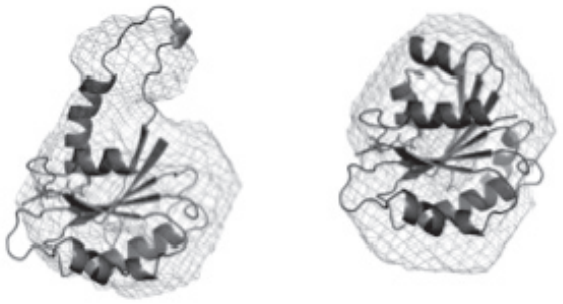
Arf1 and Arf6 also have distinctive biochemical properties *in vitro*, for which no straightforward structural explanation has been put forward.

Activation of Arf proteins is a multi-step event, which involves the recruitment of Arf-GDP from the cytosol to membranes, followed by GDP/GTP exchange. We show that a truncated Arf6 mutant, a cytosolic construct that mimics membrane-bound Arf6-GDP, is partially unfolded in the crystal compared to full-length Arf6. This unusual conformation is the major species in solution, as shown by synchrotron SAXS analysis [1]. In contrast, the equivalent Arf1 mutant is essentially identical to full-length Arf1-GDP, as shown by NMR analysis. Taken together, these experiments suggest that the structural routes for the activation of Arf1 and Arf6 diverge at the step where GDP-bound Arf is recruited to membranes prior to nucleotide exchange [2]. These differences may account for the biochemical differences between Arf1 and Arf6, and yield their functional specificities.

[1] V. Biou, K. Aizel, P. Roblin, A. Thureau, E. Jacquet, S. Hansson, B. Guibert, E. Guittet, C. van Heijenoort, M. Zeghouf, J. Perez, J. Cherfils, *J Mol Biol.* **2010** *402*, 696-707. [2] V. Buosi, J.P. Placial, J.L. Leroy, J. Cherfils, É. Guittet, van C. Heijenoort, *J Biol Chem.* **2010** 285:37987-94.



The figure shows the remarkable fit of the unfolded region as seen in the Δ13Arf6-GDP crystal with a protrusion in the 3D envelope calculated *ab initio* from SAXS data in solution (left). The fit of full-length Arf6 with the SAXS envelope is shown on the right panel.

Keywords: small GTPases, X-ray crystallography, SAXS

MS.51.4

Acta Cryst. (2011) A**67,** C121

Structural and energetic mechanisms of cooperative autoinhibition and activation of vav1

Diana R. Tomchick,[a] Bingke Yu,[a,b] Ilídio R.S. Martins,[a,b,c] Pilong Li,[a,b] Gaya K. Amarasinghe,[a,b,e] Junko Umetani,[a] Martin E. Fernandez-Zapico,[d] Daniel D. Billadeau,[d] Mischa Machius,[a,f] Michael K. Rosen,[a,b] [a]*Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas TX (USA).* [b]*Howard Hughes Medical Institute.* [c]*Departamento de Bioquímica, Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Coimbra (Portugal).* [d]*Department of Immunology and Division of Oncology Research, Mayo Clinic, Rochester MN (USA).* [e]*Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames IA (USA).* [f]*University of North Carolina School of Medicine, Chapel Hill NC (USA).* E-mail: Diana.Tomchick@UTSouthwestern.edu

Vav proteins are guanine nucleotide exchange factors (GEFs) for Rho family GTPases. They control processes including T cell activation, phagocytosis, and migration of normal and transformed cells. We report the structure and biophysical and cellular analyses of the five-domain autoinhibitory element of Vav1. The catalytic Dbl homology (DH) domain of Vav1 is controlled by two energetically coupled processes. The DH active site is directly, but weakly, inhibited by a helix from the adjacent Acidic domain. This core interaction is strengthened 10-fold by contacts of the calponin homology (CH) domain with the Acidic, pleckstrin homology, and DH domains. This construction enables efficient, stepwise relief of autoinhibition: initial phosphorylation events disrupt the modulatory CH contacts, facilitating phosphorylation of the inhibitory helix and consequent GEF activation. Our findings illustrate how the opposing requirements of strong suppression of activity and rapid kinetics of activation can be achieved in multidomain systems.

Here we report the crystal structure of the five-domain regulatory element of Vav1, CADPZ. The structure shows that the CH domain and the N terminus of the Acidic element bind to each other and to a platform formed by the PH domain and a C-terminal extension of the DH domain. Nuclear magnetic resonance (NMR), biochemical, and cell biological analyses show that these interactions suppress GEF activity by modulating the core helix-DH equilibrium, shifting it toward the inhibited state by approximately 10-fold, likely by restraining the inhibitory helix to the DH domain. Phosphorylation of Tyr142 and Tyr160 relieves the modulatory interactions, making Tyr174 more accessible to kinases, suggesting a sequential activation mechanism for full-length Vav1. The layered construction of Vav1 provides a means of achieving strong suppression of activity while still maintaining a route to rapid activation, features that are probably general among multidomain systems in biology.

The NMR approach we developed here provides a means to directly measure the populations of different states across regulatory equilibria and has allowed us to quantitatively characterize the energetic landscape of Vav1. This in turn has established coupled equilibria as a major mechanism of interdomain cooperativity in this system. Application of these methods to other systems should reveal how widespread cooperative inhibition through coupled equilibria is in multidomain proteins.

Keywords: inhibition, activation, cooperativity

MS.51.5

Acta Cryst. (2011) A**67,** C121-C122

The Effect of Ligand-Stabilized Oligomerization on Rio1 Kinase Activity

Nicole LaRonde-LeBlanc and Irene Kiburu, *Department of Chemistry and Biochemistry, Center for Biomolecular Structure and Organization, University of Maryland, College Park, MD, (USA).* E-mail: nlaronde@umd.edu

The Rio1 kinases belong to a family of atypical protein kinases for which there is little information about molecular function [1]. Rio1 kinases are essential and involved in ribosome biogenesis and cell cycle progression [2, 3]. We have identified a small molecule antibiotic, toyocamycin, which binds tightly to Rio1 in its ATP-binding pocket, confirmed by crystallographic studies. However, determination of the steady state kinetic parameters showed that toyocamycin inhibits Rio1 via mixed inhibition, and ATP inhibits Rio1 above micromolar concentrations. This lead to the hypothesis that Rio1 accesses multiple oligomeric states that impact its catalytic activity, which allows the inhibitor to influence activity via allosteric effects. In order to address this, we analyzed the oligomeric states of Rio1 in both the autophosphorylated and unphosphorylated forms, and in the absence and presence of toyocamycin and ATP. Sedimentation equilibrium analysis shows that Rio1 forms higher order oligomers, including dimer, trimer and tetramer, under all conditions except when autophosphorylated and unliganded. Analysis of crystal packing