Application of ADAR-mediated RNA editing to modulate protein-protein interactions

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Summary

- With PRISM™, Wave can generate stereopure AIMers (A-to-I editing oligonucleotides) that promote RNA editing with endogenous ADAR (adenosine deaminase acting on RNA) enzymes in cellular and animal models.[1]
- We have previously demonstrated that AIMers incorporating stereopure design as well as PN backbone chemistry have overall higher editing efficiencies compared to stereorandom AIMers lacking PN.[1]
- We developed AIMers designed to disrupt the NRF2-KEAP1 protein-protein interaction interface and applied them *in vitro*, showing that we can elicit robust RNA editing that corresponds with NRF2-dependent gene activation, which is consistent with a disruption of the interaction interface.
- We developed GalNAc-conjugated AIMers that target human NRF2 and show that they promote NRF2 editing and NRF2-dependent gene expression in primary human hepatocytes.
- To evaluate GalNAc-AIMers in mice, we first tested the mouse AIMers in primary mouse hepatocytes to confirm that they direct RNA editing and gene activation. In mice, these GalNAc-AIMers led to ~50% editing of Nrf2 mRNA in the liver and increased Nrf2-dependent gene expression as evaluated by RT-PCR and RNA-seq.
- Together, these data provide preclinical proof of concept that AIMers can be applied to modulate protein-protein interactions *in vivo*.

Introduction

- We apply PRISM™, our discovery and drug development platform, to generate stereopure AIMers with controlled sequence, chemistry, and stereochemistry **(Figure 1A)**.[1,2]
- Wave has developed chemically modified oligonucleotides, called AIMers, which facilitate RNA base editing by recruiting endogenous ADAR enzymes **(Figure 1B)**.[3]
- Here, we apply our PRISM platform to generate AIMers designed to modulate protein-protein interactions using the Nrf2-Keap1 system.

Figure 1. Introduction to AIMers, PRISM, and PN chemistry

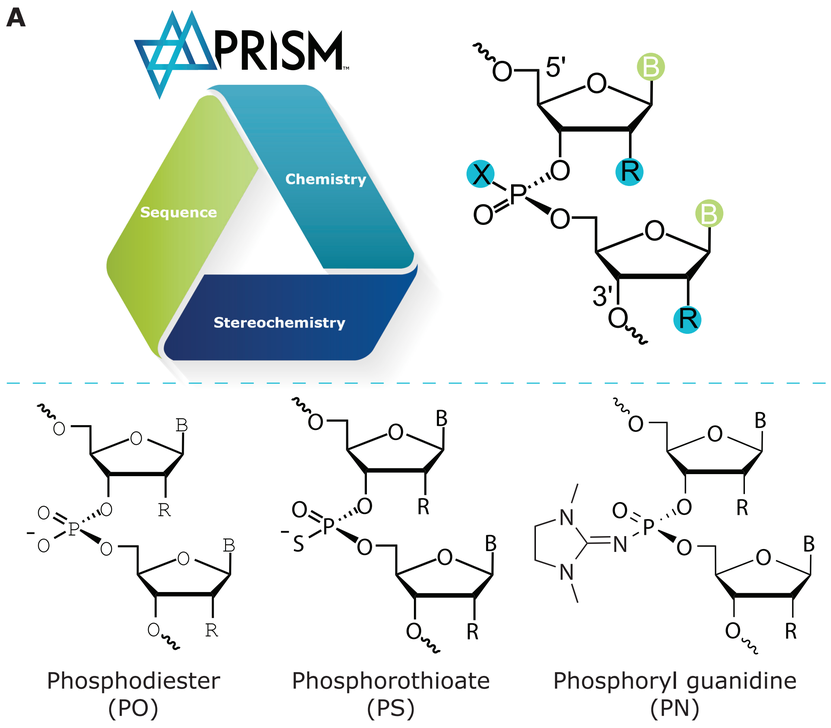
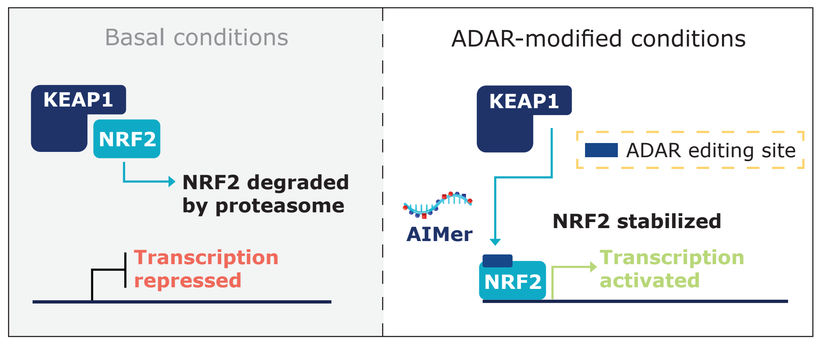
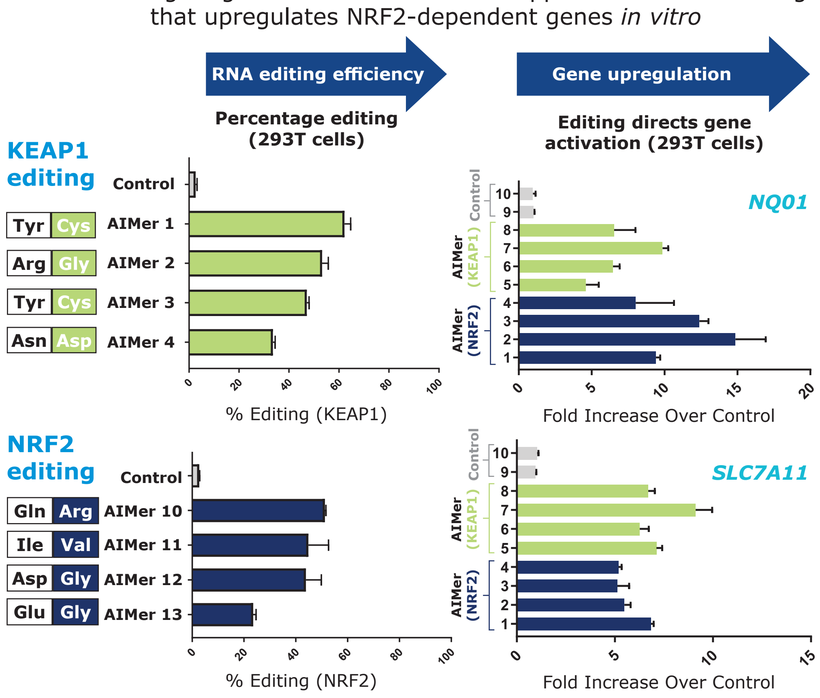


Figure 2. Applying AIMers to modify protein-protein interactions *in vitro*



B

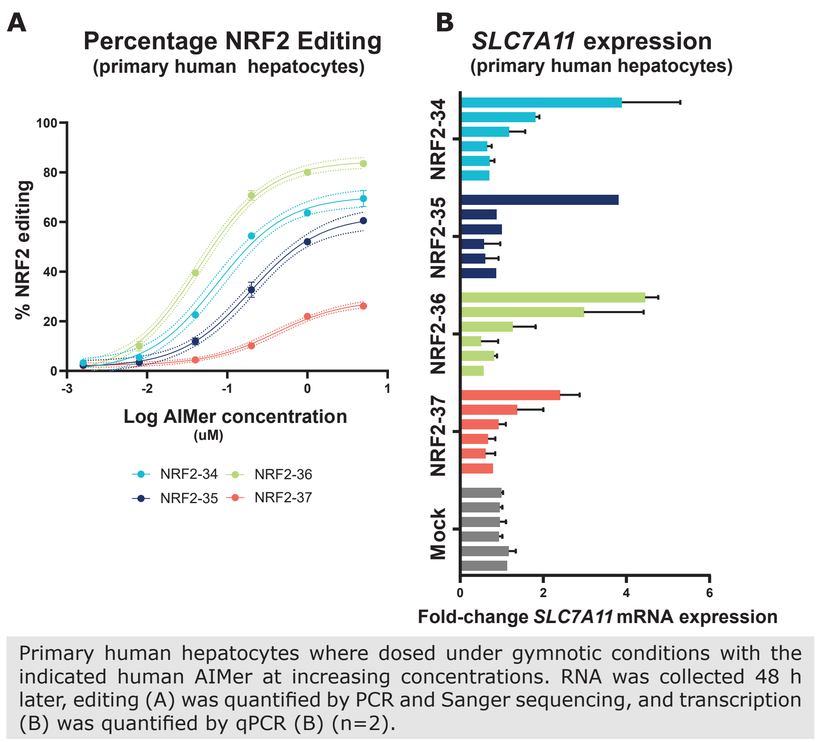
AIMers targeting NRF2-KEAP1 interface support RNA based editing that upregulates NRF2-dependent genes *in vitro*



(B) 293T cells transfected with 25 nM AIMer and either ADAR p110 or p150 plasmid. RNA was collected 48 h later. Editing was quantified by PCR and Sanger sequencing. Gene expression was quantified by qPCR (n=2). Concentration range 0.0016-5 μM.

- KEAP1 interacts directly with NRF2, leading to its degradation. When this protein-protein interaction is disrupted, NRF2 translocates to the nucleus and activates gene expression **(Figure 2A)**.
- We generated AIMers designed to edit NRF2 or KEAP1 mRNAs leading to amino acid changes predicted to disrupt the NRF2-KEAP1 interface.
- AIMer treatment led to **KEAP1** (green) and **NRF2** (blue) RNA editing *in vitro* in 293T cells **(Figure 2B)**, with the best AIMers supporting >50% editing.
- RNA editing of **KEAP1** and **NRF2** corresponds with increased expression of genes regulated by NRF2 (**Figure 2B)**, indicating that the edits resulted in amino acid changes that disrupted the NRF2-KEAP1 interface.

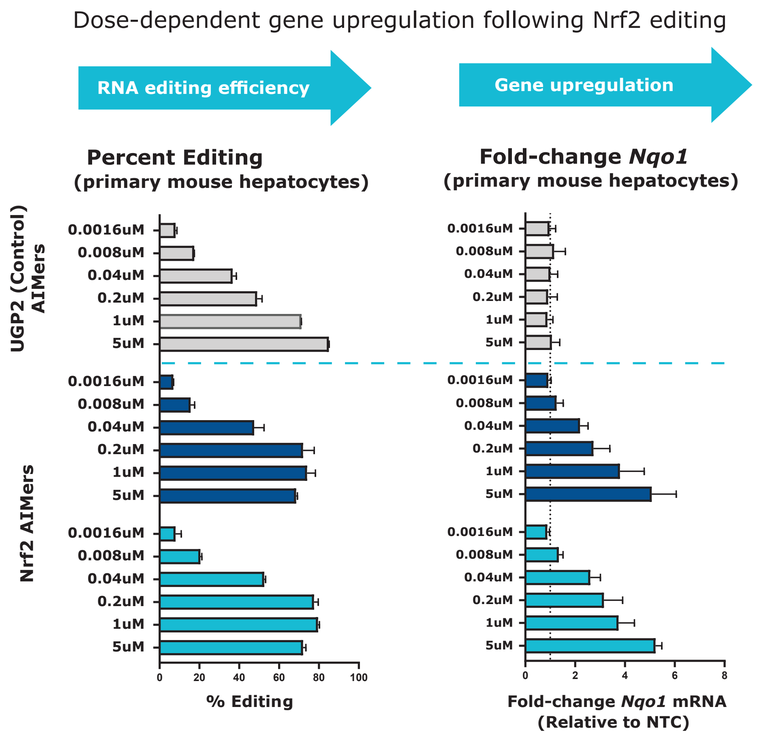
Figure 3. Editing of NRF2 in primary human hepatocytes increases NRF2-dependent gene expression



Primary human hepatocytes where dosed under gymnotic conditions with the indicated human AIMer at increasing concentrations. RNA was collected 48 h later, editing (A) was quantified by PCR and Sanger sequencing, and transcription (B) was quantified by qPCR (B) (n=2).

- We generated GalNAc-conjugated human NRF2-targeting AIMers to evaluate editing in primary human hepatocytes.
- In cultured primary human hepatocytes, multiple AIMers support robust and dose-dependent NRF2 RNA editing **(Figure 3A)**, with the best AIMers editing >50% of the mRNA.
- In the same cells, the treatment led to dose-dependent increases in expression of *SLC7A11* mRNA, a gene downstream of NRF2 **(Figure 3B)**, once again indicating successful disruption of the NRF2-KEAP1 interface in human cells.

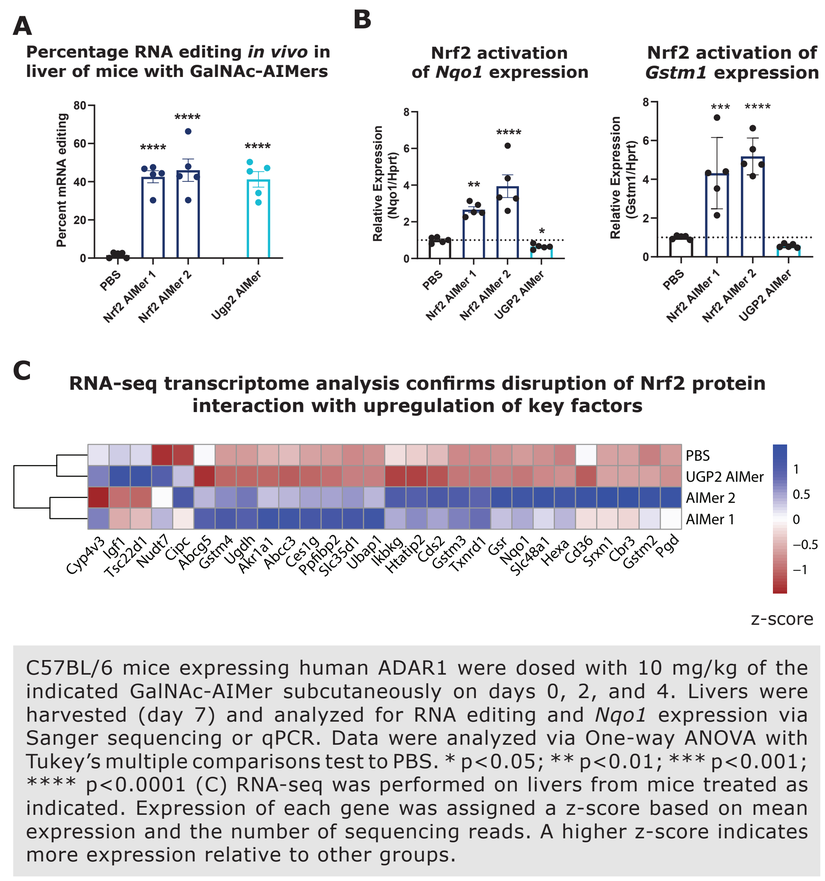
Figure 4. Dose-dependent modulation of protein-protein interactions



As described for Figure 3 only in primary mouse hepatocytes.

- In order to test AIMers *in vivo* in a mouse model, we generated comparable GalNAc-AIMers for evaluating editing in a mouse system. The AIMers are designed to target mouse Nrf2 mRNA, altering amino acids at the Nrf2-Keap1 interface.
- In cultured primary mouse hepatocytes, AIMers supported dose-dependent editing of either mouse Ugp2 (control RNA) or Nrf2 mRNA **(Figure 4)**. In the same cells, treatment with AIMers targeting Ugp2 had no effect on expression of *Nqo1*, a gene downstream, of Nrf2 **(Figure 4)**.
- The AIMers targeting Nrf2 led to dose-dependent increases in *Nqo1* expression **(Figure 4)** consistent with disruption of the protein-protein interaction in mouse cells.

Figure 5. AIMers enable activation of gene pathway *in vivo* with a single edit



C57BL/6 mice expressing human ADAR1 were dosed with 10 mg/kg of the indicated GalNAc-AIMer subcutaneously on days 0, 2, and 4. Livers were harvested (day 7) and analyzed for RNA editing and *Nqo1* expression via Sanger sequencing or qPCR. Data were analyzed via One-way ANOVA with Tukey's multiple comparisons test to PBS. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$ (C) RNA-seq was performed on livers from mice treated as indicated. Expression of each gene was assigned a z-score based on mean expression and the number of sequencing reads. A higher z-score indicates more expression relative to other groups.

- In mouse liver 1-week after treatment onset, GalNAc-AIMers led to ~50% editing of the targeted mRNA (Nrf2 or Ugp2) **(Figure 5A)**.
- At the same time, expression of Nrf2-dependent RNAs *Nqo1* and *Gstm1* increased significantly after GalNAc-Nrf2 AIMer treatment compared with PBS, whereas expression of these same RNAs did not increase after treatment with GalNAc-Ugp2 AIMer **(Figure 5B)**.
- RNA-seq evaluation of liver gene expression 1-week after treatment onset shows that Nrf2-dependent genes generally increased after GalNAc-Nrf2 AIMer treatment, whereas the expression of these same genes did not increase in response to PBS or GalNAc-Ugp2 AIMer treatment **(Figure 5C)**.
- These Nrf2-Keap1 data provide preclinical proof-of-concept that AIMer-mediated RNA base editing can be applied to modulate protein-protein interactions in mice.

References: 1. Monian et al., 2022. Nature Biotechnology 2. Kandasamy et al., 2022. Nuc Acids Res 3. Kandasamy et al., 2022. Nuc Acids Res 4. Woolf et al., 2022. PNAS Vol. 92, pp. 8298-8302, 1995 **Acknowledgments:** The authors are grateful to Amy Donner (Wave Life Sciences) and Nicole Wolf for editorial and graphical support, respectively.

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