



Centro Regionale di
Competenza

Diagnostica e
Farmaceutica
Molecolari

www.crdc-dfm.it



Unione Europea



Assessorato all'Università e Ricerca Scientifica,
Innovazione Tecnologica e Nuova Economia,
Sistemi Informativi e Statistica

The Players

The *CRdC DFM* consists up of six subjects belonging to five public research groups located across the Campania region in an integrated scientific structure.



Institute of Biostructures and Bioimaging (Group leader)

CNR

Interdepartmental Center for Instrumental Analysis Services

University of Naples "Federico II"

Department of Pharmaceutical Science

University of Salerno

Department of Neurological Science

Second University of Naples

Department of Experimental Oncology

National Cancer Institute "Fondazione G.Pascale"

Department of Biomorphological and Functional Science

University of Naples "Federico II"

The objectives: a brief introduction.

One of the scientific revolution of the XX century is the transition from an “organicistic” to a “molecular” approach to the pathology.

The main assumption underlying this revolution is:

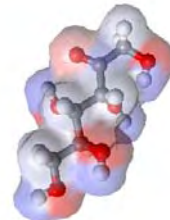
A given pathology can be described as an alteration in the molecular phenotype of an individual (molecular dysfunction).

1. The target for a drug/diagnostic is not the “sickness” in general terms...



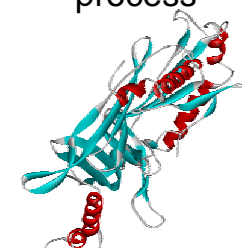
... but one or more bioactive molecules

2. The drug/diagnostic is not an extract or a mix of unknown active “principia” ...



... but a defined molecule or a formulation of different molecules, where any molecule has a known and predetermined action.

3. The action of a drug/diagnostic is mediated by a molecular recognition process

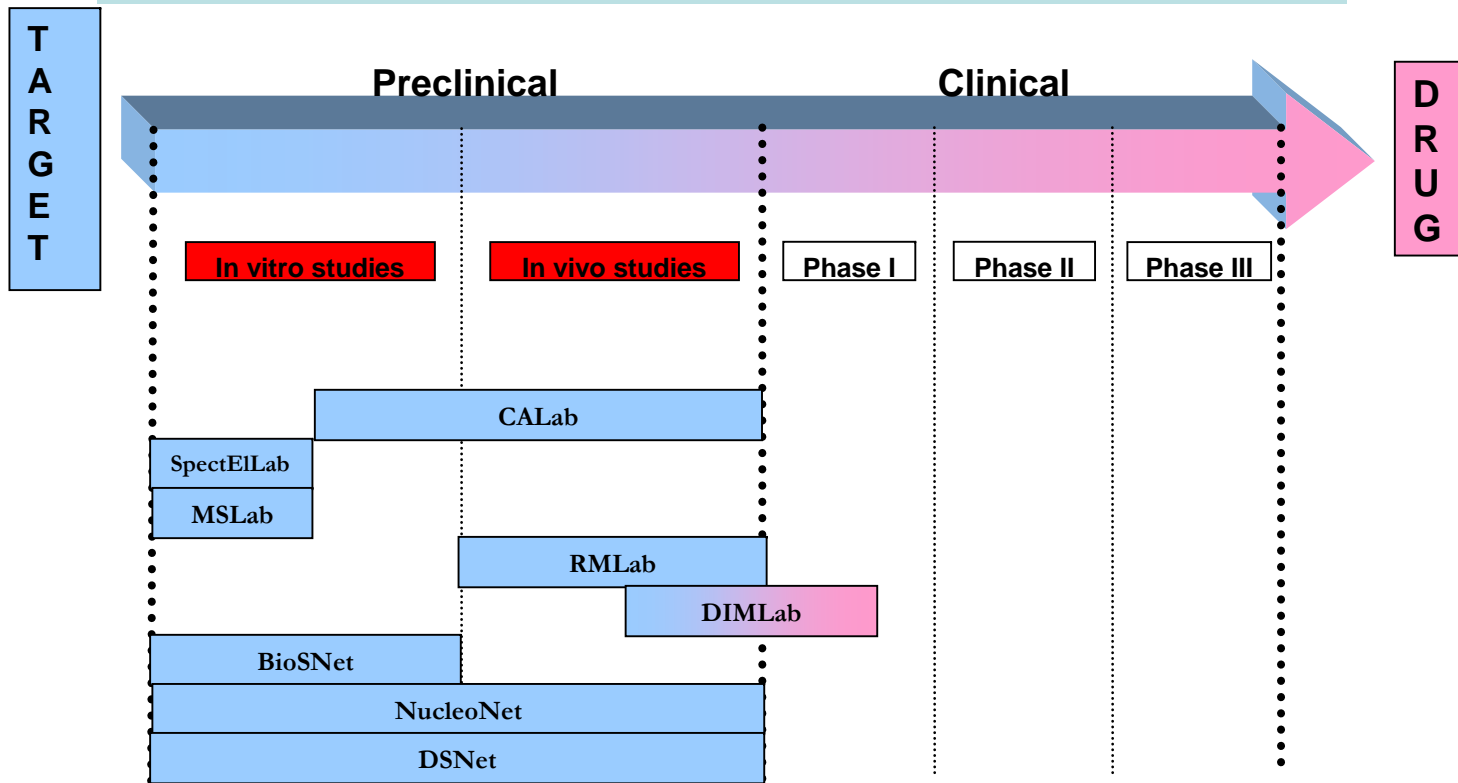


An aptamer binds a viral protein (Pdb code: 7MSF)

Main goals of the CRdC DFM

- *Identifying and defining at a molecular level new diagnostic and therapeutic targets;*
- *Finding new bioactive ligands able to modulate the activity of the identified targets;*
- *Improving the transport, specificity, biodistribution, toxicity of identified active compounds by the design of new drug delivery molecular systems.*

CRdC DFM R&D pipeline



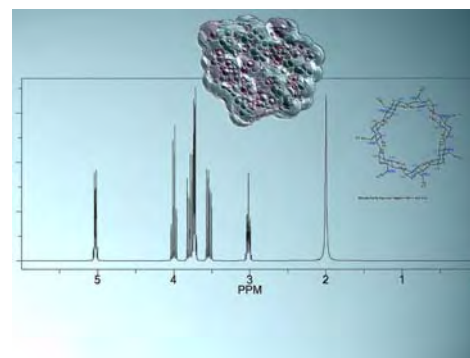
CALab – Cells and Animals

- **Aims:**
To run in a single facility cellular and animal tests.
- **Main technologies:**
Cell line library (mostly from cancer lines);
Animal facility (mouse);
High-throughput microscopy;
Fluorescence cytology lab;
Primary human cell lines (mostly from cancer patients).



SpectELab – Structural Formula

- **Aims:**
To determine the structural formula of bioactive organic compounds.
- **Main technologies:**
NMR Factory (700 MHz Varian UnityInova with ColdProbe, 500 MHz Varian UnityInova, 400 MHz Varian MercuryPlus, 400 MHz Varian Mercury VX)
Mass Spectrometry Factory (Magnetic VG Prospec FISON, ESI-triple quadrupole Applied Biosystems API2000, ESI-Ion Trap Termoquest LCQ, GC-MS Hewlett-Packard)



MSLab – Mass Spectrometry

- **Aims:**

To exploit the potential of Mass Spectrometry in proteomic, ligand screening, innovative applications.

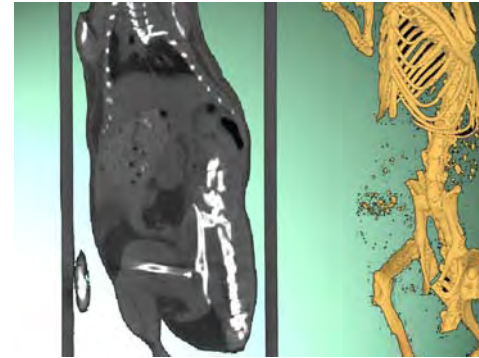
- **Main technologies:**

Mass Spectrometry Factory (LC-MS Thermoquest LCQ-Deca with source ESI and PCI and ionic trap, LC-MS Thermoquest LCQ with source ESI (Z-spray) and ionic trap, GC-MS Thermoquest GCQ with source EI and PCI, Triple quadrupole LC-MS Applied Biosystems API-2000 with source ESI and PCI, Capillary electrophoresis connected to Thermoquest LCQ systems, MALDI-TOF Reflectron, LC-MS hybrid quadrupole-TOF).



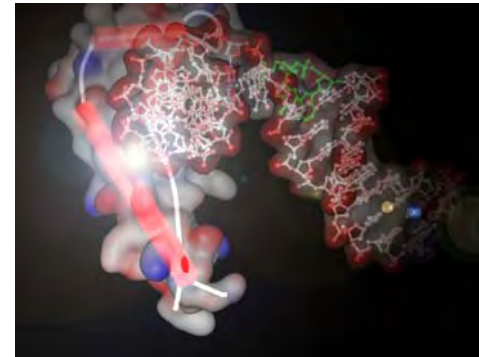
RMLab – Magnetic Resonance

- **Aims:**
Morpho-functional and non-conventional Magnetic Resonance Studies on small animals.
- **Main technologies:**
NMR resonance on small animals *in vivo* (mouse, rat, rabbit head)



BioSNet – Structural Biology

- **Aims:**
To obtain useful structural data on the target of a drug/diagnostic and to describe the binding of a lead compound to its target
- **Technologies:**
X-Ray diffraction
Multidimensional NMR
Mass spectrometry
Bioinformatic



DIMLab – Diagnostic and Imaging

- **Aims:**

Morpho-functional studies, imaging and biodistribution of labelled and unlabelled molecules in human.
New diagnostic development.

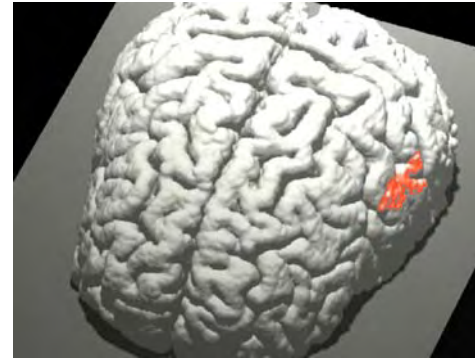
- **Main technologies:**

PET-TAC

PET-SPET Ultrasound

Tissue Imaging

Morphometric analysis



NucleoNet – Nucleic Acids

- **Aims:**

To exploit the diagnostic and therapeutic potential of oligonucleotides and related molecules.

- **Main technologies:**

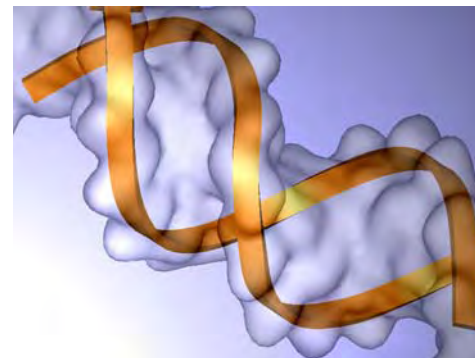
Oligonucleotides and analogues chemistry

Antisense technology

Aptamers, SELEX and libraries

Nucleic Acid structure

Nucleic Acid based materials



DSNet – Drug Screening

- **Aims:**

To screen different molecular repertoires for identifying ligands toward a defined target or group of targets, both at cellular and molecular level

- **Main technologies:**

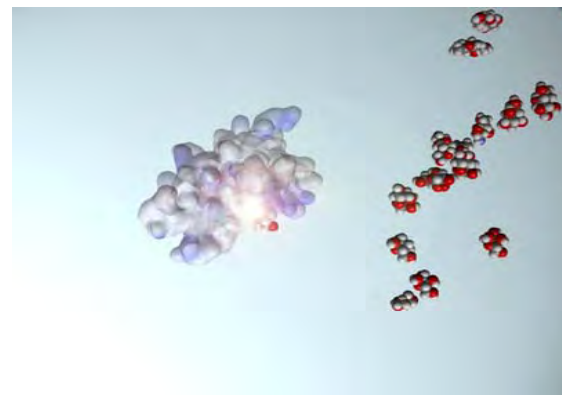
Automated HTS system

Biacore

Combinatorial and microwave-assisted chemistry

Biopolymer libraries

Virtual screening



Patents

- 1) B. Alfano; A. Prinster; M. Quantarelli: Process for realising a biomorphic, stereolithographed phantom, which is multicompartamental and suitable for multianalytical examinations, and relevant device- WO2004029881-08/04/2004
- 2) A. Leone; M.C. Turco: BAG3 nucleotide and protein sequences to be used in research, diagnostics and therapy for cell death-involving diseases, and for modulation of cell survival and/or death- US2005176660-11/08/2005
- 3) P. Abrescia, L.D. D'Andrea, C.Pedone: Method for identifying agents modulating the metabolism of steroid molecules and agents identified by the method- WO2006008775- 26/01/2006 (*)
- 4) A. Verdoliva; P. Bellofiore; G. Cassani; D. Marasco; C. Pedone; S. Monti; M. Ruvo: Peptide ligands specific to immunoglobulins- EP1621546-01/02/2006 (*)
- 5) S. Aime; E. Gianoli; G. Morelli; C. Pedone; D. Tesauro; L. Lattuada; M. Visigall; P. Lucio Anelli: Peptides conjugates, their derivatives with metal complexes and use thereof for magnetic resonance imaging (MRI) US2005008573- 13/1/05 (*)

(*) Industrially partnered

- 6) M. Saviano; S. De Luca; G. Morelli; D. Tesauro ; C. Pedone: Preparation and use of cyclic and branched peptides and their labelled derivatives as therapeutic agents, cholecystinin agonists or antagonists, and diagnostic agents to identify and locate tumours -US2004254339- 16/12/2004
- 7) E. Novellino; P. Grieco; P. Rovero: Cyclic peptides acting as urotensin-II antagonists- WO2005023845 – 17/03/2005 (*)
- 8) D. G Barone; Marco E Bianchi; E. M Bucci; S. Fumero; M. Valente; Roberto Sapio; D. Musumeci Therapeutic agents for the treatment of HMGB1-related pathologies WO2006002971-12/01/2006 (*)
- 9) G. Basile, U. Benatti, S. Bruzzone, G. Damonte, A. De Flora, E. Fattorusso, L. Franco, L. Guida, O. Taglialtela-Scafati e E. Zocchi Nuovi Dinucleotidi Adenilici con Attività Antitumorale e Procedimento per la loro Preparazione Ital. Patent TO 2005A000298 (dep. 2/5/2005) (*)
- 10) G. Bringmann, G. Lang, H. Tsuruta, e. Fattorusso, A. Aiello, M. D'Esposito, M. Menna, W. E. G. Mueller, H.C. Schroeder, S. Petrovic-Ottstadt, New medically insertable pyrrole alkaloids: Procedure for its isolation, synthesis, it containing drug and their use. Ger. Offen. (2005)-DE 102004002885 (*)

(*) Industrially partnered

- 11) G. Campiani, S. Gemma, M. De Angelis, B. P. Joshi, G. Kukreja, L. Savini, E.Fattorusso, O. Taglialatela-Scafati, C. Fattorusso Novel Polycyclic compounds as antimalarial agents Eur. Patent 06 005 306.3 (app 15/3/2006) (*)
- 12) G. Campiani, S. Gemma, M. De Angelis, B. P. Joshi, G. Kukreja, E.Fattorusso, C. Fattorusso Antimalarial agents having polyaromatic structure Eur. Patent 06 005 307.1 (app 15/3/2006) (*)
- 13) W.E.G. Muller, H. C. Schroeder, E. Fattorusso, M.L. Menna, A. Aiello and A. Giordano Isolation Structure Determination, Synthesis and Bioactivity of Damipipecolin and Damituricin Eur. Patent 06 005 564.7 (app. 17/3/2006) (*)
- 14) L.D. D'Andrea, C. Pedone, G. Iaccarino, B. Trimarco, C.M. Turco, A. Leone: Compounds based on VEGF helix 17-25 as modulator of angiogenesis - Provisional n°US60/694671 - 29/6/2005
- 15) L. Vitagliano, L. Zaccaro, G. Morelli, E. Martegani: Heterodimeric peptide compounds displaying NGF activity and their use to treat neurodegenerative disorders" EPO Applications n. 05012643.2 -13.6.05 (*)

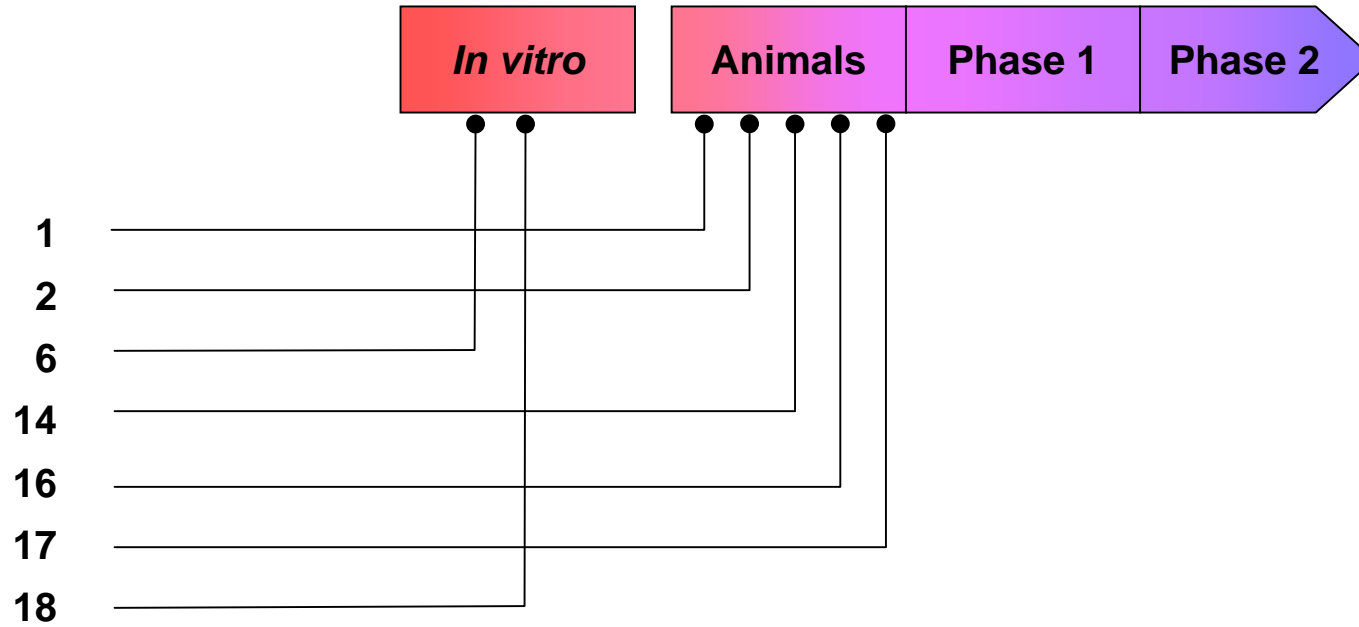
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16. A. Del Gatto, L. Zaccaro, C. Pedone, M. Saviano: Novel and Selective Ligand of the Integrin $\alpha_v\beta_3$ Receptor: Design, Synthesis and Biological Behaviour" Provisional n°US60/7296294 -12/8/2005
17. A. Accardo, D. Tesauro, L. Paduano, C. Pedone, L. Aloj, G. Morelli: Supramolecular aggregates containing chelating agents and bioactive peptides as effective and selective delivery tools for drugs and contrast agents in MRI or nuclear medicine. Provisional n°US60/685538-13/05/2005
18. R. Bisogni, A. Lamberti, A. Petrella, M.F. Romano, M.C. Turco, S. Venuta Nucleotide sequences and protein(s) encoded by such nucleotides for modulation of apoptosis WO 02/055553 A2 July 18th, 2002
19. Ada Yonath, Anat Bashan, Raz Zarivach, Francois Franceschi, Frank Schluenzen, Joerg Harms, Renate Albrecht, Rita Berisio. Methods of growing crystals of free, antibiotic complexed, and substrate-complexed large ribosomal subunits, and methods of rational designing or identifying antibiotics using structure coordinate data derived from such crystals. WO 03/026562 A2. April 3th, 2003 (*)

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What is in the pipeline?

Patents



Current research activities at CRdC DFM

Ongoing activities follow two main directions:

- 1) Further development of the molecules described in the patents listed above.**
- 2) Design, synthesis, and characterization of new compounds of therapeutic or diagnostic interest.**

The first line also includes activities aimed at setting up phase I clinical trials for compounds # 14 and # 16 of the patent list.

Most relevant subjects of the ongoing research lines:

1) Therapy of hypertension: modulation of HDL apolipoprotein A-I activity

Synthetic molecules which induce high activity of the HDL apolipoprotein A-I in the reverse cholesterol transport and in the endothelium response to inflammatory cytokines have been identified.

2) Carbonic anhydrase inhibitors for the treatment of glaucoma

Starting from X-ray crystallography of the various carbonic anhydrase isozymes in complex with inhibitors/activators, we are currently working on the drug design of better drugs useful to treat glaucoma.

3) New tools for earlier diagnosis of cancer

Novel compounds, based on radiolabeled peptides with high affinity towards membrane receptors overexpressed by tumour cells, have been developed.

4) Peptide containing aggregates as selective nanocarriers for therapeutics or diagnostics

The objective of this project is develop peptides containing aggregates, such as micelles and vesicles, able to vehicle selectively drugs on tumour cells

5) Structural requirements for proinflammatory activity of porin P2 from Haemophilus influenzae type b (Hib)

Insights into the role played by porin P2 in the pathogenesis induced by Hib have been obtained. We designed and characterized synthetic peptides from protein P2 that activate JNK and p38 mitogen-activated protein kinase (MAPK) pathways, and induce release of TNF- α and IL-6.

6) New targets for the development of compounds for tuberculosis treatments

Tuberculosis is the main cause of infectious disease mortality in the world after AIDS as it causes from 2 to 3 million deaths per year. Our main efforts are devoted to the understanding the mechanisms that regulate the route through the bacterial dormant state as well as those responsible for drug resistance. Several protein responsible for these processes are under study.

7) Antiviral peptides blocking herpes simplex virus type 1 entry into cells

We are developing innovative Human herpes simplex virus 1 (HSV-1) therapies, based on the use of viral peptides; we have identified four promising targets present on the surface of the viral particle which are known to mediate cell attachment and entry.

8) Cripto-Alk4 antagonists for tumor treatment

We discovered tetrameric peptides that are Cripto-Alk4 receptor antagonists (IC50 about 100 nM). These peptides seem to bind to mouse Cripto. In vitro evaluation on cell fate modulation on a Cripto^{-/-} ES murine cells is ongoing.

9) PIGF-Flt1 antagonists as anti-tumor agents

We characterized tetrameric peptides that are PIGF-Flt1 antagonists receptor antagonists (IC50 about 3000-5000 nM). Peptides do bind to Flt-1 with a selectivity for human variant over the murine one. In vitro evaluation on capillary tube formation on HUVE cells positive (same IC50). Evaluation of *in vivo* capillary formation assay is ongoing.

10) New strategies for the purifications of macromolecules of biotechnological interest

We found two a1AT ligands with low affinity for the protein. These were used as synthetic ligands for affinity purification of a1AT from biological fluids. Evaluation of purification capacity is ongoing. We also discovered two IgG ligands with low affinity for the protein. KD in the low micromolar range (about 20 micromolar, ELISA). Synthetic ligand for the affinity purification of IgG from biological fluids.



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Diagnostica e Farmaceutica Molecolari***

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