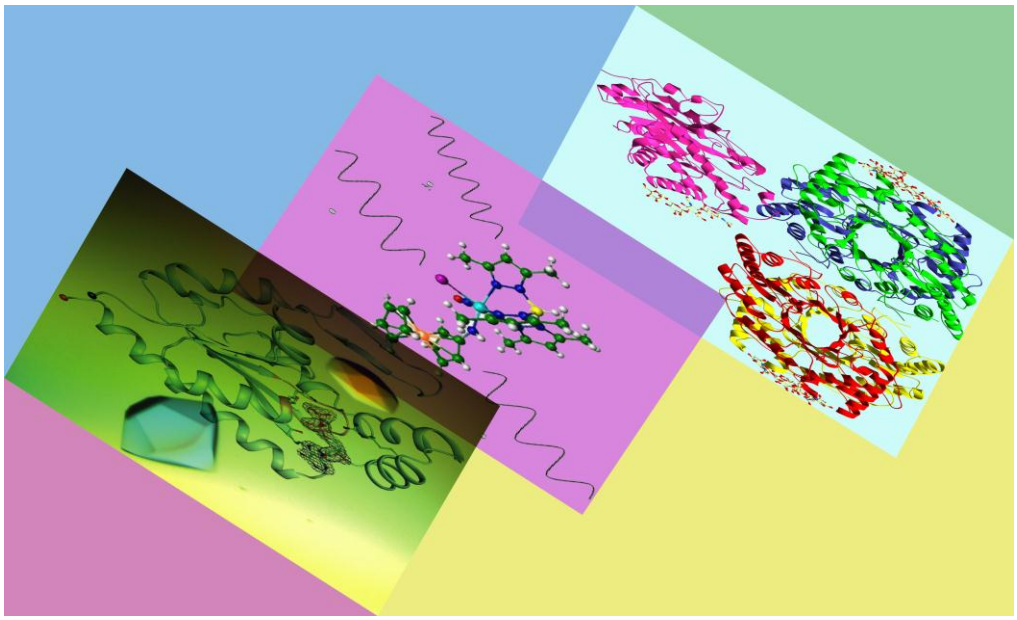
Structure- & Computer-aided Design Workshop: Bioactive Molecules & Materials



ABSTRACT BOOK

7 – 11 November, 2011

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LIST OF SPEAKERS

Pavlos AGIANIAN	Democritus University of Thrace
Joanna ANDREADOU	National and Kapodistrian University of Athens
Lucia BANCI	University of Florence, Italy
Sarah BUTCHER	University of Helsinki, Finland
Jose Maria CARAZO	Spanish National Center for Biotechnology
Zoe COURNIA	Biomedical Research Foundation, Academy of Athens
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Elias ELIOPOULOS	Agricultural University of Athens
Elspeth GARMAN	University of Oxford, UK
Darren HART	European Molecular Biology Laboratory, France
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George KONTOPIDIS	University of Thessaly
Georgios LEONIS	National Hellenic Research Foundation
Josep Maria LUIS	University of Gerona, Spain
Thomas MAVROMOUSTAKOS	National and Kapodistrian University of Athens
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Christiana MITSOPOULOU	National and Kapodistrian University of Athens
Masha NIV	The Hebrew University of Jerusalem, Israel
Giannis PAPAEFSTATHIOU	University of Athens
Andreas PAPAPETROPOULOS	University of Patras
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Thomas STEINBRECHER	Universität Karlsruhe, Germany
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National Hellenic Research Foundation,
Institute of Organic & Pharmaceutical Chemistry

Structure– & Computer– Aided Design Workshop: Bioactive Molecules & Materials

7 – 11 November, 2011

	MONDAY, 7 November 2011	
9.00 – 10.15	Registration	
10.15 – 10.30	Welcome & opening remarks	
10.30 – 11.15	**Lucia Banci** Structural-based design of new vaccines: towards Structural Vaccinology	
11.15 – 12.00	**Matthias Wilmanns** How bioactive nitrogen incorporation in metabolites is regulated in glutamine amidotransferases	
12.00 – 12.30	Coffee break	
12.30 – 13.15	**Jose Maria Carazo** Challenges in Three-dimensional Electron Microscopy	
13.15 – 14.00	**Sarah Butcher** Discovery of new drug targets by cryo-electron tomography and image reconstruction	
14.00 – 15.00	Lunch break & Poster session	
15.00 – 15.45	**Georgios Spyroulias** NMR Structural Biology at University of Patras & The SEE-DRUG project	
15.45 – 16:45	**Practical session** virtual screening **Manos Mikros (Group A)**	**Instruct-ELLAS meeting of current and potential users**
16.45 – 17.15	Coffee break	
17.15 - 18.15	**Practical session** virtual screening **Manos Mikros (Group B)**	**Instruct-ELLAS meeting of current and potential users**

	TUESDAY, 8 November	
09.30 – 10.15	**Darren Hart** ESPRIT: Library-based construct screening for difficult-to-express proteins	
10.15 – 11.00	**Patrick Shaw Stewart (Douglas Instruments)** Random micro-seeding: a theoretical and practical exploration of seed stability and seeding techniques for successful protein crystallization	
11.00 – 11.30	**Coffee break**	
11.30 – 12.15	**Elspeth Garman** From hot to cool and more for less.	
12.15 – 13.00	**George Kontopidis** Optimise outcome from ligand soaking: consideration and results	
13.00 – 14.00	**Lunch break & Poster session**	
14.00 – 15.30	**Practical session Robotic Crystallization** **Patrick Shaw Stewart (Group A)**	**Practical session X-ray data collection** **Elspeth Garman (Group B)**
15.30 – 16.00	**Coffee Break**	
16.00 – 17.30	**Practical session Robotic Crystallization** **Patrick Shaw Stewart (Group B)**	**Practical session X-ray data collection** **Elspeth Garman (Group A)**
19.00	**Reception Dinner**	

	WEDNESDAY, 9 November
9.30 – 10.15	**Thomas Steinbrecher** Free Energy Calculations in Ligand Protein-Binding Studies
10.15 – 11.00	**Rod Hubbard** Structure and Fragment-Based Ligand Discovery Methods and Applications
11.00 – 11.30	**Coffee break**
11.30 – 12.15	**Michael Szardenings** Exploring Proteins with Peptide Phage Display
12.15 – 13.00	**Masha Niv** Computational Design of Protein Kinase-Inhibiting Peptides and Peptidomimetics

13.00 – 14.00	**Lunch break & Poster session**
14.00 – 14.45	**Elias Eliopoulos** Designing inhibitors for Rheumatoid arthritis. A "hit to lead" approach
14.45 – 15.15	**Joe Hayes** Molecular modeling targeting glycogenolysis control

	THURSDAY, 10 November
09.30 – 10.15	**Josep Maria Luis** Exploring the exohedral reactivity and selective encapsulation of fullerene compounds
10.15 – 11.00	**Giannis Papaefstathiou** Assembling Polynuclear Metal Complexes into Supramolecular Architectures
11.00 – 11.30	**Coffee break**
11.30 – 12.15	**Zoe Cournia** Nanoparticle-lipid bilayer interactions: Insights from Molecular Dynamics and free energy calculations
12.15 – 12.45	**Haralambos Tzoupis** Binding of Novel Fullerene Inhibitors to HIV-1 Protease: Insight through Molecular Dynamics and Molecular Mechanics Poisson–Boltzmann Surface Area Calculations
12.45 – 13.45	**Lunch break & Poster session**
13.45 – 14.30	**Thomas Mavromoustakos** Rational Drug Design
14.30 – 15.15	**Georgios Leonis** Molecular Dynamics and Binding Free Energy Calculations in Protein Systems: Advancements on Hypertension Treatment
15.15 – 16.00	**Christiana Mitsopoulou** Dithiolene Complexes: Properties and applications in material science and bioinorganic/biological chemistry.
16.00 – 16.30	**Coffee Break**
16.30– 18.30	**Practical session: Desmond software *Interactive Session*** **Istvan Kolossvary (Group A)**

	FRIDAY, 11 November
09.30 – 11.30	**Practical session: Desmond software *Interactive Session*** **Istvan Kolossvary (Group B)**

11.30 – 12.00	**Coffee break**
12.00 – 12.45	**Kostas Iatrou** Odorant binding protein-based screens for discovery of natural compounds effecting mosquito olfactory responses
12.45 – 13.30	**Pavlos Agianian** Direct insecticide binding to malaria mosquito GSTs
13.30 – 14.30	**Lunch break & Poster session**
14.30 – 15.15	**Joanna Andreadou** Novel targets as pharmacological tools for the development of new therapeutic strategies for the coronary heart disease
15.15 – 16.00	**Andreas Papapetropoulos** Hydrogen sulfide a novel signalling molecule in mammalian cells
16.30 – 17.15	**Ioannis Emiris** Geometric algorithms for modeling molecular structure
17.15 – 17.30	**Closing remarks**

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SEVENTH FRAMEWORK PROGRAMME

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Structural-based design of new vaccines: towards Structural Vaccinology

Lucia Banci

CERM & Department of Chemistry, University of Florence,
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The knowledge of the structural properties of biomolecules is essential not only for the design of new small molecules towards a given target but also, at wider level, for developing new therapeutics in general and, specifically, new vaccines which are efficient, safe and with very broad protection.

A detailed and deep characterization of the structural and dynamical properties of selected antigens and the analysis of the antigen-antibody recognition is essential for the optimization of the use of the antigens as starting point in designing new vaccines, through an innovative and very effective approach in vaccine development. Indeed, the knowledge of the structural features of the various, non cross protective antigens of different pathogen variants, and their interaction sites with antibodies, allowed us the design of chimera proteins with a broad protective efficacy with respect to all the variants.

This structural-based approach in designing a new generation of vaccines will be presented and discussed.

"How bioactive nitrogen incorporation in metabolites is regulated in glutamine amidotransferases".

Matthias Wilmanns

European Molecular Laboratory, Hamburg, Germany

"Nitrogen is incorporated into various metabolites by multifunctional glutamine amidotransferases via reactive ammonia generated by glutaminase hydrolysis of glutamine. Although this process is generally tightly regulated by subsequent synthase activity, little is known about how the glutaminase is inhibited in the absence of an activating signal. We use imidazoleglycerolphosphate synthase as a model to investigate the mechanism of glutaminase regulation. A structure of the bienzyme–glutamine complex reveals that the glutaminase active site is in a catalysis-competent conformation but that the ammonia pathway towards the synthase active site is blocked.

The mutation of two residues blocking the pathway leads to a complete uncoupling of the two catalyzed reactions and to a 2800-fold amplification of glutaminase activity. Our findings provide insight into how enzymatic incorporation of nitrogen into chemical compounds, which is an important process in biotechnology, could be exploited efficiently."

Challenges in Three-dimensional Electron Microscopy

José-Maria Carazo, Sjors Scheres and Carlos O.S. Sorzano

National Center for Biotechnology, CNB-CSIC,

and INSTRUCT Associated Center for Image Processing in Microscopy

The study of large and potentially flexible macromolecular complexes is not an easy task for any experimental technique. Still, three-dimensional electron microscopy (3DEM) offers the potential to analyze in three-dimensions the structure of large complexes without imposing restrictive size limits or requiring the growth of any type of crystals.

The principles of 3DEM are rooted in the field of medical tomography, where a set of 2D projection images of a 3D object obtained from different projection directions are combined into a quantitative estimation of the 3D structure of the object. In the case of 3DEM the images are obtained with the help of an electron microscope, achieving a typical spatial resolution between 1 and 2 nm. It should be noted that the interaction between electrons and matter is relatively strong, forcing the use of very low electron doses in the images of the complexes, which results is very poor electron statistics in the images and, consequently, a very strong noise, typically many times more than the signal coming from the macromolecule itself.

The unique characteristic of 3DEM as compared to other experimental techniques in structural biology is that the experimental information, the 2D images, are obtained from each of the complexes individually rather than representing an "ensemble" information. However, the experimental 2D images are very noisy, requiring some level of averaging to obtain a reliable 3D structure. It is the trade-off between the unique capacity to visualize individual complexes and the need to average them that determines the 3DEM capacity to classify the images being obtained into structural classes, and, in this way, to better understand the flexibility of the complex. In this presentation we will review a number of examples in which 3DEM has successfully provided new structural information on the structure of large and flexible macromolecular complexes, presenting in each case the methods that have been used for their determination.

Discovery of new drug targets by cryo-electron tomography and image reconstruction

Butcher Sarah

University of Helsinki, Finland

Rational drug discovery aided by structural information often concentrates on isolated proteins as targets. However, in developing new antiviral targets it is important to identify steps in the replication cycle which do not necessarily involve enzymatic activities. Such steps can include receptor binding, viral uncoating and assembly. Measles virus is a highly infectious, enveloped, pleomorphic virus. We combined electron cryotomography with subvolume averaging and immunosorbent electron microscopy to characterize the three-dimensional ultrastructure of the virion. We show that the matrix protein forms helices coating the helical ribonucleocapsid rather than coating the inner leaflet of the membrane as previously thought. As such we have uncovered two new potential areas for drug development, those that prevent uncoating of the ribonucleocapsid during entry, and conversely those that would prevent matrix-nucleocapsid interactions during assembly.

NMR Structural Biology at University of Patras & The SEE-DRUG project

Georgios A. SPYROULIAS

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Although the research in chemistry, biology and pharmacology is moving towards new horizons targeting complex biological systems, structural and dynamical views on individual proteins, key-players in biological pathways, is still a major challenge in structural biology. Biomolecular NMR Spectroscopy offers atomic-level insights into the conformational dynamics of biomolecules and biomolecular complexes. NMR is also unique in coupling structural information about biomolecular architecture in concert with data about the dynamical properties of the biomolecules, such as mobility, molecular interaction, chemical/conformational exchange, etc.

Our group at University of Patras, Pharmacy Department, exhibits a long-term expertise to the application of modern NMR techniques for the conformational dynamics and structure-activity correlation studies of peptides, proteins and their complexes. The activities of the group encompasses the protein production in suitable forms for NMR studies (uniform labeling in ^{2}H, ^{13}C, ^{15}N nuclei, and specific labeling of residues or chemical groups in ^{13}C, ^{15}N), the analysis of NMR data and the determination of the 3D structures and the relaxation properties of proteins with molecular weight ranging between 7 and 25 kDa.
Among our current research interests is the NMR-driven conformational dynamics study of disease-related enzymes and proteins, such as: (i) various E3 ubiquitin ligases involved in ubiquitination pathway, which are enzymes that catalyzes the ubiquitination of proteins targeted for destruction through the proteasome, (ii) extracellular domains of ligand-gated ion channels of the cys-loop family that involved in various neurodegenerative disorders, and (iii) Zn(II)-proteases that proteolyse signaling polypeptides regulating thus vital processes during the cell-cycle.

Recently, our group coordinated a NMR-oriented proposal, with other research teams from the Department of Pharmacy at University of Patras (UPAT) and the participation of groups from Medicine, Chemistry and Biology departments. This proposal has been successfully evaluated and granted with a FP7 Research Potential fund. The project aims to upgrade the Structural Biology capacities of UPAT and to the establishment of a Center of Excellence for Structure-Based Drug Design efforts in South-Eastern EU region (***SEE-DRUG project)***. Through this grant it is anticipated the installation of a **high-field 700 MHz, NMR** instrument in 2012, optimized for Biomolecular NMR applications. This instrument will be the highest NMR field in Greece, and one of the highest in the entire Eastern EU region.

ESPRIT: Library-based construct screening for difficult-to-express proteins

Dr Darren J. Hart

High Throughput Protein Technologies, EMBL, Grenoble, France.

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Expression of sufficient quantities of soluble protein for structural biology and other applications can be a difficult task, especially when multimilligram quantities are required. To improve yield, solubility or crystallisability of a protein, it is common to subclone shorter genetic constructs corresponding to single or multidomain fragments. However, it is not always clear where domain boundaries are located, especially when working on novel targets with little or no sequence similarity to other proteins. The ESPRIT (Expression of Soluble Proteins by Random Incremental Truncation) construct screening technology has been developed at EMBL to identify soluble constructs of "difficult-to-express" protein targets that resist the classical approach of bioinformatics and PCR cloning. In each experiment, 30,000 individual constructs are assayed in *E. coli* for yield and solubility using a highly automated colony array format. Here we show several examples to provide a view on its capabilities, plus recent developments for addressing protein complexes.

Virtual Screening – Practical session

Manos Mikros

University of Athens

Microseed it! A theoretical and Practical Exploration of Seed Stability and Seeding Techniques for Successful Protein Crystallization

Patrick Shaw Steward

Traditionally, microseeding has been used as an optimization step, i.e. seed crystals are dispensed or transferred into crystallization solutions that are similar to the solution that gave the original hit [e.g. 1]. A novel, more systematic approach, referred to as random Microseed Matrix Screening (rMMS), was introduced by Ireton and Stoddard [2]. This method was automated and further improved by D'Arcy *et al.*[3], who first seeded into commercial random screening kits. Experience has shown that rMMS used in this way not only produces more hits, it also generates better-diffracting crystals - because crystals are more likely to grow in the metastable zone [4]. Systematic users of the method report that it gives a useful improvement in about 75% of cases [5].

The theory and practice of the rMMS method will be described with case studies, and novel approaches to rMMS by Douglas Instruments [6] will also be presented, including using the method with protein complexes and predicting whether the seed stocks will be stable in various solutions. Other topics covered will include the scaling up of crystallization experiments from nano-drops to micro-drops.

References

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[2] Gregory Ireton and Barry Stoddard. 'Microseed matrix screening to improve crystals of yeast cytosine deaminase". Acta Crystallographica section D60 (2004), 601–605. Available on-line athttp://scripts.iucr.org/cgi-bin/paper?S0907444903029664
[3] Allan D'Arcy, Frederic Villarda, May Marsh. 'An automated microseed matrix-screening method for protein crystallization'. Acta Crystallographica section D63 (2007), 550–554. On-line at http://scripts.iucr.org/cgi-bin/paper?S0907444907007652
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[5] Personal communication, Paris Ward, Thomas Malia, Galina Obmolova, Allan D'Arcy.
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See also http://www.douglas.co.uk/mms.htm

From hot to cool and more for less.

Elspeth F. Garman

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Structural biology relies on X-ray crystallography to provide much of the three dimensional information on macromolecules that informs biological function. To enable problems not previously accessible to structure solution to be tackled, improved methods must be developed. A notable example of this has been the progress in finding protocols to cryocool protein crystals [1,2,3] prior to data 100K collection to reduce the rate of radiation damage by around a factor of 70 [4] compared to that at room temperature (RT): **from hot to cool and more for less.** Cryocooling is now routine in macromolecular crystallography and last year 97% of the new structures in the PDB were determined at 100 K. Much anecdote is attached to its use, with some folklore developing over the last 15 years about what does and does not work.

In this talk I will present methods to search for optimum protocols for cryocooling, point out some common pitfalls with it in practice, and suggest some strategies if nothing appears to work. I will also mention some aspects of radiation damage to the sample. This damage is an inherent problem when utilising ionising X-radiation in MX. It is now known that radiation damage can also be a limiting factor for MX at even 100K [for a review see 5] and mitigation strategies are being actively sought (e.g. [6]).

References

[1] Teng, TY. *Journal of Applied Crystallography* (1990) 23, 387
[2] Garman, EF & Schneider, TR *Journal of Applied Crystallography* (1997) 30, 211-237
[3] Garman, EF *Acta Cryst D* (1999) D55, 1641-1653
[4] Garman, EF & Nave, C *Journal of Synchrotron Radiation* (2005), 12, 257-260
[5] Garman, EF *Acta Cryst D* (2010) 66, 339-351
[6] De la Mora, E, Carmichael & Garman EF *Journal of Synchrotron Radiation* (2011) 18, 313-317

Optimise outcome from ligand soaking: consideration and results

Dr. George Kontopidis

Head of the biochemistry Laboratory, Faculty of Veterinary Medicine,
University of Thessaly, Greece

Protein-Peptide crystal complexes presented here were obtained through the novel technique of ligand exchange within protein crystals. This method may find general application for obtaining complex structures of proteins with surface-bound ligands.

References

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Free Energy Calculations in Ligand Protein-Binding Studies

Dr. Thomas Steinbrecher

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Cells contain a multitude of different chemical reaction paths running simultaneously and without interference next to each other. This amazing feat is enabled by molecular recognition, the ability of biomolecules to form stable and specific complexes with each other and with their substrates.

A better theoretical understanding of this process would be invaluable in the study of biological systems. In addition, as the mode of action of many pharmaceuticals is based upon their inhibition or activation of biomolecular targets, predictive models of ligand-receptor binding are important tools in rational drug design.

Therefore, the prediction of binding constants has always been one of the major goals in the field of computational chemistry. Among a varied set of binding energy prediction methods, Free Energy Calculations represent one of the most accurate, but also computationally expensive. While they are still not suited for large scale screenings, they are increasingly put to use for the detailed study of protein ligand interactions.

After a brief overview of other popular methods for the calculation of free energies, recent advances in methodology and exemplary studies of molecular dynamics simulation based free energy calculations are presented.

Structure and Fragment-Based Ligand Discovery Methods and Applications

Roderick E. Hubbard

YSBL, University of York and Vernalis (R&D) Ltd, UK

The past ten years has seen the increasing use of structural methods in support of drug discovery. Series of compounds are now entering the clinic which have been discovered using the methods, and they are becoming widespread amongst both industrial and academic drug discovery organisations.

In this lecture, I will review the principle methods used in Structure-Based Discovery. The computational methods are virtual screening and molecular docking. The experimental methods are X-ray crystallography and an increasing range of biophysical methods for probing protein-ligand interactions, including SPR, ITC, NMR and DSF. I will give a brief overview of the principles behind the methods and discuss our experiences with using them in a number of drug discovery campaigns. A particularly powerful variant of the approach is Fragment-Based Lead Discovery (FBLD). The central idea is to identify fragments (MW 120-250Da) that bind to the target from a small library (1000-2000 molecules), usually by biophysical techniques. Such libraries notionally represent a massive chemical space of much larger compounds and are more likely to contain cores that fit into the target binding site. Fragments are then evolved to larger compounds, either by growing the fragments to pick up additional interactions or linking or merging fragments together

Exploring Proteins with Peptide Phage Display

Dr. Michael Szardenings

Head Ligand Development Unit, Vice Head Department of Immunology
Fraunhofer Institute for Cell Therapy and Immunology (IZI), Germany

Peptide Phage display has been widely used to identify peptides that bind to proteins or the mapping of antibodies. In most cases the results of such approaches are hardly more than one or two good peptide ligands, although theoretically hundreds of different peptide structures could possibly bind the same site. Here as in many other cases the size of the libraries used for screening is only allowing to present a very small fraction of the possible variety of a randomized peptide sequence of given length. The use of libraries larger than many of those commercially or otherwise available is already resulting in more different peptide ligand than usual. The recombination of peptide genes allows obtaining results that would only be comparable to the application of libraries beyond 10E15 sequence variants, which cannot be generated for physical reasons. Examples are presented how the results of such phage display selections allow an improved understanding of the binding pockets. It would be desirable to use these results also for improving the modelling of critical drug targeting sites

Computational Design of Protein Kinase-Inhibiting Peptides and Peptidomimetics

Masha. Y. Niv

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Protein kinases constitute one of the largest gene families in the human genome and are major targets for drug discovery and development. Specific inhibition is crucial for elucidating the role of a kinase of interest in cell signaling and for developing novel drugs with low off-target reactivity. However, specificity is difficult to achieve via classical targeting of the ATP-binding site, which is common to all kinases. We therefore aim for non-ATP inhibitors, such as peptides and peptidomimetics [1].

The highly flexible nature of peptides presents a challenge for standard computational drug design tools. To overcome this challenge, we have developed a simulated annealing anchor-driven peptide docking protocol [2]. Anchoring points on the surface of the kinase catalytic domain were identified and used to rationalize the preference of kinases towards substrate consensus sequences3 and to provide novel insights into peptidomimetic structureactivity relations [4].

Several peptidomimetics were shown to specifically inhibit the target kinase *in-vitro* and *in-vivo*. The approaches presented here are applicable to other proteins and contribute to the computational toolbox for designing peptidic inhibitors of protein-protein interactions.

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Designing inhibitors for Rheumatoid arthritis. A "hit to lead" approach.

E. Eliopoulos[1], G. Kollias[2], G. Kontopidis[3], E.Couladouros[1], E. Douni[2,1], T. Papakyriakou[1], K. Alexiou[1], T.Thireou[1], D.Fimereli[1], V.Kostourou[2], V. Rinotas[1,2], P. Papadaki[2], A.Afantitis[2], M. Armaka[2], V. Ntougkos[2], V. Koliaraki[2], Ch. Papaneophytou[3], A. Mettou[3], M.Denis[4], N.Karagianni[4], S. Chaitidou[5], S. Gouma[5], I. Katsoulis[5], F. Liepouri[5], A.Strongilos[5]

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Rheumatoid arthritis (RA) is a devastating, chronic inflammatory disorder that affects approximately 1% of the worldwide population, and is characterized by persistent active inflammation with concurrent tissue destruction. TheRAlead project aims to exploit the **"hit to lead"** concept beginning from several validated **target** molecules involved in the pathogenesis of RA, to bridge the gap between target identification and pre-clinical validation exploiting a **"hit to lead" pipeline,** in an interdisciplinary approach consisting of structure based drug design, organic synthesis of designed derivatives, combinatorial chemistry for the development of small libraries of targeted bioactive compounds and pharmacophoric scaffolds, *in vitro* screening, cellular assays, *in silico* optimisation, and validation in animal models of arthritis. Targets include TNF/TNFR1 and RANKL.

Molecular modeling targeting glycogenolysis control

J.M. Hayes[1*], V.G. Tsirkone[1], V.T. Skamnaki[1], G. Archontis[2], S.E. Zographos[1], D. Komiotis[3], D.D. Leonidas[3]

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Type II diabetes is characterized by insulin resistance and the inability to control blood glucose levels. In order to treat this pathological condition, we have targeted enzymes that play a significant role in regulating blood glucose levels. Glycogenolysis involves the breakdown of glycogen towards glucose. Two key enzymes in this pathway are phosphorylase kinase (PhK) and glycogen phosphorylase (GP). Structure based drug design (SBDD) efforts in our laboratory targeting PhK and GP have therefore focused on compounds that might prevent unwanted glycogenolysis under high glucose conditions. An essential component in the design of new and effective inhibitors is the role of computation exploiting a multidisciplinary approach. An overview of some recent molecular modeling results for PhK and/or GP inhibition will be presented.

Exploring the exohedral reactivity and selective encapsulation of fullerene compounds

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Since fullerene discovering in 1985 by Kroto, Smalley and Curl, the interest on this new type of molecules has only grown because of their potential applications in medicine (e.g. as a drug transporter) and technological (interesting electronic properties) fields. But until now, about 25 years after their discovering, reactivity of fullerene compounds and their behavior are still largely unknown. The aim of this study is twofold. First, to shed some light on the reactivity of fullerene compounds by the computational study of the Diels-Alder cycloaddition reaction on the Ti2C2@-D3h-C78 metallofullerene. Second, to perform a computational study about host-guest interactions between recently synthesized 3D nanostructures and C60 fullerene to discuss whether these metalloporphyrin-based nanocages are able to selective encapsulate fullerene molecules.

Assembling Polynuclear Metal Complexes into Supramolecular Architectures

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Polynuclear metal complexes (clusters) based on paramagnetic metal ions continue to attract attention due to their fascinating structures and magnetic properties. Over the last years, polynuclear metal complexes have also emerged as building blocks for the construction of polymeric complexes known as Metal-Organic Frameworks (MOFs). Although only a handful of pre-isolated clusters have been utilized as building blocks for the construction of such materials, there have been developed some exceptional synthetic strategies which lead to cluster-based MOFs where the clusters present in the frameworks have been isolated in the past.

To this end, we are exploiting the possibility of utilizing pre-isolated polynuclear metal complexes as starting materials – building blocks for the construction of discrete and infinite supramolecular architectures [1-3]. The development of such a synthetic strategy forms the basis for constructing either discrete architectures (i.e. polygons, polyhedra) or polymeric complexes (i.e. coordination polymers, MOFs) which will carry the properties of the cluster-starting materials (e.g. magnetic, optical, etc.). Pre-isolated cluster-based MOFs, may also provide a convenient method to define size and function of the resulting cavities by replacing – modifying the intracluster ligands instead of modifying the ligands that bridge between the clusters. We will present here our attempts to assemble clusters with pre-determined structures and single-molecule magnets into discrete and infinite architectures.

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Nanoparticle-lipid bilayer interactions: Insights from Molecular Dynamics and free energy calculations.

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The increasing applications of nanotechnology in medicine rely on the fact that engineered nanomaterials, such as diagnostic and therapeutic nanoparticles (NPs) [1], will come in contact with human cells without damaging essential tissues. The entry point of an NP to a cell is the plasma membrane. Thus, the first step into assessing the NP cytotoxicity requires a thorough understanding of the NP-membrane interaction mechanism. In the present study, extensive coarse-grained Molecular Dynamics (CG-MD) simulations and free-energy calculations were employed [2-4], providing insights into the significance of NP surface chemistry and cholesterol concentration of the membrane in the NP-membrane interplay. Our CG-MD simulations show that a NP with an ordered distribution of hydrophobic and hydrophilic groups on its surface exhibits a low barrier for penetration of approximately 20 kTs and is able to translocate across the membrane with a direct mechanism without disrupting it. On the other hand, a NP with a random distribution of hydrophobic and hydrophilic groups features one strongly pronounced minimum at the center of the lipid bilayer, in agreement with our equilibrium MD simulations. PMF calculations [5] in a cholesterol-containing lipid bilayer showed that increase in the cholesterol concentration leads to a higher energy barrier of NP translocation across the membrane. In particular, this energy barrier is 55 kTs for cholesterol-free membranes or membranes with 10% cholesterol concentration, 128 kTs for 20% and 30% cholesterol concentration and 160 kTs for concentrations of 40% and 50%. Overall, the results of the present study provide valuable information on correlating NP surface characteristics with specific membrane interaction patterns and provide insights for the design of NPs with tailored functionalities, for example direct cellular entry. Moreover, our results indicate that cholesterol concentration plays a crucial role on NP-membrane interactions.

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Binding of Novel Fullerene Inhibitors to HIV-1 Protease: Insight through Molecular Dynamics and Molecular Mechanics Poisson–Boltzmann Surface Area Calculations

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The objective is the design of a series of novel fullerene-based inhibitors for HIV-1 protease (HIV-1 PR), by employing strategies that can also be applied to the design of inhibitors for any other target. Also analysis of the interactions which contribute to the observed exceptionally high binding free energies was performed. In particular, we investigated: (i) hydrogen bonding (H-bond) interactions between specific fullerene derivatives and the protease, (ii) the regions of HIV-1 PR that play a significant role in binding, (iii) protease changes upon binding and (iv) various contributions to the binding free energy, in order to identify the most significant of them. The techniques employed include docking, CoMFA/CoMSIA 3D-QSAR models, molecular dynamics (MD) simulations and the molecular mechanics Poisson–Boltzmann surface area (MM–PBSA) method. The computed binding free energies are in satisfactory agreement with the experimental results of anti-viral drugs employed for HIV therapy. Finally, the suitability of specific fullerene derivatives as drug candidates was further enhanced, by estimating ADMET (absorption, distribution, metabolism, excretion and toxicity) properties. The analysis revealed important protein-ligand interaction patterns that may lead towards the development of novel, potent HIV-1 PR inhibitors.

Rational Drug Design

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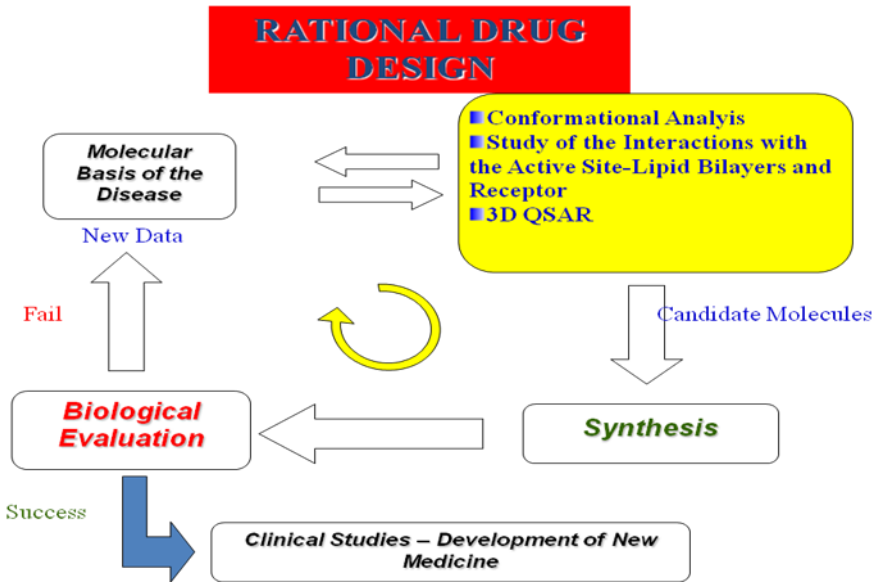
Rational design is applied in the discovery of novel lead drugs. Its rapid development is mainly attributed to the tremendous advancements in the computer science, statistics, molecular biology, biophysics, biochemistry, medicinal chemistry, pharmacokinetics and pharmacodynamics experienced in the last few decades. The promising feature that characterizes the application of rational drug design is that it uses for developing potential leads in drug discovery all known theoretical and experimental knowledge of the system under study. The utilization of the knowledge of the molecular basis of the system ultimately aims to reduce human power cost, time saving and laboratory expenses in the drug discovery.

Examples will be given from my research experience in which the limitations and advantages of the rational drug design will be outlined.



Molecular Dynamics and Binding Free Energy Calculations in Protein Systems: Advancements on Hypertension Treatment

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Aliskiren is the first orally active, direct renin inhibitor to be approved for the treatment of hypertension. Its structure elucidation and conformational analysis were discussed using random search and molecular dynamics (MD) simulations. For the first time, MD calculations of aliskiren have been also performed at the receptor site, in order to reveal its molecular basis of action. It is suggested that aliskiren binds in an extended conformation and is involved in several stabilizing hydrogen bonding interactions with active site (Asp32/255, Gly34) and other binding-cavity (Arg74, Ser76, Tyr14) residues. Of paramount importance is the finding of a loop consisting of residues around Ser76 that determines the entrapping of aliskiren into the active site of renin. Molecular mechanics Poisson-Boltzmann surface area (MM–PBSA) free energy calculations for the aliskiren-renin complex provided insight into the binding mode of aliskiren by identifying van der Waals and nonpolar contribution to solvation as the main components of favorable binding interactions.

Dithiolene Complexes: Properties and applications in material science and bioinorganic/biological chemistry.

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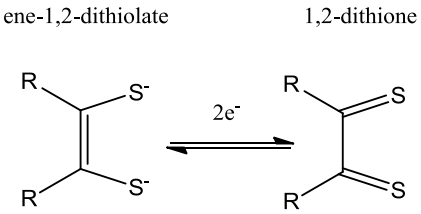
Dithiolene complexes display unusual structural, electronic photophysical, photochemical and reactivity features. Numerous metallo-dithiolenes have been synthesized and, depending on the choice of the transition metal and the nature of the dithiolene, possess lumophoric, magnetic and conducting properties. One of the most fascinating function of transition metal dithiolenes is their ability to catalyze a variety of formal oxygen atom transfer reactions in specific model systems and at the active sites of pyranopterin molybdenum and tungsten enzymes.

The numerous properties of metallo-dithiolene complexes are a direct consequence of a fantastic redox interplay between the redox active metal and the dithiolene ligand. A considerable amount of experimental evidence over the last forty years has pointed the fact that dithiolenes are highly noninnocent ligands. That is, they may be viewed in a valence bond description as existing somewhere between the extremes of neutral (dithione) and dianionic (dithiolate) forms, according to the following scheme.



Thus, determining the electronic structure of metallo-dithiolenes is pivotal in developing a detailed understanding in their role in bioinorganic chemistry as well as in material science. In this context, herein a combined experimental and theoretical study will be presented referring to some dithiolene complexes and their applications in diverse areas such as light energy conversion , non linear optics, DNA binding and anticancer activity.

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Desmond 3.0 Tutorial

Kolossvary, Istvan

D.E. Shaw Research, USA

The tutorial will include hands-on exercises in preparing structure files, simulating the system, and analyzing results. We will start with simple protein simulations as well as basic workflow issues, followed by configuring advanced options, preparing a membrane protein simulation, an example of FEP relative binding free energy calculation, and a metadynamics example. For interested participant we can arrange dedicated remote servers running Desmond interactively on the Amazon EC2 cloud.

Odorant binding protein-based screens for discovery of natural compounds effecting mosquito olfactory responses

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To identify mosquito repellents of natural origin, an *in vitro* binding competition assay was employed as screening tool for identification of essential oils containing ligand-like binding activities for specific odorant binding proteins (OBPs) of the mosquito malaria vector *Anopheles gambiae*. The binding competition assay monitored the displacement of a generic fluorescent marker, 1-NPN, from the binding pockets of a number of selected OBPs. Binding competition screens for an initial set of more than 100 essential oils (EOs), derived from terrestrial, mostly aromatic, plant specimens collected in Greece against 6 recombinant OBPs expressed at high levels and with a female bias in the antennae of *A. gambiae* revealed the presence of OBP ligand-like compounds in ~60% of the examined oils. EOs containing OBP-binding activities were also subjected to behavioral screens for the presence of mosquito repellent and/or attractant bioactivities, and oils displaying repellent or attractant properties were identified and selected for further studies. Analysis of a limited number of bioactive oils by gas chromatography (GC)-coupled electroantennography and mass spectroscopy (MS) led to the identification of several compounds displaying strong mosquito repellent activities, which could conceivably be developed into control measures for the mosquito vector, as well as others displaying weaker repellent or some attractant activities. Interestingly, several simple derivatives of a natural compound displaying weak repellent activity were found to be stronger repellents than the parental compound. Finally, to deduce whether the strength of behavior-modifying, repellent or attractant, activity may be correlated with OBP binding strength, we tested several representative compounds with some of the selected OBPs in the 1-NPN binding competition assay. These studies revealed that most identified ligands were bound by the selected OBPs with a lower affinity than 1-NPN, a finding consistent with conclusions drawn from the analysis of crystallographic data from a limited number of mosquito OBPs. Therefore, although OBP binding may be used as an indicator for the possible presence of olfaction-related bioactive compounds in complex mixtures, strength of binding of specific ligands by specific OBPs cannot be a predictive criterion for the magnitude of the ensuing physiological and bahavioral responses, which are probably determined by cognate olfactory receptors (ORs) residing in the same olfactory sensilla as some of the cognate OBPs.

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Direct insecticide binding to malaria mosquito GSTs

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Malaria kills more than 1 million people (mostly children) worldwide. Preventing the rapidly growing resistance of malaria mosquitoes to various insecticides is essential in ensuring effective vector control programs. Together with other defense proteins, mosquito glutathione S-transferases (GSTs) are implicated in conferring insecticide resistance, via metabolism or sequestration. However, the exact role of GSTs in this process has remained elusive, in particular their physical interaction with insecticide molecules.

I will present data from a preliminary assessment of direct insecticide binding to resistance-related GST isoforms from *Anopheles dirus* and *Anopheles gambiae*, the main malaria vectors in SA-Asia and Africa respectively. Representative insecticides of three chemical classes (pyrethroids, organophosphates and organochlorinates) were screened, using two technologies, Surface Plasmon Resonance (SPR) and Differential Scanning Fluorimetry (DSF). Interpretation of the data shows differential selectivity, end-point affinities and binding kinetics for the various insecticide classes, suggesting a remarkable adaptability in insecticide recognition by GSTs across mosquito species, characterized for the first time.

Novel targets as pharmacological tools for the development of new therapeutic strategies for the coronary heart disease.

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Despite current optimal treatment, the morbidity and mortality of coronary heart disease remain significant worldwide. In the last two decades, a remarkable scientific effort has focused on the limitation of infarct size. Important input from experimental studies has led the way in this direction. However, clinical and preclinical results using various cardioprotective strategies to attenuate reperfusion injury have generally not been applicable for every day clinical practice. Protection of the ischemic myocardium is known to occur as a result of ischemic preconditioning (PC), in which repetitive brief periods of ischemia protect the heart from a subsequent prolong ischemic insult. Although PC is a powerful form of protection, it is of limited clinical application for obvious ethical and practical reasons. Another endogenous form of cardioprotection, termed postconditioning (PostC), has been currently described. Short series of repetitive cycles of brief reperfusion and re-occlusion of the coronary artery applied at the onset of reperfusion, reduce the infarct size and coronary artery endothelial dysfunction. The mechanisms of PC and PostC are common and the whole procedure targets lethal reperfusion injury by reducing oxidative stress, decreasing intracellular Ca^{2+} overload, delaying the restoration of neutral pH, and reducing neutrophil accumulation, hence attenuating apoptotic cardiomyocyte death and improving endothelial function. All those effects are accomplished by the activation of specific RISK kinases, PI3, Akt and ERKs, which prevent the mitochondrial PTP opening. Several studies concern the role of glycogen- synthase- kinase 3β (GSK3-β) as a common target proximal to mPTP opening. We summarize the recent research efforts on novel therapeutic strategies and on the design of new compounds based on the knowledge of the ligands, receptors, and intracellular signaling pathways of PC and PostC.

Hydrogen sulfide a novel signalling molecule in mammalian cells

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University of Patras

Geometric algorithms for modeling molecular structure

Ioannis Emiris

University of Athens

We discuss efficient computational methods for expressing and studying 3D structure, namely rigid sub-structures and degrees of freedom between them. Our main tools model distances and angles, and are particularly suited for NMR data, such as those obtained from NOE and RDC experiments. We mention applications in modeling transmembrane proteins, as well as in conformational search.

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