RNAct

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

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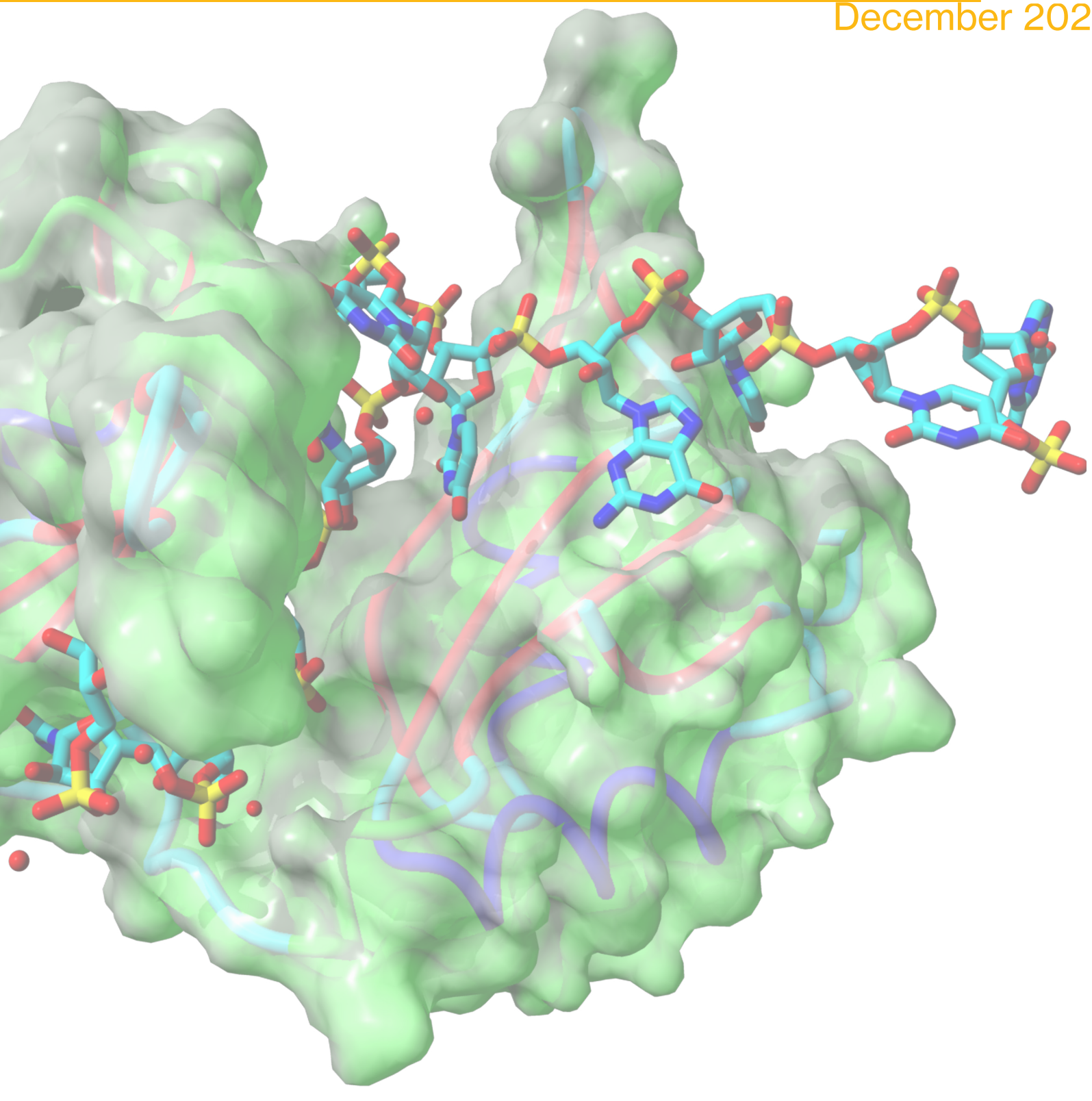
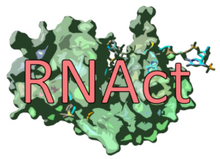
December 2020





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This issue of the newsletter has been edited by Stefano Mocci (ESR 5) and Guillermo E. Pérez Ropero (ESR 10).

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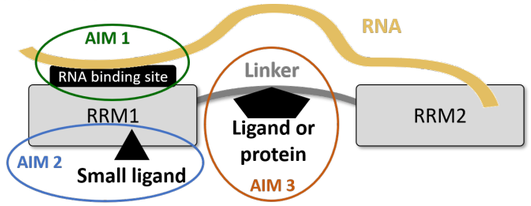
RNAct at a glance

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:

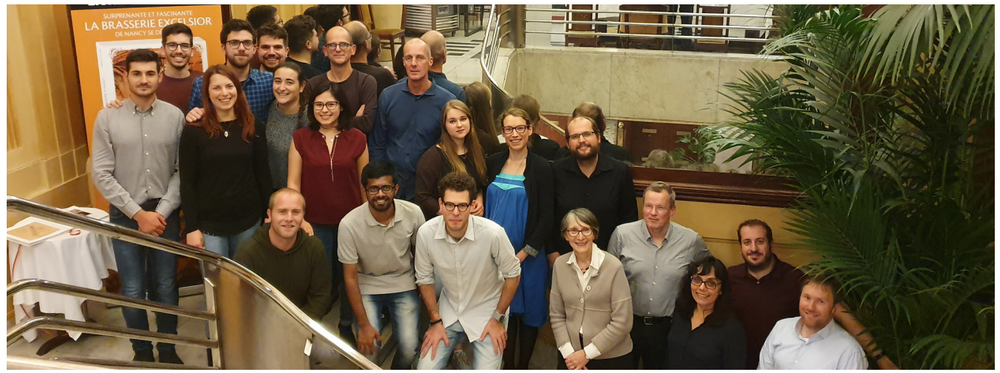
Work Package 1. Creation and characterisation of functional RRMs.
Work Package 2. Representation and design of dynamic proteins.
Work Package 3. Bio-analytics and synthetic biology.
Work Package 4. Training and education.
Work Package 5. Coordination and management.
Work Package 6. Dissemination and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



Meet the PIs

Dr. Jos Buijs

Jos Buijs is the Chief Executive Officer of Ridgeview Instruments AB.

Jos attained his PhD in the Netherlands (WAU) studying antibody interactions and continued as a Post Doc in Salt Lake City (UoU). In 1997 Jos moved to Sweden and became senior researcher at the physics department at Uppsala University developing new analytical methods to study protein structure and interactions. In 2001, Jos moved to industry; first at Biacore (GE Healthcare) starting with smaller R&D projects and ending with being responsible for the overall design of new Biacore systems such as the T200 and 8K interaction analysis instruments and, since 2013, at Ridgeview Instruments as CTO. In 2008, Jos was appointed as adjunct Professor in Biotechnology at Mälardalen University, and since 2016 Jos is affiliated as senior lecturer at the Department of Immunology, Genetics and Pathology of Uppsala University. In 2020, Jos became CEO of Ridgeview Instruments

How is a normal working day at Ridgeview?

The interesting part of working in a small company is that there is no normal working day except for the start; a cup of coffee (or two) and a quick look at e-mails to see what requires immediate attention. This usually takes untill it's time for "fika"; the Swedish word for a coffee break. After fika we usually have meetings to align our work; the development of soft -and hardware products, research, and the ways we get our products to our customers. The rest of the time can be spent on anything from soldering contacts on electronic circuit boards to filling US tax forms.

What is the role of Ridgeview Instruments in RNAct?

As a company providing tools and knowledge to help our customers to understand interaction on and in live cells; our role is pretty obvious…

How do you see the computational-experimental collaborations?

Interesting but challenging: Too often, various research areas work on the same topic with the same overall goal but without taking full advantage of the discoveries made in the individual areas. In order to leverage the input from the various areas we need to learn the "language" used in the various fields and get an understanding of how knowledge from one research area translates to other areas. By combining the expertise from computational and experimental oriented researchers, RNAct created a unique opportunity to make this knowledge transition happen.

How did your find your passion for biophysics?

I have always been the person that liked to push a button to see what happens and wanted to understand life. I just followed my curiosity and discovered that you can actually make a job as a biophysicist out of it.

What is your role in this project?

Most often, my role in the project comes down to discussing with Guillermo on the strategies to characterize how molecules go into cells and interact with other intracellular components. Otherwise, my role is to contribute with knowledge transfer, organization and administrative tasks that make RNAct a consortium rather than a collection of individual research projects.

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

One of the strong-points of RNAct is of course the computational-experimental collaborations mentioned earlier. Using biology to synthesize the molecules we need is an obvious way to create a more sustainable society. The mutual goal of all partners in the RNAct consortium to advance on ways to tweak metabolic and regulation pathways is a crucial step to get there.

Why Real Time Cell Assays are a key technology to understand RNA-protein interactions?

The complexity of nature always keeps surprising us. So, no matter how good our computational models are or how well we are able to characterize interactions between the isolated molecules, studying how molecules interact in real-life is still required. As humans, we are usually better in sensing changes than static events. The same applies when trying to understanding how cells react on RNA binding events. In other words; monitoring effects in a real-time manner tells us more and more precisely than just looking at the final outcome.

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

To create a sustainable society, it is crucial that we are able to produce (bio)chemicals in a resource efficient and renewable manner. As it is hard to beat nature when it comes to producing compounds that are biodegradable, renewable and in an energy efficient way, the logical think to do is to let nature produce the compound that we need. As this is easier said then done, I'm sure that this research field is just in its infancy.

About - Ridgeview Instruments

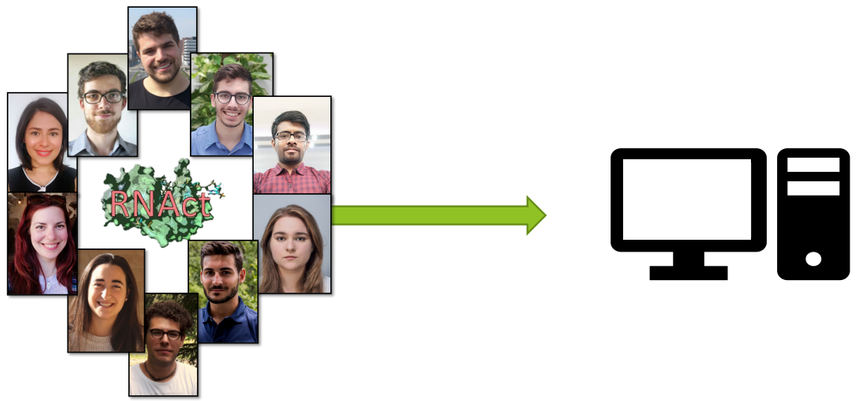
Ridgeview Instruments develops, manufactures and sells analytical instruments (LigandTracer) and evaluation software (TraceDrawer) for interaction analysis between proteins and live cells to academic life science research and pharmaceutical companies. These products are typically applied when developing pharmaceuticals or imaging agents to treat and monitor cancer. Moreover, Ridgeview supports external partners in the development of *in vitro* medical device software and assays; most recently by helping setting up a COVID-19 PCR test facility (a23lab).

Online projects

Three different online projects were implemented during the lockdown period. These were organized and coordinated by ESRs.

Data collection project

Coordinated by ESR 1 (Joel Roca), this was a one-week joint project involving the 10 ESRs in the RNAct ITN. The main goal was to gather experimental information about RRM-RNA binding as well as about ligands that bind RRMs. Biophysical information about binding affinities between both RRM-RNAs and RRM-ligands was collected. The information was stored in the protein framework and it is crucial for the development of the binding affinity predictor. Additionally, the ESRs also learned about the different biophysical tests that can be conducted to determine binding affinities and how these can be compared.



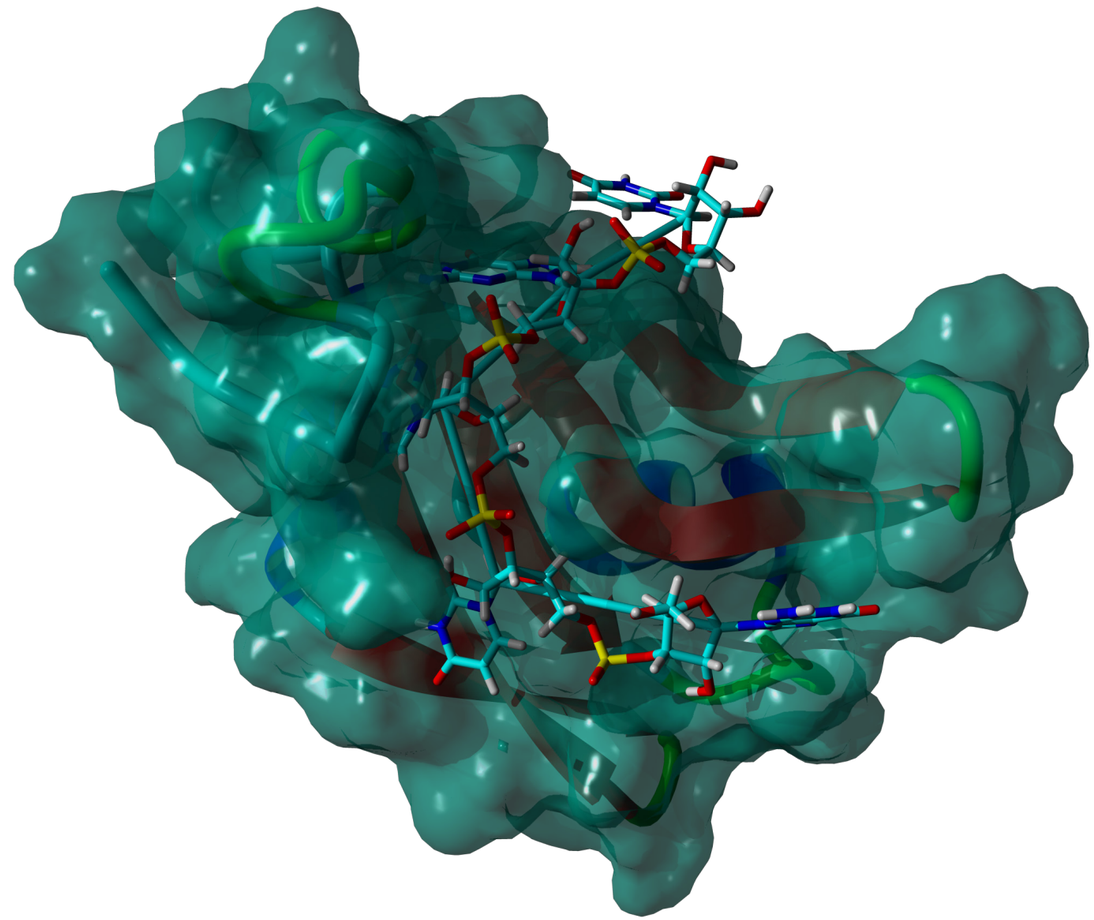
Python course

Jose Gavaldá chaired an “Introduction to Python” course to show all ESRs a coding and powerful approach to data analysis. The content of the course was specially fitted to improve the computational-experimental collaboration, giving to the experimentals ESRs a general overview about how to apply new data analysis methods to their data.

python™

RNA-RRM interface data collection

ESR 3 (Hrishikesh Dhondge) and ESR 4 (Anna Kravchenko), both based on Loria (Lorraine Research Laboratory in Computer Sciences and its Applications) coordinated a joint data collection project wth a focus on specific residues and structures involved in RRM-RNA interactions between different proteins and RNA strands.



Project progress

Meetings

Mid-term check

The members of the RNAct network met with the Project Officer from the Research Executive Agency on 24th March 2020to discuss on the project implementation during its first 15 months.

Board meetings

The supervisory and management boards met online on 24th March and 18th Setpember 2020 during the 2nd and 3rd workshops. The meetings focused on the general management of the network, with special emphasis on the fellows' updated personal career plans (PCDPs). Jose Gavaldá (ESR 1) attended the meetings as ESR representative.

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.

Deliverables and milestones (Mar.-Dec. 2020)

Deliverables

D2.1 Tool for interpretation of RRMs in biophysical space
D4.2 First report on ESR research and PCDP progress, including summary of network-wide training events

Milestones

M16 Project check
M9 Prototype RNA biochip
M6 Test pipeline for RRM model generation

Contribution at scientific meetings

Gavalda-Garcia J., Roca-Martinez J., Vranken W. DynaMine v2, an updated version of the sequence-to-dynamics predictor. Poster and flash presentation at BIOSB2020, 27-28 October 2020, Online.

Gavalda-Garcia J., Roca-Martinez J., Vranken W. DynaMine v2, an updated version of the sequence-to-dynamics predictor. Oral presentation at the ISCB Regional Student Group - Belgium Symposium, 4 December 2020, Online.

Workshop 2 - Experiments and Data

The second RNAct workshop, "Experiments and Data" took place in March 2020 and was organized by VUB (Brussels). Due to the COVID-19 pandemic, the workshop was held online. The 10 ESRs attended for a week to seminars and courses aimed at increasing their research and communication skills.

The seminars and courses included in the second workshop were:

- **Molecular interactions in drug development.** Dr. Jos Buijs (Ridgeview, Sweden)
- **Open Science and FAIR data.** Prof. Dr. Lennart Martens (Ghent University, Belgium)

In addition, the workshop included ESRs progress presentations, the board meetings, and the mid-term check meeting with the Project Officer from the Eropean Commission.

The ESRs attended the scientific seminar on "Molecular interactions in drug development", covering a wide range of important biophysical techniques in drug development. Several biophysical topics related to drug discovery were discussed. The lecture included an overview of available techniques, from the more conventional SPR and ITC to the more innovative approaches, such as Ligand Tracer.

During the session of Open Science and FAIR data the fellows learned the relevance of making scientific research and its dissemination accessible to everyone.

Workshop 3 - Proteins computation and design

The third RNAct workshop, "Proteins computation and design" took place in September 2020 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 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 included also 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.

Project progress

Workshop 4 - Science in industry

The fourth workshop, “Science in industry”, will take place in June 2020 in Brussels (Belgium). It will include the following seminars and courses:

- **Academic/industry collaborations**. Prof. Helena Danielson (Uppsala Universitet, Sweden)
- **Intellectual property rights**. Dr. Geoffrey Aerts (Vrije Universiteit Brussel, Belgium)
- **Programming**. Prof. Dr. Wim Vranken (Vrije Universiteit Brussel, Belgium)
- **Industry R&D, quality control and project management**. Dr. Jos Buijs (Ridgeview, Sweden) and Dr. Wolfgang Kaiser (Dynamic Biosensors, Germany).
- **Bioanalytics**. Speaker to be confirmed.
- **Biophysical sample preparation, do’s and don’ts practical**. Dr. Tommaso Martelli (Giotto Biotech, Italy)
- **Presenting with impact**. Hans Van de Water (The Floor is Yours, Belgium)

In addition, the workshop will include ESRs progress presentations, and the management and supervisory board meetings.

Webinars

The fellows presented a series of webinar discussing topics related to the project.

1st Online seminar:

Date: 16/04/2020
Title: Biological NMR
Link: https://bit.ly/3bqkWZS
Presenter: Luca Sperotto (ESR 6)

2nd Online seminar:

Date: 20/04/2020
Title: Transcription and translation
Link: https://bit.ly/3dHPQOU
Presenter: Anna Pérez i Ràfols (ESR 7)

3rd Online seminar:

Date: 22/04/2020
Title: Regulation of gene expression
Link: https://bit.ly/3dHPQOU
Presenter: Roswitha Dolcemascolo (ESR 8)

4th Online seminar:

Date: 22/04/2020
Title: Biophysical methods
Link: https://bit.ly/3cuvrwm
Presenters: Guillermo Pérez Ropero and Anahí Higuera (ESR 10 and ESR 9)

Journal club

The fellows meet online periodically to discuss about articles relevant to the project.

3rd Journal club session:

Date: 07/05/2020
Article: Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids.
DOI: https://doi.org/10.1038/nbt.2149
Chair: Roswitha Dolcemascolo (ESR 8)

4th Journal club session:

Date: 02/10/2020
Article: Molecular basis for the increased affinity of an RNA recognition motif with re-engineered specificity: A molecular dynamics and enhanced sampling simulations study.
DOI: http://doi.org/10.5281/zenodo
Chair: Joel Roca Martínez (ESR 2)

5th Journal club session:

Date: 17/11/2020
Article: Using a specific RNA–protein interaction to quench the fluorescent RNA spinach.
DOI: https://doi.org/10.1021/acschembio.7b00332
Chair: Guillermo Pérez Ropero (ESR 10)

Stay tuned!

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Linkedin
https://tinyurl.com/tmeqjww

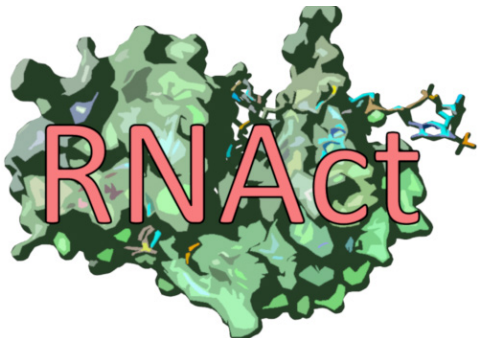
Instagram
https://tinyurl.com/utdvkb8

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

Youtube
https://tinyurl.com/s22x9x5

Researchgate
https://tinyurl.com/rdpogqb

RNAct Newsletters
https://tinyurl.com/wftv37p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