Thesis for Doctor of Philosophy
in Natural Science with a
specialization in Chemistry

Development of automated nanopore-based biosensor platform:

ATCUN-peptide functionalization enables highly sensitive detection of Cu(II) ions in fluids

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Abstract

Copper is essential for energy production, antioxidant defense, and neurotransmitter synthesis; its dysregulation is implicated in disorders such as Wilson's, Menkes, and Alzheimer's disease. This thesis addresses the need for sensitive, rapid copper(II) detection to improve clinical monitoring and diagnosis.

The thesis focuses on the development of an automated nanopore-based biosensor platform for copper(II) detection to address the clinical need for rapid and accurate copper monitoring. The results of this thesis are summarized in three papers.

Paper I reports the development of an efficient synthetic protocol to the fluorescently labelled Amino-Terminal Cu(II) and Ni(II) binding (ATCUN) peptides. Various coupling conditions are investigated and improved coupling protocols significantly increase yields. Physico-chemical properties of the rhodamine-labelled ATCUN peptide were investigated, resulting in optimized quenching and binding efficiencies at pH 6.5.

Paper II describes about platform with multiplexed voltage-to-current (IV) and fluorescence measurements of the samples. This automated platform was called Bio-Sensei, which incorporates a robotic pipetting arm, picoampermeter and fluorescence measurement diode. This platform enables measurement, collection of data from instruments and storage of the data. The platform is tested with ATCUN functionalized nanopore membranes in Cu(II) spiked MES/KCL buffer at pH 6.5. Results show the ability to detect Cu(II) up to femtomolar concentrations in buffer.

Paper III presents the integration of reconfigurable microfluidics with the automated platform, and measurements in human serum mimics samples, by spiking them with Cu(II). This is followed by data analysis using neural network algorithms to predict Cu(II) concentrations in the solution. Results of this study show that nanopores functionalized with rhodamine labelled ATCUN peptide showed improved sensitivity to Cu(II) ions than previously used FAM labelled ATCUN.

The results of this thesis contribute to the development of the platform with the potential to enable the measurement of Cu(II) in clinical samples, such as Wilson's disease diagnosis or monitoring. Future directions include expansion to other metal ions, integration with wearable technology, and further refinement of machine learning algorithms to enhance clinical utility.