# FROM BASIC RESEARCH TO TECHNOLOGY TRANSFER

## IBPM Annual Meeting

**Rome, June 9th 2016**
**Aula Convegni CNR**


LA LUCE DELLA SCIENZA CERCO E L BENEFICIO
Consiglio Nazionale delle Ricerche
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# SUMMARY

# Programme

09.00 - 09.30 ***Registration of participants***

09.30 - 09.45 ***Authorities' greetings***

**Prof. Clara Balsano**
*Introduction of the IBPM Director*

09.45-10.30 **Prof. Gioacchino Natoli**
*Inflammation insights by a genomic approach*
European Institute of Oncology (IEO), Milan

## Session I: Cellular transformation (IBPM)

10.30 - 10.45 **Elisa Caffarelli**
*The neural long noncoding RNA, linc-NeD125, functions as a competing endogenous RNA to modulate the expression of Group 4 medulloblastoma driver genes*

10.45 - 11.00 **Cecilia Mannironi**
*MicroRNAs as regulators of stress-related behavior: the case of mir-135a*

11.00-11.15 **Barbara Illi**
*Functional association between Myc and the Protein Arginine Methyltransferase5 (PRMT5) in glioblastoma cells*

11.15 -11.45 ***Coffee break***

11.45 - 12.00 **Maria Grazia Giansanti**
*GOLPH3 and COG7, two proteins involved in cytokinesis and human diseases*

12.00 - 12.15 **Patrizia Lavia**
*Importin beta and the GTPase ran have global roles in mechanisms of genetic instability in cancer cells*

12.15 - 12.30 **Maria Patrizia Somma**
*Int6 is required for proper spindle formation and genome stability in Drosophila and human cells*

## Session II: Antitumoral therapy (IBPM)

12.30 - 12.45 **Pierpaolo Ceci**
*Human Ferritins: selective nanocarriers for antitumor payloads*

12.45-13.00 **Gianni Colotti**
*Sorcin induces resistance to chemotherapeutics in tumors*

13.00-13.15 **Laetitia Gonzalez**
*Amorphous silica nanoparticles alter microtubule dynamics and cell migration*

13.15 - 13.30 **Sergio Nasi**
*Resetting cancer stem cell regulatory nodes upon MYC inhibition*

13.30 -15.00 ***Lunch and Poster session***

## Session III: Inflammation (IBPM)

15.45- 16.00 **Paola Fragapane**
*Response to acute corticosterone treatment of hippocampal neurons in vitro is reduced in dystrophic mdx mice compared to wild type*

16.00 - 16.15 **Andrea Ilari**
*Structural and functional studies of trypanothione reductase (TR) and tryparedoxin peroxidase (TXNPx) two good targets to find new drugs against Leishmaniasis*

16.15- 16.30 **Alessandro Giuffrè**
*Hydrogen sulfide, cell respiration and the interplay between gasotransmitters at human cystathionine $\beta$-synthase*

## Session IV: Model systems (IBPM)

16.30 - 16.45 **Maura Cardarelli**
*Hormonal control of reproductive development and response to abiotic stress in Arabidopsis thaliana*

16.45 - 17.00 **Monica Carabelli**
*Role of the Arabidopsis HD-Zip II transcription factors HAT3 and ATHB4 in flower development*

17.00 - 17.15 **Patrizia Filetici**
*Epigenetic from budding yeast to human cancer*

17.15 - 17.30 **Giulia Guarguaglini**
*The informational content of single-cell time-lapse imaging to study dynamic biological processes*

17.30 - 17.45 **Prof. Clara Balsano**
*Conclusions of the IBPM Director*

# INTRODUCTION

*Prof. Clara Balsano*

**Annual conference IBPM "From Basic Research to Technology Transfer"**

Prof. Clara Balsano, Director of IBPM-CNR

Here we present an overview of the main research programs and technology transfers that are currently underway and in development at the Institute of Molecular Biology and Pathology at the CNR (IBPM-CNR).

The presence, in the Auditorium, of Professor Eugenio Gaudio, the Rector Magnificus of the Sapienza University of Rome, is proof of the close and virtuous scientific relationship which exists between the IBPM Institute and the Sapienza University of Rome. Today we also have present Professor Tullio Pozzan, the Director of the Department of Biomedical Sciences, who by being here demonstrates, once again, the special "attention" granted towards the Institute, that I have the honor to lead.


The large participation of researchers, both young and old, belonging to the Institute and other prestigious research institutions, such as: Sapienza University, the University of Tor Vergata, L'Aquila University, the University of Rome 3-biomedical Campus, the National Cancer Institute Regina Elena and finally the European Institute of Oncology, has allowed for a fruitful exchange of information and experiences. Again, those who do research are well aware of the significance of belonging to a community and know the importance of team effort

in order to achieve a common goal.

So I hope we have succeeded in creating the basis for new networks of excellence, by connecting an important critical mass to provide all the participants with food for thought and the opportunity to share ideas, hoping, in the near future, for a further qualitative leap in the activities of IBPM's research.

At the gathering, the heads of the Institute's research projects have presented some of their latest research. Many of the topics presented are of great interest: In the **first session** there was talk of the pathogenetic mechanisms of cell transformation, starting with the non-coding RNAs and miRNAs and continuing with studies on the involvement of importin-beta in the induction of GTPase RAN in inducing genetic instability in cancer cells and studies on the pathogenetic role GOLPH3 oncogene in several tumor types, such as breast cancer, ovarian cancer, lung cancer and prostate cancer, providing knowledge for identifying new cancer biomarkers. The **second session** was devoted to research focused on innovative anti-cancer therapies. Among the most interesting topics were the results obtained by the group coordinated by Dr. Ceci for "personal and precision medicine" for various types of cancer: they created innovative nanoparticles (known as nanoferritins). Moreover, innovative approaches been reported for the neutralization of the resistance of adult stem cells to chemotherapy drugs. Furthermore, I would like to highlight the **third session** devoted to the study of inflammation, oxidative stress and innovative treatments for the neglected infection by Leishmania parasites. The **fourth session** focused on the results obtained using different model systems in vitro and in vivo in place at the IBPM Institute (primary and stem cells, yeast, plants and model mouse models).

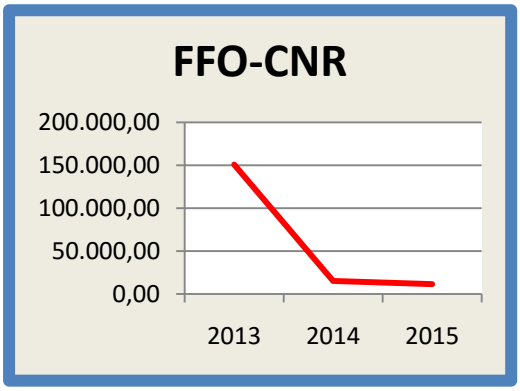
Below, let me describe the structural and management characteristics of the Institute, which make it unique in the CNR research scenario.

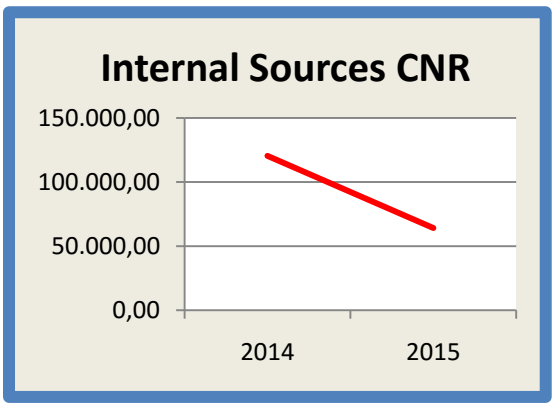
The IBPM has, in fact, all the skills from basic research to biotechnology applications. The Institute consists of 77 members of staff: 73 are researchers/technicians and 4 administrative staff. In 2014/2015 the Institute's researchers, despite the difficulties that, from year to year, are multiplied by the net reduction of institutional funding for research by the Italian government and the managerial/administrative complication switch caused significant internal problems, have published 67 articles with peer review, gaining a total impact factor of 280. The scientific productivity is in line with that of 2014, the year in which researchers published 69 articles in peer review, obtaining an impact factor of 281. Below are the research areas of the Institute:

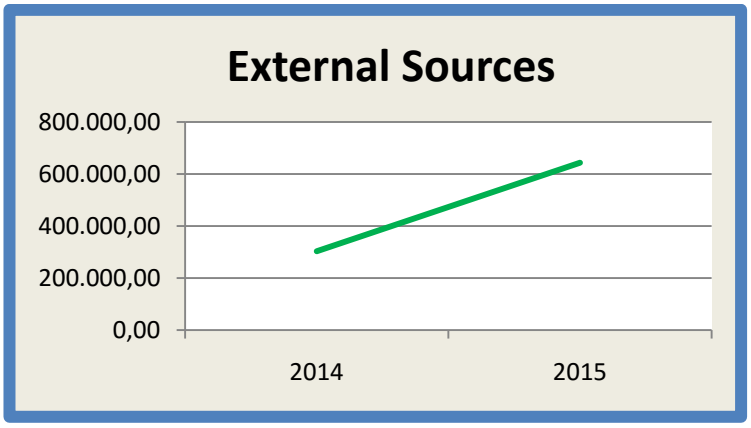
- GENETICS
- NUCLEIC ACIDS
- PROTEIN STRUCTURAL BIOCHEMISTRY AND BIOINFORMATICS
- MOLECULAR PATHOLOGY
- ORGANIC MOLECULES SINTHESIS

Added value obtained thanks to the presence of the Biocrystal facility that supports scientific groups interested in studying and developing the structural aspects of biology and which provides advanced and specific advisory services to perform protein expression, also giving the opportunity to obtain their purification, characterization, crystallization and determination of their structures.

In these years, the Institute has also shown great scientific liveliness, doubling the capacity to attract external funding (Fig. 1) by achieving external sources mainly from "charities", such as: the Italian Association for Cancer Research (AIRC) and the Telethon Foundation.




Finally, the result obtained by the evaluation of the Institutes, carried out by CNR in 2014, in which the IBPM was positioned in sixth position, gaining 2 points compared to the 2009 assessment, reflects the energetic and profitable research of IBPM. The success obtained is also a sign of the prevailing widespread recognition that the knowledge of the mechanisms underlying life has not only an

intrinsic cognitive value, but has also many potential and significant applications in biotechnology and biomedical technologies, so much so that all the most significant research institutions in developed countries have decided to fund and significantly support the current called "Life Sciences".

At this point, let me make a few remarks.

Development of technologies makes technology and science increasingly strictly interdependent, so much that every technological advance makes new scientific findings possible. This in turn implies the need to adapt the research structures and their management to the current historical context.

Unfortunately, in recent years, Italy has not shone for its financial investment on basic research because of the country's financial difficulties that make uncertain the survival not only of research organizations, but also of the University.

Finally, despite the fall in the amount allocated by the Italian Government for basic research, we should not despair!!! Young researchers should be aware that, out of the current crisis, it is necessary to follow new paths, identifying new models, covering sustainability assumptions previously inexistent or simply managed wrongly. Scientific research is something in itself excellent and desirable for a Nation, but it is expensive and hardly produces immediate results. So despite the fact that *art. 9* of the Italian Constitution (which this year celebrated its seventy year anniversary) states that: "The Republic promotes the development of culture and scientific and technical research. Protecting the landscape and the historical and artistic heritage of the Nation", we must resolve the ambiguity between the right to wish for freedom and creativity of scientific thought and the need to address the rising costs imposed by innovative research. Thus, the integration of basic and applied research is certainly necessary, but we must always keep in mind that only an open and dynamic public research system will ensure keeping up with ever-changing scientific knowledge.

What I have said above brings to mind the sentences of two great writers of the nineteenth century: Oscar Wilde and Frances Hodgson Burnett, which will hopefully serve as a warning to young researchers. They are outlined below:

- "**Progress is the realisation of Utopias**." (Oscar Wilde, 1854-1900)

- "**At first people refuse to believe that a strange new thing can be done, then they begin to hope it can be done, then they see it can be done-then it is done and all the world wonders why it was not done centuries ago.**" (Frances Hodgson Burnett, 1849-1924).

So never forget that you have to fight! Please do not stop believing in the future!

Finally, we believe it is important to remind young researchers of the Mertonian norms coined by Robert King Merton in the mid-twentieth century, which in our opinion remain, to this day, relevant and indispensable.
The rules are mentioned below and bring this brief introduction to a close.

1. **Universalism**: All scientists can contribute to science regardless of their race, nationality, culture, gender.

2. **Communism**: All scientists must have equal access to scientific assets (intellectual property) and there must be a sense of common ownership in order to promote collective cooperation; secrecy is the opposite of this rule.

3. **Disinterestedness**: It is assumed that scientists act for the benefit of the scientific community rather than for personal gain.

4. **Originality**: The scientific results should represent a fresh contribution, be it a new problem, a new approach, new data, a new theory or a new explanation.

5. **Organized skepticism**: Before being accepted, the scientific results must be subjected to critical scrutiny.

# ORAL PRESENTATIONS

**The neural long noncoding RNA, linc-NeD125, functions as a competing endogenous RNA to modulate the expression of Group 4 medulloblastoma driver genes**

Pietro Laneve[1], Jessica Rea[2], Annarita Favia[3], Agnese Po[4], Vincenzo Alfano[4], Elisabetta Ferretti[5], Elisa Caffarelli[3]

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Long noncoding RNAs (lncRNAs) are emerging as main nodes of regulatory networks underlying developmental processes in Eukaryotes. Their action is particularly relevant in the central nervous system, whose wide variety of cells are highly transcriptionally active and express almost half of the lncRNAs detected in the human brain [1]. In addition, their potential activity as oncosuppressors or oncogenes and their association with cancer subtypes and clinical prognosis is also emerging [2].

We identified a novel human neuronal lncRNA, linc-NeD125, as the host gene for the neuronal-enriched miR-125b-1. Linc-NeD125 is induced in response to the neuronal differentiation stimulus both in tumor cell lines and in mouse embryonic stem cells. We demonstrated that it controls medulloblastoma (MB) cell proliferation and apoptosis, thus contributing to create the conditions needed for cells undergoing differentiation [3].

Notably, it is significantly and specifically upregulated in Group 4 MB primary tumors, suggesting it may work as a novel cancer biomarker. We also dissected its mechanism of action at the molecular level. We found that linc-NeD125 may act as a competing endogenous RNA (ceRNA), which may sequester an handful of miRNAs, namely miR-19a, miR-19b and miR-106a, that negatively control the expression of several MB Group 4 driver genes. Therefore, we suggest that linc-NeD125, by modulating miRNA activity, has a tumorigenic potential and might serve as a novel target in anti-medulloblastoma therapy.

**References:**

1. Briggs JA, Wolvetang EJ, Mattick JS, Rinn JL, Barry G. Mechanisms of long non-coding RNAs in mammalian nervous system development, plasticity, disease, and evolution. Neuron, 2015, **88:**861-877

2. Huarte M. The emerging role of lncRMAs in cancer. Nat Med, 2015, **21:**1253-1261

3. Bevilacqua V, Gioia U, Di Carlo V, Tortorelli AF, Colombo T, Bozzoni I, Laneve P, Caffarelli E. Identification of linc-NeD125, a novel long non coding RNA that hosts miR-125b-1 and negatively controls proliferation of human neuroblastoma cells. RNA Biol, 2015, **12:**1323-1337

**MicroRNAs as regulators of stress-related behavior: the case of miR-135a.**

Samyutha Rajendran[1], Antonio Biundo[2], Francesca De Vito[3], Silvia Caioli[3], Cristina Zona[3,4], Teresa Ciotti[5], Emerald Perlas[6], Giorgia Del Vecchio[1],Irene Bozzoni[1], Arianna Rinaldi[1,5], Andrea Mele[1,5], Carlo Presutti[1], Cecilia Mannironi[7]

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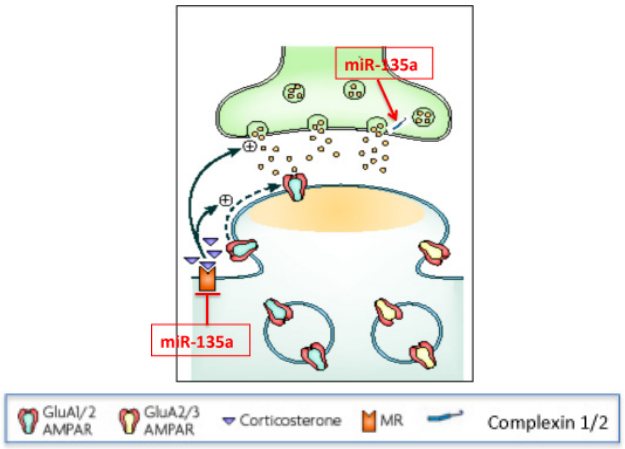


MicroRNAs (miRNAs) are a class of non coding RNAs with a growing significance in regulatory mechanisms of gene expression related to brain function and plasticity. Recent studies indicate that they potentially orchestrate any complex phenomena sustained by structural and functional plasticity. In this respect, we have shown that miRNAs have a functional role inspatial memory formation and neuronal response to homeostatic challenges[1,2]. Our findings pointed to a role of the amygdalar miR-135a in acute stress, as regulator of the Mineralocorticoid Receptor (MR), an important effector of the early stress response. Interestingly, in the midbrain raphe nuclei miR-135a has emerged to be essential for stress resiliency [3]. In the present study, we have examined the role of amygdalar miR-135a in the context of stress-related behavior. We found that the depletion of miR-135a in the basolateral and the central amygdala of

adult mice induced an increase in anxiety-like behavior. Furthermore, by *in vitro* studies with neuronal primary cultures, we demonstrated its role in the regulation of synaptic transmission. We characterized, as direct targets complexin-1 and -2 (Cpx1 and Cpx2), key regulators of synaptic vesicle fusion. Specific interactions between miR-135a,Cpx1 and Cpx2 mRNAs were demonstrated. Our findings pinpoint to miR-135a as a general modulator of synaptic plasticity. Overall, our results unravel a previously unknown miRNA-dependent mechanism in amygdala in controlling anxiety-like behavior, suggesting a physiological role of miR-135a in the modulation of stress-related behavior. Possible implications in the onset and susceptibility of stress-related disorders will be discussed.

**References:**


1. Capitano F, Camon J, Ferretti V, Licursi V, De Vito F, Rinaldi A, Vincenti S, Mannironi C, Fragapane P, Bozzoni I, Oliverio A, Negri R, Presutti C, Mele A. microRNAs modulate spatial memory in the hippocampus and in the ventral striatum in a region-specific manner. Mol Neurobiol, 2015, 1-13
2. Mannironi C, Camon J, De Vito F, Biundo A, De Stefano ME, Persiconi I, Bozzoni I, Fragapane P, Mele A, Presutti C. Acute stress alters amygdala microRNA miR-135a and miR-124 expression: inferences for corticosteroid dependent stress response. PloS one, 2013, **8:**e73385
3. Issler O, Haramati S, Paul ED, Maeno H, Navon I, Zwang R, Gil S, Mayberg HS, Dunlop BW, Menke A, Awatramani R. MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity. Neuron, 2014, **83:**344-360

**Functional association between Myc and the Protein Arginine Methyltransferase 5 (PRMT5) in glioblastoma cells.**

Maria Patrizia Mongiardi[1], Mauro Savino[2], Laura Bartoli[2], Sara Beji[2], Annarita Favia[2], Andrea Levi[1], Sergio Nasi[2], Barbara Illi[2]

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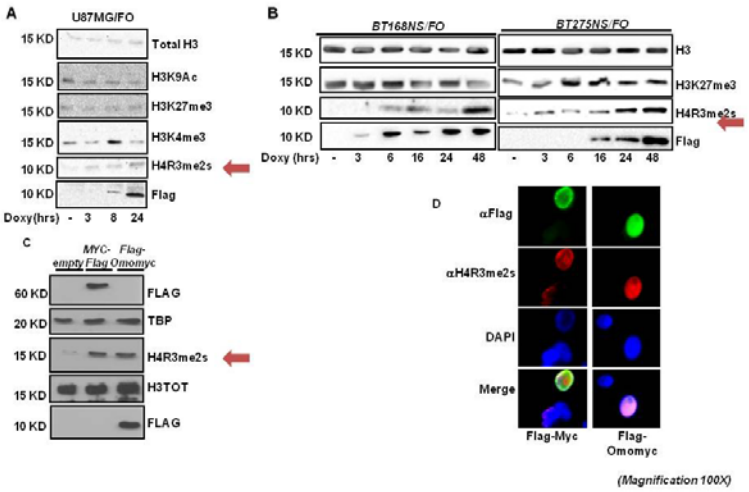
The c-Myc protein is dysregulated in many human cancers and its function has not been fully elucidated yet. The c-Myc inhibitor Omomyc displays potent anticancer properties in animal models. It perturbs the c-Myc protein network, impairs c-Myc binding to the E-boxes, retaining transrepressive properties and inducing histone deacetylation. Here we have employed Omomyc to further analyse Myc activity at the epigenetic level. We show that both Myc and Omomyc stimulate histone H4 symmetric dimethylation of arginine (R) 3 (H4R3me2s), in human glioblastoma and HEK293T cells (fig. 1). Consistently, both associated with protein Arginine Methyltransferase 5 (PRMT5) – the catalyst of the reaction - and its co-factor Methylosome Protein 50 (MEP50). Confocal experiments showed that Omomyc co-localized with c-Myc, PRMT5 and H4R3me2s-enriched chromatin domains. Finally, interfering with PRMT5 activity impaired target gene activation by Myc whereas it restrained Omomyc-dependent repression (Mongiardi et al., Sci Rep, 2015).

The identification of a histone-modifying complex associated with Omomyc represents the first demonstration of an active role of this miniprotein in modifying chromatin structure and adds new information regarding its action on c-Myc targets. More importantly, the observation that c-Myc may recruit PRMT5-MEP50, inducing H4R3 symmetric di-methylation, suggests previously unpredictable roles for c-Myc in gene expression regulation and new potential targets for therapy.

Fig. 1



**GOLPH3 and COG7, two proteins involved in cytokinesis and human diseases.**

Stefano Sechi[1], Anna Frappaolo[1], Michael Tiemeyer[2], Roberto Piergentili[1], Roberta Fraschini[3], Giorgio Belloni[4], Maria Grazia Giansanti[1]

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In animal cell cytokinesis, assembly and constriction of an actomyosin ring must be finely coordinated with plasma membrane remodeling and vesicle trafficking at the cleavage furrow. Studies in mammalian cells and in animal model systems indicate that targeted vesicle transport is necessary for cleavage furrow ingression as well as during abscission. In addition, cytokinesis depends on a special lipid composition at the cleavage site. Phosphoinositide lipids provide critical membrane signals that control contractile ring assembly and dynamics, as well as new membrane addition.

The highly conserved Golgi phosphoprotein 3 (GOLPH3) protein, characterized as a Phosphatidylinositol 4-phosphate [PI(4)P] effector at the Golgi, is commonly amplified in several solid tumors and associated with more aggressive tumors. We have demonstrated that GOLPH3 accumulates at the cleavage furrow and is essential for cytokinesis in *Drosophila melanogaster*. In spermatocyte and neuroblast dividing cells, GOLPH3 is essential for maintenance of centralspindlin and Rho1 at cell equator and stabilization of Myosin II and Septin rings. We have shown that GOLPH3 function during cytokinesis is dependent on its binding to PI(4)P. Mutations that affect interaction with PI(4)P, disrupt localization of GOLPH3 at both the Golgi stacks and the cleavage furrow. Telophase spermatocytes from mutants with defective GOLPH3-PI(4)P interaction, also fail to accumulate PI(4)P-and Rab11-associated secretory organelles at the cleavage site. We hypothesize that GOLPH3 might act as a key molecule in coupling vesicle trafficking with actomyosin ring assembly and stability during cytokinesis. Consistent with our hypothesis we found that GOLPH3 protein forms a complex with Rab11 GTPase and directly binds to the centralspindlin component Pavarotti. Because cytokinesis failures have been associated with premalignant disease and cancer, the novel connection between *GOLPH3* and cytokinesis, imposes new fields of investigation in cancer biology and therapy.

The conserved oligomeric Golgi (COG) complex functions as a vesicle-tethering factor for intra-Golgi retrograde trafficking, playing a crucial role in maintaining the glycosylation enzymes across the Golgi cisternae. Mutations in human *COG7* and other *COG* genes cause distinct forms of inherited, autosomal recessive, congenital disorders of glycosylation (CDG) associated with multisystemic deficiencies. We have shown that *Drosophila* Cog7 localizes to Golgi stacks and is required for normal cytokinesis in both spermatocytes and larval neuroblasts. Cytokinesis failures have been proposed to affect neurogenesis thus contributing to microcephaly, one of the neurological trait of COG7-CDG patients. We are dissecting the molecular interactions involving Cog7, GOLPH3 and other vesicle trafficking proteins involved in brain cytokinesis. In addition, we are collaborating with the Tiemeyer laboratory (Georgia, USA) for profiling glycoproteins and glycolipids from fly heads of wild type and *Cog7* mutants by the use of multi-dimensional ion trap mass spectrometry. Overall this analysis will shed light on the molecular circuits involving Cog7 in brain cytokinesis and will be probably suggest new therapeutic avenues.

**Importin Beta and the GTPase RAN have global roles in mechanisms of genetic instability in cancer cells**

Annalisa Verrico[1], Laura Di Francesco[2], Valeria de Turris[3], Alessandro Rosa[4], Erica Di Cesare[1], Eugenia Gilistro[1], Paola Rovella[1], Miriam Carbo[1], Andrea Ilari[1], Patrizio Di Micco[1], Veronica Morea[1], Maria Eugenia Schininà[2], Patrizia Lavia[1]

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Importin beta is the major effector of the GTPase RAN and has global roles in mammalian cells: it acts as the major vector of protein import in the nuclei of interphase cells, and - when nucleo-cytoplasmic transport ceases at mitotic onset - it regulates the organization and function of the mitotic apparatus. Importin beta is expressed at abnormally elevated levels in several cancer types (cervix, gastric and breast cancer, as well as myeloma), associated with high levels of genetic instability in these cancers. These observations have led to propose that importin beta might act as a novel mechanistic player and a potential therapeutic target in cancer; specific inhibitors are indeed being developed.

Genetic instability is a characterizing and evolving feature of cancer. By combining proteome-wide interactomics studies, time-lapse imaging, proximity ligation assays and functional approaches, we are seeking to identify processes that are sensitive to importin beta /RAN network dysfunction. These approaches have enabled us to pinpoint multiple processes through which importin beta ensures ordered progression through mitosis and hence regulated chromosome segregation. These studies contribute to disentangle the mechanisms through which high importin beta levels can influence genetic instability in cancer cells.

**Mitotic functions of the SF3A2 and PRP31 splicing factors and INT6 in *Drosophila* and human cells**

Fioranna Renda[1], Claudia Pellacani[1],Elisabetta Bucciarelli[2],Elisa Maria Turco[1], David J. Sharp[3], Maurizio Gatti[1,2], Maria Patrizia Somma[2]

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[2]Institute of Molecular Biology and Pathology, National Research Council, Rome
[3]Department of Physiology and Biophysics, Albert Einstein College of Medicine, New York, USA

An RNAi-based screen performed in our laboratory identified several *Drosophila* proteins required for chromosome integrity, mitotic spindle assembly and functioning, and cell cycle progression. Most of these proteins are conserved, providing information on the putative function of their human orthologues. Using this information, we developed a biological signature that predicts survival in breast cancer patients.

We identified many genes required for chromosome segregation, including several highly conserved splicing factors (SFs). A detailed analysis of the role of two of these SFs (Sf3A2 and Prp31) showed that their loss causes a metaphase arrest phenotype in both *Drosophila* and HeLa cells. Both SFs bind strongly the spindle microtubules (MTs) and the Ndc80 complex, the key factor in kinetochore-MT interaction. In addition injection of antibodies against these SFs into *Drosophila* embryos arrested cell division within a minute. Together, these results strongly suggest that Sf3A2 and Prp31directly regulate interactions between kinetochores, Ndc80 and spindle MTs.

Another gene required mitotic progression identified in our screen was *int6*, which encodes a translation initiation complex subunit, known to interact with both the 26S proteasome and the COP9 signalosome. Int6-depleted *Drosophila* S2 cells exhibit a high frequency of cells arrested in metaphase, short spindles and distortion of centromere/kinetochore region. FRAP analysis suggested that loss of Int6 specifically enhances microtubule plus-end dynamics at the spindle equator. This phenomenon is due to an accumulation near the kinetochores of the MT depolymerase Klp67A, which is not properly ubiquitinated and degraded. We examined spindle morphology and mitotic progression also in INT6-depleted HeLa cells. We observed frequent multipolar spindles and massive chromosome mis-segregation. Intriguingly a similar phenotype has been previously observed in cells overexpressing KIF18A, a Klp67A homologue overexpressed in several

cancers. Collectively, our results suggest that Int6 regulates degradation MT-depolymerizing motors ensuring proper expression and activity of these motors during the mitotic process.

## Human Ferritins: selective nanocarriers for antitumor payloads

Pierpaolo Ceci[1], Elisabetta Falvo[1,2], Alberto Boffi[2], Patrizio Giacomini[3], Elisa Tremante[3], Gianni Colotti[1], Veronica Morea[1], Alessandro Arcovito[4], Giamaica Conti[5], Giulio Fracasso[6]

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Selective delivery to malignant lesions is particularly critical for many current cancer therapeutics which distribute non-specifically in the body, thereby affecting both normal and cancer cells. In this context, tumor targeting of nanomedicines has emerged as a promising approach to overcome the lack of specificity of conventional chemotherapeutic agents [1].

Among the reported nano-carriers for targeted delivery, protein-cage molecules based on human ferritin (HFt) are attracting growing interest due to their exceptional characteristics, namely biodegradability, lack of immunogenicity, solubility, functionalization versatility and remarkable capacity to encapsulate different types of drugs inside the protein cavity[2] (Figure 1).

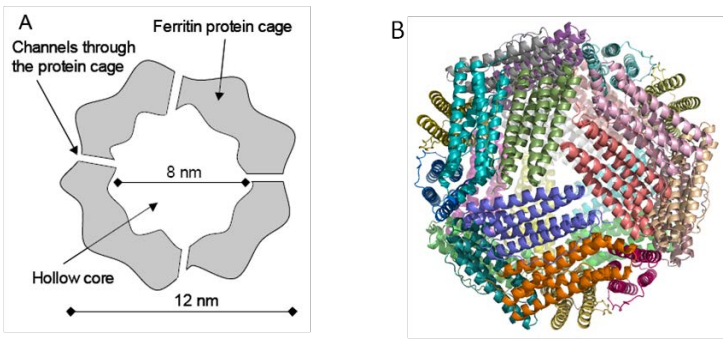


Figure 1. Ferritins are composed of 24 structurally identical subunits surrounding an internal cavity of 8 nm.

Further, HFt is one the few natural NPs that has *per se* the ability to effectively and selectively bind cancer cells. In fact, HFt was shown to be internalized by using one of the most attractive molecule for the targeted therapy of cancer, the transferrin receptor 1 (TfR1), a receptor that is upregulated on the surface of many cancer types (up to 100-fold higher than in normal cells) and is efficiently internalized [3].

In the present communication we report the use of a modified HFt-based nanocarrier loaded with Doxorubicin as payload. This novel HFt version is endowed with a higher blood stability and a better doxorubicin encapsulation capacity with respect to the native HFt (Figure 2) [4].

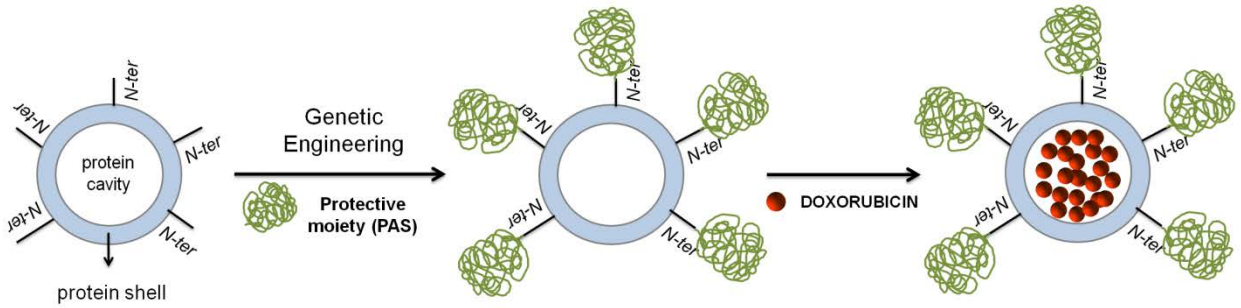


Figure 2. A sketch to briefly summarize the structure and the synthesis of our new HFt based nanocarriers.

This novel HFt nanocarrier is composed of 24 polypeptides-ferritin fusion protein monomers, autoassembling in a modified 24-mer protein cage. The nanocarrier is formed by distinct modules to increase blood stability and drug-loading due to the presence of an inert polypeptide rich in Proline, Alanine and Serine (PAS). The genetic fusion protein construct HFt-PAS was obtained and purified as recombinant protein in *Escherichia coli* at high yields (100 mg per *E. coli* liter). HFt-MMP-PAS nanocages were fully assembled and highly soluble and stable in buffer and plasma. Size-exclusion chromatography, dynamic light scattering and transmission electron microscopy experiments indicated that HFt-PAS is highly monodispersed with an average diameter of 18 nm. In addition, we observed a significant improvement in the Doxorubicin loading yields (about 6 fold) with respect to the native HFt, likely due to the higher solubility of this PASylated version of the HFt.

Then, the presence of the PAS sequences considerably prolonged circulation of the HFt-PAS loaded with doxorubicin in healthy mice *in vivo*. For these reasons we decided to investigate the therapeutic ability of HFt-PAS in different cancer

models-bearing mice. The results are very promising showing our HFt-based construct to possess a higher therapeutic efficacy also with respect to a novel formulation of doxorubicin currently in clinical phase III, the pro-drug Aldoxorubicin (INNO-206) [5].

**References:**


1. Sun TM, Zhang YS, Pang B, Hyun DC, Yang MX, Xia NY. Engineered nanoparticles for drug delivery in cancer therapy. Angewandte Chemie-International Edition, 2014, **53:**12320-12364
2. Falvo E, Tremante E, Fraioli R, Leonetti C, Zamparelli C, Boffi A*, et al.* Antibody-drug conjugates: targeting melanoma with cisplatin encapsulated in protein-cage nanoparticles based on human ferritin. Nanoscale*,* 2013, **5:**12278-12285
3. Fan K, Gao L, Yan X. Human ferritin for tumor detection and therapy. Wiley Interdiscip Rev Nanomed Nanobiotechnol, 2013, **5:**287-298
4. Falvo E, Tremante E, Arcovito A, Papi M, Elad N, Boffi A*, et al.* Improved doxorubicin encapsulation and pharmacokinetics of ferritin-fusion protein nanocarriers bearing proline, serine, and alanine elements. Biomacromolecules, 2016, **17:**514-522
5. Kratz F, Azab S, Zeisig R, Fichtner I, Warnecke A. Evaluation of combination therapy schedules of doxorubicin and an acid-sensitive albumin-binding prodrug of doxorubicin in the MIA PaCa-2 pancreatic xenograft model. Int J Pharm, 2013, **441:**499-506

## Sorcin induces resistance to chemotherapeutics in tumors

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Sorcin (SOluble Resistance-related Calcium binding protein) is an essential penta-EF hand calcium binding protein, which participates in the modulation of calcium homeostasis (and of heart contraction) and in calcium-dependent cell signalling in normal and cancer cells. Sorcin is able to regulate Endoplasmic Reticulum (ER) calcium levels and to control ER stress. Sorcin silencing blocks cell cycle progression in mitosis and induces cell death by triggering apoptosis.

The X-ray structures of Sorcin in the apo (apoSor) and in calcium bound form (CaSor) reveal the structural basis of Sorcin action: calcium binding to the EF1-3 hands promotes a large conformational change, involving a movement of the long D-helix joining the EF1-EF2 sub-domain to EF3 and the opening of EF1. This movement determines the exposure of a pocket, which can accommodate hydrophobic sorcin target domains as well as organic hydrophobic molecules.

The gene for sorcin (SRI) is located in chromosome 7, in the same amplicon of other genes involved in the resistance to chemotherapeutic agents in cancer cells as the ABC transporters ABCB4 and ABCB1 (Mdr1, or P-glycoprotein 1). In recent years, an increasing number of data have demonstrated a role of sorcin in Multi Drug Resistance (MDR), pointing sorcin out as an oncoprotein. Sorcin is highly expressed in chemoresistant cell lines, and its overexpression confers MDR. The level of sorcin expression in leukemia patients inversely correlates with patients' response to chemotherapies and overall prognosis. Sorcin overexpression by gene transfection resulted in increased drug resistance to a variety of chemotherapeutic agents, while inhibition of sorcin expression by sorcin-targeting RNA interference led to reversal of resistance to drugs in several cell lines. Sorcin induces MDR by efficiently binding chemotherapeutic agents, thus decreasing their cellular effects and impairing cell death.

**Amorphous silica nanoparticles alter microtubule dynamics and cell migration**

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Amorphous silica nanoparticles ($SiO_2$-NPs) have been studied for their toxic and genotoxic potential. Although contradictory data have been reported and the possible modes of action are not fully elucidated, aneugenic events have been reported, indicating the microtubule (MT) network as a potential target. A thorough understanding of the modes of action of $SiO_2$-NPs, and nanomaterials in general, is urgently needed for the elaboration of a robust regulatory framework for nanomaterials, as well as adequate monitoring systems. To investigate MTs as potential targets, we examined the effects of 59 nm (10 mg/ml) and 174 nm (7.5 mg/ml) $SiO_2$-NPs on MTs in mitotic and interphase A549 human lung carcinoma cells. No gross morphological changes of the mitotic spindle or induction of multipolar spindles were observed upon $SiO_2$-NPs treatment. The influence of $SiO_2$-NPs on the interphase MTs network dynamics was investigated by in situ depolymerisation/repolymerisation experiments. Results showed a clear increase in MT dynamics after $SiO_2$-NP treatment. Consistent with this, reduced levels of MT acetylation were observed. In addition, live cell microscopy demonstrated that $SiO_2$-NP treatment reduced A549 cell motility. The $SiO_2$-NP doses and conditions (serum-free) used in this study did not induce significant cell toxicity or MN frequencies. Therefore, the effects on MT dynamics, MT acetylation and migration observed, are direct effects of the $SiO_2$-NPs and not a consequence of NP overload or toxic or genotoxic effects.

## Resetting cancer stem cell regulatory nodes upon MYC inhibition

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MYC deregulation is common in human cancer and has a role in sustaining the aggressive cancer stem cell populations. MYC mediates a broad transcriptional response controlling normal biological programs but its activity is not clearly understood. We address MYC function in cancer stem cells through the inducible expression of the interfering miniprotein Omomyc, taking as model the most lethal brain tumour, glioblastoma. Omomyc bridles the key cancer stem cell features and affects tumour microenvironment, inhibiting angiogenesis. This occurs because Omomyc broadly replaces MYC on the genome, leading to selective repression of master transcription factors for glioblastoma stem cell identity like POU3F2, SOX2, and OLIG2, upregulation of effectors of tumour suppression and differentiation such as PTEN, ID4, MIAT, and modulation of the expression of microRNAs that target molecules implicated in glioblastoma growth and invasion like EGFR and ZEB1. Data support a novel view of MYC as a network stabiliser that strengthens the regulatory nodes of the gene expression programs controlling cell phenotype and highlight Omomyc as model molecule for targeting cancer stem cells.

**Response to acute corticosterone treatment of hippocampal neurons *in vitro* is reduced in dystrophic *mdx* mice compared to wild type**

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One aspect of the response to stressful events is the activation of the autonomic nervous system and the hypotalamus-pituitary-adrenal axis, with consequent increase in glucocorticoid (GC) synthesis and release [1,2]. Important brain areas (i.e. hippocampus, prefrontal cortex, and amygdala) are particularly susceptible to stressful stimuli, and their alteration may determine behavioral and neurological disturbances. In these regions, both acute (single) and chronic (repeated) stress stimuli induce prominent changes in neuronal activity and synaptic functions, which rely on neuron circuit remodeling (i.e. dendrite shortening and pruning). These stress-induced changes are largely mediated by modifications in gene expression [3,4]. In human diseases, GCs are used to suppress inflammation and their use in a variety of inflammatory and autoimmune diseases makes them among the most frequently prescribed classes of drugs. GCs represents standard medications for treating Duchenne muscular dystrophy [5]. This is a lethal, X-linked disease, characterized by muscular wasting due to lack of dystrophin, a 427 KDa protein (Dp427). Dp427 is also expressed in brain and DMD patients experience different degrees of neurological disorders [6].

Our research interest is to better understand the consequences of GC treatments on hippocampal neuron physiology, with particular attention to gene expression modulation of the GC receptor (GR). In fact, except for the anti-inflammatory effect, little is known on GC molecular mechanism(s) that yield to so many important and well-described side effects. In this project, we used wild type (wt) and dystrophic *mdx* mice to uncover the effects that GC exert on hippocampal neurons, which are both affected in DMD and particularly sensitive to stress [7,8]. We analyzed GR mRNA (real time RT-PCR, qPCR) and protein levels (Western immunoblot) in hippocampal neuron cultures from E18 WT and *mdx* mice, following incubation (1 h, 37°C) with either 1 μM or 10 μM CORT. In WT mouse hippocampal cells, qPCR analysis revealed an increase in GR mRNA levels after both CORT treatments (1.5 fold), compared to control (CNTR, vehicle

alone). Differently, in *mdx* mouse cells, mRNA levels increased slightly (0.5 folds) only after 1 μM CORT incubation, and decreased significantly with 10 μM CORT. Accordingly, GR protein levels in WT mouse hippocampal cells increased significantly, compared to control, after 1μM CORT incubation, with *p*GR levels having the same tendency. In *mdx* mouse neurons, instead, both GR and pGR do not vary. We hypothesize that *mdx* mouse neurons could be "pre-sensitized" by blood-derived GC in pregnant mothers, also afflicted by mild myodegeneration and inflammation. This could interfere with further GC signaling *in vitro* in terms of GR mRNA synthesis, post-transcriptional modifications and translation, as well protein synthesis/degradation ratio [9]. This may also explain why the increase in GR mRNA in the WT samples was not linear, being the 10 μM concentration the limit after which no further changes are triggered. Moreover, as in *mdx* mice and DMD patients neuronal alterations begin during development, steps of the GC-GR signaling could also be directly affected.

**References:**


1. de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. Nature Rev, 2005, **6:**463-475
2. de Kloet, ER. Brain corticosteroid receptor balance and homeostatic control. Frontiers in neuroendocrinology, 1991, **12:**95-164
3. McEwen BS. Stress and hippocampal plasticity. Annu Rev Neurosci, 1999, **22:**105-122
4. Rinaldi A, Vincenti S, De Vito F, Bozzoni I, Oliverio A, Presutti C, Fragapane P, Mele A. Stress induces region specific alterations in micro RNA expression in mice. Behav Brain Res, 2010, **208:**265-269
5. de Kloet ER. From receptor balance to rational glucocorticoid therapy. Endocrinology, 2014, **155:**2754-2769
6. Anderson JL, Head SI, Rae C, Morley JW. Brain function in Duchenne muscular dystrophy. Brain, 2002, **125:**4-13
7. De Stefano ME, Zaccaria ML, Cavaldesi M, Petrucci TC, Medori R, Paggi P. Dystrophin and its isoforms in a sympathetic ganglion of normal and dystrophic mdx mice: immunolocalization by electron microscopy and biochemical characterization. Neuroscience, 1997, **80:**613-624
8. De Stefano ME, Leone L, Lombardi L, Paggi P. Lack of dystrophin leads to the selective loss of superior cervical ganglion neurons projecting to muscular targets in genetically dystrophic mdx mice. Neurobiol Dis, 2005, **20:**929-942
9. Ayroldi E, Riccardi C. Glucocorticoid induced leucine zipper (GILZ): a new important mediator of glucocorticoid action. FASEB J, 2009, 23:3649-3658

**Structural and functional studies of trypanothionereductase (TR) and tryparedoxin peroxidase (TXNPx) two good targets to find new drugs against Leishmaniasis.**

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Leishmaniasis is a neglected disease killing 60,000 people worldwide, caused by the protozoan *Leishmania* a trypanosomatid family member. The parasite developed a unique metabolism based on a dithiol, the trypanothione to defend itself from the reactive oxygen species produced by the host immune system during the infection. Trypanothione, synthesized by trypanothione synthetase (TryS) and reduced by trypanothione reductase (TR), is used as a source of electrons by the tryparedoxin/tryparedoxin peroxidase system (TXN/TXNPx) to reduce the hydroperoxides produced by macrophages. We solved the X-ray structure of TR, TXN and TXNPx with the aim to understand the structure-function relationships in these proteins and use this knowledge to design new lead compounds against leishmaniasis. The X-ray structure of reduced TR in complex with Sb(III) allowed us to disclose the molecular basis of TR inhibition by antimonial compounds, which are the first choice drugs against leishmaniasis. Sb(III) binds to the residues of the active site pocket involved in catalysis, namely Cys52, Cys57, Thr335 of one subunit and His461' of the two-fold symmetry related subunit, thereby blocking enzymatic activity [1]. The X-ray structures of TR in complex with silver and gold showed that Ag (I) and auranofin inhibit TR activity by binding to the catalytic cysteine residues as well, but, as shown by kinetic studies, with significantly higher affinity than Sb(III) (Ki = 50 nM, 155 nM and 1.5 µM, respectively) [2,3]. Additionally, silver and gold containing compounds have been shown to be able to kill the parasite in both the amastigote and promastigote stages. These results indicate that silver- and gold-containing compounds are strong inhibitors of TR and active against Leishmania parasites in vitro, and are therefore worth investigating as potential lead compounds for the development of novel metal-based anti-leishmanial agents. The structures of TXN and TXNPx from *Leishmania major*, solved by X-ray crystallography, offer the unique opportunity to study the peroxide reduction in Leishmania parasite at a molecular level and lay the basis of multienzyme inhibition-based drug discovery [4]. The analyses of the electrostatic surfaces of the two proteins unveiled the structural elements allowing the interaction between TXN and TXNPx. This finding allows us to build a complete model for the

interactions between the two proteins which is important to understand the mechanism of peroxide reduction catalyzed by the couple. The X-ray structures of TXNPx in both fully folded (reduced) and locally unfolded (oxidized) conformations have been used for high throughput docking experiments that led us to the identification of the first non covalent inhibitors of this enzyme [5].

## References


1. Baiocco P, Colotti G, Franceschini S, Ilari A. Molecular basis of antimony treatment in leishmaniasis. J Med Chem, 2009, **52:**2603-2612
2. Ilari A, Ceci P, Orsini S, Gramiccia M, Di Muccio T, Colotti G. Inhibitory effect of silver nanoparticles on trypanothione reductase activity and Leishmania infantum proliferation. ACS Med Chem Lett, 2011, **2:**230-233
3. Ilari A, Baiocco P, Messori L, Fiorillo A, Boffi A, Gramiccia M, Di Muccio T, Colotti G. A gold-containing drug against parasitic polyamine metabolism: the X-ray structure of trypanothione reductase from Leishmania infantum in complex with auranofin reveals a dual mechanism of enzyme inhibition. Amino Acids, 2012, **42:**803-811
4. Fiorillo A, Colotti G, Boffi A, Baiocco P, Ilari A. The crystal structures of the tryparedoxin-tryparedoxin peroxidase couple unveil the structural determinants of Leishmania detoxification pathway. PLoS Negl Trop Dis, 2012, **6:**e1781.
5. Brindisi M, Brogi S, Relitti N, Vallone A, Butini S, Gemma S, Novellino E, Colotti G, Angiulli G, Di Chiaro F, Fiorillo A, Ilari A, Campiani G. Structure-based discovery of the first non-covalent inhibitors of Leishmania major tryparedoxin peroxidase by high throughput docking. Sci Rep, 2015, **5:**9705

**Hydrogen sulfide, cell respiration and the interplay between gasotransmitters at human cystathionine β-synthase**

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Merely considered as a toxic gas in the past, hydrogen sulfide ($H_2S$) is currently viewed as the third 'gasotransmitter' in addition to nitric oxide (NO) and carbon monoxide (CO). $H_2S$ plays a key signalling role in human (patho)physiology that crucially depends on the bioavailability of this gaseous molecule and its crosstalk with NO and CO. We found that the $H_2S$-producing human enzyme cystathionine β-synthase (CBS), recently shown to sustain proliferation in colorectal cancer cells, mediates the interplay between gasotransmitters [1,2]. The enzyme was indeed recently found to be negatively modulated by physiological concentrations of CO and NO, particularly in the presence of the allosteric activator S-adenosyl-l-methionine (AdoMet). Besides acting as a signaling molecule, sulfide is a potent inhibitor of respiratory oxidases, such as mitochondrial cytochrome *c* oxidase (reviewed in [3]). This led us to raise the hypothesis that in sulfide-rich environments, like our gut, bacteria can accomplish $O_2$-dependent respiration due to sulfide-insensitive oxidases. The hypothesis was recently validated working on *Escherichia coli* [4]. The physiological significance and potential impact of this discovery will be discussed.

**References:**


1. Vicente JB, Colaço HG, Mendes MI, Sarti P, Leandro P, Giuffrè A. NO binds human cystathionine beta-synthase quickly and tightly. J Biol Chem, 2014, **289:**8579-8587

2. Vicente JB, Colaço HG, Sarti P, Leandro P, Giuffrè A. S-adenosyl-L-methionine modulates CO and NO binding to the human $H_2S$-generating enzyme cystathionine beta-synthase. J Biol Chem, 2016, **291:**572-581

3. Vicente JB, Malagrinò F, Arese M, Forte E, Sarti P, Giuffrè A. Bioenergetic relevance of hydrogen sulfide and the interplay between gasotransmitters at human cystathionine beta-synthase. Biochim Biophys Acta, 2016, **1857:**1127-1138

4. Forte E, Borisov VB, Falabella M, Colaço HG, Tinajero-Trejo M, Poole RK, Vicente JB, Sarti P, Giuffrè A. The terminal oxidase cytochrome *bd* promotes sulfide-resistant bacterial respiration and growth. Sci Rep, 2016, **6:**23788.

**Hormonal control of reproductive development and response to abiotic stress in *Arabidopsis thaliana***

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The main focus of the research carried out in the lab is on the hormonal control of late stamen development in the model species *Arabidopsis thaliana*. Late stamen development consists of three simultaneous processes: pollen maturation, anther opening (dehiscence) and filament elongation. These processes are crucial in controlling the self-fertilization of autogamous plants, such as the model plant *Arabidopsis thaliana* and several crops such as rice, soybean, and tomato. It is known that in animals concentration gradients of morphogens regulates organ development and similarly in plants the hormone auxin may play the role of a morphogen. We showed that auxin has a key inhibitory role in pollen maturation and anther dehiscence, whereas it promotes filament elongation [1]. Very recently we have shown the establishment of an auxin maximum in the anther tissue middle layer, and provided strong evidence that it regulates auxin distribution between the different tissues, to coordinate anther and pollen maturation [2]. We are currently studying also the role of the auxin response factors ARF6 and ARF8 in late stamen development. We have shown that ARF8 is subjected to alternative splicing and that the three isoforms have different effects on stamen elongation. In particular, ARF8.3 has a main role in stamen filament elongation and early events of anther dehiscence. Our results suggestthat alternative splicing of specific ARFs may be among the mechanisms through which auxin regulates the growth of the reproductive organs.

The other research line in the lab focuses on the role of phytochelatins (PCs) in heavy metal tolerance in Arabidopsis and tobacco. Plants can be used for decontamination of heavy metal polluted soils and enhanced metal tolerance and accumulation is a desirable trait forphytoremediation. We have shown the involvement of the ABC transporter ABCC3 in the compartmentalization of cadmium- PCs complexes into the vacuole. We are currently studying the role of ABCC3 in arsenic tolerance and accumulation.

## References


1. Cecchetti V, Altamura MM, Falasca G, Costantino P, Cardarelli M. Auxin regulates *Arabidopsis* anther dehiscence, pollen maturation, and filament elongation. Plant Cell, 2008, vol. 20, p. 1760-1774
2. Cecchetti V, Celebrin D, Napoli N, Ghelli R, Brunetti P, Costantino P, and Cardarelli M, 2016 (manuscript in preparation)

## Role of the Arabidopsis HD-Zip II transcription factors HAT3 and ATHB4 in flower development

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The flower in angiosperms is formed by four concentric whorls of defined organs: from outside inwards, sepals, petals, stamens (male organs), gynoecium (female organ). The number of organs may vary among species, but the basic organization is fixed. Through the use of direct and reverse genetics in several plant systems, it has been formulated the (A)BCE genes model, which, at the moment, explains flower development in most angiosperms. In Arabidopsis, the sepals are specified by class A genes, [APETALA1 and APETALA2 (AP2)]; the petals are specified by class A and class B gene (PISTILLATA and APETALA3); the stamens are specified by class B and class C [AGAMOUS (AG)] genes; the gynoecium is specified by the sole class C. The expression of class E genes (SEPALLATA 1-4) in all whorls establishes a “flower” background in which the ABC genes can specify flower organs [1].

Our laboratory is involved in the elucidation of the function of the homeodomain-leucine zipper (HD-Zip) class II genes in plant development and recently demonstrated that HD-Zip II transcription factors control apical embryo development, meristem function and organ polarity [2,3]. Flower development is also altered in *hd-zip II* loss-of-function mutants. In particular, in the double *homeobox arabidopsis thaliana 3* (*hat3*) *arabidopsis thaliana homeobox 4* (*athb4*) mutant background, we observed phenotypes in the gynoecium, as the valves appear severely splitted, instead of being tightly fused [4]. This phenotype is resembling the loss of function of the SPATULA (SPT) gene, which is indeed involved in the formation of the style and stigma, the upper part of the gynoecium. In the wild type the apical style region undergoes a transition from a bilaterally symmetric stage to a radially symmetric structure during gynoecium development. Two transcription factors, INDEHISCENT and SPT, are both necessary and sufficient for the radialization process and control style symmetry by directly regulating auxin distribution [5]. We are evaluating the genetic interactions between HAT3/ATHB4 and SPT by crossing the respective mutant lines and by expression analysis of HAT3 and SPT marker lines in the *spt* and *hat3 athb4* mutants, respectively. Moreover, we are studying auxin dynamics in

the *hat3 athb4* mutant background in order to evaluate if auxin distribution is also regulated by HAT3 and ATHB4.

**References:**


1. Sablowski R. Control of patterning, growth, and differentiation by floral organ identity genes. J Exp Bot, 2015, **66:**1065-1073
2. Turchi L, Carabelli M, Ruzza V, Possenti M, Sassi M, Peñalosa A, Sessa G, Salvi S, Forte V, Morelli G, Ruberti I. Arabidopsis HD-Zip II transcription factors control apical embryo development and meristem function. Development, 2013, **140:**2118-2129
3. Turchi L, Baima S, Morelli G, Ruberti I. Interplay of HD-Zip II and III transcription factors in auxin-regulated plant development. J Exp Bot, 2015, **66:**5043-5053
4. Carabelli M, Turchi L, Ruzza V, Morelli G, Ruberti I. Homeodomain-Leucine Zipper II family of transcription factors to the limelight: central regulators of plant development. Plant Signal Behav, 2013, **8:**e25447
5. Moubayidin L, Ostergaard L. Dynamic control of auxin distribution imposes a bilateral-to-radial symmetry switch during gynoecium development. Curr Biol, 2014, **24:**2743-2748

## Epigenetic from budding yeast to human cancer

Claudia Canzonetta[1], Manuela Leo[2], Serena Di Vito[1], Giulia Fanelli[1], Antonella Stoppacciaro[4], Elisa Cocco[4], Antonello Mai[3], Dante Rotili[3] and Patrizia Filetici[1]

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Post-translational modifications (PTMs) such as Acetylation Ubiquitylation, Methylation regulate the genome architecture and expression. We study epigenetic regulatory pathways in the simple yeast with the longlasting goal to translate our discoveries for the comprehension of pathways involved in oncogenic transformation and cancer progression.

**SAGA/STAGA complex; Acetylation and Deubiquitylation**

Acetylation is a PTM spanning from chromatin and histones to a vast number of non histone proteins, the so called Acetylome, engaged in signal transduction and affecting protein functions/localization. In past, we have extensively studied the key K-acetyltransferase Gcn5. Among these study the identification and 3D characterization of the Ac-Lys chromatin-reader Bromodomain [2], the identification of Gcn5 role in mitosis [3] and in genome stability at centromere [4] and kinetochore.

Collectively our results highlight a broader role for Gcn5 not only devoted to transcriptional activation but directly required for cell signaling through acetylation. We recently identified a single histone H3-Lys exclusively acetylated by KAT-Gcn5. This H3-Lys is an important diagnostic/prognostic mark in the classification of selected human cancer types, following this evidence we are currently investigating this mark and its putative use in the diagnosis of tumors at early stage. We are also involved in a collaborative project aiming to investigate the role of acetylation and KATs Gcn5/p300 in the progression of the aggressive renal clear cell carcinoma.

**Acetylation and metabolism**

Signaling pathways affected by genetic mutations and tumour microenvironment have a profound effect on core metabolism. This is one of the most intense area of research in cancer biology. In the simple yeast we are currently investigating a novel link of acetylation, sugar metabolism and mitochondria. In particular, we are carrying out a study focused on the role of SAGA complex and selected components such as KAT-Gcn5 and DUB-Ubp8 in the

regulation of respiration and mitochondria activity.

**KAT inhibitors as anticancer drugs**

Ultimately, from our past research we obtained an active Gcn5/p300 KAT-inhibitor, CPTH2 [1,5], from this lead compound we are developing novel molecules with the aim to increase their efficacy. Our current focus is centered on the use of CPTH2 and the analysis of its efficacy on differentiation and proliferation of cancer cell cultures and its effects on global histone acetylation.

**References:**


1. Vernarecci S, Tosi F, Filetici P. Tuning acetylated chromatin with HAT inhibitors: a novel tool for therapy. Epigenetics, 2010, **5:**105-111
2. Owen DJ, Ornaghi P, Yang JC, Lowe N, Evans PR, Ballario P, Neuhaus D, Filetici P, Travers AA. The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. EMBO J, 2000, **19:**6141-6149
3. Vernarecci S, Ornaghi P, Bâgu A, Cundari E, Ballario P, Filetici P. Gcn5p plays an important role in centromere kinetochore function in budding yeast. Mol Cell Biol, 2008, **28:**988-996
4. Canzonetta C, Vernarecci S, Iuliani M, Marracino C, Belloni C, Ballario P, Filetici P. SAGA DUB-Ubp8 deubiquitylates centromeric histone variant Cse4. G3:Genes, Genomes Genetics, 2015, **6:**287-298
5. Chimenti F, Bizzarri B, Maccioni E, Secci D, Bolasco A, Chimenti P, Fioravanti R, Granese A, Carradori S, Tosi F, Ballario P, Vernarecci S, Filetici P. A novel histone acetyltransferase inhibitor modulating Gcn5 network: cyclopentylidene-[4-(4'-chlorophenyl)thiazol-2-yl)hydrazone. J Med Chem, 2009, **52:**530-536

## The informational content of single-cell time-lapse imaging to study dynamic biological processes

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Microscopy is rapidly evolving from a qualitative to a more quantitative approach, representing a valuable methodology in cell biology. Most complex biological processes (proliferation, cell death, cell differentiation, infection, host/pathogen interactions, cell adhesion and migration and others) have an inherent dynamic dimension. Of relevance is the development of time-lapse video-recording of live cells to visualize the unfolding of these processes, highlighting features that would remain unnoticed with analyses of fixed samples or whole cell population approaches.

We have developed quantitative high-resolution imaging methods in two major fields of application: 1) the drug design field, and 2) the study of single proteins, protein domains or post-translational modifications.

In the first type of application, live imaging is employed to record the behaviour, over days, of single cells treated with molecules of potential therapeutic value in cancer treatment. Paradigmatic examples are microtubule-targeting molecules of innovative design and inhibitors of the mitotic kinase Aurora-A. The informational content from the recorded videos can be extracted to gain information on dynamic parameters, temporal sequence and identification of stochastic events. Overall, our studies show that the response of a cell population to anti-cancer drugs is in fact a “profile of cell fates”. They pinpoint specific events occurring in single cells, and/or the requirement for particular factors, that may influence the outcome of the treatment. This is relevant to identify molecular determinants underlying the heterogeneity in cell response and to improve the design/efficacy of treatments.

In parallel applications, we have imaged the fate of cells forced to overexpress, or on the contrary, lacking, specific mitotic regulatory proteins, or expressing dominant-negative mutants, or mutants defective for particular post-

translational modifications. Time-lapse recording has revealed unexpected roles for some of these proteins in apparently unrelated processes, or in unexpected cell cycle windows, that could not have been appreciated with other approaches. Recording of functional rescue experiments, by co-expressing in the same cells putative partners or targets of the protein under examination, identified by distinct fluorochromes, is proving a powerful tool to reconstruct complex pathways.

The wide range of applications of time-lapse microscopy suggests the need for standardization and data storage in automated databases. We have therefore created the "time-lapse imaging" database, which includes different experiments performed at our microscopy platform: this has enabled us to extract the most relevant parameters and devised acquisition protocols for each application. The database is an effective and dynamic tool that facilitates the retrieval, comparison and sharing of microscopy data within the scientific community to maximally exploit the resources of the imaging platform.

# POSTER SESSION

## P-1: The Biocrystal Facility at the CNR-IBPM: a support to determine the three dimensional structure of biological macromolecules

Carmelinda Savino[1], Adele Di Matteo[1], Veronica Morea[1], Gianni Colotti[1], Andrea Ilari[1]

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The Biocrystal Facility at the CNR Institute of Molecular Biology and Pathology in Rome offers users, either internal or external to the CNR, access to the production of protein or nucleic acid crystals. This is a required step to solve the three-dimensional structures of biological macromolecules or their complexes by X-ray crystallography. In turn, structural information is essential to understand the molecular basis of essential biological events.

The first support offered by the CNR personnel affiliated to the Biocrystal Facility consists in the assessment of the feasibility of structural biology projects and identification of objectives and critical points. To this end, expert protein crystallographers, biochemists, and molecular and computational biologists interact to evaluate features such as expected protein solubility, homology to available structures, possibility to produce large amounts of highly purified proteins, and other requirements of crystallization trials. As a result of this analysis, modifications ranging from single-point mutations to production of protein fragments may be suggest to increase the chance of success.

Once sufficient amounts of purified protein are obtained, experienced crystallographers carry out robotic crystallization trials, analysis of results and optimization of crystallization conditions using in-house equipment.

In addition, the Facility offers support for applications to the European Biostruct-X initiative to access synchrotron light sources.

The Biocrystal Facility has been set up in 2012 by the CNR Department of Life Sciences in collaboration with the Department of Biochemistry of the “Sapienza” University of Rome. One of the initial aims of the Facility was to increase the number of Italian users of European facilities such as the European Synchrotron Radiation Facility (ESRF) and, as a consequence, the impact of the CNR in the field of structural biology. This action is in line with the aims of the “Horizon 2020" initiative, which include the access to and development of European infrastructures for advanced research.

**http://www.biocrystalfacility.it/** **mail: info@biocrystalfacility.it**

**P-2: Mesophilic and thermophilic genetic systems for heterologous and xenologous expression of of "extremozyme" genes from hyperthermophilic source.**

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Three sequences encoding putative extracellular endoglucanase (*sso1354*, *sso1949* and *sso2534)* have been found on the complete genome sequence of *S. solfataricus* P2. These enzymes which can be classified as members of glycoside hydrolases family 12, have been only partially studied. SSO2534 [1] and SSO1949 have been so far characterized differently from SSO 1354. The present work has been focused on the cloning and the expression of *sso1354* and *sso2534* both in xenologous and heterologous fashions and on the characterization of the recombinant products. Due to the documented difficulties to express cellulase genes from hypertermophilic organisms in phylogenetically distant conventional hosts only sso1949 has been expressed at low levels in *E. coli*; similar attempts for *sso1354* and *sso2534* were unsuccessful. To overcome these obstacles and to allow the study of gene product *in vivo,* for *sso1354* a homologous expression system based on the *E. coli – Sulfolobus solfataricus* pMSSV shuttle vector was used [2,3]. The development of an effective expression vector for archaeal organisms is a key step to study their characteristics in depth [4]. The genome sequencing progress provides a large quantity of DNA sequences and the function of the putative encoded proteins has only been assumed by similarity analysis for some of them. In this contest an effective transformation-expression system can be very useful, allowing the gene product to be studied *in vivo* by homologous expression. The *sso1354* gene was cloned under the control of the promoter of the *glcS* gene encoding the glucose binding protein, which is inducible by glucose, and expressed in *Sulfolobus solfataricus* cells [3]. Moreover both *sso1354* and *sso2534* were expressed in the mesophilic hosts *E. coli* and *Kluyveromyces lactis* with the aim to optimize the yield of the active recombinant enzymes and to compare the proteins obtained from the different sources. After the unsuccessful first attempts of cloning the whole coding sequences in *E. coli* good results were obtained with the exclusion of the N-terminal hydrophobic sequence which interfered with the correct folding of the protein. This sequence shows a significant similarity with corresponding regions of *S. solfataricus* sugar binding proteins AraS, GlcS and TreS and is

responsible for the anchoring of the polypeptides to the membrane. The results of the expression of both protein in the different hosts show that they present mainly an endo β1-4 glucanase activity and specifically hydrolyze cellulose; however for sso1354 were also detected activities toward several other polymers such as lichenan, xilan, debranchedarabinan, pachyman and curdlan. Moreover, as expected they are optimally active at low pH and high temperature showing also an elevated termostability; e.g. sso13254 expressed in *K. lactis* showed a half life of 180 min at 90°C.

**References:**


1. Limauro D, Cannio R, Fiorentino G, Rossi M, Bartolucci S. Identification and molecular characterization of an endoglucanase gene, *celS*, from the extremely thermophilic archaeon *Sulfolobus solfataricus*. Extremophiles, 2001, **5:**213-219
2. Aucelli T, Contursi P, Girfoglio M, Rossi M, Cannio R. A spreadable, non-integrative and high copy number shuttle vector for Sulfolobus solfataricus based on the genetic element pSSVx from *Sulfolobus islandicus*. Nucleic Acids Res, 2006, **34:**e114
3. Girfoglio M, Rossi M, Cannio R. Cellulose degradation by *Sulfolobus solfataricus* requires a cell-anchored endo-β-1-4-glucanase. J Bacteriol, 2012, **194:**5091-5100
4. Contursi P, Fusco S, Cannio R, She Q. Molecular biology of fuselloviruses and their satellites. Extremophiles, 2014, **18:**473-489

## P-3: Structural and functional characterization of a P450 epoxidase involved in Oleandomycin biosynthesis

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Cythocrome P450s are heme-containing enzyme that catalyze the oxidation of non-reactive C-H bonds and are involved in numerous metabolic reactions of physiological compounds and xenobiotic molecules [1].

The P450 epoxidase OleP from *Streptomyces antibioticus* is active during the tailoring steps of antibiotic oleandomycin biosynthesis, catalyzing the epoxidation at the C-C8a of the macrolactone ring. Given the paucity of structural and functional data *in vitro*, many doubts still remain regarding both the timing of the OleP reaction and the mechanism of epoxidation itself catalyzed by this P450, since discordant findings have been reported [2,3,4].

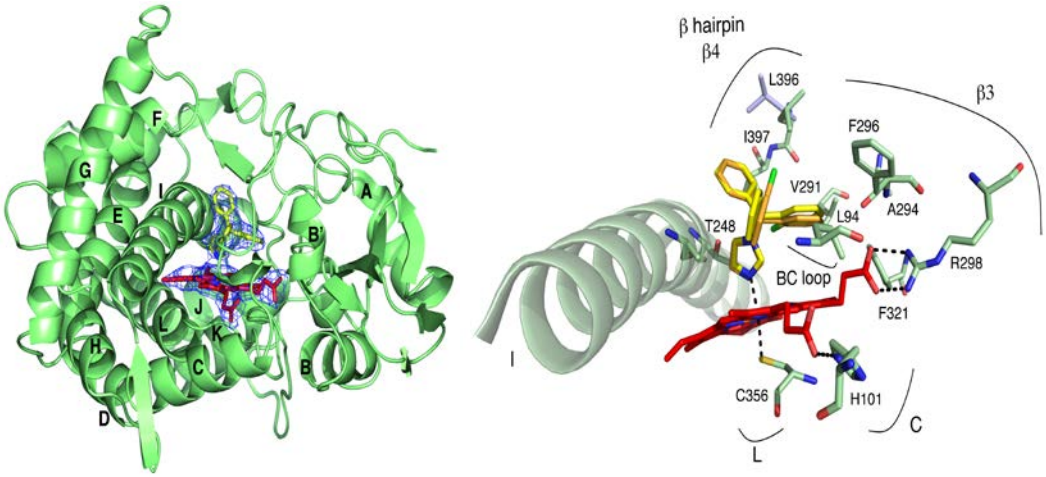
We solved the structure of OleP in complex with a substrate analogue, 6DEB, and in complex to a common P450 inhibitor, clotrimazole (CTZ). In complex with CTZ, OleP crystallizes as a dimer in the asymmetric unit, both monomers arranged in an open conformation [5]. The 6DEB-bound form is organized in the crystallographic lattice as an octamer in the asymmetric unit. Six monomers adopt an open conformation, but two were found in a closed form, suggesting that, in complex with the substrate, OleP may explore an open and closed structure.

OleP is the only P450 that introduces an epoxide on a non-activated C–C bond. The data here presented are necessary to understand the rare chemistry carried out by OleP, to engineer it and to design more selective and potent P450-targeted drugs.

### References:

1. Denisov IG, Makris TM, Sligar SG, Schlichting I. Structure and chemistry of cytochrome P450. Chem Rev, 2005, **105:**2253-2277
2. Tatsuta K, Gunji H, Tajima S, Ishiyama T, Imai S, Okuyama S, Fukatsu S. Biosynthetic studies on oleandomycin by incorporation of the chemically synthesized aglycones. J Antibiot, 1990, **43:**909-911
3. Shah S, Xue Q, Tang L, Carney JR, Betlach M, McDaniel R. Cloning,

characterization and heterologous expression of a polyketide synthase and P-450 oxidase involved in the biosynthesis of the antibiotic oleandomycin. J Antibiot, 2000, **53:**502-508
4. Rodriguez AM, Olano C, Méndez C, Hutchinson CR, Salas JA. A cytochrome P450-like gene possibly involved in oleandomycin biosynthesis by Streptomyces antibioticus. FEMS Microbiol Lett, 1995, **127:**117-120
5. Montemiglio LC, Parisi G, Scaglione A, Sciara G, Savino C, Vallone B. Functional analysis and crystallographic structure of clotrimazole bound OleP, a cytochrome P450 epoxidase from Streptomyces antibioticus involved in oleandomycin biosynthesis. BBA, 2016, **1860:**465-475




On the left: ribbon representation of OleP (green) in complex with clotrimazole, CTZ, (yellow stick), heme group is in red stick and the electronic density at 1 Å is shown in blue. On the right: the clotrimazole (CTZ) in the active site of OleP. The imidazole moiety of CTZ coordinates the heme iron in sixth position at a distance of 2 Å, while the N1 substituent group establishes van der Waals interactions with the side chains of specific residues exposed on the active site and reported in figure. CTZ adopts two different orientations in the active site of monomer A (yellow) and B (orange).

## P-4: Neurotrophin level changes induced by QMT, a new cognitive-motor training.

Micaela Caserta[1], Sabrina Venditti[2], Valerio Vetriani[2], Tal Dotan Ben-Soussan[3], Loredana Verdone[1]

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### Summary and Results

The *Quadrato Motor Training* (QMT) is a new whole-body training paradigm, which was found to improve neuronal synchronization and to increase creativity, reflectivity, attention, as well as neuroplasticity [1].

Neuroplasticity implies remodeling of neuronal structures [2], which in turn involves molecular modifications. Maintaining neuroplasticity is an important goal, which can be stimulated through training, by activating molecular mechanisms, for example, regulation of growth factors.

The aim of the current exploratory study was to examine the link between structural and neurotrophic changes following the QMT, providing novel insights regarding the possible underlying neural and molecular mechanisms.

We analyzed by western blot the protein levels of two neurotrophins, BDNF and NGF, in saliva samples of healthy volunteers who practiced QMT for 1-3 months [3] [4]. The results show that the training induces an increase of proBDNF , and a decrease of proNGF, relative to controls (Figure 1).

These molecular changes were correlated with increased creativity, as assessed by the alternate uses task, and with increased cerebellar volume, including synaptogenesis and dendritic arborization, as shown by multimodal magnetic resonance imaging (MRI) (Figure 2).

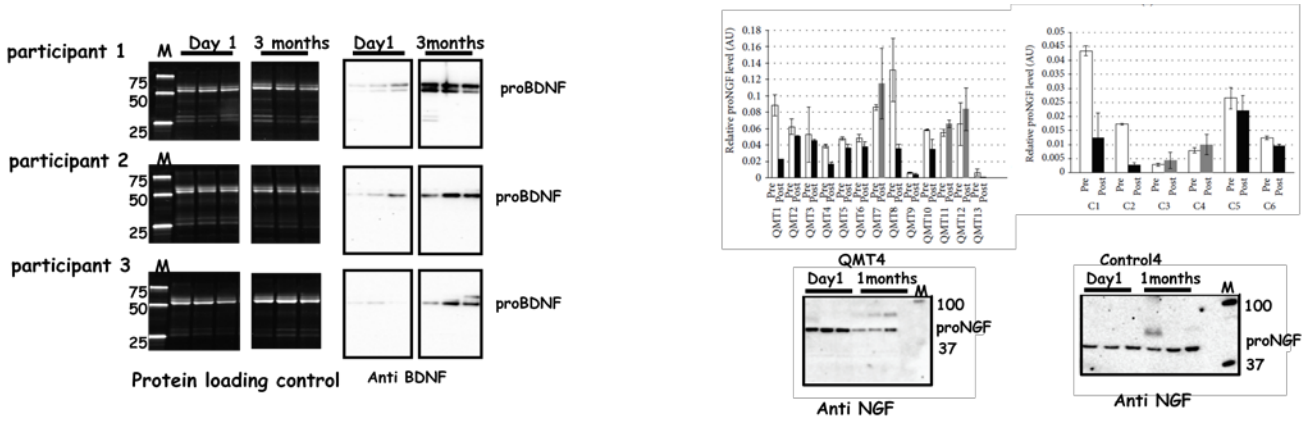


Figure 1: Effects on molecular markers after 3 months (left panel) and after 1 month (right panel) of Quadrato Motor Training.

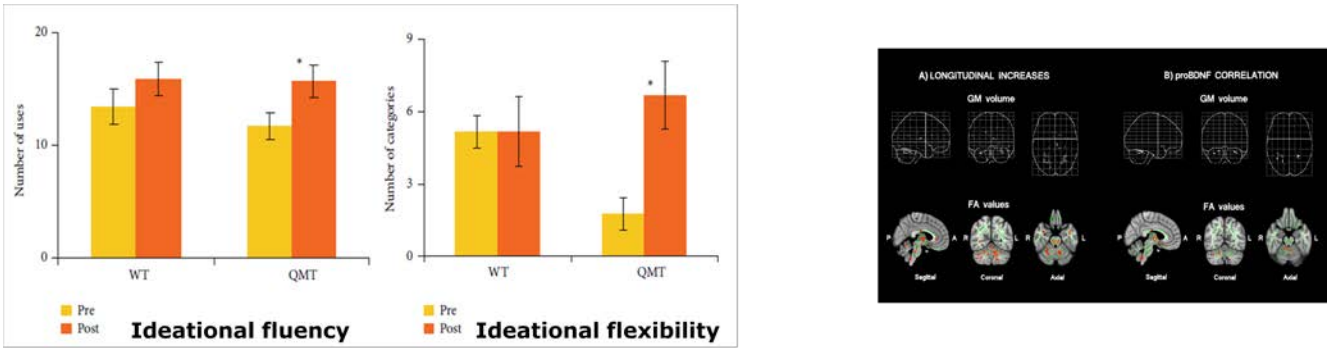


Figure 2: Cognitive (left panel) and structural (right panel) effects of Quadrato Motor Training.

## Conclusion

Changes in functional connectivity, cerebellar activity, and cognitive changes, namely increased creativity and improved spatial cognition, are specifically induced by QMT. Our studies show that there is a dynamic relationship between stuctural and neurotrophic changes following training. The increase of proBDNF level in saliva is associated with a size increase of specific brain areas [3]; the decrease of salivary proNGF is correlated with increased cognitive performance both in children and adults [4].

**Future aims**

To study how QMT influence the **expression** of **genes** coding for neurotrophins and the underlying **epigenetic mechanisms**; **a)** To analyze other molecules whose level could be affected by QMT (**neurotrophin receptors**); **b)** To approach early stages of **neurodegenerative diseases**; **c)** To extend the analysis to **other** movement-based **mindfulness** practices (Tai Chi, Yoga, Qi Gong).

**References:**


1. Ben-Soussan TD, Glicksohn J, Goldstein A, Berkovich-Ohana A, Donchin O. Into the square and out of the box: the effects of Quadrato Motor Training on creativity and alpha coherence. PLoS One, 2013, **8:**e55023
2. Ben-Soussan TD, Piervincenzi C, Carducci F. Increased corona radiata and cerebellar tracts following Quadrato Motor Training: a pilot diffusion tensor imaging study. International Journal of Clinical & Medical Imaging, 2014, **1:**1000192.
3. Ben-Soussan TD, Piervincenzi C, Venditti S, Verdone L, Caserta M, Carducci F. Increased cerebellar volume and BDNF level following quadrato motor training. Synapse, 2015, **69:**1-6
4. Venditti S, Verdone L, Pesce C, Tocci N, Caserta M, Ben-Soussan TD. Creating well-being: increased creativity and proNGF decrease following Quadrato Motor Training. Biomed Res Int, 2015, **2015:**275062.

## P-5: Role of nArgBP2 in different neuropsychiatric disorders

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According to the World Health Organization neuropsychiatric disorders together represent worldwide a spectrum of diseases significatively more frequent than cardiovascular and cancer diseases. It has been demonstrated that *de novo* mutations provide a significant contribution to several different intellectual disabilities and that these genetic alterations were preferentially enriched in postsynaptic proteins of excitatory synapse. We have previously studied the postsynaptic enriched adaptor protein nArgBP2 (neural Abelson-related gene binding protein 2), which belongs to a family of adaptor proteins that are involved in the regulation of cell adhesion, actin cytoskeleton organization, and signaling downstream of growth factor receptors. In this work we demonstrate a direct involvement of nArgBP2 in the regulation F-actin dynamics in dendritic spines and in the formation of excitatory synaptic contacts. nArgBP2 physically interacts with SAPAP3 and Shank3. Mice lacking SAPAP3 protein show defects in cortico-striatal synapses associated to anxious-like and compulsive behaviors. Shank3 overexpression causes manic-like behaviors in both humans and mice, while Shank3 loss was linked to autism spectrum disorder through studies of both human genetics and knockout mice. Thus nArgBP2-mediated regulation of the SAPAP3/SHANK3 represents a very interesting opportunity to unveil the relationship between actin polymerization and synapse plasticity in the group of different neuropsychiatric disorders.

## P-6: Characterization of DPR interactors to unveil C9orf72 toxicity in ALS

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Expansion of GGGGCC repeats in the first intron of C9orf72 gene is the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia. A major priority in the field is to understand whether neurotoxicity is caused by repeated RNA itself or if dipeptide repeat proteins (DPRs), generated by a non-ATG translation of this sequence, are responsible of the disease. Recent evidence coming from in vivo experiments in *Drosophila melanogaster* suggests that a relevant toxic effect is exerted by the expression of poly-glycine-arginine and poly-proline-arginine peptides, generated by a non-ATG translation of the repeats. Accordingly, DPRs were also found to cause neurodegeneration in cell culture and in animal models and, most importantly, DPR inclusions were found in tissues from patients. In our laboratory we have identified DPR-interacting proteins that specifically bind to the more toxic poly-glycine-arginine and poly-proline-arginine peptides and we are studying their functional involvement in C9orf72-mediated neurodegeneration in *Drosophila melanogaster* and in cell culture systems.

## P-7: Nucleophosmin and cancer: structural and functional investigations

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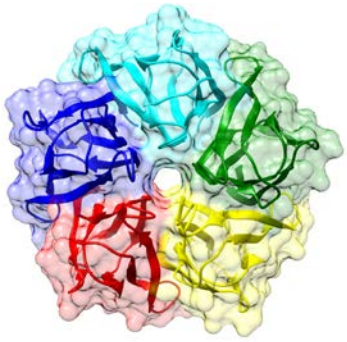
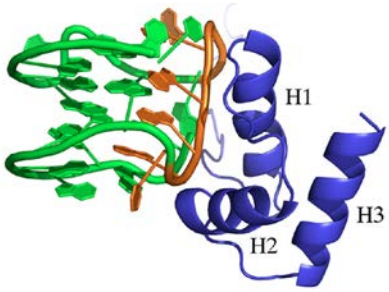
Nucleophosmin (NPM1) is an abundant and ubiquitously expressed protein, mainly localized in nucleoli but built to shuttle between nucleus and cytoplasm to fulfil its many functions, such as ribogenesis, histone chaperoning, DNA damage repair andresponse to stress stimuli. NPM1 is overexpressed in a variety of solid tumors of diverse histological origin and the *NPM1* gene is mutated in 50-60% of normal karyotype acute myeloid leukemia (AML) patients; the mutants are aberrantly and stably localized in the cytoplasm because of destabilization of the NPM1 C-terminal domain and the presence of a new nuclear export signal [1].

Our work has provided important clues on NPM1 functions, localization/mislocalization and interactions with molecular partners. We showed that the C-terminal region of the protein (C-NPM1) binds oligonucleotide sequences with G-quadruplex structure found at the non-coding strands of ribosomal DNA [2]; the interactions between C-NPM1 and a prototypical G-quadruplexes sequence from the c-MYC gene promoter have been characterized by NMR [3].

Given that in the most common NPM1 leukemic variant the C-terminal domain is completely unfolded, we showed that destabilization impairs the ability to bind G-quadruplexes, providing a structural interpretation for the NPM1 nucleolar localization and its impairment by AML-associated mutations [2,4].



The N-terminal region of NPM1 assembles as a pentamer




Structural analysis of the complex between C-NPM1 and a G-quadruplex

NPM1 also interacts with several protein partners such as the tumor suppressors p14Arf, Fbw7γ, p53; it is known that NPM1 mutants co-translocate in the cytosol also the partners. Recently we focused on the interaction between NPM1 and Fbw7γ, a nucleolar E3-ubiquitin ligase controlling c-MYC levels. In leukemic blasts, as a consequence of Fbw7γ degradation, c-MYC is stabilized [5]. We identified the domains of both proteins crucial to binding and characterized key residues implicated. We suggest that the NPM1 exposed surface that interacts with Fbw7γ, which is involved in the interaction of NPM1 with other protein partners, may be a possible target for the specific treatment of AML linked to NPM1 mutations.

**References:**

1. Di Matteo A, Franceschini M, Chiarella S, Rocchio S, Travaglini-Allocatelli C, Federici L. Molecules that target nucleophosmin for cancer treatment: an update. Oncotarget, 2016, Apr 5
2. Chiarella S, De Cola A, Scaglione GL, Carletti E, Graziano V, Barcaroli D, Lo Sterzo C, Di Matteo A, Di Ilio C, Falini B, Arcovito A, De Laurenzi V, Federici L. Nucleophosmin mutations alter its nucleolar localization by impairing G-quadruplex binding at ribosomal DNA. Nucleic Acids Res, 2013, **41:**3228-3239
3. Gallo A, Lo Sterzo C, Mori M, Di Matteo A, Bertini I, Banci L, Brunori M, Federici L. Structure of nucleophosmin DNA-binding domain and analysis of its complex with a G-quadruplex sequence from the c-MYC promoter. J Biol Chem, 2012, **287:**26539-26548
4. Federici L, Arcovito A, Scaglione GL, Scaloni F, Lo Sterzo C, Di Matteo A, Falini B, Giardina B, Brunori M. Nucleophosmin C-terminal leukemia-associated domain interacts with G-rich quadruplex forming DNA. J Biol Chem, 2010, **285:**37138-37149
5. Bonetti P, Davoli T, Sironi C, Amati B, Pelicci PG, Colombo E. Nucleophosmin and its AML-associated mutant regulate c-Myc turnover through Fbw7 gamma. J Cell Biol, 2008, **182:**19-26.

**P-8: Isolated peptides from mt-leucyl-tRNA synthetase as therapeutic instruments against mitochondrial diseases caused by mt-tRNA point mutations.**

Elena Perli[1], Annarita Fiorillo[2], Carla Giordano[1], Annalinda Pisano[1], Arianna Montanari[3,5], Paola Grazioli[4], Antonio F. Campese[4], Patrizio Di Micco[2], Helen A. Tuppen[6], Ilaria Genovese[2], Elena Poser[2], Carmela Preziuso[1], Robert W. Taylor[6], Veronica Morea[7], Gianni Colotti[7], Giulia d'Amati[1,5]

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**BACKGROUND**

Mitochondrial (mt) diseases are multi-system disorders due to mutations in nuclear or mtDNA genes. Among the latter mutations, more than 50% are located in transfer RNA genes and are responsible for a wide range of syndromes, for which no effective treatment is available at present.

**RATIONALE**

Defects due to point mutations in several mt-tRNAs are rescued by the cognate human mt-aaRS in both the yeast model and human cells.

In 3D structures, tRNALeu interacts extensively with the carboxy-terminal domain of LeuRS (LeuRS-Cterm), and in particular with residues within β-strands β30, β31, β32 and β33.

The isolated human mtLeuRS-Cterm rescues defects due to point mutations in several mt-tRNAs aminoacylated by aaRSs belonging to either class I or class II in the yeast model.

Peptides β30-31 and β32-33 derived from LeuRS-Cterm rescue defects due to point mutations in mt-tRNAs aminoacylated by aaRSs belonging to either class I or class II.

**RESULTS**

LeuRS-Cterm: i) rescues the pathological phenotype of cybrids carrying the severe MELAS m.4234A>G point mutation in mt-tRNALeu(UUR); ii) localizes to mitochondria even in the absence of a canonical N-terminal mt targeting

sequence; iii) directly and specifically interacts with human mt-tRNALeu(UUR) in vitro with high affinity and stability; iv) rescues the pathological phenotype of cybrids carrying the relatively mild m.4277T>C and m.4300A>G mutations in mt-tRNAIle; v) interacts with human mt-tRNAIle in vitro; vi) rescues the pathological phenotype of cybrids carrying the severe MERRF m.8344A>G point mutation in mt-tRNALys.

Peptides β30-31 and β32-33: i) ameliorate viability and energetic proficiency of human cells carrying either m.3243A>G mutation in mt-tRNALeu(UUR) or m. 8344A>G mutation in mt-tRNALys; ii) interact in vitro with: human mt-tRNALeu(UUR) with high affinity and with the m.3243A>G mutant with lower affinity; and mt-tRNALys; iii) restore the stability and structure of mt-tRNALeu(UUR), which are strongly impaired by the m.3243A>G mutation.

**CONCLUSIONS**

We have demonstrated that β30-31 and β32-33 peptides derived from LeuRS are able to rescue the pathological phenotype in cellular models of two severe mt diseases:

(1) MELAS syndrome due to the m.3243A>G point mutation in the mt-tRNALeu(UUR) gene, which is the most prevalent tRNA mutation identified to date;

(2) MERRF syndrome due to the m.8344A>G in mt-tRNALys.

Therefore, these peptides represent attractive new candidates for future therapeutic applications against syndromes associated with mt-tRNA mutation.

**References:**


1. Perli E, Giordano C, Pisano A, Montanari A, Campese AF, Reyes A, Ghezzi D, Nasca A, Tuppen HA, Orlandi M, Di Micco P, Poser E, Taylor RW, Colotti G, Francisci S, Morea V, Frontali L, Zeviani M, d'Amati G. The isolated carboxy-terminal domain of human mitochondrial leucyl-tRNA synthetase rescues the pathological phenotype of mitochondrial tRNA mutations in human cells. EMBO Mol Med, 2014, **6:**169-182
2. Di Micco P, Fazzi D'Orsi M, Morea V, Frontali L, Francisci S, Montanari A. The yeast model suggests the use of short peptides derived from mt LeuRS for the therapy of diseases due to mutations in several mt tRNAs. Biochim Biophys Acta, 2014, **1843:**3065-3074.
3. Perli E, Fiorillo A, Giordano C, Pisano A, Montanari A, Grazioli P, Campese AF, Di Micco P, Tuppen HA, Genovese I, Poser E, Preziuso C, Taylor RW, Morea V, Colotti G, d'Amati G. Short peptides from leucyl-tRNA synthetase rescue disease-causing mitochondrial tRNA point mutations. Hum Mol Genet, 2016, **25:**903-915.

## P-9: New strategy for the synthesis of benzofuran derivatives

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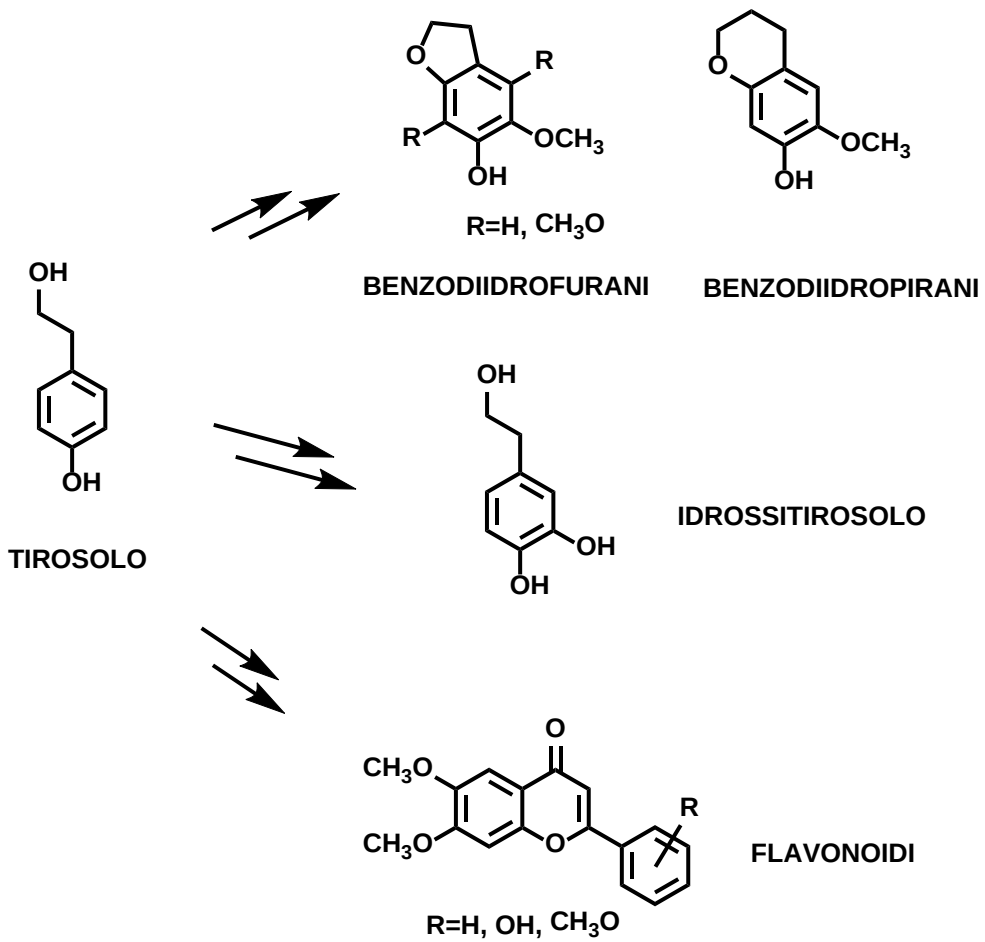
Polyphenols are natural antioxidants present in plants that have several biomedical effects, including the arrestation of tumor growth. Despite having different structures, all polyphenols are characterized by aromatic rings and one or more hydroxyl substituents. In this context, our aim is to use a multistep procedure we have developed to prepare new analogs of flavonoids, tyrosol and resveratrol, with high degrees of oxygenation and/or functionalized with lipophilic chains.

For the synthesis of aromatic polyhydroxylated compounds we exploited a protocol developed in our laboratories consisting of 1) selective and environmentally sustainable bromination of easily available aromatic compounds, 2) methoxylation with MeONa in the presence of CuBr [1,2], and 3) eventual demethylation with $BBr_3$, through oxidative demethylation with IBX or Oxone, via reduction with hydrides, or by means of enzymatic conversion with lipase to obtain the final product.

In addition, our attention is focused on developing a synthetic strategy for the preparation of benzofuran and benzopyran derivatives, a group of biological active heterocycles that are extracted from different plants and used in traditional medicine in certain countries. To synthesize 2,3-diidrobenzofuranes, benzofuranes, and benzopyranes scaffolds for the preparation of more complex molecules, we are investigating the use of catalysts of copper-free ligands as an alternative to ruthenium, palladium, and other transition metals, which must be assisted by complex ligands in any event. The use of copper(I) salts is particularly advantageous in terms of cost and ease of use. A procedure recently developed in our laboratory will be used to prepare analogs of resveratrol. The procedure is based on an easy and environmentally-friendly formation of a phenonium ion, which exploits the ability of phenethyl alcohol carboxymethyl esters to rearrange [3].

The reactivity of this cation, which is highly unstable, towards nucleophiles, which are also weak, allows the synthesis of a large number of phenethyl derivatives, including stylbenoid structures, using anisole derivatives as nucleophile.




**References:**


1. Barontini M, Proietti Silvestri I, Nardi V, Crisante F, Pepe G, Pari L, Gallucci F, Bovicelli P, Righi G. Synthesis and biological evaluation of gallic acid analogs. Medicinal Chemistry Research, 2013, **22:**674-680
2. Righi G, Proietti Silvestri I, Barontini M, Crisante F, Di Manno A, Pelagalli R, Bovicelli P. Efficient synthesis of scutellarein. Natural Product Research, 2012, **26:**1278-1284
3. Barontini M, Proietti Silvestri I, Nardi V, Bovicelli P, Pari L, Gallucci F, Spezia R, Righi G. Easy eco-friendly phenonium ion production from phenethyl alcohols in dimethyl carbonate. Tetrahedron Lett, 2013, **54:**5004-5006

## P-10: Stereocontrolled synthesis of iminosugars

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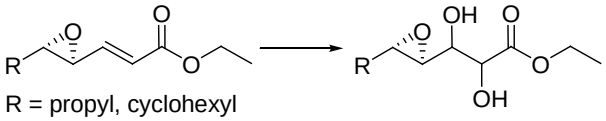
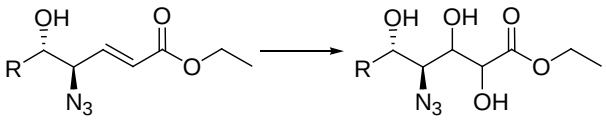
Iminosugars are carbohydrate analogues in which the endocyclic oxygen atom is replaced by a nitrogen. They are the most attractive class of sugar mimics due to their interesting glycosidase and glycosyl transferase inhibitor activity, which enables their high therapeutic potential in a wide range of diseases such as diabetes, viral infections, lysosomal storage disorders and tumor metastasis [1].

Our research group has recently developed a procedure to perform the asymmetric dihydroxylation reaction [2] on optically active *trans* α,β-insaturated esters and derivatives [3] and the conditions were optimized in order to modulate the diastereomeric ratio of the diols depending on which Cinchona type chiral ligand was used.

The most interesting results have been obtained on epoxide and azido alcohol derivatives:



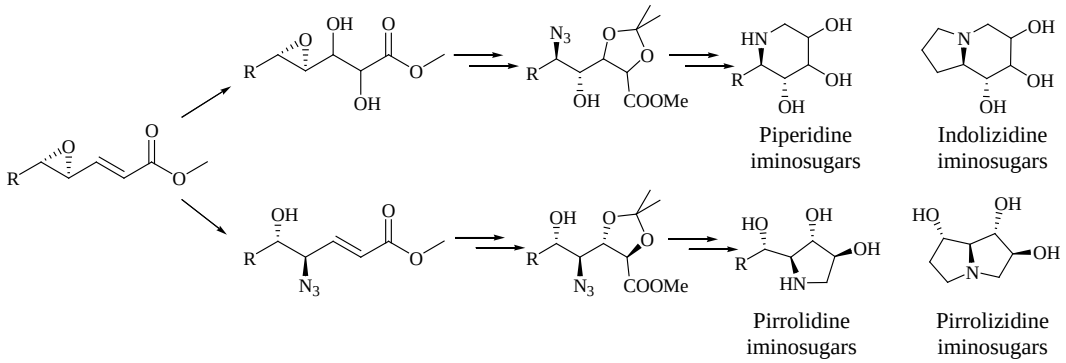

R = propyl, cyclohexyl




R = propyl, cyclohexyl

| Ligand | *syn/anti* | Yield | Ligand | *syn/anti* | Yield |
|---|---|---|---|---|---|
| - | 60:40 | 89% | - | 90:10 | 84% |
| $(DHQ)_2PHAL$ | 15:85 | 90% | $(DHQ)_2PHAL$ | >95:5 | 74% |
| $(DHQD)_2PHAL$ | 83:17 | 84% | $(DHQD)_2PHAL$ | 70:30 | 76% |

These reactions, which allow to obtain four adjacent chiral centres with full stereochemistry control, are the key step in our approach for achieving the total synthesis of pyrrolidine [4] and piperidine iminosugars and their bicyclic derivatives.




**References:**


1. Afarinkia K, Bahar A. Recent advances in the chemistry of azapyranose sugars. Tetrahedron: Asym, 2005, **16:**1239-1287

2. Kolb HC, Van Nieuwenhze MS, Sharpless KB. Catalytic asymmetric dihydroxylation. Chem Rev, 1994, **94:**2483-2547

3. a) Righi G, Bovicelli P, Mandic' E, Naponiello GCM, Tirotta I. Study on the stereochemical control of dihydroxylation of vinyl epoxides and their derivatives. Tetrahedron, 2012, **68:**2984-2992. b) Righi G, Bovicelli P, Mandic' E, Sappino C. Double stereodifferentiation in the asymmetric dihydroxylation of optically active olefins. Chirality (in press)

4. Righi G, Mandic' E, Tirotta I, Naponiello GCM, Sappino C, Marucci C, Tomei M, Bovicelli P. Stereoselective synthesis of (+)-1-deoxyaltronojirimycin. Nat Prod Res (in press)

## P-11: Synthesis of chiral functionalized nanoparticles as new chiral catalysts for the asymmetric reactions

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Recently, the use of functionalized nanoparticles has led to new catalysts that combine advantages of both homogeneous and heterogeneous catalysis [1]. Considering the presence of the β-amino alcohol motif in the structure of numerous chiral catalysts used in asymmetric synthesis [2], we were recently involved in the development of a novel versatile, magnetically recoverable β-amino alcohol 'nanocatalyst'.

We focused on the design and synthesis of ligands bearing, in addition to a fine-tunable catalytic site, a functionality (an alkoxysilane group) for their covalent anchoring to magnetite nanoparticles (Figure 1-A).

Prior to immobilizing the catalyst on the nanoparticles we addressed the design and the optimization of the ligand. After extensive fine-tuning, we selected the structure **1** (Figure 1-B) as an excellent chiral ligand in the addition of diethylzinc to benzaldehyde (yields: >95%, *ee*: >95%). We finally confirmed the validity of the optimization process employing the selected ligand in the addition of diethylzinc to a family of aldehydes leading to outstanding results (Figure 1-C).

In light of such excellent results, we are now focusing on employing the selected ligand in various organic asymmetric reactions (*i.e.,* Henry reaction, conjugated addition) and, in parallel, on studying its covalent immobilization on different materials, including silica and superparamagnetic core-shell nanoparticles.

### References:

1. Shylesh S, Schuneman V, Thiel WR. Magnetically separable nanocatalysts: bridges between homogeneous and heterogeneous catalysis. Angew Chem Int Ed, 2010, **49:**3428-3459
2. Vicario JL, Badia D, Carrillo L, Reyes E, Etxebarria J. α-Amino acids, β-amino alcohols and related compounds as chiral auxiliaries, ligands and catalysts in the asymmetric aldol reaction. Curr Org Chem, 2005, **9:**219-235

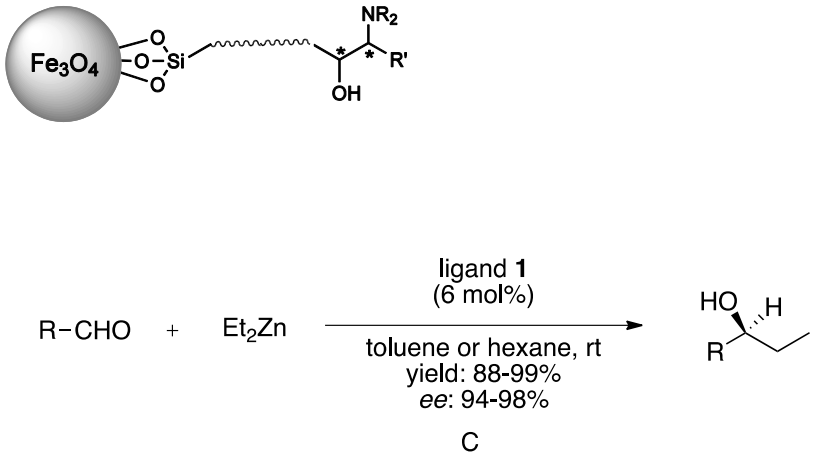


Figure 1: A) Structure of amino alcohol catalysts supported on magnetic nanoparticles. B) Optimized ligand. C) Addition of diethylzinc to a family of aldehydes employing **1**.

## P-12: Genes controlling centrosome number and structure in *Drosophila*

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Centrosomes are specialized organelles that act as the main microtubule (MT) organizing centers in most animal cells. They consist of a pair of centrioles, which are cylindrical structures usually composed of nine microtubule triplets, surrounded by a fibrous meshwork called pericentriolar material. Centrosomes/centrioles loss or overduplication lead to aberrant mitotic divisions and chromosome instability, an established hallmark of cancer. Moreover, defects in centriole/basal body formation and function are associated with a variety of human diseases, including ciliopathies.

We are currently pursuing the identification and characterization of genes controlling centriole/centrosome structure and behavior using *Drosophila* as a model system. Besides its sophisticated molecular genetics, *Drosophila* provides several cell systems that are particularly suitable for studies on centrioles/centrosomes, such as the larval brains and the male germ line. In particular, *Drosophila* primary spermatocytes possess unusually long (up to 2 mm) centrioles that during spermiogenesis nucleate sperm axonemes, making these cells an ideal system for studying centriole biogenesis and behavior and the transition from a centrosome to a cilium templating function.

Recently, we have characterized two new genes required for centriole stability called *fragile centrioles (fract)* and *broken centrioles (broc),* which are identified by male-sterile mutations. During the first meiotic division of *fract* mutant males one centriole rapidly degenerates, giving rise to monopolar secondary spermatocytes and eventually to spermatids devoid of the basal body. This phenotype is dominantly suppressed by mutations in *Klp61F* (encoding a kinesin homologous to Eg5) or in *Dhc64C* (encoding a dynein heavy chain), suggesting that the centrioles of *fract* mutants are intrinsically fragile and undergo rapid degradation when subjected to the forces generated by the meiotic spindle. *broc* mutant spermatocytes exhibit abnormally long and fragile centrioles that appear to degenerate at the onset of meiosis, giving rise to aberrant spindles and defective chromosome segregation. *broc* encodes a zinc finger protein that is specifically expressed in testes and whose function has not yet been defined.

## P-13: Cytokinesis failure, chromosomal instability and tumorigenicity: HIPK2 role in cancer

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The oncosuppressor HIPK2 is a kinase involved in the cell fate decisions in the development and response to stress [1]. Recently, we have demonstrated the relevance of HIPK2 in the control of cytokinesis and in prevention of tetraploidy-mediated chromosomal instability (CIN). HIPK2depleted cells accumulate aberrant midbodies and fail abscission, the final step of cytokinesis, leading to CIN and increased tumorigenicity in vitro and in vivo[2-3]. Furthermore, we found a significant correlation among reduced HIPK2 expression, high grade of malignancy, and high nuclear size, a ploidy marker, in pancreatic cancer, a human tumor in which cytokinesis failure is considered the main mechanism underlying tetraploidization. These findings led us to hypothesize that HIPK2 dysfunctions might play a key role in pancreatic cancer and contribute to the emergency of more aggressive aneuploid karyotypes during pancreatic cancer progression[3].We are analyzing the molecular basis of specific HIPK2 functions in ploidy maintenance and assessing the relevance of HIPK2 dysfunctions in pancreatic cancer development in well characterized mouse models and in patient samples. During studies on the molecular basis of HIPK2 role in cytokinesis and in ploidy maintenance, we found that HIPK2 directly regulates the protein levels of the microtubule severing protein spastin, whose activity is essential for the abscission. We will show our findings on the molecular mechanisms underlying HIPK2 regulation on spastin and its effects on microtubule stability, cytokinesis failure and CIN.

Furthermore, we will present unexpected implications of the HIPK2/spastin cross talk in Hereditary Spastic Paraplegia (HSP), a neurodegenerative disorder mainly due to heterozygous mutations in the SPG4 gene, encoding spastin.

**References:**


1. D'Orazi G, Rinaldo C, Soddu S. Updates on HIPK2: a resourceful oncosuppressor for clearing cancer. J Exp Clin Cancer Res, 2012, **31:**63
2. Rinaldo C, Moncada A, Gradi A, Ciuffini L, D'Eliseo D, Siepi F, Prodosmo A, Giorgi A, Pierantoni GM, Trapasso F, Guarguaglini G, Bartolazzi A, Cundari E, Schininà ME, Fusco A, Soddu S. HIPK2 controls cytokinesis and prevents tetraploidization by phosphorylating histone H2B at the midbody. Mol Cell, 2012, **47:**87-98
3. Valente D, Bossi G, Moncada A, Tornincasa M, Indelicato S, Piscuoglio S, Karamitopoulou ED, Bartolazzi A, Pierantoni GM, Fusco A, Soddu S, Rinaldo C. HIPK2 deficiency causes chromosomal instability by cytokinesis failure and increases tumorigenicity. Oncotarget, 2015, **6:**10320-10334.

## P-14: The Aurora-A/TPX2 complex in chromosome stability, tumorigenesis and anti-cancer therapy.

Italia Anna Asteriti[1], Valentina Sterbini[1], Alessandra Russo[1], Fabiola De Mattia[1], Edmilson Ozoriodos Santos[1,2], Giulia Guarguaglini[1]

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The Aurora-A mitotic kinase controls several aspects of spindle assembly and function, and hence chromosome stability. It is overexpressed in cancer, and inhibitors directed to the ATP-binding pocket (ATP-competitors) have been developed for therapeutic purposes, and are being tested in clinical trials. The microtubule binding protein TPX2 is the best characterised activator of Aurora-A, controlling its activity, stability, and localisation to the spindle.

We have investigated the effects of Aurora-A inhibition in human cells by RNA-interference and small molecule inhibitors. By these means we characterised the role of the kinase in the control of spindle pole integrity, and highlighted a novel function in the regulation of mitotic spindle orientation. Altogether, results showed that Aurora-A inhibition by small molecules has highly heterogeneous effects, including induction of a potentially pro-tumorigenic aneuploidy condition, thus raising concern on the use of these compounds in therapy. Analyses to investigate how the observed spindle defects generate chromosomal instability in daughter cells are ongoing.

We have found evidence in the literature, and through database mining, of TPX2 overexpression in cancer, and most notably of co-overexpression of Aurora-A and TPX2: the Aurora-A/TPX2 complex may therefore act as an oncogenic unit. We are currently addressing directly the transforming activity of the complex: results indicate that indeed overexpression of the whole complex in non-transformed cells induces more severe defects than overexpressing the single components.

From all these observations, we propose that the Aurora-A/TPX2 complex may constitute a novel promising target in anti-cancer therapy, providing an effective alternative to ATP-competitor inhibitors of Aurora-A. To this aim we are developing protein-protein interaction inhibitors: promising hits identified in *in vitro* assays are being assayed in cultured cells.

## P-15: The kinetochore protein Hec1 as a molecular target in cancer therapy

Manuela Ferrara[1], Gaetana Sessa[1], Mario Fiore[1], Lia Asteriti[1], Enrico Cundari[1], Francesca Degrassi[1]

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Aneuploidy is strongly involved in tumorigenesis but massive aneuploidy has been found to be a tumor suppressive mechanism. Consequently, the idea of promoting cell death by inducing massive aneuploidy at mitotic division has been proposed as a therapeutic strategy to selectively eliminate highly proliferating tumor cells. Highly Expressed in Cancer protein 1 (Hec1) is a subunit of the kinetochore (KT)-associated Ndc80 complex, which ensures proper alignment and segregation of sister chromatids during mitosis and is highly expressed in many malignancies. Recently, our lab has been focused in exploring Hec1 as a molecular target for cancer therapy with the final goal to produce massive chromosome aneuploidy and cell death in cancer cells. We have shown that expression of a N-terminus modified Hec1 massively kills cancer cells both in vitro and in xenograft tumors. Live cell imaging of N-terminus modified Hec1 expressing cells demonstrated that cancer cell death is triggered by a prolonged mitotic arrest due to an attempted chromosome segregation within multipolar spindles followed by cell death at mitosis.

Structural reconstitution of Ndc80 complexes bound to microtubules (MTs) has revealed that a region of the Hec1 calponin homology domain with positively charged amino acid residues binds a task on the wall of a MT, interacting with negatively charged residues at the intra-and inter-tubulin interface. In collaboration with Dr. A. Brancale (Welsh School of Pharmacy, Cardiff University) we have performed a virtual screening and identified 15 small molecules that potentially bind at the interaction domain between MTs and Hec1. Among the 15 molecules, one compound able to induce alterations in the mitotic process and produce cancer cell death ($IC_{50}$= 20.18 μM) in HeLa cells has been identified. On the basis of these results, Dr. Brancale has selected a series of analogues through the addition of functional groups or chemical modifications, in order to increase the affinity for the target, optimize pharmacokinetic parameters and possibly increase the biological potency.

We have investigated the biological activity of the lead compound and the analogue molecules on human cancer cells and identified two highly cytotoxic small molecules (SMs) that produce a G2/M cell cycle block. Immunofluorescence studies demonstrated that the two SMs inhibit the progression of mitotic division

and produce chromosome segregation defects, suggesting that the compounds interfere with the MT-KT interaction. Live imaging analysis of treated cells, together with the analysis of specific markers, demonstrated apoptosis as the modality of induced cell death preceded by the presence of autophagic vacuoles. The totality of cells entering mitosis died trough mitotic catastrophe. However, a clear inhibition of mitotic entry associated with the occurrence of cell death from interphase was also recorded in time lapse experiments, as the SMs could exert their cytotoxic action also outside mitosis. Immunofluorescence experiments addressing the resistance of treated cells to cold-induced MT depolymerization demonstrated that SM treatment renders both mitotic spindle MTs and interphase MTs more stable than control MTs, suggesting that a SM-dependent hyper-stabilization of mitotic MTs could impede the correction of erroneous MT-KT interactions. Interphase cell death could be associated to the hyperstabilization of interphase MTs. In conclusion, our work has identified two SMs showing promising anti-cancer activity in *in vitro* cell assays, which may work by stabilizing the MT-KT interaction or the MT itself. Further studies will discriminate between these two possibilities.

## P-16: Role of oxidative modifications of proteins in Human Papilloma Virus-driven cervical carcinogenesis

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[1]Institute of Molecular Biology and Pathology, National Research Council, Rome
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One of the main interests of our research group is related to the role played by oxidative stress (OS) in the pathogenesis and progression of many diseases, including neurodegenerative disorders and cancer. In particular, we have focused our attention on the analysis of oxidative modifications of proteins and how dysfunction of selected proteins translates into pathological features of a disease state. By following this approach we aim to shed light on critical molecular determinants underlying cognitive dysfunction and carcinogenesis process.

Our proteomics studies indicated that OS is an early event in Down Syndrome pathogenesis and is involved in the occurrence of relapses in Multiple Sclerosis.

In particular, we focus on the role played by OS in cervical cancer, the second most common neoplastic disease among women worldwide. The initiating event is the infection with certain types of Human Papilloma Virus (HPV), a very common condition in the general population. However, the majority of HPV infections is subclinical and transitory and is resolved spontaneously. Intriguingly, viral oncogene expression, although necessary, is not per se sufficient to promote cervical cancer and other factors are involved in the progression of infected cells to the full neoplastic phenotype. In this perspective we are interested in investigating the interplay between the viral mechanisms modulating cell homeostasis and redox sensitive mechanisms. Results obtained either from cell culture models and human tissues led us to hypothesize the mechanisms by which HPV exploits host cell survival mechanisms, through modulation of redox homeostasis. We suggest that tumor cells adapt their metabolism in order to support their growth and survival, likely creating a paradox of high ROS production in the presence of high antioxidant levels, to fit well with stress conditions.

Recently, we are investigating on specific proteins found to be oxidized in HPV-infected cervical tissues, such as retinal dehydrogenase. This enzyme catalyzes the synthesis of retinoic acid (RA), compound recognized to play a major role in a wide range of pathways, such as cell cycle regulation, differentiation and morphogenesis. Among its properties, RA is recognized as a suppressor of HPV-16 oncogenes transcription. Ongoing studies on cellular

models of HPV infection aim to determine if the severity of infection correlates with the degree of oxidation (inhibition) of the enzyme. Preliminary results suggest that the enzyme is inhibited in the presence of ROS. We are also studying the integrity of the signalling pathways related to RA, such as AP-1, transcription factor playing a central role in regulation of viral oncogene expression, whose induction by some factors, among which OS, is repressed by RA. The finding of altered metabolic pathway, due to oxidative modification of specific proteins, could shed light on the molecular mechanisms that may contribute to cell transformation and cancer development.

## P-17: Nitric oxide-dependent epigenetic signals in glioblastoma stem cells neural differentiation

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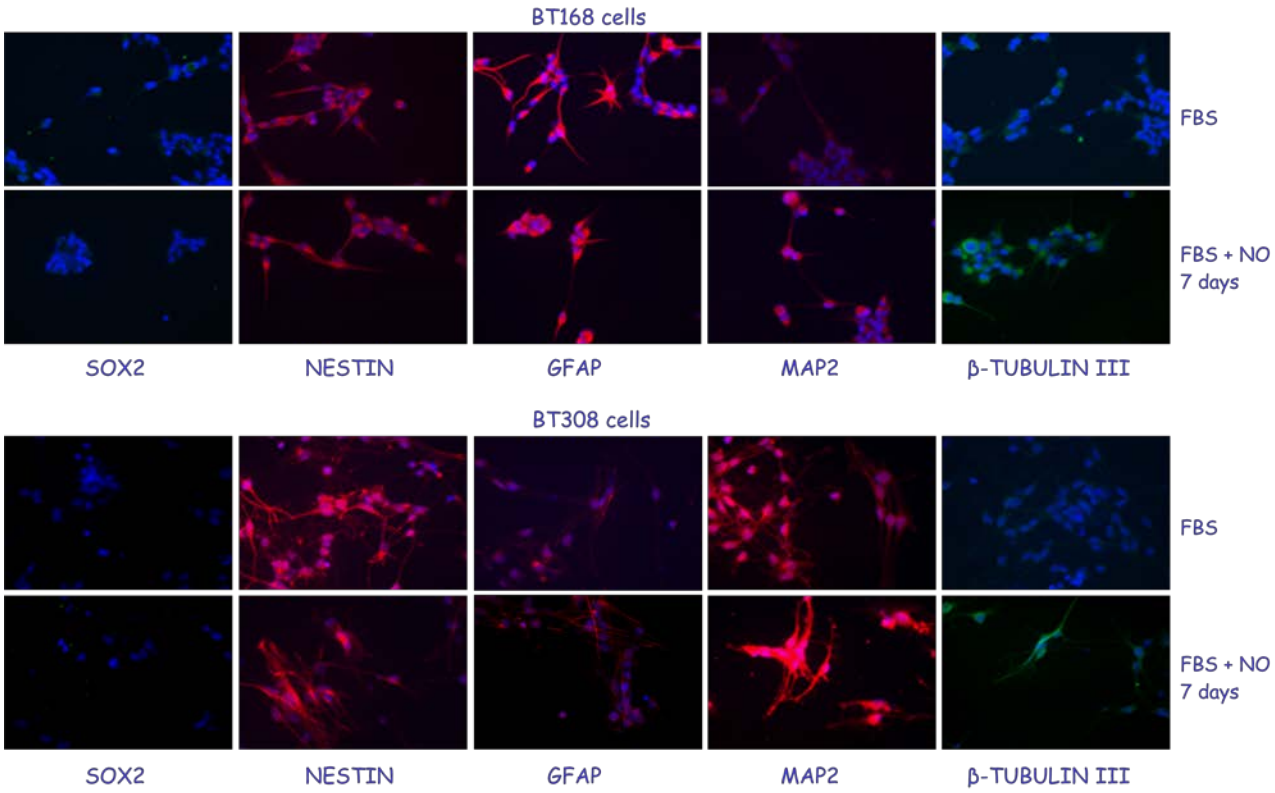
Glioblastoma multiforme (GBM) is a relatively rare, very aggressive and incurable brain tumor, associated with high mortality and recurrence. As in other tumors, glioblastoma onset, progression and spread depends on a small subpopulation of cancer stem cells (glioblastoma stem cells, GSCs). Accordingly, depletion of GSCs through controlled, drug-induced differentiation could have profound therapeutic implications. Nitric oxide (NO) is a small diffusible molecule that promotes the neurons' survival and differentiation. Moreover, at high concentrations, NO treatment induces apoptosis, radio- and chemosensitization in malignant glioma cells and increases permeability of the blood brain barrier. Therefore, the use of NO-releasing molecules holds great potential as an adjuvant in the multimodal treatment of GBM. In addition, NO is an epigenetic molecule, being able to exert a regulatory function on chromatin remodeling and gene expression.

Currently, no information are available about the role of NO in brain cancer stem cells. Therefore, we evaluated the effects of 100 μM diethylenetriamine/nitric oxide adduct (DETA/NO) on two patient-derived GSC populations, to test NO therapeutic potential through the evaluation of its pro-differentiation activity. In particular, we aimed to highlight changes in molecular pathways after NO-induced GSCs' differentiation, with main attention to the epigenetic modifications on GSCs' chromatin landscape and transcriptional output.

Our preliminary results showed that NO markedly affected the stem-like phenotype of GSCs, as it enhanced the effects of FBS in inducing GSCs' differentiation along a time course up to 7 days. In particular, immunofluorescence and western blot analyses showed a decrease of the levels of SOX2 and nestin (markers of stem cells and neural progenitors, respectively), further enhanced by NO exposure, in GSCs growing in differentiating conditions. Conversely, a broad increase of MAP2 and β-tubulin III expression, typical

markers of mature neurons, was shown. To note, the expression of GFAP, a marker of astrocytes, was inhibited by NO, consistently with NO-specific promotion of the neuronal lineage. Interestingly, after 7 days of NO exposure, GSCs displayed clearly evident, MAP-positive, axon-like, cell processes and dendritic spines. In parallel, western blot analysis revealed that 6 h of NO exposure was able to promote histone H3 deacetylation and the increase of HDAC2 expression. Overall, these results suggest an epigenetic role of NO on GSCs' differentiation. To explore this possibility, further studies will be performed to analyze the effect of NO in regulating the activity of chromatin remodeling enzymes (e.g. HDACs) and reshaping the histone modification profile within the promoter of NO target genes required for GSCs neuronal differentiation.

Understanding the NO-associated epigenetic effects on GSCs' chromatin landscape, transcriptional output and phenotypic changes could allow the development of specific therapeutic tools which can be used as epigenetic drugs for GBM treatment.



## P-18: MicroRNA profiling in E6/E7 HPV-transformed human keratinocytes and exosomes.

Gianna Fiorucci[1,2], Maria Vincenza Chiantore[2], Giorgio Mangino[3], Marco Iuliano[3], Gabriele Vaccari[4], Rosita Accardi[5], Massimo Tommasino[5], Sandra Columba Cabezas[6], Maurizio Federico[7], Giovanna Romeo[3]

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**Background:** Human Papilloma Viruses (HPVs) include more than 100 different genotypes, divided into mucosal or cutaneous HPVs. Mucosal HPVs are the causative agents of cervical cancer and are also associated with other types of cancers. On the other hand, the involvement of cutaneous HPV genotypes in non melanoma skin cancer is still not established.

Cancer cells show a specific pattern of microRNA (miRNA) expression and HPVs are able to modulate miRNAs expressed by infected cells. The production of membrane vesicles, in particular exosomes, is deregulated in cancer, and the cargo delivered by exosomes to the microenvironment can promote tumor growth and progression.

Keratinocytes transduced with the E6 and E7 oncoproteins from mucosal HPV-16 or cutaneous HPV-38 (K16 and K38) were studied to analyze the involvement of HPV oncoproteins in miRNA expression in cells and exosomes.

**Methods:** MiRNAs were analyzed by using the TaqMan Array Human MicroRNA Cards, followed by Real Time RT-PCR assay for specific miRNAs. Selected miRNA targets were analyzed by Western blot and correlated to the HPV oncoproteins by specifically silencing E6 and E7 expression. To isolate exosomes, K16 and K38 supernatants underwent differential centrifugations and exosomes

were quantified through the vesicle-associated acetylcholinesterase activity.

**Results:** MiRNA profiling led to the identification of miRNAs deregulated in K16 and K38 cells. HPV-16 and/or HPV-38 E6 and E7 single proteins can modify the expression of miRNAs selected on the basis of their role in the tumorigenesis, in particular miR-18a, -19a, -34a, and -590-5p. The analysis of the content of exosomes isolated from HPV-positive cells revealed the presence of E6 and E7 mRNAs and few miRNAs. Interestingly, miR-222, a key miRNA deregulated in many cancers, was identified in exosomes from K16 cells.

**Conclusions:** HPV infection can induce the deregulation of some miRNAs through mechanisms that appear to involve E6 and/or E7 oncoprotein expression. Moreover, through the production and function of exosomes, HPV oncogenes as well as HPV-deregulated miRNAs can potentiate the virus oncogenic effects in the tumor cell microenvironment.

## P-19: A simple tool for macromolecule interfaces analysis

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**Background**

Analysis of interfaces in macromolecular complexes is essential to unveil the mechanisms underlying molecular recognition. However, currently available interface analysis tools provide important results, such as contact maps, in non-editable formats. Additionally, many of them do not provide relevant information.

**Results**

We have developed a novel fast and comprehensive tool to analyse the interfaces between any two macromolecules (i.e., either proteins or nucleic acids).

The program takes as an input the coordinates of two macromolecule chains in PDB format and calculates a number of structural parameters by using both internal routines and external programs such as Naccess (for solvent accessible surface area) or DSSP (for secondary structures).

As a result, the program provides extensive information about the analysed interface, including: interacting residues and atoms; polar vs. non polar nature of the interaction; and number of polar and non-polar contacts (as a rough measure of the interaction strength). These data are provided both as simple text and in formats that can be easily parsed or ready to be imported in spreadsheet applications or PyMol for further structure analysis and visualization.

We have applied the program to the analysis of two different types of interfaces: i) between tRNA molecules and the cognate aminoacyl-tRNA synthetases; and ii) between the subunits of multimeric protein assemblies. In the first case, based on the results of our analysis we designed small molecule peptides endowed with therapeutic properties against defective phenotypes arising from single-point mutations in human mt-tRNA, which could be used as lead molecules to develop therapeutic agents. In the second case, elucidation of the structural difference between subunit interfaces in the decamer and double-decamer forms of *Schistosoma mansoni* peroxiredoxin I allowed us to understand the structural events causing the shift between the two assemblies and their relative functions.

**Conclusion**

We have developed a fast and interactive program to analyse interfaces involving protein and/or nucleic acid molecules whose output can be easily handled and parsed by the user. The program will be soon available to users as a web server.

## P-20: The HD-Zip II transcription factor HAT3 acts via recruitment of a chromatin remodeling complex

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In recent years transcriptional repressors have emerged as important elements essential for establishing intricate spatio-temporal patterns of gene expression during plant development and plant responses to stress and hormonal signals. Transcriptional repressors, estimated as 6% of the Arabidopsis proteome, are classified as active or passive. Active repressors generally contain a distinct, small and portable repression motif(s) that inhibits activation of transcription either by modifying chromatin structure or by interacting with and inhibiting the functions of components of the basal transcription machinery. Transcriptional repression mediated by the ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motif is emerging as one of the principal mechanisms of plant gene regulation as it is conserved in negative regulators functioning in a broad range of developmental and physiological processes and across evolutionarily diverse plant species. The discovery that the EAR motif interacts with TOPLESS (TPL) and related TPL-proteins (TPR) together with the demonstration of genetic interaction between TPL and histone deacetylase 19 (AtHDA19) as well as complex formation between TPR1 and AtHDA19 support a model where EAR repressors, via recruitment of chromatin remodelling factors, facilitate epigenetic regulation of gene expression [1].

Among the *Arabidopsis* transcription factors containing an EAR motif are the homeodomain-leucine zipper II (HD-Zip II) proteins [2, 3]. In Arabidopsis the HD-Zip II transcription factors form a protein family consisting of 10 members which can be grouped into four clades (α-δ) [2]. HD-Zip II γ (*ATHB2, HAT1, HAT2*) and δ (*ATHB4, HAT3*) genes, known to be involved in plant response to light quality changes that induce the shade avoidance in most of the angiosperms [4], have been recently implicated in embryonic apical patterning, shoot apical meristem function and organ polarity hinting toward a role for these proteins in plant development per se [3, 5]. From the molecular point of view, there are several evidence that the HD-Zip II proteins act as negative regulators of gene expression [6, 7, 8], and recent work demonstrated that HAT3/ATHB4 directly repress the expression of the *ATHB2* gene [3]. However, the molecular

mechanism underlying the repressive activity of HAT3/ATBH4 is unknown. The presence of the EAR repression motif in the N-terminal region of these proteins led to hypothesize a repression mechanism acting via TPL and/or TPR proteins. Consistent with this hypothesis, recent work has demonstrated that HAT3 and TPL physically interact and that the HAT3 EAR motif is essential for this interaction. Molecular and phenotypic analysis will be presented to discuss the centrality of the HAT3/TPL complex to HAT3 function.

**References:**


1. Kagale S, Rozwadowski K. EAR motif-mediated transcriptional repression in plants: An underlying mechanism for epigenetic regulation of gene expression. Epigenetics, 2011, **6:**141–146

2. Ciarbelli AR, Ciolfi A, Salvucci S, Ruzza V, Possenti M, Carabelli M, Fruscalzo A, Sessa G, Morelli G, Ruberti I. The Arabidopsis homeodomain-leucine zipper II gene family: diversity and redundancy. Plant Mol Biol, 2008, **68:**465-478

3. Turchi L, Carabelli M, Ruzza V, Possenti M, Sassi M, Peñalosa A, Sessa G, Salvi S, Forte V, Morelli G, Ruberti I. Arabidopsis HD-Zip II transcription factors control apical embryo development and meristem function. Development, 2013, **140:**2118-2129

4. Ruberti I, Sessa G, Ciolfi A, Possenti M, Carabelli M, Morelli G. Plant adaptation to dynamically changing environment: the shade avoidance response. Biotechnol Adv, 2012, **30:**1047-1058

5. Turchi L, Baima S, Morelli G, Ruberti I. Interplay of HD-Zip II and III transcription factors in auxin-regulated plant development. J Exp Bot, 2015, **66:**5043-53

6. Steindler C, Matteucci A, Sessa G, Weimar T, Ohgishi M, Aoyama T, Morelli G, Ruberti I. Shade avoidance responses are mediated by the ATHB-2 HD-zip protein, a negative regulator of gene expression. Development, 1999, **126:**4235-4245

7. Ohgishi M, Oka A, Morelli G, Ruberti I, Aoyama T. Negative autoregulation of the Arabidopsis homeobox gene ATHB-2. Plant J, 2001, **25:**389-398

8. Sawa S, Ohgishi M, Goda H, Higuchi K, Shimada Y, Yoshida S, Koshiba T. The HAT2 gene, a member of the HD-Zip gene family, isolated as an auxin inducible gene by DNA microarray screening, affects auxin response in Arabidopsis. Plant J, 2002, **32:**1011-1022

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