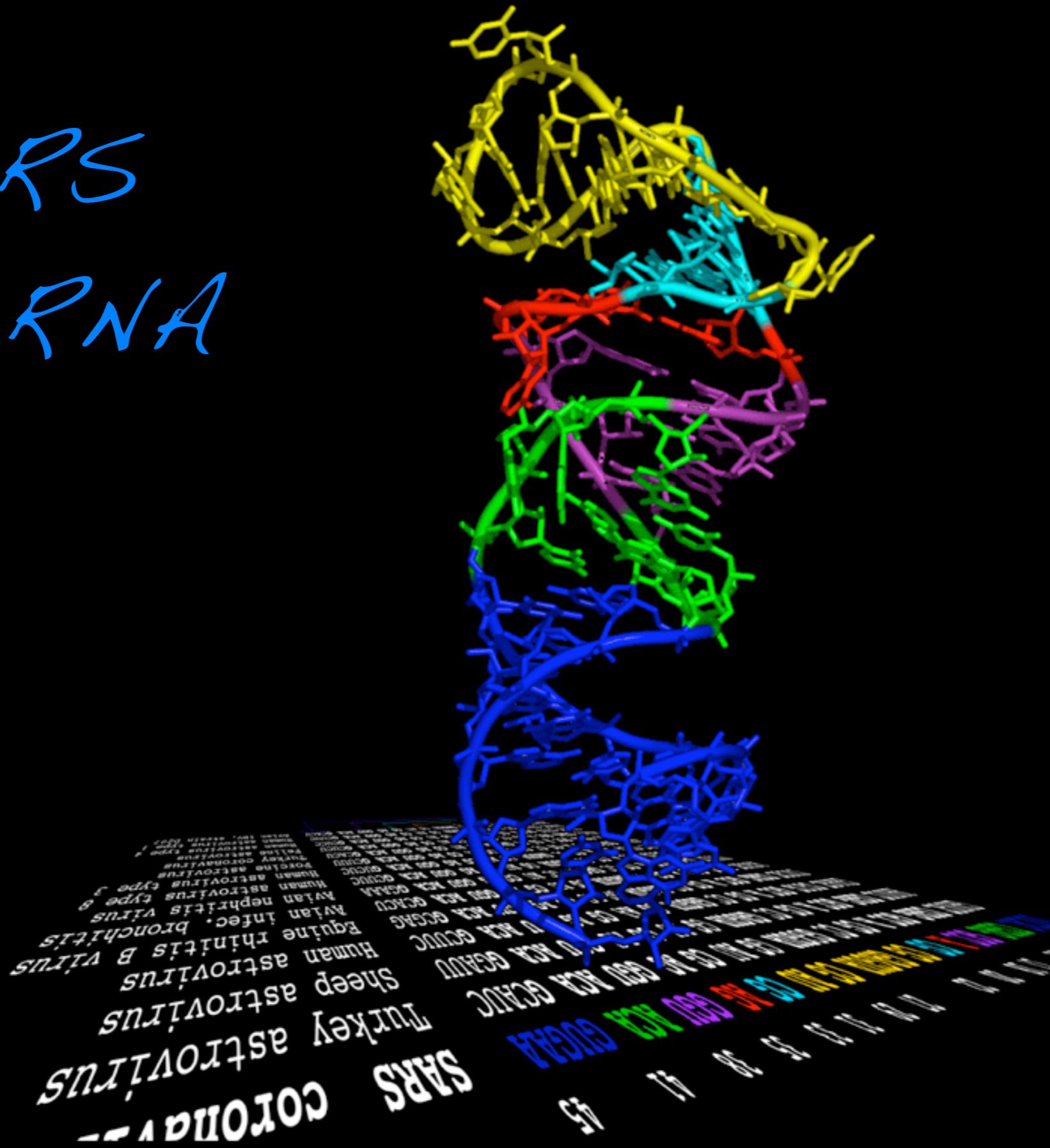


*Scott Lab*

# *Scott Lab*

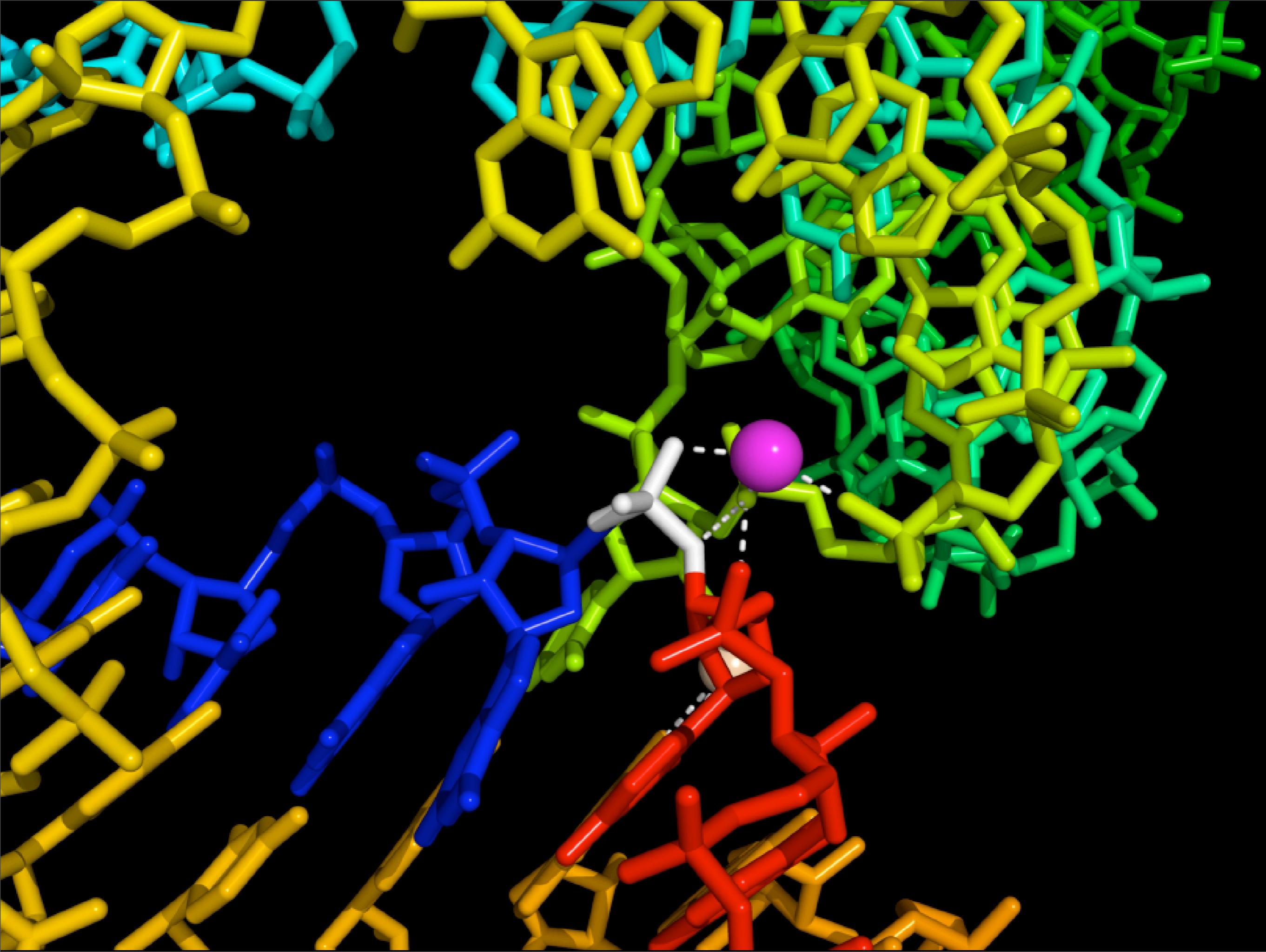
*"We are trying to  
understand RNA,  
catalysis and the origin  
of life."*

SARS  
5' m RNA



# *Hammerhead Ribozyme*





# The Structural Basis of Ribozyme-Catalyzed RNA Assembly



*Dr. Michael P. Robertson*



*Dr. Michael P. Robertson*





*Dr. Michael P. Robertson*





*Dr. Michael P. Robertson*



# The Central Dogma of Molecular Biology

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DNA → RNA → Proteins

# The RNA World Hypothesis

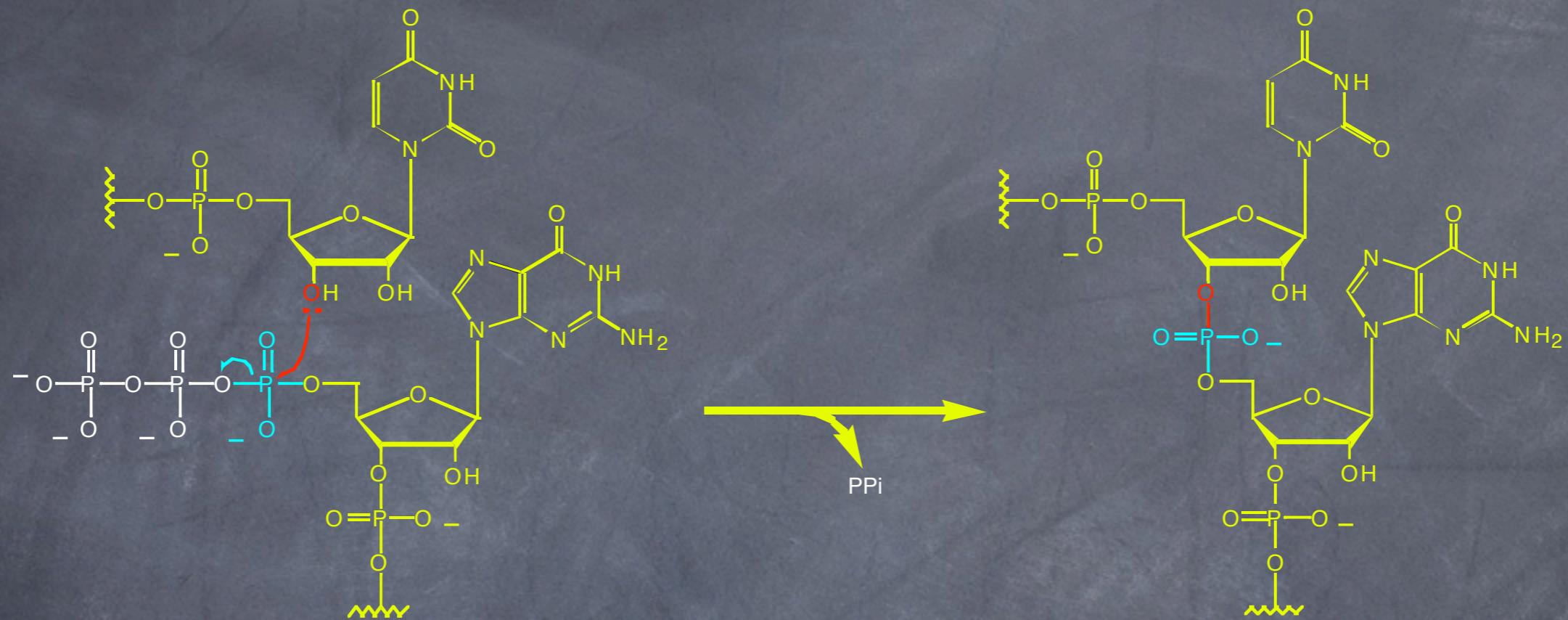
RNA

# The RNA World Hypothesis

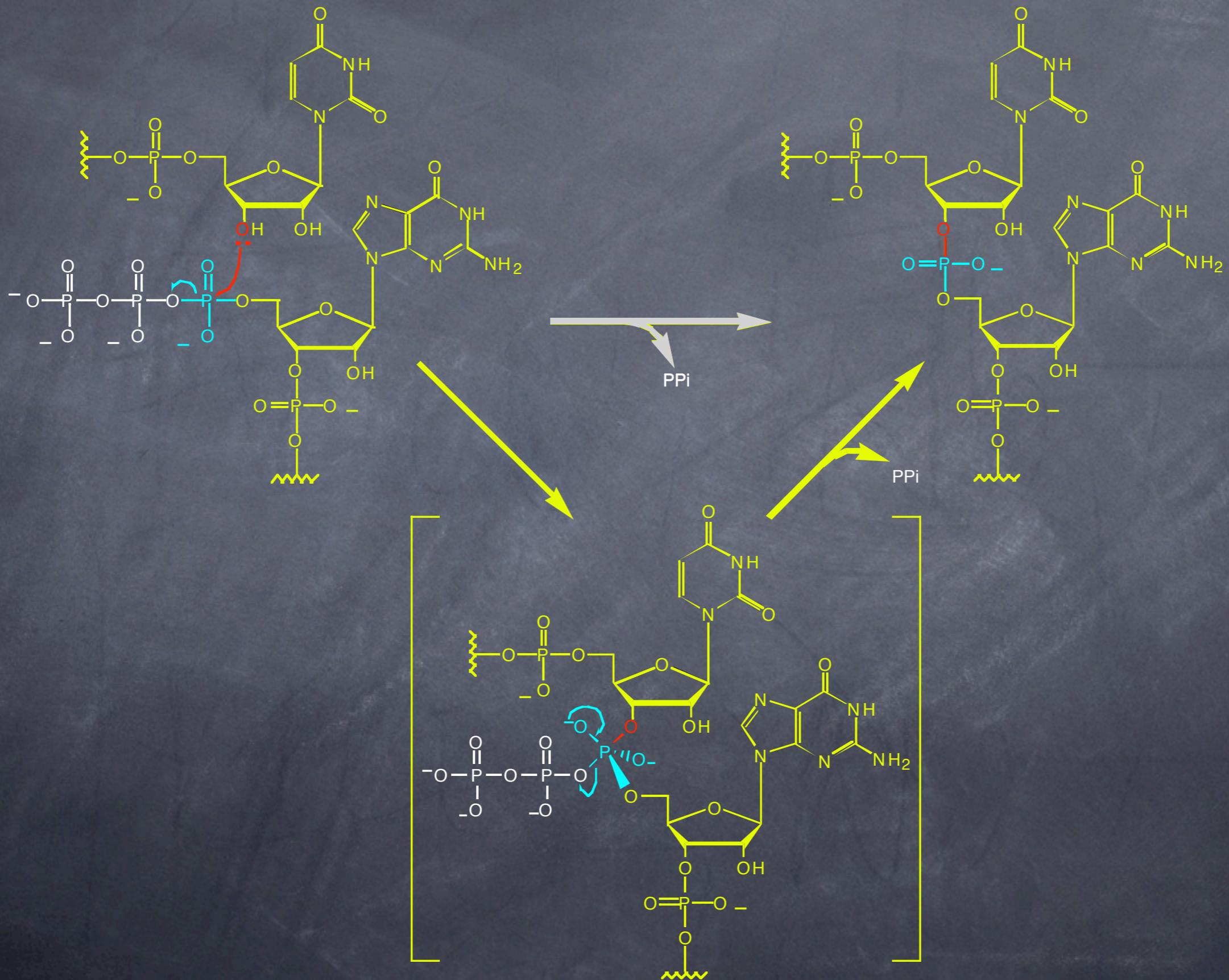
DNA → RNA → Proteins

# *RNA Ligation Mechanism*

# RNA Ligation Mechanism



# RNA Ligation Mechanism



# *in vitro selection*

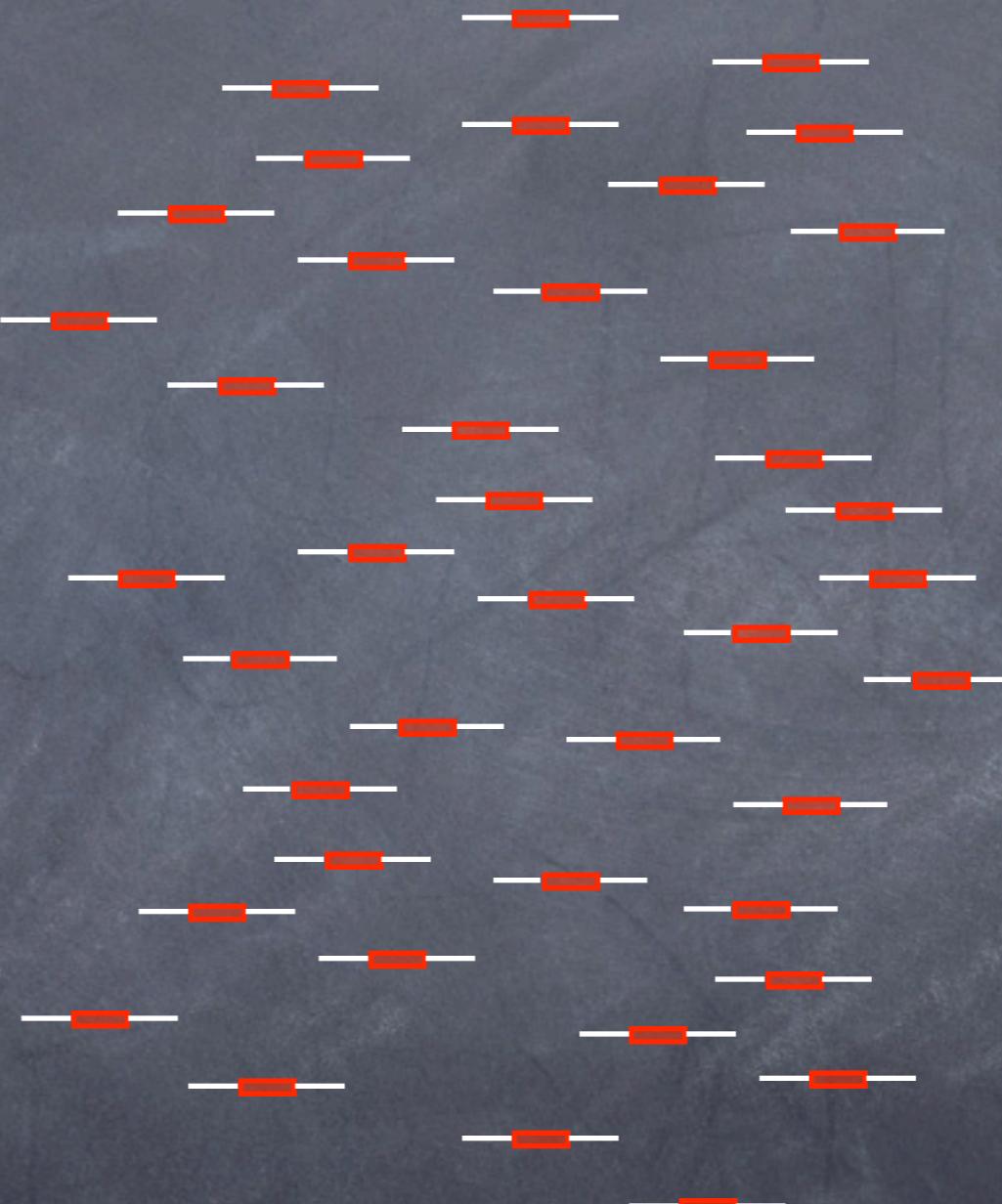
ggacuuucgguccaggagugcucgug - N90 + cucgugauguccagucgc



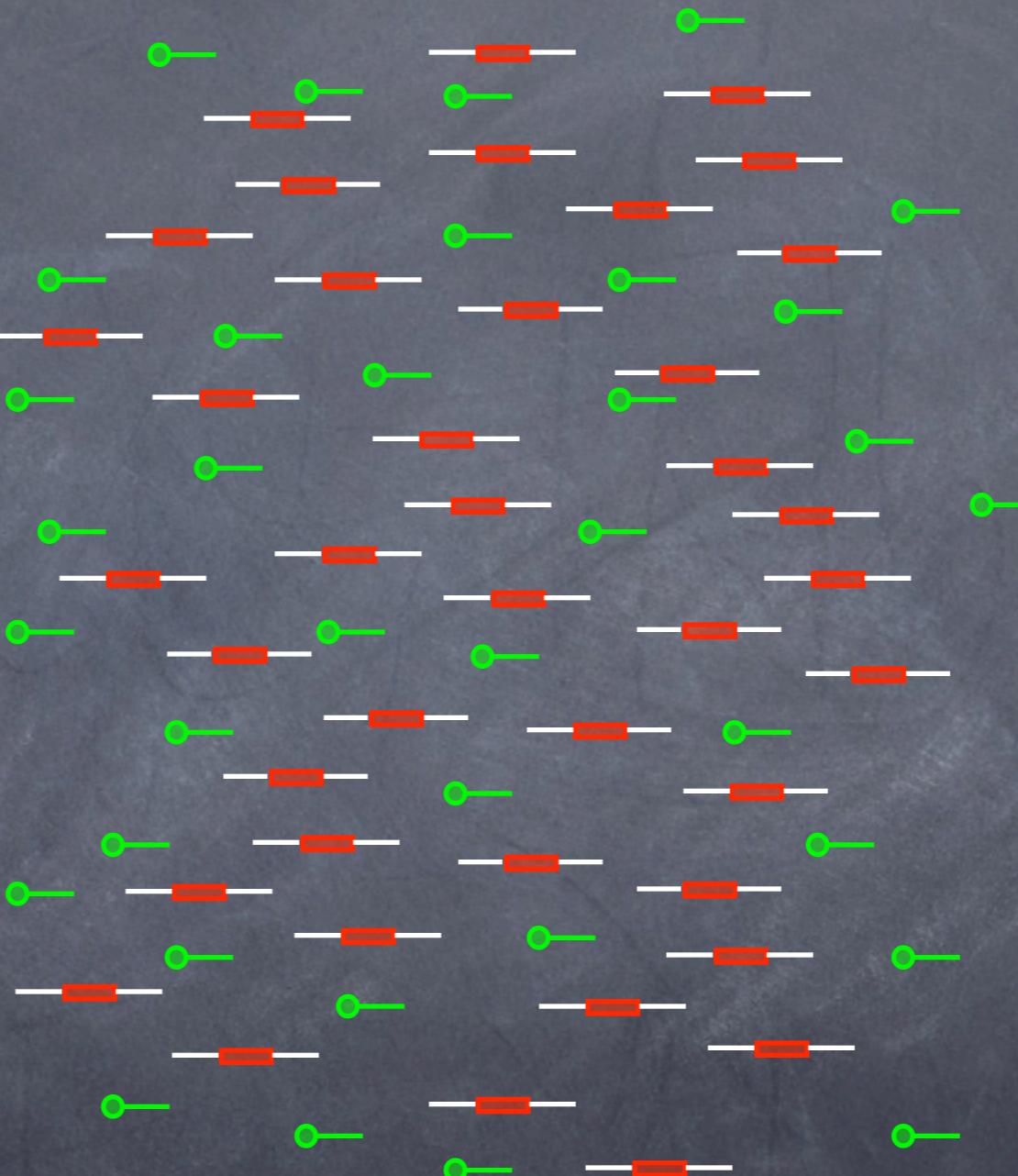
*in vitro selection*



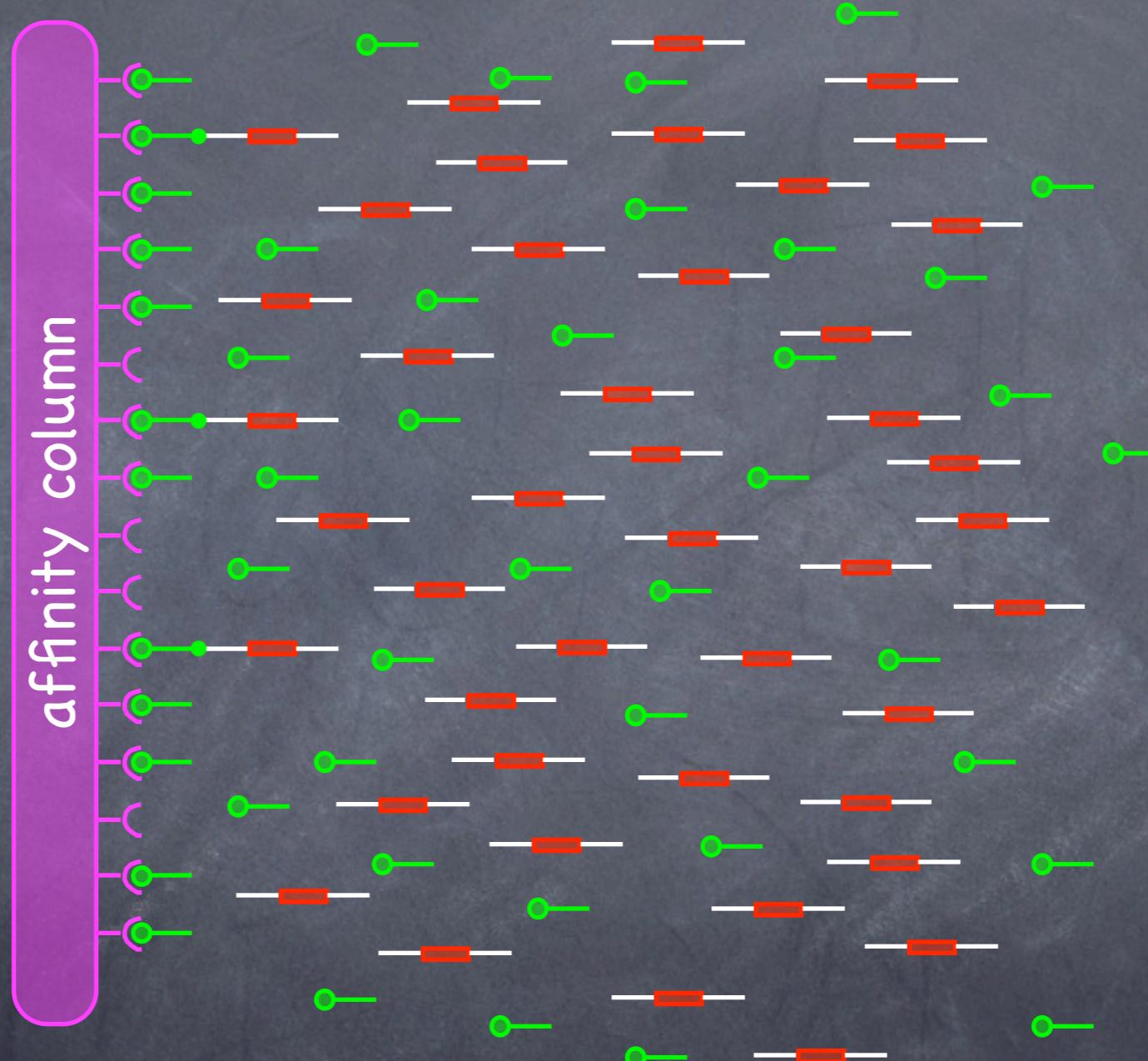
# *in vitro selection*



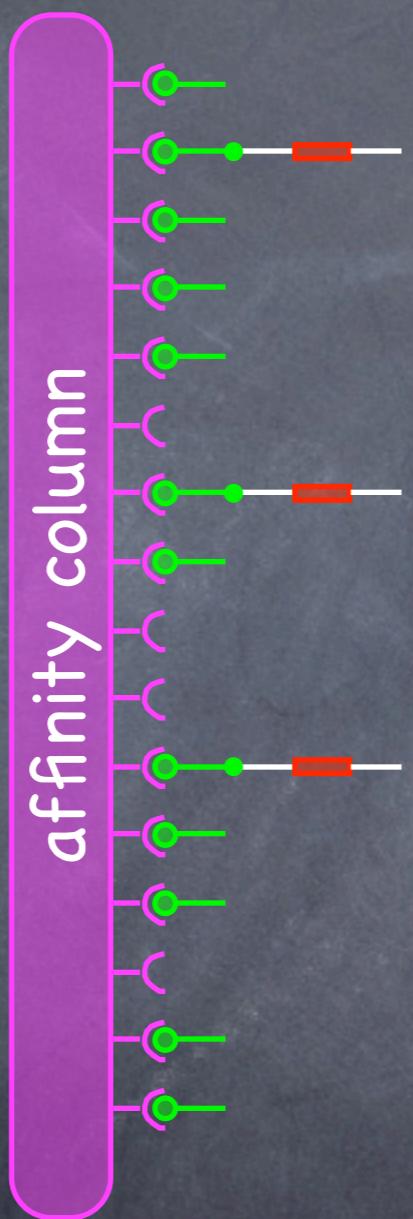
# *in vitro selection*



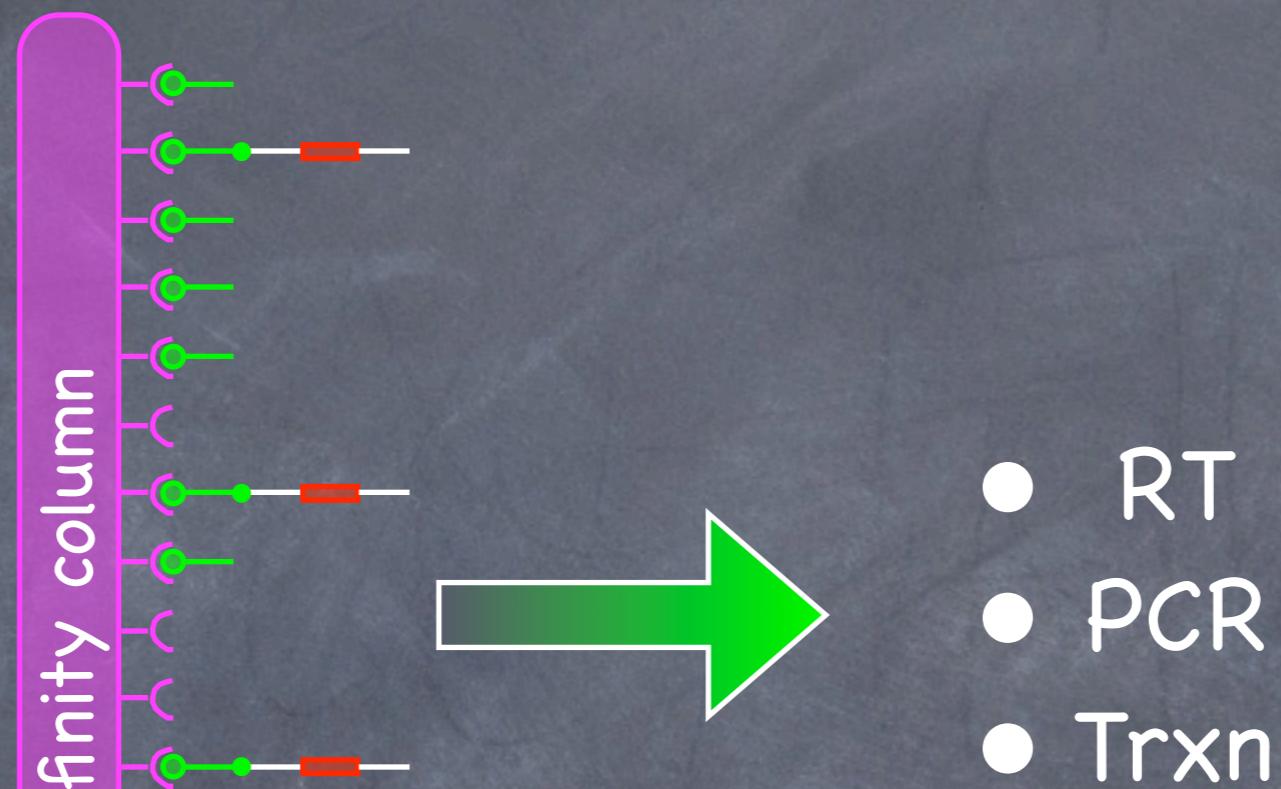
# *in vitro selection*



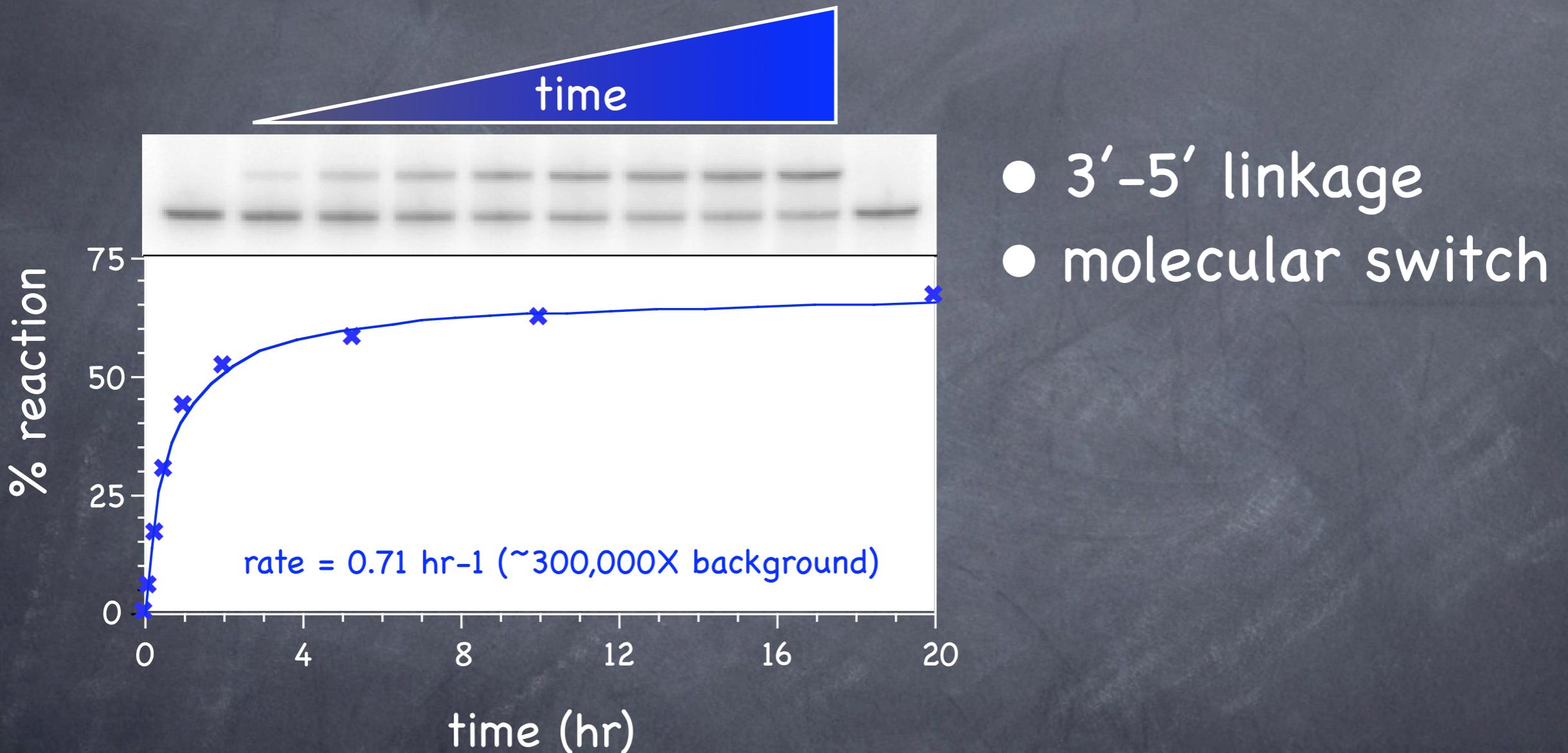
# *in vitro selection*



# *in vitro selection*



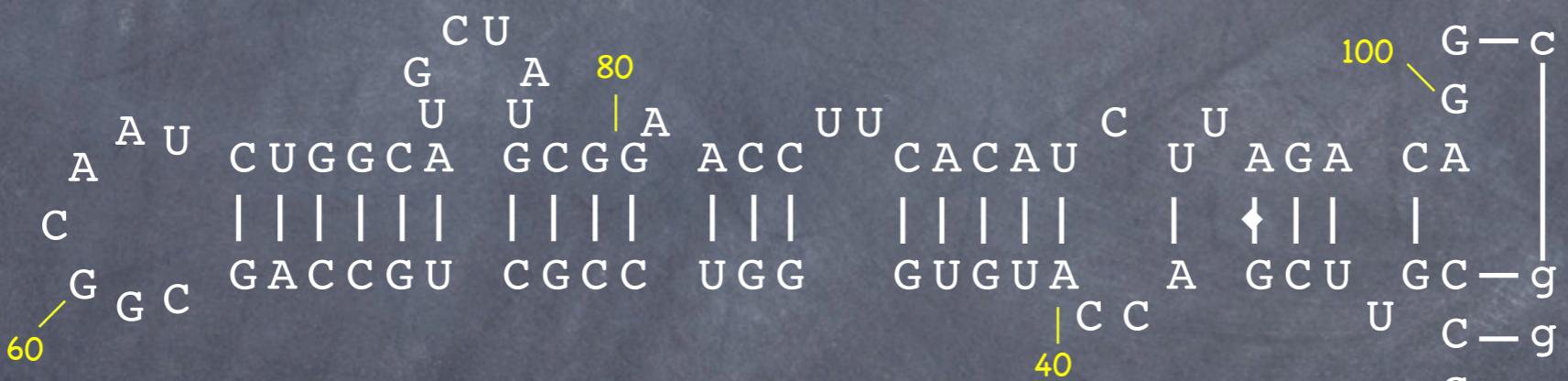
# Li ligase



# Li Ligase

2° structure

## Stem C



## Stem A

A ← g — 1  
U ← g  
U — a  
G — c  
G ← u  
A — u

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

## Stem B

a    g  
a    a

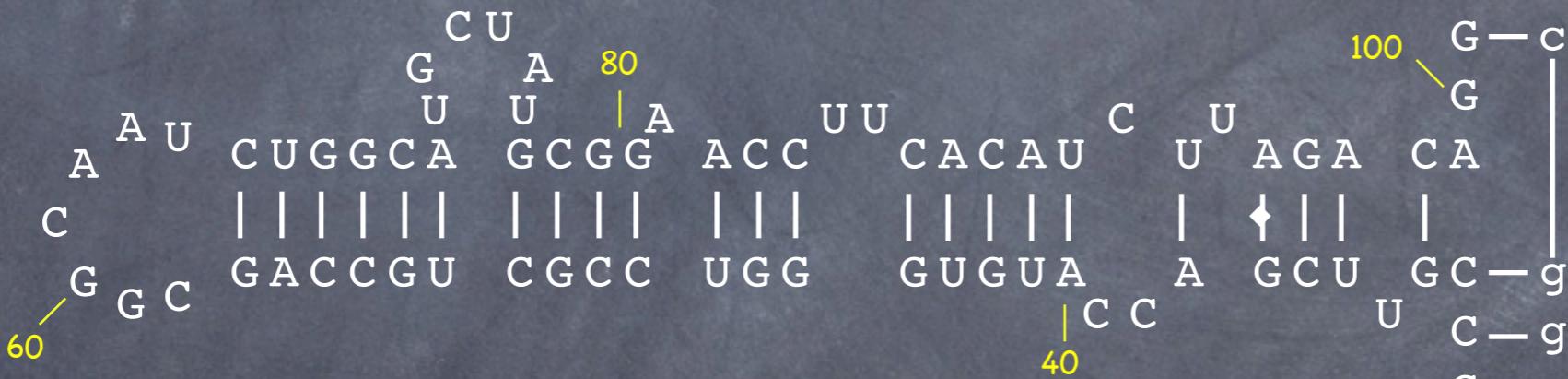
20 /



# Li Ligase

2° structure

## Stem C



## Stem A



## Stem B

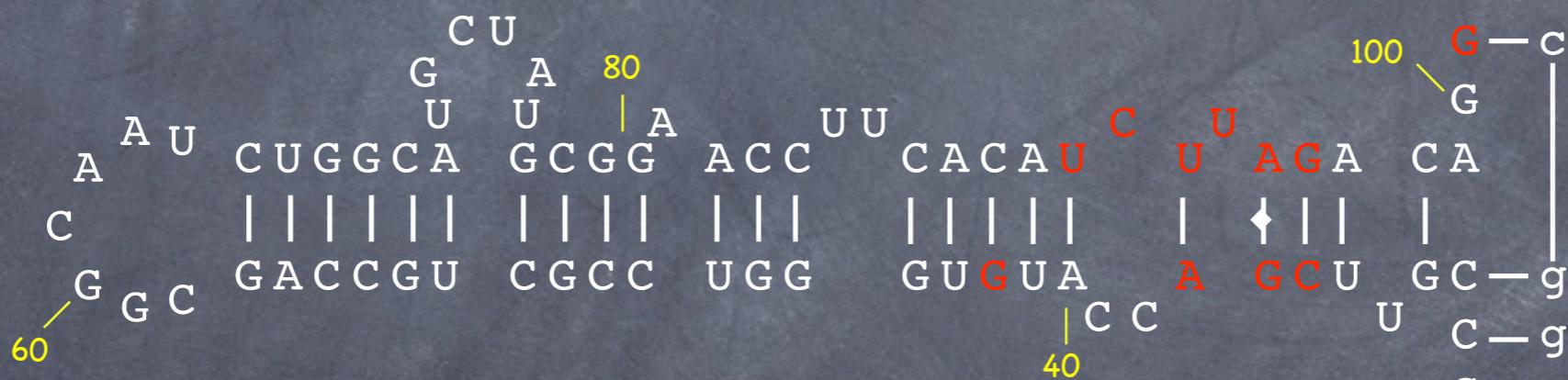
a g  
a a



# LI Ligase

conserved positions

## Stem C



100% conserved

## Stem A



## Stem B

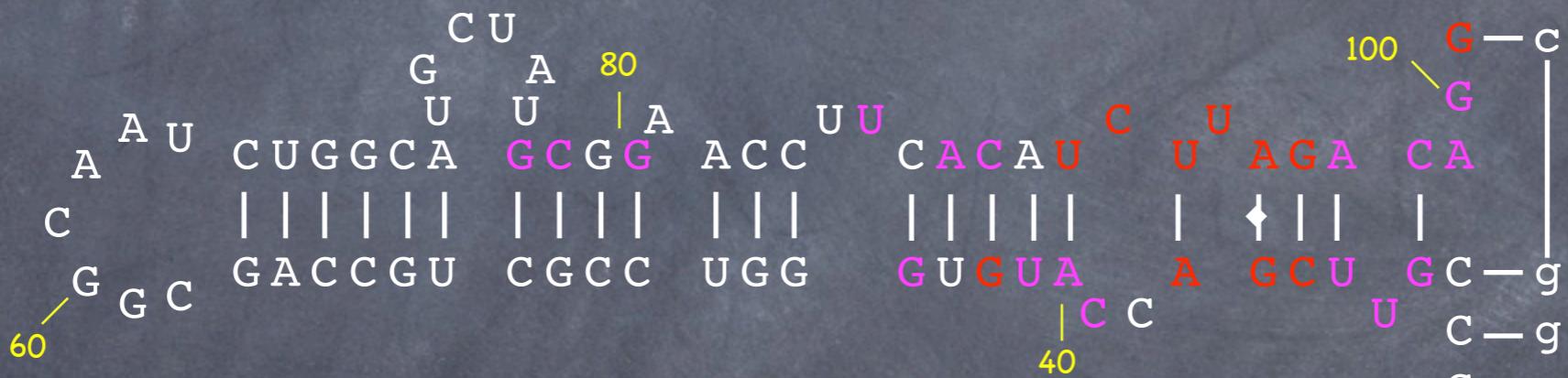
a a g  
a a a



# LI Ligase

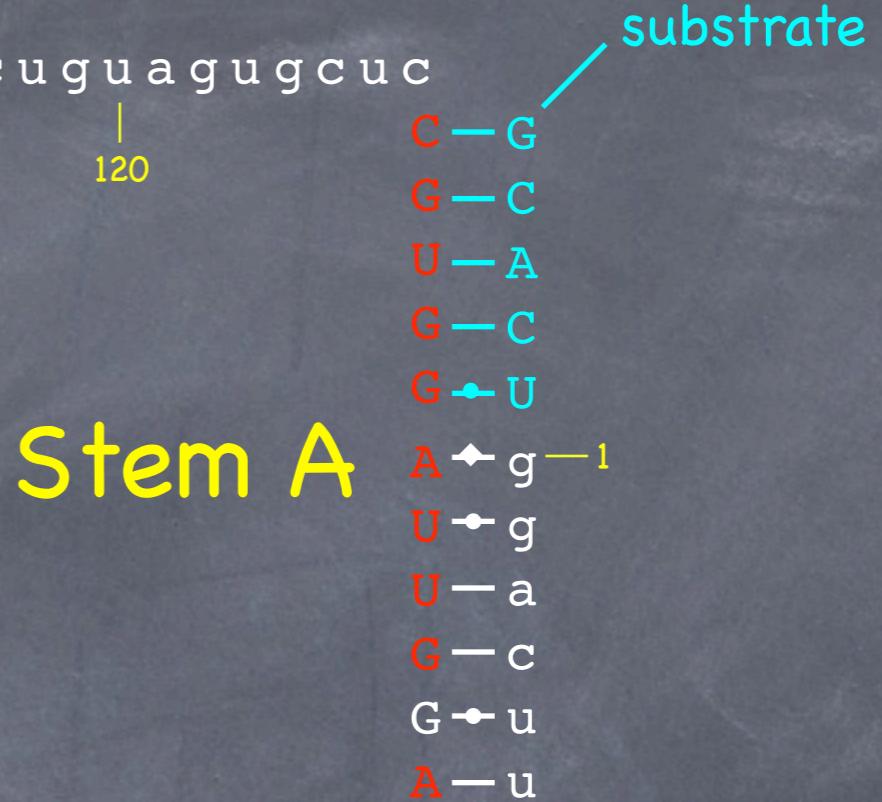
conserved positions

## Stem C



100% conserved  
≥85% conserved

## Stem A



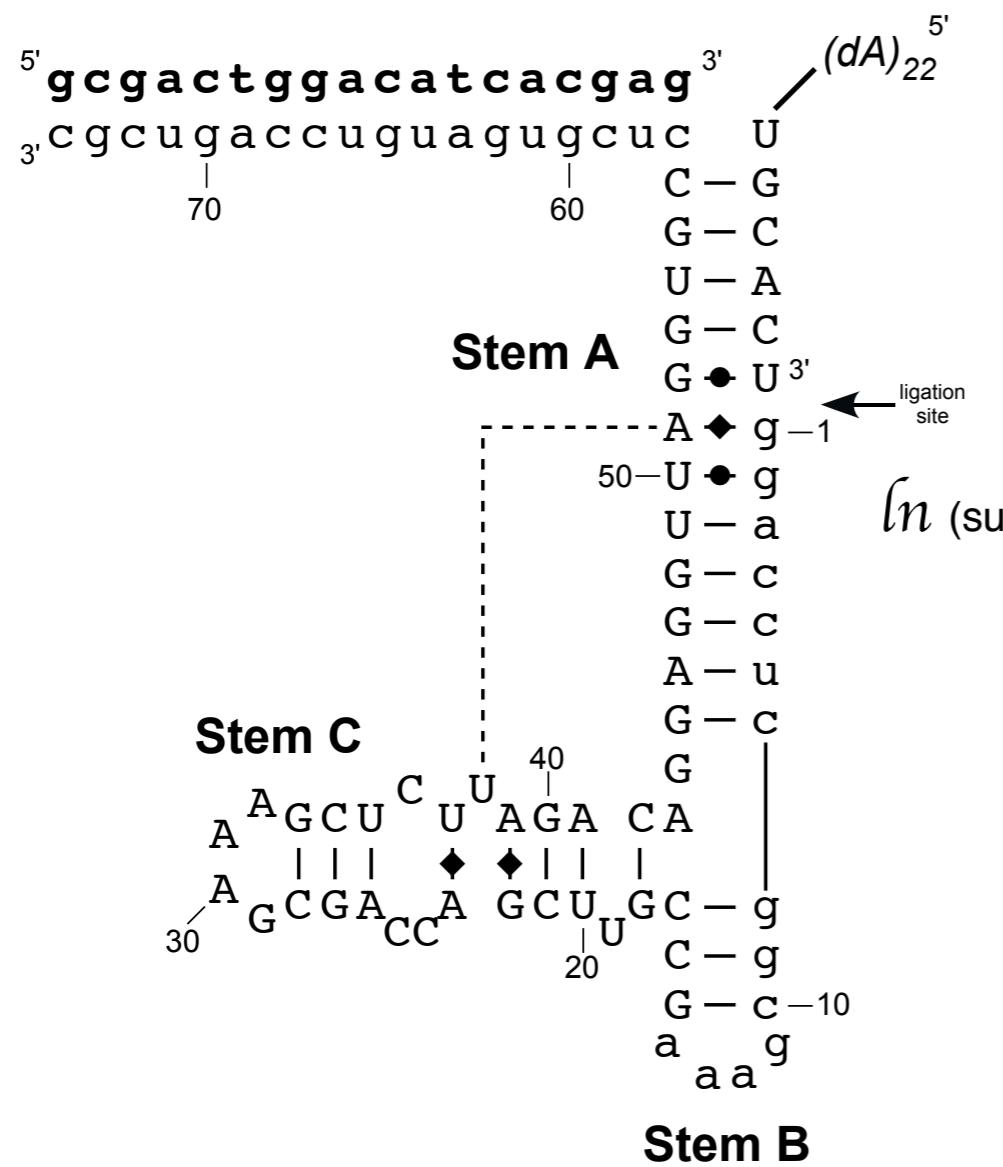
## Stem B

a a a g g  
20 /



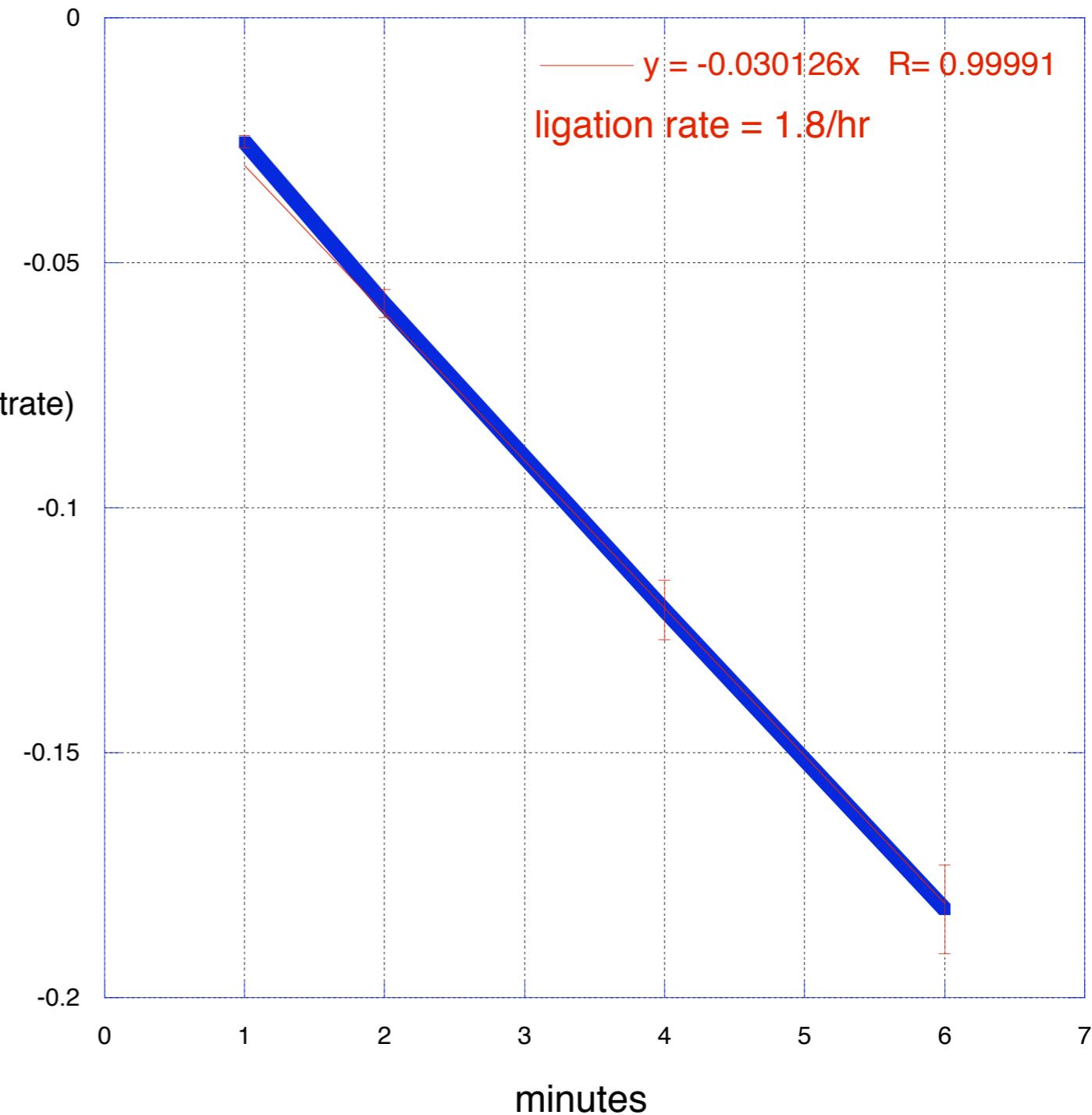
# A smaller ligase ribozyme

A



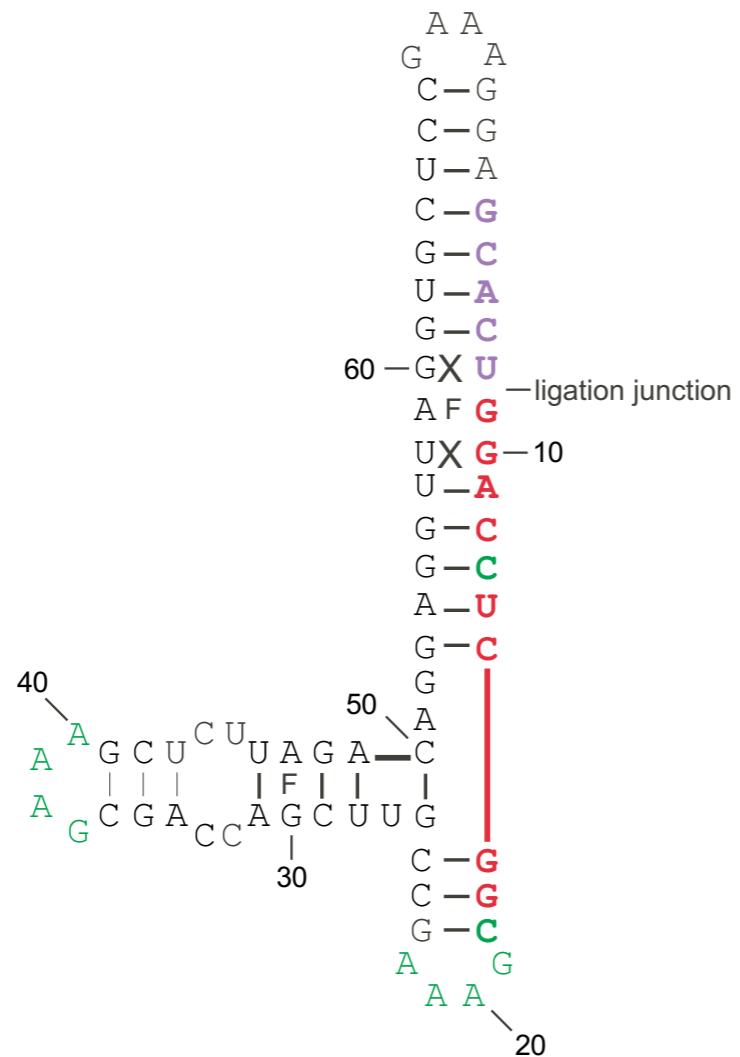
B

ribozyme ligation rate



# A unimolecular variant

L1X6c



115 bp DNA

71 nt transcript

71 nt circularL1 RNA construct

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

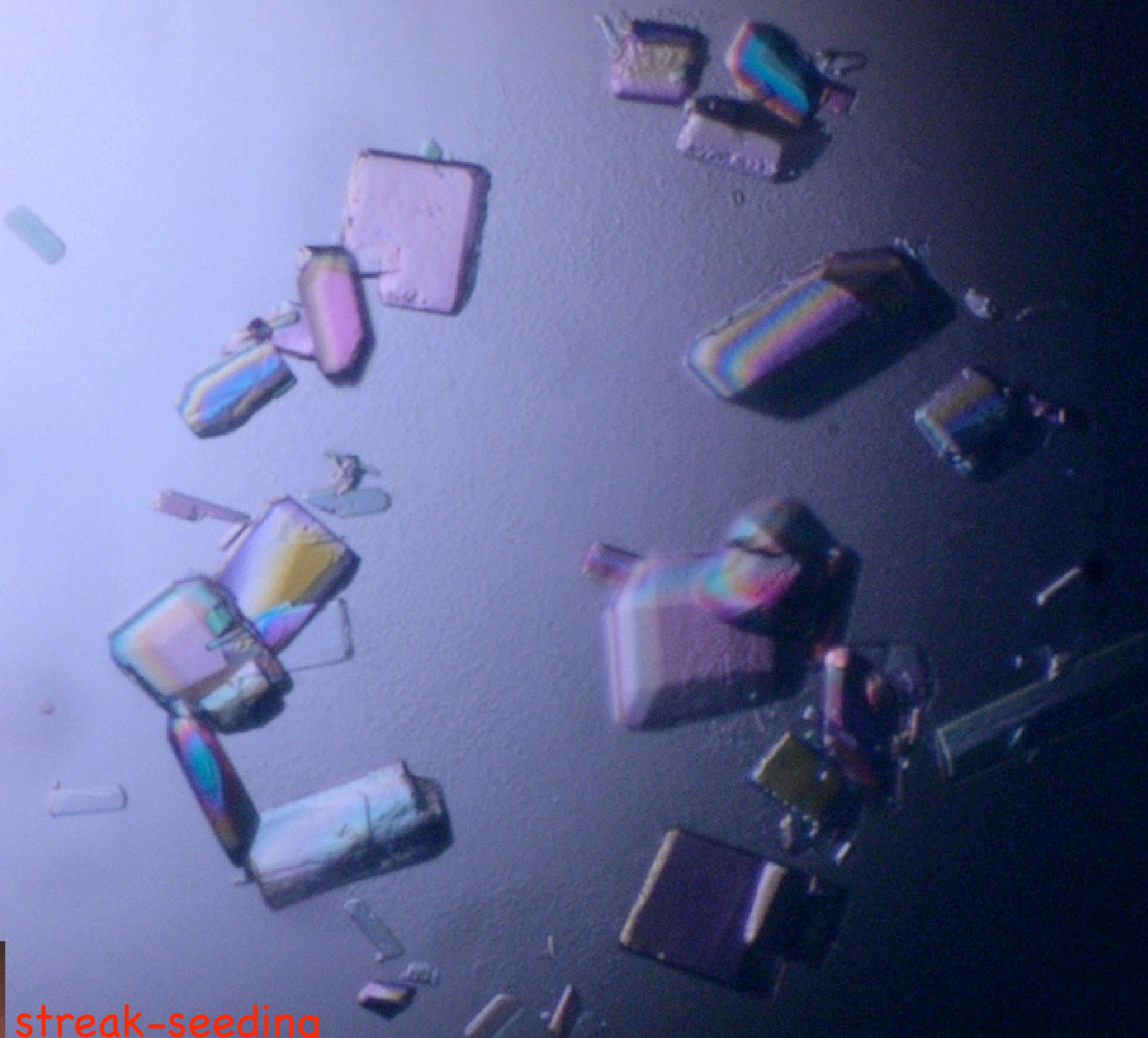
Amplify L1X2 template with  
44.L1X2.5'/43.L1X6c.3' primer set

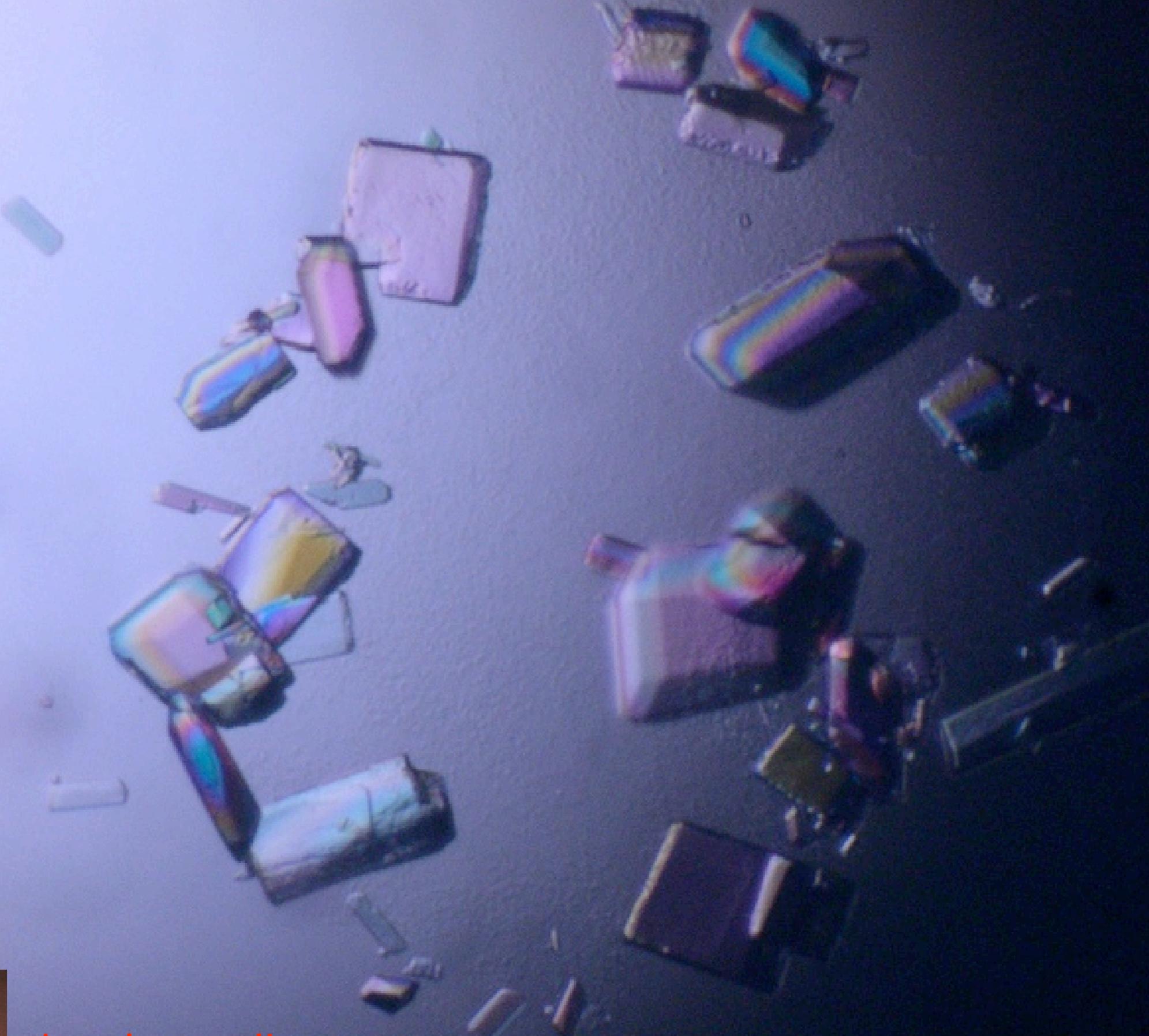
CG**GAATT**C TTCTAATACGACTCACTATA**GGACC**  
**TCGGCGAA**AGCCGTTGACCAAGC**GAAA**GCTCTT  
AGACAGGAGGTTAGGTGCTCCGAAAGGAGCACT  
**GGAGAC**TG**GGATCC**CG

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
$R_{\text{merge}}$	0.044	0.467
$R_{\text{meas}}$ (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 <sup>2</sup>	

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 <sup>2</sup>	

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 <sup>2</sup>	
Side-Chain Angle Refined Atoms	1.857 Å <sup>2</sup>	

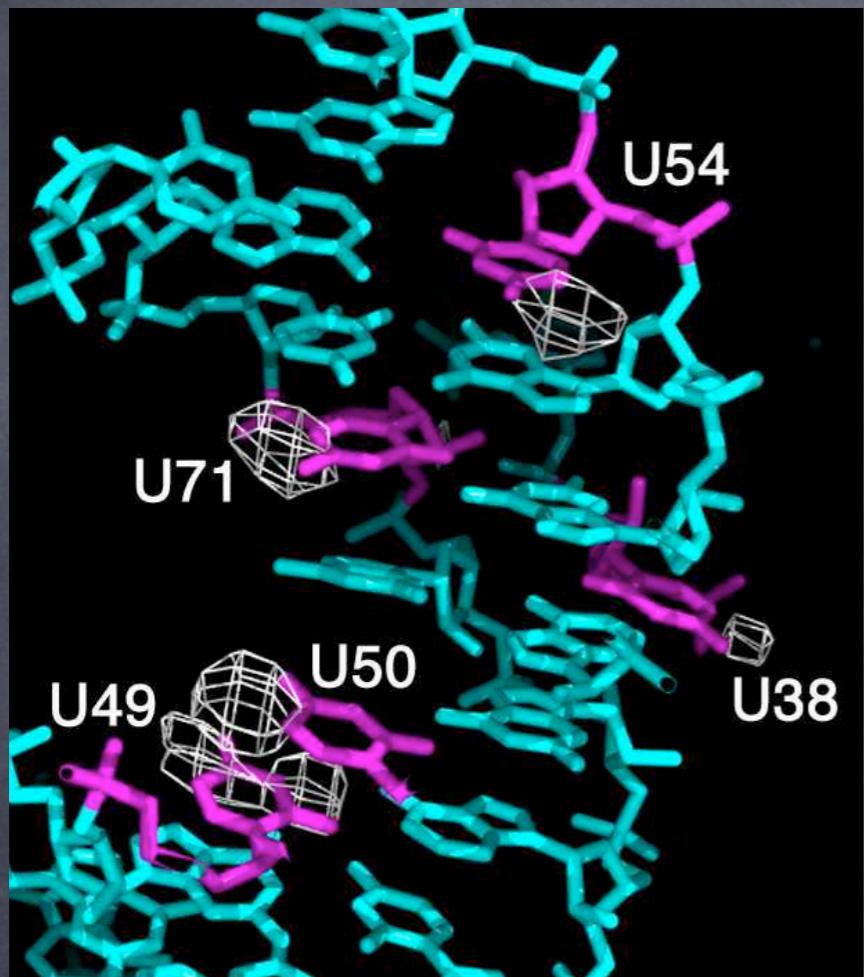
2.6 Å structure

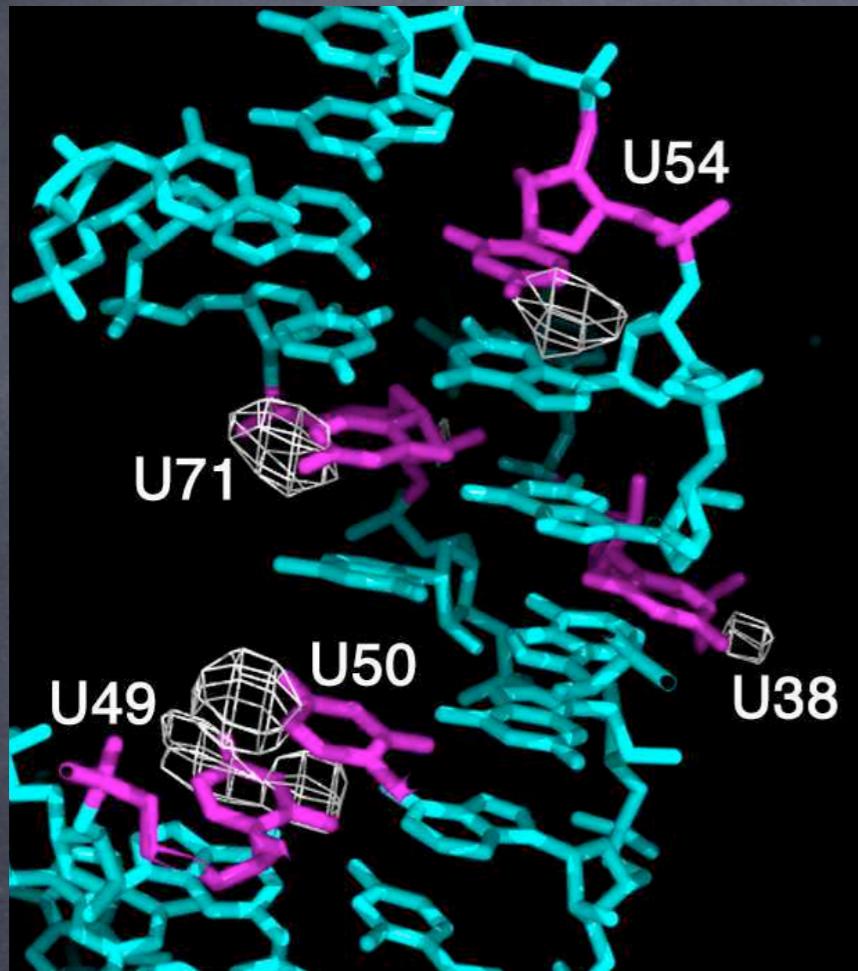
$R_{\text{free}} = 23.9\%$

*rmsd bonds = 0.01 Å*

142 nucleotides  
in asymm. unit

phase problem solved  
w/o derivatives

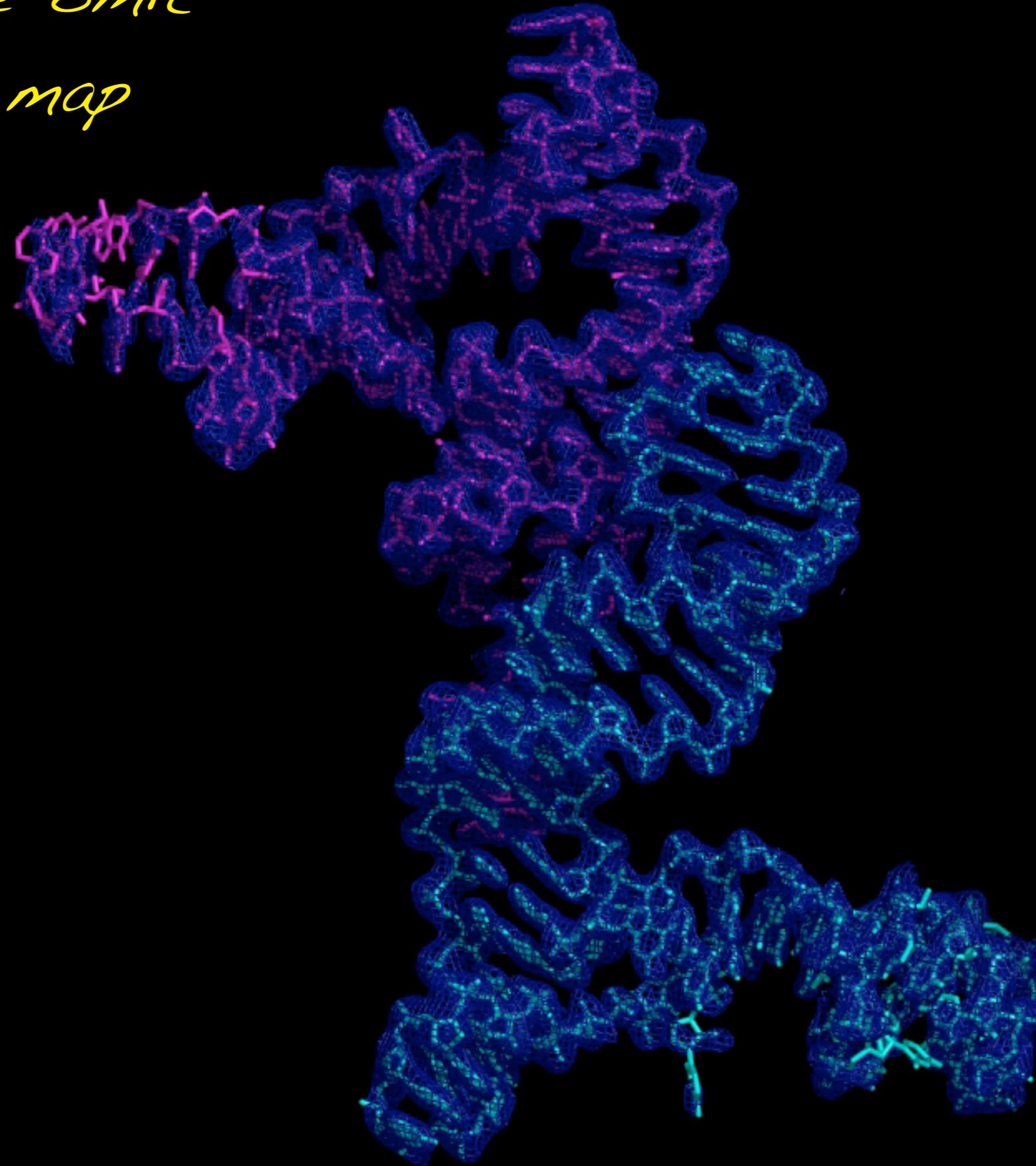


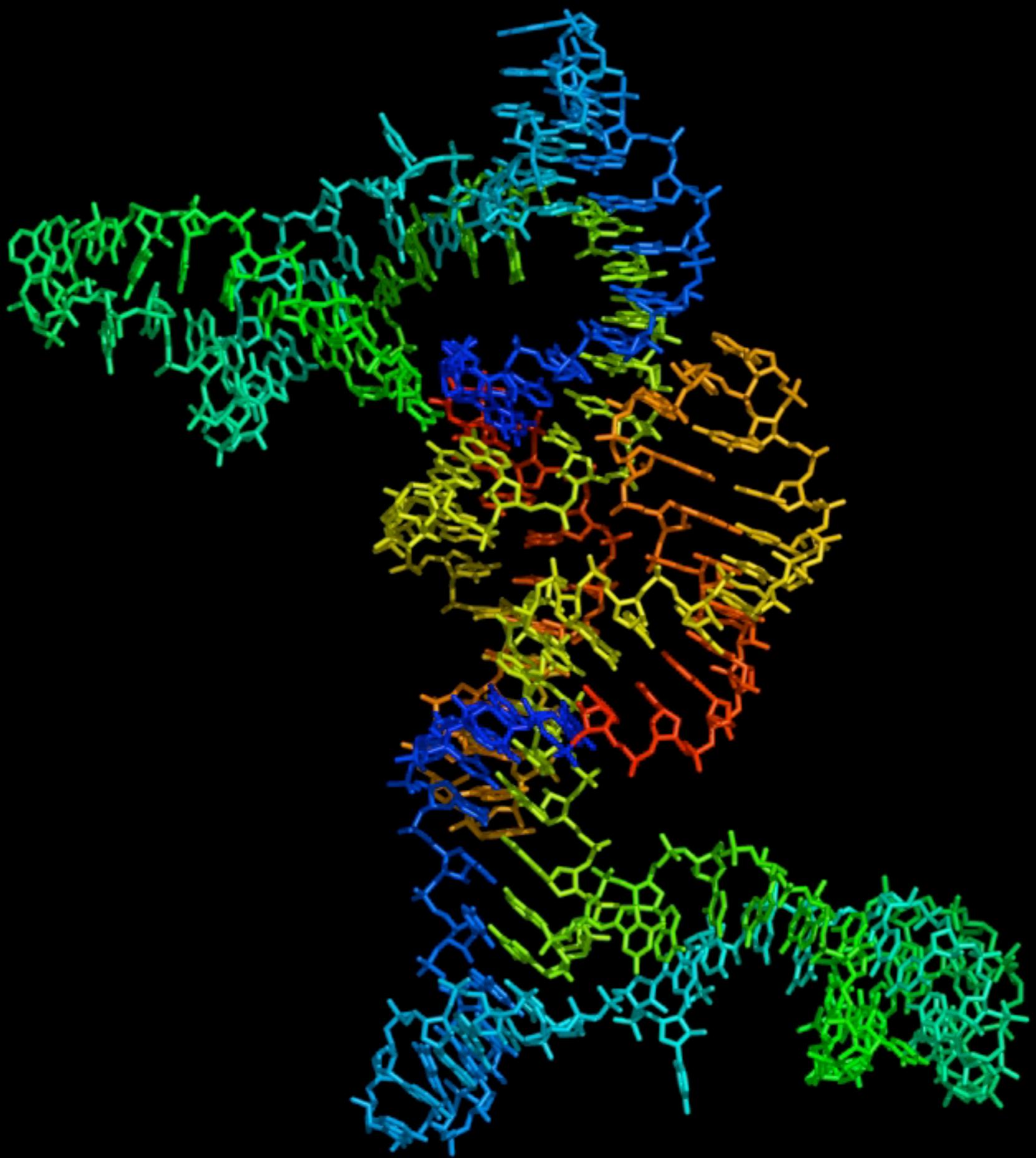


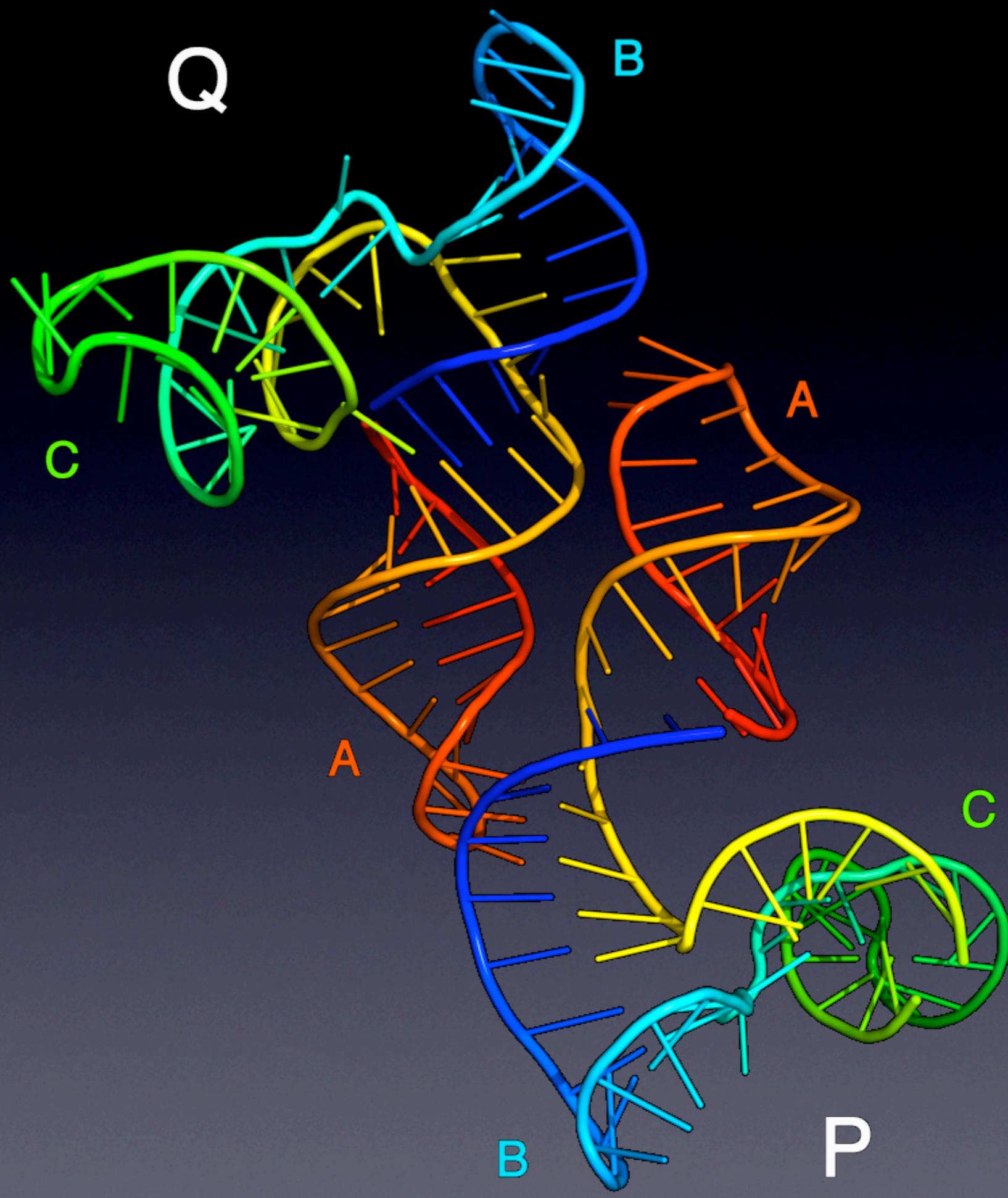
5-Br-U  
transcription  
product:

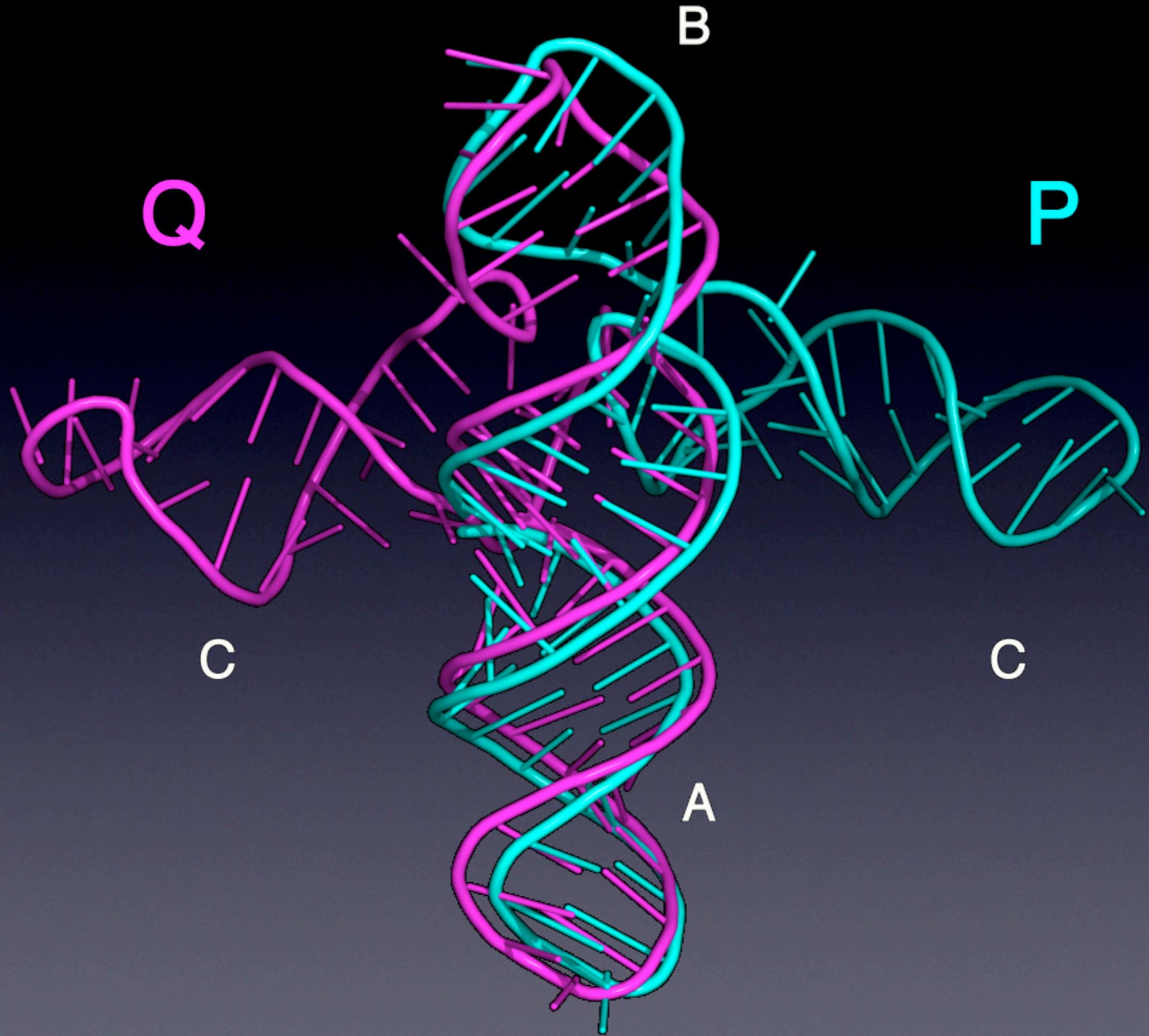
difference Fourier  
confirms trace

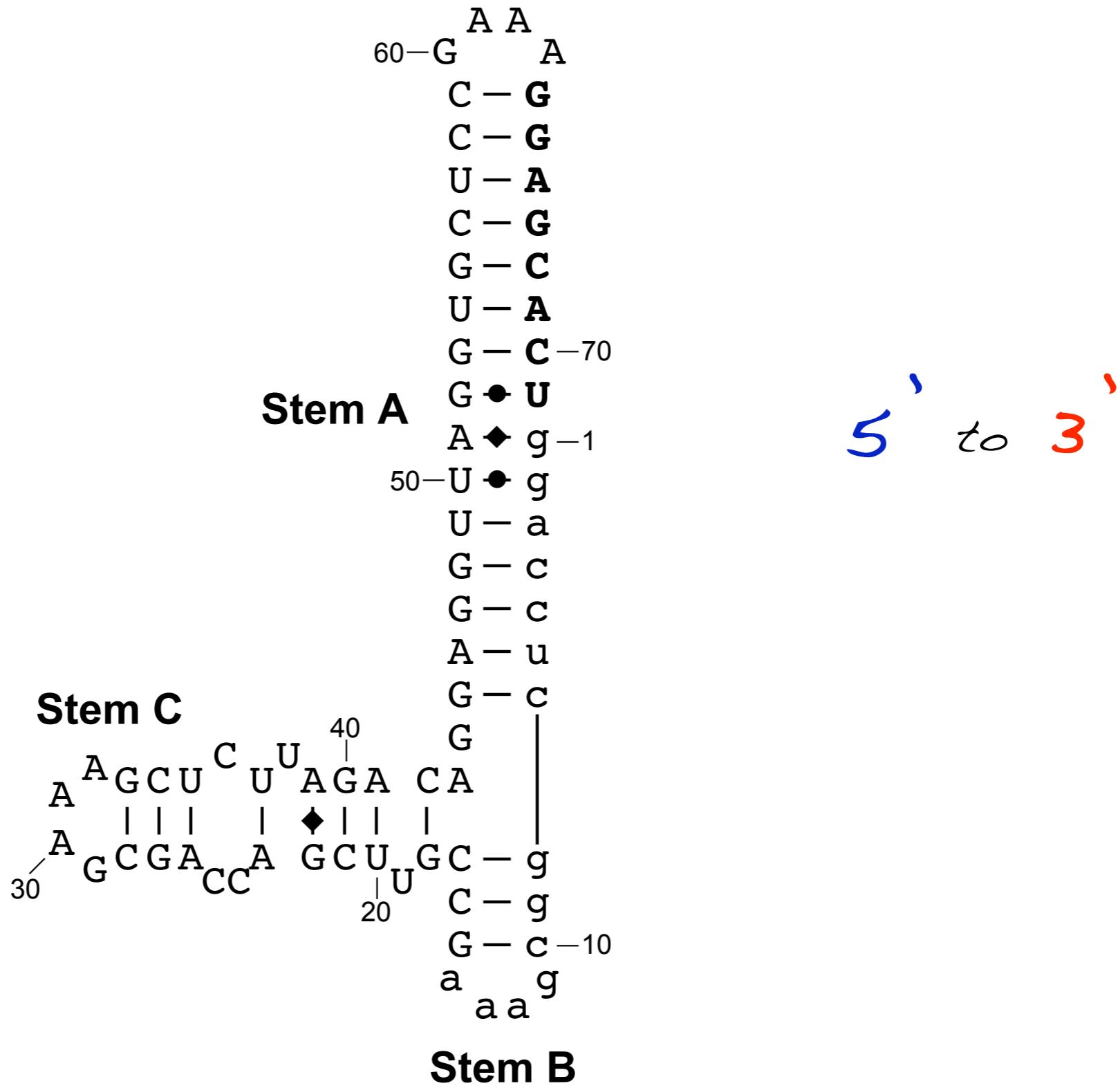
composite-omit  
2Fo-Fc map

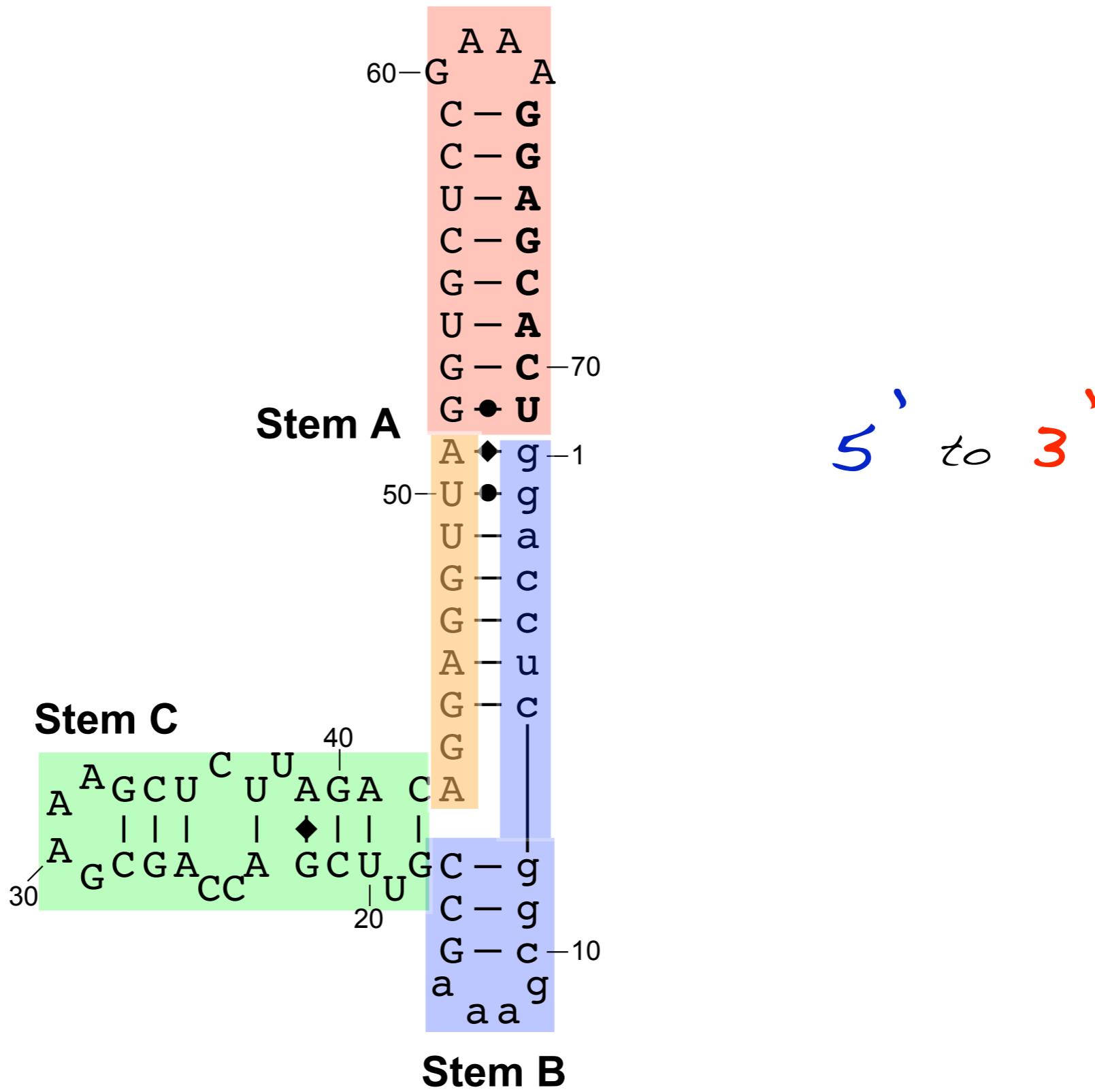






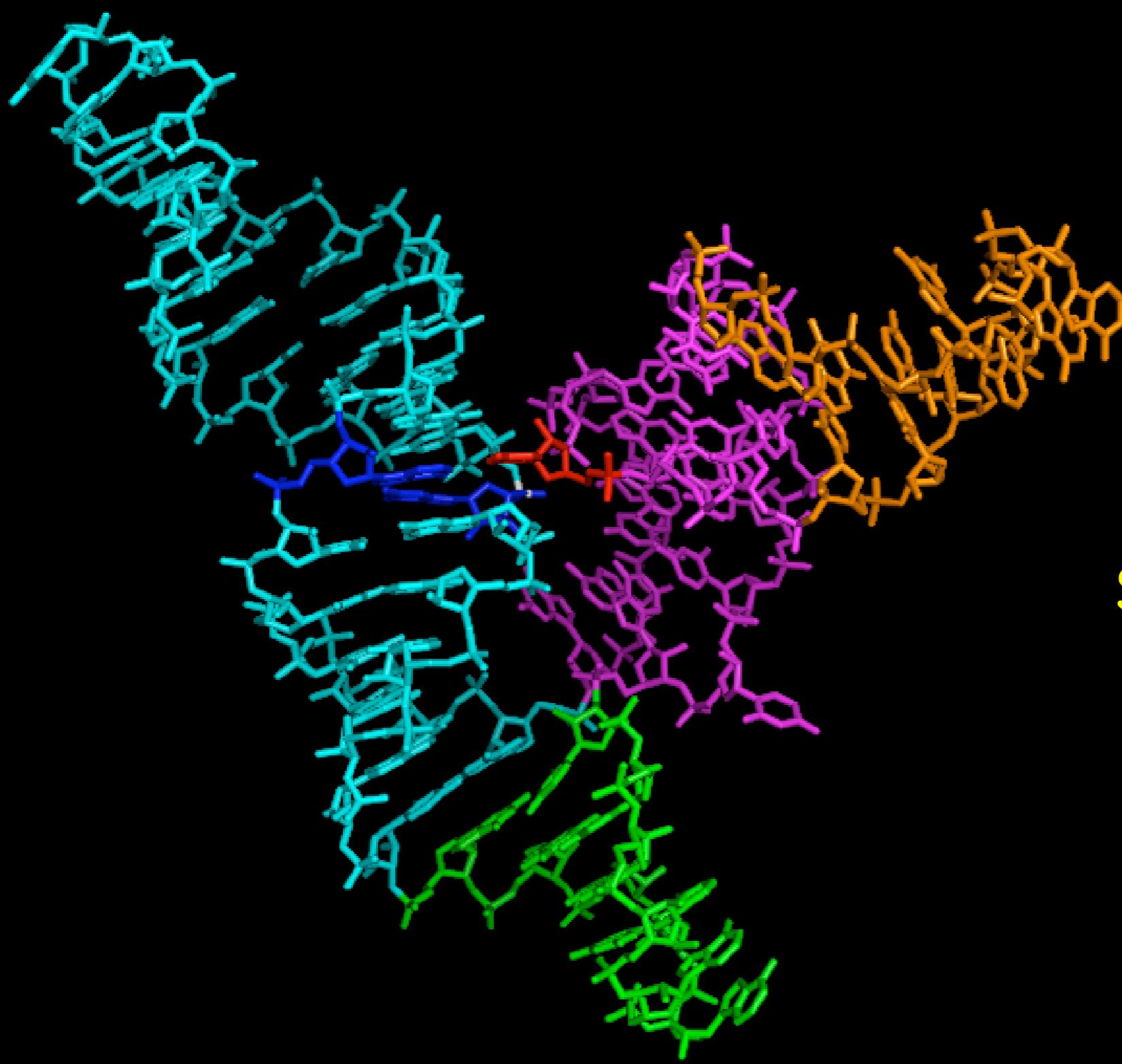






*Molecule Q*

# Molecule Q



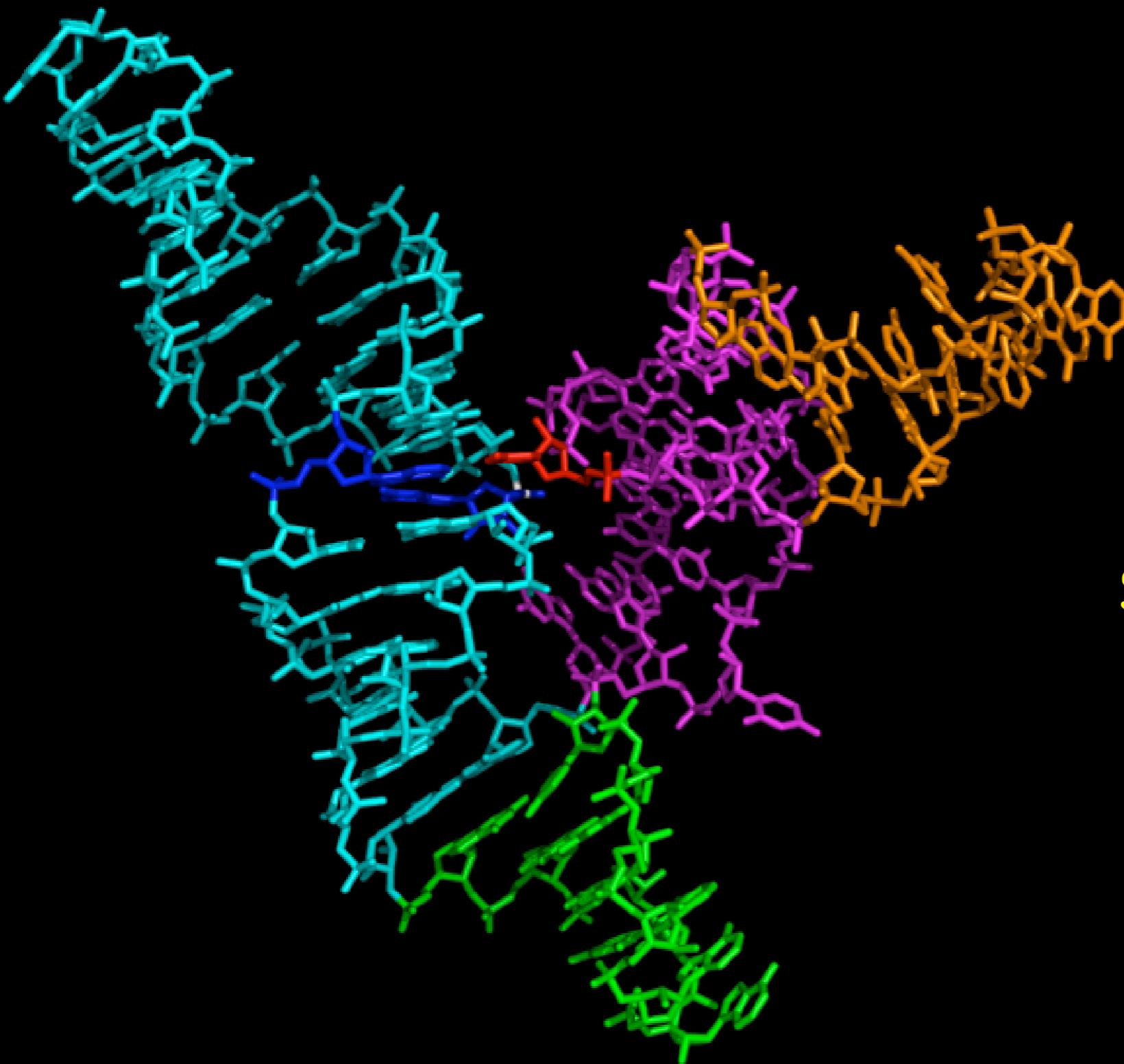
Stem A

Stem C

Stem B

A A  
60 — G A  
C — G  
C — G  
U — A  
C — G  
G — C  
U — A  
G — C — 70  
G • U  
A ♦ g — 1  
50 — U • g  
U — a  
G — c  
G — c  
A — u  
G — c  
G  
A  
A GCU C U AGA CA  
A A CGA ||| A GCU GC — g  
G C C ||| A | U C — g  
30 / | | | | | | 20 | G — c — 10  
a a g a a a

# Molecule Q



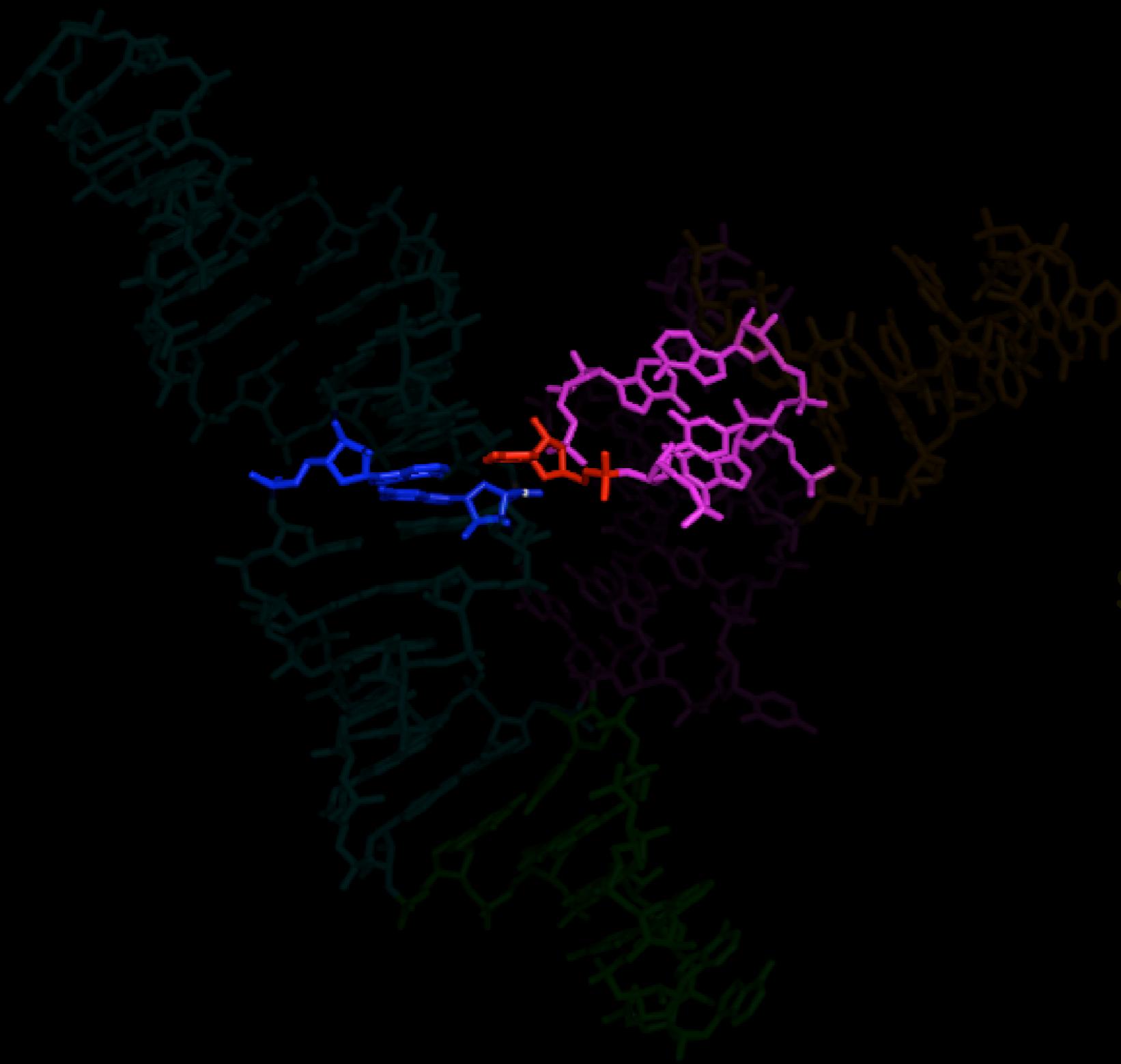
Stem A

Stem C

Stem B

A A		
60 — G	C — G	A
C — G	U — A	
U — A	C — G	
C — G	G — C	
G — C	U — A	
U — A	G — C — 70	
G — C	G — U	
50 — A — g — 1	A — g — 1	
U — g	U — a	
U — a	G — c	
G — c	G — c	
G — c	A — u	
A — u	G — c	
G — c	G	
40	U	
A G C U C	U AGA CA	
A G C U C G	U G C U G C — g	
A G C U C G	U C — g	
A G C — 10	G — c — 10	
a g	a a	

# Molecule Q



Stem A

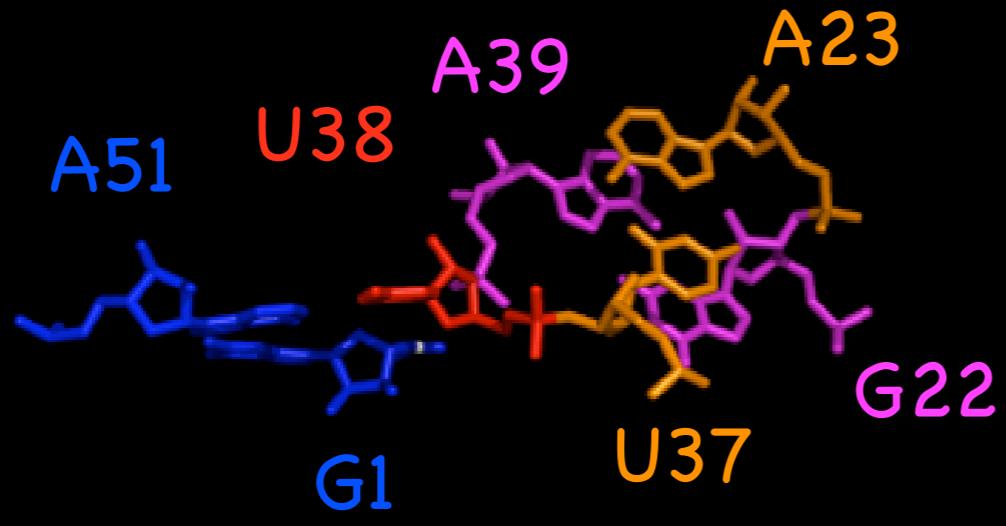
Stem C

Stem B

30'     A G C U     C  
        | | | |  
        A G C U     U G C - g  
              | | | |  
              A G C U     C - g  
                          G - c - 10  
                          a g  
                          a a

60 — G A  
      C — G  
      C — G  
      U — A  
      C — G  
      G — C  
      U — A  
      G — C — 70  
      G — U  
      A — g — 1  
50 — U — g  
      U — a  
      G — c  
      G — c  
      A — u  
      G — c  
      G

# *stem A/C bridge*



Stem A

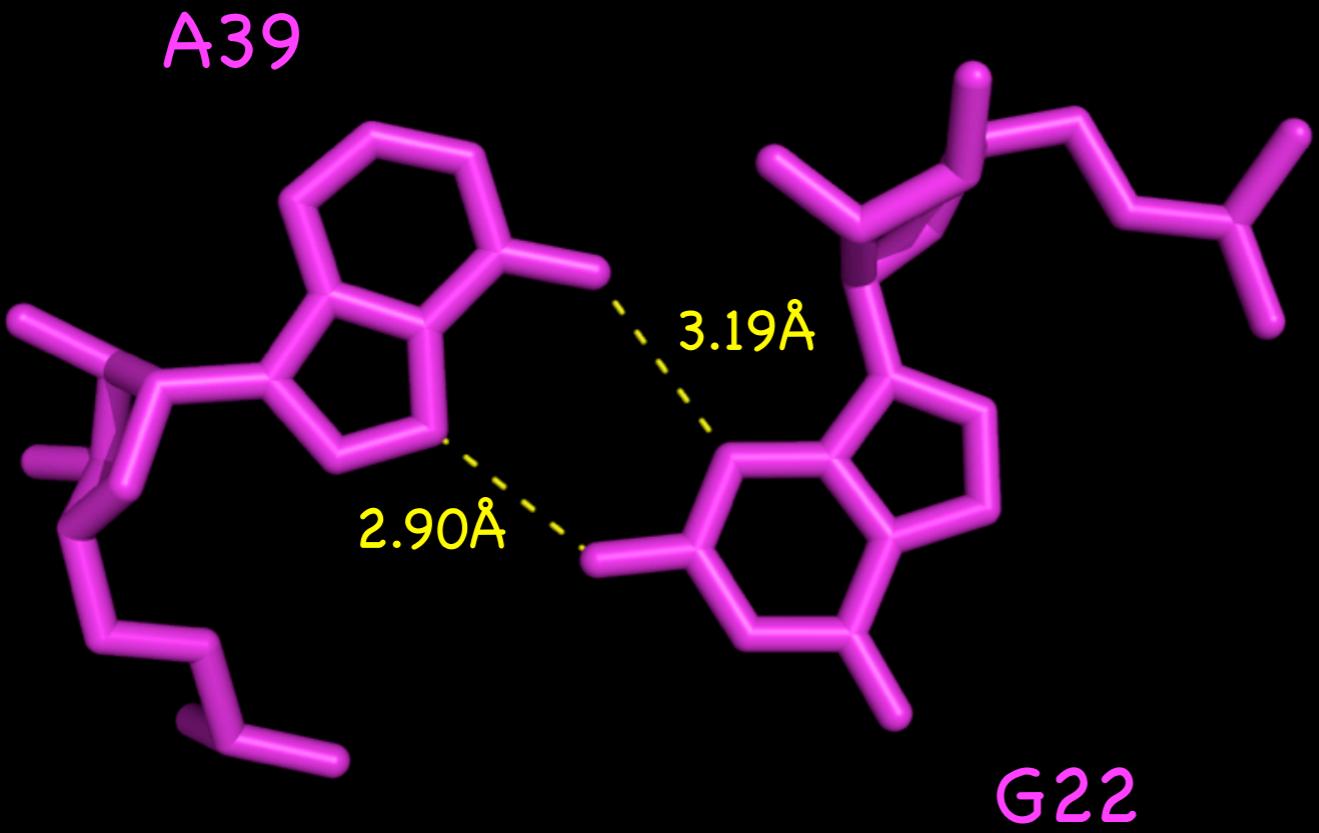
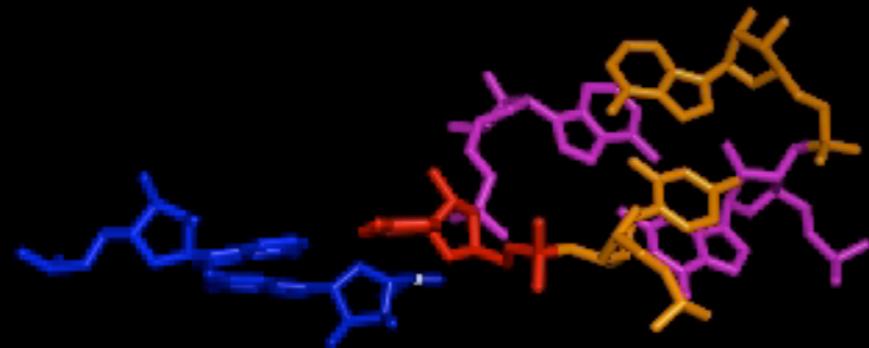
A	A
60 — G	A
C — G	
C — G	
U — A	
C — G	
G — C	
U — A	
G — C — 70	
G — U	

Stem C

A	GCU	C	G
A		U	
A	CGA	AGA	CA
30 / G		A	
A	GCU	GA	CA
20 / U		G	C — g
		C — g	
		G — c — 10	
a		a	g
		a	a

Stem B

# *stem A/C bridge*



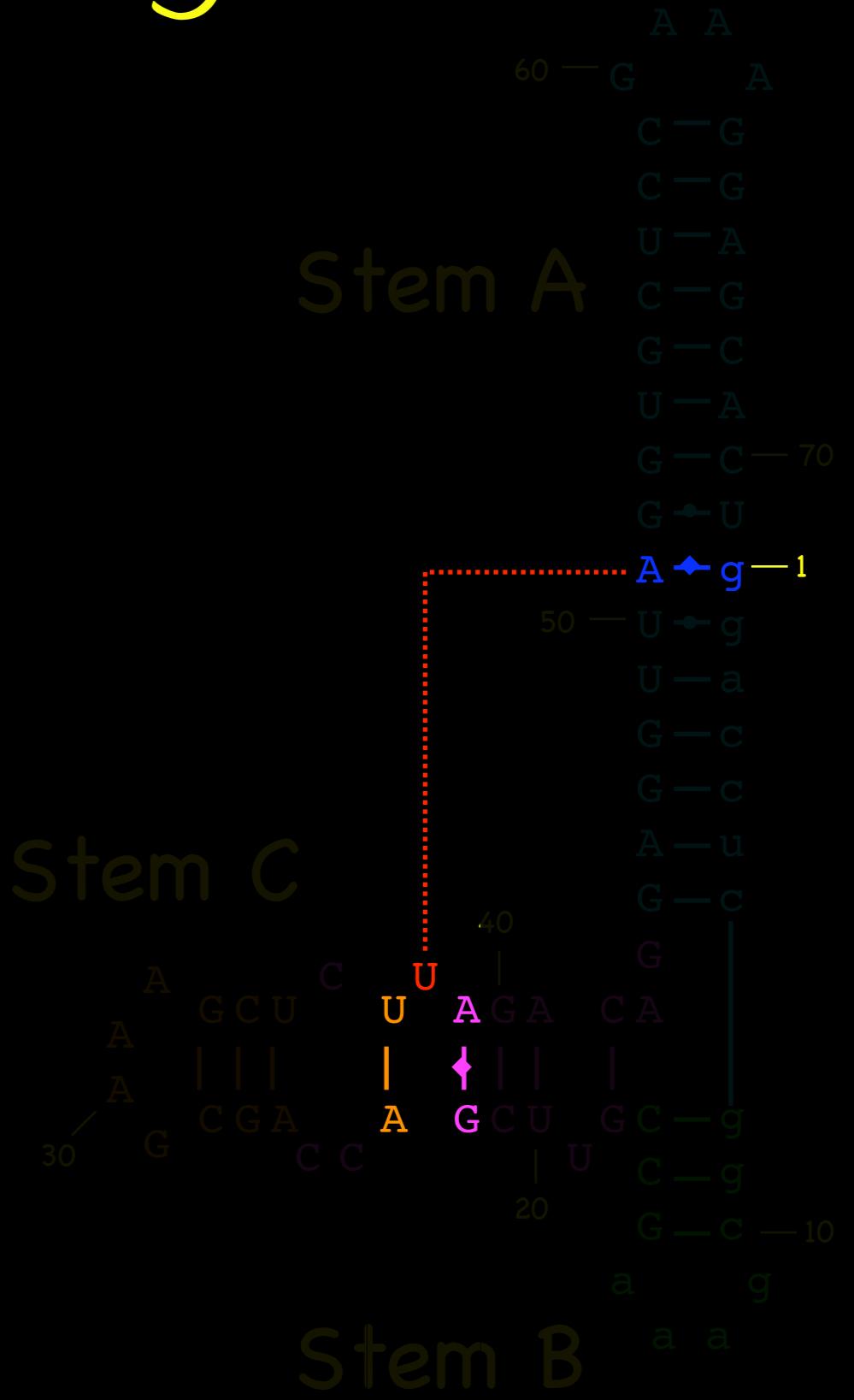
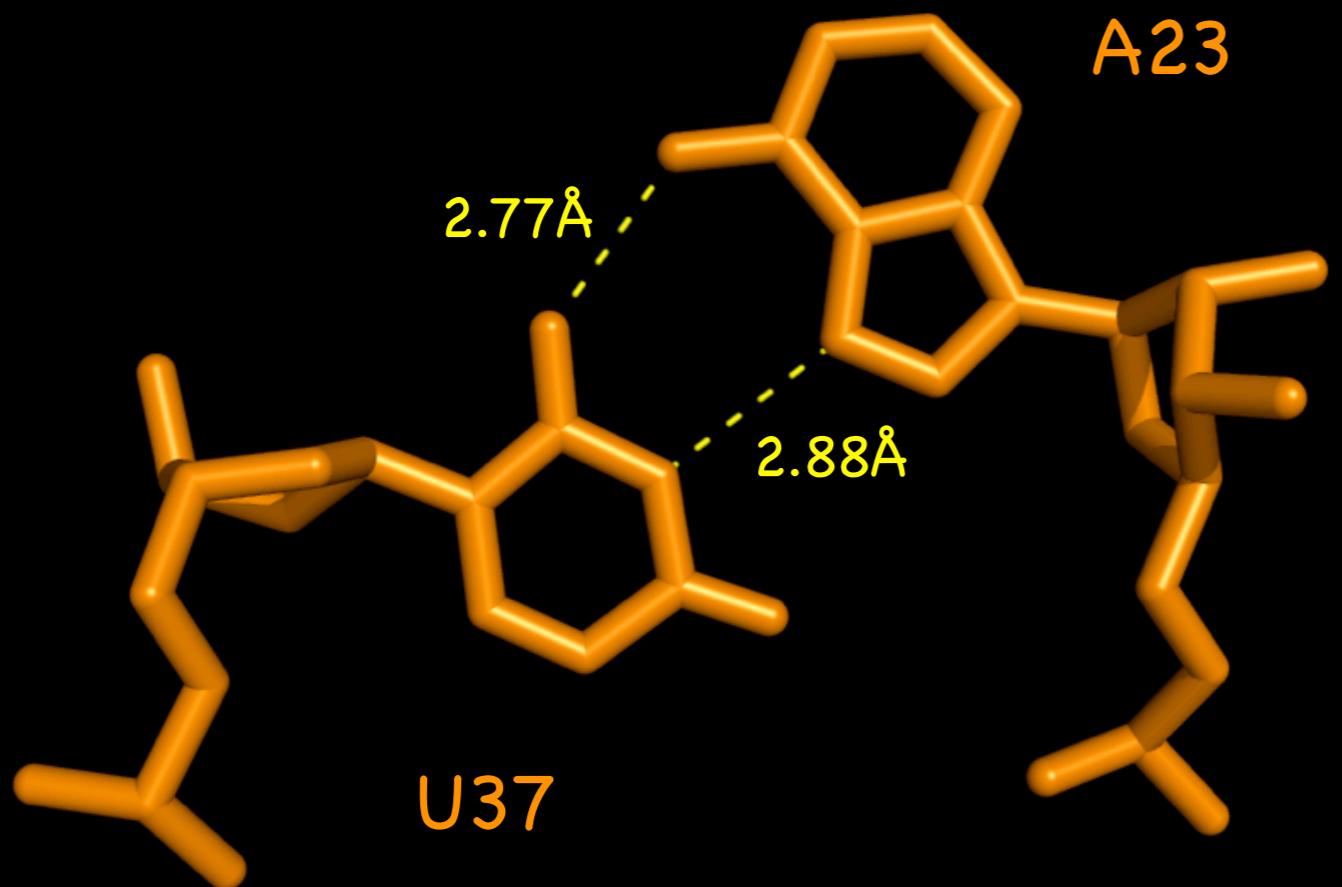
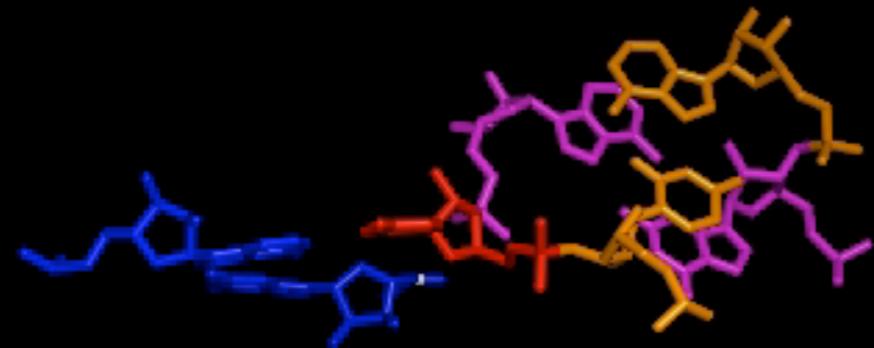
Stem A

Stem C

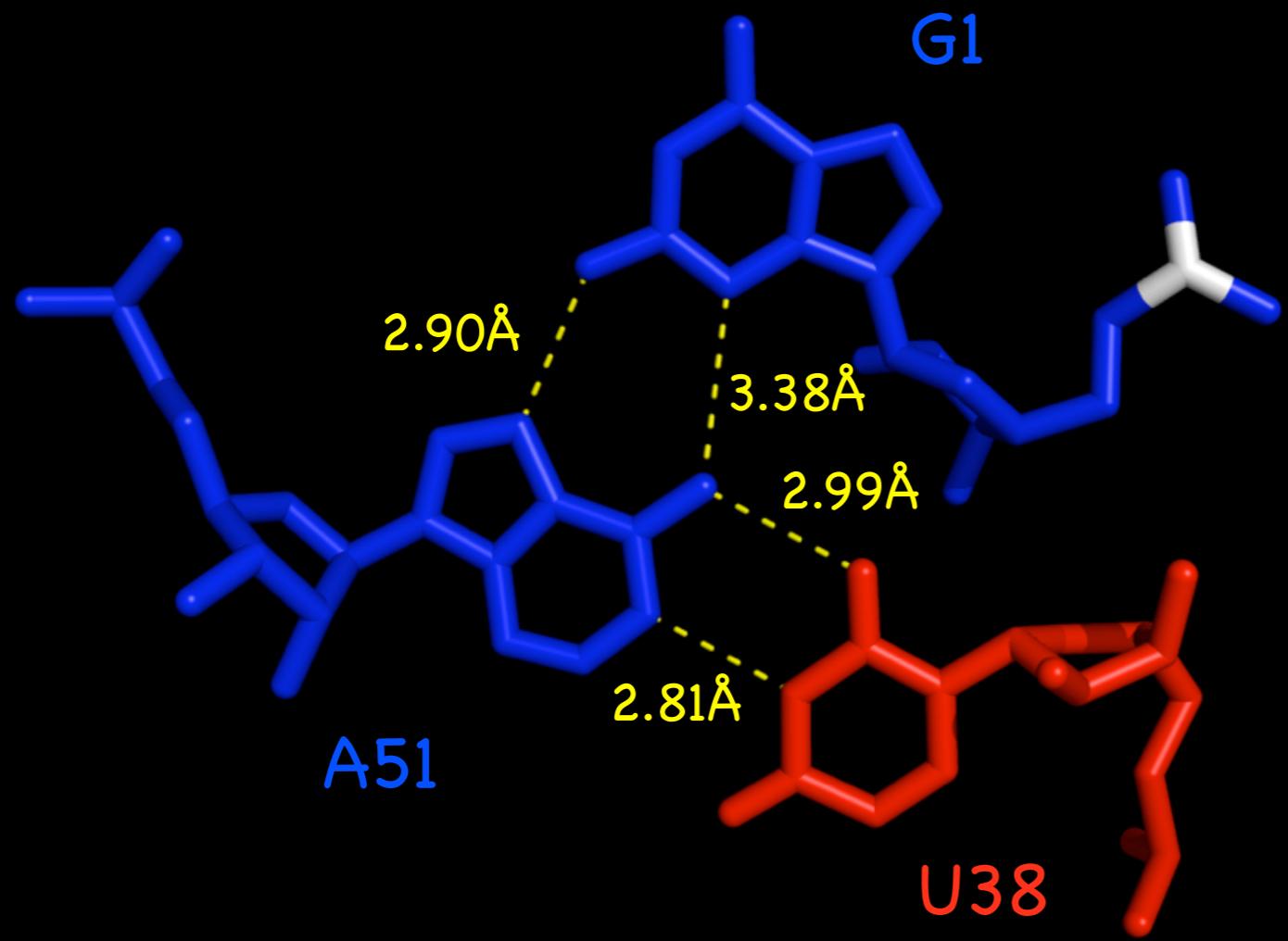
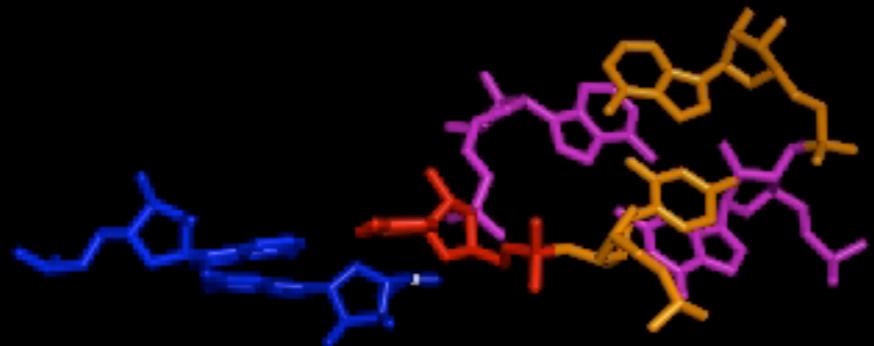
Stem B

A A	60 — G	A
C — G	C — G	
C — G	U — A	
U — A	C — G	
C — G	G — C	
G — C	U — A	
U — A	G — C — 70	
G — C — 70	G — U	
G — U	A ♦ g — 1	
A ♦ g — 1	50 — U — g	
50 — U — g	U — a	
U — a	G — c	
G — c	G — c	
G — c	A — u	
A — u	G — c	
G — c	G	
G	40	
	U	C
	A G A	CA
A		
A		
G C U		
G C U	U	G C — g
G C — g	20	C — g
C — g		G — c — 10
G — c — 10	a	g
a	a a	

# *stem A/C bridge*



# *stem A/C bridge*



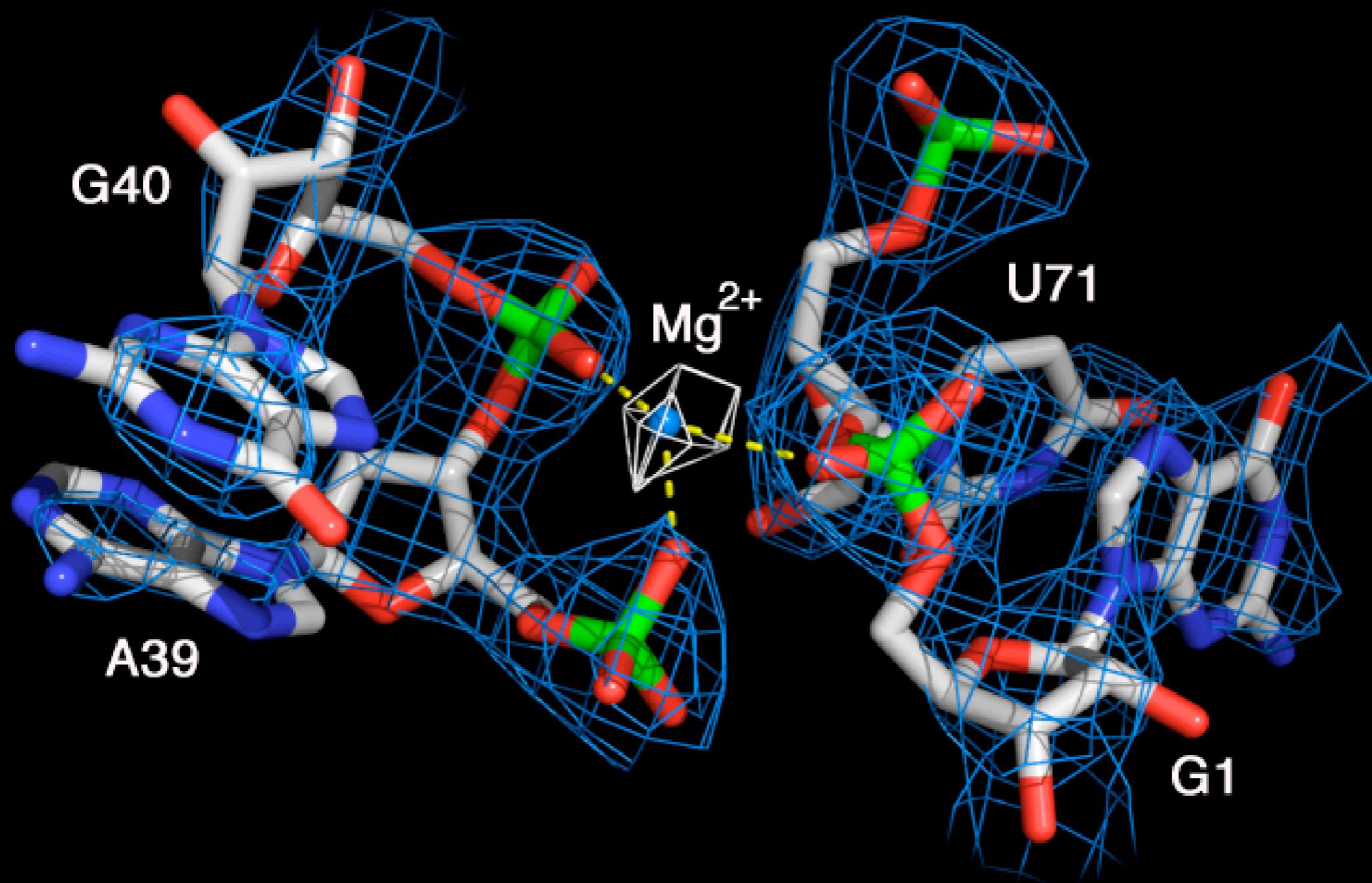
Stem A

Stem C

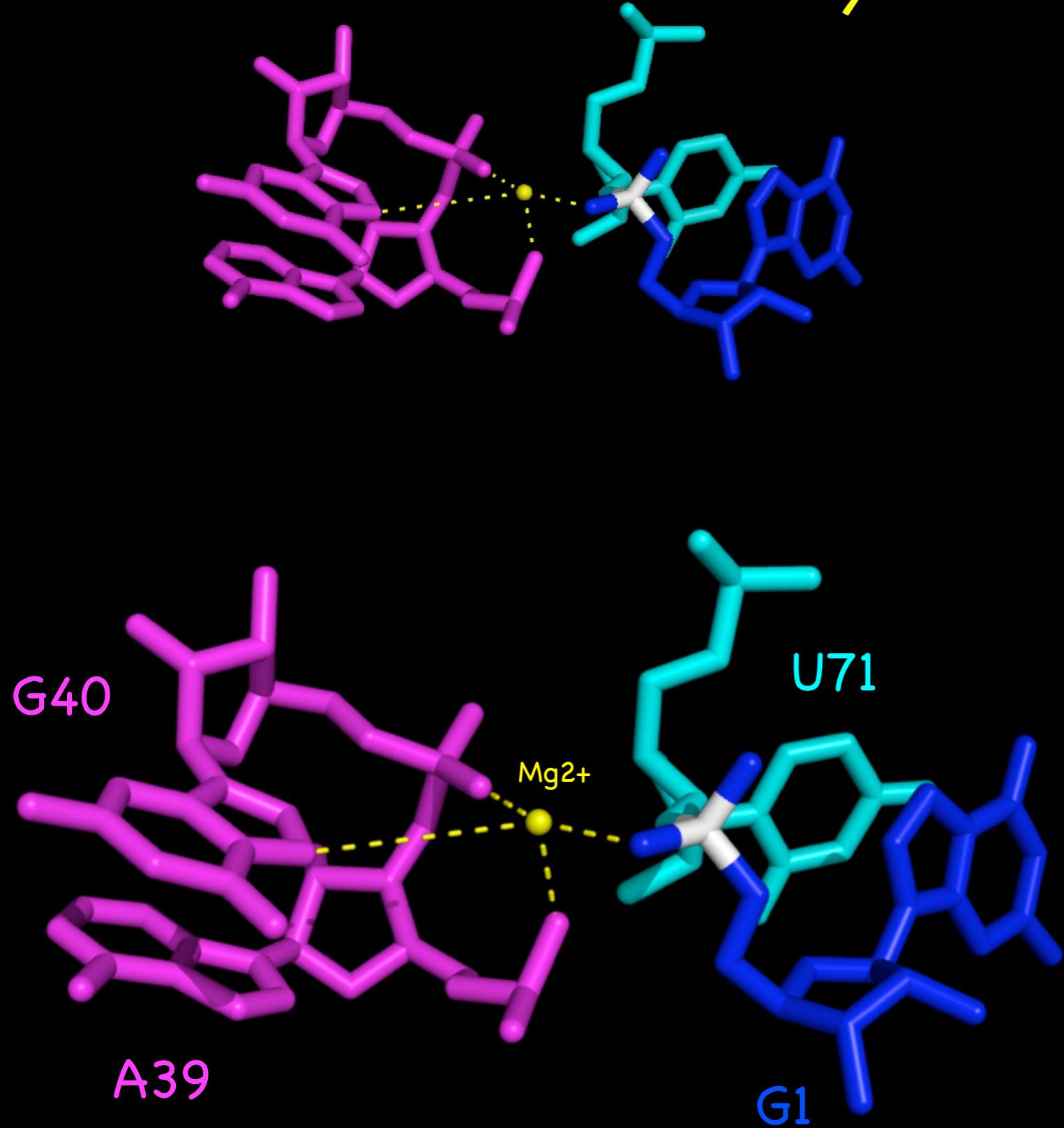
Stem B

A A	60 — G	A
C — G	C — G	
C — G		
U — A	U — A	
C — G		
G — C		
U — A		
G — C — 70		
G — U		
A ♦ g — 1		
50 — U — g		
U — a		
G — c		
G — c		
A — u		
G — c		
G		
40	U	
A	GCU	C
A		CA
G	CGA	CA
A		G
	A	CG
A	GCU	— g
	U	C — g
20		G — c — 10
		a g
		a a

*catalytic*  $Mg^{2+}$



*catalytic*  $Mg^{2+}$



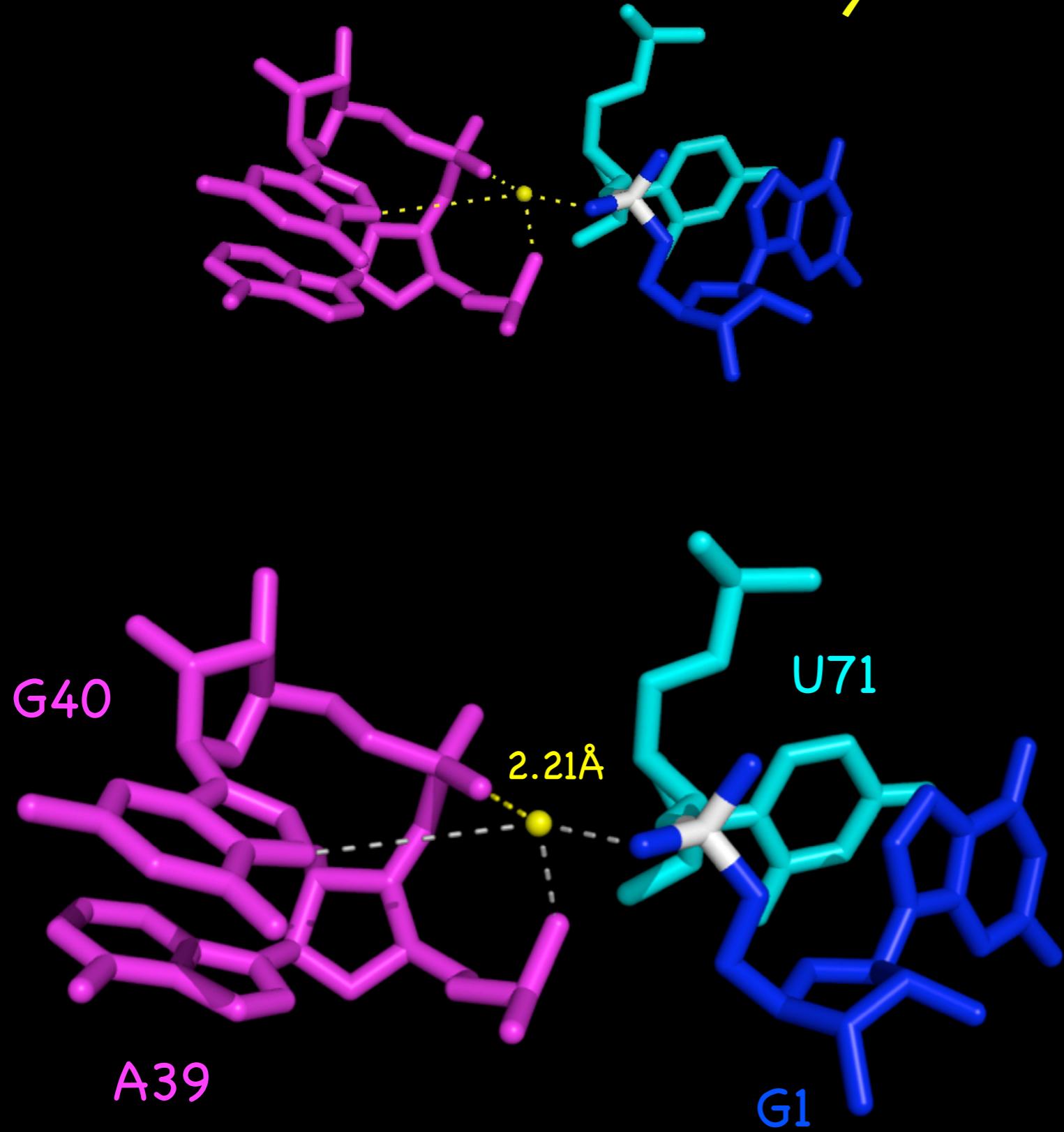
Stem A

Stem C

Stem B

A A	60 — G	A
C — G	C — G	
C — G	U — A	
U — A	C — G	
C — G	G — C	
G — C	U — A	
U — A	G — C — 70	
G — U	G — U	
A — g — 1	A — g — 1	
50 — U — g	50 — U — g	
U — a	U — a	
G — c	G — c	
G — c	A — u	
A — u	G — c	
G — c	G	
G	A G A	C
A G A	G C U	U
A		U
A	G C U	CA
G C U		
	A	A
A	G C — g	G C — g
G C — g	C — g	G — c — 10
C — g	a	a
G — c — 10	g	a a

*catalytic*  $Mg^{2+}$



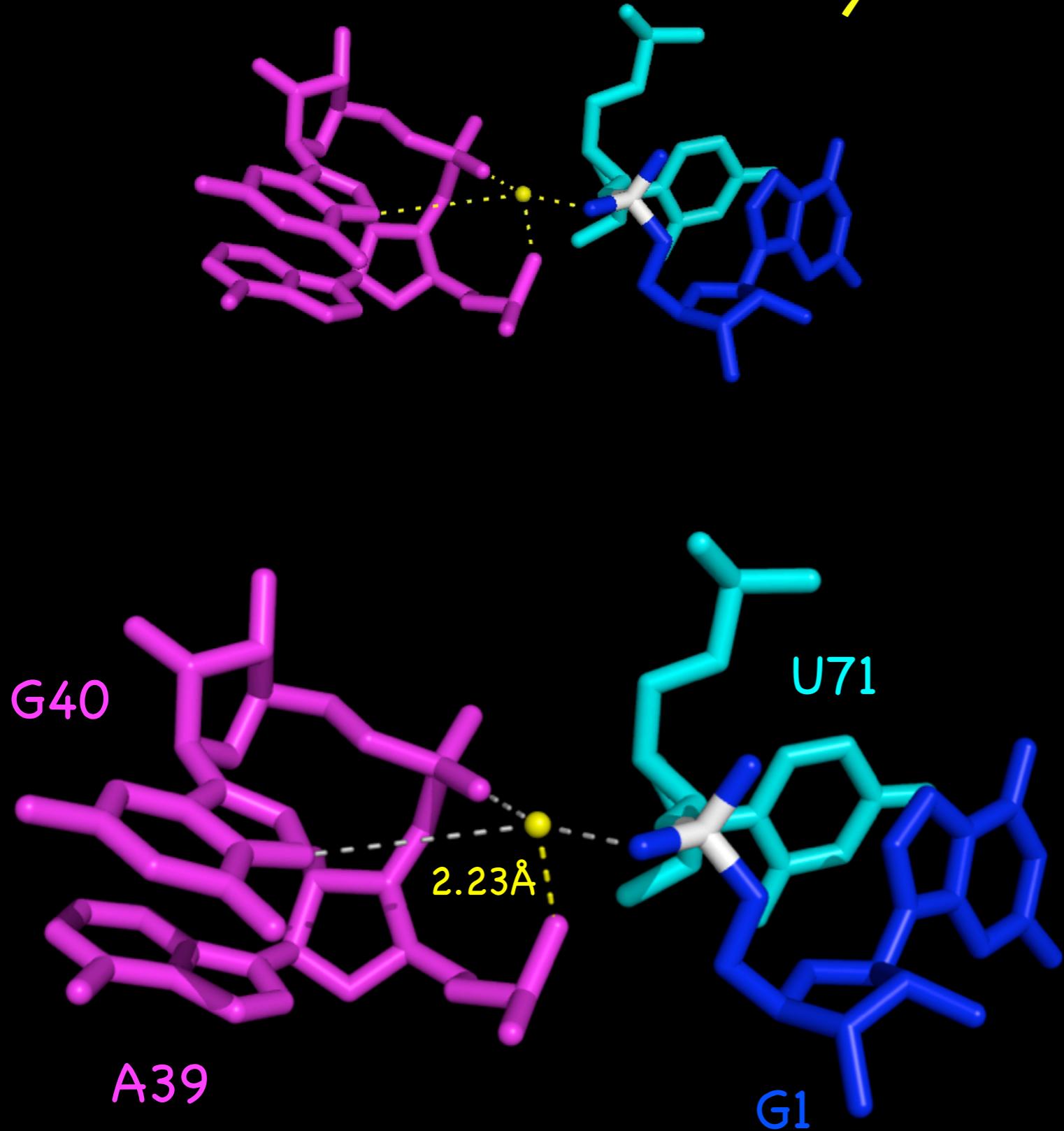
Stem A

Stem C

Stem B

A A	60 — G	A
C — G	C — G	
C — G	U — A	
U — A	C — G	
C — G	G — C	
G — C	U — A	
U — A	G — C — 70	
G — U	G — U	
A — g — 1	A — g — 1	
50 — U — g	50 — U — g	
U — a	U — a	
G — c	G — c	
G — c	A — u	
A — u	G — c	
G — c	G	
G	A G A	C
A G A	G C U	U
G C U	C G A	A
C G A	C C	C C
C C	30 / G	20 / U
30 / G	a	a
a	g	g
g	a a	a a
a a	10	10

*catalytic*  $Mg^{2+}$



Stem A

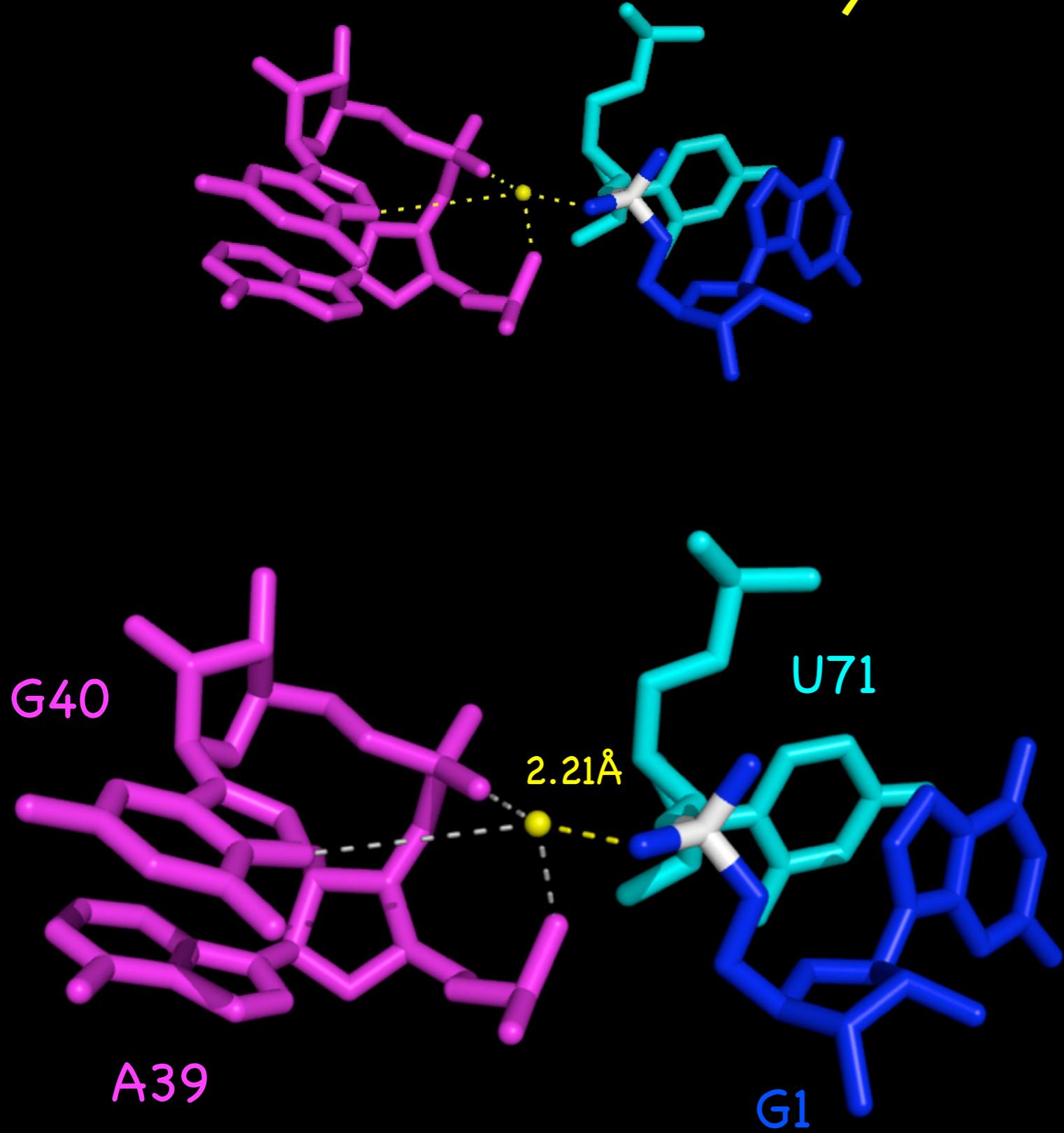
Stem C

Stem B

40 |  
A G C U C U U  
A | | | A G C U U  
A G C U G C g  
G C g  
G C 10  
a g  
a a

60 — G A A  
C — G  
C — G  
U — A  
C — G  
G — C  
U — A  
G — C — 70  
G — U  
A — g — 1  
50 — U — g  
U — a  
G — c  
G — c  
A — u  
G — c  
G

*catalytic*  $Mg^{2+}$

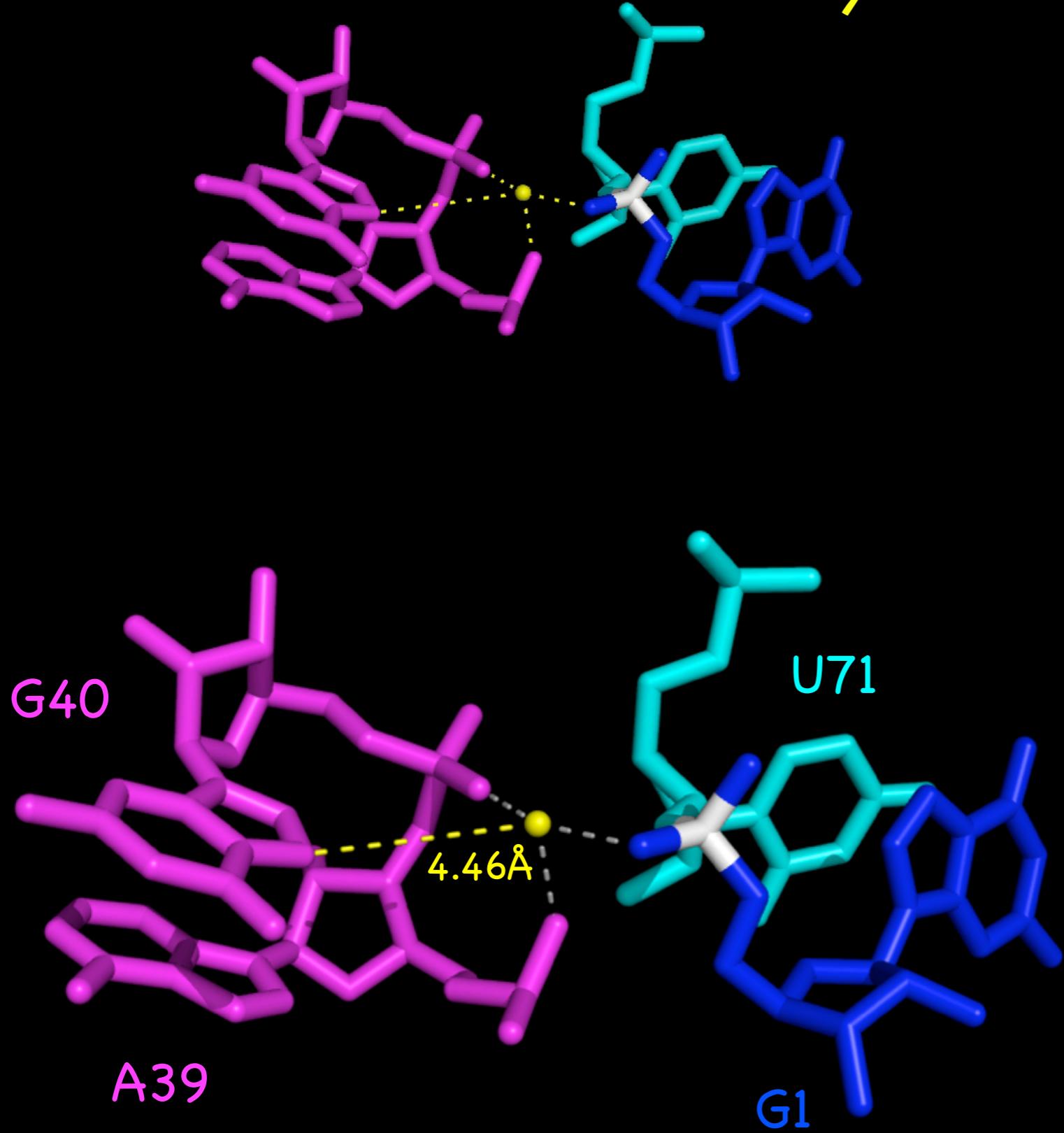


Stem A

Stem C

Stem B

*catalytic*  $Mg^{2+}$



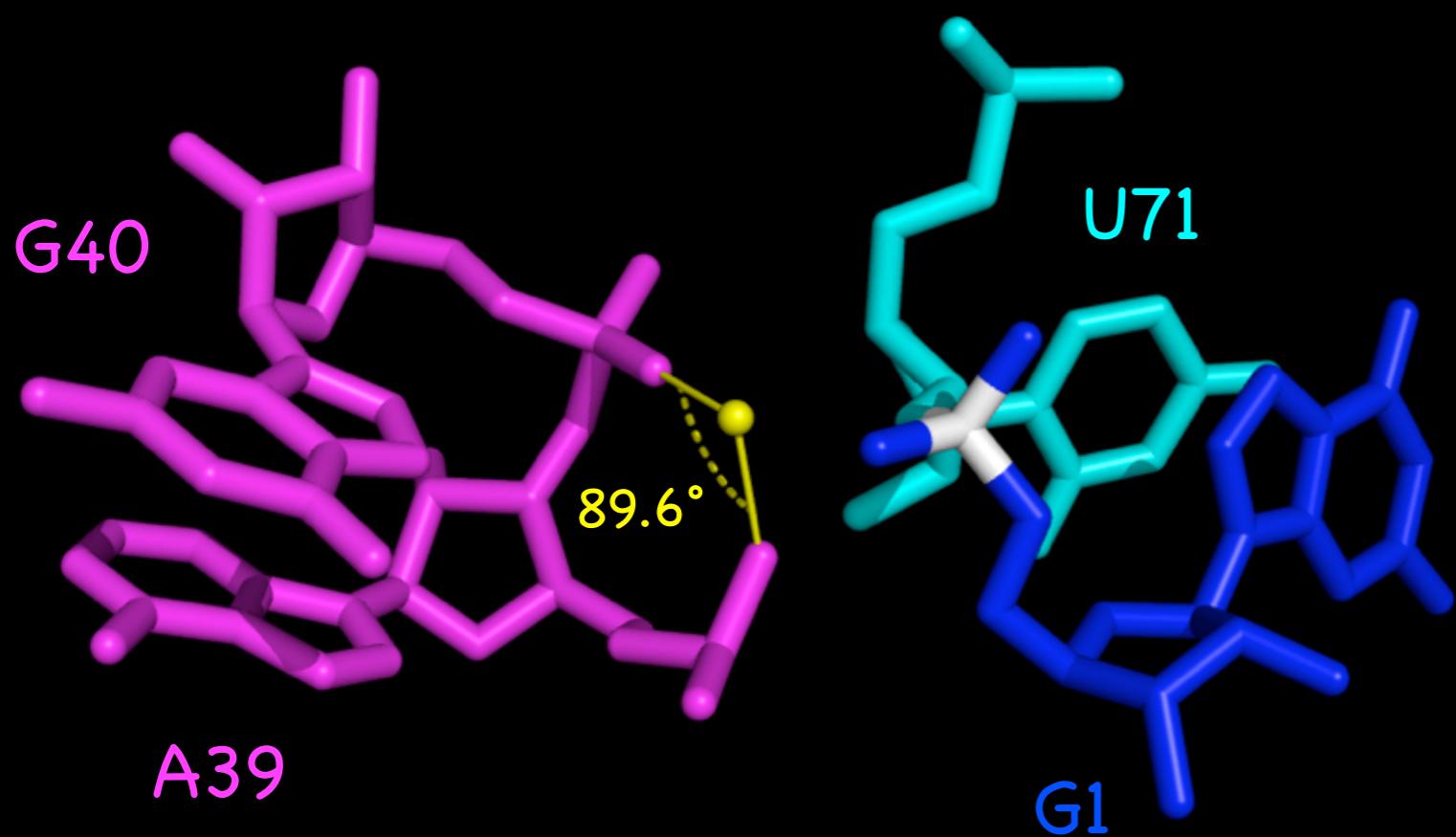
Stem A

Stem C

Stem B

A A	60 — G	A
C — G	C — G	
C — G	U — A	
U — A	C — G	
C — G	G — C	
G — C	U — A	
U — A	G — C — 70	
G — U	G — U	
A $\bullet$ g — 1	A $\bullet$ g — 1	
50 — U $\bullet$ g	U — a	
U — a	G — c	
G — c	G — c	
A — u	A — u	
G — c	G — c	
G	G	
40	A G A	C
A	G C U	U
A		U
A	G C U	C C
G		A
30 /	G	A
20	C G — g	CA
a	C — g	G
g	G — c — 10	a a

*catalytic*  $Mg^{2+}$



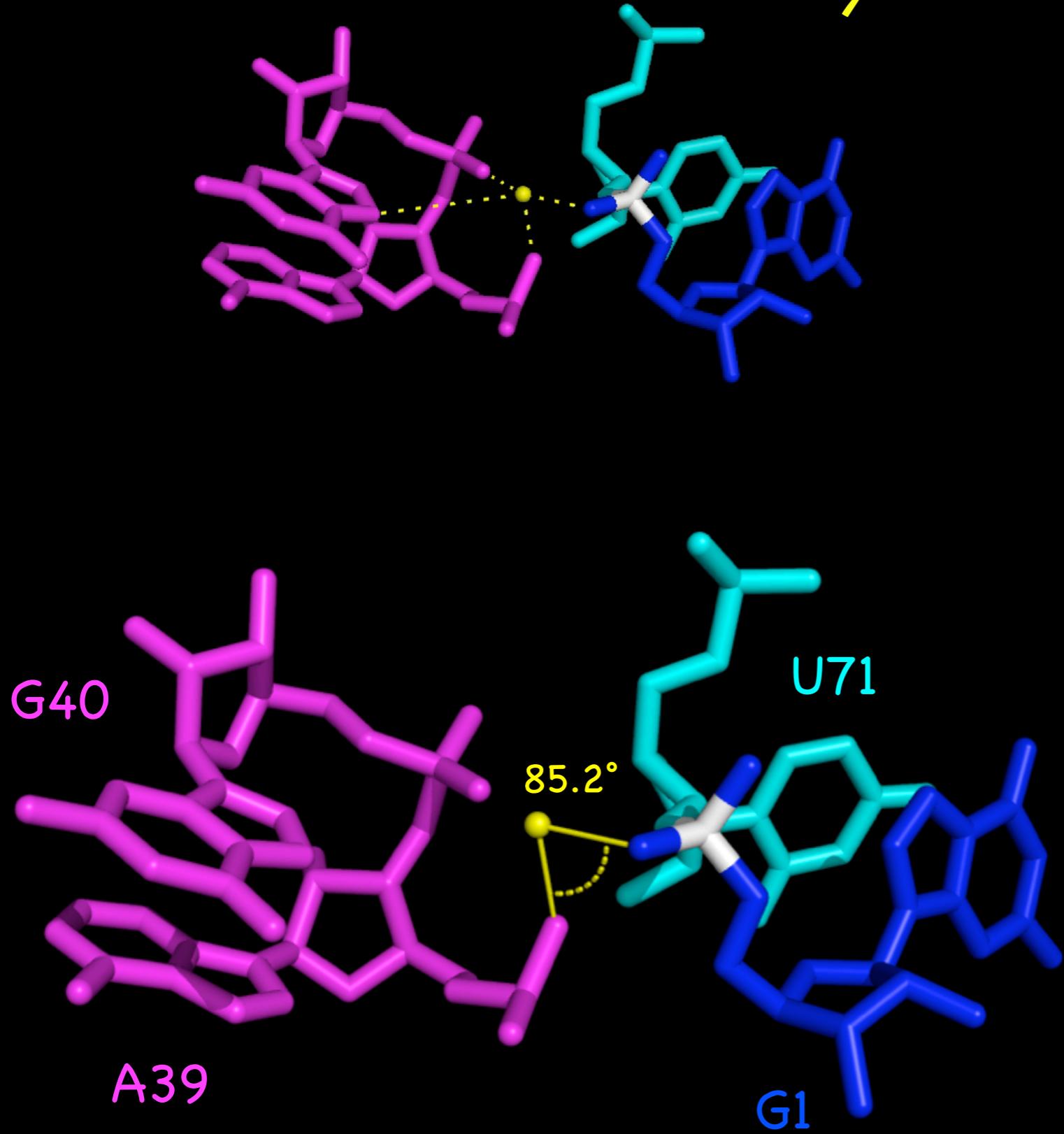
Stem A

Stem C

Stem B

A	A	
C	—G	
C	—G	
U	—A	
C	—G	
G	—C	
U	—A	
G	—C	70
G	—U	
A	♦ g	1
50	—U ♦ g	
U	—a	
G	—c	
G	—c	
A	—u	
G	—c	
G		
40		
A	GCU	C
A		U
A	CGA	A
G		GCU
	CC	U
		G
		C
		CA
		CA
		G
		CG — g
		C — g
		G — c — 10
a	g	
a	a	

*catalytic*  $Mg^{2+}$



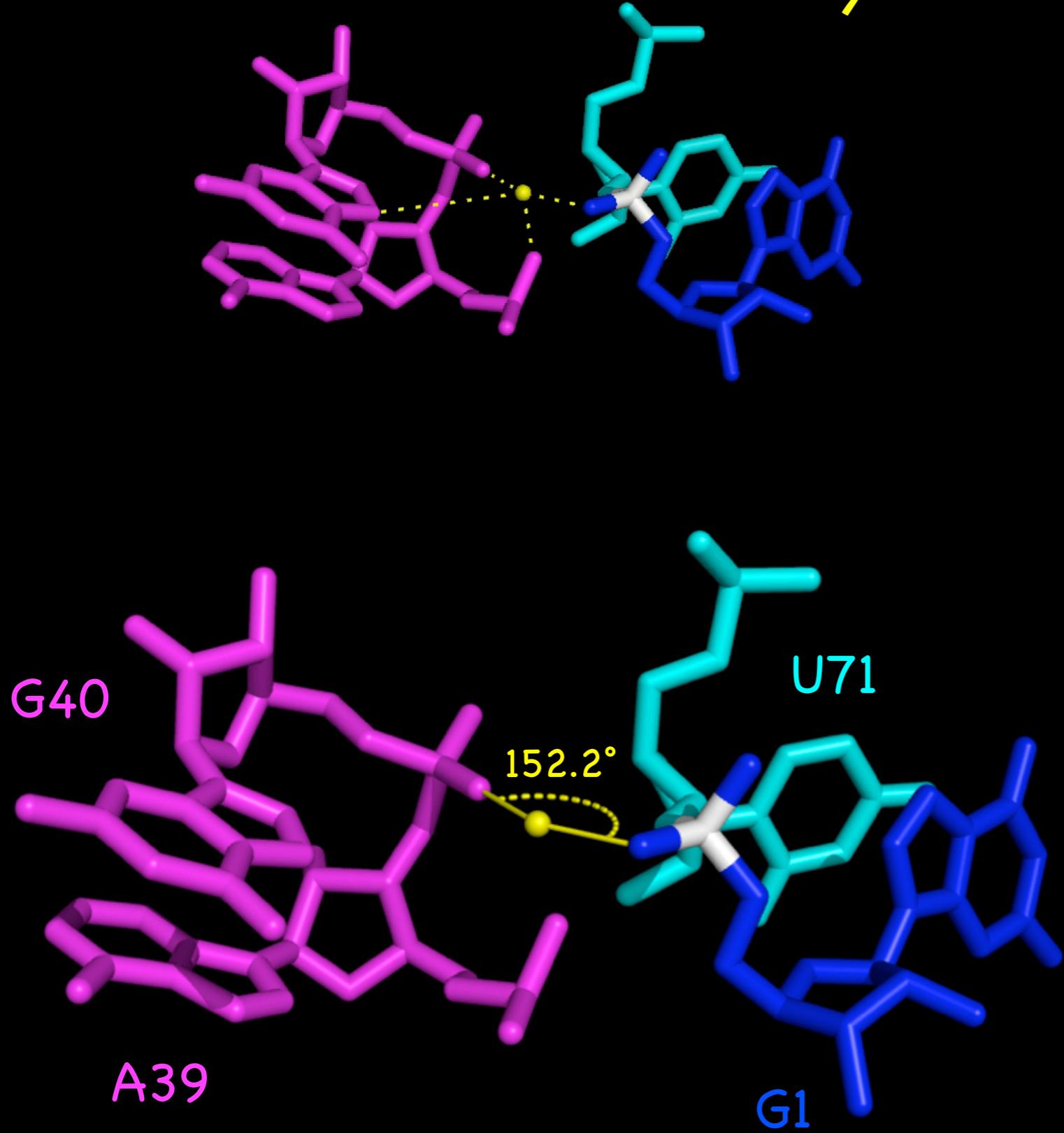
Stem A

Stem C

Stem B

A	A	60 — G	A
C	— G	C — G	
C	— G	C — G	
U	— A	U — A	
C	— G	C — G	
G	— C	G — C	
U	— A	U — A	
G	— C — 70	G — C — 70	
G	— U	G — U	
A	♦ g — 1	A ♦ g — 1	
50 — U	— g	50 — U — g	
U	— a	U — a	
G	— c	G — c	
G	— c	G — c	
A	— u	A — u	
G	— c	G — c	
G		G	
40		A G A	CA
A		G C U	C
A			U
G		C G A	A
30	/		A
A		G C U	G C — g
A			C — g
G		U	G — c — 10
a		G C — g	
a		C — g	
g		G — c — 10	

*catalytic*  $Mg^{2+}$



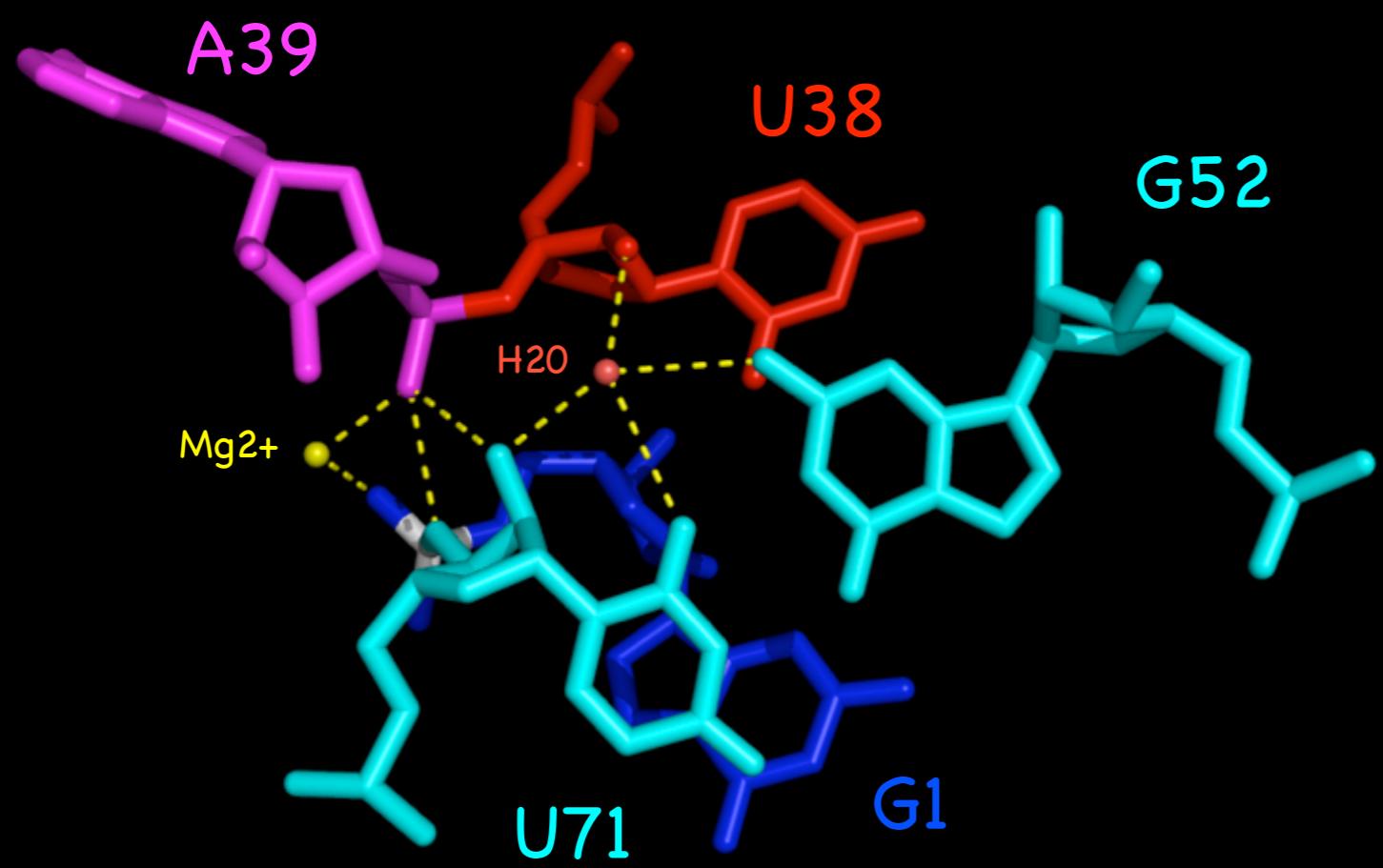
Stem A

Stem C

Stem B

A A	60 — G	A
C — G	C — G	
C — G	U — A	
U — A	C — G	
C — G	G — C	
G — C	U — A	
U — A	G — C — 70	
G — U	G — U	
A — g — 1	A — g — 1	
50 — U — g	50 — U — g	
U — a	U — a	
G — c	G — c	
G — c	A — u	
A — u	G — c	
G — c	G	
G	A G A	C
A G A	G C U	U
A		U
A	G C U	U
G C U		G C — g
	G C — g	C — g
30 / G	20 / U	G — c — 10
A A G	a a a	

# *regiospecificity*



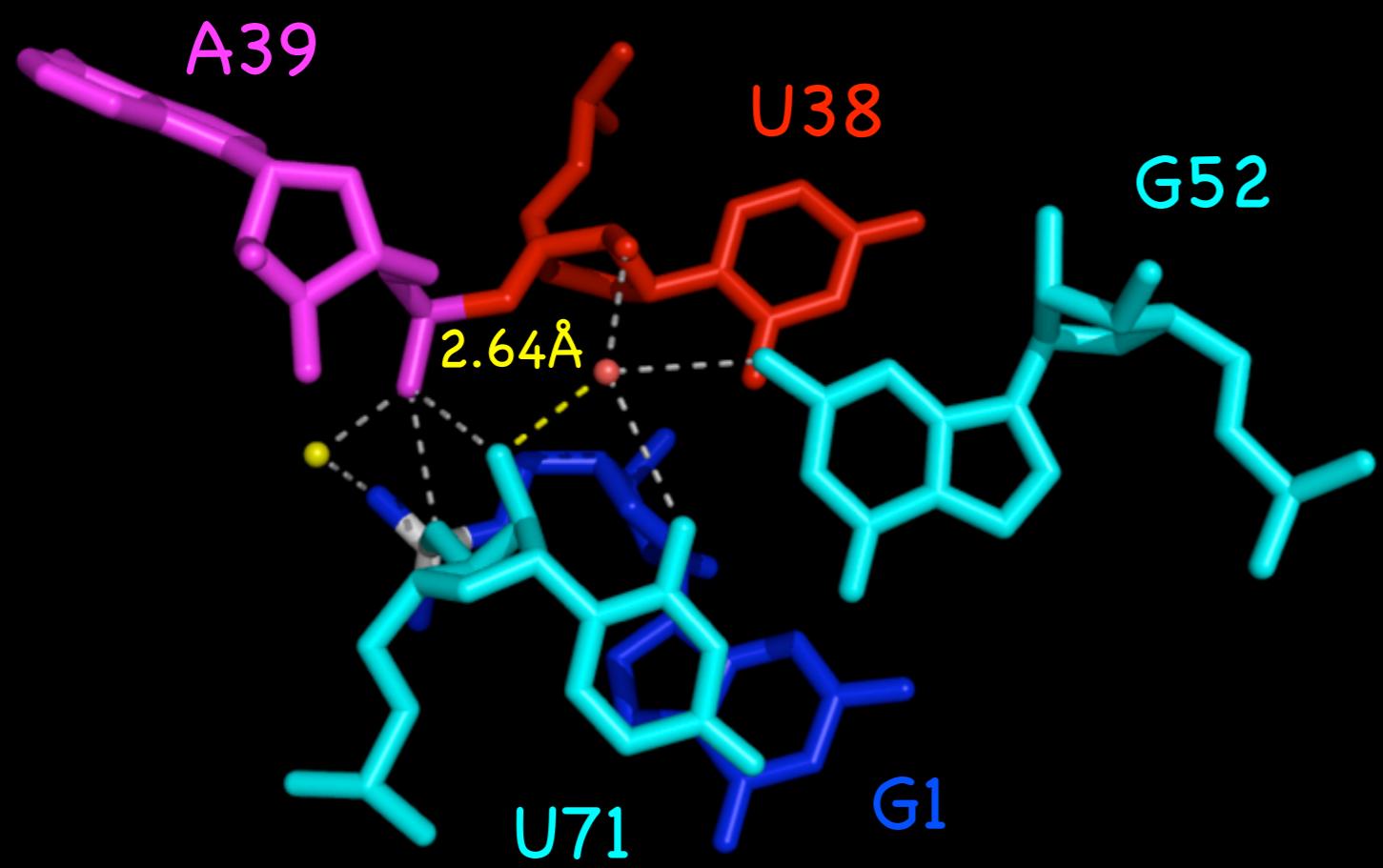
Stem A

Stem C

Stem B

A A	60 — G	A
C — G	C — G	
C — G	U — A	
U — A	C — G	
C — G	G — C	
G — C	U — A	
U — A	G — C — 70	
G — C — 70	G — U	
G — U	A — g — 1	
A — g — 1	50 — U — g	
50 — U — g	U — a	
U — a	G — c	
G — c	G — c	
G — c	A — u	
A — u	G — c	
G — c	G	
G	40	
	U	
	A G A	
	G C U	C
		U
	A C G A	A
		C C
	A G C U	
	G C — g	
	C — g	
	G — c — 10	
	a g	
	a a	

# *regiospecificity*



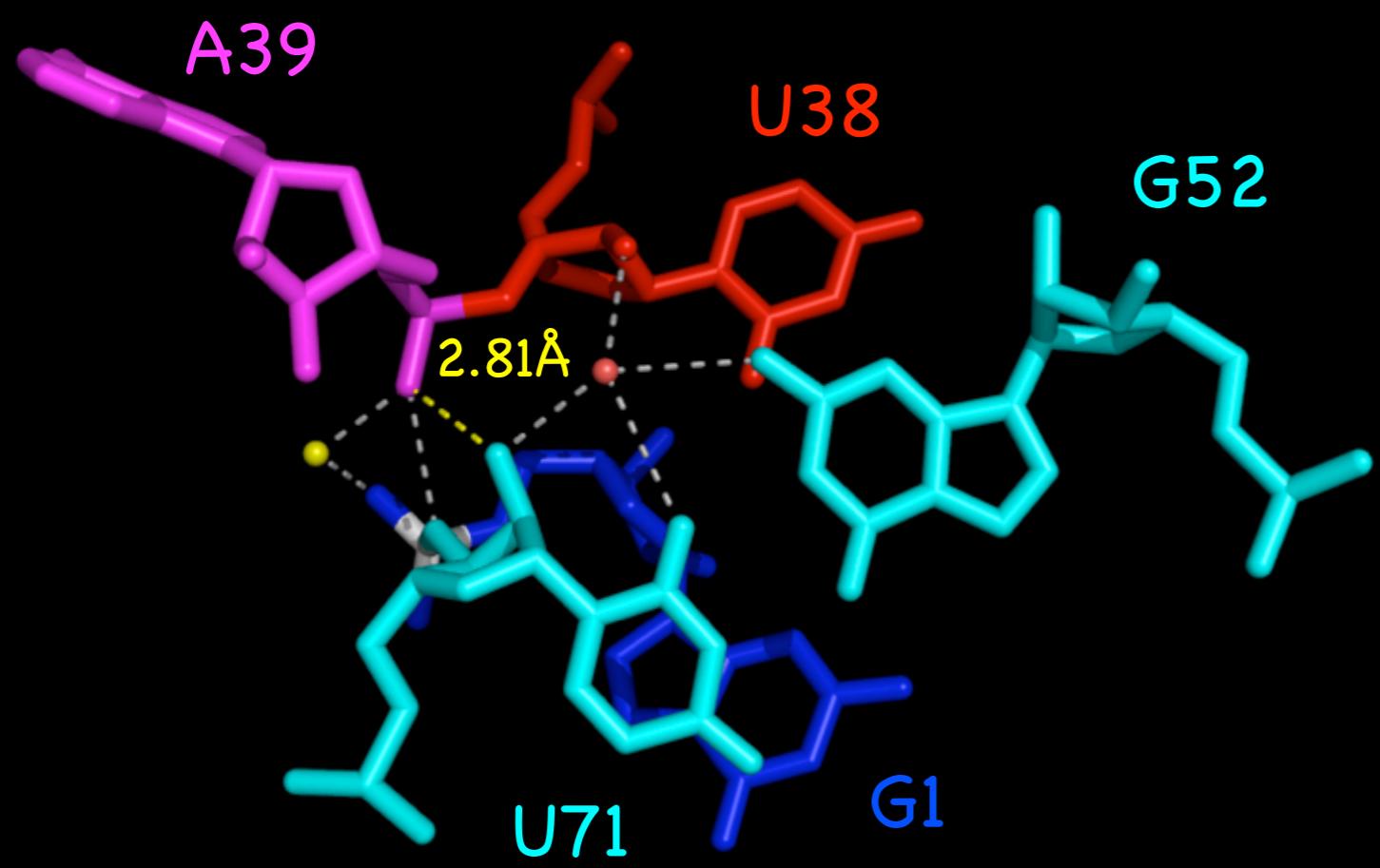
Stem A

Stem C

Stem B

A A	60 — G	A
C — G	C — G	
C — G	U — A	
U — A	C — G	
C — G	G — C	
G — C	U — A	
U — A	G — C — 70	
G — C — 70	G — U	
G — U	A — g — 1	
A — g — 1	50 — U — g	
50 — U — g	U — a	
U — a	G — c	
G — c	G — c	
G — c	A — u	
A — u	G — c	
G — c	G	
G	40 — U — A	
40 — U — A	G — A — G — A	
G — A — G — A	G — C — G — C — g	
G — C — g	CA	
CA	CA	
CA	G — C — g	
G — C — g	C — g	
C — g	G — C — 10	
G — C — 10	a — g	
a — g	a — a	

# *regiospecificity*



30' / A A G C U C U A G C U C C A G C U U G C - g C - g G - c - 10 a a g a a a

40 | U A G A | || | A G C U | U G C - g C - g G - c - 10

50 | U - g U - a G - c G - c A - u G - c G | CA |

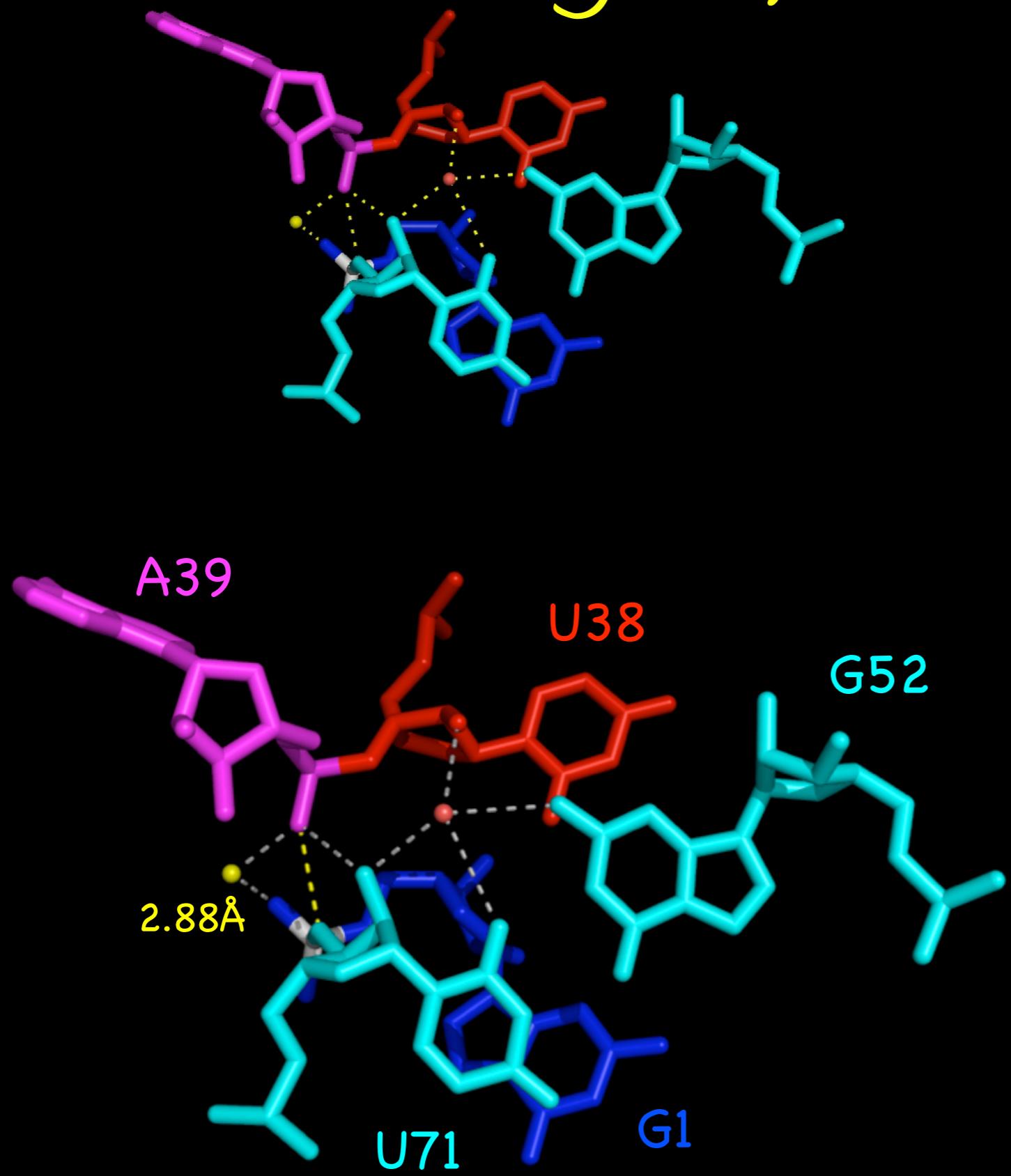
60 — G C — G C — G U — A C — G G — C U — A G — C — 70 G — U — 1 A — g — 1

Stem A

Stem C

Stem B

# *regiospecificity*

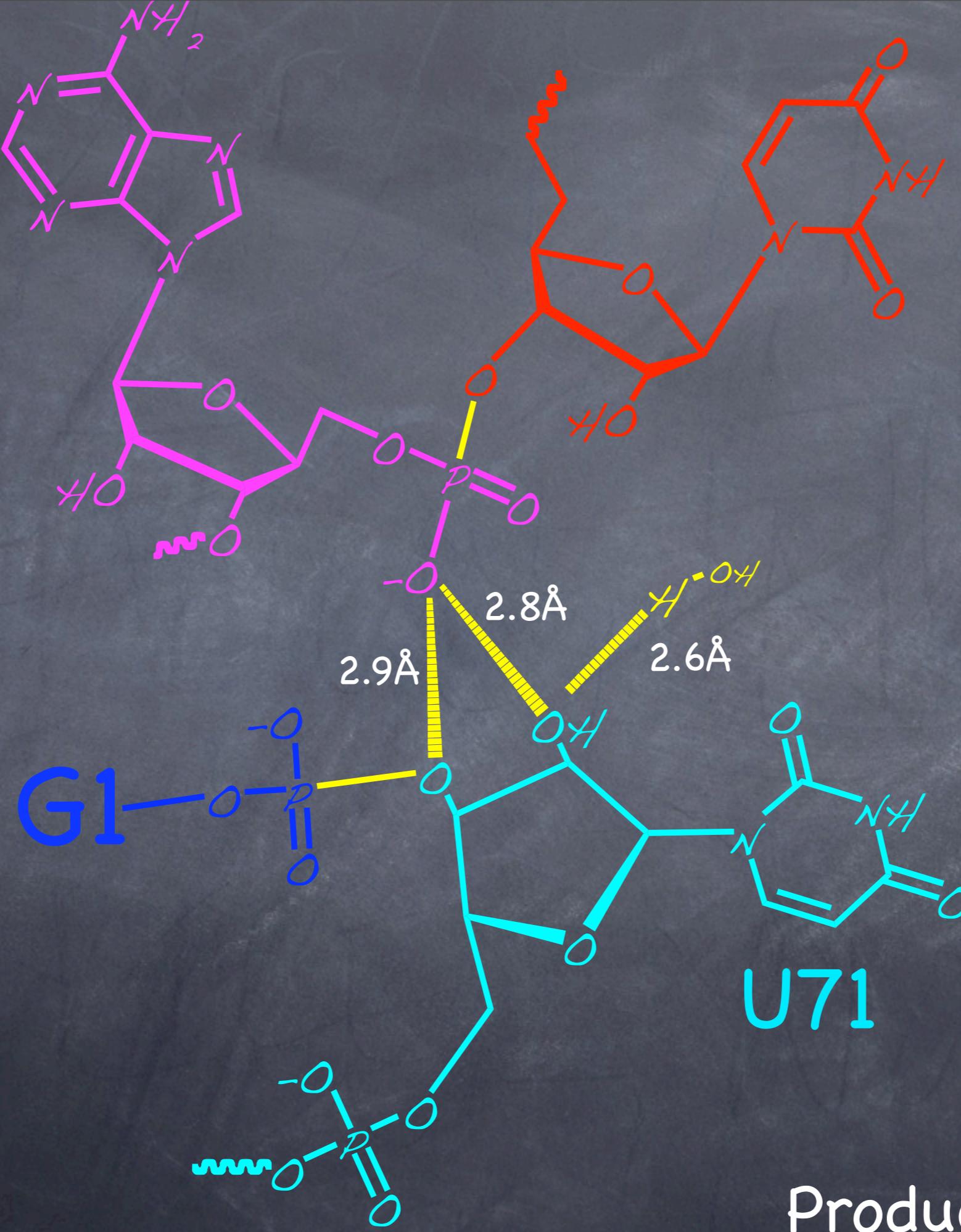


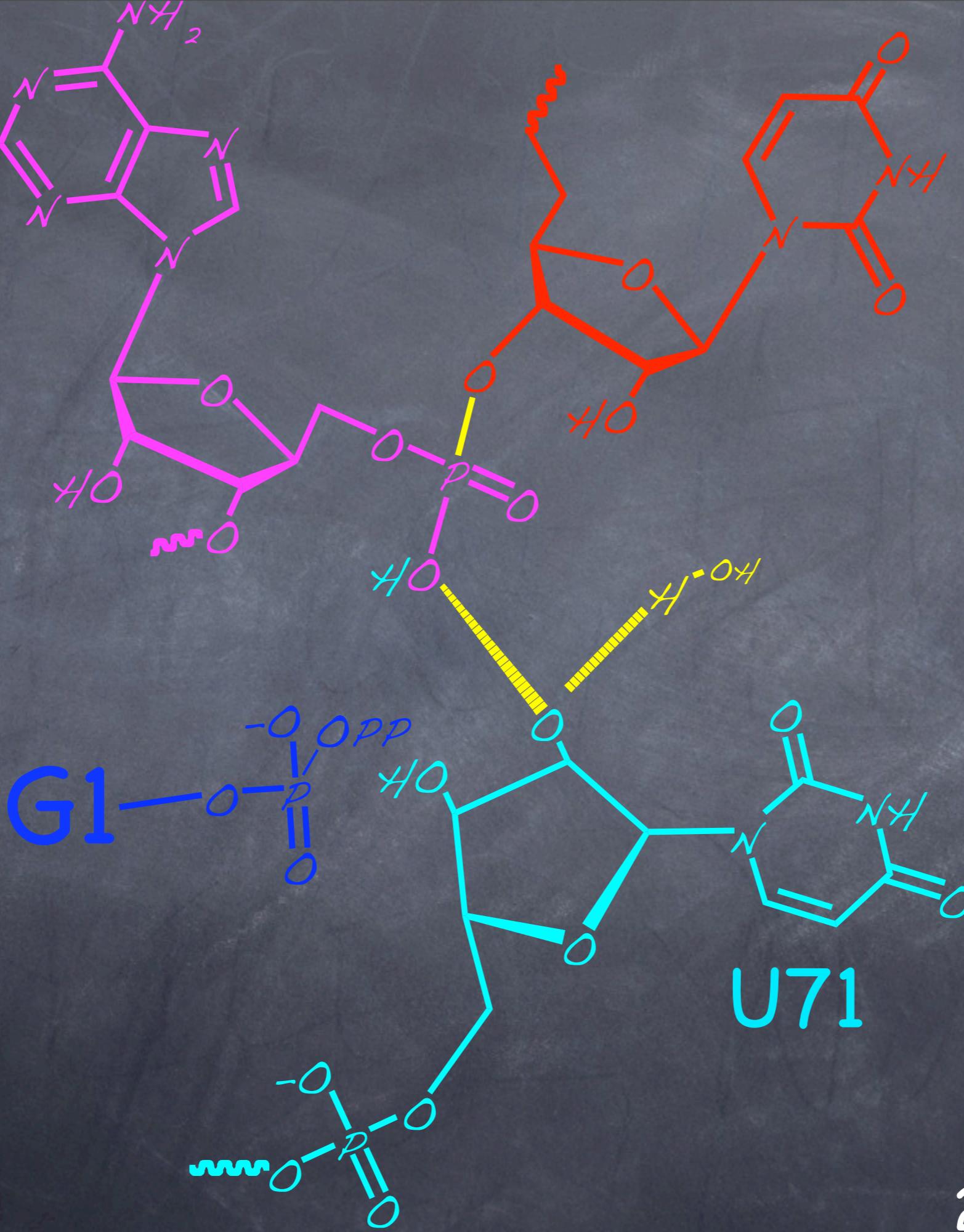
Stem A

Stem C

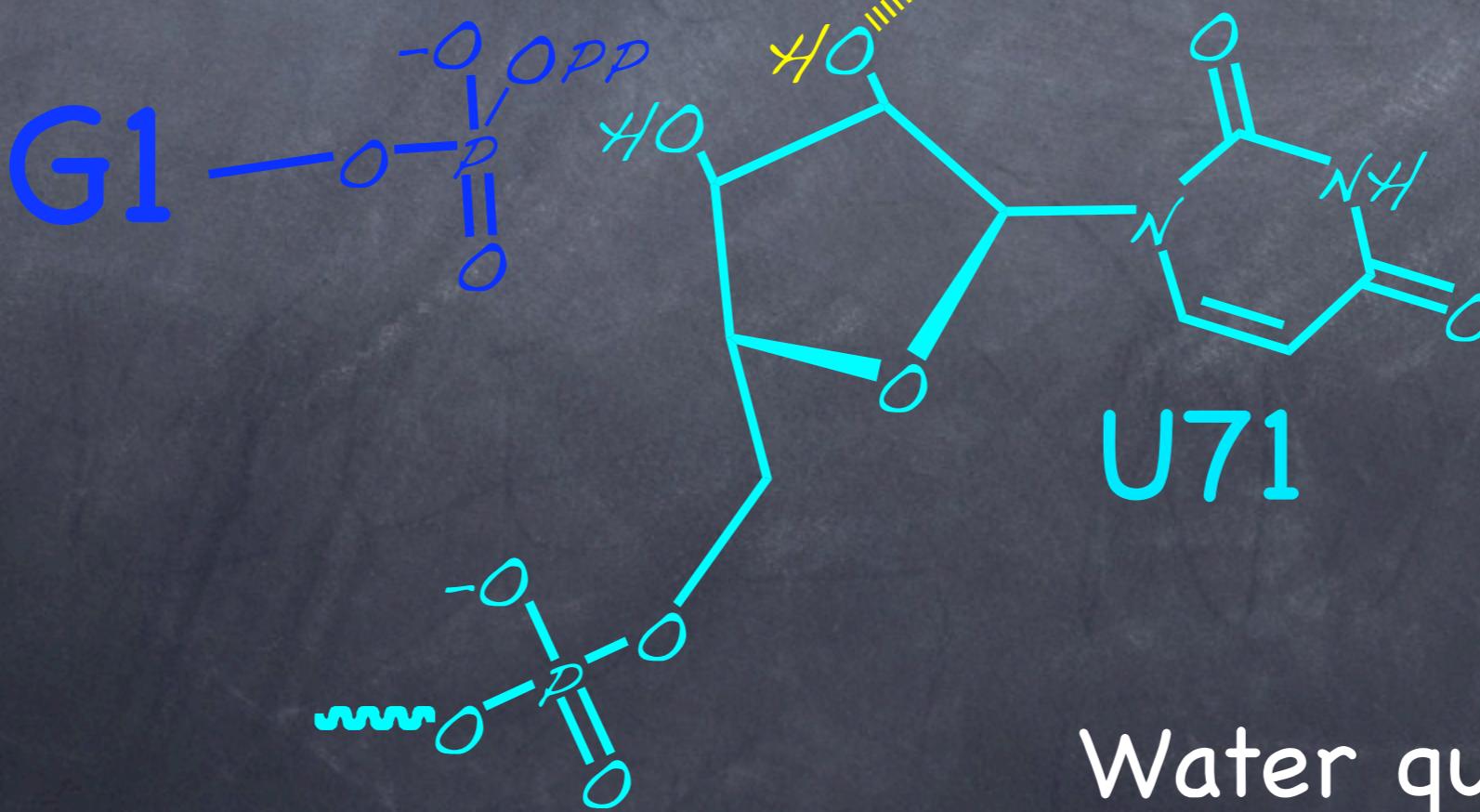
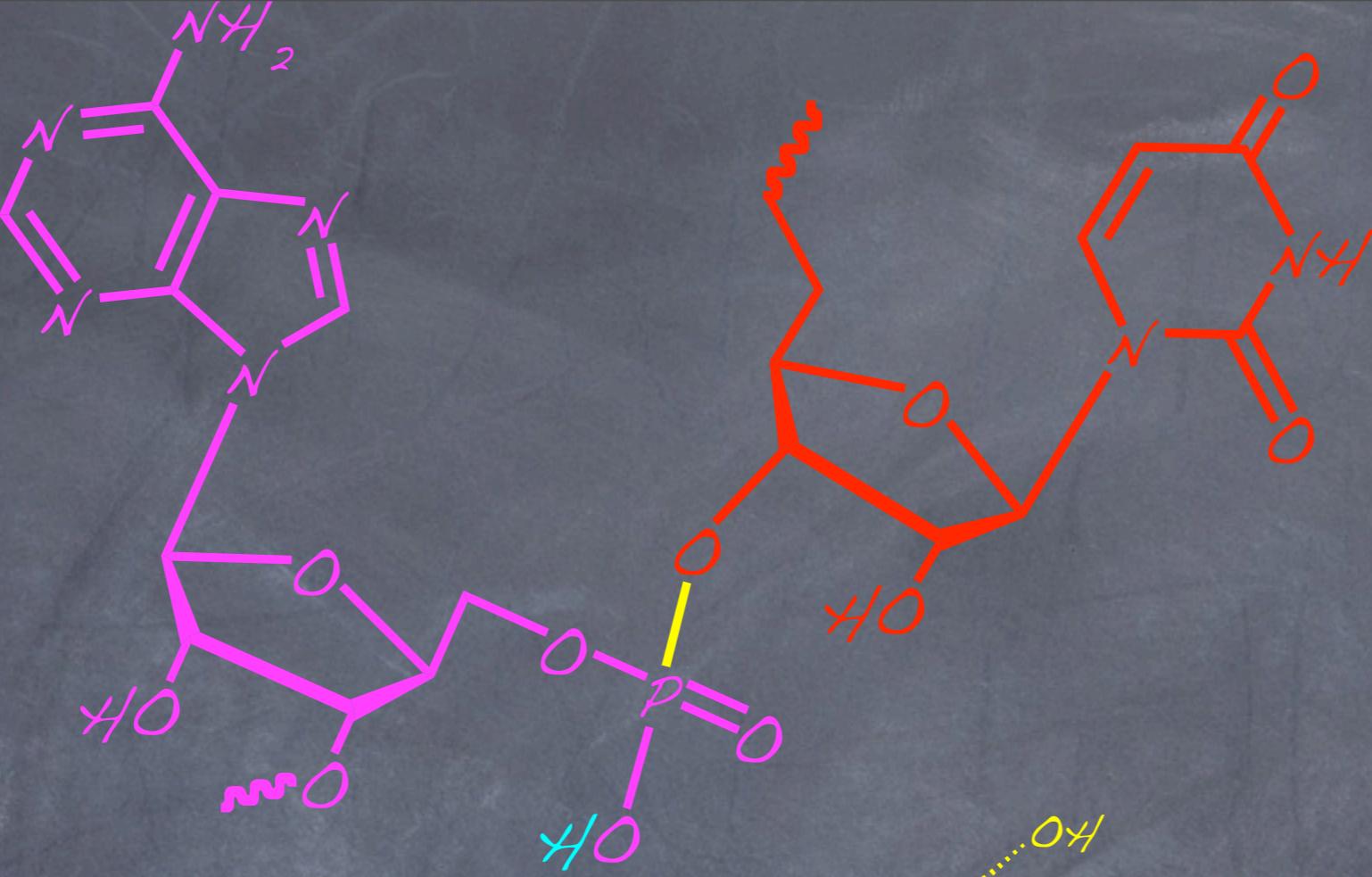
Stem B

A A	60 — G	A
C — G	C — G	
C — G		
U — A		
C — G		
G — C		
U — A		
G — C — 70		
G — U		
A — g — 1		
50 — U — g		
U — a		
G — c		
G — c		
A — u		
G — c		
G		
A G C U C U A G G C U G C — g	40	20
A A G C G A C C A G C U U G C — g		
A G C G A C C A G C U U G C — g		
A G C G A C C A G C U U G C — g		
a g		
a a		

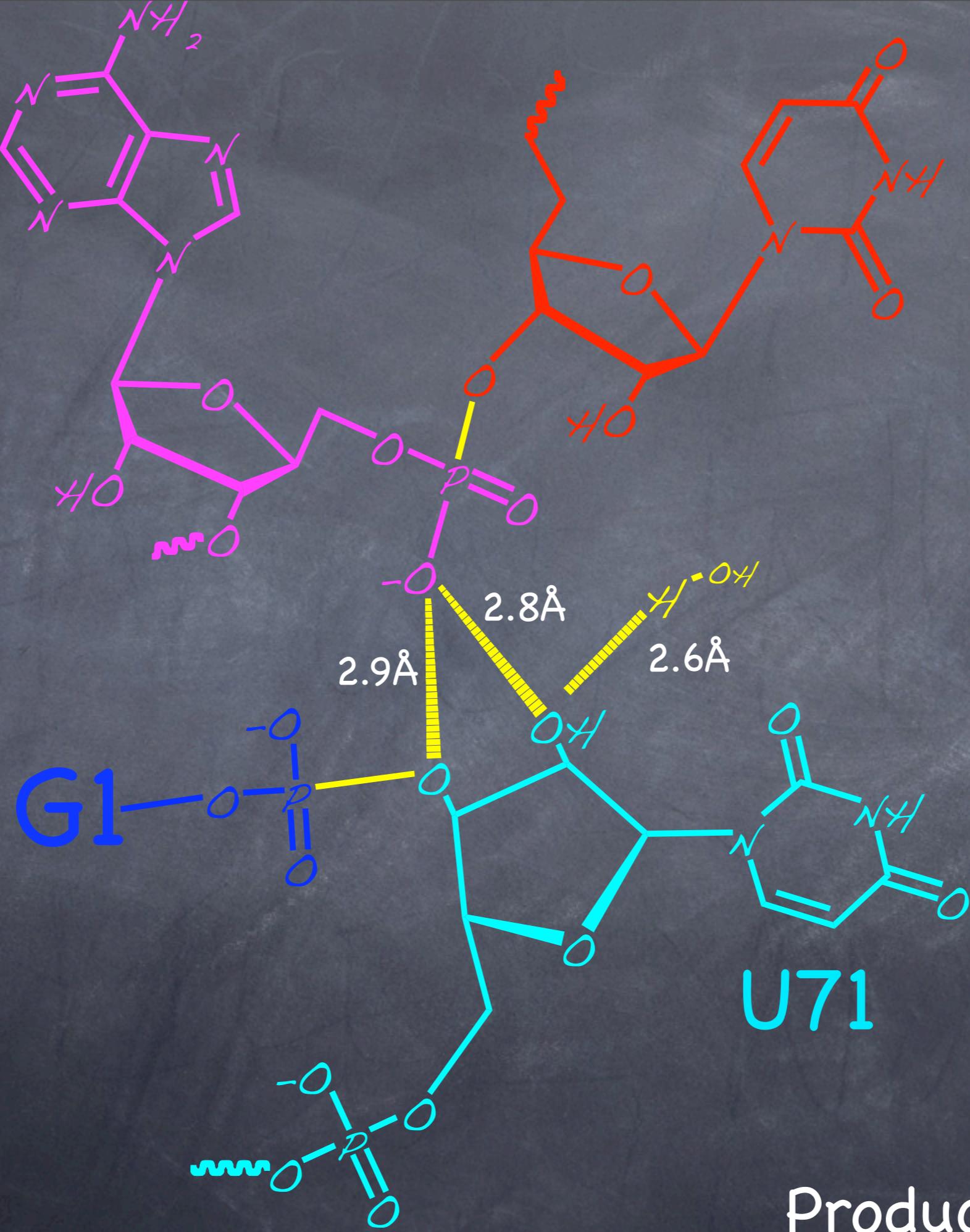


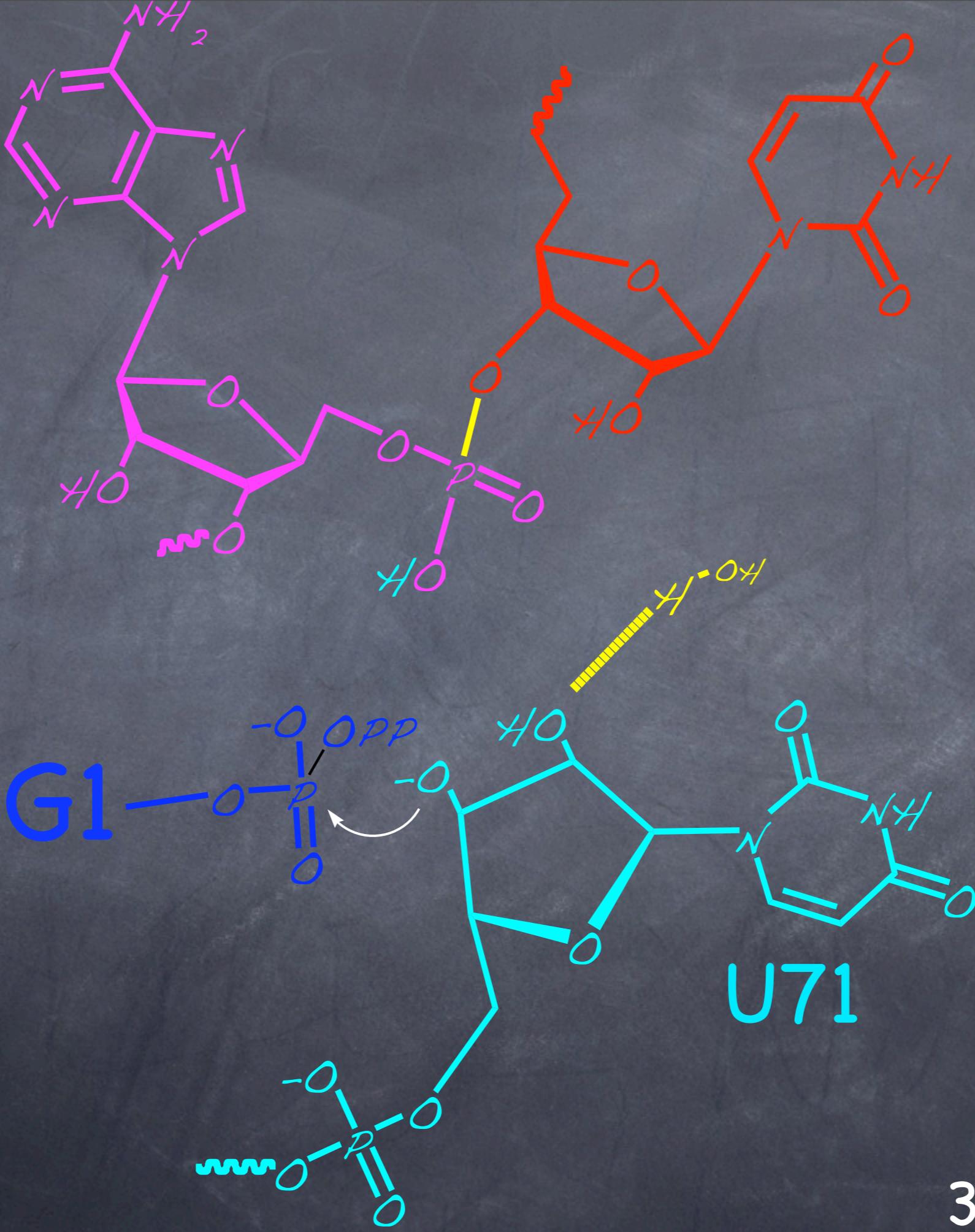


2'H transfer



Water quenches  $2'\text{O}^-$





3'H transfer

*The moment of bond creation ...*



*(Imaginary deity not shown to scale.)*



Many thanks to

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• Harry Noller and UCSC RNA Center

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- Harry Noller and UCSC RNA Center
- Andy Ellington

