University of Fribourg / Faculty of Science and Medicine
Department of Oncology/Microbiology/Immunology (OMI)

DNA-based nanodevices for sensitive and specific detection of nucleic acids

Ivana Domljanovic

The main cause of cancer-related death is due to cancer cell spreading and the formation of metastasis. Recently, it has been shown that microRNAs (miRNAs) in the blood can be used as cancer biomarkers. However, the detection of miRNAs in the blood remains a challenge in clinical diagnostics. The development of DNA nanotechnology provides a promising approach for the creation of DNA origami biosensors that can detect cancer biomarkers, including miRNA, with high sensitivity. Therefore, the main focus of this thesis was to develop dynamic DNA origami biosensors based on amplification-free fluorescence signals for the rapid detection of multiple miRNAs as cancer biomarkers. The demonstration of the detection of two targets (miRNA) was done in two experimental settings at a single molecule level by high-resolution fluorescence microscopy and in bulk by fluorescence spectrometry. The obtained results showed that detecting targets of interest is possible within 10 minutes and with a detection limit of 1-10 pM. Overall, our results showed that the DNA origami biosensor is highly sensitive and specific for detecting synthetic target oligonucleotides and natural miRNAs extracted from cancer cells

Based on that, we foresee that our biosensor may be developed into a diagnostic point-of-care device for the specific and sensitive detection of various DNAs and RNAs in biofluids.

The second part of the thesis was focused on developing an assay that reveals details of DNA and RNA strands displacement mechanisms. Stands displacement is fundamentally important in biology and is also becoming an essential mechanism in nanotechnology applications. Thus, we designed a FRET-based assay to investigate the kinetics of strand displacement of natural DNA, natural RNA, and locked nucleic acid (LNA) modified DNA *in vitro* in PBS with or without molecular additives/crowders such as diethylene glycol dimethyl ether (deg), polyethylene glycol (peg), and polyvinylpyrrolidone (pvp). The LNA bicyclic-modified nucleotide is usually incorporated within a nucleic acid to advance its target binding affinity and specificity. The results indicated that the kinetics of three displacement strands (DNA, RNA, and LAN-DNA) are dissimilar in pure PBS buffer: the LNA-DNA showed the fastest displacement in PBS, followed by DNA and then by RNA. On the other hand, different molecular crowders affect the displacement mechanism, depending on the crowder's nature and shape. Our data show that the environment affects the stand displacement mechanism, particularly hydrophobic interactions, and crowding. They also suggest that the effect of molecular additives may have an essential role in stand displacement.

This study helps us understand that the type of strand (DNA, RNA, LAN-DNA) and the nature of the molecular cowders are important for strand displacement. Gained knowledge could improve the sensitivity of DNA-based structures for nucleic acid detection.

Jury:

Prof. Jens Stein, UniFR, Fribourg	President of the jury
Prof. Dr. Curzio Rüegg, UniFR, Fribourg	Thesis director
Prof. Guillermo Acuna, UniFR, Fribourg	Internal co-examiner
Prof. Johan Jofkens, KU Leuven, Belgium	External o-examiner