

# Macromolecular Crystallography Group

## Summary

The focus of our Group is the molecular understanding of the role played by macromolecules involved in oncogenic processes. To achieve this goal we work on the structural determination of these biomolecules and their complexes.

The human genome is a sophisticated and complex coding system capable of producing thousands of different proteins in a tightly controlled manner. Proteins interact with other macromolecules forming assemblies that perform particular cellular tasks. The structural determination of these complexes will help us decipher the molecular mechanisms that rule these processes.

## Strategic Goals

- Unravel macromolecular machines involved in cell cycle dynamics and control
- Design structurally homing endonucleases for gene targeting

## Guillermo Montoya *Group Leader*

Guillermo Montoya was born in Madrid in 1967 and obtained his Bachelor degree in Biochemistry from the *Universidad del País Vasco* in 1990, and his PhD in Chemistry from the *Universidad de Zaragoza* in 1993.

He obtained both a European Molecular Biology Organisation (EMBO) and Federation of European Biochemical Societies (FEBS) Fellowship and moved to the *Max Planck Institut für Biophysik in Frankfurt am Main, Germany*, where he worked on membrane protein crystallisation in the Group of the Nobel laureate H. Michel.

Montoya later obtained a Marie Curie Fellowship and spent nine years at the European Molecular Biology Laboratory (EMBL), Heidelberg, Germany, working in I. Sinning's Group where he focused on the crystallisation of the cytochrome bc<sub>1</sub> membrane protein complex and later pioneered the study of the structure of the signal recognition particle (SRP), an essential ribonucleoprotein complex involved in protein targeting.

In 1998 he was appointed as Researcher at the *Consejo Superior de Investigaciones Científicas (CSIC)* and was awarded a Peter und Traudl Engelhorn Foundation Research Fellowship. Since 2003 he has been an Honorary Professor in Biochemistry at the *Universidad Autónoma de Madrid* and Member of the working group in charge of the design of the biocrystallography beamline at the Spanish Synchrotron.

Montoya has been Head of the CNIO's Macromolecular Crystallography Group since February 2002 and was acting Director of the Structural Biology and Biocomputing Programme from November 2003 to January 2006. During this year he has been awarded with the National Prizes from the *Fundación Mutua Madrileña* and *Caja Rural de Granada, Ministerio de Sanidad*.





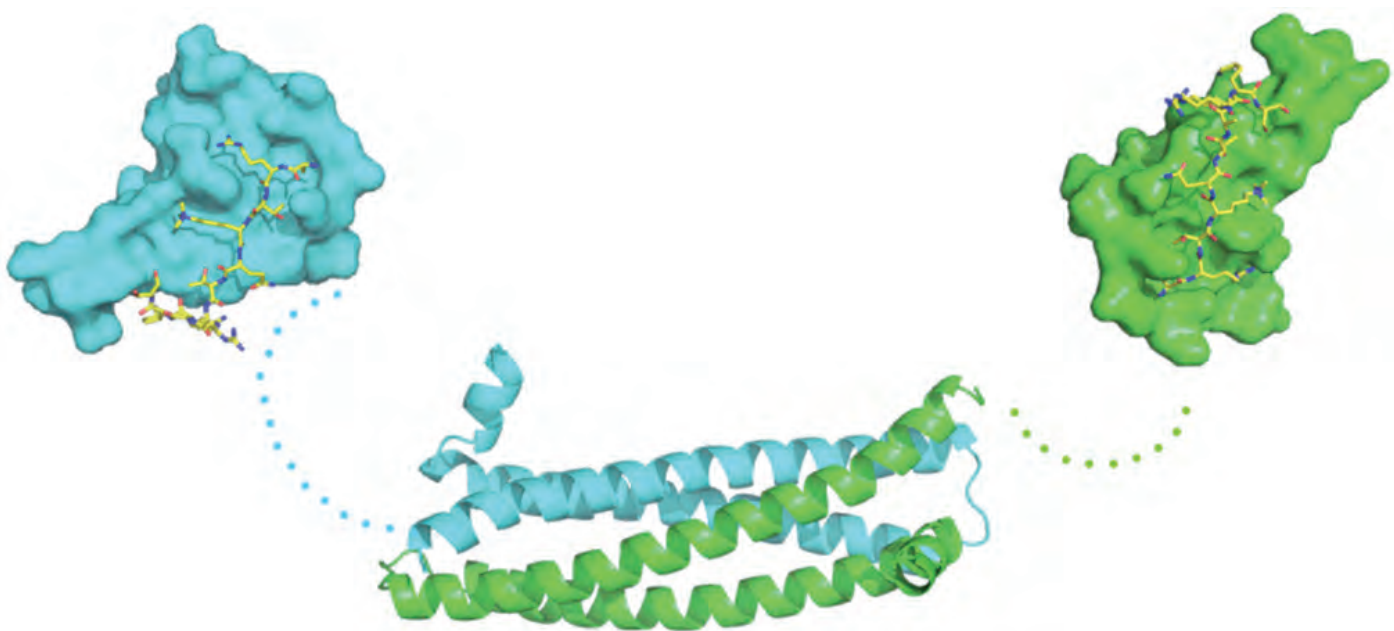
**Staff scientists:** Jasminka Boskovic, Gulnahr Mortuza, Inés G. Muñoz, Francisco J. Prieto. **Post-doctoral fellows:** María J. Marcaida, Marco Mazzorana, Sunita Subramanian. **Graduate students:** Javier Coloma, Ana M. Garrote (since October), Jaime Martínez (since October), June Sánchez. **Technicians:** Elisabeth R. Bragado, Sonia Ibáñez, Pablo Mesa, Juan G. Pedrero, M. Pilar Redondo.

## Highlights

### S-phase and replication

DNA replication is an essential process during cell division. The identification of the DNA helicase(s) involved in eukaryotic DNA replication is still a matter of much debate. Recently, the helicase activity of the hexameric MCM complex has been revealed as being responsible for the unwinding of DNA during S phase in association with two partners: initiation factor Cdc45 and a four-subunit complex

called GINS. In conjunction they form the CMG complex which contains ATP dependent helicase activity. We aim to unravel the molecular mechanisms of this cellular machinery essential for eukaryotic DNA replication. During this year we have been able to obtain all these components using co-expression techniques. Thus, we are now able to study them by combining X-ray crystallography and EM studies to decipher the structure of this complex and its components.



**Figure 1:** Model of methylated histone binding protein complex based on the crystal structure of its dimerisation domain.

## Mitotic complexes

Cellular growth and division are regulated by an integrated protein network which ensures the genomic integrity of all eukaryotic cells during mitosis. This cell cycle stage witnesses a massive reorganisation of cellular architecture. All these events need the assistance of different proteins to ensure their proper protein folding and performance. One protein that performs this function is a eukaryotic macromolecular complex that allows tubulin, an essential cytoskeletal molecule, to fold properly and form the mitotic spindle among other structures. We have solved the structure of this macromolecular machine, in complex with tubulin, one of its main substrates. Our objective is to obtain high resolution information regarding the atomic structure and the regulation of these molecules.

## Structural design of homing endonucleases for gene targeting

Homing endonucleases or meganucleases are sequence-specific enzymes which recognise large (12–45 bp) DNA target sites. These enzymes are often encoded

by introns or inteins behaving as mobile genetic elements. They recognise sites that usually correspond to intron-free or intein-free genes, where they produce a DNA double-strand break (DSB). Eventually, DSB repair by homologous recombination with an intron – or intein – containing gene results in the insertion of the intron or intein where DSB occurred in specific loci in living cells.

These results present new perspectives in a wide range of applications, such as the correction of mutations linked with monogenic inherited diseases. Our Group has participated in the development of a chimaeric enzyme that could target mutations in the RAG gene promoting its repair. In addition we have shown that repair of the gene can be done in its locus in human cells, opening avenues to possible therapeutic applications. The crystal structure of monomeric meganuclease I-Dmol in complex with its target DNA has allowed us to turn this endonuclease into a nicking enzyme, providing us with

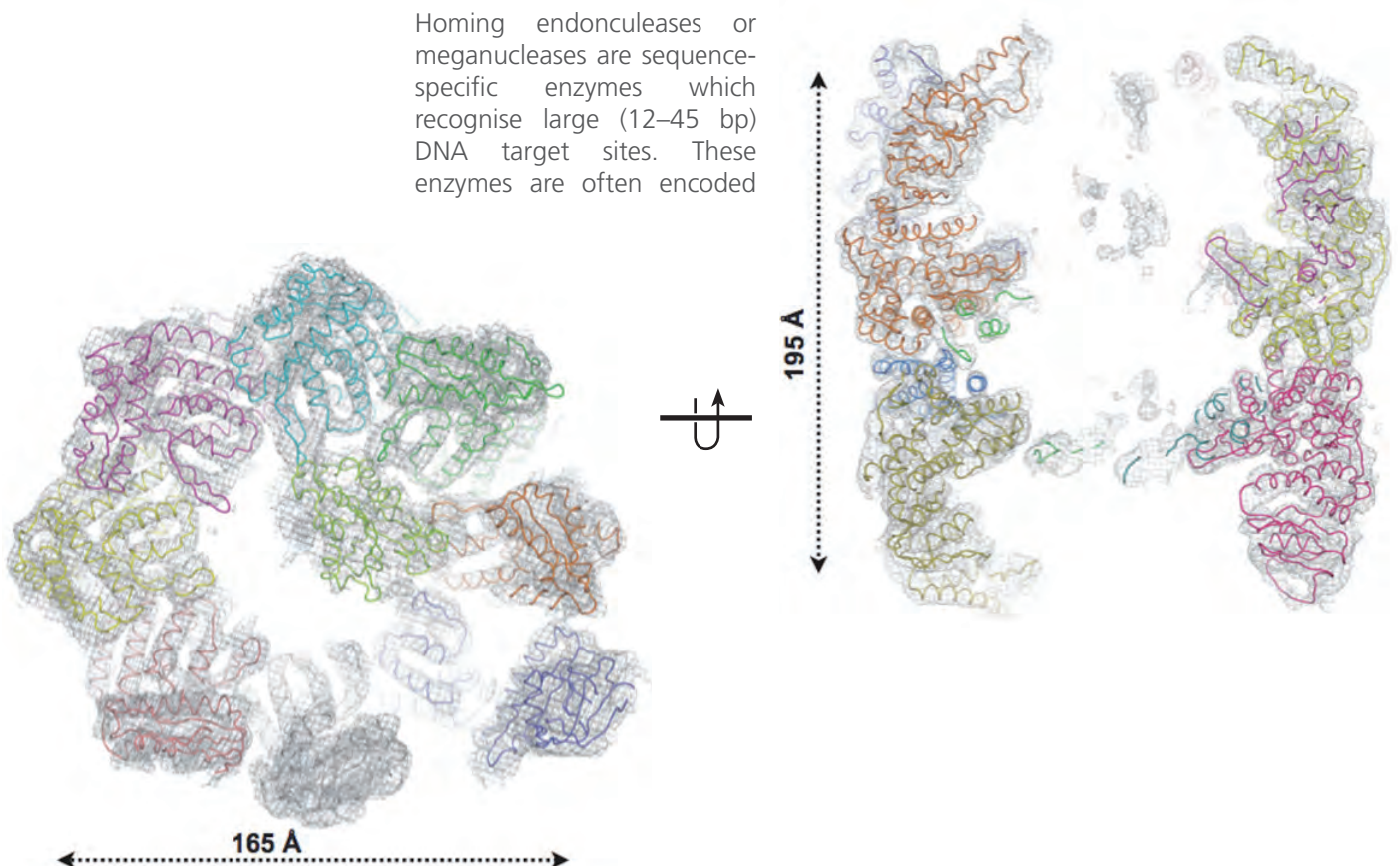
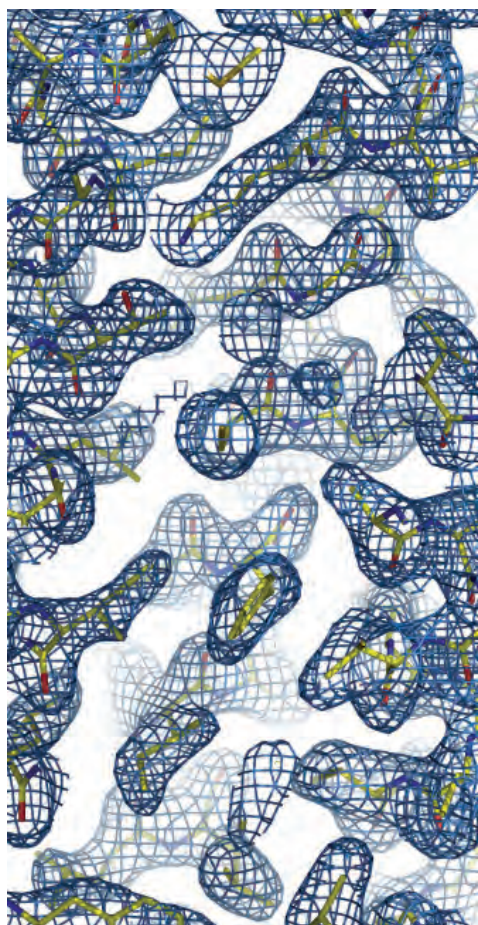


Figure 2: Crystal structure of a 1MDa complex essential for microtubule growth.



a new tool to repair genes preferentially using DSB homologous recombination. DNA nicks are favourably repaired using this route to avoid the unsafe non-homologous end joining (NHEJ) pathway that promotes loss of genetic information.

Figure 3: Electron density map of the dimerisation domain of a histone code reader protein.

## Publications

Lara E, Mai A, Calvanese V, Altucci L, Lopez-Nieva P, Martínez-Chantar ML, Varela-Rey M, Rotili D, Nebbioso A, Roperio S, Montoya G, Oyarzabal J, Velasco S, Serrano M, Witt M, Villar-Garea A, Imhof A, Mato JM, Esteller M, Fraga MF (2009). Salermide, a Sirtuin inhibitor with a strong cancer-specific proapoptotic effect. *Oncogene* 28, 781-791.

Aparicio T, Guillou E, Coloma J, Montoya G, Méndez J (2009). The human GINS complex associates with Cdc45 and MCM and is essential for DNA replication. *Nucleic Acids Res* 37, 2087-2095.

Grizot S, Smith J, Daboussi F, Prieto J, Redondo P, Merino N, Villate M, Thomas S, Lemaire L, Montoya G, Blanco FJ, Pâques F, Duchateau P (2009). Efficient targeting of a SCID gene by an engineered single-chain homing endonuclease. *Nucleic Acids Res* 37, 5405-5419.

Martínez-Cruz LA, Encinar JA, Kortazar D, Prieto J, Gómez J, Fernández-Millán P, Lucas M, Arribas EA, Fernández JA, Martínez-Chantar ML, Mato JM, Neira JL (2009). The CBS domain protein MJ0729 of *Methanocaldococcus jannaschii* is a thermostable protein with a pH-dependent self-oligomerization. *Biochemistry* 48, 2760-2776.

Neira JL, Roman-Trufero M, Contreras LM, Prieto J, Singh G, Barrera FN, Renart ML, Vidal M (2009). The transcriptional repressor RYBP is a natively unfolded protein which folds upon binding to DNA. *Biochemistry* 48, 1348-1360.

Pozo-Dengra J, Martínez-Rodríguez S, Contreras LM, Prieto J, Andújar-Sánchez M, Clemente-Jiménez JM, Las Heras-Vázquez FJ, Rodríguez-Vico F, Neira JL (2009). Structure and conformational stability of a tetrameric thermostable N-succinylamino acid racemase. *Biopolymers* 91, 757-772.

Martínez-Rodríguez S, Encinar JA, Hurtado-Gómez E, Prieto J, Clemente-Jiménez JM, Las Heras-Vázquez FJ, Rodríguez-Vico F, Neira JL (2009). Metal-triggered changes in the stability and secondary structure of a tetrameric dihydropyrimidinase: a biophysical characterization. *Biophys Chem* 139, 42-52.

## Awards and Recognition

Editorial Board Member, *Encyclopedia of Life Sciences*

Board Member, EC-funded PCUBE platform

National Prize "Best Biomedical Research Work" from the *Fundación Mutua Madrileña*, Spain

Health Sciences Prize from *Caja Rural de Granada, Ministerio de Sanidad*, Spain