# Biophysics Party

An one-day meeting on biophysics with a sunset party

July 6th 2018 ITQB-NOVA

# Abstract book

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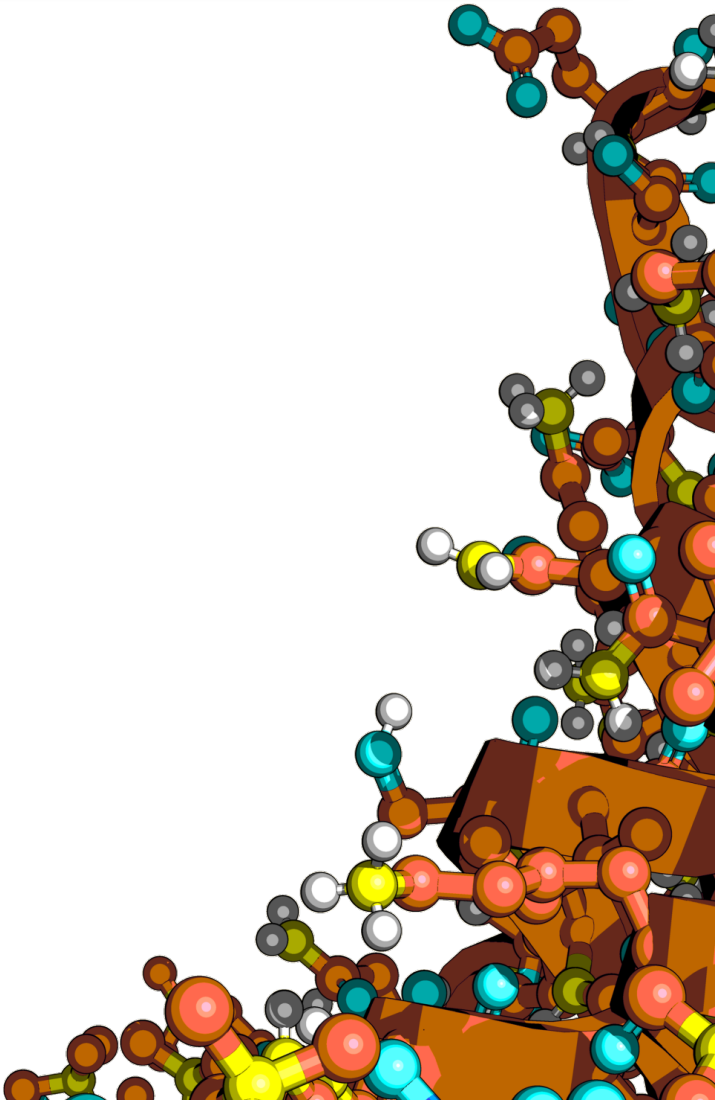
# Welcome Message



Dear participants,

On behalf of the Portuguese Young Biophysicists (PYBphy), we would like to take this opportunity to sincerely appreciate your participation and contribution to the first Meeting of the PYBphy group, the *1st Biophysics Party*, 6th July 2018, Oeiras, Portugal.

First of all, we would like to introduce the PYBphy group to all of you, recently founded (2017) and inserted in the activities of the Portuguese Biophysics Society (Sociedade Portuguesa de Biofísica, SPBf). This group comprises graduate, master or *Ph.D.* students, and early Post-Docs (6 years from their *Ph.D.* graduation) members of SPBf, with the aim of promoting events that could be useful for their career or professional profile. Our main objective is simple: **networking**. We consider that is through this basis that scientific and personal ties can be made.

To celebrate and divulgate the creation of the PYBphy group, we choose a one-day conference, that besides the opportunity to present the current work (through oral, flash or poster communications), it has also time and opportunities for people to interact. A sunset party seemed to be the perfect scenario for that to happen.

During the day, modern *Biophysics* and the new challenges that the field is facing nowadays will be the main focus of discussion, mostly due to the multidisciplinary of techniques and methods of our area of work. Prof. Manuel Prieto, experienced biophysicist, linked to the SPBf roots and very active in science outreach activities, will for sure give us his perspective of what else is need it to communicate with society. On the contrary, Marta Marques, winner of the 2018 young biophysicist award of SPBf, will explain us how *Biophysics* can be useful in the current science picture. Finally, Prof. Alexandre Quintanilha, scientist and member of Portuguese Parliament, will clarify why interdisciplinarity is so important in 21st century.

It will be a day full of science, but also full of fun, two words that need to be constantly together.

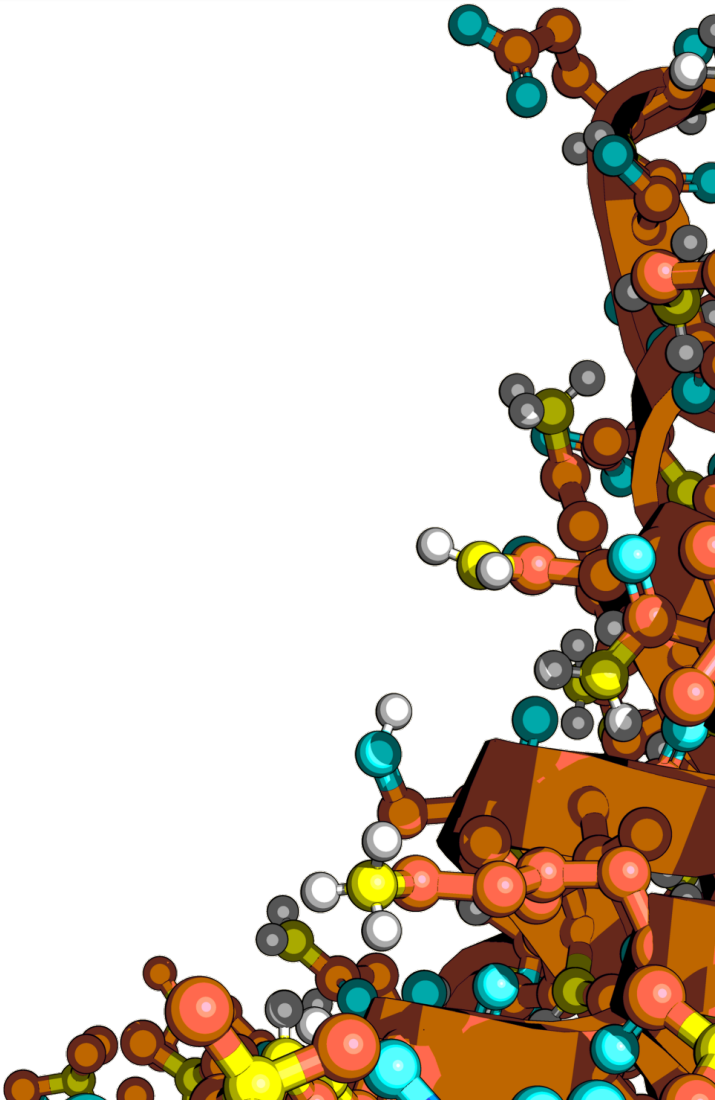
Sincerely, thank you all for this opportunity.

Oeiras, 6th July 2018,

Organizing committee of *1st Biophysics Party*

*Portuguese Young Biophysicists (PYBphy)*

# List of Communications



## Oral communications

**OC1 –** Carmen Montoya

**OC2 –** Filipa Trovão

**OC3 –** Francisco Leisico

**OC4 –** Márcia Alves

## Flash communications

**FC1 –** Catarina Pereira-Leite

**FC2 –** Ana Melo

**FC3 –** Luís Borges-Araújo

**FC4 –** Marino Santos

**FC5 –** Bruno Carneiro

**FC6 –** Bárbara Sousa

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**FC8 –** Joana Gonçalves

**FC9 –** Rui Loureiro

**FC10 –** Cláudia Godinho

## Poster communications

**PC1 –** Lucinda Bessa

**PC2 –** Luca Bighinati

**PC3 –** Patrícia Carvalho

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**PC7 –** Sara Félix

**PC8 –** Giulia Fellegara

**PC9 –** Tomás Fernandes

**PC10 –** Hugo Filipe

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**PC12 –** Catarina Lopes

**PC13 –** Giulio Marchello

**PC14 –** Marta Marques

**PC15 –** Ana Martins

**PC16 –** Andreia Mòsca

**PC17 –** Bárbara Mota

**PC18 –** Ana Paiva

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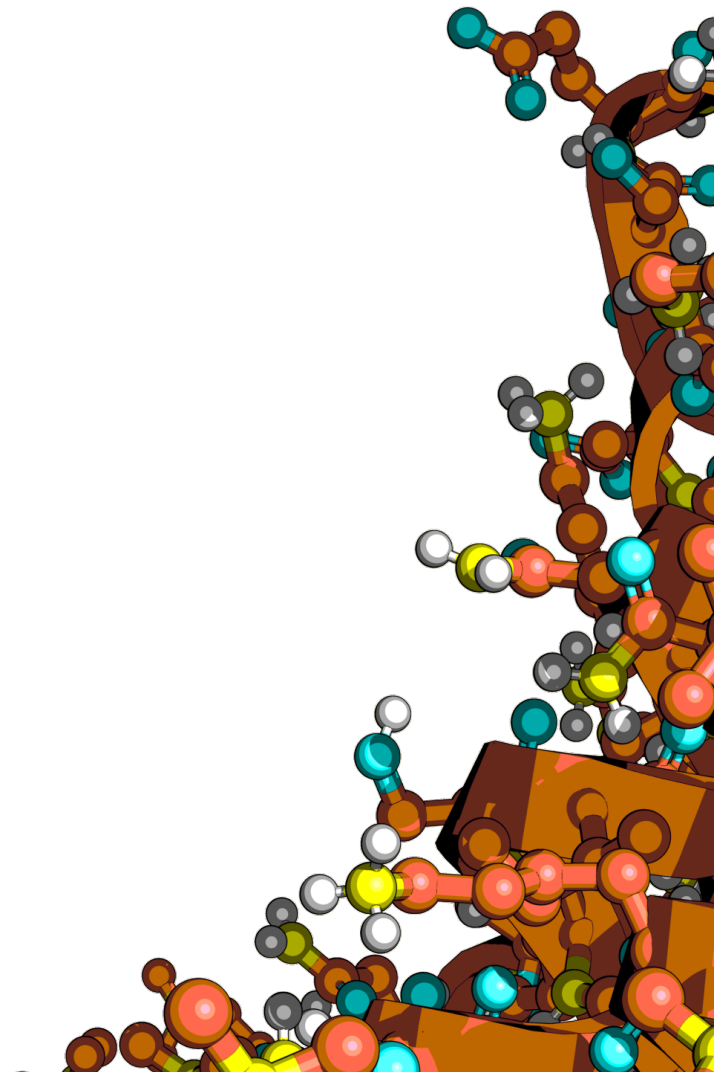
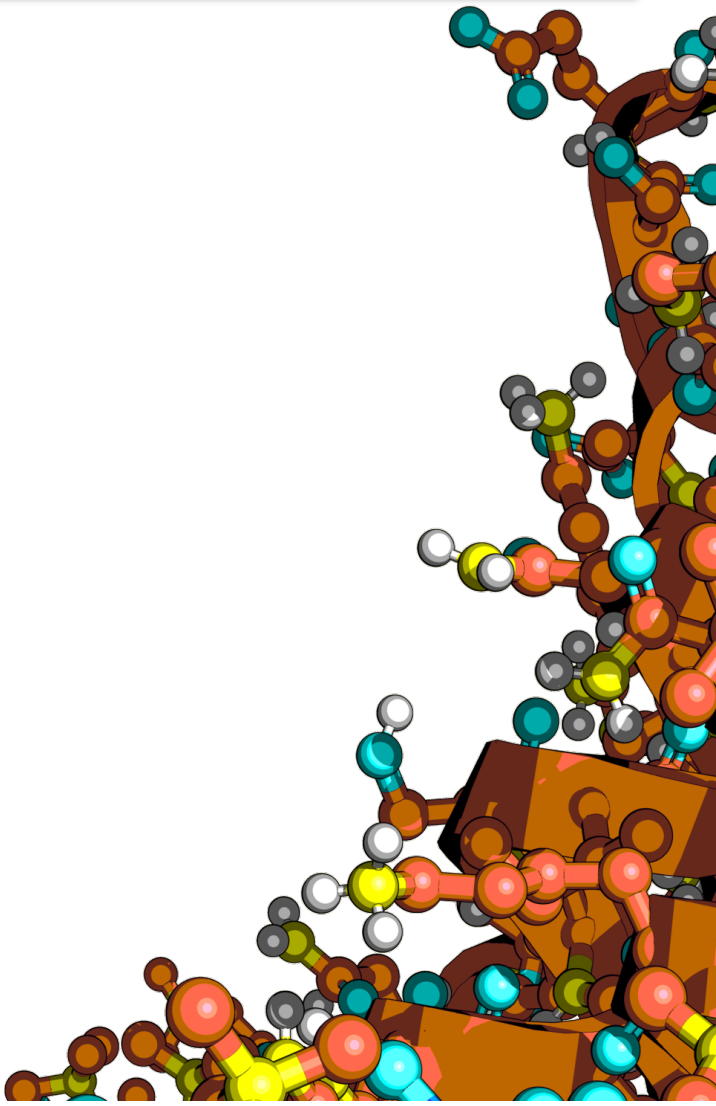


# Programme



**9:45 Registration**

**10:15 Opening session**

**10:30 Opening Lecture**
*To define*
**Manuel Prieto – IST**

**Coffee Break and Poster session**

**11:15 Oral Talk – OC1:**
*Complete study of Protein GB1 encapsulated in a Reverse micelle system by Nuclear Magnetic Resonance*
**Carmen Montoya, FCT – UNL**

**11:30 Flash Talks (FC1 – FC5):**
*Putting membrane lipids on the radar of drug development: the case of NO-indomethacin*
**Catarina Pereira-Leite, LAQV-REQUIMTE, FFUP**

*Untangling Tau: Exploring the Conformational Plasticity of Tau-Tubulin Complex by Single-Molecule FRET*
**Ana M. Melo, CQFM-IN and IBB, IST**

*Impact of Ca2+ -dependent PI(4,5)P2 clustering on the properties of PI(4,5)P2 binding proteins*
**Luís Borges Araújo, IST**

*Structural and biophysical characterization of protein-vanadium interactions by X-ray crystallography and SAXS*
**Marino Santos, UCIBIO-REQUIMTE, FCT – UNL**

*Functional and structural characterization of MsmK*
**Bruno Carneiro, UCIBIO-REQUIMTE, FCT - UNL**

**12:00 Oral Talk – OC2:**
*Structural evidence for the loss of DNA binding function of a relevant mutant of Human p53*
**Filipa Trovão, UCIBIO-REQUIMTE, FCT - UNL**

**LUNCH and Poster session**

**14:00 Lecture**
*The direct role of selenocysteine in [NiFeSe] hydrogenase maturation and catalysis*
**Marta C. Marques – iMM-JLA, FMUL**
**Winner of the 2018 Young Biophysicist Award**

**14:30 Oral Talk – OC3:**
*Multitask ATPases in bacteria – msmX from Bacillus subtilis as a case study*
**Francisco Leisico, UCIBIO-REQUIMTE, FCT – UNL**

**14:45 Flash Talks (FC6 – FC10):**
*Biochemical and Biophysical characterization of human BMX, a therapeutic target for prostate cancer*
**Barbara Sousa, ITQB, UNL**

*Molecular and Structural details of a diagnostic tool for Chronic Myeloid Leukemia*
**Teresa Santos Silva, UCIBIO-REQUIMTE, FCT – UNL**

*Structural and biophysical characterization of PtpA-inhibitors complexes: a possible target against tuberculosis*
**Joana Gonçalves, UCIBIO-REQUIMTE, FCT – UNL**

*The importance of unstructured termini in the aggregation cascade of beta-2-microglobulin: insights from molecular simulations of D76N mutant*
**Rui João Loureiro, BioISI, FCUL**

*Pdr18 is involved in yeast response to acetic acid stress counteracting the decrease of plasma membrane ergosterol content and order*
**Cláudia P. Godinho, IBB, IST**

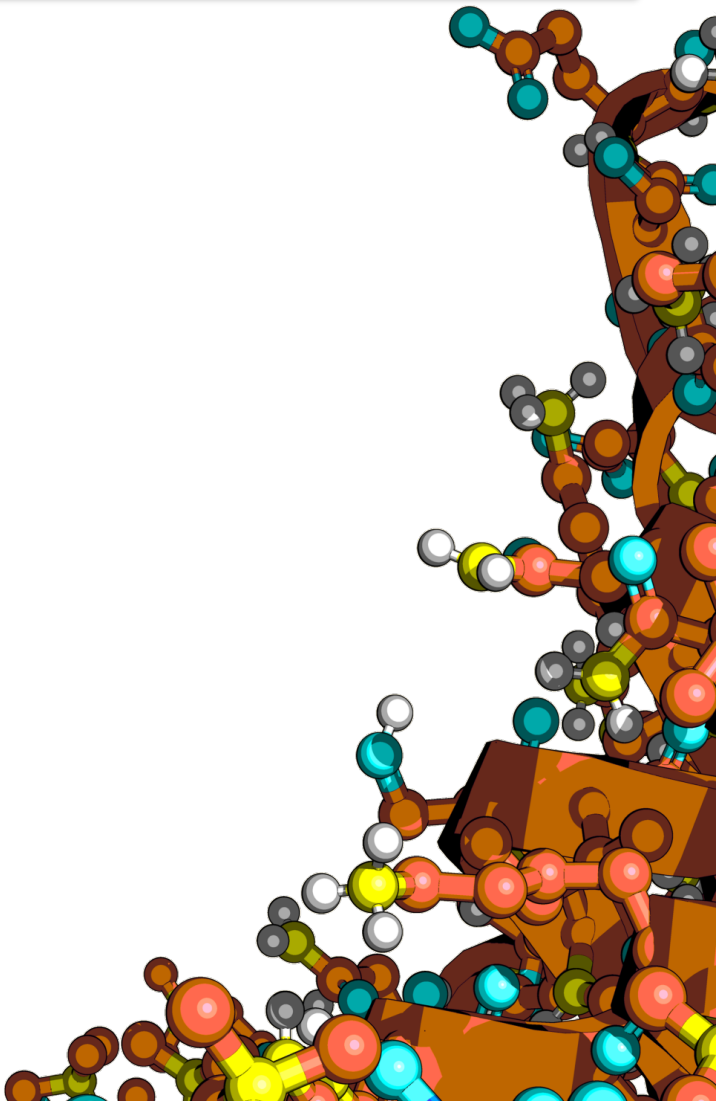
**15:15 Oral Talk – OC4:**
*Encapsulation of Two Model Proteins by Fluorinated Ionic Liquids: Their potential as Drug Delivery System*
**Márcia Alves, ITQB, UNL**

**Coffee Break and Poster Session**

**16:30 Closing Lecture**
*A importância da Interdisciplinaridade*
**Alexandre Quintanilha – i3S - UP, AR**

**PARTY**

# Invited Speakers



## Prof. Manuel Prieto

Manuel Prieto is Full Professor of IST, Universidade de Lisboa, and his PhD was obtained at the same institution (1981), on research on fluorescence, colloidal systems, and spectroscopy.

Later he centered his work on biological systems, and his present research interests are deeply focused on Membrane Biophysics specifically lipid domains (rafts), lipid-protein interaction, amyloid fiber formation, lipid phase diagrams, oxidized lipids and sphingolipids.

For the above studies, the group has a high expertise and attained international recognition on time-resolved fluorescence methodologies, and advanced microscopy approaches, such as FCS, FLIM, FAIM, FLIM/FRET.

Manuel Prieto was a founder element of the Portuguese Biophysical Society and has collaborated intensively with international organizations. He is the former President of EBSA (European Biophysical Societies' Association) and is the President-Elect of IUPAB (International Union of Pure and Applied Biophysics). He is also the recipient of several national and international awards, and is very active on Science outreach activities, both in Portugal and Latin-America.

Manuel Prieto research units are: CQFM-IN and iBB Institute for Bioengineering and Biosciences, at IST, ULisboa.

## Dr.ª Marta Marques

Marta C. Marques obtained her Ph.D. in Biochemistry from New University of Lisbon in 2014, working at Instituto de Tecnologia Química e Biológica (ITQB), under the joint supervision of Dr. Pedro Matias and Dr. Inês Cardoso Pereira. Her research focused on the functional, structural and spectroscopic characterization of the highly active bacterial enzymes. International collaborations included CSIC in Madrid, Spain and CNRS in Marseille, France. In 2015, she started a post-doctoral (PostDoc) research fellowship at Instituto de Medicina Molecular (iMM), Faculdade de Medicina, Universidade de Lisboa. The main objective of the work was the development of innovative potent membrane-targeted peptide fusion inhibitor applied to antiviral therapies. Since November 2016, she works as PostDoc at Dr. Gonçalo Bernardes Lab, also at iMM.

In 2018, she won the 2018 Young Biophysicist Award with her paper entitled in “The direct role of selenocysteine in [NiFeSe] hydrogenase maturation and catalysis” (Nature Chemical Biology, 2017, doi:10.1038/nchembio.2335). This work unrevealed the essential role played by a selenocysteine amino acid at the active site, which gives some enzymes a very high activity and tolerance to oxidative damage. It also showed that, unexpectedly, this amino acid residue is essential for the incorporation of the Ni atom into the active site.

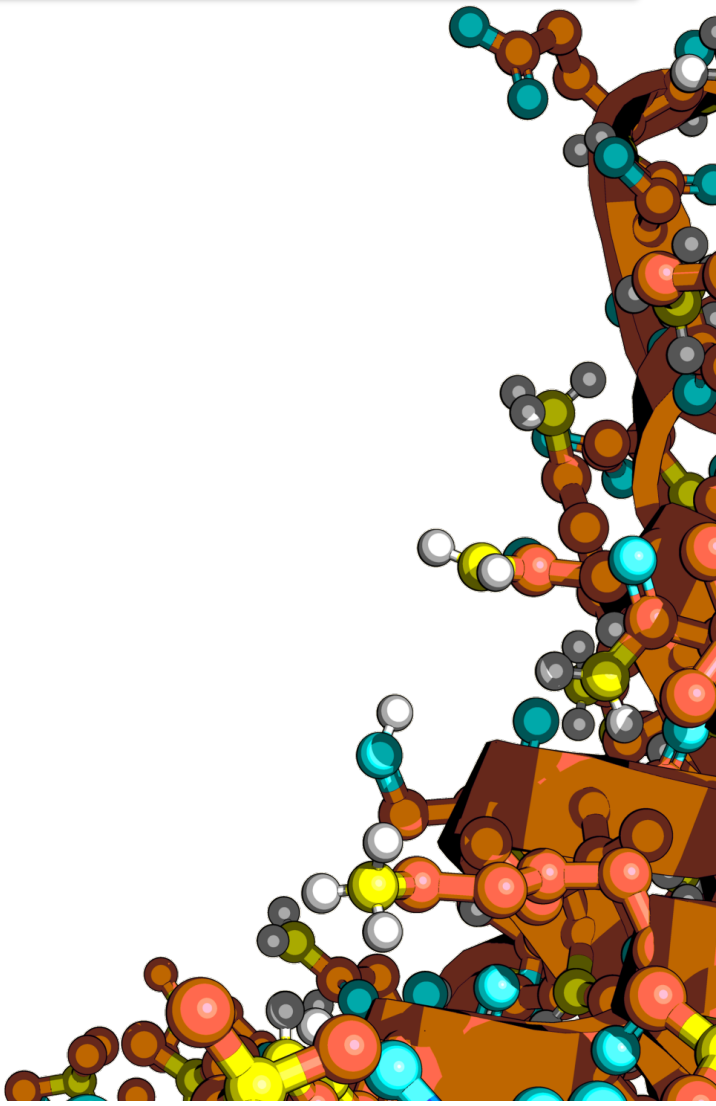
## Prof. Alexandre Quintanilha

Alexandre Quintanilha graduated in Physics and obtained his PhD in solid state Physics, at the University of the Witwatersrand, in Johannesburg, South Africa.

In 1972, he started working in University of California at Berkeley and the Lawrence Berkeley national Laboratory, where he oriented is research toward the Life Sciences. At this University, Alexandre Quintanilha was Professor of Physiology and Biophysics in the Molecular & Cell Biology Department, Assistant Director of the Energy and Environment Division and Director of a Center for Environmental Studies. In 1991, Alexandre Quintanilha started working as a Professor at ICBAS, University of Porto. In Portugal, he was director of the Instituto de Biologia Molecular e Celular (IBMC) and President of the Instituto de Engenharia Biomédica (INEB).

Alexandre Quintanilha is currently a researcher at the Instituto de Investigação e Inovação em Saúde (i3S) and a Member of the Portuguese Parliament, He is currently the President of the parliamentary committee for Education and Science. His areas of scientific interest include physiological stress in organisms, cellular and molecular targets, defense mechanisms, adaptation and regulation. With six books and over one hundred scientific articles published over the years, Alexandre Quintanilha gave a great contribution for the Biophysics field.

# Oral Communications



# OC1

## Complete study of protein GB1 encapsulated in a reverse micelle system by nuclear magnetic resonance

Carmen Montoya[1,2], Teresa Casimiro[2], Marta Corvo[3] and Eurico J. Cabrita[1]

[1]UCIBIO, REQUIMTE, DQ; [2]LAQV, REQUIMTE, DQ; [3]CENIMAT, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

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Protein encapsulation into reverse micelles water cores tumbling in a low viscous solvent has emerged as a powerful tool in NMR for the determination of biologically relevant protein structures [1]. Water cores inside reverse micelles provide a protective environment for proteins. In this non-bulk water environment, intrinsic to biologic systems, the dynamics of water are distinctly different from those of water in pure liquid [2], allowing to study the interaction of biological macromolecules with water, which are fundamental to understand their structure, dynamics and function [3].

In this work, the globular domain B1 of the protein G (GB1) was successfully encapsulated in a reverse micelle formed by the anionic surfactant AOT, water, and the low viscosity solvent isooctane. A completed structural and dynamic study of the system was performed by NMR. Comprehensive structural and dynamic information of the protein encapsulated was obtained for different sizes of reverse micelle nucleus cores. Distinct types of interactions between non-bulk water molecules inside the cores and the surface of the protein were characterized and the clustering of different hydration-dynamics over the protein structure was mapped.

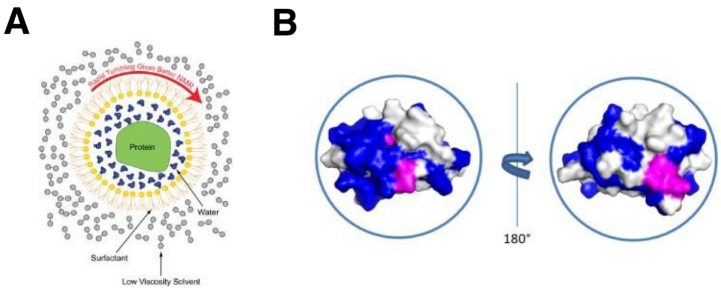


Figure 1. Protein encapsulated in a reverse micelle tumbling in a low viscosity solvent (A) [3]. Mapping of different water-protein interactions inside an AOT revese micelle water core (B).

### References

1. Charles R. Babu, Peter F. Flynn, and A. Joshua Wand, JACS 2001, 123, 2691-2692
2. Michael D. Fayer.Physiolog 2011, 26, 6, 381-392
3. Nathaniel V Nucci, Maxim Pometun, A Joshua Wand, Nature Structure and Molecular Biology, 2011, 18, 2

# OC2

## Structural evidence for the loss of DNA binding function of a relevant mutant of human p53

Filipa Trovão[1], Ana S. Gomes[2], Benedita A. Pinheiro[1], Sara Gomes[2], Carla Oliveira[3], Lucília Domingues[3], Maria J. Romão[1], Lucília Saraiva[2] and Ana L. Carvalho[1]

[1]UCIBIO-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

[2]LAQV-REQUIMTE, Faculdade de Farmácia, Universidade do Porto, 4050-313 Porto, Portugal

[3]CEB-Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal

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The p53 tumor suppressor protein regulates cell proliferation, DNA repair, differentiation and death. This protein is widely found mutated in human cancer. The loss of p53 transcriptional activity may lead to tumor development and maintenance [1,2]. For these reasons, p53 is seen as a promising target for therapeutic strategies to halt cancer. The first crystal structure of mutant p53 R280K DNA binding domain (DBD) has been determined in the absence of DNA, with a resolution of 2.0 Å. The final model was refined to a final R factor of 19.4% (Rfree = 23.7%) and contains four molecules of p53 R280K DBD in the asymmetric unit, four zinc ions and 339 water molecules. This structure was compared with the wild-type p53 core domain structures, alone and in complex with DNA. These comparisons contributed to a deeper understanding of mutant p53R280K structure. The increased distance and weaker binding of lysine 280 (mutation site) to DNA disables the formation of stabilizing interactions with DNA, explaining p53 R280K loss of binding [3]. The structural information resultant from this study may also lead to further rational design of new potential anticancer therapeutic approaches, such as p53 reactivation molecules.

### References

1. Vousden KH, Prives C. Blinded by the Light: The Growing Complexity of p53. Cell. 2009;137(3):413–31.
2. Kim MP, Lozano G. Mutant p53 partners in crime. Cell Death Differ [Internet]. Nature Publishing Group; 2017;1–8.
3. Gomes A, Trovão F, Andrade Pinheiro B, Freire F, Gomes S, Oliveira C, et al. The Crystal Structure of the R280K Mutant of Human p53 Explains the Loss of DNA Binding. Int J Mol Sci [Internet]. 2018;19(4):1184.

# OC3

## Multitask ATPases in bacteria – msmX from Bacillus subtilis as a case study

Francisco Leisico[1], Bruno Carneiro[1,2], Maria João Romão[1], Isabel Sá-Nogueira[2] and Teresa Santos-Silva[1]

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ABC transporters are one of the most diverse superfamily in nature and it is widespread in all domains of life, being responsible for the primary transport of molecules against a chemical gradient through ATP hydrolysis. They are composed of different proteins specifically related to the substrate they transport, including the cytoplasmic ATPase domain. However, some bacteria show a different approach, like *Bacillus subtilis* that uses MsmX protein as a multitask ATPase to interact with several transporters responsible for the uptake of different oligosaccharides[1]. Since, multitask ATPases are involved in carbohydrate uptake that mediates bacteria colonization and pathogenesis in the hosts[2,3], we aim to characterize MsmX protein structural and functionally. In this work, we solved the first crystal structure of MsmX at 1.9Å. Potential structural determinants for the promiscuity of this protein are being functionally tested *in vivo* through mutagenesis approaches and correspondent mutants produced *in vitro* for further biophysical characterization. The structural, biophysical and *in vivo* insights into MsmX multitask ability derived from this multidisciplinary project (Fig. 1) will integrate future structure-based drug design efforts to develop new drugs to fight bacterial infections, since *B. subtilis* is a well-known model to study Gram-positive pathogenic bacteria like *Streptococcus*, *Staphylococcus* and *Clostridium*.

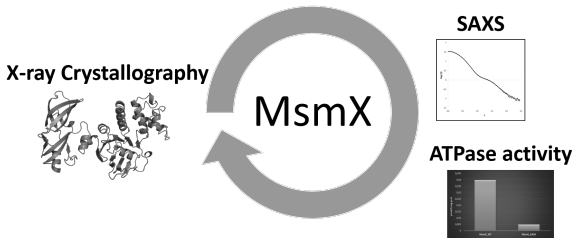


Figure 1. An integrative approach combining X-ray crystallography, SAXS experiments and biophysical/functional studies was used to identify the molecular determinants involved in MsmX multitask ability.

### References

1. M. J. Ferreira, I. de Sá-Nogueira, 2010. *J Bacteriol*, 192(20), 5312-8.
2. C. Marion *et al*, 2011. *Infect Immun*, 79(10), 4193-200.
3. M. F. Tan *et al*, 2015. *PLoS One*, 10(7), e0130792.

# OC4

## Encapsulation of two model proteins by fluorinated ionic liquids: their potential as drug delivery system

Márcia Alves[1,3], N.S.M. Vieira[1,3], H.D.T. Mertens[2], D.I. Svergun[2], L.P.N. Rebelo[1], J.M.M. Araújo[3], A.B. Pereiro[3] and M. Archer[1]

[1]ITQB NOVA, Oeiras, Portugal; [2]EMBL Hamburg Outstation, Hamburg, Germany; [3]FCT NOVA, Caparica, Portugal

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A diversity of proteins, e.g. insulin, coagulation factors, interferons and antibodies are currently available for pharmaceutical use to treat a wide range of diseases, such as diabetes, hemophilia, multiple sclerosis and cancers. However, problems such as dosage, protein instability and degradation still need to be fully addressed. The development of biocompatible drug delivery systems (DDS) capable of overcoming these issues is very promising and can improve not only the effectivity of the therapy but patient compliance as well. Ionic liquids (ILs) are salts/fluids composed of ions which present low melting points. The possibility of tuning IL ions to reach the desired properties and interactions represents a major advantage compared to surfactants and other artificial membrane-mimetic solvents. Ionic liquids spontaneously self-assembly above critical aggregation concentration (CAC) [1]. Our aim is to investigate the potential use of fluorinated ionic liquids (FILs) as drug delivery systems for therapeutic proteins. This work evaluates the effect of FILs on the stability, function, structure and aggregation state of hen egg white lysozyme (HEWL) [2] and bovine serum albumin (BSA). Different techniques were used for this purpose, such as differential scanning fluorimetry (DSF), spectrophotometric assays, circular dichroism (CD), dynamic light scattering (DLS), isothermal titration calorimetry (ITC) and small angle x-ray scattering (SAXS).

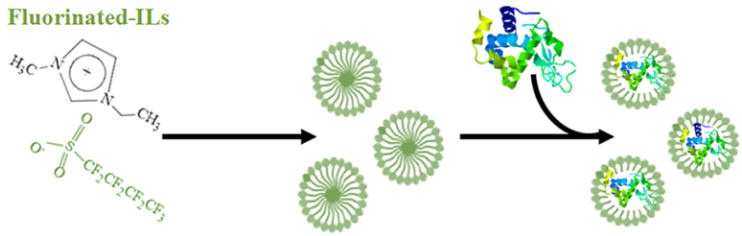
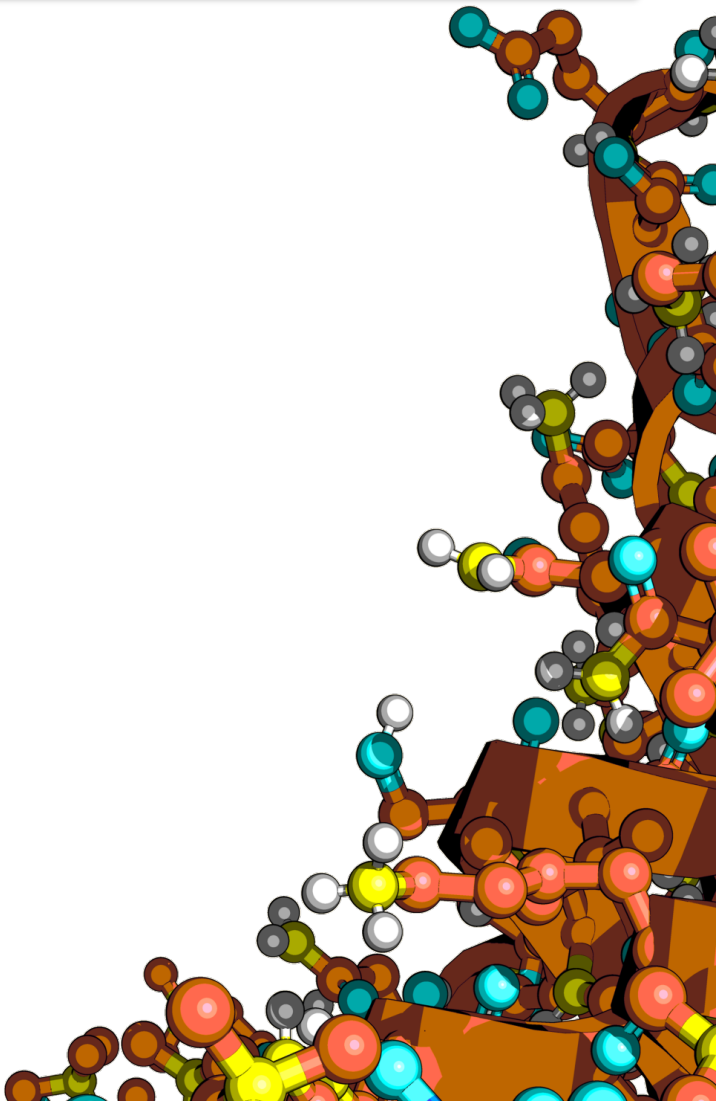



Figure 1. Mechanism of encapsulation of proteins by the FIL

### References

1. Pereiro, A. B., et al., 2015. Aggregation Behavior and Total Miscibility of Fluorinated Ionic Liquids in Water. Langmuir 31, 1283-1295.
2. Alves, M,, Vieira, N.S.M., Rebelo, L.P.N., Araújo, J.M.M., Pereiro, A.B., Archer, M., 2017. Fluorinated ionic liquids for protein drug delivery systems: investigating their impact on the structure and function of lysozyme. Int J Pharm 526, 309-320.

# Flash Communications



# FC1

## Putting membrane lipids on the radar of drug development: the case of NO-indomethacin

Catarina Pereira-Leite[1,2], Cláudia Nunes[1], José C. Bozelli Jr.[2,3], Shirley Schreier[2], Christina S. Kamma-Lorger[4], Iolanda M. Cuccovia[2] and Salette Reis[1]

[1]LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal

[2]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil

[3]Department of Biochemistry and Biomedical Sciences, McMaster University, Canada

[4]ALBA Synchrotron, Consorcio para la Construcción, Equipamiento y Explotación del Laboratorio de Luz de Sincrotrón (CELLS), Cerdanyola del Vallès, Barcelona, Spain

*Corresponding author: catarinapl12@gmail.com*

Nitric oxide (NO)-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) have been developed to overcome the toxicity of NSAIDs [1]. Since evidences support that NSAIDs toxicity is related to their topical actions in membrane lipids [2], this work aims at evaluating the impact of adding a NO-releasing moiety to parent NSAIDs regarding their effect on lipid bilayers. Thus, the interactions of NO-indomethacin and indomethacin with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayers were described. Diverse experimental techniques were combined to characterize the partitioning and location of drugs in DMPC bilayers, and to analyze their effects on the lipid phase transition and the bilayer structure. Both drugs inserted the DMPC bilayer and modified their biophysical properties. Drug-membrane interactions were dependent on drug ionization state, drug:lipid molar ratio, temperature and lipid phase. It is noteworthy that NO-indomethacin induced more pronounced alterations in DMPC bilayers than indomethacin. Such modifications may have *in vivo* implications, particularly in the gastric mucosa, where NO-NSAIDs-induced changes in the protective phospholipid layers may contribute to the occurrence of toxicity.

### References

1. Pereira-Leite C, *et al.* Med Res Rev, 2017;37(4):802-59.
2. Pereira-Leite C, *et al.* Prog Lipid Res, 2013;52:571-584.

# FC2

## Untangling Tau: exploring the conformational plasticity of Tau-Tubulin complex by Single-Molecule FRET

Ana M. Melo[1,2], Juliana Coraor[3], Garrett Cobb[3], Shana Elbaum-Garfinkle[3] and Elizabeth Rhoades[1]

[1] Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA

[2] Present address: CQFM-IN and IBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

[3] Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, CT, USA

*Corresponding author: anammelo@tecnico.ulisboa.pt*

Tau is a neuronal intrinsically disordered protein (IDP), whose aggregation is linked to several tauopathies, including Alzheimer's disease. Additionally, the loss of its native interaction with microtubules is thought to contribute for pathology. Despite intense study, structural details of the tau-tubulin complex are lacking, in part due to its highly dynamic nature and the capacity to promote tubulin polymerization. Here, we use intramolecular single-molecule Förster Resonance Energy Transfer (smFRET) to determine topological features of tau bound to soluble tubulin heterodimers. Tau adopts an overall extended conformation upon tubulin binding, in which the long-range of contacts between both termini and the microtubule binding region (MTBR) that characterize its compact solution structure are diminished. Surprisingly, the individual repeats within MTBR that directly interface with tubulin undergo an expansion in order to accommodate tubulin binding without changing the overall dimensions. Notably, it suggests the formation of such a "fuzzy complex", in which tau displays significant flexibility to allow for local changes in conformation while preserving global features. Moreover, our results contrast differences in tau isoforms and a conformational ensemble of tubulin-bound state distinct from its aggregation- prone structure. This work draws attention to the importance of the role of tau's conformational plasticity in function.

# FC3

## Impact of $Ca^{2+}$-dependent $PI(4,5)P_2$ clustering on the properties of $PI(4,5)P_2$ binding proteins

L. Borges-Araújo[1], M.E. Monteiro[1], D. Mil-Homens[2], N. Bernardes[2], M.J. Sarmento[1], A. Coutinho[1,3], M. Prieto[1] and F. Fernandes[1,4]

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Phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) is a key modulator of eukaryotic plasma membrane associated signalling events. Studies on the impact of physiological divalent cation concentrations on $PI(4,5)P_2$ clustering, suggest that protein anchoring to the membrane through $PI(4,5)P_2$, is not defined solely by a simple (monomeric $PI(4,5)P_2$)/(protein bound $PI(4,5)P_2$) equilibrium, but instead involve interactions with $PI(4,5)P_2$ clusters. Nevertheless, the impact of the complex $PI(4,5)P_2$ organization on its interactions with binding proteins is largely unknown.

Using advanced spectroscopic methodologies (FRET, FCS and PCH), we characterized the impact of calcium on the dynamics of pleckstrin homology (PH) domains tagged with a fluorescent protein. We show that in Giant Unilamellar Vesicles (GUVs) presenting $PI(4,5)P_2$, the membrane diffusion properties of PH-FP are affected by the presence of $Ca^{2+}$, suggesting interaction of the protein with $PI(4,5)P_2$ clusters. Importantly, PH-FP is found to dimerize in the membrane in the absence of $Ca^{2+}$ and this oligomerization is inhibited in the presence of physiological concentrations of the cation. Furthermore, $Ca^{2+}$ induced clustering of $PI(4,5)P_2$ enhanced protein sequestration of the phosphoinositide, depleting the levels of free $PI(4,5)P_2$. These results confirm that $Ca^{2+}$-dependent $PI(4,5)P_2$ clustering has the potential to influence affinity, oligomerization and organization of PI(4,5)P2 binding proteins in the plasma membrane.

**FC4**

# Structural and biophysical characterization of protein-vanadium interactions by X-ray crystallography and SAXS

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Vanadium (V) plays different biological functions and its therapeutic potential (anti-diabetic and anti-cancer) has been suggested [1, 2, 3]. Herein, we present a structural and biophysical study focused on the interactions of inorganic ($V^{IV}OSO_4$ and $NaV^{V}O_3$) and organic ($V^{IV}O(acac)_2$) vanadium compounds with proteins: Human Serum Transferrin (HTF) and Hen Egg White Lysozyme (HEWL) [4, 5]. HTF is a metal ion blood carrier and Small Angle X-ray Scattering (SAXS) was firstly used to confirm the protein-ligand binding. Different datasets – native apoHTF, apoHTF-$V^{IV}O(acac)_2$, apoHTF-$V^{IV}OSO_4$ and apoHTF-$NaV^{V}O_3$ – were collected at beamline BM29 (ESRF) suggesting a partial conformational change of HTF upon vanadium binding. Other experimental and theoretical methodologies – EPR, Circular Dichroism, MALDI-TOF, electrochemical methods and DFT calculations – have been also used confirming such protein-ligand interactions. The three compounds were used in soaking experiments and a 1.34Å resolution HEWL-$V^{IV}OSO_4$ structure was obtained at beamline BM30A (ESRF). Metal adducts were found next to Asp52 (Figure 1), Asp87 and Leu129 revealing different occupancies and geometries. The $V^{IV}$=O bond distances confirm the metal oxidation state. In conclusion, the studied vanadium-based compounds are able to interact with proteins. The binding to plasma proteins modulates their distribution in the organism and an accurate elucidation of the process is critical for a successful application of V-compounds as therapeutic agents.

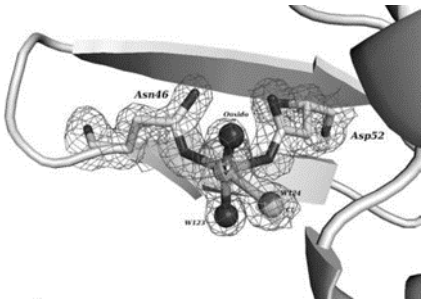


Figure 1. Representaion of the V adduct bound to Asp52.

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# FC5

## Functional and structural characterization of MsmK

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*Streptococcus pneumoniae* is an opportunistic human pathogen and the causal agent of several diseases, namely pneumonia, the deadliest infection in children under 5 years. Sugars are the main carbon source for *S. pneumoniae*, thus ABC importers represent a crucial point in pneumonia progression[1]. MsmK is an energizing multiple ABC importer responsible for carbohydrate uptake (Fig. 1) and the deletion of this protein attenuates the pathogen virulence[2]. In this work, we combine *in vivo* and *in vitro* assays to characterize structurally and functionally MsmK. We optimized the overexpression (*E. coli*) and purification (IMAC, SEC) of MsmK obtaining stable and active protein at 30mg/mL. SAXS data was collected at P13 (PETRA III) and the analysis shows that the protein presents a monomeric form *in vitro* with an ATPase typical shape. *In vivo* experiments suggest that MsmK is able to dimerize without its transmembrane domain. Integration of these results will help to understand the enzymatic mechanism of MsmK and its importance in pneumonia development helping to find therapies against this disease.

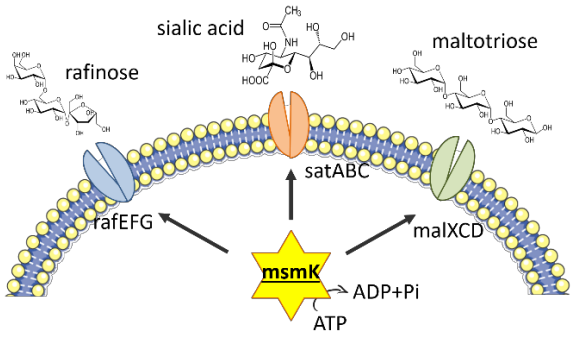


Figure 1. Illustration of an ABC importer energized by MsmK with sugars that are possible importer.

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# FC6

## Biochemical and Biophysical characterization of human BMX*, a therapeutic target for prostate cancer*

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Bone marrow tyrosine kinase in chromosome X (BMX) is a member of the TEC family kinases and has been implicated in tumorogenecity, motility, proliferation and differentiation [1-5]. BMX is highly overexpressed in prostate cancer and it is involved in the adaptive compensatory mechanism of castrate-resistance prostate cancer to androgen deprivation therapy [6]. Besides, it suppresses a core component of the intrinsic apoptotic pathway, granting tumor cells the ability to escape apoptosis induced by chemotherapeutic drugs [7]. BMX knockout mice have a normal life span without any obvious altered phenotype, suggesting that therapies based on BMX inhibition might have limited side effects [8]. We have developed a series of BMX-IN-1 analogues that showed an increased inhibitory capacity when compared to BMX-IN-1 [9]. Since crystallographic information is essential to get a molecular view of BMX ATP binding pocket and how it binds to irreversible inhibitors we developed a baculovirus expression system to produce recombinant human BMX. Here we report the biochemical and biophysical characterization of human BMX (SDS-PAGE, CD, Thermofluor and DLS). Moreover, we set up the crystallization trials to generate crystals for diffraction analysis. The structure of the BMX alone and in complex with our inhibitors will encourage further studies aiming to develop therapeutically active molecules.

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# FC7

## Molecular and structural details of a diagnostic tool for Chronic Myeloid Leukemia

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Chronic myeloid leukemia affects 1-1.5 in 100000 adults per year, representing 15% of all types of leukemia in adults (data from IPO-Lisboa). The aim of this project is to elucidate the molecular and structural details of a FRET-based two-component molecular beacon as a diagnostic tool for Chronic Myeloid Leukemia relying on different biophysical techniques. Using Small-angle X-ray Scattering (SAXS), we characterized the biological components of the biosensor separately: hairpin(A), target sequence(B) and revelator(C)) and also the interaction between them (AB and ABC) – Fig. 1. The Rg, $I_0$ and $D_{max}$ obtained are in agreement with the expected values for this type of biomolecules and the *ab initio* bead-models are consistent with the bioinformatics simulations, and the results obtained using Microscale Thermophoresis, Differential Electrophoretic Mobility assays and Steady state Fluorescence Spectroscopy.

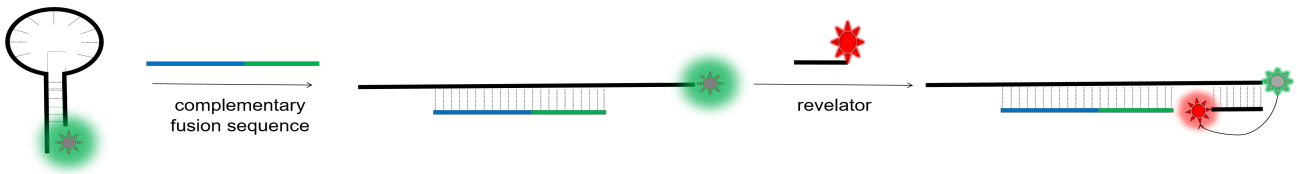


Figure 1. Schematic representation of the recognition principle used in the developed biosensor.

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# FC8

## Structural and biophysical characterization of PtpA-inhibitors complexes: a possible target against tuberculosis

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Mycobacterium tuberculosis (Mtb) is the main agent of tuberculosis [1,2]. The survival of Mtb in its host is directly related to the release of protein tyrosine phosphatases, particularly protein tyrosine phosphatase A (PtpA), since it interferes in macrophage cell signalling (Fig. 1) [3]. The crystal structure of the native protein was solved in 2005 [4].

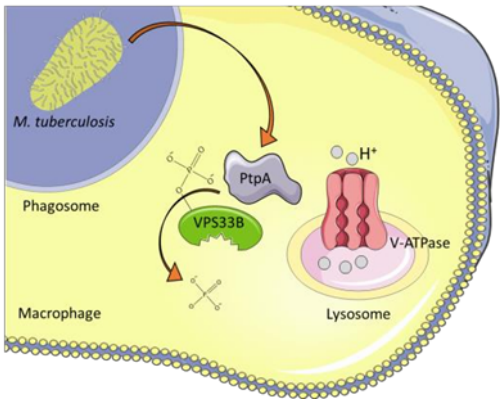


Figure 1. Schematic representation of PtpA action mechanism. PtpA is secreted by Mtb to cytosol macrophage, during infection, where the substrate, protein VPS33B, is located. Substrate desphosphorilation affects phagosome maturation, since it blocks V-ATPase rec.

Chalcones and thiosemicarbazones have been identified as potential competitive inhibitors of PtpA. Preliminary studies revealed that the inhibitor's predominant factor is the molecule planarity/hydrophobicity and the nature of the substituents that establish hydrogen bonds with the residues in the active site of PtpA [3,5].

In this work, the inhibitory properties of six compounds are under study by a combined biophysical and structural approach. PtpA was successfully overexpressed, purified and biophysically characterized by thermal shift assays. Crystallization and soaking trials were conducted and a complete protein-inhibitor dataset was collected at 2.9Å resolution. Structural analysis is still in progress, but preliminary analysis of the electron density map suggests the inhibitor binds to PtpA active site.

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# FC9

## The importance of unstructured termini in the aggregation cascade of beta-2 microglobulin: insights from molecular simulations of D76N mutant

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The identification of folding and aggregation intermediate states is important, both from a fundamental standpoint and for the design of new therapies for conformational disorders. Here, we use the single point mutant (D76N) of β2m, the causing agent of a hereditary systemic amyloidosis affecting visceral organs, as a model system to study the aggregation mechanism of β2m using molecular simulations. We present our predictions on the early molecular events triggering the amyloid cascade for the D76N mutant. Folding simulations highlight the existence of an aggregation-prone intermediate called I1 which presents an unstructured C- terminus and of an aggregation-prone intermediate featuring two unstructured termini called I2. Additionally, Monte Carlo docking simulations suggest that both intermediates have high aggregation-propensity, particularly at acidic pH.

These simulations support an essential role of the DE and EF-loops and the termini in the dimerization of both intermediates. The relevance of the C-terminus is higher at the acidic pH 5.2 while the N-terminus become important at pH 6.2. At physiological pH, the DE and EF-loops are the crucial regions for dimerization. These predictions rationalize experimental results that support the involvement of $Lys^{19}$, $Phe^{56}$, $Trp^{60}$ and $Tyr^{63}$ in amyloidogenesis in the wild-type and other model systems of β2m.

# FC10

## Pdr18 is involved in yeast response to acetic acid stress counteracting the decrease of plasma membrane ergosterol content and order[1]

Cláudia P. Godinho, Catarina S. Prata, Sandra N. Pinto, Carlos Cardoso, Narcisa M. Bandarra, Fábio Fernandes and Isabel Sá-Correia

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The ability of Saccharomyces cerevisiae to overcome the stress induced by a wide range of structurally and functionally unrelated toxic compounds is partially due to the action of plasma membrane transporters from the ATP-binding cassette (ABC) superfamily. Although the role of these transporters in multidrug/multixenobiotic resistance (MDR/MXR) in yeast is traditionally associated to their putative ability to catalyse the active efflux of toxic compounds, this biological activity as efflux pump has been recently questioned.

This study provides new insights into the biological role and impact in yeast response to acetic acid stress of the MDR/MXR determinant Pdr18, proposed herein to mediate ergosterol transport at yeast plasma membrane. Taking advantage from the use of fluorescence microscopy, it was found that Pdr18 expression counteracts the decrease of plasma membrane lipid order, the increase in non-specific permeability, and the decrease in transmembrane electrochemical potential, induced by acetic acid stress. A coordinated activation between PDR18 and several ergosterol biosynthetic pathway genes was registered during acetic acid-adaptation period. Collectively, results presented in this study support the notion that Pdr18-mediated MDR/MXR is closely linked to the status of plasma membrane lipid environment related with ergosterol content and associated plasma membrane properties.

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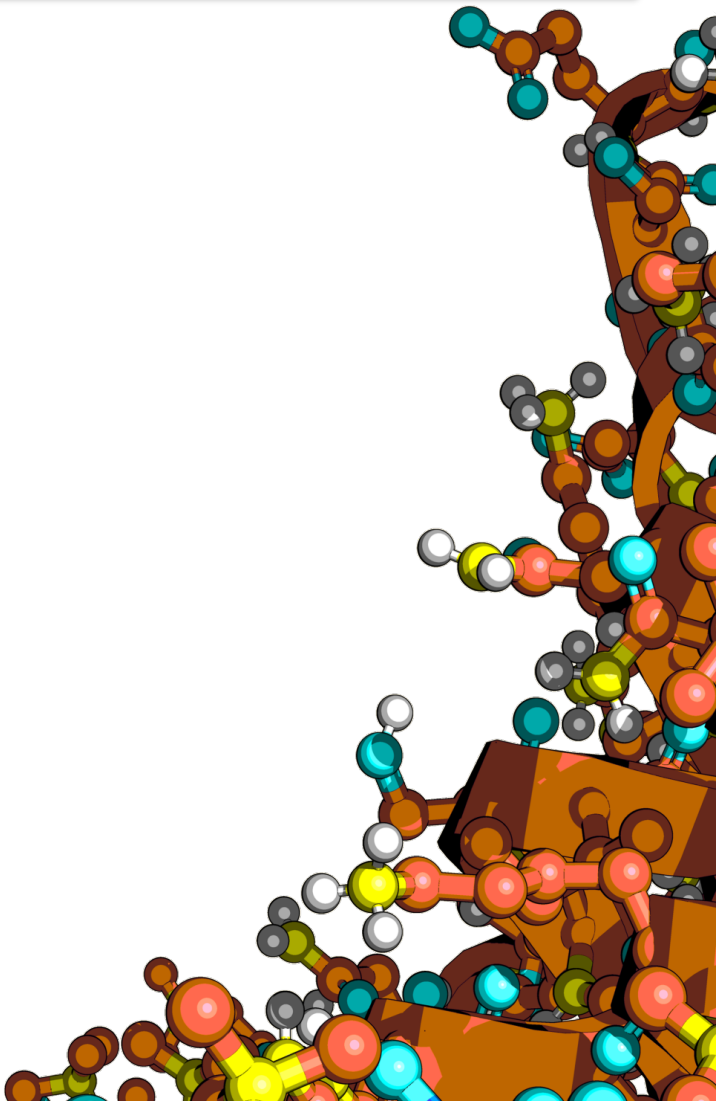
# Poster Communications



# PC1

## Membrane biophysical properties of multidrug-resistant isolates of *Escherichia coli* and *Staphylococcus aureus*

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The ability of most compounds (nutrients and antibiotics) and ions to cross the bacterial cytoplasmic membrane by diffusion and active transport is highly dependent on cytoplasmic membrane fluidity. It was our aim to study possible differences in the membrane fluidity i) of multidrug-resistant (MDR) isolates in comparison to a susceptible strain and ii) in absence and presence of antibiotics (ceftazidime and ciprofloxacin), by measuring biophysical properties of their membranes, such as membrane anisotropy and membrane polarization, which were assessed through the use of fluorescent probes, DPH and Laurdan, respectively. Fluorescence anisotropy of DPH-labelled cells as well as Laurdan Generalized Polarization (GP) measurements were performed at 24-h intervals up to 6 days.

The anisotropy values as well as the Laurdan GP values of all three *E. coli* strains studied were quite similar, suffering an equal similar trend throughout the 6 days. Nonetheless, in the case of *S. aureus*, both anisotropy and Laurdan GP values were higher in MDR isolates when compared to the reference strain, meaning they have a less fluid membrane.

The exposition of MDR isolates of both *E. coli* and *S. aureus* to sub-inhibitory concentrations of both antibiotics tested did not affect the membrane fluidity.

# PC2

## Development of extemporaneous formulation of diazepam: comparison of Avicel RC581 and Vivapur MCG 811P

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Diazepam for pediatric use is a drug to prevent and control seizures and status epilepticus; yet, no commercial formulations are available for them. The substance is insoluble in water so the need to develop an aqueous suspension, without any problematic co-solvents, is required. Three parameters were studied: viscosity, particles size, and stability. Kinexus Lab+ Malvern was used to study the viscous behavior, Mastersizer 3000 Malvern for the particles' dimensions and UltiMate3000 HPLC for drug's degradation. Three batches of extemporaneous formulations were prepared with diazepam's tablets and the polymers Avicel RC581 at 0.45% and Vivapur MCG 811P at 1.2%, storing at 25ºC and 5ºC. Shear rate ramp test and Herschel-Bulkley theoretical model shown for Avicel 0.45% a K factor of 0.062 ± 0.004 with 9000 rpm while for Vivapur 1.2% are 1.063±0.676 with 3000 rpm. Sizes measured were: tablets in Vivapur got Dv50 equal to 83.16 ± 2.17 μm; tablets in Avicel got Dv50 equal to 22.08 ± 0.43 μm. HPLC measurement (on-going) showed the stability of at least 14 days for Vivapur in both conditions (25ºC = 97.51 ± 6.68; 5ºC = 98.49 ± 0.63) and Avicel in 5ºC (95.76 ± 2.18). Although the smallest particles were found in Avicel, higher stability and viscosity has been shown with Vivapur; hence, Vivapur may be better for diazepam liquid extemporaneous preparations.

# PC3

## Liposome encapsulation of a fibrinolytic agent and its effect on clot degradation

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There is increasing evidence for a consistent association of denser fibrin clot structure and higher resistance to degradation (fibrinolysis) with cardiovascular diseases (CVDs). CVDs account for nearly one-third of deaths worldwide and there is an urgent need to overcome this scenario. We aim to develop an encapsulated fibrinolytic lipid nanoparticle strategy with lower bleeding risk, to be incorporated in the clot structure. We studied the impact of the nanoparticle on clot formation and lysis and observed that the nanoparticles do not affect clot properties. Also, we concluded that the nanoparticle is stable over time without any measurable aggregation or change in surface charge. Turbidimetry studies showed that the presence of the nanoparticles reflected a non-significant small increase in fibrin fiber radius, protofibril packing and protein content with increasing lipid concentrations. Two methods of tPA encapsulation in lipid nanoparticles were tested, with one achieving 90% encapsulation efficiency. Ultracentrifugation was used to separate non-encapsulated material without triggering nanoparticle aggregation. Preliminary results have already demonstrated a controlled release of tPA in a solid emulation of a clot, without activity loss. Future work will focus on optimizing the targeting element incorporation in the liposome surface.

# PC4

## $^{19}$F-NMR spectroscopy shows that GalNAc-Ts glycosylation mechanism follows an induced-fit mechanism

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GalNAc-Transferases (GalNAc-Ts) are a large family of glycosyltransferases responsible to initiate protein *O*-glycosylation [1]. Deficiencies and dysregulation of individual GalNAc-Ts have been related to cancer and metabolic diseases. In particular, it was demonstrated that a GALNT2 mutant ($F_{104}S$) leading to the inactivation of the enzyme, induces low high-density lipoprotein cholesterol (HDL-C) levels in humans [2]. GalNAc-Ts have one *N*-terminal catalytic domain connected to a *C*-terminal lectin domain by a short flexible linker [3]. Furthermore, in the catalytic domain there is a flexible loop, which in the case of GalNAc-T2 comprises residues $Val_{360}$ to $Gly_{372}$ [4]. Structural studies on GalNAc-Ts can be employed to infer how mutations on these enzymes might lead to loss-of-function. Herein, we will report the molecular basis for F104S mutant inactivation by a multidisciplinary structural approach that combines saturation transfer difference NMR experiments, molecular dynamics (MD) simulations, X-Ray crystallography and $^{19}$F-NMR experiments. The present research unveiled new insights into the catalytic mechanism of the large family of GalNAc-Ts and how these enzymes orchestrate protein *O*-glycosylation [5].

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# PC5

## Discovery of novel anti-Influenza agents: *in silico* fragment-based screening and experimental validation by NMR

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Influenza viruses are major human pathogens responsible for respiratory diseases affecting millions of people worldwide and characterized by high morbidity and significant mortality. Several structural studies of influenza non-structural protein 1 (NS1) have proposed this protein as a potential therapeutic target. NS1 has two distinctive structural domains, a double stranded RNA-binding domain (RBD) and a C-terminal effector domain (ED).

In this work we have first characterized the RBD using distinct techniques, such as Nuclear Magnetic Resonance (NMR). A workflow comprising both computational and experimental fragment-based approaches have been devised.

The $^{1}H$-$^{15}N$ HSQC spectrum of RBD-NS1 shows large chemical shift dispersion and thus presents high potential to be used in compound screening based on chemical shift perturbation experiments. Lastly, we tested eight fragments and the preliminary results show the success of the protocol. The innovative computational protocol designed is a new approach that can be used to rank and determine fragments to be used in posterior phases of the fragment-based lead design.

# PC6

## Structural basis for human macrophage galactose-type lectin recognition

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Glycans have an important role on immune modulation [1]. In this context, the human Macrophage Galactose-type Lectin (MGL), exclusively expressed by macrophages and dendritic cells (DCs) within the immune system, specifically bind with high affinity the N-acetylgalactosamine motif (GalNAc) [2]. GalNAc-containing glycoconjugates are present on pathogens, self-glycoproteins and tumor cells and bind to MGL tuning immune cell responses [3]. Particularly in cancer, MGL seems to induce suppressive immune responses [4]. Thus, targeting of MGL could provide promising novel therapeutic approaches to elicit anti-tumor immunity. For that purpose, structural insights into the glycan/MGL complexes are essential for a rational design of potential glycan-based therapies. Indeed, the binding modes of tumor-associated mucin Tn-antigens by MGL were previously elucidated by us[4]. Herein, we will report new advances on the structural elucidation of MGL recognition process unveiled by NMR spectroscopy.

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# PC7

## Formation of membraneless organelles by liquid-liquid phase separation of intrinsically disordered proteins

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Subcellular compartmentalization allows the organization of complex biochemical reactions in space and time, underlying vital cell processes such as homeostasis, division and development [1]. Several compartments, however, lack a physical barrier. These nn-membrane-bound organelles are usually an assembly of stalled mRNA and proteins, that undergo liquid-liquid phase separation (LLPS), being termed as ribonucleoprotein (RNP) granules [2]. These proteinaceous liquids likely provide a microenvironment that allows selective particle partition and promotes specific nucleic-acid processing [3].

Proteins that drive LLPS process normally exhibit an overall unstructured composition, being referred as intrinsically disordered proteins (IDPs). Fused in sarcoma (FUS) is a ubiquitously expressed 526 amino-acid protein, containing a disordered low complexity (LC) domain, a RNA recognition motif (RRM), and three arginine-glycine-glycine (RGG) boxes. In certain stress conditions, FUS can undergo LLPS in the cytoplasm through a variety of processes at the LC region and RGG boxes. Formation of these RNP granules increases the risk of self- templating protein fibrils, that underpin fatal neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) [4].

Although cell environment is known to play a crucial role in the RNP granules formation, the mechanisms and determinants that drive LLPS are still unclear. In this context, we studied the influence of different abundant cellular osmolytes on the degree of FUS LLPS under different pH's. FUS concentration dependence of RNP granule formation was followed by microscopic assays. 2D $^{1}H$-$^{15}N$ HSQC was carried out in the phase-separated and in the liquid dispersed phase of FUS to evaluate the differences in the overall protein conformation. Moreover, 2D $^{1}H$- $^{15}N$ HSQC spectra were acquired under different temperatures, to determine the influence of temperature on the LLPS process.

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# PC8

## Development of a Carbopol 940 mucoadhesive oral gel for the treatment of recurrent aphthous stomatitis: rheological and mucoadhesive properties

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Recurrent aphthous stomatitis is an inflammatory disease that causes ulcerations on the oral mucosa inducing pain, difficulty in speaking and eating. The objective was to create a Carbopol 940 (CP) mucoadhesive oral gel with triamcinolone acetonide and hyaluronic acid, well-known synergic drugs for the management of this disease. This topical pharmaceutical form allows a greater contact time with the ulcers and better patient compliance. To optimize the formulation, a screening of the concentration of CP was measured by taking into consideration the rheological and mucoadhesive properties. The viscosity was measured with the Kinexus lab+ rheometer Malvern (frequency table strain control: T 20ºC, start frequency 10.00 Hz, end frequency 0.01 Hz, shear strain 0.39%) and the mucoadhesion was measured using the TA.XT2iHR Texture Analyser Stable Micro Systems, with a mucoadhesive rig and pork buccal mucosa (withdrawal speed 0.50 mm/s, applied force 1N, contact time 150 s). At frequency 1 Hz, the average viscosity for CP gel at 0.5%, 1%, 1.5% was, respectively, 170.51, 153.31 and 155.84 Pa□s. The work of adhesion (WOA) was, respectively, 0.089 ± 0.018, 0.167 ± 0.030 and 0.231 ± 0.061 N/mm. WOA results, analysed with ANOVA and Tuckey post hoc test, showed significant differences for the three formulations. The concentration of CP that delivered the best result was 1.5%.

# PC9

## Functional characterization of the periplasmic triheme cytochrome PpcA from *G. metallireducens*

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In the recent years, *G. metallireducens* bacterium has been presented as an microrganism of high interest in the bioremediation and bioenergy fields, due to its ability to oxidize a wide range of organic and inorganic substances, as well as for its high current density production in microbial fuel cells. This bacterium has a family of five periplasmic triheme cytochromes that are crucial to bridge the electron transfer between the cytoplasmic donors and the extracellular acceptors. One of these cytochromes (PpcA) was recently characterized [1]. The unique biochemical and structural features displayed by this cytochrome prompted us to undertake its detailed functional characterization. Using a complementary approach of NMR and visible spectroscopic techniques, we determined the individual heme redox potentials, their redox and redox-Bohr interactions. These thermodynamic parameters revealed unprecedent features compared to other members of this class, which included the significant difference between the heme reduction potentials and their functional working potential ranges. It was also found that the order of oxidation of the hemes is pH independent. Nonetheless, the cytochrome has the necessary properties to perform $e^-/H^+$ coupled transfer at physiological pH.

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# PC10

## Quantitative assessment of methods used to obtain rate constants from molecular dynamics simulations - translocation of cholesterol across a lipid bilayer

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Calculation of rate constants of chemical processes from molecular dynamics (MD) simulations has been a long-sought but elusive goal. This problem is particularly relevant in processes occurring in biological systems, such as the translocation across biomembranes, the rate limiting step in the permeation of most drugs. While several formalisms have been proposed to calculate these rate constants, their applicability has not been critically evaluated. This work presents this assessment. To this end, we first used unbiased coarse-grained MD simulations to generate a large set of spontaneous events for the translocation of cholesterol across 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine bilayers, allowing us to determine the reaction coordinate, the rate, and the molecular mechanism of cholesterol's translocation in great detail. In this context, a novel procedure was also employed to obtain an effective rate constant, based on transitions between different states along the reaction coordinate. These quantitative data were then compared with the predictions of several available formalisms to assess their accuracy. While most of the tested formalisms lead to results in reasonable agreement with the effective rate constant, one of the methods was superior. This technique is based on explicit relaxation frequencies from the transition state in the forward- and backward-directions along the reaction coordinate [1].

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# PC11

## A comprehensive computational analysis of the structural and functional impact of interface Non-Synonymous Single Nucleotide Polymorphisms (nsSNPs) on Bcl2:Bax interaction

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The present study aims to investigate the distribution of interface nsSNPs in anti-apoptotic Bcl-2 protein and understand their impact on pro-apoptotic Bax interaction, using computational approaches. Bcl-2 family of proteins regulates the apoptotic process by either induction (pro-survival) or inhibition (pro-death) maintaining the balance between Pro-apoptotic and Anti-apoptotic members [1-2]. Non-synonymous Single Nucleotide Polymorphisms (nsSNP) are point mutations which can alter the sequence of amino acid residues and these alterations lead to the pathogenic phenotypes. In recent years, the consequence of interface nsSNPs upon protein–protein interactions (PPIs) has also been examined, giving a greater perception into the mechanisms by which nsSNPs can lead to disease. An identification of these nsSNPs responsible for a specific pathogenic state with experimental techniques is a costly, time-consuming and cumbersome process. Thus, Bioinformatics approaches were attempted to identify and prioritize the possible deleterious interface nsSNPs of Bcl-2 and their impact on Bax binding was studied. Our *in silico* analysis showed that R207Q interface nsSNP (Accession No: rs369294037) could be the most promising deleterious variant which affects the Bcl-2:Bax interactions significantly. This study provided strong insights to understand the impact of other inhibitors/binding partners towards Bcl-2, and thus opens new direction for anti-cancer therapeutics.

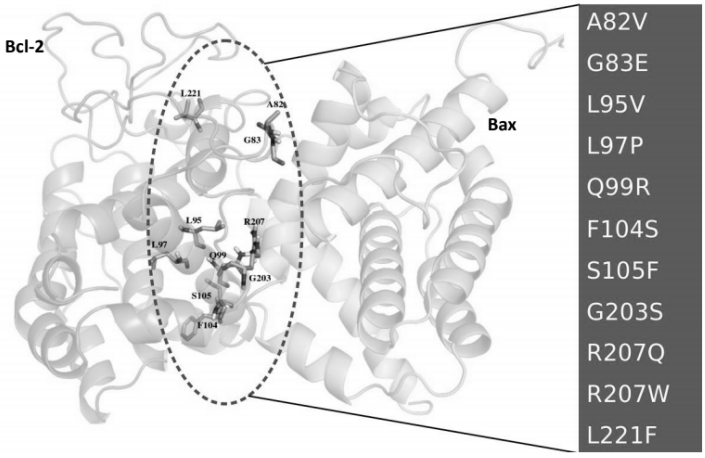


Figure 1. Distribution of interface nsSNPs in Bcl-2:Bax complex.

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# PC12

## Changes on elasticity and morphology of erythrocytes from amyotrophic lateral sclerosis patients

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Patients' complications, such as venous thromboembolism (VTE), promote changes in haemodynamic properties and abnormalities in red blood cells (RBC) membrane and on its lipid content. Our main goal was to evaluate changes in the elastic and morphological properties of RBCs in ALS and compare them with the erythrocytes from healthy donors. By atomic force microscopy (AFM), RBC membrane roughness, elasticity and morphological parameters were analysed for both groups. Patients' RBCs are stiffer, have higher penetration depth and are more capable to deform, presenting an increased membrane roughness. Morphological changes on RBCs from ALS patients were also assessed by AFM, showing lower thickness and higher cell area. Zeta-potential analysis showed that the surface of patients' RBCs is less negatively charged, which may be due to a lower density of sialic acid residues. Fluorescence spectroscopy showed that RBC membranes from ALS patients are more fluid. This may be associated with changes on membrane lipid composition and packing. We conclude that ALS disease leads to significant electrostatic and morphologic changes in RBC membranes. These findings may contribute to understand the complex interplay between ALS disease progression rate and RBC lipid profile.

# PC13

## Development of a Poloxamer 407- based thermosensitive gel for the treatment of Eosinophilic Esophagitis: focus on the rheological properties

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Eosinophilic Esophagitis is an immune, food-based antigen-mediated, chronic and progressive disease characterized by symptoms related to esophageal dysfunction and Eosinophil-predominant inflammation. Nowadays, lacking endpoint treatments, management is achieved with specific diet, dilation when structuring occurs and pharmacological administration. Topical glucocorticoids have shown to be the most promising drugs to control it, although the actual formulations are still not approved by the regulatory agencies and they are so used as off-label treatment. The study aims to develop a new formulation for the administration of clobetasol in the form of a mucoadhesive and thermosensitive Poloxamer 407-based gel to obtain optimization of the contact time with the esophageal mucosa and achieve a sustained release. To properly target the affected tissue, a liquid-solid transition trough body thermal activation is achieved by screening a specific concentration of Poloxamer (20, 15, 14 and 13% w/w). The gelling point was measured with a Kinexus Lab+ Rheometer Malvern (temperature ramp: 20ºC to 40ºC, 1ºC/min ramp rate, 1.0 Hz frequency, 0.08% shear strain) obtaining, respectively, the following results: 25.04 ± 0.16ºC, 29.59 ± 0.96ºC, 32.63 ± 0.59ºC, 34.90 ± 0.50ºC. The 13% concentration gave the closest gelification point to the aimed 37ºC.

# PC14

## Towards understanding the complexity of molecular interactions: a structure-based approach to develop high affinity leads

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The pharmaceutical industry and academic research laboratories are constantly facing challenging obstacles in their pursuit for novel and effective therapies. Over the past years, remarkable discoveries in the Chemical Biology field improved the design and synthesis of molecules that ultimately will lead to novel medicines. Structural characterization of ligand-protein complexes provides a powerful insight about crucial aspects such as mechanisms of action, intrinsic dynamics and function. This approach becomes highly important for our currently research, which aims to investigate the role of biologically relevant proteins and identify and characterize the binding mode of several inhibitors. In one remarkable example, we identified a water-mediated motif present in the protein tyrosine phosphatase from *Mycobacterium tuberculosis*, that modulates the accessibility to the catalytic pocket. In another notable study, we assessed the binding activity of two new inhibitors of the bromodomain (BRD) family, the "epigenetic readers" that recognize acetylated lysine marks on histones and potential drug targets in cancer and inflammation. In this study, we were able to disclose the complexity of the molecular interactions between BET proteins and the new molecules we have developed in our lab. To gain insight into the different binding modes of these potent inhibitors we determined the crystal structures of several inhibitors in complex with two different BRD-containing proteins (BDR2 and BDR4). The results clearly demonstrate new interactions that stabilize the protein-ligand complexes and the existence of structured water molecules between the ligands and the target proteins. Ultimately, we aim to optimize ligand affinity and develop novel chemotypes with increased potency and specificity.

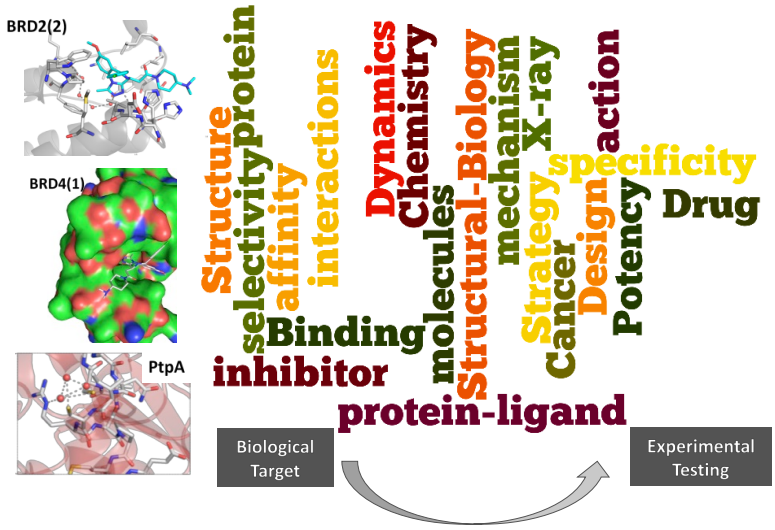


Figure 1. Exploiting structural biology as a tool to accelerate drug discovery.

# PC15

## Comparison of Zika virus capsid protein with related *Flavivirus* capsid proteins and their ability to interact with host lipid systems

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Zika (ZIKV), Dengue (DENV) and West Nile (WNV) viruses are related mosquito-borne flaviviruses. ZIKV infection was associated with severe congenital microcephaly in newborns and with Guillain-Barré syndrome. Although there is a lack of knowledge on basic aspects of the viral life cycle, much can be inferred from the closely related DENV and WNV. For example, DENV C interaction with host lipid droplets (LDs) is essential for viral replication, having been studied in detail by us. Thus, here, we investigated ZIKV C binding to host lipid systems via biophysical approaches. Zeta potential shows that ZIKV C interacts with intracellular LDs. However, ZIKV C-LDs interactions do not require potassium ions, as previously shown by us for DENV and WNV C. Dynamic light scattering measurements indicate that ZIKV C interacts with plasma lipoproteins, namely VLDL and LDL. ZIKV, WNV and DENV C proteins display similar predicted hydrophobicity, α-helical propensity and tertiary structure, which can thus be targeted via similar approaches. Combining this with our background on DENV C studies and pep14-23 development (an inhibitor of DENV C binding to host lipid systems, designed and patented by us), we will use this information in drug development strategies against ZIKV and related flaviviruses.

# PC16

## Short-term regulation of Aquaporin-5 by combined phosphorylation and pH

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Aquaporin-5 (AQP5) is a membrane water channel widely distributed in human tissues that was found up-regulated in different tumors and considered implicated in carcinogenesis. AQP5 phosphorylation was reported to increase its expression and trafficking, resulting in increased membrane abundance. Interestingly, AQP5 is mostly found phosphorylated in tumor tissues. However, the effects of phosphorylation on channel activity as well as its sensitivity to extracellular acidification have not been investigated so far.

In this work, using a yeast model of heterologous expression, we investigated the effect of protein phosphorylation and pH sensitivity as a mechanism of AQP5 short-term regulation. We observed that external acidification alone (pH 5) does not affect AQP5 activity. However, AQP5 phosphorylation induced by intracellular cAMP renders the protein channel prone to pH sensing, resulting in marked increase in water permeability when pH is raised to 7.4.

The mechanism of gating may involve intracellular phosphorylation of AQP5 with consequent change in protein conformation and channel pore widening. In this new open configuration, deprotonation of residues occurring at pH 7.4 may support an increased water transport capacity of the AQP5 protein.

**PC17**

## Modelling circadian rhythms in *Drosophila melanogaster*

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We propose an activation-repression model with autoactivation and delay for the circadian rhythms taking place in Drosophila melanogaster. In comparison with previous models, our model was entirely obtained by applying the mass-action law. We resort to numerical analysis to show that the model has oscillatory solutions. The equations of our model are, in themselves, interesting from the point of view of the dynamical systems theory: for some values of its parameters the system undergoes a Hopf bifurcation, after which a limit cycle appears. We found that away from the Hopf bifurcation the period of the oscillations is linearly dependent on the introduced delay, which is an advantage if a calibration of the model with experimental data is to be performed.

# PC18

## From cell extract to chips: bypassing protein purification in drug discovery

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Identifying and characterizing drug-target protein interactions is a key step in the process of drug research and development; however, there is a large number of proteins with potential pharmaceutical application whose expression and purification remains extremely difficult. The aim of this project is to develop a method that bypasses protein purification by using *E. coli* cell lysates enriched in biotinylated proteins, to kinetically characterize their interaction with lead molecules via surface plasmon resonance (SPR). As proof of concept, the well characterized human Cyclophilin D (CypD) was biotinylated both *in vitro* and *in vivo*, immobilized onto a streptavidin surface chip and studied with respect to its interaction with high affinity reference compounds. The kinetic profile revealed to be comparable regardless of the sample heterogeneity. The same approach was successfully applied on two other potential drug-targets, TBP and RPAP3, previously known to be stably purified only in their truncated forms. The kinetic characterization of these proteins, in their full-length form, with reference molecules or known protein partners was performed and highlights the potential of this approach applied to the early stages of drug research and development.

# PC19

## Screening of Avicel RC581 and polymer activation to optimize the viscosity of a Warfarin suspension

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Warfarin in UE is only sold in the form of 5 mg tablets, which may not respond of the needs of every patient. Doses and pharmaceutical forms need to be personalized in base of each clinical necessities. Pharmaceutical compounding provides individual extemporaneously manufacturing to improve effectiveness, safety and compliance. The viscosity of liquid formulations has direct implications on the sustainability of the homogenous distribution of the particles during the drug administration. Avicel RC581 was used as the main suspending agent. To optimize the formulation, the rheology properties of different concentration of Avicel (0.70%, 0.90%, 1.20% and 1.60%) and different rates of polymer activation (3000rpm, 6000rpm, 9000rpm) was studied using “Share Rate Ramp” test in the Malvern Kinexus Lab+ rheometer and the Herschel- Bulkley theoretical model, which allows to compare the consistency of the different preparations. The K factors of 0.70% Avicel activated with 3000rpm, 6000rpm and 9000rpm are respectively 0.045, 0.035, 0.026; of 0.90% Avicel are respectively 0.066, 0.089, 0.804; of 1.2% are respectively 0.067, 0.124, 0.804; of the 1.6% are respectively 0.073, 0.148, 2.396. The aim is to acquire proper viscosity using the lowest polymer concentration and the lowest energy possible, which is obtained with 0.9% Avicel activated with 9000 rpm.

# PC20

## Molecular modeling study of pH effects on β-lactoglobulin

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Milk and its derivatives are an important worldwide food source, particularly for infant nutrition, but their use faces a major health complication: some of their proteins are allergens, especially β-lactoglobulin (BLG), a major component of the bovine milk. The fate of BLG upon human ingestion remains unsettled, being unclear how extensive BLG proteolysis is and how it relates to allergenicity. The fact that its proteolytic resistance and antigenic response remain related even in the case of non-oral administration [1] suggests that they are not causally related but rather reflect an underlying common feature. This feature may be the formation of dimers, which can hinder proteolysis and seems to facilitate the binding of protein allergens to IgE antibodies; indeed, BLG is dimeric when complexed with IgE Fab fragments [2] and shows lower antigenicity when in the monomeric form [3]. As shown in experimental studies, this form is predominant at pH below 3 and above 8 and between these there's the formation of a reversible dimer at a moderate ionic strength [4]. The changes in pH are also associated to the Tanford transition, that is, a change in the conformation in a loop near the binding site, allowing or inhibiting the binding of ligands, regulated by the protonation of Glu89 [5]. Previous studies have shown that the dimerization involves electrostatic interactions, for which a better understanding at a molecular-level is essential. In this study, we intended to analyse the effect of the pH in conformational alterations on the monomer and dimer and its dissociation process. For that, Constant pH molecular dynamics (CpHMD) simulations were performed for the monomer and dimer, which allows us to treat pH as an explicit parameter and couples the MM/MD and Poisson-Boltzmann/Monte Carlo (PB/MC) algorithms.

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# PC21

## AQP5-mediated hydrogen peroxide transport improves cell resistance to oxidative stress

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Aquaporins (AQPs) facilitate water and glycerol permeation through membranes and a few isoforms can also permeate $H_2O_2$ (*peroxiporins).* Increased $H_2O_2$ levels misbalance cell redox reactions and may induce tumorigenesis. Recently, we evaluated $H_2O_2$ permeability of mammalian AQPs individually expressed in yeast and reported the ability of the rat AQP5-transformed yeast strain to conduct $H_2O_2$. Sequence alignment of human and rat AQP5 isoforms show a sequence identity of 91%. Therefore, we next investigated $H_2O_2$ permeation by human AQP5 and related cell resistance to oxidative stress. The results indicate that, similarly to AQP3, and AQP8, human AQP5 also permeates $H_2O_2$ and importantly, improves cell resistance under oxidative stress. In addition, the effect of anti-oxidant compounds on hAQP5-dependent $H_2O_2$ accumulation and cell growth was examined. Curcumin and naringenin enhanced cell resistance in AQP5-transformed yeast cells and anti-proliferative properties known for these compounds were attenuated in yeast cells expressing human AQP5. These data suggest an important role of AQP5 in oxidative stress resistance and point to a novel mechanism explaining AQP5 involvement in cancer.

# PC22

## Physico-Chemical properties of atypical sphingolipids in model and cell membranes

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Sphingolipids (SLs) participate in many cellular events. Their building blocks, long chain sphingoid bases, are formed from L-serine and palmitoyl-CoA. However, alanine and glycine can also be used, forming atypical 1-deoxy-sphingoid bases that lack the OH at the C1 position. Elevated levels of 1-deoxySLs are associated with the development of HSAN1 and diabetes type II. Nevertheless, their biological significance and the molecular mechanisms underlying their pathological role remain elusive.

Using fluorescence-based methodologies we showed that, in contrast to their canonical counterparts, 1-deoxySLs failed to form highly-ordered gel domains in fluid model membranes. Moreover, elevated cellular levels of 1-deoxySLs increased the overall membrane fluidity compared to control cells. To investigate if this was a consequence of impaired H-bond network due to the lack of the C1-OH group, the biophysical properties of 1-methoxy-SLs were studied. The ability to form gel domains and decrease membrane fluidity was reestablished, although to a less extent. These results indicate that C1 headgroup of the SLs determines the formation of tightly packed domains.

In conclusion, canonical and 1-deoxySLs lead to opposite changes in membrane biophysical properties, suggesting a possible mechanism to mediate the distinct biological actions of these species.

# PC23

## Biophysical characterization of lipid-tagged peptides as fusion inhibitors for respiratory viruses

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Human parainfluenza viruses (HPIV) and respiratory syncytial virus (RSV) are paramyxoviruses and are among the most common respiratory pathogens affecting infants and children worldwide. Nowadays, acute respiratory infections are the leading cause of mortality in children, accounting for nearly 20% of childhood deaths worldwide (nearly 3 million children each year). There are no effective treatments available. Consequently, there is an urgent demand for efficient antiviral therapies. Infection of healthy cells by these respiratory viruses requires fusion of the viral membrane with the target cell membrane, a process mediated by a trimeric viral fusion protein, F protein. Inhibitory peptides inhibit viral fusion by binding to F's transient intermediate, preventing it from advancing to the next step in membrane fusion. Here we assessed variants of lipid-tagged F-derived peptides to search for properties that may associate with efficacy and broad-spectrum activity. Fluorescence spectroscopy was used to study the interaction of the peptides with biomembrane model systems, using partition assays. Using acrylamide, a quencher of tryptophan fluorescence, was possible to understand the preferential localization of the peptides in lipid bilayers. The interaction of the peptides with human blood cell-binding was also evaluated using the dipole potential probe, di-8-ANEPPS. Understanding the membrane biophysics processes involved in enveloped viruses entry will enable the development of new inhibition strategies.

# PC24

## Deciphering a charged metabolite effect on protein structure and stability

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Different environmental factors can change the composition of the protein environment and have been associated with protein misfolding and, ultimately, with neurodegenerative diseases such as Alzheimer's or Parkinson's [1]. One very important aspect is that the intracellular environment is extremely crowded with small charged metabolites that can form ion-pairs and have the potential to act on the folding and stability of proteins.

Inspired by the high concentrations of organic charged metabolites found in the cell milieu, specifically the choline cation and the glutamate anion, and in our previous studies with imidazolium-based ionic liquids (IL), that disclosed the effects of specific P-IL interactions on protein stability [2,3], we studied the effect of the biocompatible IL [Ch][Glu] on the stability of the domain B1 of protein G (GB1), a globular and highly stable protein [4].

In this communication, through the combination of different NMR techniques and the determination of protein stability by fluorescence and calorimetric studies, we will present results concerning the discrimination of the nature of the contacts established by ion-pairs and the ions alone. We expect to contribute to an understanding of how changes in the cellular homeostasis may control the protein folding landscape.

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3. M. Silva, A. M. Figueiredo, E. J. Cabrita, *Phys. Chem. Chem. Phys.* 2014, *16*, 23394–23403.
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# PC25

## Biophysical assessment of pancreatic aquaporins as water and glycerol channels and their implication in obesity-induced inflammation

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Obesity leads to inflammation and subsequent loss of function and secretion of proinflammatory cytokines by the injured tissues. Aquaporins (AQPs) facilitate the permeation of water and glycerol across membranes. AQP7, the main aquaglyceroporin in pancreatic β-cells, is responsible for glycerol uptake and is involved in insulin secretion. AQP12, with unclear permeability features, was shown upregulated in pancreatitis.

In this work, AQPs expression and function in pancreatic β-cells as well as their involvement in cell proliferation, migration and adhesion were investigated. The contribution of AQP7 and AQP12 to inflammation in endocrine pancreas was evaluated using a loss-of-function/gain-of-function strategy in the pancreatic RIN-m5F cell line.

Our results confirm AQP7 as a water and glycerol channel in β-cells and revealed its implication in cell proliferation. AQP7 and AQP12 overexpressing cells show improved cell adhesion and migration. The implication of AQPs in inflammation was evaluated by assessing their mRNA expression levels as well as of markers of inflammation (TNFα, IL-1β and IL6) in a pro-inflammatory condition (TNFα). TNFα-induced dysfunction upregulates AQP7, AQP12 and proinflammatory markers. In AQP12-overexpressing cells proinflammatory markers (TNFα, IL-1β and IL-6) are downregulated while in AQP12- silenced ones we observe the opposite effect. Our data suggests a protective role of AQP12 in inflammation.

# PC26

## Partition of Ciprofloxacin and its copper complex in bacterial membrane: a theoretical and experimental study

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Ciprofloxacin (Cpx) is one of the most used Fluoroquinolones in clinical therapy. Numerous studies report a high rate of resistance to Cpx, with critical prevalence in developing countries. Since Cpx acts inside the bacteria, its permeation is a crucial aspect when studying resistance to this drug. Two mechanisms of internalization are known: diffusion through the lipid membrane or translocation through the porin channel. At physiological conditions (pH≈7.4), Cpx is mainly zwitterionic and thus its ability to diffuse through the membrane should be compromised. Ternary copper complexes, composed by one copper coordinated with one Fluoroquinolone and one Phenanthroline (CuFQPhen), have been studied as alternatives to free-FQ. They show a higher membrane partition, suggesting an increased ability to cross the lipid bacterial membrane, promoted by favourable drug-lipid interactions.

In this work, we studied the permeation of Cpx and CuCpxPhen in a phosphatidylglycerol membrane that mimics the negatively charged bacterial membrane. Fluorescence Spectroscopy and Molecular Dynamics were used to determine the drug's partition coefficient and to further explore the interactions that are involved in the permeation process. The investigation of CuCpxPhen permeation provide insights that will be important to proceed with the study of metal-complexes of fluoroquinolones as alternatives in resistant infections.

# PC27

## Antimicrobial peptides *Pa*MAP2 and *Pa*MAP1.9 reveal anticancer activity

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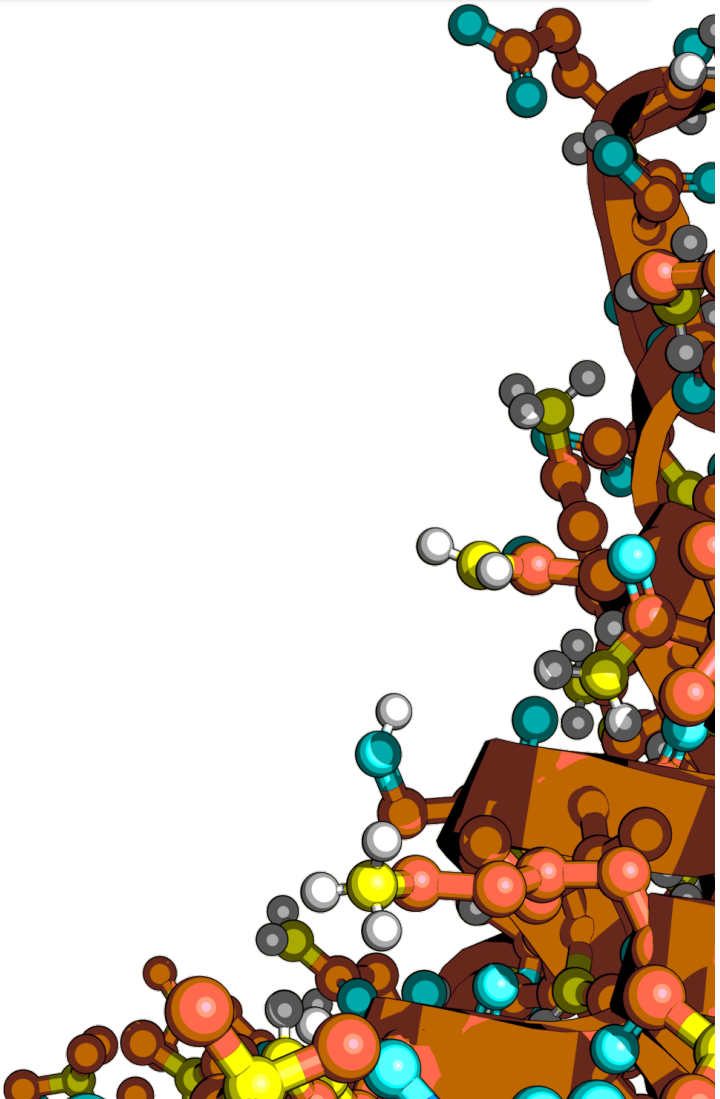
Antibiotic resistance is a major public health problem that is expected to lead pharmaceutical companies to a new paradigm, where conventional molecules will need to be replaced. As a matter of fact, the World Health Organization has already pointed out the urgency in finding new molecules against different pathogens, named the *ESKAPE* group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Enterobacter* species). Additionally, in cancer therapy, the number of resistance cases in patients has increased, with associated infections being a cause of death in many of those cases.

Antimicrobial peptides (AMPs) are considering a new promising alternative for infectious and cancer therapies. Being small, cationic and hydrophobic, their major advantage is the difficulty for pathogens to acquire resistance. We studied *Pa*MAP2 and *Pa*MAP1.9, two synthetic AMPs that showed effectiveness against multiresistant clinical isolated bacteria. Using biophysical techniques (fluorescence spectroscopy, flow cytometry and confocal microscopy), focused on peptide-membrane interactions, we tested if these AMPs could be efficient against cancer cells. Our data demonstrate that, besides antimicrobial activity, *Pa*MAP1.9 (but not *Pa*MAP2) can also target cancer cells, showing that AMPs can have a dual activity, which is important for cancer therapy patients.

# Committees



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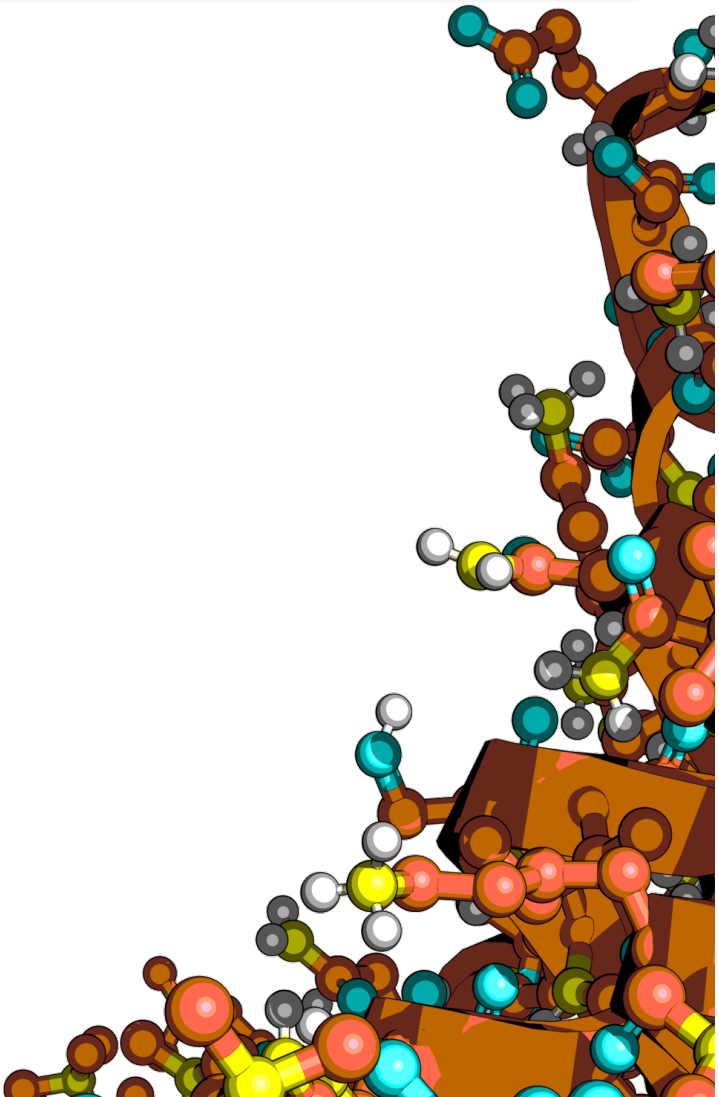

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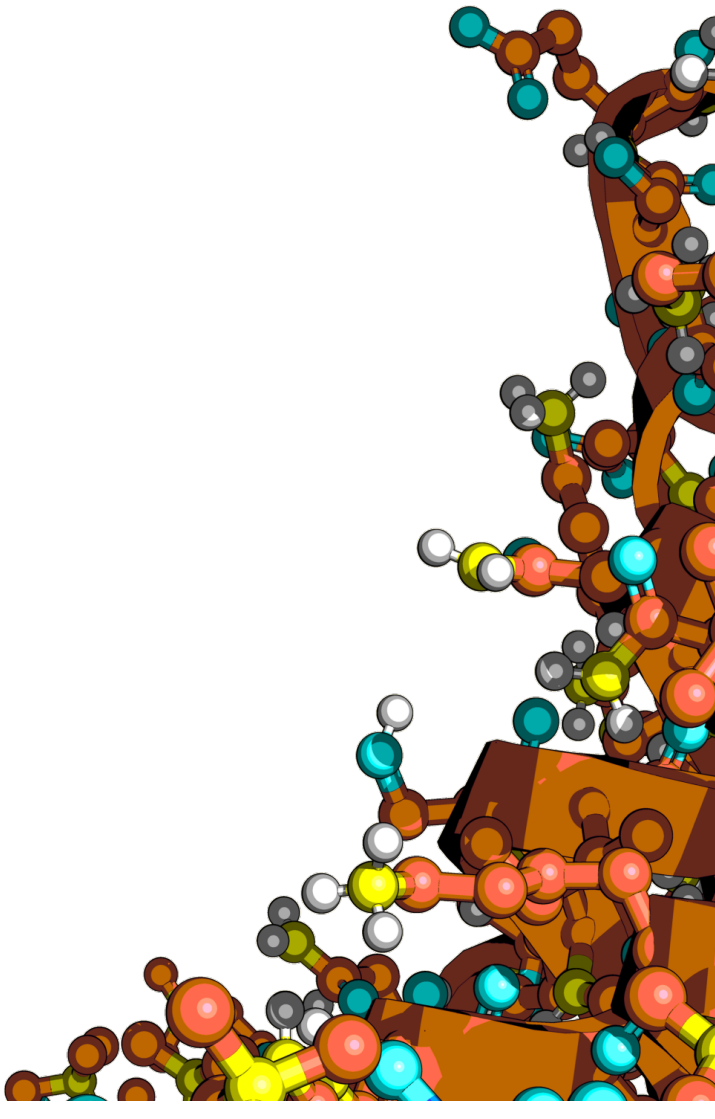

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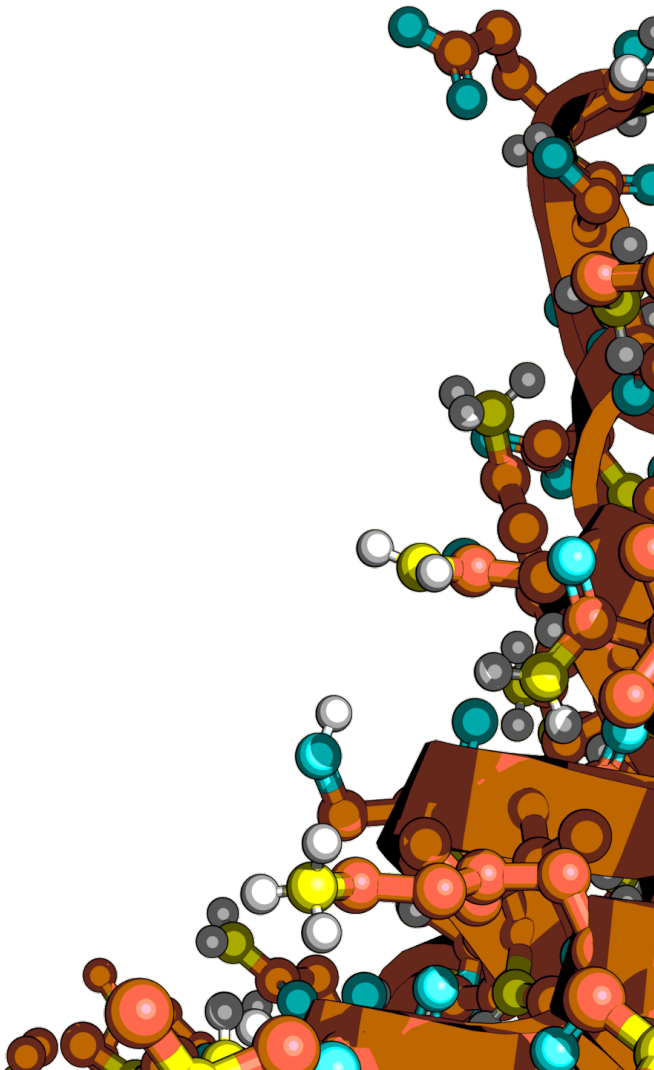

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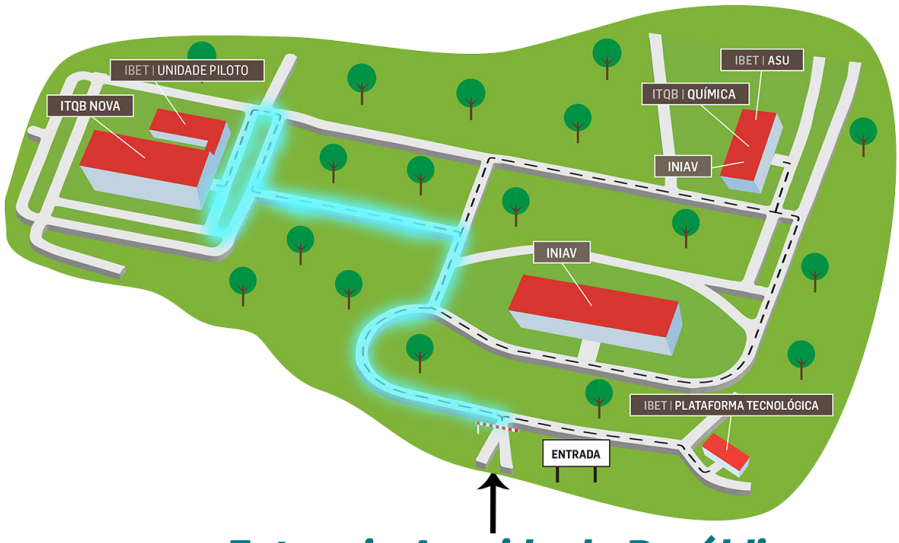


***Entry via Avenida da República***

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## *Departure after 8:00pm:*

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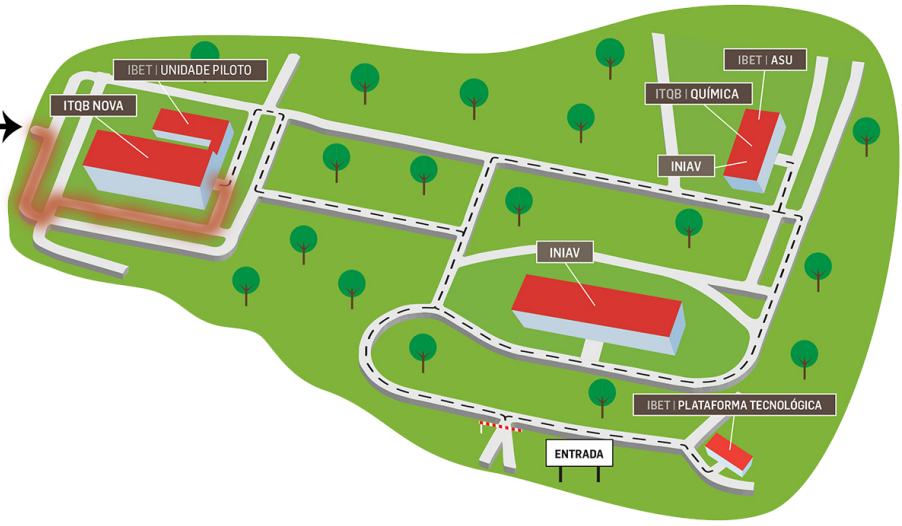

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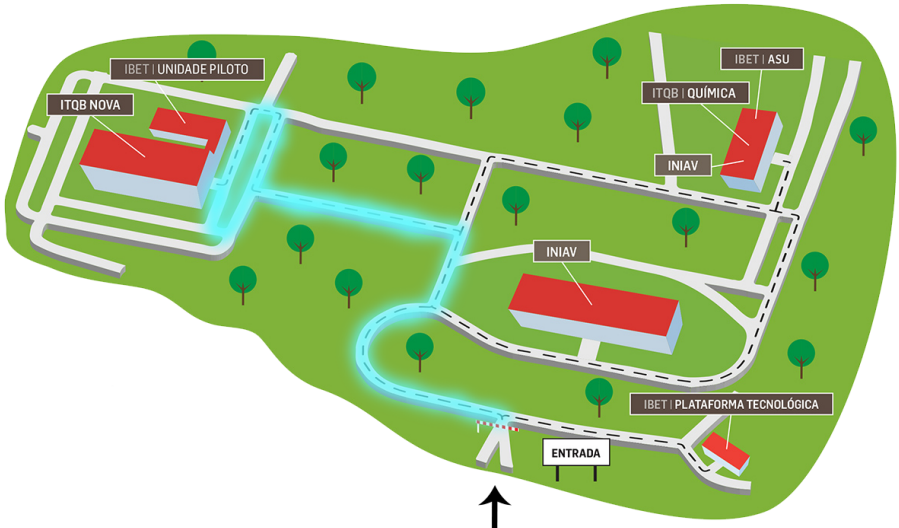


***Entry and exit via Avenida da República***