



University of  
Zurich<sup>UZH</sup>

Department of Chemistry

# NMR spectroscopy of RNA G-quadruplexes

Swiss NMR Symposium

Silke Johannsen

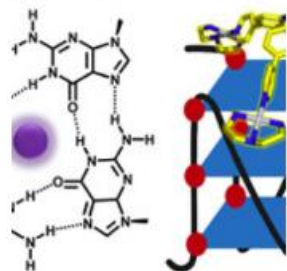
Group of Prof. Roland Sigel



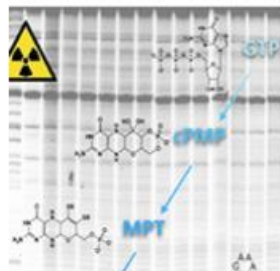
# Interaction of Metal Ions with Nucleic Acids

## (RNA) Systems Under Study

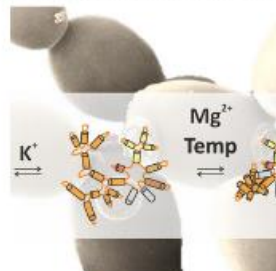
Quadruplexes



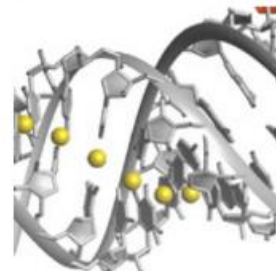
Riboswitches



Ribozymes

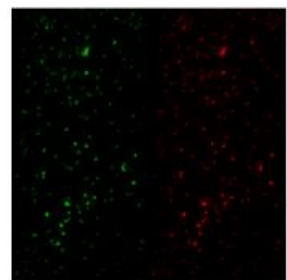


Metal-Modified Bases

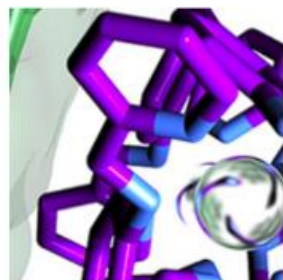


## Methodology and Computational Approaches

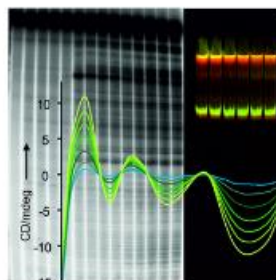
Fluorescence Spectroscopy



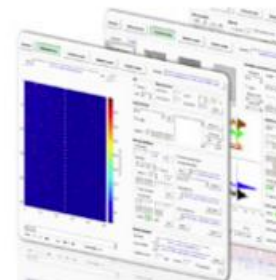
Structure Determination



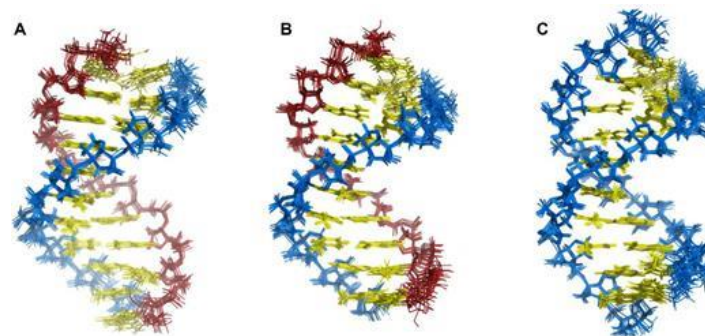
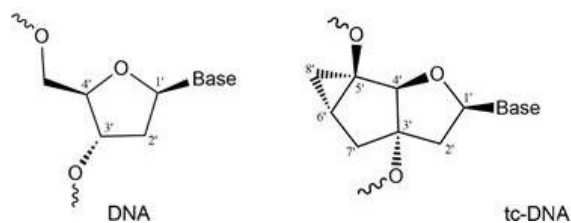
Chemical Biology



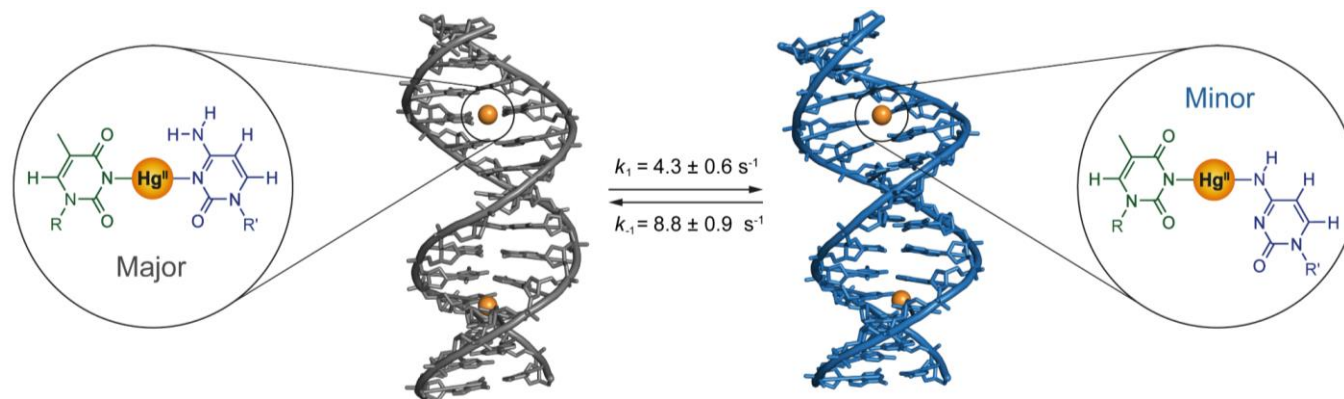
Software Development



# Modified Nucleic Acids



A. Istrate, S. Johannsen, A. Istrate, R.K.O. Sigel, C.J. Leumann, *Nucleic Acids Res.* 2019, 47, 4872.

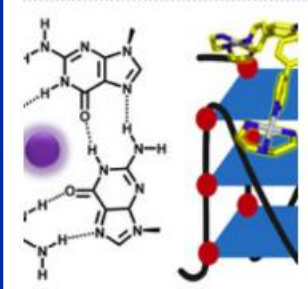
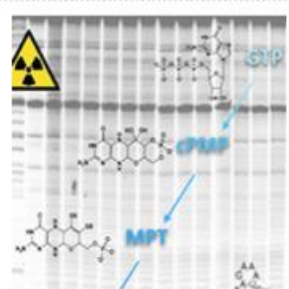
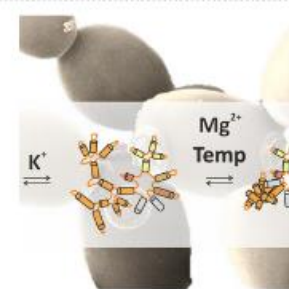
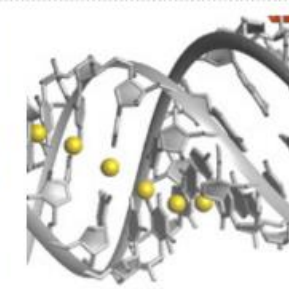


O.P. Schmidt, S. Jurt, S. Johannsen, A. Karimi, R.K.O. Sigel, N.W. Luedtke, *Nat. Comm.* 2019, 10, 4818.

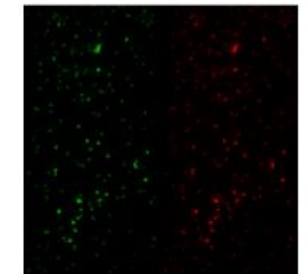
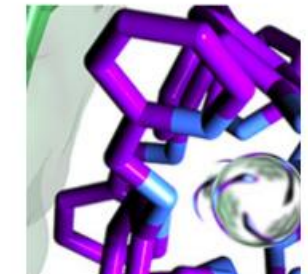
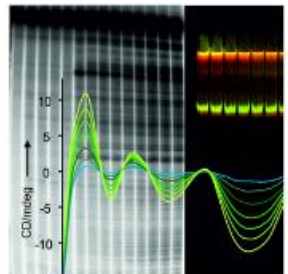
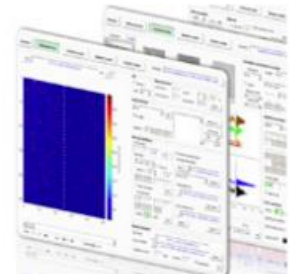


# Interaction of Metal Ions with Nucleic Acids

## (RNA) Systems Under Study

Quadruplexes	Riboswitches	Ribozymes	Metal-Modified Bases
			

## Methodology and Computational Approaches

Fluorescence Spectroscopy	Structure Determination	Chemical Biology	Software Development
			



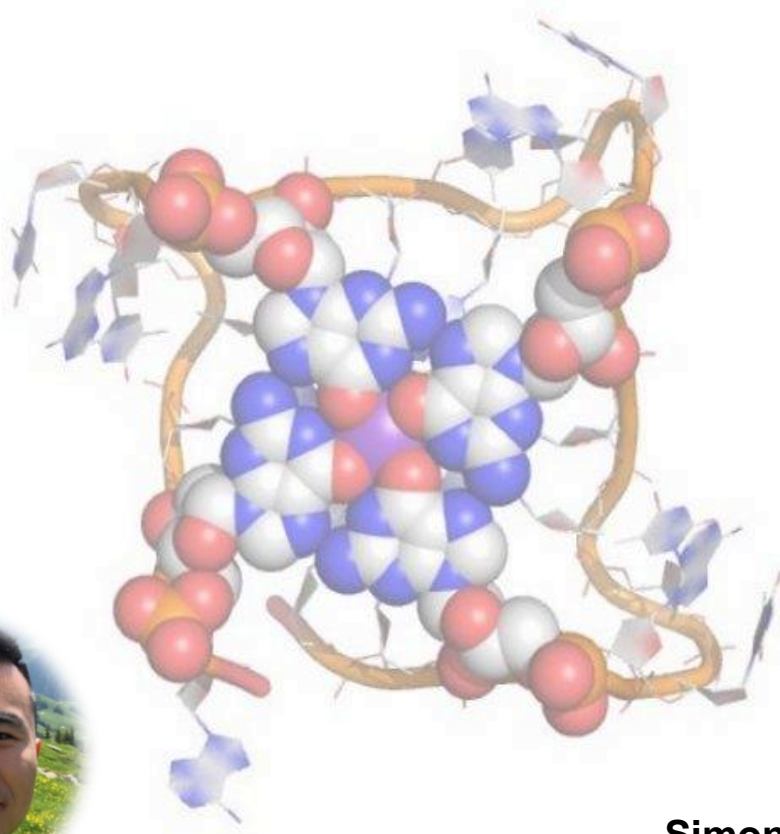
# RNA Quadruplexes



Dr. Alicia Dominguez-Martin



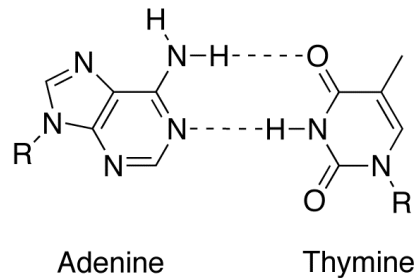
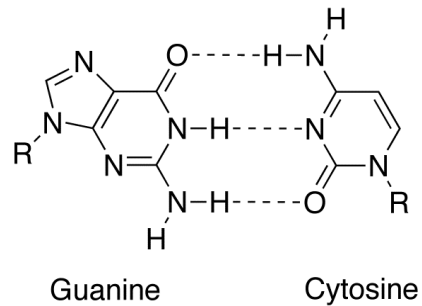
Zenghui Wang



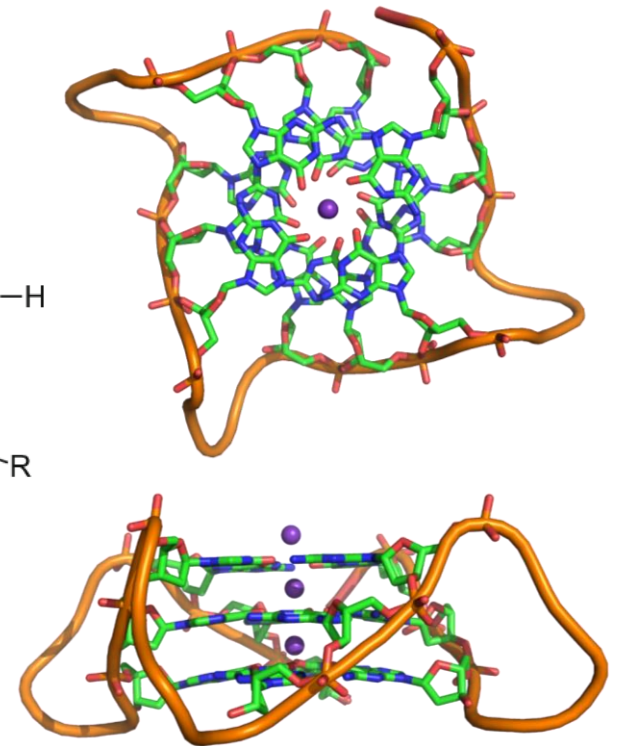
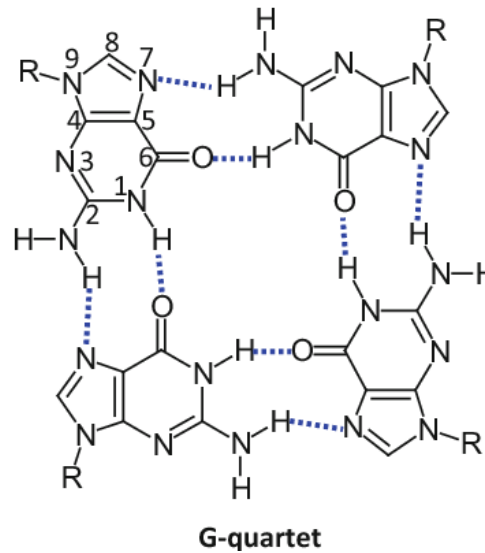
Simon Jurt

# G-Quadruplex (G4)

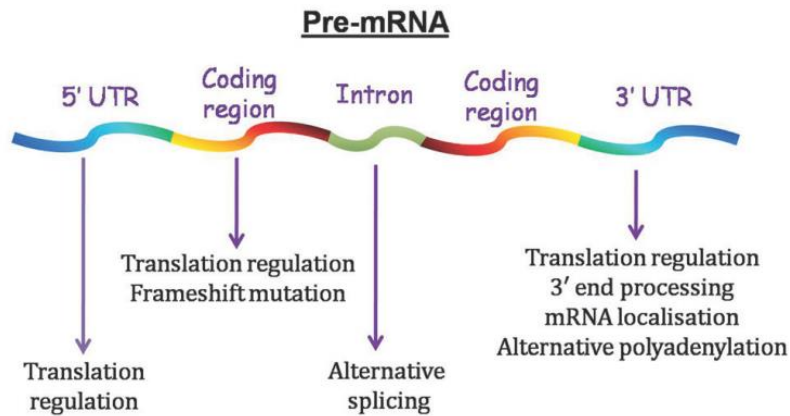
## Watson-Crick base pairing



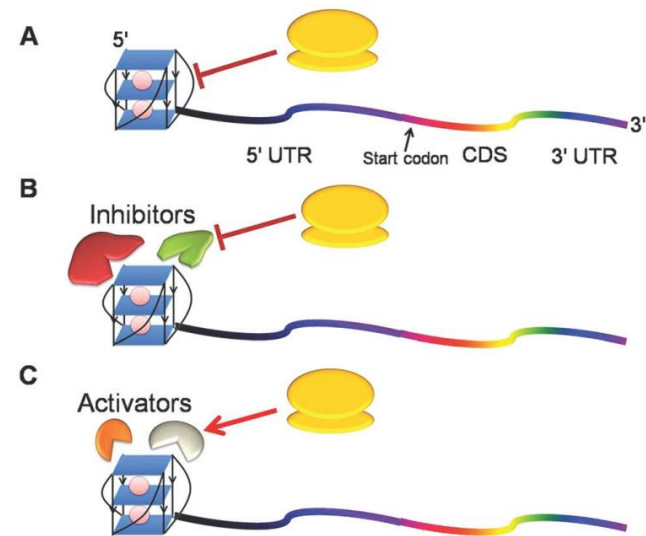
## Hoogsteen base pairing



# The role of RNA G4

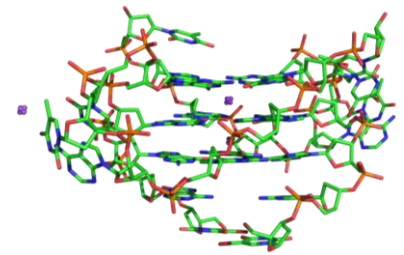
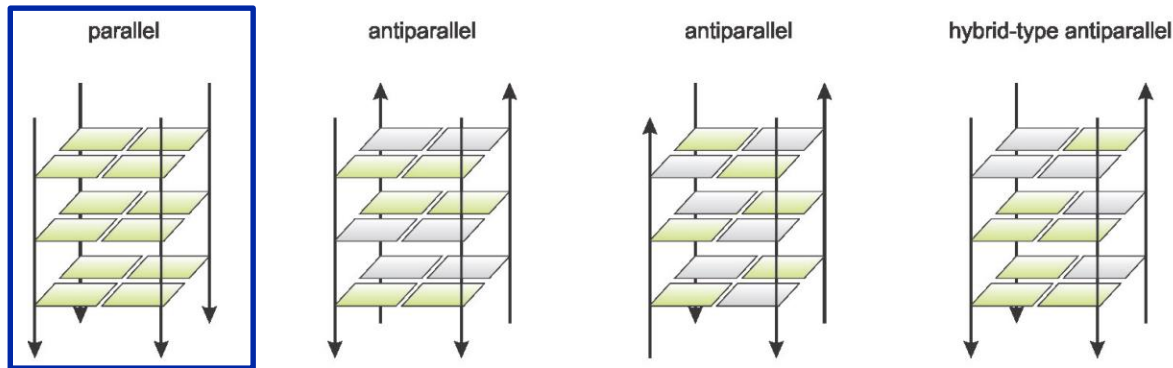


- translational regulation
- 3' end processing
- transcription termination
- alternative splicing
- mRNA localization
- protein binding
- telomeric RNA biology



Probable mechanism for regulation of gene expression by 5' UTR mRNA

# Diverse Topologies and Structures of G4

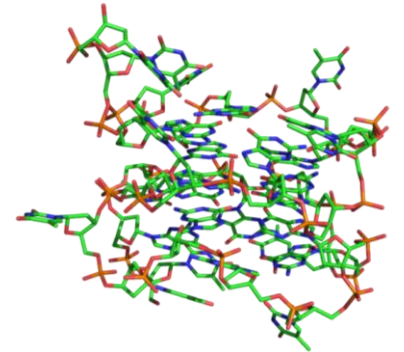
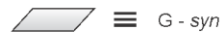
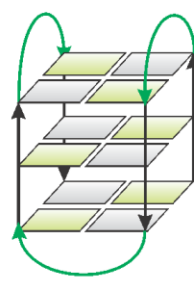
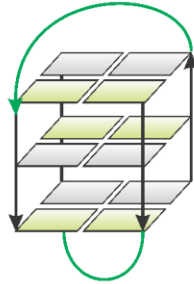
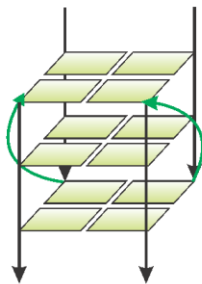


PDB : 2ms9

Double chain reversal loops

Diagonal loops

Edgewise loops



PDB : 1k8p

Gdaniec *et al.*, *Acta Biochimica Polonica*, 2016, 63, 609.





## Structural studies of RNA G4s

Sequence	PDB ID	Type	Topology	NMR/X-ray	Reference
r(UGGGGU)	1RAU	Tetramolecular	Parallel	NMR	Cheong and Moore 1992
r(UGGGGU)	1J8G	Dimer of tetramolecular G-quadruplexes	Parallel	X-ray	J. Deng, Xiong, and Sundaralingam 2001
r(UGGGGU)	4RKV 4RJ1 4RNE	Tetramolecular	Parallel	X-ray	Fyfe et al. 2015
l(TGGGT)	1S9L	Tetramolecular	Parallel	NMR	Randazzo et al. 2004
l(TGGGT)	4L0A	Tetramolecular	Parallel	X-ray	Russo Krauss et al. 2014
r(UAGGGUUAGGGU)	2KBP	Bimolecular	Parallel	NMR	Martadinata and Phan 2009
r(GGGUUAGGGU)	2M18	Dimer of bimolecular G-quadruplexes	Parallel	NMR	Martadinata and Phan 2013
r(UAGGGUUAGGGU)	3MIJ	Bimolecular			
r <sup>(Br)</sup> UAGGGUUAGGGU)	3IBK	Dimer of bimolecular G-quadruplexes			
r(GGAGGUUUUGGAGG)	1MY9	Bimolecular	Parallel	NMR	Liu et al. 2002
r(GGAGGAGGAGGA)	2RQJ	Bimolecular	Parallel	NMR	Mashima et al. 2009
d <sup>(Br)</sup> U)r(GAGGU)	1J6S	Tetramolecular	Parallel	X-ray	Pan et al. 2003
d <sup>(Br)</sup> U)r(GIGGU)	2GRB	Tetramolecular	Parallel	X-ray	Pan et al. 2006b
r(U)d <sup>(Br)</sup> G)r(AGGU)	1MDG	Dimer of tetramolecular G-quadruplexes	Parallel	X-ray	Pan et al. 2003a
r(U)d <sup>(Br)</sup> G)r(GUGU)	2AWE	Dimer of tetramolecular G-quadruplexes	Parallel	X-ray	Pan et al. 2006a
r(U)d <sup>(Br)</sup> G)r(UGGU)	1P79	Tetramolecular	Parallel	X-ray	Pan et al. 2003b

Until today, there is no unimolecular RNA G4 structure reported.



# Breast Cancer Lymphoma-2 Proto-oncogene (BCL-2)

BCL-2 wt sequence

5'- GGGGG C C G U GGGG U GGG A G C U GGG G -3'

BCL-2 short sequence

5'- GGG C C G U GGGG U GGG A G C U GGG -3'

BCL-2 6A8U17U mut

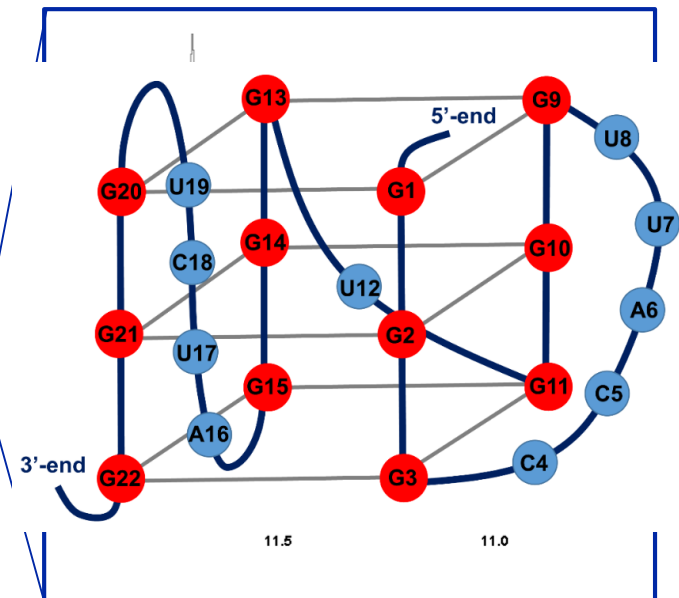
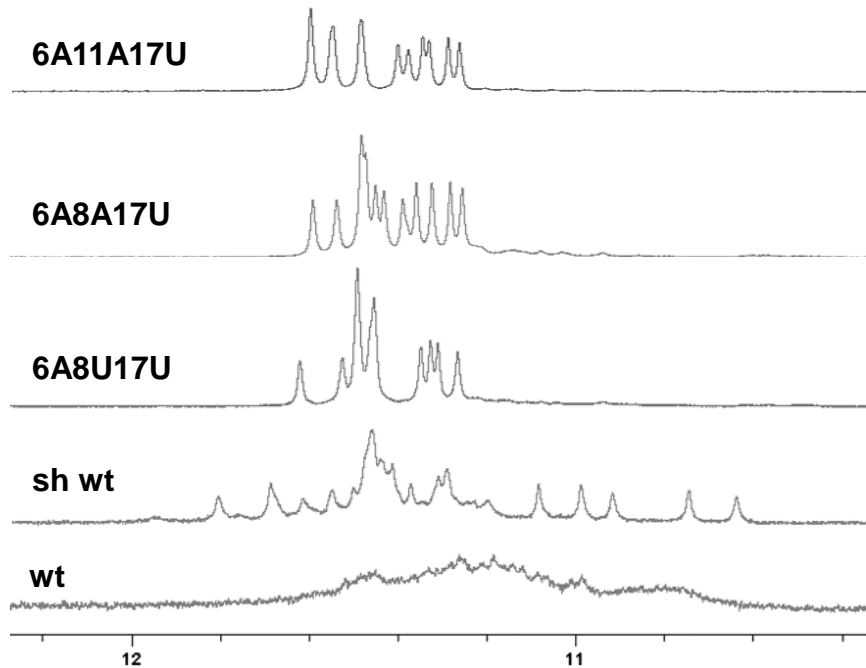
5'- GGG C C A U U GGG U GGG A U C U GGG -3'

BCL-2 6A8A17U mut

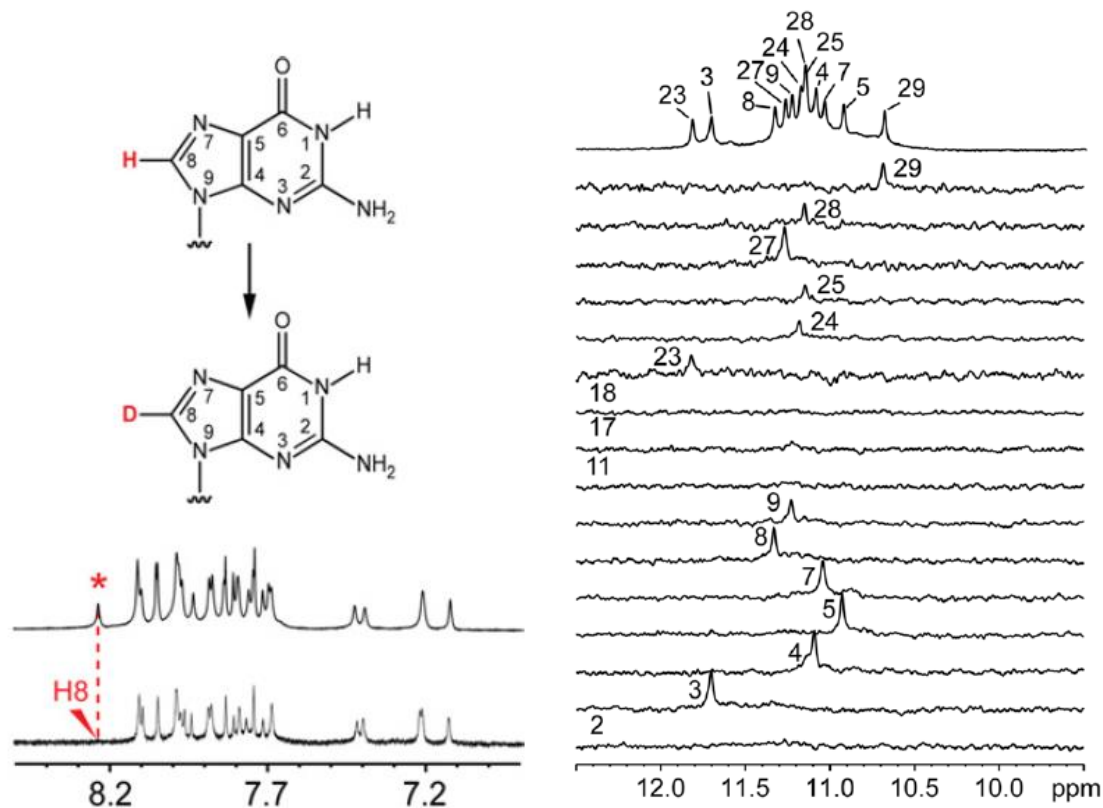
5'- GGG C C A U A GGG U GGG A U C U GGG -3'

BCL-2 6A11A17U mut

5'- GGG C C A U GGG A U GGG A U C U GGG -3'



## Most assignment strategies rely on site-specific labeling



RNA :

No site-specific labeling,

but

Nucleotide-specific  
labeling possible

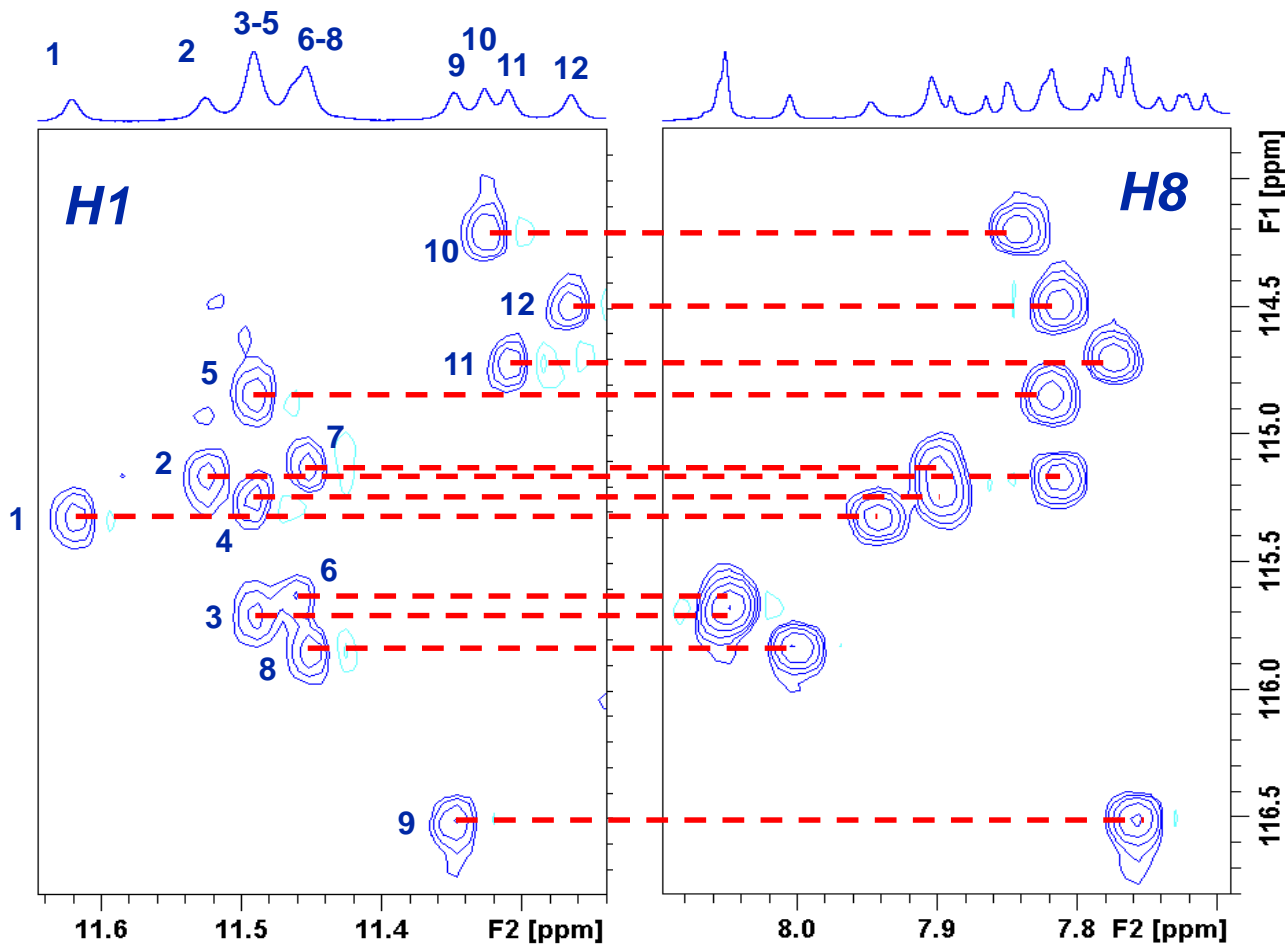
D. J. Patel *et al.*, *J. Am. Chem. Soc.*, 2002, 124, 1160

Anh Tuân Phan *et al.*, *Methods*, 2012, 57, 11

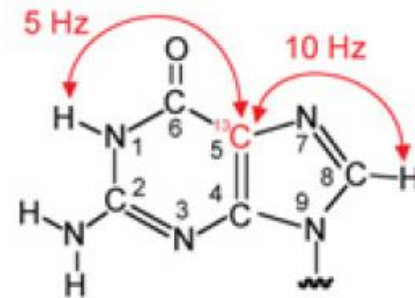
D. Yang *et al.*, *J. Am. Chem. Soc.* 2014, 136, 1750



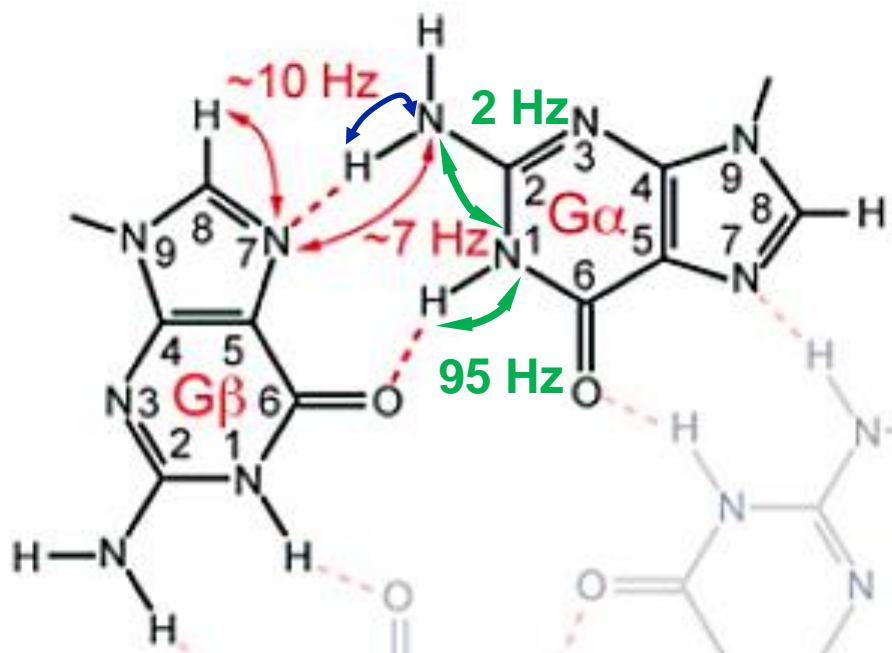
# Intra-residue H1 to H8 correlation



JR-HMBC



## Inter-residue H1 to H8 correlation



**HNN-COSY-H8N2:**

$H8(G\beta)$  to  $N7(G\beta)$   
 $N7(G\beta)$  to  $N2(G\alpha)$

**$^1H$ ,  $^{15}N$ -COSY:**

$H2(G\alpha)$  to  $N2(G\alpha)$

**HNN-COSY-H1N2:**

$H1(G\alpha)$  to  $N1(G\alpha)$   
 $N1(G\alpha)$  to  $N2(G\alpha)$

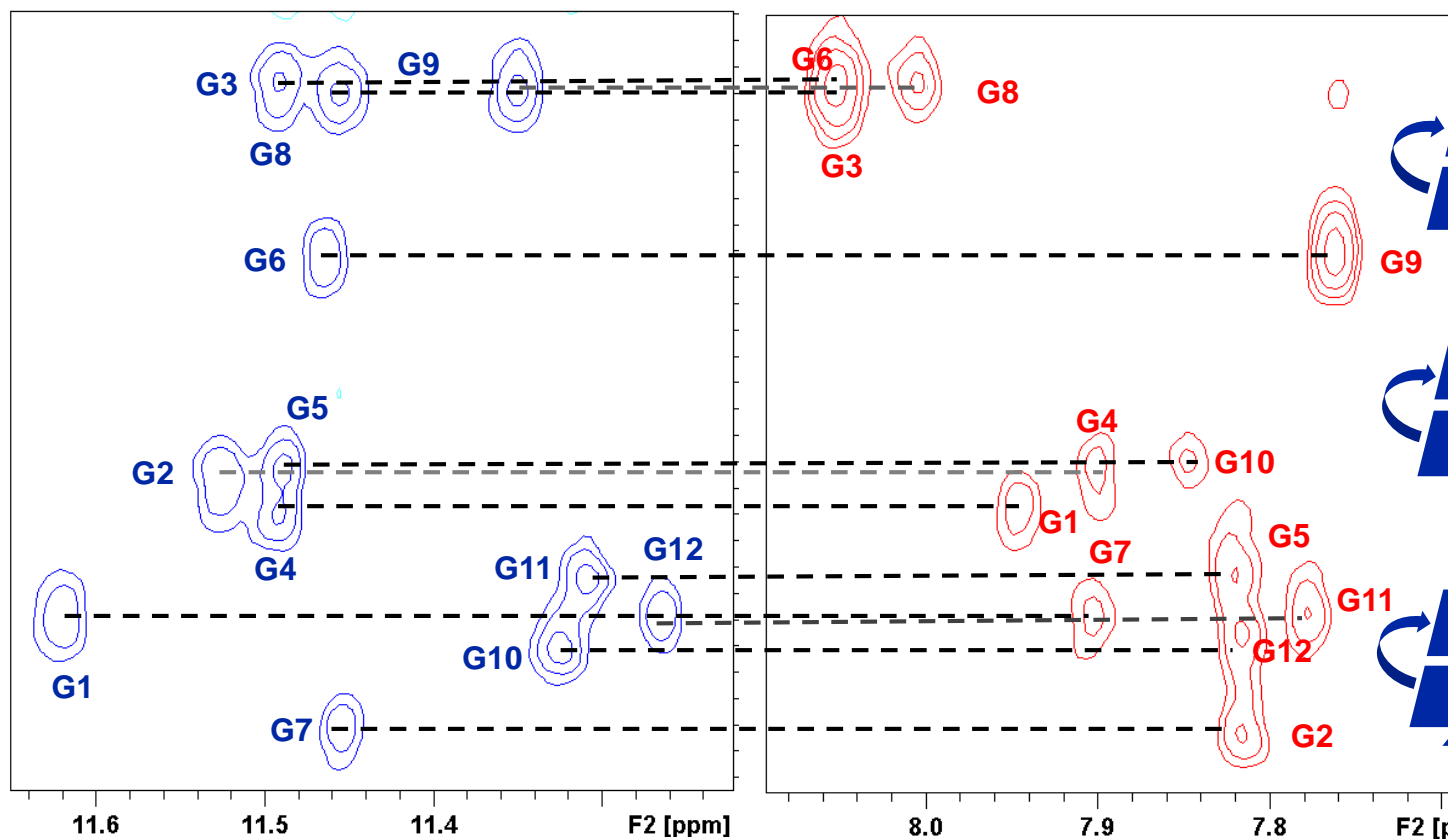
Adapted from D. J. Patel et al., *J. Biomol. NMR*, 1999, 15, 207.



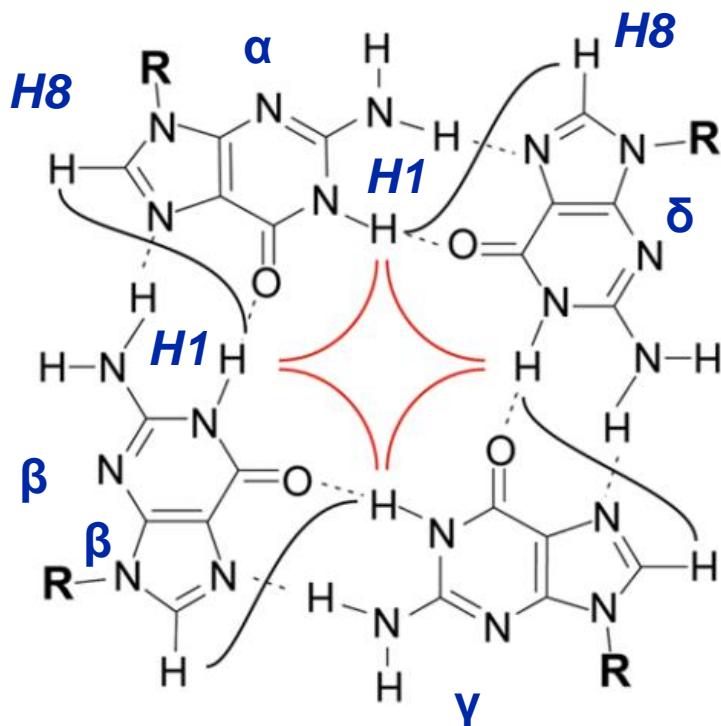
# Inter-residue H1 to H8 correlation

H1 of G $\alpha$  *HNN-COSY-H1N2*

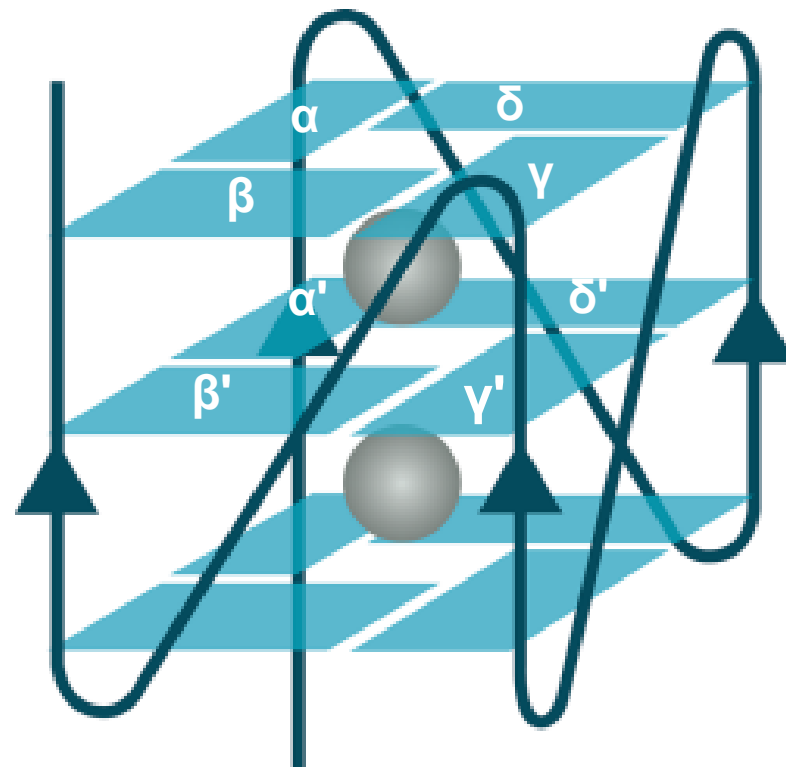
H8 of G $\beta$  *HNN-COSY-H8N2*



## H1-H8 NOE along the arrangement of G-quartet

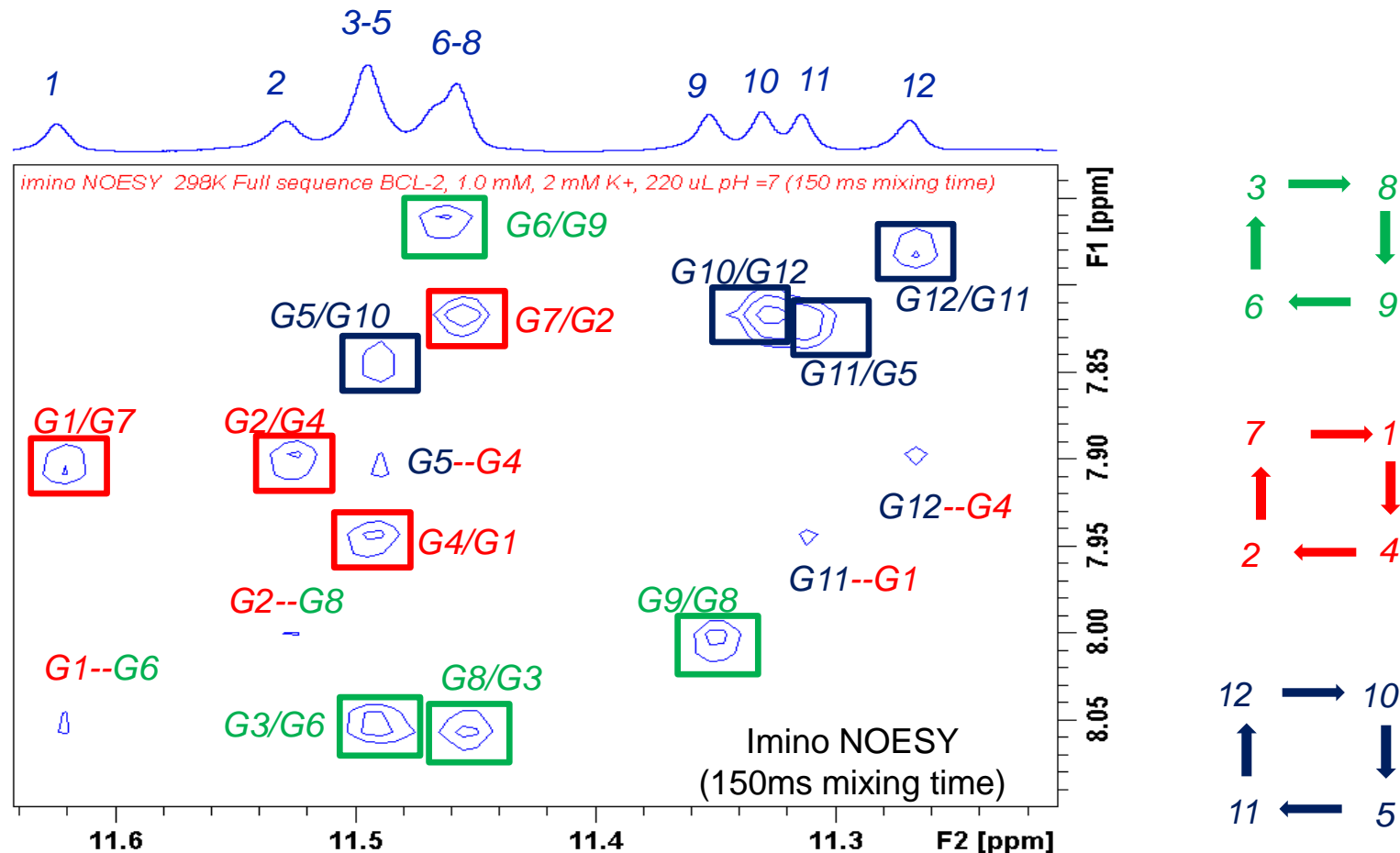


Stronger:  $\alpha$  to  $\beta$   
Weaker:  $\alpha$  to  $\beta'$



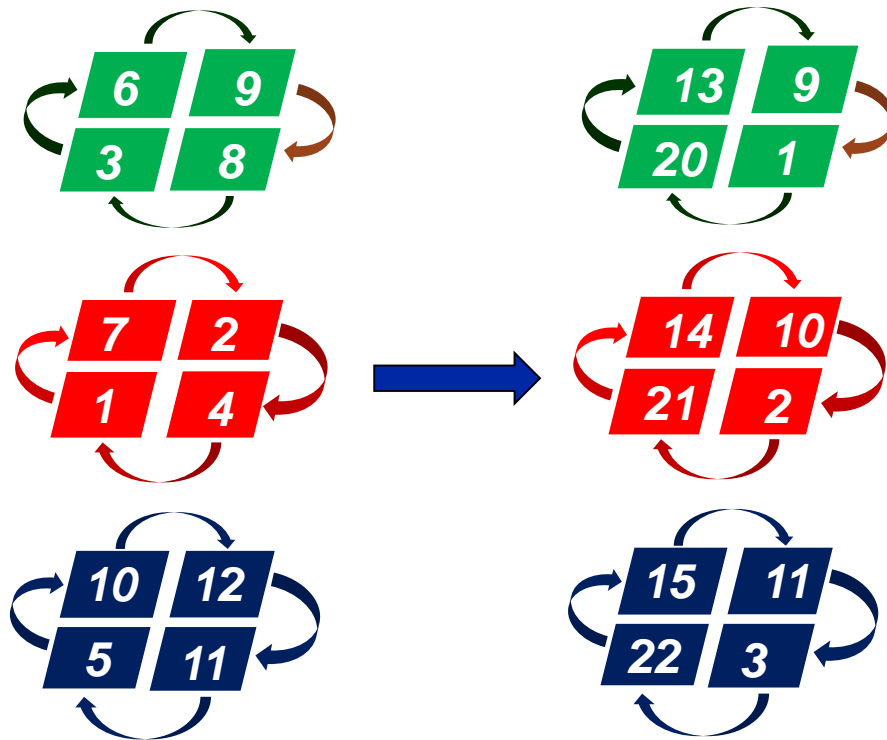


# H1-H8 NOE along the arrangement of G-quartet

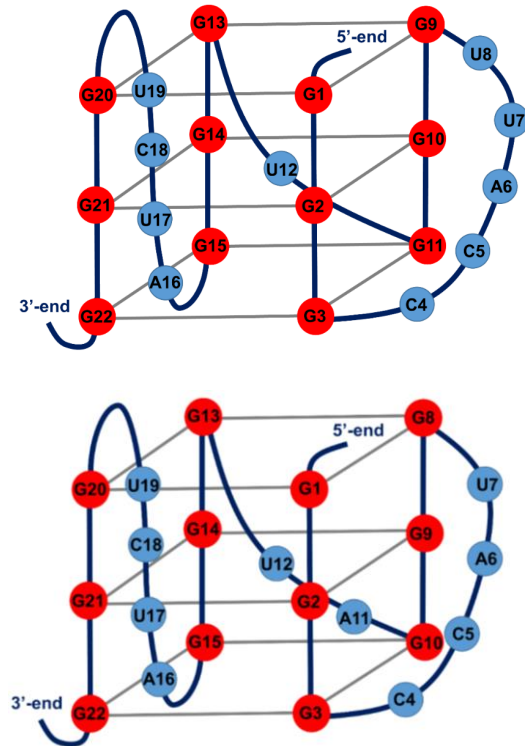




## Assignment of RNA G4s



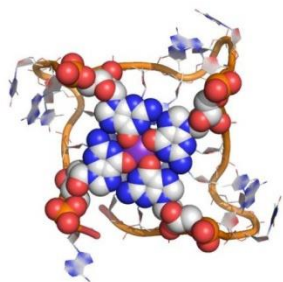
6A8U17U Mutant





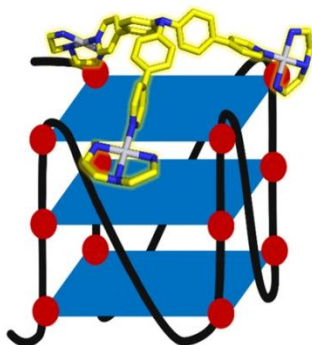
## Next steps and further directions

### Determination of the G4 structures



- First structure of unimolecular RNA G4
- Structure / dynamics of the loops
- Apply general strategy to more mutants

### Interaction of metal- complexes with G4



- Investigation of complex binding
- Structure G4 / complex



# Acknowledgements

## Sigel-Group



## Financial support



FONDS NATIONAL SUISSE  
SCHWEIZERISCHER NATIONALFONDS  
FONDO NAZIONALE SVIZZERO  
SWISS NATIONAL SCIENCE FOUNDATION



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