Structure-based computational approach for optimizing oligonucleotides for A-to-I editing

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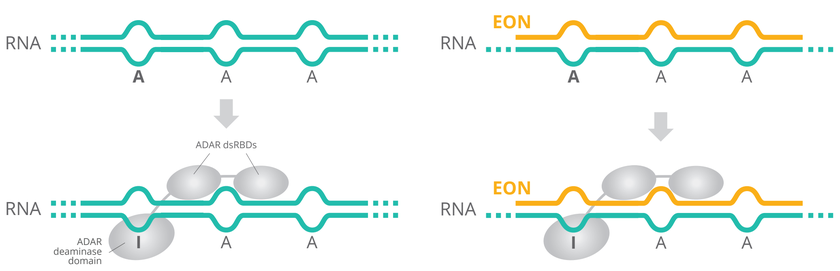
Axiomer® technology: Editing oligonucleotides (EONs)

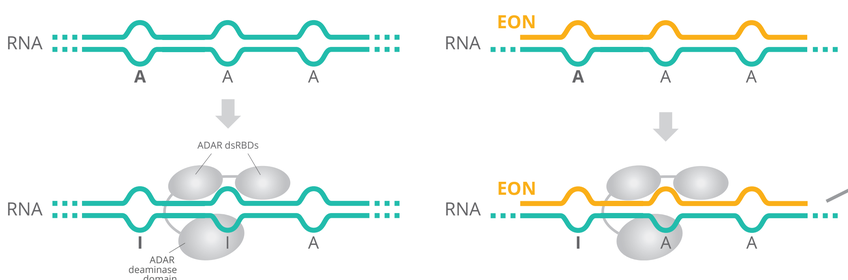
ADAR deaminates the target A in the EON-target RNA helix



Endogenous editing

EON-directed therapeutic editing

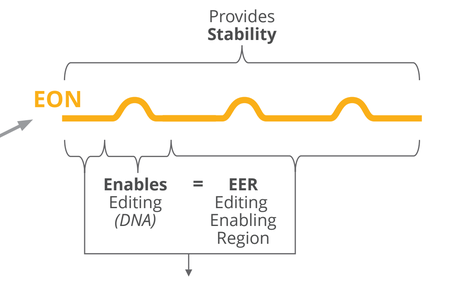
Advantage over RNA guides: specificity



Endogenous editing
Editing site with RNA guide is flexible

EON-directed therapeutic editing
Editing site with EONs is precise. No off-target editing upon protein repositioning

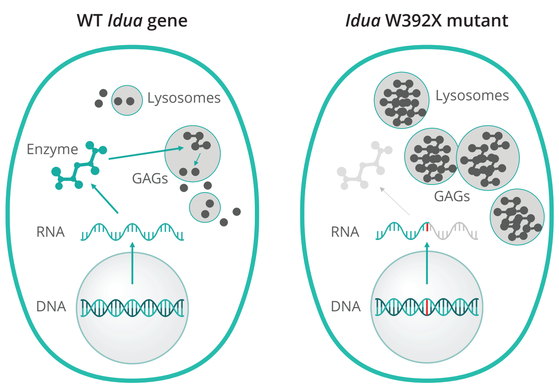
Sequence defines target RNA binding, while EON chemical modifications define functional regions



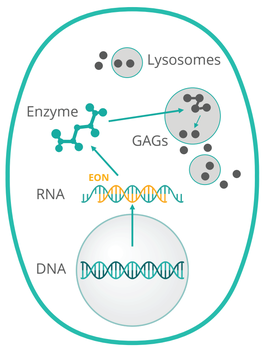
Backbone modifications enable ADAR binding, and disable off-target editing
(2'-OMe and/or 2'-MOE, and PS in undisclosed positions)

Background

Mouse model of the Hurler syndrome for targeted editing

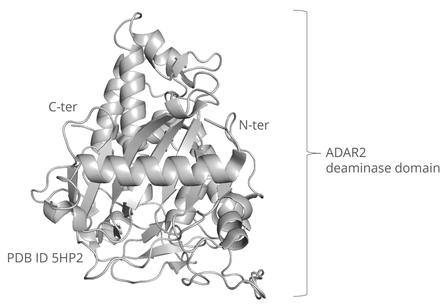


Idua W392X mutant and EON



The Idua gene encodes for an enzyme called the iduronidase which is essential for the hydrolysis of large sugar molecules called glycosaminoglycans (GAGs). The enzyme is located in lysosomes. The autosomal recessive disorder mucopolysaccharidosis type I-Hurler (MPS I-H) or Hurler syndrome is the most severe deficiency of iduronidase. The Idua-W392X mutation is analogous to the human IDUA-W402X[1] and the mouse model provides an important tool to develop a therapeutic approach based on the Axiomer® technology.

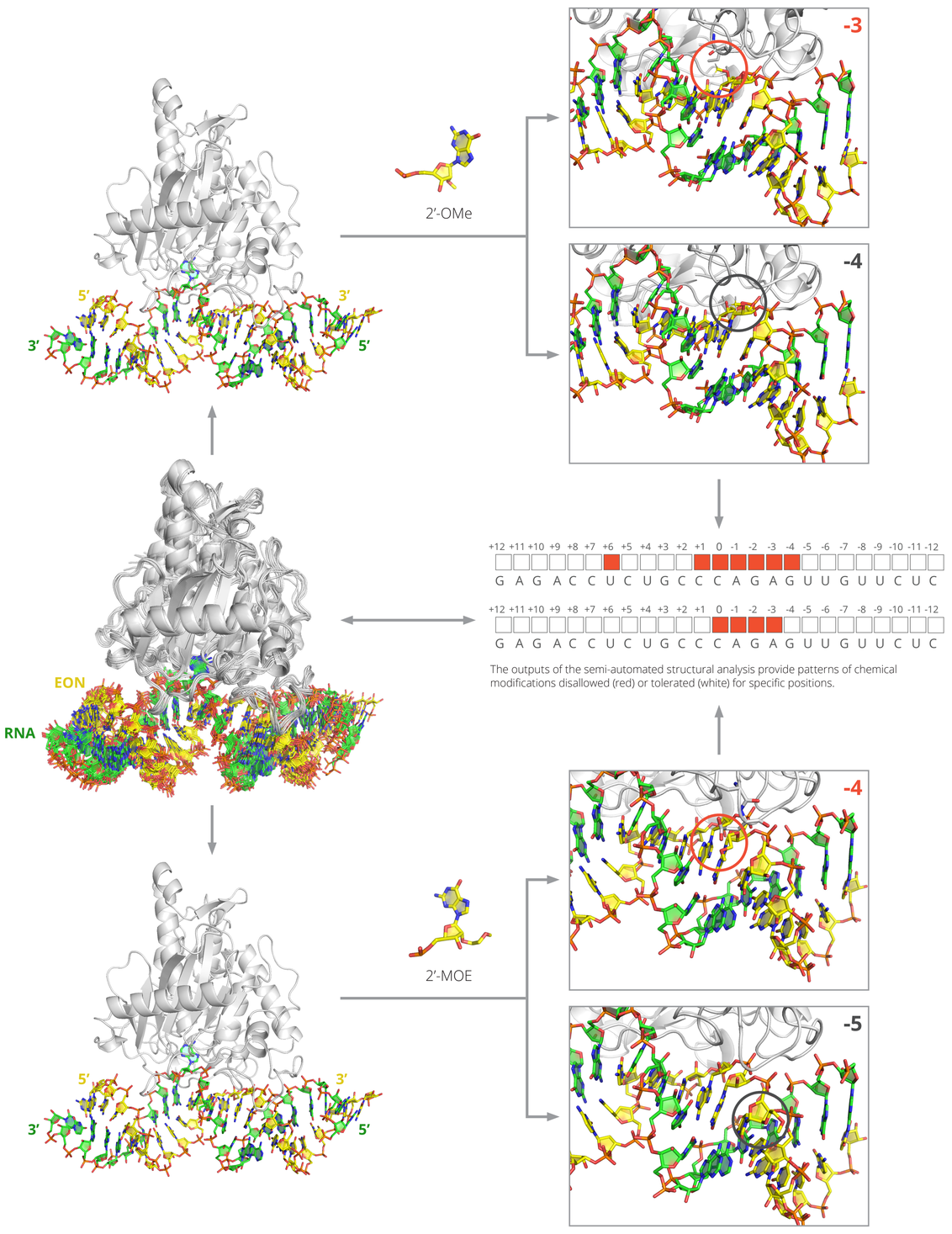
Structural requirements for computational modelling



The modelled system is constituted with a structural template derived from the RNA-bound X-ray structure of the human ADAR2 deaminase domain[2] (grey cartoon). The double-stranded oligonucleotides has been calculated with distance restraints derived from a standard A RNA. Networks of distance restraints are commonly used for solution structure determination. Numbering of the EON nucleotides has been defined relative to the target site in the RNA, which defined as the 0 position. Nucleotides numbers are negatively and positively incremented towards the 5' and the 3' ends of the target RNA, respectively, with the complementary nucleotides on the EON being given the same number.

Computational Methods and Analysis

Multiple cycles of *de novo* structures calculations



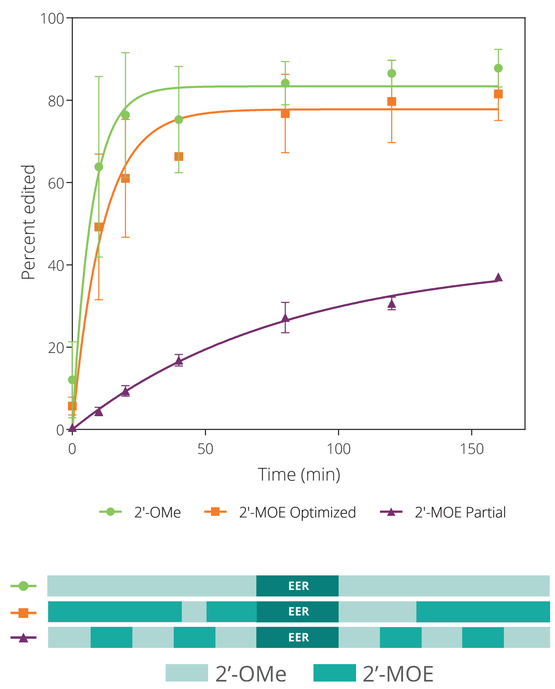
The outputs of the semi-automated structural analysis provide patterns of chemical modifications disallowed (red) or tolerated (white) for specific positions.

We implemented a library of chemical modifications which are integrated in the structure calculation of the protein/RNA complex. Backbone chemical modifications are successively inserted in the different positions within our EON annealed to the Hurler RNA target. For each cycle, 200 structures are calculated with the CYANA software[3] and the 20 lowest energy structures are refined under the AMBER14 force-field[4] (structure ensemble in the centre). A semi-automated analysis allowed us to determine the positions in which chemical modifications of the ribose 2' positions do not generate steric hindrances with the protein side-chains (extended regions on the left boxes), indicating positions compatible with ADAR2 binding. EONs and RNA targets are shown with yellow and green carbon atoms, respectively. The ADAR2 deaminase domain is displayed in light grey.

Experimental Results

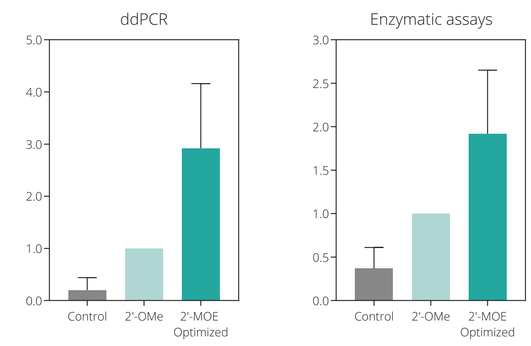
Modelled 2'-MOE pattern is compatible with editing with purified ADAR2

Experiments with EONs containing different ratios of 2'-OMe and 2'-MOE were performed under single-turnover conditions with saturating full length ADAR2 concentration[5]. Fraction of edited RNA (y axis) is indirectly measured with digital droplet PCR assays to quantify the number of restored WT RNA targets, with the mean from 2 replicates shown. Data have been collected at different time points (indicated in minutes on the x axis). Error bars are reported for each point and indicate standard deviations.



Modelled 2'-MOE pattern is compatible with editing in cells

Different EONs with 2'-OMe and 2'-MOE chemical modifications were transfected in mouse embryonic fibroblast cells harbouring the Idua W392X reporter construct. Restoration of the WT RNA target and iduronidase activity were monitored with digital droplet PCR (left panel) and standardized enzymatic assays[6] (right panel), respectively. An EON with 2'-OMe modifications is compared to a 2'-MOE Optimized EON. 2'-MOE modifications inserted in this EON followed the pattern defined with our computational approach. Digital droplet PCR assays (in triplicates) and enzymatic activity (in duplicates) are normalized to 2'-OMe EON.



References

- [1] Wang D. *et al.*, Characterization of an MPS I-H knock-in mouse that carries a nonsense mutation analogous to the human IDUA-W402X mutation, Mol. Genet. Metab., 2010
- [2] Matthews M.M. *et al.*, Structures of human ADAR2 bound to dsRNA reveal base-flipping mechanism and basis for site selectivity, Nat. Struct. Mol. Biol., 2016
- [3] Herrmann T. *et al.*, Protein NMR structure determination with automated NOE assignment using the new software CANDID and the torsion angle dynamics algorithm DYANA, J. Mol. Biol., 2002
- [4] Case D.A. *et al.*, The Amber biomolecular simulation programs, Comput. Chem., 2005
- [5] Mizrahi R.A. *et al.*, Nucleoside Analog studies indicate mechanistic differences between RNA-editing adenosine deaminases, Nucleic Acids Res., 2012
- [6] Ou L. *et al.*, Standardization of α-L-iduronidase enzyme assay with Michaelis-Menten kinetics, Mol. Genet. Metab., 2014

Conclusion & Outlooks

- **A robust computational approach is developed to screen for compatible interactions between backbone chemical modifications of EONs and the side-chains of the ADAR2 deaminase domain. Our data have been successfully correlated with docking studies performed with the Autodock Vina program**
- **Experimental results show that the modelled patterns of specific chemical modifications are compatible with enzymatic activity**
- **We demonstrate the applicability of a structure-based modelling approach as a tool for in silico screening of oligonucleotides for targeted RNA editing**
- **We will extend our structure-based computational tools to other domains of ADARs and combine in silico screening with data from high-throughput biochemical assays**

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