iNEXT workshop on Integrated methodologies and approaches for structural biology

Name of Speaker: **Marcin Nowotny**
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Title of Lecture: **Indirect dynamic readout of nucleic acid structure and sequence in DNA repair and reverse transcription**

Abstract:

Protein-nucleic acid complexes are often considered as interactions of rigid bodies. However, large or very subtle conformational changes are very often required for the proteins to achieve specific recognition of the nucleic acids and perform enzymatic reactions. In this talk, I will describe several examples of such mechanisms. The first concerns UvrA – a DNA damage sensor in bacteria. Its unique feature is the ability to detect DNA lesions of various, unrelated chemical structures. We reported the first crystal structure of UvrA in complex with modified DNA, which showed that the protein detects the deformations of the DNA caused by the damage. It also induces further conformational change of the DNA exploiting the increased flexibility of damaged nucleic acid (Jaciuk M, 2011). Only the deformed DNA is complementary with the molecular surface of UvrA. Thus, the protein uses indirect readout of the DNA damage that allows it to achieve its broad specificity.
RuvC is a nuclease that specifically cleaves four-way DNA structures termed Holliday junctions (HJ). HJs arise for example in homologous recombination. RuvC is a dimeric enzyme that cleaves the HJ by introducing two symmetric cuts in the DNA (Górecka K, 2013), each at the 5′-A/TTT↓G/C-3′ cognate sequence. We recently solved a 3.4 Å crystal structure of RuvC-HJ complex, which showed that the protein introduces conformational tension at the center of the HJ, which displaces the substrate from both active sites. Molecular Dynamics calculations showed that for the cuts to occur the tension needs to be released by flipping out of a base opposite of scissile phosphate. This is only possible for TG/C dinucleotide explaining the readout of the DNA sequence around the cut site. The mechanism constitutes an important and interesting example of dynamic readout of DNA sequence by short-lived high-energy conformational states of a protein-nucleic acid complex.
Reverse transcription is a conversion of single-stranded RNA to double-stranded DNA – an essential step of retrovirus life cycle. It is catalyzed by reverse transcriptases (RTs) with DNA polymerase and RNase H activities. A key step of reverse transcription of HIV is the generation and usage of the polypurine tract (PPT) primer. It is a fragment of the viral RNA with a stretch of adenines and guanines, which is not degraded by the RNase H activity and is used to prime the synthesis of the second DNA strand. Using Molecular Dynamics and kinetic studies we showed that the RNA/DNA substrate of HIV RT needs to undergo a conformational change (mostly unwinding) for the RNase H cut to occur (Figiel M, 2017). Later, we also demonstrated that the stretch of adenines in the PPT cannot undergo this conformational change protecting the body of PPT from cleavage (Figiel M,

2018). This is another example of dynamic conformational readout of the nucleic acid sequence with important consequences for the mechanism of HIV RT.

Jaciuk M, Nowak E, Skowronek K, Tanska A, Nowotny M.. ***Nat. Struct. Mol. Biol.*** 2011; 18:191-197
Górecka KM, Komorowska W, Nowotny M.. ***Nucleic Acids Res.*** 2013; 41(21):9945-55
Figiel M, Krepl M, Poznanski J, Golab A, Šponer J, Nowotny M. ***Nucleic Acids Res.*** 2017 3341-3352
Figiel M, Krepl M, Park S, Poznański J, Skowronek K, Gołąb A, Ha T, Šponer J, Nowotny M. ***J Biol Chem***. 2018, 293(1):191-202

Research Profile:

Laboratory of Protein structure focuses on structural and biochemical studies of proteins involved in nucleic acid processing – DNA repair, RNA processing, and reverse transcription. We use protein crystallography as our primary method and we recently introduced cryo-EM to our group. We are interested in particular in the mechanism specific recognition of nucleic acids by proteins.

Three selected publications:

1. Razew M, Warkocki S, Taube M, Kolondra A, Czarnocki-Cieciura M,1 Nowak E, Labedzka-Dmoch K, Kawinska A, Piatkowski J, Golik P, Kozak M, Dziembowski A, Nowotny M. Structural analysis of mtEXO mitochondrial RNA degradosome reveals tight coupling of nuclease and helicase components, ***Nat. Commun.*** 2018, *9*(1):97
2. Nowak E, Miller JT, Bona MK, Studnicka J, Szczepanowski RH, Jurkowski J, Le Grice SFJ§, Nowotny M§. Ty3 reverse transcriptase complexed with an RNA-DNA hybrid shows structural and functional asymmetry. ***Nat. Struct. Mol. Biol.*** 2014; 21(4):389-96
3. Rychlik MP, Chon H, Cerritelli SM, Klimek P, Crouch RJ, Nowotny M. Crystal Structures of RNase H2 in complex with nucleic acid reveal the mechanism of RNA-DNA junction recognition and cleavage. ***Mol. Cell*** 2010; 40:658-670

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