Overview of the second super-resolution microscopy session of the joint Asian Biophysics Association/Australian Society for Biophysics meeting

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On Wednesday, December 5, the second super-resolution microscopy session of the joint Asian Biophysics Association/Australian Society for Biophysics meeting welcomed speakers covering topics spanning individual nanoparticles and viral proteins to whole cell membrane dynamics and damage response pathways. The speakers in this session were Assoc. Prof. Olga Shimoni from the University of Technology in Sydney, Dr. Niall Geoghegan from the Walter and Eliza Hall Institute of Medical Research in Melbourne, Mr. Max Baker from the University of Sydney, and Dr. Donna Whelan from La Trobe University in Bendigo.

The session commenced with a presentation by Assoc. Prof. Olga Shimoni on the benefits of using upconversion nanoparticles (UCNPs) for long-term quantitative analysis of single nanoparticles. UCNPs contain thousands of photon sensitizers (i.e., Yb³⁺ ions) and activators ions (i.e., Tm³⁺ and Er³⁺ ions) to form an energy transfer network capable of strong fluorescence emissions (Wang et al. 2018). Assoc. Prof. Shimoni described a library of monodisperse and emission-tuneable UCNPs that are bright and photostable enough to enable long-term imaging and tracking, as well as some of the approaches for surface functionalization that she has developed with her colleagues (Duong et al. 2018; Sun et al. 2018). UCNPs engender low phototoxicity, making use of low-intensity near-infrared excitation and, as such, are ideal candidates for live cell, low-power multiphoton, and deep tissue microscopy. Assoc. Prof. Shimoni went on to detail

several successful applications of these UCNPs including high-precision single particle tracking in vivo and nanoscale measurements of local viscosity and forces within the intracellular environment.

Dr. Niall Geoghegan was the second speaker in the session, describing his recent work to visualize the dynamic process of *Plasmodium* parasite invasion of red blood cells (RBCs) which causes malaria. This process involves a series of ligand-receptor interactions resulting in physical alteration of the RBC membrane allowing parasite penetration and parasitophorous vacuole formation. Once inside the RBC, the parasite can reproduce asexually to create a new generation of RBC-invading merozoites. Previously, our understanding of parasite invasion had been based on theoretical simulations of RBC membrane-wrapping mechanisms; however, an in vivo experimental approach had not been achieved due to the highly dynamic nature of the parasite penetration and the limitations of microscopic techniques (Dasgupta et al. 2014). Dr. Geoghegan and his colleagues have overcome this to visualize *Plasmodium* invasion of RBCs using state-of-the-art 4D lattice light sheet microscopy (Chen et al. 2014). This approach, paired with innovative labeling approaches, has afforded an unprecedented level of insight into the real-time dynamics of parasite contact, penetration, and resealing of the RBC membrane. In particular, the research team has been able to map the distribution of cholesterol in the membrane, pointing to its potential key role in formation of the parasitophorous vacuole. Dr. Geoghegan's talk impressed with many stunning images and movies, convincingly demonstrating the enormous potential of lattice light sheet microscopy.

The next speaker was Mr. Max Baker discussing his PhD thesis work using a range of biophysical techniques to characterize a novel viral Receptor Homotypic Interaction Motif (RHIM)-containing protein—ORF20—and its role in inhibiting programmed cell death (Baker et al. 2018). Four known human RHIM proteins are responsible for mediating

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the amyloid structure of the heteromeric signaling complex known as the necrosome which induces cell death by programmed necrosis. More recently, the necrosome has also been implicated in other cell death pathways (Li et al. 2012). Using a combination of fluorescence and electron microscopy, along with single molecule spectroscopy, Mr. Baker described the mechanism by which viral ORF20 drives formation of homomeric amyloid fibrils, as well as its interaction with human RHIM proteins. Mr. Baker went on to describe how ORF20 inhibits apoptosis within the context of the host organism, but not necroptosis, in contrast to other viral RHIMs studied by the group (Pham et al. 2019). His work also revealed that ORF20's activity is not solely dependent on RHIM interactions, uncovering another potential aspect of the viral strategy for overcoming host organism defensive processes.

The final speaker for the session was Dr. Donna Whelan who presented her recent work combining single-molecule localization microscopy (SMLM) with synchrotron-sourced Fourier transform infrared (FTIR) spectroscopy to detect and characterize low levels of DNA damage in fixed cells. Dr. Whelan and colleagues had previously used FTIR spectroscopy to quantify DNA and elucidate its conformation in single live cells (Whelan et al. 2013) and had applied SMLM to visualize DNA damage and repair at the individual double-strand break level (Whelan et al. 2018). Combining these two powerful approaches, Dr. Whelan demonstrated their complementarity in capturing both holistic and highly specific information from cells, including label-free live information which can be used to determine the perturbations caused by cell fixation (Whelan and Bell 2015). Dr. Whelan's data demonstrate the immediate recruitment of DNA repair proteins to single stressed and broken replication forks, alongside the wide-ranging signaling response of the cell. To end the session, Dr. Whelan showed pictures of the new advanced fluorescence microscope she has built at La Trobe University for her future single-molecule research.

Compliance with ethical standards

Conflict of interest Donna R. Whelan declares that she has no conflicts of interest.

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

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