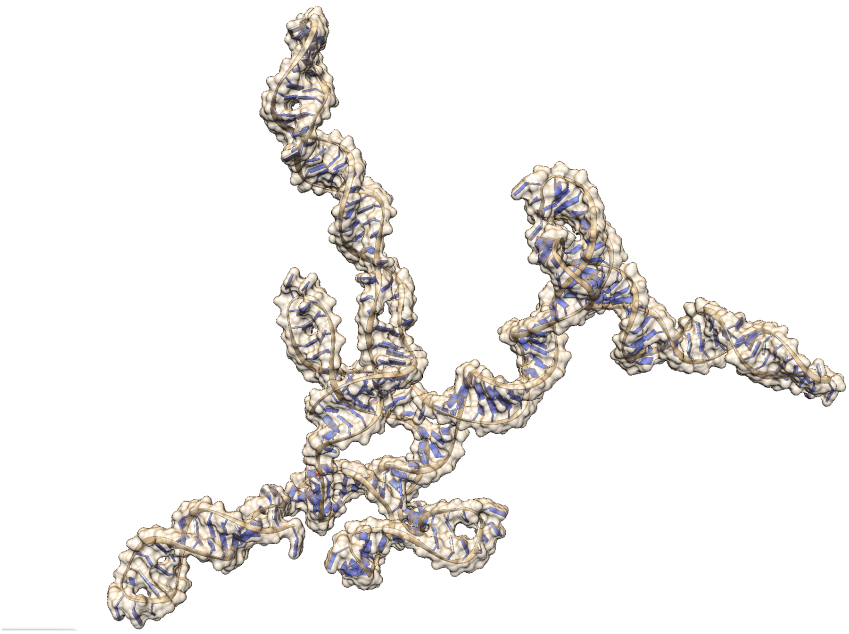
STRUCTURE AND TOPOLOGY OF RNA IN LIVING SYSTEMS

BOOK OF ABSTRACTS



JANUARY 30 – FEBRUARY 2, 2023

ECT*, TRENTO, ITALY

LIST OF ABSTRACTS

MONDAY 30. 1., SESSION I, 9:00 – 10:30

SELECTIVE PACKAGING OF HIV-1 GENOMIC RNA

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The assembly of HIV-1 virions consists of a series of well-coordinated steps involving a series of protein-protein and protein-RNA interactions between viral components, and also between host-cell factors and viral proteins. Packaging of the retroviral genome is a highly regulated process that is mostly coordinated between the structural protein Gag and the genomic RNA (gRNA). It is widely accepted that the specificity of this process depends on a cis-acting RNA signal (packaging signal or Y); however, there are several unanswered questions about the exact identity of Y as well as the molecular mechanism that confers packaging specificity to the dimer form of the gRNA. Here we will discuss the complexity of studying HIV-1 Y, the possible sequences that comprise this sequence, and most importantly, the role of the specific and non-specific interaction during Gag-RNA binding. Furthermore, we will show how to dissect the contribution of these interactions to binding specificity and to the in vitro assembly of HIV-1 immature virus-like-particles. Finally, we will propose a mechanism by which the gRNA is selectively packaged during virion assembly.

MONDAY 30. 1., SESSION II, 11:00 – 12:30

USING SYNTHETIC BIOLOGY TO UNRAVEL RNA TERTIARY INTERACTIONS

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The relationship between structure and function of single RNA molecules and large molecular complexes composed of several RNA molecules is poorly understood, despite the ubiquitous utilization of the former and frequent occurrence of the latter within various cellular contexts. In this work, we present an oligo library (OL) and modelling based approach to study RNA tertiary interaction. We demonstrate the efficacy of our method via two examples, which attributes the experimentally detected phenomenon to weak tertiary-scale interaction. Our method consists of

setting up a translational regulatory massively parallel reporter (TR-MPRA), where the RNA aptamer that we wish to characterize is positioned in regulatory hot-spot locations on the mRNA. We then scan many variants of the aptamer using the TR-MPRA, and analyze the results via thermodynamic modelling, machine-learning-based modelling, or some combination thereof. Our results demonstrate a tertiary-interaction dependent cooperative regulatory effect, structure dependent up- or down- regulation of translation, and a tertiary-interaction induced liquid-gel phase separation. Consequently, I present a new experimental tool which together with thermodynamic and machine-learning based models allows us to gain an insight into the role that tertiary interaction plays on RNA's structure-function relationship.

COMPUTATIONAL MODELING OF ICOSAHEDRAL SHELL ASSEMBLY AND ENCAPSULATION OF FLEXIBLE POLYMERS

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Bacterial microcompartments are proteinaceous 'organelles' found in bacteria, consisting of large icosahedral protein shells that assemble around collections of enzymes, scaffolding proteins, and other components involved in particular metabolic processes. In this talk, I will present results from coarse-grained computational modeling and theory, which elucidate factors that control the assembly pathways, size, structure, and function of microcompartments. In particular, we find that the properties of the scaffolding proteins, such as their length and binding affinities, play key roles in determining assembly pathways and the size of the assembled shell.

MONDAY 30. 1., SESSION III, 14:30 – 16:00

SPIDERS, ENTANGLEMENTS, AND RNA STRUCTURE PREDICTION

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A growing interest in RNAs, especially in the context of RNA therapeutics, led to increased efforts to develop new models and computational methods that support the study of these molecules.

The recent decade resulted, among other things, in new systems for predicting the three-dimensional structure of RNAs based on their sequence. However, most of these systems in their first published version do not have mechanisms to verify the correctness of the generated structural models. It seems that not only the users of these systems, but also their authors, are not always aware of this fact. This is shown, for example, by analyses of predictions submitted to the RNA-Puzzles and CASP-RNA competitions. In 3D computer models of RNAs, one can find groups of overlapping atoms, excessively long bonds, incorrect torsion angles, entanglements of structural elements, etc. During the lecture, we will address the latter. The types of entanglements of structural elements found in predicted 3D RNA structures will be presented [1]. We will look at the statistics of the entanglements. We will consider why entanglements appear in RNA 3D models, how to detect them, and whether they should all be treated as topologically incorrect. We will learn about RNAspider [2] which automatically identifies and visualizes entanglement sites.

[1] Popenda et al. (2021) Entanglements of structure elements revealed in RNA 3D models, *Nucleic Acids Res* 49(17):9625-9632.

[2] Luwanski et al. (2022) RNAspider: a webserver to analyze entanglements in RNA 3D structures, *Nucleic Acids Res* 50(W1):W663-W669.

PROBING THE STRUCTURE AND FUNCTION OF LONG VIRAL RNAS

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RNA viruses must efficiently encode large amounts of information within compact genomes. A common data compression strategy is the synthesis of sub-genomic RNAs through alternative start sites, termination sites and/or alternative splicing. These transcript isoforms may have different RNA structures within their common regions that could have importance consequences for their function. Measurements of RNA structure can be obtained using reagents that react with unpaired bases, leading to adducts that can be identified by mutational profiling on next

generation sequencing machines. One drawback of these experiments is that short sequencing reads can rarely be mapped to specific transcript isoforms. Consequently, information is acquired as a population average in regions that are shared between transcripts thus blurring the underlying structural landscape. We have developed nanopore dimethyl-sulfate mutational profiling (Nano-DMS-MaP), a method that provides isoform resolved structural information of highly similar RNA molecules. We use Nano-DMS-MaP to resolve the complex structural landscape of HIV-1 transcripts in infected cells. We show that unspliced and spliced transcripts have distinct structures at the packaging site within their the common 5'UTR, likely explaining why spliced viral RNAs are excluded from viral particles. Thus, Nano-DMS-MaP can be used to resolve biologically important transcript-specific RNA structures that were previously hidden in short read ensemble analyses.

MONDAY 30. 1., SHORT TALK SESSION I, 16:30 – 17:30

MOLECULAR DYNAMICS SIMULATIONS OF MODIFIED RNA MATCHING DENATURATION EXPERIMENTS

V. Piomponi[1], G. Bussi[1]
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Post-transcriptional modifications of RNA are the object of growing interest in the community, since they have been shown to be functional in a variety of biological processes, and consist of biochemical alterations of nucleotides that can directly impact RNA structure and/or dynamics. Although the RNA community widely studies RNA modifications, the number of applications of molecular dynamics (MD) simulations to modified RNA is still limited. MD is a powerful tool to access the structural dynamics of RNA at the atomistic level, but the accuracy of the simulations strongly relies on the quality of the force-field parameters used. For this reason, it is often useful to combine simulations with experiments, by fitting force fields against experiments or by refining ensembles predicted by the simulations in order to enforce experimental averages. In this talk I will show how we used these strategies to characterize the dynamics of two modified RNA systems. First, I will address our effort to improve the quality of the N6-methyladenosine (m^6A) force field by fitting partial charges and a torsional potential to denaturation experiments performed on m^6A-containing duplexes [1]. The fitting also allowed us to correctly estimate the populations of m^6A syn/anti isomers as predicted by NMR measurements. Our fitting strategy makes use of alchemical free-energy calculations, which allows us to estimate the destabilization

induced by the methylation on duplexes. Next, I will show how we made use of ensemble refinement methods to investigate the structure of a 20-bp RNA helix containing Inosines. In particular, we used the maximum entropy principle, together with advanced enhanced sampling techniques, in order to generate an ensemble of structures compatible with NMR data, showing how the presence of Inosines increases flexibility in the helix and allows sugar puckering to be observed in the C2-endo conformation, which is not expected in ds-RNA [2]

[1] Piomponi et al, *ACS Cent. Sci.,* 8, 8 (2022).
[2] Collaboration with M. Sattler group, work in preparation

WEBTETRADO, YOUR ASSISTANT IN THE G4 SPACE

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[2]*Poznan Supercomputing and Networking Center, 60-965 Poznan, Poland.*

[3]*Institute of Bioorganic Chemistry, Polish Academy of Sciences, 61-704 Poznan, Poland.*

WebTetrado is a web server to analyze DNA and RNA 3D structures containing quadruplexes. On input, it accepts PDB and PDBx/mmCIF files, uploaded from a local drive, downloaded automatically based on a PDB id, or selected from the predefined examples. The input form allows also for configuring the quadruplex searching parameters. The computational pipeline identifies nucleotide base-base contacts to build an interaction graph in which tetrads are recognizable as cycles of four vertices. Next, based on stacking interactions, WebTetrado finds the ordering of tetrads constructing the quadruplex. Finally, it runs the classification procedures, computes both tetrads- and quadruplexes-related metrics, and prepares visualizations. The output contains a list of identified quadruplexes, each with its type (uni-, bi-, or tetramolecular), and topology classification separately for loops (Webba da Silva), tetrads (Webba da Silva and ONZ) and quadruplexes (ONZM). The final view has a two-line dot-bracket representation with three specialized 2D and 2.5D static visualizations and one interactive 3D view -- all similarly color-coded to improve comprehension. Furthermore, WebTetrado summarizes in tables several crucial quadruplex-related measures - rise, twist, and tetrad's planarity deviation -- together with tetrads, loops, chi angles, base pairs, and nucleotide details. In addition, all results are downloadable in formats promoting reuse: SVG for graphics and CSV for tables.

TUESDAY 31. 1., SESSION IV, 9:00 – 10:30

PHYSICAL MODELS FOR THE DEGRADATION RESISTANCE OF XRRNAS

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RNA genomes of Zika and other flaviviruses contain short pseudoknotted elements that protect them from exonuclease degradation. These elements, known as xrRNAs, are ca 80-nucleotide long and have a non-specific resistance to exonucleases. To clarify the physical basis of the latter, we modeled the enzymatic degradation of Zika xrRNA as a driven translocation through a cylindrical pore. We studied the process using implicit-solvent atomistic simulations with a native-centric force field. We observed a dramatic directional resistance to translocation, with a significantly higher activation barrier at the 5' end compared to the 3' one. The remarkable directional effect originates from the way that mechanical tension propagates in the pseudoknotted xrRNAs, and can account for the observed degradation resistance [1]. Our study further establishes that nanopore setups could harness the time-resolved profile of translocation velocity, which is non-uniform and directional-dependent, to infer the secondary and tertiary organization of RNAs [1,2].

[1] A. Suma, L. Coronel, G. Bussi and C. Micheletti, *Nature Commun.*, **11**, art no. 3749 (2020)

[2] M. Becchi, P. Chiarantoni and C. Micheletti,*J. Phys. Chem. B*,**125**, 1098-1196 (2021)

RNA AS A RANDOMLY BRANCHED POLYMER

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[1]*Department of Theoretical Physics, Jožef Stefan Institute, Ljubljana, Slovenia*

[2]*Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy*

[3]*Department of Physics, University of Trento, Trento, Italy*

Formation of both short- and long-range base pairs between the nucleotides of an RNA sequence gives rise to an often highly branched secondary and tertiary RNA structure. While numerous studies have demonstrated the importance of the high degree of branching of RNA structure for some of its functions—most notably in the genomes of positive-sense, single-stranded RNA (+ssRNA) viruses—the nature of the RNA branching topology remains largely unexplored. We use the theory of branched polymers to determine the scaling properties of both random RNA sequences as well as of genomes of +ssRNA viruses by mapping their secondary structures onto planar graphs. We derive scaling exponents ε and ϱ, related to the topology of branching, in two different ways, not only from simple scaling relationships but also from distributions of the related topological quantities of individual RNAs. This allows us to compare the general scaling behaviour of RNA to known classes of branched polymers, and to compare the scaling behaviour of the genomes of +ssRNA viruses to random RNA sequences. In this way, we are able to elucidate the branching properties of RNA within the framework of branched polymers and simultaneously point out where this description becomes insufficient. Understanding the scaling properties of RNA related to its branching structure aims to improve our understanding of the principles which give rise to it, opening up the possibility for de novo design of RNA sequences with desired topological properties.

TUESDAY 31. 1., SESSION V, 11:00 – 12:30

TUESDAY 31. 1., SESSION VI, 14:30 – 16:00

CONFORMATIONAL SPACE EXPLORATION AND REACTIVITY OF MINIMAL RIBOZYMES FOR AUTOCATALYTIC NETWORKS

Sélène Forget[1], Marie Juillé[1,2], Damien Laage[2], Élise Duboué-Dijon[1] and Guillaume Stirnemann[1]

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[2]*Chemistry Department, Ecole Normale Supérieure - PSL, CNRS, Paris, France*

A key question for the RNA world hypothesis is the emergence of autocatalytic networks in abiotic conditions without protein-based enzymes. Ribozymes (RNA-enzymes) are likely components. However, large ribozymes, which are known to form self-replicating networks, cannot have self-assembled from the short RNA fragments available in abiotic conditions, and

typical short ribozymes tend to favor the cleavage reaction over the required ligation for the formation of larger molecules. A promising direction is to adapt specific environmental conditions (temperature, ions), strand sequence and length, which were shown by experiments to favor ligation e.g. in the small hairpin ribozyme, but an understanding of these factors' impact on the catalytic steps is still missing.

Our aim is to provide a molecular understanding of the tertiary structure effect on the ligation/cleavage equilibrium using an all-atom molecular dynamics approach, and to clarify the reaction thermodynamics and mechanism for these systems. We will present our first results obtained on different variants of the hairpin ribozyme, that highlight the crucial importance of using specific enhanced sampling techniques to provide a reliable conformational sampling of the reactant and product states, that is typically not achieved even with microsecond, brute force simulations. We will also discuss our investigation of the catalyzed reaction free-energy barrier and free-energy difference using mixed quantum-classical approaches and alchemical transformations, respectively.

SIMULATIONS OF RNA STRUCTURAL DYNAMICS AND LIGAND ASSOCIATION PATHS: THE CASE OF AN AMINOGLYCOSIDE-SENSING RIBOSWITCH

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[2]*Interdisciplinary Doctoral School, University of Warsaw, Warsaw, Poland.*

[3]*Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland.*

[4]*RIKEN Center for Biosystems Dynamics Research, Kobe, Japan.*

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[6]*RIKEN Cluster for Pioneering Research, Wako, Japan.*

[7]*RIKEN Center for Computational Science, Kobe, Japan.*

Riboswitches are regulatory fragments of mRNA that exert their function upon binding of a ligand or upon change of temperature or pH. Thus, the activity of riboswitches is related to their structural dynamics. We focus on a synthetic 27-nucleotide riboswitch, named N1, that binds

aminoglycosides (Duchardt-Ferner, E. et al., Angew. Chem. Int. Ed., 55:1527, 2016). Experiments show that some mutations, especially A17G, change the riboswitch activity (Weigand, J. E. et al., ChemBioChem, 15:1627, 2014). We apply both conventional and two-dimensional replica-exchange all-atom molecular dynamics simulations (generalized replica-exchange with solute tempering and umbrella sampling, varying both the temperature and ligand distance). We investigate (i) how mutations in N1 riboswitch affect its internal dynamics and aminoglycoside-N1 complexes (Chyży P. et al., Front. Mol. Biosci., 8:633130, 2021), (ii) why N1 riboswitch responds differently to neomycin than paromomycin, which are similar ligands (Kulik M. et al. NAR, 46:9960, 2018), and (iii) neomycin association pathways with N1 riboswitch, observing a two-step binding mechanism (Chyży P. et al., in preparation). The N1 dynamical picture corroborates the experimental aminoglycoside dissociation constants for mutants. The two-dimensional free-energy surfaces show two stable low-energy minima along the pathway.

We thank the National Science Centre Poland (Harmonia, DEC-2017/26/M/NZ1/00827) for funding.

WEDNESDAY 1. 2., SESSION VII, 9:00 – 10:30

RNA STRUCTURAL ENSEMBLES FROM ENERGY LANDSCAPE EXPLORATIONS

K. Röder[1]

[1]*Randall Centre for Cell & Molecular Biophysics, King's College London, United Kingdom.*

The versatile functions fulfilled by non-coding RNAs are encoded in the polymorphic structural ensembles adopted by these molecules. As these ensembles are highly dynamic, it is difficult to obtain accurate descriptions of them in experiment and simulation. At the same time, the centrality of RNAs in many regulatory processes, for example in viruses, makes them a key target in guiding drug-development efforts through structural biology.

In this talk, I will discuss how computational explorations of RNA energy landscapes lead to detailed descriptions of structural ensembles (1,2). The approach can be used in combination with other simulation methods (3), to study peptide-NA association (1,4) and to probe a variety of perturbations (e.g. chemical modifications (2), sequence mutations (1), and applied forces). I will conclude this discussion by highlighting future research directions and challenges.

[1] K Röder, G Stirnemann, AC Dock-Bregeon, DJ Wales, S Pasquali, *Nucleic Acids Res*. 48 (1), 373-389, 2020

[2] K Röder, AM Barker, A Whitehouse, S Pasquali, *PLOS Comput. Biol*. 18 (5), e1010150, 2022

[3] K Röder, G Stirnemann, P Faccioli, S Pasquali, *QRB Discovery* 3, e21, 2022

[4] KC Liu, K Röder, C Mayer, S Adhikari, DJ Wales, S Balasubramanian, *J. Amer. Chem. Soc.* 142 (18), 8367-8373, 2020

WEDNESDAY 1. 2., SESSION VIII, 11:00 – 12:30

INTEGRATIVE MODELING STRATEGIES AND RNA POLYMORPHISM

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[1]*Laboratoire de Biologie Foncionelle et Adaptative, Université Paris Cité, Paris, France*

RNA molecules are characterized by the existence of a multitude of stable states that result in a frustrated energy landscape, where the observed structures depend sensibly on experimental conditions and can depend on the initial, unfolded, structure [1,2]. In this context guiding the modeling process with experimental data and placing the molecule in the environment sampled in experiments is essential.

As proof or principle, the coarse-grained model we develop [3] is a useful starting point to couple simulations with experimental data as one has full control over its force field and sampling methods. We have recently developed a simulation technique allowing to bias MD coarse-grained simulations with SAXS data onthe-fly [4], and a theoretical framework to perform fast constant pH simulations where we can model the system considering the exchange of charges with the solvent [5]. These developments allow us to account for the environment to obtain reasonable structures to then be studied more thoroughly with high-resolution modeling, introducing also the comparison with SHAPE data from first principles [6].

[1] K Röder, G Stirnemann, AC Dock-Bregeon, DJ Wales, S Pasquali, Structural transitions in the RNA 7SK 5′ hairpin and their effect on HEXIM binding, Nucleic Acids Research 48 (1), 373-389 (2020)
[2] K Röder, AM Barker, A Whitehouse, S Pasquali, Investigating the structural changes due to adenosine methylation of the Kaposi's sarcoma-associated herpes virus ORF50 transcript, PLOS Computational Biology 2022, 18(5):e1010150, doi: 10.1371/journal.pcbi.1010150, PMID: 35617364
[3] T. Cragnolini, Y. Laurin, P. Derreumaux, S. Pasquali, The coarse-grained HiRE-RNA model for de novo calculations of RNA free energy surfaces, folding, pathways and complex structure predictions, J. Chem. Theory Comput., 11, 3510 (2015)
[4] L Mazzanti, L Alferkh, E Frezza, S Pasquali, Biasing RNA coarse-grained folding simulations with Small-Angle X-ray Scattering (SAXS) data, J. Chem. Theory Comput., 17, 6509-6521 (2021)
[5] S. Pasquali, E. Frezza, F.L. Barroso da Silva, Coarse-grained dynamic RNA titration simulations, Interface Focus 9: 20180066 (2019)
[6] E. Frezza, A. Courban, D. Allouche, B. Sargueil, S. Pasquali, The interplay between molecular flexibility and RNA chemical probing reactivities analyzed at the nucleotide level via an extensive molecular dynamics study, Methods 162-163:108-127 (2019).

RNACOMPOSER-BASED MODELING OF RNA 3D STRUCTURES IN CASP15

M. Antczak[1,2], M. Popenda[2], J. Sarzynska[2], T. Zok[1], M. Szachniuk[1,2]

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[2]*Insitute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland.*

CASP15 is the 15th edition of an international experiment advancing the state of the art in modeling protein structure. In the last round, the RNA tertiary structure modeling category was also considered, in which our group participated. The structure prediction workflow, used by our group, considers three major stages: (1) RNA secondary structure prediction, (2) building of an ensemble of RNA 3D structures, and (3) finally, selecting the potentially promising submissions. As RNA secondary structures, we first used the consensus structures obtained from the literature or Rfam database [1]. Next, if needed we predicted them using selected tools incorporated into the RNAComposer [2,3]. RNA 3D models were predicted by the RNAComposer system in a fully-automated and expert modeling manner. In the latter one, we searched through the RNA FRABASE 2.0 repository [4] to find 3D structure elements of low sequence homology which were next clustered to allow us to select the representative candidates

for expert modeling. Resultant RNA 3D predictions were ranked using the total energy coefficient computed by XPLOR [5]. Next, we used RNAspider [6] to identify entanglements of structural elements and rejected all the models that included them. Finally, we performed model clustering based on the RMSD measure to select group representatives as our submissions ensuring high consistency with the found literature data.

[1] Kalvari, Ioanna, et al. "Rfam 14: expanded coverage of metagenomic, viral and microRNA families" *Nucleic Acids Research* 49.D1 (2021): D192-D200.
[2] Popenda, Mariusz, et al. "Automated 3D structure composition for large RNAs" *Nucleic Acids Research* 40.14 (2012): e112-e112.
[3] Antczak, Maciej, et al. "New functionality of RNAComposer: an application to shape the axis of miR160 precursor structure" *Acta Biochimica Polonica* 63.4 (2016): 737-744.
[4] Popenda, Mariusz, et al. "RNA FRABASE 2.0: an advanced web-accessible database with the capacity to search the three-dimensional fragments within RNA structures" *BMC Bioinformatics* 11.1 (2010): 1-12.
[5] Schwieters, Charles D., et al. "The Xplor-NIH NMR molecular structure determination package" *Journal of Magnetic Resonance* 160.1 (2003): 65-73.
[6] Luwanski, Kamil, et al. "RNAspider: a webserver to analyze entanglements in RNA 3D structures" *Nucleic Acids Research* 50.W1 (2022): W663-W669.

WEDNESDAY 1. 2., SESSION IX, 14:30 – 16:00

DIFFERENTIAL SHAPE PROBING TO SCREEN COMPUTATIONALLY DESIGNED RNA AND TO DETECT PSEUDOKNOT AND NON-CANONICAL INTERACTIONS

P. Hardouin[1], J. Cossio[4], FX Lyonnet du Moutier[1], A. Giocchino[4], G. de Bisschop[1], E. Frezza[1], B.Masquida[2], S.Will[3], Y. Ponty[3], S. Cocco[4], R. Monasson[4], B. Sargueil[1]
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The development of reliable RNA design processes requires experimental validation. RNA structure modelling from chemical probing experiments has made tremendous progress, however accurately predicting large RNA structures is still challenging for several reasons. In particular interactions such as pseudoknots and non-canonical base pairs which are not captured by the available incomplete thermodynamic model are hardly predicted efficiently. To identify nucleotides involved in pseudoknots and non-canonical interactions, we scrutinized the SHAPE reactivity of each nucleotide of a benchmark RNA under multiple conditions. We show that probing at increasing temperature was remarkably efficient at pointing to non-canonical interactions and pseudoknot pairings. The SHAPE probing technology was then use to screen for RNA computationally designed to interact with a small molecule. RNA Aptamers to S-adenosyl methionine (SAM) were designed by different processes of artificial intelligence trained on natural sequences. Their properties were analyzed and compared to the variety of SAM aptamers found in the bio-diversity.

THURSDAY 2. 2., SESSION X, 9:00 – 10:30

PREDICTION OF NCRNA STRUCTURE: THE CASE OF LNCRNA DLEU2

Margherita Matarrese[1,2,3], Grazia Cottone[4], Francesca Guerrieri[5], Letizia Chiodo[1]

[1]*Department of Engineering, Campus Bio-Medico University of Rome, Rome, Italy*

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[3]*Department of Bioengineering, The University of Texas at Arlington, Arlington, TX, USA*

[4]*Department of Physics and Chemistry- Emilio Segrè, University of Palermo, Palermo, Italy*

[5]*Cancer Research Center of Lyon (CRCL), UMR Inserm 1052 CNRS 5286 Mixte CLB, Université de Lyon 1 (UCBL1), Lyon, France.*

The RNA structure is critical for understanding RNA molecular function. Due to the RNA intrinsic features (i.e., flexible structure and variety of functions) and the lack of massive experimental structural data, the in-silico prediction of secondary and tridimensional structures is highly relevant to the structure-function paradigm. Yet, prediction is extremely challenging, especially for large RNA molecules. Here, we first evaluate a selection of freely accessible web-server tools to identify the best approach(es) for prospective lncRNAs investigations and to shed light on specific biological questions. Then, as a practical example of the successful application of in-silico protocols to guide and interpret biomolecular and cellular biological assays, we analyze

the case of the human lncRNA DLEU2, which is expressed in the liver, with increased expression in human hepatocellular carcinoma (HCC). DLEU2 physiologically interacts with zeste homolog 2 (EZH2) in the PRC2 complex for transcription repression. The hepatitis B viral protein HBx activates DLEU2 expression and directly binds it to modulate transcription and boost HBV replication. We model the complexes involving DLEU2/EZH2/HBx and predict a protein competition, experimentally confirmed, impacting EZH2/PRC2 functions.

[1] Salerno D, et al., Gut 2020, doi:10.1136/gutjnl-2019-319637
[2] Matarrese MAG, et al., JBSD 2022, doi:10.1080/07391102.2022.2116110

DECIPHERING THE MECHANISM OF INFLUENZA A GENOME PACKAGING USING GENOME-WIDE APPROACHES

Anne-Caroline Jousset[1#], Hardin Bolte[2#], Antoine Hache[1#], Celia Jakob[2#], Béatrice Chane-Woon-Ming[1], Damien Ferhadian[1], Daniel Desiro[3], Gabriel Lencioni Lovate[3], Anne Schweigert[1], Manja Marz[3], Martin Schwemmle[2], Roland Marquet[1]

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Segmentation of the genome of Influenza A (IAV) viruses favours rapid evolution by genetic reassortment, but complicates packaging as IAV particles must contain one copy of each of the eight vRNAs to be infectious. Regions containing packaging signals have been identified at the termini of each vRNA and the current model proposes that the eight vRNAs are packaged as a supramolecular complex held together by base-pairing interactions between the packaging signals, but the evidence supporting this model remains limited (for a review see [1]). Recently, several groups identified inter-vRNA interactions using genome-wide cross-linking studies. However, most of these interactions are located in the central regions of the vRNAs, rather than in the regions containing known packaging signals, and except for one case, viruses in which interactions were disrupted displayed no phenotype [1]. Furthermore, we showed that sequential disruption of SPLAH-identified RNA interactions failed to identify RNA sequences involved in

RNA packaging. Using SHAPE-MaP, a genome-wide chemical probing approach, we observed that mutations can affect packaging by inducing either unfolding or aberrant folding of the terminal packaging signals, which are likely involved in inter-vRNA interactions. Mutating a single packaging signal results in a complex rearrangement of the RNA-RNA interaction network, which leaves a footprint in the RNA structure after dissociation of the viral particles.

[1] Jakob, C., Paul Stansilaus, R. *et al.* (2022) ***Nucleic Acids Res.*** **50**, 9023-9038.

THURSDAY 2. 2., SHORT TALK SESSION 2, 11:00 – 12:30

RNA ABSTRACTIONS FOR STRUCTURAL COMPARISON AND CLASSIFICATION

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RNAs adopt three-dimensional conformations to enable their biological functions. Therefore, the comparison and classification of RNAs play fundamental roles in understanding their behaviour and in grouping similar organisms. The literature has faced the problem with several approaches whose results strongly depend on molecular representations and codified information. Most of them focus on the secondary structure.

We present an algebraic approach to compare RNA secondary structures with arbitrary pseudoknots. Each molecule is associated with a unique arc-annotated sequence (AAS), which consist of a nucleotide sequence equipped with arcs representing the hydrogen bonds (Figure 1-a). We formalize the relations among the bonds as concatenation, nesting and crossing (Figure 1-b) to abstract an AAS into a particular tree (Figure 1-c). The alignment of these trees determines a distance.

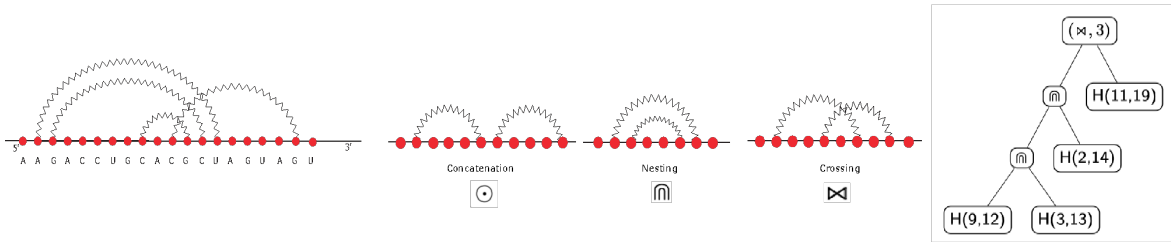

Figure 1 a. An example of RNA Secondary Structure represented as arc-annotated sequence; b. the three operators c. the structural tree accociated to the molecules in part a.

We also present an application of this approach to study the phylogenies of 5S, 16S and 23S rRNAs of Archaea, Bacteria and Eukaryota. Finally, we briefly introduce an extension of this approach to compare three-dimensional conformations.

[1] Michela Quadrini, Luca Tesei, and Emanuela Merelli (2020). ASPRAlign: a tool for the alignment of RNA secondary structures with arbitrary pseudoknots. Bioinformatics, 36(11), 3578-3579.
[2] Michela Quadrini, Luca Tesei, and Emanuela Merelli (2019). An algebraic language for RNA pseudoknots comparison. BMC Bioinformatics, 20(4), 1-18.

COMPUTATIONAL 3D STRUCTURE CHARACTERIZATION OF CCMV RNA2 FRAGMENT

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An increasing amount of structural information about the protein capsid of viruses has been collected and is now available in the Protein Data Bank; yet, the intrinsically disordered nature of viral ssRNA structures poses a challenge for experimentalists [1], this being critical on account of the impact that the RNA structure has on its function [2]. In this concern, molecular dynamics simulations might be powerful tools to achieve (putative) structural and dynamical insights on the behaviour of systems that are poorly characterised [3].

In this talk, I will discuss the main results of our ongoing project on the development of new methodologies to characterise the unrestrained dynamics of a viral RNA fragment [4] as well as under spatial constraints mimicking the protein capsid around it. I will illustrate the features of the secondary and tertiary structure that emerge from coarse-grained simulations, performed

with the oxRNA force-field [5]. I will also describe a simple and general protocol to generate an initial configuration of the whole virion particle to perform all-atom MD simulations, starting from a PDB structure of the capsid [6] and the sequence of the RNA fragment. This approach will provide detailed information about the structure and dynamics of the viral RNA, involving a description of the interactions between the nucleotides and the tails of the capsid, which is hardly explorable via experimental techniques [2]. Finally, I will comment on how this simulation framework can be instrumental to collect insights into the mechanisms of the self-assembly process.

[1] Smyth *et al*., *Front. Immunol.,* 9:2097 (2018)
[2] Strobel *et al*., *Curr. Op. Biotech.,* 39, 182-191 (2016)
[3] Šponer, Bussi *et al*., *Chem. Rev.* (2018), 118, 8, 4177–4338
[4] Allison *et al*., *Virology* (Sept. 1989), 172(1):321-30
[5] Matek *et al*., *J. Chem. Phys.* 143, 243122 (2015)
[6] Speir *et al*., *Structure* (1995), 3(1), 63-78