

Final project report

Project ID: 2002/1.02
Title: Physiological, biochemical and molecular bases of the evolutionary adaptation of Antarctic Teleostei

Principal investigator: Ennio Cocca
Institution: Istituto di Biochimica delle Proteine (IBP) - CNR
Email: e.cocca@ibp.cnr.it

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Activities and results

The Project was focused on the study of molecular processes underlying the biochemical and physiological adaptations of Antarctic teleosts.

In the framework of the study of the structure and function of Hb from Antarctic fish, it has been undertaken the analysis of various systems from Notothenioidei living in different habitats. The results indicate that Hbs from sub-Antarctic fish have higher multiplicity and their modulation of oxygen affinity is more pronounced.

This characterization has been carried out through various techniques: crystallography, UV and Visible Resonance Raman Spectroscopy, O₂ and CO kinetic binding, phylogenetic analysis.

The Root effect is characteristic of fish Hbs. It has been performed the analysis of crystallographic structures of deoxy forms of Hb1 from *Trematomus bernacchii* and of HbC from *T. newnesi*, at different pHs. The results support the emerging theory that structural determinants, at least for a portion of the Root effect, are specific to each Hb presenting this property.

The Hb1 of the Arctic skate *Raja hyperorbea* has been compared to the Hb1 from the Antarctic skate *Bathyraja eatonii*. The Hbs were characterized by molecular modeling. This study shows that evolutionary convergence has brought these two species, geographically very distant, to adopt similar physiological mechanisms in their extreme environments.

In the framework of the study on globin genes of Antarctic fish, the Hb1 genes of *B. eatonii* were characterized. It has been isolated and analyzed a genomic fragment containing the alpha- and beta-globin genes linked 3'-5' each other.

Ferritins extracted from various organs of *T. bernacchii* and *T. newnesi* were analyzed. The ferritins from liver of both species appear to contain two types of subunits, M and H, in similar amounts.

The comparative study of adaptation of cardiovascular system in Antarctic teleosts has been carried out through the analysis of the role of nitric oxide (NO) in cardiac and vascular modulation. The activity of NOSs (nitric oxide synthase) and the localization of their isoforms have been analyzed in the heart of *Chionodraco hamatus* and *T. bernacchii*.

In the study of the regulation of metallothionein (MT) genes and the 3D structure of the proteins from Antarctic fish, it was performed the comparison with counterparts from mammalian homologs. The Antarctic MT show different characteristics in several respects: evolution, pattern of expression, structure and function.

The characterization of the transmembrane helix region of the heavy chains of Ig of Antarctic fish was carried out using the approach of Molecular Modeling. Models of the dimeric domain of *T. bernacchii* and *C. hamatus* were constructed and then subjected to molecular dynamics to identify the amino acids residues responsible for the helix-helix interactions.

The cytogenetic characterization of Notothenioidei has focused on chromosome mapping for genes and sequences that are interesting for the perspective of evolution, and for karyotypic diversification. It were undertaken the analysis of chromosomal organization of genes coding for the 28S ribosomal protein and for Ig heavy chains.

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In the study of isoforms of carbonic anhydrase from Antarctic fish it has been addressed the characterization of the protein from *C. hamatus*. This protein shows structural changes for the conformation of the reaction center and for its overall tertiary structure, that are related to cold adaptation.

Products

A – papers in scientific magazines

1. Capasso C, Carginale V, Crescenzi O, Di Maro D, Parisi E, Spadaccini R, Temussi PA (2003) Solution structure of MT_nc, a novel metallothionein from the Antarctic fish *Notothenia coriiceps*. *Structure* 11, 435-443 IF 5,993
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3. Corda M, Tamburrini M, De Rosa MC, Sanna MT, Fais A, Olianias A, Pellegrini M, Giardina B, di Prisco G. (2003) Whale (*Balaenoptera physalus*) haemoglobin: primary structure, functional characterization and computer modeling studies. *Comp Biochem Physiol B Biochem Mol Biol.* 134(1):53-62 IF 1,579
4. Imbrogno S, Cerra MC, Tota B. (2003) Angiotensin II-induced inotropism requires an endocardial endothelium-nitric oxide mechanism in the in-vitro heart of *Anguilla anguilla*. *J Exp Biol.*206:2675-84 IF 2,271
5. Maffia M., Rizzello A., Acierno R., Verri T., Rollo M., Danieli A., Döring F., Daniel H., Storelli C. (2003) Characterisation of intestinal peptide transporter of the Antarctic haemoglobinless teleost *Chionodraco hamatus*. *J Exp Biol.* 206, 705-714 IF 2,271
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14. Verde C, Parisi E, di Prisco G (2003) The evolution of polar fish hemoglobin: a phylogenetic analysis of the ancestral amino acid residues linked to the Root effect. *J Mol Evol* 57: S258-S267 IF 3,114
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42. Tota B, Amelio D, Pellegrino D, Ip YK, Cerra MC (2005) NO modulation of myocardial performance in fish hearts. *Comp Biochem Physiol A Mol Integr Physiol* 142:164-77 IF 1,351
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B – book chapters

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2. Pisano E, Ghigliotti L, Mazzei F, Ozouf-Costaz C (2003) Cytogenetics of *Notothenia angustata* Hutton, 1875, an Antarctic fish living in non-Antarctic waters. In: *Antarctic Biology in a Global Context*, A.L Huiskes, W.W.C. Gieskes, J. Rozema, R.M.L. Schorno, S-M. Van der Vies, W.J. Wolff (eds.), Backhuys Publishers, Leiden, The Netherlands: 117-121
3. Pisano E, Ozouf-Costaz C (2003) Cytogenetics and Evolution in extreme environment: the case of Antarctic Fishes. In : *Fish Adaptations* (A. Val e G. Kapoor Eds), Oxford 6 IBH Publishing, New Delhi: pp 311-338
4. Verde C, Parisi E, de Pascale D, Riccio A, di Prisco G (2003) The hemoglobin system of the Arctic spotted wolffish *Anarhichas minor*: comparison of northern and southern polar marine environments. In *Proceedings of the SCAR 8th International Biology Symposium "Antarctic Biology in A Global Context"* (AHL Huiskes, WWC Gieskes, J Rozema, RML Schorno, SM van der Vies, WJ Wolff, eds). Backhuys Publishers, Leden, The Netherlands, pp 187-192
5. Acierno R, Rizzello A, Maffia M (2005) Transport protein adaptation in Antarctic teleosts. In: P. Luporini & M. Morbidoni (eds) *Proceedings of the Fifth PNRA Meeting on Antarctic Biology*. Polarnet Technical Report, pp 106-110.
6. Capasso C, Carginale V, Parisi E, Scudiero R, Temussi PA (2005) Metallothionein from Antarctic fish: structure, function and evolution. In: P. Luporini & M. Morbidoni (eds) *Proceedings of the Fifth PNRA Meeting on Antarctic Biology*. Polarnet Technical Report, pp 84-89
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9. Elia L, Gavazzo P, Moran O (2005) Molecular characterization of sodium and potassium channels of the Antarctic fish *Trematomus bernacchii*. In: P. Luporini & M. Morbidoni (eds) *Proceedings of the Fifth PNRA Meeting on Antarctic Biology*. Polarnet Technical Report, pp 100-105
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11. Giardina B, Mosca D, De Rosa MC (2005) Oxygen binding in hemoglobins from Arctic and Antarctic species: structural basis of the low enthalpy change. In: P. Luporini & M. Morbidoni (eds) Proceedings of the Fifth PNRA Meeting on Antarctic Biology. Polarnet Technical Report, pp 139-146
 12. Mazzarella L, Vergara A, Franzese M, Vitagliano L, Merlino A, Verde C, di Prisco G (2005) Structural peculiarity of Antarctic fish hemoglobins. In: P. Luporini & M. Morbidoni (eds) Proceedings of the Fifth PNRA Meeting on Antarctic Biology. Polarnet Technical Report, pp 127-134
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C - proceedings of international conferences

1. Pellegrino D, Tota B (2003) Control of cardiovascular function in the icefish *Chionodraco hamatus*: involvement of nitric oxide and cGMP. Sixth International Congress of Comparative Physiology and Biochemistry, La Trobe, Mt Buller, Australia
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13. Cheng C-H, Zhu T, Silic S, Nicodemus J, Devries CA, Ghigliotti L, Pisano E (2005) Genomic analyses of AFGP evolution in Antarctic notothenioid fish. XXIX SCAR Open Science Conference, Curitiba. Abstracts, 1 p.
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17. Mazzei F, Ghigliotti L, Lecointre G, Ozouf-Costaz C, Coutanceau J-P, Detrich WH III, Pisano E (2005) Karyotypes of basal lineages in notothenioid fishes: the genus *Bovichtus*. The ICEFISH Cruise Symposium, Walpole. Abstracts: 18
18. Mazzei F, Ghigliotti L, Lecointre G, Ozouf-Costaz C, Detrich WH III, Pisano E (2005) Looking for the ancestral karyotype of notothenioid fishes. XXIX SCAR Open Science Conference. Curitiba, Abstracts, 1 p.
19. Pellegrino D, Amelio D, Garofalo F, Tota B, Cerra MC (2005) The NOS system in the heart of white- and red-blooded Antarctic teleosts. Acts of Annual Main Meeting of the Society for Experimental Biology, published in Comparative Biochemistry and Physiology, Barcelona, Spain
20. Pellegrino D, Angelone T, Tota B (2005) Inotropic effects of nitrite in the vertebrate heart. National Institute of Health "Role of Nitrite in Physiology, Pathophysiology, and Therapeutics Meeting", Bethesda, Maryland, USA
21. Tota B, Amelio D, Garofalo F, Pellegrino D (2005) Non-uniformity of fish heart ventricle in the nitric oxide era. Annual Main Meeting of the Society for Experimental Biology, published in Comparative Biochemistry and Physiology, Barcelona, Spain
22. Tota B, Imbrogno S, Pellegrino D (2005) Nitric oxide and the plasticity of cardiac performance to temperature change in frog and fish. Annual Main Meeting of the Society for Experimental Biology, published in Comparative Biochemistry and Physiology, Barcelona, Spain
23. Vitagliano L, Bonomi G, Franzese M, Merlino A, Vergara A, Verde C, di Prisco G, Mazzarella L (2005) Structural characterization of the oxidation pathway of Antarctic fish hemoglobins. XX Congress of the International Union of Crystallography, Firenze, Italy
24. Cerra M C, Pellegrino D, Amelio D, Garofalo F, Tota B (2006) Cardiac NO system in the extremes: the example of antarctic teleosts. XIV International Conference on Dioxxygen Binding and Sensing Proteins, Stazione Zoologica "A. Dohrn", Naples, Italy
25. Garofalo F, Parisella ML, Gattuso A, Mule' F, Tota B, Pellegrino D (2006) Cardiovascular biology of nitric oxide in Antarctic fish. 57° Meeting of The Physiology Society of Italy, published in *Acta Physiologica*", Ravenna, Italy
26. Pellegrino D, Angelone T, Tota B (2006) Inotropic effects of nitrite in the vertebrate heart. XIV International conference on Dioxxygen Binding and Sensing Proteins", Stazione Zoologica "A. Dohrn", Naples, Italy
27. Pellegrino D, Angelone T, Tota B, Gladwin M (2006) Influence of nitrite on the mechanical performance of fish and mammalian hearts. The Ninth International Symposium on Fish Physiology, Toxicology, and Water Quality, Capri, Italy
28. Tota B (2006) Cardiac nitric oxide (NO) in naturally occurring hemoglobin genetic knockouts Antarctic fish. Marine Biotechnology, Now and Future, Miyazaki, Japan

D - proceedings of national meetings and conferences

1. Abelli L, Coscia MR, De Santis A, Zeni C, Oreste U (2004) Hepato-biliary transport of immunoglobulin in the Antarctic teleost *Trematomus bernacchii*. VI Incontro Scientifico della Società Italiana di Immunobiologia Comparata e dello Sviluppo, Padova, Italy. *Invert Surv J* 1:49
2. Coscia MR, Furino L, Oreste U (2004) Immunoglobulin light chain isotypes from the Antarctic teleost *Trematomus bernacchii*. VI Incontro Scientifico della Società Italiana di Immunobiologia Comparata e dello Sviluppo, Padova, Italy. *Invert Surv J* 1:49-50
3. Ghigliotti L, Mazzei F, Christiansen JS, Fevolden S-E, Pisano E (2004) From Antarctic to Arctic polar fishes: first cytogenetic analyses of three gadid species (*Arctogadus glacialis*, *Boreogadus saida* and *Gadus morhua*). Meeting della Biologia Antartica del PNRA, Messina, Italy
4. Maglione M, Giacomelli S, Coscia MR, Oreste U (2004) Sequence diversity of Antarctic fish IgTM exons. VI Incontro Scientifico della Società Italiana di Immunobiologia Comparata e dello Sviluppo. Padova, Italy. *Invert Surv J* 1:51
5. Motta CM, Tammaro S, De Santis A, Coscia MR, Filosa S, Oreste U (2004) Immunoglobulin localization in the skin of the Antarctic notothenioid *Trematomus bernacchii*. VI Incontro Scientifico della Società Italiana di Immunobiologia Comparata e dello Sviluppo. Padova, Italy. Riassunti pag. 16
6. Oreste U, Maglione M, Giacomelli S, Coscia MR (2004) Evolutionary adaptation of membrane-bound immunoglobulins of Antarctic teleost. Meeting della Biologia Antartica del PNRA, Messina, Italy
7. Oreste U, Mazzei F, Alfieri V, Ghigliotti L, Coscia MR, Ozouf-Costaz C, Pisano E (2004) Mapping of Igh locus on Antarctic fish chromosomes. VI Incontro Scientifico della Società Italiana di Immunobiologia Comparata e dello Sviluppo. Padova, Italy. Riassunti pag. 19
8. Pisano E, Mazzei F, Ghigliotti L (2004) Cytogenetics of Antarctic teleosts: new insight into chromosome structure, diversification and evolution. Meeting della Biologia Antartica del PNRA, Messina, Italy

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- Rizzello A, Acierno R, Verri T, Zizzo I, Storelli C, Maffia M (2004) Partial sequencing and tissutal distribution of a H+/Peptide transporter in the haemoglobinless Antarctic teleost *Chionodraco hamatus*. 54° Congresso Nazionale della Società Italiana di Fisiologia. Chieti, Italy, Pflügers Archiv - European Journal of Physiology. 448(6), p. R55
- Borrelli L, De Stasio R, Filosa S, Parisi E, Riggio M, Scudiero R, Trinchella F (2005) Evoluzione dei geni codificanti le aspartico proteasi: il caso delle notepsine. 66° Congresso Nazionale UZI, Roma, Italy
- Ghigliotti L, Cardamone L, Mazzei F, Fevolden S-E, Pisano E (2005) Analisi dei geni ribosomali 5S nel teleosteo artico *Arctogadus glacialis* (Gadidae). 66° Congresso Unione zoologica Italiana (UZI) Roma, Italy. Abstracts: 117
- Vergara A, Vitagliano L, Bonomi G, Franzese M, Merlino A, Verde C, di Prisco G, Mazzarella L (2005) Inter-aspartic hydrogen bond at the $\alpha 1\beta 2$ interface functions as a proton sink motif in fish hemoglobins. XXXIV Congresso Nazionale di Chimica Fisica, Siena, Italy
- Vergara A, Vitagliano L, Bonomi G, Franzese M, Merlino A, Verde C, di Prisco G, Mazzarella L (2005) Studi cristallografici di emoglobine di pesci antartici rivelano caratteristiche strutturali inusuali. Giornate Scientifiche del polo della vita, Napoli, Italy
- Quintieri A, Angelone T, Filice E, Tota B, Gladwin M, Pellegrino D (2006) Cardiomodulatory action of nitrite on the rat Langendorff perfused heart. XIII Congresso Nazionale della Società Italiana di Ricerche Cardiovascolari (S.I.R.C.), Imola (BO), Italy

F - patents, prototypes and data bases

Crystal structures deposited in Protein Data Bank (PDB)

- 1S5X - crystal structure of *Trematomus bernacchii* hemoglobin oxidized by air. Deposited on 04/05/2004
- 1S5X - crystal structure of *Trematomus bernacchii* hemoglobin oxidized by ferricyanide. Deposited on 04/05/2004
- 2AA1 - crystal structure of the cathodic hemoglobin isolated from the Antarctic fish *Trematomus newnesi* in a deoxy state. Deposited on 13/07/2005

Nucleotide sequences deposited in Gene Data Bank (GeneBank)

- AY772716 - *Bathyraja eatonii* alpha 1 globin mRNA, complete cds. Deposited on 07/10/2004
- AY772717 - *Bathyraja eatonii* beta 1 globin mRNA, complete cds. Deposited on 07/10/2004
- AY773131 - *Amblyraja hyperborea* alpha globin mRNA, partial cds. Deposited on 08/10/2004
- AY773132 - *Amblyraja hyperborea* beta globin mRNA, partial cds. Deposited on 08/10/2004

G -- exhibits, organization of conferences, editing and similar

Speeches

- C Verde: The hemoglobin of *Pseudaphritis urvillii*, a primitive notothenioid fish of temperate latitudes: evolutionary implications (2004) Invited Speaker. Ecology of the Antarctic Sea Ice Zone, Final Symposium, Korčula, Croatia
- C Verde: Structure and function of the Gondwanian hemoglobin of *Pseudaphritis urvillii*, a primitive notothenioid fish of temperate latitudes. The structure of the heme environment by molecular modelling (2004) Speaker. XXVIII SCAR & COMNAP XVI: Evolution and Biodiversity of life in polar regions, Bremen, Germany
- C Verde: L'evoluzione nei pesci polari: struttura, funzione e filogenesi molecolare dell'emoglobina (2005) Keynote Speaker. XVI riunione della sezione Sardegna della Società Italiana di Biochimica e Biologia Molecolare, Sassari, Italia
- C Verde: Structure Function and Molecular Evolution of the Hemoglobins of Polar Fishes (2005) Invited Speaker. IX SCAR International Antarctic Biology Symposium, Curitiba, Brasil
- C Verde: The adaptive evolution of hemoglobins in the fish family Bovichtidae (2005) Invited Speaker. The ICEFISH Symposium, Walpole, Maine, USA
- C Verde: The adaptive evolution of polar fish: structure, function and molecular phylogeny of hemoglobin (2005) Invited Speaker. Third International Symposium on the Arctic Research and Seventh Ny-Ålesund Scientific Seminary, Tokyo, Japan
- A Vergara: Studi cristallografici di emoglobine di pesci antartici rivelano caratteristiche strutturali inusuali (2005) Speaker. Giornate Scientifiche del polo della vita, Napoli, Italy
- A Vergara: Inter-aspartic hydrogen bond at the $\alpha 1\beta 2$ interface functions as a proton sink motif in fish hemoglobins (2005) Speaker. XXXIV Congresso Nazionale di Chimica Fisica, Siena, Italy

H - formation (PhD thesis, research fellowships, etc.)

Degree Thesis

Programma Nazionale di Ricerche in Antartide (PNRA)

- L Cardamone: Caratterizzazione molecolare dei geni ribosomali 5S (5S rDNA) nelle specie *Boreogadus saida*, *Arctogadus glacialis* e *Gadus morhua*. Anno Accademico 2005. Università di Genova, Corso di Laurea in Scienze Biologiche
- M Garibaldi: Citogenetica di teleostei artici della famiglia Gadidae. Anno Accademico 2005. Università di Genova, Corso di Laurea in Scienze Biologiche
- L Grassi: Il sistema di trasporto di ossigeno del teleosteo Nototenoideo sub-antartico *Cottoperca gobio*. Anno Accademico 2006. Università Federico II di Napoli, Corso di Laurea in Scienze Biologiche

PhD Thesis

- F Garofano: Il sistema NOS/NO nel cuore dei teleostei antartici: uno studio comparato. Anno Accademico 2004. UNICAL, Dottorato in Biologia Animale. Tutor: Prof B Tota
- F Mazzei: Il genoma dei teleostei antartici: studio citochimico e citotassonomico. Anno Accademico 2004. Università di Pavia, Dottorato in Biologia Cellulare. Tutor: Dott E pisano
- A Rizzello: Adattamenti ecofisiologici dei teleostei antartici. Anno Accademico 2005. Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali. Università del Salento. Tutor: Prof M Maffia
- D Giordano: Struttura, funzione ed evoluzione di emoglobine di pesci polari. Anno Accademico 2006. Dipartimento di Chimica. Università Cattolica del Sacro Cuore di Roma. Tutori: Prof B Giardina e Dott C Verde
- M Franzese: Indagini strutturali su metalloproteine mediante diffrazione dei raggi X. Anno Accademico 2006. Dipartimento di Chimica. Università Federico II di Napoli. Tutor: Prof L Mazzarella

Fellowships

- F Mazzei: Studio citogenetico dei teleostei antartici e subantartici della campagna ICEFISH 2004. (2004) Università di Genova. Duration: 6 months
- L Ghigliotti: Studio molecolare e citogenetico di geni ribosomali 5S in teleostei polari. (2004) Università di Genova. Duration: 6 months

Research units

LUP-CAC

Responsabile: Dr. Marcello Cacace
Istituto di afferenza: Ist. per lo Studio dei Materiali Nanostrutturati, CNR Bologna
e-mail: cacace@unisi.it

Marcello Cacace	Primo Ricercatore	CSCG/CNR
G Stefanachi	Ricercatore	CSCG/CNR
G Margheri	Ricercatore	IROE/CNR, Firenze

LUP-CHI

Responsabile: Prof. Emilia Chiancone
Istituto di afferenza: Istituto di Biologia e Patologia Molecolari, CNR; e Dip. di Scienze Biochimiche, Università La Sapienza Roma
e-mail: emilia.chiancone@uniroma1.it

Emilia Chiancone	Prof Ordinario	Univ La Sapienza
Laura Giangiacomo	Ricercatore	Univ La Sapienza

LUP-GIA

Responsabile: Prof. Bruno Giardina
Istituto di afferenza: Istituto di Biochimica e Biochimica Clinica, Università Cattolica; e Istituto per la Chimica del Riconoscimento Molecolare, CNR
Indirizzo: Largo Francesco Vito 1, Roma I-00168
e-mail: b.giardina@uniserv.ccr.rm.cnr

Bruno Giardina	Prof ordinario	CSCG/CNR; Univ Cattolica
Maria Cristina De Rosa	Ricercatore	CSCG/CNR; Univ Cattolica
Roberto Scatena	Ricercatore	CSCG/CNR; Univ Cattolica
Francesco Misiti	Ricercatore	CSCG/CNR; Univ Cattolica
Maria Teresa Sanna	Ricercatore	Univ di Cagliari
Alvaro Mordente	Prof Associato	CSCG/CNR; Univ Cattolica
Claudia Bertonati	Dottoranda	CSCG/CNR; Univ Cattolica

Programma Nazionale di Ricerche in Antartide (PNRA)

LUP-MAF

Responsabile: Prof. Michele Maffia

Istituto di afferenza: Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Univ. di Lecce

e-mail: m.maffia@physiology.unile.it

Michele Maffia	Prof associato	Univ di Lecce
Carlo Storelli	Prof ordinario	Univ di Lecce
Raffaele Acierno	Tecnico	Univ di Lecce
Antonia Rizzello	Dottorando	Univ di Lecce

LUP-MAR

Responsabile: Prof. Bruno Maresca

Istituto di afferenza: Universita' di Salerno, Facolta' di Farmacia, Dip. di Scienze Farmaceutiche

e-mail maresca@iigbna.iigb.na.cnr.it

Bruno Maresca	Prof Ordinario	Univ di Salerno
Anna Maria D'Ursi		
Amalia Porta		
Marina Luongo		
Alfredo Franco	Tecnico	IIGB/CNR

LUP-MAZ

Responsabile: Prof. Lelio Mazzarella

Istituto di afferenza: Univ. Federico II di Napoli, Dip. Di Chimica

e-mail: mazzarella@chemistry.unina.it

Lelio Mazzarella	Prof Ordinario	Univ di Napoli
Adriana Zagari	Prof Ordinario	Univ di Napoli
Filomena Sica	Prof Associato	Univ di Napoli
Luigi Vitagliano	Ricercatore	Centro Biocristallografia, CNR
Alessandro Vergara	Borsa post-dottorato	Univ di Napoli
Giovanna Bonomi	Dottoranda	Univ di Napoli
Antonello Merlino	Dottorando	Univ di Napoli
Giosuè Sorrentino	Tecnico	Univ di Napoli

LUP-MOR

Responsabile: Dr. Oscar Moran

Istituto di afferenza: Istituto di Cibernetica e Biofisica, CNR Genova

e-mail: moran@barolo.icb.ge.cnr.it

Oscar Moran	Ricercatore	ICB/CNR
Franco Conti	Ricercatore	ICB/CNR
Michael Puschparai	Ricercatore	ICB/CNR

LUP-ORE

Responsabile: Dr. Umberto Oreste

Istituto di afferenza: Istituto di Biochimica delle Proteine, CNR Napoli

e-mail: u.oreste@ibp.cnr.it

Umberto Oreste	Primo Ricercatore	IBP/CNR
Maria Rosaria Coscia	Ricercatore	IBP/CNR
Biagio Pucci	Laureato	IBP/CNR
Giuseppina Meo	Studentessa	IBP/CNR

LUP-PAR

Responsabile: Prof. Elio Parisi

Istituto di afferenza: Istituto di Biochimica delle Proteine, CNR Napoli

e-mail: e.parisi@ibp.cnr.it

Elio Parisi	Dirigente di Ricerca	IBP/CNR
Clemente Capasso	Ricercatore	IBP/CNR
Rosaria Scudiero	Ricercatore	Univ di Napoli
Vincenzo Carginale	Ricercatore	IBP/CNR

Programma Nazionale di Ricerche in Antartide (PNRA)

LUP-PIS

Responsabile: Dr. Eva Pisano
Istituto di afferenza: Dipartimento di Biologia, Univ. di Genova
Indirizzo: Viale Benedetto XV 5, Genova I-16132
e-mail: pisano@unige.it

Eva Pisano	Ricercatore	Univ di Genova
Federico Mazzei	Laureato	Univ di Genova
Laura Ghigliotti	Laureato	Univ di Genova

LUP-PRI

Responsabile: Prof. Guido di Prisco
Istituto di afferenza: Istituto di Biochimica delle Proteine, CNR Napoli
e-mail: g.diprisco@ibp.cnr.it

Guido di Prisco	Dirigente di Ricerca	IBP/CNR
Ennio Cocca	Ricercatore	IBP/CNR
Cinzia Verde	Ricercatore comandato	IBP/CNR
M.Antonietta Ciardiello	Ricercatore	IBP/CNR
Donatella de Pascale	Assegnista	IBP/CNR
Antonio Riccio	Dottorando	IBP/CNR
Vito Carratore	Tecnico	IBP/CNR

LUP-TOT

Responsabile: Prof. Bruno Tota
Istituto di afferenza: Dip. di Biologia Cellulare, Univ. della Calabria
e-mail: tota@pobox.unical.it

Bruno Tota	Prof Ordinario	Univ della Calabria
Maria Carmele Cerra	Ricercatore	Univ della Calabria
Alfonsina Gattuso	Ricercatore	Univ della Calabria
Daniela Pellegrino	Assegnista	Univ della Calabria
Sandra Imbrogno	Assegnista	Univ della Calabria
Rosa Mazza	Borsa post-dottorato	Univ della Calabria
Raffaele Acierno	Tecnico	Univ di Lecce
