Abstract

In the first part of this thesis, I developed reactive DNA probe for selective cross-linking with lysine residues of DNA-binding proteins. I synthesized 2'-deoxycytidine 5'-*O*-mono- and triphosphate bearing squaramate moiety tethered to the position 5 via propargylamine linker. The monophosphate was used as a model compound to test the reactivity of this mixed squaramate in cross-linking reactions with lysine and short lysine containing peptides. Squaramate modified 2'-deoxycytidine 5'-*O*-triphosphate was found to be suitable substrate for KOD XL polymerase in both PEX and PCR synthesis of modified DNA. Squaramate modified DNA forms stable diamide linkage with primary amines. I tested the reactivity of this DNA probe in bioconjugation reactions with sulfo-Cy5-amine and lysine containing peptides. Afterwards, squaramate-linked DNA was successfully cross-linked with lysine rich histone proteins. This reactive squaramate modified nucleotide showed potential for following bioconjugation reactions of nucleic acids with amines or lysine containing peptides and proteins without the need of external reagent.

Based on positive results of experiments with squaramate modified DNA, in the second part of the thesis I developed and synthesized squaramate modified ribonucleotide to study cross-linking with RNA binding proteins. After optimisation of reaction conditions, squaramate modified cytidine 5'-*O*-triphosphate was incorporated into the RNA by T7 RNA polymerase in *in vitro* transcription reaction. Next, the modified RNA was used for post-synthetic labelling with sulfo-Cy-5-amine. Finally, I tested the squaramate modified RNA probe in reaction with various model proteins (i.e., JEV and YFV NS5, SARS-CoV-2 RdRp, SARS-CoV-2 nucleoprotein and HIV-rt). The modified ribonucleotide was also used in RNA extension reactions catalysed by RNA dependent RNA polymerases (JEV NS5 and SARS-CoV-2 RdRp). Synthesized modified RNA cross-linked during polymerase reaction with JEV NS5 polymerase, which was confirmed by PAGE and immunodetection. Proteomic analysis of polymerase reaction mixtures identified three lysine residues (K269, K462, K463) of the protein cross-linked with squaramate modified RNA.