ProQR®

AXIOMER® TECHNOLOGY

Therapeutic oligonucleotides for directing site-specific A-to-I editing by endogenous ADAR enzymes

Presenter: Antti Aalto

Forward-looking statements

This presentation contains forward-looking statements that involve substantial risks and uncertainties. All statements, other than statements of historical facts, contained in this presentation, including but not limited to, statements regarding our strategy, future operations, future pre-clinical and clinical trial plans and related timing of trials and results, research and development, future financial position, future revenues, projected costs, prospects, therapeutic potential of our products, plans and objectives of management, are forward-looking statements. The words "aim," "anticipate," "believe," "estimate," "expect," "intend," "may," "plan," "predict," "project," "target," "potential," "will," "would," "could," "should," "continue," and similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words.

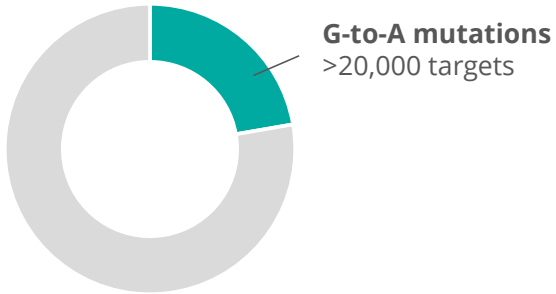
Forward-looking statements represent our management's beliefs and assumptions only as of the date of this presentation. We may not actually achieve the plans, intentions or expectations disclosed in our forward-looking statements, and you should not place undue reliance on our forward-looking statements. Actual results or events could differ materially from the plans, intentions and expectations disclosed in the forward-looking statements we make. The forward-looking statements contained in this presentation reflect our current views with respect to future events, and we assume no obligation to update any forward-looking statements except as required by applicable law. These forward-looking statements are subject to a number of risks, uncertainties and assumptions, including those that may be described in greater detail in the annual report filed on Form 20-F for the year ended December 31, 2017 that we have filed with the U.S. Securities and Exchange Commission (the "SEC") and any subsequent filings we have made with the SEC. We have included important factors in the cautionary statements included in that annual report, particularly in the Risk Factors section, and subsequent filings with the SEC that we believe could cause actual results or events to differ materially from the forward-looking statements that we make.

Key messages

- Axiomer® technology can introduce precise A-to-I modifications in endogenous RNA transcripts
- This RNA editing enables *e.g.* the correction of G-to-A mutations, since inosines are interpreted as guanosines
- RNA editing is achieved by Axiomer® Editing Oligonucleotides (EONs) that can direct site-specific deamination by endogenous ADARs (Adenosine Deaminases Acting on RNA)
- Rational EON design relies both on computational and empirical approaches to achieve high potency and drug-like properties

Therapeutic potential

Unmet need for genetic diseases caused by G-to-A point mutations



Human disease-causing substitution mutations

ProQR®

Axiomer®

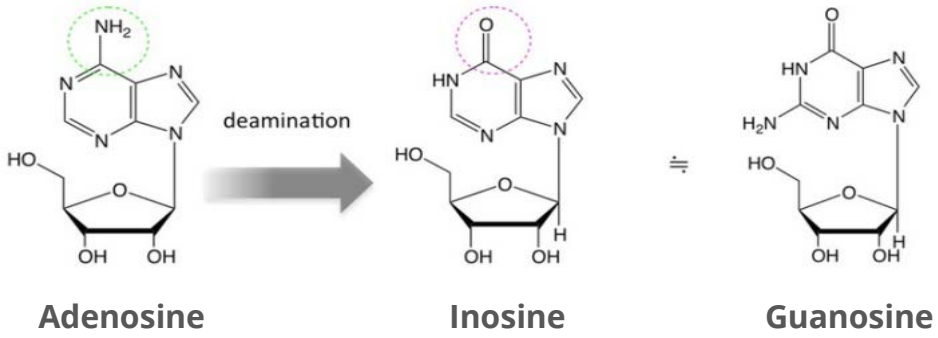
A-to-I editing

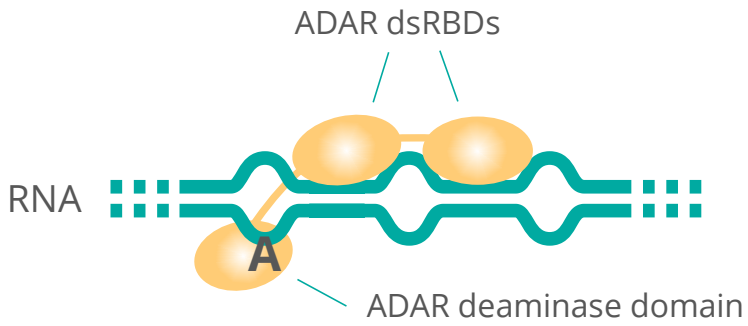
Original sequence restored

- Most G-to-A mutations require correction of the specific nucleotide for functional restoration
- A-to-I results in a functional A-to-G change, providing means for correcting G-to-A mutations

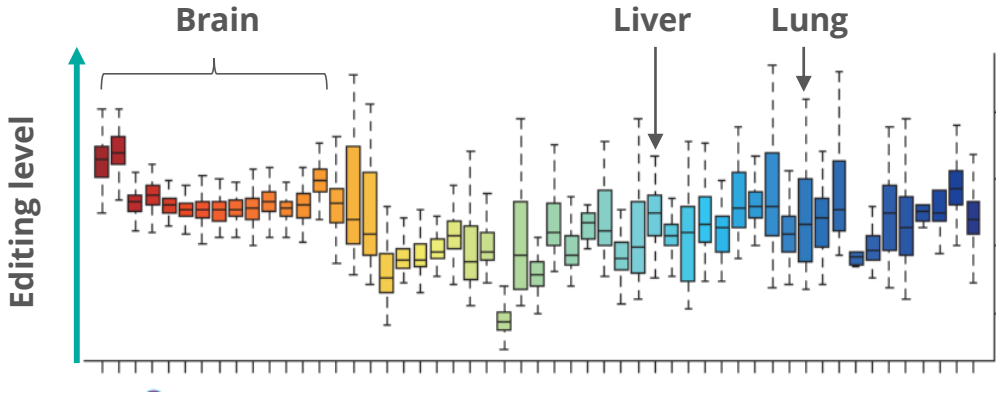
A-to-I editing: Therapeutic opportunity

The most prevalent editing event in human tissues





- Human catalytic ADARs: ADAR1 and ADAR2
- A-to-I editing occurs in both nucleus and cytoplasm

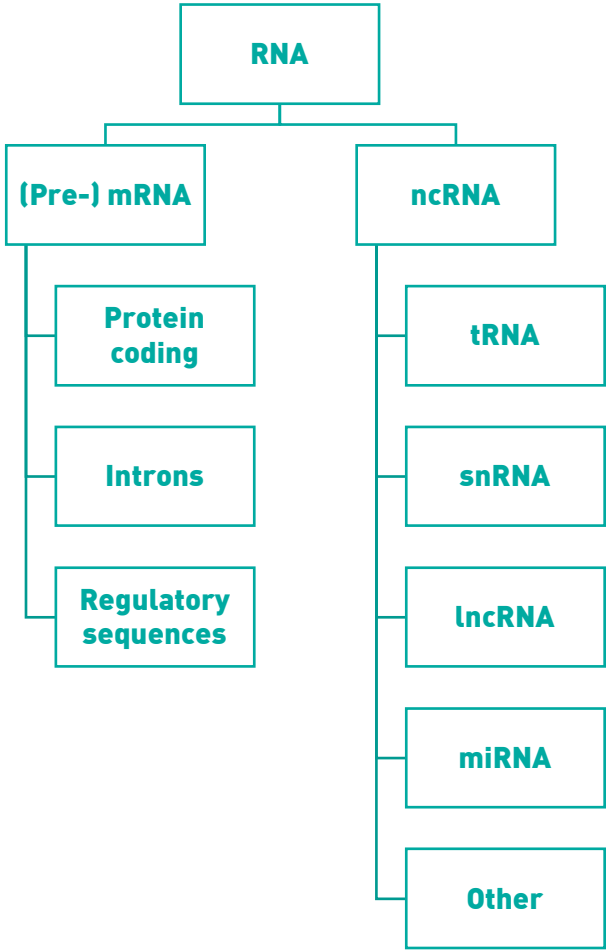


Adapted from Tan et al. 2017 Nature 550:249-254

- No sequence dependence
- 4 million ADAR sites in the human transcriptome
- Extent of editing similar in most human tissues, making therapeutic editing feasible in all disease areas

Axiomer® is widely applicable

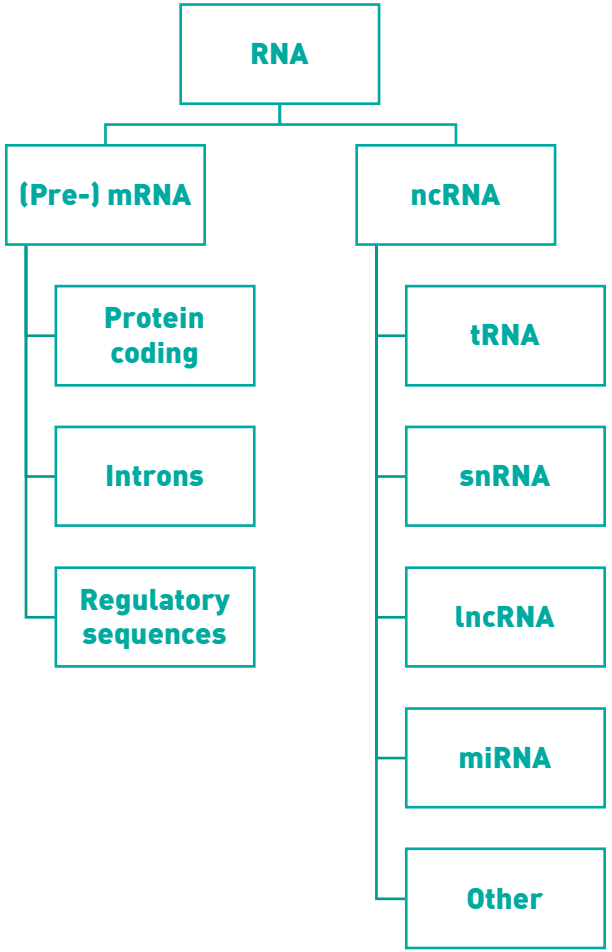
Examples of different target RNAs



Effect on RNA	Effect of therapy
Correct nonsense mutations	Restore translation
Correct missense mutations	Restore protein
Correct splice sites	Restore splicing of mRNA
Modify codon	Alter protein function
Modify protein binding	Alter expression
Modify 2° RNA structure	Alter expression
Alter regulatory sequence	Modify target RNA/DNA/ protein binding

Axiomer® is widely applicable

Stop codons as PoC for a wide class of disease indications



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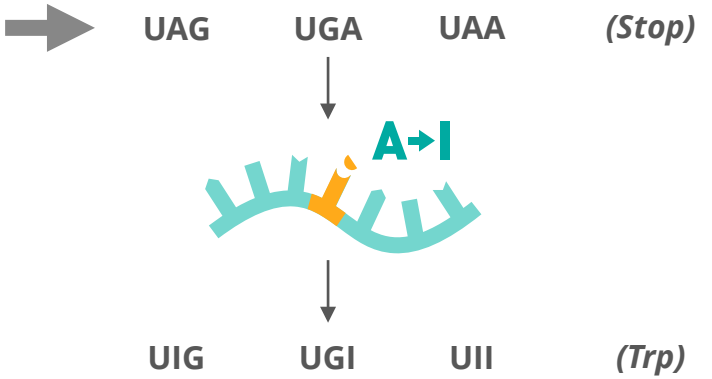


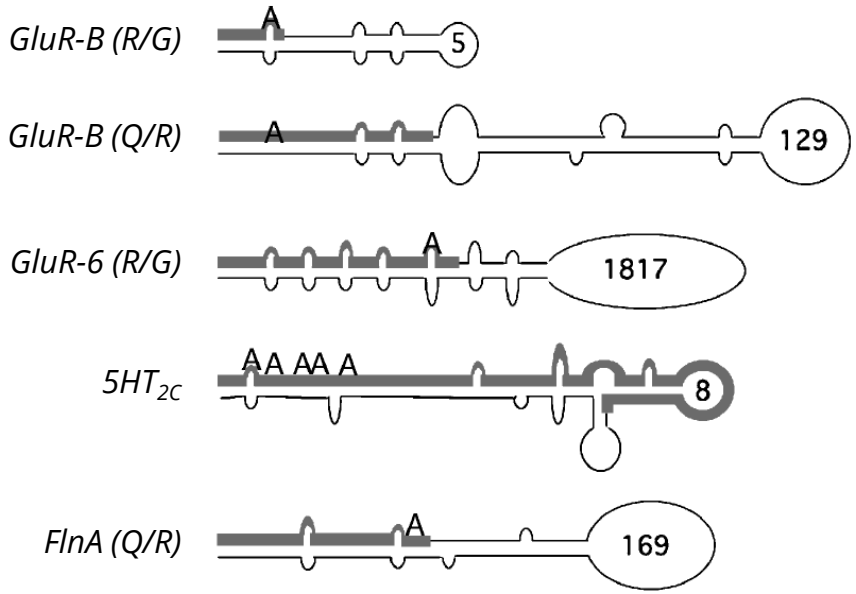
- Class of mutations resulting in complete loss of function
- First proof of concept for the Axiomer® approach

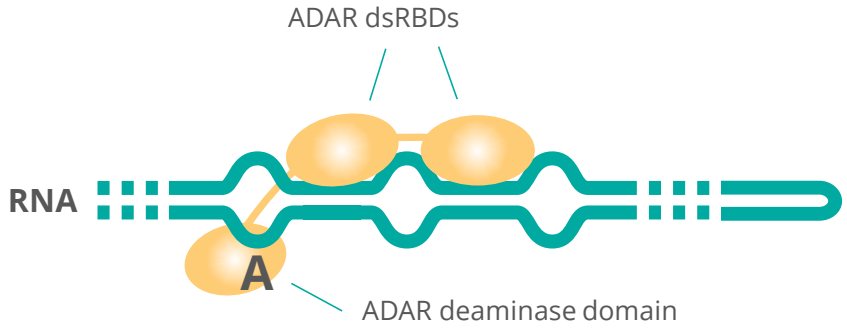
Targeted A-to-I editing

ADARs deaminate adenosine in dsRNA

Endogenous editing on natural substrates

ADAR targets:
Adenosines in dsRNAs with incomplete helices





Wahlstedt & Öhman 2011, WIREs RNA

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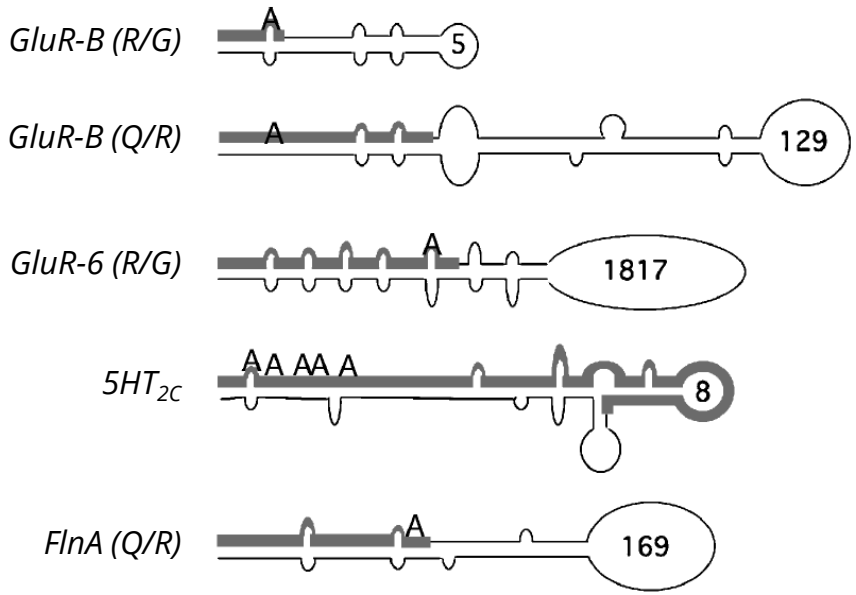
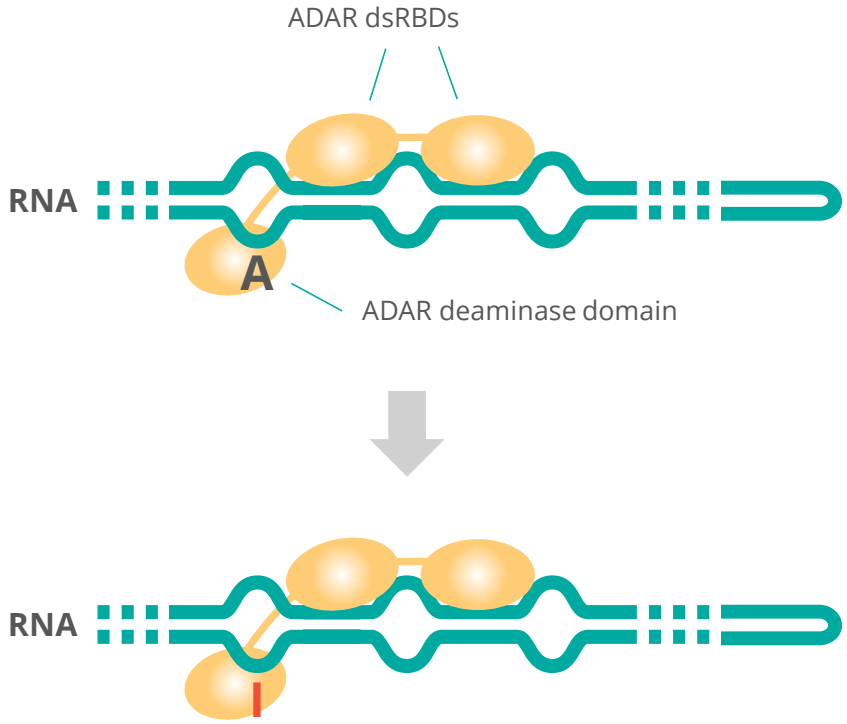
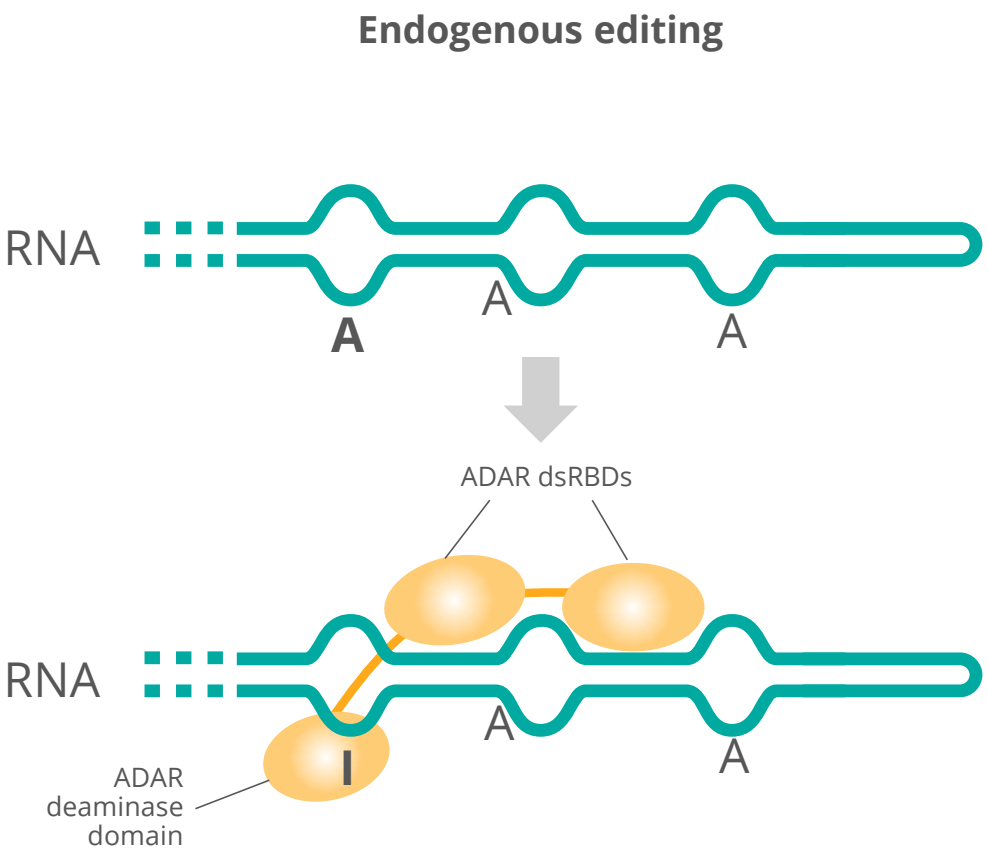




Wahlstedt & Öhman 2011, WIREs RNA

EONs designed for targeted editing (1)

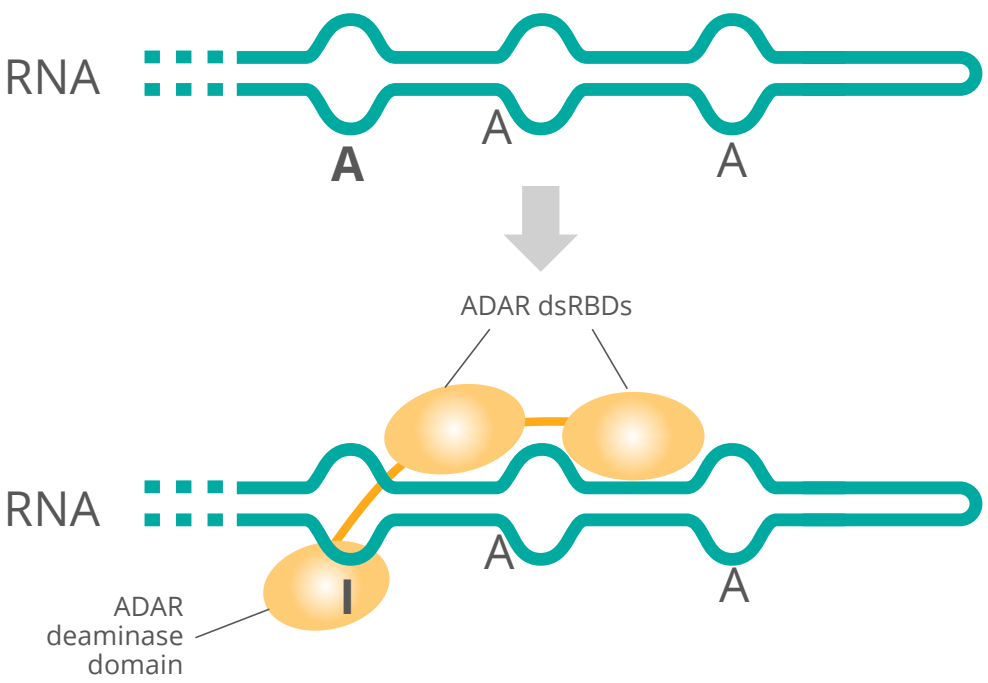
Mimicking natural RNA editing



EONs designed for targeted editing (2)

EON and the target RNA form a double helix

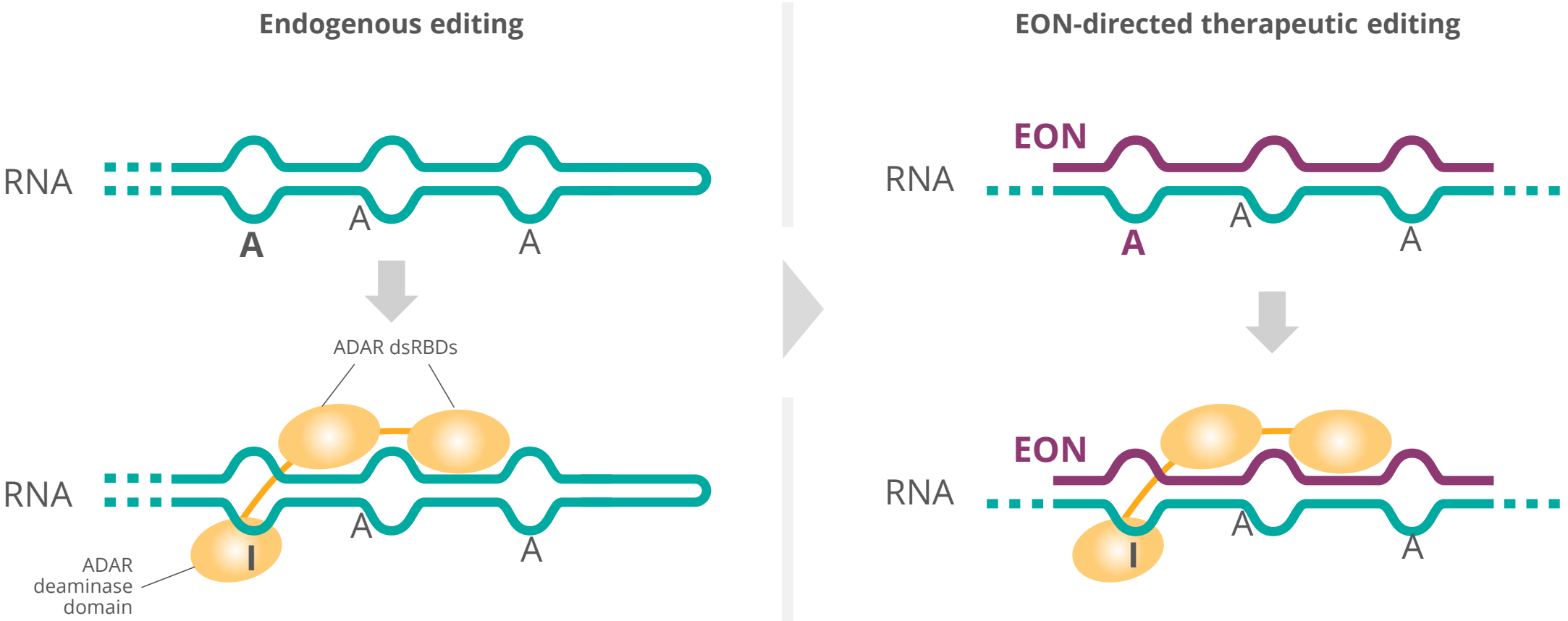
Endogenous editing



EON-directed therapeutic editing

EONs designed for targeted editing (3)

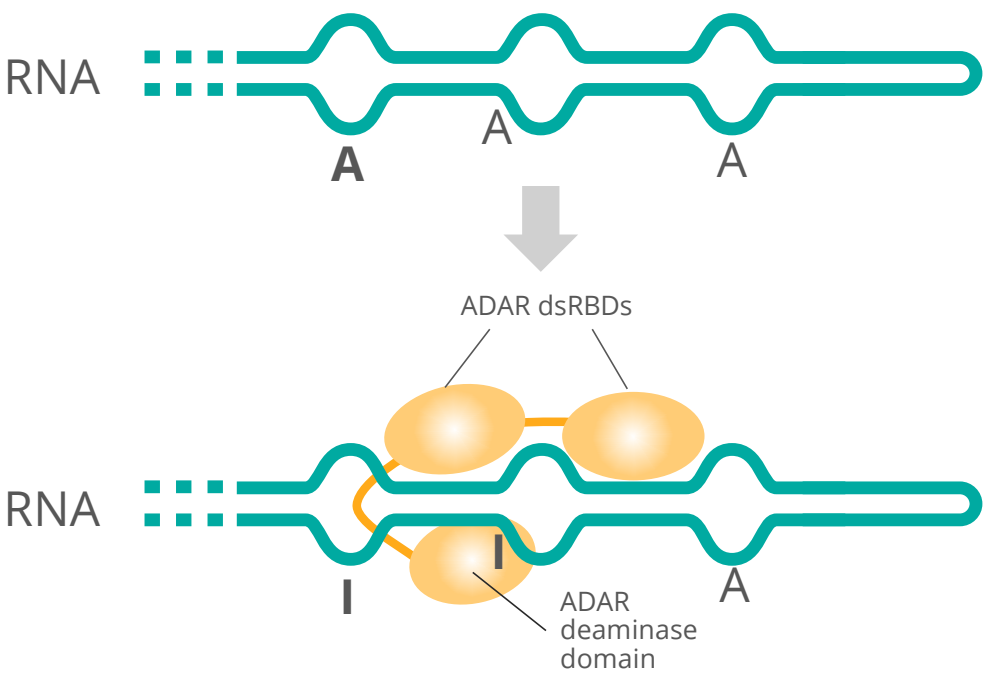
ADAR deaminates the target A in EON-target RNA helix



EONs designed for targeted editing (4)

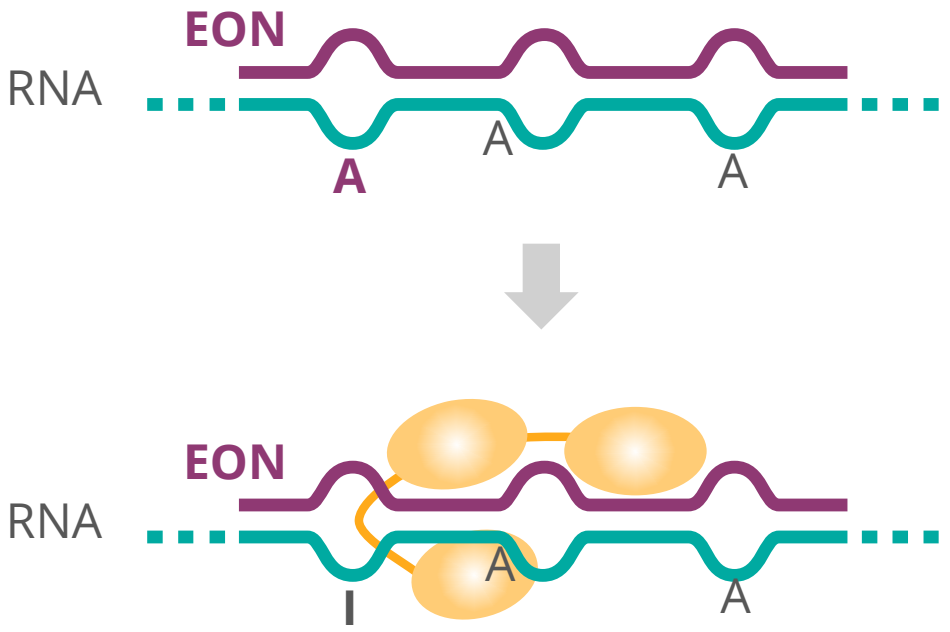
Advantage over RNA guides: Specificity

Endogenous editing



Editing site with RNA guides is flexible

EON-directed therapeutic editing



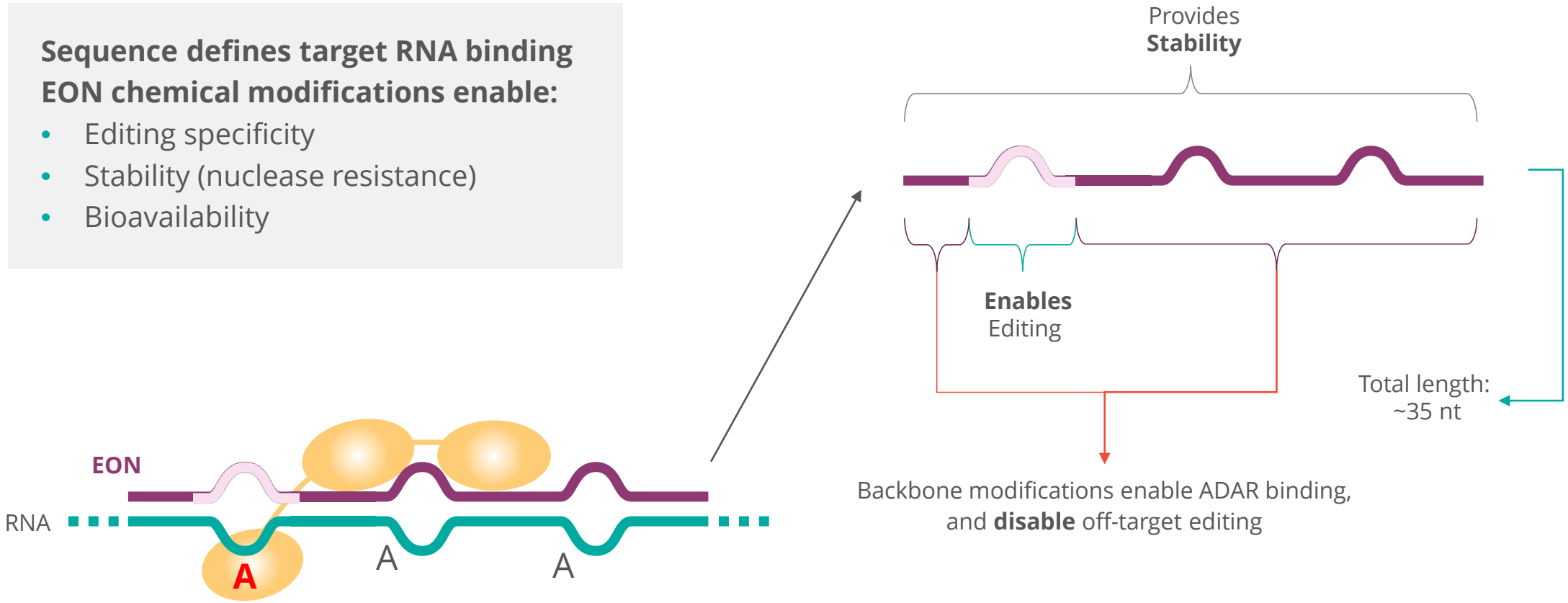
Editing site with EONs is precise:
No off-target editing even if deaminase domain shifts

EONs designed for targeted RNA editing

Functionality defined by sequence and chemistry

Sequence defines target RNA binding
EON chemical modifications enable:

- Editing specificity
- Stability (nuclease resistance)
- Bioavailability



Axiomer® EONs

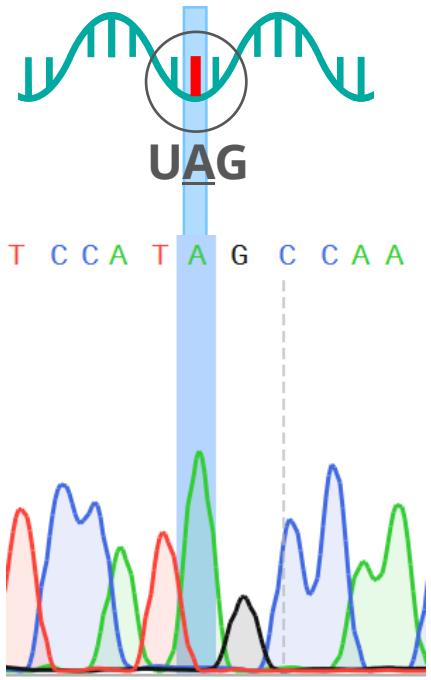
Correction of premature termination codons

EONs can restore ORFs

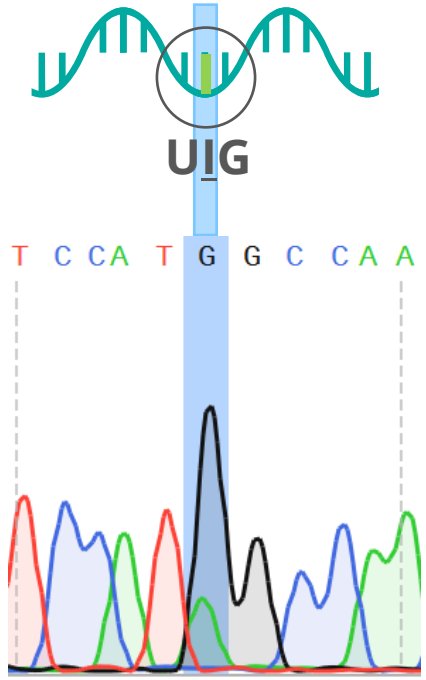
In vitro proof of concept in a GFP reporter

- GFP W57X reporter in Hepa1-6 cells
- ADAR overexpression
- Transfection with 100 nM EON
- Readout by Sanger sequencing of the RT-PCR product

W57X Mutation



Mutation treated with EON



Up to 85% of transcript corrected by editing

Source: www.mps1disease.com

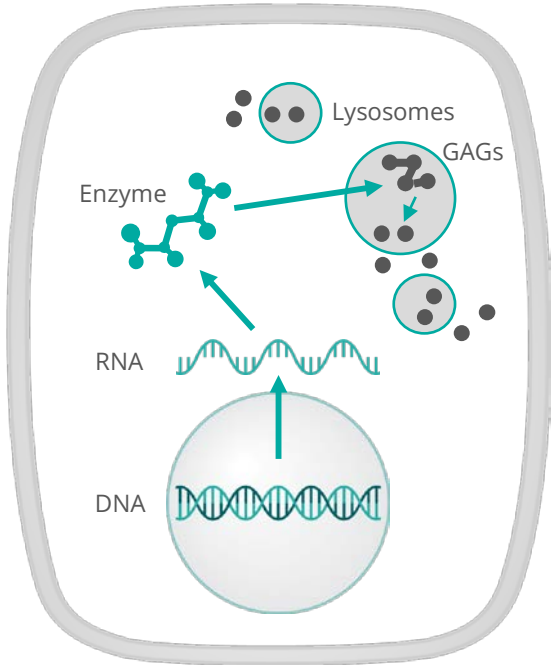
Hurler syndrome

Therapeutically relevant model for targeted RNA editing

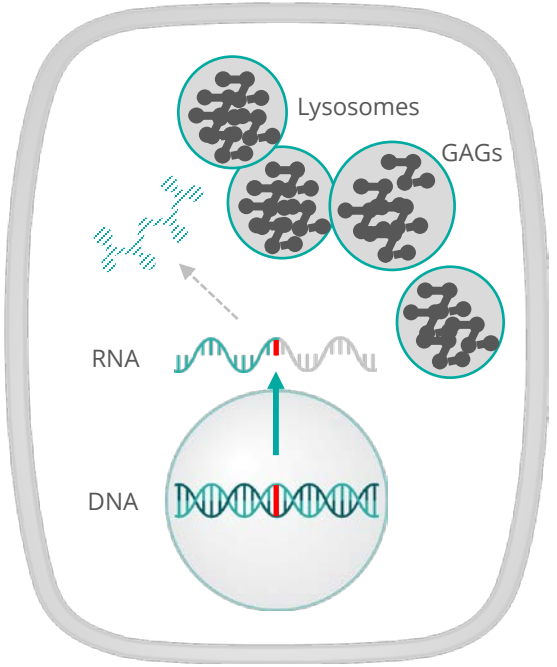
Hurler syndrome

- Mucopolysaccharidosis type I
- *IDUA* W402X mutation most common cause: **UGG -> UAG**
- Deficiency of the lysosomal **iduronidase** enzyme
- Accumulation of glycosaminoglycans (**GAGs**)

WT *IDUA* gene



***IDUA* W402X mutant**

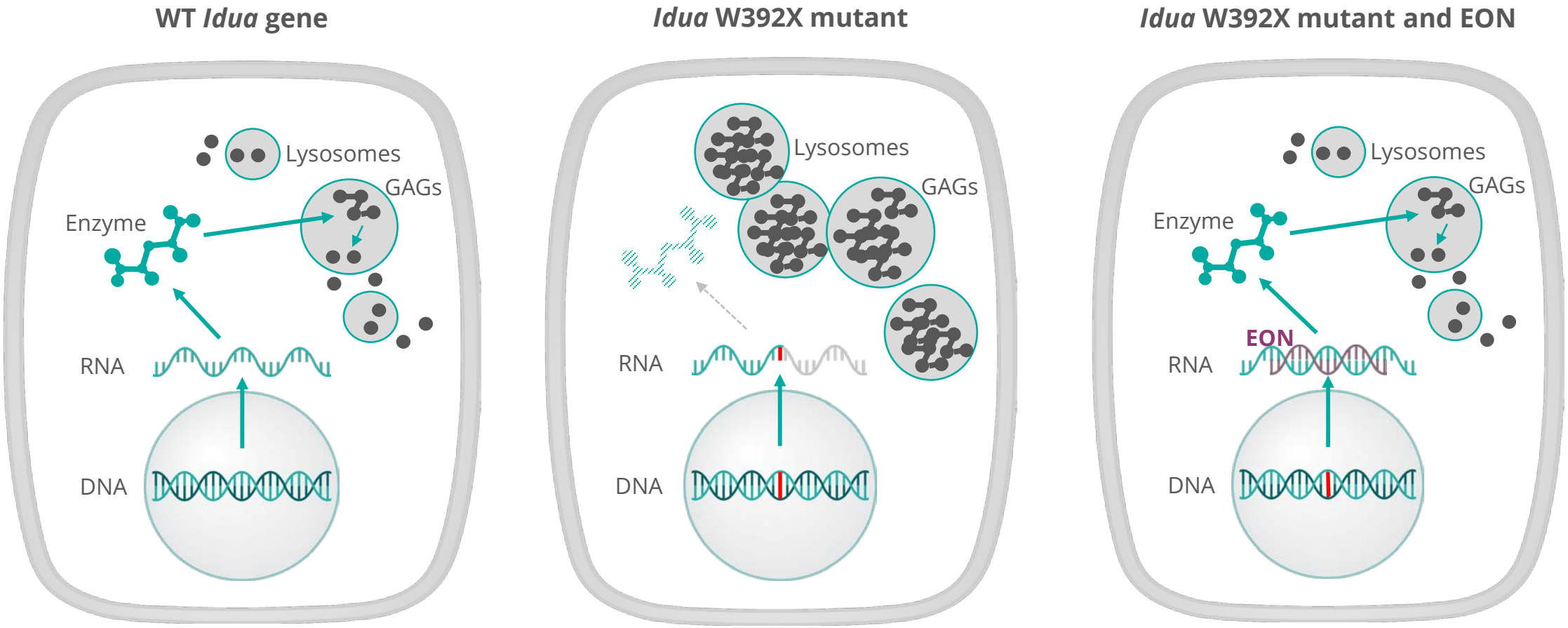


Symptom Presentation
Stiffened Joints
Skeletal Abnormalities
Carpal Tunnel Syndrome
Cardiac (Valvular) Disease
Recurrent Ear, Nose, and Throat Infections
Obstructive Airway Disease/Sleep Apnea
Corneal Clouding
Spinal Cord Compression
Hepatosplenomegaly/Splenomegaly
Inguinal or Umbilical Hernia
Hearing Loss
Cognitive Impairment
Growth Deficiencies
Coarse Facial Features
Communicating Hydrocephalus
Abnormally Shaped Teeth

Source: www.mps1disease.com

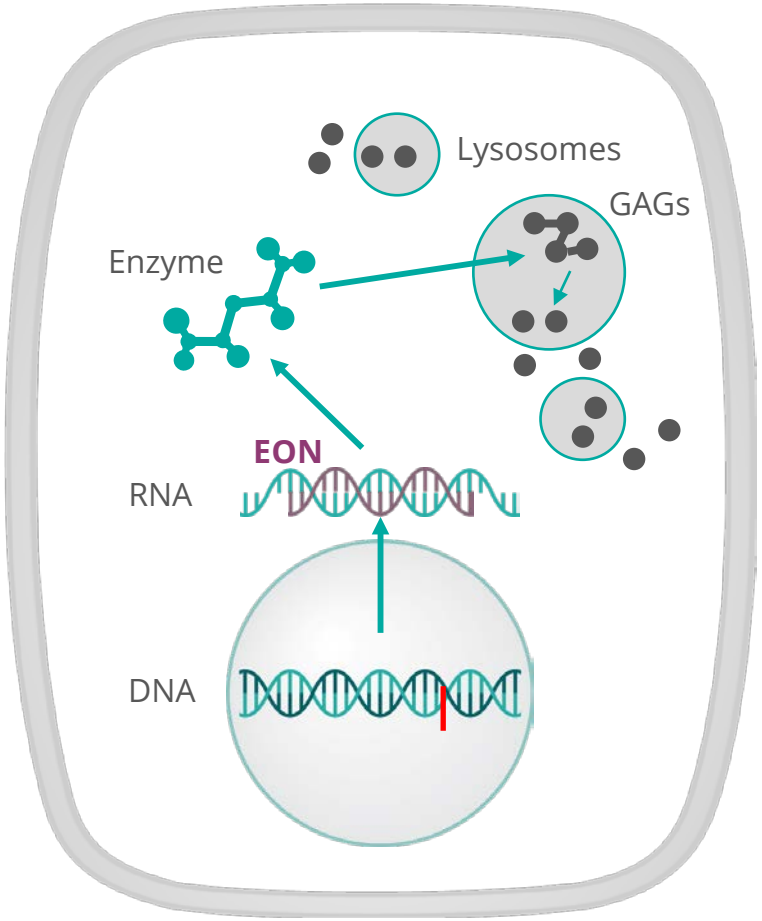
Hurler syndrome – mouse model

Therapeutic approach using Axiomer® EONs

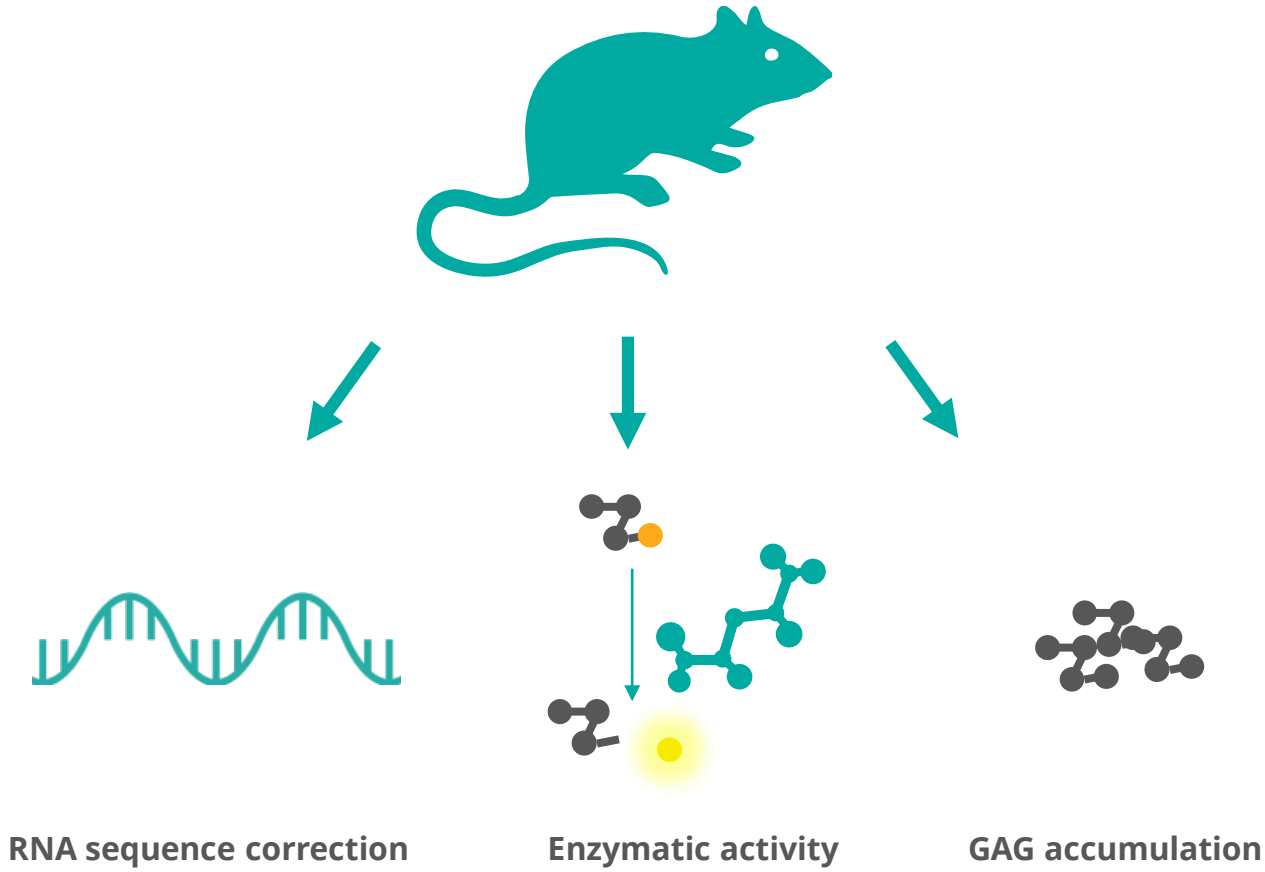


Hurler mouse model for targeted editing

***Idua* W392X mutant and EON**



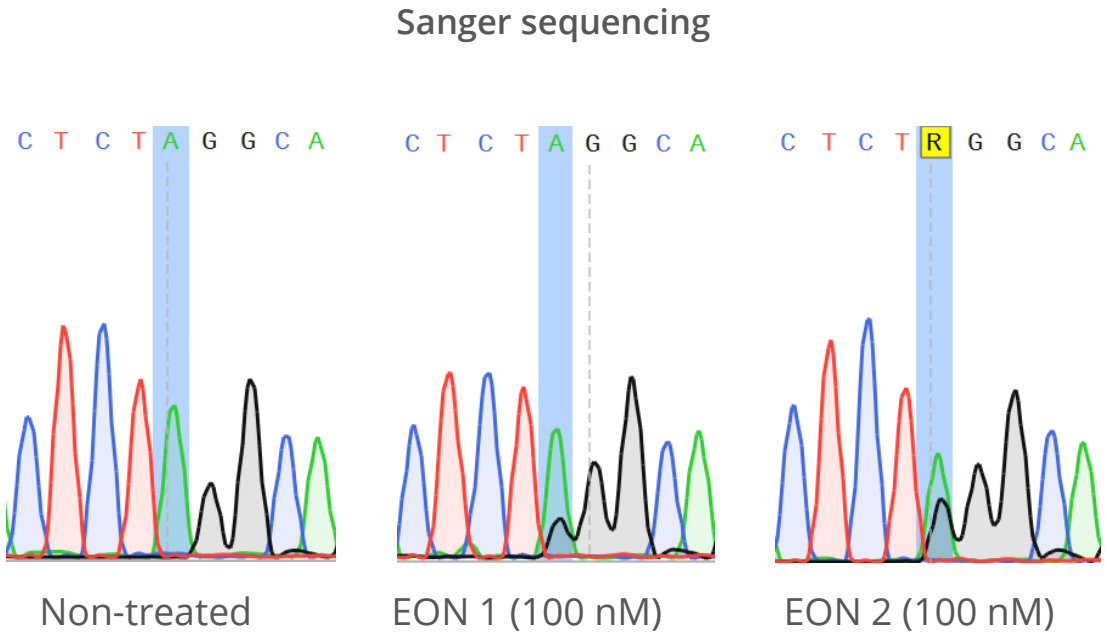
Readouts for restored function

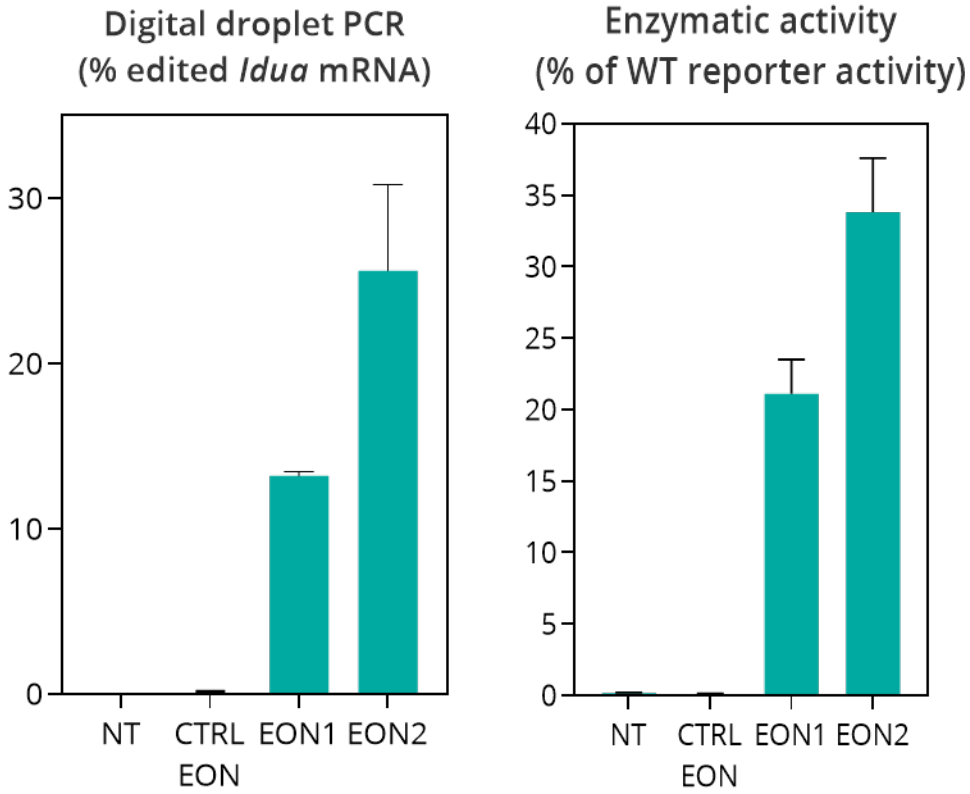


RNA sequence correction | **Enzymatic activity** | **GAG accumulation**

EONs edit *Idua* mRNA *in vitro*

Idua W392X reporter construct in MEF cells with endogenous ADAR



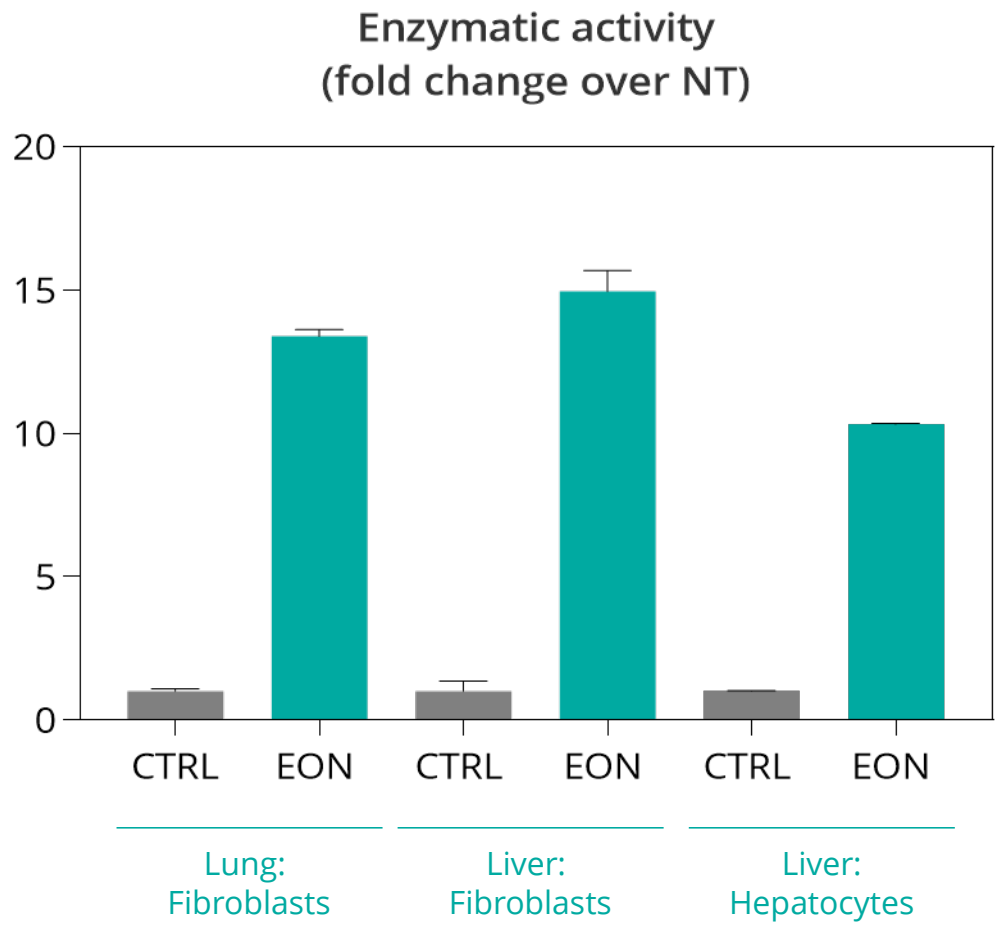


EONs restore iduronidase *in vitro*

Endogenous *Idua* in primary W392X mouse cells

Effect in cells of different origin:

Transfection of 100 nM EON into cells isolated from the W392X mouse

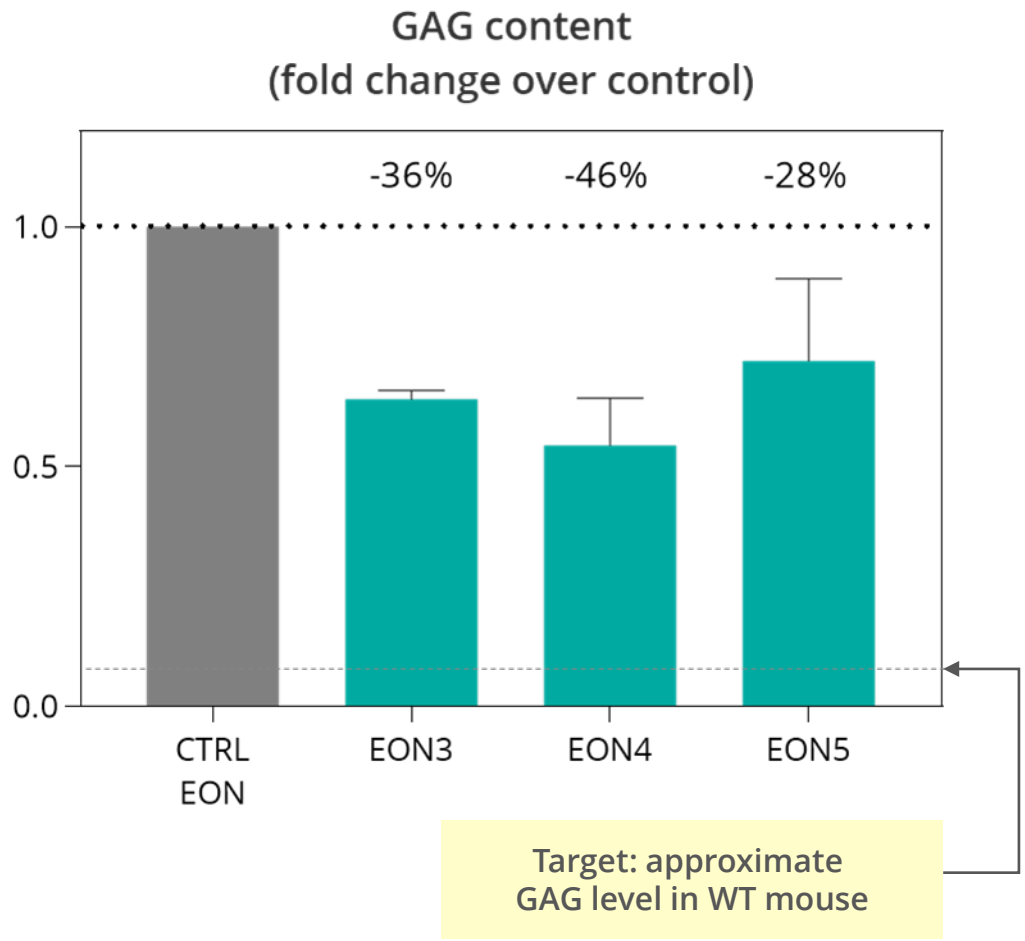


EONs restore iduronidase *in vivo*

Liver EON delivery in the W392X mouse

EON *in vivo* delivery:

- N=2
- 5 mg/kg EON
- IV (Liposomes)
- 4 doses over 8 days



EON design and optimization

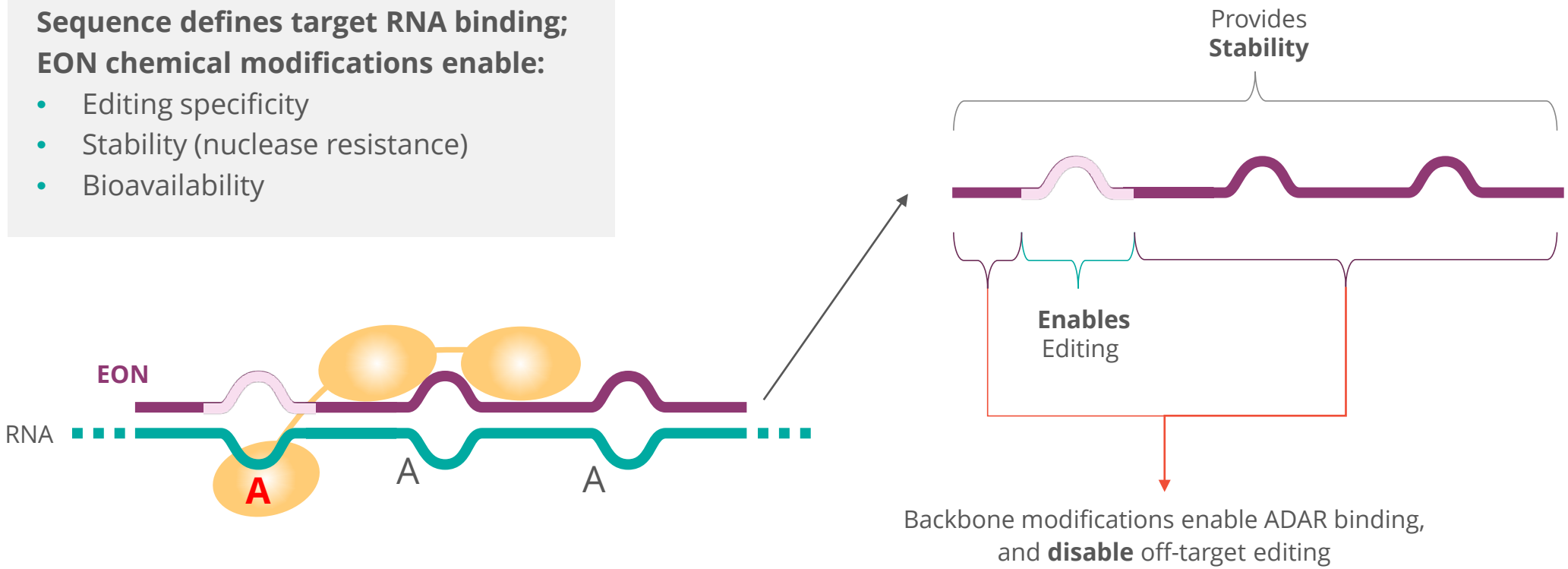
Combining computational and empirical approaches

EONs designed for targeted RNA editing

Functionality defined by sequence and chemistry

Sequence defines target RNA binding;
EON chemical modifications enable:

- Editing specificity
- Stability (nuclease resistance)
- Bioavailability



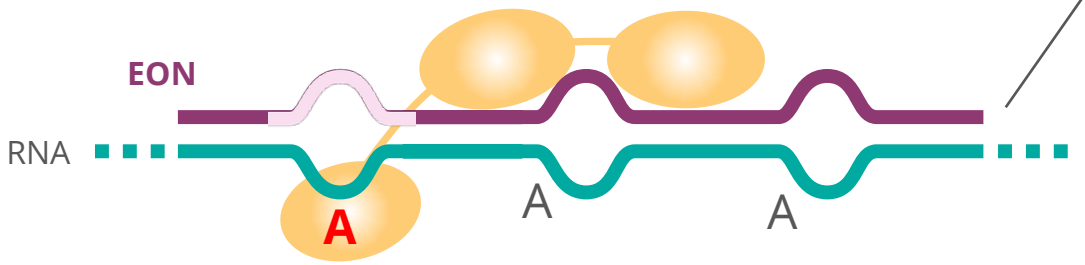
EONS are mixmers of 2'-OME, DNA and PS (and undisclosed modifications)

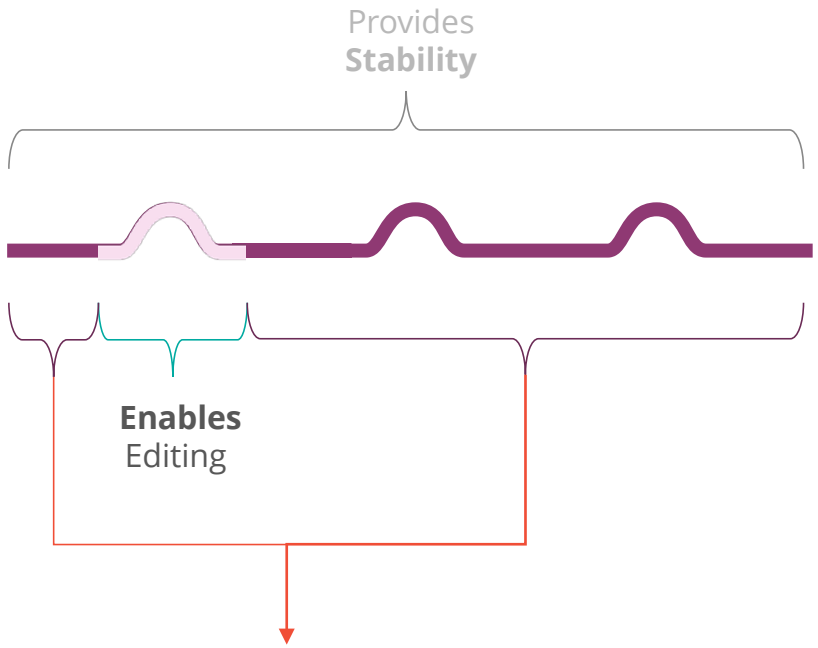
EONs designed for targeted RNA editing

Functionality defined by sequence and chemistry

Sequence defines target RNA binding;
EON chemical modifications enable:

- **Editing specificity (2'-OMe vs. DNA)**
- Stability (nuclease resistance)
- Bioavailability





Backbone modifications enable ADAR binding,
and **disable** off-target editing

Structural basis for specificity

Fit of nucleotide modifications into the catalytic site

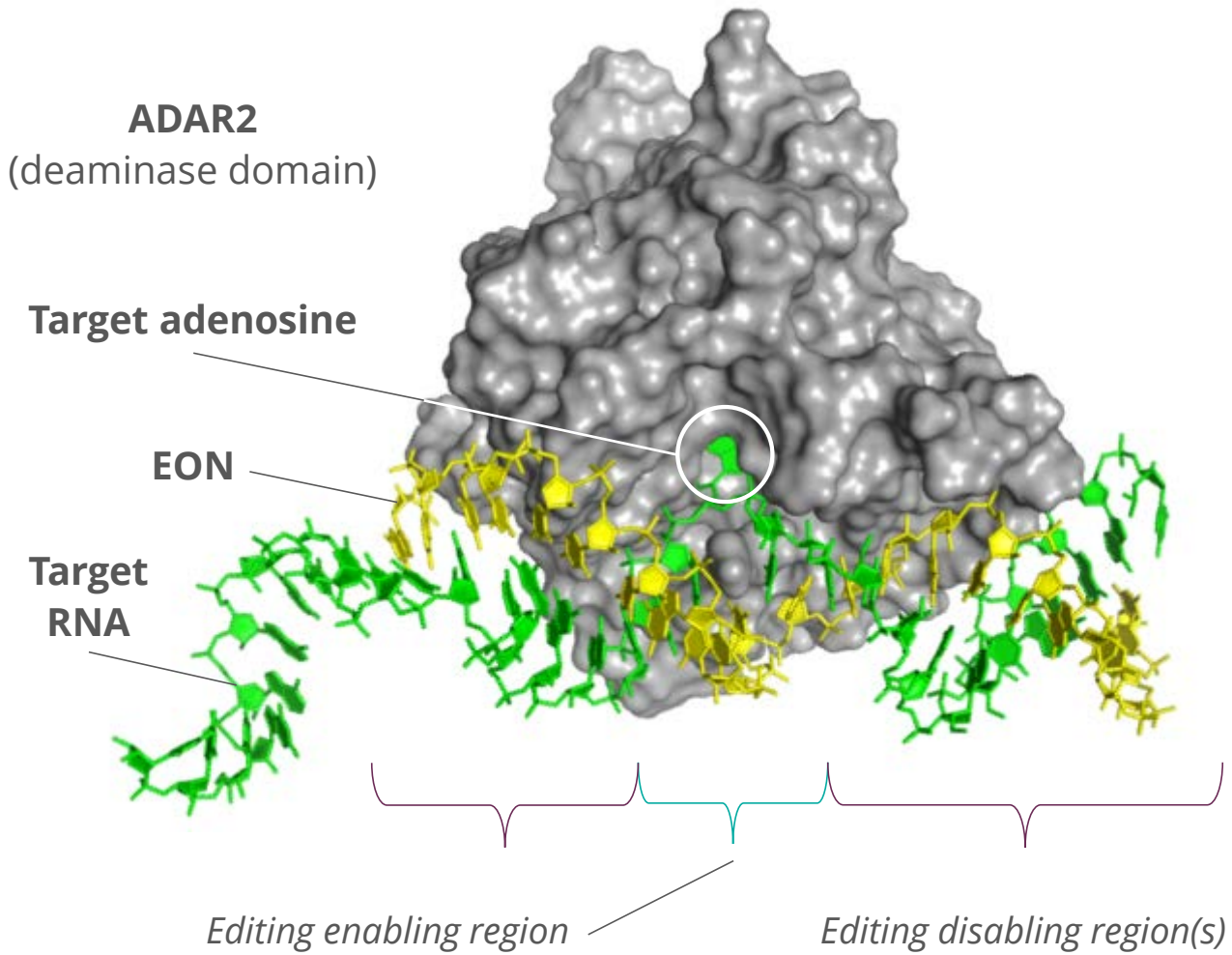
Editing disabling region

- **2’-OMe** compatible with ADAR binding

Editing enabling region

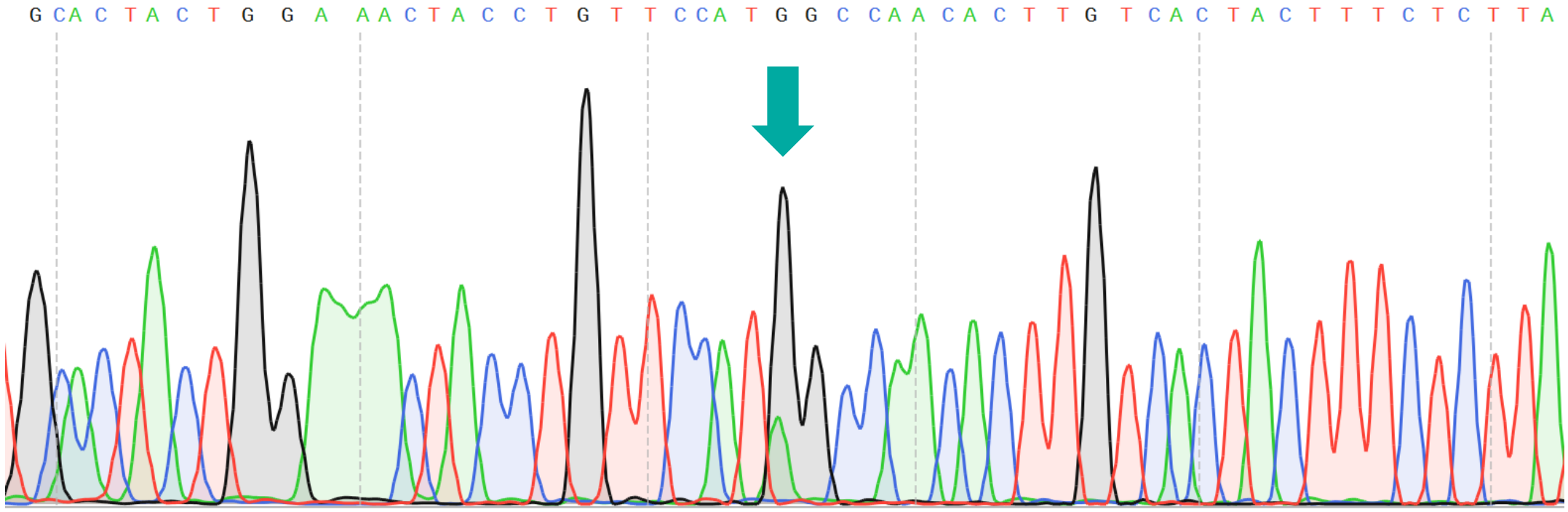
- 2’-OMe causes a steric clash with the active site
- RNA and **DNA** are compatible with catalysis

Structural modelling provides a **basis for further optimization of EONs**



EON specificity: Editing disabling region

2’-OMe prevents editing of off-target adenosines



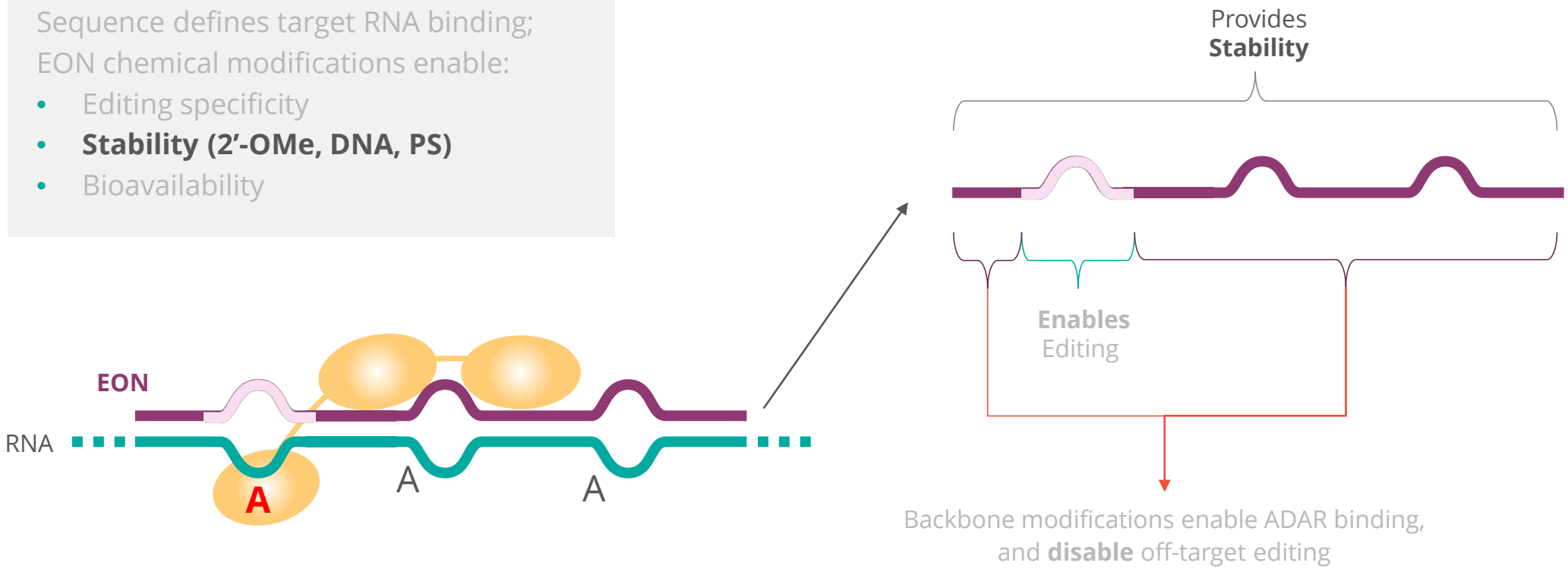
- 100 nM EON transfection with GFP W57X reporter and ADAR2 overexpression
- Readout by Sanger sequencing of the RT-PCR product
- Ongoing: Transcriptome-wide specificity analysis by RNA-seq

EONs designed for targeted RNA editing

Functionality defined by sequence and chemistry

Sequence defines target RNA binding;
EON chemical modifications enable:

- Editing specificity
- **Stability (2'-OMe, DNA, PS)**
- Bioavailability



EON stability in biological fluids

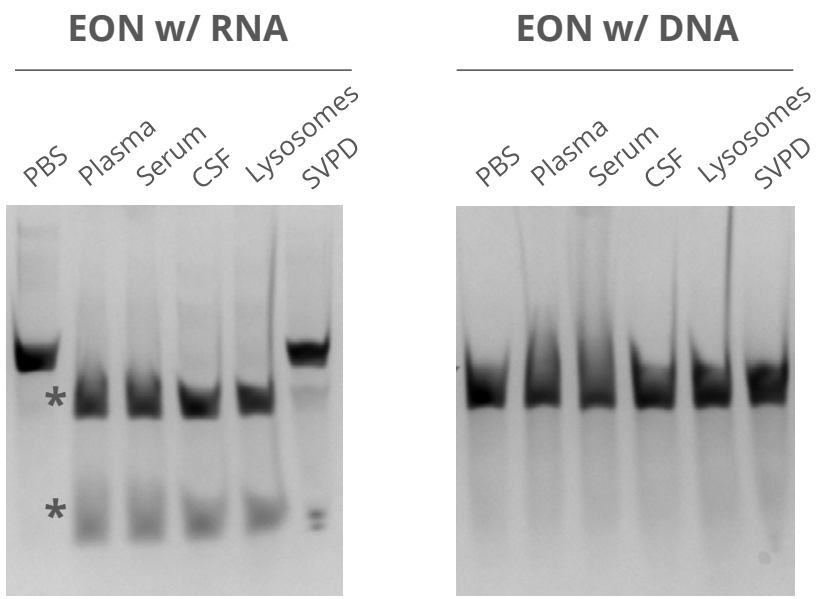
Improved endonuclease resistance by replacing RNA with DNA

Sequence defines target RNA binding; EON chemical modifications enable:

- Editing specificity
- **Stability (2'-OMe, DNA, PS)**
- Bioavailability

EON integrity analyzed by denaturing PAGE after incubation in biological fluids and nucleases *in vitro* (2h, 1d, 14d)

- PBS: Control buffer
- CSF: Human cerebrospinal fluid
- SVPD: Snake Venom Phosphodiesterase
- FBS: Fetal bovine serum



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- CSF: Human cerebrospinal fluid
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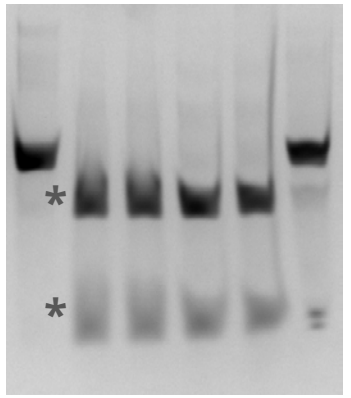
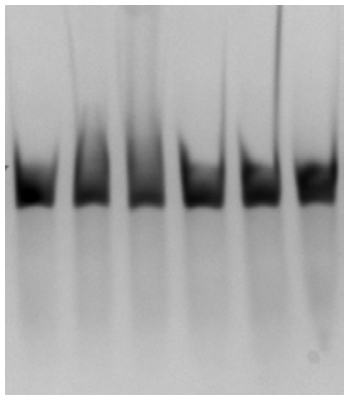
- FBS: Fetal bovine serum

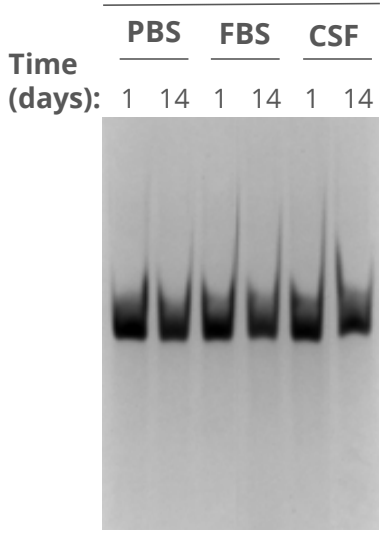
EON w/ RNA



EON w/ DNA



EON w/ DNA



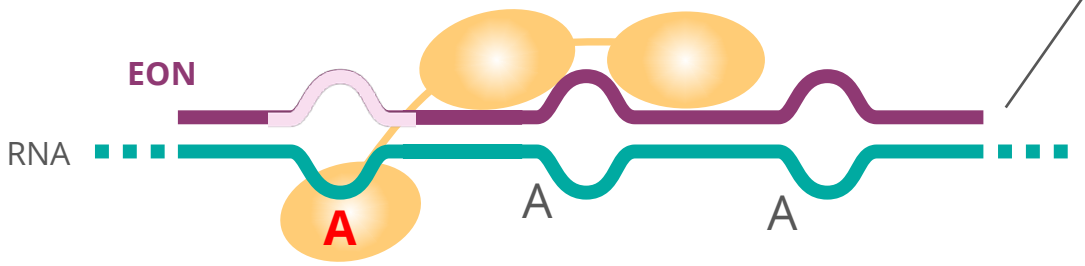
EONs additionally contain PS modifications, which are necessary for exonuclease resistance

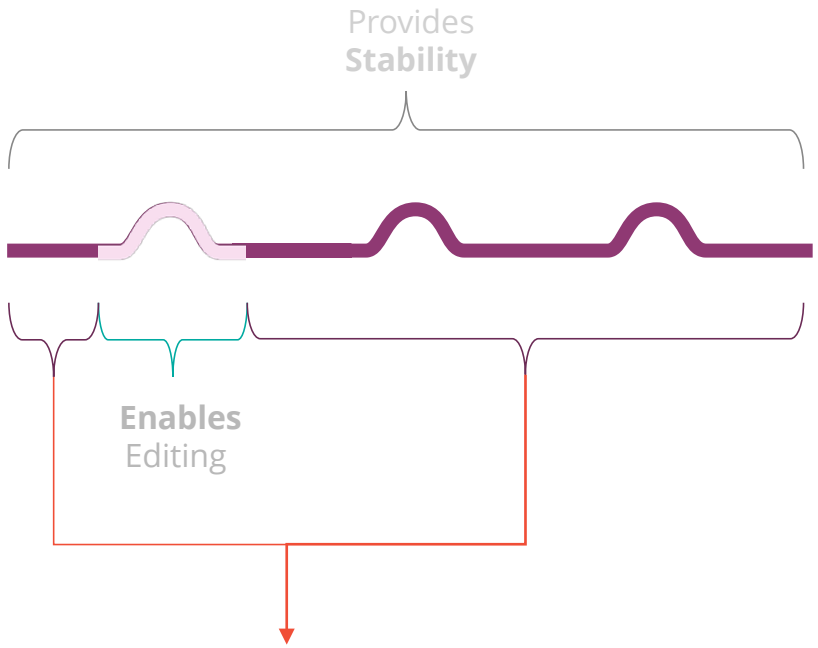
EONs designed for targeted RNA editing

Functionality defined by sequence and chemistry

Sequence defines target RNA binding;
EON chemical modifications enable:

- Editing specificity
- Stability
- **Bioavailability (PS and undisclosed)**





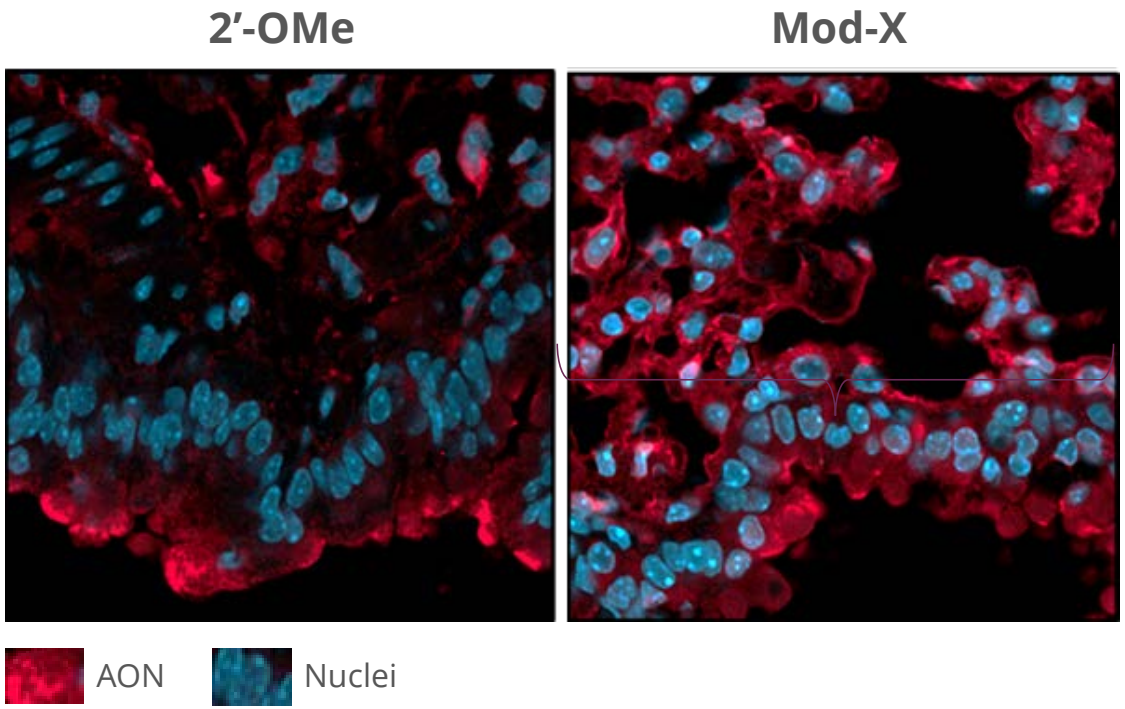
Backbone modifications enable ADAR binding,
disable off-target editing,
and enable bioavailability

EON bioavailability: Rational redesign

Changing sugar chemistry to improve uptake

Delivery with other oligo types indicates better uptake with another (undisclosed) sugar modification **(Mod-X)**

E.g. naked oligo delivery (OT) to mouse lung.

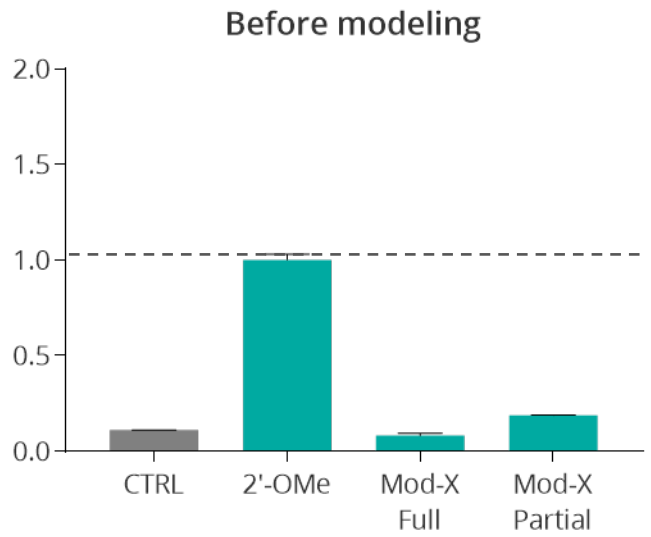


Can nucleotides with Mod-X be incorporated into EONs without compromising ADAR activity?

EON bioavailability: Rational redesign

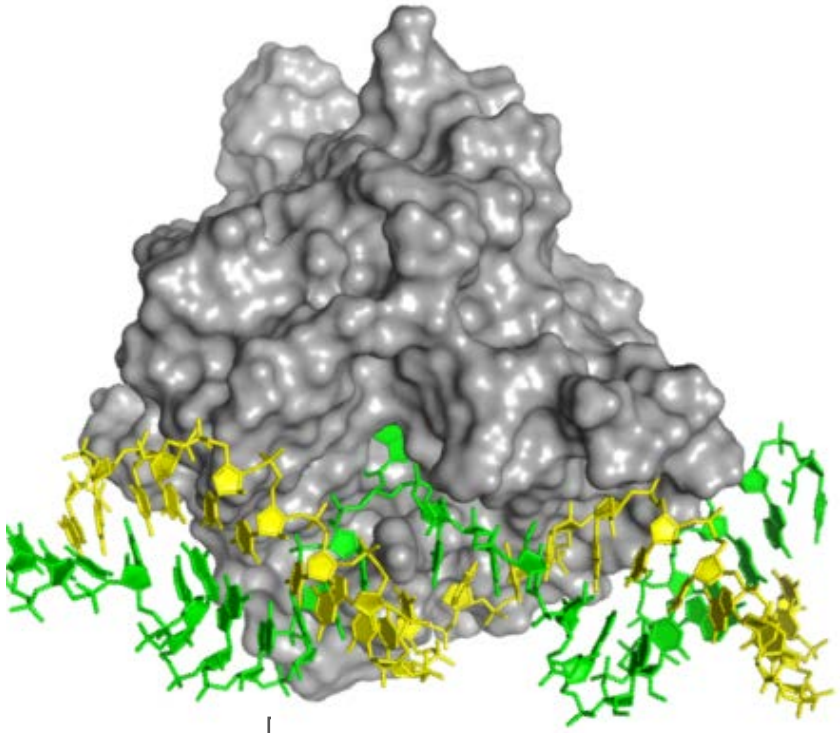
Modelling enables incorporation of improved sugar chemistry

Enzymatic activity *in vitro* (Normalized to 2'-OMe EON)

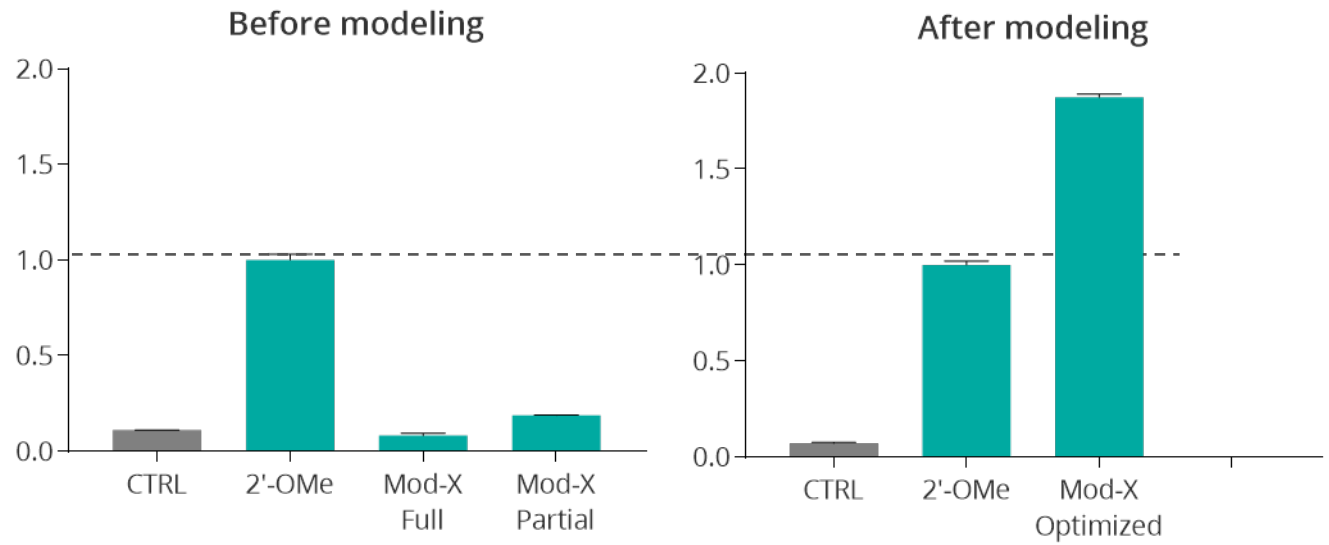


EON bioavailability: Rational redesign

Modelling enables incorporation of improved sugar chemistry



Enzymatic activity *in vitro* (Normalized to 2'-OMe EON)



Same Mod-X substitution level

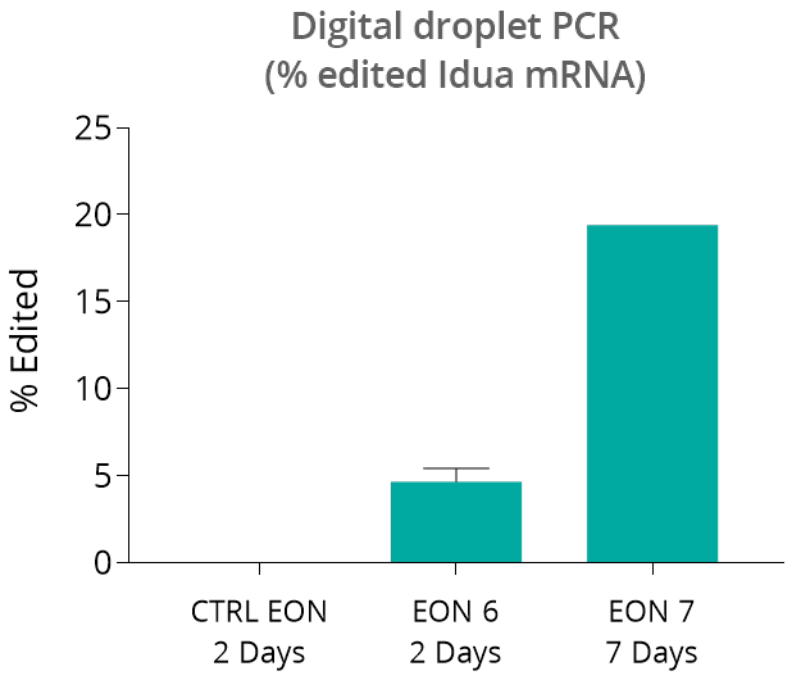
Computational modelling defines positions where Mod-X is tolerated

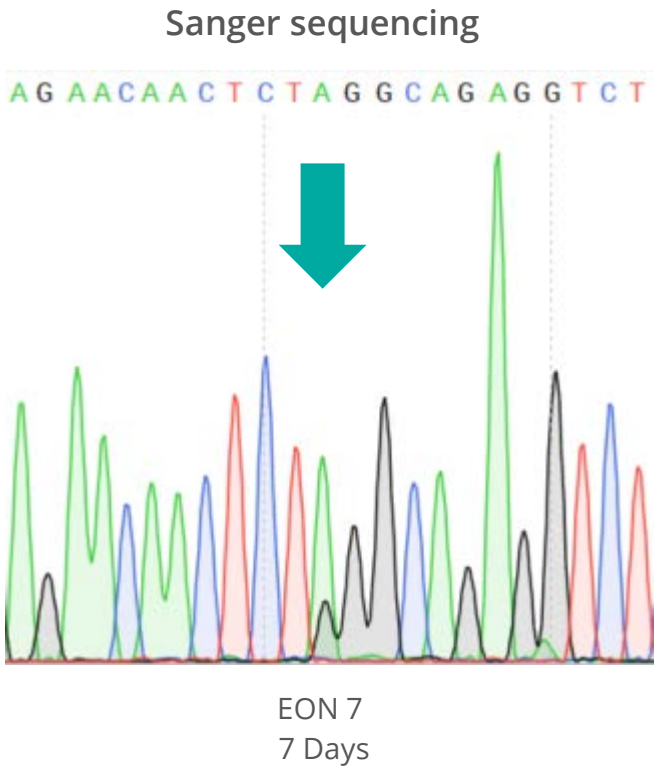
EONs restore iduronidase mRNA *in vivo*

Intravitreal delivery in the W392X mouse

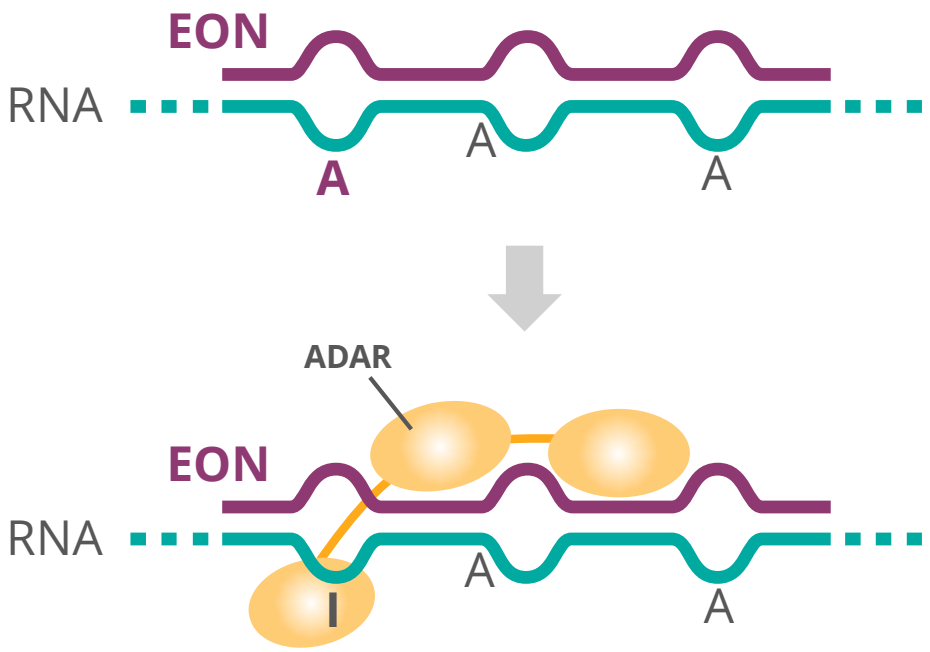
EON *in vivo* delivery:

- 50 µg EON per eye
- Single IVT dose
- Necropsy at 2 or 7 days
- ddPCR of retina





Axiomer® technology



- Editing Oligonucleotides (EONs) recruit **endogenous** ADARs to catalyze **A-to-I** editing
- Editing occurs at **specific** adenosines of **endogenous** RNA transcripts
- Axiomer® is a **single-component** technology to **reversibly** modulate cellular functions
- Rational EON design enables optimization of editing **efficiency** and **drug-like** properties

The Team

ProQR®

IT'S IN OUR RNA