Scientific Report
Biophysics Unit

2010

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Contents


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Foreword

The following pages contain a summary of the current research lines at the Unidad de Biofísica, together with the list of staff, students and visitors, and our recent publications and other activities. This Annual Report intends to serve a two-fold purpose, providing the reader with information about our activities, and supplying our sponsors with the necessary data about the end-product of their funding. We shall be glad to answer any questions or provide further information, and would be delighted to welcome you personally in our laboratory.

Presentación

Las páginas que siguen contienen un resumen de las líneas de investigación en marcha en la Unidad de Biofísica, junto con la relación de trabajadores, estudiantes y visitantes, y nuestras publicaciones y actividades recientes. Esta Memoria anual tiene un doble propósito, proporcionar información sobre nuestras actividades a los lectores en general, y transmitir en particular a nuestros patrocinadores los datos necesarios sobre los resultados producto de su generosidad. Con mucho gusto responderemos a cualquier pregunta y proporcionaremos información complementaria, y por supuesto estaremos encantados de recibirles personalmente.

Aurkezpena

Hurrengo orrietan Biofisika Unitatean gaur egun dauden ikerketa lerroen laburpena, ikertzaile, ikasle eta bisitarien zerrenda, eta gure argitarapen eta ekintzen berri eskaintzen da. Urteroko txosten honek helburu bikoitza du: irakurle orori gure ekintzen berri ematea, eta bereziki, gure laguntzaileei beraien eskuzabaltasunari esker lortutako datuak jakinaraztea. Atsegin handiz, edozein galdera erantzuteko, informazio osagarria eskaintzeko, edota pertsonalki errezibitzeko prest gaude.

News 2010

New building

2010 was an important year for our Institute. After long and complex negotiations the funds and architectural project were made available for a new and larger building. The new facilities, to be opened in 2013, should allow the *Unidad de Biofísica* to grow unhindered by space problems well into the thirties of the current century.

Last March a letter from the Presidency of the Spanish National Research Council (CSIC), a joint Patron of the *Unidad* together with the University of the Basque Country, approved our plans for developing and strengthening biophysical research. The University had already enthusiastically supported these plans. Then the generous help from the Bizkaia Provincial Government (*Diputación Foral de Bizkaia*), and the Basque and Spanish Governments enabled us to collect the necessary funds for our endeavor.

The new building, with about 9.000 m^2 of labs and offices, will be located in the new Leioa Scientific Park, an extension of the current University campus. The works will be managed and supervised by the society "*Parque Tecnológico de Bizkaia*" to whom we are indebted.

We are extremely grateful to all those who are making possible this important instrument of progress for the *Unidad de Biofísica*.

Awards

Professor **J.L.R. Arrondo** was awarded the 2010 Bruker Prize from the Spanish Biophysical Society.

Innoprot, a spin-off company of the *Unidad de Biofísica* received the 2010 Bancaja Prize for succesful technology-based companies.

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Lines of Research 2009/2010

Sphingolipids and membrane domains

(A. Alonso, F.M. Goñi)

In recent years the interest for membrane domains has increased, in particular transient domains known as rafts. The hypothesis of "rafts" suggests that these microdomains are enriched in sphingolipids and in cholesterol. Sphingomyelinases are enzymes that break down sphingomyelin into ceramides and water soluble products. Ceramides are membrane lipids, but their activity is mainly seen through cytosolic proteins. Our group studies, on the one hand, the characteristics of sphingomyelinases, and on the other hand, the changes induced by ceramides in the physical properties of membranes, in order to identify the molecular bases of the physiological activity of ceramides. We are also analysing the tendency of various different sphingolipids (ceramides, sphingosine) to form domains in the lipid bilayer plane.

References:

"Detergent-resistant membranes should not be identified with membrane rafts" D. Lichtenberg, F.M. Goñi and H. Heerklotz. *TRENDS Biochem. Sci.* **30**, 430-436 (2005).

"Biophysics of sphingolipids I. Membrane properties of sphingosine, ceramides and other simple sphingolipids" F.M. Goñi and A. Alonso. *Biochim. Biophys. Acta* **1758**, 1902-1921 (2006).

"Cholesterol displaces palmitoylceramide from its tight packing with palmitoylsphingomyelin in the absence of a liquid-disordered phase" J.V. Busto, J. Sot, J. Requejo-Isidro, F.M. Goñi, and A. Alonso. *Biophys J.* **99**, 1119-1128 (2010).

Membrane protein folding and stability. Structural motif engineering and design

(A.R. Viguera)

Protein folding has been the subject of intensive research. Well designed combinations of experimental and computational studies are enabling folding to be followed at atomic resolution, with the result that general rules are emerging. This insight, however, pertains to water-soluble proteins and it is unclear how the unifying mechanisms extend to the many proteins that reside in membranes. Understanding membrane protein folding in vitro will not only begin to overcome the problems of overexpression, purification and solubilization of membrane proteins, but also bring new techniques to membrane protein research. We have chosen the pore-forming fragment of colicin A as a model to study protein-lipid complex formation and stability. We investigate the kinetics and thermodynamics of folding aiming to obtain mechanistic detail, with an emphasis on α-helical proteins.

References:

"Par j 1 and Par j 2, the two major allergens in *Parietaria judaica*, bind preferentially to monoacylated negative lipids" R.González-Rioja, J.A.Asturias, A.Martínez, F.M.Goñi & A.R.Viguera. *FEBS J.* **276,** 1762-1775 (2009).

"NMR assignment and backbone dynamics of the pore-forming domain of colicin A" A.Ibañez de Opakua, T.Diercks, A.R.Viguera & F.J.Blanco. *Biomol NMR assign* **4**, 33-36 (2010).

Structural studies of Biomolecules using IR spectroscopy

(J.L.R. Arrondo)

In the nineteen eighties, our laboratory pioneered the application of infrared spectroscopy to the study of the structure of lipids and proteins. Currently we are developing the new technology of two-dimensional IR spectroscopy (2DCOS), applying it to the analysis of the structure and conformational changes of proteins, lipids and their complexes.

The main areas on which we are currently focussing our attention are:

- Study of sphingomyelin-colesterol mixtures
- Membran proteins and lipoproteins
- yloidogenesis
- Protein-DNA interactions

References:

"Structure and dynamics of membrane proteins as studied by infrared spectroscopy". J.L.R. Arrondo y F.M. Goñi. *Prog. Biophys. Mol. Biol.* **72**, 367-405 (1999).

"Influence of aggregation propensity and stability on amyloid fibril formation as studied by Fourier transform infrared spectroscopy and two-dimensional COS analysis". N. Cerdà-Costa, I. De la Arada, F.X. Avilés, J.L.R. Arrondo and S. Villegas. *Biochemistry* **48**, 10582-10590 (2009).

"Sphingosine-1-phosphate as an amphipathic metabolite: its properties in aqueous and membrane environments". M. García-Pacios, M.I. Collado, J.V. Busto, J. Sot, A. Alonso, J.L.R. Arrondo and F.M. Goñi. *Biophys. J.* **97**, 1398-1407 (2009).

Protein folding and the role of molecular chaperones

(A. Muga, A. Prado, F. Moro)

Living systems host a crowd of molecular chaperones that act with different mechanisms and serve to maintain protein homeostasis. Our group studies nuclear and cytosolic chaperones. Among the cytosolic chaperones we are interested in members of the Hsp60, Hsp70 and Hsp100 families. In particular, we are analysing the functional cycle of these proteins and how they interact with substrates and modulate their conformation. We are also trying to understand how Hsp70 and Hsp100 proteins collaborate, forming a productive network, to disaggregate and refold cellular protein aggregates. Among nuclear chaperones we focus on nucleoplasmin, a histone-chaperone involved in the

exchange of basic proteins bound to DNA and therefore in the regulation of the condensation state of chromatin. In particular, we are interested in the nucleoplasmin-mediated histone exchange mechanism. These studies are carried out using a combination of biochemical (expression and purification of proteins, mutagenesis, hybrid oligomeric proteins) and biophysical (fluorescence, IR spectroscopy, calorimetry,..) techniques.

References:

"Nucleoplasmin binds histone H2A-H2B dimers through its distal face". I. Ramos, J. Martín-Benito, R. Finn, L. Bretaña, K. Aloria, J.M. Arizmendi, J. Ausió, A. Muga, J.M. Valpuesta, A. Prado. *J Biol. Chem.* **285**, 33771-33778 (2010).

"Role of DnaJ G/F-rich domain in conformational recognition and binding of protein substrates". J. Perales-Calvo, A. Muga and F. Moro. *J. Biol. Chem.* **285**, 34231–34239 (2010).

"Energetics of nucleotide-induced DnaK conformational states". S.G. Taneva, F. Moro, A. Velázquez-Campoy and A. Muga. *Biochemistry* **49**, 1338-1345 (2010).

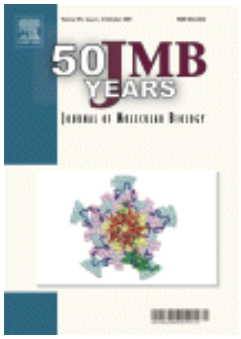
Insights into the structure, function and nucleocytoplasmic traffic of nuclear proteins

(M.A. Urbaneja, S. Bañuelos)

Nuclear chaperones are involved among other functions in the chromatin remodeling that takes place during various physiological processes such as fertilization (e.g. mediated by nucleoplasmin) and ribosome assembly and cell proliferation control (e.g. mediated by nucleophosmin). Like other nuclear proteins, they are synthesized in the cytoplasm and rely on carriers (in the case of nucleoplasmin / nucleophosmin family, importin α/β heterodimer carrier) to be imported into the cell nucleus. Nucleophosmin is a "shuttling" protein: it needs to be imported and exported continuously. Protein function is frequently regulated by cell localization, and failure in this traffic may trigger diseases. Nucleophosmin mislocalization and disfunction have been related to several types of human cancer. Based on biochemical, molecular biology and biophysical approaches we are studying nucleophosmin structure, function and interaction with nuclear transport receptors, trying to understand the basis of its pathogenic alterations.

References:

"A mechanism for histone chaperoning activity of nucleoplasmin: thermodynamic and structural models" S.G. Taneva, S. Banuelos, J. Falces, I. Arregi, A. Muga, P.V. Konarev, D.I. Svergun, A. Velazquez-Campoy & M.A. Urbaneja. *J. Mol. Biol.* **393**, 448-463 (2009).



"Recognition of nucleoplasmin by its nuclear transport receptor importin α/β: insights into a complete import complex". J. Falces, I. Arregi, P.V. Konarev, M.A. Urbaneja, D.I. Svergun, S.G. Taneva and S. Bañuelos. *Biochemistry* **49**, 9756–9769 (2010).

The role of cell membranes in bacterial pathogenesis

(H. Ostolaza, C. Martín)

In recent years, our group has been studying the phenomenon of the interaction of a pathogenic toxin, *Escherichia coli* α-haemolysin, with erythrocytes and model membranes. This is an example of protein that it is initially synthesised as a water-soluble protein, but on coming into contact with a membrane binds to it, behaving from then on as an intrinsic protein. Our efforts are focussed on studying the structural requirements of the toxin for this transformation from the soluble to the membrane-bound state to take place, as well as the parameters that may modulate the interaction with the target membrane. Recently, we have broadened the scope of our work to include another member of the RTX family, the adenylate cyclise toxin from *Bordetella pertussis*.

References:

"*Bordetella* adenylate cyclase toxin promotes calcium entry into both $CD11b^+$ and $CD11b^-$ cells through cAMP-dependent L-type-like calcium channels". C. Martín, G. Gómez-Bilbao and H. Ostolaza. *J. Biol. Chem*. **285,** 357-364 (2010).

"Interdomain Ca^{2+} effects in *Escherichia coli* α-haemolysin: Ca^{2+} binding to the C-terminal domain stabilizes both C- and N-terminal domains". L. Sánchez-Magraner, A.L. Cortajarena, M. García-Pacios, J.L.R. Arrondo, J. Agirre, D.M. Guérin, F.M. Goñi and H. Ostolaza. *Biochim. Biophys. Acta.* **1798**, 1225-1233 (2010).

Mechanism of action of peptide toxins acting at the membrane level

(J.M. González-Mañas)

Our research is focussed on the interaction of equinatoxin-II with cell and model membranes. Equinatoxin-II, a toxin produced by the anemone *Actinia equine*, forms pores in the membrane. Using site-directed mutagenesis, we are designing mutants that we hope will help us identify those residues directly involved in the insertion process. On the other hand, we are also investigating the biophysical parameters of the membrane that favour this type of interaction, and, in particular, the coexistence of lipid phases within the bilayer.

References:

"Lipid phase coexistence favors membrane insertion of equinatoxin-II, a pore-forming toxin from *Actinia equina*" A. Barlic, I. Gutiérrez-Aguirre, J.M.M. Caaveiro, A. Cruz, M.B. Ruiz-Argüello, J. Pérez-Gil and J.M. González-Mañas *J. Biol. Chem.* **279**, 34209-34216 (2004).

"Pore formation by equinatoxin, a eukaryotic pore-forming toxin, requires a flexible N-terminal region and a stable β-sandwich" K. Kristan, Z. Podlesek, V. Hojnik, I. Gutiérrez-Aguirre, G. Guncar, D. Turk, J.M. González-Mañas, J.H. Lakey, P. Macek and G. Anderluh *J. Biol. Chem*. **279**, 46509-46517 (2004).

Inter-domain relationships in integral membrane proteins

(I. Alkorta, F.M. Goñi)

The purpose of this project is to determine the role of the various protein components that are part of the bacterial conjugation system of the R388 plasmid. In particular, we are interested in the membrane protein TrwB. This protein, the first member of the coupling family of proteins to be purified, is involved in the transfer of DNA from the donor to the recipient cell. Clarifing its role in the process of conjugation will contribute to solving the problem of antibiotic resistance shown by an increasing number of bacterial strains (in collaboration with F. De la Cruz, University of Cantabria).

References:

"Role of the transmembrane domain in the stability of TrwB, an integral protein involved in bacterial conjugation" I. Hormaeche, I. Iloro, J.L.R. Arrondo, F.M. Goñi, F. de la Cruz and I. Alkorta *J. Biol. Chem.* **279**, 10955-10961 (2004).

"The transmembrane domain provides nucleotide binding specificity to the bacterial conjugation protein TrwB" I. Hormaeche, R.L. Segura, A.J. Vecino, F.M. Goñi, F. de la Cruz and I. Alkorta *FEBS Lett.* **580**, 3075-3082 (2006).

"Reconstitution in liposome bilayers enhances nucleotide binding affinity and ATP-specificity of TrwB conjugative coupling protein" A.J. Vecino, R.L. Segura, B. Ugarte-Uribe, S. Águila, I. Hormaeche, F. de la Cruz, F.M. Goñi, and I. Alkorta *BBA Biomembranes* **1798**, 2160-2169 (2010).

Mechanisms for virus-induced membrane fusion

(J.L. Nieva)

Our objective is to determine the molecular mechanism by which membrane glycoproteins of some viruses (HIV, Ebola) induce the fusion of the cell and viral membranes. Prediction tools have been developed to detect the domains that are inserted

into the target membrane. Specifically, we seek to understand their behaviour as antigenic determinants, and to develop inhibitory agents that would block their destabilizing interaction. A branch of this field consists of the characterisation of similar domains that may be involved in the infective power of the prion protein. In parallel, we are studying the mechanism of cell membrane permeabilisation, induced by certain viral products (viroporins) during infection (this work on viroporins is in collaboration with L. Carrasco, CBM, Madrid).

References:

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Mitochondrial membranes, apoptosis and cancer

(G. Basañez)

During apoptosis, mitochondrial membranes undergo dramatic changes in permeability and morphology. The principal components involved in these processes are the BCL-2 family of proteins, with assistance from an increasing number of mitochondrial protein/lipid effectors. Despite the remarkable progress made in uncovering the molecular underpinnings of apoptotic cell death in the last decade, identification of the precise mechanisms by which BCL2 family

proteins regulate the structure and functioning of mitochondrial membranes remains a key and controversial issue in the field of cell death. Given the inherent complexity of the cellular apoptotic network, we use in vitro reconstituted systems with physiologically relevant characteristics to try to elucidate the mode of action of specific members of the BCL2 family and/or their effectors at the membrane level, using a multidisciplinary approach based on biophysical techniques. Considering the important role played by BCL2 family proteins in tumorigenesis and in cellular responses to chemotherapy, the information gained in these studies may facilitate progress in the fight against cancer.

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X-ray crystallography and crystallisation of proteins and virus

(D.M.A. Guérin)

Learning about the structure of macromolecules of biological interest (enzymes, receptors, large molecular aggregates such as viruses) enables the mechanisms of the biochemical functions they perform to be interpreted. Protein crystallography, currently the most advanced and powerful technique for determining atomic structures, is used by our group to study a wide range of macromolecules. We are currently working on the resolution of the structure of several proteins of interest in the Biophysics Unit (membrane

protein Scramblase and the eukaryotic toxin FraC) and we are collaborating with national and international centres on other structural projects (Triatoma virus, Potassium Channel KcsA and Acyl-CoA binding protein, among others). In parallel with this crystallography work, we are developing new experimental devices and procedures to enhance protein crystallization.

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Identification and characterization of proteins and lipids associated to regulators of cell excitability

(Á. Villarroel)

Among ionic channels, those that are potassium selective are, by far, the most diverse group. They play a key role in processes such as immune response, cell differentiation, excitability and cell death, among others. More than 60 genes for potassium channels are known in the human genome, which together with the fact that up to four different subunits can combine to form a channel, means that there are a large numbers of variants. Despite this impressive redundancy in "potassium permeation", mutations in some subunits cause hereditary diseases, indicating that the rest of the channels cannot substitute for them. These diseases are known as channelopathies. To date, around 10 potassium channelopathies have been identified and four of these are due to mutations in genes of the KCNQ family, the gene products of which are Kv7.1-Kv7.5.

Our research is focused on the molecular study of these proteins that regulate cell excitability. In humans, mutations of these proteins cause arrhythmia, epilepsy and deafness, depending on the isoform affected and their distribution within the tissue. Our objective is to identify the network of proteins associated with these channels, by analysing the physiological consequences of these interactions through mutagenesis-function studies, and the use of electrophysiological, imaging, biochemical and high-resolution biophysical techniques. In addition, we aim to determine the role played by lipids in the regulation of these channels. Currently, we are establishing the channel regions involved in biogenesis, assembly, membrane insertion and subcellular localization, as well as in the regulation by lipid second messangers. We hope to identify the proteins and lipids that interact specifically with each of these domains, and these may become targets for the therapeutic drug development. In the longer term, our objective is to determine the three-dimensional structure of these macro-complexes. As an intermediate stage, en route to future crystallization, we are investigating new strategies for high-yield production of large quantities of water-soluble, properly folded macro-complexes.

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Advanced techniques of fluorescence spectroscopic micro and nanoscopy

(J. Requejo)

My main research interest is the development and application of novel micro and nanoscopic optical methodologies to the quantitative investigation of the spatial organisation and dynamics of inter- and intra-molecular interactions in living cells. The quantitative description of proteins behaviour and interactions in the living cell adds to the in-vitro information obtained through traditional biochemistry techniques and results in a better understanding of the molecular, cellular and physiological mechanisms of disease. Additionally I also investigate the application of these methodologies to minimally invasive medical diagnosis.

The Biophotonics lab is endowed with a multiphoton Leica TCS-SP5 microscope with advanced imaging capabilities such as Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Lifetime Imaging (FLIM). We are also currently setting up a PALM microscope for imaging at nanometer resolution.

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Systems Biophysics and Computational Biology

(J. Vilar)

Our research activity is focused on computational and mathematical analysis of biological systems at their various levels of organization, from molecular properties to cell behaviour and the role these play in the cell population dynamics. The main objective is to deepen our understanding of the underlying mechanisms and to use this information for the controlling and designing cellular processes. For this, we use the latest computational technologies with a wide range of methods including molecular dynamics, structural bioinformatics, stochastic simulation algorithms and mathematical analysis of dynamic systems. Using these computational biophysical techniques, together with corresponding experimental results, we are studying proteins, nucleic acids and lipids, as well as their interactions, their collective properties and the dynamics of their macromolecular complexes in networks of gene expression and signal transduction.

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Structural Glycobiology

(M.E. Guerin)

Glycans are not only one of the major components of the cell but also are essential molecules that modulate a variety of important biological processes in all living organisms. These oligo- and polysaccharides are used primarily as energy storage and

metabolic intermediates as well as being key structural components in bacteria and plants. Moreover, as a consequence of protein and lipid glycosylation, glycans generate a significant amount of structural diversity in biological systems. These structural features are particularly apparent in molecular recognition events including cell-cell, cell-matrix and cell-molecule interactions during critical stages of development, the immune response and host-pathogen interactions. Most of the enzymes encoded in eukaryotic/prokaryotic/archaeal genomes responsible for the biosynthesis of glycan structures are glycosyltransferases. The long-term goal of our research program is to understand how glycosyltransferases function to control health and disease at the molecular level. We are particularly interested in investigating the structural and mechanistic properties of glycosyltransferases with special emphasis on the study of integral and peripheral membrane-associated enzymes. To this end, we are using a multidisciplinary approach including molecular biology, protein biochemistry, protein biophysics and structural biology.

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Nanomechanics of cell membrane systems

(V. Frolov)

Morphological flexibility of cell membranes provides the foundation for the spatial organization of living cells. The signature morphologies of cellular endomembrane systems are created at the nanoscale where specialized proteolipid complexes assemble to control membrane curvature, shape and topology. Our main focus is on fundamental molecular mechanisms of membrane remodeling by such complexes operating at submicron scales, where pathways of membrane deformations are defined by forces applied by individual protein complexes, carefully organized in time and space, and elastic resistance of the lipid bilayer. We apply novel experimental approaches combining nanomanipulations, electrophysiology and time-resolved fluorescence, confocal and TIRF microscopy to characterize mechanical properties and dynamics of biomimetic and cell membranes at the nanoscale, with particular attention to topological membrane remodeling, fusion and fission, dynamics of the force- and geometry-induced demixing of membrane components and diffusion in complex media. We reconstitute the morphological activity of the prototype proteins controlling membrane remodeling, such as dynamin and matrix protein of enveloped viruses, using nanofabricated lipid templates to resolve subtle features of the proteolipid interactions, creation and sensing of membrane curvature by proteins, and dynamics of protein complexes on membrane surfaces.
Finally, we carry out theoretical analysis of the proteolipid interactions utilizing phenomenological membrane models and simulations.

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- "Mecanismos de regulación de proteínas BCL-2 por componentes de la maquinaría d fisión mitocondrial y por potenciales fármacos antitumorales" **Aitor Etxebarria Gallego;** Supervisor: Gorka Basañez Asúa, 20 February 2009.

- "Actividades esfingomielinasa bacterianas y de mamíferos. Caracterización y efectos estructurales en membranas" **David López Jiménez**; Supervisor: Alicia Alonso Izquierdo, 23 March 2009.

- "Toxinas bacterianas con actividad fosfolipasa C/esfingomielinasa. Interacciones con bicapas lipídicas" **Patricia Urbina Fernández;** Supervisor: Félix M. Goñi Urcelay, 24 March 2009.

- "Structural basis of the stability, infectivity and (dis)assembly process of Triatoma Virus" **Jon Agirre Hernández;** Supervisor: Diego M.A. Guérin, 29 May 2009.

- "Adenilato ciclasa de Bordetella pertussis. Estudio de su interacción con membranas" **Geraxane Gómez Bilbao**; Supervisors: Helena Ostolaza and César Martín, 17 July 2009.

- "Structural studies of membrane proteins: The coupling protein TrwB of plasmid R388 and the pore-forming toxin FraC" **Ariel E. Mechaly García;** Supervisor: Diego M. Guérin, 11 September 2009.

- "Fusión de membranas modelo inducida por fosfolipasas y por fluctuaciones térmicas" **Maitane Ibarguren Aizpitarte;** Supervisor: Félix M. Goñi, 22 Sept. 2009.

- "Estudios de formación de fibras amiloides por espectroscopía de infrarrojos" **Igor de la Arada Etxebarria;** Supervisor: José Luis Rodríguez Arrondo, 16 October 2009.

- "La reconstrucción de TrwB, un monomotor que transporta DNA, revela la importancia del dominio transmembrana de la proteína" **Ana Julia Vecino Ortega;** Supervisor: Itziar Alkorta Calvo, 30 October 2009.

- "Lipid-lipid interactions in membranes and their role in apoptosis" **Jon Busto Vega;** Supervisor: Alicia Alonso Izquierdo, 22 December 2009.

- "Reconocimiento de la nucleoplasmina por importina α, su receptor de transporte al núcleo" **Igor Arregi Vado;** Supervisors: M. Ángeles Urbaneja Arrúe and Sonia Bañuelos Rodríguez, 15 July 2010.

- "Ensamblaje y tráfico a membrana de los canales de potasio KCNQ2 y KCNQ3" **Juan Camilo Gómez Posada;** Supervisor: Álvaro Villarroel Muñoz, 26 October 2010.

- "Reactivación de agregados protéicos por ClpB. Función del dominio M" **Urko del Castillo Rojo;** Supervisor: Arturo Muga Villate, 28 October 2010.

- "Estudio de la interacción de la calmodulina con los canales KCNQ2" **Alessandro Alaimo Campi;** Supervisor: Álvaro Villarroel Muñoz, 29 October 2010.

Patents 2009/2010

INVENTORS	Luis Alberto Anel Bernal, María José Martínez Lorenzo, Luis Martínez Lostao, Gorka Basañez Asua, María Angeles Alava, Luis Larrad Mur, Martínez de Contrasta, Javier Naval Iraberri and Andrés Piñeiro Antón.
TITLE	"Liposomas recubiertos con el dominio extracelular de la proteína Apo2L/TRAIL".
APPLICANT	Universidad de Zaragoza, CSIC, UPV/EHU
APPLICATION Number	P200930618
PRIORITY DATE	21-8-2009
PRIORITY COUNTRY	Spain
EXTENSION TO OTHER COUNTRIES	Europe, PCT/ES2010/000354

INVENTORS	Olatz Landeta Diaz, Ane Landajuela Larma, Gorka Basañez Asua.
TITLE	Mutante de BAK, método asociado para la identificación de Sustancias moduladoras de BAK y péptido inhibidor de la actividad BAK.
APPLICANT	CSIC, UPV/EHU
APPLICATION Number	201031877
PRIORITY DATE	17-12-2010
PRIORITY COUNTRY	Spain

Publications 2009/2010

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- "Excitation of the M intermediates of wild-type bacteriorhodopsin and mutant D96N: temperature dependence of absorbance, electric responses and proton movements". R. Tóth-Boconádi, A. Dér, S.G. Taneva and L. Keszthelyi. *Theor. Chem. Acc.* **125**, 365–373 (2010).

- "Accurate prediction of gene expression by integration of DNA sequence statistics with detailed modeling of transcription regulation". J.M. Vilar. *Biophys J*. **99**, 2408-2413 (2010).

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- "Rational design, synthesis, and evaluation of new selective inhibitors of microbial class II (Zinc dependent) fructose bis-phosphate aldolases". R. Daher, M. Coinçon, M. Fonvielle, P.M. Gest, M.E. Guerin, M. Jackson, J. Sygusch and M. Therisod. *J. Med. Chem.* **53**, 7836-7842 (2010).

- "Fluorescence lifetime imaging reveals that the environment of the ATP binding site of myosin in muscle senses force". D. Ibañez-García, J. Requejo-Isidro, M.R. Webb, T.G. West, P. French and M.A. Ferenczi. *Biophys J*. **99**, 2163-2169 (2010).

- "ENVIRONMENT: a computational platform to stochastically simulate reacting and self-reproducing lipid compartments". F. Mavelli and K. Ruiz-Mirazo. *Phys. Biol.* **7** 036002 (13 pp). doi:10.1088/1478-3975/7/3/036002 (2010).

- "Toward understanding protocell mechanosensation". D. Balleza. *Orig Life Evol Biosph* (24 pp) DOI 10.1007/s11084-010-9225-y (2010).

- "Induced perturbations and adopted conformations in membranes by the HIV-1 fusion peptide" J.L. Nieva, N. Huarte, S. Nir and D. Weliky en *"Membrane-active peptides: methods and results on structure and function"* (M. Castanho ed.) vol. 9, pp. 565-596. IUL (International University Line) Publishers, La Jolla, California, USA (2010).

Conferences and Courses

Organization of Meetings

Bilbao, September 7-11, 2010

51st International Conference on the Biosciences of Lipids (ICBL).

This meeting was co-organized by F.M. Goñi (Unidad de Biofísica) and Antonio Gómez-Muñoz (Department of Biochemistry, UPV/EHU), and was attended by about **240** lipid scientists from **30** countries with 26 invited lecturers, 28 short oral communications and 163 poster presentations.

Madrid, January 15, 2010

Workshop UCM-FBB. Mechanics of biomembranes.

- **F.M. Goñi.** Co-organizer and Chair, together with J. Pérez-Gil (Universidad Complutense, Madrid).

Zaragoza, Spain, July 7-10, 2010

IV Spanish Portuguese Biophysical Congress

- **A Alonso.** Member of the Organizing and Scientific Committee and Chairperson.

Bilbao, Spain. July 12-13, 2010

International Workshop on Membrane Proteins, Signal TansductionTransduction and Disease.

- **D.M.A. Guérin, I. Alkorta and Á. Villarroel.** Co-organizers together with **P. Areso** (University of the Basque Country), **A. Martinez de la Cruz** (CIC bioGUNE).

Lisboa, Portugal, 13-16 September 2010

I Iberoamerican Workshop on Chagas Disease, Triatomine vectors, T. cruzi, and **O. Millet** (CiC Biogune).*Triatoma viru"*. Centro de Malária e Outras Doenças Tropicais (UCDT/CMDT), Instituto de Higiene e Medicina Tropical.

- **D.M.A. Guérin.** Organizer.

Balmaseda, Bizkaia, Spain, September 19-21, 2010

Primera Reunión de Coordinación RedTrV (CYTED)

- **D.M.A. Guérin.** Organizer.

Bilbao, Bizkaia, Spain, September 24, 2010

Neuroscience-Biophysics Encounter

- **Á. Villarroel.** Organizer.

This workshop is an effort from the *Unidad de Biofísica* to create bridges between our research unit and Neuroscientists at or near the University Campus. Our scientific experience might be in some cases complementary, and we believe that ignorance of our mutual activities is preventing possible fruitful collaborations. With this in mind, we organized a scientific workshop where five researches from the UB met a similar number of colleagues from other centers at or near the University Campus.

Baeza, Jaén, Spain, November 2-4, 2010

Ion channels and diseases of the nervous system

- **Álvaro Villarroel.** Co-Organizer.

Bilbao, November 17, 2010

II Seminario Comunicación Científica. Divulgación de la Ciencia/Comunicación científica. Salón de Actos de la Delegación del Departamento de Sanidad del Gobierno Vasco en Bilbao.

- **F.M. Goñi.** Organizer and Chair.

Invited Talks

Murcia, January 20, 2010

Ciclo de conferencias de la Facultad de Veterinaria, Murcia

- **F.M. Goñi** "El fraude científico: cómo, cuánto y por qué?"

Barcelona, January 25, 2010

Ciclo de conferencias del Instituto de Química Avanzada de Cataluña (IQAC)

- **F.M. Goñi** "Propiedades biofísicas de esfingolípidos sencillos"

Ventura, Ca, USA, February 7-12, 2010

Gordon Research Conference on: "Glycolipid & Sphingolipid Biology"

- **A. Alonso** "Ceramide and cholesterol. An unlikely couple"

San Francisco, California, USA, February 20-24, 2010

54th Annual Meeting of the Biophysical Society

- **F.M. Goñi, Co-Chair, Session:** "Membrane Dynamics & Bilayer Probes"
- **F.M. Goñi** "Solubilizing concentrations: transmembrane lipid motion, bilayer permeabilization and vesicle lysis/reassembly are independent phenomena"
- **F.M. Goñi** "Coexistence of immiscible mixtures of palmitoylsphingomyelin and palmitoylceramide in monolayers and bilayers"

Derio, Bizkaia, March 12, 2010

CIC bioGUNE, Technology Park of Bizkaia

- **M. Guerin** "A model of action for peripheral membrane-associated GT-B glycosyltransferases, an essential family of enzymes involved in glycolipid biosynthesis"

Leioa, Bizkaia, Spain, March 3, 2010

Ciclo de conferencias "Actual Research in… Biology". Facultad de Ciencia y Tecnología.

- **J.L.R. Arrondo** "Estudios estructurales de biomoléculas por espectroscopia IR"

Tokyo, Japón, March 31, 2010

Graduate School of Frontier Sciences. University of Tokyo.

- **F.M. Goñi** "Novel aspects in the structure and dynamics of biological membranes"

Madrid, Spain, April 20-23, 2010

Reunión de la Red Temática Nacional Estructura y Función de las Proteínas.

- **J.L.R. Arrondo** "2DCOS and band removal"

Bilbao, April 22, 2010

Ciclo "En tierra de nadie". Conversaciones sobre Ciencias y Letras.

- **F.M. Goñi** and **I. Azkuna** (Mayor of Bilbao) "Por el placer de vivir: comer y felicidad"

Toulouse, France, June 3, 2010

Institut de Pharmacologie et de Biologie Structurale

- **A. Muga "**ATP utilization requirements that render ClpB active as a chaperone"

Erandio, Bizkaia, Spain, June 4, 2010

Centro de Castilla y León. Concejalía de Cultura, Juventud y Deportes

- **F.M. Goñi** "Un bioquímico en la cocina"

Donostia-San Sebastián, Gipuzkoa, June 25, 2010

CIC biomaGUNE, Technology Park of Gipuzkoa

- **A. Alonso** "Membrane Domains: What you can see and what you can imagine"

Zaragoza, Spain, July 4-10, 2010

Congreso SBE. Conferencia Premio Bruker

- **J.L.R. Arrondo** "Espectroscopia de infrarrojo"

Jaca, Huesca, Spain, July 12-14, 2010

Cursos de verano de la Universidad de Zaragoza

- **J.L.R. Arrondo** "Espectroscopia de infrarrojo"

Varsaw, Poland, July 17-22, 2010

16th European Bioenergetics Conference.

- **G. Basañez** "Reconstitution of proapoptotic BAK function in liposomes reveals a dual role for mitochondrial lipids in the membrane permeabilization process"

Bilbao, Bizkaia, Spain, September 7-11, 2010

51st International Conference on the Biosciences of Lipids (ICBL).

- **M. Guerin** "A model of action for peripheral membrane-associated GT-B glycosyltransferases, an essential family of enzymes involved in glycolipid biosynthesis"
- **G. Basañez** "Role of the mitochondrial lipids in proapoptotic BAK function"

Bilbao, Bizkaia, Spain, September 24, 2010

Neuroscience-Biophysics Encounter

- **J.L.R. Arrondo** "Neuroscience-Biophysics. Infrared spectroscopy"

Dresden, Germany, September 28, 2010

Workshop on Timing and Dynamics in Biological Systems.

- **J. Vilar** "Noise propagation across molecular, cellular, and cell-population scales"

Benasque, Huesca, Spain, October 26, 2010

Noise in Life 2010

- **J. Vilar** "Noise propagation across molecular, cellular, and cell-population scales"

Madrid, Spain, November 12, 2010
First Colloquium of the Systems and Synthetic Biology Program of the CNB

- **J. Vilar** "From components to systems: lessons from gene regulation and synthetic cooperation"

Toulouse, France, November 30, 2010
Congreso: Symposium on protein misfolding and chaperones

- **A. Muga "**Allosteric interactions in ClpB"

Toulouse, France, November 30, 2010
Congreso: Symposium on protein misfolding and chaperones

- **F. Moro "**Substrate remodelling by DnaK and DnaJ"

Free Communications

Various different members of the Biophysics Unit have presented communications at conferences, **43** at international and **10** at national events, among which we should highlight contributions at the **54th** Annual Meeting of the Biophysical Society, San Francisco, California (USA), and the **XXXIII** Congress of the Spanish Society for Biochemistry and Molecular Biology, SEBBM held in Córdoba, Spain.

Bilbao Advanced Courses on Biophysics 2010

(Coordinator: **J.M. Requejo-Isidro)**

www.fundacionbiofisicabizkaia.org/**bilbaobiophysics**/

Advanced Course: "SINGLE PROTEINS MECHANICS", September 20-30, 2010.

Organizers:

Sergi García-Mañes (Columbia University, USA)

Raúl Pérez-Jiménez (Columbia University, USA)

Julio Fernández (Columbia University, USA)

Invited Speakers:

Jasna Brujic (New University, USA)

Mariano Carrión-Vázquez (Instituto Cajal, CSIC, Spain)

Hongbin Li (University of British Columbia, Canada)

Wolfgang Linke (Ruhr-Universität, Germany)

Hui Lu (University of Illinois at Chicago, USA)

The course was attended by 21 students from 4 countries.

Other Courses

Practical Course on Biotechnology:

"Modificación y expresión de proteínas en células eucariotas", May 17-30, 2010.
Sponsored by Fundación Biofísica Bizkaia.

Instructors:

Juncal Fernández Orth
Araitz Alberdi González
Covadonga Malo de la Fuente
Teresa Zamalloa Echeverria

Science Communication

A Lecture Series on ***Rare diseases*** was organised by the BBVA Foundation and CIC bioGUNE in collaboration with the **Biophysics Unit**, the British Council, the Regional Government of Biscay and the Government of the Basque Country (Bilbao, Feb-May 2010)

Bilbao, December 16, 2010
Science Week 2010 in Miguel de Unamuno Secondary School.

- **F.M. Goñi** "Obesidad, anorexia y adelgazamiento. Mitos y realidades"

Bilbao, December 16, 2010
Real Sociedad de Amigos del País
Jornada sobre Ciencia y Tecnología en el País Vasco

- **F.M. Goñi** "Biofísica: ¿de dónde venimos. adónde vamos, y para qué estamos aquí?"

A. Alonso has continued to be the organiser for the third series of *BioForo* Lectures at the Faculty of Science through 2010 with participation of the following researchers:

- **Geoffrey Burnstock** *(Autonomic Neuroscience Centre Royal Free and University College Medical School, Londres, UK)*
- **Ana Mª Cuervo** *(Albert Einstein College of Medicine. New York, USA)*
- **Adriana di Polo** *(Université de Montréal, Montreal, Quebec, Canada)*
- **Carmelo di Primo** *(INSERM. ARNA Laboratory. Université de Bourdeaux)*
- **José Antonio Enríquez** *(Universidad de Zaragoza and CNIC, Madrid)*
- **Timothy E. Kennedy** *(McGill University, Montreal, Quebec, Canada)*
- **Mikel Garcia-Marcos** *(George Palade Laboratories for Cellular and Molecular Medicine, University of California, San Diego, USA)*

- **Javier González-Maeso** *(Departments of Psychiatry and Neurology, Mount Sinai School of Medicine, New York)*
- **Elisabeth Grohmann** *(Universitäts Klinikum Freiburg, Germany)*
- **Jacqueline Gulbis** *(The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia)*
- **Thomas Harder** *(Dept. of Immunology. Sir William Dunn School of Pathology. University of Oxford, UK)*
- **Vladimir Kaberdin** *(Department of Immunology, Microbiology and Parasitology. Faculty of Science. University of the Basque Country, Bilbao)*
- **Aurora Martínez** *(Department of Biomedicine. University of Bergen (Noruega)*
- **Francois Nedelec** *(Cell biology and Biophysics Unit, European molecular biology laboratory, Heidelberg, Alemania)*
- **Walter Neupert** *(Institute of Physiological Chemistry, Munich, Alemania)*
- **José A. Obeso** *(Facultad de Medicina, Clínica Universitaria and CIMA. Universidad de Navarra)*
- **Manuel Palacín** *(Institute for Research on Biomedicine IRB, Universidad de Barcelona)*
- **Susana Rivas** *(LIPM Laboratoire Interactions Plantes Micro-organismes. INRA/CNRS, Auzeville Campus- Toulouse, France)*
- **José Mª Valpuesta** *(CNB Centro Nacional de Biotecnología – CSIC, Madrid)*
- **Angela Vincent** *(Department of Clinical Neurology, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford UK)*
- **Benedikt Westermann** *(Biología celular. Universidad de Bayreuth, Alemania)*
- **Kornelius Zeth** *(Max Planck. Institute for developmental biology. Dept. Protein Evolution. Tubinga, Alemania)*
- **José Luis Zugaza** *(Iniciativa Andaluza en Terapias Avanzadas. Junta de Andalucía, Sevilla)*

Governing Bodies and Academic Committees

I. Alkorta continued to serve as Vice-Dean of the *Facultad de Ciencia y Tecnología*, in charge of Communication, Image and Interactions with Society.

J.L. Nieva continued to serve as Head of the Department of Biochemistry and Molecular Biology.

H. Ostolaza continued to serve as the Academic Secretary of the Department of Biochemistry and Molecular Biology.

F.M. Goñi continued as Coordinator of the Ph D Programme in Molecular Biology and Biomedicine.

A. Alonso continued as a Member of the Commission for Teaching Evaluation of the Spanish National Evaluation Agency ANECA in the area of Health.

I. Alkorta continued to serve as a Member of the Academic Affairs and of the Academic Exchange Committees of the Facultad de Ciencia y Tecnología.

J.L.R. Arrondo completed her term as a member of the University Research Committee.

Scientific Societies

F.M. Goñi continued to serve as Chairman of the FEBS Publications Committee.

A. Alonso completed her term as President of the Spanish Biophysical Society (SBE) (July 2010) and was appointed as VicePresident for International Affairs of the Spanish Society for Biochemistry and Molecular Biology (SEBBM).

A. Alonso continued to serve as member of the Spanish Committee of the International Union of Pure and Applied Biophysics (IUPAB).

J.L. R. Arrondo completed his term as Member of the Spanish IUPAB Committee (October 2010)

A. Alonso continued to serve as a member of the Executive Committee of the International Union for Pure and Applied Biophysics (IUPAB).
http://iupab.org/about/officers-and-council/

F.M. Goñi was appointed Chair of the International Relations Committee of the Biophysical Society (USA) for a three-year term (July 1, 2010 – June 30, 2013).

I. Alkorta was appointed as Consul for the Basque Country of the Spanish Society of Biochemistry and Molecular Biology (SEBBM).

I. Alkorta was appointed as ordinary member of the Admissions Committee of the Spanish Society of Biochemistry and Molecular Biology (SEBBM) for a four-year term (September 1, 2010 – September 30, 2014).

Scientific Journals

F.M. Goñi continued to serve as a member of the Editorial Advisory Board of *Chemistry and Physics of Lipids* (Elsevier), the *Journal of Chemical Biology,* (Springer) and of the scientific magazine *CIC-Network*.

A. Alonso continued to serve as a member of the Editorial Committee of *Biochimica et Biophysica Acta-Biomembranes* and of *Biophysical Reviews.*

J.M.G. Vilar continued to serve as Associate Editor of *BMC Systems Biology*.

Other Activities

"Iñigo Álvarez de Toledo" Awards

F.M. Goñi was a member of the Evaluation Committee for Fundamental Research of the Iñigo Alvarez de Toledo Renal Foundation. (1 October 2010).

Novia Salcedo Foundation

F.M. Goñi is a member of its Governing Board and Working Committee.

European Science Foundation

F.M. Goñi continued to serve as a member of the ESF Pool of Reviewers of the European Science Foundation.

J.L.R. Arrondo continued to serve as a member of the ESF Pool of Reviewers of the European Science Foundation.

Department of Innovation Science and Business

F.M. Goñi was appointed evaluator of international scientific and technical projects by the Agency for Quality Assurance in Higher Education and Research of Andalusia (Department of Innovation, Science and Business).

Appearance in Parliament

F.M. Goñi held an appearance at the Basque Parlament Committee for Environment, Territorial Planning, Agriculture and Fisheries, at the request of the Hon. Carlos Gorostiza Orbañanos (Basque Socialists) to report on the current status of scientific research on the impact of electromagnetic fields on public health.

D.M.A. Guérin continued to serve as remote referee of the Ideas Specific Programme, European Research Council.

Unidad de Biofísica Visitors Programme 2010

(Coordinator: **I. Alkorta**)

Dr. Stefka G. Taneva from the Institute of Biophysics, Bulgarian Academy of Sciences, Sofia, Bulgaria, is an Ikerbasque Visiting Fellow, Nov. 2010-Oct. 2011.

Prof. **Richard Mendelsohn** from Rutgers University, Newark, New Jersey, USA, stayed with us in May 2010.

Dr. **Jesús Bernardino Velázquez** from the Universidad Autónoma de Nayarit, Tepic, Nayarit, Mexico visited from April to September 2010.

During brief visits, the following researchers gave seminars:

- **Nicola Abrescia (**CIC-Biogune, Zamudio, Biscay, Spain)
- **David Albesa-Jové (**Centre for Structural Biology; Division of Cell and Molecular Biology, Imperial College London, UK)
- **Roberto Araya (**Columbia University, New York, USA)
- **Joaquín Castilla (**CIC bioGUNE Zamudio, Biscay, Spain)
- **Jorge Cuellar (**Centro Nacional de Biotecnología (CSIC), Madrid, Spain)
- **Andrés de la Escosura (**Departamento de Química Orgánica, Universidad Autónoma de Madrid, Madrid, Spain)

- **Evan Evans (**Department of Physics and Astronomy, University of British Columbia, Vancouver, Canada)

- **Damián Garcia Olmo (**Hospital Universitario La Paz, Madrid, Spain)

- **David Giganti** (Unité de Biochimie Structurale, Institut Pasteur, Paris, France)

- **Martin Hanczyc (**Department of Physics and Chemistry, University of Southern Denmark, Odense, Denmark)

- **Oscar Millet (**CIC bioGUNE, Zamudio, Biscay, Spain)

- **Adriana Rojas (**CIC bioGUNe, Zamudio, Biscay, Spain)

- **Jamie K. Scott** (Dept. of Molecular Biology and Biochemistry, and Faculty of Health Sciences, Simon Fraser University, Vancouver, Canada)

Funding

In 2010, the Biophysics Unit received funding from the following institutions (listed alphabetically).

- European Union
- Basque Government (Department of Education, Universities and Research)
- Basque Government (Department of Industry, Commerce and Tourism)
- Ibero-American Programme for Science, Technology and Development (CYTED)
- Spanish Ministry of Science and Innovation
- Regional Government of Biscay (*Diputación Foral de Bizkaia*, Department of Innovation and Economic Promotion)
- University of the Basque Country

The Unit wishes to thank to all of these sponsors for their generous funding and ongoing support.

PhD Theses from the Biophysics Unit

(Until 1999, Biomembrane Group of the Department of Biochemistry, UPV/EHU)

- José Ignacio García Gurtubay (1979)
- Alicia Alonso Izquierdo (1981)
- Mª Carmen Barbero (1981)
- Mª Angeles Urbaneja Arrúe (1984)
- José María Valpuesta Moralejo (1985)
- Arturo Muga Villate (1988)
- Juan Manuel González Mañas (1989)
- María Aránzazu Partearroyo (1989)
- José Luis Nieva Escandón (1991)
- Ana Rosa Viguera Rincón (1992)
- José Castresana Villamor (1992)
- Helena Ostolaza Echabe (1992)
- Sonia Bañuelos Rodríguez (1995)
- Mª Asunción Requero Zabala (1995)
- Gorka Basañez Asua (1996)
- Fernando Moro (1996)
- Ana Soloaga Villoch (1997)
- Susana Rivas Cacho (1997)
- Izaskun Echabe Pérez (1997)
- Francisca Pereira Rios (1997)
- Mª Begoña Ruiz-Argüello (1998)
- José Manuel Martínez Caaveiro (1999)
- Mª Pilar Veiga Alameda (1999)
- Ana V. Villar Ramos (2000)

- Tatiana Suárez Cortés (2000)
- Asier Sáez Cirión (2001)
- Aitor Hierro Ayuela (2002)
- Aitziber López Cortajarena (2002)
- Aitziber Agirre Ruiz de Arkaute (2003)
- Asier Galán Cousillas (2003)
- Ion Gutiérrez Aguirre (2003)
- Itsaso Hormaeche Berciano (2003)
- Begoña Sot Sanz (2003)
- Ibon Iloro Manzano (2004)
- Xabier Coto Revuelta (2004)
- Jesús Sot Sanz (2005)
- Ruth Montes Burgos (2005)
- Vanesa Fernández Sáiz (2006)
- Isbaal Ramos Hernández (2006)
- Maier Lorizate Nogales (2006)
- Francesc-Xabier Contreras Gómez (2006)
- Silvia Sánchez Martínez (2007)
- Lissete Sánchez Magraner (2007)
- Marcos García Pacios (2007)
- Oihana Terrones Urio (2007)
- Aintzane Cabo Bilbao (2008)
- José Ángel Fernández Higuero (2008)
- Paloma Aivar Mateo (2008)
- Sergio Pérez Acebrón (2008)
- Nerea Huarte Arrayago (2008)
- Aitor Etxebarria Gallego (2009)
- David López Jiménez (2009)
- Patricia Urbina Fernández (2009)

- Jon Agirre Hernández (2009)
- Geraxane Gómez Bilbao (2009)
- Ariel E. Mechaly García (2009)
- Maitane Ibarguren Aizpitarte (2009)
- Igor de la Arada Etxebarria (2009)
- Ana Julia Vecino Ortega (2009)
- Jon Busto Vega (2009)
- Igor Arregi Vado (2010)
- Juan Camilo Gómez Posada (2010)
- Urko del Castillo Rojo (2010)
- Alessandro Alaimo Campi (2010)