VII International Conference on Molecular Recognition

BIFI 2016

VII International Conference on Molecular Recognition

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Zaragoza, Spain
February 1-3, 2016
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Rafael Fernandez Leiro, MRC Laboratory of Molecular Biology, U.K.
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Francisco Corzana, Universidad de La Rioja, Spain
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María Barile, Universidad de Bari, Italy
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Proteins are cell machines that carry out many biological processes essential for life. The molecular basis of how these biomolecules recognise ligands, fold and catalyze enzymatic reactions is of great importance to both basic and applied science, and in particular in several areas of science such as Medicine, Biotechnology, Microbiology, Chemistry, Pharmacy, etc.

In this conference the talks will be aimed at the dissemination of science with various aspects related with proteins/biological processes that are of great importance, and the latest techniques to study these biomolecules. It is essential that scientists can compare experiences and ideas to face the challenges provided by the studies of molecular recognition, which in turn includes various disciplines such as chemical-biology, computational methods, molecular assemblies and development of advanced techniques, etc.

The conference will address the current status of molecular recognition at the protein level, focusing on its most important current issues, and analyzing what the future perspective encompassing these studies.

The congress will be held at BIFI on February 1-3, and will be followed by another congress to be organized by "The Association of Resources for Biophysical Research in Europe (ARBRE)". This organization is made up of groups of academic and private sectors from different European countries and pretend to exchange ideas and facilitate the use of devices between groups within it.

In this congress, we will have different opinions and experiences of scientists from several European countries. At the same time it is intended to encourage cooperation between different researchers attending the conference that are joined by a common thread which is the understanding of molecular recognition.

Finally, we hope that the participants have a fruitful discussion on the topic of this conference, "Molecular Recognition", and enjoy their stay in Zaragoza.

With warmest regards,

Dr Ramón Hurtado-Guerrero
Program
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Abstracts
Talks
NMR and molecular recognition. Glycan-protein interactions

Jesús Jiménez-Barbero

Molecular Recognition and Host-Pathogen Interactions Program, CIC bioGUNE, Derio, Bizkaia, Spain.

Molecular recognition by specific targets is at the heart of the life processes. In recent years, it has been shown that the interactions between proteins (lectins, enzymes, antibodies) and carbohydrates mediate a broad range of biological activities, from fertilization, embryogenesis, and tissue maturation, to pathological processes. The elucidation of the mechanisms that govern how sugars are accommodated in the binding sites of these receptors is currently a topic of interest. Thus, the determination of the structural and conformational factors and the physicochemical features which govern the molecular recognition of these molecules is of paramount importance. This presentation is focused on the application of standard and state-of-the-art NMR methods both from the ligand and receptor’s perspective (especially chemical shift perturbation analysis, Saturation Transfer Difference, and trNOESY experiments) to the study of molecular recognition processes between a variety of polypeptides of biomedical interest and carbohydrate-based molecules, drugs and inhibitors. Lectins, antibodies, and enzymes, both wild type and mutants, have been used as receptors with the final aim to know and to evaluate the relative importance of polar (hydrogen bonding, electrostatic interactions) and non polar (van der Waals, CH-π) forces in the molecular recognition process. As examples, structural and conformational details of glycan recognition by different domains will be shown, with special emphasis in the application of novel 19F- and paramagnetic-based NMR methodologies.
Advanced Computational Developments

ARCIMBOLDO and BORGES: Supercomputing Methods for Macromolecular Crystallographic Ab Initio Phasing

Isabel Usón

Departament of Structural Biology, Instituto de Biología Molecular de Barcelona (IBMB-CSIC) Barcelona Science Park, Spain. ICREA (Spain)

Crystallographic analysis does not produce a direct image as in microscopy, as only the diffracted intensities and not the phases of the scattered X-rays are physically measurable. So far, in spite of its being computationally very demanding, crystallography has largely turned its back on supercomputing. Our group develops and implements crystallographic phasing methods exploiting massive computing [1].

ARCIMBOLDO: from atomic resolution phasing [2] to a general ab initio structure solution overcoming previous size and resolution barriers. Our method enforces secondary structure rather than atomicity through a combination of location of model fragments (alpha-helices) with PHASER [3] and density modification with SHELXE [4]. The method has been called after the Italian painter Arcimboldo [5], who composed portraits out of fruits and vegetables. While most collections of fragments remain a "still-life", but some are correct enough for density modification to reveal the protein's portrait.

BORGES [6] takes the principle ones step further, by enforcing unspecific tertiary-rather tan secondary- structure. Like in Borges' "Library of Babel", the information we need is bound to be contained already in the PDB but how to exploiting this information while the structure is still unknown? Our characteristic vector (CV) formalism, developed to extract folds from the PDB allows detailed analysis of local folds.

Active site structure and dynamics in designed and evolved enzymes

G. Jiménez-Osés, S. Osuna, K. N. Houk

Department of Chemistry and Biochemistry, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095, United States.

Understanding the enormous catalytic power of enzymes is a grand challenge for chemical biology. We have collaborated with biologists to design new enzymes with functions different from those evolved by Nature. Our successes showed that we understand which catalytic groups will accelerate the rates of reactions and that quantum-mechanical calculations predict the correct positioning of these groups. In spite of these successes, our understanding is not sufficient to rationally tune the global architecture of the protein that ultimately determines catalysis in order to achieve efficiencies rivaling those of the natural enzymes. Instead, experimental directed evolution is often used to achieve several orders of magnitude of acceleration beyond that achieved by the designed protein.

We have used molecular dynamics (MD) simulations to reveal how mutations alter the structure and organization of enzyme active sites, studying the fluctuations between active and inactive conformations normally concealed to crystallography. We first describe the importance of dynamics in evaluating a series of computationally designed and experimentally evolved enzymes for the Kemp elimination, a popular subject in the enzyme design field. We find that the dynamics of the active site is influenced not only by the original sequence design and subsequent mutations but also by the nature of the ligand present in the active site. In the second example, we show how microsecond MD has been used to uncover the role of remote mutations in the active site dynamics and catalysis of a transesterase, LovD, a useful commercial catalyst for the production of the drug simvastatin. X-ray analysis of inactive and active mutants did not reveal differences in the active sites, but relatively long time scale MD in solution showed that the active site of the wild-type enzyme preorganizes only upon binding of the acyl carrier protein (ACP) partner. In the absence of bound +ACP, a noncatalytic arrangement of the catalytic triad is dominant. Unnatural truncated substrates are inactive because of the lack of protein–protein interactions provided by the ACP. Directed evolution is able to gradually restore the catalytic organization of the active site by motion of the protein backbone that alters the active site geometry.

Referencias

CCP-SAS – a community consortium for the atomistic modelling of scattering data: example applications

Stephen J. Perkins¹, Joseph E. Curtis², Emre Brookes³, David W. Wright¹, Haiiliang Zhang², Jianhan Chen⁴, Stephen M. King⁵, Paul Butler²

1 Department of Structural and Molecular Biology, Darwin Building, University College London, Gower Street, London, U.K.,
2 NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, Maryland, U.S.A.
3 University of Texas Health Science Center, Department of Biochemistry, San Antonio, Texas U.S.A.
4 Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, Kansas U.S.A. 5 ISIS Facility, Rutherford Appleton Laboratory, Chilton, Didcot U.K.

The major infrastructural investment worldwide in multiuser X-ray synchrotrons and neutron sources during the last two decades has been immensely successful in allowing researchers to perform data collection on ever more challenging and important systems. There is however a need for the hardware investment to be matched by software developments.

In solution scattering, huge advances have been made in the throughput and accuracy of the experimental measurements. Whilst closed-source non-atomistic approaches have been most beneficial for visualizing many problems, links to atomistic modelling approaches are needed to develop novel applications, address different classes of problems, and realize the full benefit of the instrumental investments. This requires integrating the SANS and SAXS data with user-friendly, high-throughput, molecular modelling software in order to reveal how these structures change under varying experimental conditions. CCP-SAS is a joint UK/USA collaboration whose goal is to produce a new generation of open-source software for the atomistic modelling of scattering data [1].

We have implemented a web-based application incorporating the SASSIE/SCT suite of programs, developed at NIST and UCL, and made it available for open-access (beta) testing at sassie-web.chem.utk.edu/sassie2. This online workflow tool was facilitated via the development of the GenApp framework enabling automatic application generation for general scientific software programs that run on both standard and high-performance computing hardware. Inclusion of additional constraints from analytical ultracentrifugation data will also be incorporated within the same framework using the popular US-SOMO suite.

We have already completed several successful studies using the new application, and examples will be presented. At UCL, we have applied this to study the effect of hinge glycosylation on monomeric human IgA1 antibody involved in IgA nephropathy disease [2], and to a long multidomain protein termed MASP whose inter-domain flexibility is involved with activation of the complement proteins of innate immunity. Examples from other projects underway in the consortium will further illustrate the breadth of CCP-SAS. These outcomes indicate the future new directions that will become possible with atomistic modelling using the CCP-SAS package.

References

[1] Funded by a joint EPSRC (EP/K039121/1) and NSF (CHE-1265821) grant; http://www.ccpsas.org/
Talks: *Tuesday, February 2nd*

**Chemical-Biology section**

**Chemistry on Proteins for Basic Biology and Protein Therapeutics**

Gonçalo J. L. Bernardes

University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW, UK,

Our work centers on reaction engineering for site-selective chemical protein modification and its use to provide insight into biology and for the development of protein therapeutics [1]. This lecture will cover recent examples of emerging areas in our group in: (i) chemical site-selective modification of proteins through the formation of secondary amine linkages at dehydroalnine tagged proteins,2 (ii) a new traceless pH-dependent drug-delivery system for targeted delivery of drugs into the tumor microenvironment [2] (iii) a new method for histidine-metallation of proteins with a [Ru(CO)]_2^[2+] fragment that yields artificial metalloproteins that are able to deliver CO in vivo in a controlled manner [3,4] and (iv) CO-mediated immunomodulation for cancer therapy.

References

Design of novel cancer vaccines based on MUC1

Francisco Corzana, Nuria Martínez-Sáez, Iris Alicia Bermejo, Víctor Jesús Somovilla, David Madariaga, Ramón Hurtado-Guerrero, Juan Luis Asensio, Jesús Jiménez-Barbero, Jesús Héctor Busto, A. Avenoza, Jesús Manuel Peregrina

Departamento de Química, Universidad de La Rioja, Centro de Investigación en Síntesis Química, Logroño, La Rioja, Spain,

Mucin MUC1 is an O-glycoprotein overexpressed in various tumors. While in healthy tissues, the peptide sequence of this protein carries complex oligosaccharides, in cancer cells, it shows simple and truncated carbohydrates, such as the Tn antigen (α-O-GalNAc-Ser/Thr). These antigens are exposed to the immune system and can interact with it. Due to this unique characteristic, partially glycosylated MUC1 derivatives are attractive antigens for the development of therapeutic vaccines for the treatment of cancer [1].

At present, considerable effort are dedicated to synthesize MUC1 derivatives that can elicit strong immune response. However, the identification of the important structural elements involved in the recognition process of MUC1 by anti-MUC1 antibodies remains partly unclear. We are developing a multidisciplinary approach that combines synthesis, X-ray diffraction, nuclear magnetic resonance and molecular modeling to identify these structural features [2, 3]. Our results provide valuable hints for the design of efficacious cancer vaccines.

Acknowledgements: We thank the Ministerio de Economía y Competitividad (project CTQ2012-36365/FEDER), the European Commission (Curie ITN (MSCA-ITN-2015-ETN), ref: 675007) and the Asociación Española Contra el Cáncer (I. A. B. fellowship).

References


Maria Barile¹, Teresa Anna Giancaspero¹, Michele Galluccio², Cesare Indiveri²

1 Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università degli Studi di Bari, Bari, 70125 Bari, Italia;
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The primary role of the water-soluble vitamin B2, i.e. riboflavin, in cell biology is connected with its conversion into FMN and FAD, the cofactors of a large number of flavoenzymes involved in energetic metabolism, redox homeostasis, protein folding, as well as in diverse regulatory events.

Deficiency of riboflavin in men and experimental animal models has been linked to several diseases, including neuromuscular and neurological disorders and cancer. In some cases, riboflavin at pharmacological doses has been shown to play unexpected and incompletely understood regulatory roles [1]. Starting from the idea that riboflavin homeostasis derangements maybe causative of such diseases [2], this communication will deal on components of the cellular networks that ensure flavin cofactor homeostasis in humans and, in particular, with our discovery and characterization of human FAD synthase or FMN adenylyl transferase (EC 2.7.7.2) the product of FLAD1 gene. Special attention is devoted to the problem of sub-cellular compartmentalization of cofactor synthesis and flavoprotein turn-over and to the establishment of suitable models for studying human rare riboflavin-responsive mitochonrdiopathies.

In order to mimic at the organism level these pathologies, we introduced two models: Saccharomyces cerevisiae strains lacking of the mitochondrial FAD transporter gene, namely FLX1 [3] and Caenorhabditis elegans strains in which RNA interference was used to silence the single copy gene of flad-1 gene, coding for different FADS isoforms [4]. In both organisms the effects of altering flavin homeostasis on mitochondrial bioenergetics, ATP and ROS levels, and certain flavoenzyme activities/expression level were assessed in the frame of mitochondrial related phenotypical changes. The molecular rationale for riboflavin therapy will be also deal with in these systems.

Acknowledgments

This work was supported by PON 2007-2013 (project 01_00937 to M.B)

References

Fur proteins in cyanobacteria: beyond transcriptional regulation

Teresa Bes, Laura Botello-Morte, Violeta C. Sein-Echaluce, Andrés González, María Luisa Peleato and María F. Fillat

Department of Biochemistry and Molecular and Cell Biology and Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza. Zaragoza, Spain, 50009.

The Fur (ferric uptake regulator) proteins are a family of prokaryotic transcription factors that were originally described as iron-sensing repressors. Further studies evidenced that Fur proteins play a central role in heterotrophic bacteria controlling a large number of genes involved in general metabolism, electron transport, virulence and the defence against acidic and oxidative stresses. The presence of several Fur-like proteins in the same cell, exhibiting substantial differences in signal recognition and biological functions is frequent in many prokaryotes.

In cyanobacteria, iron deficiency is particularly deleterious since it causes the decline of many photosynthetic components involved in essential oxidoreductive pathways. The genome of the cyanobacterium *Anabaena* (*Nostoc*) sp. PCC 7120 contains three open reading frames coding for Fur homologues, *all1691*, *all2437* and *alr0957* (FurA, FurB and FurC). The ferric uptake regulator FurA is a global transcriptional regulator in cyanobacteria. Several experimental evidences support additional roles for FurA, emerging as a moonlighting protein in cyanobacteria.

1) Combining an in silico genome-wide predictive approach with experimental determinations allowed us to define the FurA regulon in *Anabaena* PCC 7120. 215 genes with diverse functions including iron homeostasis, photosynthesis and respiration, heterocyst differentiation, oxidative stress defences, and light-dependent signal transduction mechanisms, among others were identified as FurA targets. The in vivo FurA-mediated regulation is dependent of the environmental iron availability and the intracellular redox status.

2) FurA fulfills the characteristics of a heme-sensor protein. FurA-heme interaction impairs FurA DNA binding ability and involves C141 that undergoes a redox-dependent ligand switch.

3) FurA contains two potential redox CXXC motifs (C101VKC104 and C141PKC144). Analysis of their putative contribution to FurA functionality unveiled that FurA exhibited disulfide reductase activity, mainly due to the C101VKC104 motif, unlike other Fur homologs from heterotrophic bacteria evaluated. Redox potential values of both CXXC motifs in FurA were similar to that of bacterial cytoplasm. In vivo, FurA is present under several redox states, suggesting that the regulator can work as a CXXC-based redox sensor in *Anabaena*. Moreover, identification of FurA-interacting partners using GST-pull down and bacterial two-hybrid assays shows that FurA is able to interact with the photosynthetic electron carriers ferredoxin and flavodoxin.

On the other hand, FurB (Zur) controls zinc homeostasis and its overexpression enhances tolerance to oxidative stress imposed by peroxides. Unlike FurA, electrophoretic assays and atomic force microscopy imaging show that when present at saturating concentrations, FurB exhibits unspecific DNA-binding activity and protects DNA from cleavage produced by hydroxyl radicals or DNasel. All those data indicate that FurA and FurB, besides working as transcriptional regulators, participate in the redox-signaling networks in *Anabaena*. 
Advanced Technical Developments

The bacterial DNA replication machine caught in the act by cryo-EM

Rafael Fernández-Leiro, Julian Conrad, Sjors Scheres and Meindert H. Lamers

Structural Studies division, Medical Research Council - Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, CB2 0QH, United Kingdom.

New advances in cryo-electron microscopy (cryo-EM) have opened the window to near atomic resolution structures not only on large macromolecules but also on much smaller and challenging biological systems. Thanks to the development of new methods that can deal with compositional and conformational heterogeneity also transient and highly dynamic complexes can now be studied.

We are using cryo-EM to study the bacterial replication machinery, a large, fifteen-protein complex with speeds of up to 800 nt/s and over 150,000 base pairs synthesized per binding event. The catalytic subunit of the complex, DNA polymerase III, is a poor enzyme in isolation and relies on other replication proteins to reach full activity: the DNA sliding clamp beta, the exonuclease epsilon, and the clamp loader subunit tau. Here we present the 8Å cryo-EM structures of the tetrameric complex of the polymerase, clamp, exonuclease, and the C-terminal domain of tau in a free and DNA bound state. A large conformational change of the polymerase tail acts as a switch between DNA synthesis and DNA release. Novel contacts between the polymerase tail and the clamp are required for the synthesis mode, while clamp release is orchestrated by a 3-point contact of tau with the polymerase tail, the polymerase fingers domain, and the incoming template DNA. These structures provide a new model for how the polymerase is released from the clamp at the end of the Okazaki fragment.
New single-molecule & super-resolution tools for imaging single biomolecules

Dr Steven F. Lee

Royal Society. University of Cambridge. Department of Chemistry. Lensfield Road, CB21EW. Cambridge, UK

1) Single protein aggregate detection in human biofluids - We have developed a new single-molecule fluorescence technique that is capable of directly visualising individual amyloid oligomers in cerebrospinal fluid (CSF) samples from patients with Parkinson's disease (PD). We demonstrate that this method is able to reliably detect the presence of oligomers and observe a 3 fold increase in the oligomer concentration in CSF from PD patients compared to age-matched controls.

2) Hydrophobicity-mapping of biological samples with nanoscale resolution - the technique, named spectral-PAINT (sPAINT), uses spectrally-resolved super-resolution imaging to exploit the solvatochromic properties of a hydrophobicity-sensitive fluorophore, which shifts its emission wavelength depending on the local nano-environment of the probe. We demonstrate sPAINT is capable of achieving a spatial resolution of ~7 nm and a spectral resolution of ~2 nm, and apply it to mapping membrane asymmetries on live cells.
Microcalorimetry: A versatile tool for the characterization of biomolecular interactions

Raúl Pacheco Gómez


Higher-order biomolecular structures and their dynamic interactions with various ligands drive and regulate all biological processes; studies of biomolecular interactions are fundamentally important in all areas of life sciences. Isothermal titration calorimetry (ITC) is the ideal technique for the measurement of biological binding interactions since the data provided does not rely on the presence of chromophores or fluorophores, nor requires an enzymatic assay. ITC relies only on the detection of a heat effect upon binding and it is label-free, enabling scientists in academia and industry to better understand the conformational stability of their biomolecules and their binding to biologically relevant interactants.

This presentation covers the principles of Differential Scanning and Isothermal Titration Calorimetry (DSC and ITC) and exemplifies a broad range of applications enabled by the direct nature of the technique. The presentation will also cover examples of troubleshooting and how to obtain good data using the MicroCal ITC and DSC systems.

A special focus will be given to the benefits of PEAQ-ITC, the latest generation of MicroCal ITC instrumentation, and the solutions it offers for addressing current bottlenecks associated with the interaction analysis. Among the most recognized challenges is the need to adequately address a broad range of binding affinities and to reliably interpret the binding data, complicated by the presence of inactive protein or inherent uncertainty in the concentration of the ligand.

We will discuss the improvements in PEAQ-ITC data quality which enables increased confidence and data resolution when measuring low heats at low or uncertain sample concentrations and complex binding modes.
AFFINImeter: an advanced analytical tool to characterize molecular interactions

Eva Muñoz¹, Ángel Piñeiro¹², Juan Sabin¹, Javier Rial¹, Daniel Pérez¹, Philippe Dumas³, Eric Ennifar³

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The use of Biophysical methods for the characterization of molecular interactions has become a fundamental milestone in the area of pharmaceutical research. Here, the choice of the appropriate technique(s) together with the acquisition of high quality data are essential. Importantly, a careful and precise data analysis is indispensable to succeed in our endeavor of characterizing a particular interacting system.

AFFINImeter (http://www.affinimeter.com) is an original software for the analysis of molecular interactions, initially applied to Isothermal Titration Calorimetry (ITC) data, which introduces advanced tools specially developed for the reliable analysis of complex interacting systems. In this presentation we will show how these advanced tools can be used to characterize particular cases of complex binding; last but not least, we will present KinITC: a novel method to extract kinetic information (kon and koff) from a classical ITC titration experiment [1, 2]. Altogether, AFFINImeter is already taking the ITC technique to a whole new level.

References

Macromolecular structure, function and inborn metabolic errors: a bidirectional path to discovery

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Inborn metabolic errors are generally rare single-gene disorders. For the last 30 years, most of the effort on these disorders has been centered on genetic issues, resulting in the compilation of large repertories of missense mutations found in patients with these errors. With the frequently fragmentary single patient-based approach to genetic diagnosis, doubts on the disease-causing role of individual mutations are frequent. It is conventional wisdom that the knowledge of the structure of a protein accurately suggests the pathologic impact of each missense mutation. However, this view is in many cases too naive. Rather than having "kinetic" effects (altered substrate sites or catalytic residues), many mutations affect folding or stability, as we recently found for mutations in an entire domain of carbamoyl phosphate synthetase 1 (CPS1), rendering difficult to predict the severity of the effects for many of these mutations. Enzymes frequently have regulatory domains of poorly characterized function where the occurrence of missense mutations may have disease-causing effects of unclear nature, as for N-acetylglutamate synthase. Moonlighting and non-catalytic structural functions of macromolecular catalysts are observed with increasing frequency, as in the case of argininosuccinate lyase (ASL) for nitric acid production. Many times these functions are not explained by the mere observation of the structure of the isolated macromolecule and thus, the connection between structure, mutation and functional derangement may not be evident in these cases.

The difficulties for drawing inferences on disease causation by missense mutations should not discourage structural studies. On the contrary, more detailed studies that should consider protein complexes and different snapshots of the functional life of each macromolecule are needed. A two-snapshot study has revealed recently the importance, mechanism and pathological inferences of acetylglutamate activation of CPS1. Structural inferences have been crucial in understanding the puzzling observations that some mutations in the ALDH18A1 gene (which encodes Δ1-pyrroline-5-carboxylate synthetase) have a dominant effect while other mutations in the same gene have a recessive effect. Similarly, structural knowledge accounts for the understanding of the puzzling phenomenon of intragenic complementation that is observed for mutations of ASL. In summary, we need more rather than less structure to fill the gaps in our knowledge of molecular pathogenesis.

Conversely, the very extensive databases of clinical mutations for practically any human protein provide very important shortcuts for physical localization of functions in individual proteins. The lists of these mutations represent a small fraction of the total number of possible amino acid changes in a given protein. Since patient mutations should be expected to be detrimental, the clinical mutations can identify important residues and detrimental substitutions, guiding experimental site-directed mutagenesis studies to place on specific physical terms the functional traits of a given protein.

In summary, the structural and functional knowledge about proteins involved in metabolic processes can help much to understand the derrangements caused by specific missense mutations in individual proteins, but, in addition, the observation of the mutational spectrum found in patients can guide efforts to understant protein funtion.

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TALKS Wednesday, February 3rd

Molecular assemblies section

Recognition and Binding by Intrinsically Disordered Proteins

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There is a growing interest in understanding the properties of intrinsically disordered proteins (IDPs); however, the characterization of these states remains an open challenge. IDPs appear to have functional roles that diverge from those of folded proteins and revolve around their ability to act as hubs for protein-protein interactions. To gain a better understanding of the modes of binding of IDPs, we employ solution and solid-state NMR spectroscopy in combination with a series of biophysical and theoretical approaches to investigate the molecular mechanisms of recognition and binding of these proteins. The talk will overview recent results on the conformational recognition of the disordered protein Gab2 by the growth factor receptor-bound protein 2 (Grb2), a key interaction for normal cell signaling and cancer development, and protein-protein protein-lipid interactions of alpha-synuclein, a protein involved synaptic vesicle regulations and Parkinson's disease.
Systems biochemistry of bacterial division: Reconstructing minimal divisomes in the test tube

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The research in our laboratory aims at understanding how the elements of the bacterial division machinery - the divisome - work together as an integrated system to fulfil its essential function. To address these questions we develop and applied novel biochemical reconstitution approaches to assemble the minimal set of proteins needed to initiate division (the proto-ring complex) in systems that reproduce the spatio-temporal organization of the divisome at the cellular membrane and the crowded/confined intracellular space.

Using physical biochemistry and synthetic approaches, we study the activities, interactions and assembly properties of minimal reconstructions of the proto-ring structured in membrane-like systems, such as nanodiscs, micro-beads, bilayers, vesicles and micro-droplets. We also investigate the action of Min proteins and nucleoid-like structures (to reproduce Z-ring positioning mechanisms) on the properties of minimal divisomes.

The assembly of the divisome in the cell takes place in microenvironments characterized by the presence of high concentrations of unrelated macromolecules, often structured as soluble and/or membrane-bound dynamic networks. We apply and design synthetic reconstructions of these microenvironments to investigate the impact of the physicochemical properties of facsimile cell media on the reactivity and organization (in time and space) of minimal divisome assemblies.

These studies will contribute to define the precise conditions to build, with a minimum set of elements, functional division assemblies in the absence of cells. This integrated approach will help to complete our knowledge of how bacterial division works and will open new horizons to synthetic and biotechnological applications.

References:
Repositioning Tolcapone as a potent inhibitor of transthyretin amyloidogenesis and associated cellular toxicity

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Transthyretin (TTR) is a plasma homotetrameric protein implicated in fatal systemic amyloidoses. TTR tetramer dissociation precedes pathological TTR aggregation. Native state stabilizers are promising drugs to treat TTR amyloidoses. Here we repurpose tolcapone, an FDA-approved molecule for Parkinson’s disease, as a potent TTR aggregation inhibitor. Tolcapone binds specifically to TTR in human plasma, stabilizes the native tetramer in vivo in mice and humans and inhibits TTR cytotoxicity. Crystal structures of tolcapone bound to wild type TTR and to the V122I cardiomyopathy-associated variant show that it docks better into the TTR T4 pocket than tafamidis, so far the only drug on the market to treat TTR amyloidoses. These data indicate that tolcapone, already in clinical trials for familial amyloid polyneuropathy, is a strong candidate for therapeutic intervention in these diseases, including those affecting the central nervous system, for which no small molecule therapy exists.
Structure of TDP-43’s Q/N-rich Motif and N-terminal Domain

Douglas Laurents1, Miguel Mompeán1, David Pantoja-Uceda1, Rubén Hervás2, Mariano Carrión-Vázquez2, Valentina Romana3, Cristiana Stuani3, Francisco Baralle3, Emanuele Buratti3

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TDP-43 is an essential human RNA processing and transport protein [1]. It can putatively form functional amyloid-like structures in RNA stress granules as well as pathological aggregates implicated in ALS. We have used a variety of biophysical techniques and computational approaches to study the self-recognition of TDP-43’s C-terminal Q/N-rich motif, which is believed to form the key pathological aggregate. On this basis, we have advanced a structural model with residues 341-357 forming beta hairpins that pack in parallel with a singular beta-turn topology to form a novel amyloid-like structure [2]. Intra- and inter-molecular interactions of TDP-43’s N-terminal domain (NTD, residues 1-77) are essential for its physiological and pathological activities. On the basis of 1000+ NOE-derived constraints, we have solved the NMR solution structure of this domain [3]. The NTD has a novel fold with two highly anionic surface loops and two solvent exposed Cys residues. The domain is stable (DG° 25°C = 3.8 kcal/mol) as determined by H/D exchange and the secondary structural elements are rigid, as assessed rigorously on the basis of 1H-15N relaxation measurements. These findings contrast the poor stability, low resolution and alternative secondary structure in the model advanced by Qin et al. [4] and highlight the pitfalls of ab initio folding algorithms when attempting to model a novel fold. Other functional amyloids such as Sup35 and CPEB, whose aggregation is key for memory consolidation [5], also contain domains that govern their amyloid formation. Therefore, our elucidation of TDP-43’s NTD may represent the first structure solved of an emerging class of protein domains that regulate functional amyloid formation.

References:

Structural basis for the allosteric mechanism controlling antibiotics Resistance in PBP2A from MRSA

Juan Hermoso


The ability to resist the effect of a wide range of antibiotics makes methicillin-resistant Staphylococcus aureus (MRSA) a leading global human pathogen. A key determinant of resistance to β-lactam antibiotics in this organism is penicillin-binding protein 2a (PBP2a), an enzyme that catalyzes the crosslinking reaction between two adjacent peptide stems during the peptidoglycan biosynthesis. The recently published crystal structure of the complex of PBP2a with ceftaroline, a cephalosporin antibiotic that shows efficacy against MRSA, has revealed the allosteric site at 60-Å distance from the transpeptidase domain. Binding of ceftaroline to the allosteric site of PBP2a triggers conformational changes that lead to the opening of the active site from a closed conformation, where a second molecule of ceftaroline binds to give inhibition of the enzyme. The discovery of allostery in MRSA remains the only known example of such regulation of cell-wall biosynthesis and represents a new paradigm in fighting MRSA. In this talk we will summarize the present knowledge of the allosteric mechanism, the conformational changes allowing PBP2a catalysis and the means by which some clinical strains have acquired resistance to ceftaroline by disrupting the allosteric mechanism. The ability to resist the effect of a wide range of antibiotics makes methicillin-resistant Staphylococcus aureus (MRSA) a leading global human pathogen. A key determinant of resistance to β-lactam antibiotics in this organism is penicillin-binding protein 2a (PBP2a), an enzyme that catalyzes the crosslinking reaction between two adjacent peptide stems during the peptidoglycan biosynthesis. The recently published crystal structure of the complex of PBP2a with ceftaroline, a cephalosporin antibiotic that shows efficacy against MRSA, has revealed the allosteric site at 60-Å distance from the transpeptidase domain. Binding of ceftaroline to the allosteric site of PBP2a triggers conformational changes that lead to the opening of the active site from a closed conformation, where a second molecule of ceftaroline binds to give inhibition of the enzyme. The discovery of allostery in MRSA remains the only known example of such regulation of cell-wall biosynthesis and represents a new paradigm in fighting MRSA. In this talk we will summarize the present knowledge of the allosteric mechanism, the conformational changes allowing PBP2a catalysis and the means by which some clinical strains have acquired resistance to ceftaroline by disrupting the allosteric mechanism.

References:

Short oral presentations
Study of the molecular recognition of novel fungal transglycosylase ligands

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Fungi cell wall remodeling is controlled by the equilibrium between glycoside hydrolases, glycosyltransferases, and transglycosylases. Family 72 glycoside hydrolases (GH72) are ubiquitous in fungal organisms and are known to possess significant transglycosylase activity, producing elongated beta(1-3) glucan chains. Among them are the Gas (in S. cerevisiae), Gel (in A. fumigates) or Phr and Pga (in C. albicans), and all of them with a well conserved catalytic site. The only protein whose structure has been resolved within this family is ScGas2,[1] which will be our model for ligands and inhibitors design.

In this communication, they will be presented the design, synthesis and the study of the molecular recognition of novel beta(1-3)glucan-based ligands with ScGas2.[2,3]

To characterize the protein-ligand interactions several techniques were employed, such as saturation transfer difference NMR experiments (STD-NMR), molecular docking, molecular dynamics, non-covalent-interaction calculation (NCI) and X-ray diffraction of protein complexes.

References

Deciphering the non-equivalence of serine and threonine O-glycosilation points in biological systems.

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The Tn antigen is one of the most specific human tumor-associated structures, generally presented in cancer cells as a part of modified glycoproteins, such as mucins. In general, the Tn antigen is referred to as N-acetylgalactosamine (GalNAc) α-O-linked to Ser or Thr, without specifying which of the two amino acids the GalNAc is linked to. However, computational models had already shown the existence of different conformational behaviours in solution of the basic Ser- and Thr- containing structures [1] that could have important biological implications. Here, we present three different biological systems by which we have been able to decipher the non-equivalence of serine and threonine O-glycosilation points:

(i) A model anti-MUC1 antibody (SM3), where MUC1 is a heavily O-glycosylated membrane glycoprotein which is overexpressed and partially glycosylated in cancer cells [2].

(ii) Two different plant lectins (SBA, VVA), selected as biological targets because they specifically recognise the GalNAc carbohydrate moiety [3].

(iii) The GalNAcT2, one member of the GalNAc-transferases enzyme family, that, in humans, is responsible of the generation of the Tn antigen by the addition of an α-O-GalNAc group to a Ser/Thr residue.

References

Disordered regions in MeCP2 contribute to its structural stability

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Methyl CpG binding protein 2 (MeCP2) is a DNA binding protein involved in gene expression regulation that preferentially interacts with methylated DNA regions, though it also interacts with multiple protein partners. Certain mutations in MeCP2 are associated with Rett syndrome, an important neurodevelopmental disorder affecting young girls. Those clinically-relevant mutations in MeCP2 may alter its ability to fold and/or to interact properly with DNA, thus, hindering its multiple functions.

MeCP2 is an intrinsically disordered protein. The majority of its polypeptide chain is considered to be unstructured under physiological conditions. Unstructured regions are important because they provide the required structural plasticity for establishing multiple interactions with different binding partners, through processes where partial folding and binding are intimately coupled.

We have carried out a biophysical characterization, studying the contribution of different protein domains to MeCP2 structural stability by fluorescence and circular dichroism. The impact of methylated and unmethylated DNA interaction on that stability was also assessed. Strikingly, according to the experimental results, disordered regions in MeCP2 modulate its structural stability. In addition, large DNA stabilization effects suggest that the interaction with DNA is coupled to a structural rearrangement of the protein conformation. Therefore, the dynamics and mobility of disordered regions may strongly impact the global structural and functional features in proteins.

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How experiments and molecular simulations can help understand selective C25-hydroxylation of vitamin D by fungal peroxygenases

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25-Monohydroxylated vitamin D3 (cholecalciferol) and D2 (ergocalciferol), compounds of high interest in human health and animal feeding, can be obtained through reaction with fungal peroxygenases [1]. In this work we show the results from a combined experimental and computational study of the hydroxylation of vitamin D by Agrocybe aegerita and Coprinopsis cinerea peroxygenases [2]. To rationalize experimentally observed differences in conversion yields and regioselectivity, diffusion of D2 and D3 on the molecular structure of these two enzymes was performed with PELE software [3]. In good agreement with experimental conversion yields, simulations indicate more favorable energy profiles for the substrates’ entrance in C. cinerea than for A. aegerita enzyme. On the other hand, GC-MS analyses show that while a full regioselective conversion into the active C25 form is catalyzed by C. cinerea peroxygenase for D2 and D3, A. aegerita yielded a mixture of the hydroxylated D3 products. From the molecular simulations, relative distance distributions between the haem compound I oxygen and H24/H25 atoms (hydrogens on C24 and C25 respectively) were plotted. Results show large populations for O-H25 distances below 3 Å for D2 and D3 in C. cinerea in accordance with the high reactivity observed for this enzyme. In A. aegerita, however, cholecalciferol has similar populations (below 3 Å) for O-H25 and O-H24 which can justify the hydroxylation observed in C24. In the case of ergocalciferol, due to the bulky methyl group in position C24, very few structures are found with O-H24 distances below 3 Å and thus, as expected, reaction was only observed at C25 position.

References


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Site Directed Spin labeling EPR spectroscopy to probe recognition mechanisms between a molybdenoenzyme and its dedicated chaperone

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Molecular recognition mechanisms between proteins are essential in many biological processes. These mechanisms are associated to protein dynamics consisting in their abilities to undergo structural changes. We are interested in characterizing such mechanisms in chaperone proteins dedicated to the folding and assembly of metalloproteins.

NarJ from E. coli was taken as a model of such dedicated chaperone, being involved in the biogenesis of a molybdenoenzyme: the membrane-bound nitrate reductase complex NarGHI [1]. NarJ interacts at two different sites of the catalytic subunit NarG: at its N-terminus to prevent membrane anchoring of an immature complex and at a second site to control sequential insertion of both an iron-sulfur cluster and the molybdenum cofactor [2].

In order to obtain a detailed picture of the conformational dynamics of the NarJ chaperone upon binding to its NarG partner, we used Site-Directed Spin Labeling Electron Paramagnetic Resonance (SDSL-EPR), a powerful technique to get information on protein dynamics [3]. The technique relies on the insertion of paramagnetic labels at selected sites of a protein followed by EPR spectroscopy analyses. Using singly spin-labeled NarJ, we identified the interaction site with the N-ter of NarG. Moreover, doubly spin-labeled NarJ studied by pulsed double electron-electron resonance (DEER) techniques suggested that the recognition mechanism is governed by a conformational selection mechanism [4]. Moreover we recently demonstrated that the C-terminal region of NarJ is highly flexible and gain structural elements when interacting at the second site (unknown) site of its partner protein.

References

Characterization of protein interactions of biological interest: insights from computational models

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After sequencing the complete genomes of several organisms, one of the current biological challenges consists in unravelling their intricate protein-protein interaction networks. To this aim, one of the fundamental steps is to provide structural and energetic details at atomic level for such interactomes, which is essential to understand biological processes at molecular level and contribute to improve therapeutic intervention. Nevertheless, given the high costs and the intrinsic limitations of available experimental methods, computational modeling represent valuable tools to complement such data and thus improve our understanding of biological processes involving protein interactions.

Here we present a comparative assessment of four different computational methods for the characterization of protein-protein interfaces, such as ConSurf [1], pyDockNIP [2], per-residue pyDock binding energy and in silico Alanine-scanning [3-4]. This analysis confirms that current computational protocols aimed to model the phylogenetic, structural and energetic properties of residues within protein-protein interfaces show reasonably good predicting performance and consistency.

Moreover, we will describe the application of computational methods to the prediction and energetic characterization of host-pathogen protein interactions, which are key steps of virtually every infection process (i.e, pathogen replication and survival within the host system). Thus, we will discuss the energetic basis of the successful competition of the human pathogen Legionella pneumophila effector VipD against different endogenous ligands for the binding to GTPase Rab5 host protein [5] as well as the structural and energetic characterization of the human Rab1 deactivation by L. pneumophila SidD protein [6]. In both these examples, the predicted hot-spot residues are in substantial agreement with the experimental data and provide the basis for future development of novel therapeutic approaches.

All these findings clearly confirm that in silico protocols offer a reliable aid to the energetic characterization of protein-protein interfaces.

References

PrionW: a server for the prediction of prion-like domains and their amyloid cores

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Prions are a particular type of amyloids with the ability to self-perpetuate and propagate in vivo. Prion-like conversion underlies crucial biological processes in a growing number of species but is also connected to several human diseases including Creutzfeldt–Jakob disease and kuru. Yeast prions are the best understood transmissible amyloids. In these proteins, prion formation from an initially soluble state involves a structural conversion, often driven, by specific domains enriched in glutamine/asparagine (Q/N) residues. Importantly, domains sharing this compositional bias are also present in the proteomes of higher organisms, thus suggesting that prion-like conversion might be an evolutionary conserved mechanism. We have recently shown that the identification and evaluation of the potency of amyloid nucleating sequences in putative prion domains allows discrimination of bona fide prions. PrionW is a web application that exploits this principle to scan sequences in order to identify proteins containing Q/N enriched prion-like domains (PrLDs) in large datasets. A scan of the complete yeast proteome with PrionW identifies previously experimentally validated prions with a high success rate: a sensitivity of 0.917, a specificity of 0.949, a precision rate of 0.846, an accuracy of 0.941 and a false discovery rate of only 0.154. This website is free and open to all users who can analyze up to 10000 sequences at a time, PrLD-containing proteins are identified and their putative PrLDs and amyloid nucleating cores visualized and scored. The output files can be downloaded for further analysis. PrionW server can be accessed at http://bioinf.uab.cat/prionw/.
c.A2456C-substitution in Pck1 changes the enzyme kinetic and functional properties modifying fat distribution in pigs

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Cytosolic phosphoenolpyruvate carboxykinase, PCK1, is one of the main regulatory enzymes of gluconeogenesis and glyceroneogenesis. The substitution of a single amino acid (Met139Leu) in PCK1 as a consequence of a single nucleotide polymorphism (SNP), c.A2456C, is associated in the pig to a negative phenotype characterized by reduced intramuscular fat content, enhanced backfat thickness and lower meat quality. The p.139L enzyme shows reduced kcat values in the glyceroneogenic direction and enhanced ones in the anaplerotic direction. Accordingly, the expression of the p.139L isoform results in about 30% lower glucose and 9% lower lipid production in cell cultures. Moreover, the ability of this isoform to be acetylated is also compromised, what would increase its susceptibility to be degraded in vivo by the ubiquitin-proteasome system. The high frequency of the c.2456C allele in modern pig breeds implies that the benefits of including c.A2456C SNP in selection programs could be considerable.
Poster Session
P1. Coarse-grained discrete molecular dynamics simulations of protein-protein binding

Agustí Emperador, Modesto Orozco

Institute for Research in Biomedicine Molecular Modelling and Bioinformatics. Baldiri Reixac [IRB Barcelona], Modesto Orozco [IRB Barcelona]

Protein-protein binding is an extremely complex issue that so far has not been correctly reproduced in simulations due to inaccuracies in current atomistic force fields that are optimized using single-protein systems as test sets. Current state-of-the-art force fields give excellent results for the explicit solvent all-atom simulations of single proteins, but lead to an incorrect formation of aggregates when several proteins are included in the simulation [1]. This problem hinders the use of molecular dynamics simulations to estimate the binding propensity of two proteins.

Monte Carlo based sampling methods have given very good results in the study of protein-ligand binding, but are unfeasible for the determination of protein-protein binding. Protein-protein docking scoring functions give a very good ranking of protein-protein conformations, especially when the proteins do not undergo large conformational changes upon binding. The ability to rank correctly the different possible conformations makes docking a powerful method to estimate the experimental complex structure and to determine the interface between receptor and ligand, but protein-protein docking is unable to identify interaction partners [2]. It is necessary previous experimental evidence of the binding of the two proteins in order to ensure that the predicted conformation is actually a binding conformation.

Coarse-grained protein models using implicit solvation allow fast conformational sampling in molecular dynamics simulations. A force field whose optimization includes observables relevant to multiprotein systems, like protein-protein association/dissociation, could overcome the problems shown by standard force fields, optimized using only single-protein observables. We have used our PACSAB coarse-grained force field [3] to test the stability of experimental protein complexes and to evaluate the binding tendency of nonbinding conformations of each protein-protein pair (false positives generated in docking calculations). We have run Discrete Molecular Dynamics simulations, a method that gives increased speed as compared to standard Molecular Dynamics when implicit solvent is used.

We have used a benchmark of protein-protein complexes with a binding energy of at least 10 kcal/mol, as determined experimentally. We have found that 85% of the experimental complex structures remained stable during the simulations, and 80% of the false positive structures dissociated, as should be expected from nonbinding protein-protein conformations. We used the original parametrization of the PACSAB force field for the simulations.

PACSAB had been parametrized [3] considering the association/dissociation rate of an intrinsically disordered protein (Ab40) and the stability of a small folded protein (1FAS), therefore without considering data of protein-protein binding. The original version of PACSAB includes backbone hydrogen bonding, van der Waals and implicit solvation term, but not a Coulomb term (electrostatic effects are included via the hydrogen bonding term and the implicit solvation). We expect that the inclusion of an explicit Coulomb term and fine tuning of the force field, optimizing it with protein binding information, will improve further the ability of PACSAB to filter protein-protein docking conformations, and make it a good approach to identify protein-protein interaction partners.

References:
P2. Multivariate biophysical combinations to enhance rigid body protein-protein docking

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Protein-protein interactions are known to play key roles in the most important cellular and biological processes such as signaling, metabolism, and trafficking [1]. A full understanding of these interaction can only be gained through consideration of their atomic details [2], [3]. Currently only 7% of the known human interactome is structurally characterized (either experimentally or modeled), but computational protein-protein docking could increase up to 50% the structural coverage [4]. Protein-protein docking consist in the generation of structural poses or sampling, and the identification of the correct structures with a scoring function [5]. However, these algorithms generate a large number of incorrect predictions, and therefore the predictive success strongly depends on the accuracy of the scoring function used to evaluate the models generated [5]. To characterize the models the scoring functions are usually based on empirical, statistical or energy-based potentials [6], defined at different resolution levels (atomic, residue) [7], and then return a ranked list that aims to place the near-native solutions as close to the top as possible. We present here a procedure to select from a large pool of biophysical descriptors [8] the best Scoring functions and we explore how they can be combined to generate a new ranking scheme. In general we found combinations with large improvements in the docking success rate for decoys generated with PyDock, ZDock and Sdock protocols [6], [9], [10].

References:

P3. High-performance computational tools for the characterization of protein-protein interactions

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Protein-protein interactions mediate most cellular functions, and the detailed description of their association mechanism is needed to understand the fundamental processes that sustain life. Therefore, the development of computational tools which aim to help to model and to predict protein-protein interactions are a valuable resource for scientists.

Here we present a set of different computational tools freely available online for the scientific community. These tools are optimized to make use of high-performance computing facilities and are accessible via clean and user-friendly web interfaces and centralized in our group’s website: http://life.bsc.es/pid/pidweb/default/tools:

PyDockWEB is a web server for the structural prediction of protein-protein interactions. Starting from the unbound partners can predict protein-protein complexes 3D atomic structure. PyDockSAXS is a web server for rigid-body protein-protein docking that combines computational and experimental information (SAXS). The use of this experimental data can dramatically boost the protein-protein complex predictions (reported as up to two times the success rate compared to blind protein-protein docking predictions). CCharPPI is a web-based tool which aims to characterize the protein-protein complex interfaces in a systematic way with up to 108 different energetic descriptors.

Our tools have been widely tested and used by many users since their availability and they have shown excellent performance in community wide experiments such CAPRI, where our PyDockWEB server is in the top performing ones. It is planned in the future to develop and to integrate more tools in this platform.

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A total of six different structural alignment tools (TM-Align, TriangleMatch, CLICK, ProBis, SiteEngine and GA-SI [1-6]) were assessed for their ability to perform two particular tasks: (i) discriminating FAD (flavin adenine dinucleotide) from non-FAD binding sites, and (ii) performing an all-to-all comparison on a set of 883 FAD binding sites with the purpose of classifying them. For the first task, the consistency of each alignment method was evaluated, showing that every method is able to distinguish FAD and non-FAD binding sites with a high degree of accuracy. The results obtained for the second task revealed more significant differences among alignment methods, as reflected in the poor correlation of their results and clearly highlighted by the independent evaluation of the structural superimpositions generated by each method. The classification itself was performed using the combined results of all methods, using the best result found for each comparison of binding sites. A number of different classification schemes (Single-linkage, UPGMA, Complete-linkage, SPICKER [7] and k-Means clustering) were also used. The clusters of similar binding sites (proteins) generated by the best performing method were further analyzed in terms of local sequence identity, local structural similarity and conservation of analogous contacts with the FAD ligands. The analysis exposed that the structure-based classification had been able to separate homologous proteins, which presented only small differences in their ligand-binding sites, into different clusters. A closer look into the most populated clusters revealed that the classification had in fact separated proteins by function, being able to distinguish proteins with high sequential and structural similarities based on their ligand-binding sites. Each of the clusters was characterized by a unique set of structural features or patterns, demonstrating that the groups generated truly reflect the structural diversity of FAD binding sites.

References

P5. AGGRESHAN3D (A3D): server for prediction of aggregation properties of protein structures

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Protein aggregation has moved beyond being a mostly ignored area of protein chemistry to become a key topic in biomedicine and biotechnology. It underlies more than 40 human disorders, including neurodegenerative diseases - such as Alzheimer’s and Parkinson’s- and non-neuronal related diseases such diabetes type II or some type of cancers [1,2]. In addition, it is a pivotal factor to take into account while manufacturing protein-based therapeutics like monoclonal antibodies, growth factors or replacement enzymes [3].

With the aim of anticipate this phenomenon, the present understanding on the molecular determinants of protein aggregation has crystalized in a series of predictive algorithms to identify the aggregation-prone sites of proteins. The vast majority rely on the aminoacidic sequence. Therefore they find difficulties to predict the aggregation properties of folded globular proteins, where aggregation-prone sites are often not contiguous in sequence or buried inside the native structure. The Aggrescan 3D server overcomes these limitations by projecting onto the protein structure the experimental aggregation propensity scale from the well-established AGGRESCAN method [4, 5]. In this way, the native neighbouring tendencies modulate the aggregation propensity score for each amino acid to obtain high confident predictions. Using the A3D server, the identified aggregation-prone residues can be virtually mutated to design variants with increased solubility, or to test the impact of pathogenic mutations. Additionally, A3D server enables to take into account the dynamic fluctuations of protein structure in solution, which may influence aggregation propensity, by using the fast simulations of CABS-flex approach. The A3D server can be accessed at http://biocomp.chem.uw.edu.pl/A3D/.

Reference

P6. Screening and identification of compounds with antimicrobial activity against *S. pneumoniae*

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The final steps in the synthesis of the essential cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are catalyzed by the sequential action of two enzymatic activities, an ATP:riboflavin kinase (RFK) and an ATP:FMN adenylyltransferase (FMNAT) (Frago et al., 2008). In most eukaryotes and some archaea, both activities reside in two different enzymes; whereas most prokaryotic organisms depend on a single bifunctional enzyme, the FAD synthetase (FADS). These equivalent enzymes exhibit differential structural characteristics in prokaryotes and eukaryotes, therefore prokaryotic FADSs appear as a potential drug target for the development of inhibitors with antimicrobial activity (Serrano et al., 2013). Furthermore, the deficiency of RFK or FMNAT activities leads into FMN and FAD deficiency and consequently into the accumulation of the apo forms of a large number flavoproteins which are unable to carry out their expected functions in the cellular metabolism and other essential processes.

In this work, we have identified more than 40 potential inhibitors of the FADS from *S. pneumoniae* (commensal microbiota of the upper respiratory tract, but also opportunistic human pathogen causing diseases such as pneumonia and meningitis) from a 1040-compound commercial library by activity-based high-throughput screening. We also classified those compounds with higher inhibitory effect based on the affected enzymatic activity. Additionally we have further characterized one of these compounds through the determination of kinetic and interaction parameters, and evaluated its inhibitory activity against the equivalent human enzyme. In a further phase, SpnFADS potential inhibitors should be tested for bacteriostatic or bactericidal activity on whole-cell assays. Additionally, the effect of these compounds in other prokaryotic as well as in eukaryotic cells (cytotoxicity, reduction of viability, mutagenicity, etc.) should be characterized to assure the selectivity against the pathogen and the safety for the possible application in human therapy.

References

P7. Comparing two bacterial FAD synthetases: little variations yet big differences

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Bacterial FAD synthetases (FADS) are bifunctional proteins that carry out the flavin adenine dinucleotide (FAD) synthesis from riboflavin (RF) in two independent reactions [1]. First, RF is phosphorylated by a riboflavin kinase activity (RFK) to produce FMN and then a FMN adenylyl transferase activity (FMNAT) transforms FMN into FAD. Bacterial FADSs share function and show homologous structures organized in two different domains: the C-terminus performs de RFK activity while the N-terminus carries out the transferase one. In spite of this, FADSs from different organism present important differences. For instance, FADSs from C. ammoniagenes do not require reducing conditions to perform either of their activities and the RFK activity is inhibited when the RF concentration increases. On the contrary, the FMNAT activity of FADS from S. pneumoniae needs a reducing environment while the RFK one does not show any inhibition.

FMN and FAD are involved in processes of crucial relevance for life maintenance, and their deficiency directly leads to death. For this reason, and due to the differences between bacterial FADSs and the human homologous, these proteins appear as potential drug targets, so their deep characterization determines a problem of central relevance [3].

In this work we employ different experimental techniques, such as isothermal titration calorimetry or stopped flow to biophysically characterize the interaction of the FADS from the pathogenic organism Streptococcus pneumoniae with its ligands, which allows proposing a model for its catalytic cycle. In addition, we compare the results with those obtained for Corynebacterium ammoniagenes, which is the best known member of the family [2].

References

P8. The trimer interface in the quaternary structure of the bifunctional prokaryotic FAD synthetase from *C. ammoniagenes*

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Prokaryotic FAD synthetase (FADS) folds in two almost independent modules, each one being mainly responsible of one of its two activities. The C-terminal catalyzes the enzyme riboflavinkinase activity (RFK module and RFK activity), while the N-terminal is responsible for the FMN adenylyltransferase (FMNAT module and FMNAT activity). Crystal structure of *Corynebacterium ammoniagenes* FADS (CaFADS) predicted a dimer-of-trimers organization with the catalytic sites of two modules of neighboring protomers within the trimers approaching each other [1]. Mutational analysis additionally suggested that residues at active site of one module of the protein influence the activity at the other module of the contiguous protomer within each trimer [2]. Here we analyze at the molecular level the trimer interfaces determinants, evaluating the effect of point mutations at loops L1c-FlapI and L6c, and of helix $\alpha$1c of the RFK module of CaFADS; particularly at positions K202, E203, F206, D298, V300 and L304 predicted at the interface between protomers in the trimer. Oligomerization profiles, ligand binding and kinetic parameters for the produced mutants indicate that these residues despite not been individually critical for formation of quaternary organizations, modulate the assembly conformation as well as the binding and kinetic parameters for both of the enzyme activities; RFK and FMNAT. These results support the formation of transient oligomeric structures during the CaFADS catalytic events and substrate accommodation at each of the catalytic sites being influenced by residues of the other module of the neighbor protomer within the trimer.

References


P9. Characterization of the interaction between human Apoptosis Inducing Factor (hAIF) and DNA

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The Apoptosis Inducing Factor (AIF) is a mitochondrial NADH-dependent flavoprotein that contributes to the maintenance and stability of several respiratory chain complexes by interaction with CHCHD4 [1]. After apoptotic stimulus AIF is released to the cytosol and translocated to the nucleus where it induces partial chromatin condensation and DNA degradation into 50 kb fragments (typical chromatinolysis caspase-independent pattern) [2]. AIF interacts with DNA in a sequence-independent manner based on electrostatic interactions [3].

In healthy mitochondria, AIF is present in monomer-dimer equilibrium (being the monomer the main one), undergoing dimerization upon NADH reduction. The NADH-bound dimer is stabilized by long-lived flavin:nicotinamide charge transfer complexes, and is accompanied by conformational rearrangements of the reductase and apoptotic domains. These observations suggest some interconnection between the mitochondrial and apoptotic activities of AIF [4, 5].

In this study we further characterized the interaction between hAIF and DNA, and how it can be modulated by the redox state of the protein, by using EMSA (Electrophoretic Mobility Shift Assay), atomic force microscopy and isothermal titration calorimetry techniques.

References

P10. High Throughput Screening for modulators of Apoptosis Inducing Factor activities

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The Apoptosis Inducing Factor (AIF) was first discovered as a caspase-independent cell death promoter, which also plays a vital role in mitochondria, where it is normally confined. Several facts make of AIF a potential target for treating human disorders, as for example: i) some of the functions in which it is related are implicated in the development of diseases such as Parkinson’s disease, ischemia/reperfusion injury, amyotrophic lateral sclerosis, and Huntington’s disease; ii) six mutations on hAIF have been shown to trigger major dysfunctions in oxidative phosphorylation and cause severe illnesses related with neurodegeneration as a consequence of mitochondriopathies; iii) the role of AIF in caspase-independent apoptosis pathways opens the possibility to modulate these routes through its regulation. All these facts make of interest the search for small molecules which can bind AIF and modulate its activities. We have performed a High-Throughput Screening by FAD release thermal shift assay and tested more than 11000 compounds from two chemical libraries, with high chemical diversity and good pharmacokinetic properties. This massive screening allowed us to identify eleven compounds that bind to the apoptotic form of hAIF (hAIFΔ1-102) by reducing its melting temperature, six of them in a dose-dependent manner. We have also tested the effect of these six compounds in cellular viability, finding EC50s ranging from ~1 to ~680 µM. These compounds may serve as starting points in the search of compounds that upon interacting with AIF might modulate its activities.
P11. Studying the structural changes of human Apoptosis Inducing Factor using site-selective spin-labeling

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The Apoptosis Inducing Factor (AIF) is a mitochondrial flavoprotein that binds NADH and contributes to the maintenance and stability of several respiratory chain complexes. In mitochondria, AIF is present in monomer-dimer equilibrium, undergoing dimerization upon NADH reduction which is accompanied by conformational rearrangements of the reductase and apoptotic domains. In particular, the structure of AIF as studied by X-ray diffraction studies of the crystalline protein show that a small helix covering the entrance to the FAD pocket is not present in the dimeric form, together with a small segment of the sequence which is absent in the crystal structure of both forms. Whether this region can have different possible conformations or is totally/partially disordered is not known. After apoptotic stimulus AIF is released to the cytosol and translocated to the nucleus where it induces partial chromatin condensation and DNA degradation. There are observations which suggest some interconnection between the mitochondrial and apoptotic activities of AIF which increases the interest of the intriguing relation between redox state and structure.

EPR spectroscopy of site-directed spin labeled proteins is gaining considerable interest as a biophysical tool to study valuable structural details and conformational changes under conditions which are relevant to function. It can provide information about flexible regions of a protein though the sensitivity to the dynamics of side chains and about distances between two spin labeled side chains.

In this contribution, we present the results on two variants labeled either in one or two selected positions of the protein sequence. The combination of CW-EPR and DEER measurements on these spin labeled proteins in the different redox states allows releasing the hypothesis of a conformational change involving only some degree of structural disorder.
The aim of this work is to understand the molecular events leading to the folding of PAH. To achieve this goal, we have carried out an analysis of the urea denaturation of two different constructions of the enzyme [3]. Moreover, sedimentation velocity assays of these two enzyme constructions incubated with different urea concentration have been performed by analytical ultracentrifugation. Based on these findings, we can propose a tentative model for the distribution of the oligomeric forms in unfolding equilibrium experiments. However, there are still several undefined aspects of the denaturation pathway that we want to reveal using other techniques such as Small-Angle-X-ray Scattering (SAXS) [4].

References

P13. Structural characterization of toxic oligomers that are kinetically trapped during alpha-synuclein fibril formation

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The accumulation of abnormally aggregated proteins within the body is a common feature of several medical disorders, such as Alzheimer’s disease, Parkinson’s disease and diabetes mellitus type 2. While the specific protein found to be the major component of such deposits varies from one disease to another, the formation of the pathological aggregates seems to occur via a common process of misfolding and self-assembly of a normally soluble polypeptide chain into a series of oligomeric intermediates and, ultimately, into insoluble amyloid fibrils that accumulate within specific organs and tissues. Increasing evidence indicates that certain oligomeric protein species generated during the self-assembly of specific proteins into ordered fibrillar aggregates can be highly cytotoxic and are likely to be key players in the initiation and spreading of neurodegenerative diseases. However, little detailed structural information is currently available for these oligomeric species due to their often transient nature and, more importantly, because of their variability in terms of size and structure.

We report here the isolation and detailed characterization of an ensemble of stable toxic oligomers of alpha-synuclein, the protein whose deposition is the hallmark of Parkinson’s disease. By defining and minimizing the degree of heterogeneity of these isolated alpha-synuclein oligomers which have accumulated during the process of amyloid formation, we have identified distinct subgroups of oligomers and determined their structural properties and three-dimensional molecular architectures. This characterization has been achieved by the application of a set of complementary biophysical techniques, including a variety of spectroscopic techniques along with analytical ultracentrifugation, atomic force microscopy, and electron microscopy. Although these oligomers exist in a range of sizes, with different extents and nature of beta-sheet content and exposed hydrophobicity, all the oligomeric subgroups possess hollow cylindrical architectures with marked similarities to amyloid fibrils. This suggests that these types of oligomers are kinetically trapped during protein self-assembly and that the accumulation of at least some forms of amyloid oligomers is likely to be a consequence of very slow rates of rearrangement of their beta-sheet structures.

Our findings reveal the inherent multiplicity of pathways of protein misfolding and the key role the beta-sheet geometry acquired in the early stages of the self-assembly process plays in dictating the rates of structural conversions, and thus the kinetic stabilities and pathological nature of different amyloid oligomers. The results of this study provide the basis for a more complete understanding of the nature of the self-assembly of polypeptides into beta-sheet rich amyloid aggregates, and potentially contributes to efforts to identify specific targets for drug discovery.

Reference
P14. Toxicity and efficacy evaluation of novel compounds targeting an essential protein of Helicobacter pylori

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Helicobacter pylori, Hp, which has become the most common bacterial infection worldwide, is specialised in the lifelong colonization of the human gastric mucosa, thus affecting nearly half of the world’s population. Therefore, it has been proposed to be involved in the pathogenesis of diseases such as gastritis, peptic ulcer or gastric cancer and even, cardiovascular, hepatic or neurodegenerative disorders. Conventional therapy to treat this infection accomplishes middling success rates due to increases in resistance of the bacterium to antibiotics, so new therapeutics, which do not generally perturb the bacterial flora, are required. For this reason, many essential Hp proteins have been identified as specific targets, one of them being its redox protein flavodoxin, Hp-Fld. To develop selective antimicrobials, we follow the hypothesis that certain organic molecules could interact with a distinct pocket created in the Hp-Fld, near the cofactor binding site, interfering with flavodoxin function. This hollow is characteristic of Helicobacter pylori flavodoxin, since other similar proteins do not bear it. Accordingly, we have recently synthesized new potential compounds based on three flavodoxin inhibitors identified in a previous study. All of them are less toxic toward HeLa cells than the original lead molecules, in consonance with XTT method. These results, together with the ones from in vitro efficacy assays, have allowed us to determine the therapeutic indexes for these novel compounds. Preliminary results show that two of them stand out among the others due to their promising anti-Hp activity. Efficacy and toxicity tests are currently being undertaken in drug-resistant strains of Hp and in mice. Attending to the results of all these trials, the antibiotic effect of these new compounds toward Helicobacter pylori will be established and, ultimately, toward different diseases derived from Hp infection.

References

P14. Mechanism of water/ion exchange at the surface of *Helicobacter pylori* apoflavodoxin.

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In a theoretical study, we have continued with a series of previous works carried out in our group regarding the cofactor/apoprotein interaction phenomenon in the *H. pylori*’s flavodoxin [1-3]. The importance of studying this phenomenon itself lies in the wide variety of flavodoxins that have been identified (usually in prokaryotes microorganisms, but also in some types of eukaryotic algae), as well as in the essential role played by the cofactor FMN on the functionality of these enzymes, which, in general, are also essential for the organisms that carry them [4,5]. In those previous works, directed mutagenesis, kinetics, or even computational approaches have been implemented to study the FMN/apo-Hp-Fld interaction mechanism [1,2]. From these studies, it has been possible to extract conclusions about important aspects such as the site on the cofactor and the order by which this entity binds to the protein. However, aspects such as the observed biphasic kinetics, and the presence of anions coordinated into the binding site of the phosphate of the cofactor FMN when this has not yet been attached, are still important questions to be addressed in order to fully clarify the mentioned mechanism [1,2].

In the performed study, the last of these issues is investigated through molecular dynamics simulations. The observation of the release of a chloride anion that was imitating the phosphate group of the FMN in a number of exploratory simulations gave rise to the realization of a larger number of simulations (60 in total). That allowed us to obtain better statistics of the main events that take place and also to investigate the role of the solvent in the observed phenomenon. Our hypothesis here consisted on identifying the order of events in which the chloride release occurs. With this goal in mind, we set up some definitions and implemented accurate algorithms for registering the molecular events involved in the chloride release. Using them, we performed several analyses on the trajectories obtained. One of these analyses relied on calculating the coordination numbers of the chloride along the simulations, differentiating between the coordination due to water molecules and the coordination due to residues from protein. This approach allows to observe whether during anion release its total coordination is increased, and whether decreases in the chloride/protein coordination occurs first that the increase of the chloride/water coordination. Another analysis was based exclusively on monitoring the chloride/water interaction distances vs. the chloride/residues interaction distances; and a third one in obtaining general statistics of the temporal relativity of the events from histograms in which defined sections where a chloride/residue unbinding is observed are superimposed. Additionally, complementary analyses of the residence times of water molecules in both the first solvation shell of chloride and the binding site of the anion in the protein are carried out. All these analyses together, allowed to conclude that the chloride output into the bulk is not determined by solvent effects. Thus, a mechanism is proposed in which the interactions of the anion with apoprotein (hydrogen bonds) are successively and spontaneously broken down, and where new water molecules occupy the free valences arising both chloride and apoprotein after each of these ruptures. In this regard, and as it is consistent with the data and observations obtained from the previous studies [1,2], the proposed mechanism suggests a FMN/apo-Hp-Fld binding scenario where once the cofactor is partially bonded through its isoalloxazine moiety it should wait for any anion bound at the FMN phosphate binding site to be released to complete its binding.

References

P15. Synthesis and testing of fluorescent chemical compounds as diagnostic tools for Alzheimer’s disease

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The most common neurodegenerative disease that causes dementia in humans is Alzheimer’s disease. This degeneration of neurons and synapses in the cerebral cortex is characterized by the loss of memory and other very important human skills like reasoning, abstraction and language [1]. Nowadays, around 30 million people are affected by this disease. Histologically, Alzheimer’s disease is characterized by the presence of two microscopic lesions: extracellular senile (amyloid) plaques and neurofibrillary tangles. The senile plaques, also known as neuritic plaques, are made of deposits of amyloid beta peptide (ABeta), which derives from beta amyloid precursor protein (APP) by consecutive proteolytic cleavages. In a previous study, four chemical compounds were found to act as inhibitors of the in vitro and in vivo aggregation of ABeta peptide [2]. In order to develop novel inhibitors that, furthermore, are capable of being used as diagnostic tools in medicine, we have synthesized two compounds derived from one of them in which we have joined a fluorescent dye through two linkers of different length. Our later HTS turbidity tests have shown that this two new compounds keep the inhibitor activity of their predecessor, especially that one in which the linker is longer, what is considered a satisfactory starting point for coming studies.

References

P17. New Glycomimetics targeting Human GalNAc-T2, Synthesis, Computational and Structural Studies

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GalNAc-T2, is an enzyme belonging to the glycosyltransferase family that catalyzes the transfer of N-Acetylgalactosamine, from the donor substrate UDP-GalNAc to the acceptor hydroxyl groups in mucine-type proteins. This constitutes the most complex and well-regulated type of protein O-glycosylation, which present twenty different isoforms in the human body. Despite the important role that the protein plays, from health to metabolic disorders and disease, [1] at the present time very few ligands have been reported, [2] of which none is an effective inhibitor.

In this work we propose a synthetic method directing to a new class of glycomimetic ligands, capable to bind the protein in the active site. These ligands leading to non-hydrolisable and less polar uridine-phosphonate derivatives, in which one phosphate function was suppressed and substitute by an alkyl chain, are by far more stable than the natural substrate.

The synthetic method has been standardized on a wide pool of different carbohydrates; afterwards the protein binding capability was confirmed by a docking study and biological test too.

Finally, a crystal structure of the complex [GalNAc-T2 – ligand] confirms the predicted binding capability, validating this method as a powerful and versatile tool in order to synthetize new glycosyltransferase inhibitors.
P18. Synthesis of new transglycosylases ligands and study of the glycomimetics-SCGAS2 interactions

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Transglycosylases are crucial membrane-bound enzymes for the remodeling of the bacterial and fungal cell wall, which are indispensable to maintain the structural integrity of those microorganisms. Among all fungal transglycosylases, Gas2 of Saccharomyces cerevisiae (ScGas2)[1] is the only one whose structure has been described so far, and is an optimum model for a new class of antifungal drugs due to its active site is totally conserved with other enzymes in pathogen fungi, such as Aspergillus fumigatus and Candida albicans.

In this communication, we will report the design and synthesis of pyrrolidine-containing ligands for ScGas2; which are formed by a glycosidic part that is essential to the enzyme recognition, and a polihydroxylated pyrrolidine part, which is able to mimic sugars and could also provide the possibility of interacting with the anionic residues in the active site of the enzyme. The interaction of the glycomimetics with the protein has been evaluated by Saturation Transfer Difference NMR experiments (STD-NMR) and molecular docking calculations.

Recognition effects of modifications in the ligands structure (length of carbohydrate moiety, flexibility of pyrrolidine ring) will be discussed.

References

P19. Inferring potential zinc-binding sites of FurB/Zur from Anabaena sp. PCC 7120

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FurB is one of the three paralogs of the Fur (ferric uptake regulator) family in Anabaena sp. PCC 7120, where it has been shown to act as a Zur regulator, controlling zinc homeostasis by binding to DNA in a zinc-dependent manner [1]. Most of the already described Zur proteins contain a structural zinc atom that is necessary to maintain the proper conformation of the protein. This atom is tetracoordinated by four highly-conserved cysteine residues also present in FurB from Anabaena sp. PCC 7120. Apart from the structural atom, Zur proteins are able to bind one to two additional zinc atoms, involved in DNA binding and protein dimerization [2].

In this work, we gained insights into the zinc-binding ability of Anabaena sp. PCC 7120 FurB. We determined the presence of a structural zinc atom in recombinant FurB by Inductively Coupled Plasma spectrometry (ICP) and detected two additional zinc binding sites by Isothermal Titration Calorimetry (ITC). The comparison of a FurB structural model with the structures of crystallized Zur proteins from Streptomyces coelicolor, Mycobacterium tuberculosis and Helicobacter pylori allowed us to propose cysteines 81, 84, 121 and 124 as the most plausible ligands for the structural zinc. The structural comparison also gave us an idea of the possible ligands for the additional zinc atoms. However, protein crystallization together with mutational studies will be necessary to determine the residues involved in zinc binding.

References

P20. Development of biosensors for monitoring short-chain alkanes in marine oil spills

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Marine hydrocarbonoclastic bacteria are highly specialized microorganisms that use hydrocarbons as the sole source of carbon and energy. As a consequence, they have developed several strategies to overcome the low water solubility of hydrocarbons and facilitate their uptake prior to assimilation. These properties make marine hydrocarbonoclastic bacteria very interesting to be used as biosensors that allow the monitoring of alkanes in seawater after an oil spill. In this work, a reporter plasmid was constructed based on the alkane degradation genes of Pseudomonas oleovorans GPo1. This plasmid was introduced into five marine hydrocarbonoclastic bacteria (Marinobacter hydrocarbonoclasticus VT8, Alcanivorax borkumensis SK2, Thalassolituus oleivorans Mil-1, Oleiphilus messinensis ME102 and Cycloclasticus sp. ME7), and into Escherichia coli W3110. The ability of the reporter strains obtained to detect short chain alkanes in seawater was analysed. The marine biosensors based on Marinobacter, Alcanivorax and Thalassolituus showed a good response to short chain alkanes (C₆-C₁₀) in seawater after one hour of incubation. The biosensor based on E.coli showed a good response only if the alkanes were present in water samples of moderate ionic strength, but no response was observed in seawater samples. Moreover, the biosensors based in the hydrocarbonoclastic bacteria were about four times more sensitive than the E.coli biosensor. Finally the marine bioreporter strains were able to sense short chain alkanes present in complex mixtures of hydrocarbons such as gasoline or crude oil in seawater. Among the marine biosensors, Alcanivorax provided the best response to pure short chain alkanes and to complex mixtures, showing the lowest background fluorescence and a good sensitivity in the response.

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Salinity is an increasing serious environmental stress that affects plant survival and growth. Salt stress causes water deficit, ionic unbalance, nutritional problems and oxidative stress. Salinity tolerance implies a wide range of physiological, biochemical and molecular changes, and roots are the first organ affected, and the main responsible for response to the stress. Salt tolerance of fruit rootstocks is crucial in the adaptation of orchards to ample range of environments. In order to attempt in the future an early selection of salt-tolerant Prunus rootstocks, we aimed to identify proteins differentially expressed in response to salinity.

Isolated Prunus cerasus root apexes (10 mm) were cultured under aseptic conditions in Murashige and Skoog (1962) media with 0 and 60 mM NaCl, during 15 days, and proteomic analysis was performed in the resulting protein extracts.

2D-electrophoresis showed that salt stress causes increases and decreases in relative abundance in 13 spots, and they were identified by MALDITOF. Several isoforms of cytoplasmic fructose-bisphosphate aldolase and D-3-phosphoglycerate dehydrogenase, mitochondrial formate dehydrogenase and mandelonitrile lyase were significantly increased in salt stressed roots. Other changes affect to ferredoxin-NADP reductase, heat shock 70 kDa protein, V-type proton ATPase subunit B1 and actin. These results give clues to better understand the physiological responses of adaptation to saline conditions, and advance the search for a molecular marker for salt tolerance.
P22. Mtch1, a mitochondrial proapoptotic protein, is upregulated in response to DNA damage and transactivated by Egr-1

José Alberto Carrodeguas\textsuperscript{1,2}, María Alejandra Nelo-Bazán\textsuperscript{1,2}, Pedro Latorre\textsuperscript{1,3}, Alfonso Bolado-Carrancio\textsuperscript{4}, Flor M. Pérez-Campo\textsuperscript{4}, Pablo Echenique-Robba\textsuperscript{1,5,6}, José Carlos Rodríguez-Rey\textsuperscript{4}

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The function of mitochondrial carrier homolog 1 protein (Mtch1)/presenilin 1-associated protein (PSAP) is still starting to be unveiled. This is a mitochondrial outer membrane protein that interacts with presenilin 1 (involved in Alzheimer's disease), with homology to inner mitochondrial membrane carriers and with a yet to be understood role in apoptotic cell death. We will present our latest results demonstrating that Mtch1 expression is controlled by early response 1 (Egr-1) transcription factor and that Mtch1 is upregulated in response to DNA damage caused by doxorubicin or by ultraviolet light. These results suggest an important role for Mtch1 in the cell DNA damage response.
P23. Deciphering the non-equivalence of serine and threonine O-glycosilation points in biological systems.

Jorge Castro-López, Matilde de las Rivas, Erandi Lira-Navarrete, Jessika Valero-González Ramón Hurtado-Guerrero

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The Tn antigen is one of the most specific human tumor-associated structures, generally presented in cancer cells as a part of modified glycoproteins, such as mucins. In general, the Tn antigen is referred to as N-acetylgalactosamine (GalNAc) α-O-linked to Ser or Thr, without specifying which of the two amino acids the GalNAc is linked to. However, computational models had already shown the existence of different conformational behaviours in solution of the basic Ser- and Thr-containing structures [1] that could have important biological implications. Here, we present three different biological systems by which we have been able to decipher the non-equivalence of serine and threonine O-glycosilation points:

(i) A model anti-MUC1 antibody (SM3), where MUC1 is a heavily O-glycosylated membrane glycoprotein which is overexpressed and partially glycosylated in cancer cells [2].

(ii) Two different plant lectins (SBA, VVA), selected as biological targets because they specifically recognise the GalNAc carbohydrate moiety [3].

(iii) The GalNAcT2, one member of the GalNAc-transferases enzyme family, that, in humans, is responsible of the generation of the Tn antigen by the addition of an α-O-GalNAc group to a Ser/Thr residue.

References


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