Using DNA nanostructures as antisense delivery vehicles

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Statement of Purpose: DNA is of tremendous interest, both as a nanoscale building block and as a therapeutic molecule in gene therapy. In particular, discrete DNA nanostructures have demonstrated potential as protein carriers, capable of encapsulating or displaying various ligands. We previously demonstrated the stability of a nanoscale DNA pyramid to enzymatic digestion [1], suggesting the possibility of a different approach to non-viral gene delivery. Here we show the construction of DNA pyramids that are capable of degrading target mRNA, and of subsequently inhibiting target protein expression in vitro. Incubation of antisense pyramids with mammalian cells showed both higher uptake efficiency and gene silencing when compared to linear DNA, presumably due to enhanced stability in the presence of serum. Our results highlight the versatility of DNA nanostructures for gene / drug delivery applications.

Methods: All oligonucleotides were purchased from IDT DNA. Hybridization between component DNA strands was analyzed with native polyacrylamide gel electrophoresis. For in vitro mRNA targeting, mRNA was incubated with antisense pyramid and RNase H, and analyzed on a 2% formaldehyde agarose gel. The reaction mixture for cell-free protein synthesis followed a standard protocol [2]. EGFP expression was monitored in real-time using a fluorescence spectrophotometer. Fluorescently labeled pyramids were formed by incorporating a single Cy5-labeled strand. Mouse myoblasts (C2C12) that stably express EGFP were used for intracellular studies. The mixture of antisense pyramid and lipofectamine was added to media with 10% FBS and incubated with cells. After 24 h, uptake of Cy5 labeled antisense pyramids and EGFP expression levels were monitored at the same time using a BD LSR II flow cytometer. Fluorescence images of transfected cells were obtained with an Olympus IX71 microscope.

Results: We initially tested the antisense activity of these pyramids with in vitro transcribed EGFP mRNA and purified RNase H. We found that as the concentration of the antisense pyramid was increased, the target mRNA was progressively degraded (data not shown). Control experiments demonstrated that this was a sequence-specific effect. We next examined regulation at the protein level, using a cell-free transcription and translation system [2]. Real-time fluorescence shows that the pyramid suppresses protein synthesis to nearly the same extent as the antisense strand alone (data not shown). Control experiments also show that this protein down-regulation is sequence-specific. To demonstrate intracellular antisense activity, DNA pyramids were incubated with C2C12 cells that constitutively express EGFP. Because of the relatively low uptake of bare pyramids, samples were mixed with lipofectamine according to standard procedures.

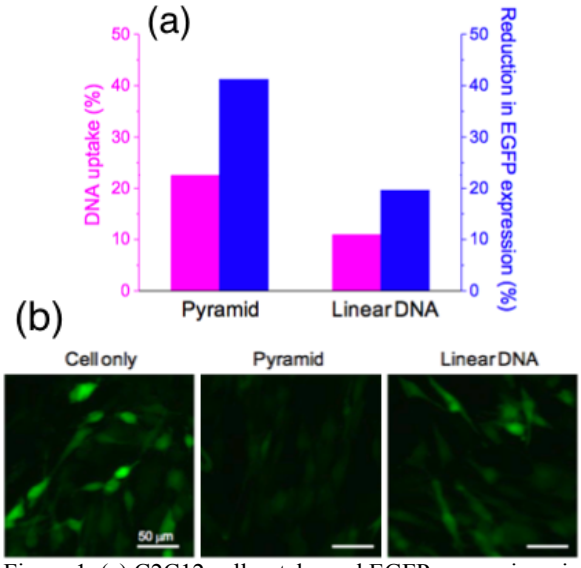

Figure 1. (a) C2C12 cell uptake and EGFP expression via flow cytometry reveals that pyramids have superior antisense properties compared to linear DNA. (b) Representative images also show that pyramids reduce EGFP expression more efficiently than linear DNA.

We found that in the presence of 10% serum, pyramids showed higher uptake than the linear DNA forms, by about a two-fold difference (**Figure 1a**). We interpret the increased uptake of pyramids to be a benefit of the enhanced nuclease resistance [1]. This improved uptake of pyramids consequently leads to more effective inhibition of EGFP: again there is a two-fold increase for the pyramids (**Figure 1a**). Representative fluorescence images agreed with the cytometry data (**Figure 1b**).

Conclusions: The stability of these pyramids with respect to nuclease degradation led to improved uptake and antisense-mediated gene knockdown in mammalian cells. The numerous approaches to functionalize DNA increase the potential of this delivery concept. The introduction of both cell-targeting and cell-penetrating motifs should be readily accomplished, and the responsive character of DNA nanostructures can be additionally exploited to release encapsulated cargo.

References: [1] Keum J-W and Bermudez H. Chem Comm. 2009; 7036-7038. [2] Ahn J. H. *et al*. Biochem Biophys Res Commun. 2005;338:1346-1352.