

# ABSTRACT

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Doctoral Thesis: **Study of azaphthalocyanines suitable for quenching of fluorescence in oligodeoxynucleotide probes**

Phthalocyanines (Pcs) and azaphthalocyanines (AzaPcs) are planar macrocyclic compounds. These synthetic compounds are derived from naturally occurring dyes – porphyrins. Pcs and AzaPcs have very interesting photophysical properties due to their extensive 18  $\pi$ -electron conjugated system. They are used in electrotechnics, material industry or in medicinal applications.

Octaalkylamino substituted tetrapyrazinoporphyrazines (TPyzPzs), which belong to AzaPc family, can quench fluorescence of other molecules due to the ultrafast intramolecular charge transfer. This thesis studies this quenching ability of TPyzPzs with the aim to develop efficient fluorescence probes. Such probes could find application in a variety of experiments across scientific disciplines i.e. – biochemistry, forensic or genetic analysis.

Synthetic part of dissertation thesis is a continuation of previous investigation of TPyzPzs in our research group. It describes design and preparation of low symmetrical TPyzPzs suitable for different binding to oligodeoxynucleotide (ODN) chain of a fluorescent probe (at 3', 5'-end or into the middle of the chain). This binding to ODN chain is enabled by various functional groups (hydroxyl, azide, and/or carboxylic acid) attached to TPyzPz. Suitable peripheral groups hinder undesirable aggregation.

Next part of the thesis assesses the effect of peripheral substitution on usability of hydrolysis probes (e.g. TaqMan<sup>®</sup> probes) in quantitative polymerase chain reaction (qPCR). First, comparison of quenching efficiency of TPyzPzs with commercially available quenchers is described. For this purpose, we developed a model method, which simulates behavior of probes during qPCR. The principle of this simplified method lays in the usage of DNase I instead of Taq polymerase employed in qPCR. We found out that J-dimers, which is a rare type of aggregation of Pcs and AzaPcs, are formed even after binding TPyzPzs to ODN chain.

Final part of the dissertation thesis deals with the detailed study of the formation of J-dimers. Influence of different factors on behavior of TPyzPzs was investigated in organic

solvent and after binding to ODN chain in hybridization buffer. Self-assembly into a unique heterotetramer formed by the J-dimer of TPyzPzs and two molecules of fluorescein was described for the first time.