Abstract

The aim of the thesis was to synthesize new nucleosides, nucleotides and the corresponding DNA probes bearing various fluorescent labels, which can be used for bioanalytical applications.

In the first part of the thesis, 2'-deoxycytidine and the corresponding nucleoside triphosphate bearing tryptophan-based imidazolinone fluorophore were synthesized by Sonogashira cross-coupling reaction. The fluorophore showed sensitivity to pH and viscosity. Nucleotide was used for the construction of modified oligonucleotides (ON) and DNA by primer extension (PEX) or polymerase chain reaction (PCR). Labelled ON probe was used for sensing interaction with single-strand binding protein, which resulted in increased fluorescence intensity of modified ON.

Next, thymidine and thymidine triphosphate labelled by bezylidene-tetrahydroxanthylium fluorophore were synthesized by copper-catalyzed azide-alkyne cycloaddition (CuAAC). Fluorescence of the fluorophore is dependent on the polarity and viscosity of the environment. Incorporation of the modified nucleotide into DNA, by PEX or PCR, led to dramatic increase of the fluorescence presumably due to the interactions of the fluorophore in the major groove. Unfortunately, the modified nucleotide was not suitable for in cellulo imaging due to its cytotoxicity. The modified dsDNA was used as fluorescent probe for sensing interactions with small molecules and proteins by change of fluorescence. Finally, the nucleotide was applied in real-time PCR as a new approach to directly visualize the DNA synthesis. Benzylidene-tetrahydroxanthylium fluorophore was also attached to 2'-deoxycytidine via triethylene glycol linker to address issues with cell experiments, however, the incorporation into DNA in live cells was not successful.

Additionally, thiazole orange (TO) modified 2'-deoxycytidine and the corresponding triphosphate were synthesized by CuAAC. TO is known to bind nucleic acids and change fluorescence intensity and lifetime depending on viscosity. The nucleotide was incorporated into DNA probes by PEX and PCR and their photophysical properties were evaluated. TO-modified nucleotide was successfully transported into live cells and incorporated into genomic DNA, what allowed real-time imaging of DNA synthesis in live cells using fluorescence lifetime imaging.

Further, 2'-deoxycytidine and its triphosphate bearing near-infrared BODIPY fluorophore tethered via propargylether or triethylene glycol linker were synthesized. New

nucleosides showed dependence of fluorescence lifetime on the viscosity of environment. Corresponding nucleoside triphosphates were used as substrates for enzymatic synthesis of DNA. Modified DNA probes were used for sensing interactions with proteins by changing fluorescence lifetime. Nucleotide bearing BODIPY via triethylene glycol linker was also incorporated into DNA in live cells.

Finally, 2'-deoxycytidine triphosphate bearing silicon rhodamine fluorophore was prepared by strain-promoted azide-alkyne cycloaddition reaction. Photophysical properties, as well as enzymatic incorporation into DNA, were studied. New nucleotide was transported into cells and incorporated into genomic DNA, what allowed super-resolution imaging of DNA.