# Alternative miRNA design for therapeutic RNAi applications

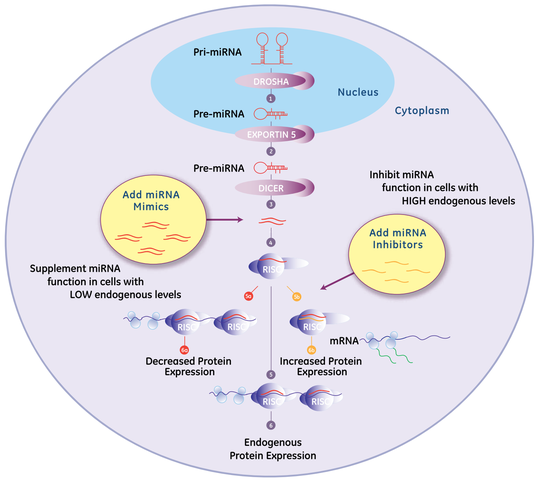
Anja van Brabant Smith, Barb Robertson, Annaleen Vermeulen, Christina Yamada, Angela Reynolds, Anastasia Khvorova, Devin Leake
Dharmacon, 2650 Crescent Drive, Suite #100, Lafayette, CO 80026, USA

## Introduction

The utility of RNA interference in therapeutic applications depends on effective delivery of highly potent molecules. While therapeutic applications of RNAi have historically been focused on introduction of siRNA molecules, microRNAs (miRNAs) have emerged as another important arena for therapeutics. miRNAs regulate gene expression through both translational attenuation and message RNA cleavage and have been shown to be important in many biologies including development, differentiation, and disease. Just as the performance of an siRNA molecule *in vivo* is heavily dependent upon its design, the design of miRNA inhibitors and miRNA mimics must be optimized for *in vivo* applications. Here we will discuss design considerations for the stability and potency of miRNA mimic molecules. We show that stabilized miRNA mimic molecules lose functionality compared to our standard miRNA mimic molecules due, in part, to the activity of the stabilized passenger strand acting as a miRNA inhibitor. We will discuss how mismatches affect the activity of the stabilized miRNA mimics, perhaps by generating a passenger strand that is less functional as an inhibitor molecule.

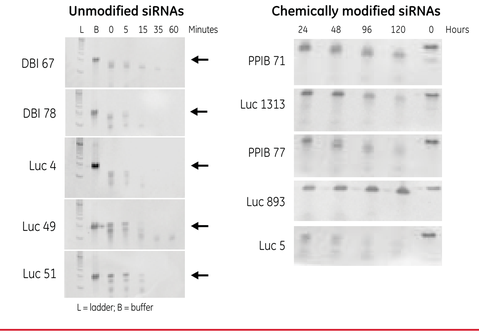
## Modulation of miRNA biology using miRNA mimics and inhibitors

miRNA activity can be modulated *in vivo* by introduction of miRNA mimics or inhibitors to supplement or inhibit, respectively, miRNA function. Considerations for effective therapeutic applications of miRNA mimics and inhibitors include stability, functionality, and delivery of the miRNA mimic and inhibitor molecules. Here we will focus on design considerations for stability and potency of miRNA mimic molecules.



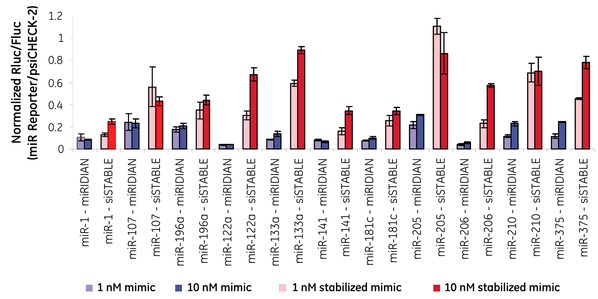

## Nuclease resistance of modified siRNAs

Both siRNAs and miRNAs are susceptible to nuclease degradation *in vivo*; thus, many researchers choose to modify the RNA molecule to generate a more nuclease resistant molecule. Here we show that unmodified siRNAs have a half-life of 1-10 minutes in 90% human serum while modified siRNAs have a half-life of approximately 85 hours in 90% human serum. Therefore, we incorporated the same modification pattern into our miRNA mimic design and tested functionality.



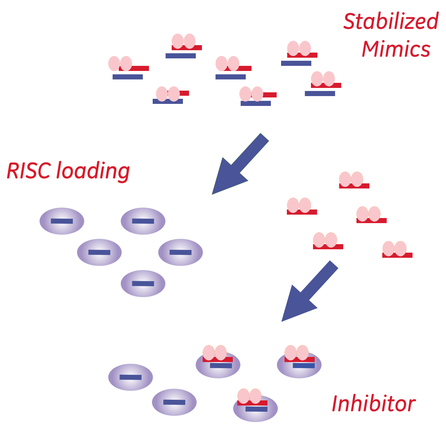

## Functionality of stabilized miRNA mimics

Stabilized miRNA mimics were designed and tested for functionality compared to our standard Dharmacon™ miRIDIAN™ miRNA mimics using a dual-luciferase reporter plasmid assay. As shown here, the stabilized miRNA mimics are less functional than the miRIDIAN miRNA mimics. In some cases, a reverse in dose response was observed for the stabilized mimics; that is, a decrease in potency was seen at the higher concentration of stabilized mimic.

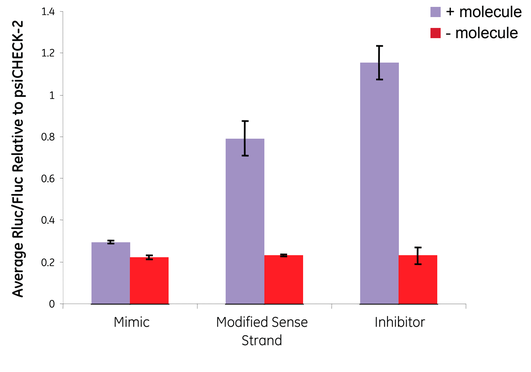



## Effect of modified sense strand on functionality of stabilized miRNA mimics

A model for how the sense strand of a stabilized miRNA mimic can act as an inhibitor for the same miRNA. This model can explain the decrease in functionality observed at higher concentrations of stabilized miRNA mimic.

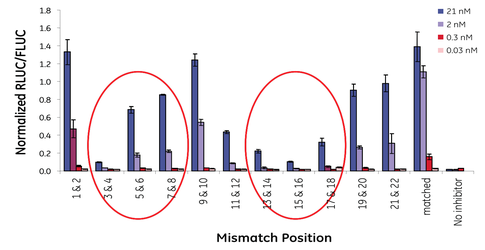



To test the functionality of the sense strand of a stabilized miRNA mimic, molecules designed against miR-122 were assayed using a dual-luciferase reporter assay in Huh-7 cells. The sense strand of the stabilized miRNA mimic decreases the endogenous activity of miR-122, and does appear to be behaving as a miRNA inhibitor molecule. Its activity, however, is not as potent as a designed inhibitor molecule. In order to reduce this effect and design a potent stabilized miRNA mimic molecule, the sense strand of the stabilized miRNA mimic should be designed so that it is a poor miRNA inhibitor.



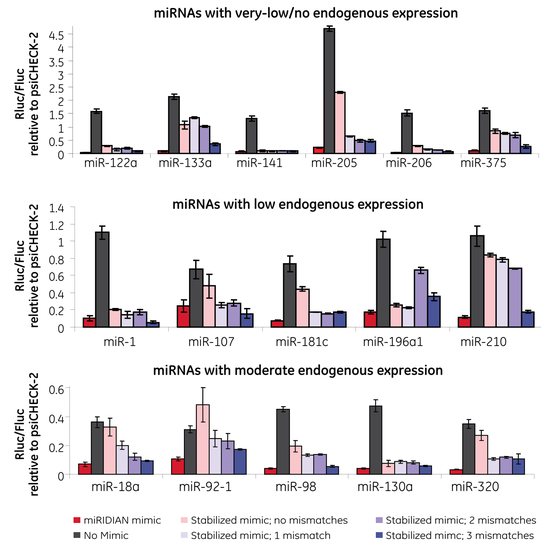

## Effect of mismatches on miRNA inhibitor functionality

miRNA inhibitors with various mismatches were designed against miR-21 and tested for functionality using a dual-luciferase reporter assay in HeLa cells. Each inhibitor contains two adjacent mismatches at the positions indicated when paired to the mature miRNA. Mismatches at the two indicated regions of the molecule significantly reduce miRNA inhibitor function; incorporation of these mismatches should improve functionality of stabilized miRNA mimics either by decreasing functionality of the sense strand as a miRNA inhibitor, or by affecting the thermodynamic properties of the stabilized miRNA mimic.




## Incorporation of mismatches in design of stabilized miRNA mimics

Incorporation of mismatches can improve the functionality of stabilized miRNA mimics for miRNAs with various levels of endogenous expression.




## Conclusions

- Introduction of chemical modifications that result in nuclease-resistant siRNA molecules into the design of stabilized miRNA mimics results in a decrease in functionality.
- Stabilized miRNA mimics can lose functionality due to the sense strand acting as a miRNA inhibitor.
- Mismatches in the design of stabilized miRNA mimics can restore functionality, presumably by making the sense strand a less functional inhibitor molecule.

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**If you have any questions directly related to this document, contact our experts:**
**UK +44 (0) 1223 976 000**
**USA +1 800 235 9880, +1 303 604 9499**
**Website horizondiscovery.com/contact-us**