DANDRITE
Danish Research Institute of Translational Neuroscience
Nordic EMBL Partnership for Molecular Medicine

AARHUS UNIVERSITY

DANDRITE Topical Seminar

Friday 28 May 2017
15.00 – 16.00

The Conference Room, building 3130, 3rd floor, room 303
Gustav Wieds Vej 10C, 8000 Aarhus C

Prof. Guillermo Montoya

Group leader and section head
The Novo Nordisk Foundation Center for Protein Research University of Copenhagen

Crystal structure of the Cpf1 R-loop complex after target DNA cleavage

Cpf1 is a single RNA-guided endonuclease of class 2 type V CRISPR-Cas system, emerging as a powerful genome editing tool [1,2]. To provide insight into its DNA targeting mechanism, we have determined the crystal structure of *Francisella novicida* Cpf1 (FnCpf1) in complex with the triple strand R-loop formed after target DNA cleavage. The structure reveals a unique machinery for target DNA unwinding to form a crRNA-DNA hybrid and a displaced DNA strand inside FnCpf1. The protospacer adjacent motif (PAM) is recognised by the PAM interacting (PI) domain. In this domain, the conserved K667, K671 and K677 are arranged in a dentate manner in a loop-lysine helix-loop motif (LKL). The helix is inserted at a 45º angle to the dsDNA longitudinal axis. Unzipping of the dsDNA in a cleft arranged by acidic and hydrophobic residues facilitates the hybridization of the target DNA strand with crRNA. K667 initiates unwinding by pushing away the guanine after the PAM sequence of the dsDNA. The PAM ssDNA is funnelled towards the nuclease site, which is located 70 Å away, through a hydrophobic protein cavity with basic patches that interact with the phosphate backbone. In this catalytically active conformation the PI and the helix-loop-helix (HLH) motif in the REC1 domain adopt a “rail shape” and “flap-on” conformations, channelling the PAM strand into the cavity. A steric barrier between the RuvC-II and REC1 domains forms a “septum” that separates the displaced PAM strand and the crRNA-DNA hybrid, avoiding re-annealing of the DNA. Mutations in key residues reveal a novel mechanism to determine the DNA product length, thereby linking the PAM and DNA nuclease sites. Our study reveals a singular working model of RNA-guided DNA cleavage by Cpf1, opening up new avenues for engineering this genome modification system

Refreshments will be served during the talk

Host: Group Leader Poul Nissen, DANDRITE, Dept. Molecular Biology and Genetics