CELL SIGNALLING AND ADHESION JUNIOR GROUP

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OVERVIEW

Our Group studies regulatory mechanisms of key signalling switches controlling growth and adhesion signals, which regulate important cellular processes such as cell proliferation, migration and survival. We use structural techniques, such as X-ray crystallography and electron microscopy, in combination with biochemical and functional studies to understand these mechanisms at atomic detail and to rationalise how oncogenic events result in their deregulation. The structural understanding allows us to design potential anti-cancer therapeutics that interfere with oncogenic deregulation.

We focus on growth and adhesion signalling systems that interact and are regulated by specific lipids in the plasma membrane. Specifically, we pursue 2 main questions:

"Using structure-based design, we generated the first irreversible and sub-nanomolar inhibitor targeting adhesion signals that trigger cancer invasion."

→ How are adhesion signals in focal adhesion complexes triggered by membrane interactions?
→ How are the levels of specific lipids regulated by the SHIP lipid phosphatase to control growth signals?

RESEARCH HIGHLIGHTS

Focal Adhesion Kinase (FAK) is a key regulator of adhesion signals and localises into a signalling layer on the plasma membrane in focal adhesion complexes. We previously discovered that FAK interacts with PIP_2 lipids in focal adhesions and this triggers its activation by inducing FAK oligomerisation, conformational changes that facilitate its autophosphorylation, Src recruitment and FAK phosphorylation by Src. Currently, we are studying the atomic architecture of FAK oligomers bound to PIP_2 membranes by electron microscopy (EM). We have obtained a 5.9 Å map, which reveals the mode of oligomerisation and large membrane induced rearrangements of FAK's regulatory FERM and kinase domains (FIGURE). The observed conformation suggests that FAK adopts a 'preactivated' primed state when bound to the membrane. We are further investigating how force, induced at focal adhesion sites by actomyosin contraction, can induce changes to these structures to fully activate focal adhesion signalling. We utilise these mechanistic insights to discover highly specific allosteric FAK inhibitors. We employ a fragment based approach to identify allosteric ligands and then use structure based drug design to develop these fragments into inhibitory lead compounds.

SHIP phosphatases remove the 5-phosphate from PIP_3 and thereby, like PTEN, negatively regulate PIP_3 levels in the plasma membrane. Despite their importance, little is known about mechanisms of SHIP regulation. We previously solved a crystal structure containing the catalytic and C2 domains of SHIP2, which, together with extensive biochemistry and cell biology experiments, showed how the C2 domain induces catalytic activation of SHIP2. Currently, we are studying the role of the PH domain flanking the catalytic domain. We find that the domain binds the PIP_3 substrate and PIP_2 product, and that this binding allosterically further activates SHIP. Together, this shows how the C2 and PH domains concertedly act to recruit SHIP to PIP_3 rich membranes in order to adopt a highly active state. ■

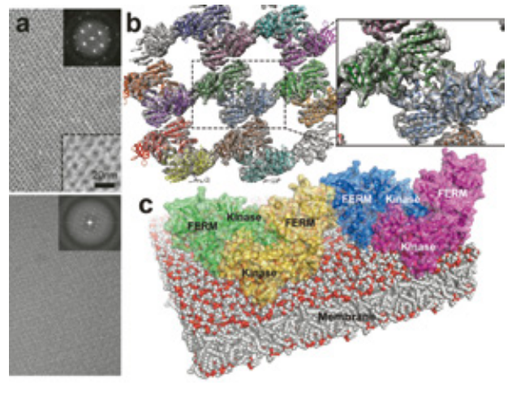

Figure **(a)** FAK 2D crystals (with Fourier transforms) formed on PIP_2 membranes imaged by negative stain (upper) or cryo-EM (lower). **(b)** EM maps at 5.9 Å fitted with FAK domains. Each colour represents one FAK molecule containing a FERM and kinase domain. **(c)** EM structure of oligomeric FAK on a lipid membrane.

PUBLICATIONS

- Toledo RA, Garralda E, Mitsi M, Pons T, Monsech J, Vega E, Otero Á, Albarran MI, Baños N, Durán Y, Bonilla V, Sarno F, Camacho-Artacho M, Sanchez-Perez T, Perea S, Álvarez R, De Martino A, Lietha D, Blanco-Aparicio C, Cubillo A, Domínguez O, Martínez-Torrecuadrada JL, Hidalgo M (2018). Exome sequencing of plasma DNA portrays the mutation landscape of colorectal cancer and discovers mutated VEGFR2 receptors as modulators of antiangiogenic therapies. *Clin Cancer Res* 24, 3550-3559.
- Bauer MS, Baumann F, Daday C, Redondo P, Durner E, Jobst MA, Milles LF, Mercadante F, Pippig DA, Gaub HE, Gräter F, Lietha D. Structural and mechanistic insights into mechanoactivation of Focal Adhesion Kinase. *Proc Natl Acad Sci USA*, doi:10.1073/pnas.1820567116.
- Yen-Pon E, Li B, Acebrón-Garcia-de-Eulate M, Tomkiewicz-Raulet C, Dawson J, Lietha D, Frame MC, Coumoul X, Garbay C, Etheve-Quelquejeu M, Chen H (2018). Structure-based design, synthesis, and characterization of the first irreversible inhibitor of Focal Adhesion Kinase. *ACS Chem Biol* 13, 2067-2073.