Nanopore blockade sensors for ultrasensitive protein detection in complex biological samples

Yanfang Wu[1], Kyloon Chuah[1], S.R.C. Vivekchand[1], Yin Yao[2], Soshan Cheong[2], Richard D. Tilley[2], Katharina Gaus[3], Peter J. Reece[4], Adam P. Micolich[4], J. Justin Gooding[1]**

[1] School of Chemistry and the Australian Centre for NanoMedicine, The University of New South Wales; [2] Electron Microscope Unit, Mark Wainwright Analytical Centre, The University of New South Wales; [3] EMBL Australia Node in Single Molecule Science, School of Medical Sciences and the ARC Centre of Excellence in Advanced Molecular Imaging, The University of New South Wales; [4] School of Physics, The University of New South Wales, Sydney, New South Wales 2052, Australia.

Email addresses: yanfang.wu@unsw.edu.au, justin.gooding@unsw.edu.au

Sensors with ultralow detection limits are highly desired as they will allow measurement of low-abundance biomarkers for liquid biopsy-based disease diagnosis and management. One promising approach is to develop single-molecule sensors.[1] The capability of detecting single molecules means the sensor could achieve quantitation at ultralow concentrations, eliminating the need for calibration and differentiating specific and nonspecific molecules in complex matrices.[2] Nanopore sensors, which use a nanoscale pore to measure single molecules passing through the pore, emerge rapidly as a promising technology for quantitative analysis.[3] However, due to the stochastic sensing nature and slow mass transport limited by diffusion, nanopore sensors typically detect analytes of nM-μM concentrations, far from the desired analysis of analytes in pM or less. Moreover, every passing molecule through the nanopore can register a signal, limiting their practical applications with complex samples. Our group has developed an effective sensing paradigm for nanopore sensors to overcome the long analysis times at low analyte concentrations and nonspecific signals in complex media.[4] The strategy involves the utility of iron oxide magnetic nanoparticles and devices with an array of 3 × 3 nanopores. The surfaces on the nanoparticles and nanopores were modified with antibodies that target a model protein analyte, prostate-specific antigen (PSA) at two different sites. The anti-PSA modified magnetic nanoparticles deliver PSA bound on the particle surface to the nanopore array under a controlled external magnetic field, blocking the pore rather than translocating through it. The sensor was able to detect sub-fM PSA in blood. Approaches including the fabrication of devices containing gold metallised nanopores at silicon nitride substrates and differential surface chemistries at nanopore interiors and exteriors have been developed to further advance the nanopore sensing technology.[5-8] Our work has demonstrated the potential in using single-molecule nanopore sensors for ultrasensitive analysis in complex sample matrices.

References:

[1] Gooding, J. J.; Gaus, K. *Angew. Chem. Int. Ed.* **2016**, *55*, 11354-11366.
[2] Wu, Y.F.; Tilley, R. D.; Gooding, J. J. *J. Am. Chem. Soc.* **2019**, *141*, 1162-1170.
[3] Wu, Y.F.; Gooding, J. J.; *Chem. Soc. Rev.* **2022**, *51*, 3862-3885.
[4] Chuah, K.; Wu, Y.F.; Vivekchand, S.R.C.; Gaus, K.; Reece, P. J.; Micolich, A. P.; Gooding, J. J. *Nat. Commun.* **2019**, *10*, 2109.
[5] Wu, Y.F.; Chuah, K.; Gooding, J. J. *Biosens. Bioelectron.* **2020**, *165*, 112434.
[6] Wu, Y.F.; Yao, Y.; Cheong, S.; Tilley, R. D.; Gooding, J. J. *Chem. Sci.* **2020**, *11*, 12570-12579.
[7] Wu, Y.F.; Lian, J.X.; Gonçales, V. R.; Pardehkhorram, R.; Tang, W.X.; Tilley, R. D.; Gooding, J. J. *Langmuir* **2020**, *36*, 5243-5250.
[8] Tang, W.X.; Wu, Y.F.; Mehdipour, M.; Chen, H.-S.; Tilley, R. D.; Gooding, J. J. *Nano Lett.* **2021**, *21*, 9374-9380.