**From Ultrasensitive to Single Molecule Biosensors that Operate in Complex Biological Fluids**

*Yanfang Wu*A*, Sharmin Hoque*A*, Padmavarthy Bakthavathsalam*A*, Danielle Bennett*A*, Roya Tavallaie*A*, Manchen Zhao*A*, Richard D. Tilley*A*, Katharina Gaus*B*, J. Justin Gooding*A*\**

ASchool of Chemistry, The Australian Centre for NanoMedicine and the ARC Centre of Excellence in Convergent Bio-Nano Science and Technology and B EMBL Australia Node in Single Molecule Science, School of Medical Sciences and the ARC Centre of Excellence in Advanced Molecular Imaging, UNSW, Sydney 2052, Australia.

One of the major opportunity in biomedical sensors is technologies that can selectively detect species at ultra-low levels [1]. This is because many of the existing pathologies, such as early detection of cancer, pathogen detection and assessment of treatment efficacy, are all required to be detected at low levels that existing commercial technologies seldom reach. We have developed a suite of technologies that are amenable to commercialisation that can detect species at femtomolar and lower levels. The suite of technologies all use the same strategy of making magnetic nanoparticle sensors collect the biomarker of interest rather than the normal approach of making the biomarker find the sensing surface. Using this strategy, the first technology will focus on the detection of ultralow levels of microRNA, as a cancer marker, in whole blood with 10 aM detection limits [2]. Next will be discussed taking this strategy down to single molecules using a unique nanopore blockade sensor that we have developed for detecting proteins at femtomolar levels [3]. This will be followed by a dark-field microscopy method for detecting viral RNA that exploits a new concept of performing quantitative analysis by counting many single-molecule events [4,5]. Taken together, the common thread in all these technologies is the use of nanoparticles to confine the measurement volume to nanolitre of lower such that a single molecule in that volume is an appreciable concentration.

**References**

1. Y.F. Wu, R.D. Tilley, J.J. Gooding, (2019). The Challenges and Solutions in Developing Ultrasensitive Biosensors, *J. Am. Chem. Soc.* 141, 1162-1170.
2. R. Tavallaie, J. McCarroll, M. Le Grand, N. Ariotti, W. Schuhmann, E. Bakker, R.D. Tilley, D.B. Hibbert,M. Kavallaris, J.J. Gooding, (2018). DNA-programmed electrically reconfigurable network of gold-coated magnetic nanoparticles enables ultrasensitive microRNA detection in blood, Nature Nanotech. 13, 1066-1071(2018).
3. K. Chuah, Y. Wu, S.R.C. Vivekchand, K. Gaus, P.J. Reece, A.P. Micolich, J.J. Gooding, (2019). Nanopore Blockade Sensors for Ultrasensitive Detection of Proteins in Complex Biological Samples, Nature Comm 10, 2109.
4. J. J. Gooding, K. Gaus, (2016). Single molecule sensors: challenges and opportunities for quantitative analysis, Angew. Chem. Int. Ed. 55, 11354-11366.
5. M. Sriram, B.M. Pouryousefi, P.R. Nicovich, D.T. Bennett, P.J. Reece, D.B. Hibbert, R.D. Tilley, K. Gaus, S.R.C. Vivekchand, J.J. Gooding, (2018). A rapid readout for many single plasmonic nanoparticles using dark-field microscopy and digital color analysis, Biosensors Bioelectronics,117, 530-536.