

### List of Abstracts

#### PL – Plenary Lectures

#### S – Symposia

- S01 Single Molecules  
 S01.1 Protein-Nucleic Acid Interactions  
 S01.2 Protein Interactions and Networks  
 S01.3 Membranes and Proteins  
 S01.4 Intrinsically Disordered Proteins  
 S01.5 Engineering and Design  
 S02 Trends in Biochemistry  
 S02.1 Genome Dynamics  
 S02.2 Transcription and Chromatin  
 S02.3 RNA Biogenesis and Processing  
 S02.4 Autophagy and Protein Homeostasis  
 S02.5 Integrated Cell Structure and Function  
 S03 Beyond Biochemistry  
 S03.1 Ageing  
 S03.2 Global Regulation and Cell Reprogramming  
 S03.3 Artificial Cells and Genomes  
 S03.4 Computing with Molecules and Cells  
 S03.5 Dealing with Errors and Evolution  
 S04 Molecular Bases of Diseases  
 S04.1 Neurodegenerative and Organ Degenerative Diseases  
 S04.2 Inflammation and Diseases  
 S04.3 Stem Cells and Their Niches  
 S04.4 Cancer Genomics and Biomarkers  
 S04.5 Role of Hypoxia in Pathogenesis of Inflammation in Cancer  
 S05 Environmental Biochemistry  
 S05.1 Oxidative Stress: Dealing with Oxygen (Portuguese-Brazilian Conference)  
 S05.2 Dealing with Osmotic Stress  
 S05.3 Life in Extreme Environments (in Memoriam of Costas Drakias)  
 S05.4 Responding to Environmental Perception  
 S05.5 Molecular Clocks and Cell Cycling  
 W – Workshops  
 W01 – W03 Omic Sciences  
 W01 Functional Genomics  
 W02 Proteomics and Signal Transduction  
 W03 Metabolomics  
 W04 Biochemistry in Medical Diagnosis and Therapy  
 W05 Forum for Bioentrepreneurs: Successful Technology Transfer: From Test Tubes to Industrial Applications  
 W06 Systems Biology

- W07 – W08 Science and Society  
 W07 AIDS: Perspectives and New Therapies  
 W08 Malaria in the Developing World  
 W09 – Science in School  
 W10 – W11 Biochemical Education  
 W10 Teaching Molecular Evolution: a Unifying Principle of Biochemistry  
 W11 Research into Effective Teaching Strategies: what Biochemistry is Learning from Other Sciences  
 W12 Women in Biochemistry: from Past to Future  
**Special Events**  
 SEBBM Congress  
 Plenary Lectures  
 Spanish-Mexican Symposium  
 NMR in Biology  
**Poster Presentations**  
 P01 Apoptosis  
 P02 Biochemical Education  
 P03 Biochemistry and Molecular Biology of Plants  
 P04 Biochemistry in Medical Diagnosis and Therapy  
 P05 Biomembranes and Bioenergetics  
 P06 Cell Signaling  
 P07 Developmental Biology  
 P08 Free Radicals and Oxidative Stress  
 P09 Genomics and Proteomics  
 P10 Glycobiology  
 P11 Membrane Transporters  
 P12 Metabolic Regulation  
 P13 Molecular Bases of Pathology  
 P14 Molecular Biorecognition  
 P15 Molecular Parasitology  
 P16 Neuroscience  
 P17 Nitrogen Biochemistry and Metabolism  
 P18 Nutritional Biochemistry  
 P19 Perinatal Biochemistry  
 P20 Protein Structure and Function  
 P21 Regulation of Gene Expression and Genome Dynamics  
 P22 RNA Biology  
 P23 Single Molecules  
 P24 Systems Biology  
 P25 Theoretical Molecular Biology  
 P26 Transgenesis in Mammals  
 P27 Others  
**Author Index**  
**Keyword Index**

its, the heavy (rBAT or 4F2hc) and the light (7 transporters in humans). The light subunit is the catalytic one. The structural information of HATs is scarce. The heavy subunits are type II membrane N-glycoproteins with a large extracellular domain, which has sequence and structural homology to bacterial glucosidases. The light subunits have 12 transmembrane domains. The present structural paradigm of the human light subunits, AdiC, the bacterial arginine/agmatine exchanger, has the LeuT-fold, which is characteristic of several transporter families. The point mutations N22A and N101A in AdiC were crucial to unveil the crystal structures of this transporter in the outward-facing conformations with bound L-arginine, and in the open and occluded states, respectively. These structures helped to unravel the mechanisms of substrate binding and substrate induced-fitting mechanisms of AdiC. Unfortunately, the low amino acid sequence identity (17–19% amino acid sequence identity to human light subunits) precludes the generation of robust structural models for the light subunits of HATs, and obviously offers no clues on the macromolecular organization of the metazoan holotransporters. To approach the atomic structure of HATs, light subunits of HATs of different origins (from fly to human) were cloned and expressed in yeast. This screening resulted in a light subunit with a melting temperature ( $T_M$ ) > 40°C and a heterodimer with a  $T_M$  > 65°C. Negative stain transmission electron microscopy and single particle analysis of this 4F2hc heterodimer was used for 3D reconstruction yielding the first structural model of a HAT at 19 Å resolution.

### P11-29

#### Cholesterol uptake in *Mycobacterium smegmatis*

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Cholesterol uptake in *Mycobacteria* has been associated to the expression of the *mce4* operon, one of the *mce* loci found in Actinobacteria that are involved in different transport functions (e.g., lipid transport, cholesterol) and that also behave as virulence factors in *M. tuberculosis*. The *mce* operons contain 8–10 genes that encode two transmembrane proteins homologous to the permease sub-units of ABC transporters, and several putative cell-surface proteins of unknown function. Since Mce are ATP-driven transport systems it has been proposed that a single ATPase, not encoded in the *mce* operons, provides the energy required for substrate uptake. In *M. smegmatis*, that contains six *mce* operons, we have proposed that the *MSMEG\_1366* gene encoding an ATPase is the one strictly required to provide the energy for cholesterol uptake by the Mce4 system. In order to study the role and the regulation of the *MSMEG\_1366* gene, we have investigated its expression by RT-PCR observing that this gene is expressed at higher levels in cells growing in cholesterol as the only carbon and energy source, when compared with the basal levels observed in glycerol containing media. Remarkably, a constructed ATPase deletion mutant was unable to grow in cholesterol, and the capacity of using this carbon source was restored by complementation of the disrupted mutant, reinforcing our hypothesis about its critical role in cholesterol uptake. These results have been confirmed by radioactive cholesterol uptake assays. Interestingly, the ATPase mutant forms clumps when growing in liquid cultures and shows significant alterations in colony morphology in solid media, suggesting that the ATPase is probably involved in other physiological phenomena through its interaction with the other Mce transporters. Given the impor-

tance of this ATPase in cholesterol active transport and its possible function in other uptake or membrane processes, the protein has been cloned and overexpressed in *E. coli*, to facilitate its purification and crystallization in order to elucidate its structural and functional properties.

### P11-30

#### Finding each other in the membrane: shall we meet in the genome?

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*Escherichia coli* as a model system, which exports cysteine and glutathione. The *cydD* and *cydC* genes are contiguous, the start codon of *cydC* follows the stop codon of *cydD*. We have created a *cydDC* knock-out strain and developed a growth assay that allows us to assess and quantify reconstitution of a functional transporter. Further, we can monitor dimer formation by Blue Native PAGE. Our results indicate that proteins synthesized from one mRNA are not committed to each other but can freely pair in the membrane. However, high subunit concentrations are needed for this to happen; at low expression levels, interactions between subunits originating from the same operon are preferred. These observations point to a scenario of concerted folding and/or reciprocal stabilization of the CydD and CydC subunits and suggest that an operon arrangement facilitates complex formation at low expression levels. Whether these observations are applicable to other membrane protein complexes remains to be studied.

### P11-31

#### Embryonic chicken and murine chondrogenic cells express TRPV1 channels that influence cartilage formation

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Proper skeletogenesis requires ability of differentiating cells to detect environmental changes. Ion transport mechanisms through plasma membrane during chondrogenesis are poorly explored, but the essential role of characteristic intracellular  $Ca^{2+}$ -concentration changes in chondrogenic cells has been reported by our laboratory. The polymodal Transient Receptor Potential Vanilloid (TRPV) subfamily of non-selective cation channels is a good candidate to translate various extracellular stimuli into intracellular ion-concentration changes.

Our experimental model is a primary chondrogenic high density cell culture system (HDC); cells are isolated from limb buds of 4-day-old and 11.5-day-old chicken and murine embryos, respectively. Spontaneous cartilage formation occurs within 6 days in HDC.

Cells of HDC expressed mRNAs of various TRPV members during the course of cartilage differentiation; nonetheless, we focused on TRPV1 characterized by high  $Ca^{2+}$  permeability. Expression of TRPV1 mRNA and proteins showed good correlation to the differential status of chondrogenic cells. Avian TRPV1 is sensitive to heat stimulus and low pH but insensitive to vanilloid-like pharmacological modulators. Therefore, we applied heat treatment on chicken HDC and vanilloid-like

pharmacoons on murine HDC. Both treatments increased the amount of metachromatically stained ECM. Intracellular calcium concentration was elevated by heat stimuli in chicken cells, indicating the presence of thermosensitive channels. This rise of  $\text{Ca}^{2+}$ -concentration did not require extracellular  $\text{Ca}^{2+}$ . Investigation of TRPV1 on murine chondrogenic cells is in progress.

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### P11-32

#### Lipids role in COPII vesicle formation

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In the cell, transport of cargoes between organelles is mediated mainly by vesicles. This vesicles are formed thanks to proteins which polymerize as coats at membrane surfaces. Coated Protein complex II (COPII) is involved in vesicle transport from Endoplasmic Reticulum (ER) to Golgi apparatus, and is considered as the way for cargoes such as proteins to leave the ER. COPII has been very well studied over the last thirty years, however many critical steps in bud formation, protein mechanics and vesicle scission remain unclear.

This project focus in the deformation of ER membrane during COPII vesicle formation and the role of lipids. We have observed that certain proteins involved in lipid metabolism can rescue mutant phenotypes in the formation of COPII vesicles. Particularly, we have found that *sec12-4*, a mutation that affects COPII proteins recruitment, can be rescued by changes in the levels of certain lipid species. The accumulation of these lipids in membranes is thought to induce changes in the local curvature of the lipid bilayer. This finding highlight the importance that lipids may play the formation of the vesicle, where membrane deformation and scission are central tasks during the formation of a mature vesicle.

We follow a lipidomic and biophysical approaches, as we are interested in which lipids are involved in the formation of a vesicle and what is the role they are playing in the membrane. Therefore, we show results from mass spectrometry, *in vitro* and *in vivo* assays where we explore variations in lipid levels and its effect in the formation of COPII vesicles.

### P11r-33

#### Trk1 is a salt-tolerance determinant in the yeast *Hansenula polymorpha*

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The study of the yeast *Hansenula polymorpha*, a member of the *Saccharomycetaceae*, has applied and basic interest for different reasons such as their ability to use methanol and nitrate as carbon and nitrogen sources respectively. Our group, in collaboration with Dr Siverio (ULL, Spain), has performed an initial study of potassium homeostasis in this yeast. We have identified two potassium transporters in the plasma membrane, Hak1 and Trk1, and we have proposed that Hak1 functions as a transporter with high affinity, which is necessary for growth at low concentrations of potassium [1].

In this work we have studied the role of Trk1 in *H. polymorpha*. By using wild type and single *trk1* mutant, we analyzed growth, cation contents, fluxes, relative membrane potential and

volume in cells under different external conditions. Our results show that *trk1* mutant is more salt sensitive than wild type and that it accumulates more sodium and lithium due to increased influx processes. In summary, the results indicate that, while Trk1 does not seem to be a relevant potassium transporter for the cell, it has a fundamental role in salt tolerance, which is consistent with the idea that the TRK transporters may have additional and/or different functions to potassium transport [2].

#### References

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- [2] Benito B, Garciadeblás B, Fraile-Escanciano A, Rodríguez-Navarro A. (2011) Potassium and sodium uptake systems in fungi. The transporter diversity of *Magnaporthe oryzae*. *Fungal Genetics and Biology* 48 (8): 812–22.

### P11-34

#### Functional significance of conserved cysteines in the arsenic/antimony antiporter Acr3 from budding yeast

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The Acr3 permease from the yeast *Saccharomyces cerevisiae* is a prototype member of the arsenical resistance-3 (Acr3) family of transporters, which are found in all domains of life. Remarkably little is known about molecular mechanism of metalloid translocation via Acr3. Members of the Acr3 family exhibit ten-transmembrane topology and lack any known sequence signatures, which could suggest mechanism of metalloid flow. It has been recently revealed that a single cysteine residue (Cys129) in the *Corynebacterium glutamicum* Acr3, which is located in the fourth transmembrane span and conserved in all members of the Acr3 family, is indispensable for As(III) efflux. We have found that the mutation of this highly conserved residue (C151A) in the *S. cerevisiae* Acr3 also led to a complete loss of transport function. This strongly suggests that interactions between As(III) and a thiol group of this residue is required to activate transport through Acr3. In addition, we mutagenized the remaining three cysteine residues C192, C283, and C344, located also in the transmembrane spans of Acr3, and found that in contrast to the C151A mutant, these mutant forms of Acr3 were able to complement metalloid sensitivity of *ACR3* deletion yeast strain and showed only a slight decrease of efflux activity. Although, all cysteine mutants of Acr3 were expressed at the wild type level, four of them, C90A, C169A, C318A and C333A, were not properly secreted to the plasma membrane and accumulated in the intracellular membranes, suggesting that these residues are required for the ER exit. C90, C169, C318, and C333 are located in the intracellular loops and might be subjected to posttranslational modifications, like palmitoylation, which are required for membrane association, protein stability and protein trafficking.