FIRST INTERNATIONAL GIBB MEETING

GRUPPO ITALIANO DI BIOMEMBRANE E BIOENERGETICA

Auditorium Giancarlo De Carlo,
Monastero dei Benedettini
University of Catania

Catania, June 14-16 2017

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GRUPPO ITALIANO DI BIOMEMBRANE E BIOENERGETICA

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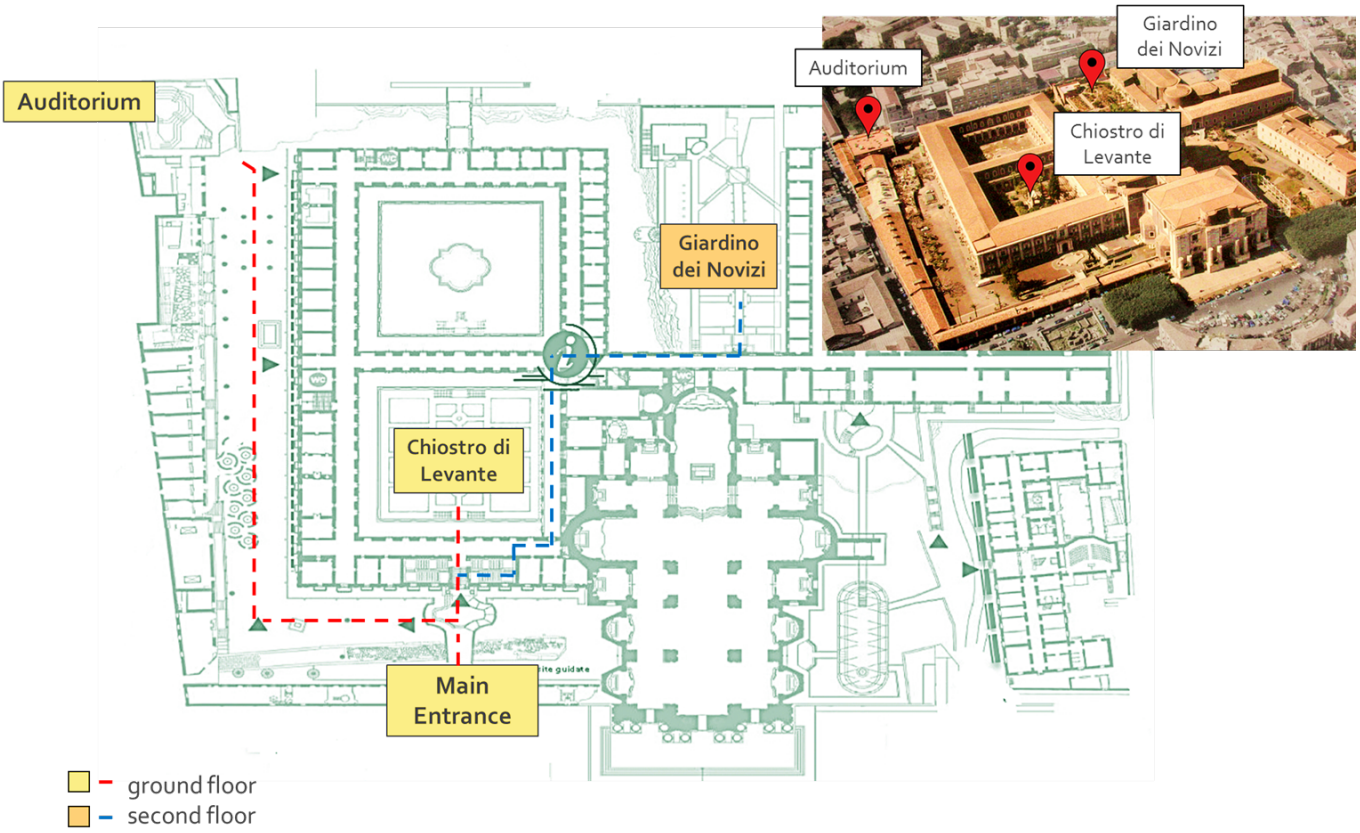
E-mail: catania2017@gibb-be.org; vdpbiofa@unict.it; fguarin@unict.it; mess@unict.it

Venue

Auditorium Giancarlo De Carlo
Monastero dei Benedettini di San Nicolò L'arena
Piazza Dante, 32 – Catania



Program

Wednesday 14th

13:00	***Registration***
15:00	***Welcome Address*** Authorities, GIBB President and the Organizing Committee
15:30	***Opening Plenary Lecture*** Chair: Paolo Bernardi (University of Padua, Italy) Sir John Walker, Nobel laureate (University of Cambridge, United Kingdom) *The respiratory chain in mitochondria: a triumph for biochemistry and biophysics*
16:30	***Session I: ATPsynthase and the complexes of respiratory chain*** *Oral Presentations* Chair: Giovanna Lippe (University of Udine, Italy), Nazareno Capitanio (University of Foggia)
16:30	Karin B. Busch (Westfälische Wilhelms-University of Münster, Germany) *Respiratory supercomplex assembly with CIV monitored by fluorescence lifetime imaging microscopy*
16:45	Clara Ferreira Pereira (University of Porto, Portugal) *Phosphoproteomic screening identifies ATP synthase beta subunit as a target of the yeast type 2A-like protein phosphatase Sit4p*
17:00	Danilo Faccenda (Royal Veterinary College, London, United Kingdom) *Control of mitochondrial bioenergetics and structure by the ATPase inhibitory factor 1: a pro-survival relay via OPA1*
17:15	Andrea Urbani (University of Padua, Italy) *Electrophysiological properties of channel formed by bovine FOF1 ATP synthase in planar lipid bilayer*
17:30	Lishu Guo (University of Padua and CNR Institute of Neuroscience, Italy) *Matrix Ca2+ is essential for opening of the mitochondrial permeability transition pore*
17:45	Marco Malferrari (University of Bologna, Italy) *The cytochrome b H291L mutation strongly impairs ubiquinol oxidation and proton translocation at the bacterial bc_1 Q_O site*

18:00	Karen Trchounian (Yerevan State University, Armenia) *Input of hydrogenases in H2 cycling and proton motive force generation in Escherichia coli during fermentation*
18:15	***End of Session***
18:30	***Welcome cocktail*** Giardino dei Novizi
20:00	***Guided tour of Monastero dei Benedettini***

Thursday 15th

9:00	***Plenary lecture*** Chair: Vito De Pinto (University of Catania, Italy) Prof. Doron Rapaport (University of Tübingen, Germany) *Biogenesis of mitochondrial outer membrane proteins in evolutionary context*
9:45	***Session II: Specialized transporters and pores, structure & function*** *Oral presentations* Chair: Cesare Indiveri (University of Calabria, Italy)
9:45	Vanessa Checchetto (University of Padua, Italy) *A new mitochondrial potassium channel involved in cardioprotection*
10:00	Simona Reina (University of Catania, Italy) *VDAC3 cysteine oxidation state and channel activity: in vitro and in cellulo studies*
10:15	Mariafrancesca Scalise (University of Calabria, Italy) *Structure/function relationships of human LAT1 amino acids transporter and screening of active-site targeted inhibitors*
10:30	Lara Console (University of Calabria, Italy) *Regulation of the mitochondrial carnitine/acylcarnitine carrier by S-nitrosylation*
10:45	Maria Carmela Di Rosa (University of Catania, Italy) *The second VDAC isoform in S. cerevisiae, an unknown protein: structural-functional characterization*
11:00	Angela Ostuni (University of Basilicata, Italy) *Structural and functional studies on multidrug resistance protein 6 (MRP6): new insights*
11:15	***Coffee break*** Chiostro di Levante

12:00	***Session III: Specialized transporters and pores, physiological and pathological roles*** *Oral presentations* Chair: Francesco Francia (University of Bologna)
12:00	Luigi Leanza (University of Padua, Italy) *Direct pharmacological targeting of a mitochondrial ion channel selectively kills tumor cells in vivo*
12.15	Daria Grobys (Adam Mickiewicz University in Poznan, Poland) *VDAC in viability and energetic coupling status of the inducible PC12 cell model of Huntington's disease*
12:30	Simona Barbato (University of Bologna, Italy) *Involvmente of miRNAs in the acquired BRAF-I resistance of metastatic melanoma cells*
12:45	Roberta Peruzzo (University of Padua, Italy) *Novel psoralen-derivatives with increased solubility in cancer treatment*
13:00	***BMR GENOMICS: Sponsor Talk***
13:30	***Lunch*** Chiostro di Levante
15:00	***Plenary lecture*** Chair: Alessandro Giuffrè (University of Rome La Sapienza, Italy) Dr. Christian Frezza (University of Cambridge, United Kingdom) *Mitochondrial dysfunction and cancer: beyond metabolism*
15:45	***Session IV: Bioenergetics metabolism and mitochondrial dysfunction*** *Oral presentations* Chair: Giancarlo Solaini (University of Bologna, Italy)
15:45	Ferdinando Chiaradonna (University of Milano-Bicocca, Italy) *Protein kinase A activation promotes cancer cell resistance to glucose starvation and anoikis*
16:00	Lara Gibellini (University of Modena and Reggio Emilia, Italy) *LonP1 differently modulates mitochondrial function and bioenergetics of primary versus metastatic colon cancer cells*
16:15	Giacomo Lazzarino (Catholic University of Rome, Italy) *The response of the mitochondrial quality control system to graded traumatic brain injury*
16:30	Francesca Malagrinò (CNR Institute of Molecular Biology and Pathology, Rome, Italy) *Novel pathogenic mechanisms from investigation of human hydrogen sulfide metabolism*

16:45	Marco Schiavone (University of Padua and CNR Institute of Neuroscience, Italy) *Alisporivir rescues mitochondrial respiratory dysfunction in Duchenne muscular dystrophy models*
17:00	***Coffee break*** Chiostro di Levante
17:30	***Session V: Reactive Oxygen Species and mitochondria*** *Oral presentations* Chair: Claudia Piccoli (University of Foggia, Italy)
17:30	Ginevra Nannelli (University of Siena, Italy) *The role of mitochondria in the maintenance of endothelial function after exposure to redox injury*
17:45	Giulia Gorini (University of Bologna, Italy) *Reactive oxygen species decrease in human cells adapted to hypoxia*
18:00	Marianna Tomasello (CNR Institute of Biostructures and Bioimaging, Catania, Italy) *Misfolded SOD1 mutant alters VDAC1-Hexokinase 1 complexes promoting mitochondrial dysfunction in an Amyotrophic Lateral Sclerosis model*
18:15	Andrea Magrì (University of Catania, Italy) *Hexokinases 1 peptide protects VDAC1 from SOD1 G93A mutant toxic interaction in an ALS model: implications for the neurodegenerative disease's treatment*
18:30	Gloria Scattolin (University of Padua, Italy) *Mitochondrial reactive oxygen species sensitize T-ALL cells to apoptosis by engaging the OMA1-OPA1 axis*
18:45	***GIBB Members Annual Meeting***
20:00	***Bus Meeting Point at piazza Dante (in front of Monastero)***
20:30	***Social Dinner*** Ristorante Feudo Delizia, Mount Etna

Friday 16th

9:15	***Plenary lecture*** Chair: Angela Messina (University of Catania, Italy) Prof. Laszlo Tretter (Semmelweis University , Budapest, Hungary) *Cyclophylin D KO protects against bioenergetic changes detected in Alzheimer's model (Tg(APPSwe)/Tg(PSEN1)) mice*

10:00	***Session VI: New and notable themes in Bioenergetics and Biomembranes*** *Oral presentations* Chair: Marcello Pinti (University of Modena & Reggio Emilia, Italy), Alexander Ruban (University of London, United Kingdom)
10:00	Alexander Ruban (University of London, United Kingdom) *Dynamic photosynthetic membrane*
10:15	Kylie A. Minor (University of Alberta, Canada) *Light-Driven Energy Production for Cell-Free Metabolic Systems*
10:30	Wolfgang Buckel (Philipps University of Marburg, Germany) *Flavin-based electron bifurcation in anaerobic Bacteria and Archaea*
10:45	Nunzio Iraci (University of Catania, Italy and of Cambridge, United Kingdom) *Extracellular vesicles are independent metabolic units with asparaginase activity*
11:00	Andonis Karachitos (Adam Mickiewicz University in Poznan, Poland) *Mitochondria monitoring in studies of successful anhydrobiosis*
11:15	***Coffee break*** Chiostro di Levante
11:45	Mary F. Rooney (Trinity College Dublin, Ireland) *Genotype variation at the MSTN locus is associated with skeletal muscle mitochondrial abundance and fibre composition in untrained Thoroughbred horses*
12:00	Claudio Laquatra (University of Padua, Italy) *Zebrafish (Danio rerio) as a model to study the pathophysiological role of the mitochondrial chaperone TRAP1*
12:15	Sarfarazhussain Farooqui (University of Vienna, Austria) *Analysis of the role of Letm1 in the development of zebrafish*
12:30	Martina La Spina (University of Padua and CNR Institute of Neuroscience, Italy) *Induction of autophagy and mitochondrial biogenesis by the natural compound Pterostilbene: mechanisms and biomedical potential*
12:45	Antonio Filippi (University of Udine, Italy) *Flavonoid transport in plant microsomes: characterization of quercetin uptake*
13:00	***Concluding remarks and awards***
13:30	***LunchBox*** Chiostro di Levante
14:30	***End of the meeting***

ABSTRACT BOOK

Professor Sir John Walker

University of Cambridge, United Kingdom

Prof. Walker studies the structure, function, regulation and assembly of the ATP synthase in mitochondria, and increasingly in bacteria. For providing the molecular basis of the rotary mechanism of the F1-catalytic domain, he was awarded the Nobel Prize in Chemistry in 1997. In 2008, he became the Founding Director of the Medical Research Council's Mitochondrial Biology Unit, in Cambridge, where he continues his research as Director Emeritus.

The respiratory chain in mitochondria: a triumph for biochemistry and biophysics

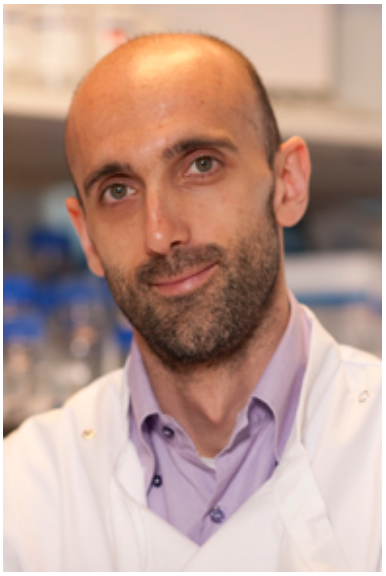
Professor Doron Rapaport

University of Tuebingen, Germany

Prof. Rapaport is studying mitochondrial biogenesis and dynamics. He graduated in Chemistry in 1988 at Tel Aviv University and obtained his PhD in Biophysics in 1995 at The Weizmann Institute of Science.
Currently, he heads a research group in the Interfaculty Institute of Biochemistry, University of Tuebingen.

Biogenesis of mitochondrial outer membrane proteins in evolutionary context

The mitochondrial outer membrane (MOM) mediates multiple interactions between the mitochondrial metabolic and genetic systems and the rest of the eukaryotic cell. Biogenesis of this membrane involves integration of newly synthesized proteins into the lipid bilayer. Among these precursor proteins are those that span the membrane once, twice or with multiple segments. Our aim is to fully define the biological processes and molecular mechanisms that underlie the biogenesis of MOM proteins. Employing *in vitro*, *in organello* and *in vivo* experiments we could characterize unique import pathways for the various families of MOM proteins. Remarkably, the biogenesis of β-barrel proteins is conserved from Gram–negative bacteria to mitochondria and this conservation allows the organelle to recognized and process bacterial substrates.



Doctor Christian Frezza

University of Cambridge, United Kingdom

Dr. Christian Frezza is working on the role of altered mitochondrial metabolism in cancer. He gained his MSc in Medicinal Chemistry at the University of Padova in 2002. He then got a PhD on mitochondrial dynamics and apoptosis in Padova. In 2008, he moved to Glasgow, where he investigated the role of mitochondrial defects in tumorigenesis. In 2012 he took up his current position as Group Leader in Cancer Metabolism at the MRC Cancer Unit in Cambridge, UK.

Mitochondrial dysfunction and cancer: beyond metabolism

Mutations of the tricarboxylic acid cycle (TCA cycle) enzyme fumarate hydratase (FH) cause the hereditary cancer syndrome Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC). FH-deficient renal cancers are highly aggressive and metastasize even when small, leading to an abysmal clinical outcome. The link between loss of FH and tumor formation is still under intense investigation. Evidence suggests that fumarate, a small molecule metabolite that accumulates in FH-deficient cells, may contribute to tumorigenesis in HLRCC. For instance, accumulation of fumarate has been associated with the stabilization of the Hypoxia Inducible Factors HIFs and to the activation of the antioxidant master regulator NRF2, via succination of its negative regulator Keap1. However, the contribution of these signaling cascades to tumorigenesis of HLRCC has been debated and the oncogenic role of fumarate still unclear. Here we used a multidisciplinary approach to investigate the consequences of the loss of FH and present evidence that FH-deficient cells undergo a fumarate-dependent epithelial-to-mesenchymal-transition, a phenotypic switch associated with cancer initiation, invasion, and metastasis. We propose that this phenotypic switch might prime cell to transformation and contribute to the tumorigenesis and metastatization of FH-deficient cancers.

Professor Laszlo Tretter

Semmelweis University, Budapest, Hungary

Prof. Tretter is interested on mitochondrial physiology, ROS homeostasis and metabolic biochemistry. He obtained M.D. and PhD degree at Semmelweis University where he is at present the head of Department of Medical Biochemisty.

Cyclophylin D KO protects against bioenergetic changes detected in Alzheimer's model (Tg(APPSwe)/Tg(PSEN1)) mice

Respiratory supercomplex assembly with CIV monitored by fluorescence lifetime imaging microscopy

Bettina Rieger[1,2], Daria Shalaeva[3], Anna-Carina Söhnel[1,2], Wladislaw Kohl[2], Patrick Duwe[1], Armen Y. Mulkidjanian[3,4,5] and Karin B. Busch[1,2]

1 Department of Biology, Westfälische Wilhelms-University of Münster, Münster (Germany); 2 School of Biology, University of Osnabrueck, Osnabrueck (Germany); 3 School of Physics, University of Osnabrueck, Osnabrueck (Germany); 4 A.N. Belozersky Institute of Physico-Chemical Biology; 5 School of Bioengineering and Bioinformatics, M.V. Lomonosov Moscow State University, Moscow (Russia).

buschkar@uni-muenster.de

Respiratory complexes CI, CIII and CIV are found in various stable supercomplex compositions when extracted from membranes, leading to the further assumption that these assemblies also have a specific functional role. Ensuing, it is of high interest to study respiratory supercomplexes also *in situ*. Here, we demonstrate that fluorescence lifetime imaging microscopy (FLIM) with a single fluorescent sensor is a versatile method for non-invasive live cell imaging of dynamic supercomplex formation. The sensor has to be localized at a position that is buried in an assembled supercomplex but exposed to the aqueous phase in a disassembled complex. According to structural information [1], Cox8a and Cox7c are subunits of CIV that fulfill this condition. Indeed, we found a significantly reduced fluorescence lifetime of a sensor placed at these subunits but not for a sensor at Cox4, where the C-terminus is localized more at the surface of CIV at the same side of the membrane [2]. Introduction of a linker between Cox8a and the fluorescent protein to lift the sensor out of the supercomplex resulted in prolonged fluorescence lifetime. The same effect was observed when the supercomplex assembly factors were downregulated. FRET between subunits of CIII and CIV confirmed the results [2]. Thus, respiratory supercomplex assembly in live cells can be monitored by use of fluorescent probes at critical positions. This complements the important biochemical and physiological studies performed with isolated or re-constituted supercomplexes and allows for following the dynamic supercomplex assembly in live cells.

[1] Letts JA *et al*, Nature 537 (2016) 644-648.
[2] Rieger B et al, Sci Rep 7 (2017) 46055.

Phosphoproteomic screening identifies ATP synthase beta subunit as a target of the yeast type 2A-like protein phosphatase Sit4p

Clara Pereira[1,2], Telma Martins[1,2], Andreia T. Pereira[2], Hugo Osório[1,4], Pedro Moradas-Ferreira[1,2,3] and Vitor Costa[1,2,3]

1 Instituto de Investigação e Inovação em Saúde, Universidade do Porto (Portugal); 2 IBMC Instituto de Biologia Celular e Molecular, Universidade do Porto (Portugal); 3 Departamento de Biologia Molecular, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (Portugal); 4 IPATIMUP Instituto de Patologia e Imunologia Molecular da Universidade do Porto (Portugal).

clara.pereira@ibmc.up.pt

Sit4p is the catalytic subunit of a type 2A-related protein phosphatase in *Saccharomyces cerevisiae*. It is involved in a wide spectrum of cellular functions, including the transcriptional repression of mitochondrial genes, which strongly impact on mitochondrial function [1]. In this study, the hypothesis that Sit4p may also regulate mitochondrial proteins post-translationally was investigated. For that, a bidimensional gel electrophoresis and immunoblotting procedure was used to identify mitochondrial proteins with increased phosphorylation in Sit4p deficient cells. Among 9 proteins identified, Atp2p, the β subunit of F1FoATP synthase complex, exhibited the most evident increase in phosphorylation. Using co-immunoprecipitation assays, we found that Sit4p and Atp2p interact physically. Using mass spectrometry, two Atp2p phosphorylation sites (Thr124 and Thr317) were identified in Sit4p deficient cells. Expressing Atp2-T124D or -T317D phosphomimetics resulted in higher Atp2p levels, accompanied by increased abundance and activity of the F1FoATP synthase complex. It also resulted in an increase in mitochondrial respiration, higher cellular ATP levels and improved growth in respiratory conditions. Our results reveal a role for Sit4p on the post-translational regulation of Atp2p which may play a role in the metabolic adaptation to distinct energetic demands.

[1] Jin C *et al*, FEBS Lett, 581 (2007) 5658-63.

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Control of mitochondrial bioenergetics and structure by the ATPase inhibitory factor 1: a pro-survival relay via OPA1

Danilo Faccenda[1,2], Junji Nakamura[3], Giulia Gorini[1], Gurtej K Dhoot[1], Rebeca Martín-Jiménez[1], Caterina Ferraina[4], Daniela Strobbe[4], Mauro Piacentini[4,5], Masasuke Yoshida[3], Claire Russell[1] and Michelangelo Campanella[1,2,4]

1 Department of Comparative Biomedical Sciences, Royal Veterinary College, London (UK); 2 UCL Consortium for Mitochondrial Research, University College London, London (UK); 3 Department of Molecular Bioscience, Kyoto Sangyo University, Kamigamo-Motoyama, Kyoto (Japan); 4 Department of Biological Sciences, University of Rome Tor Vergata, Rome (Italy); 5 National Institute for Infectious Diseases, IRCCS Lazzaro Spallanzani, Rome (Italy).

dfaccenda@rvc.ac.uk

The ATPase inhibitory factor 1 (IF_1) is a ubiquitously expressed, mitochondrial protein that is particularly abundant in high-metabolic-rate organs, such as heart, brain and liver. IF_1 is the primary endogenous inhibitor of the F_1F_o-ATPsynthase reversal. Therefore, it regulates metabolic adaptation to physiological stressors and prevents ATP depletion under ischaemia. Recent evidence suggests that the inhibitory protein plays other key roles in mitochondrial function. Indeed, several human carcinomas are characterized by an increased level of IF_1 expression, which provides survival advantage to cancer cells by enhancing evasion of apoptosis, invasion and chemoresistance. Whereas, loss of IF_1 activity is associated with alteration in mitochondrial iron assimilation and heme synthesis, leading to anaemia.
Here, we examined the effect of IF_1 on mitochondrial redox balance and cristae remodelling during apoptosis, and found that the anti-apoptotic protein maintains the antioxidant capacity of glutathione (GSH) and peroxiredoxin 3 (Prx3) systems through retention of mitochondrial ATP production. Alongside, IF_1 inhibits the stress-induced peptidase OMA1 and prevents the subsequent cleavage of the pro-fusion protein optic atrophy 1 (OPA1). IF_1-mediated preservation of the mitochondrial antioxidant status and stabilization of OPA1 are thereby key events in impeding mitochondrial derangement and completion of apoptosis.
This was further confirmed in IF_1 deficient zebrafish and mice, where the absence of functional IF_1 correlates with decreased levels of OPA1 in both brain and eye, causing neuronal loss and visual impairment.
We propose that IF_1 plays a role in the control of mitochondrial function through the dual-regulation of mitochondrial bioenergetics and structure. By promoting cell growth and survival, IF_1 not only is a relevant player in cancer, but might also be importantly involved in the physiology of highly proliferative tissue, such as the developing neural system.

Electrophysiological properties of channel formed by bovine F_OF_1 ATP synthase in planar lipid bilayer

Andrea Urbani[1], Christoph Gerle[2], Valentina Giorgio[1,3], Vanessa Checchetto[4], Paolo Bernardi[1,3] and Ildikò Szabò[4]

1 Department of Biomedical Sciences, University of Padova, Padova (Italy); 2 Laboratory of Protein Crystallography, Institute for Protein Research, Osaka University, Osaka (Japan); 3 CNR Institute of Neuroscience (Italy); 4 Department of Biology, University of Padova, Padova (Italy).

andrea.urbani@unipd.it

Permeability transition (PT) in mitochondria is triggered by Ca^{2+} and specific activators and finally leads to increased permeability to ions and solutes of the inner mitochondrial membrane (IMM). *In vitro* PT is followed by mitochondrial swelling that in turn prevents ATP synthesis. In the 1970s the idea was advanced that PT is mediated by a Ca^{2+}-regulated pore, named the Permeability Transition Pore (PTP). PTP was identified as a high-conductance channel by patch-clamping the IMM, named Mitochondrial MegaChannel (MMC). Despite many years of study and the key role of PTP opening in cell death and several diseases, the molecular nature of the Pore remains unclear. In 2013 Giorgio et al. provided evidence that dimers of F_OF_1 ATP synthase purified from native gels can form the PTP/MMC [1] but the exact mechanisms are still not clear. The present work was undertaken to further clarify the electrophysiological properties of the ATP synthase by means of planar lipid bilayer (PLB) single channel recordings, exploiting a ultra-pure preparation from bovine heart [2]. Bovine F_OF_1 ATP synthase complex was shown (i) to be intact and inclusive of all subunits, by means of SDS-PAGE and mass spectrometry; and (ii) to be active and highly sensitive to oligomycin (>95%). F_OF_1 ATP synthase was incorporated in the PLB and the channel activity was assessed in the presence of different concentrations of Ca^{2+} and Bz-423, a substance that is able to sensitize PTP to Ca^{2+}. We report evidence of channel activity with the MMC-PTP key features, including high conductance, activation by Ca^{2+}, complex gating kinetics, and inhibition by specific PTP compounds.

[1] Giorgio V *et al*, PNAS 110 (2013) 5887-9.
[2] Maeda S *et al*, Acta Cryst 69 (2013) 1368–1370.

Matrix Ca^{2+} is essential for opening of the mitochondrial permeability transition pore

Lishu Guo, Michela Carraro, Valentina Giorgio, Paolo Bernardi and Valeria Petronilli

Department of Biomedical Sciences, University of Padova and CNR Neuroscience Institute, Padova (Italy).

lishu.guo@studenti.unipd.it

Isolated mitochondria can undergo a dramatic increase of permeability to ions and solutes known as the permeability transition. The transition is mediated by the permeability transition pore (PTP), a cyclosporine A (CsA) sensitive channel, and is most easily observed after Ca^{2+} uptake in the presence of a wide variety of "inducing agents". Recently we proposed that dimers of F-ATP synthase form the permeability transition pore [1] and that Ca^{2+} binding to subunit β of F-ATP synthase induces a conformational change potentially leading to PTP opening [2]. However, although Ca^{2+} is essential for PTP opening, it has been reported that a CsA-sensitive PTP induction can be seen in the absence of added Ca^{2+} (or in the presence of EGTA) after the treatment with the SH reagent phenylarsine oxide (PhAsO) [3] or with the arginine reagent *p*-hydroxyphenylglyoxal (OH-PGO) [4]. We investigated whether these effects depend on matrix Ca^{2+} retained during the isolation of mitochondria. In this study we show that Ca^{2+} depletion with the ionophore A23187 or mitochondrial loading with the chelator BAPTA prevents PTP induction by PhAsO and OH-PGO.
Together with the finding that Ca^{2+} is an absolute requirement for opening of the megachannel in gel-purified F-ATP synthase preparations incorporated in lipid bilayers [1], these results support the conclusion that matrix Ca^{2+} is a unique "permissive" factor essential for PTP opening.

[1] Giorgio V *et al*, PNAS 110 (2013) 5887-5892.
[2] Giorgio V *et al*, EMBO Rep (2017) *in press*.
[3] Lenartowicz E *et al*, J Bioenerg Biomembr 23 (1991) 679-688.
[4] Linder MD *et al*, J Biol Chem 277 (2002) 937-942.

The cytochrome *b* H291L mutation strongly impairs ubiquinol oxidation and proton translocation at the bacterial bc_1 Q_O site

Marco Malferrari[1], Francesco Francia[2], Pascal Lanciano[3], Stefan Steimle[3], Fevzi Daldal[3] and Giovanni Venturoli[2]

1 Department of Chemistry Giacomo Ciamician, University of Bologna (Italy); 2 Department of Pharmacy and Biotechnologies, University of Bologna (Italy); 3 Department of Biology, University of Pennsylvania, Philadelphia (USA).

marco.malferrari2@unibo.it

The localization of metal ion binding sites was shown to be a valuable approach to identify proton pathways in redox membrane proteins. To this aim, the coordination of the Zn^{2+} binding site was elucidated by EXAFS spectroscopy in the avian, bovine and bacterial cyt bc_1 complex [1]. Specifically, putative ligand residues have been identified for the pseudo-octahedral site of *Rhodobacter capsulatus* and suggested to partecipate in the proton release which follows QH_2 oxidation at the Q_o catalytic site. To test this proposal, all the coordinating residues picked up by EXAFS have been mutated in non-protonatable residues. In a first screening, the H291L mutant was totally unable of photosynthetic growth. Analysis performed on native membranes showed that electron transfer from Q_o to the high potential FeS-cyt *c* chain, as well as reduction of cyt b_H, were strongly inhibited in the H291L mutant. Moreover, proton release associated with QH_2 oxidation at the Q_o site was also dramatically impaired. Based on these findings, on the putative role of His291 in liganding Zn^{2+}, and on its solvent exposed and highly conserved position, we propose that His291 of cyt *b* is critical for proton release associated to QH_2 oxidation at the Q_o site of cyt bc_1 [2].

[1] Giachini L *et al*, Biophys J 88 (2005) 2038–2046.
[2] Francia F *et al*, Biochim Biophys Acta 1857 (2016) 1796-1806.

Input of hydrogenases in H_2 cycling and proton motive force generation in *Escherichia coli* during fermentation

Karen Trchounian[1,2] and Armen Trchounian[1,2]

1 Scientific-Research Institute of Biology, Faculty of Biology, Yerevan State University, Yerevan (Armenia); 2 Department of Biochemistry, Microbiology, and Biotechnology, Faculty of Biology, Yerevan State University, Yerevan (Armenia).

k.trchounian@ysu.am

Escherichia coli has the capacity to encode four membrane bound [Ni-Fe] hydrogenases (Hyd) catalyzing the redox reaction of $2H^+ + 2e^- \rightarrow H_2$. All hydrogenases are reversible: depending on pH and carbon source they can work in H_2 uptake or producing mode. It has been proposed that the activity of Hyd enzymes and their working direction also depend on H_2 cycling through the membrane. Hyd enzymes form H_2 cycle during glucose or glycerol fermentation at pH of 5.5-7.5. Moreover, it was shown that deletion of three of the Hyd enzymes disturbs H_2 cycling. Probably, Hyd enzymes by forming H_2 cycle and redox chain to the proton F_0F_1 ATPase are working towards neutralizing the end acids, equilibrating the cytoplasmatic pH and involved in proton motive force generation.

It was shown that at pH 7.5 during glycerol fermentation in *hypF* mutant (all Hyd enzymes are absent) proton motive force generated is higher (122 mV) compared to wild type (99 mV). In contrast at pH 5.5 in *hypF* mutant it was lower than in wild type by ~20 mV. In addition, Hyd-1 and Hyd-2 activity has been shown to depend on the activity of F_0F_1 ATPase at extreme pHs (pH 5.5 and pH 7.5) during glucose or glycerol fermentation. Taken together it might be concluded that besides F_0F_1 ATPase Hyd enzymes also are involved in proton motive force generation during fermentation. H_2 cycling as new membrane-associated cycle contributes towards regulation of cytoplasmatic pH by producing or uptaking H_2. It is suggested that Hyd enzymes might be one of the main H^+ sensing systems in the cell during fermentative conditions.

A new mitochondrial potassium channel involved in cardioprotection

Vanessa Checchetto[1], Angela Paggio[2], Diego De Stefani[2], Rosario Rizzuto[2] and Ildikò Szabò[1]

1 *Department of Biology, University of Padova, Padova (Italy)*; 2 *Department of Biomedical Sciences, University of Padova, Padova (Italy).*

vanessa.checchetto@unipd.it

Mitochondrial ion channels are of great importance to ensure the proper function of this bioenergetic organelle and to regulate cell fate. However, in many cases our knowledge concerning their molecular identity and regulation is still limited. Recently, we characterized a novel protein complex located in the inner mitochondrial membrane that displays all known pharmacological and electrophysiological properties of the long-sought mitochondrial K_{ATP} (mito K_{ATP}) channel. An important role has been assigned to mito K_{ATP}, especially in the heart, in protection from I/R injury, pre-conditioning and post-conditioning but its composition, properties, regulation and function constitute the most controversial topic in the field of mitochondrial channels.

VDAC3 cysteine oxidative state and channel activity: *in vitro* and *in cellulo* studies

Simona Reina[1], Rosaria Saletti[2], Maria Gaetana Giovanna Pittalà[3], Serena Sineri[1], Federica Zinghirino[1] and Vito De Pinto[3]

1 Department of Biological, Geological and Environmental Sciences, Section of Molecular Biology, University of Catania, Catania (Italy); 2 Department of Chemical Sciences, University of Catania, Catania (Italy); 3 Department of Biomedical and Biotechnological Sciences, University of Catania, Catania (Italy).

simonareina@yahoo.it

Voltage-Dependent Anion selective Channels (VDAC) are pore-forming proteins located in the outer mitochondrial membrane. Their roles range from the formation of a hydrophilic conduit through the membrane thanks to its beta-barrel structure, to less understood functions that make them key actors in the cross-talk between the mitochondrial metabolism and the whole cell. In this regard, despite conserved sequences, similar structures, and the same gene organization, VDAC3 is the least characterized among the three isoforms that exist in mammals. Since the recent discovery of its peculiar channel activity [1], attention has been focused on unraveling the reasons of this surprising behavior. One of the most plausible hypothesis seems to reside in the greater number of VDAC3 cysteine residues compared to VDAC1. Based on bioinformatics prediction, all of the six VDAC3 cysteines would indeed be exposed towards the intermembrane space [2], making them a preferred target for oxidation by Reactive Oxygen Species. Accordingly, mass spectrometry analysis revealed that each cysteine residue exists in a set of different oxidation states within the same molecule [2, 3]. Moreover, studies based on the selective deletion of VDAC3 cysteines followed by *in vitro* conductance experiments and complementation assays in Δporin1 yeast, highlighted their role in the function of the protein [3]. All together, these data provide evidence for a direct involvement of cysteines in VDAC3 activity, raising the possibility that this isoform could not be functioning as a simple channel within the cell.

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Structure/function relationships of human LAT1 amino acids transporter and screening of active-site targeted inhibitors

Mariafrancesca Scalise[1], Lara Napolitano[1], Michele Galluccio[1], Ivano Eberini[2], Panayiotis Koutentis[3], Angelo Carotti[4] and Cesare Indiveri[1]

1 Department DiBEST (Biologia, Ecologia, Scienze della Terra) Unit of Biochemistry and Molecular Biotechnology, University of Calabria, Arcavacata di Rende (Italy); 2 Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milano (Italy); 3 Department of Chemistry, University of Cyprus, Nicosia (Cyprus); 4 Dipartimento Farmaco-Chimico, Università degli Studi Aldo Moro, Bari (Italy).

mariafrancesca.scalise@unical.it

LAT1 (SLC7A5) is a plasma membrane transporter for neutral and branched amino acids; it belongs to the Heterodimeric Amino acid Transporter group (HATs) and forms a heterodimer with the glycoprotein CD98 (SLC3A2). LAT1 mediates an antiport reaction with a random simultaneous mechanism. The great interest on LAT1 is linked to its over-expression in many cancers that are “Glutamine addicted”, needing a lot of Gln for their peculiar energy metabolism. In this scenario, LAT1 gives rise to a Gln/Leu cycle together with another transporter, ASCT2 feeding cancer cells. Then, LAT1 is considered as a pharmacological target. The design of good inhibitors for hLAT1 is challenged by the lack of 3D X-ray structure. Only homology models, built on the basis of the AdiC from *E.coli,* are available. Recently, the bacterial over-expression of recombinant hLAT1 and its reconstitution in proteoliposomes has been achieved. Key residues for substrate binding and translocation have been identified by a combined approach of site-directed mutagenesis, proteoliposome transport assay and bioinformatics: F252, S342, C335 and C407 are the most critical residues. Then, the presence of Cys residues in the binding site has been exploited to design inhibitors with higher potency and stability with respect to those so far described. A screening of a number of dithiazoles, which react with thiol groups, has been performed; among tested compounds, eight have been identified as best inhibitors with IC50 in the micromolar range. Prediction by bioinformatics suggests that inhibition is due to mixed disulfide formation with at least one Cys residue of the protein. The specific C407A mutant, indeed, showed decreased reactivity towards dithiazoles indicating that this residue is involved in binding. Interestingly, the most potent inhibitors were also able to impair viability of cancer cells expressing high levels of LAT1. These studies open new important perspectives for cancer therapy.

Regulation of the Mitochondrial Carnitine/Acylcarnitine Carrier by S-Nitrosylation

Lara Console[2], Annamaria Tonazzi[1], Nicola Giangregorio[1] and Cesare Indiveri[1,2]

1 CNR Institute of Biomembranes and Bioenergetics, Bari (Italy); 2 Department DiBEST (Biologia, Ecologia, Scienze della Terra) Unit of Biochemistry and Molecular Biotechnology, University of Calabria, Arcavacata di Rende (Italy).

consolelara@gmail.com

Nitric Oxide (NO) modulates protein functions by post-translational modifications. One of the most diffuse mechanism consists in S-nitrosylation of Cys residues of target proteins. The mitochondrial carnitine/acylcarnitine carrier (CAC) possesses six Cys, some of which are more reactive towards SH reagents. This transporter plays a central role in the mitochondrial β-oxidation of fatty acids. The possible regulation of CAC function by S-nitrosylation was studied by treating the transporter with the NO-releasing compound, nitrosoglutathione, and testing the effect on transport activity in proteoliposomes reconstituted with the native or the recombinant rat CAC. NO inhibited transport with an IC50 of 74 or 71 µM, respectively. The parallel occurrence of S-nitrosylation was revealed by an anti Cys-NO antibody by WB analysis. To obtain information of NO effect under more physiological condition, similar experiments were performed with intact mitochondria. Also in this case, inhibition of carnitine transport parallel to S-nitrosylation were found. Interestingly, after incubation of the protein with DTE, both inhibition and S-nitrosylation were abolished, indicating that the modification was reversible and that the reaction mechanism was based on Cys modification. This was then established by site-directed mutagenesis. Substitution of a specific Cys residue (C136) with Val, abolished the inhibitory effect of PTM. While, a multiple mutant containing only C136 out of the six Cys residues, behaved as the wild type. Therefore, C136 was identified as the target of NO. This residue is conserved in the CAC members of higher animals and plants but not in insects or unicellular eukaryotes. Inhibition of CAC occurs at NO concentrations present in cells during ischemic conditions, thus suggesting that nitrosylation may play an important role in controlling oxidation of fatty acids during altered cell metabolism thus limiting production of ROS.

The second VDAC isoform in *S. cerevisiae*, an unknown protein: structural-functional characterization

Maria Carmela Di Rosa[1], Andrea Magrì[1], Simona Reina[1], Carlo Guardiani[2], Matteo Ceccarelli[3], Desirèe Nicotra[1] and Vito De Pinto[4]

1 Department of Biological, Geological and Environmental Sciences, Section of Molecular Biology, University of Catania, Catania (Italy); 2 School of Engineering, University of Warwick, Coventry (UK); 3 Department of Physics, University of Cagliari, Cagliari (Italy); 4 Department of Biomedical and Biotechnological Sciences, University of Catania, Catania (Italy).

mcdirosa@unict.it

VDAC (Voltage Dependent Anion Channel) is the main channel of outer mitochondrial membrane (OMM) and mediates the exchanges of metabolites and ions between cytosol and inner of mitochondria [1]. This protein is highly conserved in the evolution and was studied in many different species. In yeast *S. cerevisiae* there are two genes encoding two VDAC isoforms: por1, encoding the main isoform (yVDAC1), homologue to human VDAC1, and most abundant on the OMM, was well characterized. The second gene por2 encodes the second isoform, a putative porin unknow molecular and functional. *S. cerevisiae* deleted of yVDAC1 (Δpor1 strain) is unable to growth on not fermentable carbon source, as glycerol, at the restrictive temperature of 37°C. This suggests the existence of another mechanism that ensures the metabolites flux through the OMM and a possible function for yVDAC2. The overexpression of yVDAC2 in Δpor1 strain complements yVDAC1 absence [2]. Overexpression is not a physiological compensative mechanism, since in Δpor1 strain the presence of por2 gene does not support the yeast growth, but an external stimulus has to be supplied to raise yVDAC2 levels and complement the growth impairment [3]. In this work we have purified and characterized the recombinant yVDAC2 by electrophysiological methods. Furthermore, *in silico* computational studies have confirmed our results, highlighting many structural differences with respect to VDAC1, that could influence the yVDAC2 own functionality. The conclusion is that yVDAC2 has similar, but not identical, features with yVDAC1.

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Structural and functional studies on multidrug resistance protein 6 (MRP6): new insights

Angela Ostuni, Rocchina Miglionico, Flavia Cuviello, Maria Francesca Armentano, Maria Antonietta Castiglione Morelli, Monica Carmosino, Magnus Monnè and Faustino Bisaccia

Department of Sciences, University of Basilicata, Potenza (Italy).

angela.ostuni@unibas.it

MRP6 protein is codified by the ABCC6 gene belonging to the ATP-binding cassette (ABC) transporters superfamily that use energy released from ATP hydrolysis to transport substrates across membranes [1]. Mutations of ABCC6 gene cause, through an unknown pathophysiological mechanism, pseudoxanthoma elasticum, a genetic disorder characterized by ectopic calcification of connective fibers. ABCC6 is mainly expressed in liver and kidney and confers low level resistance to some anticancer drugs but its natural substrate remain undefined. The MRP6 structure consists of two transmembrane domains, two nucleotide binding domains [2, 3] and an additional N-terminal transmembrane domain (TMD0) connected to core of the protein through the loop L0. TMD0 has five TMs and a Nout – Cin orientation [4]; L0 is involved in the basolateral membrane localization of the entire protein [5]. Stable ABCC6 knockdown HepG2 cells showed dysregulation of pro- and anti-mineralization genes [6], a typical feature of replicative senescence and reductive state [7]. All these findings certainly allow us to re-evaluate the role of MRP6 both in physiological and pathological conditions.

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Direct pharmacological targeting of a mitochondrial ion channel selectively kills tumor cells *in vivo*

Luigi Leanza[1], Andrea Mattarei[2], Katrin Anne Becker[3], Michele Azzolini[4,5], Lucia Biasutto[4,5], Roberta Peruzzo[1], Livio Trentin[6], Mario Zoratti[4,5], Erich Gulbins[3], Cristina Paradisi[2] and Ildikò Szabò[1]

1 Department of Biology, University of Padova, Padova (Italy); 2 Department of Chemical Sciences, University of Padova, Padova (Italy); 3 Department of Molecular Biology, University of Duisburg-Essen, Essen (Germany); 4 CNR Institute of Neurosciences, Padova (Italy); 5 Department of Biomedical Sciences, University of Padova, Padova (Italy); 6 Department of Medicine, University of Padova, Padova (Italy).

luigi.leanza@unipd.it

Mitochondria are important oncological targets due to their crucial role in apoptosis. Our work identifies a novel therapeutic tool that simultaneously exploits both the high expression of the potassium channel Kv1.3 in the mitochondria of various types of cancer cells and the characteristic altered redox state of malignant cells, thereby leading to the selective elimination of pathological cells by two mitochondria-targeted Kv1.3 inhibitors. Indeed, the inhibition of mitochondrial Kv1.3 by two novel drugs alters mitochondrial function and leads to ROS-mediated cell death. These inhibitors killed 98% of *ex vivo* primary chronic B-lymphocytic leukemia tumor cells while sparing healthy B cells. In orthotopic mouse models of melanoma and pancreatic ductal adenocarcinoma, the compounds reduced tumor size, via ROS-mediated selective apoptosis of cancer cells, by more than 90% and 60%, respectively, without causing significant side effects of healthy tissues, like immune-depression, cardiac toxicity or histological alteration. These findings thus offer the perspective of a major advance in the pharmacological treatment of some high-impact, poor-prognosis cancers.

VDAC in viability and energetic coupling status of the inducible PC12 cell model of Huntington's disease

Daria Grobys, Andonis Karachitos, Klaudia Kulczyńska, Adrian Sobusiak and Hanna Kmita

Laboratory of Bioenergetics, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznan (Poland).

dg33114@amu.edu.pl

Huntington disease (HD) is an autosomal-dominant and fatal neurodegenerative disorder characterized by a selective loss of neurons. It results from CAG trinucleotide repeat expansion in exon 1 of *HTT* gene encoding huntingtin (Htt). As the trinucleotide codes for glutamine, its repeat number higher than 35 results in an abnormally long polyglutamine tract in Htt N terminus that gives rise to its mutant form (mHtt). It is now obvious that mitochondria play a vital role in HD pathogenesis but the underlying mechanism is still elusive. Moreover, the functional relationships of Htt and mHtt to mitochondria are still uncertain. Therefore we decided to investigate the role of the voltage-dependent anion channel (VDAC) in Htt and/or mHtt effect on mitochondria. VDAC transports inorganic ions and metabolites across the outer mitochondrial membrane and is regarded as a dynamic regulator, or even governor, of mitochondrial functions. To study VDAC putative contribution, we applied PC12 model of HD, which is based on PC12 cell lines derived from a pheochromocytoma of the rat adrenal medulla and consists of PC-12HD-Q23 and PC-12HD-Q74 cells with inducible (by doxycycline) and monitored due to GFP labeling, expression of Htt and mHtt, respectively. The obtained results including analysis of reconstituted VDAC properties as well as the model cell viability and their energetic coupling status indicate that VDAC may constitute an important element of cytotoxic effect triggered by mHtt.

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Involvement of miRNAs in the acquired BRAF-I resistance of metastatic melanoma cells

Simona Barbato[1], Alessandra Baracca[1], Giancarlo Solaini[1] and Muller Fabbri[2]

1 Department of Biomedical and Neuromotor Sciences, Laboratory of Biochemistry and Mitochondrial Pathophysiology, University of Bologna, Bologna (Italy); 2 Departments of Pediatrics and Molecular Microbiology & Immunology, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Children's Center for Cancer and Blood Diseases and The Saban Research Institute, Children's Hospital Los Angeles, Los Angeles (USA).

simona.barbato3@unibo.it

Metastatic Melanoma is a highly aggressive cancer, with a median overall survival for patients with stage IV melanoma ranging from 8 to 18 months. The most common mutation $BRAF^{V600E}$, that induce a constitutive activation of the MAPK/ERK pathway, can be inhibited by small-molecule kinase inhibitors, among which Vemurafenib was approved in 2011 as the major breakthrough in the treatment of unresectable or metastatic melanoma. Unfortunately, responses to BRAF inhibitors are short lived, with evidence of disease relapse within 6-8 months after the beginning of therapy, due to the development of acquired drug resistance, along with mitochondrial bioenergetic rearrangement of resistant cells [2]. Intriguingly, several oncogenic signaling pathways support tumorigenesis by modulating micro-RNAs (miRNAs) [3] and data in the literature have strongly implicated aberrant miRNA expression in anticancer drug resistance and sensitivity. Nevertheless, only limited data exist on the implication of miRNAs in malignant melanomas and little is still known about the factors underlying the resistance to BRAF inhibition and their role in the emergence of metabolically privileged cell phenotypes.
On the basis of our preliminary results, we herein propose a miRNAs-mediated mechanism driving the acquired resistance to BRAF-I therapy and set the stage for further elucidation on the role of miRNAs as metabolic switchers in human metastatic melanoma cells.

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Novel psoralen-derivatives with increased solubility in cancer treatment

Roberta Peruzzo[.1], Michele Azzolini[2,3], Matteo Romio[4], Katrin A Becker[5], Andrea Mattarei[4], Mario Zoratti[2,3], Erich Gulbins[5], Cristina Paradisi[4], Luigi Leanza[1] and Ildikò Szabò[1,2]

1 Department of Biology, University of Padova, Padova (Italy); 2 CNR Institute of Neurosciences, Padova (Italy); 3 Department of Biomedical Sciences, University of Padova, Padova (Italy); 4 Department of Chemical Sciences, University of Padova, Padova (Italy); 5 Department of Molecular Biology, University of Duisburg-Essen, Essen (Germany).

roberta.peruzzo@studenti.unipd.it

Ion channels are emerging as new oncological targets. Indeed, several ion channels show a different expression pattern in normal and cancer cells. The potassium channel Kv1.3 has a multiple sub-cellular localization, including both in the plasma membrane and in the inner mitochondrial membrane. Pharmacological inhibition of the mitochondrial channel (mtKv1.3), but not of the plasma membrane channel, by membrane permeant blockers, Psora-4, PAP-1 and clofazimine, triggered apoptosis in different cancer cells. Cell death occurred even in the absence of Bax and Bak, by inducing mitochondrial membrane depolarization, production of mitochondrial ROS and release of cytochrome c. Downregulation by siRNA of Kv1.3 prevented all these effects, indicating specificity. Since membrane permeant Kv1.3 inhibitors are characterized by poor water solubility, in order to increase their bioavailability as well as their solubility, we have recently synthesized a few more soluble PAP-1 derivatives. The new derivatives have been found to selectively kill cancer cells and even *in vivo* in a mouse melanoma preclinical model, without inducing any side effect.

Protein Kinase A activation promotes cancer cell resistance to glucose starvation and *Anoikis*

Roberta Palorini[1,2*], Giuseppina Votta[1*], Humberto De Vitto[1], Francesca Ricciardiello[1], Karsten Hiller[2] and Ferdinando Chiaradonna [1]

1 Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan (Italy); 2 Luxembourg Centre for Systems Biomedicine, Esch-sur-Alzette (Luxembourg).
[*] Equal contribution

ferdinando.chiaradonna@unimib.it

Cancer cells often rely on glycolysis to obtain energy and support anabolic growth. Several studies showed that glycolytic cells are susceptible to cell death when subjected to low glucose availability or to lack of glucose. However, some cancer cells, including glycolytic ones, can efficiently acquire higher tolerance to glucose depletion, leading to their survival and aggressiveness. Although increased resistance to glucose starvation has been shown to be a consequence of signaling pathways and compensatory metabolic routes activation, the full repertoire of the underlying molecular alterations remain elusive. Using omics and computational analyses, we found that cAMP-PKA axis activation is fundamental for cancer cells resistance to glucose starvation and *anoikis*. Notably, here we show that such a PKA-dependent survival is mediated by parallel activation of autophagy and glutamine utilization that in concert concur to attenuate the ER stress and to sustain cell anabolism. Indeed, the inhibition of PKA-mediated autophagy or glutamine metabolism increased the level of cell death, suggesting that the induction of autophagy and metabolic rewiring by PKA is important for cancer cellular survival under glucose starvation. Importantly, both processes, together with an increased mitochondrial activity, actively participate to cancer cell survival mediated by suspension-activated PKA as well. In addition we identify also a PKA/Src mechanism capable to protect cancer cells from *anoikis*. Our results reveal for the first time the role of the versatile PKA in cancer cells survival under chronic glucose starvation and *anoikis* and may be a novel potential target for cancer treatment.

LonP1 differently modulates mitochondrial function and bioenergetics of primary versus metastatic colon cancer cells

Lara Gibellini[1], Lorena Losi[2], Sara De Biasi[2], Milena Nasi[3], Domenico Lo Tartaro[3], Simone Patergnani[4], Gianluca Carnevale[3], Luca Roncucci[5], Andrea Cossarizza[1] and Marcello Pinti[2]

1 Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Modena (Italy); 2 Department of Life Sciences, University of Modena and Reggio Emilia, Modena (Italy); 3 Department of Surgery, Medicine, Dentistry and Morphological Sciences, University of Modena and Reggio Emilia, Modena (Italy); 4 Department of Morphology, Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology and LTTA Center, University of Ferrara, Ferrara (Italy); 5 Department of Diagnostic, Clinical Medicine and Public Health, University of Modena and Reggio Emilia, Modena (Italy).

lara.gibellini@unimore.it

Mitochondrial (mt) Lon protease (LonP1) is a multi-function enzyme located in the mt matrix, able to behave as a critical regulator in several human cancer, including colorectal cancer (CRC). The mechanisms at the basis of LonP1 contribution to colorectal carcinogenesis are not fully understood. Previous data indicated that LonP1 is closely related to survival in patients with CRC, and that silencing LonP1 in colon cancer cell lines results in severe mt impairment and apoptosis. Here, we investigated the role of LonP1 in mt functions in colon primary tumour cells and in metastasis. First, LonP1 expression was quantified by western blot both in formalin-fixed paraffin-embedded CRC tissues and fresh frozen aberrant crypt foci (ACF), adenoma (Ad) and CRC. Second, the effects of LonP1 overexpression on mt structure, functions and bioenergetics, and on cell metabolism were analysed in four colon cell lines. *Ex vivo*, LonP1 was almost absent in normal mucosa, gradually increased from samples of ACF to Ad, and was most abundant in samples of CRC. *In vitro*, LonP1 overexpression leads to a significant reduction of oxygen consumption rate, and this occurred in SW620 metastatic colon cancer cells rather than in SW480 primary colon cancer cells. Overexpression of LonP1 in SW620 cells determines depolarized mitochondria, reduced levels of mt superoxide and altered mt ultrastructure. LonP1 overexpression in SW480 cells primarily affects the expression of proteins involved in glycolysis, including lactate dehydrogenase and glucose transporter-1. To investigate whether metabolism reshaping by LonP1 reflects modification in cell aggressiveness, the levels of proteins involved in epithelial-mesenchymal transition were quantified in SW480 and SW620 overexpressing LonP1. Overexpression of LonP1 in SW480 cells leads to significantly lower levels of E-cadherin and higher levels of β-ctn. Conversely, overexpression of LonP1 in SW620 cells was associated with lower levels of β-ctn.

The response of the mitochondrial quality control system to graded traumatic brain injury

Giacomo Lazzarino[1], Angela M. Amorini[1], Valentina Di Pietro[2], Antonio Belli[2] and Barbara Tavazzi[1]

1 *Institute of Biochemistry and Clinical Biochemistry, Catholic University of Rome, Rome (Italy); 2 Neuroscience and Ophthalmology Research Group, Institute of Inflammation and Ageing, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham (UK).*

g.lazzarino@libero.it

Mitochondrial dynamics are regulated by a complex system of proteins representing the mitochondrial quality control (MQC). MQC balances antagonistic forces of fusion and fission determining mitochondrial and cell fates. In chronic and acute neurodegenerations, such as traumatic brain injury (TBI), the leading cause of death below 45 years of age in Western countries, dysfunctional mitochondria are thought to contribute to the pathophysiological mechanisms of cell damage. We previously found that mild TBI (mTBI) causes a transient mitochondrial malfunctioning affecting their phosphorylating capacity, a transitory imbalance in tissue antioxidants and a temporary glucose dysmetabolism. Differently, sTBI produced permanent mitochondrial malfunctioning with decreased phosphorylating capacity and profound ATP depletion, permanent decrease in GSH [58], permanent glucose dysmetabolism with marked hyperglycolysis and excitotoxicity caused by increase in glutamate and excitatory amino acids, as well as a decrease of N-acetylaspartate.
Using brain samples from these cohorts of animals (controls, mTBI and sTBI rats), we here evaluated the main gene and protein expression involved in the MQC system. At 6, 24, 48 and 120 hours after mTBI or sTBI, gene and protein expressions of fusion and fission were measured in brain tissue homogenates.
Results showed that genes and proteins inducing fusion or fission were upregulated and downregulated, respectively, in mTBI, but downregulated and upregulated, respectively, in sTBI. In particular, OPA1, regulating inner membrane dynamics, cristae remodelling, oxidative phosphorylation, was post-translationally cleaved generating mostly long or short OPA1 in mTBI and sTBI, respectively. Corroborated by data on citrate synthase, these results confirm the transitory (mTBI) or permanent (sTBI) mitochondrial dysfunction, enhancing MQC importance to maintain cell functions and indicating in OPA1 an attractive potential therapeutic target for TBI.

Novel pathogenic mechanisms from investigation of human Hydrogen Sulfide metabolism

Francesca Malagrinò[1,2], Henrique G. Colaço[3], Karim Zuhra[1,2], Paulo E. Santo[4,5], Elena Forte[2], André Gutierres[4], Marzia Arese[2], Tiago M. Bandeiras[4,5], Paolo Sarti[2], Paula Leandro[6], José A. Brito[4], João B. Vicente[4] and Alessandro Giuffrè[1]

1 CNR Institute of Molecular Biology and Pathology, Rome (Italy); 2 Department of Biochemical Sciences, Sapienza University of Rome (Italy); 3 Instituto Gulbenkian da Ciência, Oeiras (Portugal); 4 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras (Portugal); 5 Instituto de Biologia Experimental e Tecnológica, Oeiras (Portugal); 6 Research Institute for Medicines and Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon (Portugal).

francesca.malagrino@uniroma1.it

Hydrogen sulfide (H_2S), along with nitric oxide (NO) and carbon monoxide (CO), belongs to a small group of gaseous signalling molecules, collectively termed 'gasotransmitters'. H_2S has been recognized to play a regulatory role in cell bioenergetics acting as a Janus-faced molecule. The gas is indeed able to inhibit complex IV (COX) in the mitochondrial respiratory chain at higher levels, but also to stimulate ATP synthesis at lower concentrations acting as a respiratory substrate [1]. H_2S synthesis and catabolism are accomplished, partly in the mitochondrion, by enzymes that are currently investigated by our group in collaboration with João Vicente (ITQB-UNL, Portugal). H_2S is involved in important patho-physiological processes; several pathologies are indeed associated with alterations of H_2S metabolism, including oncologic, neurodegenerative and metabolic diseases. Among these, classical homocystinuria, a not yet fully understood metabolic disease, results from mutations in the gene encoding a key enzyme controlling the homocysteine and H_2S levels in humans, the pyridoxal 5'-phosphate (PLP)-dependent and heme-containing cystathionine β-synthase (CBS). CBS is positively regulated by S-adenosyl-L-methionine (AdoMet), but fully inhibited by CO or NO• [2-3]. Here we report that the p.P49L CBS variant, as recombinantly produced in *E. coli* and purified, displays both an impaired H_2S-generating activity rescued by PLP supplementation along the protein purification and, surprisingly, a markedly increased affinity of the ferrous heme for CO, likely leading to enzyme inhibition at physiological CO concentrations [4]. We suggest that the enhanced propensity to CO inhibition documented here for the p.P49L CBS variant could represent a novel pathogenic mechanism in classical homocystinuria.

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Alisporivir rescues mitochondrial respiratory dysfunctions in Duchenne muscular dystrophy models

Marco Schiavone[1*], Alessandra Zulian[1*], Sara Menazza[1], Francesca Sardone[1], Valeria Petronilli[1], Francesco Argenton[2], Luciano Merlini[3], Patrizia Sabatelli[4] and Paolo Bernardi[1*]

1 Department of Biomedical Sciences and CNR Neuroscience Institute, University of Padova, Padova (Italy); 2 Department of Biology, University of Padova, Padova (Italy); 3 Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna (Italy); 4 CNR Institute of Molecular Genetics and Laboratory of Musculoskeletal Cell Biology, Istituto Ortopedico Rizzoli-IRCCS, Bologna (Italy).
[*]Equal contribution

marco.schiavone@unipd.it; alessandra.zulian@unipd.it

Duchenne muscular dystrophy (DMD) is a devastating muscle disease of known ethiology without effective, or generally applicable therapy. Mitochondria are affected by the disease in animal models (hamster, chicken and mouse) but whether mitochondrial dysfunction is part of the pathogenesis in patients has not been addressed before. We show that primary cultures obtained from muscle biopsies of DMD patients (i) display a dramatic decrease of respiratory reserve capacity and (ii) are prone to mitochondrial dysfunction due to opening of the permeability transition pore (PTP). Treatment with the cyclophilin inhibitor Alisporivir - a cyclosporin A derivative that desensitizes the PTP but does not inhibit calcineurin - largely restored the maximal respiratory capacity without affecting basal oxygen consumption in cell from patients, thus effectively reinstating a normal respiratory reserve. Treatment with Alisporivir, but not with cyclosporin A, led to a striking recovery of muscle structure and respiratory function matching improved survival of *sapje* zebrafish, a severe model of DMD where the ultrastructural muscle defects are close to those of DMD patients. Alisporivir has an excellent safety profile and could be used for treatment of DMD.

The role of mitochondria in the maintenance of endothelial function after exposure to redox injury

Ginevra Nannelli[1], Valentina Giorgio[2], Sandra Donnini[1], Daria Mochly-Rosen[3], Paolo Bernardi[2] and Marina Ziche[1]

1 Department of Life Sciences, University of Siena, Siena (Italy) 2 Department of Biomedical Sciences, University of Padova, Padova (Italy); 3 Department of Chemical and Systems Biology, Stanford University, Stanford (USA).

marina.ziche@unisi.it

Even though endothelial cells (ECs) appear to meet most of their energy needs anaerobically, they have an extensive mitochondrial network and consume oxygen. Their mitochondria are coupled and possess a considerable respiratory reserve, which is important in the response to oxidative stress, but the mechanistic aspects of the crosstalk between metabolism and endothelial function have only started to become appreciated, with numerous questions remaining to be addressed [1]. Mitochondrial aldehyde dehydrogenase (ALDH2) is responsible for the metabolism of acetaldehyde and other toxic lipid aldehydes. Our data documented that beta amyloid (Aβ) peptides induce a premature senescence phenotype producing alterations in endothelial function by targeting ALDH2 [2]. The aim of this study is to investigate the role of $A\beta_{1\text{-}40}$ and ALDH2 silencing on HUVECs mitochondrial function. Our preliminary results suggest that $A\beta_{1\text{-}40}$ and ALDH2 silencing reduce maximal respiration and mitochondrial reserve capacity, which is expected to lead to decreased ability to respond to secondary energetic stressors. We suggest that preserving ALDH2 activity may help preserve mitochondrial reserve capacity in stress conditions, and provide a novel strategy for the treatment of angiogenic or metabolic diseases.

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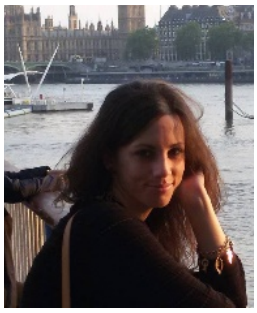

Reactive oxygen species decrease in human cells adapted to hypoxia

Giulia Gorini, Gianluca Sgarbi, Anna Costanzini, Simona Barbato, Giancarlo Solaini, Alessandra Baracca.

Department of Biomedical and Neuromotor Sciences, Laboratory of Biochemistry and Mitochondrial Pathophysiology, University of Bologna, Bologna (Italy).

alessandra.baracca@unibo.it

Hypoxia induces severe changes in cells bioenergetics, dramatically impairing mitochondrial respiration and ATP production. Indeed, we have previously demonstrated how the oxphos complexes are differently modulated when hypoxic cells are cultured in either the presence or absence of glucose [1]. When the oxygen tension decreases, the hypoxia-inducible transcription factor HIF-1α is stabilized and accumulates, inducing the activation of hundreds of genes involved in the adaptation to hypoxia [2]. Interestingly, HIF-1α is also stabilized by non-hypoxic stimuli, and ROS are hypothesized to be part of such mechanism [3]. Nevertheless, an important issue still controversial is whether ROS level increases or decreases in hypoxia, since conflicting results were reported. In a recent study [4] we addressed how moderate hypoxia (1% O_2) affected ROS content in primary human fibroblasts grown in glucose or glucose-free medium: the oxygen limitation caused a steep decrease of ROS, supported in prolonged exposure by mitophagy in cells grown in the presence of glucose and mainly by the enhancement of antioxidant enzymes in cells cultured in glucose-free medium. These results will be discussed and compared with those obtained analyzing a transformed cell model.

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Misfolded SOD1 mutant alters VDAC1-Hexokinase 1 complexes promoting mitochondrial dysfunction in an Amyotrophic Lateral Sclerosis model

Marianna Flora Tomasello[1], Francesca Guarino[2], Andrea Magrì[3], Simona Reina[3], Loredana Leggio[2] and Angela Messina[3]

1 CNR Institute of Biostructures and Bioimaging, Catania (Italy); 2 Dept. Biological, Geological and Environmental Sciences, University of Catania, Catania (Italy); 3 Dept. Biological, Geological and Environmental Sciences, University of Catania, Catania (Italy).

tomaselloflora@gmail.com

The Voltage-Dependent Anion Channel (VDAC) isoform 1 represents the main porin of Mitochondrial Outer Membrane (MOM) and allows metabolic exchanges between cytosol and mitochondria. In physiological conditions, VDAC1 interacts with the glycolytic enzyme Hexokinase 1 (HK1), letting it to gain a direct access to the synthetized ATP. Furthermore, the contact between VDAC1- HK1 stimulates cell survival by hampering VDAC1 interaction with the pro-apoptotic protein Bax.
In the neurodegenerative disease Amyotrophic Lateral Sclerosis (ALS), several misfolded forms of the antioxidant enzyme Superoxide Dismutase 1 (SOD1) were found bound to VDAC1, a situation believed to compromise the mitochondrial metabolism [1]. However, the mitochondrial accumulation of SOD1 mutants occurs exclusively in the spinal cord, a tissue characterized by lower amounts of HK1 in comparison to the brain or the liver; this suggests that in motor neurons VDAC1 could be more prone to interact with SOD1 mutants instead of interacting with HK1.
Here, by means of *in vitro* and *in cellulo* approaches, we proved the interaction between the ALS-related SOD1 mutant G93A and VDAC1 and demonstrated that SOD1 G93A is in competition with HK1 for the same VDAC1 binding site(s). Moreover, we found that SOD1 G93A is able to influence the HK1 sub-cellular distribution in ALS cell model and that its overexpression rescues the NSC34 cells from SOD1 G93A toxicity by increasing cell viability and mitochondrial membrane potential [2].
Overall, our results indicate a clear involvement of HK1 in mitochondrial dysfunctions in SOD1-mediated ALS cases and suggest a protective role of HK1 in ALS pathology.

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Hexokinases 1 peptide impairs VDAC1-SOD1 G93A mutant toxic interaction in Amyotrophic Lateral Sclerosis: implications for the neurodegenerative disease's treatment

Andrea Magrì[1], Ramona Belfiore[2], Stefano Conti Nibali[1], Maria Carmela Di Rosa[1], Loredana Leggio[1], Vito De Pinto[2] and Angela Messina[1]

1 Dept. Biological, Geological and Environmental Sciences, University of Catania, Catania (Italy); 2 Dept. Biomedical and Biotechnological Sciences, University of Catania, Catania (Italy).

andrea.magri@unict.it

Mitochondrial dysfunction in the neuromuscular disorder Amyotrophic Lateral Sclerosis (ALS) is an early key event of motor neuron degeneration and correlates with the accumulation of misfolded forms of the antioxidant enzyme Superoxide Dismutase 1 (SOD1) onto the cytosolic surface of mitochondria. The Voltage-Dependence Anion Channel (VDAC) isoform 1 is the main porin of Mitochondrial Outer Membrane (MOM) and acts as a docking site for several SOD1 mutants, as well as for other misfolded proteins in many neurodegenerative diseases. In a physiological condition, VDAC1 allows metabolic exchanges between cytosol and mitochondria and interacts with Hexokinase 1 (HK1) repressing apoptosis. However, the interaction of SOD1 G93A mutant with VDAC1 affects channel conductance and alters HK1 binding to VDAC1, indicating a competition between SOD1 G93A and HK1 binding with VDAC1.
Starting from this information, we designed and tested a short peptide based on HK1 N-terminal domain sequence (NHK1). NHK1 was able to specifically bind VDAC1 and modulate its channel activity at the Planar Lipid Bilayer, in a similar manner to that of the whole HK1. Furthermore, the addition of NHK1 peptide to the purified VDAC1 or mitochondria, strongly prevents SOD1 G93A deposition, and improves both mitochondrial function and cell viability in the ALS-cellular model [1].
Overall, our results indicate that NHK1 peptide is a promising therapeutic tool for ALS treatment.

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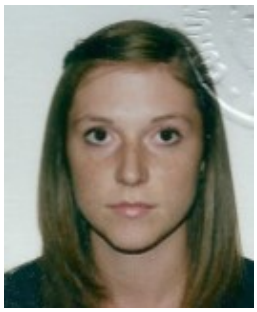
Acknowledgments: Fondazione Umberto Veronesi (Milano, Italy) for a post-doctoral fellowship to A. Magrì.



Mitochondrial reactive oxygen species sensitize T-ALL cells to apoptosis by engaging the OMA1-OPA1 axis

Gloria Scattolin[1], Micol Silic-Benussi[2], Ilaria Cavallari[2], Sonia Minuzz1[2], Samuela Francescato[3], Paola del Bianco[2], Giuseppe Basso[3], Stefano Indraccolo[2], Donna M. D'Agostino[4] and Vincenzo Ciminale[1,2]

1 Department of Surgery, Oncology, and Gastroenterology, University of Padova, Padova (Italy); 2 Istituto Oncologico Veneto-IRRCS, Padova (Italy); 3 Department of Woman and Child Health, Haemato-Oncology Division, University of Padova, Padova (Italy); 4 Department of Biomedical Sciences, University of Padova, Padova (Italy).

gloria.scattolin@studenti.unipd.it

Approximately 20% of patients with pediatric acute T-lymphoblastic leukemia (T-ALL) are refractory to current glucocorticoid-based therapies. In the present study, we investigated the possibility to selectively sensitize T-ALL cells to apoptosis by increasing their levels of mitochondrial reactive oxygen species (mtROS). To this end, we employed NS1619, a synthetic benzimidazolone derivative that increases mtROS production by opening the large conductance Ca^{2+}-activated K^+ (BK) channel and dehydroepiandrosterone (DHEA), which blunts ROS scavenging through inhibition of the pentose phosphate pathway. NS1619 and DHEA increased mtROS and induced death of T-ALL cell lines, patient-derived xenografts (PDX) and primary cells from T-ALL patients (including cases of refractory T-ALL), but did not induce death of normal human thymocytes or PBMC. The importance of depowering ROS-scavenging pathways to increase the efficacy of ROS-producing treatments was underscored by the finding that NS1619 and DHEA activated NRF2, the master regulator of antioxidant pathways. NS1619 and DHEA induced cleavage of OPA1, a mitochondrial protein that controls cristae remodeling and cytochrome *c* mobilization. OPA1 cleavage and cell death were inhibited by the ROS scavenger N-acetylcysteine and by siRNA-mediated knock-down of the mitochondrial protease OMA1, whose targets include OPA1. Importantly, NS1619 and DHEA sensitized T-ALL cells to death induced by TRAIL or by the glucocorticoid dexamethasone, both of which are known to induce mitochondrial outer membrane permeabilization (MOMP) through the activation of Bid (TRAIL) or Bim (Dexamethasone).
Taken together, our findings provide the first evidence for a role of the OMA1-OPA1 axis in sensitizing T-ALL cells to apoptosis, and suggest that effective targeting of refractory T-ALL requires a multidimensional pharmacological approach that combines increased mtROS production, inhibition of scavenging pathways and MOMP.

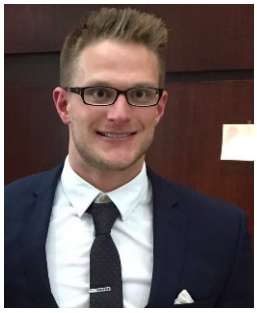
Dynamic photosynthetic membrane

Alexander V. Ruban

Department of Cell and Molecular Biology, Queen Mary University of London, London (UK)

a.ruban@qmul.ac.uk

The photosynthetic membrane is the most protein-rich biological membrane. It performs a number of energy transformation functions that include photon absorption, energy transfer between the photosynthetic light harvesting antenna complexes, excitation energy trapping by the reaction center complexes, charge separation and electron transport between a number of redox electron carriers, coupled to proton translocation across the membrane, reduction of NADP and synthesis of ATP. This sequence of coherently-coupled events is finely regulated, adjusting to changes in environmental and metabolic factors. Therefore, the study of these adaptive dynamics of the photosynthetic membrane and the factors that govern them fall increasingly in a focus of modern photosynthesis research. The light harvesting antenna of photosystem II (LHCII) of higher plants carries up to 70% of all protein in the photosynthetic membrane. It therefore inevitably governs membrane structure, dynamics and functions. Currently, a great deal of information exists on the organization of the antennas of higher plants around photosystems and attempts are being made to interpret the significance of these structures for light harvesting. However, very little is said and actually known about the properties and processes involved in the short-term dynamics of light harvesting antenna underlying the adaptations to the environment. Here, I will present a view on the role of the composition and features of the LHCII antenna in enabling the dynamics of the photosynthetic membrane that makes its light harvesting function flexible even on a minute's timescale.



Light-Driven energy production for cell-free metabolic systems

Kyle Andrew Minor[1,2], Carlo David Montemagno[1,2]

1 Ingenuity Lab, Alberta (Canada); 2 Department of Chemical and Materials Engineering, University of Alberta, Alberta (Canada).

kminor@ingenuitylab.ca

Providing and adjusting the energy needs at the nanoscale as enzymatic cofactors are the most significant and cost prohibitive limitations of Cell-free metabolic systems (CFMS). Ideally, a device must be capable of regulating cofactor balance without impacting carbon flux, only require inexpensive and abundant inputs and be compatible with any CFMS. An example of such a device is an artificial organelle which ATPase and Bacteriorhodopsin are co-reconstituted into a membrane for ATP regeneration [1, 2].

Here we introduce an engineered biotic/abiotic organelle capable of recycling NADH, merely requiring light and water [3]. This device pairs two membrane protein complexes: Photosystem II (PSII) and NADH:Ubiquinone oxidoreductase (CMI). Through controlled vectorial assembly, these two proteins function collectively to reduce NAD^+ using water as substrate and generating oxygen as the sole by-product. In this work, we show the rate of NADH production is proportional to quantum flux while coupling efficiencies near unity. Applying this technology to cell-free metabolic systems (CFMS) will permit control over NADH balance and eliminate constraints on the design of new biosynthetic pathways.

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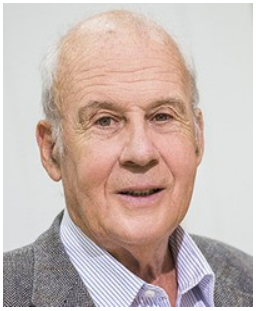

Flavin-based electron bifurcation in anaerobic Bacteria and Archaea

Wolfgang Buckel

Fachbereich Biologie, Philipps-Universität, 35032 Marburg, Germany

buckel@staff.uni-marburg.de

In complex III (cytochrome bc_1) of the mitochondrial respiratory chain, quinone-based electron bifurcation is the well-established heart of the Q-cycle. In 2008 we discovered flavin-based electron bifurcation in strict anaerobic bacteria and archaea, which amplifies the reducing power of an electron to the level of ferredoxin at the cost of the other electron from the same donor pair, such as NADH or hydrogen [1,2]. In the anaerobic bacterium *Acidaminococcus fermentans* the bifurcating system is composed of two soluble proteins, the electron transferring flavoprotein (EtfAB) and the butyryl-CoA dehydrogenase (Bcd). EtfAB and Bcd together catalyze the bifurcation of 2 NADH (E_0' = –320 mV) exergonically to crotonyl-CoA yielding butyryl-CoA (E_0' = –10 mV) and endergonically to 2 ferredoxins affording 2 reduced ferredoxins (E' = –500 mV) [3, 4]. The reduced ferredoxin is mainly used for hydrogen production and generation of electrochemical Na^+ gradients mediated by membrane-bound ferredoxin-NAD reductase (Rnf) [5]. Acetogenic bacteria or methanogenic archaea bifurcate the electrons of H_2 to ferredoxin for the reduction of CO_2, whereby NAD or heterodisulfide act as positive electron acceptors [2]. The crystal structures of EtfAB from *A. fermentans* [3] and the $(EtfAB\text{-}Bcd)_4$ complex from *Clostridium difficile* [submitted] gave insights into the mechanism of electron bifurcation.

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Extracellular vesicles are independent metabolic units with asparaginase activity

Nunzio Iraci[1,10], Edoardo Gaude[2], Tommaso Leonardi[1,3], Ana S. H. Costa[2], Chiara Cossetti[1], Luca Peruzzotti-Jametti[1], Joshua D. Bernstock[1,4], Harpreet K. Saini[3], Maurizio Gelati[5,6], Angelo Luigi Vescovi[6,7], Carlos Bastos[8], Nuno Faria[8], Luigi G. Occhipinti[9], Anton J. Enright[3], Christian Frezza[2] and Stefano Pluchino[1]

1 Wellcome Trust-Medical Research Council Stem Cell Institute, Clifford Allbutt Building, Cambridge Biosciences Campus, Department of Clinical Neurosciences-Division of Stem Cell Neurobiology, and NIHR Biomedical Research Centre, University of Cambridge, Cambridge (UK); 2 *MRC Cancer Unit, University of Cambridge, Hutchison/MRC Research Centre, Cambridge Biomedical Campus, Cambridge (UK); 3 European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Trust Genome Campus, Hinxton, Cambridge (UK); 4 Stroke Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health (NINDS/NIH), Bethesda (USA); 5 Stem Cells Laboratory, Cell Factory and Biobank, Azienda Ospedaliera 'Santa Maria', Terni (Italy); 6 IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo (Italy); 7 Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano (Italy); 8 Medical Research Council - Elsie Widdowson Laboratory, Fulbourn Road, Cambridge (UK); 9 Department of Engineering, Electrical Engineering Division, University of Cambridge, Cambridge (UK); 10 Present address: Department of Biomedical and Biotechnological Sciences (BIOMETEC), University of Catania, Catania (Italy).*

nunzio.iraci@unict.it

Extracellular vesicles (EVs) are membrane particles involved in the exchange of a broad range of bioactive molecules between cells and the microenvironment. While it has been shown that cells can traffic metabolic enzymes via EVs much remains to be elucidated with regard to their intrinsic metabolic activity. Accordingly, herein we assessed the ability of neural stem/progenitor cell (NSC)-derived EVs to consume and produce metabolites. Both our metabolomics and functional analyses revealed that EVs harbour L-asparaginase activity catalysed by the enzyme Asparaginase-like protein 1 (Asrgl1). Critically, we show that Asrgl1 activity is selective for asparagine and is devoid of glutaminase activity. We found that mouse and human NSC-derived EVs traffic ASRGL1. Our results demonstrate for the first time that NSC EVs function as independent, extracellular metabolic units able to modify the concentrations of critical nutrients, with the potential to affect the physiology of their microenvironment.

Mitochondria monitoring in studies of successful anhydrobiosis

Andonis Karachitos[1], Milena Roszkowska[2], Martyna Osiecka[1], Łukasz Kaczmarek[2], Hanna Kmita[1]

1 Laboratory of Bioenergetics, Institute of Molecular Biology and Biotechnology and 2 Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poland.

andonis@amu.edu.pl

Many tardigrade species are capable of anhydrobiosis, defined as a reversible state induced by desiccation (the lack of liquid water). The phenomenon includes entering, permanent and leaving stages corresponding to the dehydration, tun and rehydration stages, respectively. Undoubtedly, anhydrobiosis can be regarded as an organized state and as such it requires some forms of energy supply. It is clear that functional mitochondria guarantee the proper tun formation, but in the light of the available data mitochondria appear to be underestimated in studies on cellular/molecular mechanisms of successful anhydrobiosis. Therefore recovery from the desiccated stage is at present the only available indicative feature of successful anhydrobiosis. In our study we try to explain mitochondria contribution to tardigrade successful anhydrobiosis with emphasis on their role during the tun stage able to recover to the active stage. Therefore we apply functional mitochondria labeling in living individuals of *Hypsibius dujardini* (Doyère, 1840) and *Ramazzottius subanomalus* (Biserov, 1985), representing tardigrade species of different capability to undergo anhydrobiosis. The labeling is obtained by the cell-permeant, cationic, lipophilic fluorophore tetramethylrhodamine methyl ester (TMRM) which is transported into mitochondria in the presence of the inner membrane potential. The resulting labeling is monitored under the fluorescence microscope. The preliminary results indicate that this approach may clarify the role of mitochondria in the tun stage and may add to description of mitochondria functioning during different stages of successful anhydrobiosis.

Acknowledgements: the work was supported by the National Science Centre of Poland (grant no. 2016/21/B/NZ4/00131), and performed with the contribution of the Biodiversity and Astrobiology Research group (BARg) at Adam Mickiewicz University in Poznań.

Genotype variation at the *MSTN* locus is associated with skeletal muscle mitochondrial abundance and fibre composition in untrained Thoroughbred horses.

Mary F Rooney[1, 2], Emmeline W Hill[1], Lisa M Katz[3] and Richard K Porter[2]

1 UCD School of Agriculture and Food Science, University College Dublin, Belfield (Ireland); 2 School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute (TBSI), Trinity College Dublin, Dublin (Ireland); 3 UCD School of Veterinary Medicine, University College Dublin, Belfield (Ireland).

marooney@tcd.ie

Variation in the *myostatin* (*MSTN*) gene, most notably a single nucleotide polymorphism (SNP) in the first intron (g.66493737C>T) and an SINE insertion polymorphism (Ins227bp) in the promoter region, has been reported to be associated with race distance, body composition and skeletal muscle fibre composition in the horse. The aim of the present study was to test the hypothesis that *MSTN* variation influences mitochondrial phenotypes in equine skeletal muscle. In a set of N=143 horses, the two *MSTN* polymorphisms were in complete concordance. Mitochondrial abundance and skeletal muscle fibre types were measured in whole muscle biopsies from the g*luteus medius* skeletal muscle in N = 82 (n=37 CC/II, n=34 CT/IN, n=11 TT/NN) untrained Thoroughbred horses (21 ± 3 months). Mitochondrial abundance was significantly ($P < 0.01$) less in the presence of the C-allele/insertion allele and skeletal muscle fibre type proportions were significantly ($P < 0.01$) different among the three *MSTN* genotypes. CC/II horses had a lower proportion of type I fibres along with a higher proportion of type IIX fibres compared to CT/IN and TT/NN animals, indicating that variation in *MSTN* genotype correlates with mitochondrial abundance and fibre composition in untrained Thoroughbred horses [1]. Type I skeletal muscle fibres are more oxidative and contain more mitochondria than the glycolytic type IIX skeletal muscle fibres, therefore the fibre composition data described is in agreement with the observed mitochondrial abundance differences. The data described in this study is also consistent with previous published reports that the presence of the SINE insertion allele is associated with fewer type I fibres and more type IIX fibres.

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Zebrafish (Danio rerio) as a model to study the pathophysiological role of the mitochondrial chaperone TRAP1

Claudio Laquatra[1], Ionica Masgras[1], Marco Schiavone[1], Alessandra Zulian[1], Giovanni Minervini[1], Silvio Tosatto[1], Andrea Vettori[2], Francesco Argenton[2], and Andrea Rasola[1]

1 Department of Biomedical Sciences and CNR Institute of Neurosciences, University of Padova, Padova (Italy); 2 Department of Biology, University of Padova, Padova (Italy).

claudiolaquatra@gmail.com

Expression of the Hsp90-family chaperone TRAP1 is restricted to mitochondria and increased in most tumor types. We have previously shown that TRAP1 elicits the stabilization of the transcriptional factors HIF1α, thus generating a pseudohypoxic state that supports neoplastic growth. Following oncogenic activation of the Ras/ERK pathway TRAP1 is phosphorylated by ERK1/2, leading to inhibition of succinate dehydrogenase (SDH), the complex II of respiratory chain, and to the ensuing accumulation of the oncometabolite succinate, which eventually stabilizes HIF1α. However, little is known about the timing and importance of TRAP1 induction during the process of neoplastic onset and growth, as well as on the role of TRAP1 in the physiology of non-transformed cells.

To answer these questions we decided to exploit the Zebrafish model, whose bioenergetic features are still poorly investigated. We found that TRAP1 is highly expressed during the early stages of Zebrafish embryo development, but its levels are dramatically decreased at 96 hpf. Notably, an increase in TRAP1 protein level was detected after HIF1α stabilization, causing a reduction in SDH activity, and an *in silico* analysis of Zebrafish TRAP1 promotor showed the presence of hypoxic responsive elements motifs. Moreover, TRAP1 is highly expressed in a Zebrafish model of pancreatic adenocarcinoma induced by $KRas^{G12D}$ expression in Ptf1a positive cells where it causes a strong decrease of SDH activity, while it is absent in normal pancreas.

Altogether, these data suggest the existence of a positive feedback loop between HIF1α and TRAP1, in which HIF1α stabilization induces TRAP1 expression acting at the transcriptional level, and in turn TRAP1, through SDH inhibition, leads to HIF1α stabilization. This regulatory mechanism could play an important role for the adaptation of cells to fluctuating level of oxygen both in embryogenesis and during the process of neoplastic transformation.

Analysis of the role of Letm1 in the development of zebrafish

Sarfarazhussain Farooqui[1,3], Stefan Hajny[1], Eva Scheuringer[1], Friederike Schlumm[2], Kristin Tessmar-Raible[1] and Karin Nowikovsky[3]

1 Max F. Perutz Laboratories, University of Vienna, Campus Vienna Biocenter, Vienna (Austria); 2 The Research Institute of Molecular Pathology, Vienna Biocenter (VBC) , Vienna (Austria); 3 Dept. of Internal Medicine 1, Anna Spiegel Center of Translational Research, Medical University Vienna, Vienna (Austria).

sarfaraz.farooqui@univie.ac.at

Mitochondria are indispensable for the cellular energy production mostly by oxidative phosphorylation, which is very critical for energy dependent tissues like nervous system. Additionally, they have also been associated with a role in maintaining ion homeostasis (by the coordinated function of ion transporters and ion release exchange systems). LETM1 is an essential mitochondrial membrane protein regulating mitochondrial volume and cation homeostasis. Absence of LETM1 is associated with seizures in WolfHirschhorn syndrome [1]. To explore the mechanism correlating mitochondrial dysfunction with seizures and better understanding if mitochondrial ion homeostasis is involved in action potential and epilepsy we have generated a zebrafish genetic model of LETM1 deficiency using TALEN based genomeediting technique. Here, we report the results of the ongoing experiments in progress to characterize the relationship of Letm1 deficiency with epilepsy by analyzing their neuronal phenotypes in five different ways (a) analyzing the locomotor behavior under a stress inducing assay (b) calcium imaging in neuronal population [2] (c) mitochondrial dynamics using mitofish [3] (d) analyzing growth during development. The progress in these experiments will be presented in the meeting.

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Induction of autophagy and mitochondrial biogenesis by the natural compound Pterostilbene: mechanisms and biomedical potential

Martina La Spina[1,2], Michele Azzolini[1,2], Marco Schiavone[2], Mario Zoratti[1,2] and Lucia Biasutto[1,2]

1 CNR Institute of Neuroscience, Padova (Italy); 2 Department of Biomedical Sciences, University of Padova, Padova (Italy).

martina.spina@bio.unipd.it

Pterostilbene (Pt), a phenolic component of blueberries, has been proposed to be beneficial in many human pathologies such as the metabolic syndrome, neurodegenerative diseases and cancer. However, the molecular mechanisms underlying its effects have been poorly characterized. Several papers suggest that Pt may induce autophagy. This process is mainly regulated by Transcription Factor EB (TFEB), whose action is controlled by inhibitory phosphorylation by mTORC1, which retains it in the cytosol, and dephosphorylation by Ca^{2+}-dependent Calcineurin, which promotes its nuclear translocation and thus its transcriptional activity.

We confirmed that Pt at physiologically relevant (low µM) levels promotes autophagy *in vitro* by inducing TFEB nuclear migration, and traced back the upstream signaling cascade to ROS generation by mitochondria. Indeed, pre-treatment with an antioxidant reduced the TFEB nucleus/cytosol ratio. In agreement, we observed inhibition of mitochondrial respiratory chain complex I by Pt. ROS seem to be connected to an increase of cytoplasmic Ca^{2+} which activates Calcineurin. Cyclosporin A, which blocks Calcineurin, reduced Pt-induced nuclear translocation of TFEB as well.

Both ROS and Ca^{2+} are involved in AMPK activation. AMPK in turn inhibits mTORC1, facilitating TFEB migration. This metabolic sensor is known to play an important role in promoting mitochondrial biogenesis through Pgc-1α. Accordingly, we found that Pt both up-regulates Pgc-1α by acting on AMPK, and increases the mitochondrial content of cells.

These results led us to test the biomedical potential of Pt in a zebrafish model of Collagen VI myopathies characterized by a defective autophagy and accumulation of damaged mitochondria. Strikingly, this molecule partially rescued both motor and structural abnormalities in these fish at sub-µM concentrations. Preliminary experiments suggest that these effects are indeed associated with the activation of autophagy and mitochondrial biogenesis.

Flavonoid transport in plant microsomes: characterization of quercetin uptake.

Antonio Filippi, Elisa Petrussa and Enrico Braidot

Department of Agricultural, Food, Animal and Environmental Sciences, University of Udine, Udine (Italy).

enrico.braidot@uniud.it

Plant flavonoids are one of the most studied members of natural polyphenolic family due to their biochemical, physiological and pharmacological effects in both plants and animals. The biological activities of plant flavonoids depend on concentration and pH and for this reason their transport/absorption and storage systems are under strict attention, although a clear scenario is far to be elucidated. Besides transport attributable only to energized transporter systems, recent observations hypothesize that flavonoids could pass through membranes without the support of ATP hydrolysis, opening a new interesting and unknown mechanism of membrane permeation. In this work, we developed a new methodology for the analysis of *in vivo* transport of quercetin (QC) across plant bio-membranes. To this purpose, we used microsomal vesicles obtained from *Pisum sativum* etiolated stems as a model. We tested the variation of fluorescence dependent on pH, the transport modulation using oxidative\reducing agents in addition to protein crosslinker and the effective entrance of QC within the microsomes trough metaholic extractions.
This work has allowed us to propose a new easy-to-use and fast assay, aiming at monitoring flavonoid passive transport across bio-membranes.

LIST OF PARTICIPANTS

Agrimi Gennaro	*gennaro.agrimi@uniba.it*
Avetisyan Armine	*avetis@genebee.msu.ru*
Baracca Alessandra	*alessandra.baracca@unibo.it*
Barbato Simona	*simona.barbato3@unibo.it*
Barile Maria	*maria.barile@uniba.it*
Bernardi Paolo	*bernardi@biounipd.it*
Buckel Wolfgang	*buckel@staff.uni-marburg.de*
Busch Karin	*buschkar@uni-muenster.de*
Capitanio Nazareno	*nazzareno.capitanio@unifg.it*
Checchetto Vanessa	*vanessa.checchetto@unipd.it*
Chiaradonna Ferdinando	*ferdinando.chiaradonna@unimib.it*
Cocco Tiziana	*tizianamaria.cocco@uniba.it*
Console Lara	*console@hotmail.it*
Conti Nibali Stefano	*stefano92co@hotmail.it*
De Pinto Vito	*vdpbiofa@unict.it*
Di Rosa Maria Carmela	*mcdirosa@unict.it*
Faccenda Danilo	*dfaccenda@rvc.ac.uk*
Farooqui Sarfarazhussain	*sarfaraz.farooqui@univie.ac.at*
Filippi Antonio	*antoniofilippi@yahoo.it*
Forte Elena	*elena.forte@uniroma1.it*
Francia Francesco	*francesco.francia@unibo.it*
Frezza Christian	*cf366@mrc-cu.cam.ac.uk*
Galber Chiara	*chiara.galber@studenti.unipd.it*

Gibellini Lara	*lara.gibellini@unimore.it*
Giuffrè Alessandro	*alessandro.giuffre@uniroma1.it*
Gorini Giulia	*gorinigiulia@gmail.com*
Grobys Daria	*dg33114@amu.edu.pl*
Guarino Francesca	*fguarin@unict.it*
Guo Lishu	*guolsh15@gmail.com*
Indiveri Cesare	*cesare.indiveri@unical.it*
Iraci Nunzio	*nunzio.iraci@unict.it*
Karachitos Andonis	*andonis@amu.edu.pl*
Kmita Hanna	*kmita@amu.edu.pl*
La spina Martina	*martina.spina@bio.unipd.it*
Laquatra Claudio	*claudiolaquatra@gmail.com*
Lazzarino Giacomo	*g.lazzarino@libero.it*
Leanza Luigi	*luigi.leanza@unipd.it*
Leggio Loredana	*loredanaleggio@unict.it*
Lippe Giovanna	*giovanna.lippe@uniud.it.*
Magrì Andrea	*andrea.magri@unict.it*
Malagrinò Francesca	*francesca.malagrino@uniroma1.it*
Malferrari Marco	*marco.malferrari2@unibo.it*
Mancuso Francesca	*francy.mancuso87@gmail.it*
Martinelli Fabio	*biotech88@hotmail.it*
Messina Angela	*mess@unict.it*
Minor Kyle	*kminor@ingenuitylab.ca*

Nannelli Ginevra	*nannelli5@student.unisi.it*
Nicotra Desirè	*desinicotra@hotmail.it*
Nowikovsky Karin	*karin.nowikovsky@meduniwien.ac.at*
Ostuni Angela	*angela.ostuni@unibas.it*
Pacelli Consiglia	*consiglia.pacelli@unifg.it*
Pereira Clara	*clara.pereira@ibmc.up.pt*
Peruzzo Roberta	*roberta.peruzzo@studenti.unipd.it*
Piccoli Claudia	*claudia.piccoli@unifg.it*
Pinti Marcello	*marcello.pinti@unimore.it*
Pittalà Maria Gaetana G.	*marinella.pitt@virgilio.it*
Porter Richard	*rkporter@tcd.ie*
Rapaport Doron	*doron.rapaport@uni-tuebingen.de*
Reina Simona	*simonareina@yahoo.it*
Rooney Mary	*marooney@tcd.ie*
Ruban Alexander	*a.ruban@qmul.ac.uk*
Scalise Mariafrancesca	*mariafrancesca.scalise@unical.it*
Scattolin Gloria	*gloria.scattolin@studenti.unipd.it*
Schiavone Marco	*marco.schiavone@unipd.it*
Sineri Serena	*serenasineri@live.it*
Solaini Giancarlo	*giancarlo.solaini@unibo.it*
Tomasello Flora	*tomaselloflora@gmail.com*
Trchounian Karen	*k.trchounian@ysu.am*
Tretter Lazlo	*tretter.laszlo@med.semmelweis-univ.hu*

Trevisan Elena	*elena.trevisan@unipd.it*
Urbani Andrea	*andrea.urbani@unipd.it*
Vivarelli Silvia	*silvia.vivarelli7@gmail.com*
Walker John	*john.walker@mrc-mbu.cam.ac.uk*
Zinghirino Federica	*fedezingh@hotmail.it*
Zuhra Karim	*karim.zuhra@uniroma1.it*
Zulian Alessandra	*zulian.alessandra@gmail.com*