



Regione Lombardia

## **Dalla scienza dei materiali allo sviluppo di nuovi dispositivi per la diagnosi e la cura di patologie associate all'invecchiamento**

Progetto cofinanziato da Regione Lombardia tramite il "Fondo per la promozione di accordi istituzionali" nell'ambito dell'accordo quadro Regione-Università

e

dalla Fondazione Alma Mater Ticinensis nell'ambito del bando 2010-2011 "Promuovere la ricerca d'eccellenza"

Università degli Studi di Pavia  
Aula Scarpa  
Venerdì 17 giugno 2011

### **Programma**

- |             |   |
|-------------|---|
| 9.30-9.45   | Saluti del Rettore e del Direttore Università e Ricerca della Regione Lombardia |
| 9.45-11.00  | Relazioni scientifiche  |
| 11.00-11.45 | <i>Coffee break</i> e visione poster  |
| 11.45-12.30 | Relazioni scientifiche  |
| 12.30-12.45 | Intervento dei revisori e prospettive future                                    |
| 13.00       | <i>Lunch</i>  |

## **Elenco presentazioni orali**

S. Pilotto et al. "Catalysis and combinatorial assembly of histone demethylase LSD1 complexes".

A. Malara et al., "Extracellular matrix nano-mechanics determine megakaryocyte function".

P. Sommi et al., "Proteasome particle-rich cytoplasmic structures are widely present in human epithelial neoplasms. Correlative light, confocal and electron microscopy study".

C. Lanni et al., "Homeodomain interacting protein kinase 2: a target for Alzheimer's beta amyloid leading to misfolded p53 and inappropriate cell survival".

E. Saino et al., "In Vitro calcified matrix deposition by human osteoblasts onto a zinc-containing bioactive glass".

Y. Diaz-Fernandez et al., "Synthesis of star-shaped gold nanoparticles with near-IR tunable localized surface plasmon resonance for plasmonic photothermal therapy".

D. Merli et al., "Preparation and characterization of functionalized single- and multi-walled carbon nanotubes".

C. Colonna et al., "Injectable composite in situ forming gels for bone tissue regeneration".

## **Abstracts delle presentazioni poster**

Nel seguito sono allegati tutti i riassunti dei contributi presentati mediante poster.

## Stabilization and inhibition of amyloidogenesis by a new palindromic ligand of transthyretin

**Riccardo Porcari<sup>1</sup>, Patrizia Mangione<sup>1</sup>, Sofia Giorgetti<sup>1</sup>, Graham W. Taylor<sup>2</sup>, Sara Raimondi<sup>1</sup>, Irene Zorzoli<sup>1</sup>, Loredana Marchese<sup>1</sup>, Angelo Gallanti<sup>1</sup>, Cristina Soria<sup>1</sup>, Mark Pepys<sup>2</sup>, Monica Stoppini<sup>1</sup>, Vittorio Bellotti<sup>1,\*</sup>**

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Transthyretin (TTR), the wild-type (WT) plasma protein that transports thyroxine (T4) and retinol binding protein (RBP), is inherently amyloidogenic, forming microscopic, clinically silent amyloid deposits in the heart and blood vessel walls of most aged individuals. More than 80 other single-residue TTR variants cause autosomal dominant, variably penetrant hereditary amyloidosis, affecting about 10,000 people worldwide; patients symptoms can range from various combinations of peripheral and autonomic neuropathy, cardiomyopathy, renal failure, and blindness, and is fatal within 5–15 years. Currently liver transplant is the only possible cure for patients with complete cardiac and neurological functions. Another approach can be found exploiting molecules able to interfere with the fibril precursor hence arresting deposition and halting the disease development. Acknowledging Kelly results using a compound (tafamidis) able to stabilize wild-type TTR assembly which is currently in clinical trials, we have designed two palindromic ligands, mds84 and 4ajm15. This compounds are rapidly bound by wild-type TTR in whole serum and even more avidly by amyloidogenic TTR variants displacing T4 from both of its binding sites, in buffer and in serum. Mass spectrometry showed that both compounds bind TTR with an equimolar ratio even in the presence of RBP. Kinetics studies demonstrates that this process is almost irreversible and it results in a stabilisation of the homotetramer preventing the subunit exchange, stabilizing the protein in acidic pH and in unfolding conditions and inhibiting *in vitro* aggregation better than tafamidis or diflunisal. These superstabilizers are orally bioavailable and exhibit low inhibitory activity against cyclooxygenase, offering a promising platform for development of drugs to treat and prevent TTR amyloidosis.

## References

Trapping of palindromic ligands within native transthyretin prevents amyloid formation. Kolstoe SE, Mangione PP, Bellotti V, Taylor GW, Tennent GA, Deroo S, Morrison AJ, Cobb AJ, Coyne A, McCammon MG, Warner TD, Mitchell J, Gill R, Smith MD, Ley SV, Robinson CV, Wood SP, Pepys MB. *Proc Natl Acad Sci U S A*. 107(47):20483-8 (2010 Nov)

## **A preliminary study on the morphological and release properties of hydroxyapatite-alendronate nanocomposite materials**

**Priscilla Capra<sup>1</sup>, Rossella Dorati<sup>1</sup>, Claudia Colonna<sup>1</sup>, Giovanna Bruni<sup>2</sup>, Franca Pavanetto<sup>1</sup>, Ida Genta<sup>1</sup>, Bice Conti<sup>1\*</sup>**

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**Purpose:** to synthesize Hydroxyapatite-Alendronate (HA-ALN) composites as carrier for ALN that could improve drug site specific activity.

**Methods:** HA-ALN composites were prepared by co-precipitation method. Process parameters such as HA:ALN w/w ratio (1:1, 5:1, 10:1) and HA incubation times (6, 24, 48, 72 hours) were evaluated.

**Morphological characterization** by transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), **physical-chemical characterization** by Differential Scanning Calorimetry (DSC) analysis and **biocompatibility MTT tests** were performed.

**Results:** TEM and SEM analyses confirmed ALN precipitation as fine network onto HA. The results of physical-chemical characterization confirmed the presence of ALN in the composites and its interaction with HA. ALN content resulted between 60 and 80% in the HA:ALN 5:1 and 10:1 ratios composites. ALN release reached 80% from HA-ALN 10:1 composites. Biological tests revealed that complexation with HA increased by three times ALN biocompatibility. These preliminary results demonstrated that HA-ALN composites could be considered a carrier to control ALN release.

## **Injectable composite in situ forming gels for bone tissue regeneration**

**Claudia Colonna\*, Rossella. Dorati, Tiziana Modena, Bice Conti,  
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Injectable systems have been introduced for healing, repair and formation of bone through less invasive and traumatic techniques such as percutaneous administration. Different injectable systems based on bio-materials have been proposed and may be classified as follows: i) gels as pharmaceutical vehicles for bone treatment; ii) injectable ceramic/polymer composites as hybrid bone filling or grafting materials; and iii) injectable bone cements as self-setting bone substitute. Injectable hybrid bone materials have been proposed as a formulation comprising granular solids dispersed and homogenized into an organic fluid matrix.

The final purpose of this work is i) to formulate an *in situ* forming composite gel (ISFcG) consisting in a suspension of Orthoss® spongiuous granules dispersed into a *in situ* gelling polymer solution, ii) to assess the use of Orthoss® spongiuous granules to improve the mechanical properties of *in situ* forming gels and iii) to evaluate the feasibility of using the *in situ* forming composite gel as filling or grafting material suitable to support tissue regeneration in dental, plastic, cranio-maxillofacial or orthopaedic surgery.

Some of the more recent and promising results about this work will be presented. Thermosetting gels compositions were selected and Orthoss® spongiuous granules were incorporated into the thermogelling polymeric solutions; mechanical stability of ISFcGs were evaluated with satisfactory results on the stability of the composite matrix and preliminary data about ISFcGs biocompatibility and cell seeding capacity were collected. Moreover, due to the poor cell seeding capacity of Orthoss® spongiuous granules *per se*, a coating protocol for granules has been studied and different coating materials has been evaluated.

## **Integrated optofluidic device for the study of single cell mechanical properties**

**Francesca Bragheri<sup>a</sup>, Lorenzo Ferrara<sup>a</sup>, Paolo Minzioni<sup>a</sup>, Nicola Bellini<sup>b</sup>, K. C. Vishnubhatla<sup>c</sup>, R. Ramponi<sup>b</sup>, R. Osellame<sup>b</sup>, Iliaria Cristiani<sup>\*a</sup>**

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In recent years an impelling demand has arisen for the development of miniaturized devices for trapping and manipulation of single biological cells. Such devices can be usefully combined with microfluidic circuits that are becoming increasingly important for chemical, biochemical and biological analysis, as they allow reproducing complex phenomena on a micro- or sub-micro scale.

Here we present a recently developed integrated optical stretcher able to perform mechanical analysis of single cells without physical contact and with high reproducibility [1,2]. The chip is fabricated in a fused silica glass substrate by exploiting femtosecond laser micromachining, a technique that ensures extreme flexibility and accuracy. In this device optical trapping and stretching of cells is obtained through two counter-propagating beams emitted by two integrated optical waveguides. The delivery of the samples in the trapping region is accomplished through a controlled flow in a microfluidic channel. We will show that the optofluidic chip is a promising tool for the accomplishment of reliable measurements of the mechanical behaviour of single cells.

### **References**

- [1] N. Bellini, K.C.Vishnubhatla, F. Bragheri, L. Ferrara, P. Minzioni, R. Ramponi, I. Cristiani, R. Osellame *Opt. Expr.* Vol. 18, (2010) 4679-4688.
- [2] F. Bragheri, L. Ferrara, N. Bellini, K. Vishnubhatla, P. Minzioni, R. Ramponi, R. Osellame, I. Cristiani *Journal of BioPhotonics*, Special Issue on Innovative Photonic Micromanipulation Tools, Vol. 3 (2010) 234-243.

## Photonic substrates for optical biosensing devices

**Giacomo Dacarro,<sup>a,\*</sup> M. Patrini,<sup>a</sup> M. Galli,<sup>a</sup> D. Bajoni,<sup>a</sup> M. Liscidini<sup>a</sup>**

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Direct quantitation of protein analytes is an essential need in many fields of research. Measurements in a real-time, label-free manner is extremely advantageous as it reduces the need for reagents and labels, and facilitates assay development by observing bio- molecular interactions. Here we report on the design, realization and optical study of photonic substrates with sensing activity for proteins and complex molecules. We investigate all-dielectric structures, where e.m. field confinement is achieved by means of an interference effect in periodic multilayers that support Bloch surface modes (BSW). These devices are able to detect biochemical species covalently bonded or chemically/physically adsorbed on functionalized surfaces through specific recognition events. BSW are surface-bounded modes, such as surface plasmons (SP), but, unlike SP, their optical resonances can be tuned to specific target wavelengths from the visible to NIR range through proper design. Moreover, a BSW device carries the significant advantages of optical transparency and absence of absorption losses [1].

The optical response of bare and functionalized BSW substrates has been measured via micro-reflectance and fluorescence spectroscopies. We had observed increased efficiency of BSW substrates in two configurations: first, a 50-fold enhancement of the diffraction signal by a protein grating printed on the BSW structure [2]; then, a directional enhancement of the spontaneous emission by a chromophore monolayer bonded to the surface [3]. Here we propose BSW structures designed to resonate to specific label chromophores, using fluorescence detection schemes, or – with no need of labelling - to the vibrational fingerprints of the analyzed species through native or surface-enhanced Raman scattering detection.

### References

- [1] M. Liscidini and J.E. Sipe, *J. Opt. Soc. Am. B*, **26** (2009) 279.
- [2] M. Liscidini, M. Galli, M. Patrini, R.W. Loo, M. C. Goh, C. Ricciardi, F. Giorgis, and J. E. Sipe, *Appl. Phys. Lett.*, **94** (2009) 043117.
- [3] M. Liscidini, M. Galli, M. Shi, G. Dacarro, M. Patrini, D. Bajoni, and J. E. Sipe, *Opt. Lett.*, **34** (2009) 2318.

## Capillary Electrophoresis: an analytical strategy to evaluate the effects of small molecules on the toxic aggregates of Alzheimer's disease

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Alzheimer's disease (AD) is a form of amyloidosis, a disorder where the conformational changes of a protein ultimately lead to the deposition of toxic insoluble fibrils. In AD a small peptide (Abeta42) is the main responsible for the formation of amyloid fibrils in the brain. Fibrillogenesis is a dynamic process, where the formation of different intermediate species at equilibrium includes non-covalently associated soluble oligomers which are more neurotoxic than fibrils. Capillary electrophoresis (CE) can be an excellent complementary tool to the variety of spectroscopic techniques that are used to characterise this process, as it enables the separation and the detection of oligomers during their formation and just before fibril deposition.<sup>1</sup>

The CE method proved to be effective in monitoring over time the activity that co-incubated small molecules with different structure have on the kinetics of Abeta42 oligomer formation. An anthraquinone derivative with anticancer activity<sup>2,3</sup>, a bis-tacrine derivative that inhibits human acetylcholinesterase and carnosine, a physiological dipeptide with antioxidant properties have been tested. It was possible to verify if these compounds inhibit, stabilise or slow down the formation of toxic oligomers in a concentration-dependent manner. A correlation between this activity and the presence of fibrils has been investigated by transmission electron microscopy. Consistently with the results obtained their potential in the reduction of Abeta42 neurotoxicity has been assessed.

### References

- [1] S. Sabella, M. Quaglia, C. Lanni, M. Racchi, S. Govoni, G. Caccialanza, A. Calligaro, V. Bellotti, E. De Lorenzi, *Electrophoresis*, 25 (2004), 3186-3194.
- [2] R. Colombo, A. Carotti, M. Catto, M. Racchi, C. Lanni, L. Verga, G. Caccialanza, E. De Lorenzi, *Electrophoresis*, 30 (2009), 1418-1429.
- [3] R. Colombo et al. *Patent n. MI2008A000366*.



## Screening of fibrillogenesis inhibitors of beta<sub>2</sub>-microglobulin: integrated strategies by Mass Spectrometry, Capillary Electrophoresis and docking studies

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Beta<sub>2</sub>-microglobulin (β<sub>2</sub>-m) is a small protein responsible for a severe complication of long-term hemodialysis known as dialysis-related amyloidosis, in which initial β<sub>2</sub>-m misfolding leads to amyloid fibril deposition, mainly in the skeletal tissue. The stabilisation of the native form of this protein through the binding to a small molecule is one of the considered approaches aimed at preventing or even reversing the protein conformational changes that lead to amyloid deposition. As β<sub>2</sub>-m lacks a specific binding site, finding a strong ligand has always been particularly difficult.

A screening previously carried out by Affinity Capillary Electrophoresis (ACE) on a chemical library of 200 sulphonated compounds enabled the selection of a new ligand that stabilises the native form of the protein, speeds up β<sub>2</sub>-m refolding and inhibits in vitro fibril formation<sup>1,2</sup>.

Starting from this result, LTQ-Orbitrap was used for a high-throughput screening of the same library, to confirm the ACE screening and to fish out interesting hits previously discarded; QSAR analyses were instead employed for predictive purposes<sup>3</sup>. Docking studies on a β<sub>2</sub>-m dimer modelled in silico revealed those ligands that are able to interact with the active regions of β<sub>2</sub>-m and would predict their anti-amyloid activity<sup>4</sup>. The predictive ability has been used to retrieve further interesting hits, within a commercial database composed of 800000 compounds. The compounds selected were then submitted to ACE analysis, for a precise calculation of the binding affinity constants, and to in vitro fibrillogenesis tests to ultimately confirm their anti-amyloid activity<sup>4</sup>.

### References

- [1] M. Quaglia, C. Carazzone, S. Sabella, R. Colombo, S. Giorgetti, V. Bellotti, E. De Lorenzi, *Electrophoresis*, 26 (2005), 4055-4063.
- [2] C. Carazzone, R. Colombo, M. Quaglia, P. Mangione, S. Raimondi, S. Giorgetti, G. Caccialanza, V. Bellotti, E. De Lorenzi, *Electrophoresis*, 29 (2008) 1502-1510.
- [3] L. Regazzoni, L. Bertoletti, G. Vistoli, R. Colombo, G. Aldini, M. Serra, M. Carini, G. Caccialanza, E. De Lorenzi, *CHEMMEDCHEM*, 5 (2010) 1015-1025.
- [4] L. Regazzoni, R. Colombo, L. Bertoletti, G. Vistoli, G. Aldini, M. Serra, M. Carini, R. Maffei Facino, S. Giorgetti, M. Stoppini, G. Caccialanza, E. De Lorenzi, *Analytica Chimica Acta*, 685 (2011), 153-161.

## **Analysis of growth plate sulfation and cell proliferation in a mouse model with proteoglycan undersulfation**

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The sulfate transporter DTDST, also known as *SCL26A2*, is an ubiquitous expressed sulfate-chloride antiporter of the cell membrane, whose function is crucial for the uptake of inorganic sulfate, that is required for proteoglycan sulfation. Mutations in *SCL26A2* gene cause cartilage proteoglycan undersulfation resulting in a family of chondrodysplasias including phenotypes with different clinical outcomes from mild to lethal [1]. We studied the role of proteoglycan sulfation on long bone growth, in the growth plate of the *dtd* mouse, a mouse model for diastrophic dysplasia (*dtd*), reproducing the essential aspects of the DTD phenotype in humans [2]. HPLC analyses show significant undersulfation of whole *dtd* growth plates and in the resting, proliferative and hypertrophic zone, compared to those of wild-type animals at P21. Even if the overall height of the growth plate was within normal values, the relative height of the hypertrophic zone and the average number of cells per column in the proliferative and hypertrophic zones were significantly reduced compared to wild-types. Moreover, the chondrocyte proliferation rate, measured by bromodeoxyuridine labeling, was also significantly reduced in mutant mice. Immunohistochemistry combined with expression data of the *dtd* growth plate demonstrated that the sulfation defect alters the distribution pattern, but not expression, of Indian hedgehog, a long range morphogen required for chondrocyte proliferation and differentiation [3]. These data suggest that in *dtd* mice proteoglycan undersulfation causes reduced chondrocyte proliferation via the Indian hedgehog pathway, therefore contributing to reduced long bone growth.

### **References:**

- [1] J. Hästbacka, A. de la Chapelle, M.M. Mahtani, G. Clines, M.P. Reeve-Daly, M. Daly, B.A. Hamilton, K. Kusumi, B. Trivedi, A. Weaver, A. Coloma, M. Lovett, A. Buckler, I. Kaitila, E.S. Lander, *Cell*, 78 (1994) 1073-1087.
- [2] A. Forlino, R. Piazza, C. Tiveron, S. Della Torre, L. Tatangelo, L. Bonafè, B. Gualeni, A. Romano, F. Pecora, A. Superti-Furga, G. Cetta, A. Rossi, *Human Molecular Genetics*, 14 (6) (2005) 859-71.
- [3] M. Cortes, A.T. Baria, N.B. Schwartz, *Development*, 136 (10) (2009) 1697-706.

## **Gamma irradiation long term effect on functional properties and cytotoxicity of multiblock copolymer films**

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The purpose of this work was to investigate the long term effect of the gamma irradiation treatment on the functional properties of PEG-d,lPLA and PEG-PLGA films and to evaluate the biocompatibility of sterilized samples.

Chemical, thermal properties and biocompatibility of sterilized films were detected for samples at time zero and after storage at 5°C±3°C for 60 days. An *in vitro* degradation study was carried out on polymer samples to examine the effect of sterilization on the degradation performances of copolymers films. Incubated samples were characterized in terms of film surface structure (SEM), chemical (GPC) and thermal properties (DSC).

The study performed on films upon gamma sterilization showed no remarkable changes of the PEG-d,lPLA and PEG-PLGA film structure, while GPC analysis highlighted that the effect of gamma irradiation was dependent on the Mw and composition of polymers. DSC traces suggested more pronounced gamma-ray effects on the PEG-PLGA multiblock copolymer. During the stability study important changes in terms of structure surface, thermal properties and biocompatibility were observed and investigated. Data collected during the *in vitro* degradation study emphasized the need to know and investigate the degradation performances and behaviour of polymer or polymer systems (as DDS, scaffolds and bandage) treated by gamma-ray.

## Preparation and characterization of functionalized single- and multi-walled carbon nanotubes

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Recently, several functionalization schemes have extended the application spectrum of carbon nanotubes (CNTs) enabling the implementation of new functions that cannot otherwise be acquired by pristine nanotubes. Functionalization may impact on biological response to CNTs at cellular and molecular level suggesting that the toxicological profile may also be modified. In this study, aimed at (i) characterizing the physico-chemical properties, and (ii) assessing the cytotoxicity of single-walled CNTs (SW), multi-walled CNTs (MW) and functionalized MW (MW-COOH, MW-NH<sub>2</sub>) was investigated in human astrocytoma D384 cells and lung carcinoma A549 cells using MTT assay and calcein/propidium iodide (PI) staining. The tested nanomaterials were characterized by thermal analysis (TGA), infrared spectroscopy, and atomic force microscopy to assess the degree of purity and functionalization. This part reports on the physico-chemical characterization, for the second part see abstract of Roda et al.

### References

- [1] T. Coccini, E. Roda, D.A. Sarigiannis, P. Mustarelli, E. Quartarone, A. Profumo, L. Manzo, Effects of water-soluble functionalized multi-walled carbon nanotubes examined by different cytotoxicity methods in human astrocyte D384 and lung A549 cells, *Toxicology* 269 (1), (2010) 41-53.

## **A gain-of-function mutation in *PRKACB* encoding the catalytic subunit C $\beta$ of PKA causes a Carney Complex-like syndrome**

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Carney Complex (CNC) is a multiple neoplasia syndrome caused for over 60% of the cases by loss-of-function mutations of *PRKARIA*, coding for RI $\alpha$ , one of the regulatory subunits of the cAMP dependent protein kinase A (PKA). We report here a new molecular defect of the cAMP-signaling pathway associated with a Carney-like phenotype that includes multiple myxomas and a pituitary adenoma. A CNC diagnosis was entertained although the spotty skin pigmentation in the extremities and the large hypopigmented lesion were unusual for CNC. In addition, our patient had developmental delay, a feature never reported in association with CNC; the absence of any indication of adrenocortical disease despite multiple myxomas and acromegaly was also unusual. No mutation in *PRKARIA* gene was found. We identified by array-CGH a genomic amplification of *PRKACB* gene, coding for C $\beta$ , one of the catalytic subunits of PKA and causing an higher PKA activity in patient's cells. An increased *PRKACB* transcript and protein level was detected in patient's lymphoblasts and fibroblasts. Higher C $\beta$ 's expression in one of her mammary mixomas was revealed by immunohistochemistry. This is the first report showing that alteration of the catalytic subunit C $\beta$  and not only of the regulatory subunit RI $\alpha$  of the same PKA protein leads to an overlapping phenotype. The presence of extra copies of *PRKACB* gene indeed illustrates a novel mechanism leading to gain-of-function mutation, hidden by conventional Sanger sequencing.

## Mouse embryonic stem cells as an *in vitro* model to study megakaryocyte differentiation

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Megakaryocytes (MKs) undergo sequential morphological and functional transitions that culminate in the release of platelets (PLTs), highly specialized cells that represent the first defence against haemorrhage.

Some studies have demonstrated the possibility to obtain MKs from mouse and human embryonic stem cells (ESCs). This technology has opened the opportunity of producing PTLs for therapeutical purposes and to develop *in vitro* models to study MK differentiation, a process still poorly known. However, the transfer of this technology to the clinical field requires an improved knowledge of the molecular signature that underlies the ES-MKs phenotype, a necessary requirement to address the problems that are still encountered during their differentiation.

Our aim was to study the differentiation of MKs from mouse ESCs, from the very early progenitors to the formation of proplatelets and the release of platelets. When compared with the gold standard foetal liver derived-MKs (FL-MKs), ES-MKs denoted morphological and functional, differences that highlight an overall immaturity of the ES-MK population. ES-MKs were smaller and have a lower ploidy. They presented a lower efficiency in extending proplatelets than FL-MKs. Also, the proplatelets extended by ES-MKs presented shorter length, less bifurcations in secondary processes and decreased number of tips as compared to FL-MK proplatelets, but were capable to release PLTs. The great majority of PLTs from ES-MKs and FL-MKs showed the same scatter properties and fluorescence intensity (CD41<sup>+</sup>) of those isolated from the peripheral blood of a normal mouse.

Studies are in progress to determine the transcriptional and proteomic blueprint of ES-MKs.

## Defective MSCs differentiation in the murine model for Osteogenesis Imperfecta BrtlIV

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Osteogenesis imperfecta (OI) is a genetic bone disease caused mainly by collagen type I mutations. We investigated the role of osteoblast differentiation in OI pathophysiology by analyzing the ability of MSCs from the OI murine model BrtlIV to originate osteoblasts and adipocytes.

MSCs from adult mice were differentiated into adipocytes and osteoblasts. Adipocytes were stained with Oil Red O and morphological parameters were evaluated. The mineralized matrix deposited by osteoblasts was stained with silver nitrate and differentiation was assessed also by gene expression analysis. Colony Forming Unit-Fibroblasts (CFU-F) was evaluated too and the original population was analyzed by FACS for the presence of stem and precursor surface markers.

Mutant cells originated a higher number of Colony Forming Unit-Adipocytes than WT, with a higher number of adipocytes per colony, larger colonies, higher triglyceride drop number per cell and larger drops. Mutant MSCs mineralized less than WT and showed a decreased expression of various early and late osteoblastic markers. No difference was observed in CFU-F number, as well as in bone marrow content.

To improve MSC differentiation toward osteoblasts we tested *in vivo* the proteasome inhibitor Bortezomib. A significant improvement in osteoblast differentiation was observed *in vitro* and a rescue of the expression of osteoblastic markers was obtained. pQCT analysis on long bones of treated mice showed a partial improvement in some bone geometrical properties.

Our data revealed that MSCs from mutant mice had impaired osteoblasts differentiation and that promoting MSC differentiation toward bone cells could be a new therapy for OI.

## Interstitial Telomeric Repeats as regulators of gene expression

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In vertebrates, TTAGGG hexameres can be found both at the end of chromosomes and at interstitial sites [1], where they are called telomeres and interstitial telomeric repeats (ITSs), respectively. Recent work demonstrated that telomeres are transcribed into Telomeric Repeat-containing RNAs (TERRA) and that TERRA is involved in telomere length maintenance [2, 3] Whereas the function of telomeres as “guardians” of genome integrity has been established, the function of ITSs remains elusive; the recent demonstration that telomeric proteins can bind extra-telomeric sites and influence gene regulation [4] rises the question whether also ITSs have a similar function.

To investigate the involvement of ITSs in gene regulation, we carried out an extensive in-silico analysis of a subset of human ITSs (83) demonstrating that a relevant fraction of these loci (49 ITSs, 59%) is inserted within introns of validated or putative genes. The in-silico analysis of 29 informative ITS-containing genes revealed a high occurrence of alternative splicing at the exons flanking the ITS-containing intron (24 genes, 83%). Using a splicing assay in cultured human cells we detected several alternatively spliced mRNAs; the sequence of the junctions at the detected mRNAs corresponds to that of the splice sites commonly found in mammals, demonstrating that the variants derive from real events of alternative splicing and not from rearrangements of the minigene in the transfected cells. These results suggest that ITSs may be involved in the regulation of gene expression and in particular of alternative splicing.

In addition, we found that, similarly to telomeres, several extra-genic ITSs are transcribed into Telomeric Repeat-containing RNAs, demonstrating that ITS transcription contributes to the production of TERRA molecules.

In conclusion, our results suggest that interstitial telomeric sequences can play a role in gene expression regulation and in the maintenance of genome integrity.

### References

- [1] S.G. Nergadze, M.A. Santagostino, A. Salzano, C. Mondello, E. Giulotto, *Genome Biol*, 8 (2007): R260
- [2] C.M. Azzalin, P. Reichenbach, L. Khoraiuli, E. Giulotto, J. Lingner, *Science*, 318 (2007): 798-801
- [3] S.G. Nergadze, B.O. Farnung, H. Wischnewski, L. Khoraiuli, V. Vitelli, R. Chawla, E. Giulotto, C.M. Azzalin, *RNA*, 15 (2009): 2186-2194
- [4] T. Simonet, L.E. Zaragosi, C. Philippe, K. Lebrigand, C. Schouteden, A. Augereau, S. Bauwens, J. Ye, M. Santagostino, E. Giulotto, F. Magdinier, B. Horard, P. Barbry, R. Waldmann, E. Gilson, *Cell Res*, (2011) [Epub ahead of print]



## **A subtelomeric repetitive element drives transcription of human telomeres and regulates its expression**

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Vertebrate chromosome ends, called telomeres, are composed of repetitions of the conserved DNA hexamer (TTAGGG), that is bound by a series of proteins to form a higher-order structure called Shelterin. The correct function of telomeres is critical in order to maintain genomic stability and to ensure the correct replication of chromosomes allowing cells to proliferate. Telomeres were always considered as transcriptionally silent regions, but in 2007 a family of non-coding RNA stemming from telomeres, called TERRA, were discovered in human cells [1]. Recently, we have identified three repetitive CpG-island elements, present at several subtelomeric loci, that are endowed with promoter activity when tested in a gene reporter-assay. Moreover, we determined that the transcription start site of a subset of TERRA molecules lies between the repeats and the telomere; TERRA promoters activity is also regulated by cytosine methylation [2]. Here, we show that the 61bp-repeat most distal from the telomere acts as a transcription barrier when cloned between the TERRA promoter and the EGFP reporter gene. Inside this element we found three sequences with >95% identity with the consensus sequence of the insulator-binding protein CTCF [3]. Moreover, as a preliminary result, we show that expression of TERRA diminish in neck and lung tumor tissues when compared with healthy control tissues. Overall, our data suggest that TERRA expression has to be tightly regulated and one mechanism of regulation could be through transcriptional insulation of subtelomeric promoters from the rest of the chromosome.

### **References**

- [1] C.M. Azzalin, P. Reichenbach, L. Khoriauli, E. Giulotto, J. Lingner, Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends, *Science*, 318(3851), (2007), 798-801
- [2] S.G. Nergadze, B.O. Farnung, H. Wischnewski, L. Khoriauli, V. Vitelli, R. Chawla, E. Giulotto, C.M. Azzalin, CpG-island promoters drive transcription of human telomeres. *RNA*, 15(12), (2009), 2186-94
- [3] S. Cuddapah, R. Jothi, D.E. Schones, T.Y. Roh, K. Cui, K. Zhao, Global analysis of the insulator binding protein CTCF in chromatin barrier regions reveals demarcation of active and repressive domains, *Genome Research*, 19, (2009), 24-32

## APP INTRACELLULAR C-TERMINAL DOMAIN FUNCTION IS RELATED TO ITS DEGRADATION PROCESS

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**Background:** The amyloid precursor protein (APP) can be processed by either amyloidogenic and non-amyloidogenic pathway; both pathways lead to APP intracellular C-terminal domain (AICD) release. AICD involvement in signal transduction, within Fe65/Tip60 complex, is one of the most discussed mechanism thus different models have been hypothesized to explain AICD role within this complex [1]. The analysis of these models, in relation to the degradation processes, highlights the discrepancy among AICD localization, function and degradation, leading to the hypothesis that may exists a signaling mechanism which allows the APP proteolysis to generate a transcriptionally active fragment from a non active one with a different involvement of proteasome and IDE (insulin-degrading enzyme) [2].

**Aim:** Our work was aimed to analyze AICD functional role within Fe65/Tip60 complex considering the AICD degradation processes to evaluate our previous hypothesis [2].

**Results and conclusions:** In HEK-APP<sub>751</sub> cells AICD are located into nucleus where they up-regulate p53 expression which is increased by ALLN treatment, a proteasome inhibitor which promotes nuclear AICD accumulation and AICD/Fe65/Tip60 complex increase contrary to NEM, an IDE inhibitor treatments which not only do not influence nuclear AICD and consequently p53 expression, but also do not enhance AICD/Fe65/Tip60 complex. These data suggest a correlation between AICD role in gene regulation and their removal dependent by proteasome while NEM treatments underlined the presence of an alternative mechanism involved in AICD removal when they did not exert nuclear activity thus providing a clearer support to the existence of two mechanisms as we previously suggested.

### References

[1] Müller T, Meyer HE, Egensperger R, Marcus K. The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. *Prog Neurobiol* 2008; 85(4):393-406.

[2] Buoso E, Lanni C, Schettini G, Govoni S, Racchi M. beta-Amyloid precursor protein metabolism: focus on the functions and degradation of its intracellular domain. *Pharmacol Res.* 2010 Oct;62(4):308-17.

## HOMEODOMAIN INTERACTING PROTEIN KINASE 2: A TARGET FOR ALZHEIMER'S BETA AMYLOID LEADING TO MISFOLDED p53 AND INAPPROPRIATE CELL SURVIVAL

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**Background:** An unfolded state of p53 protein has been observed in tissues from patients with Alzheimer's disease (AD). The presence of an unfolded p53 form in cells from AD patients led to a lower vulnerability to stressors. The protein p53 is well known to respond to different cellular stresses and may induce cell cycle arrest or apoptosis. The induction of p53 transcriptional activity depends mainly to posttranslational modifications as well as by protein/protein interactions. Another important mechanism that controls p53 function is its conformational stability as p53 is an intrinsically unstable protein whose structure includes one zinc atom as an important co-factor for DNA-binding activity in vitro and in vivo. Among the molecules involved in p53 regulation is homeodomain-interacting-protein kinase 2 (HIPK2). HIPK2 depletion resulted in p53 misfolding, changing the wild-type conformation toward an unfolded status, which can be restored by zinc supplementation.

**Purpose:** We examined the molecular mechanisms underlying the impairment of p53 activity in two cellular models, HEK-293 cells overexpressing the amyloid precursor protein and fibroblasts from AD patients. In particular, we evaluated whether the impairment of p53 signalling pathway is a consequence of an impaired HIPK2 function and whether zinc may counteract this deregulation.

**Results:** We demonstrated that beta-amyloid 1-40 induces HIPK2 degradation and alters HIPK2 binding activity to DNA, in turn regulating the p53 conformational state and vulnerability to a noxious stimulus.

**Conclusions:** These results support the existence of a novel amyloid-based pathogenetic mechanism in AD potentially leading to the survival of injured dysfunctional cells.

## Extracellular matrix nano-mechanics determine megakaryocyte function

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Cell contact with extracellular matrices (ECMs) leads to activation of specific biochemical pathways and to cytoskeletal modifications that regulate different cellular processes. Mechanical properties of ECMs play an important role in determining cells behavior during these processes. In the bone marrow, ECMs regulate hemopoietic stem cells maturation and differentiation. Sites around the endosteal bone and vascular districts have been proposed as critical niches for stem cell differentiation into megakaryocytes (Mks).

In this scenario, fibrillar type I collagen is a key regulator of platelet release, as *in vitro* adhesion of Mks to this protein inhibits platelet release through the generation of a bulk cell contraction mediated by mechano-sensitive proteins, such as fibronectin, Rho- GTPase and myosin, that lead to Mk spreading overtime.

In this work we have used a chemical modified collagen that completely override *in vitro* collagen ligand pathways in directing Mk response in terms of cell spreading, migration, platelet formation and fibronectin assembly. This different behavior seems to be related to the different nano-mechanical properties of modified collagen with respect to native protein. Specifically, N-acetylation of lysine side chains of collagen blocks the formation of banded fibrils. Atomic force microscopy analysis of Mks interaction with collagens clearly demonstrated that absence of fibrils, despite similar integrin engagement, and different mechanical properties, regulate Mks behaviour and fate. New insights into signaling pathways and in mechano-sensing systems of Mks need to be addressed, but nanoscale mechanical properties of ECMs seem to have an important role in regulating megakaryocyte behavior *in vitro* and probably *in vivo*.

**Reference:** Malara A, Gruppi C, Rebuzzini P, Visai L, Perotti C, Moratti R, Balduini C, Tira ME, Balduini A. Megakaryocyte-matrix interaction within bone marrow: new roles for fibronectin and factor XIII-A. *Blood* 2011;117(8):2476-83

## Catalysis and combinatorial assembly of histone demethylase LSD1 complexes

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The common theme to the group's research projects is the investigation of medically and biocatalytically relevant enzymes with interesting chemical properties, such as complex multifunctional systems and proteins performing unusual catalytic functions. A main line of research investigates two human multiprotein complexes involved in chromatin remodelling. We have studied a nuclear protein complex formed by the association of histone deacetylase 1, a co-repressor protein CoREST, and lysine-specific histone demethylase LSD1 that specifically acts on Lys4 of histone H3. This LSD1/HDAC/CoREST multi-enzyme module functions as a "double-blade razor" that first eliminates the acetyl groups from acetylated Lys residues and then removes the methyl group from Lys4 [1]. Our structural studies of the CoREST/LSD1 complex highlighted a uniquely specific binding-site for the histone H3 N-terminal tail and a catalytic machinery that is closely related to that of other flavin-dependent amine oxidases. These insights have been critical for our efforts towards structure-based development of demethylase inhibitors. These newly designed inhibitors were evaluated with a cellular model of acute promyelocytic leukemia chosen since its pathogenesis includes aberrant activities of several chromatin modifiers. Marked effects on cell differentiation and an unprecedented synergistic activity with anti-leukemia drugs were observed [2]. A patent is being submitted to exploit this discovery.

It has been recently discovered that the transcription factor Snail1 binds to LSD1/CoREST and that the three proteins are over-expressed in cancer cell lines and breast tumors. Snail1 controls the epithelial-mesenchymal transition, which is essential for numerous developmental processes (including metastasis). Structure determination of the ternary complex LSD1/CoREST/Snail1 peptide has revealed that Snail1 interacts with LSD1/CoREST in a remarkable way: the N-terminal residues of this transcription factor bind in the active site cleft of LSD1 effectively mimicking the histone H3 tail. Therefore, Snail1 is a potential endogenous inhibitor of LSD1. Furthermore, this finding predicts that other members of the Snail1-related transcription factor family associate to LSD1/2 through a similar histone-mimicking mechanism [3].

A challenge for future studies will be to extend these structural investigations to visualize nucleosome binding by LSD1-containing protein complexes through biophysical methods and crystallography.

### References

- [1] Forneris F, Binda C, Battaglioli E, Mattevi A. LSD1: oxidative chemistry for multifaceted functions in chromatin regulation. *Trends Biochem Sci*, **2008**, 33, 181-189.
- [2] Binda C, Valente S, Romanenghi M, Pilotto S, et al. Biochemical, Structural, and Biological Evaluation of Tranylcypromine Derivatives as Inhibitors of Histone Demethylases LSD1 and LSD2. *J Am Chem Soc* **2010**, 285:36849-36856.
- [3] Baron R, Binda C, Tortorici M, McCammon JA, Mattevi A (2011) Molecular Mimicry and Ligand Recognition in Binding and Catalysis by the Histone Demethylase LSD1-CoREST Complex. *Structure*, **2011**, 19, 212-220

## **AL amyloidosis: investigation of the mechanisms of light chain cardiac toxicity through a functional proteomics approach**

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**Introduction.** In systemic amyloidoses, fibril deposition causes cell and organ dysfunction. A key pathogenetic role of soluble prefibrillar oligomers is emerging (1, 2). In AL amyloidosis, fibrils originate from monoclonal immunoglobulin light chains (LC); the peculiar organ tropism of each LC suggests specific interactions with cell and/or tissue components. Soluble LC causing human heart amyloidosis exert damage in rodent cardiac cells (2). We investigated the cardiotoxicity mechanisms by evaluating the interactome of amyloidogenic cardiotoxic LC in cardiac cells through functional proteomics.

**Methods.** Monoclonal LCs were purified by chromatography from urines of very well characterized patients with AL amyloidosis or multiple myeloma (controls). Ventricular rat cardiomyocytes were isolated from perfused hearts, pelleted, pooled and homogenized. Upon precleaning, LC were incubated with the homogenate, followed by immunoprecipitation (IP) with monoclonal anti-LC antibodies. After SDS-PAGE separation, eluted proteins were subjected to in-gel digestion and LC-MS/MS-based identification.

**Results.** Amyloidogenic cardiotoxic and non-amyloidogenic LC were tested in parallel. The IP system efficiently captured all LC types, with no significant leaking of antibody in the eluate. SDS-PAGE separation of eluted proteins showed multiple species in the IP of LC complexes. MS analysis identified proteins specifically captured in association with the cardiotoxic LC, including species involved in mitochondrial metabolism and calcium handling.

**Conclusions.** We developed an in-vitro system for investigating the amyloidogenic LC interactome in cardiac cells. Identification and characterization of LC protein interactors could be key to elucidate mechanisms linking extracellular misfolded proteins to cell toxicity.

1. Palladini G, Lavatelli F, Russo P, Perlini S, Perfetti V, Bosoni T, Obici L, Bradwell AR, D'Eril GM, Fogari R, Moratti R, Merlini G. Circulating amyloidogenic free light chains and serum N-terminal natriuretic peptide type B decrease simultaneously in association with improvement of survival in AL. *Blood*, 2006;107(10):3854-8.
2. Shi J, Guan J, Jiang B, Brenner DA, Del Monte F, Ward JE, Connors LH, Sawyer DB, Semigran MJ, Macgillivray TE, Seldin DC, Falk R, Liao R. Amyloidogenic light chains induce cardiomyocyte contractile dysfunction and apoptosis via a non-canonical p38alpha MAPK pathway. *PNAS*, 2010;107(9):4188-93.

## Insights on short-range mechanism for the inhibitory action of bio-mimetic capped silver nanoparticles against Gram positive and Gram negative bacteria.

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In this work we describe a simple and economic procedure to produce biomimetically-coated silver nanoparticles (Ag NPs), based on the post-functionalization and purification of colloidal silver stabilized by citrate.<sup>1</sup> Two biological capping agents have been used (cysteine CYS and glutathione GSH). The composition of the capped colloids have been ascertained by various techniques and antibacterial tests on GSH-capped Ag NPs have been carried out under physiological conditions, obtaining values of Minimum Inhibitory Concentration (MIC) of 180 µg/ml and 15 µg/ml for *S. aureus* and *E. coli* respectively. Interference on bacterial cell replication is observed for both cellular strains when exposed to GSH stabilized colloidal silver, while silver cations affect only cell replication of *E. Coli* under our experimental conditions. The GSH capped Ag NPs presented lower antibacterial activity when grafted on glass-functionalized surfaces. The obtained results suggest that the effect of GSH-capped silver NPs on *E. Coli* is more intense and can be associated with the penetration of the colloid into the cytoplasm, with the subsequent local interaction of silver with the bio-molecules, causing damages to the cells. Conversely, for *S. Aureus*, since the thick peptidoglycan layer of the cell-wall prevents the penetration of the NPs inside the cytoplasm, the antimicrobial effect is limited. Therefore, the antibacterial activity of these GSH capped NPs can be ascribed to the direct action of metallic silver NPs, rather than to the bulk release of Ag<sup>+</sup>.

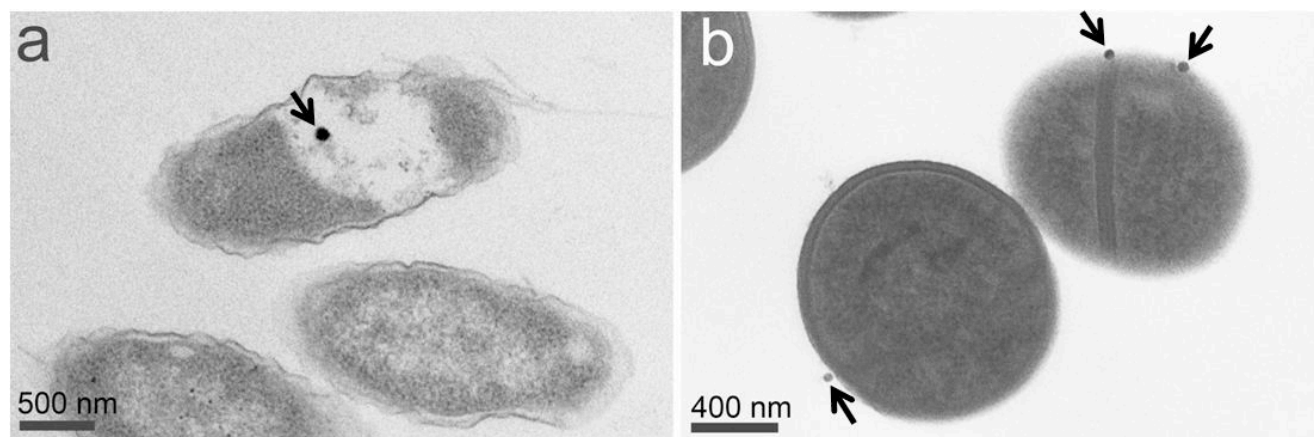


Figure: TEM imaging of the interaction of GSH-coated Ag NPs with bacteria: a) *E. coli*, penetration of the NP into the cytoplasm associated with a clearer zone of cellular damage; b) *S. aureus*, NPs outside the cellular membrane. NPs are labeled with arrows.

### References

[1] Pallavicini, P.; Taglietti A.; Dacarro, G.; Diaz Fernandez, Y.A.; Galli, M.; Grisoli, P.; Patrini, M.; Santucci De Magistris G.; Zanoni R., J. Colloid Interf. Sci., 350(2010), 110

# Monolayers of polyethylenimine on flat glass: receptors for cations and nanoparticles in the preparation of antibacterial surfaces

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This work is inscribed into a long-term research, bound to functionalize the surface of bulk material in order to produce objects capable of a slow and continuous release of metal cations with antibacterial activity.<sup>[1,2]</sup> In this case we prepared and characterized a polyethylenimine (PEI) layer covalently grafted on glass surfaces (i.e glass, quartz, silicon). Polyamines are well known ligands for transition metal cations, in particular they can bind effectively Cu(II) ions and, to a lesser extent, Ag(I) ions. Moreover, polyamines have been studied as stabilizing and capping agents for silver nanoparticles (NP), exploiting amine-metal interactions as well as electrostatic interactions between positively charged polymers and negatively charged NP. We exploited this versatility to set up a straightforward wet synthesis of a PEI monolayer able to bind both metal cations (Cu<sup>2+</sup> and Ag<sup>+</sup>) and silver nanoparticles. Cation uptake and release capabilities of the self-assembled monolayer have been studied. Morphology and physico-chemical properties of the SAM were explored through a full set of characterization techniques: UV-Visible spectroscopy, spectroscopic ellipsometry, atomic force microscopy, contact angle measurement. With a similar procedure, PEI functionalized substrates were loaded with silver NP. The quantity of loaded silver and the diameter of the grafted NP was proportional to the immersion time of the substrates in a 7 nm (diameter) NP suspension (see Figure 1). NP grafted on PEI monolayers showed a strong binding, and no NP leaking in solution was detected. However silver ions can be slowly released in a water medium thanks to the progressive oxidation of the nanostructured metal. Metal ion release from both metal complexes and metal NP monolayers was exploited to achieve a good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

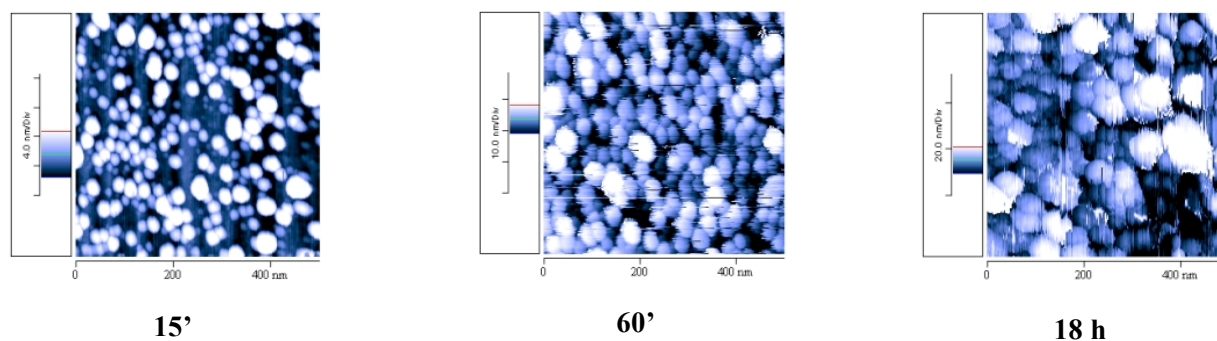


Figure 1. AFM images, glass surfaces, after 15 minutes (left), 60 minutes (center), and 18 hours (right) immersion time of the PEI-coated surfaces in a 7 nm diameter AgNP solution

## References

- [1] P. Pallavicini, A. Taglietti, G. Dacarro, Y.A. Diaz-Fernandez, M. Galli, P. Grisoli, M. Patrini, G. Santucci De Magistris, R. Zandoni, *J. Colloid Interf. Sci.*, 350 (2010), 110-116
- [2] P. Pallavicini, G. Dacarro, L. Cucca, F. Denat, P. Grisoli, M. Patrini, N. Sok, A. Taglietti, *New J. Chem.*, in the press (DOI: 10.1039/c0nj00829j)



## Synthesis of star-shaped gold nanoparticles with Near-IR tunable Localized Surface Plasmon Resonance for Plasmonic Photothermal Therapy.

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We are currently developing new seed-growth methods for preparing anisotropic gold nanoparticles with tunable Near-InfraRed Localized Surface Plasmon Resonance (NIR-LSPR). These methods lead to the formation of branched (Figure 1a) or star-shaped (Figure 1b) gold nanoparticles, thanks to the action of a zwitterionic surfactant (*N*-Dodecyl-*N*',*N*''-dimethyl-3-ammonio-1-propanesulfonate, LSB)[1] or a of non-ionic surfactant (TritonX-100), respectively. In both cases it is possible to control the shape and the optical properties of the nanoobjects through the variation of simple experimental parameters such as the concentration of the surfactant and the amount of the used reducing agent in the growth solution. In particular, the position of the LSPR is closely related to the dimension and the shape of the objects and, for star-shaped nanoparticles, to the length/base-width ratio (Aspect Ratio) of the branches. The possibility of tuning the LSPR of the nanostars in the NIR region (700nm-1300nm), where tissues, blood and water display high transmission of electromagnetic radiation, opens the way to the use in-vivo and in deep tissues of our nano-objects as plasmonic photothermal agents, capable of converting light into heat, in order e.g. to eliminate tumoral cells or deep-positioned, drug-resistant bacterial infections. Collaborative studies have already demonstrated an efficient conversion of NIR radiation in heat by Au nanostars and nanobranched objects. An exceptional stability and biocompatibility of our nanoparticles is easily achieved by functionalizing the whole surface with poly(ethylene)glycol-SH and by eliminating any trace of toxic surfactants (used in the syntheses) through consecutive cycles of ultracentrifugation. This latter feature together with the exceptionally red shifted LSPR position obtained with TritonX-100 syntheses (up to 1700 nm), represent valuable novelties with respect to the state of the art of anisotropic gold nanoobjects.

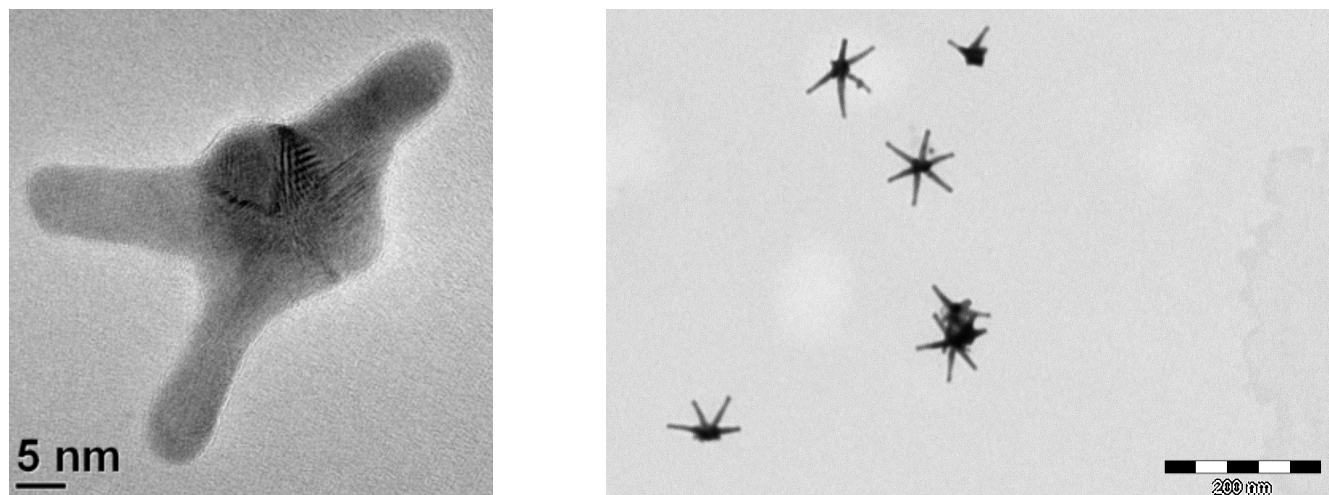


Figure 1. Left: HRTEM of a branched Au NP (scale bar = 5 nm), obtained from syntheses with LSB. Right: TEM image (scale bare = 200 nm) of nanostars obtained from syntheses with Triton X-100

### References

[1] P. Pallavicini, G. Chirico, M. Collini, G. Dacarro, A. Donà, L. D'Alfonso, A. Falqui, Y. Diaz-Fernandez, S. Freddi, B. Garofalo, A. Genovese, L. Sironi, A. Taglietti, *Chem. Commun.*, 47 (2010) 1315-1317

# A molecular fluorescent sensor for pH windows sealed in a biocompatible micellar container and its response-tuning by a polyelectrolyte

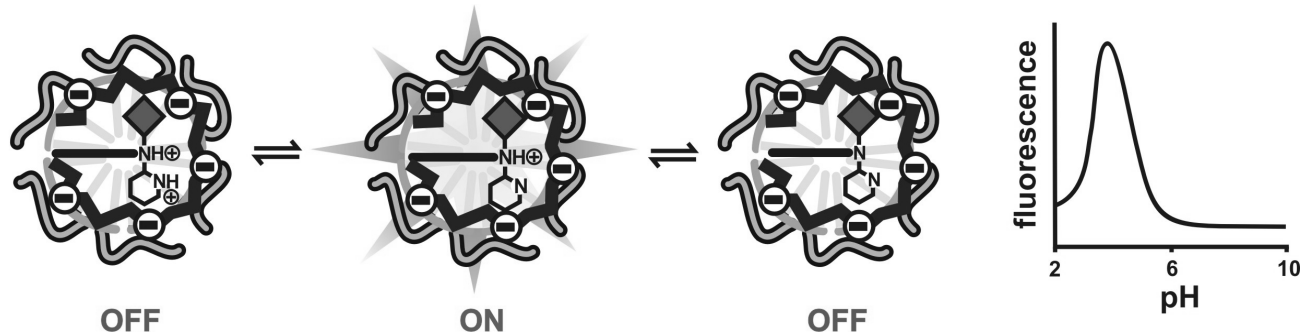
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A new approach is presented to obtain fluorescent sensors for pH-windows, that work in water and in biomimetic conditions. A single molecule is used, featuring all covalently linked components that make it capable of working as a fluorescent sensor with an OFF-ON-OFF response with pH. The components are a tertiary amine, a pyridine and a fluorophore (pyrene). The forms with both protonated bases or both neutral bases quench pyrene fluorescence, while the form with neutral pyridine and protonated amine is fluorescent. The molecular sensor is also equipped with a long alkyl chain to make it highly hydrophobic in all its protonated and unprotonated forms, i.e. either when neutral or charged. Accordingly, it can be confined at any pH value either in traditional (low molecular weight) non-ionic surfactant micelles, or inside polymeric, biocompatible micellar containers. Relevantly for future in-vivo applications, thanks to its strong hydrophobicity, no leakage of the molecular sensor is observed from the polymeric biocompatible micelles, made of polyaspartate copolymeric units, PHEA-PEG<sub>5000</sub>-C16. PHEA-PEG<sub>5000</sub>-C16 has already been proven to form biocompatible nano-dimensioned micelles (d = 12 nm)[1,2] that resist at the in-vivo dilution thanks to its very low critical micelle concentration (< 10<sup>-7</sup> M). Due to the proximity of the pyridine and amine functions in the molecular structure and to the poor hydration inside micelles, their observed pK<sub>a</sub> values are low, so that the ON window is positioned at very low pH. However, the window can be shifted to biologically relevant values by comicellization of anionic species. In particular in the micelles of the non-ionic surfactant TritonX-100, a shift of the ON window to pH 4-6 is obtained by addition of the anionic SDS surfactant, whose negative charge promotes the stability of the protonated forms of the pyridine and amine fragments. In the case of the polymeric micelles, we introduce the use of the amphiphilic PolyStyreneSulfonate anionic polyelectrolyte, whose comicellization induces a shift and a sharpening of the ON window, that is centered at pH 4.



## References

- [1] a) G. Cavallaro, M. Licciardi, G. Giammona, P. Caliceti, A. Semenzato, S. Salmaso, *J. Control. Release* 89 (2003) 285; b) G. Cavallaro, L. Maniscalco, M. Licciardi, G. Giammona, *Macromol. Biosci.* 4 (2004) 1028–1038
- [2] Y.A. Diaz-Fernandez, E. Mottini, L. Pasotti, E.F. Craparo, G. Giammona, G. Cavallaro, P. Pallavicini, *Biosensens. Bioelectron.*, 26 (2010) 29-35

## **Silk fibroin engineered 3D System for the Study of Megakaryocytes and Functional Platelet Production**

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Platelet formation occurs in the bone marrow where megakaryocytes (Mks) extend long filaments, called proplatelets (PPF), that protrude through the vascular endothelium into the sinusoid lumen to release platelets. We propose a new 3D system to gain insight into mechanisms of platelet formation as well as a new platelet production system. Bioreactor was perfused by stainless steel needles, spanned by porous silk microtubes with 500µm inner diameter, wall thickness around 50 µm and pore sizes of 2-8 µm mimicking the sinusoidal blood vessels. The silk tubes were coated with a combination of matrigel with fibrinogen (FBG), von Willebrand factor (vWF) and SDF-1 alpha and were embedded within a cell-seeded type I collagen gel which replicates the osteoblastic niche. Human cord blood derived Mks, seeded at the interface between the type I collagen gel and the coated vascular microtubes, were analyzed in terms of migration, PPF and platelet production. Mks were able to migrate in the gel enriched with FBG and vWF and, after additional 24 hours, Mks were able to adhere to the external wall of the vascular microtubes and protrude proplatelets through the pores of the vascular wall. Finally, the vascular microtubes were perfused at a rate of 32 sec-1/min and collected platelets were analyzed. The particles collected in the flow effluent of the vascular microtubes were CD61+ cells with the same scatter properties of human peripheral blood platelets. In conclusion, we validated an innovative 3D bone marrow model where the fundamental steps of thrombopoiesis can be analyzed and functional platelets can be obtained.

**Reference:** Pallotta I, Lovett M, Rice W, Kaplan DL, Balduini A. Bone Marrow Osteoblastic Niche: A New Model to Study Physiological Regulation of Megakaryopoiesis. (2009) PLoS ONE; 4: e8359

## Increasing the antibacterial effect of lysozyme by immobilization on multi-walled carbon nanotubes

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Carbon nanotubes as diagnostic or therapeutic tools in nanomedicine is arousing a widespread and increasing interest. Carbon nanotube protein conjugates have been recently considered both for the delivery of therapeutics and for their application as electrochemical biosensors. In particular, peptides were known to have a particular affinity towards CNTs, allowing their dispersion in water by increasing their solubility in aqueous medium. The entrapment of proteins into the inner space of opened CNTs has been also reported. Here we report a facile strategy to obtain multiwalled carbon nanotubes (MWCNTs) functionalized with covalently bonded lysozyme. The functionalization procedure has been investigated by means of several techniques, including thermogravimetry, Raman spectroscopy, transmission electron microscopy, and cyclic voltammetry. A functionalization of about 1 lysozyme molecule every 4000 carbon atoms is obtained. The modified lysozyme-CNTs nanocomposite shows a significant increase of the antibacterial activity towards the Gram-positive *S. aureus* if compared with lysozyme in solution.

### References

- [1] Merli D., Ugonino M., Profumo A., Fagnoni M., Quartarone E., Mustarelli P., Visai L., Grandi M.S., Galinetto P., Canton P., Increasing the Antibacterial Effect of Lysozyme by Immobilization on Multi-Walled Carbon Nanotubes, *Journal of Nanoscience and Nanotechnology*, 11 (2011) 3100-3006.

## **Proteasome particle-rich cytoplasmic structures are widely present in human epithelial neoplasms. Correlative light, confocal and electron microscopy study**

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A novel cytoplasmic structure has been recently characterized by confocal and electron microscopy in *H. pylori*-infected human gastric epithelium, as an accumulation of barrel-like proteasome reactive particles colocalized with polyubiquitinated proteins, *H. pylori* toxins and the NOD1 receptor [1,2]. This proteasome particle-rich cytoplasmic structure (PaCS), a sort of focal proteasome hyperplasia, was also detected in dysplastic cells and was found to be enriched in SHP2 and ERK proteins, known to play a role in *H. pylori*-mediated gastric carcinogenesis [1-3]. However, no information is available on its occurrence in neoplastic growths. Based on our previous findings and the mounting evidence supporting a role of proteasome in cancer development and progression [4], this study investigates human epithelial neoplasms for the presence of PaCS. Surgical specimens of gastric cancer and various other human epithelial neoplasms have been investigated by light, confocal and electron microscopy including correlative confocal and electron microscopy. PaCSs were detected in gastric cohesive, pulmonary large cell and bronchioloalveolar, thyroid papillary, parotid gland, hepatocellular, ovarian serous papillary, uterine cervix and colon adenocarcinomas, as well as in pancreatic serous microcystic adenoma. *H. pylori* bodies, their virulence factors (VacA, CagA, urease, and outer membrane proteins) and the NOD1 bacterial proteoglycan receptor were selectively concentrated inside gastric cancer PaCSs, but not in PaCSs from other neoplasms which did, however, retain proteasome (including immunoproteasome) and polyubiquitinated proteins reactivity. Particle lysis and loss of distinctive immunoreactivities were seen in some tumour cell PaCSs, possibly ending in sequestosomes or autophagic bodies. It is concluded that PaCSs are widely represented in human neoplasms and that both non-infectious and infectious factors activating the ubiquitin-proteasome system are likely to be involved in their origin. PaCS detection might help clarify the role of the ubiquitin-proteasome system in carcinogenesis.

### **References**

1. V. Necchi, P. Sommi, V. Ricci, E. Solcia. *In vivo accumulation of Helicobacter pylori products, NOD1, ubiquitinated proteins and proteasome in a novel cytoplasmic structure*. PLoS One 5 (2010): e9716.
2. J. Viala, C. Chaput, I.G. Boneca, A. Cardona, S.E. Girardin, et al. *Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island*. Nat Immunol 5 (2004): 1166–1174.
3. M. Hatakeyama. *Oncogenic mechanisms of the Helicobacter pylori CagA protein*. Nat Rev Cancer 4 (2004): 688-694.
4. D. Hoeller, I. Dikic. *Targeting the ubiquitin system in cancer therapy*. Nature 458 (2009): 438-444.

## Toxicity evaluation of differently functionalized carbon nanotubes by a set of *in vitro* and *in vivo* studies

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Increasing interest on safety evaluation of carbon nanotubes (CNTs) has risen in relation to their use in various technological fields together with the evidence of their cytotoxic effects. Functionalization schemes extended the CNTs applications, modifying their toxicological profile and thus, impacting on biological response to CNTs.

The present study assessed: (i) *in vitro*, the cytotoxicity of multi-walled-CNTs, bare (MW) and differently functionalized (MW-COOH, MW-NH<sub>2</sub>, hf-MW-NH<sub>2</sub>: highly-functionalized) on human astrocytoma (D384) and lung carcinoma (A549) cells using MTT-assay and Ca/PI-staining; (ii) *in vivo*, the onset of pulmonary effects of MW, MW-NH<sub>2</sub> and MW-COOH, intratracheally instilled in rats (200 microg/rat).

(i) Cells were exposed to CNTs (1-800µg/ml) for 24-48h. MTT data revealed a dose-dependent cytotoxicity of pristine-MW, MW-COOH, MW-NH<sub>2</sub> in both cell lines, with a decrease in MTT metabolism ranging from 50 to 75%. Contrastingly, Ca/PI-staining did not detect any CNT-mediated alterations at any concentration or exposure time.

hf-MW water-soluble CNTs, characterized by a liquid oily texture, showed dose-dependent cytotoxicity assessed by both MTT and Ca/PI-staining (30-75% loss of cell viability at 200-800µg/ml).

(ii) Rat lungs showed dark, particulate-laden macrophages, reflecting nanomaterial engulfing, 16-days after treatment with all CNTs. Lung architecture alteration was observed together with a general pulmonary toxicity coupled with inflammatory response, without fibrosis and granuloma formation.

In summary, the degree of functionalization seems to impact *in vitro* on CNT toxicity, with dissimilar responses obtained by traditional tests, due to CNT interference with the dye markers employed, while *in vivo* the observed alterations were irrespective of nanotube functionalization.

## **Effect of Electrospun Fiber Diameter and Alignment on Macrophage Activation and Secretion of Proinflammatory Cytokines and Chemokines**

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Macrophage activation can be modulated by biomaterial topography according to the biological scale (micrometric and nanometric range) [1]. In this study we investigated the effect of fiber diameter and fiber alignment of electrospun poly(L-lactic) (PLLA) scaffolds on macrophage RAW 264.7 activation and secretion of proinflammatory cytokines and chemokines at 24 h and 7 days. Macrophages were cultured on four different types of fibrous PLLA scaffold (aligned microfibers, aligned nanofibers, random microfibers and random nanofibers) and on PLLA film (used as a reference). Substrate topography was found to influence the immune response activated by macrophages, especially in the early inflammation stage. Secretion of proinflammatory molecules by macrophage cells was chiefly dependent on fiber diameter. In particular, nanofibrous PLLA scaffolds minimized the inflammatory response when compared with films and microfibrous scaffolds. The histological evaluation demonstrated a higher number of foreign body giant cells on the PLLA film than on the micro- and nanofibrous scaffolds. In summary, our results indicate that the diameter of electrospun PLLA fibers, rather than fiber alignment, plays a relevant role in influencing in vitro macrophage activation and secretion of proinflammatory molecules [2].

### **References**

[1]H. Cao, K. McHugh, S.Y. Chew, J.M. Anderson, *J Biomed. Mater. Res. A* 2010, 93, 1151-1159.

[2]E. Saino, M.L. Focarete, C. Gualandi, E. Emanuele, A.I. Cornaglia, M. Imbriani, L. Visai, *Biomacromolecules* 2011, 12(5), 1900-11.

## ***In Vitro* Calcified Matrix Deposition By Human Osteoblasts Onto a Zinc-Containing Bioactive Glass**

**Enrica Saino<sup>1,7</sup>, Stefania Grandi<sup>2,7</sup>, Eliana Quartarone<sup>2,7</sup>, Valentina Maliardi<sup>3,7</sup>, Daniela Galli<sup>3,7</sup>, Nora Bloise<sup>1</sup>, Lorenzo Fassina<sup>4,7</sup>, Maria Gabriella Cusella De Angelis<sup>3,7</sup>, Piercarlo Mustarelli<sup>2,7</sup>, Marcello Imbriani<sup>5,6</sup> and Livia Visai<sup>1,5,7\*</sup>**

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Bioactive glasses synthesized by the sol-gel technique possess many of the qualities associated with an ideal scaffold material for a bone graft substitute [1]. In view of the potential clinical applications, we performed a detailed *in vitro* study of the biological reactivity of synthesized 58S bioactive glass containing-zinc, in terms of osteoblast morphology, proliferation, and deposition of a mineralized extracellular matrix (ECM). Human Sarcoma Osteoblast (SAOS-2) cells were used to i) assess cytotoxicity by lactate dehydrogenase (LDH) release and ii) evaluate the deposition of a calcified extracellular matrix by ELISA assay and quantitative RT-PCR (qRT-PCR). In comparison with pure silica and 58S, the 58S-Zn0.4 bioglass showed a significant increase in cellular proliferation and deposition of ECM components such as decorin, fibronectin, osteocalcin, osteonectin, osteopontin, type-I and -III collagens. Calcium deposition was significantly higher than on pure silica and 58S samples. Also Alkaline phosphatase (ALP) activity and its protein content was higher with respect to pure silica and 58S. qRT-PCR analysis revealed the up-regulation of type-I collagen, bone sialoprotein and osteopontin genes. All together these results demonstrate the cytocompatibility of 58S-Zn0.4 bioglass and its capability to promote osteoblast differentiation.

### **Reference**

[1] Oki A, Parveen B, Hossain S, Adeniji S, Donahue H, *J Biomed Mater Res A*, 69 (A), 2004, 216-221.