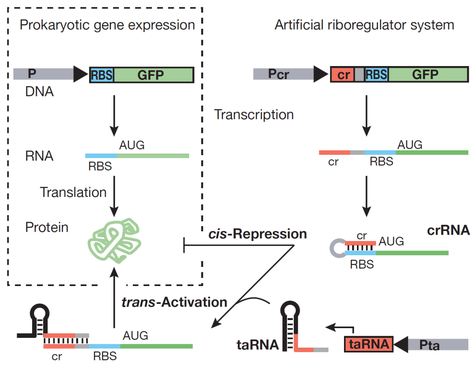
In-Vitro testing and characterization of nucleic acid structures and networks

Jonathan LEE TIN WAH and André Estévez-Torres

Laboratory for Photonics and Nanostructures, CNRS, Marcoussis, France

II. *In-vitro* implementation and testing of synthetic RNA devices

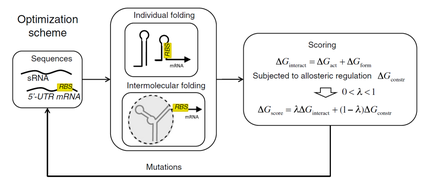
Riboregulators



Isaacs et al. Nature (2004)

- **Rational design and engineering of post-transcriptional regulation**
- **Modular riboregulator**

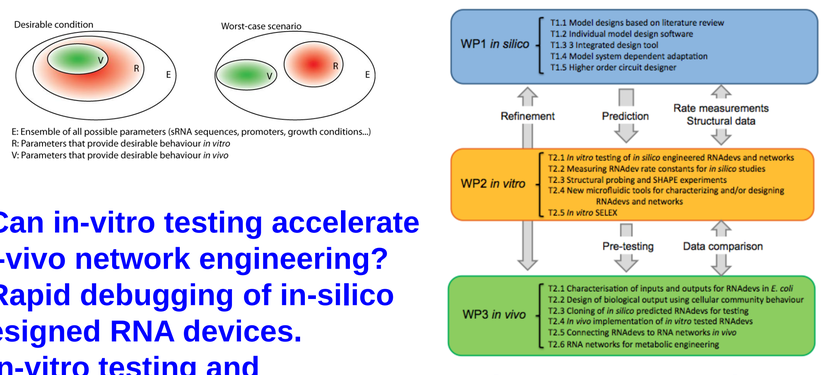
Functional de-novo RNA devices



Rodrigo et al PNAS (2012)

- **Based on trans-activation**
- **Newly generated sequences in-silico**
- **Successful implementation in-vivo**
- **Allows for modularity**

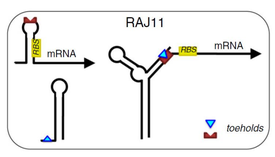
Goals and Questions



- **Can in-vitro testing accelerate in-vivo network engineering?**
- **Rapid debugging of in-silico designed RNA devices.**
- **In-vitro testing and characterization of in-silico designed RNA devices (functionality, rate constants, etc…)**

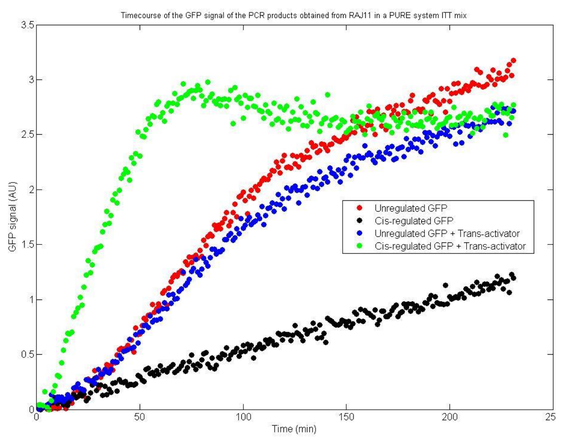
- **Joint european project**
- **Planned synergy between in-silico, in-vitro and in-vivo**

Study of a synthetic riboregulator *in-vitro*

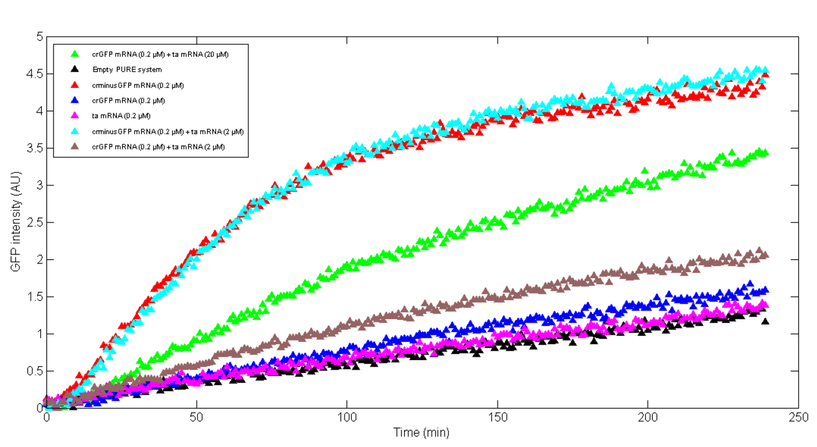


Rodrigo et al PNAS (2012)

- **Use of RAJ11 (generated de_novo)**
- **Testing performed using PCR products of the RAJ11 plasmid.**
- **GFP gene lacking a cis-repressor (unregulated, crminusGFP)**
- **Cis-regulated GFP (crGFP)**
- **Trans-activator (ta)**



- **The individual components of the RAJ11 riboregulator are functional in an in-vitro expression system.**



- **The mRNA transcribed from the PCR products are also functional.**

Work in progress

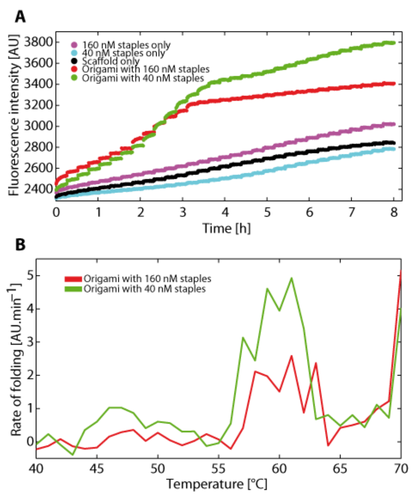
- **Monitor the trans-activation in-situ**
- **Reliable mRNA detection and quantification during transcription.**
- **Study the kinetics of the trans-activation**

II/. Folding pathway of DNA nanostructures at the single molecule level

(with Christophe David, LPN, CNRS)

Manuscript in preparation

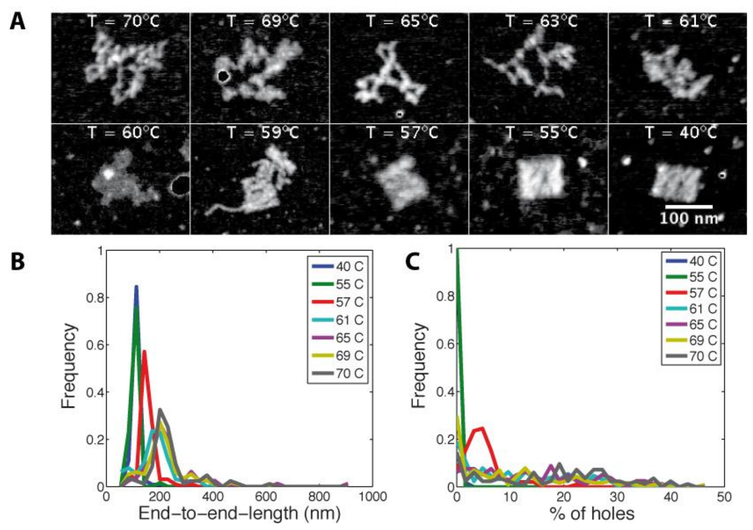
Folding rates



- **Folding occurs during a ~4° C temperature range.**
- **Consistent with the literature**
- **Allows us to identify significant points in time and temprature to perform our measurements**

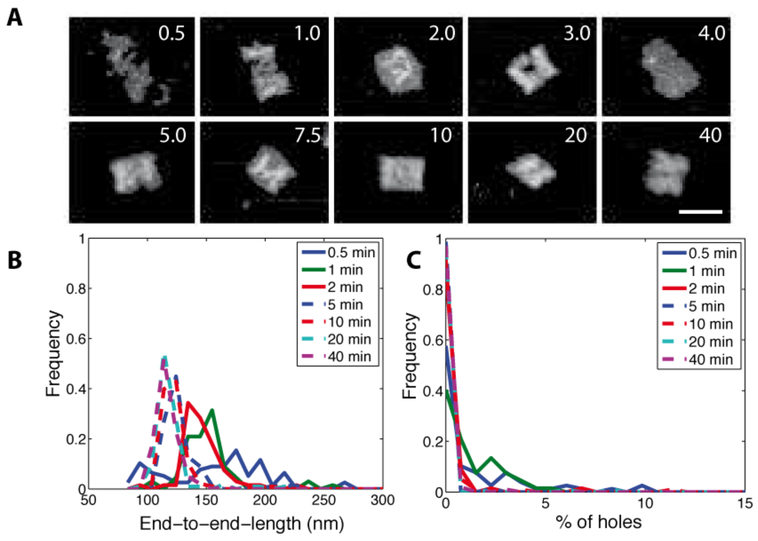
- **Rate of folding of origami built from 10 nM of M13 scaffold and 40 nM and 160 nM staples (based on an experiment from Sobczak et al Science (2012))**

Thermodynamics



- **Provides visuals of the folding process at various temperatures.**
- **Three folding 'regimes' identified after image analysis.**

Folding kinetics



- **Visualization of the folding process in isothermal conditions.**
- **Structure without holes obtained within 5 min.**
- **Fully formed origami observed after 20 min after slow reorganization.**

Acknowledgements : Ilka Axmann, Tim Kolmsee, Sven Findeiss, Christoph Flamm, Dennis Diest, Alfonso Jaramillo, Shensi Shen