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# **Viroids & the RNA World: from genomic scale (RNA) to atomic scale (ribozyme)**

Fabrice Leclerc

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# Viroids & the RNA World

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# Viroids

# &

# the RNA World

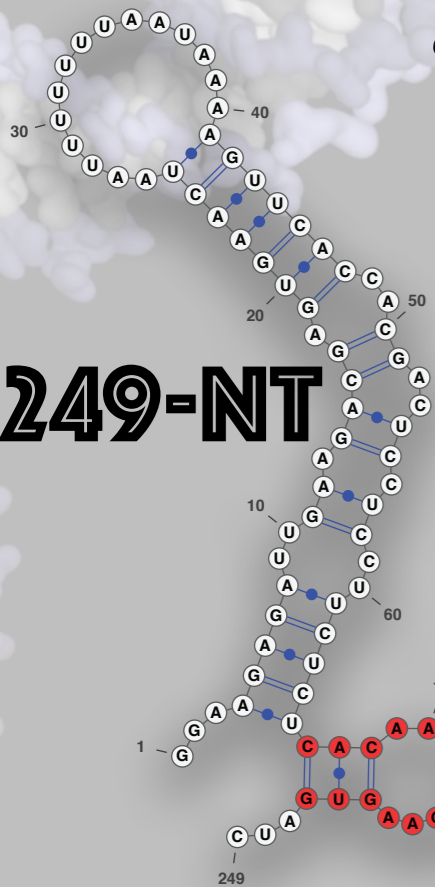
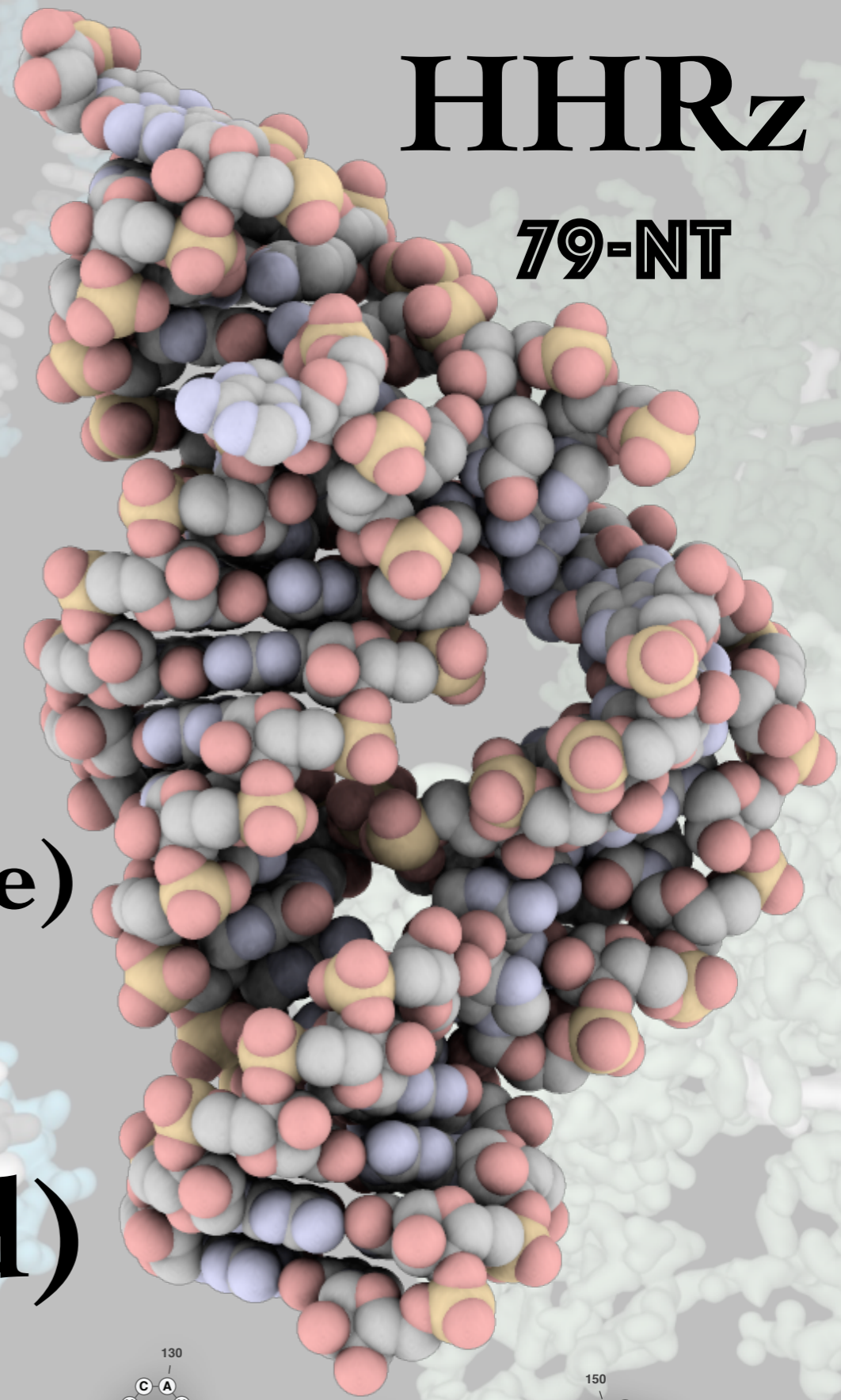
## from genomic scale (RNA)

## to

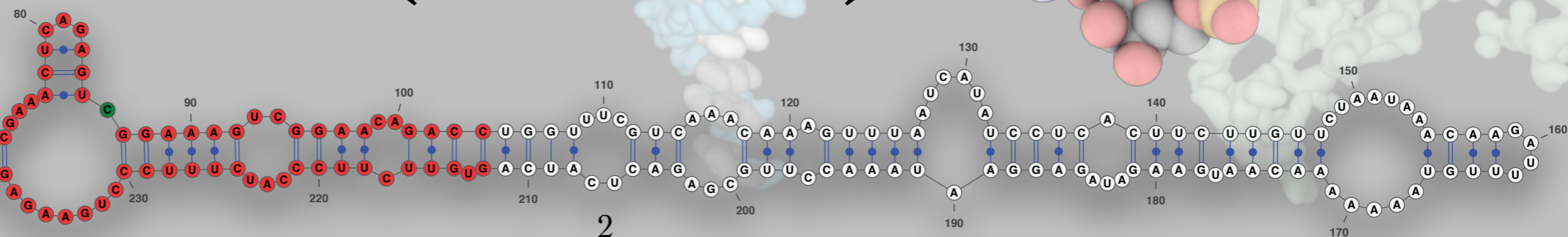
## atomic scale (ribozyme)

# HHRz

# 79-NT



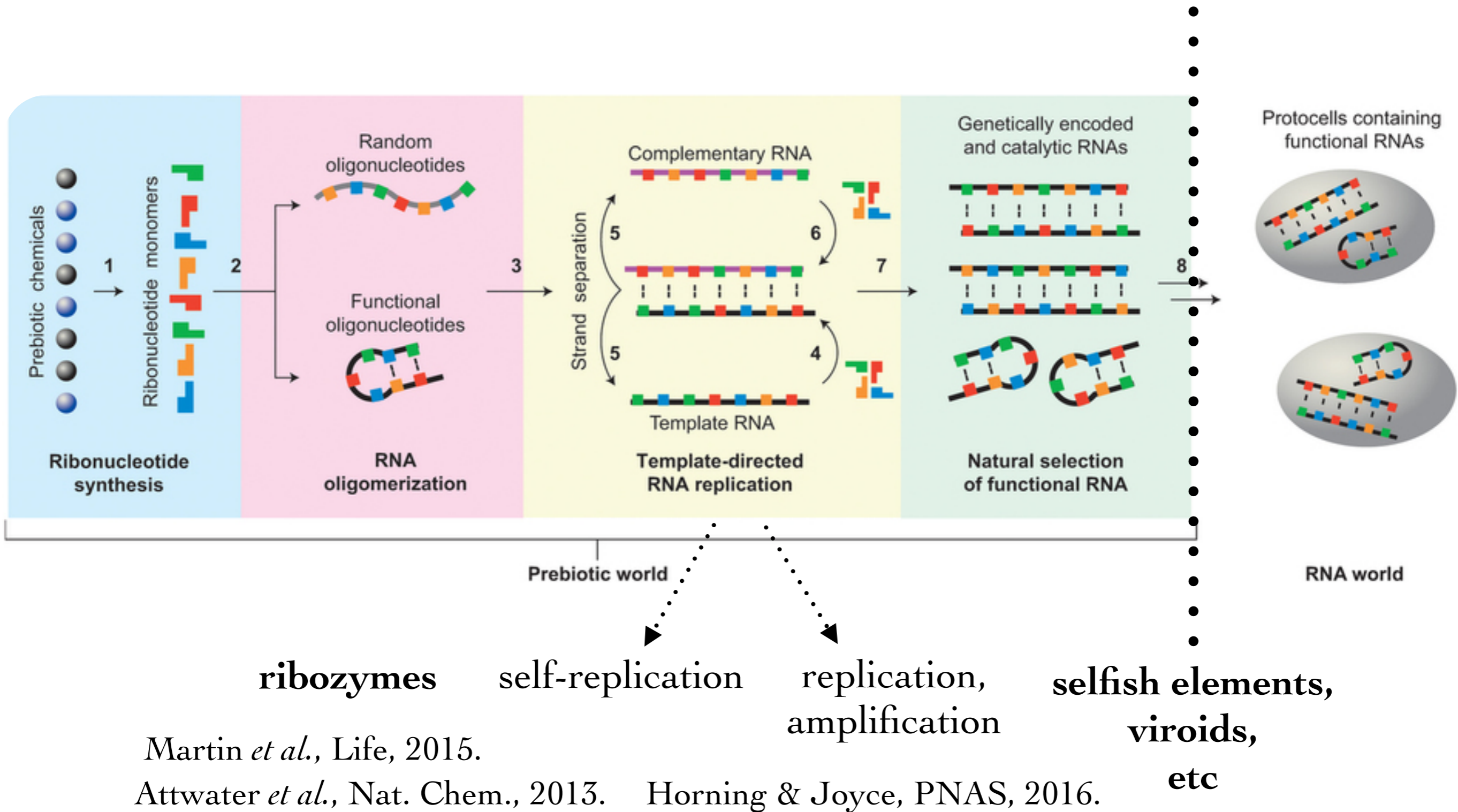
# Viroid (ASBVd)



# Introduction

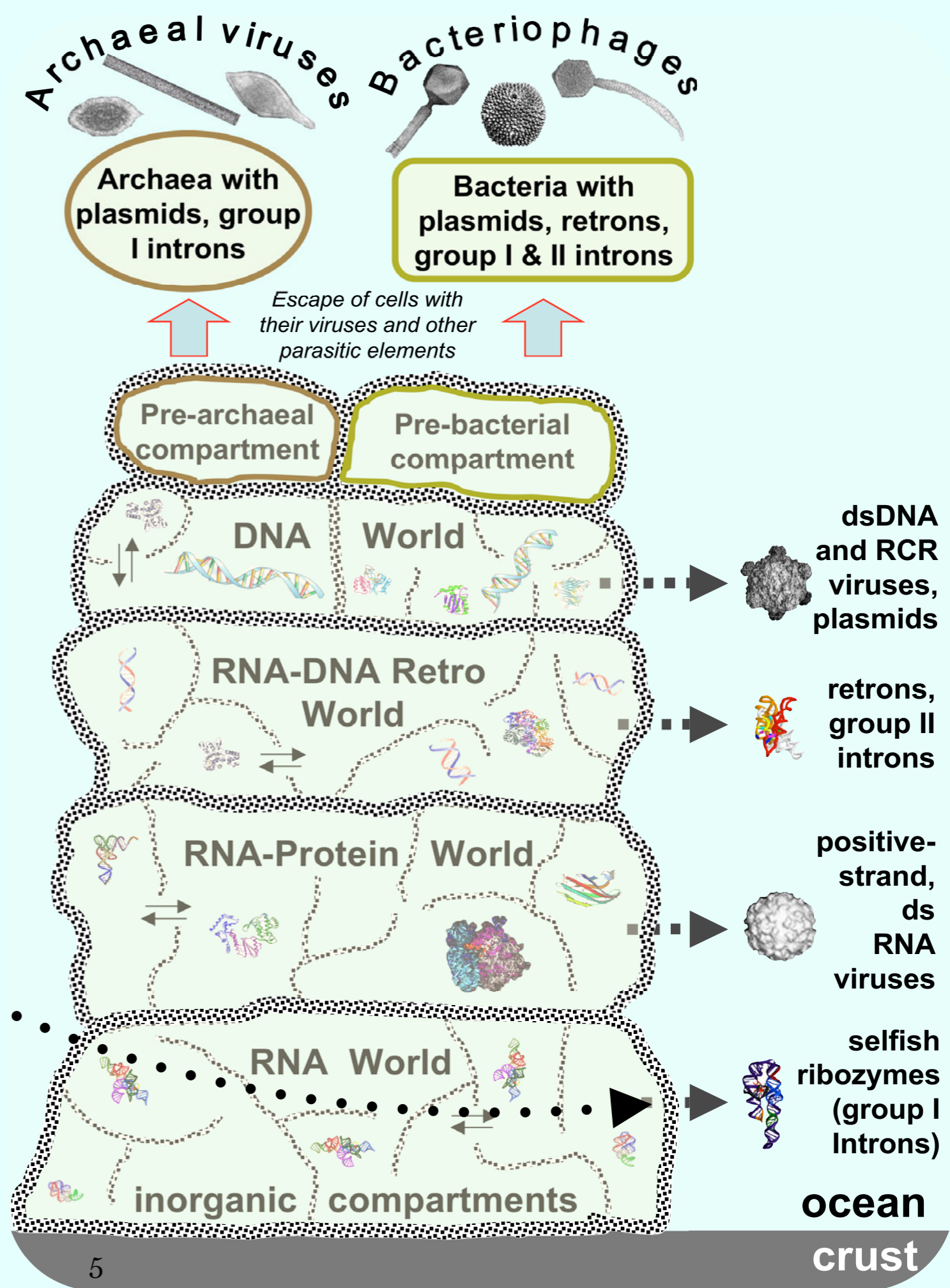
- evolution (RNA world) & genomics
- RNA biology & RNomics  
(molecular and cellular functions)
- structural biology & structural  
bioinformatics (structural basis for  
functions)
- enzymology & computational enzymology  
(catalysis)

# Prebiotic & RNA Worlds



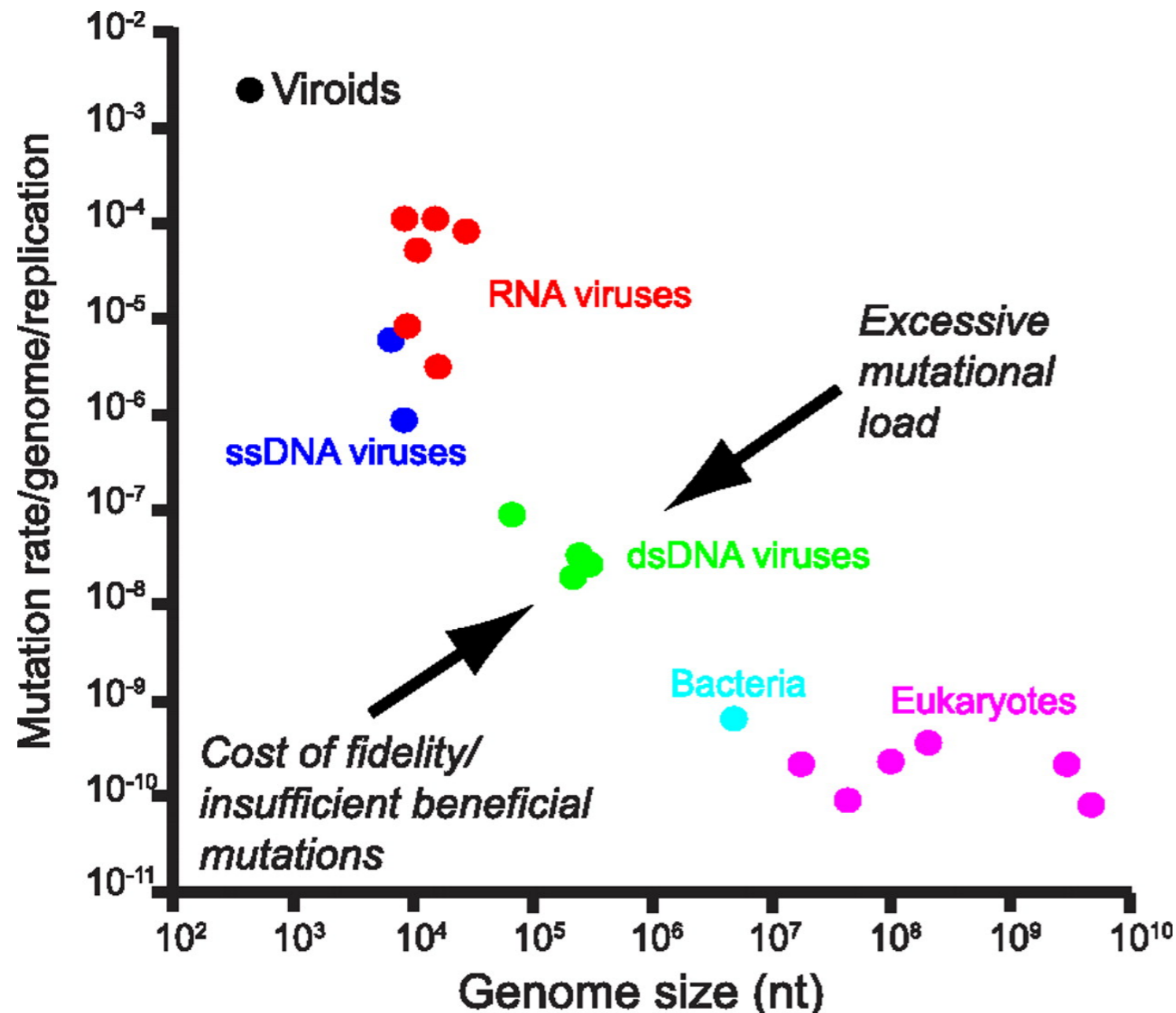
# Virus World & RNA World

viroids



« The ancient Virus World and evolution of cells »

# Viroids: « survivors from the RNA World »



Holmes, J. Virol., 2011

## functions

- error-prone replication

- small size

- high GC

- replication fidelity

- circRNA

- replication

- periodicity

- genome assembly

- no protein-coding

- ribosome-free

- ribozyme

## features

- replication

# Viroids: Families

FAMILY

SUBFAMILY

GENUS

SPECIES

*Pospiviroidae*

*Pospiviroinae*

*Pospiviroids*

**Potato spindle tuber (PSTVd)**  
 Mexican papita (MPVd)  
 Tomato planta macho (TPMVd)  
 Chrysanthemum stunt (CSVd)  
 Citrus exocortis (CEVd)  
 Tomato apical stunt (TASVd)  
 Iresine 1 (IrVd 1)  
 Columnea latent (CLVd)

*Hostuviroids*

**Hop stunt (HSVd)**

*Cocadviroids*

**Coconut cadang-cadang (CCCVd)**  
 Coconut tinangaja (CTIVd)  
 Hop latent (HLVd)  
 Citrus IV (CVd-IV)

*Apscaviroinae*

*Apscaviroids*

**Apple scar skin (ASSVd)**  
 Citrus III (CVd-III)  
 Apple dimple fruit (ADFVd)  
 Grapevine yellow speckle 1 (GYSVd 1)  
 Grapevine yellow speckle 2 (GYSVd 2)  
 Citrus bent leaf (CBLVd)  
 Pear blister canker (PBCVd)  
 Australian grapevine (AGVd)

*Coleviroinae*

*Coleviroids*

**Coleus blumei 1 (CbVd 1)**  
 Coleus blumei 2 (CbVd 2)  
 Coleus blumei 3 (CbVd 3)

*Avsunviroidae*

Flores *et al.*,  
 Arch. Virol.,  
 1998.

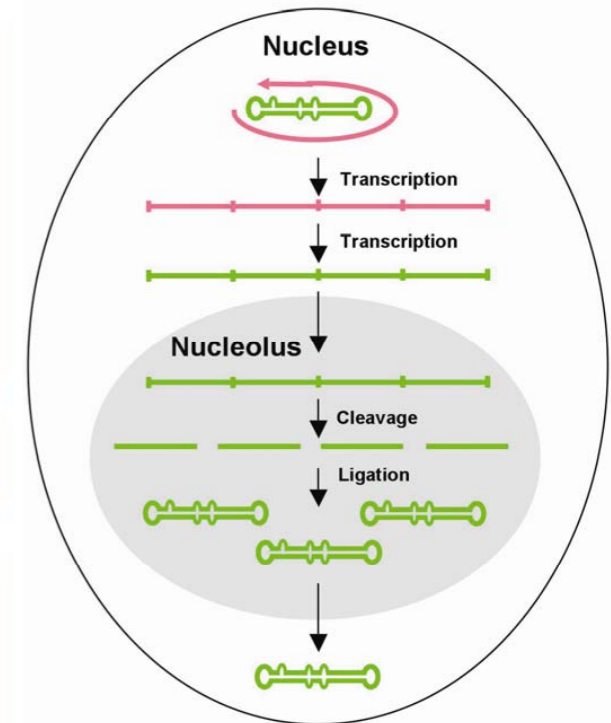
*Avsunviroids*

**Avocado sunblotch (ASBVd)**

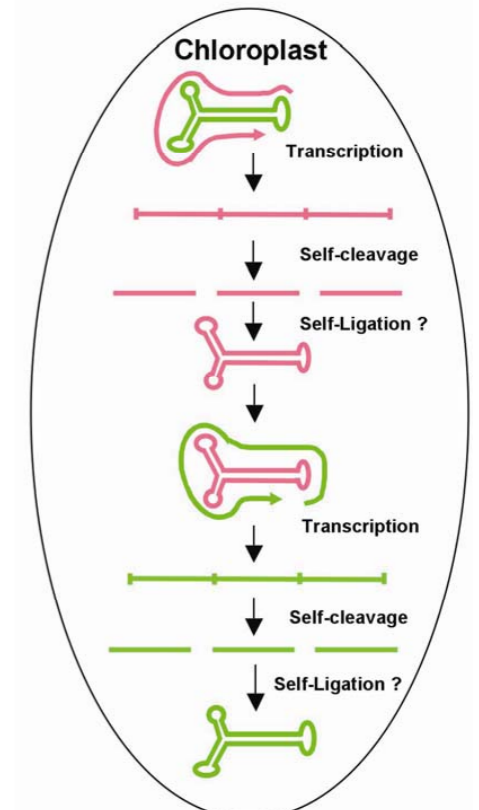
*Pelamoviroids*

**Peach latent mosaic (PLMVd)**  
 Chrysanthemum chlorotic mottle (CChMVd)

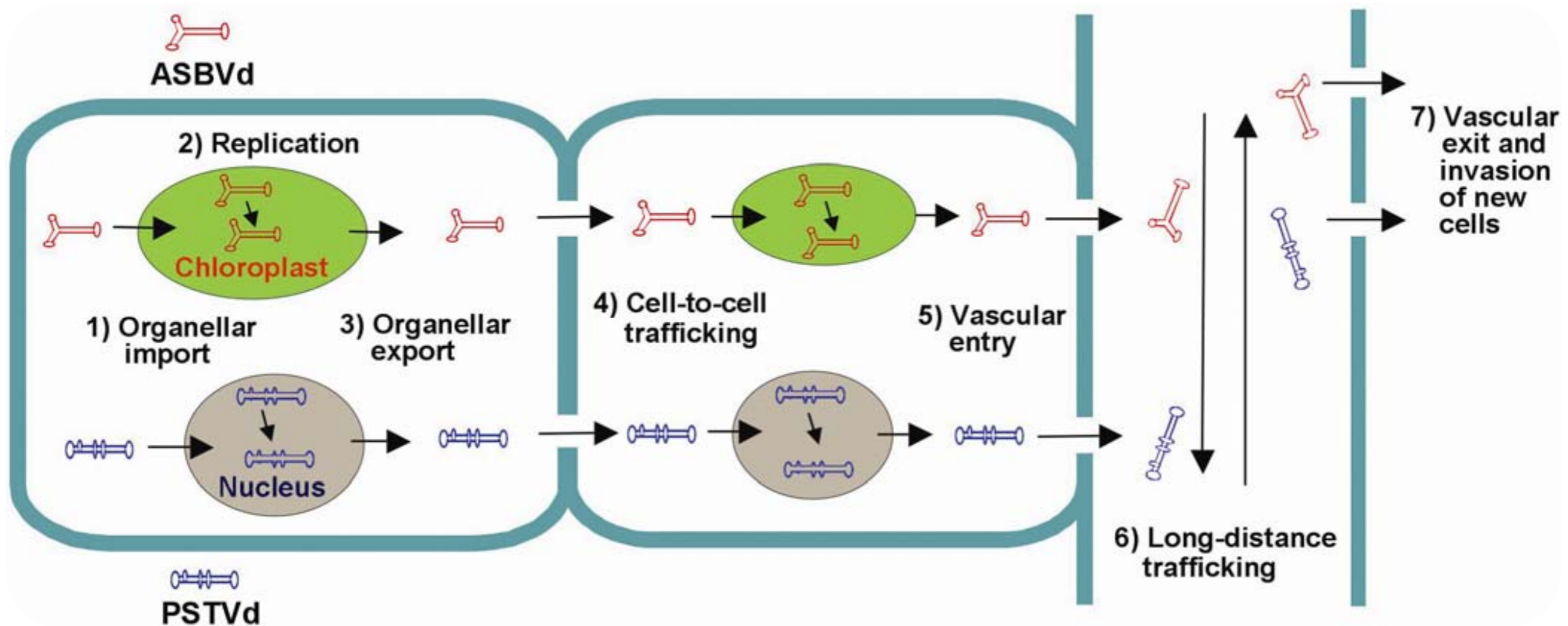
PSTVd



ASBVd



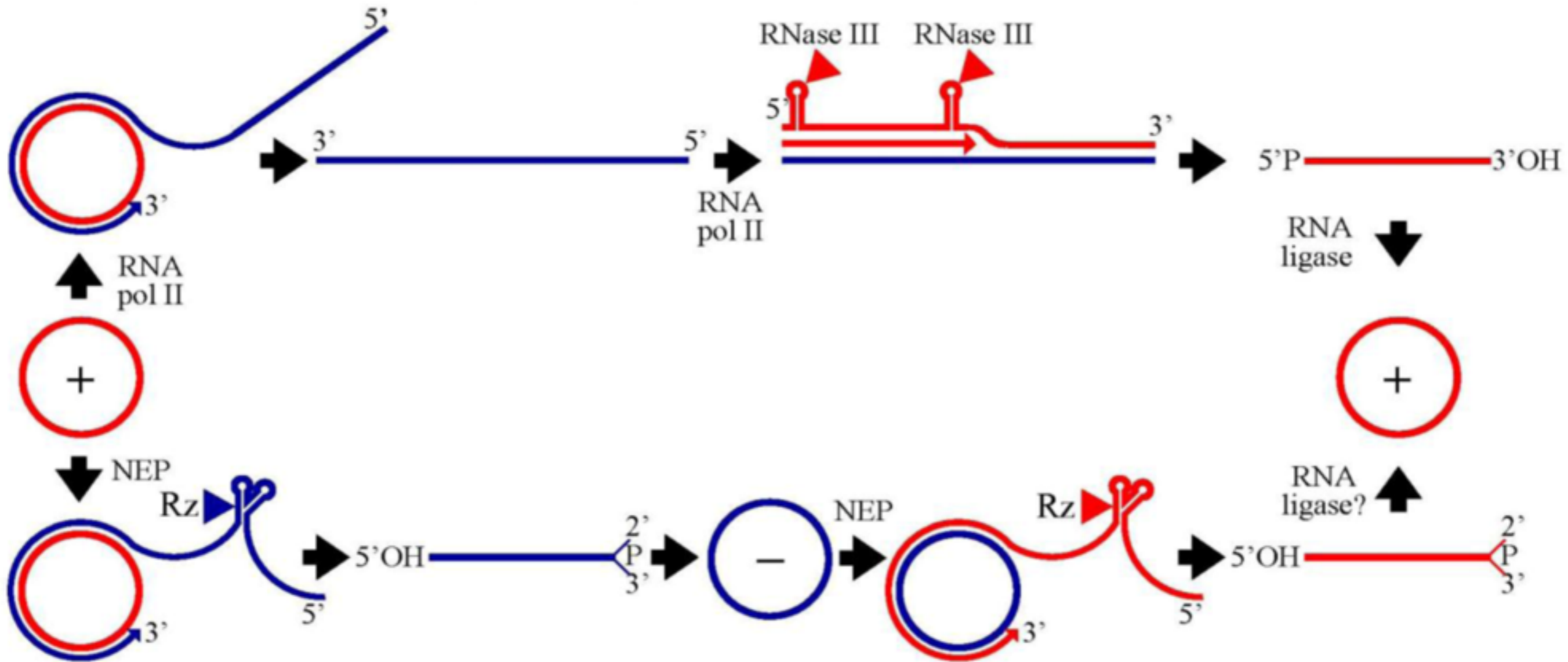
# Viroids: Plant Parasites



Ding & Itaya, Mol Plant Microbe Interact, 2007.

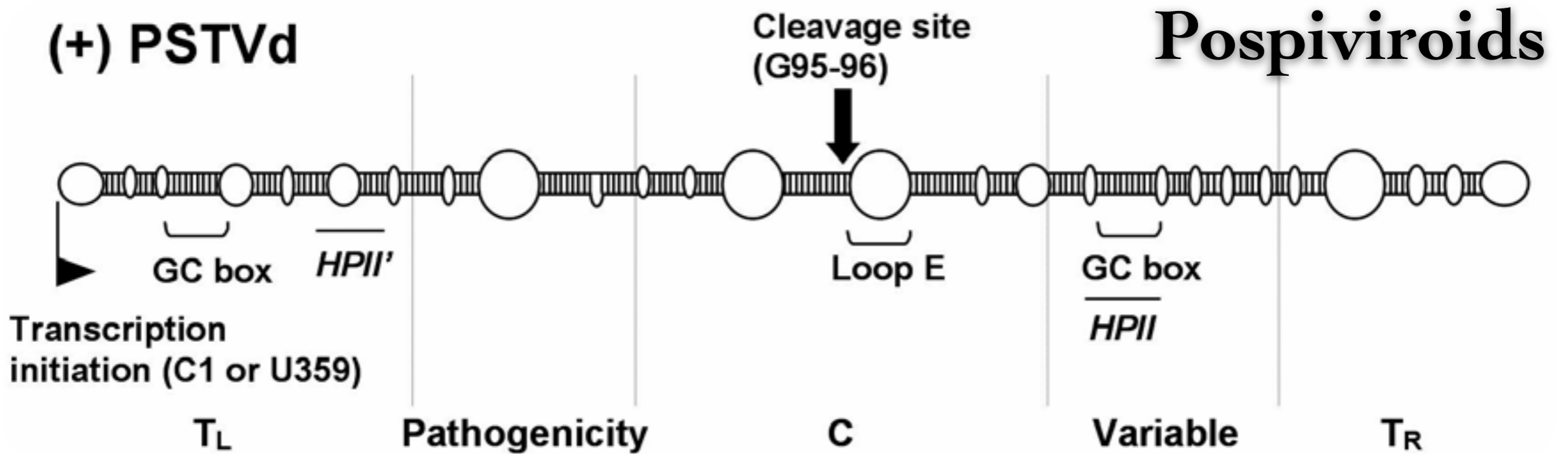
# Viroids: Replication

Asymmetric pathway (family *Pospiviroidae*)

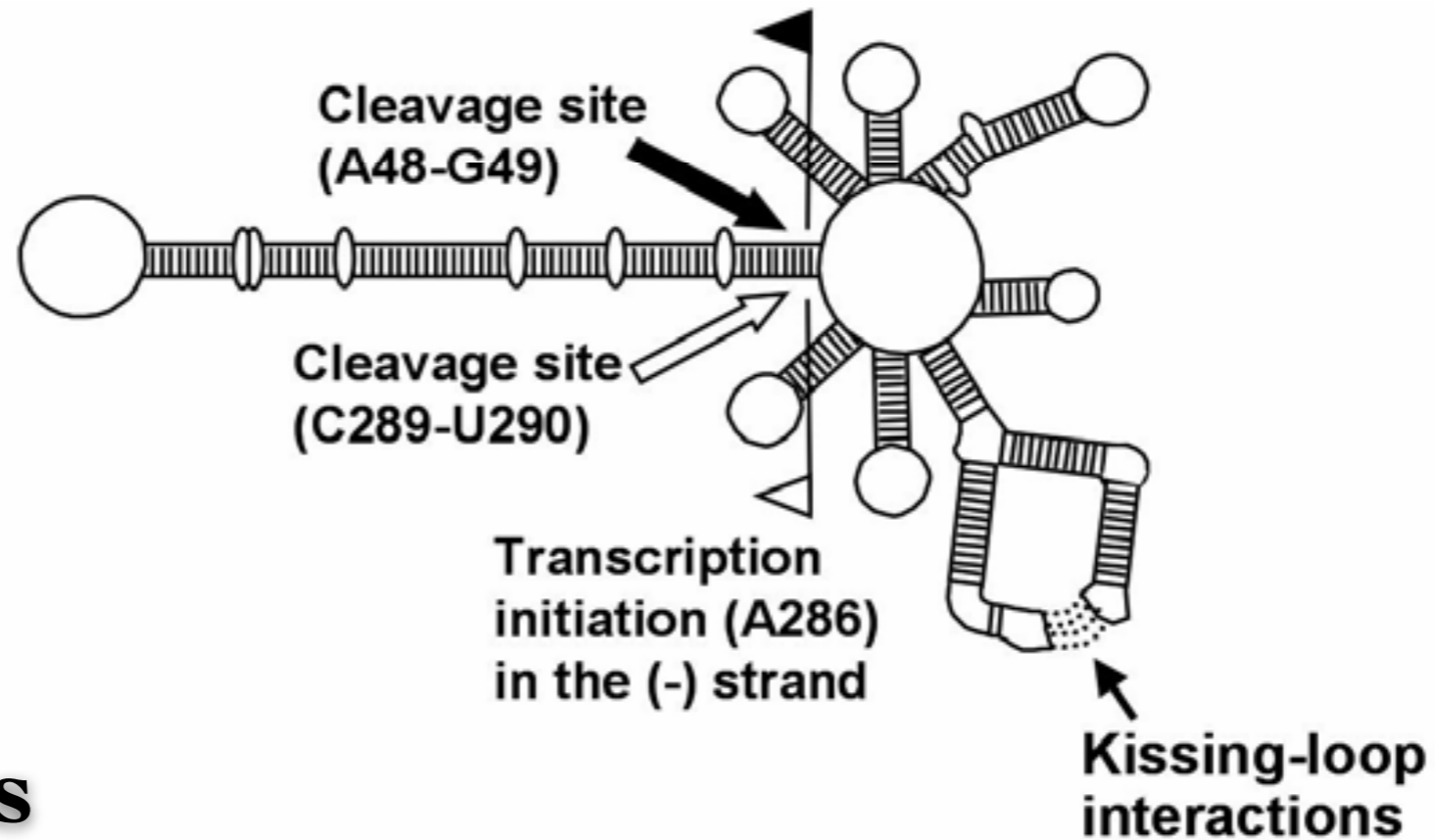


Rz: hammerhead ribozyme

# Viroids: 2D Structures



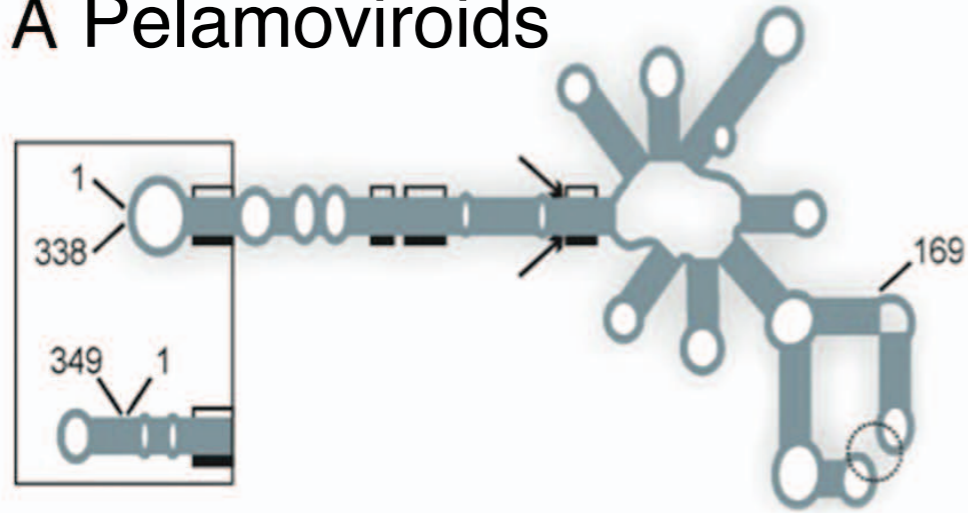
**(+) PLMVd**





# Viroids: Avsunviroids

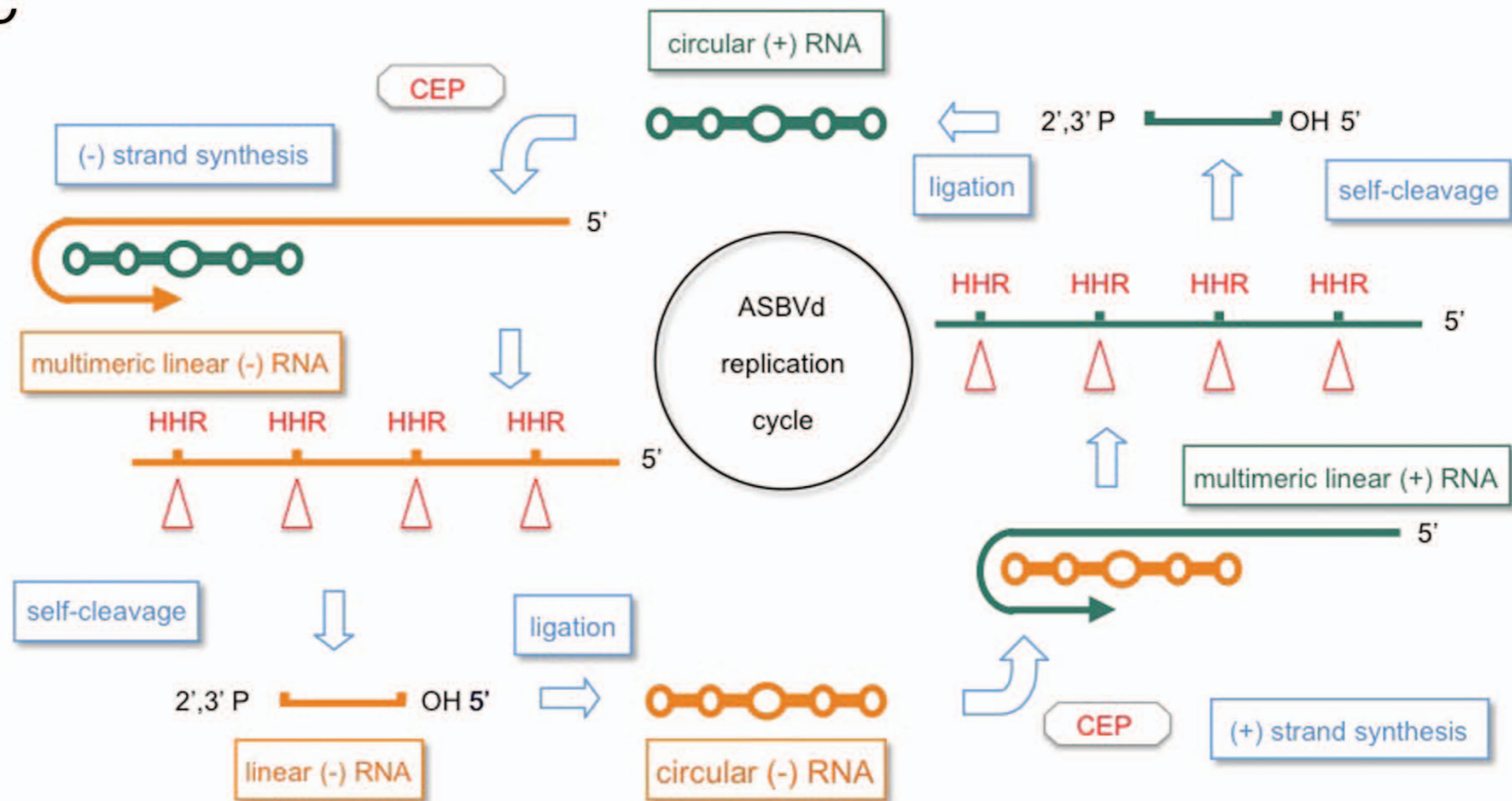
A Pelamoviroids



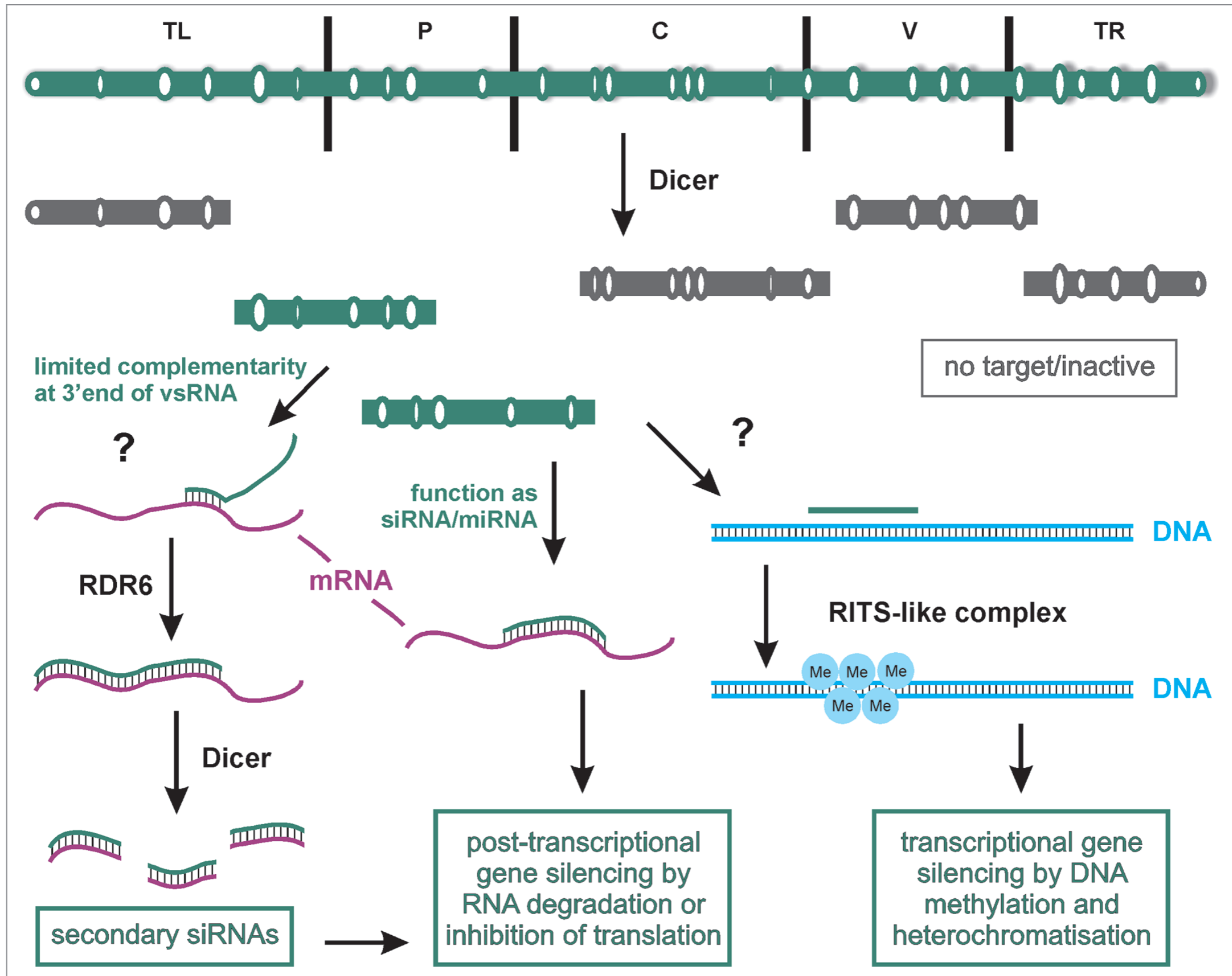
B Avsunviroids



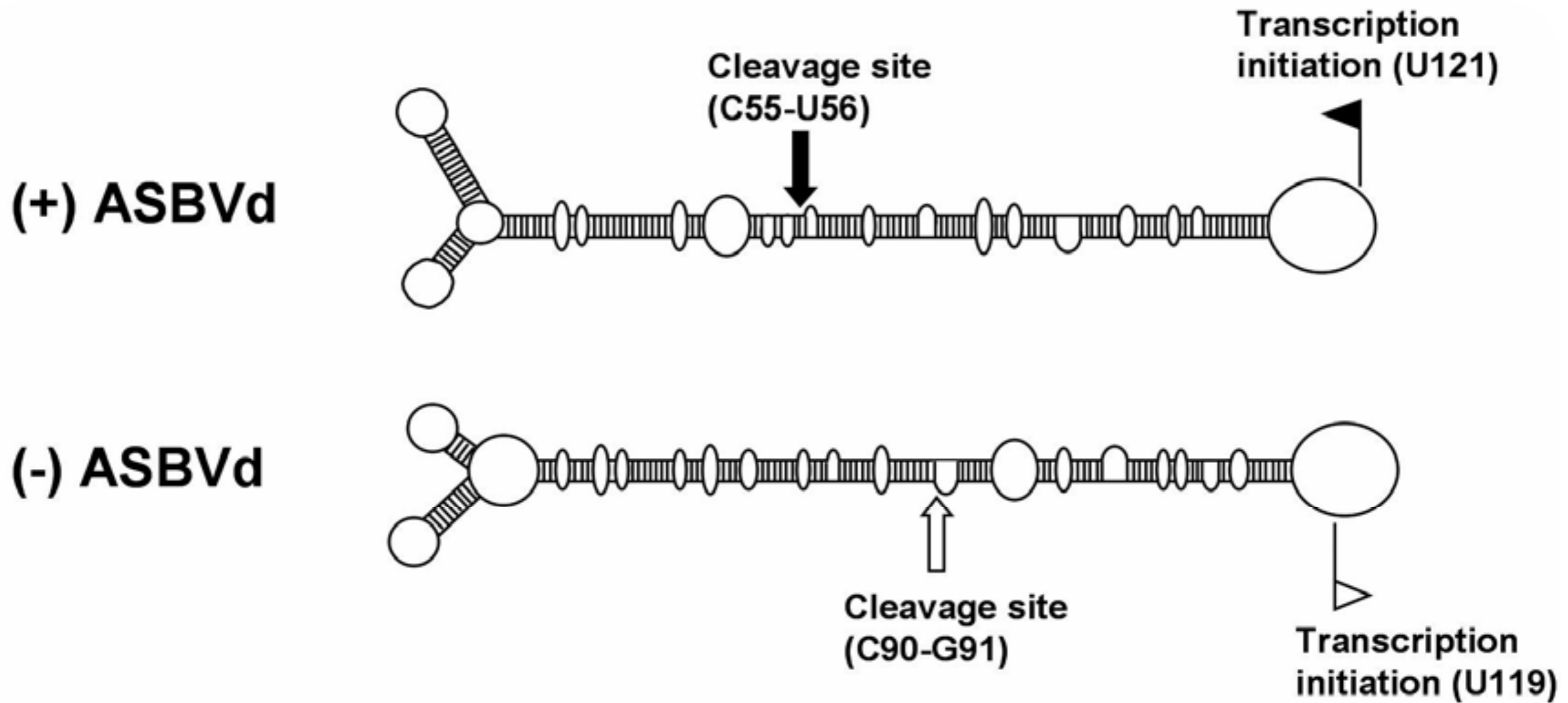
C



# Viroids & RNA silencing

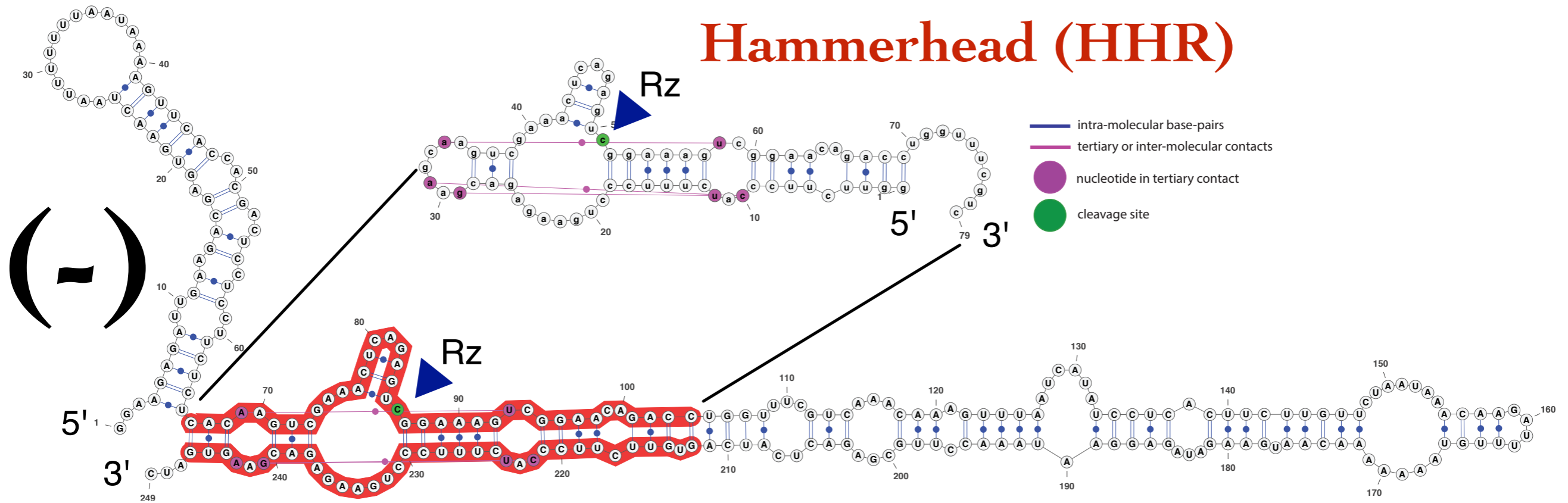


# ASBVd: 2D Structures



Ding & Itaya, Mol Plant Microbe Interact, 2007.

# ASBVd(-): 2D Structures

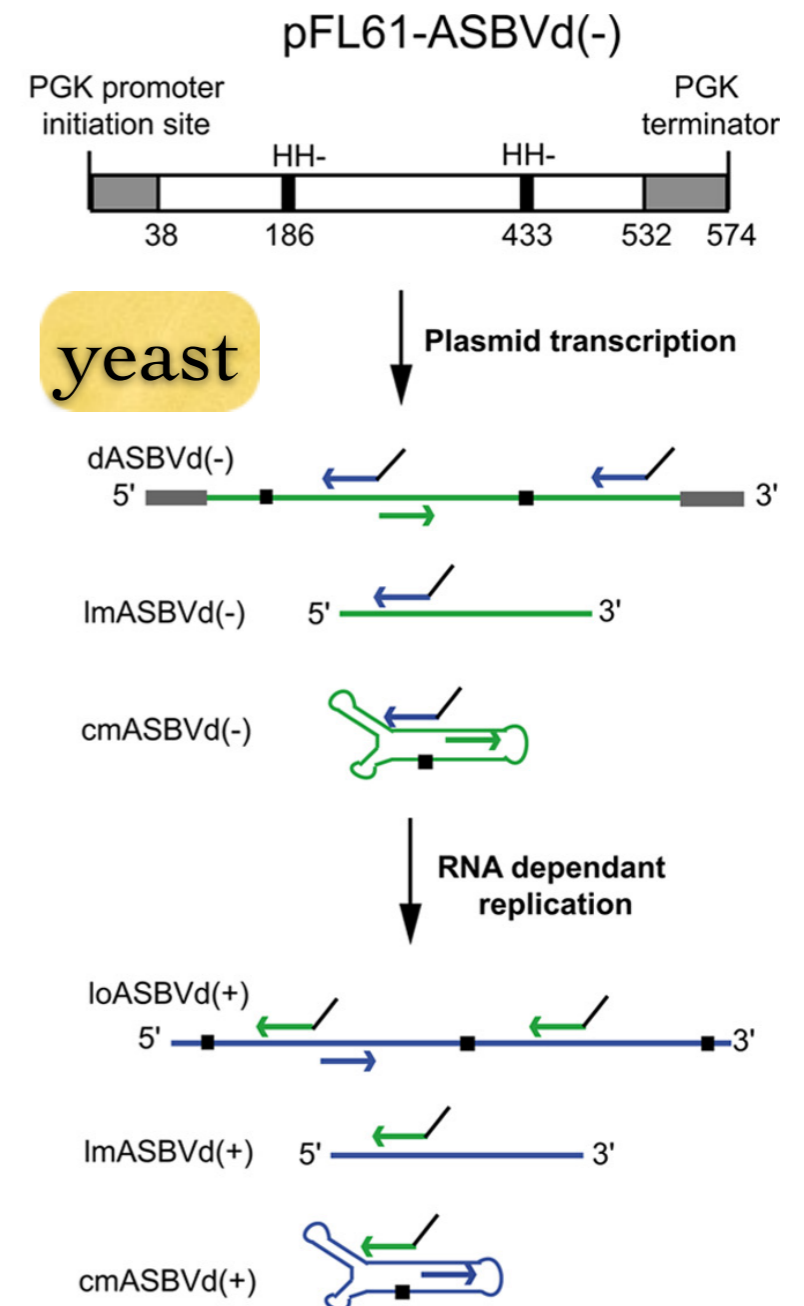
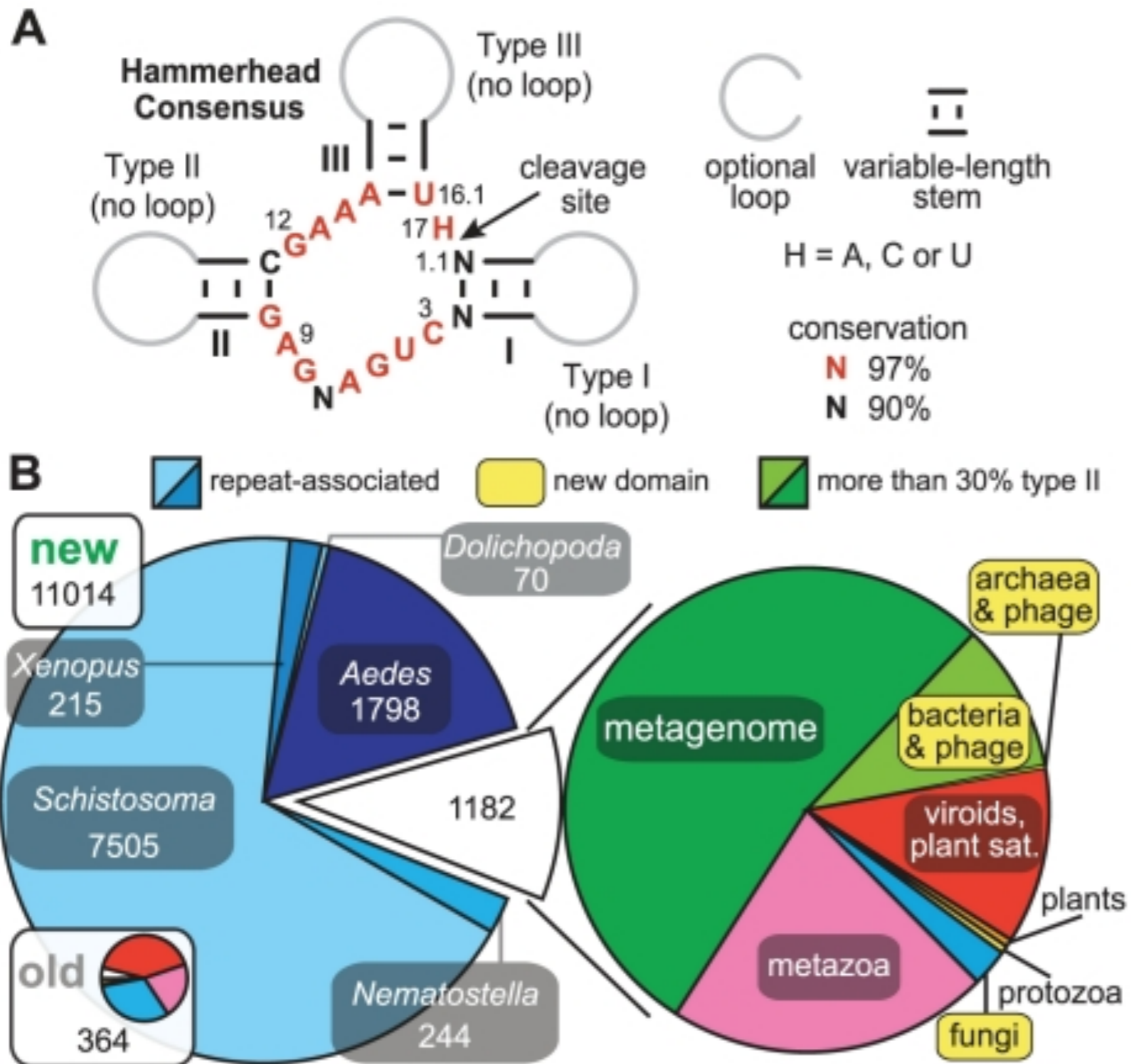


Leclerc *et al.*, Sci. Rep., 2016.



Flores *et al.*, Adv, Virus Res., 2000.

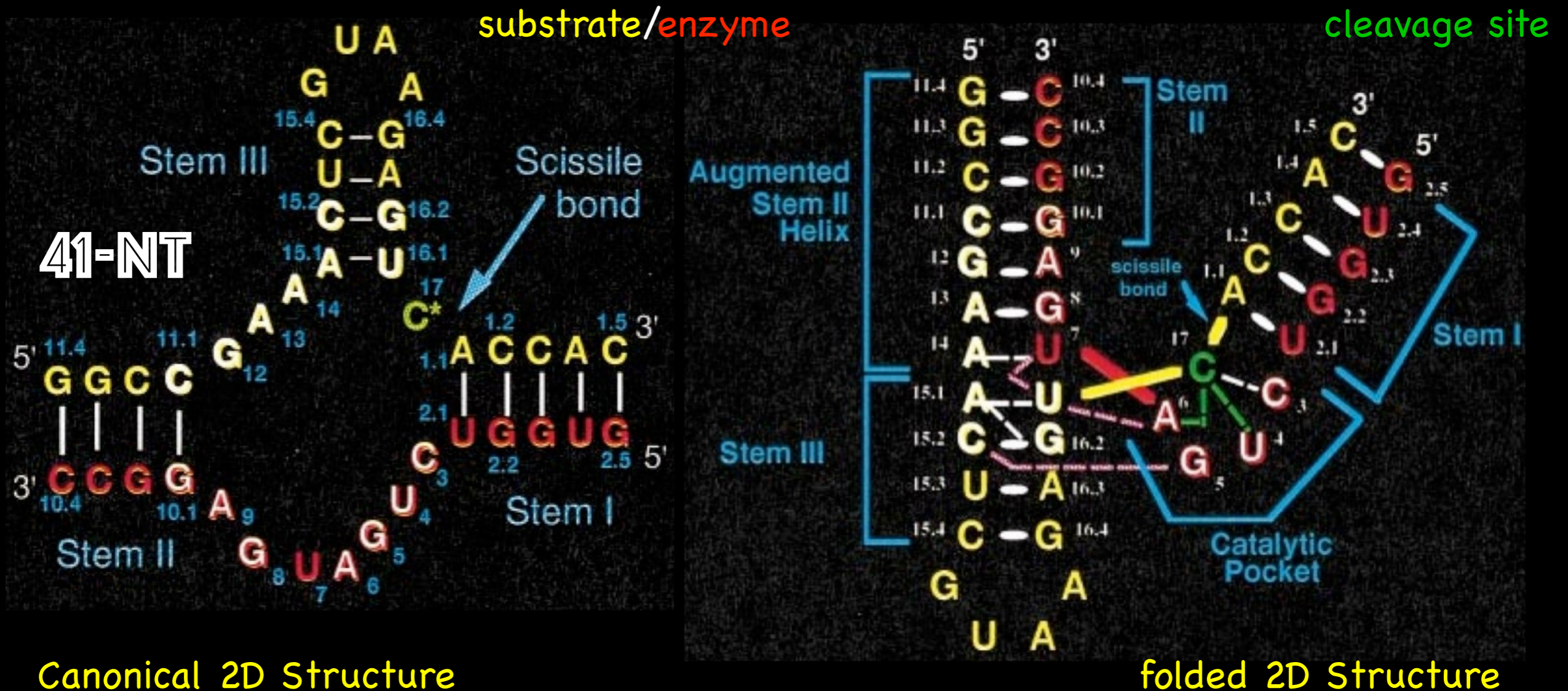
# HHH motifs in life forms



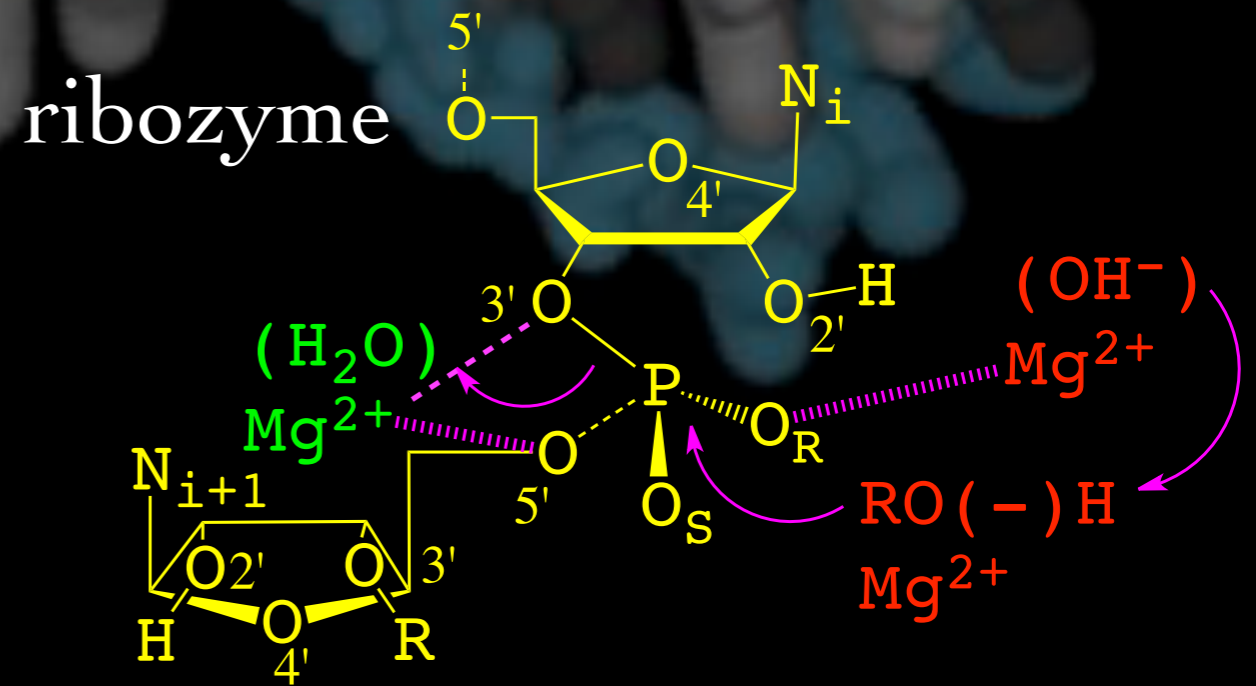
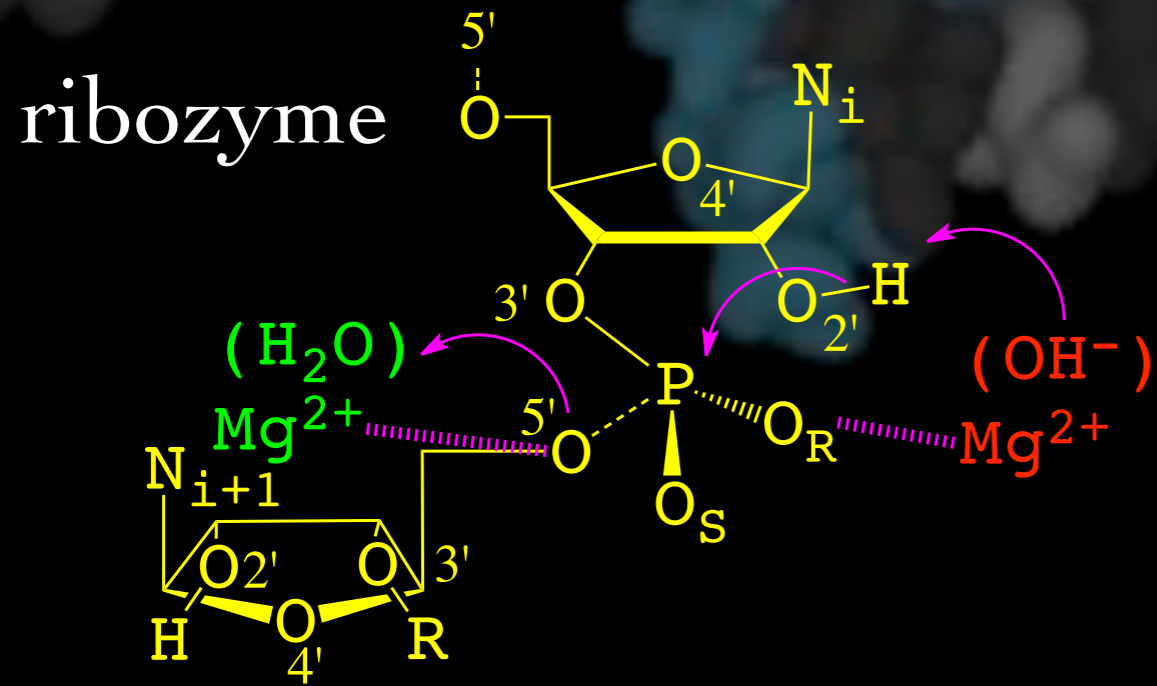
Delan-Forino *et al.*,  
 J. Virol., 2011.  
 Perreault *et al.*,  
 PLoS Comput. Biol., 2011  
 Gupta & Swati,  
 Interdiscip. Sci., 2016.

# A prototype for RNA Catalysis

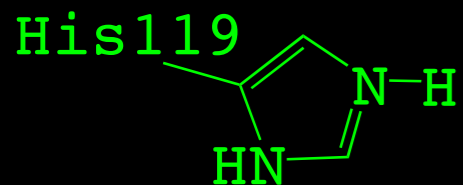
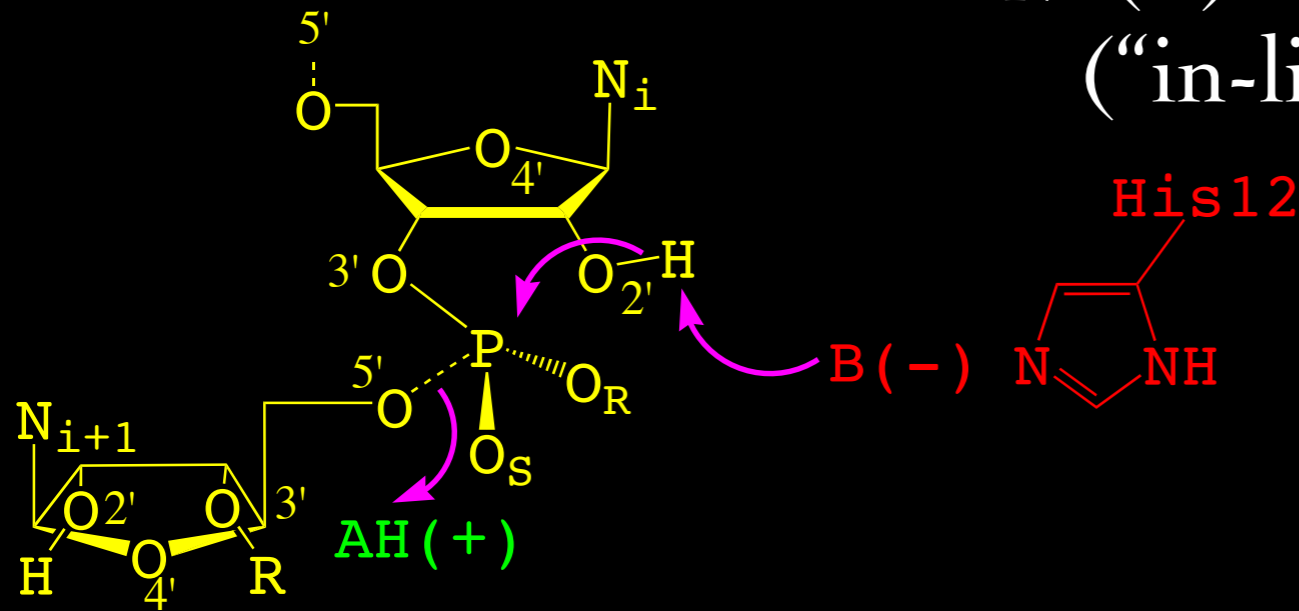
“Minimum” Hammerhead Ribozyme



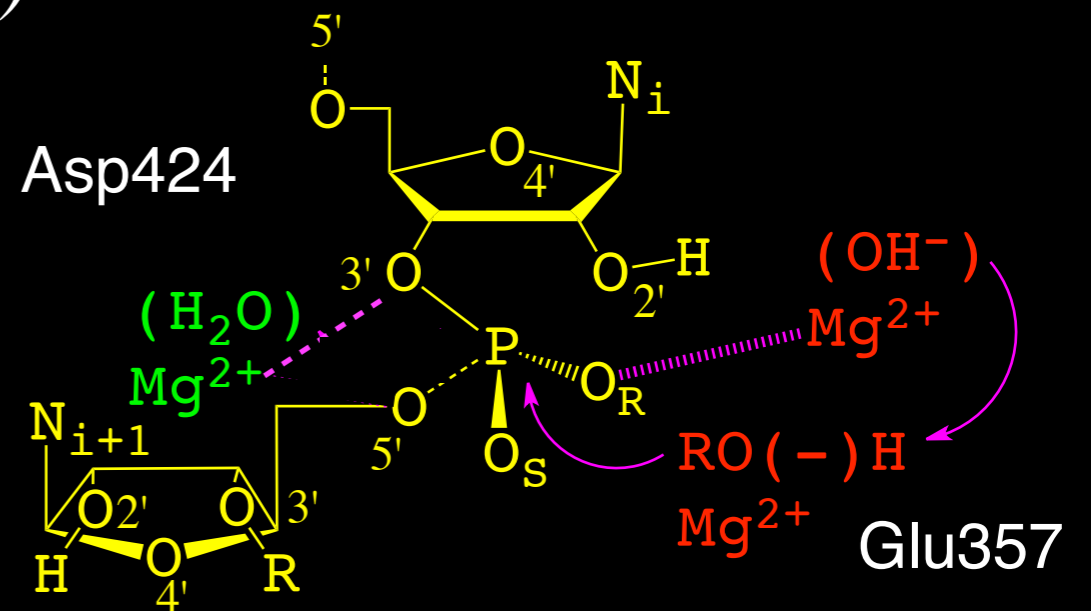
# Self-Cleaving / Self-Splicing



S<sub>N</sub>2(P) reaction  
("in-line")



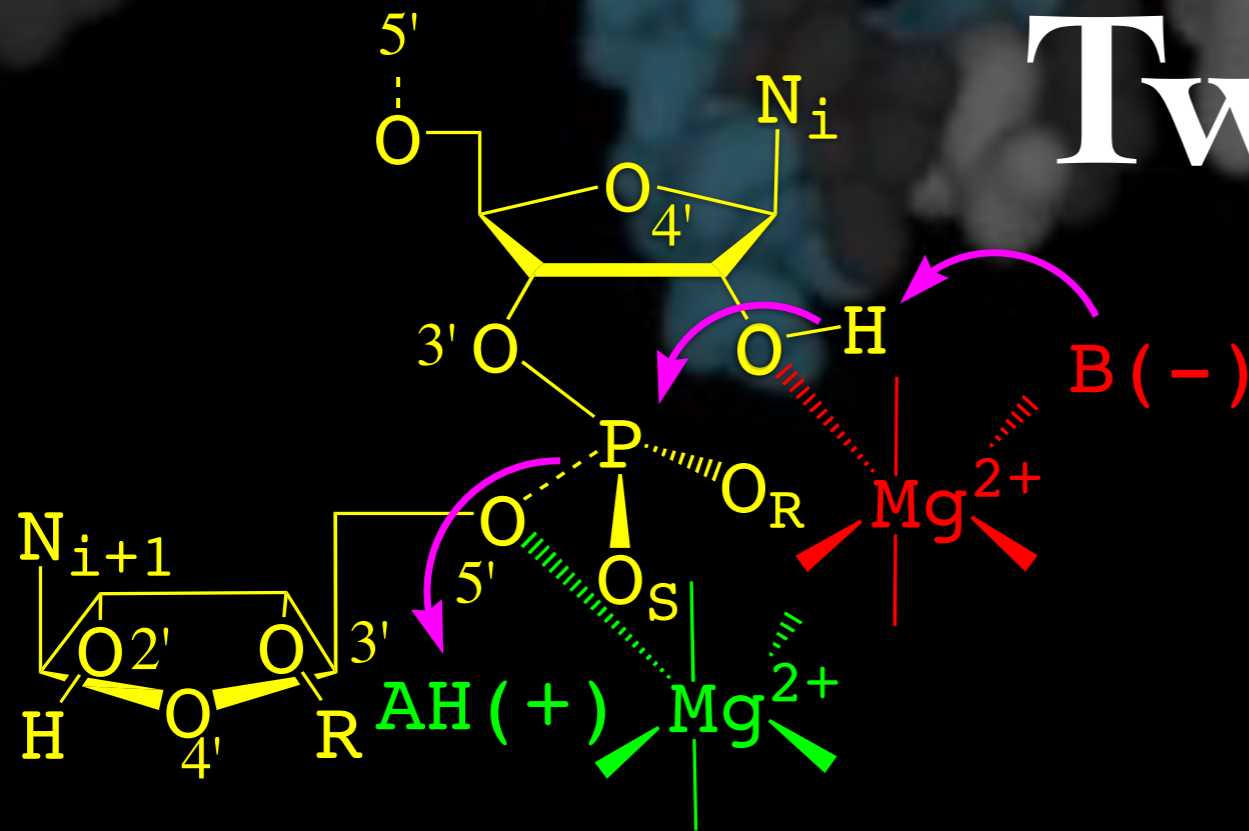
RNase A



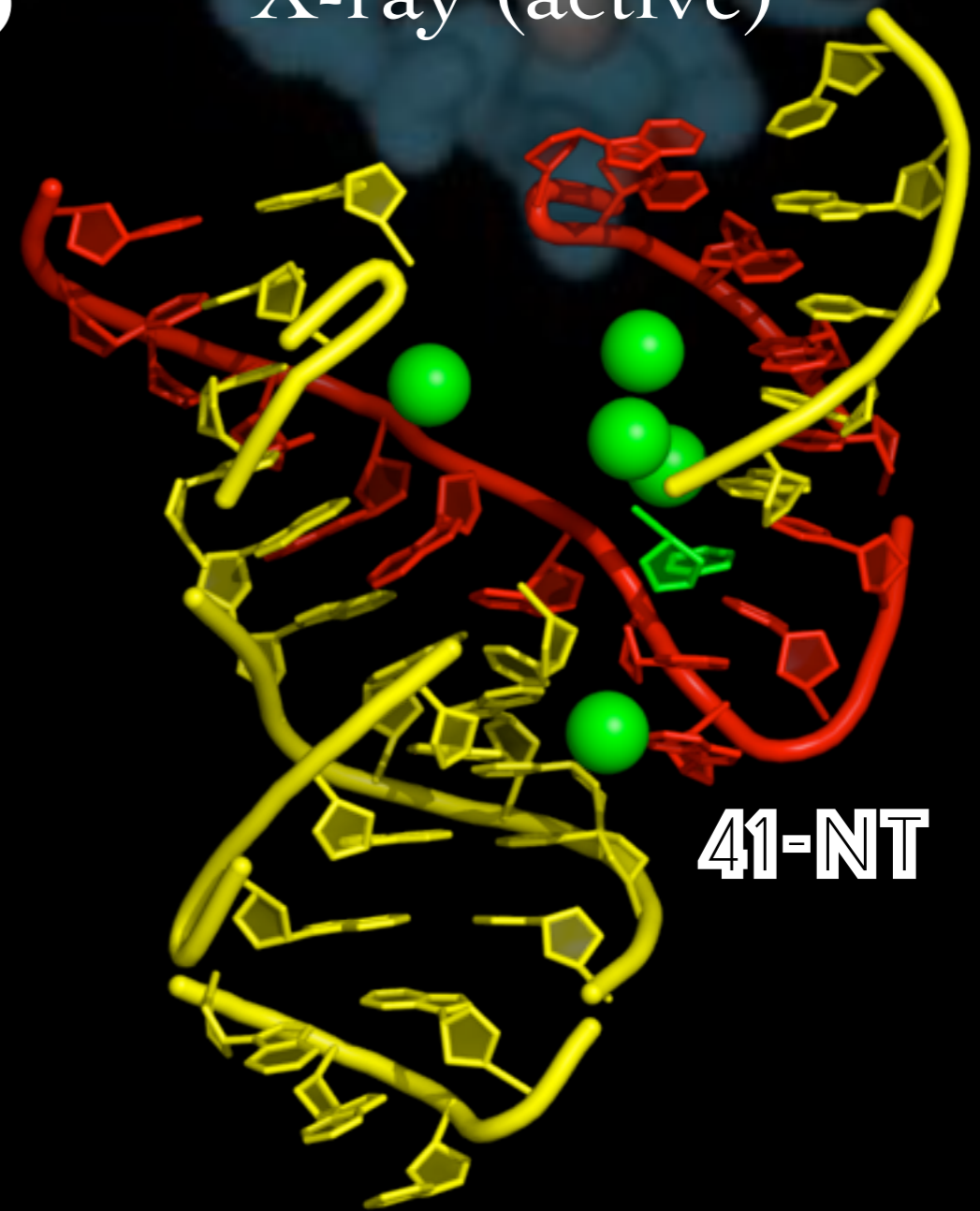
3'-5'-exonuclease

# Catalytic Metal Ions: One/

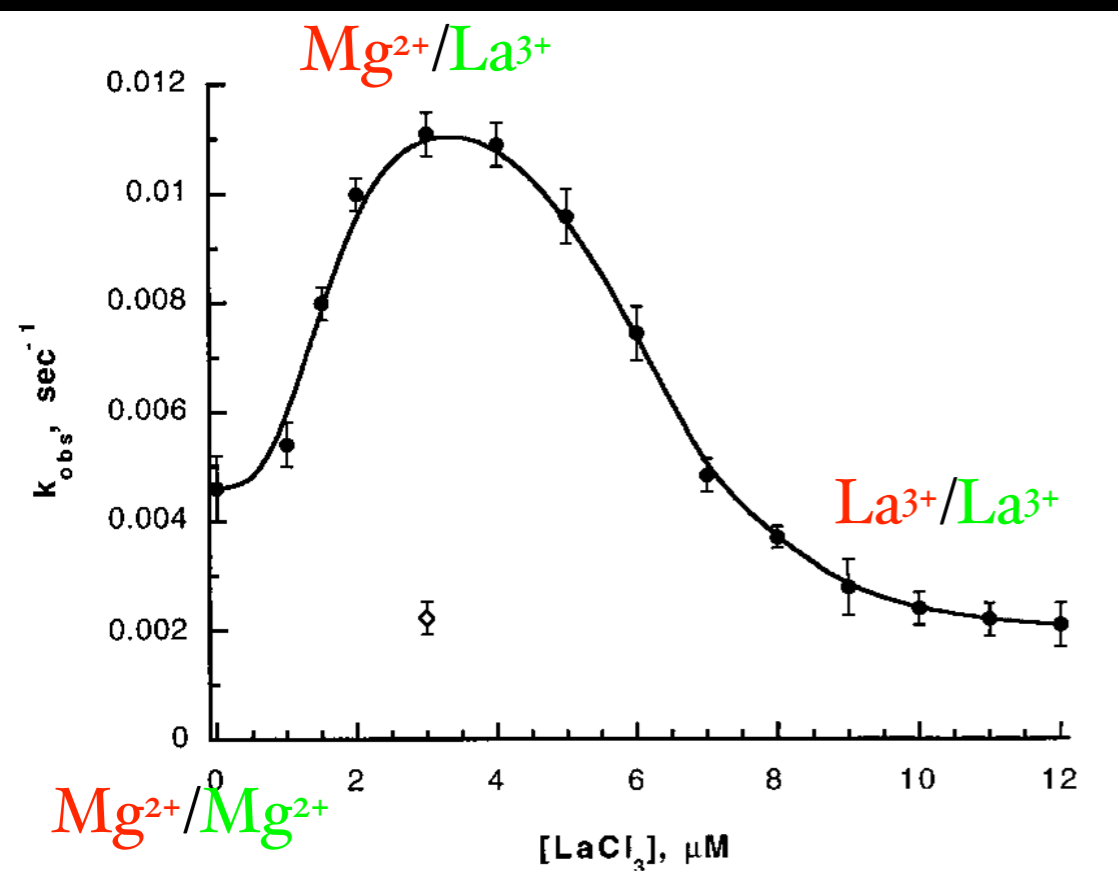
# Two



X-ray (active)



41-NT

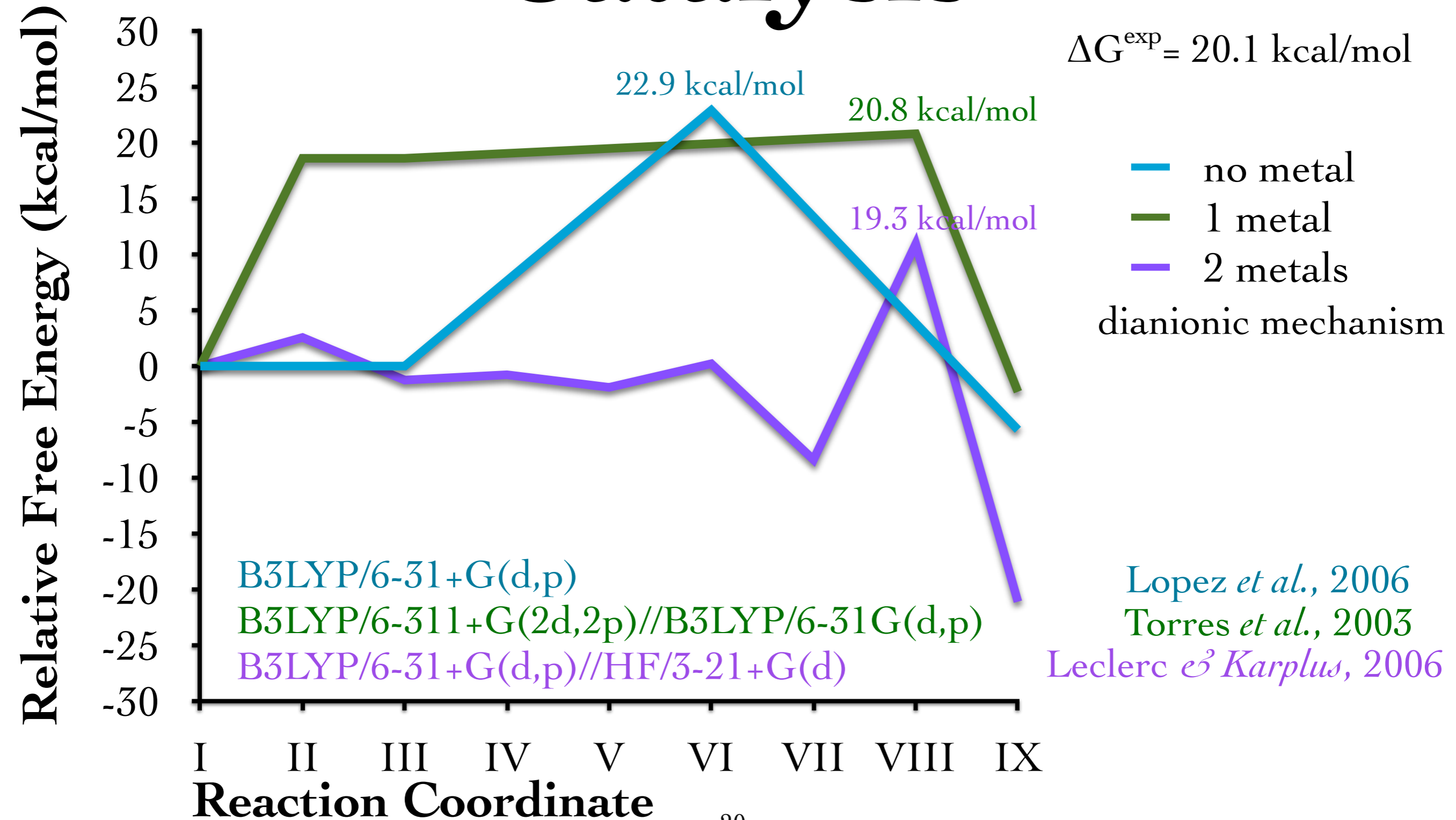


experimental: Pontius *et al.*, 1997; Lott *et al.*, 1998

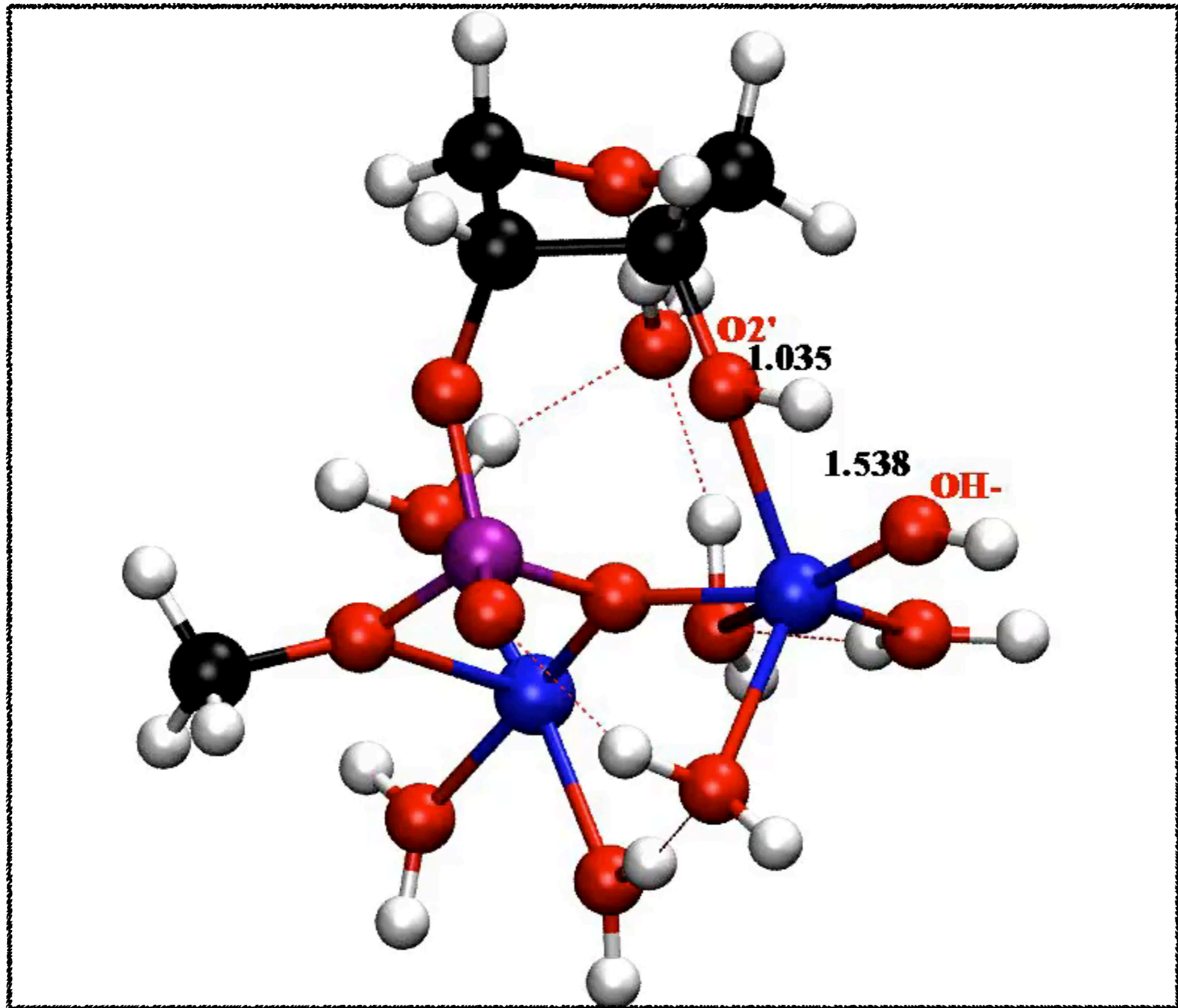
theoretical: Boero *et al.*, JCTC, 2005

theoretical: Leclerc & Karplus, JPC, 2006

# Contribution of Metals to Catalysis



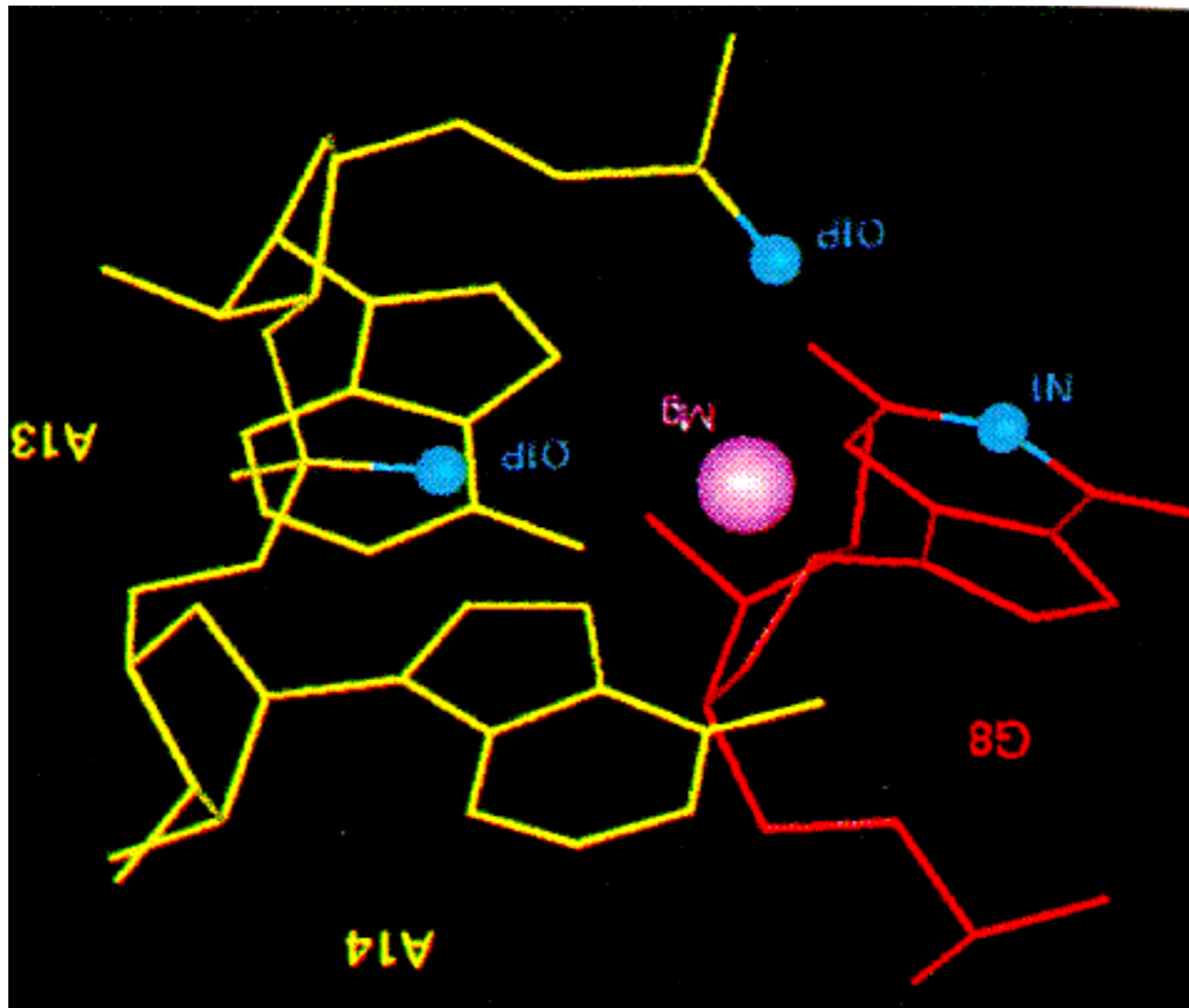
# HHR: reaction mechanism



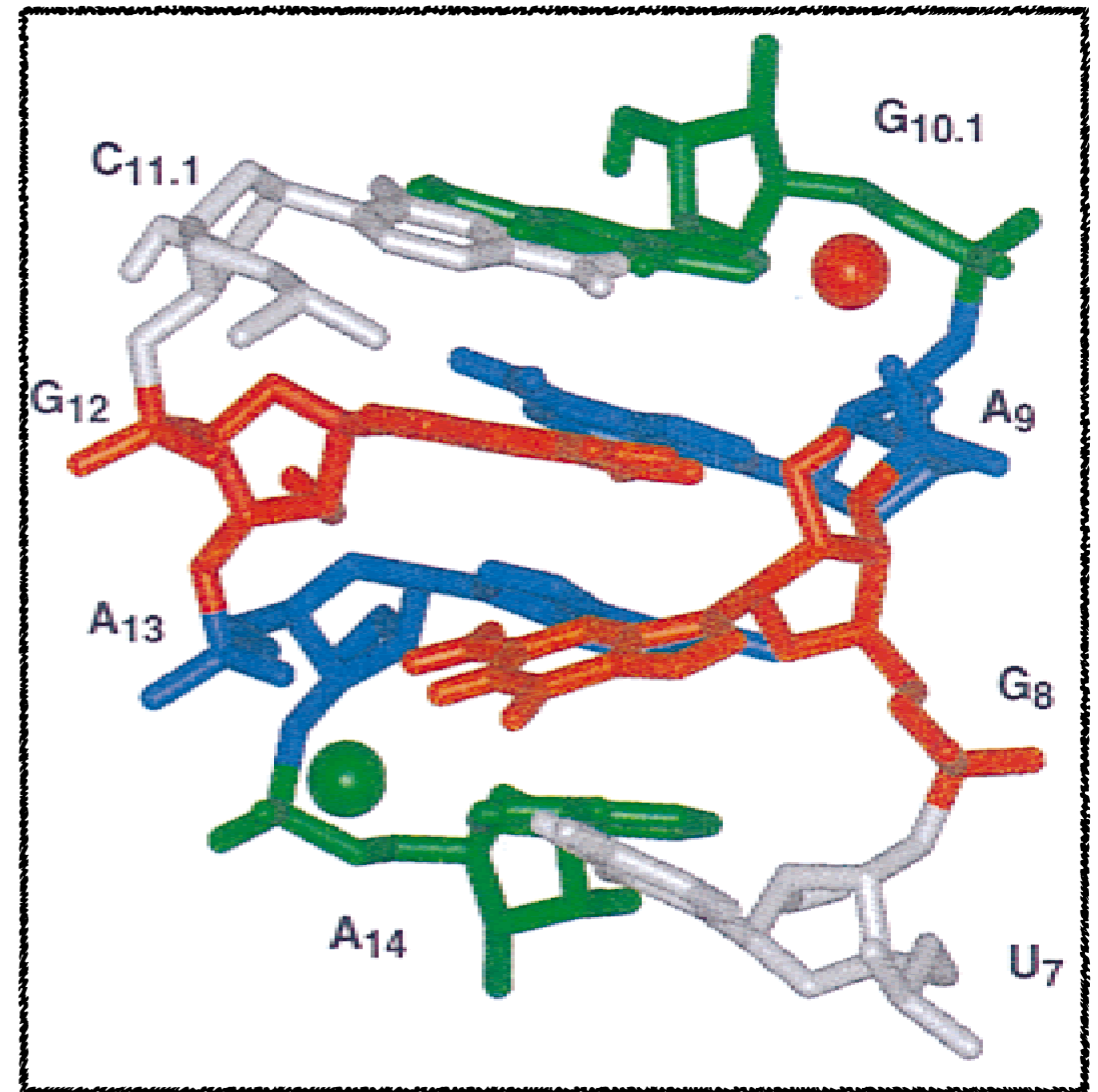
Reaction Path Following: B3LYP/6-31+G(d,p)//HF/3-21+G(d)



# Metal Binding Sites in the Hammerhead Ribozyme ?

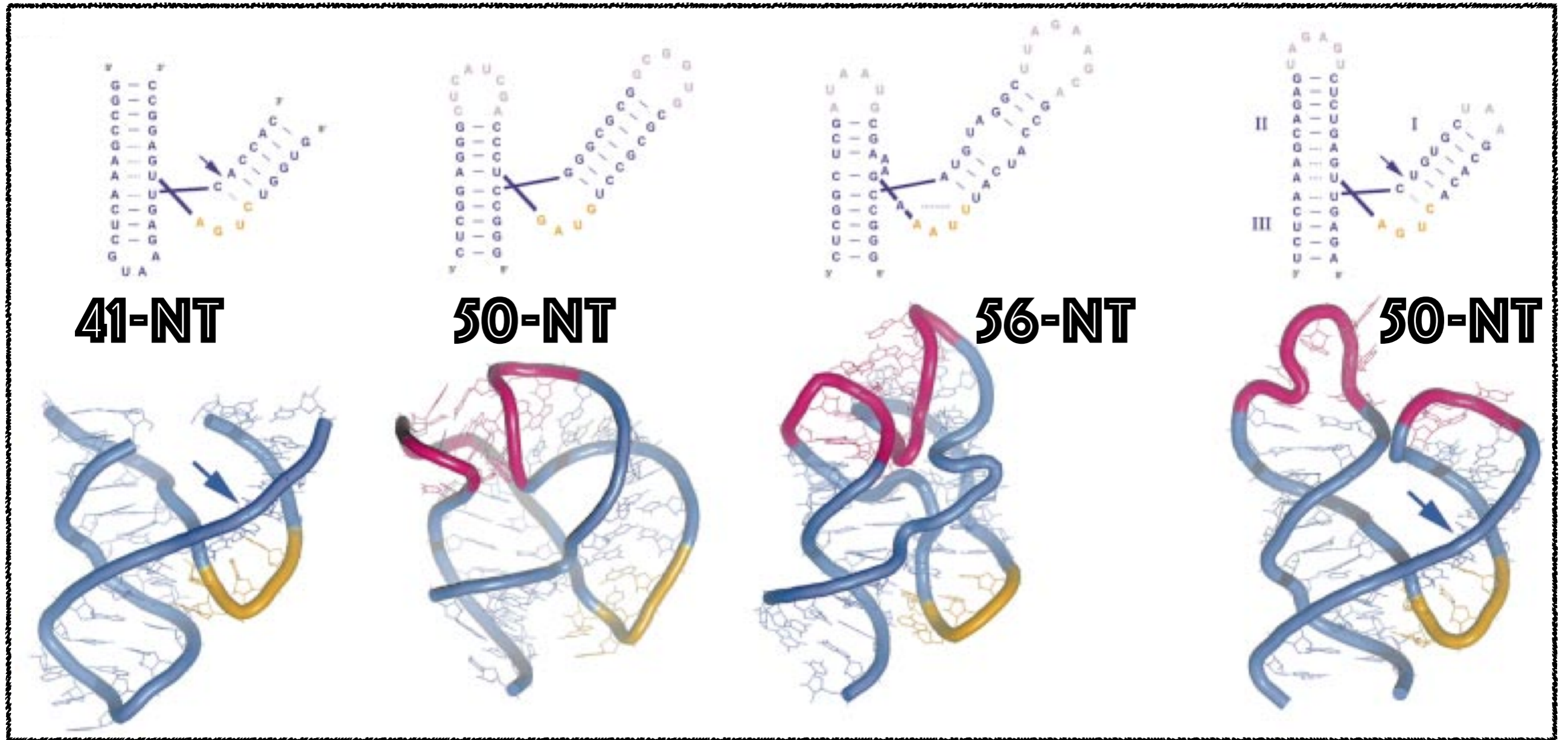


Chartrand *et al.*, RNA, 1997



Hansen *et al.*, RNA, 2008

# Minimum/Full-Length HHR



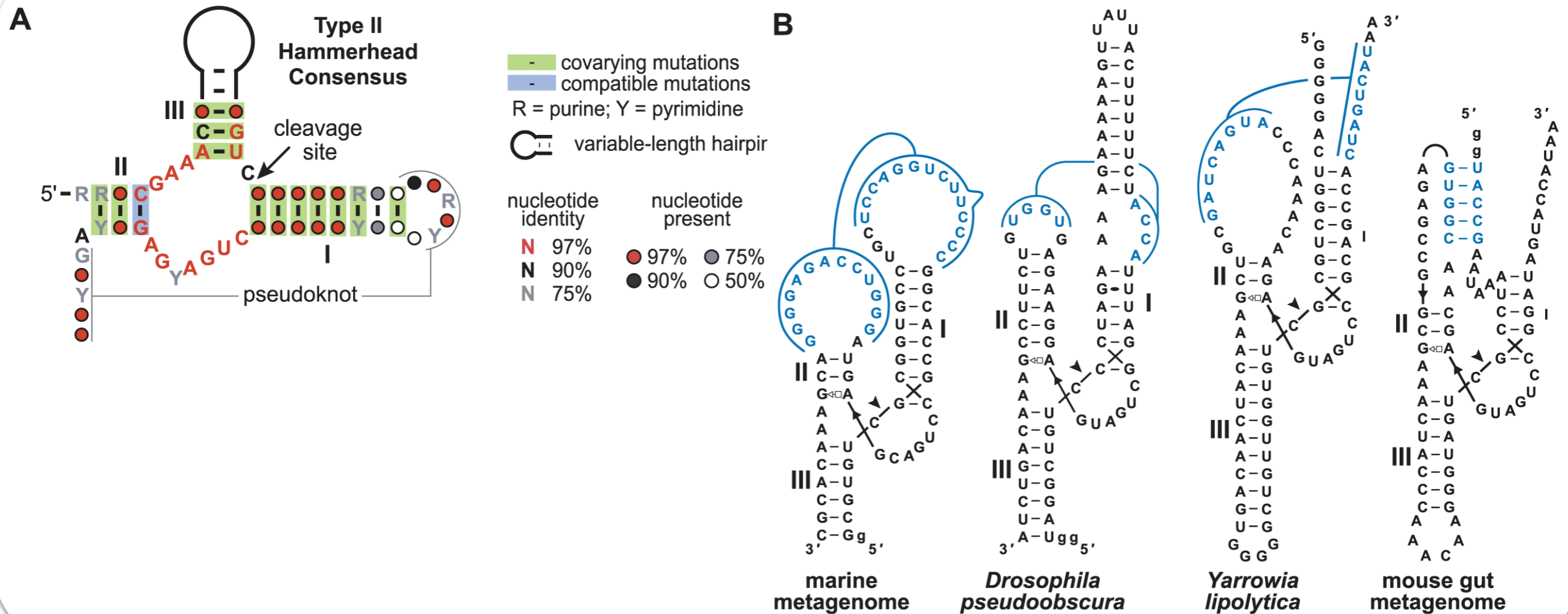
Wang *et al.*, *Biochem.*, 1999

Khvorova *et al.*, *Nat. Struct. Biol.*, 2003

de la Peña *et al.*, *EMBO J.*, 2003

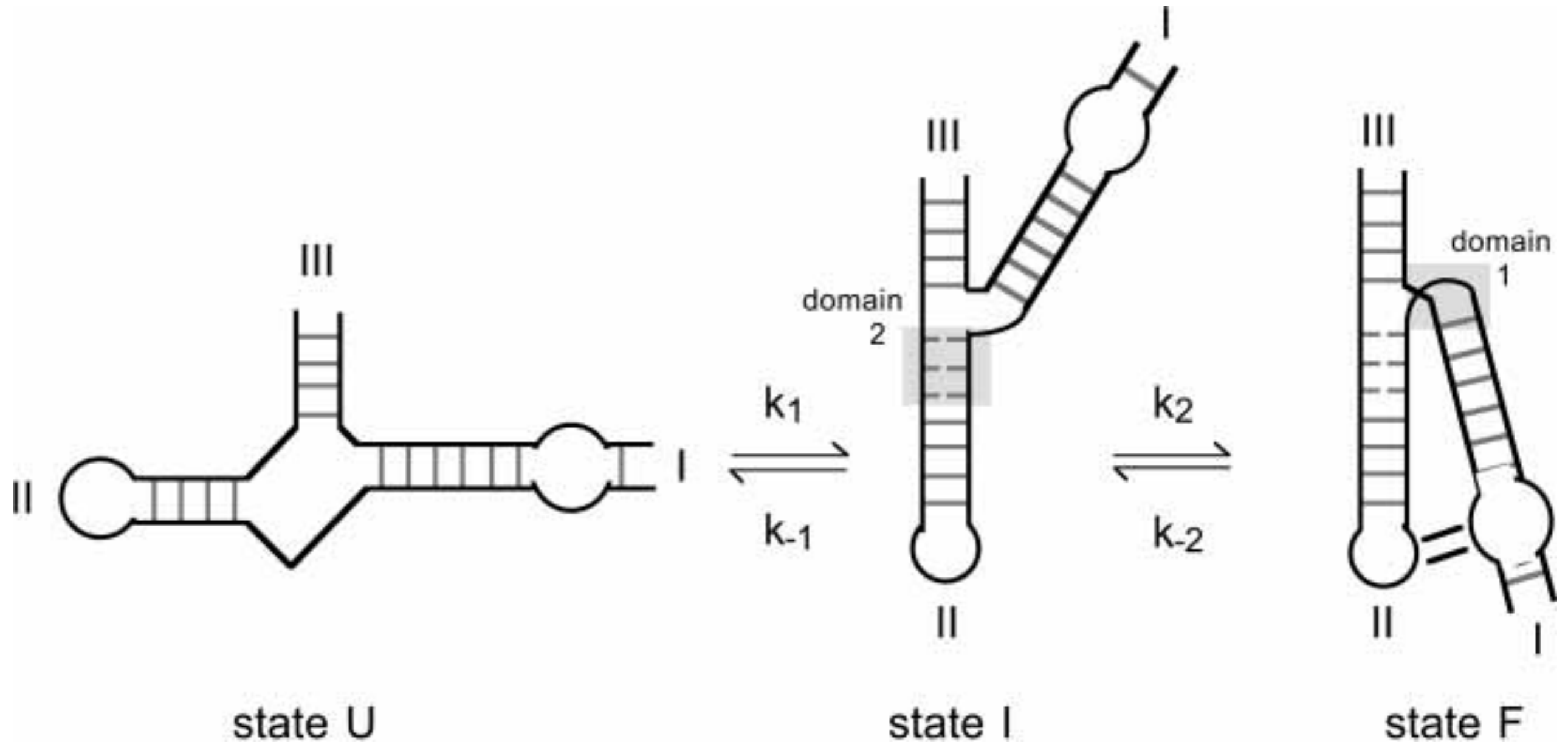
Canny *et al.*, *JACS*, 2004

# Variations in HHR motifs



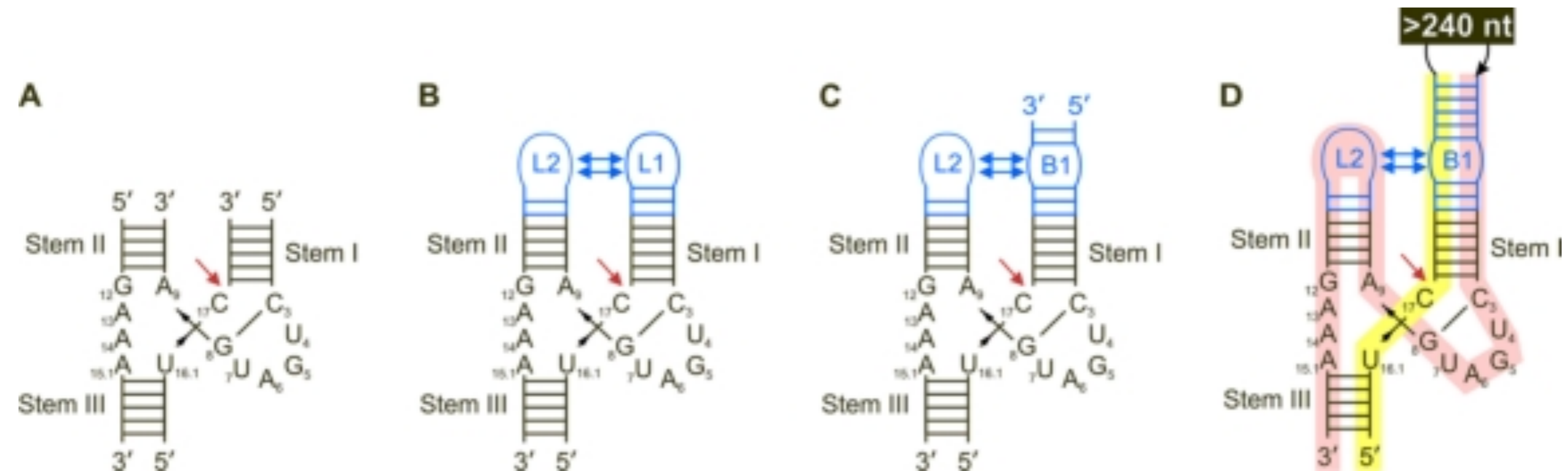
Perreault *et al.*, PLoS Comput. Biol., 2011

# Loop-Loop interactions in HHR folding

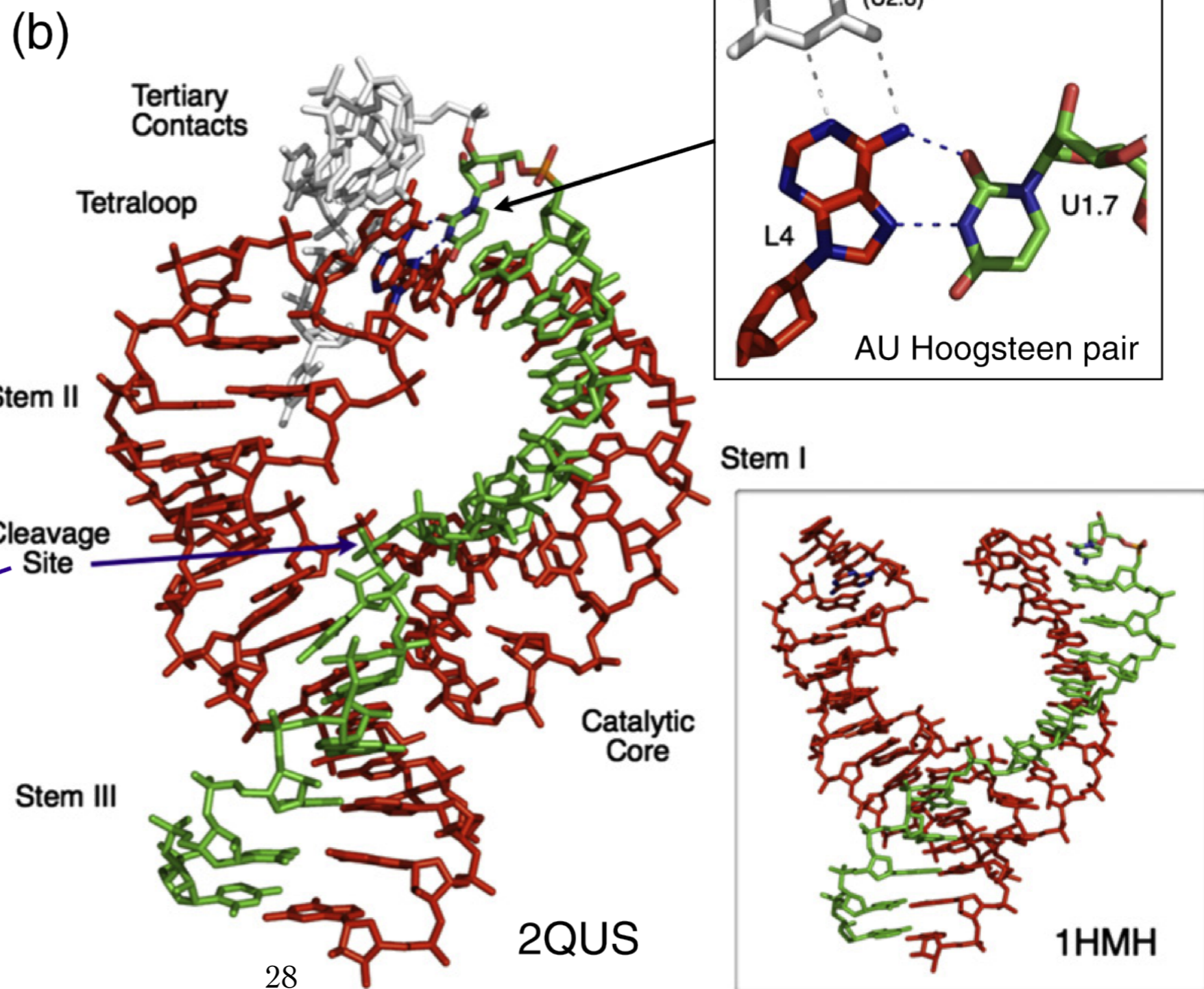
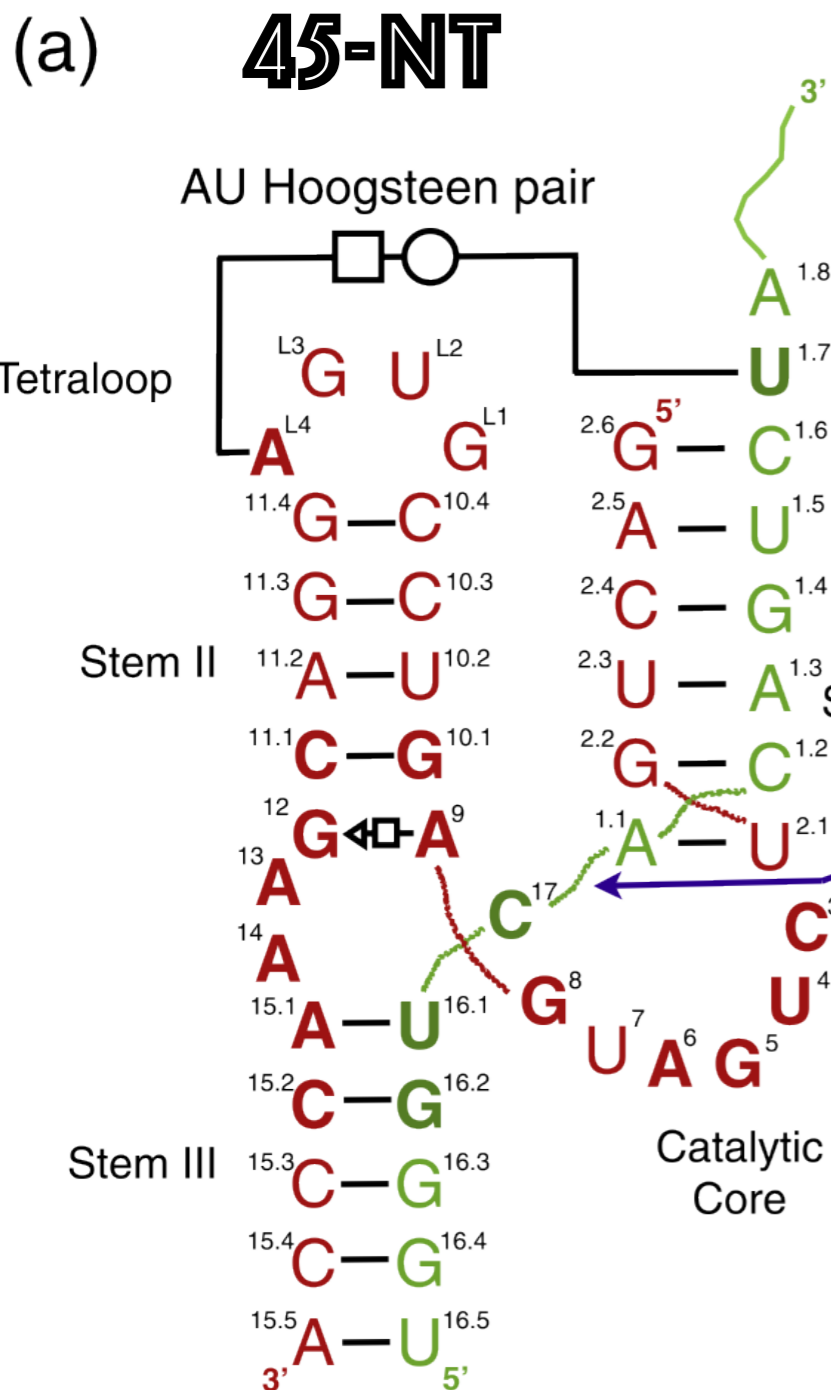


Penedo *et al.*, RNA, 2004.

# Loop-Loop interactions in HHR catalysis

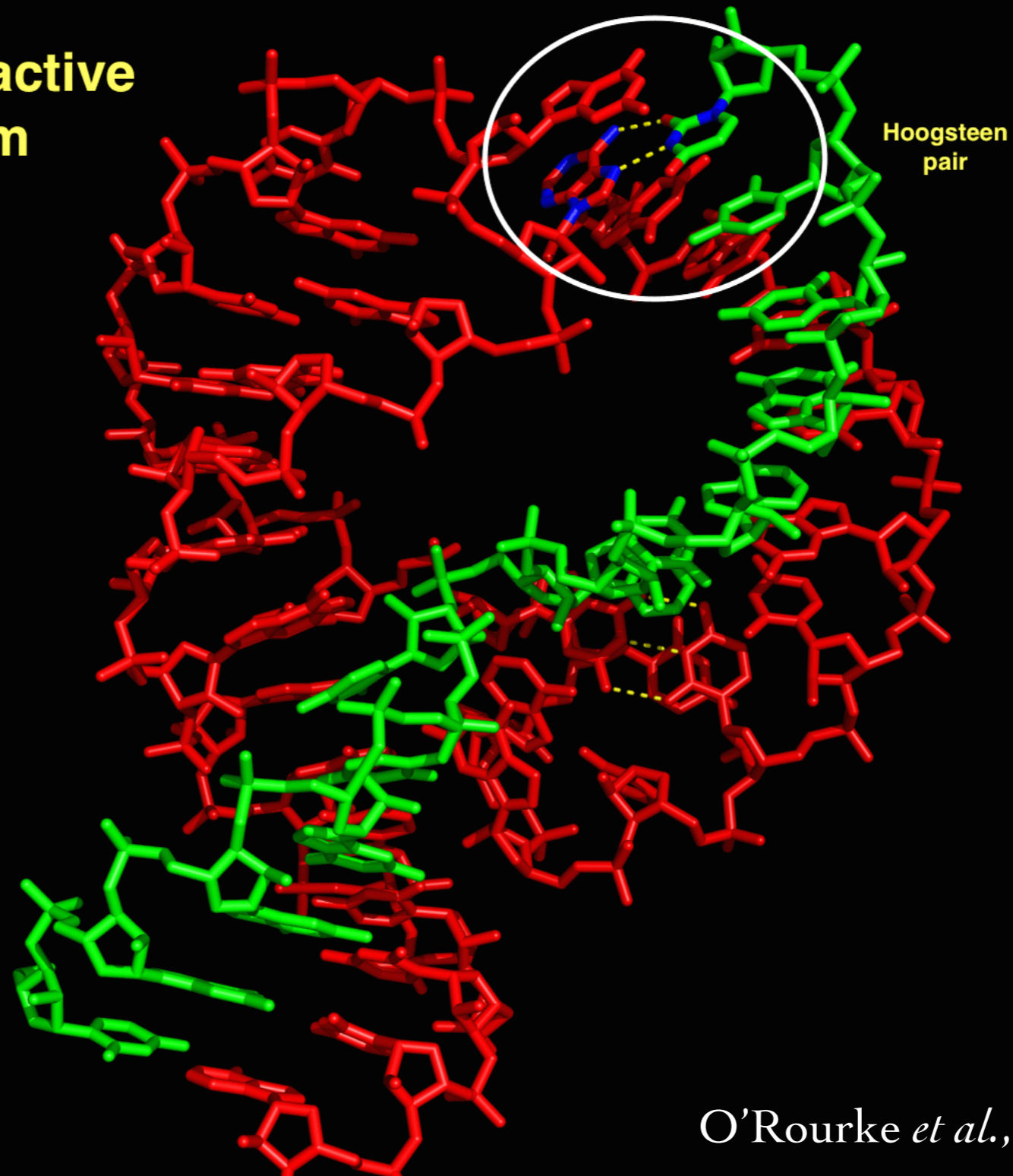


# Minimum HHR, Tertiary Contact and Catalysis



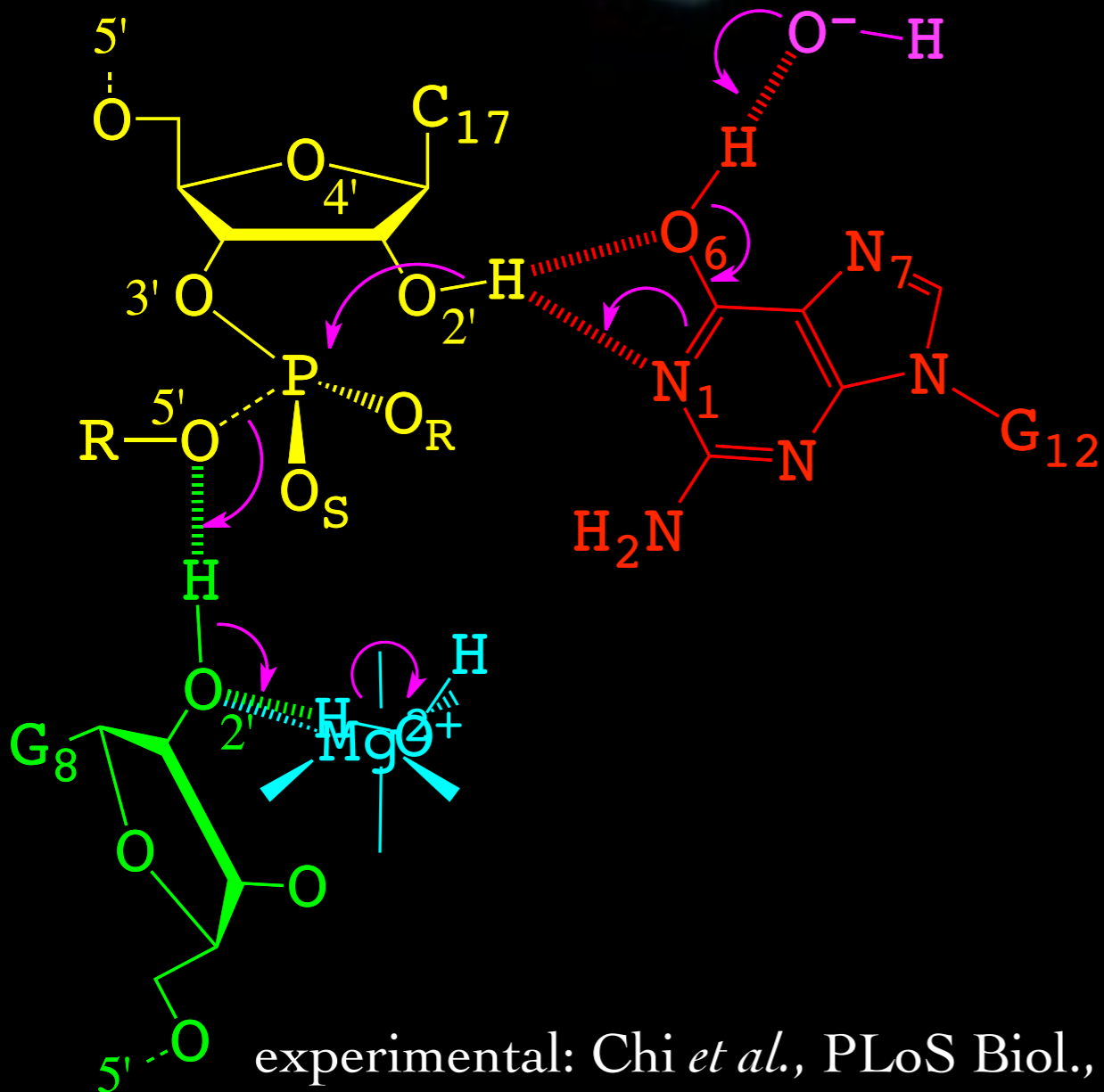
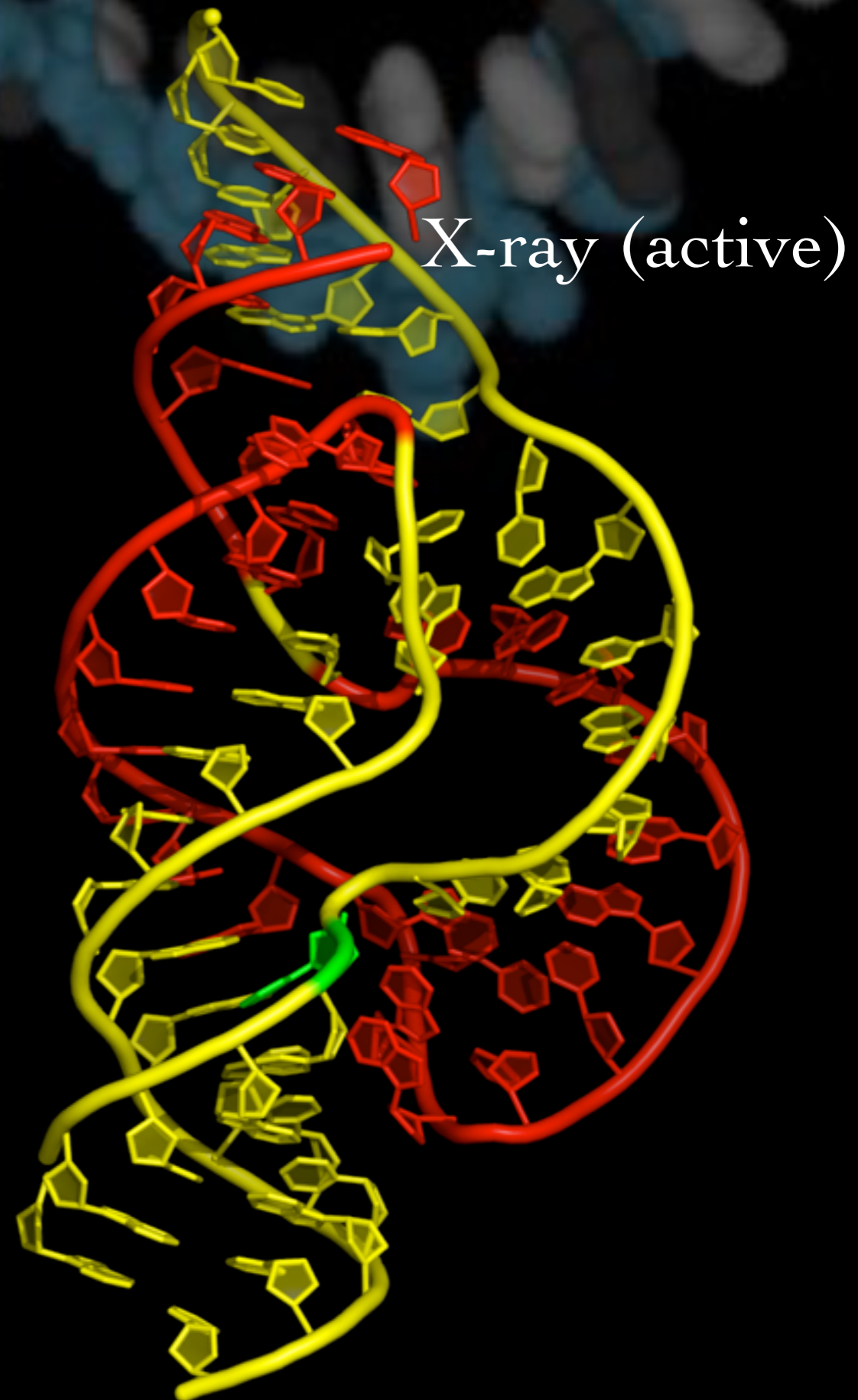
# HHR morphing

Highly active  
form



O'Rourke *et al.*, JMB, 2015

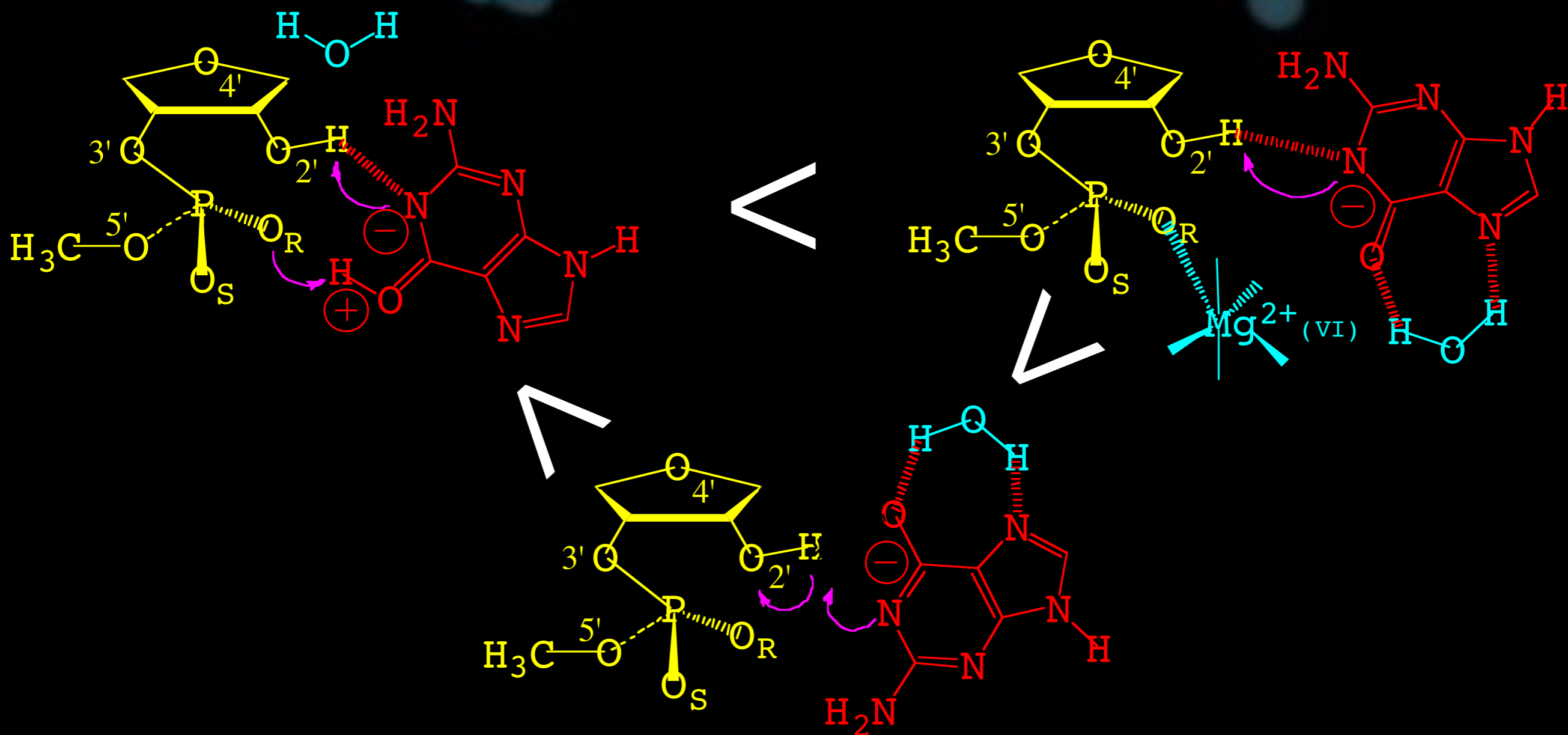
# A Nucleobase Catalyst



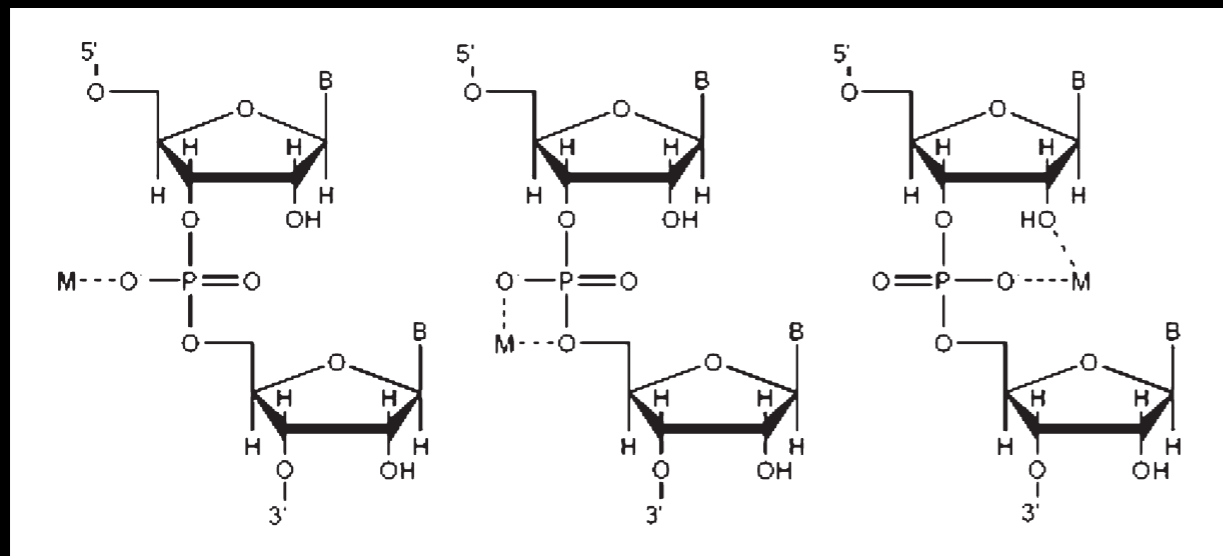
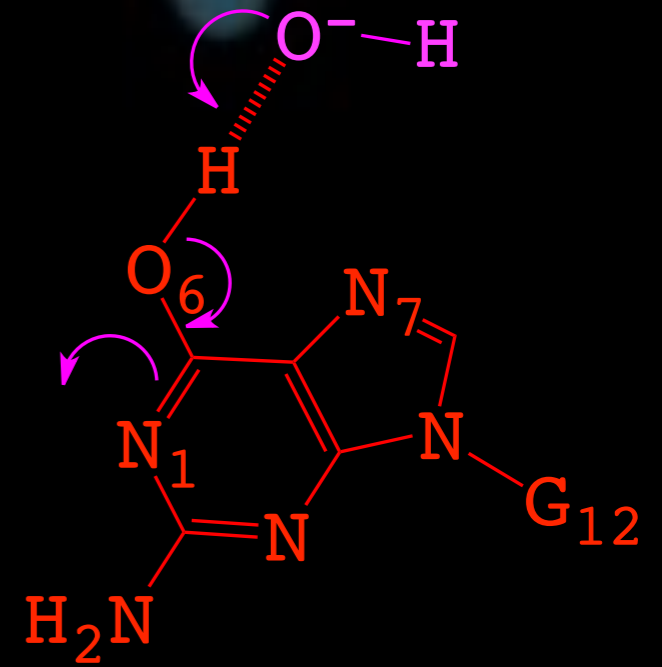
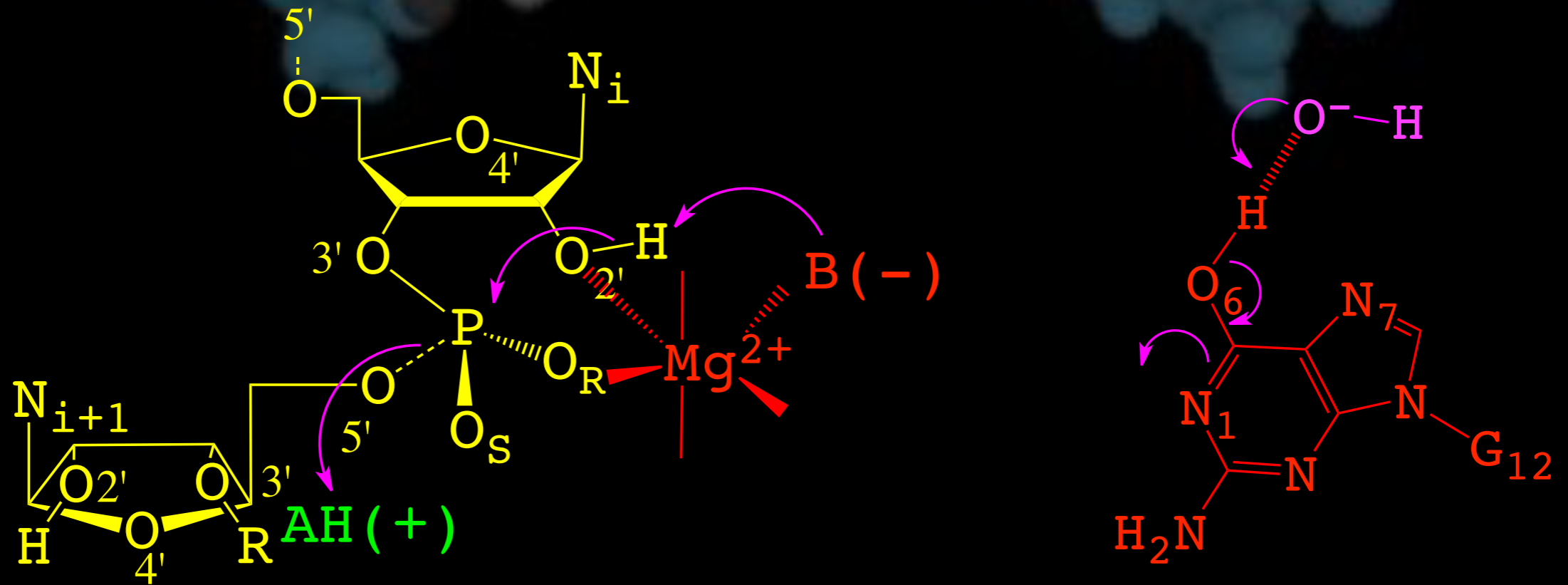
experimental: Chi *et al.*, PLoS Biol., 2008

theoretical: Lee *et al.*, JACS, 2008 30

# Metal Catalysts in the 2'OH activation ?

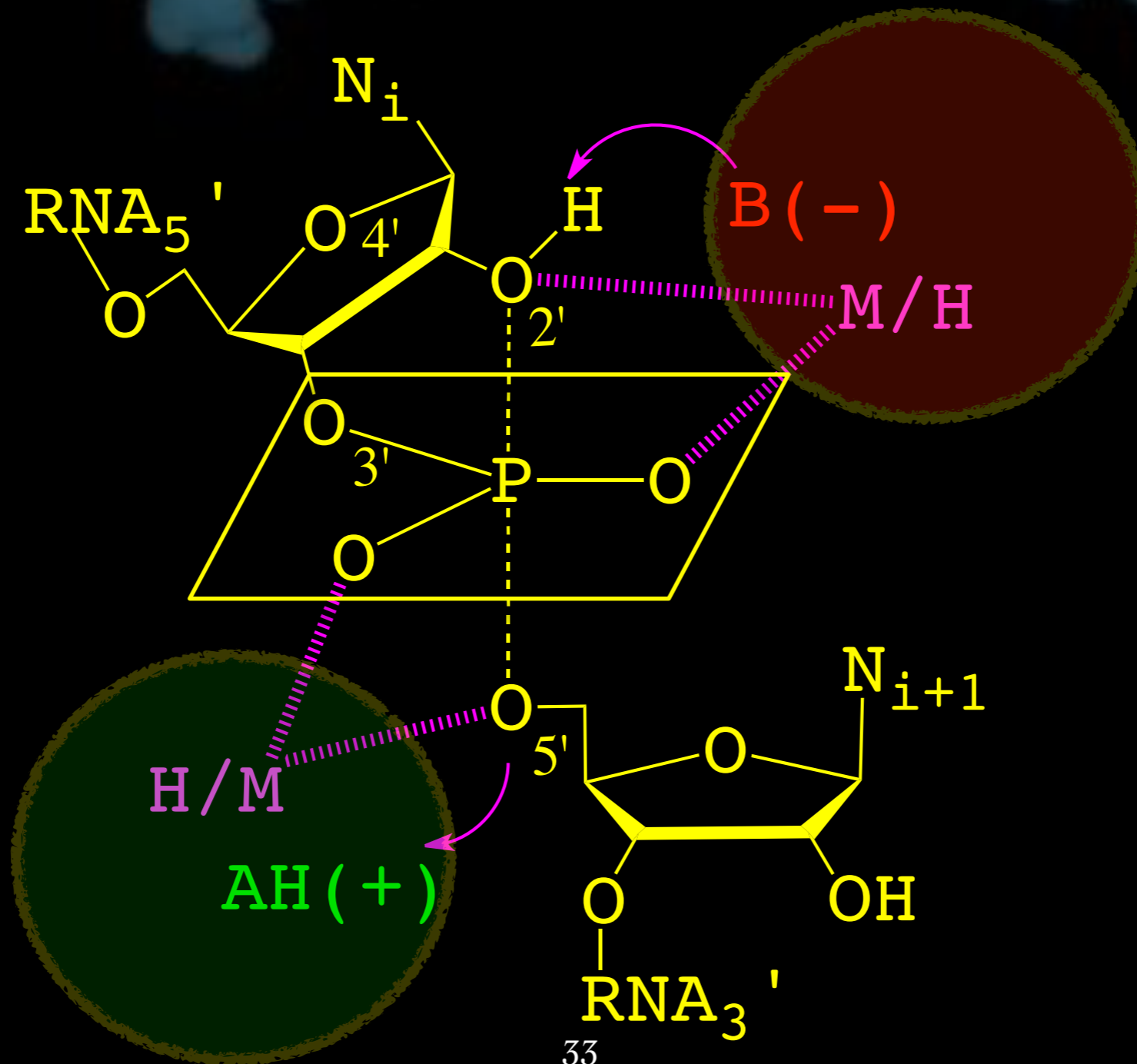


# Metal Catalysts in the Hammerhead Ribozymes ?

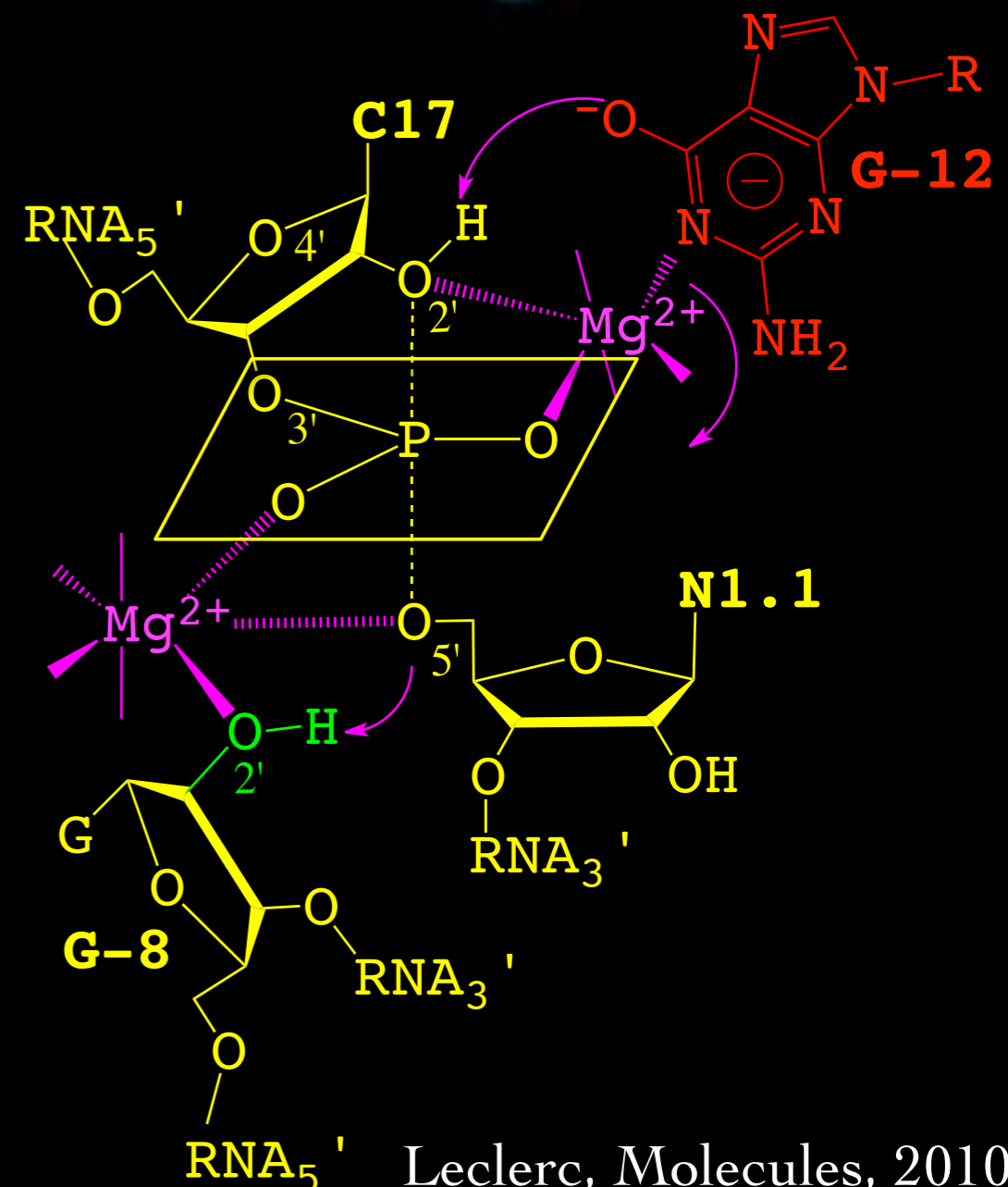
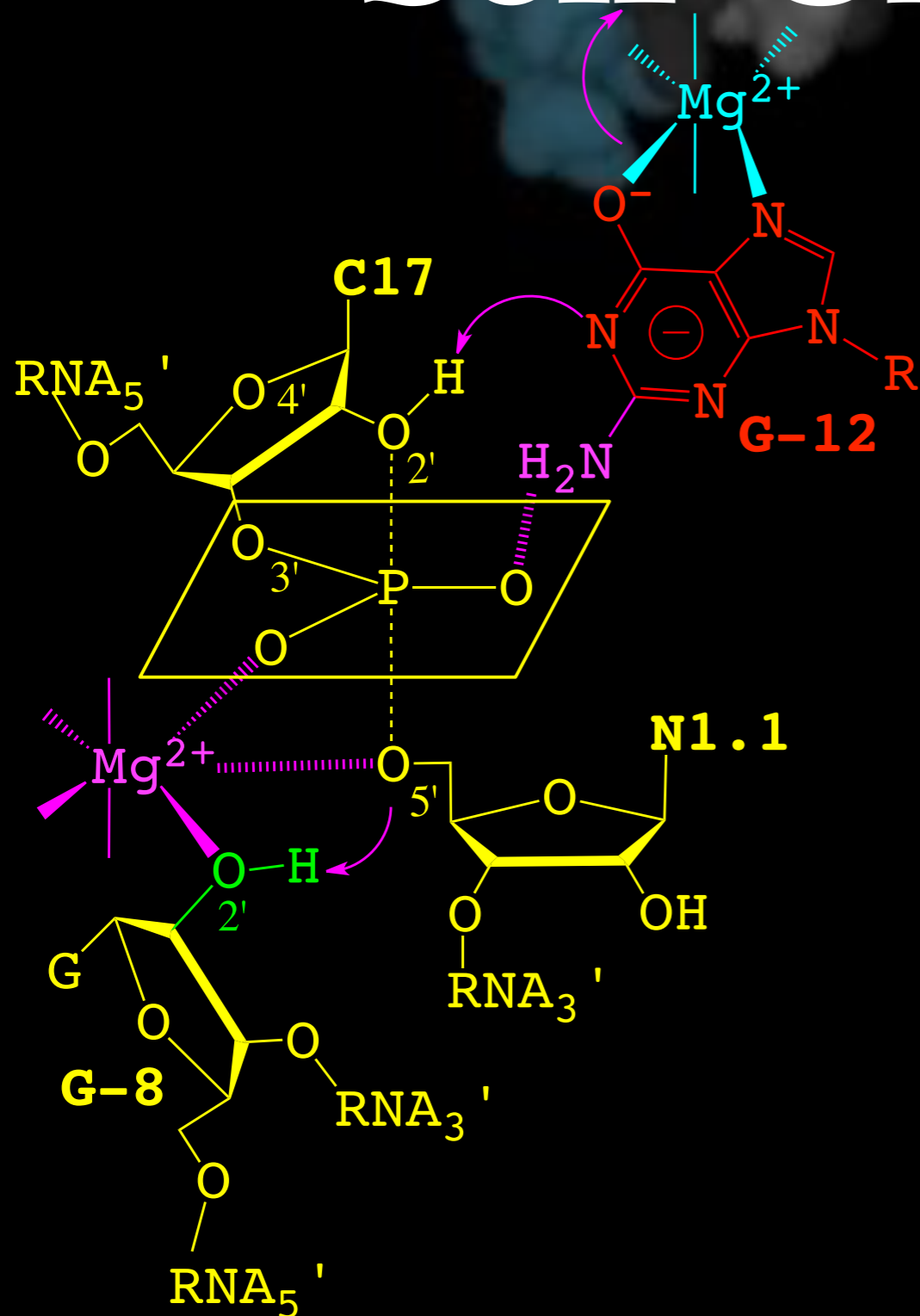


Osborne *et al.*, Biochem., 2009

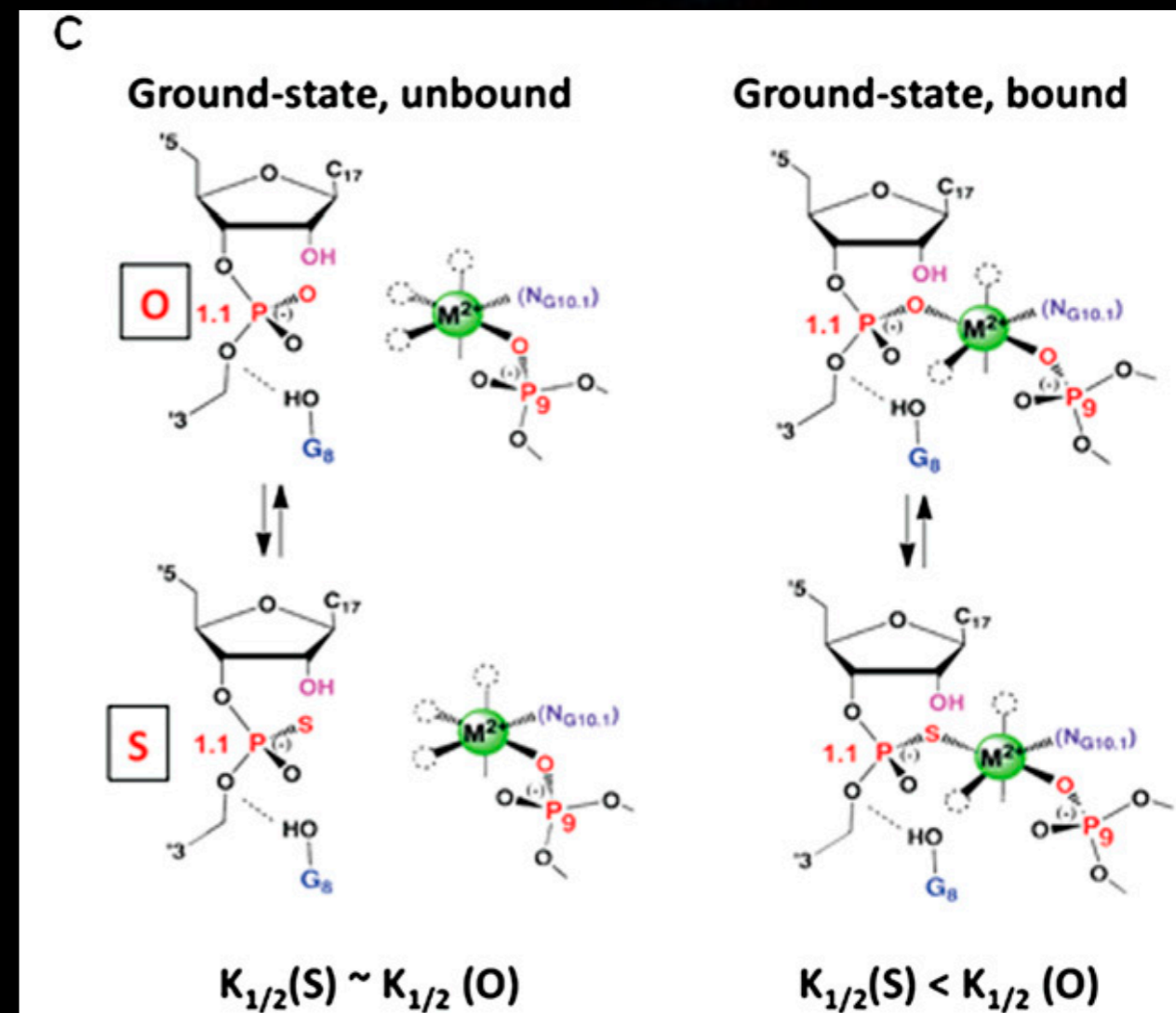
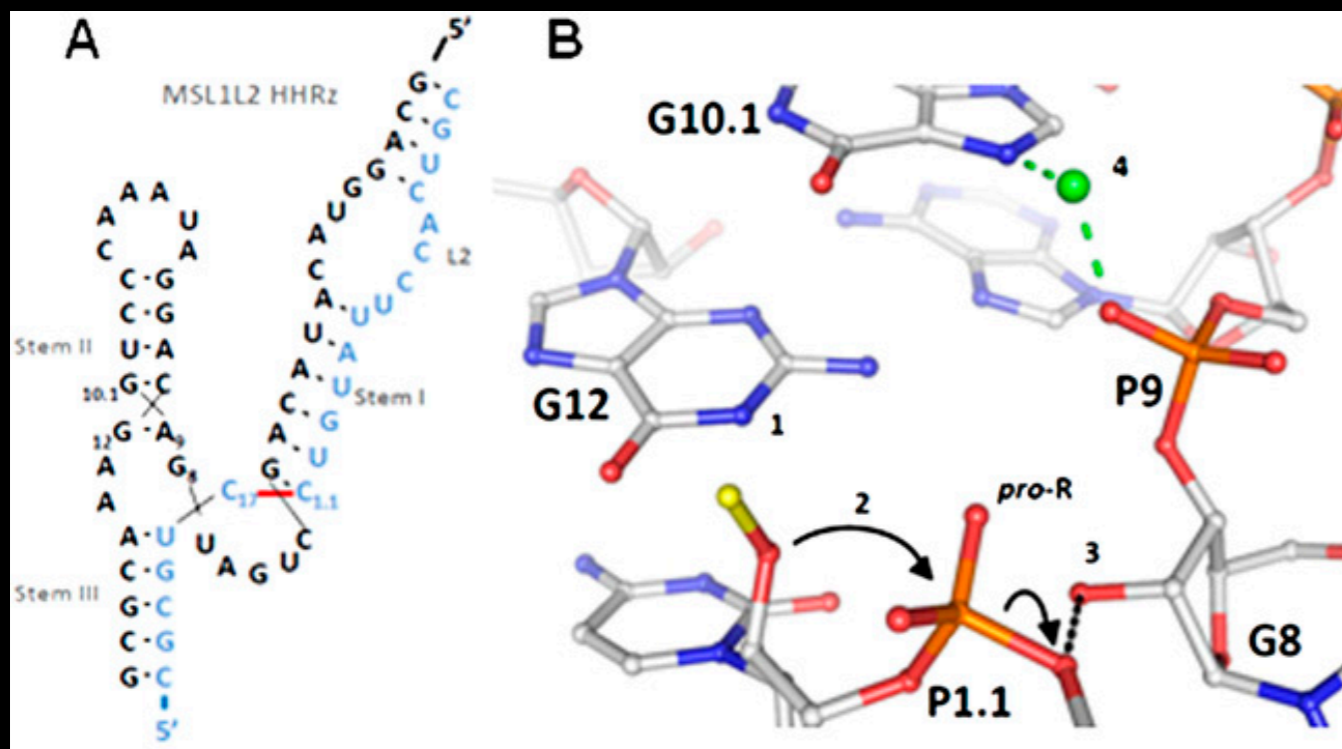
# Cooperative Models in Self-Cleaving ?



# Cooperative Models in Self-Cleaving ?

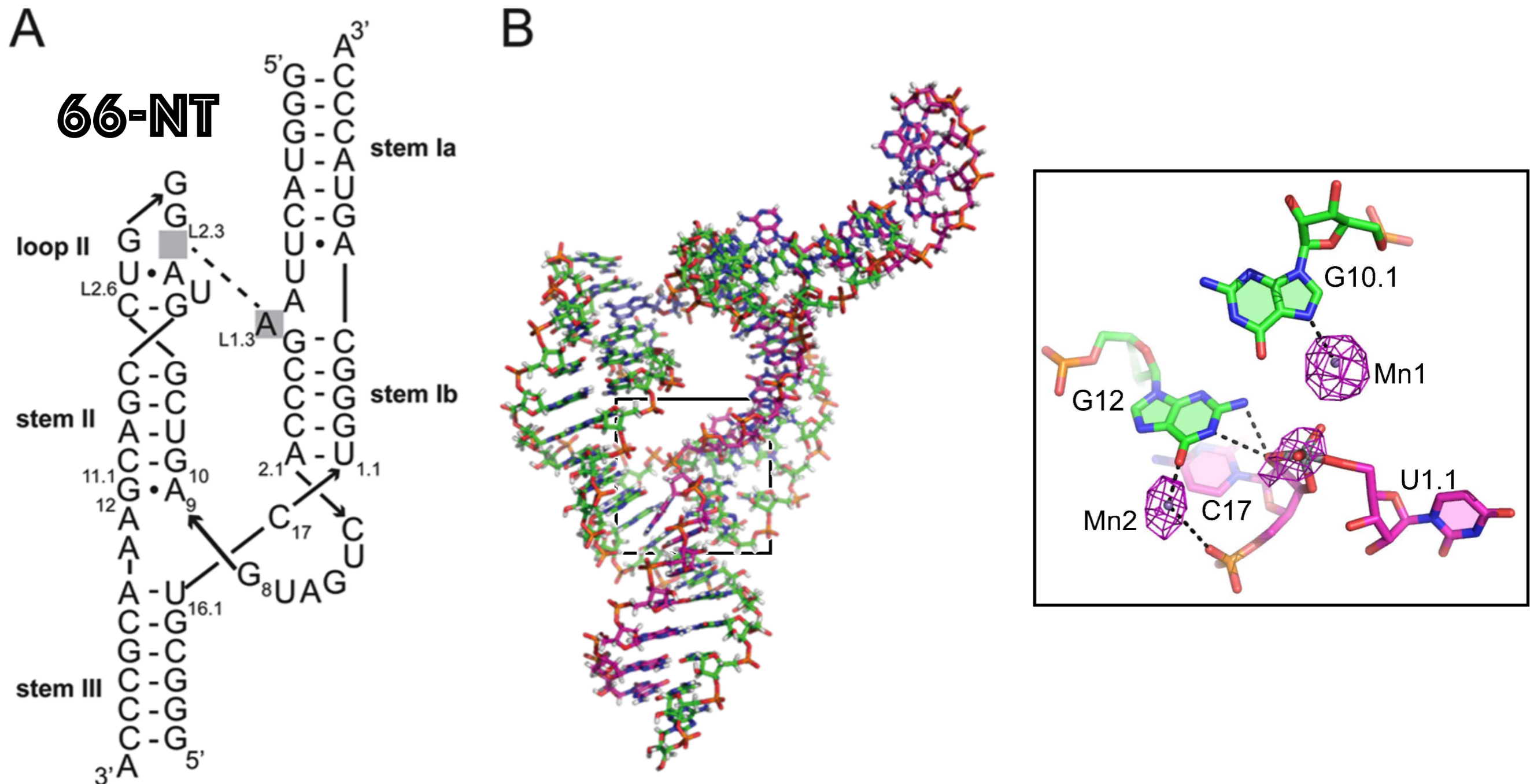


# Metal Ions back in the Hammerhead Catalysis

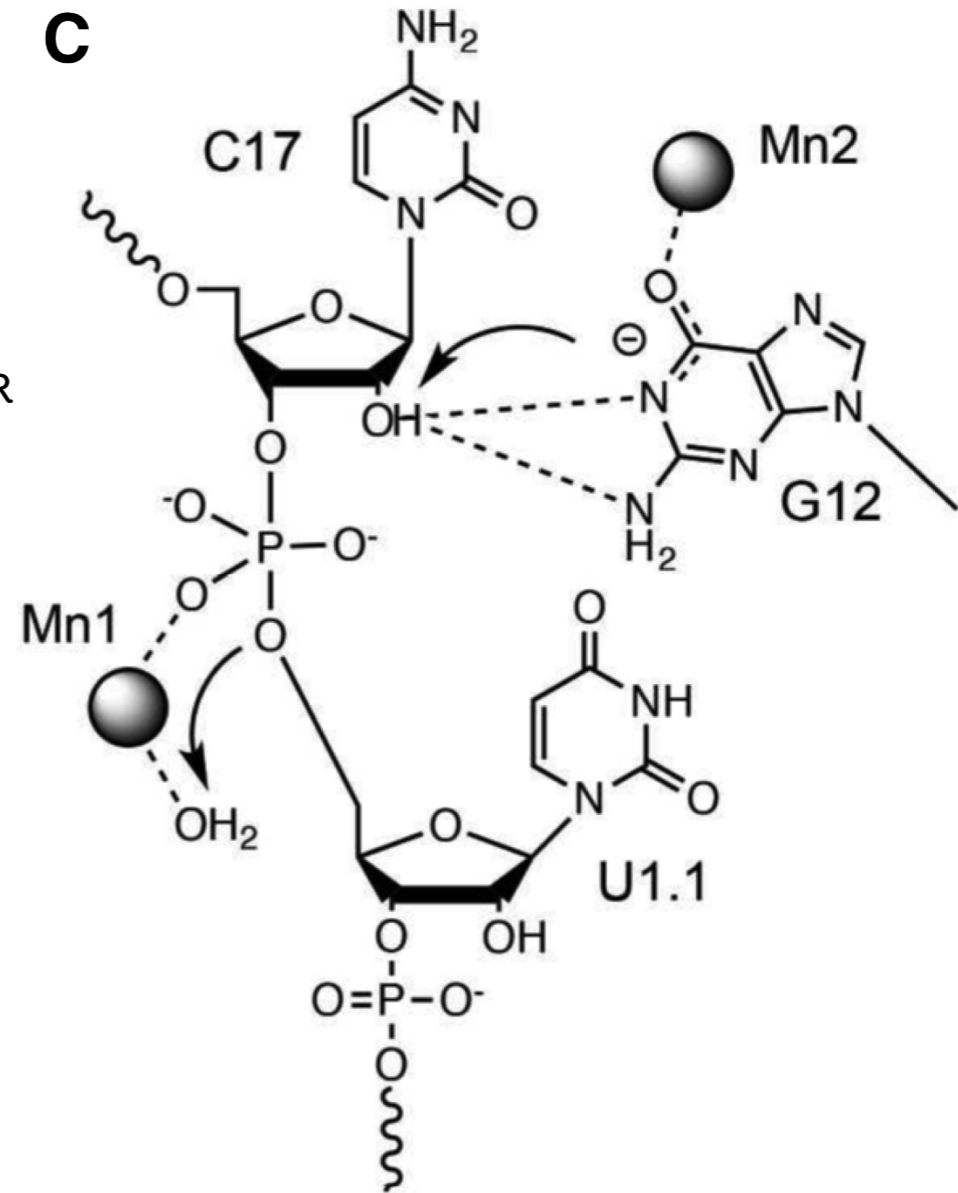
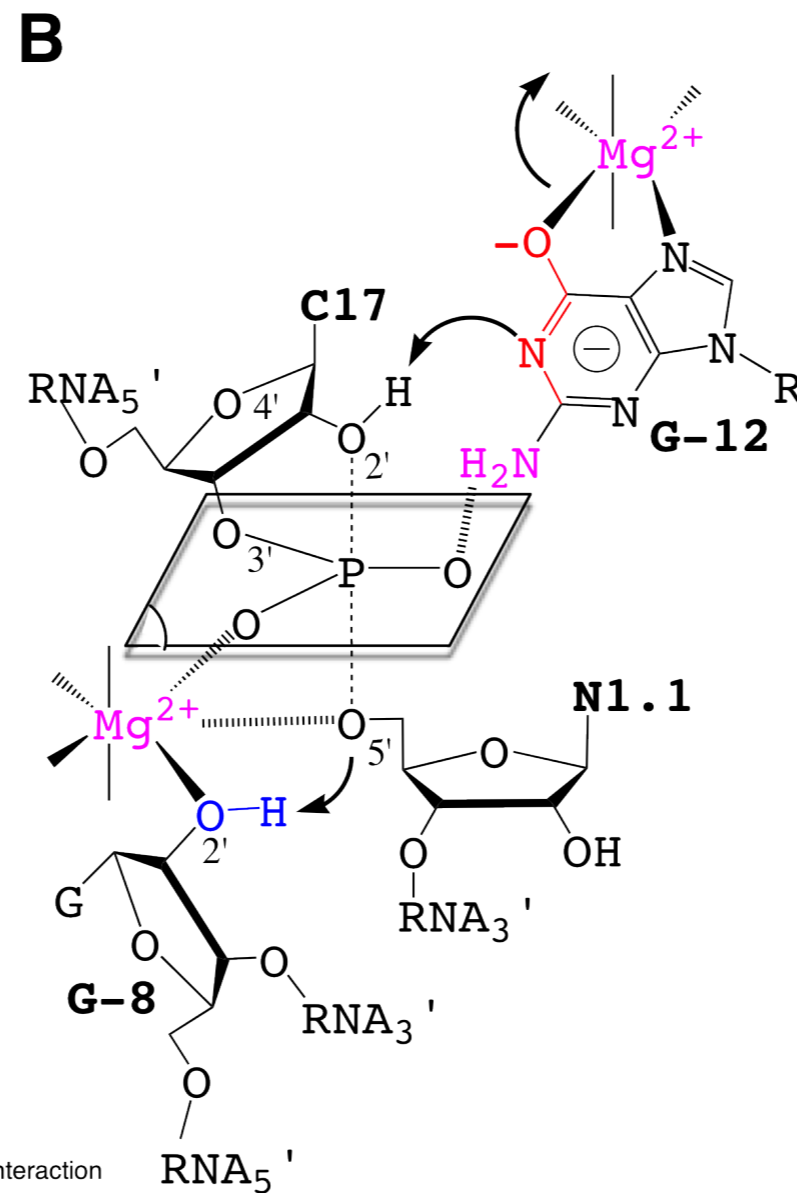
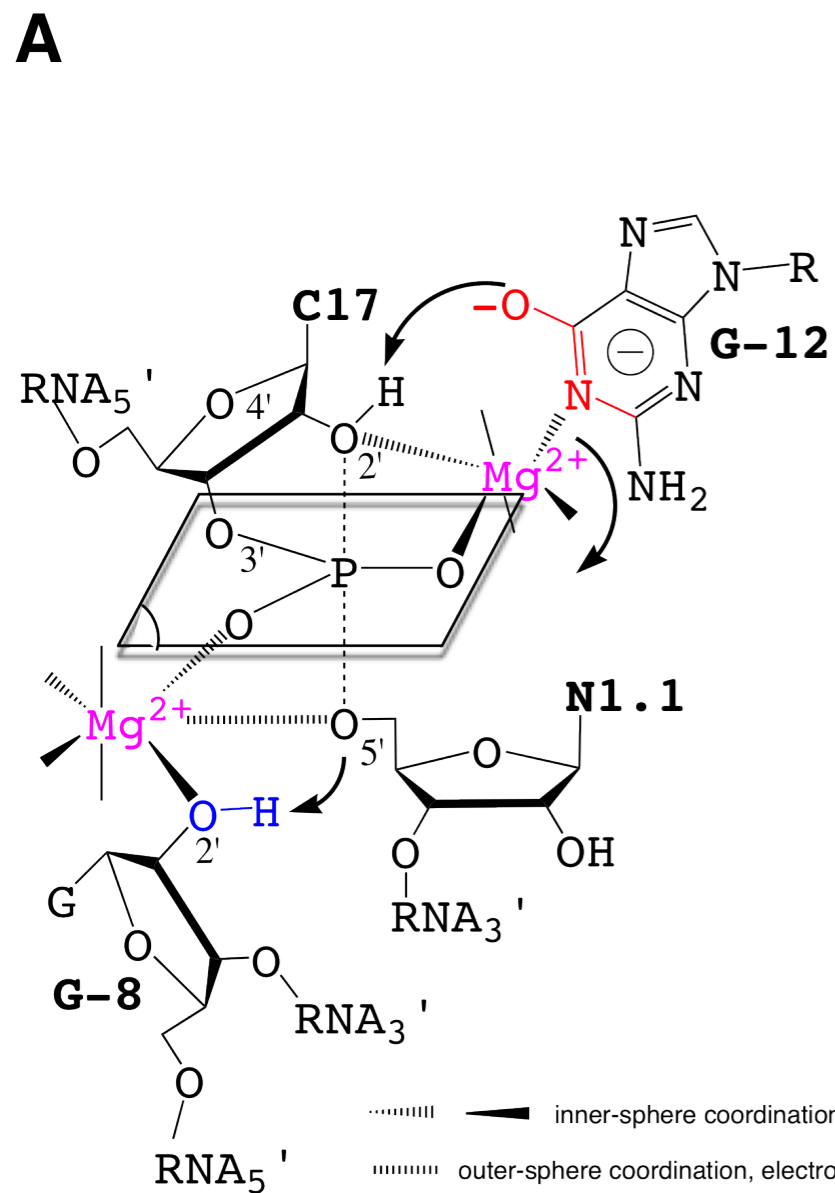


Ward & DeRose, RNA, 2011

# HHH: Active Conformation and Metal Ions



# HHR: Metal Ions and Catalysis

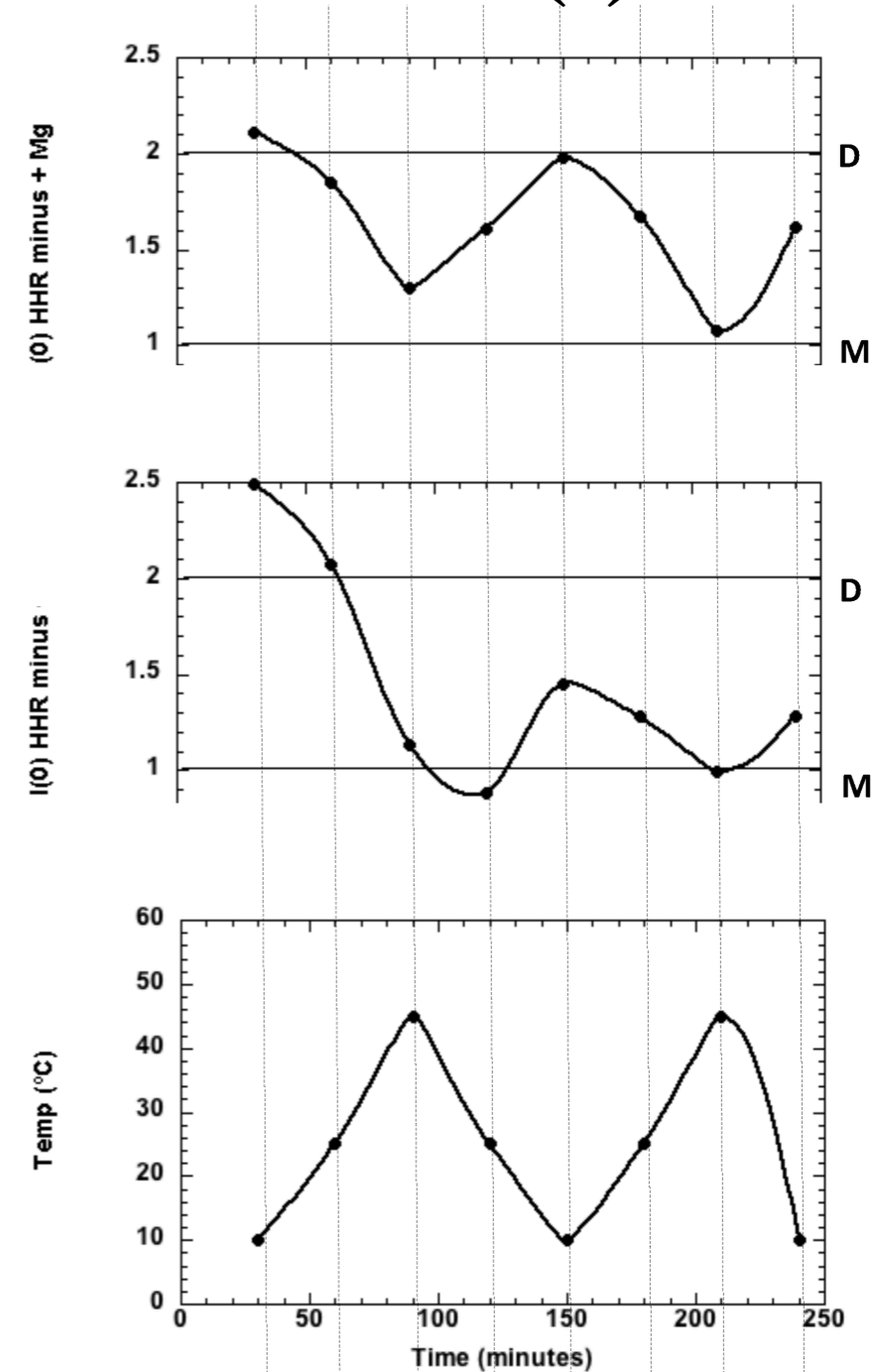
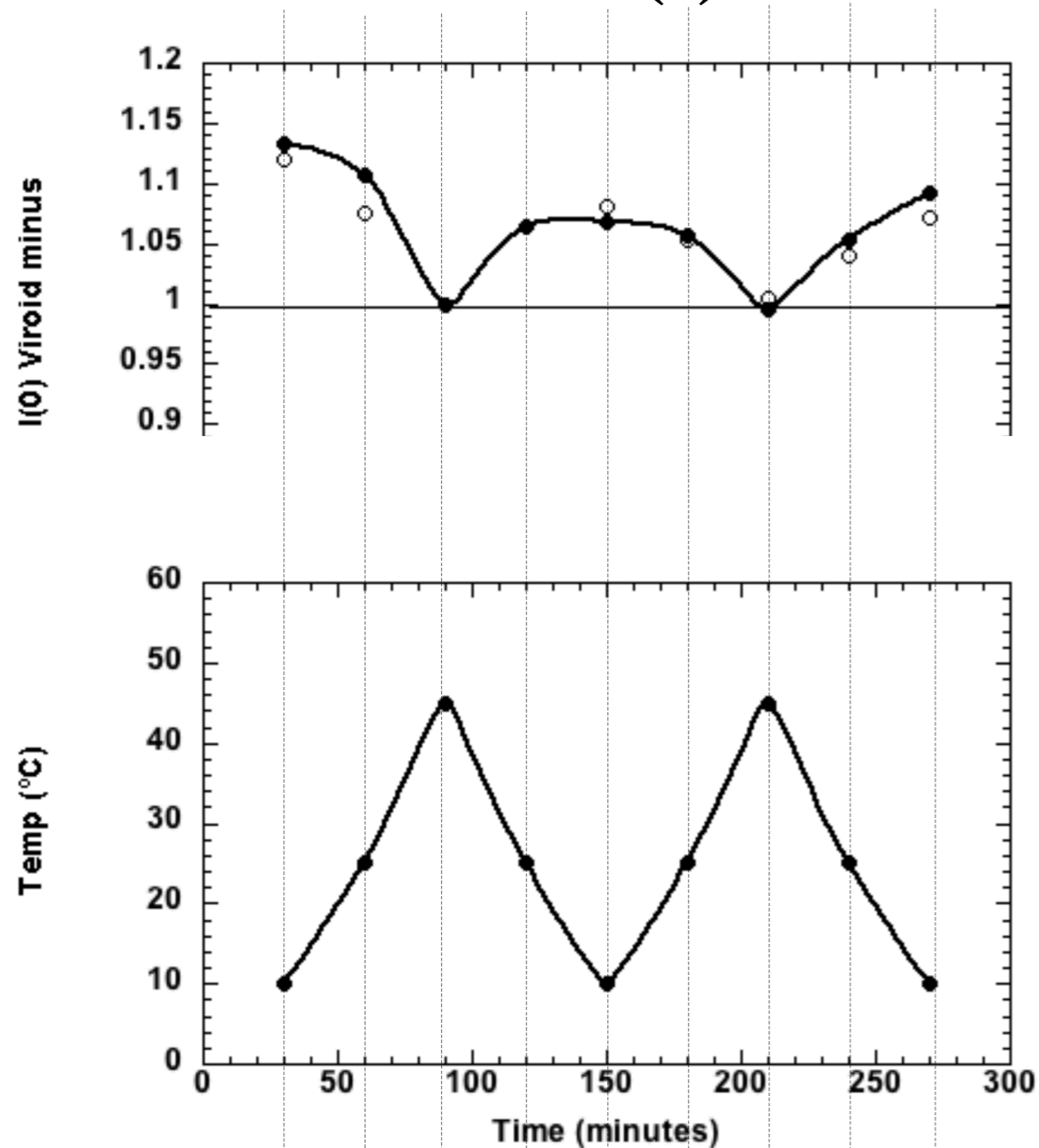


# ASBVd(-)/HHR(-): SANS

## Study

ASBVd(-)

HHR(-)



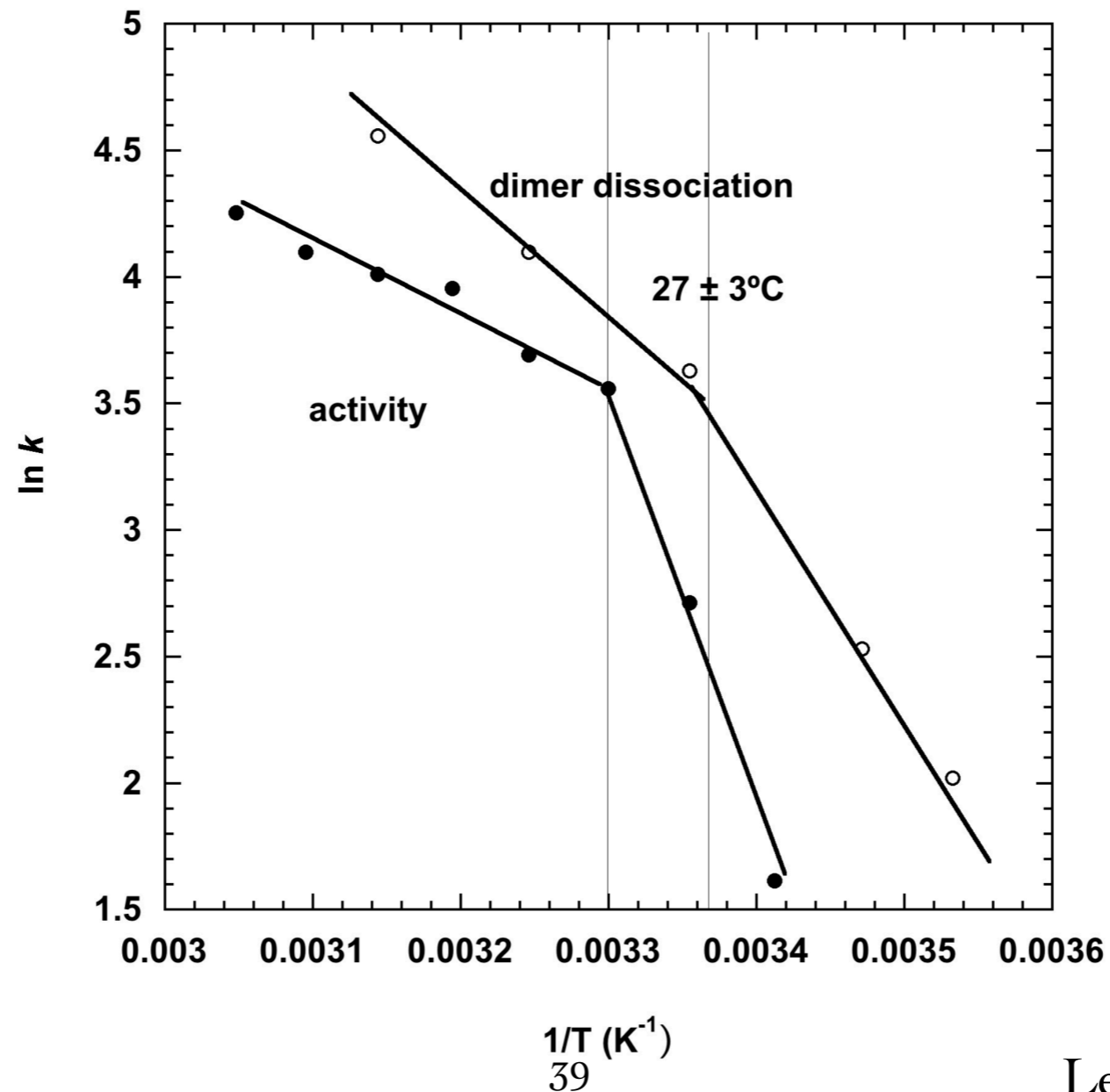
# HHR(-) dimerization/ catalysis

$E_a \sim 8\text{kcal/mol}$

$E_a \sim 6\text{kcal/mol}$

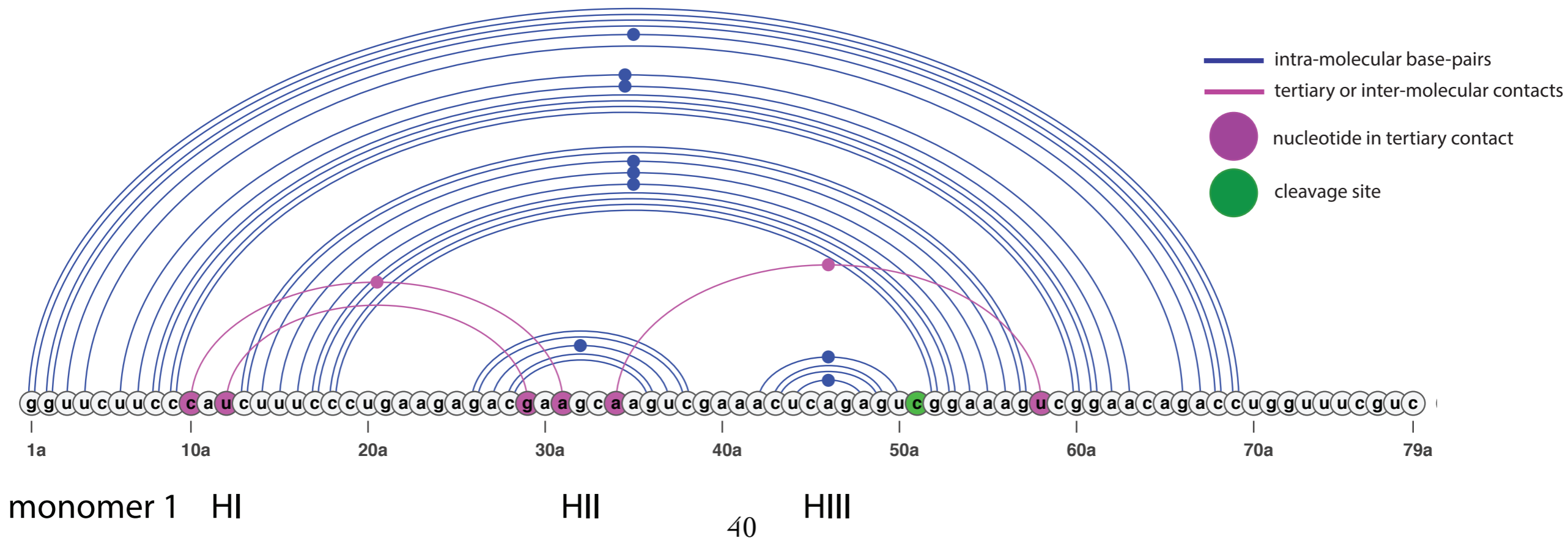
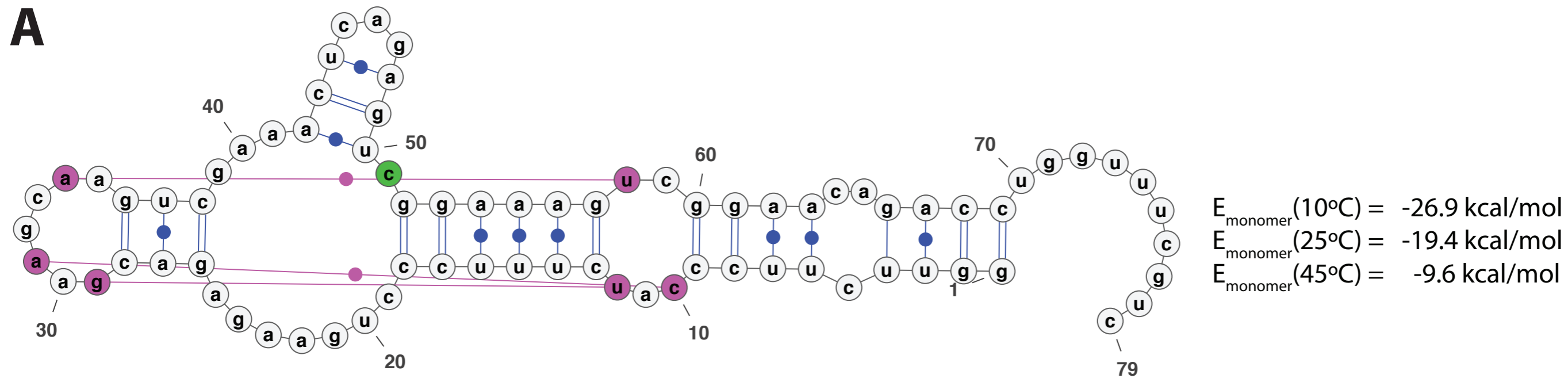
$E_a \sim 20\text{kcal/mol}$

$E_a \sim 30\text{kcal/mol}$

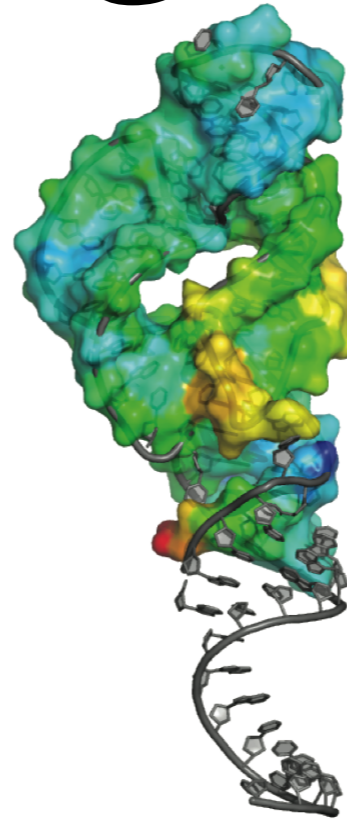
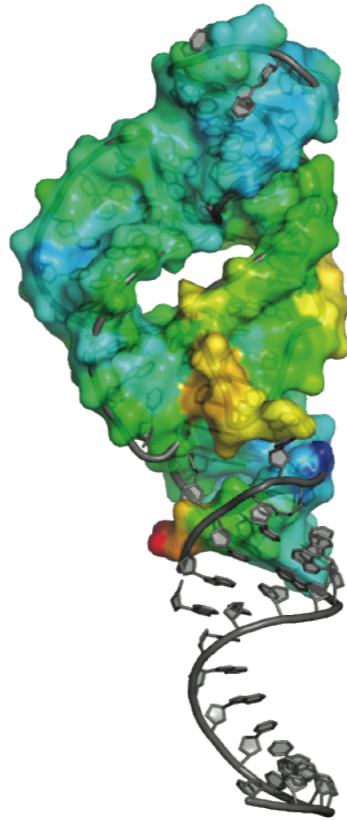
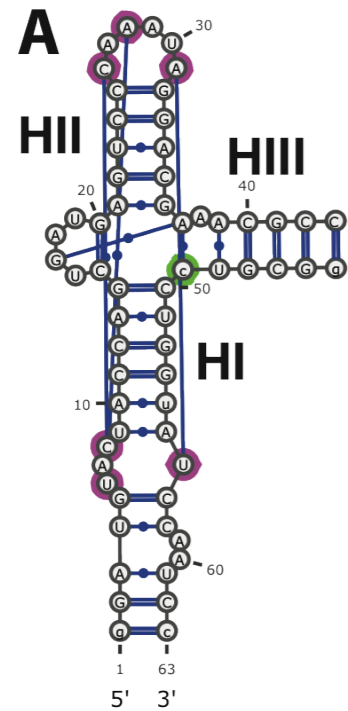


# HHR(-): 2D structures

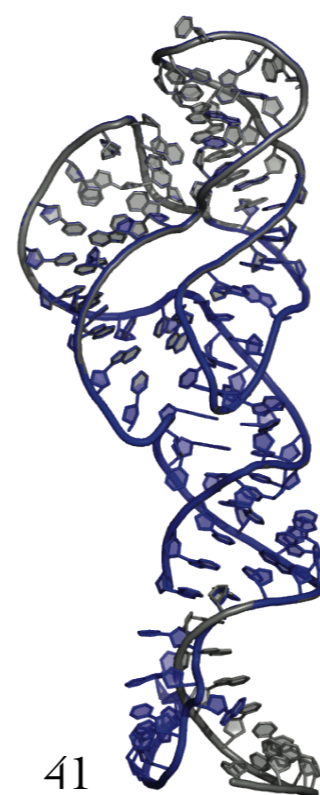
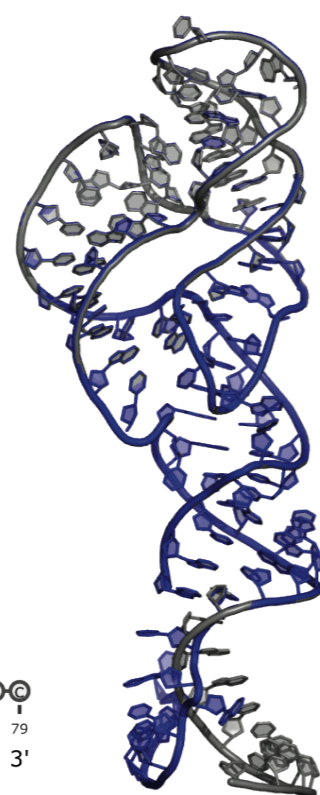
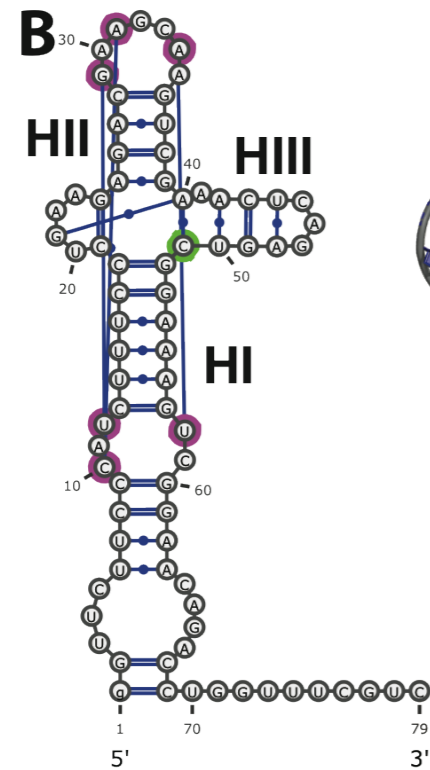
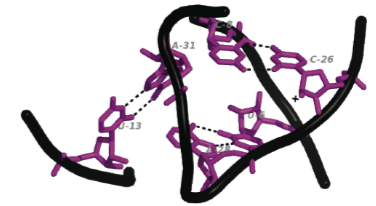
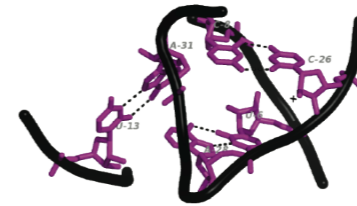
**A**



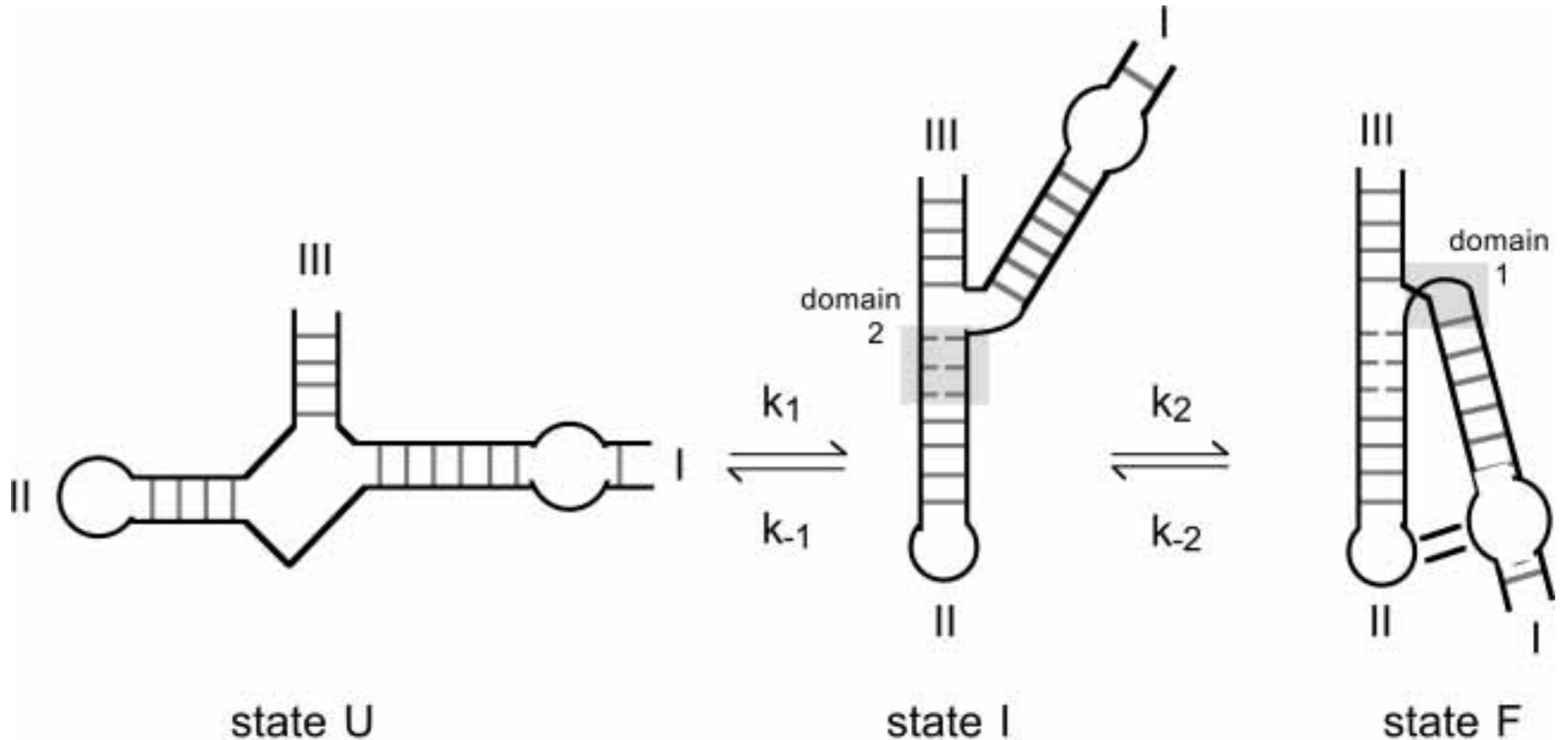
# 3D Modeling of HHR(-)



loops HI-HIII



# HHR folding



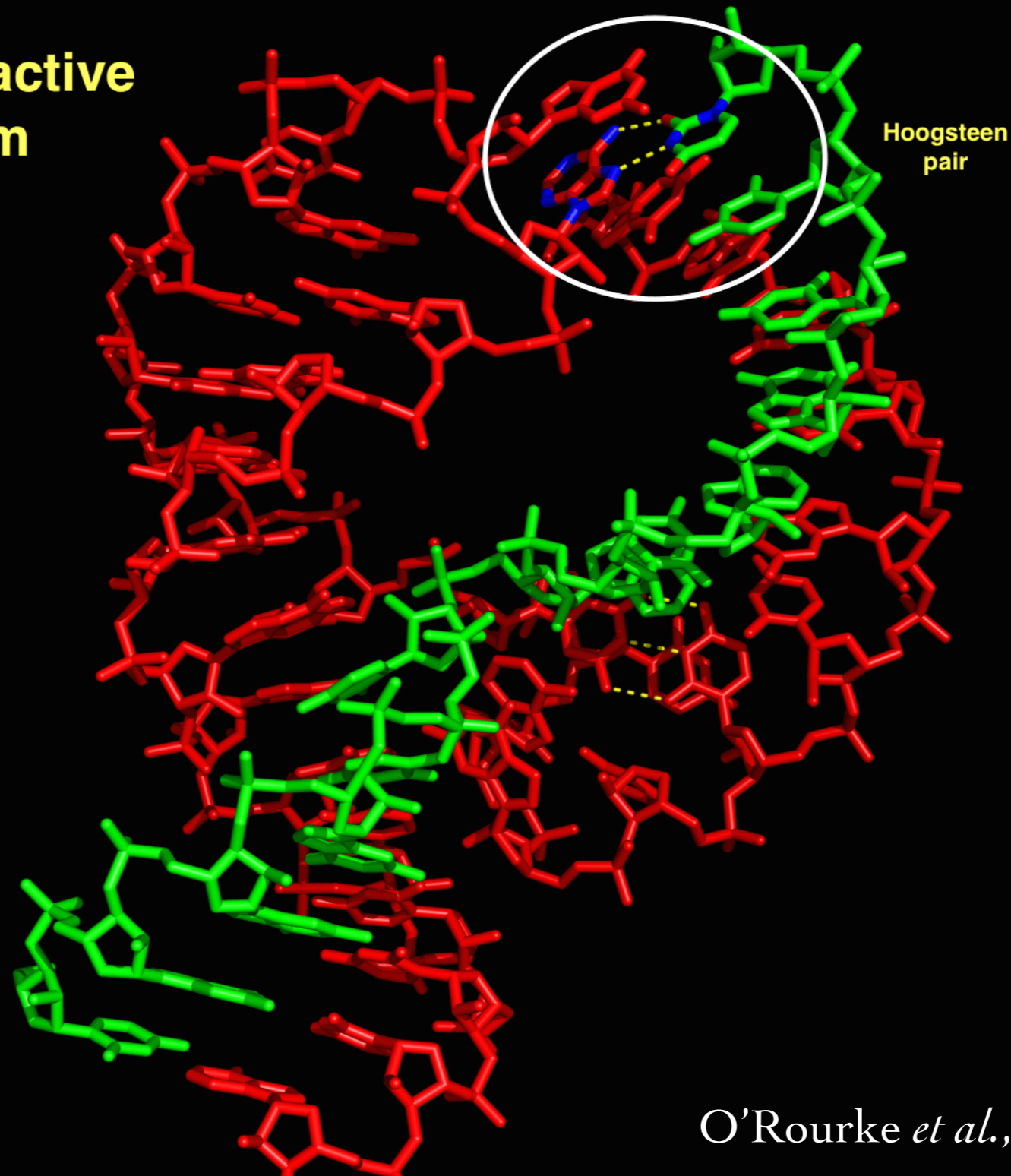
$$R_g(I)/(F) \sim 1.1$$

$$\text{ASBVd: } R_g(I)/(F) \sim 1.2$$

Penedo *et al.*, RNA, 2004.

# HHR morphing

Highly active form



O'Rourke *et al.*, JMB, 2015

$R_g(I)/(F) \sim 1.1$

43

ASBVd:  $R_g(I)/(F) \sim 1.2$

# Modeling/SANS

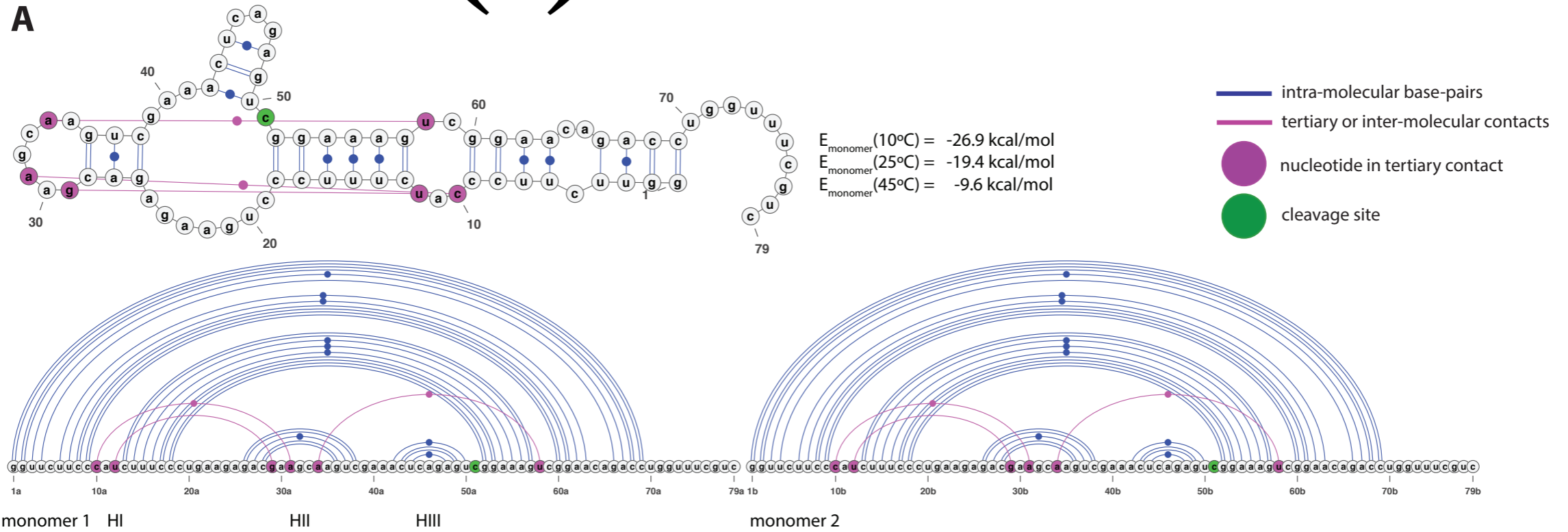
$$R_g^{\text{exp}} = 31 \text{ \AA}$$
$$R_g^{*\text{calc}} = 31 (26) \text{ \AA}$$



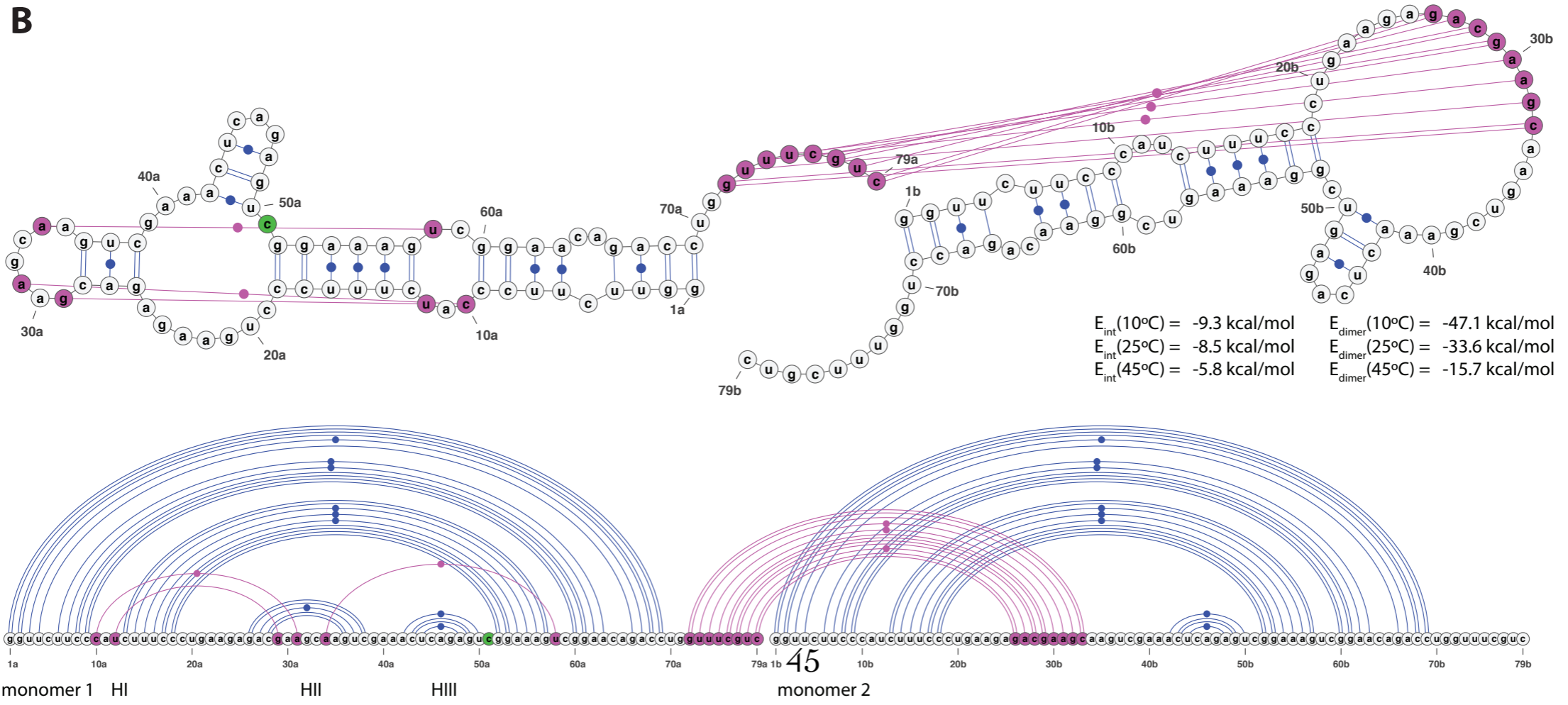
$$d^{\text{exp}} = 96.0 \text{ \AA}$$
$$d^{\text{calc}} = 96.7 \text{ \AA}$$

# HHR (-): dimerization

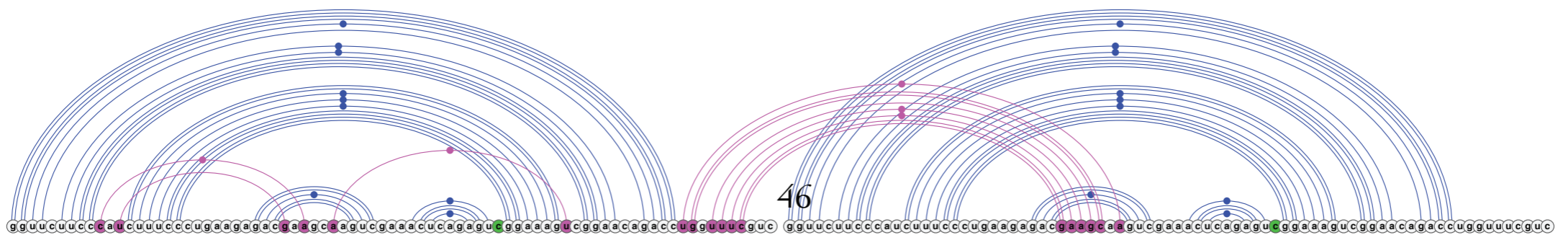
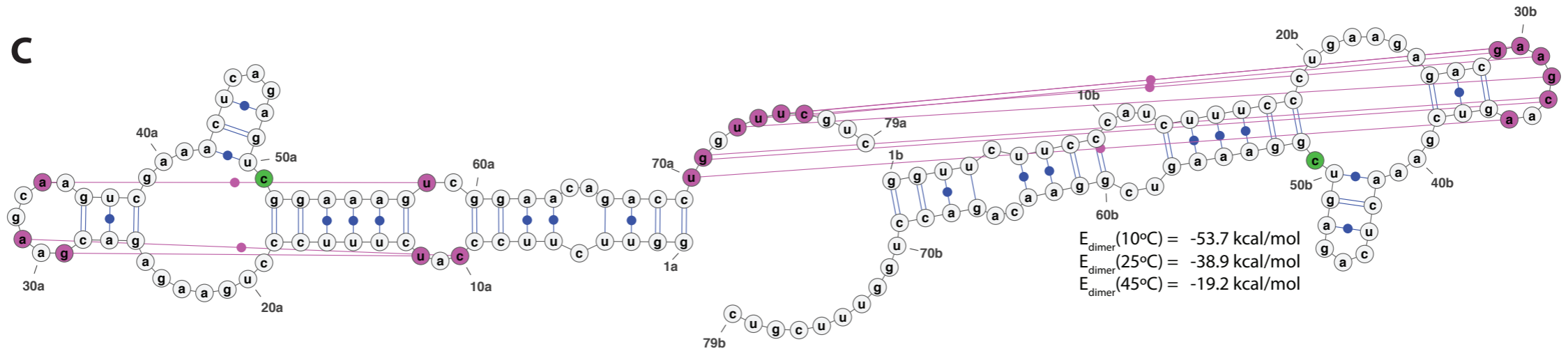
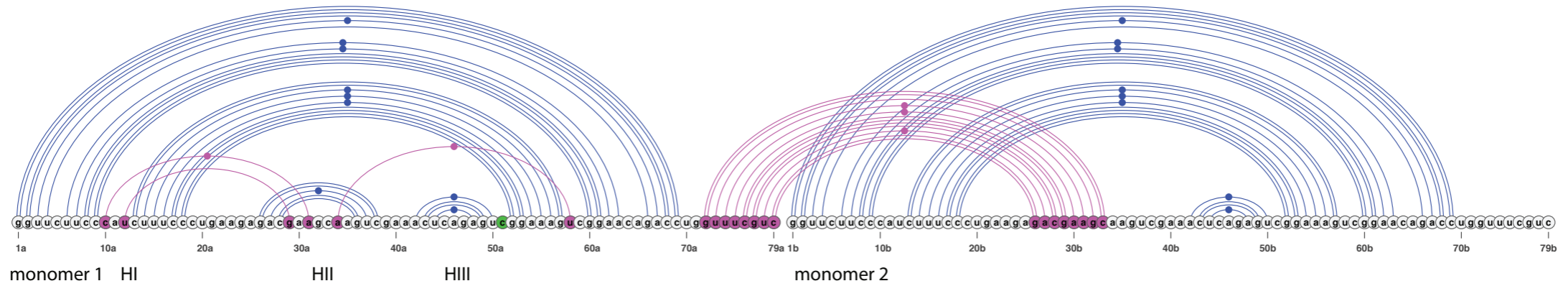
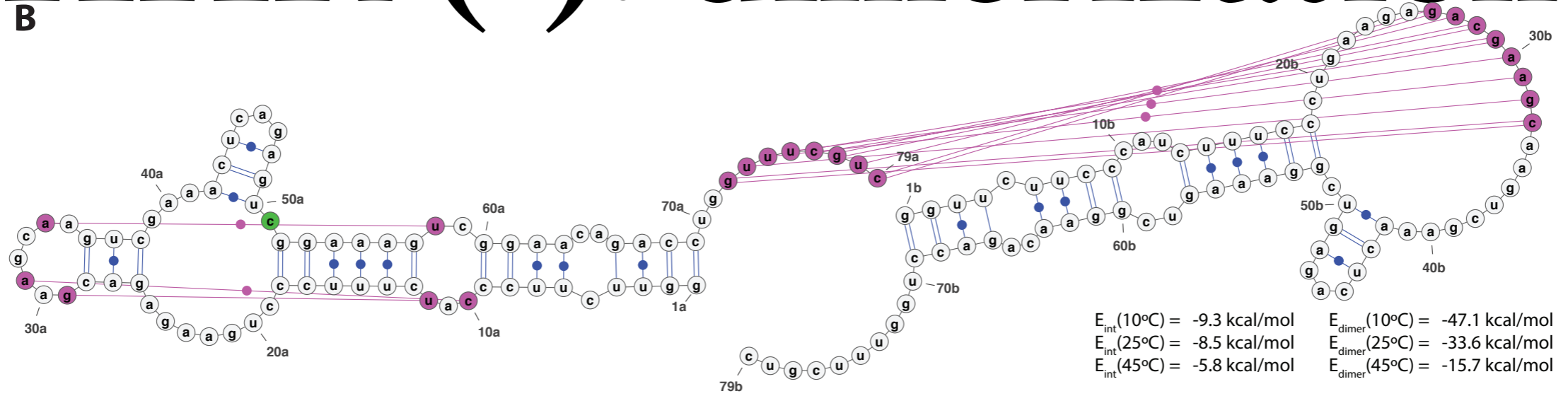
**A**



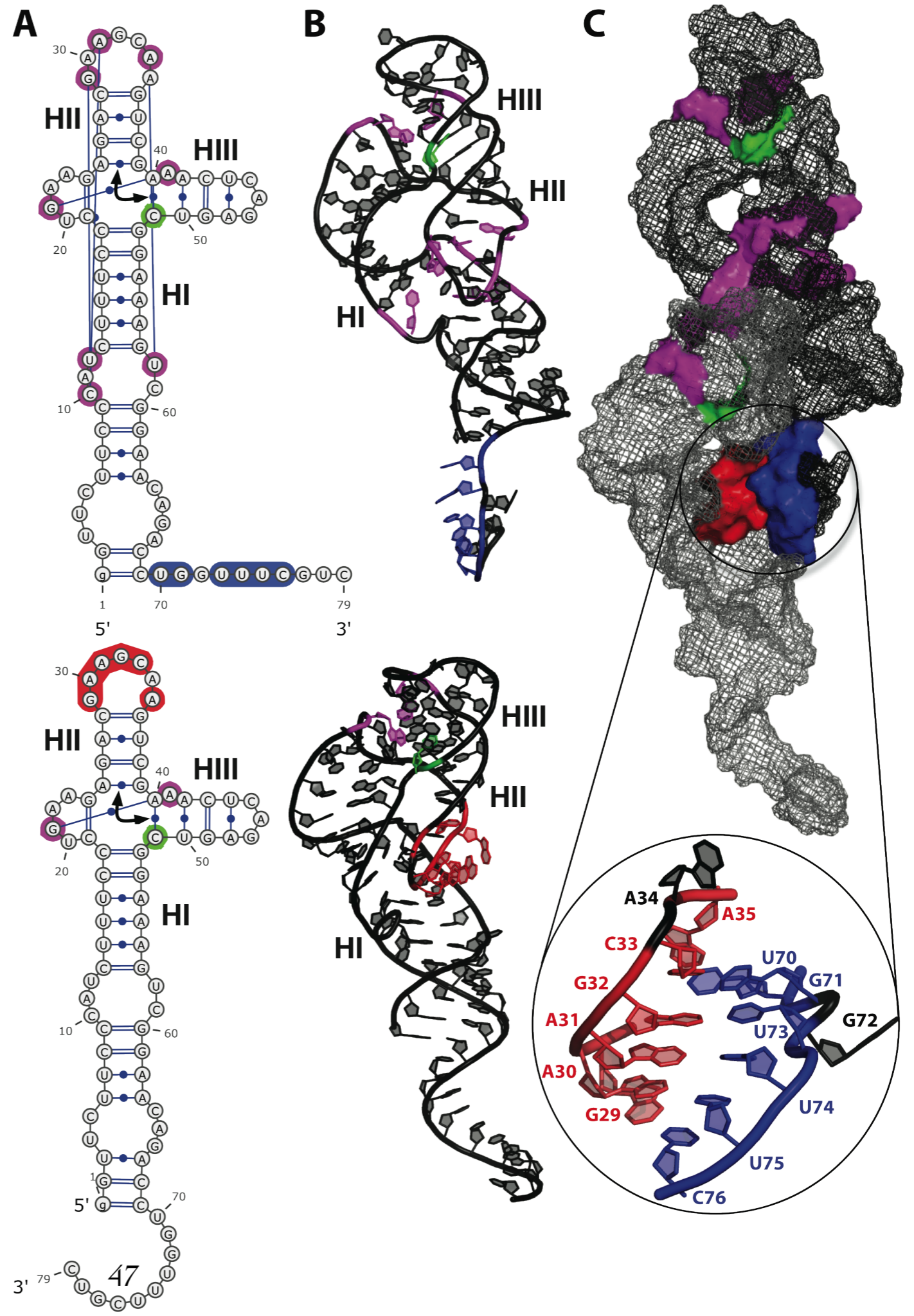
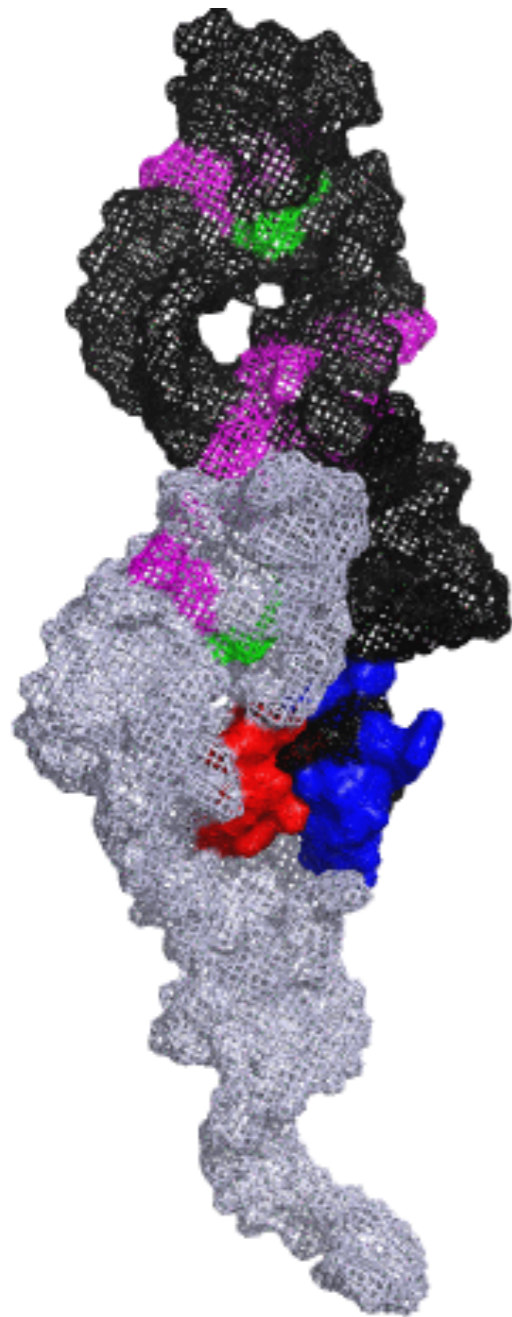
**B**



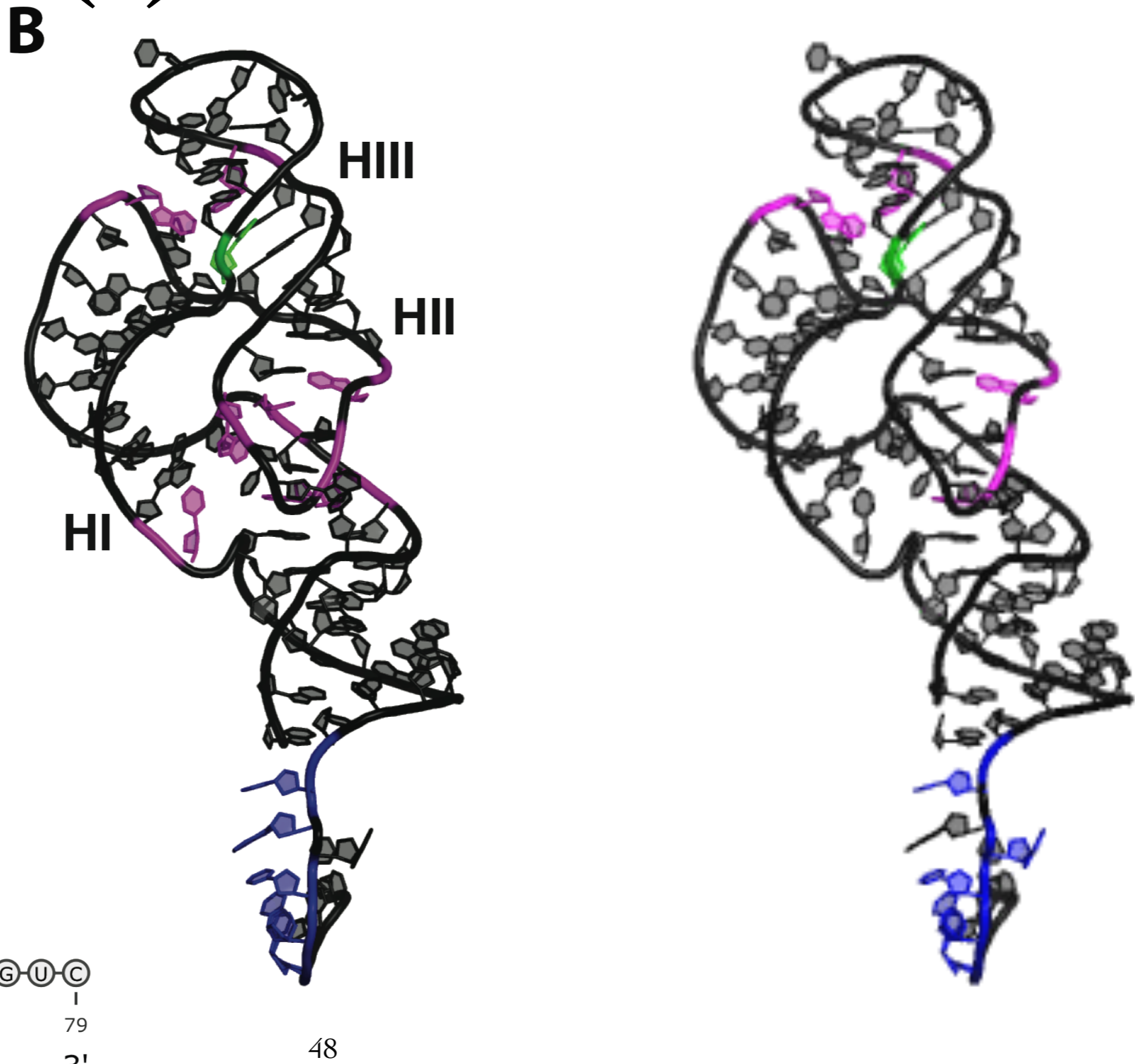
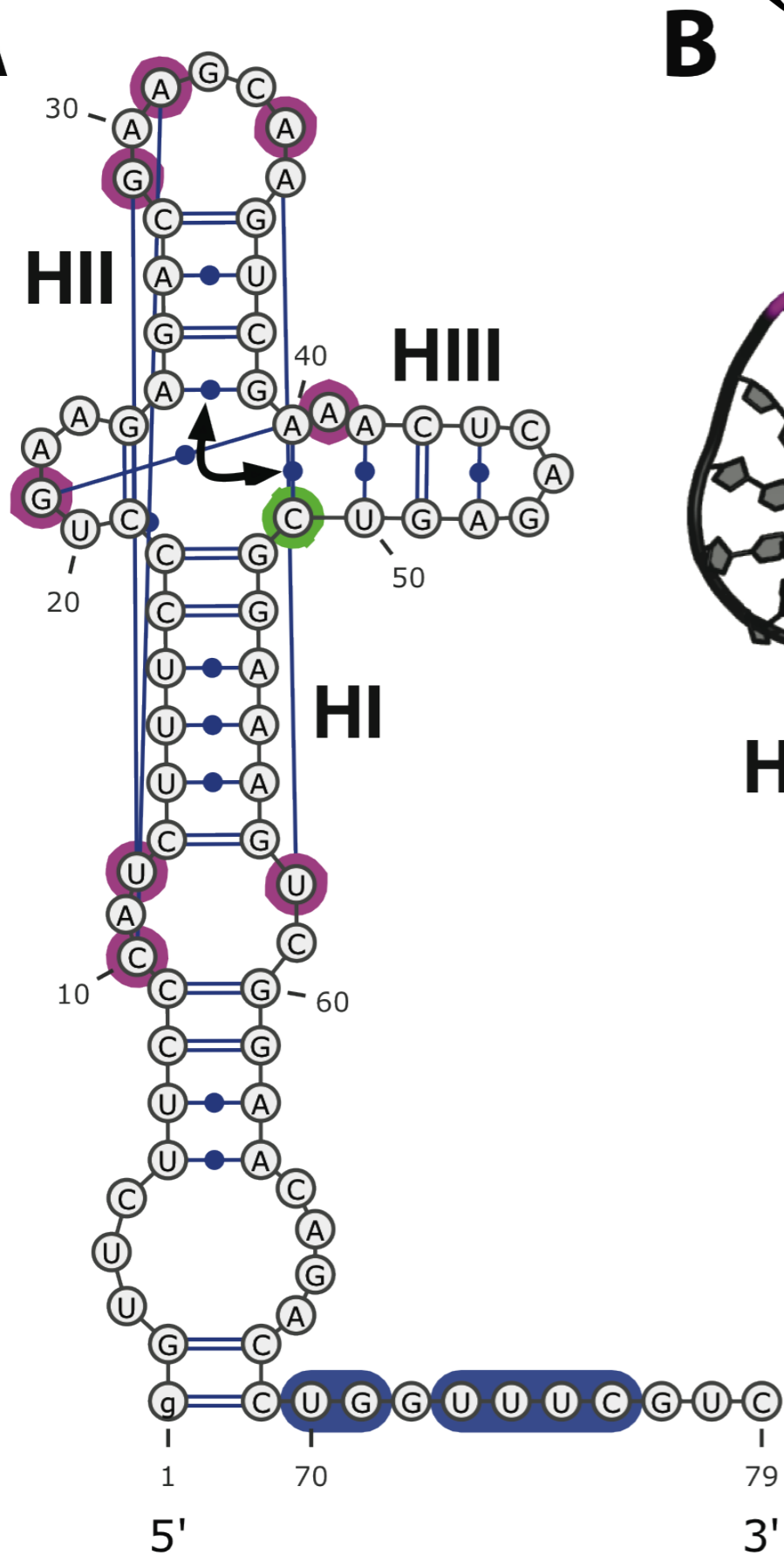
# HHR (-): dimerization



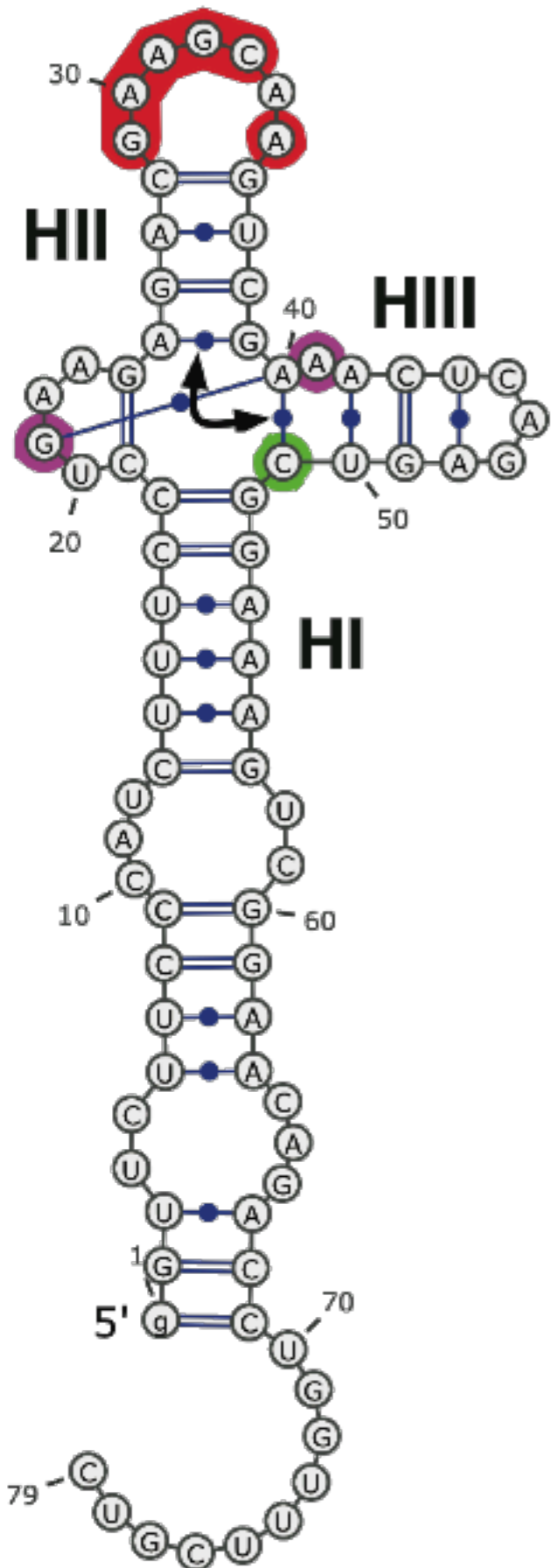
# HHR(-) dimer



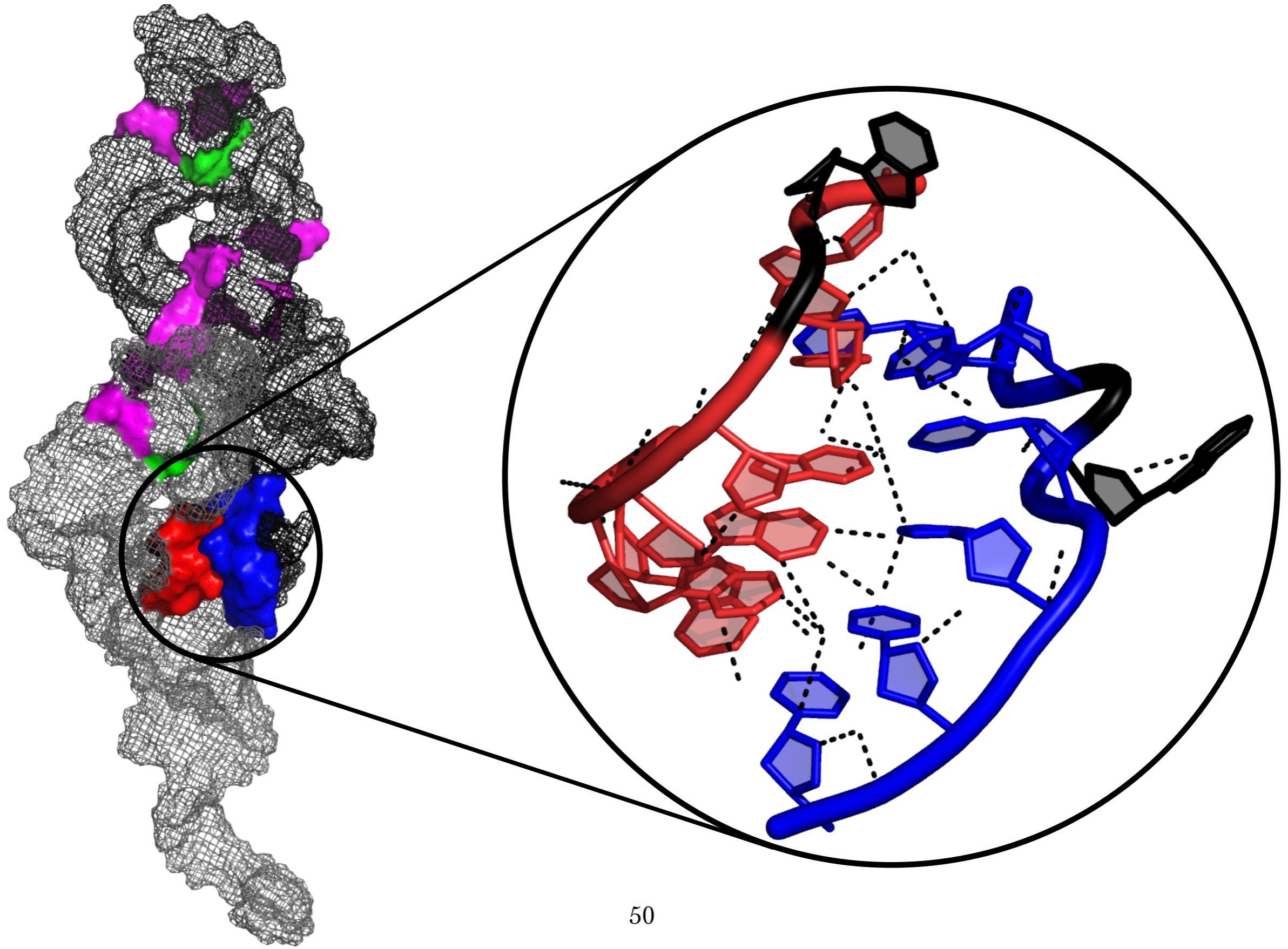
# HHR(-): monomer 1



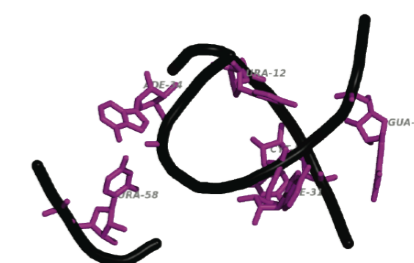
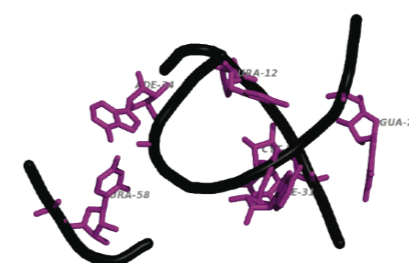
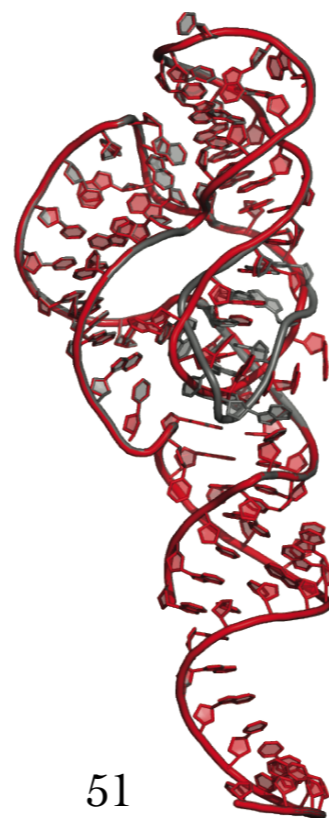
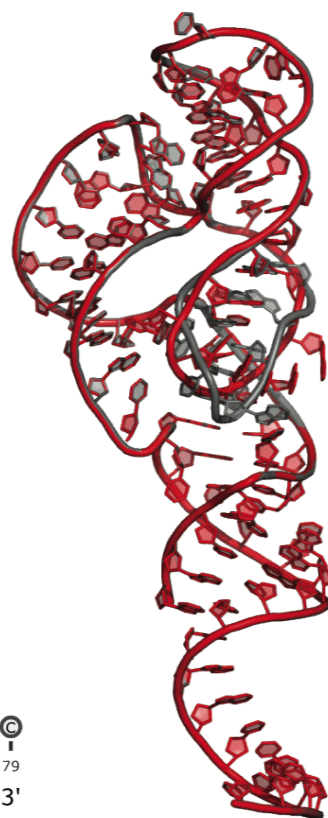
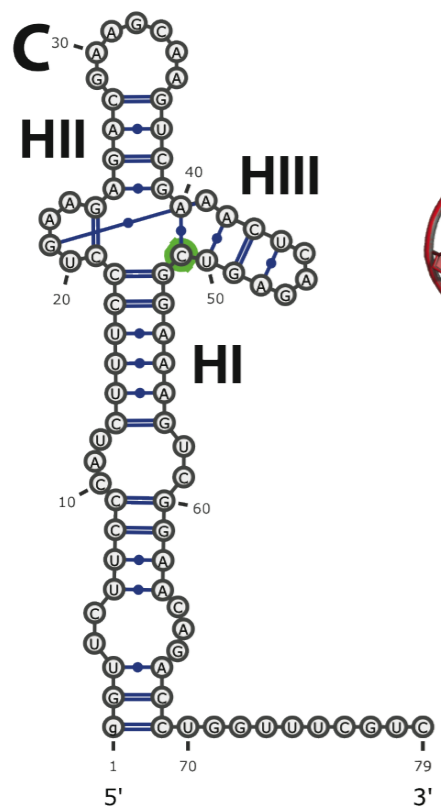
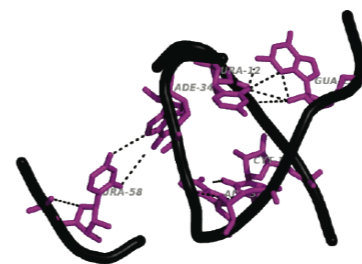
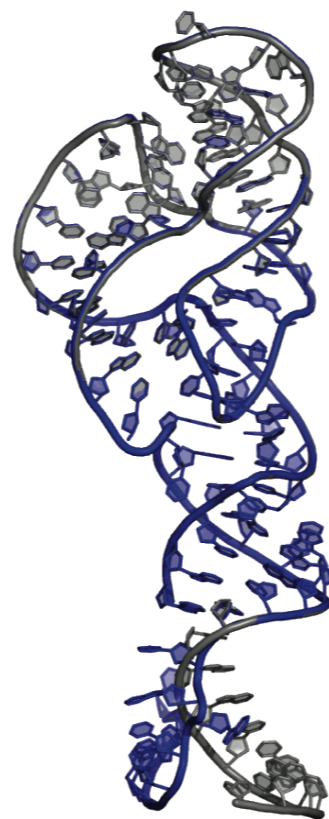
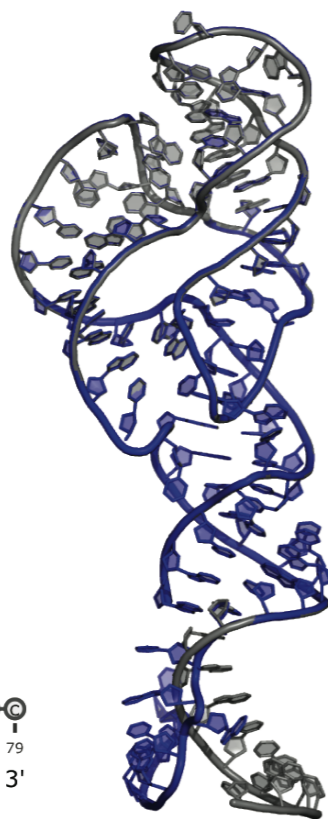
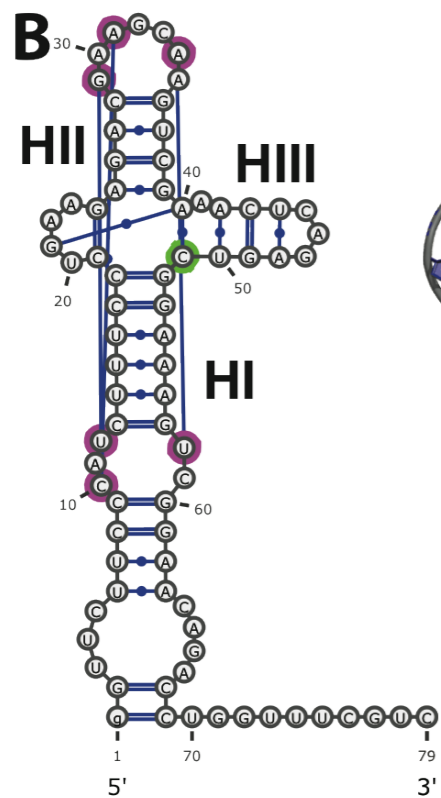
# HHR(-): monomer 2



# HHR(-): monomer seeds

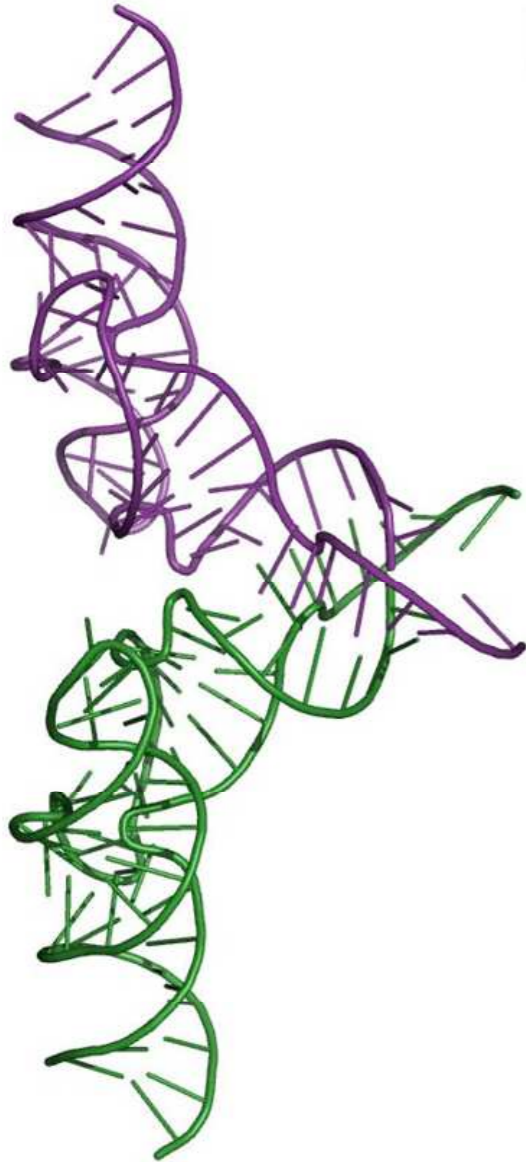


# HHR(-) monomers



# HHR: Monomer & Dimer

A



B



C



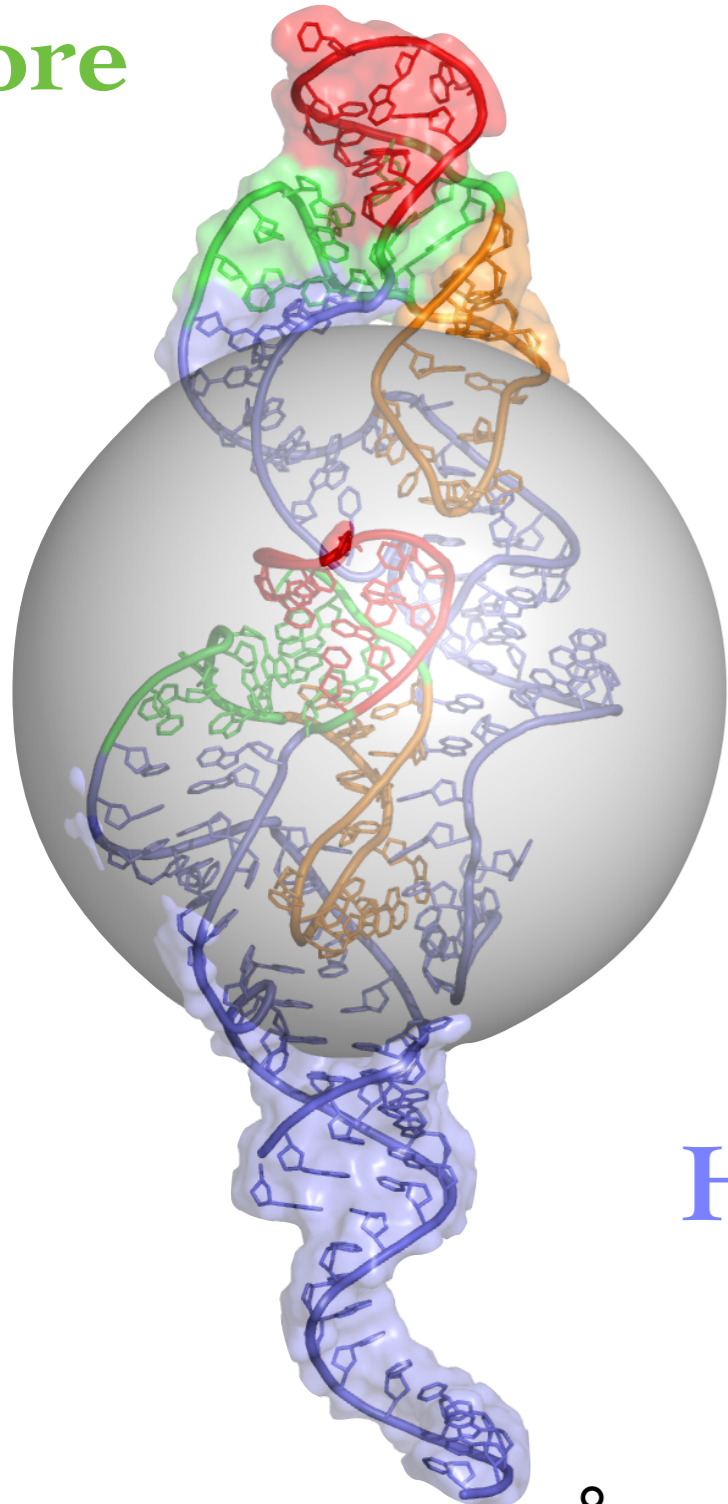
PDB ID:

# R<sub>g</sub> & self-association

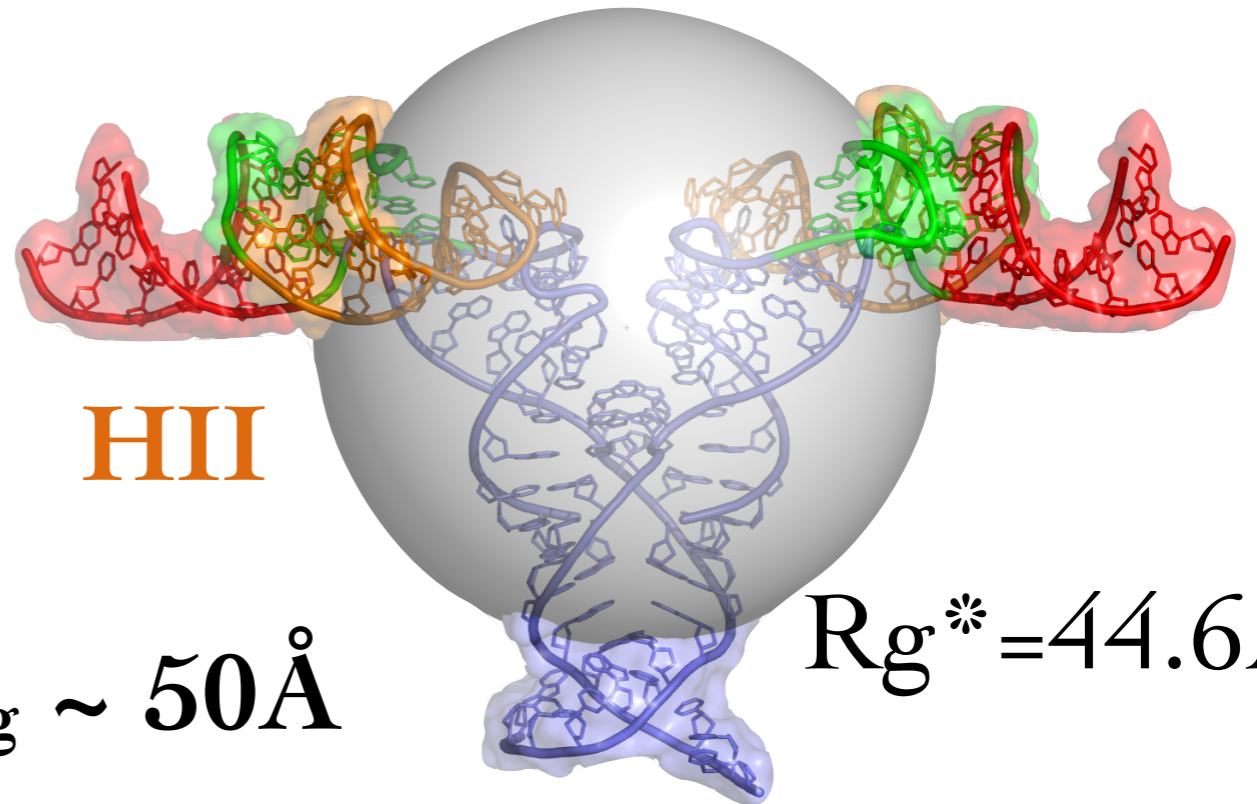
HI/HII

HI/HI

core



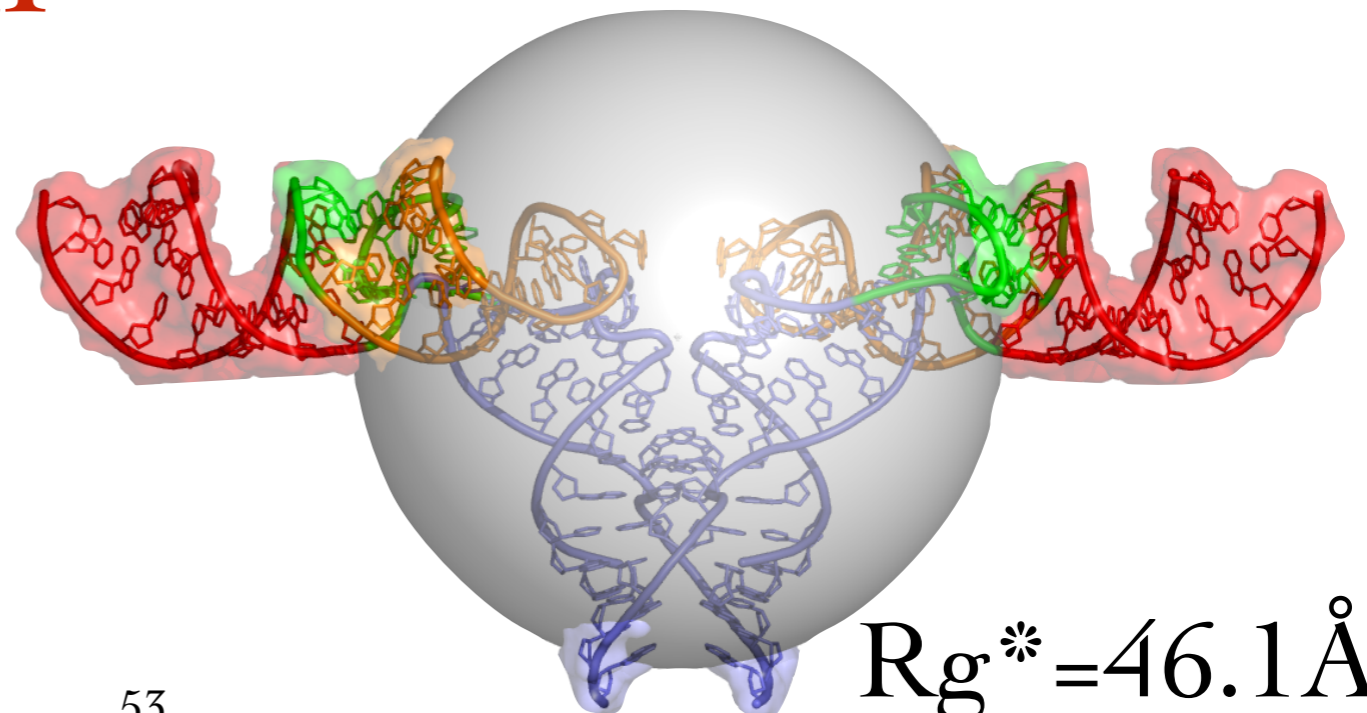
$R_g^* = 50.4 \text{ \AA}$



$R_g^* = 44.6 \text{ \AA}$

$R(\text{exp})_g \sim 50 \text{ \AA}$

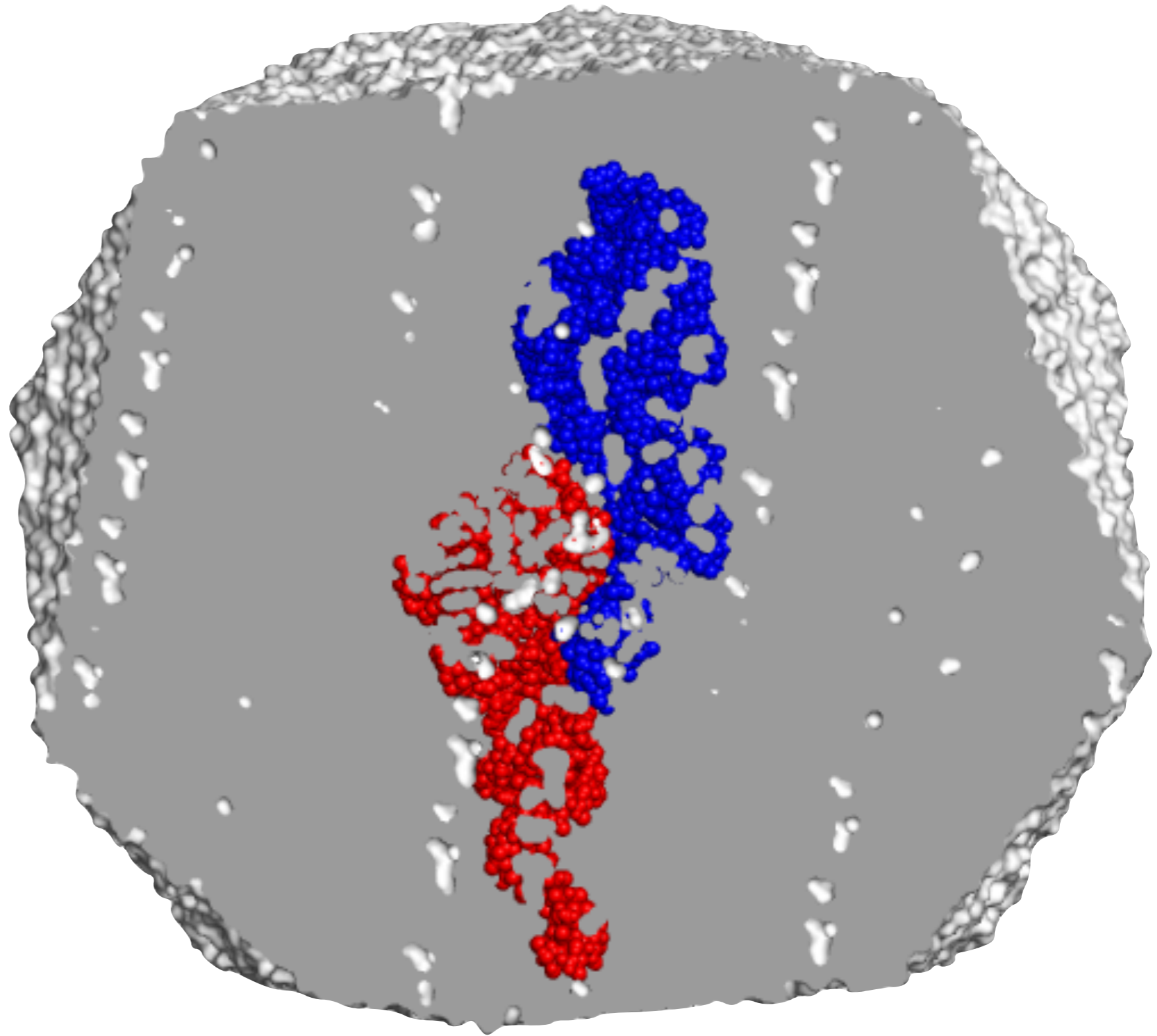
HIII



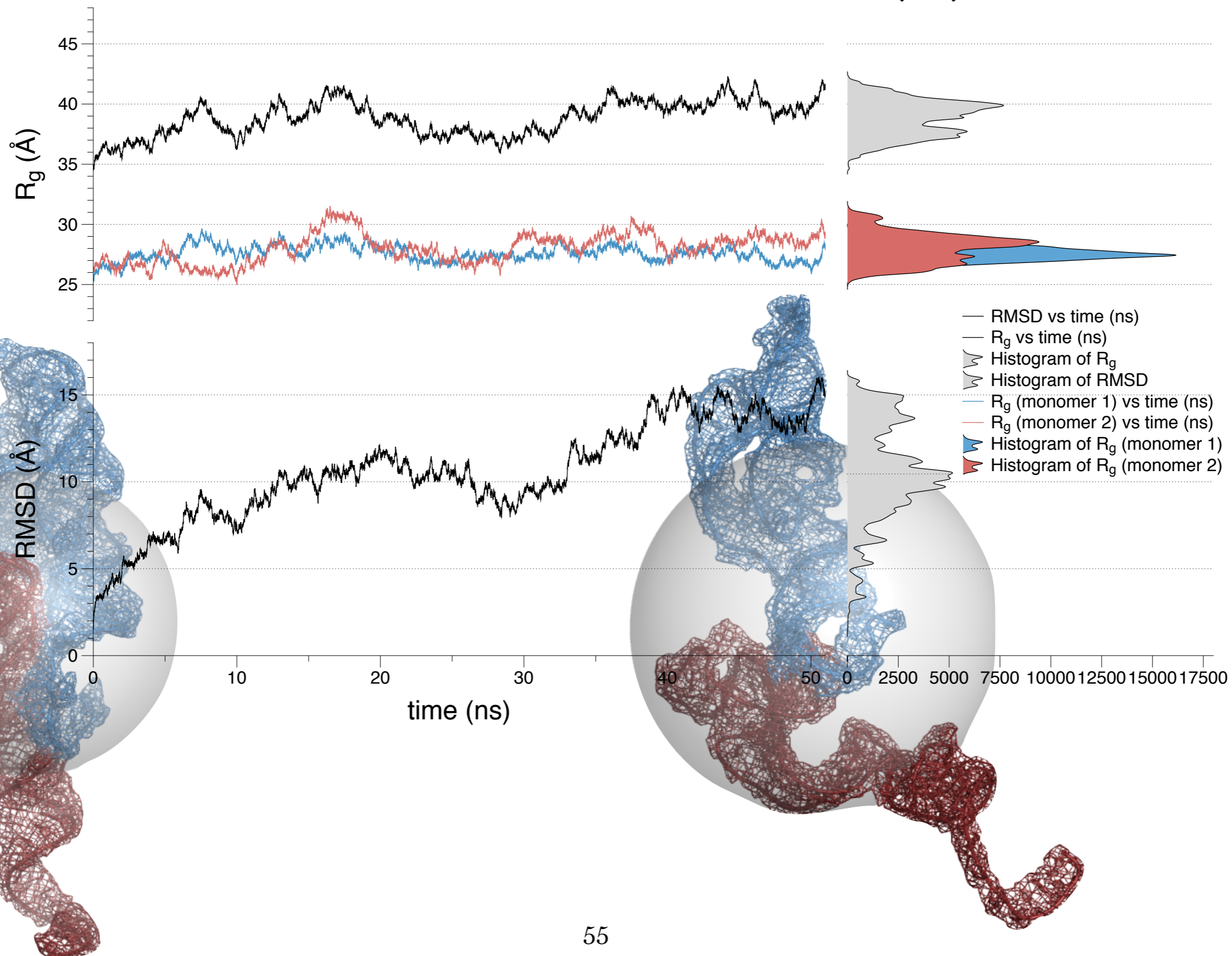
$R_g^* = 46.1 \text{ \AA}$

# MD setup for HHR dimer

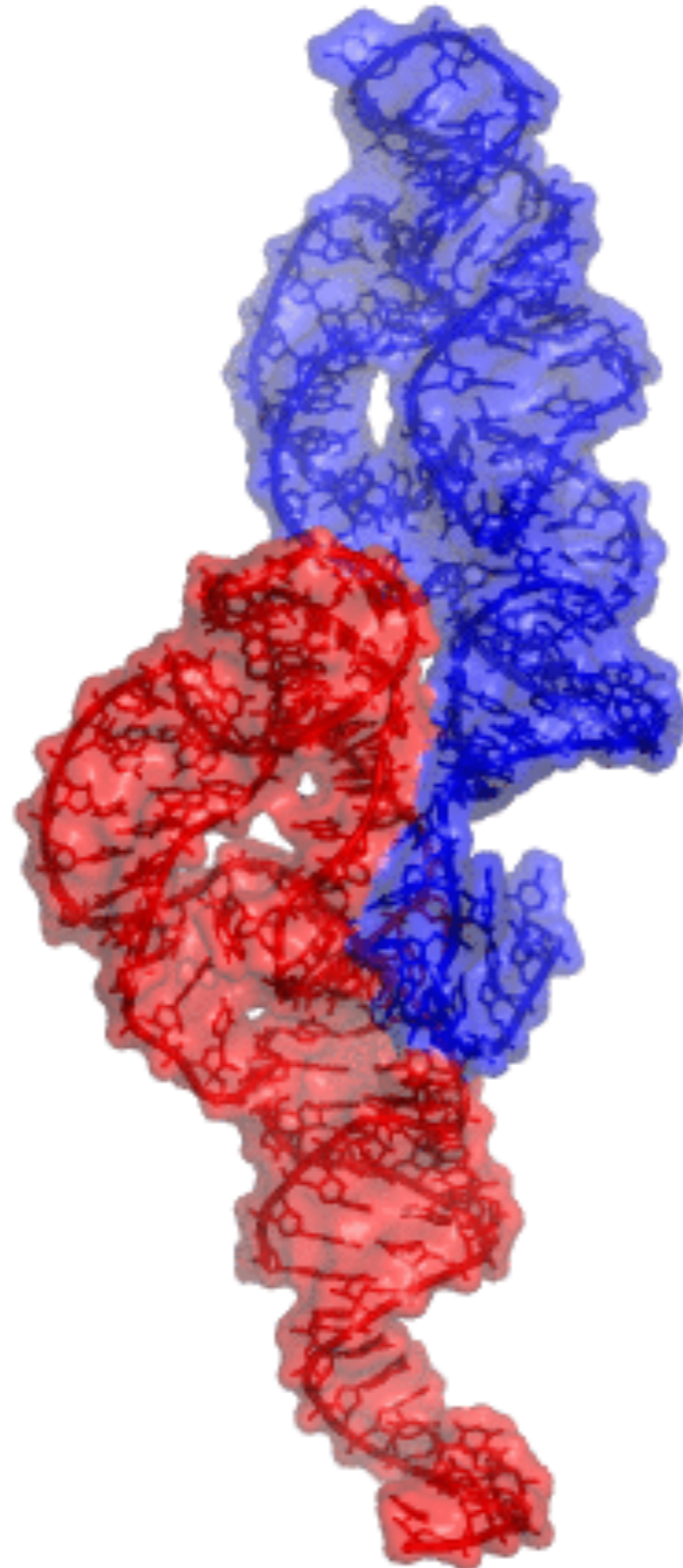
- $\sim 300\,000$  atoms
- $K^+$  &  $Cl^-$  (0.15M)
- $T = (283K, 313K)$
- NAMMD



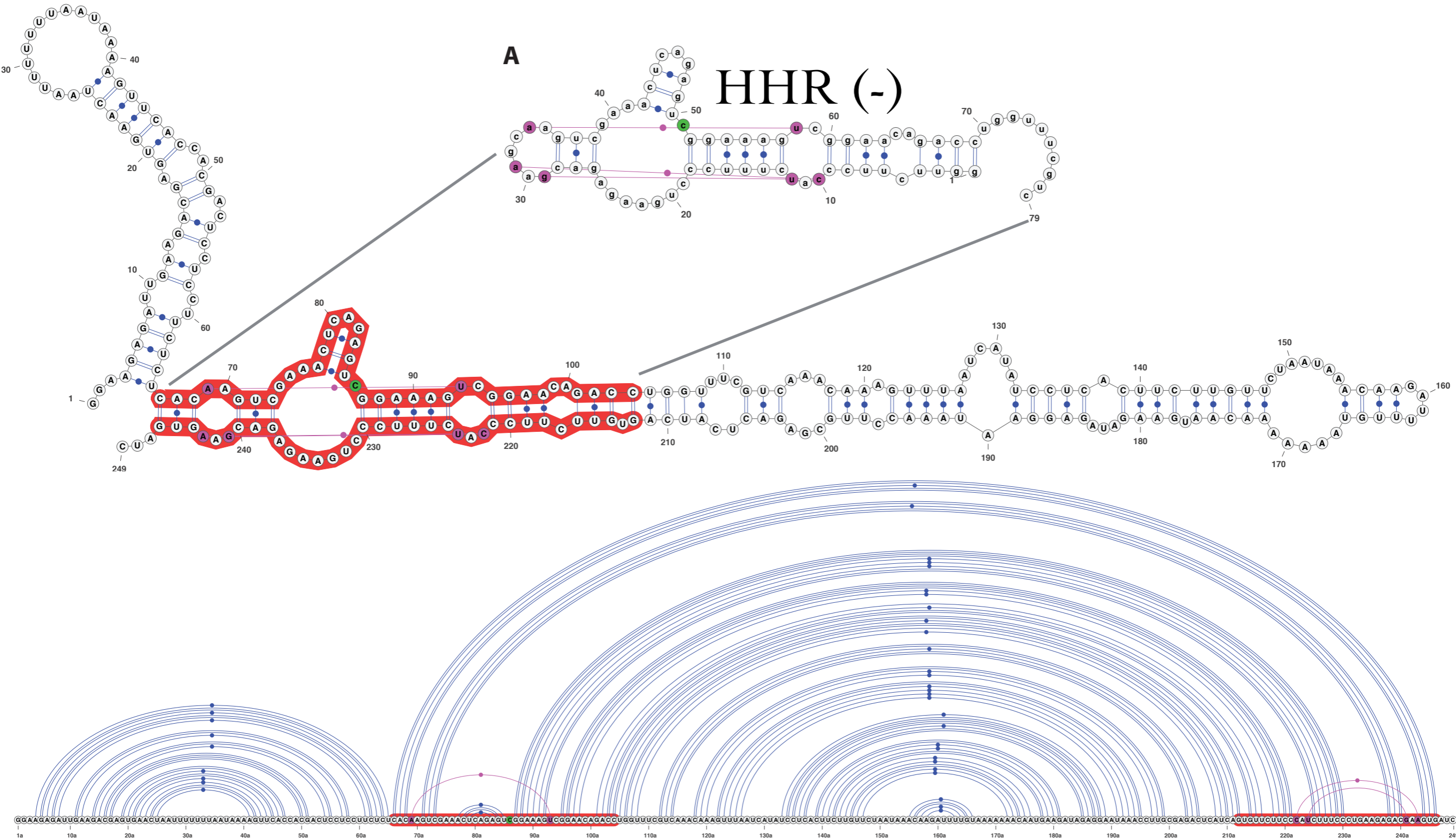
# Dynamics of HHR(-) dimer



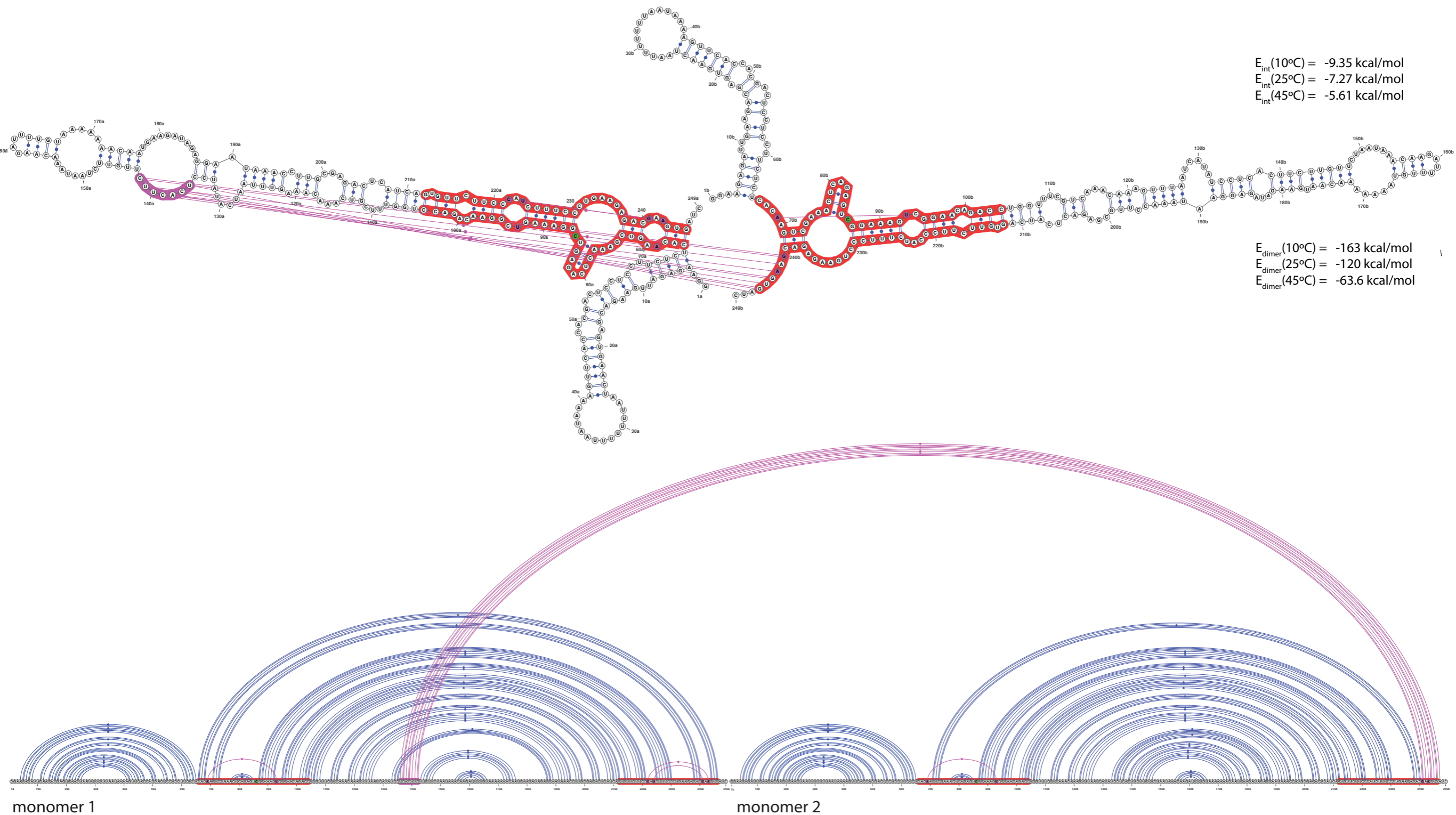
# HHH dimer: MD trajectory



# ASBVd(-): self-association



# ASBVd(-): self-association



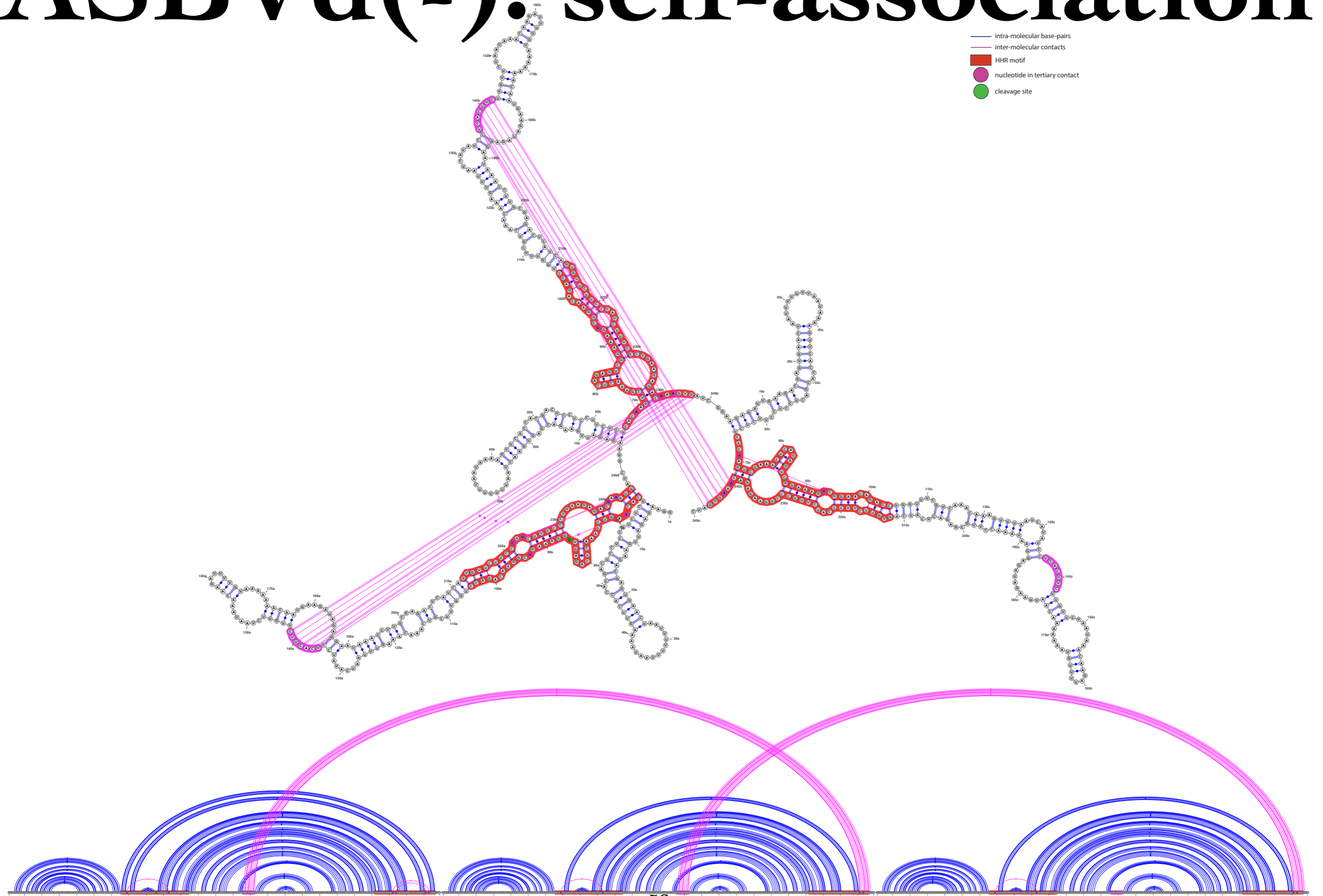
$E_{int}(10^{\circ}\text{C}) = -9.35 \text{ kcal/mol}$   
 $E_{int}(25^{\circ}\text{C}) = -7.27 \text{ kcal/mol}$   
 $E_{int}(45^{\circ}\text{C}) = -5.61 \text{ kcal/mol}$

$E_{dimer}(10^{\circ}\text{C}) = -163 \text{ kcal/mol}$   
 $E_{dimer}(25^{\circ}\text{C}) = -120 \text{ kcal/mol}$   
 $E_{dimer}(45^{\circ}\text{C}) = -63.6 \text{ kcal/mol}$

monomer 1

monomer 2

# ASBVd(-): self-association



# What did we learn ?

- for RNA and ribozymes: « too short » may be bad
- don't forget about dynamics
- SANS & modeling approaches to infer self-association modes
- theoretical approaches to explore reaction mechanisms and pathways

# Viroids: Plant Parasites

Genus *Pospiviroids*:  
PSTVd (potato spindle tuber)



Genus *Hostuviroids*:  
HSVd (hop stunt)



Genus *Cocadviroids*:  
CCCVd (coconut cadang-cadang)



Genus *Apscaviroids*:  
ASSVd (apple scar skin)



Genus *Coleviroids*:  
CbVd 1 (coleus blumei 1)



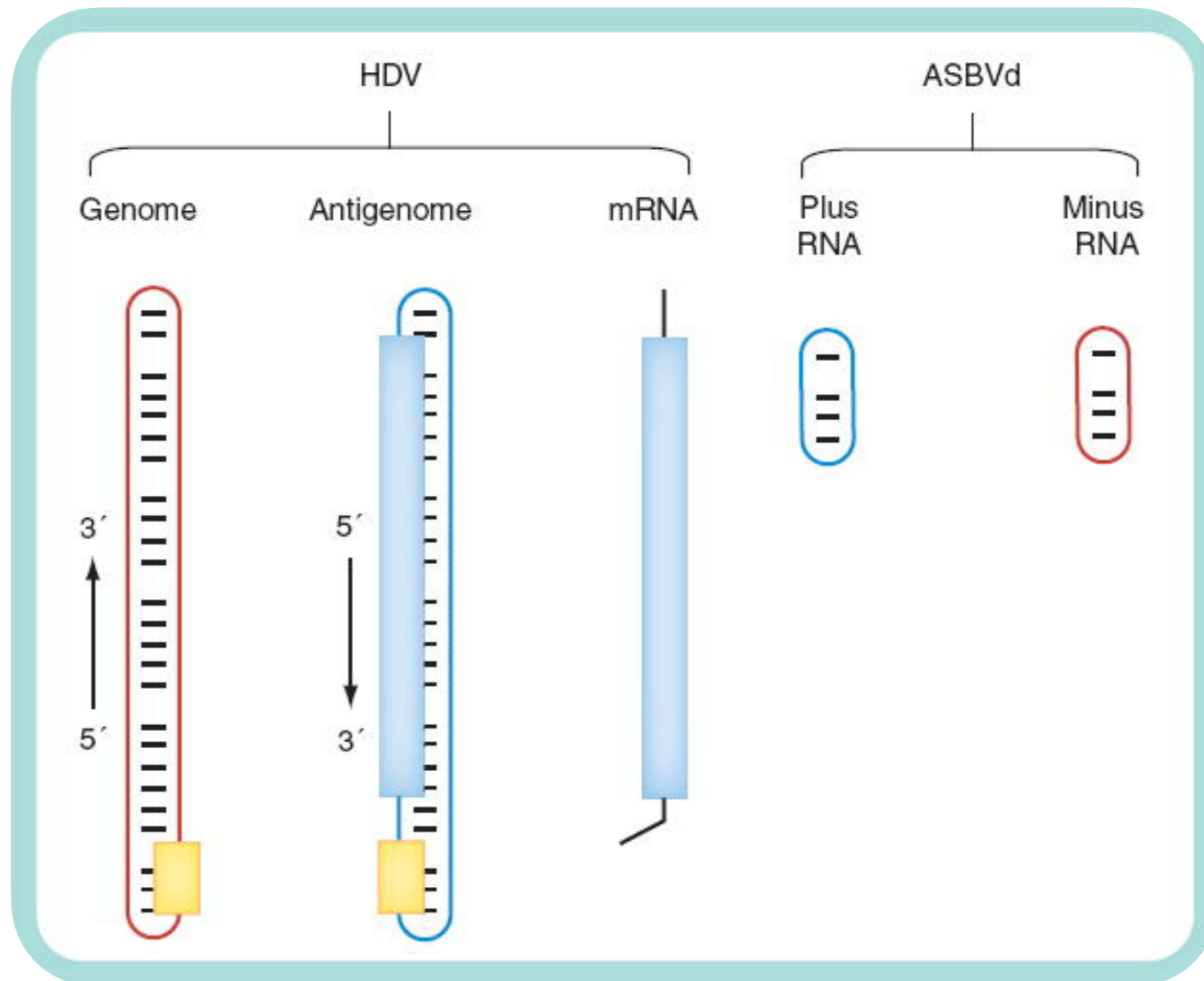
Genus *Avsunviroids*:  
ASBVd (avocado sunblotch)



Genus *Pelamoviroids*:  
PLMVd (peach latent mosaic)

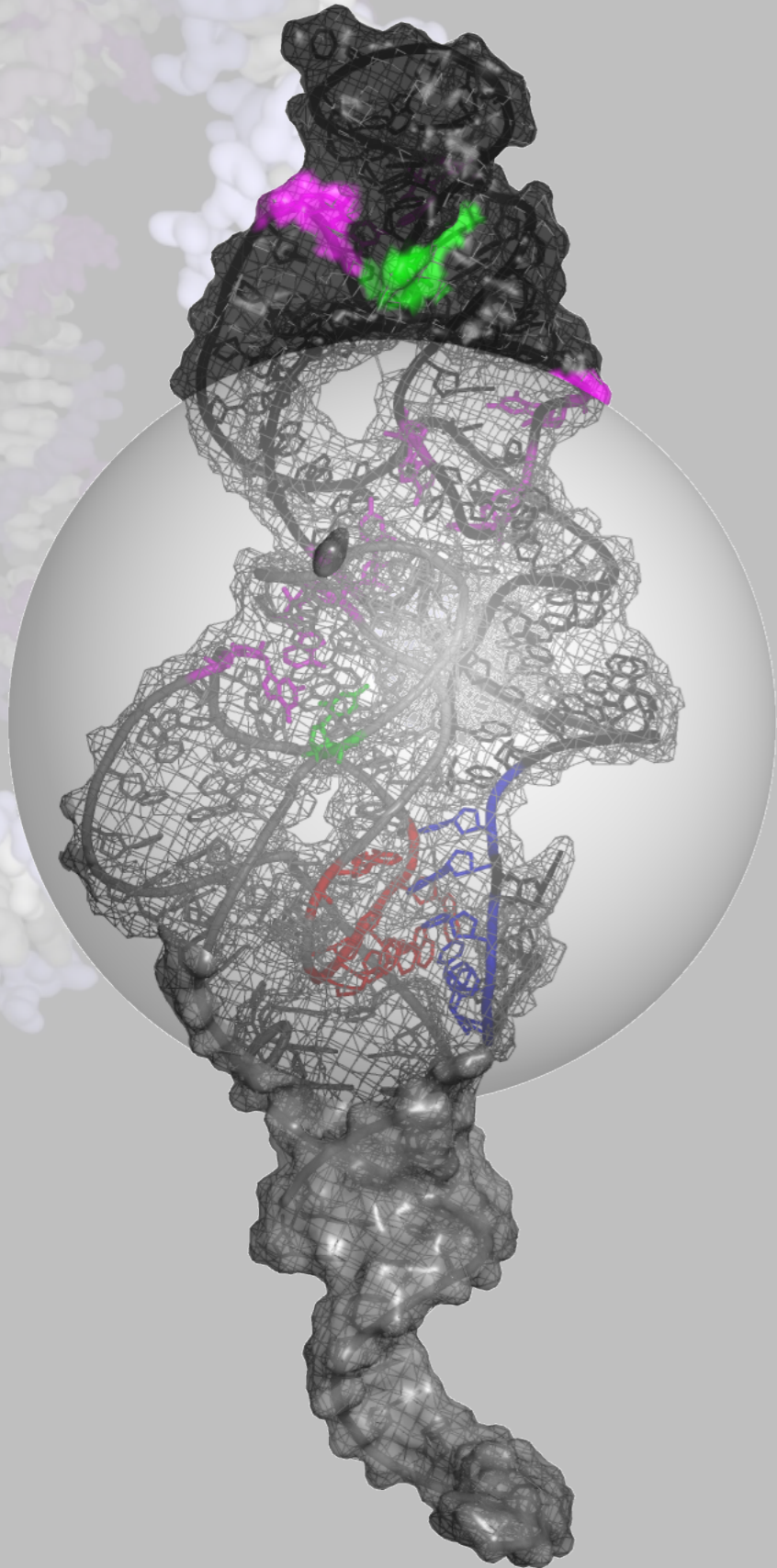


# Viroid & HDV: Hepatitis D



# Acknowledgments

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- Xavier Lopez (Euskal Herriko Unibertsitatea, SP)
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- Martin Karplus (Harvard University, USA)
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# Elements

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## Origins of Life: Transition from Geochemistry to Biogeochemistry

NITA SAHAI and HUSSEIN KADDOUR, Guest Editors

Transition from Geochemistry to Biogeochemistry

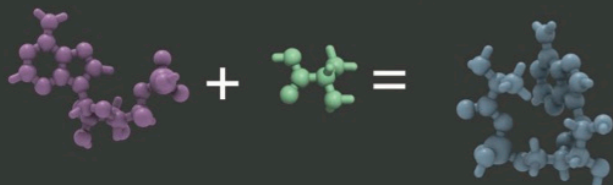
Staging Life: Early Ocean

Incubating Life: Prebiotic Sources

Foundation Stones to Life

Metal Catalysts

Protometabolism and Early Protocells



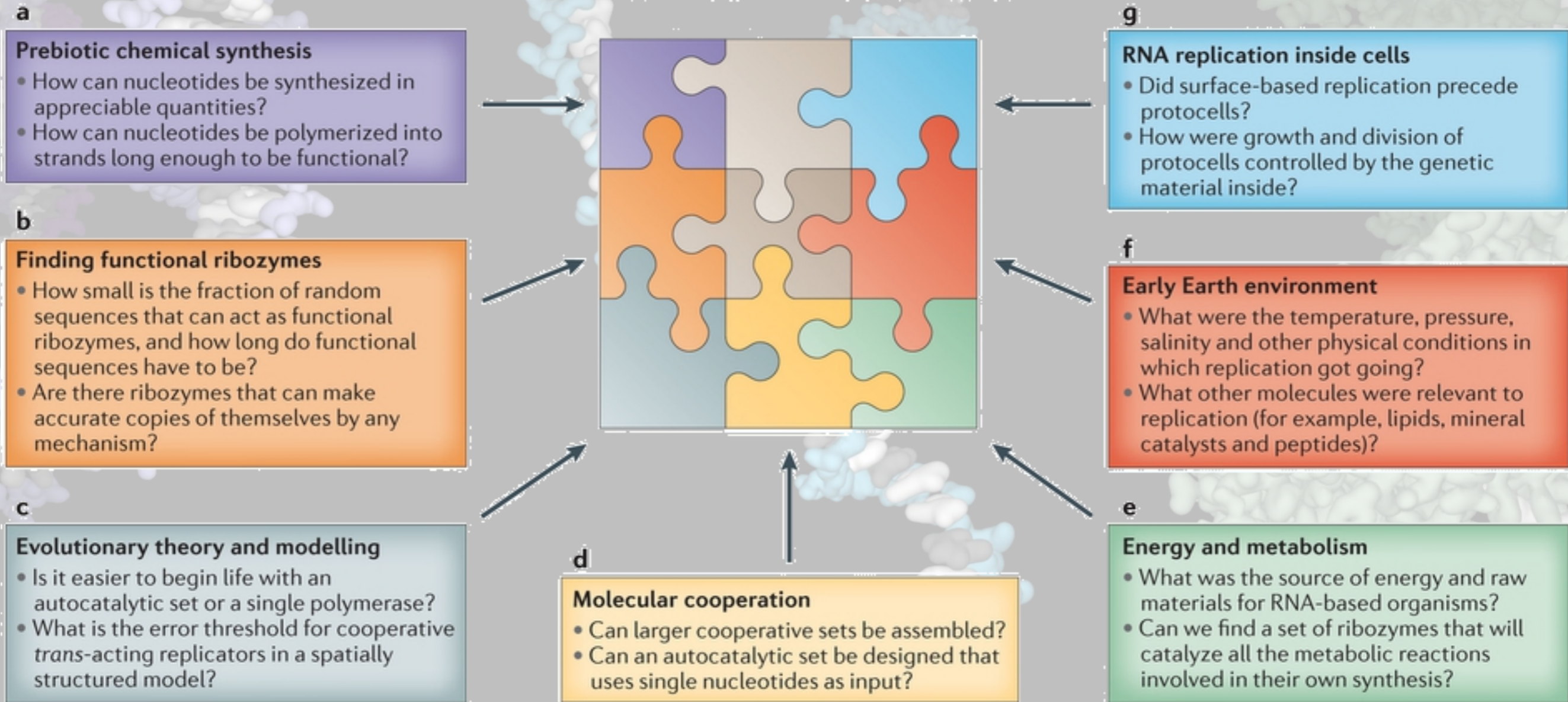
## Fabrice Leclerc, Ph.D.

I2BC / Dept. de Biologie des Génomés,  
« Séquence Structure Fonction des ARN »

SSFA (D. Gautheret)

[fabrice.leclerc@u-psud.fr](mailto:fabrice.leclerc@u-psud.fr)

# Assembling the RNA World



Higgs & Lehman, Nat. Rev. Genet., 2011

# Viroids: Phylogeny

## REPLICATION

## FAMILY

## SUBFAMILY

## GENUS

## SPECIES

## ASYMMETRIC PATHWAY

*Pospiviroidae*

*Pospiviroidae*

*Pospiviroids*

**Potato spindle tuber (PSTVd)**  
Mexican papita (MPVd)  
Tomato planta macho (TPMVd)  
Chrysanthemum stunt (CSVd)  
Citrus exocortis (CEVd)  
Tomato apical stunt (TASVd)  
Iresine 1 (IrVd 1)  
Columnnea latent (CLVd)

*Hostuviroids*

**Hop stunt (HSVd)**

*Cocadviroids*

**Coconut cadang-cadang (CCCVd)**  
Coconut tin angaja (CTIVd)  
Hop latent (HLVd)  
Citrus IV (CVd-IV)

*Apscaviroidae*

*Apscaviroids*

**Apple scar skin (ASSVd)**  
Citrus III (CVd-III)  
Apple dimple fruit (ADFVd)  
Grapevine yellow speckle 1 (GYSVd 1)  
Grapevine yellow speckle 2 (GYSVd 2)  
Citrus bent leaf (CBLVd)  
Pear blister canker (PBCVd)  
Australian grapevine (AGVd)

*Coleviroidae*

*Coleviroids*

**Coleus blumei 1 (CbVd 1)**  
Coleus blumei 2 (CbVd 2)  
Coleus blumei 3 (CbVd 3)

## SYMMETRIC PATHWAY

*Avsunviroidae*

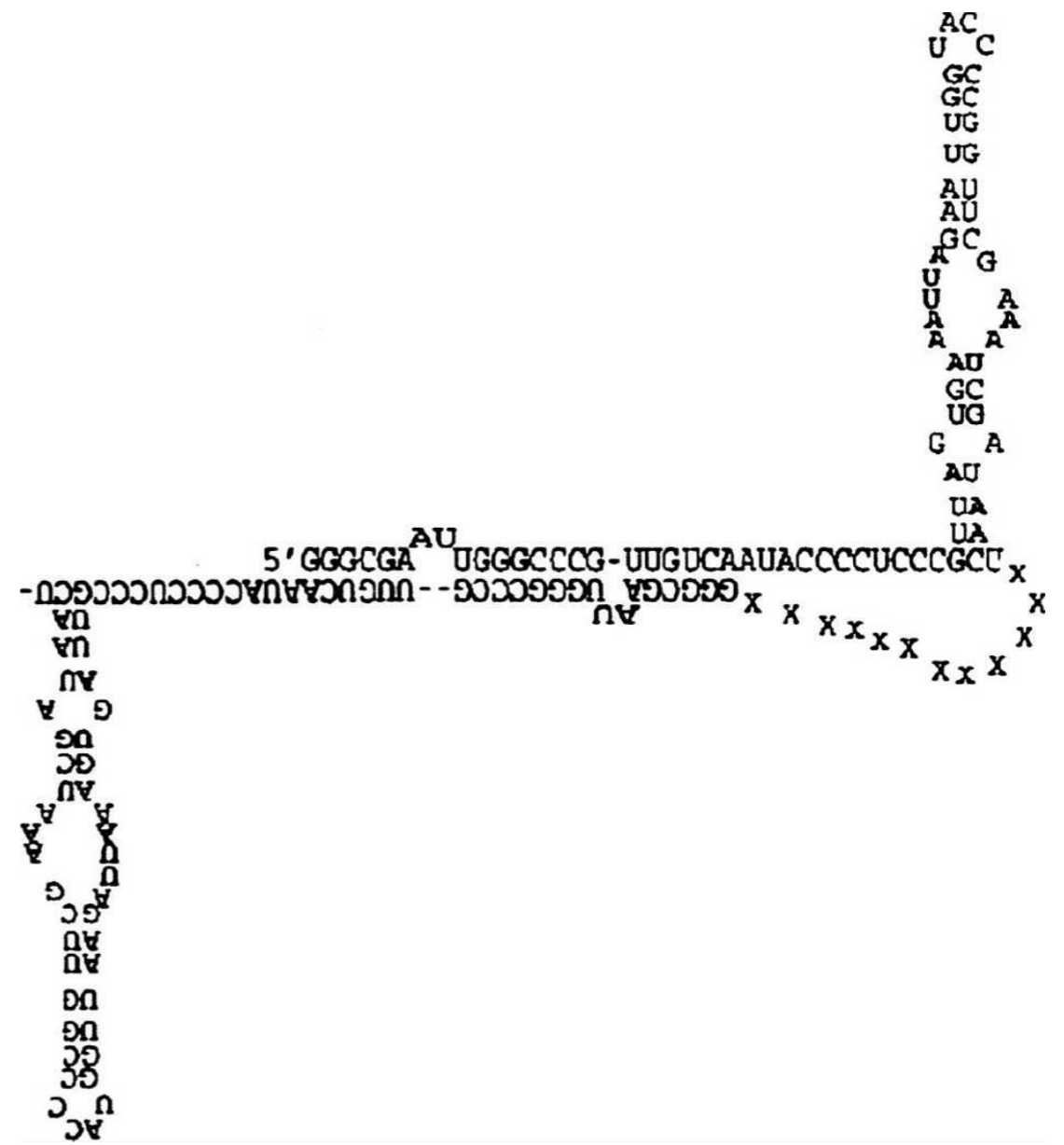
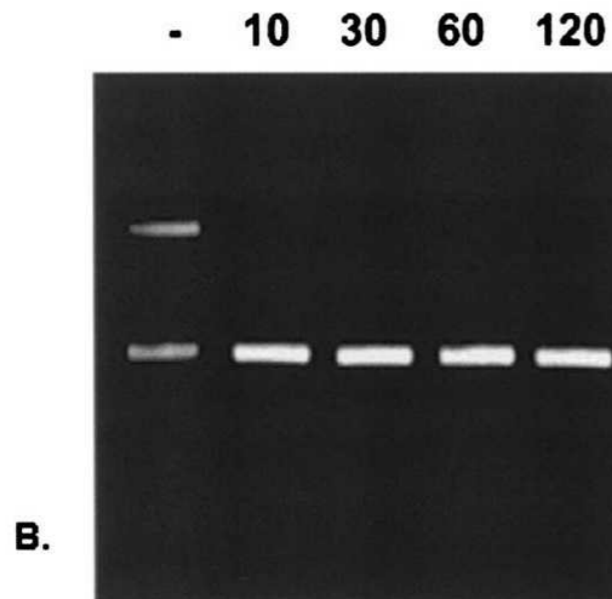
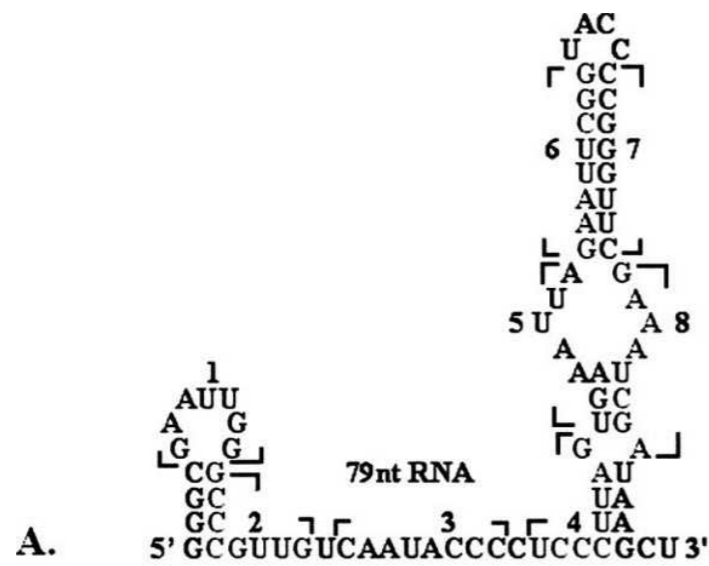
*Avsunviroids*

**Avocado sunblotch (ASBVd)**

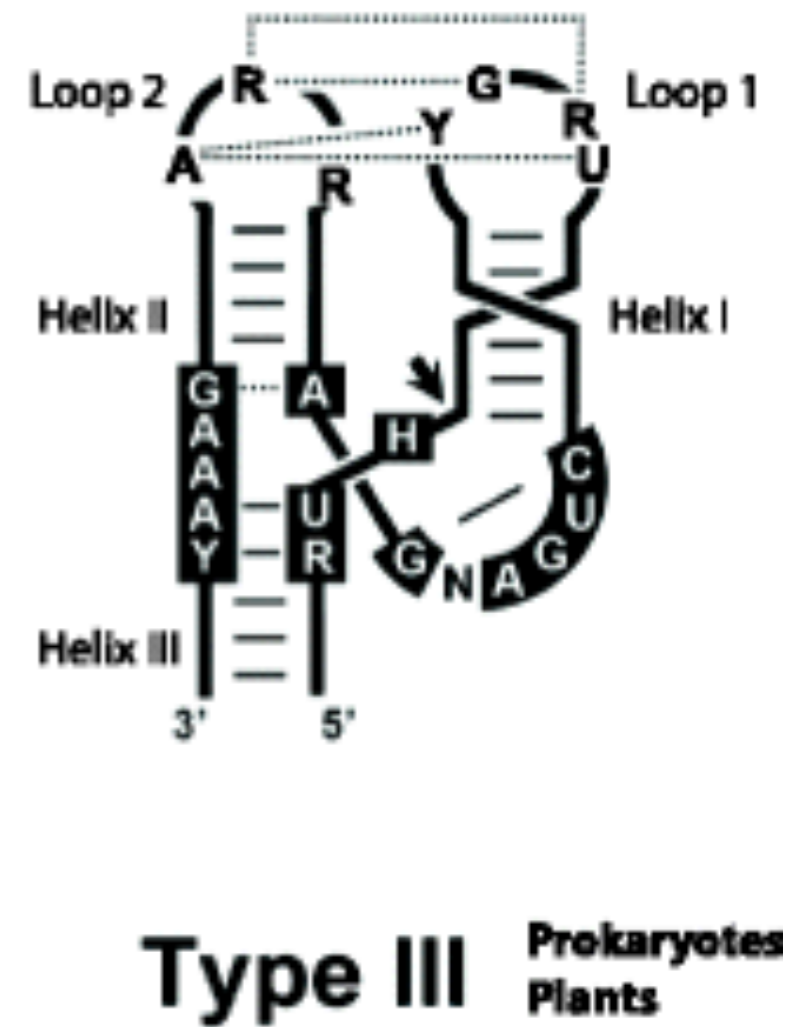
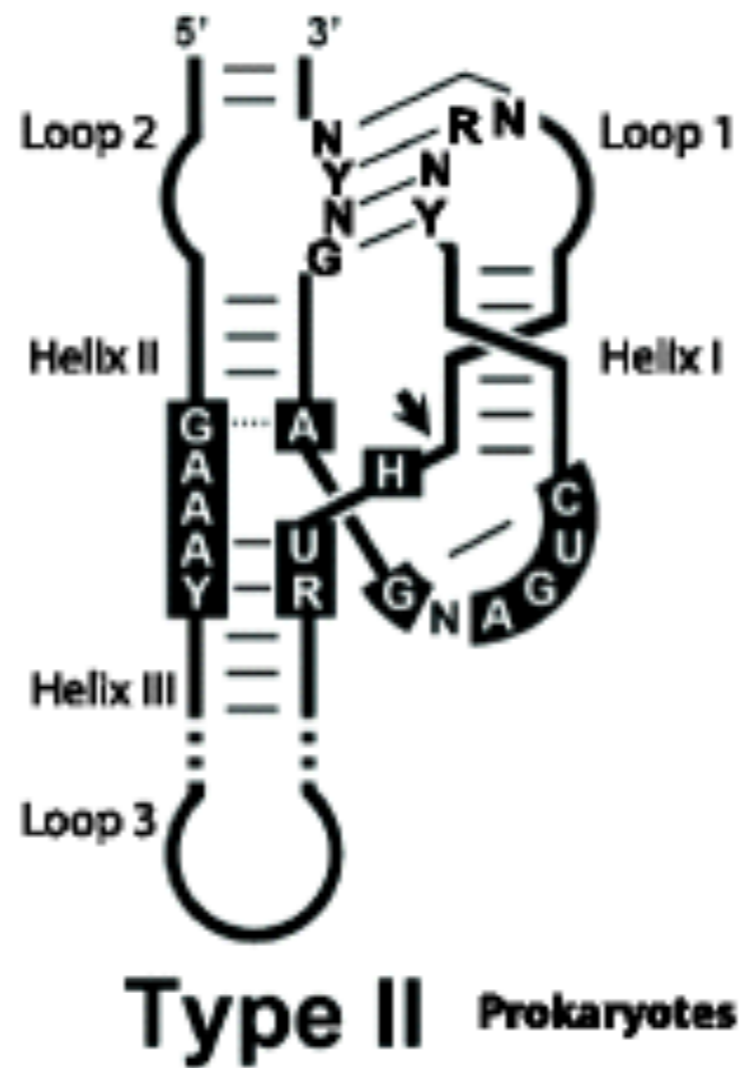
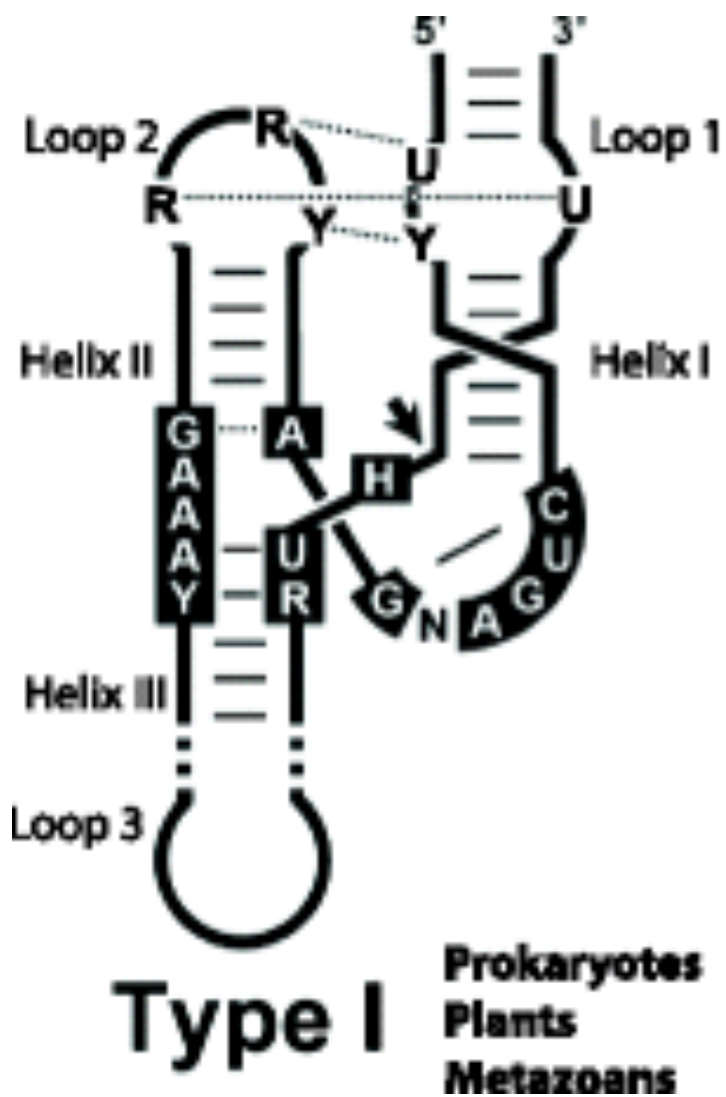
*Pelamoviroids*

**Peach latent mosaic (PLMVd)**  
Chrysanthemum chlorotic mottle (CChMVd)

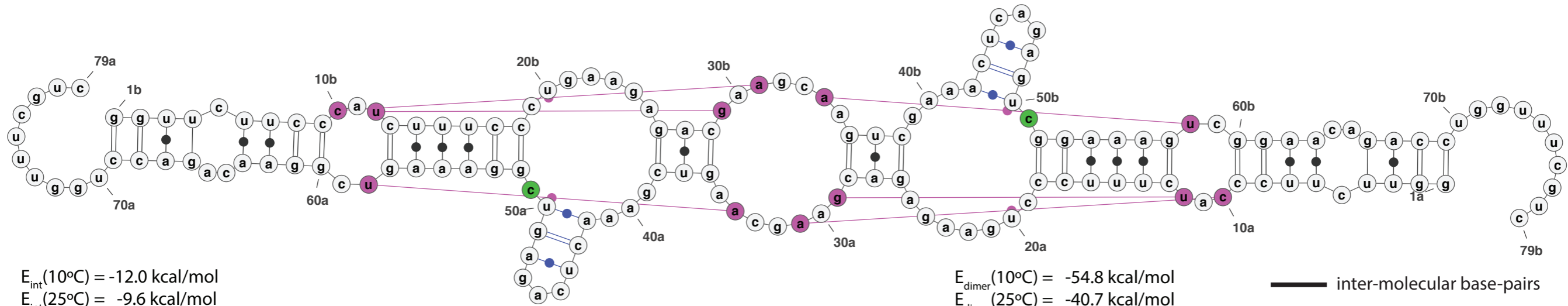
# Modeling RNA-RNA Interactions



# HHH motifs








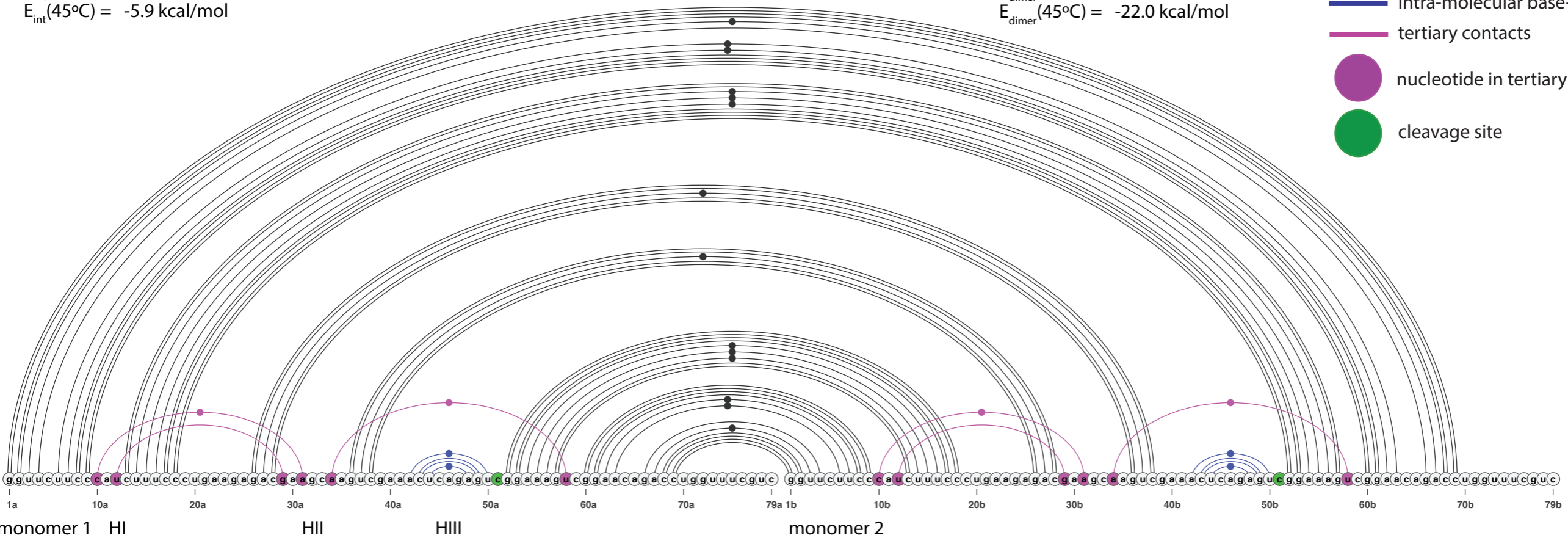
# HHR (-): symmetric dimerization



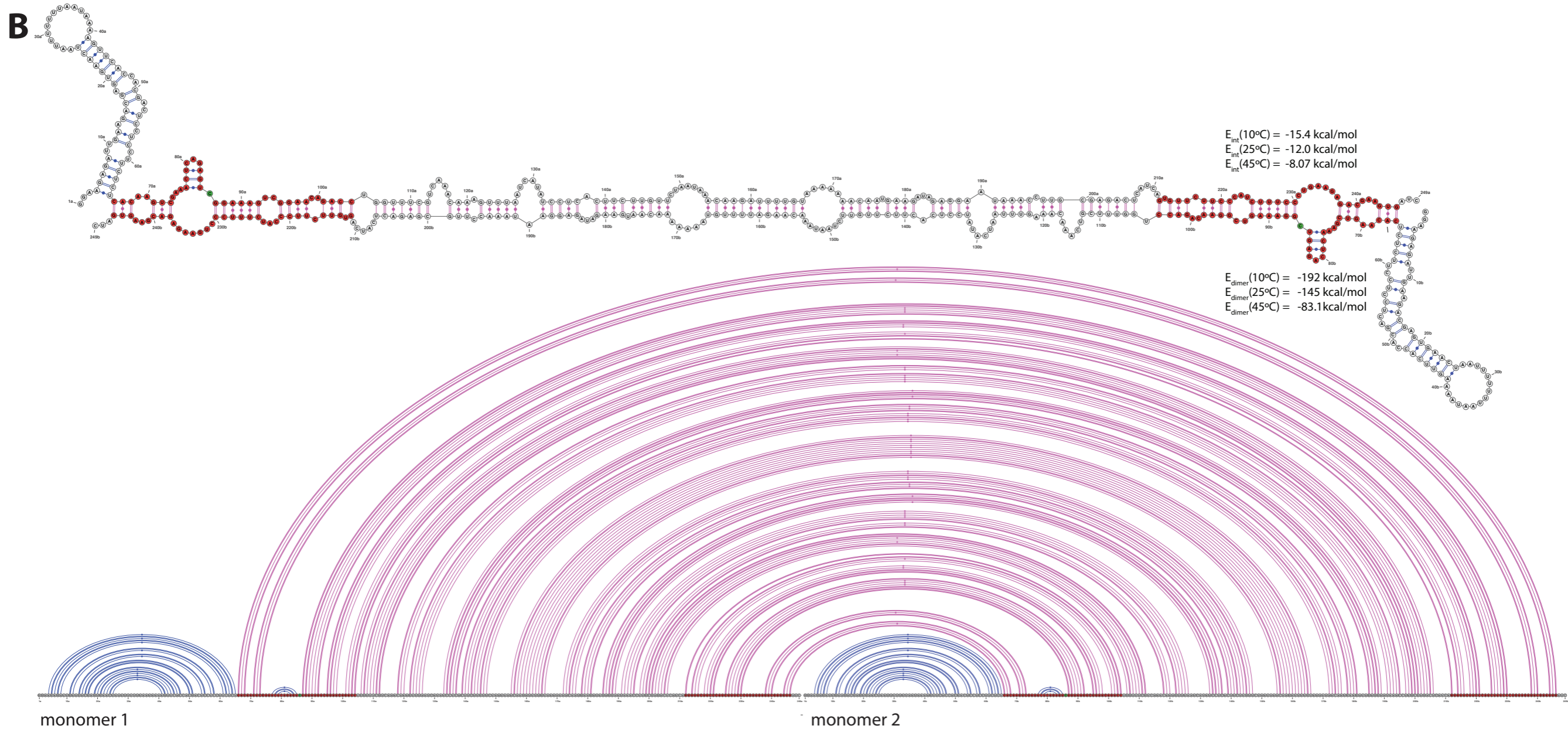
$E_{int}(10^{\circ}\text{C}) = -12.0 \text{ kcal/mol}$   
 $E_{int}(25^{\circ}\text{C}) = -9.6 \text{ kcal/mol}$   
 $E_{int}(45^{\circ}\text{C}) = -5.9 \text{ kcal/mol}$

$E_{dimer}(10^{\circ}\text{C}) = -54.8 \text{ kcal/mol}$   
 $E_{dimer}(25^{\circ}\text{C}) = -40.7 \text{ kcal/mol}$   
 $E_{dimer}(45^{\circ}\text{C}) = -22.0 \text{ kcal/mol}$

-  inter-molecular base-pairs
-  intra-molecular base-pairs
-  tertiary contacts
-  nucleotide in tertiary contact
-  cleavage site

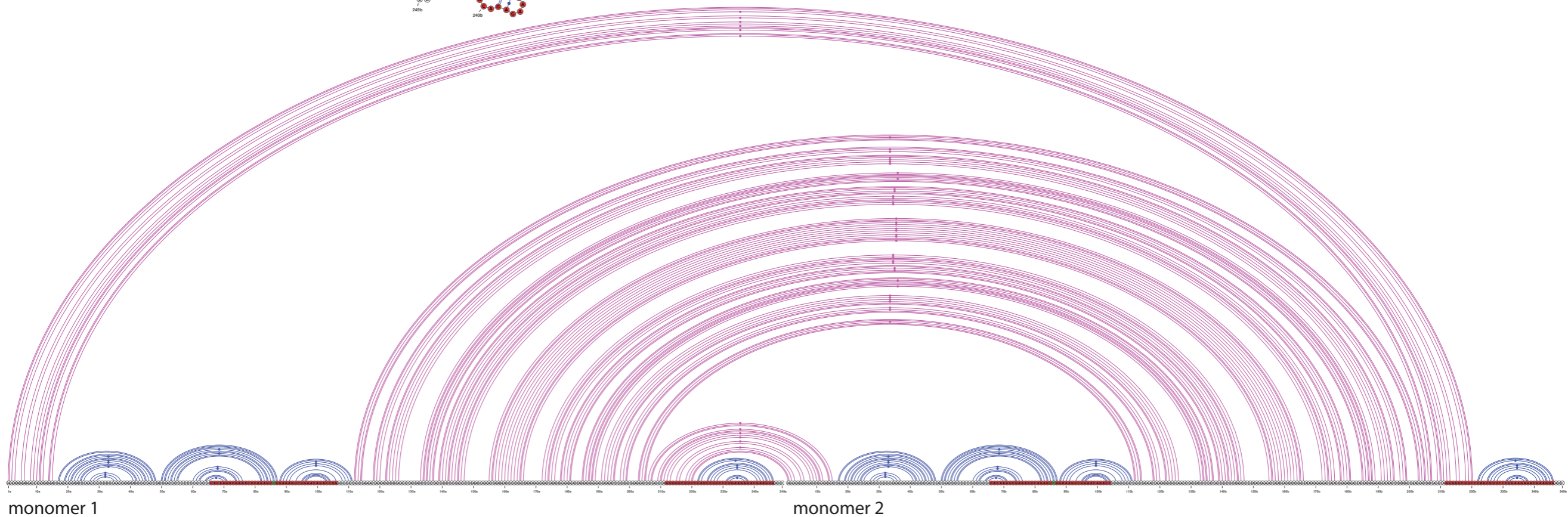
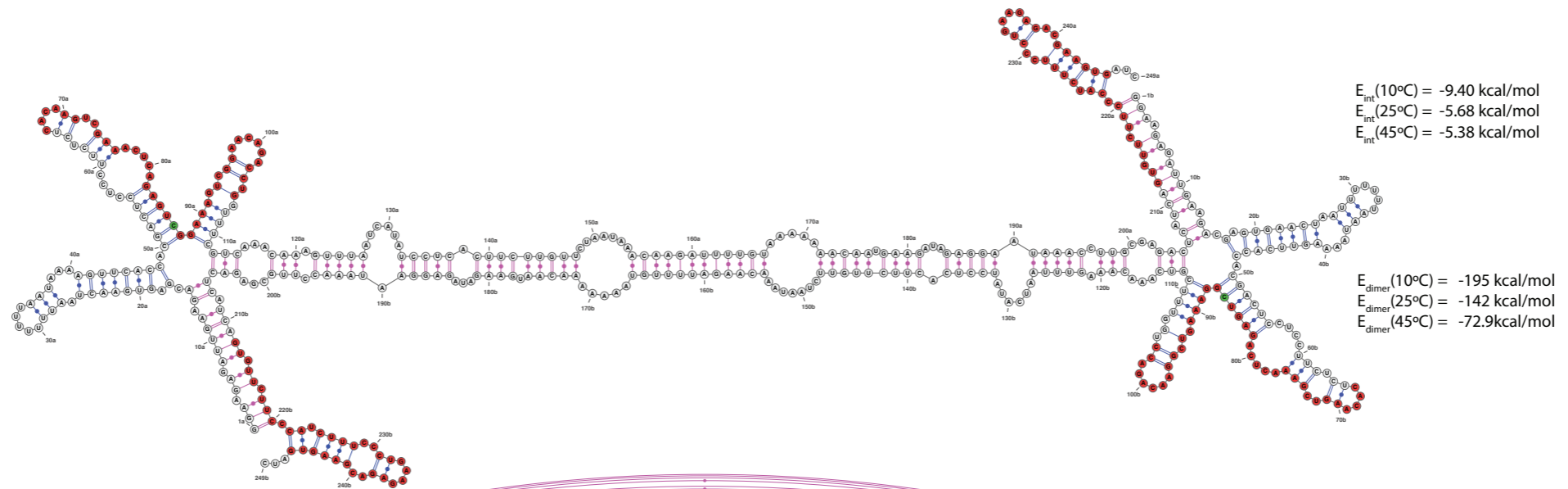


# ASBVd(-): symmetric self-association

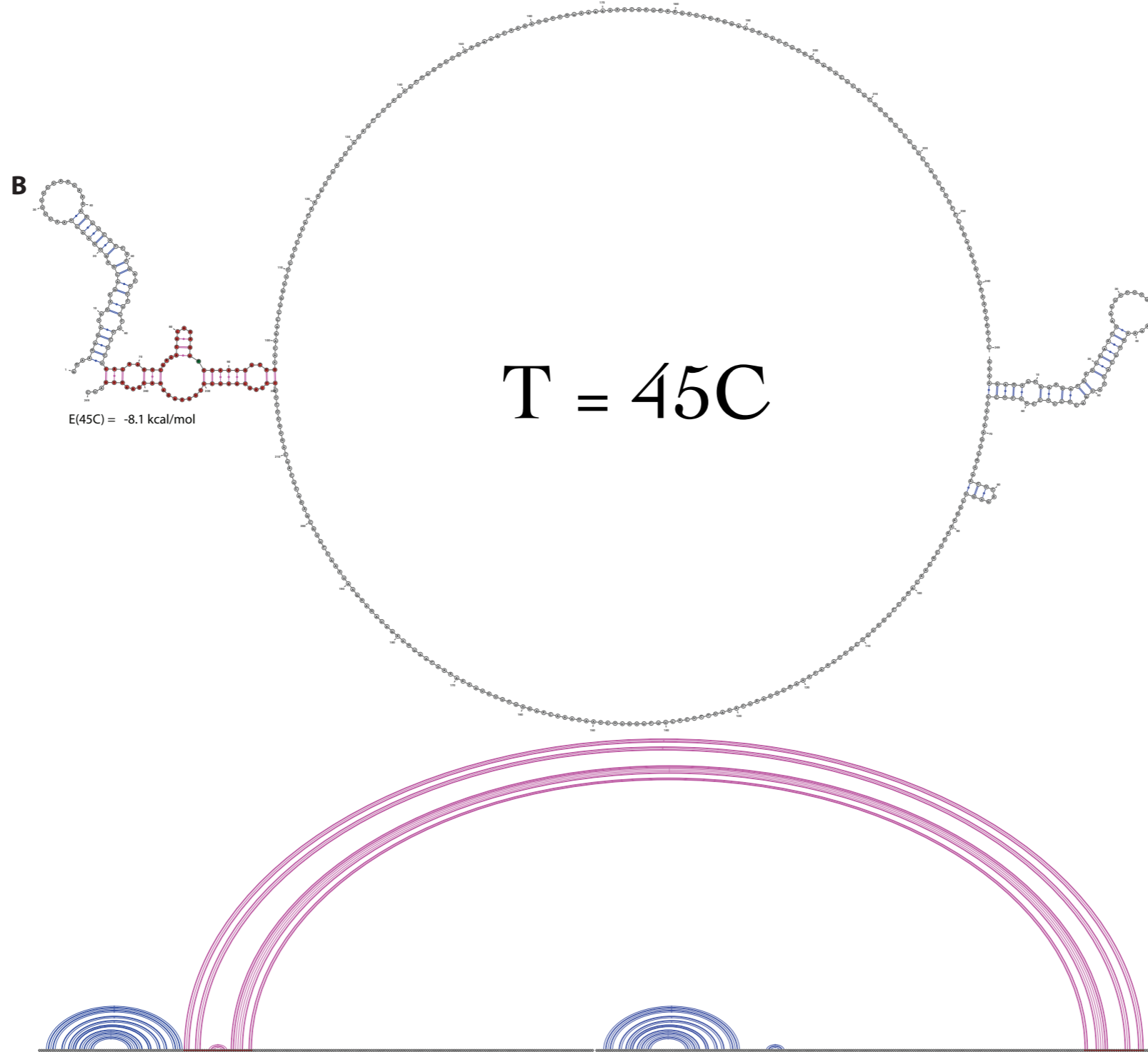


# ASBVd(-): symmetric self-association

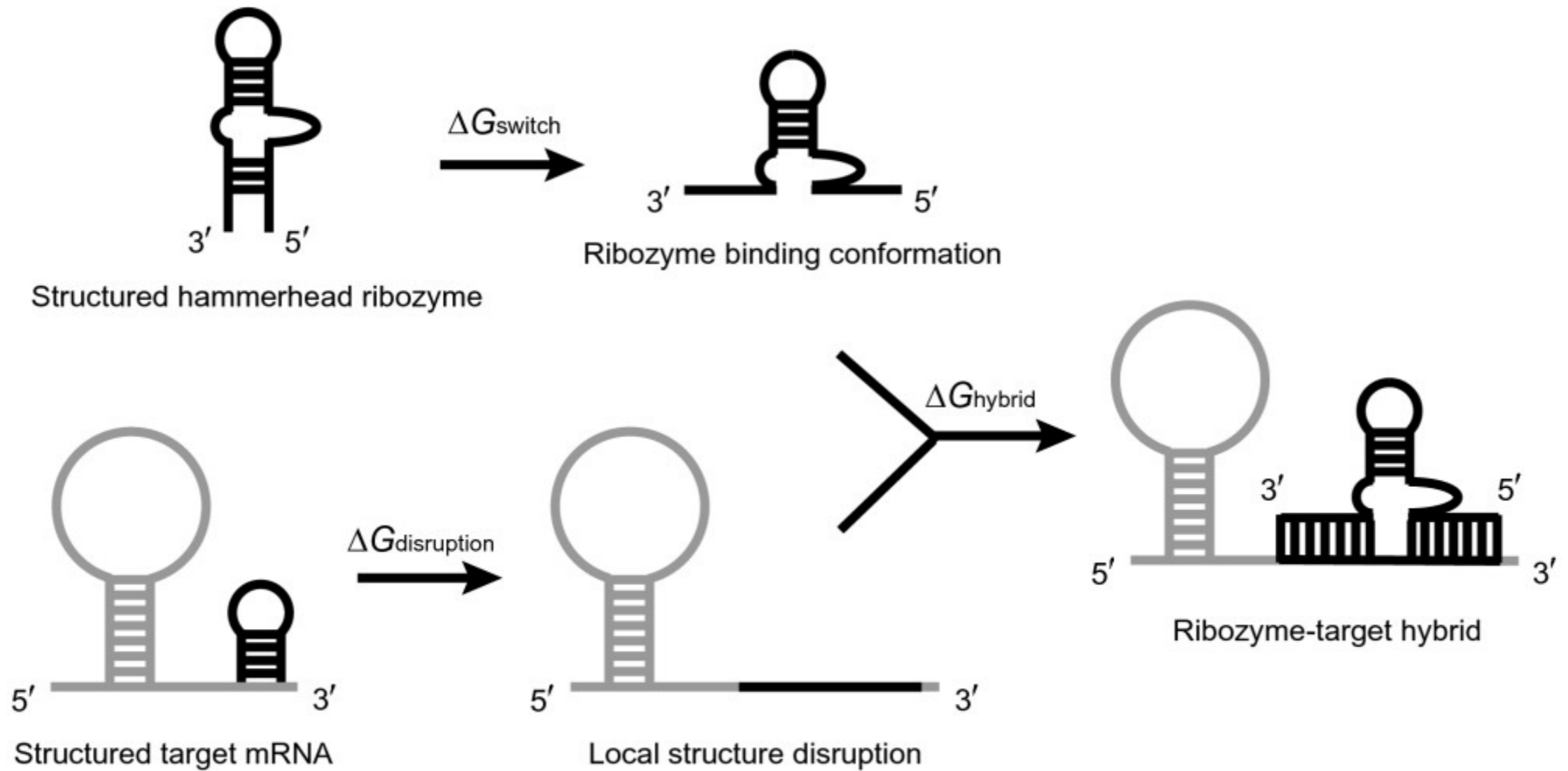
C



# ASBVd(-): symmetric self-association

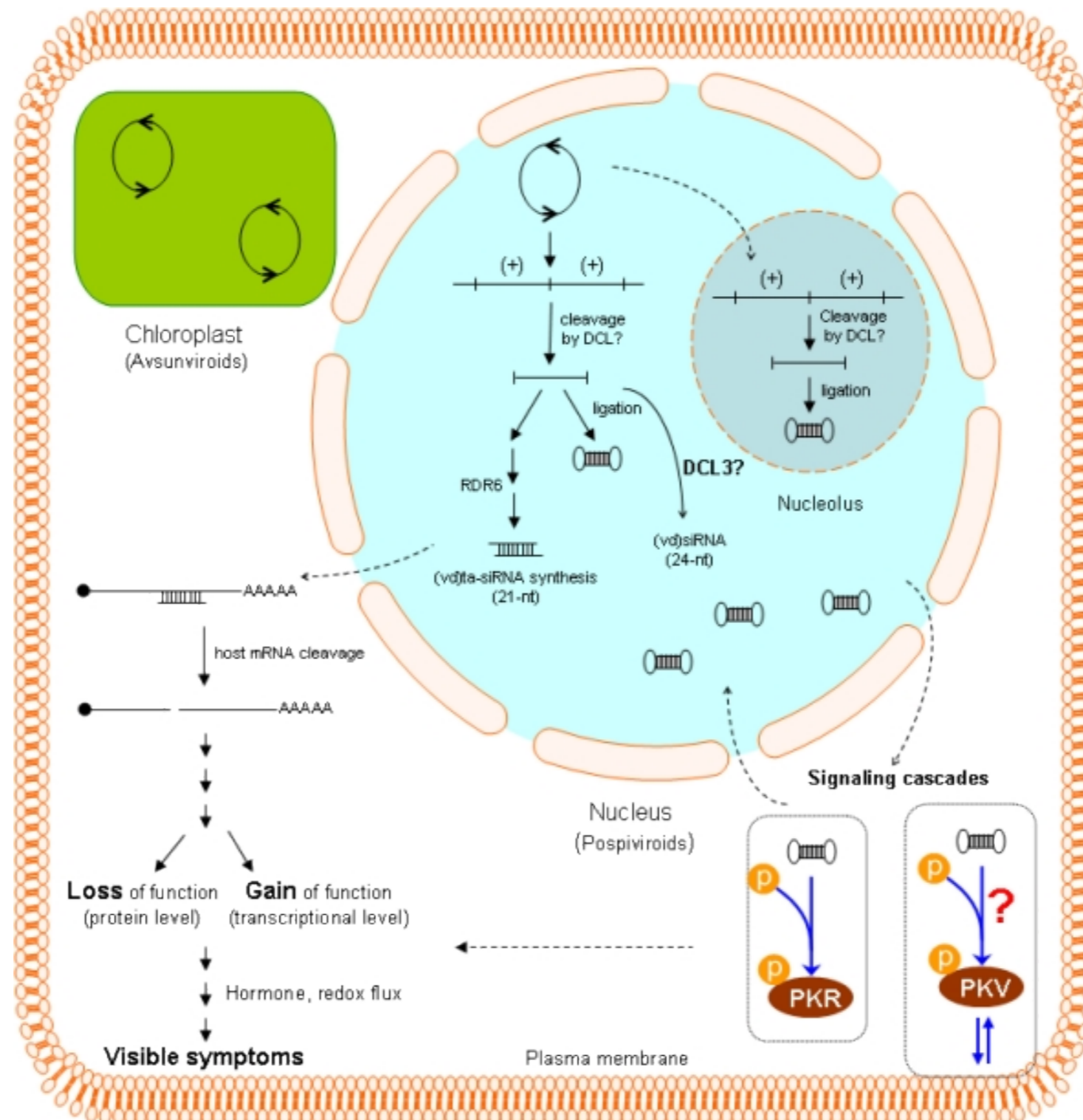


# HHR design & engineering

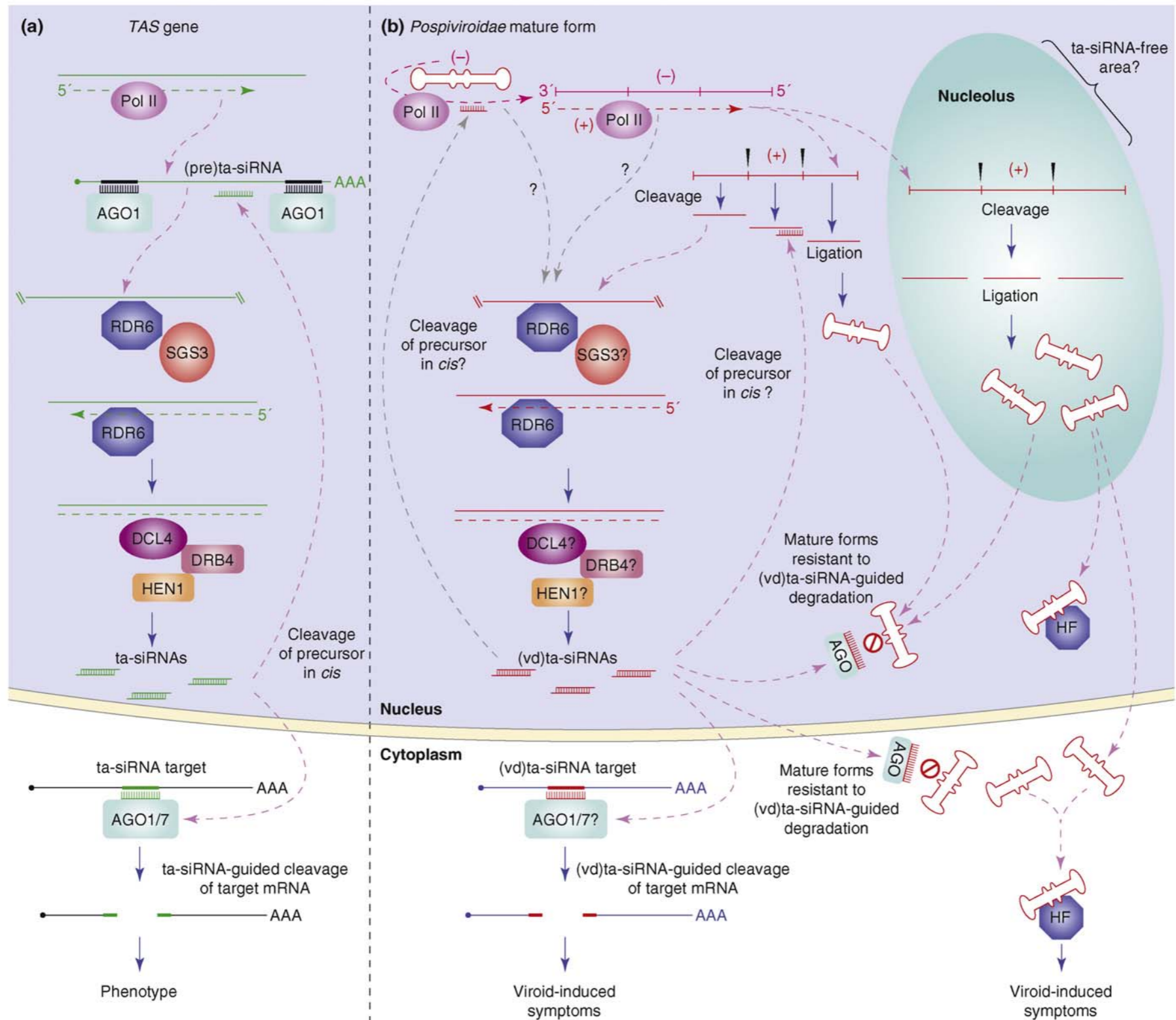




# Viroid Pathogenicity & Localization

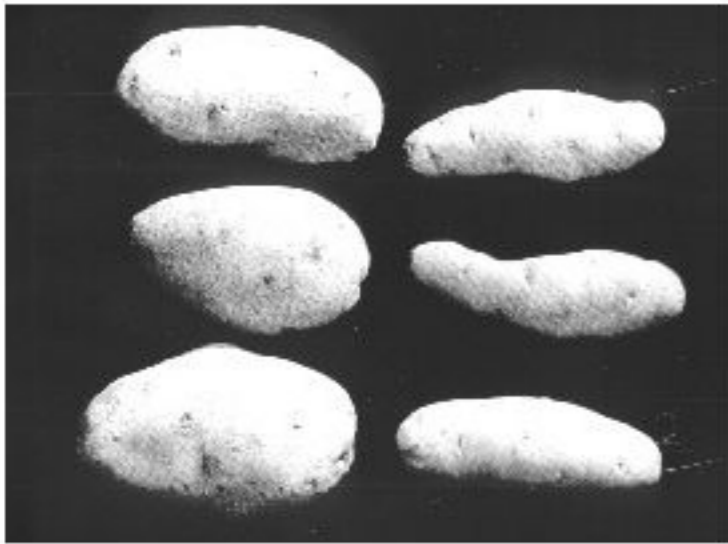


# Viroid & RNA silencing



TRENDS in Plant Science

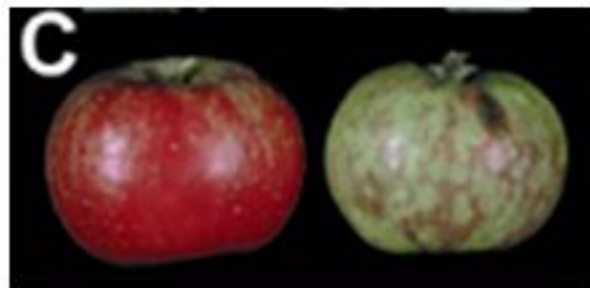
# Viroid disease symptoms



Potato spindle tuber viroid



chrysanthemum  
stunt viroid



Apple viroid disease



Avocado  
Sunblotch Viroid



Coconut cadang-cadang viroid

