

*Referee report*

The bachelor thesis by Barbora Emmerova describes a systematic experimental analysis of fluorescence quenching of the zwitterionic fluorescence dye ATTO680 by DNA bases. The quenching efficiencies of individual DNA bases as well as the mechanism of the quenching were thoroughly investigated. The results presented on the ATTO680 dye provide data that have so far not been studied. The data presented in the thesis might contribute to development of novel FRET based techniques for structural investigations of nucleic acids. In addition, the experimentally determined quenching efficiencies of individual DNA bases are necessary to further improve accuracy of FRET-based investigations of oligonucleotides.

In general, the experimental data presented are well controlled and sound. However, there are several minor points that should be addressed by the student during the thesis defense:

a) Why the left side of the equation 1.1 is multiplied by 0? I suppose this is just a typographic error.

b) Equations 1.5 and 1.6 are, in my eyes, identical. If not what is a difference?

c) Ms. Emmerova states that the main motivation for the experiments was to provide the data for development of novel structural assay based on quenching phenomena, which would scan interaction between fluorescent probe and its nano-environment. However, the concept of such a method is not included in the thesis. Although, this method is not a subject of the thesis - it would be useful to outline at least general principles of this method as the experimental setup employed in the thesis directly assumes sequence-nonspecific noncovalent interactions of the ATTO680 with double stranded DNA. I was wondering if Ms. Emmerova could address application potential of experimentally determined quenching efficiencies for non-canonical forms of DNA and discuss limitations in their application for covalently attached ATTO680 to non-terminal positions along the DNA helix?

Altogether, the thesis by Barbora Emmerova fulfills requirements for bachelor thesis. I evaluate the thesis with A-.

In Utrecht, 13. 6. 2010

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