

Biochemistry Section



Jesús M. Fominaya

Section Head

Born in Madrid in 1960, Jesús Fominaya studied Biology at *Universidad Complutense de Madrid* and obtained his PhD in Biochemistry at the same University in 1989, investigating the RNase-RNase Inhibitor system. In 1990 he moved to the *Friedrich Miescher Institut Novartis*, Basel (Switzerland) as a Postdoctoral Fellow to extend his studies on the regulation of ribonucleolytic activities. He then moved to Freiburg (Germany) to join the *Institut für Experimentelle Krebsforschung*. During this time he worked in the field of Gene Therapy and developed a new concept of versatile recombinant fusion proteins for the target cell-specific delivery of DNA.

He joined the *Centro Nacional de Biotecnología* (CNB-CSIC) in Madrid at the beginning of 1997, extending his concept of modular gene delivery systems to multifunctional peptides. From 1999 to 2001 he established the protein purification facility at INGENASA, a Company that focuses on molecular diagnostics. He joined the CNIO in February 2001 as a Staff Scientist in the Assay Development Group. In April 2006, Fominaya accepted the position as Head of the Biochemistry Section in the Experimental Therapeutics Programme.

Summary

The main goal of the Section is to provide biochemical support for all drug discovery projects in the Experimental Therapeutics Programme. This includes the generation of reagents as well as the cloning, expression and purification of selected proteins. The final yields of purified proteins need to fulfil quality requirements (purity, specific activity, homogeneity) established in accordance with their specific end purpose (enzymatic assay, structural studies).

A second important activity of the Section is the development of a panel of different biochemical assays and their optimisation for the proteins used in our screening campaigns. The Section evaluates the different available technologies to adapt the most accurate platform (sensitivity, robustness, simplicity, and cost effectiveness) for every specific situation. Finally, the group also focuses on the elucidation of biochemical mechanisms of action for selected low molecular weight inhibitors.

Main Objectives

- Express and purify proteins and enzymes to be used for drug discovery
- Biochemically characterise therapeutically relevant enzymes (targets) and develop activity assays as tools to search for active inhibitors on HTS platforms
- Elucidate the biochemical mechanism of action of selected low molecular weight inhibitors



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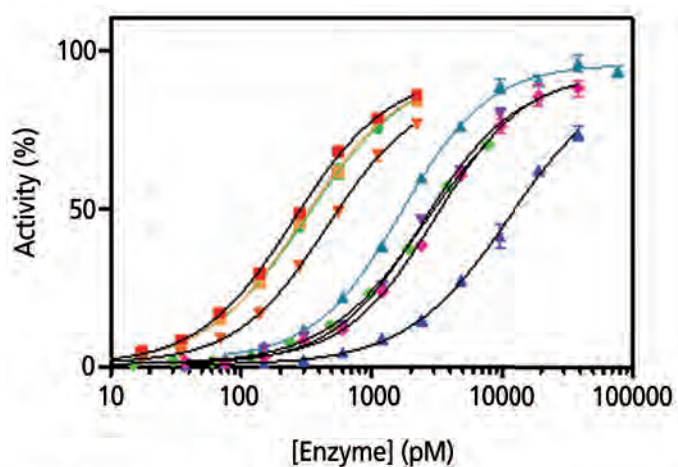
Highlights

The Biochemistry Section provides the expertise for the production of high quality, fully active protein reagents using different expression systems. Five FPLC platforms allow the automatic purification of the expressed proteins ensuring reproducibility and facilitating the isolation of sufficient homogeneous active material to support the different screening campaigns. We have expressed and purified a large number of different proteins, including substrates, enzymes and mutants to ensure the proper development of common projects in the Programme.

Well-validated assays increase the likelihood of identifying genuine inhibitors during screening and are also crucial for developing good Structure Activity Relationships. We have therefore carried out detailed biochemical characterisation to select the optimal assay and establish accurate conditions for screening for

low molecular weight inhibitors with minimal cost and maximal quality to ensure clear and reliable results. We have currently established more than a dozen different types of biochemical assays mainly focused on kinase activities and covering a broad range of sensitivities and technologies (FI, FP, FRET, TR-FRET, chemiluminescence, coupled enzymatic reactions). Recently, we have incorporated binding assays to search for inhibitors against low-activity kinases (such as purified cdk8-cyclin C) thus allowing us to identify both type I and II kinase inhibitors. These assays have been applied to 45 different targets and others are ongoing (Figure).

The Section is also responsible for the characterisation of mechanism of action for selected inhibitors, including determination of kinetic parameters and mechanism of inhibition. Additionally, we provide high quality material for structural studies including the crystallisation of target-compound complexes to obtain detailed structural information of spatial compound orientation and interaction with specific residues of the protein.



- cdk1/cyc B
- cyc5/p25
- cdk9/cyc T1
- cdk2/cyc A
- cdk6/cyc D1
- GSK3β
- cdk4/cyc D1
- cdk7/cyc H/MAT1
- DYRK1A

Figure: Comparison of cdk activities (and family members) on a common assay platform (TR-FRET).

Publication

Oyarzabal J, Zarich N, Albarran MI, Palacios I, Urbano-Cuadrado M, Mateos G, Reymundo I, Rabal O, Salgado A, Corrionero A, Fominaya J, Pastor J, Bischoff JR (2010). Discovery of mitogen-activated protein kinase-interacting kinase 1 inhibitors by a comprehensive fragment-oriented virtual screening approach. *J Med Chem* 53, 6618-6628.