— X NATIONAL CONFERENCE —

BIFI 2021

3rd – 5th

FEBRUARY

ZARAGOZA / SPAIN

Content

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Foreword

Welcome to the X National Conference BIFI 2021, held in the Institute for Biocomputation and Physics of Complex Systems (BIFI), located in Zaragoza, Spain.

As usual with our bi-annual series of National Conferences, the main aim of this meeting is to offer BIFI members a public forum to present their work and network with their fellow researchers, promoting the establishment of new collaborations among the different research lines and areas of the Institute, as well as with external colleagues.

Due to the exceptional circumstances imposed by the COVID19 pandemics, this year we have conceived the conference as a virtual meeting. The program will include plenary lectures by the principal Investigators of all the active research lines of the Institute and its joint units, as well as short talks given by students and trainees, and two poster sessions. All the talks of the conference will be streamed through Zoom.

The last year has been especially challenging for many of us, both from a personal, and a scientific point of view. After this difficult period, we hope this conference will be an excellent scenario for our community to catch up, engage in exciting and fruitful discussions, sparking new ideas and seeing new collaborations come out.

The BIFI2021 Organizing Committee
Zaragoza, 2021

Program

February 3rd

09:30-09:45 **Opening Ceremony (BIFI Director)**

Biochemistry and MCB / Chair: Jesús Clemente-Gallardo

Gene regulation and physiology of cyanobacteria.

09:45-10:15 **Maria F. Fillat,** FUR proteins from Anabaena sp. PCC7120: beyond the control of metal Homeostasis. Ref. 01PI.

10:15-10:30 **Jorge Guío,** Lin genes in Anabaena sp. PCC7120: experimental bases for the development of a lindane biosensor. Ref. 01T.

Apoptosis and Metabolism.

10:30-11:00 **José A. Carrodeguas,** Cellular models to investigate Parkinson's alfa-synuclein aggregation and cell-to-cell transmission. Ref. 02PI.

11:00-11:15 **Diego de la Fuente,** Crosstalk between alpha-synuclein and ubiquitin in Parkinson's disease. Ref. 02T.

11:15-11:45 Break

Physics and Computation / Chair: Jesús Clemente-Gallardo.

Physical modelling of biomolecules.

11:45-12:15 **Alessandro Fiasconaro,** Overview and updates on the Physical Modelling of Biomolecules research line. Ref. 03PI.

12:15-12:45 **Antonio Rey,** Simulation of protein folding in confined and/or crowded environments. Ref. 04PI.

12:45-13:00 **David Luna,** Some strategies to test and refine a statistical inference model for antibody sequences. Ref. 03T.

Theoretical and applied modeling of complex systems

13:00-13:30 **Jesús Gómez-Gardeñes**, Modeling and analysis of COVID-19 diffusion by integrating multiple interactions and mobility patterns. Ref. 05PI.

13:30 - 13:45 **Sergio Faci**, A framework for joint explosive percolation and synchronization Transitions. Ref. 04T.

13:45 - 14:00 **Adriana Reyna**, Virus spread versus contact tracing: Two competing contagion processes. Ref. 05T.

14:00-15:30 Lunch Break.

Biophysics / Chair: Nunilo Cremades

Protein folding & Molecular design.

15:30 - 16:00 **Javier Sancho**, Proteins as molecules to understand and modulate. Ref. 06PI.

16:00 - 16:15 **Juan José Galano Frutos**, PirePred: linking Bioinformatics to clinical diagnosis. Ref. 06T.

16:15 - 16:30 **Helena García Cebollada**, Computational pathogenicity prediction in Rett Syndrome using 2DRMSD clustering. Ref. 07T.

Signal transduction & membrane protein therapies

16:30 - 17:00 **Javier García-Nafría**, Signal transduction & membrane protein therapies. Ref. 07PI.

Structural biology of brain receptor complexes

17:00 - 17:15 **Beatriz Herguedas**, Structural biology of brain receptor complexes. Ref. 08PI.

17:15-18:30 **Poster Session 1.**

Ref. P01 **Teresa Bes**, Exploring FurA interaction with photosynthetic electron carrier proteins in *Anabaena* sp. PCC7120

Ref. P02 **Sergio Boneta**, Computational insights on the hydride transference of the human Apoptosis Inducing Factor (hAIF) enzyme

Ref. P03 **José D. Camino**, Towards a physiologically relevant aggregation pathway of α-synuclein in Parkinson's disease

Ref. P04 **Javier Casado**, The ArsR response regulator: a novel and validated therapeutic target against *Helicobacter pylori* infection

Ref. P05 **Pablo Gracia**, Dual Color Time-Resolved Fluorescence Spectroscopy: a Framework for Studying Small Molecule-Amyloid Interactions

Ref. P06 **Jorge Guío**, Identification of regulatory networks performed by the Ferric Uptake Regulator FurA in Anabaena sp. PCC7120

Ref. P07 **Marta Martínez-Júlvez**, Biochemical characterisation of MurA: an essential component in peptidoglycan cell wall biosynthesis

Ref. P08 **Francho Nerín**, Expanding a Molecular Dynamics-based method for the calculation of protein stability from first principles

Ref. P09 **Asier Urriolabeitia**, Rh-NHC catalyzed head-to-tail alkyne dimerization enhanced by metal-ligand cooperation.

18:30 **BIFI Council Meeting**

February 4th

Physics and Computation / Chair: Joaquín Sanz

Spin glasses.

9:30 - 10:00 **Javier Moreno-Gordo**, Temperature chaos is present in off-equilibrium spin-glass dynamics. Ref. 09PI.

Molecular structure and electronic dynamics

10:00 - 10:15 **Carlos Bouthelier**, Entropy and canonical ensemble of hybrid quantum classical systems. Ref. 08T.

Computation Science

10:15 - 10:45 **Daniel Martínez**, Systems, Support and Supercomputing Ref. 10PI.

Citizen Science

10:45 - 11:15 **Jesús Clemente-Gallardo.** Citizen Science in Zaragoza: recent collaborations of BIFI and Ibercivis Foundation. Ref. 11PI.

11:15-11:45 Break

Biophysics / Chair: Javier García Nafría.

Biomolecular interactions.

11:45 - 12:30 **Adrián Velázquez-Campoy**, Biomolecular interactions, drug discovery, LACRIMA and ZCAM. Ref. 12PI.

12:30 - 12:45 **David Ortega-Alarcón**, Translational research in MeCP2-related diseases. Ref. 09T.

Flavoenzymes: action mechanisms & Biotechnology

12:45 - 13:15 **Milagros Medina**, Flavoenzymes as versatile catalysts: gaining basic knowledge to develop Biotech and Biomed tools. Ref. 13PI.

13:15 - 13:30 **Nerea Novo**, W483 in the apoptosis inducing factor: thermodynamic, kinetic and dynamic implications for NADH oxidation. Ref. 10T.

13:30 - 13:45 **Martha Minjarez-Sáenz**, The flavoproteome of Brucella ovis in the search of antimicrobial targets: MurB, a promising candidate. Ref. 11T.

13:45-15:15 Lunch Break.

Biophysics / Chair: Nunilo Cremades.

Clinical Diagnosis and Drug Delivery.

15:15 - 15:45 **Olga Abián**, Clinical Diagnosis and Drug Delivery. Ref. 14PI.

15:45 - 16:00 **Ana Jiménez-Alesanco**, Drug discovery for inhibiting 3CLPro: an essential protease of SARSCoV-2. Ref. 12T.

Biochemistry and MCB / Chair: Nunilo Cremades.

***Mycobacterium tuberculosis*.**

16:00 - 16:30 **Jesús Gonzalo Asensio**, Some reasons to study the adaptation of Mycobacterium tuberculosis to its human host. Ref. 15PI.

16:30 - 16:45 **Juan Calvet Seral**, *Mycobacterium tuberculosis* PhoP-reporter strains for testing potential anti-virulence molecules. Ref. 13T.

Development of antimicrobials and mechanisms of resistance

16:45 - 17:15 **José A. Aínsa**, Antimicrobial "magic bullets" in the 21st century. Ref. 16PI.

17:15 - 17:30 **José Manuel Ezquerra**, Strategies to elucidate the mode of action of avermectins against mycobacteria. Ref. 14T.

17:30-19:00 **Poster Session 2.**

Ref. P10 **Martha Minjárez-Sáenz**, The flavoproteome of *Brucella ovis* in the search of antimicrobial targets: MurB, a promising candidate.

Ref. P11 **Violeta Morcuende**, A novel approach for early cancer detection based on dendrimer nanoparticles .

Ref. P12 **Irene Oliván**, Unraveling biofilm formation in the filamentous cyanobacterium Anabaena sp. PCC7120.

Ref. P13 **Cristina Sarasa-Buisán**, Expanding the FurC regulon from *Anabaena* sp. PCC7120: *in silico* approach and experimental validation of novel FurC targets.

Ref. P14 **David Soriano-Paños**, Influence of recurrent mobility patterns on the spread of vector-borne diseases.

Ref. P15 **Ernesto Anoz-Carbonell**, The Catalytic Cycle of Human NQO1: Hydride Transfer, Conformational Dynamics, and Functional Cooperativity.

Ref. P16 **Sonia Hermoso-Durán**, Thermal Liquid Biopsy (TLB) applied to Pancreatic Cyst Diagnosis.

Ref. P17 **Nerea Novo**, W483 in the apoptosis inducing factor: thermodynamic, kinetic and dynamic implications for NADH oxidation.

Ref. P18 **David Polanco**, Characterization of the aggregation pathway of Parkinson´s related amyloid protein alpha-synuclein under liquid-liquid phase separation.

Ref. P19 **Patricia Bruñén**, Design and synthesis of FMN derivatives for covalent binding to *Anabaena* apoflavodoxin.

February 5th

Physics and Computation / Chair: Joaquín Sanz.

Complex systems & Networks.

09:30 - 10:00 **Yamir Moreno**, Research lines @ COSNET Lab. Ref. 17PI.

10:00 - 10:15 **Carlos Gracia**, Game Theory: Modeling from experimental results. Ref. 15T.

10:15 - 10:30 **Alberto Aletá**, Modeling COVID-19: A Quest for Data. Ref. 16T.

Biophysics / Chair: Joaquín Sanz.

Protein misfolding & amyloid aggregation.

10:30 - 11:00 **Nunilo Cremades**, Pathological protein self-assembly mechanisms and single-molecule experimental tools for their study. Ref. 18PI.

11:00 - 11:15 **Pablo Gracia**, Dual Color Time-Resolved Fluorescence Spectroscopy: a Framework for Studying Small Molecule-Amyloid Interactions. Ref. 17T.

11:15-11:45 Break

Biophysics / Chair: Nunilo Cremades.

Protein glycosylation and its role in disease.

11:45 - 12:15 **Ramón Hurtado**, Why glycosylation matters in health and disease? Ref. 19PI.

12:15 - 12:30 **Ana García-García**, Structural basis for substrate specificity and catalysis of 1,6- fucosyltransferase. Ref. 18T.

Associated Units / Chair: Nunilo Cremades.

12:30 - 13:00 **Inmaculada Yruela** *(Aula Dei-CSIC / Structural and Computational Biology),* The RDI Unit 'GBsC' (BIFI-Unizar) associated to the CSIC: a holistic practice in the investigation of genes and proteins. Ref. 20PI.

13:00 - 13:30 **Juan Hermoso** *(IQF"Rocasolano"-CSIC / Structural Biology and Crystallography),* From Fundamental Processes to Biotechnology and Biomedicine. A Glimpse of Structural Research at CBE-IQFR. Ref. 21PI.

13:30 - 14:00 **Douglas V. Laurents** *(IQF"Rocasolano"-CSIC / Protein NMR group)*, Ongoing Research in the "Manuel Rico" NMR Laboratory. Ref. 22PI.

14:00-15:30 Lunch Break

Biochemistry and MCB / Chair: Javier García Nafría.

Functional genomics of the OXPHOS system.

15:30 - 15:45 **Patricio Fernández**, How hot are mitochondria? Ref. 23PI.

15:45 - 16:00 **Raquel Moreno-Loshuertos**, Real-Time Intracellular Temperature Imaging Using Lanthanide-Bearing Nanoparticles. Ref. 24PI.

16:00 - 16:15 **Ruth Soler-Agesta**, Mechanism of dichloroacetate-induced cell death in highly tumorigenic and glycolytic cancer cells. Ref. 19T.

Computational genomics and systems Bio-medicine

16:15 - 16:45 **Joaquín Sanz**, Computational genomics and systems Bio-medicine: a new research line at BIFI. Ref. 25PI.

16:45 - 17:00 **Mario Tovar Calonge**, A Bayesian approach to minimize bias and uncertainty in impact evaluation of tuberculosis vaccines. Ref. 20T.

17:00 - 17:15 **Ignacio Marchante Hueso**, Using single-cell RNA-sequencing to characterize genetic ancestry effects on the response to viral infection. Ref. 21T.

Plant evolutionary biology

17:15 - 17:45 **Pilar Catalán**, Gradual polyploid genome evolution of model Brachypodium grasses revealed by pangenomic analysis. Ref. 26PI.

17:45 - 18:00 **Rubén Sancho**, PhyloSD: Phylogenomic detection of known and ghost subgenomes of polyploid plants. Ref. 22T.

18:00 **Concluding remarks and poster prizes**

Talks

Wednesday, February 3rd

Ref. Number: 01PI

FUR proteins from Anabaena sp. PCC7120: beyond the control of metal Homeostasis.

María F. Fillat[1,2].

1. Department of Biochemistry and Molecular and Cellular Biology, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.

Presenting author: fillat-at-unizar.es

FUR (ferric uptake regulator) proteins constitute a superfamily of prokaryotic transcriptional regulators that usually control the homeostasis of different metals. In the cyanobacterium *Anabaena* sp. PCC7120 (herein *Anabaena*), this family expands to three members that correspond to the FurA (iron response), FurB (zinc response) and FurC (peroxide response) paralogs. Multi-approach analyses evidence that in *Anabaena,* FUR are multifunctional proteins which coordinate the regulation of metal homeostasis with the antioxidant response and the photosynthetic and nitrogen metabolisms. FurA is a redox protein that beyond the direct transcriptional control of regulated genes can bind small molecules, namely heme and 2-oxoglutarate, modulating its affinity for DNA. FurB (Zur), in addition to control zinc homeostasis, regulates a set of genes crucial for cellular defense against reactive oxygen species. FurB integrates the response to zinc availability with the redox environmental conditions through thiol-mediated redox modulation and/or heme binding. The FurC (PerR) regulon includes a set of genes involved in the response to peroxide, some components of the phycobilisome and cell division genes, including the major thylakoid membrane protease *ftsH*, among others. Direct regulation by FurC of key genes involved in nitrogen metabolism and heterocyst development has been verified, expanding the regulon of a PerR orthologue.

Ref. Number: 01T

Lin genes in *Anabaena* sp. PCC7120: experimental bases for the development of a lindane biosensor.

Jorge Guío[1,2], Hernández-Sancho JM[1,2], Fillat MF[1,2], Peleato ML[1,2] and Sevilla E[1,2]

1. Department of Biochemistry and Molecular and Cellular Biology, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.

Presenting author: Jorge Guío: jguio-at-unizar.es

Lindane (γ-hexachlorociclohexane, γ-HCH) is a persistent pesticide that triggers environmental and health problems. Although it is durable and recalcitrant, some microorganisms such as *Sphingomonas paucimobilis* are capable of degrading it. *S. paucimobilis* contains in its genome several enzymes involved in HCH degradation, which are encoded by the catabolic *lin* genes and are dispersed across the genome. As previous studies showed that the cyanobacterium *Anabaena* sp. PCC7120 was also able to degrade lindane, we sought to investigate if this cyanobacterium contained genes involved in lindane degradation.

A comparative genomic study with *S. paucimobilis* identified potential *lin* genes in its genome, whose expression was analyzed in the presence of γ-HCH and 2,5-DCHQ (a degradation intermediate that acts as an inductor of *lin* genes expression in other organisms). Our results showed that the expression of one of them increases more than 15 times in the presence of lindane and consequently could be a good candidate for the development of a whole-cell lindane biosensor. The degradative capacity and tolerance of *Anabaena* sp. PCC7120 to α-HCH, β-HCH and δ-HCH isomers were also studied, proving that this cyanobacterium is able to tolerate and possibly degrade other HCH isomers.

Ref. Number: 02PI

Cellular models to investigate Parkinson's alfa-synuclein aggregation and cell-to-cell transmission.

Diego de la Fuente[1], Beatriz Sáenz de Buruaga[1], Nunilo Cremades[1, 2] and **José A. Carrodeguas[1, 2]**

1. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.
2. Department of Biochemistry and Molecular and Cellular Biology, University of Zaragoza.

Presenting author: José A. Carrodeguas: carrode-at-unizar.es

Parkinson's disease is one of the prevalent neurodegenerative diseases in our society. At the molecular level it is characterized by misfolding of an intrinsically disordered protein, α -synuclein (aSyn), which generates neurotoxic insoluble amyloid fibers that accumulate mainly in the substantia nigra of the midbrain, although soluble oligomers appear to be more toxic. aSyn adopts an α-helical secondary structure upon interaction with membranes in order to perform its main physiological function, related to vesicle trafficking. Under certain unknown conditions, however, the protein misfolds and adopts a α-sheet structure upon self-assembly, which is able to promote the misfolding of new aSyn molecules that can migrate from neuron to neuron in a way similar to prions. We are trying to uncover the initial events that lead to aSyn misfolding and self-assembly using different cellular models cultured both in two and in three dimensions (3D cultures), including neuronal cells differentiated *in vitro*, co-culture of neurons and glial cells and knockout cells deficient in autophagy (a process involved in clearing misfolded proteins). We are also interested in exploring the role of the interaction of aSyn with other proteins like tau and also recently discovered mechanisms that place a possible origin of the disease at the gut, since transmission from gut to brain of misfolded aSyn has been demonstrated. We will discuss the different approaches we are using at the lab.

Ref. Number: 02T

Crosstalk between alpha-synuclein and ubiquitin in Parkinson's disease.

Diego De La Fuente[1,2], José A. Carrodeguas[1,2], Nunilo Cremades[1,2]

1. Department of Biochemistry and Molecular and Cellular Biology, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.

Presenting author: Diego de la Fuente: dfuente-at-bifi.es

Alpha synuclein (AS) is the major protein component of Lewy Bodies (LBs), the characteristic intracellular neuronal inclusions associated with Parkinson's disease (PD) and other neurodegenerative disorders. Indeed, the process of AS oligomerization and aggregation has been associated with the pathogenesis of LBs diseases and is believed to play a central role in the etiology of PD. However, the mechanisms by which AS starts to aggregate into toxic oligomers and fibers in neuronal cells remain largely unknown.

AS suffers extensive post-translational modifications, including ubiquitination, which is highly frequent in the protein found in the LBs, although it is not clear if any of these modifications are relevant for the initiation of the aggregation process.

p62 is a key protein involved in the degradation of ubiquitinated proteins either by their presentation to the proteasome or their sequestration into large condensates generated by phase separation and directed to autophagy. Interestingly, p62 is highly abundant in LBs and mutations in the p62 gene have been associated with a number of neurodegenerative diseases due to the accumulation of misfolded proteins.

In this work we present a preliminary assessment of a potential novel interaction between AS and p62.

Ref. Number: 03PI

Overview and updates on the Physical Modelling of Biomolecules research line.

Alessandro Fiasconaro[1,2]

1. Department of Condensed Matter Physics, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.

Presenting author: afiascon-at-unizar.es

In this talk, we will outline the different research lines connected to Physical Modelling of Biomolecules, that are developed at the BIFI institute.

These include the study of the energy landscape involved in folding/unfolding problems, for example applied to the guanine quadruplex (secondary DNA structure) and their conformations; the analysis of different models of polymer stretching and of the properties of polymer translocation with active (eventually ATP fueled) driving, also considering the flexibility of membrane and channels, with their implication in protein translocation; finally, we will mention the study of the statistical, species-dependent properties of antibody sequences, and of the strategies to model them, and the application of coarse-grained models to protein and polymer folding, in different environmental conditions (crowded/confined space, different temperatures and pressures, aggregating conditions).

In our research, we apply the theoretical and computational methods of statistical and non-linear physics, from analytic calculations to molecular dynamics or monte-carlo numerical simulations, where microscopic and mesoscopic spatial scales are explored. In the same line, the Force Spectroscopy approach has been also recently addressed with the purpose to both propose its use in mechanisms other than structure unfolding, and generalize its formal construction and derivation.

Computer simulations are typically executed with homemade programs to run in clusters, and occasionally with the use of packages for classical molecular dynamics as Gromacs.

Ref. Number: 04PI

Simulation of protein folding in confined and/or crowded environments.

Antonio Rey[1,2]

1. Department of Physical Chemistry, Universidad Complutense de Madrid.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.

Presenting author: areygayo-at-ucm.es

In biological conditions, proteins do not acquire their folded structures in a "clean", homogeneous dilute medium, as it happens *in vitro*. On the contrary, in a cellular environment a high fraction of the volume is occupied by other macromolecules, structures, membranes, and so on, with different sizes, shapes, and interaction features. Even more, some proteins only fold inside dedicated cavities in the cell, named chaperonins. Both situations affect the thermodynamic and kinetic features of the folding process, and have been the aim of recent active research.

In this talk, I summarize the molecular simulations we are presently doing in our group to describe these processes, using very simple, coarse-grained models. The folding of several proteins inside cavities does not only depend on the cavity size, but also on the interactions between the wall and some residues of the protein. As a matter of fact, our results varying the hydrophobic/hydrophilic nature of these interactions can help to understand, in a very simple way, the folding cycle inside a chaperonin barrel.

In a different, but related, project, I describe our work on a simple representation of the crowded conditions. A high density of crowders, specially of small size (comparable to the size of the folded protein), can enhance the stability of the folded state. However, the combined effects of crowders and confinement walls can induce a certain ordering in the crowders structure, which in turn affects the folding equilibrium.

Ref. Number: 03T

Some strategies to test and refine a statistical inference model for antibody sequences.

David Luna[1,2]

1. Department of Theoretical Physics, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.

Presenting author: luna.cerralbo.david-at-gmail.com

A Multivariate Gaussian Model has been proposed to perform a Bayesian inference of the human antibody sequences. Despite the good results obtained in classification and humanization tasks, the model has not been thoroughly studied and tested yet in controlled conditions, also due to the intrinsic difficulties entailed by the high dimensionality of the probability space. In this talk I report our efforts to design strategies to perform compelling tests on the model, discussing some (perhaps) surprising results.

Ref. Number: 05PI

Modeling and analysis of COVID-19 diffusion by integrating multiple interactions and mobility patterns.

Jesús Gómez-Gardeñes[1,2]

1. Department of Condensed Matter Physics, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.

Presenting author: gardenes-at-unizar.es

The spread of COVID-19 is posing an unprecedented threat to health systems worldwide. The fast propagation of the disease combined with the existence of covert contagions by asymptomatic individuals make the controlling of this disease particularly challenging. Here, we propose a metapopulation model that integrates into a single framework the different mobility and interaction patterns that coexist in our societies. These mixing and mobility patterns are the main drivers behind SARS-CoV-2 diffusion and the impact of COVID-19 impact on our health systems. This tailored epidemic model allows us to monitor and explain the advance of the disease and to find an analytical expression for the effective reproduction number, R, as a function of mobility restrictions and confinement measures. The expression for R is an extremely useful tool to design containment policies that are able to suppress the epidemics allowing us to determine the precise reduction of mobility needed to bend the curve of epidemic incidence.

Ref. Number: 04T

A framework for joint explosive percolation and synchronization transitions.

Sergio Faci Lázaro[1,2]

1. Department of Condensed Matter Physics, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.

Presenting author: sergiofacilazaro-at-gmail.es

Abrupt and explosive phase transitions are one of the most important results in Statistical Physics and in Complex Networks of the last few decades. The examples more commonly studied are the Explosive Percolation (EP) and the Explosive Synchronization (ES) transition. Our objective in this work is the development and derivation of a rule or set of rules that allow a system to display an explosive behavior in the percolation transition as well as in the synchronization transition. With this aim in mind, let us consider a system of N non-identical Kuramoto oscillators running on top of a network, whose macroscopic behavior is captured by the modulus of the Kuramoto order parameter, r. By making a series of assumptions [such as: (i) the system tends to maximize the synchronization, (ii) we have limited information, i. e. the process is decentralized, (iii) the percolation is adiabatic compared to the synchronization, and (iv) the system is closed to the synchronization attractor] we are able to derive a rule for the order in which the links of the network have to be added (removed) that delays the critical point of the percolation and the synchronization transition, giving them an abrupt and explosive nature, respectively.

Ref. Number: 05T

Virus spread versus contact tracing: Two competing contagion processes.

Adriana Reyna Lara[1,2]

1. Department of Condensed Matter Physics, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.

Presenting author: adriana.ryl-at-gmail.es

After the blockade that many nations suffered to stop the growth of the incidence curve of COVID-19 during the first half of 2020, they face the challenge of resuming their social and economic activity. The rapid airborne transmissibility of SARS-CoV-2, and the absence of a vaccine, calls for active containment measures to avoid the propagation of transmission chains. The best strategy up to date, popularly known as Test-Track-Treat (TTT), consists in testing the population for diagnosis, tracking the contacts of those infected, and treating by quarantine all these cases. The dynamical process that better describes the combined action of the former mechanisms is that of a contagion process that competes with the spread of the pathogen, cutting off potential contagion pathways. Here we propose a compartmental model that couples the dynamics of the infection with the contact tracing and isolation of cases. We develop an analytical expression for the effective case reproduction number Rc(t) that reveals the role of contact tracing in the mitigation and suppression of the epidemic. We show that there is a tradeoff between the infection propagation and the isolation of cases. If the isolation is limited to symptomatic individuals only, the incidence curve can be flattened but not bended. However, if contact tracing is applied to asymptomatic individuals too, the strategy can bend the curve and suppress the epidemics. Quantitative results are dependent on the network topology. We quantify, the most important indicator of the effectiveness of contact tracing, namely its capacity to sharply reverse the increasing tendency of the original epidemic curve, causing its bending.

Ref. Number: 06PI

Proteins as molecules to understand and modulate.

Javier Sancho[1,2,3,4]

1. Department of Biochemistry and Molecular and Cellular Biology, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.
3. Joint Units BIFI-IQFR (CSIC) and GBs-CSIC, University of Zaragoza, Spain.
4. Aragon Health Research Institute (IIS Aragón), Zaragoza, Spain.

Presenting author: jsancho-at-unizar.es

Proteins are versatile biological molecules, key to understanding cell function and highly valuable in medical and biotechnological contexts. In many cases, existing proteins are not optimal for a given task, and protein engineering embarks on the design of suitable variants by making the necessary changes to the original amino acid sequence. As protein folding is a well-defined problem, computational simulation based on the physico-chemical principles that govern the structure of proteins is a useful and mature approach. Our group combines theory, experimentation and simulation, to investigate the principles and details of protein stability and try to devise reliable strategies for protein stabilization. We also investigate the impact of amino acid replacements on the stability and function of human proteins in hopes of generating computational tools for more precise genetic interpretation. Following talks by Helena García-Cebollada and Juan José Galano-Frutos will illustrate our recent activity in genetic interpretation. On the other hand, the structure, stability and function of proteins can be modulated by small binding molecules. Having discovered by HTS several interesting hits that modify the activity of proteins related to human diseases, we are implementing medical chemistry programs to refine those hits and bring them closer to clinical trials. I will highlight where we are and where we want to go in genetic interpretation and protein stabilization and show examples of our commitment to creating web servers to facilitate the use of current knowledge by other groups. I will also present the latest developments in our medical chemistry programs aimed at obtaining new anti-microbials, pharmacological chaperones, and protein aggregation inhibitors of interest for *Helicobacter pylori* infection, phenylketonuria, and Parkinson's disease.

Ref. Number: 06T

PirePred: linking Bioinformatics to clinical diagnosis.

Juan José Galano Frutos[1,2,3,4]

1. Department of Biochemistry and Molecular and Cellular Biology, University of Zaragoza.
2. Institute BIFI for Biocomputation and Physics of Complex Systems, University of Zaragoza.
3. Joint Units BIFI-IQFR (CSIC) and GBs-CSIC, University of Zaragoza, Spain.
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PirePred is a bioinformatics server designed as an interpretation tool for clinicians interested in the genotype/phenotype relationships of clinical variants found in 58 genes related to conditions investigated in neonatal screening programs. Missense, nonsense and frameshift Single Nucleotide Variants (SNVs) annotated in the ClinVar database are retrieved in real time and presented in the structural context of the original protein. For each variant, binary classifications (tolerated/deleterious) from 16 popular predictors are shown and taken for issuing a consensus ternary classification (tolerated/uncertain/deleterious) by using a Majority vote algorithm. The consensus classification, applied to reliably annotated ClinVar variants, is as accurate as the best predictors and largely increases their coverage. At the user level, a gene, protein or disease can be selected, the output being presented in two panels, one displaying a list of SNVs and the other showing a ribbon interactive representation of the protein. The proteins are described by quality structures or homology models of the expected oligomers so that target variants can be interpreted in its structural context. Further structural and functional data, e.g. occurrence of variation at monomer/monomer interfaces, catalytic sites, or other functional sites, are also included which can help to interpret the phenotype. SNVs not documented in ClinVar (e.g., a newly found variant) are also included and classified by Pirepred. This server will be a great help to neonatal screening units and pediatricians in general and will provide them an inviting, appealing gate into the structural/functional consequences of genetic variants.

Keywords: neonatal screening, genetic interpretation, bioinformatic tool, single nucleotide variation, genotype/phenotype relationships, clinical diagnosis.

Ref. Number: 07T

Computational pathogenicity prediction in Rett Syndrome using 2DRMSD clustering.

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Rett syndrome is a genetic developmental disorder affecting 1 out of 8500 chromosomally female babies, caused by a mutation in the MECP2 gene, located on the X chromosome. Current Next Generation Sequencing methods are efficient finding variants of this gene, including single nucleotide ones (SNVs), but they lack a direct interpretation of the variant, unless previously found. Predictive computational methods seek to cover for this gap, usually focused on missense SNVs. Most methods only use information from sequence homology or few structural static measures, reaching a maximum accuracy up to 85% in commonly used predictors. However, the increasing availability of three-dimensional structures and the improvement of the methods used in Molecular Dynamics simulations can improve the accuracy of these predictions. In this work, relaxation Molecular Dynamics simulations are performed for variants in the Methyl Binding Domain of MECP2. Their analysis with a clustering algorithm sorting different conformations on the trajectory based on two-dimensional Root Mean Square Deviation of the structure allows to qualitatively predict the effect of the mutation and estimating the change in free Gibbs energy upon mutation ($\Delta\Delta G$). The improvement and fine tuning of this method may result in an accurate and simple pathogenicity predictor for missense SNPs.

Ref. Number: 07PI

Signal transduction and membrane protein therapies.

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G protein-coupled receptors (GPCRs) are the largest family of membrane proteins in the human body, they control the function of all major organs and are also the most prolific pharmacological targets. I will present the group´s current research to understand new structural and functional mechanisms of the GPCR family taking as model system the human dopamine receptors. Dopamine receptors modulate the motor function, reward and cognition and also the target for anti-Parkinson and anti-psychotic drugs. I will introduce our approach to understand drug specificity, signaling bias and oligomerization of dopamine receptors and how that can translate into improved anti-Parkinson therapeutics. For this purpose we use state-of-the-art cryo-electron microscopy, biophysics, biochemistry and cell assays while developing new tools and approaches in protein engineering and receptor oligomerization.

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Ref. Number: 08PI

Structural biology of brain receptor complexes.

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In the brain, chemical synapses allow the communication between presynaptic neurons (which release neurotransmitters) and postsynaptic neurons, which contain receptors in their surface that detect neurotransmitters and induce a response in the postsynaptic neuron. Among these neurotransmitter receptors, Ionotropic Glutamate Receptors (iGluRs) bind Glutamate, the main excitatory neurotransmitter in the brain, and are involved in synaptic plasticity, the molecular mechanism underlying memory formation. Disfunction of iGluRs is associated with neurological disorders, including epilepsy, Alzheimer's or Amyotrophic Lateral Sclerosis. Our aim is to understand the molecular diversity of AMPA-type iGluRs, focusing on Ca2+-permeable AMPA receptor complexes, which can be abnormally incorporated into synapses and cause excitotoxicity. We are currently working on complexes with two families of AMPAR auxiliary proteins, type-2- TARPs and CKAMP proteins, focusing on complex production and purification, cryo-EM structure determination and conformational dynamics characterization.

Ref. Number: 09PI

Temperature chaos is present in off-equilibrium spin-glass dynamics.

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Spin glasses exhibit a fragile behavior in response to perturbations such as temperature changes. Specifically, arbitrary small changes in the temperature would lead to a complete reorganization of the equilibrium configurations of the spin glass. This phenomenon has been called Temperature Chaos [1,2].

This equilibrium definition has focused the research effort on small system sizes that can be equilibrated. We have observed a phenomenon that closely mimics the Temperature Chaos in large non-equilibrium spin-glass and we provide a quantitative description [3]. By invoking the static-dynamic equivalence principle we find that the key quantity which is ruling the non-equilibrium Temperature Chaos phenomenon is the correlation length ξ. Also, a rare-event analysis is needed to deal with the strong spatial-heterogeneity of the non-equilibrium Temperature Chaos. We find a crossover between weak and strong chaos regime controlled by a crossover length ξ^* whose analysis reveals the close relation with its equilibrium counterpart: the chaotic-length [2].

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[3] J. Moreno-Gordo et al Work submitted (https://arxiv.org/abs/2011.09419)

Ref. Number: 08T

Entropy and canonical ensemble of hybrid quantum classical systems

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In this work we generalize and combine Gibbs and von Neumann approaches to build, for the first time, a rigorous definition of entropy for hybrid quantum-classical systems. The resulting function coincides with the two cases above when the suitable limits are considered. Then, we apply the MaxEnt principle for this hybrid entropy function and obtain the natural candidate for the hybrid canonical ensemble (HCE). We prove that the suitable classical and quantum limits of the HCE coincide with the usual classical and quantum canonical ensembles since the whole scheme admits both limits, thus showing that the MaxEnt principle is applicable and consistent for hybrid systems.

Ref. Number: 10PI

Systems, Support and Supercomputing.

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During my talk, I will give you an overview of the services and available infrastructures that are currently managed by the team of computational systems and support at BIFI to assist researchers, users and scientific projects in the Institute. Those include HPC (High Performance Computing), Cloud Computing, server administration, Housing and external services, datacenter management, network administration and user support. The team nowadays is in charge of a variety of tasks, from the deployment of parallel computing systems to the installation of programs or printers on user computers, as well as monitoring of environmental data from the datacenter.

Ref. Number: 11PI

Citizen Science in Zaragoza: recent collaborations of BIFI and Ibercivis Foundation

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In this talk we will summarize the most recent collaborations of our group with Ibercivis Foundation, one of the main Spanish actors in the Citizen Science landscape. We will focus in two recent projects: the European project BRITEC which is studying the potential of Citizen Science as a didactic resource in secondary education and the study of the citizen science expansion and evolution in terms of the properties of the graphs which encode relations between scientists by studying co-authorship and the consequent networks of collaboration.

Ref. Number: 12PI

Biomolecular interactions, drug discovery, LACRIMA and ZCAM

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Physical interaction is a requirement for all biological molecules to function. The ensemble of conformational and functional states in a protein constitutes the conformational-functional landscape for that protein, which represents the molecular basis of its function and its allosteric control. Different types of protein interactions will be reviewed with experimental examples.

LACRIMA (Laboratorio de CRibado e Interacciones Moleculares de Aragón), the Biophysics experimental facility at BIFI, has been established along the last few years thanks to individual and collective efforts from BIFI researchers in the drug discovery and development field. Many local, national and international projects have benefitted from this experimental infrastructure. Information on LACRIMA and its participation in two European initiatives, ARBRE-MOBIEU and MOSBRI, will be given.

Ref. Number: 09T

Translational research in MeCP2-related diseases.

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As the field of epigenetics is rapidly growing as a research field, the efforts in elucidating these transcription regulation mechanisms are being focused not only on the molecules that create these epigenetic marks (such as DNA methylation, histone post-translational modifications...) but also the readers that transform those signals into an actual regulation of gene expression and cellular differentiation or development.

Methyl-CpG Binding Protein 2 (MeCP2) is an intrinsically disordered protein (IDP) which acts as one of those epigenetic readers. Thanks to its structural and functional plasticity MeCP2 is able to bind DNA, discriminate among different epigenetic marks and recruit activators or repressors of gene expression.

Basic research concerning MeCP2 structure and binding to different partners will allow us not only to have a better understanding of epigenetic mechanisms but it will also have a great impact on human health, as MeCP2 upregulation or dysfunction are directly related to different neurodevelopmental diseases (as Rett Syndome or MeCP2 duplication syndrome) and it's also indirectly involved in some kinds of cancer when transcriptional regulation is lost.
Taking these considerations into account, our goal was to start from basic research to comprehend the implications of the different domains and mutations of the protein in stability and DNA binding at a structural and thermodynamic level using Circular Dichroism, fluorescence measurement of thermal denaturation and isothermal titration calorimetry.

Afterwards, we have used this knowledge to approach a translational research, and obtain a set of chemical compounds via high throughput screening based thermal shift assay (TSA) to tune the MeCP2 function. Finally, we have started to scale it to cellular level using neuronal precursor cell lines with RETT-related mutations, introduced by CRISPR-Cas9, and studying the effect of the compounds on the expression of genes related to Rett and MeCP2 duplication syndrome, employing RT-qPCR and ChIP methodologies.

Ref. Number: 13PI

Flavoenzymes as versatile catalysts: gaining basic knowledge to develop Biotech and Biomed tools.

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Flavoenzymes are versatile and diverse biomolecules that contribute to maintain and build cellular structures, to move molecules among cellular compartments, to eliminate toxins, to facilitate chemical transformations in biosynthetic pathways, while being particularly in charge of cellular bioenergetics and signalling. Their particular feature is that they have as cofactors the riboflavin (RF, vitamin B_2) derivatives flavin mononucleotide (FMN) and/or flavin adenine dinucleotide (FAD), which confer them unique and versatile properties as catalysts. All organisms contain key flavoenzymes, and many of them are becoming interesting as therapeutic targets or biotechnological tools. Implementation of the use of flavoenzymes in such actions requires to understand the relationships between the pathological and molecular mechanisms in which they might be involved, as well as the mechanisms for their catalytic versatility under different conditions. This is the main goal of the "Flavoenzymes: mechanisms of action and biotechnology" Lab at BIFI. Here we will present some of the particular topics we are researching on at the moment, as well as the different methodologies applied in our research.

Ref. Number: 10T

W483 in the Apoptosis Inducing Factor: Thermodynamic, Kinetic and Dynamic Implications for NADH Oxidation

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The human apoptosis inducing factor (hAIF) is a moonlighting flavoenzyme that fulfils a vital function in the bioenergetics and redox metabolism of mitochondria in healthy cells, but which may also trigger caspase-independent cell death upon pro–apoptotic stimuli. The FAD cofactor of hAIF can be reduced by NADH, prompting dimerization and the formation of a remarkably stable $FADH^{-}$:NAD^{+} charge transfer complex (CTC). The allosteric binding of a second non-catalytic NADH molecule ($NAD(H)_B$) further arbitrates the resulting monomer–dimer equilibrium. Tryptophan 483 of hAIF is involved in an extensive H–bond network that stabilizes the conformation of the catalytic NADH ($NAD(H)_A$) while simultaneously T–stacking between the flavin isoalloxacine ring and the nicotinamide of $NAD(H)_B$. Consequently, W483 plays a potentially critical role in the formation of a catalytically–competent complex for hydride transfer between the FAD and $NAD(H)_A$, determining the efficiency of hAIF as an oxidoreductase. Three W483 mutations expected to alter the environment of the isoalloxacine ring (W483G, W483L and W483Y) had been previously generated in our group, and significant effects were observed in both hydride transfer and dissociation rates. Said mutants are further characterised in the present work, demonstrating the critical aftermath in hydride transfer efficiency and dynamics, redox potential and thermodynamic stability.

Ref. Number: 11T

The flavoproteome of *Brucella ovis* in the search of antimicrobial targets: MurB, a promising candidate.

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Flavoproteins are key in the energetic metabolism in bacteria, participating in processes such as metabolism of fats, carbohydrates and proteins, oxidative stress response, photosensitization, peptidoglycan wall synthesis and activation of other vitamins as folate and pyridoxine. Bacterial genomes encode for around 2% of flavoproteins, many of which are probably essential and species specific (1-3).

We have characterized the flavoproteome content in the bacterial model *Brucella ovis*, a pathogen of ovine brucellosis, by using a combination of NCBI, Uniprot, PDB, BRENDA and Pfam Family databases. We have identified around 78 proteins of a total of 2850 proteins distributed in 2 chromosomes, almost 2.7% correspond to putative proteins with flavin-binding motifs, which have been classified with respect to their possible activity and biological role.

We find with special interest the flavoenzyme encoded by the gene BOV_1386, MurB or UDP-N-acetylenolpyruvoylglucosamine reductase, involved in the synthesis of the bacterial cell wall, which reduces EP-UDP-GlcNac to UDP-MurNac using NADPH and FAD, which is an attractive target for antimicrobials design and is absent in eukaryotic organisms (4,5). We have successfully cloned and produced the MurB enzyme from *B. ovis*. Its integrity and structural characteristics have been evaluated by spectroscopy, fluorescence, circular dichroism, and thermal stability, while Stopped Flow spectrophotometry has been used to evaluate its activity under different conditions.

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Ref. Number: 14PI

Clinical Diagnosis and Drug Delivery

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Thermal Liquid Biopsy (TLB) refers to the analysis of serum samples using differential scanning calorimetry (DSC). The precise determination of the thermally-induced conformational transitions of biomolecules can be performed using DSC due to its high sensitivity. In particular, the thermodynamic parameters of protein thermal denaturation (unfolding) can be determined directly by this technique. Our previous studies have contributed to TLB development and now it is closer to its clinical application in cancer diagnosis and monitoring. Our main objective is to implement and evaluate the ability of TLB in health programs (for example, colorectal screening program). We will present a general overview of the research line, projects and ongoing studies and the main results obtained up today.

Ref. Number: 12T

Drug discovery for inhibiting 3CLPro: an essential protease of SARSCoV-2

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The global health emergency generated by coronavirus disease 2019 (COVID-19) has resulted in the search for preventive as well as therapeutic treatments for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogen which causes this global outbreak. Many are the efforts focus on the potential targets of the virus for drug discovery and development in order to tackle this disease. One of these targets is the main protease, 3CLpro, which is an essential player in the viral replication cycle, processing the large viral polyproteins and rendering the individual proteins functional.

In our group, a characterization of both the structural stability and the catalytic activity of *3CLpro from SARS-CoV-2 was performed using biophysical techniques in order to perform a suitable in vitro molecular screening to identify compounds which are able to inhibit the protease activity of the protein.* By screening of a chemical library consisting of about 150 compounds, few compounds have shown inhibition effects on the protease activity and, among them, the natural product quercetin was identified as a reasonably potent inhibitor of SARS-CoV-2 3CLpro. Quercetin shows that interacts with 3CLpro using biophysical techniques and, regarding to molecular simulations, binds to the active site. This compound, with well-known pharmacokinetic and ADMET properties, is currently been testing on Vero cells infected with the virus, so it can be considered as a good candidate for inhibiting this essential protease of SARS-CoV-2.

Once our group designed a reliable and successful method from a small chemical library to identify potential inhibitors for 3CLpro from SARS-CoV-2, we decided to look for more potential inhibitors by using bigger chemical libraries (~11,000 compounds). *The selected compounds could be considered as* good candidates for further optimization and development, or repositioned for COVID-19 therapeutic treatment.

Ref. Number: 15PI

Some reasons to study the adaptation of *Mycobacterium tuberculosis* to its human host

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The *Mycobacterium tuberculosis* Complex (MTBC) comprises a variety of species and ecotypes, each specialized to infect different mammals. *M. tuberculosis* and *M. africanum* infect humans, while other species, as *M. bovis*, infect cows and entail a treat for human zoonosis. In our research line, we consider that understanding the host-pathogen interactions of pathogenic mycobacteria implies studying the *MTBC* as a whole. Accordingly, we have recently conducted the following investigations involving different members of the MTBC: First, we have demonstrated that the live attenuated vaccine candidate against tuberculosis, MTBVAC, protects mice against infection with the more widespread lineages causing this disease in humans. This result has important implications for future clinical trials of efficacy with the MTBVAC vaccine. Second, we have demonstrated that polymorphisms between *M. tuberculosis* and *M. bovis* in the regulatory proteins PhoP and PknH, impact on the functionality of these proteins and consequently on host adaptation. Third, we have demonstrated that transposition of IS*6110* is differentially regulated between MTBC species. Accordingly, *M. tuberculosis* contains a high copy number of this element, in contrast with animal-adapted species. Finally, we advise against the use of "*M. tuberculosis* laboratory strains" paradigmatic representatives of the Complex. As an example, a WhiB6 polymorphism exclusive of the H37Rv laboratory strain, causes decreased secretion of ESAT-6, the main virulence factor of *Mycobacterium*.

Ref. Number: 13T

Mycobacterium tuberculosis PhoP-reporter strains for testing potential anti-virulence molecules

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Tuberculosis is a major cause of death and disease. In 2019, an estimated 10 million new cases and 1.4 million deaths were caused by *Mycobacterium tuberculosis* (Mtb). Even more alarming, drug-resistant Mtb strains, which are difficult or even impossible to treat, are progressively spreading worldwide (WHO, 2020). With this scenario, anti-virulence therapies are promising. They consist on small molecule inhibitors of bacterial virulence factors, which co-work with the immune system and reduce pathogen survival in the host (Dickey, *et al.* 2017).

PhoPR is a key virulence regulator of Mtb and an attractive candidate to develop anti-virulence strategies. We have constructed several reporter systems of the PhoPR activity by placing GFP variants under the control of well-known PhoPR-regulated promoters (*mcr7* and *pks2*), which are strongly regulated by PhoPR. Proof-of-concept of these reporters were established with ethoxzolamide (ETZ), a documented PhoPR inhibitor (Johnson, et al. 2015). Results showed a gradual inhibition of GFP fluorescence, without altering bacterial growth, in the presence of increasing concentrations of ETZ. These assays were robustly reproduced with different strains from the more widespread lineages of Mtb. In addition, transcriptomic experiments, demonstrated that treatment with ETZ resulted in downregulation of selected genes from the PhoPR regulon. Altogether, these reporter strains are valuable tools for downstream High Throughput Screening of anti-virulence molecules against tuberculosis.

Ref. Number: 16PI

Antimicrobial “magic bullets” in the 21st century

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In our days, more than 100 years after Paul Ehrlich defined the concept of ‘magic bullets’ for antimicrobial chemotherapy, it is still needed to find and develop novel antibiotics and antimicrobial agents, giving the rising incidence of multi-drug resistant infections. In our research team, we are focused on antimicrobial drug discovery and pre-clinical development, mainly for *Mycobacterium tuberculosis*. In particular, we are interested on discovering new antituberculosis compounds and regimens, from medicinal chemistry efforts or from natural sources; along with this, we are implementing novel dynamic PK/PD models. Combinations of antimicrobial agents and repurposing of already approved drugs for novel antimicrobial uses are two basic concepts on the research strategy that we are carrying out, as well as the development of novel delivery methods for antimicrobial drugs. Along with that, investigation of acquired and intrinsic mechanisms of drug resistance constitutes another major research project in our laboratory. In addition to *M. tuberculosis*, we are interested in non-tuberculous mycobacteria (such as *M. abscessus*, *M. kansasii* and *M. ulcerans*) and a selection of Gram-positive and Gram-negative bacterial pathogens.

Ref. Number: 14T

Strategies to elucidate the mode of action of the avermectins against mycobacteria

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New active compounds are urgently required for the treatment of tuberculosis (TB). Repurposing, i.e., finding new applications for existing drugs, can be a quick alternative to the traditional discovery process. By using this approach, the avermectins, a family of anthelmintic compounds previously believed to be inactive against bacteria, were found to be effective against *Mycobacterium tuberculosis* and other mycobacteria (PMID: 23165468, 26270480). However, their antimycobacterial mode of action remains unknown.

We first characterized the *in vitro* antimicrobial activity of selamectin, a model avermectin, against the non-pathogenic *Mycolicibacterium smegmatis* and attempted isolation of resistant mutants with the wild-type strain and a pool of transposition mutants. However, only when the hypermutator strain *M. smegmatis* Δ*nucS* (PMID: 28128207) was used, we were able to isolate low-level selamectin-resistant mutants showing a 2-fold increase of the MIC (Minimal Inhibitory Concentration) of selamectin. Subsequent rounds of selection using the previously isolated low-level selamectin resistant strains yielded high-level resistant mutants (>8-fold MIC changes).

In this presentation we aim to shed light on the mode of action of the avermectins and will report the current progress on the characterization of their antimycobacterial activity.

Ref. Number: 17PI

Research lines @ COSNET Lab

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The Complex Systems and Networks (Cosnet) Research line at BIFI was established in 2003 as part of the Physics of Complex Systems area within the Institute. In this presentation, we revise the body of work that the group has produced in the last 17 years, which includes around 200 publications and 5 European projects. Specifically, through different examples, we will illustrate how we characterize the structure and dynamics of complex networks and systems, model the spreading of diseases like Flu, Covid-19 and Tuberculosis and of information, analyze the behavior of humans when facing a social dilemma and quantify network effects in ecology and other biological systems. We round off the talk with a summary of the challenges we are currently undertaking.

Ref. Number: 15T

Game Theory: Modeling from experimental results

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Game theory constitutes a powerful tool to model, through a decision-making approach, systems of very different nature and involving agents of a diverse range, from bacteria to humans. When dealing with human interactions, some ingredients of the models can be checked and refined through behavioral experiments. In this talk, we will show a sample of experiments performed in our group about different topics, from trading in networks to cooperative behavior. Then, we will expose the subsequent models built up relying on the experimental findings.

Ref. Number: 16T

Modeling COVID-19: A Quest for Data

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The modeling of the spreading of communicable diseases has experienced significant advances in the last decades. This has been possible due to the proliferation of data and the development of new methods to gather, mine, and analyze it. A key role has also been played by the latest advances in new disciplines like network science. Nonetheless, any model can only be as good as the data used to build and calibrate it. In this talk, we will explore the work that our group has done to understand the past and the future of COVID-19, ranging from the simplest models focused on the early spreading in China, to highly sophisticated agent-based models to forecast the future. We will show how integrating data coming from many different sources can provide answers to our questions, while raising many others. Even more important, we will discuss the limitations of these models, both due to their mathematical definition or to the availability of data. The dynamics of an epidemic are completely intertwined with the behavior of the population and, as such, we will only be able to provide answers as long as we can effectively predict the human component of the disease.

Ref. Number: 18PI

Pathological protein self-assembly mechanisms and single-molecule experimental tools for their study

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Many neurodegenerative disorders are characterised by the conformational change of normally soluble proteins or peptides into pathological conformers by a process of misfolding and self-assembly that leads to the formation of amyloid aggregates. Our recent studies on alpha-synuclein, the protein whose aggregation and deposition are associated with a number of neurodegenerative diseases collectively referred to as synucleinopathies, including Parkinson´s disease, support the idea that multiple aggregated species can be generated through diverse misfolding pathways during the process of amyloid aggregation of a given polypeptide, which possess different degrees of neuronal toxicity and infectivity. We are elucidating some of the different mechanisms that lead to alpha-synuclein self-assembly and the formation of structurally different amyloid polymorphs, some of which could be involved in distinct pathologies and/or stages of the development of a particular disease. In addition, we are developing single-molecule-based experimental approaches to study protein self-assembly processes and the interaction of ligands with particular types of protein species within heterogeneous complex systems with the possibility of characterizing the stoichiometry of heterocomplexes. Some of these tools can help us to develop rational strategies for the early detection and treatment of synucleinopathies.

Ref. Number: 17T

Dual Color Time-Resolved Fluorescence Spectroscopy: a Framework for Studying Small Molecule-Amyloid Interactions

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Amyloid protein aggregation is widely involved in a number of neurodegenerative diseases for which novel therapeutic and diagnostic strategies are still needed. Owing to the complex and heterogeneous nature of the aggregated species responsible for toxicity in these disorders a detailed characterization of the interaction of molecules of interest with the amyloid aggregates is a challenging endeavor. Here, we present the experimental and analytical steps of a protocol which combines dual-color fluorescence cross-correlation spectroscopy (dcFCCS) and dual-color single particle fluorescence spectroscopy (dcSPFS) to quantify the binding of an inhibitor of α-synuclein amyloid aggregation. This approach allows studying the interaction in detail and through two independent analytical methods, thus yielding a remarkably robust tool that could be extended to investigating the interaction of molecules of interest to other pathogenic protein aggregates. As an example, we show results that revealed strong and selective binding of the aggregation inhibitor to the cytotoxic species of α-synuclein while being inert to the physiologically functional monomeric form of the protein.

Ref. Number: 19PI

Why glycosylation matters in health and disease?

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Glycosylation is a reaction in which a carbohydrate unit is attached to proteins, carbohydrates, small organic molecules, and lipids. It plays key roles in many diverse biological functions and is present in all organisms. Here, I will summarize the importance of this process, focusing on its relevance in posttranslational modifications (PTMs). Finally, I will show three examples of our lab that evidence Glycosylation's relevance in two different O-glycosylation pathways and cancer.

Ref. Number: 18T

Structural basis for substrate specificity and catalysis of 1,6-fucosyltransferase

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Core-fucosylation is an essential biological modification of the N-glycan core in eukaryotes (except for plants and fungi) that is performed by the inverting α1,6- fucosyltransferase (FUT8). FUT8 transfers a L-fucose residue from GDP-β-L-fucose onto the innermost GlcNAc of N-glycan to form an α1,6-linkage. Core-fucosylation is important for regulation of the immune system. In addition, FUT8 is up-regulated in numerous types of cancer suggesting that blocking its activity could be a promising strategy for improving anti-tumor immune responses. The human FUT8 apo form has been previously reported revealing that FUT8 is a multi-domain enzyme that contains an N-terminal coiled-coil domain, a catalytic domain, which adopts a GTB fold, and a C-terminal SH3 domain. The lack of solved ternary complexes has impeded to obtain mechanistic insights into the glycosyl transfer reaction or reveal the molecular basis of the requirement for a terminal GlcNAc moiety on the α1,3 arm for optimal catalysis. Here, we report the first crystal structure of FUT8 complexed with GDP and a biantennary complex N glycan (G0), uncovering the molecular basis of FUT8 catalysis and recognition of acceptor substrates. FUT8 follows a SN2 mechanism and deploys a series of mobile loops and an α-helix contributing to the binding site. An exosite is responsible for the branching sugars and in particular the α1,3 arm GlcNAc recognition, a feature required for catalysis. This structure serves as a framework for inhibitor design.

Ref. Number: 20PI

The RDI Unit 'GBsC' (BIFI-Unizar) associated to the CSIC: a holistic practice in the investigation of genes and proteins.

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The Research, Development and Innovation (RDI) Unit "Biochemistry, Biophysic and Computational group 'GBsC' (BIFI, University of Zaragoza)" associated to the CSIC was constituted and approved in March 2016, and subsequently removed in March 2019. The protocol establishes that it needs to be removed each three years.

The RDI Unit 'GBsC' is a multidisciplinary group of researchers and professors of BIFI-Unizar and Estación Experimental de Aula Dei-CSIC who work in chemistry of proteins, molecular biology, biophysics, genomic, botany, evolution, computation and bioinformatics. The activity of the Unit is focused on maintaining and reinforcing the collaborations between both Institutes through ongoing and future projects, as well as promoting new collaborations. The objectives include also the organization of courses, workshops and outreach activities that favor the collaboration.

The scientific objectives are focused on: (1) structural and computational analysis of flavoenzymes and regulatory proteins (i.e., Fur, transcription factors); (2) computational analysis of plant pangenomes; (3) phylogenetic and evolutionary analysis of genomes and proteomes (i.e. *Brachypodium distachyon*, legumes and related species).

Ref. Number: 21PI

From Fundamental Processes to Biotechnology and Biomedicine. A Glimpse of Structural Research at CBE-IQFR

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The main objective of the CBE-IQFR Department is to interpret biological phenomena in terms of structural studies at the atomic, molecular and supramolecular level, trying to understand the basic processes of life and to use this knowledge to solve biotechnological and biomedical problems. To achieve this objective, we combine and develop molecular biology techniques, high throughput crystallization technologies, crystallographic techniques and data-base mining. The research of the Department is focused in several interrelated lines, including structural enzymology, structure and disease, protein-lipid interaction and structure of complex modular systems. In the talk we will present some of our most recent research breakthroughs. As it will be detailed in the talk, in-depth structural study of fundamental processes on different biological systems allowed high-impact results with strong implications in applied fields such as biotechnology or biomedicine.

Ref. Number: 22PI

Research In the "Manuel Rico" NMR Laboratory to Understand Protein and Nucleic Acid Conformation, Dynamics and Function

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Dr. Mª Ángeles Jiménez investigates structure-dynamics-function relationships in peptides and proteins. In recent years, her research has uncovered peptides that fascinatingly transform from a-helices to b-strands depending on the solution conditions [1], as well as remarkably high dynamics in a Histone protein [2]. Dr. Douglas V. Laurents studies intrinsically disordered proteins involved in pathologies and alternatively memory consolidation [3]. As many of these proteins are glycine rich, their ability to adopt polyproline II helical bundles [4] for condensate formation and biotech applications is being pursued. Dr. José Manuel Pérez Cañadillas investigates interactions involving RNA binding proteins revealing novel recognition modes [5] as well as the effects of PTMs like phosphorylation [6] and methylation. Dr. Javier Oroz investigates the role of extremely large chaperone complexes in amyloid pathologies [7,8] as well as functional amyloids [3]. Together with Dr. Miguel Treviño, he characterizes the Phox2B protein whose expanded Alatract is linked to Ondine's syndrome. Dr. Miguel Mompeán uses liquid state and solid-state NMR spectroscopies and computational methods to study pathological amyloid formation and stability [9] and heteroamyloids involved in viral defense signaling [10]. Prof. Subramanian Padmanabhan studies protein structure and function in Myxococcus to reveal how bacteria see [11], in close collaboration with Prof. Montserrat Elías Arnanz (Univ. Murcia). These studies led to the discovery of a long-sought orphan desaturase enzyme [12]. All of this research depends on close teamwork with students Belén Chávez Arquero and Daniel Ramírez de Mingo, skilled technicians: Daniel Calvo, Dr. Sergio Carreras, Dr. Miguel Treviño [4] and Dr. David Pantoja Uceda [2,13] for production of isotopically labeled proteins, advanced pulse sequences and data analysis. Prof. Carlos González has pioneered the application in Spain of NMR for studying nucleic acid structure, particularly those adopting non-canonical conformations such as the G-quadruplex and i-motif [14]. Chemically modified nucleic acids [15] and the search of conformation-specific ligands [16] are studied by Prof. González and his team of Ph.D. students: Israel Serrano-Chacón [16] and Cristina Cabrero, technician: Dr. Irene Gómez Pinto and postdoc: Dr. Miguel Garavís [15]. NMR experiments were performed in the "Manuel Rico" NMR Laboratory (LMR) of the Spanish National Research Council (CSIC), a node of the Spanish Large-Scale National Facility (ICTS R-LRB).

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Ref. Number: 23PI

How hot are mitochondria? Temperatures above 43^{0}C induce the degradation of respiratory complexes and supercomplexes independently of respiratory activity

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Mitochondrial function generates heat contributing to the maintenance of the organismal temperature, but the actual values reached inside the organelles are a matter of debate. A recent report concluded that, physiologically, mitochondria maintain close to 50^{0}C, more than 10^{0}C warmer than the external temperature. This difference was abolished when mitochondrial function was prevented by OXPHOS inhibitors. We have examined the stability of mitochondrial complexes and supercomplexes (SCs), structures located in the inner mitochondrial membrane, at different temperatures with and without OXPHOS inhibitors. Our findings contradict the values reported in the mentioned publication, showing that at values above 43^{0}C, and after relatively short times, respiratory complexes and SCs are unstable both in intact cells and in isolated mitochondria even in the absence of respiratory activity. This temperature-dependent de-stabilization is similar but not identical in liver and cultured cells and also differs for the various complexes and their associations, being CI and CI-containing SCs particularly sensitive to heat stress. The main effects of high temperatures on complexes and SCs observed in whole cells are reproduced in isolated mitochondria, indicating that they are not dependent on other cell compartments.

Ref. Number: 24PI

Real-Time Intracellular Temperature Imaging Using Lanthanide-Bearing Nanoparticles.

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Besides their prominent role in energy metabolism through the oxidative phosphorylation system (OXPHOS), mitochondria participate in many other relevant cell functions. As a consequence of the OXPHOS system function, mitochondria are also an important source of heat, so much so that there are specialized tissues such as brown adipose tissue, whose mitochondria are mainly involved in thermoregulation. On the other hand, hyperthermia generates ROS and alters the activity of mitochondrial respiratory complexes and is a potential tool to selectively kill tumour cells.

The actual heat generation power/activity of mitochondria is still a question of controversy that we are trying to answer by using lanthanide-bearing nanoparticles as molecular nanothermometers. In order to determine thermal processes associated to mitochondrial activity we have analyzed the effect of different metabolic fuels and OXPHOS inhibitors on intracellular temperature. Although our results are preliminary, we have been able to detect temperature changes due to mitochondrial function inhibition, accompanied by changes in ROS production and SCs stability, which could help to understand the role of mitochondria in thermal processes in cells.

Ref. Number: 19T

Mechanism of dichloroacetate-induced cell death in highly tumorigenic and glycolytic cancer cells

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Cancer cells are known to redirect their energy metabolism preferentially towards fermentation instead of using OXPHOS to produce energy, even in the presence of oxygen, a phenomenon named 'Warburg effect'. This unique feature can thus be exploited as a target for cancer therapy. The PDK1 inhibitor dichloroacetate, which favours OXPHOS, has shown preliminary success *in vitro* against tumour cells with defective mitochondria. However, its mechanism of induced cell death has not been fully characterized yet. In the present work, we used L929dt cells, derived from the mouse fibroblast parental cell line L929 and which harbour mutations in the *ND2* subunit from complex I, as a model to elucidate dichloroacetate mechanism of action.

Our results demonstrate that DCA induces ROS production and decreases mitochondrial membrane potential only in OXPHOS-deficient cells. However, absence of caspase-3 processing and insensitivity to apoptosis inhibitor Z-VAD-fmk or necroptosis inhibitor necrostatin-1, indicate that DCA-induced cell death does not follow an apoptotic or necroptotic pathway.

In addition, L929dt cells showed to have a tendency towards autophagy, further driven by dichloroacetate, which in turn could eventually lead to non-canonical cell death. We hypothesize that starvation induced by DCA promotes Bim phosphorylation and, consequently, Beclin-1 release to activate autophagy. Furthermore, upregulation of Bcl-x_L and Mcl-1 expression in L929dt cells would prevent them from dying by apoptosis.

Ref. Number: 25PI

Computational genomics and systems Bio-medicine: a new research line at BIFI

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The study of inter-individual variation in immune responses to pathogens constitutes a highly instrumental approach for understanding immune pathology and infectious disease epidemiology. Combining functional genomics assays with bio-informatics and mathematical modeling has unlocked the identification of both genetic and environmental determinants associated with significant gradients in the intensity of immune responses, that can in turn be linked to inter-individual differences in disease risk for a number of pathologies. These causal factors and their distributions across human populations often constitute a valuable asset for understanding the transmission patterns of infectious diseases, as they translate into the observed heterogeneity in many key epidemiological parameters.

During my talk, I will present the main goals and active research threads of the lab in computational genomics and systems Bio-medicine, created in the institute in 2020. Our research activity revolves around three thematic poles: the study of inter-individual variation in immune responses using NGS data, the development of improved computational methodologies for the analysis of single-cell -omics data, and the systems Biology of tuberculosis disease.

Ref. Number: 20T

A Bayesian approach to minimize bias and uncertainty in impact evaluation of tuberculosis vaccines

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Tuberculosis (TB) is one of the most complex diseases from the perspective of mathematical epidemiology. It arises that, when evaluating epidemiological interventions whose mechanisms of action are unknown, an observation of reduced risk of infection or disease does not hold a unique description in terms of transmission models. An example of this situation is the evaluation of TB vaccines that confer prevention of disease (POD) in trials conducted on participants previously exposed to the pathogen (IGRA+). In those trials, the potential heterogeneity among the enrolled participants makes difficulty to pinpoint specific vaccine mechanisms at place. As a consequence, a readout of vaccine-mediated POD can emerge as a consequence of at least three basic mechanisms: 1. A delay in the ongoing progression to disease, for the participants undergoing progression to active TB at the moment of vaccination, 2. an arrest of reactivation, for the LTBI carriers or 3. A risk reduction against primary TB upon reinfection, for all individuals who are exposed to eventual reinfections. Moreover, combinations of those basic mechanisms can occur, making the ambiguity even bigger.

In this work, we identify this ambiguity, and describe how it compromises the chances of achieving accurate descriptions of novel TB vaccines from the results of multi-centric phase 2b efficacy trials conducted on IGRA+ volunteers. Then, we introduce a novel Bayesian approach to interpret the data that emanate from such trials that allows us to estimate the likelihood of observing each of the mentioned vaccine mechanisms, as a function of trials settings and outcomes. Finally, leaning on a combination of compartmental models and in-silico simulations of clinical trials, we show how properly characterizing the mechanism of action of TB vaccines is necessary, since the expected impact of the vaccine when introduced to the general population is significantly subject to it.

Ref. Number: 21T

Using single-cell RNA-sequencing to characterize genetic ancestry effects on the response to viral infection

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Susceptibility to infectious disease is variable across individuals, and one of the reasons is the heterogeneity that can be observed in the immune responses following infection. These differences stem from both environmental and genetic causes [1], and, among the latter, ancestry profiles have been found to exert a significant effect [2]. In this project, we are using single cell RNA-seq data to quantify the genetic contribution to this variation in peripheral blood mononuclear cells. Our samples come from a panel of admixed individuals with different levels of African and European ancestry, and have been infected in vitro with influenza.

The usage of single-cell transcriptomics in our experimental design, beyond serving for the identification of common, though highly cell-type specific ancestry effects on the magnitude of immune responses to influenza infection [3], unlocks the interrogation of ancestry effects on gene expression coherence across cells, and the study of the interactions between ancestry and tissue composition on the responses of particular genes and pathways.

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Ref. Number: 26PI

Gradual polyploid genome evolution of model *Brachypodium* grasses revealed by pangenomic analysis

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Our understanding of polyploid genome evolution is constrained because we cannot know the exact founders of a particular polyploid. To differentiate between founder effects and post polyploidization evolution, we use a pan-genomic approach to study the allotetraploid *Brachypodium hybridum* and its diploid progenitors. Comparative analysis suggests that most *B. hybridum* whole gene presence/absence variation is part of the standing variation in its diploid progenitors. Analysis of nuclear single nucleotide variants, plastomes and k-mers associated with retrotransposons reveals two independent origins for *B. hybridum*, ~1.4 and ~0.14 million years ago. Examination of gene expression in the younger *B. hybridum* lineage reveals no bias in overall subgenome expression. Our results are consistent with a gradual accumulation of genomic changes after polyploidization and a lack of subgenome expression dominance. Significantly, if we did not use a pan-genomic approach, we would grossly overestimate the number of genomic changes attributable to post polyploidization evolution.

Ref. Numbers: 22T

PhyloSD: Phylogenomic detection of known and ghost subgenomes of polyploid plants

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Current approaches to detect subgenomes within polyploid genomes require comparisons to putative diploid progenitor genomes. This is problematic when they are unknown. To address this challenge, we developed a Phylogenomic Subgenomic Detection (PhyloSD) pipeline with three sequential nearest diploid species node, bootstrapping refinement, and subgenome assignment algorithms. It was validated in *Triticum* polyploids with known progenitor genomes and used to infer the identities of three extant and four 'ghost' subgenomes in six *Brachypodium* polyploids, of which five contain undescribed homeologous subgenomes. Our method improves previous phylogenetic-based approaches and is able to uncover the subgenomes of both allo and autopolyploids.

Posters

Ref. Number: P01

Exploring FurA interaction with photosynthetic electron carrier proteins in *Anabaena* sp. PCC7120

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Photosynthesis is one of the main cellular processes in cyanobacteria. In this process electrons extracted from water by photosystem II (PSII) are transferred through several electron carriers to photosystem I (PSI) and ultimately to ferredoxin or flavodoxin to produce NADPH via FNR. Previous studies from our group showed that the Ferric Uptake Regulator FurA from the cyanobacterium *Anabaena* sp. PCC7120 regulates several genes involved in photosynthesis and revealed that its overexpression has a strong effect on the photosynthetic activity. It has also been shown that FurA can interact with small molecules (heme or 2-oxoglutarate), affecting its DNA binding activity. Consequently, we sought to investigate the interaction of FurA with soluble photosynthetic electron carriers, namely plastocyanin, ferredoxin, flavodoxin and ferredoxin:$NADP^+$ reductase (FNR).

Cross-linking assays and bacterial two hybrid assays (BATCH) showed that FurA is able to interact with ferredoxin and flavodoxin, both *in vitro* and *in vivo*. This interaction does not appear to affect FurA DNA binding activity or to have a redox implication, since none of these proteins interfere on FurA-DNA interaction or are able to reduce FurA. Conversely, the observed interaction seems to modulate electron transport from FNR to flavodoxin and ferredoxin, as seen by *in vitro* enzymatic assays.

Ref. Number: P02

Computational insights on the hydride transference of the human Apoptosis Inducing Factor (hAIF) enzyme

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The regulation of the programmed death process in human cells is closely tight to the apoptosis-inducing factor (hAIF). This enzyme, usually found in the mitochondria, is responsible of triggering the chromatin condensation and DNA fragmentation without caspase intervention. It is believed that the redox balance of the cell environment can activate this response through the reduction of its FAD cofactor with NADH to form a stable charge transfer complex (CTC) that leads to structural rearrangements and alterations in the monomer-dimer equilibrium of the protein. Thus, the study of the hydride transfer between ligands including the enzymatic environment offers valuable information on the regulatory mechanism of this enzyme and can give insights on the related known disease-associated mutations. The results obtained using quantum mechanics/molecular mechanics (QM/MM) calculations allow us to describe the reaction at an atomic scale and can reveal the interacting role of every residue during the catalyzed pathway. Kinetic properties are also computed to be directly compared with experimental data.

Ref. Number: P03

Towards a physiologically relevant aggregation pathway of α-synuclein in Parkinson's disease

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α-Synuclein (α-Syn) is a presynaptic intrinsically disordered neuronal protein whose misfolding and aggregation in the form of amyloid fibrils is the hallmark of different neurodegenerative disorders known as synucleinopathies, including Parkinson's disease. Recent findings have shown that the typical α-Syn amyloid structures that have been generated in vitro until now differ from those that have been obtained from patient brain extracts. Amyloid nucleation has been observed to be initiated in vitro at hydrophobic/hydrophilic interfaces by heterogeneous nucleation generating parallel β-sheet aggregates, although no such interfaces have yet been identified in vivo. With the aim of understanding the different *in vivo* possible amyloid relevant α-Syn mechanisms, we have characterised the amyloid pathways of α-Syn under a variety of experimental conditions and we have discovered that α-Syn can self-assemble into amyloid aggregates by homogeneous nucleation, without the need of an active surface, and with a preference for an antiparallel β-sheet arrangement. This newly discovered amyloid pathway might occur under limited hydration conditions such as those encountered in the interior of membrane-less organelles.

Ref. Number: P04

The ArsR response regulator: a novel and validated therapeutic target against *Helicobacter pylori* infection

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The World Health Organization has included *Helicobacter pylori* on its "high-priority" list of antibiotic-resistant bacteria that pose at present the greatest threat to human health, for which new antibiotics are urgently needed [1-3]. A major challenge in this antibiotic crisis is the identification of novel microbial targets whose inhibitors can overcome the currently circulating resistome [4]. In the present study, we have validated the use of the essential response regulator ArsR as a novel and promising therapeutic target against *H. pylori* infections. A high-throughput screening of a repurposing chemical library using a fluorescence-based thermal shift assay identified several ArsR binders. At least four of these low-molecular weight compounds noticeably inhibited the DNA binding activity of ArsR and showed bactericidal effects against antibiotic-resistant strains of *H. pylori*. Among the ArsR inhibitors, a human secondary bile acid, lithocholic acid, quickly destroyed *H. pylori* cells and exhibited partial synergistic action in combination with clarithromycin or levofloxacin, while the antimicrobial effect of this compound against representative members of the normal human microbiota such as *Escherichia coli* and *Staphylococcus epidermidis* appeared irrelevant. Our results enhance the battery of novel therapeutic tools against refractory infections caused by multidrug resistant *H. pylori* strains.

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Ref. Number: P05

Dual Color Time-Resolved Fluorescence Spectroscopy: A Framework for Studying Small Molecule-Amyloid Interactions

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Amyloid protein aggregation is widely involved in a number of neurodegenerative diseases for which novel therapeutic and diagnostic strategies are still needed. Owing to the complex and heterogeneous nature of the aggregated species responsible for toxicity in these disorders a detailed characterization of the interaction of molecules of interest with the amyloid aggregates is a challenging endeavor. Here, we present the experimental and analytical steps of a protocol which combines dual-color fluorescence cross-correlation spectroscopy (dcFCCS) and dual-color single particle fluorescence spectroscopy (dcSPFS) to quantify the binding of an inhibitor of α-synuclein amyloid aggregation. This approach allows studying the interaction in detail and through two independent analytical methods, thus yielding a remarkably robust tool that could be extended to investigating the interaction of molecules of interest to other pathogenic protein aggregates. As an example, we show results that revealed strong and selective binding of the aggregation inhibitor to the cytotoxic species of α-synuclein while being inert to the physiologically functional monomeric form of the protein.

Ref. Number: P06

Identification of regulatory networks performed by the Ferric Uptake Regulator FurA in *Anabaena* sp. PCC7120

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FUR (ferric uptake regulator) proteins are metalloregulators present in most prokaryotes. The cyanobacterium *Anabaena* sp. PCC7120 contains three FUR paralogues: FurA, FurB and FurC. Previous studies carried out in our research group showed that the misregulation of FurA in this cyanobacterium produced significant alterations in the transcription of almost 200 genes with regulatory functions in *Anabaena* sp. PCC7120. This suggests that FurA regulates gene expression both in a direct way and in an indirect way by means of other transcriptional regulators.

In this work we have analyzed several genes with regulatory functions that presented a transcriptional change in a *furA*-turning off strain and/or contained a predicted FurA box. We have performed electrophoretic mobility shift assays (EMSA) which have allowed us to identify several genes such as adenylate cyclases, transcriptional regulators, sensor kinases and response regulators which are directly regulated by FurA. These results open the door to a better understanding of regulatory networks in cyanobacteria, in particular those controlled by FUR proteins.

Ref. Number: P07

Biochemical characterisation of MurA: an essential component in peptidoglycan cell wall biosynthesis

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Peptidoglycan (PG) is an important component of bacterial cell walls. Any enzyme involved in its synthesis is supposed to be a good target for selective inhibition and therefore for antibacterial therapy. One of our research lines focus on *Brucella ovis*, a pathogen of sheep and ram that causes brucellosis, an infectious disease bringing about reproductive failure. With the aim of finding a drug to treat brucellosis, we have identified in the genome of *Brucella ovis* one gene for Mur A by homology with similar sequence proteins from other organisms. This enzyme is the responsible for the first step of the biosynthesis of PG: the transfer of enolpyruvate from phospoenolpyruvate (PEP) to UDP-N-acetylglucosamine (UNAG) to yield UDP-N-acetylglucosamine enolpyruvate (UNAGEP). The lack of a counterpart of MurA in mammals becomes MurA a potential drug against brucellosis. We have cloned, produced and initiated the biochemical and biophysical characterization of this enzyme by spectroscopy and thermal stability. Additionally, its activity has been measured by the malachite green colorimetric assay, optimising the assay conditions to get the higher yield of the product. Modelling and crystallization experiments for MurA are in progress.

Ref. Number: P08

Expanding a Molecular Dynamics-based method for the calculation of protein stability from first principles

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The energy data that can be collected from Molecular Dynamics (MD) simulations has previously been used to accurately calculate the stability (ΔGunfolding) of two model proteins, barnase and SNase. In this work we extend the method to a protein with a more complex unfolding energy landscape that includes an intermediate -the apoflavodoxin from *Anabaena* sp.- and to two other model proteins with one-step unfolding -T4 lysozyme and barley chymotrypsin inhibitor 2-, to further validate the applicability of the method. The thermodynamic parameters of these proteins are reproduced in great agreement -within the error margin- with those determined *in vitro*, even though the experimental mean temperatures of denaturation (T_m) are used for the stability calculation ("semi-computational" method). The current MD-based method constitutes an accurate and easily attainable approach for predicting relevant unfolding thermodynamics quantities of proteins, which may offer to protein designers and biologists an advantageous tool for the study and design of therapeutic and commercial proteins. These results are also a fair advance towards the goal of predicting protein stability completely *in silico*, which we will address by calculating the entropy change upon unfolding, thus avoiding the need to use the experimentally determined T_m in the stability calculation.

Ref. Number: P09

Rh-NHC catalyzed head-to-tail alkyne dimerization enhanced by metal-ligand cooperation

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Dimerization of terminal alkynes provides a simple and atom-economic route for the synthesis of 1.3-enynes. This structure makes possible a great variety of functionalizations that yield a conjugated skeleton with the desired functional groups [1]. Thus, these products are highly sought-after in pharmaceutical and organic synthesis. However, this process usually suffers from poor regio- and chemoselectivity. One of the most effective solutions to this problem is the use of organometallic homogeneous catalysts in cooperation with a ligand. Metal Ligand Cooperation (MLC) can improve both the high catalytic efficiency and control of the selectivity of the catalyst [2].

In this work, we present a theoretical study based on density functional theory (DFT) of the *gem* selective mechanism of dimerization of terminal alkynes. The pyridine present in the

catalyst, Rh{κ^2-O,N-(Opy)}(η^2-HC≡CPh)(IPr), is shown to play a central role in the process enabling the formation of the product via Ligand Assisted Proton Shuttle [3]. This mechanism presents a lower activation energy and higher selectivity than other processes, like the insertion of the alkyne in the Rh—H bond, described for similar systems.

Keywords: Homogeneous catalysis, N-Heterocyclic Carbene, Metal-Ligand Cooperation, Proton Shuttle, DFT Calculations.

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Ref. Number: P10

The flavoproteome of *Brucella ovis* in the search of antimicrobial targets: MurB, a promising candidate.

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Flavoproteins are key in the energetic metabolism in bacteria, participating in processes such as metabolism of fats, carbohydrates and proteins, oxidative stress response, photosensitization, peptidoglycan wall synthesis and activation of other vitamins as folate and pyridoxine. Bacterial genomes encode for around 2% of flavoproteins, many of which are probably essential and species specific [1-3].

We have characterized the flavoproteome content in the bacterial model *Brucella ovis*, a pathogen of ovine brucellosis, by using a combination of NCBI, Uniprot, PDB, BRENDA and Pfam Family databases. We have identified around 78 proteins of a total of 2850 proteins distributed in 2 chromosomes, almost 2.7% correspond to putative proteins with flavin-binding motifs, which have been classified with respect to their possible activity and biological role.

We find with special interest the flavoenzyme encoded by the gene BOV_1386, MurB or UDP-N-acetylenolpyruvoylglucosamine reductase, involved in the synthesis of the bacterial cell wall, which reduces EP-UDP-GlcNac to UDP-MurNac using NADPH and FAD, which is an attractive target for antimicrobials design and is absent in eukaryotic organisms [4,5]. We have successfully cloned and produced the MurB enzyme from *B. ovis*. Its integrity and structural characteristics have been evaluated by spectroscopy, fluorescence, circular dichroism, and thermal stability, while Stopped Flow spectrophotometry has been used to evaluate its activity under different conditions.

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Ref. Number: P11

A novel approach for early cancer detection based on dendrimer nanoparticles

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Over the last years, the application of nanotechnology to biomedicine has emerged as a promising strategy for the treatment of various clinical diseases, particularly cancer. Among nanomaterials, dendrimers (three-dimensional hyperbranched polymers) have gathered increasing attention because of their unique structural properties. Monodispersity, internal molecular cavities, tunable size and numerous peripheral functional groups for chemical modifications make them excellent nanocarriers for drug and contrast agent delivery for cancer treatment and diagnostic imaging. Previous studies have demonstrated the potential of using nanoparticles for developing a diagnostic system based on the interaction of nanomaterials with serum components. When nanoparticles interact with a biological fluid, proteins spontaneously bind to surface, forming a biomolecular layer which is commonly referred to as the protein corona. The formation of this coating depends on the physicochemical properties of nanoparticles and characteristics of the biological environment. As protein pattern in serum from cancer patients differs from that of healthy donors, the molecular composition of the protein corona formed around nanoparticles change, offering the opportunity of using dendrimers and dendritic derivatives as a tool for cancer diagnosis.

Ref. Number: P12

Unraveling biofilm formation in the filamentous cyanobacterium *Anabaena sp.* PCC7120.

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Cyanobacterial biofilms are considered promising chelating agents for biosorption and removal of metal-contaminated waters, aquaculture or hydrogen production. Exopolysaccharides (EPSs) constitute the major component of the extracellular polymeric matrix that establishes the functional and structural integrity of biofilms and protects cells against environmental stresses. EPSs not only work as a nutrient sink, but also play an important role in the sequestration of metal cations. Considerable amount of work on biofilms composed by heterotrophic microorganisms has been done. However, pathways of EPSs export and assembly in cyanobacteria, the factors determining the formation of cyanobacterial biofilms, their regulation, as well as the influence of abiotic stresses in biofilm formation and composition, remain to be characterized.

We have set in our laboratory procedures to obtain biofilms from cultures of *Anabaena sp.* PCC7120. We have observed that under either nitrogen deficiency or salt stress, extracellular EPSs production in Anabaena is enhanced. On the other hand, genome-mining bioinformatics approaches to identify key proteins for biofilm formation in *Anabaena sp*. PCC7120, together with previous comparative transcriptomic analysis of this strain with *Anabaena* variants affected in the synthesis of the transcriptional regulators FurA and FurC, allowed us to define novel players in the synthesis of phototrophic biofilms.

Ref. Number: P13

Expanding the FurC regulon from *Anabaena* sp. PCC7120: *in silico* approach and experimental validation of novel FurC targets.

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FurC from *Anabaena* sp. PCC7120 is a key transcriptional regulator previously described as the Peroxide Stress Regulator (PerR). However, recent studies revealed that FurC regulation goes beyond the control of the oxidative stress response, modulating the expression of genes involved photosynthesis [1], iron homeostasis and nitrogen metabolism (unpublished results). In this work, *in silico* approaches and its experimental validation were performed to extend our knowledge of FurC regulon. Briefly, a putative 19 bp FurC-box was inferred through MEME analyses performed with the promoter regions of known FurC direct targets. The FurC-box revealed that FurC presumably associates to DNA at a 19 bp-consensus sequence 5′-CAAAATCATAACGACTTTG-3′ that shared 58% homology with the classical 9-1-9 *E.coli* Fur consensus 5′-GATAATGATAATCATTATC-3′ [2]. The position-weight-matrix derived from the FurC-box was scanned in the upstream regions of all genes from *Anabaena* sp. PCC7120 genome revealing 141 new putative FurC targets. These genes belonged to the expected categories such as iron metabolism, photosynthesis, oxidative stress and nitrogen metabolism but also to novel categories highlighting many genes involved in carbon metabolism and regulatory functions. Finally, the prediction was validated by EMSA and RT-qPCR comparing the expression of novel FurC targets between a *furC*-overexpressing strain EB2770FurC and the wild-type strain *Anabaena* sp. PCC7120.

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Ref. Number: P14

Influence of recurrent mobility patterns on the spread of vector-borne diseases

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In light of the crucial role that human mobility has played on the spread of many diseases, we here extend the Ross-Macdonald model to account for the impact of recurrent human mobility patterns. Based on the equations previously developed for directly transmitted diseases between humans [1], we propose a new formalism [2] to assess the effects of human mobility on the spread of vector-borne diseases. The formalism is composed of a set of Markovian equations which allows for tracking the spatio-temporal evolution of this type of diseases.

To illustrate a real application of the model, we address the spread of Dengue across an endemic region like Santiago de Cali in Colombia. In particular, we derive a theoretical indicator by linearizing the equations, the epidemic risk ER, which enables to quantify the exposure of different areas inside a city to vector-borne diseases. Finally, we show that this indicator is strongly correlated with the spatial distribution of cumulative cases reported from 2015 to 2016 in Cali.

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Ref. Number: P15

The Catalytic Cycle of Human NQO1: Hydride Transfer, Conformational Dynamics, and Functional Cooperativity

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Human NQO1 [NAD(P)H:quinone oxidoreductase 1] is a multi-functional and stress-inducible flavoprotein involved in the antioxidant defense and the stabilization of oncosupressors. In this work, we provide a comprehensive analysis of the NQO1 catalytic cycle using rapid mixing techniques, including multiwavelength and spectral deconvolution studies, kinetic modeling, and temperature-dependent kinetic isotope effects (KIEs). Our results systematically support the existence of two pathways for hydride transfer throughout the NQO1 catalytic cycle, likely reflecting that the two active sites in the dimer catalyze two-electron reduction with different rates. Analysis of KIEs and their temperature dependence also show significantly different contributions from quantum tunneling, structural dynamics and reorganizations to catalysis at the two active sites. Our work will improve our understanding of the effects of cancer-associated single amino acid variants and post-translational modifications in this protein of high relevance in cancer progression and treatment.

Ref. Number: P16

Thermal Liquid Biopsy (TLB) applied to Pancreatic Cyst Diagnosis

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Background: Current efforts in the identification of new biomarkers are directed towards an accurate differentiation between benign and premalignant cysts. Thermal Liquid Biopsy (TLB) has been previously applied to inflammatory and tumor diseases and it could offer an interesting point of view in this type of pathology.

Methods: In this work, twenty patients (12 males and 8 females, average ages 62) diagnosed with a pancreatic cyst benign (10) and premalignant (10) cyst lesions were recruited, and biological samples were obtained during the endoscopic ultrasonography procedure.

Results: Proteomic content of cyst liquid samples was studied and several common proteins in the different groups were identified. TLB cyst liquid profiles reflected protein content. Also, TLB serum score was able to discriminate between healthy and cysts patients (71% sensitivity and 98% specificity) and between benign and premalignant cysts (75% sensitivity and 67% specificity).

Conclusions: TLB analysis of plasmatic serum sample, a quick, simple and non-invasive technique that can be easily implemented, reports valuable information on the observed pancreatic lesion. These preliminary results set the basis for a larger study to refine TLB serum score and move closer to the clinical application of TLB providing useful information to the gastroenterologist during patient diagnosis.

Keywords: pancreatic cysts; thermal liquid biopsy; differential scanning calorimetry; diagnosis; generalized linear models

Ref. Number: P17

W483 in the Apoptosis Inducing Factor: Thermodynamic, Kinetic and Dynamic Implications for NADH Oxidation

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The human apoptosis inducing factor (hAIF) is a moonlighting flavoenzyme that fulfils a vital function in the bioenergetics and redox metabolism of mitochondria in healthy cells, but which may also trigger caspase-independent cell death upon pro–apoptotic stimuli. The FAD cofactor of hAIF can be reduced by NADH, prompting dimerization and the formation of a remarkably stable $FADH^{-}$:NAD^{+} charge transfer complex (CTC). The allosteric binding of a second non-catalytic NADH molecule ($NAD(H)_B$) further arbitrates the resulting monomer–dimer equilibrium. Tryptophan 483 of hAIF is involved in an extensive H–bond network that stabilizes the conformation of the catalytic NADH ($NAD(H)_A$) while simultaneously T–stacking between the flavin isoalloxacine ring and the nicotinamide of $NAD(H)_B$. Consequently, W483 plays a potentially critical role in the formation of a catalytically–competent complex for hydride transfer between the FAD and $NAD(H)_A$, determining the efficiency of hAIF as an oxidoreductase. Three W483 mutations expected to alter the environment of the isoalloxacine ring (W483G, W483L and W483Y) had been previously generated in our group, and significant effects were observed in both hydride transfer and dissociation rates. Said mutants are further characterised in the present work, demonstrating the critical aftermath in hydride transfer efficiency and dynamics, redox potential and thermodynamic stability.

Ref. Number: P18

Characterization of the aggregation pathway of Parkinson´s related amyloid protein alpha-synuclein under liquid-liquid phase separation

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Liquid-liquid phase separation (LLPS) also referred to as demixing, has been long observed within certain aqueous mixtures of two or more components such as synthetic polymers, proteins, salts, nucleic acids or polysaccharides. Recently, growing experimental evidence indicates that this process can also occur inside cells giving rise to what has been referred to as membrane-less organelles. A number of proteins that have been reported to undergo this process are well known amyloidogenic proteins related to different types of neurodegenerative disorders and, in those cases, the formation of protein droplets has been suggested to precede their aggregation *in vivo*. Last year, it was reported that alpha-synuclein, the protein that accumulates into the form of amyloid aggregates in Parkinson´s disease, forms such liquid droplets and that the protein aggregates in this particular environment both *in vitro* and in cells. We have reproduced the formation of alpha-synuclein droplets *in vitro* and our preliminary characterization of the liquid-to-solid transition of the protein into amyloid aggregates in such environment suggests that this process takes place through a new amyloid pathway that leads to the formation of a novel amyloid polymorph.

Ref. Number: P19

Design and synthesis of FMN derivatives for covalent binding to *Anabaena* apoflavodoxin

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Many flavoproteins are involved in vital metabolic transformation. They play essential biological roles in both small organisms such as bacteria and in more complex organisms such as humans. Additionally, they are of high biotechnological relevance. Flavodoxins [1] are electron-transfer flavo-proteins that contain one molecule of non-covalently bound flavin mononucleotide (FMN) as the redox active component.

Although the apoprotein-cofactor complexes of flavoproteins often exhibit high binding constants, their strengths are influenced by solution conditions [2], which may result in cofactor dissociation, leading to destabilization of the protein moiety and to irreversible loss of catalytic activity. In the industrial context this represents a great economic burden.

In order to develop a new strategy for the rational increase of flavoproteins´ conformational stability, we have been focused on getting variants of the *Anabaena* flavodoxin bearing covalently bound [3] catalytically active FMN. For the success of the research project it is essential to have some novel FMN derivatives containing electrophilic and reactive groups[4].

In this sense, we have designed two such FMN derivatives and appropriate synthetic routes to obtain them and we have performed a brief computational study in order to assess that those compounds are suitable to establish covalent links with flavodoxin variants, and that the links are compatible with maintaining the native orientation of the isoalloxacin moiety relative to the apoprotein. One of those FMN derivatives has already been synthetized with good yields and, at present, we are working on the synthesis of the second FMN derivative. Suitable flavodoxin variants have also been designed, expressed and purified.

Keywords: flavoproteins, flavodoxin, Anabaena, covalent, FMN.

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