

Strategies in RNA X-ray crystallography

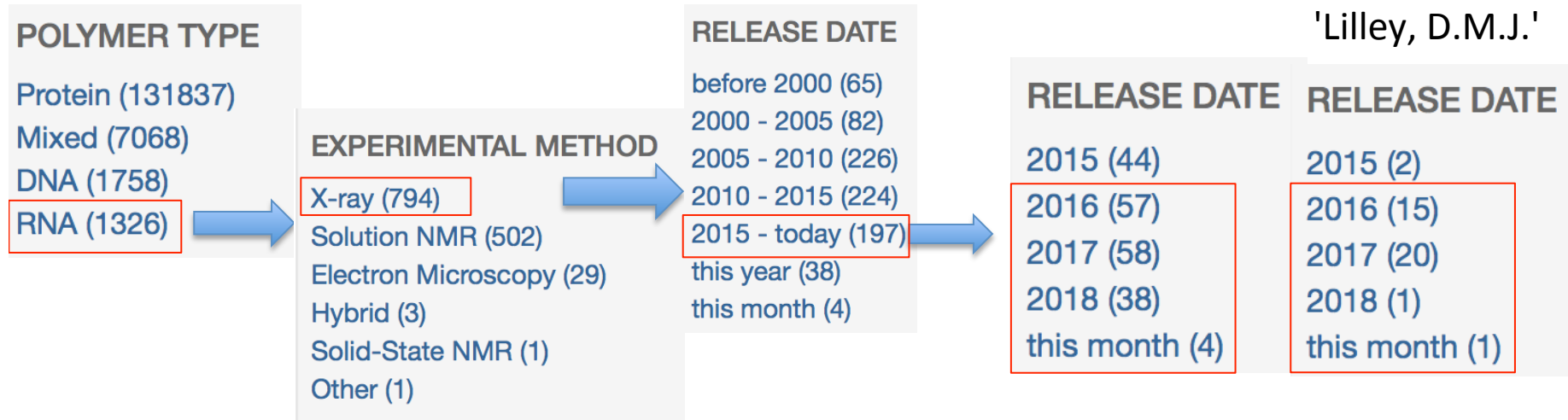
Lin Huang
David Lilley lab
University of Dundee, UK

PDB Statistics



142015 Biological
Macromolecular Structures
Enabling Breakthroughs in
Research and Education

Author name starts with
'Lilley, D.M.J.'



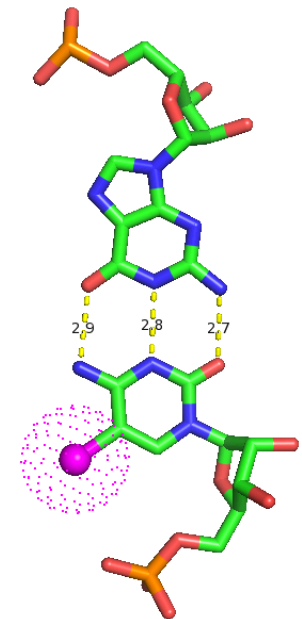
- 20180717 from PDB

RNA structures determined in Lilley's lab

- Small RNA motifs
 - Kink turns (28 PDBs)
 - double sheared basepair and triple sheared basepair (5 PDBs with 30+ unpublished)
- Ribozymes and riboswitches
 - Twister ribozyme (1 PDB)
 - Twister-sister ribozyme (1 PDB)
 - Guanidine II riboswitch (8 PDBs with 3 unpublished)
 - Guanidine III riboswitch (8 PDBs)
 - SAM-V riboswitch (1 PDB with 2 unpublished)

Strategies in RNA crystallography

- Rational designs
 - Kink turns
 - double sheared basepair and triple sheared basepair
- 5-Bromo-cytidine
 - double sheared basepair and triple sheared basepair
 - Twister ribozyme
 - Twister-sister ribozyme
 - Guanidine II riboswitch (Rational designs, Motif)
 - Guanidine III riboswitch (Rational designs, Jalview)
 - SAM-V riboswitch (Rational designs, Jalview)



Small RNA motifs

Kink turn

L Huang, DMJ Lilley (2013) The molecular recognition of kink-turn structure by the L7Ae class of proteins. RNA (2 PDBs)

L Huang, DMJ Lilley (2014) Structure of a rare non-standard sequence k-turn bound by L7Ae protein Nucleic acids research 42 (7), 4734-4740 (1 PDB)

SA McPhee, L Huang, Lilley DM (2014) A critical base pair in k-turns that confers folding characteristics and correlates with biological function. Nature Communication (1 PDB)

L Huang, DMJ Lilley (2016) A quasi-cyclic RNA nano-scale molecular object constructed using kink turns. Nanoscale (5 PDBs)

L Huang, J Wang, DMJ Lilley (2016) A critical base pair in k-turns determines the conformational class adopted, and correlates with biological function. Nucleic Acids Research (15 PDBs)

Double sheared basepair and triple sheared basepair

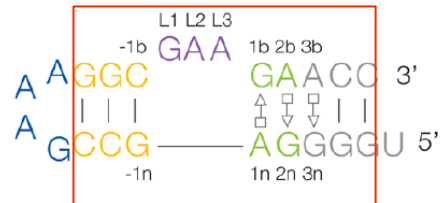
L Huang, S Ashraf, J Wang, DMJ Lilley (2017) Control of box C/D snoRNP assembly by N6-methylation of adenine. EMBO report (5 PDBs)

Rational designs for k-turns

8 ways to determine k-turn structure

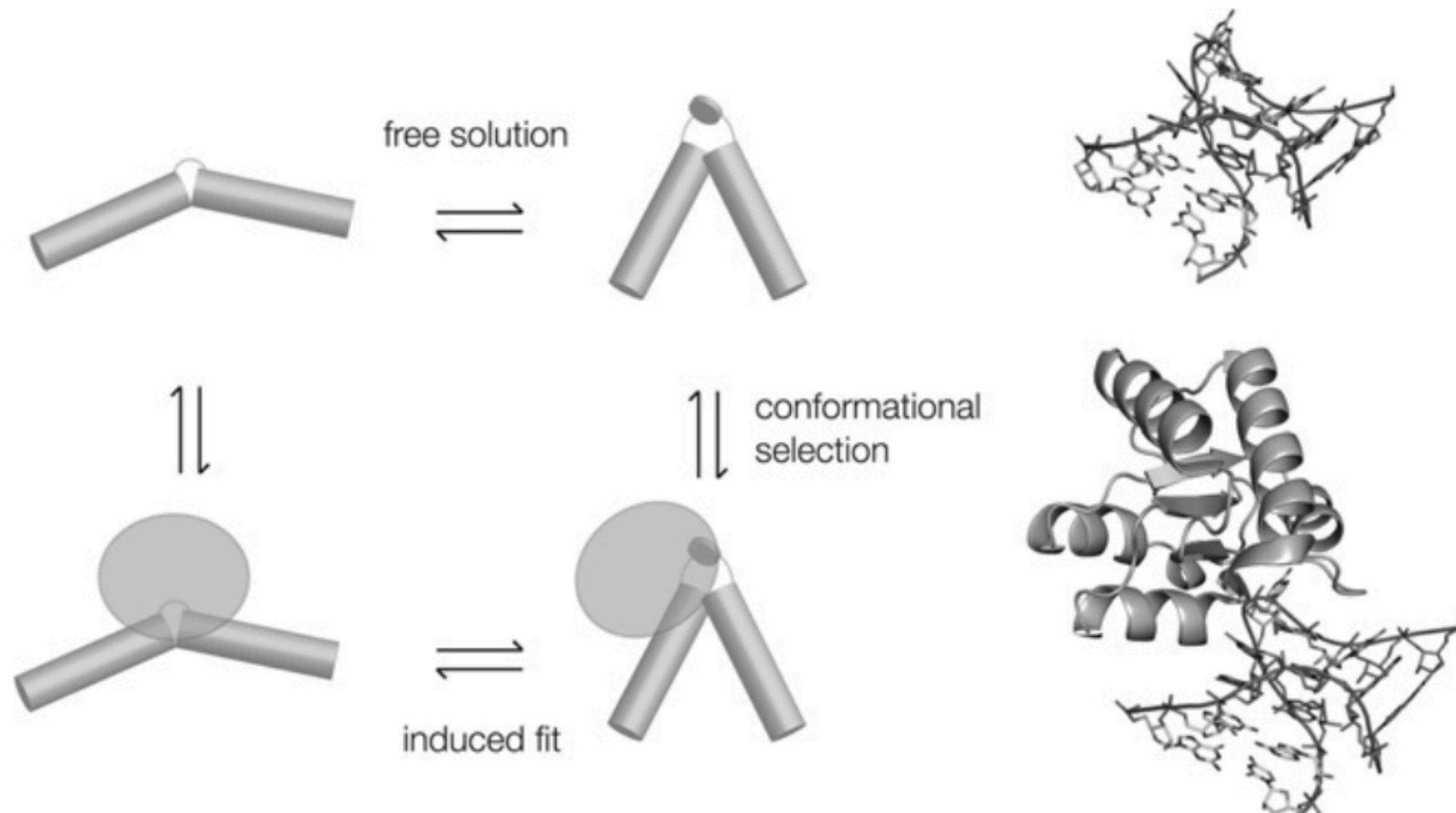
A

design (a)
stem loop
24nt
PDB 5FJ1



X-ray crystallography

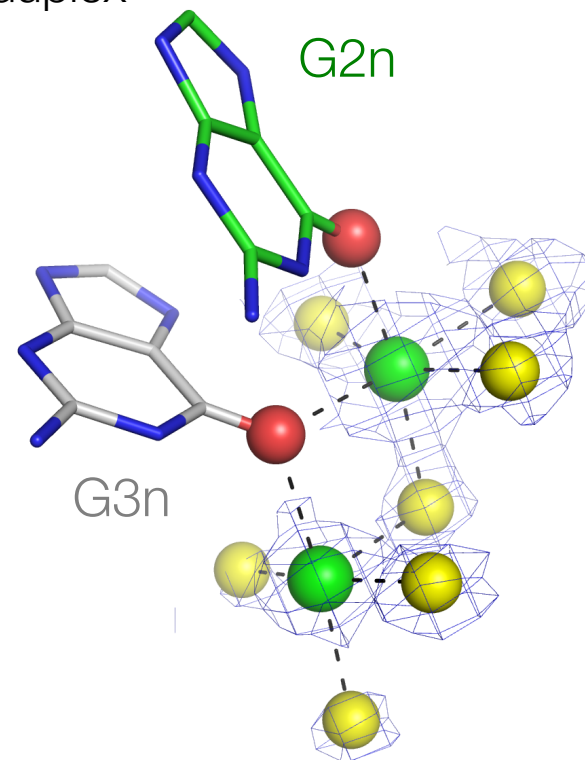
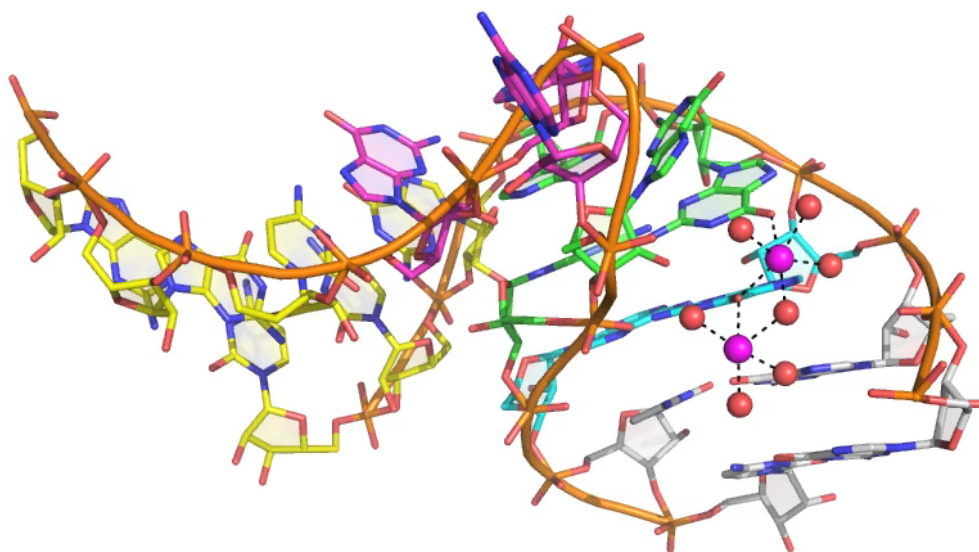
The molecular recognition of kink-turn structure by the L7Ae class of proteins



Best resolution of free k-turns

HmKt-7

Two metal ions bound to G2n, G3n in Kt-7 duplex

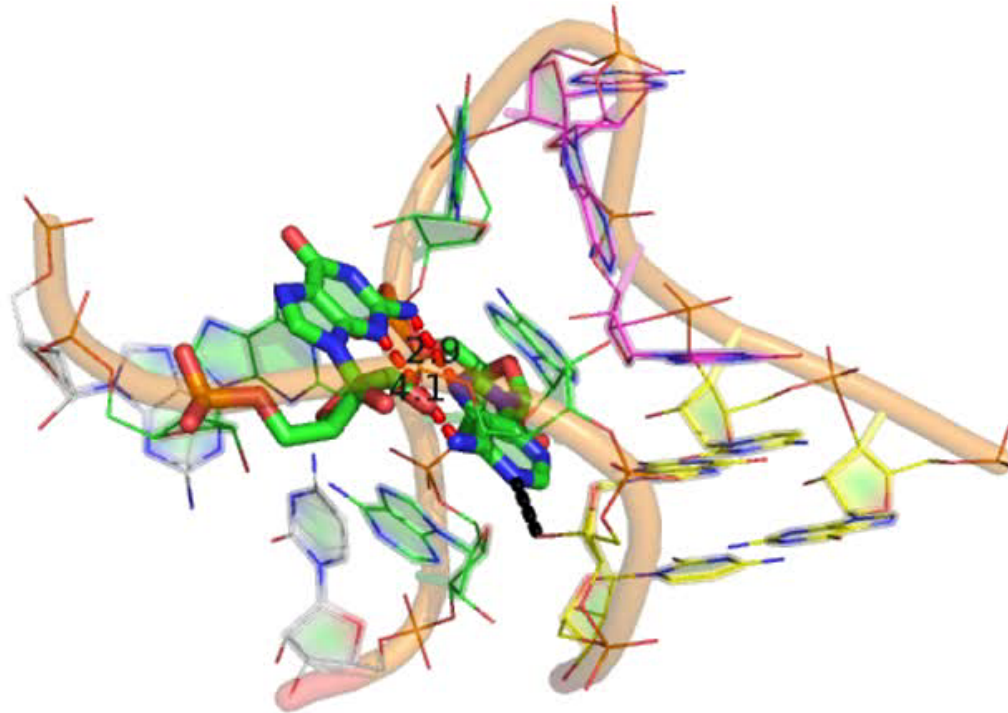


$2F_o - F_c$ 1σ

2.0 Å resolution

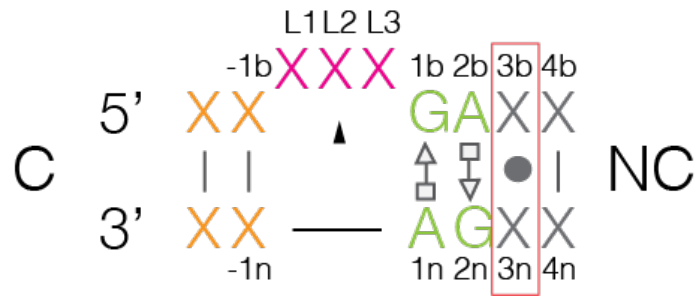
A number of environmental factors can influence the conformation adopted by a given k-turn

morph
N3 <> N1 class structures



RNA 2013, NAR 2014

The conformation of k-turns as a function of the 3b:3n sequence



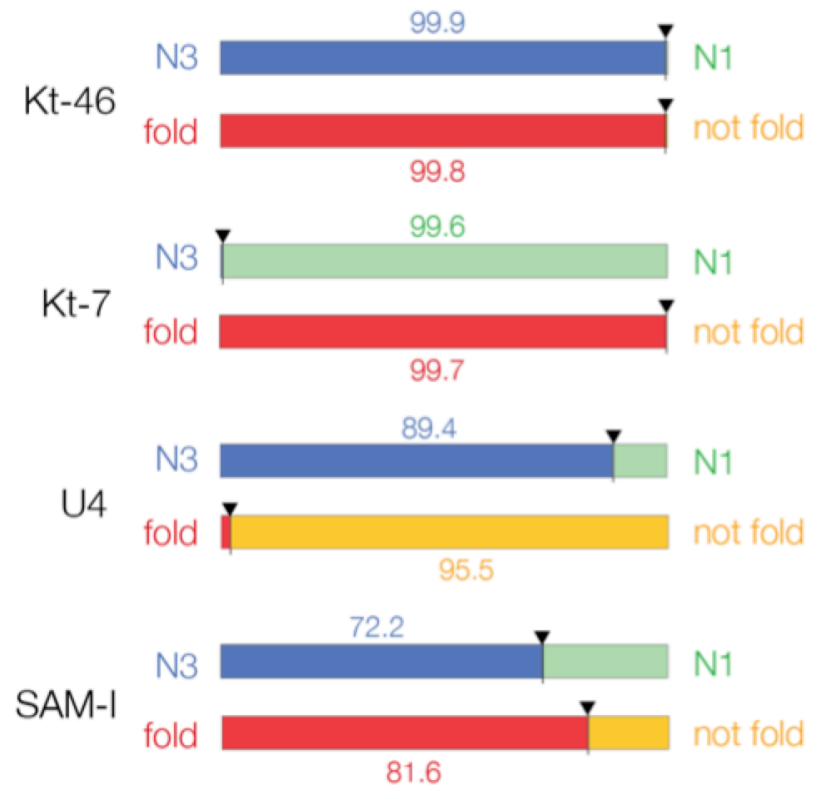
	3n				
	A	C	G	U	
A	3 3	3	33 3		
C	1	1	3	1	riboswitches
G		3 3	1	3	ribosome bound to L7Ae
U	1	1	1 1	3 3	Kt7 variants

L Huang, J Wang, DMJ Lilley (2016) A critical base pair in k-turns determines the conformational class adopted, and correlates with biological function. *Nucleic Acids Research*

The influence of the 3b:3n sequence on conformation and folding of k-turns

More than ten thousands of sequences been analyzed

		3n			
		A	C	G	U
3b	A	N3	N3	N3	(N3)
	C	N1	N1	N3	N1
	G	N3	N3	N1	N3
	U	N1	N1	N1	N3



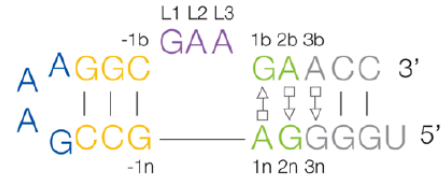
SA McPhee, **L Huang**, Lilley DM (2014) A critical base pair in k-turns that confers folding characteristics and correlates with biological function. Nature Communication

L Huang, J Wang, DMJ Lilley (2016) A critical base pair in k-turns determines the conformational class adopted, and correlates with biological function. Nucleic Acids Research

Also apply to other RNA motifs

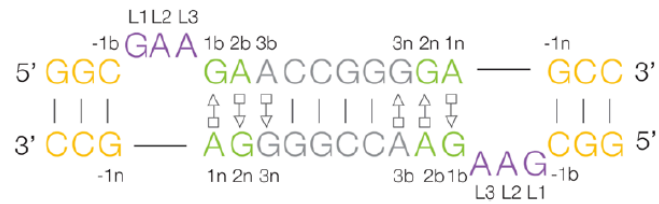
A

design (a)
stem loop
24nt
PDB 5FJ1



B

design (b)
simple duplex
19nt
PDB 5FJ0



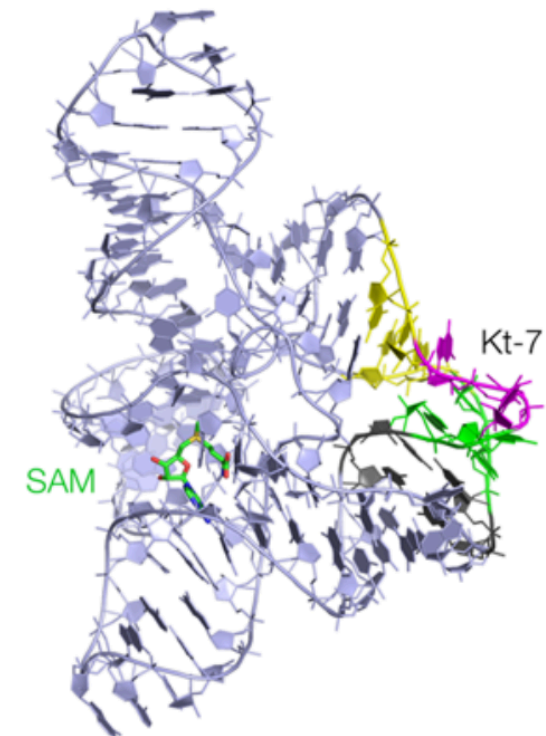
C

HmKt-7 bound to
L7Ae + U1A
35nt
PDB 5FJ4



X-ray crystallography

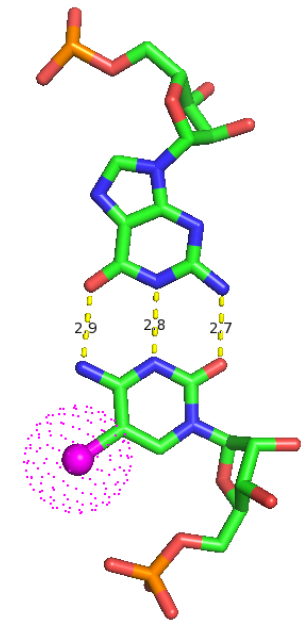
D



5-Bromo-cytidine advantages

From sequence design to structure determination

- Twister ribozyme (1 PDB) 3 month+2month
- Twister-sister ribozyme (1 PDB) 3 month
- Guanidine II riboswitch (8 PDBs) 1 month
- Guanidine III riboswitch (8 PDBs) 3 month
- SAM-V riboswitch (1 PDB) 2 month

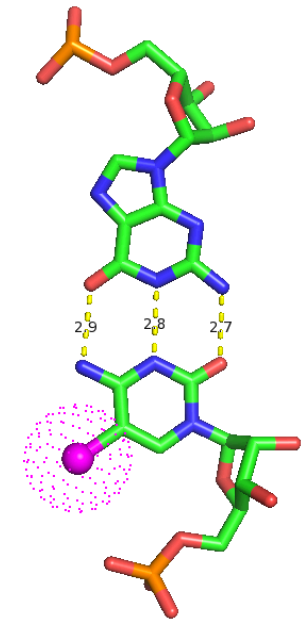
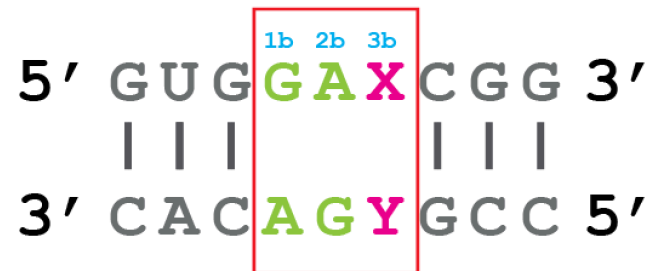
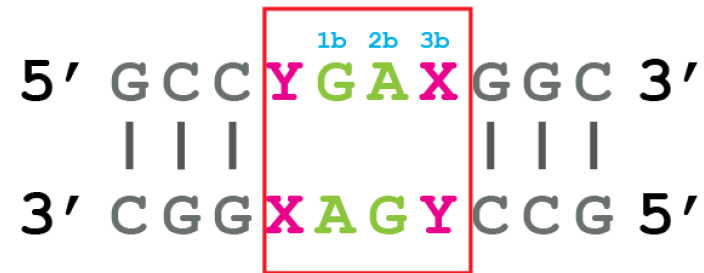


1. Fast = time saving, money saving, less laborious =
2. Better quality

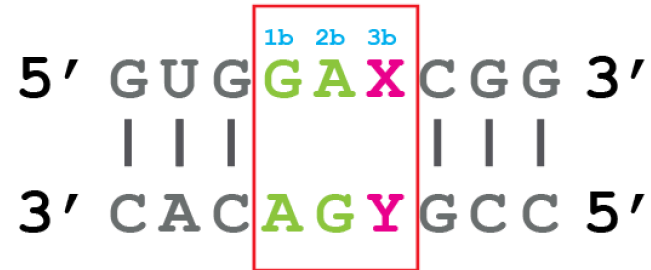
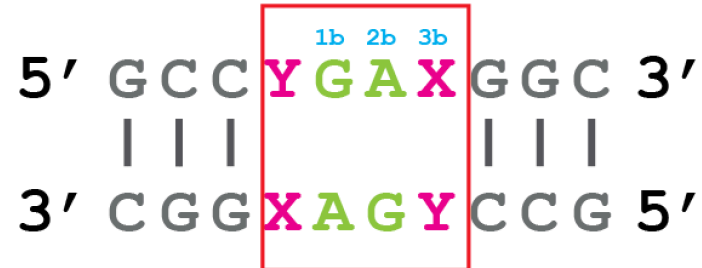


Double sheared motif and triple sheared motif

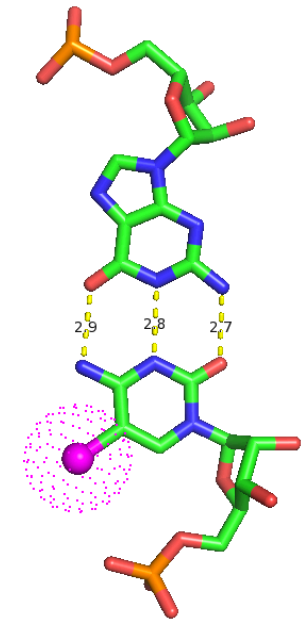
5-Bromo-cytidine Symmetry design



Double sheared motif and triple sheared motif



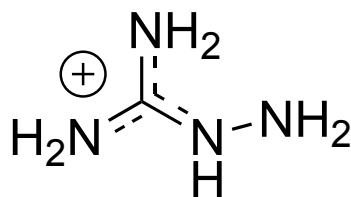
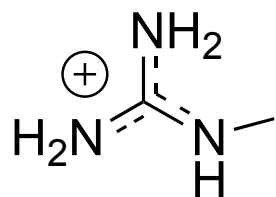
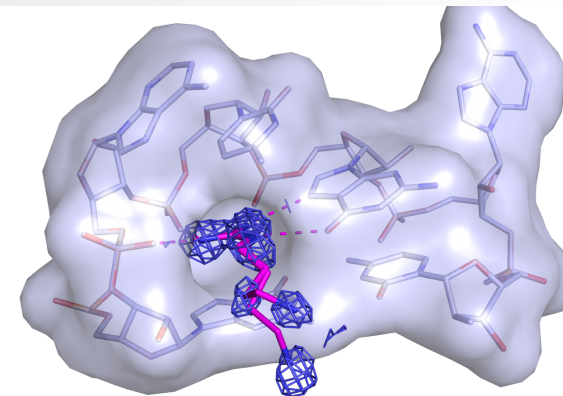
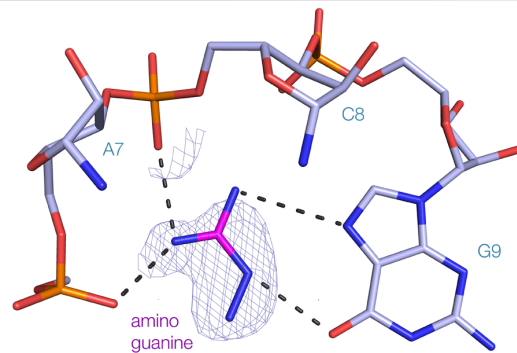
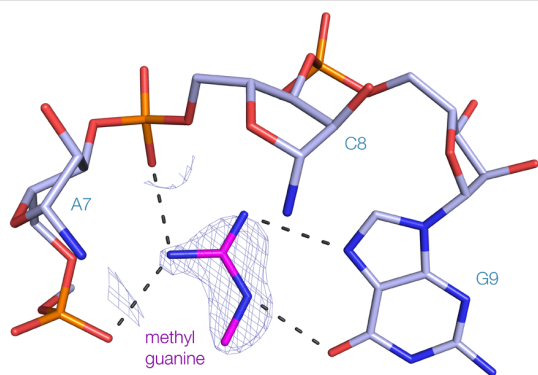
2 PDBs published with 30+ unpublished
5-Bromo-cytidine make this possible



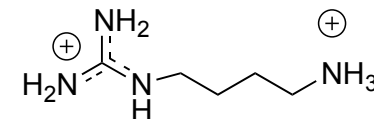
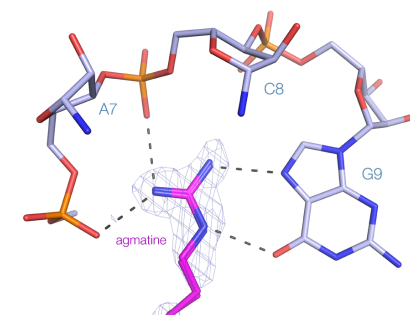
L Huang, S Ashraf, J Wang, DMJ Lilley (2017) Control of box C/D snoRNP assembly by N6-methylation of adenine. EMBO report

5-Bromo-cytidine advantages---Better quality

Guanidine II riboswitch binding pocket



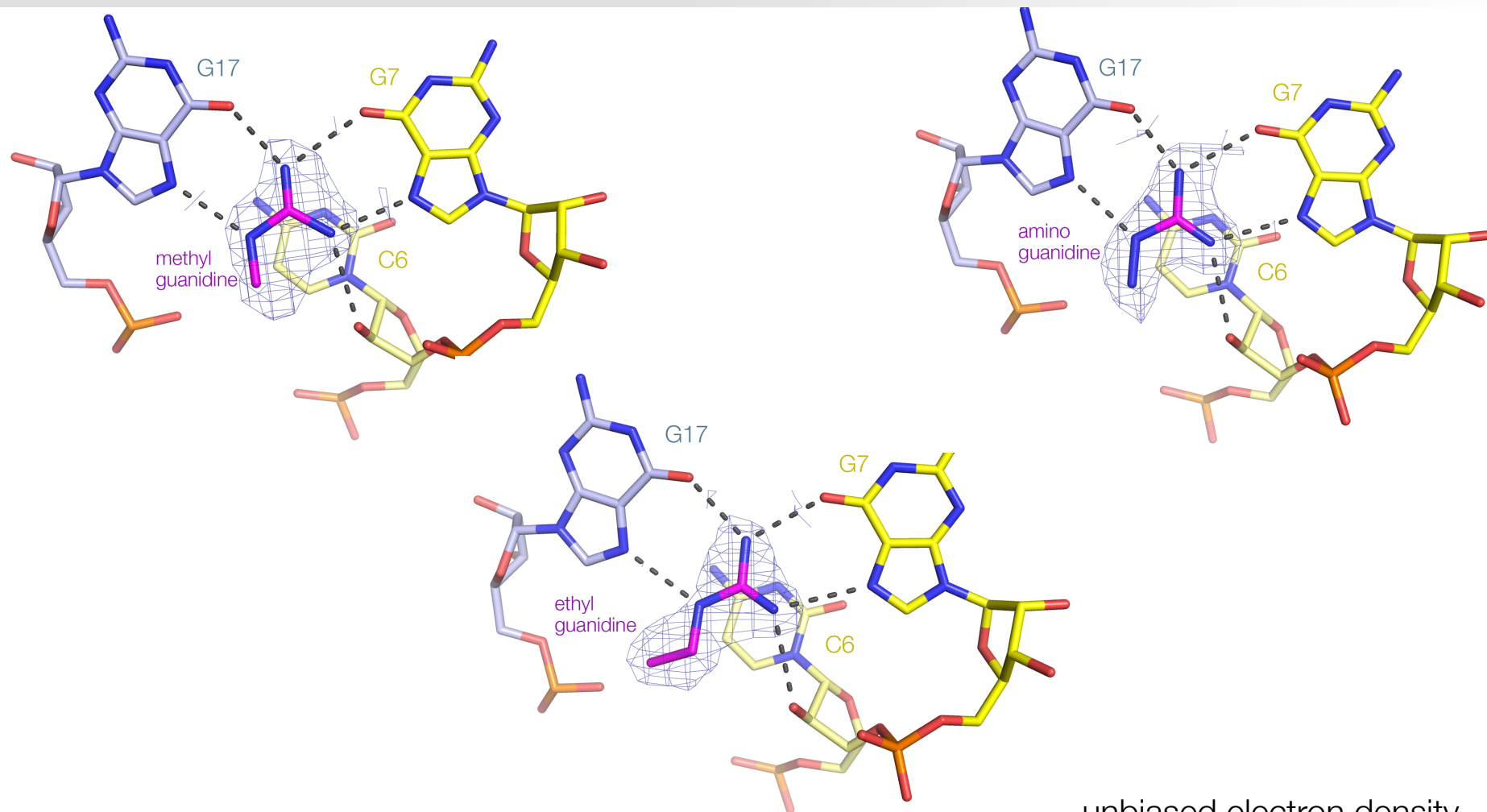
composite omit maps contoured at 1.2σ



unbiased electron density map contoured at 1.2σ

5-Bromo-cytidine advantages---Better quality

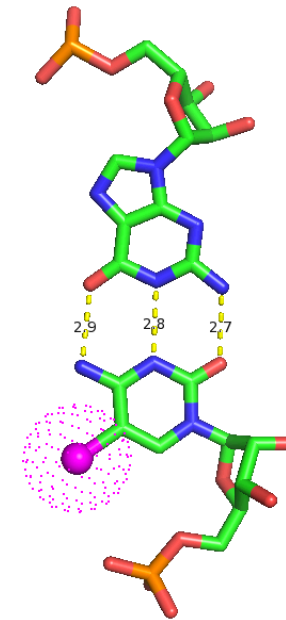
Guanidine III riboswitch binding pocket



unbiased electron density
maps contoured at 1.2σ

5-Bromo-cytidine disadvantages

- Minor or major groove
- Where to use the Br-C



The SAM-V riboswitch (Breaker)

A variant riboswitch aptamer class for *S*-adenosylmethionine common in marine bacteria



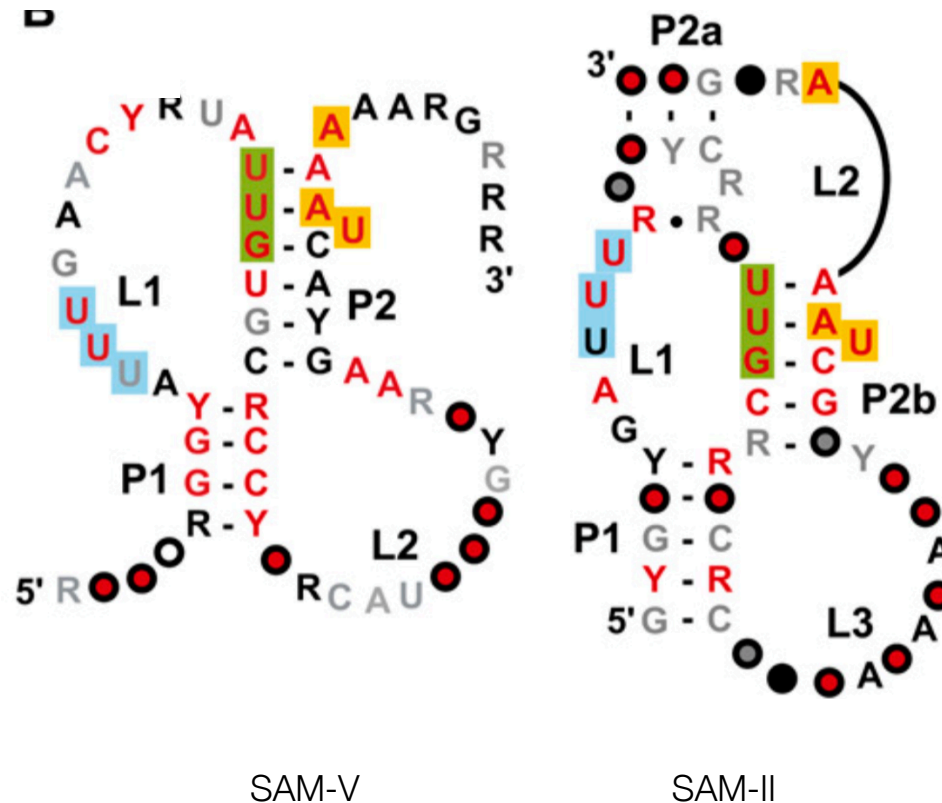
Ron Breaker

ELENA POIATA,¹ MICHELLE M. MEYER,¹ TYLER D. AMES,¹ and RONALD R. BREAKER^{1,2,3}

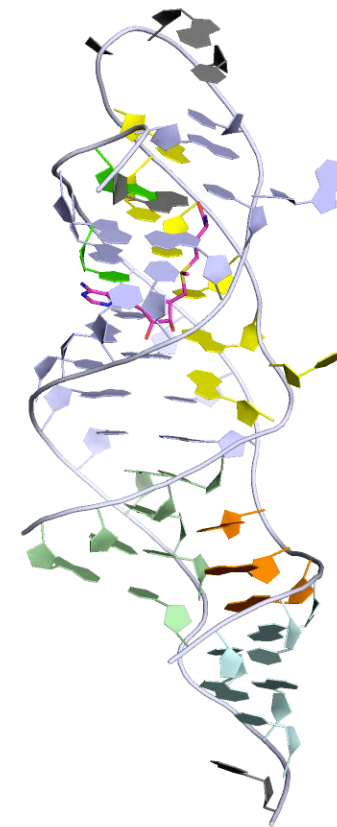
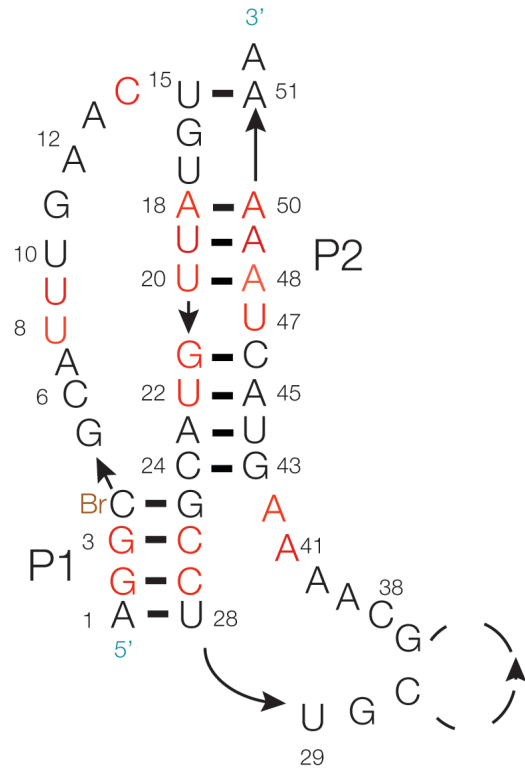
¹Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520, USA

²Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA

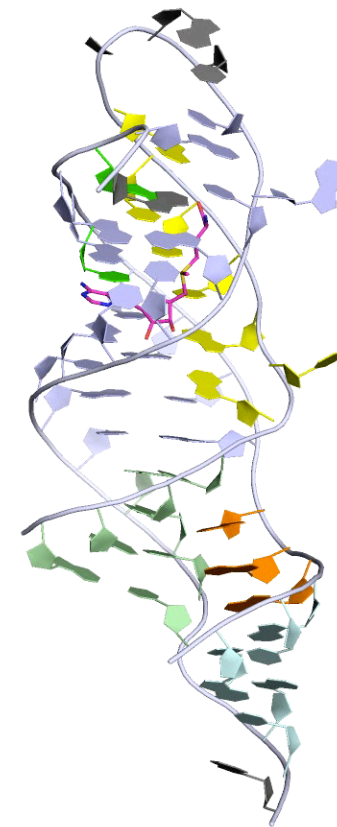
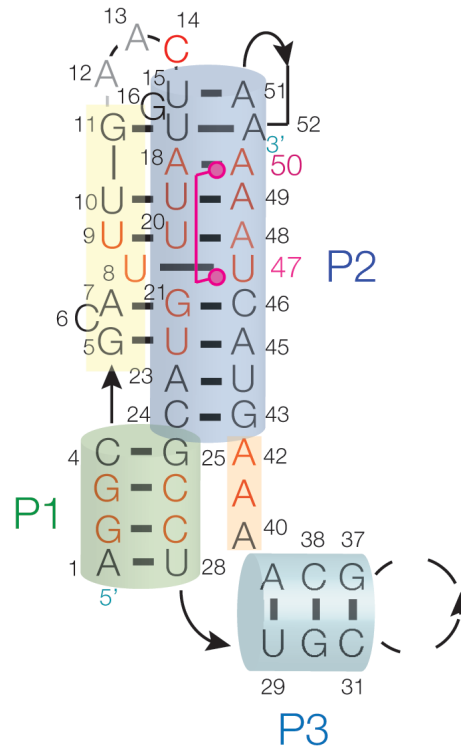
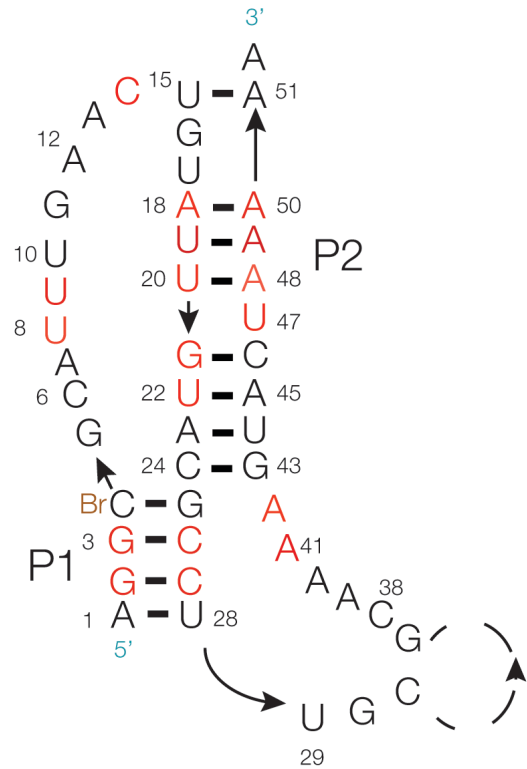
³Howard Hughes Medical Institute, Yale University, New Haven, Connecticut 06520, USA



The SAM-V riboswitch structure



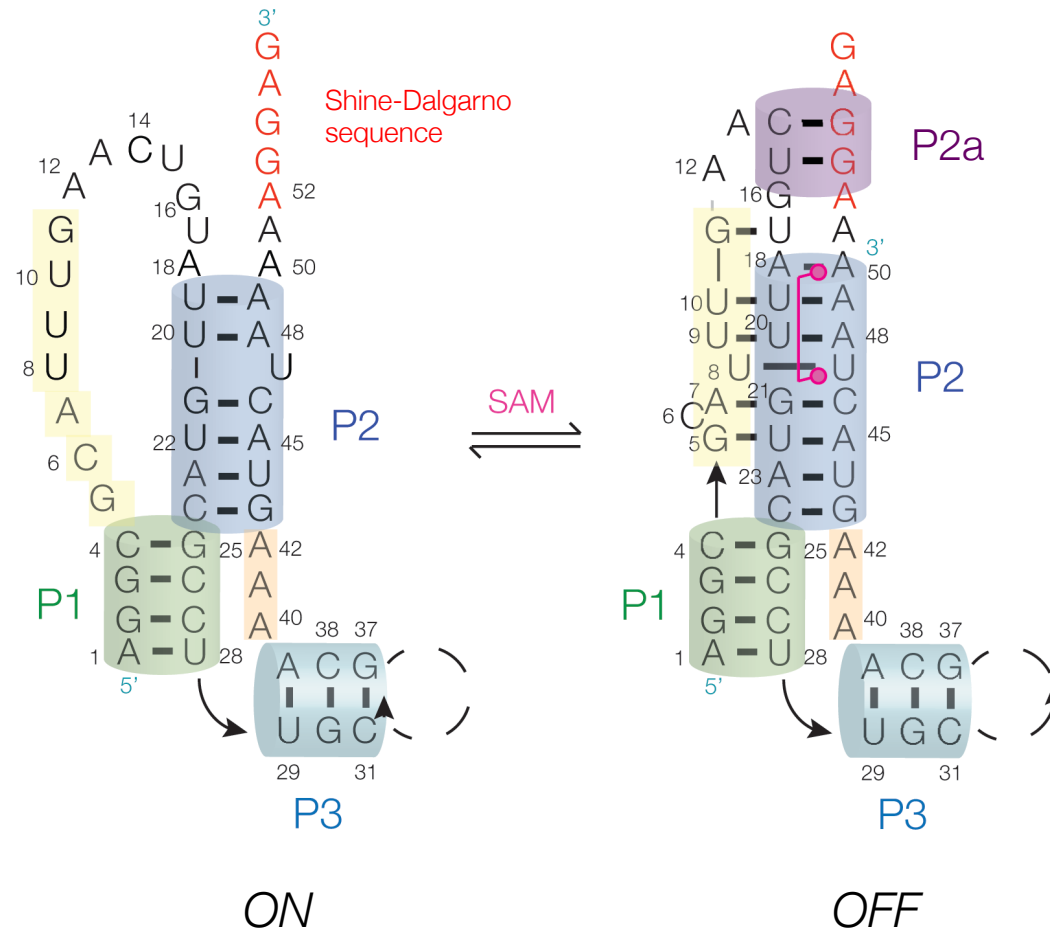
The SAM-V riboswitch structure



Rational design

- Secondary structure prediction
- 3D structure prediction
- Sequence selection (by Jalview)

A proposed mechanism of action for the SAM-V riboswitch



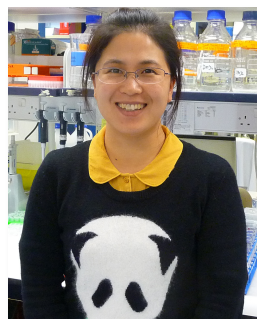
Summary

Rational designs + 5-Bromo-cytidine

Efficient strategy in RNA x-ray crystallography



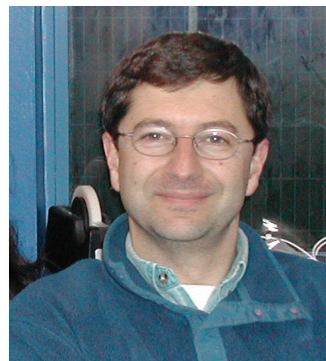
CR-UK
Nucleic Acid Structure
Research Group



Jia Wang



Saira Ashraf

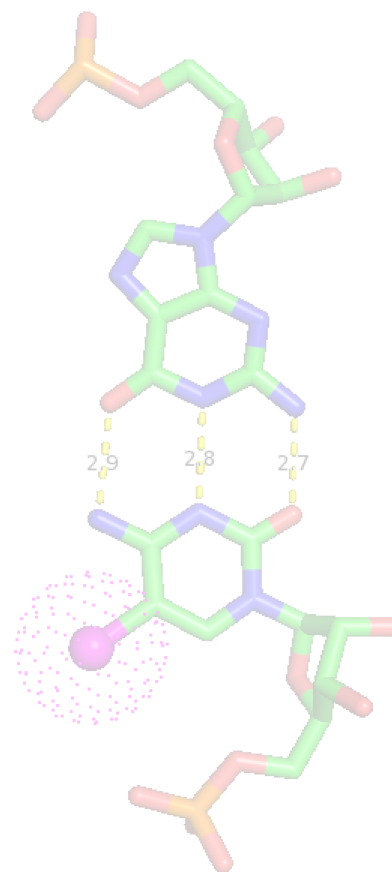


Tim Wilson



David Lilley

Jalview



Dr Jim Procter



Prof. Geoff Barton