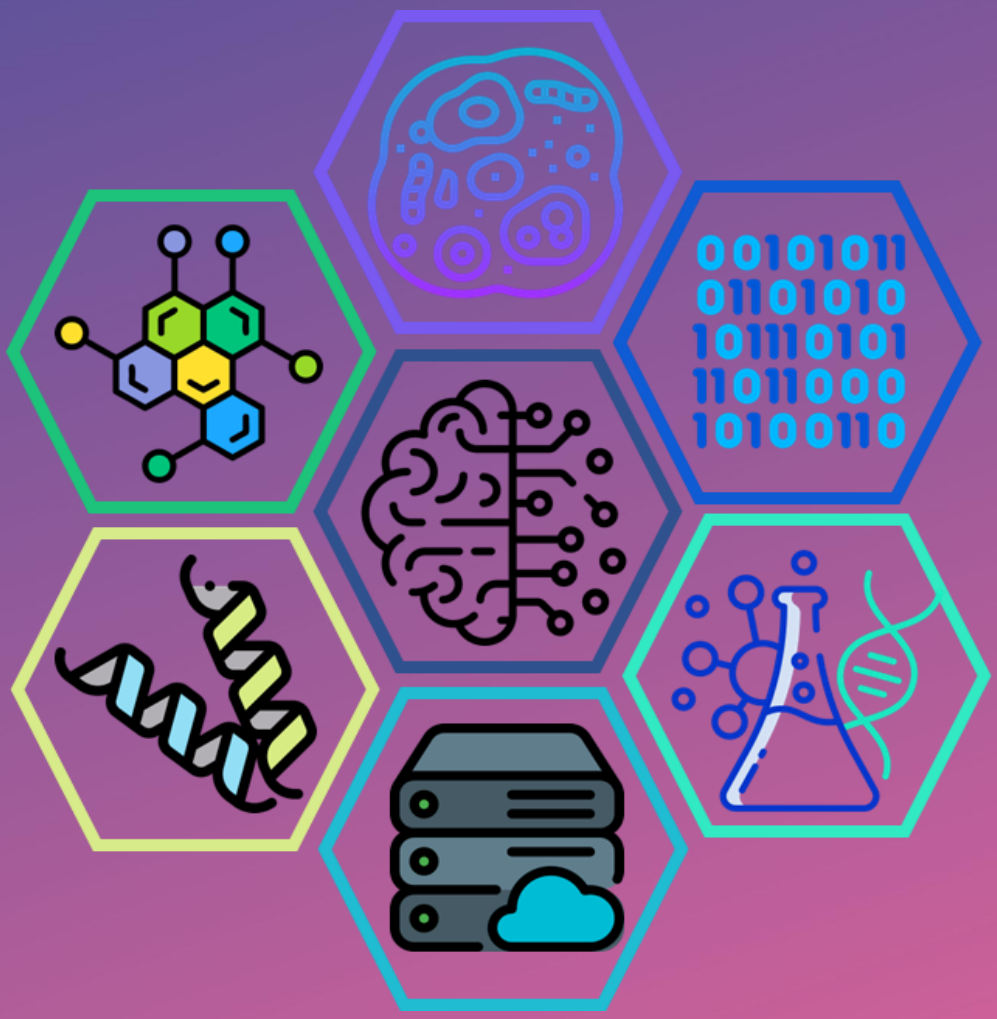
YOUNG MINDS AT WORK: BLENDING BIOCHEMISTRY AND BIOINFORMATICS

Abstract Book



On-line workshop

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Abstracts

Angiogenesis and vascular patterning: a calibrated mathematical model using the chicken chorioallantoic membrane

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Sprouting angiogenesis is the fundamental biological process responsible for the development of the vascular systems in adult individuals of superior eukaryotes. Capillaries undergo sprouting angiogenesis by altering the phenotype of their epithelial cells and proliferating toward starving or hypoxic cells [1]. Because of its importance in restoring tissue homeostasis, it has a relevant role during wound healing and is also employed by tumors to sustain their development. Considering its impact on human health, many efforts have been put into modeling angiogenesis [2]. While models may efficiently reproduce the mechanism of a biological process, the reliability of their prediction depends on the fitness they show with respect to the empirical evidence [3]. In the present work, we present our effort in calibrating a mathematical phase-field model originally proposed by Travasso et al. in 2011. The work took advantage of the 2022 implementation of the original model, included in the open-source package Mocafe, and employed experimental data of sprouting angiogenesis measured with the chicken chorioallantoic membrane system (CAM).

The calibration of the model's parameters followed a forward approach consisting in subsequent rounds of simulations employing a wide array of values. The calibration aimed at finding the optimal parameter set with respect to the fitness of the simulation with the empirical data. In our preliminary results the optimal values of two important model parameters were found to differ significantly from the ones originally proposed in 2011, and a useful modification of the model's framework has been proposed.

Although there is room for improvement, the preliminary results obtained are promising, and the model developed is now proposed for permanent implementation.

References

1. G. Eelen, L. Treps, X. Li, e P. Carmeliet, «Basic and Therapeutic Aspects of Angiogenesis Updated», Circ. Res., vol. 127, fasc. 2, pp. 310–329, lug. 2020, doi: 10.1161/CIRCRESAHA.120.316851.
2. J. A. Flegg, S. N. Menon, H. M. Byrne, e D. L. S. McElwain, «A Current Perspective on Wound Healing and Tumour-Induced Angiogenesis», Bull. Math. Biol., vol. 82, fasc. 2, p. 23, gen. 2020, doi: 10.1007/s11538-020-00696-0.
3. M. Cardiff e P. K. Kitanidis, «Efficient solution of nonlinear, underdetermined inverse problems with a generalized PDE model», Comput. Geosci., vol. 34, fasc. 11, pp. 1480–1491, nov. 2008, doi: 10.1016/j.cageo.2008.01.013.

Understanding Complex Systems through Differential Causal Networks

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Background: In the evolving landscape of data science and computational biology, Causal Networks (CNs) have emerged as a robust framework for modelling causal relationships among elements of complex systems derived from experimental data [1][2]. CNs can efficiently model causal relationships emerging in a single system while comparing multiple systems, allowing to understand rewiring in different cells, tissues, and physiological states with a deeper perspective [8].

Despite differential networks have been used to compare coexpression and correlation structures [4], introducing causality in differential analysis is essential to understand information flows and improve interventions, for example for agricultural or pharmacological purposes.

Methods: To achieve the ambitious goal of identifying the differences in causal relationships between two conditions, we introduce Differential Causal Networks (DCNs). DCNs are a novel framework that represents differences between two existing CNs. A DCN is obtained from experimental data by comparing two CNs, and it is a power tool for highlighting differences in causal relations. We also compare differences among three possible definitions of DCNs to highlight similarities and differences, with a focus on those of biological importance in our case, but generally to identify significant differences.

Results: After a careful definition and design of DCNs, we test our algorithm to model possible differential causal relationships between genes [5] responsible for the onset of type 2 diabetes mellitus-related pathologies [6] considering patients' sex at the tissue level [3][7]. DCNs allowed us to shed light on causal differences between sexes across nine tissues. Code, Data and Supplementary Information are available at https://github.com/hguzzi/DifferentialCausalNetworks [9][10][11].

Conclusion: The primary aim of researching DCNs and other network-based methodologies in a biological context is to enhance clinical care by personalizing treatment for patients with different conditions, such as the two sexes, and examining the differences in causal mechanisms influencing certain pathologies. Given this, the proposed method opens the doors to using DCNs in the genetic field, with the potential to expand into the proteomic and metabolomic domains. The clinical validation of the information drawn from this type of analysis remains of fundamental importance.

References

1. Pearl, J. Causal inference in statistics: An overview. Stat. Surv. 3, 96–146 (2009).
2. Glymour, C., Zhang, K. & Spirtes, P. Review of causal discovery methods based on graphical models. Front. Genet. 10, DOI: 10.3389/fgene.2019.00524 (2019).
3. Guzzi, P. H. et al. Differential network analysis between sex of the genes related to comorbidities of type 2 mellitus diabetes. Appl. Netw. Sci. 8, 1–16 (2023).
4. Ideker, T. & Krogan, N. J. Differential network biology. Mol. systems biology 8, 565 (2012).
5. Lonsdale, J. et al. The genotype-tissue expression (gtex) project. Nat. genetics 45, 580–585 (2013).
6. Rani, J. et al. T2diacod: a gene atlas of type 2 diabetes mellitus associated complex disorders. Sci. Reports 7, 1–21 (2017).

7. Guzzi, P. H. et al. Analysis of age-dependent gene-expression in human tissues for studying diabetes comorbidities. Sci.Reports 13, 10372 (2023).
8. Molak, A. Causal Inference and Discovery in Python: Unlock the secrets of modern causal machine learning with DoWhy, EconML, PyTorch and more (Packt Publishing, Birmingham, 2023), 1. edn. https://amzn.to/3RebWzn.
9. Doncheva, N. T., Morris, J. H., Gorodkin, J. & Jensen, L. J. Cytoscape stringapp: network analysis and visualization of proteomics data. J. proteome research 18, 623–632 (2018).
10. Zhang, K. et al. gcastle: A python toolbox for causal discovery (2021). 2111.15155.
11. Consortium, G. O. The gene ontology resource: 20 years and still going strong. Nucleic acids research 47, D330–D338 (2019).

Computational Benchmarking of Aptamers for Selective Inhibition of HuR

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Background: RNA-binding proteins (RBPs) are post-transcriptional regulators of gene expression, affecting RNA localization and translation. HuR (ELAVL-1) is a RBP that binds AU-rich elements (AREs) in the 3' UTR of target mRNAs through its three RNA recognition motifs (RRMs). Primarily nuclear, HuR dimerizes through its RRM3 domain under specific stimuli, stabilizing target mRNAs for cytoplasmic export, where it enhances translation. Nuclear HuR affects splicing and stability, while its cytoplasmic form increases mRNA translation, making HuR a potential therapeutic target due to its role in promoting tumor growth. While several small molecule inhibitors target HuR, they often lack specificity. For this reason, RNA-based aptamers, which offer high affinity and specificity, represent a promising alternative.

Methods: 12 RNA aptamer sequences - divided in five classes: A, B, C, D and orphan - were retrieved from the study of König et al. to be analyzed. The HuR-aptamers systems were modeled using AlphaFold3. The topology and coordinates files were prepared with AmberTools22, using the AMBER ff19SB and OL3 force fields for protein and RNA, respectively. Each complex was simulated for 500 ns of Gaussian-accelerated Molecular Dynamics (GaMD). The MD trajectories were analyzed with modules of GROMACS 2022 suite. The MM/GBSA method was used to quantify the free energy of interaction between HuR and the aptamer.

Results: RMSD analysis revealed aptamer A4 to be the least stable, while aptamers A1, B1, B3, and C exhibited the highest structural stability with an average RMSD below 1.5 Å. RMSF results indicated increased flexibility in the RRM3 region (residues 244-322) of the HuR protein for complexes HuR-A4 and HuR-B3, suggesting potential inhibition of dimerization. PCA analysis demonstrated restricted conformational space exploration for HuR when bound to aptamers A1, B2, and B3, contrasting with A2 and A4, which showed wider exploration.

Conclusion: The benchmark analysis identified five aptamers (B2, A2, orphan, A1, and B1) as the most promising candidates for further optimization. These selected aptamers will be refined to create a super-inhibitor. Subsequent molecular docking and MD simulations will be employed to design and optimize the most selective aptamers for in vitro and in vivo testing.

References

1. N. Ripin, et al.: Molecular basis for AU-rich element recognition and dimerization by the HuR C-terminal RRM, Proceedings of the National Academy of Sciences, 2019, 116, 2935-2944.
2. C.W. Schultz, et al.: Understanding and targeting the disease-related RNA binding protein human antigen R (HuR), Wiley Interdisciplinary Reviews: RNA, 2020, 11, e1581.
3. J. König, et al.: Combining SELEX and the yeast three-hybrid system for in vivo selection and classification of RNA aptamers, RNA, 2007, 13, 614-622.
4. J. Abramson, J. Adler, J. Dunger, et al.: Accurate structure prediction of biomolecular interactions with AlphaFold 3, Nature, 2024, 630, 493–500.
5. R. Salomon-Ferrer, et al.: An Overview of the Amber Biomolecular Simulation Package, WIREs Computational Molecular Science, 2013, 3, 198–210.
6. J. Wang, et al.: Gaussian accelerated molecular dynamics: principles and applications, WIREs Computational Molecular Science, 2021, e1521.
7. D.A. Case, et al.: The Amber biomolecular simulation programs, Journal of Computational Chemistry, 2005, 26, 1668–1688.
8. M.J. Abraham, et al.: Gromacs: High performance molecular simulations through multi-level parallelism from laptops to supercomputers, SoftwareX, 2015, 1–2, 19–25.

Intrinsically Disorder Ensemble Tools (IDPET), A Python Package for Studying Conformational Ensembles of Disordered Proteins

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Intrinsically Disordered Proteins (IDPs) and Intrinsically Disordered Regions (IDRs) play critical roles in a range of biological and pathological processes across both prokaryotic and eukaryotic organisms. Unraveling the structures and dynamics of IDPs/IDRs can thus offer valuable insights into cellular functions such as cell signaling, division, circadian biology, genome maintenance, and cellular homeostasis. Due to the highly dynamic nature of IDPs/IDRs, which arises from their unique sequence compositions, they are best represented by conformational ensembles—collections of rapidly interconverting structures that are statistically weighted to reflect the intrinsic dynamics and significance of each conformation.(1)

Here, we present IDPET (Intrinsically Disordered Protein Ensemble Tool), a Python software package designed for the analysis and visualization of IDP/IDR structural ensemble features. IDPET enables simultaneous loading of multiple ensembles, allowing extraction of diverse local and global properties, alongside data visualization. It includes powerful tools for in-depth analysis, such as dimensionality reduction, clustering, and ensemble comparison techniques, and can retrieve data directly from relevant databases, like the Protein Ensemble Database (PED) (2), to streamline the analysis process.

IDPET facilitates detailed exploration of structural distributions within ensembles for the same protein or regions generated by different methods. Additionally, it includes statistical tools to detect similarities and differences between ensembles, providing a robust framework for comparative analysis. We demonstrate IDPET's capabilities across multiple example cases and have prepared comprehensive documentation to support users in harnessing the package's full potential.

References

1. Holehouse, Alex S., and Birthe B. Kragelund. "The molecular basis for cellular function of intrinsically disordered protein regions." Nature Reviews Molecular Cell Biology 25.3 (2024): 187-211.
2. Ghafouri, Hamidreza, et al. "PED in 2024: improving the community deposition of structural ensembles for intrinsically disordered proteins." Nucleic acids research 52.D1 (2024): D536-D544.

EPS-urine derived extracellular vesicles: multi-omics profiling for prostate cancer risk stratification

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Introduction: The need for novel biomarkers to improve the performance of available tests for prostate cancer (PCa) diagnosis remains critical (1). Extracellular vesicles (EVs) have emerged as promising tools for improving PCa diagnosis and patient stratification. While extensive research has explored the protein composition of EVs isolated from expressed prostatic secretion (EPS)-urine (2), their metabolomic and lipidomic profile is yet to be fully understood. This study seeks to address this knowledge gap through an untargeted LC-MS/MS metabolomic and lipidomic analysis of EVs derived from EPS-urine samples of PCa patients.

Methods: EVs were isolated from the EPS-urine of 42 prostate cancer (PCa) patients, categorized into high-risk (HR) PCa (n=21) and low-risk (LR) PCa (n=21) according to EAU guidelines. Size analysis and classical protein marker identification were performed using nanoparticle tracking analysis (NTA) and Western blotting, respectively. A global extraction protocol was used to isolate metabolites and lipids, which were analysed by LC-MS/MS. Data Independent Acquisition (DIA) and Data Dependent Acquisition (DDA), implemented with Hermes, were combined (3). Omics data were integrated using the DIABLO model from the MixOmics R package.

Results: Separate single-omics analyses revealed 287 lipids and 89 metabolites. Enrichment analysis underscored the significance of amino acids synthesis, along with sphingolipid and glycerophospholipid metabolism. Furthermore, a notable reduction in sphingolipids in HR patients compared to LR patients (p 0.8) capable of distinguishing HR from LR PCa patients. By integrating the results from the t-test and DIABLO output with multivariate ROC analysis we constructed an optimized panel of 7 key features, demonstrating a potential robust diagnostic performance (AUC = 0.875; accuracy = 78.1%).

Conclusions: This multi-omics analysis unveiled significant lipidomic and metabolomic alterations associated with PCa risk stratification. The use of DIABLO model data demonstrated a robust correlation between lipidomic and metabolomic profiles, facilitating the identification of key features for distinguishing HR from LR prostate cancer.

References

1. Eskra, J. N., Rabizadeh, D., Pavlovich, C. P., Catalona, W. J., & Luo, J. (2019). Approaches to urinary detection of prostate cancer. In Prostate Cancer and Prostatic Diseases (Vol. 22, Issue 3, pp. 362–381). Nature Publishing Group. https://doi.org/10.1038/s41391-019-0127-4
2. Principe, S., Jones, E. E., Kim, Y., Sinha, A., Nyalwidhe, J. O., Brooks, J., Semmes, O. J., Troyer, D. A., Lance, R. S., Kislinger, T., & Drake, R. R. (2013). In-depth proteomic analyses of exosomes isolated from expressed prostatic secretions in urine. Proteomics, 13(10–11), 1667–1671. https://doi.org/10.1002/pmic.201200561

3. Giné, R., Capellades, J., Badia, J. M., Vughs, D., Schwaiger-Haber, M., Alexandrov, T., Vinaixa, M., Brunner, A. M., Patti, G. J., & Yanes, O. (2021). HERMES: a molecular-formula-oriented method to target the metabolome. Nature Methods, 18(11). https://doi.org/10.1038/s41592-021-01307-z

Integration of mass spectrometry and mass cytometry imaging for spatially resolved single cell profiling

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Background: Spatial mapping tools offer an approach to study tissue complexity and heterogeneity in (patho)physiology. However, due to specific platform-to-platform incompatibility, protocols that maximize output from a single tissue section are lacking. Here, we present a workflow for the sequential imaging of archival material using matrix assisted laser desorption ionization (MALDI) and multiplexed ion beam imaging (MIBI). MnM enables the acquisition of untargeted molecular composition (MALDI) and targeted single-cell phenotype (MIBI) from a single tissue section.

Methods: FFPE sections from lymphatic tissues were sectioned onto a new organic polymer-coated slide (MIBIblue). The tissue was first imaged with a MALDI. The matrix was washed off and sections were stained with a mix of metal-labelled antibodies targeted to immune phenotypic markers. Data was acquired with a MIBIscope and sections were counterstained with H&E. Bilinear interpolation was used for pixel up-sampling of the MALDI dataset to match the MIBI pixel size. Alignment of the two modalities into a shared coordinate system was achieved by registration between MALDI-H&E and MIBI-H&E using the H&E as ground truth. Registered MnM imaging data were combined into a single dataset where each pixel retains bimodal information from N-glycans and MIBI probes.

Results: Here, we show that dual modality MALDI and MIBI (MnM) imaging is possible on the same tissue section, demonstrating the proof of concept of human lymphatics. The number of ionized species and N-glycan annotations were comparable between the two slides, indicating that MIBIblue can be used for N-glycan MALDI imaging. Then, we used MIBI to measure the expression of metal-tagged immune markers across the tissue area previously rastered with MALDI. MIBI imaging data from MALDI-treated regions were comparable to MALDI-free areas, suggesting that laser ablation by MALDI does not affect MIBI acquisition.

Conclusion: We present a novel sequential imaging and registration strategy enabling mass spectrometry imaging by MALDI, high-definition spatial proteomics by MIBI and traditional H&E on the same tissue section.

Multi-omics data analysis to characterize age-specific metabolic shifts during embryo development

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Background: Recent findings suggest that cell metabolism may be key to understanding miscarriages and developmental abnormalities in early embryonic development [1-6]. To explore metabolic differences between pre- and post-implantation embryonic cells, we analyzed single-cell RNA-seq data from various 3D culture models, integrating data from two studies: one using Inner Cell Mass Cells [7] and another using human Embryonic or induced Stem Cells [8]. Using metabolic network reconstructions, constraint-based methods [9], and machine learning techniques [10], we estimated metabolic fluxes and assessed shifts in cellular metabolic activity.

Methods: Metabolic fluxes are calculated using COBRA-based models. Transcriptomic data are preprocessed and denoised using the MAGIC imputation algorithm. The processed data are used to calculate Reaction Activity Scores (RAS), which, along with Flux Variability Analysis (FVA) results, define the metabolic model constraints. Metabolomics data constrain exchange reactions before flux calculation. The model is sampled using the Constrained-Based Sampling (CBS) technique with randomly fixed objective functions. The fluxes are averaged to generate a flux distribution representing the metabolic activity of each cell.

Results: Preliminary findings revealed significant variations in biomass production and glucose consumption between stages. However, inconsistencies across experiments indicate the need for further investigations.

The same analyses can now be performed using COBRAxy, a Galaxy-based tool that extends the Marea platform [11]. COBRAxy enables users to analyze cellular metabolism using transcriptomic and metabolomic data, comparing gene expression and metabolite differences through the Reaction Activity Score (RAS) and Reaction Propensity Score (RPS), visualized on a metabolic map. It also allows users to calculate metabolic fluxes and compare these across different cell groups, offering insights into metabolic changes during development.

Conclusion: This project aims to develop dynamic metabolic maps by integrating multi-omics data, including transcriptomics, proteomics, and exo-metabolomics, to study stage- and sex-specific metabolic changes during human embryonic development. Methods such as single-cell Flux Balance Analysis (scFBA), solution space sampling, and geometric neural networks will be employed to characterize metabolic shifts across pre-, peri-, and post-implantation stages. This integrative approach could provide valuable insights into the metabolic landscape of early development, enhancing our understanding of growth anomalies and embryonic viability.

References

1. Mangu, SVVS Ravi, et al. "Maternal high-cholesterol diet negatively programs offspring bone development and downregulates hedgehog signaling in osteoblasts." Journal of Biological Chemistry 298.9 (2022).
2. Blom, Henk J., et al. "Neural tube defects and folate: case far from closed." Nature Reviews Neuroscience 7.9 (2006): 724-731.
3. Winkel, Louise, et al. "Fetal Programming of the Endocrine Pancreas: Impact of a Maternal Low-Protein Diet on Gene Expression in the Perinatal Rat Pancreas." International Journal of Molecular Sciences 23.19 (2022): 11057.
4. Takashima, Yasuhiro, et al. "Resetting transcription factor control circuitry toward ground-state pluripotency in human." Cell 158.6 (2014): 1254-1269.

5. Dearden, Laura, Sebastien G. Bouret, and Susan E. Ozanne. "Sex and gender differences in developmental programming of metabolism." Molecular metabolism 15 (2018): 8-19.
6. Schulz, Edda G., et al. "The two active X chromosomes in female ESCs block exit from the pluripotent state by modulating the ESC signaling network." Cell stem cell 14.2 (2014): 203-216.
7. Molè, Matteo A., et al. "A single cell characterisation of human embryogenesis identifies pluripotency transitions and putative anterior hypoblast centre." Nature communications 12.1 (2021): 3679.
8. Zorzan, Irene, et al. "TGF-beta dynamically controls epithelial identity in a 3D model of human epiblast." bioRxiv (2023): 2023-12.
9. Damiani, Chiara, et al. "Integration of single-cell RNA-seq data into population models to characterize cancer metabolism." PLoS computational biology 15.2 (2019): e1006733.
10. Alghamdi, Norah, et al. "A graph neural network model to estimate cell-wise metabolic flux using single-cell RNA-seq data." Genome research 31.10 (2021): 1867-1884.
11. Damiani, Chiara, et al. "MaREA4Galaxy: Metabolic reaction enrichment analysis and visualization of RNA-seq data within Galaxy." Computational and structural biotechnology journal 18 (2020): 993-999.

Molecular determinants of response to neoadjuvant chemotherapy in esophageal adenocarcinoma at single-cell spatial resolution

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Background: Standard of care for locally advanced esophageal adenocarcinoma (EAC) is neoadjuvant chemotherapy (nCT) followed by surgery. However, less than 30% of treated patients achieve a pathological complete response associated with better survival. Understanding the mechanisms of response to nCT is pivotal to better stratify patients and inform therapies. Our preliminary analysis [1] on treatment naive EAC tumor and blood indicates a correlation between pre-existing immunity and response to nCT and molecular pathways of resistance that may be targeted to improve clinical outcomes.

Methods: We are now investigating in-depth the biological mechanisms correlated to nCT response by single-cell spatial transcriptomics (CosMX spatial molecular imager) on treatment-naïve EAC biopsies of n=6 patients, comparing n=3 complete responders (CR) versus n=3 non responders (NR), with 1000x panel of human transcripts for cell typing, cell state, ligand-receptor interactions and hormone activities.

Results: We defined clusters of tumor cells, adjacent healthy squamous epithelial cells, stromal cells (the majority fibroblasts and endothelial cells), and immune cells (macrophages, B and T lymphocytes, plasmacells). We identified also niches characterized by different cellular compositions.

Tumor cells of NR tumors showed an enrichment of genes related to proliferation, hypoxia, tumor necrosis factor and angiogenesis. Moreover, NR showed an enrichment of protumoral M2 TAM and inflammatory CAF, promoting inflammation and tumor growth.

Notably, B lymphocytes and plasma cells, potentially contributing to the anti-tumoral immune response, were found at higher frequencies in CR esophageal adenocarcinomas (EACs). According to neighbor analysis, these type of are more in contact in CR with tumor cells. Finally, intercellular communication analysis revealed distinct ligand-receptor interactions and neighboring patterns between tumor and microenvironment cell types in CR and NR samples.

Conclusions: Collectively, these results suggest an overall association between nCT response and active immune infiltration, increased cell proliferation, and vulnerability to chemotherapy. Further differential expression analysis for cell populations, and their correlation with spatial location (nearest neighbour), will allow to identify novel molecular mechanisms associated with nCT resistance.

References

1. Preexisting immunity drives the response to neoadjuvant chemotherapy in esophageal adenocarcinoma, Arbore G. et al., Cancer Research (2023).

Exploring the molecular mechanisms that influence Retinoic Acid responsiveness or resistance in cancer stem cells within patient-derived cancer organoids.

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Retinoic acid (RA), a potent derivative of vitamin A, is crucial for regulating cell growth and differentiation during early stages of embryonic development. However, as cancer progresses, its cellular traits shift, displaying less differentiation and increasingly stem cell-like characteristics, which contribute to tumor growth and resistance to treatment. Despite extensive research, the precise impact of RA signaling on cancer growth arrest and progression remains unclear.

RA mediates its effects by binding to nuclear receptors called retinoic acid receptors (RARs), which target specific regions within genes known as RA response elements (RAREs). These RAREs generally consist of direct repeats of a core motif "(A/G)G(G/T)TC(A/G)" and are classified according to spacing as DR0, DR1, DR2, and DR5. Studies suggest that in both embryonic development and tumor microenvironments, some cells resist differentiation cues, preserving stem-like characteristics that make them challenging to treat.

Our objective is to identify transcription factors (TFs) regulated by RA within cancer stem cell environments. We achieve this by using computational algorithms to identify RAREs in promoter sequences, focusing on different DR classifications. Next, we integrate an in silico Gene Regulatory Network (GRN) model of cancer stem cells with Master Regulator Analysis (MRA) to pinpoint critical RA-responsive TFs, known as master regulators (MRs), that influence transcriptional changes associated with cancer.

Given that ligand-dependent transcription factors regulate gene networks responsible for differentiation and self-renewal, RA-responsive MRs are potentially valuable targets for cancer therapy. By modulating these MRs and applying single-cell RNA sequencing (scRNA-seq) in patient-derived cancer organoids (PDOs), we aim to identify cellular subpopulations with differential sensitivity to RA. This will deepen our understanding of RA's role in cancer and highlight new avenues for targeted therapies.

References

1. Meier AB, Zawada D, De Angelis MT, et al. Epicardioid single-cell genomics uncovers principles of human epicardium biology in heart development and disease. *Nat Biotechnol*. 2023;41(12):1787-1800. doi:10.1038/s41587-023-01718-7.
2. Zawada D, Kornherr J, Meier AB, et al. Retinoic acid signaling modulation guides in vitro specification of human heart field-specific progenitor pools. *Nat Commun*. 2023;14(1):1722. Published 2023 Apr 3. doi:10.1038/s41467-023-36764-xdoi:10.1038/s41556-022-00899-8.
3. Parrotta EI, Scalise S, Taverna D, et al. Comprehensive proteogenomic analysis of human embryonic and induced pluripotent stem cells. *J Cell Mol Med*. 2019;23(8):5440-5453. doi:10.1111/jcmm.14426.
4. De Angelis MT, Parrotta EI, Santamaria G, Cuda G. Short-term retinoic acid treatment sustains pluripotency and suppresses differentiation of human induced pluripotent stem cells. *Cell Death Dis*. 2018;9(1):6. Published 2018 Jan 5. doi:10.1038/s41419-017-0028-1.

Exploring synthetic SIL1 protein reintroduction to treat Marinesco-Sjogren Syndrome.

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Introduction: Marinesco-Sjogren Syndrome (MSS) is a rare autosomal recessive disease caused by alterations in the SIL1 gene, encoding a nucleotide exchange factor for the ER chaperone BiP (1,2). Individuals with MSS display a complex phenotype, with cerebellar ataxia, progressive myopathy, and early-onset congenital cataracts amongst other symptoms. A spontaneous recessive mutation in the mouse Sil1 gene results in cerebellar ataxia and muscular atrophy, with the woozy (wz-/-) mouse representing a valid preclinical model to investigate MSS and explore pharmacotherapeutic approaches (3). We here analysed the adenovirus-mediated administration of an engineered human SIL1 protein in wz-/- mice, investigating whether the treatment could act on the involved tissues and reestablish the healthy phenotype.

Methods: An AAV8-carried human SIL1 was IV injected in the tail of 5-week-old wz-/- mice. The beam walking and rotarod tests were weekly used to test mice's muscular strength and motor coordination until the 12th and 14th week, respectively. Mice were sacrified 4 and 9 weeks after the treatment, and tissues were collected for molecular analysis. Both virus' genome copies and human SIL1 protein quantification were performed in different tissues.

Results: Both the rotarod and beam walking tests revealed a slower manifestation of the pathological phenotype in the AAV-treated mice compared to the control group (saline), with an increased latency to fall and a reduced contralateral foot falls in the rotarod and beam walking tests. The molecular analysis revealed the presence of human SIL1 in the quadriceps of treated mice, one of the most involved tissues in the disorder, confirming the ability of the purified protein to reach the peripheral tissues.

Conclusions: The presence of human SIL1 in the quadriceps of woozy mice confirmed the ability of the protein to reach tissues mainly affected by the syndrome. The AAV-mediated protein reintroduction seems to be a potential treatment to compensate for the protein deficit in the tissues mainly involved in the pathology. Further studies are needed to deepen the pharmacokinetics of the engineered protein and whether this treatment may act by regressing the affected phenotype or slowing down the course of MSS.

References

1. Anttonen AK, Mahjneh I, Hämäläinen RH, et al. The gene disrupted in Marinesco-Sjögren syndrome encodes SIL1, an HSPA5 cochaperone. Nat Genet. 2005;37(12):1309-1311. doi:10.1038/ng1677
2. Ezgu F, Krejci P, Li S, et al. Phenotype-genotype correlations in patients with Marinesco-Sjögren syndrome. Clin Genet. 2014;86(1):74-84. doi:10.1111/cge.12230
3. Zhao L, Longo-Guess C, Harris BS, Lee JW, Ackerman SL. Protein accumulation and neurodegeneration in the woozy mutant mouse is caused by disruption of SIL1, a cochaperone of BiP. Nat Genet. 2005;37(9):974-979. doi:10.1038/ng1620.

Two new members of Ros/MucR family: MucR2 and MucR3 from Sinorhizobium meliloti

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Ros/MucR proteins, expressed in alpha-proteobacteria comprising symbiotic and pathogenic species, regulate the expression of symbiotic and virulence genes (1). These proteins are characterized by an N-terminal oligomerization domain (NTD) and a C-terminal DNA-binding domain (DBD), which can fold around a zinc ion or in absence of any metal as in the case of Ml5 from M. loti (1, 2). In all the Ros/MucR proteins, the NTD allows the formation of higher-order oligomers and the DBD binds AT-rich DNA sequences. All these features are typical of Histone-like Nucleoid Structuring (H-NS) proteins playing the role to condense the nucleoid (1). By combing cryo-EM, NMR and AlphaFold2 modeling, we showed that two prototypes of the family form circular oligomers shaped in a truncated cone structure formed by NTDs of the monomers connected by flexible linkers to DBDs protruding from the wider rim of the truncated cone (fig.1). Structural and functional studies led to classify Ros/MucR proteins as a new sub-family of H-NS- like proteins .

In Sinorhizobium meliloti, MucR (MucR1) regulates the expression of genes involved in symbiosis with the plant host. We identified by mass spectrometry two new Ros/MucR family members, MucR2 and MucR3, sharing a high sequence identity with MucR1, but showing a prolonged N-terminus (MucR2) or a prolonged C-terminus (MucR3). Both MucR2 and MucR3 differs from MucR1 in the zinc coordination sphere. Key residues for oligomerization are conserved in MucR2 and MucR3 NTD, with the exception of Thr64 substituting a valine playing a role in MucR1 oligomerization. We demonstrated that both MucR2 and MucR3 can bind AT-rich DNA sequences. MucR2 has a lower affinity than MucR3, which has a lower affinity than MucR1 to bind the same DNA target. Light scattering (LS) analysis shows MucR2 cannot form high-order oligomers. To investigate the structural elements impairing oligomerization of MucR2, we produced a deleted version of this protein lacking the prolonged N-terminus. LS analysis shows that the lack of the prolonged N-terminus does not restore the ability to oligomerize. Work in progress is LS analysis for other MucR2 mutants and for MucR3. Finally, we will carry out NMR studies on the DBDs.

References

1. Baglivo, I., Malgieri, G., Roop, R.M., Barton, I.S., Wang, X., Russo, V., Pirone, L., Pedone, E.M. and Pedone, P.V. (2024) MucR protein: Three decades of studies have led to the identification of a new H-NS-like protein. Mol Microbiol.
2. Baglivo, I., Russo, L., Esposito, S., Malgieri, G., Renda, M., Salluzzo, A., Di Blasio, B., Isernia, C., Fattorusso, R. and Pedone, P.V. (2009) The structural role of the zinc ion can be dispensable in prokaryotic zinc-finger domains. Proc Natl Acad Sci U S A, 106, 6933-6938.

Early biochemical alterations of the endocannabinoid system in the retina of Alzheimer's disease mice

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Background: The retina is considered as a "window to the brain" due to the onset of extra-cerebral manifestations of brain diseases, including Alzheimer's disease (AD). Recently, dysregulation of the endocannabinoid (eCB) system (ECS) in AD brain has been observed. Here, we explored the possible alterations of ECS and the onset of gliosis in the retina of AD-like mice at an early stage of AD phenotype (before the onset of hippocampal β-amyloid plaques).

Methods: Tg2576 (TG) mice over-expressing the amyloid precursor protein (APP) were used. Analysis of receptors and enzymes of the ECS was performed through Western-Blotting (WB) and immunofluorescence (IF), and endogenous levels of major eCBs, 2-arachidonoylglycerol (2-AG) and anandamide (AEA), were quantified through ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS). β-amyloid plaques in brain and retina were investigated through immunohistochemistry on cryosections. Oxidative stress was quantified as mean of Acrolein fluorescence intensity and retinal degeneration was assessed by analysing retinal thickness. Excitotoxicity was evaluated by quantifying glutamate using a bioluminescent Glutamate dehydrogenase/NADH detection system.

Results: At the age of 12 months, TG retinas displayed up-regulation of cannabinoid receptor 2 (CB2) and of monoacylglycerol lipase (MAGL), the enzyme responsible for the degradation of 2-AG. Consistently, UPLC–MS/MS demonstrated a significant reduction of 2-AG in TG retinas, while a trend toward increase was found for the other eCB AEA. No statistically significant differences were found for the other enzymes/ receptors of the ECS under study. However, linear regression analysis for individual animals showed a significant correlation between CB2 and fatty acid amide hydrolase (FAAH), diacylglycerol lipase α/β (DAGLα/β), and APP; instead, a significant negative correlation was found between MAGL and APP. Moreover, TG retinas showed a significant increase in the number of IBA1 (+) microglia cells. Gliosis was not associated with hippocampal or retinal β-amyloid plaques, evident retinal degenerative signatures or excitotoxicity; instead, oxidative stress burden was observed as increased acrolein levels.

Conclusion: Overall, our data indicate that ECS dysregulation – in particular of CB2, MAGL and 2-AG – and microglia reactivity in the retina of AD-like mice are early events of the pathology, occurring well before the development of hippocampal β-amyloid plaques.

References

Tisi, A., Scipioni, L., Carozza, G., Di Re, L., Cimino, G., Di Meo, C., Palaniappan, S., Valle, F. D., Fanti, F., Giacovazzo, G., Compagnone, D., Maccarone, R., Oddi, S., & Maccarrone, M. (2024). Alterations of endocannabinoid signaling and microglia reactivity in the retinas of AD-like mice precede the onset of hippocampal β-amyloid plaques. Journal of Neurochemistry, 00, 1–15. DOI: 10.1111/jnc.16256 (in production).

OCTN1 (SLC22A4) displays two different transport pathways for organic cations or zwitterions

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Background: OCTN1 (SLC22A4) is a polyspecific transporter able to mediate the transport of many organic cations, among which the cation prototype TEA (tetraethylammonium) and the neurotransmitter acetylcholine, but also zwitterions, such as carnitine and the mushroom metabolite ergothioneine. Many functional aspects of OCTN1, as well as it's physiological significance, are still not understood.

Methods: In this work, the in vitro experimental model of proteoliposomes reconstituted with the recombinant human OCTN1 was used to we investigate OCTN1-mediated uptake mechanisms of two model substrates: the cation TEA and the zwitterion carnitine. A computational analysis of OCTN1-mediated uptake of TEA and carnitine was also exerted.

Results: Transport assays, performed using [14C]-TEA or [3H]-Carnitine, revealed that TEA and carnitine did not inhibit each other. The effect of Na+ or Benzyltriethylammonium on [14C]-TEA or [3H]-Carnitine uptake was tested, revealing that the two compounds only inhibited TEA transport. Differently, carnitine transport was stimulated by 75 mM Na+. The homology model of OCTN1 was built using the human OCT3 structure as a template. The obtained structure was used to perform docking analysis, revealing that TEA, Na+, and carnitine interact with E381 in the OCTN1 substrate site. In the presence of Na+, only carnitine is able to enter the transporter binding site, interacting with R469.

Conclusions: Experimental and computational evidences are in line with the existence of two transport pathways for cations and zwitterions. This work sheds new light into the structure/function relationships of the transporter OCTN1, widening the knowledge about OCTN1 role in body homeostasis. Furthermore, the knowledge of the molecular determinants for substrate binding can be exploited for drug design and drug screening. [1]

References

1. L. Pochini, F. Barone, L. Console, C. Brunocilla, M. Galluccio, M. Scalise, C. Indiveri, OCTN1 (SLC22A4) displays two different transport pathways for organic cations or zwitterions, Biochim Biophys Acta Biomembr, 1866 (2024) 184263. 10.1016/j.bbamem.2023.184263

Shwachman-Diamond Syndrome: different signalling pathways driving chronic inflammation

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Background: Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive disorder. In many cases, SDS is caused by mutations in the Shwachman-Bodian-Diamond syndrome (SBDS) gene that codes for the SBDS protein, involved in ribosome biogenesis. For this reason, SDS is considered a ribosomopathy and, as such, its manifestations fall on a systemic level. What most compromises the quality of life of patients, however, consists of bone marrow failure that often degenerates into myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML) which represent the main causes of death [1, 2]. In addition, recent studies demonstrate how SBDS is involved in important signaling pathways, such as those of Signal Transducer and Activator of Transcription (STAT)-3 and Mammalian Target of Rapamycin (mTOR) [3, 4]. Between its roles, STAT3 regulates the transcription of inflammatory markers, including IL-6 which in turn may activate the STAT3 pathway generating a positive, self-perpetuating loop, often found to be overactivated in AML [4].

Methods: Control and SDS-derived samples were analyzed through flow cytometry and Bio-Plex assay to quantify the expression of both phosphoproteins and inflammatory markers supposed to be involved in SDS pathophysiology. Bio-Plex assay was also performed on SDS-derived primary cell cultures treated with Everolimus, an mTOR inhibitor currently used in cancer treatment [5], to evaluate the direct involvement of mTOR in SDS chronic inflammation.

Results: We confirmed mTOR and STAT3 hyperactivation in SDS, along with the dysregulation of two associated phosphoproteins, ERK1/2 and AKT.

Additionally, we detected elevated levels of various soluble inflammatory markers in SDS-derived samples: G-CSF, IL-6, IL-8, IL-12, and MIP-1α.

Finally, the in vitro treatment with Everolimus demonstrated a general reduction of various inflammatory factors, including IL-6, confirming the implication of mTOR in the SDS-related chronic inflammation [6].

Conclusions: The dysregulated levels of ERK1/2 and AKT indicate their potential role in amplifying mTOR and STAT3 hyperactivation, contributing to SDS pathophysiology. In addition, our results suggest that chronic inflammation in SDS may be a major driver of leukemogenesis. However, treatment with Everolimus demonstrates that targeting molecules within these signaling pathways can disrupt the previously described self-perpetuating inflammatory loop, thereby interfering with the pathogenic mechanisms of SDS.

References

1. Kawashima N, Oyarbide U, Cipolli M, Bezzerri V, Corey SJ. Shwachman-Diamond syndromes: clinical, genetic, and biochemical insights from the rare variants. Haematologica. 2023 Oct 1;108(10):2594-2605.
2. Boocock GR, Morrison JA, Popovic M, et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. Nat Genet. 2003;33(1):97-101.
3. Bezzerri V, Vella A, Calcaterra E, et al. New insights into the Shwachman-Diamond Syndrome-related haematological disorder: hyper-activation of mTOR and STAT3 in leukocytes. Sci Rep. 2016;6:33165.
4. Vella A, D'Aversa E, Api M, et al. mTOR and STAT3 Pathway Hyper-Activation is Associated with Elevated Interleukin-6 Levels in Patients with Shwachman-Diamond Syndrome: Further Evidence of Lymphoid Lineage Impairment. Cancers (Basel). 2020;12(3):597.
5. Moreau-Bachelard C, Robert M, Gourmelon C, et al. Evaluating everolimus for the treatment of breast cancer. Expert Opin Pharmacother. 2023;24(10):1105-1111.
6. Sabbioni G., D'Aversa E., Breveglieri G., et al. Constitutive systemic inflammation in Shwachman-Diamond Syndrome. Submitted to Molecular Medicine.

Alpha&ESMhFolds: a web server for comparing 42,942 AlphaFold2 and ESMFold models of the human reference proteome

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Background: Results from CASP15 confirm the relevance of AI-based modelling on the accuracy of protein structure prediction. The best performances are reported by different methods based on DeepMind's AlphaFold2. Alternatively, methods such as ESMFold take advantage of Protein Language Models, allowing for faster computations. To compare the two approaches, we develop a novel database storing AlphaFold2 and ESMFold models for 42,942 proteins covering the Human Reference Proteome.

Methods: We collect 42,942 proteins from the human Reference Proteome available at UniProt. For 2,900 of them, we additionally collect PDB structures with high coverage to the UniProt sequence. We adopt Foldseek to evaluate the structural similarity between paired models, computing a similarity metric called TM-score.

Results: When comparing pairs of models to the experimental structure, in 81% of the available 2,900 proteins we observe that AlphaFold2 and ESMFold perform similarly, with TM-scores differing by less than 0.1. For the remaining proteins, AlphaFold2 tends to perform better than ESMFold. This is expected, as the first method retrieves known templates during the prediction phase.

On the remaining 40,042 proteins, we compare the similarity between the two predicted models to their self-assessed quality, as measured by the pLDDT. The two measures are strongly correlated, with pLDDT values decreasing at decreasing TM-score. We identify a region of 23,440 proteins having model-to-model TM-score < 0.6, in which no method is clearly superior and either one should be adopted more carefully. It is especially in this region that the ability to carefully compare different predicted structures can help to identify high-quality models to be adopted for further analysis.

Conclusions: Our database is freely accessible as a web server at https://alpha-esmhfolds.biocomp.unibo.it/, allowing users to easily compare models for 42,942 human proteins. From the Home page, it is possible to search a protein of interest. For all entries, the corresponding page shows multiple information, including general protein data, the structure superimposition between the AlphaFold2 and ESMFold models, the sequence alignment, and several statistics based on the quality of each model and their similarity. When a PDB structure is available, each model is similarly compared to the experimental structure.

References

Matteo Manfredi, Castrense Savojardo, Georgii Iardukhin, Davide Salomoni, Alessandro Costantini, Pier Luigi Martelli, Rita Casadio, Alpha&ESMhFolds: A Web Server for Comparing AlphaFold2 and ESMFold Models of the Human Reference Proteome, Journal of Molecular Biology, Volume 436, Issue 17, 2024, 168593, ISSN 0022-2836, https://doi.org/10.1016/j.jmb.2024.168593.

Bionformatic neoantigens prediction from Next generation sequencing data

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Neoantigens are tumor specific proteins arising from various mechanisms like somatic mutations and transcriptomic alterations. They are not present in healthy cells, so they could represent elective targets for cancer immunotherapy triggering T-cell activation. However, mutations and neoantigens are strictly individual and their identification requires a combination of high throughput omics bioinformatic analysis. To address to these challenges, the aim of our study is the development of a bioinformatic pipeline for neoantigens prediction. It is structured in three main parts: variant calling, HLA typing, MHC binding prediction. Currently, our case study are 15 patients with Pancreatic Neuroendocrine Tumors (PNETs). We perform a comparative analysis of Whole Exome Sequencing from PNETs tissues and PBMCs to identify somatic mutations. BWA MEM-aligned reads to hg38 has been subjected to rigorous quality filtering with a focus on retaining only those with a mapping quality>=60. To enhance reliability of somatic variant calls we use a combination of three variant callers: Mutect2, Strelka2, Varscan2. Only variants identified by three VC with a minimum coverage of 10X are considered. Each patient has an average of 20 missense mutations predominantly located in same genes across patients, found not only in well-known drivers genes such as DAXX, but also in other genes with potential biological relevance. In the second step, we use HLAscan tool, which utilizes IMGT database to genotype HLAI and HLAII loci on chromosome 6. Neoantimon R package is used to predict a list of potential peptides based on their binding affinity with MHC Class I and II, classifying them into strong binders with a Rank Threshold (RT) below 0.5% and weak binders with RT<2.0%. Currently, we have integrated RNA-seq data from tumor transcriptome with the corresponding gene expression levels in TPM to prioritize peptides with a higher likelihood of being expressed. Furthermore, we are exploring transcriptomic alterations in process such as RNA editing and alternative splicing as additional sources of neoantigens. Computationally predicted neoantigens that demonstrate the ability to stimulate T cells derived patients in vitro could be considered for immunotherapeutic strategies including CAR-T cells or cancer vaccines offering novel therapeutic alternatives to traditional cancer treatments.

References

1. Xie, N., et al. "Neoantigens: Promising Targets for Cancer Therapy." Signal Transduction and Targeted Therapy, vol. 8, 2023, p. 9.
2. Huang, R., et al "DNA Damage Repair: Historical Perspectives, Mechanistic Pathways and Clinical Translation for Targeted Cancer Therapy." .Signal Transduction and Targeted Therapy, vol. 6, no. 1, 9 July 2021, p. 254.
3. Gurung, H. R., et al. "Systematic Discovery of Neoepitope-HLA Pairs for Neoantigens Shared Among Patients and Tumor Types." Nature Biotechnology, vol. 42, no. 7, July 2024, pp. 1107-1117.
4. Ka, S., et al. "HLAscan: Genotyping of the HLA Region Using Next-Generation Sequencing Data." BMC Bioinformatics, vol. 18, no. 1, 12 May 2017, p. 258.
5. Hasegawa, T., et al. "Neoantimon: A Multifunctional R Package for Identification of Tumor-Specific Neoantigens." Bioinformatics, vol. 36, no. 18, 15 Sept. 2020, pp. 4813-4816.

Simulation of bacteria interaction networks: from topology to species abundances

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Background: Microorganisms frequently coexist and establish complex relationships within their environments. Recent advancements in high-throughput 16S rDNA sequencing techniques have significantly improved our capacity to explore the factors that influence bacterial community organization. However, despite the development of numerous inference methods, the lack of a well-established biological truth presents challenges in validating the obtained results. Therefore, in-silico solutions are critical for simulating gold standards.

Methods: We introduce N2SIMBA, a modular algorithmic approach that begins with a known directed and weighted graph, which is assumed to represent microbial interactions. Assuming that microbial interactions are mediated by produced and consumed metabolites, this graph is used to generate the rules of a Microbial Consumer Resource Model (Goldford et al., 2018). This model describes the bacterial community dynamic evolution through a set of Ordinary Differential Equations (ODEs). We also simulate the sequencing process leveraging on a previously published tool, metaSPARSim (Patuzzi et al., 2019).

Results: There is no clear consensus in the literature regarding which network topology best describes microbial interactions, although the scale-free model is often suggested. Notably, N2SIMBA accepts an adjacency matrix as input, allowing to simulate any microbial interaction network whose topology is provided. Results indicate that the N2SIMBA generates count data of realistic distribution. Specifically, as network parameters vary, the distribution of species abundances mirrors the topology of the network used to model the microbial community. In particular, the simulation of varying sizes of microbial interaction network modeled with a scale-free network topology under rich-environment conditions leads to a power-law distribution of species abundances, with the most interconnected species thriving and the others contributing only marginally to the bacterial community as individuals.

Conclusions: The modular nature of N2SIMBA allows the investigation of the effect of different network and environmental parameters on bacterial community evolution. Thus, offering a robust methodology for systematically evaluating network inference methods using simulated bacterial communities.

References

1. Marsland R, Cui W, Goldford J, Mehta P. The Community Simulator: A Python package for microbial ecology. PLOS ONE. 2020 03;15(3):1–18
2. Marsland III R, Cui W, Goldford J, Sanchez A, Korolev K, Mehta P. Available energy fluxes drive a transition in the diversity, stability, and functional structure of microbial communities. PLoS computational biology. 2019;15(2):e1006793.
3. Patuzzi I, Baruzzo G, Losasso C, Ricci A, Di Camillo B. metaSPARSim: a 16S rRNA gene sequencing count data simulator. BMC Bioinformatics. 2019;20.
4. Cappellato M, Baruzzo G, Di Camillo B. Investigating differential abundance methods in microbiome data: A benchmark study. PLoS Computational Biology. 2022;18(9):e1010467
5. Albert R, Barab´asi AL. Statistical mechanics of complex networks. Reviews of Modern Physics. 2002;74:47–97.

Biochemical characterization of the feedforward loop between CDK1 and FOXM1 in epidermal stem cells

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Epidermal stem cells orchestrate epidermal renewal and timely wound repair through a tight regulation of self-renewal, proliferation, and differentiation (1-3) . In culture, human epidermal stem cells (EPSCs) generate a clonal type referred to as holoclone (1), which give rise to transient amplifying progenitors (meroclone and paraclone-forming cells) eventually generating terminally differentiated cells (4). Leveraging single-cell transcriptomic data, we explored the FOXM1-dependent biochemical signals controlling self-renewal and differentiation in epidermal stem cells aimed at improving regenerative medicine applications. FOXM1 is one of the main players in this network (5), but the upstream signals regulating its activity remain to be elucidated.

Mass spectrometry identified CDK1 as a key hub in a stem cell-associated protein network, showing its upregulation and interaction with essential self renewal-related markers. Hampering CDK1 and FOXM1 activity by means of specific inhibitors and CRISPR-Cas9 technology, we pointed out CDK1 as one of the major kinases responsible for FOXM1 phosphorylation and stabilization. CDK1 phosphorylates FOXM1 at specific residues, stabilizing the protein and enhancing its nuclear localization and transcriptional activity, promoting self-renewal. Additionally, FOXM1 binds to the CDK1 promoter, inducing its expression. We identify the CDK1-FOXM1 feedforward loop as a critical axis sustaining EPSCs during in vitro cultivation. Understanding the upstream regulators of FOXM1 activity offers new insights into the biochemical mechanisms underlying self-renewal and differentiation in human primary keratinocytes.

References

1. Hirsch T, Rothoeft T, Teig N, Bauer JW, Pellegrini G, De Rosa L, et al. Regeneration of the entire human epidermis using transgenic stem cells. Nature. 2017;551(7680):327–32.
2. Pellegrini G, Golisano O, Paterna P, Lambiase A, Bonini S, Rama P, et al. Location and clonal analysis of stem cells and their differentiated progeny in the human ocular surface. J Cell Biol. 1999;145(4):769–82. 5.
3. Rochat A, Kobayashi K, Barrandon Y. Location of stem cells of human hair follicles by clonal analysis. Cell. 1994;76(6):1063–73.
4. Barrandon Y, Green H. Three clonal types of keratinocyte with different capacities for multiplication. Proc Natl Acad Sci U S A. 1987;84(8):2302–6.
5. Enzo E, Secone Seconetti A, Forcato M, Tenedini E, Polito MP, Sala I, et al. Single-keratinocyte transcriptomic analyses identify different clonal types and proliferative potential mediated by FOXM1 in human epidermal stem cells. Nat Commun. 2021;12(1):2505.

Glutamate enrichment in myelofibrosis tumor microenvironment induces mesenchymal stromal cells senescence by promoting intracellular fumarate accumulation.

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Background: Myelofibrosis (MF) is a BCR/ABL1 myeloproliferative disorder, characterized by JAK2V617F, MPL, and Calreticulin mutations, characterized by bone marrow fibrosis, splenomegaly, eventually shifting to acute myeloid leukemia (1).

Its pathogenesis is modulated by malignant clone synergism with tumor microenvironment (TME), where mesenchymal stromal cells (MSCs), usually supporting clonal hematopoiesis, gain senescent traits, enriching TME with several proinflammatory cytokines (2). This process is supported by metabolic reprogramming, which we aim to dissect in this work.

Methods: Using high-performance liquid chromatography (HPLC) we screened the metabolic profile of MF and healthy donors (HD). We established a healthy MSC in vitro model and a coculture system. We performed, specific assays to evaluate fumarate hydratase (FH) activity, quantitative PCR, western blot, different flow cytometry analysis, immunohistochemistry, and immunocytochemistry assays, and we assessed b-galactosidase activity to validate our in vitro models.

Results: HPLC analysis showed an accumulation of Glutamate in MF patients' sera (3). Glutamate might replenish the TCA cycle by transamination, a process leading to a-ketoglutarate formation. Therefore, we supplemented glutamate to a healthy MSCs in vitro model. qPCR analysis on different TCA cycle genes showed a significant upregulation of succinate dehydrogenase subunits, while FH expression was decreased. Corroborating this we detected intracellular fumarate accumulation and a concomitant decreased FH activity. Of note, intracellular fumarate accumulation enforced OXPHOS, exposing cells to robust oxidative stress. Corroborating this, glutamate and fumarate supplementation leads to ROS accumulation inducing senescence, as proved by b-galactosidase activity, and bone marrow fibrosis. Interestingly, it has been demonstrated that fumarate mediates chromatin methylation by TETs and KDM inhibition (4). Corroborating this, fumarate supplementation increases chromatin methylation in our in vitro model, evidence mirrored on primary MF MSC. Corroborating the incidence of TME on MF progression we showed similar outcomes by coculturing healthy MSCs with in vitro MF cell models.

Conclusion: Glutamate metabolism has a central role in PMF progression. According to our data, its conversion to fumarate is crucial for establishing an MSC senescent profile, eventually promoting the proinflammatory and fibrotic state characterizing PMF bone marrow. Further studies will be needed to unveil novel therapeutic strategies against PMF.

References

1. Mughal TI, Vaddi K, Sarlis NJ, Verstovsek S. Myelofibrosis-associated complications: pathogenesis, clinical manifestations, and effects on outcomes. Int J Gen Med. 2014;7:89-101.
2. La Spina E, Giallongo S, Giallongo C, Vicario N, Duminuco A, Parenti R, et al. Mesenchymal stromal cells in tumor microenvironment remodeling of BCR-ABL negative myeloproliferative diseases. Front Oncol. 2023;13:1141610.
3. Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E, et al. Glutaminolysis and Fumarate Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity. Cell Metab. 2016;24(6):807-19.
4. Wentzel JF, Lewies A, Bronkhorst AJ, van Dyk E, du Plessis LH, Pretorius PJ. Exposure to high levels of fumarate and succinate leads to apoptotic cytotoxicity and altered global DNA methylation profiles in vitro. Biochimie. 2017;135:28-34.

Addressing the Biological Interpretation of High-Dimensional Cell-Cell Communication Data from Single Cell Transcriptomics Through an Interactive Web Application

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Background: Biological systems rely on coordinated cell activity to adapt and respond to external stimuli. In multicellular organisms, cell-cell communication (CCC) is crucial for tissue and organ function, with disruptions resulting in pathological conditions. Therefore, understanding CCC is fundamental for comprehending biological systems' physiopathology[1].

In recent years, significant advances have been made in the computational inference of CCC from single-cell RNA sequencing data[2,3]. However, interpreting the biological implications of these analyses remains challenging, requiring both computational and biological expertise.

Methods: To address this, we introduce CClens, a web application designed for laboratory scientists to efficiently analyze predicted CCC data, without requiring programming skills. Implemented in R/Shiny[4], CClens uses reactive programming and is available as R package on GitLab (https://gitlab.com/sysbiobig/cclens). A docker image is also provided to ensure reproducibility. The package includes vignettes and case-studies datasets to familiarize users with the platform. It accepts input files in CSV, TSV, and RData formats from major CCC tools. Users can upload intercellular (ligand-receptor) and intracellular (downstream signal transduction activation) scores, statistical significance data, and user-defined scores for flexible CCC results analysis.

Results: CClens provides a comprehensive and user-friendly platform for visualizing CCC inference results from single or comparative biological conditions. The app guides CCC interpretation through three main sections: a broad global perspective, a cell-type centered analysis, and a molecular-level focus. A first panel allows interactive data filtering with custom thresholds for scores, and selections of specific cell types and molecules.

The first visualization panel offers a summary of ongoing communications, including interactive heatmaps, chord-diagrams, and network plots. Another tab focuses on single cell-types, showing their roles as either sender (ligand-expressing) or receiver (receptor-expressing). For molecular-level insights, interactive bubble plots display ligand-receptor interactions, allowing users to query specific communications of interest, as shown in figure.

Conclusions: Overall, CClens enhances the interpretation of CCC data by providing an interactive framework tailored to laboratory scientists. The platform's intuitive design and visualizations support detailed exploration of CCC dynamics, allowing researchers to focus on biologically significant interactions case-study specific. By facilitating in-depth analyses and flexible data querying, CClens empowers the formulation of data-driven hypotheses on CCC mechanisms.

References

1. Armingol, E., Officer, A., Harismendy, O. & Lewis, N. E. Deciphering cell-cell interactions and communication from gene expression. Nat. Rev. Genet. 22, 71–88 (2021).
2. Almet, A. A., Cang, Z., Jin, S. & Nie, Q. The landscape of cell-cell communication through single-cell transcriptomics. Curr. Opin. Syst. Biol. 26, 12–23 (2021).

3. Dimitrov, D. et al. Comparison of methods and resources for cell-cell communication inference from single-cell RNA-Seq data. Nat. Commun. 13, 3224 (2022).
4. Winston Chang and Joe Cheng and JJ Allaire and Carson Sievert and Barret Schloerke and Yihui Xie and Jeff Allen and Jonathan McPherson and Alan Dipert and Barbara Borges. shiny: Web Application Framework for R. (2023).

Role of Integrins in Maternal-Embryonic Communication: Proteomic Analysis of Early Pregnancy in Alpacas (Vicugna pacos)

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In South American camelids, a striking reproductive trait is that 98% of pregnancies occur in the left uterine horn (LUH), despite ovulations occurring almost equally from both ovaries. It is hypothesized that the right uterine horn (RUH) may be less supportive of pregnancy, forcing embryos originating from the right ovary to migrate to the LUH for implantation. Effective communication between the uterus and embryo during this period is critical for pregnancy establishment, yet the molecular mechanisms involved remain poorly understood. This study investigated the proteomic landscape of both uterine horns and pre-implantation embryos, just before implantation, to identify factors influencing maternal-embryo communication and implantation success. Three alpacas were mated and sacrificed on day 15 post-mating. Uterine horns and embryos were preserved in RNAlater, lysed in 4% SDS buffer, and proteins were analyzed using SDS-PAGE, in-gel digestion, and mass spectrometry (LC-MS/MS). The data obtained were processed using ProteomeDiscoverer, Perseus, Metascape, Proteomaps, and Cytoscape. We identified 1,728 proteins in the LUH, 1,983 in the RUH, and 510 in embryos. Proteins associated with implantation were significantly enriched across all samples, with key biological processes identified as reproductive function, growth, immune response, and locomotion. Among these, integrins were prominently expressed in both uterine horns and embryos. The embryo proteome showed the presence of integrins Alpha 6 (α6) and Beta 1 (β1). In endometrial samples, integrins αV, α1, α5, α6, β1, β2, and β4 were detected, with integrin β1 showing higher abundance. RT-PCR further confirmed the expression of ITGAV, ITGA1, ITGA5, ITGA6, ITGB1, and ITGB3 in all samples. Integrin sequences were aligned with human homologs using T-Coffee, and a phylogenetic tree was constructed. Structural domains, conserved motifs, and ligand-binding regions were analyzed to identify key similarities and differences. The analysis revealed a high degree of conservation, suggesting that despite species differences, integrins likely play conserved roles in processes such as implantation and tissue remodeling in alpacas, as observed in humans. These findings highlight the critical role of integrins in facilitating the molecular interactions necessary for maternal recognition of pregnancy and implantation in alpacas, offering new insights into early pregnancy establishment.

References

1. Fernandez-Baca, S., Hansel, W., Saatman, R., Sumar, J., Novoa, C., (1979). Differential luteolytic effects of right and left uterine horns in the alpaca. Biol. Reprod., 20:586–595.
2. Silva M, Urra F, Ratto MH. (2018). Uterine endometrial vascularization during ovarian growth in llamas: The effect of estradiol plasma concentration. Theriogenology 106, 164-169.
3. Sumar J, Adams GP, (2006) Reproductive anatomy and life cycle of the male and female llama and alpaca. In: Youngquist RS, Threlfall WR (eds), Current Therapy in Large Animal Theriogenology. W B Saunders, Philadelphia, PA, USA, pp.
4. Urra F., Ratto M.H., Silva M. (2018) Evaluation of mating, intrauterine deposition of raw seminal plasma or seminal plasma purified β-NGF on endometrial vascularization in llamas. Theriogenology 125, 18-23.
5. Vaughan J, Tibary A, (2006). Reproductive physiology and infertility in male South American camelids: a review and clinical observations. Small Rumin Res 61, 283–298.

Large-scale analysis of structured tandem repeat proteins across the tree of life

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Proteins containing repetitive motifs named tandem repeat proteins are widespread in genomes across the tree of life [1, 2]. Structured Tandem Repeat Proteins (STRPs) are specific subset of the tandem repeat proteins with well-defined structures [3]. STRPs have distinctive features and their complexity, conservation and flexibility in terms of structure and function makes them an attractive topic to study and characterize the biological meaning of them [4]. Our study focused on large-scale, in-depth analysis of 39,185 STRPs obtained from the RepeatsDB database (https://repeatsdb.org). RepeatsDB remains as a fundamental repository for STRPs annotation and classification [5]. It contains STRPs annotated on experimental structures and models from PDB [6] and AlphaFoldDB [7] databases, respectively. Here we performed comparative analysis of experimental structures and models of STRPs. We analyzed the distribution of different types of STRPs through the tree of life in different levels of classification described by Kajava [1]. Separate analysis of human STRPs with clinical importance done to determine quality of predicted repeated regions in STRPs. Co-occurence of different types of repeats in the same STRPs identified and interesting examples of new STRPs are highlighted. The preliminary results show alpha-solenoid, TIM-barrel and beta-barrel repeats containing proteins are most abundant across species and some species show significantly high frequency of specific STRPs.

References

1. Kajava, A. V. (2011). Tandem repeats in proteins: From sequence to structure. Journal of Structural Biology, 179(3), 279–288. https://doi.org/10.1016/j.jsb.2011.08.009
2. Marcotte, E. M., Pellegrini, M., Ng, H., Rice, D. W., Yeates, T. O., & Eisenberg, D. (1999). Detecting Protein Function and Protein-Protein Interactions from Genome Sequences. Science, 285(5428), 751–753. https://doi.org/10.1126/science.285.5428.751
3. Monzon, A. M., Arrías, P. N., Elofsson, A., Mier, P., Andrade-Navarro, M. A., Bevilacqua, M., Clementel, D., Bateman, A., Hirsh, L., Fornasari, M. S., Parisi, G., Piovesan, D., Kajava, A. V., & Tosatto, S. C. (2023). A STRP-ed definition of Structured Tandem Repeats in Proteins. Journal of Structural Biology, 215(4), 108023. https://doi.org/10.1016/j.jsb.2023.108023
4. Mac Donagh, J., Marchesini, A., Spiga, A., Fallico, M. J., Arrías, P. N., Monzon, A. M., Vagiona, A., Gonçalves-Kulik, M., Mier, P., & Andrade-Navarro, M. A. (2024). Structured tandem repeats in protein interactions. International Journal of Molecular Sciences, 25(5), 2994. https://doi.org/10.3390/ijms25052994
5. Paladin, L., Bevilacqua, M., Errigo, S., Piovesan, D., Mičetić, I., Necci, M., Monzon, A. M., Fabre, M. L., Lopez, J. L., Nilsson, J. F., Rios, J., Menna, P. L., Cabrera, M., Buitron, M. G., Kulik, M. G., Fernandez-Alberti, S., Fornasari, M. S., Parisi, G., Lagares, A., . . . Tosatto, S. C. E. (2020). RepeatsDB in 2021: improved data and extended classification for protein tandem repeat structures. Nucleic Acids Research, 49(D1), D452–D457. https://doi.org/10.1093/nar/gkaa1097
6. Varadi, M., Anyango, S., Armstrong, D., Berrisford, J., Choudhary, P., Deshpande, M., Nadzirin, N., Nair, S. S., Pravda, L., Tanweer, A., Al-Lazikani, B., Andreini, C., Barton, G. J., Bednar, D., Berka, K., Blundell, T., Brock, K. P., Carazo, J. M., Damborsky, J., . . . Velankar, S. (2021). PDBe-KB: collaboratively defining the biological context of structural data. Nucleic Acids Research, 50(D1), D534–D542. https://doi.org/10.1093/nar/gkab988
7. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. a. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., . . . Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. Nature, 596(7873), 583–589. https://doi.org/10.1038/s41586-021-03819-2

Computational strategies for the design of novel TAAR1 agonists

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Background: TAAR1 (trace-amine associated receptor-1) is a class-A GPCR primarily expressed in the CNS. TAAR1 regulation is currently regarded as a promising strategy to treat a plethora of diseases, such as neuropsychiatric disorders, metabolic syndromes, and neurodegenerative diseases [1].

Aims: The aim of the study was the discovery of novel chemotypes active towards TAAR1, as well as the elucidation of their binding mode to the target.

Methods: In silico study of the binding mode and structure-activity relationship (SAR) of known TAAR1 agonists were carried out. The analyses revealed pharmacophore features for TAAR1 agonists, leading to explore the potential bioactivity of a new series of compounds (1a-10a and guanfacine). The candidates were evaluated via Ligand-Based (LB) techniques and docking in the TAAR1 AlphaFold predicted structure. The series was submitted to in vitro test. The best compounds were further analyzed in silico via extensive docking and Molecular Dynamics (MD) relying on the recently solved structures of TAAR1.

Results: The study of known TAAR1 agonists via molecular docking elucidated key interactions to enable TAAR1 agonism: these turn in the presence of proper ligand features such as an aromatic core, as well as a H-bond donor group to establish the key H-bond to D103. LB and Structure-based (SB) methods were utilized to evaluate a set of in-house pyrimidinone-benzimidazoles (1a-10a) and the compound guanfacine. In vitro tests revealed a maximum potency (EC50) of 526.3 nM for compound 1a [2], and of 20 nM for guanfacine [3]. These two compounds were selected for further investigation using the experimental structures of TAAR1 (Fig.1A-B). A third chemotype (1b) (Fig.1C), bearing intermediate features between guanfacine and 1a, was designed and tested in vitro (EC50 = 1 μM). Analogs of compound 1b were proposed with the aim of optimizing the TAAR1 affinity.

Conclusion: Novel TAAR1 agonists were identified (the pyrimidinone-benzimidazoles, guanfacine and compound 1b). The binding mode of 1a, 1b, and guanfacine were investigated in silico, and an optimization strategy was applied to the 1b prototype.

References

1. Tonelli, M.; Cichero, E. Trace amine associated receptor 1 (TAAR1) modulators: a patent review (2010-present). Expert Opin. Ther. Pat. 2020, 30, 137–145.
2. Grossi, G.; Scarano, N.; Musumeci, F.; Tonelli, M.; Kanov, E.; Carbone, A.; Fossa, P.; Gainetdinov, R.R.; Cichero, E.; Schenone, S. Discovery of a Novel Chemo-Type for TAAR1 Agonism via Molecular Modeling. Molecules 2024, 29, 1739.
3. Cichero, E.; Francesconi, V.; Casini, B.; Casale, M.; Kanov, E.; Gerasimov, A.S.; Sukhanov, I.; Savchenko, A.; Espinoza, S.; Gainetdinov, R.R.; et al. Discovery of Guanfacine as a Novel TAAR1 Agonist: A Combination Strategy through Molecular Modeling Studies and Biological Assays. Pharmaceuticals 2023, 16, 1632.

3D structure of the ABC transporter MRP2 by Cryo-EM. Insights Post-translational modifications

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Background: Multidrug Resistance-Associated Protein 2 (MRP2/ABCC2) is a crucial ATP-binding cassette (ABC) transporter that plays a pivotal role in the efflux of various organic anions, including bilirubin glucuronides and numerous drugs. Its overexpression in cancer cells has been shown to be determinant for drug resistance and limits the efficacy of chemotherapy. Dysfunction of MRP2 leads to Dubin-Johnson syndrome.

Methods: To elucidate the structural basis of MRP2 function and regulation, we employed cryo-electron microscopy (cryo-EM) to determine the high-resolution structure of rat Mrp2. Additionally, biochemical and functional assays were conducted to investigate the impact of phosphorylation on MRP2 activity.

Results: The cryo-EM structure reveals a unique autoinhibited conformation, characterized by a U-folded regulatory domain within the transmembrane domain cavity. Mass spectrometry analysis identified Ser922 and Ser926 as critical phosphorylation sites. In vitro phosphorylation and functional assays demonstrated that phosphorylation of these residues triggers a significant increase in MRP2's ATPase and transport activity, measured in proteoliposomes using the fluorescent substrate 5(6)-Carboxy-2′,7′-dichlorofluorescein. The probenecid-bound structure uncovered two distinct drug-binding sites, providing insights into the mechanism of drug inhibition.

Conclusion: Our findings provide a comprehensive understanding of the structural and functional dynamics of MRP2. The identification of the autoinhibited state and the role of phosphorylation in activating the transporter offer novel insights into the molecular mechanisms underlying drug resistance. These findings have significant implications for the development of strategies to overcome drug resistance and enhance the efficacy of cancer therapies.

References

Mazza, T., Roumeliotis, T.I., Garitta, E. et al. Structural basis for the modulation of MRP2 activity by phosphorylation and drugs. Nat Commun 15, 1983 (2024). https://doi.org/10.1038/s41467-024-46392-8

Cryo-EM structures of hemoglobin-IsdH c omplexes: paving the way for novel protein-protein interaction inhibitors as antibiotics

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Background: Staphylococcus aureus depends on iron to establish infections in the host, with hemoglobin (Hb) being its main iron source [1]. To capture heme, the bacterium exploits the nine-protein iron-surface determinant (Isd) system that is highly expressed under iron-limiting conditions [2]. The initial steps involve two surface receptors, IsdB and IsdH, which bind to Hb and extract heme. Targeting the IsdB/IsdH-mediated heme acquisition pathway to induce iron starvation in S. aureus represents a promising approach for the development of new antimicrobial agents [3].

Methods: Cryo-EM grids were prepared by mixing the minimal functional IsdH construct (IsdHN2N3, comprising two of its three native NEAT domains) and carboxyhemoglobin (HbCO) at equimolar concentrations. 11,000 micrographs were collected with a Titan Krios microscope (Diamond, Oxford) and Single-Particle Analysis (SPA) was performed using RELION.

Results: Structural insights on the complex between IsdHN2N3 and HbCO were obtained exploiting cryo-EM, capturing key snapshots of the protein-protein interaction (PPI) before heme extraction. The SPA analysis involved multiple rounds of 2D and 3D classifications and allowed to isolate subsets of particles belonging to different binding stoichiometries. The complex formed by a Hb dimer bound to two IsdHN2N3 (2to2 complex) was found to be heterogeneous. In fact, within the structures having this stoichiometry, two different complexes (2to2α and 2to2β, Figure 1) could be identified, with the NEAT3 more stably bound to the α or to the β Hb chain, respectively, while the second NEAT3 domain exhibits a lower local resolution. These data are in agreement with previously published molecular dynamics simulations which suggested that the linker region between the two NEAT domains in IsdHN2N3 is endowed with a certain degree of flexibility [4]. This behaviour was not observed in the IsdB:Hb cryo-EM map, in which the hemophore appeared firmly bound to Hb [5].

Conclusion: The determination of the structures of IsdH/IsdB:Hb complexes represents the groundwork for a targeted drug discovery campaign aimed at identifying novel PPI inhibitors as promising antibiotic candidates.

References

1. Marchetti, Marialaura et al. "Iron Metabolism at the Interface between Host and Pathogen: From Nutritional Immunity to Antibacterial Development." International journal of molecular sciences vol. 21,6 2145. 20 Mar. 2020, doi:10.3390/ijms21062145
2. Grigg, Jason C et al. "Structural biology of heme binding in the Staphylococcus aureus Isd system." Journal of inorganic biochemistry vol. 104,3 (2010): 341-8. doi:10.1016/j.jinorgbio.2009.09.012
3. Cozzi, M., Failla, M., Gianquinto, E. et al. Identification of small molecules affecting the interaction between human hemoglobin and Staphylococcus aureus IsdB hemophore. Sci Rep 14, 8272 (2024). https://doi.org/10.1038/s41598-024-55931-8
4. Clayton, Joseph et al. "Directed Inter-domain Motions Enable the IsdH Staphylococcus aureus Receptor to Rapidly Extract Heme from Human Hemoglobin." Journal of molecular biology vol. 434,12 (2022): 167623. doi:10.1016/j.jmb.2022.167623

5. De Bei, Omar et al. "Cryo-EM structures of staphylococcal IsdB bound to human hemoglobin reveal the process of heme extraction." Proceedings of the National Academy of Sciences of the United States of America vol. 119,14 (2022): e2116708119. doi:10.1073/pnas.2116708119

Repurposing of approved drugs to fight M. abscessus infections.

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Mycobacterium abscessus (Mab) is an emerging opportunistic pathogen responsible for pulmonary infections, particularly in people with cystic fibrosis and chronic pulmonary disease [1]. It is highly resistant to currently used antimicrobial agents, so the therapies, mostly based on old drugs, have very poor success rates [2]. In this picture, we focused on the inhibition of the Salicylate Synthase Mab-SaS by repurposing already approved FDA drugs exploiting an in silico approach. Mab-SaS is a virulence factor catalyzing the first reaction of the biosynthetic pathway of mycobactins which is is not present in human [3].

For this work, the chemical structures of approved drugs have been retrieved from three databases (Enamine, DrugBank and PubChem) and optimized with Maestro, a software of the Schrödinger Suite. The obtained ~3400 conformers have been docked in the Mab-SaS model using Glide, a tool integrated in Maestro [4]. Results have been filtered according to their docking score and MM-GBSA and a selection of 11 drugs have been evaluated in vitro against the enzyme.

Five molecules resulted to be effective against Mab-SaS. Labetalol and levolansoprazole showed a mild inhibition of the enzyme activity at 100 μM of 53.2 ± 1.1% and 37.0 ± 0.5%, respectively. Esomeprazole, fostamatinib and hydroxystilbamidine completely blocked the enzyme at the same concentration, exhibiting an IC50 of 13.6 ± 1.1 μM, 21.0 ± 1.3 μM and 11.5 ± 1.1 μM, respectively.

The steady-state kinetic analysis of Mab-SaS in the presence of different concentrations of the inhibitors demonstrated that the three molecules behave as competitive inhibitors, with Ki values in the low μM range, reflecting the preliminary results obtained from the in silico analysis, whereas these molecules docked in the same position of the chorismic acid, the natural substrate of Mab-SaS.

In conclusion, exploiting the so called "repurposing strategy", a combination of in silico tools and in vitro analysis identified three good inhibitors of Mab-SaS showing an IC50 in the lower micromolar. These molecules will be the starting point for the rational design and optimization of novel inhibitors of Mab-SaS, possibly fastening the drug development process thus reducing the cytotoxicity effects.

References

1. Degiacomi, G., Sammartino, J. C., Chiarelli, L. R., Riabova, O., Makarov, V. & Pasca, M. R. (2019) Mycobacterium abscessus, an Emerging and Worrisome Pathogen among Cystic Fibrosis Patients, Int J Mol Sci. 20.
2. Degiacomi, G., Chiarelli, L. R., Riabova, O., Lore, N. I., Munoz-Munoz, L., Recchia, D., Stelitano, G., Postiglione, U., Saliu, F., Griego, A., Scoffone, V. C., Kazakova, E., Scarpa, E., Ezquerra-Aznarez, J. M., Stamilla, A., Buroni, S., Tortoli, E., Rizzello, L., Sassera, D., Ramon-Garcia, S., Cirillo, D. M., Makarov, V. & Pasca, M. R. (2024) The novel drug candidate VOMG kills Mycobacterium abscessus and other pathogens by inhibiting cell division, Int J Antimicrob Agents. 64, 107278.
3. Mori, M., Stelitano, G., Cazzaniga, G., Gelain, A., Tresoldi, A., Cocorullo, M., Roversi, M., Chiarelli, L. R., Tomaiuolo, M., Del Re, P., Mangiatordi, G. F., Griego, A., Rizzello, L., Cassetta, A., Covaceuszach, S., Villa, S. & Meneghetti, F. (2023) Targeting siderophore-mediated iron uptake in M. abscessus: a new strategy to limit the virulence of non-tuberculous mycobacteria, Pharmaceutics. 15, 502.
4. Friesner, R. A., Murphy, R. B., Repasky, M. P., Frye, L. L., Greenwood, J. R., Halgren, T. A., Sanschagrin, P. C. & Mainz, D. T. (2006) Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes, J Med Chem. 49, 6177-96.

X-MAP: Explainable Mutation Analysis Platform for Predicting Genetic Variant Impacts on Protein Structure, Stability, and Human Health

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Background: Genetic variants, especially missense mutations, can significantly impact protein function by altering structure, stability, and interactions [1]. Investigating these mutations is crucial for understanding disease mechanisms. Existing predictive tools often struggle with protein folding complexity [1]. Traditional methods like SIFT and Polyphen2 predict variant pathogenicity [2,3], but deep learning techniques, particularly protein language models (pLMs), have revolutionized the field by capturing complex protein attributes [4,5].

Methods: X-MAP integrates data from public sources like Humsavar and UniProt [6] to create a comprehensive database of genetic mutations with pathogenicity annotations. Initial scoring uses tools like DDGun [2] and PhD-SNPg [3]. To enhance accuracy, X-MAP incorporates pLMs such as ESM2, ESM-1v [7] and ProtTrans [4], generating embeddings that capture detailed protein attributes. By combining traditional scores with pLM insights, X-MAP provides a comprehensive assessment of mutation impacts.

Results: We analyzed 72,585 genetic variants across 12,664 human proteins, with variants being 54.8% benign (B), 45.2% pathogenic (P). Embeddings were generated for a randomized subset of 4000 proteins with P and B variants; a preliminary 5-fold cross-validated multilayer-perceptron model achieved 89% accuracy, 89% F1-score, and 96% ROC AUC in the binary classification. We are currently integrating additional scores from dbNSFP [8], and further pLM embeddings to improve the prediction reliability.

Conclusion: The X-MAP project successfully integrates traditional bioinformatics tools and deep learning techniques to enhance variant analysis. By combining different approaches predicting the impact of protein variants at functional and structural levels with pLMs, we are building a comprehensive framework that captures complex protein attributes.

References

1. Diwan GD, Gonzalez-Sanchez JC, Apic G, Russell RB. Next Generation Protein Structure Predictions and Genetic Variant Interpretation. J Mol Biol. 2021;433(20):167180. doi:10.1016/j.jmb.2021.167180
2. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003;31(13):3812-3814. doi:10.1093/nar/gkg509
3. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7(4):248-249. doi:10.1038/nmeth0410-248
4. Elnaggar A, Heinzinger M, Dallago C, et al. ProtTrans: Toward Understanding the Language of Life Through Self-Supervised Learning. IEEE Trans Pattern Anal Mach Intell. 2022;44(10):7112-7127. doi:10.1109/TPAMI.2021.3095381
5. Marquet C, Heinzinger M, Olenyi T, et al. Embeddings from protein language models predict conservation and variant effects. Hum Genet. 2022;141(10):1629-1647. doi:10.1007/s00439-021-02411-y
6. UniProt Consortium. UniProt: the Universal Protein Knowledgebase in 2023. Nucleic Acids Res. 2023;51(D1):D523-D531. doi:10.1093/nar/gkac1052
7. Lin Z, Akin H, Rao R, et al. Evolutionary-scale prediction of atomic-level protein structure with a language model. Science. 2023;379(6637):1123-1130. doi:10.1126/science.ade2574
8. Liu X, Li C, Mou C, Dong Y, Tu Y. dbNSFP v4: a comprehensive database of transcript-specific functional predictions and annotations for human nonsynonymous and splice-site SNVs. Genome Med. 2020;12(1):103. Published 2020 Dec 2. doi:10.1186/s13073-020-00803-9

Identifying disease biomarkers using a novel data analysis pipeline based on multi-view learning

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Background: MicroRNAs (miRNAs) play a key role in regulating gene expression and are involved in the onset and progression of many diseases [1].

Our goal is to develop a Machine Learning model able to identify miRNA as early disease biomarkers using heterogeneous biological data [2,3]. To illustrate the effectiveness of our approach, we have analyzed data from patients with Mild Cognitive Impairment (MCI) and Alzheimer's Disease (AD) using publicly available datasets. Preliminary results show a significant involvement of some potential miRNA biomarkers in neurodevelopment and neurodegenerative processes.

Methods: We adopt a multi-view learning approach, which exploits various patient-related aspects (views), including miRNA expression values, sequences and metadata. The novelty lies in the simultaneous use of these three views, complemented by a fourth view representing miRNA interactions.

The analytical pipeline started with the pre-processing and integration of four datasets from the Gene Expression Omnibus (GEO) archive, which were then fed by a Random Forest multi-view classifier. Explicability techniques based on significance and feature permutation were adopted to identify candidate biomarkers. Finally, we conducted miRNA-target interaction (multiMIR) and functional pathway (DAVID, KEGG) analyses to interpret the results from a biological perspective.

The predicted high-scored disease biomarkers will be validated by nanofluid qPCR analysis of miRNAs extracted from blood samples of AD and MCI patients under clinical observation.

Results: The analysis of the first 50 predicted high-scored miRNAs revealed a significant enrichment of targeted genes in Alzheimer's disease and neurodegenerative disorders pathways, with 86% of the analysed miRNAs showing connections to these disease processes.

Conclusions: By the analysis of the literature, high scored miRNAs have not previously been associated with neurological diseases. Therefore, this surprising enrichment suggests the existence of a largely unexplored level of molecular regulation. The substantial presence of already known targets in neurodegenerative diseases indicates that these miRNAs may function as key 'upstream' molecular regulators, controlling disease-associated gene expression. Our findings open new perspectives both for the understanding of pathogenetic mechanisms and the identification of novel potential diagnostic biomarkers and therapeutic targets.

References

1. Rashidi SK, Kalirad A, Rafie S, Behzad E, Dezfouli MA. The role of microRNAs in neurobiology and pathophysiology of the hippocampus. Front Mol Neurosci. 2023 Sep 4;16:1226413. doi: 10.3389/fnmol.2023.1226413. PMID: 37727513; PMCID: PMC10506409.

2. Tarca AL, Carey VJ, Chen XW, Romero R, Drăghici S. Machine learning and its applications to biology. PLoS Comput Biol. 2007 Jun;3(6):e116. doi: 10.1371/journal.pcbi.0030116. PMID: 17604446; PMCID: PMC1904382.
3. Hu X, Yin Z, Zeng Z, Peng Y. Prediction of miRNA-Disease Associations by Cascade Forest Model Based on Stacked Autoencoder. Molecules. 2023 Jun 27;28(13):5013. doi: 10.3390/molecules28135013. PMID: 37446675; PMCID: PMC10343850.
4. Akiyama M, Sakakibara Y. Informative RNA base embedding for RNA structural alignment and clustering by deep representation learning. NAR Genom Bioinform. 2022 Feb 22;4(1):lqac012. doi: 10.1093/nargab/lqac012. PMID: 35211670; PMCID: PMC8862729.

Biochemical Characterivzation of a New Class of Selective Haspin kinase Inhibitors as potential anticancer drugs

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Background: This study investigates Haspin, an emerging kinase involved in tumor growth via mitotic regulation, by presenting the design, synthesis, and evaluation of new Haspin inhibitors. Through FRET analysis, the synthesized derivatives showed potent Haspin inhibition, with compounds 47 and 60 identified as lead candidates. These two compounds enhanced the antitumor effect of paclitaxel twofold in both 2D and 3D models, with compound 60 demonstrating high selectivity across a panel of 70 kinases. In-silico studies provided key structural insights valuable for the future design of Haspin inhibitors, positioning compounds 47 and 60 as promising for further exploration.

Methods: A virtual library of approximately 14 million compounds was generated through in-silico modifications of a lead compound to screen potential Haspin inhibitors. Selected derivatives were tested for kinase inhibition with a focus on Haspin via FRET-based assays. Cell viability was evaluated using an in-house cellular panel (Raji, HeLa, A375, MiaPaca2, MCF7) known for Haspin expression. Apoptosis and cell cycle alterations were assessed via flow cytometry, and histone extraction with western blotting confirmed inhibition of Haspin-specific histone H3Thr3 phosphorylation. Confocal microscopy further revealed abnormal mitotic spindle formations post-treatment. Using 3D models, tumor-like environments were simulated for chemosensitivity testing. LC-MS analysis confirmed the in vitro pharmacokinetic stability of the compounds.

Results: This study presents a series of Haspin kinase inhibitors developed through virtual screening and synthetic accessibility. Of the 29 derivatives synthesized, 22 showed significant in vitro potency, with potencies ranging from micromolar to subnanomolar. Comprehensive cell-based testing identified compounds 47 and 60 as promising leads that synergistically enhanced paclitaxel's antitumor effects, particularly doubling its efficacy in 3D HeLa models. LC-MS further confirmed the chemical and metabolic stability of compounds 47 and 60, underscoring their potential as drug candidates.

Conclusion: This study provides a thorough characterization of novel indole-based compounds targeting Haspin, clarifying structural features essential for kinase inhibition. Considering the scarcity of effective Haspin modulators and their potential in impacting tumor growth, these findings form a solid starting point for future drug development.

References

1. Liu Y, Yang H, Fang Y, Xing Y, Pang X, Li Y, Zhang Y, Liu Y. Function and inhibition of Haspin kinase: targeting multiple cancer therapies by antimitosis. J Pharm Pharmacol. 2023 Apr 7;75(4):445-465. doi: 10.1093/jpp/rgac080.

2. Vestuto V, Ciaglia T, Musella S, Di Sarno V, Smaldone G, Di Matteo F, Scala MC, Napolitano V, Miranda MR, Amodio G, Novi S, Pepe G, Basilicata MG, Gazzillo E, Pace S, Gomez-Monterrey IM, Sala M, Bifulco G, Tecce MF, Campiglia P, Ostacolo C, Lauro G, Manfra M, Bertamino A. A Comprehensive In Vitro Characterization of a New Class of Indole-Based Compounds Developed as Selective Haspin Inhibitors. J Med Chem. 2024 Aug 8;67(15):12711-12734. doi: 10.1021/acs.jmedchem.4c00718.

[FeFe] hydrogenases as promising biocatalysts for H2 applications

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Bacterial [FeFe]-hydrogenases represent promising biocatalysts for H2 high efficiency production or consumption of hydrogen in bio-electrolysers or bio-fuel cells.

So far, the [FeFe] hydrogenase from Clostridium beijerinckii, named CbA5H, seems to be the only one with a unique self-protection mechanism against irreversible damage caused by oxygen and other oxidants [1,2].

Herein two new [FeFe] hydrogenases, not previously studied, were selected through sequence alignment based on conserved regions and three key amino acid substitutions identified so far.

The new enzymes were successfully recombinantly produced in E. coli through co-expression of maturases, followed by aerobic purification and characterization. Both hydrogenases demonstrated consistent production of molecular hydrogen, as detected by gas chromatography, highlighting the enzyme's tolerance to O2. Moreover, spectroscopic measurements showed, upon exposure to oxidants, such as the aerobic atmosphere, thionine or DCIP, the characteristic signal, called Hinact.

In parallel, the molecular dynamics approach could provide "direct" information that led to model the structural rearrangements related to the oxygen protection mechanism.

Identifying and characterizing novel [FeFe]-hydrogenases presents an opportunity to fill the existing gaps in information about these enzymes. Moreover, those biocatalysts could address the growing demand for producing H2 at high rates and low costs.

These results contribute to the expansion of a particularly robust enzyme library, with the potential to identify new critical residues responsible for this distinctive trait.

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References

1. Previously published in: Winkler M. et al. (2021) Nat Commun 12, 756
2. Morra S. et al. (2016) Biochemistry, 55, 42, 5897–5900

A genome-wide association study in European advanced cancer patients treated with opioids identified variants regulating the expression of OPRL1 as possible modulators of pain intensity

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Background: Cancer pain causes suffering and lowered quality of life, thus advanced cancer patients often require analgesic therapy [1,2]. Opioids of step III of the WHO-defined analgesic ladder are the standard of care for the treatment of cancer pain. However, 20-30% of patients do not receive adequate pain relief from opioids[3,4]. Literature suggests that genetics plays a role in predisposing patients to a good or poor response to opioids [5] and, herein, we investigated it by performing a genome-wide association study (GWAS).

Methods: We individually genotyped 2,060 European advanced cancer patients treated with morphine, buprenorphine, fentanyl, and oxycodone. We performed a whole-genome regression model (using REGENIE software) between genotypes and the opioid response phenotype, defined as a numerical score measuring pain intensity based on patients' responses to the Brief Pain Inventory Questionnaire.

Results: The GWAS identified five non-coding variants on chromosome 20 at P-value < 5.0x10-8. For all of them, the minor allele was associated with a lower pain intensity. These variants were intronic of PCMTD2 gene and were 200 kbp downstream of OPRL1, the Opioid Related Nociceptine Receptor 1. Interestingly, four of them acted as expression quantitative trait loci, modulating the expression of OPRL1, according to eQTLGen database.

Conclusion: This is the largest GWAS performed in this field, so far. Our results strengthen the evidence for a role of genetics in opioid response. Further functional analyses are needed to validate the results obtained and to understand the biological mechanism underlying the observed associations.

References

3. 1. Caraceni A et al (2012) Use of opioid analgesics in the treatment of cancer pain: evidence-based recommendations from the EAPC. Lancet Oncol 13:e58–68

4. 2. Teunissen SCCM et al (2007) Symptom prevalence in patients with incurable cancer: a systematic review. J Pain Symptom Manage 34:94–104
5. 3. Klepstad P et al (2005) Pain and pain treatments in European palliative care units. A cross sectional survey from the European Association for Palliative Care Research Network. Palliat Med 19:477–484
6. 4. Oosten AW, Oldenmenger WH, Mathijssen RHJ, Van Der Rijt CC (2015) D. A Systematic Review of Prospective Studies Reporting Adverse Events of Commonly Used Opioids for Cancer-Related Pain: A Call for the Use of Standardized Outcome Measures. J Pain 16:935–946
7. 5. Angst MS et al (2012) Aversive and reinforcing opioid effects: a pharmacogenomic twin study. Anesthesiology 117:22–37

A Machine Learning model for diagnosis of Inflammatory Bowel Disease

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Background: The integration of bioinformatics, machine learning (ML), and molecular biology offers opportunities for discovering biomarkers for the diagnosis and prognosis of various diseases. This study focuses on inflammatory bowel disease (IBD), a chronic condition characterized by inflammation of the gastrointestinal tract. Recent studies have linked IBD to epigenetic molecules, such as miRNAs, whose dysregulation is associated with key signaling pathways [1,2].

Methods: Given the availability of extensive biological data, we delve into the application of ML techniques to identify potential biomarkers for disease prediction and patient. In our analysis, we considered six microarray datasets sourced from the Gene Expression Omnibus, which include comprehensive miRNA expression profiles from patients diagnosed with ulcerative colitis (UC) alongside profiles from healthy individuals for comparison.

We employed a range of classification models, including Logistic Regression, Support Vector Machines, Random Forest, Naive Bayes, K-Nearest Neighbors and XGBoost, to assess the predictive power of these miRNAs.

Results and Conclusion: Preliminary results show that our best model achieves an average F1 score of approximately 0.7 across 30 train-test splits, indicating a reliable capacity for distinguishing between disease states and healthy controls. This multifaceted approach not only enhances our understanding of miRNA dynamics in UC but also sets the stage for further exploration of tailored treatment strategies based on individual patient profiles.

Additionally, we conducted analyses on miRNAs that influence predictions, revealing that some of these are differentially expressed. Some of them are already recognized as biomarkers for UC, using the HMDD database [3].

References

1. Feng Wu, Michelle Zikusoka, Anil Trindade, Themistocles Dassopoulos, Mary L Harris, Theodore M Bayless, Steven R Brant, Shukti Chakravarti, and John H Kwon. Micrornas are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2α. Gastroenterology, 135(5):1624–1635, 2008
2. Stamatia Papoutsopoulou, Barry J. Campbell, Epigenetic Modifications of the Nuclear Factor Kappa B Signalling Pathway and its Impact on Inflammatory Bowel Disease, Current Pharmaceutical Design; Volume 27, Issue 35, Year 2021. DOI: 10.2174/1381612827666210218141847
3. Cui C, Zhong B, Fan R, Cui Q. HMDD v4.0: a database for experimentally supported human microRNA-disease associations. Nucleic Acids Res. 2024;52(D1):D1327-D1332. doi:10.1093/nar/gkad717

A new approach against cancer: translational readthrough inducing drugs rescuing nonsense mutated TP53

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Stop mutations (nonsense) are gene mutations characterized by the substitution of a single nucleotide in the coding sequence of a gene, which causes the onset of a premature stop codon (PTC) within the reading frame of the mRNA, resulting in the formation of a truncated and non-functional protein. Stop mutations are the cause of approximately 11% of genetic diseases (Cystic Fibrosis and Duchenne Muscular Dystrophy) [1] and can also be present in the coding sequence of tumor suppressor genes such as TP53; in fact, 10% of TP53 mutations are stop mutations [2]. The TP53 gene encodes the transcriptional factor p53, which regulates numerous pathways such as cell cycle arrest and DNA damage repair when cells are under certain stress conditions [3]. Mutations of TP53 can lead to the formation of tumors; in fact, it is the most frequently mutated gene in human tumors [4]. Today there is no therapy for the pathologies caused by this type of mutation, but an approach that has proven to be particularly effective is represented by molecules with readthrough activity (TRIDs; Translational Readthrough Inducing Drugs) which intervene on the ribosome allowing the overcoming of the PTC and the restoration of the synthesis and subsequent functionality of the protein [5]. This work evaluated the readthrough potential of these molecules and the recovery of p53 expression, following the induction of DNA damage on cells presenting the PTC UGA in the TP53 cDNA. The cellular localization of p53 was evaluated by immunofluorescence assay, the transcript of p53 and two of its target genes, p21, and GADD45, by Real-Time RT PCR, and the protein levels of p53 by Western blot. Treatment with TRIDs involves a partial localization of p53 in the nucleus following the induction of damage and the recovery of its expression in cells presenting the stop mutation and treated with the molecules. Through these results, we can, therefore, conclude that the molecules have a readthrough potential that allows the recovery of p53 expression and its correct localization at the nuclear level.

References

1. Martins-Dias P, Romão L. Nonsense suppression therapies in human genetic diseases. Cell Mol Life Sci. 2021 May;78(10):4677-4701. doi: 10.1007/s00018-021-03809-7. Epub 2021 Mar 22. PMID: 33751142.
2. Wang H, Guo M, Wei H, Chen Y. Targeting p53 pathways: mechanisms, structures, and advances in therapy. Signal Transduct Target Ther. 2023 Mar 1;8(1):92. doi: 10.1038/s41392-023-01347-1. PMID: 36859359; PMCID: PMC9977964.
3. Bykov VJN, Eriksson SE, Bianchi J, Wiman KG. Targeting mutant p53 for efficient cancer therapy. Nat Rev Cancer. 2018 Feb;18(2):89-102. doi: 10.1038/nrc.2017.109. Epub 2017 Dec 15. PMID: 29242642.
4. Palomar-Siles M, Heldin A, Zhang M, Strandgren C, Yurevych V, van Dinter JT, Engels SAG, Hofman DA, Öhlin S, Meineke B, Bykov VJN, van Heesch S, Wiman KG. Translational readthrough of nonsense mutant TP53 by mRNA incorporation of 5-Fluorouridine. Cell Death Dis. 2022 Nov 25;13(11):997. doi: 10.1038/s41419-022-05431-2. PMID: 36433934; PMCID: PMC9700717.
5. Pibiri I, Melfi R, Tutone M, Di Leonardo A, Pace A, Lentini L. Targeting Nonsense: Optimization of 1,2,4-Oxadiazole TRIDs to Rescue CFTR Expression and Functionality in Cystic Fibrosis Cell Model Systems. Int J Mol Sci. 2020 Sep 3;21(17):6420. doi: 10.3390/ijms21176420. PMID: 32899265; PMCID: PMC7504161.

A Preliminary Experimental Analysis of Dynamical Properties on PPI Networks by Deep Graph Networks

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Background: The scope and detail of Protein-Protein Interaction Networks (PPINs) have expanded in last decades, thanks to advances in high-throuput technlogies and techniques that allow functional associations among proteins. However, PPINs remain static shapshots that hare rarely associated with the dynamics of processes represented by their arcs. Biochemical Pathways (BPs), on the other hand, allow to describe cellular processes over time and can be modeled as complex networks of chemical reactions. Analyzing BPs via numerical simulations allows for insight into dynamical properties. Here, we examine the potential of studying dynamical properties directly on PPINs, focusing on sensitivity - a global property that reveals how an input molecular species' concentration impacts an output species at a steady state.

Methods: We simulate BPs through ODE simulations to compute sensitivities across species pairs. These sensitivities were then mapped onto PPINs using BioGRID and UniPROT, creating the a dataset that describe sensitivities for pairs of proteins, instead of pairs of molecules as it would be done for BPs. Then we train a neural model in the family of Deep Graph Networks (DGN) for predicting sensitivity relationships within PPINs.

The trained model can be used to predict sensitivities between protein selecting any subgraph from the PPIN.

Results: Preliminar results show that this model predicts sensitivity relationships effectively across various scenarios. We also found that the PPIN structure itself is crucial for inferring sensitivity.

Conclusion: This study proposes a novel approach to sensitivity analysis on PPINs. Our findings could support applications in drug discovery, repurposing, and personalized medicine without requiring detailed process models.

References

1. Jing, X., Zhou, Y., Shi, M.: Dynamic graph neural network learning for temporal omics data prediction 10, 116241–116252 https://doi.org/10.1109/ACCESS.2022.3218027;
2. Costello, Z., Martin, H.G.: A machine learning approach to predict metabolic pathway dynamics from time-series multiomics data 4(1), 19 https://doi.org/10.1038/s41540-018-0054-3;
3. Jeong, H., Tombor, B., Albert, R., Oltvai, Z.N., Barab´asi, A.-L.: The large-scale organization of metabolic networks 407(6804), 651–654 https://doi.org/10.1038/35036627;
4. antolini, M., Barab´asi, A.-L.: Predicting perturbation patterns from the topology of biological networks 115(27), 6375–6383 https://doi.org/10.1073/pnas.1720589115;
5. Bove, P., Micheli, A., Milazzo, P., Podda, M.: Prediction of dynamical properties of biochemical pathways with graph neural networks:. In: Proceedings
6. of the 13th International Joint Conference on Biomedical Engineering Systems and Technologies, pp. 32–43. SCITEPRESS - Science and Technology Publications. https://doi.org/10.5220/0008964700320043;
7. Podda, M., Bove, P., Micheli, A., Milazzo, P.: Classification of biochemical
8. pathway robustness with neural networks for graphs. In: Ye, X., Soares, F.,De Maria, E., G´omez Vilda, P., Cabitza, F., Fred, A., Gamboa, H. (eds.) Biomedical Engineering Systems and Technologies. Communications in Computer and Information Science, pp. 215–239. Springer. https://doi.org/10.1007/978-3-030-72379-8_11;
9. Fontanesi, M., Micheli, A., Milazzo, P., Podda, M.: Exploiting the structure of biochemical pathways to investigate dynamical properties with neural

10. networks for graphs. Bioinformatics 39(11), 678 (2023) https://doi.org/10.1093/bioinformatics/btad678;
11. Campolongo, F., Saltelli, A., Cariboni, J.: From screening to quantitative sensitivity analysis. a unified approach 182(4), 978–988 https://doi.org/10.1016/j.cpc.2010.12.039

ABCC6: A Key Regulator of Tumor Aggressiveness in Hepatocellular Carcinoma

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Emerging evidence implicates ATP-binding cassette (ABC) transporters in tumorigenesis, with roles beyond xenobiotic detoxification to include disrupting normal cellular functions, promoting cancer hallmarks, and enhancing metastatic potential (1). ABCC6, a key ABC transporter, has been linked to cytoskeletal rearrangements, migration, and altered purinergic signaling via extracellular ATP modulation in HepG2 hepatocarcinoma cells. These findings suggest ABCC6 as a therapeutic target in hepatocellular carcinoma (HCC) (2,3).

To investigate ABCC6's role in HCC, RNA sequencing on ABCC6-silenced HepG2 cells was performed, followed by differential gene expression analysis through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, with key findings in focal adhesion genes validated by qPCR. Functional assays confirmed ABCC6's role in enhancing cellular adhesion, migration, invasion, and matrix metalloproteinase (MMP) activity. Seahorse metabolic assays revealed a shift in energy metabolism upon ABCC6-knockdown, indicating reprogramming of central carbon metabolism that could support a less aggressive tumor phenotype. In vitro studies using the ABCC6 inhibitor Probenecid demonstrated effects consistent with ABCC6 silencing, notably reducing cellular adhesion, migration, and invasion while decreasing the aggressive phenotype via inhibition of the p38 MAPK pathway. In vivo, Probenecid reduced tumor engraftment in an orthotopic murine model of liver cancer. This decrease was associated with lower plasma vascular endothelial growth factor (VEGF) levels, measured by ELISA, and reduced MMP2 and MMP9 activity, as confirmed through zymography.

These results underscore ABCC6's influence on HCC biology, showing that its down-regulation disrupts cellular adhesion, invasion, and metabolic processes, influencing signaling pathways such as AKT/mTOR and WNT/β-catenin to reduce tumor aggressiveness. Targeting ABCC6 could thus represent a viable therapeutic approach in HCC, addressing its roles in cytoskeletal dynamics, purinergic signaling, and metabolic reprogramming to mitigate liver cancer progression.

References

12. 1. Duvivier, Laurent, Louise Gerard, Adriana Diaz, and Jean-Pierre Gillet. 2024. 'Linking ABC Transporters to the Hallmarks of Cancer'. Trends in Cancer 10 (2): 124– 34. https://doi.org/10.1016/j.trecan.2023.09.013;
13. 2. Ostuni, Angela, Monica Carmosino, Rocchina Miglionico, Vittorio Abruzzese, Fabio Martinelli, Daniela Russo, Ilaria Laurenzana, Agata Petillo, and Faustino Bisaccia. 2020a. 'Inhibition of ABCC6 Transporter Modifies Cytoskeleton and Reduces Motility of HepG2 Cells via Purinergic Pathway'. Cells 9 (6). https://doi.org/10.3390/cells9061410;
14. 3. Abruzzese, Vittorio, Ilenia Matera, Fabio Martinelli, Monica Carmosino, Prashant Koshal, Luigi Milella, Faustino Bisaccia, and Angela Ostuni. 2021. 'Effect of Quercetin on ABCC6 Transporter: Implication in HepG2 Migration'. International Journal of Molecular Sciences 22 (8). https://doi.org/10.3390/ijms22083871.

A cloud-based, open-access platform for comprehensive metagenomic data analysis

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Background: The future Italian Node of the European Microbial Resource Research Infrastructure MIRRI-ERIC (MIRRI-IT) will be leading advancing microbial research in Italy. In 2022, the Italian government dedicated €17 million from the NextGeneration EU-funded PNRR program to support the SUS-MIRRI.IT project. Due to the vast amount of metadata and high-throughput sequencing datasets associated with microbial resources, the SUS-MIRRI.IT project has been focusing on creating bioinformatics analysis workflows and offering them as an online service. This service provides scientists with remarkable computing power, typically beyond the reach of most laboratories, and supports their research efforts with an interactive and intuitive environment.

Methods: Our service integrates multiple microbial workflows within the high-performance computing (HPC) architecture of the University of Turin. To enhance user accessibility, we built a web interface using Next.js. The workflows are managed by StreamFlow [1], a Workflow Management System that adheres to the Common Workflow Language standard, and are executed within the HPC environment using SLURM [2], shown in Fig. 1A/1B. This setup allows for the simultaneous execution of multiple workflows.

Results: To demonstrate our approach's effectiveness, here we present two distinct workflows. The first workflow focuses on microbial genome assembly. It supports long-read sequencing by utilising various assembly tools [3][4][5]. Following the initial assembly, the workflow applies polishing and merging steps to enhance the quality of the assembled genomes. The second workflow is designed for metagenomic analysis, incorporating taxonomic annotation and microbial population characterisation. This workflow includes: (i) read alignment for host genome removal and taxonomic classification using Kraken2 [6] and MetaPhlAn4 [7]; (ii) post-processing steps such as normalization, decontamination, filtering, abundance estimation, and diversity calculation; and (iii) metadata analysis and regression model computation. Using our Infrastructure, we achieved a significant acceleration in the analysis, as demonstrated in Fig. 1C, which will be of great value for researchers and users.

Conclusions: Both implemented workflows demonstrate a fivefold increase in speed compared to a personal computer, highlighting our service's scalability as it adapts efficiently to different computational resources. This performance boost also enhances the quality of our results, enabling the integration of additional tools and finer adjustments.

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References

1. I. Colonnelli, B. Cantalupo, I. Merelli and M. Aldinucci, "StreamFlow: Cross-Breeding Cloud With HPC," in IEEE Transactions on Emerging Topics in Computing, vol. 9, no. 4, pp. 1723-1737, 1 Oct.-Dec. 2021, doi: 10.1109/TETC.2020.3019202.
2. Georgiou, Y., Hautreux, M. (2013). Evaluating Scalability and Efficiency of the Resource and Job Management System on Large HPC Clusters. In: Cirne, W., Desai, N., Frachtenberg, E., Schwiegelshohn, U. (eds) Job Scheduling Strategies for Parallel Processing. JSSPP 2012. Lecture Notes in Computer Science, vol 7698. Springer, Berlin, Heidelberg.
3. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res. 2017 May;27(5):722-736. doi: 10.1101/gr.215087.116. Epub 2017 Mar 15. PMID: 28298431; PMCID: PMC5411767.
4. Kolmogorov, M., Yuan, J., Lin, Y. et al. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol 37, 540–546 (2019). https://doi.org/10.1038/s41587-019-0072-8.
5. Ruan, J., Li, H. Fast and accurate long-read assembly with wtdbg2. Nat Methods 17, 155–158 (2020). https://doi.org/10.1038/s41592-019-0669-3.
6. Wood, D.E., Lu, J. & Langmead, B. Improved metagenomic analysis with Kraken 2.Genome Biol 20, 257 (2019). https://doi.org/10.1186/s13059-019-1891-0.
7. Blanco-Míguez, A., Beghini, F., Cumbo, F. et al. Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. Nat Biotechnol 41, 1633–1644 (2023). https://doi.org/10.1038/s41587-023-01688-w.

AI strategies for Genome-Wide Association Studies

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Background: Through the comparison of single nucleotide polymorphisms (SNPs) between healthy and affected subjects, genome-wide association studies (GWASs) it's now able to identify genetic variants associated with complex diseases, clarifying their genetic architecture. However, despite its potential, GWASs encounter significant limitations, including high false-positive rates, low reproducibility, and consequently having difficulty distinguishing causal SNPs from those in linkage disequilibrium (LD) (i.e., those only physically associated with the phenotype and not truly causal) [1,3,4]. These and more challenges limit the accuracy of GWAS interpretation in studies of complex phenotypes [2,3].

Methods: Artificial intelligence (AI) can improve the quality of GWAS results through an automated machine learning approach (AutoML) for selecting SNPs of real interest, model tuning, and the ability to analyze large-scale genomic data [3]. However, this approach faces serious challenges, such as high dimensionality, when millions of SNPs are analyzed in small sample sizes. Problems related to overfitting or interpretability (especially when a deep learning approach is used) may then result [6]. This therefore requires human-in-the-loop approaches that inherently incorporate expert knowledge of AI processes to guide decision-making in clinical genomics.

Results: Our ongoing review assessed the current applications and potential of AI in GWAS by analyzing 116 articles and 17 biobanks. The main findings relate to predictive models, feature selection methods, and the relative advantages and limitations of different AI approaches, thus providing guidelines for researchers in choosing an optimal AI strategy based on data and sample characteristics [3].

Conclusion: Also, we provide a preview of our project: a framework based on Tsamardino's work for SNP risk identification [3]. This is a framework that uses meta-features in the configuration of feature selection and preprocessing techniques. This involves parallel processes for identifying optimal algorithms with hyperparametric settings. Simultaneously, feature selection informs the targeting of genes and molecular pathways to enable cross-validation of results. The goal of our project is very broad: it seeks to contribute to the improvement of GWAS through the development of scalable, robust, and interpretable AI models, with a view to advancing AI that can be aimed at personalized medicine.

References

1. Zeggini, E., et al. (2019). Translational genomics and precision medicine: Moving from the lab to the clinic. Science (New York, N.Y.), https://doi.org/10.1126/science.aax4588.
2. Potamias, G., et al. (2014). Deciphering next-generation pharmacogenomics: an information technology perspective. Open biology, https://doi.org/10.1098/rsob.140071
3. Lakiotaki, K., et al. (2023). Automated machine learning for genome wide association studies. Bioinformatics (Oxford, England), https://doi.org/10.1093/bioinformatics/btad545
4. Schaid, D. J., et al. (2018). From genome-wide associations to candidate causal variants by statistical fine-mapping. Nature reviews. https://doi.org/10.1038/s41576-018-0016-z
5. https://www.ebi.ac.uk/training/online/courses/gwas-catalogue-exploring-snp-trait-associations/what-is-gwas-catalog/what-are-genome-wide-association-studies-gwas/
6. Lin, J., & Ngiam, K. Y. (2023). How data science and AI-based technologies impact genomics. Singapore medical journal. https://doi.org/10.4103/singaporemedj.

An interactive Shiny app for micro-RNA-target analysis

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Currently, there are several biological databases containing information on miRNAs. Among these, HMDD [1] and Mir2Disease [2] are the two most commonly used manually curated databases that provide a comprehensive resource on miRNA deregulation in various human diseases. MiRTarBase [3], on the other hand, is a curated database of MicroRNA-Gene Target Interactions that offers the most up-to-date collection by comparing it with other similar, previously developed databases.

Since these databases are often difficult to access, a user-friendly web application has been developed to provide an overview of the role of miRNAs in the literature and their interactions with genes and diseases. Being web-based, the applications can be used without the need to install additional software.

To create this web app, the "Shiny" library from RStudio was utilized. The web app is divided into three main sections: documentation, miRNA-disease interaction, and miRNA-gene interaction. In the documentation section, users can find detailed information on how to use the app and the methods used to collect and analyze the data. Subsequently, the user can choose to explore starting from one or more miRNAs or from one or more diseases. Depending on the selected option and the specified variables, a filtered table will be presented containing the miRNA-disease interactions, along with relevant references to the literature. The same logic will be applied in the third section, where a network will also be available to graphically visualize specific miRNA-gene interactions.

References

1. Cui C, Zhong B, Fan R, Cui Q. HMDD v4.0: a database for experimentally supported human microRNA-disease associations. Nucleic Acids Res. 2024;52(D1):D1327-D1332. doi:10.1093/nar/gkad717
2. Jiang Q., Wang Y., Hao Y., Juan L., Teng M., Zhang X., Li M., Wang G., Liu Y., (2009) miR2Disease: a manually curated database for microRNA deregulation in human disease. Nucleic Acids Res 37:D98-104.
3. Hsu SD, Lin FM, Wu WY, Liang C, Huang WC, Chan WL, Tsai WT, Chen GZ, Lee CJ, Chiu CM, Chien CH, Wu MC, Huang CY, Tsou AP, Huang HD (2011). "miRTarBase: a database curates experimentally validated microRNA-target interactions". Nucleic Acids Research. 39 (Database issue): D163-9. doi:10.1093/nar/gkq1107. PMC 3013699. PMID 21071411.

Landscape on apolipoproteins mutations

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Critical studies have unwaveringly established the importance of peculiar SNPs in APO genes as genetic risk factors for dyslipidemias and its related comorbidities. Low-density lipoproteins (LDL) have different apolipoproteins cargo, depending on their electronegativity levels which in turn can be influenced by SNPs with amino-acid substitution neutral or acidic to basic. It is worth mentioning that even if pharmacological strategies to lower plasma LDL concentrations, the atherosclerotic process persists, highlighting therapeutic inadequacy. Therefore, there is a need to quest to identify molecules that could decrease LDL electronegativity or disturb negative-positive interaction with APO proteins useful in both basic lipid research and clinical medicine. Herein, using in silico approaches we performed an overview of the mutations of the APO proteins (focusing on E and C3) that could better fit with electronegative interactions. The computational tools used included SIFT, PolyPhen-2, FATHMM, SNPs&Go, mCSM, DynaMut2, MAESTROweb, PremPS, MutPred2, and PhD-SNP. The analysis showed that mutations occurring in the minimally frustrated residues of APOE could disrupt stability, affecting the protein's function and potentially contributing to their fit with L5. Therefore APOC3 A43T mutation can be “friend”, while R163C mutation “foe”.

ASO design for the disruption of the NSP1-5'UTR interaction of SARS-CoV-2

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Background: SL1 to SL3 are the most conserved stem-loops of SARS-CoV-2 5' UTR and are involved in its interaction with NSP1, to favour the translation of viral RNA [1].

Two modifications can be found in viral 5'UTR, the pseudouridilation of U54 [2] and m6A modification on A74 [3]; these affect the RNA structure and influence its interaction propensity.

This study aims to design antisense oligonucleotides able to bind the SL1-SL3 stem loops, to affect the recognition and binding of NSP1.

Methods: Methods are depicted in the attached image [4][5][6][7][8][9][10][11].

Results: Among the 51 designed ASOs, eight were selected and docked primarly agains SARS-CoV-2 5'UTR SL1-SL3, in unmodified, single and double (54-Ψ, 74-m6A) modification forms. RNA-ASO complexes were docked against NSP1 in a second step and results were evaluated by MD simulation. Results were compared to ASO4, obtained from literature [5], as reference.

RNA local flexibility is reduced in all complexes bound to ASO_12-36, as well as to ASO4. Binding of ASO_12-36 reduces NSP1 C-term flexibility in all the conditions, while ASO_15-39 in unmodified and 54-Ψ forms, and ASO_43-67 in 74-m6A and double modified forms. Considering complexes conformations, ASO_14-38 and 15-39 bind the NSP1 C-term preventing the formation of the α-helix required for the protein interaction with the host ribosome.

Overall, all proposed ASOs display higher binding affinity for SL1-SL3 5'UTR than reference ASO4. ASOs presence reduce the affinity for NSP1, in particular, ASO_17-41 induce a lower affinity between 5'UTR and NSP1 in all conditions, like ASO4. Instead, if we consider a possible chelating effect of the ASO reducing NSP1 availlability, ASO_15-39 and 43-67 display a high binding energy among the molecules.

Conclusion: All tested ASOs display characteristics potentially usefull for their therapeutic usage. In particular, ASO_12-36 and 14-38 reduce C-term mobility; while, ASO_15-39 and 43-67 display a high binding affinity for NSP1 reducing its availability. Instead, ASO_17-41 interfere with 5'UTR-NSP1 binding. Results will be experimentally validated in cellular models.

References

1. Cell 2020, 183, 1325–1339
2. Molecular Therapy: Nucleic Acids, 2023, 34
3. bioRxiv 2022. 10.1101/2022.10.17.512569
4. RNA Biol. 2021; 18(4): 447–456
5. PNAS, 2022; Vol. 119, Issue 9
6. Nucleic Acids Research, 2004, Vol. 32; Web Server issue: W135–W141
7. Nucleic Acids Research, 2006, Vol. 34(Issue suppl_2): W451–W454
8. PLoS One. 2016; 11(9): e0163454
9. J Chem Theory Comput. 2021 Mar 9; 17(3): 1842–185

10. Nucleic Acids Res. 2017 Jul 3; 45(Web Server issue): W365–W373
11. J Phys Chem Lett. 2020 Nov 19;11(22):9659-9668

Assessing functional signature embeddings for drugs mechanism of action comparison

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Background: Characterizing the mechanisms of action (MoA) of compounds is critical for advancing drug discovery and repurposing efforts. Transcriptomic signatures, which reflect a compound's impact on gene expression, are used widely to assess MoA, thanks to resources like the Connectivity Map (CMap) and the Library of Integrated Network-Based Cellular Signatures (LINCS), which provide extensive transcriptomic datasets for numerous compounds [1]. However, drug transcriptomic signatures can vary significantly across cell lines and conditions, potentially limiting their efficacy in reliably predicting MoA. Additionally, transcriptomic data alone, may be insufficient for accurate MoA description, suggesting the need for integrative approaches.

This study aimed to test the effectiveness of Functional Representation of Gene Signatures (FRoGS) method, which integrates functional information into transcriptomic signatures to compare drugs MoA [2]. Compared to the original application of FRoGS, which was focused on drug target prediction, this analysis assessed the drug distances computed using FRoGS-derived transcriptomic signature embeddings against those obtained from a baseline method based on z-score distance.

Methods: We obtained the LINCS level 5 perturbagen datasets from the LINCS CLUE platform and selected the moderated z-score for 10174 genes classified as Best INferred Genes (BING).

We defined benchmark scenarios for drug-drug similarity assessments involving drugs with shared targets, pathways, as well as genetic perturbagens.

For each drug or genetic perturbagen signature, we computed the FRoGS embedding using the top 200 modulated genes and GO gene embeddings.

Perturbagens MoAs were compared using cosine distance using both raw LINCS z-scores (baseline method) and FRoGS signature embeddings.

Results: In all tested scenarios, the embedding-based distances of drugs sharing the same target and pharmacological action consistently showed lower values compared to z-score-based distances, suggesting that FRoGS approach identifies more subtle similarities between compounds.

Conclusions: The outcomes show that functional integration in signature embeddings improves MoA comparisons. Therefore, this approach could provide a robust framework for embedding-based drug similarity assessments, informing broader drug discovery and repurposing efforts.

References

1. Subramanian A, et al. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell. 2017;171:1437–1452.e17. doi: 10.1016/j.cell.2017.10.049.
2. Chen H, et al. Drug target prediction through deep learning functional representation of gene signatures. Nat Commun. 2024;15:1853. doi: 10.1038/s41467-024-46089-y

Automatic Classification of Units in Tandem Repeat Proteins

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Background and motivation. Tandem repeat proteins (TRPs) are a widespread class of non-globular proteins involved in various biological processes, including cell adhesion and molecular recognition. A TRP consists of Repeat Regions made up of Insertions and Repeat Units, the smallest structural units. Classifying these units is crucial for phylogenetic studies and understanding sequence-structure-function relationships. While many manual classifications exist, automatic classifications are increasingly valuable due to the growing number of TRP structures.

Method and Results. In the talk, we introduce an automatic method to classify units in protein tandem repeats. The method abstracts the units by non-covalent interactions at the atomic level [1] and exploits Algebraic Structural Alignment (ASA) distance to quantify the structural dissimilarity between units [2]. The protein abstraction consists of five chemical interactions extracted by exploiting geometrical parameters. Such an abstraction is formalized as arc-annotated sequences (AASs), a base sequence equipped with arcs representing the chemical interactions. The structural differences between two units are quantified by ASA distance, a computational dissimilarity measure, which maps the AASs into structural trees that formalize arcs and relations among them by exploiting classical tree alignment. The hierarchical Clustering algorithm infers an automatic classification by grouping similar objects into clusters.

The pipeline related to the method is implemented in Python by exploring RING and STAlign, two tools to extract protein interactions and qualify the structural difference based on ASA distance, respectively. Moreover, the pipeline uses the implementation of the Hierarchical Clustering method provided in scikit-learn [3].

The approach has been tested on subsets of molecules of REPEAST DB [4], a database of annotated TRPs, and compared with the TM-ALIGN tool in order to classify the Class and Topology. The results, evaluated with the clustering methods, show that our approach overcomes the results obtained with the TM-ALIGN tool.

Conclusions. This study introduces an automatic annotation method for TRP units that can be extended to other bioinformatics applications.We are working on the method extension by considering other protein abstractions and machine learning approaches combining sequences and structures.

References

1. Clementel, Damiano, et al. "RING 3.0: Fast generation of probabilistic residue interaction networks from structural ensembles." Nucleic Acids Research 50.W1 (2022): W651-W656
2. https://github.com/bdslab/STAlign
3. Scikit-learn: Machine Learning in Python, Pedregosa et al., JMLR 12, pp. 2825-2830, 2011.
4. Paladin, Lisanna, et al. "RepeatsDB in 2021: improved data and extended classification for protein tandem repeat structures

Benchmarking differential expression pipeline from Nextflow

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Background: Recent advancements in genome sequencing have significantly improved our ability to identify gene variants associated with human disorders, yet many patients with rare diseases remain undiagnosed. Sequencing studies allowed the identification of causal variants in 36% of neurodevelopmental disorder (NDD) cases, with an additional 23% classified as variants of uncertain significance (VUS) [1]. In this context, whole-exome sequencing (WES) tends to be the predominant technology employed for diagnosis, yet RNA-sequencing (RNA-seq) has proven to aid the identification of rare-disease variants. Indeed, the latter approach can elucidate disease-related changes in gene expression as well as alternative splicing [2]. Therefore, we sought to validate the use of the nf-core/rnaseq and the nf-core/differentialabundance pipelines on our infrastructure.

Methods: To test the RNAseq pipeline combination, we processed 10 replicates from a previously employed and publicly available human benchmark dataset (from SEQC and ENCODE) containing external ERCC synthetic RNA. Based on methods utilized in a previous study for a similar pipeline combination [4], fold-change in expression was compared between two groups of samples containing different concentrations of the ERCC standard.

Results: With respect to a line mapping expected log2 fold values 1-to-1 (assuming 100% accuracy), the regression line mapping observed to expected log2 fold values is shifted by approximately 0.5. Therefore, the obtained linear regression line established that most fold-change values overestimated the actual ERCC spike-in concentrations.

Conclusion: The differential expression results obtained for the external standard are congruent with a previous analysis of the same samples using a similar pipeline configuration.

References

1. Sanchis-Juan, A., Megy, K., Stephens, J., Armirola Ricaurte, C., Dewhurst, E., Low, K., French, C. E., Grozeva, D., Stirrups, K., Erwood, M., McTague, A., Penkett, C. J., Shamardina, O., Tuna, S., Daugherty, L. C., Gleadall, N., Duarte, S. T., Hedrera-Fernández, A., Vogt, J., … Carss, K. J. (2023). Genome sequencing and comprehensive rare-variant analysis of 465 families with neurodevelopmental disorders. American Journal of Human Genetics, 110(8), 1343–1355. https://doi.org/10.1016/j.ajhg.2023.07.007
2. Montgomery, S. B., Bernstein, J. A., & Wheeler, M. T. (2022). Toward transcriptomics as a primary tool for rare disease investigation. Cold Spring Harbor Molecular Case Studies, 8(2), a006198. https://doi.org/10.1101/mcs.a006198

Bioinformatics analysis of HDL-microRNAs cargo

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Background: The high-density lipoprotein (HDL), are traditionally known for their role in reverse cholesterol transport and cardiovascular protection. Recent findings have identified HDL as a carrier of microRNAs, small non-coding RNAs that regulate gene expression, suggesting a potential novel functional role for HDL-microRNA cargo. Methods: Here we conduct in-depth bioinformatics analysis of HDL-microRNA cargo to uncover their molecular mechanisms and therapeutic potential. First, using Gene Expression Omnibus (GEO), we performed computational analysis on public Taqman Human MicroRNA Array datasets (GSE 25425 platform GPL11162) obtained by highly-purified fractions of HDL, from human plasma (last update date: Mar 25, 2019) in order to identify miRNAs cargo [1]. Results: The cargo was also analysed using a bioinformatics approach to recognize their validated target genes. The plugin "BiNGO" of Cytoscape applied Gene Ontology enrichment analysis. The key genes mainly enriched in the biological process of cellular regulation were identified. Finally, the protein-protein interaction and co-expression network were analysed using the STRING and GeneMANIA Cytoscape plugins. Conclusion: This study explores a novel field of HDL by analyzing their role as carriers of miRNAs, small non-coding RNAs that influence gene expression.

References

1. Vickers, K. C., Palmisano, B. T., Shoucri, B. M., Shamburek, R. D., & Remaley, A. T. (2011). MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nature cell biology, 13(4), 423–433. https://doi.org/10.1038/ncb2210

Chestnut Burrs as Natural Source of Antimicrobial Bioactive Compounds: A Valorisation of Agri-Food Waste

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Background: Currently, one-third of global food production, accounting for 1.3 billion tons, goes wasted due to major humanitarian and environmental challenges. In such a scenario, the circular bioeconomy model stands as an innovative solution by promoting sustainable production, utilizing agri-food waste, and converting non-renewable products into valuable resources.

Methods: Here, the circular bioeconomy concept was applied to a previously obtained chestnut burr extract (agri-food waste) composed of gallic acid, quinic acid, protocatechuic acid, brevifolin carboxylic acid, and ellagic acid to evaluate its antimicrobial activity against four bacterial opportunistic pathogens (Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli).

Results : Our results evidenced a modest but measurable antibacterial activity against Enterococcus faecalis, with a minimum inhibitory concentration (MIC) of 64 μg/mL. In silico studies allowed for identifying the potential molecular target, supporting the underlying antibacterial activity of the active principle and providing useful molecular findings regarding their interaction.

Conclusion: In this study, we show a robust and comprehensive in vitro and in silico pipeline aimed at the identification of novel antibacterial scaffolds taking advantage of agri-food waste.

References

Trezza, A.; Barletta, R.; Geminiani, M.; Frusciante, L.; Olmastroni, T.; Sannio, F.; Docquier, J.-D.; Santucci, A. Chestnut Burrs as Natural Source of Antimicrobial Bioactive Compounds: A Valorization of Agri-Food Waste. Appl. Sci. 2024, 14, 6552

Computational analysis of interfering compounds in the HuR-mRNA interaction

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Background: RNA-binding proteins (RBPs) are post-transcriptional regulators that control RNA localization, stability, and translation. Human Antigen R (HuR) is a RBP that binds to AU-rich elements (AREs) in the 3' untranslated regions (UTRs) of target mRNAs through its three RNA recognition motifs (RRMs). Primarily nuclear, HuR dimerizes through its RRM3 domain in response to various stimuli, stabilizing target mRNAs and facilitating their cytoplasmic export. The nuclear HuR influences splicing and mRNA stability, while cytoplasmic HuR enhances mRNA translation. Dysregulation of HuR is associated with pro-inflammatory phenotypes. Several small-molecule inhibitors are known to inhibit HuR, but their molecular mechanism is not clear. This study aims to identify and characterize the mechanism of HuR-mRNA disruption caused by three known inhibitors. activity.

Methods: Molecular docking simulations of the inhibitors MS-444 (PubChem ID: 132904), SRI-42127 (PubChem ID: 163415849) and STK018404 (Pubchem ID: 57672012) in complex with a 3D full-length HuR model, generated through advanced computational techniques, were performed using Autodock Vina. Three replicas for each HuR-mRNA-inhibitor were performed with Gaussian accelerated Molecular Dynamics (GaMD) simulations of 500 ns duration were conducted for each system. A 500 ns GaMD was also performed for a HuR-mRNA system to use as reference. The MD trajectories were analyzed using modules of the GROMACS 2022 suite. The MM/PBSA method was employed to quantify the free energy of interaction between HuR and the inhibitors.

Results: The results show that all three inhibitors stiffen HuR, specifically within the RRM2-RRM3 interdomain linker and the RRM3 domain (residues 187-322). This flexibility decrease is particularly evident in the collective motions of the first principal component (PC1) extracted from PCA analysis. This result suggests that the reduced flexibility in these regions destabilizes mRNA binding and, consequently, the dimerization process.

Conclusion: Overall, these findings can provide valuable insights for the design of new HuR inhibitors, expanding our understanding of RBPs and their potential therapeutic applications. Further MD simulations and in vitro studies will be employed to validate the effect of these inhibitors on HuR-mRNA interactions.

References

1. Assoni, G., La Pietra, V., Digilio, R., Ciani, C., Licata, N.V., Micaelli, M., Facen, E., Tomaszewska, W., Cerofolini, L., Pérez-Ràfols, A., et al. (2022). HuR-targeted agents: An insight into medicinal chemistry, biophysical, computational studies and pharmacological effects on cancer models. Advanced Drug Delivery Reviews 181, 114088.
2. Brennan, C.M., and Steitz, J.A. (2001). HuR and mRNA stability: CMLS, Cell. Mol. Life Sci. 58, 266–277.
3. Nasti, R., Rossi, D., Amadio, M., Pascale, A., Unver, M.Y., Hirsch, A.K.H., and Collina, S. (2017). Compounds Interfering with Embryonic Lethal Abnormal Vision (ELAV) Protein–RNA Complexes: An Avenue for Discovering New Drugs: Miniperspective. J. Med. Chem. 60, 8257–8267.
4. Ottoz, D.S.M., and Berchowitz, L.E. (2020). The role of disorder in RNA binding affinity and specificity. Open Biol. 10, 200328.
5. [5] Ripin, N., Boudet, J., Duszczyk, M.M., Hinniger, A., Faller, M., Krepl, M., Gadi, A., Schneider, R.J., Šponer, J., Meisner-Kober, N.C., et al. (2019).
6. Molecular basis for AU-rich element recognition and dimerization by the HuR C-terminal RRM. Proc. Natl. Acad. Sci. U.S.A. 116, 2935–2944.
7. R. Salomon-Ferrer, et al.: An Overview of the Amber Biomolecular Simulation Package, WIREs Computational Molecular Science, 2013, 3, 198–210.

8. J. Wang, et al.: Gaussian accelerated molecular dynamics: principles and applications, WIREs Computational Molecular Science, 2021, e1521.
9. D.A. Case, et al.: The Amber biomolecular simulation programs, Journal of Computational Chemistry, 2005, 26, 1668–1688.
10. M.J. Abraham, et al.: Gromacs: High performance molecular simulations through multi-level parallelism from laptops to supercomputers, SoftwareX, 2015, 1–2, 19–25.

Critical assessment of protein intrinsic disorder prediction (CAID) - Round 3

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Protein intrinsic disorder (ID) is a complex phenomenon encompassing a spectrum ranging from fully disordered to folded states with long dynamic regions. The absence of a universally applicable ground truth for all ID variations and the potential for order-to-disorder transitions under specific conditions pose challenges in ID prediction.

The CAID challenge assesses the performance of different ID predictors across diverse benchmarks, utilizing the annotation provided by the DisProt database, manually curated to store the ID regions when experimental evidence is available in the literature.

This study presents the results from Round 3 of the CAID challenge. CAID3 uses unreleased DisProt data as ground truth. DisProt has improved the quality and depth of annotations compared to previous versions, especially the functional annotations associated with ID regions. Over 19 new predictors were submitted for evaluation during this round, with a significant portion utilizing information from protein language models.

The performance of the top method has improved by more than 10% for binding and more than

8% for linker prediction compared to CAID2. The analysis of CAID3 methods shows that the top 10 positions are mainly populated by new methods using embedding from protein language models, demonstrating that embedding information could be helpful in solving tasks related to disordered proteins.

The CAID challenge reveals varying performance among different prediction methods across different benchmarks, emphasizing the ongoing need to develop more versatile and efficient prediction software on one end and extend the benchmarking to all high-quality data available on the other.

References

Conte AD, Mehdiabadi M, Bouhraoua A, Miguel Monzon A, Tosatto SCE, Piovesan D. Critical assessment of protein intrinsic disorder prediction (CAID) - Results of round 2. Proteins. 2023 Dec;91(12):1925-1934. doi: 10.1002/prot.26582. Epub 2023 Aug 25. PMID: 37621223.

Alessio Del Conte, Adel Bouhraoua, Mahta Mehdiabadi, Damiano Clementel, Alexander Miguel Monzon, CAID predictors , Silvio C E Tosatto, Damiano Piovesan, CAID prediction portal: a comprehensive service for predicting intrinsic disorder and binding regions in proteins, Nucleic Acids Research, Volume 51, Issue W1, 5 July 2023, Pages W62–W69, https://doi.org/10.1093/nar/gkad430

Necci M, Piovesan D; CAID Predictors; DisProt Curators; Tosatto SCE. Critical assessment of protein intrinsic disorder prediction. Nat Methods. 2021 May;18(5):472-481. doi: 10.1038/s41592-021-01117-3. Epub 2021 Apr 19. PMID: 33875885; PMCID: PMC8105172.

Deep Diving into Phylogenetic Inference of SARS-CoV-2 Spike Gene

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The continuous evolution of SARS-CoV-2 has led to the emergence of several variants representing significant challenges for public health. Many studies highlight the phylogenetic analysis relevance of either complete genomes (1,2) or the spike gene sequences (3,4) of SARS-CoV-2 as potential method to discriminate among the virus variants. Here we describe a preliminary phylogenetic analysis framework to assess the capacity of SARS-CoV-2 Spike gene amino acid sequences to resolve viral variants evolutionary classification. To this aim, we considered the major variants of concern (VOCs) and the Wuhan-Hu-1 (NCBI-accession: NC_045512.2) reference sequences, as well as those appeared more recently namely XBB, EG and BA. The representative complete viral genome sequences of the VOCs were retrieved from COVID19 data portal 5 , while the sequences of XBB, EG and BA were downloaded from GISAID (6). Using hmmsearch (HMMer 3.3 package) (7) , the spike amino acid sequences HMM profile of references were searched against the genomes of the other variants to annotate and extract the relevant spike region. The extracted amino acid sequences were then aligned by hmmalign (HMMer 3.3 package) (7) against Spike gene references. Using ad-hoc python script the sequences were de-replicated considering only site variations represented by at least one sequence. To conduct the phylogenetic analysis ModelFinder (IQ-TREE package) (8) algorithm was used to obtain the best molecular evolution model. The consensus phylogenetic tree was obtained using Maximum likelihood method in IQ-TREE where node support statistical significance was calculated using non-parametric ultrafast bootstrap analysis with 1000 replicates. The first and preliminary results obtained from this study highlight a potential and important use of Spike gene evolutionary information to rapidly track the virus at molecular level. However, additional investigations are needed, also on bigger datasets, to demonstrate the capacity of Spike gene to substitute the phylogenomic approach with the aim to draw the evolutionary history and relationship of SARS-CoV-2 variants.

References

1. Chakraborty, C., Sharma, A. R., Bhattacharya, M., Agoramoorthy, G., & Lee, S.-S. (2021). 'Evolution, Mode of Transmission, and Mutational Landscape of Newly Emerging SARS-CoV-2 Variants', (S. P. Goff, Ed.)mBio, 12/4: e01140-21. DOI: 10.1128/mBio.01140-21
2. (2)Cui, J., Li, F., & Shi, Z.-L. (2019). 'Origin and evolution of pathogenic coronaviruses', Nature Reviews Microbiology, 17/3: 181–92. DOI: 10.1038/s41579-018-0118-9
3. Glenet, M., Lebreil, A.-L., N'Guyen, Y., Meyer, I., Baud, S., & Andreoletti, L. (2024). 'Structural impact of a new spike Y170W mutation detected in early emerging SARS-CoV-2 Omicron variants in France', Virus Research, 343: 199354. DOI: 10.1016/j.virusres.2024.199354
4. Ito, J., Suzuki, R., Uriu, K., Itakura, Y., Zahradnik, J., Kimura, K. T., Deguchi, S., et al. (2023). 'Convergent evolution of SARS-CoV-2 Omicron subvariants leading to the emergence of BQ.1.1 variant', Nature Communications, 14/1: 2671. DOI: 10.1038/s41467-023-38188-z
5. Harrison, P. W., Lopez, R., Rahman, N., Allen, S. G., Aslam, R., Buso, N., Cummins, C., et al. (2021). 'The COVID-19 Data Portal: accelerating SARS-CoV-2 and COVID-19 research through rapid open access data sharing', Nucleic Acids Research, 49/W1: W619–23. DOI: 10.1093/nar/gkab417; https://www.covid19dataportal.org/
6. https://gisaid.org/
7. http://hmmer.org/

8. Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). 'IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era', (E. Teeling, Ed.)Molecular Biology and Evolution, 37/5: 1530–4. DOI:10.1093/molbev/msaa015

DisProt: The Manually Curated Resource for Intrinsically Disordered Proteins

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Background: DisProt (https://disprot.org/) is the gold standard as a manually curated database of intrinsically disordered proteins (IDPs) and regions (IDRs), along with their functions. A central activity of DisProt is the meticulous annotation of IDPs/IDRs from pertinent research articles, ensuring high quality, accuracy, relevance, and coverage of data.

Methods: To achieve these standards, DisProt has improved its curation processes and engaged community curators. Community curators receive support through regular online training sessions and updated multilingual e-learning courses. In addition, a thorough review process conducted by expert curators helps maintain the reliability of annotations. To ensure consistency, DisProt emphasizes the use of controlled vocabularies, such as Gene Ontology (GO) and Evidence and Conclusion Ontology (ECO), alongside the adoption of standards like the Minimum Information About Disorder (MIADE) guidelines.

Results: In its effort to expand coverage, DisProt focuses on thematic datasets that highlight biological areas where IDPs play key roles. Recent collections include those based on molecular activities, such as RNA-binding proteins, and proteins involved in critical biological processes and diseases, like neurodegenerative disorders. With regular biannual releases, DisProt continually enhances its offerings.

Conclusion: DisProt remains a vital resource for the biocuration and research community, providing standardized and up-to-date information on IDPs across a wide range of organisms.

References

Aspromonte, M. C., Nugnes, M. V., Quaglia, F., Bouharoua, A., Tosatto, S. C., & Piovesan, D. (2024). DisProt in 2024: improving function annotation of intrinsically disordered proteins. Nucleic Acids Research, 52(D1), D434-D441.

Quaglia, F., Chasapi, A., Nugnes, M. V., Aspromonte, M. C., Leonardi, E., Piovesan, D., & Tosatto, S. C. (2024). Best practices for the manual curation of intrinsically disordered proteins in DisProt. Database, 2024, baae009.

DOME Registry: Implementing community-wide recommendations for reporting supervised machine learning in biology

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Background: Supervised machine learning is widely used in biology and deserves more scrutiny. DOME recommendations focus on key aspects including data, optimization, evaluation, and model interpretability, aiming to establish standards of supervised machine learning validation in biology. Formulated as questions, DOME recommendations are intended to enhance the reproducibility and clarity of ML methods, representing an initial iteration of a consensus-based community discussion. In addition to the DOME Registry, a website (https://www.dome-ml.org/) is available for providing continuous updates regarding DOME recommendations.

Methods: DOME Registry, a platform dedicated to accessing and submitting reports on supervised ML publications. Each entry consists of an annotated DOME recommendations report, accompanied by essential article details such as journal, title, authors, DOI, and PubMedID, along with a DOME score and a unique identifier within the registry. Communication between the user interface and the database is facilitated through a REST API, with access regulated through ORCID authentication. By assigning a unique identifier to each publication and providing a DOME score, the registry facilitates the dissemination and adoption of DOME recommendations among data scientists and practitioners in biological science fields. The registry integrates external resources like ORCID, Data Stewardship Wizard (DSW) and APICURON enhancing user access management and data input. Through a user-friendly web interface, users can search, browse, and submit annotations, while administrators have full control over managing and publishing annotations.

Results: We present the database structure, implementation details, and a use case demonstrating the integration of DOME recommendations and Registry into the article publishing process. The DOME Registry serves as a vital resource for advancing reproducibility and transparency in ML applications within biological sciences.

Conclusion: As the field of ML in biological sciences continues to grow, there is an increasing need for standardized reporting practices to ensure that ML is transparent and reproducible. The DOME recommendations aim to enhance the reproducibility and clarity of ML methods, serving as an initial framework for a consensus-based community discussion.

References

1. Attafi, O. A., Clementel, D., Kyritsis, K., Capriotti, E., Farrell, G., Fragkouli, S. C., ... & Tosatto, S. C. (2024). DOME Registry: Implementing community-wide recommendations for reporting supervised machine learning in biology. arXiv preprint arXiv:2408.07721.
2. Walsh, I., Fishman, D., Garcia-Gasulla, D. et al. DOME: recommendations for supervised machine learning validation in biology. Nat Methods 18, 1122–1127 (2021). https://doi.org/10.1038/s41592-021-01205-4

Effects of mutated TNPO3 in LGMDD2 Zebrafish Model

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Bachground: Limb-Girdle Muscular Dystrophy Type 2 (LGMDD2) is a rare autosomal dominant neuromuscular disease caused by mutation in the TNPO3 gene, encoding Transportin-3, which facilitates nuclear protein transport like SR proteins1, essential in RNA metabolism and splicing2. The role of TNPO3 in disease mechanisms and its effects on myogenesis, regulated by muscle-specific myomiRs, remains unclear3. For this purpose, we developed a mutated TNPO3 in vivo model based on zebrafish (Danio rerio)4 and analyzed myogenic regulatory factors genes (MRF such as myf5, myod, myog, mylpfa, smyhc1)5, muscle proteins (α-actinin, MyHC, SRSF1), and key myomiRs (miR-1, miR-206, miR-133a, miR-133b)6 to better understand TNPO3 role in muscle formation and disease mechanism.

Methods: LGMDD2 in vivo model was developed by microinjecting wild-type (WT) or mutated (MUT)7 human TNPO3 mRNAs into zebrafish embryos. Embryos were maintained at 24 hour post fertilization (hpf) and 48 hpf, including non-injected embryos as controls (NI). After RNA and protein extractions, RNA and protein analysis were performed by RT-qPCR and western blotting respectively.8

Results: Myf5 and myod gene expressions, typically active in early-to-mid myogenesis and deactivated soon after, were alterated in WT and MUT zebrafish. In particular, at 48 hpf, myf5 and myod levels significantly increased in WT and/or MUT samples, while myog expression at 24 hpf, relative to NI at same stage. However, mylpfa levels significantly decreased in WT embryos at 24 hpf, while smyhc1 significantly increased at 48 hpf. Protein analysis showed a significant increase of α-actinin and SRSF1 in 48 hpf MUT embryos, and MyHC in all different condition. Finally, an upregulation of miR-206, miR-1 and miR-133a (not miR-133b) in MUT embryos at 24 hpf, with a decrease in all four myomiRs (significant for miR-206) in WT embryos at the same hpf were observed; while at 48 hpf, MUT embryos showed decreased miR-206 and miR-1 levels and increased miR-133a and miR-133b levels compared to NI and WT embryos, where myomiR levels were similar.

Conclusion: These findings suggest that TNPO3 mutation affects myogenesis, indicating its contribution in LGMDD2 pathogenesis. Additionally, our zebrafish model methodology could advance understanding of TNPO3 function in muscle formation and the mechanisms underlying LGMDD2.

References

1. Maertens et al.Proc Natl Acad Sci U S A. 2014;111(7):2728-33
2. Costa R et al. Front Neurol. 2022;13:840683
3. Poyatos-García et al. Molecular therapy. Nucleic acids. 2023; vol. 31 324-338
4. Bradford et al. ILAR journal. 2017; vol. 58,1: 4-16

5. Sandmann et al. Developmental Cell. 2006; Volume 10, Issue 6 Pages 797-807
6. Horak M et al. Dev Biol. 2016;410:1
7. Palenzuela et al. Neurology. 2003; 61:404–6; Gamez et al. Neurology. 2001; 56:450–4
8. Costa et al. Molecular and cellular biochemistry.2021;vol.476,4:1797-1811

The role of bioninformatics in identifying anticancer potential of Avermectin compounds via Molecular Docking

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While avermectins are traditionally known for their antiparasitic properties, recent research has indicated their potential role as anticancer agents by disrupting microtubule dynamics. This study provides a novel, detailed molecular docking analysis of avermectin compounds against β-tubulin, elucidating interactions that may contribute to their high anticancer potential. Using advanced in silico methodologies, including the CB-Dock 2 platform and PyMol visualization, we discovered that specific avermectins (notably ivermectin B1a, selamectin, and doramectin) exhibit exceptionally high binding affinities to tubulin, with binding scores surpassing that of taxol (-18.0, -9.1, and -8.9 kcal/mol, respectively). This analysis uniquely identifies key β-tubulin residues involved in the binding of avermectin compounds, suggesting these compounds may stabilize microtubules via a mechanism distinct from conventional taxanes. Our findings underscore the therapeutic promise of avermectins as alternative anticancer agents, offering new insights into their binding modes and laying the groundwork for future structural studies and preclinical testing.

References

Ashraf, S., Beech, R. N., Hancock, M. A., & Prichard, R. K. (2015). Ivermectin binds to Haemonchus contortus tubulins and promotes stability of microtubules. International Journal for Parasitology, 45(9–10), 647–654. https://doi.org/10.1016/J.IJPARA.2015.03.010

Ashraf, S., & Prichard, R. (2016). Ivermectin exhibits potent anti-mitotic activity. Veterinary Parasitology, 226, 1–4. https://doi.org/10.1016/J.VETPAR.2016.06.015

Batiha, G. E. S., Alqahtani, A., Ilesanmi, O. B., Saati, A. A., El-Mleeh, A., Hetta, H. F., &Beshbishy, A. M. (2020a). Avermectin derivatives, pharmacokinetics, therapeutic and toxic dosages, mechanism of action, and their biological effects. Pharmaceuticals, 13(8), 1–37. https://doi.org/10.3390/ph13080196

C. Campbell, W. (2012). History of Avermectin and Ivermectin, with Notes on the History of Other Macrocyclic Lactone Antiparasitic Agents. Current Pharmaceutical Biotechnology, 13(6), 853–865. https://doi.org/10.2174/138920112800399095

Exploring Microbial Communities in PDO and Non-PDO Parmigiano Reggiano Using Shotgun Metagenomics

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Background: Microbial communities in long-ripened hard cheeses, such as Parmigiano Reggiano, shape the product's organoleptic qualities through complex metabolic interactions(1), notably involving Lactic Acid Bacteria (LAB)(2-3). Characterizing these microbial communities is crucial for understanding species interactions during ripening and identifying potential differences influenced by the cheese's origin, especially in Protected Designation of Origin (PDO) cheeses. This study uses shotgun metagenomics to examine microbial compositions in 12-month ripened PDO and non-PDO Parmigiano Reggiano, aiming to uncover taxonomic structures and pinpoint distinct microbial profiles between these categories.

Methods: 15 PDO and 15 non-PDO 12-month ripened Parmigiano Reggiano cheese samples were selected for this study (the earliest age for commercial marketing). To represent the cheese's microbial load accurately, aliquots were obtained from the core, middle, and near-rind areas of each sample. Each sample was homogenized with 100 ml of 2% tri-sodium citrate in 5 g of cheese, and DNA was extracted using the Maxwell® RSC PureFood Pathogen Kit on a Maxwell® RSC DNA/RNA extractor. Total DNA was quantified via the QuantiFluor® ONE dsDNA System (Promega, USA). Sequencing was conducted on the Illumina NovaSeq X Plus PE150 platform, providing approximately 9 gigabases per sample.

FASTQ files from shotgun sequencing were trimmed with Adapter Removal software(4) and analyzed in Bowtie2(5) to exclude Bos taurus sequences. MetaPhlAn4(6) was then applied to assess microbial composition down to the species level.

Results: Both PDO and non-PDO samples were dominated by LAB, with Lactobacillus delbrueckii, Lactobacillus helveticus, Lacticaseibacillus rhamnosus, Lacticaseibacillus paracasei, and Streptococcus thermophilus as the most prevalent species. Relative abundances varied among samples, with two non-PDO samples exhibiting high levels of Streptococcus uberis (76%) and Lactococcus carnosus (36%).

Conclusions: Shotgun metagenomics effectively captures the complexity of microbial communities in cheese, providing insights into both taxonomic composition and functional potential(7-8). Here, LAB dominated across samples with notable inter-sample variations. Further genome reconstruction and functional analyses will be essential to unravel interaction networks within these communities and assess their influence on the ripening process.

References

1. Bottari, B., Levante, A., Bancalari, E., Sforza, S., Bottesini, C., Prandi, B., De Filippis, F., Ercolini, D., Nocetti, M., & Gatti, M. (2020). The Interrelationship Between Microbiota and Peptides During Ripening as a Driver for Parmigiano Reggiano Cheese Quality. Frontiers in microbiology, 11, 581658.
2. Neviani, Erasmo, Alessia Levante, and Monica Gatti. 2024. "The Microbial Community of Natural Whey Starter: Why Is It a Driver for the Production of the Most Famous Italian Long-Ripened Cheeses?" Fermentation 10, no. 4: 186. https://doi.org/10.3390/fermentation10040186
3. Gatti, M., Bottari, B., Lazzi, C., Neviani, E., & Mucchetti, G. (2013). Invited review: Microbial evolution in raw-milk, long-ripened cheeses produced using undefined natural whey starters. Journal of Dairy Science, 97(2), 573–591. https://doi.org/10.3168/jds.2013-7187
4. Schubert, M., Lindgreen, S., & Orlando, L. (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Research Notes, 9(1). https://doi.org/10.1186/s13104-016-1900-2

5. Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. Nature Methods, 9(4), 357–359. https://doi.org/10.1038/nmeth.1923
6. Blanco-Miguez, A., Beghini, F., Cumbo, F., McIver, L. J., Thompson, K. N., Zolfo, M., Manghi, P., Dubois, L., Huang, K. D., Thomas, A. M., Piccinno, G., Piperni, E., Punčochář, M., Valles-Colomer, M., Tett, A., Giordano, F., Davies, R., Wolf, J., Berry, S. E., . . . Segata, N. (2022). Extending and improving metagenomic taxonomic profiling with uncharacterized species with MetaPhlAn 4. bioRxiv (Cold Spring Harbor Laboratory). https://doi.org/10.1101/2022.08.22.504593
7. Walsh, A. M., Macori, G., Kilcawley, K. N., & Cotter, P. D. (2020). Meta-analysis of cheese microbiomes highlights contributions to multiple aspects of quality. Nature Food, 1(8), 500–510. https://doi.org/10.1038/s43016-020-0129-3
8. Somerville, V., Berthoud, H., Schmidt, R. S., Bachmann, H., Meng, Y. H., Fuchsmann, P., Von Ah, U., & Engel, P. (2021). Functional strain redundancy and persistent phage infection in Swiss hard cheese starter cultures. The ISME Journal, 16(2), 388–399. https://doi.org/10.1038/s41396-021-01071-0

Functional characterization and network analysis of genes associated with neurodevelopmental disorders

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Background: Neurodevelopmental disorders (NDDs) represent a complex group of conditions characterised by impairments in brain functions that affect at various levels the social, motor and cognitive abilities of the patient [1]. Despite the growing recognition of these disorders, due to their genetic heterogeneity and broad phenotypic spectrum [2], significant challenges remain in understanding the complex molecular mechanism underlying them, hindering the advancement of diagnostic processes and the development of therapeutic strategies.

Methods: This work involved a comprehensive multi-step approach to identify genes associated with NDDs. We first integrated genes related to NDDs from several databases, including: i) Orphanet [3], ii) SFARI [4] and iii) gene lists retrieved from recent publications on NDDs [5, 6]. Then, after homogenising the data, a score based on the consistency of annotations across the different sources was used to divide it into different sets with varying degree of association to NDDs. The obtained dataset was then subjected to a gene enrichment analysis exploiting EnrichR [7], in order to pinpoint overrepresented functions, processes, and pathways. In the last step, a network analysis was performed to examine protein-protein interactions derived from IntAct [8] and assess the roles of key nodes within the network.

Results: We retrieved 8,611 genes related to NDDs, of which 2,323 showed a particular strong association. The gene enrichment analysis identified several enriched biological processes and pathways related to nervous system development, synaptic processes, t-RNA aminoacylation and, somewhat surprisingly, cancer. Meanwhile, the network analysis highlighted highly connected proteins in our networks, with the main ones in the set with the strongest association to NDDS being MAPT, LRRK2, and CUL3, identifying them as potential targets for further research.

Conclusion: This work provides new insights into the complex genetic landscape of NDDs, by advancing the understanding of NDD-associated genes and their interactions, offering a foundation for future research aimed at improving diagnostic accuracy and developing targeted therapies for these challenging disorders. Moreover, the suggested shared biological basis between NDDs and cancer, recently proposed by a few other authors as well [9], offers an intriguing subject for future research.

References

1. Doernberg E, Hollander E. Neurodevelopmental Disorders (ASD and ADHD): DSM-5, ICD-10, and ICD-11. CNS Spectr. 2016;21:295–9.
2. Morris-Rosendahl DJ, Crocq M-A. Neurodevelopmental disorders-the history and future of a diagnostic concept. Dialogues Clin Neurosci. 2020;22:65–72.
3. Rath A, Olry A, Dhombres F, Brandt MM, Urbero B, Ayme S. Representation of rare diseases in health information systems: The orphanet approach to serve a wide range of end users. Hum Mutat. 2012;33:803–8.
4. Abrahams BS, Arking DE, Campbell DB, Mefford HC, Morrow EM, Weiss LA, et al. SFARI Gene 2.0: a community-driven knowledgebase for the autism spectrum disorders (ASDs). Mol Autism. 2013;4:36.
5. Leblond CS, Le T-L, Malesys S, Cliquet F, Tabet A-C, Delorme R, et al. Operative list of genes associated with autism and neurodevelopmental disorders based on database review. Mol Cell Neurosci. 2021;113:103623.
6. Sanchis-Juan A, Megy K, Stephens J, Armirola Ricaurte C, Dewhurst E, Low K, et al. Genome sequencing and comprehensive rare-variant analysis of 465 families with neurodevelopmental disorders. Am J Hum Genet. 2023;110:1343–55.
7. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. 2013;14:128.

8. Orchard S, Ammari M, Aranda B, Breuza L, Briganti L, Broackes-Carter F, et al. The MIntAct project--IntAct as a common curation platform for 11 molecular interaction databases. Nucleic Acids Res. 2014;42 Database issue:D358-63.
9. Nussinov R, Yavuz BR, Demirel HC, Arici MK, Jang H, Tuncbag N. Review: Cancer and neurodevelopmental disorders: multi-scale reasoning and computational guide. Front Cell Dev Biol. 2024;12.

Harnessing chromosomal instability to uncover novel therapeutic vulnerabilities of colorectal cancer stem cells

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Background. Cancer stem cells (CSCs) are poorly differentiated cancer cells driving tumor initiation, propagation, and dissemination and contributing to therapy resistance and relapse1,2. Recent evidence indicates that CSCs exhibit significant plasticity and heterogeneity, at least in part due to their tendency to replication defects (so-called replication stress) and chromosomal instability (CIN)3.

Methods. To this end, we take advantage of colorectal cancer patient-derived models enriched for CSCs (spheroids) or derived from CSCs (organoids or tumoroids). We modulate CIN through experimental strategies, including whole genome duplication (WGD) and mitotic checkpoint abrogation via MPS1 inhibitors. Then, we perform RNA-seq to characterize primary spheroids/organoids with distinct CIN levels for their transcriptional and epigenetic profiles.

Results. Preliminary findings suggest that CIN induces transcriptional rewiring in CSCs, triggering an intracellular stress response that facilitates CSC adaptation. Specifically, in primary spheroids, we observe a correlation between WGD and dysregulation of immune response pathways and several epigenetic factors, particularly of the MLL histone methyltransferase complex.

Conclusion. By integrating complementary approaches, we are unveiling the transcriptional impact of CIN in CSCs and identifying specific vulnerabilities for the development of new strategies to eradicate colorectal cancer.

References

1. Yu Z, et al. Int J Biochem Cell Biol. 2012;44(12):2144-51
2. Dalerba P, et al. Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A. 2007 ;104(24):10158-63.
3. Loh JJ and Ma S. Hallmarks of cancer stemness. Cell Stem Cell. 2024;31(5):617-639.

Structural and fuctional characterization of Klebsiella p. phage depolymerase

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Antimicrobial resistance (AMR) is nowadays a significant public health issue. A group of six pathogens—Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.—collectively known as "ESKAPE" can evade conventional antibiotics, and the World Health Organization predicts their impact will grow in the coming decades. This has driven global scientific efforts to find ways to prevent infections caused by multidrug-resistant ESKAPE bacteria.

Among these, Klebsiella pneumoniae is particularly challenging; this bacterium uses various mechanisms to resist immune responses and antibiotics. A crucial part of its defense is the cell envelope, which includes capsular polysaccharides (CPS) and lipopolysaccharides (LPS). Bacteriophages, which naturally target bacteria, possess enzymes called depolymerases that can break down CPS, suggesting potential use in weakening bacterial defenses. As engineered proteins, these depolymerases constitute a promise for biotechnology and medicine. My research aims to understand their structural characteristics and interactions to develop new approaches against hypercapsulated, multidrug-resistant bacteria like Klebsiella. X-ray crystallography is a valuable technique for revealing protein structures, but it requires specific crystallization conditions, which can be a major limitation. Other techniques with a key-role in macromolecules structure determination like NMR and Cryo-EM also have their challenges. Combining these methods with computational models can help overcome these hurdles. Advances in artificial intelligence, such as AlphaFold, have significantly sped up progress in structural biology and drug discovery.

In a recent study on the depolymerase KP34gp57, we used single-wavelength anomalous diffraction for crystal resolution, which involved complex preparation and crystallization. However, AI tools could now simplify this process. For example, using Molecular Replacement with AlphaFold's model could help to resolve crystal structures without extensive experimental work.The previous unavailability of a proper search model was due to the extremely low sequence identity (<15%) of this enzyme with proteins of known structure. Our findings underscore the importance of computational methods in complementing experimental research, providing deeper insights into the molecular processes involved in disease.

References

1. Mulani M.S., Kamble E.E., Kumkar S.N., Tawre M.S., Pardesi K.R. (2019) Front Microbiol. 10:539.
2. De Oliveira D.M.P., Forde B.M., Kidd T.J., Harris P.N.A., Schembri M.A., Beatson S.A., Paterson D.L., Walker M.J. (2020) Clin Microbiol Rev. 33(3).
3. Majkowska S.G., Łątka A., Berisio R., Maciejewska B., Squeglia F., Romano M., Lavigne R., Struve C., Drulis-Kawa Z. (2016) Viruses. 8(12):324.
4. Majkowska-Skrobek G, Latka A, Berisio R, Squeglia F, Maciejewska B, Briers Y, Drulis-Kawa Z. (2018) Front Microbiol. 9:2517.
5. Maciejewska B., Squeglia F., Latka A., Privitera M., Olejniczak S., Switala P., Ruggiero A., Marasco D., Kramarska E., Drulis-Kawa Z., Berisio R. (2023) mBio 14(5).

Impact of Imidazolium-Based Ionic Liquids on Carbnic Anhydrase II: Insights from Molecular Dynamics

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Carbonic anhydrases (CA) are gaining attention in industrial biotechnology for CO_2 capture and conversion, offering an efficient biocatalytic solution to reduce greenhouse gas emissions. However, CA industrial application requires stabilizing enzyme structure and activity under operational conditions, which involves optimizing process parameters and exploring alternative solvents like ionic liquids (ILs) for a more favourable environment. Therefore, the objective of this study was to evaluate the impact of imidazolium-based ionic liquids (ILs) on the structure and activity of carbonic anhydrase type II (CA-II) through Molecular Dynamics (MD). The MD simulations were conducted using bovine CA-II in the presence of ILs ([C4mim][Tos] and [C4mim][HSO4]) at a concentration of 0.5M. All calculations were performed using Yasara (version 21.12.19) with the AMBER14 force field. The validation of MD results obtained was carried out using CA activity assays. The stability of CA was analysed by Root Mean Square Deviation (RMSD), Radius of Gyration (Rg), Root Mean Square Fluctuation (RMSF) and Solvent Accessible Surface Area (SASA). According to results obtained, CA showed highest stability in presence of [C4mim][Tos], that display similar values of RMSD, Rg, RMSF and SASA with the system without IL. However, highest values were found in presence of [C4mim][HSO4], indicating the impact of IL on CA-II structure and flexibility. In fact, the values of enzymatic activity measured showed the improvement of CA-II activity in presence of [C4mim][Tos] (306%). On the other hand, a strong inhibition of were founded with [C4mim][HSO4] (0.37%). Based on MD calculations and enzymatic activity, it can be concluded that CA-II microenvironment was altered by interaction with the ILs. These findings suggest that MD simulations could be useful tool for elucidating and predicting the behaviour of other ILs in CA-II activity to improve CO2 capture.

In silico characterization of different Phytophthora capsici isolates infecting different crops to identify host-specific molecular signatures, and revealing structural and functional variability in their effectors

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Phytophthora capsici is an important plant pathogen, which poses a serious threat to many important crop species including vegetable, fruit and forest crops worldwide. It shows an extensive variability in their host range and virulence patterns, which makes associated disease management practices very difficult. This study utilized various sequence-based molecular signatures including SNPs localized within the ITS1-5.8S rRNA-ITS2 regions and Ras-related GTP binding protein (Ypt1) genes to evaluate the haplotype variability and phylogenetic relationship between different P. capsici isolates infecting different crops worldwide, having significant epidemiological implications. Moreover, various effector protein sequences including 101 RxLR, were studied to reveal their functionally associated sub-cellular organelles, signal peptide's cleavage sites and peptide disorder patterns, to determine their potential roles in pathogenicity. Twelve different ITS-based evolutionary lineages specific to particular hosts and/or geographical regions and differed by several SNPs, and 30 haplotypes with maximum diversity in India and Pakistan, were identified in this study. It includes country-specific SNP lineages Pc-I and Pc-II in India with multiple host ranges, capsicum-specific lineages Pc-IV, Pc-V and Pc-VIII from Pakistan; papaya-specific lineages Pc-VI from China, and watermelon-specific lineages Pc-VII and Pc-XI from USA. Many lineages showed cross-border infections such as Pc-III and Pc-X in India and Pakistan, Cocoa-specific lineage Pc-IX in Brazil and Cote d'Ivoire, and Capsicum-specific multi-continental lineage Pc-XII. Similarly, 11 different SNP-lineages and 32 haplotypes with maximum diversity in China were identified for the Ypt1 gene. Sequence analysis of the RxLR effectors showed variability in their functionally targeted organelles in the host plants such as most of the RxLRs function in the cytoplasm with lowest intrinsically distorted peptide regions, while the effector DVH05_001149 containing mitochondria-specific signal peptide, and DVH05_00143, PcREK6, Avh1, RxLR145, DVH05_011137 and DVH05_010186 effectors with nuclear localization signals, had maximum intrinsically distorted peptide regions. Some of the RxLRs also contained trans-membrane signals such as DVH05_004789 and DVH05_010188, which could have a crucial role in pathogen recognition by host plants. These findings would be very useful for epidemiological surveillance of P. capsici strains worldwide, understanding population and evolutionary dynamics, and also deciphering the molecular basis of effector-specific disease development phenomenon in different host plants.

References

Blair, J.E., Coffey, M.D., Park, S.Y., Geiser, D.M. and Kang, S. (2008). A multi-locus phylogeny for Phytophthora utilizing markers derived from complete genome sequences. Fungal Genet Biol, 45(3): 266–277.

Chakraborty, A., Hussain, A. and Sabnam, N. (2023). Uncovering the structural stability of Magnaporthe oryzae effectors: a secretome-wide in silico analysis. J Biomol Struct Dyn, 1-22.

Chen, Y. and Roxby, R. (1996). Characterization of a Phytophthora infestans gene involved in vesicle transport. Gene. 181(1-2):89–94.

Kumar, S. and Singh, A. (2024). In silico analysis to understand genetic variability, phylogenetic and phylogeographic relationships between Phytophthora capsici isolates infecting various crops. Archives of Phytopathology and Plant Protection, 1-22.

Inferring microbial ecological networks from metagenomics data, when should we trust the results?

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Background: The interaction network of a microbial community plays a fundamental role in determining community functionality and evolution. Given the importance of microbial communities in various fields [1-3] much effort has been devoted to inferring these interaction networks, a necessary requirement to modify the community functionality to obtain a desired output or restore from a dysbiosis.

Many methods have been proposed to infer these interaction networks from quantitative metagenomics data; however, they have found scarce applications in real-world studies due to concerns about their reliability. Assessing tool performance is challenging, mainly because of the lack of a ground truth dataset for evaluation. To solve this problem, in this study, we use a new microbial community simulator [4] to obtain a synthetic ground-truth and exploit it to extensively benchmark inference methods.

Methods: To simulate the ground truth network and the corresponding metagenomics count data, we used N2SIMBA simulator [4]. N2SIMBA allows to set a network topology, then use it to model bacteria interactions, simulates the evolution of the bacterial community and generates the corresponding 16S count table.

We benchmarked 18 inference methods [5-13] across 210 simulated scenarios, varying network topology, number of species, number of samples, etc. We then assessed methods' performance by measuring precision, recall and MCC, evaluating the resemblance of the inferred network to the true one.

Results: We observed that method's performance increases with the number of samples, while performance drops sharply as the network density increases. This is likely due to the sparsity assumption made by the methods which limits the number of inferred edges causing low recall in denser networks. Interestingly, network topology determines statistically different performance for some methods, in particular straggling with power law degree distributions.

Conclusions: From this extensive benchmark, we can conclude that microbial interaction inference methods show in general poor performances and that they struggle to reproduce the true network topology. We identified a narrow field of application: high number of samples and low node degree, where some of the methods achieve good performance. Outside this field of applications methods performance drops sharply and inference results are not reliable.

References

1. Kaijian Hou, Zhuo-Xun Wu, Xuan-Yu Chen, et al. Microbiota in health and diseases. Signal Transduction and Targeted Therapy, 7:135, 2022.
2. Berg G, Rybakova D, Grube M, Koberl M. The plant microbiome explored: implications for experimental botany. Journal of Experimental Botany. 2015;67(4):995– 1002. doi:10.1093/jxb/erv466.
3. Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, et al. Structure and function of the global ocean microbiome. Science. 2015;348(6237):1261359.doi:10.1126/science.1261359.
4. Baldan Matteo, Ada Rossato, Marco Cappellato, Giacomo Baruzzo, and Barbara di Camillo. N2BPC: an algorithmic approach from Networks to Bacteria's metabolite Production and Consumption. In Proceedings of the 18th Conference on Computational Intelligence Methods for Bioinformatics and Biostatistics, 2023.

5. Friedman J, Alm EJ. Inferring Correlation Networks from Genomic Survey Data. PLOS Computational Biology. 2012;8(9):1–11. doi:10.1371/journal.pcbi.1002687.
6. Fang H, Huang C, Zhao H, Deng M. CCLasso: correlation inference for compositional data through Lasso. Bioinformatics. 2015;31(19):3172–3180. doi:10.1093/bioinformatics/btv349.
7. 26. Kurtz ZD, Muller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA. Sparse and Compositionally Robust Inference of Microbial Ecological Networks. PLOS Computational Biology. 2015;11(5):1–25. doi:10.1371/journal.pcbi.1004226.
8. Yoon G, Gaynanova I, Muller CL. Microbial Networks in SPRING - Semi-parametric Rank-Based Correlation and Partial Correlation Estimation for Quantitative Microbiome Data. Frontiers in Genetics. 2019;10.doi:10.3389/fgene.2019.00516
9. Fang H, Huang C, Zhao H, Deng M. gCoda: Conditional Dependence Network Inference for Compositional Data. Journal of Computational Biology. 2017;24(7):699–708. doi:10.1089/cmb.2017.0054.
10. Tackmann J, Matias Rodrigues JF, von Mering C. Rapid Inference of Direct Interactions in Large-Scale Ecological Networks from Heterogeneous Microbial Sequencing Data. Cell Systems. 2019;9(3):286–296.e8. doi:https://doi.org/10.1016/j.cels.2019.08.002.
11. Jiang S, Xiao G, Koh AY, Chen Y, Yao B, Li Q, et al. HARMONIES: A Hybrid Approach for Microbiome Networks Inference via Exploiting Sparsity. Frontiers in Genetics. 2020;11. doi:10.3389/fgene.2020.00445.
12. Ha MJ, Kim J, Galloway-Pe˜na J, Do KA, Peterson CB. Compositional zero-inflated network estimation for microbiome data. BMC Bioinformatics. 2020;21:581. doi:10.1186/s12859-020-03911-w.
13. Lin H, Eggesbø M, Peddada SD. Linear and nonlinear correlation estimators unveil undescribed taxa interactions in microbiome data. Nature Communications. 2022;13:4946. doi:10.1038/s41467-022-32243-x

In-silico investigation of nonsynonymous single nucleotide polymorphisms in BCL2 apoptosis gene to design novel protei-based drug against cancer

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Urtica dioica (stinging nettle) has been traditionally used in Chinese medicine for the treatment of joint pain and rheumatoid arthritis. This study aims to elucidate the active compounds and mechanisms by which it acts against gout arthritis (GA). Gout-related genes were identified from the DisGeNet, GeneCards, and OMIM databases. These genes may play a role in inhibiting corresponding proteins targeted by the active compounds identified from the literature, which have an oral bioavailability of ≥ 30% and a drug-likeness score of ≥ 0.18. A human protein-protein interaction network was constructed, resulting in sixteen clusters containing plant-targeted genes, including ABCG2, SLC22A12, MAP2K7, ADCY10, RELA, and TP53. The key bioactive compounds, apigenin-7-O-glucoside and kaempferol, demonstrated significant binding to SLC22A12 and ABCG2, suggesting their potential to reduce uric acid levels and inflammation. Pathway enrichment analysis further identified key metabolic pathways involved, highlighting a dual mechanism of anti-inflammatory and urate-lowering effects. These findings underscore the potential of U. dioica in targeting multiple pathways involved in GA, combining traditional medicine with modern pharmacology. This integrated approach provides a foundation for future research and the development of multi-target therapeutic strategies for managing gout arthritis.

References

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INTERACT: A novel approach for continuous Genotype-Phenotype association analysis

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Genetic variants are associated with a wide array of diseases and physical traits across diverse populations [1]. However, associating rare variants with phenotypes remains a significant challenge due to limited statistical power, often necessitating large sample sizes [2], [3]. Existing tools focus on dichotomous phenotypes and utilize linear models, while only a few addresses continuous phenotypes [4], [5], which can restrict flexibility and introduce bias in the analysis. To address these limitations, we propose INTERACT, an innovative method that leverages on ABACUS [6] set-based analysis to enhance the detection of associations with continuous phenotypes, improving our understanding of genetic contributions to complex traits.

INTERACT provides a method for linking single nucleotide polymorphisms (SNPs) to continuous phenotypes by the distribution of the phenotype derived from actual data to the genotype distribution. In particular, a bivariate statistic is designed to measure the strength of association between SNP pairs, assessing how well the phenotype distribution for specific genotypes matches the phenotype distribution. This statistic is represented as a graph adjacency matrix, where nodes represent SNPs and edges indicate the strength of associations, facilitating efficient exploration of SNP relationships. To handle large datasets efficiently, INTERACT leverages parallel computation and optimized memory usage, allowing large SNP-sets without increasing memory footprint. To reduce the false positives rates, INTERACT employs a Bonferroni correction, adjusting the significance threshold based on the number of tests conducted. It also uses bootstrapping to generate null hypotheses, ensuring reliable statistical results while remaining scalable, making INTERACT a useful tool for uncovering the genetic basis of complex traits.

Preliminary results indicate that INTERACT effectively controls false positive rates in SNP-set associations, demonstrating a conservative approach due to the Bonferroni correction. The empirical type I error rate at the SNP level closely aligns with the overall SNP-set size, reinforcing the method's robustness in association testing.

In conclusion, INTERACT is a valuable tool for associating SNPs with continuous phenotypes, addressing limitations of existing methods. By effectively controlling false positive rates and offering scalable performance, INTERACT enhances the reliability of genetic association studies and supports exploration of the genetic basis of complex traits.

References

1. P. P. Kuksa et al., "Scalable approaches for functional analyses of whole-genome sequencing non-coding variants," Hum Mol Genet, vol. 31, no. R1, p. R62, Oct. 2022, doi: 10.1093/HMG/DDAC191.
2. M. C. Wu, S. Lee, T. Cai, Y. Li, M. Boehnke, and X. Lin, "Rare-Variant Association Testing for Sequencing Data with the Sequence Kernel Association Test," The American Journal of Human Genetics, vol. 89, no. 1, pp. 82–93, Jul. 2011, doi: 10.1016/J.AJHG.2011.05.029.
3. P. F. O'Reilly et al., "MultiPhen: joint model of multiple phenotypes can increase discovery in GWAS," PLoS One, vol. 7, no. 5, p. e34861, May 2012, doi: 10.1371/journal.pone.0034861.
4. H. R. Morris, H. Houlden, and J. Polke, "Whole-genome sequencing," Pract Neurol, vol. 21, no. 4, p. 322, Aug. 2021, doi: 10.1136/PRACTNEUROL-2020-002561.
5. C. Eitan et al., "Whole-genome sequencing reveals that variants in the Interleukin 18 Receptor Accessory Protein 3'UTR protect against ALS," Nat Neurosci, vol. 25, no. 4, pp. 433–445, Apr. 2022, doi: 10.1038/S41593-022-01040-6.

6. B. Di Camillo, F. Sambo, G. Toffolo, and C. Cobelli, "ABACUS: an entropy-based cumulative bivariate statistic robust to rare variants and different direction of genotype effect," Bioinformatics, vol. 30, no. 3, pp. 384–391, Feb. 2014, doi: 10.1093/BIOINFORMATICS/BTT697.

Molecular basis of the crosstalk between energy-stressed adipocytes and breast cancer cells

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Background: Obesity is a risk factor for breast cancer and a predictor of poor prognosis, and in literature there is evidence suggesting that the adipocyte secretome, particularly in the form of extracellular vesicles, play a key role in the crosstalk between adipocytes and cancer by promoting cancer cell growth and invasiveness. However, the molecular basis of the crosstalk between energy-stressed adipocytes and cancer is still poorly investigated. Our long-term aim is elucidating the molecular mechanisms that control the remodeling of the adipocyte secretome in response to obesity and oxidative stress, and investigating their role in modulating the crosstalk between adipocytes and cancer cells, with the goal of identifying innovative therapeutic targets.

Methods: Cell culture in DMEM+CALF serum, Adipocyte-Differentiation with ATCC protocol, RNA extraction with RNeasy Plus Mini Kit - QIAGEN or miRNeasy Mini Kit -QIAGEN, cDNA making with iScript, RT-qPCR Sybr Green ThermoFisher, Exosomes extraction with EXO-Spin mini Purification Kit

Results: As a first objective, we established which is/are the best model(s) of adipocytes to use for in-vitro experiments and omics profiling. Experiments have been performed to compare the transcriptome and stress response in adipocytes differentiated from murine Adipose-Derived Stromal Vascular Fraction (SVF), and in vitro differentiated 3T3-L1 cells - an established pre-adipocyte murine cell line. Expression of genes involved in the endoplasmic reticulum and mitochondrial unfolded protein response (ER-UPR and mt-UPR) was upregulated after treatment with different mitochondrial stressors and used to identify optimal time points for RNA sequencing. We also optimized the protocols for extraction and quantification of extracellular vesicles released by adipocytes (adipocyte-derived exosomes) and their miRNA content.

Conclusions & Future Perspectives: Key transcription factors and their downstream molecules involved in ER-UPR and mt-UPR are upregulated upon mitochondrial stress and their contribution to regulating the cargo content of exosomes released by energy-stressed adipocytes will be studied in the cellular model and with the kinetics defined by these preliminary studies. Our future includes comparing the profiling of cellular transcriptomes with the profiling of proteins and RNA included in exosomes released under corresponding experimental conditions to dissect the effect of mitochondrial stress on the remodeling of the adipocyte secretome.

References

- Zhao C. et al., Cancer-associated adipocytes: Emerging supporters in breast cancer, J Exp Clin Canc Res, 2020
- Gao X. et al., Extracellular vesicles from adipose tissue—a potential role in obesity and type 2 diabetes?, Front Endocrinol, 2017
- Cao Y., Adipocyte and lipid metabolism in cancer drug resistance, J Clin Investig, 2019
- Ferrante S. C. et al., Adipocyte-derived exosomal miRNAs: a novel mechanism for obesity-related disease, Pediatr Res, 2015
- Cardamone M. D. et al., Mitochondrial retrograde signaling in mammals is mediated by the transcriptional cofactor GPS2 via direct mitochondria-to-nucleus translocation, Mol Cell, 2018
- English J. et al., GPS2-mediated regulation of the adipocyte secretome modulates adipose tissue remodeling at the onset of diet-induced obesity, Mol Metab, 2023
- Prof. Valentina Perissi's talk at 2024 Nature Conferences in Beijing (China)

- Quirós, Pedro M., et al. "Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals." Journal of Cell Biology 216.7 (2017): 2027-2045'

Molecular origins of the rare disease reviewed with orpha.net: structural bioinformatics investigation with Orphanetta

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Rare diseases (RDs), predominantly genetic in origin [1], affect a small part of the population and are associated with significant disability and reduced life expectancy. Due to the lack of comprehensive knowledge and experimental protein structures linked to these conditions, addressing RDs remains a challenge [2].

This works aims to explore the molecular mechanisms of Mendelian Rare Diseases (MRDs) using structural bioinformatics approaches. The project integrates data from the Orpha.net database [3] with protein structure models obtained with AlphaFold AI system [4], focusing on missense variants implicated in MRD. The bioinformatics tool developed, called Orphanetta, combines data from Orpha.net, ClinVar [5] and UniProtKB [6] to analyse pathogenic protein mutations. Using Python programming and advanced structural analysis tools (POPScomp)[7], the study identifies and categorises the topological locations of mutation sites and provides insights into the effects of these mutations on protein function and stability.

The findings contribute to the understanding of the structural basis of pathogenicity in MRDs and could guide the development of targeted therapies. The results highlight the potential of structural bioinformatics in uncovering the molecular origins of rare diseases and underline the need for continued research to support therapeutic advances.

References

1. S. Nguengang Wakap et al., 'Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database', European Journal of Human Genetics, vol. 28, no. 2, pp. 165–173, Feb. 2020.
2. Schieppati, A.; Henter, J.-I.; Daina, E.; Aperia, A. Why Rare Diseases Are an Important Medical and Social Issue. The Lancet 2008, 371 (9629), 2039–2041.
3. Orphanet: The Portal for Rare Diseases and Orphan Drugs. Reference Reviews 2009, 23 (8), 45–4.
4. J. Jumper et al., 'Highly accurate protein structure prediction with AlphaFold', Nature, vol. 596, no. 7873, pp. 583–589, Aug. 2021.
5. M. J. Landrum et al., 'ClinVar: Public archive of interpretations of clinically relevant variants', Nucleic Acids Res, vol. 44, no. D1, pp. D862–D868, 2016.
6. A. Bateman et al., 'UniProt: the Universal Protein Knowledgebase in 2023', Nucleic Acids Res,vol. 51, no. D1, pp. D523–D531, Jan. 2023.
7. L. Cavallo, J. Kleinjung, and F. Fraternali, 'POPS: A fast algorithm for solvent accessible surface areas at atomic and residue level', Nucleic Acids Res, vol. 31, no. 13, pp. 3364–3366, Jul. 2003.

Multiomic profiling and neuroprotective bioactivity of salvia hairy root-derived extracellular vesicles in a cellular model of parkison's disease

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Background: Extracellular vesicles (EVs) have gained attention as potential nanomedicine tools, but mammalian-derived EVs face challenges in purification and safety. Plants offer a promising alternative, and hairy roots (HRs) of medicinal plants are emerging as biotechnological platforms for producing therapeutic EVs [1,2]. This study focuses on EVs derived from Salvia sclarea and Salvia dominica HRs and their bioactivity in a Parkinson's disease model. Furthermore, a detailed proteomic bioinformatic analysis was conducted to gain insight into the protein composition of these EVs.

Methods: HRs were generated by transforming S. sclarea leaf explants with Agrobacterium rhizogenes. EVs were isolated from HR-conditioned media using differential ultracentrifugation (cUC) and size exclusion chromatography (SEC). Nanoparticle tracking analysis (NTA), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) characterized EV size and structure. Proteomic and metabolomic profiles were assessed using mass spectrometry. Gene ontology analysis of identified proteins was carried out using the ShinyGo 0.80. The bioactivity of HR EVs was tested in a Parkinson's disease model with SH-SY5Y cells treated with 6-hydroxydopamine (6-OHDA). Confocal microscopy tracked EV uptake, while spectrophotometric assays analyzed the biochemical response of SH-SY5Y cells treated with EVs and 6-OHDA.

Results: EVs from S. sclarea and S. dominica HRs were successfully purified and displayed distinctive biophysical and proteomic profiles. Metabolomic analyses showed a conserved cargo, rich in triterpenoids with antioxidant properties. Proteomic bioinformatics analysis revealed that EVs carried a unique set proteins, including ATP synthase subunits, ribosomal constituents, cytoskeleton elements, elongation factors, and molecular chaperones. These findings expand our understanding of plant EV biomarkers and provide new anchor proteins for EV bioengineering approaches. In SH-SY5Y cells, HR EVs were non-toxic, entered cells efficiently, and significantly inhibited cell death induced by 6-OHDA. In-cell metabolomics revealed that EVs maintained SH-SY5Y metabolic homeostasis and mitigated oxidative stress. Mechanistically, HR EVs reduced the oxidative products of 6-OHDA, preventing its autoxidation and associated toxicity [3].

Conclusions: Our study demonstrates that EVs from Salvia HRs represent a safe and effective non-mammalian platform with potential for therapeutic applications in neurological disorders, such as Parkinson's disease. These findings underscore the potential of plant-derived EVs as alternative therapeutic agents in nanobiotechnology.

References

1. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as a next-generation drug delivery platform. Nat Nanotechnol. Jul 2021;16(7):748–59.
2. Boccia E, Alfieri M, Belvedere R, Santoro V, Colella M, Del Gaudio P, et al. Plant hairy roots for the production of extracellular vesicles with antitumor bioactivity. Commun Biol. Aug 2022;5(1):848.
3. Vestuto V, Conte M, Vietri M, Mensitieri F, Santoro V, Di Muro A, Alfieri M, Moros M, Miranda MR, Amante C, Delli Carri M, Campiglia P, Dal Piaz F, Del Gaudio P, De Tommasi N, Leone A, Moltedo O, Pepe G, Cappetta E, Ambrosone A. Multiomic Profiling and Neuroprotective Bioactivity of Salvia Hairy Root-Derived Extracellular Vesicles in a Cellular Model of Parkinson's Disease. Int J Nanomedicine. 2024 Sep 11;19:9373-9393.

Patient-specific modeling and simulation of Tumor-Induced Angiogenesis in the Human Retina

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Background: Retinal Hemangioblastoma (RH) is the most frequent and the earliest manifestation of the von Hippel-Lindau disease (VHL) [1]. As with many other VHL-related tumors, it is highly vascular, a feature that could recently be observed with unprecedented detail thanks to the introduction of Optical Coherence Tomography (OCT) [2]. However, studying RH development in time remains challenging, especially without an animal model. Mathematical models are rising as a valid alternative to study cancer dynamics, and, among all, Phase Field Models (PFMs) have proved remarkable results in reproducing tumor growth and angiogenesis [3].

Methods: We selected OCT images from three patients presenting an early-stage RH. We used the images to feed a deterministic PFM, simulated using the FEniCS computational platform and our recently developed software, Mocafe. We estimated the model's parameters from experimental evidence reported in the literature.

Results: We could derive several key observations from our simulations. First, we confirmed that our PFM model can recapitulate sprouting angiogenesis as observed in RH. Second, using different parameter combinations, we could assess that sprouting angiogenesis starts upon reaching a minimal dimension in RH. We also estimate such a minimal dimension to be around 200 um. Third, our simulations show that angiogenesis in RH occurs quickly, leading to the formation of stable vascular structures in the order of weeks. The latter fact might explain the discouraging results of antiangiogenic therapy for RH (e.g., anti-VEGF), suggesting that at the moment of diagnosis, tumor vascularization is already too stable to be treated with such a therapy.

Conclusion: To our knowledge, this is the first study presenting a mathematical model of RH. Our results align with the agreed RH pathology and experimental observations. Our estimates on the minimal RH dimension and the speed of vascularization are coherent with several studies. Moreover, our results provide a new perspective on the applicability of AF inhibition therapy for RH. In conclusion, our work suggests that combining PFMs with OCTA provides a valuable tool for studying RH, partially filling the absence of an animal model.

References

1. P. Sagar, R. Rajesh, M. Shanmugam, V. K. Konana, D. Mishra, Comparison of optical coherence tomography angiography and fundus fluorescein angiography features of retinal capillary hemangioblastoma. Indian J. Ophthalmol. 66, 872–876 (2018).
2. S. Karimi, A. Arabi, T. Shahraki, S. Safi, Von Hippel-Lindau Disease and the Eye. J. Ophthalmic Vis. Res. 15, 78 (2020).
3. Rockne RC, Scott JG. Introduction to Mathematical Oncology. JCO Clin Cancer Inform. 2019 Apr;3:1-4.

Rational Design of Aptamer Splitting Techniques for Advanced Biosensor Development

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Nucleic acid sequences exhibit exceptional versatility, being capable of forming diverse complex structures that are proven to be fundamental in bioanalytical applications [1]. Split aptamers, composed of two or more short nucleic acid fragments, have emerged as a particularly promising tool in the development of advanced biosensors. This innovative approach involves strategically splitting an aptamer into independent fragments that reassemble upon recognizing a specific target molecule [2].

This study focuses on optimizing aptamer design for enhanced suitability as biosensors, specifically investigating the effects of structural modifications on an ATP-binding aptamer. The aptamer model (PDB ID: 1AW4), capable of binding two ATP molecules, was modified by introducing poly-T loops of varying lengths (8, 16, and 32 bases) at three equidistant modification sites within its sequence.

Molecular dynamics simulations assessed the resulting structural and binding changes using root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), hydrogen bond analysis and MM/GBSA free energy calculations.

The MM/GBSA analysis revealed that the incorporation of poly-T loops generally decrease the aptamer's binding affinity for ATP, indicating that such modifications can induce destabilizing effects on the interaction. However, an 8-base poly-T loop inserted at the fourteenth position demonstrated comparable binding free energies to the unmodified aptamer and fostered enhanced hydrogen bonding with ATP, suggesting this configuration supports a favorable interaction profile.

Further analysis showed that longer loops, particularly the 32-base insertion, substantially increased flexibility and structural deviation from the initial conformation, as indicated by elevated RMSD and RMSF values. In contrast, shorter loops (8 and 16 bases) yielded minor structural perturbations, with the 8-base loop at the fourteenth site imparting the least destabilizing effect.

These findings highlight the potential of controlled structural modifications to improve binding stability and affinity in aptamers, thereby enhancing their applicability in biosensing contexts. Future studies will experimentally validate these computational insights and explore additional structural adjustments to further optimize aptamer performance for therapeutic and diagnostic applications.

References

1. A. Ottaviani, F. Iacovelli, A. Idili, M. Falconi, F. Ricci, A. Desideri: Engineering a responsive DNA triple helix into an octahedral DNA nanostructure for a reversible opening/closing switching mechanism: a computational and experimental integrated study, Nucleic Acids Res., 2018, 46, 9951–9959.
2. D. Mariottini, A. Idili, M. A. D. Nijenhuis, T. F. A. de Greef, and F. Ricci: DNA-Based Nanodevices Controlled by Purely Entropic Linker Domains, Journal of the American Chemical Society, 2018, 140 (44), 14725-14734.

Relevance of DNA tridimensional shape in RNA:DNA:DNA triple helix formation

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Long non-coding RNAs (lncRNAs) play key roles in regulating gene expression by interacting with DNA through various mechanisms. One such mechanism is the formation of RNA:DNA:DNA triple helices (triplex), where a single strand of RNA forms non-canonical hydrogen bonds with double-stranded DNA, interacting with the major groove of the genomic helix (Maldotti 2022). Predicting these triplexes computationally has gained significant attention recently, but consensus on optimal prediction methods remains elusive (Cicconetti 2023). This study investigates the influence of DNA 3D shape features — helix twist (HelT), minor groove width (MGW), propeller twist (ProT), and roll (Roll) — on the formation of triplexes and their potential to improve prediction models.

To explore this, we collected experimentally validated triplex targets and estimated the values for the four shape features at binding sites and control regions. We employed nested logistic regression models to assess whether incorporating these DNA shape features, alongside traditional sequence-based data, enhances prediction accuracy.

Our models demonstrated that adding 3D shape parameters increased the area under the curve (AUC) of the predictions by 17.5%, representing a significant improvement in predictive performance, even after controlling for chromatin openness, a factor known to enrich triplex sites. Further analysis revealed that the most significant contributions came from the HelT and ProT features, which showed the greatest difference between triplex-forming and non-forming regions. The MGW and Roll parameters, while still contributing to the model, had a smaller but noticeable impact. Importantly, the 3D DNA shape features improved triplex prediction across various lncRNAs, demonstrating that these features offer independent and complementary information to traditional sequence-based predictions.

These findings indicate that the tridimensional structure of DNA plays a critical role in determining RNA:DNA:DNA triplex formation. By incorporating DNA shape features into triplex prediction algorithms, such as 3plex (Cicconetti 2023), we can achieve more accurate and reliable results, potentially opening up new avenues for understanding the regulatory roles of lncRNAs in gene expression and their broader implications in health and disease.

References

Maldotti M, Lauria A, Anselmi F, Molineris I, Tamburrini A, Meng G, Polignano IL, Scrivano MG, Campestre F, Simon LM, Rapelli S, Morandi E, Incarnato D, Oliviero S. The acetyltransferase p300 is recruited in trans to multiple enhancer sites by lncSmad7. Nucleic Acids Res. 2022. doi: 10.1093/nar/gkac083.

Cicconetti C, Lauria A, Proserpio V, Masera M, Tamburrini A, Maldotti M, Oliviero S, Molineris I. 3plex enables deep computational investigation of triplex forming lncRNAs. Comput Struct Biotechnol J. 2023. doi: 10.1016/j.csbj.2023.05.016.

Spatial-transcriptomics algorithmic learning methods to unravel Host-Pathogen interaction: a preliminary approach

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Background: Spatial transcriptomics (ST) enables spatially resolved gene expression analysis within tissue samples, providing information about gene activity in the context of tissue structure. For the study of Host-Pathogen interaction, ST represents a promising asset allowing to characterize the interplay among the two players at the spatial level by using custom bacterial probes to detect the bacterial activity in addition to host ones. This project aims to develop a machine-learning model for analyzing Host-Pathogen ST data, enhancing bacterial gene expression detection and allowing to dissect the host-pathogen interplay.

Methods: Visium-technology records spatial gene expression of tissue slides across thousands of spots of 55um diameter. We designed M. abscessus (MABSc) specific probes and performed ST experiments on mouse tissues subjected to controlled MABSc chronic infection. Bacterial gene expression was detected in infected tissues but was sparse and underestimated. We improved its analysis by leveraging host gene expression and spatial coordinates. To dissect the MABSc-Host interplay, we enhanced bacterial gene expression exploiting host gene expression and spatial coordinates of transcriptional capturing spots. Preliminary models were developed using neural network architectures, including auto-encoders (AE) for data denoising and low-dimensional representation, and graph neural networks (GNN) for leveraging spatial relationships. We additionaly implementedlinear algorithms like Lasso regression for benchmarking purposes. The data used for this work include a set of 7 consecutive FFPE mouse lung slices infected by MABSc.

Results: The preliminary trained models implementing GNNs, AEs and linear regressions were able to predict the MABSc genes expression in high-inflamed regions enlightening different bacterial behaviours among spatial domains. The predicted bacterial genes were then used for clustering analysis identifying bacterial gene sets associated to specific tissue domains and increasing the original resolution obtained by the Host transcriptional cluster analysis.

Conclusions: The designed preliminary models were able to predict the MABSc genes expression in high-inflamed regions enlightening different bacterial behaviours among spatial domains. The ability to impute bacterial transcripts will expand the applicability of ST in studying Host-Pathogen interactions enabling a retrospective analysis of samples without custom probes. This approach could lead to the discovery of interspecies gene networks increasing our understanding of host-pathogen interactions.

References

- Di Marco, F., Nicola, F., Giannese, F., Saliu, F., Tonon, G., de Pretis, S., Cirillo, D. M., & Lorè, N. I. (2024). Dual spatial host-bacterial gene expression in Mycobacterium abscessus respiratory infections. Communications Biology 2024 7:1, 7(1), 1–8. https://doi.org/10.1038/s42003-024-06929-5
- D Dar, N. D. L. C. D. N. (2021). Spatial transcriptomics of planktonic and sessile bacterial populations at single-cell resolution. Science, 373, eabi4882.

- Long, Y., Ang, K. S., Li, M., Chong, K. L. K., Sethi, R., Zhong, C., Xu, H., Ong, Z., Sachaphibulkij, K., Chen, A., Zeng, L., Fu, H., Wu, M., Lim, L. H. K., Liu, L., & Chen, J. (2023). Spatially informed clustering, integration, and deconvolution of spatial transcriptomics with GraphST. Nature Communications 2023 14:1, 14(1), 1–19. https://doi.org/10.1038/s41467-023-36796-3
- Sounart, H. (2023). Dual spatially resolved transcriptomics for human host-pathogen colocalization studies in FFPE tissue sections. Genome Biol., 24.

Structural and functional insights into Androgen Receptor and pVHL interactions in ccRCC

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The androgen receptor (AR) plays a significant role in the development and progression of various cancers, particularly prostate cancer. However, recent studies reveal that its involvement in cell proliferation and survival extends to organs beyond those traditionally associated with male physiology, including the breast, bladder, lungs, and kidneys. In this context, we focus on kidney cancer, specifically clear cell renal cell carcinoma (ccRCC), the most common histological subtype. Mutations in the VHL tumour suppressor gene are present in approximately 80% of ccRCCs, identifying it as one of the key genetic driver for initiation and progression. VHL is part of the E3 ubiquitin ligase complex and negatively regulates hypoxia-inducible factor (HIF) signalling by targeting HIF-1/2α for degradation. VHL loss of function mutations leads to HIF-1/2α accumulation, activating transcription of genes involved in metabolism, cell cycle regulation, and angiogenesis. From existing literature, it is known that AR and pVHL interact through specific regions: Asp551-Asn706 of AR and Leu63-Pro154 of pVHL30. Our experimental data suggest that pVHL modulates AR activity, with pVHL30 enhancing AR turnover, while pVHL19 increases AR stability and transcriptional activity. To understand how AR is modulated under various conditions, we analyzed multiple ccRCC RNA-seq datasets. We found the greatest difference in AR expression in the wt VHL vs. mutant VHL dataset, where AR expression was higher when VHL was lost. This dataset was then used to investigate the expression of AR, EPAS1, and HIF-1α target genes. Additionally, we performed a comprehensive structural investigation of the molecular complexes formed between AR and VHL using the AlphaFold 3 server, such as VCB and CRL2-VHL and AR-foldosome (early, intermediate and mature stages) aiming to decipher the nature of the interaction understudy. AR expression is significantly increased when VHL is loss. Overexpression of AR and EPAS1 upregulated glycolytic enzymes and survival-promoting proteins, while downregulating cell cycle regulators. Structural analysis using AlphaFold3 revealed that pVHL30 binds AR for degradation, while pVHL19 stabilizes AR and enhances ligand-binding (DHT) activity. Specific hydroxylation sites for pVHL19, absent in pVHL30, support experimental findings: pVHL30 promotes AR turnover, whereas pVHL19 increases AR stability and transcriptional activity.

References

1. Hu, C., Fang, D., Xu, H., Wang, Q. & Xia, H. The androgen receptor expression and association with patient's survival in different cancers. Genomics 112, 1926–1940 (2020).
2. Foersch, S. et al. Prognostic relevance of androgen receptor expression in renal cell carcinomas. Oncotarget 8, 78545–78555 (2017).
3. Michmerhuizen, A. R., Spratt, D. E., Pierce, L. J. & Speers, C. W. ARe we there yet? Understanding androgen receptor signaling in breast cancer. npj Breast Cancer 6, 1–19 (2020).
4. Huang, Q. et al. Androgen receptor modulates metastatic routes of VHL wild-type clear cell renal cell carcinoma in an oxygen-dependent manner. Oncogene 39, 6677–6691 (2020).
5. Chen, S., Chen, K., Zhang, Q., Cheng, H. & Zhou, R. Regulation of the transcriptional activation of the androgen receptor by the UXT-binding protein VHL. (2013).
6. Huang, Q. et al. Androgen receptor modulates metastatic routes of VHL wild-type clear cell renal cell carcinoma in an oxygen-dependent manner. Oncogene 39, 6677–6691 (2020).
7. Wang, J. et al. The Von Hippel-Lindau Protein Suppresses Androgen Receptor Activity. Mol Endocrinol 28, 239–248 (2014).
8. Abramson, J. et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature 630, 493–500 (2024).
9. von Roemeling, C. A. et al. Neuronal Pentraxin 2 Supports Clear Cell Renal Cell Carcinoma by Activating the AMPA-Selective Glutamate Receptor-4. Cancer Research 74, 4796–4810 (2014).

10. Transcriptomic characterization and innovative molecular classification of clear cell renal cell carcinoma in the Chinese population | Cancer Cell International | Full Text. https://cancerci.biomedcentral.com/articles/10.1186/s12935-020-01552-w.
11. Kajdasz, A. et al. Identification of RCC Subtype-Specific microRNAs-Meta-Analysis of High-Throughput RCC Tumor microRNA Expression Data. Cancers (Basel) 13, 548 (2021).
12. Ho, T. H. et al. A Multidisciplinary Biospecimen Bank of Renal Cell Carcinomas Compatible with Discovery Platforms at Mayo Clinic, Scottsdale, Arizona. PLOS ONE 10, e0132831 (2015).
13. Yao, X. et al. VHL Deficiency Drives Enhancer Activation of Oncogenes in Clear Cell Renal Cell Carcinoma. Cancer Discov 7, 1284–1305 (2017).
14. Lucarelli, G. et al. Integrated multi-omics characterization reveals a distinctive metabolic signature and the role of NDUFA4L2 in promoting angiogenesis, chemoresistance, and mitochondrial dysfunction in clear cell renal cell carcinoma. Aging 10, 3957–3985 (2018).
15. Eckel-Passow, J. E. et al. Somatic expression of ENRAGE is associated with obesity status among patients with clear cell renal cell carcinoma. Carcinogenesis 35, 822 (2013).
16. Gerlinger, M. et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet 46, 225–233 (2014).
17. Liep, J. et al. Cooperative Effect of miR-141-3p and miR-145-5p in the Regulation of Targets in Clear Cell Renal Cell Carcinoma. PLOS ONE 11, e0157801 (2016).
18. Nam, H.-Y. et al. Integrative Epigenetic and Gene Expression Analysis of Renal Tumor Progression to Metastasis. Mol Cancer Res 17, 84–96 (2019).
19. Thibodeau, B. J. et al. Characterization of clear cell renal cell carcinoma by gene expression profiling. Urologic Oncology: Seminars and Original Investigations 34, 168.e1-168.e9 (2016).
20. Lobo, N. C. et al. Efficient generation of patient-matched malignant and normal primary cell cultures from clear cell renal cell carcinoma patients: clinically relevant models for research and personalized medicine. BMC Cancer 16, 485 (2016).

Structural characterization of Hypoxia Inducible Factor a-Prolyl Hydroxylases interaction through MD simulations

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The Prolyl Hydroxylases (PHDs) are an enzymatic family that regulates cell oxygen-sensing pathway. Under normoxia condition, PHDs hydroxylate the hypoxia-inducible transcription factors α (HIFs-α), driving their proteasomal degradation. Hypoxia inhibits PHDs activity, stabilizing HIFs-α, which initiate the transcription of target genes involved in cell metabolism adaptation to hypoxia [1]. As a main hallmark of cancer, hypoxia promotes neo-angiogenesis, cell proliferation, and survival [2]. The PHD isoforms are thought to have a variable and cell-dependent impact on tumor progression. All isoforms hydroxylate HIF-α (HIF-1,2,3α) with different affinities. However, what determines these differences and how they pair with tumor growth is poorly understood [3,4].

Here, molecular dynamics (MD) simulations were used to characterize the PHD2 [5] and PHD3 isoforms binding properties in complexes with HIF-1α and HIF-2α. In parallel, conservation analysis and binding free energy calculations were performed to better understand PHDs substrate affinity. The results showed distinct affinities of PHD2 and PHD3 for HIFs-α substrates with the PHD2 C-terminus playing a critical role in substrate binding and stabilization. MD simulations revealed that the PHD2 C-terminus oscillates between folded and unfolded states, impacting its affinity: in its unfolded conformation, PHD2 showed enhanced interactions with HIF-1α, while the folded state preferentially bound HIF-2α. These findings challenge previous assumptions that PHD2 preferentially binds HIF-1α, showing conditions under which it favors HIF-2α. For PHD3, interactions with HIF-1α were primarily stabilized by the β2β3-loop, while its C-terminal region contributed to HIF-2α binding. Interestingly, binding free energy calculations indicated a stronger affinity of PHD3 for HIF-1α, contrasting with prior reports suggesting its primary regulation of HIF-2α in hypoxia [6]. The identification of specific binding sites in PHDs that impact substrate specificity without altering enzymatic activity could have implications for cancer research.

Collectively, our findings suggest that the PHDs C-terminus may act as a molecular regulator of PHD's activity, highlighting differences in PHD2 and PHD3 substrate specificity.

References

1. Ohh, M.; Taber, C.C.; Ferens, F.G.; Tarade, D. Hypoxia-Inducible Factor Underlies von Hippel-Lindau Disease Stigmata. eLife 2022, 11, e80774, doi:10.7554/eLife.80774.
2. Shi, R.; Liao, C.; Zhang, Q. Hypoxia-Driven Effects in Cancer: Characterization, Mechanisms, and Therapeutic Implications. Cells 2021, 10, 678, doi:10.3390/cells10030678.
3. Strocchi, S.; Reggiani, F.; Gobbi, G.; Ciarrocchi, A.; Sancisi, V. The Multifaceted Role of EGLN Family Prolyl Hydroxylases in Cancer: Going beyond HIF Regulation. Oncogene 2022, 41, 3665–3679, doi:10.1038/s41388-022-02378-8.
4. Yu, M.; Lun, J.; Zhang, H.; Zhu, L.; Zhang, G.; Fang, J. The Non-Canonical Functions of HIF Prolyl Hydroxylases and Their Dual Roles in Cancer. Int. J. Biochem. Cell Biol. 2021, 135, 105982, doi:10.1016/j.biocel.2021.105982.
5. Camagni, G.F.; Minervini, G.; Tosatto, S.C.E. Structural Characterization of Hypoxia Inducible Factor α—Prolyl Hydroxylase Domain 2 Interaction through MD Simulations. Int. J. Mol. Sci. 2023, 24, 4710, doi:10.3390/ijms24054710.
6. Place TL, Domann FE. Prolyl-hydroxylase 3: Evolving Roles for an Ancient Signaling Protein. Hypoxia (Auckl). 2013 Oct 1;2013(1):13-17. doi: 10.2147/HP.S50091. PMID: 24672806; PMCID: PMC3963164.

Supporting Machine Learning Model in the Treatment of Chronic Pain

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Conventional therapy options for chronic pain are still insufficient and patients most frequently request alternative medical treatments, such as medical cannabis. Although clinical evidence supports the use of cannabis for pain [1-3], very little is known about the efficacy, dosage, administration methods, or side effects of widely used and accessible cannabis products. A possible solution could be given by pharmacogenetics, with the identification of several polymorphic genes that may play a role in the pharmacodynamics and pharmacokinetics of cannabis [4]. Based on these findings, data from patients treated with medical cannabis and genotyped for several candidate single-nucleotide polymorphisms (SNPs) were collected, and integrated. The data were then analyzed through the XGBoost model to demonstrate that the reduction in pain intensity is closely related to gene polymorphisms [5]. Dynamic and static patient features, the ones that changed over the treatment period and the ones that remained constant respectively, were considered to predict the optimal combination of THC and CBD doses. The analysis revealed that specific gene variants—such as ABCB1 rs1045642, TRPV1 rs8065080, UGT2B7 rs7438135, and COMT rs4680—along with patient age and pain severity, significantly influenced the effectiveness of cannabis treatment. The XGBoost model achieved a mean absolute error (MAE) of 1.01 mg in predicting the optimal cannabis dose, highlighting its accuracy in guiding personalized treatment plans. Our findings suggest that machine learning models demonstrate significant potential in predicting personalized cannabis therapy based on genetic profiles. By incorporating pharmacogenomics into clinical practice, these models can help clinicians optimize treatment, improving pain relief and minimizing side effects, thereby advancing precision medicine in chronic pain management.

References

1. Pattnaik, F.; Nanda, S.; Mohanty, S.; Dalai, A.K.; Kumar, V.; Ponnusamy, S.K.; Naik, S. Cannabis: Chemistry, Extraction and Therapeutic Applications. Chemosphere 2022, 289, 133012.
2. Mucke, M.; Phillips, T.; Radbruch, L.; Petzke, F.; Hauser, W. Cannabis Based Medicines for Chronic Neuropathic Pain in Adults. Cochrane Database Syst. Rev. 2018, 3, CD012182.
3. Chung, M.; Kim, H.K.; Abdi, S. Update on Cannabis and Cannabinoids for Cancer Pain. Curr. Opin. Anaesthesiol. 2020, 33, 825–831.
4. Hryhorowicz, S.; Walczak, M.; Zakerska-Banaszak, O.; Słomski, R.; Skrzypczak-Zielinska, M. Pharmacogenetics of Cannabinoids. Eur. J. Drug Metab. Pharmacokinet. 2018, 43, 1–12.
5. Visibelli, A.; Peruzzi, L.; Poli, P.; Scocca, A.; Carnevale, S.; Spiga, O.; Santucci, A. Supporting Machine Learning Model in the Treatment of Chronic Pain. Biomedicines 2023, 11, 1776.

Suppression therapy against nonsense diseases: using the PURE-LITE in vitro model system to evaluate Translational Readthrough Inducing Drugs mechanism of action.

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Precision medicine represents a new insight in genetic medicine to treat the mutation profile of a patient1. Nonsense mutations represent a severe genetic defect in 11% the rare genetic diseases. Currently, suppression therapy is focused on addressing nonsense mutation diseases, for which no treatments that stimulate readthrough are approved. This approach uses translational readthrough-inducing drugs (TRIDs) against premature termination codons (PTCs) occurring in mRNA2. Our studies are centered on understanding the mechanism of action (MoA) of three TRID (NV848, NV914, and NV930) molecules. For this purpose, we determined NV TRIDs' effect on readthrough using the reconstituted in vitro model system PURE-LITE, which allows for the separate determination of TRIDs' impact on both readthrough and termination activities. We find that all three NVs induce readthrough in the PURE-LITE system and that their MoA differs from Ataluren, which is, actually, the only approved TRID. Our results exclude eRF1 and eRF3 as targets, but further efforts will be necessary to establish the precise MoA.

References

1. Goetz LH, Schork NJ. Personalized medicine: motivation, challenges, and progress. Fertil Steril. 2018 Jun;109(6):952-963. doi: 10.1016/j.fertnstert.2018.05.006. PMID: 29935653; PMCID: PMC6366451.
2. Martins-Dias P, Romão L. Nonsense suppression therapies in human genetic diseases. Cell Mol Life Sci. 2021 May;78(10):4677-4701. doi: 10.1007/s00018-021-03809-7. Epub 2021 Mar 22. PMID: 33751142; PMCID: PMC11073055.

The hydroalcoholic extract of olive leaves alleviates non-alcoholic fatty liver disease

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Background: The alteration of lipid metabolism is closely associated with the onset of non-alcoholic fatty liver disease (NAFLD), which is the leading cause of morbidity and mortality associated with the liver. Therefore, the effort to understand new agents capable of preventing the pathogenesis of non-alcoholic steatosis is fundamental [1,2]. Our aim was to verify if an extract of olive leaves (OI) could prevent NAFLD and clarify its underlying molecular mechanism. HepG2 cells exposed only to free fatty acids (FFA) showed intracellular lipid accumulation, endoplasmic reticulum (ER) stress, and disrupted expression of proteins, lipids, and lipid synthesis-related genes, as demonstrated by omics analyses and biochemical assays. However, co-treatment with OI attenuated and partially reversed the expression of these markers, restoring levels closer to the physiological state. The bioinformatic analysis of OI key components suggested potential binding targets.

Methods: HepG2 cells were treated with a mixture of FFA OA/PA (oleic acid: palmitic acid, 2:1) for 24 hours to induce NAFLD. To verify the reduction of lipid accumulation in HepG2 cells, phenotypic assays, western blots, q-PCR and cellular lipidome analyses were performed. Additionally, bioinformatic tools (Swiss Target Prediction, SuperPred) were employed to identify potential protein targets.

Results: Our findings revealed significant improvements in cell viability, mitochondrial membrane potential, and a reduction in lipid droplets and steatosis markers through the modulation of AMPK and SREBP1/FAS pathways by alleviating ER stress. A detailed lipidomic analysis was conducted to explore the molecular mechanisms driving the anti-steatotic effects showing a significant decrease in lipid metabolites, diacylglycerols and triacylglycerol. Metabolic pathway analysis revealed that these metabolites were closely associated with the metabolism of glycerophospholipids and glycerolipids, the regulation of lipolysis, fat digestion and absorption. Additionally, bioinformatic predictions based on the most abundant components in the OI extract, identified using the RP-UHPLC-PDA-ESI-MS/MS platform, suggested potential binding interactions between key flavonoids and components of lipid metabolism.

Conclusion: Exploring natural compounds and their derivatives offers valuable insights into the biochemical mechanisms involved in liver disease signaling pathways. These results suggest that OI can potentially be used as a therapeutic agent to ameliorate NAFLD through regulation of lipid metabolism.

References

1. Pouwels, S., Sakran, N., Graham, Y., Leal, A., Pintar, T., Yang, W., Kassir, R., Singhal, R., Mahawar, K., Ramnarain, D. (2022). Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC Endocr Disord. 14, 22(1), 63. 10.1186/s12902-022-00980-1.
2. Novi, S., Vestuto, V., Campiglia, P., Tecce, N., Bertamino, A., Tecce, M.F. (2023). Anti-Angiogenic Effects of Natural Compounds in Diet-Associated Hepatic Inflammation. Nutrients. 14, 15(12), 2748. 10.3390/nu15122748.

Decoding Microbial systems Dynamics in Complex Environments

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Background: Constraint-based metabolic models (CMB) apply to elucidate biological properties in variable environments. However, achieving accurate predictions remains arduous without comprehensive experimental data. Conversely, at the core of unveiling the functionalities in complex systems lies the use of Ordinary Differential Equations (ODEs), which detail the evolution of system's entities. Expanding knowledge of such systems through ODEs requires an in-depth understanding of the implicit dynamics of experimentally unobserved variables and obtaining ODEs unknown parameters. Nevertheless, parameter estimation is inherently challenging, as data are often limited or difficult to achieve, particularly for large-scale, complex models.

Methods: We proposed a systems-biology-informed hybrid modeling approach called UnifiedGreatMod [1]. The general framework GreatMod [2] implements our approach. Our approach harmonizes ODEs and Flux Balance Analysis (FBA), which allows to state the dynamic model represented by an ODEs system, the FBA-based model, and the thier coupling [5]. Our method integrates the precision of ODEs-based kinetics with the broad applicability of genome-scale metabolic models (GEMs) for analyzing metabolic networks and predicting cellular growth and flux distributions (Fig. 1A). We illustrate this with cross-feeding interaction within the intestinal microbiota model SIHUMIx [4], a community-level model comprising eight species designed to simulate dynamic environments and replicate metabolic behaviors. By integrating transcriptomics-guided data with knowledge of media conditions given GEMs as data integration platforms [3], we incorporate ODE systems into multi-species-specific GEMs, adding constraints to the optimization procedure in CMB (Fig. 1B). The automation and scalability of our hybrid models expanded the framework's general applications.

Results: Our hybrid modeling framework accurately predicts microbial growth concerning extracellular short-chain fatty acids (SCFAs) concentrations. The framework quantitatively predicts community growth rates and SCFAs, preserving higher-order community properties. The transcriptome-guided integration into GEMs captures context-specific metabolism, revealing the metabolic interplay among bacterial species in digesting and producing resources.

Conclusion: Our modeling approach provides insights into microbial metabolic interactions, particularly under host and environmental influences on the microbiota ecosystem. By refining GEM constraints through dynamic ODE resolution, the framework accurately identifies underlying dynamics, captures the context-specific metabolism and circumvents the limitation of data availability, representing an advancement in Systems Biology models or biological engineering projects.

References

1. Riccardo Aucello et al. "UnifiedGreatMod: A New Holistic Modeling Paradigm for Studying Biological Systems on a Complete and Harmonious Scale". In: under review, Bioinformatics (2024). DOI: 10. 1101/2024.09.18.613635.
2. P. Castagno et al. "A computational framework for modeling and studying pertussis epidemiology and vaccination." In: BMC Bionformatics (2020). DOI: 10.1186/s12859-020-03648-6.
3. ML Jenior et al. "Transcriptome-guided parsimonious flux analysis improves predictions with metabolic networks in complex environments". In: PLoS Comput Biol 16.4 (2020), e1007099. DOI: 10.1371/ journal.pcbi.1007099.
4. Jannike Krause et al. "Following the community development of SIHUMIx – a new intestinal in vitro model for bioreactor use". In: Gut Microbes 11 (2020), pp. 1–14. DOI: 10.1080/19490976.2019. 1702431.
5. S Pernice et al. "Integrating petri nets and flux balance methods in computational biology models: a methodological and computational practice". In: Fundamenta Informaticae (2019). DOI: 10.3233/FI- 2020-1888.

Using Archetypal Analysis for scRNAseq data clustering and trajectory identification

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Background: This study evaluates archetypal analysis (AA) as a tool for the analysis of single-cell RNA sequencing (scRNA-seq) data. Archetypal Analysis (AA) [1] is an unsupervised learning method that selects k vertices within the convex hull of the dataset, representing exemplary extreme points called archetypes. AA then represents observations as a mixture of archetypes, facilitating result interpretation when data does not consist of clearly distinct clusters. This method is formulated as a constrained optimization problem to best approximate the dataset through convex combination of archetypes [1]. In this work we apply AA to analyze single cell RNA sequencing data with the objective of characterizing each cell as a mixture of archetypes, thus being able to describe cell clusters and cell trajectories.

Methods: Few implementations of this method exist. In this study, we use the R package archetypal [2], which provides an implementation of AA, solving the constrained optimization problem with the projected gradient method and the FurthestSum initialization. [3].

Two scRNAseq datasets were analyzed: the Tirosh et. al dataset [4], snapshot of a human Melanoma sample (~4.000 cells), and the Yuzwa et. al dataset [5], which provides cell's genes expression, at different developmental stages, of murine cerebral cortex (~6.000 cells). These datasets were chosen to examine cases where clustered and cell-types boundaries are not well-defined, providing opportunities to test AA capabilities in single-cell transcriptional data analysis.

Results: We assessed cell assignment accuracy against reference classification using UMAP [6] and Sankey plots to visually compare and quantify the alignment between predicted and reference clusters. Results from AA were promising across both datasets. In the Tirosh dataset, AA effectively identified cell types, including malignant cells, while in the Yuzwa dataset, it showed potential in recognizing developmental trajectories. Performance analysis showed that FurthestSum is a viable initialization strategy; however, AA computation on these datasets was time intensive.

Conclusion: AA shows strong potential for scRNA-seq data, capturing complex relationships between cell types and developmental trajectories. While computational optimization is needed for larger datasets, the AA approach could enrich scRNA-seq analysis with interpretable models.

References

1. A. Cutler and L. Breiman, 'Archetypal Analysis', Technometrics, vol. 36, no. 4, pp. 338–347, Nov. 1994, doi: 10.1080/00401706.1994.10485840.
2. D. Christopoulos, D. Midgley, S. Venaik, and I. F. France, archetypal: Finds the Archetypal Analysis of a Data Frame. (May 23, 2024). Accessed: Aug. 07, 2024. [Online]. Available: https://cran.r-project.org/web/packages/archetypal/index.html
3. M. Mørup and L. K. Hansen, 'Archetypal analysis for machine learning and data mining', Neurocomputing, vol. 80, pp. 54–63, Mar. 2012, doi: 10.1016/j.neucom.2011.06.033.
4. I. Tirosh et al., 'Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq', Science, vol. 352, no. 6282, pp. 189–196, Apr. 2016, doi: 10.1126/science.aad0501.
5. S. A. Yuzwa et al., 'Developmental Emergence of Adult Neural Stem Cells as Revealed by Single-Cell Transcriptional Profiling', Cell Rep., vol. 21, no. 13, pp. 3970–3986, Dec. 2017, doi: 10.1016/j.celrep.2017.12.017.
6. L. McInnes, J. Healy, and J. Melville, 'UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction', Sep. 17, 2020, arXiv: arXiv:1802.03426. doi: 10.48550/arXiv.1802.03426.