Mini Review

Single-Molecule Studies of Small Molecule Drugs through Nanopore Detection

Song Shaojiao, Yuan Ziwei, Liang Huanyi, Zhao Qiuyue*, Li Ting*

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*Corresponding author: Li Ting and Zhao Qiuyue, Mianyang normal university, Mianyang, China

Introduction

During the last decades, nanopore single-molecule analysis has shown great potential in detection of DNA [1-3], RNA [4], proteins [5-8], polymers [9], organic molecules [10-11], and chemical reactions [12-13], and has been further extended to high-throughput nucleic acid sequencing. The detection of drug interactions [14] is crucial to evaluate the effect and safety of drugs, and it is also an indispensable part in the process of drug development and clinic treatment [15]. Over the past few years, the nanopore worked as a new platform for the discovery of drug interactions. Nanopore sensor, with the advantages of simple operation, speed and portability, can allow single molecule to pass through.

Discussion

a) Direct detection of small molecule drugs using nanopore technology

Applied to nanopore, Li-Qun Gu and Hagan Bayley were first to propose stochastic sensing to detect drug molecules, including Promethazine and Imipramine [16]. The paper shows that stochastic sensing of drug molecules can be procured from a-haemolysin by equipping the channel with an internal, non-covalently molecule adapter which mediates channel blocking by the drug molecule. Through hybrid a-hemolysin protein pore and the channel blocking characteristics, another interesting paper was reported by Tudor Luchian [17]. This work can distinguish between antibiotic molecules of different size and charge, belonging to the b-lactam family, including ampicillin, amoxicillin and azlocillin.

b) Detection of nucleic acid-small molecule interactions using nanopore technology

Most studies of interactions between nucleic acid and small molecules has accomplished by monitoring changes in nanopore current patterns of nucleic acids or binding with drug molecules. Yao et al. performed nanopore-based detection of DNA-doxorubicin (Dox) drug interactions using a-HL pores [18]. The DNA-Dox adduct showed a distinctive electronic signal pattern compared to original DNA. In contrast to the hairpin DNA event, DNA-Dox adduct generated prolonged events with a 10-fold longer duration. This result suggests that the nanopore sensor can be used to examine interactions between anticancer drug and DNA, and can be applied to functional analysis and Pharmacological analysis. Recently, our research group investigated DNA shearing action caused by bleomycin, accompanied by detection of bleomycin with high selectivity and ultra-low detection limit [19]. A dumbbell-based DNA probe based on the nanopore current signal was designed, which can produce blockage current signals. however, the addition

of BLM-Fe (II)would make the dumbbell DNA probe undergo an irreversible strand break, resulting and releasing the short-chain oligonucleotide fragment, which lead to the disappearance of the blockage event. Soon afterwards, a new method related to DNA oxaliplatin adduct was proposed [20], which enables real-time monitoring of DNA oxaliplatin condensation process. Specifically, signals with specific current characteristics are observed during this process. Typical high-frequency signals were obtained by recording the resulting designed DNA sequences. Furthermore, the generation of these signals was confirmed to be independent of the homologous adducts. Suggesting that DNA oxaliplatin adducts can serve as potential sensors to detect oxaliplatin lesions and multiple types of molecules.

Conclusions

Taken together, nanopore could be a promising platform for the ultrasensitive, label-free and single-molecule analysis for drug interactions and screening research. Through nanopore technology, we can accurately and efficiently evaluate drug interactions, providing an important reference for drug development and disease diagnosis and treatment. There are still some challenges and urgent problems to be solved in the application of nanopore technology in drug interaction detection. For example, the preparation method and stability of nanopore need to be further improved to improve the accuracy and reproducibility of detection. Meanwhile, the application of nanopore technology in large-scale screening of drug interactions needs to be further explored. Finally, we suggest that single-molecule-based nanopore applications would be useful for high-throughput screening in drug discovery. In future, nanopore technology open up a new path for single-molecule-level drug discovery, which will provide guidance for drug discovery and pharmacological research.

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