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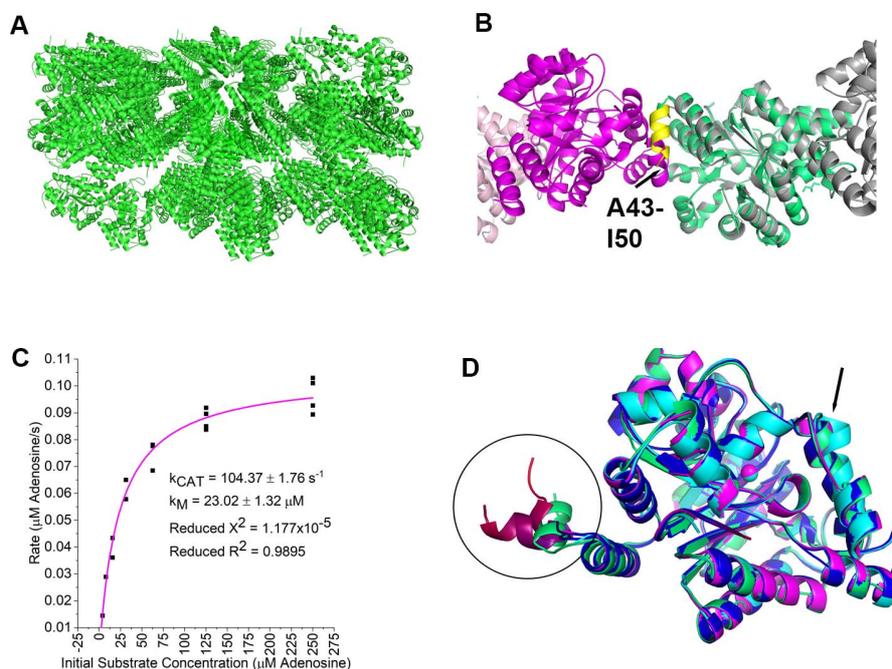
**Catalytically active holo *Homo sapiens* adenosine deaminase I adopts a closed conformation**

**Minh Thu Ma, Maria Rain Jennings, John Blazeck and Raquel L. Lieberman**

sp_P56658_ADA_BOVINE	1	AQTPAF	KPKVEL	HVLDG	AIKPE	TILY	YGR	KRGIALPADTPEELQNIIGMDKPL	LP	FLAKFDYYMP	70		
1VFL_BOVINE	1	AQTPAF	KPKVEL	HVLDG	AIKPE	TILY	YGR	RRGIALPADTPEELQNIIGMDKPL	LP	DFLAKFDYYMP	69		
sp_P03958_ADA_MOUSE	1	MAQTPAF	NKPKVEL	HVLDG	AIKPE	TILY	FGK	KRGIALPADTVEELRNIIGMDKPL	SLP	GF	FLAKFDYYMP	70	
3MVI_Mouse	1	--T	PAFNKPKVEL	HVLDG	AIKPE	TILY	FGK	KRGIALPADTVEELRNIIGMDKPL	SLP	GF	FLAKFDYYMP	67	
sp_P00813_ADA_HUMAN	1	MAQTPAF	KPKVEL	HVLDG	SIKPE	TILY	YGR	RRGIALPANTABGLLNVIIGMDKPL	LP	DFLAKFDYYMP	70		
sp_P56658_ADA_BOVINE	71	AIAGCREA	V	KRIAYEFVEMKAKDGVVYVEVRYS	PHLLANSKVE	PI	PWNQAEGLDTPDEVVSLV	NQGLQEG			140		
1VFL_BOVINE	70	AIAGCRDA	I	KRIAYEFVEMKAKDGVVYVEVRYS	PHLLANSKVE	PI	PWNQAEGLDTPDEVVSLV	NQGLQEG			139		
sp_P03958_ADA_MOUSE	71	VIAGCREAI	KRIAYEFVEMKAKEG	VVYVEVRYS	PHLLANSKVD	PMPWNQTE	EGDVT	PDVVDLV	NQGLQEG		140		
3MVI_Mouse	68	VIAGCREAI	KRIAYEFVEMKAKEG	VVYVEVRYS	PHLLANSKVD	PMPWNQTE	EGDVT	PDVVDLV	NQGLQEG		137		
sp_P00813_ADA_HUMAN	71	AIAGCREA	I	KRIAYEFVEMKAKEG	VVYVEVRYS	PHLLANSKVE	PI	PWNQAEGLDTPDEVVALV	QGGLQEG		140		
sp_P56658_ADA_BOVINE	141	ERDFGV	KVRSILCCMRHQPS	WSE	VVELCKKY	REQ	TVVAID	LAGDETI	EGSSLP	PGHV	YAYAEAVKSGVH	210	
1VFL_BOVINE	140	ERDFGV	KVRSILCCMRHQPS	WSE	VVELCKKY	REQ	TVVAID	LAGDETI	EGSSLP	PGHV	YAYAEAVKSGVH	209	
sp_P03958_ADA_MOUSE	141	EQA	FGIKVRSILCCMRHQPS	WSE	LVLELCKKY	NQK	TVVAMD	LAGDETI	EGSSLP	PGHV	EAYEGAVKNGIH	210	
3MVI_Mouse	138	EQA	FGIKVRSILCCMRHQPS	WSE	LVLELCKKY	NQK	TVVAMD	LAGDETI	EGSSLP	PGHV	EAYEGAVKNGIH	207	
sp_P00813_ADA_HUMAN	141	ERDFGV	KARSILCCMRHQPN	WSP	KVVELCKKY	QQQ	TVVAID	LAGDETI	PGSSLP	PGHV	YAYQEA	AVKSGIH	210
sp_P56658_ADA_BOVINE	211	RTVHAGEVGS	ANVVK	EAVDTLKTERLGHGYHTLED	ATLYNRLRQENMHFE		CPWSSYL	TGAWKPDTEHPV			280		
1VFL_BOVINE	210	RTVHAGEVGS	ANVVK	EAVDTLKTERLGHGYHTLED	TTLYNRLRQENMHFE		CPWSSYL	TGAWKPDTEHAV			279		
sp_P03958_ADA_MOUSE	211	RTVHAGEVGS	PEVV	REAVDILKTERVGHGYHTIED	EALYNRL	LKENMHFE	VCPWSSYL	TGAWDPKTTHAV			280		
3MVI_Mouse	208	RTVHAGEVGS	PEVV	REAVDILKTERVGHGYHTIED	EALYNRL	LKENMHFE	VCPWSSYL	TGAWDPKTTHAV			277		
sp_P00813_ADA_HUMAN	211	RTVHAGEVGS	AEVV	KEAVDILKTERLGHGYHTLED	QALYNRLRQENMHFE		CPWSSYL	TGAWKPDTEHAV			280		
sp_P56658_ADA_BOVINE	281	RFKNDQV	NYSLNTDDPLIFKSTLTDYQMTK		MGFT	EEEFKRLNINA	AKSSFLPEDEK	KELLDL	LYKA		350		
1VFL_BOVINE	280	RFKNDQV	NYSLNTDDPLIFKSTLTDYQMTK		MGFT	EEEFKRLNINA	AKSSFLPEDEK	KELLDL	LYKA		349		
sp_P03958_ADA_MOUSE	281	VRFKNDK	ANYSLNTDDPLIFKSTLTDYQMTK	KD	MGFT	EEEFKRLNINA	AKSSFLPEE	EKKELLER	LYRE		350		
3MVI_Mouse	278	VRFKNDK	ANYSLNTDDPLIFKSTLTDYQMTK	KD	MGFT	EEEFKRLNINA	AKSSFLPEE	EKKELLER	LYRE		347		
sp_P00813_ADA_HUMAN	281	RLKNDQA	NYSLNTDDPLIFKSTLTDYQMTK		MGFT	EEEFKRLNINA	AKSSFLPEDEK	RELLDL	LYKA		350		
sp_P56658_ADA_BOVINE	351	Y	MPS	PASAEQCL							363		
1VFL_BOVINE	350	Y	MPS	PA-----							356		
sp_P03958_ADA_MOUSE	351	Y	Q	-----							352		
3MVI_Mouse	348	Y	Q	-----							349		
sp_P00813_ADA_HUMAN	351	Y	MPS	SASAGQNL							363		

**Supplementary Figure S1. PROMALS3D(1) alignment of human, bovine, and mouse ADA sequences and structures.** Yellow highlight, e.g. **G**, 1VFL/Bovine differs from Uniprot sequence and/or matches human residue at that position (8 total). Orange font, e.g. **S**, non-

identical residues across sequences. Blue highlight, e.g. **H**, metal binding residue. Grey shadow, e.g. **L**, residues involved in helix gate. Green shadow, e.g. **A**, residues missing from 7RTG structure (this study).



**Supplementary Figure S2. Crystal packing of HsADA1 monomer A.** *A*, cartoon representation of the crystal lattice. *B*, Packing of monomer A (*magenta*) with its symmetry mates (*grey*). A symmetry mate is overlaid with 3iar (*lime green*) shows incompatibility with monomer A residues Ala43-Ile50 (*yellow*). *C*, Michaelis-Menten kinetic parameters for C-terminally truncated HsADA1 following histidine tag cleavage. *D*, AlphaFold predictions for BtADA and HsADA1 colored from high confidence (*blue*) to low confidence (*red*) show low confidence in terminal residues only ordered in 3iar (*lime green*, circle). In addition, the closed conformation for the structural gate helix is predicted for BtADA, as visualized by superposition with 1vfl (arrow).



## References

1. Pei J, Grishin NV. PROMALS3D: multiple protein sequence alignment enhanced with evolutionary and three-dimensional structural information. *Methods Mol Biol.* 2014;1079:263-71. Epub 2013/10/31. doi: 10.1007/978-1-62703-646-7\_17. PubMed PMID: 24170408; PMCID: PMC4506754.