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# SOMMARIO
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Conference

# Supra and Sub Molecular Investigation of Pathologic Tissues by X-Ray Scanning Microscopy.

Cinzia Giannini,[a,*] Davide Altamura,[a] Teresa Sibillano,[a] Alberta Terzi,[a] Francesco Scattarella,[a] Dritan Siliqi,[a] Rocco Lassandro,[a] Massimo Ladisa,[a] Liberato De Caro.[a]

X-ray Small and Wide Scattering scanning microscopies have been adopted to inspect morphological and structural properties of collagen-based tissues at the atomic and nano scale[1]. Examples will be discussed on specific pathologies:

- osteoarthritis of the hip, also named osteoarthrosis of the hip or coxarthrosis, which is a chronic degenerative disorder of the hip joint, causing growing articular pain that can bring the patient to lifestyle limitations until surgical intervention is needed[2]
- keratoconus, a pathology affecting cornea, which causes progressive thinning of the stroma and consequently abnormal curvature, inducing irregular astigmatism and myopia, corneal fibrosis, and distortion of vision, due to the modification in the organization of the corneal collagen[3]
- abdominal aortic aneurysm, that occurs in the major artery from the hearth that supplies blood to the abdomen, and popliteal aneurysm, that takes place in the legs, behind the knees, characterized by alteration of collagen structure into vessel's wall of the aneurysm tissues, heterogeneous grade of inflammation related to infiltrating cells and extracellular matrix changes, in particular disruption of elastic fibers, fibrosis and calcifications[4]
- diabetes mellitus, a metabolic disorder characterized by high blood sugar levels over a prolonged period due to defects in insulin action or secretion, which causes collagen to have a fixed orientation, stiffen the tissue and is likely to disrupt the normal cell interactions.[5]

## References

1 C. Giannini, D. Altamura, M. Aresta, S. Sibillano, Teresa, Dritan, L. De Caro, Inorganic micro-and nanomaterials: synthesis and characterization, Edited by A. Dibenedetto and M. Aresta, Published by Walter de Gruyter, 2013.

2 C. Giannini, D. Siliqi, M. Ladisa, D. Altamura, A. Diaz, A. Beraudi, T. Sibillano, L. De Caro, S. Stea, F. Baruffaldi, O. Bunk, Scanning SAXS–WAXS microscopy on osteoarthritis-affected bone – an age-related study, Journal of Applied Crystallography 47 (1) (2014) 110–117. doi:10.1107/S1600576713030215.

3 T. Sibillano, L. De Caro, F. Scattarella, G. Scarcelli, D. Siliqi, D. Altamura, M. Liebi, M. Ladisa, O. Bunk, C. Giannini, Interfibrillar packing of bovine cornea by table-top and synchrotron scanning SAXS microscopy, Journal of Applied Crystallography 49 (4) (2016) 1231–1239. doi:10.1107/S1600576716010396.

4 C. Giannini, M. Ladisa, V. Lutz-Bueno, A. Terzi, M. Ramella, L. Fusaro, D. Altamura, D. Siliqi, T. Sibillano, A. Diaz, F. Boccafoschi, O. Bunk, X-ray scanning microscopies of microcalcifications in abdominal aortic and popliteal artery aneurysms, IUCrJ 6 (2) (2019) 267–276. doi:10.1107/S2052252519001544.

5 C. Giannini, L. De Caro, A. Terzi, L. Fusaro, D. Altamura, A. Diaz, R. Lassandro, F. Boccafoschi, O. Bunk, Decellularized pericardium tissues at increasing glucose, galactose and ribose concentrations and at different time points studied using scanning X-ray microscopy, IUCrJ 8 (4) (2021) 621–632. doi:10.1107/S2052252521005054.

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# Structure-based prediction of hERG-related cardiotoxicity: a benchmark study.

Giuseppe Felice Mangiatordi,[a,*] Pietro Delre,[a,b] Teresa Maria Creanza,[c] Nicola Ancona,[c] Giovanni Lentini,[d] Michele Saviano,[a]

Drug-induced blockade of the human ether-à-go-go-related gene (hERG) channel is today considered the main cause of cardiotoxicity in post-marketing surveillance. Hence, several ligand-based approaches were developed in the last years and are currently employed in the early stages of a drug discovery process for in silico cardiac safety assessment of drug candidates. The first structure-based classifiers able to discern hERG binders from non-binders will be presented. LASSO regularized Support Vector Machines were applied to integrate docking scores and protein-ligand interaction fingerprints. 396 models were trained and validated based on: i) high-quality experimental bioactivity information returned by 8,337 curated compounds extracted from ChEMBL (version 25[1]) and ii) structural predictor data. Molecular docking simulations were performed by using GLIDE and GOLD software programs and four different hERG structural models, namely the recently published structures obtained by cryo-electron microscopy (PDB codes: 5VA1[2] and 7CN1[3]) and two published homology models selected for comparison. Interestingly, some classifiers return performances comparable to ligand-based models in terms of accuracy (AUCMAX = 0.86±0.01) and negative predictive values (NPVMAX = 0.81±0.01) thus putting forward the herein presented computational workflow as a valuable tool for predicting hERG-related cardiotoxicity without the limitations of ligand-based models, typically affected by low interpretability and a limited applicability domain. From a methodological point of view, the study represents the first effort to develop classifiers integrating docking scores and protein-ligand interaction fingerprints, an approach ensuring significantly better accuracies than those returned by classifiers based on docking scores only ($p < 0.05$). Finally, the study highlights the importance of using hERG structural models accounting for ligand-induced fit effects and allowed us to select the best performing protein conformation to be employed for a reliable structure-based prediction of hERG-related cardiotoxicity.

## References

1 M. Davies, M. Nowotka, G. Papadatos, N. Dedman, A. Gaulton, F. Atkinson, L. Bellis, J. P. Overington, ChEMBL web services: streamlining access to drug discovery data and utilities, Nucleic Acids Research 43 (W1) (2015) W612–W620. doi:10.1093/nar/gkv352.

2 W. Wang, R. MacKinnon, Cryo-em structure of the open human ether-à-go-go-related k+ channel herg, Cell 169 (3) (2017) 422–430.e10. doi:https://doi.org/10.1016/j.cell.2017.03.048.

3 T. Asai, N. Adachi, T. Moriya, H. Oki, T. Maru, M. Kawasaki, K. Suzuki, S. Chen, R. Ishii, K. Yonemori, S. Igaki, S. Yasuda, S. Ogasawara, T. Senda, T. Murata, Cryo-em structure of k+-bound herg channel complexed with the blocker astemizole, Structure 29 (3) (2021) 203–212.e4. doi:https://doi.org/10.1016/j.str.2020.12.007.

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Conference

# Structural Insights into the Intracellular Region of the Human Magnesium Transport Mediator CNNM4. †

Dritan Siliqi,[a,*] Paula Giménez-Mascarell,[b] Iker Oyenarte,[b] Luis Alfonso Martínez-Cruz,[b]

The four member family of "Cyclin and Cystathionine-synthase (CBS) domain divalent metal cation transport mediators", CNNMs, are the least-studied mammalian magnesium transport mediators. CNNM4 is abundant in the brain and the intestinal tract, and its abnormal activity causes Jalili Syndrome. Recent findings show that suppression of CNNM4 in mice promotes malignant progression of intestinal polyps and is linked to infertility.

The association of CNNM4 with phosphatases of the regenerating liver, PRLs, abrogates its $Mg^{2+}$-efflux capacity, thus resulting in an increased intracellular $Mg^{2+}$ concentration that favors tumor growth. Here we present the crystal structures of the two independent intracellular domains of human CNNM4, i.e., the Bateman module and the cyclic nucleotide binding-like domain (cNMP).[1] We also derive, by Small Angle X ray Scattering (SAXS) as shown in Figure 1, a model structure for the full intracellular region in the absence and presence of MgATP and the oncogenic interacting partner, PRL-1.[1] We find that only the Bateman module interacts with ATP and $Mg^{2+}$, at non-overlapping sites facilitating their positive cooperativity. Furthermore, both domains dimerize autonomously, where the cNMP domain dimer forms a rigid cleft to restrict the $Mg^{2+}$ induced sliding of the inserting CBS1 motives of the Bateman module, from a twisted to a flat disk shaped dimer.

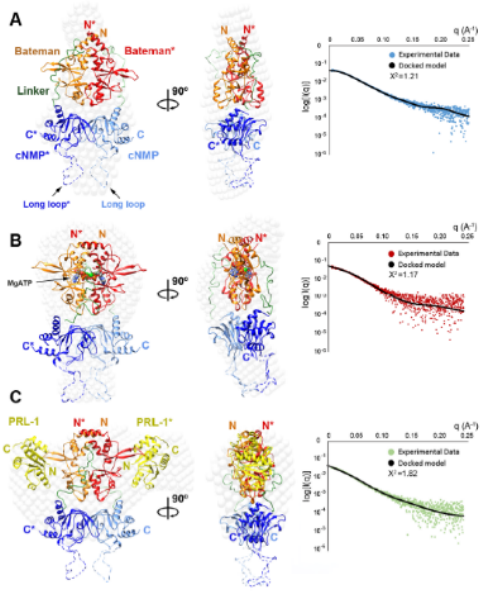


**Fig. 1** Solution structure of $CNNM4_{BAT-cNMP-Ctail}$ alone and in complex with PRL-1. SAXS derived models for A) $CNNM4_{BAT-cNMP-Ctail}$ in the absence of MgATP, B) $CNNM4_{BAT-cNMP-Ctail}$ in the presence of MgATP, C) the complex formed by PRL-1 and $CNNM4_{BAT-cNMP-Ctail}$ in the presence of MgATP.

## References

1 P. Giménez-Mascarell, I. Oyenarte, I. González-Recio, C. Fernández-Rodríguez, M. A. Corral-Rodríguez, I. Campos-Zarraga, J. Simón, E. Kostantin, S. Hardy, A. Díaz Quintana, M. Zubillaga Lizeaga, N. Merino, T. Diercks, F. J. Blanco, I. Díaz Moreno, M. L. Martínez-Chantar, M. L. Tremblay, D. Müller, D. Siliqi, L. A. Martínez-Cruz, Structural insights into the intracellular region of the human magnesium transport mediator cnnm4, International Journal of Molecular Sciences 20 (24) (2019) 6279. doi:10.3390/ijms20246279.

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Conference

# Synthesis of Porphyrin-Peptide conjugates and interaction with A$\beta_{42}$: potential use of the systems as theranostic agents in Alzheimer's disease.

Rita Tosto,[a,b,*] Giuseppe Di Natale,[a] Tiziana Campagna,[a] Francesco Bellia,[a] Giuseppe Pappalardo,[a]

Alzheimer's disease (AD) is the most common cause of senile dementia affecting more than 50 million people worldwide. AD is a disorder of the central nervous system, clinically characterized by progressive loss of memory and other cognitive skills. Unfortunately, there is no cure for AD.

The major pathophysiological hallmark is the formation of amyloid deposits with a common $\beta$-sheet structure[1]. Amyloid plaques are the results of a long process initiated with the seeding and production of smaller aggregated soluble forms of A$\beta$ called oligomers. They are the underlying toxic species responsible for synaptic dysfunction in the brains of AD patients.

Our research focus on development of new compounds able to interfere with the early stages of the aggregation process of the A$\beta$ peptides. Our compounds could be of interest as potential drug candidates AD therapy. We designed and synthesized a series of di-functional systems explicating synergic and/or additive actions to counteract the adverse effects of A$\beta$ aggregated forms.[2,3]

In particular, we covalently linked a cationic metallo-porphyrin with the well-known A$\beta$-recognizing KLVFF amino acid sequence.[4] The peptide conjugation to the porphyrin macrocyclic was first accomplished via the formation of an amide. A second generation was obtained in good yields using a click chemistry approach.

In this communication, we describe the different synthesis approach and the ability of the conjugated porphyrin peptides to interact with A$\beta$, together with the role of metal ions (Zn or Cu) at the core of porphyrin macrocycle in assisting the recognition process.[5]

The interaction between the peptide conjugate and A$\beta$ was studied by using an array of different biophysical techniques including Dynamic Light Scattering (DLS), far-UV, Circular Dicroism (CD), Fluorescence spectroscopy and MALDI-TOF-MS.

## References

1 W. M. Tay, D. Huang, T. L. Rosenberry, A. K. Paravastu, The alzheimer's amyloid-$\beta$(1–42) peptide forms off-pathway oligomers and fibrils that are distinguished structurally by intermolecular organization, Journal of Molecular Biology 425 (14) (2013) 2494 – 2508. doi:https://doi.org/10.1016/j.jmb.2013.04.003.

2 P. De Bona, M. Laura Giuffrida, F. Caraci, A. Copani, B. Pignataro, F. Attanasio, S. Cataldo, G. Pappalardo, E. Rizzarelli, Design and synthesis of new trehalose-conjugated pentapeptides as inhibitors of a$\beta$(1–42) fibrillogenesis and toxicity, Journal of Peptide Science 15 (3) (2009) 220–228. doi:10.1002/psc.1109.

3 A. Sinopoli, A. Giuffrida, M. F. Tomasello, M. L. Giuffrida, M. Leone, F. Attanasio, F. Caraci, P. De Bona, I. Naletova, M. Saviano, A. Copani, G. Pappalardo, E. Rizzarelli, Ac-LPFFD-Th: A Trehalose-Conjugated Peptidomimetic as a Strong Suppressor of Amyloid-$\beta$ Oligomer Formation and Cytotoxicity, ChemBioChem 17 (16) (2016) 1541–1549. doi:10.1002/cbic.201600243.

4 V. Villari, R. Tosto, G. Di Natale, A. Sinopoli, M. F. Tomasello, S. Lazzaro, N. Micali, G. Pappalardo, A Metalloporphyrin-Peptide Conjugate as an Effective Inhibitor of Amyloid-$\beta$ Peptide Fibrillation and Cytotoxicity, ChemistrySelect 2 (28) (2017) 9122–9129. doi:10.1002/slct.201701148.

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5 S. Lazzaro, N. Ogrinc, L. Lamont, G. Vecchio, G. Pappalardo, H. R. M. A., Ion mobility spectrometry combined with multivariate statistical analysis: revealing the effects of a drug candidate for Alzheimer's disease on A$\beta$1-40 peptide early assembly, Analytical and Bioanalytical Chemistry 411 (2019) 6353–6363. doi:10.1007/s00216-019-02030-7.

Conference

# PNAs: a versatile synthetic tool in miRNAs targeting.

Maria Moccia,[a,*] Concetta Avitabile,[a] Michele Saviano,[a]

MiRNAs, small, highly conserved non coding RNAs, directly regulate more than 60% of the entire human mRNAs.[1] They are involved in the regulation of many biological processes as well as their dysregulation can lead to various human diseases.[2] As a result, miRNAs represent an attractive class of molecules in drug development and diagnostic. Peptide Nucleic Acid (PNA)[3] is an unnatural mimic of DNA/RNA made of a pseudo peptide backbone of N-aminoethyl glycine (*aeg*) unit instead of the sugar phosphate one. PNA showed high affinity/specificity binding to DNA/RNA and demonstrated excellent enzymatic resistance. We will show the employment of PNAs based molecules in two case studies:

a) PNAs as a new class of analogues of tumor suppressive miRNA-34a[4]
b) PNAs as smart probes for multiple detection of circulating miRNAs.

In the first case, through an silico and experimental integrated approach new PNA based analogues of different length of tumor suppressive miRNA-34a, were realized (Figure 1A). Their interaction with two binding sites on the target MYCN mRNA was investigated by molecular dynamics simulation and spectroscopic techniques (CD, UV, NMR). Intake assay and confocal microscopy of PNA sequences were also carried out in vitro on neuroblastoma Kelly cells. PNA/RNA hetero duplexes, despite the presence of multiple mismatches, showed very interesting features in terms of stability/affinity as well as of cellular uptake. In the second case PNA based smart probes (fluorescent and electrochemical), were designed, synthesized and characterized by CD, UV, fluorescence spectroscopy. The smart probes were developed for the early diagnosis of pancreatic cancer and coeliac disease, for which a prompt and non-invasive diagnosis methodology is still missing.

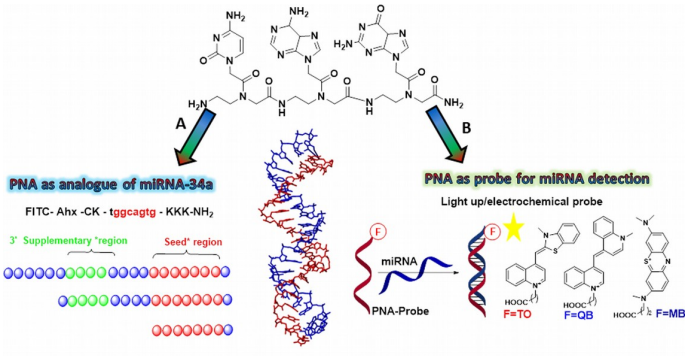


**Fig. 1**

## Riferimenti

1 D. P. Bartel, Micrornas: Target recognition and regulatory functions, Cell 136 (2) (2009) 215 – 233. doi:https://doi.org/10.1016/j.cell.2009.01.002.

2 A. L. Kasinski, F. J. Slack, MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy, Nature Reviews Cancer 11 (12) (2011) 849–864. doi:10.1038/nrc3166.

3 P. Nielsen, M. Egholm, R. Berg, O. Buchardt, Sequence-selective recognition of dna by strand displacement with a thymine-substituted polyamide, Science 254 (5037) (1991) 1497–1500. doi:10.1126/science.1962210.

4 V. Piacenti, E. Langella, I. Autiero, J. C. Nolan, O. Piskareva, M. F. Adamo, M. Saviano, M. Moccia, A combined experimental and computational study on peptide nucleic acid (pna) analogues of tumor suppressive mirna-34a, Bioorganic Chemistry 91 (2019) 103165. doi:https://doi.org/10.1016/j.bioorg.2019.103165.

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Conference

# Visible light active$TiO_2$/Au nanorods for photocatalytic environmental remediation.

Francesca Petronella,[a,b,*] Alessandra Truppi,[b] Tiziana Placido,[b] Valerio Margiotta,[c] Giovanni Lasorella, [b] Marinella Striccoli,[b] Cinzia Giannini,[d] Teresa Sibillano,[d] Angela Agostano,[d] Maria Lucia Curri,[d] Roberto Comparelli.[d]

Nanostructured materials exhibit outstanding size/shape dependent properties that make them extremely promising in several application fields. Fundamental features that include (but are not limited to) their optical response, thermodynamic behaviour, plasmonic, magnetic and catalytic properties can be modulated by varying nanocrystal size and shape, without altering their chemical composition.[1] Furthermore, synthesis routes as well as characterization techniques have rapidly evolved thus enabling to rationally design, synthesize, process and organise nanomaterials for specific application fields.[1] In the area of environmental remediation, nanomaterial with photocatalytic properties are gaining increasing attention. In particular, metal/semiconductor thanks to their original photocatalytic properties can be exploited to address several environmental concerns. Indeed, the nanometal moiety can induce the photoactivation of the nanocomposite under visible light irradiation because of several effects, promoted by the Surface Plasmon Resonance (SPR) phenomenon that is strongly size and shape dependent.[2] The present work, aims at synthesizing a visible light active plasmonic photocatalyst on a gram scale by means of promptly scalable procedure. The synthesis of $TiO_2/Au\ NRs$ hybrid nanocomposites has been performed and their photocatalytic properties investigated. The choice of Au NRs arises from their unique plasmonic properties that enable, to efficiently exploit the UV, Visible and NIR ranges of the solar spectrum. The $TiO_2/AuNRs$ nanocomposites have been prepared by a conventional co-precipitation technique and their textural and structural properties have been investigated. The photocatalytic efficiency of the $TiO_2/AuNRs$ has been evaluated by carrying out a set of experiments including the photodegradation reactions under UV light irradiation of an organic model compound, Methylene Blue, the photodegradation of the Nalidixic acid as a model real pollutant under visible light irradiation. Finally, the $TiO_2/AuNRs$ calcinated at 450 °C resulted to be the most efficient photocatalyst among the investigated nanocomposites, thus holding a great promise as a versatile candidate for real scale applications.[3]

## References

1 M. Curri, R. Comparelli, M. Striccoli, A. Agostiano, Emerging methods for fabricating functional structures by patterning and assembling engineered nanocrystals, Physical Chemistry Chemical Physics 12 (37) (2010) 11197–11207. doi:10.1039/B926146J.

2 A. Truppi, F. Petronella, T. Placido, M. Striccoli, A. Agostiano, M. L. Curri, R. Comparelli, Visible-light-active tio2-based hybrid nanocatalysts for environmental applications, Catalysts 7 (4) (2017) 100. doi:10.3390/catal7040100.

3 A. Truppi, F. Petronella, T. Placido, V. Margiotta, G. Lasorella, L. Giotta, C. Giannini, T. Sibillano, S. Murgolo, G. Mascolo, A. Agostiano, M. Curri, R. Comparelli, Gram-scale synthesis of uv–vis light active plasmonic photocatalytic nanocomposite based on tio2/au nanorods for degradation of pollutants in water, Applied Catalysis B: Environmental 243 (2019) 604 – 613. doi:https://doi.org/10.1016/j.apcatb.2018.11.002.

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Conference

# A survey of different approaches using ESI Mass Spectrometry for the characterization of metal binding sites in amyloid peptide fragments.

Giuseppe Di Natale,[a,*]

Neurodegenerative disorders (NDs) such as Alzheimer's disease (AD), Parkinson's disease (PD) and prion diseases are some of the most common forms of age-related diseases. Even if pathogenesis of these neurodegenerative diseases remains unclear, increasing evidences point out a common critical molecular process involving the assembly of various aggregated protein with a $\beta$-sheet conformation, termed amyloids.[1] The inhibition of this process could be a viable therapeutic strategy for the treatment of neurodegenerative diseases. Metal ions, especially copper, zinc and iron play a very important roles in neurodegeneration having impact on both protein structure and oxidative stress.[2] For a better comprehension of the structural features of the metal-amyloid protein complexes, our previous studies aimed at determining the stoichiometry, the affinity and the location of metal binding sites as well as the coordination environment around the metal ions.[3–6] In particular, potentiometric investigations were used to determine the stoichiometry and quantify the metal binding affinity of protein and/or peptide complex species. Moreover, the speciations resulting from thermodynamic data were coupled with spectroscopic information (UV–Vis, CD, ESR, NMR, and MS) in order to clarify the binding modes of various species. One of the main hindrances in amyloid protein investigation concerns the low peptide solubility at the concentrations needed to perform potentiometric titrations and spectroscopic studies. In this contest, the high sensitivity of mass spectrometry may overcome this limit. In this presentation, i will discuss the different mass spectrometry approaches used in the characterization of metal ion complexes with different amyloid peptides such as prion,[4] A$\beta$,[5] Tau[6] and IAPP fragments. In particular, high-resolution ESI-MS assignments can be used to obtain direct information on stoichiometry of different metal complex species existing in dilute solutions. Tandem mass spectrometry investigations provide additional information regarding the metal interaction with amyloid peptide fragments. In particular, HCD (High energy Collision Dissociation) of copper(II) complexes, selected as precursor ions, provides interesting information about the amino acid residues involved in the copper(II) coordination. In order to evaluate the involvement of specific aminoacid residues in the formation of metal complexes, i will show some examples of limited proteolysis experiments together with ESI mass spectrometric analysis of peptide complexes.[4,5]

## Riferimenti


1 C. A. Ross, M. A. Poirier, Protein aggregation and neurodegenerative disease, Nature Medicine 10 (2004) S10–S17. doi:10.1038/nm1066.

2 Y. Miller, B. Ma, R. Nussinov, Metal binding sites in amyloid oligomers: Complexes and mechanisms, Coordination Chemistry Reviews 256 (19) (2012) 2245 – 2252. doi:https://doi.org/10.1016/j.ccr.2011.12.022.

3 G. Arena, D. L. Mendola, G. Pappalardo, I. Sóvágó, E. Rizzarelli, Interactions of $Cu^{2+}$ with prion family peptide fragments: Considerations on affinity, speciation and coordination, Coordination Chemistry Reviews 256 (19) (2012) 2202 – 2218. doi:https://doi.org/10.1016/j.ccr.2012.03.038.

4 G. Di Natale, I. Turi, G. Pappalardo, I. Sóvágó, E. Rizzarelli, Cross-Talk Between the Octarepeat Domain and the Fifth Binding Site of Prion Protein Driven by the Interaction of Copper(II) with the N-terminus, Chemistry - A European Journal 21 (10) (2015) 4071–4084. doi:10.1002/chem.201405502.

5 G. Di Natale, A. Sinopoli, Á. Grenács, D. Sanna, I. Sóvágó, G. Pappalardo, Copper (ii) coordination properties of the a$\beta$ (1–16) 2 peptidomimetic: experimental evidence of intermolecular macrochelate complex species in the a$\beta$ dimer, New Journal of Chemistry 40 (12) (2016) 10274–10284. doi:10.1039/C6NJ02354A.


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6 M. Lukács, G. Szunyog, A. Grenács, N. Lihi, C. Kállay, G. Di Natale, T. Campagna, V. Lanza, G. Tabbi, G. Pappalardo, I. Sóvágó, K. Várnagy, Copper(II) Coordination Abilities of the Tau Protein's N-Terminus Peptide Fragments: A Combined Potentiometric, Spectroscopic and Mass Spectrometric Study, ChemPlusChem 84 (11) (2019) 1697–1708. doi:10.1002/cplu.201900504.

Conference

# The Interdepartmental S&T Foresight Project.

Cecilia Bartolucci,[a,b,*]

## WHY FORESIGHT?

The Science and Technology Foresight Project has carried out initiatives of great relevance, in order to define research strategies able to address crucial social problems related to energy, food, health, water, as well as the cross-sectoral topic of breakthrough innovative materials, complexity of systems and data science. Both, the holistic approach applied to the analysis of the topics, as well as the innovative format of the invitation only workshops, enticed the participation of internationally acknowledged experts. This framework guaranteed all participants the necessary conditions to carry out an open interactive debate, consolidating a collective intelligence, which assisted in achieving a consensus on research priorities, knowledge gaps, and funding needs.

## ACTIVITY

The Foresight Project has involved the international scientific community in the effort of identifying innovative, scientifically based, medium/long term solutions of problems within the four areas mentioned above. The current social needs have been identified as the drivers for change and the proposed solutions must address them. Each problem is a big challenge by itself, and it becomes even more substantial when considering that all issues are connected. Looking for a proper identification of both the relevant features of each problem, as well as the current knowledge gaps, which must be overcome, we adopted a systems approach. The goal is to move beyond a linear view of cause and effect, towards the development of a systemic and integrated view.

## References

1 http://www.foresight.cnr.it/.

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Conference

# A year in antarctica.

Massimiliano Catricalà,[a]

More than 1000km from the coast and at 3200m of altitude, the Concordia base is one of the 3 bases in the middle of the Antarctic plateau.

Temperatures that go down to -80 degrees have a great effect on activities.

During the long 4-month winter night a small group of people maintain the base and carry out measures and observers.

the National Research Program in Antarctica, PNRA, and the French Polar Institute, IPEV, are the bodies for the management and implementation of scientific and technological research programs in Concordia.

Sensations and observers in the Italian-French station of Concordia during the winterover 2019. Isolation and activities of the next 2022 campaign.

What does it mean to work in an extreme environment.

## References

1 https://www.ilmessaggero.it/scienza/la_spedizione_italo_francese_in_antartide-4370008.html.

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Conference

# Advanced X-ray Characterization of Hierarchical Systems: from Biotissues to Electronic Devices.

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Label-free Multiscale X-ray Imaging can be performed by Table-Top equipment, exploiting both Absorption (usually referred to as X-ray Transmission Microscopy "XTM") and Scattering contrast. X-ray scattering techniques can provide a large amount of structural and morphological information, both at the atomic and nano-scale, and are thus particularly suited to study composite/nanostructured materials. The crystalline components are mainly studied by Wide Angle X-ray Scattering (WAXS), providing information on the crystallinity and crystalline phases, as well as on possible texture. The nanoscale structure/morphology can be assessed based on the Small Angle X-ray Scattering (SAXS) signal, and related to the possible crystallinity through combined SAXS/WAXS mapping. SAXS/WAXS/XT Microscopy is particularly suited to study biological tissues (or any structured material) with nano and/or atomic scale periodicity, such as calcified healthy or pathological tissues.[1] In the biomedical field, this approach allows for example to study mineralized bio-scaffolds, or reveal osteogenic differentiation of stem cells through the analysis of nanocrystalline differentiation products.[2] The availability of high brilliance X-ray micro-sources for laboratory equipment allows nowadays to perform the aforesaid advanced X-ray characterization, in both transmission and reflection geometries, in the home laboratory, being such X-ray sources considered as "synchrotron-class". Moreover, suitable data treatment can further enhance the performances of the experimental equipment, returning in several cases results comparable to those obtained at a synchrotron beamline. A in-house developed package (SUNBIM) for the collection and analysis of X-ray microscopies with absorption and/or diffraction contrast, as well as data reduction for transmission and reflection geometries is freely available at http://www.ba.ic.cnr.it/softwareic/sunbimweb/, and constantly updated. The synchrotron-class micro-source combined with the SAXS/WAXS system installed at IC-Bari (XMI-L@b) has been successfully applied to the study of free-standing bio- and composite materials, as well as to polymer nanofibers for optoelectronic devices. Moreover, it has been successfully applied in grazing incidence reflection geometry (GISAXS/-GIWAXS) for the study of several nanostructured films for advanced electronic applications, in particular based on 2D nanocrystals.[3]

## References

1 C. Giannini, M. Ladisa, V. Lutz-Bueno, A. Terzi, M. Ramella, L. Fusaro, D. Altamura, D. Siliqi, T. Sibillano, A. Diaz, F. Boccafoschi, O. Bunk, X-ray scanning microscopies of microcalcifications in abdominal aortic and popliteal artery aneurysms, IUCrJ 6 (2) (2019) 267–276. doi:10.1107/S2052252519001544.

2 A. Procopio, E. Malucelli, P. Alexandra, C. Cappadone, G. Farruggia, A. Sargenti, S. Castiglioni, D. Altamura, A. Sorrentino, C. Giannini, E. Pereiro, P. Cloetens, J. A. M. Maier, S. Iotti, Chemical Fingerprint of Zn–Hydroxyapatite in the Early Stages of Osteogenic Differentiation, ACS Cent. Sci. 5 (8) (2019) 1449–1460. doi:10.1021/acscentsci.9b00509.

3 F. Scattarella, E. Altamura, P. Albanese, D. Siliqi, M. Ladisa, F. Mavelli, C. Giannini, D. Altamura, Table-top combined scanning x-ray small angle scattering and transmission microscopies of lipid vesicles dispersed in free-standing gel, RSC Advances 11 (1) (2021) 484–492. doi:10.1039/D0RA08581B.

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Conference

# Structural Basis for Inhibition of Copper Trafficking by Platinum Anticancer Drugs.

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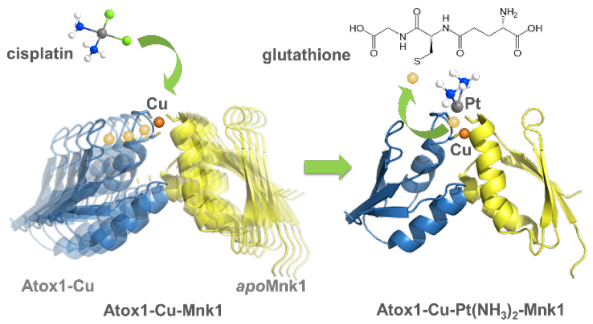
Proteins involved in copper transport and regulation, such as the human Antioxidant 1 copper chaperone (Atox1), are able to mediate the cellular uptake, sequestration, and efflux from cell of platinum-based drugs, affecting their anticancer activity.[1] X-ray crystallographic investigations have disclosed the Pt binding sites of Atox1, and shown that the metal binding site of the Atox1 dimer is partially occupied by a $Pt2^{+}$ ion, with $Cu^{+}$ ions completing the site occupancy.[2] Thus, the platinum ion is able to replace partially the copper ion, leading to a disruption of the delicate process that regulates copper level in the cell.

A further recently published study has revealed the structural mechanisms underlying the interaction between Pt-based drugs and two proteins involved in Cu trafficking (Atox1 and the first domain of Menkes ATPase, Mnk1).[3] Crystallographic and nuclear magnetic resonance investigations demonstrated that the kinetic inertness of the Pt(II) derivative imparts a bullet time effect to the fast process of copper exchange between Atox1 and Mnk1, by freezing the Cu(I) ion or hijacking it to glutathione, a physiological antioxidant in the biological matrix. Thus, the anticancer drugs cisplatin and oxaliplatin can interfere with the rapid exchange of Cu between Atox1 and Mnk1, with possible consequences on cancer cell viability and migration.




*Acknowledgement*

The Italian Ministry for Education and Research (MIUR) is kindly acknowledged for financial support through Project PRIN 2017 (grant number 2017WBZFHL)

## Riferimenti

1 I. H. Hung, R. L. B. Casareno, G. Labesse, F. S. Mathews, J. D. Gitlin, HAH1 is a copper-binding protein with distinct amino acid residues mediating copper homeostasis and antioxidant defense, Journal of Biological Chemistry 273 (3) (1998) 1749–1754. doi:0.1074/jbc.273.3.1749.

2 B. D. Belviso, A. Galliani, A. Lasorsa, V. Mirabelli, R. Caliandro, F. Arnesano, G. Natile, Oxaliplatin binding to human copper chaperone atox1 and protein dimerization, Inorganic chemistry 55 (13) (2016) 6563–6573. doi:10.1021/acs.inorgchem.6b00750.

3 A. Lasorsa, M. I. Nardella, A. Rosato, V. Mirabelli, R. Caliandro, R. Caliandro, G. Natile, F. Arnesano, Mechanistic and structural basis for inhibition of copper trafficking by platinum anticancer drugs, Journal of the American Chemical Society 141 (30) (2019) 12109–12120. doi:10.1021/jacs.9b05550.

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Conference

# Design and fabrication of biosensors for environmental and food safety monitoring.

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Modern environmental and food monitoring practices require reliable, facile, and on-site detection of hazardous compounds potentially affecting human health. By satisfying these requirements, sensors represent complementary screening tools with a lower impact on the environment compared to conventional chromatographic-based methods. Specifically, biosensor technology offers the possibility to combine and tailor the selectivity of biological recognition elements to the sensitivity of transducing platforms giving rise to analytical devices providing specific situation awareness. The exploitation of disposable scree-printed electrodes and microfluidic systems prompted the development of miniaturized, portable systems, while the integration of nanostructured materials enabled to improve selectivity and performance of electrical detectors. Besides these advancements, keeping the proper functionality of the biological component in a hostile environment, such as solid-state devices, is still a challenge.

In the quest to identify physiological parameters correlated with more efficient phenotypes, we studied the structure/function/dynamics relationships occurring in microalgae strains hosting single aminoacidic substitutions in the photosystem II D1 protein. The mutants were produced by combining *in silico* studies with a *in vitro* directed evolution approach followed by site-directed mutagenesis experiments[1,2]. Biophysical and physiological studies enabled the identification of phenotypes having enhanced stability and photosynthetic performance under stressful conditions, and improved sensitivity to different classes of environmental contaminants[3]. As a further step, we set-up immobilization procedures to preserve the activity of algal cells on screen-printed electrodes[4] and enhanced the electrochemical response by exploiting bi-metallic nanoparticles. Finally, neutron spectroscopy studies on algal mutants shed a light on dynamics properties of photosynthetic reaction centre underlying an improved biosensor performance[5,6].

The construction of amperometric photosinthesis-based biosensors will be presented alongside the development of a label-free impedimetric immunosensor for the detection of micotoxins,[7] that will be included in a novel multitransduction electrochemical biosensoristic platform. This work was supported by the Lazio project FACILE, n. 85-2017-15256.

## References

1 G. Rea, F. Polticelli, A. Antonacci, V. Scognamiglio, P. Katiyar, S. A. Kulkarni, U. Johanningmeier, M. T. Giardi, Structure-based design of novel chlamydomonas reinhardtii d1-d2 photosynthetic proteins for herbicide monitoring, Protein Science 18 (10) (2009) 2139–2151. doi:10.1002/pro.228.

2 G. Rea, M. Lambreva, F. Polticelli, I. Bertalan, A. Antonacci, S. Pastorelli, M. Damasso, U. Johanningmeier, M. T. Giardi, Directed evolution and in silico analysis of reaction centre proteins reveal molecular signatures of photosynthesis adaptation to radiation pressure, PLoS One 6 (1) (2011) e16216.

3 M. D. Lambreva, M. T. Giardi, I. Rambaldi, A. Antonacci, S. Pastorelli, I. Bertalan, I. Husu, U. Johanningmeier, G. Rea, A Powerful Molecular Engineering Tool Provided Efficient Chlamydomonas Mutants as Bio-Sensing Elements for Herbicides Detection, PLOS ONE 8 (4) (2013) 1–9. doi:10.1371/journal.pone.0061851.

4 X. Xu, D. Wang, J. Liu, P. Sun, Y. Guan, H. Zhang, Y. Sun, F. Liu, X. Liang, Y. Gao, G. Lu, Template-free synthesis of novel $In_2O_3$ nanostructures and their application to gas sensors, Sensors and Actuators B: Chemical 185 (2013) 32 – 38. doi:10.1016/j.snb.2013.04.078.

5 A. Antonacci, M. D. Lambreva, A. Margonelli, A. P. Sobolev, S. Pastorelli, I. Bertalan, U. Johanningmeier, V. Sobolev,

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I. Samish, M. Edelman, V. Havurinne, E. Tyystjärvi, M. T. Giardi, A. K. Mattoo, G. Rea, Photosystem-ii d1 protein mutants of chlamydomonas reinhardtii in relation to metabolic rewiring and remodelling of h-bond network at qb site, Scientific reports 8 (1) (2018) 1–14. doi:10.1038/s41598-018-33146-y.

6 D. Russo, M. D. Lambreva, C. A. Simionesco, P. Sebban, G. Rea, Dynamics properties of photosynthetic microorganisms probed by incoherent neutron scattering, Biophysical Journal 116 (9) (2019) 1759 – 1768. doi:10.1016/j.bpj.2019.03.029.

7 R. Cancelliere, D. Albano, B. Brugnoli, K. Buonasera, G. Leo, A. Margonelli, G. Rea, Electrochemical and morphological layer-by-layer characterization of electrode interfaces during a label-free impedimetric immunosensor build-up: The case of ochratoxin a, Applied Surface Science 567 (2021) 150791. doi:https://doi.org/10.1016/j.apsusc.2021.150791.

Conference

# Multiple scale characterisation of ultrasmall nanocrystals by X-ray Total Scattering.

Antonietta Guagliardi[a,b]

Fundamental aspects and an overview of applications of cutting-edge nanocrystallography tools grounded on Wide Angle X-ray Total Scattering (WAXTS) techniques (mainly synchrotron-based) and the use of the Debye Scattering Equation (DSE) for data modeling in reciprocal space will be presented. [1,2]

The approach overcome the limits of conventional diffraction-based methods for characterizing engineered ultrasmall nanocrystals and, by exploiting the full (Bragg and diffuse scattering) information in the experimental data, is able to quantitatively recover atomic and nanometer scales properties (structural distortions, defects of various kind, lattice strains, surface relaxation, size, morphology and their relative dispersion) suitably encoded in the same atomistic model. Combining WAXTS with Small Angle X-ray scattering (SAXS) data fruitfully exploits their complementarity for a more robust, quantitative multiple-scale analysis and/or for disentangling size/shape from structural defects-induced effects.

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## References

1 A. Cervellino, R. Frison, N. Masciocchi, A. Guagliardi, X-Ray Powder Diffraction Characterization of Nanomaterials, Springer Berlin Heidelberg, Berlin, Heidelberg, 2016, pp. 545–608. doi:10.1007/978-3-662-48606-1_10.

2 A. Cervellino, R. Frison, F. Bertolotti, A. Guagliardi, *DEBUSSY 2.0*: the new release of a Debye user system for nanocrystalline and/or disordered materials, Journal of Applied Crystallography 48 (6) (2015) 2026–2032. doi:10.1107/S1600576715020488.

3 F. Bertolotti, D. N. Dirin, M. Ibáñez, F. Krumeich, A. Cervellino, R. Frison, O. Voznyy, E. H. Sargent, M. V. Kovalenko, A. Guagliardi, et al., Crystal symmetry breaking and vacancies in colloidal lead chalcogenide quantum dots, Nature materials 15 (9) (2016) 987–994. doi:10.1038/nmat4661.

4 F. Bertolotti, L. Protesescu, M. V. Kovalenko, S. Yakunin, A. Cervellino, S. J. L. Billinge, M. W. Terban, J. S. Pedersen, N. Masciocchi, A. Guagliardi, Coherent nanotwins and dynamic disorder in cesium lead halide perovskite nanocrystals, ACS Nano 11 (4) (2017) 3819–3831. doi:10.1021/acsnano.7b00017.

5 D. Moscheni, F. Bertolotti, L. Piveteau, L. Protesescu, D. N. Dirin, M. V. Kovalenko, A. Cervellino, J. S. Pedersen, N. Masciocchi, A. Guagliardi, Size-dependent fault-driven relaxation and faceting in zincblende cdse colloidal quantum dots, ACS Nano 12 (12) (2018) 12558–12570. doi:10.1021/acsnano.8b07092.

6 F. Bertolotti, G. Nedelcu, A. Vivani, A. Cervellino, N. Masciocchi, A. Guagliardi, M. V. Kovalenko, Crystal structure, morphology, and surface termination of cyan-emissive, six-monolayers-thick cspbbr3 nanoplatelets from x-ray total scattering, ACS Nano 13 (12) (2019) 14294–14307. doi:10.1021/acsnano.9b07626.

7 J. M. Delgado-López, R. Frison, A. Cervellino, J. Gómez-Morales, A. Guagliardi, N. Masciocchi, Crystal size, morphology, and growth mechanism in bio-inspired apatite nanocrystals, Advanced Functional Materials 24 (8) (2014) 1090–1099. doi:https://doi.org/10.1002/adfm.201302075.

8 R. Frison, G. Cernuto, A. Cervellino, O. Zaharko, G. M. Colonna, A. Guagliardi, N. Masciocchi, Magnetite–maghemite nanoparticles in the 5–15 nm range: Correlating the core–shell composition and the surface structure to the magnetic properties. a total scattering study., Chemistry of Materials 25 (23) (2013) 4820–4827. doi:10.1021/cm403360f.

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Conference

# The XRD1 Synchrotron hard X-ray light source beamline as a tool for chemistry and science and technology of materials..

Luisa Barba,[a,*] Alberto Cassetta,[a] and Giuseppe Chita[a]

The Italian synchrotron Elettra was one of the first 3rd generation accumulation rings designed and built with the aim of producing photon beams to be used in radiation-matter interaction experiments, in which photons constitute a probe to investigate electronic, magnetic and structural properties of matter in solid, liquid and gaseous state. Elettra houses four beamlines dedicated to crystallography, and of these the first to be designed and built, in partnership with the CNR, was XRD1.[1] This beamline, originally designed for X-ray diffraction experiments in macromolecular crystallography, has shown over the years to be very versatile and able to adapt to a great variety of geometries and experimental techniques.[2] Its source is a permanent magnet wiggler that offers a continuous range of emission from 4 to 21 keV, its experimental station is equipped with a two-dimensional high dynamic range X-ray detector coupled to a diffractometer for alignment and rotation about various axes of the samples in the beam. At the experimental station, it is possible to perform X-ray diffraction experiments ranging from macromolecular crystallography to studies of surfaces and thin films in grazing incidence, from powder diffraction to the use of low energies for solving the phase problem. Here a brief presentation of the experimental station is given along with some examples of investigations in materials sciences performed there, in particular for applications in electronic (organic semiconductors) energy conversion (solar cell prototypes), food science (ex-situ DSC-XRD combined investigations of lipidic matrices) and of phase transitions and structural properties of high Tc superconductors.

## References

1 S. Bernstorff, E. Busetto, C. Gramaccioni, A. Lausi, L. Olivi, F. Zanini, A. Savoia, M. Colapietro, G. Portalone, M. Camalli, A. Pifferi, R. Spagna, L. Barba, A. Cassetta, The macromolecular crystallography beamline at elettra, Review of Scientific Instruments 66 (2) (1995) 1661–1664. doi:10.1063/1.1145875.

2 A. Lausi, M. Polentarutti, S. Onesti, J. Plaisier, E. Busetto, G. Bais, L. Barba, A. Cassetta, G. Campi, D. Lamba, et al., Status of the crystallography beamlines at elettra, The European Physical Journal Plus 130 (3) (2015) 1–8. doi:10.1140/epjp/i2015-15043-3.

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Conference

# Disclosing the Major Structural Determinants Essential for Proneurotrophins Biological Functions.

Sonia Covaceuszach,[a,*] Leticia Yamila Peche,[a] Petr Valeryevich Konarev,[b], Doriano Lamba,[a,c]

Nerve Growth Factor, Brain-Derived Neurotrophic Factor, Neurotrophin 3 and Neurotrophin 4 play a range of crucial functions in the developing and adult peripheral and central nervous systems. Initially synthesized as precursors, named proneurotrophins (proNTs), that are cleaved to release C-terminal mature forms, they act through two types of receptors, the specific Trk receptors and the pan-neurotrophin receptor p75NTR, to promote differentiation, neuronal survival, synaptogenesis and modulating synaptic plasticity. Recently, all the proNTs but proNT4 have been demonstrated to be not just inactive precursors, but biologically active signaling ligands that mediate opposing actions in fundamental aspects of the nervous system with respect to the mature counterparts through dual receptor complexes formation, involving a member of the VPS10 family and p75NTR. Despite the functional relevance, the molecular determinants underpinning the interactions between the pro-domains and their receptors are still elusive probably due to their intrinsically disordered nature. Here we present an evolutionary approach coupled to an experimental study aiming to uncover the structural and dynamical basis of the biological functions displayed by proNGF, proBDNF and proNT3 but missing in proNT4. A bioinformatic analysis allowed elucidating the functional adaptability of the proNTs family in vertebrates, identifying conserved key structural features. The combined biochemical and SAXS experiments shed lights on the structure and dynamic behavior of the human proNTs in solution, giving insights on the evolutionary conserved structural motifs, essential for the multifaceted roles of proNTs in physiological as well as in pathological contexts.[1]

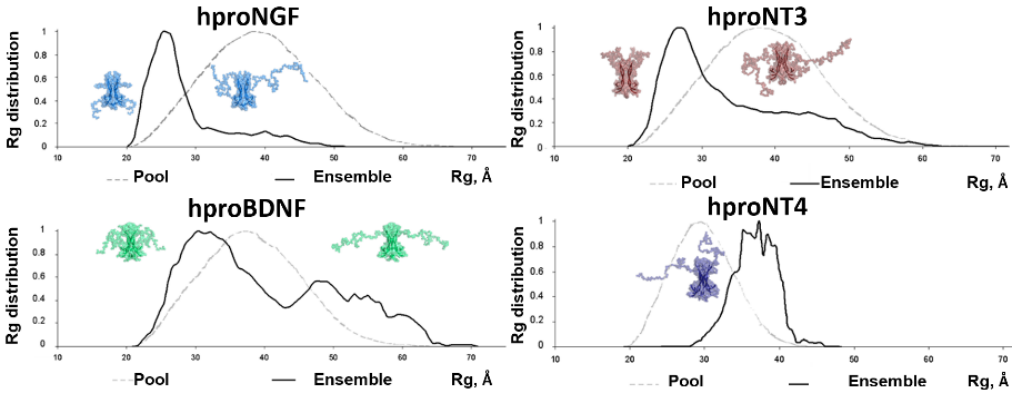


**Fig. 1** The distributions of the radius of gyration ($R_g$) obtained by the Ensemble Optimization Methos for hproNGF, hproBDNF, hproNT3 and hproNT4. Initial random pools of the models (grey dot lines) and for the selected ensembles (black solid lines) are shown as well as the representative compact (on the left) and extended (on the right) conformations (semitransparent surfaces).

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## References

1 S. Covaceuszach, L. Peche, P. Konarev, D. Lamba, A combined evolutionary and structural approach to disclose the primary structural determinants essential for proneurotrophins biological functions, Computational and Structural Biotechnology Journal 19 (2021) 2891–2904. doi:https://doi.org/10.1016/j.csbj.2021.05.007.

Conference

# Solving polycrystalline structures: from data collection to the development of innovative crystallographic methods.

Rosanna Rizzi,[a,*]

Although the crystal structure solution from powder diffraction data is limited respect to the single crystal case, its interest has surprisingly increased in the last twenty-five years. The difficult chance to get a correct interpretation of the experimental powder diffraction pattern, due to peak overlap, background estimation, and preferred orientation, is addressed with improvement in modern instrumentation, as well as development of innovative theories and software.

Our research activity is mainly devoted to the solution by powder diffraction data of organic, inorganic, metal-organic crystal structures of different scientific and technological interest.

The process of structure solution starts from powder diffraction data collection by a Rigaku laboratory diffractometer (18Kw) equipped with an asymmetric Ge(111) monochromator and a silicon strip detector.

The core of the research activity is developing advanced methodological approaches and computational abilities aiming at successfully and automatically carrying out the full pathway of the solution process, from the indexation to the Rietveld refinement[1].

Regarding the methodologies, we develop computational approaches using Direct Methods, working in the reciprocal space, and Simulated Annealing, working in the real space and both[2]. They have differences: Direct Methods are fast and require minimal information, depend on the efficiency of the pattern decomposition, on the experimental resolution and structure complexity. Simulated Annealing methods can be very slow and need additional information on the expected molecular geometry, are independent from the accuracy of the extracted structure factor moduli and do not need atomic resolution.

The decision on what should be the best solution strategy to apply, mainly depends on data quality, experimental resolution and peak overlap; structure complexity and/or degrees of freedom of the structure[2].

The new methodological and computational procedures are implemented in the software EXPO[3], free for academic institution, based on a user-friendly graphic interface, able to adopt default and non-default solution strategies, all innovative approaches for attaining the complete and correct crystal structure solution.

The different requested conditions and the most appropriate algorithm to apply for a successful powder solution by Direct Methods and Simulated Annealing with some examples will be discussed.

## References

1 International Tables for Crystallography, Volume H, Powder Diffraction; C.J. Gilmore, J.A. Kaduk, H. Schenk Eds., Wiley: New York, 2019, 978-1-118-41628-0.

2 A. Altomare, N. Corriero, C. Cuocci, A. Falcicchio, R. Rizzi, Solving a Structure in the Reciprocal Space, Real Space and Both by Using the EXPO Software, Crystals 10 (1) (2019) 16. doi:10.3390/cryst10010016.

3 A. Altomare, C. Cuocci, C. Giacovazzo, A. Moliterni, R. Rizzi, N. Corriero, A. Falcicchio, *EXPO2013*: a kit of tools for phasing crystal structures from powder data, Journal of Applied Crystallography 46 (4) (2013) 1231–1235. doi:10.1107/S0021889813013113.

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Conference

# The role of stereochemistry in the inhibition of A$\beta$ Amyloid growth and toxicity by silybins.

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The self-assembling of the amyloid $\beta$ (A$\beta$) is considered an hallmark in the pathogenesis of Alzheimer's disease (AD). Many efforts have been devoted in designing molecules able to halt disease progression by inhibiting A$\beta$ self-assembly. We combine biophysical, biochemical and computational techniques to investigate the capacity of four optically pure components of the natural product silymarin (silybin A, silybin B, 2,3-dehydrosilybin A, 2,3-dehydrosilybin B) to inhibit A$\beta$ aggregation. TEM analysis demonstrated that all the four investigated flavonoids prevent the formation of mature fibrils, however ThT assays, WB and AFM investigations showed that only silybin B was able inhibit the formation of small protofiber (considered the most toxic species) diverting the aggregation toward the formation of large amorphous aggregates. By using molecular dynamics (MD) simulations we observed that silybin B interacts mainly with the C-terminal hydrophobic segment $^{35}$MVGGVV$^{40}$ of A$\beta$40 and the peptide conformation remains predominantly unstructured along all the simulations. By contrast, silybin A interacts preferentially with the segments $^{17}$LVFF$^{20}$ and $^{27}$NKGAII$^{32}$ of A$\beta$40 which shows a high tendency to form bend, turn, and $\beta$-sheet conformation in and around these two domains. Both 2,3-dehydrosilybin enantiomers bind preferentially the segment $^{17}$LVFF$^{20}$ but lead to the formation of different small-sized, ThT-positive A$\beta$ aggregates. Finally, in vivo studies in a transgenic *Caenorhabditis elegans* strain expressing human A$\beta$ indicated that silybin B is the most effective of the four compounds in counteracting A$\beta$ proteotoxicity. This study underscores the pivotal role of stereochemistry in determining the neuroprotective potential of silybins and points to silybin B as a promising lead compound for further development in anti-AD therapeutics.

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Conference

# Structural investigation of calcium phosphates.

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Calcium phosphates (CaP) materials, employed in different biomedical applications because of their density very close to those of CaP mineralized phases of human bones, are largely investigated in many scientific fields.[1] The most known CaP are tricalcium phosphate $Ca_3(PO_4)_2$ (TCP) and hydroxyapatite $Ca_5(PO_4)_3OH$ (HAp). In the present work, we investigated two sets of substituted $\beta$-TCP materials, coming from solid-state reactions at T = 1200°, respectively with rare earths cations (*RE*),[2] and with divalent transition metals (Mn, Ni, Cu).[3] All TCP were subject to a multi-methodological crystal chemical investigation based on SEM-EDS microscopy, powder X-ray diffraction (PXRD), FTIR, Raman and luminescence spectroscopies. Investigation was completed with the structural refinement (Rietveld method). SEM morphological analyses revealed the presence of subspherical microcrystalline aggregates. Refinement of site occupancies showed the tendency of *RE* cations to replace Ca in the largest structural sites; in the smaller octahedral M5 site, low *RE* (La→Gd) are not present, contrary to high *RE* (Dy→Lu) present in this site, while divalent cations showed a strong tendency to occupy this energetically favourable site. FTIR and Raman spectra show slight band shifts of the phosphate modes correlated to the evolving size of the replacing element, as well as strong luminescence properties were found in many *RE*- TPC (Eu and Gd phases) and in Mn-TCP.

HAp is the primary mineral component of human bones and teeth. We investigated HAp, coming from high-temperature syntheses, doped with different cations at different concentrations, e.g. bismuth-HAp as possible biomaterial,[4] and *RE* (Eu, Gd)-HAp as phosphor materials. Multi-methodological characterization was completed by dielectric measurements for Bi-HAp: morphological analysis revealed a decrease in crystallite size by bismuth-addition in HAp lattice, while the relative permittivity, dielectric loss and alternating current conductivity change with increasing frequency, the alternating conductivity gradually decreases with the addition of Bi. For (Eu, Gd)-HAp, FTIR spectra showed slight band shifts of the phosphate modes correlated to the evolving size of the replacing cation, while significant luminescence properties were found.

## References

1 S. V. Dorozhkin, Calcium orthophosphate bioceramics, Ceramics International 41 (10, Part B) (2015) 13913 – 13966. doi:10.1016/j.ceramint.2015.08.004.

2 F. Capitelli, M. Rossi, A. ElKhouri, M. Elaatmani, N. Corriero, A. Sodo, G. D. Ventura, Synthesis, structural model and vibrational spectroscopy of lutetium tricalcium phosphate $Ca_9Lu(PO_4)_7$, Journal of Rare Earths 36 (11) (2018) 1162 – 1168. doi:10.1016/j.jre.2018.02.014.

3 A. Altomare, R. Rizzi, M. Rossi, A. El Khouri, M. Elaatmani, V. Paterlini, G. Della Ventura, F. Capitelli, New $Ca_{2.90}(Me^{2+})_{0.10}(PO_4)_2$ $\beta$-tricalcium Phosphates with $Me^{2+} = Mn, Ni, Cu$: Synthesis, Crystal-Chemistry, and Luminescence Properties, Crystals 9 (6) (2019) 288. doi:10.3390/cryst9060288.

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4 A. E. Khouri, A. Zegzouti, M. Elaatmani, F. Capitelli, Bismuth-substituted hydroxyapatite ceramics synthesis: Morphological, structural, vibrational and dielectric properties, Inorganic Chemistry Communications 110 (2019) 107568. doi:10.1016/j.inoche.2019.107568.

Conference

# Natural polysaccharides from microalgae for the protection of cultural heritage.

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Cultural heritage, whatever their composition and location (indoor or outdoor), are susceptible to deterioration triggered by several factors, such as thermo-hygrometric conditions, mechanical stress, electromagnetic radiation and biodeterioration. One of the main challenges in this sector is the promotion of innovative products for the restoration/protection of artistic value surfaces.

In this context, we propose the development of a new product based on polysaccharides, extracted from unicellular algae, compatible with ancient materials and not harmful to humans and the environment. In particular, the interesting qualities of algal polysaccharides have been recently described, for their significant antioxidant, antifungal, cosmeceutical and nutraceutical activities in the medical, agri-food and cosmetics fields, but still little is known in the cultural heritage sector. Hence, the goal of our research is to utilize the microalgal polysaccharidic extract to foster an innovative restoration and conservation of artistic work (paper, wooden and mortar artworks), promoting mechanical restoration, as well as hindrance the onset of biotic colonization.

For these reasons, specific strains of microalgae (wild type and mutants) were selected and grown to induce a natural accumulation of carbohydrates. Furthermore, an extraction protocol of the polysaccharide mixture was optimized and its composition determined by FT-IR, NMR, elemental chemical analysis, as well as its antioxidant capacity. Moreover, antifungal and antibacterial capacities on organic and inorganic materials will be tested. The consolidating and regenerative properties of polysaccharides will be analysed over the time by MO, FTIR, SEM-EDS, imaging UV, UV-VIS-NIR, in comparison with conventional treatments.

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Conference

# GHK-Hyaluronic acid conjugates affect the wound closure in the presence of copper ions.

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Wound healing is a complex, efficient and highly regulated biological process that consists of four phases: hemostasis, inflammation, proliferation and migration of cells.[1] Copper-dependent stimulation of vessel formation during the wound healing has been mainly attributed to its regulation of vascular endothelial growth factor (VEGF) and angiogenin.[2] The human copper-binding peptide GHK (glycyl-l-histidyl-l-lysine) is a small, naturally occurring tri-peptide present in human plasma that can be released from tissues in the case of an injury and is able to control the fibrinogen biosynthesis in liver tissue.[3,4] Most authors attribute effects of GHK to its ability to bind copper (II) ions that can affect not only the copper metabolism but also regulate a number of human genes.[5] Hyaluronic acid (HA) is currently used in tissue regeneration either alone or conjugated with bioactive molecules.[6,7] 200 kDa HA affects tissue regeneration and pro-angiogenic and wound closure processes.

In the present work, we report on the protective and regenerative actions of the HA-GHK conjugate on mouse embryonic fibroblasts cell line (NIH/3T3) in the presence and in the absence of Cu(II) ion. Dose-response experiments show no significant effect on cell viability/proliferation with or without 1 $\mu$M copper. The effect of HA-GHK conjugates on wound healing was investigated; as expected, HA200 treatment slightly increases the wound closure, while the addition of HA-GHK with different % of GHK loading results in higher % of wound closure than with HA200. Addition of 1 $\mu$M copper or 50 $\mu$M copper chelator BCS, modify this effect. Altogether, our findings pinpoint that GHK-HA is a good candidate as new molecular entity in wound healing and skin repairing.

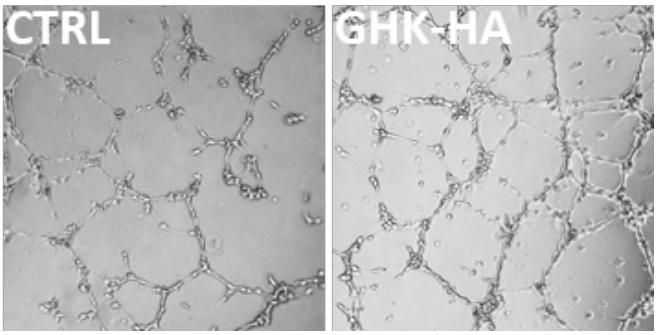


**Fig. 1** Tube formation by HUVECs on Matrigel induced by GHK-HA

## References

1 S. Guo, L. DiPietro, Factors affecting wound healing, Journal of Dental Research 89 (3) (2010) 219–229. doi:10.1177/0022034509359125.

2 A. P. Kornblatt, V. G. Nicoletti, A. Travaglia, The neglected role of copper ions in wound healing, Journal of Inorganic Biochemistry 161 (2016) 1–8. doi:https://doi.org/10.1016/j.jinorgbio.2016.02.012.

3 L. Pickart, The human tri-peptide ghk and tissue remodeling, Journal of Biomaterials Science, Polymer Edition 19 (8) (2008) 969–988. doi:10.1163/156856208784909435.

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† oral communication at 1 st Conference on Crystallography, Structural Chemistry and Biosystems, (Catania) 04-06/10/2021

4 L. Pickart, J. M. Vasquez-Soltero, A. Margolina, The human tripeptide ghk-cu in prevention of oxidative stress and degenerative conditions of aging: implications for cognitive health, Oxidative medicine and cellular longevity 2012. doi:10.1155/2012/324832.

5 L. Pickart, J. M. Vasquez-Soltero, A. Margolina, Ghk and dna: resetting the human genome to health, BioMed research international 2014. doi:0.1155/2014/151479.

6 M. G. Neuman, R. M. Nanau, L. Oruña-Sanchez, G. Coto, Hyaluronic acid and wound healing, Journal of Pharmacy & Pharmaceutical Sciences 18 (1) (2015) 53–60. doi:10.18433/J3K89D.

7 S. Sciuto, V. Greco, E. Rizzarelli, F. Bellia, V. Lanza, S. Vaccaro, L. Messina, Derivatives obtained from hyaluronic acid and carnosine, PCT/IB2015/055782 (2015).

Conference

# Rescuing proteostasis by small molecules.

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Many apparently unrelated diseases, including Alzheimer's Disease (AD), Parkinson Disease (PD) and type II diabetes mellitus (T2DM), result from protein misfolding and abnormal accumulation of toxic amyloid deposits in affected tissues. According to the "Amyloid Hypothesis", targeting protein misfolding and self-assembly into toxic amyloid aggregates would prevent the diseases. However, the failure of all clinical trials focusing on anti-aggregating drugs has clearly demonstrated that a deeper understanding of the mechanisms involved in proteome maintenance is needed.

Our group focuses on the intertwined biochemical mechanisms that control protein homeostasis (proteostasis). We employ an interdisciplinary approach to screen small molecules (e.g. natural compounds, bioconjugates, and repurposed drugs) for their ability to restore proteome integrity by a multi-target strategy. Our work spans from fundamental topics related to thermodynamics of protein stability, amyloid aggregation, and ligand-protein interactions to applications in medicinal chemistry focusing on the development of bioactive compounds, involving chemically tailored contacts between lipids, proteins, nucleic acids, small molecules, and metal ions. [1–3]

## References

1 F. Bellia, V. Lanza, S. García-Viñuales, I. Ahmed, A. Pietropaolo, C. Iacobucci, G. Malgieri, G. D'Abrosca, R. Fattorusso, V. Nicoletti, et al., Ubiquitin binds the amyloid $\beta$ peptide and interferes with its clearance pathways, Chemical science 10 (9) (2019) 2732–2742. doi:10.1039/C8SC03394C.

2 D. Milardi, E. Gazit, S. E. Radford, Y. Xu, R. U. Gallardo, A. Caflisch, G. T. Westermark, P. Westermark, C. L. Rosa, A. Ramamoorthy, Proteostasis of islet amyloid polypeptide: A molecular perspective of risk factors and protective strategies for type ii diabetes, Chemical Reviews 121 (3) (2021) 1845–1893. doi:10.1021/acs.chemrev.0c00981.

3 G. Grasso, A. M. Santoro, V. Lanza, D. Sbardella, G. R. Tundo, C. Ciaccio, S. Marini, M. Coletta, D. Milardi, The double faced role of copper in A$\beta$ homeostasis: A survey on the interrelationship between metal dyshomeostasis, UPS functioning and autophagy in neurodegeneration, Coordination Chemistry Reviews 347 (2017) 1–22. doi:https://doi.org/10.1016/j.ccr.2017.06.004.

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# Cross talk between Amyloid $\beta$ peptides and Ubiquitin: new perspective in Alzheimer's disease.

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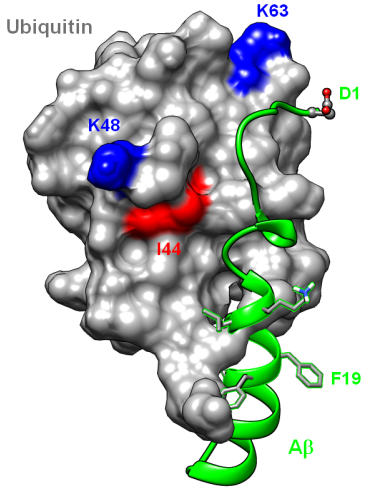


Alzheimer's disease (AD), the most common form of dementia worldwide, is an age-related, fatal neurodegenerative disorder. A hallmark of AD is the presence of extracellular proteinaceous deposits (senile plaques) in the brain of affected people.[1] The prevalent component of senile plaques are $\beta$-amyloid (A$\beta$) peptides but it has been underlined the presence of ubiquitin. A reduced Ubiquitin Proteasome System (UPS) activity has been found in patients affected by AD and many reports suggest that the UPS malfunction plays a significant role in A$\beta$ accumulation and, in turn, in AD progress.

Here we set out to test whether Ub may bind the A$\beta$ peptide and have any effect on its physiological clearance pathways.

We demonstrated that A$\beta$40 binds Ub with a 1:1 stochiometry and Kd in the low micromolar range, using an integrated array of MALDI-TOF/UPLC-HRMS, fluorescence, NMR, SPR and molecular dynamics studies.

In particular, we show that the N-terminal domain of A$\beta$ peptide (through residues D1, E3 and R5) interacts with the C-terminal tail of Ub (involving residues K63 and E64), inducing the central region of A$\beta$ (14HQKLVFFAEDVGSNK28) to adopt a mixed $\alpha$-helix/$\beta$-turn structure. In neuroblastoma cell lysates, we have shown that A$\beta$ competitively binds Ub also in the presence of the entire pool of cytosolic Ub binding proteins. Ub-bound A$\beta$ has a lower tendency to aggregate into amyloid-like fibrils and is more slowly degraded by the Insulin degrading Enzyme (IDE). Finally, we observe that the water soluble fragment A$\beta$1-16 significantly inhibits Ub chain growth reactions.

These results point out how the non-covalent interaction between A$\beta$ peptides and Ub may have relevant effects on the regulation of the upstream events of the UPS.

## References

1 A. Ciechanover, P. Brundin, The ubiquitin proteasome system in neurodegenerative diseases: Sometimes the chicken, sometimes the egg, Neuron 40 (2) (2003) 427 – 446. doi:10.1016/S0896-6273(03)00606-8.

2 V. Lanza, F. Bellia, E. Rizzarelli, An inorganic overview of natural aβ fragments: Copper(ii) and zinc(ii)-mediated pathways, Coordination Chemistry Reviews 369 (2018) 1 – 14. doi:10.1016/j.ccr.2018.04.004.

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# Software for the Characterization of Polycrystalline Compounds.

Nicola Corriero,[a]

Innovative methodologies have been developed and implemented in computer programs aiming at providing efficient computational tools for a wide range of applications in the field of the characterization of polycrystalline materials. The software, based on sound theoretical principles, developed according to the most recent and advanced programming languages and supported by a high level of automatism which makes its use simple also to non-expert in crystallographic knowledge, is worldwide used.
The developed software are:

EXPO[1]: a computing program devoted to structure solution of materials available in the form of microcrystalline powder, by using X-ray diffraction data. The chemical formula and the experimental diffraction profile are the only information necessary to run it. EXPO is able to investigate small molecules, inorganic, organic and metal-organic. It can execute, in a complete automatic way, all the steps characterizing the structure solution process and consisting of: 1) identification of crystal cell parameters; 2) determination of space group; 3) solution of crystal structure by locating the correct atom positions; 4) refinement of the final crystal model.

QUALX[2]: a software for identifying the crystal phase(s) present in a powder sample. It automatically performs: 1) the location of peaks in the experimental diffraction profile, 2) the subtraction of the background noise, 3) the search in crystal phase databanks. At the end of the process, QUALX brings out the chemical phase(s) which best match the peaks in the experimental pattern. In addition, it can query two databases: one commercial (PDF-2) and one free (POW_COD).

OChemDb[3](Open Chemistry Database): a new free web portal which has been developed for assisting in the crystal structure determination process by searching and analyzing crystal chemical information of organic and inorganic compounds. OChemDb is dedicated to collect, to make available by statistical tools and to manage crystal-chemical information coming from the CIF files contained in the Crystallography Open Database (COD, http://www.crystallography.net/cod/). OChemDb can be used for searching and analyzing crystal-chemical information (bond distance, bond angle, space group) of structures already solved, to be used for different scientific purposes. It provides statistics on desired distances, bond angles, torsion angles and space groups. OChemDb uses a suitably designed database of solved crystal structures. The use of OChemDb requires only a web browser and an internet connection.

For each software, examples of challenging applications by users around the world covering a wide range of scientific interests will be presented.

## References

1 A. Altomare, C. Cuocci, C. Giacovazzo, A. Moliterni, R. Rizzi, N. Corriero, A. Falcicchio, *EXPO2013*: a kit of tools for phasing crystal structures from powder data, Journal of Applied Crystallography 46 (4) (2013) 1231–1235. doi:10.1107/S0021889813013113.

2 A. Altomare, N. Corriero, C. Cuocci, A. Falcicchio, A. Moliterni, R. Rizzi, *QUALX2.0*: a qualitative phase analysis software using the freely available database POW_COD, Journal of Applied Crystallography 48 (2) (2015) 598–603. doi:10.1107/S1600576715002319.

3 A. Altomare, N. Corriero, C. Cuocci, A. Falcicchio, A. Moliterni, R. Rizzi, OChemDb: the free online Open Chemistry Database portal for searching and analysing crystal structure information, Journal of Applied Crystallography 51 (4) (2018) 1229–1236. doi:10.1107/S1600576718008166.

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# Competitive effectors of alpha-synuclein.

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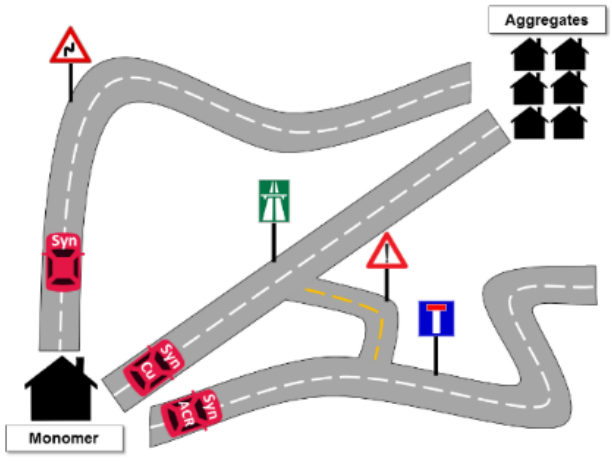
e ottenere il codice latex delle equazioni.

Parkinson's disease (PD) and $\alpha$-synucleinopathies are characterized by the progressive loss of neuronal cells and the decline of cognitive and motor functions. Biochemical and neuropathological evidence supports the role of oxidative stress, metal dyshomeostasis and $\alpha$-synuclein ($\alpha$Syn, a presynaptic and intrinsically disordered protein), in the development of these disorders[1,2]. Mounting evidence suggests that the aggregation of $\alpha$Syn is a crucial event in the pathogenesis of $\alpha$-synucleinopathies.

Metal-protein interactions play an important role in $\alpha$Syn aggregation and might represent a link between the pathological processes of protein aggregation, oxidative damage, and neural death. High Copper concentration is detected the cerebrospinal fluid of PD patients, as well as in the Lewy bodies, the intracellular aggregates of $\alpha$Syn. Moreover, Copper regulates $\alpha$Syn intracellular localization and cytotoxicity[3].




Lipoxidation and carbonylation have also been observed in neurodegenerative diseases. $\alpha$Syn seems to induce lipid peroxidation and, conversely, $\alpha$Syn carbonylation has been found in PD. Lipoxidation leads to the formation of the so-called Reactive Carbonyl Species (RCS); in particular, acrolein (ACR) and 4-hydroxy-nonenal (HNE) have been reported to affect the aggregation process of $\alpha$Syn[4,5]. The adducts ACR-$\alpha$Syn have been less explored and characterized. Moreover, the interplay between ACR, copper, and $\alpha$Syn has never been investigated.

Therefore, we explored more thoroughly the dose- and time-dependent effects of ACR on $\alpha$Syn using an approach based on Ultra Performance Liquid Chromatography coupled with High-Resolution Mass spectrometry. Moreover, we evaluated the effects of $Cu^{2+}$ ions on these chemical modifications, and the influence of His carbonylation on $Cu^{2+}$-binding. Finally, we investigated the effects of ACR and $Cu^{2+}$ ions on $\alpha$Syn aggregation by a fluorescence assay and dynamic light scattering (DLS).

## References

1 K. Jomova, D. Vondrakova, M. Lawson, M. Valko, Metals, oxidative stress and neurodegenerative disorders, Molecular and cellular biochemistry 345 (1-2) (2010) 91–104. doi:10.1007/s11010-010-0563-x.

2 V. Lanza, F. Bellia, E. Rizzarelli, An inorganic overview of natural a$\beta$ fragments: Copper(ii) and zinc(ii)-mediated pathways, Coordination Chemistry Reviews 369 (2018) 1 – 14. doi:https://doi.org/10.1016/j.ccr.2018.04.004.

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3 A. Binolfi, L. Quintanar, C. W. Bertoncini, C. Griesinger, C. O. Fernández, Bioinorganic chemistry of copper coordination to alpha-synuclein: Relevance to parkinson's disease, Coordination Chemistry Reviews 256 (19) (2012) 2188 – 2201. doi:https://doi.org/10.1016/j.ccr.2012.05.004.

4 T. Näsström, T. Fagerqvist, M. Barbu, M. Karlsson, F. Nikolajeff, A. Kasrayan, M. Ekberg, L. Lannfelt, M. Ingelsson, J. Bergström, The lipid peroxidation products 4-oxo-2-nonenal and 4-hydroxy-2-nonenal promote the formation of $\alpha$-synuclein oligomers with distinct biochemical, morphological, and functional properties, Free Radical Biology and Medicine 50 (3) (2011) 428 – 437. doi:https://doi.org/10.1016/j.freeradbiomed.2010.11.027.

5 Y.-T. Wang, H.-C. Lin, W.-Z. Zhao, H.-J. Huang, Y.-L. Lo, H.-T. Wang, A. M.-Y. Lin, Acrolein acts as a neurotoxin in the nigrostriatal dopaminergic system of rat: involvement of $\alpha$-synuclein aggregation and programmed cell death, Scientific reports 7 (2017) 45741. doi:10.1038/srep45741.

Conference

# Antimicrobial peptides (AMPs): Analysis, synthetic design and biological analysis.

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The increase of resistance to antibiotics,[1] also due to a systematic and widespread misuse and abuse of these drugs, is a tremendous problem of healthcare systems and society. Multiple resistance to antibiotics is a global threat aggravated by the lack of novel alternative and effective therapeutic agents.[2] The most worrying multidrug-resistant pathogens are listed by the World Health Organization under the acronym "ESKAPE",[3] (i.e., *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.*) as needing urgent and prompt discovery of new antimicrobials.

Antimicrobial peptides (AMPs) are potentially suitable to alternatives to conventional antibiotics[4] or effective adjuvant drugs allowing conventional antibiotics to overcome resistance .[2,5] In this scenario, our research activity focuses on identifying, analyzing, synthesizing and testing new AMPs of natural origin, and their optimized synthetic variants. Here, we present a hierarchical approach applied to *Taenia solium* peptides (TSO8), whose sequence is compared with other native AMPs, analyzed to determine potential active fragments, and then synthesized to obtain preliminary functional (MIC, cytotoxicity) and structural (CD) characteristics.

## References

1 M. D. Torres, S. Sothiselvam, T. K. Lu, C. de la Fuente-Nunez, Peptide design principles for antimicrobial applications, Journal of Molecular Biology 431 (18) (2019) 3547–3567, the molecular basis of antibiotic action and resistance. doi:https://doi.org/10.1016/j.jmb.2018.12.015.

2 N. Mookherjee, M. A. Anderson, H. P. Haagsman, D. J. Davidson, Antimicrobial host defence peptides: functions and clinical potential, Nature reviews Drug discovery 19 (5) (2020) 311–332. doi:10.1038/s41573-019-0058-8.

3 D. M. De Oliveira, B. M. Forde, T. J. Kidd, P. N. Harris, M. A. Schembri, S. A. Beatson, D. L. Paterson, M. J. Walker, Antimicrobial resistance in eskape pathogens, Clinical microbiology reviews 33 (3) (2020) e00181–19. doi:10.1128/CMR.00181-19.

4 M. Magana, M. Pushpanathan, A. L. Santos, L. Leanse, M. Fernandez, A. Ioannidis, M. A. Giulianotti, Y. Apidianakis, S. Bradfute, A. L. Ferguson, A. Cherkasov, M. N. Seleem, C. Pinilla, C. de la Fuente-Nunez, T. Lazaridis, T. Dai, R. A. Houghten, R. E. W. Hancock, G. P. Tegos, The value of antimicrobial peptides in the age of resistance, The Lancet Infectious Diseases 20 (9) (2020) e216–e230. doi:https://doi.org/10.1016/S1473-3099(20)30327-3.

5 K. Sharma, S. Aaghaz, K. Shenmar, R. Jain, Short antimicrobial peptides, Recent patents on anti-infective drug discovery 13 (1) (2018) 12–52. doi:10.2174/1574891X13666180628105928.

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# Free radicals induced DNA damage: Chemical, Analytical, Biological, and Diagnostic Aspects.

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DNA damage caused by free-radicals includes a large variety of base and sugar lesions leading to DNA breaks and DNA-protein cross-links. Among free radicals, the diffusible hydroxyl radicals ($HO^{\cdot}$) are key oxidant species responsible for reacting with DNA either by hydrogen abstraction from 2-deoxyribose units or by addition to the base moieties. The purine 5',8-cyclo-2'-deoxynucleosides (cPu) are an important class of lesions exclusively generated by the $HO^{\cdot}$ attack to DNA purine nucleotides, forming C5' radicals followed by an internal cyclization giving 5',8-cyclo-2'-deoxyadenosine (cdA) and 5',8-cyclo-2'-deoxyguanosine (cdG) identified as products, in two possible diastereomeric forms, 5'*R* and 5'*S* (Fig. 1).[1,2]

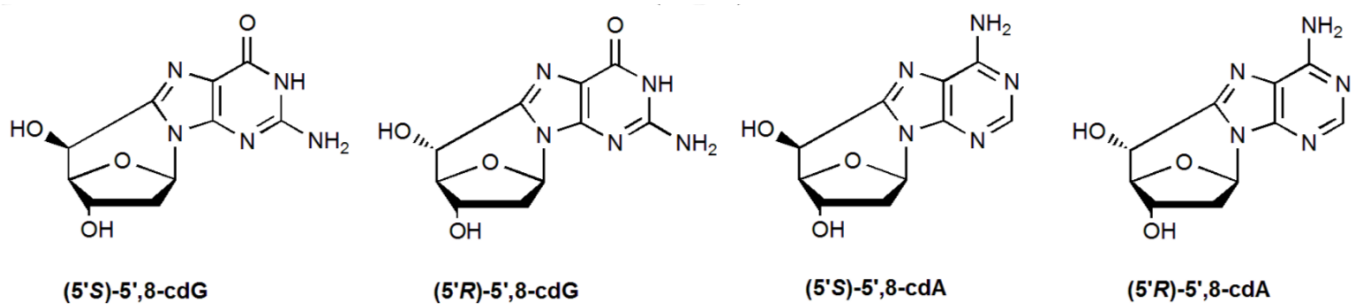


**Fig. 1**

These lesions are exclusively repaired by the Nucleotide Excision Repair pathway (NER) with low efficiency in comparison with other bulky DNA lesions.[3]

Nowadays cPu are used as radical stress biomarkers of DNA damage thanks to their specific formation mechanism and their chemical stability during work-up.

The diastereomeric 5'*S*- and 5'*R*-cPu are discussed in terms of:

(i) Synthetic and analytical protocols for the availability and characterization of the diastereoisomeric 5'S- and 5'R-cPu lesions, also as isotopic labelled references;
(ii) Physical-chemical studies on specific oligonucleotide models as: MD simulations, NMR, Melting Temperature;
(iii) Biological and Clinical studies for the investigation of the relationships between the levels of lesions and human health, disease, and aging is a matter of investigation.[4]

## References

1 C. Chatgilialoglu, C. Ferreri, N. E. Geacintov, M. G. Krokidis, Y. Liu, A. Masi, V. Shafirovich, M. A. Terzidis, P. S. Tsegay, 5',8-cyclopurine lesions in dna damage: Chemical, analytical, biological, and diagnostic significance, Cells 8 (6) (2019) 513. doi:10.3390/cells8060513.

2 C. Chatgilialoglu, L. A. Eriksson, M. G. Krokidis, A. Masi, S. Wang, R. Zhang, Oxygen dependent purine lesions in double-stranded oligodeoxynucleotides: Kinetic and computational studies highlight the mechanism for 5', 8-

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cyclopurine formation, Journal of the American Chemical Society 142 (12) (2020) 5825–5833. doi:10.1021/jacs.0c00945.

3 K. Kropachev, S. Ding, M. A. Terzidis, A. Masi, Z. Liu, Y. Cai, M. Kolbanovskiy, C. Chatgilialoglu, S. Broyde, N. E. Geacintov, V. Shafirovich, Structural basis for the recognition of diastereomeric 5',8-cyclo-2'-deoxypurine lesions by the human nucleotide excision repair system, Nucleic Acids Research 42 (8) (2014) 5020–5032. doi:10.1093/nar/gku162.

4 A. Masi, P. Fortini, M. G. Krokidis, E. F. Romeo, C. Bascietto, P. De Angelis, V. Guglielmi, C. Chatgilialoglu, Increased levels of 5',8-cyclopurine dna lesions in inflammatory bowel diseases, Redox Biology 34 (2020) 101562. doi:https://doi.org/10.1016/j.redox.2020.101562.

Conference

# Carnosine and trehalose-carnosine tunesthe activity and expression of endogenous protection factors and their crosstalk with metal homeostasis.

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Carnosine ($\beta$-alanyl-L-histidine) is a natural dipeptide widely distributed in mammalian tissues and presented at high concentrations (0.7–2.0mM) in the brain.[1] As reported previously, carnosine augmented the secretion and expression of various neurotrophic factors (for example, BDNF), leading to the induction of neurite growth in SY-SY5Y cells.[2] Moreover, carnosine glial release and neuronal utilization in CNS have been described;[3] carnosine intercepts the regulatory routes of Cu homeostasis in nervous cells and tissues. Cu dysregulation imply the oxidative stress, free-radical production and functional impairment leading to neurodegeneration. Barca et al showed that the extracellular carnosine exposure influenced intracellular Cu entry and affected the key Cu-sensing system (SP1 and CTR1).[4] On this basis, carnosine, its derivate with trehalose and potential role of copper ions were investigated in the present study. First of all, we demonstrate that trehalose-carnosine crosses the cell membrane better than carnosine and its translocation does not depend on copper ions. On the next step, we analyzed a role of carnosine and its derivative in the modulation of CREB functions in the normal and in the copper ions deprivation conditions. Previously, it has been shown that carnosine and copper alone induce CREB phosphorylation[5,6]. Here we found that 30 min of PC12 cells incubation with trehalose-carnosine stimulates CREB phosphorylation more than carnosine alone and the level of phospho-CREB depends on the presence of copper ions in the medium. To compare the influence of trehalose-carnosine and carnosine alone on copper homeostasis, a measure of the copper transporter CTR1 and transcriptional factor SP1 expression in culture of PC12 cells was carried out.

## References

1 A. R. Hipkiss, Carnosine, diabetes and alzheimer's disease, Expert Review of Neurotherapeutics 9 (5) (2009) 583–585. doi:10.1586/ern.09.32.

2 K. Kadooka, K. Fujii, T. Matsumoto, M. Sato, F. Morimatsu, K. Tashiro, S. Kuhara, Y. Katakura, Mechanisms and consequences of carnosine-induced activation of intestinal epithelial cells, Journal of Functional Foods 13 (2015) 32 – 37. doi:10.1016/j.jff.2014.12.024.

3 K. Bauer, Carnosine and homocarnosine, the forgotten, enigmatic peptides of the brain, Neurochemical research 30 (10) (2005) 1339–1345. doi:10.1007/s11064-005-8806-z.

4 A. Barca, S. Ippati, E. Urso, C. Vetrugno, C. Storelli, M. Maffia, A. Romano, T. Verri, Carnosine modulates the Sp1-Slc31a1/Ctr1 copper-sensing system and influences copper homeostasis in murine CNS-derived cells, American Journal of Physiology-Cell Physiology 316 (2) (2019) C235–C245. doi:10.1152/ajpcell.00106.2018.

5 K. Fujii, K. Abe, K. Kadooka, T. Matsumoto, Y. Katakura, Carnosine activates the creb pathway in caco-2 cells, Cytotechnology 69 (3) (2017) 523–527. doi:10.1007/Fs10616-017-0089-0.

6 I. Naletova, C. Satriano, A. Pietropaolo, F. Gianì, G. Pandini, V. Triaca, G. Amadoro, V. Latina, P. Calissano, A. Travaglia, V. G. Nicoletti, D. La Mendola, E. Rizzarelli, The Copper(II)-Assisted Connection between NGF and BDNF by Means of Nerve Growth Factor-Mimicking Short Peptides, Cells 8 (4) (2019) 301. doi:10.3390/cells8040301.

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Conference

# Insights into the PPAR$\gamma$ phosphorylation and its inhibition mechanism.

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Peroxisome proliferator-activated receptor gamma Ligand Binding Domain (PPAR$\gamma$-LBD) represents a key target for the treatment of type II diabetes and metabolic syndrome. This receptor is the target of thiazolidinediones, a class of antidiabetic drugs, which improve insulin sensitization and regulate glycemia in type 2 diabetes. Unfortunately, despite the beneficial effects of synthetic drugs, their use is associated with serious undesirable side effects[1,2] related to their agonism. By contrast, a promising activation-independent mechanism that involves the inhibition of cyclin-dependent kinase 5 (Cdk5)-mediated PPAR$\gamma$ phosphorylation (CMPF) has been related to the insulin-sensitizing effects induced by these drugs.[3,4] For this reason, the search for new inhibitors of CMPF represents an opportunity for the development of an improved generation of anti-diabetic drugs acting through this nuclear receptor. Thus, with the aim to identify novel drug-like inhibitors of CMPF capable of interacting with PPAR$\gamma$ but that lack agonist properties we adopted a multi-disciplinary approach, including protein-protein docking, X-ray, NMR, HDX, MD simulations and site-directed mutagenesis to investigate conformational changes in PPAR$\gamma$ that impair the ability of Cdk5 to interact with this nuclear receptor and hence inhibit its phosphorylation.

## References

1 M. Bodmer, C. Meier, M. E. Kraenzlin, C. R. Meier, Risk of Fractures with Glitazones, Drug safety 32 (7) (2009) 539–547. doi:10.2165/00002018-200932070-00001.

2 R. W. Nesto, D. Bell, R. O. Bonow, V. Fonseca, S. M. Grundy, E. S. Horton, M. Le Winter, D. Porte, C. F. Semenkovich, S. Smith, et al., Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association, Diabetes care 27 (1) (2004) 256–263. doi:10.2337/diacare.27.1.256.

3 J. H. Choi, A. S. Banks, J. L. Estall, S. Kajimura, P. Boström, D. Laznik, J. L. Ruas, M. J. Chalmers, T. M. Kamenecka, M. Blüher, et al., Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPAR$\gamma$ by Cdk5, Nature 466 (7305) (2010) 451–456. doi:10.1038/nature09291.

4 J. H. Choi, A. S. Banks, T. M. Kamenecka, S. A. Busby, M. J. Chalmers, N. Kumar, D. S. Kuruvilla, Y. Shin, Y. He, J. B. Bruning, et al., Antidiabetic actions of a non-agonist PPAR$\gamma$ ligand blocking Cdk5-mediated phosphorylation, Nature 477 (7365) (2011) 477–481. doi:10.1038/nature10383.

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# Peptide therapeutics: $\beta$-sheet breaker conjugates for neuroprotection and amyloid detection.

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Peptides may represent an opportunity for therapeutic intervention that closely mimics natural pathways.[1] As a unique class of pharmaceutical compounds peptides are endowed with biochemically and therapeutically distinct properties with respect to small molecules or proteins. Peptides' favourable pharmaceutical properties include high specificity and potency for their target, minimal potential for drug-drug interactions, lack of accumulation in tissues, and effectively metabo- lized by endogenous enzymes to non-toxic metabolites. On the other hand, peptide therapeutics may suffer unfavourable pharmaceutical drawbacks. These comprise instability and short duration of action, inability to cross cellular membranes, and potential for immunogenicity. Recent research in peptide chemistry has made advances to resolve these critical aspects. In particular, bio-conjugation can prolong peptides' plasma stability by preventing their degradation by exopeptidases. Bio-conjugated peptides play an important role in several fields of biomolecular and medicinal chemistry.[2] In particular, peptide-based epitopes with covalently attached other moieties able to explicate additional or complementary functions, including BBB permeation, metal chelation or aggregates disassembling, targeted imaging and treatment, hold a promising potential for applications in Alzheimer's disease (AD). AD has been proving to be extraordinarily refractory to any attempt aimed at halting or slowing the progression of the disease. In our laboratory, we have been synthesizing a variety of small peptides bio-conjugates differing by the peptide epitope or the conjugated scaffold.[3,4] A range of molecular details, together with measured biological effects, have been listed with these systems, all of them accounting for the observed neuroprotection against the toxic insult induced by A$\beta$ aggregation in primary cortical neurons. In this communication an overview of the design principles of the peptide conjugates, their neuroprotective activity and their capability in detecting A$\beta$ peptide in solution are described in terms of potential use of these compounds as theranostic agents and for the targeted drug delivery.

## References

1 J. L. Lau, M. K. Dunn, Therapeutic peptides: Historical perspectives, current development trends, and future directions, Bioorganic & Medicinal Chemistry 26 (10) (2018) 2700 – 2707. doi:10.1016/j.bmc.2017.06.052.

2 S. B. Gunnoo, A. Madder, Bioconjugation – using selective chemistry to enhance the properties of proteins and peptides as therapeutics and carriers, Org. Biomol. Chem. 14 (2016) 8002–8013. doi:10.1039/C6OB00808A.

3 S. Lazzaro, N. Ogrinc, L. Lamont, G. Vecchio, G. Pappalardo, R. M. Heeren, Ion mobility spectrometry combined with multivariate statistical analysis: revealing the effects of a drug candidate for alzheimer's disease on a$\beta$1-40 peptide early assembly, Analytical and bioanalytical chemistry 411 (24) (2019) 6353–6363. doi:10.1007/s00216-019-02030-7.

4 G. Di Natale, S. Zimbone, F. Bellia, M. Tomasello, M. Giuffrida, G. Pappalardo, E. Rizzarelli, Potential therapeutics of alzheimer's diseases: New insights into the neuroprotective role of trehalose-conjugated beta sheet breaker peptides, Peptide Science 110 (5) (2018) e24083. doi:10.1002/pep2.24083.

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# Reverse engineering and 3D additive manufacturing from research to the clinical assistance.

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## 1 Introduction

This contribution aims to present the design and implementation of an innovative image treatment and 3D printing lab for the production of customized medical devices within a hospital facility.

## 2 Methods and Materials

In 2016 a charity from Bank of Italy to Fondazione Santobono Pausilipon (active in assistance, research, training and improvement of the quality of life of pediatric patients) was spent in a project to create in reverse engineering and 3D printing personalised plastic casts to be experimented at Orthopaedics and Traumatology Dep. of the Santobono Pediatric Hospital. To develop this study the know-how and multidisciplinary competences from different CNR Institutes (IC now ICCSB, IBB, IPCB) has been involved to produce the proof of concept and the consequent clinical application. Following the design phase[1] in 2017 the Ethics Committee authorized a clinical trial of the developed device on 40 patients[2]. In the meanwhile the project was awarded by Italian Convention of Public Health (ForimPA Sanità). The success of clinical trial[3] brought researcher and hospital management to awareness that the scientific outcomes are ready to be transferred to the real clinical use.

This led to the establishment of an innovative company (Santobono Innovatin srl) operating within the Santobono-Pausilipon Foundation in wich CNR was consultant to optimize manufacturing workflow. The company structured two models of 3D printed devices ad submitted itself to the certification process for the production of medical devices. In June 2019 the ISO 13485 certification and the exposure on market of public companies (MEPA).

## 3 Conclusion

The collaboration between researcher from CNR and clinical practitioners inside a health facility produces real innovations and represent a great opportunity to promote new collaborations and research projects among stakeholders. Indeed the introduction of image processing, reverse engineering and 3D printing techniques is rapidly spreading to exploit the advantages given by the design flexibility and low-cost production of prostheses, medical devices and anatomical models.

## References

1 F. Clemente, F. Colella, M. D'albore, A. Gloria, P. Guida, A. Casaburi, Esoscheletri prototipali personalizzati in materiale plastico per immobilizzazioni prolungate. applicazioni cliniche in pediatria., 1° Congresso Nazionale IDBN, Bologna (2017), abstract n. IDBN 8001-08.

2 Ethics Commetee AA.OO.R.N "A.Cardarelli/Santobono Pausilipon, clinical trial:. FSP1/2016 and Italian Ministry of Health, clinical trial: 0002535-13/01/2017-DGDMF-MDS-P.

3 P. Guida, A. Casaburi, T. Busiello, D. Lamberti, A. Sorrentino, L. Iuppariello, M. D'Albore, F. Colella, F. Clemente, An alternative to plaster cast treatment in a pediatric trauma center using the cad/cam technology to manufacture

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customized three-dimensional-printed orthoses in a totally hospital context: a feasibility study, Journal of Pediatric Orthopaedics B 28 (3) (2019) 248–255. doi:10.1097/BPB.0000000000000589.

Conference

# Privacy @CNR: panoramica generale su argomento, organizzazione e adempimenti.

Caterina Chiarella.[a]

Con la entrata in vigore del Regolamento generale sulla protezione dei dati UE 2016/679 (RGPD), il CNR ha delineato il proprio assetto organizzativo per la gestione degli adempimenti da questo previsti e posto in essere le attività da svolgere, con il fine di assicurare l'applicazione delle disposizioni in materia di protezione dei dati personali.

Si daranno i principali riferimenti normativi sull'argomento, si riporterà l'assetto organizzativo che si è dato il CNR e si metteranno in evidenza gli adempimenti cui sono tenuti gli Istituti, con particolare riferimento alla istituzione del "Registro delle attività di trattamento dei dati" e suo aggiornamento.

L'aggiornamento deve avvenire prima di avviare un trattamento, sia esso per finalità gestionali-amministrative che di ricerca. Tutte le attività condotte in Istituto sono quindi interessate.

L'obiettivo è quello di informare e sensibilizzare il personale sulla tematica. Considerata la trasversalità dell'argomento, è necessaria una ottimale collaborazione tra tutti i ruoli e profili presenti in Istituto.

## References

1 Regolamento (UE) 2016/679 del Parlamento europeo e del Consiglio del 27 aprile 2016 .

2 Garante per la Protezione dei Dati Personali (GPDP) https://www.garanteprivacy.it/.

3 Circolari CNR n.10/2018, n.24/2019, 34/2019, 12/2020 https://www.urp.cnr.it/documenti.php.

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Conference

# Toward Personalized Medicine: RGD-Peptide as Scaffold for the Comprehension of Structural Determinants for Integrin Specific Recognition.

Michele Saviano,[a] Annarita Del Gatto,[b] Laura Zaccaro,[b] Luigi Russo,[c] Roberto Fattorusso.[c]

A great challenge in cancer therapy is the selective delivery of anti cancer agents or radiotherapeutic to tumour cells reducing the side effects on normal cells. To further improve delivery efficiency and cancer specificity, great efforts have been dedicated to the development of effective systems that can actively target tumours through the molecular recognition of unique cancer-specific "markers" that are overexpressed in the cancerous tissues.

The use of peptides as targeting tools has been validated in a number of applications. Examples are radio-labelled peptides used to deliver radio-therapeutic doses to cancer tissues. In this case a cytotoxic entity, such as a radionuclide or a chemotherapeutic drug is driven by the peptide to the transformed cells with a higher efficacy than to normal cells. Typically such conjugates, at lower doses, have useful applications for diagnostic applications whereby they are used to visualize even small lesions due to the overexpression of tumour genes.

In this lecture, we will present our most recent results on a project that aim at the rational design of targeting peptidomimetic ligands to specific tumour receptors. More specifically, the design of novel anticancer drugs and/or new class of diagnostic non invasive tracers as suitable tools for $\alpha_v\beta_3$-targeted therapy and imaging. Since integrins are involved in the regulation of physiological processes, as well as in pathological ones, the development of integrin subtype-selective antagonists is highly desirable. Integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$, key mediators of cell adhesion, differentiation, proliferation, angiogenesis and tumor growth, have been considered very promising targets for theranostic application. Due to the similarity of the RGD binding regions in these integrins, the development of small synthetic molecules with high activity and selectivity for the different subtypes is a tricky goal to pursue. In the last decade, we designed and characterized the chimeric molecule RGDechi, a peptide selective for $\alpha_v\beta_3$.[1] We demonstrated anti-adhesive and proapoptotic effects on tumor cells and antiangiogenic activity in vivo.[2–4] In addition, SPECT and PET imaging studies with $^{111}In$ and $^{18}F$-labelled RGDechi in a xenograft model confirmed the ability of peptide to selectively visualize this integrin. Recently, NMR and computational analyses on cell membranes allowed a detailed understanding of $\alpha_v\beta_3$GDechi recognition mechanism.[5] Herein we have investigated the capability of the peptide to bind $\alpha_5\beta_1$ integrin and characterized the molecular determinants governing this interaction through a combined experimental and computational approach.

The detailed comparison of RGDechi/$\alpha_5\beta_1$ structural model with that, previously, determined of RGDechi in complex with $\alpha_v\beta_3$ has shown how the chimeric nature of the peptide renders the molecule an important scaffold to recognize integrins with different recognition modalities, providing insight on the structural requirements needed to design novel peptides selective for $\alpha_5\beta_1$ to use in theranostics.[6]

## References

1 A. Del Gatto, L. Zaccaro, P. Grieco, E. Novellino, A. Zannetti, S. Del Vecchio, F. Iommelli, M. Salvatore, C. Pedone, M. Saviano, Novel and selective $\alpha_v\beta_3$ receptor peptide antagonist: Design, synthesis, and biological behavior, Journal of medicinal chemistry 49 (11) (2006) 3416–3420. doi:10.1021/jm060233m.

2 A. Zannetti, S. Del Vecchio, F. Iommelli, A. Del Gatto, S. De Luca, L. Zaccaro, A. Papaccioli, J. Sommella, M. Panico, A. Speranza, P. Grieco, E. Novellino, M. Saviano, C. Pedone, M. Salvatore, Imaging of $\alpha_v\beta_3$ expression by a bi-

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functional chimeric rgd peptide not cross-reacting with $\alpha_v\beta_5$, Clinical Cancer Research 15 (16) (2009) 5224–5233. doi:10.1158/1078-0432.CCR-08-3270.

3 G. Santulli, M. F. Basilicata, M. De Simone, C. Del Giudice, A. Anastasio, D. Sorriento, M. Saviano, A. Del Gatto, B. Trimarco, C. Pedone, et al., Evaluation of the anti-angiogenic properties of the new selective $\alpha_v\beta_3$ integrin antagonist rgdechihcit, Journal of translational medicine 9 (1) (2011) 1–10. doi:10.1186/1479-5876-9-7.

4 M. PISANO, I. DE PAOLA, V. NIEDDU, I. SASSU, S. COSSU, G. GALLERI, A. DEL GATTO, M. BUDRONI, A. COSSU, M. SAVIANO, G. PALMIERI, L. ZACCARO, C. ROZZO, In vitro activity of the $\alpha_v\beta_3$ integrin antagonist rgdechi-hcit on malignant melanoma cells, Anticancer Research 33 (3) (2013) 871–879.
URL https://ar.iiarjournals.org/content/33/3/871

5 B. Farina, I. de Paola, L. Russo, D. Capasso, A. Liguoro, A. D. Gatto, M. Saviano, P. V. Pedone, S. Di Gaetano, G. Malgieri, L. Zaccaro, R. Fattorusso, A combined nmr and computational approach to determine the rgdechi-hcit-$\alpha_v\beta_3$ integrin recognition mode in isolated cell membranes, Chemistry - A European Journal 22 (2) (2016) 681–693. doi:https://doi.org/10.1002/chem.201503126.

6 B. Farina, A. Del Gatto, D. Comegna, S. Di Gaetano, D. Capasso, C. Isernia, M. Saviano, R. Fattorusso, L. Zaccaro, L. Russo, Conformational studies of rgdechi peptide by natural-abundance nmr spectroscopy, Journal of Peptide Science 25 (5) (2019) e3166. doi:https://doi.org/10.1002/psc.3166.

Conference

# Protein Crystallization in Hydrated Deep Eutectic Solvents.

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Deep Eutectic Solvents (DESs) are mixtures of Lewis or Brønsted acids and bases whose melting point is much lower than that of the individual components.[1] Several applications are known in the literature for such neoteric solvents which include the usage of DESs in extraction processes, in biodiesel production and purification, as proton conductors for fuel cells, in metal-, organo- and biocatalyzed transformations, as well as the in polishing procedures of metal surfaces.[2–7] Ten years ago, it was found that deep eutectic mixtures spontaneously form in plants as a consequence of the accumulation in cells of metabolites like sugars, alcohols, amines, amino acids, and organic acids,[8,9] an evidence that aroused the curiosity of the experts. Indeed, such these mixtures appeared to be an ideal solvent for the transport of macromolecules of diverse polarities inside the plants, hence they could be used as component of the intra cellular medium in synthetic biology. Thus, the quest for reliable structural information about the interactions between DESs and proteins is of the utmost importance and the first step towards such target is to obtain high quality crystals of protein molecules in the presence of DESs.

Here, we show, for the first time, that protein molecules are able to crystallize in the presence of DESs.[10] Our crystallization experiments have shown that hen-egg white lysozyme crystallizes according to the DES components and the hydration level. Successful crystallization have been achieved by using choline chloride:urea, choline chloride:glycerol, and choline chloride:glutamic acid eutectic mixtures at a 1:2 molar ratio. X-ray diffraction experiments have provided details about the binding sites of DES components on lysozyme protein surface and precise information about the intriguing non-covalent bond network by which protein, DES components and water molecules interact each other. A non-covalent bond network between DES components mediated by water molecules has also been unveiled. DESs appear to have negligible effects on protein conformation. On the other hand, DESs significantly reduce solvent evaporation from the crystallization drop, a key property to increase the dissolution time of the protein crystals. Moreover, DESs could tune protein solubility because it appears to affect hydration shell of the protein.

Our results prove that high-quality protein crystals can be obtained in the presence of DESs and that such crystals can be used to get precise structural information about the interaction between DESs and proteins, which is still lacking in the literature. Moreover, the ability of DESs to protect protein crystals from their rapid dissolution (which has been observed in this work) could be exploited in biotechnological applications involving enzymes in crystalline form.

## References

1 B. B. Hansen, S. Spittle, B. Chen, D. Poe, Y. Zhang, J. M. Klein, A. Horton, L. Adhikari, T. Zelovich, B. W. Doherty, et al., Deep eutectic solvents: A review of fundamentals and applications, Chemical reviews 121 (3) (2020) 1232–1285. doi:10.1021/acs.chemrev.0c00385.

2 M. Hayyan, F. S. Mjalli, M. A. Hashim, I. M. AlNashef, A novel technique for separating glycerine from palm oil-based biodiesel using ionic liquids, Fuel Processing Technology 91 (1) (2010) 116–120. doi:10.1016/j.fuproc.2009.09.002.

3 A. Hayyan, M. A. Hashim, M. Hayyan, F. S. Mjalli, I. M. AlNashef, A novel ammonium based eutectic solvent for the treatment of free fatty acid and synthesis of biodiesel fuel, Industrial Crops and Products 46 (2013) 392–398.

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doi:10.1016/j.indcrop.2013.01.033.

4 E. Habibi, K. Ghanemi, M. Fallah-Mehrjardi, A. Dadolahi-Sohrab, A novel digestion method based on a choline chloride–oxalic acid deep eutectic solvent for determining cu, fe, and zn in fish samples, Analytica Chimica Acta 762 (2013) 61–67. doi:10.1016/j.aca.2012.11.054.

5 S. O. Edeki, H. Okagbue, A. Opanuga, S. Adeosun, A semi-analytical method for solutions of a certain class of second order ordinary differential equations, Applied mathematics 2014 (5) (2014) 2034–2041. doi:10.4236/am.2014.513196.

6 F. M. Perna, P. Vitale, V. Capriati, Deep eutectic solvents and their applications as green solvents, Current Opinion in Green and Sustainable Chemistry 21 (2020) 27–33. doi:10.1016/j.cogsc.2019.09.004.

7 L. Cicco, G. Dilauro, F. M. Perna, P. Vitale, V. Capriati, Advances in deep eutectic solvents and water: applications in metal-and biocatalyzed processes, in the synthesis of apis, and other biologically active compounds, Organic & Biomolecular Chemistry 19 (12) (2021) 2558–2577. doi:10.1039/D0OB02491K.

8 Y. H. Choi, J. van Spronsen, Y. Dai, M. Verberne, F. Hollmann, I. W. Arends, G.-J. Witkamp, R. Verpoorte, Are Natural Deep Eutectic Solvents the Missing Link in Understanding Cellular Metabolism and Physiology?, Plant Physiology 156 (4) (2011) 1701–1705. doi:10.1104/pp.111.178426.

9 Y. Dai, J. van Spronsen, G.-J. Witkamp, R. Verpoorte, Y. H. Choi, Natural deep eutectic solvents as new potential media for green technology, Analytica Chimica Acta 766 (2013) 61–68. doi:10.1016/j.aca.2012.12.019.

10 B. D. Belviso, F. M. Perna, B. Carrozzini, M. Trotta, V. Capriati, R. Caliandro, Introducing protein crystallization in hydrated deep eutectic solvents, ACS Sustainable Chemistry & Engineeringdoi:10.1021/acssuschemeng.1c01230.

Conference

# Surface Plasmon Resonance, a powerful tool for ligand binding investigation.

Davide Capelli,[a,*]

Selection of promising, well characterized hits and leads is essential for success in the drug discovery process. Furthermore, information on the interaction of potential drug candidates with the targeted biomolecule is important as a basis for the understanding of more complex schemes. Binding assays are the type of assays that provide such information on affinity, kinetics and thermodynamics.[1] Biophysical binding assays are alternative tools in generating label free, high quality data on the interaction between a target and a potential drug candidate. Label free screening methods include mass spectroscopy, nuclear magnetic resonance spectroscopy, isothermal titration calorimetry (ITC) and biosensor.

Biosensors based on optical detection principles are the type of sensors most often used. They offer a rapid way to determine relevant binding data without the need for labeling of the interacting molecules. These biosensors measure in real time the quantity of complex formed between a molecule immobilized on the sensor surface and a molecule in solution, determining small changes in refractive index induced at the interface upon binding. A huge number of different optical techniques to monitor such refractive index changes have already been introduced in the past and this number is still increasing. Among them, Surface Plasmon Resonance (SPR) is currently the most widely used due to the wide versatility and sensibility of the method.

Here we present the results obtained using SPR with various types of assay involving different biological targets. In particular, kinetics data from single compound binding assay,[2] surface competition assay[3] and screening of small compounds library[4] will be shown.

## Riferimenti

1 J. S. Marks, D. S. Burdette, D. A. Giegel, Homogeneous Techniques for Monitoring Receptor–Ligand Interactions, Humana Press, Totowa, NJ, 2002, pp. 51–63. doi:10.1385/1-59259-180-9:051.

2 D. Capelli, C. Parravicini, G. Pochetti, R. Montanari, C. Temporini, M. Rabuffetti, M. L. Trincavelli, S. Daniele, M. Fumagalli, S. Saporiti, E. Bonfanti, M. P. Abbracchio, I. Eberini, S. Ceruti, E. Calleri, S. Capaldi, Surface plasmon resonance as a tool for ligand binding investigation of engineered gpr17 receptor, a g protein coupled receptor involved in myelination, Frontiers in Chemistry 7 (2020) 910. doi:10.3389/fchem.2019.00910.

3 F. Souvalidou, D. Boi, R. Montanari, F. Polverino, G. Marini, D. Capelli, G. Pochetti, R. Contestabile, D. Trisciuoglio, A. Tramonti, P. Carpinelli, C. Ascanelli, C. Lindon, A. De Leo, R. Di Santo, R. Costi, G. Guarguaglini, A. Paiardini, A focused small-molecule screen identifies pha-680626 as an amphosteric inhibitor disrupting the interaction between aurora-a and n-myc, bioRxiv (preprint2021.03.10.433854). doi:10.1101/2021.03.10.433854.
URL https://www.biorxiv.org/content/early/2021/03/10/2021.03.10.433854

4 R. Montanari, D. Capelli, A. Tava, A. Galli, A. Laghezza, P. Tortorella, F. Loiodice, G. Pochetti, Screening of saponins and sapogenins from medicago species as potential pparγ agonists and x-ray structure of the complex pparγ/caulophyllogenin, Scientific reports 6 (2016) 27658. doi:10.1038/srep27658.

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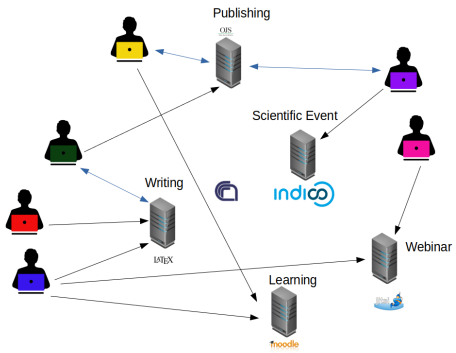
Conference

# ICT solutions for Scientific Communication and Learning.

Guido Righini,[a] Marco Simonetti,[b] Augusto Pifferi.[a]

One of the institutional tasks of public research bodies is to promote the dissemination of knowledge through editorial initiatives and training courses. Technological progress, in the field of communication, and the diffusion of the Internet makes it possible for the scientific community to produce and distribute academic publishing products on its own. The self- publication of scientific content takes place through the use of IT platforms dedicated to the management of the editorial process of production[1] and distribution of both magazines and digital monographs. Specialized IT platforms for collaborative scientific writing[2] are also available to complete the editorial process. With this platform it is possible to create scientific publishing products of high typographical quality in a collaborative way, multiple authors working simultaneously on the same document, even on different parts of the same. The software keeps track of all changes and also manages a workgroup chat. The results of the experimentation and the advantages found on the use of the IT platforms created will be exposed.




Also for training, various IT solutions were tested to the creation of an integrated system of IT platforms to carry out e-learning. The results of Learning Contest Manager System[3] and Web services integration will be exposed.

Another area of scientific communication is the organization and distribution of the contents of conferences and scientific seminars. Also in this area, a specific IT platform[4,5] was tested with which to manage the entire organizational process of scientific events: from the collection of contributions, to the realization of the scientific program of the event and the management of registrations.

## References

1 G. Righini, M. Simonetti, C. Ricci, L. Ianniello, A. Pifferi, Indico: Software per l'organizzazione e la gestione di eventi accademici, SMART eLAB 12 (2018) 5–10. doi:10.30441/smart-elab.v12i0.217.

2 G. Righini, A. Pifferi, A. Lora, Scrittura Collaborativa Accademica: metodiche e applicazioni tecnologiche, Smart eLab 8 (2016) 23–26. doi:10.30441/smart-elab.v8i0.196.

3 G. Righini, L. Ianniello, G. Nantista, A. Lora, A. Pifferi, Progetto Minerva: La Piattaforma di E-Learning dell"Area della Ricerca RM 1., Smart eLab 1 (2013) 13–25. doi:10.30441/smart-elab.v1i0.24.

4 G. Righini, L. Ianniello, G. Nantista, C. Ricci, A. Pifferi, PROGETTO CALLIOPE: La Piattaforma di e-Publishing dell'Area della Ricerca RM 1., Smart eLab 1 (2013) 33–37. doi:10.30441/smart-elab.v1i0.27.

5 V. G. Muzzini, R. Calandrelli, L. Leonardo, M. Simonetti, G. Righini, A. Pifferi, L. Ianniello, A. De Simone, M. Di Claudio, G. Giardini, B. Benedetti, F. Filippone, Realizzazione di un sistema di videoconferenza utilizzando il software open source Jitsi., SMART eLAB 13 (2019) 2–8. doi:10.30441/smart-elab.v13i0.222.

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Conference

# The syntetic eptapeptide Semax, a fragment of the ACTH hormone, sustains differentiated neuroblastoma, by improving the cellular bioenergetic: investigations toward New Therapeutic Solutions.

Maria Carmela Di Rosa,[a,c] Marianna Flora Tomasello,[a,*] Mariangela Amorini,[b] Miriam Saab,[b], Giuseppe Lazzarino,[b] Irina Naletova,[a] Francesco Attanasio.[a]

Semax is the active component of a drugs developed in Russia in the1982 and originally used as a treatment for brain hypoxia and ischemia, brain traumas, and to facilitate adaptive processes to extreme situations. Semax is now used as a nootropic for mental enhancement in healthy people and for treating many cognitive disorders. Semax is a syntetic eptapeptide consisting of the Met-Glu-His-Phe fragment of ACTH and the C-terminal tripeptide Pro-GlyPro. N-terminal fragments of the adrenocorticotropic hormone (ACTH) are well known for their potent neuro-regenerative and cognitive activities.[1] The C-terminal PGP fragment provide resistance to enzymatic cleavage. Semax is devoid of hormonal activity but is still able to stimulate learning and memory formation in rodents and humans.[2] However, the molecular mechanisms underlying the action of Semax, are still unknown. At the cellular level, Semax was shown to prevent the death of cultured neurons, and to increase the expression of neurotrophine and their receptors,[3] thus implying that Semax might modulate brain functions by influencing neurotrophins functions. Here we investigated the mechanism by which Semax acts and describe the effects at the cellular level on RA-differentiated SHSY5SY cells. Interestingly, when differentiated in the presence of Semax, cells present improved mitochondrial functions and mass, increased ATP levels and improved resistance to stressors. Furthermore, we found that Semax increase the BDNF expression and release, trough the activation of the p-CREB signalling pathway. We suggest that Semax promotes cognitive brain functions by modulating the expression of the BDNF/trkB system which in turn stimulate the mitochondrial function. Thus, Semax provide neurons with the ability to better exploit feuls, either under basal or stressful conditions, which results in the improvement of neurons viability as demonstrated by the means of several experimental approaches. The finding that Semax, by modulating neurotrophins levels, improve the mitochondrial functions, has important implications for neurodegenerative and psychiatric diseases. Therefore, the therapeutic potential of Semax is far from being exhausted and new indications for its application could be discovered by an in-depth study of its mechanism of action.

## References

1 D. de Wied, Behavioral pharmacology of neuropeptides related to melanocortins and the neurohypophyseal hormones, European Journal of Pharmacology 375 (1) (1999) 1 – 11. doi:10.1016/S0014-2999(99)00339-8.

2 I. Ashmarin, V. Nezavibat'ko, N. Myasoedov, A. Kamenskii, I. Grivennikov, M. Ponomareva-Stepnaya, L. Andreeva, A. Y. Kaplan, V. Koshelev, A nootropic analog of adrenocorticotrophic hormone 4-10–semax (experience of 15 years of development and investigation), Zh. Vyssh. Nerv. Deyat 47 (1997) 420–430.

3 T. Kolomin, M. Shadrina, P. Slominsky, S. Limborska, N. Myasoedov, et al., A new generation of drugs: synthetic peptides based on natural regulatory peptides, Neuroscience & Medicine 4 (04) (2013) 223.

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Conference

# In vitro and in vivo approaches to the study of Alzheimer's disease.

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Alzheimer's disease (AD) is a progressive brain disorder that slowly leads to memory loss and cognitive decline. It is considered the most common cause of dementia in the elderly population, with an incidence and related cost of medical care that are expected to increase in the next future. However, none of the pharmacologic treatments available today for AD dementia is able to stop the neurodegeneration that causes the common symptoms of the disease.

This lack of effective cure has led to the need of new biomarkers to be used in early diagnosis and new molecular target to address interventions.

We have recently focused our work on the study of HSP60 as a new potential target for AD. By the use of in vitro and in vivo techniques we found that AD conditions affect the expression level and localization of the protein, in primary neuronal cultures. We also observed that decreased level of HSP60 in neuronal cells, positively correlate with IGF-1Rs expression, which are known to be downregulated in AD post-mortem brains.[1]

According to recent data of the literature, we investigated the modulation of HSP60 in an in vitro model of insulin resistance, in order to better understand its involvement in the downregulation of IGF1R observed in AD brain and in the other molecular mechanisms shared by Alzheimer's and Type II diabetes.[2,3]

Starting from these new data and in the light of our previous results, the main point of this presentation, will be the relevance of IGF-1 receptor and its downstream effectors in AD progression, with particular focus on the experimental techniques commonly used by our group to study Alzheimer's disease.

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## References

1 K. Talbot, H.-Y. Wang, H. Kazi, L.-Y. Han, K. P. Bakshi, A. Stucky, R. L. Fuino, K. R. Kawaguchi, A. J. Samoyedny, R. S. Wilson, Z. Arvanitakis, J. A. Schneider, B. A. Wolf, D. A. Bennett, J. Q. Trojanowski, S. E. Arnold, Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline, The Journal of Clinical Investigation 122 (4) (2012) 1316–1338. doi:10.1172/JCI59903.

2 de la Monte, S.M., Contributions of Brain Insulin Resistance and Deficiency in Amyloid-Related Neurodegeneration in Alzheimer's Disease., Drugs 72 (2012) 49–66. doi:10.2165/11597760-000000000-00000.

3 M. L. Giuffrida, F. Tomasello, F. Caraci, S. Chiechio, F. Nicoletti, A. Copani, Beta-Amyloid Monomer and Insulin/IGF-1 Signaling in Alzheimer's Disease., Mol Neurobiol 46 (2012) 605–613. doi:10.1007/s12035-012-8313-6.

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# New insight into the physiological activities of Amyloid Beta monomers.

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Alzheimer's disease (AD) is one of the most common form of dementia in the elderly, characterized by a progressive neurodegeneration associated with synaptic dysfunction, pathological accumulation of $\beta$-amyloid (A$\beta$) in plaques, and neuronal loss. The self-association of A$\beta$ monomers into soluble oligomers seems to be crucial for the development of neurotoxicity.[1]

Some of the toxic effects of A$\beta$ are mediated by its adverse effect on neurotrophic factor expressions. In particular, A$\beta$ oligomers have been found to decrease both phosphorylated CREB and BDNF mRNA in the neuroblastoma cell line, SH-SY5Y, suggesting that oligomeric A$\beta$ could compromise neuronal functions in AD by downregulating BDNF.[2] Accordingly, phosphorylated CREB and CREB-regulated BDNF are recently shown to be reduced in the brain of AD patients and Tg2576 mice.[3]

We previously reported a neuroprotective activity of monomeric A$\beta$ involving the activation of a PI3K/Akt survival pathway.[4] Here we demonstrate that A$\beta$ monomers are specifically able to activate CREB, a converging point for mechanisms and pathways involved in memory formation.[5] Our data suggest a new model whereby A$\beta$ monomers may preserve cognitive decline.

## References

1 D. M. Walsh, D. J. Selkoe, A$\beta$ Oligomers – a decade of discovery, Journal of Neurochemistry 101 (5) (2007) 1172–1184. doi:10.1111/j.1471-4159.2006.04426.x.

2 D. J. Garzon, M. Fahnestock, Oligomeric Amyloid Decreases Basal Levels of Brain-Derived Neurotrophic factor (BDNF) mRNA via Specific Downregulation of BDNF Transcripts IV and V in Differentiated Human Neuroblastoma Cells, Journal of Neuroscience 27 (10) (2007) 2628–2635. doi:10.1523/JNEUROSCI.5053-06.2007.

3 S. Pugazhenthi, M. Wang, S. Pham, C.-I. Sze, C. B. Eckman, Downregulation of CREB expression in Alzheimer's brain and in A$\beta$-treated rat hippocampal neurons, Molecular neurodegeneration 6 (1) (2011) 60. doi:10.1186/1750-1326-6-60.

4 M. L. Giuffrida, F. Caraci, B. Pignataro, S. Cataldo, P. De Bona, V. Bruno, G. Molinaro, G. Pappalardo, A. Messina, A. Palmigiano, D. Garozzo, F. Nicoletti, E. Rizzarelli, A. Copani, $\beta$-Amyloid Monomers Are Neuroprotective, Journal of Neuroscience 29 (34) (2009) 10582–10587. doi:10.1523/JNEUROSCI.1736-09.2009.

5 A. F. Teich, R. E. Nicholls, D. Puzzo, J. Fiorito, R. Purgatorio, O. Arancio, Synaptic therapy in Alzheimer's disease: a CREB-centric approach, Neurotherapeutics 12 (1) (2015) 29–41. doi:10.1007/s13311-014-0327-5.

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Conference

# CNR Hard X-ray diffraction activities at Elettra 2.0.

Alberto Cassetta,[a,*] Luisa Barba,[a] Giuseppe Chita,[a] Doriano Lamba.[a]

Elettra, the Italian third generation synchrotron source, has been in operation since 1994 and is currently planning a major upgrade of both the storage ring and the beamlines in the framework of a project named Elettra 2.0. A new machine is foresaw, which the current beam parameters[1] are very much improved, and planned to be completed in 2026, making Elettra competitive with the most up-to-date European synchrotron radiation facilities. While the new machine will be still operating at 2-0-2.4 GeV and with an unchanged ring diameter, the brilliance will be greatly increased, with a substantial gain for the entire Elettra users' community. Indeed, such an improvement in machine performance will be significant also for all the X-ray diffraction beamlines[2] currently operating at Elettra.

CNR has been contributing to the scientific growth and technical development of the Elettra research infrastructure as the main institutional partner in the last 24 years. CNR staff and associates have been involved in a variety of different experimental techniques: from X-ray macromolecular crystallography to VUV photoemissions. The CNR community has been essential in supporting the Elettra users' activities and extremely active in proposing novel experiments. In view of Elettra 2.0, the CNR community working at the Elettra beamlines have been proposing its strategic view of the Elettra evolution, which has been shared with the Elettra management and the entire scientific community.

The research group of the Istituto di Cristallografia operating in Trieste has been involved in the development of the XRD1 crystallographic beamline since the very beginning of Elettra activities. XRD1 is a multipurpose diffraction facility that has been used for macromolecular and small-molecule crystallography as well as for grazing-incidence X-ray diffraction and X-ray scattering experiments. While XRD1 has been for a long time the one and only hard X-ray diffraction beamline at the facility, owing to a strong request from the respective users' community, a number of more specialized X-ray diffraction beamlines have been recently established. With Elettra 2.0, the performances of the X-ray diffraction beamlines will be further strengthened making them more competitive to those already operational at various radiation sources. In view of the improved storage ring, we have been involved in the re-thinking of the X-ray diffraction beamlines at Elettra, especially the ones on which are focused the research activities of the CNR-IC group: XRD1 but also the macromolecular crystallography beamline currently denominated XRD2, developed by Elettra in partnership with the Indian Institute of Science.

## References

1 https://www.elettra.trieste.it/lightsources/elettra/elettra-2-0.html?showall.

2 A. Lausi, M. Polentarutti, S. Onesti, J. R. Plaisier, E. Busetto, G. Bais, L. Barba, A. Cassetta, G. Campi, D. Lamba, A. Pifferi, S. C. Mande, D. D. Sarma, S. M. Sharma, G. Paolucci, Status of the crystallography beamlines at Elettra., Eur. Phys. J. Plus 130 (2015) 43. doi:10.1140/epjp/i2015-15043-3.

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Conference

# Algae-Based Biosensors.

Viviana Scognamiglio,[a,*] Amina Antonacci.[a]

Biosensors are interesting devices arising from a synergistic combination of well-established scientific knowledge and cutting-edge technologies, including nanotechnology, biotechnology, and materials science. This cross-disciplinary approach actively contributes to the customization of algae-based biosensors with improved analytical performance (e.g. sensitivity, reproducibility, and fast response) in environmental and agrifood applications.[1–4] The main advantages in using whole algae cells and their sub-components as biorecognition elements relies on their adaptability to diverse environments, easy immobilizations procedures, and sensitivity towards specific target analytes. In addition, algae and their sub-components can be integrated into both optical and electrochemical transduction, allowing analysis in complex matrices with different turbidity and analytes concentrations. Moreover, extensive sample pre-treatments are not required, unless filtration or concentration to enhance the sensitivity.

In this scenario, we present examples of biosensors designed exploiting *Chlamydomonas reinhardtii* algae, in combination with advanced sustainable materials (e.g. paper) and nanomaterials (e.g. carbon black), for the detection of diverse target analytes (e.g. photosynthetic herbicides and chemical weapons). An optical eco-designed paper-based device was developed for nanoencapsulated-atrazine detection, where algae were immobilised on a paper substrate soaked with agar thin film. This biosensor provided detection limit in the picomolar range in tap water, intriguing results on interference, recovery and stability studies.[5] Then, an amperometric biosensor combining nanomaterials with algae was realised for the detection of photosynthetic herbicides in river water. C. reinhardtii cells were immobilised on carbon black modified screen-printed electrodes and atrazine was detected in the nanomolar range, with satisfactory results of working/storage stability, as well as in the presence of interferents.[6] The algae versatility in biosensor applications was further proved with an optical bioassay for the detection of chemical warfare agent simulants for application in security sector.[7] The bioassay was tested towards bis-2-chloroethyl amine and 2-chloroethyl ethyl sulphide in the millimolar range, demonstrating capability to discriminate chemical weapons from herbicides, if present in the same water sample. More recently, a dual opto-electrochemical biosensing platform was realised based on 28 strains of *C. reinhardtii*, immobilised on paper-based screen-printed electrodes nanomodified with carbon black, for the detection of atrazine, terbuthylazine, and diuron in tap and surface waters with detection limits in the picomolar ranges.[8] Pathogen detection was also accomplished by exploiting CC125 strain of *C. reinhardtii* based electrochemical biosensor associated to a dual electro-optical transduction prototype ad hoc designed for algal photosynthetic process. *Escherichia coli* was exploited as case study pathogen to assess the algae capability to sense their presence in wastewater. Indeed, aerobic bacteria can promote algal growth and thus oxygen evolution by reducing the photosynthetic oxygen tension within the microenvironment of the algal cells. *E. coli* was analysed in a concentration range from 100 to 2000 CFU / 100 mL, and an increase of the current signals and thus of the oxygen evolution of 10 % was registered in the presence of 1000 CFU / 100 mL and 25 % in the presence of 2000 CFU / 100 mL of pathogen concentration. A detection limit of 92 CFU/mL was achieved (LOD = 3×sd/slope).[9]. Moreover, an optical biosensor based on D1 bioinspired peptidomimetics functionalised with quantum dots nanoparticles was designed for the detection of atrazine, a case study herbicide widely exploited in agriculture and often found in WWT. This biosensor showed excellent sensitivity toward the atrazine target, with detection limits in the $\mu$g/L concentration range, meeting the requirements of E.U. legislation.[10]

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## References

1 A. Antonacci, F. Arduini, D. Moscone, G. Palleschi, V. Scognamiglio, Nanostructured (bio)sensors for smart agriculture, TrAC Trends in Analytical Chemistry 98 (2018) 95–103. doi:10.1016/j.trac.2017.10.022.

2 A. Antonacci, V. Scognamiglio, Biotechnological advances in the design of algae-based biosensors, Trends in Biotechnology 38 (3) (2020) 334–347. doi:10.1016/j.tibtech.2019.10.005.

3 A. Antonacci, V. Scognamiglio, Photosynthesis-based hybrid nanostructures: Electrochemical sensors and photovoltaic cells as case studies, TrAC Trends in Analytical Chemistry 115 (2019) 100–109. doi:10.1016/j.trac.2019.04.001.

4 C. Bartolucci, A. Antonacci, F. Arduini, D. Moscone, L. Fraceto, E. Campos, R. Attaallah, A. Amine, C. Zanardi, L. M. Cubillana-Aguilera, J. M. Palacios Santander, V. Scognamiglio, Green nanomaterials fostering agrifood sustainability, TrAC Trends in Analytical Chemistry 125 (2020) 115840. doi:10.1016/j.trac.2020.115840.

5 V. Scognamiglio, A. Antonacci, F. Arduini, D. Moscone, E. V. Campos, L. F. Fraceto, G. Palleschi, An eco-designed paper-based algal biosensor for nanoformulated herbicide optical detection, Journal of Hazardous Materials 373 (2019) 483–492. doi:10.1016/j.jhazmat.2019.03.082.

6 R. Attaallah, A. Antonacci, V. Mazzaracchio, D. Moscone, G. Palleschi, F. Arduini, A. Amine, V. Scognamiglio, Carbon black nanoparticles to sense algae oxygen evolution for herbicides detection: Atrazine as a case study, Biosensors and Bioelectronics 159 (2020) 112203. doi:10.1016/j.bios.2020.112203.

7 A. Antonacci, M. D. Lambreva, F. Arduini, D. Moscone, G. Palleschi, V. Scognamiglio, A whole cell optical bioassay for the detection of chemical warfare mustard agent simulants, Sensors and Actuators B: Chemical 257 (2018) 658–665. doi:10.1016/j.snb.2017.11.020.

8 A. Antonacci, R. Attaallah, F. Arduini, A. Amine, M. T. Giardi, V. Scognamiglio, A dual electro-optical biosensor based on chlamydomonas reinhardtii immobilised on paper-based nanomodified screen-printed electrodes for herbicide monitoring, Journal of nanobiotechnology 19 (1) (2021) 145. doi:10.1186/s12951-021-00887-4.

9 A. Antonacci, V. Scognamiglio, et al., Water, in press (2021).

10 M. T. Giardi, D. Zappi, M. Turemis, G. Varani, F. Lo Celso, G. Barone, P. Calandra, A. Antonacci, V. Scognamiglio, Quantum dots functionalised artificial peptides bioinspired to the d1 protein from the photosystem ii of chlamydomonas reinhardtii for endocrine disruptor optosensing, Talanta 224 (2021) 121854. doi:10.1016/j.talanta.2020.121854.

Conference

# Cockayne syndrome: a DNA repair defective syndrome with neurodegenerative features.

Barbara Pascucci,[a] Mariarosaria D'Errico,[b]

Cockayne syndrome (CS) is a rare genetic progeroid disorder characterized by growth and development defects, severe cutaneous photosensitivity, cachectic dwarfism, progressive neurological dysfunction, and precocious aging. CS is due to mutations in two genes, CSA and CSB. Both gene products are involved in transcription-coupled repair, subpathway of nucleotide excision repair (NER). Although CS-defective cells show hypersensitivity to UV light, impaired repair of bulky DNA lesions, delayed recovery of RNA synthesis after UV-damage, and enhanced apoptosis after transcription blockage (reviewed in[1]), patients with CS do not present increased cancer risk as expected in DNA repair defective syndromes. Furthermore, the characteristics of CS patients are hardly attributable to the NER impairment only, since some of them do not occur in XP-A patients, which show a complete NER deficiency.[2]

A growing body of evidence indicate that, besides the typical NER lesions, CS cells are defective in the repair of a broad range of DNA damage that may account for the clinical symptoms of CS (reviewed in[3]). For instance, it is well established that, upon oxidative stress, CS cells accumulate oxidatively induced DNA damage. In particular, CS cells are defective in the repair of 8-oxoguanine, 5-hydroxycytosine and cyclopurines.[4] Moreover, also a correct repair of DNA breaks is of potential great relevance for the clinical features of CS patients (defective DNA break repair is involved in neurodegeneration and neurogenesis).[5] We have showed that CS proteins have a role in single and double strand break repair.[6] Moreover, we and others have shown that CS cells present increased levels of intracellular reactive oxygen species, an intense glycolytic metabolism and mitochondria abnormalities with excessive mitochondrial fragmentation due to hyper-phosphorylation of the mitochondrial fission protein (DRP1).[7–10]

Since the hyper-phosphorylation of DRP1, we wondered if DRP1 inhibition was capable to rescue the CS pathological phenotype. When enzymatic activity of DRP1 is inhibited by MDVI, a mitochondrial fission inhibitor, the high ROS levels are reduced, and the dysfunctional mitochondrial, apoptotic phenotype of CS cells is recovered. Moreover, CS cells present a significant reduction of nitric oxide levels and interestingly, CS cells are characterized by an overexpression of the S-nitrosoglutathione reductase, suggesting that nitrosative stress might play a role in CS. All these preliminary data indicate that the modulation of enzymatic activity of DRP1 is critical in CS cells, suggesting DRP1 as a potential therapeutic target as well as its inhibitors as potential therapeutic tools.

## References

1 M. D'Errico, B. Pascucci, E. Iorio, B. Van Houten, E. Dogliotti, The role of csa and csb protein in the oxidative stress response, Mechanisms of Ageing and Development 134 (5) (2013) 261–269, special Issue on the segmental progeria Cockayne syndrome. doi:10.1016/j.mad.2013.03.006.

2 K. Kraemer, N. Patronas, R. Schiffmann, B. Brooks, D. Tamura, J. DiGiovanna, Xeroderma pigmentosum, trichothiodystrophy and cockayne syndrome: A complex genotype–phenotype relationship, Neuroscience 145 (4) (2007) 1388–1396, genome Dynamics and DNA Repair in the CNS. doi:10.1016/j.neuroscience.2006.12.020.

3 B. Pascucci, M. D'errico, E. Parlanti, S. Giovannini, E. Dogliotti, Role of nucleotide excision repair proteins in oxidative dna damage repair: an updating, Biochemistry (Moscow) 76 (1) (2011) 4–15. doi:10.1134/S0006297911010032.

4 M. D'errico, E. Parlanti, M. Teson, P. Degan, T. Lemma, A. Calcagnile, I. Iavarone, P. Jaruga, M. Ropolo, A. Pedrini,

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† oral communication at 1 st Conference on Crystallography, Structural Chemistry and Biosystems, (Catania) 04-06/10/2021

et al., The role of csa in the response to oxidative dna damage in human cells, Oncogene 26 (30) (2007) 4336–4343. doi:10.1038/sj.onc.1210232.

5 S. Katyal, P. J. McKinnon, Dna strand breaks, neurodegeneration and aging in the brain, Mechanisms of Ageing and Development 129 (7) (2008) 483–491, dNA damage, repair, ageing and age-related disease. doi:10.1016/j.mad.2008.03.008.

6 B. Pascucci, A. Fragale, V. Marabitti, G. Leuzzi, A. S. Calcagnile, E. Parlanti, A. Franchitto, E. Dogliotti, M. D'Errico, Csa and csb play a role in the response to dna breaks, Oncotarget 9 (14) (2018) 11581. doi:10.18632/oncotarget.24342.

7 B. Pascucci, T. Lemma, E. Iorio, S. Giovannini, B. Vaz, I. Iavarone, A. Calcagnile, L. Narciso, P. Degan, F. Podo, V. Roginskya, B. M. Janjic, B. Van Houten, M. Stefanini, E. Dogliotti, M. D'Errico, An altered redox balance mediates the hypersensitivity of cockayne syndrome primary fibroblasts to oxidative stress, Aging Cell 11 (3) (2012) 520–529. doi:10.1111/j.1474-9726.2012.00815.x.

8 M. Scheibye-Knudsen, M. Ramamoorthy, P. Sykora, S. Maynard, P.-C. Lin, R. K. Minor, I. Wilson, David M., M. Cooper, R. Spencer, R. de Cabo, D. L. Croteau, V. A. Bohr, Cockayne syndrome group B protein prevents the accumulation of damaged mitochondria by promoting mitochondrial autophagy, Journal of Experimental Medicine 209 (4) (2012) 855–869. doi:10.1084/jem.20111721.

9 E. F. Fang, M. Scheibye-Knudsen, L. E. Brace, H. Kassahun, T. SenGupta, H. Nilsen, J. R. Mitchell, D. L. Croteau, V. A. Bohr, Defective mitophagy in xpa via parp-1 hyperactivation and nad+/sirt1 reduction, Cell 157 (4) (2014) 882–896. doi:10.1016/j.cell.2014.03.026.

10 B. Pascucci, M. D'Errico, A. Romagnoli, C. De Nuccio, M. Savino, D. Pietraforte, M. Lanzafame, A. S. Calcagnile, P. Fortini, S. Baccarini, et al., Overexpression of parkin rescues the defective mitochondrial phenotype and the increased apoptosis of cockayne syndrome a cells, Oncotarget 8 (61) (2017) 102852. doi:10.18632/Foncotarget.9913.

Conference

# 3D electron and X-ray powder diffraction multi-technique approach for structural investigation of new challenging lead chalcohalide nanocrystals.

Anna Moliterni,[a,*] Stefano Toso, [b] Enrico Mugnaioli,[c] Julien Ramade,[d] Sara Bals,[d] Mauro Gemmi,[c] Liberato Manna,[b] Cinzia Giannini.[a]

The promising, fascinating, and unique optoelectronic properties of lead-halide and lead-chalcogenide nanocrystals motivated, during the last decade, an always increasing scientific interest. The excellent performances of $CsPbX_3$ (X = Cl,Br,I) and PbE (E=S,Se) as nanocrystalline semiconductors naturally raised the attention about the potential advantages of lead-chalcohalides, namely compounds within the Pb-E-X ternary diagram. However, this ternary diagram is mostly unexplored, with only two metastable materials being known through high-pressure and temperature solid-state synthesis.[1,2]

In the presented work, the versatility of colloidal chemistry gave us access to a whole series of Pb-S-X compounds. At the same time, it posed the challenge of elucidating the structure of a yet unknown material, namely $Pb_4S_3Br_2$, that was obtained in the form of small nanocrystals. This challenge was tackled by a combination of techniques: 3D electron diffraction (3D-ED),[3] X-ray powder diffraction (XRPD), and 3D electron tomography (3D-ET).[4] The extremely small size of crystallites called for a synergic combination of such techniques, which ensured a successful structure solution and proved to be a useful approach for overcoming the limits related to each individual technique. First, 3D-ED provided a plausible structural model by the application of Direct Methods with *XSIR2019*.[5] Then, 3D-ET confirmed the extracted cell parameters, and helped ruling out the presence of geminates or heterostructures. Finally, the reliability of the structural model was tested by an ab-initio structure solution process by XRPD that was performed through *EXPO2014*.[6] Due to the broad diffraction peaks typical of nanocrystals, the indexing process by XRPD found many plausible candidates. At this stage, the prior information on the cell parameters provided by 3D-ED and 3D-ET was essential. In addition, the prior knowledge on the space group identified by 3D-ED (i.e., Pnma) compensated the failure of the space group determination by XRPD induced by the unavoidable errors on the integrated intensities. The structure determined by XRPD and refined by Rietveld method via Fullprof[7] was very similar to the one provided by 3D-ED: the similarity between the two structure models further validated the structural solution. The equivalence of the two structural models was further proved via DFT calculations (see Figure 1) that, applied to both, converged to the same crystal structure.

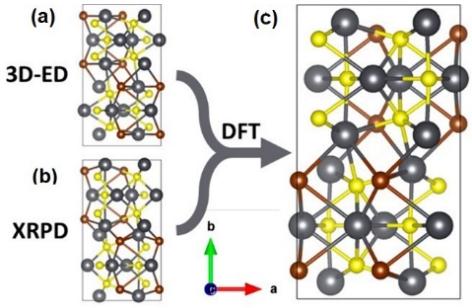


**Fig. 1** $Pb_4S_3Br_2$: a view along c of the crystal packing in case of the structure model determined by 3D-ED (a) and XRPD (b). The two structure models were strongly overlapping and converged to the same crystal structure upon DFT relaxation (c).

The main crystallographic results are here presented.

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## References


1 D. Ni, S. Guo, Z. S. Yang, K. M. Powderly, R. J. Cava, Pb4s3i2–a high-pressure phase in the pbs-pbi2 system, Solid State Sciences 91 (2019) 49–53. doi:10.1016/j.solidstatesciences.2019.03.012.

2 D. Ni, S. Guo, K. M. Powderly, R. Zhong, R. J. Cava, A high-pressure phase with a non-centrosymmetric crystal structure in the pbse–pbbr2 system, Journal of Solid State Chemistry 280 (2019) 120982. doi:10.1016/j.jssc.2019.120982.

3 M. Gemmi, E. Mugnaioli, T. E. Gorelik, U. Kolb, L. Palatinus, P. Boullay, H. S. , J. P. Abrahams, 3d electron diffraction: the nanocrystallography revolution, ACS central science 5 (8) (2019) 1315–1329. doi:10.1021/acscentsci.9b00394.

4 S. Toso, Q. A. Akkerman, B. Martín-García, M. Prato, J. Zito, I. Infante, Z. Dang, A. Moliterni, C. Giannini, E. Bladt, et al., Nanocrystals of lead chalcohalides: A series of kinetically trapped metastable nanostructures, Journal of the American Chemical Society 142 (22) (2020) 10198–10211. doi:10.1021/jacs.0c03577.

5 M. C. Burla, R. Caliandro, B. Carrozzini, G. L. Cascarano, C. Cuocci, C. Giacovazzo, M. Mallamo, A. Mazzone, G. Polidori, Crystal structure determination and refinement viasir2014, Journal of Applied Crystallography 48 (1) (2015) 306–309. doi:10.1107/S1600576715001132.

6 A. Altomare, C. Cuocci, C. Giacovazzo, A. Moliterni, R. Rizzi, N. Corriero, A. Falcicchio, *EXPO2013*: a kit of tools for phasing crystal structures from powder data, Journal of Applied Crystallography 46 (4) (2013) 1231–1235. doi:10.1107/S0021889813013113.

7 J. Rodriguez-Carvajal, J. Gonzalez-Platas, T. Roisnel, N. A. Katcho, C. Frontera, L. Chapon, O. Baltuano, FullProf Suite 2020, https://www.ill.eu/sites/fullprof.

Conference

# Kinetic analysis for the new enzymology.

Alessandro Pesaresi,[a,*]

Enzyme kinetics relies on a bulky corpus of knowledge which has been consolidated along with more than 100 years of biochemical research. The studies on sucrose hydrolysis by invertase made by Leonor Michaelis and Maud Menten led to the publication in 1913 of their homonymous equation, regarded as the fundamental equation of biochemistry. Since then, enzymologists have devised many different ways to measure and analyze kinetic data, and many different enzyme or inhibition mechanisms were identified.

The Michaelis-Menten equation and the relative “steady state” model however provide only an oversimplified description of the real enzyme behavior. The recent development of computer software for the global fitting of enzymatic reaction progress curves by numerical integration represents a fundamental breakthrough in the century-old field of enzymology. The resolution of kinetic mechanisms by computer simulation will foster a shift in the paradigm from the “steady state” to “transient state”, simplifying the study of complex mechanisms and pushing toward a next level understanding of enzyme catalysis. Here a brief introduction will be given on this new method for the study of enzyme kinetic.

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# Functional disorder in complex materials.

Gaetano Campi,[a]

Structural and electronic disorder at nano and mesoscale plays an important role in the functionality of complex materials. Here local heterogeneity and weak interactions developing between structural units cause dynamical spatio-temporal configurations with dynamical correlated disorder. Visualizing these configurations is fundamental for understanding the physical properties of complex matter and requires advanced experimental methodologies. We discuss the connections between the dynamical correlated disorder and functionality in different fields from material science to biology.

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Conference

# X-ray multilevel investigation of collagen in tissue engineering and pathology.

Alberta Terzi,[a,*] Teresa Sibillano,[a] Davide Altamura,[a] Dritan Siliqi,[a] Liberato De Caro,[a] Cinzia Giannini.[a]

Type I collagen is the main fibril-forming protein of the extracellular matrix (ECM), that provides mechanical support to tissues and organs. The protein distribution and organization is tissue-specific, depending on the biomechanical function of the tissue itself. From the molecular order, up to supramolecular scale, type I collagen is organized in triple helices assembled in fibrils and fibers, in accordance with a liquid crystalline arrangement at nanoscale. Thanks to its hierarchical structure and functional domains, collagen supplies physical support to cells attachment and growth, influencing tissue development. Thus its biocompatibility, bioactivity and biodegradability make this protein so attractive in tissue engineering, as biomaterial for implantable medical devices. Type I collagen can be extracted from different collagen-rich tissues of distinct animal species (bovine, equine, fish etc.) by chemical and/or enzymatic processes, that lead to structural alteration of the fibrillary arrangement. Since most of the structural features of type I collagen were assessed by classic X-rays investigations during last decades[1,2] in our studies we demonstrate the worthwhile contribution of Wide and Small Angle X ray Scattering (WAXS, SAXS)[3] techniques in the structural evaluation of sub and supramolecular changes of the protein, during the biomaterial fabrication steps from fresh collagen-rich tissues (bovine dermis, equine tendon, fish skin) to the final scaffolds. The evidences show the impact of processing conditions on both molecular scale and fibrillary arrangement at nanoscale. Moreover, the demonstration that further manufacturing protocols deeply affect the features of the biomaterial itself, allow to screen the suitable protocols according to the tissue to regenerate.[4,5]

The X-ray structural evaluation of collagen has been proved to be useful when applied not only to the biomaterials of engineered tissues, but even when directly applied to pathologic tissues. In particular it was demonstrated the possibility to assess the morphological alteration of collagen through scanning X-ray microdiffraction (SAXS/WAXS), when the tissue is placed under specific pathologic conditions, such as high glucose concentration typical of diabetes. The study permitted to characterize intra and intermolecular parameters alteration of collagen network with picometer precision, opening the possibility to inspect the effect of diabetes on the connectives and the impact of therapies on them.[6]

## Riferimenti

1 A. Rich, F. Crick, The molecular structure of collagen, Journal of molecular biology 3 (5) (1961) 483–IN4. doi:10.1016/S0022-2836(61)80016-8.

2 K. Okuyama, X. Xu, M. Iguchi, K. Noguchi, Revision of collagen molecular structure, Peptide Science: Original Research on Biomolecules 84 (2) (2006) 181–191. doi:10.1002/bip.20381.

3 D. Altamura, R. Lassandro, F. Vittoria, L. De Caro, D. Siliqi, M. Ladisa, C. Giannini, X-ray microimaging laboratory (xmi-lab), Journal of Applied Crystallography 45 (4) (2012) 869–873. doi:10.1107/S0021889812025733.

4 A. Terzi, E. Storelli, S. Bettini, T. Sibillano, D. Altamura, L. Salvatore, M. Madaghiele, A. Romano, D. Siliqi, M. Ladisa, et al., Effects of processing on structural, mechanical and biological properties of collagen-based substrates for regenerative medicine, Scientific reports 8 (1) (2018) 1–13. doi:10.1038/s41598-018-19786-0.

5 A. Terzi, N. Gallo, S. Bettini, T. Sibillano, D. Altamura, L. Campa, M. L. Natali, L. Salvatore, M. Madaghiele, L. De Caro, et al., Investigations of processing–induced structural changes in horse type-i collagen at sub and supramolecular

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levels, Frontiers in bioengineering and biotechnology 7 (2019) 203. doi:10.3389/fbioe.2019.00203.

6 C. Giannini, A. Terzi, L. Fusaro, T. Sibillano, A. Diaz, M. Ramella, V. Lutz-Bueno, F. Boccafoschi, O. Bunk, Scanning x-ray microdiffraction of decellularized pericardium tissue at increasing glucose concentration, Journal of biophotonics 12 (10) (2019) e201900106. doi:10.1002/jbio.201900106.

Conference

# Wide Angle and Small Angle X-ray Scattering for the structural characterization of fibers.

Teresa Sibillano,[a,*] Alberta Terzi,[a] Liberato De Caro,[a] Francesco Scattarella,[a] Rocco Lassandro,[a] Cinzia Giannini.[a]

Over the years, there has been a growing interest in the development of nanofibrous structures with natural (collagen, silk fibroin, elastin, chitosan, alginate) and synthetic (polylactic acid - PLA, poly-lactic-co-glycolic acid – PLGA, polycaprolactone – PCL) polymers as fibrous structures. Thanks to tunable specific features linked to their nanostructure, the application of nanofibers in several fields has shown to be suitable for the solution of practical problems. Wide-Angle (WAXS) and Small-Angle (SAXS) X-ray Scattering are two techniques available at the MicroImaging Laboratory (XMI-Lab) of the Institute of Crystallography in Bari[1] equipped with by a table top high brilliance (synchrotron class) X-ray micro-source. Here, we report our work on natural polymeric fibers such as type I collagen[2,3], silk fibroin from Bombix Mori, cellulose[4] but also on engineered fibers, such as peptide-based supramolecular architectures.[5]

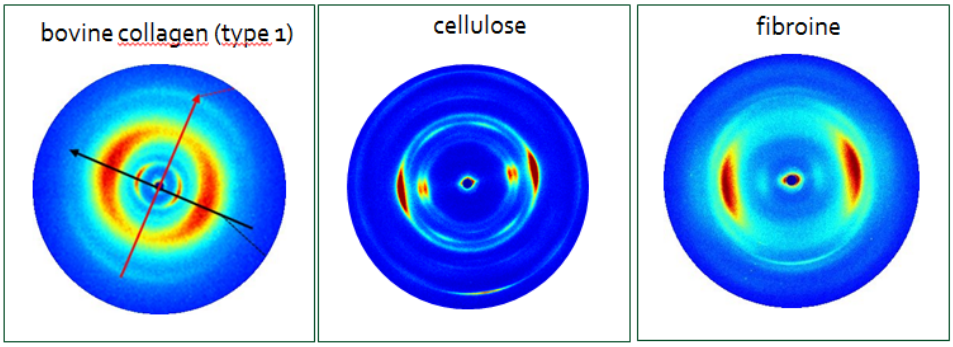


**Fig. 1** Wide-Angle X-ray Scattering (WAXS) patterns measured on natural fibers.

## References

1 D. Altamura, R. Lassandro, F. Vittoria, L. De Caro, D. Siliqi, M. Ladisa, C. Giannini, X-ray microimaging laboratory (xmi-lab), Journal of Applied Crystallography 45 (4) (2012) 869–873. doi:10.1107/S0021889812025733.

2 A. Terzi, E. Storelli, S. Bettini, T. Sibillano, D. Altamura, L. Salvatore, M. Madaghiele, A. Romano, D. Siliqi, M. Ladisa, et al., Effects of processing on structural, mechanical and biological properties of collagen-based substrates for regenerative medicine, Scientific reports 8 (1) (2018) 1–13. doi:10.1038/s41598-018-19786-0.

3 A. Terzi, N. Gallo, S. Bettini, T. Sibillano, D. Altamura, L. Campa, M. L. Natali, L. Salvatore, M. Madaghiele, L. De Caro, L. Valli, A. Sannino, C. Giannini, Investigations of processing–induced structural changes in horse type-i collagen at sub and supramolecular levels, Frontiers in bioengineering and biotechnology 7 (2019) 203. doi:10.3389/fbioe.2019.00203.

4 L. De Caro, C. Giannini, R. Lassandro, F. Scattarella, T. Sibillano, E. Matricciani, G. Fanti, X-ray dating of ancient linen fabrics, Heritage 2 (4) (2019) 2763–2783. doi:10.3390/heritage2040171.

5 C. Diaferia, N. Balasco, T. Sibillano, M. Ghosh, L. Adler-Abramovich, C. Giannini, L. Vitagliano, G. Morelli, A. Accardo, Amyloid-like fibrillary morphology originated by tyrosine-containing aromatic hexapeptides, Chemistry–A European Journal 24 (26) (2018) 6804–6817. doi:10.1002/chem.201800351.

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# Molecular Replacement, new procedures and applications.

Maria Cristina Burla,[a] Benedetta Carrozzini[a] Giovanni Luca Cascarano,[a,*] Rocco Caliandro,[a], Carmelo Giacovazzo,[a] Giampiero Polidori.[a]

Molecular Replacement is the most used technique to solve macromolecules; an original algorithm called REMO09[1] has been developed and implemented in the software produced by Institute of Crystallography. In order to get a final model as complete and refined as possible, we have developed a pipeline based on the synergy between our phase refinement algorithms[2] and some Automated Model Building programs (AMB) distributed by different scientific teams. Among them we have selected the program Buccaneer,[3] a well known fast and efficient automatic model building program, using it also as a tool for phase refinement: indeed input phases are used for calculating electron density maps which are interpreted in terms of molecular model, from which new phase estimates may be obtained in a cyclic way. This procedure, called CAB,[4] has been implemented in a modified version of Sir2014.[5]

CAB has been tested on 81 protein structures, solved *via* Molecular Replacement, anomalous dispersion and *ab initio* methods. As it is usually done the phases so obtained were submitted to phase refinement and then they have been used as input for CAB. The experimental results were compared with those obtained with the use of Buccaneer alone: it is shown that CAB improves Buccaneer results, both in completeness and in accuracy.

The work is in progress in order to apply such a philosopy to DNA/RNA structures using Nautilus[6] as AMB program.

An example of application to a protein of Molecular Replacement + CAB will be presented.

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The Italian Ministry for Education and Research (MIUR) is kindly acknowledged for financial support through Project PRIN 2017 (grant number 2017WBZFHL).

## Riferimenti

1 R. Caliandro, B. Carrozzini, G. L. Cascarano, C. Giacovazzo, A. Mazzone, D. Siliqi, Molecular replacement: the probabilistic approach of the program *REMO09* and its applications, Acta Crystallographica Section A 65 (6) (2009) 512–527. doi:10.1107/S0108767309035612.

2 M. C. Burla, G. L. Cascarano, C. Giacovazzo, G. Polidori, Synergy among phase-refinement techniques in macromolecular crystallography, Acta Crystallographica Section D 73 (11) (2017) 877–888. doi:10.1107/S2059798317014590.

3 K. Cowtan, The *Buccaneer* software for automated model building. 1. Tracing protein chains, Acta Crystallographica Section D 62 (9) (2006) 1002–1011. doi:10.1107/S0907444906022116.

4 M. C. Burla, B. Carrozzini, G. L. Cascarano, G. Polidori, C. Giacovazzo, *CAB*: a cyclic automatic model-building procedure, Acta Crystallographica Section D 74 (11) (2018) 1096–1104. doi:10.1107/S2059798318013438.

5 M. C. Burla, R. Caliandro, B. Carrozzini, G. L. Cascarano, C. Cuocci, C. Giacovazzo, M. Mallamo, A. Mazzone, G. Polidori, Crystal structure determination and refinement *via SIR2014*, Journal of Applied Crystallography 48 (1) (2015) 306–309. doi:10.1107/S1600576715001132.

6 Collaborative Computational Project, Number 4, The *CCP*4 suite: programs for protein crystallography, Acta Crystallographica Section D 50 (5) (1994) 760–763. doi:10.1107/S0907444994003112.

7 M. C. Burla, B. Carrozzini, G. L. Cascarano, C. Giacovazzo, G. Polidori, How far are we from automatic crystal structure solution via molecular-replacement techniques?, Acta Crystallographica Section D 76 (1) (2020) 9–18. doi:10.1107/S2059798319015468.

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Conference

# Structural characterization of halide perovskites by X-ray measurements and advanced analysis.

Rocco Caliandro,[a,*] Davide Altamura,[a] Benny D. Belviso,[a] Cinzia Giannini.[a]

The advent of new-generation X-ray sources as well as more sensitive and fast detectors discloses the possibility of deeper static and dynamic structural investigations. X-ray powder diffraction (XPD) and pair distribution function (PDF) measurements are sensitive to long and short-range order, which can be modelled by fitting procedures. Subtle structural changes induced in situ by varying external parameters (temperature, light) can be also detected by processing efficiently multiple measurements. In this case, the traditional approach to fit each measurement independently can be coupled with the new approach to apply multivariate methods to the whole dataset.[1] Single and multiple XPD and PDF measurements have been here analyzed for the high-sensitivity structural characterization of halide perovskites. We have investigated the role played by cyclodextrins (CD) to generate a hybrid perovskite-soft material, demonstrating that the interaction between CDs and perovskite precursors leads to the formation of a supramolecular organic–inorganic hybrid framework that modifies solution chemistry and properties of the perovskite film.**(author?)**[2] The multivariate analysis approach has been applied to *in situ* experiments to reveal under illumination the reversible generation of paramagnetic $Pb^{3+}$ defects in $CH_3NH_3PbI_3$ perovskite (Figure 1),[3] and to get new insights into its tetragonal-to-cubic phase transition under temperature changes.[4]

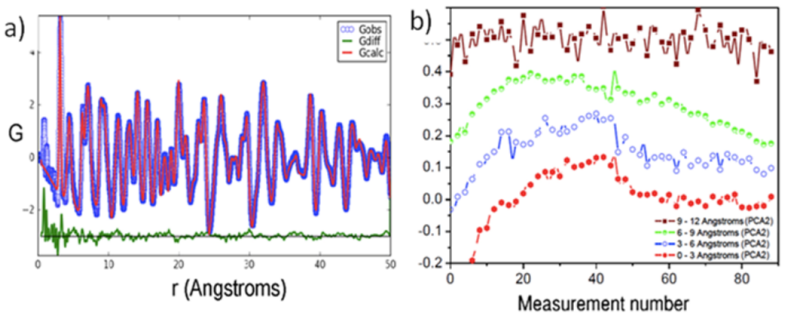


**Fig. 1** Fit of the PDF profile (a) and scores obtained by PCA applied to the PDF data matrix in slices of 3Å, for increasing interatomic distances (b).

## Riferimenti

1 R. Caliandro, D. B. Belviso, Rootprof: software for multivariate analysis of unidimensional profiles, Journal of Applied Crystallography 47 (3) (2014) 1087–1096. doi:10.1107/S1600576714005895.

2 S. Masi, F. Aiello, A. Listorti, F. Balzano, D. Altamura, C. Giannini, R. Caliandro, G. Uccello-Barretta, A. Rizzo, S. Colella, Connecting the solution chemistry of pbi 2 and mai: a cyclodextrin-based supramolecular approach to the formation of hybrid halide perovskites, Chemical science 9 (12) (2018) 3200–3208. doi:10.1039/C7SC05095J.

3 S. Colella, M. Todaro, S. Masi, A. Listorti, D. Altamura, R. Caliandro, C. Giannini, E. Carignani, M. Geppi, D. Meggiolaro, G. Buscarino, F. De Angelis, A. Rizzo, Light-induced formation of pb3+ paramagnetic species in lead halide perovskites, ACS Energy Letters 3 (8) (2018) 1840–1847. doi:10.1021/acsenergylett.8b00944.

4 R. Caliandro, D. Altamura, B. D. Belviso, A. Rizzo, S. Masi, C. Giannini, Investigating temperature-induced structural changes of lead halide perovskites by *in situ* X-ray powder diffraction, Journal of Applied Crystallography 52 (5) (2019) 1104–1118. doi:10.1107/S160057671901166X.

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Conference

# The Structure of the Pro-domain of Mouse proNGF in Contact with the NGF Domain.

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Nerve growth factor (NGF) is an important neurotrophic factor involved in the regulation of cell differentiation and survival of target neurons. Expressed as a proNGF precursor, NGF is matured by furin-mediated protease cleavage.

Increasing evidence suggests that NGF and proNGF have distinct functional roles. While the structure of mature NGF is available, little is known about that of the pro-domain because of its dynamical structural features.

We exploited an ad hoc hybrid strategy based on nuclear magnetic resonance and modeling validated by small-angle X-ray scattering to gain novel insights on the pro-domain, both in isolation and in the context of proNGF.[1]

We show that the isolated pro-domain is intrinsically unstructured but forms transient intramolecular contacts with mature NGF and has per se the ability to induce growth cone collapse, indicating functional independence.

Our data represent an important step toward the structural and functional characterization of the properties of proNGF.

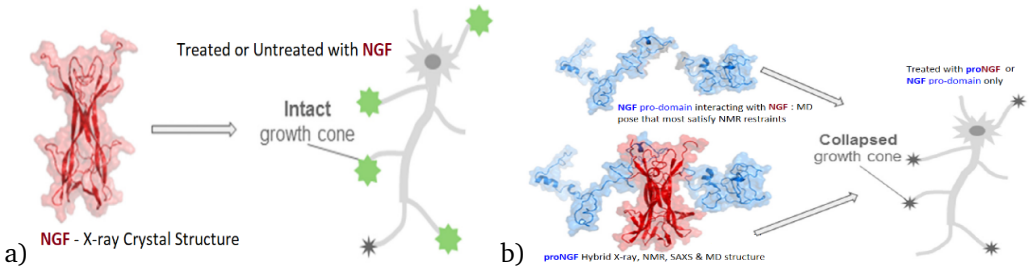


**Fig. 1** a) Structure and Function of proNGF; b) NGF pro domain and mature NGF

## Riferimenti

1 R. Yan, H. Yalinca, F. Paoletti, F. Gobbo, L. Marchetti, A. Kuzmanic, D. Lamba, F. L. Gervasio, P. V. Konarev, A. Cattaneo, et al., The structure of the pro-domain of mouse prongf in contact with the ngf domain, Structure 27 (1) (2019) 78–89. doi:10.1016/j.str.2018.09.013.

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Conference

# SUNBIM: a package for X-ray imaging of nano- and biomaterials using SAXS, WAXS, GISAXS and GIWAXS techniques.

Francesco Scattarella,[a,*] Dritan Siliqi,[a] Liberato De Caro,[a] Massimo Ladisa,[a] Annamaria Mazzone,[a] Davide Altamura,[a] Teresa Sibillano,[a] Cinzia Giannini.[a]

SUNBIM (supramolecular and submolecular nano- and biomaterials X-ray imaging)[1] is a suite of integrated programs which, through a user-friendly graphical interface, are optimized to perform a number of functions, such as: centering, q-scale calibration, two-dimensional to one-dimensional folding of small- and wide-angle X-ray scattering (SAXS/WAXS) data, also in grazing-incidence (GISAXS/GIWAXS), indexing of two-dimensional GISAXS frames and extraction of one-dimensional GISAXS profiles along specific cuts, quantitative scanning microscopy.

SUNBIM consists of five main programs:

1. Calibration package, a set of functions allow one to find all of the geometrical parameters needed to extract a one-dimensional profile out of a two-dimensional image;
2. Batch Script & 2D Mesh Composite, to prepare batch script files (ASCII files) to run a sequential acquisition of two-dimensional frames (in scanning mode) and to perform a composite of the as-collected two-dimensional SAXS frames into a single image;
3. Multi-scan SAXS and WAXS data analysis, to fold each two-dimensional frame of the mesh into a one-dimensional profile and extract all the relevant features of the sample with a multi-modal imaging approach[2];
4. Single-scan (GI)SAXS and (GI)WAXS data analysis, to calibrate and fold the two-dimensional data, in order to extract relevant information from the experimental data and to fold 2D data into 1D profiles;
5. One-D Data Analysis Manager, to manage with one dimensional profiles and import, trigger, save and export plots.

SUNBIM combines in the same package both originally developed algorithms (denoising, beam centering etc.) and reliable methods documented in the literature (multi-modal imaging, GISAXS three-dimensional frame indexing). SUNBIM is developed in the MATLAB language and it will be distributed free of charge to the academic user (downloadable after a valid registration from http://www.ba.ic.cnr.it/softwareic/sunbim/).

## Riferimenti

1 D. Siliqi, L. De Caro, M. Ladisa, F. Scattarella, A. Mazzone, D. Altamura, T. Sibillano, C. Giannini, *SUNBIM*: a package for X-ray imaging of nano- and biomaterials using SAXS, WAXS, GISAXS and GIWAXS techniques, Journal of Applied Crystallography 49 (3) (2016) 1107–1114. doi:10.1107/S1600576716006932.

2 O. Bunk, M. Bech, T. H. Jensen, R. Feidenhans'l, T. Binderup, A. Menzel, F. Pfeiffer, Multimodal x-ray scatter imaging, New Journal of Physics 11 (12) (2009) 123016. doi:10.1088/1367-2630/11/12/123016.

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Conference

# Crystallographic investigation on whole anti-CD20 mAbs.

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Molecules in crystal form represent nowadays one of the preferred formulation for bio-therapeutics. In addition, protein crystallization ensures high stability and a high purity level of the active ingredients and it is potentially able to reduce downstream manufacturing costs by improving the efficiency of the biomolecule purification step with respect to conventional chromatographic methods.[1,2] However, this process is often not trivial, particularly in case monoclonal antibodies (mAbs), which are recalcitrant to form ordinate solid state due to their flexibility and complex structure.

mAbs are Y-shaped molecules, whose arms are made of one Fc-domain and two Fab-domains loosely connected by a very flexible polypeptide chain. Such great flexibility prevents mAb crystallization, making really difficult to get their structure determination at atomic resolution. This is the case of anti-cluster of differentiation 20 (anti-CD20), a mAb belonging to IgG1 family that is used in autoimmune disease therapy and against CD20-expressing non-Hodgkin's lymphoma.[3] Although anti- CD20 is the first antibody approved for clinical use and one of the most sold antibodies for therapeutic purposes, it shows a low reproducibility of the published crystallization conditions and its crystal structure is still not available.

Recently, many efforts were made to optimize crystallization conditions for producing anti-CD20 crystals in terms of yield, starting material, speed, and crystal quality.[4] Due to the diffraction quality of mAb crystals obtained applying the meso batch crystallization protocol, low resolution methods for powder diffraction data were applied and exploited to assess that crystals were formed by the antibody molecules. The size of the whole mAb crystal cell, and its crystallographic symmetry, were also reasonably extrapolated from powder X-ray diffraction patterns. This study represents the first crystallographic characterization of the whole anti-CD20 mAb,[4] and is an important step toward the unraveling of its full-length crystal structure.

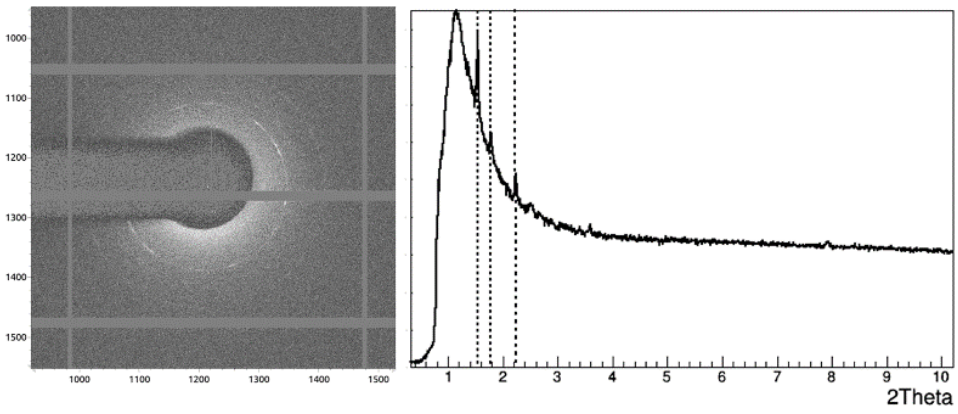


**Fig. 1** Anti-CD20 diffraction image.

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## Riferimenti

1 H. Yang, W. Chen, P. Peczulis, J. Y. Heng, Development and workflow of a continuous protein crystallization process: A case of lysozyme, Crystal Growth & Design 19 (2) (2019) 983–991. doi:10.1021/acs.cgd.8b01534.

2 L. Govada, N. E. Chayen, Choosing the method of crystallization to obtain optimal results, Crystals 9 (2) (2019) 106. doi:10.3390/cryst9020106.

3 M. D. Pescovitz, Rituximab, an anti-cd20 monoclonal antibody: history and mechanism of action, American Journal of Transplantation 6 (5p1) (2006) 859–866. doi:10.1111/j.1600-6143.2006.01288.x.

4 H. Yang, B. D. Belviso, X. Li, W. Chen, T. F. Mastropietro, G. Di Profio, R. Caliandro, J. Y. Heng, Optimization of vapor diffusion conditions for anti-cd20 crystallization and scale-up to meso batch, Crystals 9 (5) (2019) 230. doi:10.3390/cryst9050230.

Conference

# Single-crystal synchrotron X-ray diffraction study of new anthracene derivative compounds.

Anna Moliterni,[a] Davide Altamura,[a,*] Rocco Lassandro,[a] Vincent Olieric,[b] Andrea Camposeo,[c] Dario Pisignano,[c] Devin B. Granger,[d] Sean R. Parkin,[d] John E. Anthony,[d] Cinzia Giannini.[a]

Anthracene derivative compounds have recently known an increasing scientific interest thanks to their unique physical properties (*i.e.*, bright luminescence and large charge mobility)[1], making them ideal candidates for applications in new optoelectronic devices.

A structural study of new anthracene derivative compounds was carried out by single-crystal synchrotron X-ray diffraction to have insights on the structure-property relationships, characterize the crystal packing and the main aromatic interactions, and detect the presence of stacking arrangements (see, for example, Figure 1).

Synchrotron data were collected at room temperature at the Swiss Light Source (SLS), Villigen, Switzerland, at the beamline X06DA-PXIII[2]. Structure solution was carried out by Direct Methods using *SIR2019*[3] and refined by *SHELXL2014/7* **(author?)**[4]. All non-hydrogen atoms were refined anisotropically; the carbon-bound H atoms were placed on geometrically calculated positions and refined using a riding-model approximation.

The main crystallographic results are here presented.

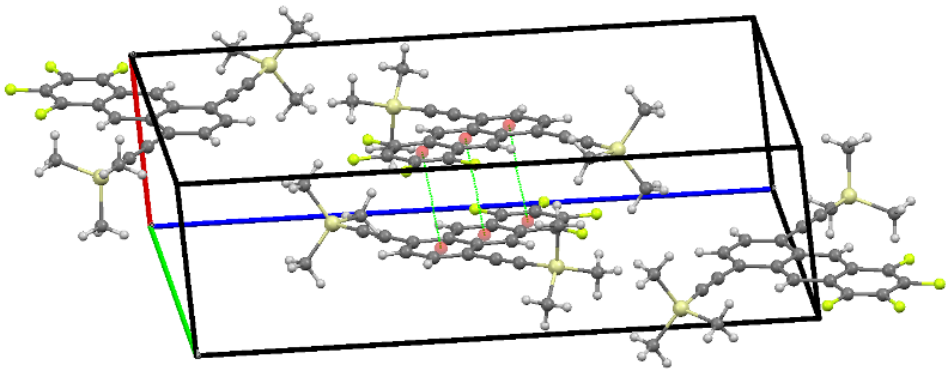

**Fig. 1** 1,2,3,4-Tetrafluoro-5,8-bis(trimethylsilylethynyl)anthracene: a view of the crystal packing. The parallel-offset $\pi - \pi$ interactions are indicated by broken lines between centroids.

## References

1 A. Camposeo, D. B. Granger, S. R. Parkin, D. Altamura, C. Giannini, J. E. Anthony, D. Pisignano, Directed functionalization tailors the polarized emission and waveguiding properties of anthracene-based molecular crystals, Chemistry of Materials 31 (5) (2019) 1775–1783. doi:10.1021/acs.chemmater.8b05361.

2 X06DA-PXIII, http://www.psi.ch/sls/pxiii/.

3 M. C. Burla, R. Caliandro, B. Carrozzini, G. L. Cascarano, C. Cuocci, C. Giacovazzo, M. Mallamo, A. Mazzone,

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† poster presented at 1 st Conference on Crystallography, Structural Chemistry and Biosystems, (Catania) 04-06/10/2021

G. Polidori, Crystal structure determination and refinement viasir2014, Journal of Applied Crystallography 48 (1) (2015) 306–309. doi:10.1107/S1600576715001132.

4 G. M. Sheldrick, Crystal structure refinement with *SHELXL*, Acta Crystallographica Section C 71 (1) (2015) 3–8. doi:10.1107/S2053229614024218.

5 C. R. Martinez, B. L. Iverson, Rethinking the term “pi-stacking”, Chemical Science 3 (7) (2012) 2191–2201. doi:10.1039/C2SC20045G.

Conference

# VEGF bioactive fragments and their copper (II) complexes in angiogenesis.

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Angiogenesis plays a crucial role in numerous physiological and pathological phenomena and consists in a multi-regulated process in which many factors are involved, including growth factors and metals. In particular, Copper stimulation of growth factors, such as vascular endothelial growth factor (VEGF), is involved in the formation of new blood vessels from pre-existing ones[1]. Although the angiogenic process is intensely investigated the role played by copper(II) ions in angiogenic mechanisms is still unclear. Certainly, their ability to modulate molecular mechanisms involved in angiogenesis plays a crucial role in numerous diseases, including cancer and cardiovascular disease. Within this frame, new classes of metal-binding peptides have been designed and studied, as ligands able to modulate angiogenesi.[2] Recently, we reported the pro-apoptotic activity of VEGF73-101[3], the protein fragment directly involved in the VEGF receptor (VEGFR2) interaction. This peptide combines the properties of beta hairpin sequence 73-85, rich in hydrophobic residues, with the sequence 85-101, characterized by the presence of three histidines, known sites of anchoring for Cu(II).

In order to establish a relationship between the structure of the ligand fragment and the cytotoxic activity of the resulting copper(II) complexes, specific structural modifications were introduced to VEGF73-101 fragment, obtaining two singly mutated peptides.

Herewith we extended the study of peptide VEGF73-101 to deepen its apoptotic activity on HUVEC cells in comparison with the singly mutated peptides, with particular attention to the ionophoric behavior hypothesized. To this aim, we studied the effects of these peptides and of their copper (II) complexes on membrane model system, the cellular uptake and the related anti-proliferative activity on HUVEC cells, by MTS and cytofluorimetry analysis.

In addition, spectroscopic UV-Vis, CD-Vis and spectrometric ESI-MS measurements to characterize the metal coordination mode of peptides, were reported. The obtained results suggest that VEGF73-101, is an effective copper ionophore, and represents a promising tool in the regulation of angiogenetic pathways.

## References

1 L. Finney, S. Vogt, T. Fukai, D. Glesne, Copper and angiogenesis: unravelling a relationship key to cancer progression, Clinical and Experimental Pharmacology and Physiology 36 (1) (2009) 88–94. doi:10.1111/j.1440-1681.2008.04969.x.

2 A. Henninot, J. C. Collins, J. M. Nuss, The current state of peptide drug discovery: back to the future?, Journal of medicinal chemistry 61 (4) (2018) 1382–1414. doi:10.1021/acs.jmedchem.7b00318.

3 G. Grasso, A. M. Santoro, A. Magrì, D. La Mendola, M. F. Tomasello, S. Zimbone, E. Rizzarelli, The Inorganic Perspective of VEGF: Interactions of Cu2+ with Peptides Encompassing a Recognition Domain of the VEGF Receptor, Journal of inorganic biochemistry 159 (2016) 149–158. doi:10.1016/j.jinorgbio.2016.03.004.

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Conference

# Closing or opening proteasome doors by porphyrins.

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The role of proteasome in the regulation of all cellular functions, is so relevant that its modulation became a useful therapeutic strategy for a large variety of diseases.[1] Besides the potential clinical usefulness, proteasome regulators provide interesting and important tools for cell and molecular studies. Some years ago we have proposed cationic porphyrins as a new class of proteasome inhibitors,[2] extending a new regulatory function to the broad-spectrum activities of these "multi-purpose" molecules. The external face of the 20S CP, the $\alpha$ ring, has its own regulation system consisting of dynamic gate that constantly switches between closed and open state. These physiological receptorial regions of canonical regulatory particles are characterized by a regular arrangement of charged aminoacids that represent a sort of "electrostatic code" regulating the "state" of the gate; the peripheral porphyrin charges represent a key able to interfere with the gate "door lock".[3] Thus, porphyrins behave as gatekeepers of 20S CP,[4] either inducing a partial gate occlusion (e.g., $H_2$T4) or allosterically, triggering a conformational change that affect the open-closed equilibrium (e.g., pTMPyPP4).[3] Finally, in the case of tricationic porphyrin Tris-T4, 20S CP activation has been observed, as the result of a new proteasome functional state characterized by a much higher substrate affinity and a higher catalytic efficiency. According to our hypothesis, supported by NMR and computational data, the h20S activation observed upon Tris-T4 binding, might simulate to some extent the allosteric activation by regulatory proteins. These results coupled with porphyrin's versatile chemistry, position porphyrins as a novel class of CP conformational modulators of proteasome with a significant pharmacological potential.

## References

1 D. Finley, X. Chen, K. J. Walters, Gates, channels, and switches: elements of the proteasome machine, Trends in biochemical sciences 41 (1) (2016) 77–93. doi:10.1016/j.tibs.2015.10.009.

2 A. M. Santoro, M. C. Lo Giudice, A. D'Urso, R. Lauceri, R. Purrello, D. Milardi, Cationic porphyrins are reversible proteasome inhibitors, Journal of the American Chemical Society 134 (25) (2012) 10451–10457. doi:10.1021/ja300781u.

3 A. Di Dato, A. Cunsolo, M. Persico, A. M. Santoro, A. D'Urso, D. Milardi, R. Purrello, M. Stefanelli, R. Paolesse, G. R. Tundo, et al., Electrostatic map of proteasome $\alpha$-rings encodes the design of allosteric porphyrin-based inhibitors able to affect 20s conformation by cooperative binding, Scientific reports 7 (2017) 17098. doi:10.1038/s41598-017-17008-7.

4 A. M. Santoro, A. Cunsolo, A. D'Urso, D. Sbardella, G. R. Tundo, C. Ciaccio, M. Coletta, D. Diana, R. Fattorusso, M. Persico, et al., Cationic porphyrins are tunable gatekeepers of the 20s proteasome, Chemical science 7 (2) (2016) 1286–1297. doi:10.1039/C5SC03312H.

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Conference

# Hyaluronan-carnosine conjugates inhibit Amyloid-$\beta$ aggregation and toxicity.

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Hyaluronic acid (Hy) is a glycosaminoglycan widely distributed in humans and it is the main component of the extracellular matrix[1]. The wide range of physiological functions includes hydration and turgidity maintenance of tissue, extracellular matrix structure, regulation of innate immunity, and protection and lubrication of joints. The molecular and mechanical properties make this biocompatible polymer very used for several applications, such as tissue engineering, treatment of osteoarthritis and drug targeting[2,3].

The potential applications of Hy has been recently widen by the conjugation with carnosine (Car), a multifuntional dipeptide widely distributed in several animal species[4]. Also this dipeptide exerts a variety of physiological properties, including antioxidant, antiglycating, and antiaggregant abilities[5]. he biocojugation of carnosine effectively inhibits the rapid dipeptide degradation in serum catalysed by carnosinase[6], making the new HyCar derivative[7] a promising compound to better treat several diseases and/or prevent their onset.

Based on these data, a series of Hy-Car derivatives have been synthesized and patented by us in recent years, by using two different molecular weights (200 and 700 kDa) and different loading percentages of carnosine. All of them have been structurally characterized. The ability of the HyCar derivatives to inhibit the aggregation of the amyloid-$\beta$ peptide (whose dyshomeostasis is involved in the onset of Alzheimer's disease) and the relative toxicity has been tested by using several assays and methodological approaches.

## References

1 J. R. E. Fraser, T. C. Laurent, U. Laurent, Hyaluronan: its nature, distribution, functions and turnover, Journal of internal medicine 242 (1) (1997) 27–33. doi:10.1046/j.1365-2796.1997.00170.x.

2 G. Kogan, L. Šoltés, R. Stern, P. Gemeiner, Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications, Biotechnology letters 29 (1) (2007) 17–25. doi:10.1007/s10529-006-9219-z.

3 A. K. Yadav, P. Mishra, G. P. Agrawal, An insight on hyaluronic acid in drug targeting and drug delivery, Journal of drug targeting 16 (2) (2008) 91–107. doi:10.1080/10611860701794296.

4 A. A. Boldyrev, G. Aldini, W. Derave, Physiology and pathophysiology of carnosine, Physiological reviews 93 (4) (2013) 1803–1845. doi:10.1152/physrev.00039.2012.

5 F. Bellia, G. Vecchio, S. Cuzzocrea, V. Calabrese, E. Rizzarelli, Neuroprotective features of carnosine in oxidative driven diseases, Molecular aspects of medicine 32 (4-6) (2011) 258–266. doi:10.1016/j.mam.2011.10.009.

6 F. Bellia, G. Vecchio, E. Rizzarelli, Carnosine derivatives: new multifunctional drug-like molecules, Amino acids 43 (1) (2012) 153–163. doi:10.1007/s00726-011-1178-6.

7 S. Sciuto, V. Greco, E. Rizzarelli, F. Bellia, V. Lanza, S. Vaccaro, L. Messina, Derivatives obtained from hyaluronic acid and carnosine,WO2016016847A (2016).

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[b] *Dipartimento di Scienze Chimiche, Università degli Studi di Catania, A. Doria 6, 95125 Catania, Italy*

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Conference

# Intrinsically disordered proteins (IDP) and metal ions: the case of amylin (HiAPP).

Antonio Magrì,[a,*] Giovanni Tabbì,[a] Francesco Attanasio,[a] Giuseppe Di Natale,[a] Diego La Mendola,[b] Enrico Rizzarelli.[a]

Intrinsically disordered proteins (IDPs) do not have rigid 3D structures, showing changes in their folding depending on the environment or ligands. Intrinsically disordered proteins are widely spread in eukaryotic genomes, and these proteins participate in many cell regulatory metabolism processes. Some IDPs, when aberrantly folded, can be the cause of some diseases such as Alzheimer's, Parkinson's, diabetes and prionic, among others. In these diseases, there are modifications in parts of the protein or in its entirety. A common conformational variation of these IDPs is misfolding and aggregation, forming, for instance, neurotoxic amyloid plaques. Some metal ions play an important role in the aggregation processes of these IDPs as showed in several papers present in literature.

Amylin is a 37-residue peptide hormone produced by the islet $\beta$-cells of pancreas and the formation of amylin aggregates is strongly associated with b-cell degeneration in type 2 diabetes, as demonstrated by more than 95% of patients exhibiting amylin amyloid upon autopsy. It is widely recognized that metal ions such as copper(II) and zinc(II) have been implicated in the aggregation process of amylin.[1,2]

Here we reported our experimental studies (thermodynamic and spectroscopic) on the interaction between Cu(II) and Zn(II) with several isoforms of amylin (human, porcine, murine) and their fragments.[3,4]

## References

1 G. Pappalardo, D. Milardi, A. Magrì, F. Attanasio, G. Impellizzeri, C. La Rosa, D. Grasso, E. Rizzarelli, Environmental factors differently affect human and rat iapp: Conformational preferences and membrane interactions of iapp17–29 peptide derivatives, Chemistry – A European Journal 13 (36) (2007) 10204–10215. doi:10.1002/chem.200700576.

2 A. Sinopoli, A. Magrì, D. Milardi, M. Pappalardo, P. Pucci, A. Flagiello, J. J. Titman, V. G. Nicoletti, G. Caruso, G. Pappalardo, G. Grasso, The role of copper(ii) in the aggregation of human amylin, Metallomics 6 (2014) 1841–1852. doi:10.1039/C4MT00130C.

3 A. Magrì, D. La Mendola, V. G. Nicoletti, G. Pappalardo, E. Rizzarelli, New insight in copper-ion binding to human islet amyloid: The contribution of metal-complex speciation to reveal the polypeptide toxicity, Chemistry – A European Journal 22 (37) (2016) 13287–13300. doi:10.1002/chem.201602816.

4 A. Magrì, A. Pietropaolo, G. Tabbì, D. La Mendola, E. Rizzarelli, From peptide fragments to whole protein: Copper(ii) load and coordination features of iapp, Chemistry – A European Journal 23 (71) (2017) 17898–17902. doi:10.1002/chem.201704910.

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Conference

# Antioxidant properties of Hyaluronate–Carnosine bioconjugates and their copper(II) complexes.

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Hyaluronic acid (Hy), is a polyanionic linear nonsulfated glycosaminoglycan (GAG).[1] It is widely distributed throughout mammalian cells and tissues and its biomechanical and biochemical properties support its involvement in myriad physiological functions, including hydration and turgidity maintenance of tissue, extracellular matrix structure, regulation of innate immunity, and protection and lubrication of joints. Due to this versatility, Hy represents a promising bio-indicator of pathophysiology and inflammation, and has consequently been targeted for disease-specific diagnostics.[2] Moreover, the excellent biocompatibility makes Hy useful as a drug delivery system, to which pharmacologically active compounds can be covalently conjugated.[3]

Carnosine (Car) is a multifuntional dipeptide widely distributed in several animal species[4]. The variety of physiological properties, including antioxidant, antiglycating, antiaggregant and metal binding abilities, account for the distribution in several tissues and the relative high concentration reached in many cerebral areas.[5] The promising role as a drug is limited by the rapid degradation in serum catalyzed by carnosinase. The bioconjugation of Car through the amino on the carboxylic groups has been proposed as a promising strategy to overcome this limitation.[6]

Based on these data, a series of Hy-Car derivatives have been synthesized and patented by us in recent years,[7] by using two different molecular weights (200 and 700 kDa) and different loading percentages of carnosine. All of them have been structurally characterized. The antioxidant property of the HyCar derivatives and that of their copper(II) complexes has been tested by using several assays and methodological approaches.

## References

1 J. R. E. Fraser, T. C. Laurent, U. Laurent, Hyaluronan: its nature, distribution, functions and turnover, Journal of internal medicine 242 (1) (1997) 27–33. doi:10.1046/j.1365-2796.1997.00170.x.

2 G. Kogan, L. Šoltés, R. Stern, P. Gemeiner, Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications, Biotechnology letters 29 (1) (2007) 17–25. doi:10.1007/s10529-006-9219-z.

3 A. K. Yadav, P. Mishra, G. P. Agrawal, An insight on hyaluronic acid in drug targeting and drug delivery, Journal of drug targeting 16 (2) (2008) 91–107. doi:10.1080/10611860701794296.

4 A. A. Boldyrev, G. Aldini, W. Derave, Physiology and pathophysiology of carnosine, Physiological reviews 93 (4) (2013) 1803–1845. doi:10.1152/physrev.00039.2012.

5 F. Bellia, G. Vecchio, S. Cuzzocrea, V. Calabrese, E. Rizzarelli, Neuroprotective features of carnosine in oxidative driven diseases, Molecular aspects of medicine 32 (4-6) (2011) 258–266. doi:10.1016/j.mam.2011.10.009.

6 F. Bellia, G. Vecchio, E. Rizzarelli, Carnosine derivatives: new multifunctional drug-like molecules, Amino acids 43 (1) (2012) 153–163. doi:10.1007/s00726-011-1178-6.

7 S. Sciuto, V. Greco, E. Rizzarelli, F. Bellia, V. Lanza, S. Vaccaro, L. Messina, Derivatives obtained from hyaluronic acid and carnosine, WO2016016847A1 (2016).
URL https://patents.google.com/patent/WO2016016847A1/en

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Conference

# Structural determinants of quorum sensing specificity vs promiscuity.

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Quorum sensing (QS) is a bacterial cell-to-cell communication mechanism based on the production and detection of chemical signals called autoinducers (AIs) to regulate gene expression in response to changes in population density. QS allows bacteria to behave as a cohesive group under differing environments by means of microbe–microbe and host–microbe interactions[1]. Interestingly, bacteria employs QS not only to coordinate collective behaviors such as bioluminescence production, but also to synchronize microbial activities essential for infection and survival in the host, such as biofilm formation, genetic exchange and virulence factor expression. This key role in bacterial pathogenicity suggests that quenching microbial QS (namely quorum quenching[2]) can be a promising disease control strategy, aiming at the attenuation of virulence, instead of imposing a 'life-or-death' selection pressure, in order to contain infectious diseases and to prevent antibiotic resistance in microbial communities.

In Gram-negative bacteria, AI molecules are mainly comprised of N-acyl homoserine lactones (AHLs) that differ in the length, in the saturation levels and the oxidation states of the acylic tail. Generally, AHL QS involves two regulatory proteins encoded in the same operon, a member of the LuxI family of AHL synthase and a member of the LuxR family of AHL-responsive transcriptional regulator. Recently several studies and the sequencing of an increasing number of bacterial genomes have evidenced the widespread distribution of *luxR*-type genes that are unpaired to a cognate *luxI* (therefore defined solos), which are considered major players in bacterial cell–cell communication, not only in intra-species but also in inter-species and inter-kingdom signaling.

All LuxR regulators are composed of two distinct domains: an N-terminal ligand binding domain (LBD) and a C-terminal DNA-binding domain. While most QS LuxR-type receptors display high specificity to the AHL ligand produced by their cognate LuxI-type synthetase, others are quite promiscuous in signal detection. The structural basis of the different degree of specificity of these pharmacological targets is still poorly understood.

Our study is focused on the structural characterization of a recently described LuxR solo, LoxR from *Kosakonia* KO348. We demonstrated that it is a very promiscuous LuxR whose ensemble of conformations is dependent on the type of the bound AHL. The solution of its crystal structure in complex with a long acylic chain AHL together with the structural information on promiscuous and specific members of LuxR family in the PDB allowed to elucidate the structural determinants mediating LuxR specificity towards AHLs with long acylic chains.

## References

1 W. C. Fuqua, S. C. Winans, E. P. Greenberg, Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators., Journal of bacteriology 176 (2) (1994) 269. doi:10.1128/jb.176.2.269-275.1994.

2 M. Schuster, D. Joseph Sexton, S. P. Diggle, E. Peter Greenberg, Acyl-Homoserine Lactone Quorum Sensing: From Evolution to Application, Annual Review of Microbiology 67 (1) (2013) 43–63. doi:10.1146/annurev-micro-092412-155635.

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# A painful dance between Insulin degrading enzyme and two substrates: Insulin and OFQ/N.

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Neurodegenerative disorders are mainly protein aggregation diseases widespread in the elderly, yet onsets in youngsters are not unheard of. The complexity also arises from their association with each other. For example, a linkage was established between Alzheimer's disease (AD) and Diabetes mellitus type 2 (T2DM). Studies have shown the increased prevalence of dementia in diabetic vs. non diabetic patients. Cognitive dysfunction is associated with hyperglycemia, hypoglycemia and insulin resistance all of which are factors associated with T2DM. Both diseases decrease the quality of life and impose a huge financial burden on the healthcare system. [1]

The Insulin degrading enzyme (IDE) is a zinc metallopeptidase[2,3] that targets proteins with a size limit of 40-50 amino acid residues. This enzyme is involved in protein clearance which is a crucial step in avoiding the pathological consequences of protein aggregation. IDE is linked to T2DM[4] as it mainly targets insulin, but it is also able to degrade other endogenous small peptides. One of them is Nociceptin/orphanin (OFQ/N)[5,6], a 17 amino acid neuropeptide involved in pain transmission and brain disorders such as AD. Interestingly, amyloid beta (A$\beta$) one of the main proteins linked to AD is also degraded by IDE[7]. Hence, a possible indirect link between T2DM and AD can be established through studies of IDE, insulin and OFQ/N.

In this study, mass spectrometric approaches were utilized to establish the hydrolysis pattern of OFQ/$N_{1\text{-}16}$ peptide in the presence of IDE. Moreover, the effect of the insulin co-incubation on the hydrolysis of OFQ/$N_{1\text{-}16}$ peptide was monitored. The experiment revealed the clear preference of insulin over OFQ/N as a substrate of IDE, and the potential role of T2DM in pain transmission.

## Riferimenti

1 T. Kawamura, T. Umemura, N. Hotta, Cognitive impairment in diabetic patients: Can diabetic control prevent cognitive decline?, Journal of Diabetes Investigation 3 (5) (2012) 413–423. doi:10.1111/j.2040-1124.2012.00234.x.

2 S. K. Sharma, E. Chorell, P. Steneberg, E. Vernersson-Lindahl, H. Edlund, P. Wittung-Stafshede, Insulin-degrading enzyme prevents $\alpha$-synuclein fibril formation in a nonproteolytical manner, Scientific reports 5 (1) (2015) 1–10. doi:10.1038/srep12531.

3 S. K. Sharma, E. Chorell, P. Wittung-Stafshede, Insulin-degrading enzyme is activated by the c-terminus of $\alpha$-synuclein, Biochemical and biophysical research communications 466 (2) (2015) 192–195. doi:10.1016/j.bbrc.2015.09.002.

4 W.-J. Tang, Targeting insulin-degrading enzyme to treat type 2 diabetes mellitus, Trends in endocrinology & metabolism 27 (1) (2016) 24–34. doi:10.1016/j.tem.2015.11.003.

5 J.-C. Meunier, C. Mollereau, L. Toll, C. Suaudeau, C. Moisand, P. Alvinerie, J.-L. Butour, J.-C. Guillemot, P. Ferrara, B. Monsarrat, H. Mazarguil, G. Vassart, M. Parmentier, J. Costentin, Isolation and structure of the endogenous agonist of opioid receptor-like orl 1 receptor, Nature 377 (6549) (1995) 532–535. doi:10.1038/377532a0.

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6 R. K. Reinscheid, H.-P. Nothacker, A. Bourson, A. Ardati, R. A. Henningsen, J. R. Bunzow, D. K. Grandy, H. Langen, F. J. Monsma, O. Civelli, Orphanin fq: a neuropeptide that activates an opioidlike g protein-coupled receptor, Science 270 (5237) (1995) 792–794. doi:10.1126/science.270.5237.792.

7 F. Bellia, V. Lanza, S. García-Viñuales, I. Ahmed, A. Pietropaolo, C. Iacobucci, G. Malgieri, G. D'Abrosca, R. Fattorusso, V. Nicoletti, et al., Ubiquitin binds the amyloid $\beta$ peptide and interferes with its clearance pathways, Chemical science 10 (9) (2019) 2732–2742. doi:10.1039/C8SC03394C.

Conference

# A re-investigation of copper coordination mode in the N-terminal 1-14 fragment of human Ctr1 protein.

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Copper (Cu) is an essential micronutrient for most organisms and serves mainly as a redox-active catalytic centre in enzyme cycling between $Cu^{+}$ and $Cu^{2+}$. In mammalian cells the membrane transporter Ctr1 regulates the import of Cu into the cytosol (Fig. 1).

Even though the Cu is imported by Ctr1 as $Cu^{+}$, it may be transferred as $Cu^{2+}$ to the high-affinity N-terminal $Cu^{2+}$ binding site of the h-Ctr1 extracellular domain (ATCUN) and then reduced, for instance by ascorbate or a STEAP reductase on the cell membrane.[1] The critical nature of Ctr1 in human health has spurred interest in structure and function; however details on Ctr1-dependent Cu uptake and transport have to be elucidate.[2] Several studies on Ctr1 model peptides shed light on the identity of the Cu uptake through the extracellular binding site motif, but unresolved questions are yet opened.[3] We examined the copper coordination mode of the $Ctr_{11-14}$ fragment, a most used Ctr1 model peptide, in the presence of an excess of copper (II) (1:2 peptide/copper ratio). Taking in consideration that silver ion may adopt the typical $Cu^{+}$ coordination modes we used $Ag^{+}$ to study the possible formation of ternary complex species with $Cu^{2+}$. A combined potentiometric, spectroscopic and redox study, revealed that, at physiological pH, no ternary complex species are present, suggesting a crucial involvement of the His 3 as an anchoring point in the coordination of the $Cu^{2+}$ and/or $Ag^{+}$

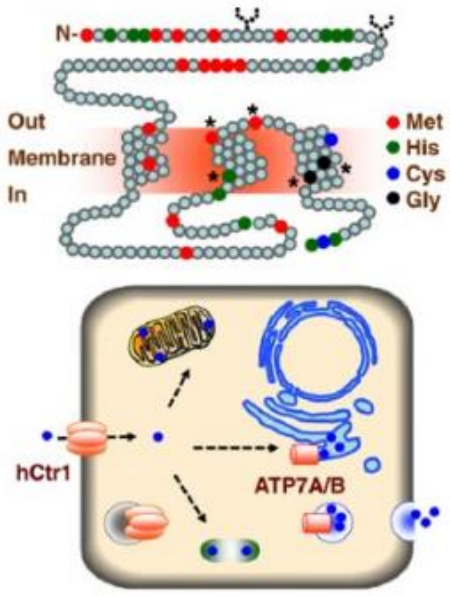


**Fig. 1** Schematic illustration of the structure, localization, and function of human CTR1.

## References

1 K. L. Haas, A. B. Putterman, D. R. White, D. J. Thiele, K. J. Franz, Model peptides provide new insights into the role of histidine residues as potential ligands in human cellular copper acquisition via ctr1, Journal of the American Chemical Society 133 (12) (2011) 4427–4437. doi:10.1021/ja108890c.

2 F. Ren, B. L. Logeman, X. Zhang, Y. Liu, D. J. Thiele, P. Yuan, X-ray structures of the high-affinity copper transporter ctr1, Nature communications 10 (1) (2019) 1–9. doi:10.1038/s41467-019-09376-7.

3 E. Stefaniak, D. Płonka, S. C. Drew, K. Bossak-Ahmad, K. L. Haas, M. J. Pushie, P. Faller, N. E. Wezynfeld, W. Bal, The N-terminal 14-mer model peptide of human Ctr1 can collect Cu(ii) from albumin. Implications for copper uptake by Ctr1, Metallomics 10 (12) (2018) 1723–1727. doi:10.1039/c8mt00274f.

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Conference

# Ceftazidime and other cephalosporins inhibit bacterial NAD biosynthesis by targeting NadD enzymes. Implication for the treatment of chronic infections.

Alessandro Pesaresi,[a,*] Rosanna Caliandro.[b]

It is known since 40's that the rate of killing by antibiotic is in strict correlation with the bacterial growth rate, and that for non growing bacteria antibiotics result ineffective. The ability of some bacteria to enter and endure in a highly depressed non-replicating metabolic state has been recognize as a frequent cause of failure in the treatment of chronic infections, and the need for new targets whose inhibition kills both replicating and non-replicating bacteria has been highlighted.[1]

Genomics-based reconstruction and experimental data showed that in bacteria the last two steps of NAD biosynthesis from a nicotinate mononucleotide (NaMN) precursor *via* nicotinate adenine dinucleotide (NaAD) intermediate are irreplaceable. The respective enzymes, NaMN adenyltransferase (NadD) and NAD-synthase (NadE), are conserved in most bacterial species, being quite distant from their human counterparts in term both of kinetic properties and structural features. The inhibition of NadD enzymes was shown to kill bacteria both in the replicating and non-replicating state,[2,3] validating NadD as a target for the development of new antibiotics.

We report on a structure-based virtual screening which indicated some cephalosporins as potential inhibitors of NadD enzymes. Ceftazidime, and other third generation cephalosporins, were confirmed to inhibit NadD of *M. tuberculosis*, *E. coli* and *P. aeruginosa* with $K_i$ in the $\mu$M range. It was also found that Ceftazidime and Cefquinome speed-up the cell death rate in non replicating cultures of *E. coli* and *P. aeruginosa*. The same cultures were insensitive to high concentrations of Ampicillin and other cephalosporins which do not inhibit NadD. Showing that the observed toxicity depends on the impairement of NAD biosynthesis by Ceftazidime and Cefquinome. Altough their potency is probably not sufficient to suggest an alternative clinical use of these drugs, they might serve as lead compound to develop better NadD inhibitors and possibly new antibiotics.

## References

1 E. M. Windels, J. E. Michiels, M. Fauvart, T. Wenseleers, B. Van den Bergh, J. Michiels, Bacterial persistence promotes the evolution of antibiotic resistance by increasing survival and mutation rates, The ISME journal 13 (5) (2019) 1239–1251. doi:10.1038/s41396-019-0344-9.

2 J.-H. Kim, K. M. O'Brien, R. Sharma, H. I. M. Boshoff, G. Rehren, S. Chakraborty, J. B. Wallach, M. Monteleone, D. J. Wilson, C. C. Aldrich, C. E. Barry, K. Y. Rhee, S. Ehrt, D. Schnappinger, A genetic strategy to identify targets for the development of drugs that prevent bacterial persistence, Proceedings of the National Academy of Sciences 110 (47) (2013) 19095–19100. doi:10.1073/pnas.1315860110.

3 I. A. Rodionova, B. M. Schuster, K. M. Guinn, L. Sorci, D. A. Scott, X. Li, I. Kheterpal, C. Shoen, M. Cynamon, C. Locher, E. J. Rubin, A. L. Osterman, Metabolic and bactericidal effects of targeted suppression of nadd and nade enzymes in mycobacteria, mBio 5 (1). doi:10.1128/mBio.00747-13.

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Conference

# Geant4, a toolkit to simulate the interaction of particles with matter dedicated to nuclear medicine.

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Understanding of how radiation emitted by a particular radiotracer distributed in various organs, deposits energy in different tissues through various mechanisms of radiation interaction with matter, relies on the correct description of the chemical composition of the scattering medium

and of the involved radiation transport processes. MC methods allow the simulation of the interaction between particle and matter thus providing a fundamental method to study the physics of nuclear medicine, radiology, and radiation therapy.

The concepts of deposited energy and absorbed dose are of particular interest not only for radio-therapy applications[1] but also for imaging applications[2] involving ionizing radiations.

The accurate assessment of the absorbed dose distribution throughout the organs and tissues of interest is required in radiation therapy (RT) treatment planning whatever the RT approach (e.g., using photons, electrons, protons, carbon beams, radioisotopes) and the different delivery conditions (broad beam, pencil beam, scanning, rotational, brachytherapy, and targeted radionuclide therapy).

In diagnostic imaging applications involving ionizing radiation (e.g., computed tomography, positron emission tomography, or single photon emission tomography) the assessment of the absorbed doses is important to better analyze the risk-benefit of the procedure.

Hence the need for a MC simulation platform supporting radiation transport modeling for imaging and dosimetry applications. Among the MC simulation tools that have been developed for imaging or dosimetry, the GEANT4[3] toolkit will be presented. It is a powerful tool which allows simulating the interactions between particles and matter, while different geant4 projects provides additional high-level features to facilitate the design of GEANT4-based simulations.

## References

1 N. Reynaert, S. Van der Marck, D. Schaart, W. Van der Zee, C. Van Vliet-Vroegindeweij, M. Tomsej, J. Jansen, B. Heijmen, M. Coghe, C. De Wagter, Monte carlo treatment planning for photon and electron beams, Radiation Physics and Chemistry 76 (4) (2007) 643–686. doi:10.1016/j.radphyschem.2006.05.015.

2 M. J. Murphy, J. Balter, S. Balter, J. A. BenComo Jr, I. J. Das, S. B. Jiang, C.-M. Ma, G. H. Olivera, R. F. Rodebaugh, K. J. Ruchala, H. Shirato, F. Yin, The management of imaging dose during image-guided radiotherapy: report of the aapm task group 75, Medical physics 34 (10) (2007) 4041–4063. doi:10.1118/1.2775667.

3 S. Agostinelli, J. Allison, K. a. Amako, J. Apostolakis, H. Araujo, P. Arce, M. Asai, D. Axen, S. Banerjee, G. . Barrand, et al., Geant4—a simulation toolkit, Nuclear instruments and methods in physics research section A: Accelerators, Spectrometers, Detectors and Associated Equipment 506 (3) (2003) 250–303. doi:10.1016/S0168-9002(03)01368-8.

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Conference

# ICT methods and tools for health.

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**Introduction.**

An actual challenge of health systems is the practical implementation of ICT. It is useful to introduce new organizational models and guide decision makers towards an appropriate use of resources. Thanks to a strictly cooperation with healthcare units using ICT techniques and technologies, innovative applications have been studied, designed and implemented.

**Telemedicine.**

Telemedicine is the provision of healthcare services, through use of ICT. It has been proposed in a variety of health care fields with significant advantages: As a matter of fact today at national level there are patchworked telemedicine initiatives not well coordinated. The ICCSB has collaborated in the design of new teleconsultation, telemonitoring and teleassistance services.[1] These projects involve territorial and specialist hospitals from different regions. Particular experience dealt with teleconsultation and sharing of clinical documentation for second-opinion, As technology is beyond clinical practice, making operative telemedicine it is necessary building confidence in and acceptance of new services. Last but not least it is necessary to bring legal clarity in management sensitive data. In practice, the design of each project must consider functional and ethical requirements, organizational requirements, technological requirements, legal requirements (GDPR). Some active telemedicine services will be presented.

**Home care.**

A theme ancillary to telemedicine and care outside of hospital is related to the high intensity home care. The use of medical devices at patient's home by personnel who are not healthcare practitioners introduces new potential risks. A methodological approach to investigate potential failures and define improvement actions to address the dangerous potential situations in home care has been developed.[2]

**Process Modelling.**

A third application regards healthcare process modelling and simulation. The use of software tools allows the representation of the system (As Is) to study the performances and the design of suggested variations (To Be) to predict the effect of designed changes. Application has been developer for home care and to study the emergency department optimization.[3]

**H2020 Project.**

An effective study on the security, privacy data protection in e-health has been carried out in the framework of the participation at H2020 KONFIDO project devoted to cross border data exange.[4]

## References

1 E. Papaleo, G. Faiella, R. Orofino, F. Borrometi and F. Clemente, "TELPASS Project: B2B Teleconsultation for Pediatric Hospices," 2019 E-Health and Bioengineering Conference (EHB), Iasi, Romania, 2019, pp. 1-4. doi:10.1109/EHB47216.2019.8969882.

2 F. Clemente, G. Faiella, G. Rutoli, P. Bifulco, M. Romano, M. Cesarelli, Critical failures in the use of home ventilation medical equipment, Heliyon 5 (12) (2019) e03034. doi:10.1016/j.heliyon.2019.e03034.

3 G. Faiella, M. Simonetti, A. D'Uffizi, V. Tipo, M. Cesarelli, F. Clemente, Use of modelling simulation to monitor the

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performance of a pediatric emergency department, in: L. Lhotska, L. Sukupova, I. Lacković, G. S. Ibbott (Eds.), World Congress on Medical Physics and Biomedical Engineering 2018, Springer Singapore, Singapore, 2019, pp. 567–572.

4 M. Nalin, I. Baroni, G. Faiella, M. Romano, F. Matrisciano, E. Gelenbe, D. M. Martinez, J. Dumortier, P. Natsiavas, K. Votis, V. Koutkias, D. Tzovaras, F. Clemente, The european cross-border health data exchange roadmap: Case study in the italian setting, Journal of Biomedical Informatics 94 (2019) 103183. doi:10.1016/j.jbi.2019.103183.

Conference

# Cyclic synthetic peptide mimicking NGF enhances PC12 differentiation and BDNF/VEGF expression.

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The neurotrophins (NTs) are a structurally and functionally related family of growth factors that regulate cell survival, differentiation, neurite outgrowth and regeneration, and synaptic plasticity in both the central and the peripheral nervous system. NTs include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5, -6, -7.[1] A few small molecules have been designed and synthesized to achieve the functional and structural mimicry of NTs.[2–4] Cyclic peptides have gained increasing attention in recent years as an alternative scaffold. The structural constraints provided by cyclization not only helps to resist to a degradation by proteases but also facilitates passage through the cell membrane, thus broadening the potential use of cyclic peptides beyond extracellular targets to include intracellular targets[5]

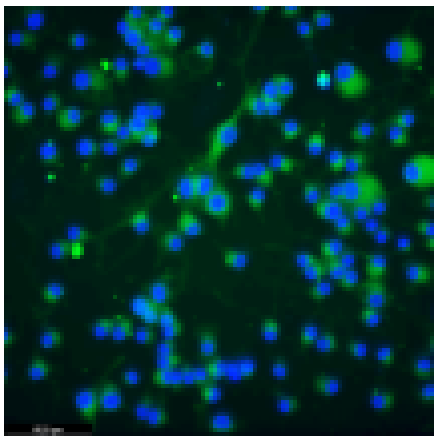

**Fig. 1** NGF cyclic peptide induces synapsin expression

In this work we characterized cyclic peptide of N-terminal domain of NGF. The biological properties of this peptide were tested on the PC12 cell line. Our results demonstrate that peptides activate phosphorylation of TrkA, VEGFR1 and VEGFR2 receptors, induce the phosphorylation of CREB, cells differentiation and BDNF, VEGF protein release. It is well known that metal ions may affect the neurotrophin activity and that the alteration of copper homeostasis is a prominent factor in the development of neurological pathologies. Incubation of cells with peptide and copper chelator BCS leads to markedly decrease the effect of peptide. This indicates that the activation of BDNF and VEGF protein release by the peptide is not strictly related to Trks recognition but also includes pathways that are somehow linked to copper.

## References

1 N. Khan, M. T. Smith, Neurotrophins and neuropathic pain: Role in pathobiology, Molecules 20 (6) (2015) 10657–10688. doi:10.3390/molecules200610657.

2 A. Travaglia, D. La Mendola, A. Magrì, V. G. Nicoletti, A. Pietropaolo, E. Rizzarelli, Copper, bdnf and its n-terminal domain: Inorganic features and biological perspectives, Chemistry – A European Journal 18 (49) (2012) 15618–15631. doi:https://doi.org/10.1002/chem.201202775.

3 I. Naletova, G. I. Grasso, C. Satriano, A. Travaglia, D. La Mendola, G. Arena, E. Rizzarelli, Copper complexes of synthetic peptides mimicking neurotrophin-3 enhance neurite outgrowth and CREB phosphorylation, Metallomics 11 (9) (2019) 1567–1578. doi:10.1039/c9mt00045c.

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4 I. Naletova, C. Satriano, A. Pietropaolo, F. Gianì, G. Pandini, V. Triaca, G. Amadoro, V. Latina, P. Calissano, A. Travaglia, V. G. Nicoletti, D. La Mendola, E. Rizzarelli, The copper(ii)-assisted connection between ngf and bdnf by means of nerve growth factor-mimicking short peptides, Cells 8 (4) (2019) 301. doi:10.3390/cells8040301.

5 D. Gang, D. W. Kim, H.-S. Park, Cyclic peptides: Promising scaffolds for biopharmaceuticals, Genes 9 (11) (2018) 557. doi:10.3390/genes9110557.