

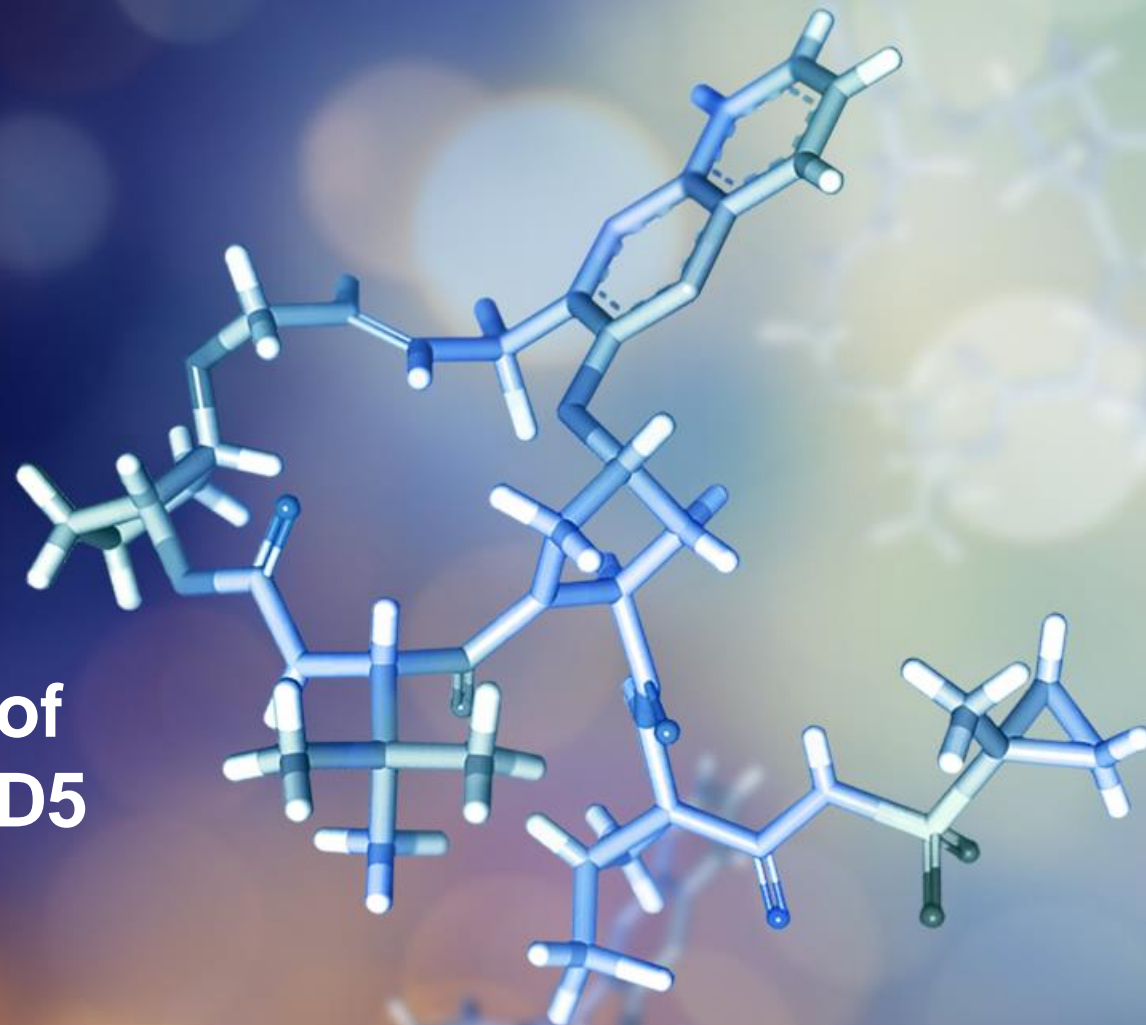
ENANTA

Pharmaceuticals

CREATING SMALL MOLECULE DRUGS
FOR VIRAL INFECTIONS AND LIVER DISEASES

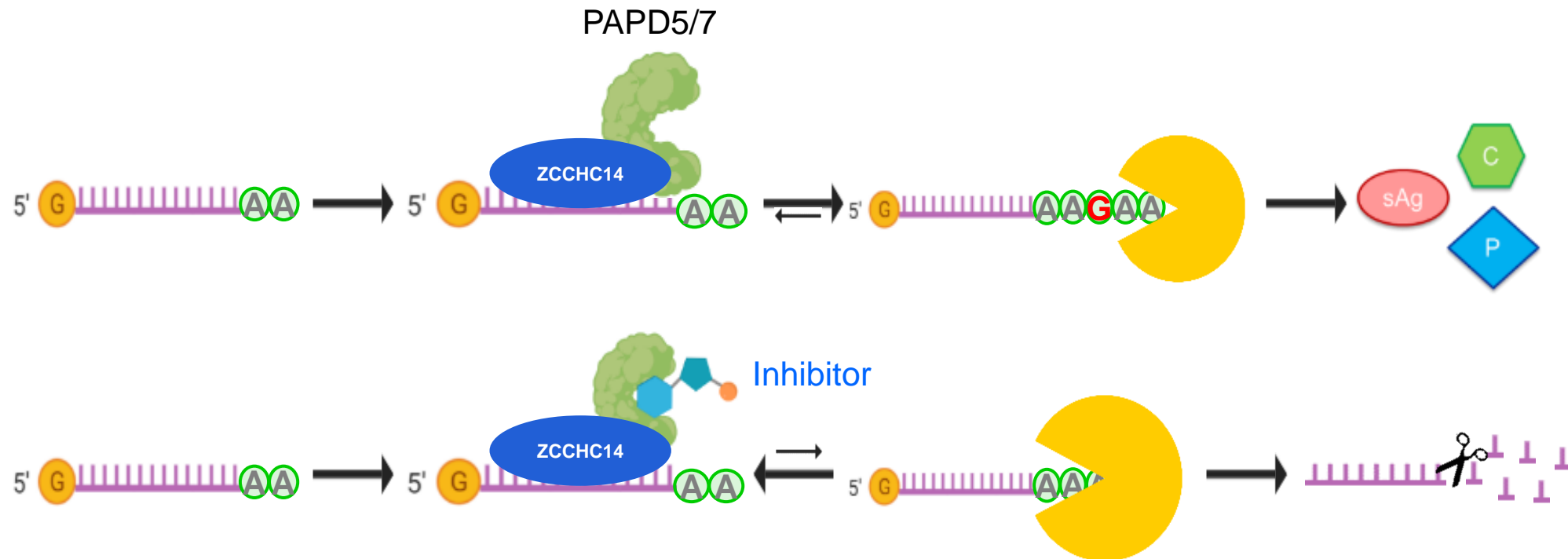
**Mechanism and structural basis of
small molecule inhibition of PAPD5
and PAPD7**

September 27, 2021



Mode of Action of RNA Destabilizers in HBV Infected Cells

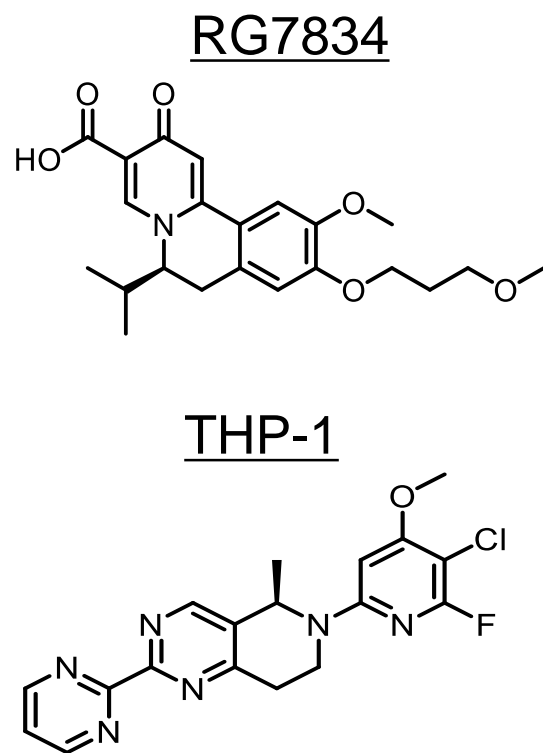
- HBV utilizes PAPD5 and PAPD7, related host noncanonical poly(A) polymerases, to stabilize mRNAs
- RNA destabilizers reduce HBV mRNA stability and viral proteins via PAPD5/7 inhibition in cells.



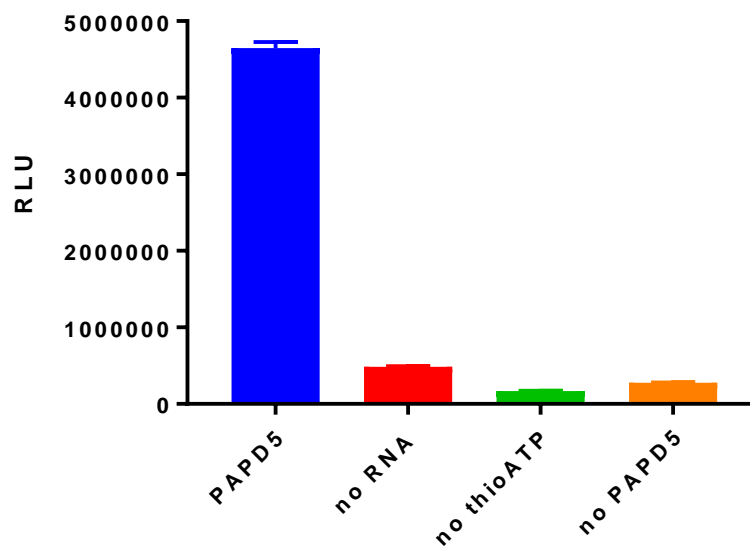
- Biochemical mechanism and structural basis of PAPD5/7 inhibition by RNA destabilizers is unreported

Potency and Selectivity of RNA Destabilizers

- Two distinct chemical series of PAPD5/7 inhibitors have been described in the literature



Pyrophosphate Release Assay



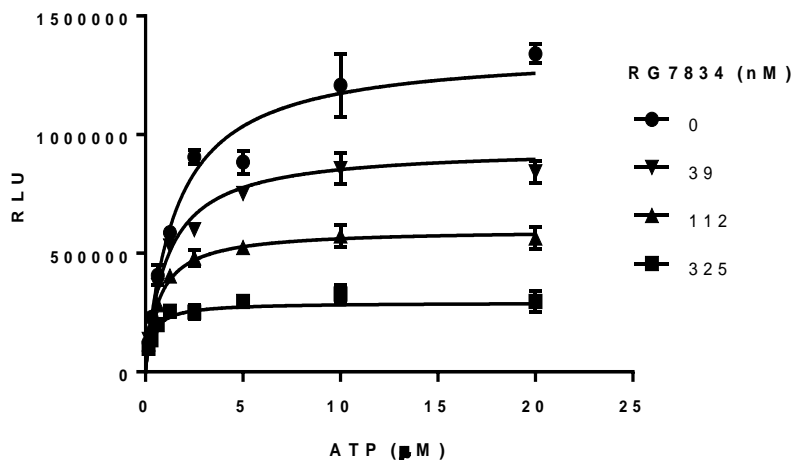
RNA-A15 $K_m = 0.3 \mu M$
 α -S-ATP $K_m = 2.7 \mu M$

	IC ₅₀ (nM)	
	RG7834	THP-1
2.2.15 HBsAg	2.3	1.4
PAPD5	112	73
PAPD7	2080	81

- Despite similar cell based activity, RG7834 and THP-1 possess different potencies against PAPD5 and PAPD7

RNA Destabilizers Share a Common Kinetic Mechanism of PAPD5/7 Inhibition

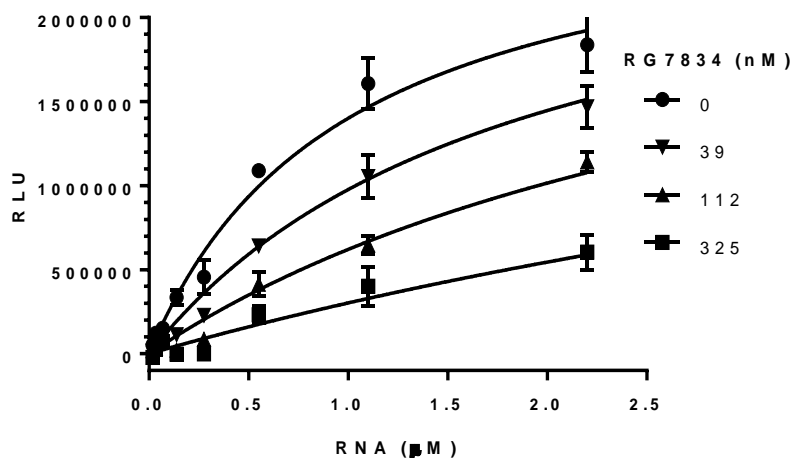
RG7834 vs ATP PAPD5



Uncompetitive Inhibition wrt ATP

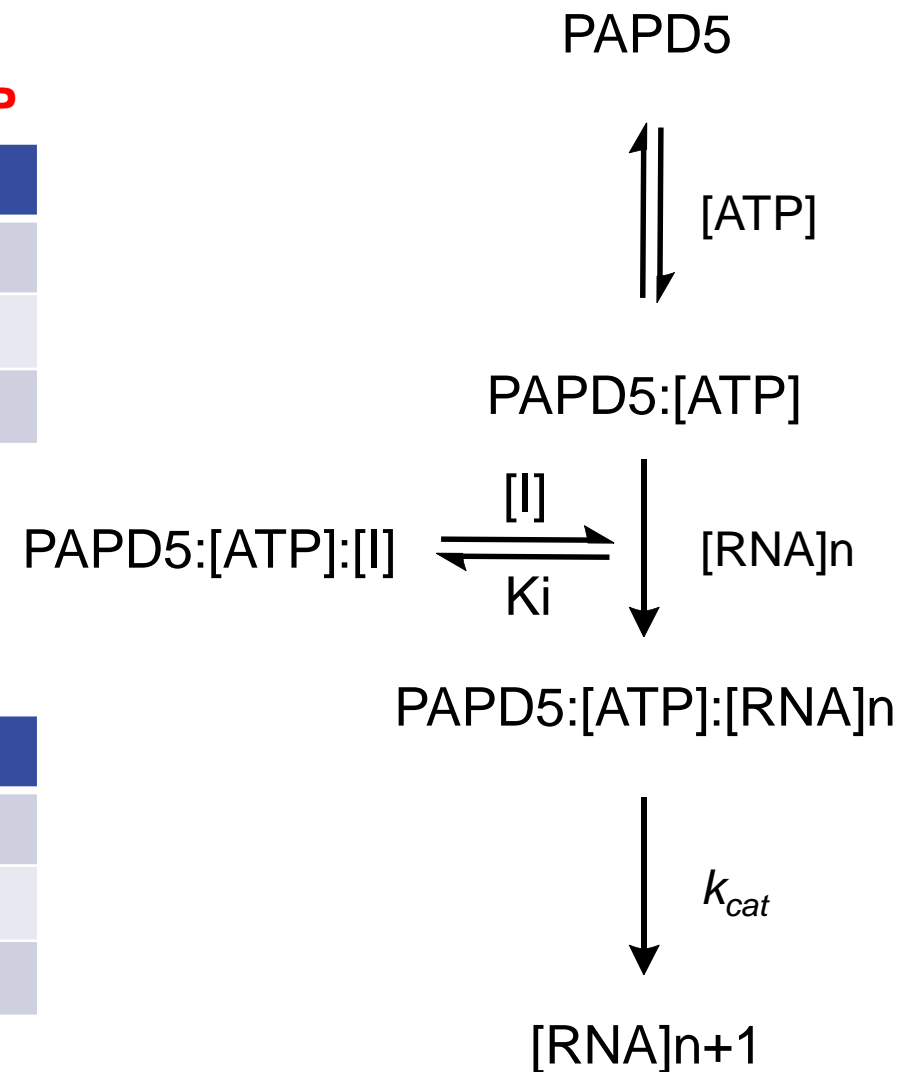
	K_{iu} (nM)	
	RG7834	THP-1
PAPD5	89	69
PAPD7	1697	65

RG7834 vs RNA PAPD5



Competitive Inhibition wrt RNA

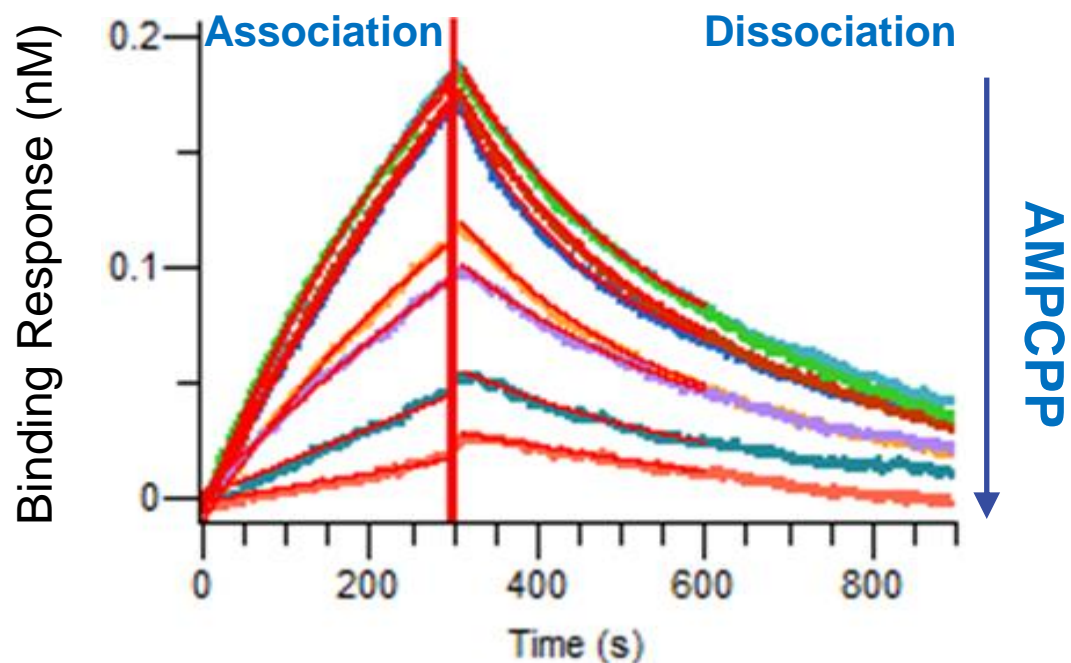
	K_{ic} (nM)	
	RG7834	THP-1
PAPD5	45	38
PAPD7	514	26



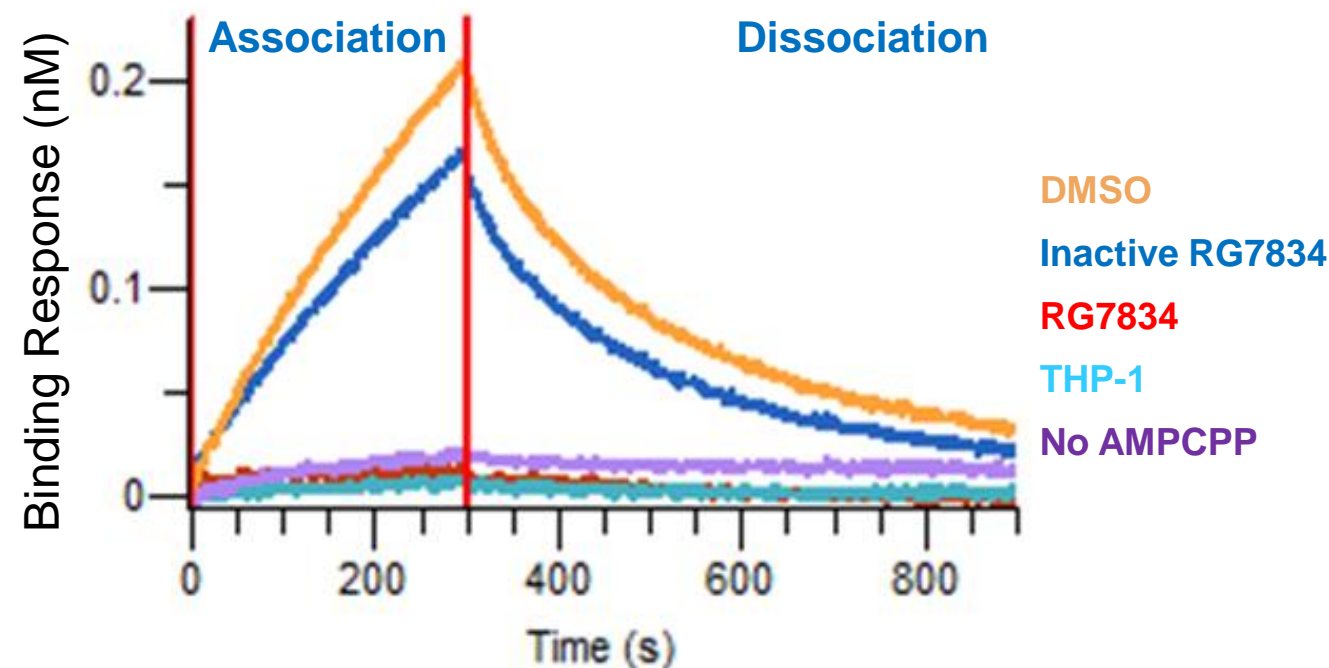
RG7834 and THP-1 Inhibit RNA Binding to PAPD5

- Developed BLI binding assay that monitors enzyme binding to RNA immobilized sensor

RNA Binding Nucleotide Dependence



PAPD5 Binding Kinetics by BLI

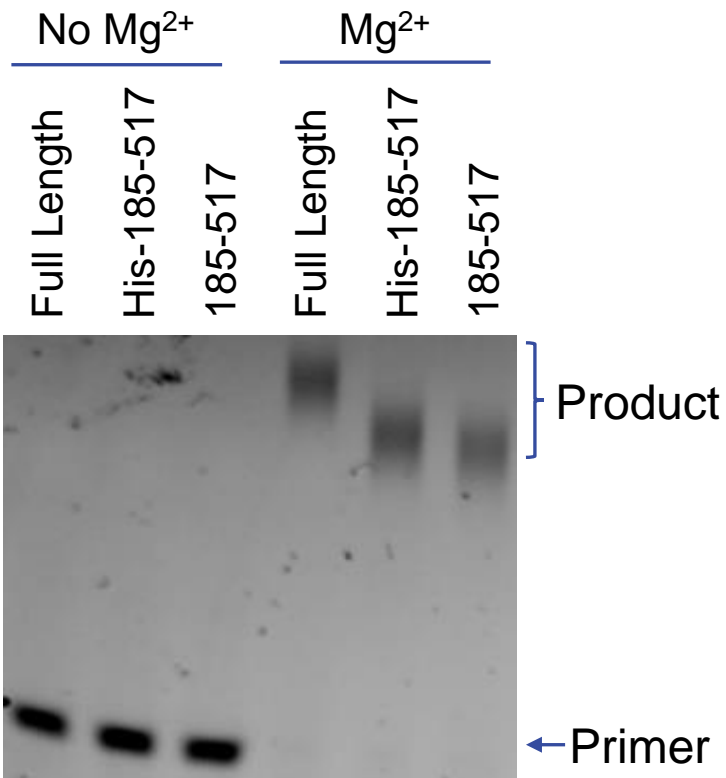


- PAPD5 binds RNA only in presence of AMPCPP
- Compound inhibits binding of RNA to the PAPD5:AMPCPP complex

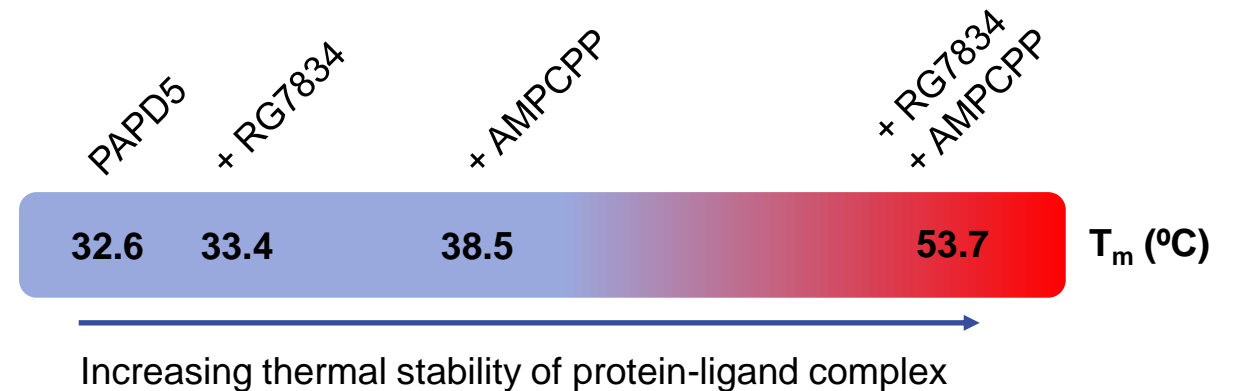
PAPD5 Crystallography Constructs and Characterization



RNA synthesis Assay

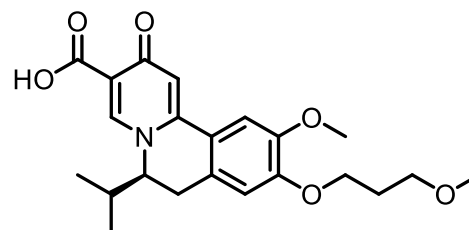


Thermal Stability by DSF Assay



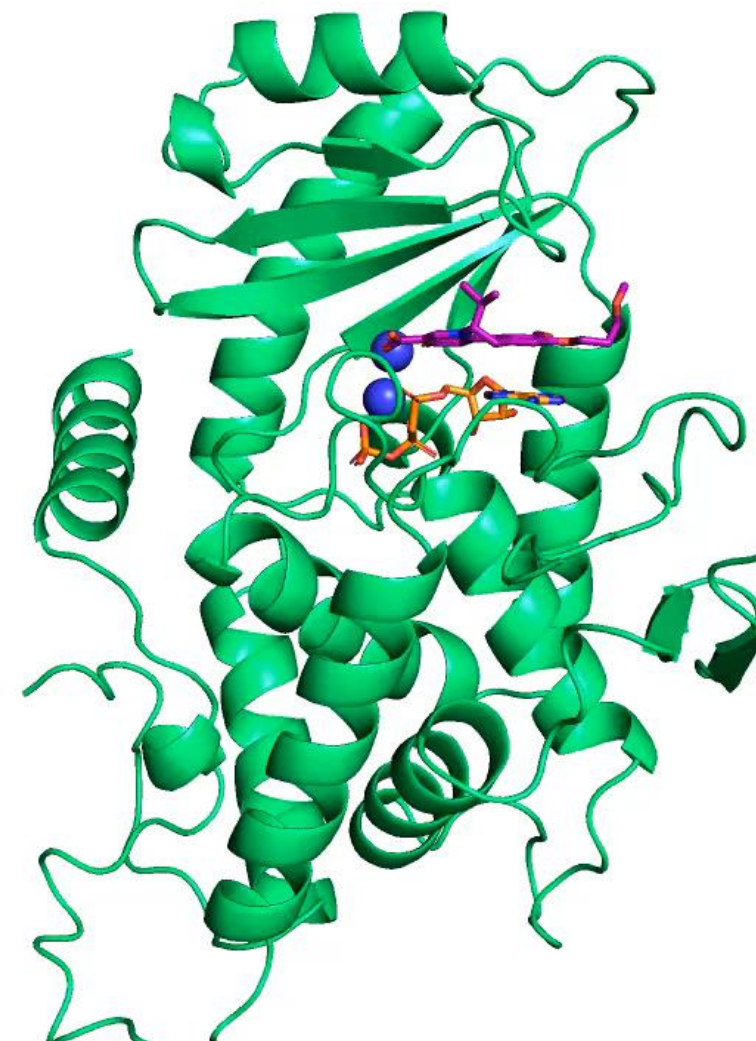
PAPD5 Crystallized in Presence of RG7834 and ATP analog

- Structure refined to 2.0 Å
- RG7834 binds in active site stacked between AMPCPP and PAPD5 anchor loop
- Minimal hydrogen bonding between RG7834 and PAPD5
- RG7834 occupies position expected to accommodate -1 nucleotide of incoming RNA substrate
- RG7834 β -ketoacid motif mediates ionic interaction with Mg^{2+}
- Van der Waals interaction between anchor loop and RG7834 isopropyl group



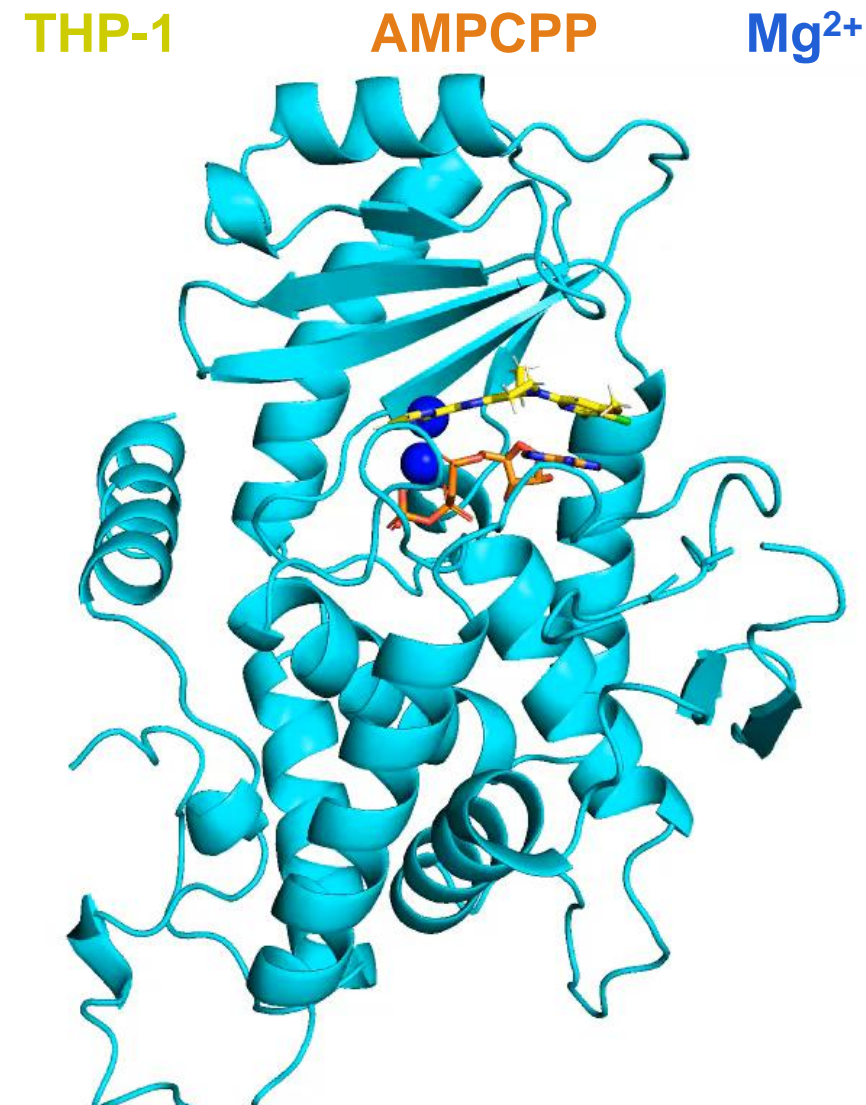
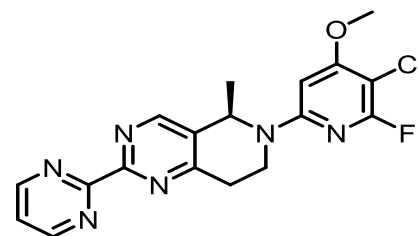
RG7834

AMPCPP

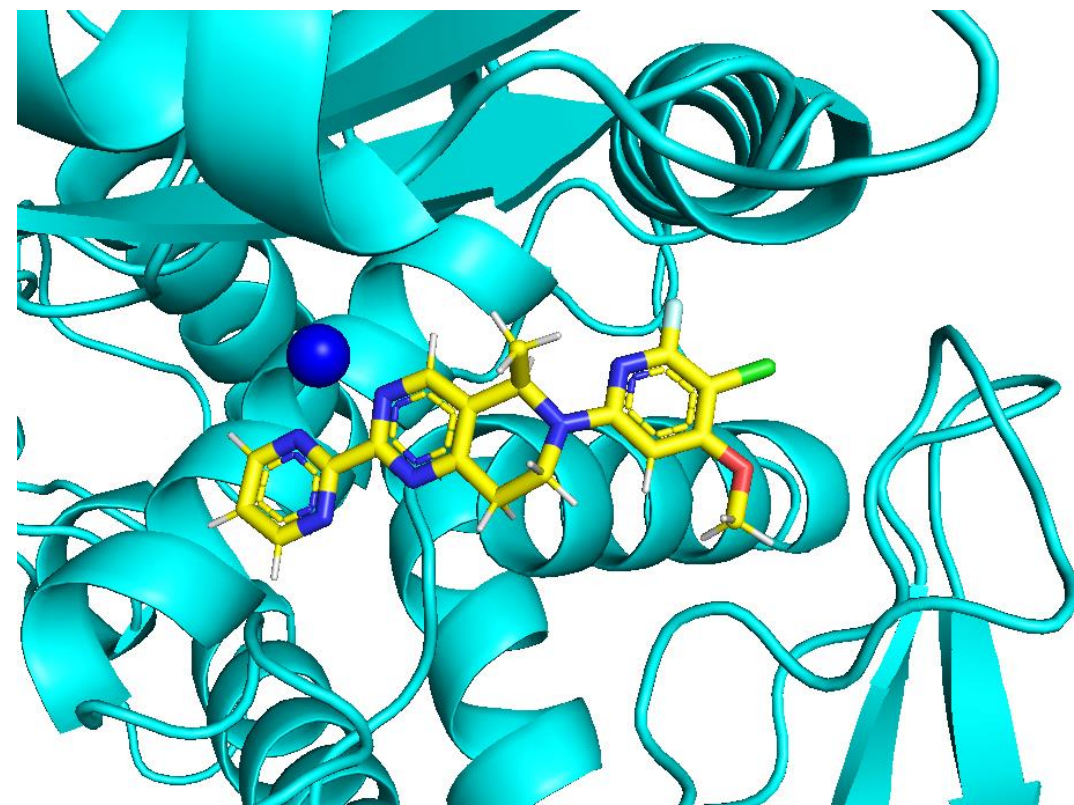
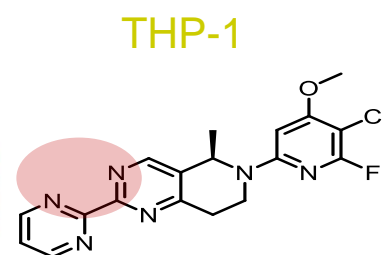
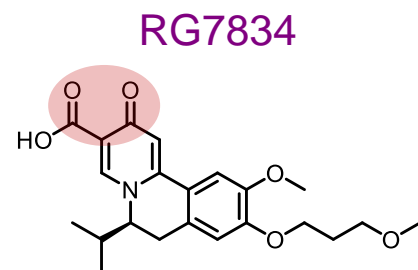
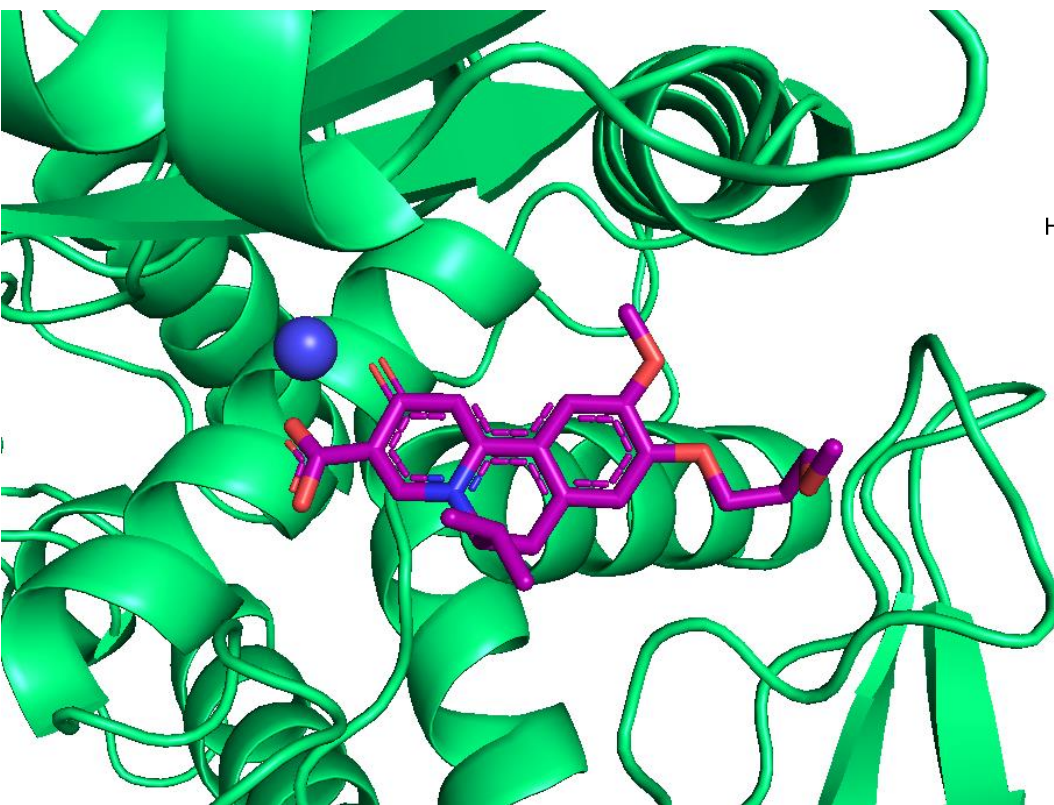
 Mg^{2+} 

PAPD5 Crystallized in Presence of THP-1 and ATP Analog

- Structure refined to 1.8 Å
- THP-1 binds in active site stacked between AMPCPP and PAPD5 anchor loop
- Minimal hydrogen bonding between THP-1 and PAPD5
- THP-1 occupies position expected to accommodate the -1 nucleotide of incoming RNA substrate
- THP-1 dipyrimidine motif mediates ionic interaction with Mg^{2+}
- Limited Van der Waals interaction between anchor loop and THP-1
- Pi interaction between THP-1 and AMPCPP



RG7834 and THP-1 Bound Structures Reveal Similarity in Binding Modes



Conclusions

- RG7834 and THP-1 are two PAPD5/7 inhibitors with distinct chemical scaffolds but highly overlapping mechanistic profiles
- THP-1 is equipotent against both PAPD5 & 7 while RG7834 has decreased potency against PAPD7
- Biochemical studies reveal an unprecedented ATP uncompetitive and RNA competitive mode of inhibition
- Both RG7834 and THP-1 bind by stacking in the PAPD5 active site between the nucleotide and anchor loop
- Both RG7834 and THP-1 interact with active site Mg^{2+}
 - RG7834 uses a β -ketoacid motif
 - THP-1 uses a dipyrimidine motif

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