Supplemental material for "Structural and Mechanistic Studies on *Klebsiella pneumoniae* 2-Oxo-4-hydroxy-4-carboxy-5-ureidoimidazole Decarboxylase" by Jarrod B. French and Steven E. Ealick.

Figure S1. Ribbon diagram of unliganded KpOHCU decarboxylase colored by B-factor. Allantoin is shown in green for reference. The colors indicate the magnitude of the values from low (blue-cyan-green) to high (yellow-orange-red).

Figure S2. Sequence alignment of KpOHCU decarboxylase, *A. thaliana* OHCU decarboxylase (2Q37), and the OHCU decarboxylase from zebrafish (2O70). The secondary structure elements shown above the alignment correspond to those observed in KpOHCU decarboxylase and the blue arrows indicate residues responsible for making hydrogen bonding contacts to the ligand.

Figure S3. His67 movement in the active site. *A,* Superposition of unliganded KpOHCU decarboxylase (gold) and KpOHCU decarboxylase with allantoin bound (green). His67 is shifted to accommodate the ligand in the KpOHCU decarboxylase-allantoin complex structure. Modeling of the substrate, OHCU (*B*), and the reaction intermediate (*C*) showing two of the predicted low energy conformations of His67. One of the low energy conformers (blue) is positioned to deprotonate the hydroxyl of OHCU, while a second conformer (yellow) is poised to protonate the prochiral carbon of the reaction intermediate. Comparison of the modeled reactant (*B*) and intermediate (*C*) also reveals that, after release of the carboxy moiety, there is a shift of the imidazoline ring towards His67.

Figure S4. Structure and crystal packing of KpOHCU decarboxylase in tetragonal crystal form. Ribbon representations of two molecules are shown with a semi-transparent space fill model of allantoin to mark the active site. One molecule (green) packs into the active site of the other (blue) where helix 6 of the enzyme is seen in the unliganded (*P*1) and liganded structures.

Figure S5. Size exclusion chromatography of KpOHCU decarboxylase. A) Chromatogram of KpOHCU decarboxylase run on an ACTA Explorer FPLC with a HiLoad 26/60 Superdex prep grade G200 column showing the predicted molecular weight of KpOHCU decarboxylase (20 900 Da). The KpOHCU decarboxylase monomer calculated molecular weight is 20 802 Da. B) The standard curve used to predict the molecular weight. The standards used were HpxT (*K. pneumoniae* HIU hydrolase, tetramer), SpNic (*S. pneumoniae* nicotinamidase, tetramer), Nc2Pur (*Neurospora crassa* bifunctional purine biosynthetic enzyme, dimer), and GDH (Bovine glutamate dehydrogenase, hexamer).

Table S1. Results of DALI search for structural homology

PDB ID	Z-Score	RMSD	% identity	Description
2O70	19.1	2.4	22	OHCU Decarboxylase
2Q37	17.1	1.8	29	OHCU Decarboxylase
2O8I	14.1	2.8	23	Hypothetical Protein ATU2327

Figure S1.

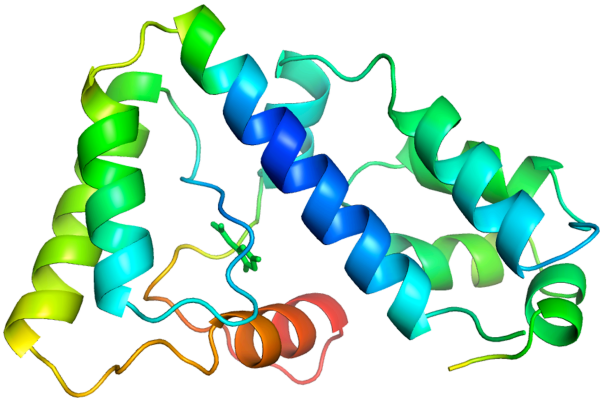


Figure S2.

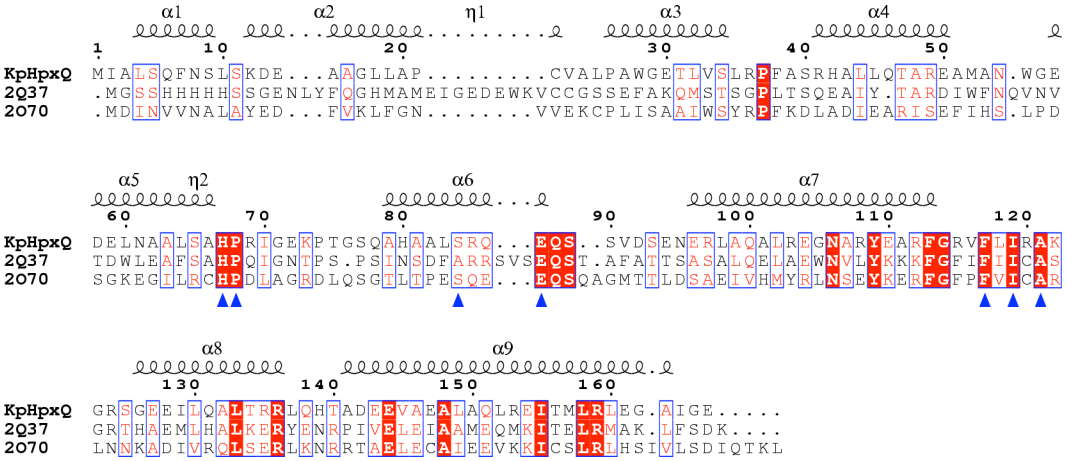

α1 α2 η1 α3 α4
KpHprQ MIALSQFNSLSKDE...AAGLLAP.....CVALPAWGETLVSLRPFASRHALLQTARDAMAN.WGE
2Q37 .MGSSHHHHHSSGENLYFQGHMAMEIGEDEWKVCCGSSEFAKQMSTSGPLTSQEAIY.TARDIWFNQVNV
2070 .MDINVVNALAYED...FVKLFGN.....VVEKCPLISAAIWSYRPFKDLADIEARISEFIHS.LPD
α5 η2 α6 α7
KpHprQ DELNAALSAHPRIGEKPTGSQAHAALSRQ...EQS..SVDSENERLAQALREGNARYEARFGRVFLIRAK
2Q37 TDWLEAFSAHPQIGNTPS.PSINSDFARRSVSEQST.AFATTSASALQELAEWNVLYKKKFGFIFIICAS
2070 SGKEGILRCHPDLAGRDLQSGTLTPESQE...EQSQAGMTTLDSAEIVHMYRLNSEYKERFGFPFVICAR
α8 α9
KpHprQ GRSGEEILQALTRRLQHTADEEVAEALAQLREITMLRLEG.AIGE.....
2Q37 GRTHAEMLHALKPRYENRPIVELEIAAMEQMKITELRMAK.LFSDK....
2070 LNNKADIVRQLSERLKNRRTAELECAIEEVKKICSLRLHSIVLSDIQTKL

Figure S3.

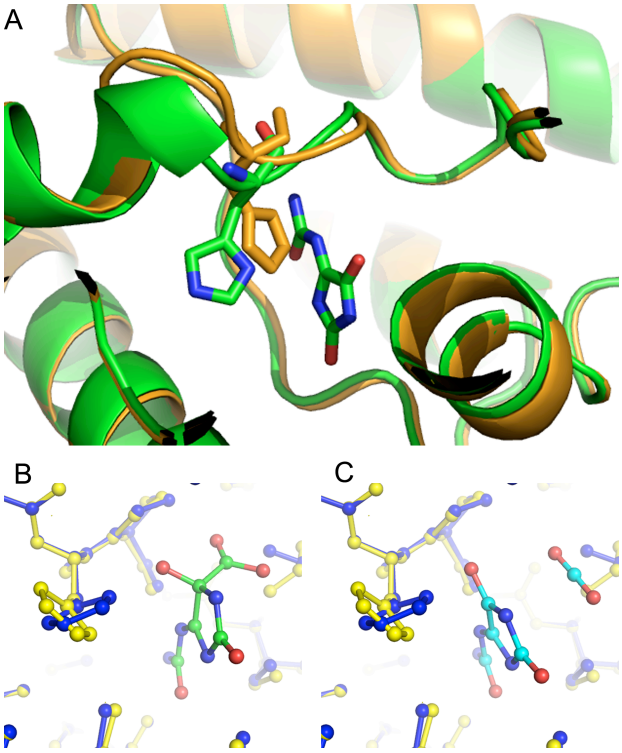


Figure S4.

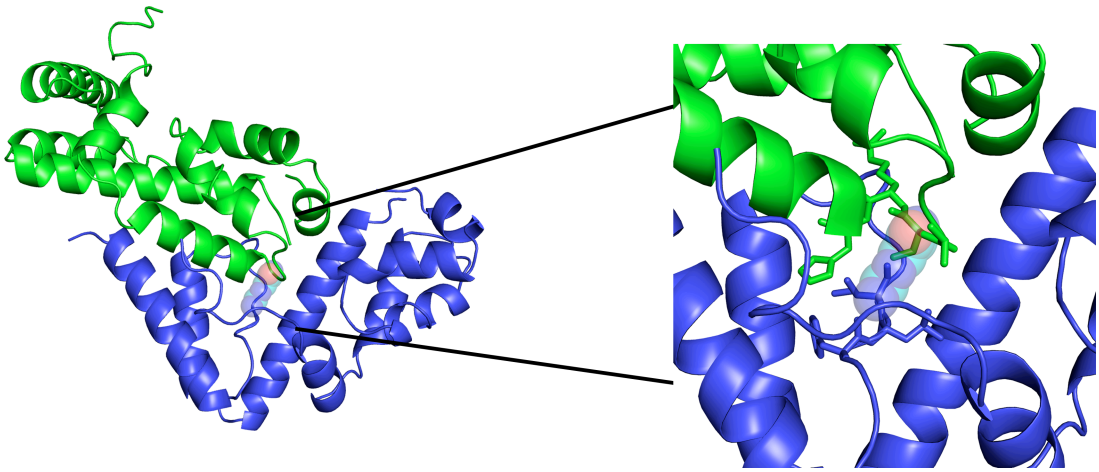
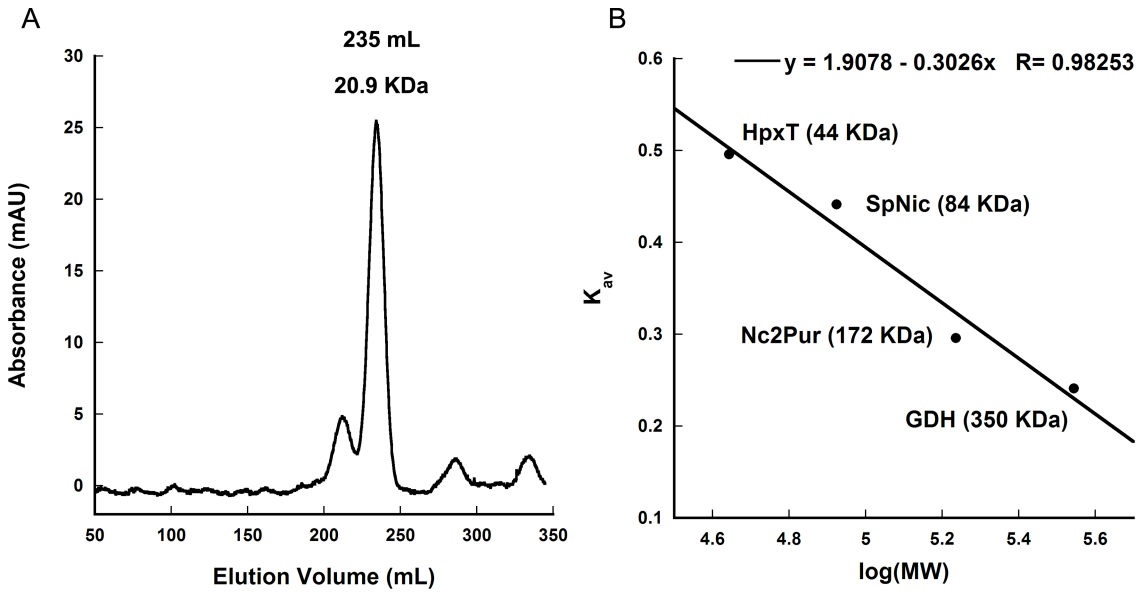


Figure S5.


A
235 mL
20.9 KDa
Absorbance (mAU)
Elution Volume (mL)
B
y = 1.9078 - 0.3026x R= 0.98253
HpxT (44 KDa)
SpNic (84 KDa)
Nc2Pur (172 KDa)
GDH (350 KDa)
K_av
log(MW)