Lost in missing residues

Challenges while working with RNA structures from the Protein Data Base

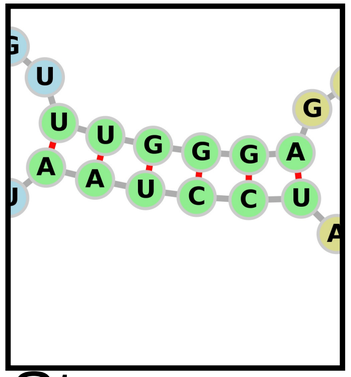
Bernhard Thiel, Bled 2018

What is RNA?

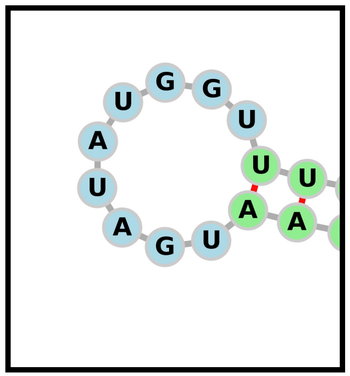
*) Polymer-chain of Adenine, Uracil, Guanine and Cytosine

AUAGUGCUGACUGACUGACUCGAUCGUCAGUCAGCA

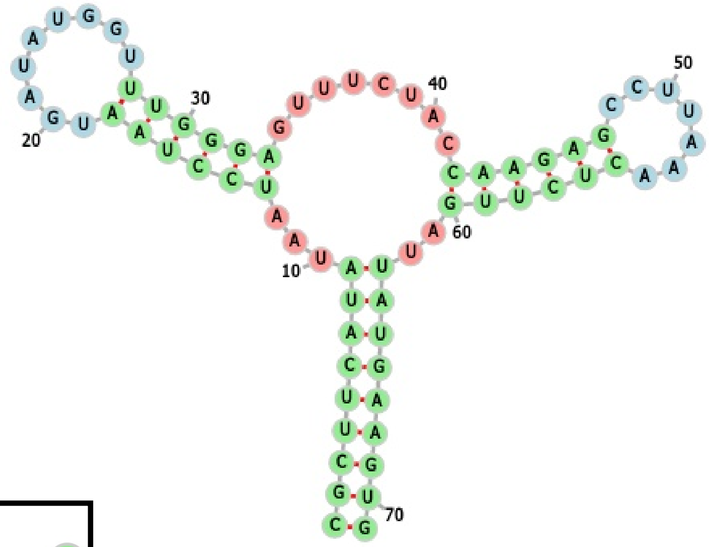
The **forgi** representation of RNA?

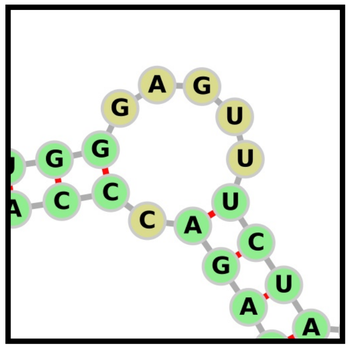


Stem

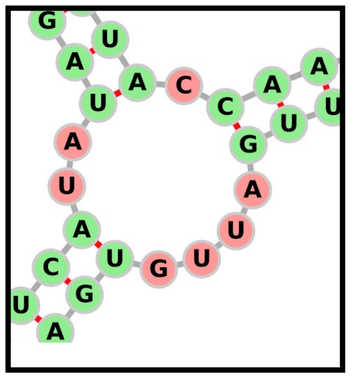


Hairpin





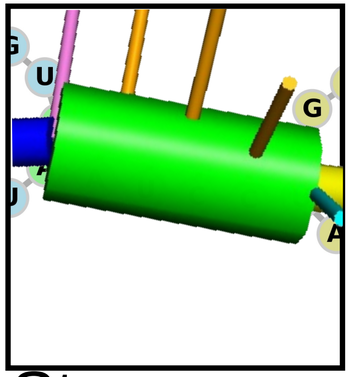
Interior loop



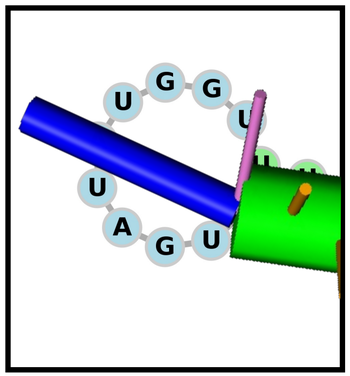
Multiloop

P.Kerpedjiev, B.Thiel, I.Hofacker & contributors
www.github.com/ViennaRNA/forgi

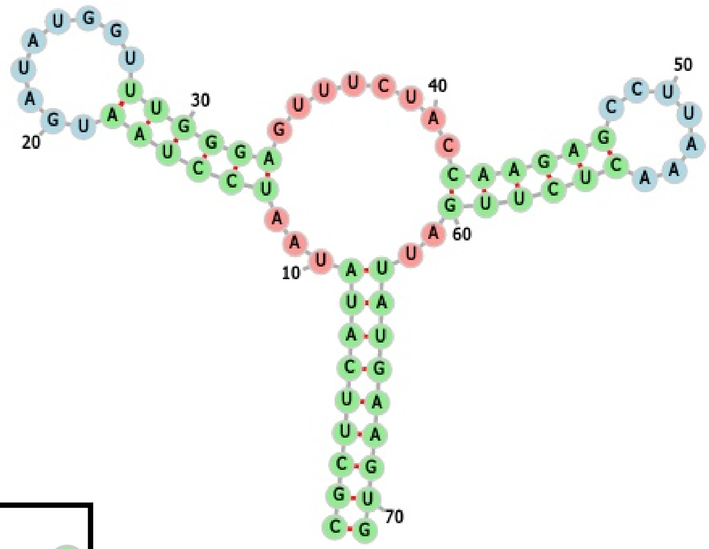
The **forgi** representation of RNA?

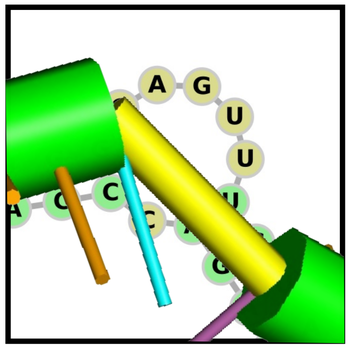


Stem

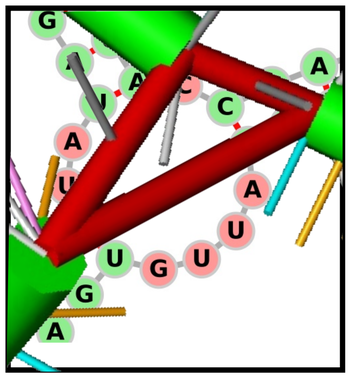


Hairpin





Interior loop



Multiloop

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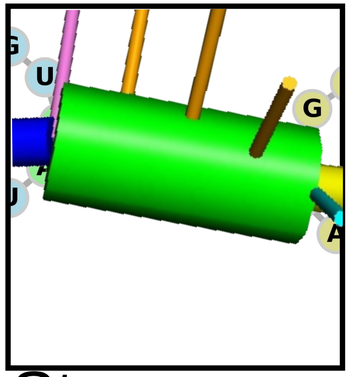
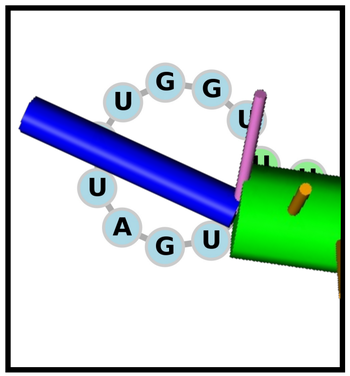
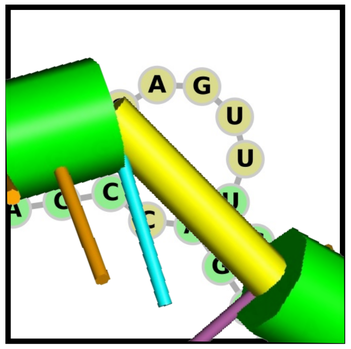
The **forgi** representation of RNA?



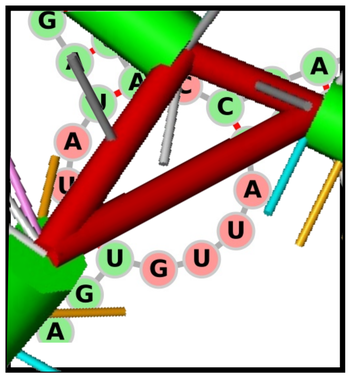
Stem



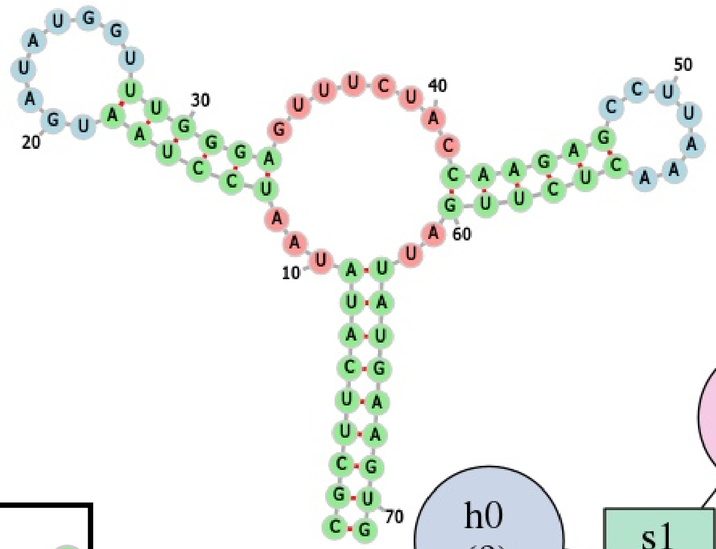
Hairpin

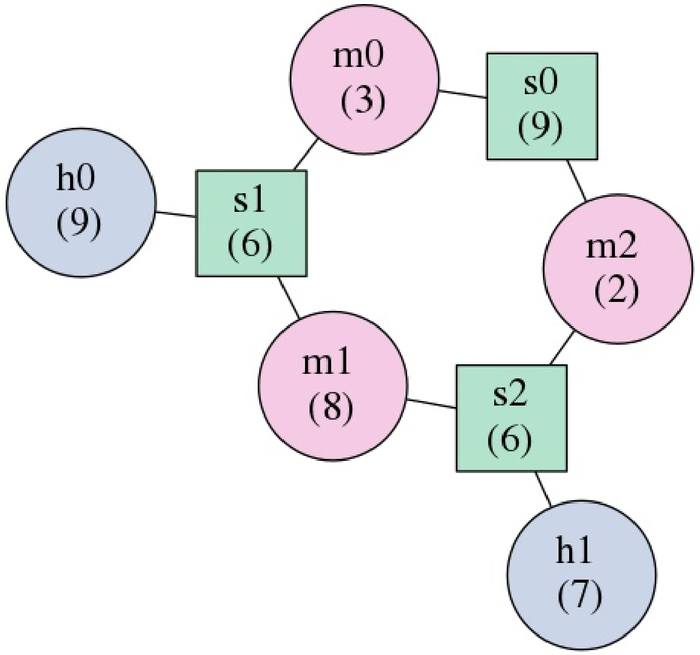


Interior loop



Multiloop





P.Kerpedjiev, B.Thiel, I.Hofacker & contributors
www.github.com/ViennaRNA/forgi

What is the PDB?

*) Experimentally
solved structures
- XRAY, NMR, Cryo-EM

PDB-File Format

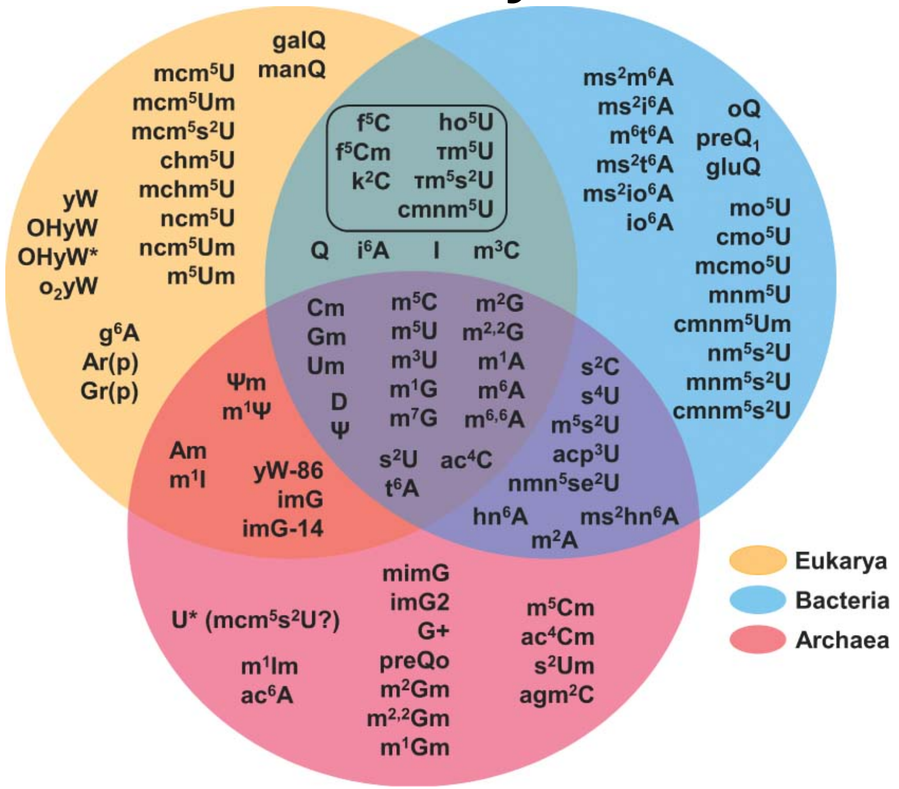
Some header information and ATOM records

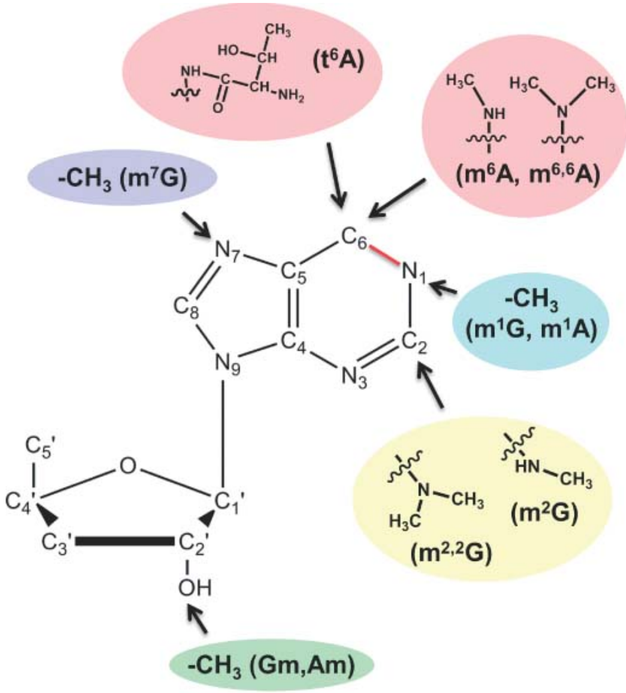
For each atom, the following is stored:
* the residue name, e.g. A for Adenine
* the number in the sequence
* the Atom type and its coordinates

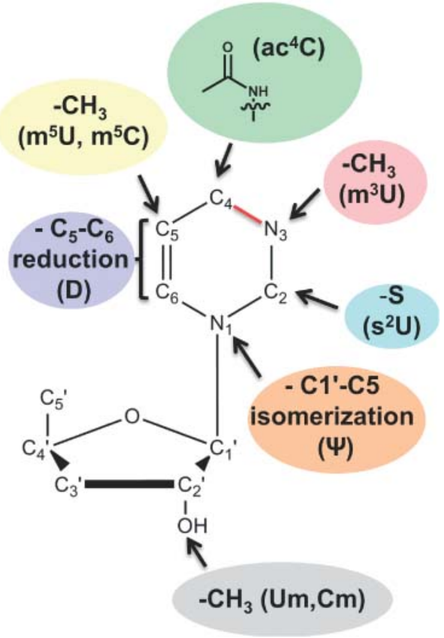
```
          1         2         3         4         5         6         7         8
12345678901234567890123456789012345678901234567890123456789012345678901234567890
MODEL        1
ATOM      1  N   ALA A   1      11.104   6.134  -6.504  1.00  0.00           N
ATOM      2  CA  ALA A   1      11.639   6.071  -5.147  1.00  0.00           C
…
…
…
ATOM    293 1HG  GLU A  18     -14.861  -4.847   0.361  1.00  0.00           H
ATOM    294 2HG  GLU A  18     -13.518  -3.769   0.084  1.00  0.00           H
TER     295      GLU A  18
```

RNA Modifications

tRNA is heavily modified



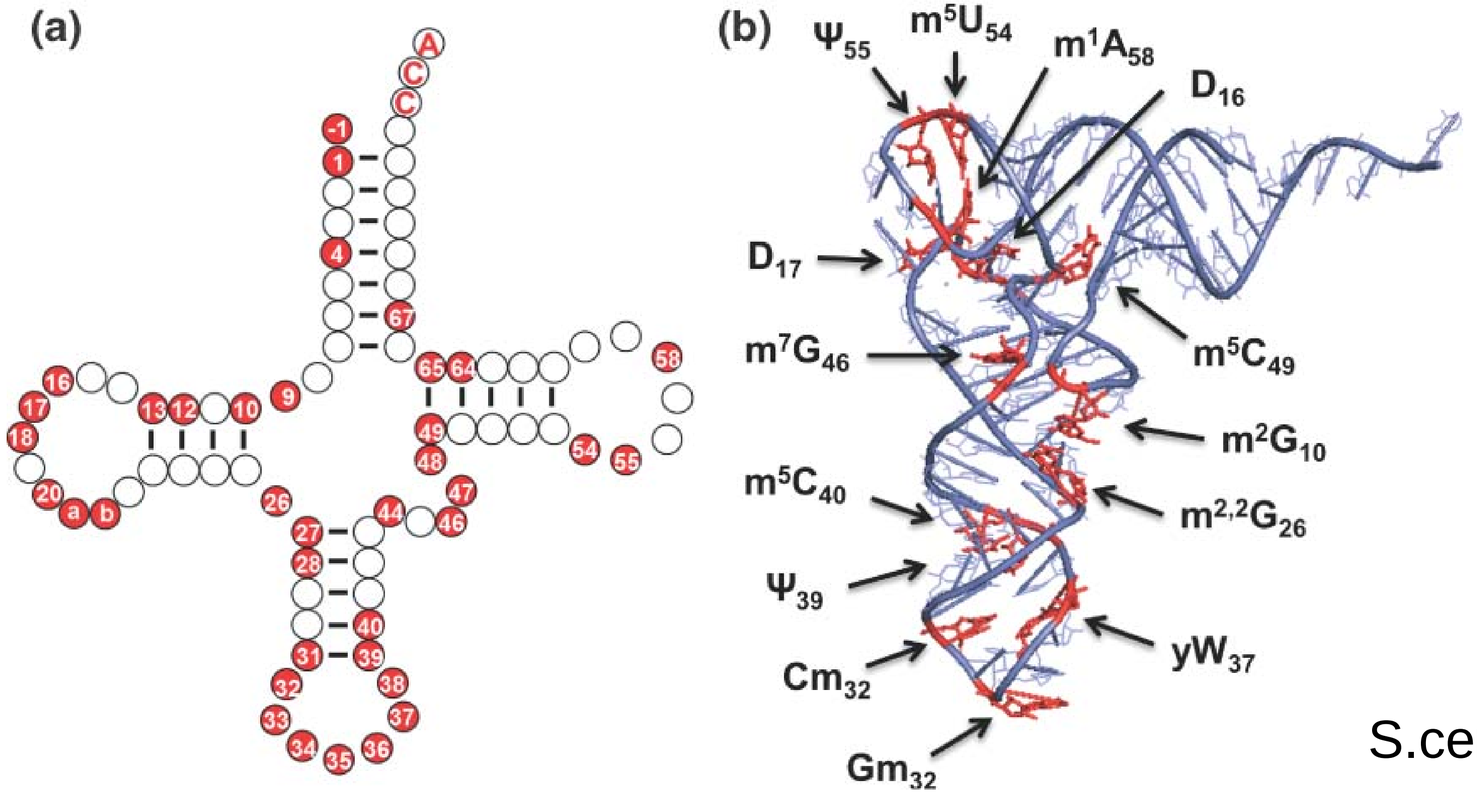




Images:
Transfer RNA modifications: nature's combinatorial chemistry playground
Wiley Interdisciplinary Reviews: RNA
Volume 4, Issue 1, pages 35-48, 8 NOV 2012 DOI: 10.1002/wrna.1144

RNA Modifications

tRNA is heavily modified

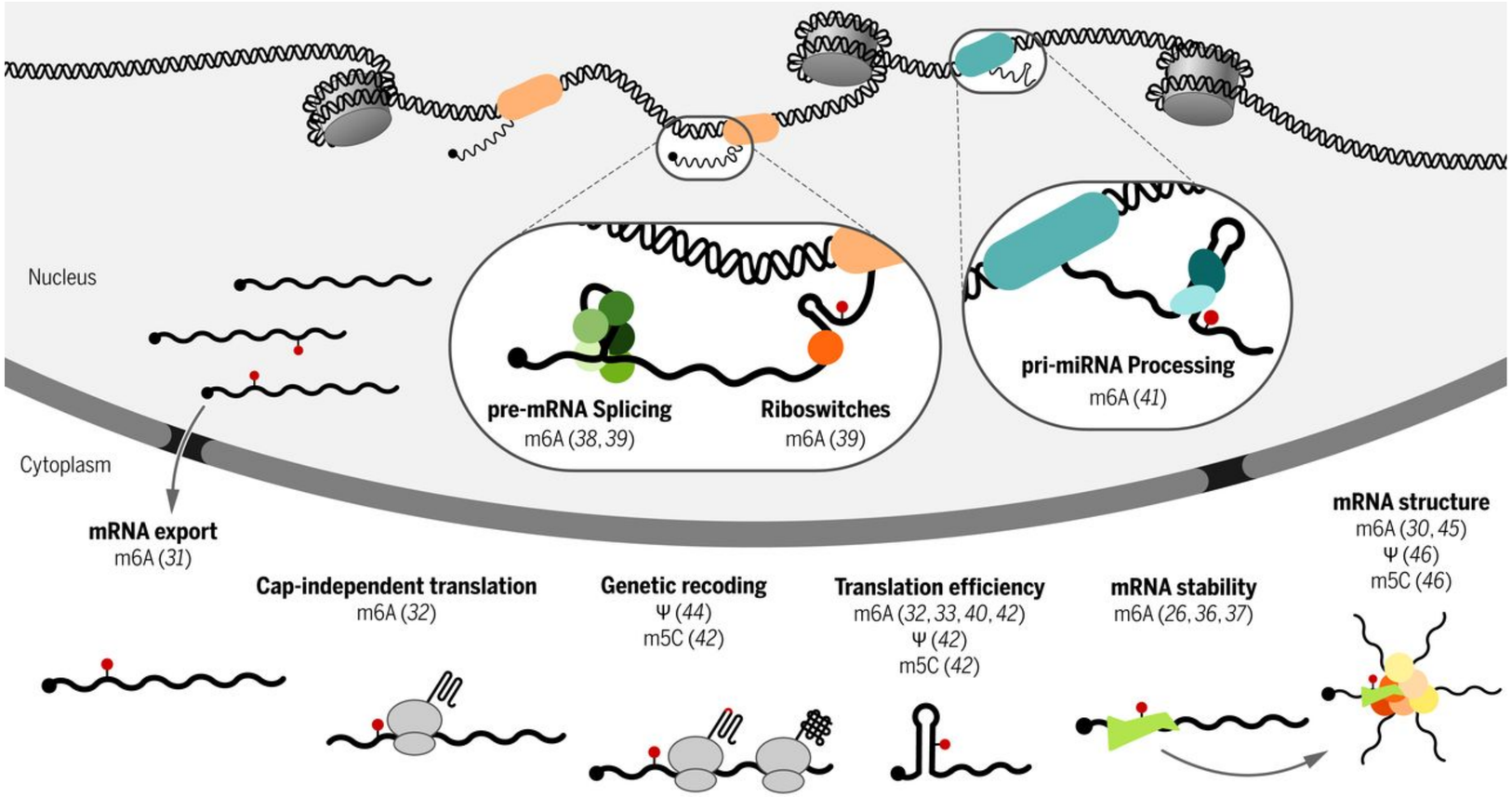


S.cerevisiae tRNA

Image:
Transfer RNA modifications: nature's combinatorial chemistry playground
Wiley Interdisciplinary Reviews: RNA
Volume 4, Issue 1, pages 35-48, 8 NOV 2012 DOI: 10.1002/wrna.1144

RNA Modifications

"Widespread sparse modification of messenger RNAs"[1]



Quote and image:
Gilbert et al, 2016, DOI: 10.1126/science.aad8711 ([1]: Quote from Abstract)

RNA Modifications

A-to-I editing
*) in double stranded RNA
*) by enzyme ADAR
*) I base-pairs like G

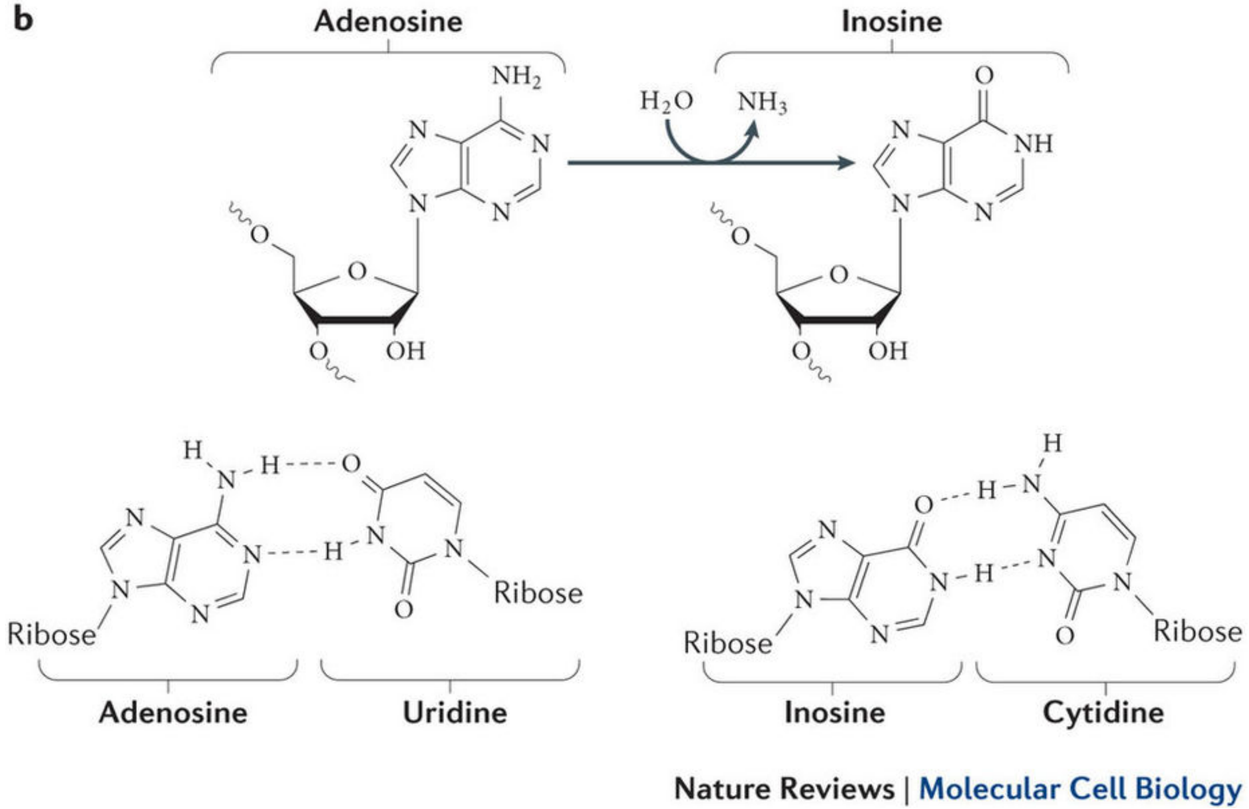


Image:
Nishikura, 2016, DOI: 10.1038/nrm.2015.4

RNA Modifications

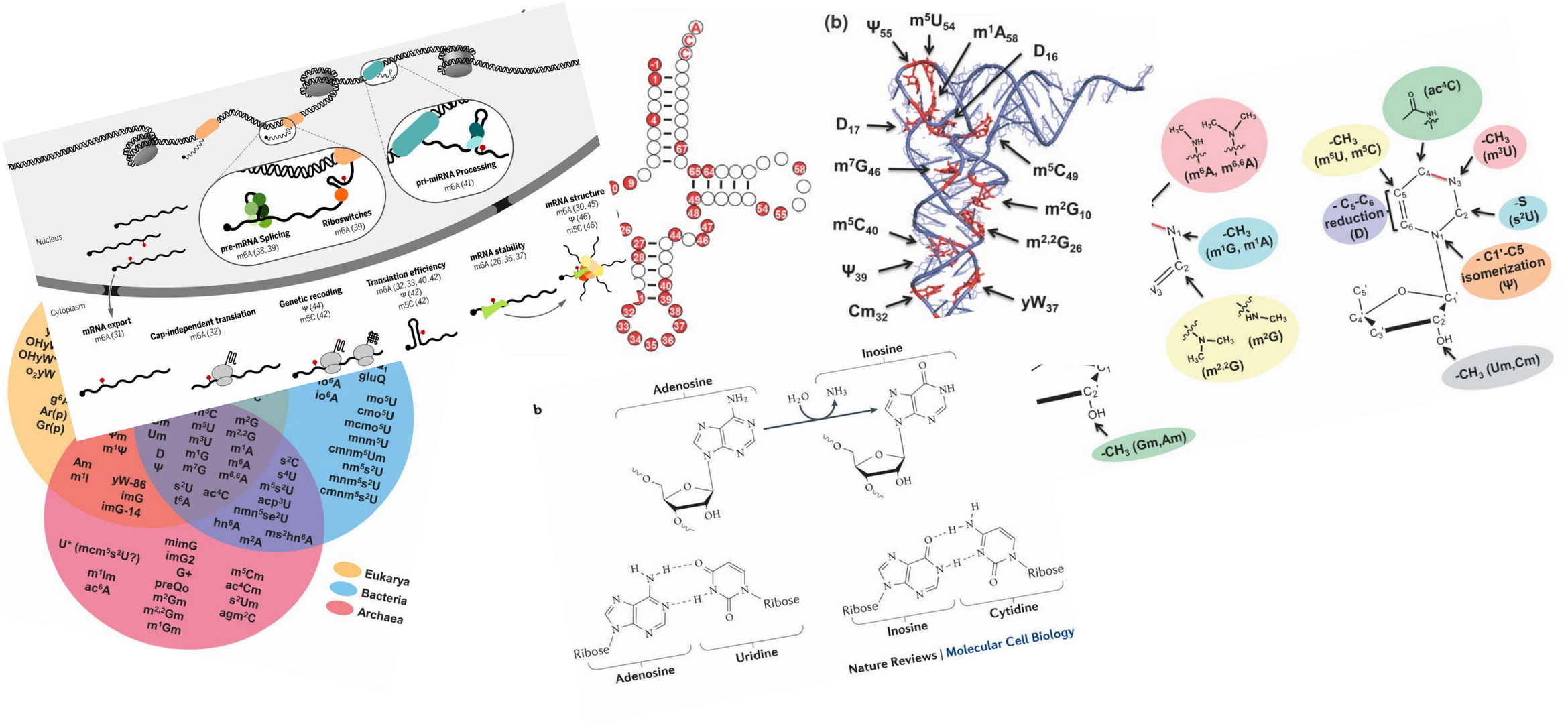
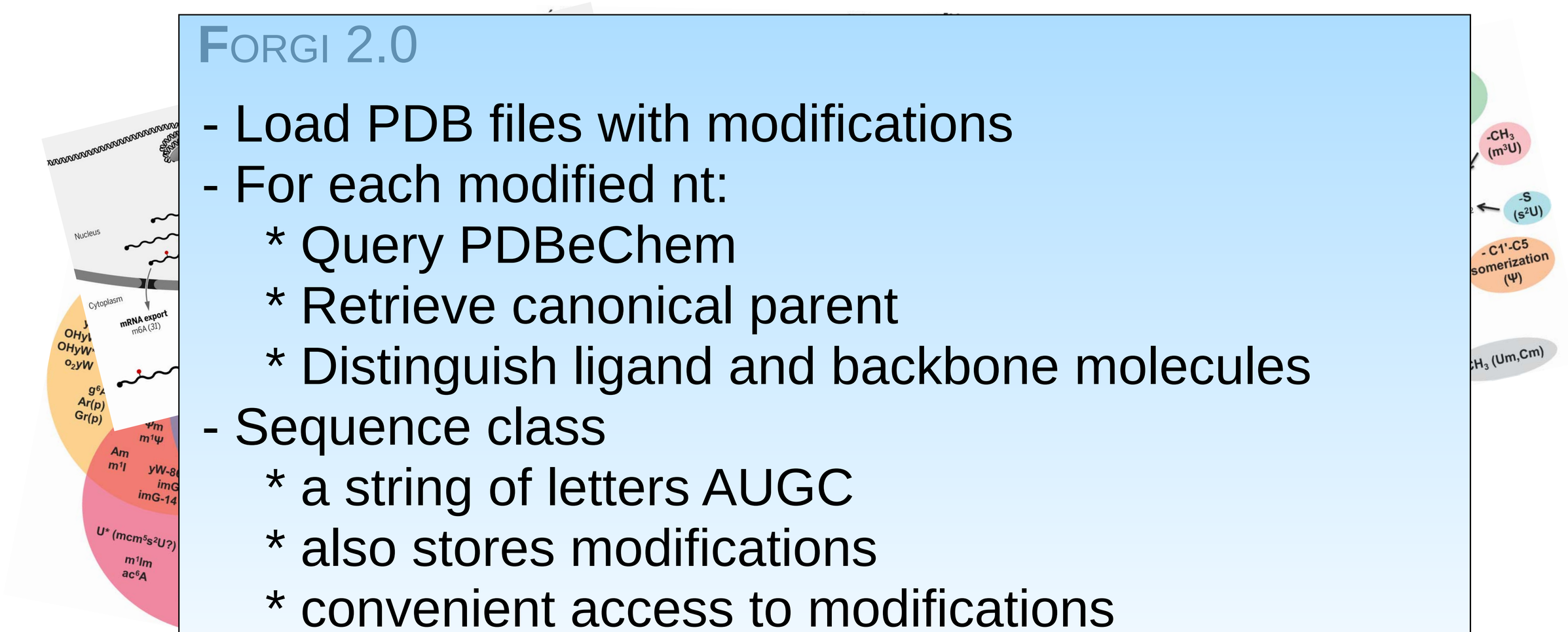


Image sources: See previous slides

RNA Modifications



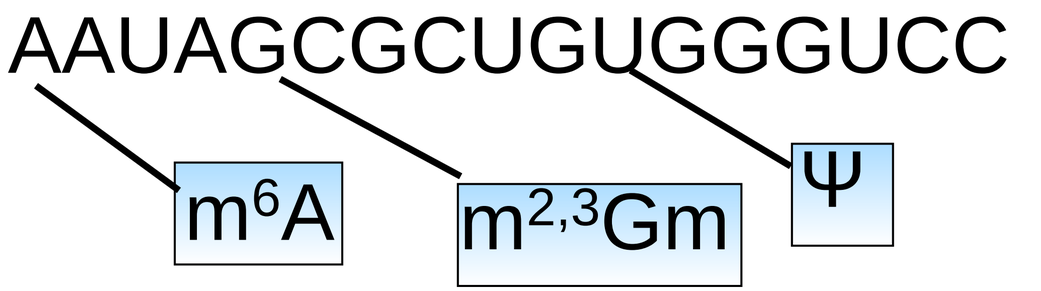
FORGI 2.0

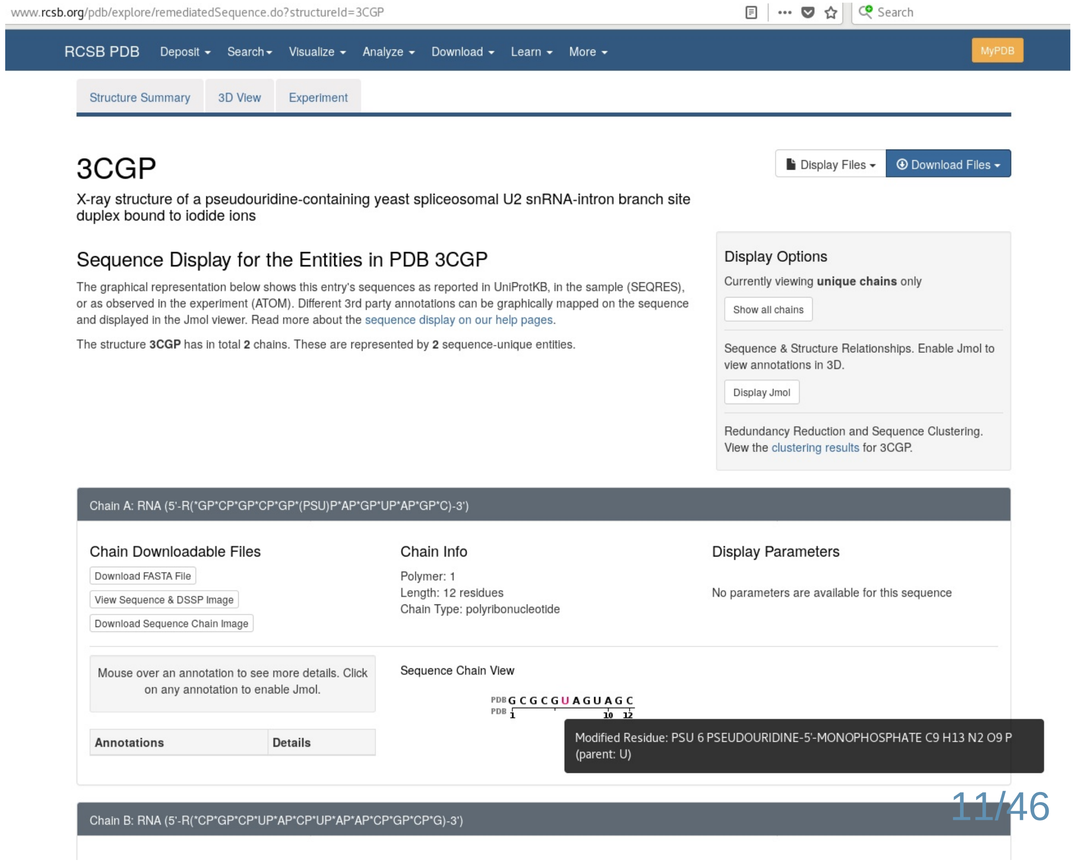
- Load PDB files with modifications
- For each modified nt:
  * Query PDBeChem
  * Retrieve canonical parent
  * Distinguish ligand and backbone molecules
- Sequence class
  * a string of letters AUGC
  * also stores modifications
  * convenient access to modifications

Image sources:

What is RNA?

*) Polymer-chain of Adenine, Uracil, Guanine and Cytosine with annotations for modifications





Residue number

Author provided
Number 1 is usually biologically meaningful,
but can be anywhere
Numbers can be missining
-) Deletions
-) No coordinates determined
Nucleotides can be inserted
Nucleotides can have negative numbers

Residue number

A -3
U -2
G 1
C 2
U 2B
A 2C
A 3
G 7
U 8
G 9

Residue number

A -3
U -2
G 1
C 2
U 2B
A 2C
A 3
G 7
U 8
G 9

FORGI 2.0

Sequence class supports 2 kinds of indices:
*) consecutive, 1-based
*) corresponding to PDB
Indices are distinguished by type

Missing residues

Residues present in experiment, for which no coordinates were determined.

PDB-File contains information at 3 places:

SEQRES: The sequence of the molecule used for the experiment, without numbers. -> parse with Bio.SeqIO
ATOM: atom-coordinates, nucleotide number, nt-name
REMARK 465: nt-number and nt-name for residues without coordinates

Missing residues

PDB-File contains information at 3 places:
SEQRES, ATOM, REMARK 465

```
         1         2         3         4         5         6         7         8
12345678901234567890123456789012345678901234567890123456789012345678901234567890
MODEL        1
ATOM      1  N   ALA A   1      11.104   6.134  -6.504  1.00  0.00           N
ATOM      2  CA  ALA A   1      11.639   6.071  -5.147  1.00  0.00           C
…
…
…
ATOM    293 1HG  GLU A  18     -14.861  -4.847   0.361  1.00  0.00           H
ATOM    294 2HG  GLU A  18     -13.518  -3.769   0.084  1.00  0.00           H
TER     295      GLU A  18
```

```
SEQRES  10 C  129  ILE PRO SER ALA ILE ALA ALA ASN SER GLY ILE TYR
SEQRES   1 R   14    U   C   G   C   C   A   A   C   A   G   G   C   G
SEQRES   2 R   14    G
SEQRES   1 S   14    U   C   G   C   C   A   A   C   A   G   G   C   G
SEQRES   2 S   14    G
FORMUL   6  HOH   *90(H2 O)
                                                      26  GLY A   28  5                                        3
                                                      98  LEU A  111  1                                       14
```

```
REMARK 350   BIOMT3  60  0.934172 -0.127323 -0.333334        0.00000
REMARK 465
REMARK 465 MISSING RESIDUES
REMARK 465 THE FOLLOWING RESIDUES WERE NOT LOCATED IN THE
REMARK 465 EXPERIMENT. (M=MODEL NUMBER; RES=RESIDUE NAME; C=CHAIN
REMARK 465 IDENTIFIER; SSSEQ=SEQUENCE NUMBER; I=INSERTION CODE.)
REMARK 465
REMARK 465   M RES C SSSEQI
REMARK 465       U S     1
REMARK 465       C S     2
REMARK 465       G S    14
REMARK 470
REMARK 470 MISSING ATOM
REMARK 470 THE FOLLOWING RESIDUES HAVE MISSING ATOMS(M=MODEL NUMBER;
                                                      532,1         11%
```

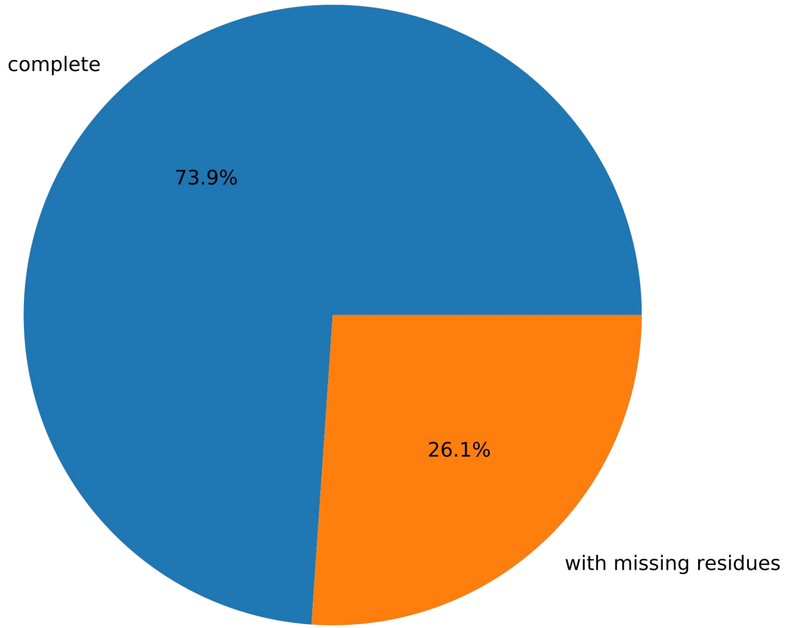
Missing residues

FORGI 2.0

* Parse REMARK 465 (Biopython PR)
* Get (sub-) sequences with or without missing residues.
* Identify coarse-grained element (stem/...) which contain missing residues.

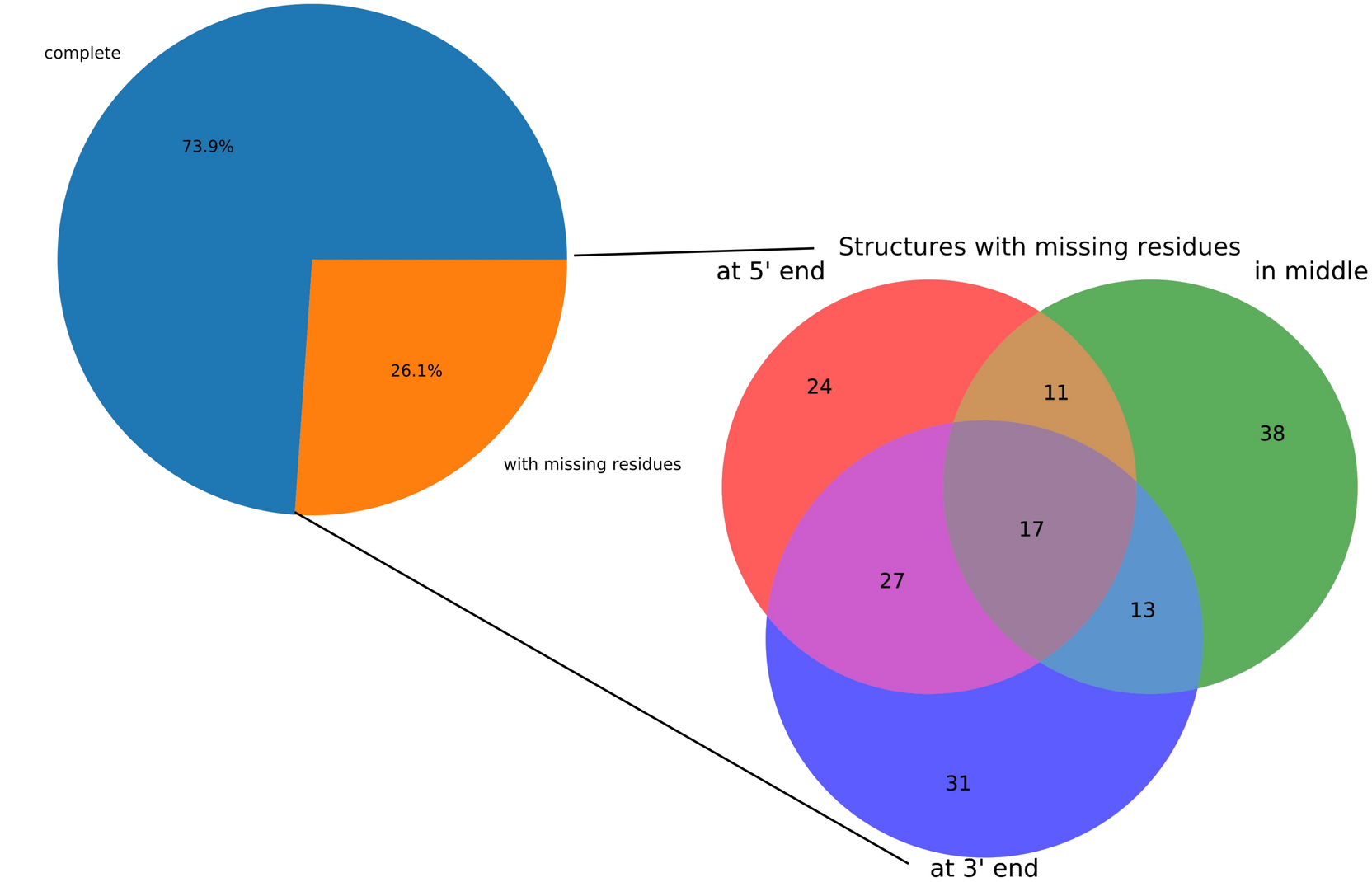
Missing residues

Large PDB structures in representative set

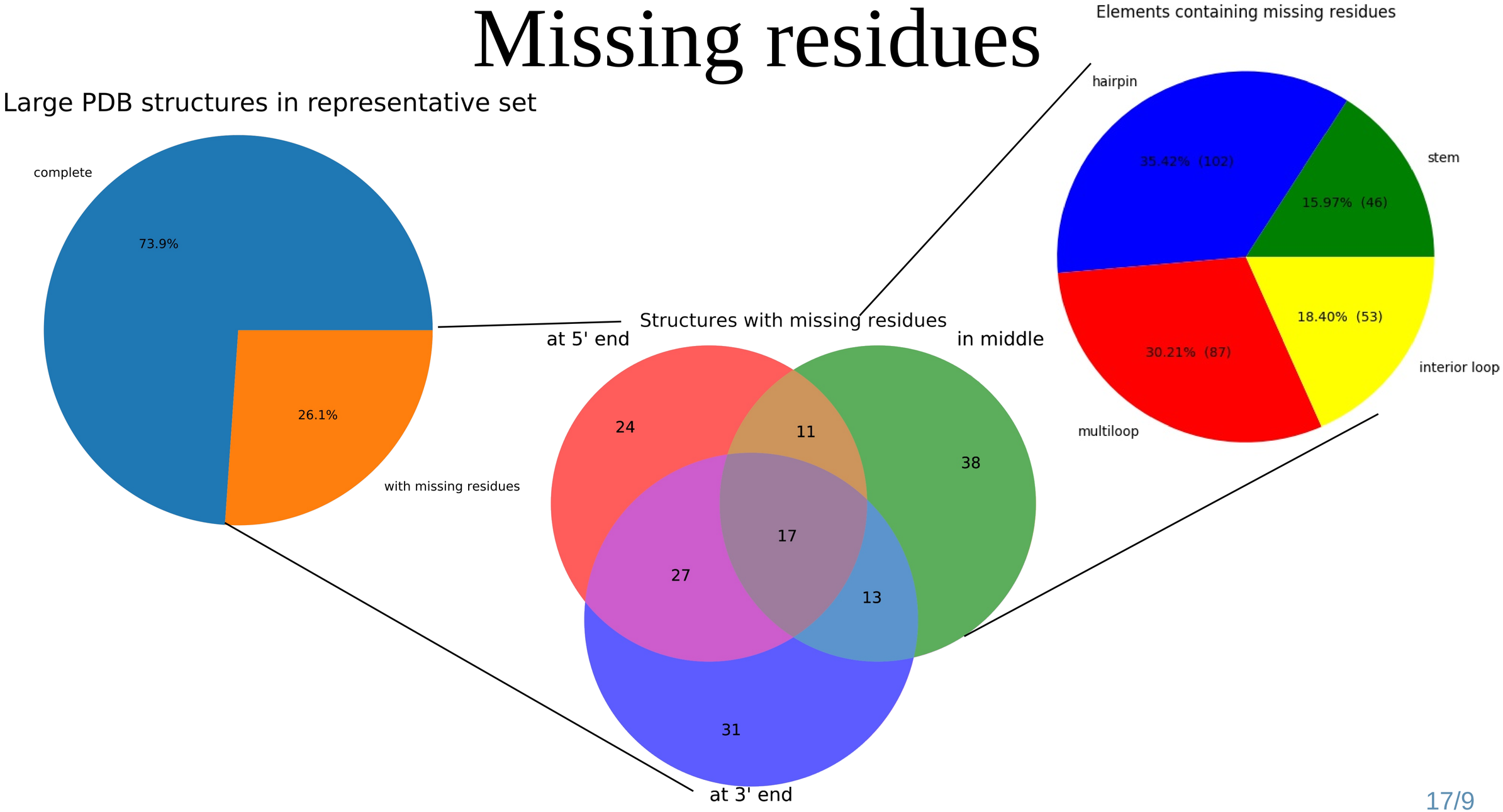


Missing residues

Large PDB structures in representative set



Missing residues



Co- and Multi-fold structures

Base-pairs between multiple chains

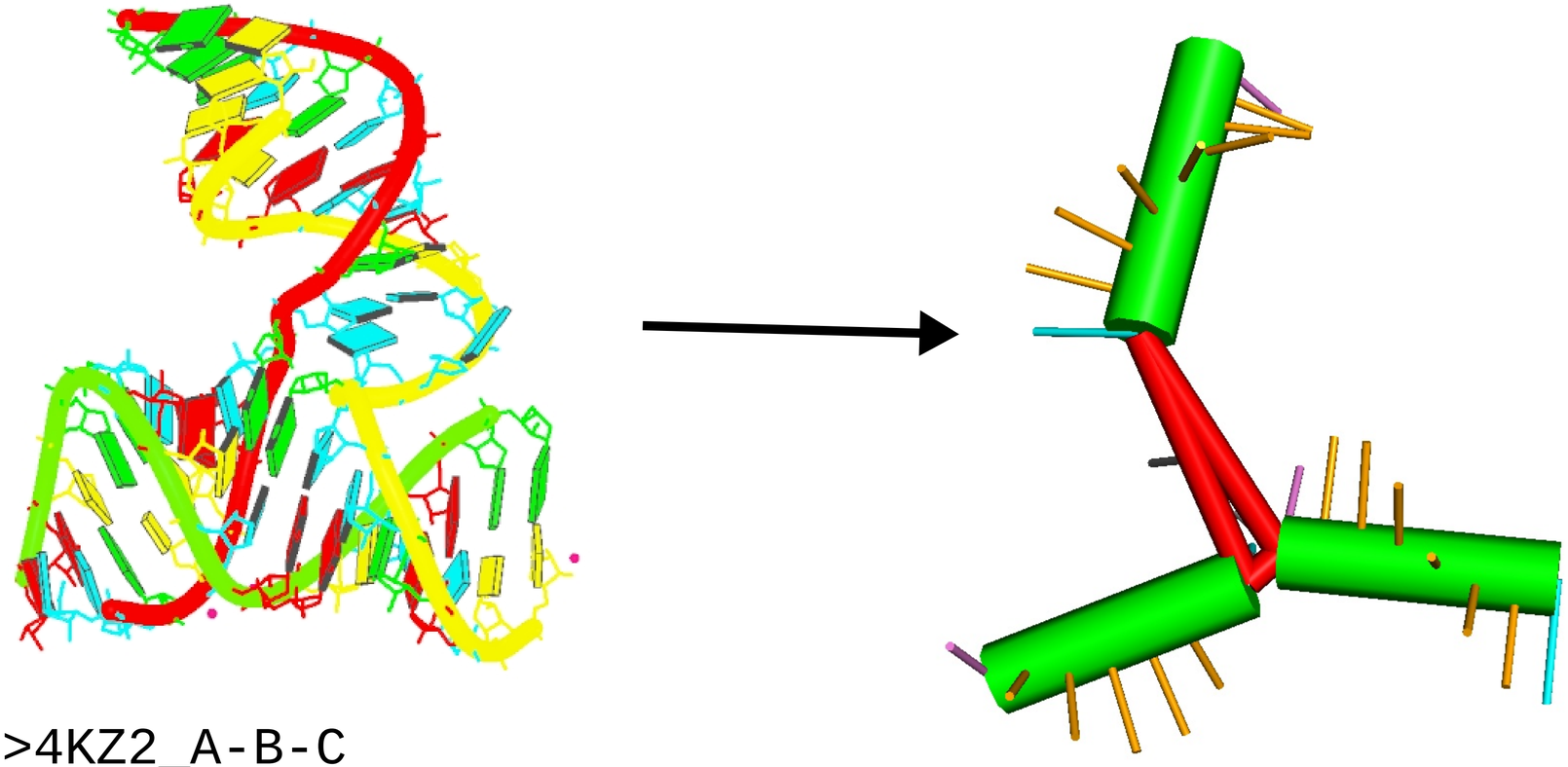
Co- and Multi-fold structures

FORGI 2.0

* Arbitrary number of cutpoints ('&') in sequence
* Indexing and slicing works
* Each Bulge-Graph object represents one connected component

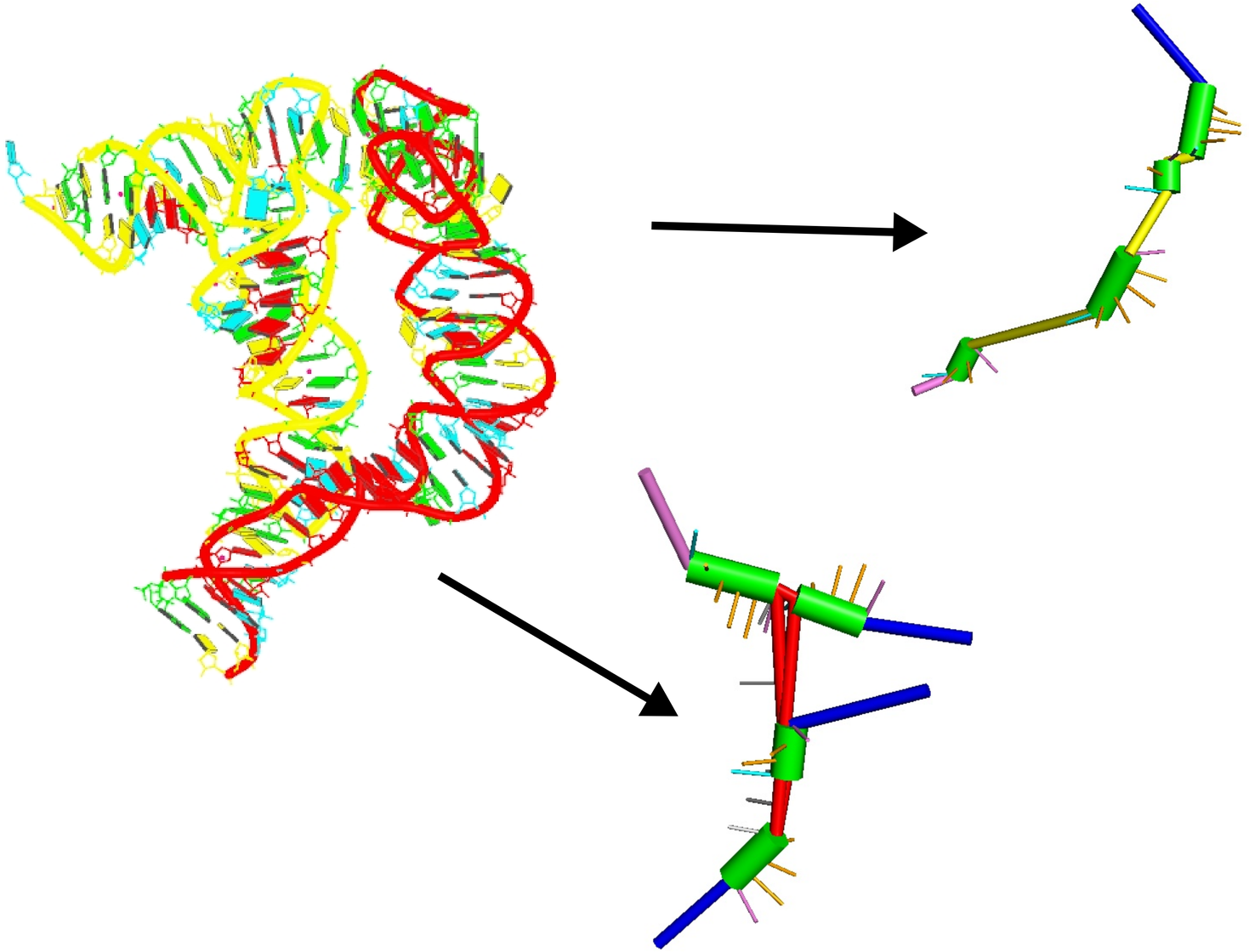
Co- and Multi-fold structures

Base-pairs between multiple chains



```
>4KZ2_A-B-C
UUGCCAUGUGUAUGUGGG&CCCACAUACUUUGUUGAUCC&GGAUCAAUCAUGGCAA
(((((((((.(((((((((&))))))))).((((((((&))))))))))))))))
```

Multiple connected components



FORGI 2.0

* 1 connected component per BulgeGraph object

Other improvements

*) All scripts use the same commandline arguments for RNA parsing.
*) Choose between MC-Annotate and DSSR
*) Full support of MMCIF file format (coming soon)

What can forgi do for YOU?

FORGI 2.0

* Convert between RNA file formats (bpseq, fasta, pdb, dotplot)
* Simplified 3D structure operations:
    RMSD, angle between stems, ...
* Select secondary structure elements: bulges, interior loops, ...

Thank you

Peter Kerpedjiev
Gregor Entzian
Irene Beckmann
Ivo Hofacker
TBI

FORGI 2.0

www.github.com/ViennaRNA/forgi