

Cell Signalling and Adhesion *Junior Group*

Summary

We are studying the molecular downstream mechanisms of growth factor receptors and cell/matrix adhesion signalling and how these signals converge to activate Focal Adhesion Kinase. The aim is to obtain an atomic-resolution understanding of these signalling events using X-ray crystallography in combination with detailed biochemical and functional analysis. This insight will provide direct input for the structure based design of new potential therapeutics to correct inappropriate signalling emanating from growth and adhesion cues.

Strategic Goals

- Define the molecular mechanisms that lead from integrin and growth factor receptor signalling to activation of Focal Adhesion Kinase (FAK)
- Apply structure based drug design to develop ATP competitive FAK inhibitors
- High-throughput screening for the discovery of allosteric inhibitors of FAK using a FRET based conformational biosensor of FAK

Daniel Lietha *Junior Group Leader (since September)*



Daniel Lietha was born in 1970 in Chur, Switzerland. He received his undergraduate degrees in Chemistry in 1995 from the *Zürcher Hochschule für Angewandte Wissenschaften*, Switzerland and in Biotechnology in 1998 from the University of Teesside, UK.

In 2003 he obtained his PhD degree in Protein Crystallography from Birkbeck College at the University of London, UK. He carried out his graduate research at the MRC Laboratory of Molecular Biology in Cambridge, UK, where he focused on structural and mechanistic aspects of signalling through Hepatocyte Growth Factor/Scatter Factor and its receptor c-Met. For his postdoctoral training he joined the laboratory of M.J. Eck at the Dana Faber Cancer Institute, Harvard Medical School, Boston, USA. His work led to the discovery of the molecular mechanisms that regulate the non-receptor tyrosine kinase, Focal Adhesion Kinase.

In September 2009 Daniel Lietha joined the CNIO as Junior Group Leader in the Structural Biology and Biocomputing Programme, where he is setting up a research group that will continue to focus on molecular mechanisms of cell signalling and adhesion, downstream of growth factor receptor and integrin signalling.

Daniel Lietha was granted a predoctoral award from the Roche Research Foundation and a postdoctoral fellowship from the National Cancer Institute (NCI), Bethesda (USA). He has authored several high-profile peer-reviewed articles and his graduate work has led to a patent on agonistic c-Met ligands for use in tissue and organ regeneration.



Technician: Guillermina Goñi (since December).

Highlights

Focal Adhesion Kinase (FAK) is a central signalling component in focal adhesions. FAK is activated downstream of integrin and growth factor receptor signalling and it is likely to be the key molecule that integrates growth and adhesion signals.

One main focus of our research is to understand the mechanisms that regulate FAK at atomic resolution. We have recently elucidated the molecular mechanisms of FAK autoinhibition by structural and biochemical means (Figure). We demonstrated that docking of the N-terminal FERM domain onto the kinase domain leads to catalytic inhibition and sequesters regulatory phosphorylation sites. We have also recently discovered that the lipid phosphatidylinositol 4,5-bisphosphate (PIP2) directly interacts with the FERM domain of FAK and induces activation. We are currently studying the detailed mechanism of PIP2 induced FAK activation using biochemical techniques in combination with X-ray crystallography.

Based on the crystal structure of autoinhibited FAK, we have designed a FRET based conformational biosensor of FAK that can report the activation state of FAK. We have utilised this biosensor to monitor FAK activation in living cells

and found that FAK is specifically activated when localised to focal adhesions, but not when it resides in the cytosol. We have now successfully purified this FAK biosensor and plan to use it firstly to study the conformational switch of FAK activation *in vitro*, and secondly – in collaboration with the CNIO Experimental Therapeutics Programme – in a high-throughput screen to discover non-ATP competitive FAK inhibitors. We are also working with the CNIO Computational Biophysics Group to perform *in silico* modelling and docking procedures, which collectively with our X-ray crystallographic analysis will facilitate the design of specific FAK inhibitors.

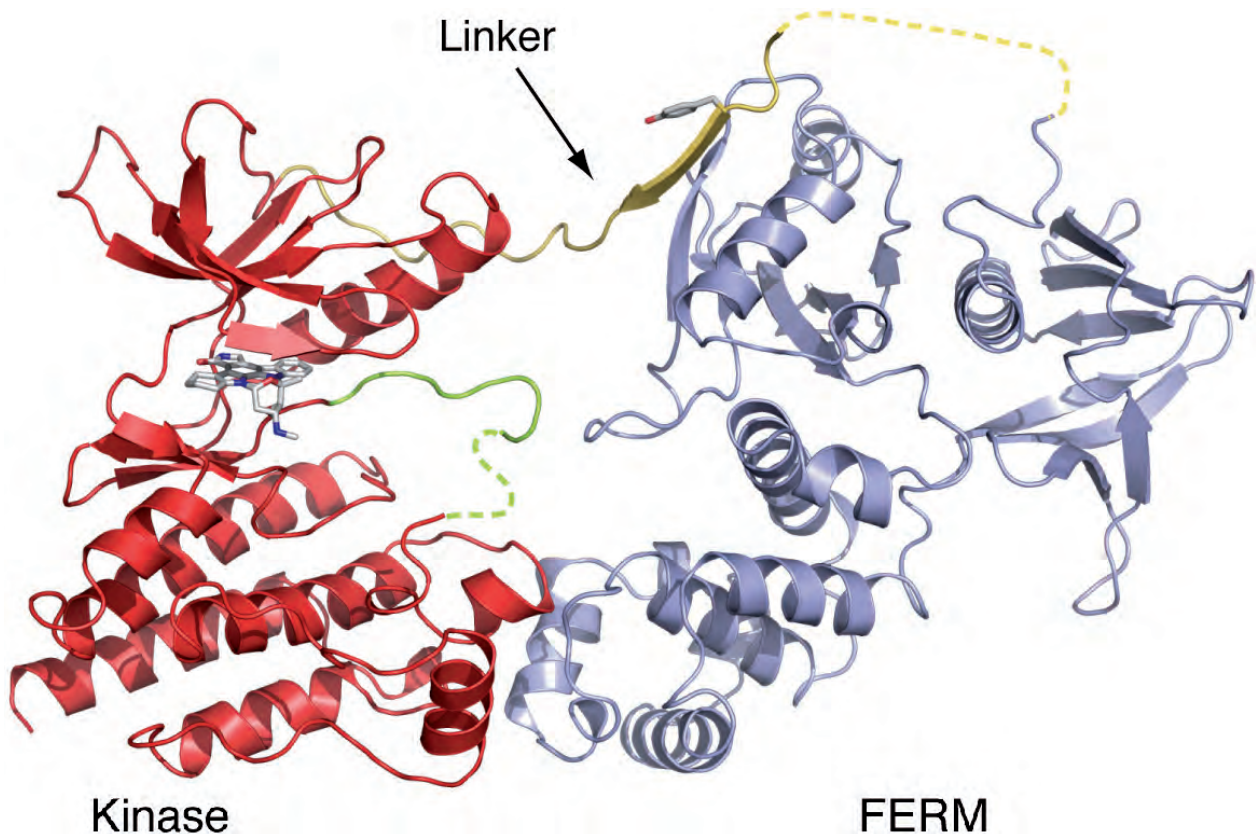


Figure: Crystal structure of FAK containing the FERM and kinase domains. The structure reveals the mode of autoinhibition: the FERM domain (blue) docks onto the kinase domain (red) and thereby inhibits the kinase catalytically and protects regulatory tyrosine phosphorylation sites. The linker is shown in yellow and the activation loop within the kinase domain in green.