Caco-2 cells through exploitation of the natural receptor governed processes involved in VB12 absorption.

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## A26

## Four-wave mixing imaging to study protein entry and release in mammalian cells

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Optical microscopy is a powerful tool for tracking the binding, internalisation and subcellular trafficking of delivery vectors to mammalian cells. By exploiting multiphoton processes, subcellular structures can be imaged with intrinsic three-dimensional (3D) spatial resolution. Common fluorescent labels in multiphoton microscopy include organic fluorophores, which suffer from photobleaching, and quantum dots which are more photostable but contain cytotoxic elements (such as Cd or In). Gold nanoparticles (GNPs) are ideal optical labels in terms of photostability and bio-compatibility, but emit weak fluorescent signal. We have developed a novel multiphoton microscopy technique that exploits the third-order nonlinearity called four-wave mixing (FWM) of GNPs in resonance with their surface plasmon. In terms of imaging performances, FWM microscopy features a spatial resolution better than the one-photon diffraction limit and optical sectioning capabilities. We show high-contrast background-free imaging of gold-labels (down to 5 nm size) and sensitivity to the single particle level. We are also able to demonstrate a directed dissociation of the GNP from bound proteins at their surface. These results pave the way for active tracking of conjugated nanoparticles, before the controlled release of therapeutically relevant proteins to a localised site of interest.

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## A27

## Efficient gene delivery using acid-responsive lipid envelopes for adenovirus

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Gene therapy involves the delivery of a functional gene by a vector into target cells, resulting in a desired therapeutic effect. Adenovirus (Ad) has shown a great promise in gene therapy [1,2]. However, *in vivo* studies have reported an immunogenic response and an overwhelming accumulation and gene expression in the liver resulting in significant hepatoxicity. These issues currently inhibit the use of this vector for use in clinical therapies. Such limitations have been overcome by engineering artificially enveloped Ad using zwitterionic and cationic lipid bilayers [3,4]. However, this resulted in a significant reduction of gene expression *in vitro*. We observed that this may be due to poor release of the Ad from its lipid envelope. In the present work, we have explored the use of pH-sensitive DOPE:CHEMS lipid-envelopes to stimulate the virus release from the envelope and consequently result in higher levels of gene expression. Artificially enveloped Ad (DOPE:CHEMS:Ad) were prepared by lipid film hydration followed by sonication. The physicochemical characteristics of the resulting hybrid biomaterials were characterised by transmission electron microscopy, atomic force microscopy, dot blot, dynamic light scattering and zeta potential measurements. The enveloped viruses exhibited good stability at physiological pH (7.4) but immediately collapsed and released naked virions at pH 5.5. Furthermore, recombinant Ad encoding for beta-galactosidase (β-gal) enveloped in DOPE:CHEMS showed comparable levels of gene expression to naked Ad in different cell lines. These transfection results were further confirmed by studying the intracellular trafficking of fluorescently labelled, Cy3-Ad using confocal laser scanning microscopy (CLSM). Interestingly, Cy-3 Ad enveloped in DOPE:CHEMS showed a uniform fluorescence distribution within the cytoplasm indicating Ad endosomal release. In addition, pH-sensitive enveloped Ad injected directly into human cervical adenocarinoma (C33a) xenografts grown on the flank of nude mice showed similar levels of gene expression to naked Ad. In conclusion, this type of artificially enveloped Ad offers a promising tool in gene delivery since high level of Ad gene expression can be maintained while one can expect to dramatically improve the innate Ad immunogenicity and hepatotoxicity *in vivo*.

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## A28

## *In vitro* silencing of TGFβ1 in a corneal epithelial cell line using nanoparticles

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*Introduction*: Severe ocular inflammatory disorders constitute a sight-threatening group of diseases that present treatment difficulties due to the intrinsic barriers of the ocular surface. Previous work in our group has demonstrated that epithelial cells from human cornea (HCE cell line) basally secrete TGFβ1 (a commonly detected cytokine in ocular inflammatory diseases). At present, gene therapy (including siRNA-based therapies) holds promise for the treatment of several diseases, including ocular disorders. However, the development of safe and effective delivery vehicles still remains a major challenge for its clinical application. *Purpose*: This work is a proof-of-concept study meant to evaluate the efficacy of the *in vitro* gene silencing technique for different siRNAs targeting relevant pro-inflammatory cytokines