Characterization of Selective, Allosteric Inhibitors of Human XRN1

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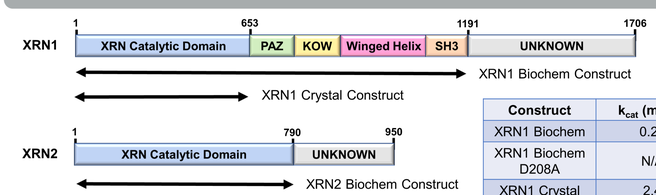
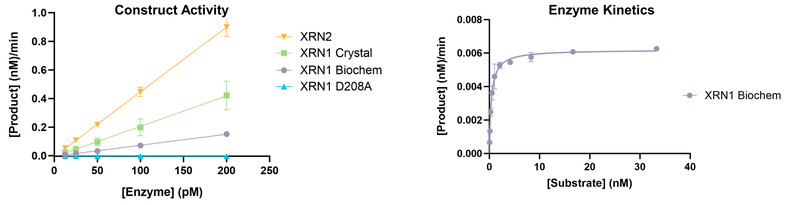
XRN1 is a Target for Cancer Therapeutics

- 5′→3′ exoribonuclease 1 (XRN1) is integral for normal mRNA turnover in the cell and plays an important role in innate immunity by preventing double stranded RNA (dsRNA) accumulation and pPKR induction in virus-infected cells[1].
- XRN1 has emerged as a potential target for drug discovery efforts as tumors with high interferon signaling are sensitive to XRN1 loss using genetic tools[2].
- 3′,5′-adenosine diphosphate (pAp) is a non-selective nuclease inhibitor and has been shown to be active against XRN1[3], but full biochemical characterization has not been published. To date, no XRN1-selective inhibitors have been disclosed.
- This study details *in vitro* characterization through biochemical, biophysical and structural analysis of two small-molecule inhibitors of XRN1: pAp and Compound 1.

XRN1 Construct Design and Assay Characterization



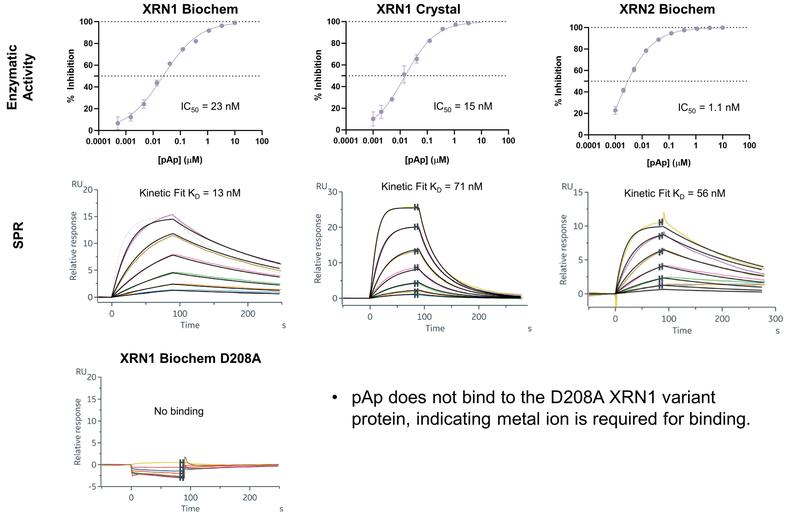
Construct	k_{cat} (min $^{-1}$)	RNA K_M (nM)
XRN1 Biochem	0.21	0.4
XRN1 Biochem D208A	N/A	N/A
XRN1 Crystal	2.4	44.5
XRN2 Biochem	3.6	5.1



- To enable drug discovery efforts, constructs of human XRN1 and counter target XRN2 were designed and characterized for enzymatic activity.
- The D208A mutation ablates a catalytic magnesium ion and is therefore inactive[4].
- Assays were developed under balanced conditions for active proteins.

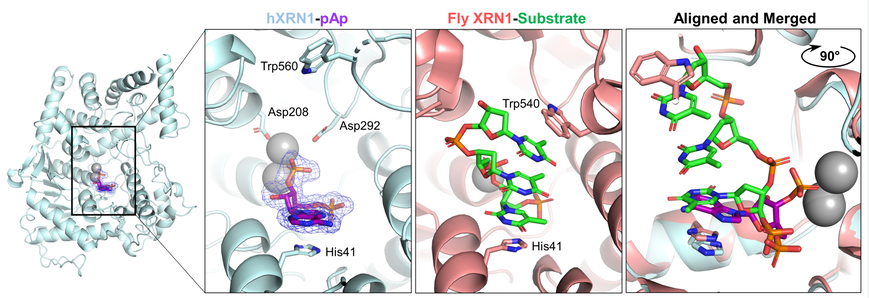
pAp is a Potent Inhibitor of XRN1 and XRN2

- Characterization by biochemical and SPR assays reveals pAp is a nM inhibitor of both XRN1 and XRN2.



- pAp does not bind to the D208A XRN1 variant protein, indicating metal ion is required for binding.

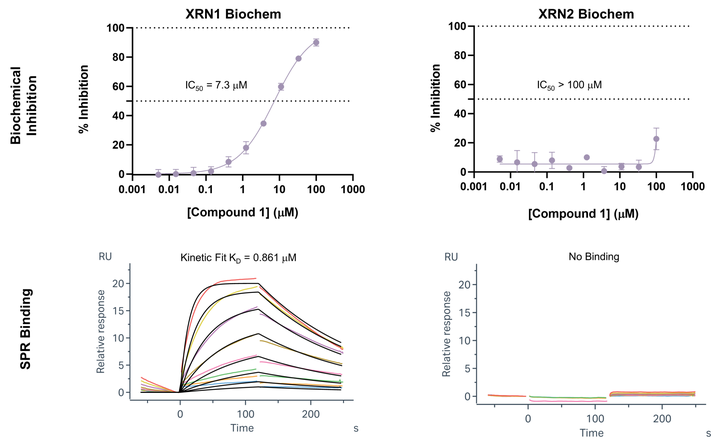
Human XRN1 Crystal Structure Solved with pAp Bound in the Active Site



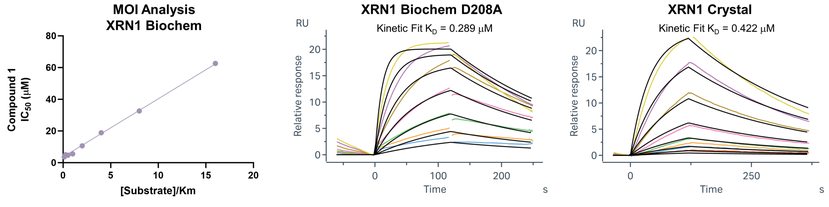
- A crystal structure of human XRN1 (hXRN1) nuclease domain in complex with pAp at 2.1 Å was solved.
- pAp binds in the RNA binding site. The phosphate moieties interact with both magnesium ions. The adenine base forms a π-stacking interaction with His41.
- 5′-phosphate and nucleobase of pAp overlaps with first nucleotide of the substrate previously solved in *Drosophila melanogaster* XRN1 (PDB: 2Y35)[5].

Compound 1 is a Selective, Substrate-Competitive Inhibitor of XRN1

- Compound 1 was identified as an inhibitor of XRN1 that did not inhibit or bind to XRN2.

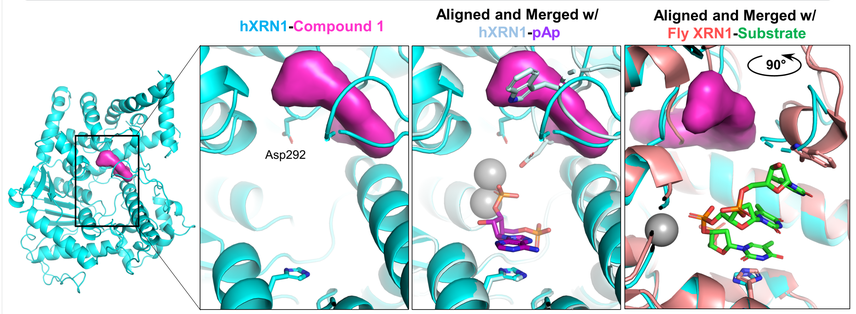


- Compound 1 is competitive with RNA as indicated by the linear relationship with increasing substrate, as shown in the MOI analysis plot.
- The compound is also able to bind D208A mutant XRN1 with comparable affinity to wild-type XRN1. This result indicates the compound is unlikely to interact with the metal ion-containing active site of XRN1 and binds to a distinct site.



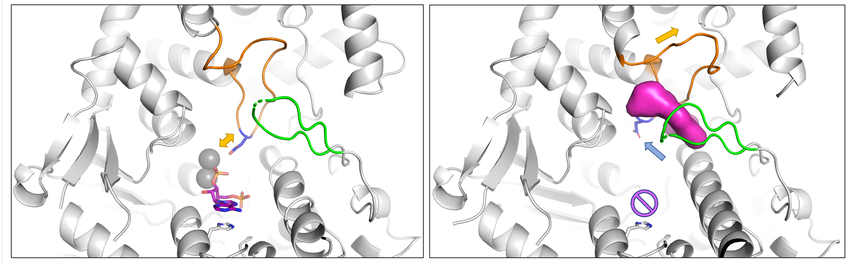
- Equivalent binding of Compound 1 to the XRN1 crystallography construct enabled initiation of structural studies to determine the compound binding site.

Compound 1 Binds in an Allosteric Pocket Adjacent to the Active Site



- The XRN1-Compound 1 cocomplex was solved to 1.96 Å resolution. The compound binds to an allosteric pocket proximal to the active site. The Compound 1 binding site is distinct from the pAp and oligonucleotide substrate binding site. No density is visible for the catalytic metal ions.
- While the XRN1 and XRN2 active sites are highly conserved, the allosteric Compound 1 binding site has low sequence identity, thus driving the selectivity seen in biochemical and biophysical assays.

Conformational Changes Explain Substrate-Competitive MOI of Compound 1



Purple Sticks = pAp
Magenta Surface = Compound 1
Grey Spheres = Magnesium Ions
Orange = Magnesium Coordinating Loop
Blue = Asp292
Green = RNA-stacking Loop

- Compound binding induces a conformational rearrangement of the Mg^{2+}-coordinating loop (orange), disrupting metal binding.
- Compound 1 binding also coordinates/facilitates interactions with the RNA-stacking loop (green), perturbing substrate binding.

Conclusions

- A toolkit consisting of biochemical and biophysical assays and human XRN1 crystallography has been generated to support identification, characterization and validation of inhibitors for XRN1. This suite was used to characterize binding of a non-selective tool compound, pAp.
- An XRN1-selective, RNA substrate-competitive compound was identified and validated. A binding site distinct from the metal active site was predicted from biophysical assays and confirmed by crystallography.
- The compound induces large conformational changes upon binding that disrupt the substrate binding site, revealing plasticity in the XRN1 active site that that was previously unknown.

Acknowledgements

- The authors thank Jim Valentine, the compound management team at Accent Therapeutics, our CRO partners and current and former Accent employees for project support .

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