we formulated CTS-RNAs (siRNA or microRNA) nanoparticles by direct complexation. The nanoparticles with sizes of 120–200 nm and surface charge of ~20 mV showed complex stability and efficiency of protecting RNAs from RNase degradation. These nanoparticles can both transfer RNAs into cells and protect entrapped intracellular RNAs, in 2–4 hours without apparent critical cytotoxicity. Moreover, cell adhesive peptide GRGDY has been grafted to CTS by photosensitive crosslinker [3], and PEGylation has been carried out for target transportation to tumor cells with overexpressed integrin receptors and for efficient delivery of drugs or RNA therapeutics.

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A13
Incorporation of 2,3-diaminopropionic acid in linear cationic amphipathic peptides produces pH sensitive vectors

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Non-viral vectors that harness the change in pH in endosomes are increasingly being used to deliver cargoes, including nucleic acids, to mammalian cells. Here we present evidence that the pK_a of the β-NH2 in 2,3-diaminopropionic acid (Dap) is sufficiently lowered, when incorporated in peptides, that its protonation state is sensitive to the pH changes that occur during endosomal acidification. The lowered pK_a around 6.3 is stabilised by the increased electron withdrawing effect of the peptide bonds by inter-molecular hydrogen bonding and from contributions arising from the peptide conformation, including mixed polar/apolar environments, Coulombic interactions and inter-molecular hydrogen bonding. Changes of the charged state are therefore expected between pH 5 and 7 and large-scale conformational changes are observed in Dap rich peptides, in contrast with analogues containing lysine or ornithine, when the pH is altered through this range. These physical properties confer a robust gene delivery capability on designed cationic amphipathic peptides that incorporate Dap. Recent results investigating the link between hydrophobicity, number of charges, Coulombic interactions and side chain pK_a are considered in terms of the efficiency of gene delivery.

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A14
Octaarginine mediated delivery of fluorescent cargo to human smooth muscle cells

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The high incidence and severity of diseases involving smooth muscle dysfunction, which include cardiovascular diseases and premature labour, dictates the need for our continued search for novel therapeutic strategies to treat these conditions. Cell penetrating peptides (CPP) are a class of non-viral vectors that show considerable promise for drug delivery purposes yet their suitability for uptake, and delivery of biologically active cargo, to human native cells and tissues remains unresolved. For any new drug delivery strategy, including the use of CPPs, to reach fruition this needs to be elucidated. We have begun to explore this issue for CPPs applied to human uterine cells and tissues (including myometrium and blood vessels) obtained from biopsies collected, following LREC-approved written informed consent, from patients undergoing elective Caesarean section at the end of pregnancy. Primary cultured human myometrial cells were prepared on glass-bottomed culture dishes, grown to 80–90% confluence and exposed to serum-reduced conditions overnight before exposure to CPP (or, separately, were methanol-fixed for subsequent immunofluoresence staining of protein localisation). Cellular uptake of fluorescently labelled (Alexa 488) D-Octaarginine (R8, 2 μM) was assessed in the first series of experiments for 24, 48 and 72 hours ($n = 2$). At each time point, z-section confocal microscopy revealed punctate intracellular fluorescence (indicative of vesicular compartmentalisation) particularly dense in the perinuclear area. A second series of experiments assessed the time-course of intracellular delivery up to 24 hours. Punctate intracellular loading was observed by 4 hours. More dense perinuclear and plasma membrane-localised fluorescence was observed at later time points. Immunofluoresence labelling revealed that human myometrial cells possessed expected cytoskeletal (α-smooth muscle actin, tubulin), plasma membranous and perinuclear localised components of endocytotic pathways (Caveolin-1, Clathrin Heavy Chain, Early Endosomal Antigen-1, Lysosomal Associated Membrane Protein-1 and 2 and Flotillins). Next, small segments of native (non-cultured), human uterine tissue were incubated with 2 μM D-R8 and nuclear dye Hoechst 33342 (1 μM) for 4 hours. Confocal microscopic examination revealed peptide entry into smooth muscle cells of both the myometrium and uterine blood vessels with homogenous intracellular fluorescence in many cells but some with more punctate perinuclear/nuclear fluorescence. In uterine tissues incubated with a similar, putatively cell-impermeant, Alexa488 control peptide $(GS)_4GC$, no intracellular fluorescence was observed. These preliminary investigations illustrate that an octameric cationic CPP can successfully enter primary cultured and native human smooth muscle cells and tissues. This opens up a new avenue for targeted delivery of cellular therapeutics in human tissues and in particular to human smooth muscles.

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