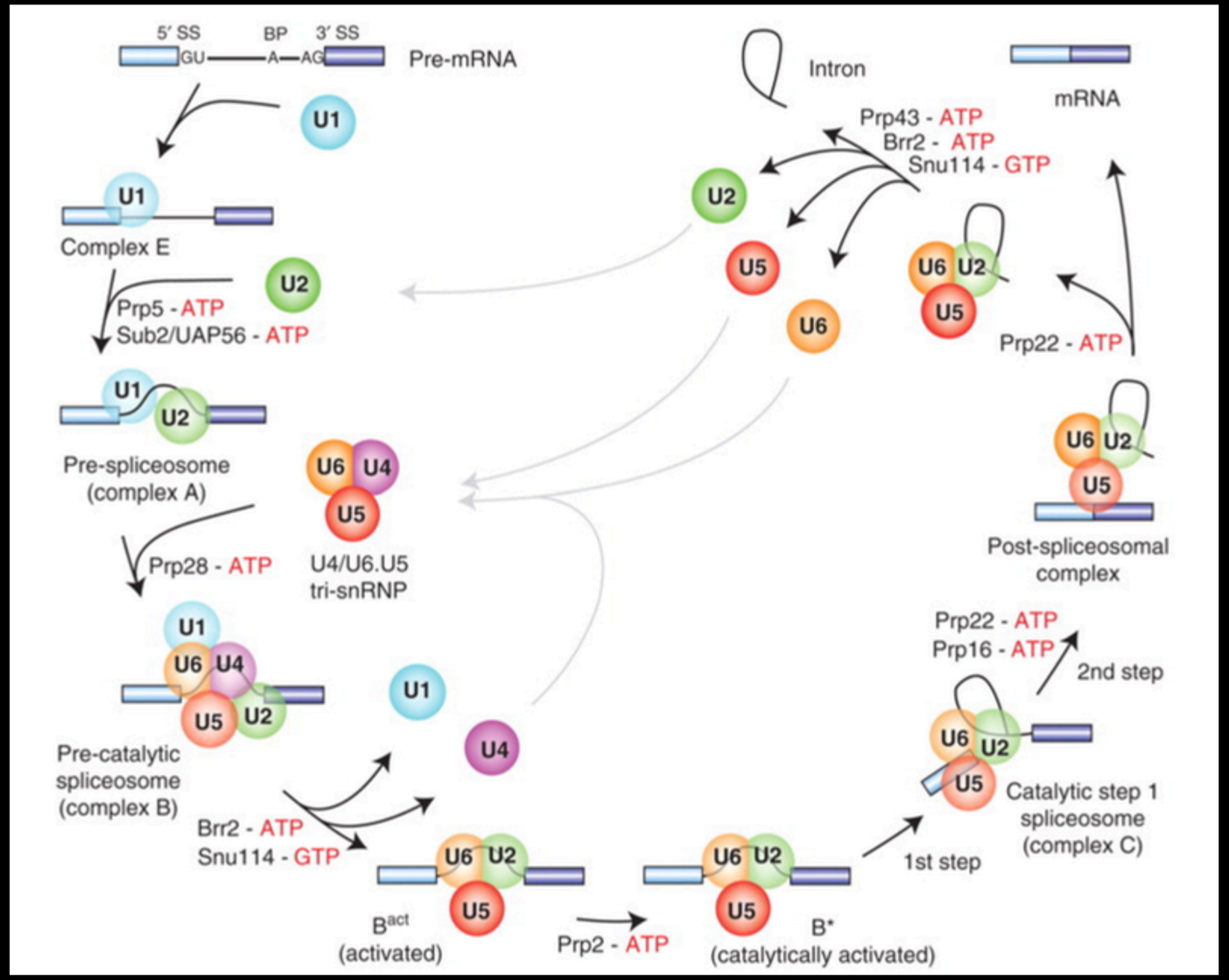
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RNA + ring of proteins



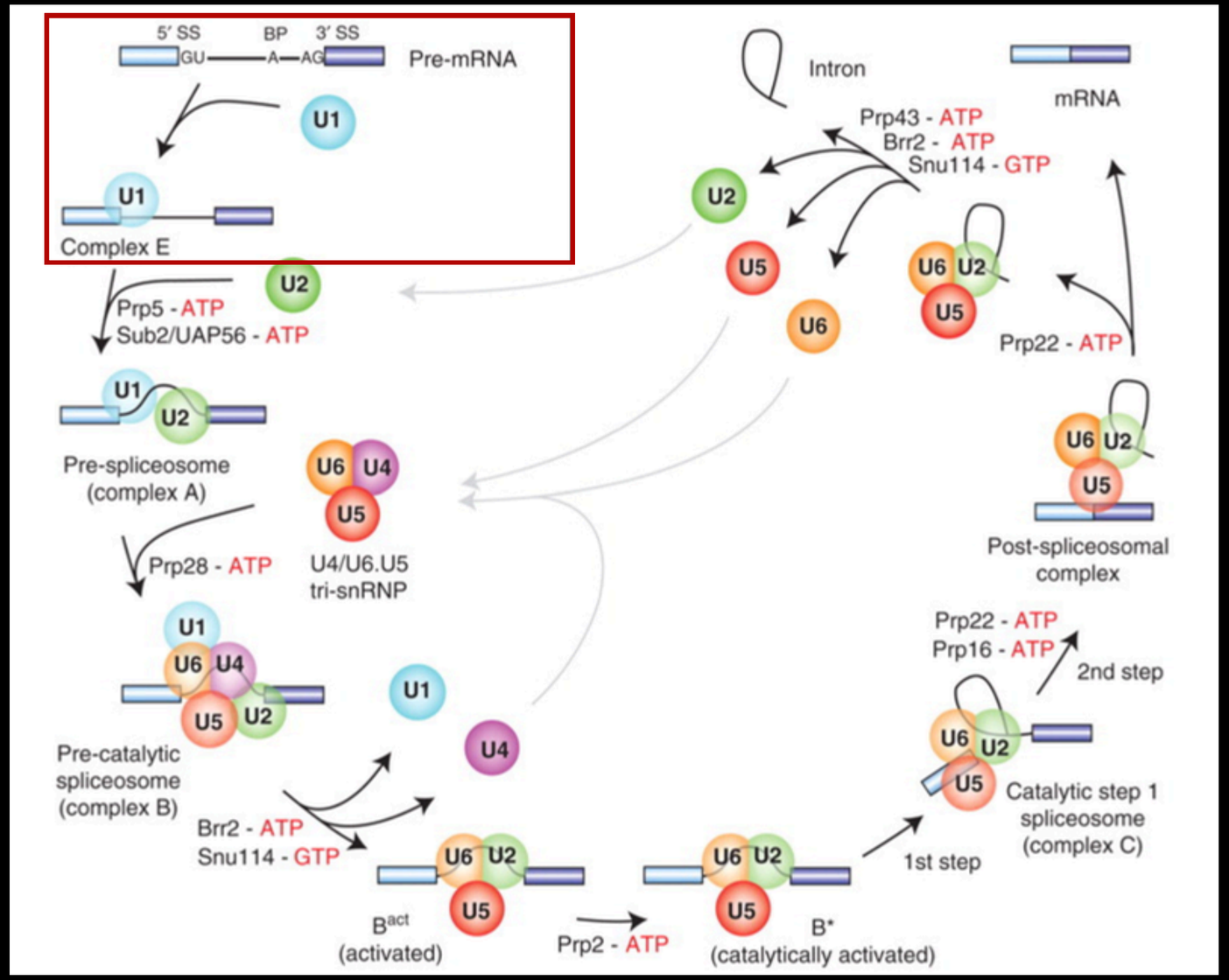
Created with biorender

Splicing mechanism



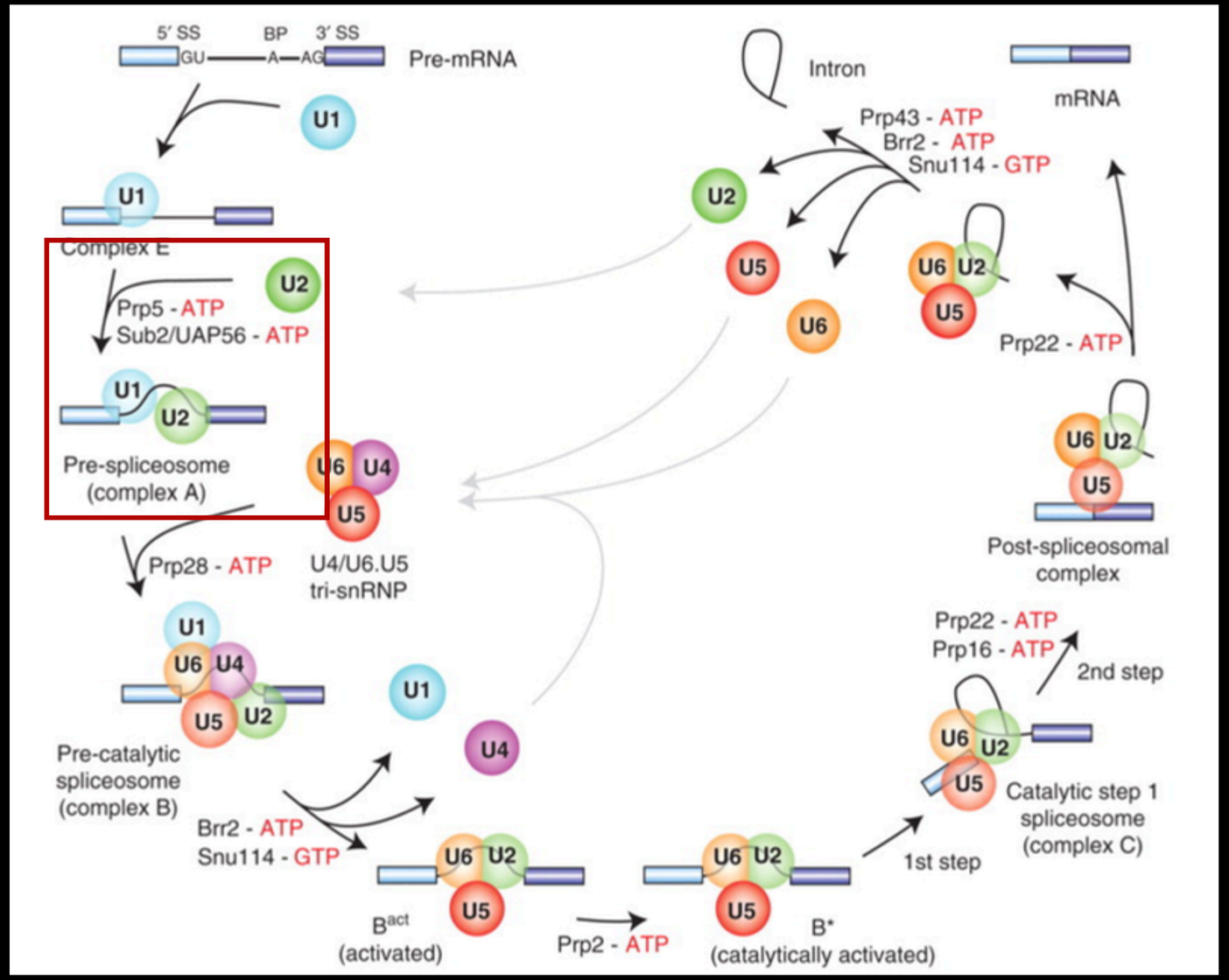
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Splicing mechanism



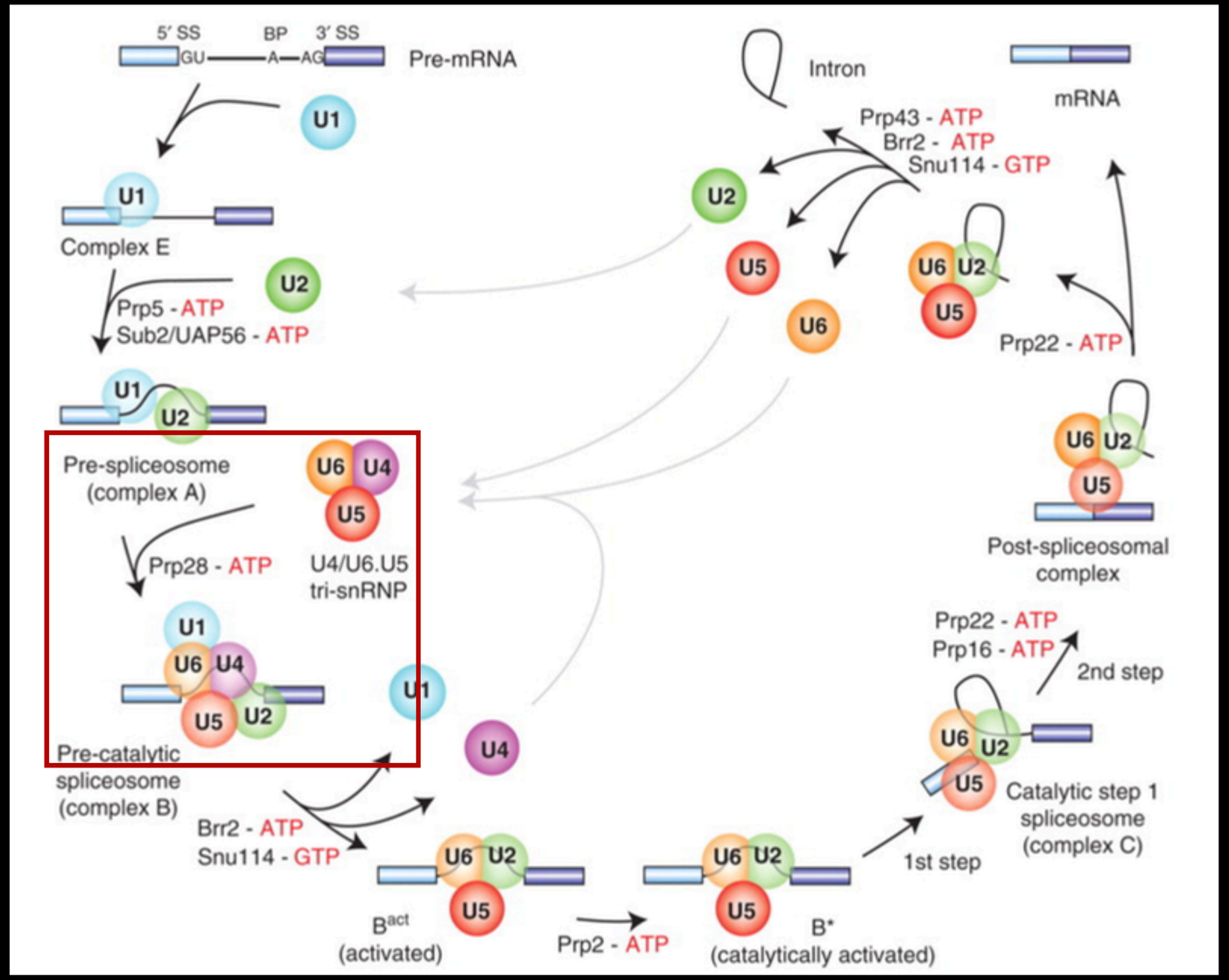
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Splicing mechanism



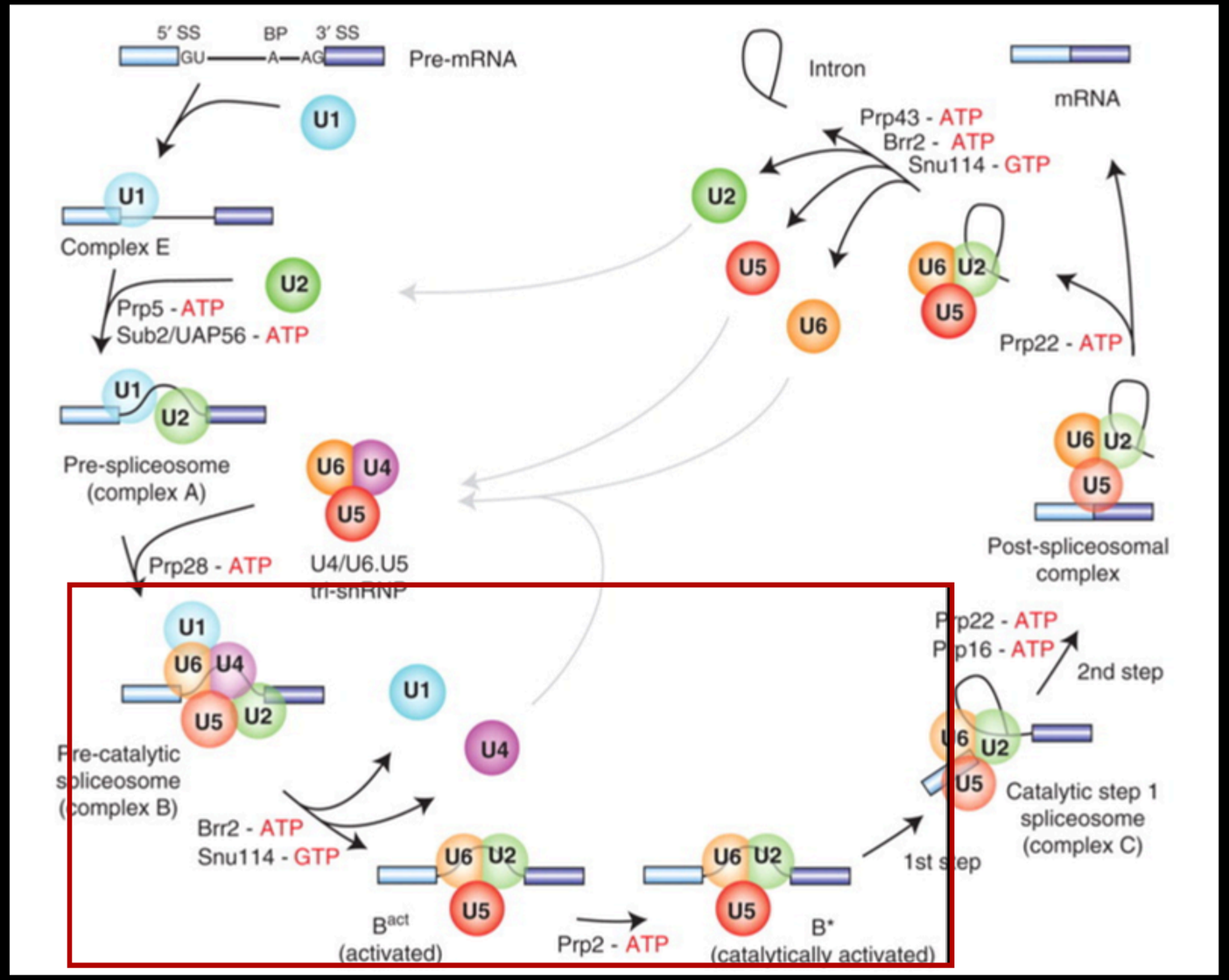
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Splicing mechanism



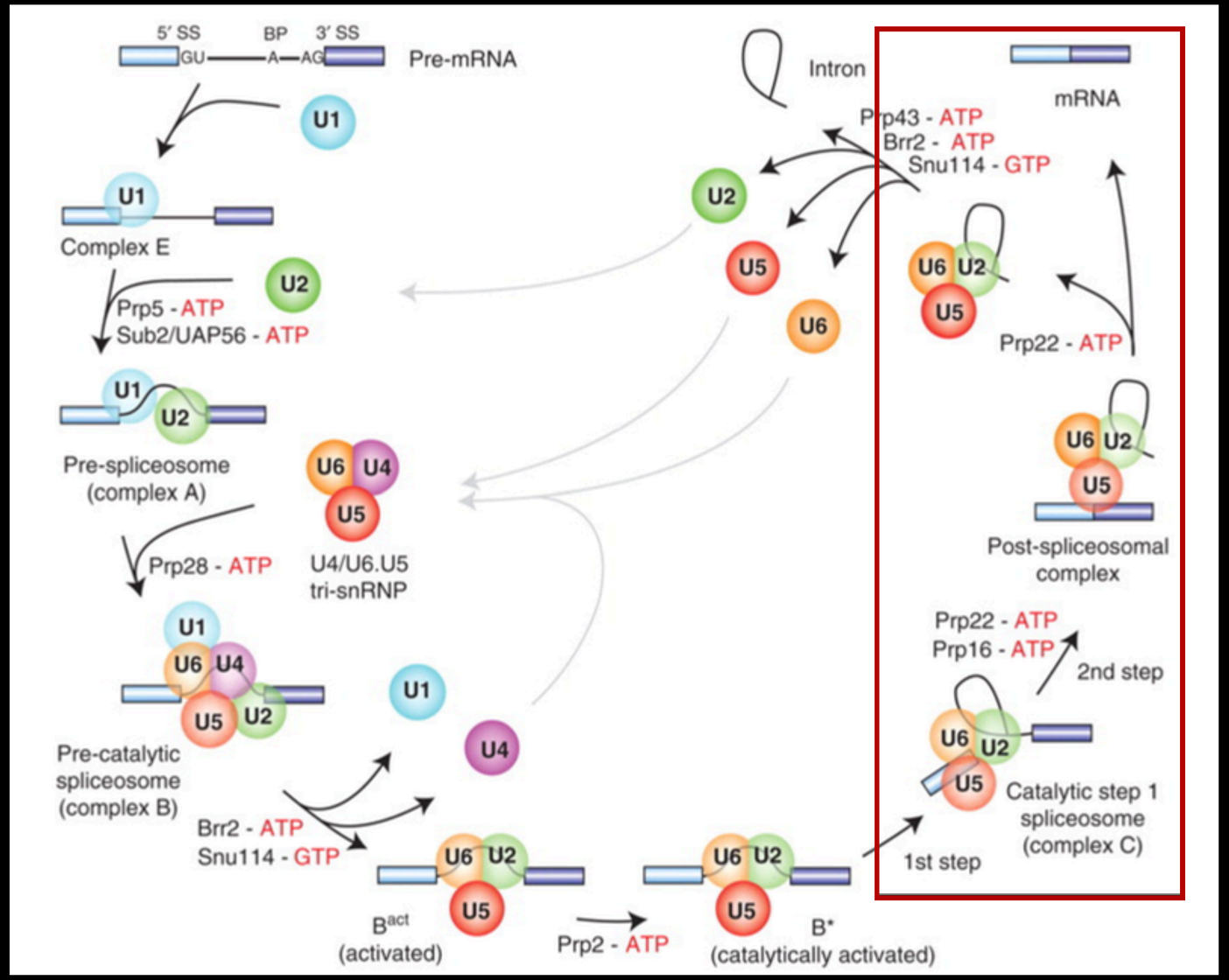
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Splicing mechanism



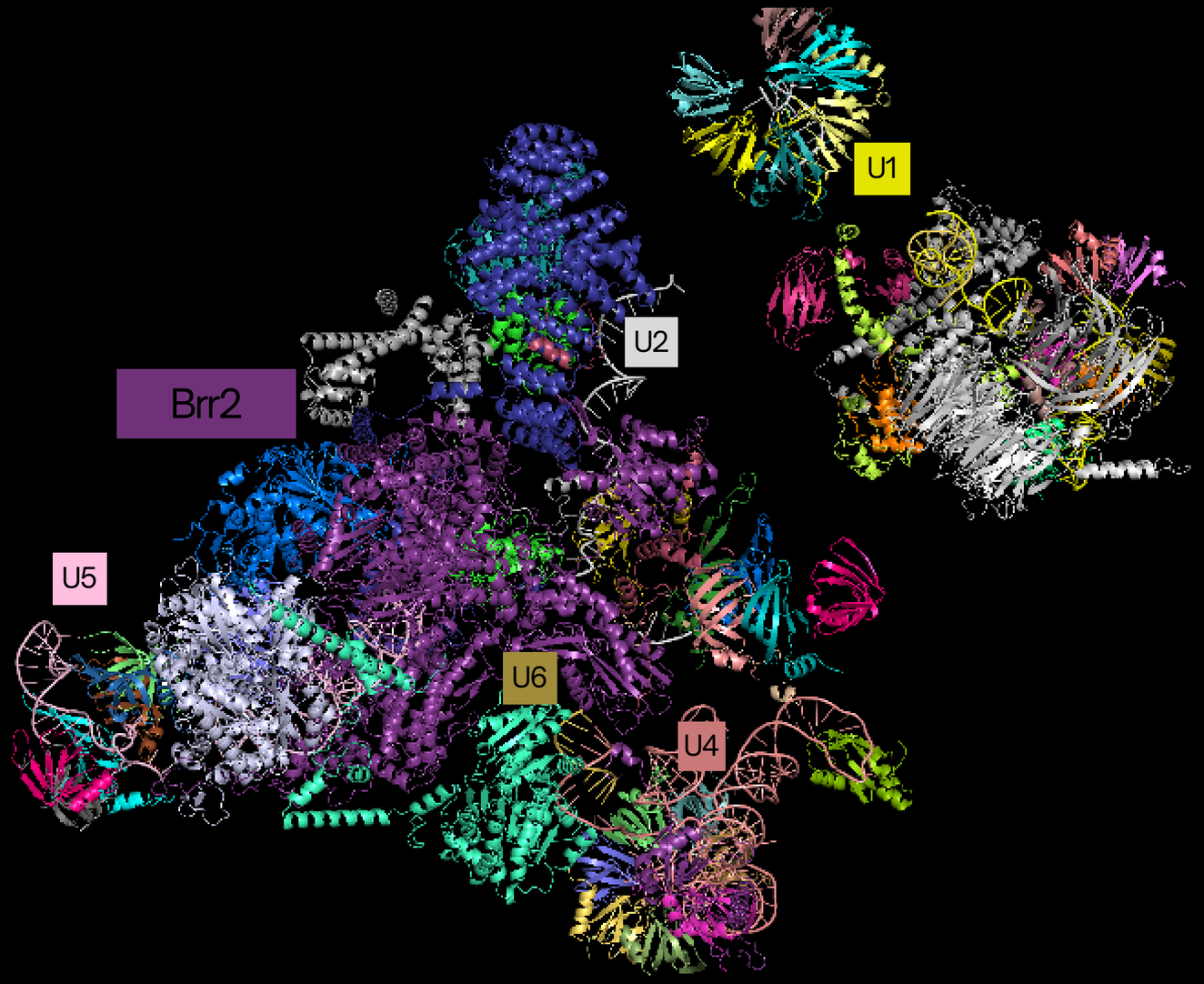
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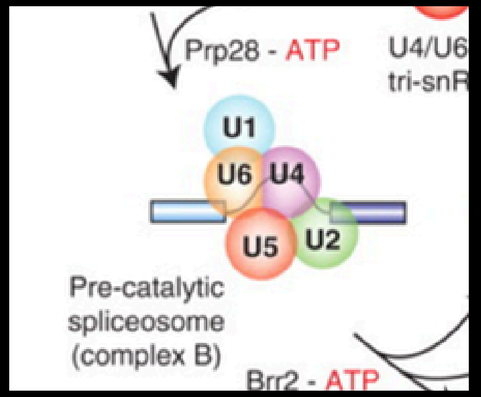
Splicing mechanism



Will, C. L., & Luhrmann, R. (2010b). Spliceosome Structure and Function. Cold Spring Harbor Perspectives In Biology

Complex pre-B

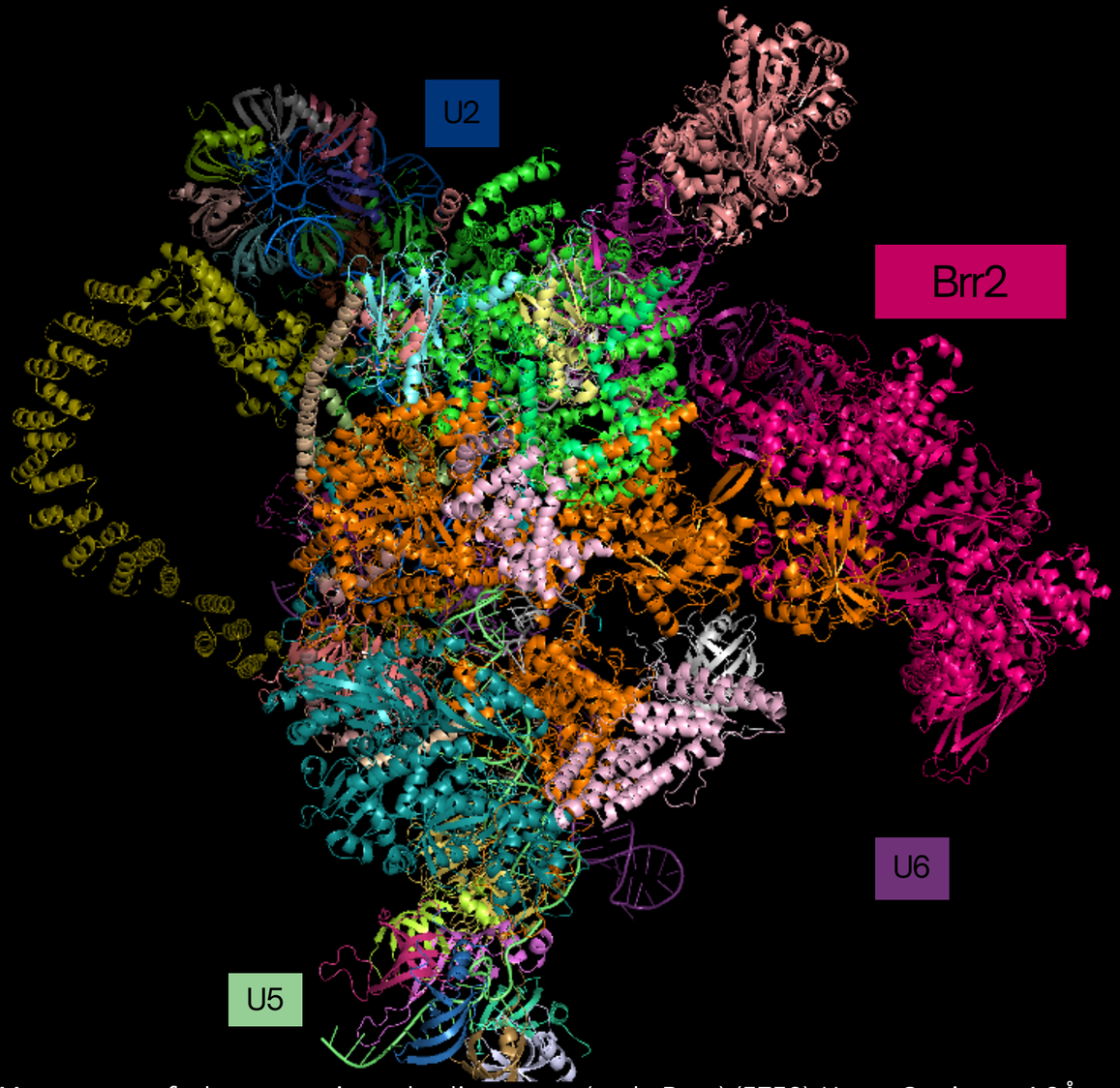




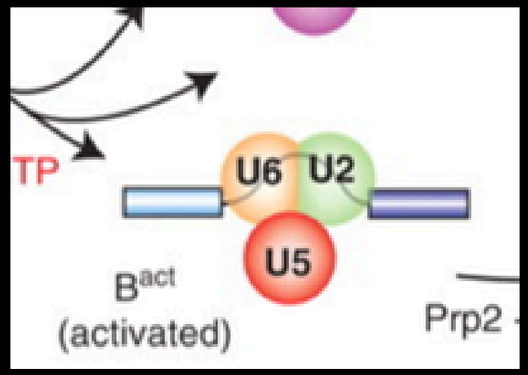
Will, C. L., & Luhrmann, R. (2010b). Spliceosome Structure and Function. Cold Spring Harbor Perspectives In Biology

Structure of a human fully-assembled precatalytic spliceosome (pre-B complex) (6QX9) *Homo Sapiens* - 3.28Å

Complex B act



Cryo-EM structure of a human activated spliceosome (early Bact) (5Z58) *Homo Sapiens* - 4.9Å



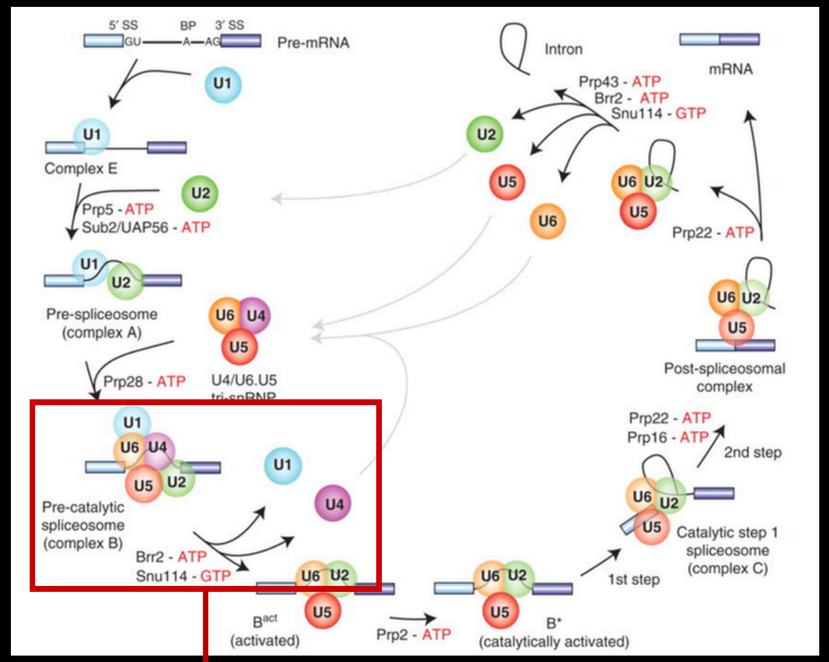
Will, C. L., & Luhrmann, R. (2010b). Spliceosome Structure and Function. Cold Spring Harbor Perspectives In Biology

SCOP CLASSIFICATION

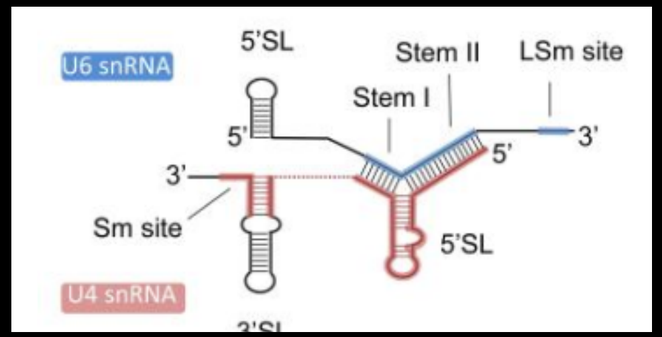
Lineage for Protein: RecA protein, ATPase-domain

1. Root: SCOPe 2.08
2.  Class c: Alpha and beta proteins (a/b) [51349] (148 folds)
3.  Fold c.37: P-loop containing nucleoside triphosphate hydrolases [52539] (1 superfamily)
   3 layers: a/b/a, parallel or mixed beta-sheets of variable sizes
4.  Superfamily c.37.1: P-loop containing nucleoside triphosphate hydrolases [52540] (27 families) S
   division into families based on beta-sheet topologies
5.  Family c.37.1.11: RecA protein-like (ATPase-domain) [52670] (22 proteins)
   core: mixed beta-sheet of 8 strands, order 32451678; strand 7 is antiparallel to the rest

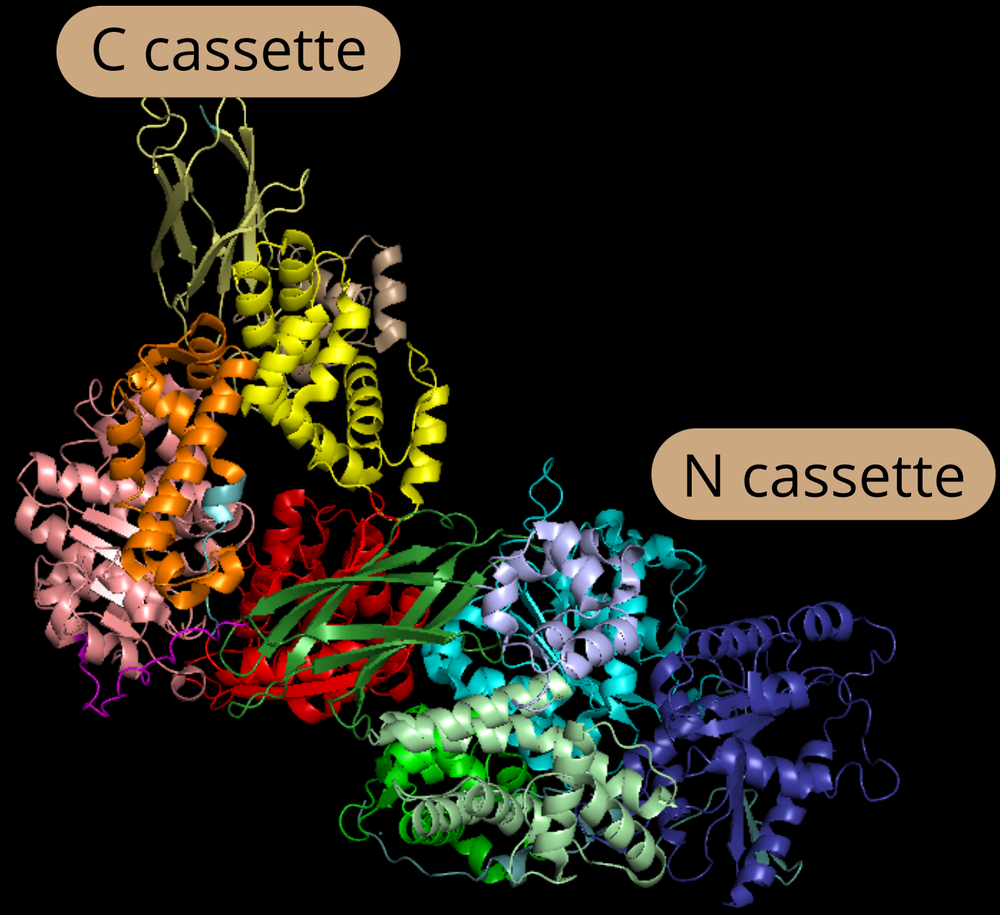
Brr2



Will, C. L., & Luhrmann, R. (2010b). Spliceosome Structure and Function. Cold Spring Harbor Perspectives In Biology

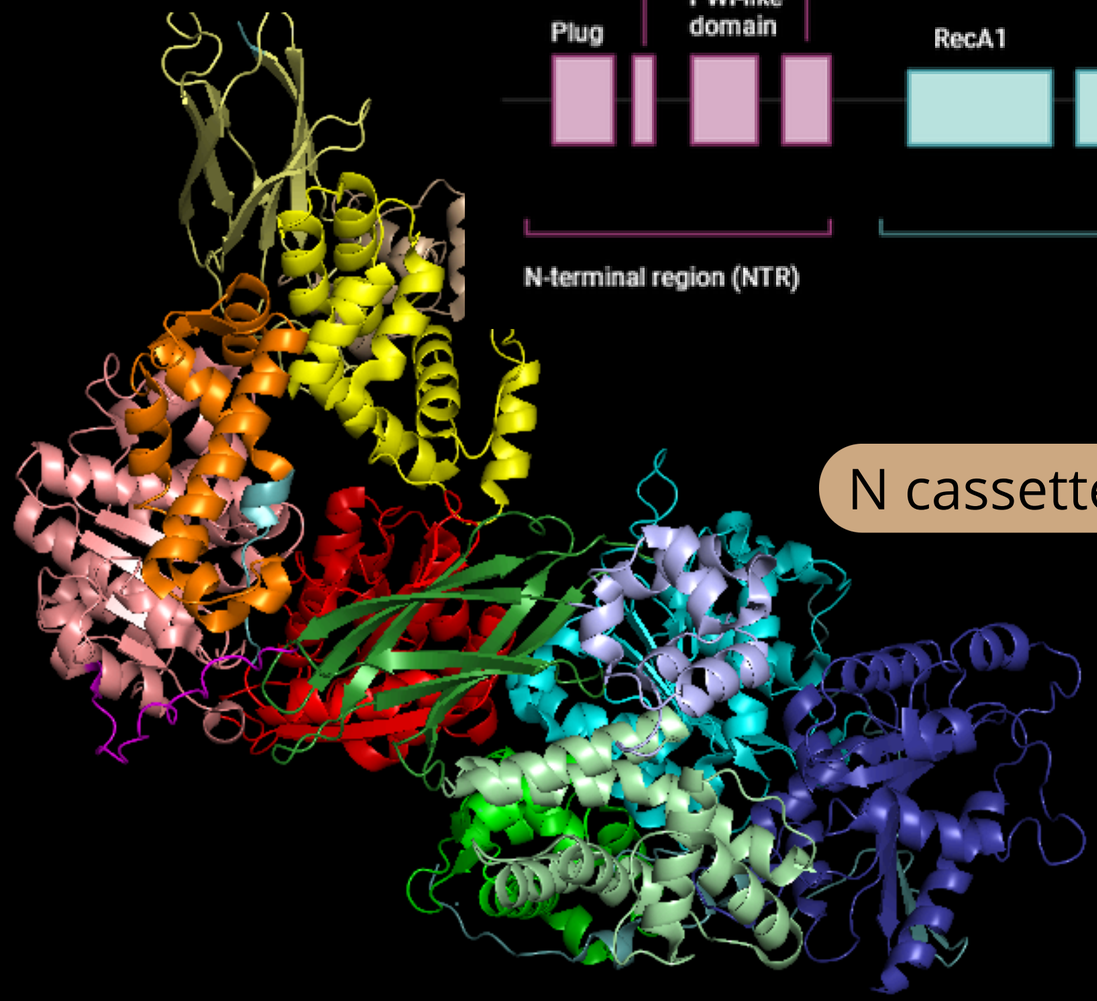


Adapted from Nguyen TH, Galej WP, Bai XC, Savva CG, Newman AJ, Scheres SH, Nagai K. The architecture of the spliceosomal U4/U6.U5 tri-snRNP. Nature. 2015 Jul 2;523(7558):47-52. doi: 10.1038/nature14548. Epub 2015 Jun 24. PMID: 26106855; PMCID: PMC4536768.

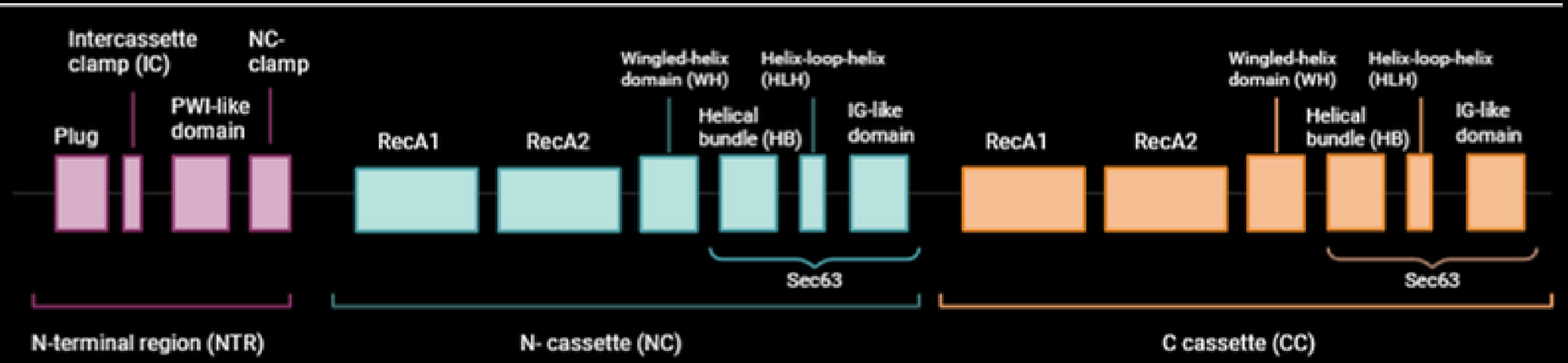


Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

C cassette

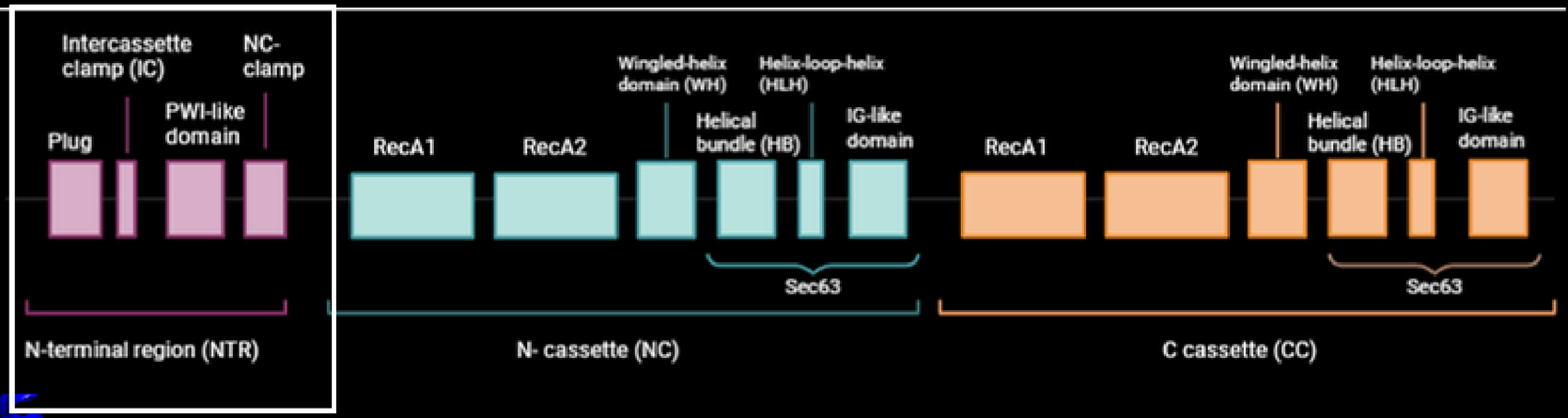


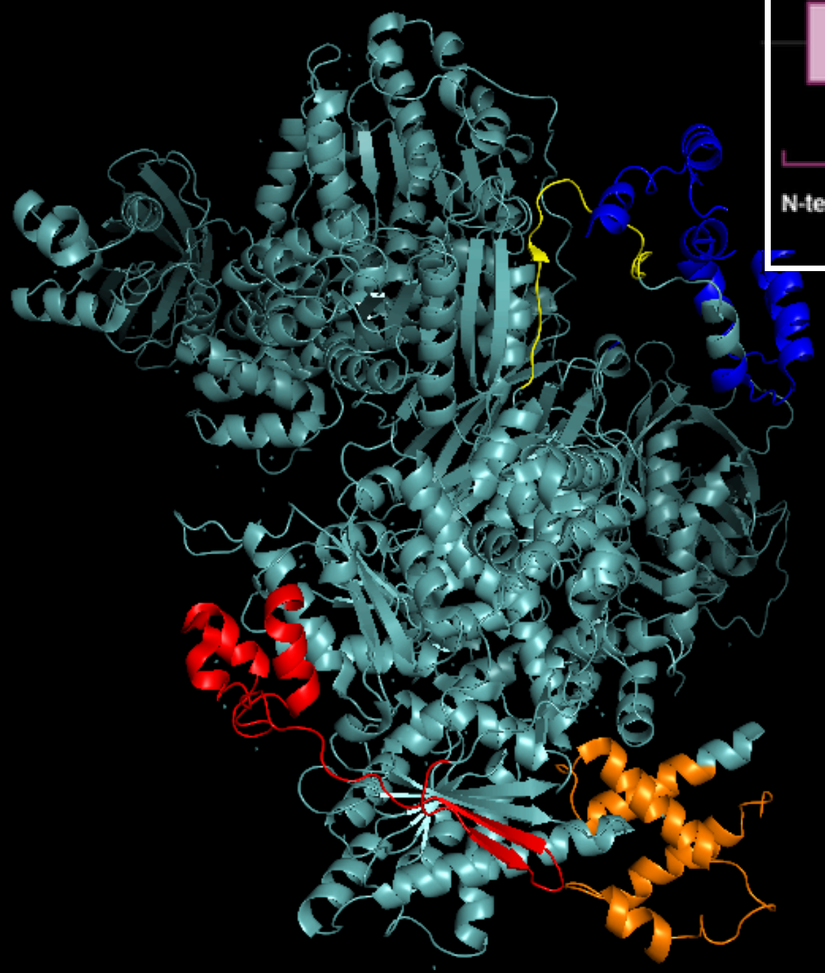
N cassette



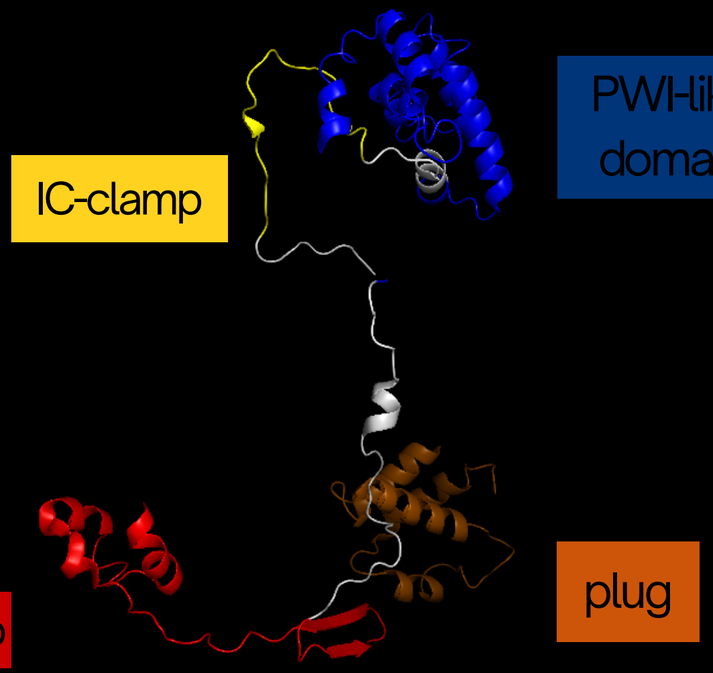
Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

N-terminal region (NTR)



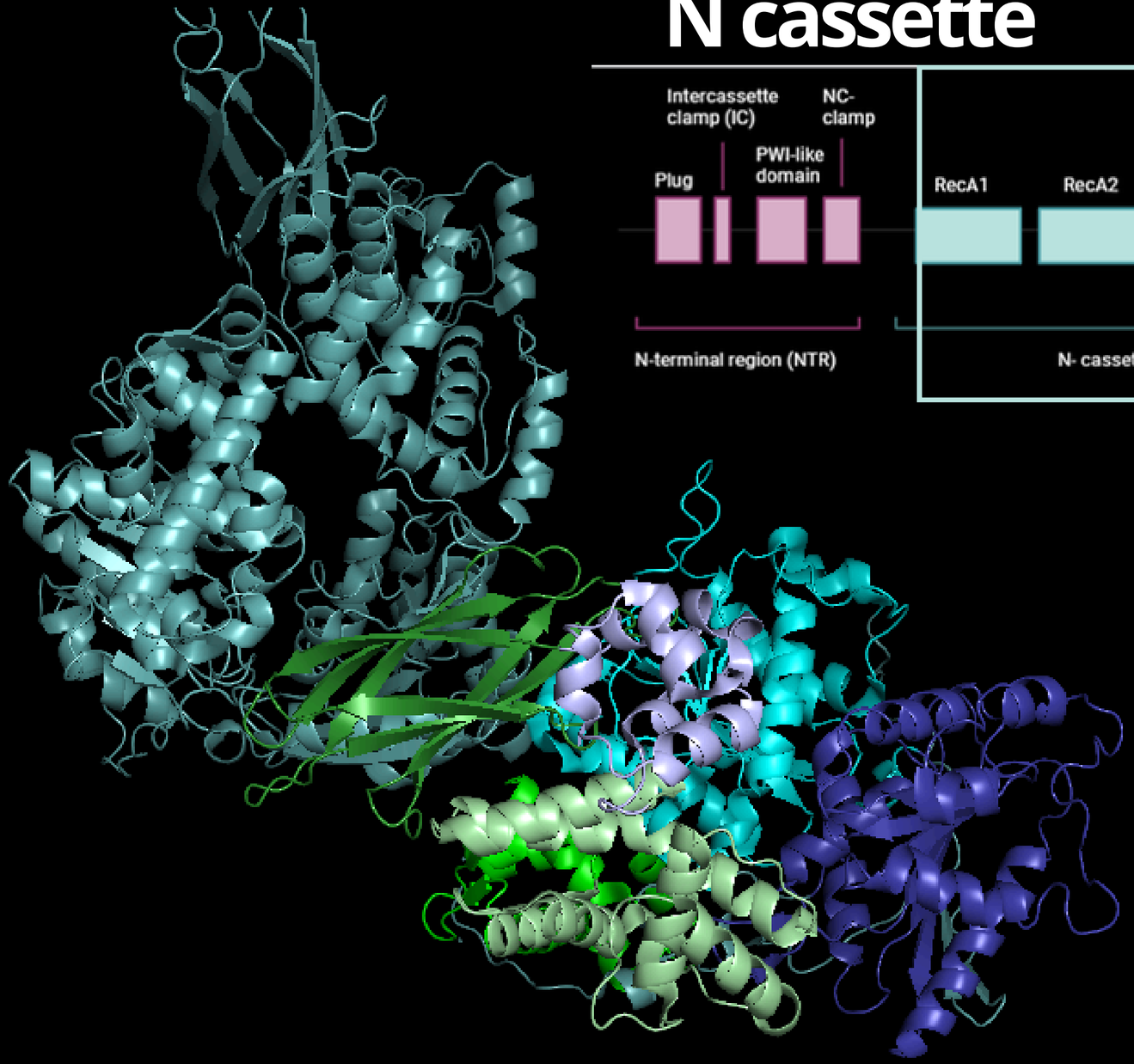


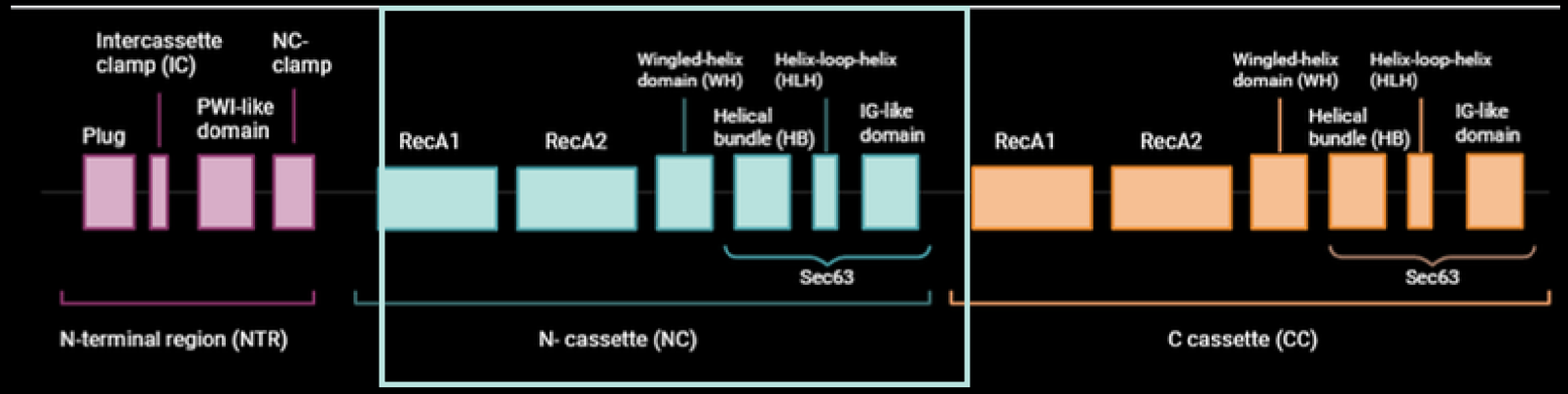
UNKNOWN FOLD



Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

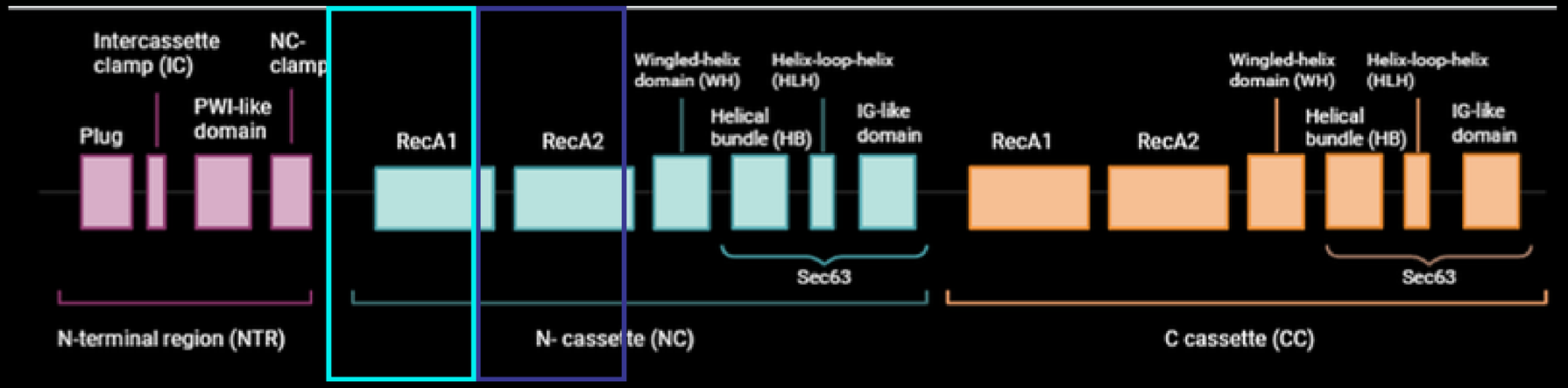
N cassette

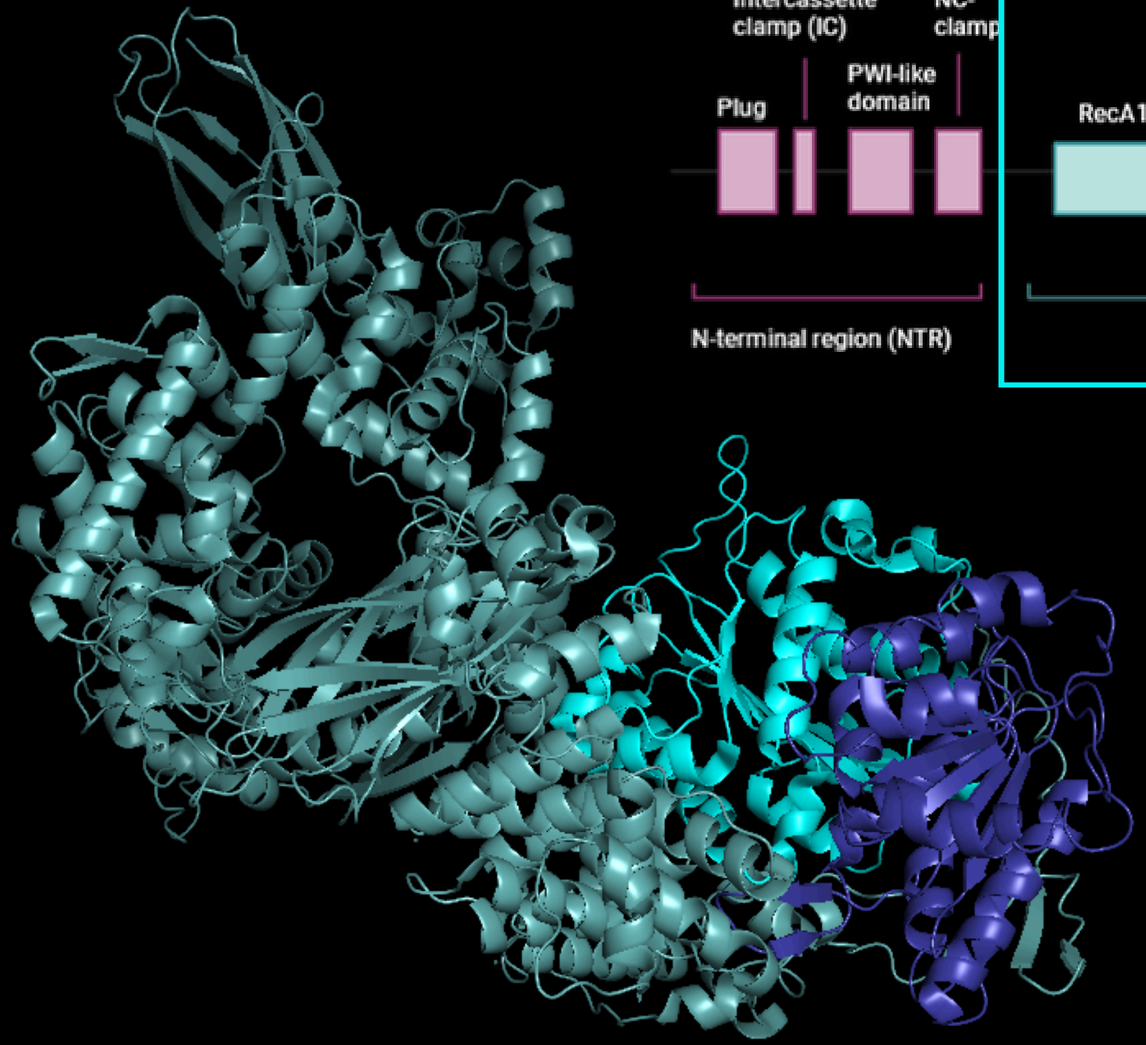




Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

RecA1 and RecA2



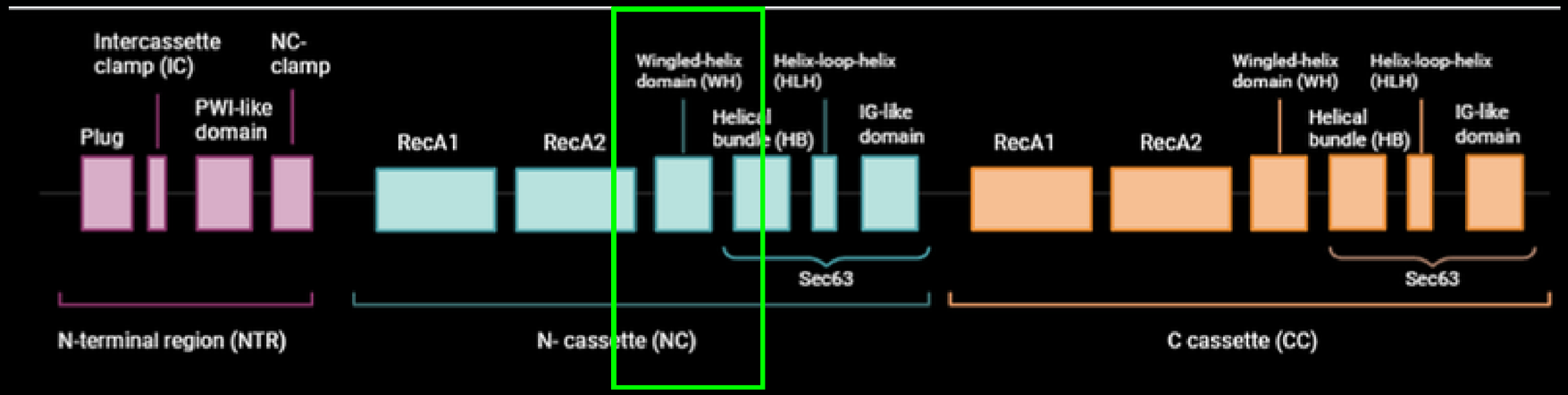


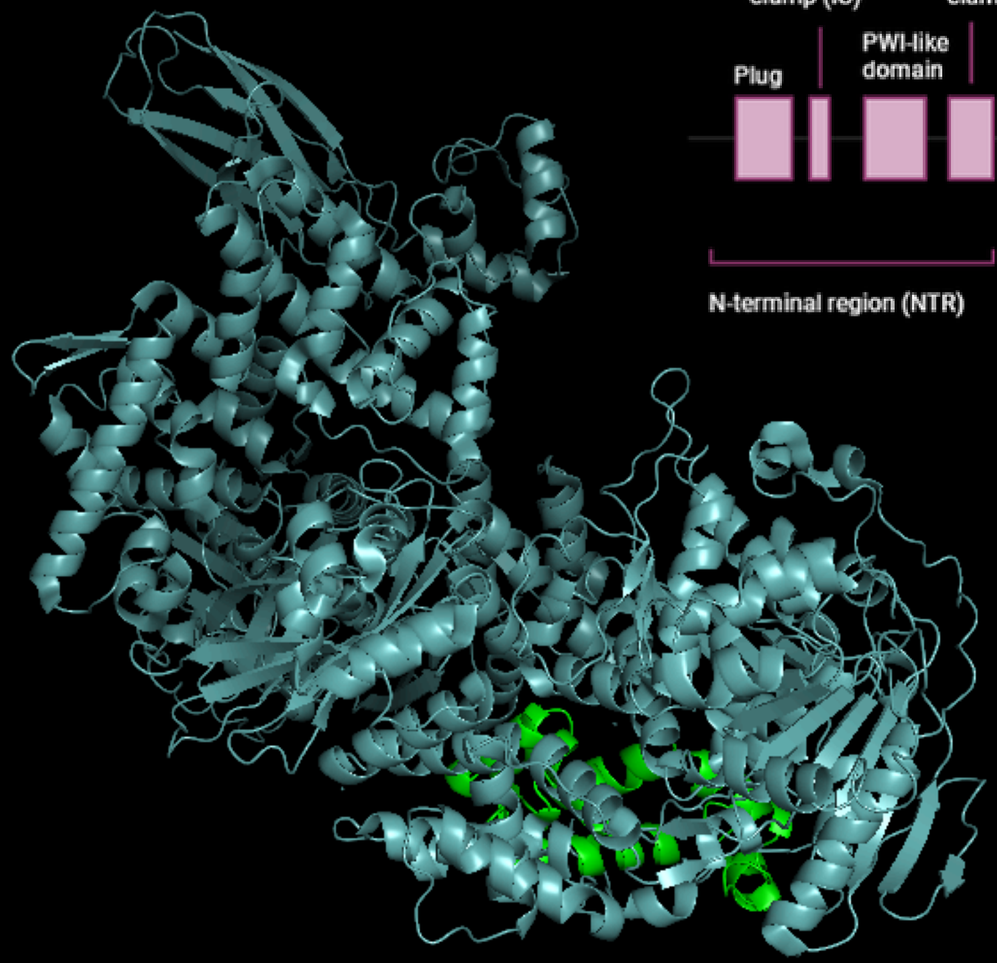


Rossmann fold

Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

WH



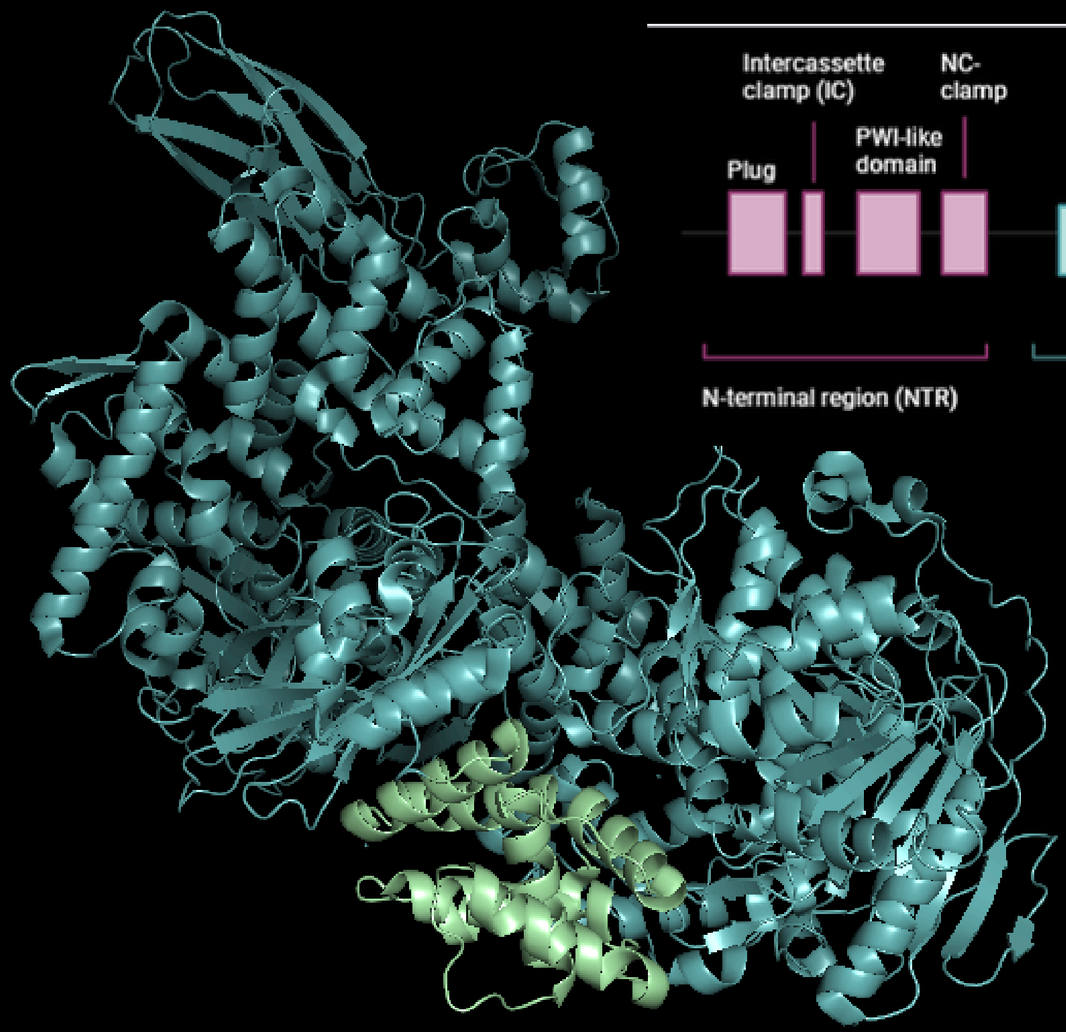


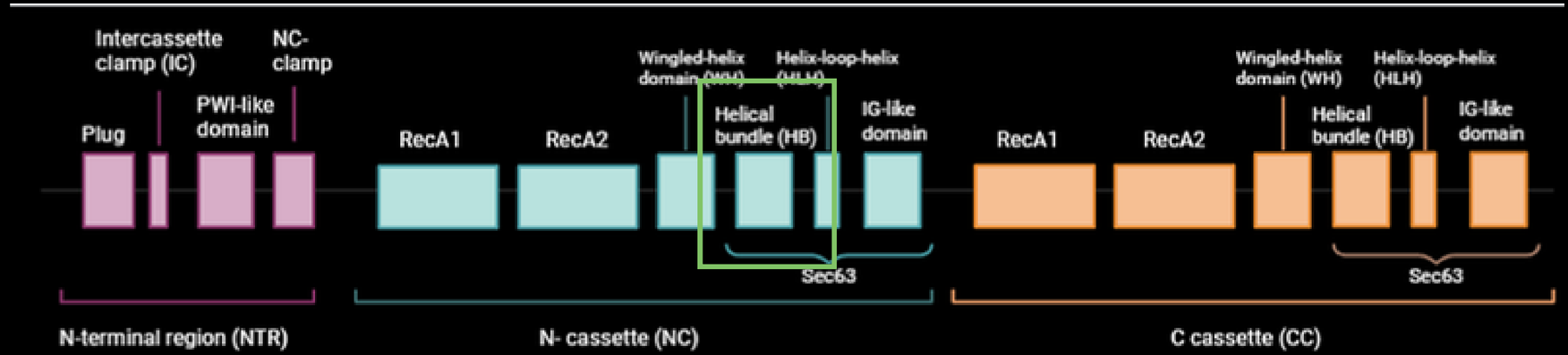


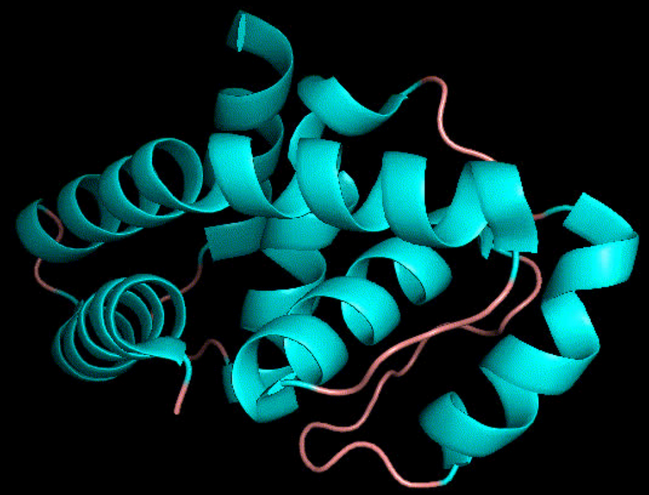
bundle of alpha-helices followed by a small antiparallel beta-sheet.

Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

HB



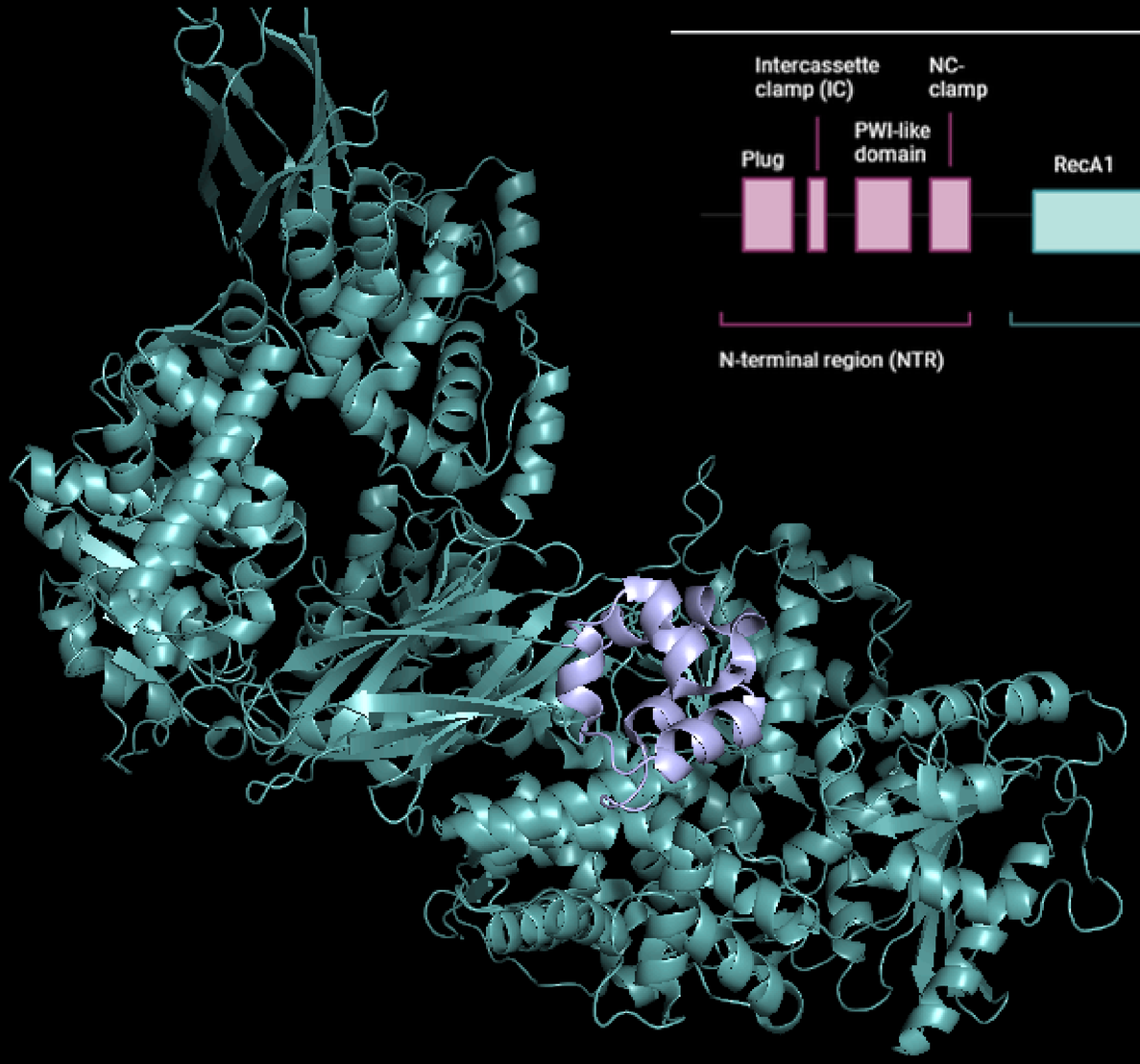




Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

alpha-helix bundle

HLH



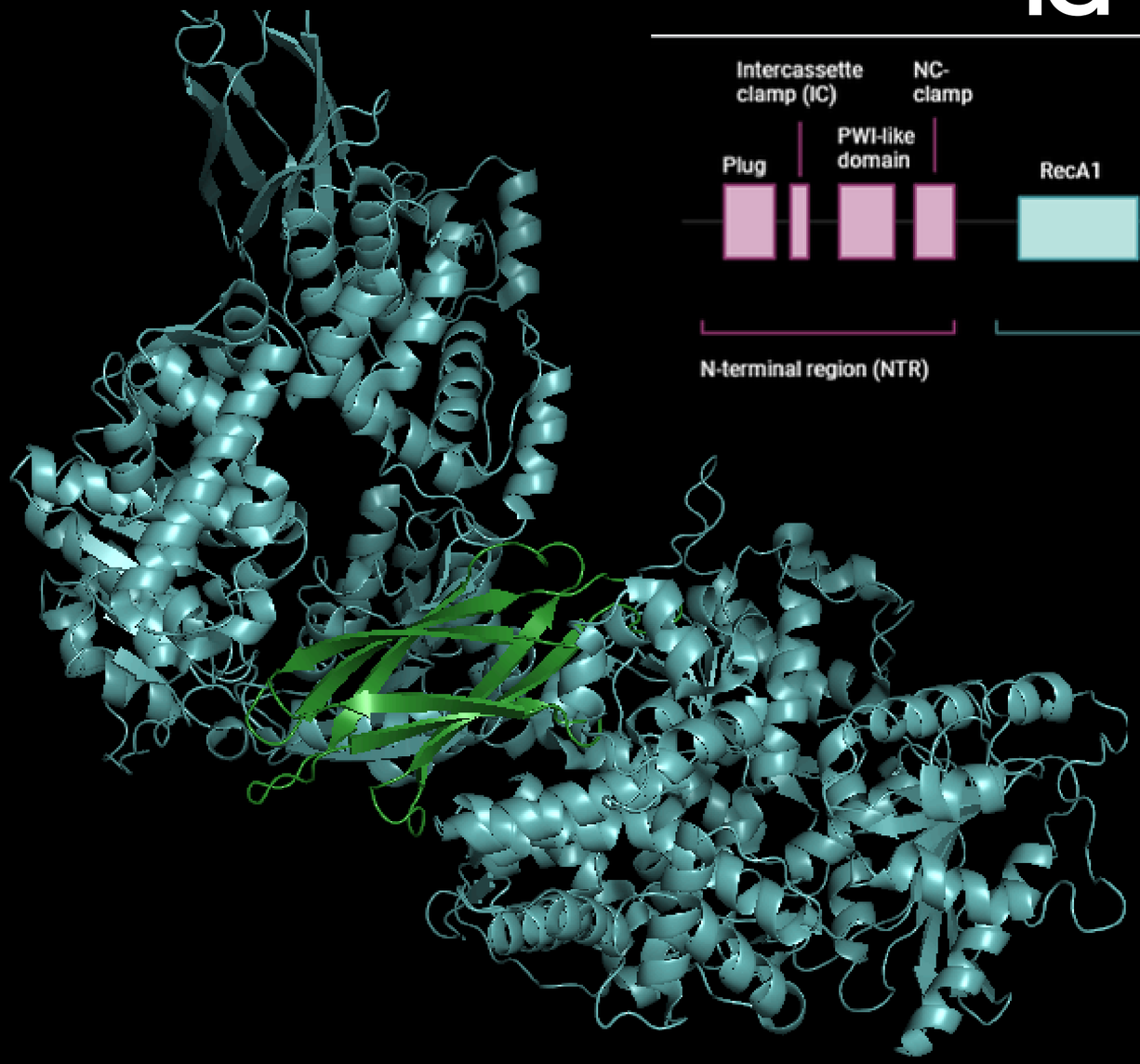


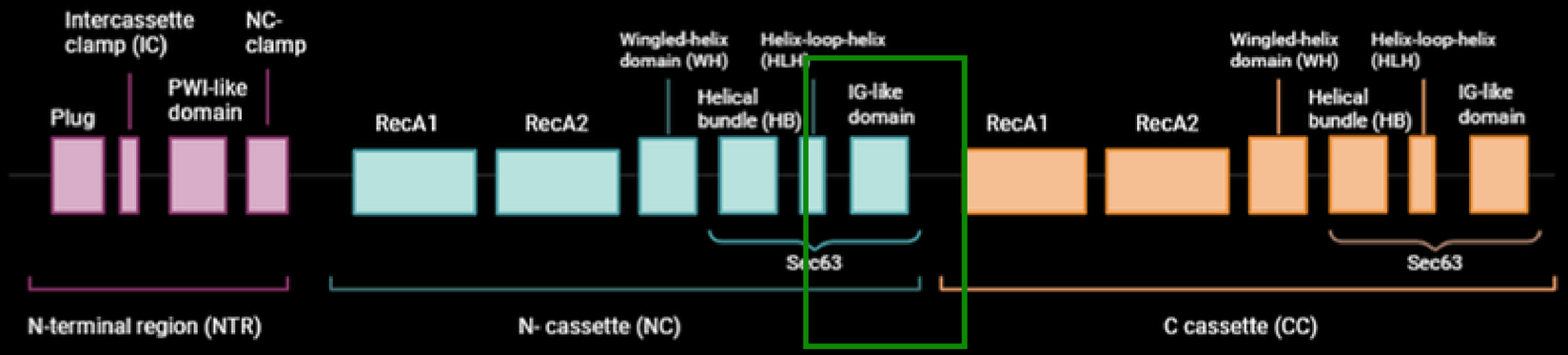


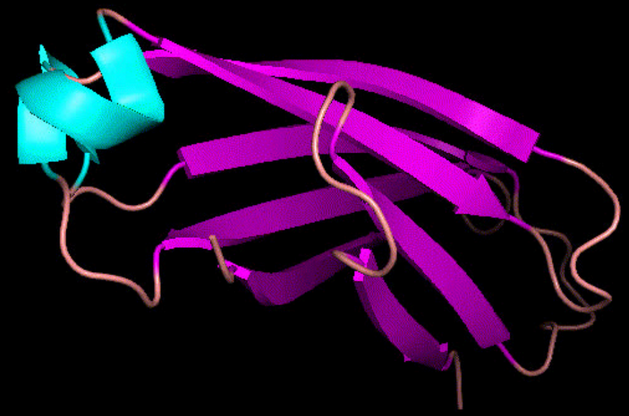
Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

alpha helices connected by loops

IG





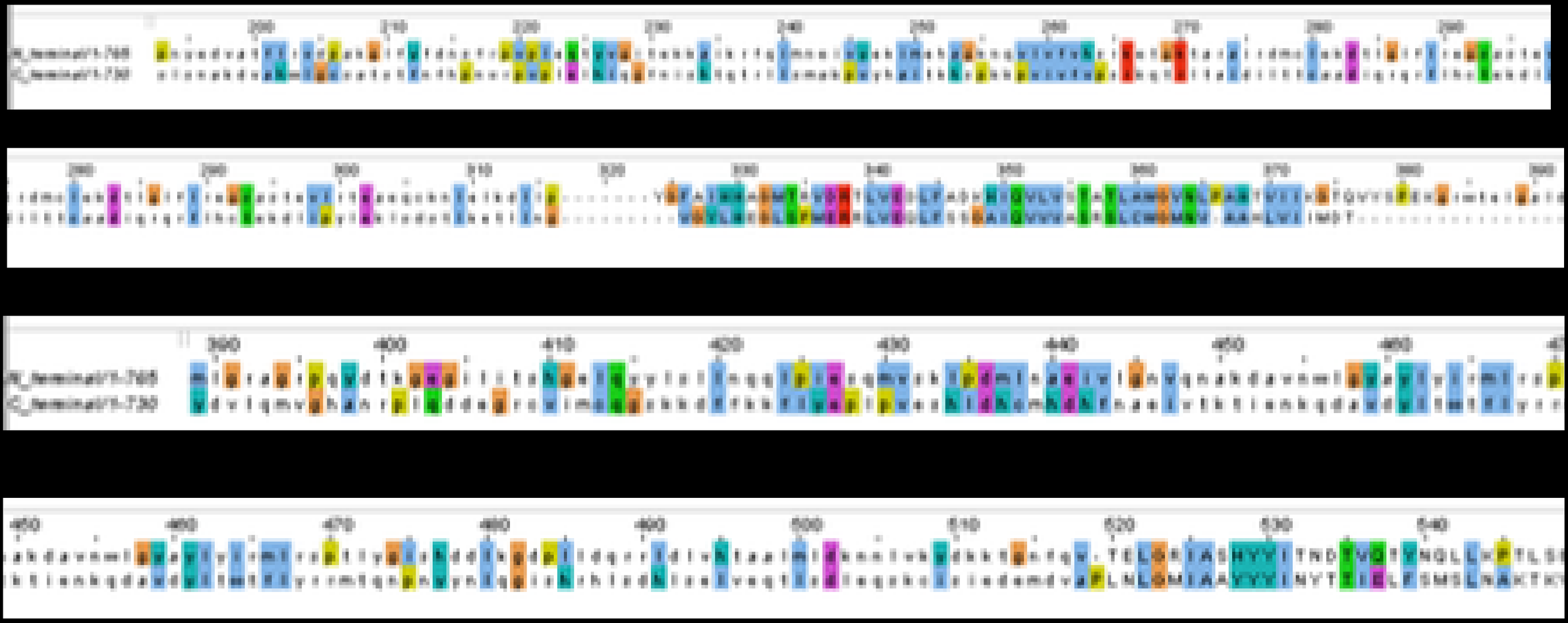


barrel of antiparallel beta-sheets

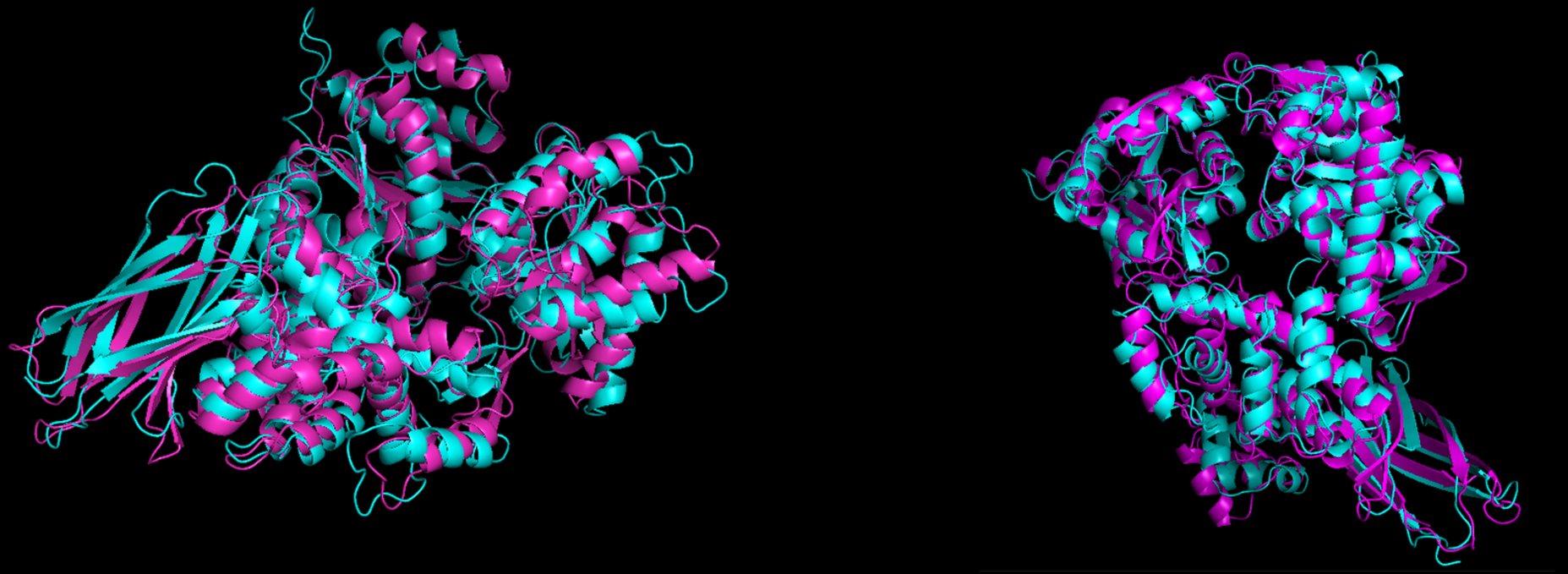
Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

C-cassette and N-cassette comparison

Comparison between N-cassete and C-cassette



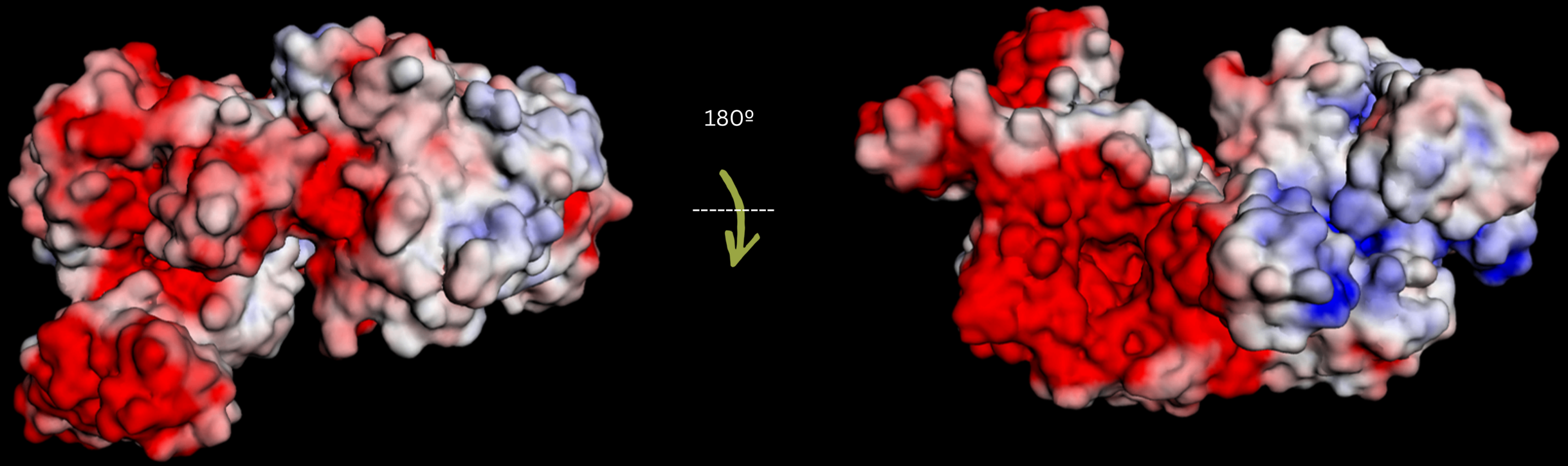
NC and CC structural superimposition



Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å
RMSD: 2.15

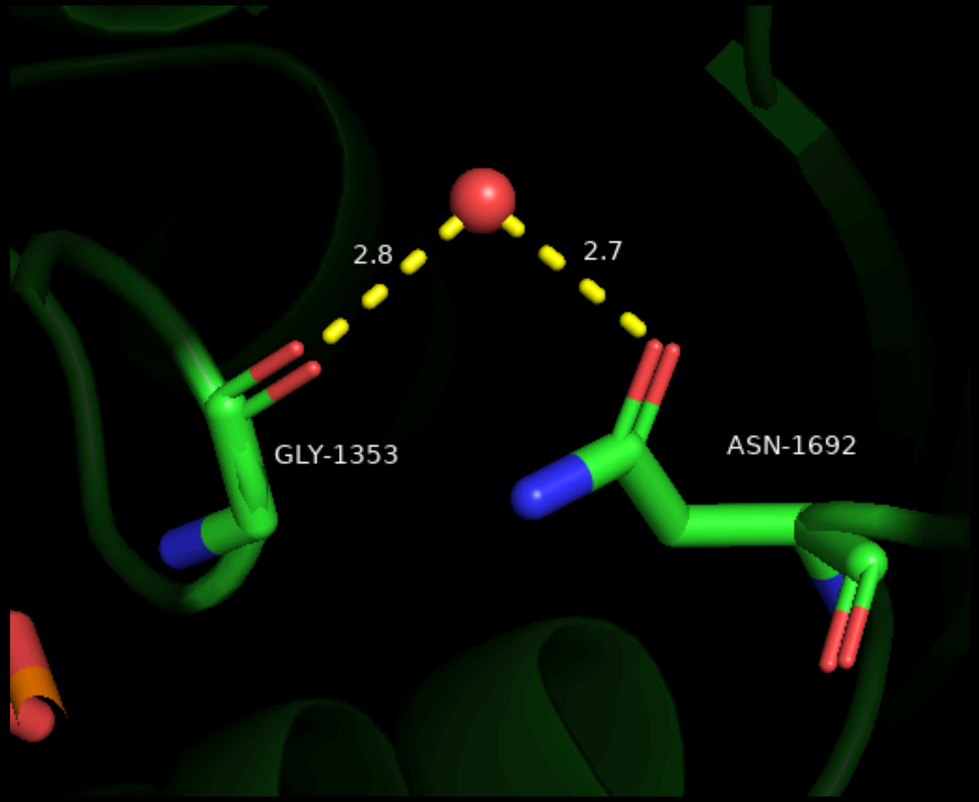
```
     No.  Domain1         Domain2         Sc     RMS    Len1 Len2  Align NFit Eq. Secs.   %I   %S   P(m)
Pair   1  4f91_C_cassete  4f91_N_cassette 6.37   2.15    814  826   862  663 636    0  30.19 100.00 2.44e-45
Reading in matrix file cassettes.mat...
Doing cluster analysis...
Cluster:  1 (4f91_C_cassete  & 4f91_N_cassette ) Sc  6.37 RMS   2.16 Len 862 nfit 665
 See file cassettes.1 for the alignment and transformations
```

NC and CC comparison

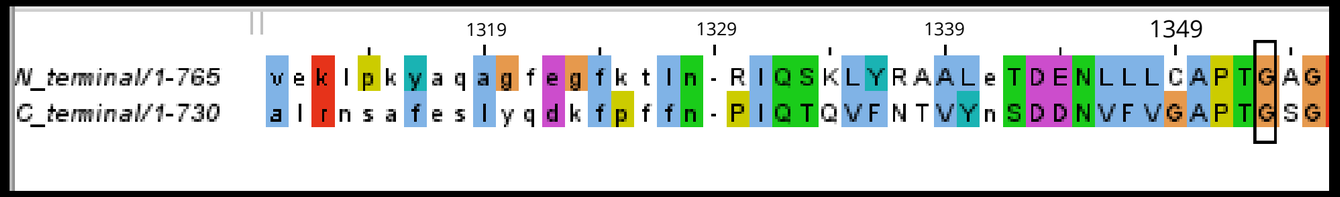


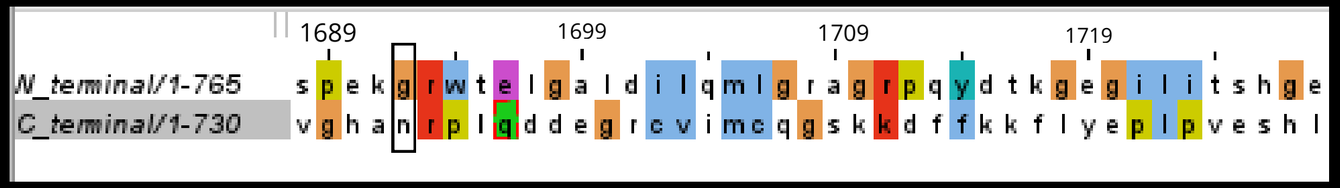
Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

C-cassette mutation

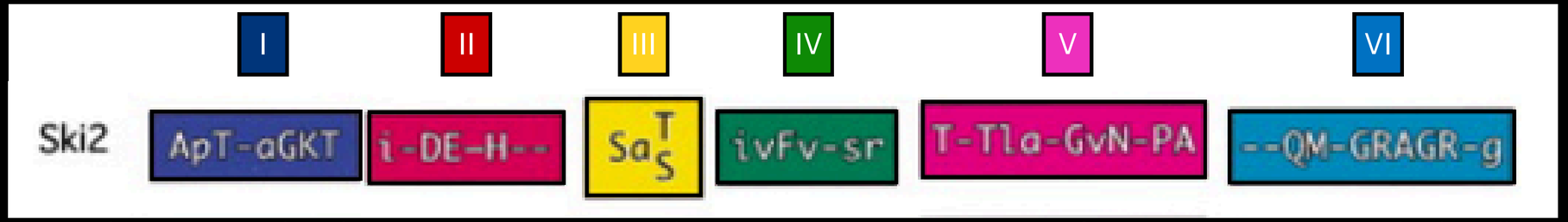


Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å

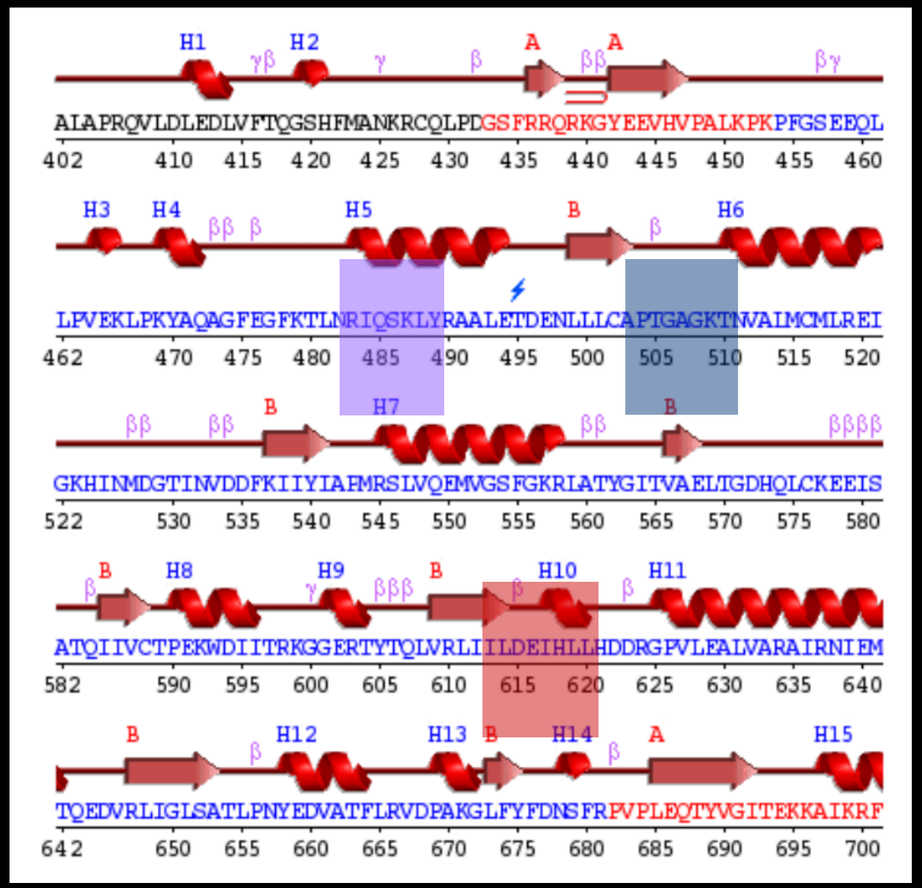


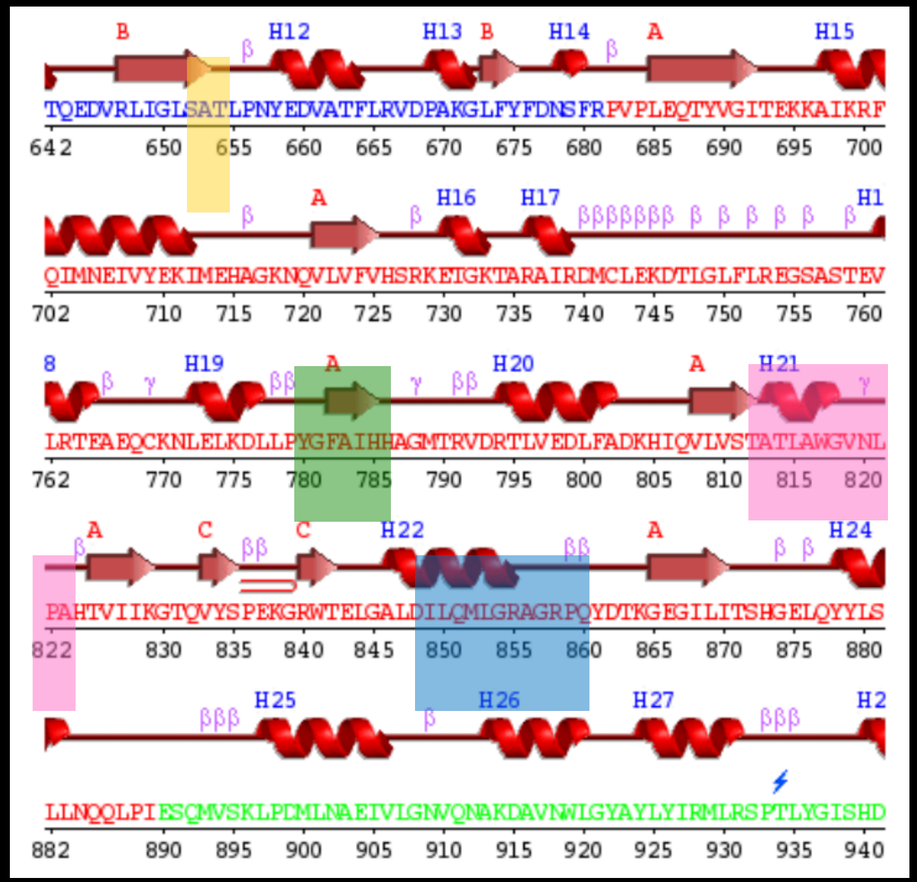


Ski2-like motifs

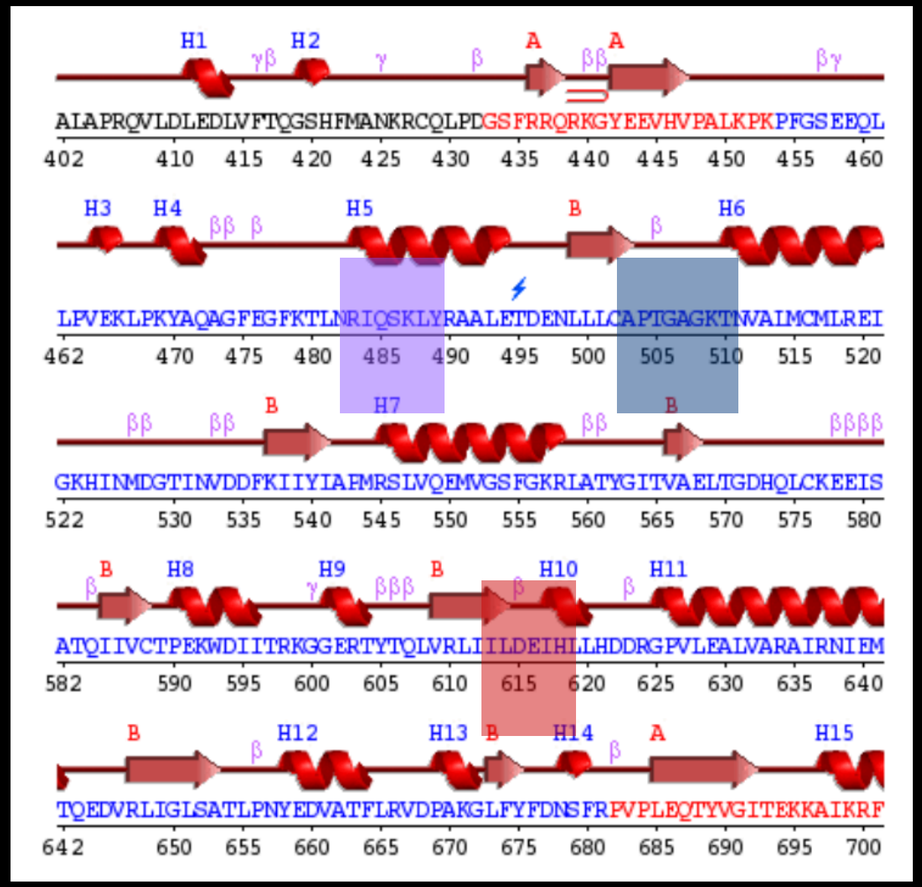


N.Kyle Tanner, Patrick Linder (2001). DExD/H Box RNA Helicases: From Generic Motors to Specific Dissociation Functions. *Molecular Cell*



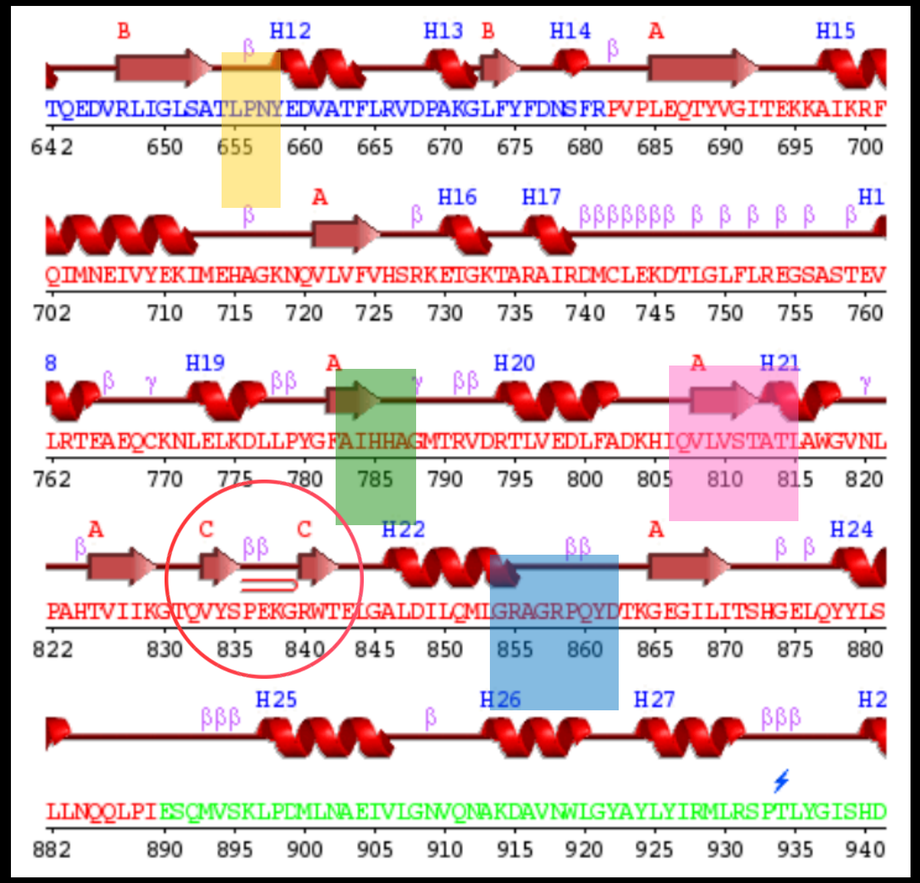


Ski2-like motifs



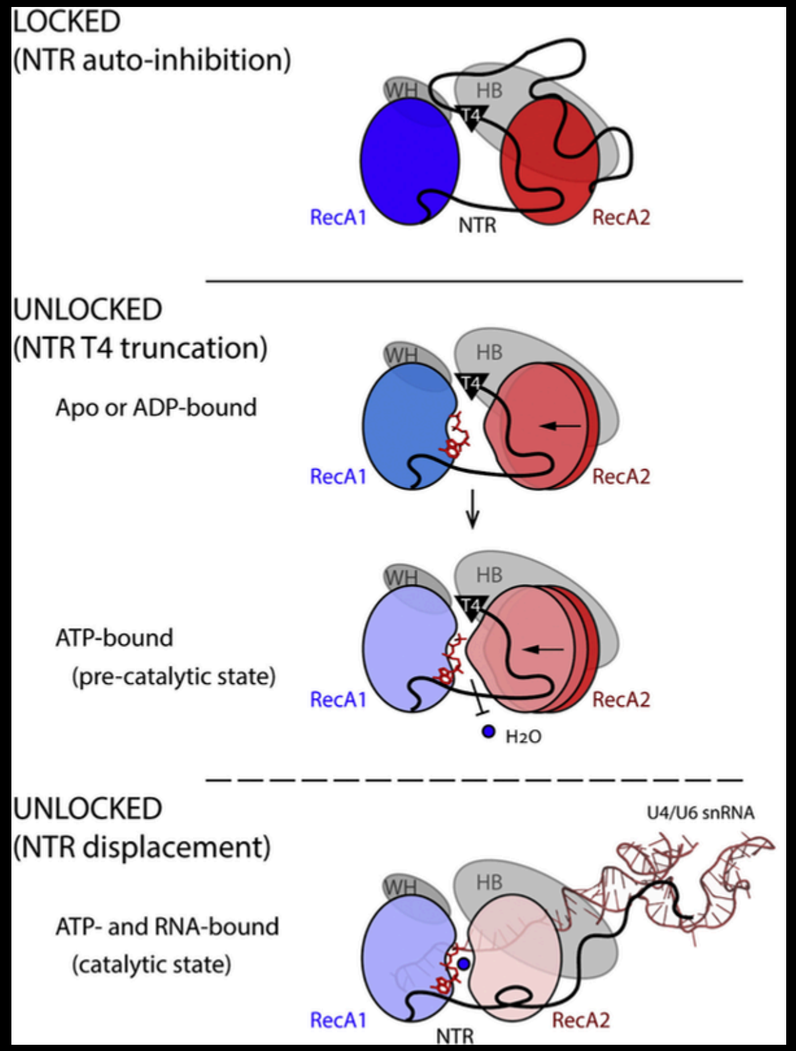
- Q motif Binds adenine's N6 and N7
- Motif I **ApT-aGKT** P-loop, Walker A motif, binds phosphates of NTP
- Motif II **i-DE-H--** Walker B, binds β and γ phopshate through Mg2+, coordinates hydrolysis of NTP with water molecule

Ski2-like motifs

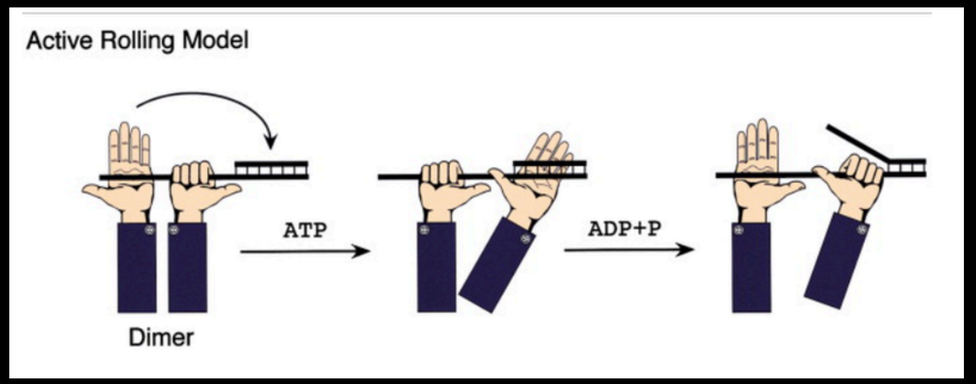


- Motif III **SaT/S** Comunication between RecA 1 and RecA 2
- Motif IV **ivFv-sr** Substrate binding
- Motif V **T-Tla-GvN-PA** Binds substrate through sugar-phosphate backbone
- Motif VI **--QM-GRAGR-g** Binds γ phosphate, converts NTP binding/hydrolysis with domain 1 and 2 movement

Movement of RecA1 and RecA2 with ATP



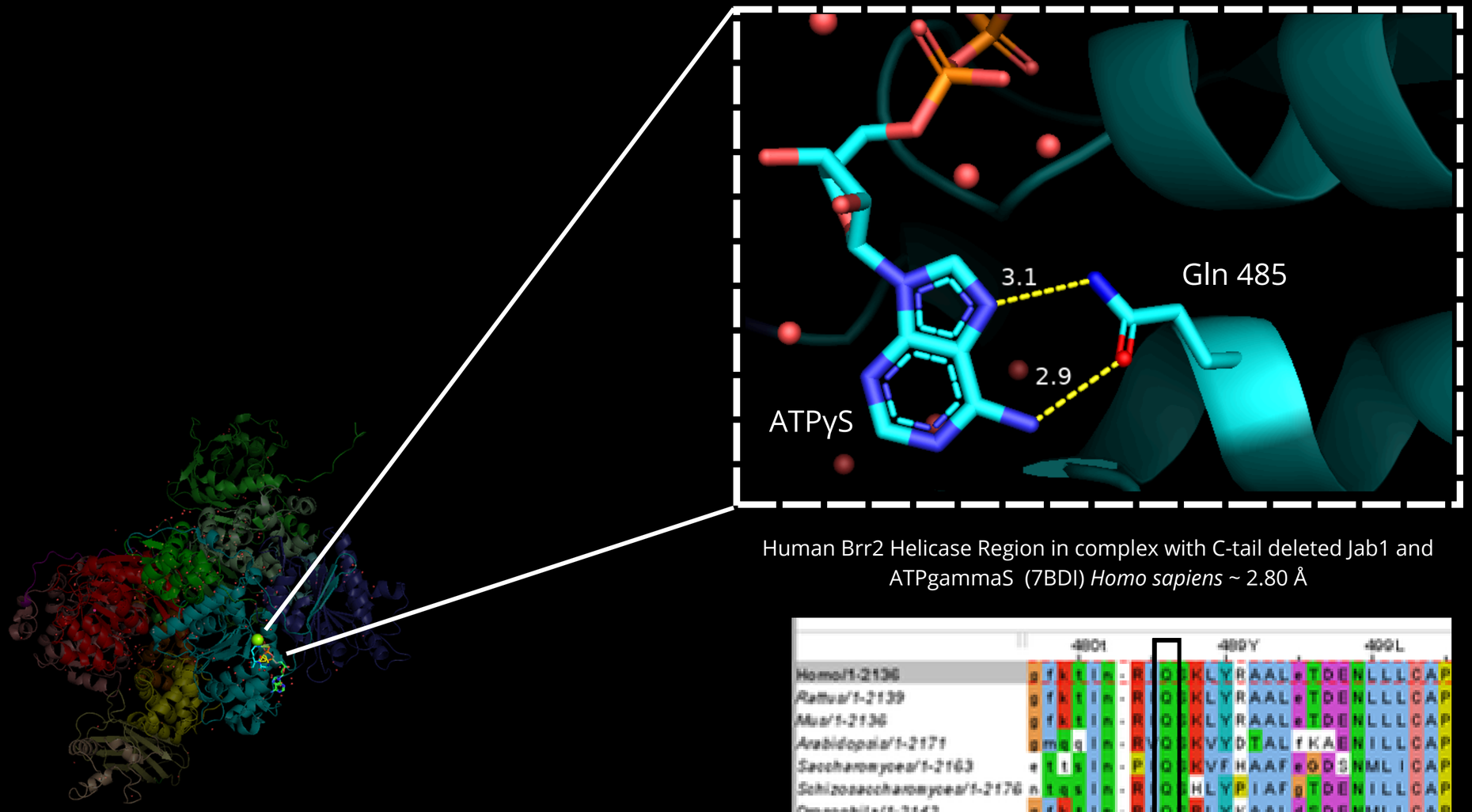
Johnson, S. J., & Yim, M. K. (2020). Closing in on ATPase activity by an RNA helicase. Structure (London, England: 1993)



N.Kyle Tanner, Patrick Linder, DExD/H Box RNA Helicases: From Generic Motors to Specific Dissociation Functions, Molecular Cell, Volume 8, Issue 2, 2001, Pages 251-262.

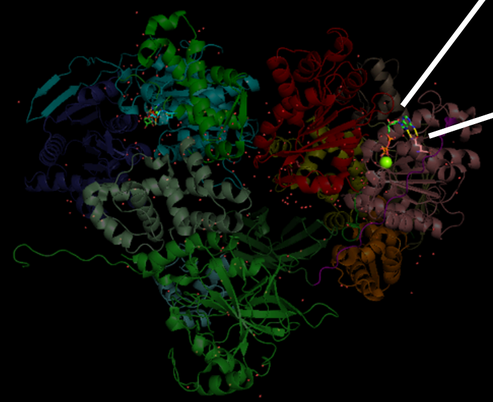
ATP-motifs interaccions

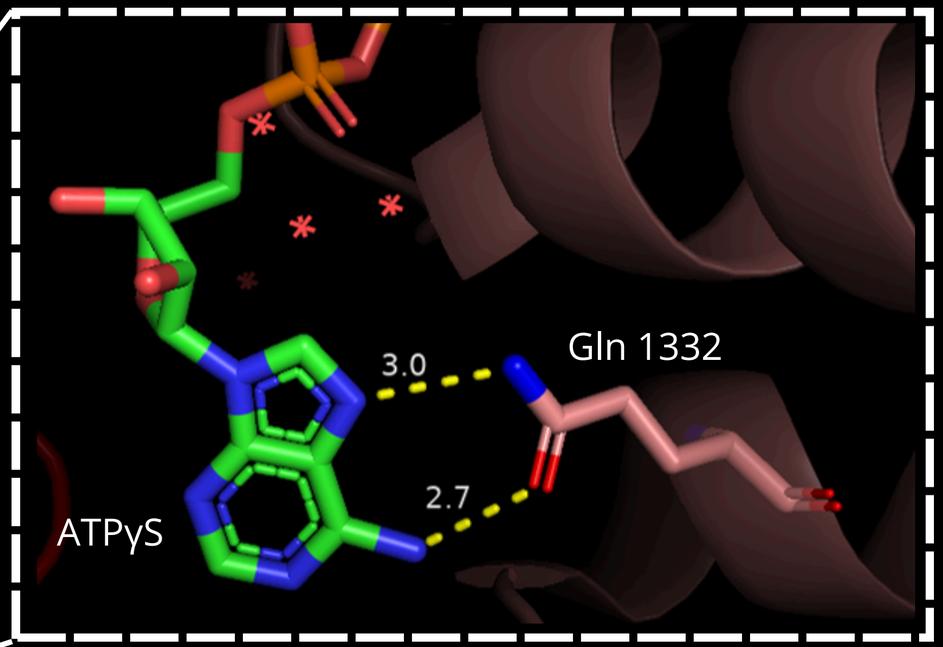
Q motif N-cassette



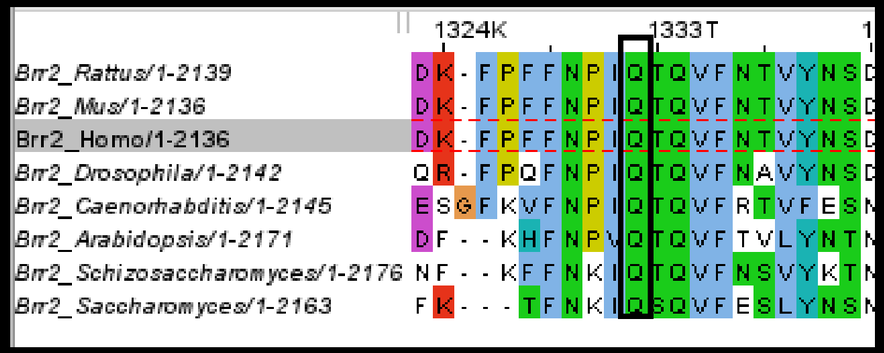
Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å

Q motif C-cassette

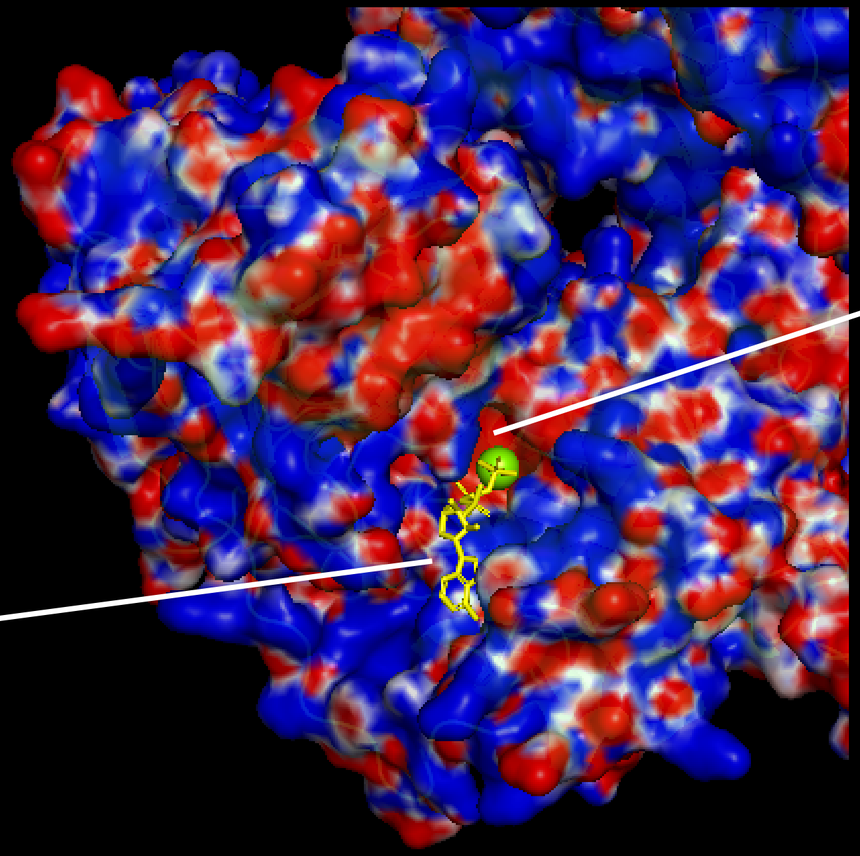




Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å



MOTIF I → ATP binding pocket

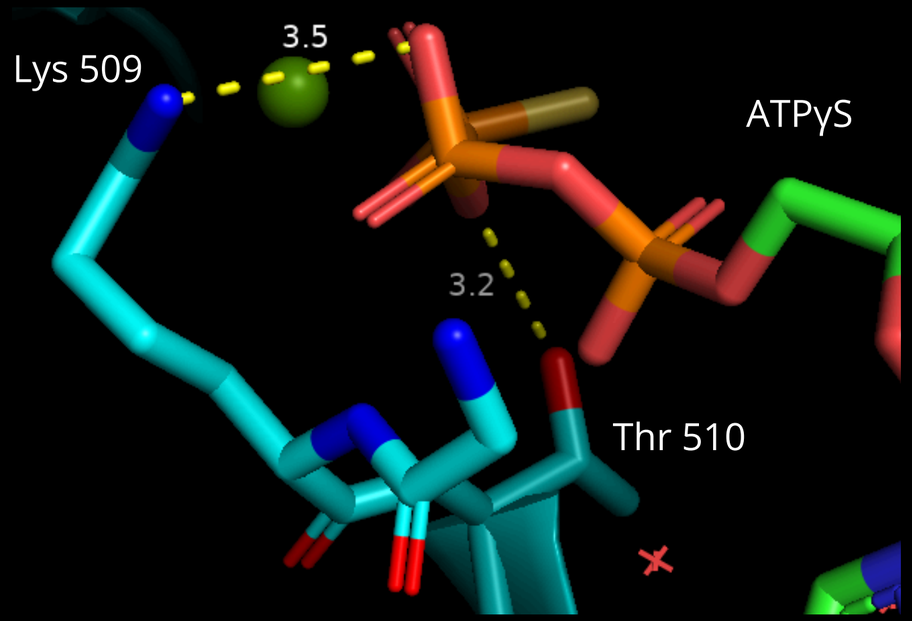


Negatively charged surface

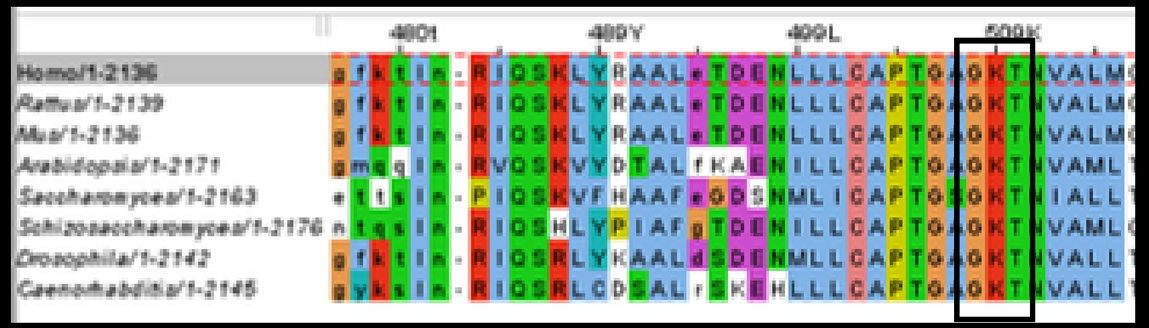
Positively charged surface

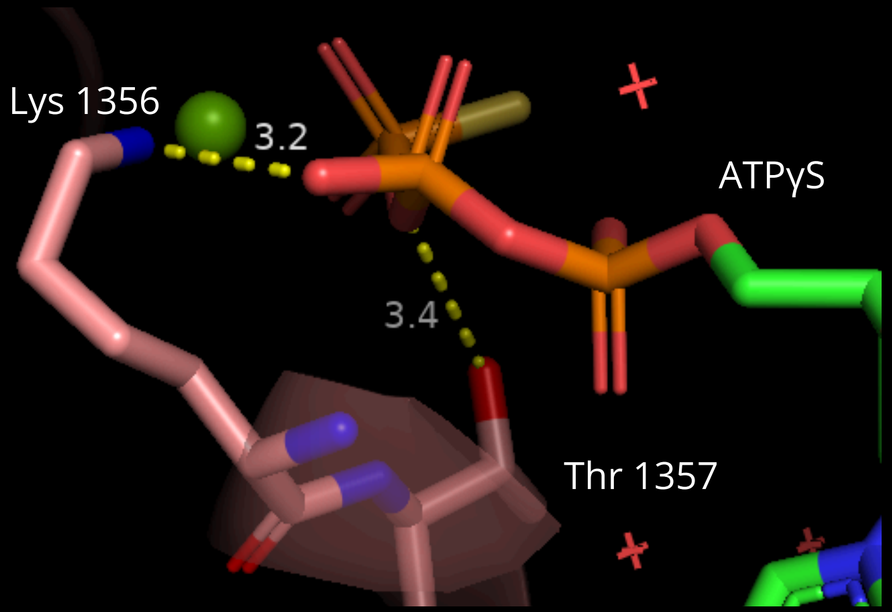
ATP binding pocket (7BDI) *Homo sapiens* ~ 2.80 Å

motif I

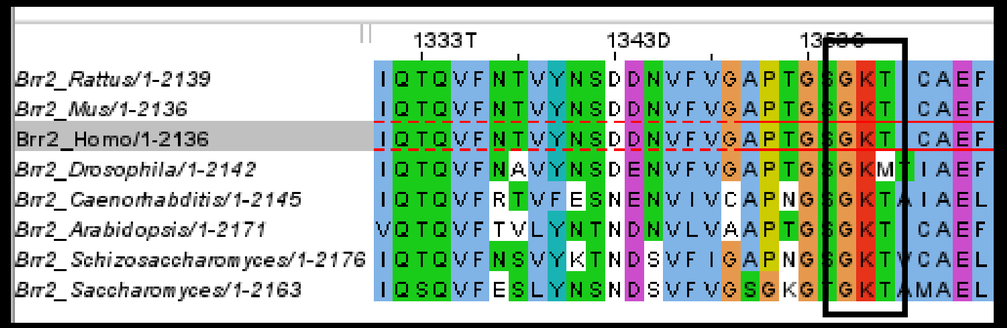


Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å



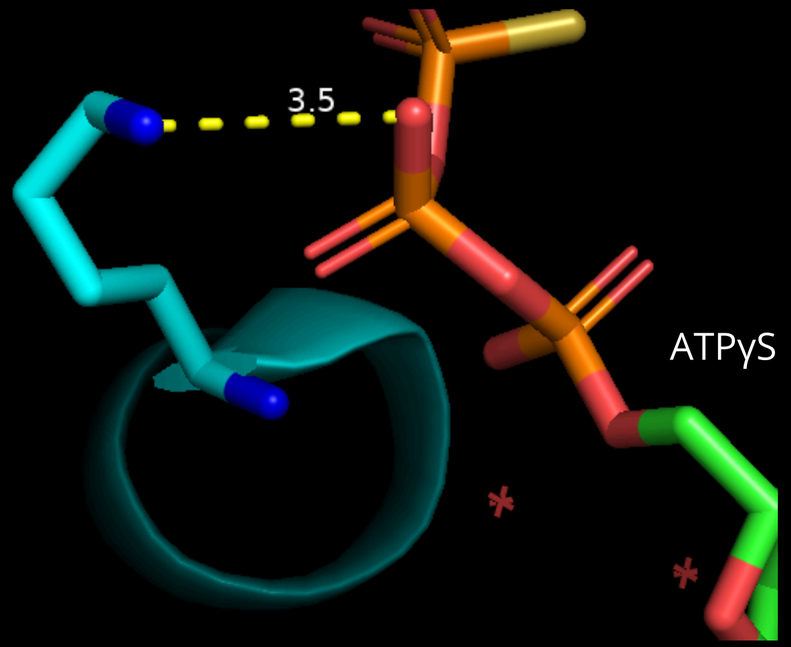


Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å

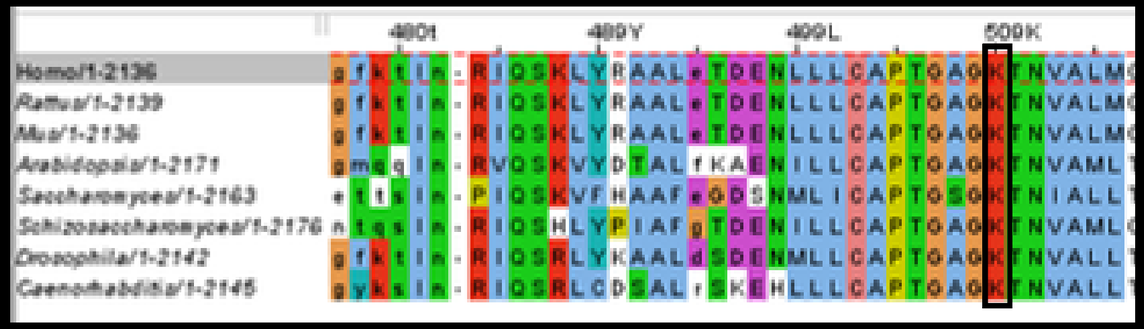


motif I

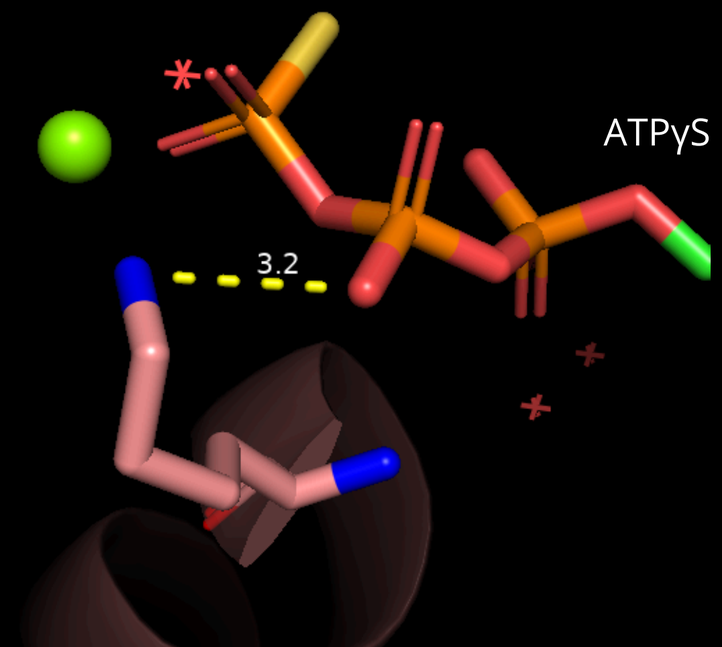
Lys 509



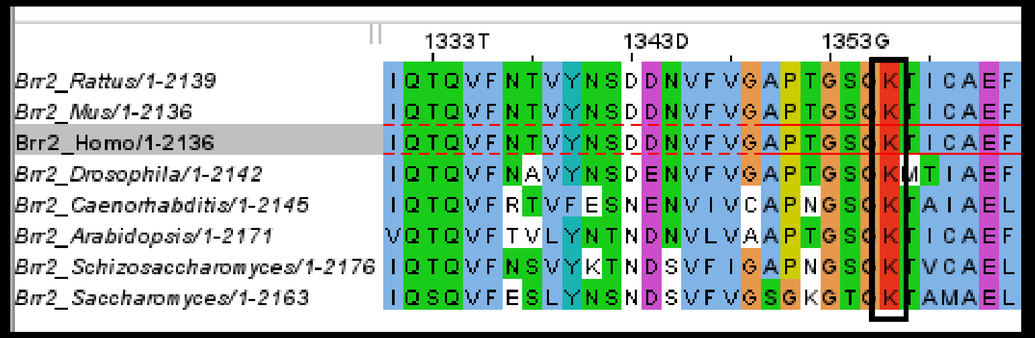
Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å



Lys 1356

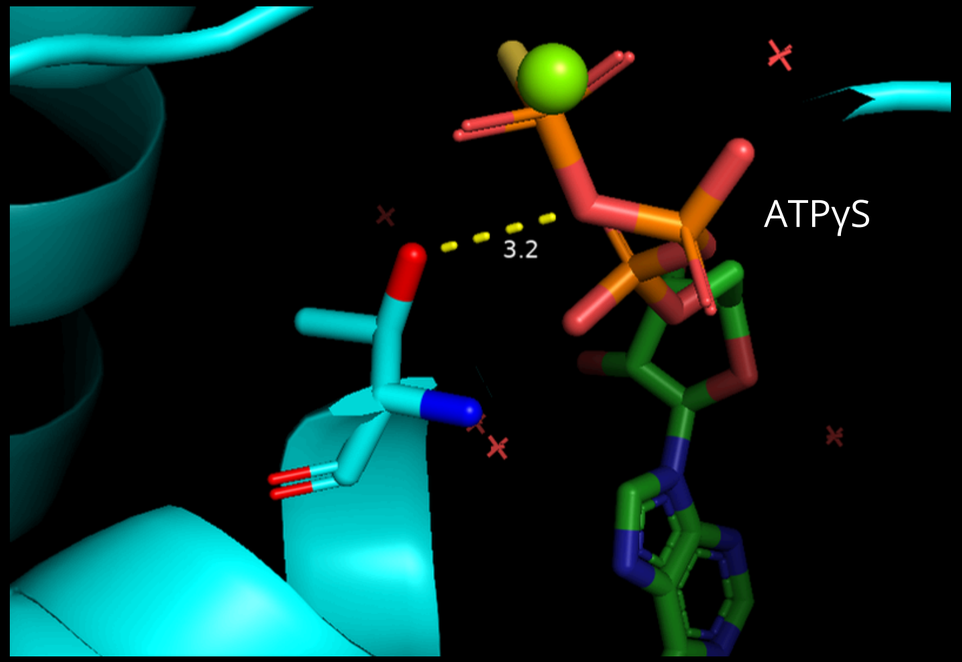


Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å

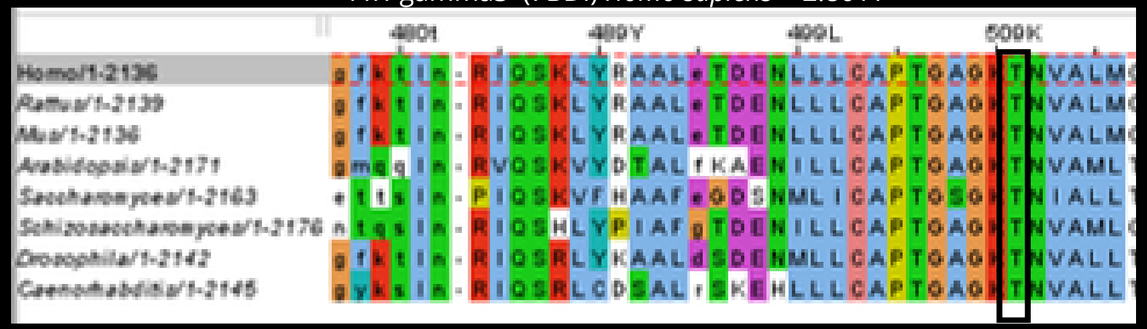


motif I

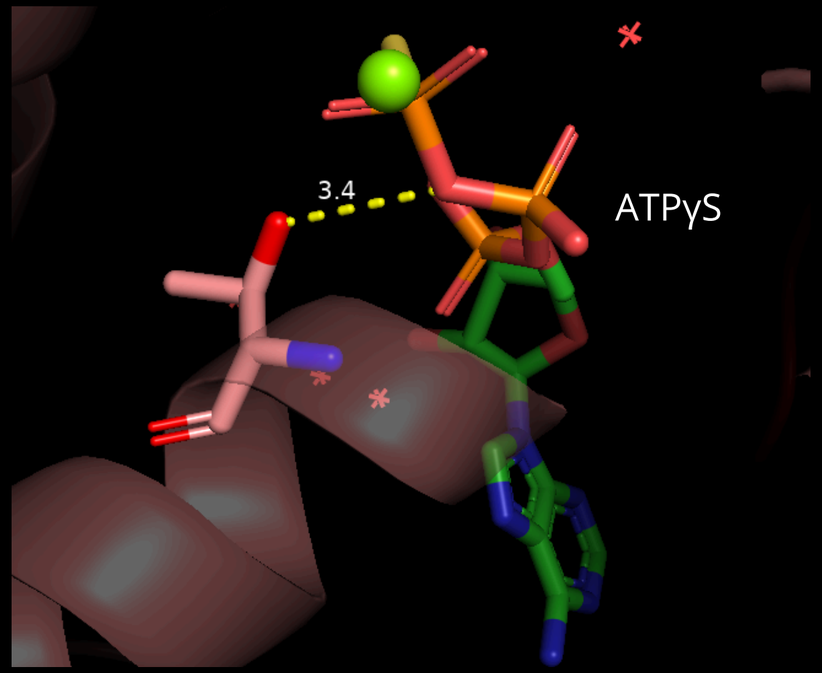
Thr 510



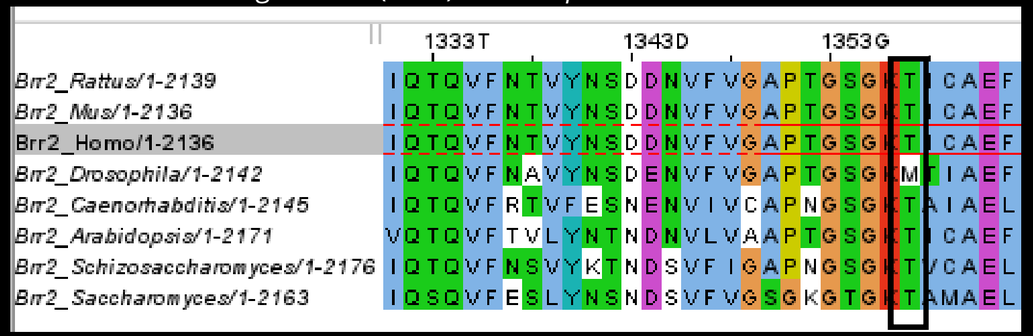
Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å



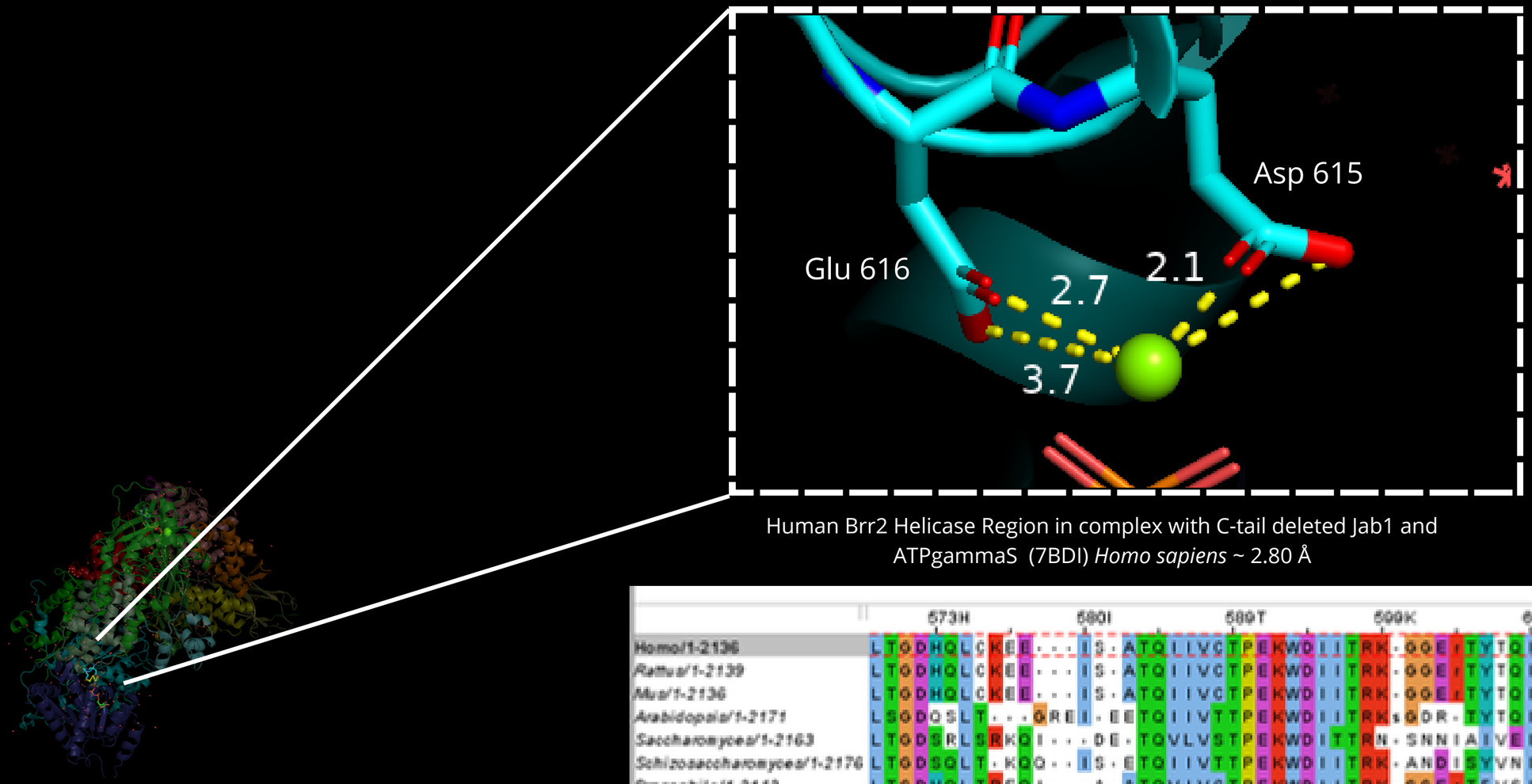
Thr 1357



Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å

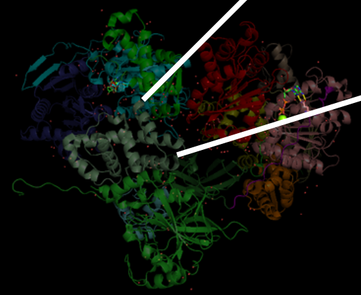


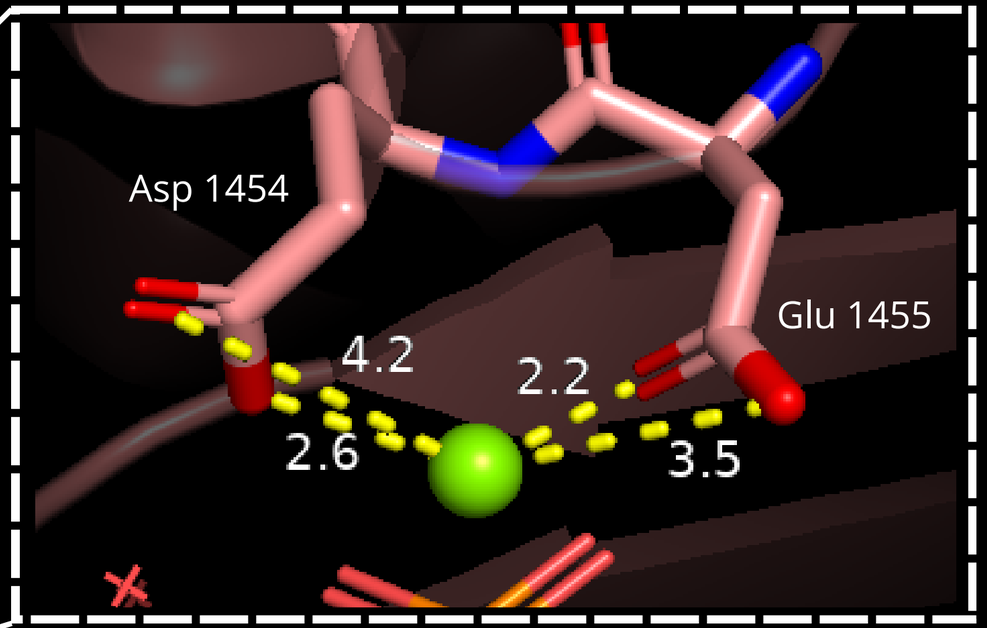
motif II N-cassette



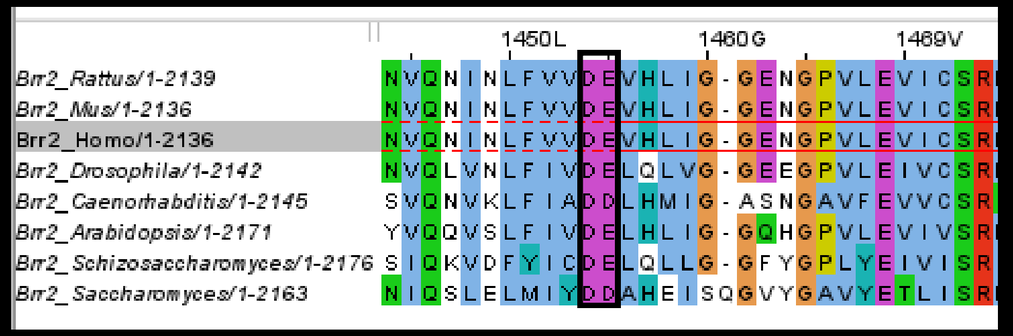
Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å

motif II C-cassette

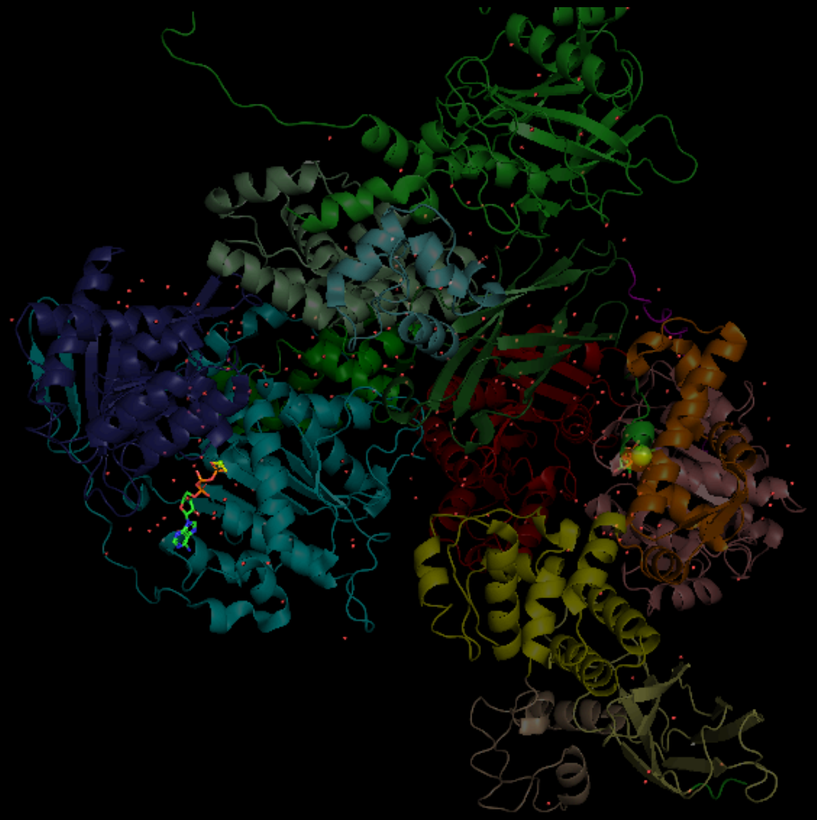




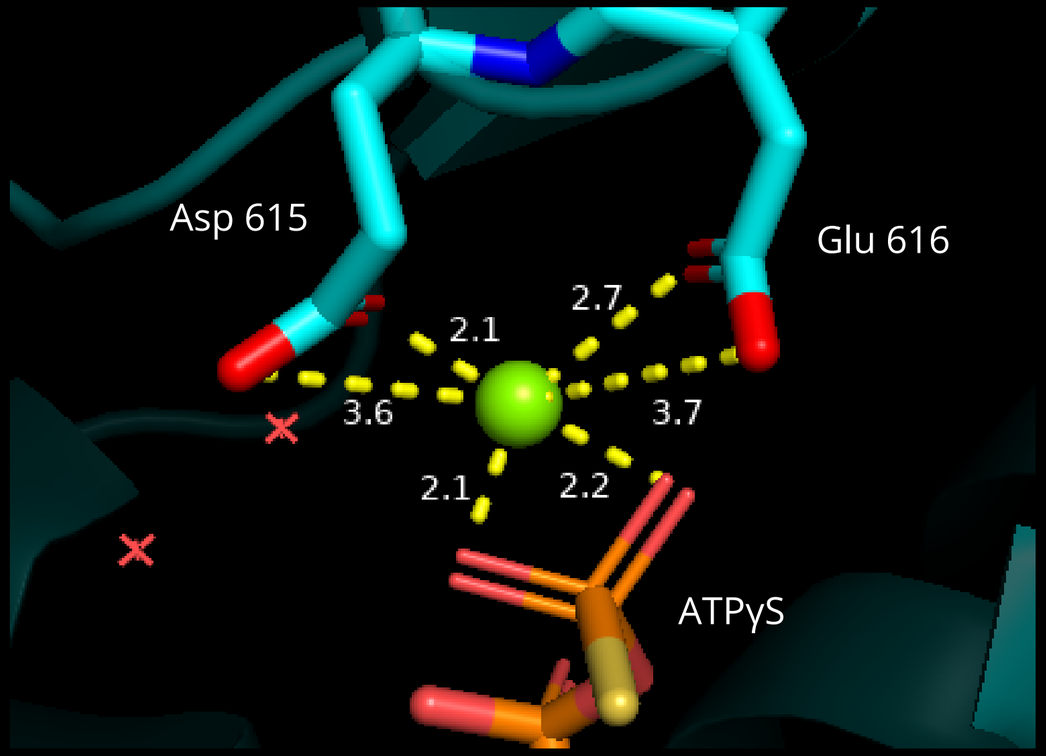
Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å



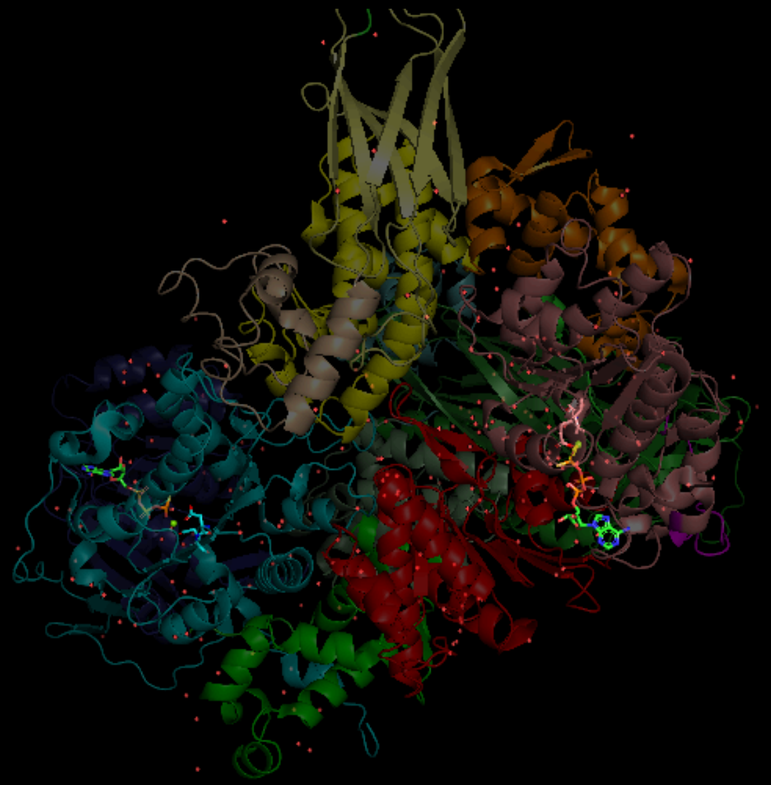
Interactions between Mg and N-cassette



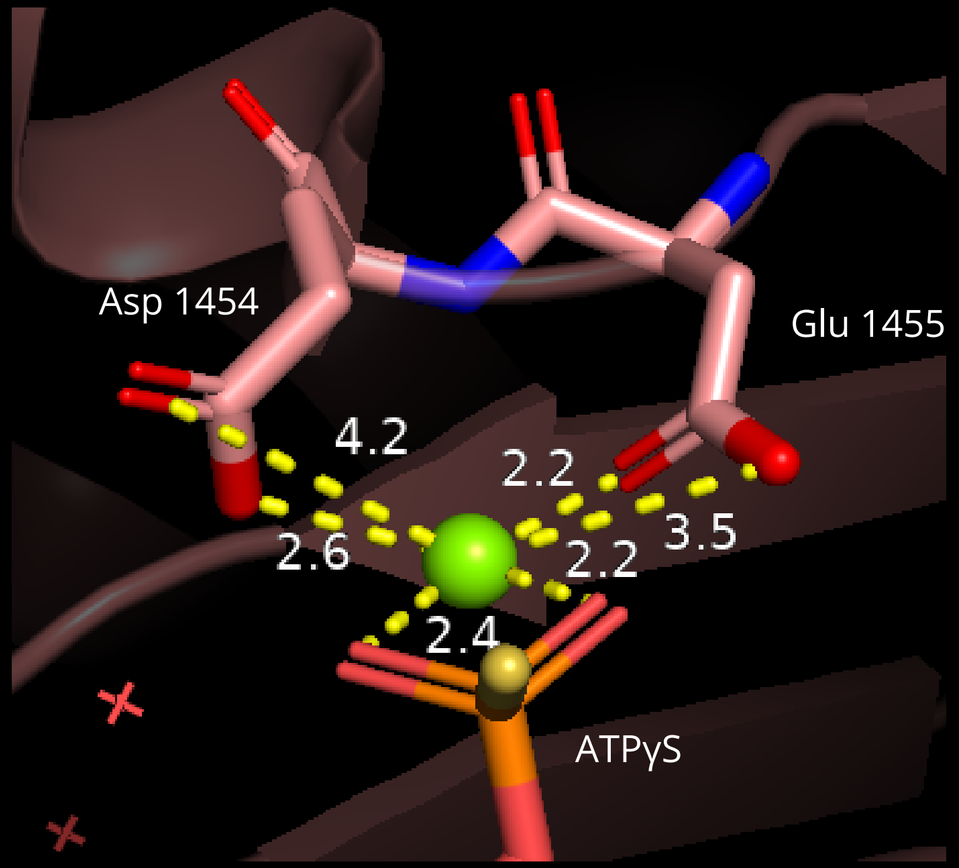
Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å



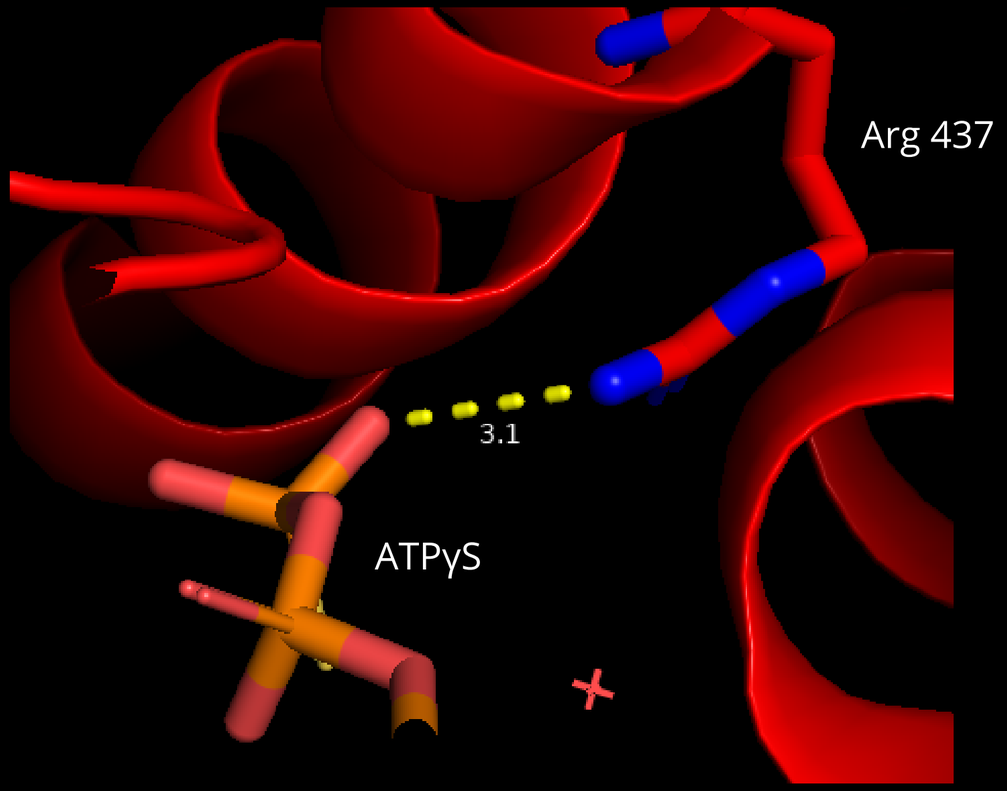
Interactions between Mg and C-cassette



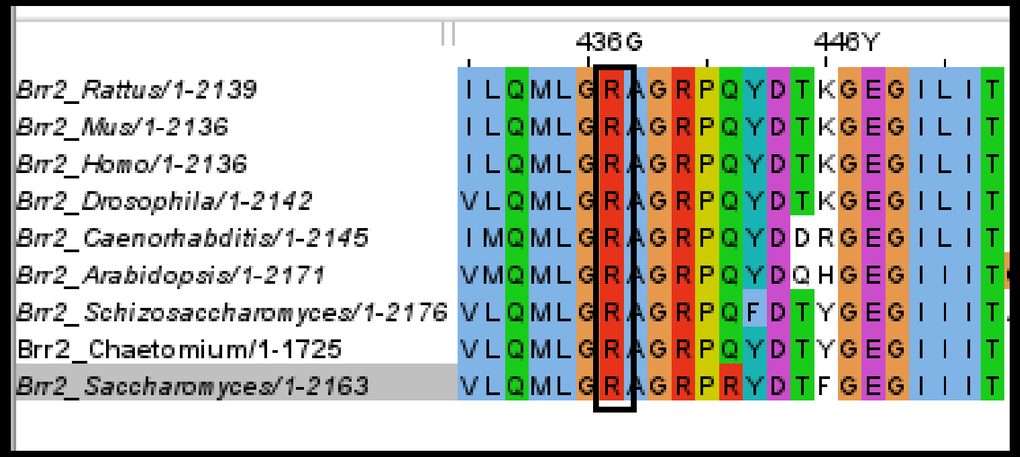
Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å



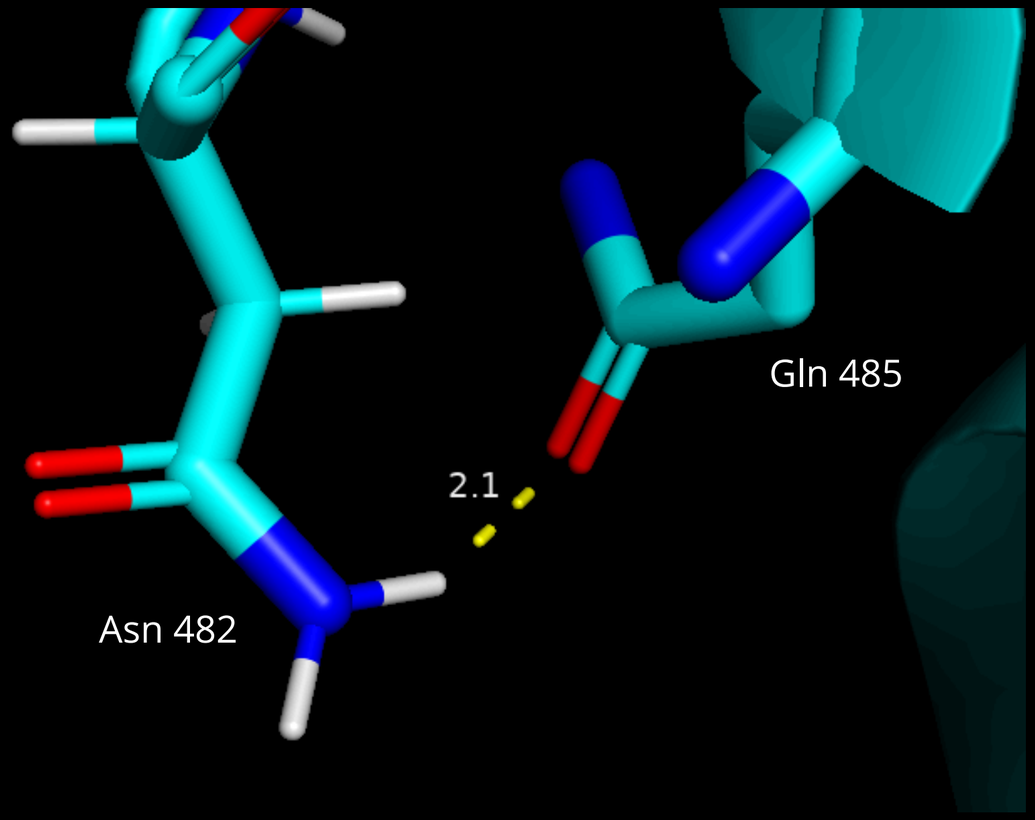
motif VI



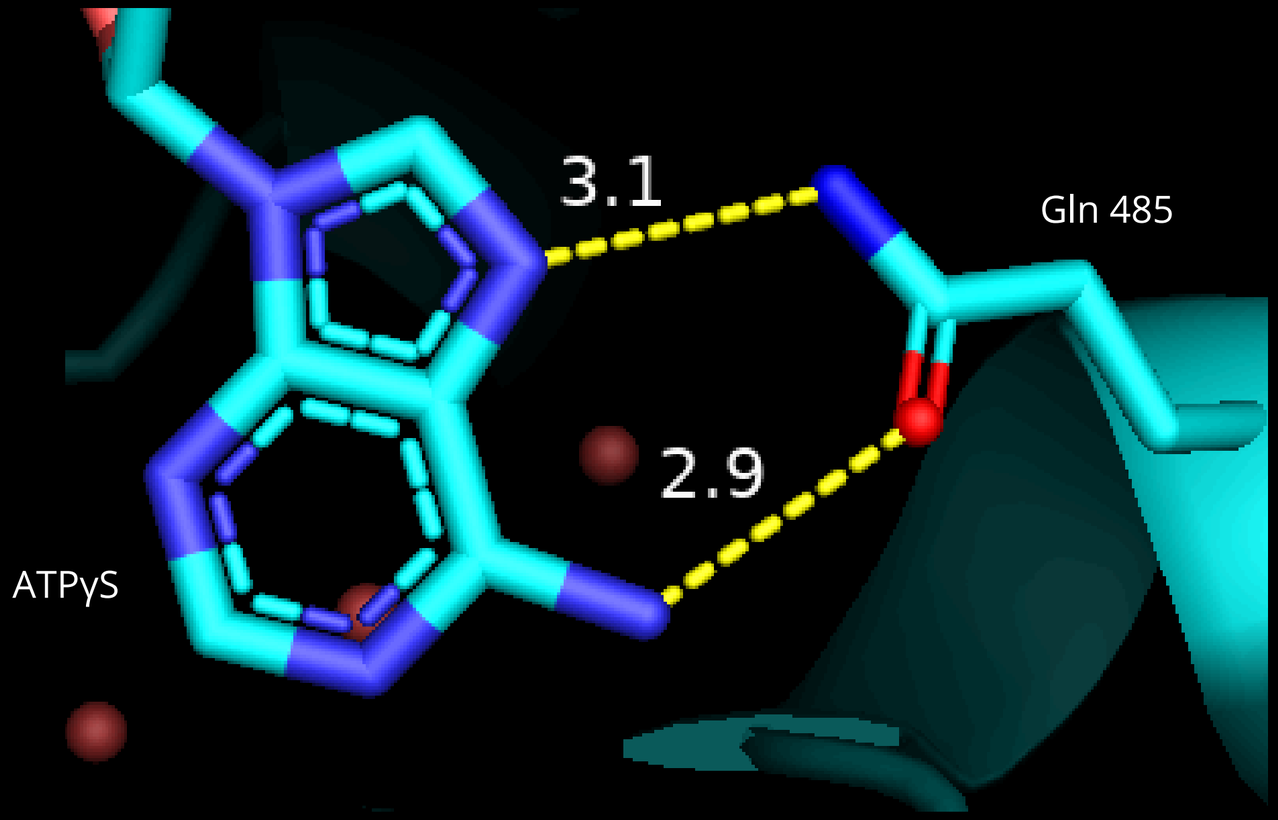
Crystal structure of the Ski2 RNA-helicase Brr2 from Chaetomium thermophilum bound to ATP-gamma-S (6QV4) *Chaetomium thermophilum* ~ 2.80 Å



Q motif inhibition

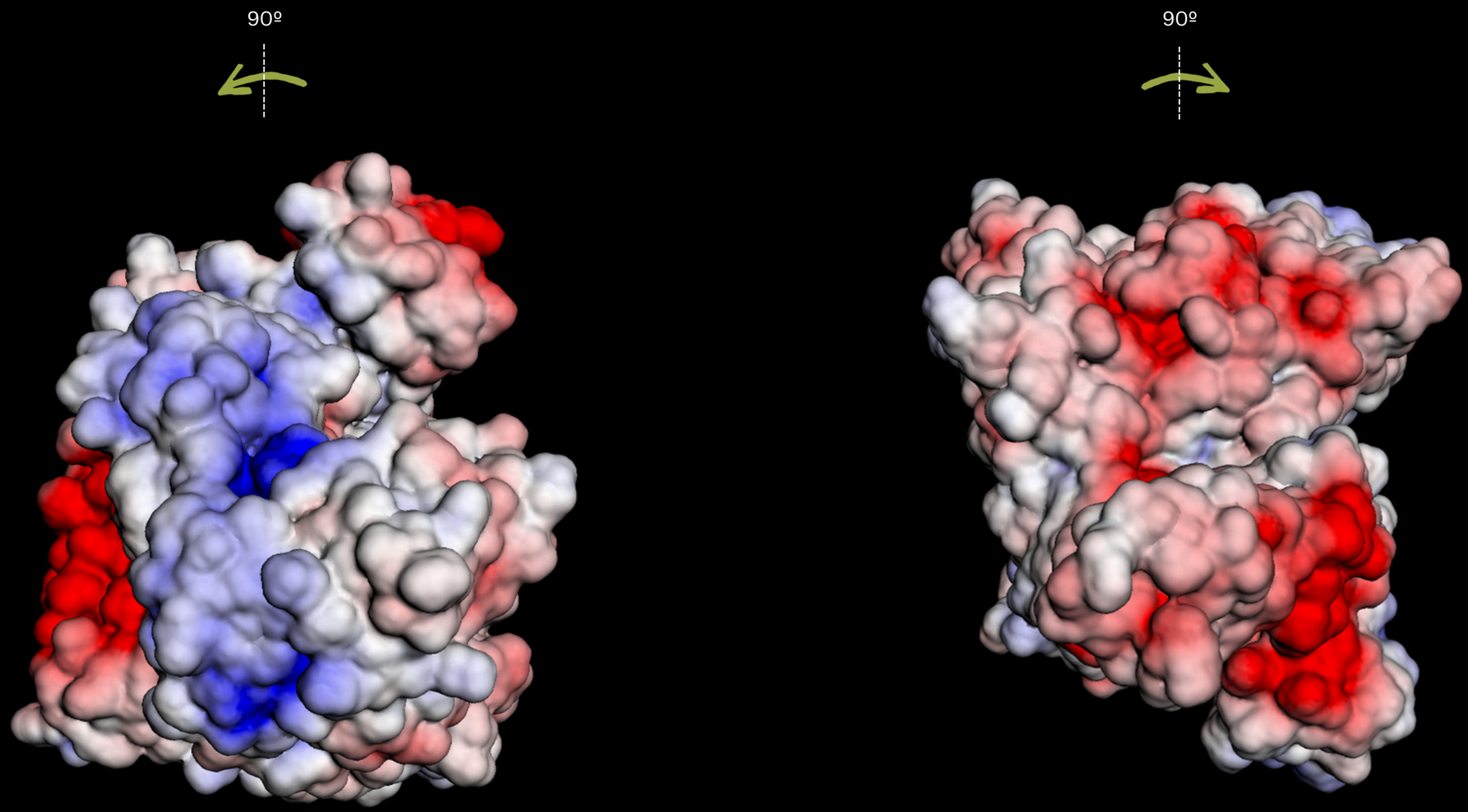


Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å



Human Brr2 Helicase Region in complex with C-tail deleted Jab1 and ATPgammaS (7BDI) *Homo sapiens* ~ 2.80 Å

NC - CC interaction



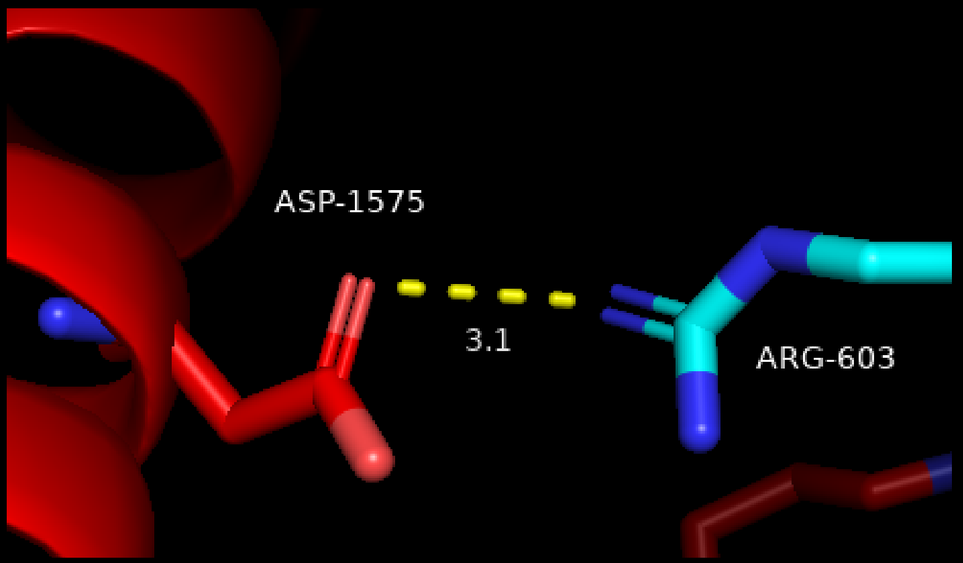
C-CASSETTE

N-CASSETTE

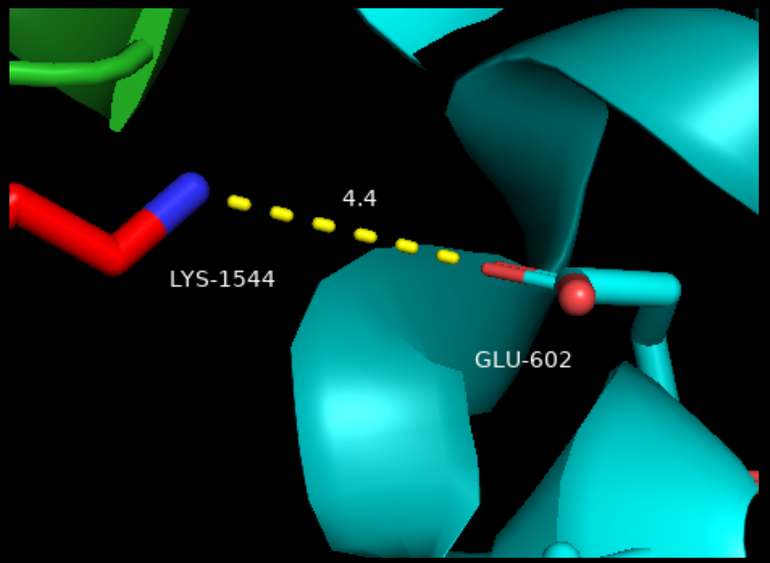
Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

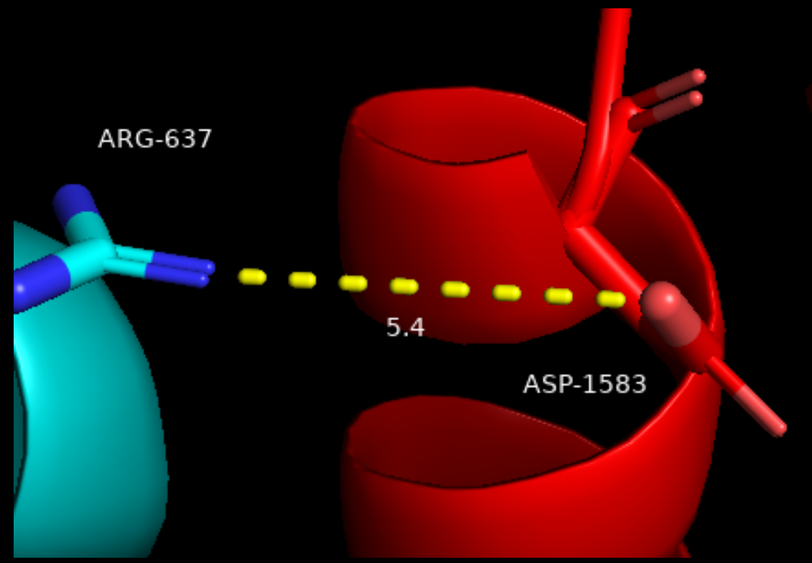
Interactions between NC-CC

Possible salt bridge



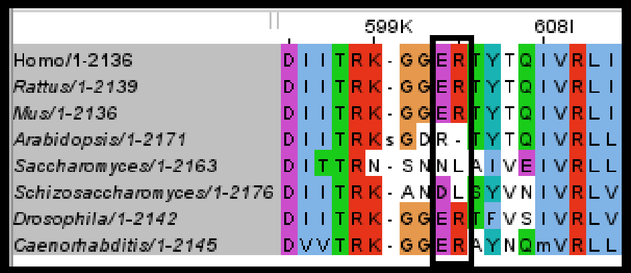
Electrostatic interactions





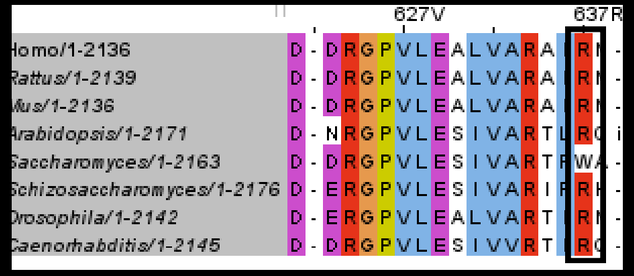
Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

ARG 603



GLU602

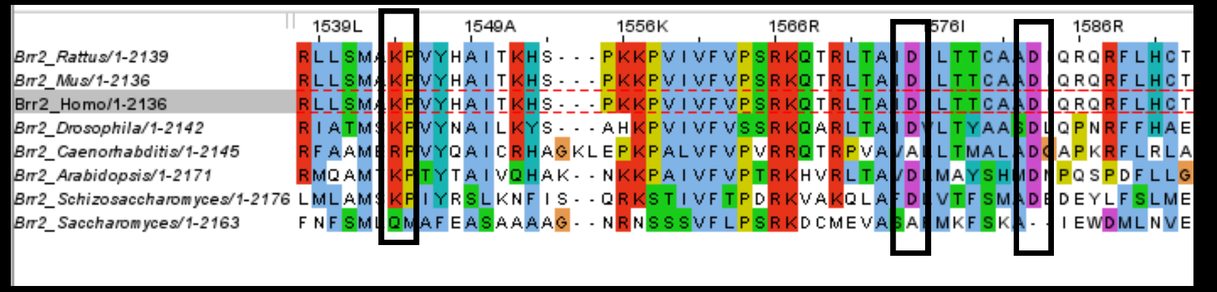
ARG 637



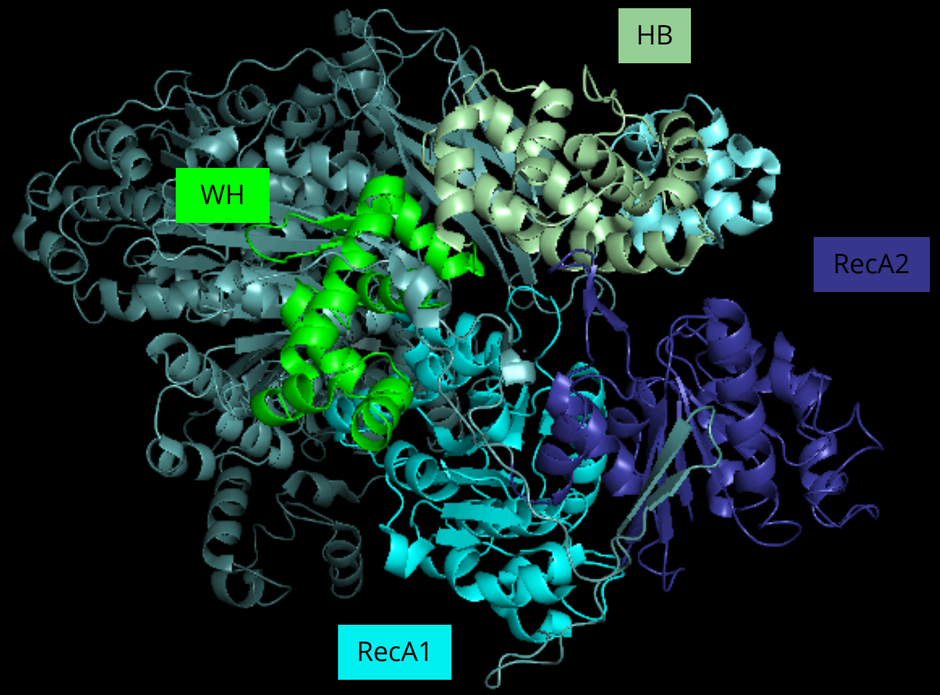
LYS 1544

ASP 1575

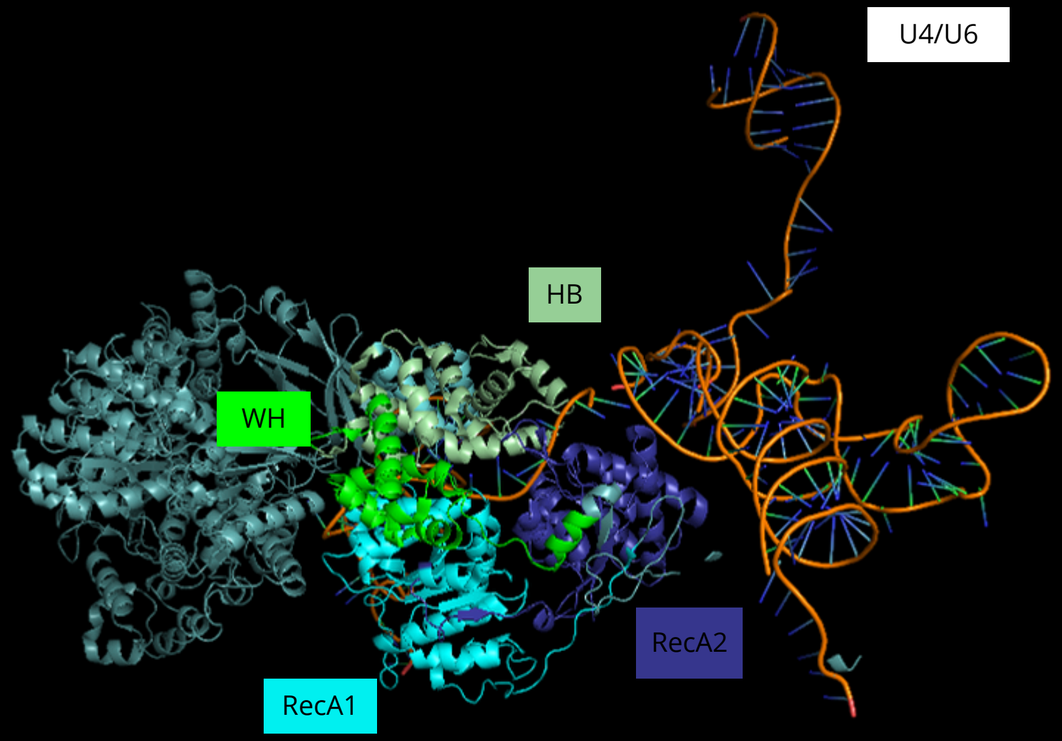
ASP 1583



Tunnel-like structure of NC

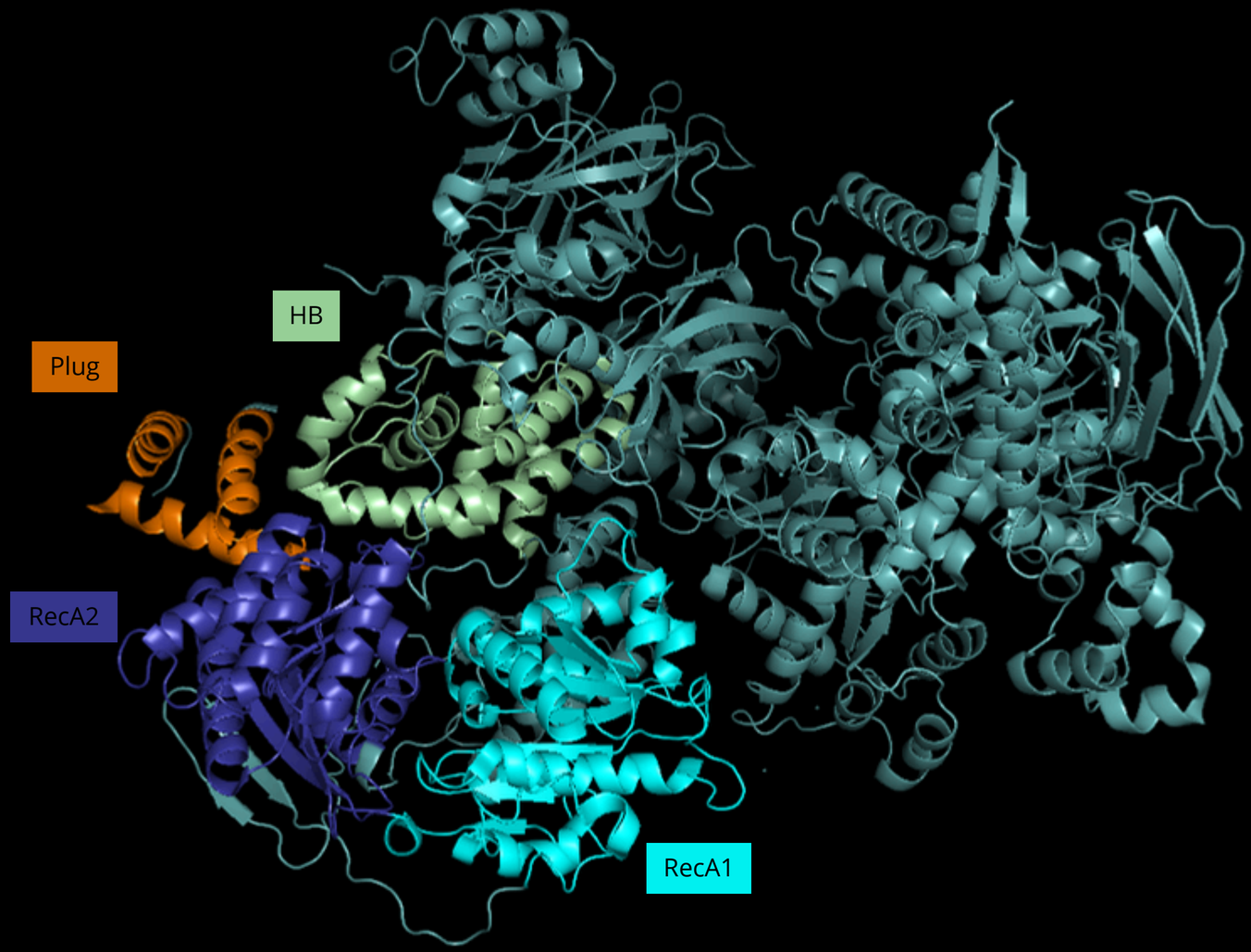


Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å



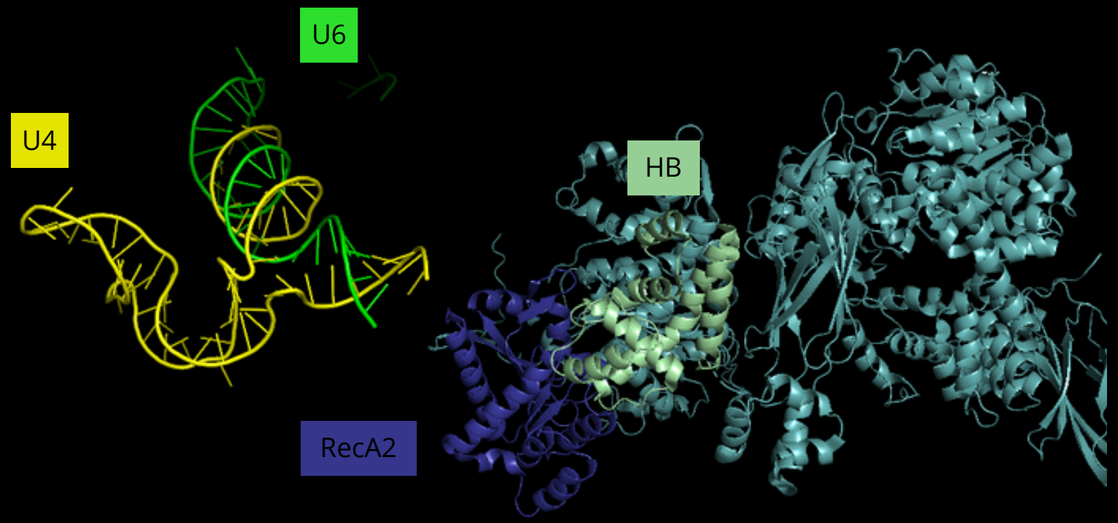
The overall structure of the yeast spliceosomal U4/U6.U5 tri-snRNP (5GAN)
Saccharomyces cerevisiae ~ 3.70 Å

NTR auto-inhibition

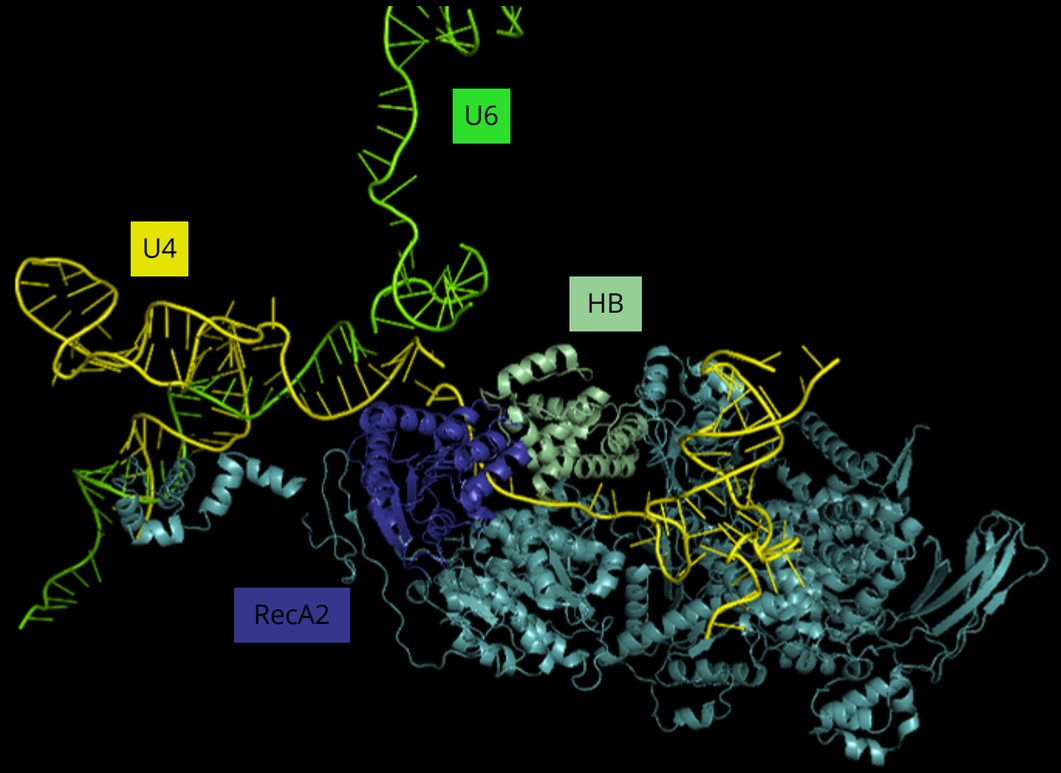


Crystal structure of yeast Brr2 full-lenght in complex with Prp8 Jab1 domain
(5M52) *Saccharomyces cerevisiae* ~ 3.40 Å

RNA movement

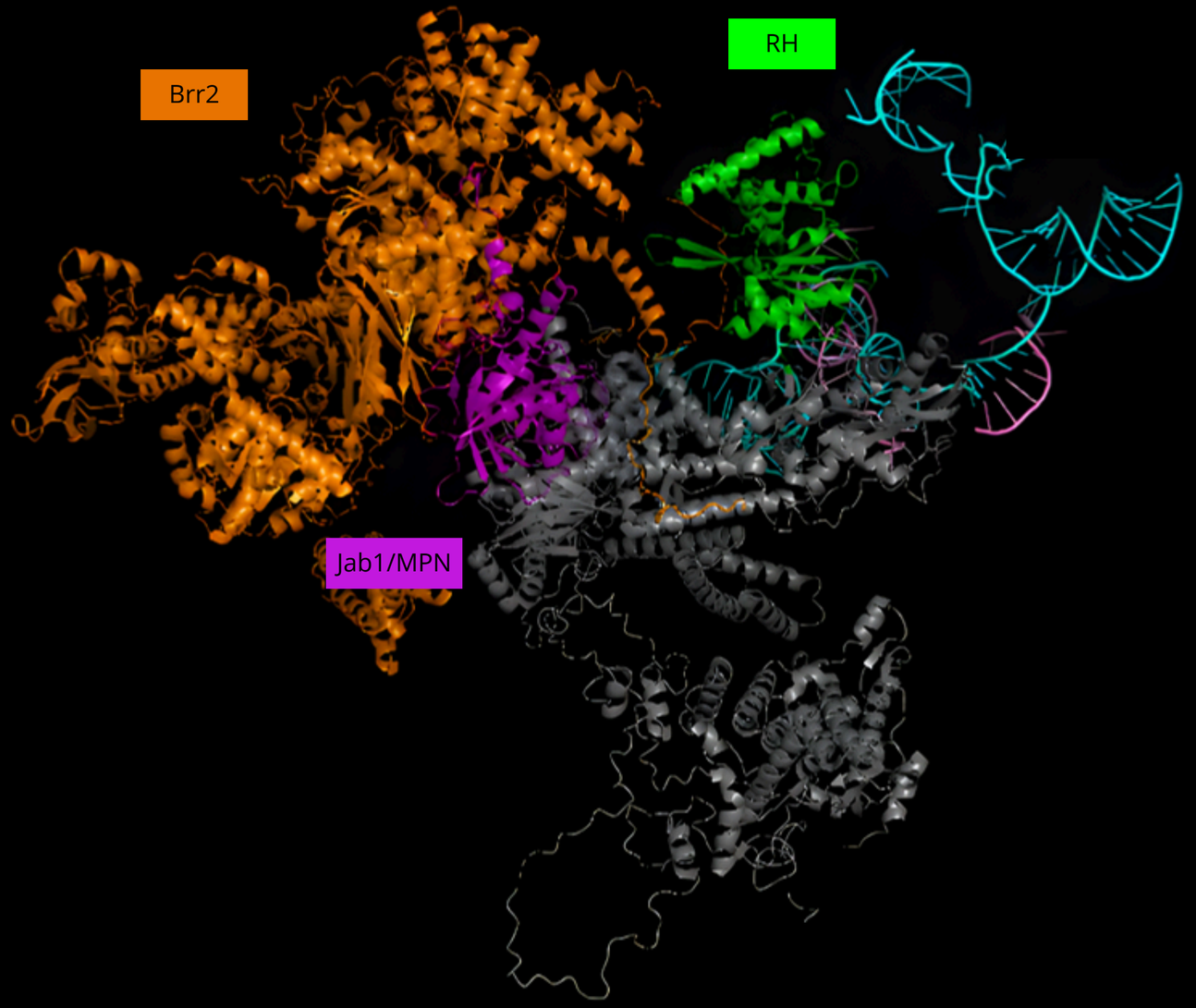


Cryo-EM structure of the spliceosomal U4/U6.U5 tri-snRNP (3JCM) *Saccharomyces cerevisiae* ~ 3.80 Å



The overall structure of the yeast spliceosomal U4/U6.U5 tri-snRNP (5GAN)
Saccharomyces cerevisiae ~ 3.70 Å

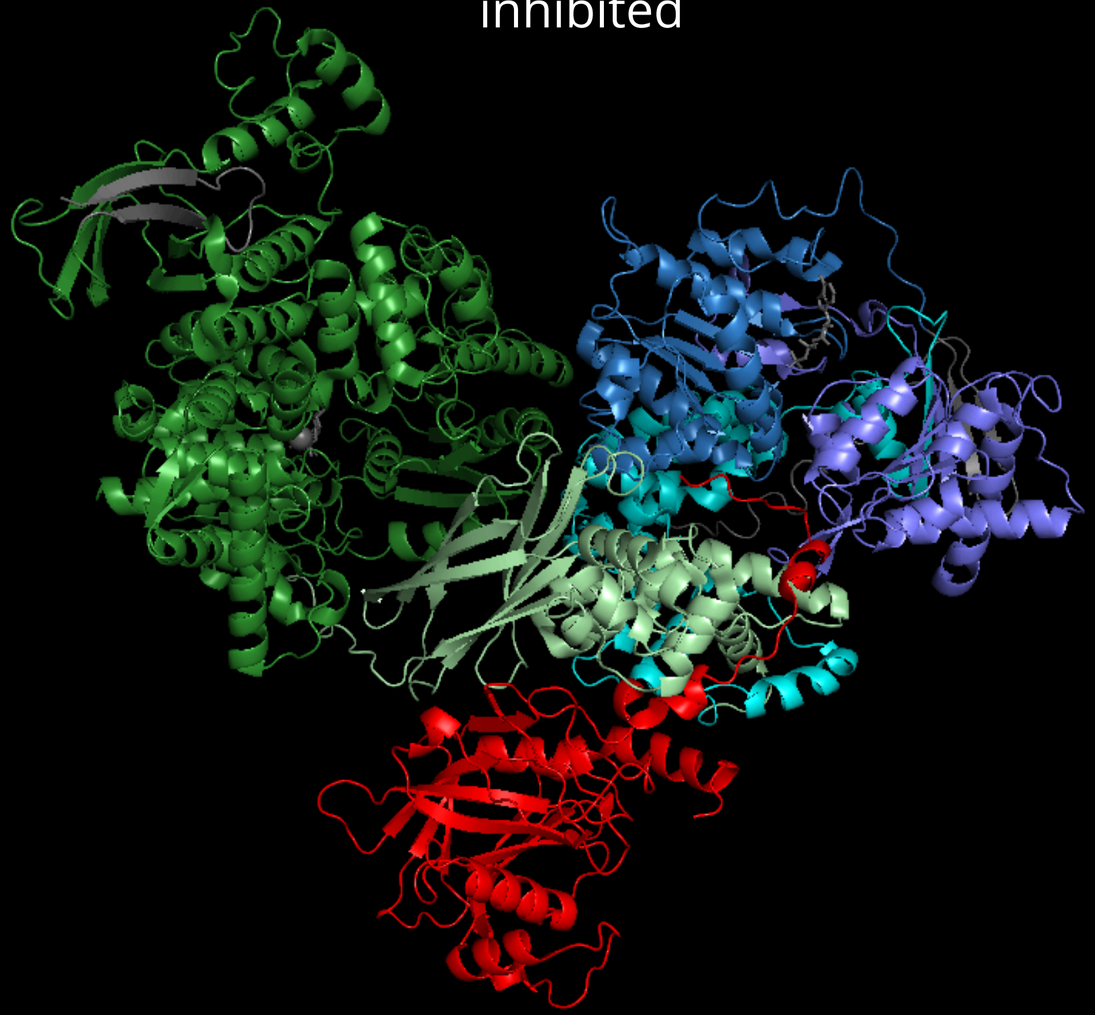
Inhibition through Prp8



Structure of the human U5.U4/U6 tri-snRNP (6QW6) *Homo sapiens* ~ 2.9Å

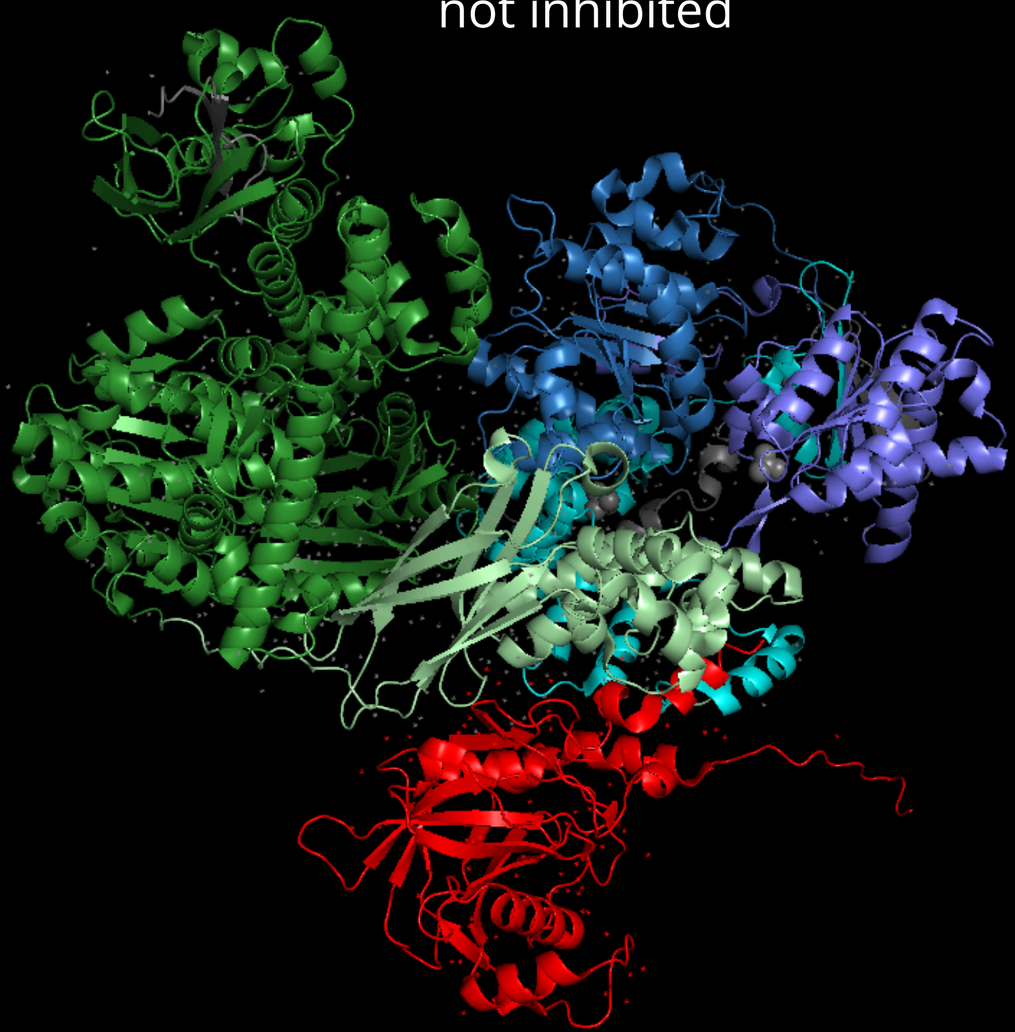
Jab1/MPN c-terminal domain

inhibited



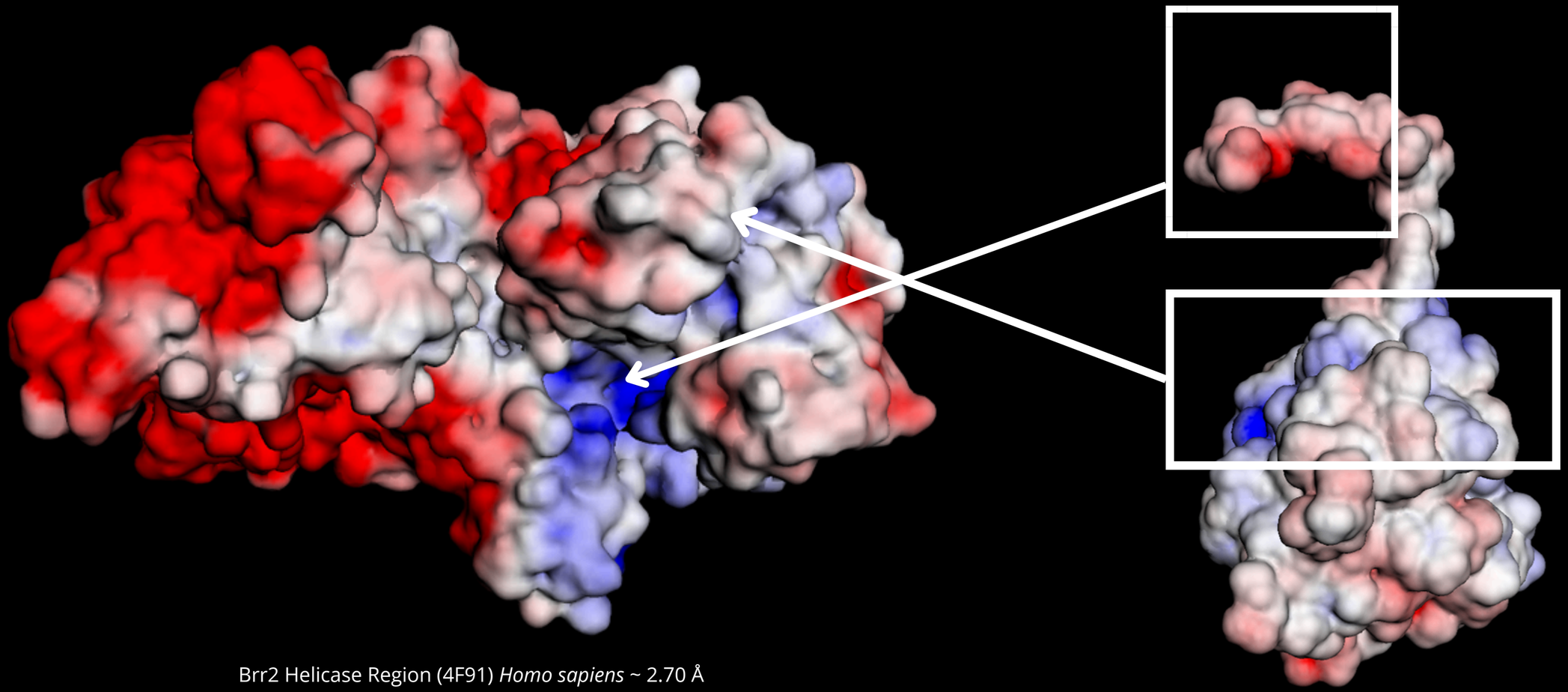
Crystal structure of human Brr2 in complex with the Prp8 Jab1/MPN domain (4KIT) *Homo sapiens* ~ 3.6 Å

not inhibited



Human Brr2 Helicase Region in complex with C-tail deleted Jab1 (6S8Q) *Homo sapiens* ~ 2.39 Å

Jab1/MPN - Brr2 interactions



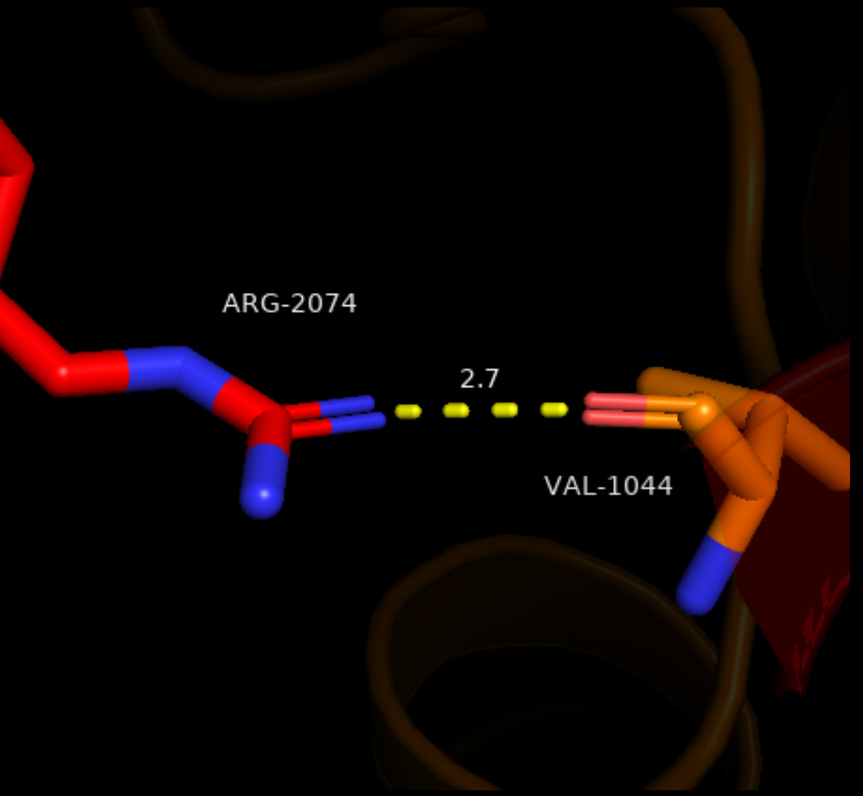
Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

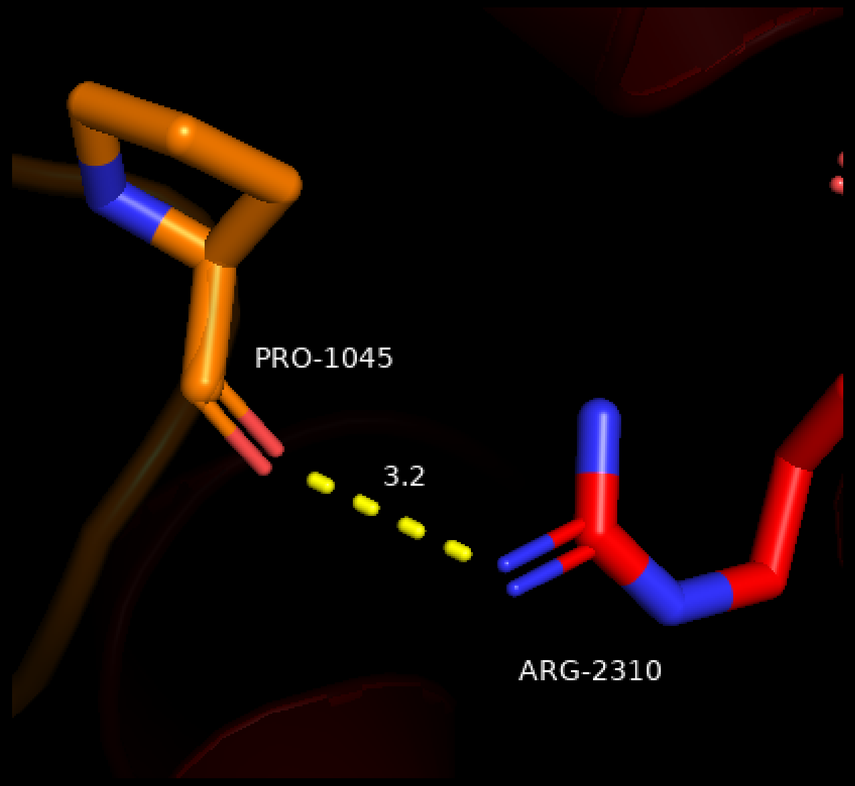
(bottom view)

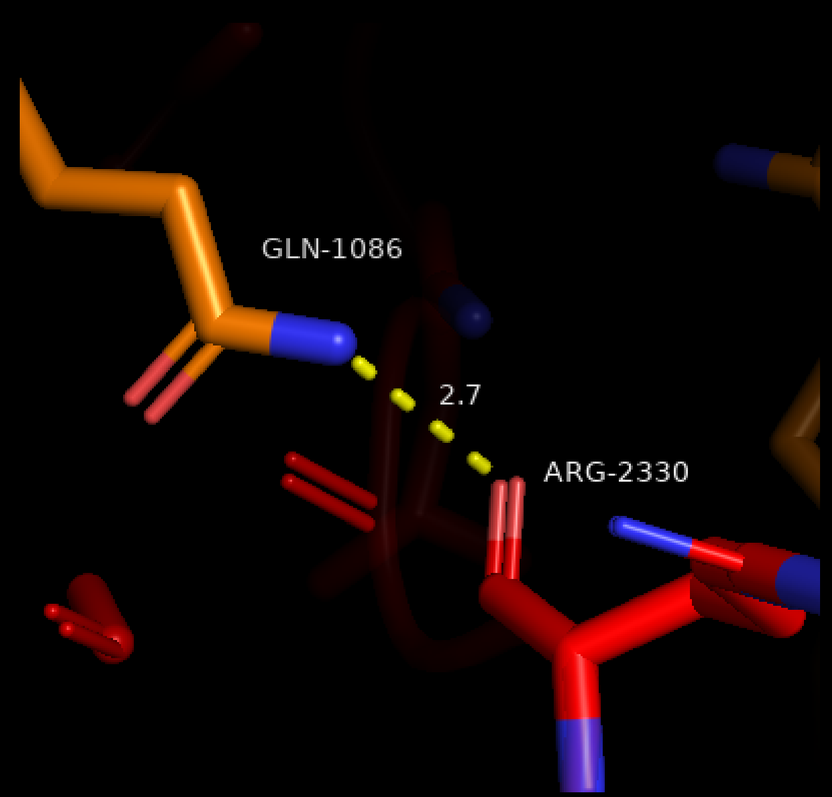
Prp8 Jab1/MPN domain (4KIT) *Homo sapiens* ~ 3.6 Å

Jab1/MPN - Brr2 interactions

> 20 hydrogen bonds





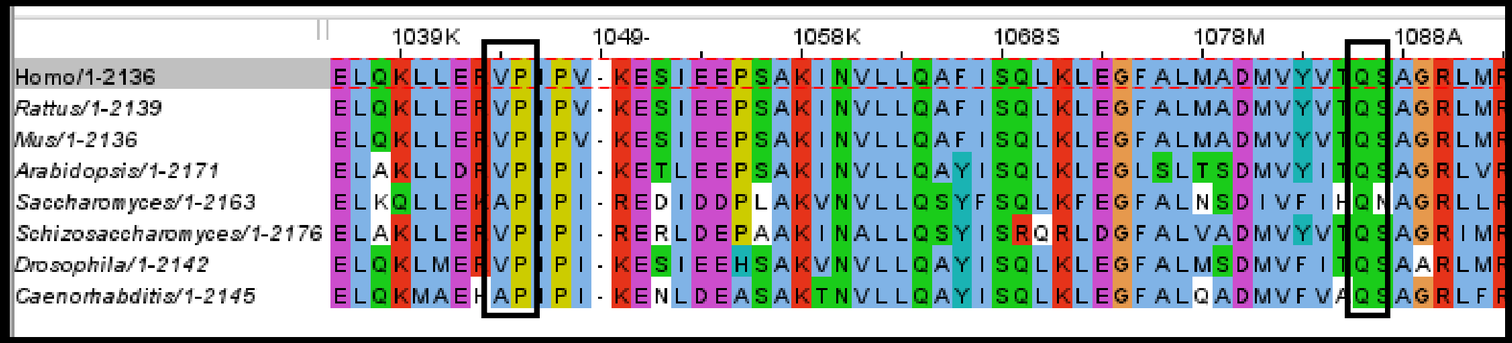


Crystal structure of human Brr2 in complex with the Prp8 Jab1/MPN domain (4KIT) *Homo sapiens* ~ 3.6 Å

Sequence alignment

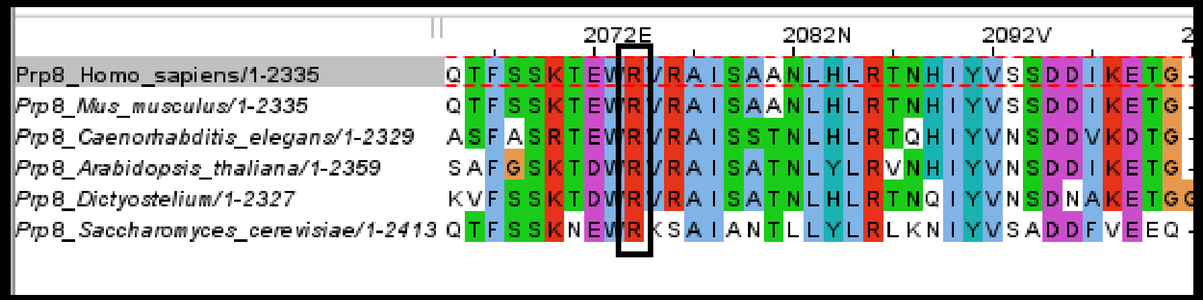
VAL 1044

GLN 1086



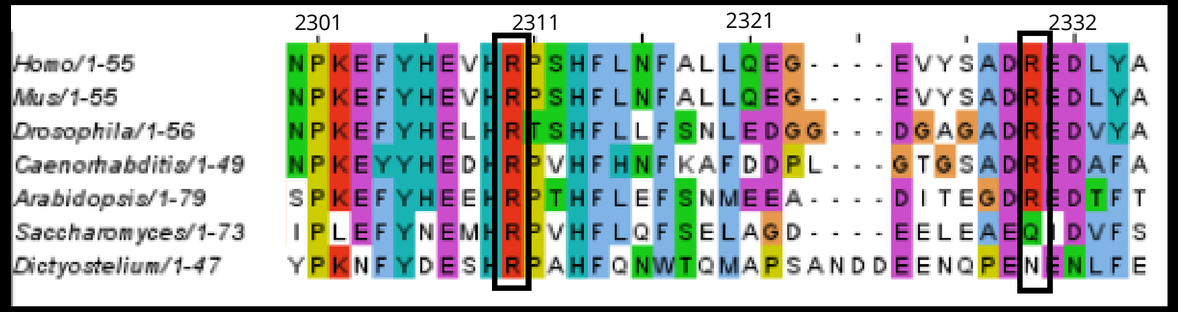
PRO 1045

ARG 2074



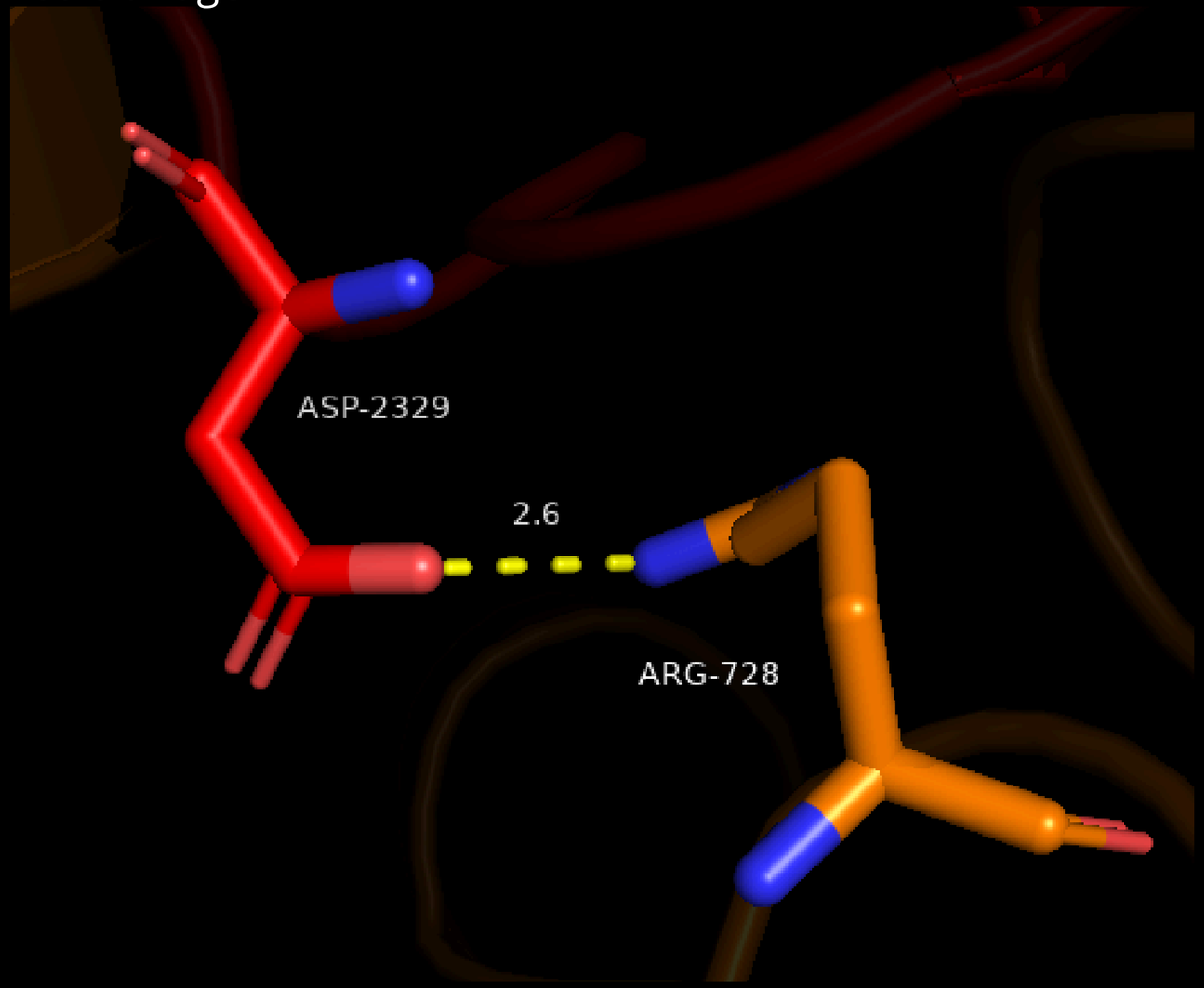
ARG 2310

ARG 2330

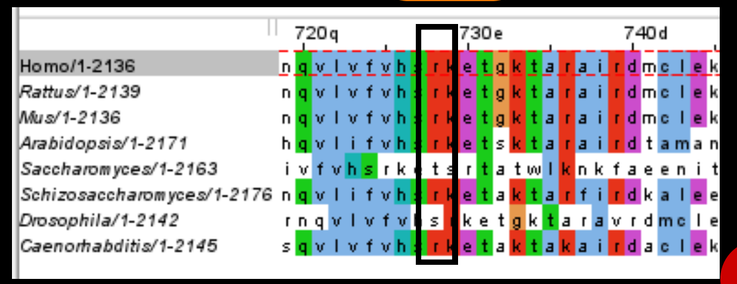


Jab1/MPN - Brr2 interactions

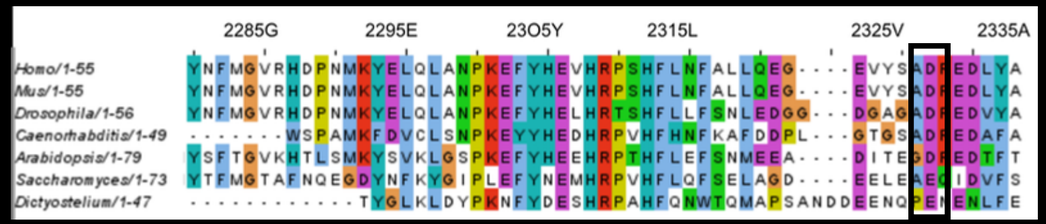
Salt bridge



ARG 728



ASP 2329



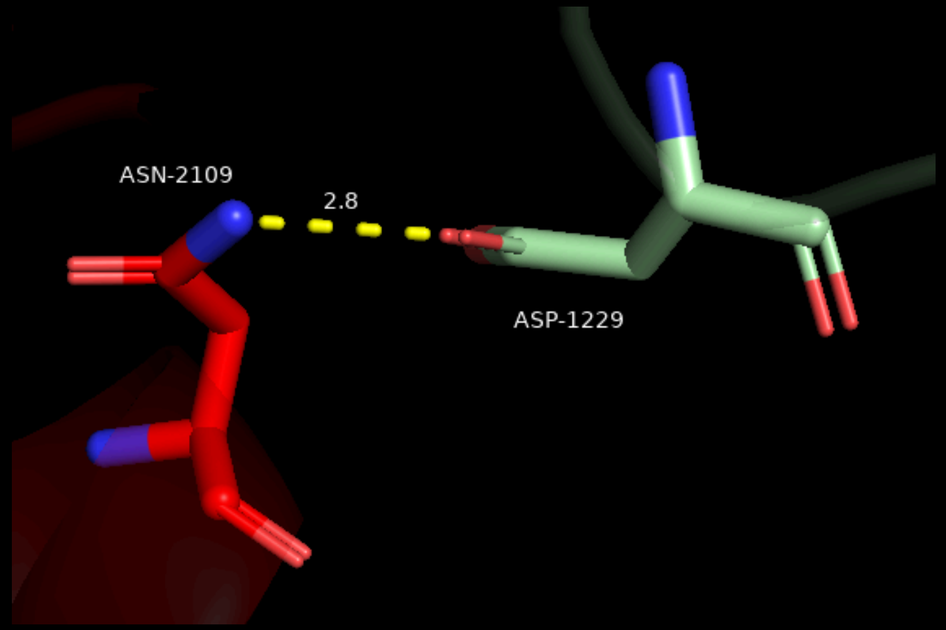
Salt bridges XML

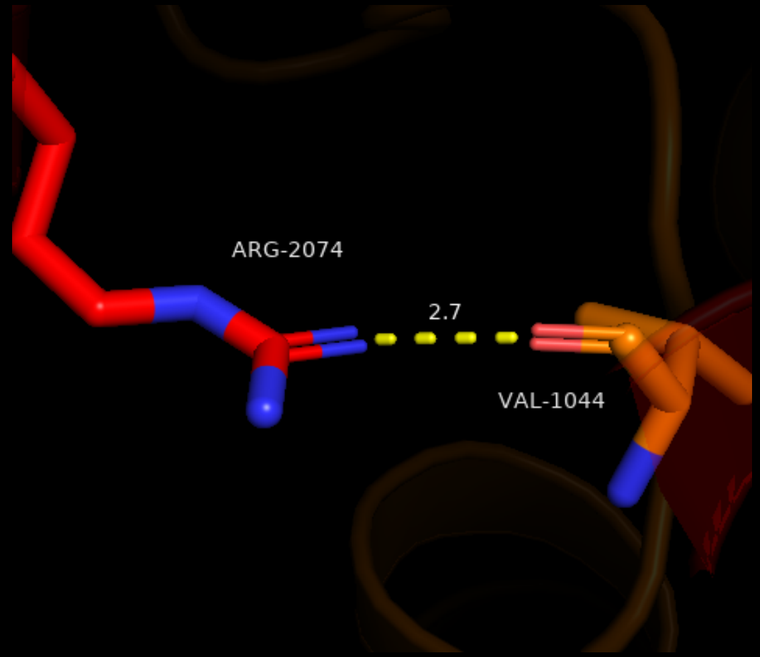
##	Structure 1	Dist. [Å]	Structure 2
1	C:ARG2074[NE]	3.98	B:GLU1042[OE1]
2	C:ARG2074[NE]	3.73	B:GLU1042[OE2]
3	C:ARG2074[NH1]	3.64	B:GLU1042[OE1]
4	C:ASP2329[OD1]	3.83	B:HIS 726[NE2]
5	C:ASP2329[OD2]	3.95	B:ARG 728[NE]
6	C:ASP2329[OD2]	2.63	B:ARG 728[NH1]
7	C:ALA2335[O]	3.33	B:ARG 545[NH1]
8	C:ALA2335[O]	3.98	B:ARG 545[NH2]
9	C:ALA2335[OXT]	3.71	B:ARG 545[NH1]

Crystal structure of human Brr2 in complex with the Prp8 Jab1/MPN domain (4KIT) *Homo sapiens* ~ 3.6 Å

PDBe PISA (4KIT) *Homo sapiens ~ 3.6 Å*

Jab1/MPN - Brr2 interactions

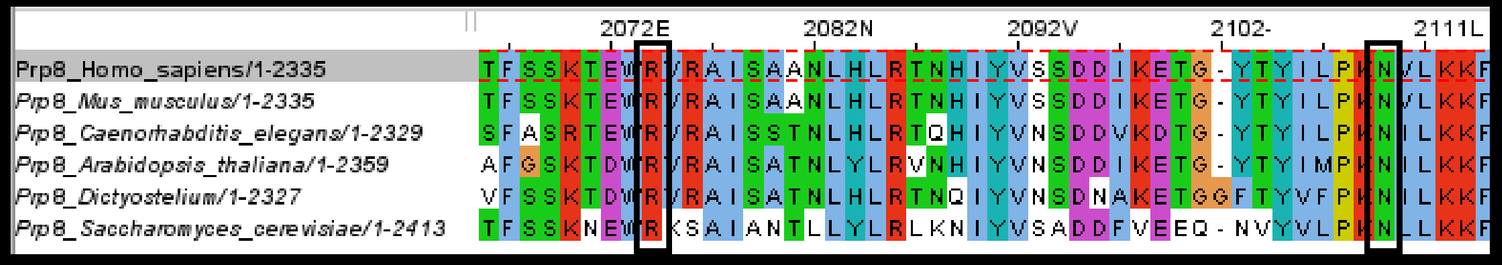




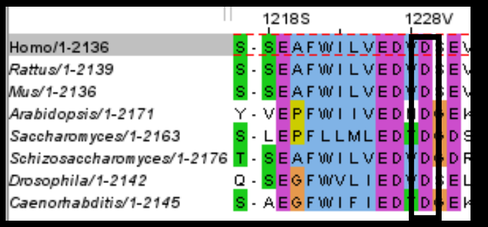
Human Brr2 Helicase Region in complex with C-tail deleted Jab1 (6S8Q) *Homo sapiens* ~ 2.39 Å

VAL 1044

ASP 1229



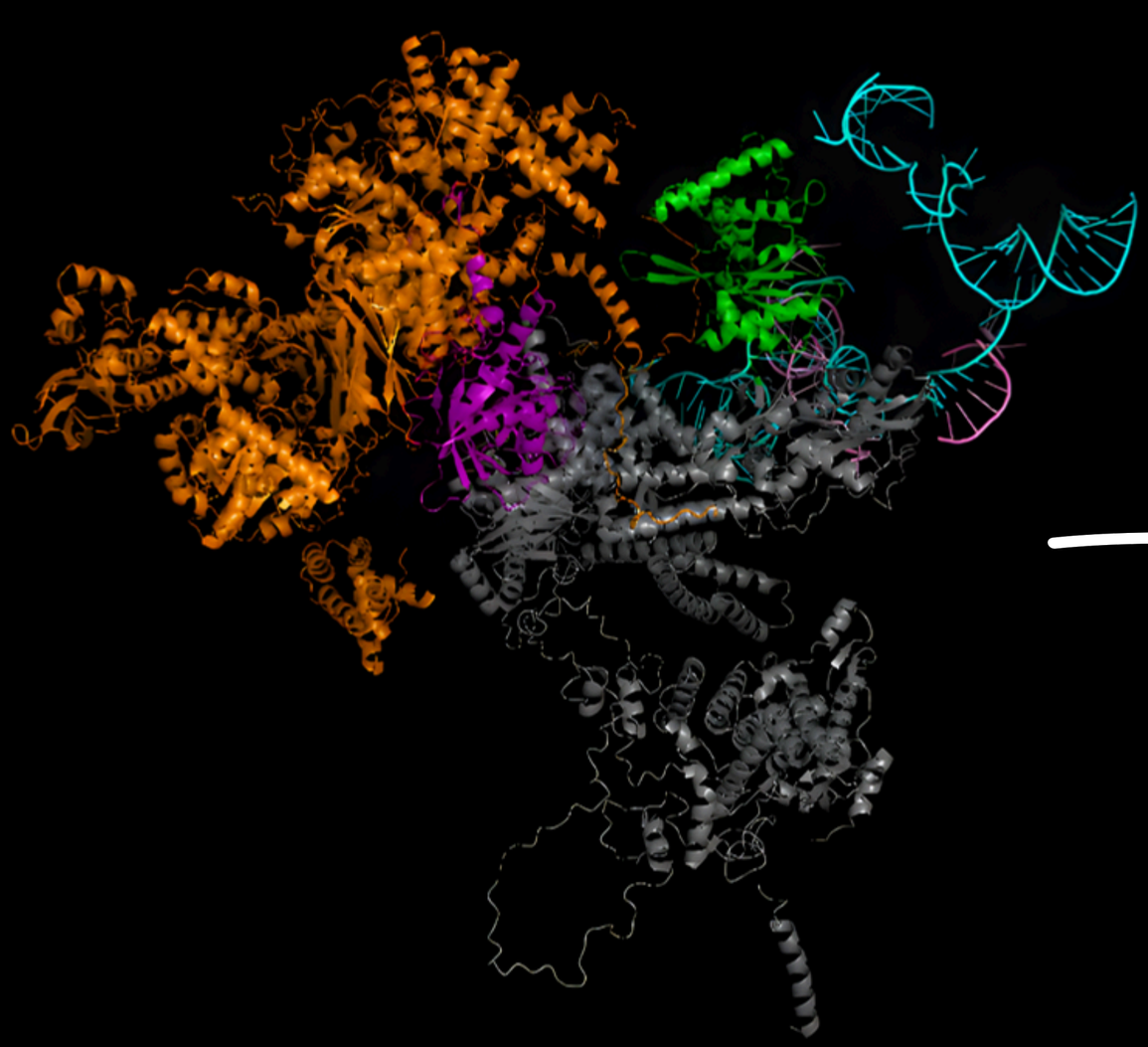




ARG 2074

ASN 2109

RH inhibition

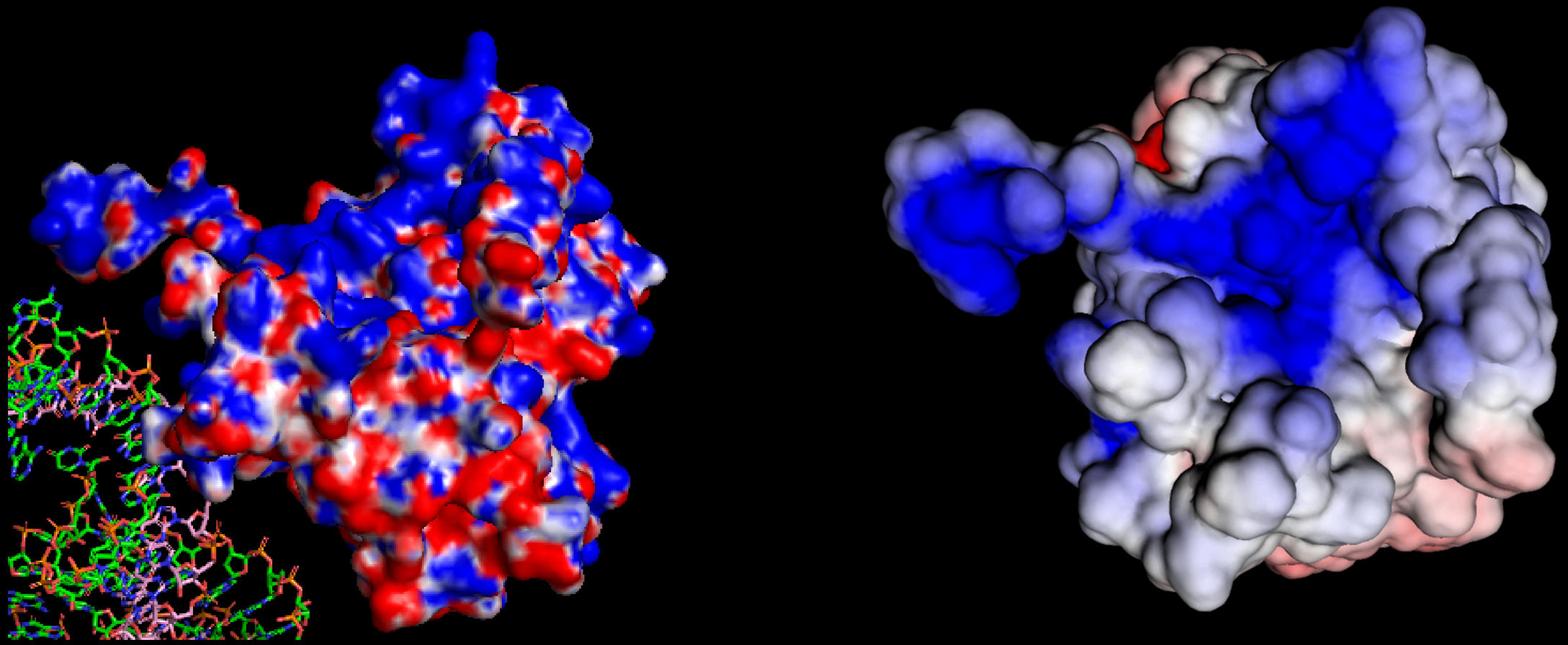


Structure of the human U5.U4/U6 tri-snRNP (6QW6) *Homo sapiens* ~ 2.9Å



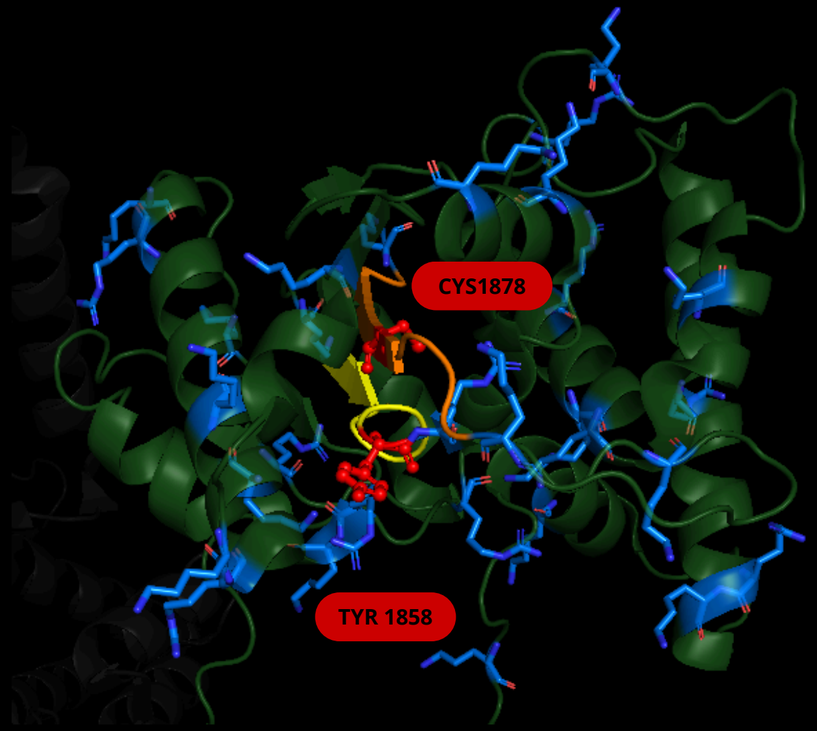
The overall structure of the yeast spliceosomal U4/U6.U5 tri-snRNP (5GAN) *Saccharomyces cerevisiae* ~ 3.70 Å

RH-RNA interaction

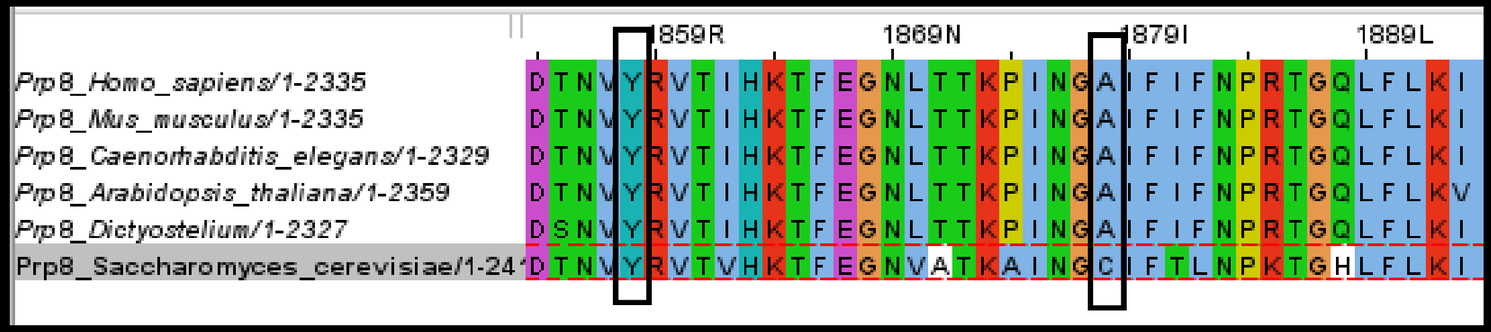


RH domain (5GAN) *Saccharomyces cerevisiae* ~ 3.70 Å

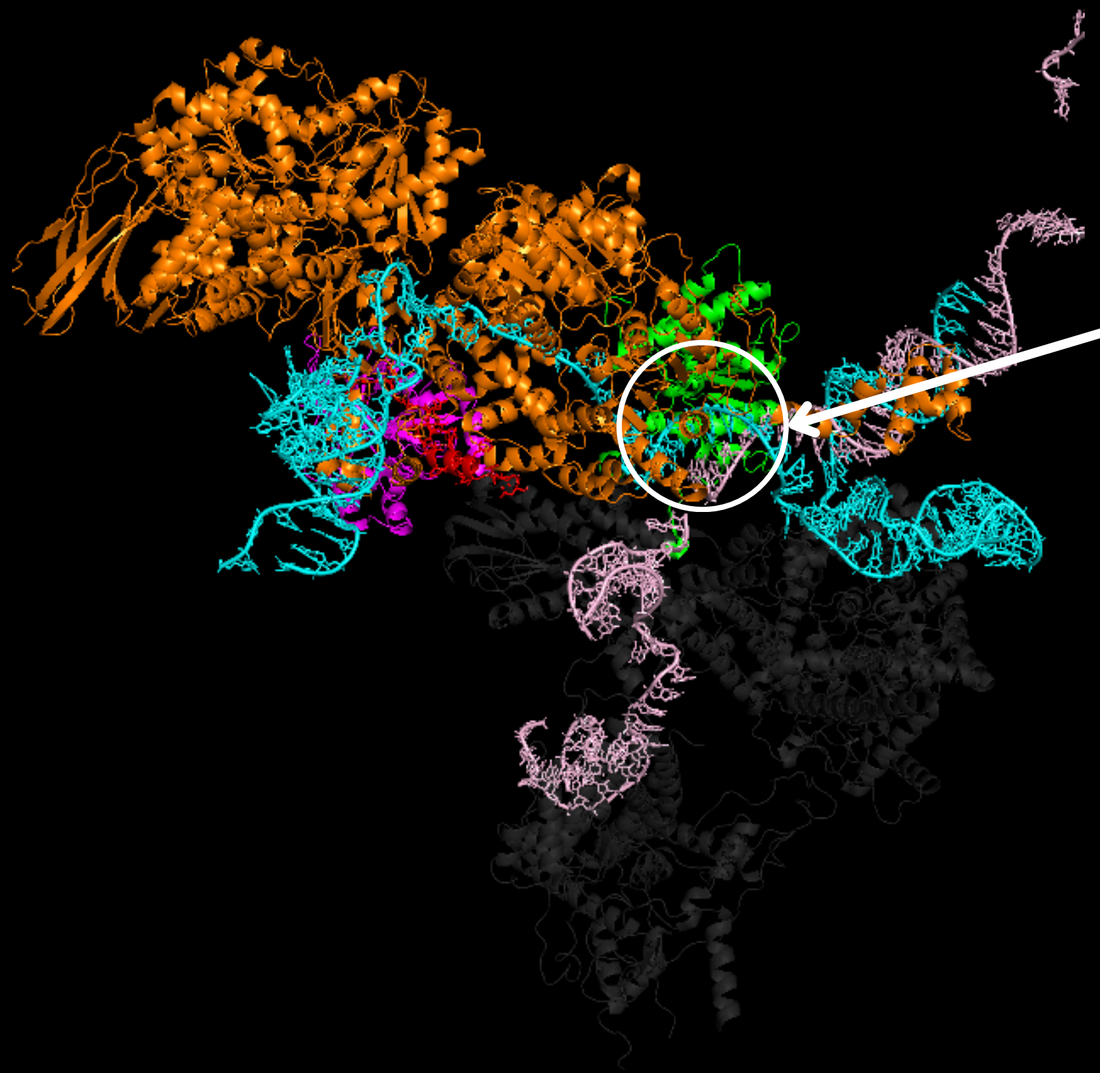
RH-RNA interaction

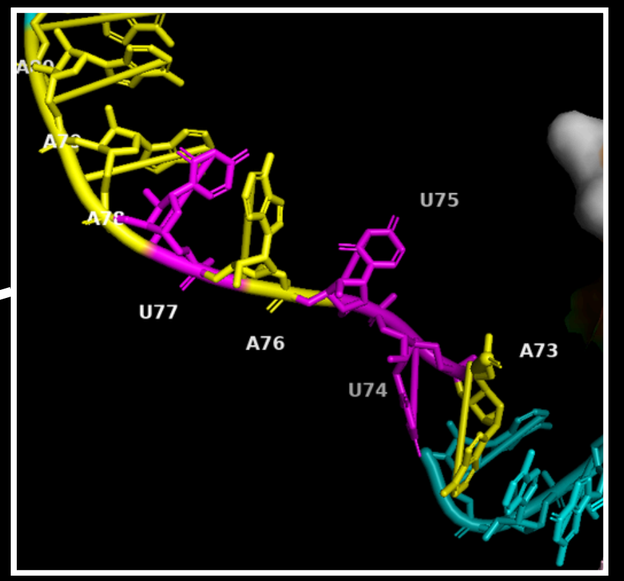


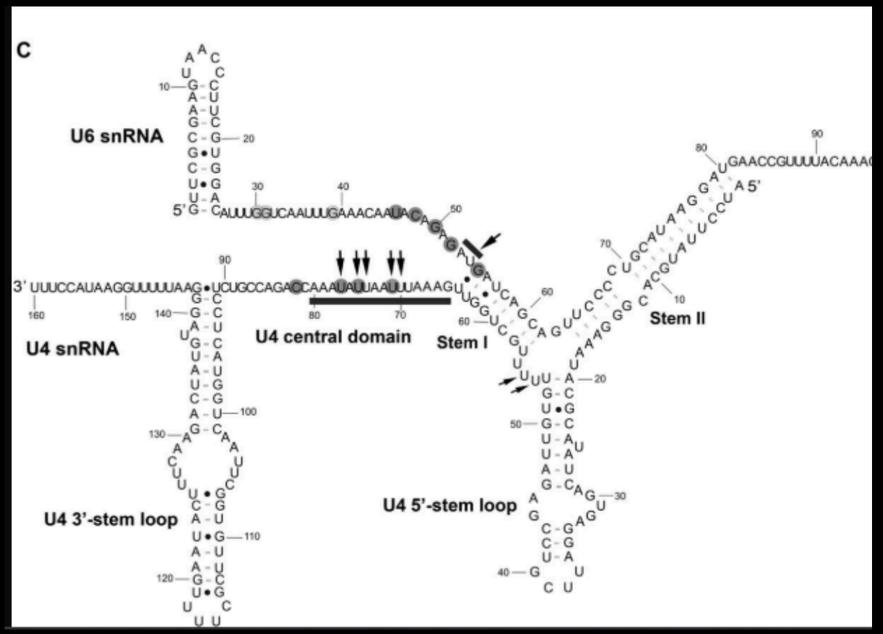
RH domain (5GAN) *Saccharomyces cerevisiae* ~ 3.70 Å



RH inhibition



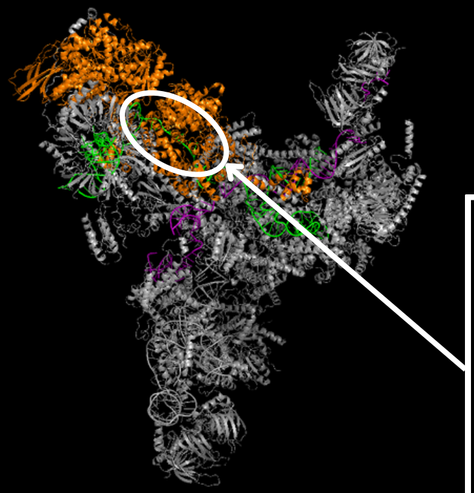


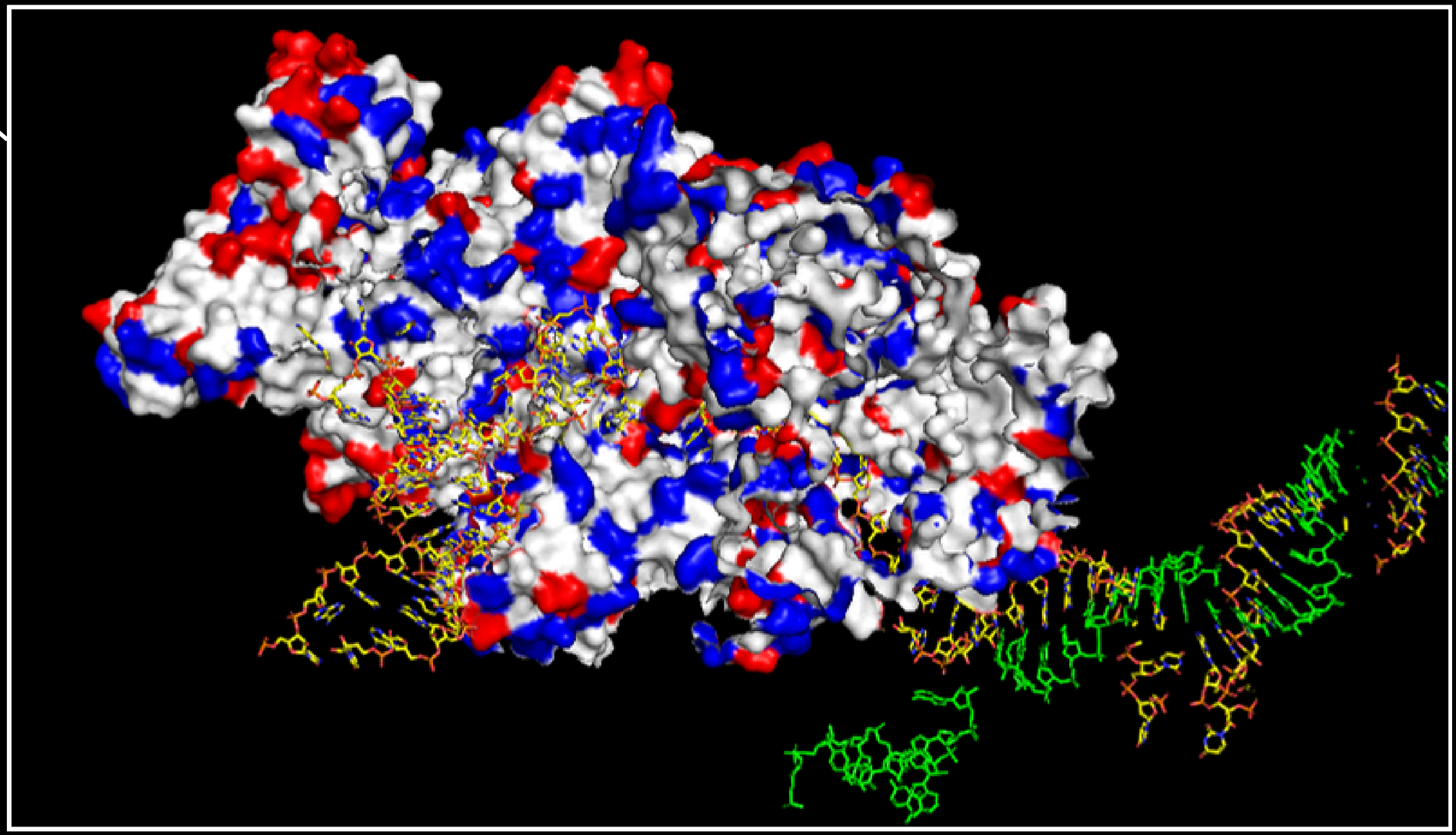


The overall structure of the yeast spliceosomal U4/U6.U5 tri-snRNP (5GAN)
Saccharomyces cerevisiae ~ 3.70 Å

Mozaffari-Jovin, S., Santos, K. F., Hsiao, H.-H., Will, C. L., Urlaub, H., Wahl, M. C., & Lührmann, R. (2012). The Prp8 RNase H-like domain inhibits Brr2-mediated U4/U6 snRNA unwinding by blocking Brr2 loading onto the U4 snRNA. *Genes & Development.*

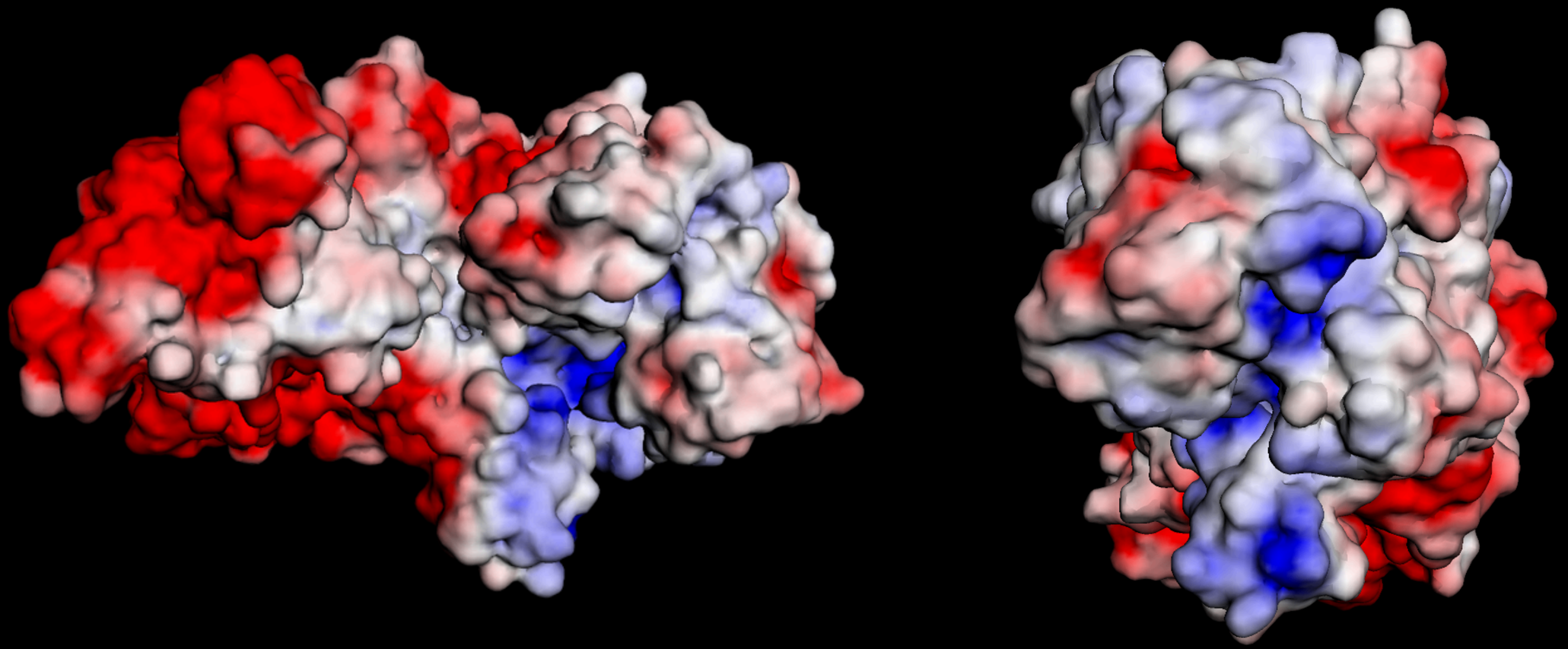
RNA - Brr2 interaction





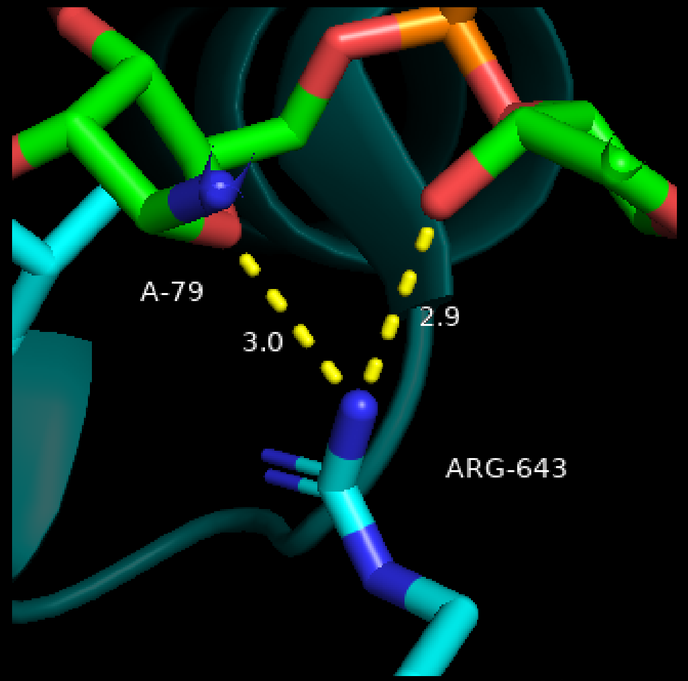
The overall structure of the yeast spliceosomal U4/U6.U5 tri-snRNP (5GAN) *Saccharomyces cerevisiae* ~ 3.70 Å

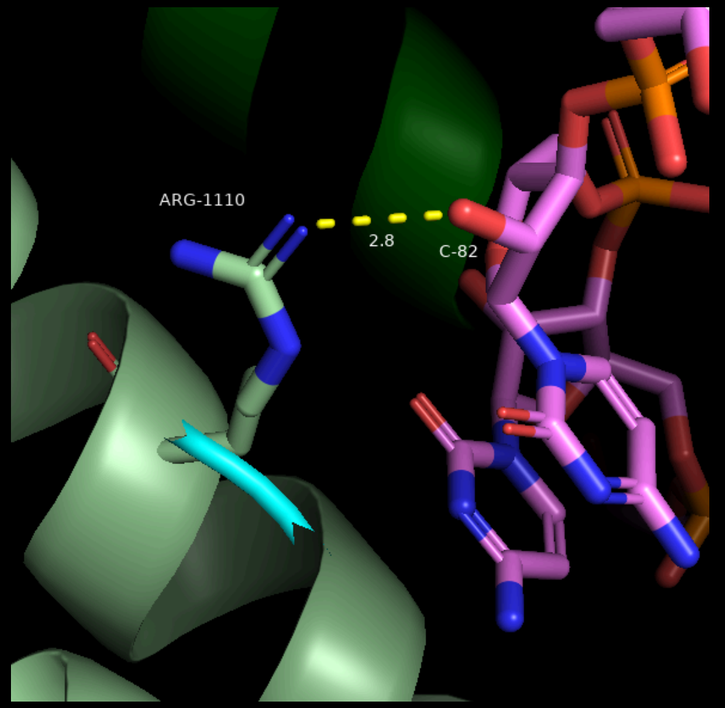
RNA interaction



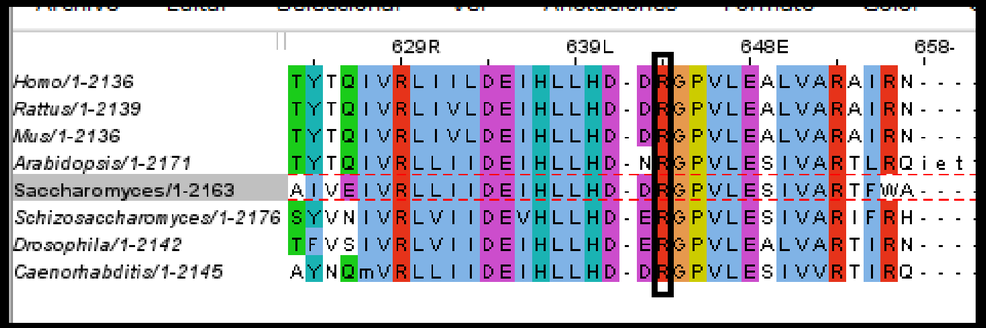
Brr2 Helicase Region (4F91) *Homo sapiens* ~ 2.70 Å

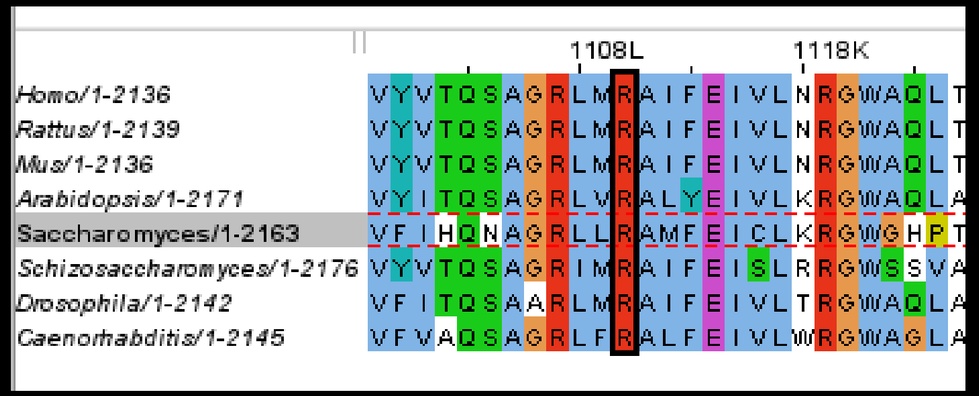
RNA interaction



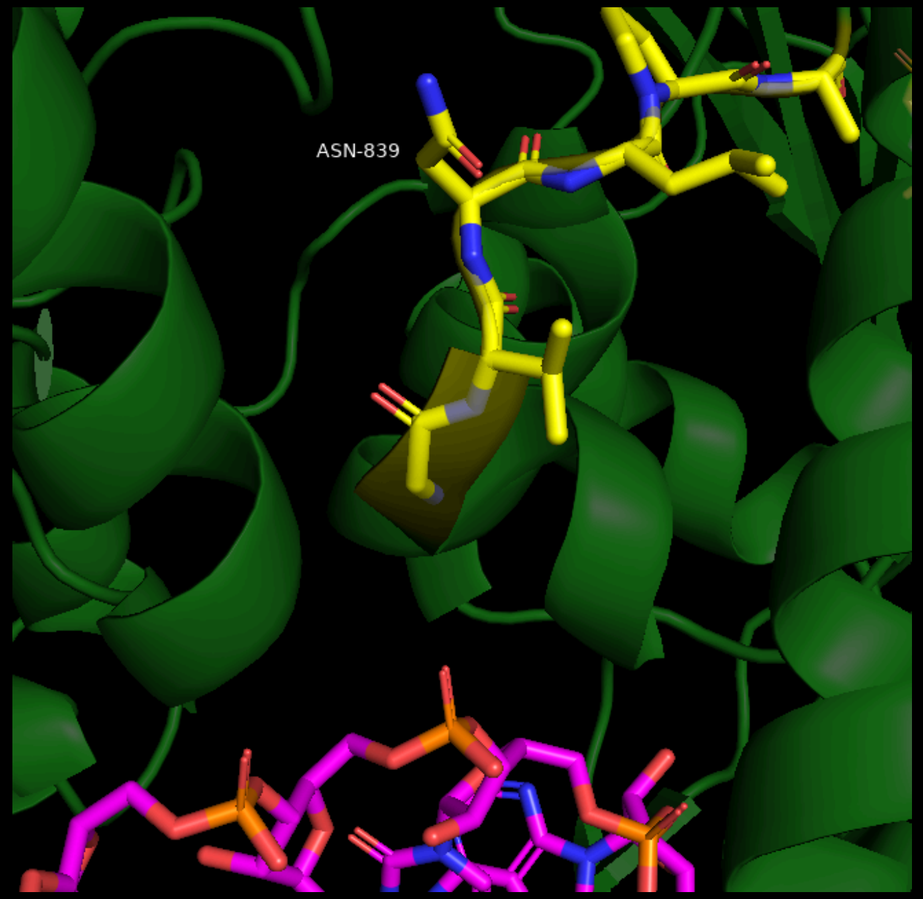


RNA interaction (5GAN) *Saccharomyces cerevisiae* ~ 3.70 Å

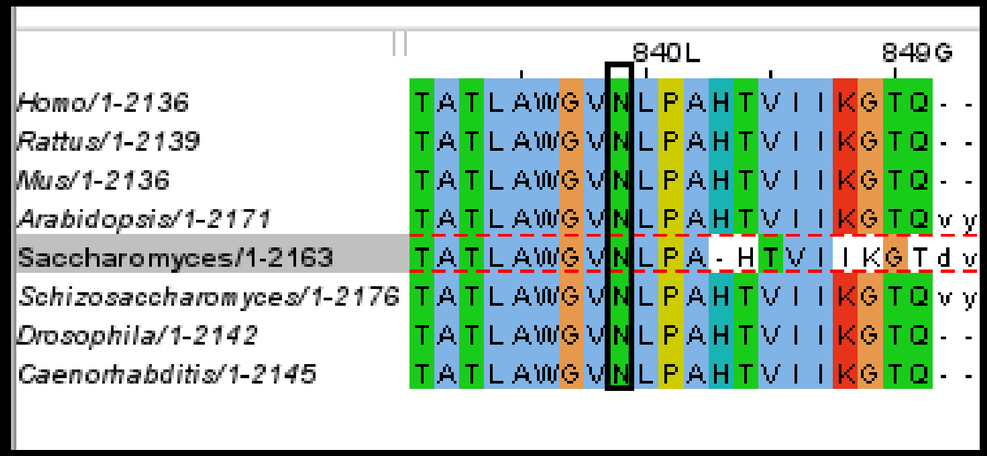




RNA interaction and movement



RNA interaction (5GAN) *Saccharomyces cerevisiae* ~ 3.70 Å



EVOLUTION

```
Database: uniprot_sprot.fasta
           537,505 sequences; 190,795,113 total letters



Query= 200 kDa helicase Homo sapiens

Length=2136

                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value

sp|O75643|U520_HUMAN U5 small nuclear ribonucleoprotein 200 kDa h...  4437    0.0
sp|Q9VUV9|U520_DROME Putative U5 small nuclear ribonucleoprotein ...  3383    0.0
sp|Q9U2G0|U520_CAEEL Putative U5 small nuclear ribonucleoprotein ...  2757    0.0
sp|Q55CI8|HELCL_DICDI Activating signal cointegrator 1 complex su...  2224    0.0
sp|Q9UT24|BRR2_SCHPO Pre-mRNA-splicing factor brr2 OS=Schizosacch...  2102    0.0
sp|F1NTD6|ASCC3_CHICK Activating signal cointegrator 1 complex su...  1623    0.0
sp|E1BNG3|ASCC3_BOVIN Activating signal cointegrator 1 complex su...  1594    0.0
sp|E9PZJ8|ASCC3_MOUSE Activating signal cointegrator 1 complex su...  1579    0.0
sp|Q8N3C0|ASCC3_HUMAN Activating signal cointegrator 1 complex su...  1575    0.0
sp|F1LPQ2|ASCC3_RAT Activating signal cointegrator 1 complex subu...  1548    0.0
sp|P32639|BRR2_YEAST Pre-mRNA-splicing helicase BRR2 OS=Saccharom...  1533    0.0
sp|Q54G57|HELC1_DICDI Activating signal cointegrator 1 complex su...  1490    0.0
sp|E7F8F4|ASCC3_DANRE Activating signal cointegrator 1 complex su...  1414    0.0
sp|O60072|MUG81_SCHPO Putative helicase mug81 OS=Schizosaccharomy...  1380    0.0
sp|P53327|SLH1_YEAST Antiviral helicase SLH1 OS=Saccharomyces cer...  1274    0.0
sp|A2RUV5|HFM1_XENTR Probable ATP-dependent DNA helicase HFM1 OS=...  335     1e-92
sp|A2PYH4|HFM1_HUMAN Probable ATP-dependent DNA helicase HFM1 OS=...  331     4e-91
sp|P51979|HFM1_YEAST ATP-dependent DNA helicase MER3 OS=Saccharom...  271     1e-72
sp|Q9V0A9|HELS_PYRAB Putative ski2-type helicase OS=Pyrococcus ab...  188     6e-48
sp|O59025|HELS_PYRHO Putative ski2-type helicase OS=Pyrococcus ho...  179     6e-45
sp|Q97VY9|HELS_SULSO Putative ski2-type helicase OS=Sulfolobus so...  175     1e-43
sp|O73946|HELS_PYRFU Putative ski2-type helicase OS=Pyrococcus fu...  175     1e-43
sp|Q3IU46|HELS_NATPD Putative ski2-type helicase OS=Natronomonas ...  171     2e-42
sp|Q9HMV6|HELS_HALSA Putative ski2-type helicase OS=Halobacterium...  171     6e-42
sp|B0R7Q2|HELS_HALS3 Putative ski2-type helicase OS=Halobacterium...  171     6e-42
sp|Q974S1|HELS_SULTO Putative ski2-type helicase OS=Sulfolobus to...  169     8e-42
sp|Q4JC00|HELS_SULAC Putative ski2-type helicase OS=Sulfolobus ac...  167     5e-41
sp|Q5UYM9|HELS_HALMA Putative ski2-type helicase OS=Haloarcula ma...  166     2e-40
sp|Q9HJX7|HELS_THEAC Putative ski2-type helicase OS=Thermoplasma ...  164     3e-40
```

ANIMALIA

O75643 → Human

F1LNJ2→ Rattus norvegicus

Q6P4T2→ Mus musculus

Q9VUV9 → Drosophila

Q9U2G0 → C.Elegans

PLANTAE

Q9SYP1→ Arabidopsis Thaliana

FUNGI

Q9UT24 → SACCHAROMYCES POMBE

P32639 → SACCHAROMYCES CEREVISAE

PROCARIOTAS

ATP

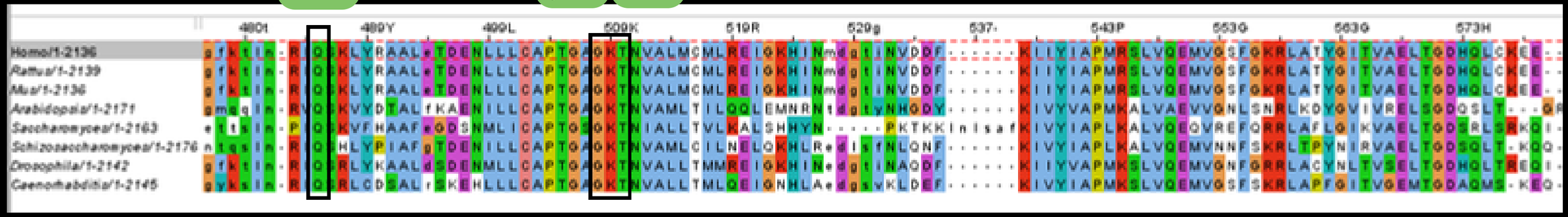
Q MOTIF

MOTIF I

Gln 485

Lys 509

Thr 510



MOTIF II

MOTIF III

Asp 615

Glu 616

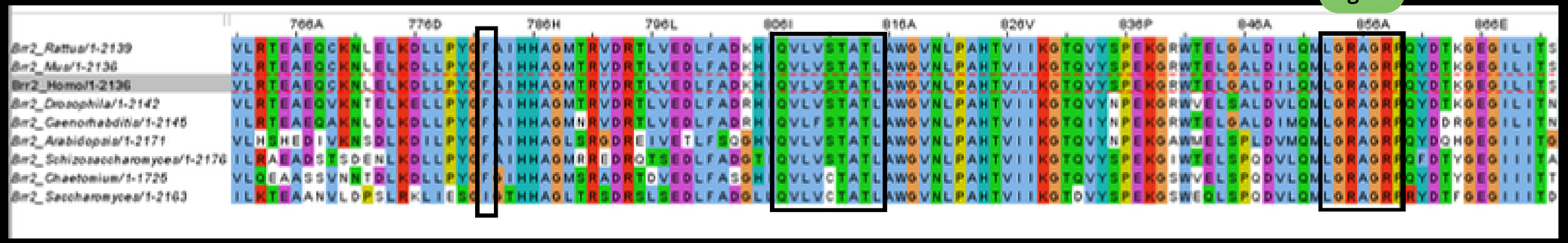


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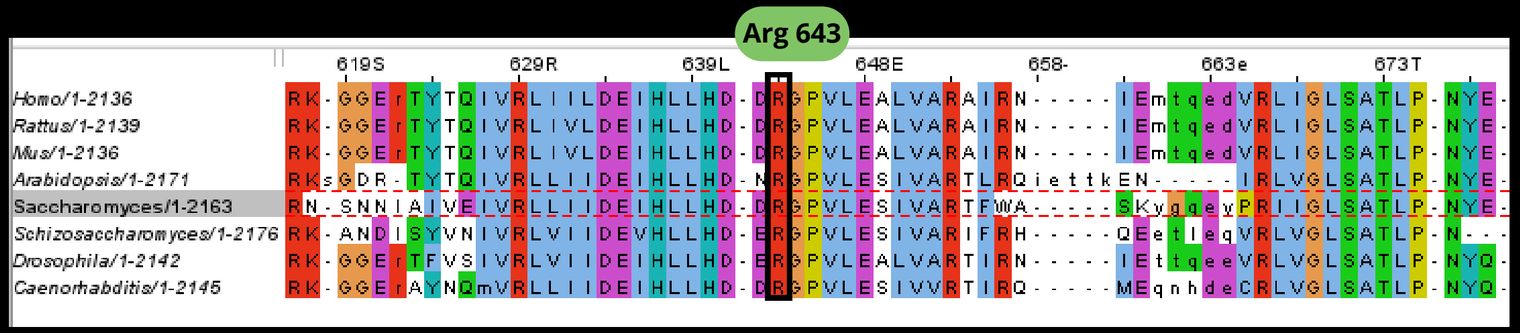
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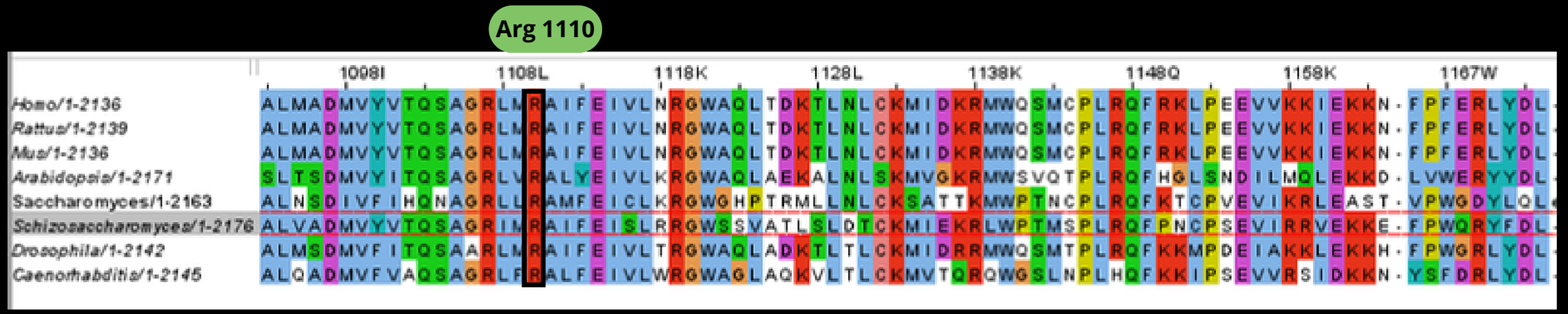
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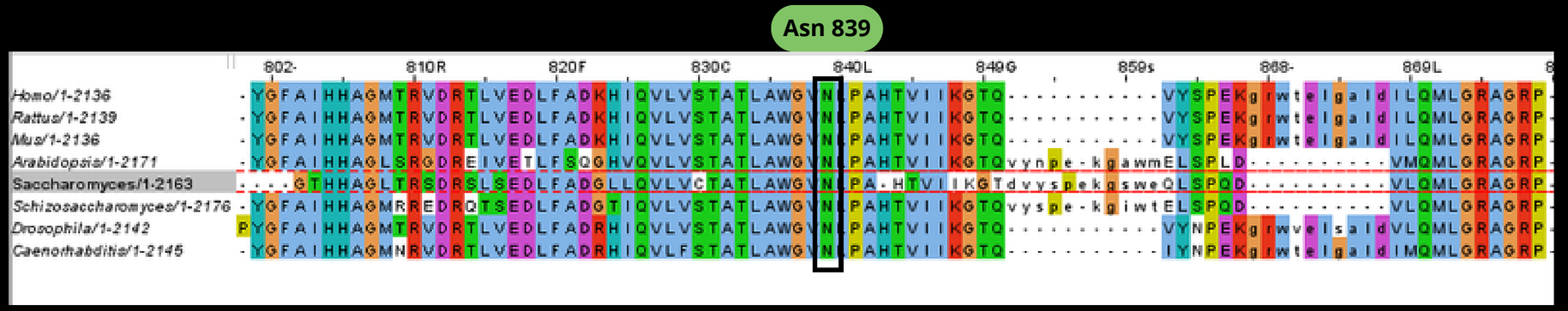
Arg 855



RNA


Arg 643
619S
629R
639L
648E
658-
663e
673T
Homo/1-2136
Rattus/1-2139
Mus/1-2136
Arabidopsis/1-2171
Saccharomyces/1-2163
Schizosaccharomyces/1-2176
Drosophila/1-2142
Caenorhabditis/1-2145


Arg 1110
1098I
1108L
1118K
1128L
1138K
1148Q
1158K
1167W
Homo/1-2136
Rattus/1-2139
Mus/1-2136
Arabidopsis/1-2171
Saccharomyces/1-2163
Schizosaccharomyces/1-2176
Drosophila/1-2142
Caenorhabditis/1-2145


Asn 839
802-
810R
820F
830C
840L
849G
859s
868-
869L
Homo/1-2136
Rattus/1-2139
Mus/1-2136
Arabidopsis/1-2171
Saccharomyces/1-2163
Schizosaccharomyces/1-2176
Drosophila/1-2142
Caenorhabditis/1-2145

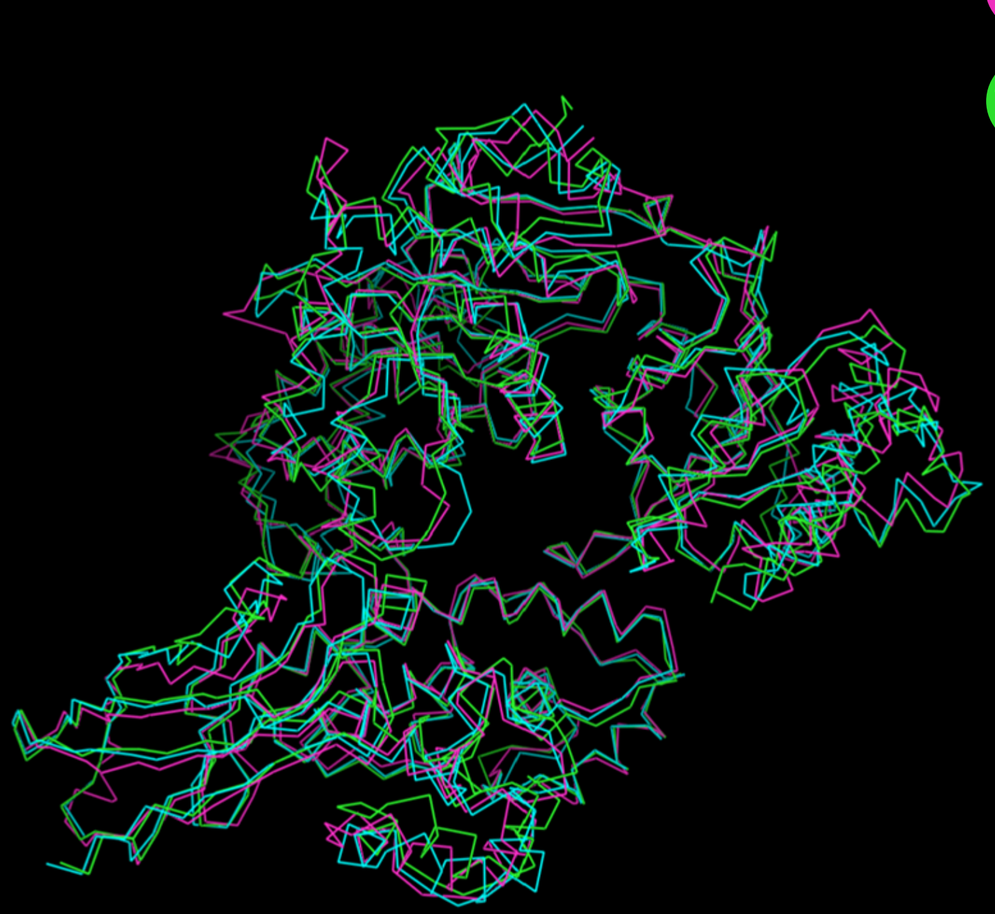
N cassette

C cassette

Homo sapiens

Saccharomyces cerevisiae

Thermochaetoides thermophila

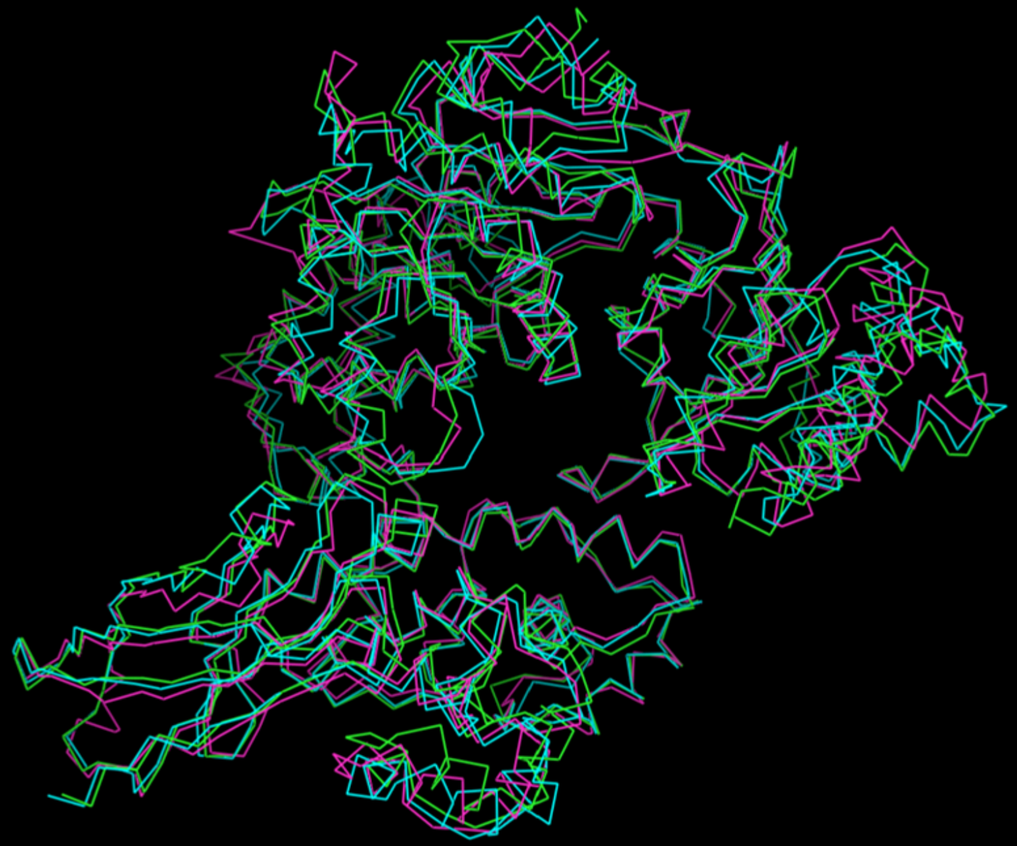




Brr2 Helicase Region (4F91) *Homo Sapiens* ~ 2.70 Å
Crystal structure of Brr2 in complex with the Jab1/MPN domain of Prp8 (4BGD) *Saccharomyces cerevisiae* ~ 3.10Å
Crystal structure of the Ski2 RNA-helicase Brr2 from Chaetomium thermophilum in the apo state (6QWS) *Thermochaetoides thermophila* ~ 3.30Å

N cassette

Homo sapiens ***Saccharomyces cerevisiae*** ***Thermochaetoides thermophila***



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Version 4.4 (May 2010)
 by Robert B. Russell & Geoffrey J. Barton
 Please cite PROTEINS, v14, 309-323, 1992

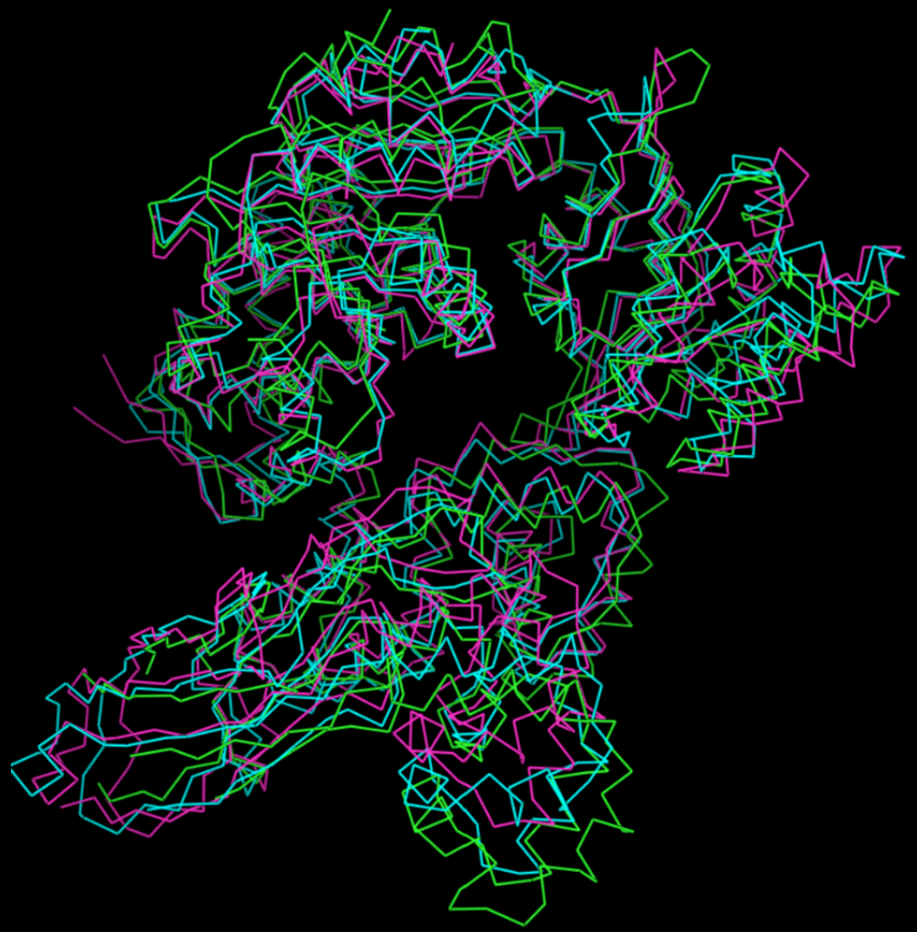

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    Len1, Len2 = length of domain, Nfit = residues fitted
    Secs = no. equivalent sec. strucs. Eq = no. equivalent residues
    %I = seq. identity, %S = sec. str. identity
    P(m)  = P value (p=1/10) calculated after Murzin (1993), JMB, 230, 689-694
            (NC = P value not calculated - potential FP overflow)

     No.  Domain1         Domain2         Sc     RMS    Len1 Len2  Align NFit Eq. Secs.   %I   %S   P(m)
Pair   1  4F91B           4BGDA           8.37   1.36    826  831   844  789 778    0  51.67 100.00 0.00e+00
Pair   2  4F91B           6QWSA           8.24   1.53    826  831   839  791 778    0  63.88 100.00 0.00e+00
Pair   3  4BGDA           6QWSA           8.24   1.54    831  831   843  792 786    0  56.62 100.00 0.00e+00
Reading in matrix file nc2.mat...
Doing cluster analysis...
Cluster:  1 (   4F91B  &    4BGDA ) Sc  8.37 RMS   1.36 Len 844 nfit 789
 See file nc2.1 for the alignment and transformations
Cluster:  2 (   6QWSA  &    4F91B    4BGDA ) Sc  9.25 RMS   1.42 Len 851 nfit 796
 See file nc2.2 for the alignment and transformations
```

Brr2 Helicase Region (4F91) *Homo Sapiens* ~ 2.70 Å
Crystal structure of Brr2 in complex with the Jab1/MPN domain of Prp8 (4BGD) *Saccharomyces cerevisiae* ~ 3.10Å
Crystal structure of the Ski2 RNA-helicase Brr2 from Chaetomium thermophilum in the apo state (6QWS) *Thermochaetoides thermophila* ~ 3.30Å

C cassette

Homo sapiens ***Saccharomyces cerevisiae*** ***Thermochaetoides thermophila***



```
STAMP Structural Alignment of Multiple Proteins

Version 4.4 (May 2010)
 by Robert B. Russell & Geoffrey J. Barton
 Please cite PROTEINS, v14, 309-323, 1992


    Sc = STAMP score, RMS = RMS deviation, Align = alignment length
    Len1, Len2 = length of domain, Nfit = residues fitted
    Secs = no. equivalent sec. strucs. Eq = no. equivalent residues
    %I = seq. identity, %S = sec. str. identity
    P(m)  = P value (p=1/10) calculated after Murzin (1993), JMB, 230, 689-694
            (NC = P value not calculated - potential FP overflow)

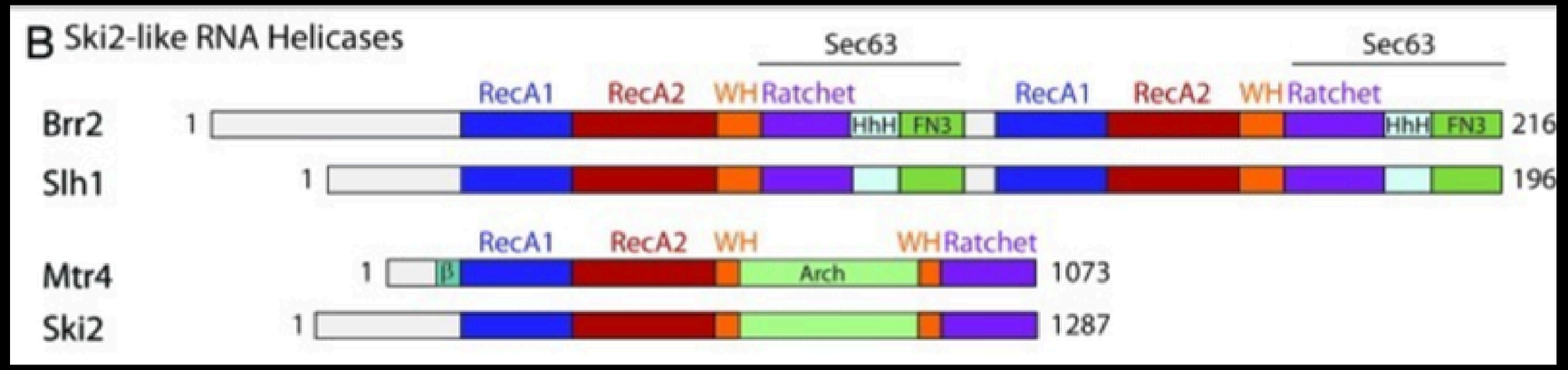
     No.  Domain1         Domain2         Sc     RMS    Len1 Len2  Align NFit Eq. Secs.   %I   %S   P(m)
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Pair   2  4F91B           6QWSA           7.59   1.88    814  796   828  746 739    0  49.66 100.00 0.00e+00
Pair   3  4BGDA           6QWSA           6.61   2.07    819  796   847  684 665    0  41.50 100.00 4.92e-100
Reading in matrix file cc2.mat...
Doing cluster analysis...
Cluster:  1 (   4F91B  &    4BGDA ) Sc  7.61 RMS   1.72 Len 828 nfit 721
 See file cc2.1 for the alignment and transformations
Cluster:  2 (   6QWSA  &    4F91B    4BGDA ) Sc  8.75 RMS   1.99 Len 839 nfit 732
 See file cc2.2 for the alignment and transformations
```

Brr2 Helicase Region (4F91) *Homo Sapiens* ~ 2.70 Å

Crystal structure of Brr2 in complex with the Jab1/MPN domain of Prp8 (4BGD) *Saccharomyces cerevisiae* ~ 3.10Å

Crystal structure of the Ski2 RNA-helicase Brr2 from Chaetomium thermophilum in the apo state (6QWS) *Thermochaetoides thermophila* ~ 3.30Å

Paralogs of Brr2



Johnson, S. J., & Jackson, R. N. (2013). Ski2-like RNA helicase structures: common themes and complex assemblies: Common themes and complex assemblies. RNA Biology

RecA1 + **RecA2** + **WH** + **Ratchet**

nucleous

citoplasm

Mtr4 → exosoma nuclear

Ski2 → exosoma citoplasmático

} 1 cassette

Slh1 → encharge of the Ribosome-associated Quality Control

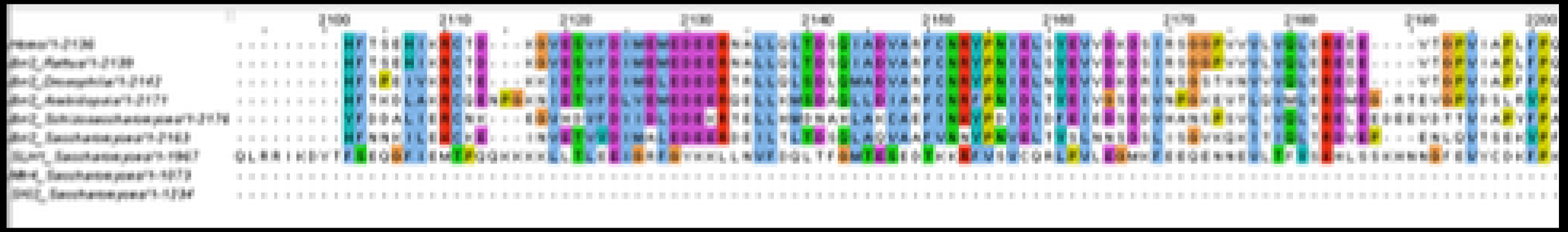
Brr2 → Splicing

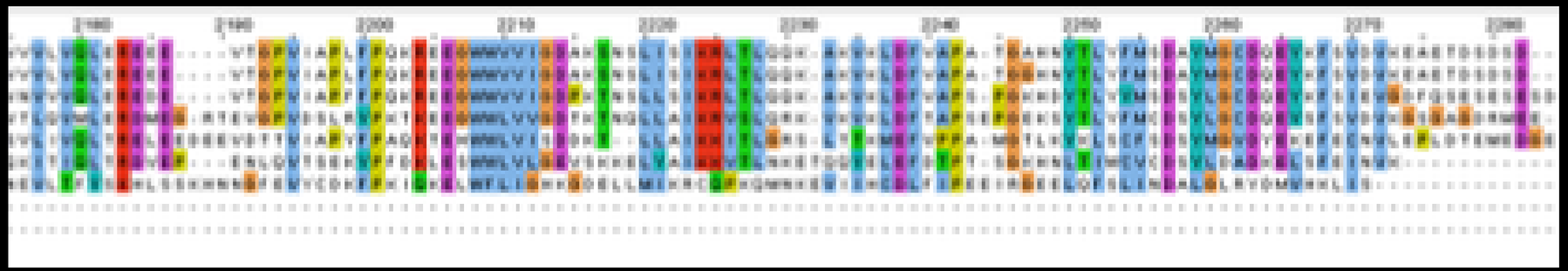
} 2 cassetts

N- terminal Region

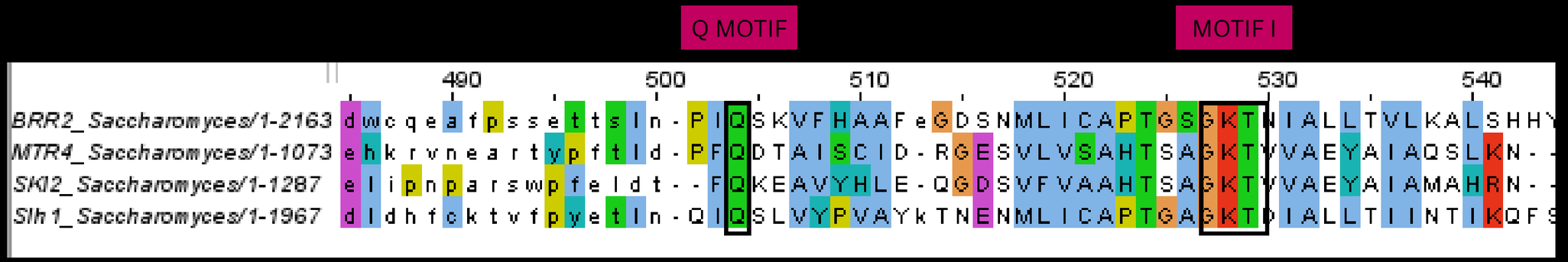


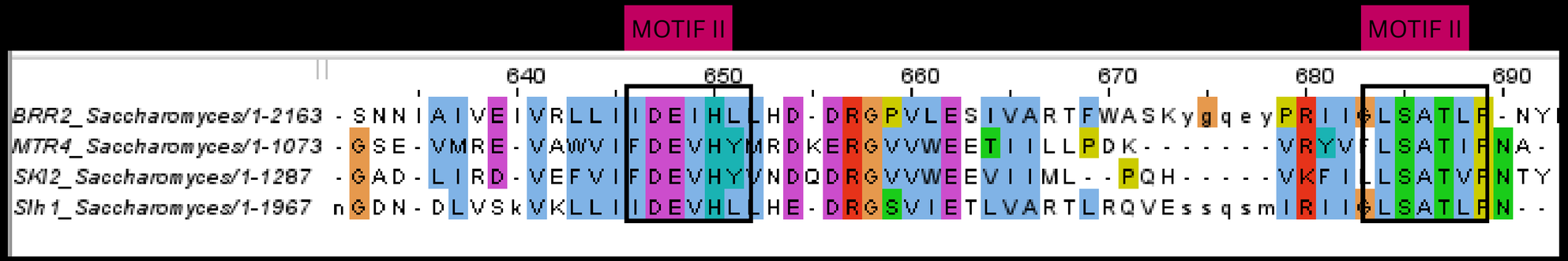
C-terminal of the C cassette



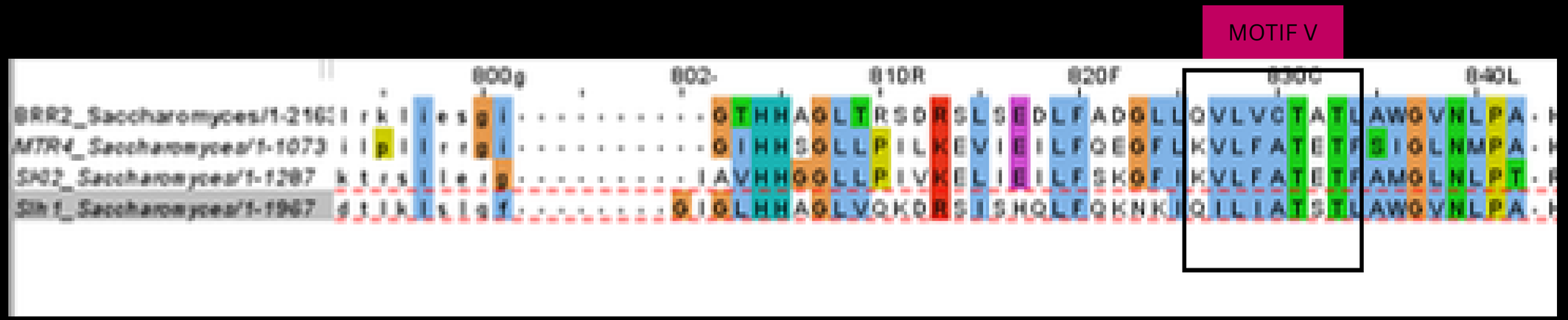


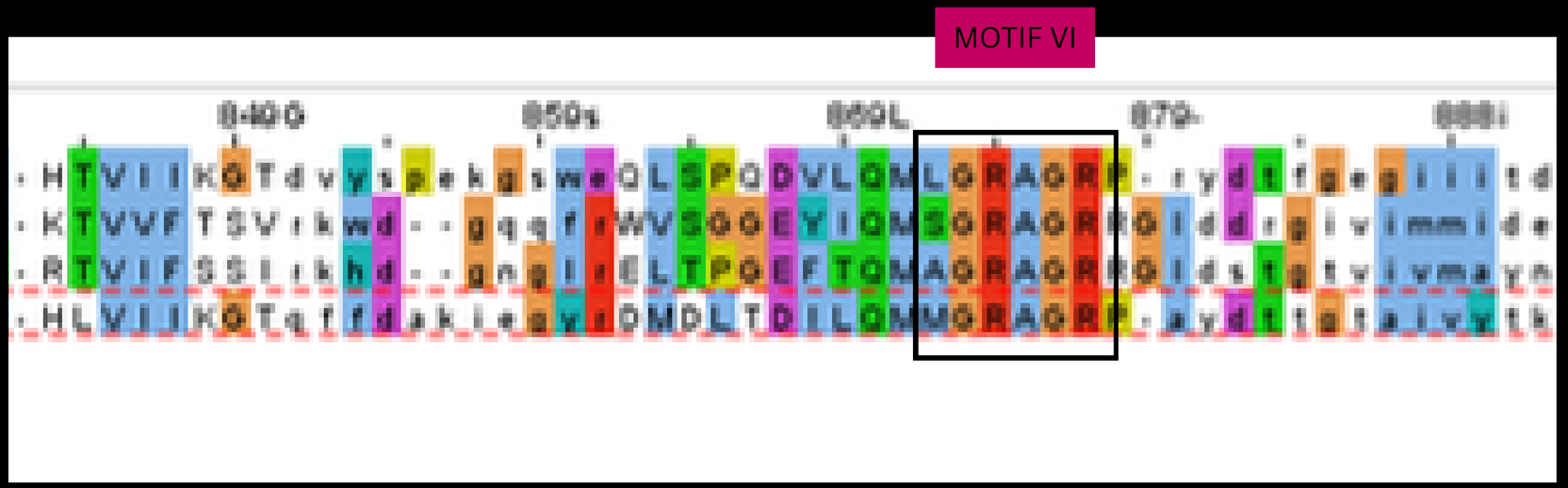
RecA1 domain





RecA2 domain





Structural superimposition

RECA1 DOMAIN

Mtr4 **Ski2** **Brr2**



```
STAMP Structural Alignment of Multiple Proteins

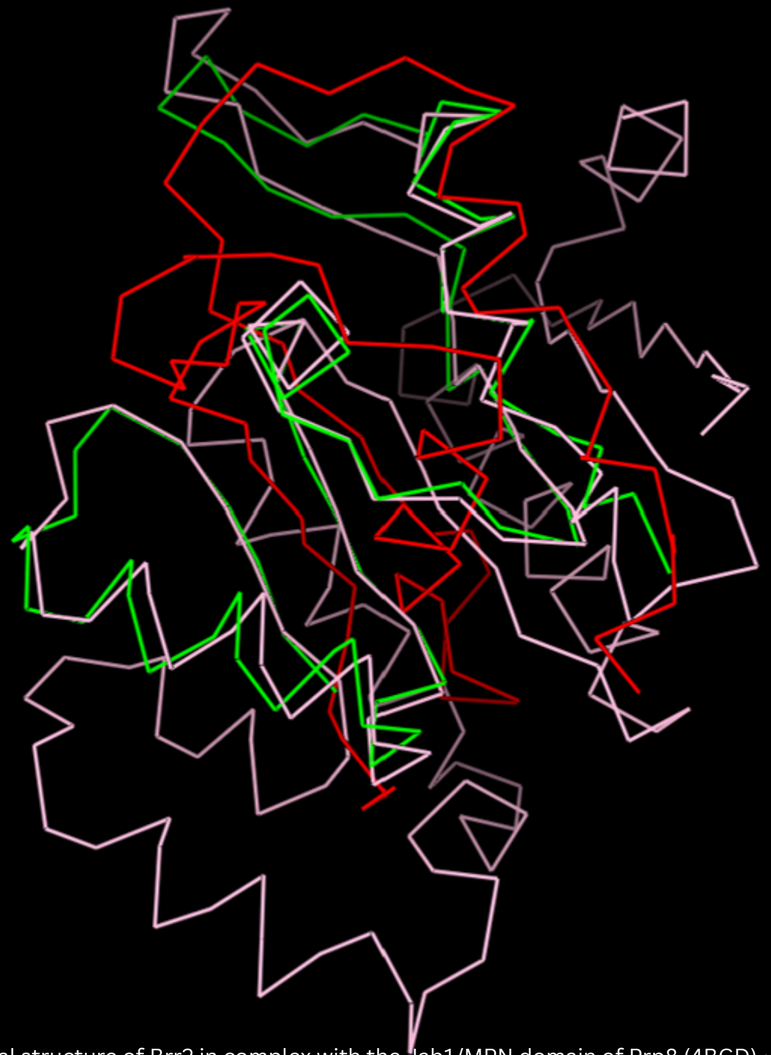
Version 4.4 (May 2010)
 by Robert B. Russell & Geoffrey J. Barton
 Please cite PROTEINS, v14, 309-323, 1992

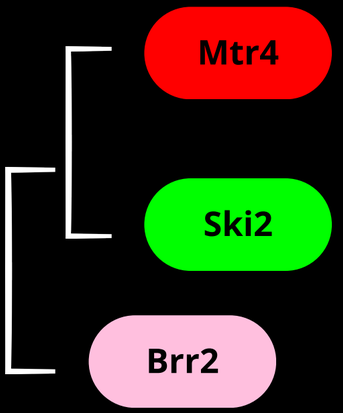

    Sc = STAMP score, RMS = RMS deviation, Align = alignment length
    Len1, Len2 = length of domain, Nfit = residues fitted
    Secs = no. equivalent sec. strucs. Eq = no. equivalent residues
    %I = seq. identity, %S = sec. str. identity
    P(m)  = P value (p=1/10) calculated after Murzin (1993), JMB, 230, 689-694
            (NC = P value not calculated - potential FP overflow)

     No.  Domain1         Domain2         Sc      RMS    Len1 Len2  Align NFit Eq. Secs.   %I   %S   P(m)
Pair   1  4BGDA          4QU4A          4.08    1.39     223  122   153  107 104    0  28.85 100.00 4.83e-08
Pair   2  4BGDA          4A4ZA          4.71    1.82     223  147   180  125 121    0  28.93 100.00 3.75e-09
Pair   3  4QU4A          4A4ZA          6.00    1.29     122  147   115  106 102    0  61.76 100.00 3.90e-37
Reading in matrix file paralogos_RecA1.mat...
Doing cluster analysis...
Cluster:  1 (   4QU4A  &    4A4ZA ) Sc  6.00 RMS   1.29 Len 115 nfit 106
 See file paralogos_RecA1.1 for the alignment and transformations
Cluster:  2 (   4BGDA  &    4QU4A    4A4ZA ) Sc  5.91 RMS   1.53 Len 188 nfit 101
 See file paralogos_RecA1.2 for the alignment and transformations
```

Crystal structure of Brr2 in complex with the Jab1/MPN domain of Prp8 (4BGD) ~ 3.10Å
Improved refinement of the Mtr4 apo crystal structure (4QU4) ~ 3.39Å
CRYSTAL STRUCTURE OF THE S. CEREVISIAE DEXH HELICASE SKI2 BOUND TO AMPPNP (4A4Z) ~ 2.40Å

RECA2 DOMAIN






```
STAMP Structural Alignment of Multiple Proteins

Version 4.4 (May 2010)
 by Robert B. Russell & Geoffrey J. Barton
 Please cite PROTEINS, v14, 309-323, 1992


    Sc = STAMP score, RMS = RMS deviation, Align = alignment length
    Len1, Len2 = length of domain, Nfit = residues fitted
    Secs = no. equivalent sec. strucs. Eq = no. equivalent residues
    %I = seq. identity, %S = sec. str. identity
    P(m)  = P value (p=1/10) calculated after Murzin (1993), JMB, 230, 689-694
            (NC = P value not calculated - potential FP overflow)

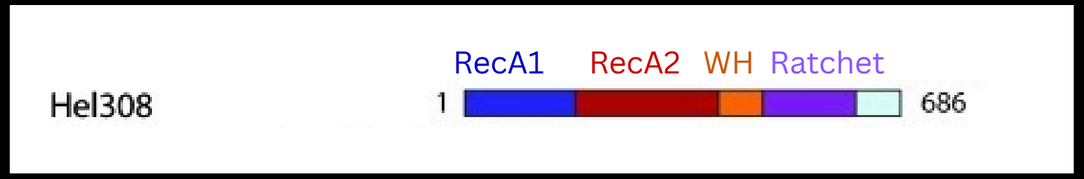
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Pair   2  4BGDA          4QU4A          1.75   1.17    201   44    45   41  41    0  46.34 100.00 2.41e-09
Pair   3  4A4ZA          4QU4A          4.64   0.88     77   44    45   40  40    0  75.00 100.00 2.96e-22
Reading in matrix file paralogos_RecA2.mat...
Doing cluster analysis...
Cluster:  1 (   4A4ZA  &    4QU4A ) Sc  4.63 RMS   0.88 Len  45 nfit  40
 See file paralogos_RecA2.1 for the alignment and transformations
Cluster:  2 (   4BGDA  &    4A4ZA    4QU4A ) Sc  3.99 RMS   0.96 Len  80 nfit  43
 See file paralogos_RecA2.2 for the alignment and transformations
```

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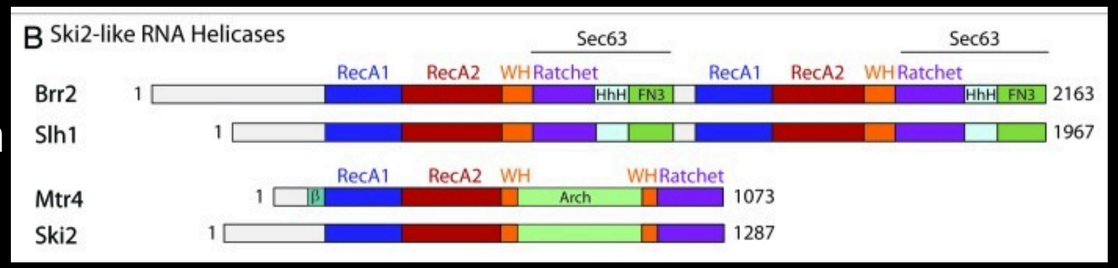
SKI2-LIKE FAMILY

MRCA

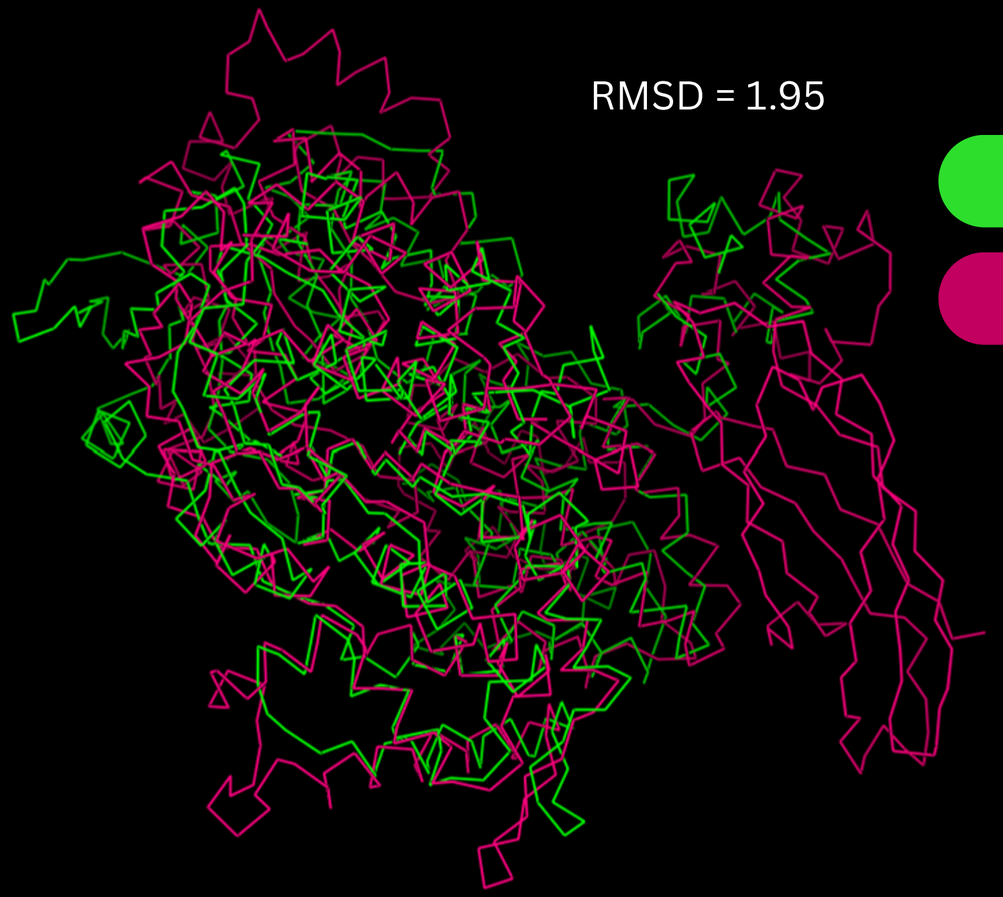
Archea



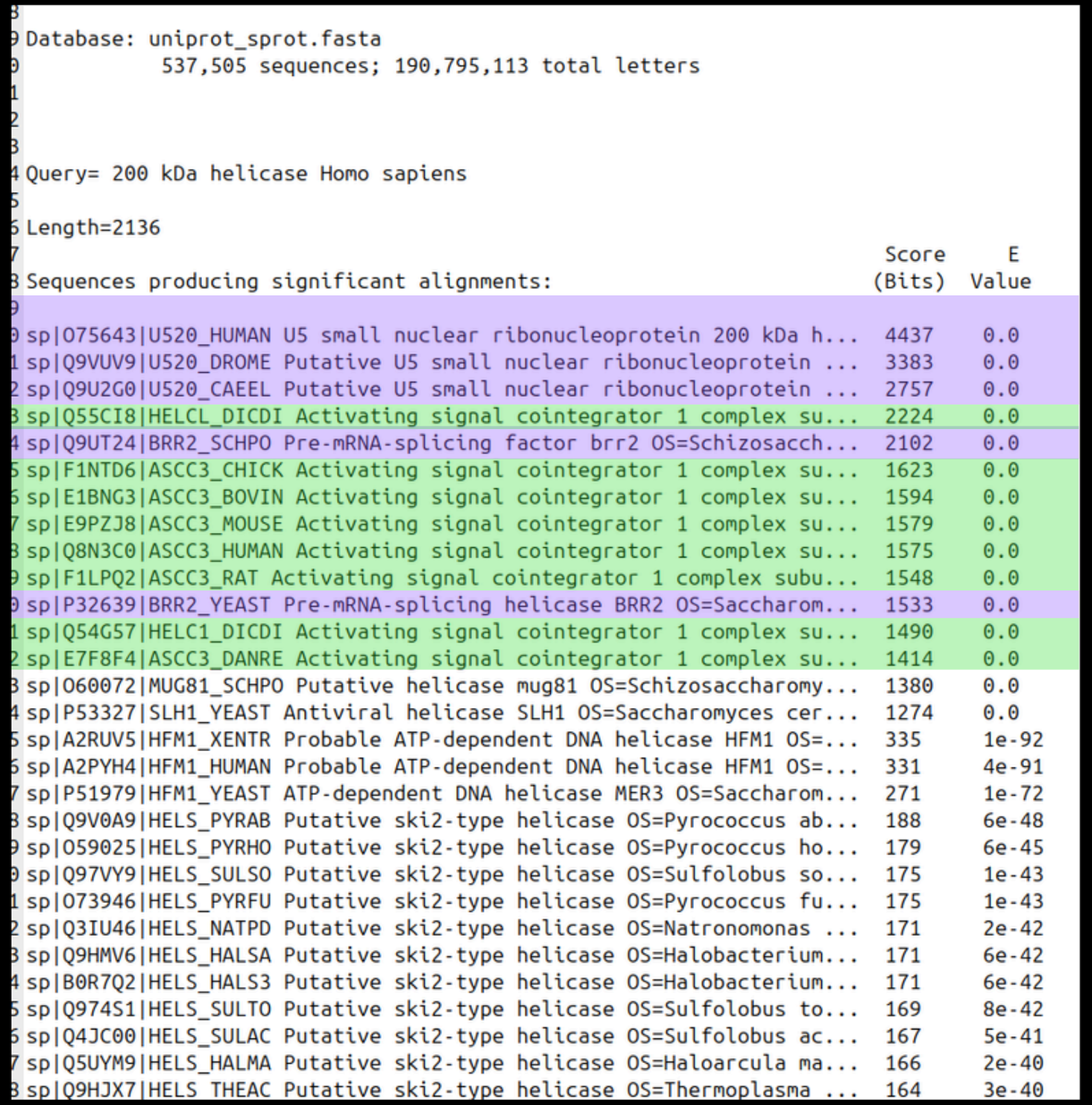
Eucarya



Johnson SJ, Jackson RN. Ski2-like RNA helicase structures: common themes and complex assemblies. RNA Biol. 2013 Jan;10(1):33-43. doi: 10.4161/rna.22101. Epub 2012 Sep 20. PMID: 22995828; PMCID: PMC3590235.



Crystal structure of Brr2 in complex with the Jab1/MPN domain of Prp8 (4BGD) ~ 3.10Å
Crystal structure of superfamily 2 helicase Hel308 in complex with unwound DNA (2P6R) ~ 3.00 Å



```
Database: uniprot_sprot.fasta
           537,505 sequences; 190,795,113 total letters


Query= 200 kDa helicase Homo sapiens

Length=2136
                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value

sp|O75643|U520_HUMAN U5 small nuclear ribonucleoprotein 200 kDa h...  4437    0.0
sp|Q9VUV9|U520_DROME Putative U5 small nuclear ribonucleoprotein ...  3383    0.0
sp|Q9U2G0|U520_CAEEL Putative U5 small nuclear ribonucleoprotein ...  2757    0.0
sp|Q55CI8|HELCL_DICDI Activating signal cointegrator 1 complex su...  2224    0.0
sp|Q9UT24|BRR2_SCHPO Pre-mRNA-splicing factor brr2 OS=Schizosacch...  2102    0.0
sp|F1NTD6|ASCC3_CHICK Activating signal cointegrator 1 complex su...  1623    0.0
sp|E1BNG3|ASCC3_BOVIN Activating signal cointegrator 1 complex su...  1594    0.0
sp|E9PZJ8|ASCC3_MOUSE Activating signal cointegrator 1 complex su...  1579    0.0
sp|Q8N3C0|ASCC3_HUMAN Activating signal cointegrator 1 complex su...  1575    0.0
sp|F1LPQ2|ASCC3_RAT Activating signal cointegrator 1 complex subu...  1548    0.0
sp|P32639|BRR2_YEAST Pre-mRNA-splicing helicase BRR2 OS=Saccharom...  1533    0.0
sp|Q54G57|HELC1_DICDI Activating signal cointegrator 1 complex su...  1490    0.0
sp|E7F8F4|ASCC3_DANRE Activating signal cointegrator 1 complex su...  1414    0.0
sp|O60072|MUG81_SCHPO Putative helicase mug81 OS=Schizosaccharomy...  1380    0.0
sp|P53327|SLH1_YEAST Antiviral helicase SLH1 OS=Saccharomyces cer...  1274    0.0
sp|A2RUV5|HFM1_XENTR Probable ATP-dependent DNA helicase HFM1 OS=...  335     1e-92
sp|A2PYH4|HFM1_HUMAN Probable ATP-dependent DNA helicase HFM1 OS=...  331     4e-91
sp|P51979|HFM1_YEAST ATP-dependent DNA helicase MER3 OS=Saccharom...  271     1e-72
sp|Q9V0A9|HELS_PYRAB Putative ski2-type helicase OS=Pyrococcus ab...  188     6e-48
sp|O59025|HELS_PYRHO Putative ski2-type helicase OS=Pyrococcus ho...  179     6e-45
sp|Q97VY9|HELS_SULSO Putative ski2-type helicase OS=Sulfolobus so...  175     1e-43
sp|O73946|HELS_PYRFU Putative ski2-type helicase OS=Pyrococcus fu...  175     1e-43
sp|Q3IU46|HELS_NATPD Putative ski2-type helicase OS=Natronomonas ...  171     2e-42
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sp|B0R7Q2|HELS_HALS3 Putative ski2-type helicase OS=Halobacterium...  171     6e-42
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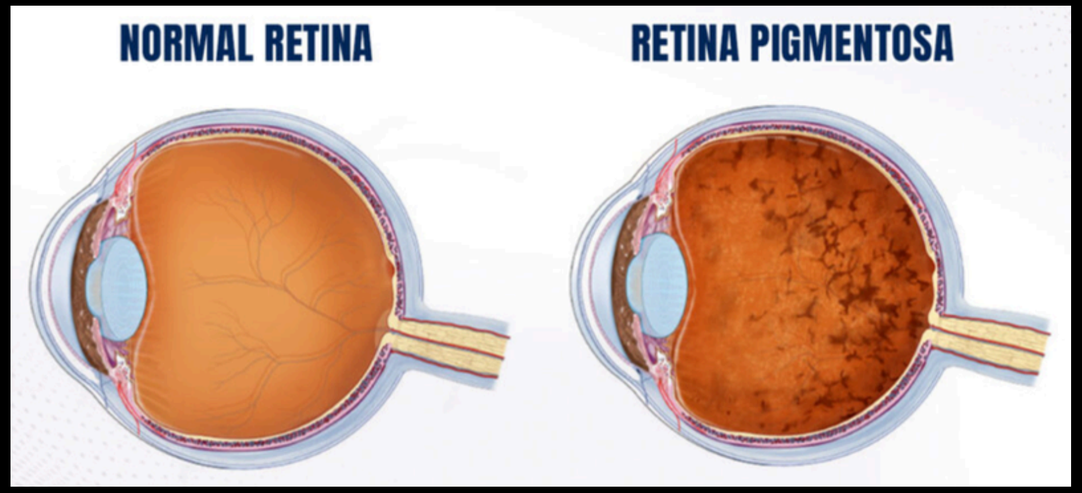
ORTHOLOGS

PARALOGS

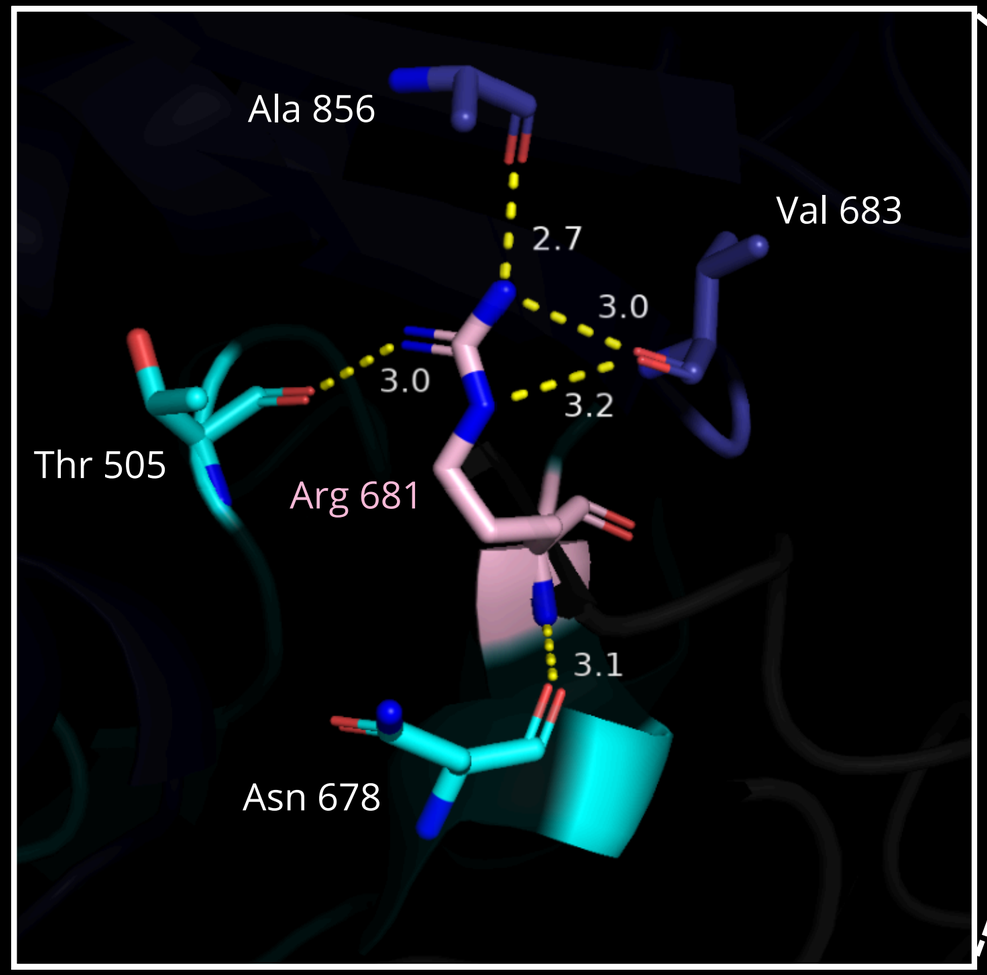
MUTATION AND DISEASE

Retinitis pigmentosa type 33

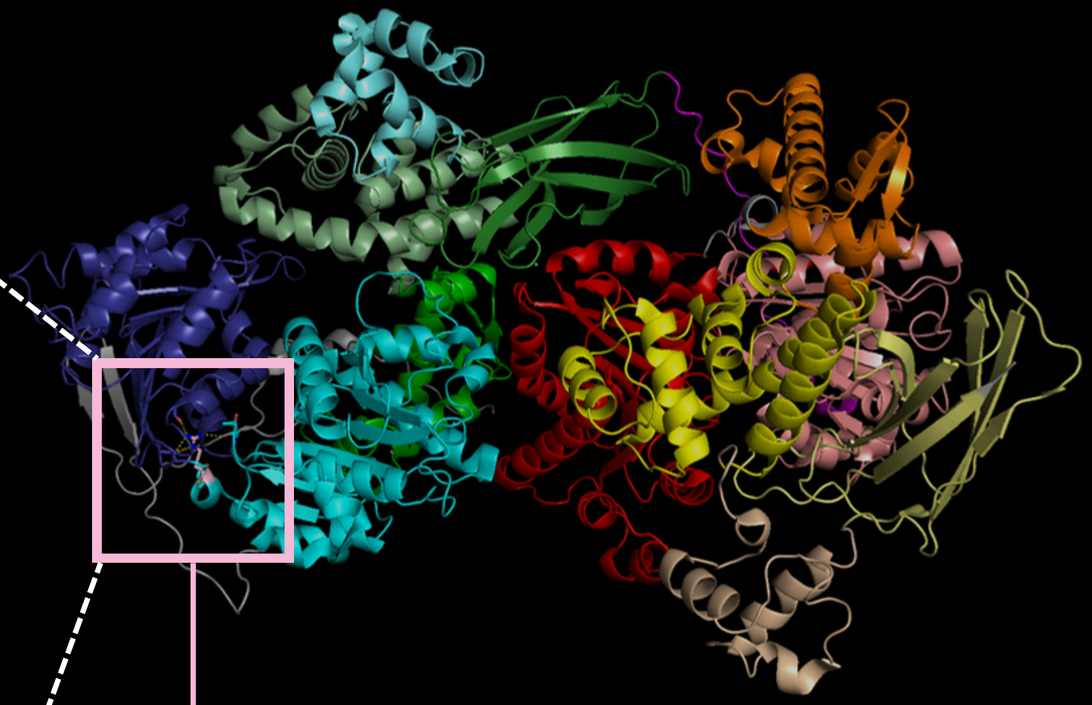
- Hereditary retinal diseases
- Progressive degeneration of rod and cone photoreceptors
- Autosomal dominant
- 2q11.2
- exon 16 of the SNRNP200 gene
- c.2041C>T
- **R681C**



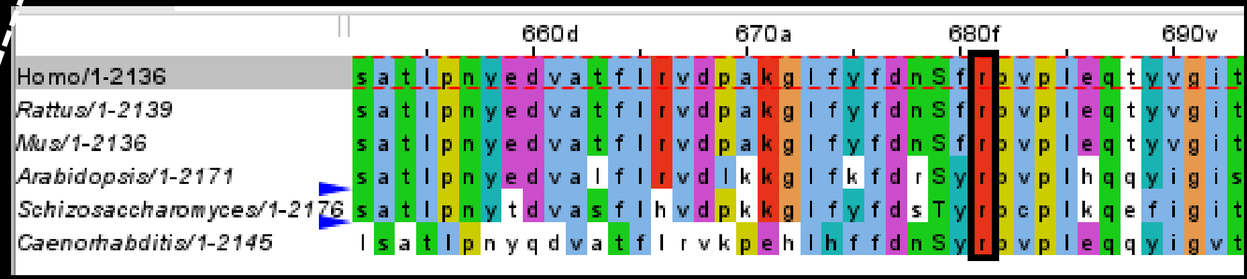
LJ Eye Institute. (2024). Retina Pigmentosa: Causes, Symptoms, and Treatments.



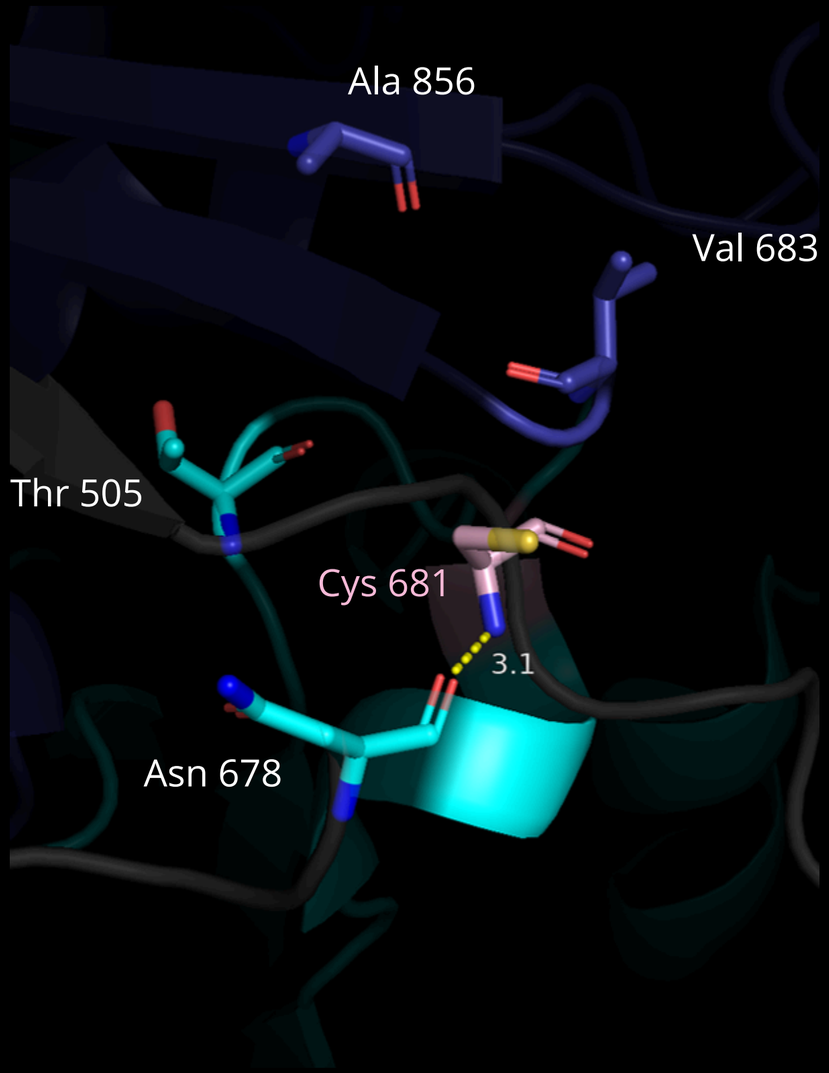
Brr2 Helicase Region (4F91) *Homo Sapiens* ~ 2.70 Å

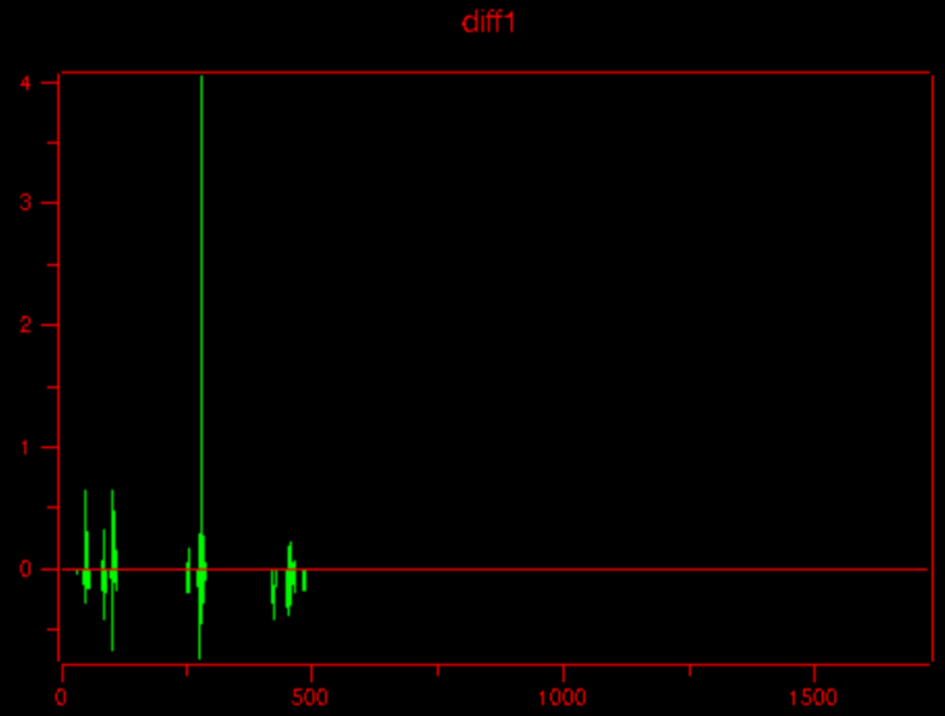


Brr2 Helicase Region (4F91) *Homo Sapiens* ~ 2.70 Å



R681C





Brr2 Helicase Region (4F91) *Homo Sapiens* ~ 2.70 Å


THANK YOU!

QUESTIONS PEM

1. Which fold has the RecA domain from Brr2?
a. **rossmann fold**
b. greek-key
c. beta propeller
d. all alpha helix bundle
e. falta alguna opcio aqui

2. The Q motif is capable of binding...
a. The N6 of the ATP
b. The N7 of the ATP
c. **the two previous answers are correct**
d. The gamma phosphate
e. The beta phosphate

3. Which is the mutation responsible for the incapacity of the C-cassette to hydrolyze ATP?
a. G1353N
b. **G1692N**
c. G452T
d. N1284G
e. N1983G

4. Identify the Brr2 mutation responsible for Retinitis Pigmentosa 33 that exhibits a significant energetic shift compared to the wild-type protein.
a. A561L
b. R681A
c. **R681C**
d. R732C
e. R732V

5. Which coordination number does magnesium have in Brr2 of the spliceosoma?
a. 14
b. **6**
c. 9
d. 2
e. 1

6. Which family does Brr2 belong to?
a. DEAD box
b. SF2
c. **Ski-2 like**
d. Hemagglutinin
e. Kinases

7. How does RH domain of Prp8 inhibit Brr2?
a. by physical inhibition only
b. when Jab1/MPN domain is not inhibiting
c. the two previous are correct
d. by physical inhibition and allosteric inhibition
e. all the above are correct

8. Which of these subunits is not a component of the spliceosome?
a. U1
b. U2
c. **U3**
d. U4
e. U5

9. The C cassette has the function of:
a. catalytic domain
b. **Regulatory domain**
c. Forms the tunnel where the RNA is going to enter
d. Structural support of the N cassette
e. Inhibitory by blocking the entrance of RNA in the tunnel.

10. Which motifs are in charge of binding and hydrolize the ATP:
a. I,II, III
b. I, III, IV
c. I, III, V
d. **I, II, VI**
e. I, II, IV

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