Chimica dei Sistemi Biologici

CSB-KN-01 Il trasporto intracellulare target-selettivo

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Negli ultimi anni numerosi gruppi di ricerca hanno rivolto il loro interesse agli studi dei meccanismi di trasporto di farmaci per il trattamento di gravi patologie: è noto che la membrana plasmatica è caratterizzata da una elevata selettività che la rende impermeabile alle molecole che non utilizzano meccanismi di riconoscimento specifici e/o meccanismi passivi. Per migliorare il delivery intracellulare, sono apparsi in letteratura numerosi studi riguardanti l'identificazione di sistemi innovativi di natura peptidica ma, in particolare, è l'utilizzo di nano particelle che ha attratto molta attenzione per le loro potenziali applicazioni nel trasporto di principi attivi. Verranno presentati studi sulla progettazione, sintesi e caratterizzazione di una nuova classe di molecole capaci di target specifico su cellule tumorali e/o di attraversare in maniera efficace le membrane cellulari; in particolare l'attenzione è stata rivolta ad aggregati sopra-molecolari di natura peptidica e liposomiale utilizzabili come vettori per il rilascio intracellulare di molecole bioattive volte al miglioramento della tecniche terapeutiche e diagnostiche attualmente in uso.

CSB-KN-02 Design and Synthesis of DC-SIGN antagonists

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DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule 3-Grabbing Nonintegrin), a specific C-type lectin recognizing pathogen-cell surface glycoproteins, is probably the first trans-membrane receptor on immature dendritic cells (DC) which encounters invading pathogens and binds a number of diverse pathogen-associated molecular patterns. Normally, this binding event triggers internalization of the DC-SIGN-pathogen complex followed by lysosomal degradation of the pathogen and conjugation of the resulting fragments with MHC-II to initiate an adaptive immune response from T cells. Some pathogens, however, have been reported to take advantage of this mechanism, as they appear to deter DC maturation through DC-SIGN-mediated signalling and inhibit antigen presentation to T cells. In particular, van Kooyk's group has shown that HIV-1 enters DC via DC-SIGN avoiding lytic degradation [1]. By doing so, HIV-1 not only escapes the host immune system, but also is presented directly to T cells, which enables fully disseminated HIV-1 infection. Inhibition of pathogen interaction using DC-SIGN specific antagonists is considered as a plausible concept for the development of novel anti-infective agents. Several groups have recently demonstrated that inhibition of DC-SIGN, either by designed glycoconjugates or by antibodies, prevents pathogen attachment to DC and inhibits the infection of other immune cells at its earliest steps [2,3].
Our group has been active in this area and, in collaboration with the European network Carmusys [4], has developed two groups of mannose-based and fucose-based glycomimetic ligands that inhibit DC-SIGN mediated HIV infection in cellular and tissue models.
The presentation will deal with the design and synthesis of these molecules, as well as on the structural studies detailing their interaction with the target lectin.

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CSB-KN-03 Target therapy in anticancer treatment: the HDACi journey.

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In anticancer research, during the last 15 years, more and more efforts have been devoted to the identification and development of NCE targeting specific pathways. The development challenge is the identification of medicines with less toxicity, being specific, and to be used in combination with really cytotoxic products. The biological targets, however, were proteins/enzymes involved in several basic function of the cell like damage repair (i.e. HSP90 or PARP). metabolism (i.e. mTOR, PI3K or AKT) or replication (HGAC).
Among these specific targets, HDACi was until a few years ago one of the main area of interest. Two molecules have been approved by the FDA, Zolinza (Vorinostat by Merck) in 2006 and Istodax (Romidepsin by Glouchester Pharmaceutical in 2009). Both products have been approved in the same niche area, single agents against Cutaneous T-cell lymphoma. The scientific community is still waiting the first approved combination of these drugs.
The research activities on HDACi started when the biological function of HDAC was not completely clear as well as the mode of action of these enzymes. There is still ongoing a debate on the in vitro screening tests and on the pan (3 class of Zn(II), 11 enzymes) HDAC inhibitors versus the selective ones. We are going to discuss Sigma-tau journey in the fields of zinc(II) HDAC inhibitors [1a-h].

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CSB-KN-04 Identification the molecular determinants of amyloid fibril formation *in vivo*

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In 2003 it was reported for the first time that the effect of mutations on the conversion of fully or partially unfolded proteins into amyloid fibrils can be rationalised, and even predicted, on the basis of a number of physico-chemical factors including hydrophobicity, propensity to form β-sheet structure and charge. Since then, ca. 15 algorithms have been published, with predictions largely consistent with experimental data.
The next challenge was to test the validity of these principles in living algorithms and identify the additional physico-chemical and biological factors that determine the aggregation behaviour of proteins *in vivo*. Following the increasing evidence that inclusion bodies accumulating in *E. coli* consist of amyloid-like fibrils, we expressed in *E. coli* cells a C-terminally truncated variant of the N-terminal domain of the HypF protein from E. coli (HypF-N). Expression was carried out at low levels, in a controlled manner and resulted in a folding-incompetent form, due to the lack of the C-terminal residues (as desired). Western blotting assays allowed the measurement of the protein fractions remained soluble and aggregating after expression. The analysis was repeated on 20 mutants of the same C-terminally truncated form of HypF-N, each carrying a single amino acid substitution. This allowed the effect of the mutation on the *in vivo* aggregation to be measured.
The experimentally obtained data were compared with theoretical predictions using algorithms previously tested and validated using only *in vitro* data. The analysis showed a good correlation between the experimentally measured aggregation propensities of the mutants *in vivo* and of the corresponding values calculated from the algorithms. The analysis was thus extended to variants of the amyloid β peptide (Aβ) previously expressed in *E. coli* as peptides fused to the green fluorescent protein (GFP) to measure quantitatively their tendency to aggregate after expression in *E. coli*. The experimental data of aggregation propensity in vivo were again consistent with all tested algorithms, raising the possibility that algorithms previously tuned from *in vitro* data are also largely applicable for aggregation data *in vivo*.

CSB-OR-01 Dopamine receptor agonists and Levodopa modulate protein levels in human T lymphocytes.

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Dopamine receptor agonists and Levodopa are used to treat symptoms in patients affected by Parkinson's Disease (PD) to counteract dopamine (DA) loss at the striatum level [1]. (T)-Lymphocytes express some features of the dopaminergic system (they express tyrosine hydroxylase, dopamine receptors and transporters, and store catecholamines into vesicles [2]), so their functions or activation can be regulated by dopamine. Several effects of dopamine on immune cells have been described [3,4], but none of these studies focus on proteome modifications of human T-cells.

In the attempt to establish if T-cells undergo modifications at the protein level after administration of DA agonists or Levodopa in human subjects, we enrolled 9 PD patients under DA agonist therapy and 5 PD patients DA agonist free. We compared two-dimensional electrophoresis maps of total proteins from T-cells and we identified 7 proteins whose level was significantly different in the two groups considered. Eleven among these patients were treated with Levodopa; indeed, we found levels of 4 spots correlating with the dose of Levodopa assumed daily by patients.

These findings demonstrate that DA stimulation (either by Levodopa or DA agonists) has important effects on T-cell proteome in patients under long term treatment. Therefore, therapies acting on the dopaminergic system have additional effects on the immune system that cannot be neglected. In this view, studies that assert alterations in lymphocytes of PD patients have to take into account the therapy administered to patients.

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CSB-OR-02 RGD-based peptides targeting $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ integrin receptors: an NMR point of view

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Integrins are transmembrane receptors that link the cytoskeleton to the extracellular matrix (ECM),[1] mediating cell–cell and cell–matrix adhesion and providing the traction for cell mobility and invasion.[1] Integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ expression is correlated with disease progression in various tumor types (melanoma, prostate, glioblastoma) while the platelet integrin $\alpha_{IIb}\beta_3$ plays a key role in adhesion of platelet to protein-coated surfaces and in platelet/platelet aggregation (thrombus formation). The two integrins show a similar drug-receptor site and bind to the Arg-Gly-Asp (RGD) motif of ligands as primary recognition sequence. The conformation of the RGD sequence is critical for the specificity of this recognition. Since the RGD motif occurs in many extracellular matrix ligands, the recognition specificity is expected to be modified by other residues and to depend on the conformation of the RGD sequence.
Detailed comparison to different ligands of the same integrin binding site could shed light on the essential elements that determine their interaction, specificity and affinity, and allow the rational design of new antagonists.
Our work focuses on the application of NMR techniques[2] (STD and tr-NOESY) for understanding at atomic level the diverse mechanisms of recognition between peptidomimetics and the binding sites of integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$. The data allow us to identify (by STD) the portions of the ligands in closest contact with the protein and to define (by trNOESY) the preferred conformation of the bound ligands.
The properties of integrins embedded into cell membranes may differ from those of purified receptors, therefore, we show that the interaction between a small library of RGD-peptidomimetics[3] and membrane-bound proteins can be observed by NMR directly in a H_2O-buffer-suspension of living cells, without the need of isolating the protein receptor. We performed the NMR experiments on a suspension of ECV304, bladder cancer cells in which the integrin $\alpha_v\beta_3$ is highly expressed, or in the presence of whole human platelets (where integrin $\square_{IIb}\square_3$ is the most abundant platelet cell surface glycoprotein).
The NMR data are interpreted with the aid of docking calculations affording an improved understanding of integrin-ligand interactions.

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CSB-OR-03 Molecular and supramolecular structure of elastin model peptides containing (2S,4R)-4- hydroxy-proline

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The hydroxylation of some proline residues by prolyl-4-hydroxylase to form (2S,4R)-4-hydroxyproline (Hyp) (Figure 1) is the most common post-translational modification in animals. In particular, it is fundamental in the biosynthesis of collagen, where it plays a critical role in stabilizing the triple helix structure, conferring the correct structure and mechanical strength to collagen fibers. Conversely, in elastin where up to 33 % of proline are Hyp, the possible role for this modification has not been identified.

Recent studies obtained by enzymatic digestion were able to identify the position of proline hydroxylation in skin elastin [1]. In order to investigate the possible role of Hyp in the molecular and supramolecular structure of elastin, we chemically synthesized some elastin–related model peptides containing Hyp. We analyzed them by Circular dichroism (CD), Nuclear Magnetic Resonance (NMR) spectroscopy in order to found out the possible role of Hyp in defining the conformational ensemble populated by elastin peptides and compared them to the related proline-containing peptides. Furthermore, supramolecular studies highlighting the ultrastructure of the self-assembled aggregates, carried out by AFM and TEM microscopies, were presented.

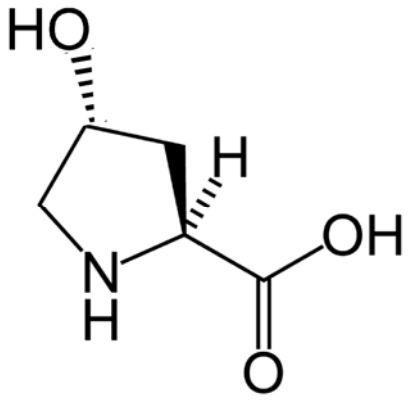

Figure 1: (2S,4R)-4-hydroxyproline

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CSB-OR-04 The Fully-Extended, Peptide Conformation: in Search of Stabilizing Features

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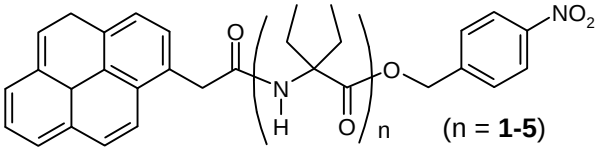
The highly crystalline nature of the peptides rich in C^{α}-tetrasubstituted residues was exploited to characterize in detail the fully-extended (2.0_5-helix) by X-ray diffraction analyses. Multiple, consecutive C_5 conformations were observed in homo-peptides made up of residues with both side chains longer than a methyl. This is for instance the case of $C^{\alpha,\alpha}$-diethylglycine (Deg), the residue used in this study. Interestingly, the axial translation per residue in the 2.0_5-helix is about 3.70 Å, the longest possible for a single amino acid, thus making this conformation extremely attractive for its use as a spacer or a bridge [1]. In this communication we describe our recent efforts aimed at detecting conditions and features able to stabilize the 2.0_5-helix. We synthesized and characterized in solution and in the crystal state a variety of Deg homo-peptides. Our findings can be summarized as follows: (i) either an ester or a tertiary amide (both lacking the N-H group) at the peptide C-terminus is compatible with the 2.0_5-helix; (ii) a primary or a secondary amide at the C-terminus promotes 3_{10}-helix formation; (iii) the nature of the solvent is crucial for biasing the peptide secondary structure towards the 2.0_5- or the 3_{10}-helix. This latter conclusion, in particular, was obtained through time-resolved fluorescence experiments on Deg homo-peptides bearing a pyrenylacetyl fluorophore at the N-terminus and a *para*-nitrobenzyloxy quencher at the C-terminus.



Our analysis shows that the peptides are predominantly fully extended in $CHCl_3$, but folded in MeCN or MeOH.

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CSB-OR-05 Diiron-oxo protein models: the role of turns in stabilizing α-helical harpins

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Turns represent portions of protein chains, where the structure doubles back on itself in a hairpin-like conformation. Important for molecular recognition, turns significantly help to stabilize the fold of proteins. Numerous studies have been carried out on the role of turns in four-helix bundle protein structures, showing that amino acid sequences of inter-helical turns influence the stability and even the fold of helical proteins [1].

Previously, we developed minimal models of diiron-oxo proteins, named DFs, using a *de novo design* strategy. The first model, DF1, idealizes the approximate *C*2 symmetry of the parent natural proteins. It is made up of two helix-loop-helix (α2) motifs, able to assembly into an antiparallel four-helix bundle, with the loops on opposite sides of the bundle. DF1 contains a Glu4His2 liganding environment for the diiron center, housed within the center of the bundle [2]. The structural analysis carried out on DF1 and several variants revealed that these minimal models are rigid scaffolds able to tolerate significant changes in the amino acid sequence, without affecting their overall fold. In fact, all the DFs share the same four-helix bundle structure and active site ligand environment as in natural diiron-oxo proteins [3]. Therefore, they could provide an excellent framework for understanding the factors that stabilize inter-helical turns. In this respect, we have designed two new DF variants, DF3 [4] and L9G/L13G-DF1 that differ in the loop sequence connecting the two helices.

The structural and thermodynamic characterization of these models will be presented. The analysis of all the data shows that the turn conformation is dictated by the structural context within the protein, whose overall stability is, in concert, strictly related to the turn conformation.

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CSB-OR-06 Structural and functional aspects of a metallopeptidase from "Mycobacterium tuberculosis" involved in pathological processes

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Zinc metallopeptidases of bacterial pathogens are widely distributed virulence factors and represent promising pharmacological targets. The impact of zinc metallopeptidases on the pathogenesis of *Mycobacterium tuberculosis* (Mtb) has been addressed only recently, identifying Zmp1 as a virulence factor of *Mycobacterium tuberculosis*.
We have cloned, expressed and purified Zmp1, a zinc metallopeptidase belonging to the neprilysin (M13) family. Substrate specificity of Zmp1 was investigated by peptide array method. Several sequences derived from biologically relevant proteins were identified as possible substrates, including the neuropeptides bradykinin, neurotensin and neuropeptide FF. Further, subsequences of other small bioactive peptides were found among most frequently cleaved sites, e.g. insulin and apelin. We determined the specific cleavage site within neuropeptides by mass spectrometry techniques. Hydrophobic amino acids, mainly phenylalanine and isoleucine, were overrepresented at position P1'.
X-ray structure of Zmp1 has been solved, unraveling an oval shape with dimensions of about 78 Å for the major axis and 60 Å for the minor one. The overall structure is composed by two mainly α-helical lobes (red and blue); the enzyme catalytic site is located between the two lobes and is accessible via two oppositely positioned small opening on the protein surface.
Interestingly, this enzyme shows an optimum activity toward a synthetic substrate at moderately acidic pH values (i.e. 6.3), which corresponds to those reported for Mtb phagosome where this enzyme should exert its pathological activity. In addition, the enzymatic mechanism of Zmp1 toward these neuropeptides has been characterized, displaying some differences with respect to the synthetic substrate and indicating that the enzyme adapts its enzymatic action to different substrates.

CSB-OR-07 γ sulphate PNA (S-PNA): a new PNA analogue with strong antigéne activity

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The research of stable and biologically active DNA mimics has promoted studies on Peptide Nucleic Acids (PNAs), nucleic acid analogues showing high stability to enzyme degradation and strong affinity and specificity of binding toward DNA and RNA [1]. Several PNA analogues have been obtained so far in the attempt to overcome solubility, uptake and aggregation issues [2,3]. Studies on PNA oligomers demonstrated that preorganization is a requisite to increase the affinity of binding toward complementary nucleic acids while the presence of positive charges on the backbone increases their cellular uptake. Encouraged by these results and with the aim to develop a new PNA analogue more DNA-like in terms of polarity, charge and solubility we undertook studies on PNAs having a sulphate moiety in the gamma position of the backbone. Studies on sulphate PNA add a new brick to our knowledge on the effect of charge on the secondary structure, stability of the hybrids and potential of the modified oligomer to be biologically active. The sulphate group is, in fact, very similar to the phosphate of DNA, in geometry, steric hindrance, polarity. In this work, we investigated sulphate PNAs. We set up protocols for the synthesis of sulphate PNA monomers and oligomers. The conformational preferences of the monomers and the oligomers were explored, together with the ability and selectivity in the DNA binding. We focused on a homopyrimidine oligomer designed to interfere with the transcription of the proto-oncogene ErbB2, a cell membrane surface-bound receptor tyrosine kinase normally involved in the signal transduction pathways leading to cell growth and differentiation. Interestingly, the sulphate PNAs were found to be potent antigéne molecules, inhibiting the transcription of the target gene.

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CSB-OR-08 Catalytic specificity of heme-protein models

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Mimicking enzymes with alternative molecules represents an important objective in synthetic biology, aimed to obtain new chemical entities for specific applications[1].New biocatalysts, designed to mimic the main structural and functional features of enzymes, may find applications in different fields, such as biocatalysis, biosensor technology, decontamination and detoxification of industrial wastes, drug and food processing. Moreover, they are basic in elucidating structure-function relationship of enzymes. The design of metalloenzymes has been recognized as being an intricate challengesince both the requirements of protein structure and metal ion coordination should be fulfilled[2]. Moreover, metalloenzymes with asymmetrical coordination centre have rarely been mimicked.

Peptide-based models seem valuable candidates for mimicking metalloenzymes [3]. Their structures are smaller than native proteins, making them easier to be used for practical applications. However, their size is such to allow sufficient chemical diversity for the construction of functional sites.In this perspective, aclass of heme-peptide conjugates, named Mimochromes, have been developed in our laboratory to investigate the effects of peptide chain composition and folding in modulating the properties of the metal ion into the porphyrin ring [4]. The main features of these molecules are the covalent structure and the sandwich motif, with two helical peptides surroundings the heme on both faces. Here we presentan evolution, through design and redesign, of this class of molecules, Fe(III)-Mimochrome VI [5].This molecule embodies some of the key elements for peroxidase-like activity: it is stable, water soluble and it exhibits mono-hystidyl-coordination to the heme. Studies on its peroxidase-like activity and specificity, using various reducing substrates, will be presented.

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CSB-OR-09 New neutral liposomal delivery systems of genetic material

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Complexes of DNA with lipid vesicles or synthetic polymers offer an attractive alternative to viral vectors in genetic material delivery. As a matter of fact it is today current opinion among scientists that synthetic carriers must be considered as the future of a safe and efficient Human Gene Therapy. With the aim of developing new lipid-based DNA carriers, in recent years we have studied structure and phase behavior of ternary water suspensions of self assembled complexes composed by zwitterionic lipids, DNA and metal divalent ions[1-3]. Divalent metal cations promote the DNA condensation with liposomes, acting as bridges between the phosphate groups of both DNA and polar heads of lipids. The ternary complexes obtained show structures similar to the ones of the lipoplexes, with the important advantage of the absence of toxicity, being formulated with phospholipids that naturally occur in the cell membrane. In order to improve the ability of liposomes to complex DNA, our strategy has been to develop liposomal systems containing neutral lipids functionalized with complexing agents able to coordinate bivalent metals. The presence of this moiety allows to obtain more stable complexes of plasmidic DNA and ensures a higher control of the surface charge of liposomes. In this perspective we have prepared and studied mixtures of neutral commercial lipids with some newly synthesized amphiphilic lipids, which have been characterized by means of syncrothron x–ray diffraction, dynamic light-scattering and zeta potential.

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CSB-OR-10 3D-Structure Activity Relationships of new simple antimalarial endoperoxides.

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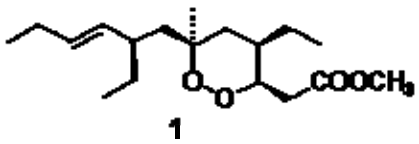
Although the association of chloroquine derivatives with analogues of artemisinin is giving some good results, the therapeutic choices are still too limited for the large and poor malaria market. Indeed, artemisinin therapeutic value is limited by the modest oral availability and high cost of synthesis. Therefore, there is an urgent need of new and economically affordable antimalarial drugs with high efficacy against resistant strains and broad stage mode of action. In this context, it is our aim to contribute to the development of novel antimalarial agents possessing the endoperoxide pharmacophore. The lack of a definitive proof about these drugs modus operandi limits the design of new synthetic derivatives. In the course of our ongoing search for antimalarial lead compounds from marine sources, we have reported that plakortin (**1**), simple endoperoxide containing polyketides, possessed a significant in vitro antimalarial activity particularly on CQ resistant Pf strains [1]. On the basis of a multidisciplinary approach including chemical and computational studies as well as in vitro pharmacological assays, we refined our knowledge on the putative antimalarial action mechanism of molecules belonging to the plakortin family [2]. The hypothesized mechanism of action accounts for the structure-activity relationships of other endoperoxyketal polyketides [3] and newly synthesized derivatives [4]. Thus, our study not only allowed the identification of the structural requirements necessary for antimalarial activity but also provided a tool for subsequent structural modifications.



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CSB-OR-11 Insulin-degrading-enzyme is an heat shock protein and affects neuroblastoma proliferation.

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Insulin-degrading-enzyme (IDE) is an highly conserved and ubiquitous zinc metalloprotease involved in the degradation of substrates such as insulin and β-amyloid (Aβ)[1-3]. In the last decade, the identification of novel substrates (*i.e.* ubiquitin) and its involvement in several biological functions, as steroid signaling and Varicella Zoster virus infection, has cast light that IDE is a multifunctional protein with relevant roles in several physiopathological processes [4-6].

In this work we demonstrate that IDE level is upregulated after cell exposure to different stress conditions, envisaging an Heat Shock Protein-like behavior. We also show that IDE is highly expressed *in vivo* in tumor biopsy of the Central Nervous System (SNC). Additionally, IDE-silencing specifically inhibits neuroblastoma proliferation, triggering cell death. Therefore, we propose a novel role as heat shock protein for IDE, involved in the regulation of tumorigenesis in SNC.

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CSB-OR-12 Advanced Drug Screening platforms by Inkjet printing

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In this work, we show a low-cost, speed, microarray-based drug screening platform that employs inkjet printing drug dispensing on an enzymatic-rich surface.

Mixtures of a model substrate (D-glucose)/inhibitor (D-glucal) couple have been inkjet printed on a target enzymatic monolayer (glucose oxidase) linked to a functionalized silicon oxide solid surface [1].

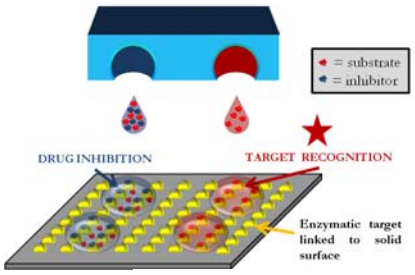

Figure 1.

It has been possible to fabricate microarrays with quality factors as high as those of conventional pin printing spotting. By a simple horseradish-based colorimetric enzymatic assay, the detection of biological activity at the single spot has been proved. The figure shows a scheme of the platform: molecular inks of the enzymatic substrate or a substrate/inhibitor mixture are dispensed on the enzymatic-rich surface with detection at the single spots. Optical intensity measurements showed a competitive inhibition mechanism at the solid-liquid interface, along with overcompeting effects at lower inhibitor concentrations [2]. This methodology is extended to CYP450 enzymes like CYP3A4, one of the main targets for the phase I drug metabolism. In this respect, sol-gel enzymatic encapsulation strategies inside a polymer matrix prepared by MTMOS (methyltrimethoxysilane) precursors [3] or alginate are envisioned. The evaluation of the biological activity is realized via a fluorescent-based assay. In conclusion, we show how inkjet printing methodologies may investigate interesting physico-chemical activities of functional biomolecules at a solid surfaces including their interaction and reaction behavior. Moreover, if coupled with a simple and generalized detection method they may satisfy speed, low-cost, miniaturized and high-throughput screening needs by dispensing entire chemical libraries on solid supported biological targets.

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CSB-OR-13 Amyloidogenesis of EX30-derived human tropoelastin polypeptide sequences

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Amyloid fibers are associated to a large number of diseases such as, for example, Alzheimer's dementia (AD). Many evidences link AD to vascular diseases, while only few data connect amyloids, atherosclerosis and aging via deposits in the aortic intima. Recent results demonstrate that the C-terminal region of human tropoelastin sequence is able to give rise to amyloid-like fibers in vitro, when isolated from the entire protein [1]. Furthermore, it has been demonstrated that the process is favored by sodium taurocholate presence, mimicking the presence of cholesterol. On this basis, it is tempting to speculate that in vascular diseases, under high concentration of lipids, some N- and C-terminal sequences, released from elastin by enhanced proteolytic activity, could aggregate because of mutated microenvironment to form amyloids (whose formation is also favored by lipids) that constitutes the "elastotic material" described in several reports. In order to demonstrate this hypothesis, we synthetized and studied four polypeptide sequences, resulted from the cleavage of human skin elastin by enzymes such as MMP-12 and pepsin [2], all of them self-contained in human tropoelastin gene exon30 coded sequence (EX30D), in order to assess their potential amyloidogenic behavior. We demonstrated that the longest EX30D polypeptide, among those synthetized, is amyloidogenic.

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CSB-OR-14 Corroles: interaction with Polynucleotides.

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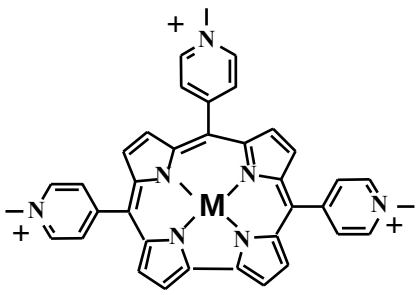
Synthetic porphyrins have been a useful tool to fully understand the complex reactions performed by these macrocycles in biological systems.[1] Furthermore, the attempt to mimic the peculiar chemistry operated by natural porphyrins opened the way to their possible exploitation in a wide range of practical applications. [2]
However, a similar possibility is rarely found with regards to porphyrin analogues, macrocycles having some modification in the molecular skeleton with respect to that of the parent porphyrin.[2] Distinguishable from the core structure of porphyrin by the absence of one of the four meso-positions, corroles contain three "pyrrole-type" hydrogens and a direct pyrrole–pyrrole link.



M = 3H, Ge

The synthesis of pyridyl-substituted corrole has been rarely reported [3]. There are only a few reports about a corrole with positively charged substituents. [4] The corrole with three remote positive charges displayed significantly better efficacy in inhibiting tumour progression and metastasis in animal models than analogous porphyrins. CD, UV-visible absorption and Fluorescence studies with tricationic water-soluble corrole, (TMPC), show that this species can serve as probes to discriminate between single and double strand conformations of polynucleotides poly A and poly C. Furthermore the Germanium (GeTMPC) derivate is able to discriminate between single strand of polyadenilic acid and polycytosine acid, forming extended assemblies in presence of polyA single strand.

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CSB-OR-15 G-quadruplexes and their interactions: a physico-chemical approach

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Guanosine-rich nucleic acid sequences can adopt unusual DNA secondary structures with biological significance, the G-quadruplexes [1]. These structures are four-stranded helical complexes, composed of stacks of G-tetrads, cyclic arrays of four guanine bases which are connected by Hoogsteen hydrogen bonding. The formation of G-quadruplexes requires the presence of metal cations that selectively bind to guanine O6 carbonyl groups in the central cavity generated by the stacked layers of G-tetrads.
Sequences with propensity to form G-quadruplexes have been identified in many regions of human genome. Several pieces of experimental evidence have proven that the 3′ single-stranded overhang of telomeric DNA in eukaryotic cells may adopt this peculiar structure [2]. It has been shown that the formation of G-quadruplexes by telomeric DNA inhibits telomerase activity, with implications in apoptosis and cancer. In addition, some small molecules able to bind G-quadruplex structures alter telomere functions, leading to marked inhibition of tumour cells growth [3]. Recent studies have shown the high density of GC-rich sequences in the promoter region of oncogenes that can transiently unwind and form single-stranded tracts, eventually folding into G-quadruplexes, thus supporting the biological importance of these structures in the control of gene expression [4].
In view of biomedical applications, the understanding of the energetic aspects concerning the structure and stability of G-quadruplexes has achieved new importance in the last years. Moreover, design and development of drugs that selectively bind to a G-quadruplex structure, can be greatly enhanced by detailed knowledge of the thermodynamics of binding to the target [5,6].
In this context, we investigated, with a physico-chemical approach, the G-quadruplex structures and their interaction with proteins and a variety of molecules with high pharmacological interest.

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CSB-OR-16 Site-specific protein double labeling by expressed protein ligation: applications to repeat proteins

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In the last years, the use of labeled proteins has expanded significantly in life sciences. Labeled proteins are nowadays indispensable tools for a wide spectrum of applications in biophysics and chemical biology. The ability to introduce into proteins new functionalities such as post-translational modifications, biophysical and biochemical probes provides a means by which to characterize and modulate protein function or to endow these macromolecules of new useful properties. So far, different protein engineering approaches, based on the use of both chemistry and/or molecular biology, have been developed. We describe a synthetic strategy, based on expressed protein ligation [1], to prepare proteins in high purity and homogeneity in which two different molecular probes are incorporated specifically at any desired position. Proteins are sequentially labeled in solution, with the advantage that large excess of probes are not required and the labeled fragments are not restricted to peptide synthesis length-limitation. This strategy was applied to selectively label a repeat protein, CTPR3 (*Consensus* TetratricoPeptide Repeat) [2], with a fluorophores pair in different positions along the protein sequence. The doubly labeled proteins were prepared at high purity and homogeneity as required for single molecule FRET studies. Remarkably, this approach can be adapted to the introduction of more than two molecular probes.

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CSB-OR-17 siRNAs bearing aromatic residues in the 3'-overhang region

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RNA interference (RNAi) is a biological process whereby small interfering RNA (siRNA) and microRNA (miRNA) silence gene expression in a sequence-specific manner [1]. These effectors regulate gene expression through the RNA-induced silencing complex (RISC). It has been suggested that RISC preferentially selects and incorporates one of two strands of the siRNA duplex depending on its thermodynamic features and that the off-target effects of siRNAs can be correlated to the T_m of the duplex [2]. The problem of unwanted incorporation of the passenger strand into RISC could be address altering the thermodynamic asymmetry of the duplex by using specific chemical modifications [3, 4]. Structural studies have revealed that the 3'-overhang region of the guide strand of siRNA is recognized by the PAZ domain and is accommodated into its hydrophobic binding pocket. We expected that aromatic-based modifications in 3'-overhang would enhance RISC selection of antisense strands of siRNA duplexes, reducing off-target effects induced by sense strands.
In this study, we report the synthesis of siRNAs bearing diphenylpropylamine, tyramine and tryptamine units at the 3'-end of sense and antisense strands. We found thermodynamic stability of the conjugates was increased by these modifications. Furthermore, but not surprisingly, the modified duplexes were found to retain RNA-like A-type conformation. We also assessed the nuclease resistance of the modified siRNAs and found it was similar to those of unmodified siRNAs. These results prompted us to investigate the silencing activity of the siRNAs possessing the aromatic moiety in the 3'-end by *in vitro* experiments in mammalian cells.

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CSB-OR-18 Characterization of copper(II) and zinc(II) complexes with the N-terminal domain of Nerve Growth Factor

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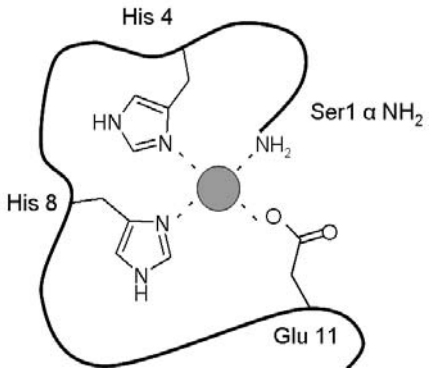
Nerve growth factor (NGF) is a protein involved in development and survival of specific neuronal populations [1]. In Alzheimer's Disease (AD), the lack of trophic support guaranteed by NGF has been observed in the same areas involved in learning and memory. Some d-block metal ions have been proposed to play a crucial role in AD and, intriguingly, NGF performs its activity in the same brain areas affected by metal dys-homeostasis in pathological conditions [2].
As N-terminal residues of NGF are crucial for the activity of this protein, the peptide fragment encompassing the sequence 1-14 of the human NGF, named NGF(1-14), was synthesized and its copper(II) and zinc(II) complexes characterized by means of potentiometric and spectroscopic (UV-vis, CD, NMR and EPR) techniques. The predominant Cu^{2+} complex species in the pH range 5.5-7.4 is the $[CuLH_{-1}]$, in which Cu^{2+} is bound to an amino, an amide and an imidazole nitrogen atom donors (NH_2, N^-, N_{Im}) in a highly distorted environment, due to the presence of an apical oxygen atom of the carboxylate and/or an imidazole nitrogen. Otherwise, Zn^{2+} is bound to two imidazole nitrogen atoms and to the Glu11 carboxylate group in acidic region, whereas Ser1 amino group is involved in the metal coordination above pH 6.5 (Figure 1). Beside to be the first anchoring site for Cu^{2+} and to be involved in Zn^{2+} coordination at physiological pH, the free amino group plays a key role which is stressed by biological essays. Indeed, a synergic proliferative effect has been observed after co-treatment with NGF(1-14) and Cu^{2+} or Zn^{2+} on SHSY5Y neuroblastoma cell line, but this effect was not observed after co-treatment with metals and the N-acetylated form of the peptide fragment, suggesting an important correlation between biological activity and coordination environment.



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CSB-OR-19 Stabilization of G-quadruplex DNA structures by specific ligands and study of their binding affinity and selectivity with mass spectroscopy

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G-quadruplexes are a family of nucleic acids secondary structures stabilized by G-tetrads, coplanar quartets of guanines held together by a cyclic arrangement of eight unconventional hydrogen bonds (Hoogsteen bonds).[1] DNA G-quadruplex can be formed by G-rich strands as the telomeric sequences. Telomeric single-strand DNA is the substrate of telomerase, an enzyme necessary for telomeric replication, which is over-expressed in most cancer cells and participates in tumors genesis. The formation and stabilation of a telomeric G-quadruplex blocks telomerase activity and offers an original strategy for new anti-cancer agents. In the last 25 years, several families of compounds have been identified which specifically bind to the telomeric quadruplex. These derivatives, called "G-quadruplex DNA ligands", are able to block telomeric replication in cancer cells and to cause replicative senescence and/or apoptosis after a few cell cycles. All these molecules are characterized by an aromatic core, which favours stacking interactions with the G-tetrads, and, in most cases, by basic side chains (positively charged under physiological conditions), which interact with the quadruplex loops and helix grooves.[1].

In our research group, a great interest is devoted to study the stabilization of telomeric G-quadruplex structures by small organic molecules, in particular perylene [2] and coronene [3] derivatives, aza-truxenes [4] and alkaloids related to natural compounds such as berberine [5] and taspine.

The affinity of these ligands at various concentrations towards different DNA structures has been studied by ESI-MS measurements [6,7]. This technique allows the transfer of non covalently bound complexes into the gas phase without the disruption of the complex itself. So, the determination of stoichiometry and modes and energies of interaction can be carried out performing mass spectra of samples containing both DNA and varying their ratio. We have found that these molecules are able to bind and stabilize G-quadruplex DNA oligomers.

The selectivity for these structures with respect to duplex DNA, a fundamental topic for the biological evaluation and the pharmacological application of these ligands as potential chemotherapeutic agents, has also been investigated by competition experiments in which there were both quadruplex and duplex DNA structures [7].

Significant biological data about *in vitro* and *in vivo* activity of these ligands will be presented. The correlation between these data and ESI-MS measurements will be also discussed.

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CSB-OR-20 Understanding secreted aspartic proteinase 2 from *C. Albicans* binding with bicyclic peptidomimetic inhibitors by molecular docking

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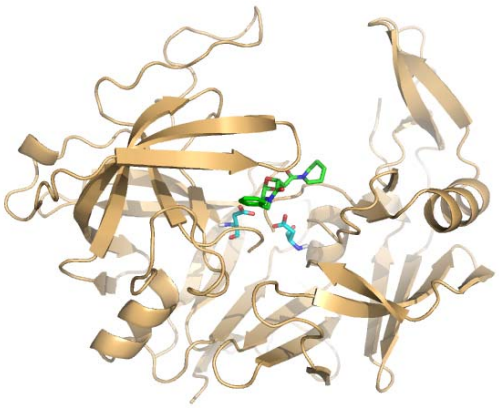
Candida albicans is an opportunistic fungal pathogen that causes even severe systemic infections especially in immunodeficient individuals [1]. Although a certain number of antifungal agents are available, the need for new drugs against *C. albicans* is escalating due to the development of resistance against available drugs. Secreted Aspartic Proteinases (SAPs) activity appears to be a main virulence factor of this fungus, allowing it to adhere and invade host tissues, therefore this family of enzymes offers a potential target for drug intervention in infections. Particularly, SAP2 has been recognized as a crucial virulence factor for vaginal infection and both reversible and irreversible inhibitors have been reported accordingly [2].



We recently reported on the identification of a novel class of small-molecule peptidomimetic SAP2 inhibitors based on 6,8-dioxa-3-azabicyclo[3.2.1]octane scaffold, which showed antifungal activity against *C. Albicans* [3]. In particular, we selected two hit candidates, which proved to be effective both *in vitro* and *in vivo* against drug-resistant *C. albicans* strains. With aim to give more insight into the binding mode and the structural requirements of these compounds for the inhibitory activity towards SAP2, we carried out a structural study by molecular modelling and enzyme inhibition assays, also taking into account the four possible stereoisomers of the two lead compounds.
Through this computational analysis, performed using the automated docking program AutoDOCK 4.0, we gain insight into the possible orientation of these compounds in enzyme catalytic site and the various structural factors and interactions responsible for their inhibitory potency.

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CSB-OR-21 SOLID-PHASE SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL NUCLEOSIDE-TETHERED DINUCLEAR PLATINUM(II) COMPLEXES

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Cis-diamminedichloroplatinum(II), commonly known as cisplatin, is a well-known antitumour drug currently used in the treatment of testicular, ovarian, bladder head/neck, lung, and cervical cancers. Unfortunately it has several major drawbacks.
Recent studies concluded that it is possible to overcome the cross-resistance to cisplatin and its analogues using polinuclear compounds. For these reasons, in the last decades some research groups focused their attention on the design and synthesis of new polinuclear platinum complexes where the metal centres are separated by an aliphatic unbranched amine linking ligand. In the light of these studies, we chose to prepare, according to a solid phase approach already employed by us,[1] three new dinuclear platinum complexes, where both platinum moieties are installed on a modified nucleoside acting as ligand. The dinuclear platinum compounds were obtained in good yields starting from a commercially available nucleoside (inosine) and were tested against four different human tumor cell lines. One complex proved to be more cytotoxic than cisplatin against MCF7 cancer cell line in a short-term exposure assay.[2]

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CSB-OR-22 Porphyrins as Proteasome Inhibitors: a new activity for an old molecule.

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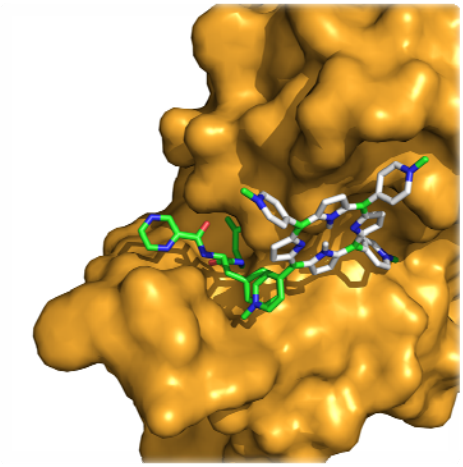
Inhibition of proteasome activity represent a promising anticancer strategy [1]. Proteasome plays a critical role in modulating intracellular levels of proteins involved in cell cycle regulation, it regulates tumor suppressor genes, oncogenes, and the activity of signal transduction pathways.



Porphyrins, very versatile molecules, are also used in PDT cancer therapy [2]. In this study we have tested their efficiency to inhibit proteasome. Chymotriptic, caspasic and tryptic activities on purified 20S, cell extract of 20 S and 26S samples have shown that cationic porphyrins are efficient reversible proteasome inhibitors. Their IC50 values are, in fact, in the range of 10^{-7}M; i.e. they are 100 times more active than lactacystin and MG132.
Spectroscopic results parallel the enzymatic behaviour and molecular modeling suggests that active porphyrins can effectively dock into the proteasome chymotryptic site by electrostatic interactions bridging the residues Thr1 with porphyrin positive groups.

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CSB-OR-23 Clioquinol Glycoconjugates as Potential Anti-Cancer Prodrugs

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Clioquinol (CQ) is a lipophilic chelator of copper, zinc and iron. Several recent studies have generated interest in CQ as a modulator of metal homeostasis in neurodegenerative disorders and cancer. *In vitro* and *in vivo* studies have demonstrated that CQ shows an anti-cancer activity at low micromolar concentration. The mechanism by which it induces cell death in the malignant cells is not completely understood, although some experimental evidences suggest a role of CQ as a copper dependent and/independent proteasome inhibitor [1].
The glycoconjugation has been investigated as a potential strategy to achieve selectivity and the drug glycoconjugates can be recognized by specific receptors such as glucose transporters or galectins, over-expressed in the cancer cells [2].
Thus, following preferential up-take of glycoconjugate compounds, they are subject to hydrolysis by specific β-glycosidases, allowing to liberate the active aglycone part which exerts its anticancer activity [3].
In this scenario, we report the synthesis of conjugates of 8-hydroxyquinoline and clioquinol with glucose and galactose. The derivatives were synthesized starting from protected α-anomer glycosyl bromide by a biphasic reaction, using a phase transfer catalyst. The conjugates obtained were characterized by NMR and UV spectroscopy and ESI-MS.
To evaluate the effect of the glycoconjugation, the clioquinol derivatives were tested in a neuroblastoma cell line, SH-SY5Y. The biological activity of the compounds was analysed by viability test like MTT assay and the selectivity of these compounds to induce cancer cells death was also tested.

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CSB-OR-24 Short Peptaibiotics as New Antimicrobial and Antitumor Agents: Synthesis, Conformational Analysis, Antimicrobial and Cytotoxic Evaluations of Trichogin GA IV and Selected Analogues thereof

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Trichogin GA IV, isolated from the fungus *Trichoderma longibrachiatum* [1], is the prototype of lipopeptaibols, a sub-class of short-length peptaibiotics exhibiting membrane-modifying properties. Its primary structure is as follows:

n-Oct-Aib^{1}-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile^{10}-Lol

where *n*-Oct is *n*-octanoyl, Aib is α-aminoisobutyric acid, and Lol is the 1,2-amino alcohol leucinol. Using a variety of techniques we demonstrated that this peptaibol is predominantly folded in a mixed 3_{10}-/α- helical conformation with a clear, albeit modest, amphiphilic character [2]. In this work, we synthesized by solution and solid-phase methodologies a set of trichogin GA IV analogues in which the four Gly residues, lying on the poorly hydrophilic face of the helical structure, are substituted by one, two, three, and four strongly hydrophilic Lys residues. The conformational preferences of these analogues were assessed by CD and 2D-NMR techniques in aqueous, organic, and membrane-mimetic environments. We tested the role played by the markedly increased amphiphilicity on the peptide bioactivity, performing fluorescence leakage experiments in model membranes, and checking protease resistance, antibacteral and antifungal activities, cytotoxicity, and hemolysis. The cytotoxicity of trichogin GA IV and its analogues was tested using three *in vitro* cell-based assays: (i) Human red blood cells lysis. (ii) Cell mortality assays in total human blood leukocytes and in separate sub-populations. (iii) Cell mortality of three tumor-derived stable cell lines (HeLa, A541, A431). Our data show that some of our trichogin analogues are active against tumor cells, leaving the leukocytes unaffected.

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CSB-OR-25 Interaction of cisplatin with copper transport proteins: in vitro and in-cell NMR studies

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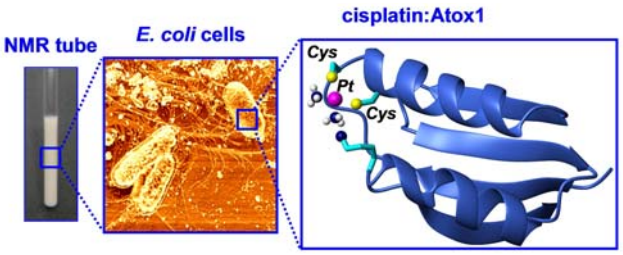
The anticancer drug cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) exploits the cellular transport routes for essential copper (Cu) ions. The Cu permease Ctr1, located on the plasma membrane, is involved in cisplatin uptake, whereas the Menkes and Wilson Cu ATPases regulate cisplatin efflux and vesicular sequestration. In addition, the cytosolic Cu chaperone Atox1 has been found to translocate to the nucleus where it acts as a Cu-dependent transcription factor, thus representing a candidate nuclear carrier for cisplatin. In the nucleus, cisplatin forms adducts with DNA which are at the basis of its antitumor activity [1].

We investigated the coordination properties of Cu(I)-binding motifs of Ctr1 and Atox1 towards different Pt complexes by NMR spectroscopy and circular dichroism, and we determined the stoichiometry of adducts by ESI-MS [2,3]. Cisplatin binds to methionine-rich motifs of Ctr1 and to cysteine motifs of Atox1, but only in the latter case the drug retains its ammine ligands essential for antitumor activity. A combined approach using *in-cell* NMR and ICP-MS was used to probe intracellular drug delivery and the interaction of cisplatin with the dithiol motif of Atox1 in living *E. coli* cells, aiming to obtain detailed molecular information in a physiological environment.

Atox1 overexpression is shown to have a cytoprotective role against cisplatin toxicity by reducing the extent of DNA platination and subsequent cell morphology alterations, and improving cell viability.

The structural characterization of adducts of cisplatin with Cu transport proteins provides the basis for unraveling the effect of Pt-based drugs on Cu homeostasis and may contribute to the rational design of novel and more effective anticancer agents overcoming drug resistance and adverse side-effects.



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CSB-OR-26 Molecular recognition in fatty acid binding proteins: NMR interaction studies with lipids, lipophilic drugs, and liposomes

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Lipid trafficking in cells is a complex and dynamic process that affects many aspects of cellular function. The distribution of bioactive lipids to different cellular compartments appears to be coordinated by lipid chaperones known as fatty acid binding proteins (FABPs), a group of molecules that is also strongly linked to metabolic and inflammatory pathways [1].

FABPs have been shown to reversibly bind hydrophobic ligands, such as saturated and unsaturated long-chain fatty acids, bile acids, eicosanoids and other lipids. Some of the proteins have also displayed ability to bind a variety of exogenous molecules. Although the overall three-dimensional structure is conserved between isoforms, significant differences in ligand selectivity, binding affinity and binding mechanism are found [2].

The binding features of FABPs appear difficult to be captured, although recent studies have set important milestones in the atomic-level description of FABP/ligand interactions. NMR spectroscopy in particular proved an extremely powerful method to characterize these systems in terms of structure, dynamics, binding mechanisms and chemical equilibria, revealing very intriguing features of some members of the family such as binding cooperativity and site-selectivity [3].

The interactions of bile salts to specific FABP carriers have been extensively investigated in our laboratory applying a variety of NMR techniques and are here summarized. Recent results from studies addressing the role of bio-membranes in regulating ligand uptake and release are presented [4]. Finally, we describe lipid-based drugs which were found to be specifically bound by FABPs and displayed attractive features for applications in magnetic resonance imaging [5].

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CSB-OR-27 NMR based studies of stability and dynamics of different forms of BS-RNase

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The development of drugs that can overcome tumor resistance and minimize toxic effects to normal cells is one of the main targets of cancer therapy [1]. Several ribonucleases seem to be a good candidates because of their strong cytotoxic activity towards malignant tumour cells.

Among ribonucleases, bovine seminal ribonuclease (BS-RNase) is the one that shows some of the most interesting features. This is the only mammalian dimeric ribonuclease and it has been proposed that the interchange (swapping) of N-terminal helices is often associated with new biological functions including cytotoxic activity.The current hypothesis linking the swapping to the antitumor activity is that the entanglement of N-terminal tails hinders the neutralizing effect of the cytosolic ribonuclease inhibitor (RI), a protein extremely abundant in mammalian cells that protects endogenous RNA by binding with very high affinity endogenous ribonucleases. Thanks to two disulfide bonds bridging the two identical subunits of BS-RNase, the native protein exists as an equilibrium mixture of two isoforms, MxM and M=M, with and without exchange respectively, which show only minor structural differences in their X-ray structures, located essentially at level of the 16-22 hinge regions, *i.e*. the loop connecting the dislocating arm to the main body of the protein. High resolution NMR experiments allow a fine characterization of the structural and dynamical properties of the different forms that the BS-RNase adopts in solution, i.e. monomer, swapped and un-swapped dimers and can be helpful to define the mechanism of interconversion and, perhaps, to design mutants with improved biological activity. Here we present recent results based on relaxation, H/D exchange and chemical stability NMR experiments acquired on both monomeric and dimeric forms of BS-RNase. Our data suggest a possible mechanism for domain swapping of natural BS RNase.

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CSB-OR-28 Unfolding pathway characterization in prokaryotic zinc-finger domains.

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We have recently characterized the prokaryotic Cys_2His_2 zinc-finger domain[1,2], identified in Ros protein from *Agrobacterium tumefaciens*, demonstrating that, though possessing a similar zinc coordination sphere, this domain is structurally very different from its eukaryotic counterpart (Figure 1).

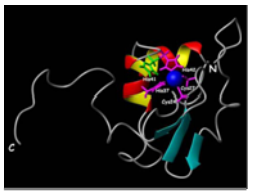

Fig 1 : The prokaryotic zinc-finger domain

A large number of Ros homologues have been found in different bacteria, having mostly a high sequence identity with Ros protein, which, surprisingly, does not comprise the zinc coordination sphere. We have demonstrated that the prokaryotic zinc-finger domain in Ros homologues can either change the coordination sphere or lose the metal while still preserving the DNA binding activity[3,4]. Here we report a thermodynamic and kinetic study of the Ros protein and of one of its zinc lacking homologues unveiling interesting differences in the mechanism of folding of the two proteins. In light of these findings, the role of the zinc ion in the stability and folding of the prokaryotic zinc-finger domains is discussed.

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CSB-PO-01 DNA G-quadruplex binders with a xanthene scaffold

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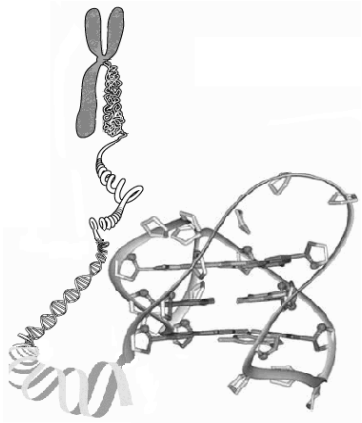
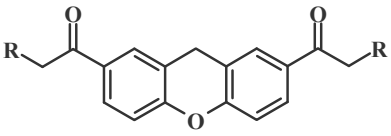
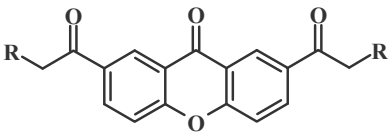


G-quadruplex structures at telomeres (region of repetitive DNA sequence at the end of a chromosome) are likely to play a relevant role, both from the structural and the functional point of view [1]. In fact, molecules able to induce G-quadruplex structures are intensively studied for their ability to inhibit telomerase, as possible therapeutic antitumoral agents [2]. In our research group, a great interest is devoted to study the formation of telomeric G-quadruplex structures by small organic molecules, in particular perylene [3,4] and coronene [5] derivatives and alkaloids related to the natural compound berberine [6]. Recently, we have synthesized the proposed xanthene and xanthone derivatives to test an additional simple aromatoid moiety. The affinity of these ligands at various concentrations towards different DNA structures, was study by ESI-MS measurements [5]. This technique allows the transfer of non covalently bound complexes into the gas phase without the disruption of the complex itself and therefore the determination of the stoichiometry and, in particularly favourable cases, modes and energies of interaction [7]. So, we have found that these molecules are able to bind and stabilize G-quadruplex DNA oligomers. Moreover, we have also studied the selectivity of these compounds for G-quadruplex over duplex DNA. In fact, the selectivity is surely a highly relevant topic and could be related to the specificity of the biological activity of these compounds.



Disubstituted xanthene



Disubstituted xanthone

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CSB-PO-02 Studies on Temporin B analogues

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Temporins are antimicrobial peptides produced and secreted by the granular glands of the European red frog *(Rana temporaria*).[1] They are amphipathic□□ helical peptides, 10–14 amino acids long, containing only 1 or 2 positively charged amino acids (R or K). Most of them selectively interact with bacterial membranes, being active against Gram positive bacteria, and are not toxic to eukaryotic cells.These peptides, as most of the AMPs, are highly membrane-active and have been hypothesized to fold upon interaction with bacterial membranes. The mechanism of action hypothesized for TL and TB after studies carried out on membrane models, leads to the hypothesis that the peptides first associate to the bacterial membrane; upon interaction, the peptides fold, intercalate the phospholipid bilayer and aggregate forming toxic oligomers, following a pathway similar to that observed the formation of amyloid fibers. [2]
In this study we focused on analogues of temporin B, obtained either after substitution of one or two amino-acids by an alanine and by lengthening the sequence, with the aim to widen our understanding of the factors which determine the interaction of the peptide with the bacterial membrane and their influence on the peptide secondary structure and antimicrobial activity. We investigated the antimicrobial activity of the peptides against Gram positive and Gram negative bacteria, their secondary structure by CD. Interestingly the analogue named G6AKK, with a +4 charge, was found active also against Gram negative bacteria. The interaction of this peptide with the LPS from Salmonella and E.coli was investigated by CD, fluorescence and NMR. The results obtained argue for a close relationship between the composition of the bacterial membrane and the strength of the peptide-membrane interaction which in turn determines the peptide folding and antimicrobial activity.

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CSB-PO-03 Turning ubiquitin into a glass-adhesive and lipid-soluble amyloid

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Ubiquitin (Ub) is involved in the pathways for eliminating misfolded proteins. Failure of this process leads to formation of amyloid aggregates enriched with Ub, that are a hallmark of most neurodegenerative disorders [1]. Amyloid fibril formation is preceded by structural changes in native proteins leading to sticky, partially misfolded intermediates. Although these species are considered to be the primary toxic species, very little is known about their conformational features.
The early steps of the aggregation process can be catalyzed by metal ions. We have shown by NMR that Cu^{II} destabilizes Ub [2] and triggers its aggregation [3] and we were able to determine the crystal structure of Ub in the presence of Zn^{II} [4]. On the basis of the detailed knowledge of the effects of metal coordination, we produced a Ub mutant (E16V), specifically designed to neutralize a negative charge at one edge β-strand, which, to some extent, can mimic metal binding. In water at 37 °C E16V is soluble and folded. In contrast, in water/trifluoroethanol (80:20, v/v) E16V undergoes a time-dependent increase in β-sheet content which drives the protein to interact with ANS hydrophobicity probe and to form amyloid-like deposits on a quartz surface. When added to anionic (but not to zwitterionic) phospholipid liposomes, E16V forms β-rich oligomers able to penetrate the bilayer and to react with the A11 antibody, which specifically recognizes amyloid oligomers [5].
The trapping of E16V oligomers prospects the possibility of using the Ub mutant as a model system for elucidating the structural features of amyloid most toxic species.

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CSB-PO-04 Hydroxymethylferrocene/β-Cyclodextrin inclusion complex: preparation and characterization in aqueous solution and in solid state

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Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six (α-CD), seven (β-CD), eight (γ-CD) or more D-glucopyranose units linked by α-1,4 bonds in a toroidal structure. The inside cavity of β-CD is hydrophobic and the outside is hydrophilic. Due to their structural features, they are capable to include a wide variety of molecules of appropriate size and polarity into their cavity forming inclusion complexes [1]. CDs-drugs inclusion complexes have recently gained interest in the pharmaceutical field due to the enhanced solubility, stability and bioavailability of the drug [2]. Molecular encapsulation may occur both in solid and in solution state.

Ferrocene (Fc) is an organometallic compound consisting of two planar and parallel cyclopentadienyl rings and an iron atom. The biorganometallic and medicinal chemistry of ferrocene has been extensively reviewed recently [3]: the synthesis, characterization and antitumor activities of different CD inclusion complexes containing one of the ferrocene derivatives have been recently described [4].

In this work, we report the preparation and caracterization of inclusion complex between hydroxymethylferrocene (FeMeOH) and β-CD in solid state and in aqueous solution. A solid binary system was prepared in a 1:1 stoichiometric by different techniques such as physical mixture, co-precipitation, kneading, and freeze-drying. These products were characterized using powder X-ray diffractometry (XRPD) and Fourier transform-infrared spectroscopy (FT-IR).

The results obtained indicate the formation of an inclusion complex of FeMeOH with β-CD in the solid state. The effect of β-CD on the aqueous solution and dissolution rate of FeMeOH were also investigated. Our results indicate that the solubility of FeMeOH is significantly increased in presence of β-CD and its phase solubility profile is classified as AL- type, indicating a stoichiometry 1:1 inclusion complexes [5].

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CSB-PO-05 β-hairpin stabilization through click chemistry

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The β-hairpin is an especially interesting naturally occurring scaffold used by many proteins for biomolecular recognition, and thus is an attractive tool for mimetic design [1], in fact the β-Hairpins constitute an important class of connecting protein secondary structures, such structures are the simplest form of β -sheets, the motif consists of two strands that are adjacent in primary structure oriented in an antiparallel arrangement, where the N-terminus of one sheet is adjacent to the C-terminus of the next, and linked by a short loop of two to five amino acids. In this review we explore the possibility to stabilize a β -hairpin conformation through an intramolecular side-chain to side-chain cyclization which will should also improve resistance to proteolytic degradation. We focus on the well know click chemistry reaction which brings to the formation of a 1,2,3, triazole linkage between alkyne and azide reactive groups. Furthermore, we analyzed the relationships between β -hairpin stability and side chain length of the reacting groups. The β -hairpin model system we chosen was the Trpzip2 peptide, and we analyzed the contribution to β -hairpin stability of the 1,2,3 triazole linkage in a nonhydrogens bonded position (NHB). The alkyne and azide amino acids were introduced in position 4 and 9 of Trpzip2 respectively. Trp11 was replaced with valine in order to assess the contribution in absence of aromatic contribution and Trp2 was left as spectroscopic probe. As alkyne amino acids were inserted propargylglycine (Pra), homopropargylglycine (Hpg) and bishomopropargylglycine (Bpg). On the other strand the following azide amino acids were introduced: L-beta azidoalanine, L-gamma-azidohomoalanine and L-delta-azidoornithine. The synthesized and purified linear peptides, were cyclized by the CuAAC reaction and purified prior to the spectroscopic characterization. Linear and cyclic peptides were analyzed by a combination of CD and NMR techniques.

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CSB-PO-06 A novel dual function peptide for gold nanoparticles stabilization and integrin targeting

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Over the past decade gold nanoparticles have attracted much interest since they are versatile agents with a variety of biomedical applications including drug and gene delivery[1]. Several stabilizing agents, that interact with nanoparticle surface, are usually added to improve their stability. Recently, we reported peptide sequences displaying thiol group (GC) as capping agents for gold nanoparticles preparation [2]. A special feature of these peptides is that they do not lead to particle aggregation by cross-linking.
In this framework we designed a novel dual function peptide (RGD-$(GC)_2$) encompasses a RGD motif for $\alpha_v\beta_3$ targeting [3] and a GC motif to stabilise the gold nanoparticles. The nanoparticles Au@RGD-$(GC)_2$ functionalized with the peptide were characterised by UV-visible, ATR-FTIR and NMR spectroscopies, confocal and TEM microscopies. The cellular uptake of Au@RGD-$(GC)_2$ gold particles into U87 cells were investigated by confocal microscopy in comparison with Au@$(GC)_2$ as control. A quantitative determination of the uptaken nanoparticles were carried out by measuring the brightness of the images of both systems that highlighted the importance of the RGD sequence of the peptide. In order to understand if the receptor-mediated entrance was favourite, TEM experiments with Au@RGD-$(GC)_2$ and Au@$(GC)_2$ nanoparticles were carried out.

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CSB-PO-07 Harpin oligonucleotides forming G-quadruplexes: new aptamers with potential anti-HIV activity

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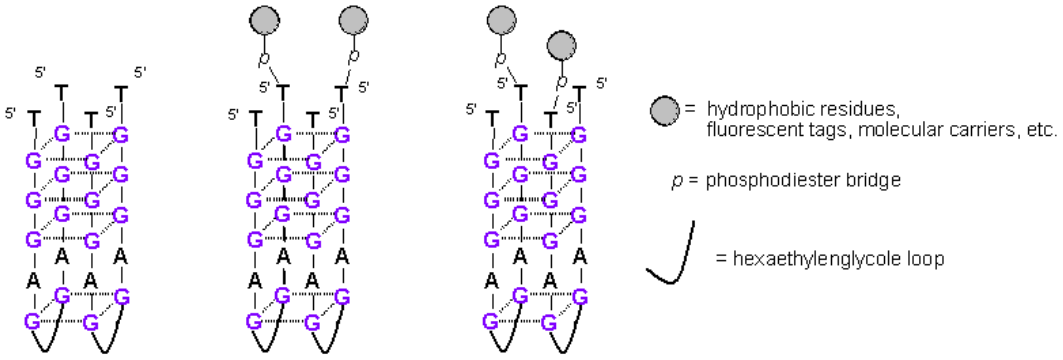
Several G-rich synthetic oligodeoxyribonucleotides (ODNs) have shown promising biological properties, ranging from anticancer to anti-HIV activities. G-quadruplex formation was found to be a crucial prerequisite in determining these biological effects [1]. Aptamers exhibiting anti-HIV activity represent an important class of potential therapeutics [2]. Recently we described the synthesis and characterization of new d(TGGGAG) ODNs, conjugated with different aromatic groups at the 5'-end through a phosphodiester bond [3]. The modified sequences showed a parallel stranded tetramolecular G-quadruplexes CD profile and a pronounced anti-HIV-1 activity.

Herein, with the aim to use d(TGGGAG) as a lead sequence for a more effective anti-HIV agent, we propose the fully automated synthesis of new ODNs containing two d(TGGGAG) sequences whose 3-ends are joint by an hexaethylenglycole loop. CD analysis was undertaken on the 3'-3' linked d(TGGGAG) *hairpins* in comparison with the corresponding unmodified oligomers. Besides, in order to study the influence of the conjugation at the ends of the *harpin* chains on their ability to stabilize quadruplex structures and on their anti-HIV activity, different conjugated oligomers have been studied.



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CSB-PO-08 Synthesis and characterization of a mini-library of new conjugated d(TGGGAG) oligonucleotides with potential anti-HIV activity.

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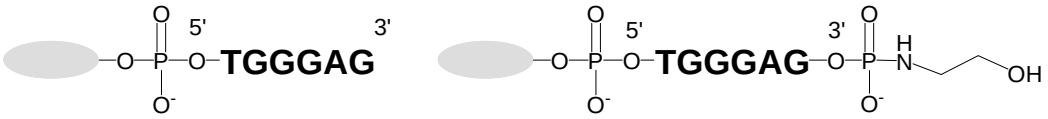
In the search for ODNs endowed with relevant antiviral properties, Hotoda and coworkers [1] investigated a series of G-quadruplex-forming ODNs, finally focusing on modified d(TGGGAG) ODNs conjugated with aromatic residues at the 5-end. These were found to exhibit potent anti-HIV activity associated with low cytotoxicity when carrying at the 5′-end bulky aromatic residues. Recently we described a general approach to obtain a mini library of new d(TGGGAG) ODNs, conjugated with different aromatic groups at the 5'-end through a phosphodiester bond [2]. Several modified sequences showed pronounced anti-HIV-1 activity and they showed high binding affinities for the HIV-1 envelope gp120 and gp41. In these structures the 5-end residues play a major role on the G-quadruplex stability, dramatically enhancing stability of the quadruplex complexes (ΔTm>20°C).

With the final goal to expand the repertoire of accessible end-modified G-rich ODNs, and to get a more complete picture of their structure-activity relationships, we describe herein the synthesis and characterization of a mini-library of new d(5'TGGGAG3') carrying hydrophobic groups at the 5'-end and 2-hydroxyethylphosphate group at the 3'-end, connected through phosphodiester and phosphoramidate bonds, respectively. In order to study the influence of the conjugation at the ends of the oligonucleotide chains on their ability to form quadruplex structures, a CD analysis was undertaken on the conjugated oligomers in comparison with the corresponding unmodified d(TGGGAG) oligomer.



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CSB-PO-09 Characterization of VEGFR-2 extracellular domains: a tool for new VEGF receptors binder molecules

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Angiogenesis is the process characterized by the formation of new blood capillaries from preexisting vessels, resulting from a complex balance between positive and negative regulators. The most important pro-angiogenic factor is the Vascular Endothelial Growth Factor (VEGF) that regulates blood and lymph vessel formation through activation of two receptor tyrosine kinases, VEGFR-1 and VEGFR-2 [1]. The extracellular portion of VEGF receptors consists of seven immunoglobulin homology domains, which, upon ligand binding, promote receptor dimerization. Dimerization involves domain 4 (D4) and initiates transmembrane signaling, which activates the intracellular tyrosine kinase domains of the receptors [2, 3]. Furthermore, there are structural and biochemical evidences demonstrating that homotypic contacts between the most membrane-proximal Ig-like domain of the extracellular portion (D7) of VEGF receptors play a critical role in VEGF-induced activation and cell signaling *via* VEGF receptors [4]. VEGFR-2 is considered to be the major mediator of several effects of VEGF-A on endothelial cells (ECs) and the predominant transducer of signals required for physiological and pathological angiogenesis [5]. VEGFR-2 regulates ECs proliferation, survival, migration and vascular permeability. In case of pathological conditions associated with angiogenesis, the inhibition of the VEGF/VEGFRs pathway is a promising anti-angiogenic treatment which has already found therapeutic application for example in oncology. Compounds targeting VEGF receptors could be employed in the antiangiogenic therapy as well as in angiogenesis imaging [6]. In this respect, new molecular entities as peptides have already been reported to bind to the extracellular region of VEGF receptors, acting as agonists or antagonists of their activity [7, 8]. In this report we present the expression and purification of D4 and D7 extracellular domains of VEGFR-2 and their preliminary structural characterization, in order to identify new molecules able to bind and inhibit VEGF receptors signaling.

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CSB-PO-10 Heparin-binding hemagglutinin HBHA from Mycobacterium tuberculosis affects actin polymerisation

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Mycobacterium tuberculosis (Mtb) is the etiological agent of tuberculosis (TB), the leading cause of death in the world from a bacterial infectious disease.
Dissemination from the site of primary infection involves interactions of Mtb with epithelial cells through a virulence factor called heparin-binding haemagglutinin, HBHA [1]. This is a surface exposed protein, that mediates binding of mycobacteria to epithelial cells through its C-terminal lysine rich domain, which interacts with heparan sulphate proteoglycans at the cell surface [2]. Also HBHA can cross epithelia cell layers and enter the cytoplasm. Once inside the cytosol, HBHA, but not its truncated from (HBHAΔC) induces a reorganisation of actin cytoskeleton [3]. This finding is very important in light of the newly recognised ability of Mtb to escape from the phagosome into the cytosol, where it replicates and causes cell damage that is instrumental for bacterial spread and infection of other cells. Indeed, HBHA-deficient strains are hampered in their ability to disseminate from the lungs to other tissues. Previously, using Single Molecule Force Spectroscopy, it was shown that both HBHA and HBHAΔC are able to bind actin [4], a finding that does not explain why only full-length HBHA is able to induce cytoskeleton reorganisations [3]. We here reported the actin binding capabilities studies of both HBHA and HBHAΔC. To this aim, we investigated actin filament nucleation and polymerisation, two crucial steps in intracellular organelle movements, in response to HBHA and HBHAΔC [5]. Results provide an explanation to the ability of HBHA to affect cytoskeleton morphology and strengthen the concept that HBHA is involved in Mtb pathogenesis at different dissemination levels.

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CSB-PO-11 Clickable functionalization of liposomes with gH625 peptide from Herpes simplex virus type I for intracellular delivery

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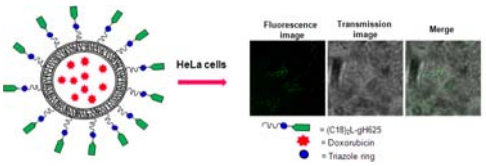
Liposomes externally modified with the nineteen residues gH625 peptide, previously identified as a membrane-perturbing domain in the gH glycoprotein of *Herpes simplex* virus type I [1], have been prepared in order to improve intracellular uptake of an encapsulated drug. An easy and versatile synthetic strategy, based on click chemistry [2], has been used to bind, in a controlled way, several copies of the hydrophobic gH625 peptide on the external surface of DOPG based liposomes. DLS measurements indicate an increase of liposomes diameter of approximately 30% after peptide introduction, and confirm the positioning of the coupled peptides on the liposome external surface. Liposomes have been loaded with the cytotoxic drug doxorubicin. Their ability to penetrate inside cells, promoted by gH625 peptide, has been evaluated by confocal microscopy experiments. Results suggest that liposomes functionalized with gH625 may act as promising intracellular targeting carriers for efficient delivery of drugs, such as chemotherapeutic agents, into tumour cells.



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CSB-PO-12 Secondary Structures in D,L-Alternating Peptides: a Model to Design Macromolecular Architectures.

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In protein structures, distinct secondary structural elements, such as helices and strand, are assembled into a compact fold that is stabilized by a complex network of tertiary interactions. In attempt to design synthetic polypeptide mimics for protein structures, a specifically designed patterning of polar and apolar residues along the synthetic sequence is generally employed in order to direct the orientation of secondary structural elements. We are pursuing an alternate approach to the construction of complex polypeptide structures in which a primary goal has been to design molecules of defined shape that are soluble in poorly interacting organic solvents. In this approach, the driving forces that determine polypeptide-chain folding are enthalpic in nature and consist primarily of hydrogen bonding and van der Waals interactions.
Alternating D,L-peptides are able to assume specific conformations including, among others, various kinds of single and double stranded β-helical structures, predicted also on theoretical ground, and α-extended chains that can aggregate through parallel or antiparallel H bonds and yeld pleated (or rippled) sheet. The stability of each structure depends on the influence of various structural factors, such as the length of the peptide chain, the nature of the lateral substituents and of the end groups, the solvents and the specific pattern of configuration (DLD or LDL) which may modify the conformational properties of the D,L-alternating peptide. The computational and spectroscopic integrated study of interparticles and collective interaction for this synthetic peptides allowed us to known the properties of different new structures.

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CSB-PO-13 A Study of Conformational Constraint Bioactive Peptides: Exploring Intermolecular Interaction and Self-assembling

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Hollow structures containing pockets and pores formed by oligopeptides and proteins are involved in numerous biological processes. Except for a small number of hollows associated with secondary structures, most voids in nature are associated with tertiary and quaternary structures of proteins. One of the most important aspect of natural hollow structures is the exquisite complementarity between their sizes and functions and those of the corresponding guest molecules, process, and reactions. With their complementarity, natural cavities and pores provide microenviroments that lead to a specific binding, catalysis, transportation, and other functions. Since the discovery of crown ether, many macrocycles have been created as host for various guests. The majority of synthetic macrocycles and their acyclic analogs have flexible backbones and thus collapsible cavities. On another front, peptidomimetic oligomers that fold into secondary structures have attracted intense interest on D,L-alternating oligopeptides. Most of these oligomers fold into secondary structures stabilized by multiple interactions that require the participation of both backbones and side chains, with overwhelming majority being helical conformations.

Hollow crescents based on backbone-rigidified oligopeptides. While most cavities and pore are associated with the tertiary and quaternary structures, some helical oligopeptides are known to contains pores. For example, the antibiotic oligopeptides Gramicidin A folds into a β-helix containing a small (~4Å across) pore. Inspired by gramicidin A and valinomicin we started a project to develop unnatural oligopeptides that folds in hollow helical conformation (β-helix) and analogues cyclic peptides that have rigid β-ring conformation. Chemical composition and surrounding medium contribute together to determine in D,L-alternating peptide specific preferences for some of the several conceivable kinds of β-helices. Looking to the example by nature pore-, or cavity-containing secondary structures, work described in this paper stemmed from the development of D,L-alternating stereo-co-oligopeptides containing any cyclic residues insert in well-defined positions of the main chain. This cyclic unit immobilize the curvature into the corresponding backbones, leads an enforced helical (or ring with cyclic peptides) conformation. As a result, a variety of reliably folded, modifiable scaffold can now be constructed. The well-defined crescent helical conformation contain noncollassable internal cavities having multiple, introverted amide bonds. Changing the backbone curvature by tuning the cyclic unit (geometry and/or position) leads to crescents ring dimension or helical with cavities of tunable sizes. We synthesized a series of molecules inserting cyclic units in well-defined position obtaining the designed, natural-like hollow structure. The computational and spectroscopic integrated study of these models allowed us to identify different new structures that will be discussed.

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CSB-PO-14 Design and synthesis of a HCA-PNA conjugates based focused mini-library

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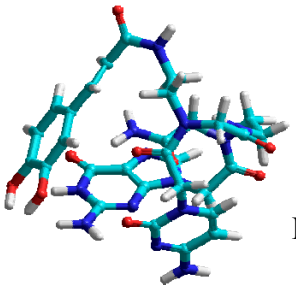
The structures and functions of natural products suggest that structural complexity of molecules is correlated with their biological activity. DOS (Diversity-Oriented Synthesis) is the facile preparation of library of structurally complex and diverse compounds from simple starting materials, typically through the use of "split-pool" combinatorial chemistry, in contrast to TOS (Target-Oriented Synthesis) which aims to prepare a specific target compound [1]. Computational chemistry and molecular modeling represent tools for the combinatorial library design and compound selection. Several virtual libraries containing all possible combinations of cores and appendages have been described [2]. Molecular descriptors are applied to virtual library in order to select the set of compounds to synthesize, for biological screening and medicinal chemistry lead generation. Hydroxycinnamic acid (HCA)-peptide conjugates exhibit a synergistically enhanced antioxidative activity [3]. Herein, we describe the design and synthesis of a focused mini-library of PNA-caffeic acid (CA) dimers conjugates. According to X^n rule (X=building blocks, n=coupling numbers), we have designed a virtual library consisting of 16 CA-PNA_2 conjugates. On the basis of calculated Log P, Log S and HyperChem analysis, we have selected the compounds with the best chemical-physical properties and positive interaction between PNA and CA residues. The selected PNA_2-CA conjugates were synthesized by conventional Fmoc chemistry on solid-phase synthesizer and characterized by NMR and MALDI-TOF analysis. Antioxidative activity studies are in progress by DPPH radical scavenging test and lipid peroxidation test with ferric thiocyanate method.



Molecular modeling of GA-HCA conjugate

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CSB-PO-15 A peptide derived from Herpes Simplex Virus type 1 glycoprotein H: membrane translocation and applications to the delivery of quantum dots

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Cell membranes are impermeable to most molecules not actively imported by living cells, including practically all macromolecules and even small molecules whose physiochemical properties prevent passive membrane diffusion. However, over the past decades, we have seen the development of increasingly sophisticated methodology for intracellular drug delivery. Cell-penetrating peptides (CPPs), representing different families of short peptides believed to enter cells by penetrating cell membranes, have attracted a great interest in the hope of enhancing gene therapy, vaccine development, and drug delivery. Nevertheless, to achieve an efficient intracellular delivery, further strategies to bypass the endocytotic pathway need to be investigated. We report on a novel peptide molecule derived from glycoprotein gH of Herpes simplex type I virus (gH625) which is able to traverse the membrane bilayer and to transport a cargo into the cytoplasm. In the present study, to accurately measure the fraction of internalised peptide, We also report confocal microscopy experiments showing the cellular uptake of gH625. In order to assess the ability of the peptide gH625 to deliver drugs inside the cell, we used quantum dots (QDs) as a model cargo. We showed that cargo molecule quantum dots, almost unable to traverse the membrane bilayer on their own, can gain constitutive access to the cell internal compartment and mainly by a non endocytotic route.

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CSB-PO-16 Copper (II) interactions with VEGF peptide fragments encompassing the VEGFR-2 binding site.

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Angiogenesis, the formation of new capillaries from existing vasculature, is a critical process in normal physiology as well as several pathophysiologies. Angiogenesis has long been associated with a heightened sensitivity to copper, but the formation of molecular species containing the metal ion with the main angiogenic agents and their roles remain elusive. The Vascular Endothelial Growth Factors (VEGFs) are the most important and extensively studied angiogenic regulators involved in multiple signaling networks. Though it has been shown that copper (II) is able to induce VEGF expression and wound healing [1] no chemical interaction results have been reported.
We investigated the copper VEGF binding features and here report the synthesis of two peptide fragments encompassing the VEGF residues, 73-101 and 84-101, that include VEGF protein β5-β6 loop aminoacid region. This domain contains the overlapping VEGF binding sites to VEGFR-2. These VEGF fragments could compete with the whole, native protein to trigger the cascade pathway involved in the angiogenesis process and contribute to clarifying the role of copper(II) in the same path. It is interesting to underline that these chosen sequences include three His residues, that are known to be preferential sites for copper(II) binding. Thus a combined spectroscopic (CD, EPR, UV, Vis) and potentiometric investigation of their copper(II) complexes was performed. Futherrmore, the functional interaction of Cu^{2+} ion with VEGF fragments and the whole VEGF protein were tested by measuring the effects on the proliferation and migration phenomena of HUVEC cells.

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CSB-PO-17 Structural characterization of proteins involved in biofilm formation

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Lactobacillus plantarum is a major component of the urogenital and intestinal microflora of most mammals, including humans. Among the probiotics it is one of the most used in processes of conservation and food processing and one of the most studied for its involvement in the immune stimulation and balancing of the intestinal microflora. We have identified within the genome of *L. plantarum* WCFS1 three unknown open reading frame proteins named Flm1, Flm2, Flm3. Interestingly, their amino acid sequences show a significant percentage of identity with the protein BrpA (biofilm regulator protein A). Chatfield et al. [1] have suggested that BrpA is located outside the cell and involved in maintaining the structure of the cell wall through the regulation of autolysines. Interestingly BrpA, as well as Flm1, Flm2, Flm3 contain a highly conserved sequence that is called *LytR-cpsA-psr*. The LytR-CpsA-Psr family of cell envelope-associated transcriptional attenuators has gained attention upon the discovery that members of this family influence various virulence factors as well as antibiotic resistance of important human pathogens. Moreover the LytR-CpsA-Psr family seems to play a role in bacterial cell envelope maintenance [1,2]. The function and structure of the LytR-CpsA-Psr domain, however, is still not known and information about this domain to date are based only on phenotypic characterizations. Here we report the preliminary structural and functional characterization of LytR-CpsA-Psr domain of the Flm1, Flm2 and Flm3 proteins in order to gain insight into the structure and the function of this interesting domain.

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CSB-PO-18 Synthesis and Antioxidant Properties of Benzoxanic Nitrones and Nitroxides

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New benzoxazinic nitrones were recently synthesized and tested as spin trapping agents [1] and it was found that they efficiently scavenge all C- and O-centered radicals. From these nitrones the corresponding nitroxides were also prepared and both nitrones and nitroxides were tested as antioxidants in biological systems. In particular, the antioxidant activity of these compounds was assessed in the oxidation of methyl linoleate micelles in aqueous dispersions induced by AAPH by following the conjugate dienes formation and in the oxidation of phosphatidylcholine liposomes by measuring the TBARS production.

1 **2**

a X = H
b X = OCH_3

Compounds **1a,b** and **2a,b** were also compared with well known antioxidants (TEMPO, PBN, Trolox) and with indolinic nitroxides whose antioxidant activity was extensively studied and demonstrated in the past by our group. [2]
The results obtained clearly indicate that nitroxides **2a,b** are very good antioxidants in both the systems used and they are much better than the indolinic ones (longer lag time in conjugated dienes formation and higher % inhibition of TBARS production). Nitrones **1a,b** are also good inhibitor of TBARS production but they have a "retardation" effect [3] when the formation of conjugated dienes is followed (no lag time, but slower rate of oxidation).

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CSB-PO-19 Evolution of classical zinc finger domains

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Cys_2His_2 zinc-finger domain is one of the most important eukaryotic structural motifs involved in protein–DNA interactions. It is a small domain consisting of 30 aa in which a zinc ion, crucial for its stability, is tetrahedrally coordinated by two cysteines and two histidines. Its amino acid consensus sequence is $(F/Y)XCX_{2-5}CX_3(F/Y)X_5\psi X_2HX_{3-5}H$, where X represents any amino acid and ψ is any hydrophobic amino acid; ψ forms with the other two hydrophobic residues (F/Y) a small hydrophobic core that together with the zinc ion stabilizes a compact 3D structure with a ββα fold.
This eukaryotic domain is also present in several prokaryotic proteins, but with some structural differences. The first prokaryotic Cys_2His_2 zinc finger domain has been identified in the transcriptional regulator Ros[1] from *A. tumefaciens.* Its globular domain consists of 58 amino acids arranged in a βββαα topology and it is stabilized by an extensive hydrophobic core (15 aa) [2]. To date, a large number of homologues have been found in different bacteria, with a high sequence identity to the Ros protein [3,4].
Here, a model for the distribution and the evolution of zinc finger domains in bacteria and eukaryotes is proposed. Zinc finger domain-containing proteins are equally distributed among diverse Alphaproteobacteria, with distinct amino acid compositions in specific families. In some cases, the zinc-finger domain can either change the coordination sphere or lose the metal while still preserving the DNA binding activity. To investigate a possible correlation between prokaryotic and eukaryotic zinc-finger domains, we conducted two separate phylogenetic analyses, one including all retrieved domain sequences and the second including only domains of prokaryotic proteins and their homologues. Our analysis allows to speculate that there is an evolutionary link between the two kingdoms, based on bacteria-to-eukaryota horizontal gene transfer (HGT).

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CSB-PO-20 Characterization of the metal binding site in prokaryotic zinc-finger: structure and dynamics of Ros87_H42A

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Ros protein from *A. tumefaciens* is the first prokaryotic classical zinc finger protein. Ros87, a mutant of Ros wild-type obtained by deletion of the first fifty-five amino acids, which is soluble and contains the zinc finger domain, is still able to bind DNA[1]. The NMR structure of Ros87[2] consists of a very well defined globular domain, in which the zinc ion is tetrahedrally coordinated by Cys-24 and Cys-27 and by His-37 and His-42, and two disordered tails at the N- and C-terminal region. Ros87 globular fold has ββαα topology and it is stabilized by an extended hydrophobic core of 15 amino acid. These new features define a novel fold never found in literature. The mutant H42A of Ros87 (in which the second coordinating histidine is mutated to alanine) is still able to bind the specific DNA sequence. Moreover, HSQC-J18 experiment demonstrated that in H42A the zinc ion is tetrahedrally coordinated by Cys-24, Cys-27, His-37 and His-41[1] because when His-42 is mutated in Ala, His-41 is able to occupy the fourth position of the zinc coordination, changing its tautomeric form from the N_{e2}-H tautomer, observed in the wild-type protein, to the $N_{\delta 1}$-H tautomer. We report here the complete NMR structural, dynamic and functional characterization of Ros87_H42A mutant in order to investigate the properties of this zinc coordination site.

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CSB-PO-21 Molecular and supramolecular structure of elastin model peptides containing (2S,4R)-4- hydroxy-proline

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The study deals essentially with the synthesis and the structural analysis of nanostructured polypeptides with potential interest in the field of biomaterials science.
Taking advantage of the unique abundance of repetitive sequences in elastomeric proteins, it is planned the synthesis of polypeptides of different size and alternative sequences, followed by crosslinking reaction in order to obtain polymeric material with elastic features. Several elastic proteins was considered as natural models for the design of elastic biomaterials. Common feature of the elastomeric proteins such as elastin, abductin, and resilin is the high content of glycine residues in their sequences, which ensures high flexibility to the monomer chains; they differ for the presence of other aminoacid residues. *[1]*.
Elastomeric protein inspired biomaterial are of paramount interest, for their intrinsic elastic properties, as well as for their straightforward design. The ease of polymer design is due to the presence of small-sized repetitive sequences in elastomeric proteins, such as for example the elastin-related repeats: VPGXG or XGGZG (x,Z= hydrophobic aminoacids).*[2]*.
In elastin they are mainly hydrophobic residues, like valine, leucina, proline, alanina, while in abductin and resilin other residues are present. Other features distinguishing the two proteins are the size (elastin and resilin are constituted by ca 750 and 621 residues respectively, while abductin by 136 aminoacids) and the type of crosslinks. The chemical syntheses of the polypeptides was be carried out by solid phase peptide synthesizer and classical solution synthesis, and in some cases polycondensations $(VPGVG)_{10}$.
We study the effect of hydroxylation on some peptide like (VPGVG) and exon 18 [(*)] of elastin change prolyne with (2S,4R)-4- hydroxy-proline.

Peptide	Sequence
$(VHypGVG)_{10}$	VHypGVG VHypGVG VHypGVG VHypGVG VHypGVG VHypGVG VHypGVG VHypGVG VHypGVG VHypGVG
Esone 18 H	GAAAGLVPGGPGFGPGVVGVPGAGVPGVGVPGAGIPVVPGAGIPGAAV (*)

Esone 18 H

AFM study effettued on exon 18 mostred fiber similar to other AFM microscopies on exon like 30_18 or exon 26. (Figure 1)

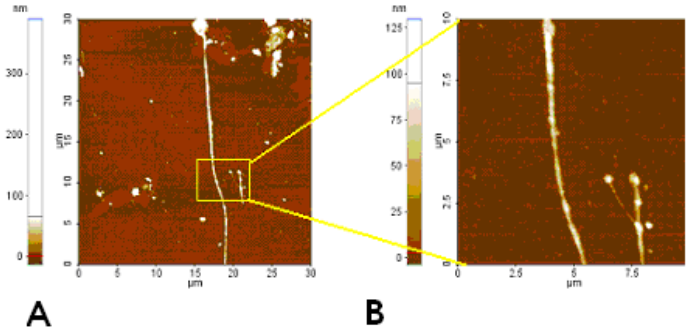

Figure 1: Exon 18. AFM images of pit refilling after water depositing *on wafer silicio (100) (A) and zoom (B).*

AFM study effettued on exon 18 H mostred aggregated dissimilar to exon 18 and elastin in general

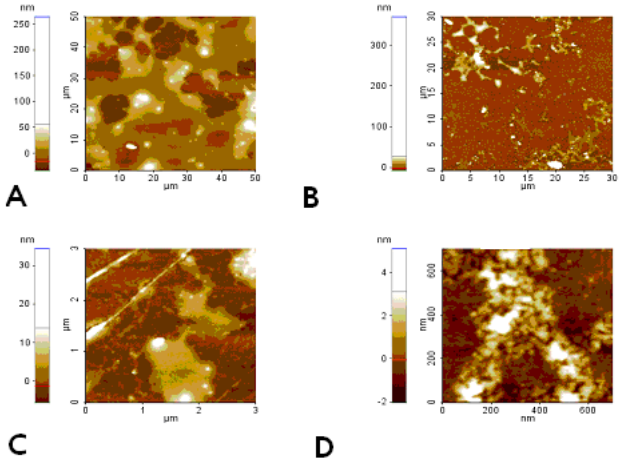

Figure 2: Exon 18 H. AFM images of pit refilling after water depositing *on wafer silicio (100) (A) and zoom (B).), (B,C,D) AFM images after incubation (50 ° for 48 h).*

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CSB-PO-22 Copper(II) and zinc(II) interaction with Aβ42: effects of metal binding on peptide's aggregation rate and morphology of the aggregates.

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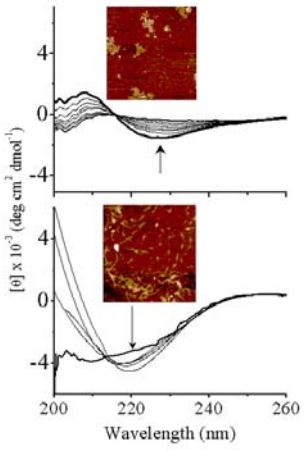
Altered levels of zinc(II) and copper(II) in different brain districts have been implicated in various aspects of Alzheimer's diseases.[1] In particular, it is well established that metal ions play a major role in the self-assembling of Aβ, but their effects on fibrillogenesis and morphology of peptide aggregates are not fully elucidated yet and conflicting results are reported in the literature.[2] In the attempt to shed light on these debated issues, two slightly different monomerization protocols were developed to mimic "seeded" and "unseeded" Aβ(1-42) assembling. Then, metal effects on the peptide aggregation and morphology were comparatively investigated by CD, ThT fluorescence and SFM techniques. Our results indicate that unlike copper(II) which promotes the formation of amorphous aggregates, zinc(II) is quite able to convert soluble Aβ peptides into amyloid-like structures. The obtained results might contribute to set up a hypothesis that correlates metals' coordination modes and different aggregate morphologies as well as in vitro toxicities towards neuronal cell cultures.



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CSB-PO-23 Insights on channel selectivity from the structural and functional characterization of the Kv1.3 channel blocker Tc32.

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The utility of toxins in biomedical research, diagnosis, and therapy is widely recognized. Unfortunately their use is limited by an inadequate target discrimination. Thus, the search for target-specific toxins is of primary relevance. The fact that despite the incredible number of toxins present in the animal kingdom, only a limited number of molecular scaffolds has been selected, is a clear evidence of the importance of the nature and spatial orientation the side chains. The description and understanding of the contact surface between the toxin and the channel entrance appears to be the target for the rationale design of selective and high affinity drugs.

Tc32 toxin from the scorpion *Tityus cambridgei* has been reported to have a clear inhibitory effect on Kv1.3 K^+ channel [1]. This channel, member of the *Shaker* family [2], carries a large proportion of the outward current not only in leucocytes [3] but also in a variety of neuronal cells [4].

In the present work, Tc32 has been cloned and expressed in a soluble and active form for the first time, employing a new protocol we devised [5]. Tc32 activity has been characterized by electrophysiological assays on a distinct subpopulation of periglomerular cells of olfactory bulb and its 3D solution structure determined by ^{1}H-NMR spectroscopy. The structure reveals it exhibits an α/β scaffold typical of the members of the α-KTx family. A structural comparison with the other members of α-KTx 18 subfamily is presented following molecular modeling calculations, and docking simulations to Kv1.1 and Kv1.3 channels.

Our data point out Tc32 as a good lead molecule for the development of new molecules suited for research, diagnosis and therapy.

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CSB-PO-24 Insight into the C-terminal domain of h-Prune: a biochemical and structural characterization

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H-prune has been identified as one of the determinants of metastasis in breast cancer [1]. This protein is a member of the DHH superfamily and its overexpression is correlated to tumor aggressiveness and development of metastasis. H-prune has a phosphodiesterase activity (cAMP-PDE) and inhibition of this activity by dipyridamole can abolish cell motility [2]. The metastatic suppressor Nm23-H1 has been characterized as one of the most important interaction partner of h-prune. In vivo data have shown that binding of Nm23-H1 to h-prune inhibits its function and induces the formation of metastasis. Sequence and limited proteolysis analyses have identified three domains in h-prune: the DHH domain (residues 10-180), the DHHA2 domain (215-330), encompassing the residues responsible for substrate binding and important for specificity, and a C-terminal domain (residues 330-453, named Prune-C), homologue to cortexillin which contains regions rich in prolines and possible coiled-coils [3]. A biochemical study was started on h-prune. Prune-C was the only domain to be obtained stable and soluble with respect to the other h-prune domains (DHHA and DHHA2). A western blotting analysis revealed that it is sufficient to bind the Nm23-H1 endogenous protein. Therefore a structural characterization has been performed by circular dichroism, light scattering and limited proteolysis. An uniformly labeled N^{15} and C^{13} C-prune sample has been prepared and used for NMR structural analyses. At last, by using NMR spectoscopy, we determined the three-dimensional structure of C-terminal Prune (residues 353-453) and mapped the minimal region of interaction with nm23-H1. By making use of a multidisciplinary experimental approach this study provides the basis for the rational design of molecules able to take part in the interaction between H-prune and Nm23-H1 for future therapeutic specific applications in cancer and can contribute to the identification, validation and development of novel anti-metastatic agents.

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CSB-PO-25 ELUCIDATION OF THE ACTION MECHANISM OF NEW ANTIMALARIAL ENDOPEROXIDES: A DFT INVESTIGATION TO SUPPORT DRUG DESIGN.

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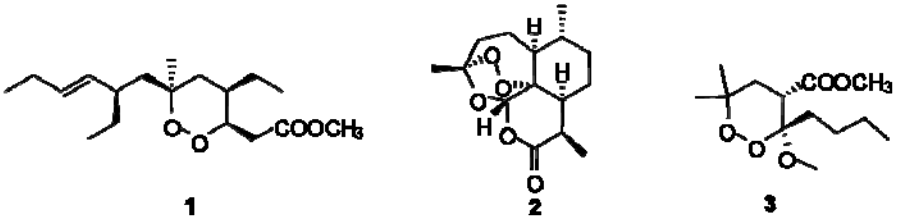
Malaria is a global health threat for about 40% of the world population, mainly located in the tropical and subtropical areas where every thirty seconds a child affected by this disease is destined to die. The therapeutic choices are still too limited for the large and poor malaria market and there is an urgent need of new and economically affordable antimalarial drugs. In this context, through a multidisciplinary approach, we designed new antimalarials based on the dioxane scaffold of plakortin (**1**).



1 **2** **3**

As the mechanism of action of antimalarial endoperoxides is not well understood yet, we performed a density functional study aiming to elucidate thermodynamic and kinetic features of our new lead compound **3** compared to reference compounds plakortin (**1**) and artemisinin (**2**). In details, the possibility of the dissociative electron transfer induced by Fe(II)-heme in the first step of the process was verified through the theoretical evaluation of the antimalarial redox potential E°. The combination of density functional theory and polarizable continuum model (PCM) of the solvent assures to obtain E° reliable values [1] against the demonstrated limits of usual experimental procedures [2]. Besides, to validate the through-space H-shift mechanism of action hypothesized in our previous studies [3], we identified the molecular species involved in the process with the aim to trace the reaction pathway. With this purpose, a DFT investigation of pre-reactive complexes, transition states and radical intermediates was performed considering all the coordination modes of iron to the endoperoxide function and different spin states of the metal.

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CSB-PO-26 The X-ray structural view of the complex between human alpha thrombin and a DNA aptamer directed to exosite II

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The aim of anticoagulant/antithrombotic therapy in cardiovascular disease is to prevent fibrin deposition and platelet aggregation, halting current and future ischemic episodes. The limitations to the effectiveness of the most commonly used agents (heparin, coumadin and aspirin) have led to the development of new anticoagulant compounds. In particular, aptamers represent an attractive approach because of their high specificity and low immunogenicity. The best known example is the Thrombin Binding Aptamer (TBA), namely $^{5'}$GGTTGGTGT-GGTTGG$^{3'}$[1]. TBA and its derivatives [2] adopt a G-quadruplex structure [2-6] and inhibit thrombin activity by blocking the fibrinogen binding site (exosite I) [3-6]. Biological properties of TBA are strictly dependent on its tertiary structure. Other antithrombotic aptamers have been identified using the SELEX process and partially characterized. Among them HD22, namely $^{5'}$GTCCGTGGTAGGGCAG-GTTGGGGTGAC$^{3'}$, is particularly interesting. It presents a 15-nucleotide core sequence that has striking similarity to TBA, and it has been reported to adopt a mixed duplex/quadruplex structure [7]. Remarkably HD22 binds thrombin with much higher affinity than TBA and has been shown to bind exosite II instead of exosite I [7]. No structural data on thrombin-HD22 complex has been reported so far. We have solved the X-ray structure of the thrombin-HD22 complex, with the aim to understand the molecular details of the interaction between the two molecules and to investigate the differences with respect to thrombin-TBA complex, whose structure we have recently determined at high resolution. These results could help the design of a new class of aptamers with an improved capability to modulate thrombin function.

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CSB-PO-27 Non-covalent interactions in organotin(IV) derivatives of 5,7-ditertbutyl- and 5,7-diphenyl-1,2,4-triazolo[1,5-a]pyrimidine as recognition motifs in crystalline self- assembly and their *in vitro* antistaphylococcal activity.

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Non-covalent interactions are known to play a key role in biological compounds due to their stabilization of the tertiary and quaternary structure of proteins [1]. Ligands similar to purine rings, such as triazolo pyrimidine ones, are very versatile in their interactions with metals and can act as model systems for natural bio-inorganic compounds [2]. A considerable series (twelve novel compounds are reported) of 5,7-ditertbutyl-1,2,4-triazolo[1,5-a]pyrimidine (**dbtp**) and 5,7-diphenyl-1,2,4-triazolo[1,5-a]pyrimidine (**dptp**) were synthesized and investigated by FT-IR and ^{119}Sn Mössbauer in the solid state and by ^{1}H and ^{13}C NMR spectroscopy, in solution [3]. The X-ray crystal and molecular structures of $Et_2SnCl_2(dbtp)_2$ and $Ph_2SnCl_2(EtOH)_2(dptp)_2$ were described, in this latter pyrimidine molecules are not directly bound to t through N(3), to the -OH group of the ethanol moieties. T aromatic interactions involving pyrimidine and phenyl rings in both complexes drives their self-assembly. Non-covalent interactions involving aromatic rings are key processes in both chemical and biological recognition, contributing to overall complex stability and forming recognition motifs. It is noteworthy that in $Ph_2SnCl_2(EtOH)_2(dptp)_2$ π–π stacking interactions between pairs of antiparallel triazolopyrimidine rings mimick base-pair interactions physiologically occurring in DNA (Fig.1).

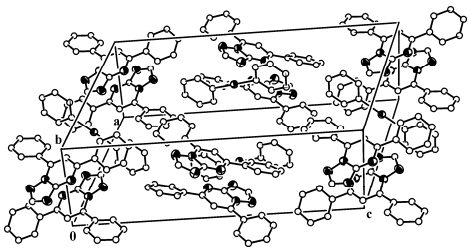

Figure 1. Edge-to-face and π-stacking interactions in 11

Mössbauer spectra suggest for $Et_2SnCl_2(dbtp)_2$ a distorted octahedral structure, with C-Sn-C bond angles lower than 180°. The estimated angle for $Et_2SnCl_2(dbtp)_2$ is virtually identical to that determined by X-ray diffraction. $Ph_2SnCl_2(EtOH)_2(dptp)_2$ is characterized by an essentially linear C-Sn-C fragment according to the X-ray all-trans structure.

The compounds were screened for their *in vitro* antibacterial activity on a group of reference staphylococcal strains susceptible or resistant to methicillin and against two reference Gram-negative pathogens [4] . We tested the biological activity of all the specimen against a group of staphylococcal reference strains (*S. aureus* ATCC 25923, *S. aureus* ATCC 29213, methicillin resistant *S. aureus* 43866 and *S. epidermidis* RP62A) along with Gram-negative pathogens (*P. aeruginosa* ATCC9027 and *E. coli* ATCC25922). $Ph_2SnCl_2(EtOH)_2(dptp)_2$ showed good antibacterial activity with a MIC value of 5 μg mL^{-1} against *S. aureus* ATCC29213 and also resulted active against methicillin resistant *S. epidermidis* RP62A.

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CSB-PO-28 Structural studies of proteins in the intracellular environment: design and characterization of suitable cell systems

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The ability to transfer complex molecules, like proteins, within cells represents a field of considerable scientific interest for both basic research and applied. Recently, the direct transfer of macromolecules in animal cells was obtained through the so-called "cell-penetrating peptides" (CPP), characterized by the property to move across cell membranes. Using advanced methods of NMR, molecular and cellular biology, it was possible to merge these peptides to proteins of interest so as to facilitate their internalization and then to obtain heteronuclear multidimensional NMR spectra of macromolecules *in vivo* at high resolution, both in eukaryotes than in prokaryotes [1]. In this way, it was possible to analyze in detail a number of intracellular events such as conformational changes and dynamic binding events between biological molecules. This study is aimed at obtaining a construct of the C-terminal domain of protein h-prune as a fusion product with a peptide for cellular internalization. The human protein h-prune, belonging to the DHH phosphoesterase protein family, induces cell motility and enhances cancer metastases and through its C-terminal region it interacts with many partners [2]. This strategy involved the use of a portion of the glycoprotein H of herpes simplex virus type 1 (HSV-1) involved in the complex mechanism of fusion between the virus envelope and host cell membrane. Biochemical and NMR spectroscopy studies showed that alpha-helical nature of this peptide is important for the interaction of the membranes and that the aromatic residues in the sequence are essential to ensure the merger [3]. The nucleotide sequence of the peptide was amplified from the genome of HSV-1 and then fused to the sequence encoding the C-terminal domain of h-prune.
Different cell strains of *E. coli*, conditions of temperature, time of induction were examined to optimize the expression levels. Size exclusion chromatography, ESI-MS spectrometry and CD analyses were used to characterize CPP-prune-C. The internalization in tumor cells was evaluated by FACS. In the future it will be essential to carry out studies of NMR *in vitro* of protein "CPP-prune-C" to obtain structural information as a starting point for subsequent experiments of *in vivo* NMR.

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CSB-PO-29 Novel supramolecular aggregates based on monoolein or diolein as target selective contrast agents.

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Magnetic Resonance Imaging (MRI) is a very powerful diagnostic imaging technique [1] giving very resolved images; unfortunately its sensitivity is very poor. During the last years, dendrimers, polymers, and supramolecular aggregates, such as micelles and liposomes, [2] all of them containing a high number of stable gadolinium(III) complexes, have been proposed as very effective contrast agents in MRI. In these high molecular weight compounds, the rotation motion of the Gadolinium complexes is slowed down with the effect of enhancing the relaxivity for each single Gd(III) complex in the compound. In order to obtain new contrast agents with enhanced properties, recently we studied supramolecular aggregates characterized by the presence of a large amount of Gadolinium complexes and of bioactive peptides exposed on their surface [3]. Here we report novel supramolecular aggregates consisting of monoolein (MO) or diolein (DO) with reversed mesophases. These particles, also termed "sponge" nanoparticles, are assumed to consist of a core of L2-(reversed micelle) phase which is stabilized by a shell of L3-(sponge) phase. In order to obtain target selective MRI contrast agents, MO or DO compositions were enriched with $(C18)_2$DTPA(Gd) monomer (from 1% to 20%) and with 3% of $(C18)_2$-Peg3000-folic acid monomer. Gadolinium amphiphilic monomer $(C18)_2$DTPA(Gd) was synthesized according to solid-phase procedures, and aggregates at several weight ratios were formulated by sonication and homogenization procedures. Structural properties of aggregates were defined by Cryo-TEM and DLS techniques. Moreover, relationship between structure and relaxometric behaviour of the sponges was clarified by HNMR and DNMR studies. Preliminary studies on the cellular uptake of sponges by confocal microscopy and by MRI are now in progress on human ovarian adenocarcinoma cell lines overespressing folate receptors.

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CSB-PO-30 Bombesin labelled Liposomes as target selective delivery system for Doxorubicin

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Conventional doxorubicin (DOX) is an established cytotoxic agent for many cancer types, in particular breast cancer. However, irreversible cardiotoxicity has been one of the primary dose-limiting toxicities for this drug [1]. A successful strategy for reducing the cardiotoxicity associated with conventional doxorubicin involves encapsulation into liposomes, which alters the tissue distribution and pharmacokinetics of these agents with the objective of maintaining efficacy and improving the therapeutic index [2]. All liposomal doxorubicin formulations (e.g. Caelyx®/Doxil®) presently marketed are based on non-specific liposomes. To increase therapeutic efficacy of the encapsulated drug and reduce potential toxic side effects on non-target cells, we suggest nanovectors decorated by Bombesin peptide able to deliver a constant dose of chemo-therapeutic agent directly and selectively to cancer cells over an extended period of time. The Bombesin receptor subtype 2 (GRPR) has been found overexpressed by tumor cell lines of several human tumors (ovarian cancers, breast cancers and prostate cancer) [3]. Many studies demonstrate that both the fourteen-residues Bombesin peptide (BN) and its eight-residues C-terminal peptide sequence ([*7-14*]BN) can be used to target these receptors. [7-14]BN containing liposomes were obtained by mixing the synthetic monomer MonY-Peg27(DTPA)-BN and two commercial phospholipid DPPC or DSPC. The development of best liposome formulation and of DOX loading procedures were reported. The cellular uptake and cytotoxicity of the targeted liposomal DOX with respect to the non-specific liposomal DOX, were evaluated in vitro on tumor PC-3 cell lines.

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CSB-PO-31 Antioxidant effect of nitroxide functionalized lipids in liposomes peroxidation

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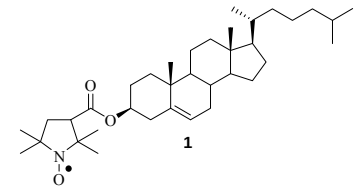
Liposomes are biodegradable and nonimmunogenic vesicular structures made of amphipatic phospholipids which can encapsulate both hydrophilic and hydrophobic materials; because of their versatility they are used as carriers in drugs and gene delivery systems. Polyunsaturated fatty acids present in the bilayer are susceptible to oxidative damage which can alter some important properties like fluidity and permeability and can cause system delivery destruction. The mechanism of lipids oxidative degradation may involve free radical reactions that lead to changes in the unsaturated systems present and eventually to the degradation of the lipid chains. In order to maintain their physical, chemical and functional properties, liposomes need to be protected by antioxidant compounds that can delay or prevent oxidation upon reaction with radical species. Nitroxide radicals are known to protect against oxidative processes in different media and under different stress conditions [1]. As part of our ongoing research in the field of non viral vectors for DNA delivery [2] we synthesized the neutral non zwitterionic lipids 1 and 2 which bear a nitroxide function able to react with radical species. Multilamellar liposomes were prepared by mixing the new lipids with PC and other commercial neutral zwitterionic lipids. Lipid peroxidation was induced by thermal decomposition of the free radical generator AAPH and evaluated using the TBARS assay. The results obtained clearly indicate that both lipids efficiently inhibit PC peroxidation and are better antioxidants than nitroxides (higher % inhibition of TBARS production). In view of a possibile use of liposomes as DNA transfection agents, the liposomes containing nitroxide lipids were complexed with DNA in the presence of bivalent Ca^{++} cations. Ternary complexes formation was investigated by means of synchrotron X-ray diffraction and the antioxidant effect of the functionalized lipids was also studied in these systems.



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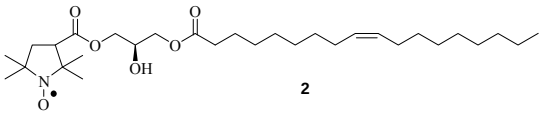

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