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Crystallographic Studies of the Nuclear Pore.
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The nuclear pore (NPCs) is one of the largest multiprotein complexes of the eukaryotic cell and controls nucleo-cytoplasmic communication. Despite recent progress in structural and biochemical characterization of the NPC and its constituents, structural understanding of nucleoporins and nucleoporin subcomplexes is still very limited. The building blocks of the NPC, called nucleoporins, are notoriously difficult to crystallize since they contain large, disordered regions, so a systematic approach is needed to find fragments suitable for crystallization. The crystal structures described in the following help to understand the architecture of the pore channel itself at the boundary between the structural scaffold of the pore and the interior of the pore which is believed to be filled by flexible, hydrophobic protein chains, as well as give an insight into the dissociation mechanism of nuclear transport complexes inside the nucleus at the nuclear basket. Nic96 is a conserved nucleoporin that recruits the Nsp1-Nup49-Nup57 complex, a module with Phe-Gly (FG) repeats, to the central transport channel of the NPC. We solved the crystal structure of a C-terminal fragment of this large structural nucleoporin (Nic96ΔN) that is thought to contribute to the scaffold of the nuclear pore [1]. Nic96ΔN is composed of three domains and is a straight molecule that—although almost entirely helical—exhibits strong deviations from a predicted α-solenoid fold. Based on structural and biochemical data we propose a model for the Nic96 subcomplex architecture in the central nuclear pore channel. Nup153 is a highly mobile, multifunctional, and essential nuclear pore protein. It contains four zinc finger motifs that are thought to be crucial for the regulation of transport-receptor/cargo interactions via their binding to the small guanine nucleotide binding protein, Ran. Our Nup153ZnF2-RanGDP complex crystal structure reveals a new type of Ran-Ran interaction partner interface [2]. This structure excludes the simultaneous formation of a complex of Ran with the transport receptor importin-β and thus suggests a local Nup153-mediated Ran reservoir at the nucleoplasmic distal ring of the NPC, where nucleotide exchange may take place in a ternary Nup153-Ran-RCC1 complex, so that import complexes are efficiently terminated.

[1] Schrader, N., Stelter, P., Flemming, D., Kunze, R., Hurt, E., Vetter, I. R., *Mol. Cell,* **2008**, 29, 46. [2] Schrader, N. , Koerner, C., Koessmeier, K., Bangert, J. A., Wittinghofer, A., Stoll, R., Vetter, I. R., *Structure,* **2008**, 16, 1116.

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The Human Cdc37-Hsp90 Complex Studied by Heteronuclear NMR Spectroscopy.
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The cell division cycle protein 37 (Cdc37) and the 90 kDa heat shock protein (Hsp90) are molecular chaperones, which are crucial elements in the protein signalling pathway. The largest class of client proteins for Cdc37 and Hsp90 are protein kinases. Predominant protein kinases include oncogenic kinases such as Raf1, Cdk4 or Akt. The catalytic domains of these kinases are stabilized by Cdc37 and their proper folding and functioning is dependent on Hsp90.
We will present the X-ray crystal structure of the 16 kDa middle domain of human Cdc37 at 1.88 Å resolution and the structure of this domain in complex with the 23 kDa N-terminal domain of human Hsp90 based on heteronuclear solution state NMR data and docking. Our results demonstrate that the middle domain of Cdc37 exists as a monomer. NMR and mutagenesis experiments reveal L205 in Cdc37 as a key residue ('Hot Spot') enabling complex formation.
Cdc37 is upregulated in cancer cells when compared to the normal tissue. Cdc37 is involved in the maturation of many oncogenic kinase clients and this was even further confirmed by extensive siRNA-mediated silencing of Cdc37. The depletion of kinases was observed in human colon, breast and prostate cancer cell lines. Therefore targeting of Cdc37 is predicted and proposed to have relatively broad-spectrum anticancer activity due to its diverse set of its client proteins. As compared to inhibition of Hsp90, the client selectivity of Cdc37 co-chaperone is predominantly directed towards protein kinases. Low molecular weight molecules that interfere with Cdc37 or Hsp90 or disrupt the Hsp90-Cdc37 complex have recently been proposed as a new class of anti-cancer agents.
Gene based-expression studies have identified the triterpene celastrol, representing a new class of non ATP-competitive inhibitors of Hsp90. Immunoprecipitation in a pancreatic cell line and docking experiments suggested that celastrol exerts its anti-proliferative activity by binding to the N-terminal domain of Hsp90 ($Hsp90_N$), thereby disrupting the complex between $Hsp90_N$ and Cdc37. In vivo, celastrol showed significant inhibition of tumor growth in nude mice with prostate or pancreatic cancer.
Here we show by NMR that celastrol, a recently identified triterpene targeting Hsp90, in fact binds to Cdc37 and disrupts the Cdc37-Hsp90 complex. Furhter, we will also present the molecular mechanism of inhibition of the human protein complex Cdc37-Hsp90, a Kinome Chaperone-Cochaperone, by triterpene celastrol.

Keywords: nuclear magnetic resonance; protein-protein interactions; inhibitor interactions