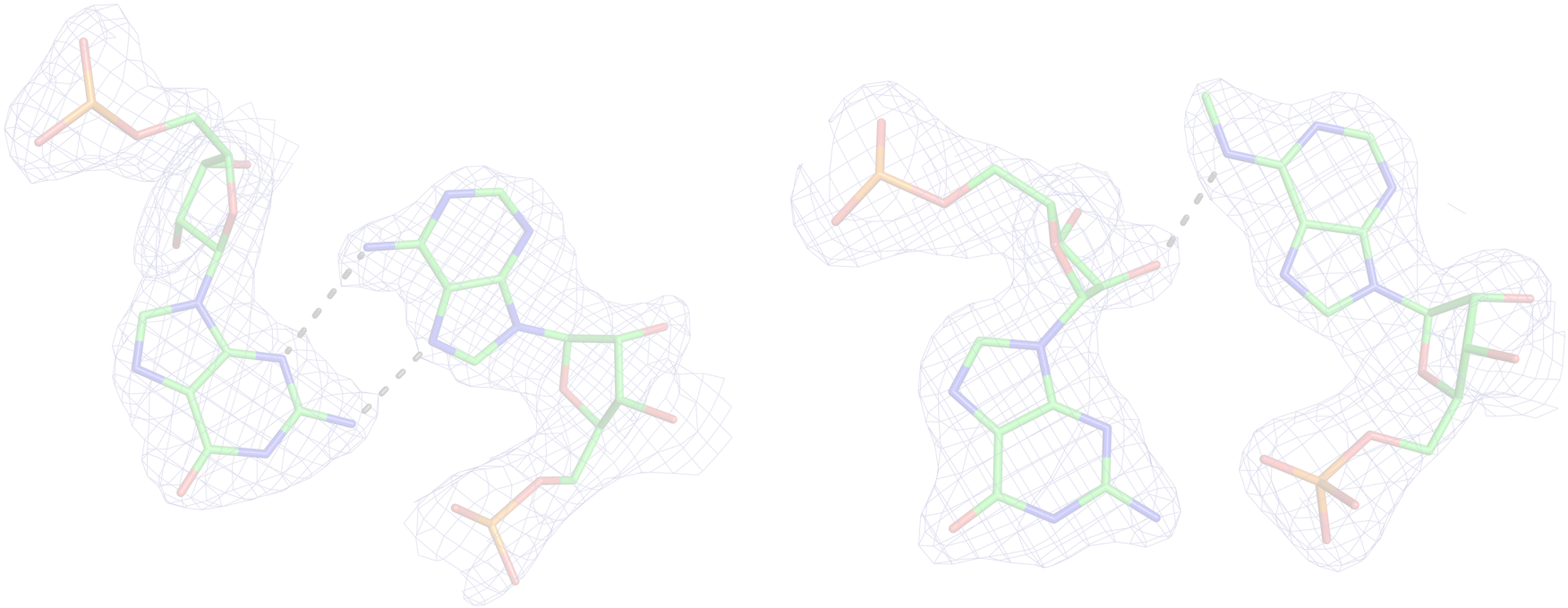
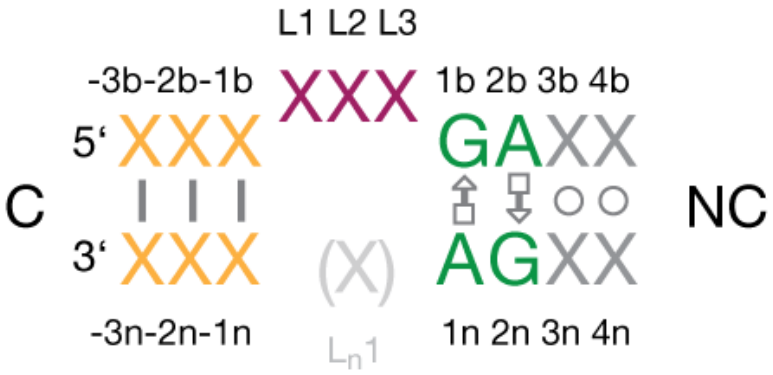
N^6-methylation of adenine in box C/D snoRNA : regulation of RNP assembly

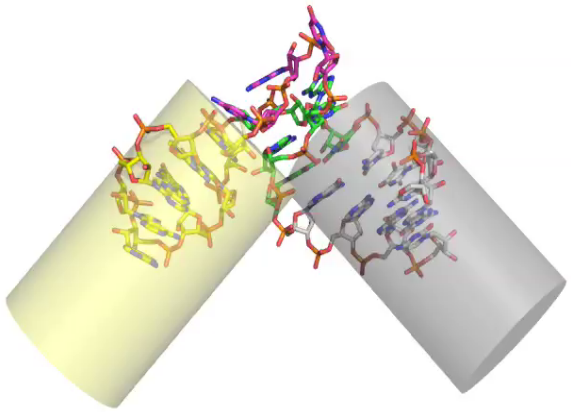


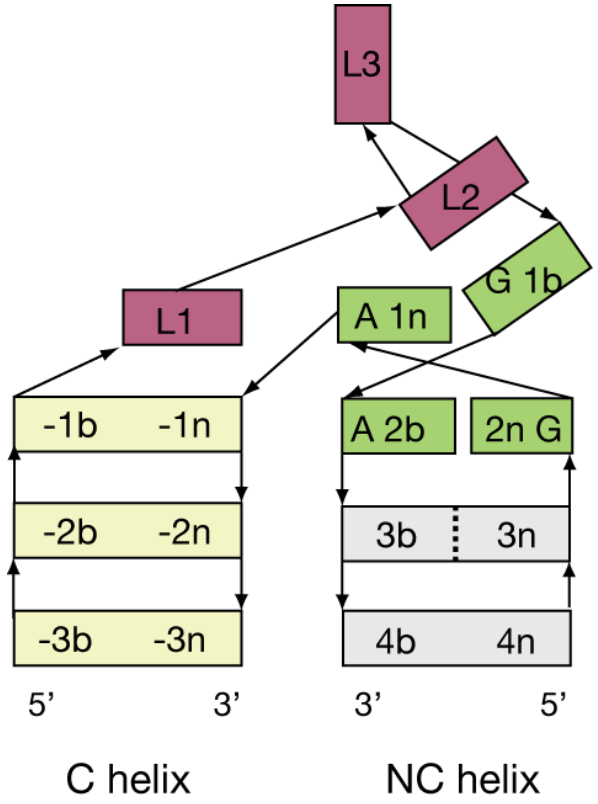
Lin Huang
David MJ Lilley Lab
Dundee University, UK

The k-turn motif - structure

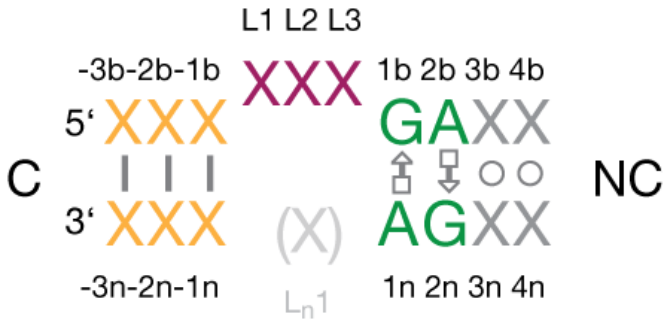
generic k-turn

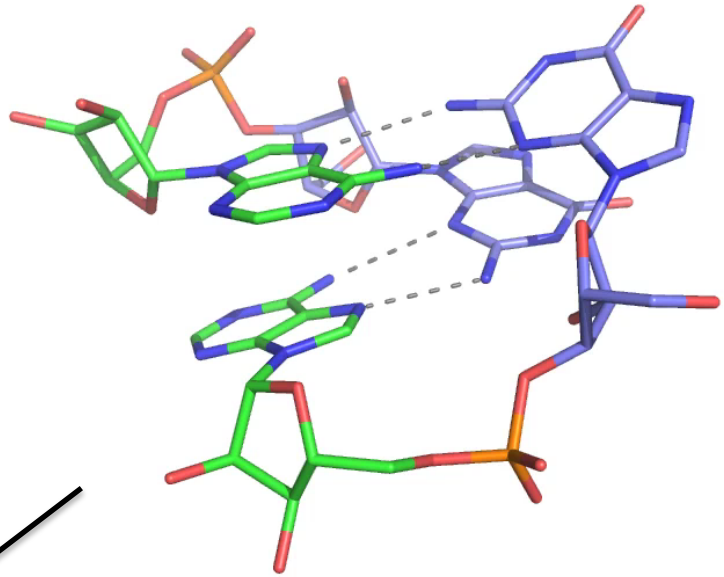


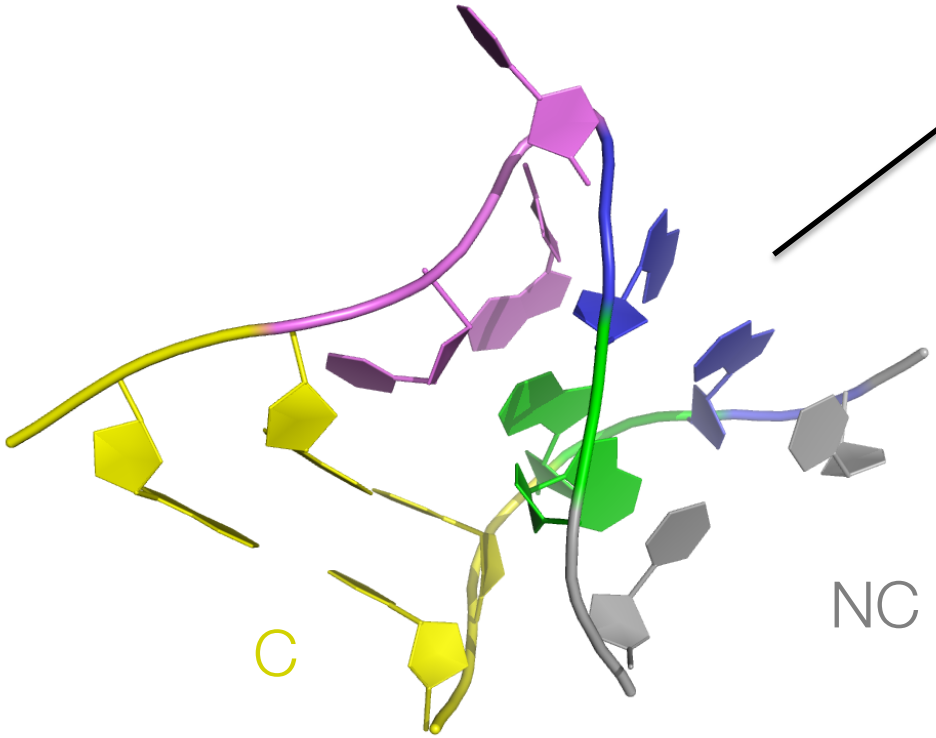


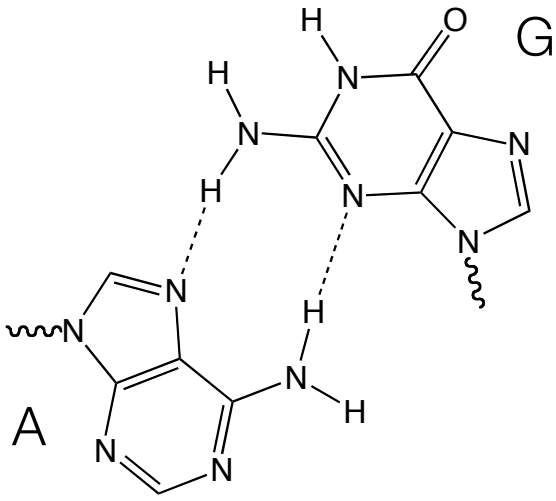


The G•A pairs

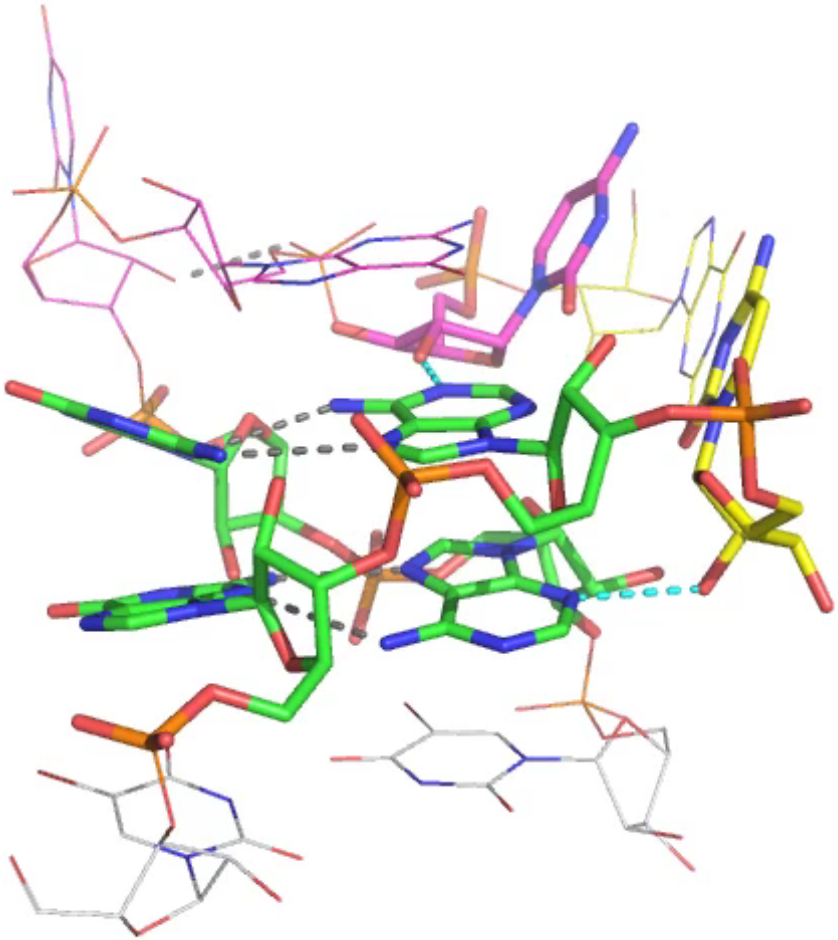

L1 L2 L3
-3b-2b-1b
XXX
1b 2b 3b 4b
5‘ XXX
GAXX
C
NC
3‘ XXX
(X)
AGXX
-3n-2n-1n
1n 2n 3n 4n
Ln1

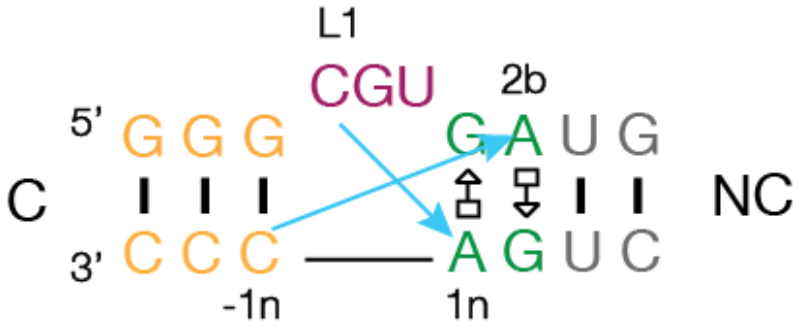



C
NC


G
A
1n / 2b

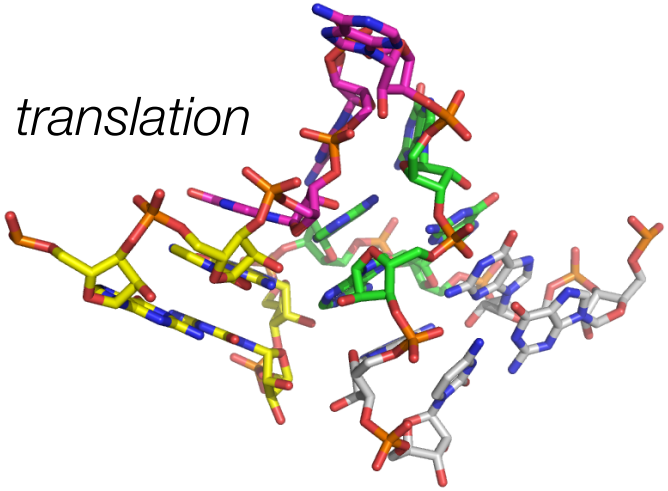
The key hydrogen bonds of a standard k-turn





The ubiquitous functions of k-turns

translation

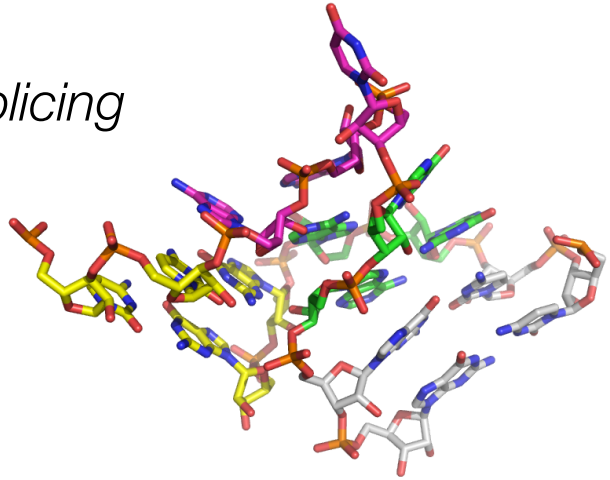


ribosome 50S Kt-7

RNA modification

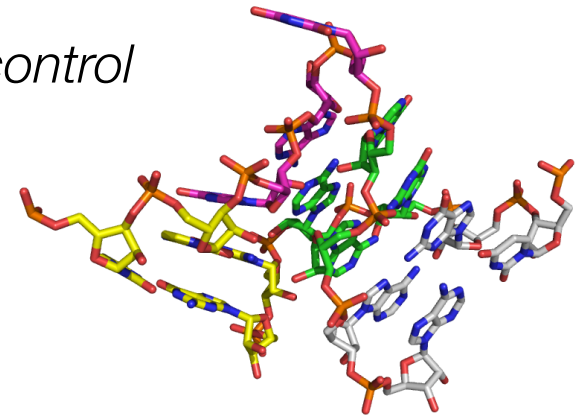
box C/D snoRNA

RNA splicing



U4 snRNA

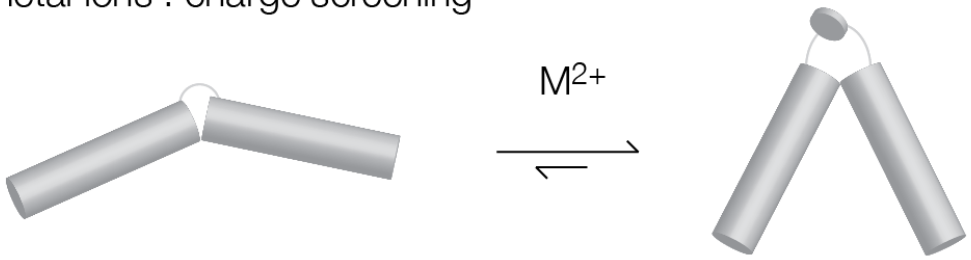
genetic control



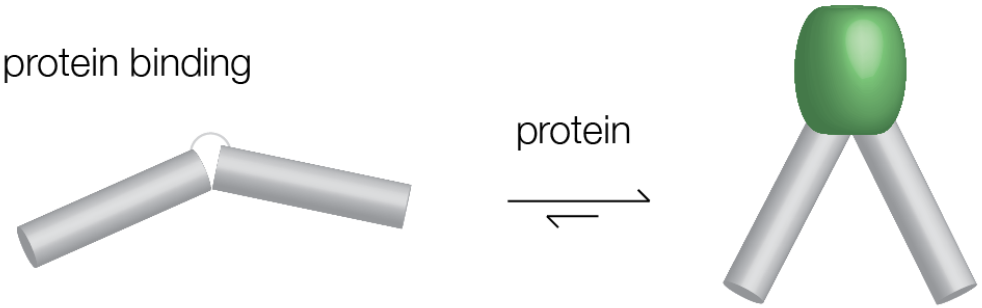
SAM riboswitch

The k-turn folding : three processes

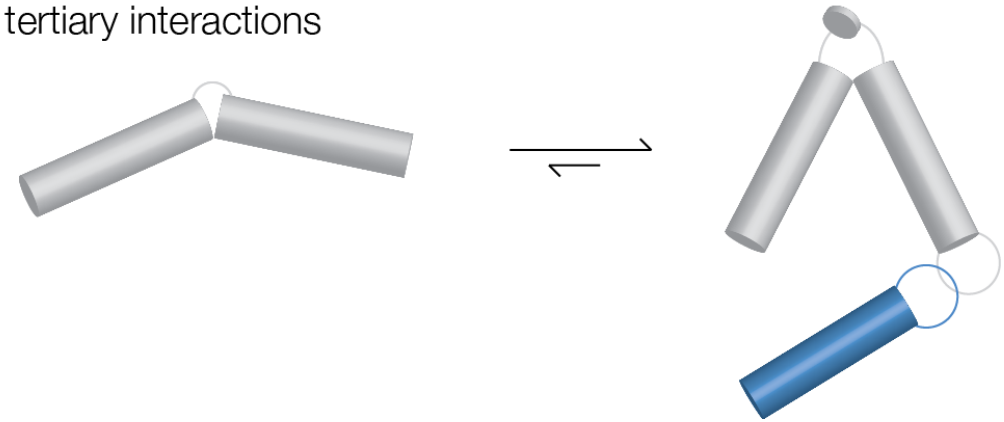
metal ions : charge screening



protein binding

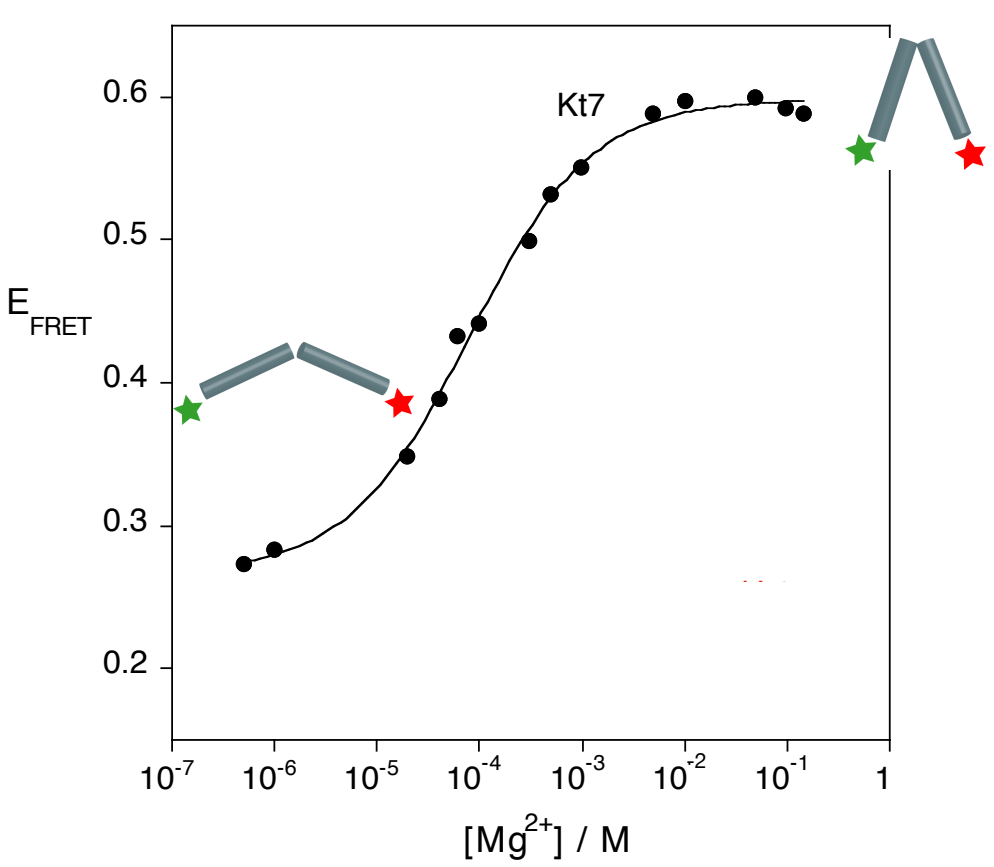


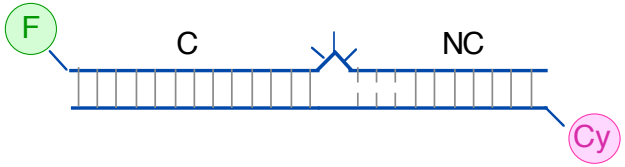
tertiary interactions



Folding of k-turns is induced by metal ions

steady-state FRET



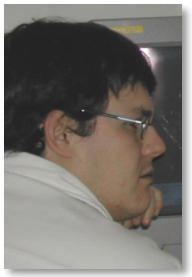


$[Mg^{2+}]_{1/2}$ ~ 80 µM

$[Na^{+}]_{1/2}$ ~ 30 mM

Kinking of K-turn RNA induced by binding of Mg^{2+} ions

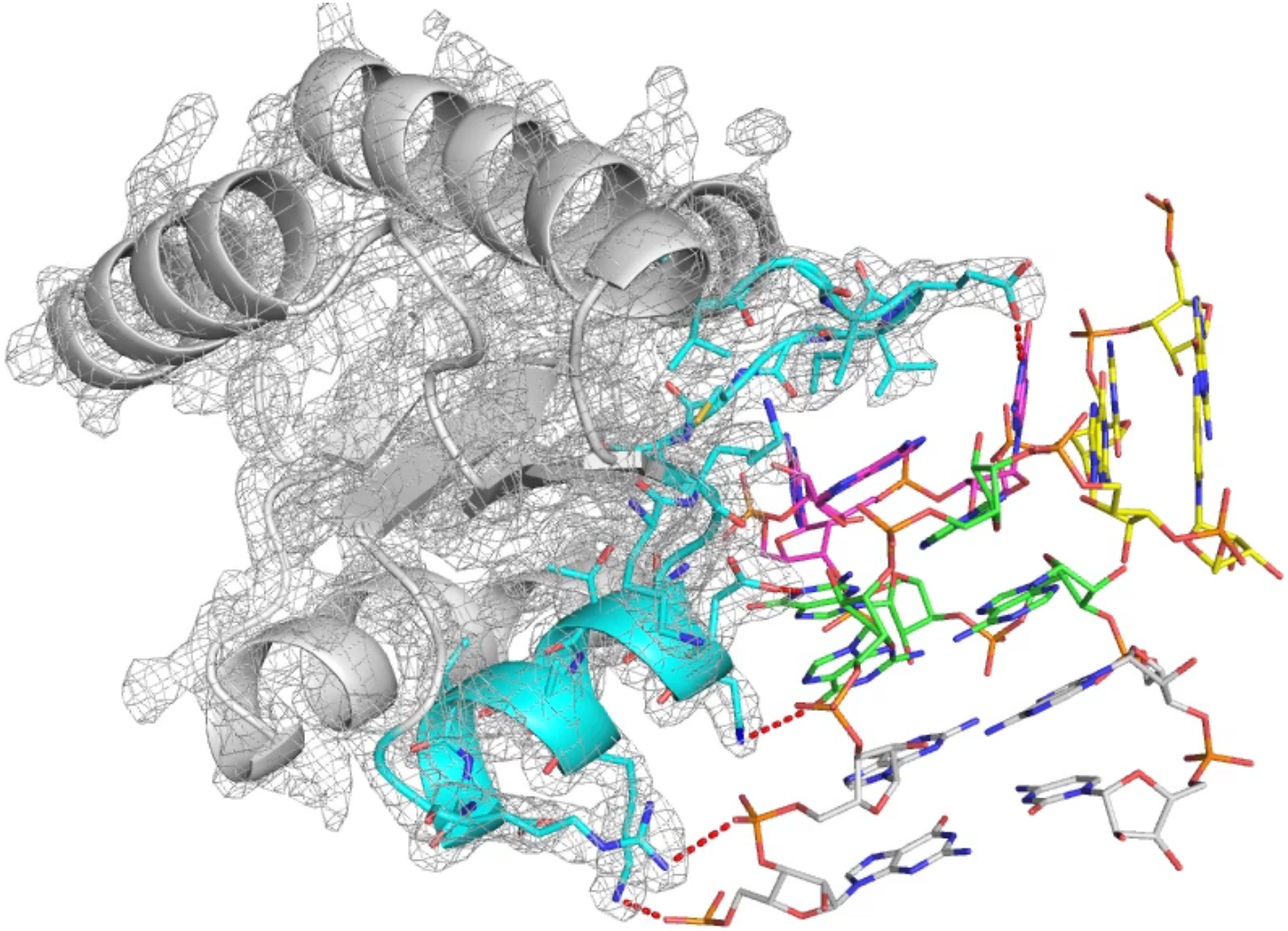
Transition fits two-state behavior



Terry Goody

Goody et al *RNA* **10**, 254–264 (2004) ; Liu & Lilley *RNA* **13**, 200-210 (2007)

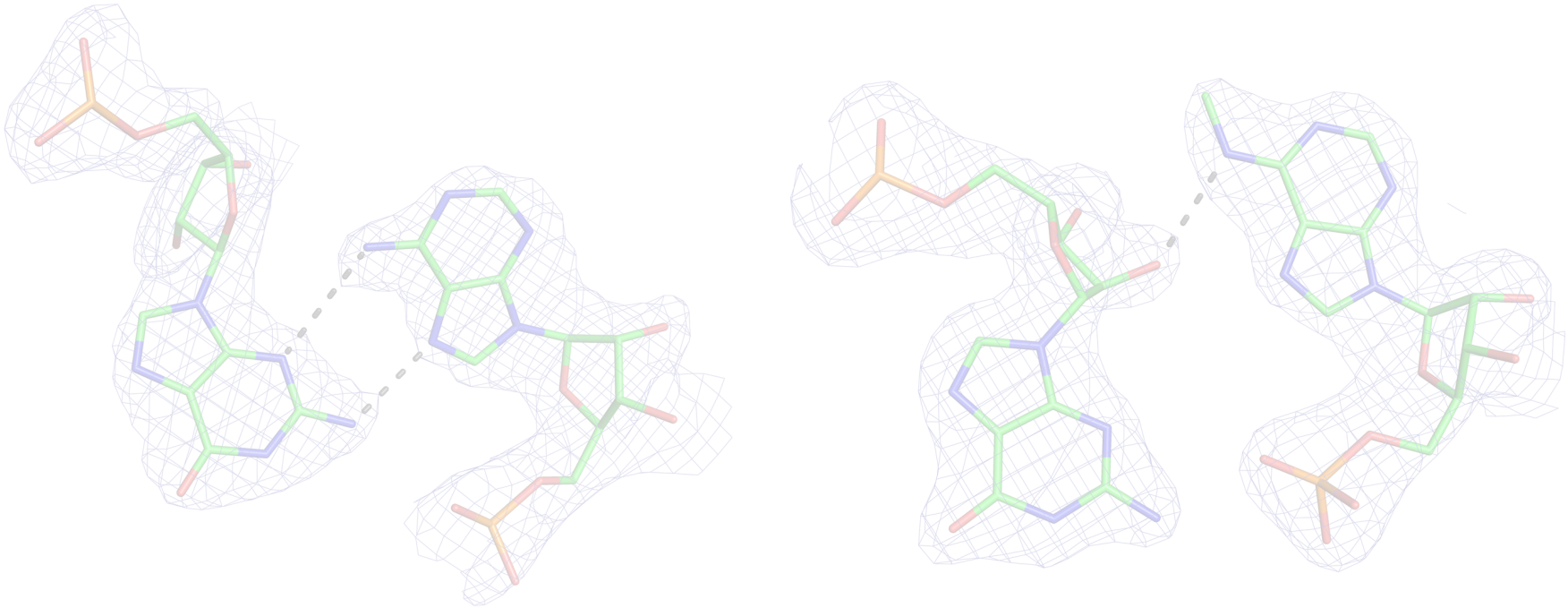
The structure of the complex of L7Ae bound to Kt-7



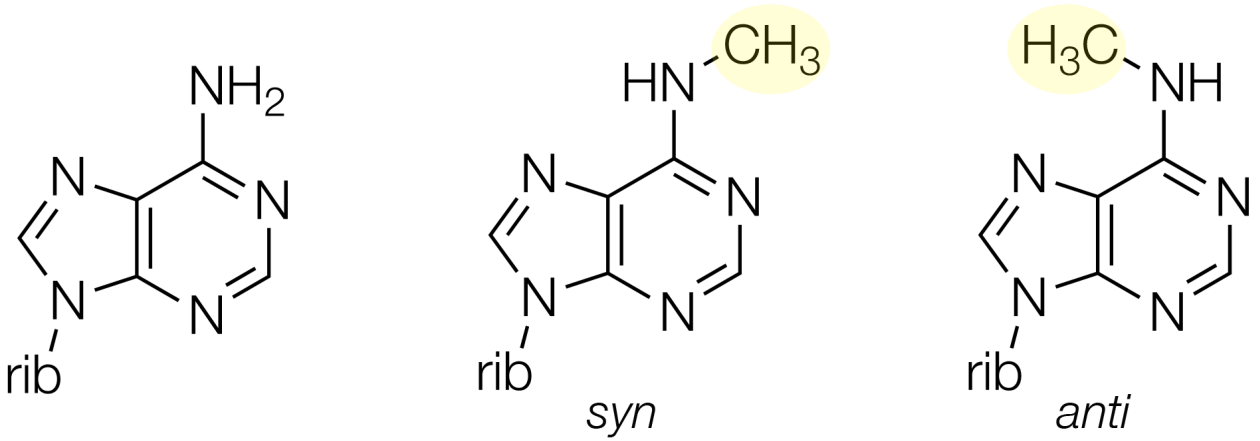
Huang, L & Lilley, *RNA* **19**, 1703-1710 (2013)

2.3 Å resolution

N^6-methylation of adenine in box C/D snoRNA : regulation of RNP assembly



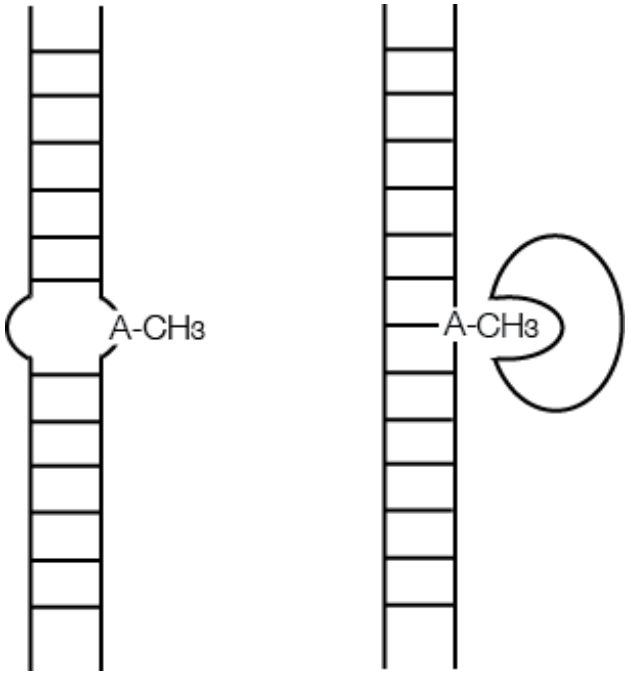
N^6-methylation of adenine in RNA



N^6-methylation of adenine is the most common modification in RNA

How is N^6mA expressed ?

1) A direct effect on local RNA structure
2) Recognition by specific binding proteins



How is N^6mA expressed ?

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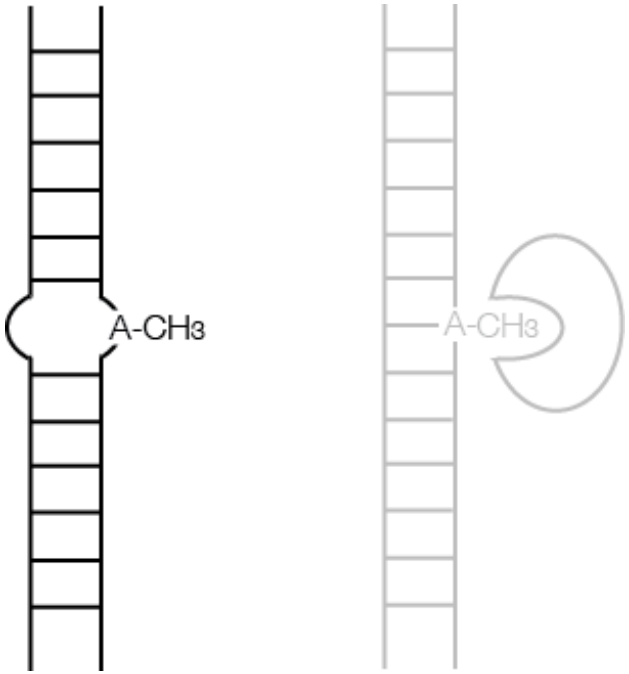
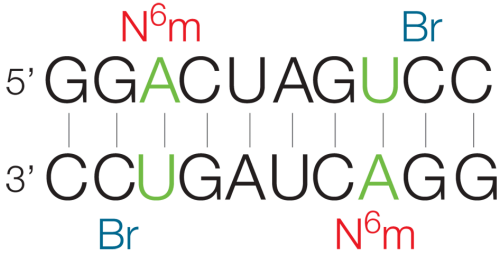
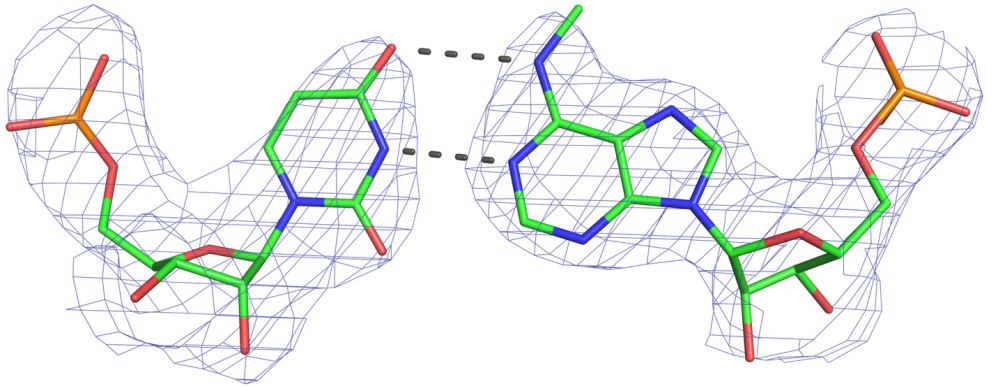
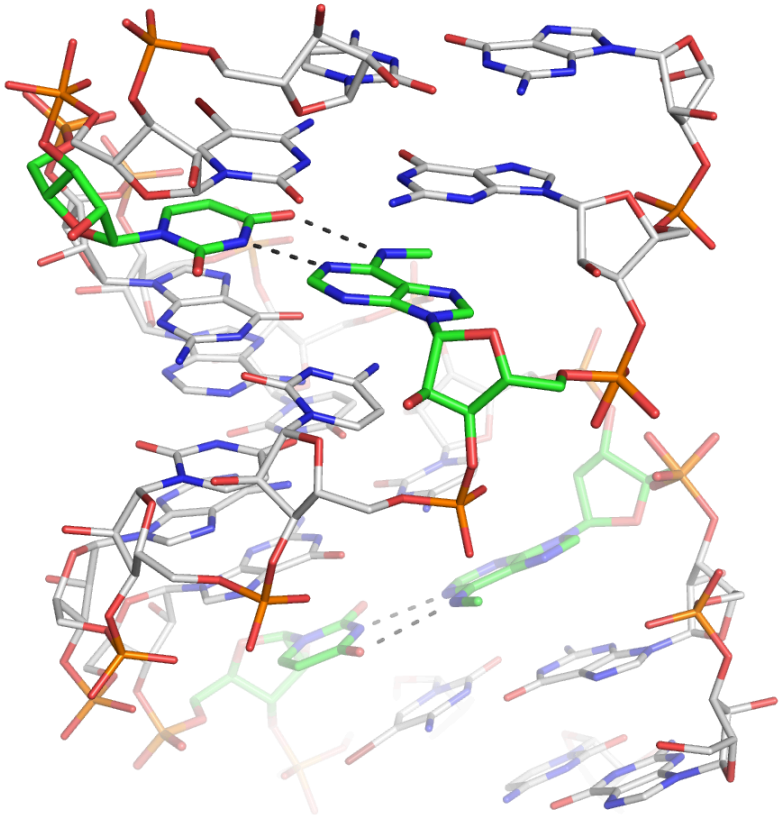


N^6-methylation of adenine is tolerated in Watson-Crick A-U basepair



$P6_5$ 2.27 Å resolution

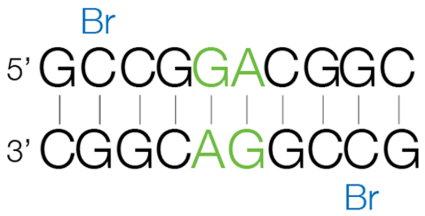


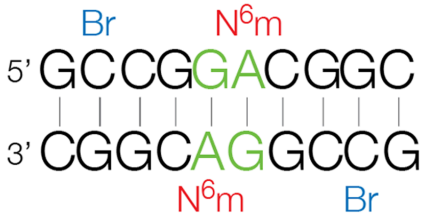


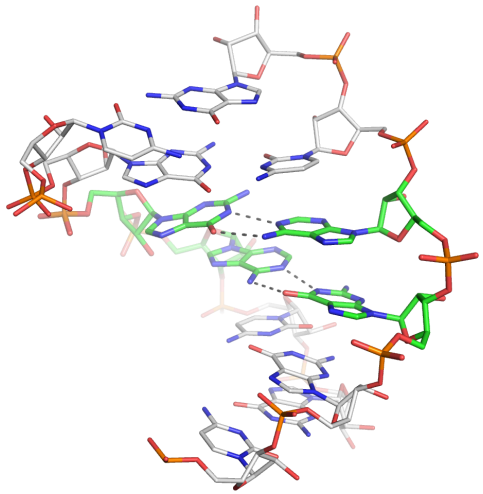
Huang, Ashraf & Lilley *EMBO rep* **18**, 1631-1645 (2017)

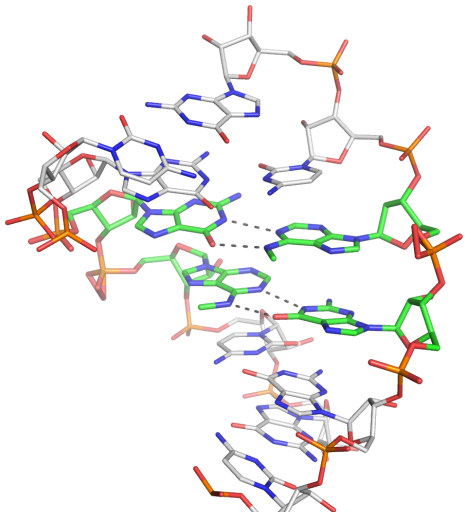
$2F_o$-F_c contoured at 1.2σ

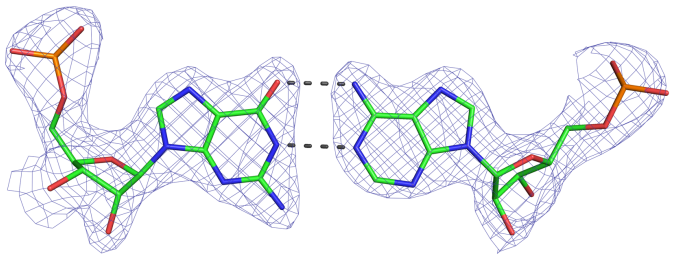
N^6-methylation of adenine is tolerated in Watson-Crick G-A basepair

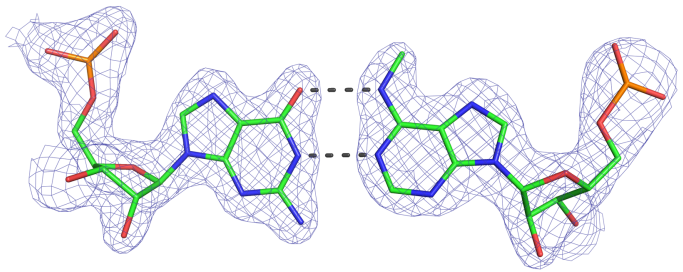












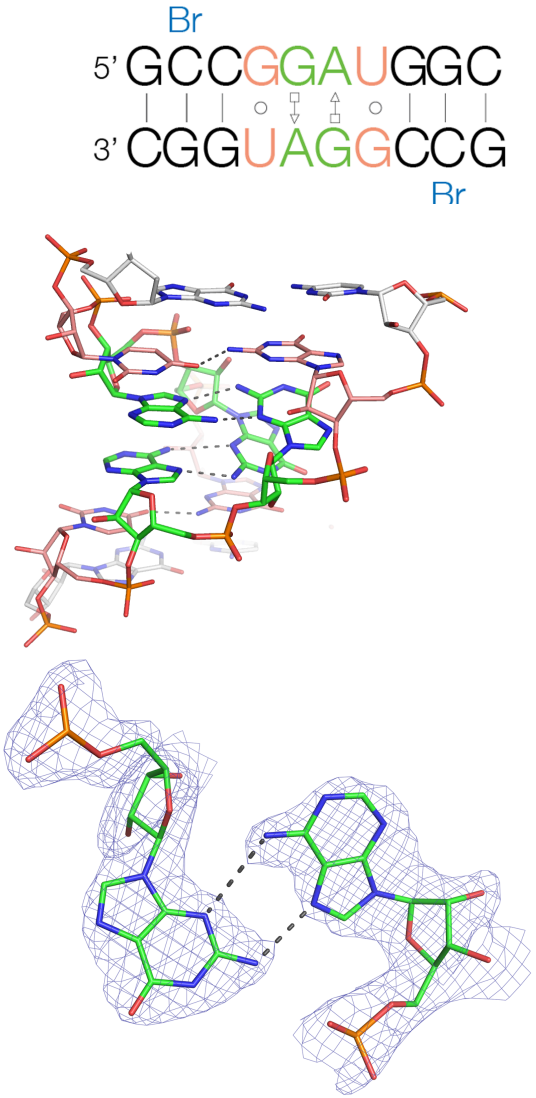
$P4_32_12$ 1.87 Å resolution

$P4_32_12$ 1.50 Å resolution

Huang, Ashraf & Lilley *EMBO rep* **18**, 1631-1645 (2017)

$2F_o$-F_c contoured at 1.2σ

N^6-methylation of adenine is NOT tolerated in sugar-Hoogsteen G-A basepair



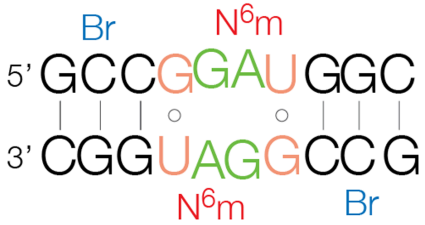
$2F_o$-F_c
contoured at 1.2σ

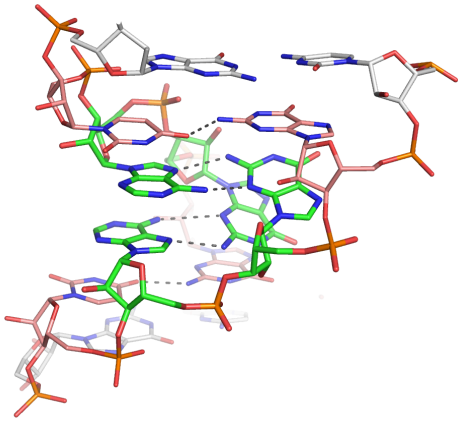
$P4_32_12$ 1.65 Å resolution

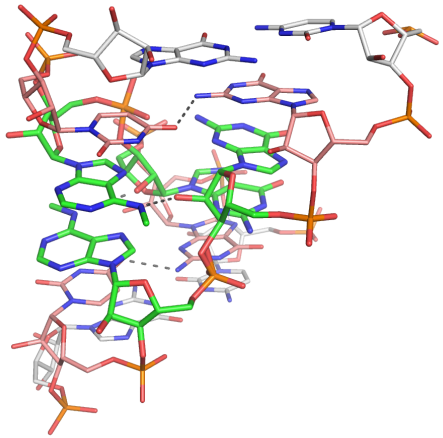
Huang, Ashraf & Lilley *EMBO rep* **18**, 1631-1645 (2017)

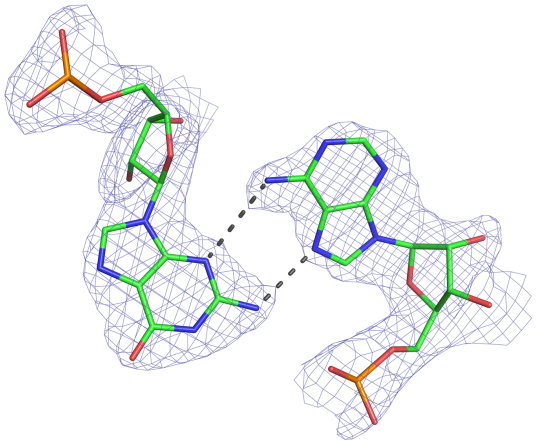
$2F_o$-F_c contoured at 1.2σ

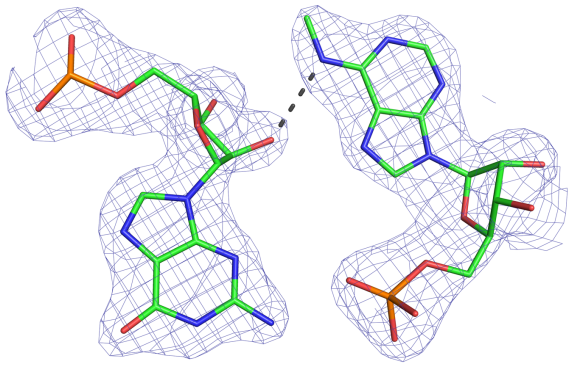
N^6-methylation of adenine is NOT tolerated in sugar-Hoogsteen G-A basepair











$2F_o$-F_c
contoured at 1.2σ

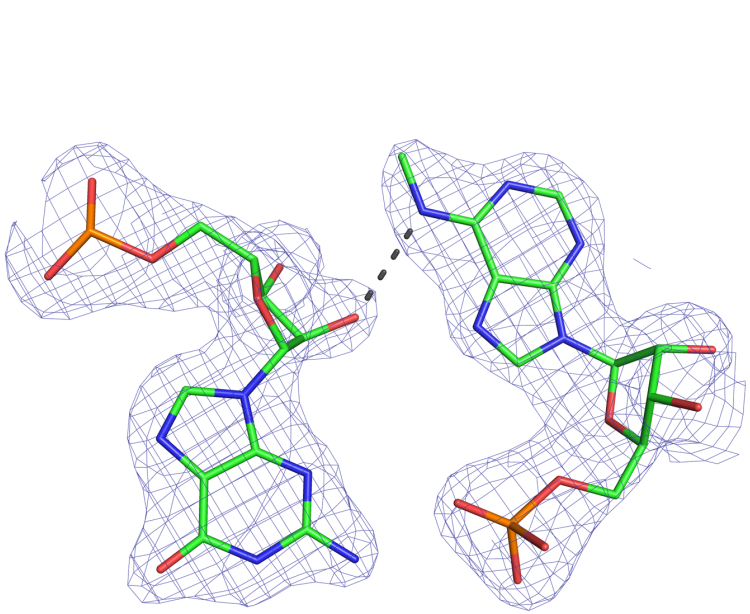
$P4_32_12$ 1.65 Å resolution

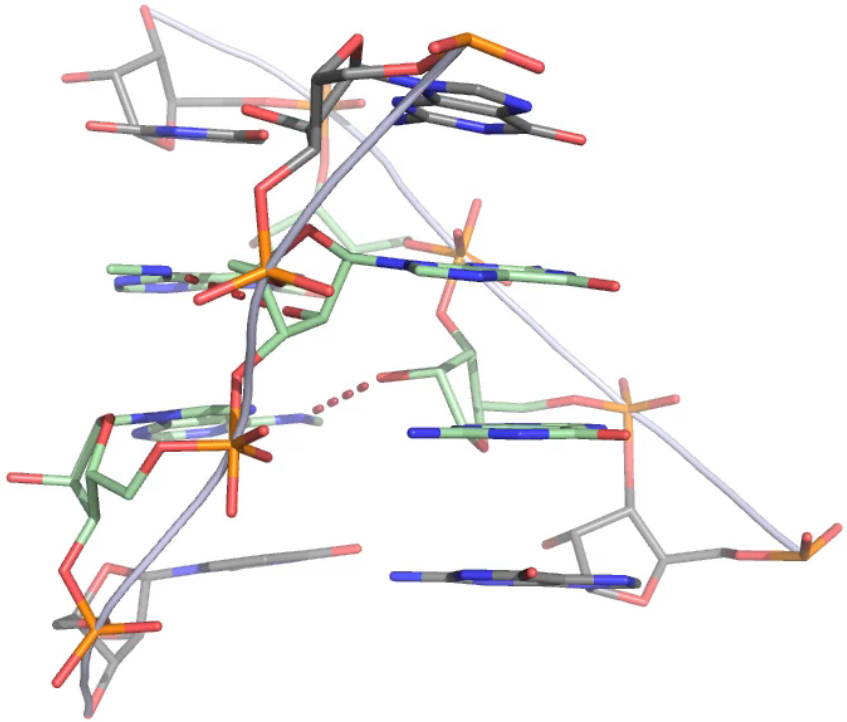
$P4_32_12$ 1.72 Å resolution

Huang, Ashraf & Lilley *EMBO rep* **18**, 1631-1645 (2017)

$2F_o$-F_c contoured at 1.2σ

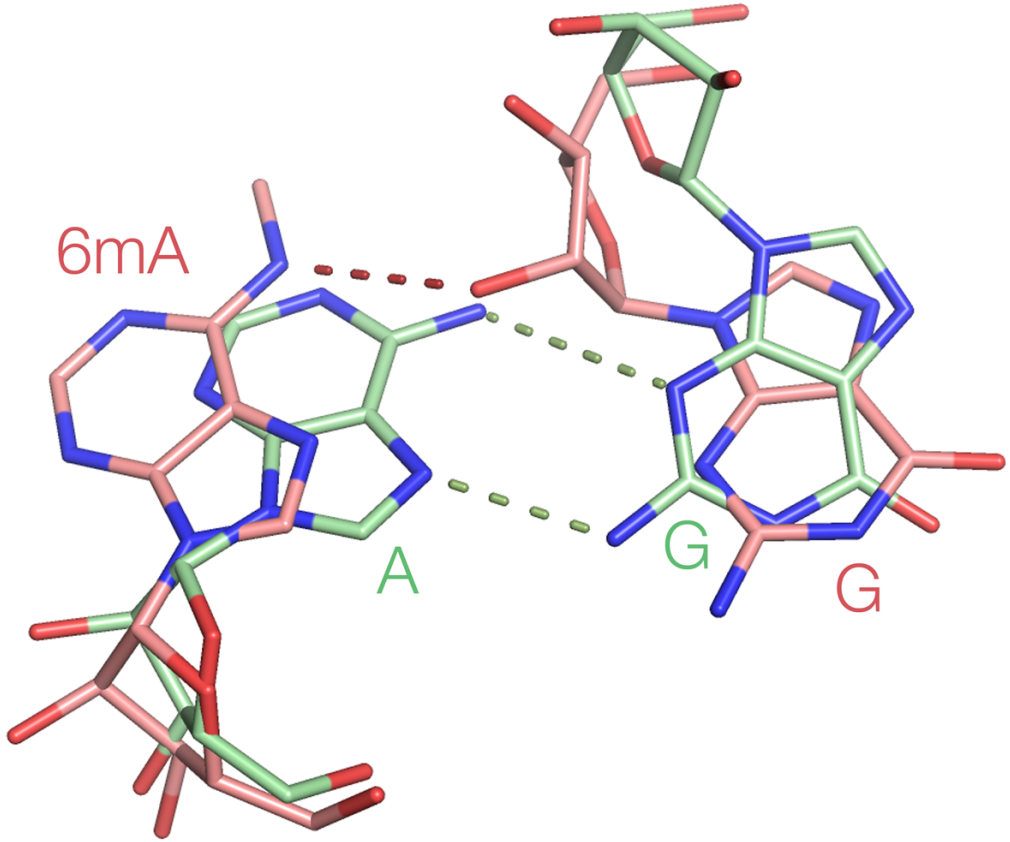
N^6-methylation of adenine disrupts sugar-Hoogsteen G-A basepair





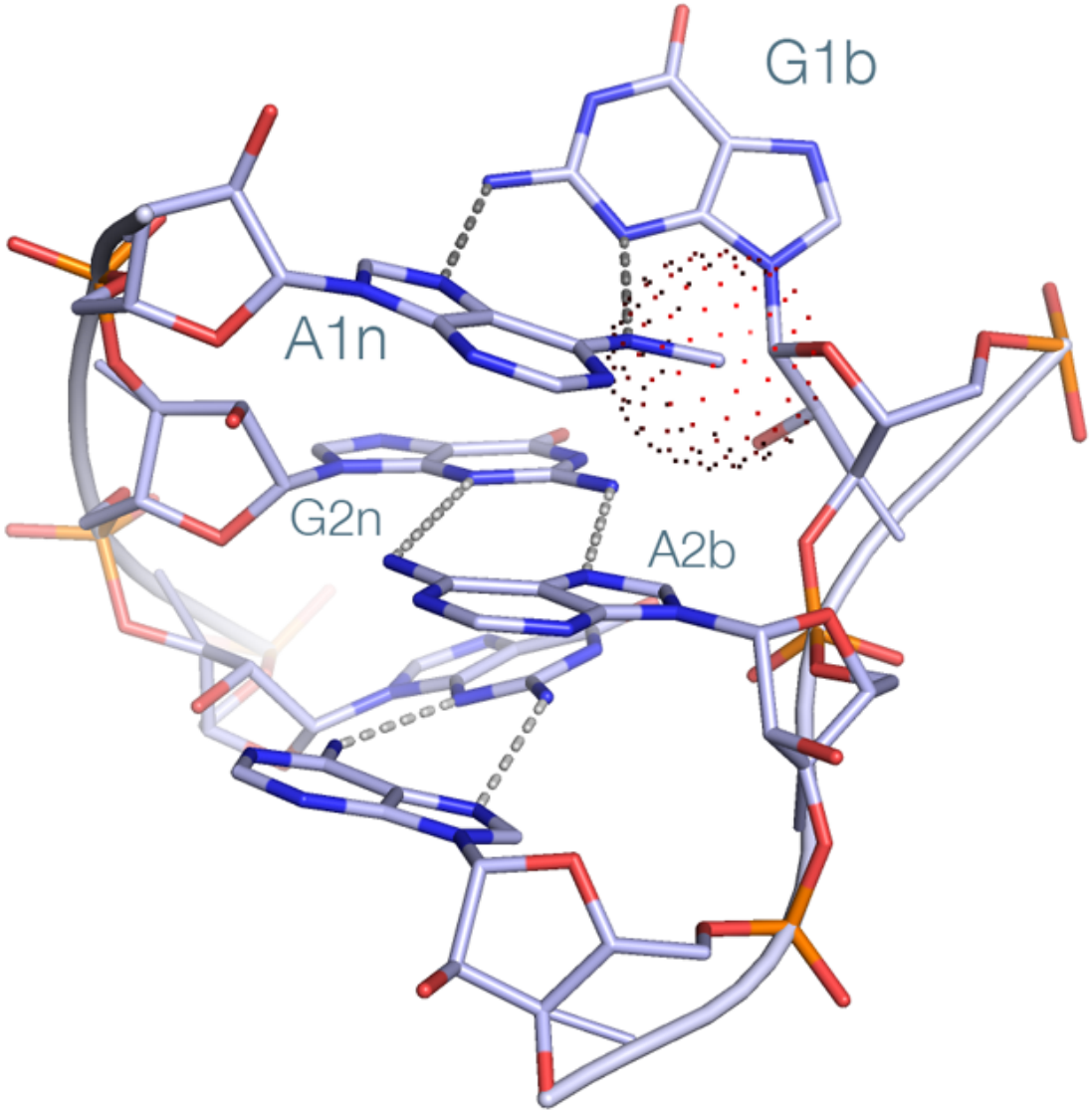
Huang, Ashraf & Lilley *EMBO rep* **18**, 1631-1645 (2017)

N^6-methylation of adenine disrupts sugar-Hoogsteen G-A basepair



Huang, Ashraf & Lilley *EMBO rep* **18**, 1631-1645 (2017)

N^6-methylation of adenine causes steric clash in sugar-Hoogsteen G-A basepair

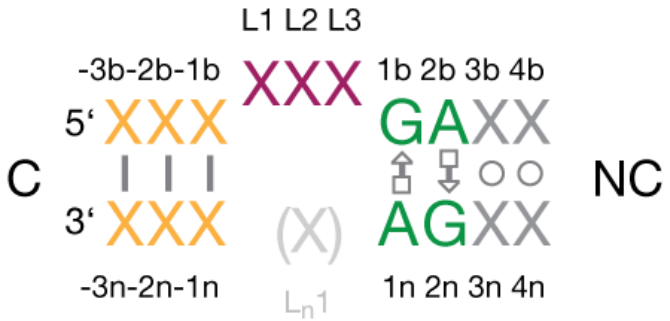


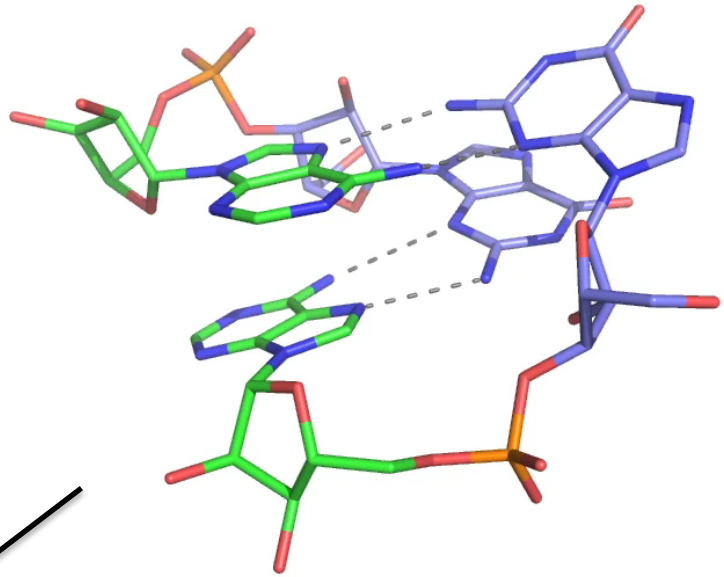
Huang, Ashraf & Lilley *EMBO rep* **18**, 1631-1645 (2017)

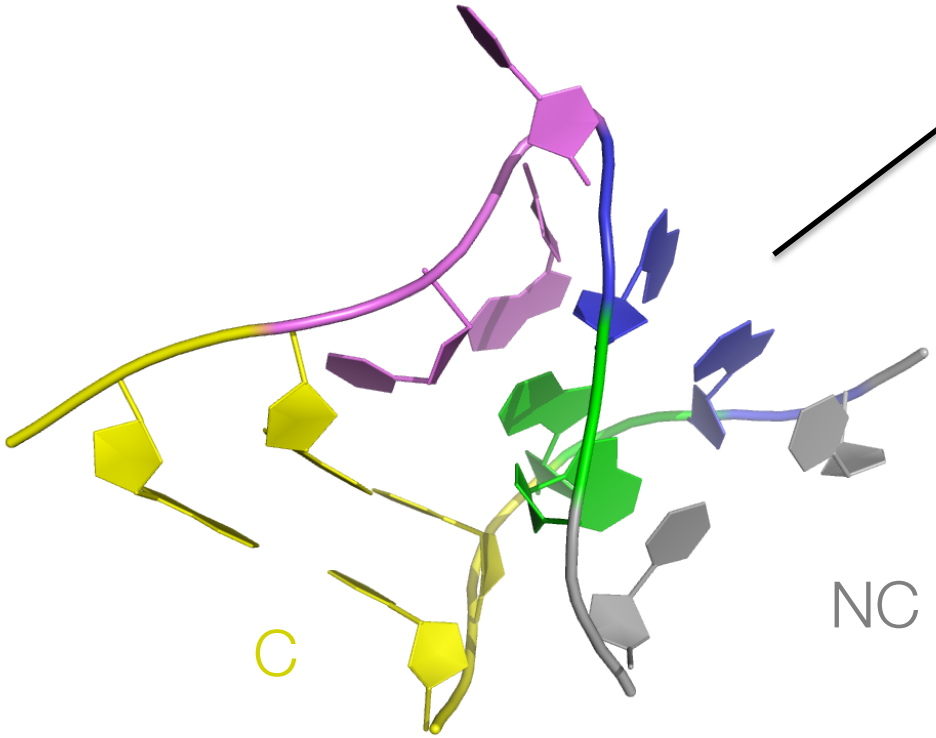
Conclusion : N^6-methylation of adenine is tolerated in Watson-Crick pairs

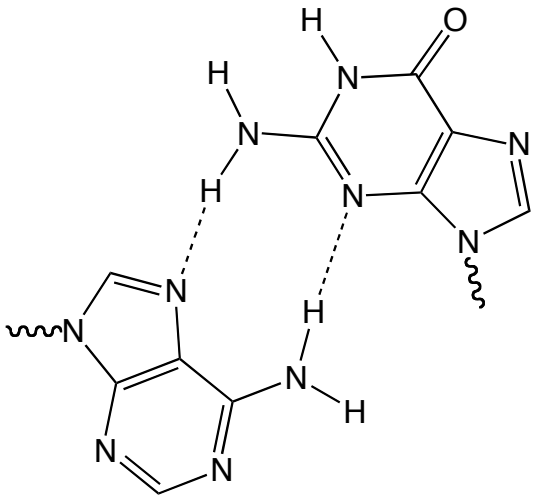
but disrupts sugar-Hoogsteen sheared G•A pairs

The G•A pairs are the core of k-turn structure



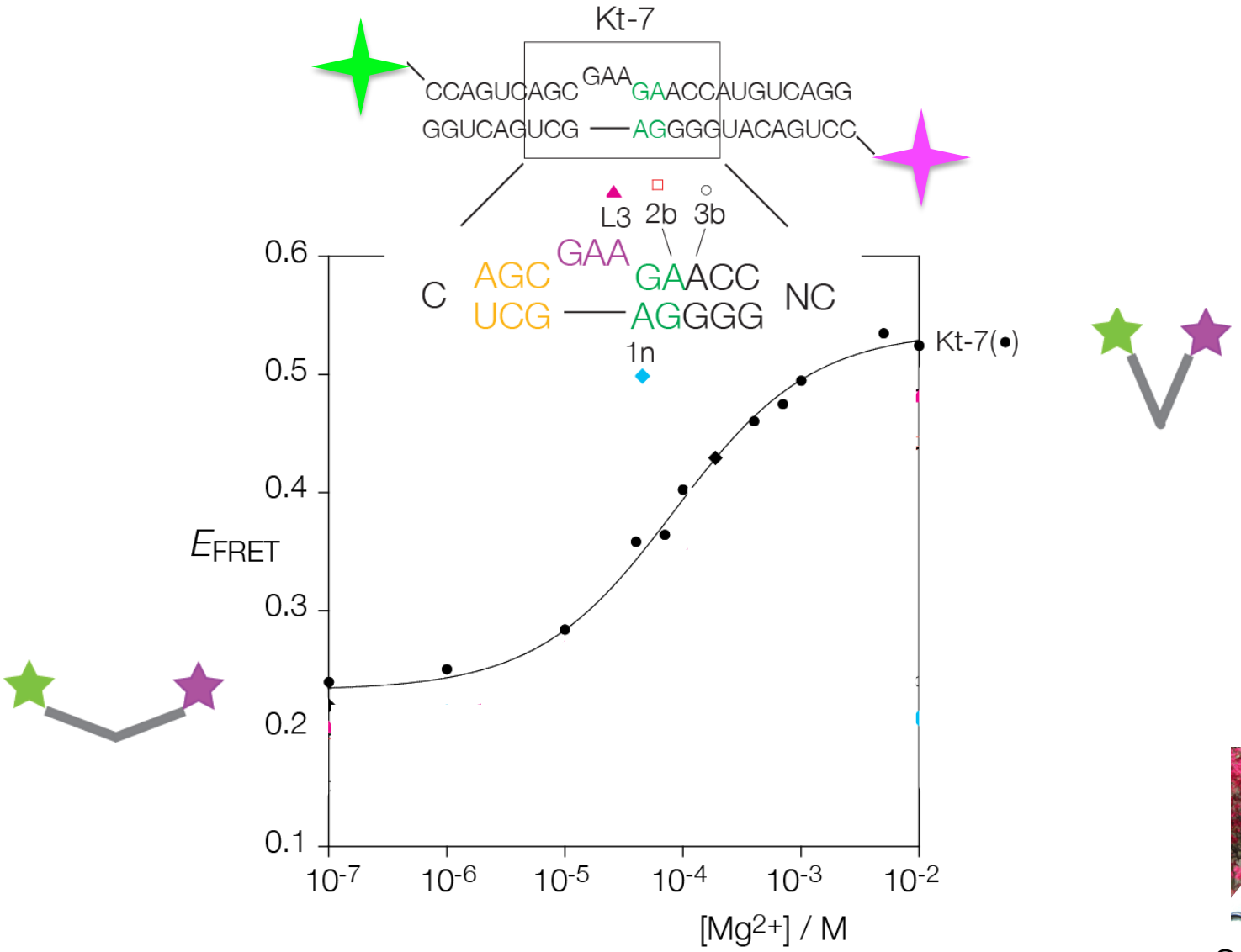






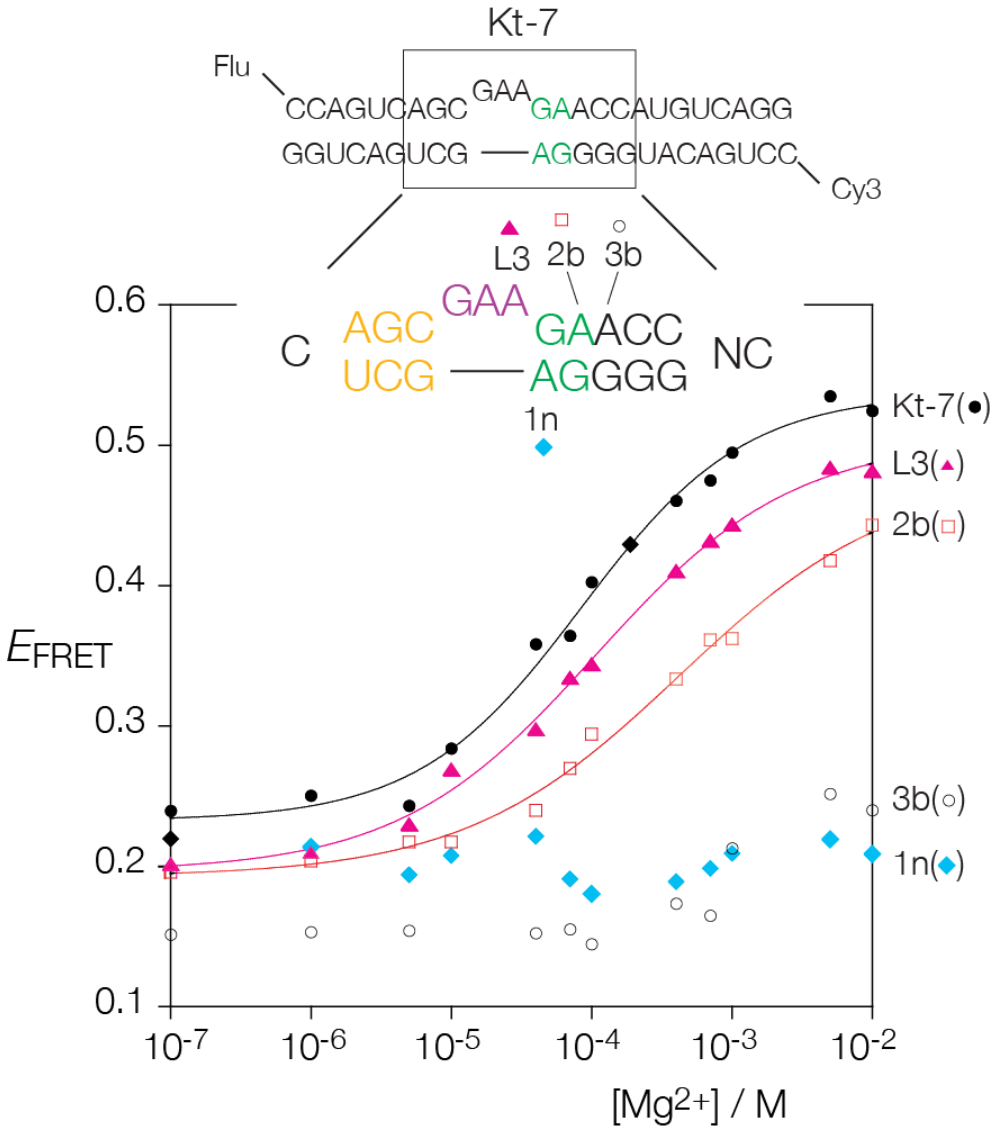
1n / 2b

Effect of N^6-methylation of adenine on the ion-induced folding of Kt-7



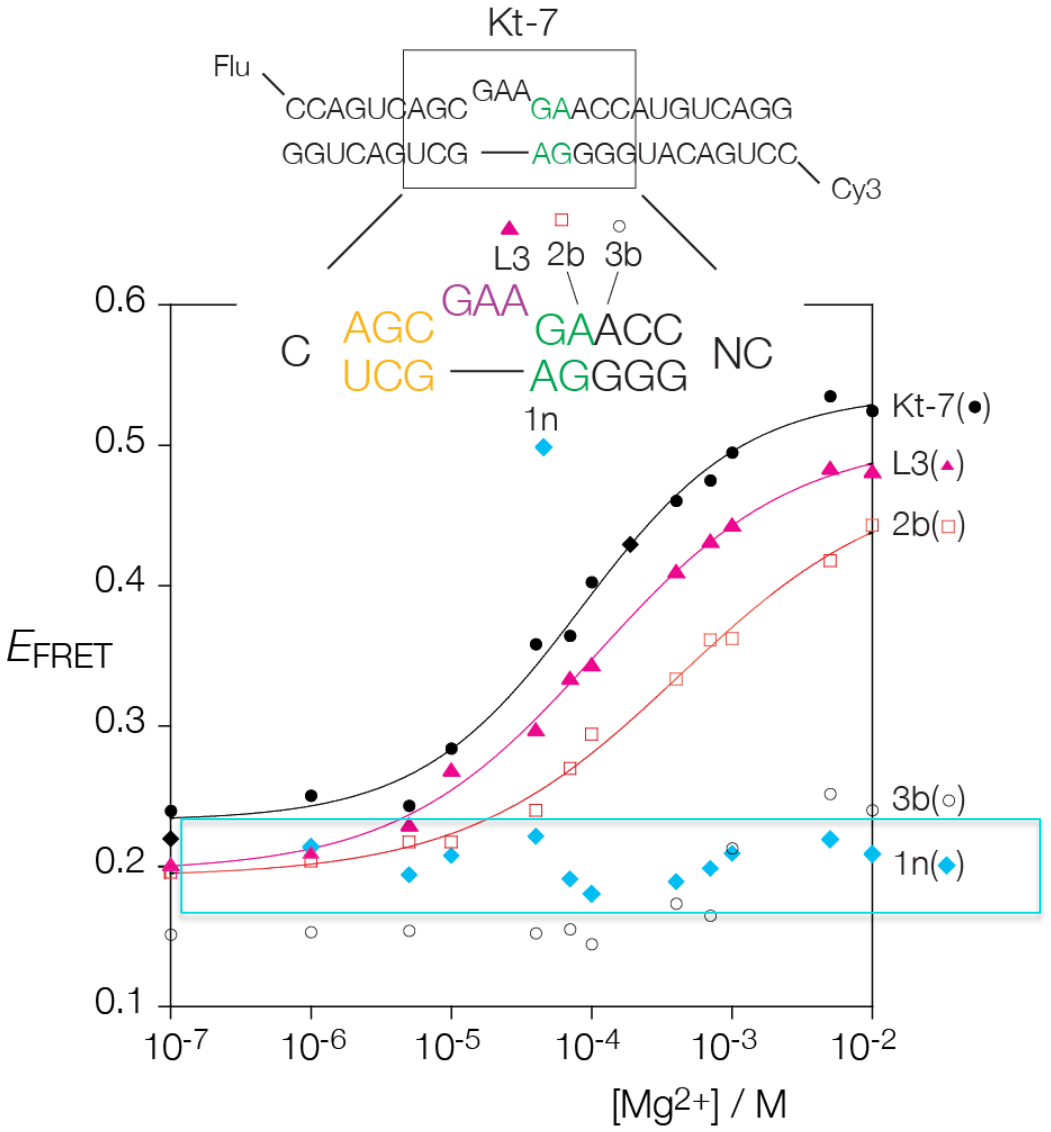
Saira Ashraf

Effect of N^6-methylation of adenine on the ion-induced folding of Kt-7



Saira Ashraf

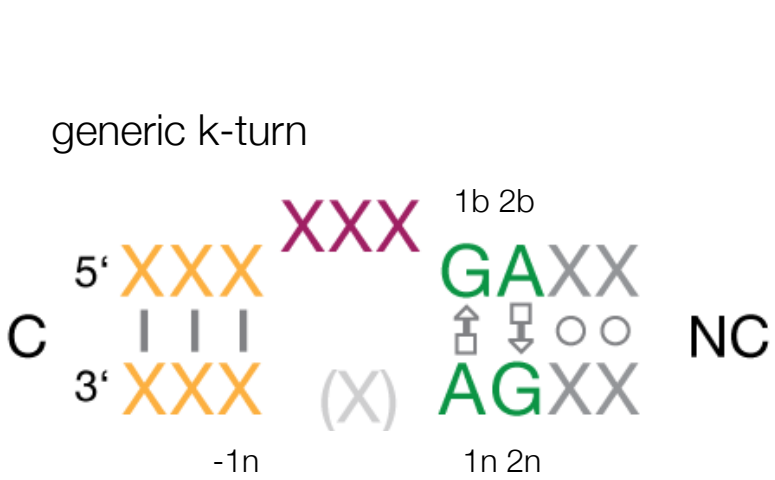
Effect of N^6-methylation of adenine on the ion-induced folding of Kt-7

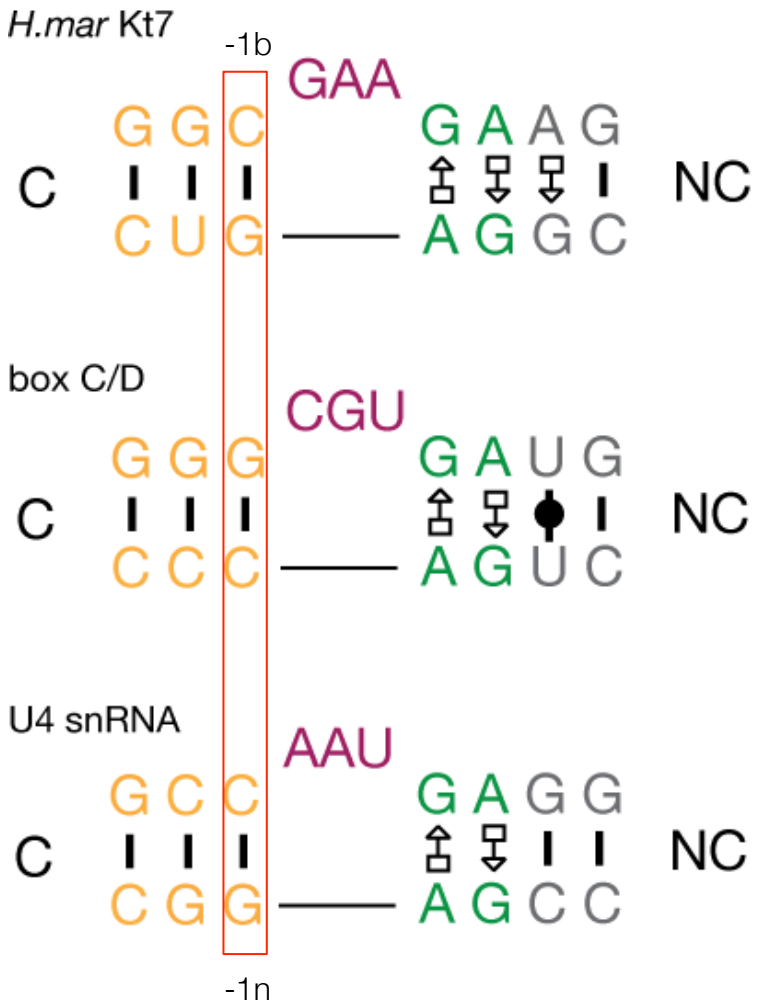


1n methylation prevents folding

Saira Ashraf

The k-turn motif

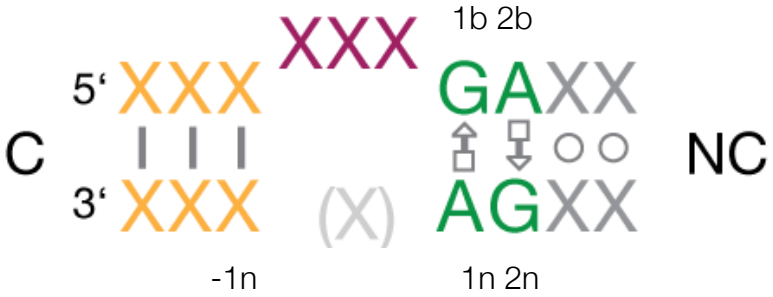


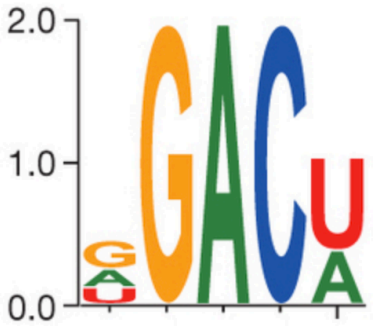


The k-turn motif

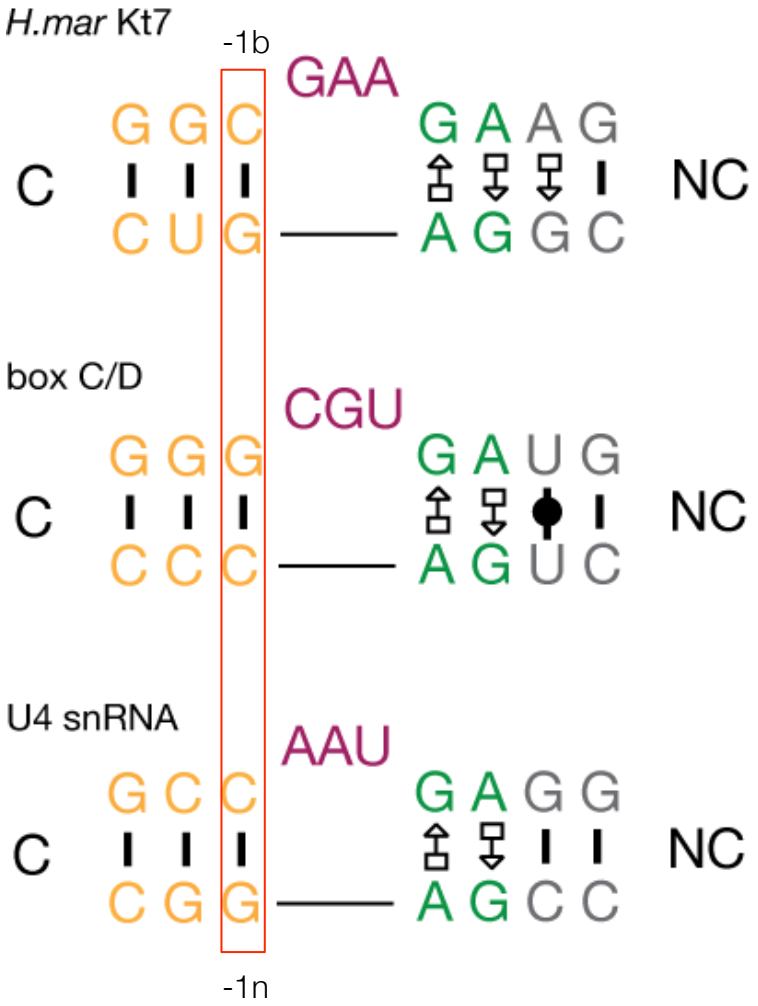
《kturn》结构基序

generic k-turn

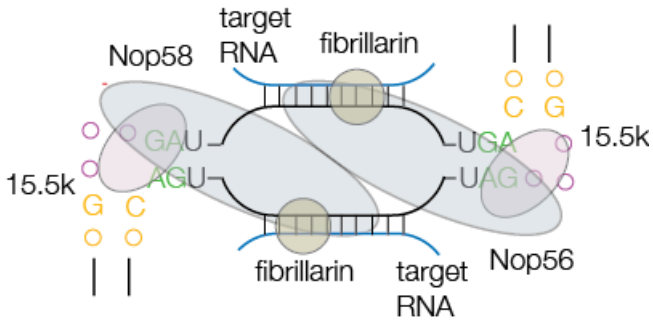


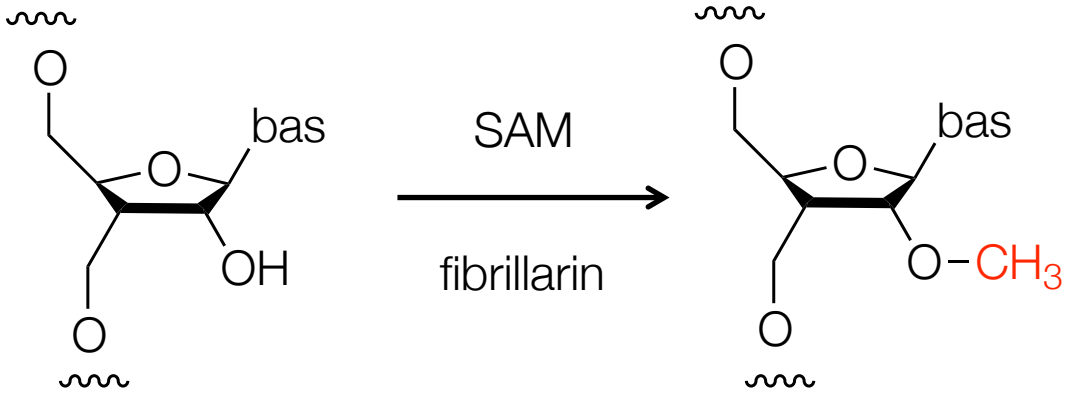


N6mA methyl transferase consensus sequence



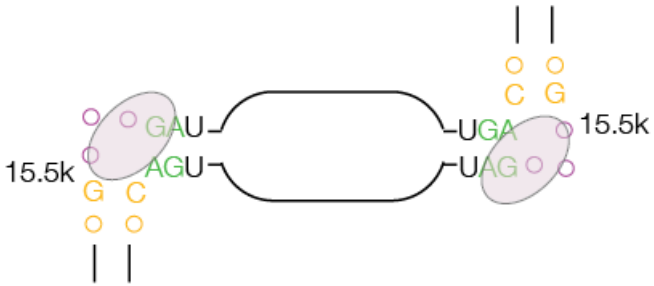
Box C/D snoRNP : site-specific O2’ methylation



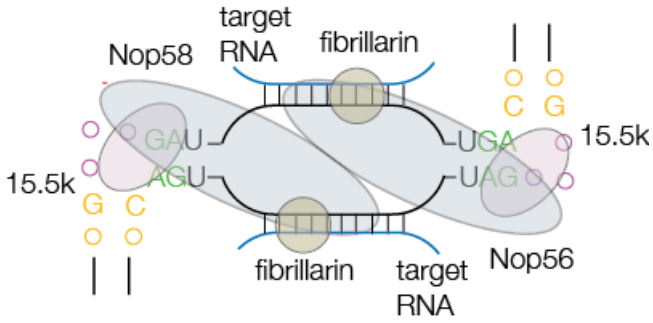


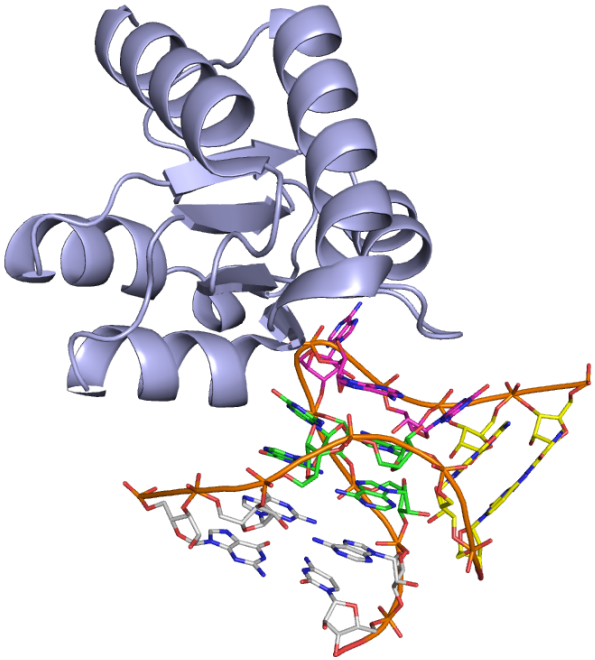
Box C/D snoRNP assembly

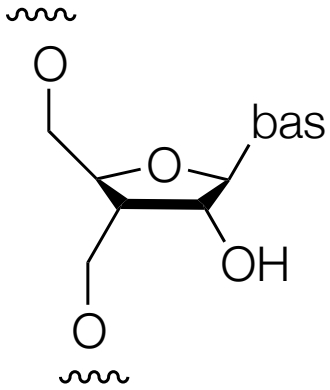
15.5k



Nop56, Nop58
Fibrillarin
target RNA

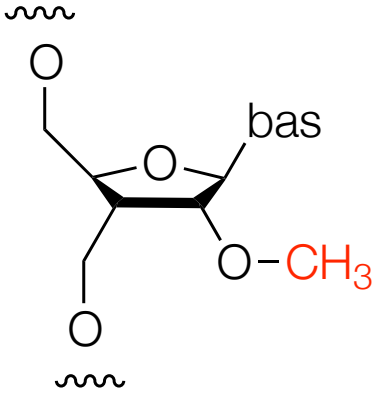






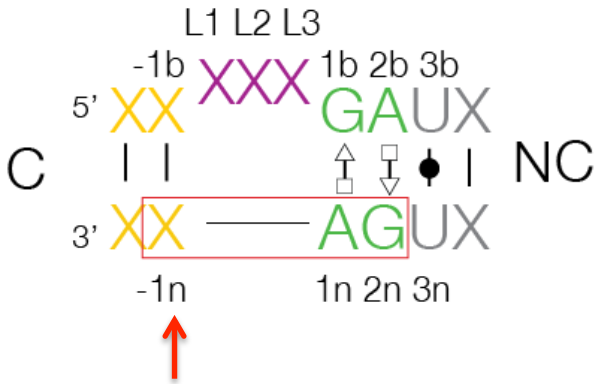
SAM

fibrillarin



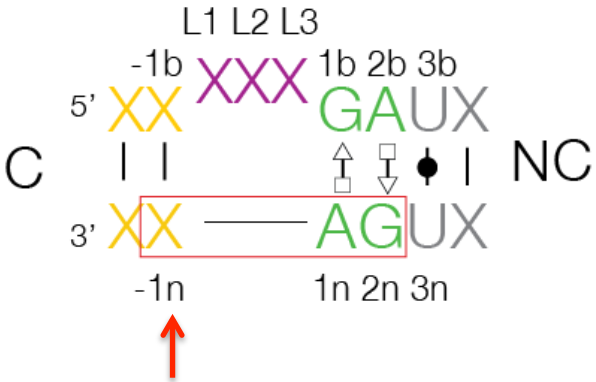
Some snoRNP sequences have a N^6A methylation sequence

Potential methylation sites in box C/D k-turn sequences



If -1n = C, it creates GAC methylation target on lower strand

Potential methylation sites in box C/D k-turn sequences



If -1n = C, it creates GAC methylation target on lower strand

In humans, how frequently is this C ?

And how often is it the A1n methylated ?

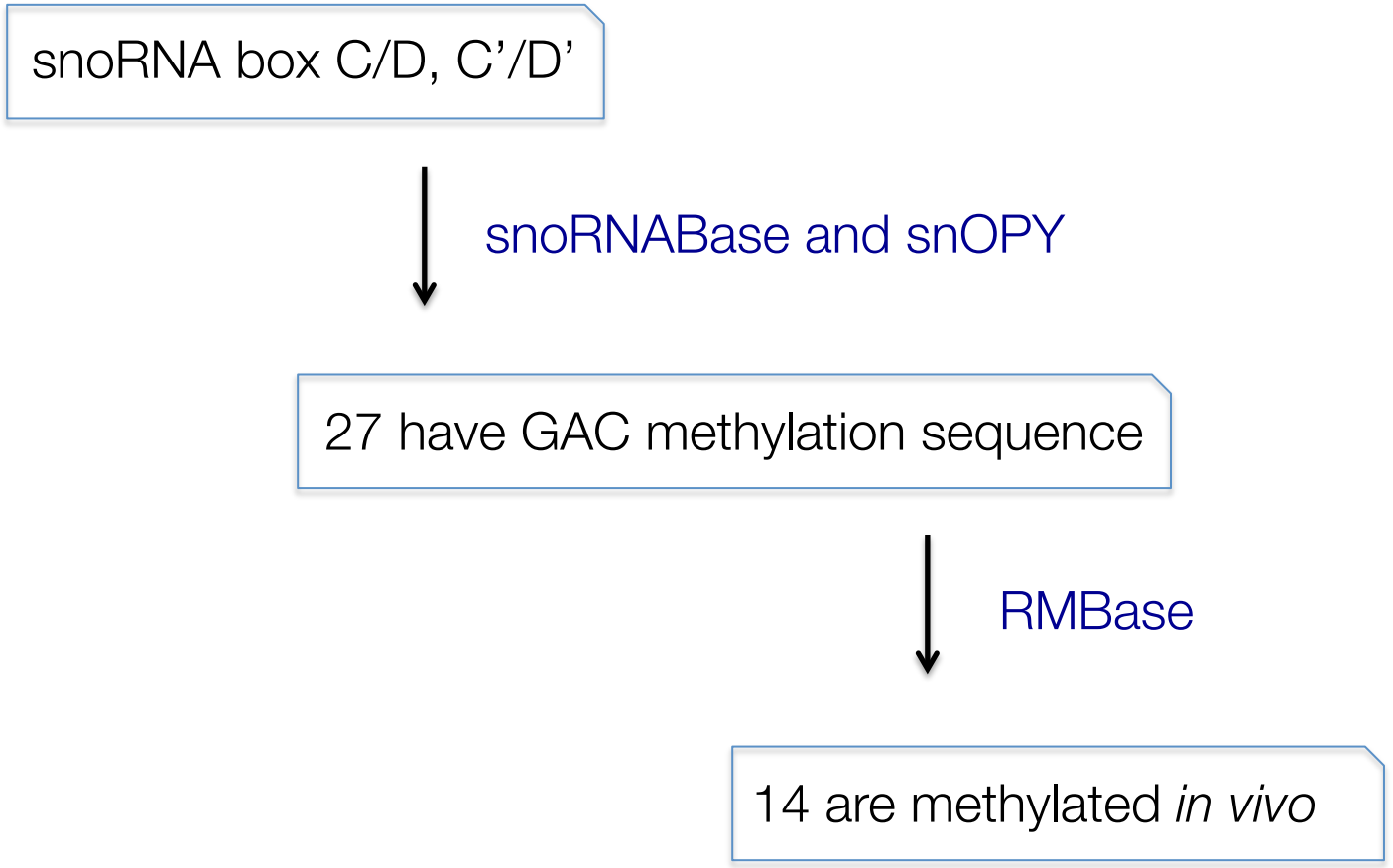
Data base mining

snoRNA box C/D, C’/D’

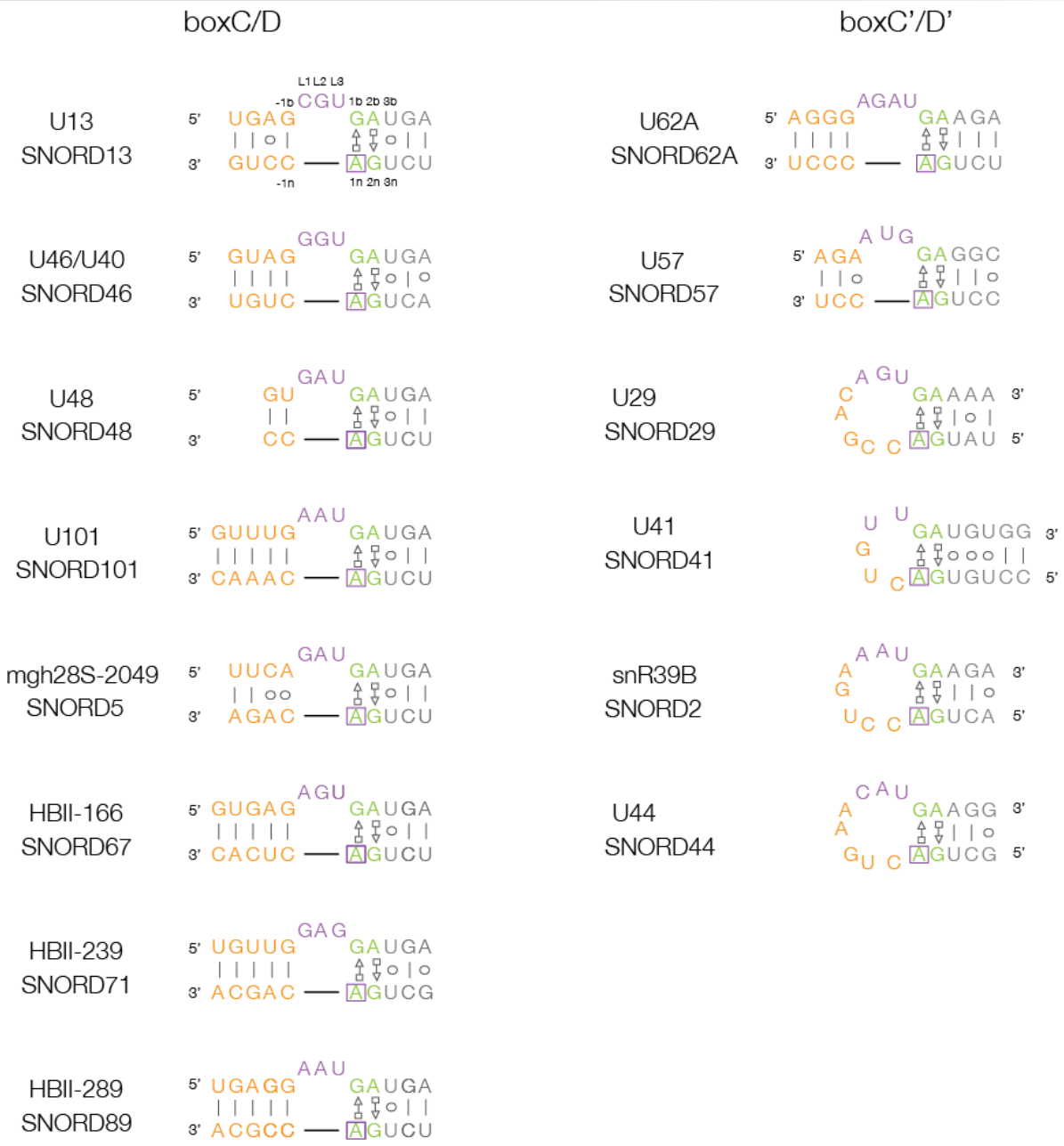
snoRNABase and snOPY

27 have GAC methylation sequence

Data base mining

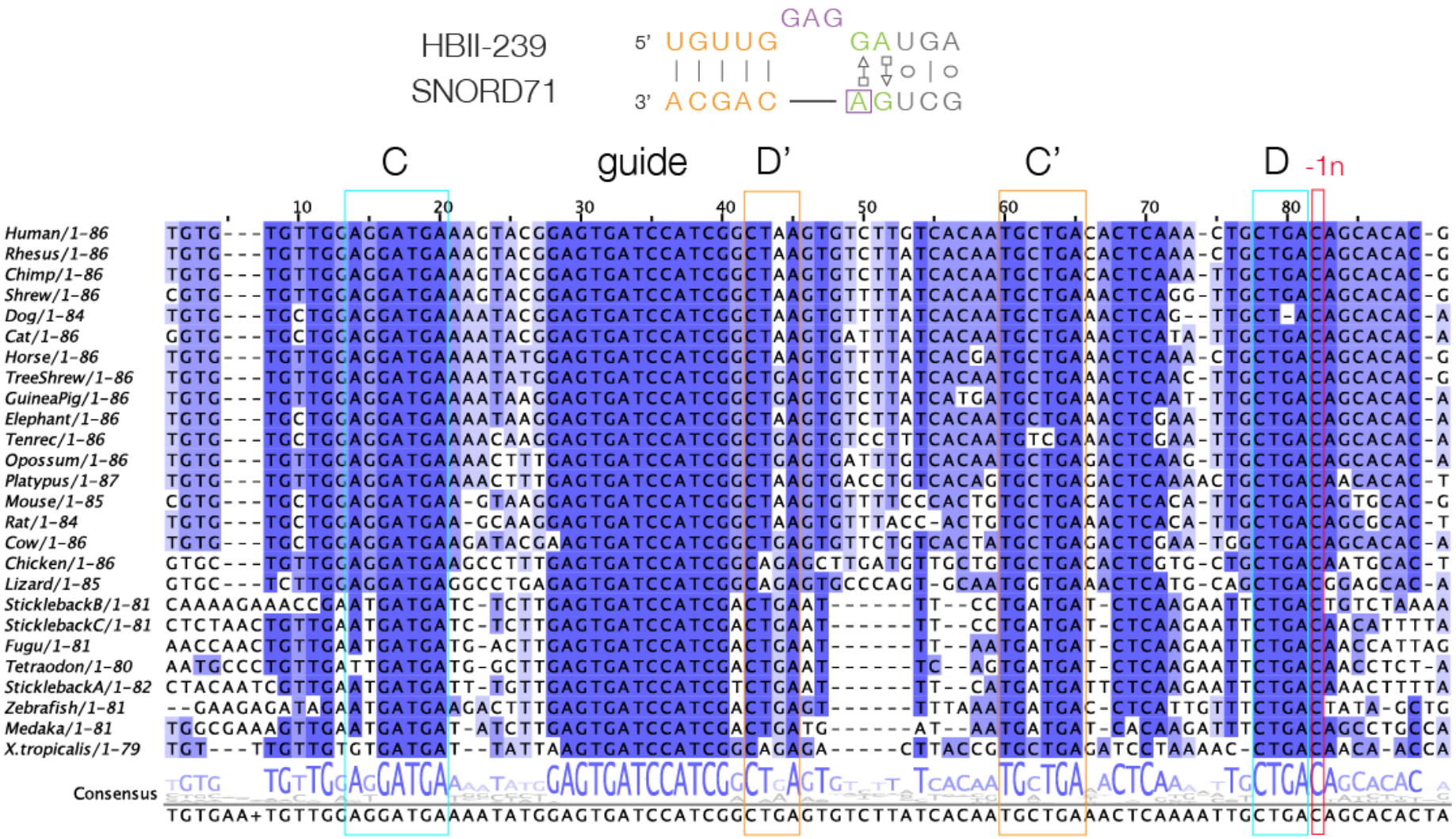


Human box C/D RNA subject to A1N N^6-methylation identified in RMBase



The methylation signals are conserved

Sequence alignment for SNORD71



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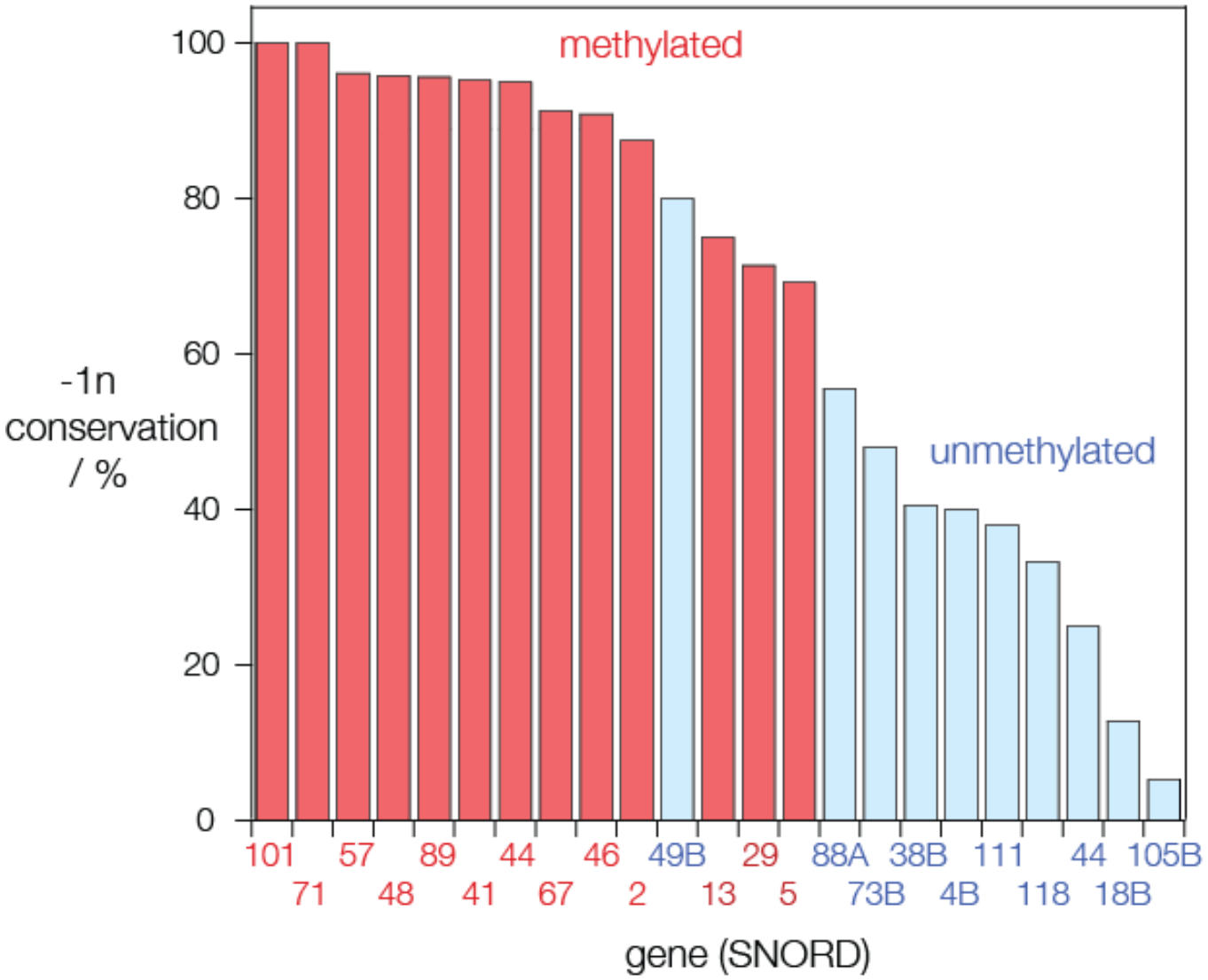
Conservation of -1n sequences (% C) for box C/D where -1n = C in humans



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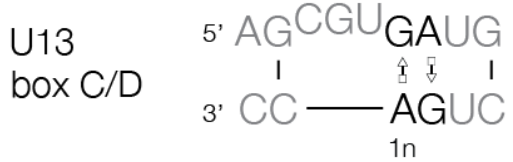
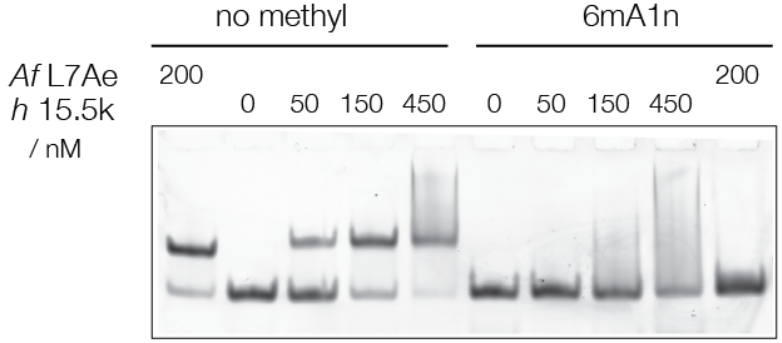
A1n N^6-methylation blocks folding/binding of the k-turn

Effect of N^6A-methylation on 15.5 kD protein binding to box C/D



15.5 kD protein

BINDING



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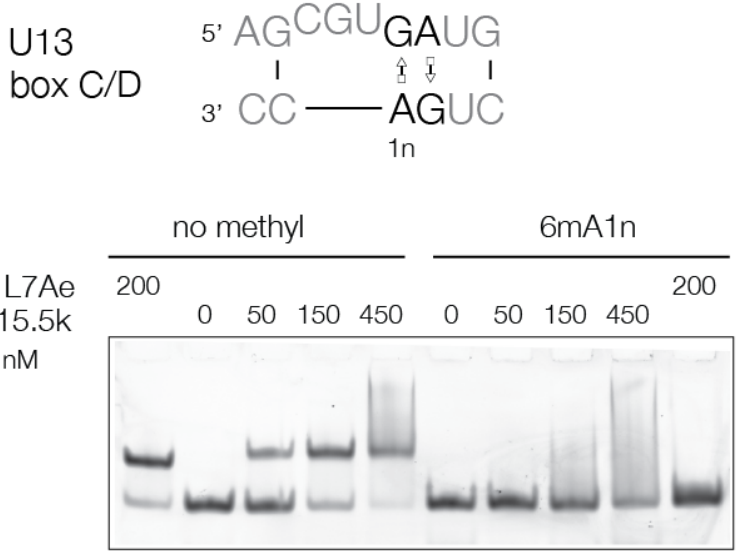
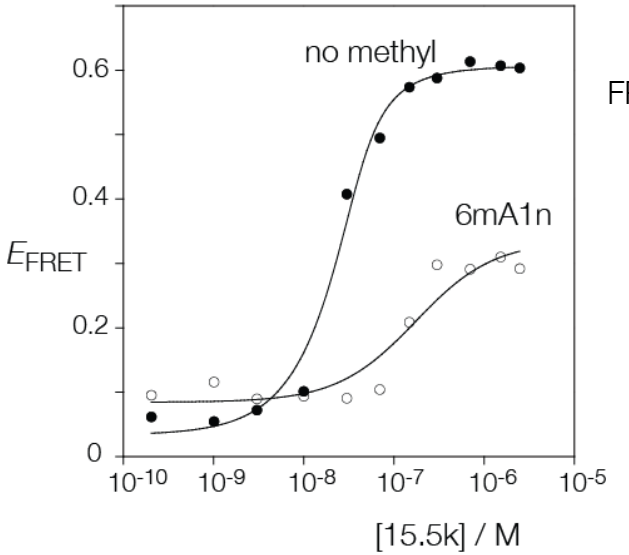
Effect of N^{6}A-methylation on 15.5 kD protein binding to box C/D

15.5 kD protein

BINDING





FRET analysis

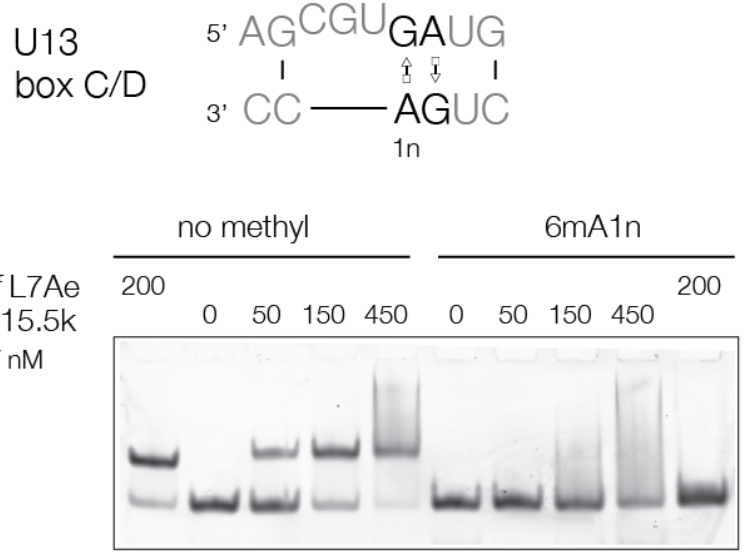
FOLDING

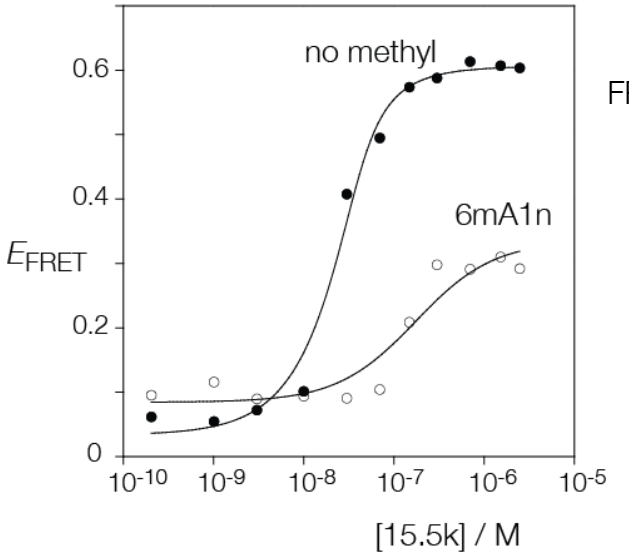
Huang, Ashraf & Lilley *EMBO rep* **18**, 1631-1645 (2017)

Effect of N^{6}A-methylation on 15.5 kD protein binding to box C/D

15.5 kD protein

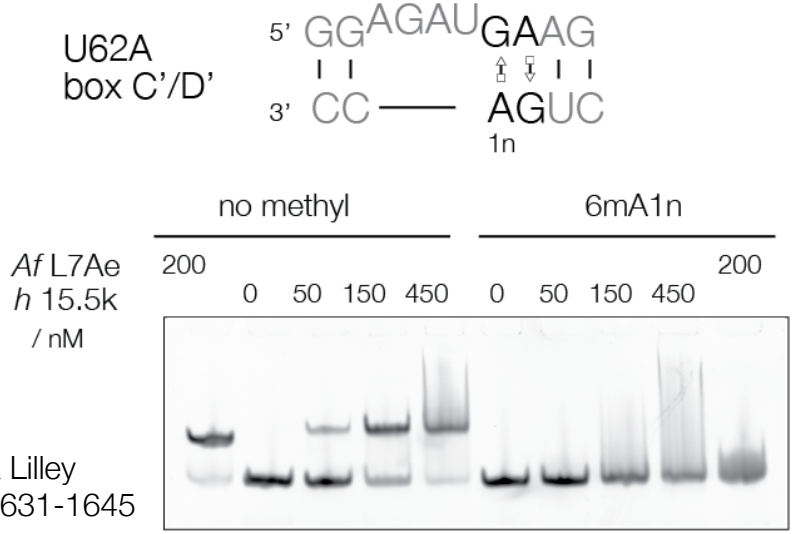
BINDING

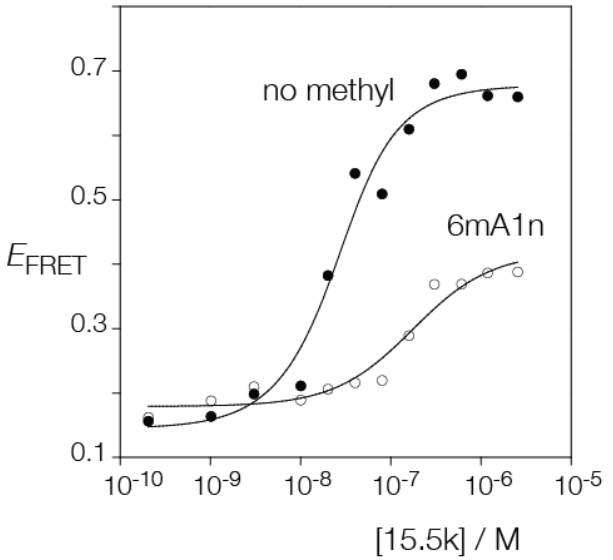




FRET analysis

FOLDING





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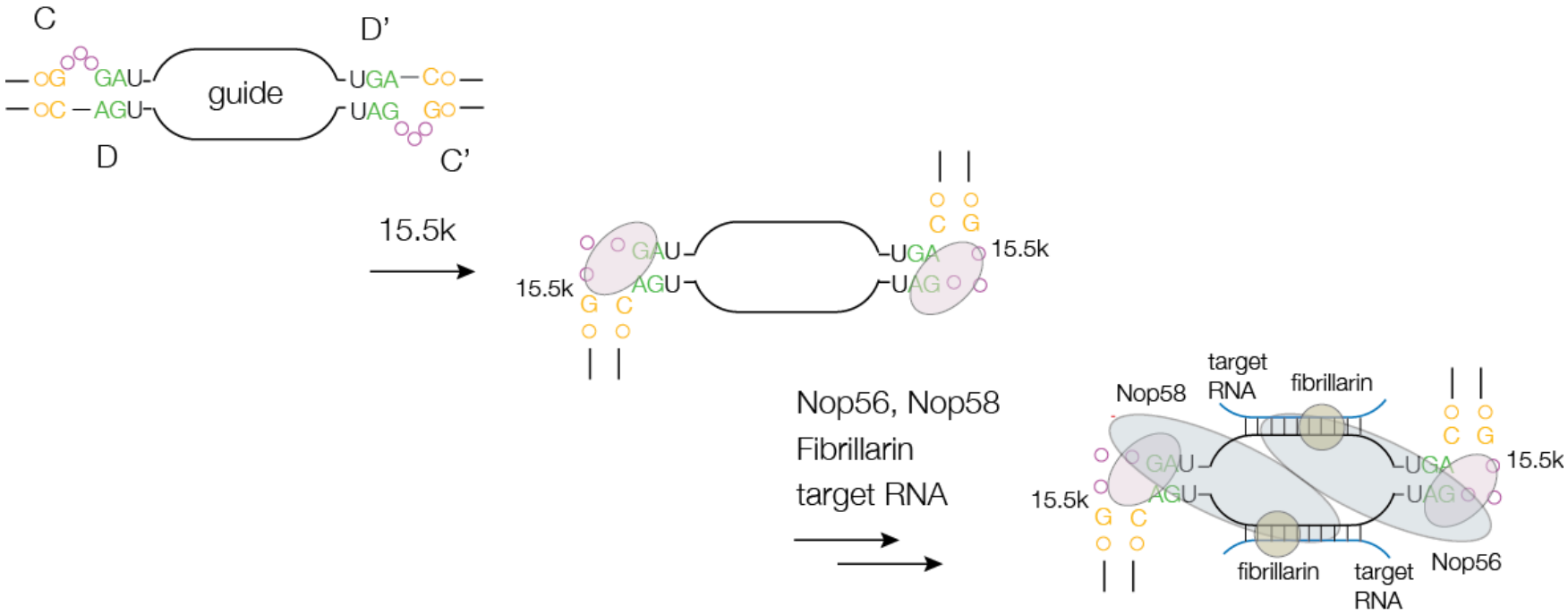
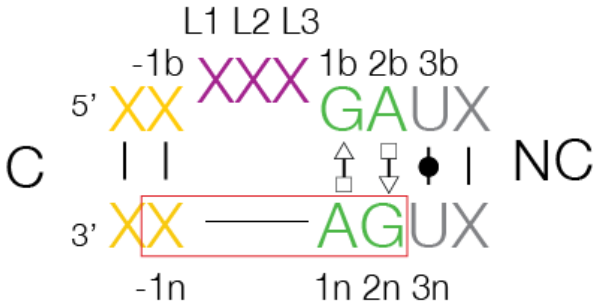
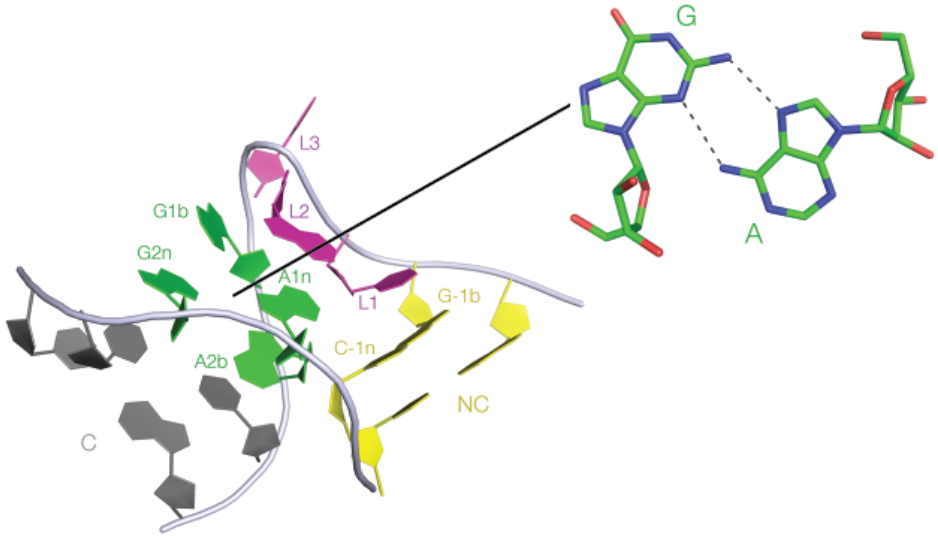
(2017)

Box C/D snoRNP assembly

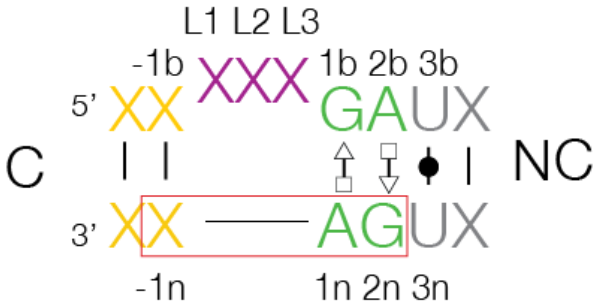


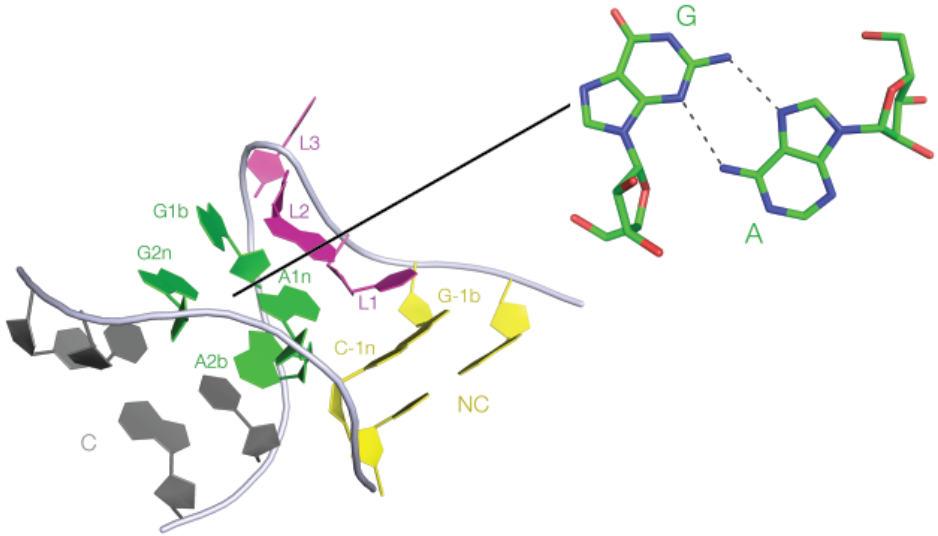




Box C/D snoRNP assembly

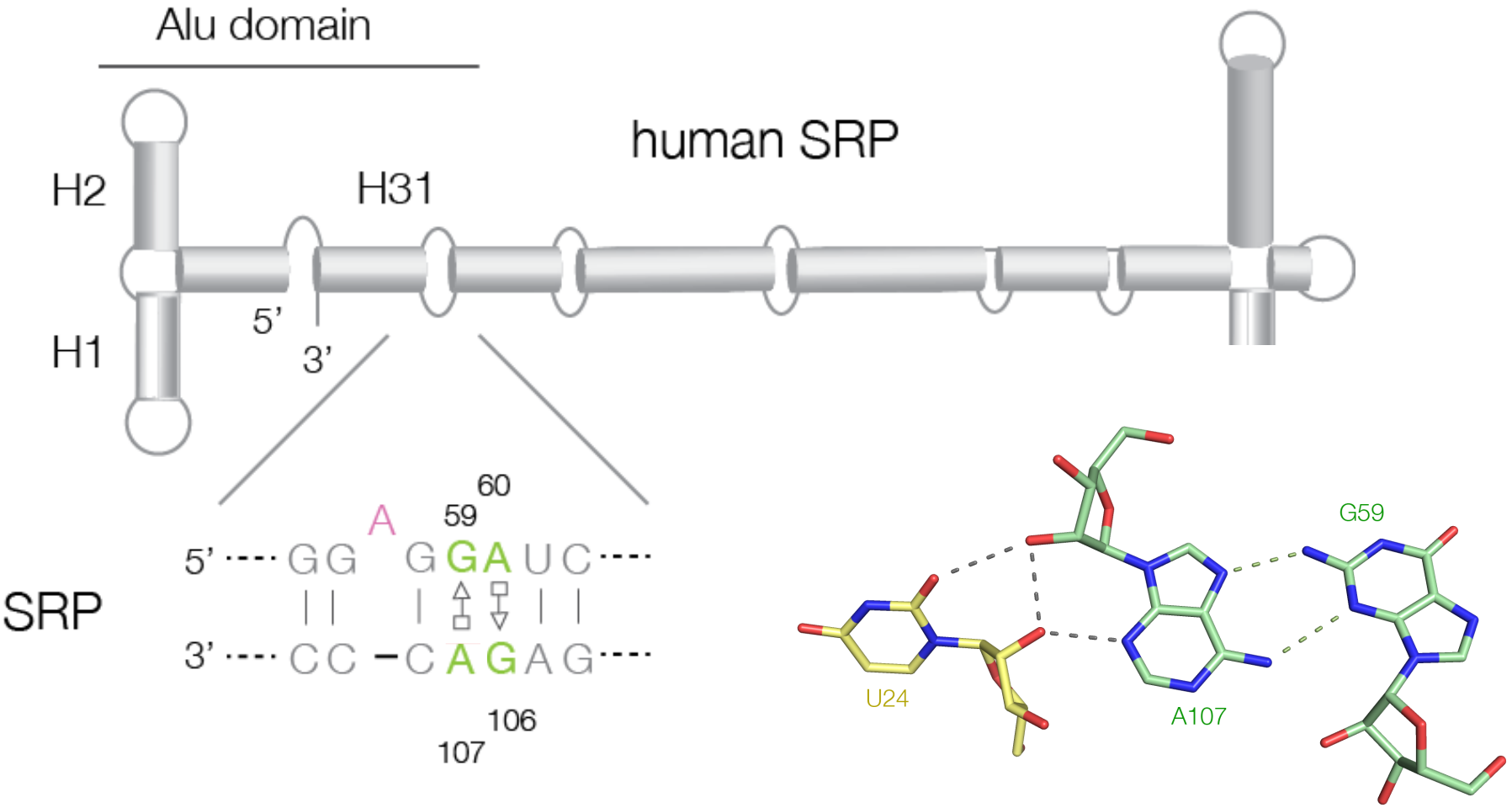
Nop56, Nop58
Fibrillarin
target RNA



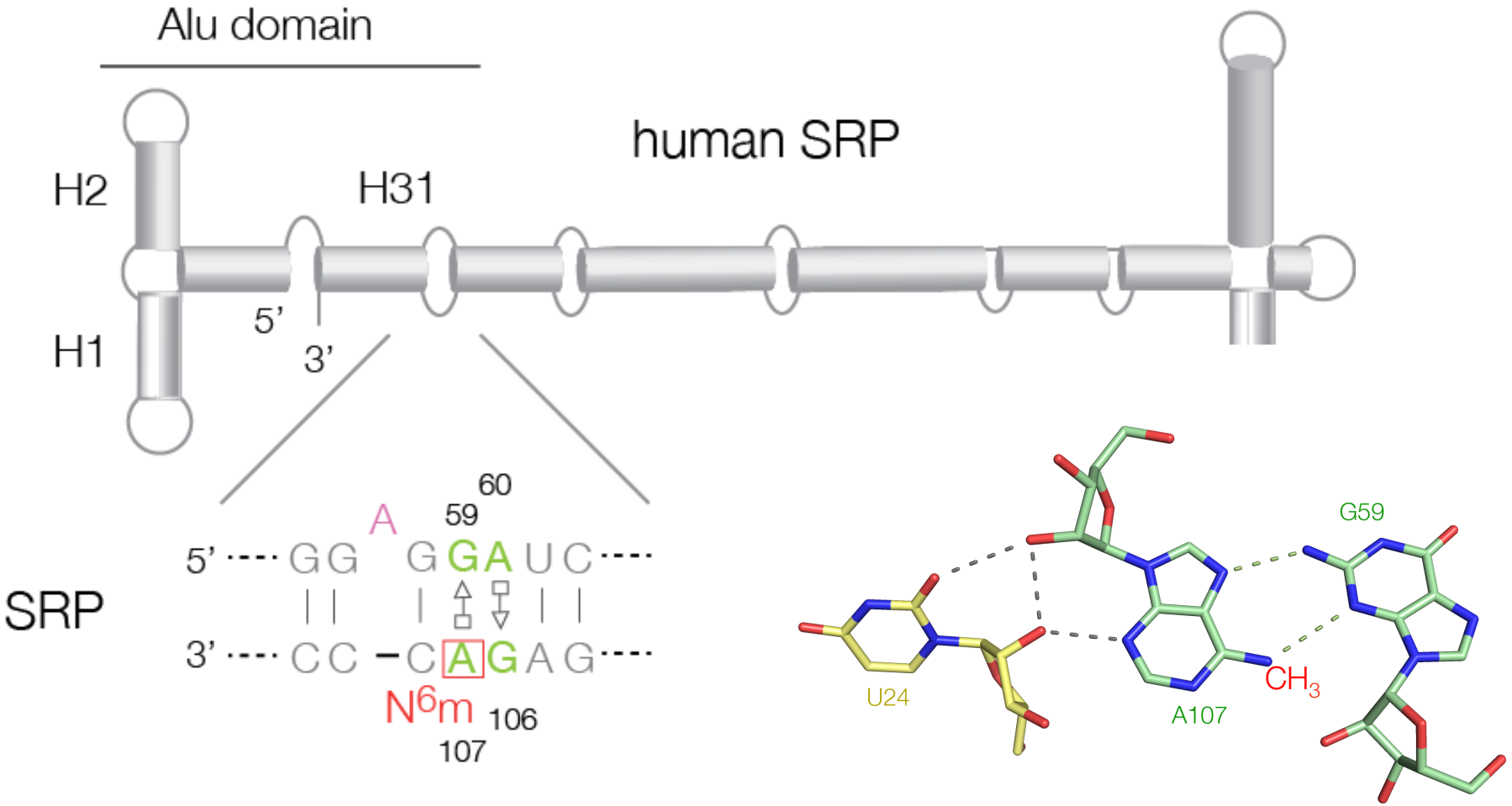


N^6A methylation in the human signal recognition particle Alu domain

Methylation in the human signal recognition particle RNA



Methylation in the human signal recognition particle RNA



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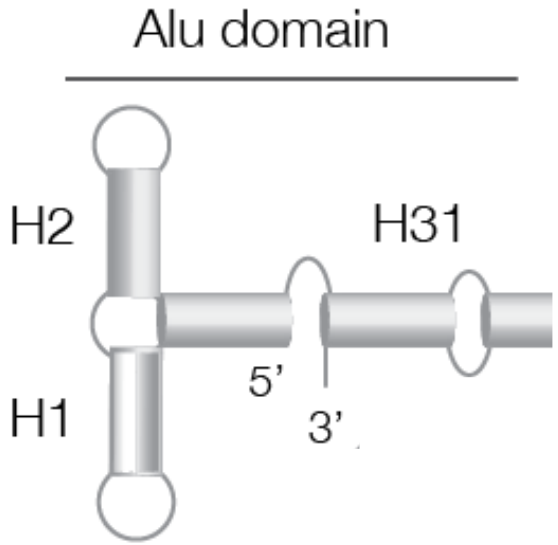
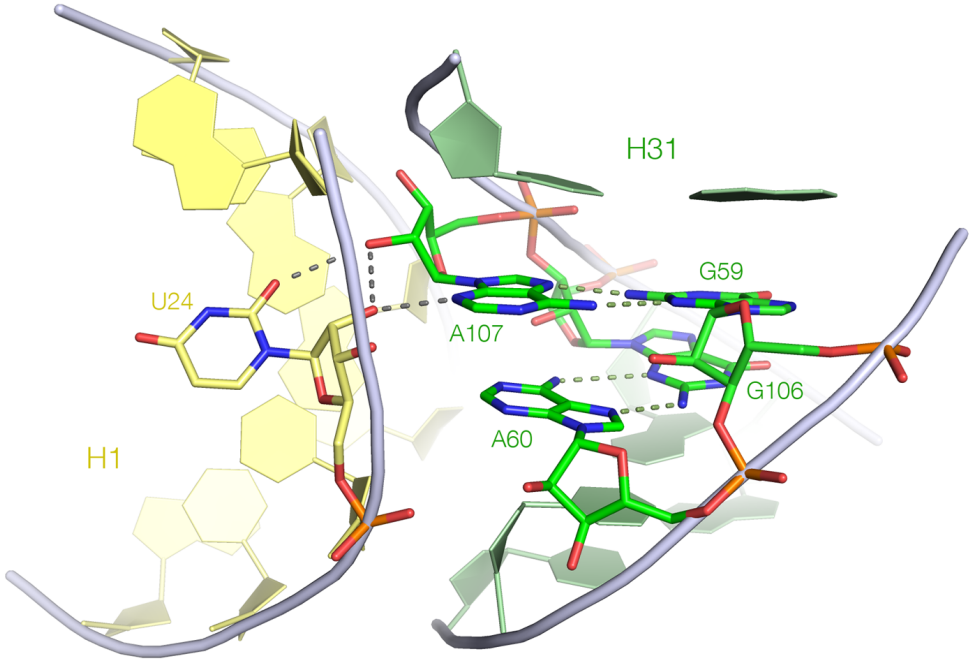
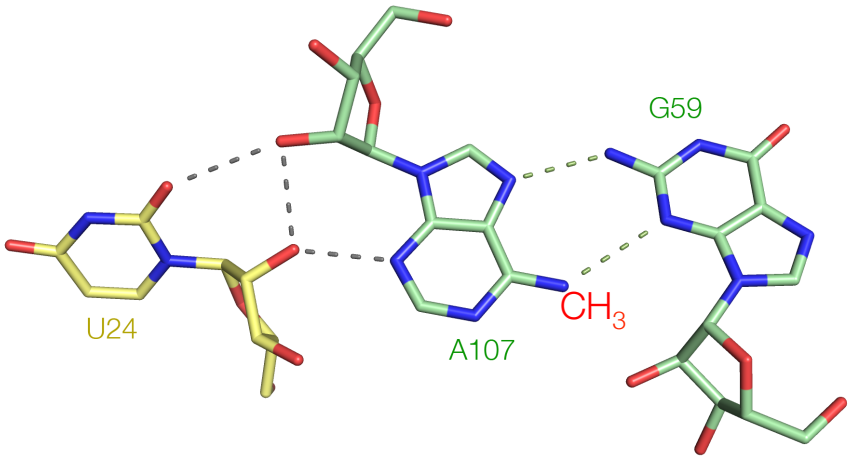
Methylation in the human signal recognition particle RNA

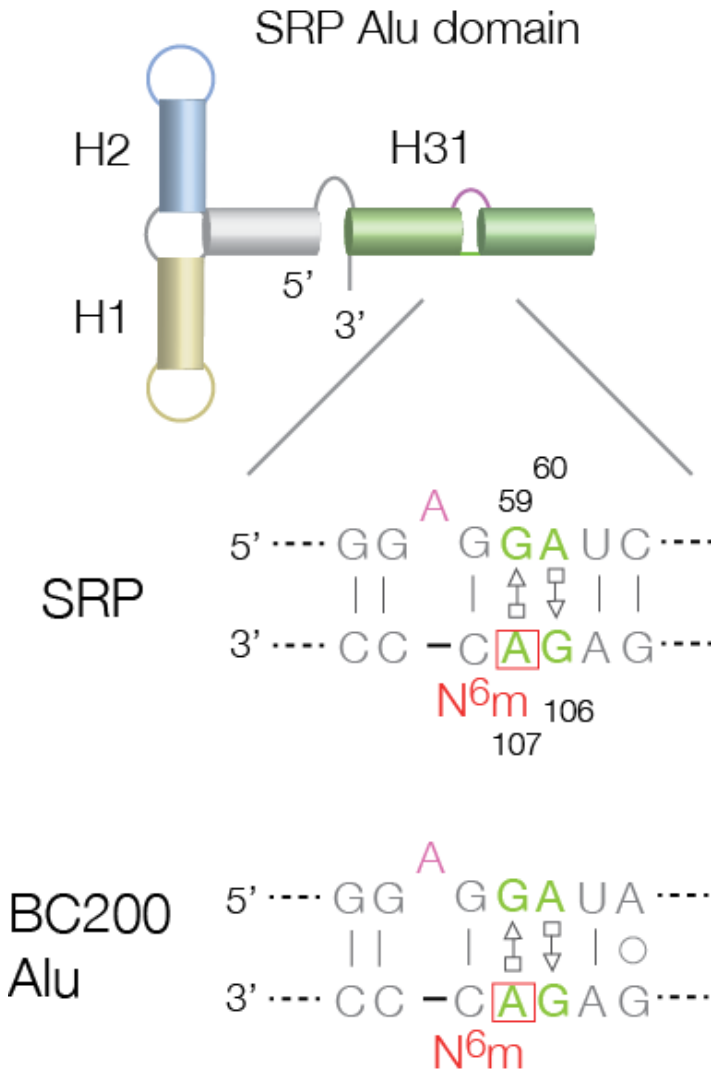






N^6A methylation in human Alu elements

N^6-methylation of Alu sequences



N^6-methylation of Alu sequences ; RMBase data

gene	mod ID	xsome	position	support no	sequence (N^6meA)
srpRNA	m6A_site_38024	chr14	50329278	14	AGCCTGAGCAACATAGCGAGACCCCGTCTCTTTTGCCCCCC
AluJo	m6A_site_12507	chr1	214372362	5	GCCTGAGCAACATAGCGAGACCCCGTCTCTAAAACAAAAA
AluSp	m6A_site_9026	chr1	154184358	4	GCCTGGGCAACAAAAGTGAAACTCTGTCTCAAAAAAAAAA
FAM	m6A_site_31320	chr12	76858065	4	AGCCTGAGCAACATAGTGAGACCCTGTCTCTAAAAAGCAAC
AluJb	m6A_site_32596	chr12	112742257	4	AGCCTGGGCAACATAGCGAGACCCCGTCTCTGCAAAAAAAT
AluJr	m6A_site_119490	chr7	70342837	4	AGCCTGAGCAACATAGCGAGACCCCGTCTCTACAAAAACTA
AluSc	m6A_site_134766	chr9	139728960	4	TCCAGCCTGGACAGAGTGAGACTCCGTCTCCAAAAAAAAGA
AluJr	m6A_site_30705	chr12	58271974	3	AGCCTGAGCAACATAGCGAGACCCCGTCTCTACAAAAAAAA
AluSx3	m6A_site_30910	chr12	66517102	3	AGCCTGGCTAATACAGTGAAACCCCATCTCTACTAAAAATA
AluSz6	m6A_site_40909	chr14	104057184	3	AGCCTGGAAGACCAAACGAGACTCTGTATCAAAAAATAATA
AluSz	m6A_site_62849	chr19	2342640	3	AGCCTGGGTGACAGAGTGAGACTCCATCTCAAAAACAAAAT
AluSz	m6A_site_64656	chr19	12986232	3	AGCCTGGGTGACAGAATGAGACTCTGTGTCAAAAAAATAAA
BC200	m6A_site_139606	chrX	138802845	1	TGCCTGGGCAATATAGCGAGACCCCGTTCTCCAGAAAAAGG

RMBase : Sun et al *Nucleic Acids res* **44**, D259 (2016).

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Summary

- N^6mA is tolerated in Watson-Crick basepairs, but prevents the formation of *trans* Hoogsteen-sugar A•G basepairs.

- A sub-set of human box C/D snoRNA species have target GAC sequences that result in the formation of N^6-methyladenine at a key *trans* Hoogsteen-sugar A•G basepair, of which half are methylated *in vivo*.

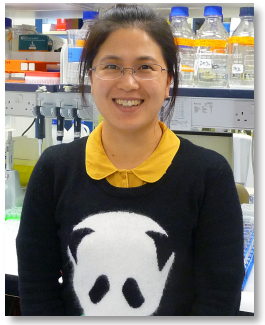
- The GAC target is conserved only in those that are methylated. Methylation prevents binding of the 15.5 kDa protein and the induced folding of the RNA. Thus the assembly of the box C/D snoRNP can be regulated by RNA methylation at its critical first stage.

- More generally, N^6-methylation of adenine occurs at sheared A•G basepairs involved in tertiary contacts in the human signal recognition particle RNA and related Alu retrotransposon RNA species.

- N^6-methylation at A•G basepairs is probably a general method of controlling conformation and RNP assembly in cellular RNA.

CR-UK
Nucleic Acid Structure
Research Group

David Lilley



Jia Wang

Saira Ashraf

Tim Wilson

http://www.dundee.ac.uk/biocentre/nasg/index.php

Thanks !