Project Details	
Project Code	MRC23IIARCa Borri
Title	Unravelling cell entry and intracellular trafficking of adenoviruses at the nanoscale using coherent optical nanoscopy
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	Adenoviruses (AdV) are promising candidates as vectors for gene therapies, yet they follow a complex intracellular journey which needs to be fully understood for the design of optimised constructs. The project will exploit a novel single virus tracking technology using photostable gold nanoparticle probes, to directly pinpoint the mechanism of cell entry and intracellular trafficking pathways of different AdV serotypes with unprecedented spatio-temporal resolution
Description	Adenoviruses (AdV) are attracting strong interest as vectors for gene therapies (including the COVID-19 vaccine) and as oncolytics for cancer treatments. To fulfil its role as a vector, an AdV needs to successfully deliver its DNA genome to the host nucleus. Notably, AdV infection is a complex process involving several steps: binding to a primary receptor at the cell surface, internalization via endocytosis, escape from the endosome, intracellular trafficking and finally genome delivery to the nucleus. AdV exist in many serotypes, some of which have been well characterised (for example Ad5) while others are unstudied and might offer exciting new avenues for “virotherapies”. Importantly, it has emerged that different serotypes undergo different trafficking pathways, but the reasons are often unclear [1]. Yet, when considering the use of AdV as vectors for therapeutic applications, understanding how they traffic inside cells and their exit efficiency from endosomes is key to the design of constructs for optimal transgene expression in target cells. Moreover, although AdV are considered not to be very dangerous, adenoviral infection is a significant cause of mortality in the immunocompromised individual, so understanding their means of infection is also critical for developing new antivirals to treat such patients. A powerful way to unravel the journey of viruses is to directly follow individual virions in space and time inside living cells, using light microscopy and single particle tracking (SPT) techniques [2]. The majority of SPT methods to date exploit fluorescent probes labelling the virion of interest. However, all organic probes are prone to photobleaching which severely limits the total time window of tracking (to at best few minutes) and the precision with which the probe can be localised in space. Moreover, photobleaching is often accompanied by cytotoxicity. The lead supervisor lab has pioneered a new SPT technology using photostable gold nanoparticle (AuNP) probes, which overcomes all limitations of fluorescent-based SPT. The technique exploits the strong and colour-selective absorption and scattering of light of a AuNP. Using a combination of short optical laser pulses to generate and detect changes in the AuNP transmission/scattering (via a process called four-wave mixing – FWM), the technique is uniquely sensitive to single small AuNPs which are detected background-free in 3D with localisation precision at the nanoscale inside cells [3]. Notably, AuNP are well established probes in electron microscopy. Hence the technique lends itself for correlative light electron microscopy (CLEM) using the same AuNP, to pinpoint the probe location within the cellular

	ultrastructure with (sub)-nm spatial resolution. This project has two main interconnected aims: 1) to develop AdV tagged with small gold nanoparticles and 2) to unravel the cell entry and intracellular trafficking of AdV by SPT with unprecedented spatio-temporal resolution using FWM-CLEM. The co-supervision (Parker) has expertise with various AdV serotypes (including Ad5, Ad10 and Ad49) and has developed biochemical tools to selective label the AdV fibre knob proteins with AuNPs. The co-supervisor in Bristol (Verkade) is a leader in CLEM. Together with the Lead supervisor (Borri) and the co-supervisor (Langbein), they have recently demonstrated a proof-of-principle FWM-CLEM workflow which will be exploited in this project. The student will be embedded in a vibrant and highly cross-disciplinary environment at the physics-life science interface. Areas in which the student will be able to take ownership and steer the project include engineering AdV serotypes with AuNP also inside the capsid (e.g. at the terminal protein), and designing experiments to gain a mechanistic understanding of the intracellular trafficking and genome delivery of new AdV serotypes. [1] 10.3390/pharmaceutics13101585 [2] 10.1021/acs.chemrev.9b00692 [3] 10.1039/C9NR08512B
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