**A Microscopy-based Assay Platform for the Measurement of Cellular Entry and Trafficking of Complex Medicines**

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For a drug molecule to be efficacious, the delivery of the molecule to its target site is crucial. Many drug targets are localized to specific subcellular compartments, yet current drug design strategies are focused on bioavailability and tissue targeting and rarely address entry of the drug to the cell and delivery of the drug to specific intracellular compartments. To this extent, quantitative measurement of internalisation of biopharmaceuticals, such as antibody-drug conjugates (ADC) and oligonucleotide medicines, and insights into how the cell traffics these compounds to different cellular locations can greatly improve drug development.

Receptor mediated internalisation is a critical characteristic of antibody-drug conjugates, with antibody-binding to cell surface proteins driving internalisation of the ADC. The rate of antibody internalisation is influenced by several factors; including epitope selectivity, binding affinity, and intracellular transportation. Thus, the selection of the antibody-epitope pair with the most suitable internalisation kinetics is an important factor in ADC development. Here we developed a microscopy-based platform to study the kinetics of internalisation of antibodies. Our assay employs the use of a fluorescent membrane dye and a fluorescently labelled antibody of interest which allow a direct measurement of internalisation kinetics. With this method, we exemplified the cellular internalisation of two well established systems; anti CD33 and anti Her2 antibodies in suspension and adherent cells respectively. Furthermore, we followed the cellular trafficking of these antibodies using immunofluorescence microscopy-based colocalization with endosomal proteins. This image-based platform provides a sensitive and robust method for quantification of the internalisation rate constant and allows the monitoring of intracellular trafficking and processing of antibodies in the endocytic pathway.