SPANISH NATIONAL
CANCER RESEARCH CENTRE

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MEETINGS 2012

# 17–19 SEPT 2012
# ALLOSTERIC REGULATION OF CELL SIGNALLING


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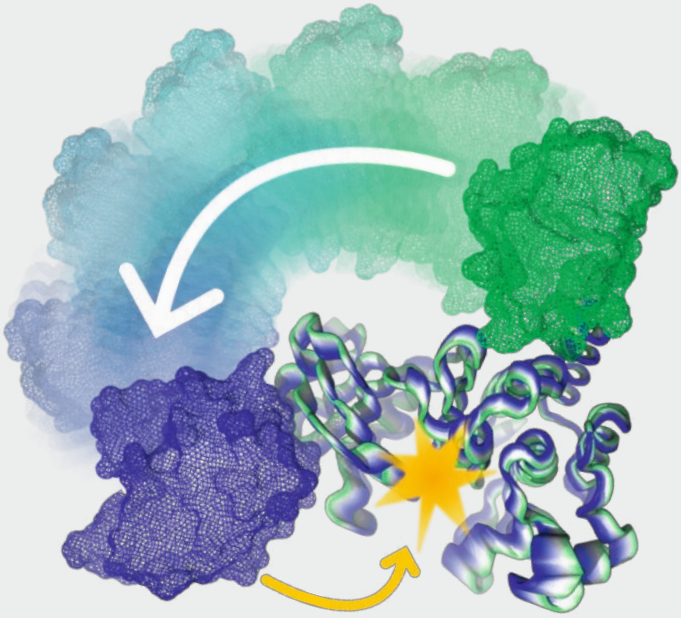

MADRID 17–19 SEPTEMBER 2012

# ALLOSTERIC REGULATION OF CELL SIGNALLING

# SUMMARY

ALLOSTERIC REGULATION OF CELL SIGNALLING

# DETAILED PROGRAMME

# MONDAY, SEPTEMBER 17TH

**09:00**
WELCOME ADDRESS

**09:20**
KEYNOTE SPEAKER:
INTRODUCTION TO ALLOSTERY.
ALLOSTERIC MECHANISMS OF SIGNAL TRANDUCTION: AN INTRODUCTION
**Jean-Pierre Changeux**, Collège de France, Pasteur Institute, Paris, France

## SESSION I
## ALLOSTERIC SIGNALLING MECHANISMS
Chair **Ermanno Gherardi** and **Daniel Lietha**

**10:20**
HORMONE-MEDIATED ACTIVATION OF G PROTEINS BY GPCRs: INSIGHTS FROM THE CRYSTAL STRUCTURE OF A GPCR-G PROTEIN COMPLEX
**Roger Sunahara**, University of Michigan Medical School, Ann Arbor, USA

**10:55**
COFFEE BREAK & BEGINNING OF POSTER SESSION

**11:15**
STRUCTURAL BASIS FOR RELAY OF ALLOSTERY IN I INTEGRINS
**Timothy A. Springer**, Harvard Medical School, Boston, USA

**11:50**
SURFACE ASSEMBLIES IN CELL GUIDANCE SIGNALLING SYSTEMS
**Yvonne Jones**, Wellcome Trust Centre for Human Genetics, University of Oxford, UK

**12:25** CONTRIBUTED TALK
ALLOSTERIC CONVERSATION IN THE HUMAN ANDROGEN RECEPTOR LIGAND-BINDING DOMAIN SURFACES
**Eva Estébanez-Perpina**, Universidad de Barcelona, Spain

**12:50**
LUNCH AND POSTER SESSION

**14:20**
DESIGNING THERAPEUTICS THAT MODULATE MULTIPROTEIN REGULATORY SYSTEMS: ALLO-TARGETING
**Tom L. Blundell**, University of Cambridge, UK

NOTE
TALKS: 30 MINUTES / SHORT TALKS: 20 MINUTES
DISCUSSION: 5 MINUTES AFTER EACH TALK /
5 MINUTES AFTER EACH SHORT TALK

**14:55**
ALLOSTERIC CONTROL OF LIPIDATED PROTEIN TRAFFIC BY THE GTP-BINDING PROTEINS Arl2 AND Arl3
**Alfred Wittinghofer**, MPI for Molecular Physiology, Dortmund, Germany

**15:30** CONTRIBUTED TALK
CRYSTAL STRUCTURE AND MECHANISM OF ACTIVATION OF TBK1
**Daniel Panne**, EMBL, Grenoble, France

**15:55**
COFFEE BREAK & POSTER SESSION

**16:15**
PKA: ASSEMBLY OF DYNAMIC MACROMOLECULAR SIGNALING COMPLEXES
**Susan S. Taylor**, Howard Hughes Medical Institute, University of California, San Diego, USA

**16:50**
ALLOSTERIC REGULATION OF OLIGOMERIC ASSEMBLY: THE ROLE OF PIP2 IN ACTIVATION OF FOCAL ADHESION KINASE
**Daniel Lietha**, CNIO, Madrid, Spain

**17:15** CONTRIBUTED TALK
FROM THE MOLECULAR MECHANISM OF REGULATION OF AGC KINASES TO THE DEVELOPMENT OF ALLOSTERIC ACTIVATORS AND ALLOSTERIC INHIBITORS
**Ricardo Biondi**, Frankfurt University Hospital, Frankfurt am Main, Germany

**17:40** CONTRIBUTED TALK
THE STRUCTURE OF THE SECOND PAIR OF FIBRONECTIN TYPE III REPEATS OF THE INTEGRIN β4 SUBUNIT SUGGESTS A MECHANISM FOR AN INTRAMOLECULAR INTERACTION
**José M. de Pereda**, Instituto de Biología Molecular y Celular del Cáncer, Salamanca, Spain

**18:05** CONTRIBUTED TALK
COORDINATION OF CRITICAL CYTOSKELETAL CHANGES BY NCK ENABLES ENDOTHELIAL CELL POLARIZATION AND MORPHOGENESIS
**Gonzalo M. Rivera**, Texas A&M University, College Station, USA

**18:30**
POSTER SESSION

# TUESDAY, SEPTEMBER 18TH

**SESSION II**
**DYNAMICS AND MODELLING OF ALLOSTERIC SYSTEMS**
Chair **Francesco Luigi Gervasio** and **Alfonso Valencia**

**09:00**
THE INS AND OUTS OF EGFR ASYMMETRY
**Daniel Leahy**, Johns Hopkins University School of Medicine, Baltimore, USA

**09:35**
CONFORMATIONAL DYNAMICS IN MULTIDOMAIN KINASES, KINASE-PHOSPHATE COMPLEXES, AND ESCRT-PROTEIN SUPERCOMPLEXES
**Gerhard Hummer**, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, USA

**10:10**
GROUP PICTURE AND COFFEE BREAK

**10:40**
ALLOSTERY AND CONFORMATIONAL CONTROL IN SIGNALING: THE UBIQUITIN E3 LIGASES
**Ruth Nussinov**, National Cancer Institute and Tel Aviv University, Israel

**11:15**
CONTROLLING ALLOSTERIC NETWORKS IN PROTEINS
**Nikolay V. Dokholyan**, University of North Carolina at Chapel Hill, USA

**11:50**
EXPLORING THE LANDSCAPE FOR PROTEINS AND THE RIBOSOME: CONNECTING SIMPLE MODELS AND DETAILED SIMULATIONS
**Jose Onuchic**, Center for Theoretical Biological Physics, Rice University, Houston, USA

**12:25** CONTRIBUTED TALK
INTRINSIC DISORDER IN WILD-TYPE EGFR KINASE UNDERLIES ONCOGENICITY OF CANCER MUTATIONS
**Yibing Shan**, D. E. Shaw Research, USA

**12:50**
LUNCH AND POSTER SESSION

**14:20**
PROTEINS IN FLAGRANTE: EXCURSIONS IN SILICO AND IN PROTEO
**Dorothee Kern**, Howard Hughes Medical Institute, Brandeis University, Waltham, USA

**14:55**
DYNAMICAL EFFECTS IN THE ALLOSTERIC CONTROL OF KINASES
**Francesco Luigi Gervasio**, CNIO, Madrid, Spain

**15:20**
CO-EVOLUTION BASED METHODS IN THE PREDICTION OF ALLOSTERIC CHANNELS AND CONTACT NETWORKS
**Alfonso Valencia**, CNIO, Madrid, Spain

**15:45**
COFFEE BREAK AND POSTER SESSION

**16:05**
THE INNATE IMMUNITY MOLECULAR MACHINERY
**Giulio Superti-Furga**, CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

**16:40**
CRYSTAL STRUCTURE OF THE 1 MDa MAMMALIAN TRiC/CCT COMPLEX WITH TUBULIN
**Guillermo Montoya**, CNIO, Madrid, Spain

**17:05** CONTRIBUTED TALK
IMPACT OF MUTATIONS ON THE ALLOSTERIC CONFORMATIONAL EQUILIBRIUM
**Patrick Weinkam**, University of California, San Francisco, USA

**17:30** CONTRIBUTED TALK
CHARACTERIZING PROTEIN CONFORMATION CHANGES THROUGH TORSIONAL LINEAR RESPONSE AND ALLOSTERIC PROFILES
**Ugo Bastolla**, Centro de Biologia Molecular Severo Ochoa CSIC-UAM, Madrid, Spain

**17:55** CONTRIBUTED TALK
ONCOGENIC MUTATIONS CORRUPT THE ORDERED AUTOPHOSPHORYLATION OF THE RET RECEPTOR TYROSINE KINASE: STRUCTURAL AND MOLECULAR BASIS OF RET-MEN2B
**Ivan Plaza Menacho**, London Research Institute, Cancer Research UK, London, UK

**18:20**
POSTER SESSION

# WEDNESDAY, SEPTEMBER 19TH

**SESSION III**
**ALLOSTERIC INHIBITION**
Chair **Giulio Superti-Furga**

**09:00**
STRUCTURAL INSIGHTS INTO THE MECHANISM JAK KINASE ACTIVATION IN MYELOPROLIFERATIVE DISEASE
**Michael J. Eck**, Dana-Farber Cancer Institute, Harvard Medical School, USA

**09:35**
ALLOSTERIC REGULATION OF INTRACELLULAR AND MEMBRANE PROTEINS BY DARPins
**Markus G. Grütter**, University of Zurich, Switzerland

**10:10**
ELUCIDATION OF THE MOLECULAR MODE OF ACTION OF SSR128129E, THE FIRST SMALL MOLECULE ALLOSTERIC INHIBITOR OF FGF RECEPTOR SIGNALING
**Françoise Bono**, Sanofi R&D, Toulouse, France

**10:45**
COFFEE BREAK

**11:05**
ALLOSTERIC KINASE INHIBITION
**Jeffrey R. Peterson**, Fox Chase Cancer Center, Philadelphia, USA

**11:40**
NON-ATP COMPETITIVE KINASE INHIBITORS: POTENTIALS AND LIMITATIONS
**Doriano Fabbro**, Novartis Pharma AG, Basel, Switzerland

**12:15**
REGULATION OF HGF/SF-MET SIGNALLING
**Ermanno Gherardi**, MRC Laboratory of Molecular Biology, Cambridge, UK

**12:50** **CONTRIBUTED TALK**
IDENTIFICATION AND CHARACTERISATION OF NOVEL ALLOSTERIC REGULATORS OF PKM2 USING FRAGMENT BASED SCREENING
**Marc O'Reilly**, Astex Pharmaceuticals, Cambridge, UK

**13:15**
Final Remarks

# KEYNOTE SPEAKER: INTRODUCTION TO ALLOSTERY. ALLOSTERIC MECHANISMS OF SIGNAL TRANDUCTION: AN INTRODUCTION

**Jean-Pierre Changeux** Collège de France, Pasteur Institute, Paris, France

Exactly 50 years ago, biochemists raised the question of the mechanism of the conformational change that mediates "allosteric" interactions between regulatory sites and biologically active sites in regulatory/receptor proteins. Do the different conformations involved already exist spontaneously in the absence of the regulatory ligands (Monod-Wyman-Changeux), such that the complementary protein conformation would be selected to mediate signal transduction, or do particular ligands induce the receptor to adopt the conformation best suited to them (Koshland-Nemethy-Filmer-induced fit)? This is not just a central question for biophysics, it also has enormous importance for drug design. Recent advances in techniques have allowed detailed experimental and theoretical comparisons with the formal models of both scenarios. Also, it has been shown that mutated receptors can adopt constitutively active confirmations in the absence of ligand. There have also been demonstrations that the atomic resolution structures of the same protein are essentially the same whether ligand is bound or not. These and other advances in past decades have produced a situation where the vast majority of the data using different categories of regulatory proteins (including regulatory enzymes, ligand-gated ion channels, G protein-coupled receptors, and nuclear receptors) support the conformational selection scheme of signal transduction.

ALLOSTERIC REGULATION OF CELL SIGNALLING

# SESSION I. ALLOSTERIC SIGNALLING MECHANISMS

SESSION I

# ALLOSTERIC SIGNALLING MECHANISMS

CHAIR: ERMANNO GHERARDI AND DANIEL LIETHA

## HORMONE-MEDIATED ACTIVATION OF G PROTEINS BY GPCRs: INSIGHTS FROM THE CRYSTAL STRUCTURE OF A GPCR-G PROTEIN COMPLEX

**Roger K. Sunahara** University of Michigan Medical School, Ann Arbor, USA

Recent advances in the structural biology of G protein-coupled receptors have helped to unravel the intricacies of ligand binding. Similarly, structural and biochemical analyses of heterotrimeric G proteins have affirmed our understanding of the mechanism underlying effector interactions and GTPase activity. We recently elucidated the crystal structure of a prototypic GPCR, the $\beta_2$-adrenergic receptor ($\beta_2$AR), in a complex with the stimulatory G protein, Gs, trapped in its nucleotide-free state. These data have helped us to understand how hormone binding to GPCRs leads to GDP release on G proteins, the principle step that precedes in GTP binding and G protein activation. The crystal structure, together with data from electron microscopy and deuterium exchange mass spectrometry, reveal dramatic changes in the G protein $\alpha$-subunit. The crystal structure also suggests that G proteins may allosterically regulate GPCRs by stabilizing a closed conformation on the extracellular face of the receptor. Consistent with these changes radioligand binding analyses suggest that G protein coupling slows ligand dissociation. These structural changes would account for the slower observed dissociation rates of agonists and likely account for their G protein-dependent high affinity binding. Cumulatively these data support a model for the mechanism for receptor-mediated nucleotide exchange, G protein activation and agonist binding.

# STRUCTURAL BASIS FOR RELAY OF ALLOSTERY IN αI INTEGRINS

**Timothy A. Springer** Harvard Medical School, Boston, USA

Integrins are complex adhesion receptors with 12 or 13 extracellular domains in their transmembrane α- and β-subunits. Half of integrin α-subunits (αI integrins) contain a ligand-binding αI domain that is inserted in their β-propeller domain, whereas αI-less integrins bind ligand at the interface between the α-subunit β-propeller domain and the βI domain. Large rearrangements in integrin ectodomains transmit signals between the membrane and the membrane-distal, ligand-binding domains. Despite hypothesized mechanisms, how allostery is relayed to integrin αI domains remains unknown. Here, we capture the relay mechanism in an $\alpha_X\beta_2$ integrin crystal structure. Complete activation of αI (open conformation) is relayed by deformation and movement of its C-terminal α7-helix toward the βI domain metal ion-dependent adhesion site (MIDAS). An invariant Glu immediately following the α7-helix moves 26 Å from its position in the closed αI domain to directly coordinate to the βI MIDAS $Mg^{2+}$. Surprisingly, the α7-helix substantially unwinds, and its C-terminal portion with conserved hydrophobic residues loses all contact with the αI domain and completely restructures to form an internal ligand. This internal ligand binds to a hydrophobic pocket formed by the $\alpha_X$ β-propeller domain and the specificity-determining and metal-binding loops of the $\beta_2$βI domain. The MIDAS touch captured here in bent $\alpha_X\beta_2$ relates to headpiece opening and extension in $\alpha_X\beta_2$ on cell surfaces, as shown by effect of mutations on ligand binding and exposure of conformation-specific epitopes.



# SURFACE ASSEMBLIES IN CELL GUIDANCE SIGNALLING SYSTEMS

**E. Yvonne Jones** Wellcome Trust Centre for Human Genetics, University of Oxford, UK

The molecular mechanisms that underpin cell-cell communication depend on receptors embedded in the plasma membrane of the cell. Many of these receptors have extracellular and cytoplasmic regions linked by a single membrane spanning helix. Crystallographic and biophysical analyses of the extracellular architecture and recognition complexes of such receptors have provided a wealth of detail on the structural determinants of ligand binding affinities and specificity. However, for many systems it is increasingly apparent that the mechanisms controlling signalling involve multiple interactions within assemblies of receptors. To analyse such systems we need to draw on an increasingly broad range of methodologies which extend from high resolution crystallographic studies to imaging the behaviour of molecules in the context of the cell. I will present some examples of the progress we are making in the study of cell surface interaction assemblies involved in the balance between cell guidance and adhesion, specifically drawing on examples from the ephrin/Eph and semaphorin/plexin families of cell guidance cues.

CONTRIBUTED TALK

## ALLOSTERIC CONVERSATION IN THE HUMAN ANDROGEN RECEPTOR LIGAND-BINDING DOMAIN SURFACES

**Eva Estébanez-Perpina** Universidad de Barcelona, Barcelona, Spain

Androgen receptor (AR) is a major therapeutic target that plays pivotal roles in prostate cancer (PCa) and androgen insensitivity syndromes.Wepreviously proposed that compounds recruited to ligand-binding domain (LBD) surfaces could regulate AR activity in hormone-refractory PCa and discovered several surface modulators of AR function. Surprisingly, the most effective compounds bound preferentially to a surface of unknown function [binding function 3 (BF-3)] instead of the coactivator-binding site [activation function 2 (AF-2)]. Different BF-3 mutations have been identified in PCa or androgen insensitivity syndrome patients, and they can strongly affect AR activity. Further, comparison of AR x-ray structures with and without bound ligands at BF-3 and AF-2 showed structural coupling between both pockets. Here, we combine experimental evidence and molecular dynamic simulations to investigate whether BF-3 mutations affect AR LBD function and dynamics possibly via allosteric conversation between surface sites. Our data indicate that AF-2 conformation is indeed closely coupled to BF-3 and provide mechanistic proof of their structural interconnection. BF-3 mutations may function as allosteric elicitors, probably shifting the AR LBD conformational ensemble toward conformations that alter AF-2 propensity to reorganize into subpockets that accommodate N-terminal domain and coactivator peptides. The induced conformation may result in either increased or decreased AR activity. Activating BF-3 mutations also favor the formation of another pocket (BF-4) in the vicinity of AF-2 and BF-3, which we also previously identified as a hot spot for a small compound. We discuss the possibility that BF-3 may be a protein-docking site that binds to the N-terminal domain and corepressors. AR surface sites are attractive pharmacological targets to develop allosteric modulators that might be alternative lead compounds for drug design.

· Mol Endocrinol. 2012 Jul;26(7):1078-90. Epub 2012 May 31.

## DESIGNING THERAPEUTICS THAT MODULATE MULTIPROTEIN REGULATORY SYSTEMS: ALLO-TARGETING

**Tom L Blundell** University of Cambridge, UK

Detailed structural knowledge of the interactions between molecules in the cell —the structural interactome— can contribute to understanding of cellular networks. It can also facilitate the selection of targets and the design of candidate therapeutics that modulate protein-protein interactions. Such molecules may target allosteric sites and mediate their actions through conformation change or electrostatics, or directly modulate protein-protein interactions involved in colocation of regulatory components; we class both as allotargeting.

I will describe databases that enable the utilisation of structural information on protein-molecule interactions. I will focus on the use of fragment-based approaches to target protein-protein interactions in order to make chemical tools and candidate therapeutic molecules. Topics will include (i) the human recombinase, Rad51, interactions with BRCA2, inhibitors of which would be helpful in modulating DNA repair during chemo— or radiotherapy and (ii) the Met receptor interaction with HGF/SF, important in regulating metastasis.

# ALLOSTERIC CONTROL OF LIPIDATED PROTEIN TRAFFIC BY THE GTP-BINDING PROTEINS Arl2 AND Arl3

**Alfred Wittinghofer** Max-Planck-Institute for molecular Physiology, Dortmund, Germany

GTP-binding (G) proteins are molecular switches that cycle between a GDP-bound OFF and a GTP-bound ON state and are regulated by Guanine nucleotide exchange factors (GEFs) and GTPase Activating Proteins (GAPs). Arl2 and 3 are members of the Arf subfamily of the Ras superfamily of G proteins. Arl3 has been implicated in the function of cilia, while not much is known about the function of Arl2. In the GTP-bound form Arl2/3 interact with a variety of proteins called downstream effectors. These are the delta subunit of phosphodiesterase PDEδ, HRG4 (human retina gene 4) also called Unc119a, which is mutated in certain rod/cone diseases, and various other proteins such as BART (Binder of Arl2).

By solving the structures of PDEδ and HRG4 and their complexes with Arl we have shown that they have a β-sandwich structure with a large hydrophobic pocket. The pocket of PDEδ is specific for farnesylated C-terminal and of HRG4 for myristoylated proteins. We have shown how the binding of cargo is allosterically regulated by Arl2 and Arl3. The specificities of interactions and the biological function will be discussed.

**Keywords**: Photoreceptor, Arl, PDEδ, HRG4



CONTRIBUTED TALK

# CRYSTAL STRUCTURE AND MECHANISM OF ACTIVATION OF TBK1

**Daniel Panne** EMBL, Grenoble, France

Members of the IKK family of kinases play a central role in the innate immune system by integrating signals from pattern recognition receptors and by mediating regulation of the inflammatory, immune and apoptotic responses. The IKK kinase family contains two canonical family members IKKα, IKKβ and two non-canonical family members IKKε and TBK1. TBK1 is a major target for drug development with applications in the treatment of cancer and a variety of inflammatory diseases including rheumatoid arthritis and obesity-related metabolic disorders. We have determined the 2.6 Å X-ray crystal structure of close to full-length TBK1 in complex with specific inhibitors. The structure reveals a dimer with a trimodular architecture containing the kinase domain (KD) a Ubiquiting-like domain (ULD) and a Scaffold dimerization domain (SDD).

The ULD packs against a hydrophobic patch at the base of the KD C-lobe and is involved in allosteric regulation of kinase activity. The kinase we have crystallized is in an inactive, autoinhibited conformation.

Comparison with the activated form shows the requirements for kinase activation. We also have analyzed the structure in solution by SAXS and determined positioning of the missing C-terminal domain. The TBK1 structure together with functional analysis indicates that dimerization is required for kinase activation. However, as such TBK1 dimers cannot autoactivate in cis, we propose that activation occurs when multiple

TBK1 dimers are brought together into higher-order signaling assemblies.

Polyubiquitination has been repeatedly associated with IKK kinase activation and one hypothesis is that polyubiquitin binding to TBK1-associated scaffold proteins triggers higher-order assembly and kinase activation. The structure described here will allow development of more specific inhibitors that might find application as anti-cancer or anti-inflammatory drugs.

# PKA: ASSEMBLY OF DYNAMIC MACROMOLECULAR SIGNALING COMPLEXES

**Susan S. Taylor**, P. Zhang, R. Ilouz, J. Wu, M. Keshwani, and A. Kornev Howard Hughes Medical Institute, University of California, San Diego, USA

cAMP-dependent protein kinase (PKA), ubiquitous in every mammalian cell, regulates a plethora of biological processes. While the PKA catalytic (C) subunit is the best understood protein kinase and serves in many ways as a prototype for the entire superfamily, the in cells it is packaged as holoenzyme with regulatory (R) subunits so that the activity of PKA is fully dependent on the second messenger, cAMP. There are four functionally non-redundant R-subunits (RIα, RIβ, RIIα, and RIIβ), and all have a conserved domain organization with a dimerization/docking (D/D) domain at the N-terminus followed by a flexible linker that contains an inhibitor site and finally at the C-terminus two tandem cyclic nucleotide binding (CNB) domains. In the absence of cAMP each R-subunit dimer is bound to two C-subunits creating and inactive tetramer. Specificity in PKA signaling is determined by the isoform diversity of the R-subunits and by targeting, which is typically mediated by A Kinase Anchoring Proteins (AKAPs), which bind through an amphipathic helix to the D/D domains of the R-subunits. In this way specific PKA holoenzymes are localized in close proximity to dedicated substrates. The functional non-redundancy of the R-subunits was not understood in molecular terms until structures of tetrameric holoenzymes were solved. Although the 1°, 2°, and 3° structures of all four isoforms are very similar, the quaternary architecture of each $R_2C_2$ tetramer is remarkably different. Formation of each tetramer creates a novel 2-fold symmetry and defines distinct isoform-specific mechanisms for allosteric regulation. (Supported in part by grants from the National Institutes of Health (GM19301, GM34921, and DK54441).

# ALLOSTERIC REGULATION OF OLIGOMERIC ASSEMBLY: THE ROLE OF $PIP_2$ IN ACTIVATION OF FOCAL ADHESION KINASE

**Daniel Lietha** CNIO, Madrid, Spain

Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase which is activated downstream of growth factor receptor and integrin signalling. FAK is believed to be the key signalling component integrating growth and adhesion signals. FAK is essential during embryogenesis and wound healing and in pathology FAK is frequently upregulated in many types of tumours and its upregulation correlates with tumour invasion and poor patent prognosis. We have previously structurally and biochemically defined the mode of FAK autoinhibition. In the autoinhibited state the N-terminal regulatory FERM domain of FAK intramolecularly interacts with the kinase domain, thereby promoting an “off-state”. As observed for other non-receptor tyrosine kinases, it was proposed that allosteric binding of activators to the regulatory domain would switch FAK to an “on-state”. Recently we identified the phosphoinositide PIP2 as a potential FAK activator. Indeed, $PIP_2$ does interact with the regulatory domain of FAK, however, the mechanism of promoting FAK activation appears to be quite unique. We show that $PIP_2$ enhances FAK autophosphorylation on a linker site via promoting the formation of FAK clusters. Supported by several lines of experiments we propose a model where in $PIP_2$ induced clusters FAK adopts an intermediate conformation where the inhibitory FERM-kinase interaction is partially maintained, but trans-autophosphorylation is promoted. Only in a subsequent step, which involves phosphorylation of the FAK kinase domain by Src, is FAK switched to a fully open conformation. Such a mechanism, where ligand binding allosterically induces a change in the oligomeric state, conceptually resembles ligand induced activation of receptor tyrosine kinases, but has not been described previously for non-receptor tyrosine kinases.

CONTRIBUTED TALK

# FROM THE MOLECULAR MECHANISM OF REGULATION OF AGC KINASES TO THE DEVELOPMENT OF ALLOSTERIC ACTIVATORS AND ALLOSTERIC INHIBITORS

**Ricardo Biondi** Frankfurt University Hospital, Frankfurt am Main, Germany

The group of AGC protein kinases includes more than 60 protein kinases in the human genome (e.g. PDK1 and isoforms of PKA, PKC, PRK, PKB/Akt, S6K, SGK, RSK, MSK, ROCK, GRK, amongst others). Human AGC kinases are involved in diverse cellular functions and are potential targets for the treatment of cancer, diabetes, obesity, neurological disorders, inflammation, viral infections, etc. Most drugs to protein kinases target the ATP-binding site, that is conserved and therefore most inhibitors to date are non-selective. Targeting non-ATP binding sites can potentially achieve high degree of selectivity that could allow protein kinase inhibitors to be used in long term treatments. We identified that a particular pocket on the kinase domain, termed the PIF-pocket, is a key mediator of the allosteric regulation of many AGC kinases. We first described the regulatory properties of the PIF-pocket on PDK1. Follow-up work verified that the PIF-pocket is also the key site that mediates the activation by Zipper/turn-motif- and hydrophobic motif- phosphorylation of PKB/Akt, S6K, RSK, SGK, PKC, PRK, and MSK. Interaction with the PIF-pocket also regulates the intramolecular inhibition of atypical PKCs by their N-terminal domains and the oligomerization that inhibits PRK2 [6]. We have developed reversible low-molecular-weight compounds that, by targeting the PIF-pocket site, allosterically activate PDK1, or allosterically inhibit atypical PKCs. As a prototype for the group of AGC kinases we showed in PDK1 that small compounds binding to the PIF-pocket produce allosteric effects that ultimately affect the ATP-binding site. Together, our work shows that the PIFpocket on AGC kinases is an allosteric regulatory site that can be targeted by small molecules to produce pharmacological activators, inhibitors and substrate-selective inhibitors of AGC kinases.



CONTRIBUTED TALK

# THE STRUCTURE OF THE SECOND PAIR OF FIBRONECTIN TYPE III REPEATS OF THE INTEGRIN β4 SUBUNIT SUGGESTS A MECHANISM FOR AN INTRAMOLECULAR INTERACTION

**José M de Pereda** Instituto de Biología Molecular y Celular del Cáncer, Salamanca, Spain

The integrin α6β4 is a component of the hemidesmosomes, protein complexes that mediate the stable anchoring of basal epithelial cells to the basement membrane. The cytodomain of β4 is unique among the integrin family and it is responsible for most of the intracellular interactions of α6β4. The cytoplasmic region of β4 contains a Calx-β domain and four fibronectin type III domains (FnIII1 to FnIII4) arranged in two pairs separated by a connecting segment (CS). A 90-residue long C-terminal tail extends after the FnIII4. An interaction between the CS and the C-tail has been observed by yeast-2-hybrid and blot overlay assays, and these two regions of β4 are in close proximity in living keratinocytes. We have combined x-ray crystallography, small angle x-ray scattering (SAXS), mutagenesis, and electron paramagnetic resonance spectroscopy (EPR) to characterize the FnIII-3,4 region of β4. The crystal structure of the FnIII3 was refined against data to 1.6 Å resolution, and the crystal structure of the FnIII4 was refined against data to 1.8 Å resolution. The structure in solution of the FnIII-3,4 region was analyzed by SAXS. The radius of gyration, calculated from the SAXS data, is 21 Å, and the maximum distance is ~63 Å. The low resolution structure of the FnIII-3,4 modelled using the SAXS data and ab initio methods reveals a heart-shaped structure with two lobes. The limited resolution of the SAXS-derived model hinders the unequivocal docking of the crystal structures of the FnIII3 and the FnIII4 into the molecular envelope. Thus, we have used site direct spin labelling (SDSL) in combination with EPR to obtain inter-domain distance restraints that will help us to elucidate the relative orientation of the FnIII3 and FnIII4 in solution. Our results have implications for the organization of the cytodomain of the integrin β4 subunit and support an intramolecular interaction between the connecting segment and the C-terminal tail.

CONTRIBUTED TALK

# COORDINATION OF CRITICAL CYTOSKELETAL CHANGES BY NCK ENABLES ENDOTHELIAL CELL POLARIZATION AND MORPHOGENESIS

**Gonzalo Rivera** Texas A&M University, College Station, USA

The establishment of cell polarity enables directional migration of endothelial cells and their organization into vascular networks. Extracellular signals that alter tyrosine phosphorylation drive vascular formation by inducing reorganization of the actin cytoskeleton. Nck, an important link between tyrosine phosphorylation and actin dynamics, has been involved in physiological responses of endothelial cells following stimulation by potent angiogenic stimuli including VEGF and integrin engagement. Nevertheless, the role of Nck in cytoskeletal remodeling during endothelial cell morphogenesis and the underlying molecular mechanisms remain largely undetermined. Here we used a combination of molecular genetics and quantitative live cell microscopy to highlight a previously unappreciated role of Nck in the coordination of endothelial cell morphodynamics. Depletion/replenishment experiments revealed an essential role for Nck in directional migration, invasion, matrix degradation, and endothelial cell morphogenesis in vitro. In confluent monolayers, Nck-dependent actin rearrangements promoted the assembly of VEcadherin-dependent cell-cell contacts. In subconfluent cultures, in contrast, Nck contributed to the acquisition of a migrating phenotype such as the one observed in tip endothelial cell. Time-lapse differential interference contrast and total internal reflection fluorescence microscopy showed that Nck couples the formation of polarized membrane protrusions with their stabilization through the assembly and maturation of cell-substrate adhesions. Measurements by atomic force microscopy showed that integrin $\alpha5\beta1$-fibronectin adhesion force and cell stiffness are also modulated by Nck. Mechanistically, the loss of front-back polarity in Nck-depleted cells was associated with altered spatiotemporal activation of FRET biosensors for the Rho GTPases Cdc42, Rac, and RhoA, as well as decreased phosphorylation of the regulatory light chain of myosin II. Collectively, these results underscore a mechanism whereby Nck orchestrates critical cytoskeletal changes that enable endothelial cell polarization and morphogenesis. Based on these findings, we postulate that Nck is an important target for therapeutic intervention in diseases associated with abnormal angiogenesis.

ALLOSTERIC REGULATION OF CELL SIGNALLING

# SESSION II. DYNAMICS AND MODELLING OF ALLOSTERIC SYSTEMS

SESSION II

# DYNAMICS AND MODELLING OF ALLOSTERIC SYSTEMS

CHAIR: FRANCESCO LUIGI GERVASIO AND ALFONSO VALENCIA

## THE INS AND OUTS OF EGFR ASYMMETRY

**Daniel J. Leahy** Johns Hopkins University School of Medicine, Baltimore, USA

The epidermal growth factor receptor (EGFR/ErbB) family of receptor tyrosine kinases comprises EGFR, HER2/ErbB2, HER3/ErbB3, and HER4/ErbB4. Each ErbB mediates cell proliferation and differentiation events essential for normal animal development, but abnormal activity of each ErbB is associated with cancer. Unregulated activity of EGFR and HER2 in particular are associated with increased cancer severity, and drugs targeting EGFR or HER2, including Trastuzumab (Herceptin®), Cetuximab (Erbitux®), Erlotinib (Tarceva®), and Lapatinib (Tykerb®), have proven successful therapies for breast, colon, lung, and head-and-neck cancers. It has long been known that ligand binding results in ErbB dimerization, stimulation of their intrinsic kinase activity, and initiation of intracellular signaling cascades. Early structural and biophysical studies of EGFR/ErbB fragments appeared consistent with this model and rationalized many aspects of ErbB behavior. Recent biochemical, biophysical, and structural studies have indicated that active, singly-ligated EGFR dimers also form, and new developments in our understanding of the molecular mechanisms governing EGFR/ErbB activation and inhibition will be presented.

# CONFORMATIONAL DYNAMICS IN MULTIDOMAIN KINASES, KINASE-PHOSPHATE COMPLEXES, AND ESCRT-PROTEIN SUPERCOMPLEXES

**Gerhard Hummer** National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, USA

We used simulation and experiment to study large-scale conformational motions in multidomain proteins involved in signaling and protein trafficking. A coarse-grained transferable energy function combined enhanced sampling methods [1] allowed us to simulate these large, dynamic and partially disordered systems. To combine the simulations with experiment, we developed ensemble refinement procedures [2,3] that incorporate data from small-angle X-ray scattering (SAXS), spin-label distance measurements (EPR), single-molecule fluorescence energy transfer (FRET), and paramagnetic relaxation enhancement (PRE) experiments as well as conventional solution NMR [2-5]. This combination with experiment allowed us not only to validate the simulation approach, but also to obtain detailed representations of the structures and motions in systems ranging from the supercomplexes of the ESCRT membrane-protein trafficking system [2,3,5] over the multi-domain protein kinase C [6] to the MAP kinases ERK2 and p38α in dynamic complexes with the phosphatase HePTP [7,8]. The dynamic character and the binding cooperativity of the protein assemblies emerge as essential elements of their function.


1 Y. C. Kim, G. Hummer, *J. Mol. Biol.* **375**, 1416–1433 (2008).
2 B. Rozycki, Y. C. Kim, G. Hummer, *Structure* **19**, 109-116 (2011).
3 E. Boura, B. Rozycki, D. Z. Herrick, H. S. Chung. J. Vecer, W. A. Eaton, D. Cafiso, G. Hummer, J. H. Hurley, *Proc. Natl. Acad. Sci. USA* **108**, 9437-9442 (2011).
4 Y. C. Kim, C. Tang, G. M. Clore, G. Hummer, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 12855-12860 (2008).
5 E. Boura, B. Rozycki, H. S. Chung, D. Z. Herrick, B. Canagarajah, D. Cafiso, W. A. Eaton, G. Hummer, J. H. Hurley, *Structure* **20**, 874-886 (2012).
6 T. A. Leonard, B. Rozycki, L. F. Saidi, G. Hummer, J. H. Hurley, *Cell* **144**, 55-66 (2011).
7 D. M. Francis, B. Rozycki, D. Koveal, G. Hummer, R. Page, W. Peti, *Nature Chem. Biol.* **7**, 916-924 (2011).
8 D. M. Francis, B. Rozycki, A Tortajada, G. Hummer, W. Peti, R. Page, *J. Am. Chem. Soc.* **133**, 17138–17141 (2011).




# ALLOSTERY AND CONFORMATIONAL CONTROL IN SIGNALING: THE UBIQUITIN E3 LIGASES

**Ruth Nussinov** National Cancer Institute and Tel Aviv University, Israel

Allostery is important for all cellular events. In E3 ubiquitin ligases, substrate binding proteins, e.g. VHL-box, SOCS-box or the F-box proteins, recruit protein substrates for ubiquitination. Ubiquitination involves several steps. The cullin-RING E3 ligase machinery is involved in one of these. In this step, ubiquitin is transferred from E2 to the substrate protein, labeling the substrate protein for degradation. However, when E3, E3-substrate and E2-ubiquitin crystal structures are modeled together, the distance between the ubiquitinated E2 and the substrate binding site is 50-59 Angstrom, raising the question how the E3 machinery bridges the distance and orients the substrate for the ubiquitin transfer? Allostery is a key factor, and the talk will address our recent work addressing this fascinating question.


1 Flexible cullins in cullin-RING E3 ligases allosterically regulate ubiquitination. **Liu J, Nussinov R.** J Biol Chem. 2011 Nov 25;286(47):40934-42.
2 Rbx1 flexible linker facilitates cullin-RING ligase function before neddylation and after deneddylation. **Liu J, Nussinov R.** Biophys J. 2010 Aug 4;99(3):736-44.
3 Molecular dynamics reveal the essential role of linker motions in the function of cullin-RING E3 ligases. **Liu J, Nussinov R.** J Mol Biol. 2010 Mar 12;396(5):1508-23.
4 The mechanism of ubiquitination in the cullin-RING E3 ligase machinery: conformational control of substrate orientation. **Liu J, Nussinov R.** PLoS Comput Biol. 2009 Oct;5(10):e1000527.
5 Allosteric effects in the marginally stable von Hippel-Lindau tumor suppressor protein and allostery-based rescue mutant design. **Liu J, Nussinov R.** Proc Natl Acad Sci USA. 2008 Jan 22;105(3):901-6.

# CONTROLLING ALLOSTERIC NETWORKS IN PROTEINS

**Nikolay V. Dokholyan** University of North Carolina at Chapel Hill, USA

We present a novel methodology for delineating allosteric pathways in proteins. We use this methodology to uncover the structural mechanisms responsible for coupling of distal sites on proteins and utilize it for allosteric modulation of proteins. We will present examples where inference of allosteric networks and its rewiring allows us to "rescue" cystic fibrosis transmembrane conductance regulator (CFTR), a protein associated with fatal genetic disease cystic fibrosis. We also use our methodology to control protein function allosterically. We design a novel protein domain that can be inserted into identified allosteric site of target protein. Using a drug that binds to our domain, we alter the function of the target protein. We successfully tested this methodology *in vitro*, in living cells and in zebrafish. We further demonstrate transferability of our allosteric modulation methodology to other systems and extend it to become ligh-activatable.

# EXPLORING THE LANDSCAPE FOR PROTEINS AND THE RIBOSOME: CONNECTING SIMPLE MODELS AND DETAILED SIMULATIONS

**José N. Onuchic** Center for Theoretical Biological Physics, Rice University, Houston, USA. *Supported by the NSF

Energy landscape theory and the funnel concept have enormously advanced our understanding of protein folding and more recently the role of global motions in protein function. Going beyond folding small and mid size proteins, the power of reduced models to study the physics of protein assembly, protein binding and recognition, and larger biomolecular machines has also proven impressive. Since energetic frustration is sufficiently small, native structure-based models, which correspond to perfectly unfrustrated energy landscapes, have shown to be a powerful approach to explore larger proteins and protein complexes, not only folding but also function associated to large conformational motions. Therefore a discussion of how global motions control the mechanistic processes in the ribosome and molecular motors will be presented. In this presentation we will focus on how the combination of detailed atomistic simulations and simplified structure-base models has allowed us to explore the entire landscape for the Ribosome. This would be impossible with one of these techniques alone. The effect of magnesium in controlling structure transitions will also be discussed mostly in the context of riboswitches.

CONTRIBUTED TALK

# INTRINSIC DISORDER IN WILD-TYPE EGFR KINASE UNDERLIES ONCOGENICITY OF CANCER MUTATIONS

**Yibing Shan** D. E. Shaw Research, New York, United States

Using molecular dynamics simulations, we find that the dimerization interface of the wild-type EGFR kinase domain is intrinsically disordered, and that it becomes ordered only upon dimerization. Our simulations suggest, moreover, that some cancer-linked mutations distal to the dimerization interface facilitate EGFR dimerization by suppressing this local disorder. Corroborating these findings, our experiments indicate that the cancer mutation causes abnormally high activity primarily by promoting EGFR dimerization. Additionally, we elucidated the conformational pathway of the deactivation of EGFR kinase and the mechanism of the cross-membrane coupling of EGFR embedded in membrane by modeling the dimers of full-length EGFR dimers.

# PROTEINS IN FLAGRANTE: EXCURSIONS IN SILICO AND IN PROTEO

**Dorothee Kern** Howard Hughes Medical Institute, Brandeis University, Waltham, USA

Understanding biological function, such as the fascinating rate acceleration of enzymes, specificity of protein/protein interactions or the delicately controlled action of signaling has been a long-standing challenge. Despite remarkable information generated using chemical tools in the last 100 years, complemented more recently with structural and computational approaches, we cannot yet identify with a complete energy inventory how ANY protein works. The secret of enzymes lies in their ability to partition energetic contributions among many atoms in a well-coordinated style. To unravel these secrets, *proteins in action* are spied on at atomic resolution to provide a comprehensive description of enzyme catalysis in the form of an energy landscape. Since the rate of catalysis is determined by the climb over a sequence of energy barriers, we focus here on the critical question of transition pathways with the highest energy state being the transition state.

I will discuss our exploration of the full energy landscape of enzyme catalysis through a combination of time-resolved NMR including high-pressure NMR, crystallography, single-molecule FRET and MD simulation. Allosteric is in play for 3 very different systems: an enzyme, a phoshorylation-mediated signaling protein and the inhibition of a kinase via protein/protein interactions. For the latter example, binding via an allosteric shift and not via an induced fit is directly demonstrated by flux measurements, the only rigorous test for selected binding.

The presented data stress the point that highly choreographed chemical integrity AND optimized conformational sampling is a prerequisite for efficient enzyme catalysis. The power of an intimate marriage between NMR and other biophysical methods and MD simulations including a variety of novel pathway algorithms will be illustrated.

# DYNAMICAL EFFECTS IN THE ALLOSTERIC CONTROL OF KINASES

N Dölker, J Juraszek and **Francesco Luigi Gervasio** CNIO, Madrid, Spain

Protein kinases (PK) are one of the largest and most functionally diverse protein families and are involved in most cellular pathways. PK malfunction is related to an important number of human diseases, such as cancer, diabetes and cardiovascular diseases. Thus, PK represent major targets for drug development.

Historically, drug discovery programs have been dominated by efforts to develop antagonists that compete for binding with endogenous ligands at orthosteric sites. However, allosteric drugs might offer several therapeutic advantages over traditional orthosteric ligands, including greater safety and/or selectivity.

Here, by combining state-of-the-art computer simulations with spectroscopy, chemical and molecular biology approaches we study in great details the role of conformational changes in the allosteric control of two pharmaceutically relevant kinases: Abl and FGFr.

In Abl a shift of the SH2 domain from the C- to the N-terminus of the catalytic domain has been found to be involved in activation [1]. The allosteric mechanism, by which the SH2 domain induces conformational changes at the active site, is still debated. We have used elastic network models, normal mode analysis, molecular dynamics simulation and mutagenesis to gain insight into the interplay between the SH2 domain and the relevant motifs at the catalytic site. We propose a mechanism, by which the SH2 domain influences the dynamics of the crucial residues directly involved in the catalytic process. In FgFr we use free energy calculations, crystallography and NMR approaches to shed light on the mode of action of a novel allosteric inhibitor. [2]

1 Nagar B, Hantschel O, Seeliger M, Davies JM, Weis WI, Superti-Furga G, Kuriyan J Molecular Cell 2006, 21, 787-798.
2 F. Bono et al., submitted to Cancer Cell

# CO-EVOLUTION BASED METHODS IN THE PREDICTION OF ALLOSTERIC CHANNELS AND CONTACT NETWORKS

**Alfonso Valencia** CNIO, Madrid, Spain

In this presentation I will describe the current status of co-evolution based methods that for the last 20 years have addressed the prediction of protein interactions (inter protein) and protein contacts (intra protein). I will emphasize the utility of methods developed by my group for the study of concerted evolution between interacting protein families (Juan et al., 2008), the detection of the residues potentially responsible of binding specificity (Rausell et al., 2010), and the potential implications of these and other developments for modeling protein complexes (Wass et al., 2011).

Together with the development of methods my group is interested the practical use of this type of computational methods for the analysis of the consequences of mutations in the context of cancer genome projects (Vazquez et al., 2012).

- Juan D, Pazos F, Valencia A (2008) High-confidence prediction of global interactomes based on genome-wide coevolutionary networks. Proc Natl Acad Sci USA. 105(3):934-9.
- Rausell A, Juan D, Pazos F, Valencia A (2010) Protein interactions and ligand binding: from protein subfamilies to functional specificity. Proc Natl Acad Sci USA. 107(5):1995-2000
- Vazquez M, et al, *in preparation*.
- Wass MN, Fuentes G, Pons C, Pazos F, Valencia A (2011) Towards the prediction of protein interaction partners using physical docking. Mol Syst Biol. 7:469.

# THE INNATE IMMUNITY MOLECULAR MACHINERY

**Giulio Superti-Furga[1],** Yazan M. Abbas[2], Andreas Pichlmair[1,3], Kumaran Kandasamy[1], Maria W Górna[1], Tilmann Bürckstümmer[1,4], Adriana Goncalves[1], Carol-Ann Eberle[1], Leonhard Heinz[1], Marielle Klein[1], Astrid Fauster[1], Adrijana Stefanovic[1], 1Christoph L Baumann[1], Alexey Stukalov[1], Andre Müller[1], Keiryn L Bennett[1], Jacques Colinge[1] and Bhushan Nagar[2] **[1]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences**, Vienna, Austria; [2]McGill University, Montreal, Canada; [3]Max-Planck Institute for Biochemistry, Martinsried / Munich, Germany; [4]Haplogen GmbH, Vienna, Austria, Vienna, Austria

Innate immune processes are very much centered on the management of molecular interactions. A variety of activities demand it: Detection of pathogens and danger signals, distinction of self- from non-self molecules, signaling to initiate both transcriptional and non-transcriptional responses, neutralization of pathogens, resolution of infection and inflammation. Using affinity proteomics coupled to mass spectrometry, bioinformatics, pathogen infections, RNAi and gene inactivation in mice for selected cases, we have step-wise mapped the cellular proteins involved in these processes and monitored interactions among themselves as well as with perturbing proteins from invading pathogens. We have used viral ORFs encoding immune modulators to map the human cell defence network. We have also used pathogen-associated molecular patterns as affinity baits to map the cell's recognition machinery. Overall, we have obtained an overview of host cellular networks involved in innate immunity as well as the dynamic assembly, disassembly and regulation of several molecular machines, such as the IFIT, TLR and NLRPs complexes as well as on the logic that a variety of different pathogens use to overturn cellular processes to their own advantage. New three-dimensional structures of components of these machines reveal new recognition mechanisms as well as allosteric movements involved in the process. From a biochemical and systems-biological point of view it is already clear that subtle calibration of accurate molecular interactions is of uttermost importance for the cell to safeguard its homeostasis in face of perilous environmental challenges such as pathogen infections.



# CRYSTAL STRUCTURE OF THE 1 MDa MAMMALIAN TRiC/CCT COMPLEX WITH TUBULIN

**Guillermo Montoya** CNIO, Madrid, Spain

Protein folding is assisted by molecular chaperones. CCT (chaperonin containing TCP-1, or TRiC) is a 1-MDa oligomer that is built by two rings comprising eight different 60-kDa subunits. This chaperonin regulates the folding of important proteins including actin, α-tubulin and β-tubulin. We used an electron density map at 5.5 Å resolution to reconstruct CCT, which showed a substrate in the inner cavities of both rings. Here we present the crystal structure of the open conformation of this nanomachine in complex with tubulin, providing information about the mechanism by which it aids tubulin folding. The structure showed that the substrate interacts with loops in the apical and equatorial domains of CCT. The organization of the ATP-binding pockets suggests that the substrate is stretched inside the cavity. Our data provide the basis for understanding the function of this chaperonin.

CONTRIBUTED TALK

# IMPACT OF MUTATIONS ON THE ALLOSTERIC CONFORMATIONAL EQUILIBRIUM

**Patrick Weinkam** University of California, San Francisco, USA

Allostery in a protein involves effector binding at an allosteric site that changes the structure and/or dynamics at a distant, functional site. In addition to the chemical equilibrium of ligand binding, allostery involves a conformational equilibrium between one protein substate that binds the effector and a second substate that less strongly binds the effector. We run molecular dynamics simulations using simple, smooth energy landscapes to sample specific ligand-induced conformational transitions, as defined by the effector-bound and unbound protein structures. We develop a set of metrics based on simulated trajectories, molecular mechanics energy functions, and stereochemical effects. Using a machine-learning algorithm on a dataset of 10 proteins and 179 mutations, we predict both the magnitude and direction of the allosteric conformational equilibrium shift by the mutation; the impact of a large identifiable fraction of the mutations can be predicted with an average unsigned error of 1 kBT.



CONTRIBUTED TALK

# CHARACTERIZING PROTEIN CONFORMATION CHANGES THROUGH TORSIONAL LINEAR RESPONSE AND ALLOSTERIC PROFILES

**Ugo Bastolla**, Helena Gomes, Javier Klett and Raul Mendez-Giraldez
Centro de Biologia Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain

An intriguing property of the functional conformation changes of proteins is that they are strongly correlated with thermal motions, despite their very different scales: tenths of angstrom for thermal motions versus several angstroms for conformation changes. This relationship is predicted by linear response theory. We develop a null model of non-functional protein deformations based on the linear response of the torsional network model (TNM) that we recently introduced, an elastic network model (ENM) that adopts main-chain and side-chain torsion angles as degrees of freedom and all-atoms contacts as interactions. Within the harmonic approximation, significant deviations from this null model have the effect to either reduce or to increase the free energy barrier opposed to the conformation change. We assess the null model and the deviations from it through a massive computational analysis of conformation changes in the PDB and several detailed case studies. We argue that this approximate analysis of energy barriers provides valuable information for characterizing conformation changes, and discuss its relationship with conformational selection and induced fit. This framework, based on the torsional linear response, naturally allows to predict protein positions that are most able to produce allosteric changes, and protein positions that perform highly robust motions. We show that active sites of proteins tend to belong to this type of sites class.

CONTRIBUTED TALK

# ONCOGENIC MUTATIONS CORRUPT THE ORDERED AUTOPHOSPHORYLATION OF THE RET RECEPTOR TYROSINE KINASE: STRUCTURAL AND MOLECULAR BASIS OF RET-MEN2B

**Ivan Plaza Menacho**[1], Barnouin K.[2], Borg A.[3], Murray-Rust J.[1], Knowles P.[1] and McDonald N. Q.[1]
[1]Structural Biology Laboratory; [2]Protein Analysis and Proteomic Laboratory and [3]Protein Production Facility, London Research Institute, Cancer Research UK, London, UK

Despite the large body of genetic and biological evidence suggesting the importance of the RET receptor tyrosine kinase in early development and neoplastic processes, the precise mechanisms of RET kinase activation and signal transduction at molecular and structural level remain unknown. In this study we investigate the temporal sequence of RET intracellular domain (ICD) autophosphorylation by using label free quantitative mass spectrometry (LFQMS) and phospho-specific antibodies. We demonstrate that upon kinase domain activation the earliest sites of RET autophoshorylation are outside the core kinase domain. In contrast, a distinct set of autophosphorylation sites are formed within the kinase domain, including RET "activation loop" tyrosines, only at later time points. Low angle X-ray scattering (SAXS) data show that the radius of gyration (Rg) of the non-phosphorylated RET ICD is smaller than when it is phosphorylated, consistent with a model where the intracellular domain of RET displays a closed conformation in the non-phoshporylated state. Upon kinase activation, phosphorylation of tyrosines residues situated within the flanking regions of the kinase domain leads to a sequential open conformation fully achieved when tyrosines in the activation loop are phosphorylated at later time points. These results further indicate that the flanking regions of RET core kinase domain can allosterically regulate kinase activity, which we prove by testing N- and terminal deletions of RET ICD by LFQMS, biochemical and structural analysis. In order to uncover the molecular mechanism driving oncogenic MEN2B-RET activation and signalling we show a perturbation in the kinetics of autophosphorylation by oncogenic RET V804M and M918T, together with higher basal levels of autophosphorylation at specific sites in the case of RET M918T. These findings were further supported with a structural analysis of oncogenic RET-MEN2B M918T kinase crystal structure solved at 2.1Å resolution.

**Other Information**

- RET (REarrange during Transfection)
- MEN2 (Multiple Endocrine Neoplasia type 2)

ALLOSTERIC REGULATION OF CELL SIGNALLING

# SESSION III. ALLOSTERIC INHIBITION

SESSION III

# ALLOSTERIC INHIBITION

CHAIR: GIULIO SUPERTI-FURGA

## STRUCTURAL INSIGHTS INTO THE MECHANISM JAK KINASE ACTIVATION IN MYELOPROLIFERATIVE DISEASE

**Michael J. Eck** Dana Farber Cancer Institute, Harvard Medical School, Boston, USA

Jak-family tyrosine kinases drive signaling from diverse growth factor and cytokine receptors. Jaks contain a pseudokinase domain, which is thought to participate in regulating the activity of the adjacent tyrosine kinase domain. Mutations within the pseudokinase domain are the cause of myeloproliferative disorders and hematologic malignancies. The V617F mutation in Jak2 leads to development of polycythemia vera, and the equivalent mutation in Jak1 (V658F) is a frequent cause of T-cell acute lymphoblastic leukemia (T-ALL). A lack of structural information for the Jak pseudokinase domains has hampered elucidation of the mechanism by which this mutation leads to constitutive kinase activation. Here we describe crystal structures of the pseudokinase domain of Jak1. Comparison of the WT structure with that of the V658F mutant reveals that the mutation induces a conformational switch that remodels one surface of the N-terminal lobe of the pseudokinase domain. A key element of this switch is a helical segment that lies just N-terminal to the pseudokinase domain; in the intact protein this segment constitutes a link to the adjacent SH2-like domain. This linker helix is also a site of activating mutations. Comparison of the two crystallographically distinct molecules in the wild type pseudokinase structure shows that the conformation adopted by the V658F mutant is also accessible in the normal protein, suggesting that the conformational switch triggered by the V658F mutation is closely related to the physiological mechanism of Jak activation in response to receptor engagement.

# ALLOSTERIC REGULATION OF INTRACELLULAR AND MEMBRANE PROTEINS BY DARPins

**Markus G. Grütter** University of Zürich, Switzerland

Repeat proteins are ubiquitous protein-protein interaction molecules fundamental to many biological processes. This feature is exploited *in vitro*, by designing ankyrin repeat proteins (DARPins) and combinatorial libraries thereof [1]. By using ribosome display DARPins having high affinity (nM range) and specificity for any target protein can be selected [2]. Their use in structural biology, in modulating the activity and in determining the role of a target protein in signaling events will be discussed. The methodology as well as structural and functional data of DARPins in complex with caspases [3], kinases [4], the membrane proteins AcrB [5] and ABC-transporters [6] will be presented. The examples illustrate the importance of specificity of recognition when analyzing the role of a particular protein in a signaling pathway but also the potential of the DARPin technology in structural biology and inhibitor design.

1 Kohl, A., Binz, H. K., Forrer, P., Stumpp, M. T., Plückthun, A. & Grütter, M. G. Designed to be stable: crystal structure of a consensus ankyrin repeat protein. Proc. Natl. Acad. Sci. USA **100**, 1700-1705 (2003).
2 Binz, H. K., Amstutz, P., Kohl, A., Stumpp, M.T., Briand, C., Forrer, P., Grütter, M. G. & Plückthun, A. High affinity binders selected from designed ankyrin repeat protein libraries. Nature Biotechnology, **22**, 575-582 (2004).
3 Schweizer, A., Roschitzki-Voser, H., Amstutz, P., Briand, C., Gulotti-Georgieva, M., Prenosil, E., Binz, HK., Capitani, G., Baici, A., Plückthun, A. & Grütter MG. Inhibition of caspase-2 by a designed ankyrin repeat protein: specificity, structure, and inhibition mechanism. *Structure* **15**, 625-636 (2007).
4 Kohl, A., Amstutz, P., Parizek, P., Binz, H.K., Briand, C., Capitani, G., Forrer, P., Plückthun, A. & Grütter, M.G. Allosteric inhibition of aminoglycoside phosphotransferase by a designed ankyrin repeat protein. Structure 13, 1131-1141 (2005).
5 Sennhauser, G., Amstutz, P., Briand, C., Storchenegger, O., & Grütter, M.G. Drug export pathway of multidrug exporter AcrB revealed by DARPin inhibitors. *PloS Biol.* **5**, e7 (ePub), (2007).
6 Mittal, A., Boehm, S., Grütter, MG., Bordignon, E. & Seeger, MA. Asymmetry in the homodimeric ABC transporter MsbA recognized by a DARPin. *J Biol Chem*. **287**, 20395-20406 (2012).

# ELUCIDATION OF THE MOLECULAR MODE OF ACTION OF SSR128129E, THE FIRST SMALL MOLECULE ALLOSTERIC INHIBITOR OF FGF RECEPTOR SIGNALING

**Françoise Bono** Sanofi R&D, Toulouse, France

The fibroblast growth factor (FGF) / fibroblast growth factor receptor (FGFR) signaling network plays an important role in cell growth, survival, differentiation and angiogenesis. Deregulation of FGFR signaling can lead to cancer development. Here we report a new FGFR inhibitor, SSR128129E (SSR), which binds to the extracellular part of the receptor, thus inhibiting FGF-induced signaling. SSR does not compete with FGF for binding to the receptor but inhibits FGF-induced signaling linked to receptor internalization in an allosteric manner as shown by crystallography studies, NMR, Fourier transform infrared spectroscopy, molecular dynamics simulations, free energy calculations and FGFR mutagenesis. SSR is the first reported small molecule allosteric inhibitor of FGF/FGFR signaling.

# ALLOSTERIC KINASE INHIBITION

**Jeffrey R. Peterson** Fox Chase Cancer Center, Philadelphia, USA

Protein kinases are an important class of drug targets in cancer due to their druggability and their central roles in promoting cell survival and proliferation. The development of ATP-competitive, small-molecule kinase inhibitors has been a key strategy in anti-cancer drug development but the evolutionary conservation of the ATP-binding pocket between different kinases generally leads to promiscuous inhibition of multiple kinases in addition to the intended target. These off-target effects can produce unwanted toxicities and complicate the interpretation of experiments using these agents. Inhibition of kinase catalytic activity by allosteric mechanisms is an alternative approach that, by targeting less-conserved binding sites, is expected to allow more selective kinase inhibition. I will discuss examples of allosteric kinase inhibition from our own work and others with an emphasis on screening approaches, mechanism of action, and target selectivity.

# NON-ATP COMPETITIVE KINASE INHIBITORS: POTENTIALS AND LIMITATIONS

**Doriano Fabbro** Novartis Pharma AG, Basel, Switzerland

Conformational bias, i.e. a shift in the equilibrium between active and inactive conformations is a key determinant in kinase regulation and can be brought about by many factors including post-translational modifications, regulatory proteins, ligand binding and pathologic kinase deregulation.

Kinase inhibitors can be viewed as particular ligands to protein kinases. As the mode of action is linked to the binding mode, the selectivity as well as the kinetics of kinase inhibitors can often be rationalized based on the target conformation. Our knowledge on the structural determinants of kinase inhibition by small molecules binding to the ATP pocket has advanced steadily in the past years. Selectivity of ATP directed kinase inhibitors and the limited set of chemotypes targeting the ATP binding site —a highly crowded area— are issues in kinase drug discovery. In contrast less is known about targeting the protein kinases outside of the "classical ATP pocket". However, a few well documented examples like the inhibitors for MEK, ABL, mTOR, AKT and IGF-1R have shown that protein kinase can be inhibited by mechanisms that are outside of the beaten tracks.

In this lecture we will review the field of non-ATP site directed inhibitors and we will discuss the potentials as well as the limitation of these approaches that should lead to improved target selectivity as well as the use of these types of inhibitors to prevent resistance protein kinase inhibition.

# REGULATION OF HGF/SF-MET SIGNALLING

**Ermanno Gherardi** MRC Centre, Cambridge and University of Pavia, Italy

Receptor tyrosine kinases are potent allosteric machines capable of initiating intracellular signals through activation of the cytoplasmic, catalytic kinase domain upon binding of specific ligands to the receptor ectodomains. Hepatocyte growth factor/scatter factor (HGF/SF) and the receptor tyrosine kinase MET control a number of developmental processes, regeneration of several epithelial organs in adult life and distant migration of a variety of cancer cells. This signalling system, therefore, has attracted considerable interest both from the point of view of the control of embryogenesis and regeneration in vertebrate organs and from a therapeutic point of view. HGF/SF is a complex multidomain protein structurally related to blood proteinase plasminogen and expressed in a tightly regulated, stage- and tissue-specific manner alongside with two truncated variants of the HGF/SF transcript known as NK1 and NK2, which act as partial receptor agonists and antagonists. We have determined several structures of the NK1 and NK2 fragments of HGF/SF either alone or in complex with heparin (a structural analogue of heparan sulphate co-receptor) and further structures of NK1, HGF/SF and anti-MET antibodies in complex with MET fragments. These studies shed some initial light on the mechanisms of ligand-induced MET activation as well as the mechanism of MET inhibition by engineered fragments of HGF/SF or anti-MET antibodies.



CONTRIBUTED TALK

# IDENTIFICATION AND CHARACTERISATION OF NOVEL ALLOSTERIC REGULATORS OF PKM2 USING FRAGMENT BASED SCREENING

**Marc O'Reilly** Astex Pharmaceuticals, Cambridge, UK

Cancer cells exhibit several unique metabolic phenotypes that are critical for cell growth and proliferation. Specifically, they over-express the M2 isoform of the tightly regulated enzyme pyruvate kinase (hPKM2), which controls glycolytic flux. This makes PKM2 an attractive target for anti-cancer therapy. A crystallographic, fragment based, screen identified multiple PKM2 ligand binding sites, including a previously uncharacterised amino acid binding pocket. I will present X-ray crystallographic, biophysical, enzymatic and cellular data that prove that the hPKM2 amino acid binding pocket represents a key hPKM2 reglulatory node that modulates hPKM2 activity via an allosteric mechanism. I will also discuss the potential biological implications for allosteric regulation of hPKM2 activity by natural amino acids.

ALLOSTERIC REGULATION OF CELL SIGNALLING

# SPEAKERS' BIOGRAPHIES

## JEAN-PIERRE CHANGEUX

Collège de France, Pasteur Institute, Paris, France

A pioneer in the field of modern neuroscience, Jean-Pierre G. Changeux is emeritus professor at the Pasteur Institute and at the Collège de France in Paris. He received a doctorate in 1964 under the tutelage of Jacques Monod of the Pasteur Institute and completed postdoctoral studies at the University of California, Berkeley, and the Columbia University College of Physicians and Surgeons before returning to the Pasteur Institute.

As a graduate student, Changeux conduct studies on the experimental basis and theoretical foundations of *allosteric* interactions between topographically distinct sites in proteins. He subsequently identifies the first protein receptor of a neurotransmitter—the nicotinic receptor of acetylcholine— and contributes to the understanding of its function in signal transduction as an allosteric membrane protein and to its role in synaptic plasticity, nicotine addiction and higher brain functions such as cognitive learning, reward mechanisms, and consciousness.

Changeux has written or co-written several books on neuroscience for general audiences, including Neuronal Man; Conversations on Mind, Matter and Mathematics; What Makes Us Think; and The Physiology of Truth.

He is a member of the U.S. National Academy of Sciences and the recipient of numerous honors including the Gairdner foundation award, the Richard Lounsbery Prize, the Wolf Prize, the Balzan Prize, the National Academy of Science's Award in Neuroscience.

## ROGER SUNAHARA

University of Michigan Medical School, Ann Arbor, USA

My graduate training with Dr. Philip Seeman (University of Toronto) and postdoctoral work with Dr. Alfred G. Gilman (University of Texas Southwestern) have provided a strong appreciation for the application of pharmacology, biochemistry and structural biology to delineate mechanisms of action. During my postdoctoral felllowship we solved the crystal structure of the catalytic core of adenylyl cyclase bound to the stimulatory G protein, Gsα, as well as activated Gsα alone. These and other data led to the elucidation of the catalytic mechanism of adenlylyl cyclase.

My laboratory focuses on understanding the mechanism by which hormone binding to G protein-coupled receptors leads to activation of signaling cascades. We develop and utilize biochemical and biophysical methodologies to elucidate how hormone binding can modulate G protein activation and/or arrestin recruitment. Indeed these approaches served to be invaluable for our recent elucidation of the crystal structure of the $\beta_2$-adrenergic receptor bound to the heterotrimeric G protein, Gs. The crystal structure and functional analyses of the GPCR-G protein complex have now provided a plausible mechanism for hormone activation of G proteins. We continue to utilize these approaches to better understand the basis for receptor-G protein specificity as well as the mechanism underlying receptor-arrestin interactions. It is our hope that by understanding the molecular mechanism and structural bases for ligand efficacy that we may engineer ligands that specifically target downstream signaling pathways. Such signaling-specific or biased ligands may provide health professionals with more selective, more potent and safer therapeutics.

## TIMOTHY A. SPRINGER

Harvard Medical School, Boston, USA

Dr. Timothy Springer is a 1971 Phi Beta Kappa graduate of the University of California, Berkeley, and earned a Ph.D. in molecular biology and biochemistry from Harvard University in 1976. He is a member of the National Academy of Sciences and the American Academy of Arts and Sciences. In 1993 he founded LeukoSite, a company through which two drugs were brought to market following a merger with Millennium Pharmaceuticals (Campath and Velcade). In 2004, he was co-recipient of the prestigious Crafoord Prize given by the Royal Swedish Academy of Sciences. Dr. Springer is one of the most highly cited scientists in the world, with his over 540 papers cited in excess of 55,000 times. He has held a 10-year NIH MERIT award not once, but twice and is responsible for many of our current concepts on how conformational changes affect integrin structure and function.

## E. YVONNE JONES

Wellcome Trust Centre for Human Genetics, University of Oxford, UK

E. Yvonne Jones is Joint Head of the Division of Structural Biology and Deputy Director of the Wellcome Trust Centre for Human Genetics at the University of Oxford. Over the last 20 or so years she has developed a programme of research into the structural biology of cell surface receptors and signalling assemblies. Her current research aims to integrate high resolution mechanistic detail with lower resolution cellular context and is focused on cell guidance cues, cell adhesion receptors and Wnt signalling.

## SIR TOM BLUNDELL

FRS, FmedSci, Department of Biochemistry at Cambridge, University of Cambridge, UK

Tom researches on molecular and structural biology of growth factors, receptor activation, signal transduction and DNA repair, important in cancer and other diseases. He has published 500 research papers, including 30 in Nature.

He was founding CEO of BBSRC 1991-1996, Chairman of Royal Commission on Environment 1998-2005, Deputy Chair of Institute of Cancer Research since 2008 and President of the UK Science Council since 2011.

He has written extensive software for structural bioinformatics and developed new approaches to structure-guided fragment-based drug discovery. In 1999 he co-founded Astex Therapeutics, an oncology company that is now public with eight drugs in clinical trials.

## ALFRED WITTINGHOFER

MPI for Molecular Physiology, Dortmund, Germany

**Academic History and Appointments**

**SINCE 2009** Emeritus (Emeritus-Group-Leader, MPI Dortmund)

**SINCE 1993** Honorary Professor of Biochemistry, Faculty of Chemistry/Biochemistry, Ruhr-University Bochum, Germany
· Director of the Dept. Structural Biology at the MPI of Molecular Physiology

**1992** Habilitation in Biochemistry, University Heidelberg, Germany

**1980** Research group leader, Max Planck Institute for Medical Research, Heidelberg, Germany

**1974-1980** Scientific employee at the Max Planck Institute for Medical Research, Heidelberg

**1971-1973** Postdoctoral fellow, University of North Carolina, USA

**1971** Ph.D., German Wool Research Institute, Aachen, Germany

**1968** Diploma in Chemistry, Technical University of Aachen, Germany

**Awards**

**2001** Louis-Jeantet Prize for Medicine

**2002** Richard-Kuhn-Medal of the Gesellschaft Deutscher Chemiker (GDCh)

**2003** Deutscher Krebspreis 2003 der Deutschen Krebsgesellschaft e.V.

**2003** Otto-Warburg-Medal of the GBM

**Major Research Interests**

We are interested in GTP-binding proteins, their regulation by Guanine Nucleotide Exchange Factors and GTPase-Activating Proteins. We also study the downstream effects of these proteins, their interaction with effectors and their biology. Recently we started looking at the function of Arl proteins in the function of cilia.

**Selected Publications:**

C. Thomas, I. Fricke, A. Scrima, A. Berken, and A. Wittinghofer. Structural evidence for a common intermediate in small G protein-GEF reactions. Molecular Cell 25, 141-149 (2007)

J.L. Bos, H. Rehmann, and A. Wittinghofer. GEFs and GAPs: Critical Elements in the Control of Small G Proteins. Cell 129, 865-877 (2007)

S. Veltel, R. Gasper, E. Eisenacher and A. Wittinghofer. The retinitis pigmentosa 2 (RP2) gene product is a GTPase-Activating protein (GAP) for Arl3. Nature Struct Mol. Biol. 15, 373-380 (2008)

B. Sot, C. Kötting, D. Deaconescu, Y. Suveyzdis, K. Gerwert, and A. Wittinghofer. Unravelling the mechanism of dual-specificity GAPs. EMBO J. 29, 1205-1214 (2010)

S. A. Ismail, Y.-X. Chen, A. Rusinova, A. Chandra, M. Bierbaum, L. Gremer, G. Triola, H. Waldmann, P.I.H. Bastiaens and A. Wittinghofer. Arl2-GTP and Arl3-GTP regulate a GDI-like transport system for farnesylated cargo. Nature Chemical Biology 7, 942-949 (2011)

## SUSAN S. TAYLOR

Howard Hughes Medical Institute, University of California, San Diego, USA

Taylor was born in Wisconsin and graduated with a major in Chemistry from the University of Wisconsin. She received her PhD in Physiological Chemistry from the Johns Hopkins University and then did postdoctoral research first at the Medical Research Laboratory of Molecular Biology with Brian Hartley and then with Nathan Kaplan in the Department of Chemistry at the newly founded University of California, San Diego. In 1972 she joined the Department of Chemistry as an Assistant Professor. In 1985 she was promoted to full Professor in the Department of Chemistry and Biochemistry and since 2006 has been a Professor in the Department of Pharmacology. In 1993 she was elected to the American Academy of Arts and Sciences, and in 1997 she was elected to the National Academy of Sciences, to the Institute of Medicine, and became a fellow of the Howard Hughes Medical Institute. She is married to Palmer W. Taylor, Professor of Pharmacology and Dean of the Skaggs School of Pharmacy and Pharmaceutical Sciences at the University of California, San Diego. They have three children, Tasha, Ashton, and Palmer Andrew and three grandchildren, Elian Vera, and Natalia and Rowan Taylor.

## DANIEL LIETHA

CNIO, Madrid, Spain

Daniel Lietha obtained his undergraduate degrees in Chemistry from the Zürcher Hochschule für Angewandte Wissenschaften (Switzerland) and in Biotechnology from Teesside University (UK). In 2003 he obtained his PhD in Protein Crystallography from Birkbeck College (UK). For his postdoctoral training he joined the laboratory of M.J. Eck at the Dana-Faber Cancer Institute (USA), where he studied signalling mechanisms triggered by cell-matrix adhesion. In 2009 Daniel joined the CNIO as Junior Group Leader in the Structural Biology and Biocomputing Programme. His research focuses on molecular mechanisms of cell signalling and adhesion, downstream of growth factor receptor and integrin signalling.

## DANIEL J. LEAHY

Johns Hopkins University School of Medicine, Baltimore, USA

My laboratory has a longstanding interest in the molecular mechanisms governing activity of cell-surface receptors in normal and disease states. Specific projects in the lab involve X-ray structural and biophysical characterizations of components of the epidermal growth factor receptor (EGFR/ErbB) and Hedgehog signaling pathways. A particular expertise of my laboratory is expression of cysteine-rich glycoproteins in eukaryotic cells in sufficient amounts and suitable forms for structural and biophysical analysis.

Professor of Biophysics & Biophysical Chemistry and Professor of Oncology

**Positions and Employment**

**1982-1984** Medical Student, Stanford University

**1984-1988** Graduate Student, Department of Chemistry, Stanford University

**1988** Postdoctoral Fellow, Dept. Physik,Technische Universität München,

**1988-1993** Postdoctoral Fellow, Dept. of Biochemistry, Columbia University,

· Assistant Professor, Johns Hopkins University School of Medicine

· Associate Professor, Johns Hopkins University School of Medicine

**2004-PRESENT** Professor, Johns Hopkins University School of Medicine

**Other Experience and Professional Memberships**

**2000-2007** Ad hoc reviewer on several NIH P01, R01, and F32 panels

**2007** Editorial Advisory Board, *Protein Science*

**2009-2012** Member, NIH MSFC study section (chair 2010-2012)

**Honors**

**1989-1992** Helen Hay Whitney Fellow Columbia University

**1994-1997** Searle Scholar Johns Hopkins University

## GERHARD HUMMER

National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, USA

Gerhard Hummer uses theory and simulations to study biomolecular systems. After receiving his Ph.D. in physics (1992; University of Vienna, Austria and Max-Planck-Institute for Biophysical Chemistry, Germany), he joined the Los Alamos National Laboratory, first as a postdoctoral fellow (1993-1996) and then as an independent researcher (1996-1999). In 1999, he moved to the National Institutes of Health where he is currently Senior Investigator, Chief of the Theoretical Biophysics Section, and Deputy Chief of the Laboratory of Chemical Physics, NIDDK. He is Fellow of the American Physical Society (2005) and received the Raymond and Beverly Sackler International Prize in Biophysics (2010).

## RUTH NUSSINOV

National Cancer Institute and Tel Aviv University, Israel

Dr. Nussinov's interests largely focus on protein folding and dynamics, protein-protein interactions, binding mechanisms and regulation, amyloid conformations and toxicity, and large multi-molecular associations with the ultimate goal of understanding the protein structure-function relationship. Among her accomplishments are the dynamic programming algorithm for the prediction of the secondary structure of RNA (1978); pioneering work in DNA sequence analysis (already in 1980); the proposition of conformational selection and population shift as an alternative binding mechanism to induced fit and the role of conformational ensembles in protein function (1999), including protein allostery (2004). More recently, she proposed new mechanistic concepts related to allostery and the roles of allosteric mechanisms and population shift in cellular functions.

## NIKOLAY V. DOKHOLYAN

University of North Carolina at Chapel Hill, USA

Dr. Dokholyan received his PhD in Physics at Boston University (advisor Prof. Gene Stanley). He completed postdoctoral training in Biophysics at Harvard University (advisor Prof. Eugene Shakhnovich). Dr. Dokholyan joined University of North Carolina at Chapel Hill Department of Biochemistry and Biophysics in 2002 and was further promoted to Associate (2008) and Full Professor (2011). Dr. Dokholyan is a member of numerous centers and programs and a member of Faculty 1000. He serves as an editor in chief for Research and Reports in Biochemistry and a book series editor "Series in Computational Biophysics". Dr. Dokholyan has published over 150 per review articles, 15 book chapters, a book, entitled "Computational Modeling of Biological Systems: From Molecules to Pathways". He studies the physical nature of interactions between atoms, molecules, cells, and organisms. The underlying question throughout his research is how these interactions shape the complex organization, behavior, and evolution of biomolecules and organisms. To approach this question Dr. Dokholyan's group has been studying structure, dynamics, function, and evolution of biological molecules. Such a broad approach is necessary to tie together the diverse pieces of knowledge of molecular properties and evolution that is to us.



## JOSÉ NELSON ONUCHIC

Rice University, Houston, USA

**JOSÉ ONUCHIC** is a Professor of Physics and Astronomy, Chemistry and Biochemistry and Cell Biology at Rice University and is the co-Director of the NSF-sponsored Center for Theoretical Biological Physics. Within the CTBP, Dr. Onuchic and his research group have led the biological physics community as it attempts to devise an integrated picture of a variety of model biochemical and biological systems. His research has expanded across the scales of molecular-level interactions to cellular systems to organized multi-cellular structures. At Rice he will move this view towards medical applications focusing on cancer. In protein folding, he has introduced the concept of protein folding funnels as a mechanism for the folding of proteins. Convergent kinetic pathways, or folding funnels, guide folding to a unique, stable, native conformation. Energy landscape theory and the funnel concept provide the theoretical framework needed to pose and to address the questions of protein folding and function mechanisms. He also works on the theory of chemical reactions in condensed matter with emphasis on biological electron transfer reactions. He is now broadening his interests to stochastic effects in genetic networks.

Dr. Onuchic did his undergraduate work at the University of São Paulo, Brazil, and received his PhD from Caltech at 1987 under the supervision of John J. Hopfield. His thesis work was on new aspects of the theory of electron transfer reactions in biology. He then spent six month at the Institute for Theoretical Physics in Santa Barbara and after that went back to Brazil at the University of São Paulo as an Assistant Professor for two and half years. During this period he continued his work on electron transfer theory as well as on the theory of chemical reactions in condensed matter and molecular electronics. He came to the University of California at San Diego in 1990. In 1989 he was awarded the International Centre for Theoretical Physics Prize in honor of Werner Heisenberg in Trieste, Italy, in 1992 he received the Beckman Young Investigator Award, and he is a fellow of the American Physical Society. In 2006 he was elected a member of the National Academy of Sciences, USA, and in 2009 he was elected a fellow of the American Academy of Arts and Sciences and of the Brazilian Academy of Sciences. In 2011 he was awarded the Einstein Professorship by the Chinese Academy of Sciences (CAS) and recently he has been elected Fellow of the Biophysical Society. In 2011, he has joined the faculty of Rice University.

## DOROTHEE KERN

Howard Hughes Medical Institute, Brandeis University, Waltham, USA

Dorothee Kern is Professor and Chair of Biochemistry at Brandeis University and an Investigator of the Howard Hughes Medical Institute. She received her PhD at the Martin Luther University in Halle, Germany followed by postdoctoral studies at UC Berkeley. She joined the faculty at Brandeis in 1999.

Dr. Kern is the recipient of the Pfizer Award in Enzyme Chemistry from the American Chemical Society, the Dayhoff Award from the Biophysical Society and the National Lecturer of the Biophysical Society. Before her professional scientific carrier, she was captain of the German National Basketball team for many years and won the MVP award.

## FRANCESCO L. GERVASIO

CNIO, Madrid, Spain

Francesco L. Gervasio was trained in physical chemistry and spectroscopy at the Molecular Spectroscopy Laboratory and the European Laboratory for Non-linear Spectroscopy, the Universitá di Firenze. He received a PhD in Chemistry in 2002 from the Universitá di Firenze and the International School for Advanced Studies in Trieste. The same year he joined the Swiss National Supercomputer Centre as a Junior Scientist.

In 2004 he joined the group of M. Parrinello as a Post-doctoral Fellow at the Eidgenössische Technische Hochschule (ETH) in Zurich (Switzerland). In 2006 he was promoted as Assistant Professor in computational chemistry at the ETH. He was appointed as Professor (2006-2009) at the Scuola Normale di Pisa (Italy).

Gervasio joined the CNIO in February 2009 to lead the Computational Biophysics Group. Since 2011 he is member of the Scientific Committe of CNIO. He is an expert in molecular modelling and simulations and has developed effective algorithms to study large-scale protein dynamics and to predict the structure-activity relationship of drug-like molecules. He has authored 57 scientific papers in international peer-reviewed journals, which have been cited more than 1500 times so far. His h-index is 25. He is part of the Innovative Medicines Initiative Open PHACTS consortium (funded by EU under the 7th Framework Programme).

## ALFONSO VALENCIA

CNIO, Madrid, Spain

Prof. Valencia is the Director of the Structural Biology and Biocomputing Programme of the Spanish National Cancer Research Centre and of Spanish Bioinformatics Institute. He is a biologist by training interested in the analysis of genomic information with particular emphasis in the evolution of protein families and protein interaction networks.

Prof. Valencia is Fellow of the International Society for Computational Biology, elected member of the EMBO, founder *"e-biolab"* science and art initiative and Professor *Honoris Causa* of the Danish Technical University. As Executive Editor of Bioinformatics (OUP) he has promoted the integration of computational and molecular biology.

## GIULIO SUPERTI-FURGA

CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences,Vienna, Austria

Scientific Director of CeMM Research Center of Molecular Medicine of the Austrian Academy of Sciences. Italian. Performed studies in molecular biology at the University Zurich, Genentech, and IMP/Vienna. Postdoctoral fellow and Team Leader at EMBL. Co-founded biotech company Cellzome, (Scientific Director). Member of: Austrian Academy of Sciences, German Academy of Sciences, EMBO, European Academy of Cancer Sciences. Chair of the EMBL Alumni Association. 2009 Advanced Investigator ERC Grant and Knight Officer Order of Merit of the Republic of Italy. 2011, Prize of the City of Vienna for Natural Sciences, and "Austria's scientist of the Year".

## GUILLERMO MONTOYA

CNIO, Madrid, Spain

Guillermo Montoya was born in Madrid in 1967 and obtained his Bachelor degree in Biochemistry from the *Universidad del País Vasco* in 1990, and his PhD in Chemistry from the *Universidad de Zaragoza* in 1993. Montoya worked at the European Molecular Biology Laboratory (EMBL) in Heidelberg (Germany), working in I. Sinning's Group, where he pioneered the study of the structure of the signal recognition particle founded by an EMBO and Marie Curie Fellowships. In 1998 he was awarded a *Peter und Traudl Engelhorn* Foundation Research Fellowship establishing his research team.

Montoya has been Head of the CNIO's Macromolecular Crystallography Group since 2002 and was acting Director of the Structural Biology and Biocomputing Programme from November 2003 to January 2006. His work has addressed the structure of different protein complexes involved in cell cycle and the redesign of protein-DNA interactions for genome modification. In 2009 he was awarded the National Prizes of the *Fundación Mutua Madrileña* and the *Fundación Caja Rural de Granada-Ministerio de Sanidad*.



## MICHAEL J. ECK

Dana-Farber Cancer Institute, Harvard Medical School, USA

Michael J. Eck MD, PhD is Professor of Biological Chemistry and Molecular Pharmacology at the Dana-Farber Cancer Institute and Harvard Medical School. He directs the Laboratory of X-ray Crystallography at the DFCI. Dr. Eck's research centers on the structural biology of signaling and cancer. His research group works to unravel the mechanisms by which mutations in kinase oncogenes alter their activity and drug sensitivity and to develop novel therapeutics that specifically target these cancer-causing proteins. Major interests include EGFR mutations in lung cancer and JAK kinase mutations in leukemia.

Dr. Eck received his Bachelors degree in Electrical Engineering from Rice University in 1985, and his M.D. and Ph.D. from the University of Texas Southwestern Medical School in 1991. He completed postdoctoral training at Children's Hospital, Boston and Harvard Medical School in 1996. He joined the faculty of Harvard Medical School and the Dana-Farber Cancer Institute in 1996.

## MARKUS G. GRÜTTER

University of Zürich, Switzerland

Markus Grütter has a PhD degree in Biophysics (University of Basel, Switzerland) and training in Protein Crystallography. He was head of a structural biology research unit at Novartis. He is full professor at the Department of Biochemistry of the University of Zürich and currently Director of the Department. Since 2001 he is directing the Swiss National Research Center (NCCR) in Structural Biology and since 2009 he is coordinating the EUprogram P-CUBE (*Infrastructure for Protein Production Platforms*). His research group focuses on the DARPin technology and the structure-function relationship of proteins involved in apoptosis and inflammation and of membrane proteins.

## FRANÇOISE BONO

Sanofi R&D, Toulouse, France

Dr. Françoise Bono is currently Vice President of Early to Candidate Unit in sanofi Research and Development in charge of a research team of 90 scientists. Dr Bono first joined Sanofi Research in 1989 as a Senior Scientist (Exploratory biochemistry) She held group leader position in Cardiovascular Thrombosis Department, (cell biology & biochemistry) in 1999 and become Vice President of the Thrombosis and Angiogenesis Department in Sanofi Aventis Research and Development between 2005 and 2008. Dr. Bono has served as the project team leader for 5 drug discovery programs and 2 of them are already in clinical evaluation. And actively contributes to the discovery of more than 10 others. She has published more than 50 research articles in various peer-reviewed scientific journals including Nature, Cell, Nature Medicine, Cancer research, Blood, and Circulation Research, and has been granted more than 30 patents.

## JEFFREY R. PETERSON

Fox Chase Cancer Center, Philadelphia, USA

Dr. Peterson is a biochemist and chemical biologist who trained at the Harvard Institute of Chemistry and Cell Biology under the direction of Drs. Marc Kirschner, Timothy Mitchison, and Stuart Schreiber. His work focuses on basic mechanisms of signal transduction and their inhibition by small molecules with a particular focus on protein kinase targets. His laboratory has developed novel allosteric inhibitors (targets include: N-WASP, Pak1 kinase, mDia1) as well as conventional active site-directed inhibitors (targets: Pak isoforms, Ack, IGF1R and others) for proteins relevant to cancer. He is currently a tenured Associate Professor at Fox Chase Cancer Center in Philadelphia.

## DORIANO FABBRO

Novartis Pharma AG, Basel, Switzerland

Doriano Fabbro received his PhD (cell biology & biochemistry, Biocenter Basel) studying the mechanisms of activation of PKC. In 1991, he joined the Oncology Research of Ciba where he contributed to the discovery of Midostaurin and Glivec. Following the merger of Ciba with Sandoz to Novartis in 1996, he was promoted Executive Director in Oncology where he was responsible for the drug discovery efforts focusing on ATP-dependent enzymes (Kinases and ATPases) and other approaches. In 2006, he was instrumental to the establishment of NIBR Expertise Platform Kinases where he serves as Head of the Kinase Biology until today.

Dr. Fabbro, a member of various professional societies and several journal editorial boards, has contributed, among many other things, to the discovery and development of kinase inhibitors for the treatment of cancer including RAD001 (Everolimus, Afinitor, launched), STI571 (Imatinib, Glivec, launched), AMN107 (Nilotinib, Tasigna, launched) and PKC412 (Midostaurin, Ph III). He has holds many patents and has written numerous publications in the area of kinase regulation, structure, screening and drug discovery.

## ERMANNO GHERARDI

MRC Centre, Cambridge and University of Pavia, Italy

Ermanno Gherardi has a degree in medicine and a Ph.D. in molecular biology from the University of Cambridge, UK. He worked at the Medical Research Council (MRC) Laboratory of Molecular Biology in Cambridge, UK, between 1987 and 2011, first on antibodies with César Milstein and subsequently as a group leader on growth factors. He has recently taken up a position at the University of Pavia, Italy. His research interests are structure-function relationships and protein engineering of antibodies and growth factors.

ALLOSTERIC REGULATION OF CELL SIGNALLING

# POSTER SESSION

# STRUCTURAL BASIS FOR THE REGULATION OF PROTEIN KINASE B

1

**Deborah Balzano** and Daniel Lietha CNIO, Madrid, Spain

Protein Kinase B (PKB) is a member of the serine/threonine AGC protein kinase family. PKB is a key signalling molecule downstream of growth factor induced PI3K signalling regulating important cellular processes such as metabolism, growth, proliferation and survival. Three highly homologous isoforms of PKB exist in mammals (α, β and γ), each containing a (N)-terminal PH domain, a kinase domain and a 21-amino acid (C)-terminal hydrophobic motif (HF). Aberrant regulation of PKB pathway is implicated in the pathogenesis of different diseases, including several human cancers and type-2 diabetes. PKB is therefore considered a good drug target and several small molecule inhibitors have already been described. A multistep process involving PIP3 and upstream kinases, such as PDK1, activates PKB. When active, PKB is located to the inner surface of the plasma membrane. Previous work has revealed that a change in the position of the PH domain relative to the kinase domain depends on the activation state of PKB. The inactive form is called the closed conformation, while the membrane-associated form is called the open conformation. Once phosphorylated, PKB is released from the membrane to the cytosol. The aim of our work is to investigate on a structural level i) the modeof PKB autoinhibition in its basal state, ii) how the binding to PIP3 and phosphorylation by PDK1 regulates the catalytic activity of PKB, and iii) understand what triggers the release of the active enzyme from the membrane. We will complement the structural studies with extensive biochemistry, like kinase activity assays as well as protein-protein and protein-lipid interaction studies. Further, new insights on PKB structure and regulation could allow the interpretation of the effects of different classes of PKB inhibitors and enable the design of inhibitors with improved selectivity.

# P38α MAP KINASE DYNAMICS IN FREE AND LIGAND-BOUND FORMS

2

**Anna Berteotti**[1], Vittorio Limongelli[2], and Michele Parrinello[3,4] [1]Istituto Italiano di Tecnologia, Genova, Italy; [2]Università di Napoli "Federico II", Naples, Italy; [3]Computational Science (ICS), Università della Svizzera Italiana, Lugano, Switzerland; [4]Computational Sciences, Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland, Faculty of Informatics, Universita' della Svizzera Italiana, Lugano, Switzerland

Kinases play pivotal roles in the cell cycle regulation and in several pathological conditions. Thus in the very recent years, a selective kinase inhibition has become an important goal for both the academia and pharmaceutical industry. In many kinases, a typical conformational change, called DFG flip characterizes the movement of three well conserved amino acids at the beginning of the activation loop. During this movement the Aspartate moves away from the ATP binding site, and its position is taken by the neighboring Phenylalanine. This transition allows the creation of an additional binding site that can be targeted by some selective inhibitors. Here the use of well-tempered metadynamics simulations[1] allowed us to enhance the sampling of the DFG flip for p38α MAP kinase in the apo form and in two ligand-bound forms[2]. The data coming from X-ray and NMR[2,3] experiments are assessed and complemented shedding light on the dynamical character of this movement. From our results the dynamical aspects of the DFG flip at atomistic level are disclosed and the data obtained here will be of precious help for future experiments and structure-based drug design.

1 A. Barducci et al., Phys. Rev. Lett. Jan 18,100, 2, 020603 (2008).
2 C. Pargellis et al., Nat. Struct. Biol. 9, 4, 268-72 (2002).
3 M. Vogtherr et al., Angew. Chem. Int. Ed. Engl. 30,45(6),993-7 (2006).

# LIGAND-DETECTED 19F NMR-BASED FRAGMENT SCREENING FOR ALLOSTERIC LIGAND DISCOVERY AGAINST SELECTED PROTEIN TARGETS

3

**Blanca López-Méndez** and **Ramón Campos-Olivas** CNIO, Madrid, Spain

Fragment-based drug discovery is an alternative approach to target-focused drug discovery based on high throughput cell or biochemical assays, which has gained widespread interest and application. It is based on the idea of building drugs piece by piece, detecting first the weak binding of low molecular weight (typically MW <150-300 Da) and low complexity compounds (fragments), that could subsequently be chemically developed into compounds with lead-like properties. The approach is particularly promising to target protein-protein interactions, and to unveil protein allosteric sites that may or may not be physiologically exploited. In our Unit we have developed tools to conduct NMR-based screening to identify and characterize the binding of small molecules to protein targets, potentially providing hits for drug discovery research and chemical tools for biophysical and functional applications. In particular, we put together a collection of ~370 fluorinated small molecule compounds with good solubility in aqueous buffer and assembled them into mixtures of eight each. Using 1D 19F NMR spectroscopy we were able to monitor the possible binding of the compounds present in each cocktail to different protein targets by monitoring the increase of 19F NMR signal linewidth upon protein addition. Typically 20 μM concentration in each CF3-containing fragment, or 50 μM for those with CF groups, are sufficient to obtain high quality spectra, and as low as 1:50 of protein equivalents produce a detectable effect on fragment binders, therefore requiring very small amounts of protein. With a sample changer and a dual H-F probe in our 700 MHz instrument, screening of the complete fluorinated fragment library can be performed in less than 2 days. Results obtained in the initial screening and the follow up of hits discovered against selected macromolecular targets, in particular in the targeting of an auto-inhibitory domain of a protein tyrosine kinase, will be presented in this poster.

## STRUCTURAL BIOLOGY OF TIM PROTEINS: A FAMILY OF CELL SURFACE PHOSPHATIDYLSERINE RECEPTORS THAT REGULATE IMMUNITY

4

**José M. Casasnovas**, Angela Ballesteros, César Santiago, Meriem Echbarthi, Gerardo G. Kaplan, Gordon J. Freeman Centro Nacional de Biotecnologia, CSIC, Madrid, Spain; Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, USA. Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA

The T-cell immunoglobulin and mucin domain (TIM) proteins are involved in the regulation of immune responses. The TIM are type I membrane proteins with an N-terminal immunoglobulin (Ig) domain followed by a heavily glycosylated mucin domain in the extracellular region, a single transmembrane region and a cytoplasmic tail with tyrosine phosphorylation motifs. The N-terminal Ig domain is engaged in ligand binding with some contribution of the mucin domain. The structures of the Ig domains determined by our group provided relevant insights on ligand recognition by the TIM proteins. A loop disulphide linked to the CFG β-sheet of the Ig domain is a distinctive structural feature of the TIM proteins. Moreover, the structures identified a unique ligand-binding pocket with a metal ion to which ligands coordinate (Metal Ion-dependent Ligand Binding Site, MILIBS). The TIM proteins use the MILIBS to bind to phosphatidylserine (PtdSer) and to mediate phagocytosis of apoptotic cells. A model for TIM protein binding to PtdSer in cellular membranes was built based on structural and functional data. In this model the tip of the N-terminal Ig domain of the protein penetrated into the membrane, whereas the hydrophilic moiety of PtdSer entered into the MILIBS. The MILIBS modulate the biology of the TIM proteins, such as the trafficking of TIM-1 to the cell surface, TIM-TIM interactions and the conformation of the proteins on the cell surface. Therefore, the MILIBS is a critical determinant of the ligand binding specificity and functional properties of the TIM family. Overall, we conclude that the unique structure of TIM IgV domains suggests that the TIM molecules evolved as a family of pattern recognition receptors for PtdSer that determine whether apoptotic cell recognition leads to immune activation or tolerance, depending on the TIM molecule engaged and the cell type it is expressed on.

## TOWARDS UNRAVELLING THE MOLECULAR MECHANISM AND REGULATION OF THE PRO/ANTI-ONCOGENE PROTEINS SHIP1 AND 2

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**Johanne Le Coq**, Luis Heredia and Daniel Lietha Cell Signalling and Adhesion Group, Structural Biology and Biocomputing Programme, CNIO, Madrid, Spain

SHIP (SH2-containing Inositol 5′-Phosphatase) proteins 1 and 2 (SHIP1/2) are two important signalling enzymes homologs from the inositol polyphosphate 5-phosphatase family, involved in several growth factor receptor-signalling pathways. They catalyse the dephosphorylation of the phospholipid phosphatidylinositol 3,4,5-triphosphate (PI(3,4,5)$P_3$) to PI(3,4)$P_2$. PI(3,4,5)$P_3$ is a key lipid second messenger which can recruit signalling proteins to the plasma membrane and subsequently initiate numerous downstream signalling pathways responsible for the regulation of a plethora of cellular events such as proliferation, growth, apoptosis, actin cytoskeletal rearrangement, to name a few. Because of its central role, three enzymes tightly regulate the levels of PI(3,4,5P)$P_3$: PI3K is responsible for its generation while PTEN, a well-characterized tumour suppressor, and SHIP1/2 are responsible for its degradation. SHIP1/2 have been heavily implicated with several serious diseases such as cancer and type 2 diabetes. The paucity in the predictability of their behaviour reflects our incomplete understanding of their exact role and the molecular mechanism by which they are regulated. Therefore it is key for cancer drug development and potentially for other diseases associated directly or indirectly with these two enzymes that we unravel their respective regulatory molecular mechanisms. To that effect we are studying both enzymes and the roles of their different domains at the structural and biochemical level. We are employing kinetic and binding studies to elucidate the regulation mechanisms of both enzymes and have recently solved the structure of SHIP2 phosphatase domain in conjunction with its C2 domain (2.2 Å).

## THE DOMAIN INTERPLAY IN PROTEIN KINASES: A STUDY ON THE ALLOSTERIC EFFECT OF THE SH2 DOMAIN ON KINASE ACTIVATION

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Protein kinases constitute one of the largest protein families and are involved in most cellular pathways. Correspondingly, kinase malfunction is related to an important number of human diseases. Protein kinases share a common canonical catalytic domain, consisting of a number of highly conserved motifs, which have to undergo distinct conformational changes to perform their catalytic activity. Most kinases also require other domains or proteins for full activation.

The Abelson tyrosine kinase (Abl) is of special interest because of its importance as an anti-cancer drug target. The fusion protein Bcr-Abl leads to over-activation of Abl, causing chronic myeloid leukemia (CML). Currently, Abl inhibitors are the only pharmaceutical treatment for CML, but a significant proportion of patients develop resistance against currently known inhibitors. As most resistance mutants are located around the active site of the catalytic domain, it would be highly desirable to develop inhibitors that bind to allosteric sites. A more detailed understanding of allosteric effects of other protein domains on the activation of Abl is, therefore, of paramount importance for the rational design of new drugs.

We focus on the protein-protein interactions of the catalytic domain with the SH2 domain, an allosteric effector involved both in auto-inhibition and activation. We have used elastic network models, normal mode analysis and molecular dynamics simulations to gain insight into the interplay between the SH2 domain and the relevant motifs at the catalytic site. We propose a mechanism, by which the SH2 domain modifies important functional modes of the catalytic domain.

This proposal was verified experimentally by introducing specific mutants the relevant motifs identified during the computational study.



## STRUCTURAL INSIGHT INTO MU TRANSPOSITION: MuB IS A AAA+ ATPASE THAT FORMS HELICAL FILAMENTS ON DNA

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DNA transposons are ubiquitous in the genomes of all forms of life and play an important evolutionary role in the generation of gene diversity and in the shaping of genomic landscapes. Although typically transposons exhibit no strong selectivity for the target DNA site, some transposons select DNA non-randomly, avoiding self-destruction; a phenomenon named "target immunity", because the presence of a copy of the transposon makes nearby DNA "immune" for additional transposon insertions. Phage Mu is one of the most complex and efficient transposable elements. Two phage-encoded proteins, MuA and MuB, are essential for efficient transposition. MuA is the transposase responsible for synapses of the two Mu ends and all the DNA cutting and joining steps. However, MuA is highly inefficient in the absence MuB, which is a small ATP-dependent non-specific DNA binding protein with slow ATPase activity. MuB is needed to (1) favor the assembly of the MuA-Mu ends complex, (2) to allosterically stimulate the activity of MuA, (3) to select and deliver the target DNA and (4) to confer target immunity. Mu. Further detailed understanding of the mechanism of action of MuB has been hampered by the bind DNA in its central channel without seemingly altering DNA B-DNA conformation. This results in a unique mismatched symmetry between protein and DNA helixes not observed previously in other nucleoprotein filaments, which forces MuB monomers to interact with DNA differently along the filament. Overall, our findings dissect the structure and functions of MuB to reveal the intricate control that an AAA+ ATPase can impose on a complex reaction system.

## MODULATION OF αVβ3 INTEGRIN ALLOSTERY BY BINDING OF ISODGR CONTAINING CYCLOPEPTIDES

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Nowadays most of pharmaceutical efforts in drug design are focused on the discovery, development and optimization of new ligands that efficiently bind to receptors involved in human disease. Even though traditional computational techniques are able to find small molecules that maximize binding free energy, they are not able to predict how small differences in ligand binding could reflect into structural and functional response of the receptor. For this reason, receptors that undergo a substantial conformational transition upon ligand binding should be precluded from structure based drug design.

Here we present a case study on Integrin avb3. One major problem with integrin ligands is their potential to activate conformational changes, which can initiate unwanted signals. Neglecting this aspect could lead to the development of drugs that induce agonist-like activities and adverse paradoxical effects. In this context, 1 we exploited a combination of computational and biochemical studies to determine the biologically active conformation of a small library of cyclopeptides, to discriminate in silico binders from non-binders, to understand at molecular level integrin allosteric changes induced by ligand binding and to identify real antagonist among the selected binders. Our study reveals the importance of ligand-receptor complex dynamics in drug design studies aiming at developing new real integrin antagonists, confirming that ligand induced conformational change of the receptor and its structure-function relationship should play a crucial step in common drug design strategies.

## IFIT PROTEINS AS NEW EFFECTORS IN ANTIVIRAL DEFENCE: INVESTIGATING RNA BINDING AND INTER-MOLECULAR INTERACTIONS IN THE IFIT COMPLEX

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Virus-derived RNA bearing a 5′ triphosphate group (ppp-RNA) and present in the cytoplasm is detected by the cell as non-self RNA, which results in strong induction of an antiviral response. So far, the best described ppp-RNA sensor is RIG-I, which initiates a signaling cascade that leads to production of the type I interferon. However, we hypothesized that there should also exist interferoninduced effector proteins that act on ppp-RNA. We previously performed an affinity-purification/mass-spectrometry screen for proteins preferentially binding to ppp-RNA and identified the interferon-induced proteins with tetratricopeptide repeats (IFIT) as top candidates. We have shown that two of the IFIT family members (IFIT1 and IFIT5) can specifically recognize ppp-RNA, and that IFIT proteins play a role in antiviral defence. IFITs also interact with each other to form a macromolecular complex. Here, we investigate in further detail which RNA features are required for efficient binding by IFITs, and dissect the proteinprotein interactions between the IFIT family members. Our data suggest that IFITs form a large multivalent molecular platform for RNA that determines the fate of certain viral RNAs.

# FOCAL ADHESION KINASE AS A CELLULAR MECHANO-SENSOR

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Focal adhesion kinase (FAK) is a crucial component of focal adhesion sites, which exerts its activity by transducing signals between the cytosol and the extracellular matrix. The translocation of FAK to focal adhesion sites and its functional activation by tyrosine phosphorylation leads to the formation of large enormous multi-molecular complexes. Theses complexes can trigger different signaling pathways, including the MAPK pathway.

The three-dimensional structure of FAK consists of a tyrosine kinase domain and two large non-catalytic regions. The N-terminal FERM domain is involved in auto-inhibition of the kinase by blocking a phosphorylation site (Tyr576/577)[1]. The exposure of this phosphorylation site induces the maximum activity of FAK. We tested if mechanical forces as they are present at focal adhesion sites can induce an allosteric switch to an active state of FAK with an exposed phosphorylation site, using Molecular Dynamics simulations.

We indeed find mechanical forces propagated onto FAK when tethered between the membrane and the cytoskeleton can remove the auto-inhibitory FERM domain from the kinase domain of FAK. This functional activation of FAK can then trigger a sequence of downstream signaling events. The mechanotransduction mechanism of FAK can explain how FAK acts as a force sensor, translating mechanical forces at the focal adhesion site into a biochemical signal.



# DISTINGUISHING BETWEEN INDUCED FIT AND CONFORMATIONAL SELECTION WITH THE TORSIONAL NETWORK MODEL

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Since the first studies on protein dynamics, it has been of interest how this event occurs upon ligand binding. Nowadays, apart form the classic “lock and key” paradigm, two new paradigms are under debate: conformational selection and induced fit models.[1][2]

In our work we aim to propose a way to distinguish whether protein motion is accounted by any of these two paradigms using our in-house elastic network model TNM.[3]

1 Bucher D, Grant BJ, McCammon JA.Biochemistry. 2011 Dec 6;50(48):10530-9. Epub 2011 Nov 10.
2 Changeux JP, Edelstein S. F1000 Biol Rep. 2011;3:19. Epub 2011 Sep 1.
3 Mendez R, Bastolla U.Phys Rev Lett. 2010 Jun 4;104(22):228103. Epub 2010 Jun 3.

# DYNAMIC FINGERPRINT OF IMATINIB SENSITIVE KINASES

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Protein kinases constitute one of the largest and most functionally diverse protein families, involved in most cellular pathways. They are of crucial importance for signal transduction, and their deregulation is related to numerous human diseases, including cancer. Among kinases, c-Abl and c-Src are of particular interest for cancer research. The BCR-Abl fusion protein, is the initial cause of chronic myeloid leukemia disease (CML) 1. CML is treated with the powerful anti-cancer drug Imatinib, which acts as ATP-competitor. Imatinib, however, is not so selective and targets numerous kinases2, beside c-Abl and c-Src, like c-KIT, PDGFR, Syk, Lck and many others, showing towards some of them a lack of activity. For instance it effectively inhibits BCR-Abl (IC50=0.2 uM) but not Src 3, no matter their high sequence homology (47%), neither that the bound structure of Src with Imatinib is very similar to that of Abl with Imatinib. In the same way, the drug inhibits c-KIT (IC50=0.41 uM) but not Syk 3, although they share the same sequence identity of 40% with both Abl and Src. Striking is the case of Lck, quite effectively inhibited by Imatinib (IC50=9 uM) 3, despite the high identity with Src kinase (70%). Recently, in our work concerning the two kinases c-Abl and c-Src, we have shown that the difference in the Imatinib activity can be attributed to a larger flexibility of c-Abl4. Now in this follow up work we have extended the analysis to the other cited kinases, analyzing their flexibility profiles. Performing classical molecular dynamic simulations, followed by RMSF analysis, we have identified regions with a higher flexibility common to all the kinases sensitive to Imatinib (c-Abl, c-KIT and also Lck) and a more rigid profile in the same regions for those not responding to the drug (c-Src and Syk). From these preliminary results we could recognize flexibility as a dynamic fingerprint of all the studied kinases sensitive to Imatinib.

1 Rowley, J.D. Nature 1973, 243, 290–293.
2 Lee SJ, Wang JYJ. Journal of Biology. 2009.
3 Deininger, M.; Buchdunger, E.; Druker, B. J. Blood 2005, 105, 2640–2653.
4 Lovera S, Sutto L, Boubeva R, et al. Journal of the American Chemical Society. 2011:8-11.

# MOLECULAR RECOGNITION MECHANISMS OF MICROTUBULE PLUS END TRACKING PROTEINS

13

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Microtubule (MT) plus-end tracking proteins (+TIPs) comprise a structurally and functionally diverse family of proteins that preferentially accumulate at MT growing ends. +TIPs form dynamic networks where most of the protein-protein interactions are mediated by End-Binding proteins (EBs). EBs were recently recognized to be master regulators of +TIP networks because: they are able to autonomously track MT tips and to recruit a large variety of other +TIPs to this location. EBs are modular proteins comprised of two functional domains connected by a long linker: a calponin-homology (CH) domain, responsible for MT binding, and a coiled-coil (CC) domain, responsible for partner binding.

Here, we present structural and functional data describing how the concerted action of the CH, linker, and CC domains of EBs accounts for the autonomous MT tip tracking, regulation of MT dynamics, and recruitment of numerous partners to MT ends. Using a combination of structural and biochemical techniques, we propose a structural model for EB proteins in solution. Furthermore, our biochemical data suggest an important role for long-range electrostatic interactions between the CC domain of EBs and the MT lattice, which allows the EBs to discriminate between the MT-lattice and MT-tips.

We also present insights into the molecular recognition mechanism of EBs by +TIPs containing a 'MT-tip localization signal' (MTLS), which comprises the short linear sequence motif SxIP. By means of an exhaustive functional analysis, we have derived the sequence determinants of a canonical MTLS and correlated the EB-binding and MT tip tracking activities of different SxIP-containing +TIPs. Moreover, we have investigated how phosphorylation regulates the EB-SxIP interaction and, consequently, MT-tip localization. Our data establish a favorable basis for computational approaches to search for novel +TIPs in entire genomes.

# A NEW PHOSPHORYLATION/ACETYLATION SWITCH IN THE REGULATION OF CORTACTIN AND ITS ROLE IN CELL SPREADING

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Cortactin is a multidomain protein that plays a central role in the remodeling of the actin cytoskeleton in virtue of its capacity to active the Arp2/3 complex and NWASP protein (1). Cortactin is an oncoprotein overexpressed in different human carcinomas and currently considered a bona fide invadopodia marker. In addition, cortactin is targeted by pathogens to invade or to adhere to host cells (2). Although cortactin was cloned as a Src substrate how this post-translational modification regulates the activity of the protein remains unclear. Moreover the protein is regulated by acetylation but how these two post-translational modifications relate to each other was unknown. Therefore to analyze the biochemical consequences of cortactin phosphorylation on tyrosines, we used a fusion-expression system that forces the interaction between cortactin and Src kinase in cells. We describe here a new regulatory mechanism: a competition between the acetylation and the tyrosine-phosphorylation of cortactin. This switch was confirmed with the endogenous protein. Furthermore, we analyzed the effect of the phosphorylation in cortactin–Focal Adhesion Kinase (FAK) complex formation during cell spreading (3). Finally we present new unpublished data of how the cortactin-FAK complex relates to N-WASP protein.

1 Martinez-Quiles N, Ho HY, Kirschner MW, Ramesh N, Geha RS. Erk/Src phosphorylation of cortactin acts as a switch on-switch off mechanism that controls its ability to activate N-WASP. Mol Cell Biol. 2004 Jun;24(12):5269-80.
2 Nieto-Pelegrin E, Martinez-Quiles N. Distinct phosphorylation requirements regulate cortactin activation by TirEPEC and its binding to N-WASP. Cell Commun Signal. 2009 May 6;7:11.
3 Meiler E, Nieto-Pelegrín E, Martinez-Quiles N. Cortactin tyrosine phosphorylation promotes its deacetylation and inhibits cell spreading. PLoS One. 2012;7(3):e33662.

# A NOVEL POTENTIAL OF MEAN FORCE TO STUDY PROTEIN FLEXIBILITY

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Understanding protein flexibility is of key importance in order to understand protein function. Any computational modeling of protein flexibility requires an appropriate energy function, which can either rely on first principles or on statistics of known protein structures. Knowledge-based potentials (KBP) are nevertheless, simpler and easier to compute than classical Molecular Mechanics force fields, but still useful to describe protein folded states. There are two main ways of obtaining KBPs: 1) Optimizing the likelihood of the protein native structure vs. the artificially generated decoys [1]; 2) Deriving Boltzmann-like statistics as Potential of the Mean Force (PMF) [2]. The former strategy can be limited because of the high dimensionality of the parameter space and/or the coarse-grained energy model adopted to cope with this problem. In the case of the PMF, the observed atomic contact frequencies must be normalized versus a reference state, defined as the one with no interactions between any two atoms. But this state is never achieved in protein structures. Here we present a novel PMF that is totally independent of any reference state. Considering only directly interacting atom pairs (such as that there are no intermediate atom), it computes the interaction energy between atoms of type a and b, as the minus log of the probability density function to find a pair of atoms of type a, b in direct contact plus a normalization constant that is determined by equaling the average interaction energy to the propensity of atoms of type a, b to establish direct contacts. In order to obtain an analytical function, a least squares non-linear fit of the computed values is performed to a quadruple exponential function (Morselike). Finally we will ensure that native protein structures are in the actual energy minimum of the DICAM force field by optimizing the parameters over a set of well determined protein structures, so that torsional fluctuations due to DICAM are minimized.

# FREE ENERGY OF THE INTERDOMAIN ASSEMBLY OF SH2 AND CATALYTIC DOMAIN IN c-Abl COMBINING METADYNAMICS AND ALL-ATOM STRUCTURE-BASED MODELING.

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We report on a computational analysis of the dynamical and structural effects due to the rearrangements of the sh2 and kinase domains of c-Abl. Our approach is based on a hybrid structure-based all-atom model [1] combined with the metadynamics method to enhance conformational sampling [2]. The combined benefits of these two approaches allow the study of events whose time and length scales are well beyond the capabilities of standard all-atom molecular dynamics simulations. This method provides us with the potential to gain new insights in the mechanisms regulating the activity of the whole c-Abl kinase in atomistic details and a free-energy landscape interpretation of some of the known cancer-driving and resistant point mutations of c-Abl.

# SEQUENCE DEPENDENT ALLOSTERY IN PDZ DOMAINS DICTATES THEIR BINDING SELECTIVITY

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The crowded and heterogeneous cellular environment demands the interactions between its numerous components to be highly specific, to avoid potentially harmful side reactions. Given the vast number of ligands competing for binding to a limited number of domain families, it is often puzzling how specificity can be achieved (1). In monomeric proteins selectivity may be modulated by intradomain allostery, whereby a remote residue is energetically connected to the functional binding site via side chain or backbone interactions. Whereas several intradomain energetic pathways have been predicted in modular protein domains, there is a paucity of experimental data to validate their existence.

Here, we have identified such functional energetic networks in one of the most common protein-protein interaction modules, the PDZ domain, involved in the control of several cellular events (2,3). Although this protein family displays a simple topology and a rather conserved binding pocket, the distinct roles of the various PDZ domains in cellular signaling require selectivity.

We used double mutant cycles (4), involving site-directed mutagenesis of both PDZ domain and peptide ligand, in conjunction with binding kinetics to unveil the fine energetic details of the specific long-range networks involved in peptide recognition. We performed the analysis on two homologous PDZ-ligand complexes and found that the energetically coupled residues differ for these complexes. This result demonstrates that amino acid sequence rather than topology dictates the allosteric pathways. Furthermore, our data support a mechanism whereby the whole domain, not only the binding pocket, is optimized for a specific ligand. Such cross-talk between binding sites and remote residues may be used to tune target selectivity.

1 Kaneko T (2011) Trends Biochem. Sci. 36, 183–90;
2 Fanning AS (1999) J. Clin. Invest. 103, 767-72;
3 Jemth P (2007) Biochemistry 46, 8701-08;
4 Horovitz A (1990) J. Mol. Biol. 214, 613–17.

# DYNAMIC PROPERTIES OF c-SRC TYROSINE KINASE BY NMR AND MOLECULAR DYNAMIC CALCULATIONS

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Tyrosine kinases (TK) play a fundamental role in cellular signaling pathways. Their deregulation is linked to several diseases including Cancer and diabetes. In particular two highly homologous TK, namely c-ABL and c-SRC, are causative of Chronic Myeloid Leukaemia (fusion gene Bcr-Abl) and metastasis (c-SRC proto oncogene). Despite the high homology (47%), they show a different sensitivity to the anticancer drug Imatinib. The catalytic domain of TK, responsible of this interaction, comprises an N-terminal lobe, containing mostly β-sheets, and a larger, α-helical C-terminal lobe. At the interface between the lobes, a number of highly conserved residues form the active site with the ATP binding pocket. Generally, kinase activation is mainly controlled by conformational changes in four conserved structural motifs at the active site: the activation loop (A-loop), the Asp-Phe-Gly (DFG) motif,the αC-helix and the P-loop. The A-loop is a highly flexible region connecting the two lobes, which can adopt an ensemble of different conformations ranging from an open form, to a closed conformation, which completely blocks the substrate binding site. The αC- helix in the N-lobe can swing out of its active conformation (so- called αC-in), interrupting a crucial salt bridge in the active site and leading to a reoriented conformation (called αC-out). The DFG motif is located at the N-terminal end of the A-loop and has been proposed to have a role in the catalytic activity of the kinase. Finally the P-loop, also located in the N-lobe, is known to adopt a distinct kinked conformation in the Abl-Imatinib complex, while in c-Src it remains unchanged upon Imatinib binding. Its importance is further underlined by the fact that many mutations which confer resistance to Imatinib are located in the P-loop.

Here we present the pico-nanosecond dynamics measured by NMR relaxation experiments on the catalytic domain of cSRC. These preliminary results will be compared with MD calculations.

# ALLOSTERIC COMMUNICATION PATHWAYS IN KIX DOMAIN-A METADYNAMICS STUDY

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Here we present an investigation on the origins of allosteric dynamical changes that are caused by the interaction between MLL protein with KIX and C-Myb domains and its biophysical characterization. In previous NMR studies [1], the dynamic ensemble of the accessible states of this system were studied using relaxation dispersion techniques. Dispersion profiles obtained for the binary state (MLL:KIX) indicated the presence of a conformational transition (on the milliseconds timescale) between two configurations: a so called "ground" state (B) which is highly populated, and an "excited" state (B*) which is scarcely populated. Interestingly, the chemical shifts of B* state were seen to be similar to the ternary complex (MLL:KIX:c-Myb). This observation suggested the pre-existence of state B*, which is likely to bind to c-Myb, in the ensemble of binary complex conformations. However, relaxation dispersion NMR techniques, could not resolve the structure of the higher energy conformation. Molecular dynamics (MD) is a natural choice for understanding the molecular origins of dynamics allostery in this system. However, this time demanding conformational transition is unaccessible for the typical MD simulation timescale. In order to avoid this limitation, we employ enhanced MD methodologies such as Metadynamics. Indeed, to achieve the sampling of the conformations ensemble, Well-Tempered Ensemble [2] combined with Parallel Tempering simulations were performed. Using this advanced technique we have properly characterized the atomistic configuration of the excited state for the KIX domain in the binary complex. Other important outcomes were obtained from the understanding of the structural nature of the communication pathway and the energetics associated with them. Indeed we shed a light on the signal transmission along the allosteric pathway and how the residues pass the information from one site to the other.

1 S. Bruschweiler, P. Schanda, K. Kloiber, B. Brutscher, G. Kontaxis, R. Konrat, and M. Tollinger, J. Am. Chem. Soc., 131, 3063-3068 (2009)
2 M. Bonomi and M. Parrinello, Phys. Rev. Lett. 104, 190601 (2010)

## DECIPHERING CAD, A 1.5 MegaDa ANTI-TUMORAL TARGET THAT CONTROLS DE SYNTHESIS OF PYRIMIDINES

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CAD is a 1.5x106 Da macromolecular complex that controls the initial steps of the de novo synthesis of pyrimidines, is implicated in tumor development, and is a target for anti-tumoral drugs. CAD is formed by a multifunctional polypeptide of 253 kDa with three enzymatic functions, Carbamyl phosphate synthetase II (CPS II), Aspartate transcarbamylase (ATC) and Dihydroorotase (DHO), each of them catalyzing one of the three initial reactions of the pathway. Up-regulation of CAD activity is needed in proliferating and tumoral cells to allow the fast synthesis of pyrimidines required for replication. Thus, CAD is a target for the design of anti-tumoral drugs like PALA, a bi-substrate inhibitor of the ATC activity. The activity of CAD is allosterically regulated, being feed-back inhibited by UTP, the final product of the pathway, and activated by the nucleotide precursor PRPP. The allosteric regulation of CAD is also modulated by phosphorylation of MAP kinase and PKA signaling cascades during cell cycle. Despite its key role in cell metabolism and its implications in cancer, there is no direct structural information about CAD or about any of its domains, other than it associates forming a homohexamer that travels between nucleus and cytoplasm depending on the functional situation or the stage of the cell cycle. The structures of CPS, ATC and DHO homologs in bacteria or archea have been determined but there is no structural information of any eukaryotic counterpart that could be applied to CAD. Given its large size and modular nature, we aim to determine the structure of CAD combining X-ray crystallography and single particle electron microscopy. Here we report the expression and purification of full-length CAD. We also present the crystal structures of the DHO and ATC domains of human CAD. These results provide the first view of eukaryotic DHO and ATC enzymes, and give us, for the first time, high-resolution information for trying to decipher the architecture of CAD.

## CRYSTAL STRUCTURES OF SCHISTOSOMA MANSONI PEROXIREDOXIN I: INSIGHT INTO THE MECHANISM OF ASSEMBLY OF STRESS-REGULATED CHAPERONES

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Schistosomiasis poses one of the greatest health challenges for countries in the tropical and sub-tropical area, where it kills 280000 people/year out of an estimate of 200 million infected. The adult worms of both sex live in the veins of the bowel and the bladder of the host, where females lay eggs causing inflammation and hemorrages, and in chronic form leads to severe complications. Praziquantel, the drug of choice against all Schistosome species, is effective only on the adult worms and unfortunately some less sensitive strains are emerging; thus it is mandatory to discover new specific drugs. The aim of our project is to obtain a complete characterization of the 3D structure of the enzymes involved in the detoxification pathway of S. mansoni. This pathway is peculiar in so far as the parasite relies on a single enzyme, thioredoxin glutathione reductase (TGR), to maintain redox homeostasis, contrary to humans that possess two separate enzymes. By X-ray crystallography we have solved the 3D structure of the following enzymes, all involved in the detoxification pathway of S. mansoni: TGR; Thioredoxin (Trx); Glutathione Peroxidase (Gpx) and Peroxiredoxin1 (Prx1). Thus specificity of new drugs may in principle be achieved by targeting this pathway. 2-Cys peroxiredoxins (Prxs) play two different roles depending on the physiological status of the cell. They are thioredoxin-dependent peroxidases under low oxidative stress and ATP-independent chaperones upon exposure to high peroxide concentrations. These alternative functions have been associated with changes in the oligomerization state from low- (LMW) to high-molecularweight (HMW) species. Here we present the structures of Schistosoma mansoni PrxI in both states: the LMW decamer and the HMW 20-mer formed by two stacked decamers. The latter is the structure of a 2-Cys Prx chaperonic form. Comparison of the structures sheds light on the mechanism by which chemical stressors, such as high H(2)O(2) as high concentration at acidic pH, are sensed and translated into a functional switch in this protein family.

## INSIGHTS ON THE MOLECULAR STRUCTURE OF THE PLANT SALT-OVERLY-SENSITIVE 1 (SOS1) NA+/H+ ANTIPORTER

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Due to their sessile nature, plants have to endure adverse environmental conditions. The Arabidopsis thaliana Na+/H+ antiporter SOS1 is essential to maintain low intracellular levels of the toxic Na+ under salt stress. Available data show that the plant SOS2 protein kinase and its interacting activator, the SOS3 calcium-binding protein, function together in decoding calcium signals elicited by salt stress and regulating the phosphorylation state and the activity of SOS1. Molecular genetic studies have shown that the activation implies a domain reorganization of the antiporter cytosolic moiety indicating that there is a clear relationship between function and molecular structure of the antiporter. To provide information on this issue, we have carried out in vivo and in vitro studies on the oligomerization state of SOS1. In addition, we have performed electron microscopy and single particle reconstruction of negatively stained full length and active AtSOS1. Our studies show that the protein is a homodimer that contains a membrane domain similar to that found in other antiporters of the family, and an elongated, large and structured cytosolic domain. Both the transmembrane and cytosolic moieties contribute to the dimerization of the antiporter. The close contacts between the transmembrane and the cytosolic domains provide a link between regulation and transport activity of the antiporter. Structural studies on the different components of Salt Overly Sensitive pathway are letting us understand how the molecular machinery for salt tolerance works and opens path towards biotechnological applications.

## RIGIDITY-BASED ALLOSTERIC COMMUNICATION

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**Adnan Sljoka** York University, Toronto, Canada

Given the 3-D structure of a protein, understanding how it functions depends in critical ways on predicting which parts are rigid and which are flexible. The rigidity and flexibility analysis of the molecular graph, using a fast combinatorial rigidity algorithm – the pebble game algorithm (flexweb.asu.edu), can rapidly decompose a protein into flexible and rigid regions.

This rigidity approach is applied to studying allostery in recently solved GPCR proteins. It is widely believed that the binding of a ligand at the allosteric site triggers a conformational change that is transmitted through the protein to cause a rearrangement and alteration of the shape of the active site. In this study we introduce several rigidity-based allostery modes of communication and corresponding algorithms which can detect rigidity and shape changes between two distant binding pockets in allosteric proteins. Starting with a GPCR structure, we will show how ligand (agonist) binding induced rigidity changes at the extracellular binding pocket triggers rigidity changes which propagate to the binding regions at the cytoplasmic domain.

## GERM-LINE DICER1 MUTATION AND ASSOCIATED LOSS OF HETEROZYGOSITY IN A PINEOBLASTOMA

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**Archana Srivastava**[1,2], Nelly Sabbaghian[1,2], Nancy Hamel[1,3], Steffen Albrecht[4], John R. Priest[5] and William D Foulkes[1,3] [1]Program in Cancer Genetics, Department of Oncology and Human Genetics, McGill University, Montreal, Quebec Canada; [2]Lady Davis Institute and Segal Cancer Centre, Jewish General Hospital, McGill University, Montreal, Quebec, Canada; [3]Research Institute, McGill University Health Centre, Montreal, Quebec, Canada; [4]Department of Pathology, Montreal Children's Hospital and McGill University, Montreal, Quebec, Canada; 5Minneapolis, Minnesota, USA

The pineal gland, so named by Galen because of its resemblance to a pine nut, is a midline structure situated on the posterior wall of the third ventricle, deep between the cerebral hemispheres. A pineoblastoma is a supratentorial primitive neuroectodermal tumour arising in the pineal gland. It is known to occur in RB-1 mutation carriers, but is rarely seen in association with other hereditary syndromes.

DICER1 is an RNAse endoribonuclease responsible for the production of microRNAs (miRNAs). In addition, DICER1 is involved in regulation of chromatin, DNA replication timing, genome stability, and cellular senescence. We and others have recently demonstrated that germ-line DICER1 mutations are associated with a cancer syndrome presenting embryonal tumours such as pleuropulmonary blastoma (PPB), ovarian sex cord stromal tumors (especially Sertoli-Leydig cell tumors), cervical embryonal rhabdomyosarcoma as well as primitive neuroectodermal tumors, Wilms tumor, cystic nephroma, pulmonary sequestration, juvenile intestinal polyps, and familial multinodular goiter. Based on this profile, we hypothesized that such mutations might also occur in children with pineoblastoma.

Here we describe a novel germ-line DICER1 mutation in a 6 year old child with a highly aggressive pineoblastoma. In addition, we observed loss of heterozygosity (LOH) of the wild-type allele, an event not previously reported in DICER1-associated tumours. Our observations suggest that: a) pineoblastoma are associated with germ-line DICER1 mutations, thus further extending the disease profile of DICER1 kindred, and b) unexpectedly, tumour cells can thrive in the absence of functional DICER1. These findings will further help identify at risk families, a clinically important task given the association between DICER1 mutations and PPB which can be fatal to affected infants.

## AN HYBRID STRUCTURE-BASED MODEL FOR LARGE SCALE CONFORMATIONAL TRANSITION IN PROTEIN KINASES

25

**L. Sutto**[1], I. Mereu[1], F.L. Gervasio[1] [1]Structural Biology and Biocomputing Programme, Spanish National Cancer Research Center (CNIO), Madrid, Spain

Structure based models are successful at conjugating the essence of the energy landscape theory of protein folding with an easy and efficient implementation. Recently their realm expanded beyond single protein structure, been used profitably to widely study large conformational transitions. Still, when dealing with conformational transition between two well-defined structures an unbiased and realistic description of the local backbone and sidechain interactions is necessary. The proposed model merges a precise description of these interactions with a structurebased non-bonded potential that takes into account different conformers. We present the results for the activation of the catalytic domain of a human kinase for which we reconstructed the transition free energy and the description of the activation loop flexibility.

CNIO FRONTIERS MEETINGS 2013

ALLOSTERIC REGULATION OF CELL SIGNALLING

# CNIO FRONTIERS MEETINGS 2013

# CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER: FROM MECHANISMS TO THERAPEUTICS

27/05/2013-29/05/2013

## ORGANISERS

Robert Benezra, Memorial Sloan-Kettering Cancer Center, New York, USA.
Ana Losada, CNIO, Madrid, Spain.
Marcos Malumbres, CNIO, Madrid, Spain.
René Medema, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

## CONFIRMED SPEAKERS

Angelika Amon, Daniela Cimini, Don W. Cleveland, Duane A. Compton, Philip Hieter, Randall King, Rong Li, David Pellman, Charles Swanton, Stephen Taylor, Jan van Deursen, Ashok Venkitaraman and Todd Waldman.

## SUMMARY

**The conference will focus on recent progress in understanding how aneuploidy contributes to initiation and progression of human cancers. Some of the subjects discussed will be:**

- Mechanisms of aneuploidy generation.
- The effect of aneuploidy on cell proliferation and survival and how cancer cells tolerate it and profit from it.
- CIN genes in human cancer.
- Modeling aneuploidy in mouse
- Targeting aneuploidy as a therapeutic opportunity.

ALLOSTERIC REGULATION OF CELL SIGNALLING

# PREVIOUS CNIO FRONTIERS MEETINGS AND CNIO CANCER CONFERENCES

## 2011

### RECAPTURING PLURIPOTENCY: LINKS BETWEEN CELLULAR REPROGRAMMING AND CANCER

07/11/2011-09/11/2011

**Organisers:** Maria A. Blasco, Konrad Hochedlinger, Manuel Serrano, Inder Verma

### CANCEROMATICS II: MULTILEVEL INTERPRETATION OF CANCER GENOME

28/03/2011-30/03/2011

**Organisers:** Søren Brunak, Stephen Chanock, Núria Malats, Chris Sander, Alfonso Valencia

### BREAST CANCER

07/02/2011-09/02/2011

**Organisers:** Joaquín Arribas, José Baselga, Miguel Ángel Piris, Lajos Pusztai and Jorge Reis-Filho

## 2010

### CANCER PHARMACOGENETICS: PERSONALIZING MEDICINE

22/11/2010-24/11/2010

**Organisers:** Javier Benítez, William E. Evans, Miguel Martín and Magnus Ingelman-Sundberg

### MOLECULAR CANCER THERAPEUTICS

08/03/2010-10/03/2010

**Organisers:** Gail Eckhardt, Roy S. Herbst and Manuel Hidalgo

## 2009

### THE ENERGY OF CANCER
02/11/2009-04/11/2009
**Organisers:** Toren Finkel, David M. Sabatini, Manuel Serrano and David A. Sinclair

### CANCER-OM-ATICS: MULTILEVELINTERPRETATION OF CANCER GENOME DATA
06/07/2009-08/07/2009
**Organisers:** Søren Brunak, Núria Malats, Chris Sander and Alfonso Valencia

### STEM CELLS AND CANCER
23/02/2009-25/02/2009
**Organisers:** Elaine Fuchs, Maria A. Blasco, Eduard Batlle and Mirna Pérez-Moreno

## 2008

### SIGNALLING UPSTREAM OF mTOR
03/11/2008-05/11/2008
**Organisers:** Dario R. Alessi, Tomi P. Mäkelä and Montserrat Sánchez-Céspedes

### STRUCTURE AND MECHANISMS OF ESSENTIAL COMPLEXES FOR CELL SURVIVAL
23/06/2008-25/06/2008
**Organisers:** Niko Grigorieff, Eva Nogales and Jose María Valpuesta

### DEVELOPMENT AND CANCER
04/02/2008-06/02/2008
**Organisers:** Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

## 2007

### LINKS BETWEEN CANCER, REPLICATION STRESS AND GENOMIC INTEGRITY
05/11/2007-07/11/2007
**Organisers:** Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez and André Nussenzweig

### MYC AND THE TRANSCRIPTIONAL CONTROL OF PROLIFERATION AND ONCOGENESIS
11/06/2007-13/06/2007
**Organisers:** Robert N. Eisenman, Martin Eilers and Javier León

### MOLECULAR MECHANISMS IN LYMPHOID NEOPLASM
19/02/2007-21/02/2007
**Organisers:** Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe and Miguel Angel Piris

## 2006

### TELOMERES AND TELOMERASE-CNIO / JOSÉF STEINER CANCER CONFERENCE
13/11/2006-15/11/2006
**Organisers:** Maria A. Blasco and Jerry Shay

### MEDICINAL CHEMISTRY IN ONCOLOGY
02/10/2006-04/10/2006
**Organisers:** Fernando Albericio, James R. Bischoff, Carlos García-Echeverria and Andrew Mortlock

### INFLAMMATION AND CANCER
22/05/2006-24/05/2006
**Organisers:** Curtis Harris, Raymond DuBois, Jorge Moscat and Manuel Serrano

### PTEN AND THE AKT ROUTE
08/05/2006-10/05/2006
**Organisers:** Ana Carrera, Pier Paolo Pandolfi and Peter Vogt

## 2005

### CANCER AND AGING
07/11/2005-09/11/2005
**Organisers:** Maria A. Blasco, Kathy Collins, Jan Hoeijmakers and Manuel Serrano

### MAP KINASES AND CANCER
30/05/2005-01/06/2005
**Organisers:** Philip Cohen, Roger Davis, Worcester, Chris Marshall and Ángel Nebreda

### ANIMAL TUMOUR MODELS AND FUNCTIONAL GENOMICS
07/03/2005-09/03/2005
**Organisers:** Allan Balmain, Mariano Barbacid, Anton Berns and Tyler Jacks

## 2004

### CADHERINS, CATENINS AND CANCER
29/11/2004-01/12/2004
**Organisers:** Amparo Cano, Hans Clevers, José Palacios and Franz Van Roy

### STRUCTURAL BIOLOGY OF CANCER TARGETS
27/09/2004-29/09/2004
**Organisers:** Ernest Laue, Guillermo Montoya and Alfred Wittinghofer

## 2003

### APOPTOSIS AND CANCER
01/12/2003-03/12/2003
**Organisers:** Gabriel Nuñez, Marisol Soengas and Scott Lowe

### SMALL GTPases IN HUMAN CARCINOGENESIS
16/06/2003-18/06/2003
**Organisers:** Juan Carlos Lacal, Channing Der and Shuh Narumiya

### TARGETED SEARCH FOR ANTICANCER DRUGS
17/03/2003-19/03/2003
**Organisers:** Amancio Carnero and David H. Beach

## 2002

### MECHANISMS OF INVASION AND METASTASIS
18/11/2002-20/11/2002
**Organisers:** Joan Massagué and Richard Hynes

### THE CELL CYCLE AND CANCER
30/09/2002-02/10/2002
**Organisers:** Marcos Malumbres, Charles Sherr and Jiri Bartek

### CANCER EPIGENETICS: DNA METHYLATION AND CHROMATIN
29/05/2002-31/05/2002
**Organisers:** Manel Esteller and Stephen B. Baylin


CNIO Distinguished Seminars
2012
06/SEP
Robert Huber
Max Planck Institute of Biochemistry, Martinsried, Germany
07/SEP
Peter Campbell
The Wellcome Trust Sanger Institute, Cambridge, UK
14/SEP
Kári Stefánsson
deCODE Genetics, Reykjavik, Iceland
21/SEP CLINICAL-TRANSLATIONAL SEMINAR
Cristóbal Belda
University Hospital Madrid Sanchinarro, Spain
05/OCT
Geoffrey Wahl
The Salk Institute for Biological Studies, La Jolla, USA
19/OCT
Eamonn Maher
University of Birmingham, UK
26/OCT
George Thomas
IDIBELL, Barcelona, Spain
16/NOV
Dan Littman
Skirball Institute of Biomolecular Medicine, New York, USA
23/NOV
Paul Nurse
Cancer Research UK, London, UK
14/DEC
Nancy Hynes
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
21/DEC
Juan Carlos Izpisúa
The Salk Institute for Biological Studies, La Jolla, USA
2013
11/JAN
Simon Boulton
London Research Institute, London, UK
18/JAN
Paul Flicek
EMBL Outstation-European Bioinformatics Institute, Cambridge, UK
01/FEB
Pedro Alonso
Institute for Global Health, Barcelona, Spain
08/FEB
René Medema
The Netherlands Cancer Institute, Amsterdam, The Netherlands
15/FEB
James Lupski
Baylor College of Medicine, Houston, USA
1/MAR CLINICAL-TRANSLATIONAL SEMINAR
Miguel Martín
General University Hospital Gregorio Marañon, Madrid, Spain
8/MAR
Allan Balmain
University of California, San Francisco, USA
15/MAR
Richard Marais
Paterson Institute for Cancer Research, Manchester, UK
22/MAR
Jan Löwe
MRC Laboratory of Molecular Biology, Cambridge, UK
05/APR
Elías Campo
Clinic Hospital, Barcelona, Spain
12/APR
Gideon Schreiber
Weizmann Institute of Science, Rehovot, Israel
26/APR
Carl Djerassi
Stanford University, Stanford, USA
17/MAY
Roel Nusse
Howard Hughes Medical Institute, Stanford University, Stanford, USA
24/MAY
Bruno Amati
IFOM-IEO Campus, Milan, Italy
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FRONTIERS IN TUMOUR HETEROGENEITY AND PLASTICITY
27/10/2013-30/10/2013
ORGANISERS
Mirna Pérez-Moreno, CNIO, Madrid.
Scott Lowe, MSKCC, New York, USA.
Erwin Wagner, CNIO, Madrid, Spain.
CO.ORGANISERS FROM NATURE PUBLISHING GROUP
Barbara Marte, Nature.
Nicola McCarthy, Nature Reviews Cancer.
Alexia-Ileana Zaromytidou, Nature Cell Biology.
KEYNOTE SPEAKERS
Kornelia Polyak and José Baselga.
CONFIRMED SPEAKERS
Cedric Blanpain, Snorri Thorgeirsson, Christoph Klein, Elaine Mardis, Charles Swanton, John Condeelis, Ruslan Medzhitov, Karen Vousden, Mike Hemann, Victoria Seewaldt, Sean Morrison, Angela Nieto, Lillian Siu, Jeff Settleman, Gail Eckhardt Nicholas Barker and Hiroyuki Mano.
a nature conference

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NOTES

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