Controlled Release of siRNA from Hydrolytically Degradable Nanomicelles for Potent Gene Knockdown

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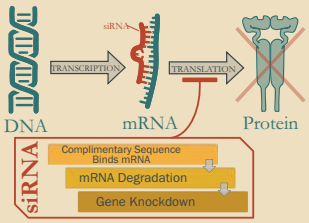
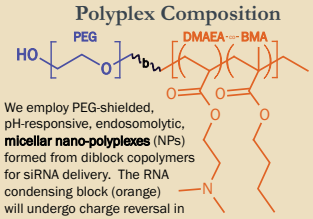
Introduction

RNA Interference



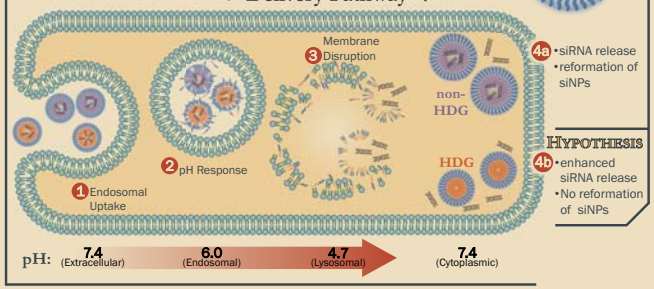
Small Interfering RNAs (siRNAs) can be used to silence almost any gene in the body, this premise allows siRNA to be utilized as a targeted therapeutic agent against disease[1]. However, intracellular release of siRNA from NPs once they have been internalized is a limiting step in siRNA bioavailability and bioactivity. Therefore, we have developed a vehicle for enhanced siRNA enhanced release post-endosomal escape.

Polyplex Composition



We employ PEG-shielded, pH-responsive, endosomolytic, **micellar nano-polyplexes** (NPs) formed from diblock copolymers for siRNA delivery. The RNA condensing block (orange) will undergo charge reversal in aqueous solution by the hydrolysis of DMAEA to acrylic acid and a benign alcohol[2]. Because of this charge reversal, we posit that hydrolytically degradable (HDG) siNPs can release siRNA into cells more effectively than previously established non-HDG NPs[3].

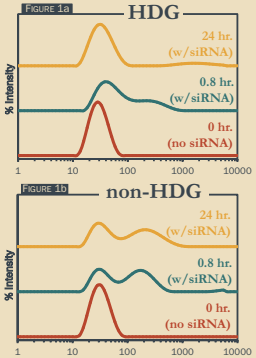
Delivery Pathway



Characterization

Polymer Library

	%BMA	DP	M_w (g/mol)	Charge (e)
40B-HDG	44.3%	113	22,800	+ 31.5
50B-HDG	55.9%	115	21,700	+ 25.5
40B	39.6%	137	23,700	+ 41.5
50B	48.3%	120	27,400	+ 31.1



Hydrolysis Mechanism

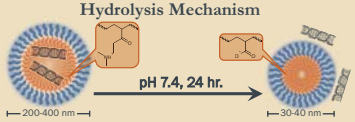


FIGURE 1: Dynamic light scattering (DLS) measurements were preformed on (1) NPs prepared from 50B polymers in pH 7.4 buffer and (2) siNPs prepared with 50B polymers and scrambled siRNA at a 5:1 NH_3^+/PO_4^- ratio. siNP solutions allowed to sit 50 min showed two particle distributions: pure polymer NPs (~30 nm) and siRNA complexed NPs (~200 nm). After 24 hours the **HDG NPs underwent self catalyzed decompexation and release of siRNA,** showing only the pure polymer peak.

Note: Curves shifted apart for clarity, Intensity = 0% at D = 1 nm for all curves.

siRNA Release

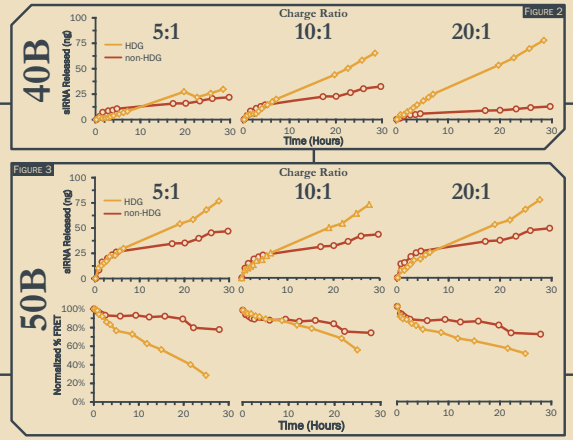


FIGURE 2: The amount of siRNA released from different siNP formulations using both HDG and non-HDG polymers was measured over time using a RiboGreen® RNA assay. The **40B-HDG siNPs show increased siRNA release over time and a faster rate of release** when compared to non-HDG siNP, especially at higher charge ratios. Furthermore, this trend gives us the ability to tune the rate of siRNA release from 40B-HDG NPs by simply controlling the charge ratio.

FIGURE 3: With 50B polymers the HDG siNPs consistently show increased siRNA release at all three charge ratios. These results were corroborated by a Förster resonance energy transfer (FRET) study*, where decrease in %FRET signal indicates decomplexation of siRNA from NPs.

*For FRET siNPs were loaded with two FRET-paired labeled DNAs (FAM & CY5) and %FRET was calculated at multiple time points by exciting the donor dye (FAM). $\left\{\%\ \text{FRET} = \frac{I_{acceptor}}{I_{acceptor} + I_{donor}}\right\}$

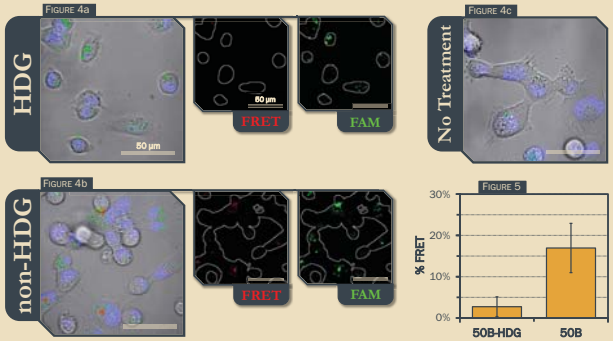
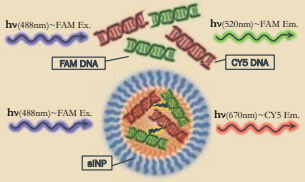


FIGURE 4: MDA-MB-231 cancer cells were treated for 9 hours with both 50B-HDG and 50B non-HDG siNPs loaded with FAM & CY5, FRET-paired DNA. The FAM signal (green) shows the presence of siRNA, while the FRET signal (red) implies that siRNA is still complexed within siNPs. Our non-HDG siNPs delivered siRNA into the cell, however stable polyplexes still remained within the cell.

FIGURE 5: Seeing a much lower %FRET signal, we know **HDG siNPs have more effective delivery and bioavailability of siRNA within cancer cells.**

FRET Background

Förster Resonance Energy Transfer



Cellular Uptake

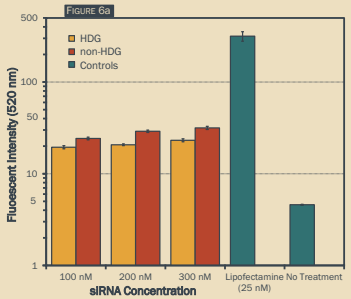
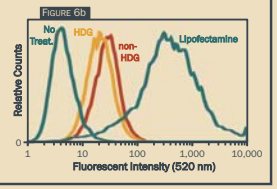
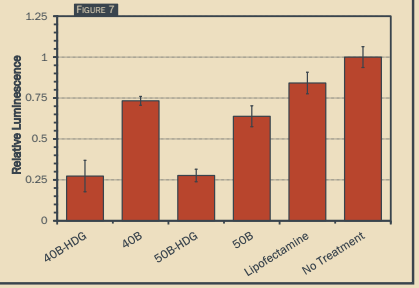


FIGURE 6: Flow cytometry was used to quantify the uptake of siRNA into cells treated with 40B HDG and non-HDG siNP's for 6 hrs. **Our HDG system shows comparable cellular uptake across multiple treatment dosages** to the established non-HDG system.



Gene Knockdown

FIGURE 7: Luciferase expressing cancer cells were treated for 12 hours with siNPs loaded with siRNA shown by previous experiments to inhibit luciferase expression. Both **40B and 50B HDG siNPs efficiently silenced ~75% of gene expression**, more than non-HDG siNPs and a standard transfection agent (Lipofectamine).



Conclusions and Future Work

HDG siNPs show enhanced siRNA release by charge reversal, and have proven to be a novel, effective platform for improved gene silencing by increasing intracellular bioavailability. The polymer chemistry of our NPs has been optimized for intravenous treatment[3]; since we have seen efficient delivery and release in cancer cells with HDG-siNPs, they prove to be an excellent candidate for a therapeutic against metastatic cancers.

Future work will asses cytotoxicity of HDG siNPs, and study the efficacy and bioactivity of siNPs delivered *in-vivo*.

References & Acknowledgements

[1] Anderson DG et. al., Nature Reviews, 2009, 10.1038/nrd2742

[2] Monteiro JT et. al., Biomacromolecules, 2011, 12 (10), pp 3540–3548

[3] Nelson CE, Kintzing JR et al. *In Review*

• Skylar Haws, Ann Hannah & the Biomaterials research group at Vanderbilt University
• Rafael Wiodarski, design reference

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