Understanding and evaluating the use of microscopy based methods for protein corona studies

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Nanoparticles (NPs) have a huge potential in biomedical and life science applications. Recently the adsorption of proteins on the surface has been found to affect the cellular transport, uptake and clearance of many NPs, as well as their ability to be effective in targeted drug delivery applications in vivo [1]. The formation of the protein coating occurs immediately upon NP contact with biological media. Reliable methods to observe and measure this **protein corona (PC)** dynamically need to be developed if NPs are to be implemented in nano-medical applications [2]. The large number and variability of proteins coupled with their low imaging and spectroscopic "contrast" make the dynamic study of the PC complex.

Our research focuses on studying the protein corona formed by serum around AU NPs (50 nm) using advanced imaging and spectroscopic techniques. Measurements via dynamic light scattering (DLS) help to obtain *in situ* size and zeta potential measurements which with careful analysis can show reproducible increases in size and altered zeta potential up to 120 min of incubation. Atomic force microscopy (AFM) can capture the dimension and mechanical properties of the protein corona at the surface of individual NPs. With transmission electron microscopy (TEM), using negative staining, we obtained contrast information of the proteins layering onto the NPs. Furthermore, we identify which proteins are mainly absorbed onto the NPs' surface depending on time using surface enhanced Raman spectroscopy (SERS). First SERS measurements show a visible change in the composition of the PC over time.

Our results indicate that special sample preparation and accessible methods can be used to understand the PC better and work towards seeing it as a tool to enhance drug delivery rather than an obstacle. We will present data of advanced imaging and spectroscopic techniques supported by proteomics to uncover new methods to visualise and understand the protein corona.

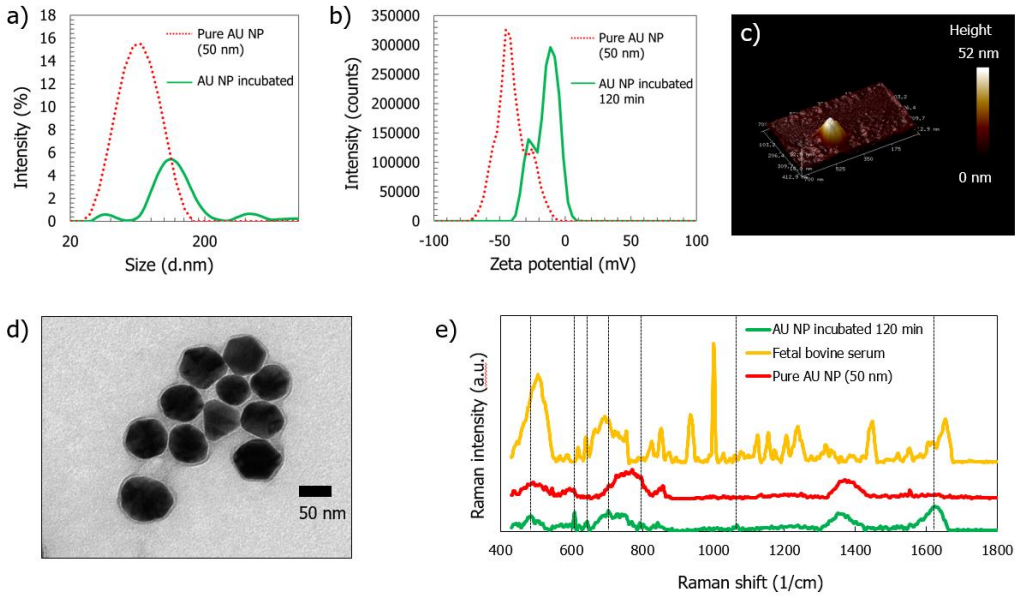

Figure 1: a) and b) show the DLS measurements of the hydrodynamic diameter and the zeta potential of pure AU NPs and AU NPs incubated in human serum (FBS) for 120 min, respectively. c) shows AFM imaging of

incubated AU NPs. d) shows TEM image (stained with 2% UCL) of incubated AU NPs. e) shows the SERS measurements of incubated AU NPs and controls (pure AU NPs, FBS). The dashed lines indicate changes in the spectra of incubated AU NPs.

This work was financed through support from the ARC Centre of Excellence for Nanoscale and BioPhotonics (CE140100003), an ARC Future Fellowship (AEGB, FT150100342).

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