Nanopore Blockade Sensors for Ultrasensitive Detection of Prostate-Specific Antigen in Human Whole Blood

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Developing a cancer into detectable levels takes time, often over one decade. A key approach to reduce deaths from these life-threatening cancers is to diagnose the cancer at early stages when treatment strategies are more effective.1 Therefore, developing ultralow detection limit bioanalytical sensors is really important because it allows ultrasensitive detection of cancer-related biomarkers after the cancer has occurred even before any biological symptoms. Moreover, these ultrasensitive biosensors would also make a substantial impact as an effective means to estimate treat efficacy and assess reoccurrence for cancer patients. Nanopore-based sensors emerge rapidly as a promising technology because nanopore sensors detect analyte molecules one by one when passing through a nanoscale pore. Thus, nanopore sensors have been extensively investigated for development of biosensing and disease diagnostics.2 This paradigm limits nanopore sensors getting detection of analytes in pM or even less, as well as nonspecific signals in complex media. Recently we developed a novel paradigm for nanopore sensing – magnetic analyte shuttling – to overcome slow transporting of molecules to the nanopore, eliminate nonspecific signals in complex media and achieve single molecule resolution at devices of multiple nanopores.3 Iron oxide magnetic nanoparticles serving as analyte carriers, are synthesised and chemically functionalised to be specific to a model protein marker of prostate cancer, prostate-specific antigen (PSA). Devices of 3×3 nanopore array are fabricated and modified with a second anti-PSA antibody (targeting to a different epitope). Only those PSA-carrying nanoparticles can specifically block the nanopore under an external magnetic field due to antigen-antibody binding events inside the pore. Nanopore blockade sensors have been effectively demonstrated to provide highly specific sub-fM detection limits, rapid response time and direct measurements of PSA in human whole blood. We also managed to spatially immobilise anti-PSA antibody just onto nanopore walls and successfully applied to detecting sub-fM PSA. Our work demonstrates a highly-sensitive nanopore sensor capable of single-molecule-level quantitation of proteins from complex media. Features including magnetic analyte shuttling, site-specific surface functionalisation and parallel single molecule detection at nanopore arrays open up the full potential of nanopore sensors for biosensing various protein and nucleic acid species.

References