From sequence to nanostructure ------

The kink turn as a building block in nano-engineering

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RNA folding and structure prediction

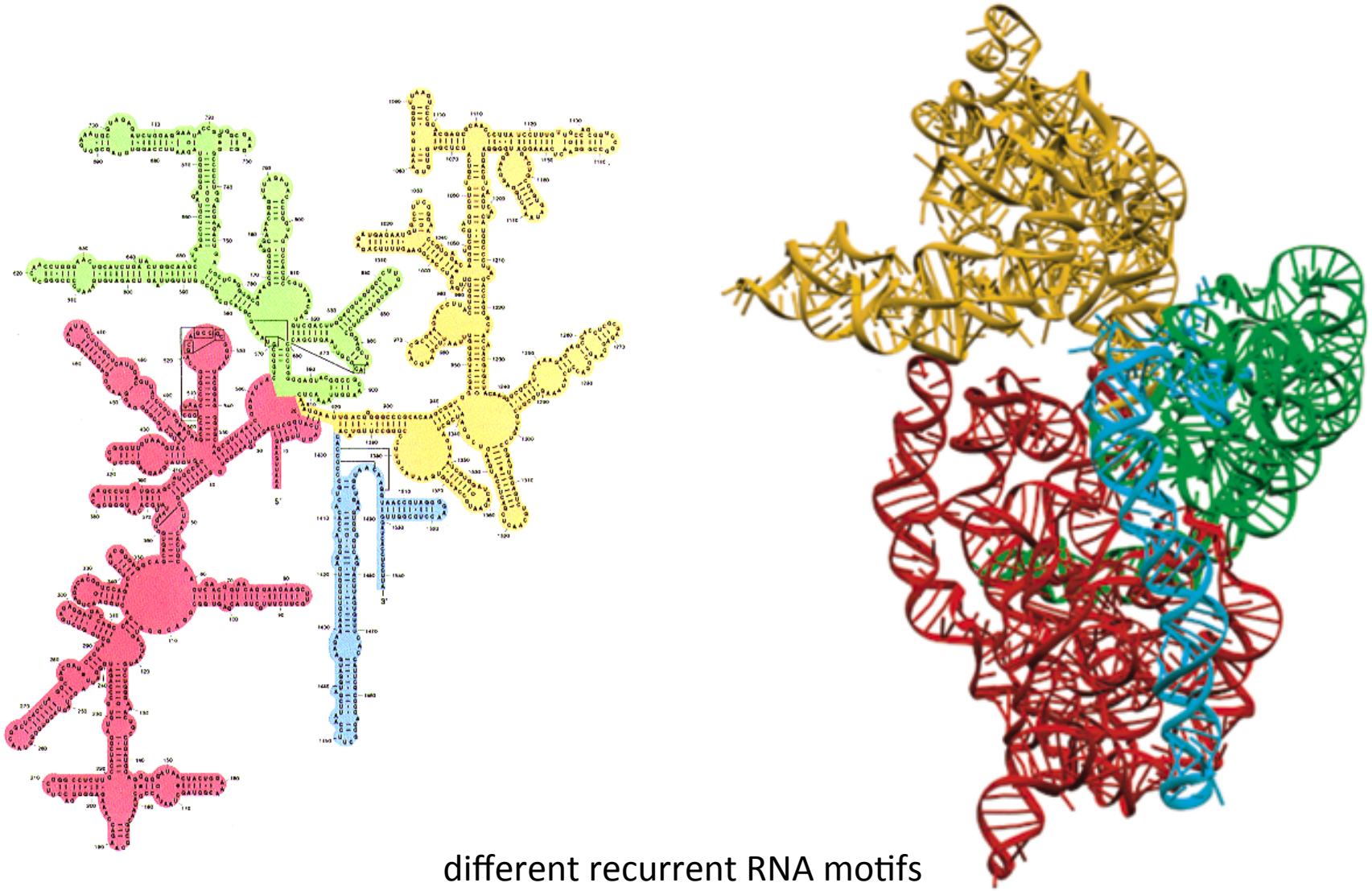
- RNAs are important.
- RNA folding.
- RNA structure prediction at Atomic Resolution.

Why Can't We Predict RNA Structure At Atomic Resolution?

Parin Sripakdeevong, Kyle Beauchamp, and Rhiju Das

N. Leontis and E. Westhof (eds.), RNA 3D Structure Analysis and Prediction, Nucleic Acids and Molecular Biology 27, Springer, 2012

16S rRNA in the 30S ribosomal subunit

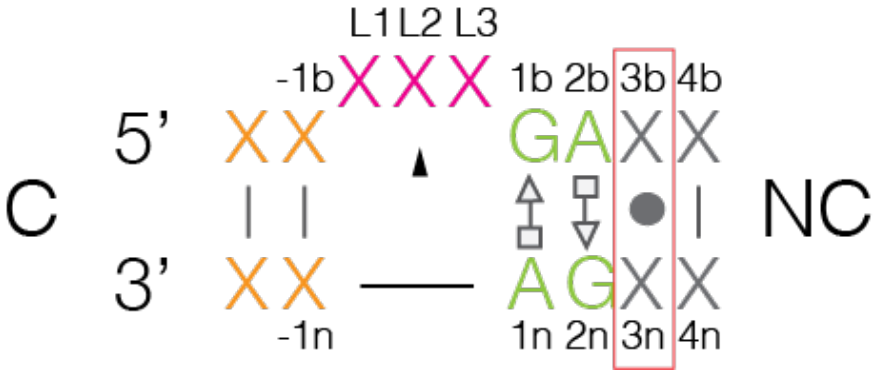


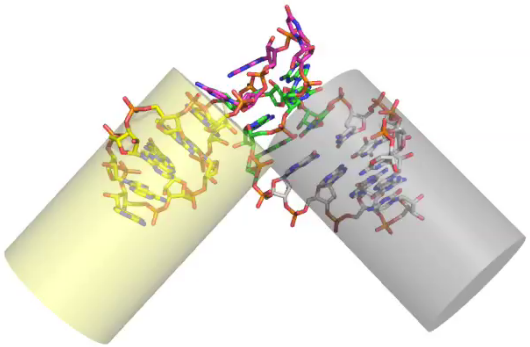
different recurrent RNA motifs

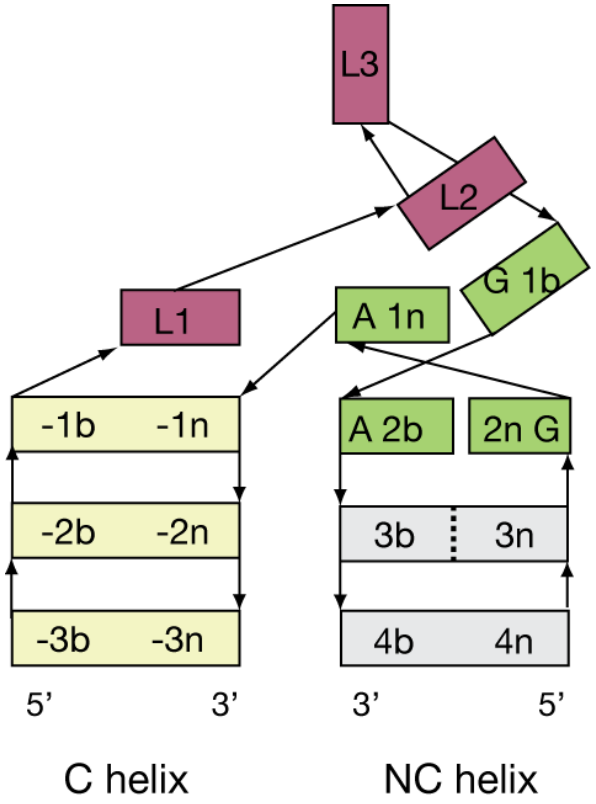
Wimberly et al *Nature* **407** 327–339 (2000).

The k-turn motif – a kinking motif

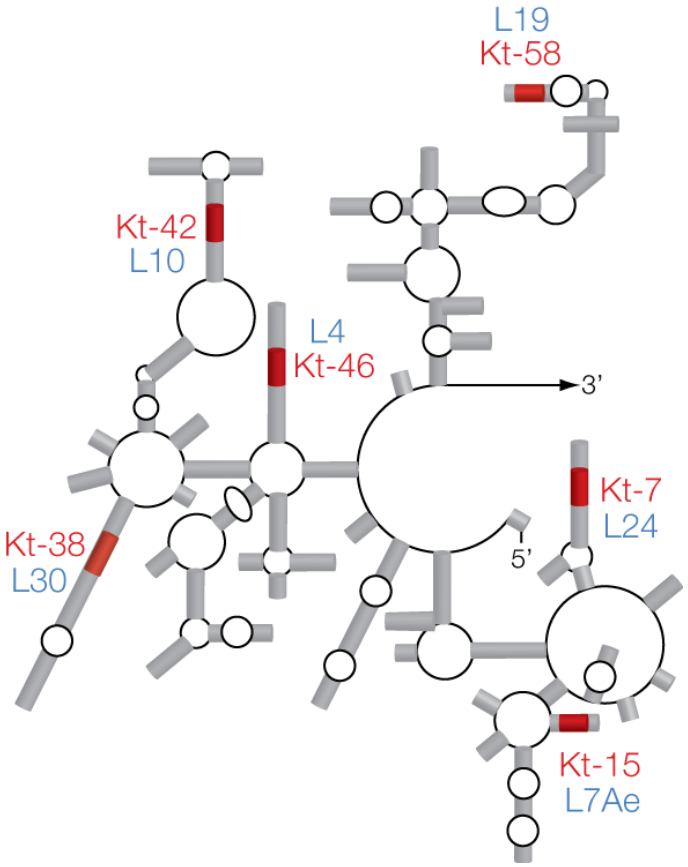
generic k-turn

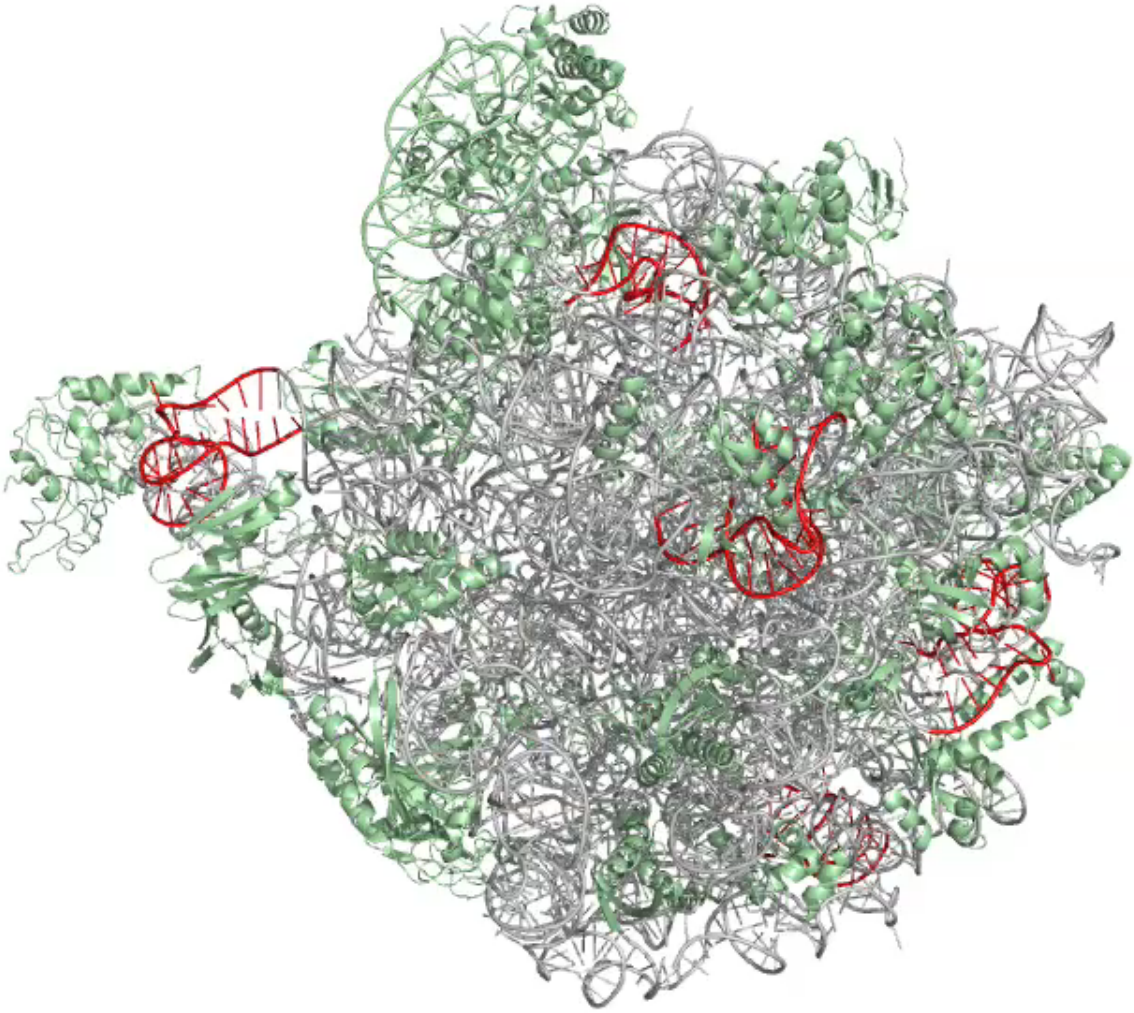






k-turns in the 50S ribosomal subunit





Ban et al *Nature* **289** 920–930 (2000).

RNA folding with metal ions

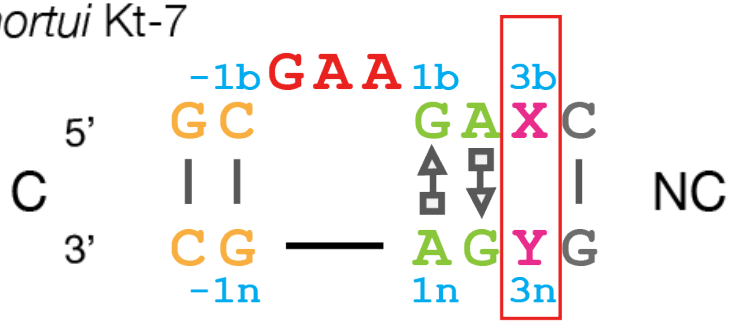
Some of k-turns' folding is induced by metal ions

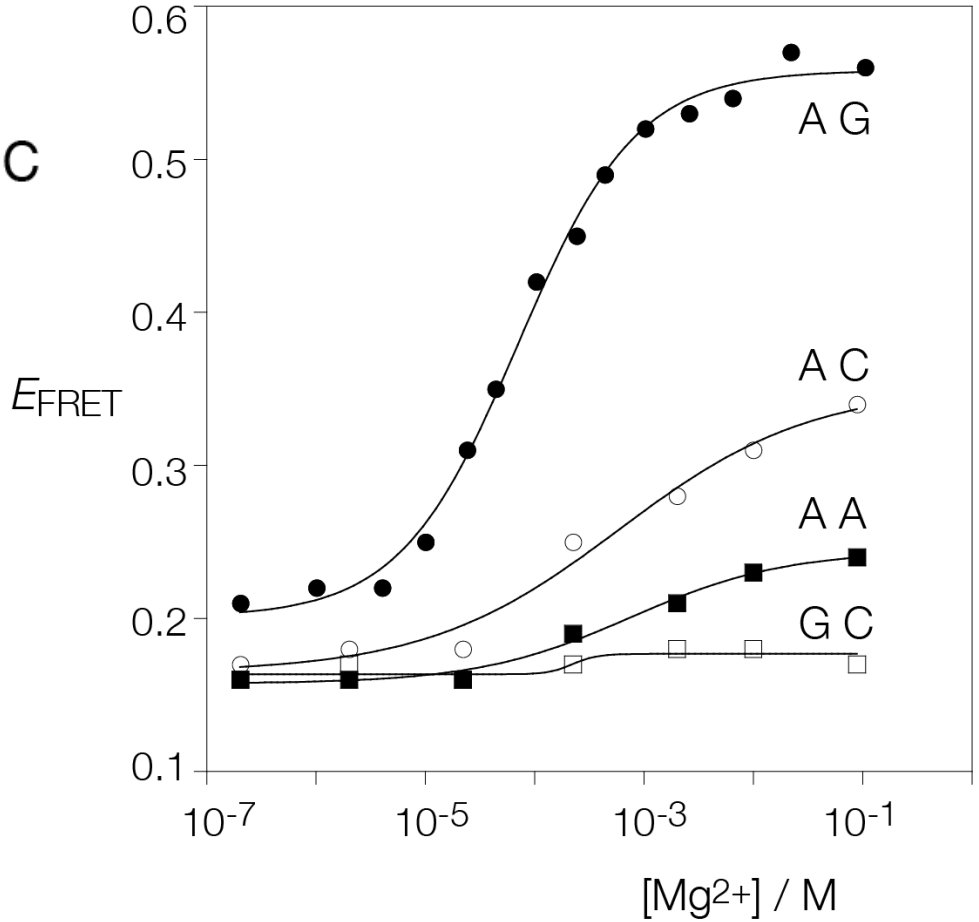
What is the sequence determinant

of the folding ability ?

Folding of Kt-7 in metal ions as a function of 3b·3n sequence

H. marismortui Kt-7





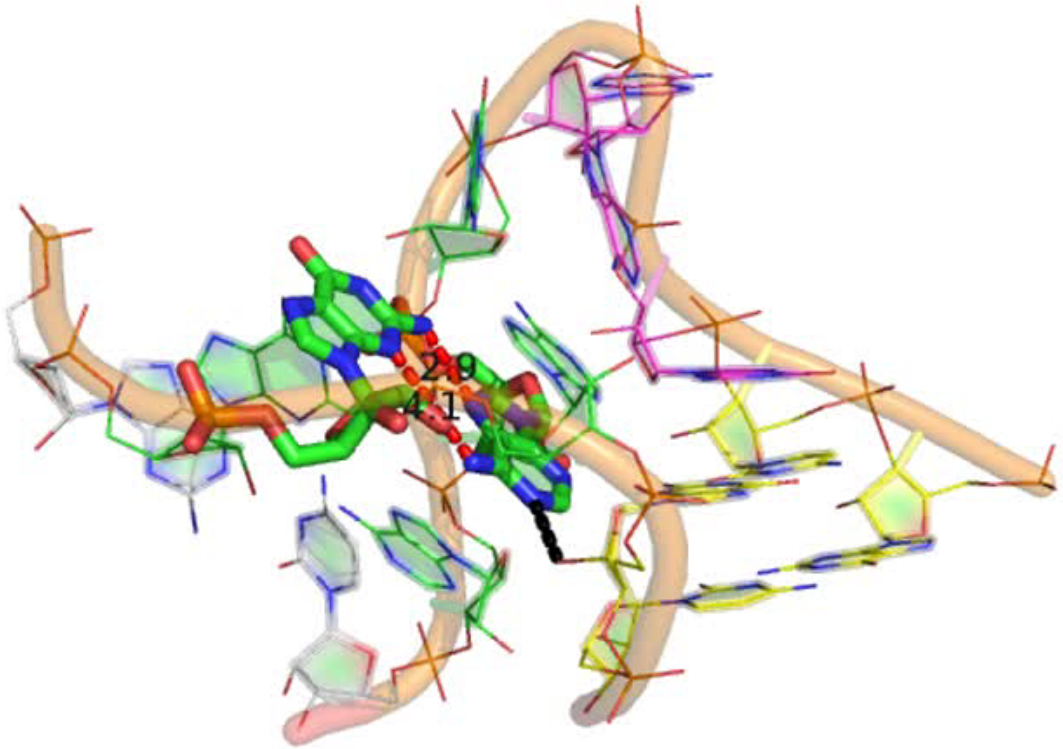
Scott McPhee

McPhee, Huang & Lilley, *Nature Commun.* **5**: 5127 (2014)

Change in global trajectory of N3 vs N1 class Kt-7

morph

N3 <> N1 class structures

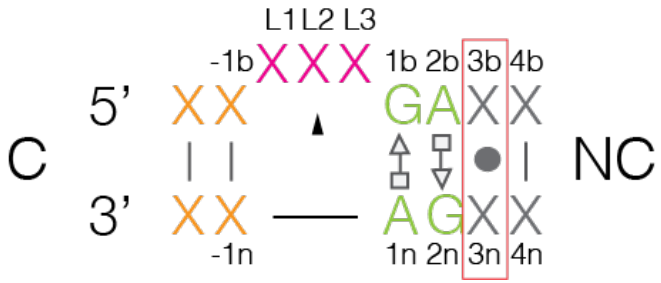


15 degree difference of
NC helical rotation

Daldrop & Lilley, *RNA.* **19 (3), 357-364** (2013)

Some simple empirical rules

1. 3b•3n = AG confers folding and N3 structure
2. Well folded combinations except AG form N1 structure



No simple pattern

a look-up table

3b \ 3n	A	C	G	U
A	N3	N3	N3	
C	N1	N1		N1
G		N3	N1	N3
U		N1	N1	N3

folded

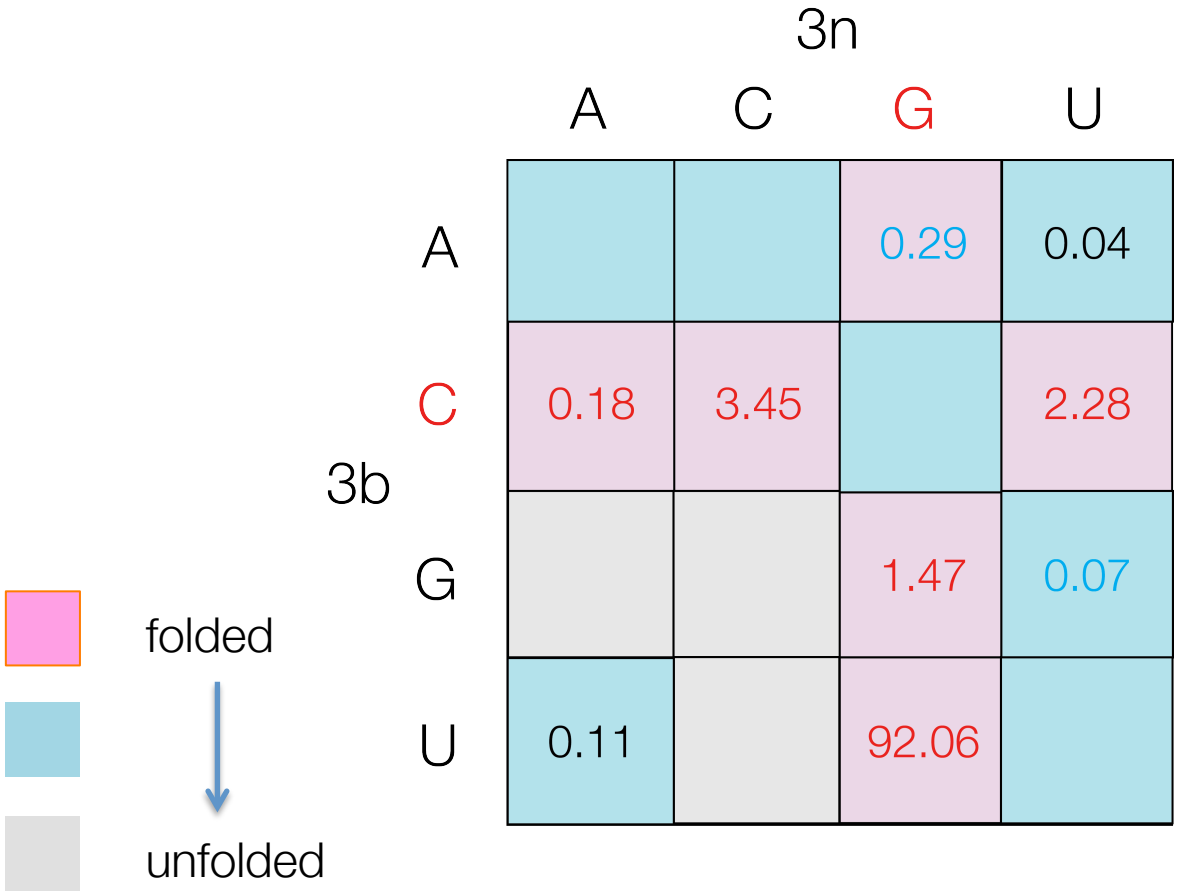
unfolded

Red = N1 structure
Blue = N3 structure

Bioinformatic analysis of the correlation between 3b•3n sequence and function

2722 kt-7 sequences from bacteria

Well folding combinations except AG form N1 structure conformation



Numbers are percentage of the 3bn combinations:
Red = N1 structure
Blue = N3 structure
Black = unknown

Bioinformatic analysis of the correlation between 3b•3n sequence and function

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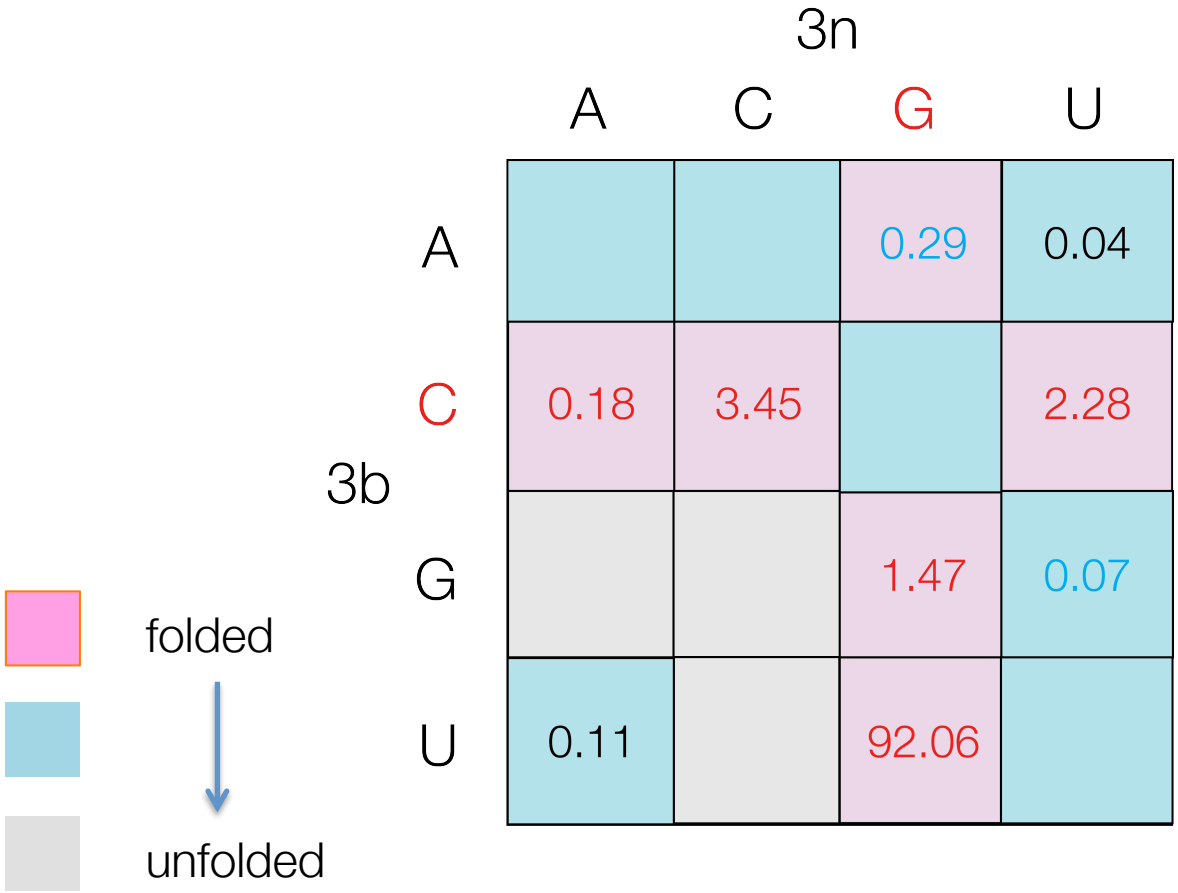


Numbers are percentage of the 3bn combinations:
Red = N1 structure
Blue = N3 structure
Black = unknown

Bioinformatic analysis of the correlation between 3b•3n sequence and function

9235 U4 spliceosomal RNA sequences were taken from the Rfam database

Prefer unfolded, N3 Structure

3b \ 3n	A	C	G	U
A	0.21	2.37	0.04	7.07
C	0.01	0.30	1.01	0.28
G	1.38	62.59	1.05	22.67
U	0.05	0.49	0.01	0.47

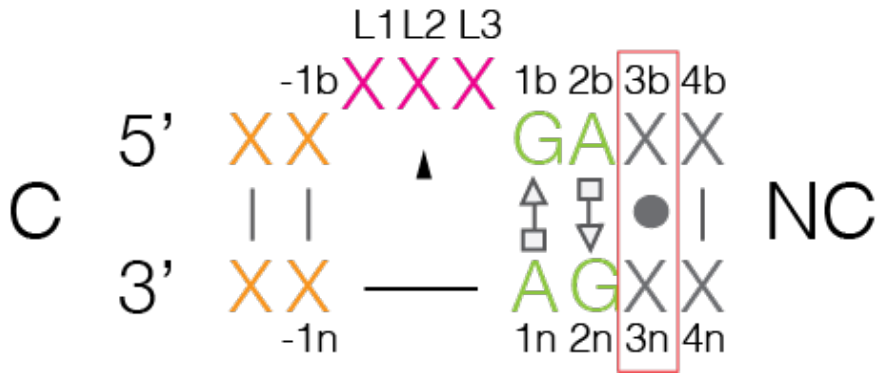
folded

unfolded

Numbers are percentage of the 3bn combinations:
Red = N1 structure
Blue = N3 structure
Black = unknown

3b \ 3n	A	C	G	U
A	N3	N3	N3	
C	N1	N1		N1
G		N3	N1	N3
U		N1	N1	N3

Application

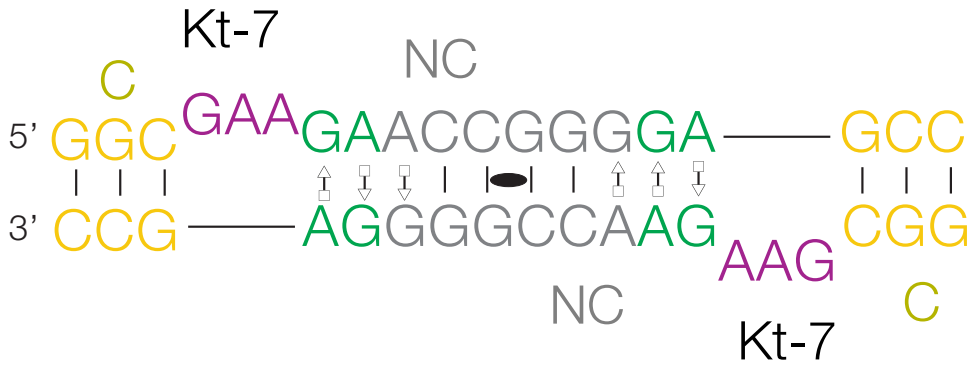


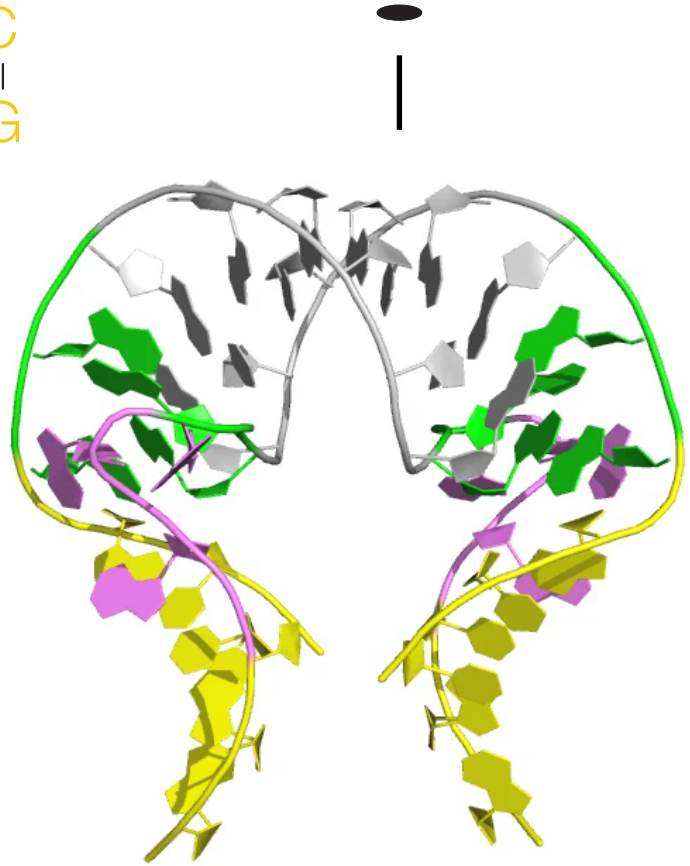
We knew folding and structural conformation.

What we can do based on those information?

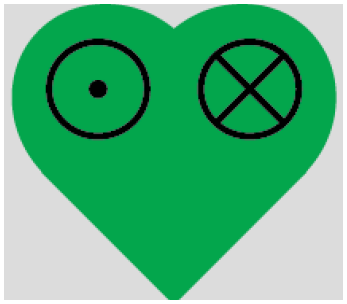
Understand RNP assembly
Rational design of artificial RNA species

The double-k-turn unit





an inverted repeat sequence
horse-shoe shape
structural building block



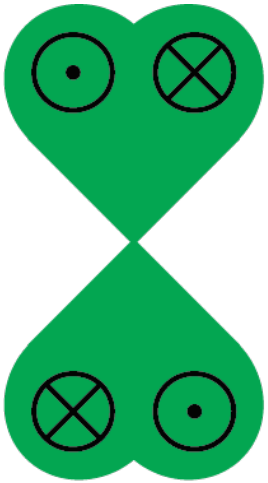
L Huang, DMJ Lilley (2016) A quasi-cyclic RNA nano-scale molecular object constructed using kink turns. Nanoscale

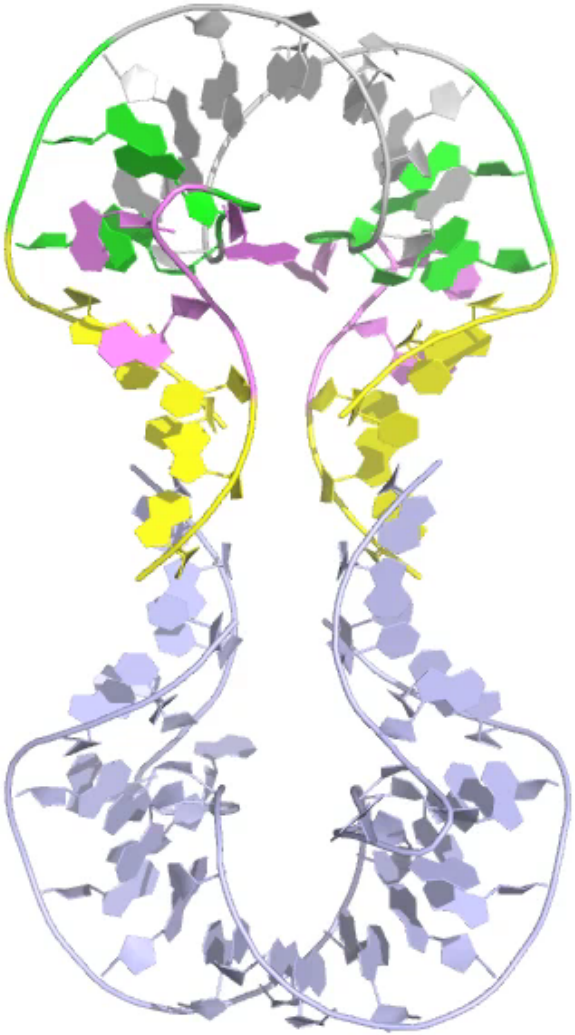
Association of 2 double-k-turn units in crystal lattice

$P4_222$

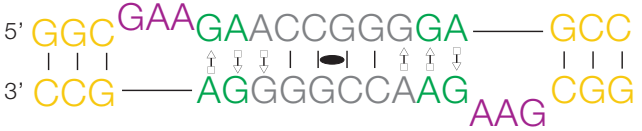
2.2 Å

5FJ0





Two-k-turn unit



dumbell shape

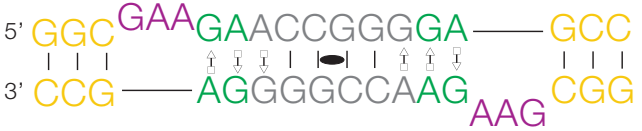
Association of 3 double-k-turn units in crystal lattice

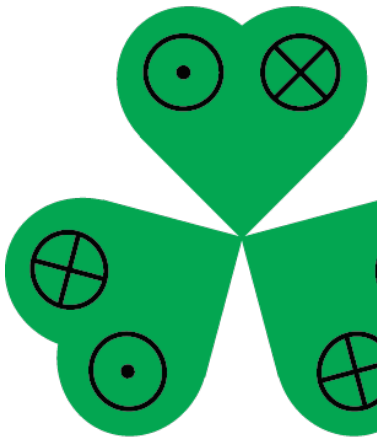
$P6_322$

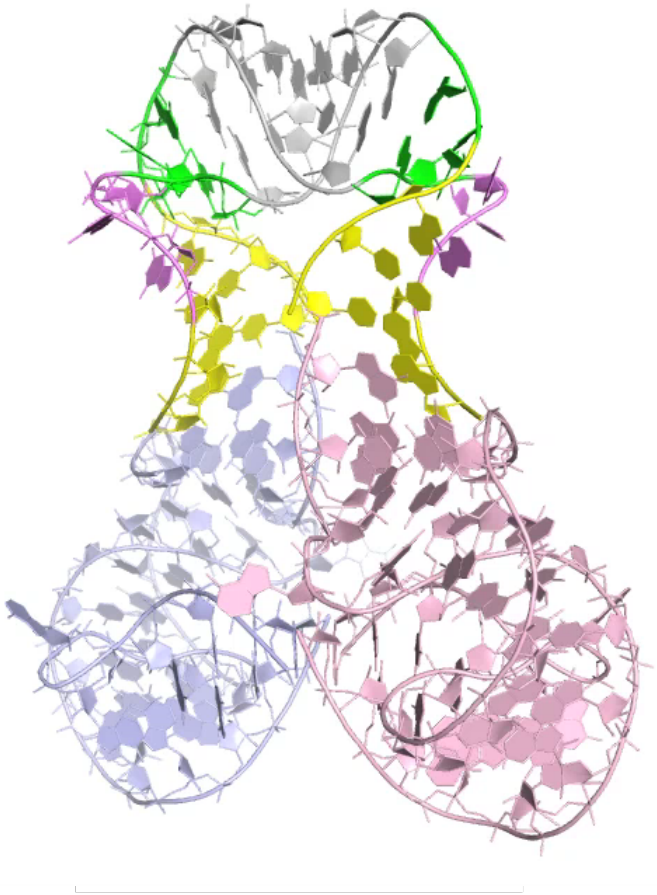
2.0 Å

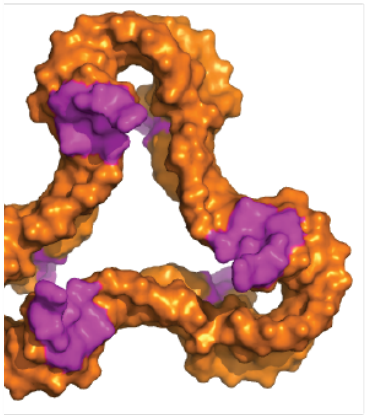
4CS1

Two-k-turn unit

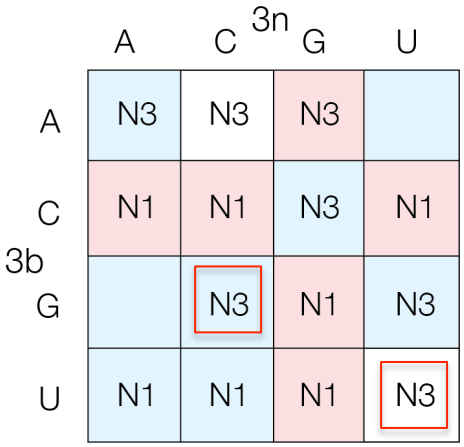








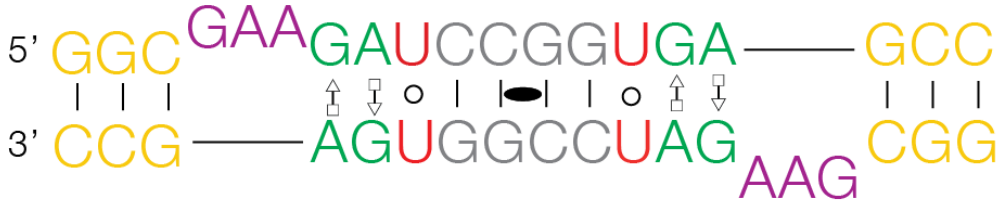
Variation of the 3b,3n sequence of Kt-7



Kt-7

N3

Kt-7 3bU 3nU



N3

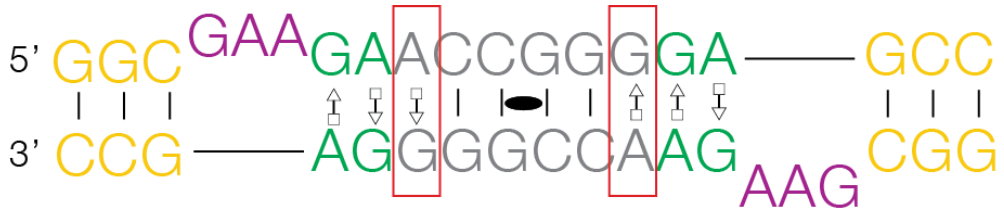
Kt-7 3bG 3nC

N3

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

Kt-7 3bU 3nU double-k-turn unit

Kt-7



N3

Kt-7 3bU 3nU

N3

Kt-7 3bG 3nC

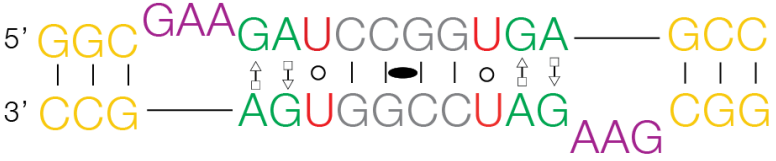
N3

Kt-7 3bU 3nU forms a protein-bound triangular association in the lattice

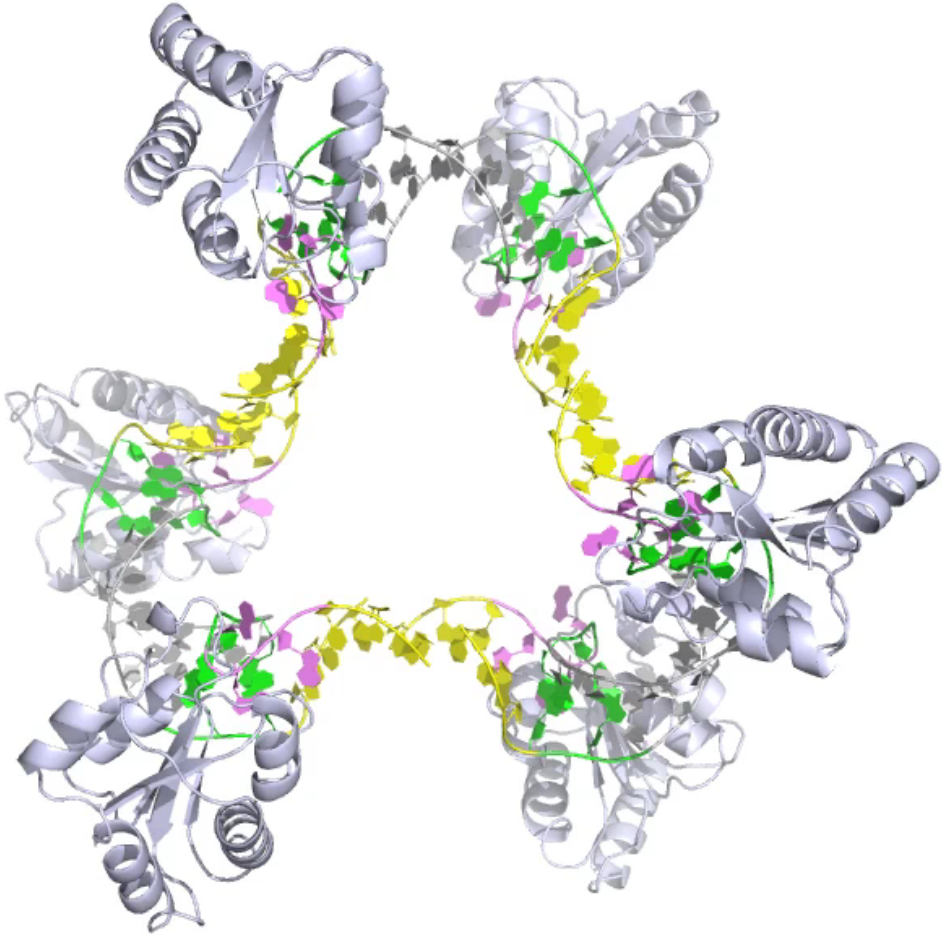
$P2_12_12_1$

2.65 Å

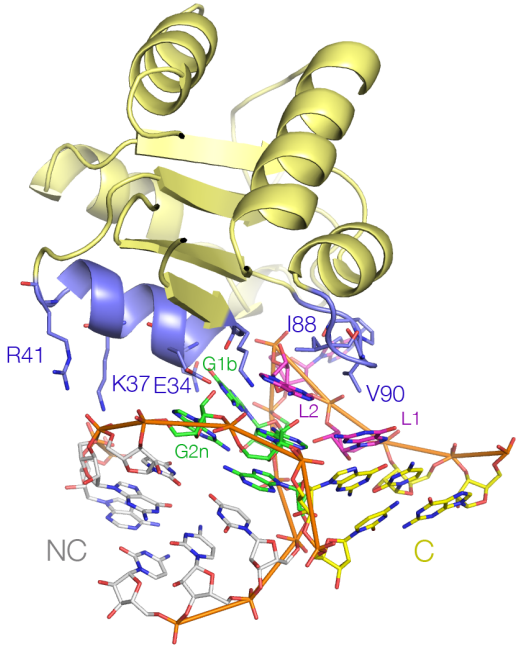
5C4U



+ L7Ae protein

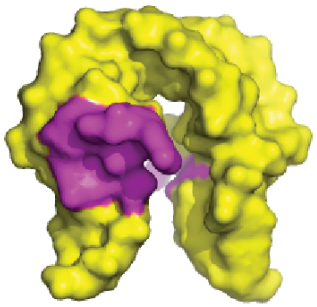


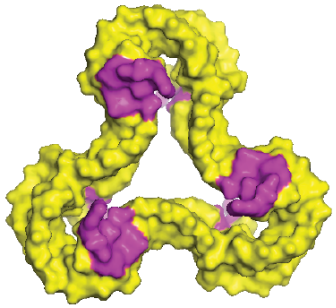
Top view



Comparison of the structures of the two triangular associations

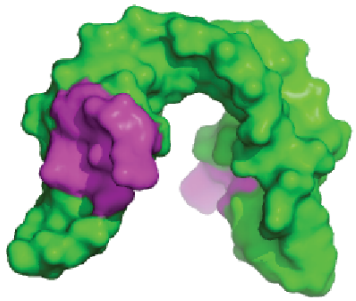
Kt-7

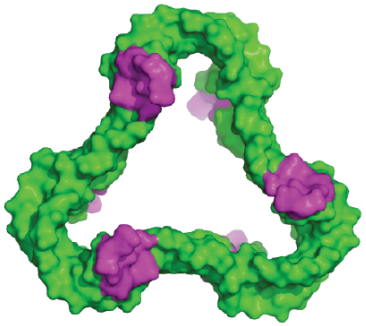


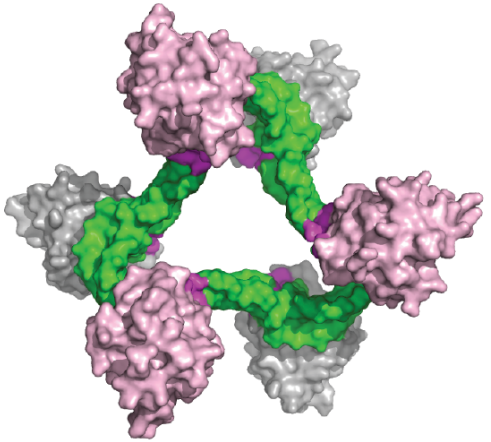


central pore = 7.5 Å

Kt-7 3bU 3nU





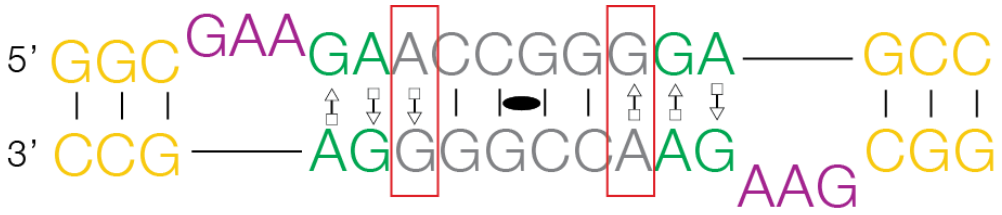


central pore = 14 Å

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

Kt-7 3bG 3nC double-k-turn unit

Kt-7



Kt-7 3bU 3nU

Kt-7 3bG 3nC

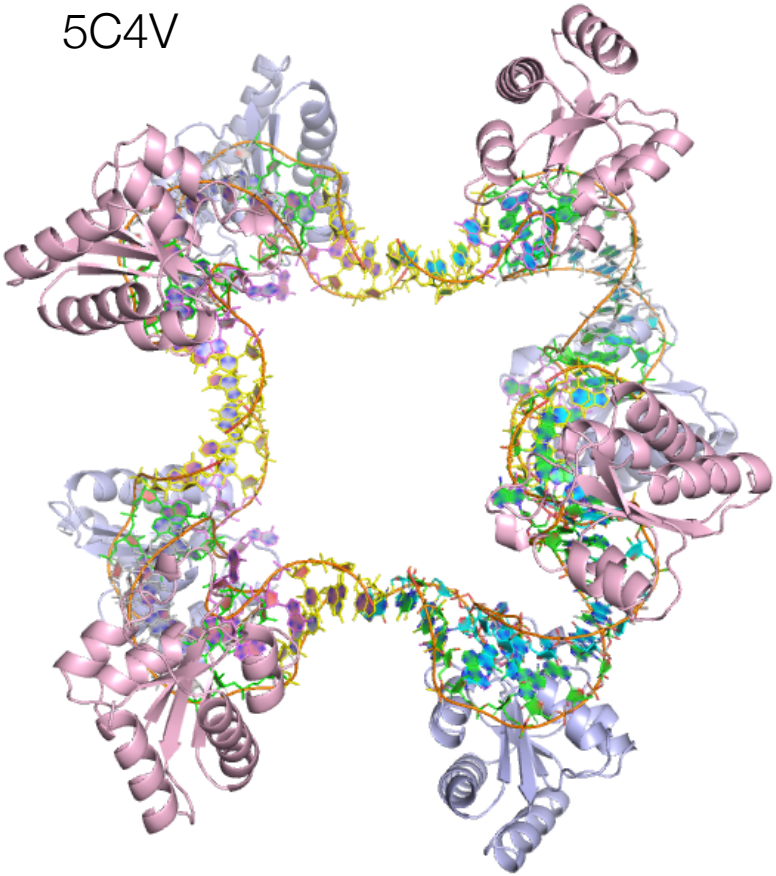
Kt-7 3bG 3nC : association of 4 double-k-turn units in crystal lattice

C1 2 1

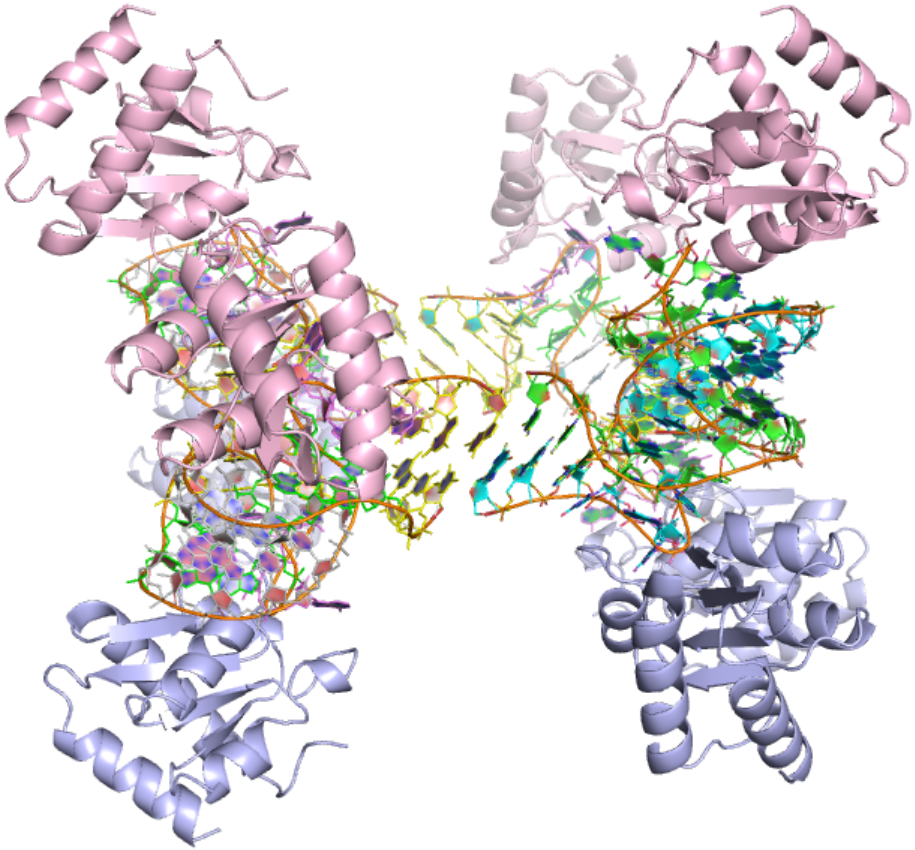
2.87 Å

5C4V

+ L7Ae protein



top



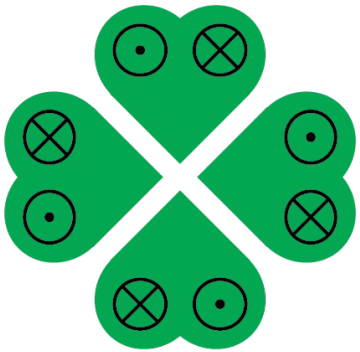
side

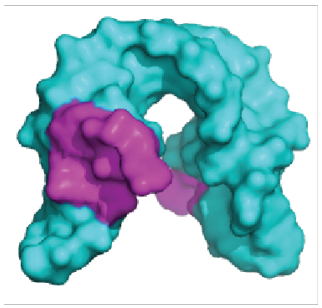
Kt-7 3bG 3nC : association of 4 two-k-turn units in crystal lattice

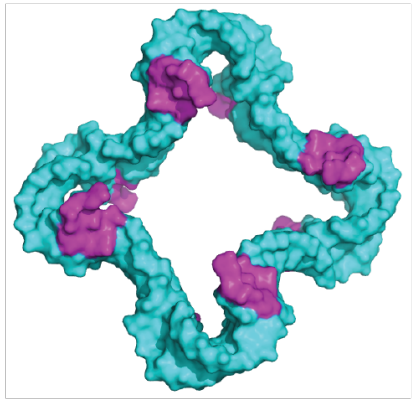
C1 2 1

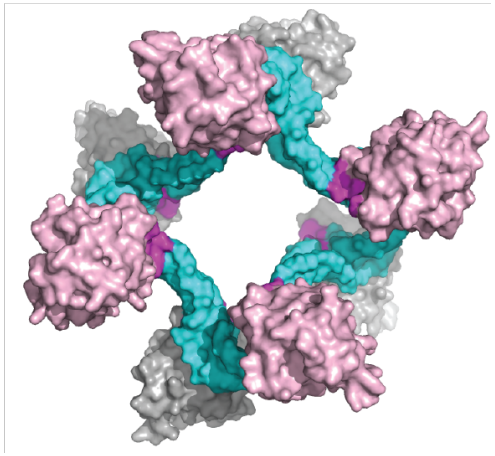
2.87 Å

5C4V





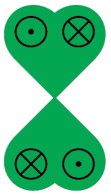


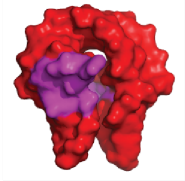


+ L7Ae protein

Crystal structure-guided design Of RNA nanoparticles

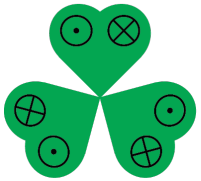
Kt-7

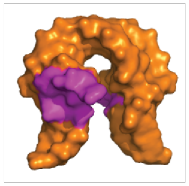


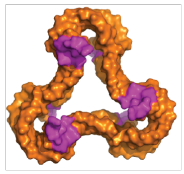


2-molecule assembly

Kt-7

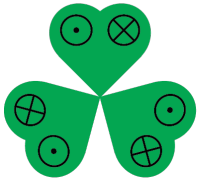


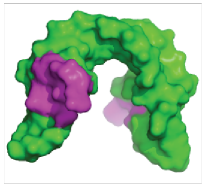


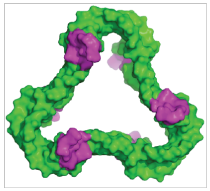


3-molecule assembly

Kt-7 3bU 3nU

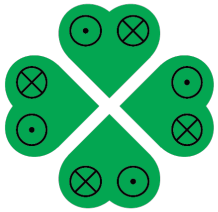


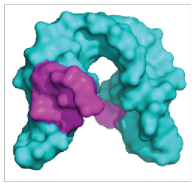


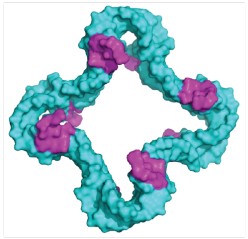


3-molecule assembly
L7Ae

Kt-7 3bG 3nC





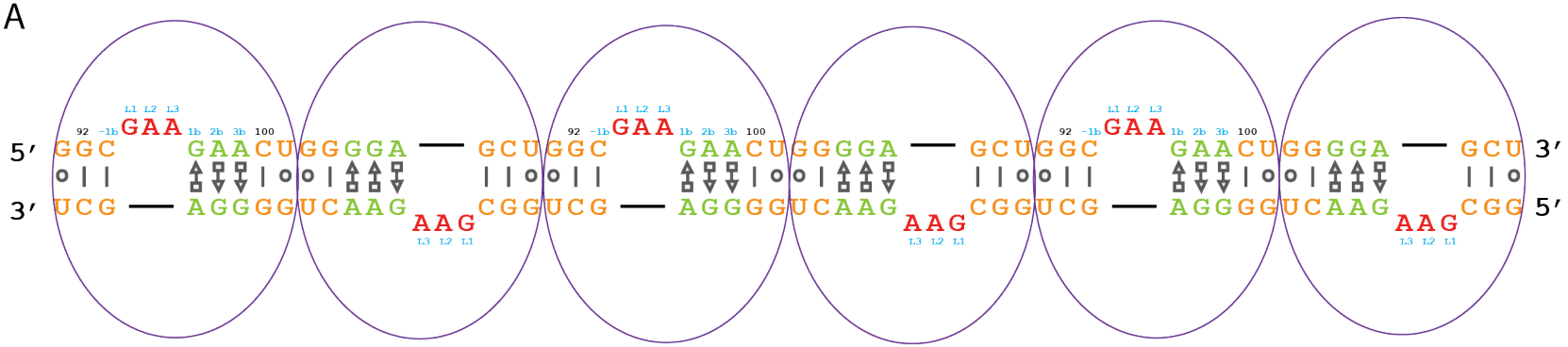


4-molecule assembly
L7Ae

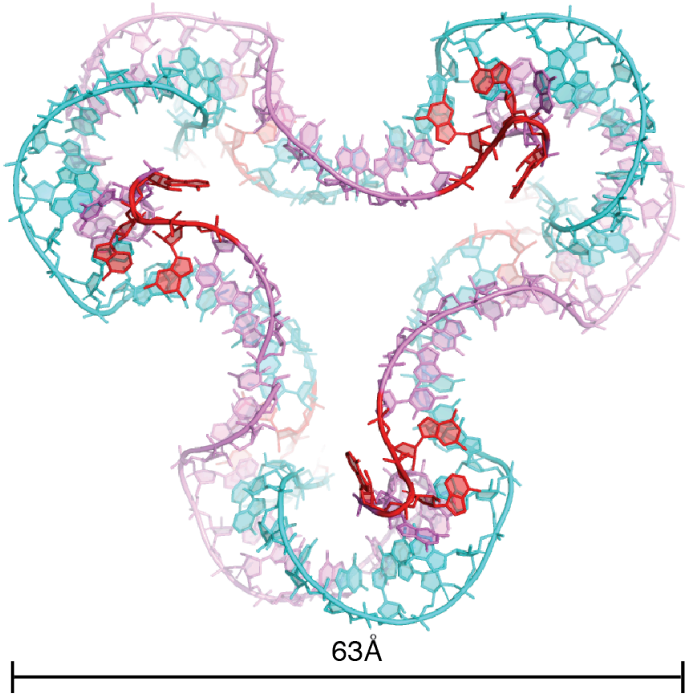
All from crystal packing

A self-complementary six k-turn molecule

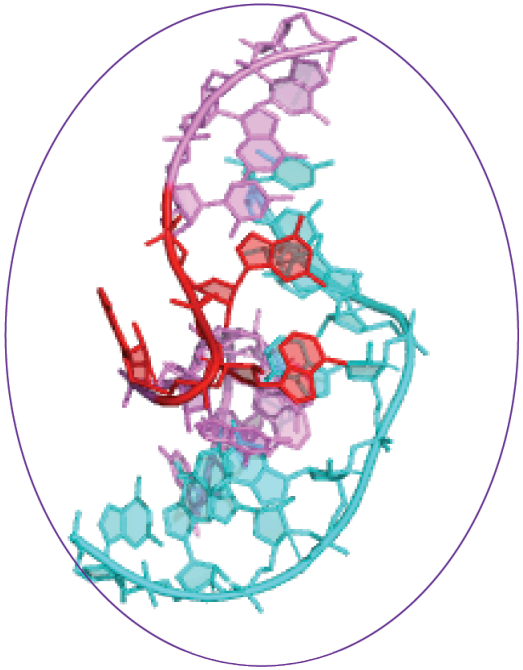
A



B



C



A six-k-turn nano-scale molecular object

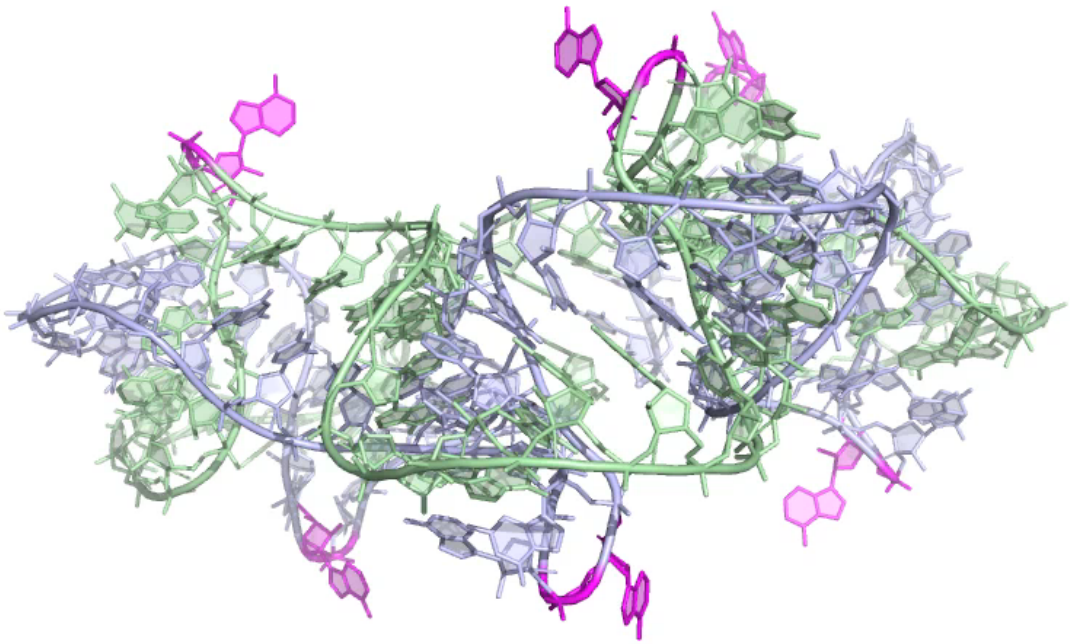
$P6_322$

2.75 Å

5G4T

Top view

A six-k-turn nano-scale molecular object

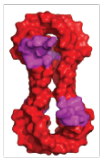


The loops of the k-turn are directed sequentially above and below the triangular plane.

Side view

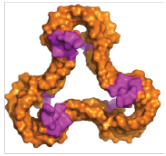
Crystal structure-guided design of RNA nanoparticles

Kt-7



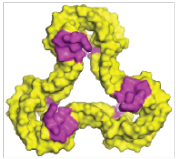
2-molecule assembly

Kt-7



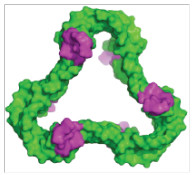
3-molecule assembly

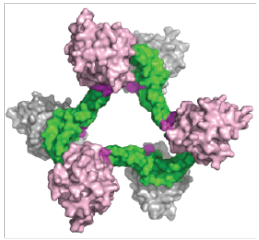
Kt-7



One molecule

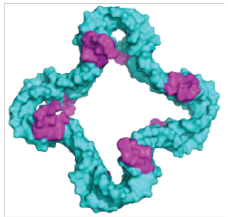
Kt-7 3bU 3nU

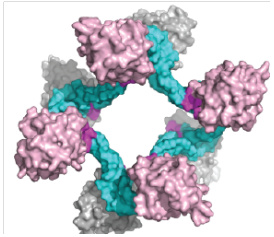




3-molecule assembly
L7Ae

Kt-7 3bG 3nC

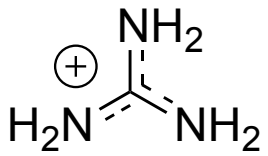




4-molecule assembly
L7Ae

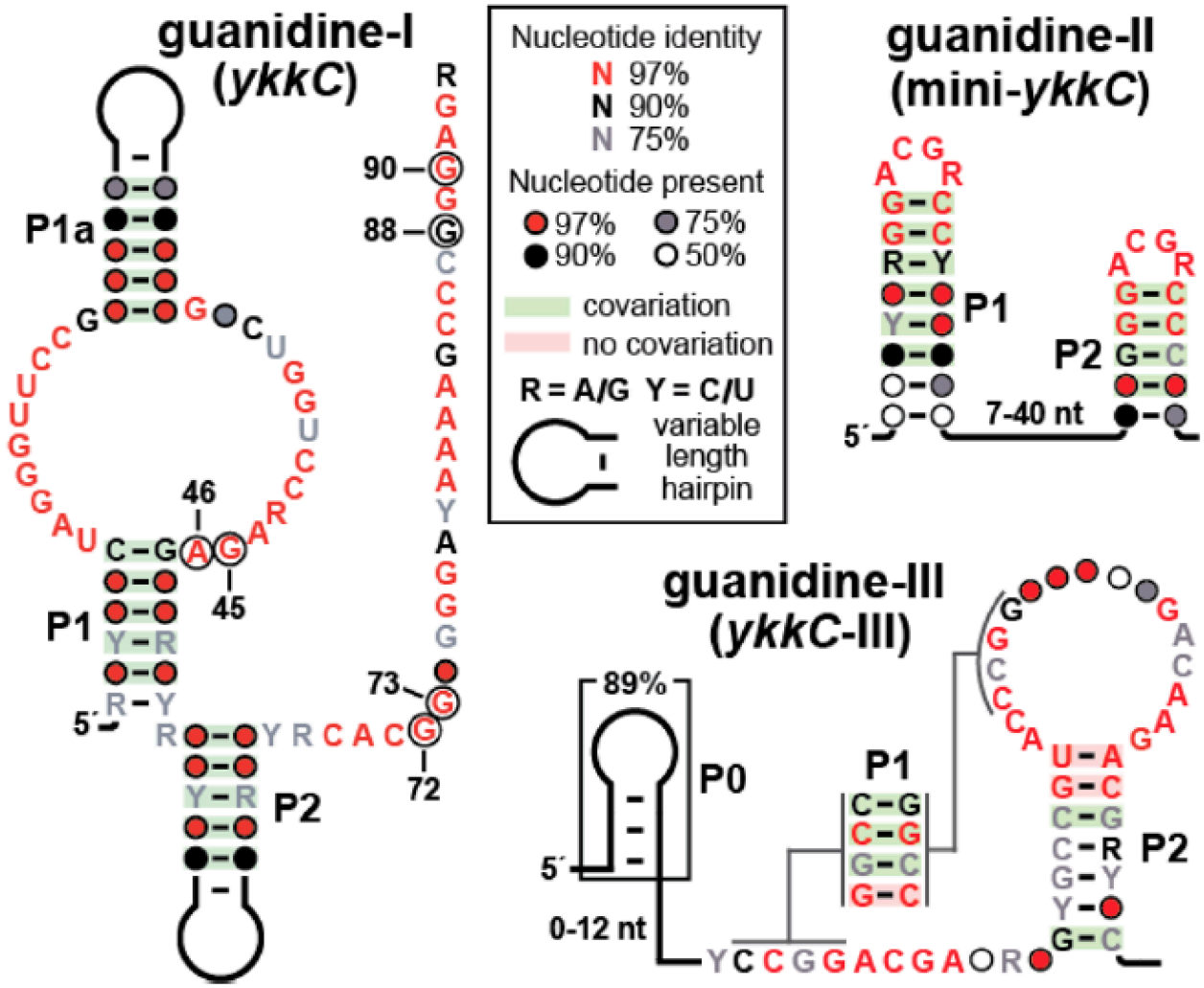
Novel RNA motifs for RNA Design

Three guanidine riboswitches (Breaker lab)



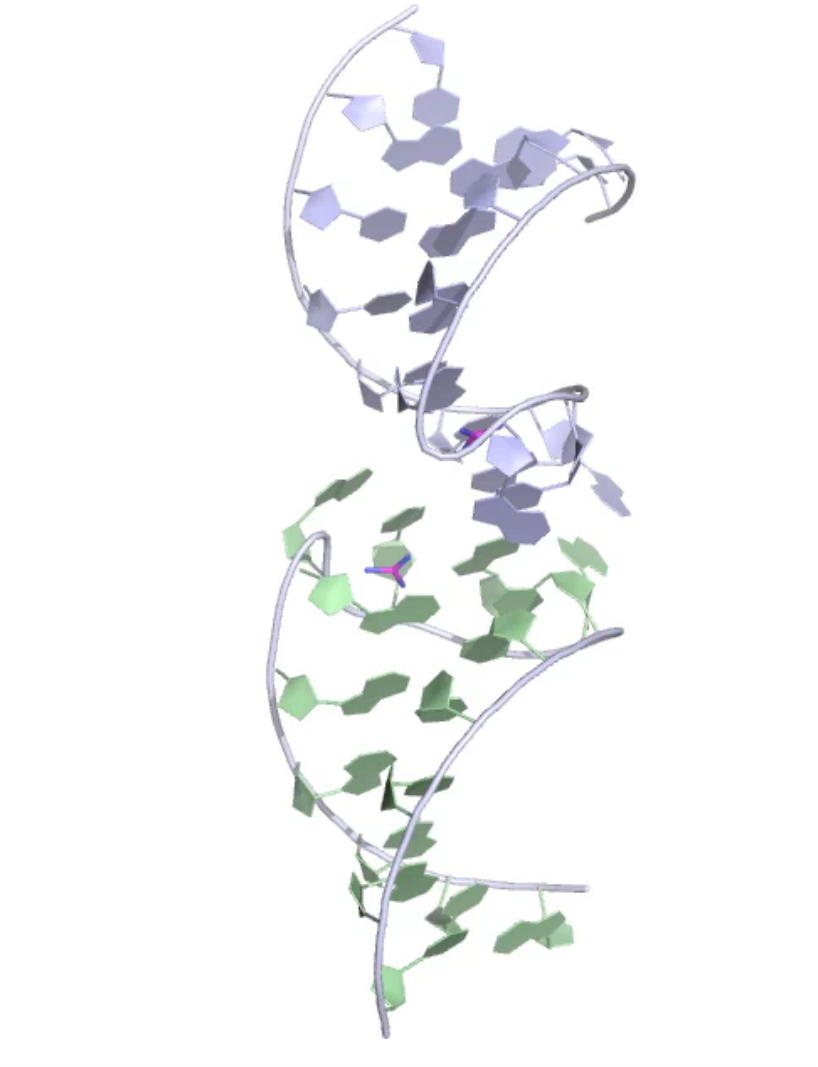
guanidinium cation

Ron Breaker



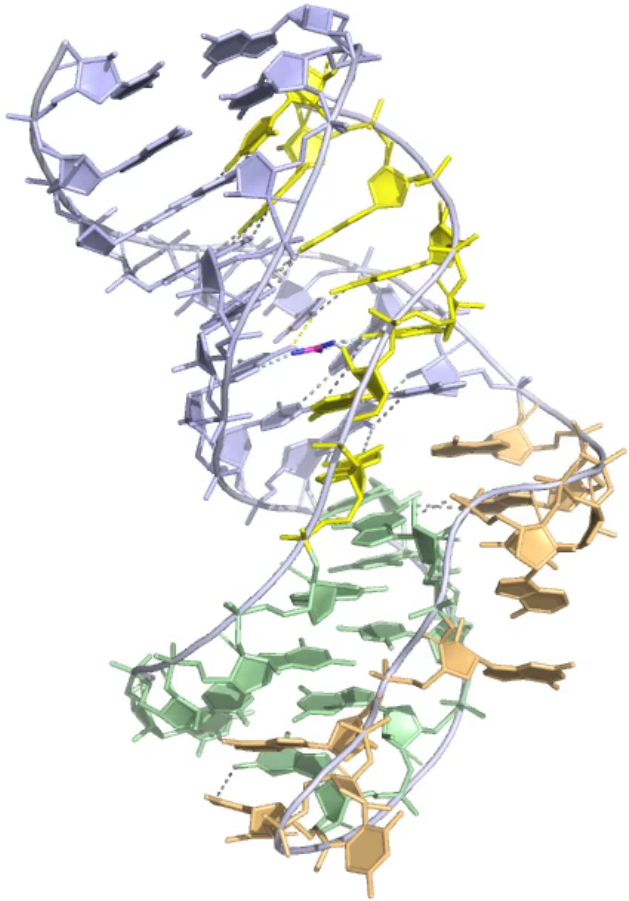
Sherlock… Breaker *Biochemistry* 56, 345-347 (2017)

Guanidine-II riboswitch

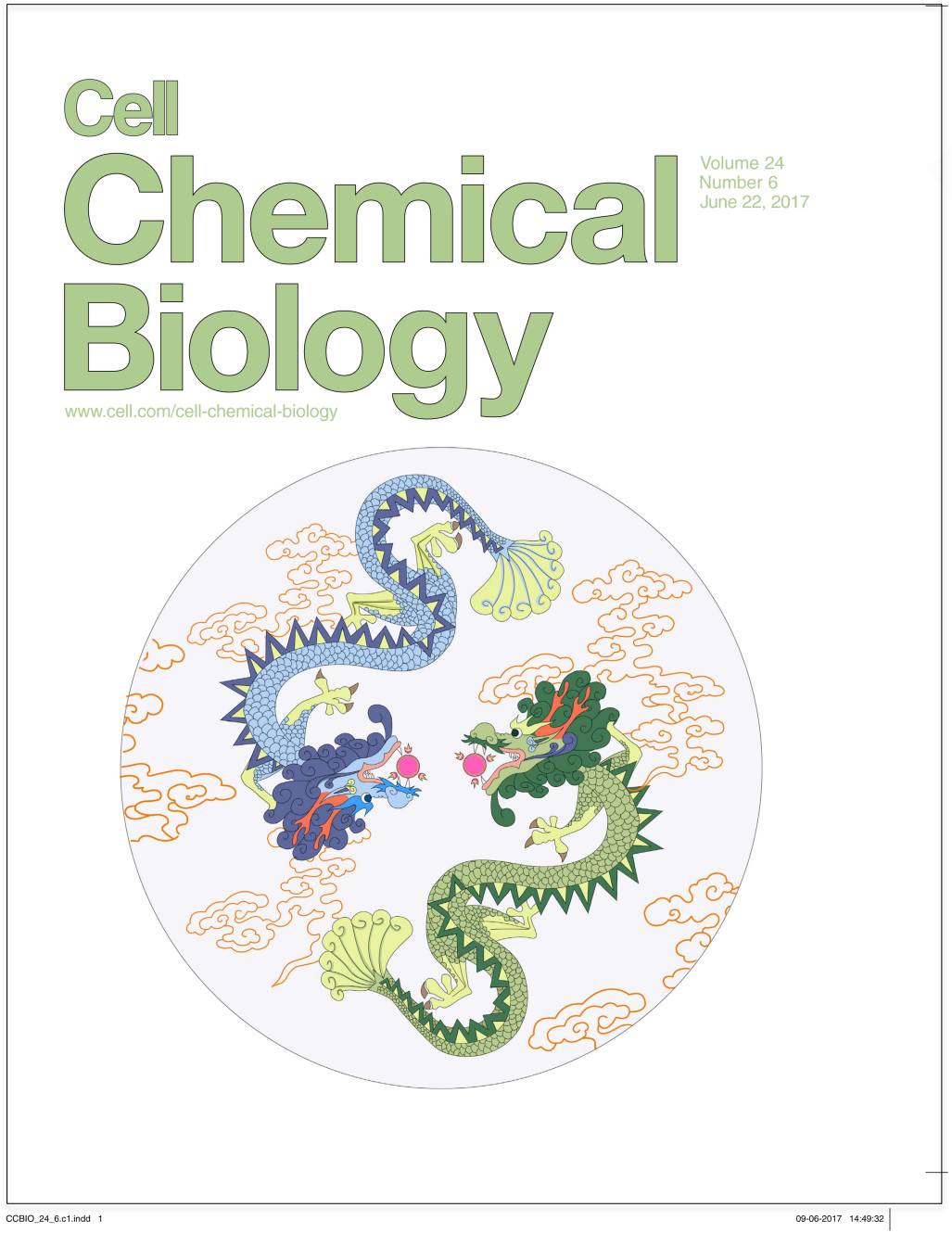


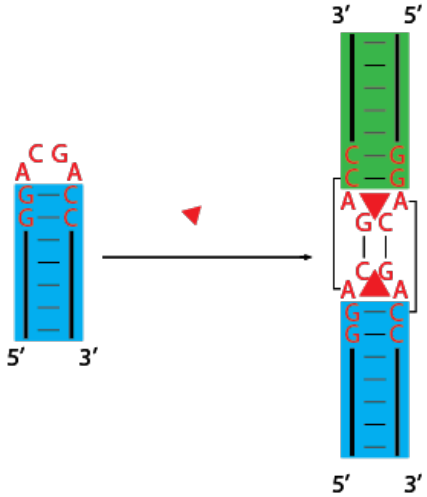
Huang, Wang & Lilley *Cell chem. biol.* **24**, 697-702 (2017)

Guanidine-III riboswitch



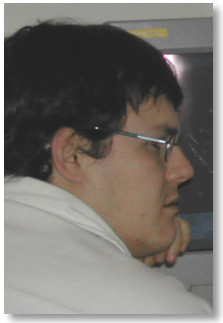
Huang … & Lilley *Cell chem. biol.* **24**, 1407-1415 (2017)





ACGR Motif

Huang, Wang & Lilley *Cell chem. biol.* **24**, 697-702 (2017)

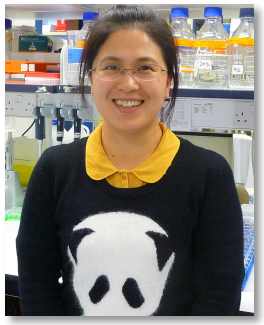


Terry Goody

Jia Liu

Kersten Schroeder

Peter Daldrop



Jia Wang

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