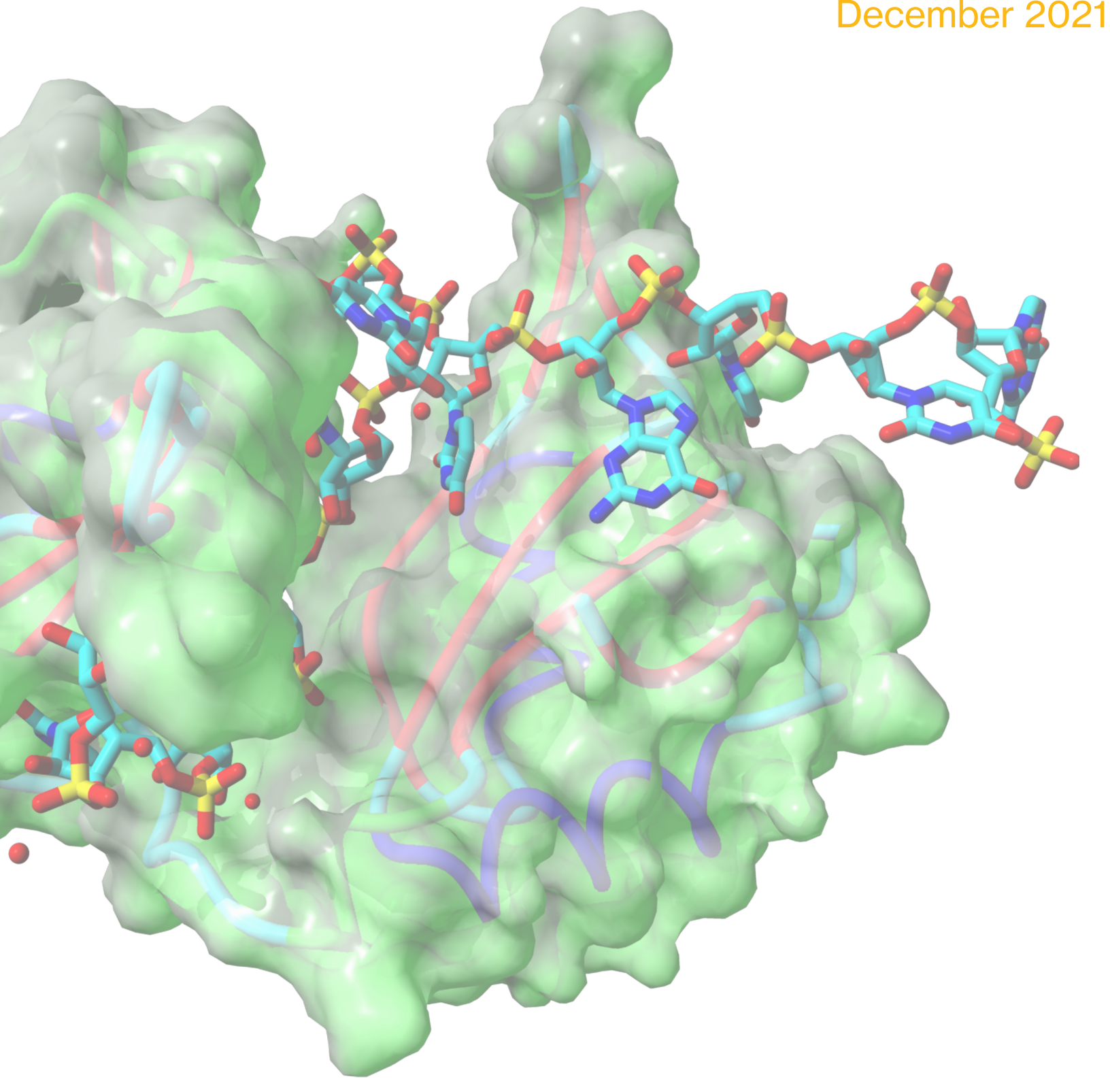
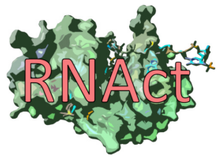
RNAct

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

4

December 2021





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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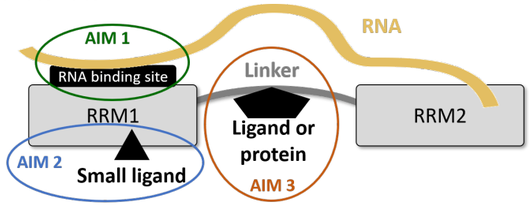
RNAct at a glance

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:

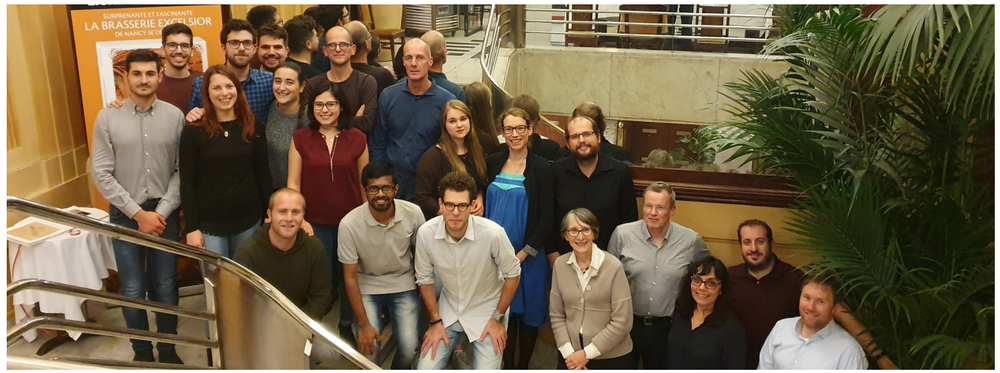
Work Package 1. Creation and characterisation of functional RRMs.
Work Package 2. Representation and design of dynamic proteins.
Work Package 3. Bio-analytics and synthetic biology.
Work Package 4. Training and education.
Work Package 5. Coordination and management.
Work Package 6. Dissemination and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

CSIC
Secondment host

Jose Gavaldá-García (ESR 1; VUB, Belgium) is currently spending three months in Valencia at the BioSystems Design Lab, directed by Guillermo Rodrigo placed at the I2SysBio (CSIC, Spain) Institute. He is using his expertise to create a binding affinity approximator for an RNA binding protein to different RNA sequences. In parallel, he is also learning to model metabolic pathways to apply such protein for complex regulation of genetic circuits in synthetic biology. Both projects work synergistically since the estimator would allow the use of completely orthogonal RNA sequences in the regulatory strategy that he is modeling. The results of the model and the affinity predictions will be later experimentally tested in vivo by Roswitha Dolcemascolo (ESR8, CSIC, Spain) and Guillermo Pérez Ropero (ESR10, RV, Sweden) who will work together again at the beginning of next year (January-March 2022) at CSIC (Valencia, Spain). We expect this work to be applicable to RRMs given sufficient training data is available in the future.

HelmholtzZentrum münchen
German Research Center for Environmental Health

HMGU
Secondment host

During this secondment, Joel Roca Martínez (ESR 2; VUB, Belgium) performed a deeper biophysical exploration of the RRMs with respect to the RRM-RNA binding, making good use of the large expertise on RNA binding proteins in Sattler's group (HelmholtzZentrum, Germany). Alongside with Niki Messini (ESR5, HelmholtzZentrum, Germany) we started integrating some preliminary phage display results into the RRMs master alignment. Finally, he was also able to follow Luca Sperotto (ESR6, Helmholtz Zentrum, Germany) on several experimental procedures, learning about Isothermal Titration Calorimetry (ITC) and phase separation assays. During his free time, Joel enjoyed sporting activities such as climbing in the German mountains. Overall, it has been an interesting experience and very useful for the progress of the project.

dynamic BIOSENSORS

DBS
Secondment host

Anna Kravchenko (ESR 4; CNRS, France) is near the end of her secondment at Dynamic Biosensors. During the past 3 months with the great support from Rosa Anahí Higuera (ESR9, DynamicBiosensors, Germany) she submerged in the world of experimental biology, learning about switchSENSE technology and measuring the kinetics herself. With the newly acquired understanding of the experimental matters, she initiated a project involving Joel Roca Martínez (ESR2, VUB, Belgium), Hrishikesh Dhondge (ESR3, CNRS, France), Niki Messini (ESR5, HelmholtzZentrum, Germany), and Rosa Anahí Higuera to measure the impact of stacking interactions on the binding kinetics. These results are expected to be useful for Anna's main project, as well as for Anahi's work.

ridgeview instruments ab

RV
Secondment host

Anna Pérez i Ràfols (ESR 7; GIO, Italy) has recently completed her secondment in a mixed academic and non-academic environment. From September to the end of November 2021; she has been working at the biotech company Ridgeview Instruments AB (RV) in Uppsala, Sweden.
During these three months, Anna has been collaborating with Guillermo Pérez Ropero (ESR10, RV) using the Ligand Tracer technology, which allows to perform real-time quantitative assays and to measure RNA-protein interactions in living bacterial cells; as well as Surface Plasmon Resonance (SPR), a technique used to monitor intermolecular interactions in real time in vitro..

Meet the PIs

Dr. Isaure Chauvot de Beauchene

Isaure Chauvot de Beauchene went to high school, then 2-years “preparatory school” in Versailles (next to Paris), and then 3 years in Veterinary school in Lyon. She then wanted to understand the molecular mechanisms of diseases rather than treating them, and obtained a Master in cellular biology in Paris.

She obtained her thesis in 2013 at the ENS-Cachan School, on molecular modeling and dynamics simulation applied to understanding the oncogenicity and drug-resistance of mutants of a tyrosine-kinase receptor. After that, she did a 3-years post-doc in Munich, on RNA-protein docking, before joining the CNRS in Nancy in 2016.

How did you find your passion for structural bioinformatics, specially docking?

I had to take some University course in parallel to the vet school to get a master 1 diploma. I was too let to register for cell biology and ended up in pharmacology. There, we had a 2 hours introductory course on molecular modeling, and I suddenly decided to work on virtual molecules rather than pets. My interest in docking came from my post-doc in the team developing ATTRACT (Prof. Zacharias, Munich).

Have you always been working on the RNA-binding proteins? How did you end up in at CNRS?

I first worked on the modeling and dynamics of membrane receptor proteins, and docking of drugs on it. I switched to RNA-protein docking in post-doc.
After the post-doc, I passed the CNRS entrance competition, in section “informatics and mathematics approaches for biology”, which was my first choice of career. There, I continued to work mostly on RNA-protein interactions.

What is your role and responsibilities at CNRS?

My role is to make and help others make scientific discoveries, by developing and applying modeling methods. This implies doing research on my own, writing publications, supervising students, but also writing projects to get PhD/post-doc grants, reviewing other’s papers.

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

Our role is to provide automated computational methods to gather, compare and analyze data on RRMs (sequence, structure, interactions), and to provide predictions on such RRM data when missing (e.g. for newly designed sequences).

What is your role in this project?

Ensure that ESRs 3 and 4 make a good thesis. Provide 3D models of RRM-RNA interactions. Participate in the set-up of the data management strategy.

How do you see the computational-experimental collaborations?

It is essential for us: to validate our methods, to feed our methods with all available data (the more input data, the better the predictions), but also to ensure that we develop methods that are useful to experimentalist in real life.

What are the strong points of RNAct? Why is RNAct important for society?

Strong points are the direct interactions and collaborations of ESRs, and the computational-experimental complementarity. RNAct aims at providing a new class of synthetic domains, that could then be used by other teams for various biological and therapeutic goals, with various benefits for society.

Why is docking a key technology to understand RNA-protein interactions?

Docking can provide almost atomic-resolution models when no experimental data are available (too long, too expensive, not possible for technical reasons...). This allows a fine understanding and predictions on the interaction. But both the accuracy and precision of docking models are always limited, and one should be careful to not over-interpret the models. Some experimental validation (s.a. mutagenesis) is always needed.

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

We are at a turning point in this field, with the recent breakthrough of AlphaFold. Now docking could technically be applied on proteins for which no structure is available, since they can be modeled with high accuracy (Only the public availability of AlphaFold is limiting there). Furthermore, it is expected that applying AI to docking will soon bring new breakthrough in that particular field.

About - CNRS

CNRS (National Center for Scientific Research) is the main public research institute in France, covering all domains. It is subdivided in thousands of labs in the whole country. Most research labs are part of both CNRS and the local university, and sometimes also another more specific national research institute. For instance, the LORIA lab _ where Isaure Chauvot de Beauchene works _ is part of CNRS, Lorraine University, and INRIA (National Research Institute for Informatics and its Applications).

The Capsid team develops algorithms and software to help study biological systems and phenomena from a structural point of view. In particular, the team aims to develop algorithms which can facilitate and improve the 3D modeling of large multi-component bio-molecular machines. While the team’s principal activity is algorithm and software development, it also tackles « real-world » biological problems through collaborations with the University of Lorraine and Nancy Hospital, and with other research teams from Inria, CNRS, INRA, INSERM, and international universities.

The team’s activities focus on two main themes:

- **Computational modeling of protein-protein and protein-RNA interactions (docking, molecular dynamics simulations)**
- **Classifying and mining protein structures and protein interactions (knowledge discovery in biological databases)**

Meet the PIs

Dr. Wolfgang Kaiser

Wolfgang Kaiser is the Head of Application Development at Dynamic Biosensors GmbH. Wolfgang studied physics at the Technical University of Munich (TUM) with a focus on semiconductor-physics and spin-electronics before starting his PhD in biophysics. In his doctoral thesis, he examined electrically actuated DNA layers and their use as biosensors (switchSENSE® technology). In 2013 he co-founded with his PhD supervisor (Ulrich Rant) and three other colleagues the company Dynamic Biosensors GmbH. He is responsible for application development and biochip production. Some milestones were: the establishment of the chip production for the DRX instruments, the development of the DRX dual-colour chip which enabled the simultaneous measurement of two interactions on the same detection spot, and in 2019 the completely new development of the heliX Adapter Biochip with many new application potentials.

What path did you follow to become Head of Applications at Dynamic Biosensors?

Actually, I did not follow a pre-defined career plan. I always did what was most interesting to me. I took on some challenges, put time end effort into the things I did, and got a few opportunities and had a lot of luck too.

Did you always know that you wanted to do a transition from academy to industry? How was the transition for you?

No, this was not clear from the start: During my physics studies, I could imagine both – working in industry or in academia. After graduation in physics, I played with the thought to go into industry to learn new things (more application-oriented), but a good friend convinced me to do a PhD and do 3 (or more) years exactly that what is most interesting. I was lucky, found a very interesting PhD offer (in the group of Uli Rant, now CEO of Dynamic Biosensors) and got a scholarship. After a few years Uli Rant, my colleagues and me founded a company based on the research we did… my way from academia to industry. The transition from academia to industry was rather smooth for me, the only thing which changed a bit, was the focus from basic research to application-/product-oriented research.

What have you learned about the challenges of commercializing a technology with a scientific/academic background?

There are many things that I learned in the commercialization of our technology: Before you can produce a prototype/start your own business you need money! Sounds trivial, but where do you get money for an idea? Ok, there are these guys called investors. Unfortunately, they do not give you money because you ask them for it or you look smart (at least this was not the case for us – or we did not look smart enough…). You need a business plan: how large is the market for the technology, what is the capital need for the product development, what can be the price for the final product… and much more. Two of my colleagues were in the lead of the business plan and took care of finances while two other colleagues and I focused on the technology (biochemistry, biochip development, instrument development). Another challenge was how to find the first (potential) customers.

What are the advantages and disadvantages of working in industry with a scientific focus?

Hmm, hard to say. I would say that in almost all cases in industry, research is aimed at a new application or product. But per se, I think that this is not a disadvantage or bad thing. I see it more as an advantage to work on something which is useful for someone else.

In a nutshell, what are your day-to-day activities in Dynamic Biosensors?

Emails, meetings, data discussions with scientists, develop new assays, chip production (planning), adapt/improve chips, discuss the outcome of experiments, brainstorming with colleagues, optimize existing assays, customer support, experiments in the lab… Actually, every day is different.

What is your role in RNAct?

As the "industrial" supervisor of Anahí Higuera my main task is to guide Anahí and discuss with her experimental results, what we can learn from the experiments and how to design new experiments to get further insights. Other tasks are organizational and administrative matters and of course meetings with other RNAct members to coordinate research.

The computational and experimental collaborations are the base of RNAct, how can Dynamic Biosensors contribute to this regard?

As an instrument manufacturer with a unique technology based on DNA and hereby easily adaptable to RNA, I hope we can contribute to the whole project by providing on one side our technology and on the other side our experimental expertise.

Why measuring binding kinetics is a key aspect to understand RNA-protein interactions?

In general, I think binding kinetics is a key aspect in understanding molecular interactions. Understanding the individual and the cooperative binding is particularly important in the case of more complex interactions such as the RNA-protein interactions where multiple binding sites are involved. However, the binding kinetics is only a part of the puzzle, other pieces are structure analysis, thermodynamics, dynamics of the molecules and much more. In my opinion, it is important to combine the different techniques (puzzle pieces) to get a complete picture of the interaction mechanism and to finally model the interaction or to further improve an existing model. Sounds like RNAct doesn't it?

Overall, why do you think is RNAct relevant for society?

In my opinion, one of the most important things of RNAct is to work together. Everyone is working on a piece of the puzzle and it is necessary to work with others as they depend on you or you depend on them. Working together is often fun and you learn new things or new aspects – for work and for life. By working together in an interdisciplinary and multicultural team you can create something that is more than the sum of individual contributions - a key to success in science. And collaboration is not only important inside RNAct, but I would also say it is important everywhere in society: in your family, in your relationship, at work, between different countries… cooperation is everywhere a benefit.

About - Dynamic Biosensors

Dynamic Biosensors is a provider of instruments, consumables, and services in the field of analytical systems for the characterization of biomolecules and molecular interactions. We are located in Martinsried, south of Munich, a vital center of the biotechnology industry in Europe. Dynamic Biosensors commercializes switchSENSE® technology, a groundbreaking platform technology for the analysis of biomolecules with applications in R&D and drug development. The switchSENSE® technology is protected worldwide and only available through Dynamic Biosensors. The company is headquartered in the south of Munich, Germany and runs offices in the United States, the United Kingdom, Japan and Singapore.

For more information about the company, please visit https://www.dynamic-biosensors.com.

Project progress

Meetings

Subcommittee meetings

The 4 dedicated subcommittees meet online monthly 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 met in-person on 30th November 2021 during the 5th Network-wide workshop. The meeting focused on general management of the network, with special emphasis on the fellow's progress against their personal career plans (PCDPs). Jose Gavaldá (ESR 1) attended the meeting as ESR representative.

Contributions at scientific meetings

Gavaldá-García Jose, Roca-Martínez Joel, **Vranken Wim F**. DynaMine v2, an updated version of the sequence-to-dynamics predictor. Poster presented at RSG Belgium online symposium 2021, July 2021, Online.

Kravchenko Anna, Smail-Tabbone Malika, de Vries Sjoerd Jacob, **de Beauchene Isaure Chauvot**. Histogram-based approach for docking parameters optimization. Poster presented at JOBIM 2021, 6-7 July 2021, JOBIM (France).

Dhondge Hrishikesh, Roca-Martínez Joel, Vranken Wim, Devignes Marie-Dominique, **de Beauchene Isaure Chauvot.** Data-driven identification of structural patterns associated with RNA binding in RNA Recognition Motif domains. 23-24 July 2021, SCS. https://doi.org/10.5281/zenodo.5146026

Kravchenko Anna, Smail-Tabbone Malika, de Vries Sjoerd Jacob, **de Beauchene Isaure Chauvot**. Histogram-based approach for docking parameters optimization. 23-24 July 2021, SCS. https://doi.org/10.5281/zenodo.5139959.

Higuera Anahí, **Sperotto Luca**, Aziz Massid, **Sattler Michael**, **Kaiser Wolfgang**. A novel and easily adaptable biochip format for the characterization of binding kinetics between RNA and proteins. Oral presentation at the 31st Anniversary World Congress on Biosensors, 26-29 July 2019, Online.

Goiriz Lucas, **Dolcemascolo Roswitha**, Montagud-Martínez Roser, **Rodrigo Guillermo**. Gene regulation by a protein translation factor at the single-cell level. Poster presented at the 2nd International BioDesign Research Conference, 6-17 December 2021, Online.

Outreach activities

Jose Gavaldá-García (ESR 1) and Joel Roca Martínez (ESR 2) represented RNAct at the European Researchers' Night 2021 edition during the WiseNight event (https://wisenight.eu/) held in Brussels on Saturday, 25 September 2021.

Workshop 5 - Engage with your future

The fifth workshop, "Engage with your future", took place recently (29th Nov-3rd Dec 2021) and was organized by Giotto Biotech (Florence, Italy). The workshop was held in-person while maintaining the RNAct bio-bubble.

The seminars and courses included in the fifth workshop were:

- **Write a research proposal.** Prof. Dr. Wim Vranken (Vrije Universiteit Brussels, Belgium) **.**
- **Marketing and product strategy.** Laura Bassani (Giotto Biotech, Italy)
- **Teaching science.** Dr. Claudia Andreini (University of Florence, Italy)
- **From Innovation to market – The LigandTracer case study.** Dr. Karl Andersson (7D-Consulting AB, Sweden)
- **Building the business Kontigo Care.** Dr. Markku Hämäläinen (7D-Consulting AB, Sweden)

In addition, the workshop included the scientific discussion, board meetings and progress presentations, in which the ESRs summarized the progress of individual projects from last workshop and the possibilities to collaborate with each other.

This workshop was important as it provides training on writing a research proposal, forcing ESRs to think about their plan and giving a positive boost in their confidence.

RNAct ESRs and supervisors in Florence.

Project progress

Workshop 6 and Final conference

The sixth workshop, “RNAct future and innovation”, will take place on 12th and 13th September 2022 in Valencia (Spain). It will include the following seminars and courses:

- **Leadership**. José Luis Garcia (CSIC, Spain).
- **Synthetic biology: where are we now?** Dr. Guillermo Rodrigo (CSIC, Spain).

The 6th Workshop will be followed by the RNAct Final Conference (12th and 13th September 2022, Valencia). The event will include an Innovation Workshop aimed at matchmaking with relevant stakeholders to establish collaborations to develop a market implementation.

Interested stakeholders may register their interest in the form using the following link to receive further information on the event. http://rnact.eu/innovationWS/.

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 the 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/9zk3fwjk
- **Hrishikesh Dhondge (ESR 3)** https://tinyurl.com/249a4346
- **Anna Kravchenko (ESR 4)** https://tinyurl.com/vwrr9mjz
- **Luca Sperotto (ESR 6)** https://tinyurl.com/2p896t6c
- **Anna Pérez i Ràfols (ESR 7)** https://tinyurl.com/yc4d7yvx
- **Roswitha Dolcemascolo (ESR 8)** https://tinyurl.com/2dw8m2av
- **Anahí Higuera (ESR 9)** https://tinyurl.com/2p8w2xkf

Reporting (June - December 2021)

Deliverables

D3.2 Approach for time-resolved RNA detection and validation
D4.3 Second report on ESR research and PCDP progress, including summary of network-wide training events
D6.2 All annual agendas of outreach activities prepared and distributed
D3.3 Bacteria with Mushashi-1 incorporated

Milestones

M8 Test data-driven RNA docking software

Journal club

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

7th Journal club session:

Date: 19/10/2021
Article: Structure of SRSF1 RRM1 bound to RNA reveals an unexpected bimodal mode of interaction and explains its involvement in SMN1 exon7 splicing
DOI: https://doi.org/10.1038/s41467-020-20481-w
Chair: Anna Pérez i Ràfols (ESR 7)

RNAct Innovation Workshop
Call for expressions of interest

RNAct infographic

Marie Skłodowska-Curie Innovative Training Network (MSCA-ITN)

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

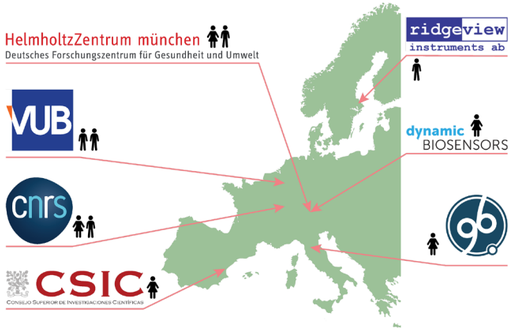
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 813239.

Synthetic biology enables us to engineer organisms so they can have new capabilities which are useful for us, like to synthesize molecules that otherwise are difficult to produce. To this end, it is necessary to regulate biological systems. Unfortunately, our understanding of regulation in organisms is not advanced enough and one of the biggest bottlenecks is the lack of professionals dedicated to this important task.

What does RNAct does to solve this issue?

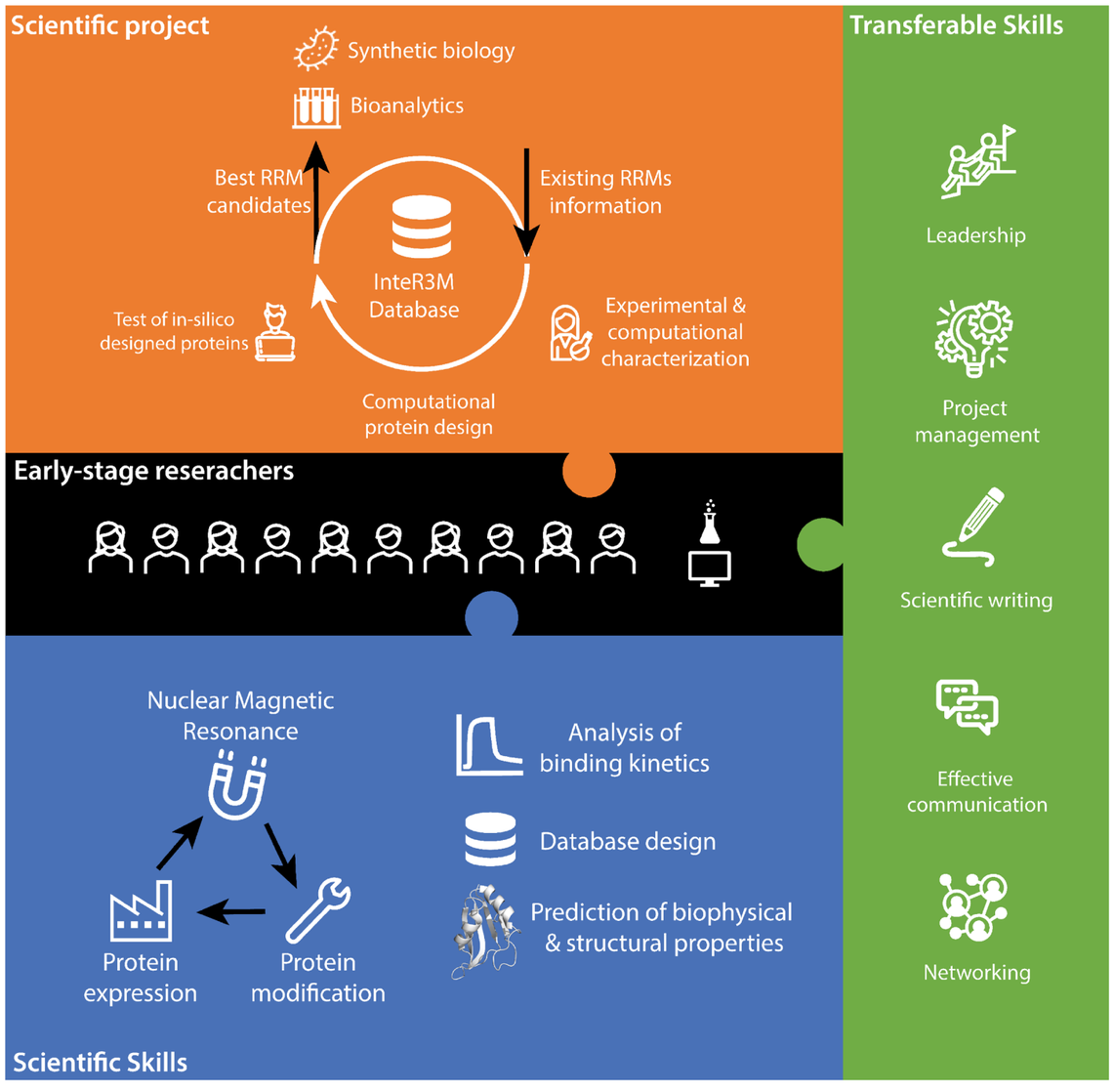
We train Early Stage Researchers (ESRs) to produce innovations at the molecular level, thereby strengthening the research capacity in Synthetic Biology, Bio-analytics and protein design within the EU borders.

ESRs study the interactions between RNA recognition Motifs (RRM) and RNA, with the aim of engineer specific binding interactions that could be applied in synthetic biology and bio-analytics.



What are the goals?

- Educate professionals qualified to work with RRMs from a diversity of specializations.
- Forge long-lasting networks of collaborators between academia and industry.
- Get a better understanding of RRM-RNA interactions.



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RNAct (MSCA-ITN)

RNAct MSCA-ITN

http://rnact.eu/

RNAct gallery

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Discussion group in Linkedin
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Researchgate
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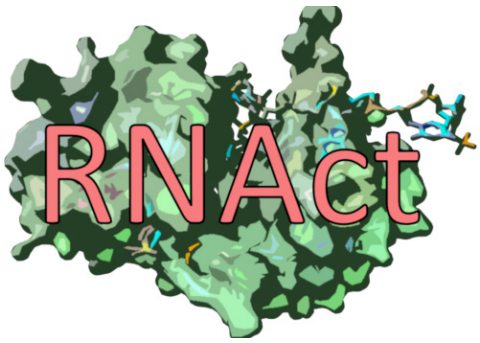
RNAct Newsletters
https://tinyurl.com/wftv37p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