

Evaluation Report on the Doctoral Thesis
New redox labels for DNA
by Anna Simonova

The presented dissertation thesis is focused on the synthesis of new nucleosides modified with redox labels that could find their application in the field of molecular diagnostics and other disciplines involved in the DNA research and medicine. For the best performance of the methodology it is desirable that all four nucleobases are labelled, each with a different redox label that ideally has a strong and differentiated electrochemical peak. This is quite a difficult task since not only the labels have peaks but nucleobases themselves may interfere. During the earlier research in Michal Hocek group, many molecules were tried and two of them fulfilled all requirements – nitrophenyl and benzofurane.

This thesis had the main aim to find the other two labels. Methoxyphenol (MOP), dihydrobenzofuran (DHB), phenothiazine (PT), and ferrocene (FC) were selected to modify the remaining bases. Nucleosides and nucleoside triphosphates were synthesized using standard cross-coupling reactions and various DNA polymerases were used to synthesize oligonucleotides which were then subjected to electrochemical studies. A combination of the new and two existing probes were also studied. As a result, MOP seems to be a good candidate and one of the modified ferrocene label seems to be the second one. On the other hand, DHB overlaps with a peak of guanine, PT has two peaks that occupy too much space and it would be difficult to fit in the fourth probe.

The thesis has a traditional structure with an introduction part followed by specific aims and rationale of the thesis, then results and discussion, conclusion, experimental part, references, and appendix. The thesis is written in a very clear language and it is easy to follow the main ideas, hypotheses, experiment arrangements and results. There is only a small amount of typos and other mistakes.

General comments to the text and content:

- As a reader, I would appreciate more information about the selected polymerases in the introduction part, especially since it seems to me, that the majority of the work of the author is about the preparation of the labelled DNAs.
- In the results and discussion part, the organic synthesis was reduced to minimum, is there really only so few to comment on? For example all compounds are fully characterized, in my opinion, there should be at least one molecule selected and shown, that the Ph.D. candidate is able to prove the structure of a new molecule by interpreting the spectral data by herself (not that all of it was interpreted by the NMR facility personnel).
- The kinetics of the analogues incorporation is evaluated just visually by looking at the gels and commented "...were incorporated with only slightly slower rate than ..." In my opinion, comparing of the k_{cat}/K_M would be more precise.

Questions:

1. Please, comment more on the polymerases. The choice of the TdT is obvious, but why did you select Pwo and KOD XL? Is there some article in the literature on how they behave during the incorporation of NTPs with bulky substituents?
2. How do you explain the formation of N+1 product by incorporating of A^{DHB} or A^{PT} by the KOD XL polymerase on *temp^{termA}* (Figure 7, 9, 32) or C^{EPT} on *temp^{termC}* (Fig. 35)?

3. On the Figure 19, the spots of radiolabeled oligonucleotides with four modified bases (Lines 7, 8, 9) have a smaller spot above them, is it a misincorporation? Do you really see only one product in the MALDI spectra of DNAs isolated (Table 3)?
4. How were the modifying molecules selected? I understand the ferrocene (it is possible to tune the peaks) but why MOP, DHB, or PT? Some earlier experience, literature precedents?

Despite of all the comments and questions mentioned above, I must state that all specific aims were completely fulfilled, the thesis shows that the author did a lot of experimental work which resulted in three publications in respected journals and in addition, she is a co-author of another publication with a slightly different topic. The thesis is a very nice multi-disciplinary piece of work and the author had to master many different techniques to achieve all of the proposed goals.

In conclusion, the presented results are original and of a high scientific value and therefore I recommend the Thesis for the defense and further proceedings for obtaining the PhD degree.

In Olomouc 27.11.2018

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