Friday Morning Session IV - September 25

L13

STRUCTURAL BASIS FOR CRM1 NUCLEAR EXPORT COMPLEX ASSEMBLY

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Nuclear transport proceeds through nuclear pore complexes and supplies cell nuclei with proteins and the cytoplasm with nuclear products such as ribosomes or tRNAs. Most nuclear transport pathways are mediated by importin -type nuclear transport receptors, which include nuclear export receptors (exportins), as well as importins.

The exportin CRM1 mediates nuclear export of numerous structurally and functionally unrelated cargoes, which carry a short leucine-rich nuclear export signal (NES) or complex export determinants. How CRM1 recognizes such a variety of cargoes and maintains versatility and specificity has been unknown so far. Here we present the 2.5 Å crystal structure of the nuclear export complex comprising the exportin CRM1, the export cargo snurportin1 (SPN1) as well as the molecular switch Ran in its GTP bound form.

CRM1 exhibits a toroid-like overall structure that engulfs the Ran molecule and binds SPN1 through its outer surface (Fig. 1). Three parts of SPN1 contact CRM1: The N-terminus of SPN1 resembling a canonical nuclear export signal (NES), the m_3G-cap binding domain and the C-terminal tail. RanGTP is enwrapped mainly by the N-terminal part of CRM1 and additionally fixed by the acidic loop of CRM1. The structure shows how CRM1 can specifically return the RNA-free form of SPN1 to the cytoplasm and suggests that RanGTP promotes cargo-binding to CRM1 solely through long-range conformational changes in the exportin.

Two perpendicular views of the complex in cartoon representation are depicted. CRM1 adopts a toroid like structure and is colored in green. RanGTP (red) is engulfed mainly by the N-terminal part and additionally bound by the acidic loop (white) of CRM1. SPN1 (purple) is bound far away from RanGTP on the outer surface of the exportin involving three different parts: The N-terminal helix resembling a canonical NES, the m_3G-cap binding domain and a C-terminal portion.

T. Monecke, T. Güttler, P. Neumann, A. Dickmanns, D. Görlich, R. Ficner, *Science*, **324**, (2009), 1087.

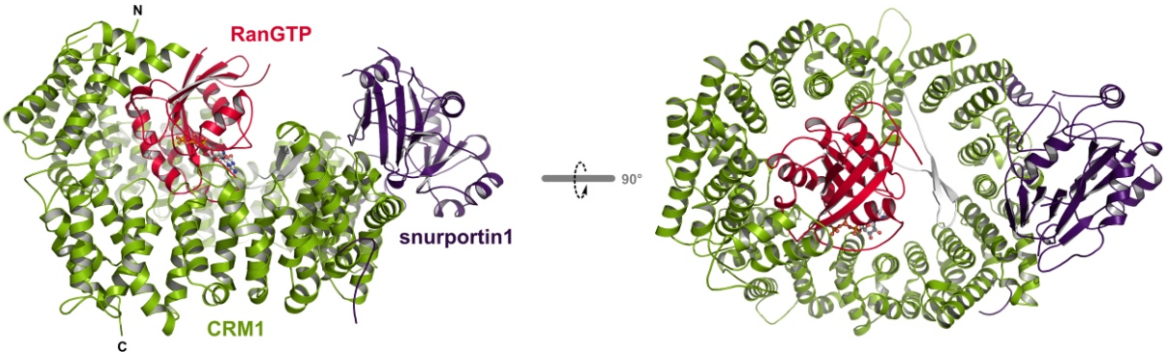

Figure 1. Structure of the CRM1·SPN1·RanGTP nuclear export complex.

L14

STRUCTURAL STUDIES OF HUMAN CARBONIC ANHYDRASE ISOENZYMES

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The human carbonic anhydrases (CAs) form a family of 14 zinc-containing enzymes that catalyze rapid interconversion between carbon dioxide and bicarbonate. This reaction is important in many physiological processes, such as respiration, regulation of pH and many other biological processes requiring carbon dioxide or bicarbonate. Human isozyme CA II is a cytosolic enzyme and belongs to the most studied isoforms. It is traditionally purified from red blood cells but it has a wide tissue distribution and is found in various organs and cell types [1]. CA II deficiency is associated with osteoporosis, renal tubular acidosis, and cerebral calcification. The transmembrane isoform CA IX has been shown to be linked with carcinogenesis. CA IX is physiologically expressed in the epithelia of the gastrointestinal tract, however it expresses ectopically in carcinomas derived from kidney, lung, cervix, uteri, oesophagus, breast, and colon. This isoform has been shown to be strongly over-expressed in hypoxic tumors, where it participates in tumor cell environment acidosis and contributes to malignant progression and poor treatment outcome. Designing isoform-selective inhibitors could thus provide potent therapeutics namely for treatment of cancer. Progress in structural studies of these two physiologically and pathophysiologically important isoenzymes in complex with selective isoquinoline inhibitors will be discussed.

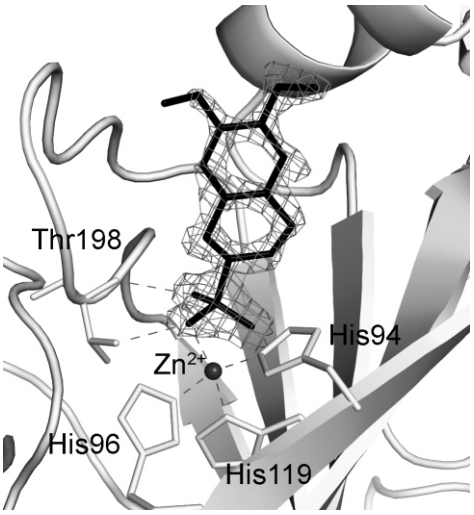

Figure 1. Isoquinoline derivative binding to active site of human CA II.

Elucidation of inhibitor binding to various isoforms could help in rational drug design of carbonic anhydrase inhibitors.

1. Sly and Hu *Annu. Rev. Biochem.* **64** (1995), pp. 375–401.

L15

THE -Me TYPE II RESTRICTION ENDONUCLEASE HPY99I WITH TARGET DNA

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The -Me restriction endonuclease Hpy99I has been identified in the human pathogen *Helicobacter pylori J99* strain. The enzyme recognizes the pseudopalindromic DNA sequence CGWCG/ (where W stands for A or T and "/" marks the cleavage site) and hydrolyzes both DNA strands. The stagger between the cuts in the DNA leads to five nucleotide 3'-overhangs, a highly unusual pattern in restriction endonuclease digestion products. The Hpy99I protein has been overexpressed from synthetic genes in *E. coli*, purified and crystallized in the presence of DNA. The structure of the Hpy99I-DNA complex (1.5 Å resolution) has been determined by x-ray crystallography. In the crystals and in solution, Hpy99I enzyme is a dimer. The Hpy99I protomer consists of an antiparallel -barrel and two repeats (Figure 1). Each repeat coordinates a structural zinc ion with four cysteine thiolates in two CXXC motifs. The -Me region of the second repeat holds the catalytic metal ion via Asp148 and Asn165 and activates a water molecule with the general base His149.

In the specific complex, Hpy99I forms a ring-like structure around the DNA that contacts DNA bases on the major and minor groove sides via the first and second repeats, respectively. Hpy99I interacts with the central base pair of the recognition sequence only on the minor groove side, where A:T resembles T:A and G:C is similar to C:G.

The Hpy99I-DNA co-crystal structure provides the first detailed illustration of the e active site in restriction endonucleases and complements structural information on the use of this active site motif in other groups of enzymes such as homing endonucleases (e.g. I-PpoI) and Holliday junction resolvases (e.g. T4 endonuclease VII).

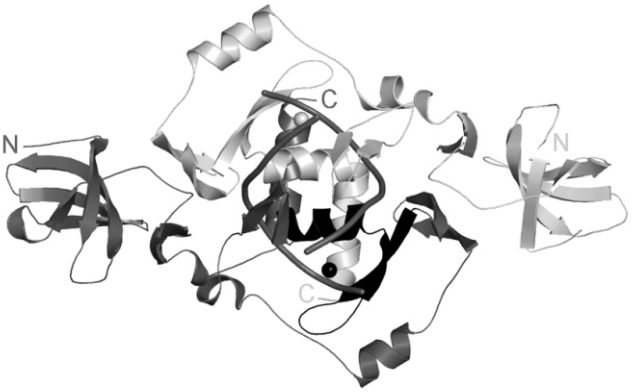

Figure 1. Crystal structure of the -Me type II restriction endonuclease Hpy99I in complex with DNA. One protomer of a dimer is shown in dark grey, the other is presented in light grey. The -Me motif of the first protomer is depicted in black. DNA is shown in dark grey.

Friday Evening Session V - September 25

L16

TURNING THE BAD INTO GOOD: A RATIONAL APPROACH TO CRYSTAL QUALITY IMPROVEMENT

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Structural genomics projects demonstrate that about half of all crystallized proteins cannot be optimized to form suitable crystals for structure determination - which translates into roughly 15% of all proteins [1,2]. While a thorough optimization of initial crystals of a particular protein is antagonistic to the high throughput idea of structural genomics projects the situation is different in most academic or industrial protein crystallization labs. After obtaining initial crystals usually great effort is invested into optimizing these "hits" into well-diffracting crystals. However, this optimization very often focuses mainly on fine-tuning of the chemical composition of the crystallization buffer (i.e. grid-screening of pH, precipitants, additives) or simply on using a large number of commercially available crystal screens. While this yields satisfactory results in some cases it does not in many others.

For optimization of initial hits one may in principle deal with either the crystallization experiment itself or - very often neglected - with the input-protein sample. In the crystallization experiment it is - alongside fine-tuning the chemistry - mainly optimization of physical/thermodynamic parameters (method/instrumentation for crystallization, temperature). In the input-protein sample it is either dealing with an already existing protein preparation (provided there is sufficient material available) or making new (better) protein by optimizing its sequence or changing the method of protein production.

In this year's HEC-Meeting we present three approaches that deal with the crystallization experiment as well as three strategies that aim at improving the input-protein sample. We show the pH-2D screen [3] that allows fine-tuning of pH from 4.0…10.0 with only one buffer system and therefore, eliminates undesired matrix effects inevitably occurring when conventional buffers are used. We discuss Hofmeister's original concept of kosmotropic and chaotropic small molecules in the context of protein crystallization [4,5] and we present a microfluidic Crystal Former^{TM} device with unique equilibration kinetics.

Regarding the input-protein, we elucidate the ready-to-use Choppy-Floppy Kit for in-situ proteolysis targeting proteins containing floppy regions or tags [2]. For cases in which the existing protein preparation does not yield sufficient material for manipulation we discuss strategies for creation of protein variants by random mutagenesis that may be more prone to crystallization [6, and references there]. Finally, we highlight the protein production platform LEXSY which is a eukaryotic protein expression system that is handled similar to *E. coli* [7,8]. By using the example of glycosylation we demonstrate that LEXSY yields highly homogenous protein preparations which is a prerequisite for successful crystallization. We discuss two structures that were determined from LEXSY-derived proteins – one by the means of X-ray crystallography and the other by NMR [9,10]. Most recently a version of LEXSY has been developed that allows cell-free *in vitro* production of up to several mg of any protein within hours [11] which will be available to the research community in late 2009.

1. http://targetdb.pdb.org
2. Dong et al. (2007) In situ proteolysis for protein crystallization and structure determination. *Nat. Methods* **4**(12):1019.
3. Newman (2004) Novel buffer systems for macromolecular crystallization. *Acta Cryst.* **D60**:610.