Developing a new bio-imaging method based on correlative light electron microscopy with gold nanoparticles

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Project description: Correlative Light Electron Microscopy (CLEM) is one of the most powerful imaging technologies as it combines the advantages of live cell imaging from light microscopy (LM) with the sub-nanometer spatial resolution of electron microscopy (EM). Using this technology, key biological questions have been answered [1].

CLEM is, however, seriously hampered by the availability of robust probes. It is highly questionable whether most bimodal probes, using a fluorophore (for LM) and an electron-dense gold nanoparticle (for EM) attached to the protein of interest, actually show the same protein pool. This is due to limitations such as photobleaching or quenching of the fluorophore, and/or detachment of the probes while trafficking inside the cell or after sample processing for EM. These are serious drawbacks that need to be addressed.

To do so, the aim of this project is to develop a novel CLEM method where a gold nanoparticle (AuNP) is used as the same probe for both LM and EM. AuNP will be visualised in LM in living cells using a novel nonlinear optical microscopy technique developed at Cardiff University. The technique uses electronically-resonant Four Wave Mixing (FWM) and exploits the strong and photostable absorption and scattering of light of a AuNP at the localised surface plasmon resonance (LSPR). Using a combination of short optical pulses to generate and detect changes in the AuNP transmission or scattering at the LSPR, the technique is uniquely sensitive to single small AuNPs which are detected background free with high spatial resolution in 3D [2]. Notably, the technique is also uniquely sensitive to the AuNP shape, which opens up the prospect of probe multiplexing via shape recognition.

In this project, spherical AuNPs and gold nanorods of various sizes will be conjugated to proteins of interest (for example the iron-binding protein transferrin) and internalised in mammalian cell lines. The aim will be to demonstrate the full CLEM workflow starting from imaging living cells with FWM (at Cardiff), to fixation, sectioning and correlative imaging of the same AuNP within the cell ultrastructure revealed by EM (at Bristol). The project will also explore the pioneering concept of assembling AuNPs directly inside living cells using proteins expressed in the cytoplasm which have the capability to bind metals and concentrate these to form electron dense particles. This could open the way to genetically tag cytoplasmic proteins with metallic NPs.

[1] DOI: 10.1038/nature14503.
[2] DOI: 10.1103/PhysRevX.7.041022

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