SAPIENZA
UNIVERSITÀ DI ROMA

Dipartimento di Chimica e Tecnologie del Farmaco

Direttore: Prof. Bruno Botta

Workshop

How computer chemistry could be exploited in current life science?

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M. C. De Rosa	Ist. di Chimica del Riconoscimento Molecolare, CNR, Roma
M. D'Abramo	Università di Roma Sapienza , Dipartimento di Chimica
C. Gargioli	Dipartimento di Biologia, Università di Tor Vergata, Roma
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7 dicembre 2016, h 9,00

Università di Roma Sapienza

Plesso Tecce della Fac. di Farmacia e Medicina, Aula D

Segreteria organizzativa:
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In recent decades computational chemistry evolved as a highly versatile discipline, moving from pure chemical applications to the adjacent fields of medicinal chemistry, biology, medicine, biotechnology and material science. In this workshop, applications of computer chemistry in the field of life science will be described, in order to highlight the potentiality and versatility of this tool and to give raise to novel possible scientific collaborations.

PROGRAM

h 9.00-9.30 The computational analysis of biomolecules and its potential impact on drug discovery

Anna Tramontano

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The synergy between experimental, computational biology and computational chemistry has greatly benefited the biomedical field, providing invaluable information in many different areas. Available method for predicting the three-dimensional structure of a protein and its interactions cannot only rely on our understanding of the basic laws of physics, because of the enormous complexity of the problem. We therefore need to rely on empirical methods for the prediction of a protein structure and of its interactions with both macromolecules and smaller compounds. I will describe some of the methods that we and others developed to this end and discuss their advantages and limitations, especially in light of their usefulness in structure based drug design.

h 9.30-10.00 In silico design and discovery of protein-protein interaction inhibitors as drug candidates against autoimmune diseases

Maria Cristina De Rosa

Istituto di Chimica del Riconoscimento Molecolare (ICRM), CNR, Roma

Autoimmune diseases are characterized by the dysfunction of T-cells which erroneously react against the body's own tissues, leading to tissue damage and destruction. The interaction of the T-cell receptors (TCRs), located on the surface of T-cells and MHC-peptide complexes on the targeted tissues, lead to these unwanted immune responses. A number of well-established immune modulatory drugs have been used in the clinic for the treatment of autoimmune diseases with significant side effects. The protein–protein interactions between TCR and autoantigen in complex with MHC can serve as targets for the development of new drugs against autoimmune diseases that may selectively block only the immune response to the self antigen, while leaving the rest of the immune system response intact and efficient. With this aim, we worked out a novel strategy to identify small-molecule inhibitors of TCR/MHCII-peptide interaction implicated in the pathogenesis of Rheumatoid Arthritis. By using computationally-generated structural information at the interface [1] and virtual screening guided by pharmacophore modeling we discovered small-molecule inhibitors which were able to selectively block proliferation of collagen-specific T cells in the contest of HLA-DR4 [2]. The compounds identified here open up new possibilities in the treatment of Rheumatoid Arthritis and preclinical and clinical studies are being pursued.

1. De Rosa, M.C. *et al. PLoS One*. 2010, 14;5(7):e11550
2. De Rosa, M.C. *et al.* Int. Patent WO2013144931, 2013.

h 10.00-10.30 Modelling of complex systems: where computation meets experiment

Marco D'Abramo

Department of Chemistry, University of Roma Sapienza, Roma

Advances in both theoretical methods and computational power give us the opportunity to model complex systems with unprecedented level of detail, making in-silico predictions quite reliable. In this talk, I will discuss some recent results on the I-DmoI endonuclease proteins, where all-atoms molecular dynamics simulations were used to shed some light on the key molecular determinants affecting the DNA phosphodiester bond break as provided by I-DmoI proteins. Our

data, compared with experimental results, strongly suggest that the catalytic activity could be affected by several factors including ions and water positioning near the cleavage site as well as the protein global dynamics.
I will also present a novel characterization of protein structural-molecular evolution by constructing a proper observable space. By combining the experimentally determined melting temperature, the structural/geometrical properties as obtained from the crystal structures and molecular dynamics simulations of a set of thioredoxins, both extant and resurrected, we were able to define a proper new protein global observable containing the main information on thioredoxin structural-molecular evolution.
Finally, I will also briefly discuss our preliminary results on the Src kinase behavior in the background of the T-Cell receptor function.

h 10.30-10.45 Intervallo

h 10.45-11.15 Strategie innovative per il recupero di tessuto muscolare danneggiato

Cesare Gargioli
Department of Biology, University of Tor Vergata, Roma, *Italy*

The skeletal muscle tissue exhibits good regenerative capabilities, which are however limited by injury size. As a matter of fact, large muscle lesions are characterized by poor recovery accompanied by scar formation and functional detriment, condition common to people suffering from volumetric muscle loss and needing reconstructive therapeutic approaches. Even if surgical autologous transplantation is a standardized procedure, the outcomes are often unsatisfactory. Hence, the pressing need to develop new therapeutic approaches to recover wasted muscle. Stem cells and regenerative medicine have greatly increased the expectations of the scientific community and the public for recovering or replacing ablated, injured, aged and diseased tissues. Reconstructing the skeletal muscle architecture and function is still a challenge requiring parallel alignment of myofibrils arranged into organized sarcomeres; moreover the new muscle must be vascularized, innervated and it must integrate with host tissue. Recently we demonstrated the great potential of a tissue engineering (TE) approach, exploiting a hybrid biomimetic matrix, namely PEG-Fibrinogen, we revealed the enhancement of myogenic cell progenitor capabilities *in vivo* by providing a suitable 3D environment for mouse muscle reconstruction. We show that an "anatomical bioreactor-like", represented by the surface of the *tibialis anterior* muscle (TA), influences maturation and alignment of fibers derived from adult muscle stem/progenitor cells embedded into a poly-ethylene-glycol-fibrinogen (PF) gel, leading to the generation of an artificial normal new muscle[1]. Furthermore by the same approach we succeeded in replacing a complete mouse TA after a massive muscle ablation, recovering morphology and function of the substituting artificial TA[1]. Skeletal muscle TE is an up-and-coming biotechnology with great potential for muscle repair, but no conclusive strategy has been demonstrated yet. Reconstructing the skeletal muscle architecture and function is still a challenge requiring the parallel alignment of myofibrils arranged into organized sarcomeres.
Starting from these observations, we developed a novel approach for the regeneration and/or reconstruction of skeletal muscle tissue segments of human-like size by exploiting a population of adult myogenic stem cells, namely pericytes, in combination with 3D bio-printing technology to guarantee a functional architecture. *In vitro* characterization of cell-laden constructs showed enhanced myogenesis and positive myostructure alignment.

1. Fuoco *et al*., *EMBO Mol Med.* 2015 Feb 25.

h 11.15-11.45 Finding the way to reach the target: the CF lung disease

Fiorentina Ascenzioni
Department of Biology and Biotechnology, University of Roma Sapienza, Roma

Drug delivery systems are as important as the active compound to optimize therapeutic efficacy, and these are particularly important for the treatment of chronic diseases, which usually oppose different barriers in the accessibility of the drug to the target tissues. Cystic fibrosis (CF) is the most common life shortening monogenetic autosomal recessive disorder affecting over 85,000

people worldwide (32,000 in Europe). CF patients develop progressive lung disease, characterized by airways mucus obstruction, bacterial infection and hyper inflammation. CF therapeutics include drugs targeting the molecular cause of the disease (dysfunctional CFTR), which enter the clinic very recently and the standard symptomatic treatments including mucolytics, to dissolve thick mucus, antibiotics and antinflammatory agents to treat infection and inflammation, respectively.

The CF lungs are obstructed by dehydrated sticky mucus, which adversely affects the absorption or action of drugs administered by the oral or pulmonary routes. Additionally, the bacteria causing chronic lung infections appear to be organized in biofilms embedded within the mucus. Based on their biophysical properties, nanoparticles have proved to be promising in pharmacotherapy. Current progress in nanoparticle engineering can improve pharmacokinetics of therapeutics while minimizing adverse effects of the drugs. By taking advantage of nanoparticles, assembled with different carriers, we got an improvement of tobramycin efficacy against *Pseudomonas aeruginosa* biofilms.

h 11.45-12.15 Efficient antagonists of SMO and GLI1 Hedgehog signaling targets by computational screening

Mattia Mori,[a] Francesca Ghirga,[a] Cinzia Ingallina,[b] Deborah Quaglio,[b] Simone Berardozzi,[a,b] Paola Infante,[a] Romina Alfonsi,[c] Lucia Di Marcotullio,[c] Bruno Botta[b]

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Hedgehog (Hh) signaling is essential for tissue development and stemness. Activating germline or somatic mutations of genes encoding Hh pathway components are found in basal cell carcinoma (BCC) and medulloblastoma (MB), while uncontrolled Hh signaling has been reported to drive tumor progression in several cancers, including lung, breast, stomach, pancreas and hematopoietic malignancies. For this reason, the development of Hh inhibitors is eliciting great interest in drug discovery.[1]

Based on the availability of structural details of SMO and GLI1, which are the most relevant upstream and downstream regulators of the Hh signaling pathway – respectively, we set up a structure-based screening strategy boosted by computational studies. In the case of SMO, the binding site of drugs and drug-candidates is well established and characterized within the heptahelical bundle of the receptor.[2] In the case of GLI1, computational and experimental efforts were first spent to clarify the structural requirements of its binding to DNA and to identify a putative ligand binding site.[3] Subsequently, an *in house* library of natural products and their derivatives was screened *in silico* against SMO and GLI1 targets by means of molecular docking, to identify novel Hh inhibitors. A synthetic chalcone derivative emerged as profitable SMO antagonist providing Hh inhibition *in vitro* on cancer (MB and BCC) and cancer stem cells (MB), and *in vivo* (BCC). The molecule proved to inhibit Hh also in the presence of a drug-resistant form of SMO.[4] Glabrescione B (GlaB), an isoflavone naturally found in the seeds of *Derris glabrescens* (Leguminosae), [3,5] emerged as efficient GLI1 antagonist that binds GLI1 zinc-finger and interferes with its interaction to DNA. Remarkably, GlaB inhibited the growth of Hh-dependent MB and BCC cells *in vitro* and *in vivo*, as well as the self-renewal ability and clonogenicity of MB cancer stem cells.

In summary, computational tools proved highly versatile and reliable in understanding the structural requirements of Hh target proteins and in identifying highly efficient small molecule modulators of pharmacological relevance.

1. Infante, P. *et al., Trends Pharm. Sci*. 2015, 36:547-58
2. Wang, C., *Nat. Commun.* 2014, 5: 4355
3. Infante, P. *et al., EMBO J.* 2015, 34:200-217.
4. Infante, P. *et al., Cell Death and Disease* 2016, 7, e2376
5. Botta, B. *et al.,* Int. Patent WO 2014207069, 2014.