CNIO Cancer Conference

Structural Biology of Cancer Targets

Madrid, September 27-29, 2004

Organisers:
Ernest Laue, University of Cambridge, Cambridge, UK
Guillermo Montoya, CNIO, Madrid, Spain
Alfred Wittinghofer, Max-Planck-Institut for Molecular Physiology, Dortmund, Germany

Auditorium
Centro Nacional de Investigaciones Oncológicas
Melchor Fernández Almagro, 3
28029 Madrid, Spain

CNIO Organisation Staff: Beatriz Ferreiro, Alma Izquierdo, Amanda Wren (ccc@cnio.es)
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Dear Colleague,

We would like to welcome you to the CNIO Cancer Conference (CCC) on Structural Biology of Cancer Targets.

This conference is following the trend being established through the CCC’s: to bring together leading scientists in a given field related to cancer research, and to provide the best possible forum for discussion. We are confident that collectively, the CNIO Cancer Conferences will be of great interest to the scientific community.

The topic “Structural Biology of Cancer Targets” is timely and of particular importance -as the knowledge of the detailed molecular structure of key players controlling tumour development is already an asset in the design of innovative treatments. It is our hope that the meeting will address the new trends in this area as well as potential strategies for exploiting this information for therapeutic benefit.

We very much hope that you will enjoy this exciting conference.

With our personal thanks,

Ernest Laue  
University of Cambridge,  
Cambridge, UK

Guillermo Montoya  
CNIO,  
Madrid, Spain

Fred Wittinghofer  
Max-Planck-Institut for Molecular Physiology,  
Dortmund, Germany

As the newest member of the European Cancer Community, the CNIO is very much open to establishing strong collaborations with you and your respective organisations. We would therefore be delighted to inform you about our Scientific Strategic Plan for the next few years, and to show you our facilities. Should you wish to meet with any member of our Faculty, please let us know and we will be delighted to introduce you.
# Programme overview

## CNIO Cancer Conference: Structural Biology of Cancer Targets
Auditorium of the Spanish National Cancer Centre, CNIO (Melchor Fernández Almagro, 3, E-28029 Madrid)  
September 27-29, 2004

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<tr>
<th>Monday, September 27</th>
<th>Tuesday, September 28</th>
<th>Wednesday, September 29</th>
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<tbody>
<tr>
<td><strong>Welcome/ 09:30h-09:45h</strong>&lt;br&gt;Mariano Barbacid. CNIO Director</td>
<td><strong>III. Computational approaches/ 09:30h-12:30h</strong>&lt;br&gt;Chair: A. Valencia</td>
<td><strong>V. Drug design/ 09:30h-11:30h</strong>&lt;br&gt;Chair: A. Meseguer</td>
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<tr>
<td>D. Barford. London (UK)&lt;br&gt;M. J. Eck. Boston (USA)&lt;br&gt;A. Wittinghofer. Dortmund (Germany)&lt;br&gt;R. Eibl. Miami (USA)</td>
<td>Coffee break and Poster Viewing (10:45h-11:15h)</td>
<td><strong>Coffee and departure (11:30h)</strong></td>
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<td><strong>Coffee break and Poster Viewing (11:30h-12:00h)</strong>&lt;br&gt;C. Müller. Grenoble (France)&lt;br&gt;J. M. Valpuesta. Madrid (Spain)&lt;br&gt;O. Llorca. Madrid (Spain)</td>
<td><strong>F. Gago. Madrid (Spain)</strong>&lt;br&gt;H. Matter. Frankfurt (Germany)&lt;br&gt;A. Ramírez. Madrid (Spain)</td>
<td><strong>Lunch (12:30h-14:00h)</strong></td>
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<td><strong>Lunch (13:15h-14:30h)</strong></td>
<td><strong>IV. Combinatorial &amp; chemical genetics strategies/ 14:00h-14:45h</strong>&lt;br&gt;Chair: F. Gago</td>
<td><strong>CNIO Building Tour:</strong> Following the close of Tuesday’s sessions, a short Tour will be conducted by CNIO staff for participants interested in CNIO activities. The meeting point will be at the Registration Desk.</td>
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<tr>
<td><strong>II. NMR/ 14:30h-17:30h</strong>&lt;br&gt;Chair: M. J. Eck</td>
<td><strong>A. Meseguer. Barcelona (Spain)</strong>&lt;br&gt;J. Poyatos. Madrid (Spain)</td>
<td>Lunches: Lunch will be provided, courtesy of the CNIO, in the main dining room of the building.</td>
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<tr>
<td>E. D. Laue. Cambridge (UK)&lt;br&gt;M. Rico. Madrid (Spain)&lt;br&gt;L. Kaustov. Toronto (Canada)</td>
<td><strong>Coffee break and Poster Viewing (14:45h)</strong></td>
<td><strong>Coffee break and Poster Viewing (15:45h-16:15h)</strong></td>
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<tr>
<td>J. Rizo. Texas (USA)&lt;br&gt;P. García. Madrid (Spain)&lt;br&gt;M. M. Zhou. New York (USA)</td>
<td><strong>CNIO Tour (15:30h)</strong></td>
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Detailed Programme

September 27

09:30-9:45 Welcome, Mariano Barbacid, CNIO Director

Session I: X-ray & EM
Chair: R. Abagyan

09:45h-10:15h  D. Barford, Chester Beatty Laboratories, ICR, London, UK
Molecular Basis for Activation of B-RAF by Oncogenic Mutations

10:15h-10:45h  M. J. Eck, Dana Farber Cancer Institute, Boston, USA
Structure of a formin homology −2 domain and insights into its mechanism of actin assembly

10:45h-11:15h  A. Wittinghofer, MPI, Dortmund, Germany
Ras as an anti-cancer drug target

11:15h-11:30h  R. Eibl, University of Miami, USA
Short talk
Atomic force microscopy measurement of SDF-1 mediated affinity modulation of single VLA-4 – VCAM-1 bonds

11:30h-12:00h  Coffee break and poster viewing

12:00h-12:30h  C. Müller, EMBL-Grenoble, France
Structural insight into transcription factor activation and nuclear transport

12:30h-13:00h  J. M. Valpuesta, CNB-CSIC, Madrid, Spain
The Eukaryotic Chaperonin CCT: A Folding Nanomachine With Many Important Roles

13:00h-13:15h  O. Llorca, CIB-CSIC, Madrid, Spain
Short talk
Structural basis for the regulation of Vav3 Rho/Rac guanosine exchange factor during normal and oncogenic activation

13:15h-14:30h  Lunch
### Session II: NMR
**Chair: M. J. Eck**

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<th>Time</th>
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<tr>
<td>14:30h-15:00h</td>
<td><strong>E.D. Laue</strong>, University of Cambridge, UK</td>
<td></td>
<td>The HP1 Protein: from Structure to Function</td>
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<tr>
<td>15:00h-15:30h</td>
<td><strong>M. Rico</strong>, IQFR-CSIC, Madrid, Spain</td>
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<td>NMR, Ribonucleases and Cancer</td>
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<tr>
<td>15:30h-15:45h</td>
<td><strong>L. Kaustov</strong>, University of Toronto, Toronto, Canada</td>
<td></td>
<td>Structural Basis and Regulatory Mechanism for the p53 Tumor Suppressor, Transactivation Domain Interaction With Replication Protein</td>
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<td>15:45h-16:15h</td>
<td><strong>Coffee break and poster viewing</strong></td>
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<tr>
<td>16:15h-16:45h</td>
<td><strong>J. Rizo</strong>, University of Texas Southwestern Medical Center, USA</td>
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<td>Mad conformational signaling in the mitotic spindle checkpoint</td>
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<tr>
<td>16:45h-17:00h</td>
<td><strong>P. García</strong>, CNIO, Madrid, Spain</td>
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<td>Structural analysis by NMR of the interactions between integrin and a peptide inhibiting angiogenesis</td>
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<td>17:00h-17:30h</td>
<td><strong>M. M. Zhou</strong>, Mount Sinai School of Medicine, NY, USA</td>
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<td>Targeting Host Transcription by Direct Histone Lysine Methylation: The Role of a Viral SET Domain</td>
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# Session III: Computational approaches

**Chair: A. Valencia**

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<tr>
<td>09:30h-10:00h</td>
<td>R. Abagyan, The Scripps Research Institute, La Jolla, USA</td>
<td>Computational Structural Proteomics and Drug Discovery</td>
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<tr>
<td>10:00h-10:30h</td>
<td>M. Orozco, University of Barcelona, Spain</td>
<td>Exploring the DNA for the design of new therapies</td>
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<td>10:30h-10:45h</td>
<td>M. Thomas, Cyclacel, Dundee, UK</td>
<td>Protein Structures in Virtual Screening</td>
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<td>10:45h-11:15h</td>
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<td>Coffee break and poster viewing</td>
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<tr>
<td>11:15h-11:45h</td>
<td>F. Gago, University of Alcalá de Henares, Madrid, Spain</td>
<td>Elongation factor eEF1A as a target for antitumour didemnin: insight from molecular modelling and computer simulations</td>
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<tr>
<td>11:45h-12:15h</td>
<td>H. Matter, Aventis Pharma Deutschland GmbH, Frankfurt, Germany</td>
<td>Target Family Landscapes: Combined Interpretation of Ligand Selectivity and Protein Binding Site Topology for Kinases and Proteases</td>
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<tr>
<td>12:15h-12:30h</td>
<td>A. Ramírez, CBM Severo Ochoa, CSIC-UAM, Madrid, Spain</td>
<td>Modeling ligand selectivity in lead optimization</td>
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<td>12:30h-14:00h</td>
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<td>Lunch</td>
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# Session IV: Combinatorial & chemical genetics strategies

**Chair: F. Gago**

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<th>Time</th>
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<th>Topic</th>
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<tr>
<td>14:00h-14:30h</td>
<td>A. Messeguer, IIQAB-CSIC, Barcelona, Spain</td>
<td>Combinatorial Chemistry approaches to cancer related pharmacological targets</td>
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<tr>
<td>14:30h-14:45h</td>
<td>J. Poyatos, CNIO, Madrid, Spain</td>
<td>Structural units of the interactome: Some concepts and their potential relevance for therapeutic situations</td>
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<td>14:45h</td>
<td></td>
<td>Coffee break and poster viewing</td>
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<tr>
<td>15:30h</td>
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<td>CNIO Tour</td>
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September 29

Session V: Drug design
Chair: A. Messeguer

09:30h-10:00h  A. Levitzki, Hebrew University of Jerusalem, Jerusalem, Israel
Tricking Cancer Cells To Die

10:00h-10:30h  G. Klebe, Institut für Pharmazeutische Chemie, Marburg, Germany
Strategies in Structure-based Drug Design

10:30h-11:00h  M. Randal, Sunesis Pharmaceuticals, Inc., San Francisco, USA
Drug Discovery at Adaptive Surfaces

11:00h-11:30h  L. T. Vassilev, Roche Research Center, Hoffmann-La Roche, Inc., Nutley, USA
Small-molecule inhibitors of p53-MDM2 binding

11:30h  Coffee and departure
Poster list (in alphabetical order of presenting author)

1. **Structural basis for substrate recognition of mannose receptor family members**  
   J. Boskovic, Centro de Investigaciones Biológicas, CSIC, Madrid, Spain

2. **Structure and dynamics of a biologically active human FGF-1 monomer, complexed to a hexasaccharide heparin analogue by NMR**  
   M. A. Canales, Centro de Investigaciones Biológicas, CSIC, Madrid, Spain

3. **The putative B7-H1 molecule is expressed in primary cell cultures generated from Saudi breast cancer patients**  
   H. Ghebeh, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia

4. **Induced-fit recognition of DNA by small circular oligonucleotides.**  
   C. González, Instituto de Química Física “Rocasolano”, CSIC, Madrid, Spain

5. **Novel non-genotoxic small molecule activators of p53**  
   S. Lain, University of Dundee, UK

6. **Tyrosine kinome re-sequencing in human cancer**  
   J. G. Páez, Broad Institute/MIT, Cambridge, MA, USA

7. **Development of novel DNA-binding chemotherapeutic agents based on flavonoid scaffolds of active ingredients isolated from Chinese herbal remedies**  
   P. Ragazzon, Open University, Milton Keynes, UK

8. **The X-ray Structure of the Recombinant Core 8D of the Nuclear Chaperone Nucleoplasmin**  
   I. G. Muñoz, Centro Nacional de Investigaciones Oncológicas, Madrid, Spain

9. **Structural basis for the redox regulation of the active-site of CDC25A protein phosphatase**  
   G. Vicentini, Institute of Cancer Research, London, UK
Abstracts-Sessions
Molecular Basis for Activation of B-RAF by Oncogenic Mutations

David Barford
Section of Structural Biology, Institute of Cancer Research, Chester Beatty Laboratories, London, UK

Over 30 mutations of the B-RAF gene associated with human cancers have been identified, the majority of which cluster to the glycine rich P-loop and activation segment of the kinase domain. We have characterised the biochemical and physiological properties of 22 B-RAF mutants and integrated these studies with crystallographic investigations of the kinase domain of wild type and oncogenic mutant forms of B-RAF in complex with the RAF inhibitor BAY43-9006. Eighteen of 22 B-RAF mutants analysed have elevated kinase activity and signal to ERK in vivo. Surprisingly, three mutants have reduced kinase activity towards MEK in vitro, but activate C-RAF in vivo enabling them to signal to ERK in cells. The crystal structure of the kinase domain of both wild type and the oncogenic mutant V599EB-RAF in complex with BAY43-9006 shows that the activation segment is held in an inactive conformation by association with the P-loop. The clustering of mutations to this region suggests that they disrupt this interaction converting B-RAF into an active conformation. The high activity mutants are able to signal to ERK by directly phosphorylating MEK, whereas the impaired activity mutants mediate MEK activation by activating endogenous C-RAF, possibly through an allosteric or transphosphorylation mechanism.

Wan et al., 2004, Cell, 116:855-67
Structure of a formin homology –2 domain and insights into its mechanism of actin assembly

Michael Eck
Dana-Farber Cancer Institute and Harvard Medical School, Boston, USA

Formin proteins participate in a wide range of cytoskeletal processes that are required for cell polarity, cytokinesis, and morphogenesis in all eukaryotes. The defining feature of formin proteins is a highly conserved ~400 residue region, the Formin Homology–2 (FH2) domain, which has recently been found to nucleate actin filaments. Unlike the Arp2/3 complex, which nucleates branched filaments, formins induce unbranched filaments required for formation of diverse actin-containing structures. The crystal structure of the *S. cerevisiae* Bni1p FH2 domain reveals a ring-shaped homodimer in which the two subunits are tied together in a head-to-tail arrangement by a unique “lasso” structure. The reciprocal tethering provided by the lasso and an adjacent linker segment in each molecule creates a stable, yet flexible dimer. Conserved patches on one surface of the FH2 dimer are likely sites of interaction with actin. The unusual tethered-dimer architecture of the FH2 domain may allow formins to nucleate actin filaments by stabilizing two actin subunits in the helical orientation found at the barbed end of actin filaments, and to stair-step on the barbed end of the nascent filament as it grows.
Ras as an anti-cancer drug target

Alfred Wittinghofer
Department Structural Biology, Max-Planck-Institute for Molecular Physiology, Dortmund, Germany

The product to the Ras oncogene is involved in the transduction of signals from the plasma membrane to the nucleus. The function of Ras as a GTP-binding protein is to switch between the GTP-bound active and the GDP-bound inactive state. Through the action of many different cell surface receptors, Ras becomes loaded with GTP and thus activated. In its GTP-bound state it interacts with effector proteins and through this interaction the effectors pick up the signal originating from the cell surface. The inability to hydrolyse GTP in oncogenic point mutations of Ras or by mutations in the NF1 gene contributes to tumour formation in many types of human neoplasia. Therefore Ras is considered to be a prime target for the development of anti-cancer drug. In addition there are other diseases such as tuberous sclerosis where a defect in a GTPase reaction (i.e. of RheB) leads to tumours.

The interaction of Ras with downstream targets such as the protein kinase Raf has been investigated in detail and three-dimensional structures of the interactions are available at high resolution. Thus the information could potentially be applied for the development of molecules inhibiting that interaction. It will be discussed that there is a large number of Ras Association or Ras Binding Domains in the database, which makes development of specific inhibitors a difficult task.

The GTPase of Ras is very slow and is stimulated $10^5$ fold by GTPase activating proteins p120-GAP or neurofibromin. The mechanism of this stimulation has been investigated both biochemically and structurally and has shown that GAP actively participates in the GTPase reaction by supplying an arginine into the active site. Oncogenic mutants of Ras have a reduced intrinsic GTPase rate and are furthermore not stimulated by GAP. Approaches to stimulate the GTPase reaction would be valuable as leads for the development of anti-Ras drugs. Biochemical and structural studies towards that goal will be discussed. They show that it should in principle be possible to develop the proper chemistry to induce GTP hydrolysis in oncogenic Ras.
Atomic force microscopy measurement of SDF-1 mediated affinity modulation of single VLA-4 – VCAM-1 bonds

Robert Eibl
Department of Physiology and Biophysics, University of Miami, Miami, FL, USA

Atomic force microscopy (AFM) measurements have recently been used to quantify cell adhesion forces of living cells down to single-molecule interactions. This study characterizes the rupture force of single VLA-4 – VCAM-1 adhesion bonds of a living cell.

At low physiologic concentration of SDF-1 the rupture force increases from 40pN to 60-80pN, whereas at higher concentrations of SDF-1 it lowers the rupture forces back to 40pN. This study confirms that arrest chemokines such as SDF-1 can rapidly modulate the affinity of an integrin receptor. To date, this is the first direct measurement of chemokine induced affinity modulation of a single receptor as well as of the desensitization of a chemokine receptor, both measured on a molecular level on a living cell. AFM has evolved into a new pharmacological test for single-molecule interactions on living cells.
Structural insight into transcription factor activation and nuclear transport

Carlo Petosa, Montserrat Soler-López, Christoph W. Müller
EMBL, Grenoble Outstation, Grenoble, France

The cellular localisation of many transcription factors including NFkB, STAT and P53 represents one important level of transcriptional regulation. Disruption or deregulation of the nuclear transport of transcription factors often result in severe cellular malfunctions and disease including different types of cancer. Soluble transport carriers named karyopherins mediate the nuclear transport of most transcription factors through the nuclear pore. Most prominent members of the karyopherin superfamily are importin β responsible for the nuclear import of NLS containing cargoes bound to importin α and CRM1/exportin1 responsible for the export of cargo through interaction with nuclear export sequences (NES). Our group uses X-ray crystallography to gain structural insight into the mechanisms, which control the nuclear transport of transcription factors and their interactions with nuclear transport receptors.

In recent years we determined structures of mammalian STAT3 bound to DNA and Dictyostelium STATa (DdSTATa) in its DNA-unbound form. DdSTATa adopts a fully extended conformation remarkably different from DNA-bound STAT3, which implies a large conformational change upon DNA recognition. Such a conformational change might be critical to ensure that only the desired species is recognized by nuclear transport factors. In DdSTATa buried hydrophilic residues predicted to destabilize the coiled coil domain suggest how hydrophobic residues may become exposed and mediate interaction with the export receptor CRM1. Other possible interactions of STAT molecules with nuclear transport receptors and implications for the design of transport inhibitors will be discussed.
The eukaryotic chaperonin CCT: a folding nanomachine with many important roles

José M. Valpuesta
Centro Nacional de Biotecnología, CSIC, Campus de la Universidad Autónoma de Madrid, Spain

Although originally characterised as a very specific molecular chaperone involved in the folding of cytoskeletal proteins, it is becoming more evident that the cytosolic chaperonin CCT plays a key role in several important cellular processes such as protein folding and association, protein degradation and control of cell signalling and recognition. In most of these roles, CCT acts in the folding or assembly of proteins that are critical in such processes. CCT shares with the rest of the chaperonins a common toroidal structure formed by the oligomerization of 60 kDa proteins. The toroid is made of two rings placed back-to-back with each ring enclosing a cavity where folding takes place. CCT is the most complex of all chaperonins known so far, and whereas the rest of chaperonins are composed of 1-3 different subunits, the eukaryotic chaperonin is composed of 8 different, albeit homologous proteins.

In most of the cases, chaperonins act on unfolded substrates using a non-specific mechanism that involves the recognition of the unfolded polypeptide by hydrophobic residues at the entrance of the chaperonin cavity, followed by folding of the polypeptide upon closure of the cavity induced by the binding of ATP and a co-chaperonin. However, CCT operates in a different manner, and this has been revealed mainly by electron microscopy studies carried out with CCT two major substrates, actin and tubulin. These studies have shown that CCT interacts with its folding substrates through defined CCT subunits and specific domains of these substrates, which have already acquired a large degree of native-like conformation before interacting with CCT. The conformational changes undergone by CCT upon nucleotide binding would be used to actively fold the two cytoskeletal proteins. This mechanism seems to be common for most of CCT-interacting proteins, although in some cases, the conformational changes are not used by CCT to fold a protein but to generate a stable and functional complex with a partner protein.
Structural basis for the regulation of Vav3 Rho/Rac guanosine exchange factor during normal and oncogenic activation

Óscar Llorca¹, Ernesto Arias-Palomo¹, José L. Zugaza², and Xosé R. Bustelo²

¹Centro de Investigaciones Biológicas, CSIC, Madrid, Spain. ²Centro de Investigación del Cáncer and Instituto de Biología Molecular y Celular del Cáncer. University of Salamanca-CSIC, Salamanca, Spain

Rho/Rac small GTPases regulate kinase cascades, transcription, cell shape and motility (Etienne-Manneville and Hall, 2002). Rho proteins are molecular switches that cycle between an inactive GDP-state and an activated GTP-bound conformation (Etienne-Manneville, 2002). GDP/GTP exchange is catalysed by Guanosine Exchange Factors (GEFs) containing tandem Dbl-homology (DH) and pleckstrin-homology (PH) domains (Cherfils and Chardin, 1999), being the Vav-family one of the best characterised (Bustelo, 2000). We have elucidated the structural basis of Vav3 regulation in the context of the whole protein, thanks to the use single-particle electron microscopy (EM) (Llorca et al., 2004). 3D structures of Vav3, activated Vav3 upon phosphorylation and an oncogenic truncated mutant have been obtained, which reveal that Vav3 activation is modulated through the concerted interaction and movement of several domains. The calponin-homology (CH) domain acts as a major player thorough its interaction with the zinc-finger region (ZF) that creates an inhibitory conformation where both the Acidic (Ac) and CH domains cooperate to block the access of the GTPases into the catalytic region. This inhibitory conformation is relieved after Lck-mediated tyrosine phosphorylation by synchronised motion of most of Vav domains. Interestingly, we have found that the conformations of phosphorylated Vav3 and N-terminally deleted Vav3 are distinct, suggesting that the activation by exchange factors is different under normal and oncogenic conditions. This model was further supported by prediction and testing of mutations with activating potential.
The HP1 Protein: from Structure to Function

Abarna Thiru, Peter R. Nielsen, Daniel Nietlispach, Helen R. Mott, Juliana Callaghan, Natasha V. Murzina, Ernest D. Laue
Cambridge Centre for Molecular Recognition, Department of Biochemistry, University of Cambridge, Cambridge, UK

HP1 and its homologues are structural components of heterochromatin that play an important role in both heterochromatin-mediated gene silencing and repression at euchromatic promoters.

Recently it has become clear that histone methylation plays a key role in the regulation of chromatin structure and thus gene expression. Methylation at lysine 9 of histone H3 strongly correlates with a repressed chromatin state. This modification is specifically recognised by the HP1 chromodomain. To understand methyllysine recognition, and to illuminate the role of other chromodomains, we recently determined the structure of the HP1b chromodomain bound to a histone H3 peptide, dimethylated at the lysine 9.

In a yeast two-hybrid screen we identified interacting partners of the C-terminal shadow chromodomain of mouse HP1. These included the large subunit of the chromatin assembly factor, CAF-1p150, the transcriptional intermediary factor, TIF1β, and IND3, a protein thought to be involved in chromosome condensation at meiosis. Here we present the first structure of a shadow chromodomain complex, namely that with the large subunit of CAF-1. The structure reveals an unusual mode of protein-peptide recognition in which a single peptide binds across the dimeric interface of the shadow chromodomain.

This new structure, in combination with biochemical studies of the localisation of structure-based HP1 mutants, defines a general model for possible interactions mediated by the chromo and shadow chromodomains in HP1, which will be discussed.
NMR, Ribonucleases and Cancer

Manuel Rico

Instituto de Química Física “Rocasolano”, CSIC, Madrid, Spain

Bovine pancreatic ribonuclease (RNaseA) has been the subject of extensive physical and biochemical studies. It is the best known member of a superfamily of secretory enzymes that operate at the crossroads of transcription and translation by catalyzing RNA degradation. Interest in the field of ribonucleases was renewed after some of these proteins, closely related to RNaseA, were shown to be much more than digestive enzymes, since in fact some of them are endowed with novel biological activities, included that of being very efficient tumour inhibitors. Angiogenin is a plasma enzyme that promotes the growth of new blood vessels, and bovine seminal ribonuclease (BSRNase), a unique dimeric homolog of RNase A, has immunosuppressive, embryotoxic, aspermatogenic and antitumour activities. Onconase, an amphibian protein, toxic to tumour cells both in vitro and in vivo, is in phase III human clinical trials as a cancer chemotherapeutic agent. The cytotoxicity of pancreatic-type ribonucleases appears to depend on their ribonucleolytic activity as well as on their ability to evade the cytosolic ribonuclease inhibitor (cRI), a 50 kDa protein containing 15 sequential leucine-rich $\beta$-$\alpha$ repeats in the shape of a horseshoe. Knowledge of the molecular structure of the complex of RNase A with its inhibitor is at the basis of a rational design of RNase variants with specific and useful cytotoxicity. BSRNase is able to evade cRI because of being a swapped dimer. A dimer of human pancreatic ribonuclease has been constructed on the basis of the structure of BSRNase that is highly cytotoxic, and that by being human does not elicit the host immune response.

Because of its potential applications as cytostatic and cytotoxic agents against some tumour cell lines, interest is growing in a well-defined family of fungal extracellular ribonucleases represented by ribonuclease T1. One of the most interesting proteins within that family is $\alpha$-sarcin, a 150-residue cytotoxic protein that inactivates ribosomes by selectively cleaving a single phosphodiester bond in a universally conserved sequence of the 28S ribosomal RNA. Interestingly, $\alpha$-sarcin is able to translocate biological membranes affected by toxic agents, virus infection or malignant transformation. $\alpha$-Sarcin is then a potent cytotoxin that promotes apoptosis of human tumour cells after internalization via endocytosis. In recent years, we have determined a highly refined solution structure of $\alpha$-sarcin and some of its mutants by using NMR methods, with the objective to have a better understanding of its high enzymatic specificity as well as its remarkable ability to enter biological membranes.
Structural Basis and Regulatory Mechanism for the p53 Tumor Suppressor Transactivation Domain Interaction With Replication Protein A

Lilia Kaustov1, Ayeda Ayed1, Jack C. C. Liao1, Andrei Okorokov2, Elena Bochkareva1,4, Jo Milner2, Alexey Bochkarev1,4, Cheryl H. Arrowsmith1

1Department of Medical Biophysics and 3Banting and Best Department of Medical Research and Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Canada. 2YCR P53 Research Group, Department of Biology, University of York, York, UK. 4Department of Biochemistry and Molecular Biology, University of Oklahoma, Health Sciences Center, Oklahoma City, Oklahoma, USA

Human replication protein A (RPA) is a heterotrimer with subunits of 70, 32 and 14 kDa (RPA70, RPA32 and RPA14, respectively), which is involved in both DNA replication and repair. RPA binds to single stranded DNA and serves as a scaffold for the assembly of the DNA processing machinery. RPA is also capable of complex formation with the tumor suppressor protein p53 in a mechanism regulated by the presence of ssDNA and phosphorylation of RPA. This regulated association is implicated in transmitting the DNA damage signal to the p53-dependent pathway of stress response. In this study, using a combination of biochemical methods, NMR Spectroscopy and X-ray crystallography we demonstrate that the N-terminal domain of the 70 kDa subunit of RPA binds specifically to residues 39-57 of p53 and report their structure at 1.6 Å resolution.

RPA70N shares the characteristic OB (oligonucleotide/oligosaccharide-binding) fold with ssDNA-binding domains (DBDs) of RPA. We performed NMR titration experiments on [15N]-labeled RPA70N with a non-labeled p53 fragment to map the RPA residues involved in p53 binding to those in the basic cleft. NMR experiments on selectively [15N,13C]-labeled p53 with a non-labeled RPA70N fragment allowed us to assign the backbone NMR resonances of RPA70N-bound p53 complex. The changes in resonance frequency for p53 residues 38-58 indicate the formation of an α-helix upon binding to RPA70N. Using further 15NHSQC NMR titration experiments we demonstrate that ssDNA and/or negatively charged peptide mimicking phosphorylated Nterminal domain of the RPA32 subunit are capable of competing p53 out of the binding site.


**Mad conformational signaling in the mitotic spindle checkpoint**

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Even chromosome segregation during mitosis is crucial to ensure that each daughter cell inherits a full complement of chromosomes. Failures in proper chromosome segregation lead to aneuploidy, which is a hallmark of many cancer cells. The fidelity of chromosome inheritance is ensured by a surveillance mechanism, called the mitotic spindle checkpoint, which is activated in response to mis-aligned sister chromatids. The checkpoint protein Mad2 is believed to act as a diffusible inhibitory signal that is activated by Mad1 and blocks the activity of the ubiquitin ligase APC/C \(^{\text{Cdc20}}\) by binding to Cdc20. Mad1 and Cdc20 bind to the same site of Mad2, yet the Mad1-Mad2 interaction is required for binding of Mad2 to Cdc20 in vivo. This apparent paradox is explained by the observation that Mad2 exhibits a striking two-state behavior. In the absence of ligands, cofactors or covalent modifications, Mad2 adopts two remarkably different tertiary structures that have comparable free energies and are in very slow equilibrium at room temperature. We refer to these two states of native Mad2 as N1 and N2. The three-dimensional structure of N2 in solution resembles the structure of Mad2 bound to Cdc20 or Mad1, whereas in the N1 structure a β-strand rearrangement results in occlusion of the Cdc20- and Mad1-binding site. N2-Mad2 is a much more potent APC/C inhibitor than N1-Mad2, but monomeric Mad2 in HeLa cells is mostly in the N1 state. Overexpression of a Mad2 mutant that can only adopt the N1 state and sequesters the N2 state through heterodimerization inhibits checkpoint signaling in a dominant negative fashion in vivo. Furthermore, the interconversion between the two Mad2 conformers is accelerated by a fragment of Mad1 that includes its Mad2-binding sequence. These results suggest a model whereby the unusual two-state behavior of Mad2 is critical for spindle checkpoint signaling. In this model, Mad1 acts as a catalyst that accelerates the conversion of the inactive (N1) state of Mad2 to its active (N2) state.
Structural analysis by NMR of the interactions between integrin and a peptide inhibiting angiogenesis.

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Tumour growth depends on angiogenesis, the recruitment of new blood vessels. Extracellular matrix proteins play a crucial role in angiogenesis. One of them is laminin, a complex protein composed of three polypeptide chains. An antiangiogenic therapy that targets laminin could be efficient for blocking tumour vascularization.

Recently, an anti-laminin antibody (called L36), that inhibits capillary morphogenesis in vivo and in vitro, has been reported. Phage display-assisted mapping permitted the identification of a peptide mimotope whose sequence corresponds to the middle part of the coiled-coil portion of laminin and also inhibits capillary morphogenesis. The fact that this peptide (called KHARS peptide) binds to the integrin alpha-2 I-Domain\(^1\) establishes a good basis for the structural study of the laminin-integrin interaction.

In a first step, the solution structure of this peptide (corresponding to the sequence: CLPKHARSCGGGS, with the cysteines forming a disulphide bond) has been determined by NMR spectroscopy. The peptide presents two populations of well defined structures, whose overall shape is driven by the conformation of the peptide bond preceding the proline residue. In the two populations of structures, the side-chains corresponding to the KHARS sequence are facing outward the cyclic peptide structure.

The binding of this peptide to the antibody L36 and the integrin alpha-2 I-Domain has been studied by the changes in their intrinsic fluorescence upon addition of the peptide. This result shows that the KHARS peptide binds to the two proteins, with different affinities (Kd=7uM for L36 and Kd=2uM for the IDomain). Using NMR spectroscopy, the residues from the protein that interact with the peptide can be detected by measuring the changes in its 1H-15N HSQC spectrum upon addition of peptide. A first step of this study consists in the assignment of all backbone amide 1H and 15N resonances, which has been performed using heteronuclear three-dimensional techniques. The residues that show the largest perturbations are located in a defined area on the surface of the integrin alpha-2 I-Domain. This region is also involved in the binding of collagen, a molecule which also adopts an elongated triple helical structure, similar to the laminin conformation.

The detailed analysis of the peptide-integrin binding will allow us to design improved peptide sequences, with stronger antiangiogenic activity.

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Targeting Host Transcription by Direct Histone Lysine Methylation: The Role of a Viral SET Domain

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Site-specific lysine methylation of histones by the SET domain-containing histone methyltransferases (HMTs) is a key epigenetic mechanism for gene regulation in all eukaryotic organisms. SET domain proteins are also encoded by viruses, but the cellular functions of these viral proteins have not been characterized. In this talk, I will present results from our recent structural and functional studies of a SET domain protein from Paramecium bursaria chlorella virus (referred to as vSET). Our previous study of the NMR-derived structure of vSET shows a butterfly-shaped head-to-head symmetric dimer with each subunit consisting of an anti-parallel beta-barrel and a three-stranded open-faced sandwich that mediates the dimer interface. vSET exhibits highly specific HMT activity for di-methylation of lysine 27 in histone H3 (H3-K27) in vitro and in vivo. Our new three-dimensional solution structure of vSET in complex with a H3-K27 peptide and cofactor S-adenosyl-L-homocysteine provides new insights into the structural and molecular basis for substrate specificity and HMT catalytic mechanism of this viral SET domain HMT. Moreover, our current study on the cellular function of vSET reveals that the protein is present in mature virions, contains a nuclear localization signal and induces transcriptional repression by selective methylation of host histone H3 at K27, a site-specific post-translational modification that has been implicated in eukaryotic gene silencing. In addition, we show that cells transfected with vSET undergo G2/M cell cycle arrest. We further demonstrate that vSET-like proteins exhibiting H3-K27 methylation activity are universally conserved in this group of chlorella viruses. Viruses are known to recruit cellular proteins in order to integrate viral genomes into host chromatin or to activate transcriptional machinery for viral DNA replication. Our study, however, argues for a novel mechanism by which virus-encoded proteins trigger transcriptional silencing by directly carrying out site-specific post-translational modification of histones.
Computational Structural Proteomics and Drug Discovery

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Structural proteomics revolutionized the process of drug discovery and generates a wealth of critical information about potential cancer drug targets. Computational methods shorten the distance between a protein target and a lead candidate dramatically.

Early prioritization of drug targets is the first important problem. After the biological significance of a target is established, we can evaluate feasibility of developing a potent and specific small molecule from a structural analysis of a 3D model of the target protein and genome-wide evolutionary analysis of potential cross-reactivity. We developed new technology for identification and evaluation of druggable sites which can also be used to prioritize the targets and direct ligand optimization.

The second group of approaches helps to build a reliable three-dimensional model of a target pocket in a state appropriate for flexible docking. It may be necessary if 1) one needs to build a model by homology, 2) parts of your receptor structure are inaccurate or missing, 3) the receptor structure is in an “unbound” state and needs to be rearranged, 4) a mutant form of a receptor needs to be targeted. New results on mutant prediction, loop prediction, sequence structure alignments, large scale flexibility simulations are presented. In addition, machine-learning methods can be used to predict sites of protein-protein interactions which can be targeted by an anti-cancer drug.

Further improvements of the ligand docking and scoring technology (virtual ligand screening) require a solution of the problem of receptor pocket changes upon compound binding. In a number of cases this problem can be partially solved by combined ligand-pocket simulations in which both side-chains and parts of the backbone are flexible. The technology can be modified and extended to the ligand optimization phase of the drug discovery process.

Finally, by predicting the ADMET properties of the compounds early in the discovery process, we can further narrow down the search and avoid frequent pitfalls associated with poor solubility, reactivity, permeability, etc.

Case studies of structure based ligand discovery projects are presented with a particular focus on kinases and nuclear receptors. Predicting structural effects of mutations can help to understand the escape mutations and potential design mutant-specific inhibitors.
Exploring the DNA for the design of new therapies

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Cancer and many other pathologies are ultimately related to the errors in the function of nucleic acids. However, even now only 1% of the drugs used to fight pathologies use the DNA as the primary target. This poor development of nucleic acid-based therapies has many origins, among them: i) the lack of clear information on when a gene can be targeted by a given oligonucleotide, and ii) the lack of accurate structural information on the physiological conformation adopted by target nucleic acids. In my talk I will summarize some of our recent advances on the use of both sequence-based and structural-based bioinformatic tools on the high-scale analysis of nucleic acids. I will show how there are three levels of information that can be obtained by a reasonable combination of theoretical methods: i) sequence based information, ii) static structural data and iii) flexibility and dynamic data. I will try to convince you how a complete picture of nucleic acids.
Protein Structures in Virtual Screening

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Introduction

Many papers have been published that describe virtual screening programs, comparisons of such programs, methods of using them (e.g. consensus scoring, consensus docking), and various theoretical aspects of them. To a large extent most of these publications have tried to define the ‘best’ method of reproducing crystallographically observed ligand poses and/or calculating theoretical binding constants (or docking scores) that bear some resemblance to (or correlation with) experimentally determined values. One variable that is rarely discussed is the effect of protein structure on the success of dockings.

One exception to this is McGovern & Shoichet (2003) who describe how a set of 95,000 small molecules, seeded with known ligands, was docked into holo, apo, and modelled conformations of ten different proteins. The main points to arise from this work were threefold. Firstly, the performance of a molecular docking screen depends on the particular conformation of the receptor used in the calculation. Secondly, the crystallographically determined holo conformation is the one most likely to yield meaningful enrichment of known ligands from a database containing mostly decoy molecules. Thirdly, if the holo structure is overspecialized, the apo or modelled structures may provide better discrimination between true ligands and decoys.

There were no kinases amongst the enzymes studied by McGovern & Shoichet (2003). Given their ubiquity, role in disease states, and potential as drug targets, this paper sets out to see if the same conclusions can be reached for kinases, specifically CDK2. More than sixty CDK2 crystal structures are publicly available. Is it possible to identify one structure that is ‘the best’ for virtual screening?

Methods

Two commonly used programs, Glide and GOLD, were used to dock, in a variety of docking modes (i.e. different speeds of docking), a set of 340 CDK2 inhibitors into twenty different CDK2 crystal structures (eighteen holo structures and two apo structures). For each program/protein structure combination, the number of docked poses that reproduce the crystallographically observed pose are reported.

Results

The results across both programs and all docking modes broadly agree: those protein structures that have a large number of correctly docked ligands with one program operating in one docking mode tend to have a large number of correctly docked ligands with both programs and all docking modes. The percentage of compounds correctly docked ranged from 0.3% to 96.2% depending on the program, docking mode and protein structure.
Discussion

Although it is not possible to say that any one structure is ‘the best’ for virtual screening there are some structures that are clearly better than others. The main determinants of this are the volume of the binding site into which the ligands are docked, and the exact orientation of the residues forming the binding site.

References

Elongation factor eEF1A as a target for antitumour didemnins: insight from molecular modelling and computer simulations

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Didemnins and tamandarins are closely related marine natural products with potent inhibitory effects on protein synthesis and cell viability. On the basis of available biochemical and structural evidence, and results from molecular dynamics simulations, a model is proposed that accounts for the strong and selective binding of these compounds to human elongation factor eEF1A in the presence of GTP. We suggest that the p-methoxyphenyl ring of these cyclic depsipeptides is inserted into the same pocket in eEF1A that normally lodges either the 3’ terminal adenine of aminoacylated-tRNA, as inferred from two prokaryotic EF-Tu·GTP·tRNA complexes, or the aromatic side chain of Phe/Tyr-163 from the nucleotide exchange factor eEF1Bα, as observed in several X-ray crystal structures of a yeast eEF1A:eEF1Bα complex. This pocket, which has a strong hydrophobic character, is formed by two protruding loops on the surface of eEF1A domain 2. Further stabilization of the bound depsipeptide is brought about by additional crucial interactions involving eEF1A domain 1 in such a way that the molecule fits snugly at the interface between these two domains. In the GDP-bound form of eEF1A this binding site exists only as two separate halves, which accounts for the much greater affinity of didemnins for the GTP-bound form of this elongation factor. This binding mode is entirely different from those seen in the complexes of the homologous prokaryotic EF-Tu with kirromycin-type antibiotics or the cyclic thiazolyl peptide antibiotic GE2270A. Interestingly, the set of interactions used by didemnins to bind to eEF1A is also distinct from that used by eEF1Bα or eEF1Bβ thus establishing a competition for binding to a common site that goes beyond simple molecular mimicry. The model presented here is consistent with both available biochemical evidence and known structure-activity relationships for these two classes of natural compounds and synthetic analogues and provides fertile ground for future research.
Target Family Landscapes: Combined Interpretation of Ligand Selectivity and Protein Binding Site Topology for Kinases and Proteases

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The organization of pharmaceutically relevant proteins into target families based on structural biology criteria provides an important framework to study the relationship of binding site motifs to privileged chemical scaffolds. It is well-documented in drug discovery that many structurally similar targets with similar interactions to ligands are implicated in different therapeutic areas. A key question is, how to generate and efficiently use this information to guide drug design across related targets.

The search for factor Xa inhibitors as relevant pharmaceutical target will be used in this contribution as one example to illustrate important aspects for target affinity and selectivity. The integration of structure-based design –stimulated by an increasing availability of high resolution X-ray structures– and statistical 3D-QSAR approaches is useful to unravel structural determinants for affinity towards one target. The extension of this integration can lead to the development of target-specific scoring functions after extracting knowledge from protein-ligand complexes and ligand information alone. In addition the attempts to control selectivity against related serine proteases like thrombin, trypsin and others will be discussed, including statistical approaches capturing information for ligands or protein binding sites.

While there are now computational approaches of increasing complexity to quantify similarity among ligands, related tasks for proteins are often not going beyond a one-dimensional sequence comparison. Here the Target Family Landscape concept –based on a multivariate description of protein binding sites using force field or knowledge-based molecular interaction fields (MIFs) in combination with consensus PCA (CPCA)– is useful to study features for unspecific interactions to an entire family and to identify determinants for interactions to only one member. Some applications of this concept will be discussed for serine proteases as well as for the family of protein kinases, where an understanding of binding affinity determinants in ATP binding pockets is key for designing more selective kinase inhibitors for applications in different therapeutic areas.
Modeling ligand selectivity in lead optimization

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An extension of COMBINE analysis is presented to consider an arbitrary number of structurally related receptors in the derivation of a single structure-activity model. Using a fully automatic approach of structural alignment, ligand docking, interaction energy decomposition and statistical modelling, highly predictive regression models for multiple receptors are obtained. This constitutes a step forward towards structure-activity models for entire protein families. Coupled to the use of homology models, this approach could be of considerable help in chemical genomics, particularly as applied to cancer targets. We will present applications to pharmacologically relevant targets where discrimination among paralogs and/or orthologs is important for the drug action.
Combinatorial Chemistry approaches to cancer related pharmacological targets

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It is widely accepted the urgent need for discovering new and efficient chemical entities for the treatment of pathological conditions such as cancer, a degenerative disease that still constitutes a challenge in many aspects. Among the approaches that have emerged from different fields of science trying to meet that challenge, medicinal chemistry has provided the combinatorial library strategies. Ten years after from its worldwide implantation, combinatorial chemistry is recognised as a highly convenient tool for exploring the chemical diversity space in a rapid and efficient manner. From the collections of libraries of non-biased, controlled mixtures, constructed basically under the split and pool format, to those of discrete molecules already addressed towards a defined pharmaceutical target, thousands of molecules have been synthesised and screened. Although it must be admitted that combinatorial libraries have not fulfilled all the initial expectations for drug discovery, it is also true that over 70 molecules identified through this approach are currently in advanced preclinical or even in clinical trials. More recently, the combichem world has found a further expansion to the virtual scenario. Actually, the in silico design and screening of combinatorial libraries has become another convenient tool to speed up drug discovery.

In the present communication, use of the above combinatorial chemistry strategies for identifying molecules exhibiting activity on targets related to tumour growth and development, will be reported. Specifically, the screening of a library of N-alkylglycine trimers (peptoids) constructed under the positional scanning format led us to identify 2 peptoids causing an intracellular accumulation of daunomycin higher than verapamil. Cellular cytotoxicity assays showed that these peptoids decreased by $\geq 3$-fold the degree of resistance of leukemia cells, presumably by blocking P-gp drug efflux activity. These peptoids fit satisfactorily into a pharmacophore model generated from the study of the interaction with P-gp. Secondly, the screening of the same library addressed to identify selective inhibitors of urokinase plasminogen activator (UPA) and more interestingly, tissue plasminogen activator (tPA), has afforded promising compounds for this latter target. In all the above cases, research efforts addressed to the synthesis of a second generation of active molecules in which the peptoid skeleton had been subjected to conformational restrictions are in due course. Finally, details on the in silico design and screening of a library of potential inhibitors of histone deacetylase will be also presented.
Structural units of the interactome: Some concepts and their potential relevance for therapeutic situations

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Drug targets are normally selected among those proteins which are thought to be relevant for a particular pathway of interest. Once a target is chosen, drug development is mainly focused on understanding how the structural biology of the target influences its drugability.

This strategy might experience two problems. First, current protein-protein interaction studies are shifting our conception of biological organization from simple pathways to complex networks. Second, altering the activity of a particular protein might have unexpected functional consequences depending on the target network location. Here, I will discuss some ideas for the characterization of protein-protein interaction networks by means of intermediate structural units or modules. By using the phylogenetic correlation, we can differentiate those modules with potentially stronger biological relevance. Finally, I will discuss a simple example of how such structural units could be used for finding antimicrobial targets in poorly studied organisms which hints at the potential relevance of understanding modular organization for other therapeutic situations.
Tricking Cancer Cells To Die

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Cancer cells develop strong anti-apoptotic signaling pathways and therefore escape many therapeutic regimens. Recognizing this feature of cancer cells, we have focused on two approaches: disarming the cancer cell from its anti-apoptotic weaponry (1, 2) and applying strategies aimed at enhancing pro-apoptotic signaling pathways selectively in the cancer cell (3). The first goal has been achieved by developing highly selective Aktstatins (1, 2) that inhibit PKB 100 times better than PKA or PKC. These inhibitors are highly non-toxic, inhibit Akt/PKB induced phosphorylation in cells and in vivo and are highly effective as anti-tumor agents in vivo.

The complementary strategy is to enhance pro-apoptotic signaling pathways, selectively in cancer cells (3). One of the key elements is to induce in the targeted cancer cells signaling pathways that induce strong by-stander effects, killing pretty fast not only the targeted cells but also the neighboring cancer cells that do not express the target, a common situation in the heterogeneous human tumor. We have achieved this goal for tumors over-expressing the EGF receptors, by targeting them with EGF guided non-viral vectors loaded with double stranded RNA. These dsRNA molecules are internalized by EGF receptor mediated endocytosis and kill only cells that over-express wild type EGFR, and neighboring tumor cells co-growing with the targeted cells. Using this targeted polyIC, we are able to cure mice bearing sizable intracranial human Glioblastoma Multiforme (GBM), without harming normal brain tissue (4).

References


Recent advances in proteomics, bioinformatics and structural genomics have opened a floodgate to thousands of possible protein targets for drug discovery. This overwhelming increase in data presently inspires the development of new technologies for lead identification. Increasingly, we learn that proteins exist in families sharing similar biochemical function. Even though, located in distinct compartments of an organism and involved in very different cell biological processes they still can be addressed by ligands showing the same recursive skeleton specifically addressing a conserved binding epitope common to all members of a protein family. This observation has strongly influenced the ideas about privileged ligand templates being central part of a compound library. Through specific decoration with appropriate side chains individual library entries can be tailored with respect to selectivity towards particular family members.

We developed a new algorithm, to infer protein function directly from the 3D structure, independently from its fold and sequence similarity with other proteins. The approach is based on the assumption that protein function is intimately connected with the recognition and chemical modification of endogenous ligands such as substrates, agonists, antagonists or allosteric effectors. Recognition and chemical transformation occurs in well-characterized clefts or cavities on protein surfaces. It is accordingly assumed that common recognition patterns (“privileged environments”) or chemical reaction conditions are conserved throughout the binding pockets of proteins of similar function. Our approach allows to compare and cluster the binding epitopes in binding pockets of proteins either with respect to similarities or differences. Examples for the design of new aspartyl protease inhibitors will be used to explain the concept.

As a complement and alternative for lead finding, virtual screening has been developed. In contrast to high-throughput screening, virtual screening is a knowledge-driven computer-based approach and it departs from the 3D structure of the target protein. It tries to screen databases of putative candidate molecules by docking and molecular similarity analyses. We have developed a strategy based on successive hierarchical filtering to reduce the initial sample of up to a million database entries to some hundred prospective hits. At first, the protein binding pocket under consideration is analyzed in terms of hot spots indicating areas that most likely accommodate functional groups of putative ligands. Once such interaction sites are detected that could anchor a ligand in the binding pocket, a protein-based pharmacophore hypothesis is developed. This hypothesis is then used to screen the ligand database. Through a series of increasingly stringent filters the original data sample of about a million commercially available compounds is gradually reduced to about 5,000 to 10,000 entries. Subsequently, they are flexibly docked and scored in the binding site. The final list of detected hits is visually inspected and the most promising 10-20 ligands are purchased for experimental binding studies including crystal structure analysis. We applied this strategy to different enzymes and a GPCR (carbonic anhydrase II, thermolysin, tRNA guanine transglycosylase, aldose reductase, NK1 receptor). In all cases, we could discover successfully micro- to sub-micromolar binding ligands. For the considered enzymes, a crystal structure has been previously determined and for the GPCR a reasonable homology model could be built.
Drug Discovery at Adaptive Surfaces

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Fragment-based methods of drug discovery are emerging as an alternative approach to traditional library screening. These methods offer the ability to access a large chemical diversity while synthesizing and screening significantly fewer compounds, and are particularly powerful when combined with structure-based drug design. At Sunesis we have developed Tethering®, a procedure for identifying low molecular weight compounds that bind to targeted sites on protein surfaces. Once identified, the challenge of assembling these fragments into viable drug leads remains. To this end, a second-generation version of the technology, Tethering® with Extenders, has been developed. This method facilitates not only the identification of fragments but also their assembly into inhibitors using dynamic combinatorial chemistry. We have used these methods to discover potent small molecule inhibitors against a variety of clinically relevant targets, and they are particularly well suited to the identification of kinase inhibitors. Tethering® with Extenders allows for the targeting of adaptive regions away from the nucleotide binding pocket, resulting in compounds with increased drug-likeness and selectivity.
Small-molecule inhibitors of p53-MDM2 binding

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Tumor suppressor p53 is a transcription factor which plays a central role in prevention of tumor development by inducing cell cycle arrest or apoptosis in response to diverse form of stress. In unstressed cells, p53 level is tightly controlled by MDM2 which binds p53 and negatively regulates its activity and stability. MDM2 is overproduced in many human cancers as a mechanism to impair p53 function. Antagonists of p53-MDM2 interaction could reactivate p53 and may offer a novel approach to cancer therapy.

We have developed the first potent and selective small-molecule inhibitors of the p53-MDM2 binding. These cis-imidazoline derivatives, named Nutlins, bind to MDM2 with high affinity and displace p53 with IC$_{50}$ in the 100-300 nM range. Crystal structures of the Nutlin-MDM2 complexes revealed that imidazolines interact with the p53-binding pocket of MDM2 by projecting functional groups that mimic critical amino acid residues from the p53 molecule.

Treatment of cancer cells harboring wild-type p53 with MDM2 antagonists stabilized p53 and activated the p53 pathway as revealed by the induction of multiple p53-regulated genes. This led to cell cycle arrest in G1 and G2 phases followed by apoptosis. All of the above occurred only in cells with wild-type but not mutant p53 confirming that the cellular activity of Nutlins is derived from intervention in MDM2-mediated p53 regulation. Human and mouse fibroblasts showed comparable sensitivity to Nutlin treatment suggesting that mouse xenograft models can assess adequately the potential therapeutic window of this new strategy.

Oral administration of MDM2 antagonists to nude mice bearing established human tumor xenografts caused dose-dependent tumor inhibition and tumor regression at non-toxic concentration. Our experiments suggest that MDM2 antagonists may have therapeutic utility in the treatment of tumors that have retained wild-type p53. They also validate the concept that small-molecule protein-protein binding inhibitors can be developed as a therapeutic modality in cancer.
Structural basis for substrate recognition of mannose receptor family members

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The Endo180 lectin receptor, also known as the urokinase plasminogen activator receptor (uPAR) associated protein (uPARAP), and the mannose receptor (MR) are members of mannose receptor family. Endo180 is implicated to play a role in extracellular matrix remodelling and is highly upregulated in different types of tumour endothelium and in some breast cancer tissues. On the other hand, MR mediates endocytosis of glycoproteins and phagocytosis of a wide variety of microbes including yeast, fungi, protozoa and bacteria (1).

Members of mannose receptor family are multidomain cell-surface receptors that share the same overall structural design (2). Their extra-cellular regions consist of an N-terminal cysteine-rich domain, that in case of MR is able to bind sulfated sugars, followed by a fibronectin type II domain (FNII), responsible for interaction with collagen, and 8 or 10 C-type lectin like domains (CTLDs). However, the majority of these CTLDs do not have C-type lectin activity. Only CTLD2 in the case of Endo180, and CTLD4 and 5 in case of MR are central for Ca2+ dependent sugar ligation. Endo180 can also associate on the cell surface in a trimolecular complex with urokinase-type plasminogen activator (uPA) and its receptor (uPAR) participating in the control of cell adhesion and migration. After a single transmembrane domain, there is a short cytoplasmatic domain essential in recycling all family members between the plasma membrane and the endosomal apparatus.

The biological properties of these receptors are mediated by the interactions of specific domains with substrates from the extra-cellular matrix. Therefore, the 3D arrangement of these ligand binding domains should influence how substrates are recognised. In order to determine the close-to-native 3D structures of two members of mannose receptor family we have purified a soluble form of the Endo180 and MR and observed them by single particle electron microscopy followed by bioinformatics analysis of the collected images. Assignment of protein domains was confirmed by docking of corresponding atomic structures.

We have demonstrated that in both proteins the cysteine-rich domain contacts the specific CTLD responsible for Ca2+ dependent sugar binding (CTLD2 in the case of Endo180 (3) and CTLD4 for MR). Therefore, we hypothesize that the other mannose receptor family members might share the similar domains disposition providing mechanism for regulation of their ligand binding properties.

References:


Structure and dynamics of a biologically active human FGF-1 monomer, complexed to a hexasaccharide heparin analogue by NMR

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The fibroblast growth factors (FGFs) constitute a family of more than twenty signaling polypeptides, that have been demonstrated to be involved in a variety of biological processes including cell proliferation and differentiation. In addition, FGFs are often detected in tumors and it has been shown that they are potent tumor-derived angiogenic factors. It is well established that FGF biological functions are triggered by binding the polypeptide to heparan sulfate glycosaminoglycans (HS-GAGs) and specific transmembrane tyrosine kinase receptors at the cell surface (FGFR). Therefore, it is posible to inhibit the angiogenic activity of this protein by disrupting the interactions with the receptors (1). It has also been proposed that FGF dimerization is a key process in the formation of the FGF:FGFR signaling complex mediated by GAGs either free or bound to the cell surface. However, using well defined synthetic HS-GAG-type oligosaccharides sequences, we have recently described sedimentation equilibrium based data that strongly suggest that GAG-induced FGF dimerization is not an absolute requirement for biological activity (2).

In particular, it was shown that a hexasaccharide in which all the sulfates groups are oriented on only one side of its helical-like structure generates monomeric species that induce the same mitogenic activity as low molecular weight heparin. Herein, we described the structure and dynamics of the biologically active monomeric complex between FGF-1 and this heparin-like hexasaccharide, as determined by NMR.

Uniformly labelled 15N and 13C, 15N double labelled recombinant FGF-1 polypeptides were expressed in E. coli for the NMR studies. Structure calculations were performed using DYANA, with 1432 NOE upper distance constraints. At each stage, 200 structures was selected based on the DYANA target function values. The selected structures were refined by restrained molecular dynamics with the AMBER program. The final structure is fairly well defined, with an average pairwise r.m.s.d. for the protein backbone atoms of 0.99 Å.

Furthermore, in order to study the hexasaccharide conformation in the bound state, we have acquired a 13C-double half filter NOESY for the complex in D2O solution. The experiment has allow us to assign the carbohydrate signals in the complex and to determined the ligand bound conformation. A ley intermolecular NOE asn chemical shift perturbation analysis also permits to position the hexasaccharide in the complex. A 3D picture of the complex can then be proposed on the basis of all experiments.

The putative B7-H1 molecule is expressed in primary cell cultures generated from Saudi breast cancer patients

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The extensive heterogeneity of breast cancer complicates the precise assessment of tumor aggressiveness making therapeutic decisions difficult and treatment inappropriate in some cases. The B7-H1 is a recently identified gene of the B7 family molecules, which encodes a transmembrane protein consisting of an Ig V-like domain and an Ig C-like domain in its extracellular portion. Upon ligation to its receptor PD-1 on T cell, it regulates the activation and the differentiation of T cells; it preferentially costimulates IL-10 production in resting cells and further induces apoptosis of activated T cells presumably by inhibiting cell cycle progression.

Moreover, direct evidence that linked the association of B7-H1 expression and immune evasion by cancer cells came form the observation that tumor-associated B7-H1 induces apoptosis of effector T cells. B7-H1 can be detected on macrophages but not on T and B cells, however, activation by various stimuli can up-regulate the expression of B7-H1 on these cells. The B7-H1 molecule has been detected in various cancers including lung, ovary, colon and melanoma, however its expression in breast cancer has not been studied. We collected breast tissues from 4 different Saudi breast cancer patients. Tissues were digested by collagenase/hyaluridinase to yield single cell suspension. Epithelial cells were separated from contaminating fibroblasts using filtration and a partial trypsinization method. Cells were analyzed for B7-H1 expression using cytometry and FACS analysis. The B7-H1 molecule was highly expressed in 4/4 primary epithelial cultures (65-92%). Currently we are seeking normal breast tissues from women undergoing plastic surgery to confirm the association of B7-H1 molecule with breast cancer. To our knowledge this is the first time to report the expression of B7-H1 in breast cancer patients. Designing biological and/or immunological tools that result in the blockade of the B7-H1 molecule should represents one approach for cancer immunotherapy.
Induced-fit recognition of DNA by small circular oligonucleotides

Núria Escaja+, Irene Gómez-Pinto+, Julia Viladoms+, Manuel Rico+, Enrique Pedroso+ & Carlos González#


During the last years, small circular oligonucleotides have emerged as promising tools in medicine as diagnostic and therapeutic agents as well as in research due to their increased nuclease resistance relative to their linear analogues (1). In addition, in many cases cyclic oligonucleotides exhibit higher binding affinities and greater specificity for the target than the linear molecule of similar structure. Cyclic oligonucleotides have been used to target single stranded DNA and RNA by forming triplexes, and double stranded DNA by forming triplexes and more complex structures, named catenanes, where a short ssDNA chain is threaded through a large circular DNA duplex. In this study, we explore a different mechanism of recognition between short linear and cyclic oligonucleotides, which may have applications to target specific regions of the genomic DNA. In this case, recognition occurs by forming a recently observed family of four stranded structures that are stabilized by tetrads resulting from the association of two Watson-Crick base-pairs.

We have investigated the molecular interaction between small cyclic oligonucleotides: d<pCAGTCCCT>, d<pCCTTCGGT>, and their lineal analogs. Complex formation was monitored by NMR and CD spectroscopy. The data indicate that the isolated octamers do not adopt a defined structure, but they can recognized to each other forming a complex, whose solution structure has been determined by NMR spectroscopy and restrained molecular dynamics. Recognition between these oligonucleotides occurs through formation of four intermolecular Watson-Crick base pairs. The resulting structure is stabilized by two tetrads, formed by facing the minor groove side of the Watson-Crick base-pairs. Overall, the three-dimensional structure is similar to those observed previously in other quadruplexes formed by minor groove alignment of Watson-Crick base pairs (2). However, in this case the complex is heterodimeric and is formed by two different tetrads (G:C:A:T and a G:C:G:C). The G:C:A:T tetrad is similar to the one observed in the dimeric structure of d<pCGCTCATT> (3), but the conformation of the G:C:G:C tetrad is different from previously characterized minor groove G:C:G:C tetrads.


**Novel non-genotoxic small molecule activators of p53**

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In approximately half of cancers P53 carries inactivating mutations, and in the remaining half wild-type p53 activity is often hampered by alterations to its regulatory mechanisms or by the presence of viral oncogenes. Induction of p53 activity in the treatment of cancer is most commonly achieved through use of DNA-damaging chemotherapeutic agents and ionizing radiation. However, such genotoxic therapies can lead to developmental problems in children and treatment-induced secondary cancers later in life.

We have developed a forward chemical genetic screen using a cell-based reporter assay for p53-dependent transcription. Following a pilot study with the 2000 compound NCI Diversity Set and a similar-sized library from Cancer Research UK, we have screened a large commercially available compound collection for activators of p53 and have identified a number of hits. We are currently investigating the mechanisms of p53 activation of the compounds and evaluating them to identify non-genotoxic activators. Details of the pilot and high-throughput screens along with preliminary results will be presented.
Tyrosine kinome re-sequencing in human cancer

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Dana-Farber Cancer Institute, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, the Howard Hughes Medical Institute and the Broad Institute/MIT, Cambridge, MA, USA, *Co-PIs*

Deregulation of protein phosphorylation, caused by mutations in genes encoding protein kinases, occurs in multiple cancer types. We are using a direct sequencing approach to discover somatically-derived mutations in protein kinases in human cancers. Each exon and flanking intronic sequences are amplified by nested PCR followed by bidirectional sequencing. 1700 primer pairs have been designed and validated to amplify the entire tyrosine kinome. To date, we have scanned the tyrosine kinase activation loop and juxtamembrane domain exons for sequence variants in three hundred cancer-derived DNA samples including lung, prostate, leukemia, melanoma and brain tumors. Thus far, we have found both known and novel mutations in several genes, including novel FLT3 activating mutations in leukemias.
Development of novel DNA-binding chemotherapeutic agents based on flavonoid scaffolds of active ingredients isolated from Chinese herbal remedies

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Herbal remedies and traditional medicines of ethnic origin have been the source of libraries of active ingredients that have found use in the development of modern medicines for a number of diseases including cancer. Such compounds have shown proven anticancer activity against various cancer cell lines according to the Developmental Therapeutics Programme of the NCI.

This project utilises a group of active ingredients isolated from Traditional Chinese herbal medicines as a scaffold (see figure below), in an attempt to devise molecules that interact with DNA selectively and can act as novel chemotherapeutic agents. The interactions of duplex genomic DNA and synthetic polynucleotides, as well as triplex and tetraplex DNA polymorphs with the unmodified compounds have been studied using different analytical techniques. This study is based on experiments including spectroscopic analysis such as UV, ethidium bromide displacement and thermal denaturation, as well as biological tests like cell viability, flow cytometry, comet assay and topoisomerase I & II assays. This battery of tests ascertained the presence of DNA binding activity for these compounds and provided an estimate of their association constants with the various forms of DNA. These were found to be in the order of 10^4 M^-1 – 10^5 M^-1 depending on the compound and the form of DNA, and provided information on the binding preference of the drug molecules.

Baicalein showed specificity for AT bases and especially pyrimidine triplex and tetraplex formations, and was characterized by activity against the MCF7 breast cancer cell line.

Quercetin showed interaction with tetraplex DNA, purine and pyrimidine triplexes, as well as AT – GC bases and also presented activity against the CCRFCEM leukemia cell line.

Daidzein has low specificity for GC bases, whereas baicalin, puerarin and rutin interact weakly with the various nucleic acid structures. As daidzein is considered a phytoestrogen, it promotes activity where estrogen receptors are located, as was shown by the promotion of growth in MCF7 cell line. In flavonoids, the planarity of the molecule allows the molecule to intercalate between the nucleobases. Furthermore, in the case of quercetin, the hydroxyl groups in R3, R5 and R6 seem to have a strong influence on the interaction with DNA.

Based on these results, we studied further modifications of the molecules and their effect on DNA-binding. We found substitutions on the A and B rings to be important as they increase the binding by an order of magnitude; the position of the B ring is also relevant, in C2 improves the binding, and in C3 decreases it. The absence of the C2-C3 double bond makes a distortion to the planarity of the molecule, which appears to decrease the activity. The acetoxy groups in positions 5 and 7 appear to reduce the binding values. These results, together with molecular modeling, will allow the synthesis of novel molecules that bind selectively to nucleic acids and have improved anticancer activity and pharmacological properties.
The efficient assembly of histone complexes and nucleosomes involves the participation of molecular chaperones, which follow the histones throughout, from their site of synthesis to their target locations where they are used and finally degraded. The histone chaperone nucleoplasmin represents the most abundant protein in the *Xenopus* oocyte nucleus. It mediates nucleosome assembly by removing basic proteins from sperm chromatin and exchanging them with histones. This function is modulated by phosphorylation at multiple sites.

The nucleoplasmin core domain and its functionality has been the object of our present work. Its structure forms a pentameric arrangement where each monomer consists of two domains: a core, that forms a stable ring-like pentamer, and a tail, which holds a polyglutamic tract and the nuclear localization signal. The lacking of the poly-Glu region, a putative binding site for basic proteins, does not affect its capacity for the binding of the sperm basic proteins and the chromatin decondensation. This activity has been reproduced artificially in a recombinant core domain through mutation of putative phosphorylation sites to aspartate, thus mimicking the charge effect of phosphorylation. The crystallographic studies of this recombinant domain (called CORE8D) at 2.2 Å resolution show the presence of these mutations which do not affect the folding of the monomer and so the formation of the pentameric structure, even though they are located in exposed flexible regions. Also, the crystal packing has revealed the formation of a nucleoplasmin-core decamer that could represent the normal biological oligomerisation state for this protein. This decamer has localized negative charges near the interface, with a network of hydrogen bond waters which serve to maintain together the opposite pentamers. Beside this, the space of the interface is also defined by the charge interactions between the residues Asp58 and Lys82 from all the opposite monomers.
Structural basis for the redox regulation of the active-site of CDC25A protein phosphatase

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Section of Structural Biology, The Institute of Cancer Research, London, UK

Protein tyrosine phosphatases (PTPs), including dual specificity phosphatases, regulate signal transduction pathways by protein dephosphorylation, and are involved in the development of cancer and other diseases including diabetes. All these enzymes are dependent on the thiol group of a catalytic cysteine which is central to the PTP signature motif located at the active site (Cys-(Xaa)_{5}Arg). Recently, the second messenger hydrogen peroxide has been implicated to modulate of PTPs via a reversible oxidation of the catalytic cysteine thus suppressing phosphatase activity.

The CDC25s are examples of cysteine-dependent dual specificity phosphatases and three homologues of CDC25 exist in humans. Here we report the crystal structure of a modified human CDC25A illustrating the oxidation of the catalytic cysteine by hydrogen peroxide. We demonstrate that oxidation by hydrogen peroxide results in the formation of a disulphide bond between the catalytic cysteine and a second cysteine residue positioned close to the active site. We hope to build on this initial observation to further understand the mechanism of redox regulation of CDC25 and its role in cell cycle regulation.
Organisers and Invited Speakers’ Portfolio

A compilation of short biographies of organisers and speakers in accordance with the order of the programme
Guillermo Montoya obtained his Ph.D. in Chemistry at the University of Zaragoza (Spain) in 1993. This research addressed the structure and function of photosystem II and was done at the Estación Experimental Aula Dei, CSIC, Zaragoza, under the supervision of R. Picorel.

After obtaining his degree he moved to the Max Planck Institut für Biophysik in Frankfurt where he worked in membrane protein crystallization in the group of H. Michel.

Montoya then spent nine years at EMBL-Heidelberg focusing on the crystallization of the cytochrome bc1 complex and later pioneering the study of the structure of the signal recognition particle. In 1998 he was appointed as Associated Researcher at the Spanish Council for Scientific Research (CSIC). Since 2003, Montoya is honorary professor of Biochemistry at the Autonomous University of Madrid.

Montoya has been head of the CNIO’s Macromolecular Crystallography Group since 2001 and Acting Director of the Structural Biology and Biocomputing Programme in this Institution since 2003.

The main focus of the group is the molecular understanding of the role played by macromolecules involved in oncogenic processes.

The research interests of his Group are as follows:

- Mitotic kinases
- DNA Methylation and Chromatin Remodelling
- Molecular Mechanisms of Metastasis.
David Barford's scientific career can be summarised as follows:

1984, D. Phil. at Oxford University, UK, under the supervision of Professor Dame L. Johnson.

1984-1988, Post-doc. at Oxford University, supervisor Professor Dame L. Johnson.

1990-1991, MRC Fellowship, University of Dundee, UK.


1994-1999, Lecturer, Oxford University, Fellow of Somerville College, Oxford, UK.

1999-present Professor of Molecular Biology, Co-chairman, Section of Structural Biology, Institute of Cancer Research, London, UK.

His research interests are concerned with understanding the structural mechanisms by which the post-translational modification of proteins regulate signal transduction pathways and the cell cycle. The aims of these studies are two-fold. Firstly, to understand the structural consequences of protein modifications, and relate these to changes in protein activity and function. Secondly, by analysing the systems which mediate these modifications, for example protein kinases and phosphatases, and E3 ubiquitin ligases, a better understanding of how these proteins function by defining their regulatory and substrate specificity mechanisms will be gained. Insights into these systems may provide a framework for rational drug design and the development of specific and potent inhibitors that may be effective for the treatment of cancer.
Michael J. Eck is an Associate Professor in the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School and the Dana-Farber Cancer Institute. His laboratory studies principles of recognition and regulation in multi-domain signal transduction proteins using X-ray crystallography. Current interests include Src and Jak-family tyrosine kinases, focal adhesion kinase, and formin proteins. Michael J. Eck received his M.D. and Ph.D. degrees from the University of Texas Southwestern Medical School in 1991. He trained as a Postdoctoral Fellow with Stephen Harrison at Children’s Hospital, Boston, and joined the Dana-Farber Cancer Institute in 1996.
Alfred Wittinghofer's scientific career and research interests can be summarised as follows:

1971, Ph.D Doctoral thesis at the German Wool Research Institute in Aachen.
Subject: total chemical synthesis of insulin.


1974-1980, scientific assistant at the Max-Planck-Institut für medizinische Forschung, Heidelberg, Germany.

Since 1980, heads his own research group. Main subjects: Studies on structure/function of EF-Tu, adenylate kinase, myosin heavy chain and the oncogene product Ras, biology of the Ras gene, interaction of Ras with regulators and effectors, studies on Ran regulated nuclear transport.

Since 1993, Director of the Department 'Strukturelle Biologie' at the Max-Planck-Institut für molekulare Physiologie, Dortmund, Germany. General subject: GTP-binding proteins in cell biology.

Wittinghofer has received several awards in the medical field, such as the Louis-Jeantet Prize for Medicine in 2001 and the German Cancer Prize of the German Cancer Society in 2003.

He is amongst others an EMBO member, a member of the Academia Europaea and Honorary Member of the Japanese Biochemical Society.
Christoph W. Müller did his Ph.D. thesis in X-ray crystallography in the group of G. E. Schulz at the University of Freiburg, Germany, where he worked on adenylate kinases. He then moved to Harvard University, Cambridge, USA, where he worked as a postdoctoral fellow together with S. C. Harrison. During this time he solved the first crystal structure of NFκB P50 bound to DNA, which at that time represented a major technical challenge and a significant advance in the understanding of NFκB transcription.

In 1995, Christoph was appointed as group leader at the EMBL Grenoble Outstation and in 2000, he became an EMBL senior scientist. He also has a joint appointment with the Gene Expression Programme at EMBL Heidelberg.

Christoph continues his research in the structural biology of transcriptional regulation.

Main interests of his group are structural studies of STAT and NFκB transcription factors, nucleosome remodeling and the structural organization of the yeast general transcription factor complex TFIIIC and yeast RNA polymerase III. His group uses a combination of X-ray crystallography, electron microscopy and biochemistry techniques.
José Valpuesta was born in Zamora, Spain. He got his Ph.D. degree in Biology at the University of the Basque Country (Bilbao) and then in 1986 he moved to the Laboratory of Molecular Biology (Cambridge, England) to join the laboratory of R. Henderson, where he worked in the structural determination of membrane proteins. He went back to Spain to work at the Centro de Biología Molecular and later at the Centro Nacional de Biotecnología, where he is now Professor.

His current interest involves the structural and functional characterisation, using mainly electron microscopy and image processing, of large macromolecular complexes, specially those involved in protein folding like the chaperonins. He is author of more than 80 scientific papers in international journals and has been awarded several honours, including the European Microscopy Society award (2004).
Ernest Laue is Professor of Structural Biology at the Department of Biochemistry at the University of Cambridge. He ran the National 800 MHz NMR facility from 1997-2002 and currently coordinates the BBSRC-funded CCPN project, which is developing a data model and software programming framework for the organization/sharing of data between different disciplines/sites in large-scale projects.

Laue's research interests lie in the structural biology of protein complexes, in particular those involved in the control of gene expression via chromatin structure. His group employs a number of biophysical and biochemical techniques (in particular NMR spectroscopy and X-ray crystallography) in order to probe the structure and function of these protein complexes.

His research is supported by a Wellcome Trust programme grant, the BBSRC, CR-UK, and the EU (NMRQUAL and TEMBLOR projects).
Manuel Rico is a Senior Researcher and Professor of the Spanish Council of Scientific Research (CSIC), and Director of the Department of Spectroscopy and Molecular Structure of the Institute of Physical Chemistry “Rocasolano” in Madrid (Spain). He is an expert in Nuclear Magnetic Resonance (NMR) Spectroscopy and in its application to protein folding, structure and dynamics. He and his group have made important contributions to the design of linear peptides with well defined conformations, either \(\alpha\)-helices, \(\beta\)-hairpins or small three-strand antiparallel \(\beta\)-sheets. These designs were based on an early discovery of theirs, according to which short linear peptides might form significant populations of secondary structure that can be detected by NMR. Analysis of the folding propensities of protein segments is now a well-established tool for getting insights into the early stages of the folding process. It is also significant their contributions in the protein folding field, in particular, their studies on folding kinetics, H/D exchange measurements, and denaturation equilibria, leading to the identification of residual structures in the random coil, and characterization of folding intermediates of several proteins, like \(\lambda\)-cro 434, Rnase A, Che-Y, apo-flavodoxin, and some fragments and cyclic permutations of cytochrome b_{562}. He and his group were able to establish meaningful relationships between structure and function of several ribonucleases, like bovine pancreatic RNase A, microbial RNase Sa and the cytotoxic ribonuclease \(\alpha\)-sarcin. The role of enzyme internal dynamics on the biological activity of these enzymes has been also the subject of their interest. His is a reference lab for NMR structural determinations of proteins and nucleic acids all over Spain, and the number of his collaborations with other chemical or biochemical groups is certainly very large. At the international level, he is currently invited to lecture in meetings, courses and symposia on the applications of NMR in Structural Biology. He is author of more than 200 research works published in international journals, five book chapters, and has been the editor of three books on NMR spectroscopy. He has been the Spanish representative in a Structural Biology Network of the CEE, and now is the co-ordinator of a National Network on protein structure and function. He has been awarded the National Research Award of the Spanish Royal Society of Chemistry and the National Award of Research in Chemical Science and Technology “Enrique Moles” of the Spanish Ministry of Science and Technology.
Josep Rizo was born in Barcelona, Spain, in 1959. He wanted to be a chemist since an early age and got a B.Sc. degree in Organic Chemistry in 1981 from the University of Barcelona. During these college studies, he became very interested in quantum field theory and statistical mechanics, which led him to obtain a second B.Sc. degree, this time in theoretical physics, from the same University in 1988. During the 1982-1988 period, he also obtained M.Sc. and Ph.D. degrees in organic chemistry from the University of Barcelona, working first on pioneering new methodology to analyze peptides attached to solid supports by gel-phase $^{13}$C nuclear magnetic resonance (NMR) spectroscopy, and later on the development of polar protecting groups for peptide synthesis. In 1989 he started postdoctoral research in the University of Texas Southwestern Medical Center at Dallas (UT Southwestern), working on conformational studies of a variety of peptides by NMR spectroscopy and molecular dynamics. In the early 1990s he shifted his interest to protein NMR and protein folding, focusing in particular on the mechanism of folding of β-sheet proteins.

Josep established his independent research group in 1995 at UT Southwestern. In search for an interesting biological system where he could apply his knowledge in protein NMR spectroscopy, he was fortunate to be at the right place and the right time, and initiated a tight collaboration with T. C. Südhof, a neurobiologist at UT Southwestern who was applying genetic and molecular biological techniques to identify and characterize proteins involved in neurotransmitter release. Since then, the main focus of the research in Josep’s laboratory has been the study of the mechanism of neurotransmitter release through the analysis of the three-dimensional structure and interactions of the proteins that control this process. The collaboration with the Südhof’s group has become a highly synergistic endeavor whereby the combined expertise of both laboratories allows them to tackle a common biological problem using a wide variety of techniques, ranging from NMR spectroscopy to mouse genetics. This collaborative work has led, among other findings, to the demonstration that the synaptic vesicle protein synaptotagmin I is the long-sought Ca$^{2+}$ sensor that triggers fast neurotransmitter release, and to the elucidation at atomic resolution of how this protein binds Ca$^{2+}$. Since 1999, Josep’s group has also been collaborating with the laboratory of Hongtao Yu, also at UT Southwestern, in studies of the mitotic spindle checkpoint that ensures proper chromosome segregation during mitosis. This work has led to the discovery that the mitotic spindle checkpoint protein Mad2 has two strikingly different native structures, which appears to be crucial for the mechanism of activation of the checkpoint. Josep is currently a Professor in the Departments of Biochemistry and Pharmacology of UT Southwestern. He has published close to 100 papers and reviews in books and scientific journals.
Ming-Ming Zhou received his Ph.D. in Chemistry from Purdue University in 1993. From 1993 to 1996, he was a postdoctoral fellow in the laboratory of S. Fesik at Abbott Laboratories (Chicago, IL), where he worked in the field of Structural Biology and Signal Transduction. In 1997, he joined the Structural Biology Program at Mount Sinai School of Medicine, New York, where he is currently an Associate Professor.

His laboratory studies the fundamental molecular mechanisms underlying a wide array of cellular processes ranging from mitogenic signal transduction to chromatin-mediated gene transcription. Through high resolution three-dimensional protein structural analysis using nuclear magnetic resonance spectroscopy, his laboratory has identified molecular functions of the evolutionarily conserved protein modules such as the bromodomain as an acetyl-lysine binding domain in chromatin remodeling and histone signaling, and the PAZ (Piwi/Argonaute/Zwille) domain as a small regulatory RNA binding domain in RNA-mediated gene silencing, including RNA interference.
Born in Obninsk, Russia, Ruben Abagyan received his Ph.D. from the Moscow State University in 1984 for his work on molecular modeling and diffraction theory under the direction of N. G. Esipova and V. G. Tumanyan. He continued to work independently at the Engelhardt Institute of Molecular Biology on molecular modeling theory, DNA structure, and sequence analysis.

In 1989 he joined J. Marcial's laboratory of protein engineering in the University of Liege in Belgium. In 1990 he moved to the European Molecular Biology Laboratory in Heidelberg, Germany where he became staff scientist in the Biocomputing Department.

In 1994 he moved to New York University where he established a computational biology laboratory at the Skirball Institute of Biomolecular Medicine directed by L. Philipson and became Associate Professor of Biochemistry (the NYU Medical School) and Mathematics (the Courant Institute of Mathematics). He also founded the Molsoft company to continue the development of the ICM program.

It was in 1999 that Ruben Abagyan settled as Professor at the Scripps Research Institute in La Jolla. From 1999 to 2001 he also served as director of computational biology at the Genomics Institute of the Novartis Research Foundation. He co-authored over 100 publications, served as director on boards of Syrrx, a structural proteomics company, and Plexus Vaccine, a vaccine design company, and received two Cap CURE awards for prostate cancer research. In 2003, he was awarded and delivered the Princess Diana honorary lecture at the V. Chang Institute in Sydney, Australia.

His main focus since 1985 was to formulate and develop a new method for macromolecular simulations which would make simulations much more predictive, efficient and easily applicable to a variety of modeling tasks, including folding, homology modeling, loop predictions and protein design, protein docking and small ligand docking and virtual screening. By 1989 he published several papers describing a new way to model macromolecular systems in an arbitrary subset of 4-types of internal coordinates and in 1991 the first version of the ICM program was applied to molecular structure prediction. A. Mazur and him were the first to describe the equations of motion for macromolecules in the internal coordinates. In 1993, with R. Wierenga, he successfully applied the ICM energy optimization method to targeted redesign of a part of protein backbone. Since 1994, with Maxim Trotov and other colleagues, he published a series of papers on energetics, global optimization algorithms, and fully flexible protein docking, peptide and small molecule docking, including the design of small molecular inhibitors for a number of important molecular targets.
Modesto Orozco obtained his Ph.D. degree in Biochemistry from the Autonomous University of Barcelona in 1990. He was Assistant Professor in Biochemistry and Molecular Biology from 1991 to 2001, and is a Full Professor since 2002 at the same University.

Since 2001, Orozco is also Director of the Molecular Modelling and Bioinformatics Unit in the Parc Científic of Barcelona.

His research activities are focused on the theoretical study of biological systems. He has published more than 200 papers in international peer-reviewed journals. Orozco is consultant of several pharmaceutical companies and serves as an expert on different scientific evaluation committees for the Spanish Government.
Federico Gago earned his degree in Pharmacy from Complutense University, Madrid, and did his Ph.D. at the University of Alcalá. Following postdoctoral research in the laboratory of W. Graham Richards at the Physical Chemistry Laboratory in Oxford, UK, he returned to the University of Alcalá where he was appointed Assistant Professor in the Department of Pharmacology.

A major focus of his research, which can be best characterized as interdisciplinary with an emphasis on simulation methods, has been the theoretical study of the interaction of short DNA oligonucleotides with proteins and small ligands, both intercalating and non-intercalating. His laboratory has pioneered the use of novel methodologies for the study of quantitative structure-activity relationships (QSAR) and works very closely with several medicinal chemistry groups in the field of drug design. In recent years he has collaborated with PharmaMar in the elucidation of the mechanism of antitumour action of several compounds of marine origin.

His current research efforts are mostly devoted to improving our understanding of mechanisms able to overcome HIV-1 resistance against reverse transcriptase inhibitors and those that could be advantageously used to achieve greater selectivity against cancer cells.

Federico Gago is presently one of the associate directors of the NFCR Centre for Computational Drug Design (Oxford), a member of the Editorial Advisory Board of Current Medicinal Chemistry - Anti-Cancer Agents, and Editor-in-Chief of Journal of Computer-Aided Molecular Design.
Hans Matter is currently working as a senior scientist in the computational chemistry group at Aventis Pharma in Frankfurt / Germany. He joined this group at former Hoechst Marion Roussel in 1996, and is involved in structure- and ligand based design applications, QSAR and ADMET modelling to support interdisciplinary project teams and protein target family research. He completed his academic studies in 1992 at the Technical University of Munich, Germany, in the group of H. Kessler, where he was working on conformational analysis of bioactive peptides and glycopeptides using NMR spectroscopy and modelling. After having obtained his Ph.D from the Technical University of Munich, he joined Tripos Inc, a company focussed on software development, services and compound libraries to support drug discovery programs as senior scientist.
Ángel Messeguer was born in Mexico in 1946. He received his bachelor's degree in chemistry from the University of Barcelona in 1969. He did his Ph.D. Thesis at the Institute for Organic Chemistry (Consejo Superior de Investigaciones Científicas, CSIC) under the supervision of F. Serratosa and received his Ph.D. degree in chemistry from University of Barcelona in 1974. In 1975 he was appointed as Associate Researcher in CSIC. He was promoted to Research Scientists in 1986 and to Full Professor in 1991 in the CSIC. In 1978-1979 he worked as postdoctoral fellow at the University of Cornell (USA) under the supervision of W. Bowers. He was an invited Professor at the University of California at Davis (1988 and 1989). He is author of 130 scientific articles, 2 books and 8 patents. He is currently President of the Catalan Society of Chemistry. His present research interests include the application of combinatorial chemistry to drug discovery, the development of new antioxidants and ageing protectants and the study of xenobiotics exhibiting toxicity to humans, with particular emphasis in the Toxic Oil Syndrome.
Alexander Levitzki, Ph.D.
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Alexander Levitzki obtained his Ph.D. degree in Chemistry (Biochemistry and Biophysics) in 1968 from the Hebrew University of Jerusalem, Israel and The Weizmann Institute of Science, Rehovot. He was a postdoctoral fellow at the department of Biochemistry, UCB, Berkeley with D. E. Koshland, Jr. during the period 1968-1971.

For the last 30 years, he has been Professor at the Hebrew University of Jerusalem. During his whole career he has held numerous appointments as Visiting Professor at different USA Research Centres and Universities. Levitzki is amongst others, an elected member of the EMBO since 1978 and member of the Israel Academy of Sciences since 1999.

Some of the scientific milestones throughout Levitzki’s career have been:
- The demonstration of the "induced fit" hypothesis using Concavalin A (1968)
- The determination of the molecular basis of "Negative cooperativity" in enzymes and receptors (1968-1980s)
- Identification of β-adrenoceptors with a radioligand binding assay (1974)
- Analysis of the mode of activation of adenylyl cyclase by the receptors (1977-19990s)
- Since the mid-1980s, the Levitzki laboratory has been developing the approach of "signal transduction therapy", combining signal transduction inhibitors and cytotoxic agents. Important signal transduction inhibitors developed by his group are the "tyrphostins" (tyrosine phosphorylation inhibitors), compounds that combat proliferative diseases. The group pioneered the development of tyrosine kinase inhibitors for the treatment of CML, which formed the basis for the development of Gleevec in the late 1990s.
- At present dsRNA technologies are being developed by his group to selectively kill cancer cells.

Alexander Levitzki has served on the editorial board of numerous scientific journal and as consultant, and founder of several biotechnology companies.
Gerhard Klebe obtained his Ph.D. degree in 1982 at the University of Frankfurt and his Habilitation at the Institute of Pharmaceutical Chemistry, University of Heidelberg, Germany.

From 1982 to 1984 Klebe held a research position at the Institute of Crystallography, University of Frankfurt with H. Fuess. From 1984 to 1996 Klebe worked at BASF-AG, Ludwigshafen, Germany in drug design, X-ray crystallography and molecular modelling.

Klebe is since 1996 Full Professor at the Institute of Pharmaceutical Chemistry, University of Marburg, Germany. His research interest go from X-ray crystallography of proteins and small organic molecules, to programme development in drug design, de-novo ligand design.
Mike Randal, Ph.D.

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Sunesis Pharmaceuticals, Inc.
South San Francisco, CA, USA

Mike Randal is a life-long resident of California, USA. After receiving his BS in Biochemistry from the University of California, Davis in 1986, he joined the crystallography group at Genentech, Inc. There he was exposed to the pioneering structural studies of A. Kossiakoff and the fledgling protein engineering work of J. Wells. Inspired by the interplay of these two disciplines, Mike left Genentech in 1991 to further his studies in the Graduate Group of Biophysics at the University of California, San Francisco under the mentorship of A. Kossiakoff and R. Stroud. He received his Ph.D. in 1997 for his x-ray crystallographic studies of interferon-gamma and its receptor. After graduation, he returned to industry with a short stint at Roche Bioscience. From there, he was recruited by J. Wells to start up the Structural Biology group at recently formed Sunesis Pharmaceuticals.

At Sunesis, Mike’s background in the structural studies of protein:protein complexes served as the springboard for a move into the field of protein:small molecule crystallography. He was part of the team that developed and validated Tethering®, Sunesis’ proprietary method for identifying low molecular weight compounds that bind to targeted sites on protein surfaces. Currently, his group is using Tethering® to develop small molecule inhibitors to a variety of medically relevant enzyme targets.
Lyubomir Vassilev, Ph.D.
Research Leader
Discovery Oncology
Hoffmann-La Roche Inc.
Nutley, NJ, USA

Lyubomir (Lubo) Vassilev is a Research Leader in the Oncology Division of Hoffmann-La Roche Inc., Nutley, New Jersey, USA. He has 12 years of experience in leading small-molecule drug discovery efforts in Oncology. Vassilev’s expertise covers the whole spectrum of activities involved in the discovery and development of cancer therapeutics: discovery and validation of molecular targets, assay development and high throughput screening, identification and optimization of drug leads. His primary research interests include signal transduction, cell cycle regulation, growth control and their dysregulation in cancer. Vassilev received his Ph.D. degree in Molecular Biology from the Institute of Molecular Biology, Bulgarian Academy of Sciences where he worked on the structure and function of chromatin. He did his post-doctoral training at the Brookdale Center for Molecular Biology, Mount Sinai School of Medicine, New York, NY, and the Roche Institute of Molecular Biology, Nutley, NJ, working on the mechanisms of initiation of mammalian DNA replication. Vassilev has co-authored 35 publications.
Invited speakers and participants
**Organisers and invited speakers**

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### 2004 CNIO Cancer Conference: Structural Biology of Cancer Targets

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## Participants

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Acknowledgements
As a non-profit organisation, we would like to thank the support from our CNIO Cancer Conferences (CCC's) collaborators. Such contribution helps to ensure that our conferences will continue to establish the CNIO as a point of reference for the international cancer research community.

For information around collaboration opportunities, please contact: Amanda Wren (awren@cnio.es)
2004 CNIO Cancer Conference: Structural Biology of Cancer Targets
Forthcoming CNIO activities
FORTHCOMING CNIO ACTIVITIES

CNIO CANCER CONFERENCES www.cnio.es/ccc

CADHERINS, CATENINS AND CANCER-CNIO CANCER CONFERENCE
Organisers: Amparo Cano, Hans Clevers, José Palacios, Frans van Roy
Dates: November 29-December 1, 2004

ANIMAL TUMOUR MODELS AND FUNCTIONAL GENOMICS-CNIO CANCER CONFERENCE
Organisers: Allan Balmain, Mariano Barbacid, Anton Berns, Tyler Jacks
Dates: March 7-9, 2005

MAP KINASES AND CANCER-CNIO CANCER CONFERENCE
Organisers: Ángel Nebreda, Philip Cohen, Chris Marshall, Roger Davis
Dates: May 30-June 1, 2005

CANCER AND AGING-CNIO CANCER CONFERENCE
Organisers: María Blasco, Kathleen Collins, Jan Hoeijmakers, Manuel Serrano
Dates: November 7-9, 2005

OTHER CNIO ACTIVITIES www.cnio.es/meetings

CÁNCER DE TIRIOIDES-JORNADA DE PATOLOGÍA MOLECULAR
Organizadores: Mercedes Robledo, Xavier Matías-Guiu, José Ángel Díaz.
Fecha: 29 octubre, 2004

NEOPLASIAS DEL SISTEMA NERVIOSO CENTRAL-JORNADA DE PATOLOGÍA MOLECULAR
Organizadores: Manel Esteller, Ricardo Yaya
Fecha: December 17, 2004
2004 CNIO Cancer Conference: Structural Biology of Cancer Targets

European School of Oncology courses in the CNIO www.cnio.es/es/cursos/cursos.htm

Curso Cáncer de Pulmón-European School of Oncology Course in Spanish
Coordinadores: Rafael Rosell, Montserrat Sánchez-Céspedes
Fecha: Winter 2005 (to be fixed)

Nuevas dianas terapéuticas y sus rutas de señalización: fundamentos y aplicaciones en cáncer
European School of Oncology Course in Spanish
Coordinadores: Hernán Cortés Funes, Hospital 12 de Octubre, Madrid, Enrique de Alava, CIC, Salamanca
Fecha: 20-22 de octubre, 2005