- the evaluation of the probability of twinned 2D nucleation on the (010) faces will be discriminating to understand the complex genetic mechanism of penetration twins.

Keywords: gypsum, twin

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Structure of the whole plakin domain of plectin

Esther Ortega Porterov,[a] Rubén Martínez Buey,[a,b] Jose M[a] de Pereda,[a] *[a]Department of Structural Biology, Instituto de Biología Molecular y Celular del Cáncer, CSIC–USAL, Salamanca, (Spain). [b]Biomolecular Reseach, Structural Biology, The Paul Scherrer Institut, (Switzerland).* E-mail:ortegape@usal.es

Plectin is a member of the plakin family of proteins that cross-links components of the cytoskeleton and link them to membrane-associated structures, such as desmosomes and hemidesmosomes. Plectin (500kDa) exhibits a multi-domain structure. The N-terminal region contains a ~ 1000-residue long sequence conserved between the members of this protein family, termed the plakin domain. The plakin domain of plectin is formed by an array of nine Spectrin Repeats (SR1 to SR9) arranged in tandem and a Src-homology 3 (SH3) domain inserted into the central SR5 [1].

We have combined X-ray crystallography and small angle X-ray scattering (SAXS) to elucidate the structure of the plakin domain. Here, we present the crystal structure of four fragments that correspond to the regions: SR3-SR4, SR4-SR5-SH3, SR7-SR8 and SR7-SR8-SR9. The SR-fold consists on three α-helices (A,B and C) connected by short loops and packed in a helical bundle with a up-down-up topology. In adjacent SRs, the helix-C of the N-terminal repeat and the helix-A of the C-terminal repeat are fused in a single helix that spans both SR, yet there is no conservation in the relative orientation of adjacent SRs. The SH3 domain of plectin shows the canonical SH3 fold, but exhibits alterations in its putative Pro-rich binding-site suggesting that this domain does not bind to Pro-rich motifs as the canonical SH3 domains [2]. Moreover, the SH3 binding-site is occluded by the SR4, making extensive contacts with it. Residues that participate in the SR4-SH3 interaction as the residues of the SH3 pseudo-binding site are conserved in other members of the plakin family. The structure of the plakin domain of plectin presented herein, serves as a structural model for other plakins.

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Conformational Plasticity of Histidine Kinases is Key for Signal Transduction

Alejandro Buschiazzo,[a] Felipe Trajtenberg,[a] Daniela Albanesi,[b] Horacio Botti,[a] Natalia Ruetalo,[a] Julia Forman,[c] Michael Nilges,[c] Diego de Mendoza,[b] Pedro Alzari[d] *[a]Unit of Protein Crystallography, Institut Pasteur de Montevideo, Montevideo (Uruguay). [b]Instituto de Biologia Molecular y Celular de Rosario, Rosario (Argentina).[c]Structural Bioinformatics and [d]Structural Biochemistry Units, Institut Pasteur, Paris (France).* E-mail : alebus@pasteur.edu.uy

DesK is a membrane-bound histidine kinase (HK) from *Bacillus subtilis*, able to sense the order of membrane lipids when cells are subjected to cold shock, ultimately behaving as a molecular thermometer. Although the relevance of sensor HKs in signal transduction is well established, we still do not understand at the molecular level how HKs transduce input signal information to regulate their output catalytic activities. We address this issue by using a combination of structural and biochemical approaches. We have determined eight crystal structures of the intracytoplasmic catalytic core of DesK, including the wild-type, the phosphorylated form, and point mutants that retain particular functional traits.

Structural analyses show that DesK has been trapped in three conformational states that correspond to alternate functions of the protein along the signaling pathway [1]. By comparing the 3D structures of a single HK in different functional configurations, we observe a remarkable plasticity in the central helical domain. Incoming signals induce helix rotations and asymmetric helical bends that modify the accessible surface of the phosphorylation site and the mobility of the ATP-binding domains, ultimately modulating the protein's catalytic activities. The transition between conformational states through helical rotational shifts, was analyzed using Targeted Molecular Dynamics simulations, further supporting their role as a functional signal transduction mechanism.

The central four-helix bundle domain includes coiled-coil structures that reach the histidine phosphorylation site. The trans-membrane sensor region seems to drive the helical rearrangements. Heptad-repeat sequence features allow for the extension or disruption of the coiled-coil towards the N-terminus of the catalytic core, ultimately serving as a signal transmission gear. In correlation with these movements, the flanking ATP-binding domains, remain either rigidly fixed to the 4-helix bundle, or otherwise free to move. We have explored the transient intradimeric autophosphorylation state by semiflexible docking algorithms, leading to a proposed mechanism working in *trans*, one monomer phosphorylating the other. Structure-based cysteine engineering lends support to the working hypotheses, allowing us to trap an intermediate state with disulfide bridges between the two domains [2]. Negative cooperativity leads to phosphorylation of only one monomer within the dimer.

Structure-based mutagenesis and protein engineering experiments *in vitro* and *in vivo*, confirm the importance of the 'coiled-coil'-mediated plasticity in the conserved central phosphotransfer domain. Similar switching mechanisms could operate in a wide range of sensor HKs. Structural studies of the interaction of DesK with its cognate response regulator DesR are currently underway.

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PLZF oncoprotein; An extensive SAXS analysis

Katrine Nørgaard Toft,[a] Mads Gravers Jeppesen,[a] Tine Kragh Nielsen,[b] Gil Prive,[c] Bente Vestergaard,[a] *[a]Department of Medicinal Chemistry, University of Copenhagen (Denmark). [b]NNF Center for Protein Research, University of Copenhagen (Denmark). [c]Department of Biochemistry, University of Toronto (Canada)* E-mail: knt@farma.ku.dk

In Acute Promyelocytic Leukemia (APL) the balance between stem cell differentiation and proliferation is disrupted. The promyelocytic zinc finger protein (PLZF) is a transcriptional repressor, and is one of six

fusion partners that play important roles in this stem cell maintenance.

The protein consists of a BTB domain, an intrinsically unfolded linker region and 9 zinc fingers. It is known that the BTB domain mediates the dimerization of the PLZF protein. Present theories suggest that a chromosomal fusion occurs with RARα and that PLZF dimer or oligomer binding causes the transcriptional repression responsible for APL. One hypothesis concerns the potential oligomerization of PLZF during DNA interaction, it is however not known which part of the protein is responsible for the protein:protein contacts.

The aim of the present SAXS study is to shed light on the dimerization and oligomerization, thus understanding the mechanisms of PLZF mediated DNA transcriptional repression. So far most studies have been concentrated on the BTB domain, since the unstructured linker region is inherently difficult to study. Using SAXS, we have the great advantage to work with proteins in solution, thus it was possible to collect data on the native-like condition of the linker region. The analysis however, is demanding and not much experience exists on data from intrinsically un-folded regions.

We have collected data on three PLZF constructs, one containing the BTB domain only, one consisting of the linker part only, and one spanning the BTB and linker part. The oligomerization process has been analyzed in a pH series spanning five pH values. Complementary analysis has been carried out using Dynamic Light Scattering and Circular Dichroism spectroscopy.

The fact that our data set is very extensive was very important for the interpretation of the data. We have successfully identified the involved species in the oligomerization process and have analysed the change of behavior of the intrinsically un-folded linker region during this process.

Keywords: SAXS, intrinsically un-folded, oligomerization

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OST1 structure provides the basis of regulation in response to stress in plants

Cristina Yunta,[a] Martín Martínez-Ripoll,[a] Jian-Kang Zhu,[b,c] Armando Albert.[a] [a]*Departament of Crystallography and Structural Biology, Instituto de Química Física "Rocasolano", CSIC, Madrid (Spain).* [b]*Department of Horticulture and Landscape Architecture, Purdue University, W. Lafayette, IN (USA).* [c]*Plant Stress Genomics Research Center, King Abdullah University of Science and Technology, Thuwal 23955-6900 (Kingdom of Saudi Arabia).* E-mail: xyunta@iqfr.csic.es

SNF1-related protein kinase 2s (SnRK2s) orchestrate the cellular stress responses by phosphorylation of transcription factors and ion channels [1]. SnRK2.6, also called Open Stomata 1 (OST1), is well characterized at molecular and physiological levels to control stomatal closure in response to abscisic acid (ABA) and osmotic stress [2], [3]. SnRK2s, protein phosphatases 2Cs (PP2Cs) and PYR/RCAR ABA receptors work together to decode environmental stress signals mediated by ABA [4], [5]. A balance between the PP2C mediated phosphorylation states of OST1 tunes the activity of the kinase [6], [7]. The structure of an inactive form of OST1 shows that the C-terminal regulatory domain of OST1 stabilizes the kinase into an unproductive conformation. Our data provide insights into the molecular activation/inactivation mechanism of the kinase and also suggest a mechanism for the ABA dependent control of the phosphatase-binding and kinase activities of OST1.

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The crystal structure of an *Arabidopsis thaliana* C2 domain

Maira Diaz,[a] Margarita Menendez,[b] Pedro Luis Rodriguez,[c] Armando Albert,[a] [a]*Departament of Crystallography and Structural Biology, Instituto de Química Física "Rocasolano", CSIC, Madrid, (Spain).* [b]*Departament of Química Física Biológica, Instituto de Química Física "Rocasolano", CSIC, Madrid, (Spain).* [c]*Instituto de Biología Celular y Molecular de Plantas CSIC, Valencia, (Spain).* E-mail: mediaz@iqfr.csic.es

Calcium represents the most versatile ion in eukaryotic organisms. It is involved in nearly all aspects of cell development and participates in many regulatory processes [1]. The C2 domains act as a calcium dependent membrane targeting module of approximately 130 amino acids. These domains either forms part of a protein or function as independent molecules [2]. We have studied a group of *Arabidopsis thaliana* C2 molecules of unknown function that may be involved in the recognition and delivery of protein molecules to cell membranes.

A representative member of the group has been expressed, purified to homogeneity and crystallized. The crystal structure at 2.2 Å resolution shows that the molecule is a dimer that folds as a canonical C2 domain with the topology of those C2 forming part of phospholipases. The electron density map reveals two calcium ions per molecule. Isothermal titration calorimetry (ITC) was used to determine the calcium binding affinity in vitro, which was similar to those observed for other sensors that work at physiological calcium concentrations [3]. The joined analyses of the biophysical and crystallographic data suggest a model of calcium-dependent membrane insertion mechanism that will involve either a dimer dissociation or a strong rearrangement of the dimeric structure.

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Mechanism of Signaling by a Receptor-Independent, Self-Activating G-Protein

Mischa Machius,[a] Jan Jones,[b] Jeff Duffy,[b] Brenda Temple,[b] Henrik Dohlman,[b] Alan Jones,[b,c] [a]*Department of Pharmacology,* [b]*Department*