Global analysis of RNA structures and their dynamics in the adaptative response of *Staphylococcus aureus*.

AZEDE Alan

PARIS-SACLAY UNIVERSITY ED SDSV– PH.D Bacteriology/Bioinformatic

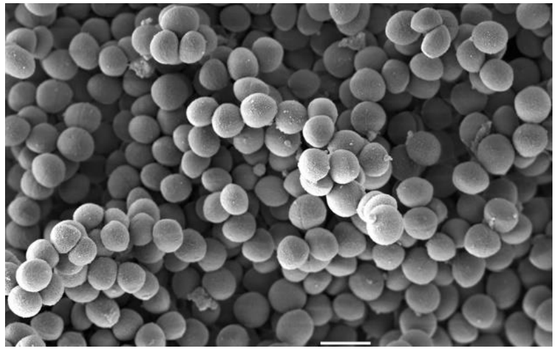
39th TBI Winterseminar

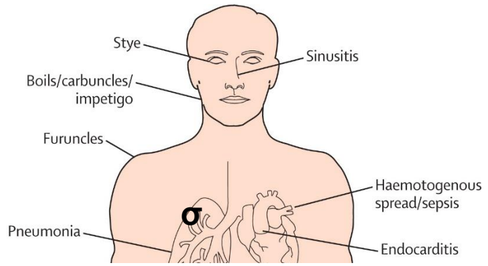
I2BC-SRRB / UMR9198

Supervisor : Philippe BOULOC

Co-tutors : Yann PONTY and Sebastian WILL(CNRS/LIX), Bruno SARGUEIL (Pharma Paris)

Introduction to the 80prime CNRS project

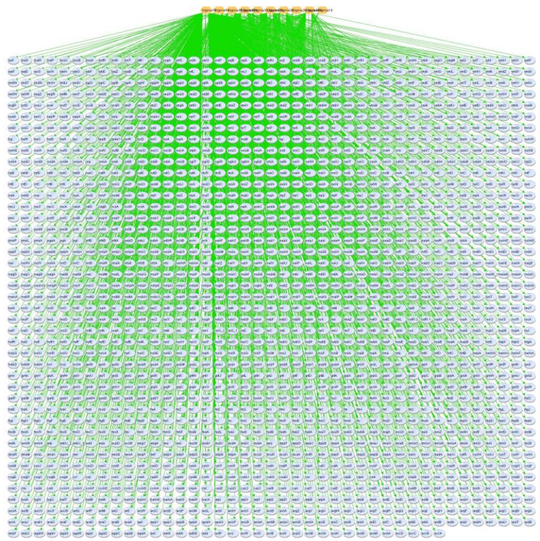




- **Major** Gram positive coagulase + **pathogen**
- Hospital/Community-acquired infections associated with **antibiotic resistants strains** (ESKAPE)

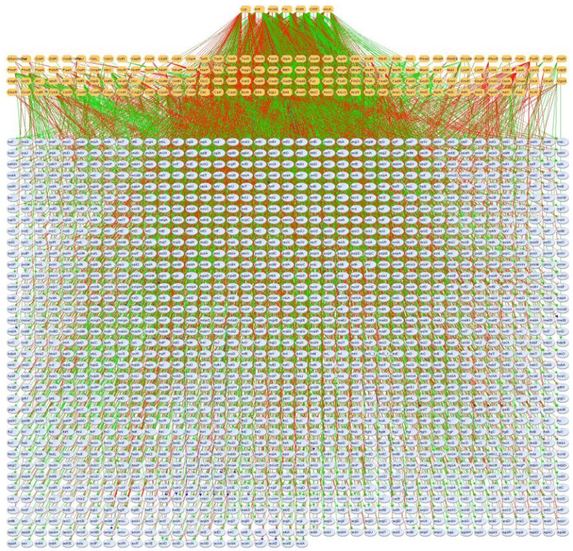
→ Bone and/or joint infection, toxin-related infection, whitlow, furonculosis, …

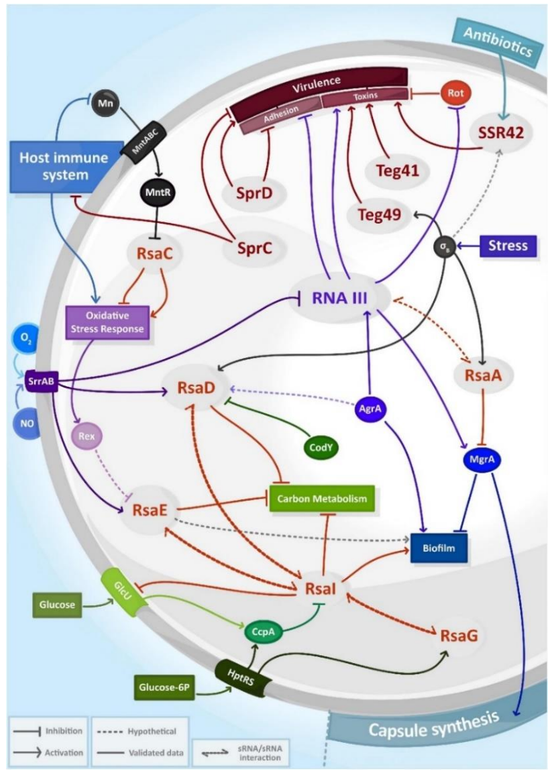
- Numerous **virulence factors**, **adaption** to various environmental conditions, **persistence** within the host, **biofilm** formation…



Martinez-Antonio *et al.*, 2003

Transcriptional factors





Barrientos *et al,* 2021

But also RNA…

My interdisciplinary Ph.D project

- Decipher **RNA structures dynamic in adaption and global physiology**

- **Classify RNA structures/motifs** into families based on position/similarities + structure function analysis

- Find or validate **novel RNA regulatory motifs**

→ Look at the RBS/TE + translational coupling between ORFs

- Study the **effect of temperature** on structures linked to pathogenicity

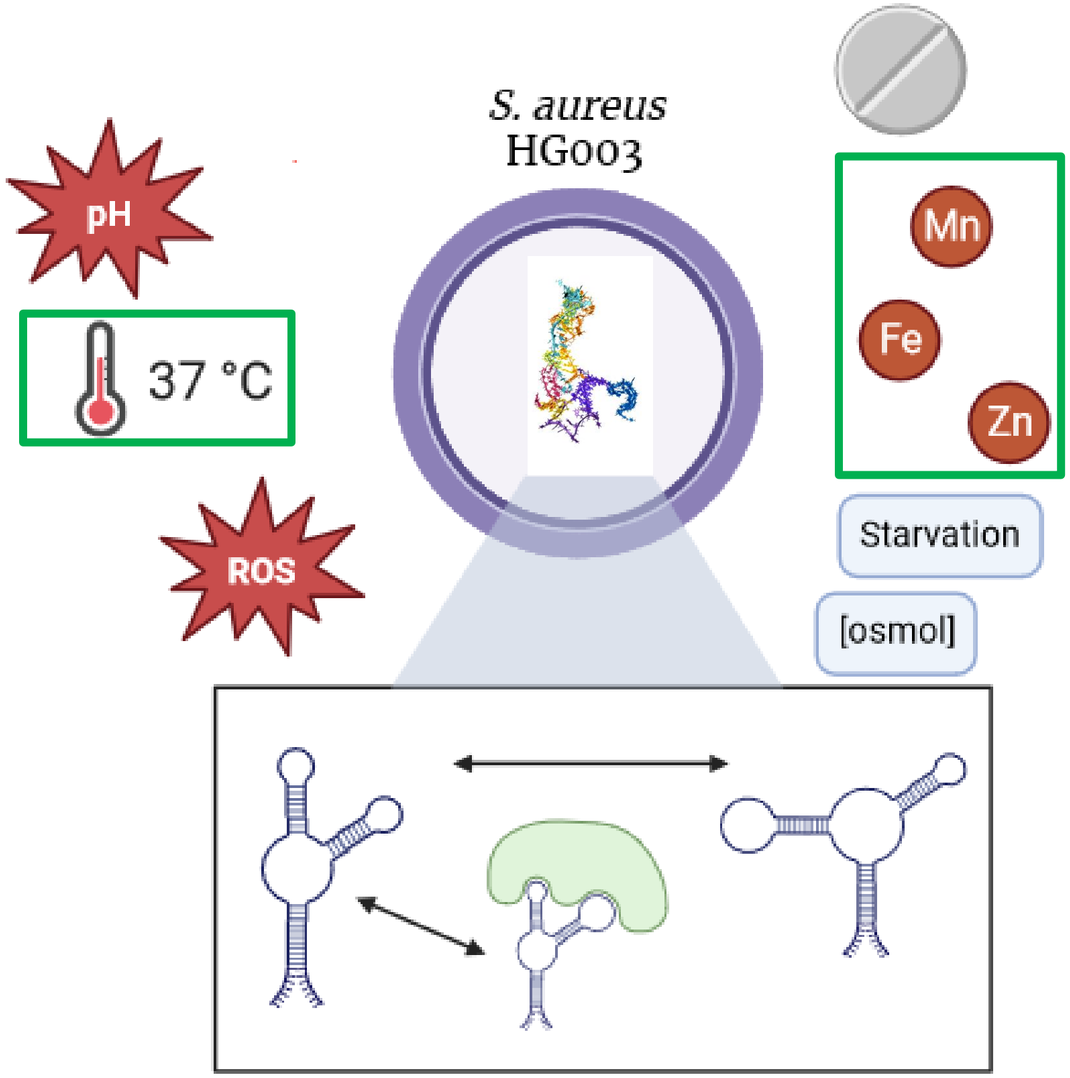
- Contribution of **RNA chaperones** on these structures

- Effect of **sRNA's induction** on mRNA targets ?

- Explore **new potentiel targets for drug design**

Mapping a « **druggable transcriptome** » in *S. aureus* ?

→Design/improve small molecules/analogs for riboswitches

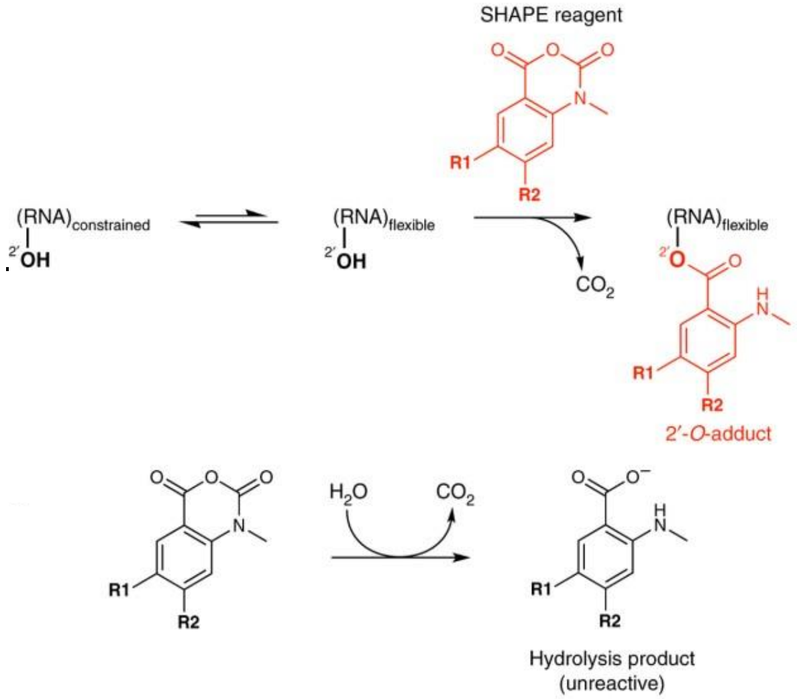


Deciphering RNA structures with the SHAPE chemistry

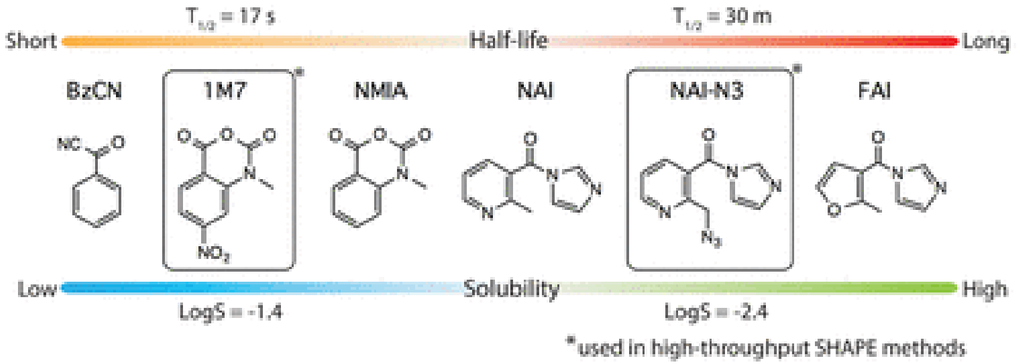
SHAPE = **Selective 2′-hydroxyl acylation** analyzed by primer extension;

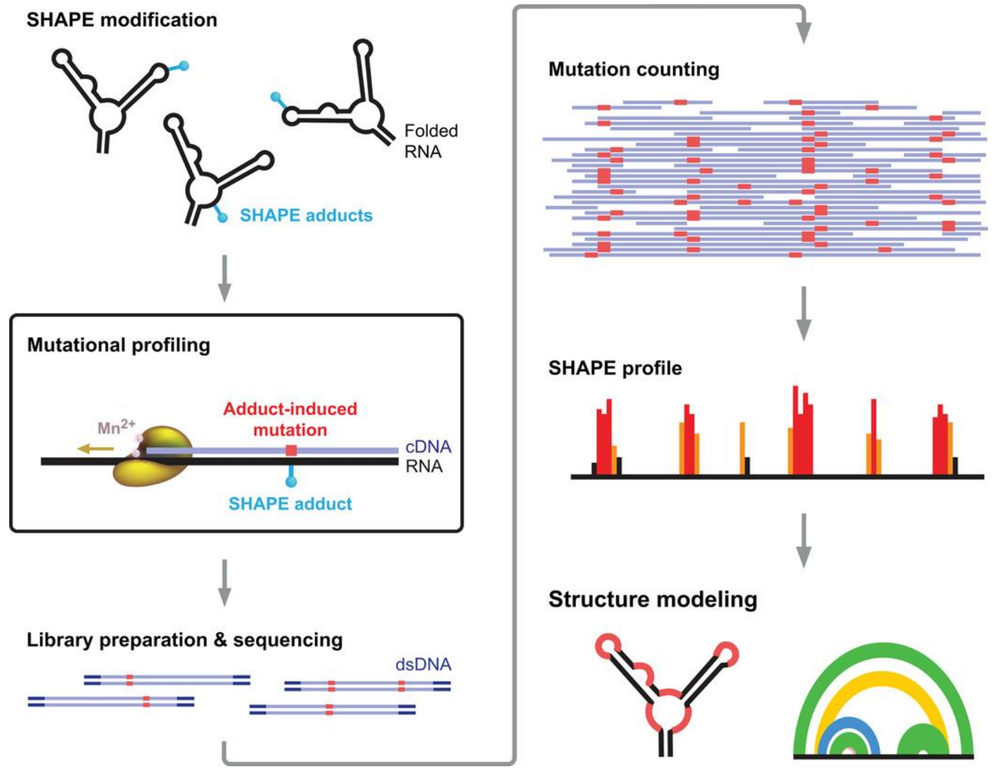
Acylation of 2’OH for a **structurally “flexible” nucleotide**;

Solving **secondary RNAs structures** ***in vitro, ex vivo and in vivo***



Matthew J Smola *et al*, 2015





Sigfried *et al*, 2014

Limitations and unknown

→ Gram positive = thick **PG**

→ Cellular **environment**

→ Conditions, probes, … → **reproductibility + robustness**

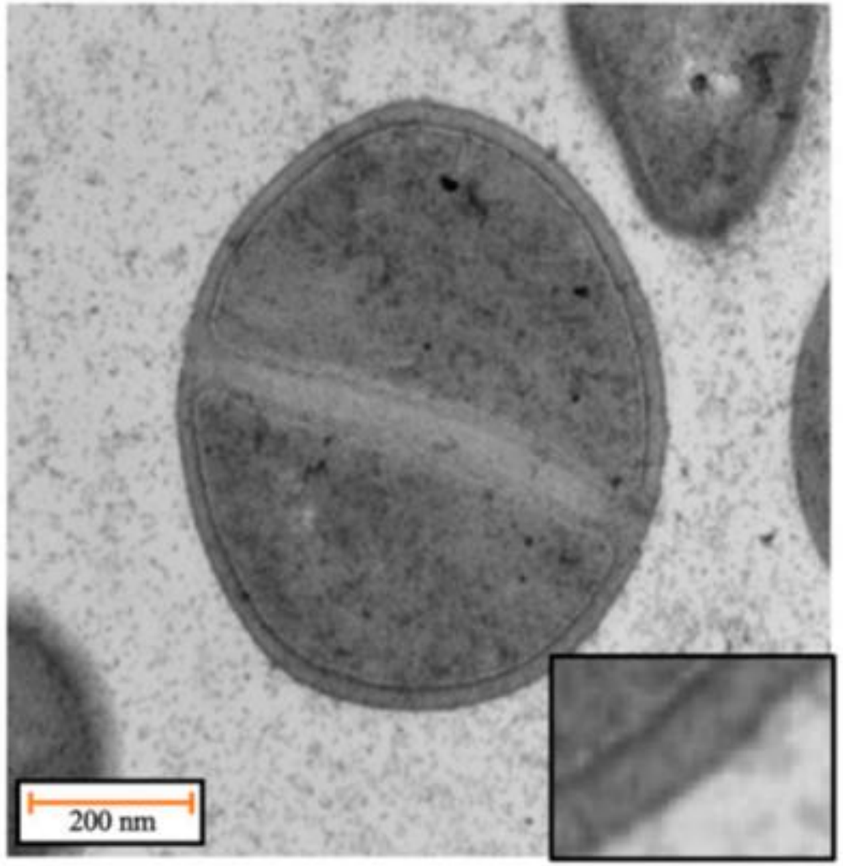
→ Modelling/predict methodologies to **detect precise conformational changes**

→ **Various species** of RNA *in cellulo*

→ Reactivity profiles at the transcriptomic level = **huge volumes of data**

→ RNA interactome and folding dynamics

How can we believe that our probing by SHAPE reagents will work *in vivo* ?



Ideas and benchmark experiments

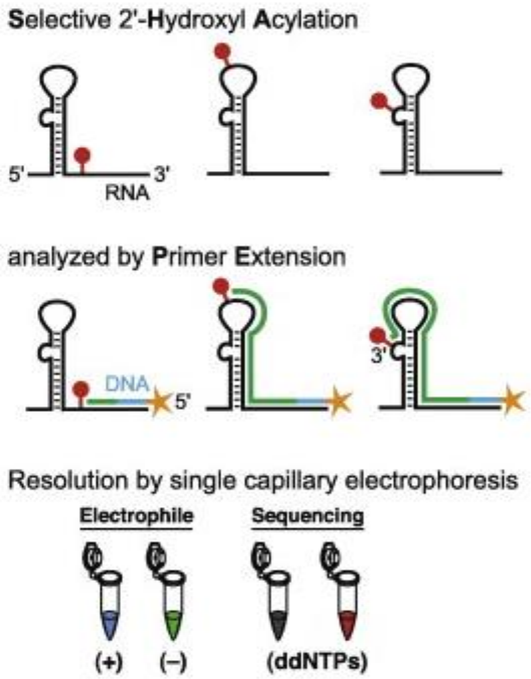
→ Use an « inert » RNA as a template for SHAPE-CE

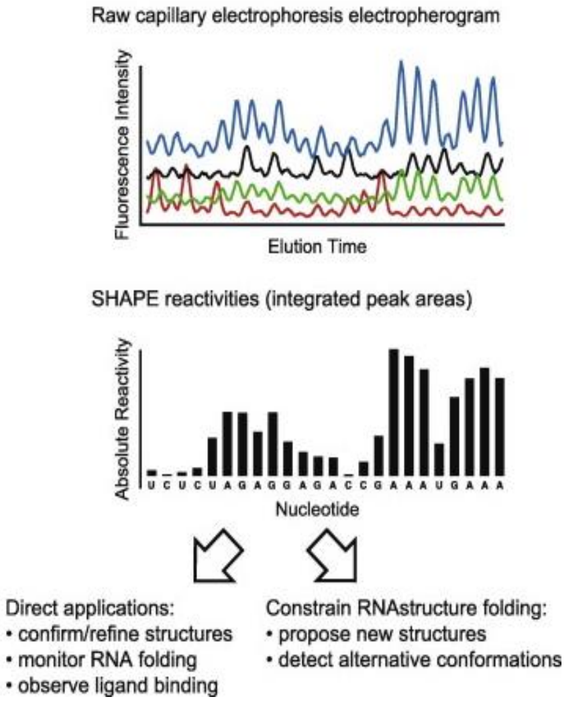
→ **Known structure** or at least predicted

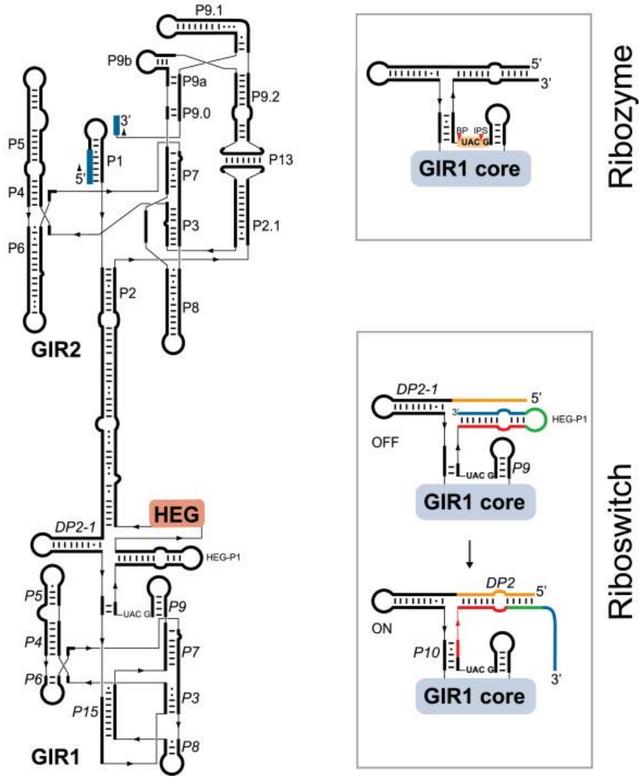
→ **Stable** and fold independantly

→ Perform a **SHAPE-CE experiment *in vivo***

Didymium iridis







Hedberg *et al,* 2013

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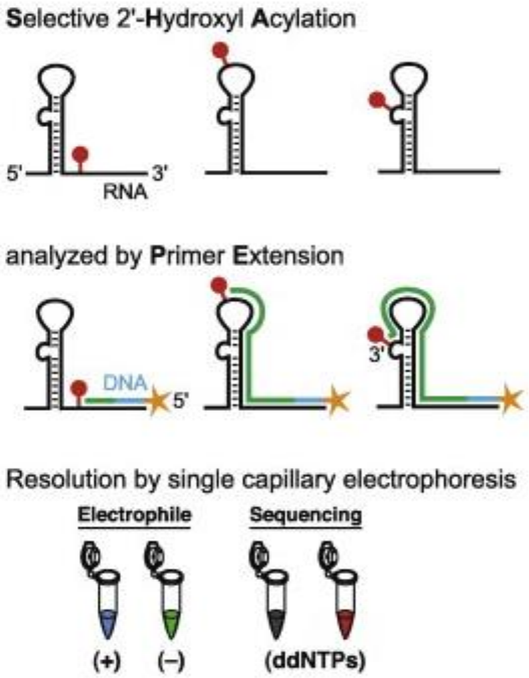

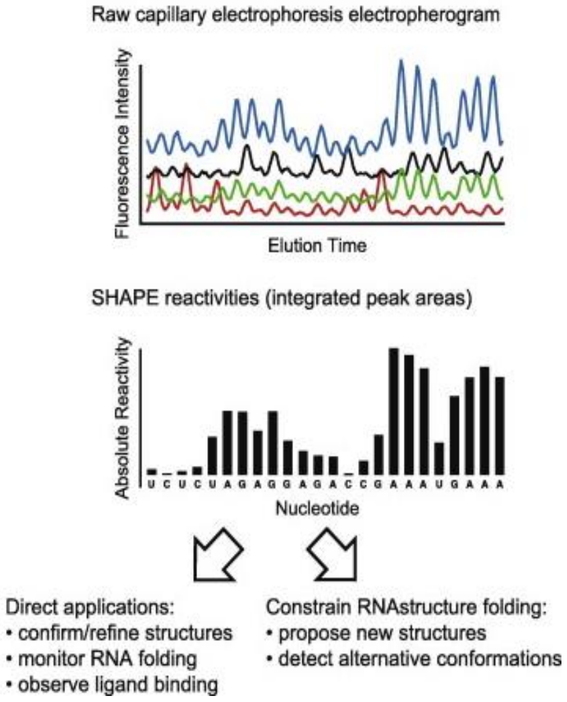

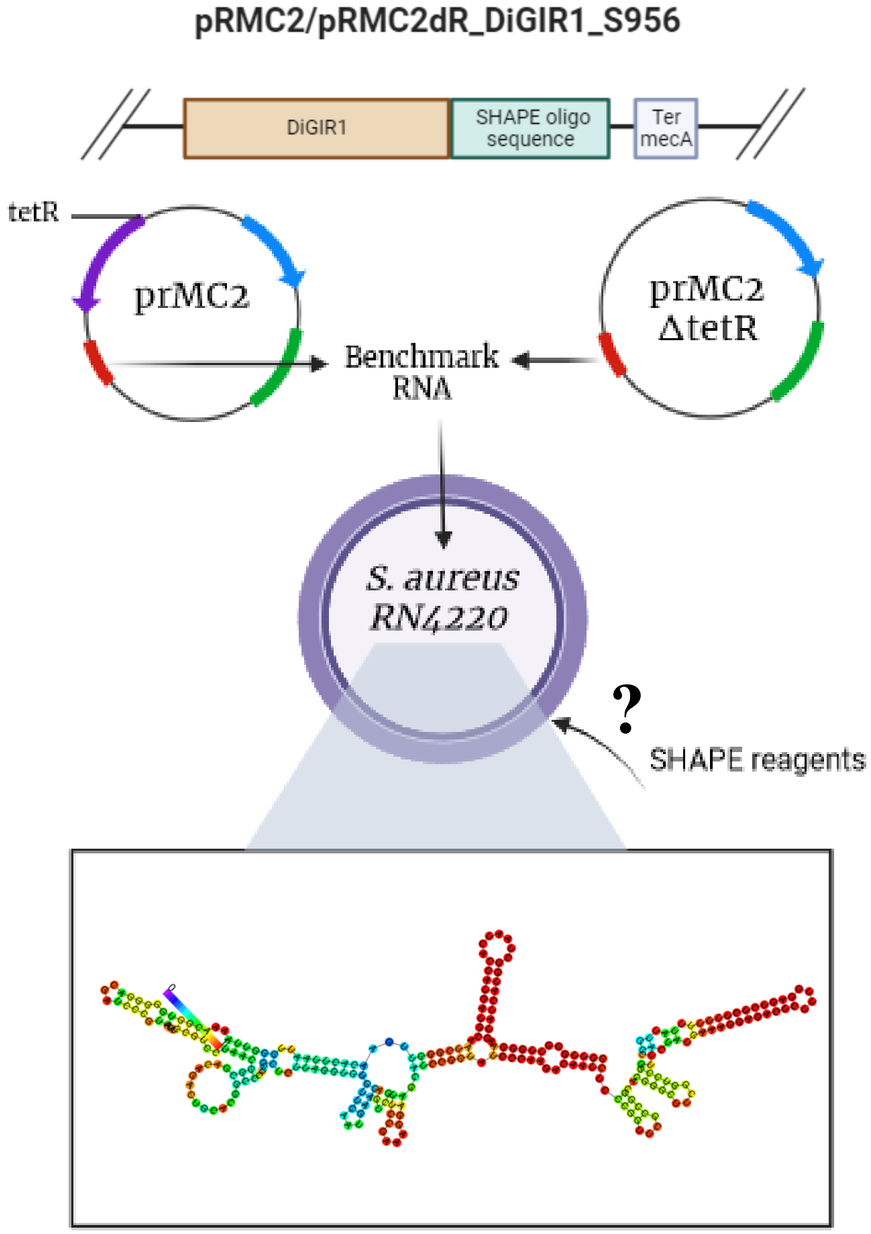

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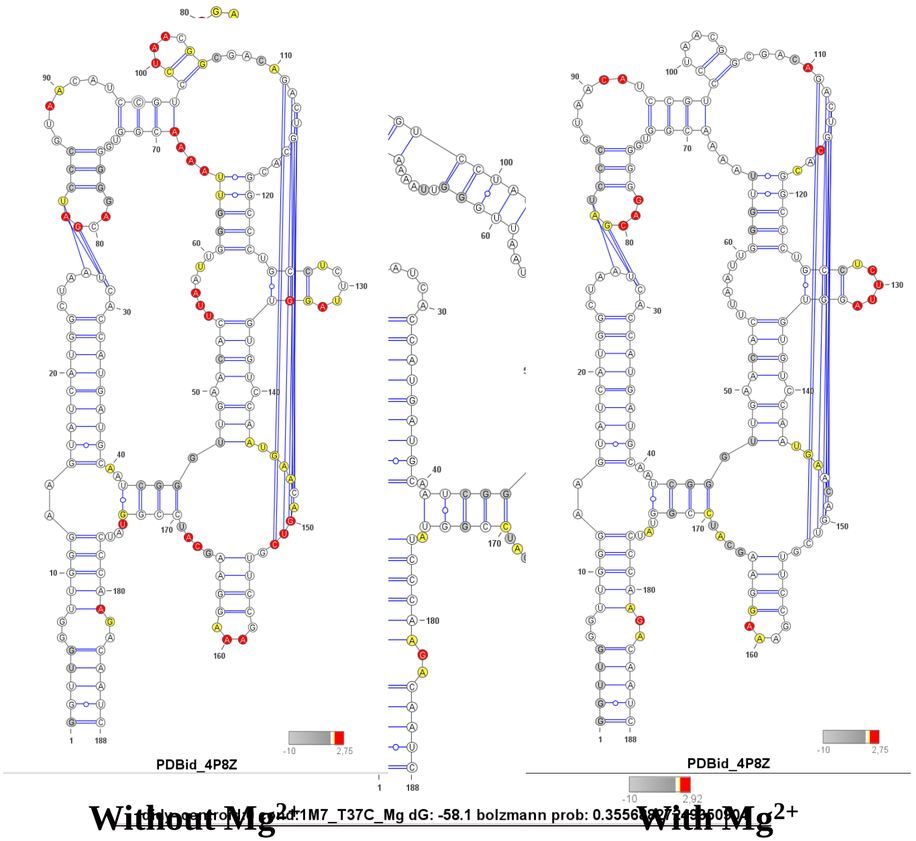
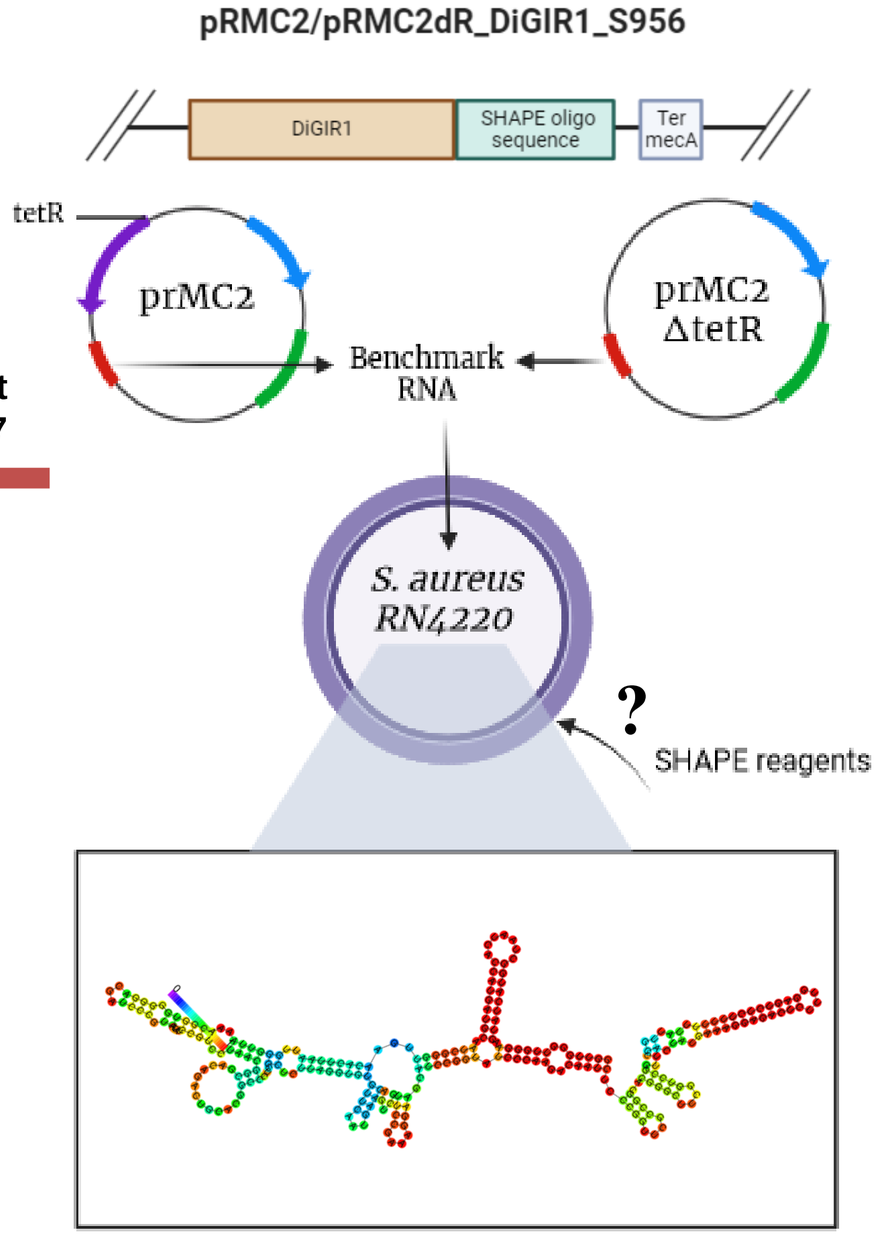


Without Mg^{2+} **With Mg^{2+}**

SHAPE-CE on the construct
In vitro Transcription + 1M7

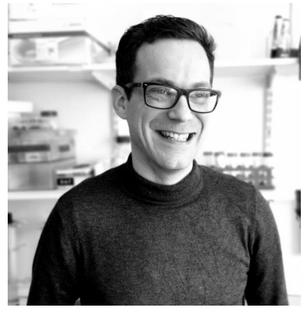


Conclusion – Issues - Perspectives

- Cloning the intron in pJET1.2 and pRMC2 → **Done**
- Transformation in *E. coli* IMO8B → **Done**
- Transformation in *S. aureus* RN4220 and HG003 strains → **Done and checked but...**
- Test few probes *in vivo/in vitro* (**5NIA**, **2A3**…) → **on going**
- SHAPE-CE *in vivo* → **Done** and **on going**
- **RNA III *in vivo* ???**

Gupta *et al., 2015*

Aknowledgement



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Nara Figueroa-Bossi

Lionello Bossi

Maxime Barrault

Etornam Kofi Kumeko

Patricia Kerboriou

AMIB(io) team :

Yann Ponty

Sebastian Will

Sarah J. Berkemer

Théo Boury

Nan Pan

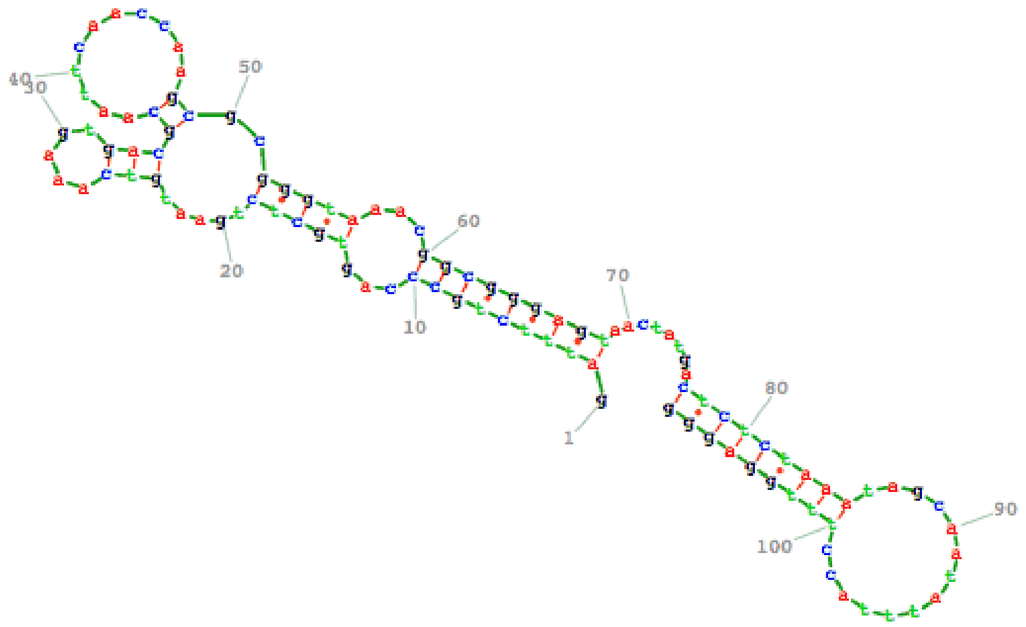
CiTCoM team :

Bruno Sargueil

Pierre Hardouin

Christelle Vasnier

Thank you for your attention

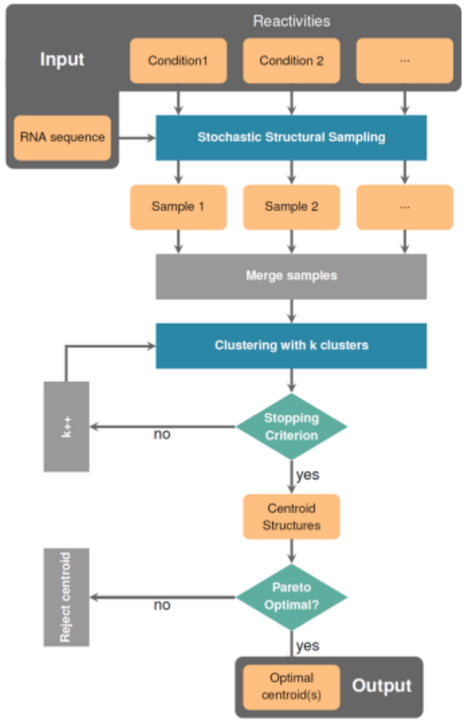


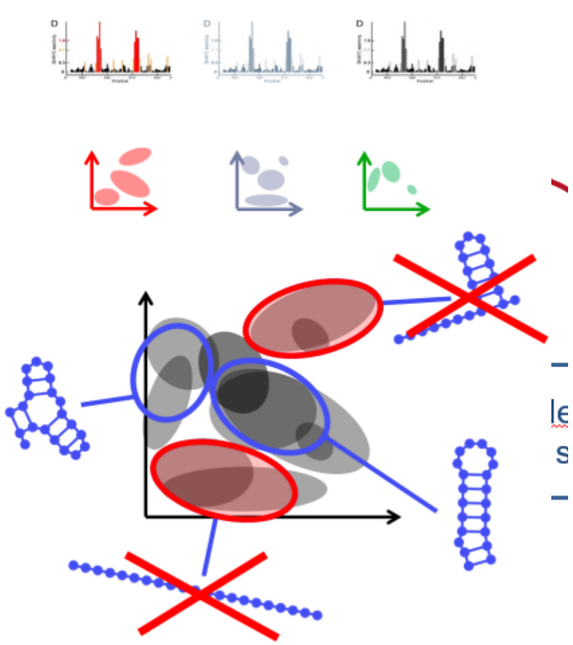
Introduction to the 80prime CNRS project

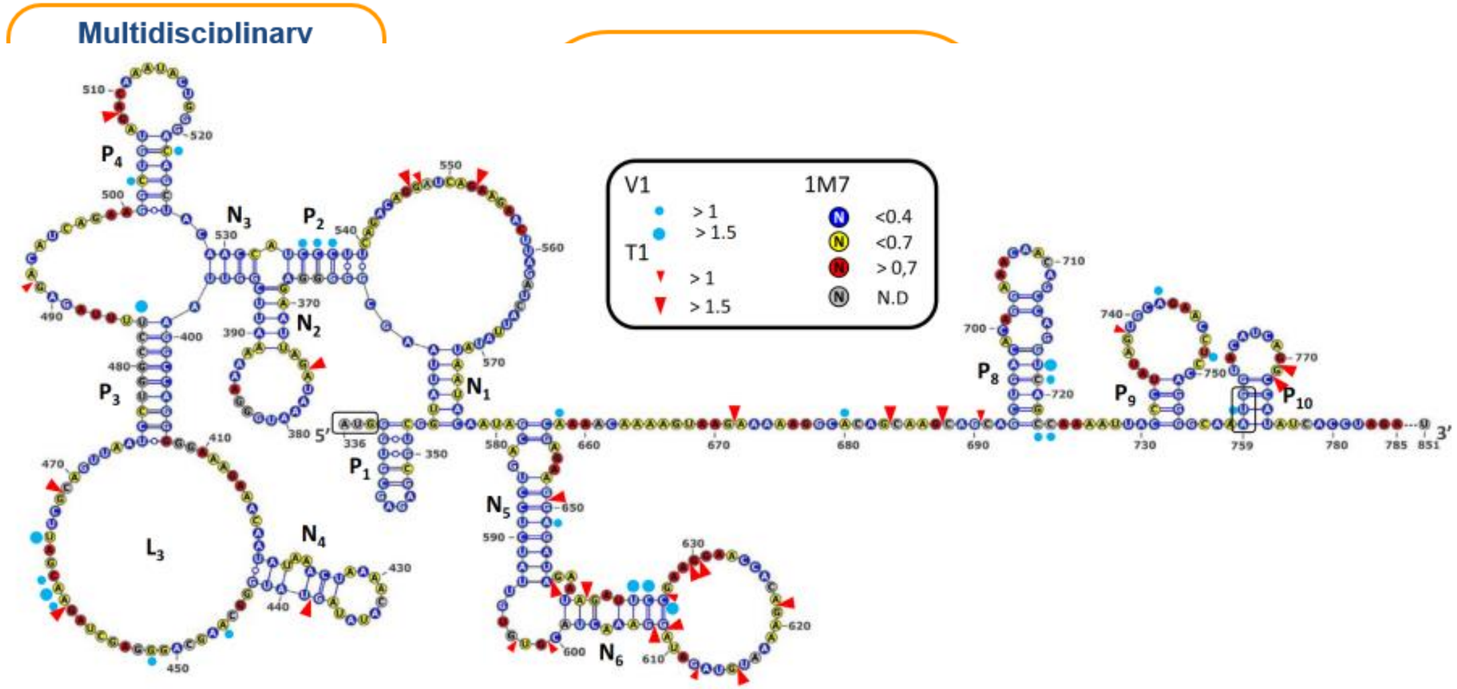
Philippe BOULOC
Signaling and Regulatory Network in Bacteria - SRRB

Bruno SARGUEIL
Molecular Mecanisms of viral RNA translation - CitCoM

Yann PONTY et Sebastian WILL
Algorithms and Models for Integrative Biology – AMIB(IO)







My research and academic related experience

BTS-BioAc

- **LncRNA** in *P. tricornatum* and phosphate stress response.

IBENS
Institut de Biologie de l'Ecole Normale Supérieure

Bachelor BR

- Unveiling new genes in ADH using NGS/WGS.

LNTS
Laboratory for Vascular Translational Science

MSc Microbiology

- Role of **MazF** during growth restart in ***E. coli***.

Institut Cochin

Post–Doc(s) ?

Assistant Professor

Ph.D Student

- **RNA structurome** in ***S. aureus*** during adaptation according to several stresses and conditions.

I2BC

&cnrs

MSc M&CB

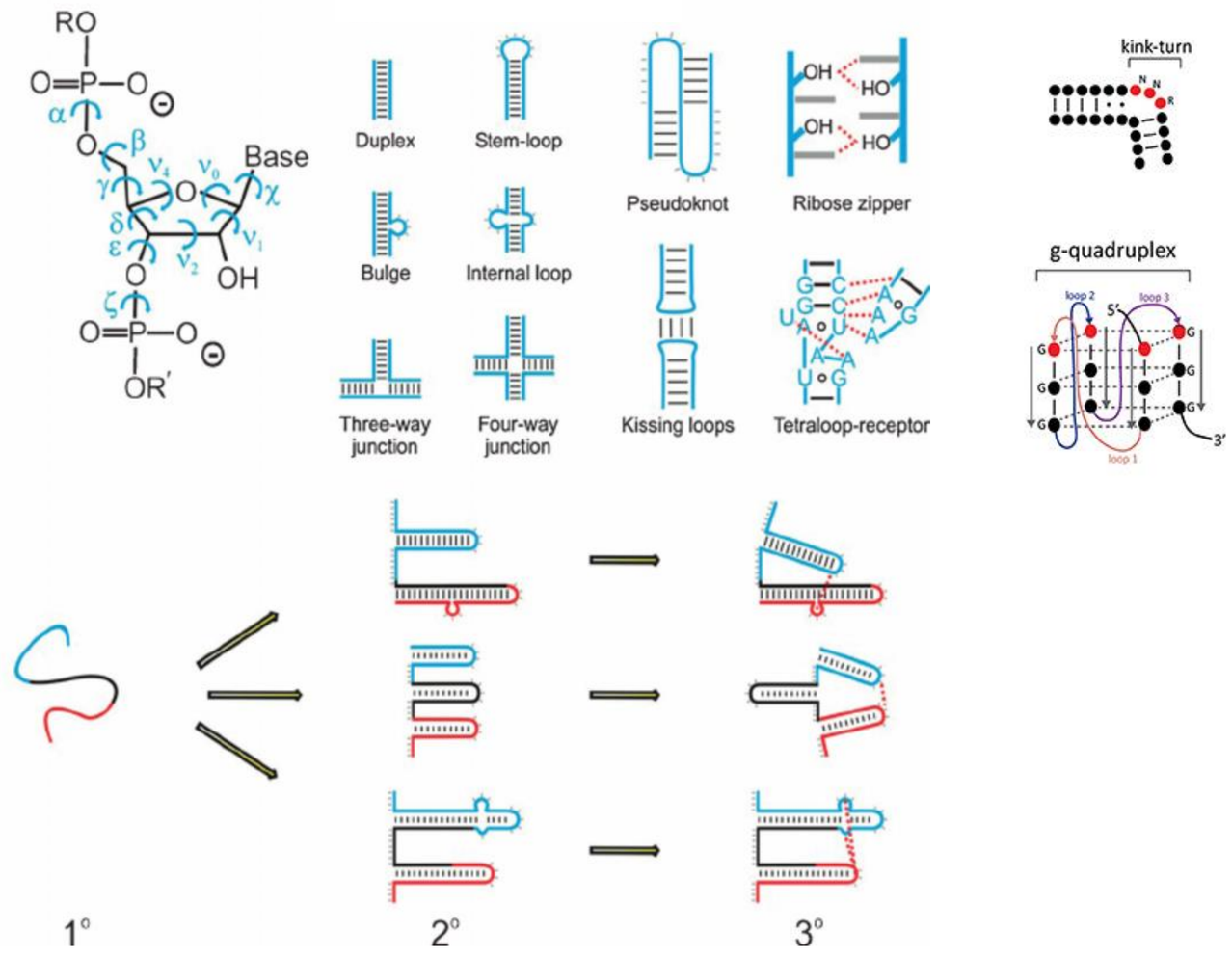
- Deciphering the putative role of the **6S RNA** in biofilm and sporulation pathways in ***C. difficile***.

INSTITUT PASTEUR

- Characterization of the **transcriptome**/proteome of the host during ***S. flexneri*** infections.

Institut Necker Enfants Malades
Centre de Recherche

Dual properties of RNA chemistry



- 2 types of information :

sequence and structure

- **4 conformational structures**;

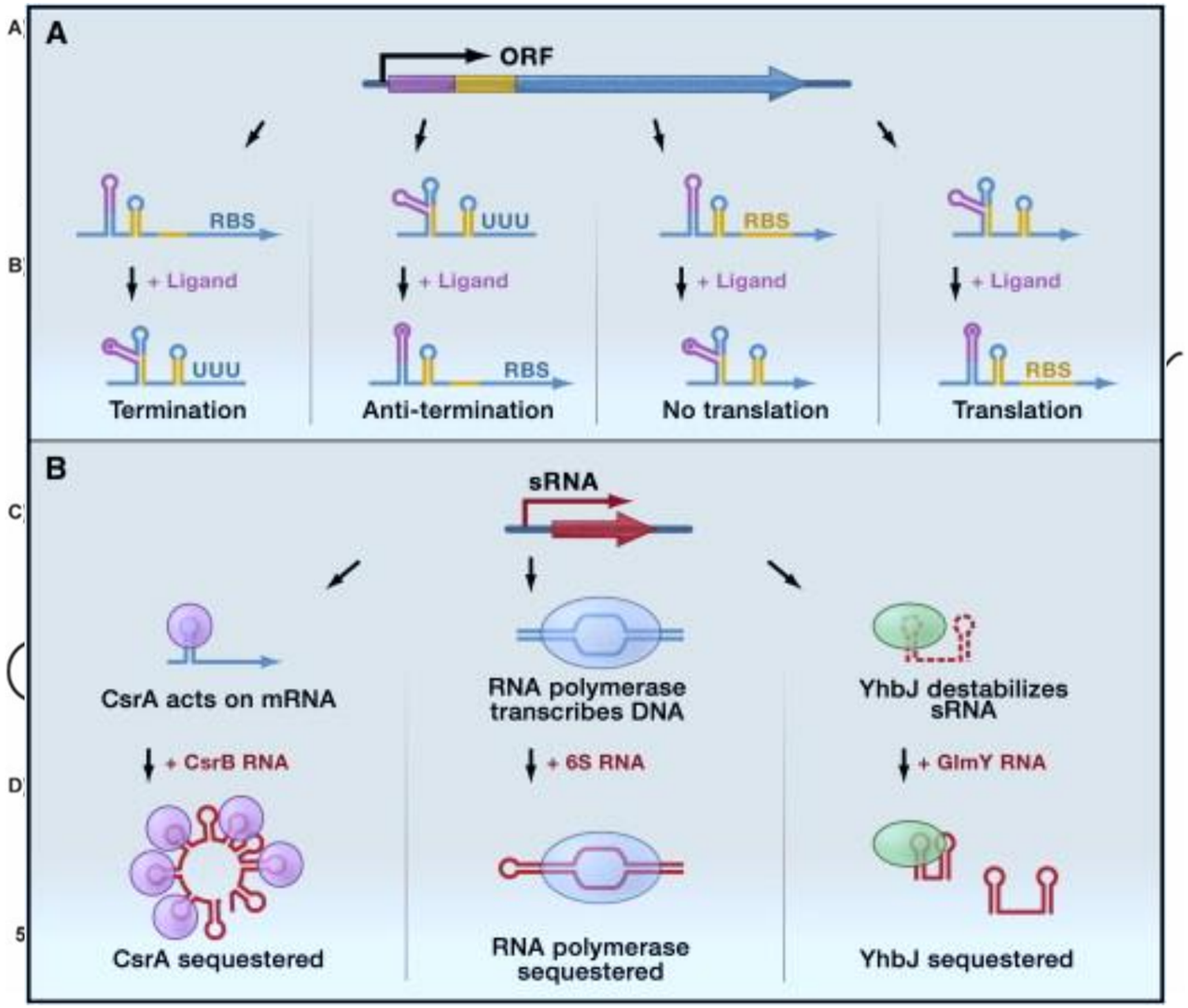
- One given sequence → **several structures**;

- Structure ⇔ function;

How can we determine these structures ?

Marek *et al*., 2011

RNA-mediated regulation in bacteria



- Plethora of basic cellular processes;

- Regulation of gene expression and translation efficiency;

- Regulation of effector proteins;

- Sensing;

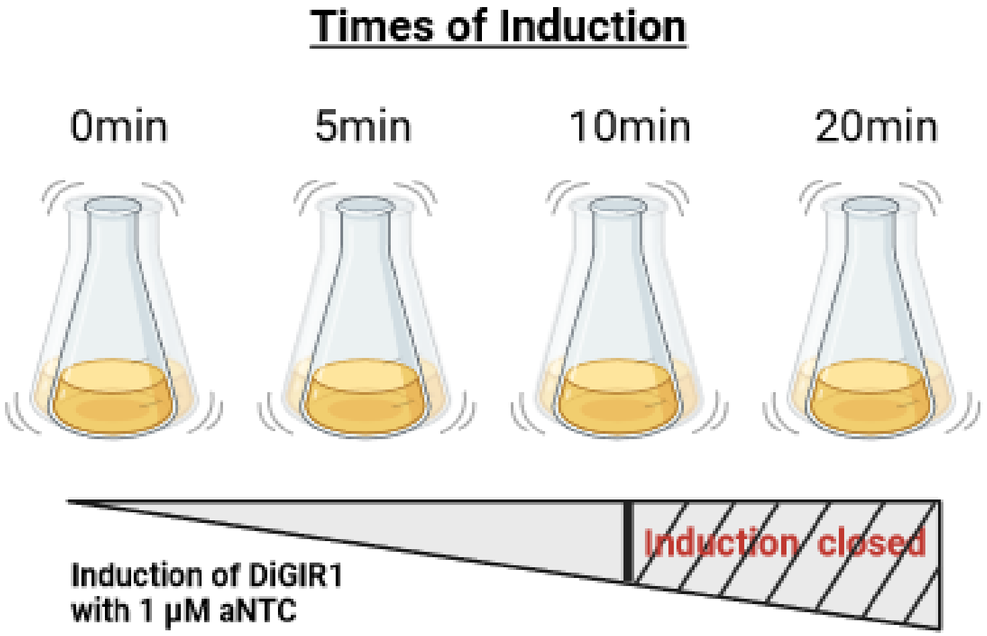
- Response to stimuli;

- Protection against MGE

Yet, a lot of these regulatory pathways need to be uncover !

Waters *et al.*, 2009 Mahendran *et al.*, 2022

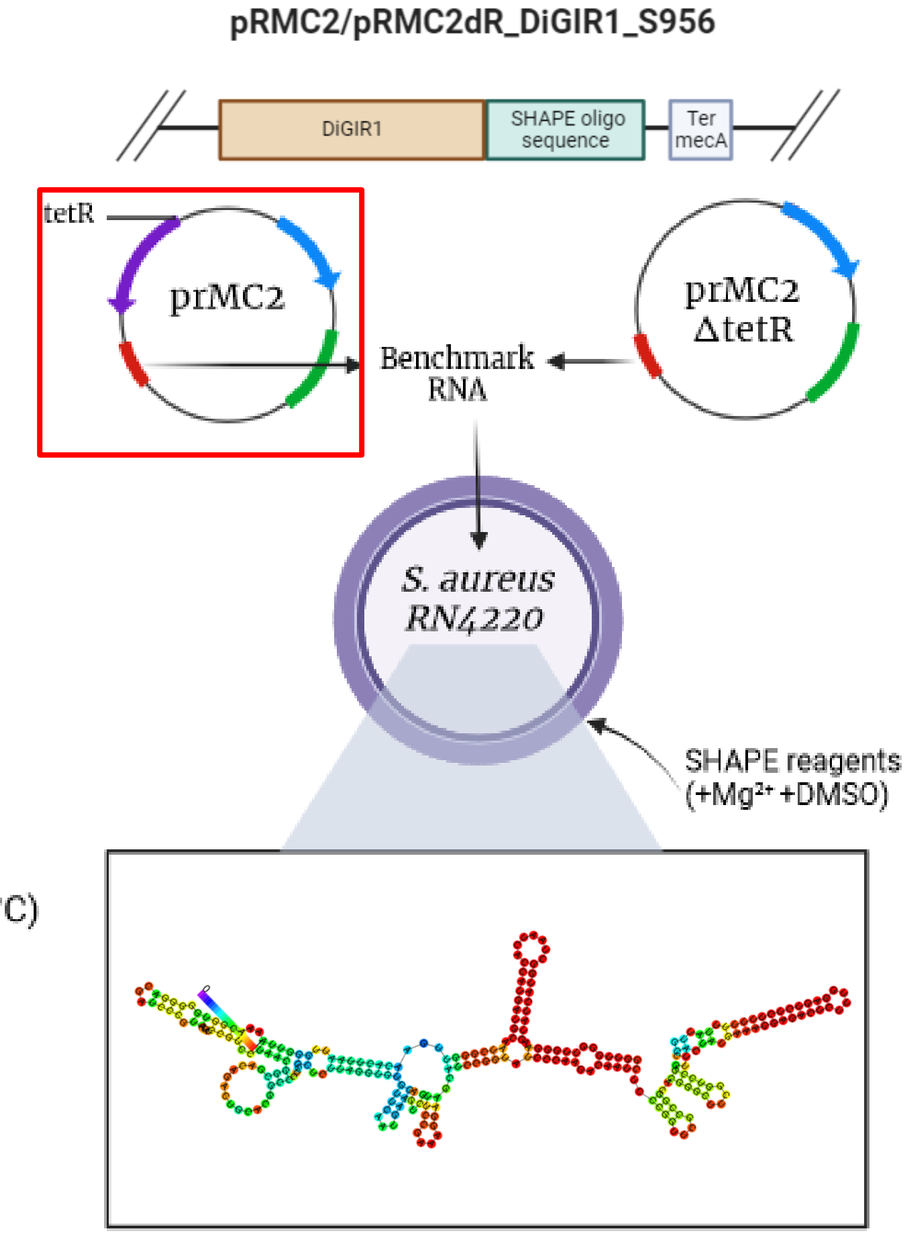
2A3 probing *in vivo* experiment (adapted)



HG003 or RN4220 pRMC2 DiGIR1
day- culture

- 2mL aliquots at OD~1-2
- Wash in PBS 7.4
- RNA probing with 2A3 (20min, 37°C) or DMSO (ctrl)

- Quenching with DTT 1M
- Lysis and heat phenol-chloroform RNA extraction
- Samples conserved at -20°C or -70°C



Accessing the RNA structure

- **Biophysic methods :**

- Cristallography

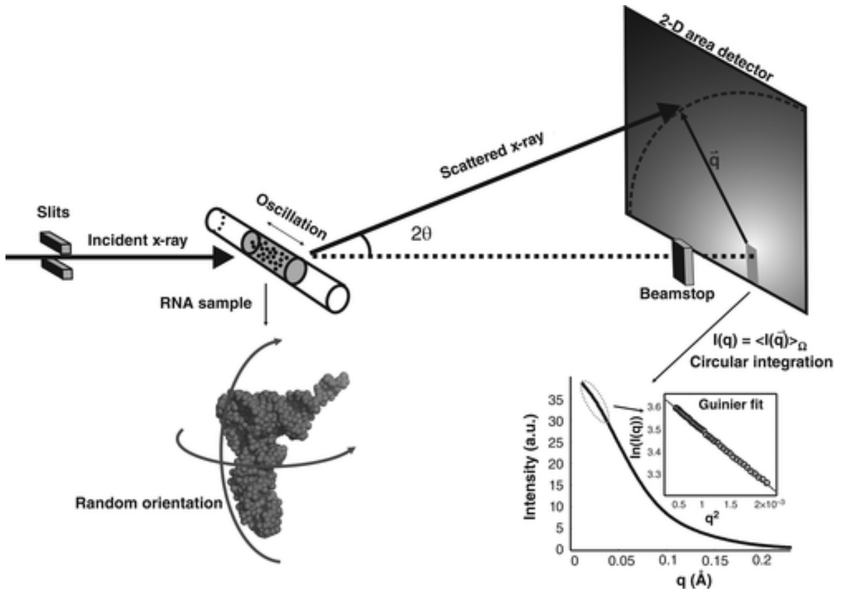
- SAXS;

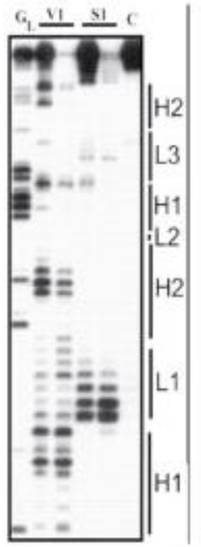
- NMR;

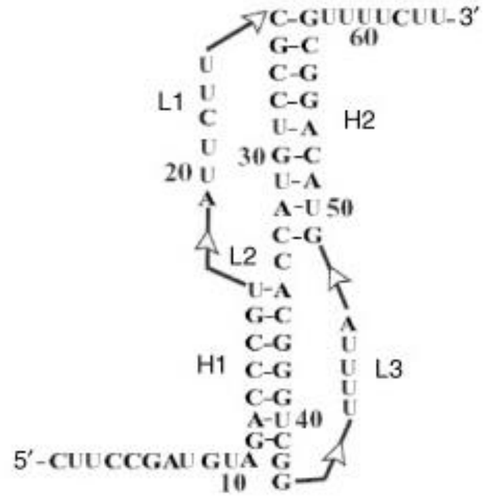
- LC-MS;

- Cryo-EM;

- FRET…







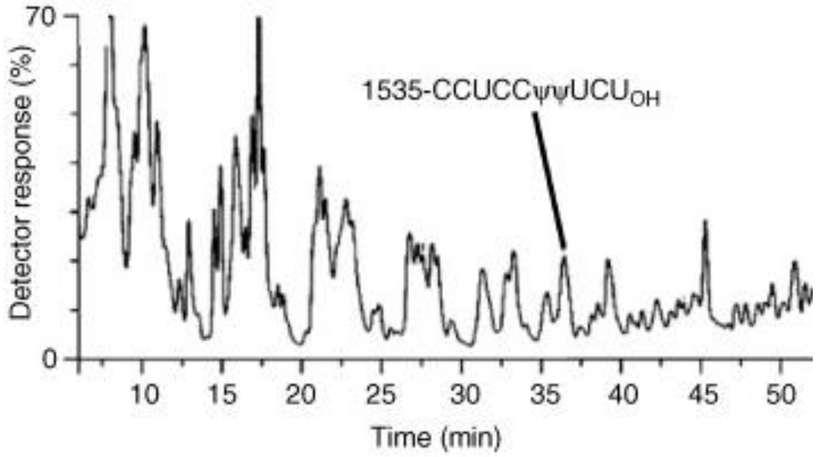
- **Structure-specific chemicals+ enzymes :**

- Nucleases;

- Rnases-mimics;

- Base-specific probes;

- **Ribose-specific probes**…



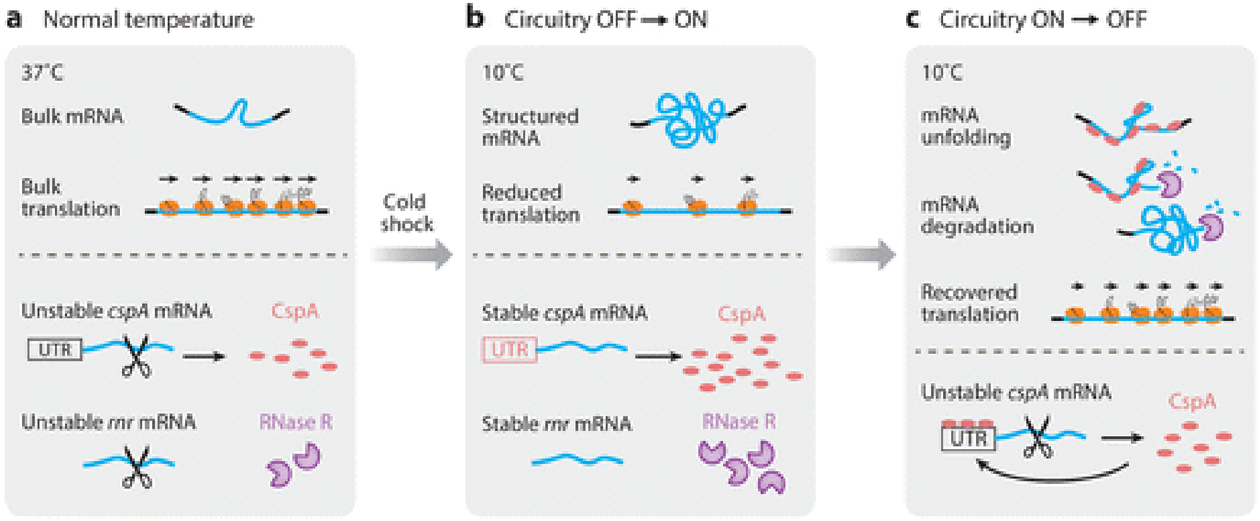
Testing temperature with thermoresponsive sequences ?

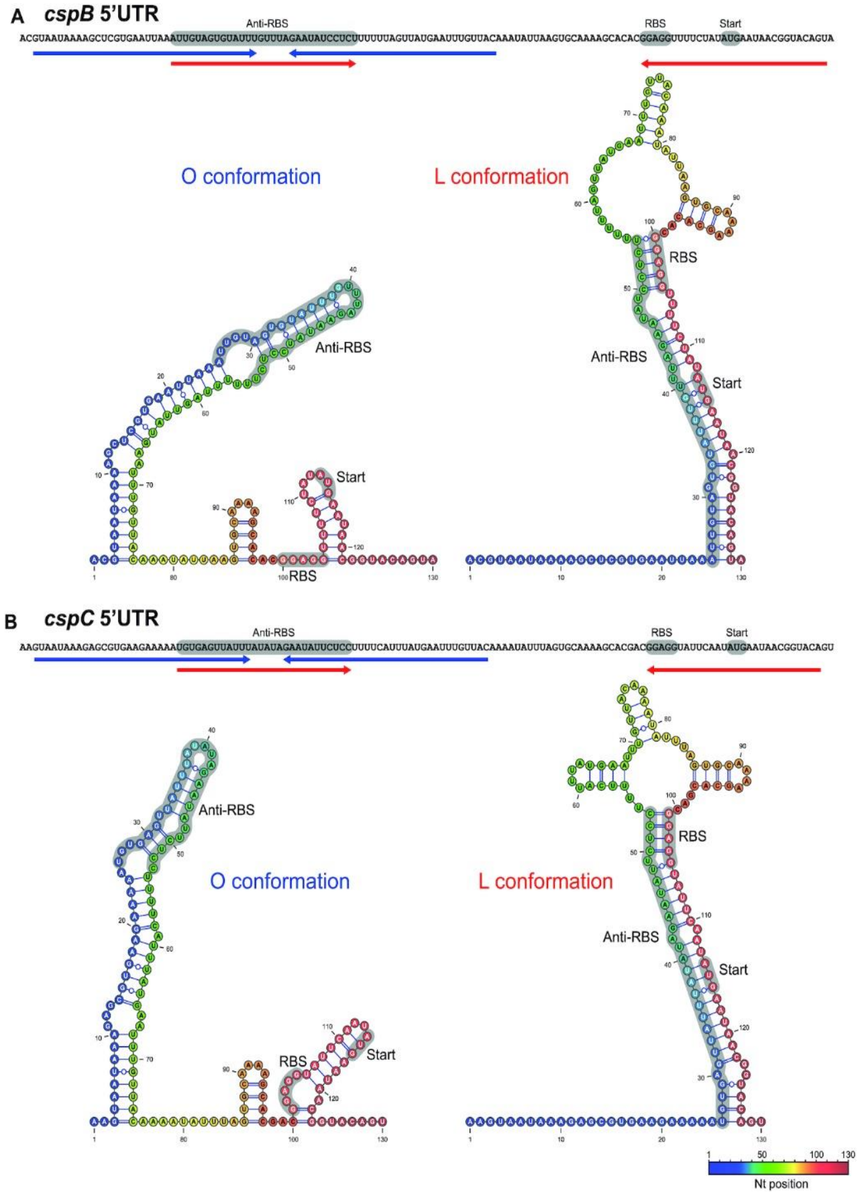
→ Use a **staphylococcal RNA**;

→ ***csp* genes** : RNA chaperones;

→ **Structure predicted** with mFold

→ Thermoresponsive éléments in **the 5'UTR** of *csp* genes





Arancha Catalan-Moreno
Ph.D

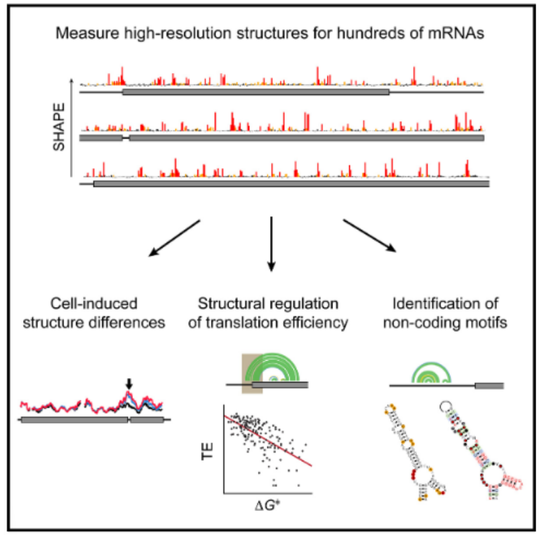
Alejandro Toledo-Arana

Relationship between TE and mRNA's structure/regulation in *E. coli*

Cell

Pervasive Regulatory Functions of mRNA Structure Revealed by High-Resolution SHAPE Probing

Graphical Abstract



Authors

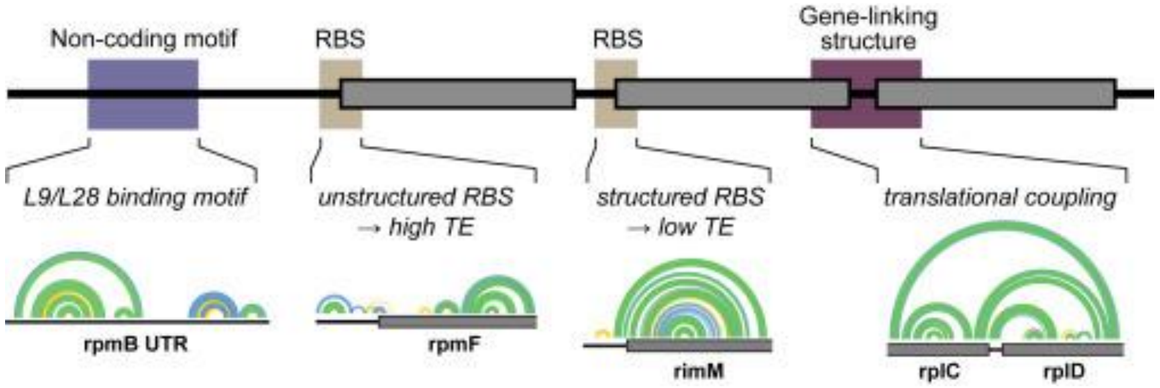
Anthony M. Mustoe, Steven Busan, Greggory M. Rice, ..., Razvan Nutiu, Jeremy L. Baryza, Kevin M. Weeks

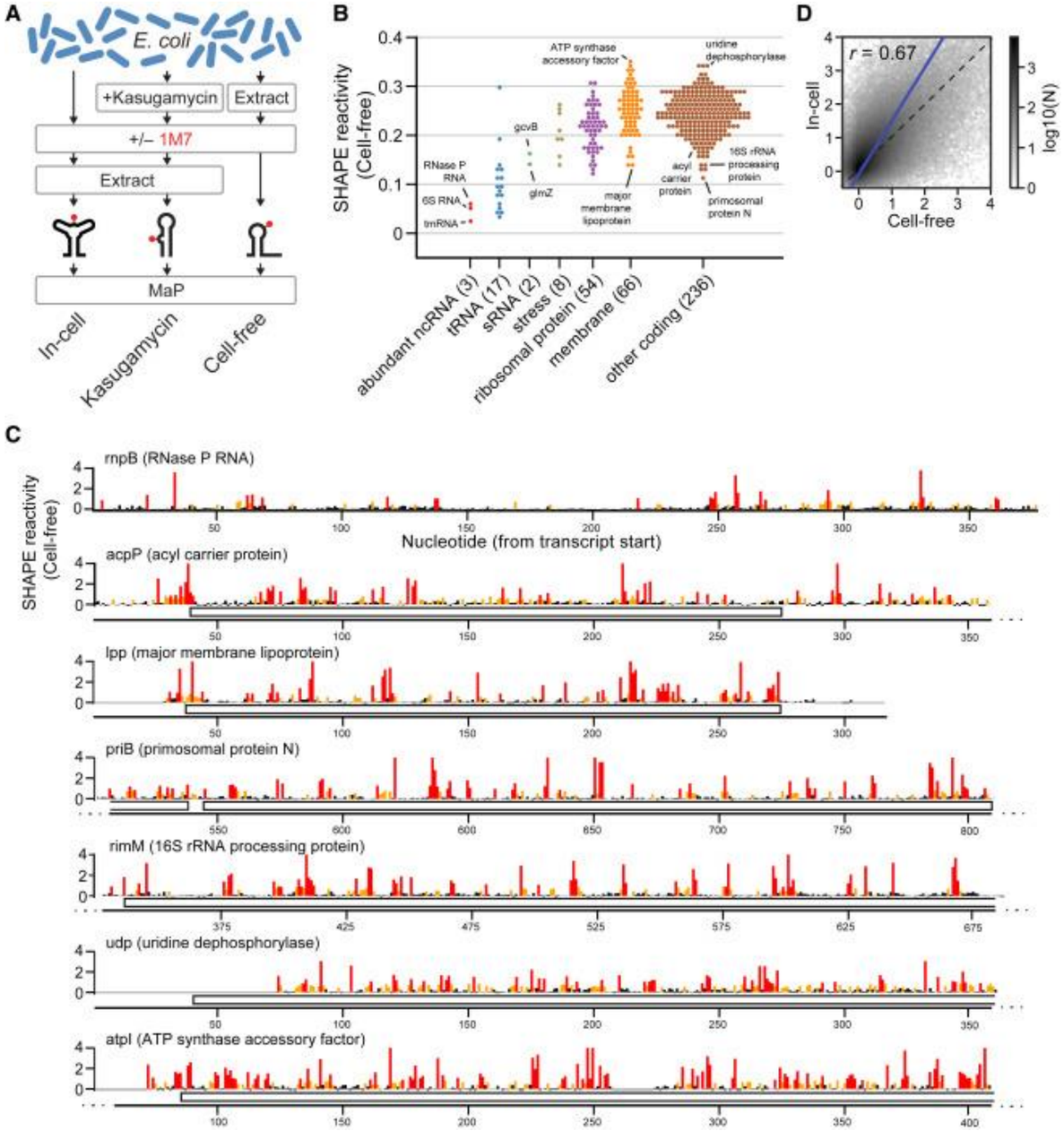
Correspondence

amustoe@unc.edu (A.M.M.), weeks@unc.edu (K.M.W.)

In Brief

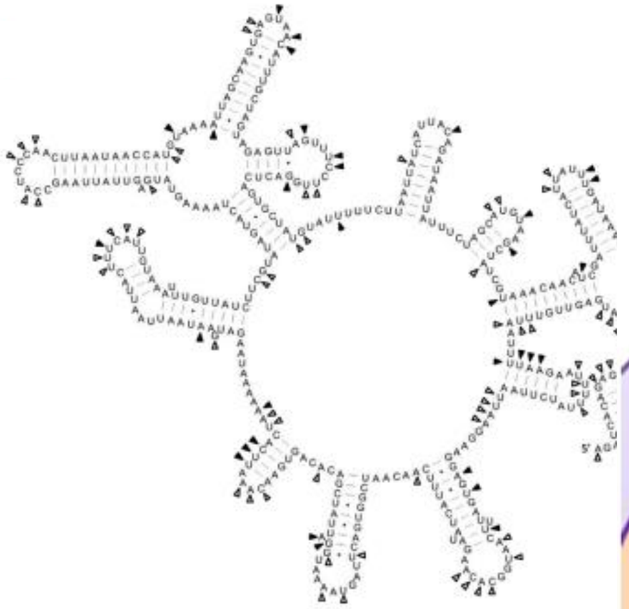
High-resolution probing of hundreds of genes in living *E. coli* cells reveals that bacterial mRNAs fold into highly diverse and complex structures and that these structures have widespread regulatory functions.

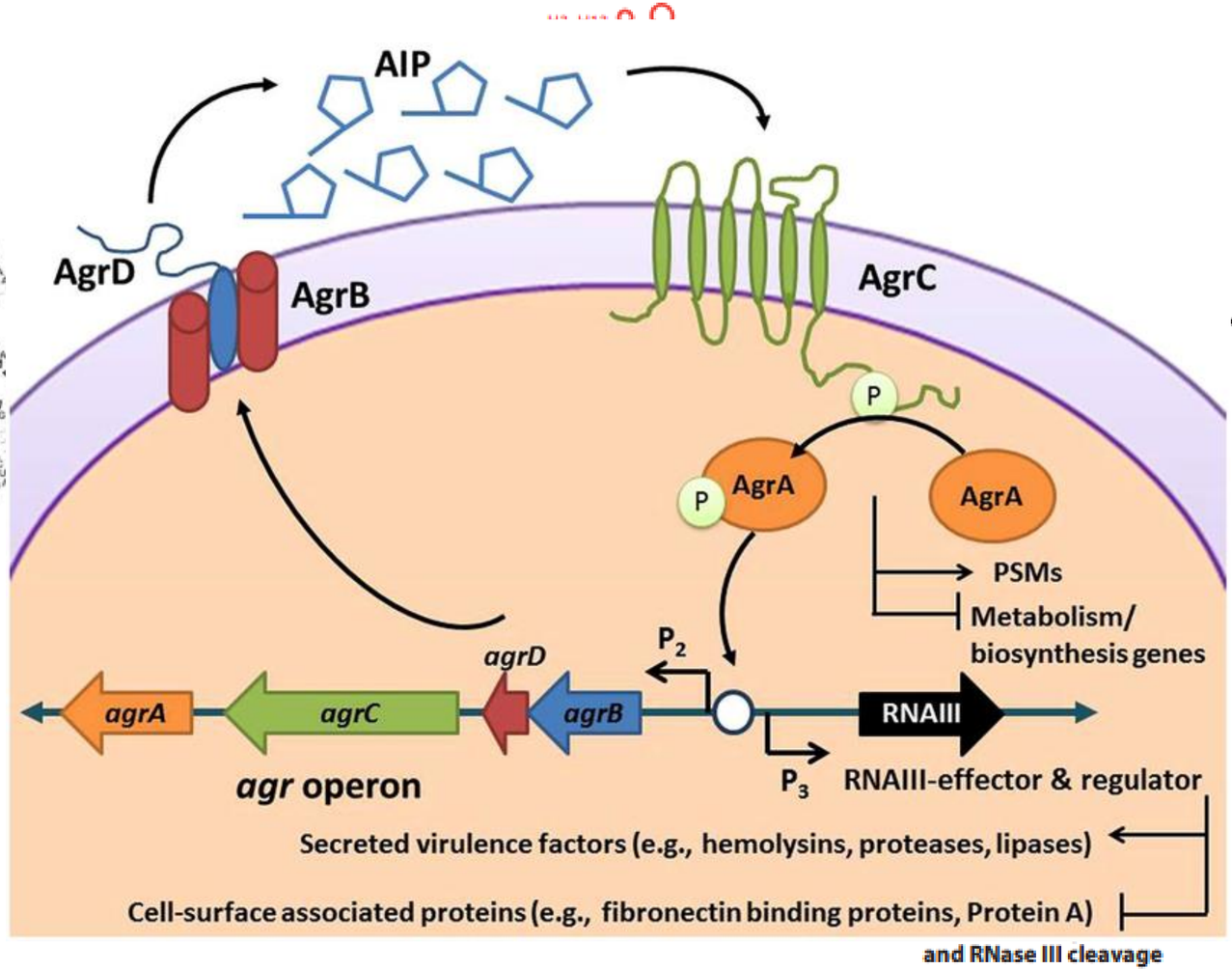


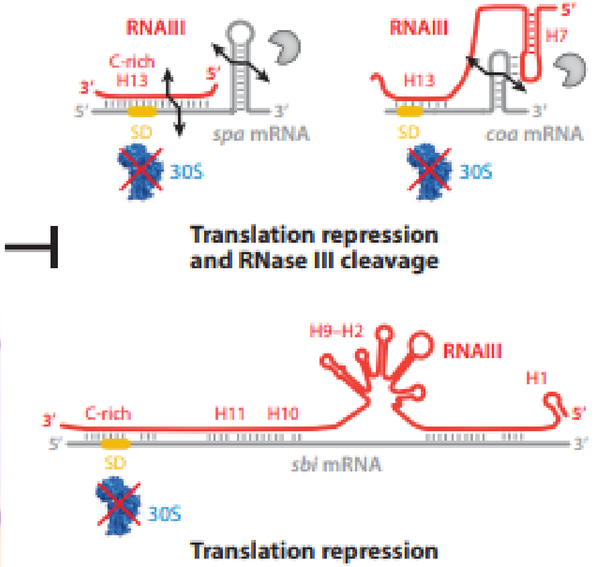


Translational coupling in bacteria

RNAIII

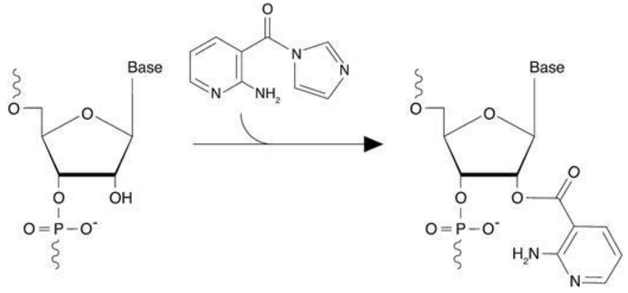
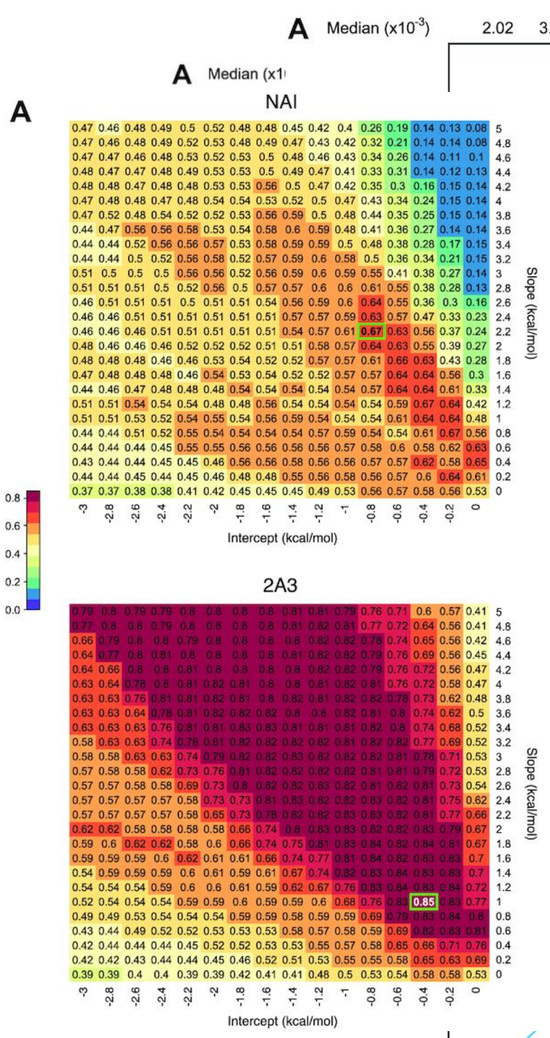
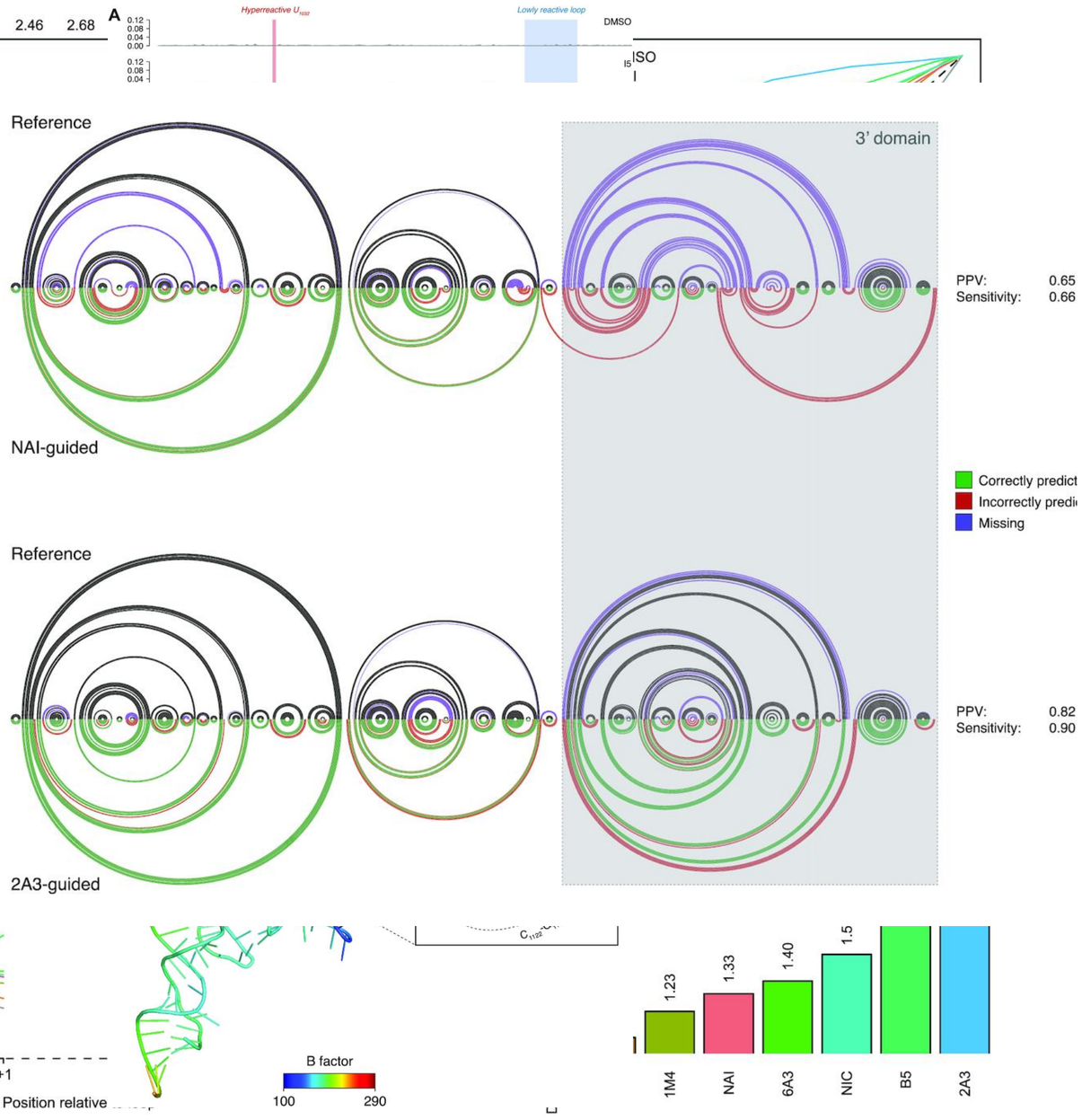
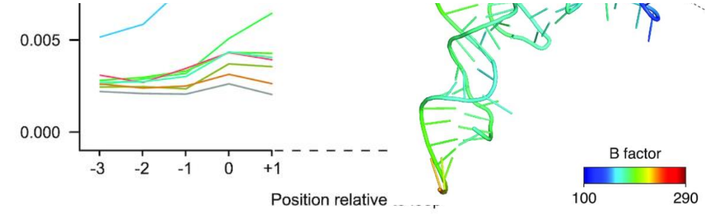






Quave CL et al. 2014,

2A3

Danny Incarnato

Tycho Marinus