s1.m5.p3 **Cellulosome assembly and the crystal structure of the cohesin-dockerin complex.** Ana L. Carvalho[a], Fernando M.V. Dias[b], José A.M. Prates[b], Tibor Nagy[c], Harry J. Gilbert[c], Gideon J. Davies[d], Carlos M.G.A. Fontes[b] and Maria J. Romao[a], [a]*REQUIMTE/CQFB, Departamento de Química, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal;* [b]*CIISA-Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Rua Prof. Cid dos Santos, 1300-477 Lisboa, Portugal;* [c]*Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK;* [d]*Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, UK. E-mail: alcarvalho@dq.fct.unl.pt*

Keywords: Cellulosome; Cohesin; dockerin; *Clostridium thermocellum*

The utilization of organized supramolecular assemblies to exploit the synergistic interactions afforded by close proximity, both for enzymatic synthesis and for the degradation of recalcitrant substrates, is an emerging theme in cellular biology. Anaerobic bacteria harness a multi-protein complex, termed the "cellulosome", for efficient degradation of the plant-cell wall. This megaDalton catalytic machine organizes an enzymatic consortium upon a multifaceted molecular scaffold whose "cohesin" domains interact with corresponding "dockerin" domains of the enzymes. Here we report the structure of the cohesin-dockerin complex from *Clostridium thermocellum* at 2.2 Å resolution. The data show that the □-sheet cohesin domain interacts predominantly with one of the helices of the dockerin. Whilst the structure of the cohesin remains essentially unchanged, the loop-helix- helix-loop-helix motif of the dockerin undergoes conformational change and ordering compared to its solution structure, although the classical 12-residue EF-hand coordination to two calcium ions is maintained. Significantly, internal sequence duplication within the dockerin is manifested in near- perfect internal 2-fold symmetry suggesting that both "halves" of the dockerin may interact with cohesins in a similar manner, and thus provide a higher level of structure to the cellulosome and may explain the presence of "polycellulosomes". The structure provides an explanation for the lack of cross-species recognition between cohesin-dockerin pairs and thus provides a blueprint for the rational design, construction and exploitation of these catalytic assemblies.

[1] Ana L. Carvalho, Fernando M.V. Dias, José A.M. Prates, Tibor Nagy, Harry J. Gilbert, Gideon J. Davies, Luís M.A. Ferreira, Maria J. Romao and Carlos M.G.A. Fontes: "Cellulosome assembly revealed by the crystal structure of the cohesin-dockerin complex: a template for nanosomal catalytic machines", P*roc. Natl. Acad. Sci.*, vol. 100, **24**, 13809-13814.

s1.m5.p4 **Oligomerization events mediated by coiled-coils as kinase regulatory mechanism.** Pilar García, Marco Marino, Olga Mayans, *Div. of Structural Biology, Biozentrum, Univ Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland. E-mail:Olga.Mayans@unibas.ch*

Keywords: Kinase activation; Coiled-coil; Oligomerization

Kinase activity can be regulated by multiple mechanisms including phosphorylation, autoinhibition by pseudosubstrate segments, inter-domain interaction, recognition of small-molecule effectors or even oligomerization events. Although the structural basis of these mechanisms have been amply investigated in a number of kinase systems, the molecular principles underlying control by oligomerization remain unidentified. Furthermore, little is known about the interplay of regulatory events taking place in the complex multi-domain kinases that control essential cell processes in higher organisms.

We are addressing these issues in the context of Myotonic Kinase (MK), a multidomain Ser/Thr kinase related to a common form of neuromuscular disorder which causes muscle hyperexcitability and degeneration. Here we report the crystal structure of the coiled-coil domain from MK at 1.6 A resolution and the complementary analysis of its oligomerization behavior by native PAGE, size exclusion chromatography and ultracentrifugation. The experimental results clearly oppose previous deductions drawn from biochemical and sequence data analysis. Moreover, our group is investigating a possible effector role for this domain in the response to small Rho-like GTP-ases. A model for the functional role of the coiled-coil domain of this kinase will be discussed, where its putative relevance as oligomerization motif and/or intermediary in protein interactions will be evaluated.

Crystallographic Methods: The domain was overexpressed as soluble product in *E.coli*. Crystals were obtained under a range of different crystallization conditions using the hanging-drop vapor-diffusion method. Native data were recorded up to 1.6 Å resolution at ID14-2, ESRF. Initially, phasing was attempted by molecular replacement in AMoRe using a distantly related search model with a sequence homology of 14.6% and corresponding to 38% of the content of the asymmetric unit. Two solutions were found resulting in good crystal packing and in good agreement with each other, each corresponding to approximately a half of the full content of the a.u. Nevertheless, the resulting phases were of insufficient quality to allow model building and refinement. In retrospect, both solutions were *misplaced* in an "up-side-down" fashion, where N- and C-termini were inverted. Finally, phasing proceeded by 3λ-MAD on Se-Met derivatives. Six sites (out of nine total) were found by anomalous difference Fourier using phases calculated from the flawed molecular replacement solutions. Phase refinement was in SHARP and density modification (including automatic NCS averaging) used DM. Phase/model improvement employed ARP/warp and model refinement CNS.