



**GT MASIM** 

## **RNA bioinformatics**

folding/interaction, stability/dynamics, thermodynamics/kinetics, ...



Fabrice Leclerc fabrice.leclerc@u-psud.fr

### I2BC - Dept. M2GB Genomics, Molecular Genetics, Bioinformatics

#### Team: RNA Sequence, Structure & function - D. Gautheret

- RNA discovery: genomics, transcriptomics (cancer) D.
  Gautheret
- RNA structure & interactions: snoRNAs, ribozymes-viroids, RNA-RNA interactions, RNA-protein interactions - F. Leclerc
- RNA function & processing: genetic code, RNA-based regulations - J. Lehmann
- 4 senior researchers (C. Toffano: vice-director of the eBio platform), 2 Ph.D. students, 2 CDI IE, (1 invited professor)

### Some recurrent problems & issues

- 2D&3D modeling of RNA variants/mutants from 3D structures
  - sparse structural data
  - experimental 3D structures & biological-physiological conditions

#### 2D&3D modeling of RNA-RNA interactions

- dense RNA-RNA interactome
- predict RNA interactions; 2D to 3D transposition
- 3D modeling of RNA/protein interactions
  - RNA binding ? binding interface binding mode binding specificity (modeling/design)

# Structural/biochemical data inconsistencies



Wang *et al.,* Biochem., 1999 de la Peña *et al.,* EMBO J., 2003 Khvorova et al., Nat. Struct. Biol., 2003

Canny et al., JACS, 2004

### **2D&3D structures of H/ACA** box s(no)RNAs (guide) Kloop guide 20 – U 5' guide sequence 3' guide sequence **Internal Loop** target 3′<sup>10</sup> <sup>50</sup>rRNA (tRNA) ANA Loop -A-C-A 3



Toffano-Nioche et al., 2013;

Toffano-Nioche et al., 2015

### 2D(3D) structural&energetic rules to classify "productive" guide RNAs



# 2D aligned structures of archaeal H/ACA box s(no)RNAs



Af4 1

Af4.2a

HACAprodfold

Af190



Af4.2b











#### **12 subfamilies**

### **RNA-RNA interactions**



### 2D structure of Hammerhead RNA



# **3D Modeling of HHR variant**



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# SANS\* & Modeling $R_{g}^{exp} = 31\text{\AA}$ $R_{g}^{calc} = 29 (26)\text{\AA}$

**SANS: Small Angle Neutron Scattering** Institut Laue-Langevin (ILL), Grenoble

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

### HHR self-association

![](_page_12_Figure_1.jpeg)

# HHR self-association & loss of catalytic activity

![](_page_13_Figure_1.jpeg)

![](_page_14_Picture_0.jpeg)

### 3D Model of HHR selfassembly

Α

HII

HII

3' <sup>79'</sup>

HIII

0000000000

HIII

ΗΙ

3'

HI

![](_page_14_Picture_2.jpeg)

Leclerc et al., 2016

# Dynamics of HHR(-) dimer

![](_page_15_Figure_1.jpeg)

### RNA Binding Proteins (RBPs): Modularity, dsRNA/ssRNA

![](_page_16_Figure_1.jpeg)

17 Lunde et al., Nat. Rev. Mol. Cell Biol., 2007

### Naive approach for modeling ssRNA ligands (fragment-linking) MCSS A U **TIS11d (CCCH)** Molpy **Zinc Finger** contraint $n \ge 8$ CHARMM 2.2Å ≤ RMSD ≤ 3.6Å **3D/NMR** PDB ID: 1RGO

### Proof of concept: fragmentbased modeling of RNA Ligands

![](_page_18_Figure_1.jpeg)

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![](_page_18_Picture_2.jpeg)

![](_page_18_Figure_3.jpeg)

### Binding strength & specificity in RBPs: RNase A

![](_page_19_Figure_1.jpeg)

Ligand	Ν	ΔH	TΔS	K <sub>d</sub> (µM)
5' <b>-AMP</b>	0.89	-17.1	-11.6	124
5' <b>-GMP</b>	1.14	-10.9	-6.4	568
3'-CMP	0.99	-13.5	-7.5	51.6
3' <b>-</b> TMP	0.99	-14.3	-7.6	15
3'-UMP	NA	NA	NA	9.7

Doucet et al., Proteins, 2010

![](_page_19_Picture_4.jpeg)

### Predict "strong" nucleotide binding sites: (fragment-growing)

![](_page_20_Figure_1.jpeg)

box setting & fragment distribution (MCSS)

### Predict nucleotide binding sites specificity

nucleobases

A, U, C, G

(PDB ID: 1RCN)

ribose, phosphate

ribose-phosphate

![](_page_21_Picture_3.jpeg)

# Predict nucleotide binding preferences in "hotspots"

![](_page_22_Figure_1.jpeg)

#### focusing on high density regions

### Clustering & scoring nucleotide binding preferences

![](_page_23_Picture_1.jpeg)

![](_page_23_Picture_2.jpeg)

PDB ID: 1RCN

Ligand	ΔH	n <sub>clust</sub>	T∆S	ΔG	$\Delta G_{exp}$
5'-AMP	-16	15	7.5	-24	-
5'-GMP	-19	21	11	-30	-
5'-CMP	-18	29	15	-33	-6.0
5'-UMP	-21	52	26	-47	-6.7

Ligand	ΔH	n <sub>clust</sub>	T∆S	ΔG	$\Delta G_{exp}$
5'-AMP	-23	27	14	-37	-5.5
5'-GMP	-24	19	10	-34	-4.5
5'-CMP	-18	31	16	-34	-
5'-UMP	-20	25	13	-33	-

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FOR SCIENCE

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