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Paestum (SA), 10-14 settembre 2017

➢Divisione di Chimica dei Sistemi Biologici

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- Luigi Russo, Università degli Studi della Campania "Luigi Vanvitelli"

Programma Scientifico

Divisione di Chimica dei Sistemi Biologici

Lunedì 11 Settembre 2017

	Sala Diana
Sessione della Divisione di Chimica dei Sistemi Biologici	
Chairpersons R. Purrello, S. Ciurli	
9.00 – 9.50	CSB PL01: M. J. Maroney, J.O. Campeciño, C.E. Carr, H.Q. Hu, H.T. Huang, Musiani F. and S. Ciurli *Bioinorganic chemistry from metals to enzymes: A nickel tour.*
9.50 – 10.10	CSB OR01: L. Mazzei, F. Musiani, G. Lente, M. Palombo, M. Cianci, S. Benini and S. Ciurli. *Biochemical and structural studies on the inhibition of urease, a nickel-dependent virulence factor.*
10.10 – 10.30	CSB OR02: C. Pozzi, S. Ciambellotti, C. Bernacchioni, F. Di Pisa, P. Turano and S. Mangani. *Structural and mechanistic insights into iron processing and biomineralization by vertebrate ferritins*
10.30 – 11.00	Coffee Break
Sessione congiunta con la Divisione di Chimica Fisica dedicata al Prof. Guido Barone	
Chairpersons C. Isernia, C. Giancola	
11.00 – 11.50	CSB PL02: M.R. Tiné. *Calorimetry and Thermoanalytical Techniques in the Study of Proteins.*
11.50 – 12.05	Divisione di Chimica Fisica: R. Oliva. *Biophysical studies of membrane perturbation induced by the antimicrobial peptide GKY20*
12.05 – 12.20	CSB OR03: A. D'Urso, C.M.A. Gangemi, S. Alaimo, A. Pulvirenti, A. Ferro and R. Purrello. *Exploiting conformation and structural analysis of endogenous miRNAs to refine gene targeting evaluation.*
12.20 – 12.35	Divisione di Chimica Fisica: A. Del Giudice. *The structural response of Human Serum Albumin to oxidation: a biological buffer to local formation of hypochlorite.*
12.35 – 12.50	CSB OR04: J. Amato. *Identification and characterization of DNA G-quadruplex interacting proteins.*

13.00 - 14.00	Intervallo Pranzo

	Sala Diana
Sessione della Divisione di Chimica dei Sistemi Biologici	
Chairpersons M.J. Maroney, A. Rosato	
15.00 – 15.50	CSB PL03: R. Fattorusso. *Protein folding pathways investigated by NMR spectroscopy.*
15.50 – 16.10	CSB OR05: D. Marasco, E. Novellino and S. La Manna. *Insights in self-recognition, misfolding and mislocalization mechanisms of Nucleophosmin 1 in Acute Myeloid Leukemia.*
16.10 – 16.30	CSB OR06: L. Russo, K. Giller, E. Pfitzner, C. Griesinger and Stefan Becker. *The molecular recognition mechanism of the coactivator NCoA-1 by STAT6.*
16.30 – 17.00	Coffee Break
Sessione della Divisione di Chimica dei Sistemi Biologici	
Chairpersons G. Fossati, A. D'Urso	

17.00 – 17.30	Premio Divisione di Chimica dei Sistemi Biologici- Italfarmaco CSB PL04: S. Sattin *Towards unconventional therapeutic approaches.*
17.30 – 17.45	CSB OR07: M. Prejanò, T. Marino, and N. Russo. *How can work methanol dehydrogenase from Methylacidiphilum fumariolicum with the alien Ce(III) ion in the active center? A theoretical study.*
17.45 – 18.00	CSB OR08: V. Maggi, F. Bianchini, A. Sartori and R. Fiammengo. *Aminoproline-RGD functionalized gold nanoparticles for targeting of integrins involved in tumor angiogenesis.*
18.00– 18.15	CSB OR09: V. Oliveri, S. Zimbone, M. L. Giuffrida, F. M. Tomasello, F. Bellia, G. Vecchio. *Functionalized cyclodextrins as modulators of Aβ cytotoxicity.*
18.15– 18.30	CSB OR10: A. Bortot, F. Munari, S. Zanzoni, M. D'Onofrio, D. Fushman and M. Assfalg. *Specific secondary interactions between ubiquitin and UBA observed in cell-mimicking crowded solution.*
18.30– 18.45	CSB OR11: M. Gaeta, R. Randazzo, D. A. Cristaldi, A. D'Urso, R. Purrello and M. E. Fragalà. *ZnTPPS demetallation: role of polyelectrolytes on aggregation after protonation in acid.*
18.45– 19.00	CSB OR12: F. Arcudi and M. Prato. *Synthesis, Separation and Characterization of Small and Highly Fluorescent Nitrogen-Doped Carbon NanoDots.*
	Sala Diana
19.00– 20.00	*Assemblea dei Soci della Divisione di Chimica dei Sistemi Biologici*

Martedì 12 Settembre 2017

	Sala Diana
Sessione congiunta con il gruppo Interdivisionale di Biotecnologie	
Chairpersons L. Cipolla, M. Coletta	
9.00 – 9.50	CSB PL05: D. Montesarchio. *Multifunctional nanosystems for theranostics.*
9.50 – 10.10	CSB OR13: M. Gobbo, F. Biscaglia, S. Rajendran, C. Benna, G. Bocchinfuso, P. Conflitti, L. Litti, R. Sommaggio, D. Nitti, A. Rosato, A. Palleschi, S. Mocellin and M. Meneghetti. *Peptide Targeted Gold Nanostructures for high effective SERRS Imaging of Colorectal Cancer Cells.*
10.10 – 10.30	Divisione di Chimica Organica: G. Oliviero, M. Marzano, A.P. Falanga, S. D'Errico, G. Piccialli, N. Borbone. *Higher order G-quadruplex-based aptamers from tetra-end-linked oligonucleotides with in vitro anti-HIV activity*
10.30 – 11.00	Coffee Break
Sessione della Divisione di Chimica dei Sistemi Biologici	
Chairpersons M. Assfalg, R. Fattorusso	
11.00 – 11.50	CSB PL06: S. C. Baffoni. *Unravelling the molecular mechanisms of iron-sulfur protein maturation.*
11.50 – 12.05	CSB OR14: D. Sala, S. Ciambellotti, A. Giachetti, P. Turano and A. Rosato. *Investigation of the iron(II) release mechanism from human ferritin as a function of pH.*
12.05 – 12.20	CSB OR15: D. Capasso, S. Di Gaetano, V. Celentano, D. Diana, L. Festa, R. Di Stasi, L. De Rosa, R. Fattorusso and L. D. D'Andrea. *Unveiling a VEGF-mimetic peptide sequence in IQGAP1 protein.*
12.20 – 12.35	CSB OR16: M. Scognamiglio and B. Schneider. *Metabolomics studies of allelopathy: unravelling chemical interactions between Mediterranean plants through an omics approach.*

12.35 – 12.50	CSB OR17: A. P. Falanga, N. Borbone, S. D'Errico, B. Pinto, M. Marzano, G. Piccialli and G. Oliviero. *Targeting of the G-quadruplex-forming bcl2G4-1 region in the human Bcl-2 gene with Peptide Nucleic Acid: an anti-gene approach for cancer treatment.*
12.50 – 13.05	CSB OR18: M. De Zotti, A. Bortolotto, I. Elmaghraby, L. Sella, F. Favaron. *Peptaibols: naturally occurring peptides as biopesticides.*

13.05 - 14.00	Intervallo Pranzo

	Sala Paestum B
14:00-15:00	*Sessione Poster 2 (CSB PO01 – CSB PO20)*

Premi
della Divisione di Chimica dei Sistemi Biologici

Premio Italfarmaco

Sara Sattin, Università degli Studi di Milano

Towards unconventional therapeutic approaches

Sara Sattin

Università degli Studi di Milano, Dipartimento di Chimica, via Golgi, 19, 20133, Milano

Complex and often multifactorial diseases, such as cancer and cystic fibrosis, but also apparently simpler microbial infections, represent an increasing burden for our society and it is becoming clear that they are increasingly difficult to control with traditional therapeutic approaches. Thus, in many areas of medicine, unconventional approaches are currently being sought out to overcome this problem.

One example of unconventional thinking consists of the idea that modulation, rather than straightforward inhibition, of hub proteins, such as the chaperone Hsp90, may represent a promising approach to achieve selective control of complex signalling pathways.(1) In a collaboration with the Colombo group, fine-tuning of Hsp90 dynamics, resulting in activation of its ATPase activity, was achieved with a family of benzofuran derivatives designed to act as allosteric modulators of the chaperone. These molecules have shown promising downstream effects.(2,3,4)

In a second example, inhibition of the first step of microbial infections,(5) *i.e.* pathogen adhesion to the host, was studied as a complementary approach to classical anti-infective agents. Anti-adhesion therapy allows clearance of the infective agent while exerting minimal selective pressure, a very important feature in order to avoid the insurgence of antimicrobial resistance. DC-SIGN is a human dendritic cells receptor that act as an adhesion factor by recognizing highly mannosylated glycoproteins present on several pathogens. Some of them (*e.g.* HIV-1, Ebola, measles, *C. albicans*, etc.) exploit this otherwise protective mechanism for host adhesion and invasion. Inhibition of DC-SIGN with aptly designed polyvalent ligands leads to effective inhibition of the infection process.(6)

References: 1. Sattin, S.; Tao, J.; Vettoretti, G.; Moroni, E.; Pennati, M.; Lopergolo, A.; Morelli, L.; Bugatti, A.; Zuehlke, A.; Moses, M.; Beebe, K.; Rusnati, M.; Neckers, L.; Zaffaroni, N.; Agard, D.A.; Bernardi, A. and Colombo, G. *Chem. Eur. J.*, 2015, *21*, 13598-13608. 2. Sattin, S.; Panza, M.; Vasile, F.; Berni, F.; Goti, G.; Tao, J.; Agard, D.; Colombo, G. and Bernardi, A.*Eur. J. Org. Chem.* 2016, *2016(20)*, 3349-3364. 3. Vettoretti, G.; Moroni, E; Sattin, S.; Tao, J.; Agard, D.A.; Bernardi, A. and Colombo, G. *Sci. Rep*. 2016, *6*, 23830. 4. Bagdany, M.; Veit, G.; Fukuda, R.; Avramescu, R.G.; Okiyoneda, T.; Baaklini, I.; Singh, J.; Sovak, G.; Xu, h.; Apaja, P.M.; Sattin, S.; Beitel, L.K.; Roldan, A.; Colombo, G.; Balch, W.; Young, J.C.; Lukacs, G. L. *Nat. Commun.* 2017, *accepted*. 5. Sattin, S. and Bernardi, A. *Trends Biotechnol.* 2016, 34(6), 483-495. 6. Mauro, N.; Ferruti, P.; Ranucci, E.; Manfredi, A.; Berzi, A.; Clerici, M.; Cagno, V.; Lembo, D.; Palmioli, A. and Sattin, S. *Sci. Rep.* 2016, *6*, 33393.

Conferenze Plenarie

- CSB PL01: Maroney M.J., University of Massachusetts
- CSB PL02: Tinè M. R., Università di Pisa
- CSB PL03: Fattorusso R., Università della Campania "Luigi Vanvitelli"
- CSB PL04: Sattin S., Università degli Studi di Milano
- CSB PL05: Montesarchio D., Università di Napoli "Federico II"
- CSB PL06: Ciofi Baffoni S., Università di Firenze

Bioinorganic chemistry from metals to enzymes: A nickel tour

Maroney M.J.[a,b], *Campeciño J.O.*[a], *Carr C.E.*[a], *Hu H.Q.*[b], *Huang H.T.*[a], *Musiani F.*[c] *and Ciurli S.*[c]

[a]*University of Massachusetts, Department of Chemistry, Amherst, MA USA 0100;*
[b]*University of Massachusetts, Graduate Program in Molecular and Cellular Biology, Amherst, MA USA 01003;*
[c]*Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, Via Fanin 40, I-40127 Bologna, Italy*

Since the discovery of nickel in jack bean urease ca. 1975, the biological roles for nickel have increased to encompass enzymes that catalyze at least eight different chemical reactions (1). Perhaps the most surprising of these are redox reactions associated with hydrogenase, CO-dehydrogenase, acetyl-coenzymeA synthase, and a nickel-dependent superoxide dismutase, NiSOD, all of which access the Ni(III) oxidation state—an oxidation state that is unstable for Ni ions in an aqueous environment. NiSOD offers the clearest view of Ni redox chemistry, since it contains no other metal ions. The Ni center employs two cysteinate ligands and three types of N-donor ligands—a histidine imidazole, a backbone amidate, and the N-terminal amine. Using a structure/function approach, the roles for these ligands in generating a redox-active Ni center will be discussed, and reveals a surprising role for the amine/amidate combination in stabilizing the desired redox chemistry (2). To supply Ni for enzymes, microorganisms have developed trafficking systems that generate specific biological responses to Ni-binding.

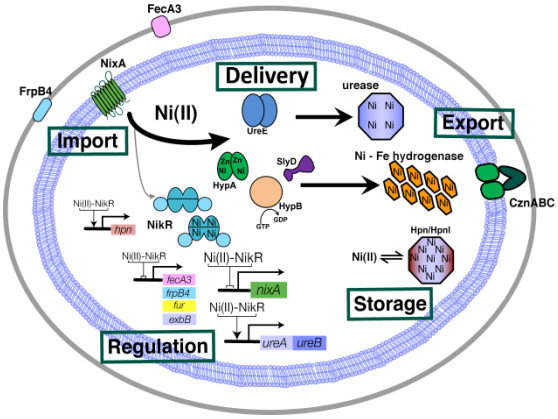

Figure 1. Overview of Ni Trafficking in *H. pylori*

These proteins include importers, exporters, transcriptional regulators, and metallochaperones, each of which must discriminate Ni(II) from the pool of available metals in the cell. The mechanisms of metal discrimination in Ni trafficking proteins will also be explored. The studies reveal the importance of the N-terminal amine in the complexes of RcnR (3), a transcriptional regulator in E. coli, and in H. pylori HypA (4), an unusual metallochaperone in this human pathogen.

References: 1. M. J. Maroney, S. Ciurli Chemical Reviews (2014), 114, 4206. 2. 2. J. O. Campeciño, M. J. Maroney, In The Biological Chemistry of Nickel; Zamble, D., Rowinska-Zyrek, M., Kozlowski, H., Eds.; The Royal Society of Chemistry: (2017) 10, 170. 3. K. A. Higgins, P. T. Chivers, M. J. Maroney, J. Am. Chem. Soc. (2012), 134, 7081. 4. H. Q. Hu, R. C. Johnson, D. S. Merrell, M. J. Maroney Biochemistry (2017,) 56, 1105.

Calorimetry and Thermoanalytical Techniques in the Study of Proteins

Maria Rosaria Tiné.

Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via G. Moruzzi 13, 56124- Pisa

Thermal analysis and calorimetry, particularly when combined with other analytical and spectroscopic techniques, offer powerful methods for studying biological macromolecules. Here, we present and discuss the application of calorimetry and thermogravimetric analysis to the study of the conformational behavior of proteins in three cases belonging to very different fields, ranging from the use as binders in tempera paintings, to medical or pharmaceutical applications.

The first issue concerns with proteinaceous materials used as paint media in order to disperse and apply pigments. Over the centuries, animal glue, egg and milk or casein have been the most common proteinaceous binders used in tempera technique. The characterization of these paints is complex because of the sample size, the high inorganic content, the degradation phenomena undergone with time, and the simultaneous presence of other organic materials. We used a combined approach (Thermogravimetric Analysis, TGA, Differential Scanning Calorimetry, DSC, Fourier Transform Infrared Spectroscopy, FTIR) to investigate the interaction occurring between selected pigments and ovalbumin, casein, and rabbit glue as well as their changes with ageing. This allowed us to characterize the molecular modifications undergone by proteins as an effect of light ageing, and depending on the pigment, in terms of amino acid side chain oxidations, cross linking/aggregation, hydrolysis, and the formation of stable complexes. We highlighted that in most cases the inorganic pigments interact with proteins by decreasing their thermal stability and their intermolecular β-sheet content, and that ageing induces aggregation.

The second issue concerns with some biocompatible nanomaterials suitable to be used in biotechnological and medical applications. We focused our attention on alloysite nanotubes (HNTs) which are considered very promising as nanocarriers, because of their low cost, high availability, biocompatibility, atoxicity, anti-inflammatory properties, and capacity to maintain the biological activity of immobilized enzymes. HNTs can be loaded with a wide range of molecules, from antioxidants to antibiotics, anticancer, and anti-inflammatory drugs and can be used for drug delivery, as tablets and capsule fillers. Therefore, to study their interaction with proteins is important because of the general concern regarding the safety of nanoparticles and the modifications that loaded biological material may undergo with alteration of their biological functions. We studied the interaction between HNTs and some proteins (bovine serum albumin, α-lactalbumin and β–lactoglobulin) loaded into HTNs, by using TGA and FTIR. These techniques enable us to assess the protein conformation and thermal stability, respectively, and to estimate the amount of protein loaded into the HNTs.

Finally, as the third issue, we show here some preliminary results on the use of protein-polymer conjugates in order to improve the properties of therapeutic proteins. It is well known that proteins and peptides exhibit great potentialities as therapeutic agents; however, they also show severe drawbacks (low solubility in water, tendency to agglomerate during storage in solution, short shelf-life, rapid kidney clearance, destruction by proteolytic enzymes, propensity to generate neutralizing antibodies). One of the most promising modification for overcoming these drawbacks is the covalent attachment of synthetic polymers (the most common being PEG) to the protein, to form protein-polymer conjugates with the aim of improving both the stability and the pharmacokinetics properties of the drug. In particular, we present some preliminary results obtained by TGA and DSC measurements on Myoglobin-PEG and myoglobin-polyphosphoesters conjugates. In fact, polyphosphoesters (PPEs) are one of the most promising new classes of polymers in biomedicine.

Protein folding pathways investigated by NMR spectroscopy

Roberto Fattorusso.

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Protein folding represents one of the most intensively studied phenomena of recent times in biology, but nonetheless the molecular mechanisms by which a peptide chain reaches its native structure have not been yet fully understood (1,2). Importantly, understanding protein folding pathways plays an essential role in the comprehension of many diseases rooted in the protein misfolding processes, also considering that every functional protein is permanently in equilibrium with its unfolded state (3). As a matter of fact, evolutionary selection favoured protein structures characterized by folding pathways preventing the formation of uncontrolled protein misfolded states, which in some peculiar conditions, either pathological or physiological, may anyhow take place.
Here, the investigation, by means of NMR and other peculiar methodologies, of protein folding mechanisms, which may help in the comprehension of misfolding molecular processes, will be described.

References : 1. S. E. Jackson *Fold. Des.* 3, R81-91 (1998). 2. S. Amani and A. Naeem *Int. J. Biol. Macromol*. 58, 104-112 (2013). 3. M. Sadqi, M, D. Fushman, & Muñoz *Nature* 442, 317-21 (2006).

Towards unconventional therapeutic approaches

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Multifunctional nanosystems for theranostics

Daniela Montesarchio.

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The development of multifunctional systems for potential applications in theranostic nanomedicine, aimed at simultaneously providing the diagnosis and treatment of a disease, is one of the hottest fields in current biomedical research.[1] For the construction of these tools, different kinds of nanosystems can be selected, decorated with multiple functional units (ranging from small bioactive molecules to targeting ligands) using various anchoring methods, and investigated as optimal scaffolds for the *in vivo* delivery of drugs and imaging agents.[2] Following this general strategy, the nanoplatforms loaded with both therapeutic and diagnostic agents can be mere carriers, in some cases, or introduce further functional activities, in others.

In this frame, a small library of multifunctional nanoparticles, differing for the nature of the nanoparticle core/coating and of the active ingredient, will be here presented.

In light of the chosen approach, the active functional agents are *ad hoc* designed and synthesized with suitable tethers so to allow their efficient incorporation into the selected nanocarrier (*i.e.*, streptavidin-coated silica nanoparticles, superparamagnetic nanoparticles or liposomes), thus exploiting different recognition schemes (*i.e.*, selective recognition, hydrophobic or electrostatic interactions).

Data on the *in vitro* activity of these multifunctional nanoparticles as theranostic agents towards specific pathologies, such as cancer and clotting disorders, will be also discussed.

References: 1. T. Lammers *et al*., *Acc. Chem. Res*. 2011, *44*, 1029-1038. 2. For a recent review, see for example: E.-K. Lim *et al*., *Chem. Rev*. 2015, *115*, 327−394. 3. G. Mangiapia *et al*., *Biomaterials* 2012, *33*, 3770–3782. 4. G. Mangiapia *et al*., *Biomacromolecules* 2013, *14*, 2549-2560. 5. G. Vitiello *et al*., *J. Mater. Chem. B* 2015, *3*, 3011–3023. 6. A. Luchini *et al*., *Nanoscale* 2016, *8*, 10078-10086. 7. C. Riccardi *et al*., *Eur. J. Org. Chem.* 2017, 1100–1119. 8. C. Riccardi *et al*., manuscript under review.

Unravelling the molecular mechanisms of iron-sulfur protein maturation

Simone Ciofi Baffoni.

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Iron-sulfur (Fe-S) clusters have long been recognized as essential and versatile cofactors of proteins involved in catalysis, electron transport and sensing of ambient conditions. Despite the relative simplicity of Fe-S clusters in terms of structure and composition, their synthesis and assembly into apo proteins is a highly complex and coordinated process in living cells. Different biogenesis machineries in both bacteria and eukaryotes have been discovered that assist Fe-S protein maturation. Molecular mechanisms of Fe-S protein maturation will be presented showing how an integrated structural biology approach can fully describe the pathways responsible of Fe-S cluster synthesis and incorporation into apo proteins (1, 2, 3). The investigation of these mechanisms will undoubtedly enhance our ability to identify and treat known disorders of Fe-S cluster biogenesis and to recognize hitherto undescribed Fe-S cluster-related diseases.

References: 1. Banci L, Ciofi-Baffoni S, Gajda K, Muzzioli R, Peruzzini R, Winkelmann J. Nat Chem Biol. 2015, 11:772-8. 2. Banci L, Camponeschi F, Ciofi-Baffoni S, Muzzioli R. J Am Chem Soc. 2015, 137:16133-43. 3. Brancaccio D, Gallo A, Piccioli M, Novellino E, Ciofi-Baffoni S, Banci L. J Am Chem Soc. 2015, 137:16133-43.

Comunicazioni Orali

Biochemical and structural studies on the inhibition of urease, a nickel-dependent virulence factor

Luca Mazzei [a], *Francesco Musiani* [a], *Gabor Lente* [b], *Marta Palombo* [a], *Michele Cianci* [c], *Stefano Benini*[d] *and Stefano Ciurli* [a].

[a] *Dept. of Pharmacy and Biotechnology, University of Bologna (Italy);*
[b] *Dept. of Inorganic and Analytical Chemistry, University of Debrecen (Hungary);*
[c] *Dept. of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche (Italy);*
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Urease is a Ni(II)-dependent enzyme that catalyzes the hydrolysis of urea to give ammonia and CO_2, determining an overall pH increase and causing negative effects for human health as well as agriculture (1). Hence, the scientific community has devoted intense efforts in the last decades for the development of efficient inhibitors of urease able to counteract its negative effects (1).

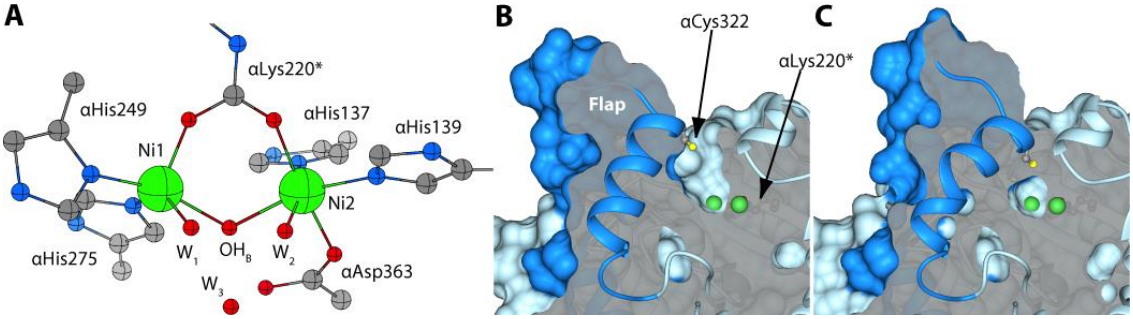

Figure 1. (A) Coordination environment of the Ni(II) ions in the active site of urease from *Sporosarcina pasteurii* (SPU). (B and C) Longitudinal section of the open (B) and closed (C) conformations of the flexible flap in SPU.

In this work, a combination of kinetic experiments and X-ray protein crystallography has been applied to the urease system in order to determine the inhibition mode of several known urease inhibitors: i) fluoride, ii) sulphite, iii) 1,4-benzoquinone (BQ), iv) catechol (CAT) and v) N-butylthiophosphotriamide (NBPT). Both fluoride and sulphite show a pH-dependent inhibition on urease, directly binding to the two Ni(II) ions in the enzyme active site (2,3). Unlike the previous cases, BQ and CAT act as time-dependent urease inhibitors covalently binding to a conserved cysteine residue located on a flexible flap that controls the access of the substrate to the active site cavity (4,5). NBPT, a commercial nitrogen stabilizer extensively used in agriculture, acts as a slow-binding inhibitor of urease. In particular, it directly interacts with the nickel ions in the urease active site, undergoing an *in situ* hydrolysis that generates a tetrahedral moiety blocking the active site and precluding the enzyme from further substrate hydrolysis (6).
All the results shown in this work will be useful to develop, through a structure-based drug design procedure, novel and more efficient urease inhibitors.

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Structural and mechanistic insights into iron processing and biomineralization by vertebrate ferritins

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Ferritins are ubiquitous multimeric protein systems showing a nanocage structure able to include thousands of iron atoms as oxoferric biomineral. In mammals, these twentyfour-mer protein shells are generally heteropolymers composed by two different types of subunits classified, according to their relative molecular weight, as heavy H and light L (183 and 175 amino acids, respectively, in human chains). The relative ratios between the two types in heteropolymers is tissue-dependent: ferritins in iron storage organs (e.g. liver and spleen) are richer in L-subunits, while those with fast iron metabolism (e.g. brain and heart) are richer in the H type. The H-subunit contains a ferroxidase center characterized by the so-called Fe1 and Fe2 sites and able to rapidly oxidize Fe^{2+} to Fe^{3+}. Besides the high structural conservation of the catalytic center, the mechanism by which the ferroxidase reaction occurs is not fully understood and different models have been proposed. We have developed a soaking/flash freezing method to allow aerobic and anaerobic addition of iron(II) to frog and human ferritin crystals (1,2,3) Multi-wavelength anomalous diffraction data have been exploited to unambiguously detect iron atoms. Through this method we have observed for the first time the iron binding sites in X-ray crystal structures of vertebrate ferritins and how they become populated with time (1,2,3). Interestingly, accessories transient metal sites have also been identified in the proximity of the ferroxidase site and demonstrated to play a key role in the reaction turnover (3,4). On the other hand, L-subunits lack the ferroxidase site, and hence iron incorporation in nanocages rich in L-chains is much slower. Nevertheless, homopolymeric L-ferritins are able to biomineralize iron. The proposed mechanism involves the presence of a putative nucleation site on the inner cage surface (5). Through a time-dependent series of X-ray crystal structures of iron-loaded homopolymeric human L-ferritin we have observed the progressive formation of a triiron cluster on the inner cage surface of each subunit (6). After 60 minutes exposure, a fully formed (μ^3-oxo)tris[(μ^2-peroxo)(μ^2-glutamato-κ*O*:κ*O*')](glutamato-κ*O*)(diaquo)triiron(III) anionic cluster was clearly visible in a structure determined at 1.98 Å resolution. The functional significance of the protein carboxylates involved in the coordination of the metallocluster for biomineralization was clearly demonstrated by the lower iron oxidation rate measured in the E60A-E61A-E64A triple variant of human L-ferritin. A similar metallocluster was also observed in the lower resolution (2.22 Å) structure of horse spleen ferritin, suggesting that it constitutes a common feature of mammalian ferritins representing the yet unobserved, nucleation site of L-type proteins. This cluster structure is unprecedent in biological systems even though it shows striking structural similarities to a synthetic hexanuclear iron cluster reported about 20 years ago and proposed as possible model of a ferritin biomineral (7). Structural data, together with stopped-flow kinetic data, provide new clues to explain the ferroxidase and biomineralization processes in vertebrate ferritins.

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Exploiting conformation and structural analysis of endogenous miRNAs to refine gene targeting evaluation

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Mature microRNAs (miRNAs) are a class of evolutionally conserved, single-stranded, small (approximately 19−23 nucleotides), endogenously expressed, and non-protein-coding RNAs that act as post-transcriptional regulators of gene expression in a broad range of animals, plants, and viruses.(1,2) The biogenesis of miRNAs is a multiple step process, which complete with the incorporation of the mature miRNA into RNA-induced silencing complex.(3) The RISC complex functions by perfectly or imperfectly matching with its complementary target mRNA, and induces target mRNA degradation or translational inhibition. Thus, alterative expression of miRNAs has been associated with a number of diseases, genetic disorders and tumors progression.(3)
We think that the knowledge of the miRNA structure may give a new insight into miRNA-dependent gene regulation mechanism and be a step forward in the understanding their function and involvement in cancerogenesis. With this aim we characterized the conformation and structures adopted by several endogenous miRNA in physiological conditions. Preliminary data obtained by CD melting experiments, using synthetic miRNA,(4) highlighted the important role played by the structures adopted by miRNA. Indeed the sequences showed a sigmoidal CD melting curves induced a significant inhibition of the luciferase activity for two of the most prominent genes associated to lung cancer, c-MET and Epidermal Growth Factor Receptor (EGFR).

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Identification and characterization of DNA G-quadruplex interacting proteins

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Guanine-rich DNA sequences can form non-canonical structures known as G-quadruplexes (G4s). These peculiar structural arrangements emerged as biologically significant due to compelling evidence that they participate in several biological processes. Experimental evidences imply that G4 DNA structures are, for example, involved in tumorigenic processes, probably with regulatory functions, and that various proteins are involved in the recognition of these structures and modulate their effect on such processes.

The analysis of the G4–protein interaction network can be considered a crucial point to clarify the elusive biological mechanisms in which such relevant DNA structures could be implicated. Some proteins are able to recognize G4 structures and some are also able to unfold them. The discovery of these proteins raises interesting questions regarding the dynamic nature and function of such structures within the genome, especially at telomere, a region of repetitive G-rich sequences at each end of chromosomes. In this frame, we decided to search for proteins able to recognize G4-forming truncations of human telomeric DNA sequence (1). In particular, we employed a chemoproteomic-driven approach, where the molecule of interest is used as a bait to fish out its interactors from nuclear extracts. In particular, we have used different G4 conformations, namely the parallel and the anti-parallel folds. Very interestingly, novel G4-interacting partners were identified, thus suggesting a possible, and so far unknown, role of these proteins. In this communication, the latest results will be presented, including a preliminary structural study of the interaction between the HMGB1 protein and the parallel telomeric G4 structure.

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Insights in self-recognition, misfolding and mislocalization mechanisms of Nucleophosmin 1 in Acute Myeloid Leukemia

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In protein misfolding processes the amyloid fibrillization is a unique ordered state governed by specific patterns of molecular interactions (1). Normally folded proteins can access to amyloidogenic states that are often considered as an ensemble of native-like conformations with locally unfolded elements. The characterization of these amyloidogenic species is crucial to elucidate potential aggregation under native conditions and for *in vivo* aggregation events (2). Nucleophosmin (NPM1) is a multifunctional protein involved in a variety of biological processes and implicated in the pathogenesis of several human malignancies, it was also identified as the most frequently mutated gene in 30% of Acute Myeloid Leukemia (AML) patients. This protein is endowed with a modular structure: the C-terminal domain (CTD) has a three helix bundle tertiary structure: H1 (243-259), H2 (264-277) and H3 (280-294) helices constitute the CTD and fold through a compact transition state and unfolds keeping a residual secondary structure at the interface between H2 and H3 helices (3). To gain insights into the role of isolated fragments in NPM1's biological functions we dissected the CTD in its helical fragments: we showed that the intrinsically unfolded regions of NPM1 significantly contribute to the binding of c-MYC G-quadruplex motif and that H1 helix is endowed with an unusual thermal stability (4). Lately we demonstrated that the H2 (5,6) and H3 AML mutated regions (7) form amyloid-like assemblies endowed with fibrillar morphology and β-sheet structure that resulted toxic in cell viability assays. Actually, our mechanistic hypothesis is that the AML-associated mutations destabilize the α-helical structure of the H3 region in the native NPM1 and disrupts the CTD tertiary structure predisposing it to the formation of toxic aggregates since it induces the exposure of the H2 and H3 regions. These findings could have implications in AML molecular mechanisms caused by NPM1 mutants.

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The molecular recognition mechanism of the coactivator NCoA-1 by STAT6

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STAT6 belongs to a family of transcription factors known as the signal transducers and activators of transcription (STAT). STAT family members share a similar protein structure, which is essential for their activation and function. STAT proteins mediate signaling from activated cytokine receptors to the nucleus(1). After phosphorylation at a specific tyrosine by a receptor associated Janus kinase, STATs form homo- or heterodimers and translocate into the nucleus where they modulate transcription by specific DNA sequence elements (1).STAT6 becomes activated in response to IL-4 and IL-13 and mediates most of the gene expression regulated by these cytokines. By direct interaction with specific parts of its transactivation domain, STAT6 recruits the co-activators p300/CDP and NCoA1 (also called steroid receptor coactivator-1, SRC-1), which are essential for transcriptional activation by IL-4 (2). In particular, the interaction between STAT6 and NCoA1 is modulated by a short region of the transactivation domain that includes the motif LXXLL (where X is any amino acid). The crystal structure of a STAT6-derived peptide (Leu^{794}-Gly^{814}) in complex with the NCoA1 PAS-B $domain^{257-385}$ revealed that the Leucine side-chains of the motif (Leu^{802}, Leu^{805} and Leu^{806}), are deeply embedded into a hydrophobic groove of the surface of NCoA1 (3). More recently, it has been demonstrated by a fluorescence polarization binding assay that additional residues (Leu^{794}, Pro^{797} and Thr^{798}), flanking the LXXLL motif in STAT6, play an important role in stabilizing the protein binding to NCoA1 (4). Here, we report the structural characterization of the complex between a STAT6-derived peptide encompassing the region from Gly^{783} to Gly^{814} and the NCoA1 PAS-B $domain^{257-385}$ using Nuclear Magnetic Resonance (NMR) and X-ray crystallography. The structural characterization of the $STAT6^{783-814}$/$NCoA1^{257-385}$complex demonstrates that $STAT6^{783-814}$peptide binds the NCoA1 PAS-B $domain^{257-385}$ by additional amino acid interactions from its N-terminal region resulting in a more extended binding interface with NCoA1 compared to that identified before in the crystal structure with the $STAT6^{794-814}$ peptide.

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How can work methanol dehydrogenase from Methylacidiphilum fumariolicum with the alien Ce(III) ion in the active center? A theoretical study

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La metanolo-de-idrogenasi (MDH), enzima appartenente alla classe delle ossidoriduttasi, è una metallo-proteina, generalmente calcio-dipendente, in grado di catalizzare efficientemente l'ossidazione del metanolo ed altri alcol primari.
Recentemente, una metanolo-deidrogenasi (MDH) contenente uno ione cerio, nel sito attivo, è stata isolata dal batterio *Methylacidiphilum Fumariolicum* (1). Con l'obiettivo di fare luce su come la sostituzione del metallo può influenzare il meccanismo catalitico, è stato effettuato uno studio teorico DFT comparativo tra i due metallo-enzimi, calcio e cerio dipendenti (rispettivamente, Ca-MDH e Ce-MDH).
La Superficie di Energia Potenziale (PES) ottenuta mostra come entrambi i metallo-enzimi preferiscano un meccanismo di addizione-eliminazione-protonazione; la barriera dello stato limitante la reazione per la Ce-MDH è stata calcolata pari a 19.4 kcal/mol.

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Aminoproline-RGD functionalized gold nanoparticles for targeting of integrins involved in tumor angiogenesis

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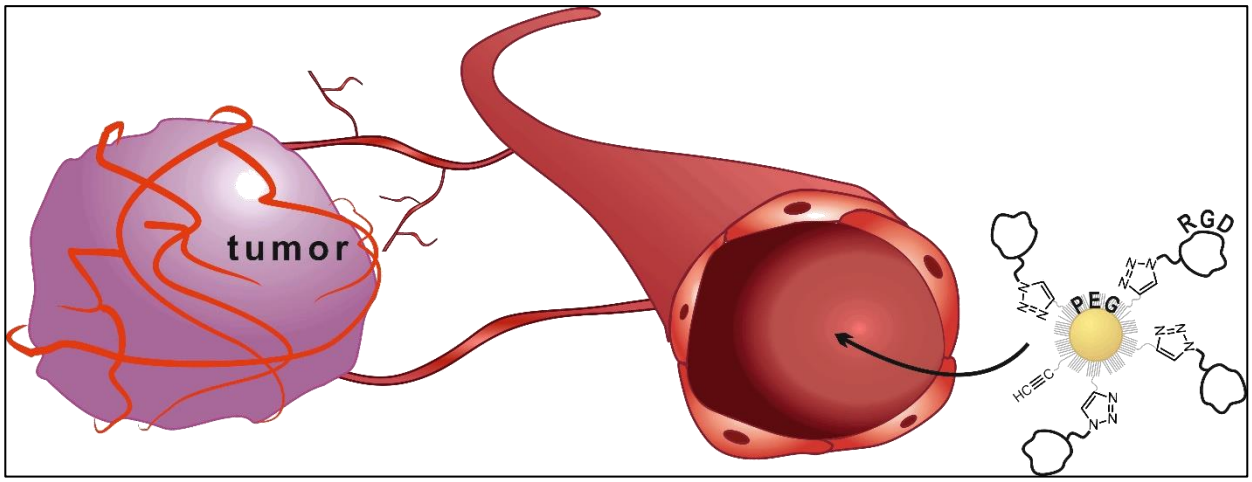
Integrin $\alpha_v\beta_3$ is a cell-adhesion molecule involved in angiogenesis, tumor invasion and metastasis which is overexpressed by tumor cells as well as by the endothelial cells of tumor neovasculature. The most potent $\alpha_v\beta_3$ ligands are cyclic peptides containing the RGD sequence. Ligand affinity can be further enhanced by utilizing multivalent scaffolds. Gold nanoparticles (AuNPs) could be ideal platforms for the multivalent presentation of RGD peptidomimetics targeting $\alpha_v\beta_3$ integrins aiming at theranostic formulations for tumor diagnosis and treatment.

We are developing novel PEGylated AuNPs (1) functionalized with multiple copies of cyclic-aminoproline RGD peptides (cAmpRGD) (2) which show high affinity and selectivity for $\alpha_v\beta_3$ integrins.

In this contribution we will discuss the molecular design, the preparation and characterization of these nanoparticles. Furthermore, we will show their excellent targeting properties from data collected using the human melanoma cell line M21 which overexpress integrin $\alpha_v\beta_3$. In fact, cAmpRGD-AuNPs target M21 cells 4 times more efficiently than control AuNPs as demonstrated by ICP-OES, selectively inhibit cellular adhesion to vitronectin (the natural, RGD containing ligand) by 50% at 1 nM concentration, and do not show any significant toxicity at concentrations as high as 10 nM after 24 h as demonstrated by Annexin V/ PI staining assays. We will also present bifunctional AuNPs functionalized with both cAmpRGD and a fluorophore useful in confocal microscopy imaging. cAmpRGD-AuNPs are expected to have great potential as novel multimodal (e.g. microSPECT/CT) tracers for diagnostic imaging (3).



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Functionalized cyclodextrins as modulators of Aβ cytotoxicity

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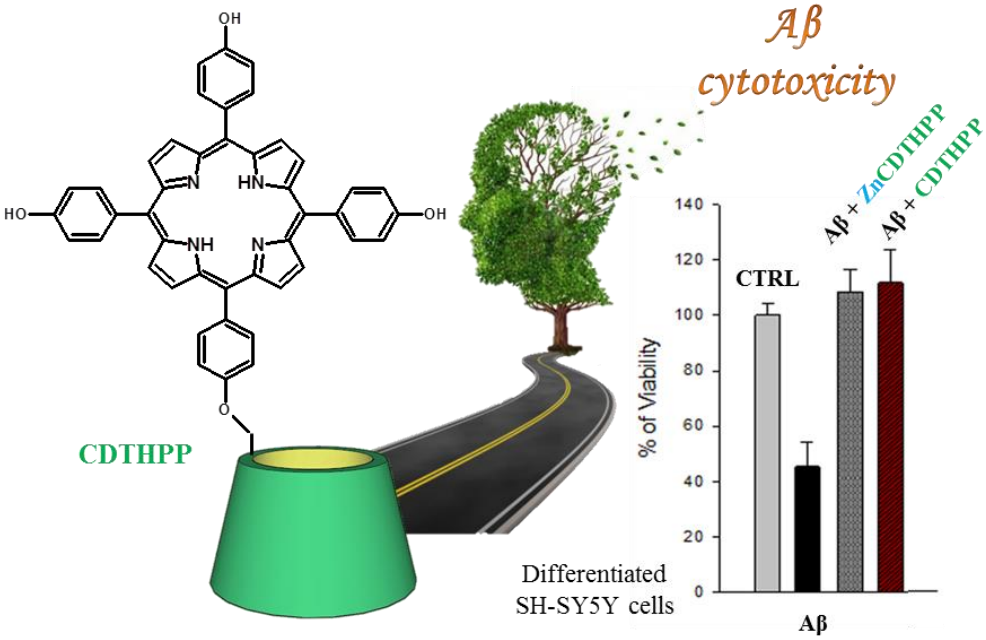
Aggregation of Amyloid-beta (Aβ) is one of the crucial events occurring during Alzheimer's disease (AD). Preventing or reducing aggregation and cytotoxicity of Aβ is one of therapeutic strategies under development or in clinical trials. Numerous studies have shown that sugars such as cyclodextrins (CyDs) provide neuroprotection in AD (1). Moreover, we have recently reported that the conjugation of cyclodextrins with aromatic moieties could provide a new avenue to the identification of novel and important modulators of Aβ aggregation (2,3,4,5). Herein, we show that a cyclodextrin compound bearing a porphyrin moiety and its zinc complex are effective in suppressing Aβ cytotoxicity. We tested the ability of the cyclodextrin-porphyrin conjugate (CDTHPP) and its zinc complex (ZnCDTHPP) to affect the toxicity of Aβ oligomers in differentiated neuroblastoma cells (SH-SY5Y). We also studied in parallel the parent compounds of the conjugates, β-cyclodextrin (CD) and 5,10,15,20-tetra(4-hydroxyphenyl)porphyrin (THPP), to demonstrate that the conjugate activity against Aβ toxicity could arise from the synergy of the THPP and CD properties.
Dot Blot analysis, UV-vis, circular dichroism, dynamic light scattering and high-performance liquid chromatography-mass spectrometry studies were performed to investigate the nature of interaction between Aβ and the porphyrin-cyclodextrin conjugates. Finally, we took advantage of the intrinsic fluorescent properties of the derivatives to verify the cell internalization of these systems. Overall, the conjugation with cyclodextrins may be a new avenue for modulating Aβ cytotoxicity.



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Specific secondary interactions between ubiquitin and UBA observed in cell-mimicking crowded solution

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Despite significant advancements in our understanding of ubiquitin-mediated signaling, the influence of the intracellular environment on the formation of transient ubiquitin-partner complexes remains poorly explored(1,2). In our work, we introduce macromolecular crowding as a first level of complexity toward the imitation of a cellular environment in the study of such interactions (3). Using NMR spectroscopy, we find that the stereospecific complex of ubiquitin and the ubiquitin-associated domain (UBA) is minimally perturbed by the crowding agent Ficoll. However, in addition to the primary canonical recognition patch on ubiquitin (4), secondary patches are identified, indicating that in cell-mimicking crowded solution, UBA contacts ubiquitin at multiple sites.

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ZnTPPS demetallation: role of polyelectrolytes on aggregation after protonation in acid

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Porphyrins are very versatile compounds whose chemical-physical properties can be tuned through a careful choice of peripheral substituent groups, which are responsible of their aggregation state.(1) In particular, in the case of water-soluble porphyrins, the spatial arrangement of chromophores, through non-covalent intermolecular interactions, can be conveniently controlled acting on medium properties such as charge repulsion, ionic strength and pH. Although the self-assembly of meso-benzyl sulfonated porphyrin is mainly driven by protonation of the porphyrin core, the presence of a metal in the porphyrin core can strongly influence the aggregation pathway.(2,3)
ZnTPPS is one of the most studied metalloporphyrins, because of the importance of zinc in biology, but also for its application in photocatalysis. Therefore, the study of its demetallation and aggregation tendency is fundamental to better understand the control of porphyrin stability (in acid media) in photocatalysis, sensing, DSSC, photodynamic therapy and many other bio- and nanotechnological fields.
Herein we investigate the behaviour of ZnTPPS at different pH values and, in particular, we study ZnTPPS demetallation(4,5) in aqueous solution and related aggregation of the demetallated/protonated forms (H_2TPPS^{4-}, H_4TPPS^{2-}) in presence of anionic and cationic polyelectrolytes. In this respect the interactions with Poly-D-Glutamate (PDG) and Poly-L-Lysine (PLL), modulated by system electrostatics and by the presence of axially coordinated central metal, trigger both protonation occurrences as well as porphyrin self-aggregation.
Herein, a detailed spectroscopic analysis in order to better understand the crucial role of electrostatic interactions experienced by metallated inner core in strong acid solution has been performed. The obtained results point to a significant role of polylysine on overall demetallation/protonation process: in fact, this cationic polypeptide makes less accessible the metal core of the porphyrin, involving a slower demetallation process, but at the same time it catalyzes formation of J-aggregates (Fig.1).

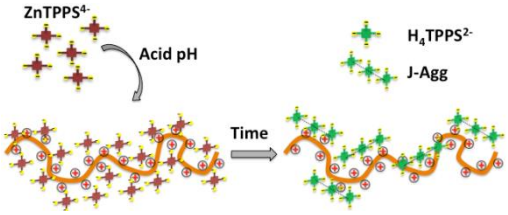

Fig.1: Templating action of the PLL on the J-aggregate formation from ZnTPPS in acidic conditions

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Synthesis, Separation and Characterization of Small and Highly Fluorescent Nitrogen-Doped Carbon NanoDots

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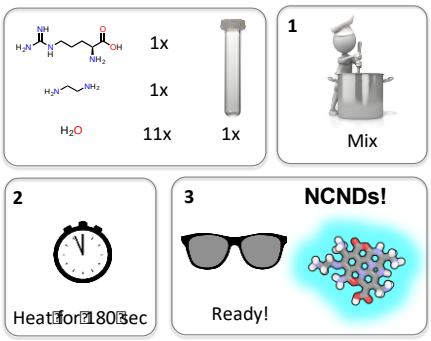
Extensive effort has been devoted to obtain non-toxic fluorescent nanomaterials, as an alternative to the popular semiconductor-based quantum dots (QDs). Carbon nanodots (CNDs) are recently discovered nanocarbons that comprise discrete, quasispherical nanoparticles with size below 10 nm and they have gradually become a prominent new member of the nanocarbon family. Compared to traditional semiconductor QDs and organic dyes, photoluminescent CNDs are potentially superior in terms of biological properties, high (aqueous) solubility, robust chemical inertness, facile modification and high resistance to photobleaching. A number of different synthetic protocols have been developed and reported (1). However, the structure and size of CNDs are still difficult to control. Therefore, a simple and cost-effective process, giving high quality and homogeneous nanodots remains a challenge.

Herein, we report a facile bottom-up approach to carbon nanodots (CNDs), using a microwave-assisted protocol under controlled conditions (2). Amino acids have been used as precursors due to their abundant, inexpensive, biocompatible and eco-friendly nature. The as-prepared nitrogen-doped CNDs (NCNDs) show narrow size-distribution, abundant surface traps and functional groups, resulting in tunable fluorescent emission and excellent solubility in water. Moreover, we present a general method for the separation of NCNDs by low-pressure size-exclusion chromatography, leading to an even narrower size distribution, different surface composition and optical properties. They display among the smallest size and the highest FLQYs reported so far. ^{13}C-enriched starting materials produced N^{13}CNDs suitable for thorough NMR studies, which gave useful information on their molecular structure. Moreover, they can be easily functionalized and can be used as water soluble carriers. This work provides a new avenue to size, surface controllable, structurally defined CNDs towards tailored properties for specific applications (3,4).



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Peptide Targeted Gold Nanostructures for high effective SERRS Imaging of Colorectal Cancer Cells

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Plasmonic nanoparticles are increasingly utilized in biomedical applications including imaging, diagnostics and therapy. Gold nanoparticles (AuNP), besides displaying useful optical properties, possess a facile surface chemistry and absence of inherent toxicity, an essential requirement for biological application. AuNP can passively target tumors by the enhanced permeability and retention effect, but active targeting by proteins (antibodies), peptides or small molecules, can further improve the pharmacokinetic and pharmacodynamics profiles of these multifunctional agents. The dodecapeptide YHWYGYTPQNVI (GE11) was recently identified as a specific ligand for the Epidermal Growth Factor Receptor (EGFR), which is overexpressed in many types of cancer (1). In the present work we have employed the enormous sensitivity of the Surface Enhanced Raman Resonance Scattering (SERRS) spectroscopy (2) to study the targeting activity of GE11-functionalized plasmonic nanostructures on different types of tumor cells. Nanoparticles were prepared, without stabilizing molecules, by laser ablation of a gold target in water, functionalized with a SERRS reporter (3), and conjugated with a number of ligands: mPEG, GE11 and different PEG-GE11 conjugates to study different aspects that can influence EGFR recognition. Full characterization of the nanostructures was performed by a combination of different techniques (TEM, UV-vis-NIR, DLS, Raman, etc). Nanoaggregates covered with PEG or with the monoclonal antibody Cetuximab were used as negative and positive control, respectively. Targeting of nanostructures to colorectal cancer cells, expressing or not EGFR, was checked recording the SERRS signals cell by cell, following incubation with the AuNP. The overall results show that a proper presentation of the targeting peptide on the surface of AuNP is very important to achieve high selectivity and sensitivity in colorectal cancer cells detection, and that the activity can be greater than that obtained using a specific antibody as targeting unit. This finding opens interesting perspectives in cancer theragnostic where the intense and characteristic signals of SERRS reporters can be used for tumor imaging and the strong absorption in the NIR spectral region of AuNP clusters allows to undertake a photothermal therapeutic approach to beat the tumor (4).

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Investigation of the iron(II) release mechanism from human ferritin as a function of pH

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Human ferritins are 24-mer nanocage structures that self-assemble from 4-helix bundle subunits. The resulting structure has octahedral symmetry. The eight C3 channels are formed by three symmetry-related motifs, specifically helix α4-loop-α3, from as many chains. Furthermore, they have been identified as the entry points of iron(II) ions. Instead, the iron release mechanism in biomineralized ferritins is less characterized.

Our kinetic measurements showed a distinct pH dependence of iron release. Lowering the pH to 4.0 resulted in significantly faster discharge of iron, accompanied by an increase of the total amount of released ions. Our MD simulations provide a detailed atomic-level view of the mechanism of iron(II) release at pH 4, which occurs through the C3 channels.

Within each individual channel, two nearby rings formed by symmetry-related Asp and Glu sidechains define the binding site to which iron ions are rapidly attracted from the internal cavity by the electrostatic gradient. Before the iron ion actually reaches the C3 site, the Asp sidechains move apart thereby allowing it to get inside the channel, coordinated by three Glu sidechains. Subsequently, the iron ion switches from three to two glutamate sidechains coordination. These two glutamates are positioned below two histidine residues, close enough to generate a salt bridge with them (Figure 1). About 5 ns before the release of the iron ion, the lifetime of the hydrogen bonds becomes significantly longer, resulting in two strictly related effects. First, a partial compensation of the negative electrostatic charge of the carboxylates that coordinate the iron ions. Second, the reinforcement of the correlation between protein dynamics and the movement of the Glu sidechains, which in turn causes an increase of the distance between the Glu sidechains. These two synergic events weaken the interaction between the metal ion and the carboxylate oxygen atoms (Figure 1). Eventually, the iron ion escapes the C3 binding site and diffuses into the bulk solution.

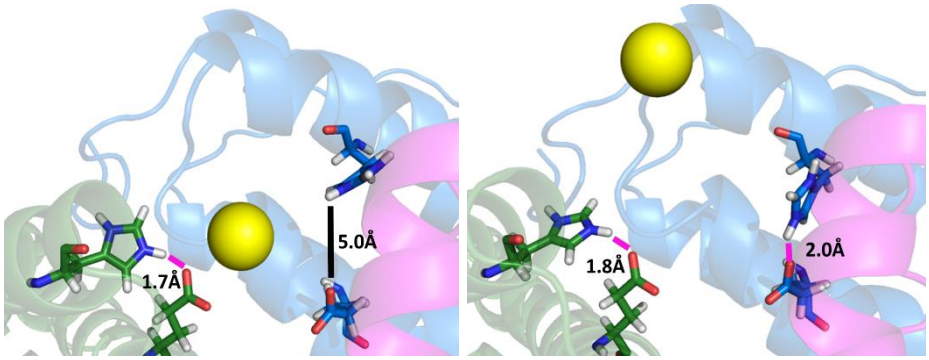

Fig. 1 Iron release after the formation of two Glu-His salt bridges.

Unveiling a VEGF-mimetic peptide sequence in IQGAP1 protein

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The ability to modulate angiogenesis by chemical tools has several important applications in fields such as medicine, biology, biomaterial science (1). In this context, vascular endothelial growth factor (VEGF) and its receptors have emerged as the main regulators of physiological and pathological angiogenesis. In the last years, much focus has been paid on the search of novel molecules with antiangiogenic activity and only few artificial proangiogenic molecules have been described. In 2005, we described a proangiogenic peptide, QK, which was designed on the N-terminal α-helix of VEGF (2). This peptide binds to and activates VEGFR2, regulates VEGF receptor and NP1 gene expression and shows a bioactivity spectrum similar to VEGF (3). In the perspective of finding novel proangiogenic molecules, we searched peptide sequences with a profile similar to QK. We found that residues 1617-1627 of the GTPase activating protein (IQGAP1) shows molecular features as QK peptide sequence. IQGAP1 is a protein that belongs to a ubiquitous expressed conserved family of scaffolins implicated in different biological processes and among the various binding partners described in literature, it was demonstrated that IQGAP1 associates with the intracellular domain of VEGFR2. In this work, we characterized the bioactivity and the structural properties of the IQGAP1-derived synthetic peptide. Structural and binding features of the peptide were analysed by means of NMR spectroscopy. The biological activity of the novel peptide was analysed on endothelial cells. The results showed that IQGAP1-derived synthetic peptide has an activity similar to VEGF and could be considered a novel tool for reparative angiogenesis.

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Metabolomics studies of allelopathy: unravelling chemical interactions between Mediterranean plants through an omics approach

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Allelopathy plays a very important role in natural and agricultural ecosystems and it has been suggested to have a great impact on vegetation of Mediterranean area (1). It is defined as any direct or indirect, harmful or beneficial effect of one plant on another through the production of chemicals released into the environment (2).
The understanding of this phenomenon has been partially constrained, among other things, by the methods available to study the secondary metabolites involved. A new method based on metabolomics has been recently developed (3, 4), and it is herewith applied to the study of allelochemicals from selected plant species of the Mediterranean region. Donor plant (*Arbutus unedo*, *Myrtus communis*, *Medicago minima* and *Daphne gnidium*) extracts were analysed by ^{1}H and 2D NMR in order to define their chemical composition. They were tested for their phytotoxicity on a receiving plant species (*Aegilops geniculata*). Morphological and metabolomics analyses were carried out on shoots and roots of *A. geniculata* plants treated with the extracts. Tests were carried out also with partially purified fractions and with the pure putative allelochemicals. The extracts of the four plant species showed a strong inhibitory activity on the receiving plant. NMR paired with multivariate data analysis of the receiving plant let to hypothesize the main metabolic pathways affected. Studies with the pure compounds confirmed in some cases the putative allelochemicals, while in other cases it was possible to determine the occurrence of synergistic effects. Some of the compounds were taken up and, in some cases, modified by the receiving plant.
The metabolomics approach proved to be a very useful tool for these studies.
Although phytotoxic activity is only one aspect of allelopathy, the identification of the active compounds lays the bases for in field studies, while the identification of the metabolic pathways affected by the allelochemicals offers new insights for the study of their mode of action.

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Targeting of the G-quadruplex-forming bcl2G4-1 region in the human Bcl-2 gene with Peptide Nucleic Acid: an anti-gene approach for cancer treatment.

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Human Bcl-2 gene contains GC-rich regions upstream of the P1 promoter that are involved in the regulation of Bcl-2 gene expression. In this region Guanine-rich DNA sequences fold in quadruplex structures under physiological concentrations of K^+. G-quadruplexes are found throughout significative regions of human genome, for example promoters of oncogenes, such as Bcl-2 of which, the abnormal overexpression is associated with many human tumours. Several oligonucleotides (ON) and ON analogues are employed as tools to counteract the expression of this oncogene. Among these the Peptide Nucleic Acids (PNAs) are the most promising. PNAs are mimic of DNA in which the back-bonds sugar-phosphate are replaced by a ethylamine glycine moiety, due these characteristic they can form, with DNA target, some structures more stable than the natural DNA/DNA complexes (1-6). To down-regulate anti-apoptotic Bcl-2 oncoproteins, here we propose an anti-gene approach based on PNA oligomers, allowing to target the bcl2G4-1 DNA sequence. Structural interactions towards the DNA target have been investigated by chemical-physical techniques. PNAs can interact with the DNA target, as shown by Circular Dichroism (CD), CD-melting and PAGE studies. Furthermore, the ability of these molecules to cross cell membranes has been studied. PNAs are able to elicit their action only when enter cell nuclei. Fluorescence microscopy also demonstrated that the suitably-FITC-labeled oligomers, specifically cross membrane and enter cell nuclei of tumor cells. These findings indicate the potential of novel PNA-based approach for the cancer therapy. This approach holds promise to improve a site specific and safe chemotherapy, reducing the unwanted toxicity to healthy tissues and organs.

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Peptaibols: naturally occurring peptides as biopesticides

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Fungi belonging to the genus *Trichoderma* are distributed worldwide and have been used successfully in field trials against many crop pathogens. They produce peptaibols, a peculiar family of peptides, as part of their defense system against other microorganisms. Such secondary metabolites are known for their plant-protection properties: they (i) possess antimicrobial activity, (ii) act as stimulants of plant defences and growth (iii) elicit plant production of volatiles to attract natural enemies of herbivorous insects. Moreover, peptides are ecofriendly compounds that are degraded by enzymes to nontoxic amino acids. With this presentation, we show our progress towards the exploitation of naturally occurring peptides of the peptaibols family as biopesticides. With such compounds, we can circumvent both the health hazards and the unreliable effectiveness in open field connected with the use of antagonistic microorganisms as biological control agents, while keeping the biomolecules responsible for their beneficial effects. Our peptides have been tested (alone or in combination) *in vitro* against the fungi *Botrytis cinerea* and *Penicillum italicum* and the bacterium *Pectobacterium carotovorum*, some of them considered priority pests for fruits, vegetables and medicinal plants across European countries. We found that an analog of the peptaibol trichogin is able to completely inhibit the growth of *B. cinerea* for over a week at low micromolar concentrations.

Comunicazioni Poster

PASTA sequence composition as a footprint of protein class identity

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PASTA domains are small modules expressed in bacteria and found in one or multiple copies at the C-terminal end of several Penicillin Binding Proteins (PBPs) and Ser/Thr protein kinases (STPKs) (1,2,3). Currently, the functional role of PASTA is not yet completely understood. At present, PASTA is annotated as a sensor domain. It has been proposed that its binding to opportune ligands, i.e. muropeptides, is able to activate the cognate proteins to their functions. However, some experimental data proved that such role might not be a general property of PASTA. Even though sharing identical folds, several studies made clear that PASTAs from different protein classes do not share the same functions. In reason of their capacity to bind cell wall fragments, PASTAs from STPKs were proposed as sensor domains for the cognate kinases. The same function associated to PASTAs from PBPs has been considered reliable until few years ago, when the binding ability *vs* cell wall fragments or their mimics did not pass the experimental verifications (4,5). For that, even though a sensor function cannot yet be discarded, it was hypothesized that PASTAs in PBPs might have a role as structure stabilizer (5,6).

A search in the PASTA PFAM family (PF03793) results in 11049 sequences, 79 architectures and 47 structures. Proteins containing PASTA domains are mostly distributed in Actinobacteria and Firmicutes, *phyla* that include some of the most dangerous microorganisms for human health like *Mycobacterium tuberculosis* (*Mtb*) and *Staphylococcus aureus* (*Sa*). Their external localization, the linking to enzymes essential for bacteria metabolisms and the fact that they are not expressed in eukaryotes, make PASTA interesting targets for new antibiotics drugs (1). PASTA domains, belonging or not to different protein classes, show wide ranges of sequences identities. Amino acid compositions, total charges and distribution of the hydrophobic/hydrophilic patches on the surface, significantly vary among PASTAs from STPKs and PBPs and seem to correlate with different functions, such as the ability/not ability to bind muropeptide (7). Here we show the analysis of amino acid composition performed on all PASTA sequences from PBPs and STPKs reported for Actinobacteria and Firmicutes in the PFAM database.

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Miniaturizing VEGF: Peptides mimicking the discontinuous VEGF receptor-binding site modulate the angiogenic response

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Angiogenesis is a physiological process during which new capillaries sprout from existing vessels but, when pathological, it contributes to the development of different types of tumors, and to the birth of metastases. VEGF, the main growth factors, plays a significant role in the the *angiogenic switch* (1) process. As part of this VEGF binds with high affinity two tyrosine kinase receptors Flt-1 and KDR (2). Three are the main interacting points of the VEGF with its own receptors: It consist in a discontinuous surface which comprises binding residues distributed in three regions belonging to both VEGF monomers (3): the N-terminal helix (residues 17-25), the loop joining strand β3 and β4 (residues 61-66) of one VEGF monomer, and the β-hairpin encompassing strand β5 and β6 (residues 79-93) of the other VEGF monomer. In order to find new active biomolecules which can modulate the interaction of VEGF discontinuous region (4) with their own receptors, we designed and synthesized a set of peptides mimicking the two secondary structure elements of VEGF α-helix (17-25) and β-hairpin (79-93) involved in the receptor recognition. The two linear amino acid segments were synthesized by solid-phase synthesis and conjugated by *native chemical ligation* (NCL) (5) through amino acid spacers of variable length and flexibility. They were analyzed by CD, NMR; LC-MS. Their biological activity has been investigated by *in vitro* and *in vivo* assays, highlighting a VEGF-like biological activity.

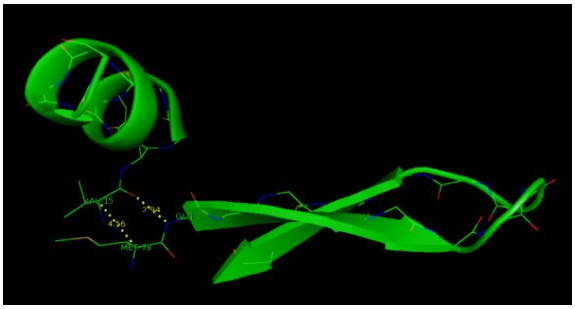

Figure 1: Molecular detail of VEGF structure highlighting the proximity between the a-helix (17-25) and the β-hairpin (79-91) of VEGF. Secondary structure element is represented as ribbon. The distance between the different aminoacids are reported in yellow

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Folding mechanisms steer amyloid fibrils formation propensity of prokaryotic zinc finger domains

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We describe the amyloidogenic propensities of three iso-structural globular proteins belonging to the prokaryotic zinc finger family (1) that possess different folding mechanisms. Particularly, the metal-free $Ml4_{52–151}$ (2) folds via classic two-state cooperative transition while the metal-binding homologues, Ros87 (2) and $Ml1_{53-149}$, exhibit more complex folding pathways, including a barrier-less downhill scenario. The results, obtained by CD and fluorescence spectroscopies, DLS, transmission and scanning electron microscopies, show that within 168 hours amyloid formation has already started in Ros87, while $Ml1_{53-149}$ has formed only amorphous aggregates and $Ml4_{52–151}$ is still monomeric in solution. Overall, this study shows how different folding mechanisms, here induced by metal binding, significantly affect amyloid fibril formation propensity of highly homologous proteins.

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Covalent Functionalization of Cotton Fabric with Antimicrobial Peptides: New Synthetic Strategies

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The onset of bacterial resistance is a worldwide problem, in particular in healthcare environments. The development of antimicrobial textiles could represent an important aid in the struggle against pathogens, bacteria and viruses and may find a variety of applications (biomedical garments, protections devices, etc,). The new materials are based on an ordinary fabrics functionalized with antimicrobial chemical species through appropriate treatments. The ultimate goal is to transfer the bioactivity of the molecule to the fabric in a durable way.
As therapeutic agents, we focused on a class of antimicrobial peptides characterized by a very short sequence, which counts just four amino acids, suitably designed to be active against certain bacterial strains. The strength of these sequences, in addition to the weighted choice of hydrophobic and cationic residues constituting the peptide sequence, stems from the presence of an aliphatic chain at one end of the peptide sequence. Once the peptide has anchored itself to the bacterial surface, by means of electrostatic interactions, such lipophilic moiety acts by disrupting the bacterial membrane.
In this presentation, we illustrate how we synthesized and anchored the above described peptides on a cotton support. Among the various strategies that can be tempted for peptide anchoring, we chose the chemoselective ligation, with the formation of an oxime bond. An aldehyde function was obtained on the peptide by oxidation of a serine residue, while the oxyamine was suitably generated on the cotton support. Functionalized cotton substrates have undergone surface spectroscopy analysis such as XPS and FT-IR-ATR. The results highlighted the presence of peptides covalently linked to the surface. The biological evaluation of the functionalized cotton fibers is underway.

Synthesis, Conformational Analysis and Biophysical Properties of Medium-Length Peptaibols

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Chalciporin A is a medium-length (14-residue) peptaibol isolated from a strain of *Sepedonium chalcipori* (1). The sequence of peptaibols is characterized by the presence of several, non-coded α-aminoisobutyric acid (Aib) residues and an uncommon C-terminal amino alcohol. Thanks to their helical structures, peptaibols display a relevant ability to interact with phospholipid bilayers. As a result, they often exhibit antimicrobial, antifungal or anticancer activity, although they sometimes target healthy human cells as well. Moreover, they are remarkably resistant to proteolysis so that the study of their mechanism of action is of interest to the design of new drugs.
In this communication, we report the total synthesis of chalciporin A and two analogs thereof, containing the paramagnetic, constrained, α-amino acid TOAC. The amino acid sequences of the three peptides are: Ac-Trp-Val-Aib-Val-Ala-Gln-Ala-Aib-Ser-Leu-Ala-Leu-Aib-Gln-Lol (chalciporin); Ac-Trp-Ala-Aib-Val-Ala-Gln-Ala-Aib-Ser-Leu-Ala-Leu-TOAC-Gln-Lol (TOAC13); Ac-Trp-Ala-TOAC-Val-Ala-Gln-Ala-Aib-Ser-Leu-Ala-Leu-TOAC-Gln-Lol (TOAC3,13).
We exploited a large combination of spectroscopic and biophysical techniques (including FT-IR absorption, CD, 2D-NMR, and fluorescence) to investigate the preferred conformation, membrane interaction, and bioactivity properties of the naturally occurring chalciporin A characterized by a relatively low (≈20%) proportion of the helicogenic Aib residue. In addition to the unlabeled peptide, we gained in-depth information from the study of the two labeled analogs. All three peptides were prepared using the SPPS methodology, which was however carefully adapted in the course of the syntheses of the poorly backbone reactive and side-chain delicate, TOAC containing analogs. Our results point to a largely predominant α-helical conformation and effective membrane affinity/penetration propensities, but to a modest antibacterial activity, for this amphiphilic peptaibol.

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Supramolecular necklace-like structures of Pluronic F127 combined with alpha and beta cyclodextrin for new topical formulation of acyclovir.

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Pluronic® F127 (PF127) is a triblock copolymer of polyethylene oxide (PEO)-b-polypropylene oxide (PPO)-b-polyethylene oxide (PEO) used in drug formulation for its capability to form thermoreversible and mucoadhesive gel in aqueous media (1). Additionally, the supramolecular necklace-like inclusion complexes resulting from interaction between cyclodextrins (CDs) and polymer chains can open new ways to address drug formulation (2). Infact, these gels combine capability to solubilize hydrophobic drugs and tunable mechanical features (3). Thus, the aim of this work was to explore the effects of combining simultaneously both αCD and βCD with PF127 at various ratios on the rheological properties of the polypseudorotaxane systems and their capability to solubilize acyclovir. Acyclovir is a synthetic drug with a similar molecular structure to the purine nucleoside and it represents the most used antiviral for the treatment of Herpes Simplex Virus infections. Nevertheless, acyclovir shows a low water solubility that compromises both bioavailabilty and antiviral performance (4).

To carry out the work, dispersions of PF127 with αCD and βCD at different concentration ratios were prepared with and without acyclovir at 5%. The complexation between PF127 and CDs was evidenced by FT-IR Spectroscopy and X-ray Powder Diffraction. Furthermore, the rheological behavior of the copolymer was investigated and the resulted changes of its gelling temperature can be interpreted as the consequence of the penetration of some PPO hydrophobic groups of PF127 inside the βCD cavities. Acyclovir solubility was evaluated after addition of the drug in excess to the dispersions, which were stored under stirring for at least three days. Then, the samples were filtered and the absorbance at 252 nm was recorded. Irritance degree of the formulations was estimated by HET-CAM assay, showing a slight bleeding in chorioallantoic membrane only for the systems in which the βCD is in concentration at 4%.

These preliminary findings indicate that the proposed approach for delivery of acyclovir can improve the physicochemical properties of the drug and represent the starting point for further experiments.

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Modified β-Cyclodextrin inclusion complex to improve the physicochemical properties of Pipemidic Acid

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The capability of cyclodextrins (CDs) to form inclusion complexes is correlated to their peculiar structure, with a relatively hydrophobic inner cavity that interacts with a variety of guest influencing their physicochemical properties, as a consequence of the complexation (1). In particular, modified cyclodextrins like Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TRIMEB) are best known to be more soluble respect to natural CDs (2) and for this reason their use in pharmaceutical formulation is desiderable (3). In order to obtain an improvement of the low solubility and bioavailability of pipemidic acid (HPPA), a quinolone derivate used as therapeutic agent for urinary tract infection active against Gram (+) and Gram (-) bacteria (4), the drug was complexed with TRIMEB. The inclusion complex was prepared in the solid state by kneading method. The formation of the inclusion complex in the solid state was confirmed by FT-IR Spectroscopy and X-ray Powder Diffraction. The association in aqueous solutions of pipemidic acid with TRIMEB was investigated by UV-Vis Spectroscopy, the 1:1 stoichiometry was established by Job plot method (5) and the binding constants were determined considering the influence of the pH (6) by UV-Vis titration, taking into account the amphoteric nature of the drug.

Furthermore, the cytotoxic activities of HPPA and its complexation product with TRIMEB was evaluated using the MTT-assay on human hepatoblastoma cell line HepG2 and human breast adenocarcinoma cell line MCF-7, revealing a higher antitumor activity of the complex respect to the drug alone.

Results obtained indicate that the formation of the complex can improve the physicochemical properties of the guest to better its bioactivity and represent the starting point for the evaluation of new pharmaceutical formulation of HPPA.

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Light Transducing Protocells: reconstituting and characterizing the bc1 complex into the membrane of giant lipid vesicles.

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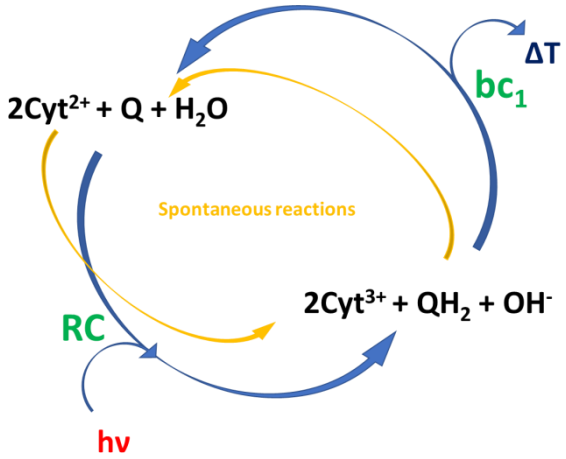
Photosynthesis is responsible for the photochemical conversion of light into the chemical energy that fuels the planet Earth. The photochemical core of this process in all photosynthetic organisms is a transmembrane protein called the reaction center (RC) (1). In purple photosynthetic bacteria a simple version of this photo-enzyme catalyzes the reduction of a quinone molecule, accompanied by the uptake of two protons from the cytoplasm and the oxidation of cyt^{2+} to cyt^{3+} from external medium. In a previous work (2), giant unilamellar vesicles (GUVs) were prepared by the phase transfer method (3) reconstituting in the lipid membrane RCs retaining the physiological orientation at 90%. These synthetic protocells (RC@GUVs) are capable of generating a photo-induced proton gradient 0.061 pH units per min across the membrane under continuous illumination and in presence of an excess of cyt^{2+} and quinone in the external solution. In this contribution, bc1 extracted both from bacteria and mitochondria are reconstituted in giant vesicle membrane (bc1@GUVs) and characterized by studying the enzymatic activity in reducing cyt^{3+} to cyt^{2+} in presence of quinole QH_2. This is a forward step towards the coupling of both RC and bc1 in in the synthetic protocells order to implement the complete photo-cycle, as shown in Figure 1:



This pave the way for the construction of more functional protocells for synthetic biology which can be ultimately harnessed to synthesize ATP(4,5).

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Structural studies on RcnR, a Ni(II) and Co(II) sensing transcription factor

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Escherichia coli RcnR (resistance to cobalt and nickel regulator, *Ec*RcnR), is a metal-responsive transcriptional regulator that represses the expression of the Ni(II) and Co(II) exporter proteins RcnAB by binding to their promoter site in the apo-form. A computational model of *Ec*RcnR suggests the presence of three α-helices in the monomer. Each monomer is in contact with a second protein chain via the three α-helices to form a dimer. The tetrameric oligomer is obtained by the interaction between the two helices α1 from one dimer with the corresponding helices from the second dimer (1). Protein-DNA release occurs when either Ni(II) or Co(II) binds to *Ec*RcnR. *Ec*RcnR also binds the non-cognate metal ions Cu(I) and Zn(II), which have no effect on protein-DNA interaction. Prior work has shown that Ni(II) and Co(II) are found in distinct sites: while both Ni(II) and Co(II) are bound to the N-terminal Cys35 and His64 residues, Co(II) is additionally bound to His3 (2). On the other hand, Cu(I) and Zn(II) have a solvent-exposed binding site and further coordinate protein ligands that do not include the N-terminus amine (3). A molecular model of apo-*Ec*RcnR revealed the presence of Glu34 and Glu63 in the vicinity of the Ni(II) and Co(II) binding site (1). The roles of Glu34 and Glu63 in Ni(II) and Co(II) binding and selectivity were further demonstrated using site-directed mutagenesis, X-ray absorption spectroscopy (XAS) and functional assays (4).
This work will involve a structural characterization of *Ec*RcnR by using a combination of structural biology techniques. X-ray protein crystallography will be exploited to depict, at an atomic level, the tridimensional structure of the transcriptional factor in both the apo- and metal-bound form. As a first approach, a large number of commercial crystallization screenings has been used to find initial conditions for the growth of RcnR crystals. A systematic variation of the initial crystallization parameters will be carried out, by the use of trial matrices, in order to optimize them and obtain crystals suitable for X-ray diffraction experiments. In parallel, solution paramagnetic NMR spectroscopy is being used to shed light on the coordination geometry of cognate metals at the *Ec*RcnR binding site under near-physiological conditions, in order to either corroborate or review previous results.

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Analysis of testosterone fatty acid esters in the digestive gland of mussels by liquid chromatography-high resolution mass spectrometry

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Several studies have indicated that up to 70% of the total steroids detected in molluscs are in the esterified form and that pollutants, by modifying the esterification of steroids with fatty acids, might act as endocrine disrupters. However, despite the strong physiological significance of this process, there is almost no information on which fatty acids form the steroid esters and how this process is modulated. This study (a) investigates the formation of fatty acid esters of testosterone in digestive gland microsomal fractions of the mussel *Mytilus galloprovincialis* incubated with either palmitoly-CoA or CoA and ATP, and (b) assesses whether the endocrine disruptor tributyltin (TBT) interferes with the esterification of testosterone. Analysis of testosterone esters was performed by liquid chromatography–high resolution mass spectrometry (UPLC-HRMS). When microsomal fractions were incubated with testosterone and palmitoly-CoA, the formation of testosterone palmitate was detected. However, when microsomes were incubated with CoA and ATP, and no exogenous activated fatty acid was added, the synthesis of 16:0, 16:1, 20:5 and 22:6 testosterone esters was observed. The presence of 100 μM TBT in the incubation mixture did not significantly alter the esterification of testosterone. These results evidence the conjugation of testosterone with the most abundant fatty acids in the digestive gland microsomal fraction of mussels.

Structural characterization of the protein FlmC from *L. plantarum*

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The interest in biofilm development has increased due to the impact that this sessile form of microbial growth may have on different aspects of the human life. Exploitation of microbial biofilm has been accomplished in different fields, including bioremediation and biotechnological production processes. Contrariwise, biofilms developed by pathogens represent a serious problem for human health.

Probiotic bacteria mainly belong to the group of the lactic acid bacteria of the genus *Lactobacillus* and *Bifidobacterium*. Among these, some strains of *Lactobacillus plantarum*, one of the most predominant species in the human gut microbiota of healthy individuals, have been defined as good performing probiotic microorganisms. In fact, while the majority of probiotic lactobacilli are highly specialized for growth in a limited number of conditions. *L. plantarum* is able to colonize a wide range of environmental niches for its high metabolic versatility.

Among genes involved in biofilm development, *flmA*, *flmB*, and *flmC* have been identified in the *L. plantarum* LM3 strain, coding respectively for the proteins named FlmA, FlmB, and FlmC (1). The three protein show a high sequence homology with the LytR-CpsA-psr (LCP) domain. The LCP family gained attention upon the discovery that some members of this family influence various virulence factors as well as antibiotic resistance of important human pathogens. Particularly, in Gram-positive bacteria, LCP proteins are responsible for ligating cell wall teichoic acids to peptidoglycan (2). Here, in order to gain insight into the structure and the function of this interesting domain, we report the functional and structural characterization of the FlmC protein.

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Metal ion replacement by Pb(II), Ni(II) and Hg(II) in the prokaryotic zinc-finger domain

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Zinc ion binding to the proteic domain is a principal event in the achievement of the correct fold in the classical zinc fingers domain since the motif is mainly unfolded in the absence of the metal cofactor. However, in the case of prokaryotic zinc finger the bigger ββββαα domain shows a hydrophobic core larger than the one found in eukaryotic zinc fingers and that plays a more relevant role in the folding mechanism. For these reasons, as great attention has been devoted to unveil the effect of metal ion replacement in zinc fingers and in zinc-containing proteins in general, the prokaryotic zinc finger domain appears to be a good model to study the interaction of exogenous metal ions with metallo-proteins.
We here explore the structural and functional consequences of the native Zn(II) substitution by Ni(II), Pb(II) and Hg(II) in Ros87, the DNA binding domain of the prokaryotic zinc finger protein Ros. Our findings will complement and extend previous results obtained for different eukaryotic zinc fingers, contributing to the evaluation of whether metal substitution in zinc fingers may be a relevant mechanism in the toxic and/or carcinogenic effects of metal ions.

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Effect of vortex on the chirality induced in porphyrins assemblies by aminoacids

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There is great interest in the chirality transfer from the molecular scale to the supra-molecular non-covalent self-assembly level.(1)
Supramolecular chirality can derive from the complex nonsymmetric arrangement of various molecular components in a noncovalent ensemble. Even symmetric molecules, may present supramolecular chirality (i) by forming intrinsically chiral assemblies, or (ii) by aggregating on to chiral polymeric templates (extrinsic chirality).(2)
Moreover vortexes are a recognized example of macroscopic chirality and one possible origin of chiral symmetry-breaking in nature,(3) the relationship between vortexes and chirality of large assemblies is a very intriguing problem which might lead to understanding fundamentals of nature and, from this, to possible technological applications.(4,5)
Porphyrins are excellent building blocks to assemble supramolecular architectures. Their remarkable and tunable spectroscopic properties have been, in fact, exploited to design and realize a paramount number of porphyrin arrays potentially useful as sensors, optoelectronic devices, antenna systems, models of metallo enzymes, etc.
The tetra-anionic 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS) is not chiral, however in the opportune conditions of pH and ionic strength self-aggregation of its protonated (zwitterionic) form induces a split Circular Dichroism (CD) signal in the absorption region. Both positive (P) and negative (M) couplets are observed randomly. However the interaction between porphyrin and a chiral inducer allow obtaining a desired organization.
The capability of aminoacids to induce supramolecular chirality in the aggregated form of the protonate TPPS was investigated.
Being aware that vortexes (obtained by mechanical stirring) play a crucial role in the selection of equilibrium structures in porphyrins aggregates solutions, and that they can have two possible effects on supramolecular chirality, both static or dynamic (3,6-10) the effect of the mechanical stirring on the obtained systems was also studied.

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Lipid synthesis model for lipid disruptors assessment using microsomal fraction of Mytilus galloprovincialis:

Analysis by High-Resolution Mass Spectrometry

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There is great interest in the chirality transfer from the molecular scale to the supra-molecular non-covalent self-assembly level.(1)
Supramolecular chirality can derive from the complex nonsymmetric arrangement of various molecular components in a noncovalent ensemble. Even symmetric molecules, may present supramolecular chirality (i) by forming intrinsically chiral assemblies, or (ii) by aggregating on to chiral polymeric templates (extrinsic chirality).(2)
Moreover vortexes are a recognized example of macroscopic chirality and one possible origin of chiral symmetry-breaking in nature,(3) the relationship between vortexes and chirality of large assemblies is a very intriguing problem which might lead to understanding fundamentals of nature and, from this, to possible technological applications.(4,5)
Porphyrins are excellent building blocks to assemble supramolecular architectures. Their remarkable and tunable spectroscopic properties have been, in fact, exploited to design and realize a paramount number of porphyrin arrays potentially useful as sensors, optoelectronic devices, antenna systems, models of metallo enzymes, etc.
The tetra-anionic 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS) is not chiral, however in the opportune conditions of pH and ionic strength self-aggregation of its protonated (zwitterionic) form induces a split Circular Dichroism (CD) signal in the absorption region. Both positive (P) and negative (M) couplets are observed randomly. However the interaction between porphyrin and a chiral inducer allow obtaining a desired organization.
The capability of aminoacids to induce supramolecular chirality in the aggregated form of the protonate TPPS was investigated.
Being aware that vortexes (obtained by mechanical stirring) play a crucial role in the selection of equilibrium structures in porphyrins aggregates solutions, and that they can have two possible effects on supramolecular chirality, both static or dynamic (3,6-10) the effect of the mechanical stirring on the obtained systems was also studied.

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Peptide Nucleic Acid dimers self assemble into highly fluorescent aggregates

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The self-assembly of nucleobases has been extensively investigated with the aim to produce new materials, electronic nanodevices and biosensors (1). Interactions between nucleobases occur not only by Watson-Crick or Hoogsteen hydrogen bonding, as observed in the DNA double and triple helices; many base pairing motifs between the four standard nucleobases characterized by two hydrogen bonds have been identified and these are at the base of self-assembling processes. In solution 5' guanine monophosphate (5' GMP) forms g quartets which are stabilized by cations such as Na+ and K+, where each cation interacts with two stacked quartets. At high 5'-GMP concentrations (18-34 wt%) 27 to 87stacked quartets generate 8-30 nm cylinders, kept together exclusively by hydrogen bonding, □-□ stacking and cation-dipole interactions (2). Self-assembly of cytosine, thymine, adenine and uracil has been explored mostly on solid surfaces, such as on Au or on Cu surfaces (3), revealing the formation of 1D and 2D supramolecular nanostructures. The assembly of nucleobases in the context of Peptide Nucleic Acids (PNA) is so far very little investigated. Recently Gazit et la. described the ability of PNA dimers to self assemble into organized structures, guided by stacking interactions and Watson-Crick base pairing (4). GC dimers exhibit interesting fluorescent and optoelectronic properties, showing promise for application as organic light emitting diodes. Encouraged by these interesting results, we initiated our investigation on the self-assembly of Peptide Nucleic Acids, focusing on the PNA dimer Fmoc-GC. Introduction of the fluorenylmethoxy carbonyl (Fmoc) moiety was hypothesized to affect the aggregation properties and likely also the optical properties of the PNA dimer. We here report the characterization of the optical and structural properties of the aggregates by fluorescence, Nuclear Magnetic Resonance and Dynamic Light Scattering. Aggregation of Fmoc GC is mediated by Watson-Crick hydrogen bonds and results in highly fluorescent compounds.

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Molecular characterization in solution of a bis-histidine-peptide complexed to Re(I)-tricarbonyl

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Radiolabeled peptides are useful in the diagnosis and therapy of a variety of human disease characterized by overexpression of peptide receptors. They present some advantages such as: high binding affinity and specificity for the cognate receptor, they are easily synthesized and modified and have rapid blood clearance. These molecules are usually composed by the targeting molecule, a linker and a bifunctional ligand which binds to the radioactive metal. Several radiometals are being used in nuclear medicine and ^{99m}Tc(I) is rapidly gaining in popularity since the introduction of mild synthetic procedure to prepare stable Tc(I)- or Re(I)-complexes $[Tc(H_2O)_3(CO)_3]$ (TcCO) or $[Re(H_2O)_3(CO)_3]$ (ReCO). In these complexes the water molecules can be replaced by ligands to obtain d6 low spin complexes. Ligands replacing all water molecules can form very stable complexes, avoiding trans-chelation reactions which may occur in vivo and the generation of free metal. Histidine is considered a good ligand for TcCO/ReCO and when positioned at the N-terminus of a peptide it acts as bidentate ligand. We reported the *in vitro* and *in vivo* characterization of the peptide CCK8 decorated with a histidine based chelator labeled with ^{99m}Tc-tricarbonyl (1). Recently, we reported the speciation, affinity and binding features of histidine and imidazole complexed to ReCO. In the present work, we analyze the solution properties of the histidine-based chelator complexed to ReCO by NMR and other spectroscopic techniques in order to highlight the molecular properties in aqueous solution of the peptide-metal complex.

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Chemical synthesis of all-D Axl domains for mirror image phage display

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Axl is a tyrosine kinases receptor belonging to the TAM family, which also includes Tyro-3 and Mer members. Axl signaling plays important role in several cellular responses, as the receptor activation promotes cellular proliferation, survival, adhesion, migration, autophagy, invasion, angiogenesis, platelet aggregation and natural killer cells differentiation. Besides, Axl is potent negative regulator of innate immune responses, thus protecting against an overzealous inflammatory response. Structurally, Axl is characterized by an extracellular domain containing two N-terminal immunoglobulin (Ig)-like domains and two fibronectin type III (FNIII) repeats, a transmembrane domain and a cytoplasmic tyrosine kinase domain. Axl can be activated through a number of different mechanisms, mainly upon ligand-induced dimerization. The vitamin K-dependent growth arrest-specific 6 (Gas6) protein is the principal natural ligand of Axl receptor. The C-terminal region of Gas6 comprising LG domains is sufficient for Axl receptor binding and activation. Only the LG region of Gas6 interacts with Ig-like domains of Axl receptor. Upon binding of Gas6 to Axl Ig-like domains, the receptor dimerizes and its tyrosine kinase domain becomes activated.
We intend develop Axl peptide for therapeutic application using the approach called "mirror phage display library". To get this aim we need to prepare the Axl interacting domains Ig1 and Ig2 with all D-amino acids. The D-protein will be immobilized on a solid support and used as bait to find binders through phage display library screening. The selected L-peptide binds to the all-D protein, this implies that the D-peptide will bind the natural L-protein. In this way a metabolically stable D-peptide binder will be developed. Here, we report the chemical strategy to prepare the all-D Axl domain by native chemical ligation.

Conformational stabilization of a β-hairpin peptide through a triazole-trpyptophan interaction

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β-hairpin peptides are useful molecules for pharmaceutical and biotechnological applications. They are especially attractive to modulate protein-protein interactions. Stability and formation of β-hairpin have been deeply analyzed and molecular tools have been developed to design conformational stable β-hairpin peptides. Recently, we have analyzed the conformational stability of a series of β-hairpin peptides presenting a triazole bridge, with variable lengths, in a non-hydrogen bonded position (NHB) (1) and in a hydrogen-bonded position (HB) (2). The "triazole bridge" is a 1-4 disubstituted 1,2,3 triazole obtained by side chain-side chain cyclization through Cu-catalyzed alkyne-azide cycloaddition (CuAAC) forming a covalent linkage between the two strands. In these previous works we have established that the formation of the intrastrand triazole bridge is an effective strategy to constrain peptides in a stable β-hairpin conformation and the optimal bridge length depends on the specific β-hairpin position (NHB or HB) of the tool.
In this work, we wish to obtain a conformational stable β-hairpin peptide combining aromatic interactions with an interstrand covalent linkage such as the triazole bridge. To get this aim we designed a series of β-hairpin peptides presenting the triazole bridge in a diagonal non hydrogen bond (DNHB) position close to the side chains of a tryptophan. Peptides were analyzed by NMR spectroscopy to evaluate their β-hairpin content

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Enzymatic Ubiquitination of Tau protein

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The deposition of protein Tau in intracellular neurofibrillary tangles is a major hallmark of Alzheimer's disease (AD) and other tauopathies. Tau is a microtubule associated protein that modulates the stability of axonal microtubules. A large variety of post-translational modifications were found in Tau, including phosphorylation, glycosylation, acetylation, truncation, ubiquitination and prolyl-isomerization (1, 2). Recent evidence indicated that, besides phosphorylation, polyubiquitin also marks Tau in paired helical filaments purified from AD brains (3). Polyubiquitination regulates fundamental cellular pathways, including protein turnover by proteasomal degradation. Due to its role in the clearance of misfolded proteins, dysfunction of the ubiquitin-proteasome system was proposed to be one of the key mechanisms of neurodegeneration. Indeed, a failure of proteasome function may cause the accumulation of ubiquitinated proteins such as Tau, exacerbating aggregation and neurotoxicity. In this context, we aim to define the effect of polyubiquitination on the structural propensities of Tau, on its aggregation pathway to fibrils, and on its clearance. The implementation of this research requires the obtainment of high amounts of Tau modified at specific lysine residues with polyubiquitin chains. We are currently focusing our attention on a enzymatic method. Ubiquitination of substrates is catalyzed in vivo by three enzymes: ubiquitin-activating (E1s), ubiquitin-conjugating (E2s), and ubiquitin ligase (E3s). Substrate specificity is given by recognition of the target protein by E3. CHIP, an E3 enzyme which targets misfolded proteins towards proteasomal degradation, can ubiquitinate Tau in vitro in combination with Ubch5 (an E2) and E1 enzymes (4). We tried to use Ubc13, an alternative E2 enzyme that was shown to interact with CHIP (5). Our preliminary results show that both combinations of E1-Ubch5b-CHIP, and E1-Ubc13-CHIP can ubiquitinate Tau. In order to determine the ubiquitination sites on Tau, we plan to perform mass spectrometry analysis. The work is in progress to control the enzymatic reaction in order to obtain homogenously modified samples, and to extend the polyubiquitin chain.

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Selective $\alpha_v\beta_3$-targeting theranostic in malignant melanoma: design, synthesis and biological studies of a new RGD peptide

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Malignant melanoma is the most aggressive form of skin cancer having strong tendency to metastasize and it is responsible for 80% of all deaths caused by tumors affecting this tissue (1). Also, melanoma is resistant to most treatment regimens such as chemotherapy and immunotherapy (2). The key objective to improve melanoma pharmacological therapy lies in the ability to early visualize and inhibit the dissemination of cancer cells that eventually contribute to the onset of secondary tumor sites. It is well documented that in melanoma high expression of $\alpha_v\beta_3$ integrin is correlated with tumor invasion and poor prognosis (3). Here we reported the design, synthesis and biological characterization of the novel peptide Ψ-RGDechi, selective for $\alpha_v\beta_3$ integrin, as theranostic agent in malignant melanoma. Respect to the parental peptide RGDechi (4,5,6,7), Ψ-RGDechi shows high resistance to proteases. Its ability to selectively inhibit cell adhesion, migration and invasion, key steps highly related in the metastatic cascade, was demonstrated in a human metastatic melanoma cell line highly expressing $\alpha_v\beta_3$ integrin. Also, labelled Ψ-RGDechi peptide was able to selectively detect human melanoma xenografts by near-infrared fluorescence.

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