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Re: PhD Thesis of Anton Slyvka

Dear Prof. Poznanski,

I am writing in response to your request for evaluation of Anton Slyvka's PhD thesis. I am doing so with great pleasure, because Anton was a bright and diligent PhD student and a very promising young scientist.

The thesis consists of a succinct Summary, which is followed by a concise, but comprehensive Introduction of DNA modifications. It then focusses specifically on 5-methylcytosine (meC) and its roles in prokaryotic restriction-modification systems (including a section discussing the structural protein domains involved in meC recognition and metabolism), and in the regulation of eukaryotic gene expression. The latter section also discusses cytosine demethylation and its enzymology. The fact that the Introduction section is followed by 310 references bears witness to the comprehensive coverage of the subject.

The following section contains four Anton Slyvka's publications, on two of which, published in Nucleic Acids Research and Scientific Reports, Anton appears as the first author.

The first publication by Czapinska *et al.* in Nucleic Acids Research, on which Anton appears as a fifth co-author, deals with the characterisation of the enzymatic activity and structure of the bacterial EcoKMcrA endonuclease, which cleaves DNA at meC and hydroxymethylcytosine (hmC) residues. The work described in this paper is of very high quality and it is also pretty extensive; the six figures in the paper are supported by 20 supplementary figures and 4 supplementary tables! In this work, the N- and C-terminal domains of the protein were studied to show that the latter was responsible for DNA binding and cleavage of the phosphodiester backbone, whereas the former contained the substrate- and flanking sequence recognition motifs. A number of different techniques, including small angle X-ray scattering (SAXS), X-ray crystallography, DNaseI footprinting and electrophoretic mobility shift assays were used in this elegant study.

The second paper by Slyvka *et al.* in Nucleic Acids Research, on which Anton is the first author, focusses on the detailed characterisation of the N-terminal domain of the EcoKMcrA protein. The DNA co-crystal structures with the N-terminal domain or with the full-length protein show that the two N-terminal domains of the homodimeric protein bind the sequences containing the methylated cytosines in the two strands of the duplex DNA and that this action does not involve flipping of the modified nucleotides out of the helix (as predicted in the first paper). The work involved similar techniques as the previous study and the paper's six figures are supported by 14 supplementary figures and 5 supplementary tables.

The third paper, in Scientific Reports, on which Anton is also the first author, deals with another topic – demethylation of meC through deoxygenation catalysed by the TET proteins, which convert meC to hmC and subsequently to formylC

and carboxyC. The individual oxidation steps of the demethylation process are well characterised, but the questions regarding the removal of the oxidised bases from DNA remained enigmatic. Thymine DNA glycosylase (TDG) has been implicated in the process, but this enzyme has limited turnover due to its tendency to remain bound to the substrate after cleaving the glycosidic bond. Anton and his co-authors were able to demonstrate that a second enzyme implicated in the removal of meC oxidation products, NEIL1, albeit unable to excise the oxidised bases, can remove the baseless sugar from the DNA backbone by β/δ-elimination. Moreover, the authors were able to show that NEIL1 and TDG interact directly and that the former enzyme stimulates the turnover of TDG. This study made use primarily of DNA cleavage assays. The results contributed substantially to our understanding of the demethylation process.

The final paper, in Journal of Molecular Biology, on which Anton is the third co-author, investigated the activity of TET enzymes on substrates containing deoxyuridines substituted at the 5-position with longer or bulkier, saturated or unsaturated, hydrocarbon chains. The study shows that the enzymes can oxidise also the β-carbon, albeit at low efficiency. The study also shows that NEIL1, but not TDG, can remove the bulkier oxidised bases from DNA.

The work carried out by Anton Slyvka is of very high standard and has, correspondingly, been accepted for publication in international peer-reviewed journals with high impact factors. I have no doubt that Anton Slyvka's output not only fulfils, but exceeds, the requirements for the award of the title of Doctor of Philosophy. Indeed, I believe that the thesis deserves an honourable mention.

Yours sincerely,

Josef Jiricny