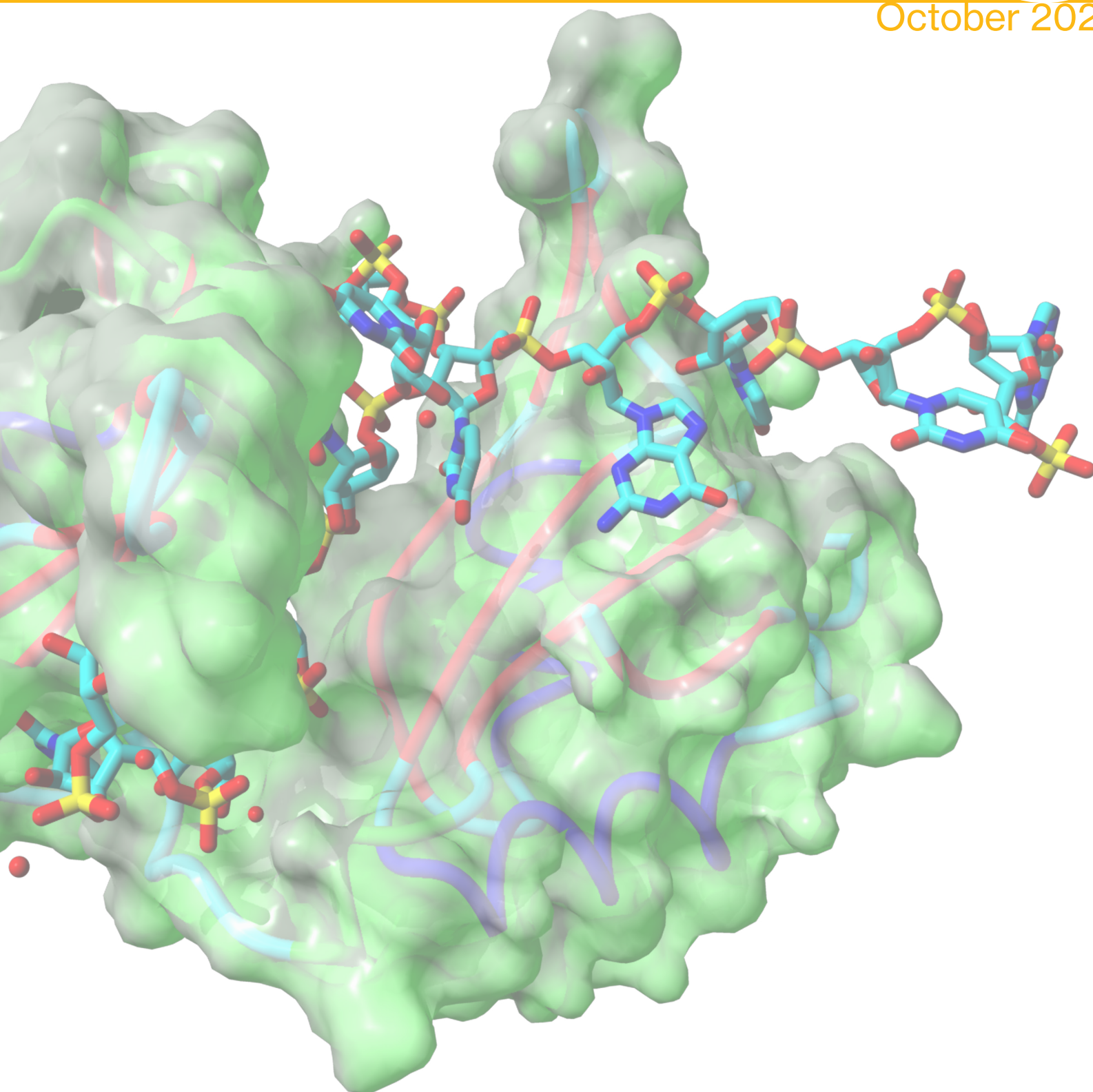


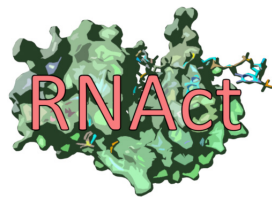
RNAAct

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

5

October 2022





This issue of the newsletter has been edited by Luca Sperotto (ESR 6) and Anna Kravchenko (ESR 4).

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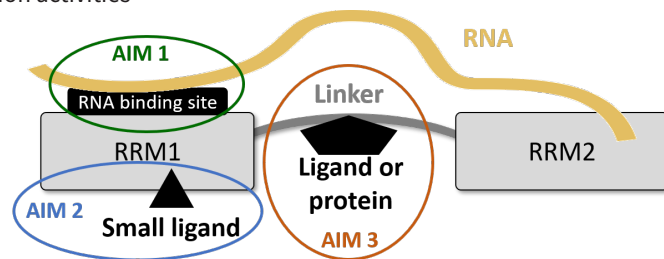
RNAct at a glance

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 RRM proteins 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 RRM proteins, 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 RRM proteins 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:

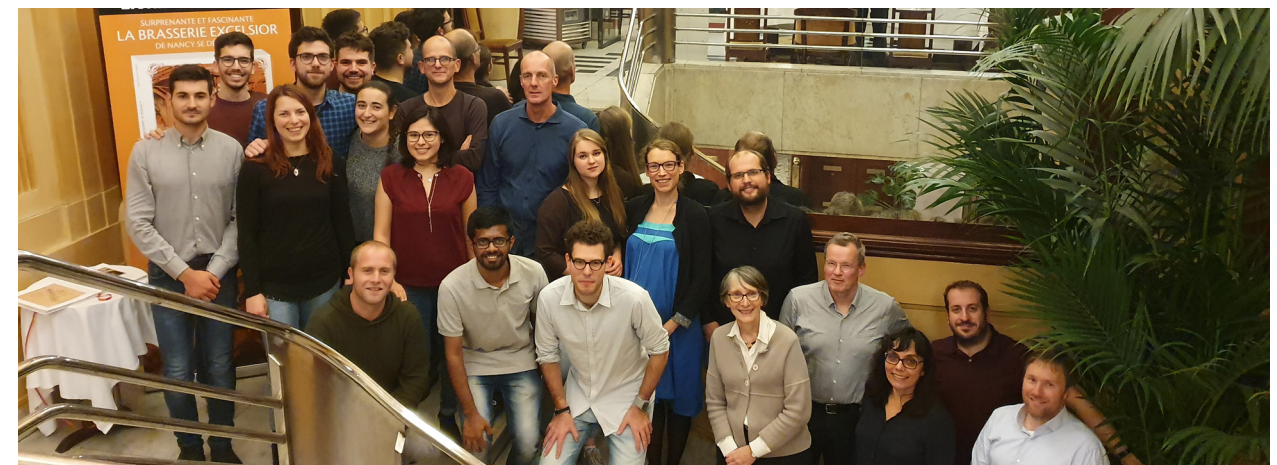
- Work Package 1.** Creation and characterisation of functional RRM proteins.
- Work Package 2.** Representation and design of dynamic proteins.
- Work Package 3.** Bio-analytics and synthetic biology.
- Work Package 4.** Training and education.
- Work Package 5.** Coordination and management.
- Work 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)
Dynamic Biosensors GmbH (DBS) Dr. Ulrich Rant / Dr. Wolfgang Kaiser	Prof. Dr. Martin Zacharias



Secondments



CSIC
Secondment host

Guillermo Pérez (ESR 10; RV, Sweden) visited Rodrigo's lab at the Institute for Systems Biology (Isys-2bio-CSIC, Valencia) for two months (January-March 2022). During that period, Guillermo collaborated with Roswitha Dolcemascolo (ESR 8) in performing experiments related to protein-RNA binding regulation in living bacteria (which was related to the work previously performed with ESR 8 and 7 during their secondments at Ridgeview Instruments). He also worked in the development of future approaches to modulate binding through the introduction of new genetic circuits in bacteria.



CNRS
Secondment host

Anahi Higuera (ESR 9; DBS, Germany) completed her secondment (February-May) at the CAPSID team, Loria University, France under the direction of Dr. Isaure Chauvot de Beauchêne, Dr. Marie-Dominique Devignes and ESR 3 Hrishikesh Dhondge. The aim of the secondment was to perform molecular dynamics (MD) simulations of the Sex-lethal protein (splicing factor controlling the sex in the fruit fly) RRM2 dissociation from its cognate RNA. The reasoning behind this project followed the results of binding kinetic experiments performed in the same biological system, which had an unexpected behavior: higher salt concentration impacted the off-rate instead of the on-rate. With MD it was expected to gain a deeper understanding of the interactions at different salt concentrations and the way these changes in the electrostatic interactions would affect the interactions of the protein towards the RNA. During her stay, Anahi learned the theoretical and practical basics of molecular dynamics (MD) simulations, as well as data analysis. The result of the secondment was an MD simulation of RRM2 dissociating faster from the RNA in high salt concentrations compared with low salt concentrations, which agreed with the experimental results.



ULG
Secondment host

Niki Messini (ESR 6; HMGU, Germany) completed her secondment at the University of Liège, Belgium. She performed phage display experiments from a large-scale random mutagenesis DNA library for RRM mutants with variant RNA targets. The library was expressed on the surface of phages and deep sequencing of the selected variants for each of the selected rounds was carried out. This delivered RRMs certified to bind particular RNA fragments for synthetic biology and bio-analytics, and large-scale protein-RNA binding data.



Project progress

Munich Workshop - Where to by end PhD, and after

Board Meetings

An extra workshop “Where to by the end PhD, and after” took place in May 2022 (from Thursday, May 19th to Friday, May 20th) organized by HMGU in Munich. During the “Career forum” session, the 10 ESRs had the chance to discuss about their career prospects, asking about their curiosities and doubts and receiving different point of views and useful advices from supervisors.



In addition, the workshop included the progress presentations, where the ESRs had a great opportunity to share their results and the progress made in the RNAct consortium. This has been the starting point for the “Scientific discussion” session, where everybody gave his/her point of view and shared ideas about the status of the project and how to proceed towards the end of the RNAct consortium.

The workshop also included the board meeting and discussion about the organization of the RNAct Final Conference.

Workshop 6 - RNAct future and innovation

The sixth and final workshop, “RNAct Future and innovation”, took place in September 2022 (Sept 12-13) in Valencia and it was organized by CSIC. The 10 ESRs had the opportunity to meet all together once again before the end of the RNAct project.

The seminars included in the sixth workshop were:

- **Synthetic biology.** Prof. Dr. Alfonso Jaramillo
- **Leadership.** Jose Luis Garcia (CSIC, Spain)

In addition, the workshop included presentations from the ESRs in preparation for the Final Conference and scientific/future of the RNAct discussion. This was a great occasion to receive useful feedbacks and look at the future after the RNAct.

RNAct Final Conference - Tailoring RNA Binding proteins and RNA targeting

The very final official RNAct event - the Final Conference titled “Tailoring RNA binding proteins and RNA targeting”, 14th-15th of September, took place in Valencia, Spain, hosted by Universidad Politécnica de Valencia, and affiliated with the International Society for Computational Biology. Many scientists, both young and senior, gathered there to share their research on RBPs. The subject was discussed from as many angles as possible, as participants were coming both from the computational and experimental sides, as well as from industry and academia. Talks about deep theoretical aspects of RNA binding were followed by talks about cutting-edge applications of the newest research results.

The Conference gathered internationally renowned speakers who gave the following keynotes:

- **Modeling RNA polymorphism.** Prof. Samuela Pasquali
- **Solving 3D puzzles of biomolecular interactions by integrative modelling.** Prof. Dr. Alexandre Bonvin
- **System biology and protein design.** Prof. Dr. Luis Serrano
- **Physics-based computational protein design.** Dr. Thomas Simonson
- **Engineered gene circuits adapting with reinforcement learning.** Prof. Alfonso Jaramillo
- **Antiviral synthetic RNA-protein granules.** Prof. Roei Amit
- **RNA-targeted therapeutics.** Dr. Leonardo De Maria
- **Frontiers in RNA simulations: challenges and expectations.** Prof. Dr. Modesto Orozco

The Conference also included a Poster Session, a Panel Session “Designing RBPs for RNA binding” and an Innovation Workshop “Prospects of RNA binding/targeting from an industrial point of view”.

In addition, the next prizes, sponsored by RNAct beneficiary Dynamic Biosensors, were awarded to:

- **Best Oral Presentation.** Joel Roca Martínez for his work entitled “Deciphering the RRM-RNA recognition code: a computational analysis”
- **Best Poster.** Philipp Innig Aguion for his work entitled “Structure determination of RNP complexes by MAS solid-state NMR spectroscopy”



Project progress

ESRs contributions at RNAct Final Conference



José Gavaldá-García
ESR 1

Conformine, a predictor of protein Conformational Variability from amino acid sequence.

Oral presentation and poster.



Joel Roca Martínez
ESR 2

Deciphering the RRM-RNA recognition code: A computational analysis.

Oral presentation and poster.



Hrishikesh Dhondge
ESR 3

CroMaSt: A workflow for domain family curation through cross-mapping of structural instances between protein domain databases.

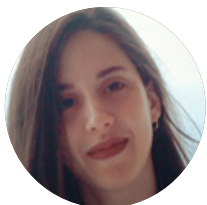
Oral presentation and poster



Anna Kravchenko
ESR 4

New strategy for optimizing knowledge-based docking parameters: application to ssRNA-RRM docking.

Oral presentation and poster.



Niki Messini
ESR 5

Unraveling the molecular basis of specific protein-RNA interactions to guide the design of novel RRMs.

Oral presentation and poster.



Luca Sperotto
ESR 6

Intramolecular interactions modulate RNA binding of hnRNP A1.

Oral presentation and poster.



Anna Pérez i Ràfols
ESR 7

Untangling the Complex Mode of Interaction of a Multi-Domain RNA Recognition Motif Protein: The Case of Human Musashi-1.

Oral presentation and poster



Roswitha Dolcemascolo
ESR 8

Repurposing the mammalian Musashi-1 as allosteric translation repressor in bacteria.

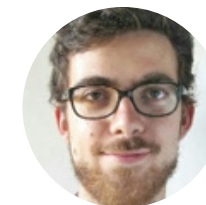
Oral presentation and poster.



Rosa Anahí Higuera
ESR 9

In union there is strength: complex mechanisms of multidomain RNA-binding proteins unraveled using binding kinetics.

Oral presentation and poster.



Guillermo Pérez Roper
ESR 10

Biophysical characterization of Musashi-1 binding to RNA.

Oral presentation and poster

Project progress

Contributions at external scientific meetings

Higuera A.R., Aziz M., **Sattler M.**, **Kaiser W.** Unravelling multidomain RNA binding protein interactions: from kinetic characterization to a complex binding mechanism. Oral presentation at the 27th RNA Society Annual Meeting, 31st May-5th June 2022, Boulder (USA).

Niki Messini, **Roca-Martínez J.**, Kachariya N., Redeghier P., **Higuera A.R.**, Morales F., Dumoulin M., **Kaiser W.**, **Vranken W.**, **Sattler M.** Understanding and designing RNA binding specificity of the RRM domain. Poster presented at the 27th RNA Society Annual Meeting, 31st May-5th June 2022, Boulder (USA).

Roca-Martínez J., **Dhondge H.**, **Vranken W.F.** Deciphering the RRM-RNA recognition code: A computational analysis. Poster presented at the Structural Biology Brussels Retreat, 1st-3rd June 2022, Blankenberge (Belgium).

Pérez-Ràfols A., **Higuera A.R.**, **Pérez-Ropero G.**, Linda Cerofolini, **Buij J.**, **Kaiser W.**, **Martelli T.** and **Fragai M.** Multi-Disciplinary Approach to Untangle the Complex Mode of Interaction of a Multi-Domain RNA Recognition Motif Protein: The Case of Human Musashi-1. Poster presented at the Chianti Workshop “Opening New Doors in Magnetic Resonance”, 19th-24th June 2022, Grosseto (Italy).

Pérez-Ràfols A., Ciani C., Bonomo I., Micaelli M., Esposito A., Zucal C., Belli R., D’Agostino V.G., Bianconi I., Calderone V., Cerofolini L., **Fragai M.***, Provenzani A. Identification and characterization of an RRM-containing, ELAV-like, RNA binding protein in *Acinetobacter Baumannii*. Poster presented at the Chianti Workshop “Opening New Doors in Magnetic Resonance”, 19th-24th June 2022, Grosseto (Italy).

Dolcemascolo R., Heras-Hernández M., Goiriz L., Montagud-Martínez R., Márquez-Costa R., Ruiz R., Requena A., **Pérez-Ràfols A.**, **Higuera A.R.**, **Pérez-Ropero G.**, **Buijs J.**, **Kaiser W.**, **Martelli T.**, and **Rodrigo G.** Repurposing the mammalian Musashi-1 as allosteric translation repressor in bacteria. Poster presentation at the Biochemical and Molecular Engineering XXII: the Dawn of a New Era, 26th-30th June 2022, Cancun (Mexico).

Pérez-Ràfols A., **Higuera A.R.**, **Pérez-Ropero G.**, Linda Cerofolini, **Buij J.**, **Kaiser W.**, **Martelli T.** and **Fragai M.** Multi-Disciplinary Approach to Untangle the Complex Mode of Interaction of a Multi-Domain RNA Recognition Motif Protein: The Case of Human Musashi-1. Poster presented at The Protein Society meeting, 6th-10th July 2022, San Francisco (USA).

Pérez-Ropero G., **Dolcemascolo R.**, **Pérez-Ràfols A.**, **Higuera A.R.**, **Buijs J.** Monitoring RNA-protein interactions in living bacteria in real time. Poster presented at The Protein Society meeting, 6th-10th July 2022, San Francisco (USA).

Sperotto L., Kang H.S., Felli I.C., **Sattler M.** Intramolecular interactions modulate RNA binding and LLPS of hnRNP A1. Poster presented at the 29th International Conference on Magnetic Resonance in Biological Systems, 21st-25th August 2022, Boston (USA).

Dhondge H., **Chauvot de Beauchene I.**, **Devignes M.D.** CroMaSt: A workflow for domain family curation through cross-mapping of structural instances between protein domain databases. Poster presented at the 21st European Conference on Computational Biology, 18th-21st September 2022, Sitges (Spain).

Roca-Martínez J., **Dhondge H.**, **Vranken W.** Deciphering the RRM-RNA recognition code: A computational analysis. Poster presented at the 21st European Conference on Computational Biology, 18th-21st September 2022, Sitges (Spain).

Gavaldá-García J., **Roca-Martínez J.**, **Vranken W.** Conformine, a predictor of protein Conformational Variability from amino acid sequence. Poster presented at the 21st European Conference on Computational Biology, 18th-21st September 2022, Sitges (Spain).

Higuera, A.R. How to... analyse catalytic rates of enzymes with switchSENSE®. Oral presentation at the Discover molecular interactions: User Meeting. 7th-9th September 2022, Munich (Germany)

Kravchenko A., **Smail-Tabbone M.**, **Chauvot de Beauchene I.**, de Vries S.J. “Optimizing scoring for ssRNA-protein docking models”. Oral presentation at the 35th Rhine-knee Regiomeeting on structural biology, 5th-7th October 2022, Gerardmer (France).

Reporting (January-October 2022)

Deliverables

D1.1 Summary report on design cycle 1 up to phage display

D1.2 Biophysical and structural biology analysis of RRM from design cycle 1

D2.3 RRM modelling & RNA-RRM docking software

D3.4 Results for allosterically regulated (Musashi-based) circuit

D4.4 Final report on ESR research and PCDP progress, including summary of network-wide training events

D5.5 Progress report year 3

Milestones

M4 Data on first newly designed RRM

M12 All ESRs presented poster at conference

M14 All ESRs discussed career choice

ESRs progress videos

The fellows recorded a series of progress videos, in which they summarised their mid-term achievements and described future projects:

- **Jose Gavaldá (ESR 1)** <https://tinyurl.com/3ytwp9yr>
- **Joel Roca (ESR 2)** <https://tinyurl.com/bdea35p5>
- **Hrishikesh Dhondge (ESR 3)** <https://tinyurl.com/28tatah6>
- **Niki Messini (ESR 5)** <https://tinyurl.com/y822yrkm>
- **Anna Pérez i Ràfols (ESR 7)** <https://tinyurl.com/25zv84b>
- **Roswitha Dolcemascolo (ESR 8)** <https://tinyurl.com/bdzex49w>
- **Anahí Higuera (ESR 9)** <https://tinyurl.com/yckrewp2>
- **Guillermo Pérez (ESR 10)** <https://tinyurl.com/a2nb4h52>

Webinars

The ESRs are developing webinars based on trainings received during the RNA workshops. Th following websinars will be made available in the [RNAct Youtube channel](#) before the end of 2022:

- **Protein design.** Joel Roca (ESR 1)
- **The beautiful complexity of RRM-RNA interactions.** Anna Kravchenko (ESR 4) and Luca Sperotto (ESR 6)
- **How to produce and store biological data to enable data-intensive research.** Jose Gavaldá (ESR 1)

Can’t wait until then? Thus go watch the webinar prepared by Roswitha Dolcemascolo (ESR 8) and Hrishikesh Dhondge (ESR 3) on “Molecular interactions in drug discovery” available at <https://tinyurl.com/2p8uk47y>.

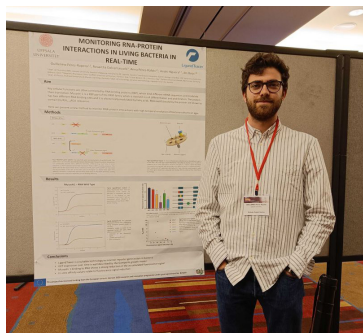
Project progress

Meetings

Board Meetings

The supervisory and management board met in-person on 20th May 2022 during the Network-wide workshop in Munich. The meeting focused on general management of the network, with special emphasis on the fellow's progress and the organization of the RNAct Final conference. Jose Gavalda-García (ESR 1) attendend the meeting as ESR representative.

News



Guillermo Pérez Ropero (ESR 10) won the Best Poster Award at the 36th Annual Symposium of the The Protein Society (San Francisco, 7th-10th July 2022).



Joel Roca Martínez (ESR 2) won the Best Presentation Award at the RNAct Final Conference “Tailoring RNA Binding proteins and RNA targeting” (Valencia, 14th-15th September 2022).

Publications

Dolcemascolo R, Goiriz L, Montagud-Martínez R, **Rodrigo G** (2022). Gene regulation by a protein translation factor at the single-cell level. *PLOS Computational Biology*, 18(5): e1010087
<https://doi.org/10.1371/journal.pcbi.1010087>

Ciani C, **Pérez-Ràfols A**, Bonomo I, Micaelli M, Esposito A, Zucal C, Belli R, D’Agostino VG, Bianconi I, Calderone V, Cerofolini L, Massidda O, Whalen MB, **Fragai M**, Provenzani A. (2022). Identification and Characterization of an RRM-Containing, RNA Binding Protein in *Acinetobacter baumannii*. *Biomolecules*, 12(7):922.
10.3390/biom12070922

Roca-Martinez J, Lazar T, **Gavalda-Garcia J**, Bickel D, Pancsa R, Dixit B, Tzavella K, Ramasamy P, Sanchez-Fornaris M, Grau I, **Vranken WF**. (2022). Challenges in describing the conformation and dynamics of proteins with ambiguous behavior. *Front Mol Biosci.*, 3;9:959956.
10.3389/fmolb.2022.959956

Journal club

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

8th Journal club session:

Date: 14/02/2022

Article: The kinetic landscape of an RNA binding protein in cells

DOI: <https://doi.org/10.1038/s41586-021-03222-x>

Chair: Anahí Higuera (ESR 9)

9th Journal club session:

Date: 12/05/2022

Article: The proteome folding problem and cellular proteostasis

DOI: <https://doi.org/10.1016/j.jmb.2021.167197>

Chair: Jose Gavalda-García (ESR 1)

10th Journal club session:

Date: 16/08/2022

Article: Aromatic side-chain conformational switch on the surface of the RNA Recognition Motif enables RNA discrimination

DOI: <https://doi.org/10.1038/s41467-017-00631-3>

Chair: Niki Messini (ESR 5)

Join the discussion!

Following the online ESRs only sessions, the debate continues in the dedicated discussion group in LinkedIn. Discussions are open to everyone interested in RRM and RNA!



<https://tinyurl.com/tx5z4bb>

RNAct gallery



Stay tuned!



Twitter
<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>

