RNAct

Enabling proteins with RNA recognition motifs for synthetic biology and bio-analytics

June 2021

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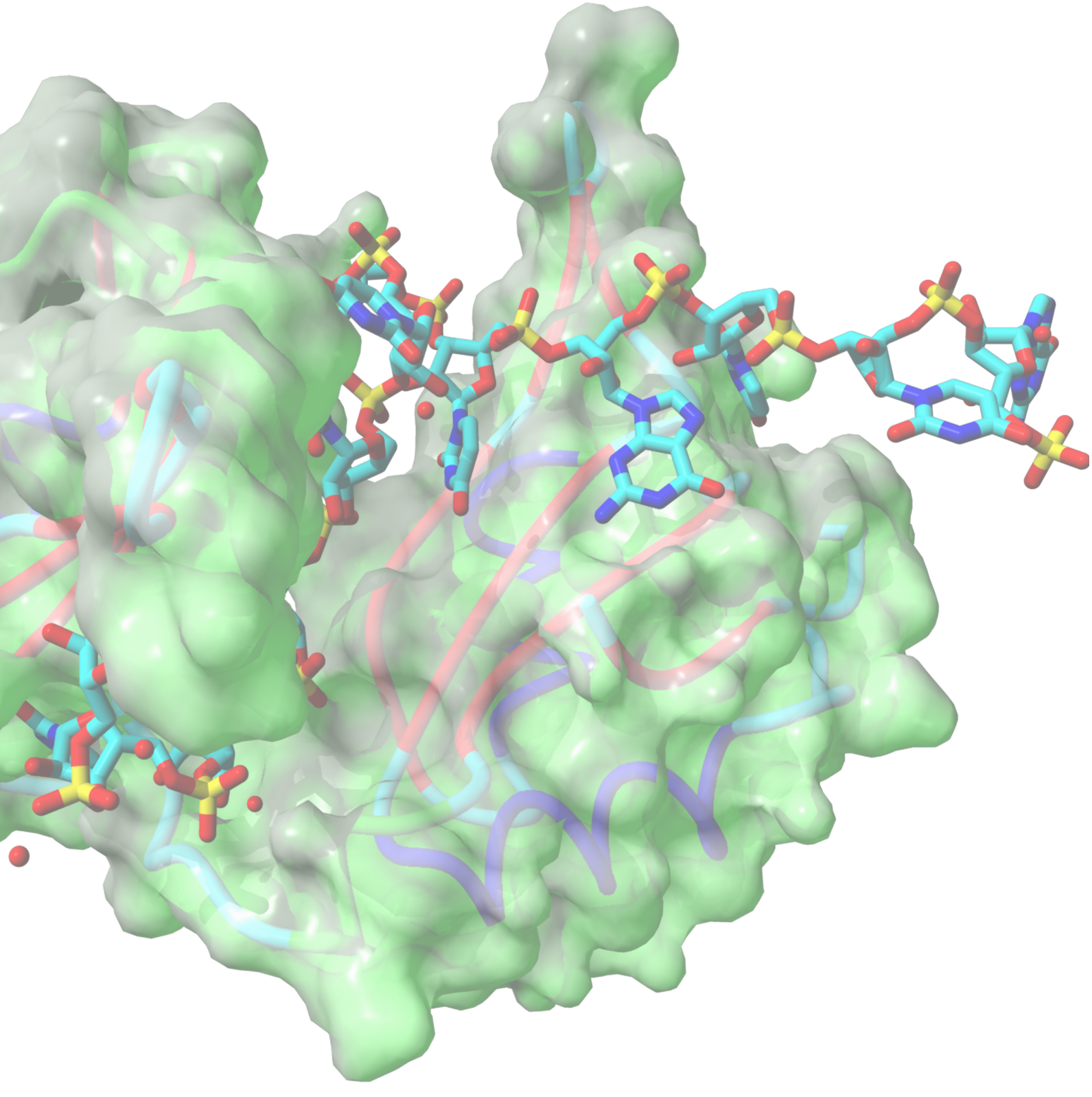

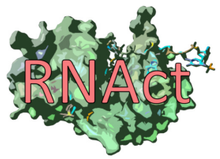

This issue of the newsletter has been edited by Hrishikesh Dhondge (ESR 3), Niki Messini (ESR 5), and Roswitha Dolcemascolo (ESR 8).

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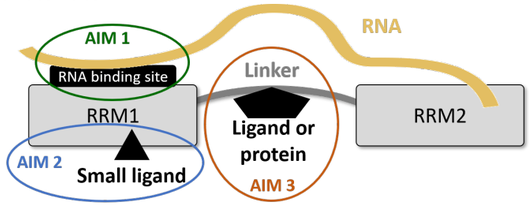
RNAct at a glance

The project in a nutshell

RNAct is a Marie Skłodowska-Curie Innovative Training Network (MSCA-ITN) project with the research aim of designing novel RNA recognition motif (RRM) proteins for exploitation in synthetic biology and bio-analytics. This is achieved through a design cycle that starts with computational approaches at the sequence and structure levels of proteins and RNA, in order to select amino acid positions and mutations for large-scale phage display experiments with RNA screening. Viable RRMs will be further investigated at the atomic level with integrative structural biology approaches, and will be applied in synthetic biology, to post-transcriptionally regulate fatty acid processing via RRMs, and in bio-analytics, to detect RNA in-cell and design RNA biochips.

RNAct creates a comprehensive, cross-disciplinary platform to train ten early-stage researchers (ESRs) with versatile computational and experimental skills, a high level of professional maturity, and an excellent academic and non-academic career opportunities. This platform includes:

- Training in molecular work for bio-analytics and synthetic biology
- Training on topical and transferable skills
- A buddy system to ensure links between computation and experiment
- Involvement in both academic and industry environments
- Engagement in dissemination and communication actions
- involvement in innovation activities



The project focuses on the following aims:

1. Modify the RNA specificity of single-domain RRMs by modulating their side-chain interactions with ssRNA motifs (3-5 nucleotides), so tuning or steering their RNA recognition while maintaining their other functions.

2. Allosterically control single-domain RRM-RNA binding via a small ligand that binds an RRM and either triggers RNA-recognition or modifies RNA specificity.

3. Design multi-domain RRM protein switches where allosteric changes in the domain linker change the RNA specificity, or where RNA binding changes the linker conformation.

The Network is organised into six Work Packages:

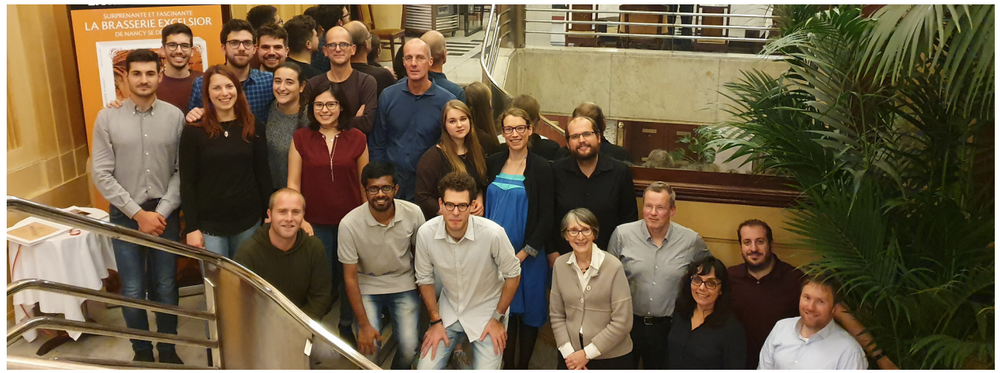
Work Package 1. Creation and characterisation of functional RRMs.
Work Package 2. Representation and design of dynamic proteins.
Work Package 3. Bio-analytics and synthetic biology.
Work Package 4. Training and education.
Work Package 5. Coordination and management.
Work Package 6. Dissemination and communication.

For more information, visit http://rnact.eu/workPackages/ .

Consortium

RNAct brings together seven beneficiary institutions from five different European countries. Four academic organisations (VUB, CNRS, CSIC, and HMGU) and three companies (Giotto Biotech, Dynamic Biosensors and Ridgeview Instruments AB) join forces with the support of six partner universities (University of Liège, Lorraine University, Technical University of Munich, University of Florence, Polytechnic University of Valencia and Uppsala University) to build up a highly interdisciplinary network to tackle the ambitious goals of the project.

Beneficiaries	Partners
Vrije Universiteit Brussel (VUB) **Prof. Dr. Wim Vranken**	Université de Liège (ULG) **Prof. André Matagne / Dr. Marylène Vandevenne**
Centre National de la Recherche Scientifique (CNRS) **Dr. Isaure Chauvot de Beauchêne** **Dr. Marie-Dominique Devignes**	Université Lorraine (UL) **Prof. Malika Smaïl-Tabbone**
Helmholtz Zentrum München (HMGU) **Prof. Dr. Michael Sattler**	Uppsala Universitet (UU) **Prof. Helena Danielson**
Consejo Superior de Investigaciones Científicas (CSIC) **Dr. Guillermo Rodrigo**	Università degli studi di Firenze (UF) **Prof. Marco Fragai**
Ridgeview Instruments AB (RV) **Dr. Karl Andersson / Dr. Jos Buijs**	Universitat Politècnica de València (UPV) **Prof. Carmelo López**
Giotto Biotech Srl (GIO) **Dr. Tommaso Martelli**	Technische Universität München (TUM) **Prof. Dr. Martin Zacharias**
Dynamic Biosensors GmbH (DBS) **Dr. Ulrich Rant / Dr. Wolfgang Kaiser**	



Secondments

Giotto biotech
Secondment host

Joel Roca (ESR2; VUB) has recently completed his secondment at Giotto Biotech where he learnt about structural biology from an experimental point of view, thus complementing his computational work in the context of the RNAct project. During this month, Joel Roca followed all the experiments that ESR7 Anna Pérez has been carrying out, from bacterial growth and protein expression and purification to much more technical experiments such as protein crystallography and NMR.
This period proved more than useful for Joel to fill some gaps between the experimental and computational work. Now, with a better understanding of the problems faced in the structural biology experiments, he will be better able to help with his computational work.

VUB
Secondment host

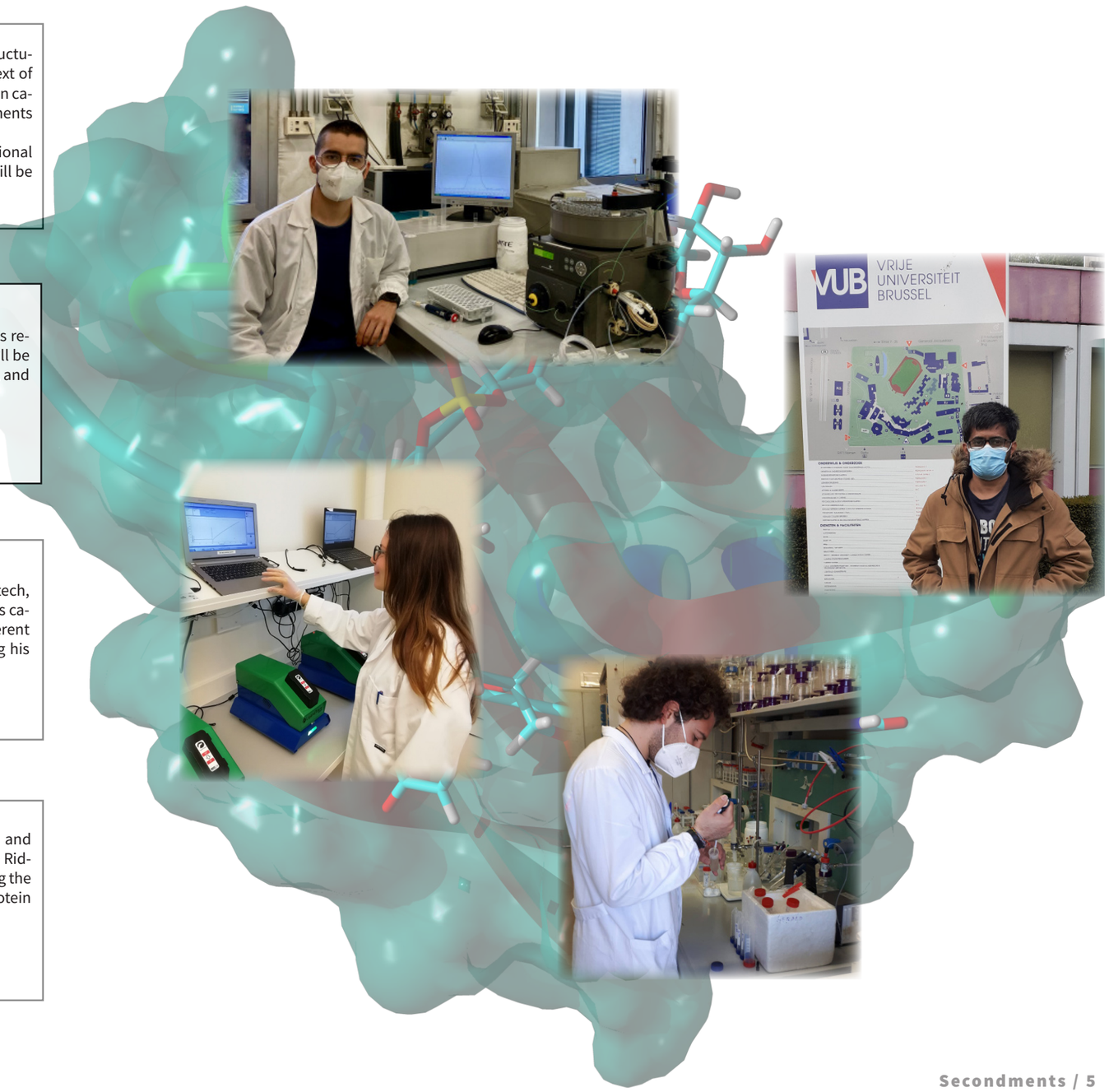
Hrishikesh Dhondge (ESR3; CNRS) is currently in Brussels (Belgium) for his secondment at VUB. During this research stay, he will investigate the differences between bound and unbound forms of RRM domain. This will be helpful for the application of sequence-based methods for protein design and analysis in synthetic biology, and the resulting data will be further incorporated into the RRM modeling and docking protocol.

Giotto biotech
Secondment host

Luca Sperotto (ESR6; HelmholtzZentrum münchen) is currently performing his secondment at Giotto Biotech, in Florence. During his 3-month secondment, he will work elucidating more about the dynamic interactions carried out by the RRM domains in the context of the proteins they are part of. With this aim, he will use a different subset of NMR experiments based on Carbon detection, along with standard NMR experiments, widening his knowledge on useful and complementary experiments for his structural biology studies.

Ridgeview Instruments
Secondment host

Roswitha Dolcemascolo (ESR8; CSIC) is developing her secondment in an excellent mixed academic and non-academic environment. From January to June 2021, she is working at the Swedish biotech company Ridgeview Instruments AB (RV) in Uppsala. There, she is collaborating with ESR10 Guillermo Pérez Ropero using the LigandTracer technology, which allows to perform real-time quantitative assays and to measure RNA-protein interactions in living bacterial cells.



Meet the PIs

Prof. Dr. Michael Sattler

HelmholtzZentrum münchen
German Research Center for Environmental Health

Michael Sattler is professor for biomolecular NMR at the Technical University of Munich and head of the Institute of Structural Biology at the Helmholtz Center Munich. He did his doctoral research with Christian Griesinger at the University of Frankfurt, Germany, focussed on the development of triple-resonance NMR methods to study biological macromolecules.
As postdoctoral research fellow with Steve Fesik at Abbott Labs, USA, he used NMR to study Bcl family proteins involved in the regulation of apoptosis. With his research group established 1997 at EMBL Heidelberg, and since 2007 in Munich, he develops and applies biomolecular NMR methods to study the structure and dynamics of proteins and RNAs in eukaryotic gene regulation (alternative splicing, (long) non-coding RNAs, and in cellular signaling). He pioneered integrative structural biology approaches, combining solution techniques (NMR, small angle X-ray and neutron scattering), highlighting the role of conformational dynamics for biomolecular function. Recent research involves NMR and structure-based drug discovery on innovative drug targets in disease-linked cellular pathways. He is an elected member of EMBO and of the German National Academy of Sciences, Leopoldina.

How did you discover your interest in biochemistry?

I always wanted to understand what the foundation of life is, both from a philosophical point of view but also how do atoms and molecules come together to make a human being…

Have you ever worked on something completely non-academic?

Of course many times, jobs to earn money: postman, wood factory, weaving mill, social service in a psychiatric day clinic and playing entertaining music at festivities on weekends.
In the end after my highschool, while during social service, I was undecided and seriously considering to study (jazz) music rather than chemistry. In the end the decision for chemistry was right I believe. But I still do play music, which is very important to me as indeed it is something completely different to enjoy and refresh mind and soul.

What path did you follow to become a Professor?

After high school (in a rural part of Germany with a nice countryside but far from metropolitan life) I studied chemistry in Frankfurt, stayed there until I received a doctoral degree in developing NMR methods for application to biomolecules. All I knew then was that I wanted to do a postdoc abroad, preferably in the US, and that, in the long term I wanted to stay in academia, and not move to an industry job. And yet, when Steve Fesik passed through Frankfurt, looking for postdoc candidates, I had an interview with him and eventually did my postdoc yet in a pharmaceutical industry lab (!). This was very exciting and successful, so I got a job as group leader at EMBL Heidelberg, an amazing international research lab, with small highly dynamic and interactive groups and truly collaborative and a multicultural atmosphere. Being a non-tenure position (people are expected to leave after 9 years), I started to look for my next job at various places in Europe and the US, and eventually found my current position at TUM and the Helmholtz Center in Munich. This allowed me to continue the research program established at EMBL and focus on new and longer term projects. This included taking advantage of our structural biology findings to develop novel therapeutic approaches using structure-based drug discovery, something that I saw being done during my postdoc at Abbott with impressing success and innovation.

What are the advantages and disadvantages of working in academia, from your perspective?

Working in academia gives you outstanding freedom to pursue your own ideas and research with little limitations (as long as funding is secured). My personal reason not to find a job in industry was the fact that I would not like to being told on a Monday morning that the project I was involved the last two years has been stopped by “upper management” for economic reasons… What is great in industry is that highly interdisciplinary teams work efficiently together to advance, for example, a drug development project from first ideas to clinical approval. This is something much more difficult to do in academia, where individual groups often focus on their own research and long term commitments (and infrastructure and funding) for a drug discovery program are more difficult to implement.

What is your role in RNAct? What do you think the RNAct project can bring to your field?

We are experimental structural biologists and contribute our skills and expertise to experimentally test the design of RRM domains with altered RNA binding specificity. Working closely together with great computational groups is very fruitful and offers unique opportunities to advance the goals of RNAct.

Why is RNAct important for society?

Understanding the RNA-binding specificity of RRMs and rationally designing and altering these has unique potential to regulate gene expression potentially in innovative therapeutic approaches in the future. This is completely out of the box and does not follow standard drug discovery strategies, and therefore has enormous potential for innovation. The great potential of targeting and using RNA and RNA-binding proteins is currently very prominently visible with RNA-based vaccines and first drugs that modulate RNA splicing (which is controlled by RNA-binding proteins, most of which harbor RRMs) to cure until recently incurable devastating diseases, such as spinal muscular atrophy.

Do you think NMR is the key to understand RNA-protein interactions? If so, why?

NMR is combined with crystallography and cryo-EM in integrative structural biology approaches. The unique toolbox NMR provides is its various unique experimental approaches to characterize biomolecular interactions at residue or even atomic level and, even more important, to study conformational flexibility and dynamics. The latter is often ignored and in the traditional view of structural biology, which was coined by static crystal structures, neglected if not overlooked. Yet, biology is absolutely 4-dimensional, and biomolecules (at the molecular level), like cells and organisms, are strongly dependent and changes over time, dynamics and conformational fluctuations, which are essential for life!

What prospects do you see for this research field in the future?

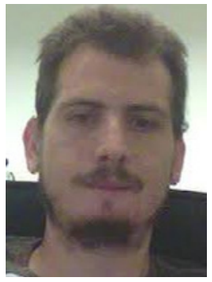
NMR is still advancing in methods and approaches, but there is now a set of experimental approaches available to study the dynamics of biomolecules from nanoseconds to days. This unique potential will be further utilized and will greatly help to advance our understanding of biomolecular function, which makes the molecular foundations of life.

About - Sattler’s research group

The main focus in the Sattler group is to understand the structural basis of protein-RNA interactions that are functionally important for various aspects of gene expression, such as the regulation of (alternative) pre-mRNA splicing by non-coding RNAs (siRNAs, miRNAs). Current projects focus on protein-protein and protein-RNA interactions that play important roles in the regulation of constitutive and alternative splicing and other aspects of RNA metabolism involving non-coding RNAs. The team is also initiating studies for the structure-based design of small molecular inhibitors. The team is implementing and developing integrated structural biology approaches combining NMR-spectroscopy ans Small Angle Neutron and/or X-ray Scattering (SAXS/SANS) data with crystallographic and other information. To investigate molecular mechanisms involving high molecular weight protein complexes in solution.

For more information about research group Sattler:
https://www.helmholtz-muenchen.de/stb/research/groups/research-group-sattler/research/index.html

Meet the PIs



Dr. Guillermo Rodrigo

Born in Valencia in 1983, Dr. Guillermo Rodrigo obtained the B.Eng. in Industrial Engineering from the Universidad Politècnica de València (UPV) in 2006, having conducted the last year of studies at ecole Polytechnique de France. He participated in the first national iGEM team. He then obtained the M.Sc. in Applied Mathematics in 2008 and the Ph.D. in Biotechnology (with Extraordinary Award) in 2011 from the UPV. He participated in the European High-Performance Computing and MIT-France programs, and also got the EMBO short-term fellowship for MIT. He followed his postdoctoral career in Paris at the CNRS, first with the EMBO long-term fellowship and then as the AXA research fund investigator. Since 2014, Guillermo is Tenured Scientist at the Spanish Research Council (CSIC), where he leads a multidisciplinary group interested in systems and synthetic biology.

Have you always been working in the synthetic biology field?

Yes. Since I am engineer by training, I was always interested in making things. Biology provides an exciting ground for applying engineering concepts.

How did you end up in I2SysBio-CSIC?

The I2SysBio is the first initiative in the CSIC to create a public institute devoted to the field of systems and synthetic biology. I was enrolled in this project together with local colleagues, so I am a founder of the institute.

How did you find your passion for synthetic biology and research?

Richard Feynman once said “What I cannot create, I do not understand”. This quote has inspired me for years.

What is the role of I2SysBio-CSIC in RNAct? And what do you think the RNAct project can bring to your field?

Our role is in the implementation of gene expression control structures by means of designer protein-RNA interactions. This is important to demonstrate the suitability of the designs. In addition, RNAct can bring to the field new computational methods and genetic elements to enlarge the synthetic biology toolbox.

What do you think about the computational-experimental collaborations?

It is quite necessary to advance in the field of molecular (re)design. The final goal is to get computational models to accurately design proteins and RNAs à la carte for several biomedical and biotechnological applications.

What are the strengths of RNAct? Why is RNAct important for society?

The predictability of intermolecular interactions (with structural and dynamic models) will allow us to assess questions such as designability and robustness of genetic systems. This will contribute in great extent to increase our knowledge on regulatory RNAs and proteins. By understanding the relationship between sequence and function, we would provide new insight into fundamental mechanisms of life with implications in biomedicine. Our results will promote collaboration and exchange of scientific ideas within the European Research Area.

What are the main perspectives of the project in your research field field?

RNAct is relevant for the field of synthetic biology because most of the previous work has focused on transcription regulation. Here, we address the engineering of gene regulation at the translational level through protein-RNA interactions. This will allow exploiting the central dogma of molecular biology to create novel biosystems with higher degree of operability and sophistication.

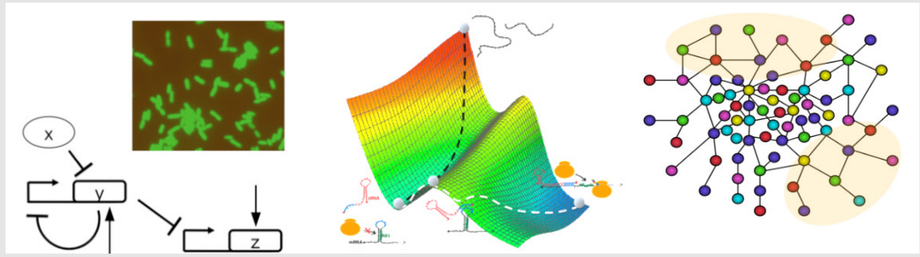
About - BioSystems Design research

Dr. Guillermo Rodrigo leads the BioSystems Design Lab. This young and competitive research group aims at understanding and engineering biology by following innovative approaches that require the combination of different disciplines, such as molecular biology, engineering, and computational science.

The main research lines focus on reprogramming living cells (using bacteria and plants as model organisms) by implementing synthetic gene circuits and genetic rewirings. In addition, the lab focuses efforts in unveiling the design principles underlying key gene regulatory networks.

The team is working at the Institute for Integrative Systems Biology (I2SysBio), a joint research center that involves the Spanish Research Council (CSIC) and the Universitat de València (UV).

For more information about the group, please visit https://biosysdesign.csic.es.



Project progress

Meetings

Subcommittee meetings

The 4 dedicated subcommittes meet online montly or bimonthly since November 2019 to follow intermediate issues with regards to training, research, data management and dissemination. Joel Roca (ESR 2) attended the dissemination subcommittee meetings, acting as subcommittee deputy manager.

Board meetings

The supervisory and management boards will meet online on 8th June 2021 during the 4th Network-wide Workshop. The meetings will focus on the general management of the network, with special emphasis on the fellows' progress against their personal career plans (PCDPs). Jose Gavaldá (ESR 1) will attend the meetings as ESR representative.

Publications

Kagami L., **Roca-Martínez J.**, **Gavaldá-García J**., Ramasamy P., Feenstra K.A., **Vranken W.F**. Online biophysical predictions for SARS-CoV-2 proteins. BMC Mol and Cell Biol 22, 23 (2021). DOI: 10.1186/s12860-021-00362-w

Kagami L.P., Orlando G., Raimondi D., Ancien F., Dixit B., **Gavaldá-García J**., Ramasamy P., **Roca-Martínez J.**, Tzavella K., **Vranken W.F**. b2bTools: online predictions for protein biophysical features and their conservation, Nucleic Acids Research, 2021; gkab425, https://doi.org/10.1093/nar/gkab425

Reporting (December 2020 - June 2021)

Deliverables

D6.4 Halfterm report about public engagement activities
D3.1 RNA biochips with variability tailored to RRM specificity

Milestones

M10 Prototype time-resolved RNA detection

Reports

Periodic Report Period 1 (Month 1 - Month 24)

Workshop 3 - Proteins: computation and design

The third workshop, "Proteins: computation and design", took place in September 2020 (Sept 14-18) and was organized by HMGU (Munich). Due to the COVID-19 pandemic, the workshop was held online. The 10 ESRs attended seminars and courses during one week.

The seminars and courses included in the third workshop were:

- **Communication strategies.** Dr. Paul Charlton (Paul Charlton Consulting, Germany).
- **Article writing.** Dr. Iain Patten (Iain Patten Scientific Writing Consultant, Spain).
- **Protein design**. Prof. Dr. Martin Zacharias (Technische Universität München, Germany).
- **Protein expression for structural biology.** Dr. Arie Geerlof (Helmholtz Zentrum München, Germany).
- **Structure-based drug discovery.** Dr. Grzegorz Popowicz (Helmholtz Zentrum München, Germany).
- **Integrated structural biology.** Dr. Florent Delhommel (Technische Universität München, Germany).
- **Biochemistry of RNA, RNA drugs and RNA targeting.** Dr. Alisha Jones (Helmholtz Zentrum München, Germany).
- **In silico screening.** Dr. Pavel Karpov (Helmholtz Zentrum München, Germany).

In addition, the workshop included the board meetings and the progress presentations, in which the ESRs summarized the results obtained during their first year in RNAct.

During the communication strategies training, the ESRs learned how to improve their communication skills, with a focus on developing a clear and effective narrative. The training also included a coaching session on how to create effective presentations.

The session of article writing has made important input on structuring the manuscript and set groundwork for the entire process.

The ESRs participated to the Race the world Team Building activity during the third workshop.

Project progress

Workshop 4 - Science in industry

The fourth workshop, “Science in industry”, will take place in June 2021 (June 1-8) in Brussels (Belgium). The ESRs will attend for a week to seminars and courses aimed at providing them with skills and knowledge relevant to science in industry. However, the workshop will combine online and physical sessions.

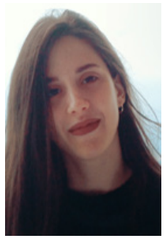
The seminars and courses included in the fourth workshop are:

- **Presenting with impact.** Hans Van de Water (The Floor is Yours, Belgium).
- **Industrial property.** Dr. Geoffrey Aerts and Prof. Dr. Thomas Crispeels (Vrije Universiteit Brussels, Belgium).
- **How to get info on proteins programatically.** Prof. Dr. Wim Vranken (Vrije Universiteit Brussels, Belgium).
- **Industry R&D, quality control and project management.** Dr. Jos Buijs (Ridgeview Instruments AB, Sweden) and Dr. Wolfgang Kaiser (Dynamic Biosensors GmbH, Germany).
- **Academy/industry collaborations.** Prof. Dr. Helena Danielson (Uppsala Universitet, Sweden).
- **Data management.** Prof. Dr. Wim Vranken (Vrije Universiteit Brussels, Belgium).
- **Bioanalysis.** Dr. Wolfgang Kaiser (Dynamic Biosensors GmbH, Germany).

In addition, the workshop will include the board meetings and the progress presentations, in which the ESRs will summarize the results obtained in the last months.

News

Niki Messini joins the RNAct family as ESR 5!



Niki was born in Athens, Greece. She has just graduated from MSc in Advanced Pharmacology at the University of Strathclyde in Glasgow, United Kingdom. Niki completed her MSc thesis in the field of neuroscience. She obtained a BSc in Physics from the University of Patras, Greece. Her BSc thesis was in the field of solid-state physics and especially on fragment screening-based drug design advised by Prof. Manfred Weiss at Helmholtz Berlin. In Berlin, she also attended the protein X-ray crystallography module for Master students of Freie Universität Berlin.
Niki is a founding member of Mindspace Patras, a nonprofit aiming to cultivate entrepreneurial mindset among students. Her dream is to help create better drugs and have a positive impact through her work to as many people as possible. She loves both experimental and computational work, and enjoys traveling, cooking, Tae Kwon Do (black belt), playing music (guitar), and mountain biking.

Contributions at scientific meetings

Márquez-Costa R, Montagud-Martínez R, **Dolcemascolo R**, **Rodrigo G.** Translation regulation with CRISPR-Cas13. Poster presented at the 1st International BioDesign Research Conference, 1-8 December 2020, Online.

Meet the ESRs - Videos

The fellows recorded a series of videos in which they introduced themselves. In these videos, they provided an overview of their background, the institution they are working at, and their individual projects within RNAct.

Would you like to know more about them? Then watch the following videos already published in YouTube:

- **Joel Roca (ESR 2)** https://tinyurl.com/3abw5khk
- **Luca Sperotto (ESR 6)** https://tinyurl.com/tbcfm6xc
- **Anna Pérez (ESR 7)** https://tinyurl.com/ysdfh59w
- **Roswitha Dolcemascolo (ESR 8)** https://tinyurl.com/yrd86237
- **Anahi Higuera (ESR 9)** https://tinyurl.com/5t92x3x3

Journal club

The fellows meet online every three months to discuss about articles relevant to the project.

6th Journal club session:

Date: 29/3/2021
Article: Protein-assisted RNA fragment docking (RnaX) for modeling RNA-protein interactions using ModelX
DOI: https://doi.org/10.1073/pnas.1910999116
Chair: Anna Kravchenko (ESR 4)

Stay tuned!

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https://tinyurl.com/vuy5kup

Facebook
https://tinyurl.com/vrzjv97

Linkedin
https://tinyurl.com/tmeqjww

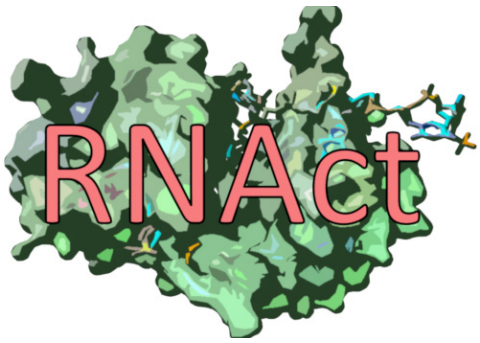
Instagram
https://tinyurl.com/utdvkb8

Discussion group in Linkedin
https://tinyurl.com/tx5z4bb

Youtube
https://tinyurl.com/s22x9x5

Researchgate
https://tinyurl.com/rdpogqb

RNAct Newsletters
https://tinyurl.com/wftv37p


RNAct