Mimetic Proteins: A Non-Toxic Cytosolic Delivery Strategy

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Introduction
- Our collective understanding of the molecular regulation of cellular homeostasis and processes such as endocytosis have increased rapidly over the last 20 years (Fig 1).
- This has allowed membrane trafficking systems to be exploited for cytosolic drug delivery. In order to assimilate cytosolic targets, many organisms have evolved virulence factors that can manipulate mammalian endomembrane trafficking and associated cargo sorting systems (Fig 1).
- Protein virulence factor toxicity may be ablated using site-specific mutagenesis whilst retaining the ability to access the cytosol without causing toxicity.
- We have exploited such a system to facilitate the cytosolic delivery of pharmacologically active antisense oligonucleotides (ASO) in vitro.

Results
- Gene knockdown in vitro: Syntaxin-5 knockdown was demonstrated both morphologically (Fig 2) and by Western blotting, after normalizing syntaxin-5 expression levels to those of a housekeeper (Derlin1) (Fig 3).
- After 48h only 43 ± 4.1% (SEM) of the protein labeled using an antibody to syntaxin-5 was observed relative to the cytosol. Of the many routes that exist, two examples are shown.
- In vitro toxicity: We have shown 88.6 ± 4.6% viability (SD; n=4) in Vero at 100 µg/ml (DDS protein concentration) after 3 days. HeLa cells were more sensitive showing 54.5 ± 3.9% viability at 100 µg/ml (DDS protein concentration) after 3 days (SD; n=4) (Fig 4).
- Under similar experimental conditions the IC50 values obtained for poly(ethyleneimine) (PEI) were between 2.4 and 7.4 µg/ml (Table 1).
- The propensity of a commercial lipid based transfection system to induce transfection artifacts was also documented in relation to the protein based system described herein (Fig 5).

Discussion
- This departure from well-characterized, non-viral ASO delivery technology, (i.e. utilizing either cationic lipids or cationic polymers), has demonstrated activity equivalent to that of nucleofection (electroporation) over a 48th time course. Further, this recombinant DDS was tested for toxicity in vitro against epithelial cell lines (Vero and HeLa) using MTT.

Methods
- Cell culture, immunofluorescence experiments and Western blotting were performed as described in the cited literature under the conditions stated.
- Recombinant reagents were from Sigma (Dorset, UK), recombiant proteins were produced using sequences obtained from GenBank, sub-cloned into the pET151 expression system (Invitrogen, Paisley, UK) using standard protocols and characterized by SDS PAGE and Coomasie staining as well as Western blotting and immunodetection after affinity chromatography using appropriate antibodies.
- Electroporation was carried out using a Nucleofector 2b (Lonza Biologics plc Cambridge, UK) following the manufacturer’s instructions.

References