

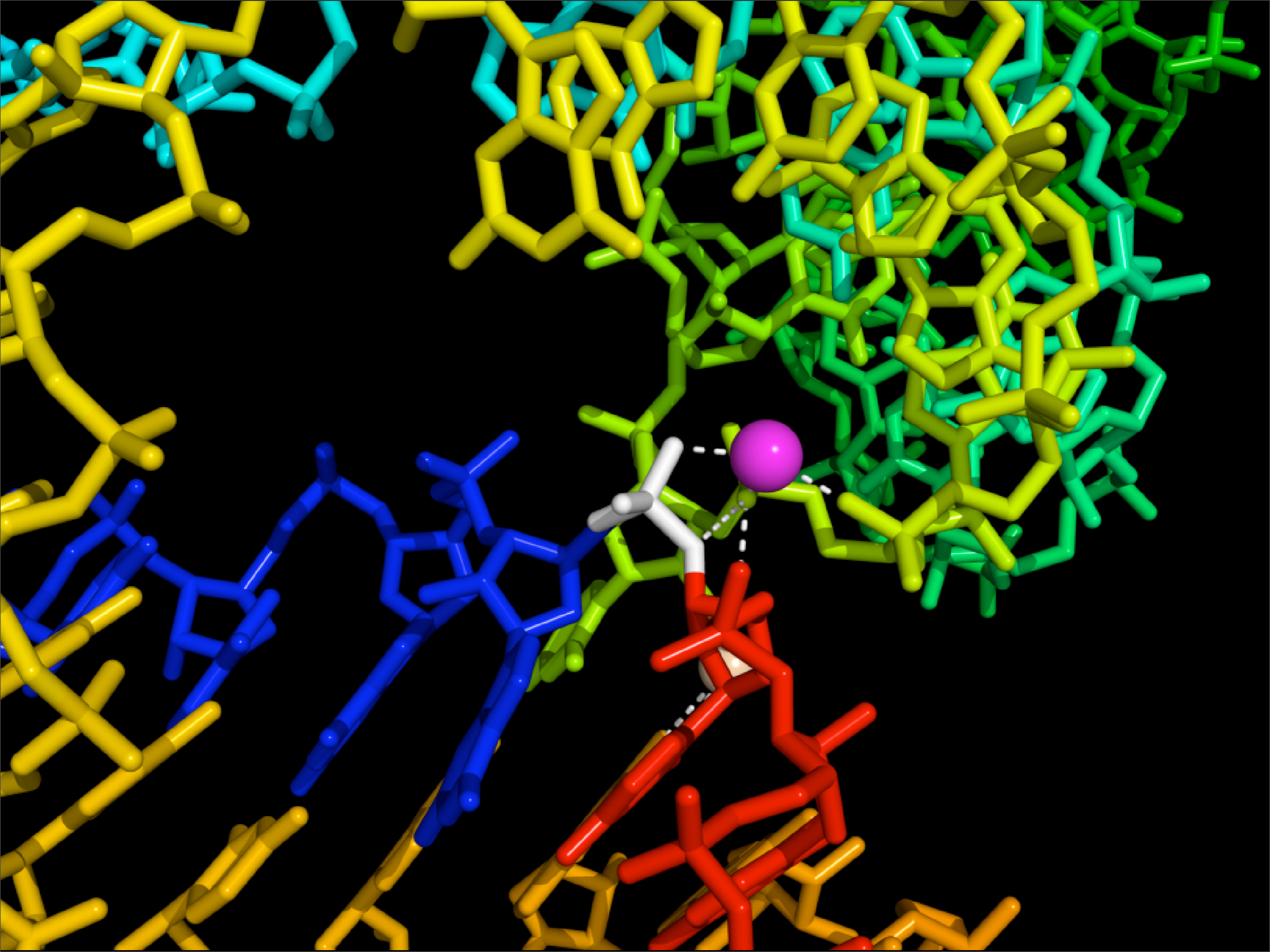
Scott Lab

Scott Lab

"We are trying to
understand RNA,
catalysis and the origin
of life."

*Hammerhead
Ribozyme*





The Structural Basis
of Ribozyme-Catalyzed
RNA Assembly



Dr. Michael P. Robertson



Dr. Michael P. Robertson



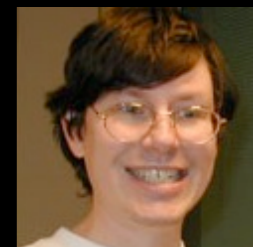


Dr. Michael P. Robertson





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The Central Dogma of Molecular Biology

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DNA → RNA → Proteins

The RNA World Hypothesis

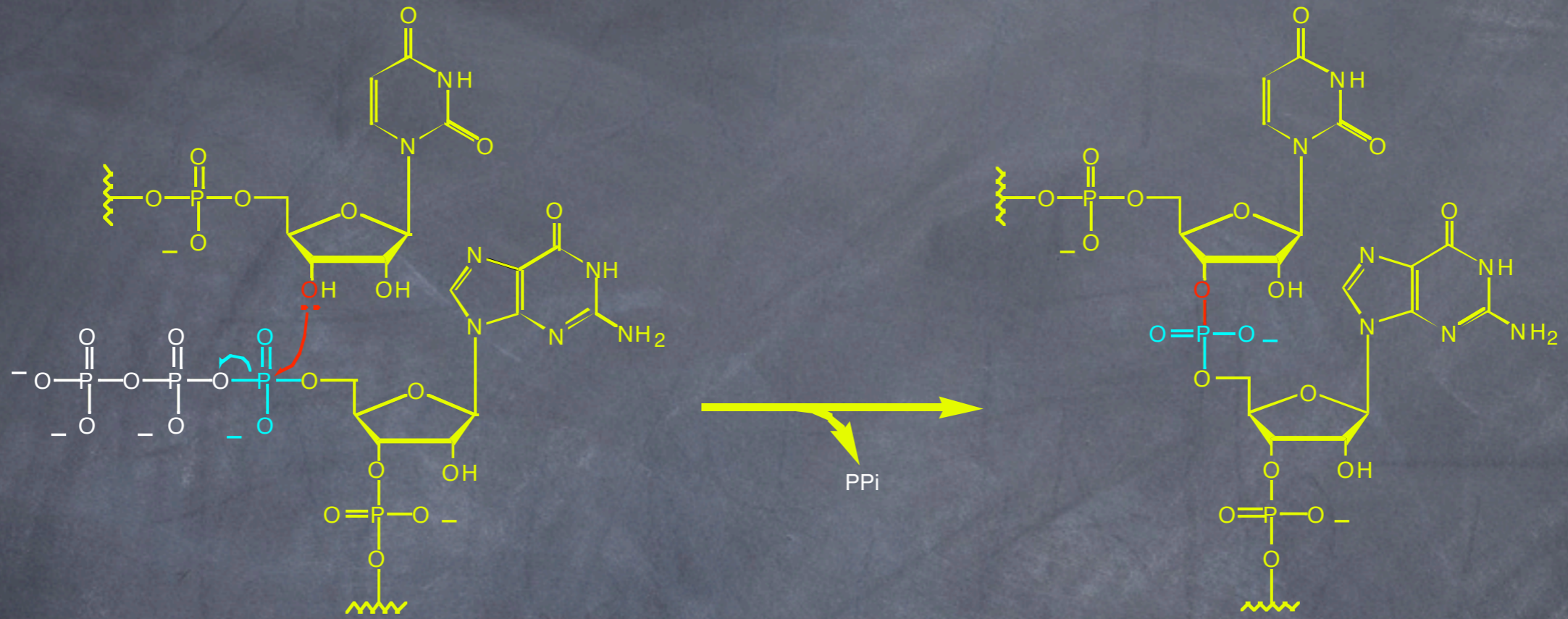
RNA

The RNA World Hypothesis

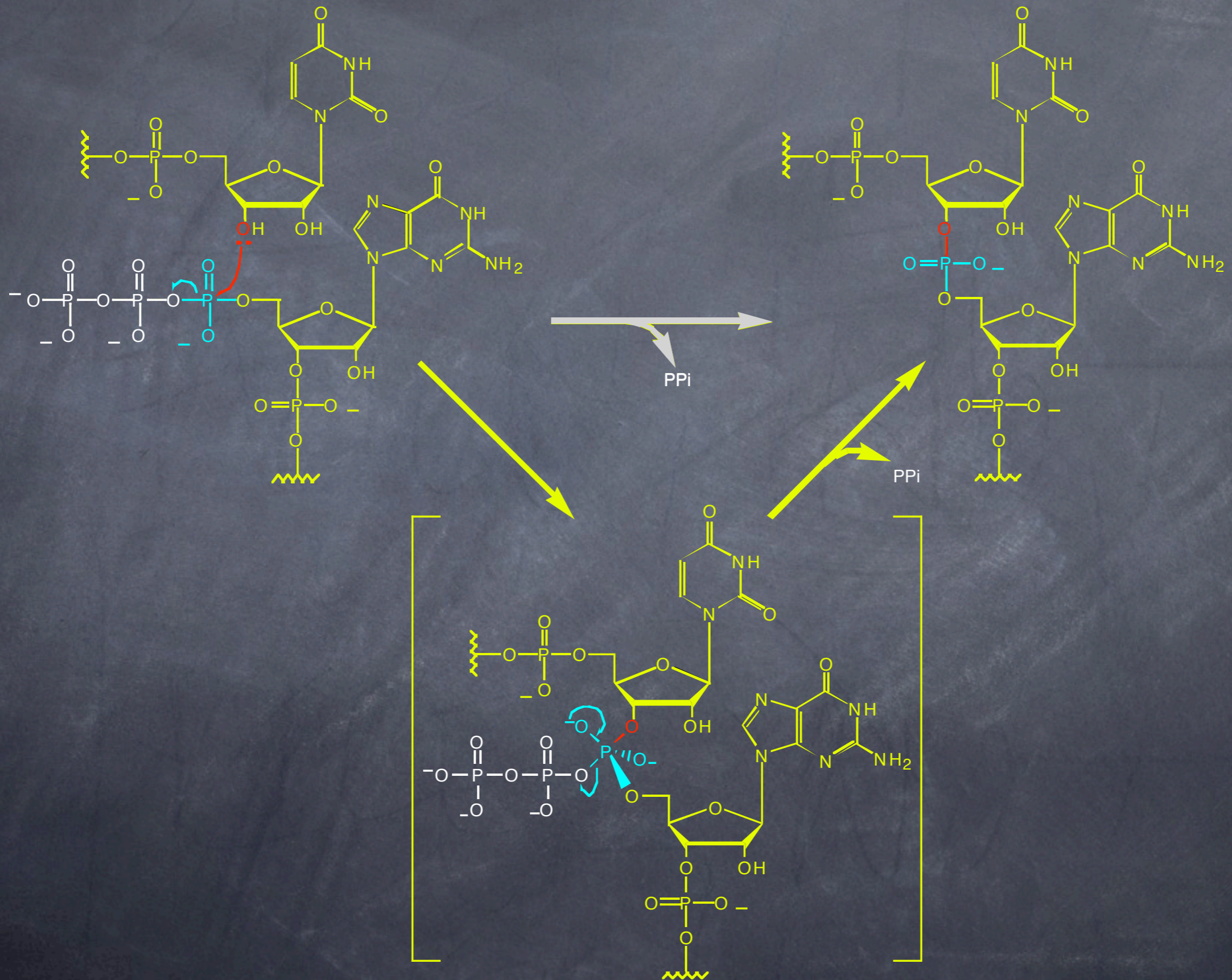
DNA → RNA → Proteins

RNA Ligation Mechanism

RNA Ligation Mechanism



RNA Ligation Mechanism

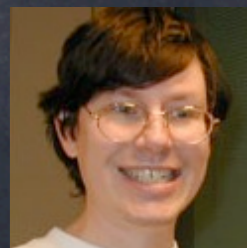


in vitro selection

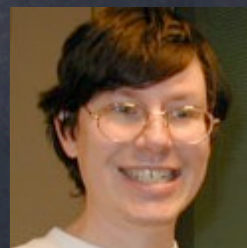
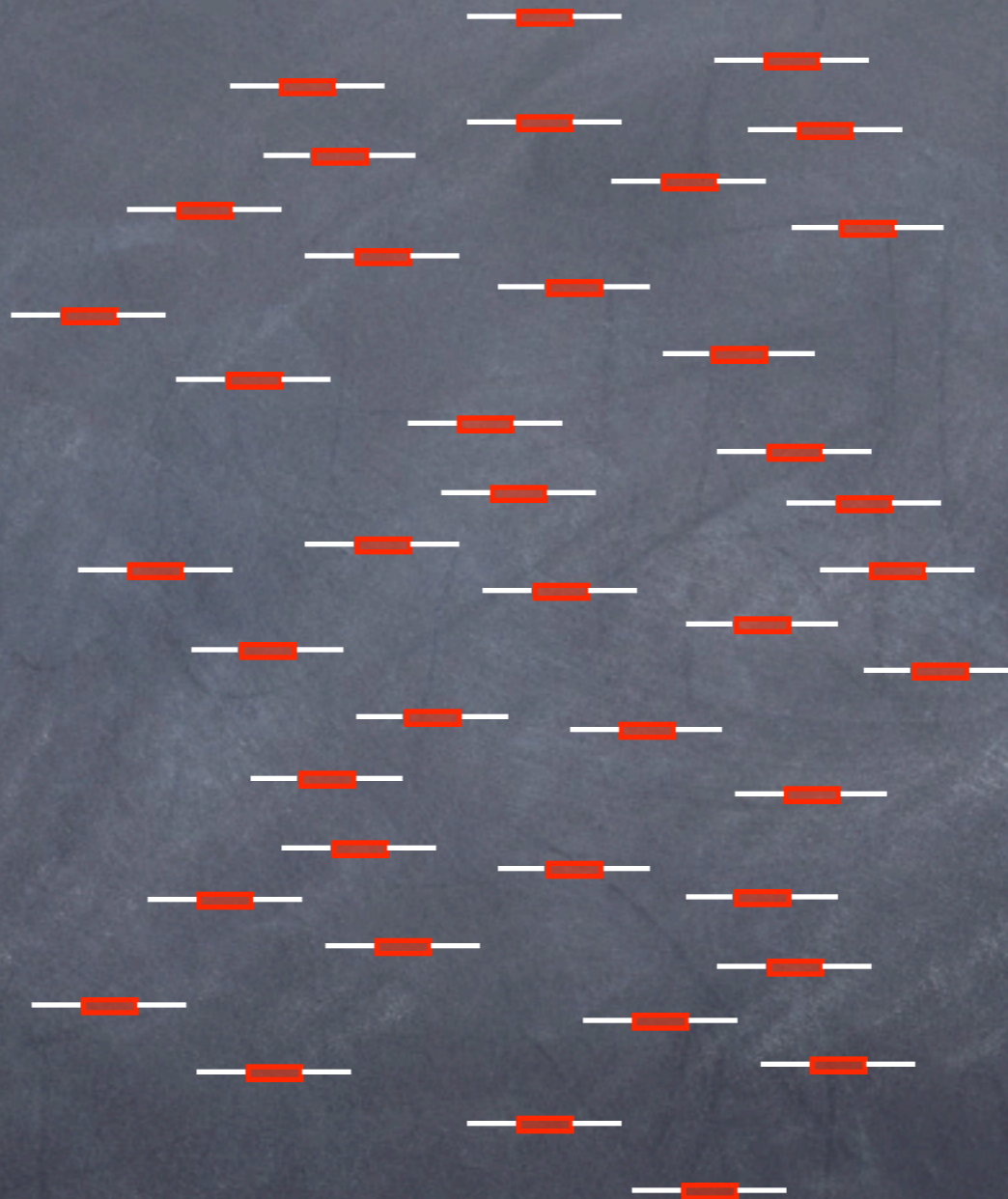
ggacuucgguccagugcucgug — N90 — cucgugauguccagucgc



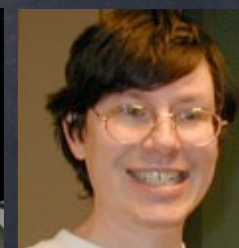
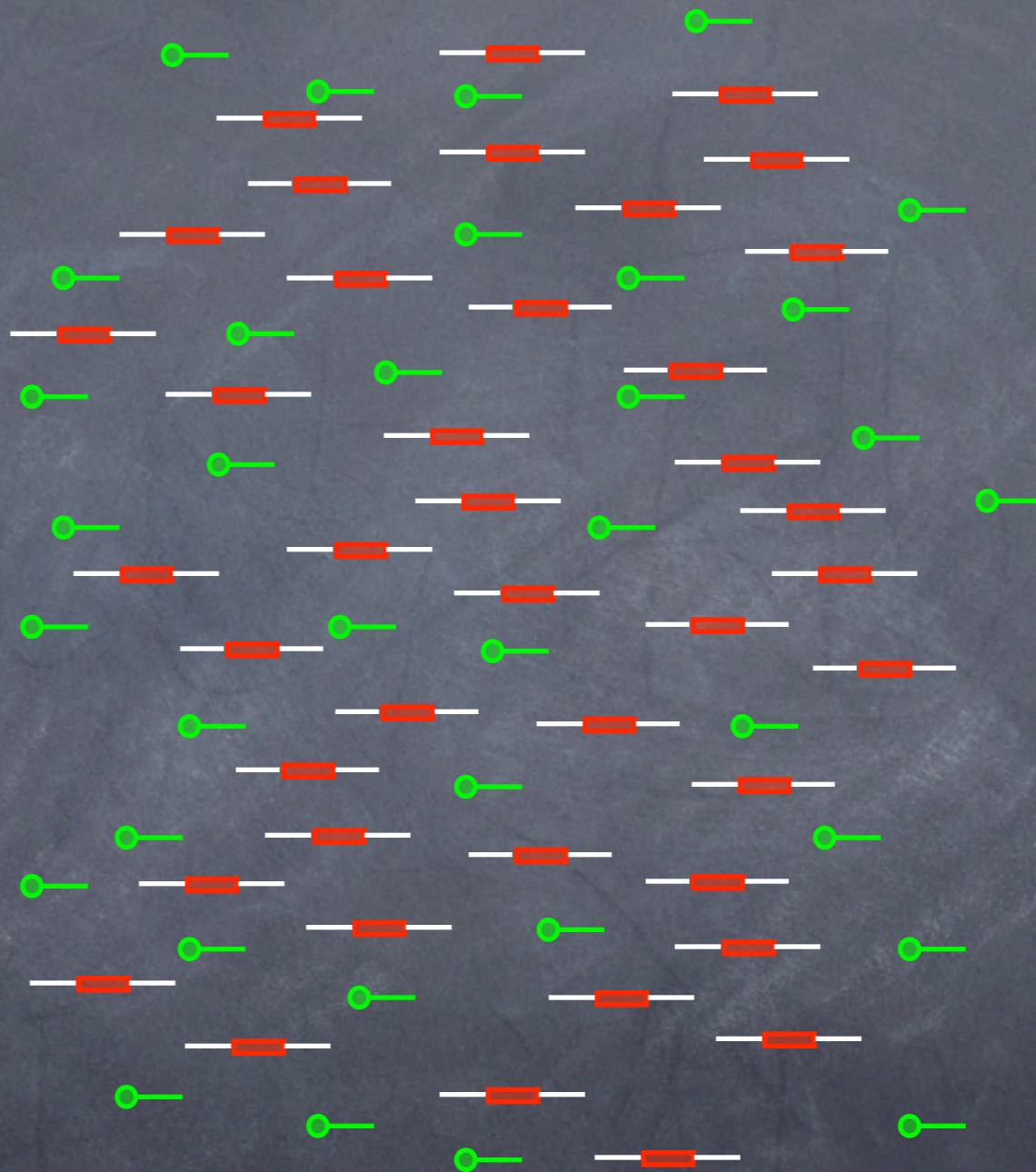
in vitro selection



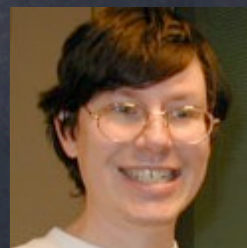
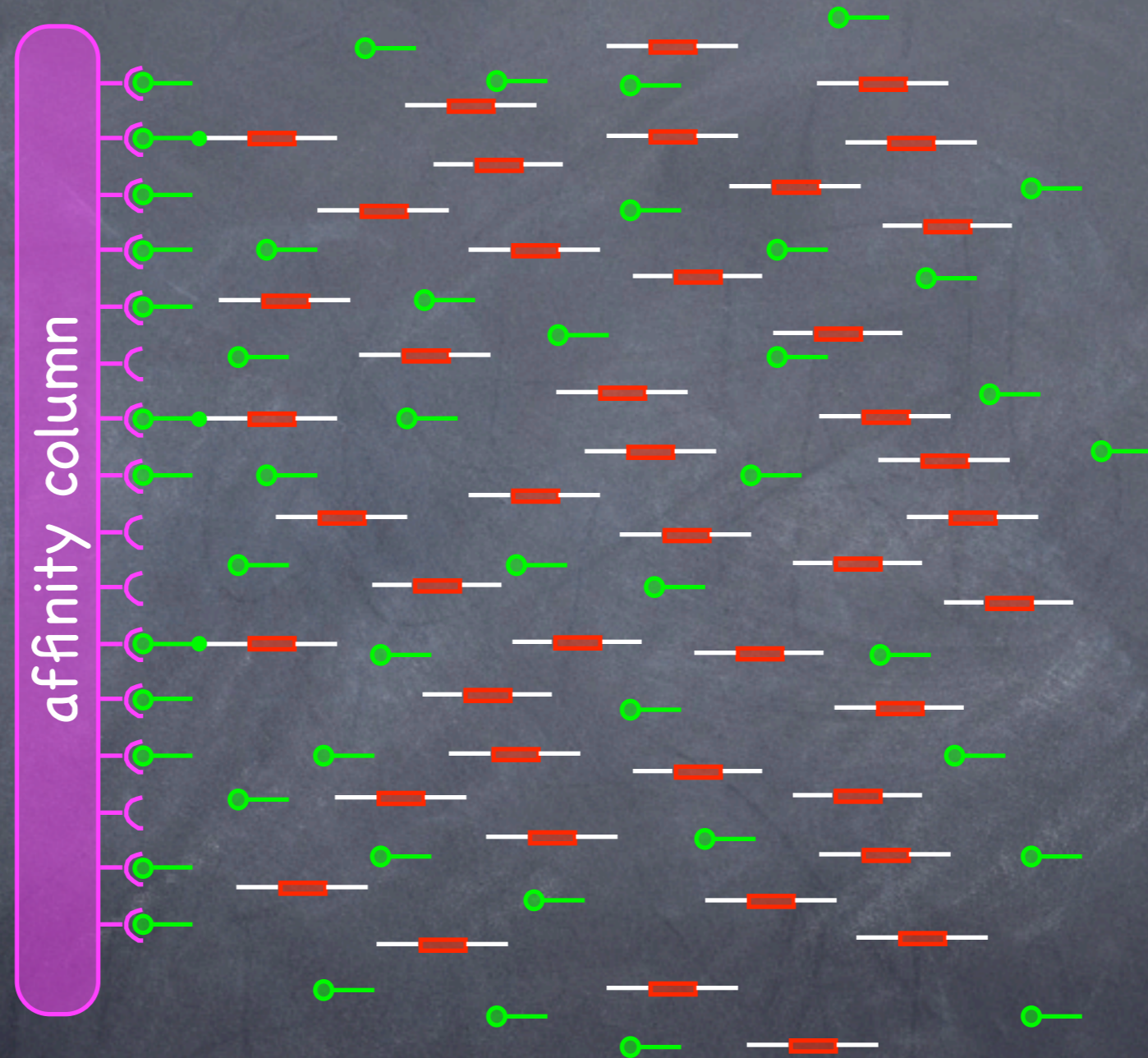
in vitro selection



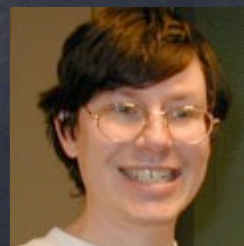
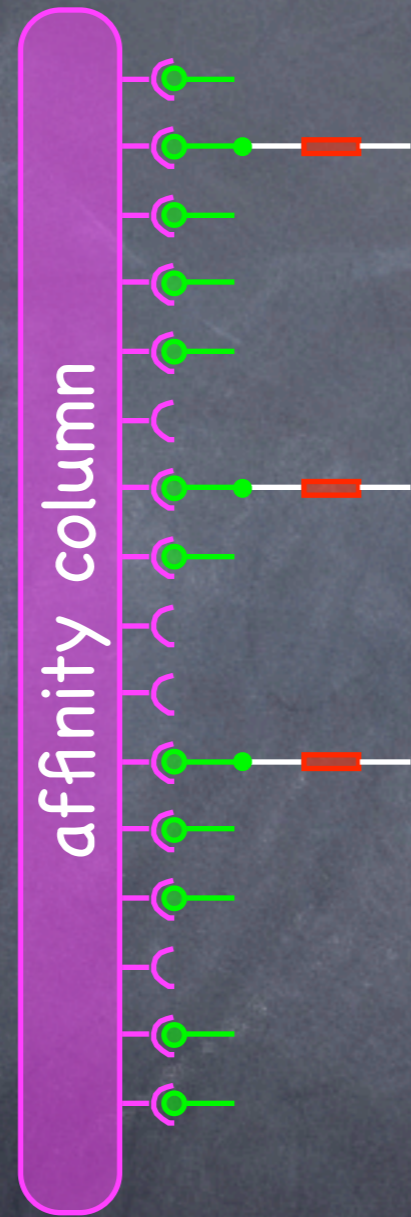
in vitro selection



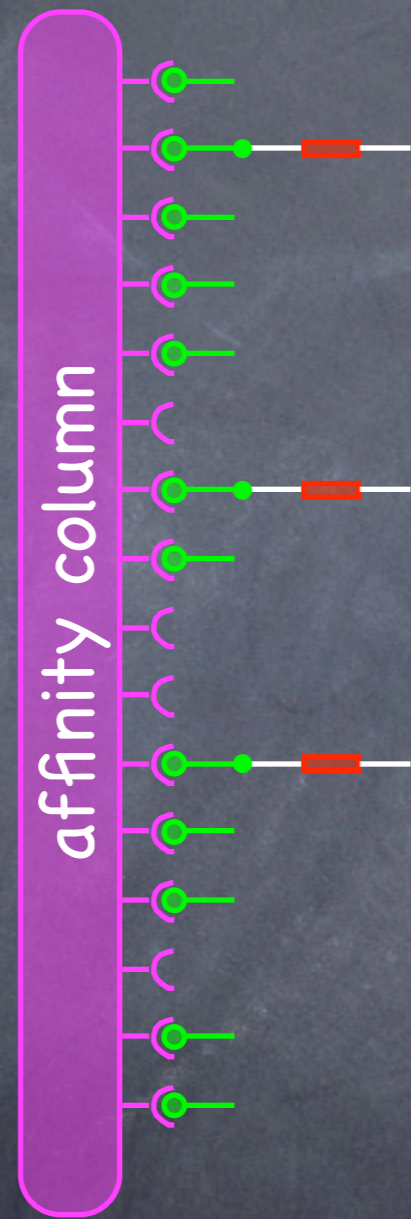
in vitro selection



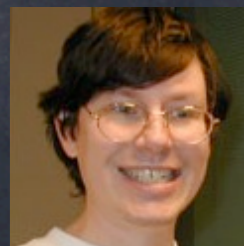
in vitro selection



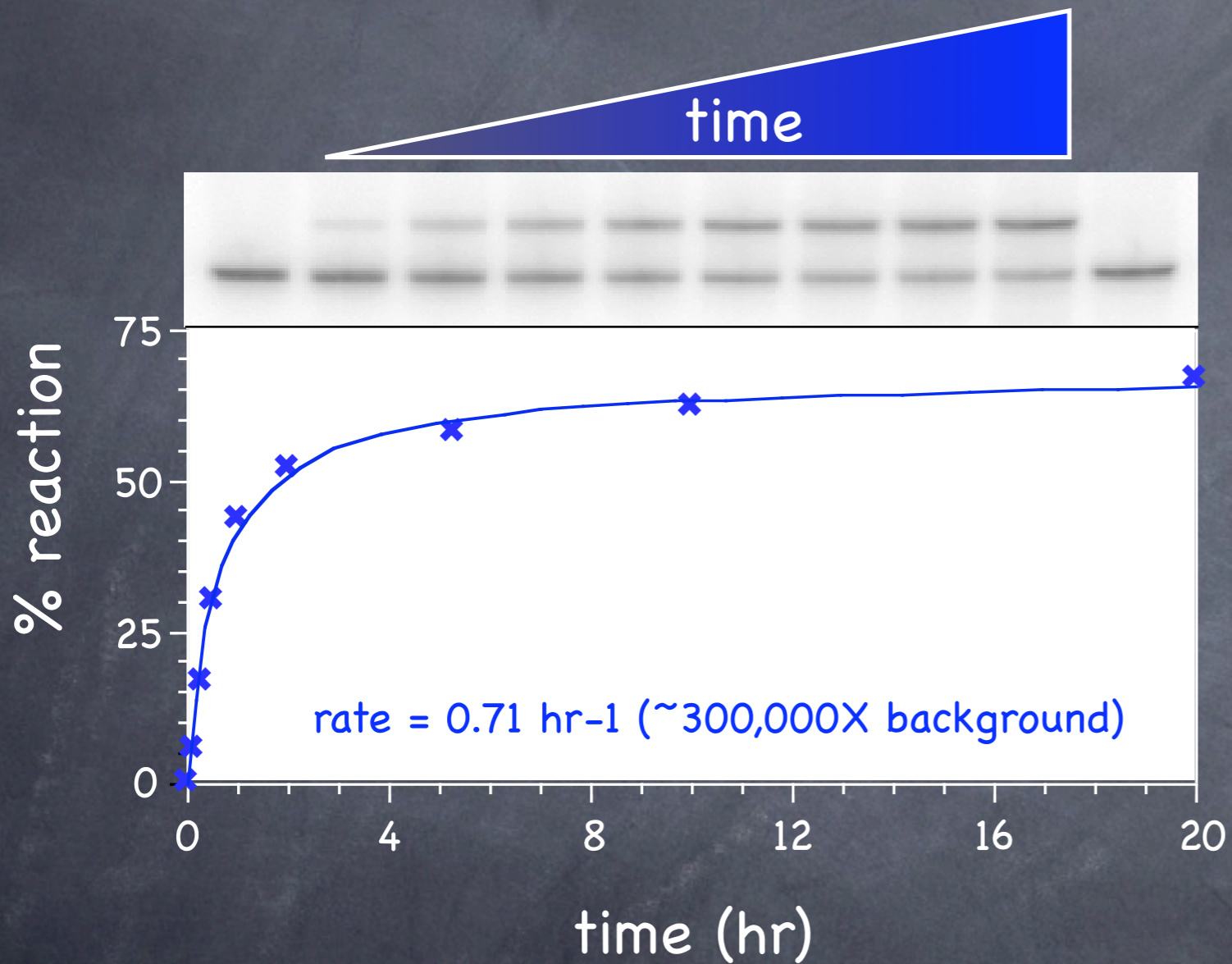
in vitro selection



- RT
- PCR
- Trxn



L1 ligase



- 3'-5' linkage
- molecular switch



L1 Ligase

2° structure

c g c u g a c c u g u a g u g c u c substrate

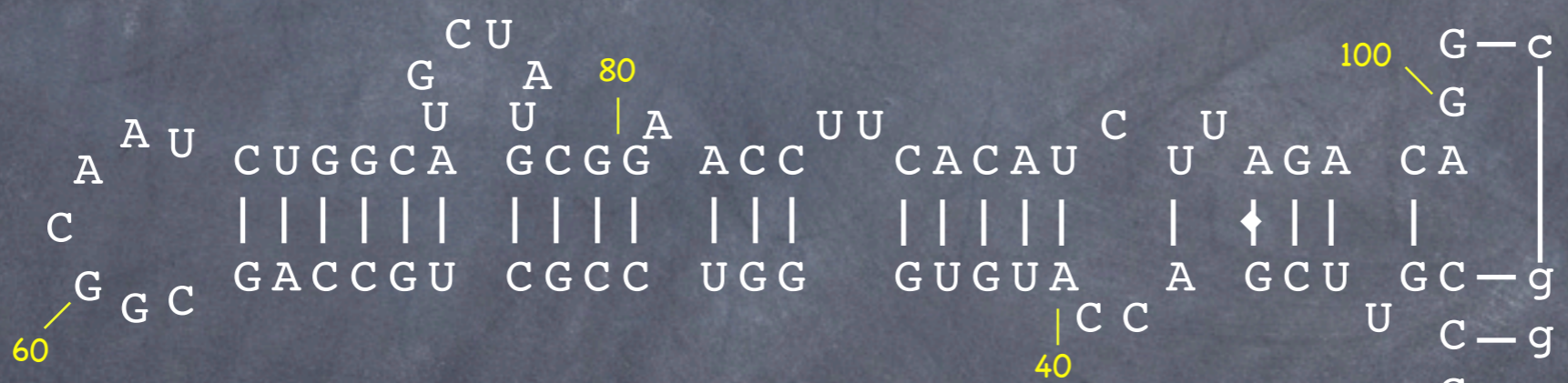
120

C—G
G—C
U—A
G—C
G—U

Stem A

A—g⁻¹
U—g
U—a
G—c
G—u
A—u

Stem C



G—c
G—c
A—c
U—a
C—g
A—u
C—g
g—c

Stem B

a g
a a

20



L1 Ligase

conserved positions

c g c u g a c c u g u a g u g c u c substrate

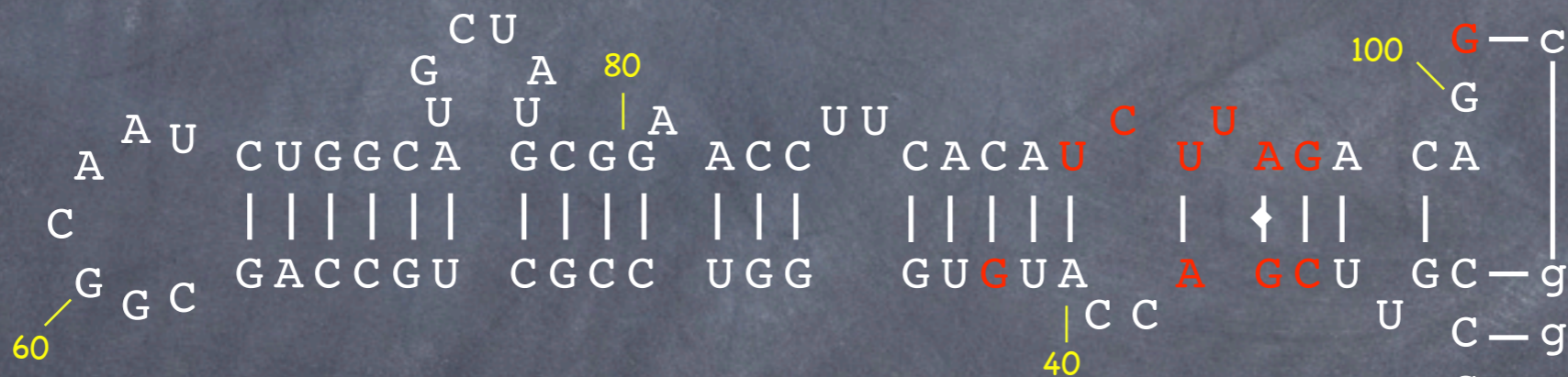
120

C—G
G—C
U—A
G—C
G—U

Stem A

A—g⁻¹
U—g
U—a
G—c
G—u
A—u

Stem C



100% conserved

Stem B

G—c
G—c
A—c
U—a
C—g
A—u
C—g
g—c
a—g
a—g
20



L1 Ligase

conserved positions

c g c u g a c c u g u a g u g c u c substrate

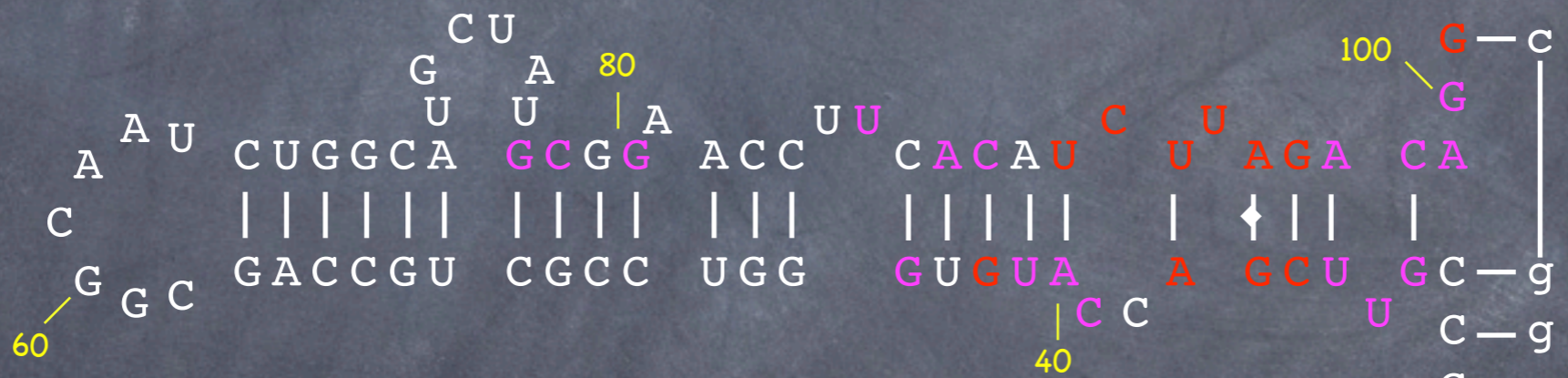
120

C—G
G—C
U—A
G—C
G—U

Stem A

A—g⁻¹
U—g
U—a
G—c
G—u
A—u

Stem C



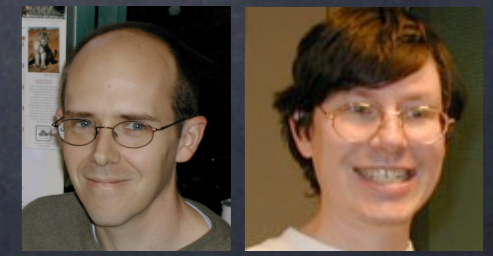
G—c
G—u
A—u
G—c
C—g
G—u
G—c
A—c
U—a
C—g
A—u
C—g
g—c

Stem B

a g
a a

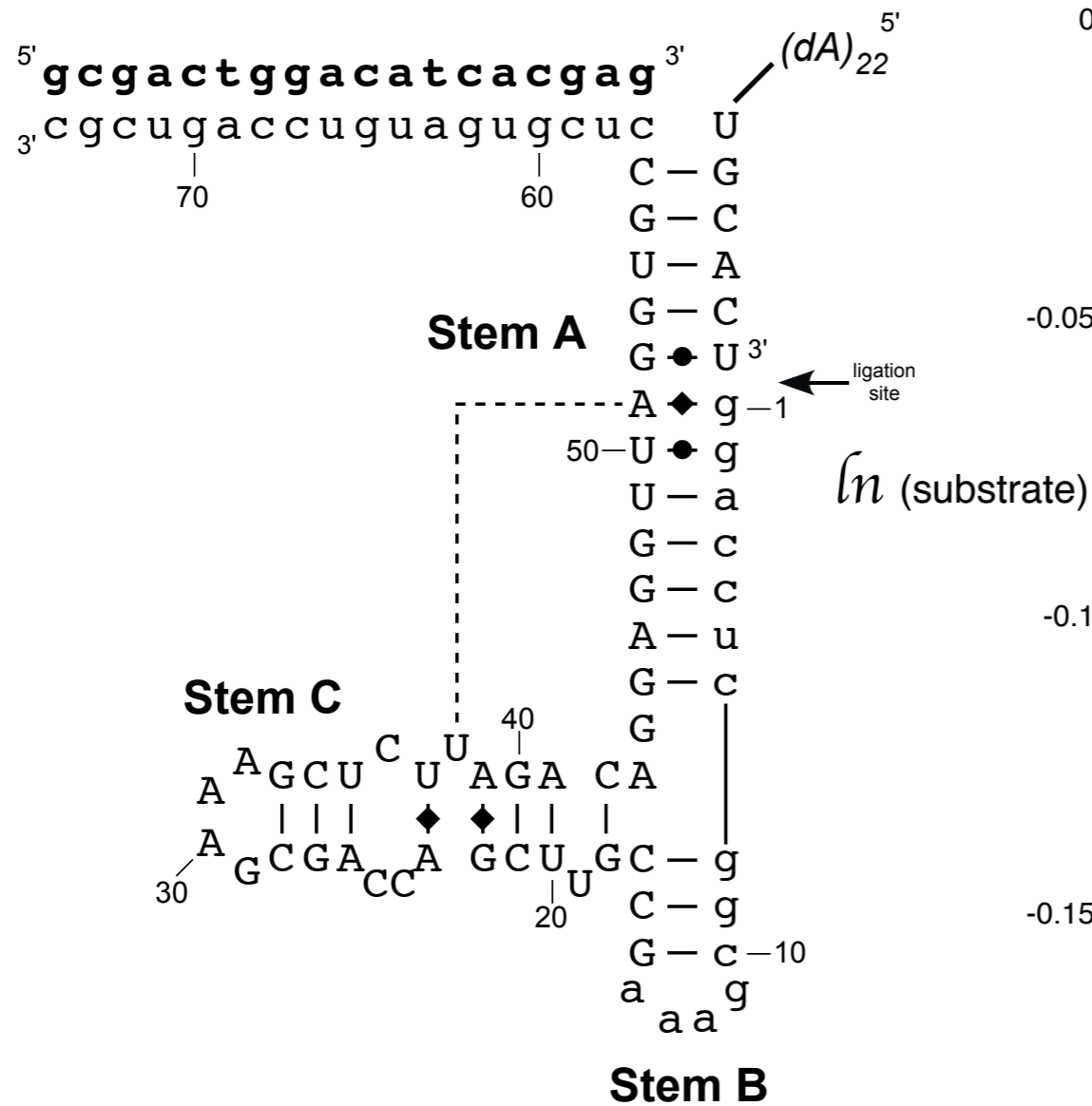
100% conserved
≥85% conserved

20



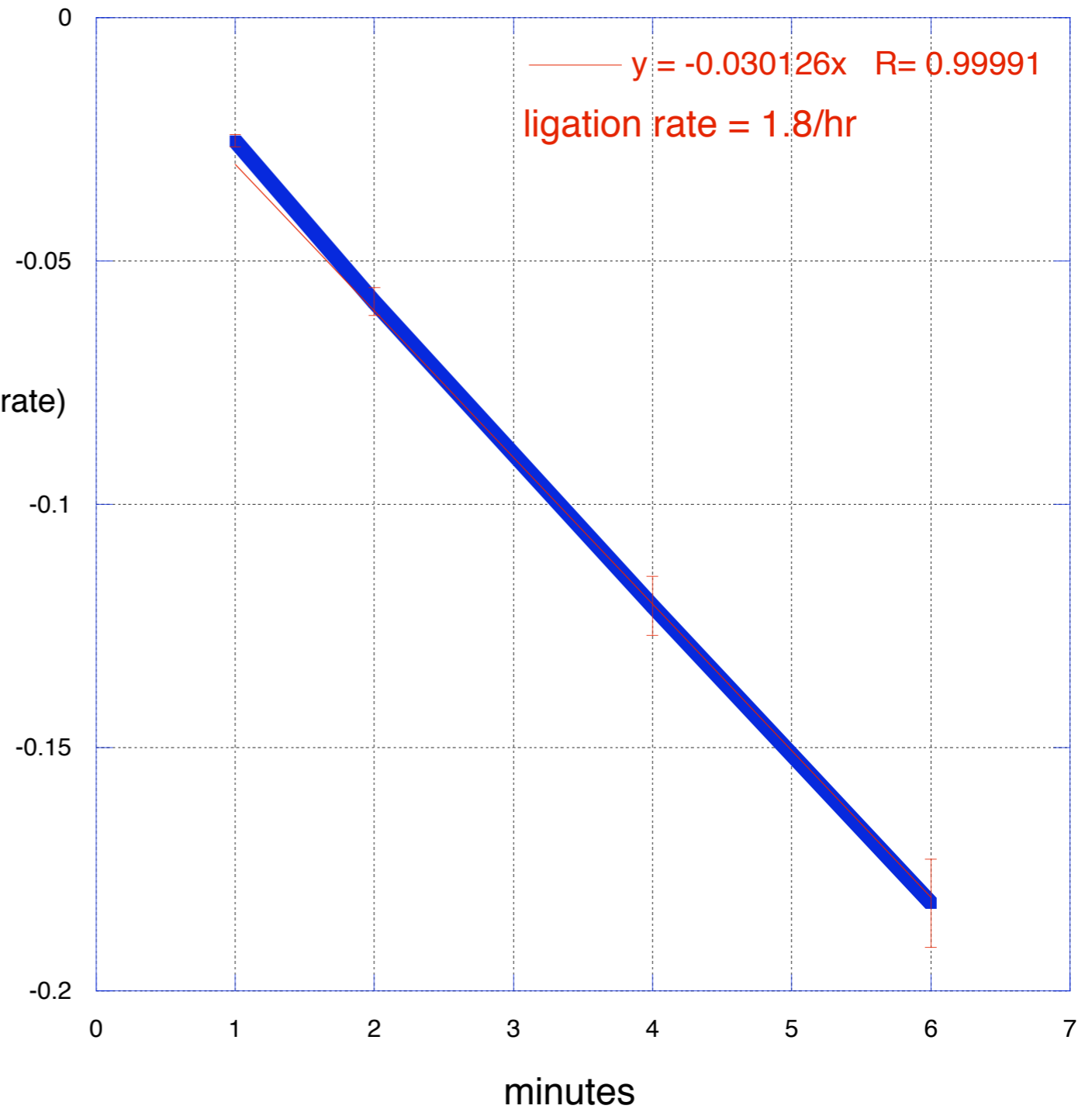
A smaller ligase ribozyme

A



B

ribozyme ligation rate



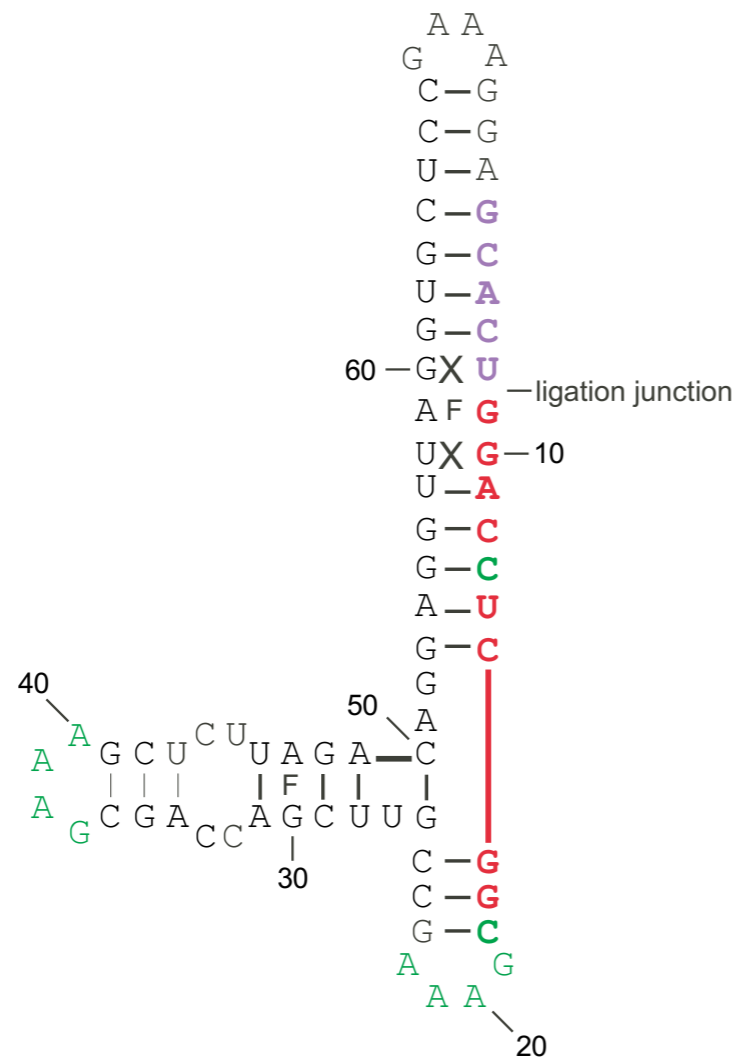
A unimolecular variant

L1X6c

115 bp DNA

71 nt transcript

71 nt circular L1 RNA construct



L1 stem B deletion (dB2); stem C deletion (dC2);
stem A U to C mutation

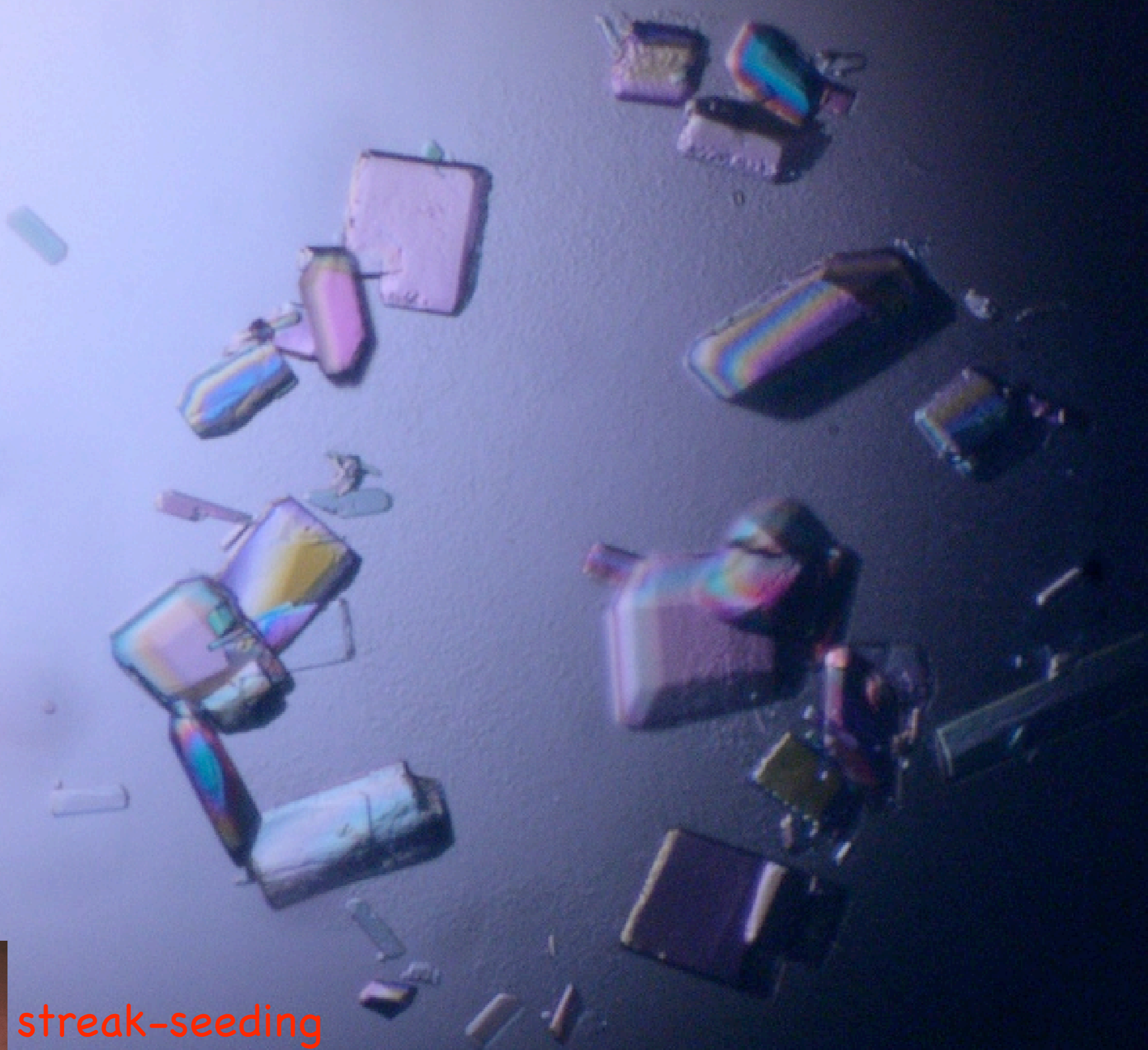
Amplify L1X2 template with
44.L1X2.5'/43.L1X6c.3' primer set

```
CGGAATTCTTCTAATACGACTCACTATAGGGACC  
TCGGCGAAAGCCGTTCGACCAGCGAAAGCTCTT  
AGACAGGAGGTTAGGTGCTCCGAAAGGAGCACT  
GGAGACTGGATCCCG
```

Ligation reaction adduct is a
circular ribozyme

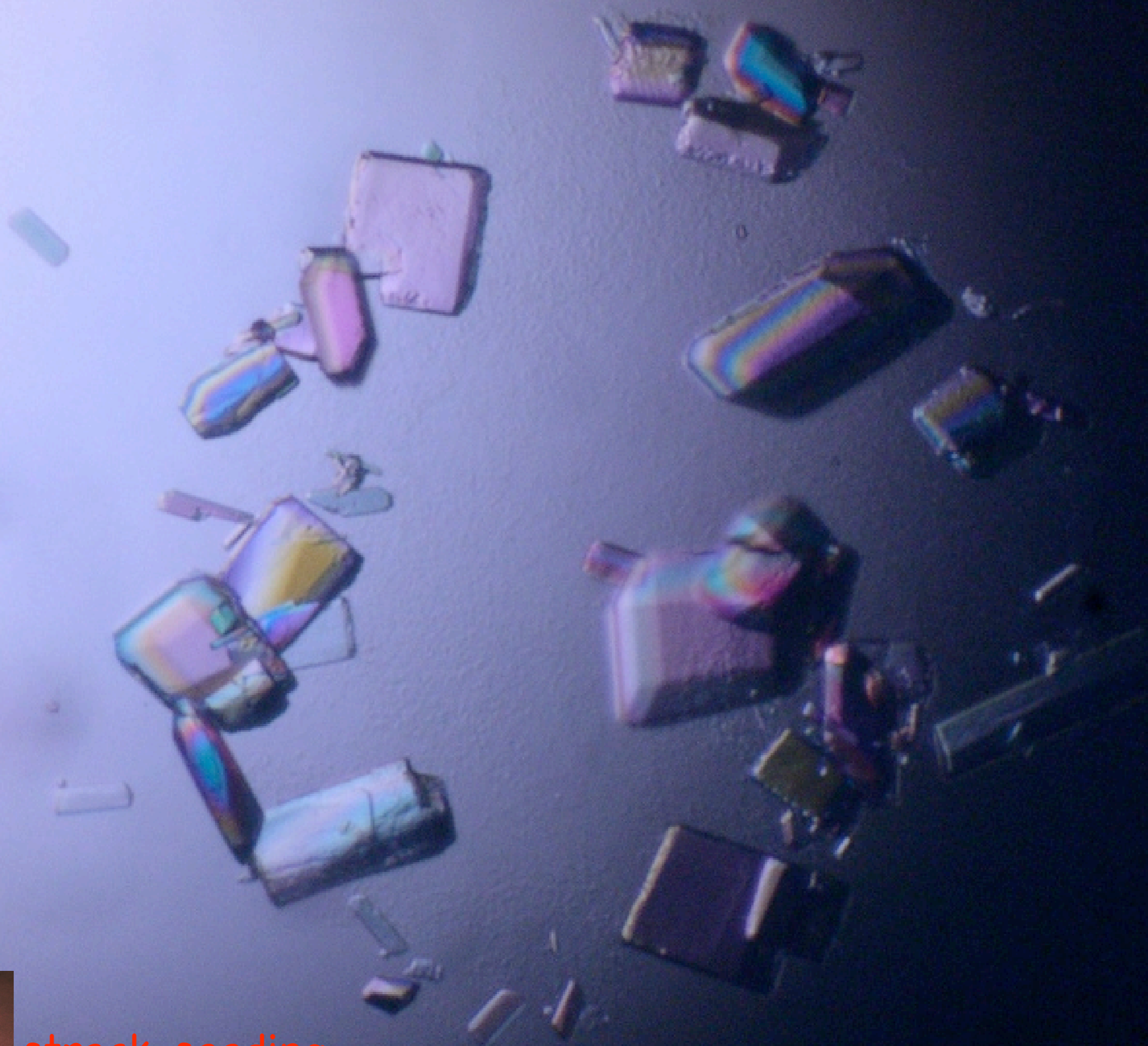


streak-seeding





streak-seeding



Data Collection

	Overall	Outer Shell
Low resolution limit	none	2.74
High resolution limit	2.60	2.60
R_{merge}	0.044	0.467
R_{meas} (all I+ & I-)	0.056	0.615
Total number of observations (all I+ & I-)	77375	11410
Total number unique (all I+ & I-)	19071	2767
$\langle I / \sigma(I) \rangle$	20.2	2.5
Completeness	99.5	99.9
Multiplicity	4.1	4.1

Refinement

Total Number of Bins Used	20	1
Resolution Range High	2.6 Å	2.6 Å
Resolution Range Low	31.34 Å	2.667 Å
Data Cutoff (Fs)	0	0
Completeness for Range	99.42 %	99.86 %
Number of Reflections	17100	1261
Goodness of Fit of Model to Data		
Cross-Validation Method	Throughout	Throughout
Free R Value (Test Set Selection)	Random	Random
R Value (Working Set)	0.20222	0.412
Free R Value	0.23876	0.42
Free R Value Test Set Size	10.2 %	12.9 %
Free R Value Test Set Count	1949	163
Number of Non-Hydrogen Atoms	3089	
Mean B Value	78.591 Å ²	
Estimated Overall Coordinate Error		
ESU Based on R Value	0.441 Å	
ESU Based on Free R Value	0.274 Å	
ESU Based on (max. likelihood)	0.252 Å	
ESU for b Values (max. likelihood)	26.738 Å ²	
RMS Deviations from Ideal Values		
Bond Lengths Refined Atoms	0.01 Å	
Bond Angles Refined Atoms	1.955°	
General Planes Refined Atoms	0.006 Å	
Non-Bonded Contacts Refined Atoms	0.204 Å	
Non-Bonded Torsion Refined Atoms	0.284 Å	
Isotropic Thermal Factor Restraints		
Side-Chain Bond Refined Atoms	1.101 Å ²	
Side-Chain Angle Refined Atoms	1.857 Å ²	

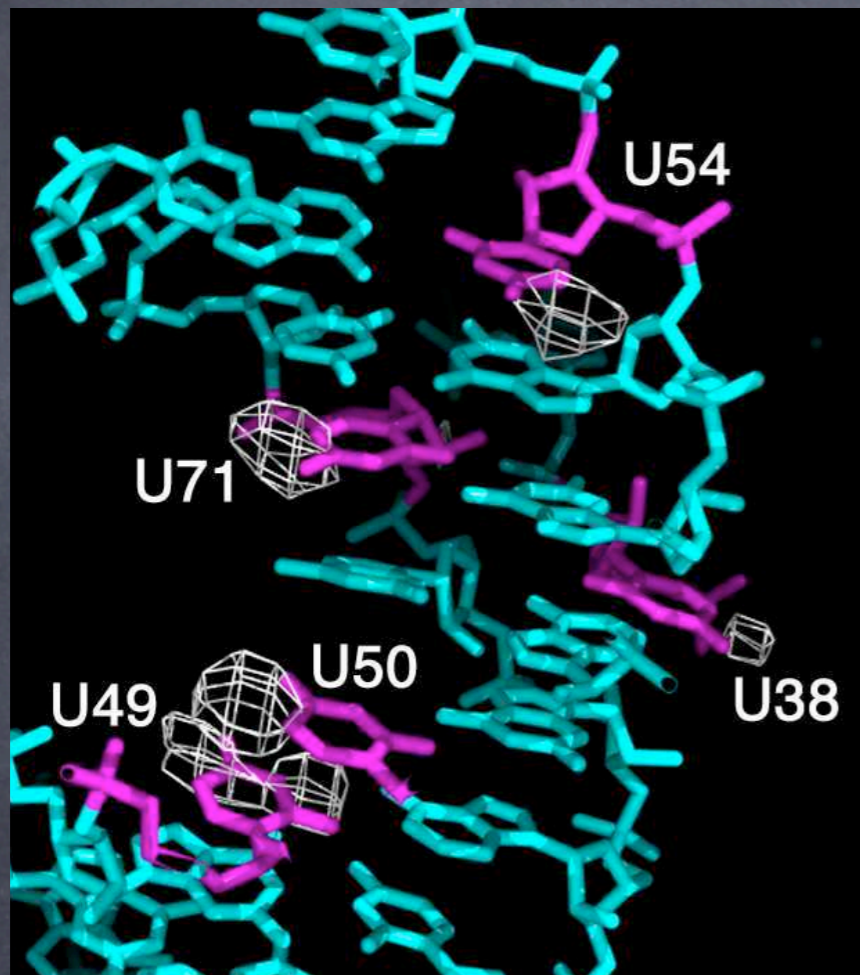
2.6 Å structure

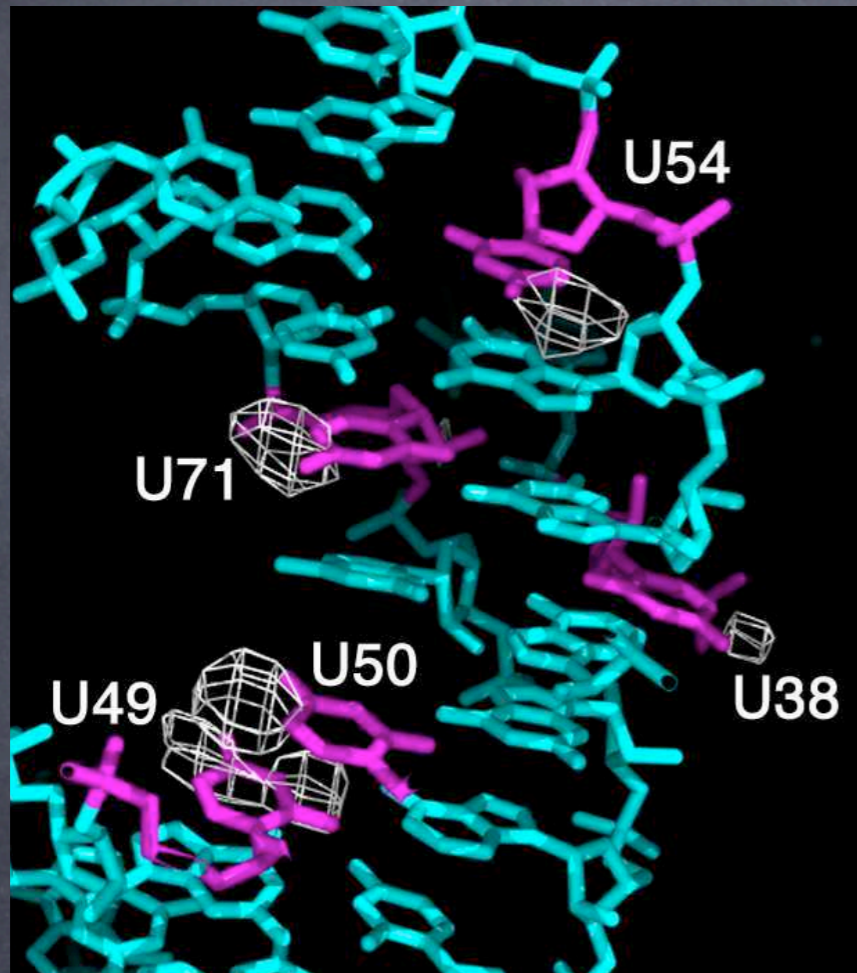
$R_{\text{free}} = 23.9\%$

$rmsd$ bonds = 0.01 Å

142 nucleotides
in asymm. unit

phase problem solved
w/o derivatives

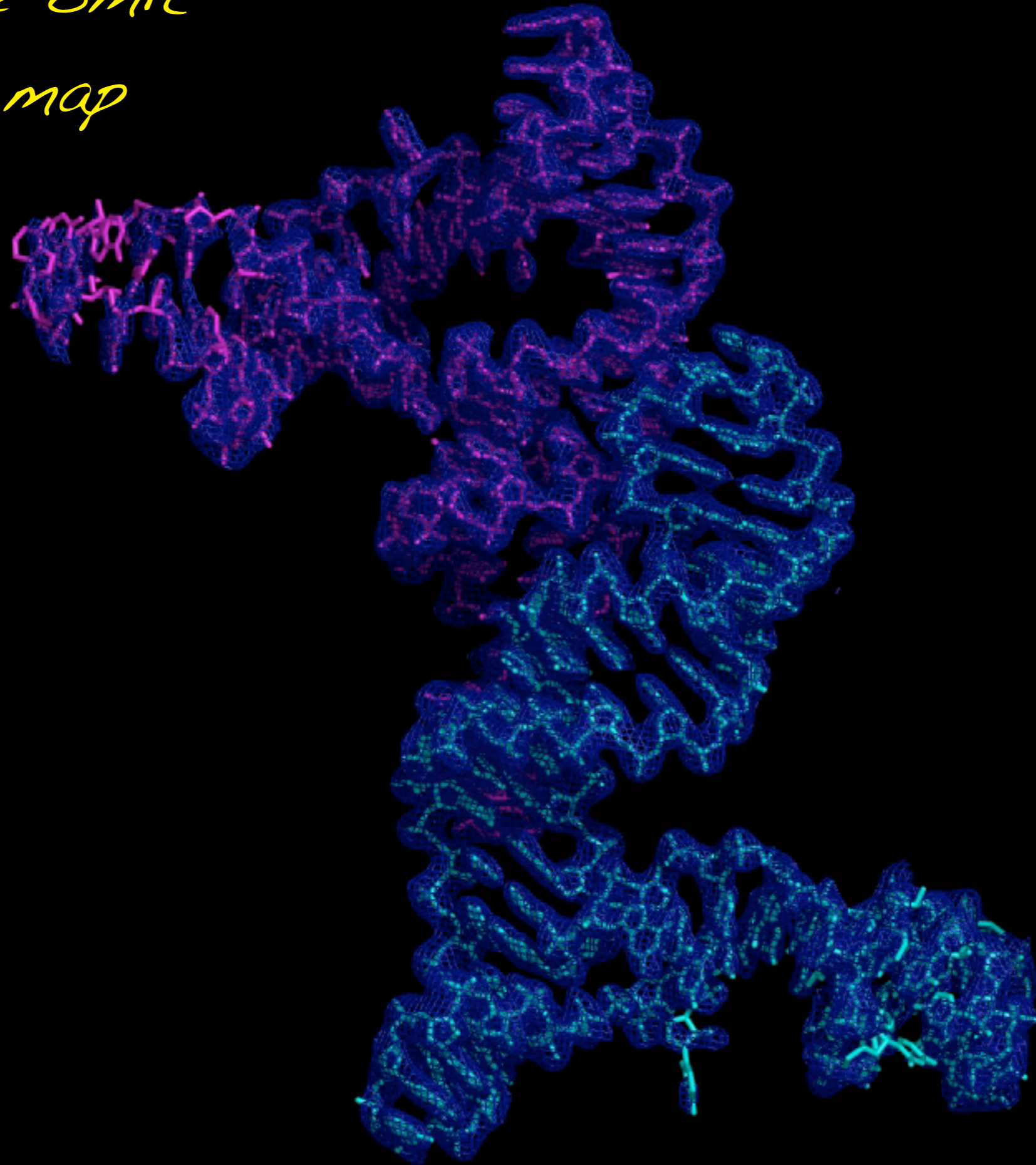


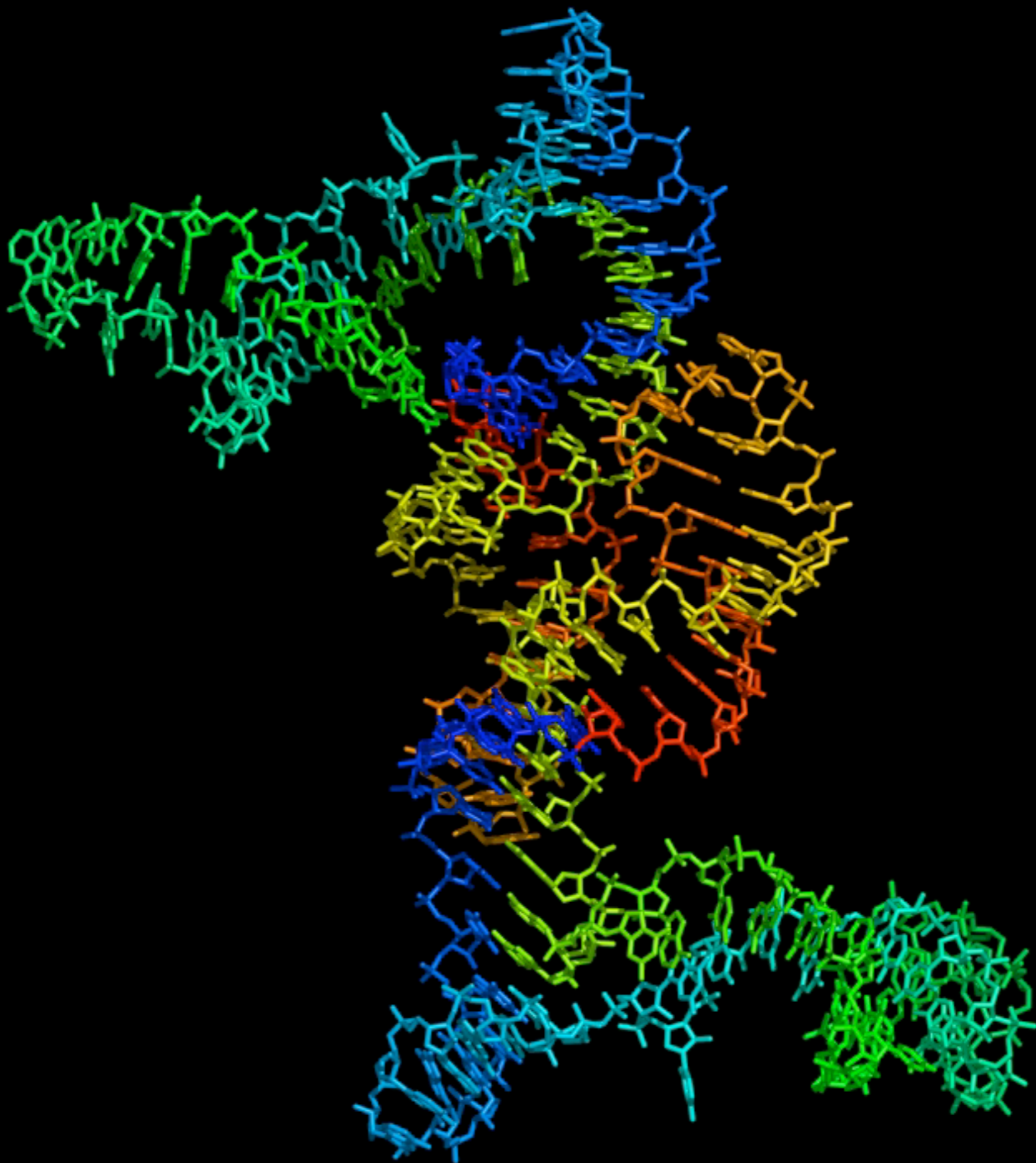


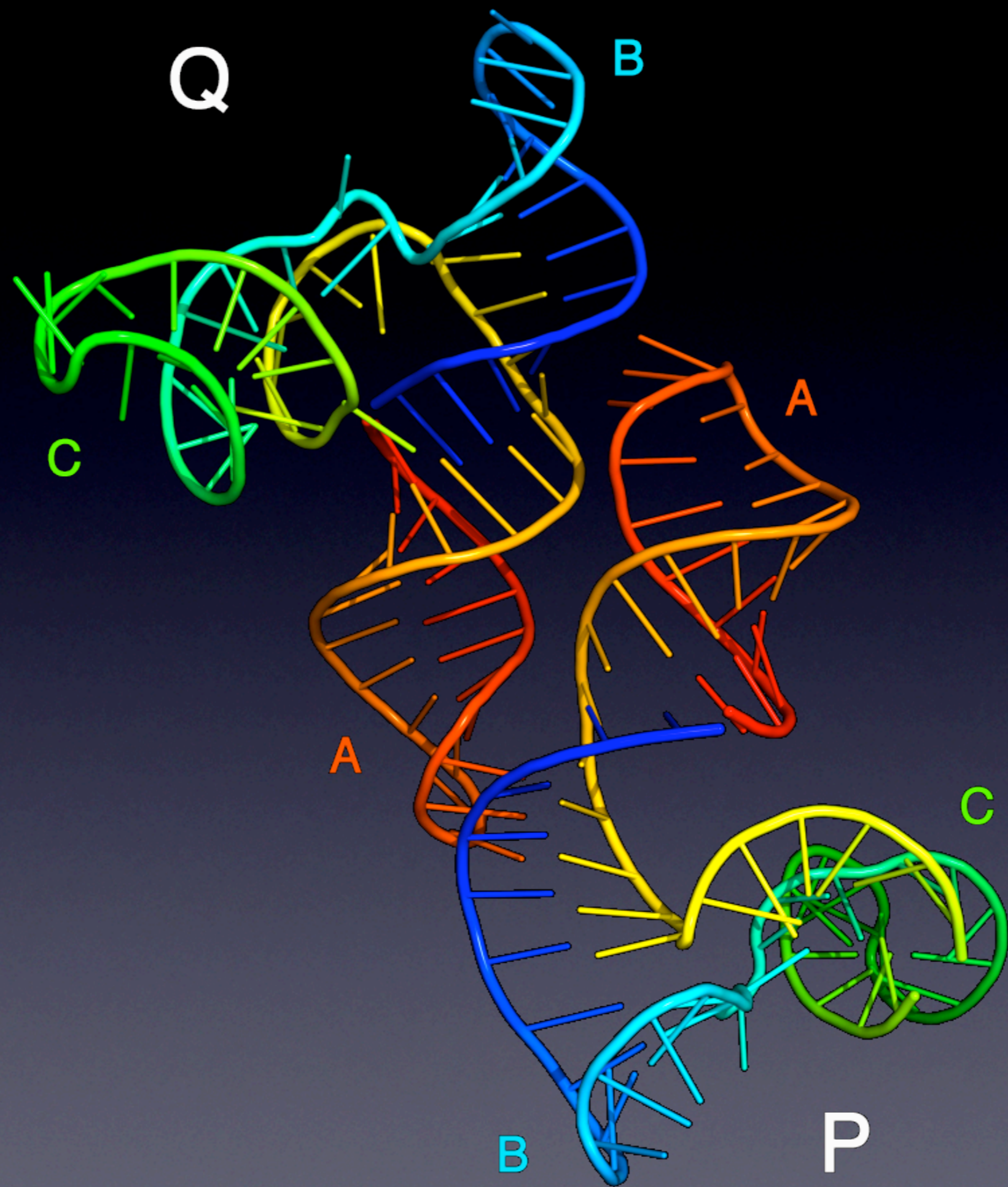
*5-Br-U
transcription
product:*

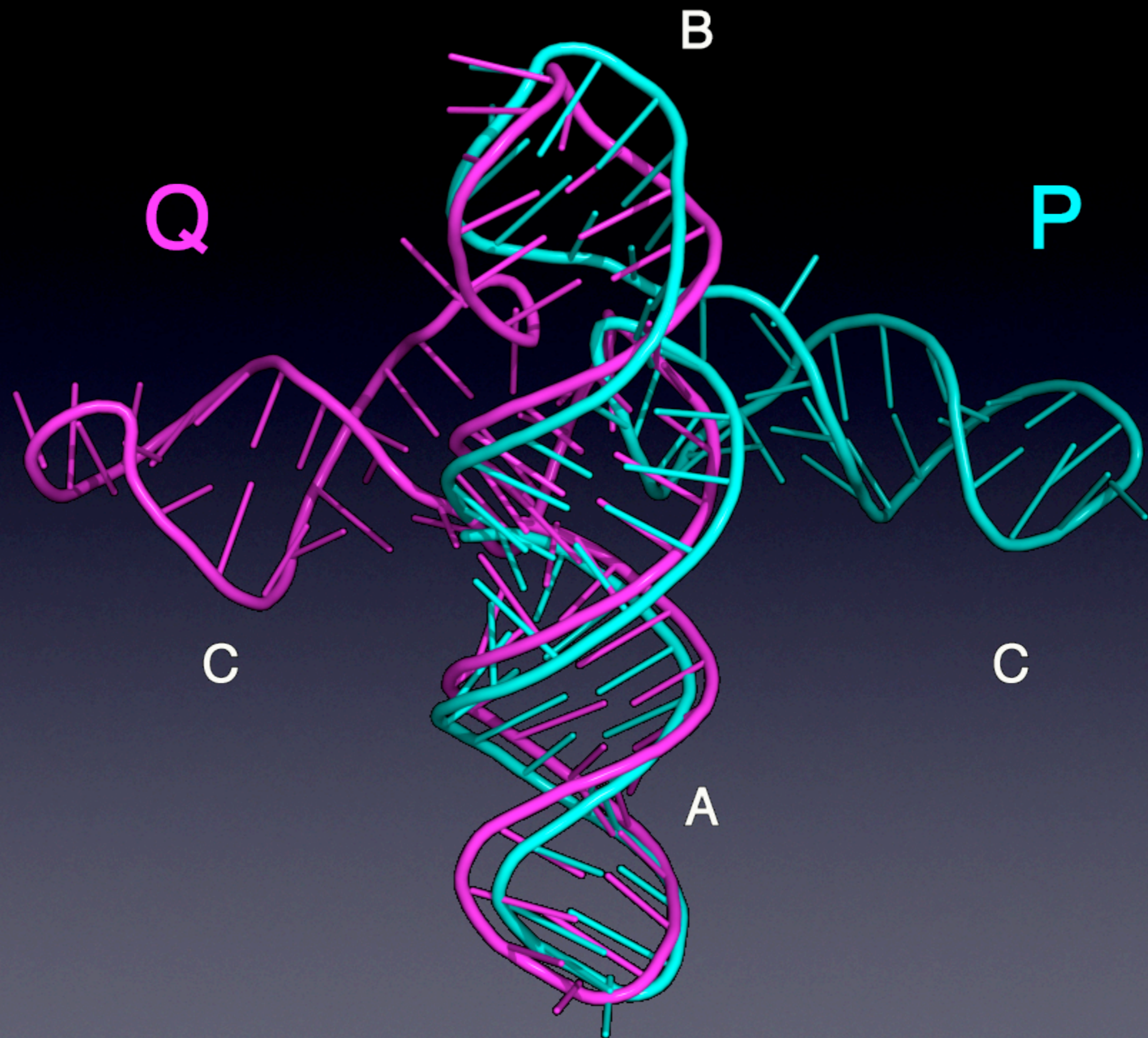
*difference Fourier
confirms trace*

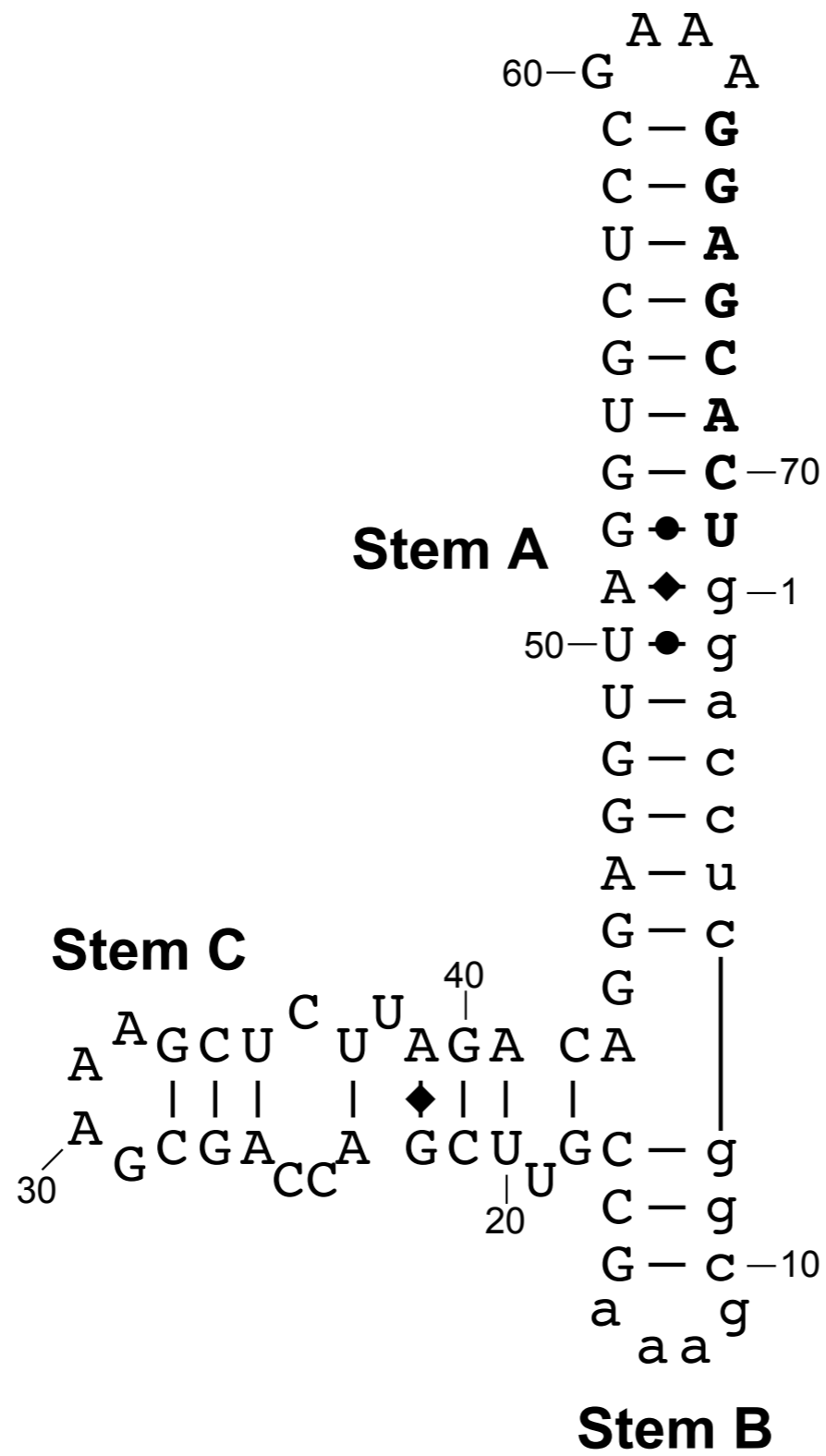
*composite-omit
2Fo-Fc map*



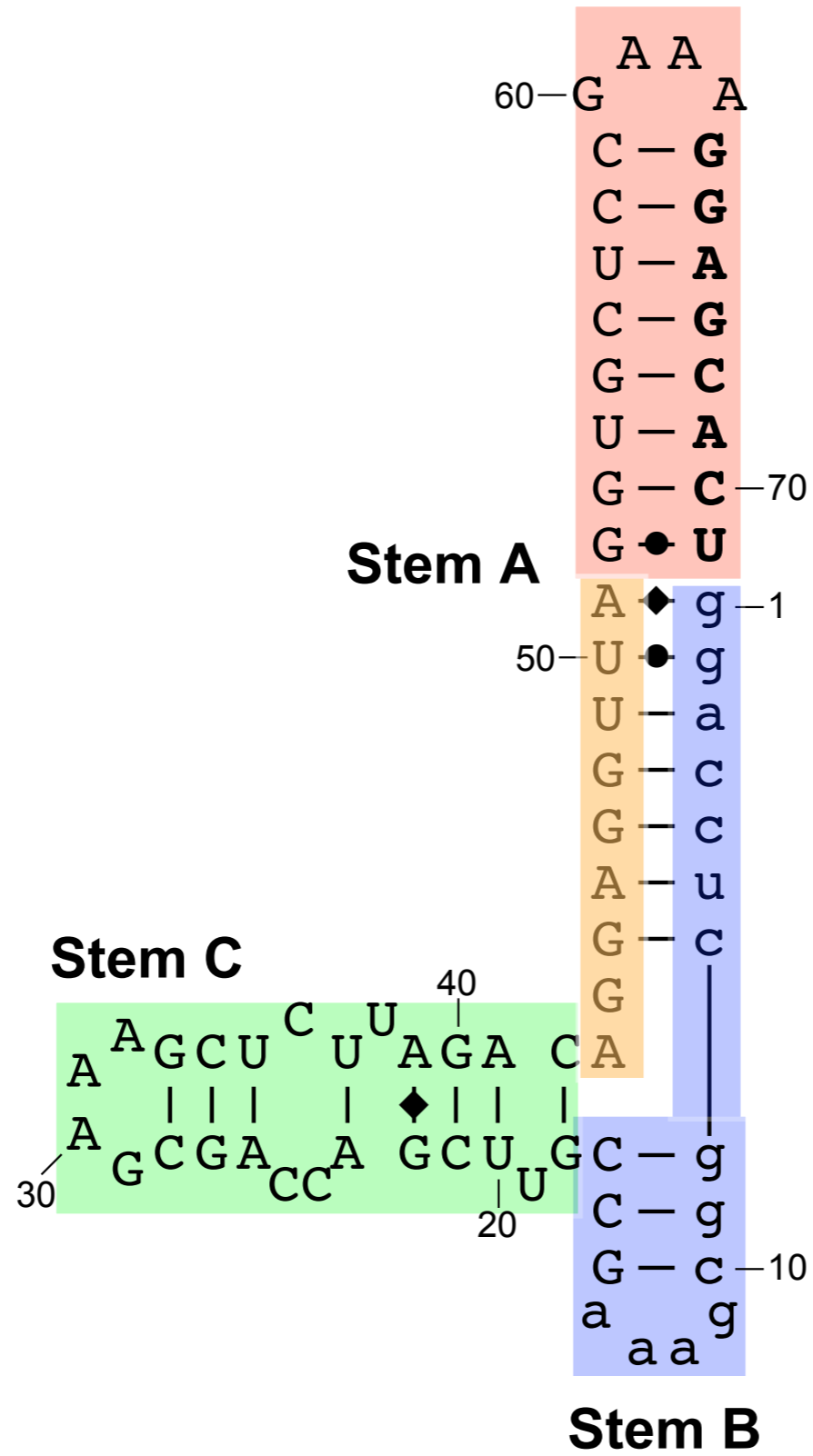








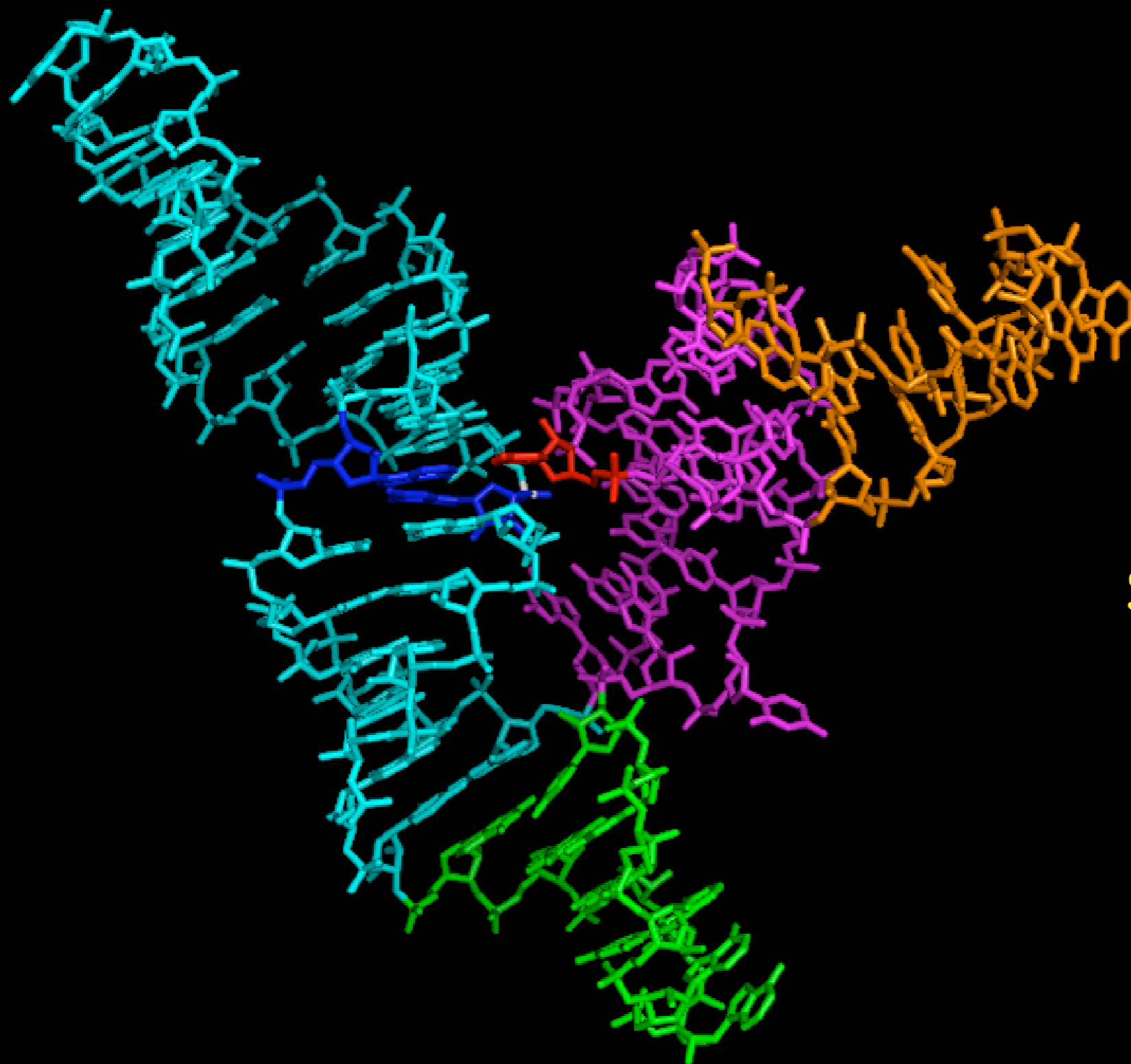
5' to 3'



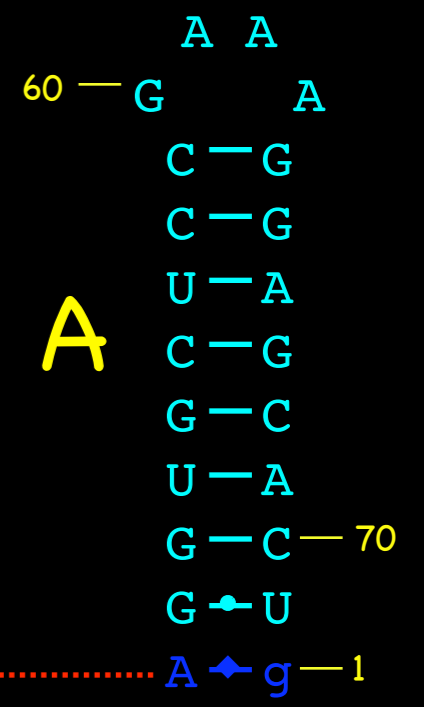
5' to 3'

Molecule Q

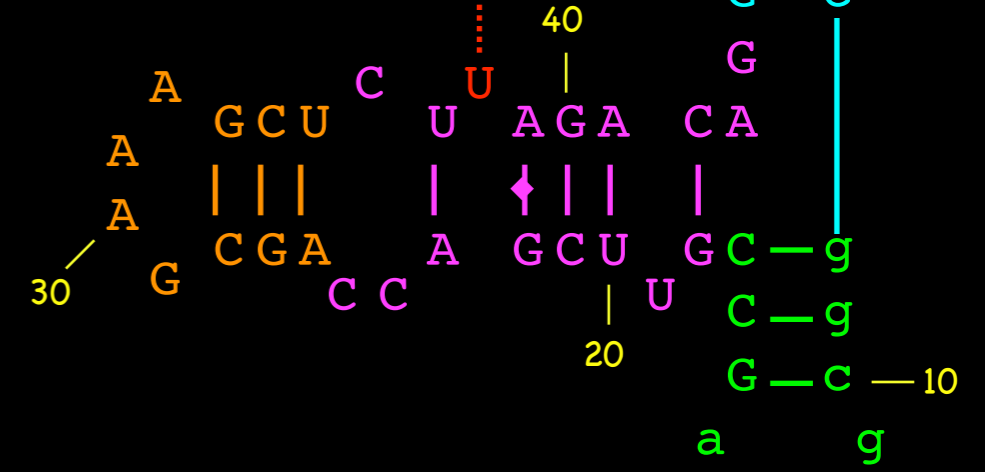
Molecule Q



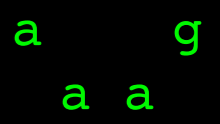
Stem A



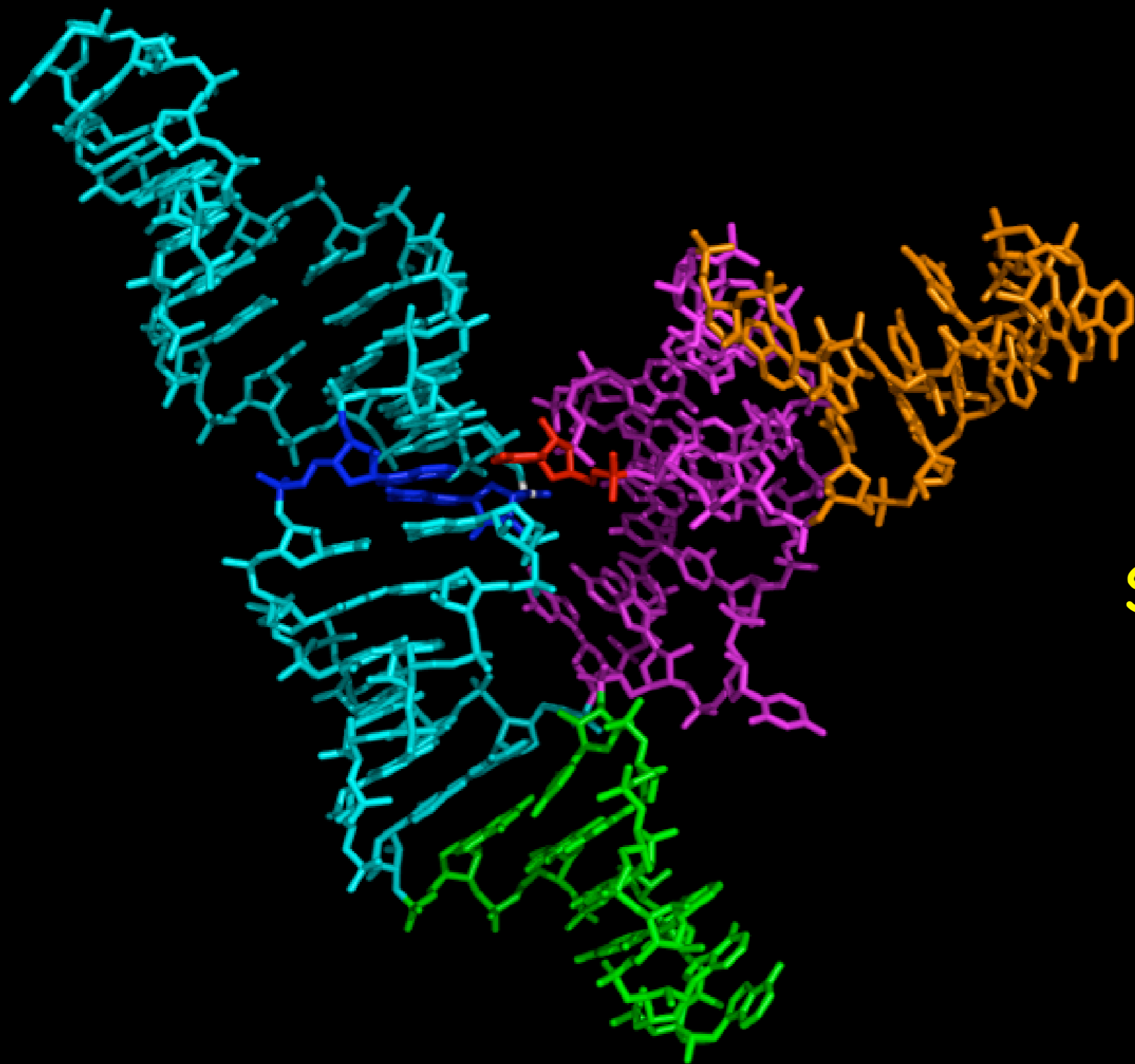
Stem C



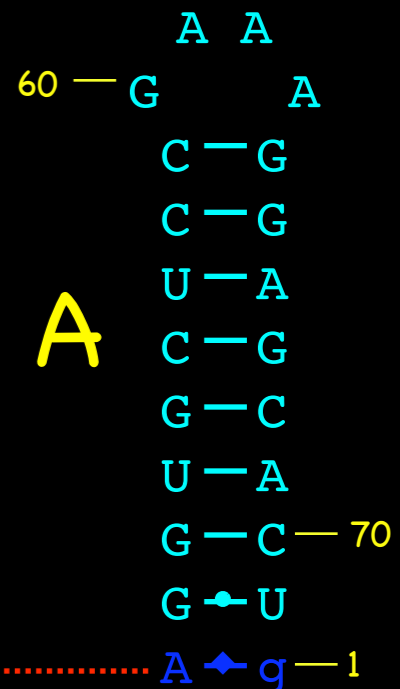
Stem B



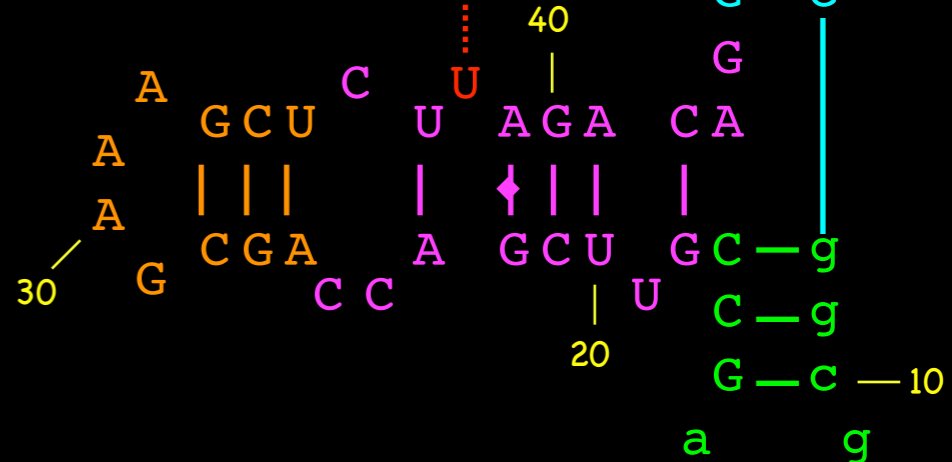
Molecule Q



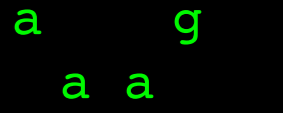
Stem A



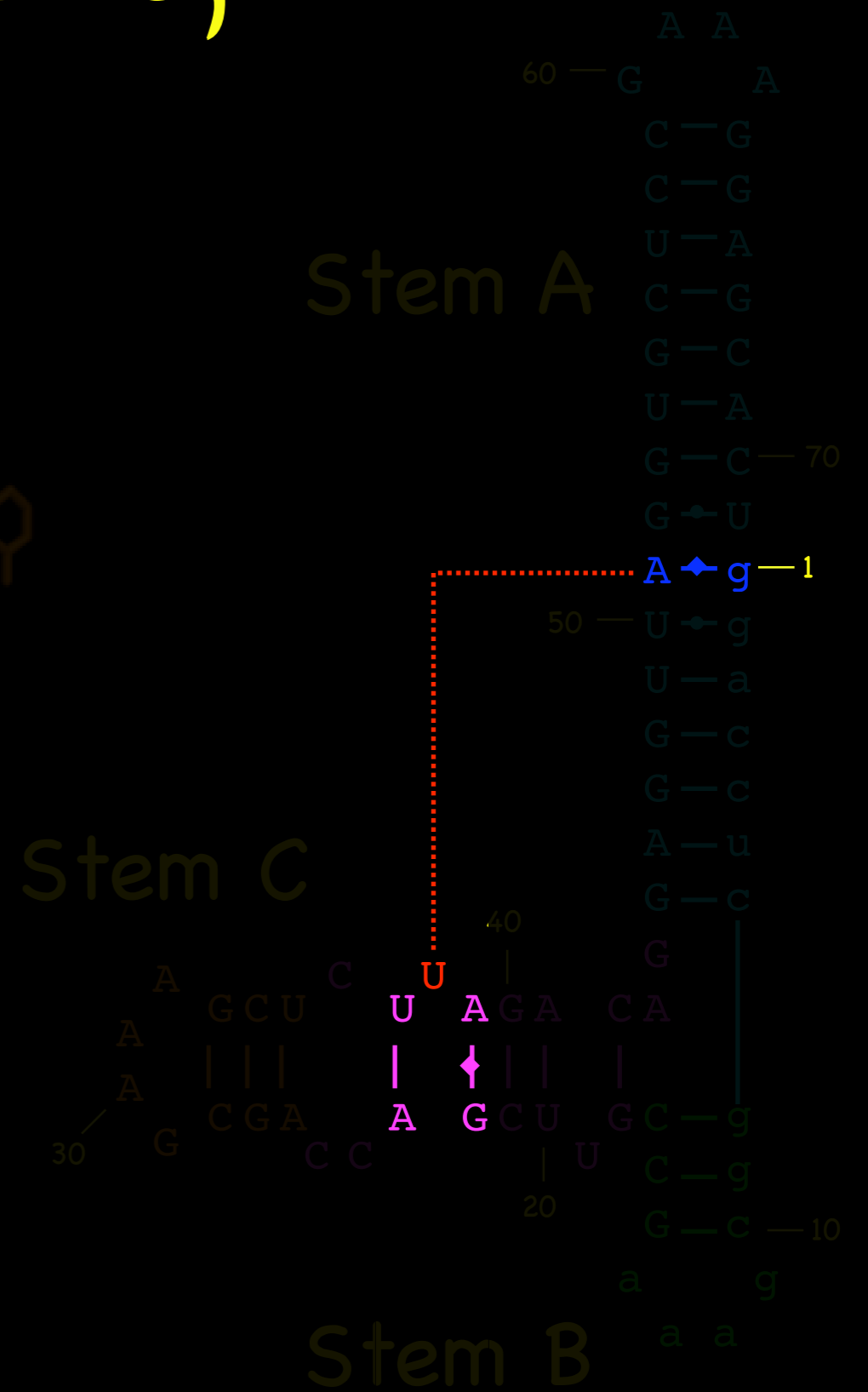
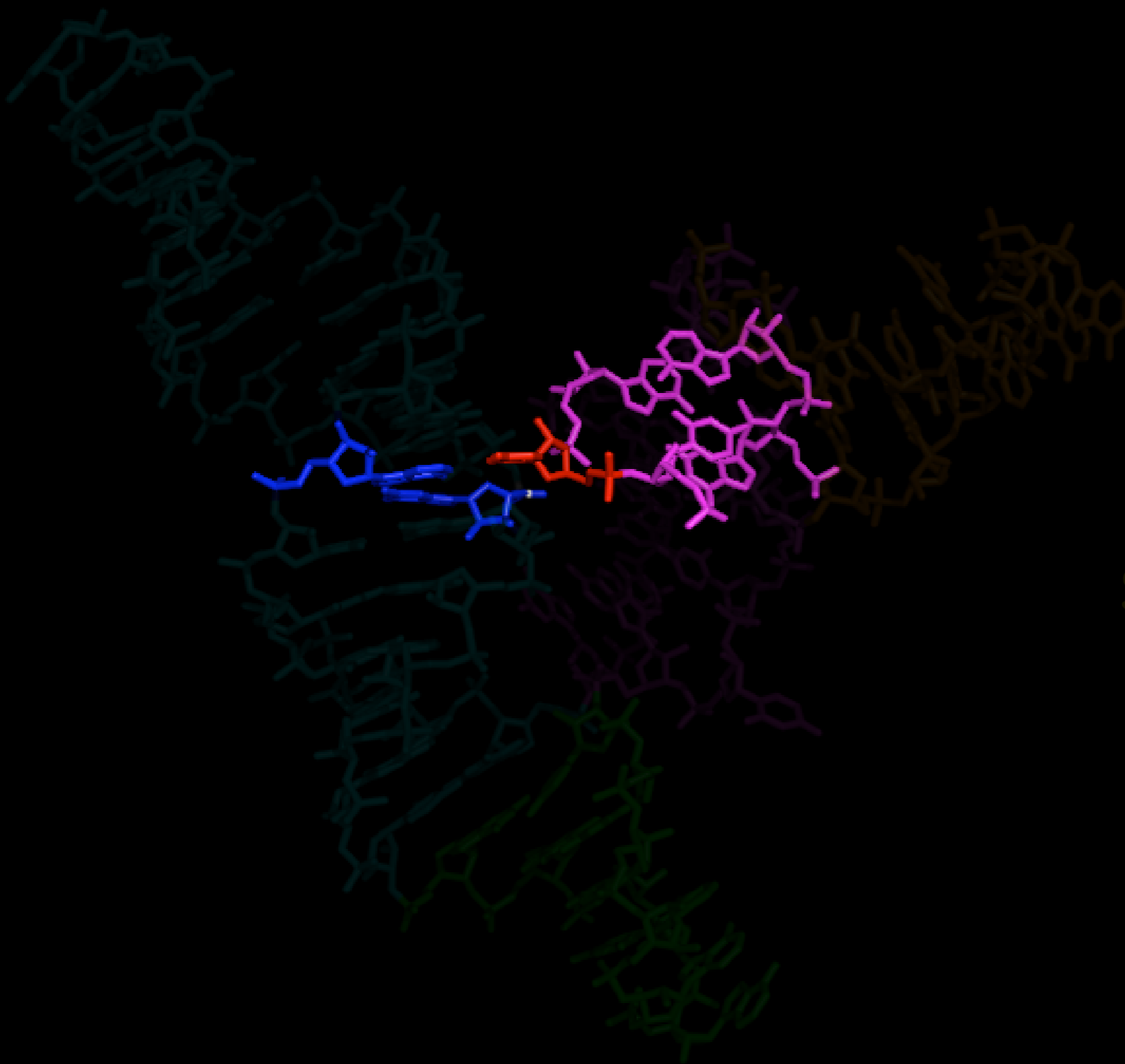
Stem C



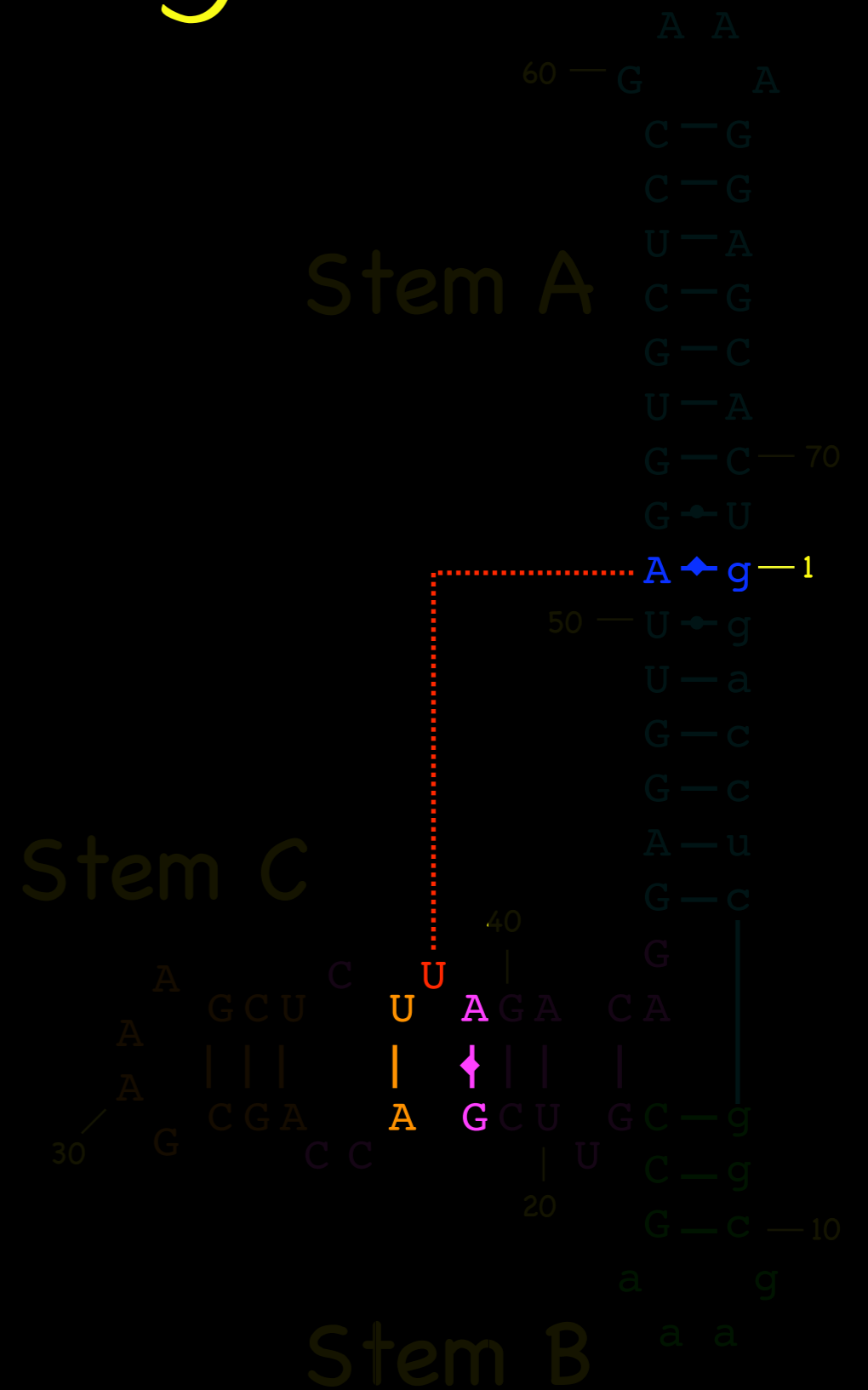
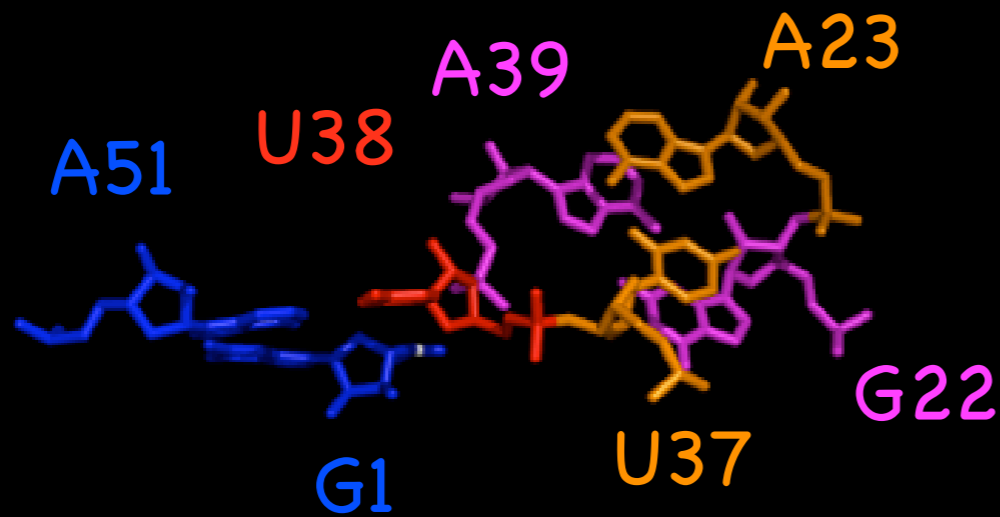
Stem B



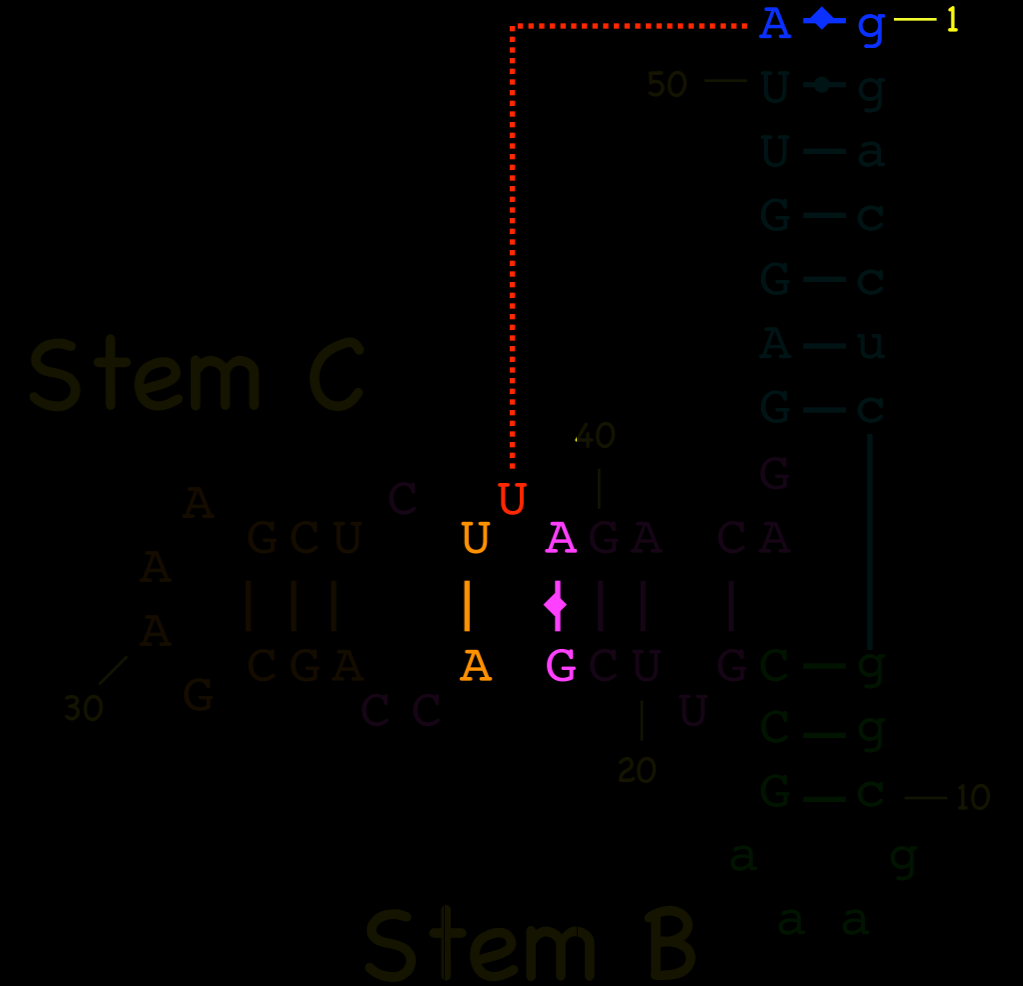
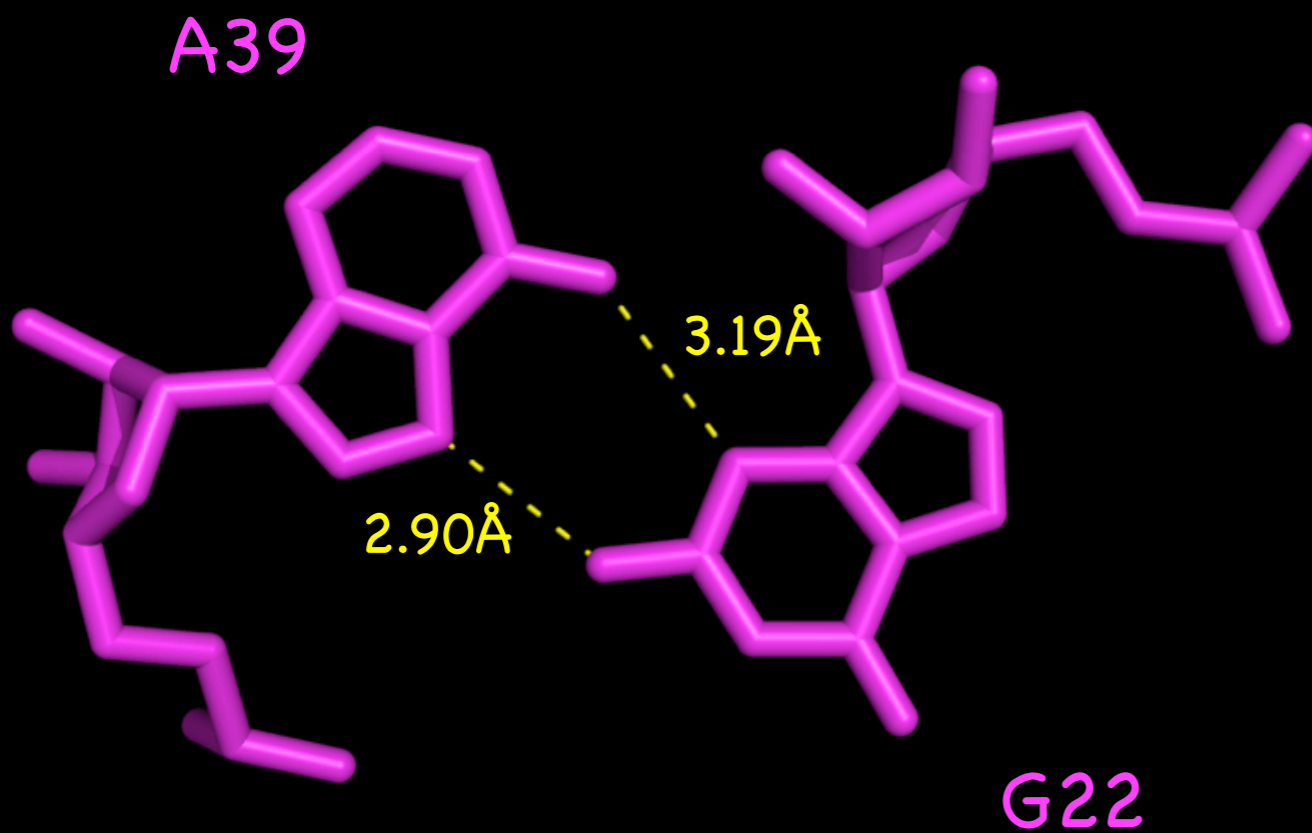
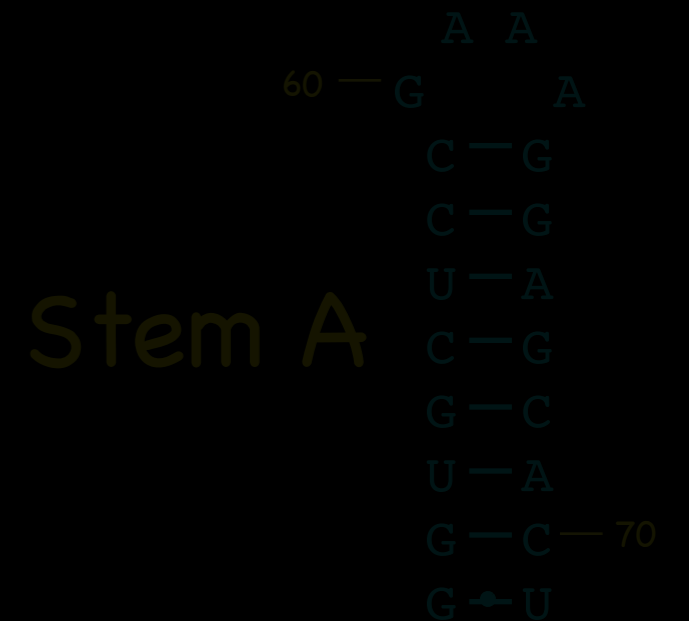
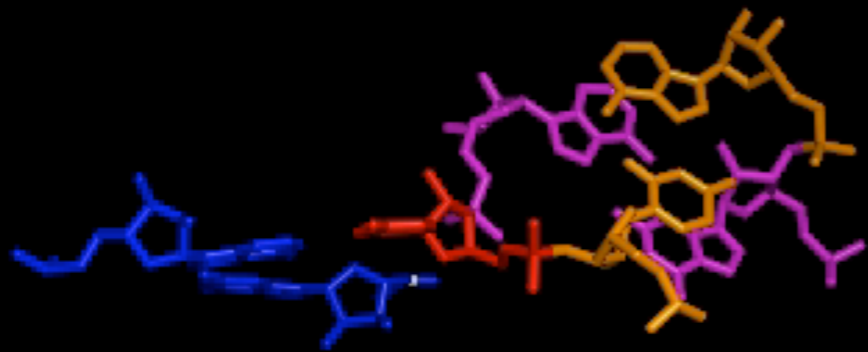
Molecule Q



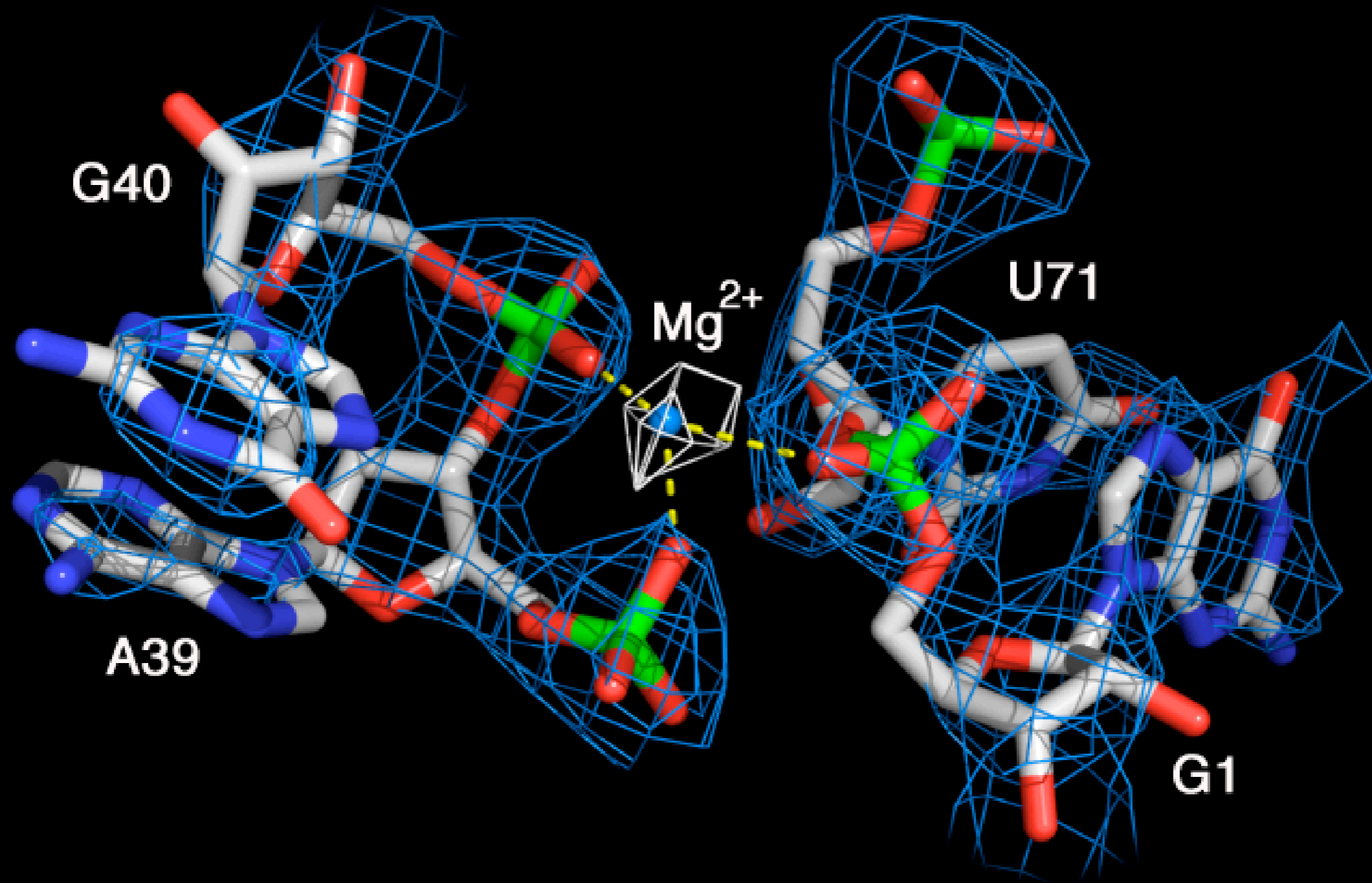
stem A/C bridge



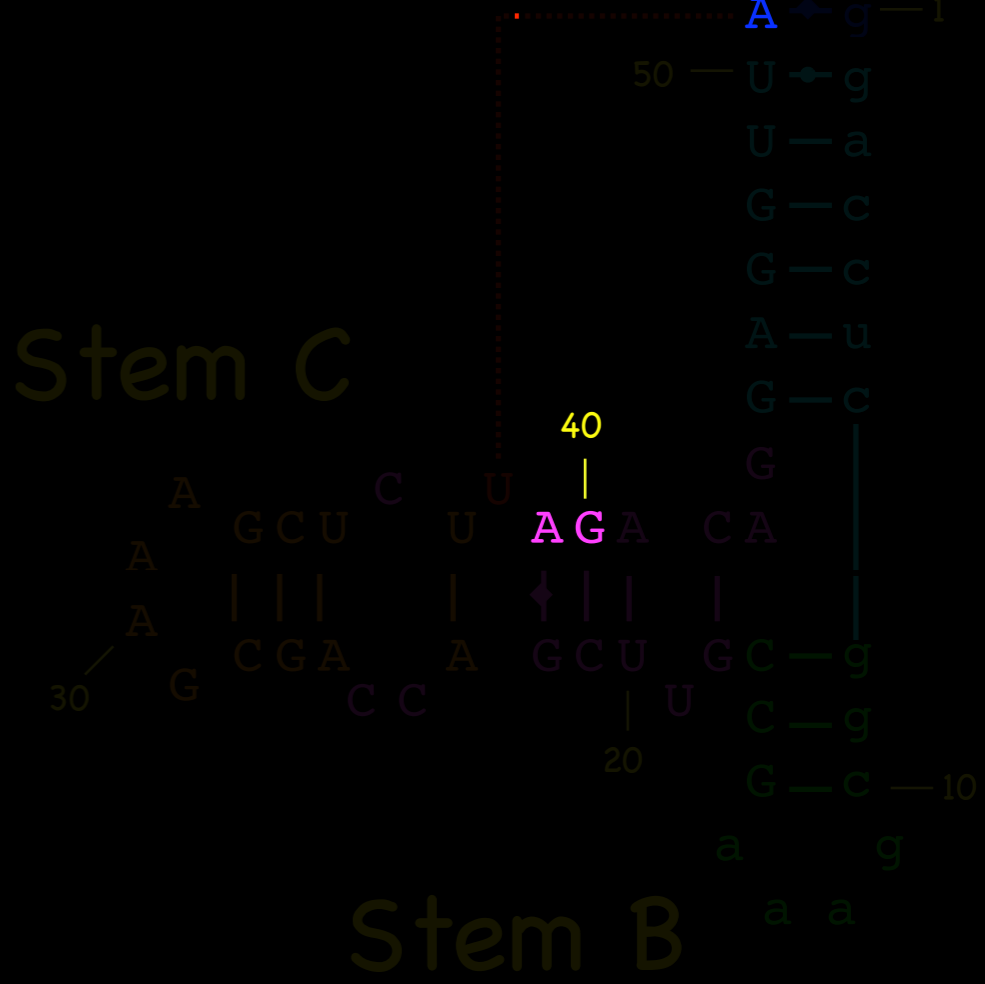
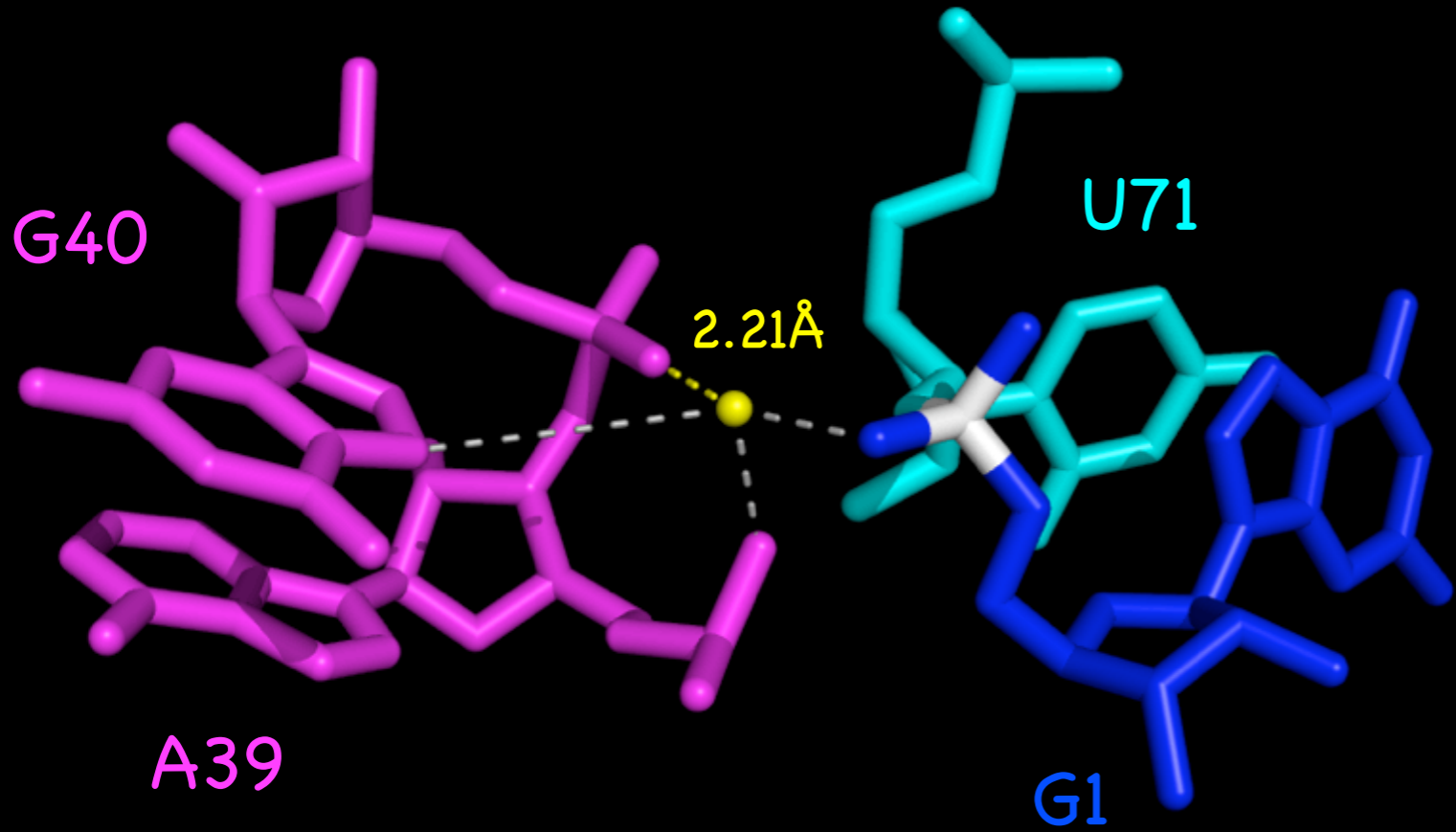
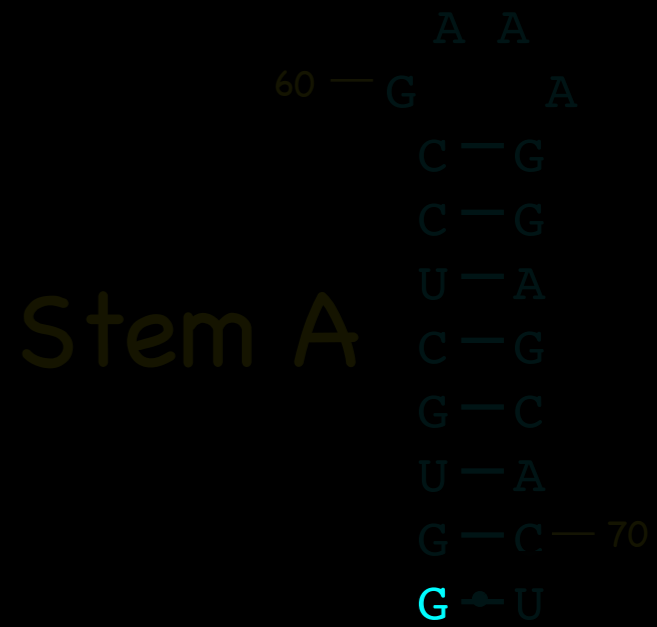
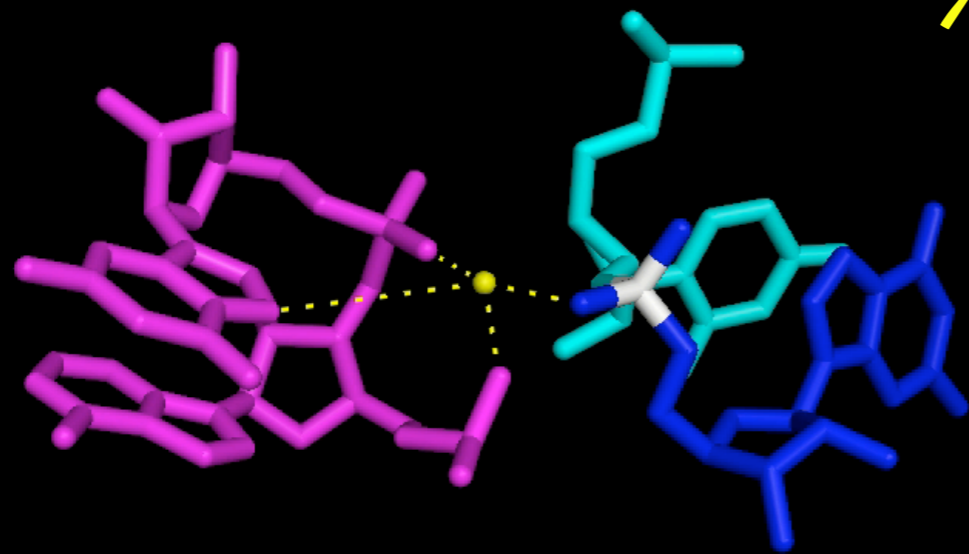
stem A/C bridge



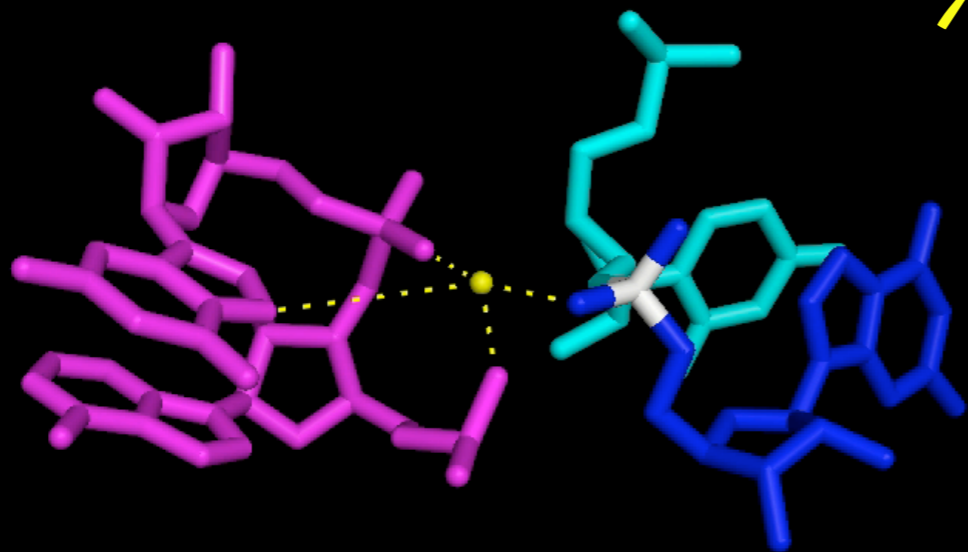
catalytic Mg^{2+}



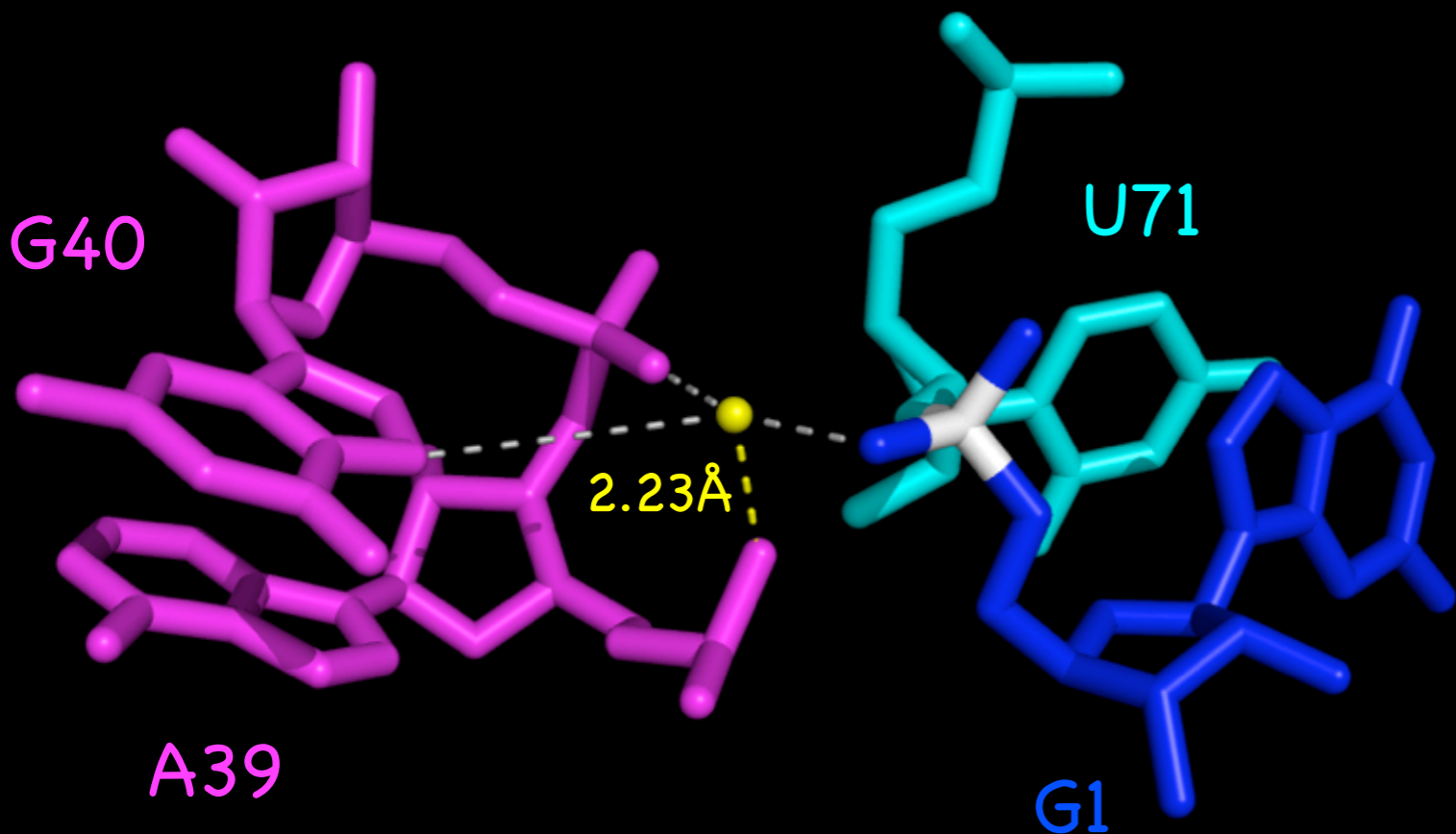
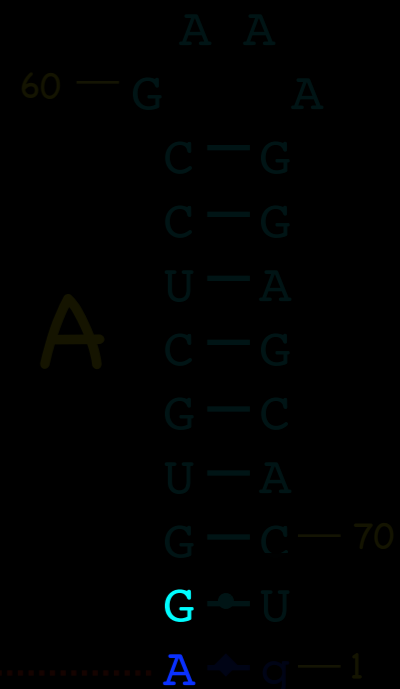
catalytic Mg^{2+}



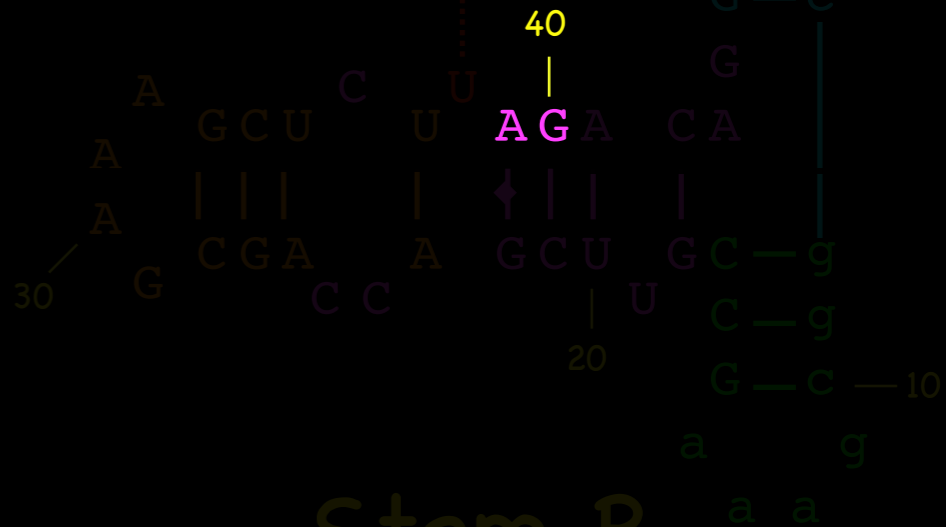
catalytic Mg^{2+}



Stem A

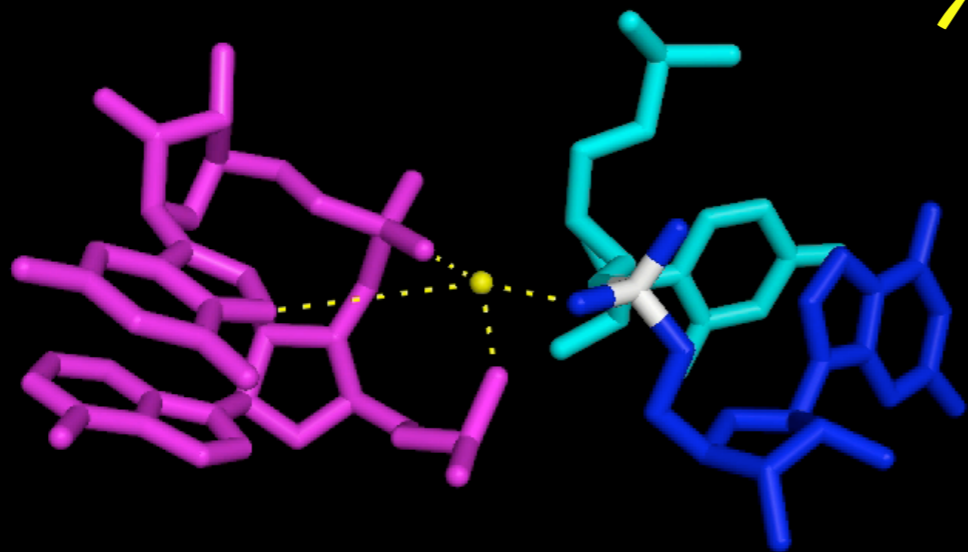


Stem C

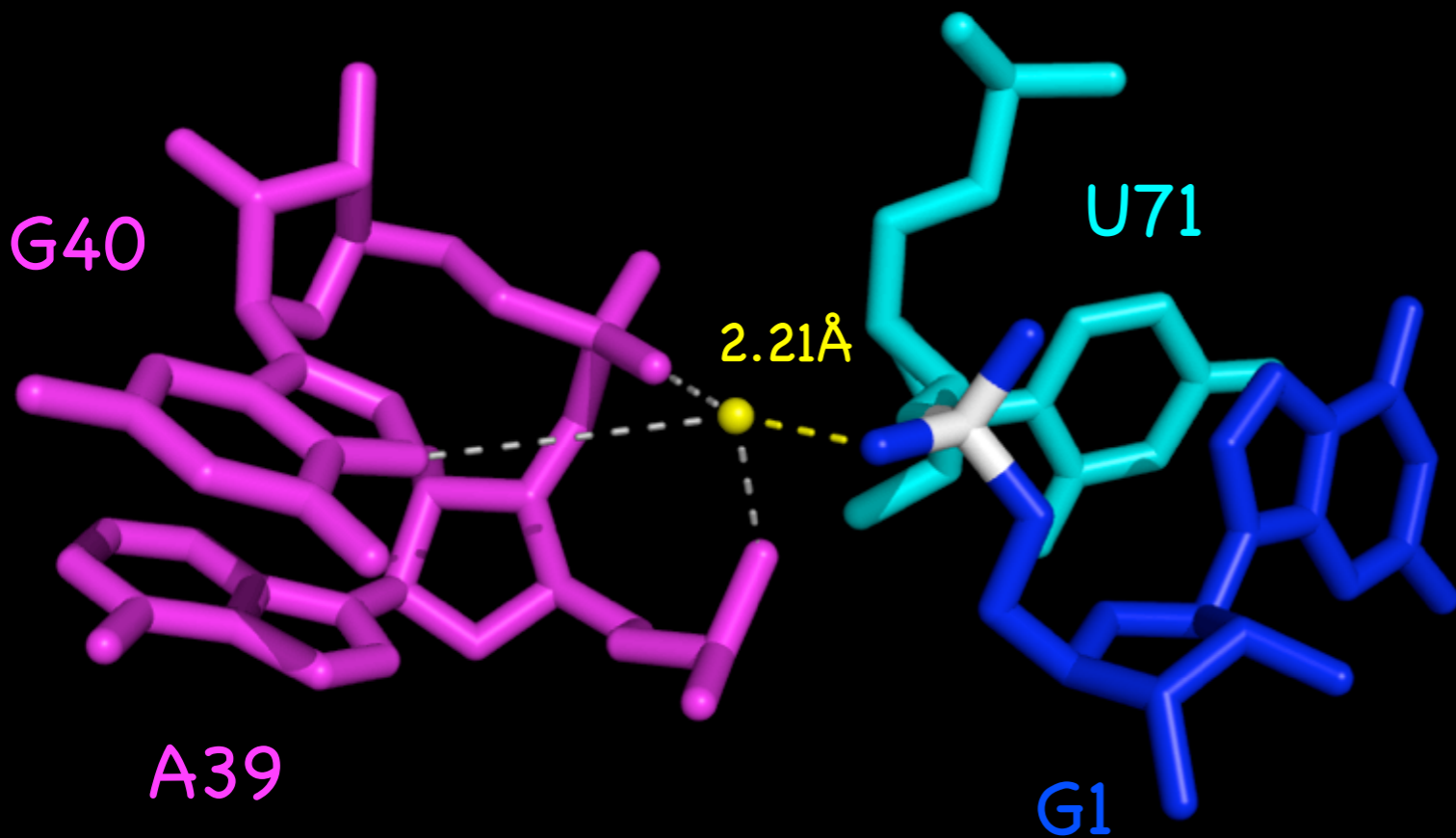
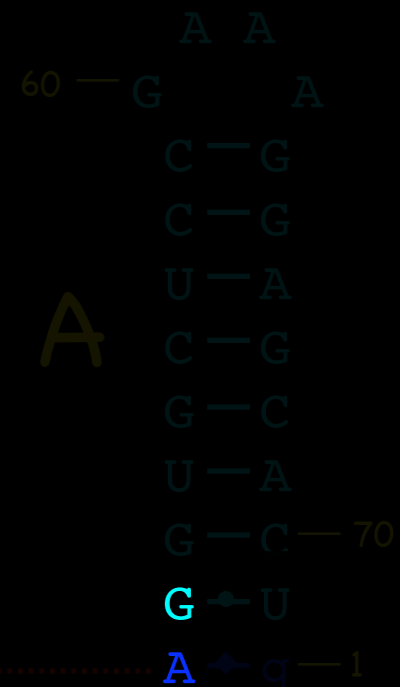


Stem B

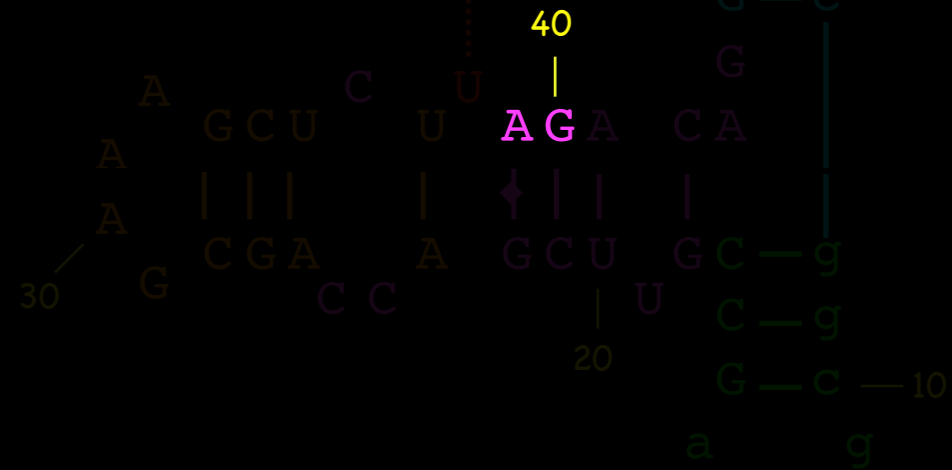
catalytic Mg^{2+}



Stem A



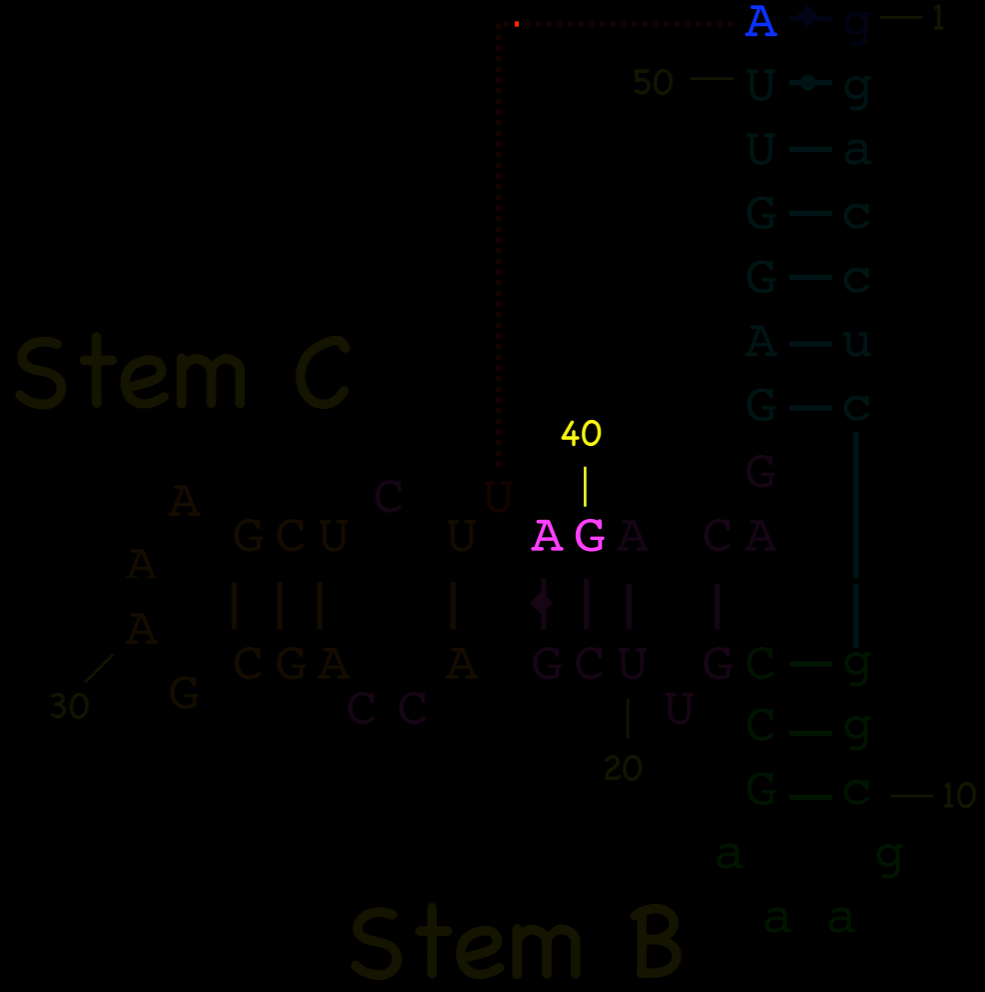
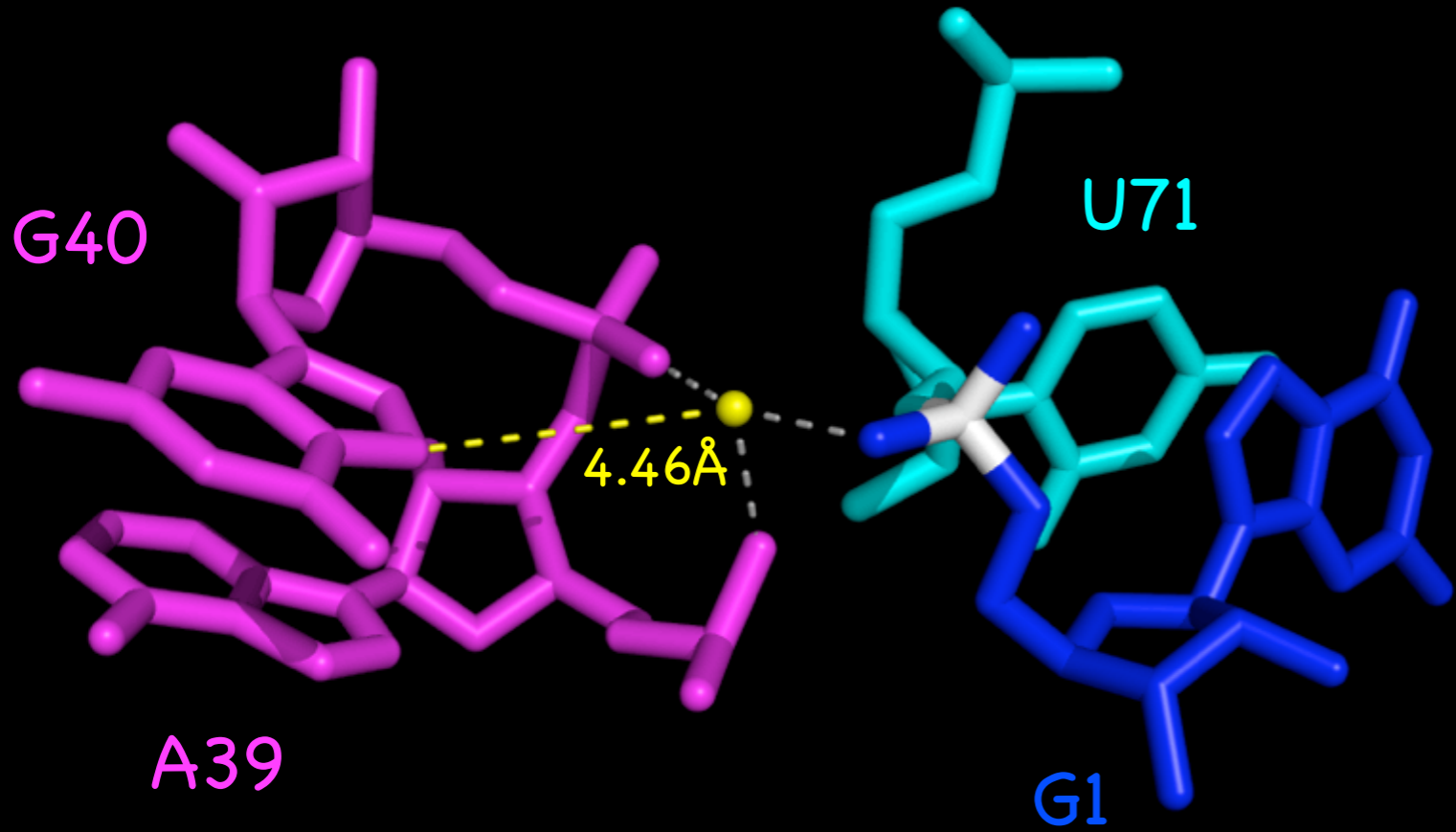
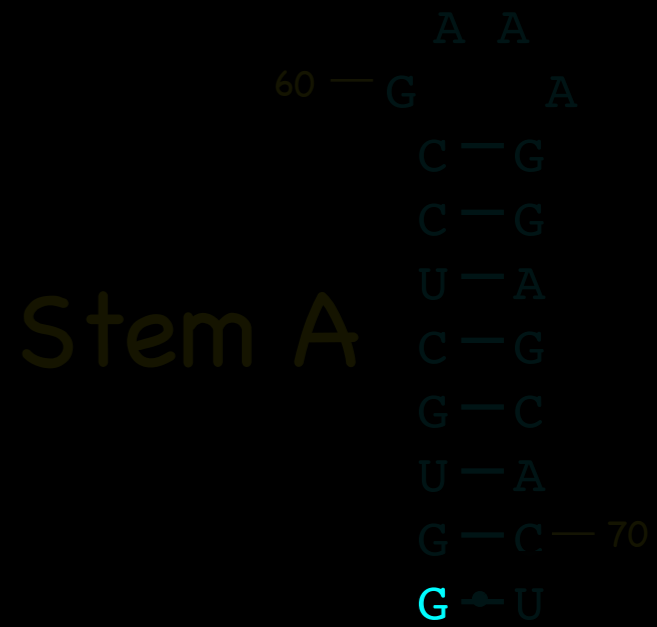
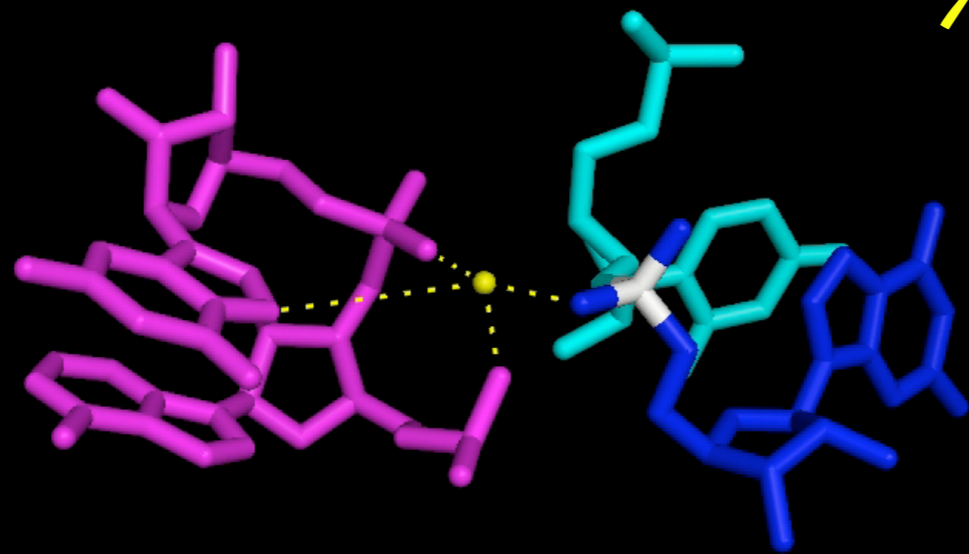
Stem C



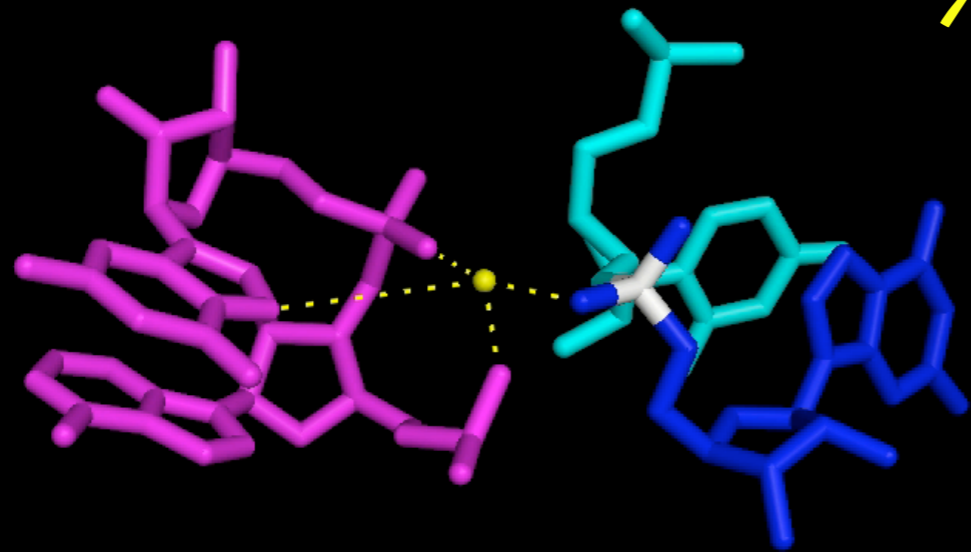
Stem B



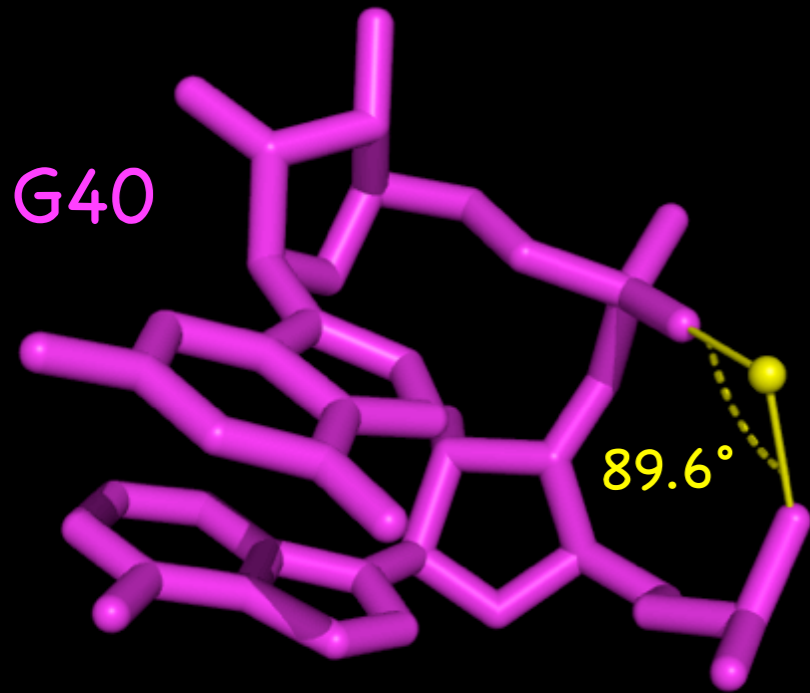
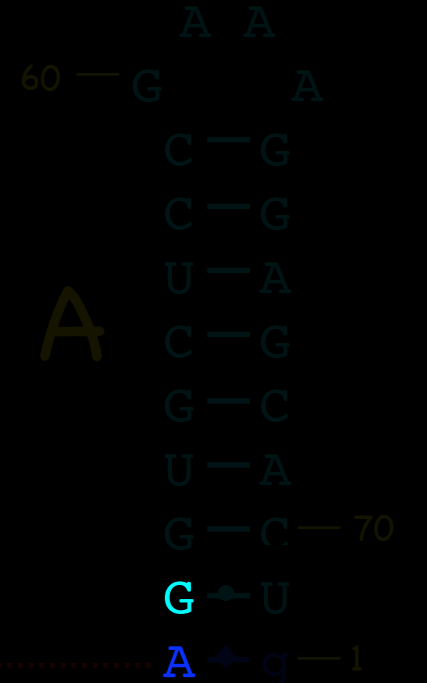
catalytic Mg²⁺



catalytic Mg^{2+}



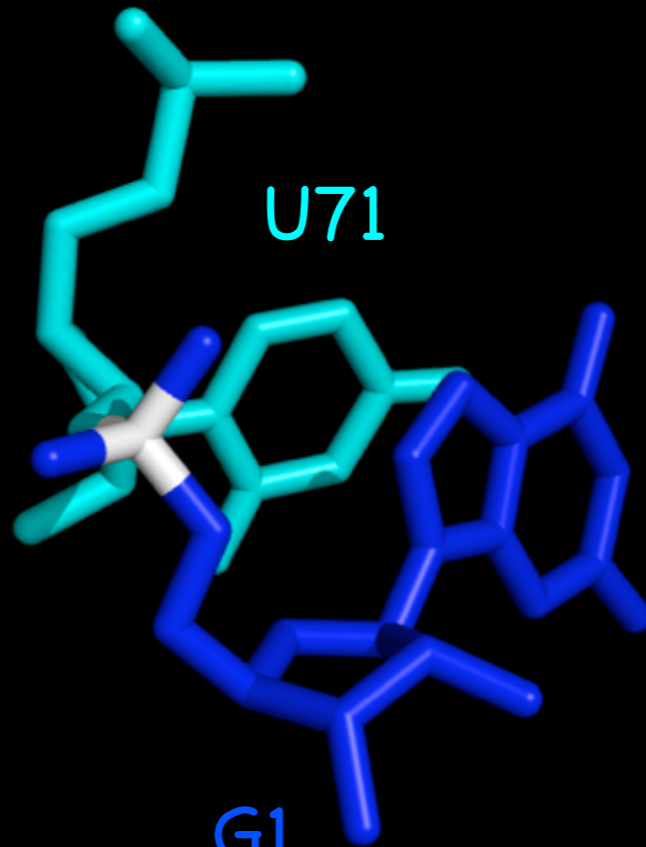
Stem A



G40

89.6°

A39



U71

G1

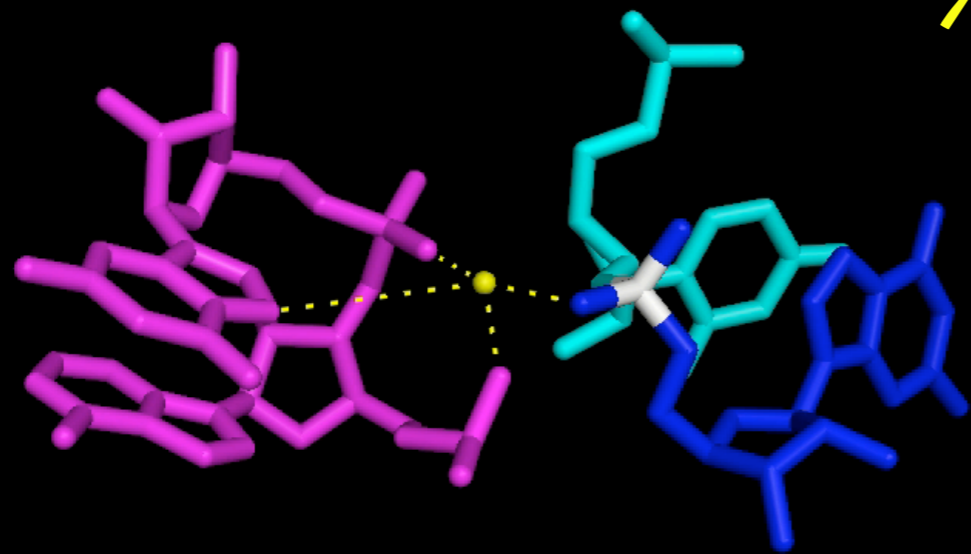
Stem C



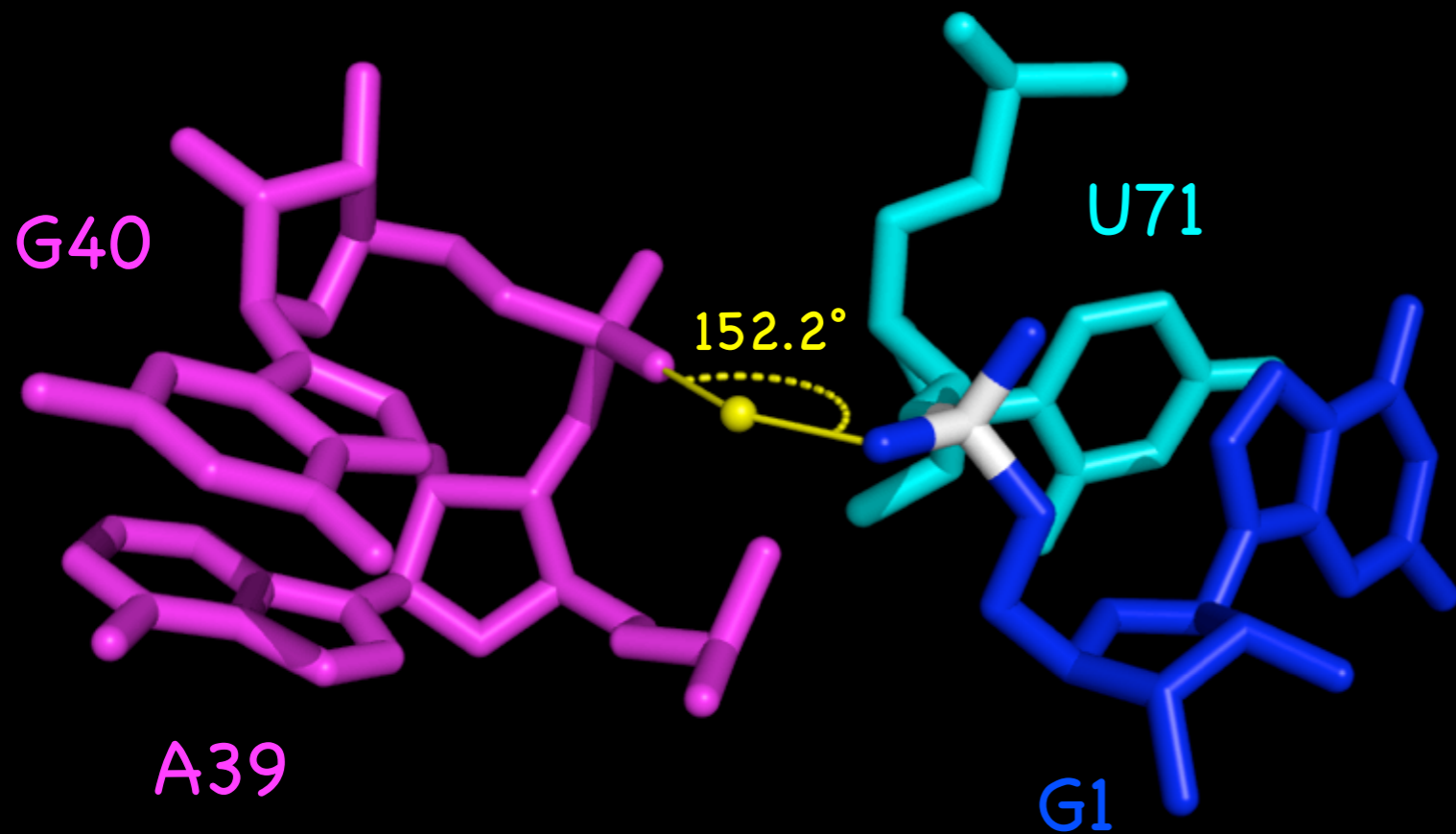
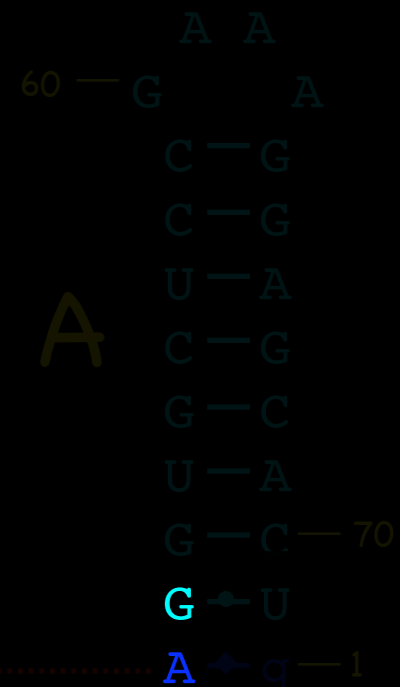
Stem B



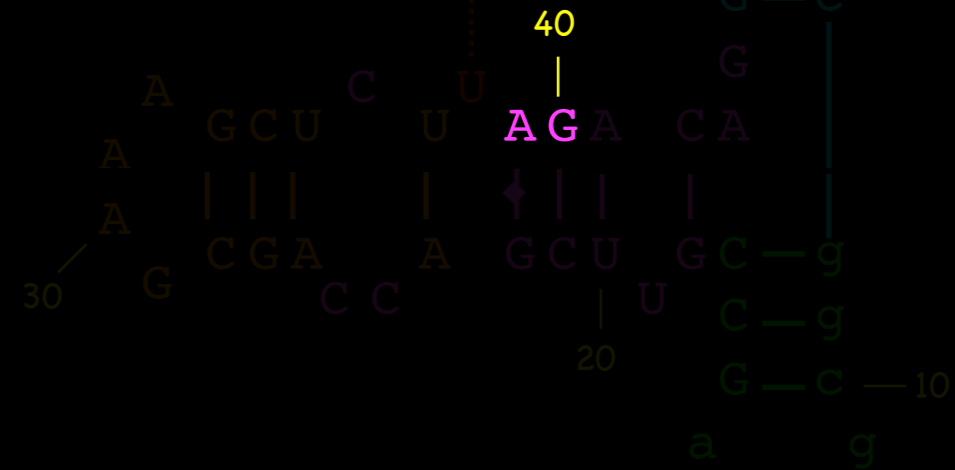
catalytic Mg^{2+}



Stem A

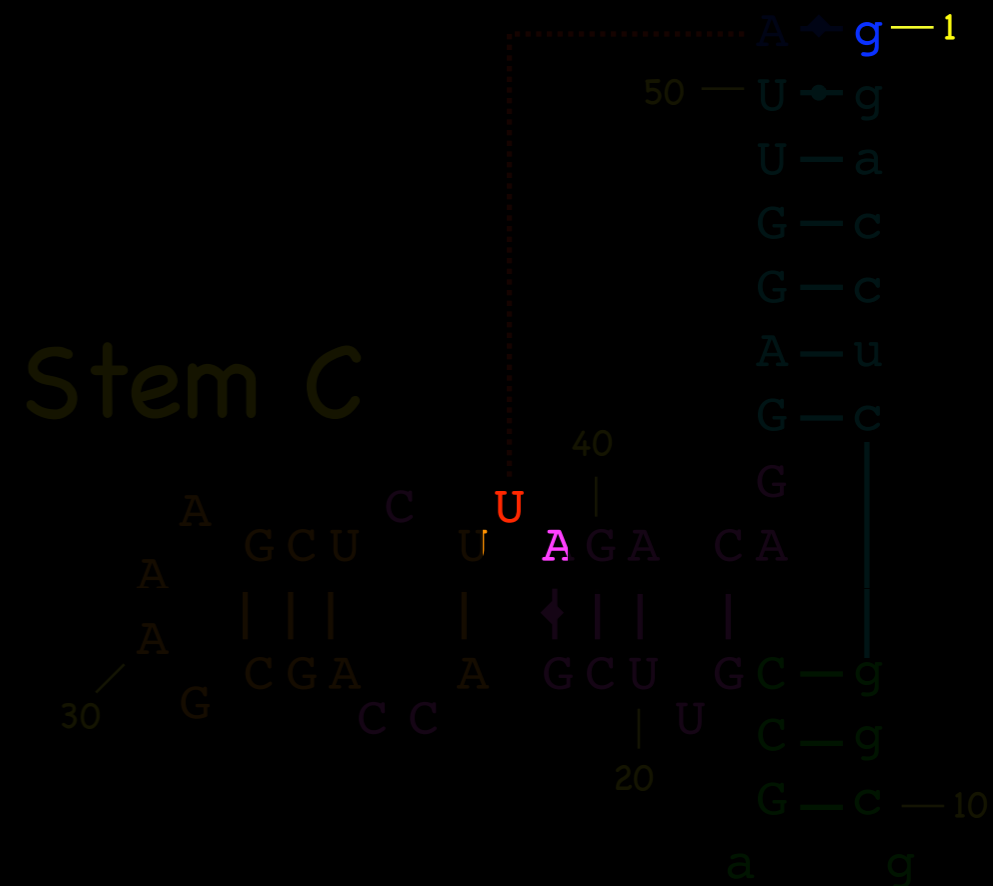
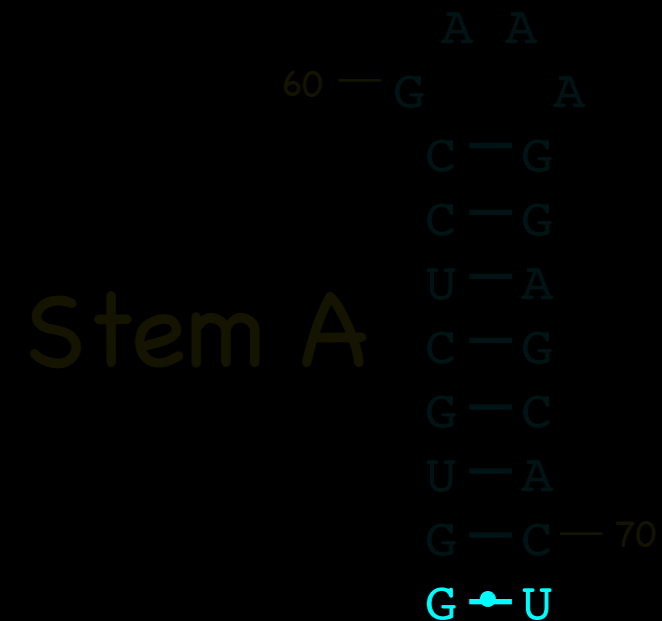
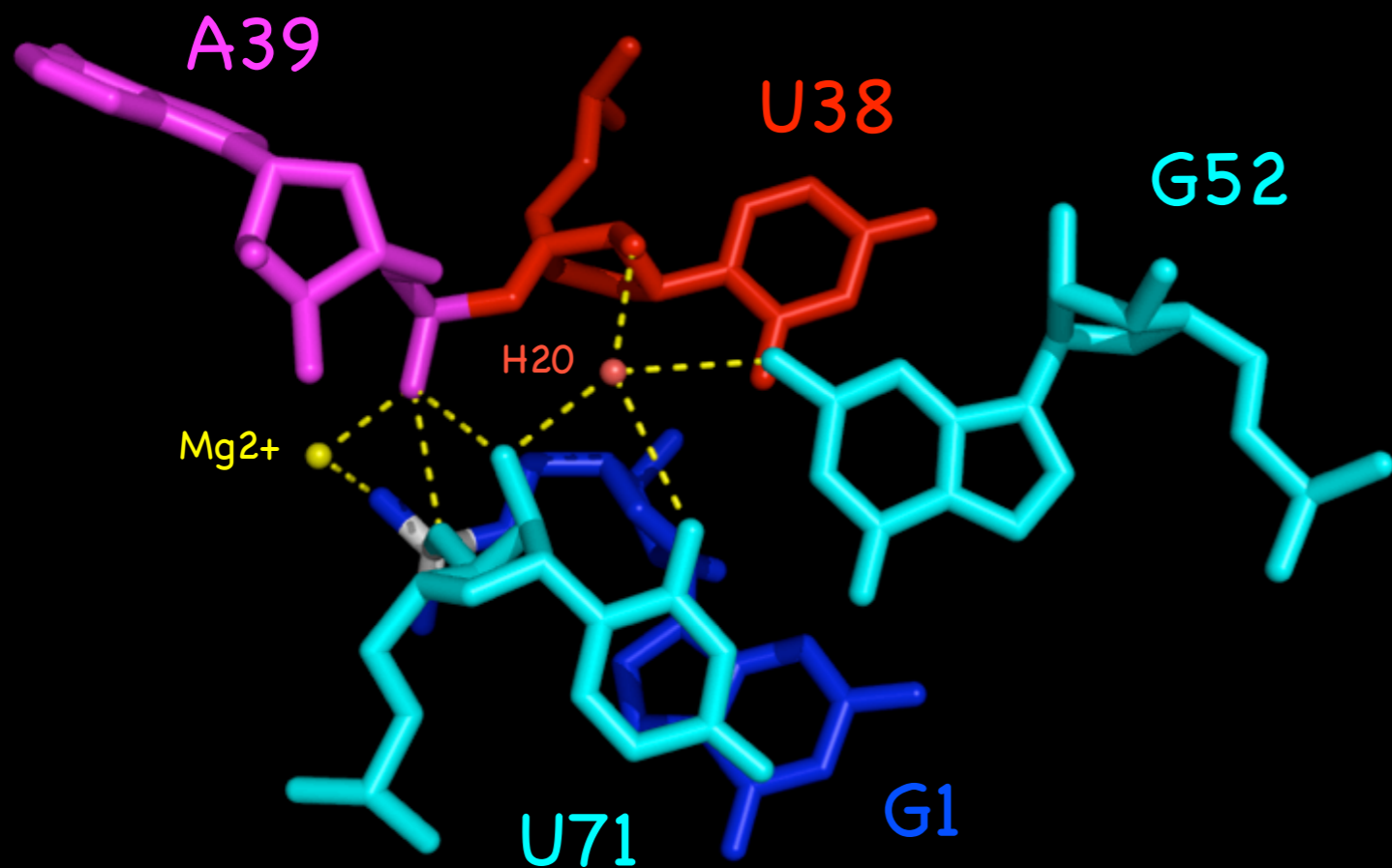


Stem C

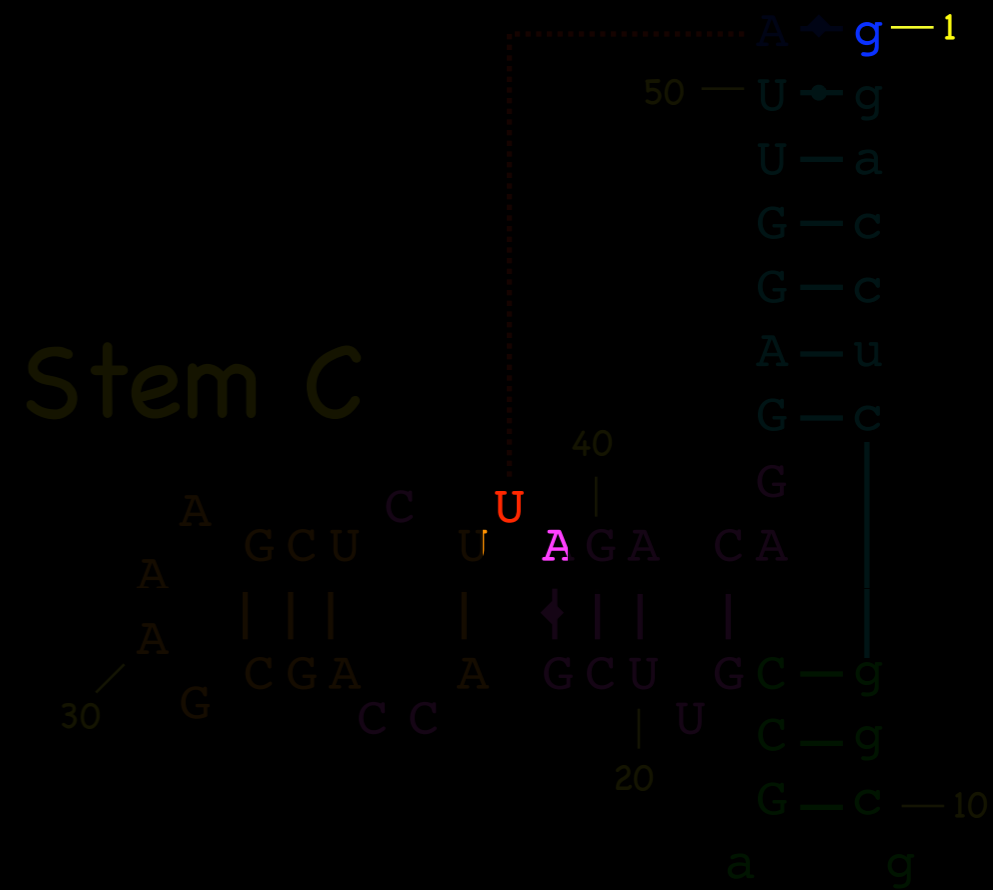
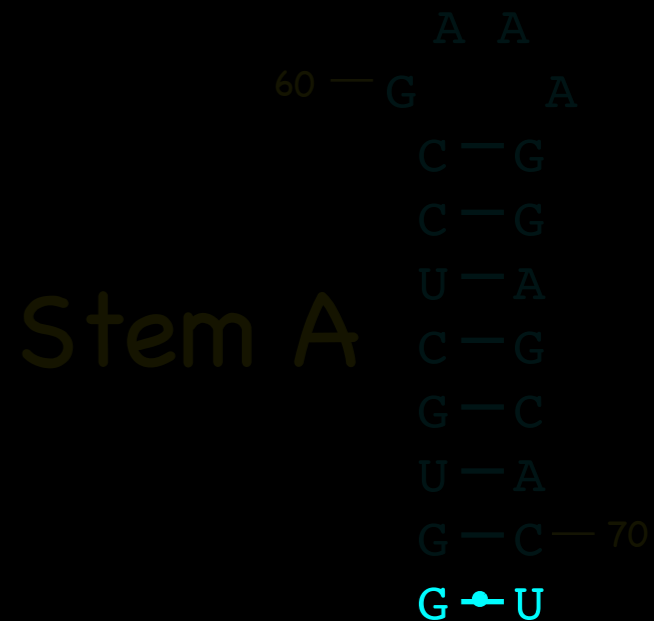
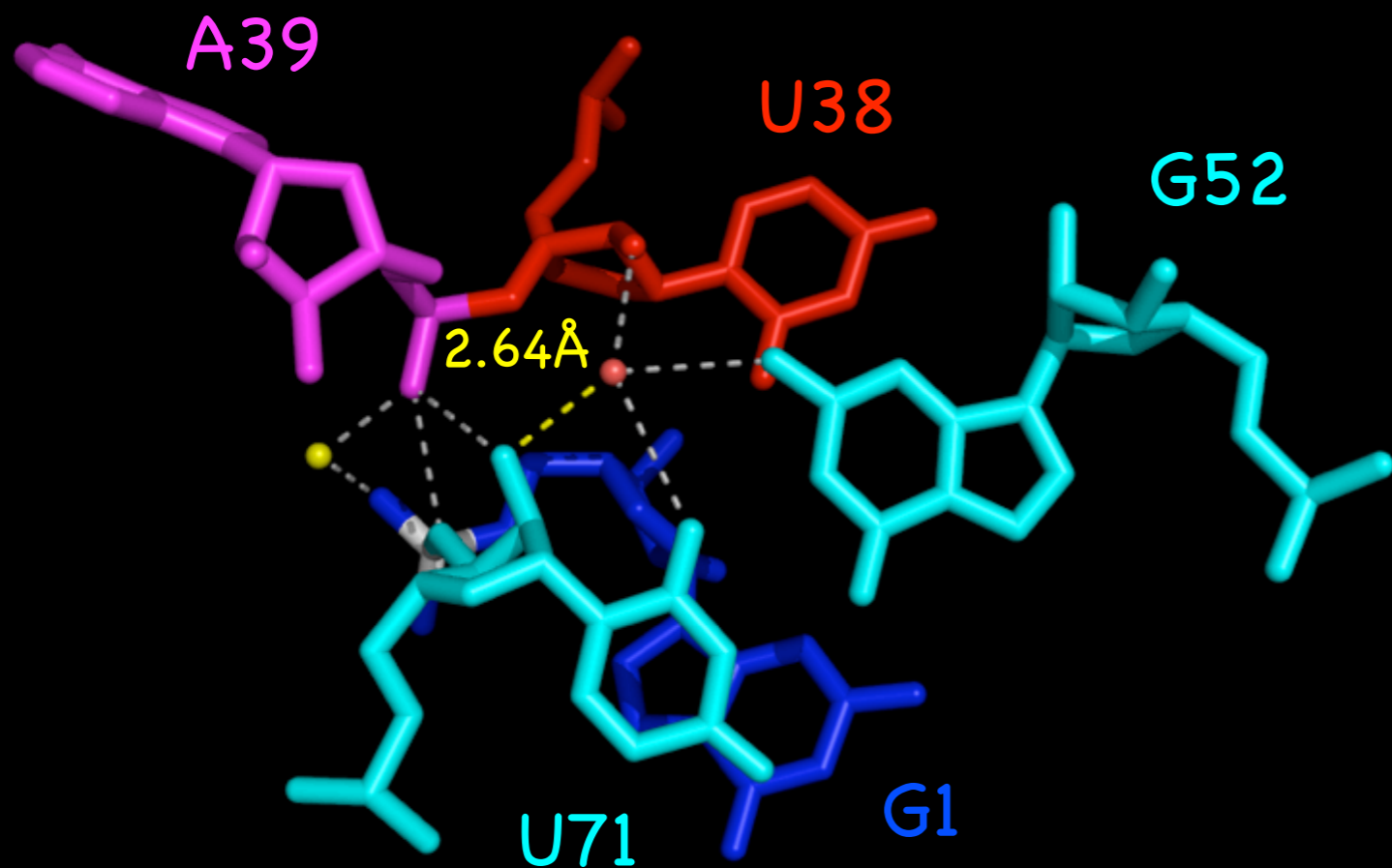
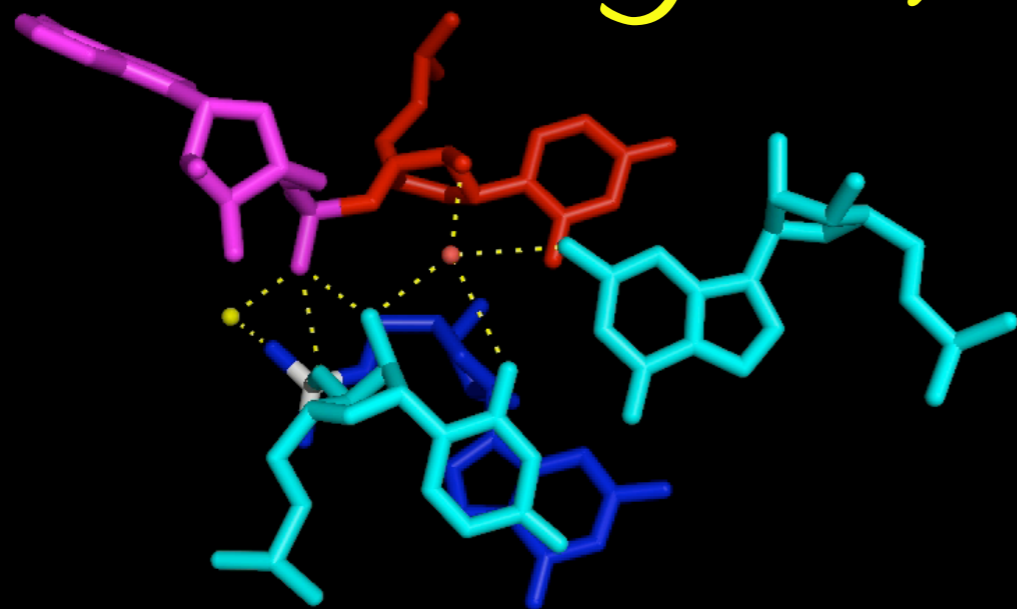


Stem B

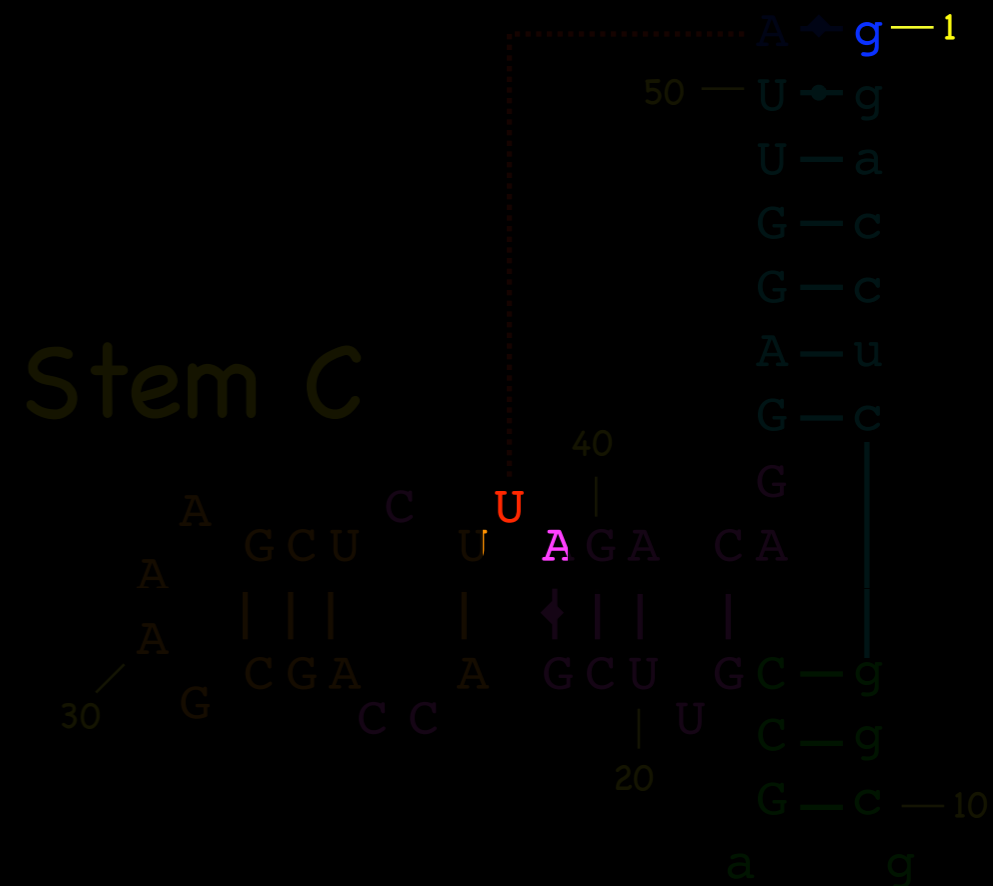
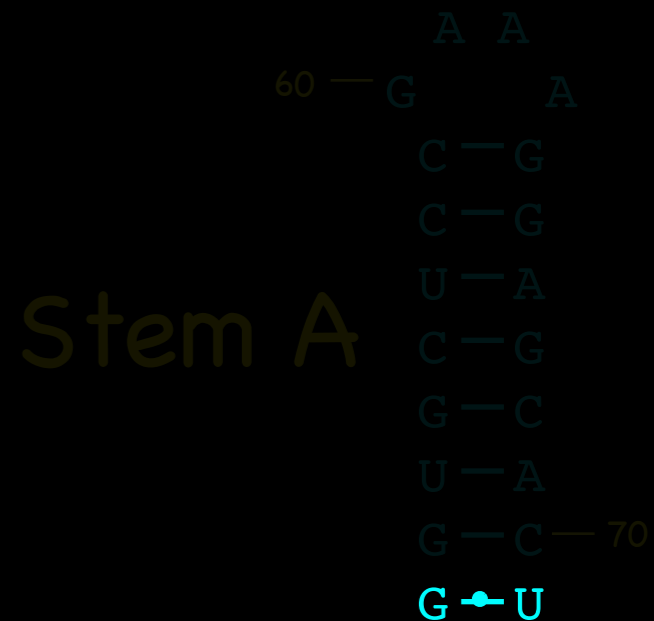
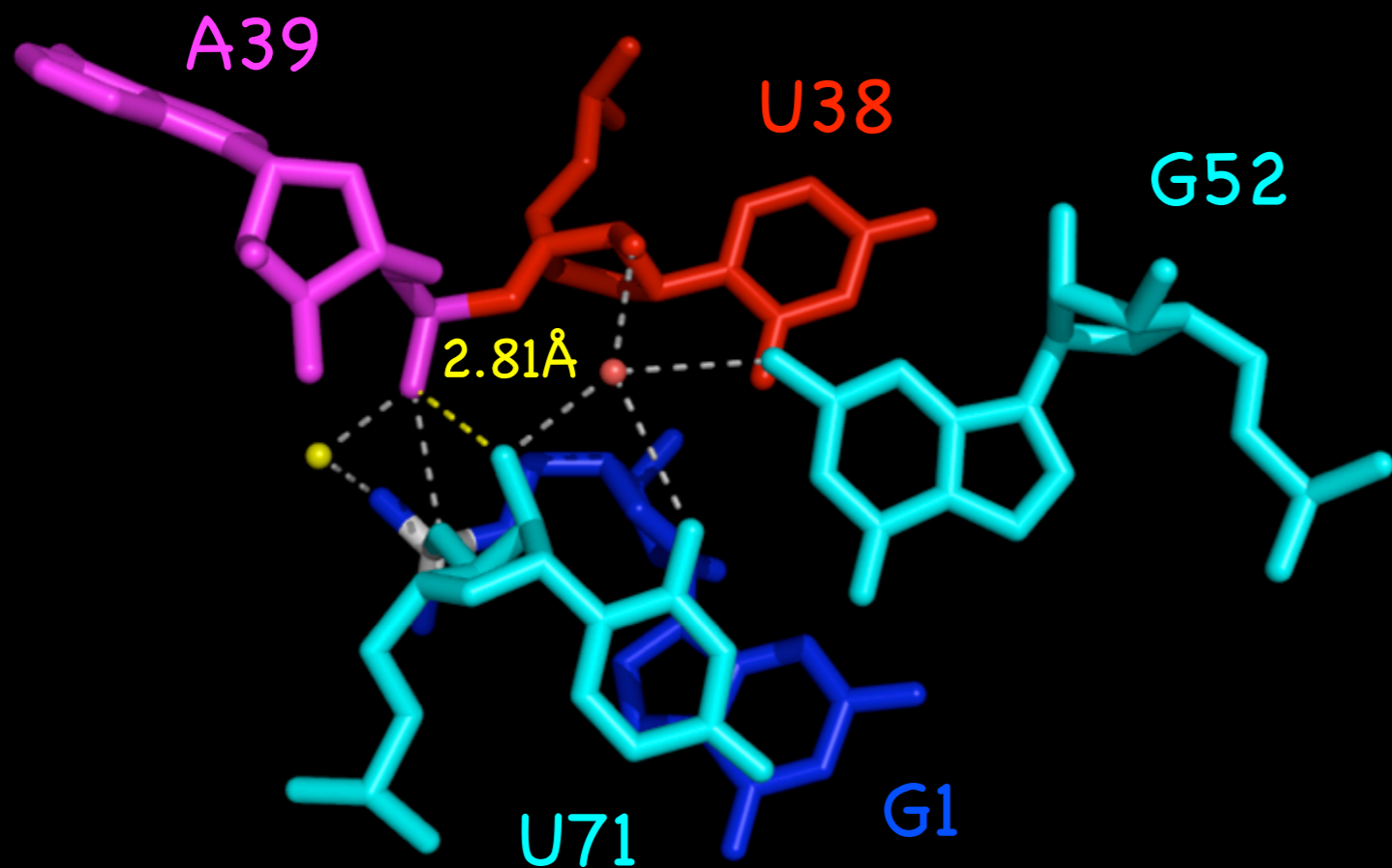
regiospecificity



regiospecificity



regiospecificity



regiospecificity



A39

U38

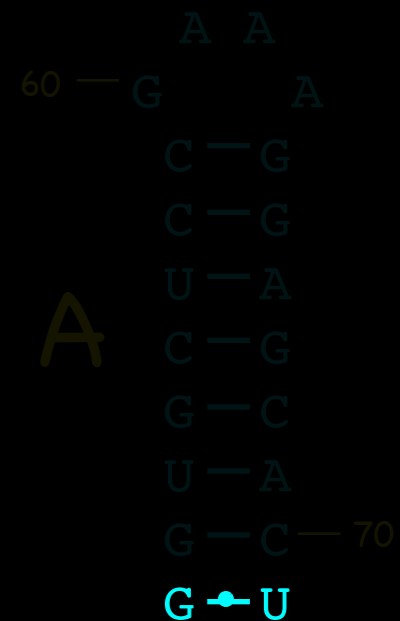
G52

2.88Å

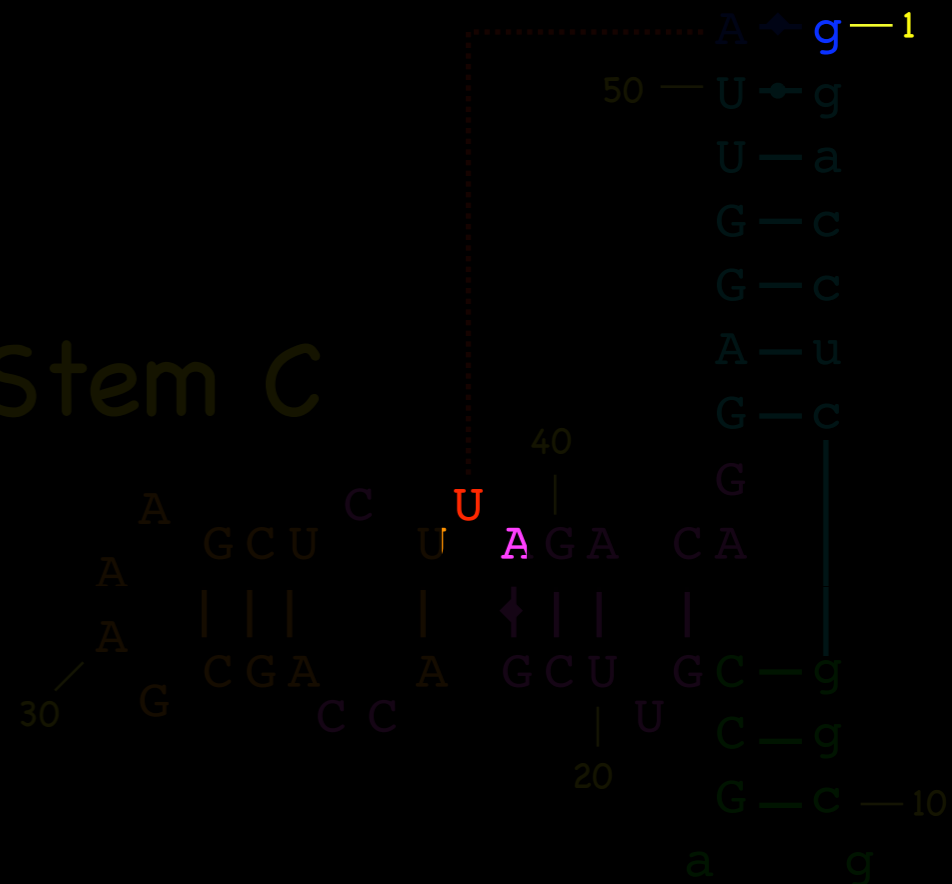
U71

G1

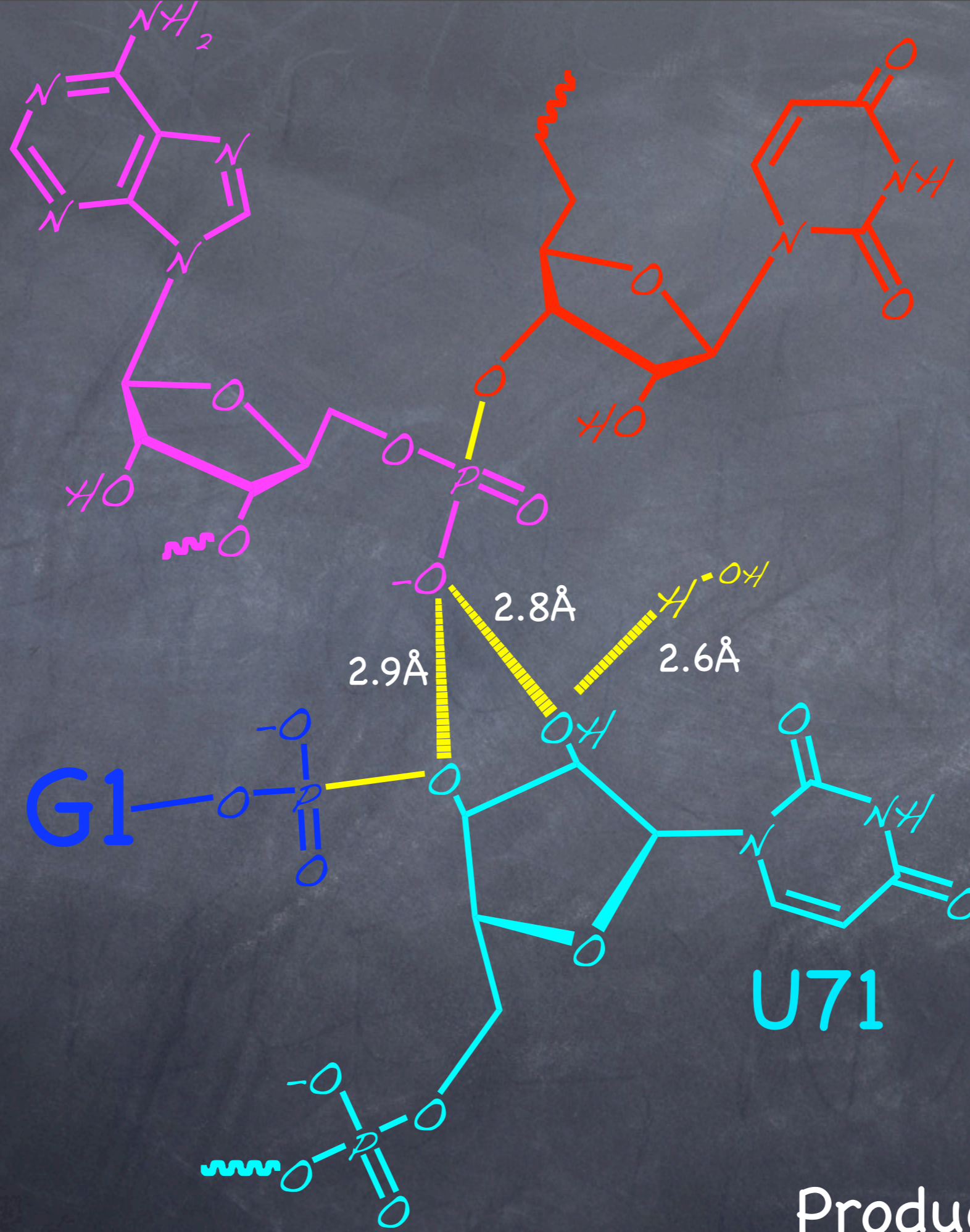
Stem A



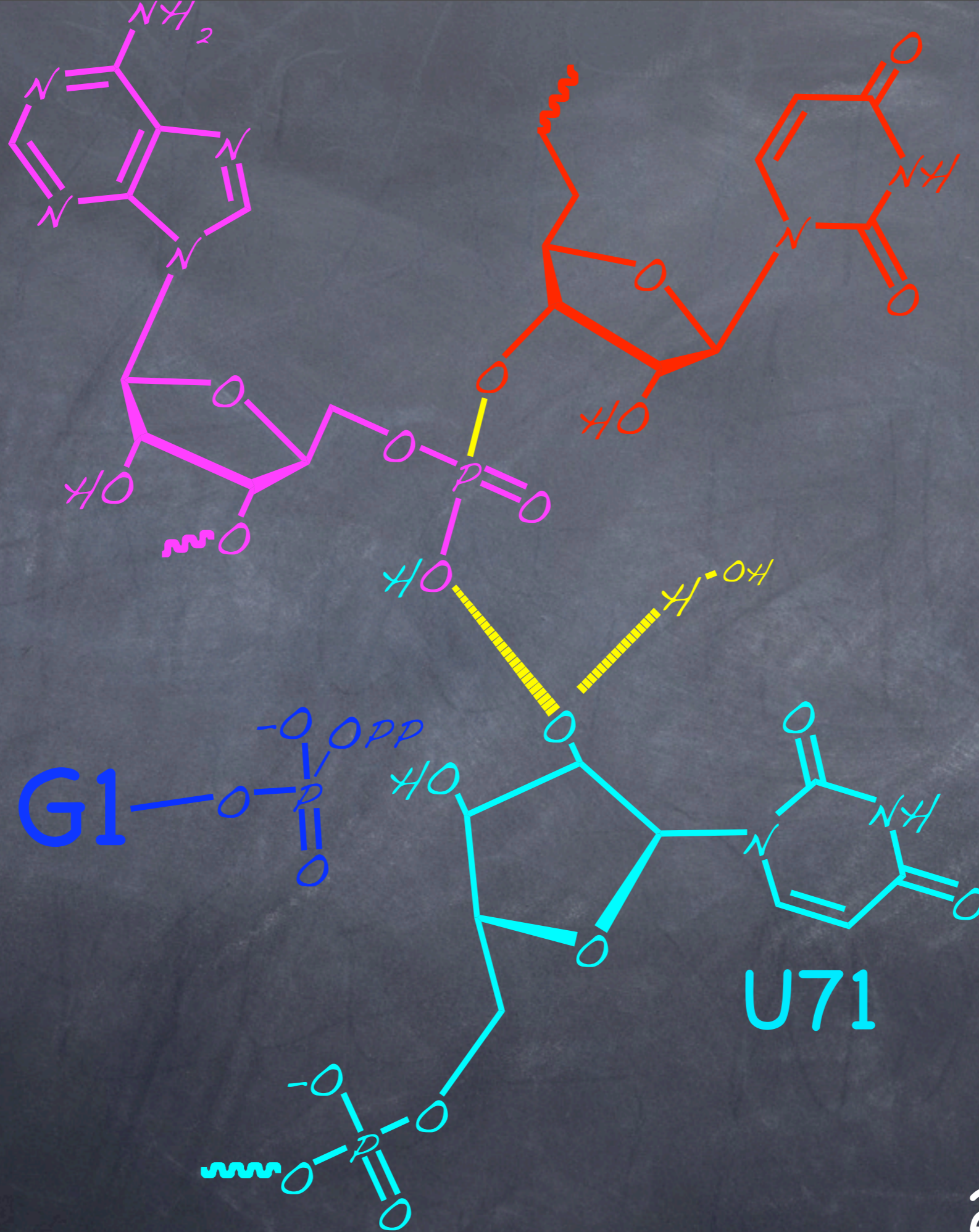
Stem C



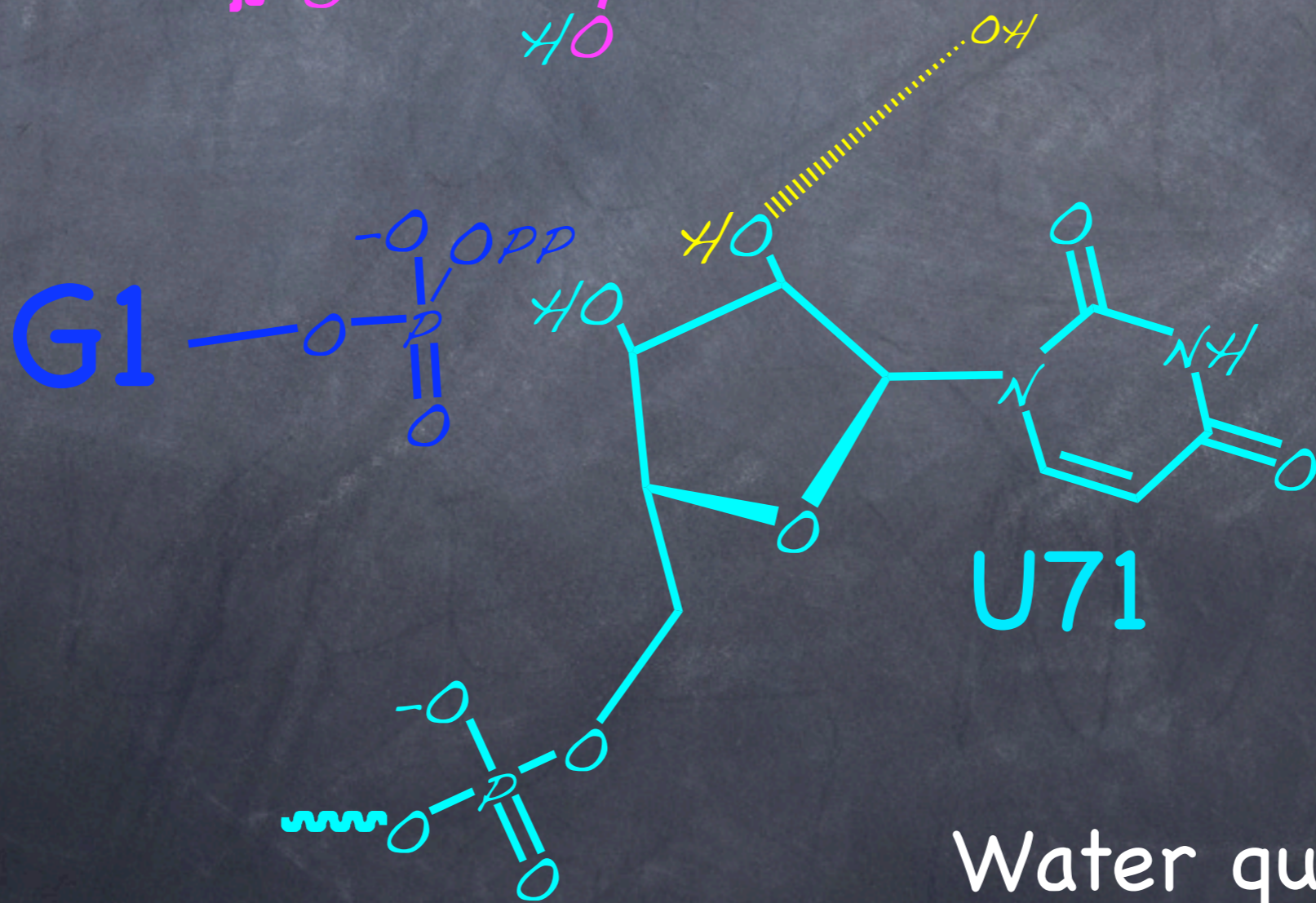
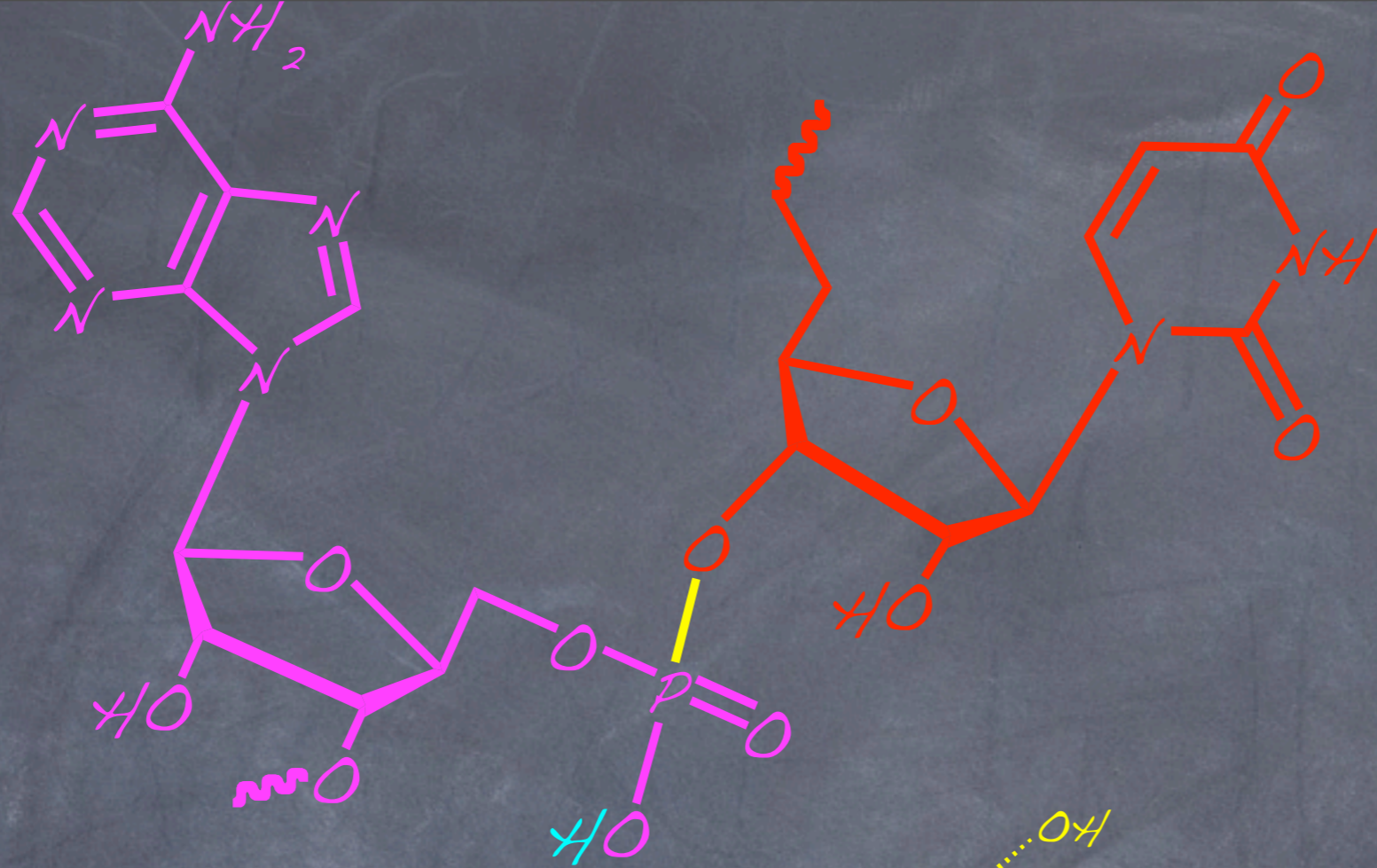
Stem B

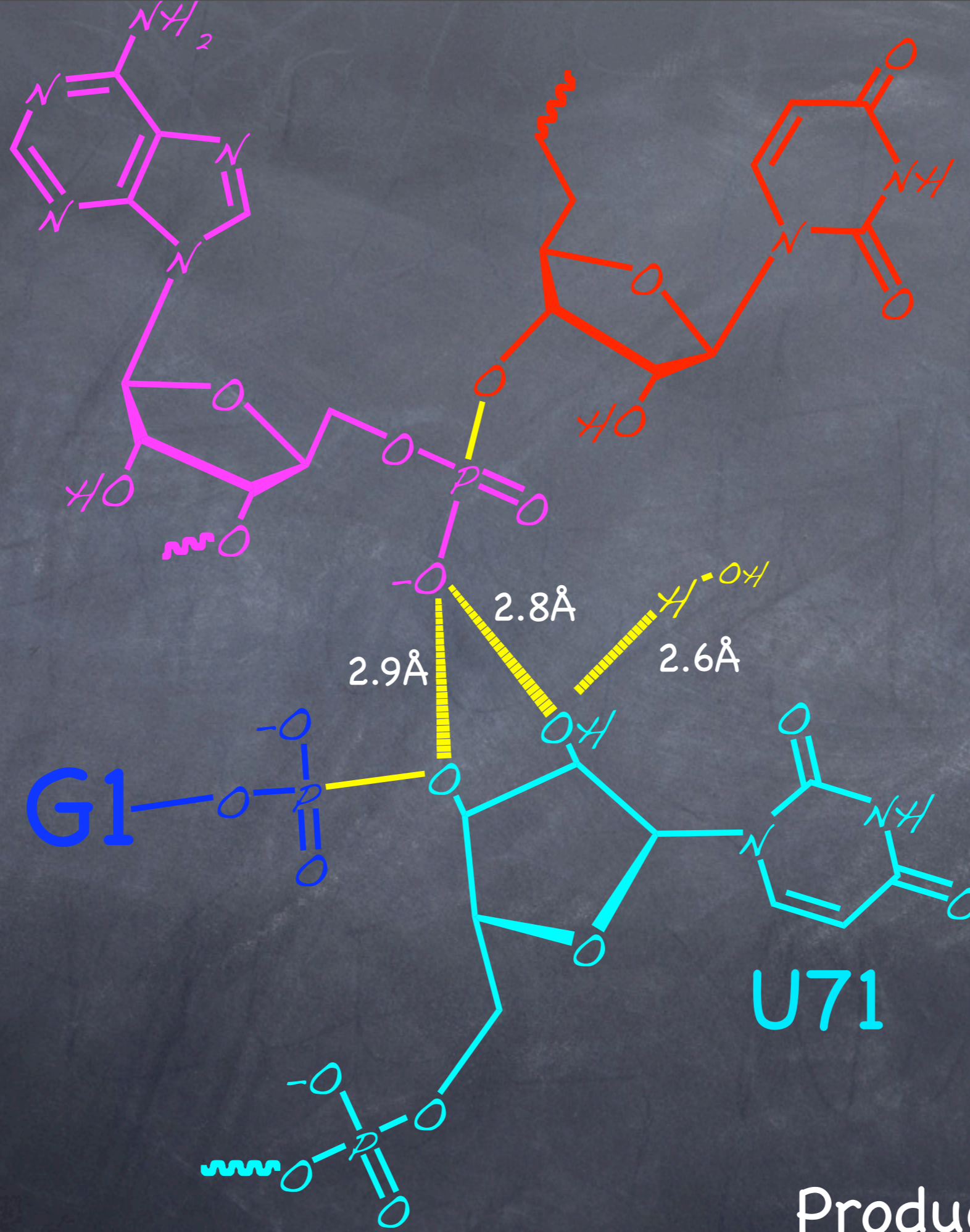


Product structure

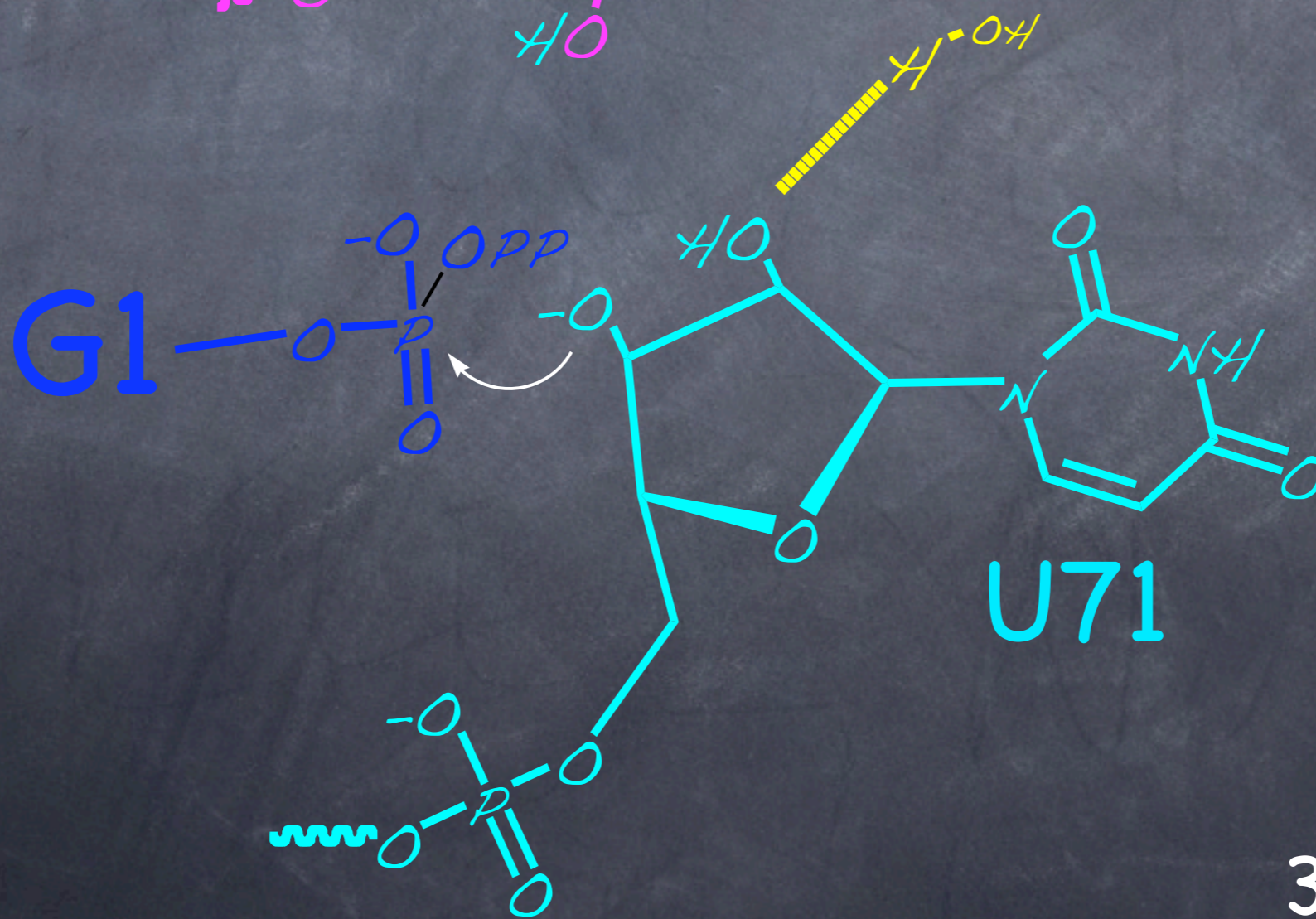
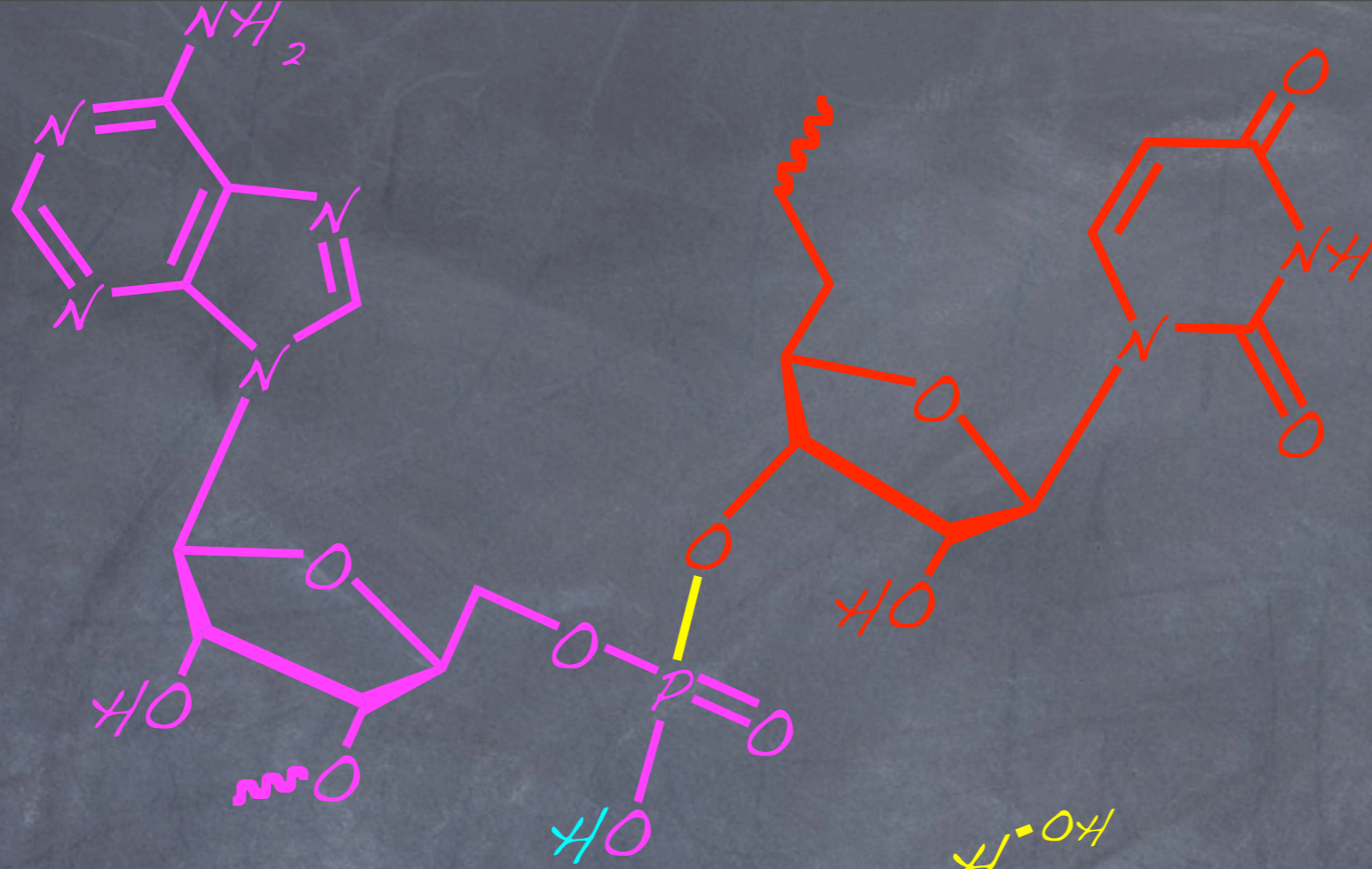


2'H transfer

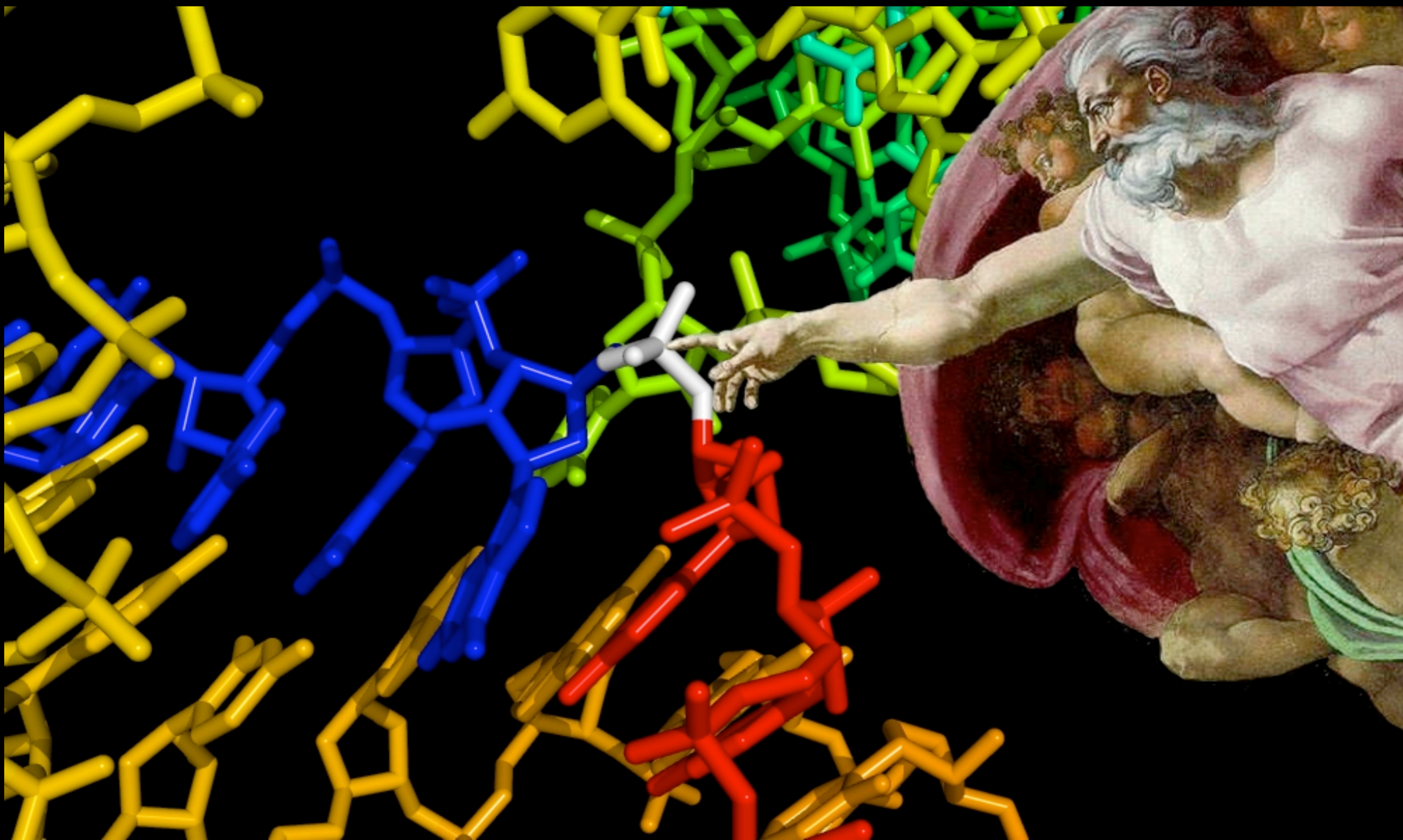




Product structure



The moment of bond creation ...



(Imaginary deity not shown to scale.)



Many thanks to

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• Andy Ellington

