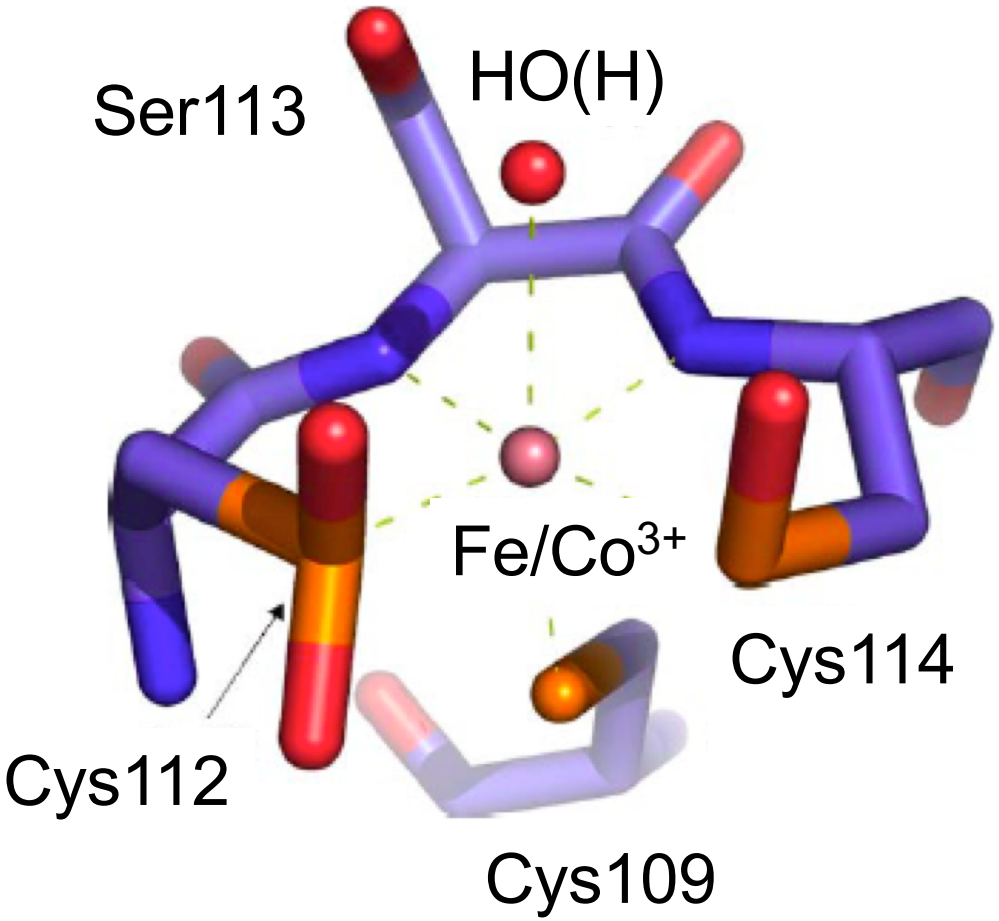
Acidity of Metal-bound Water

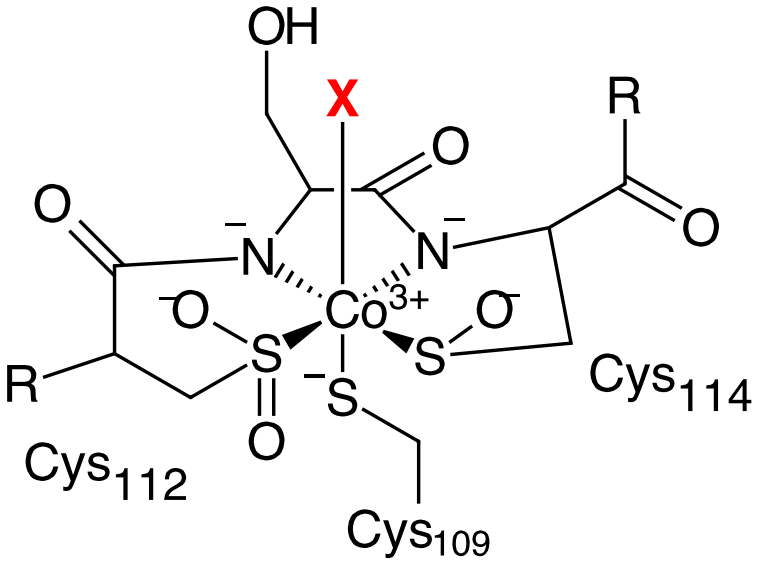
$$M(H_2O)_6^{n+} \Leftrightarrow M(H_2O)_5OH^{(n-1)+} + H^+$$

Ion	Radius(Å)	pK_a
K^+	1.52	14.5
Na^+	1.16	14.2
Ca^{2+}	1.14	12.8
Mg^{2+}	0.86	11.4
Mn^{2+}	0.97	10.6
Cd^{2+}	1.09	10.2
Fe^{2+}	0.92	9.5
Co^{2+}	0.88	9.6
Zn^{2+}	0.88	9.0
Al^{3+}	0.67	5.0
Fe^{3+}	0.78	2.2

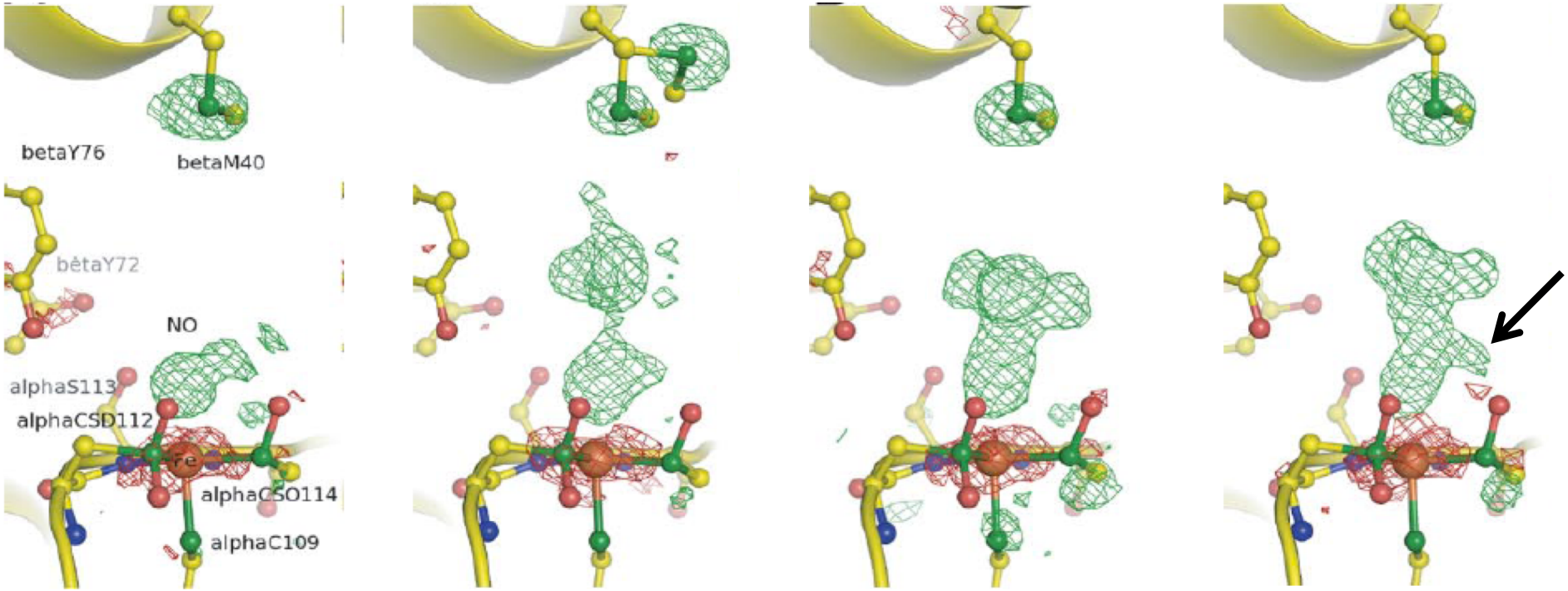
Increasing Lewis Acidity

Nitrile Hydratase





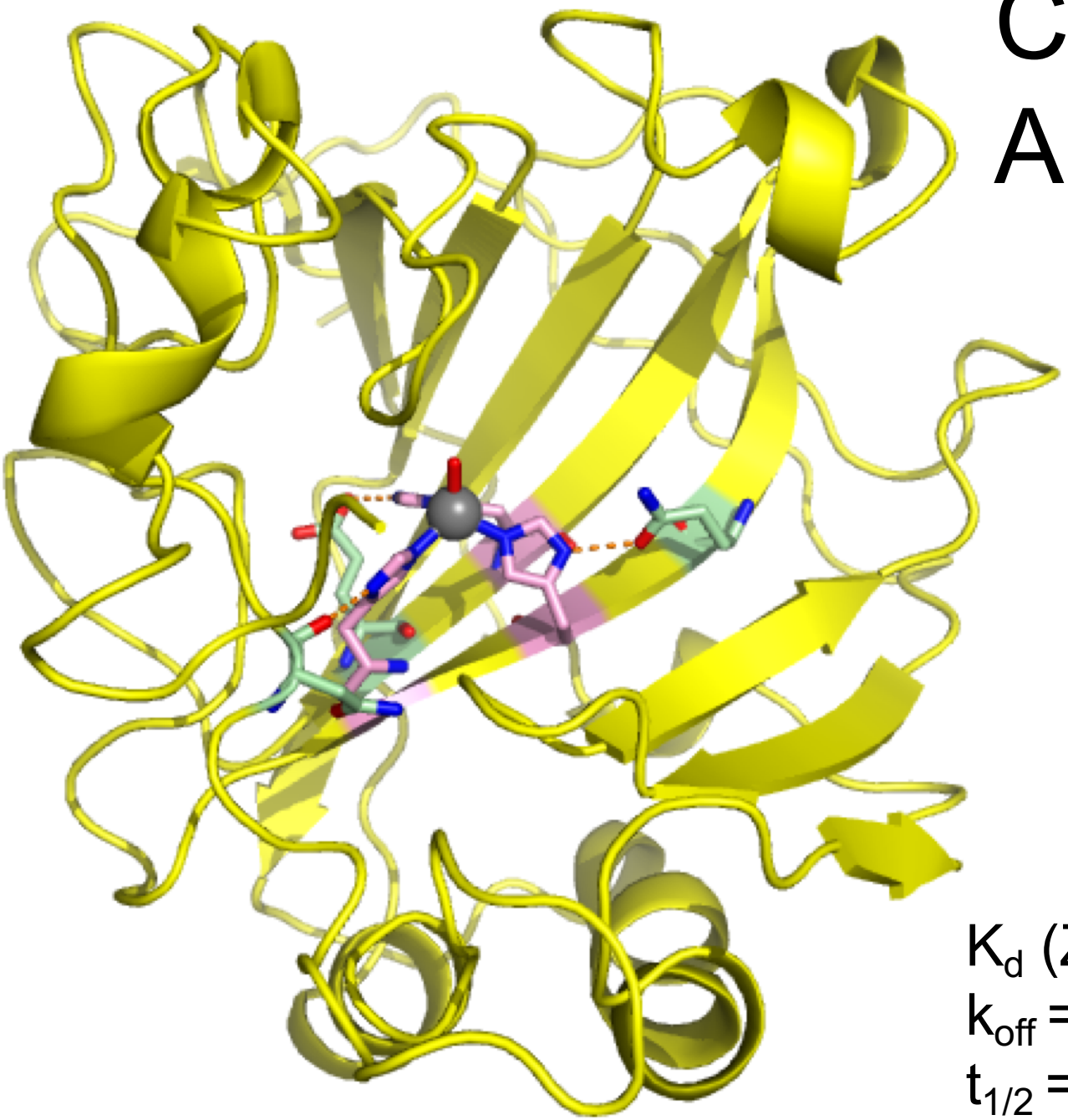
Time-Resolved Crystallography (t-butylisonitrile substrate)



t=0 t=18’ t=120’ t=440’

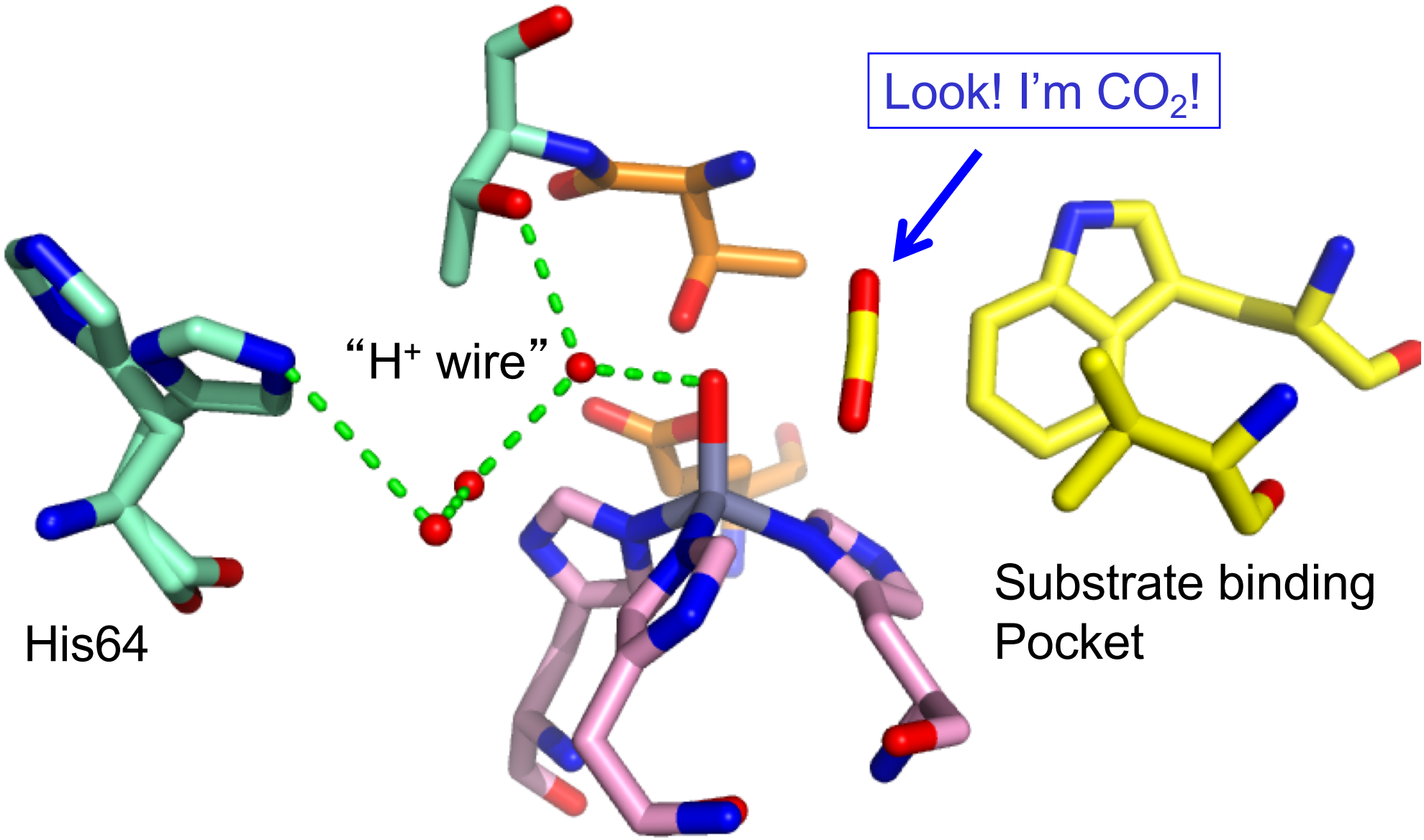
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 52, pp. 36617–36623, December 26, 2008

Carbonic Anhydrase



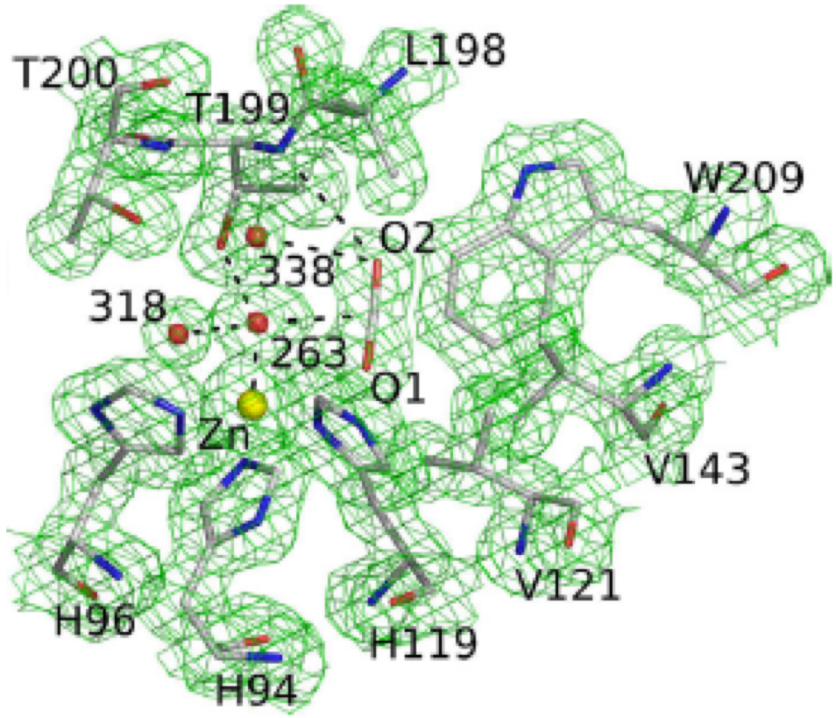
K_d (Zn^{2+}) = 4 pM
k_{off} = 8.5 x 10^{-5} min^{-1}
$t_{1/2}$ = 140 hr

Substrate Binding in Carbonic Anhydrase

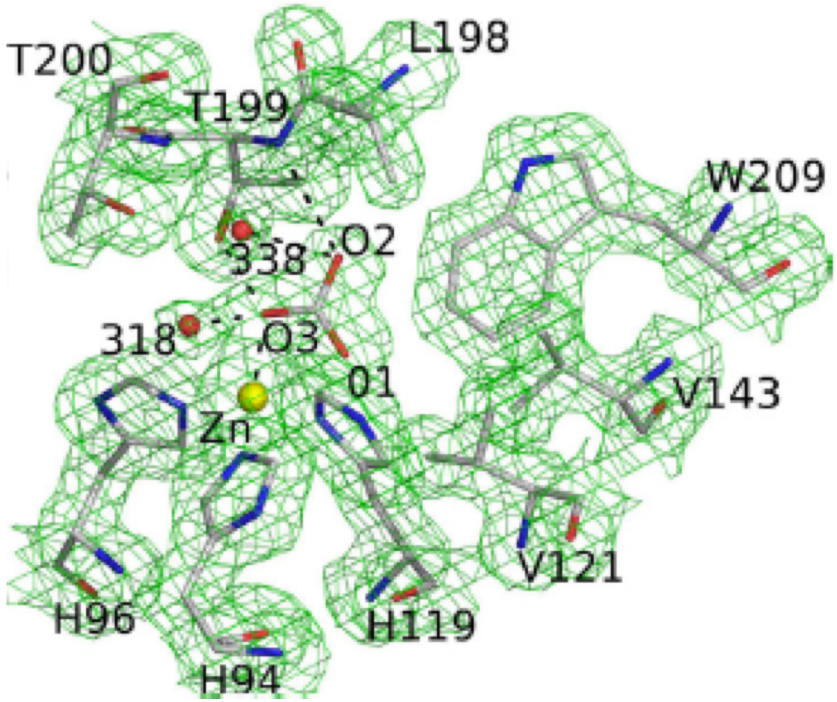


PNAS | June 30, 2009 | vol. 106 | no. 26 | 10609–10613

CA: Radiation to Reaction



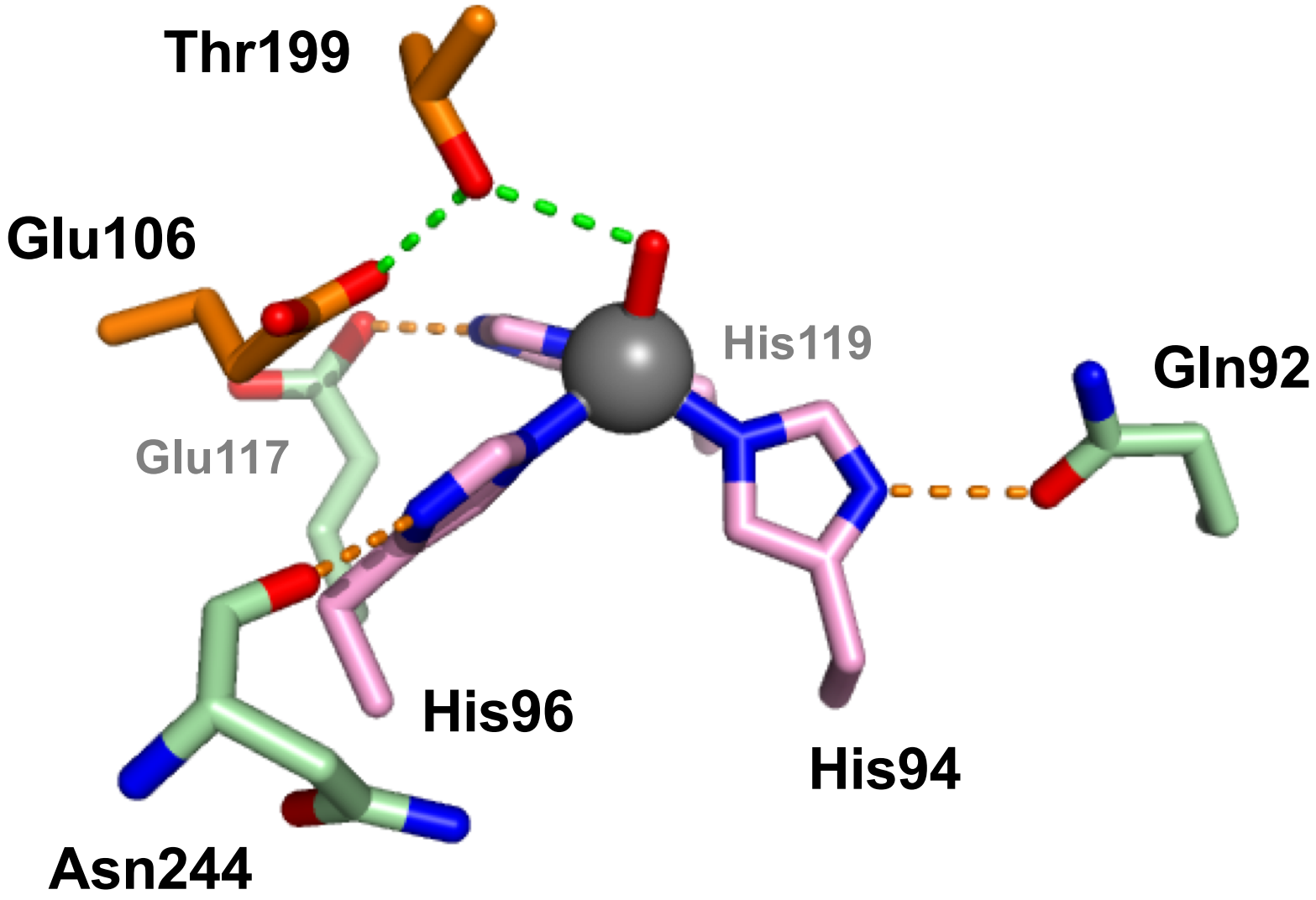
1st Data Set
CO_2 bound

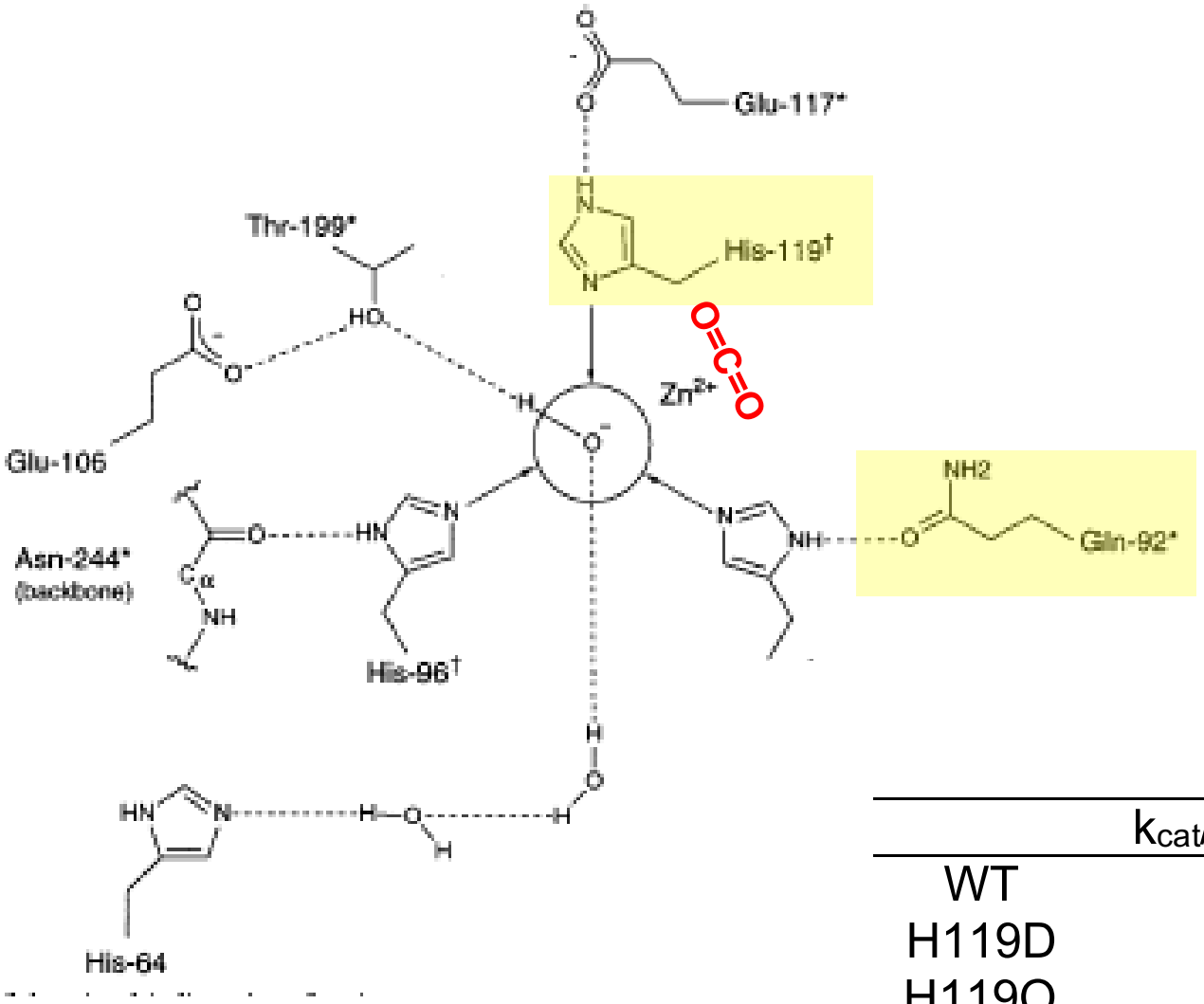


3rd Data Set
HCO_3^- bound

PNAS | June 30, 2009 | vol. 106 | no. 26 | 10609–10613

The Zinc Environment

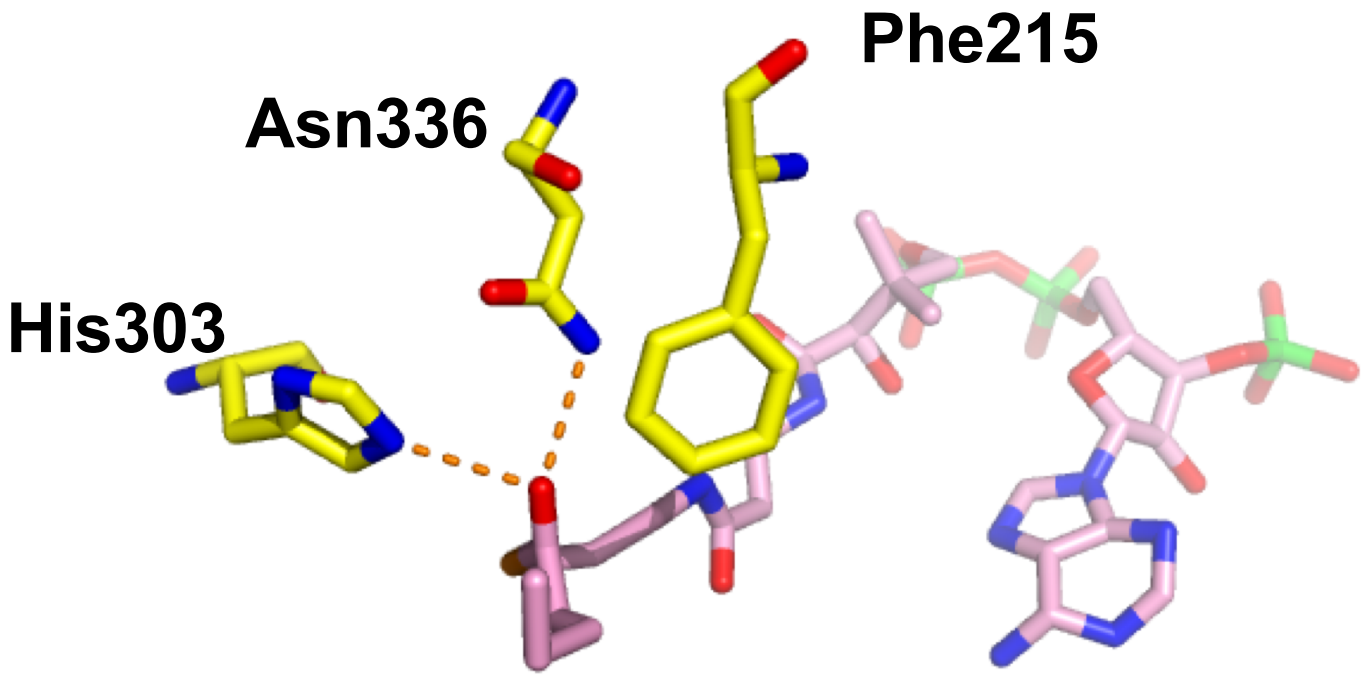




	k_{cat}/K_m ($M^{-1}s^{-1}$)	pK_a
WT	2500	6.8
H119D	830	8.6
H119Q	1490	6.9
Q92L	1750	6.4
Q92E	1362	7.7

Fierke (1994) *Biochemistry* 33, 15233

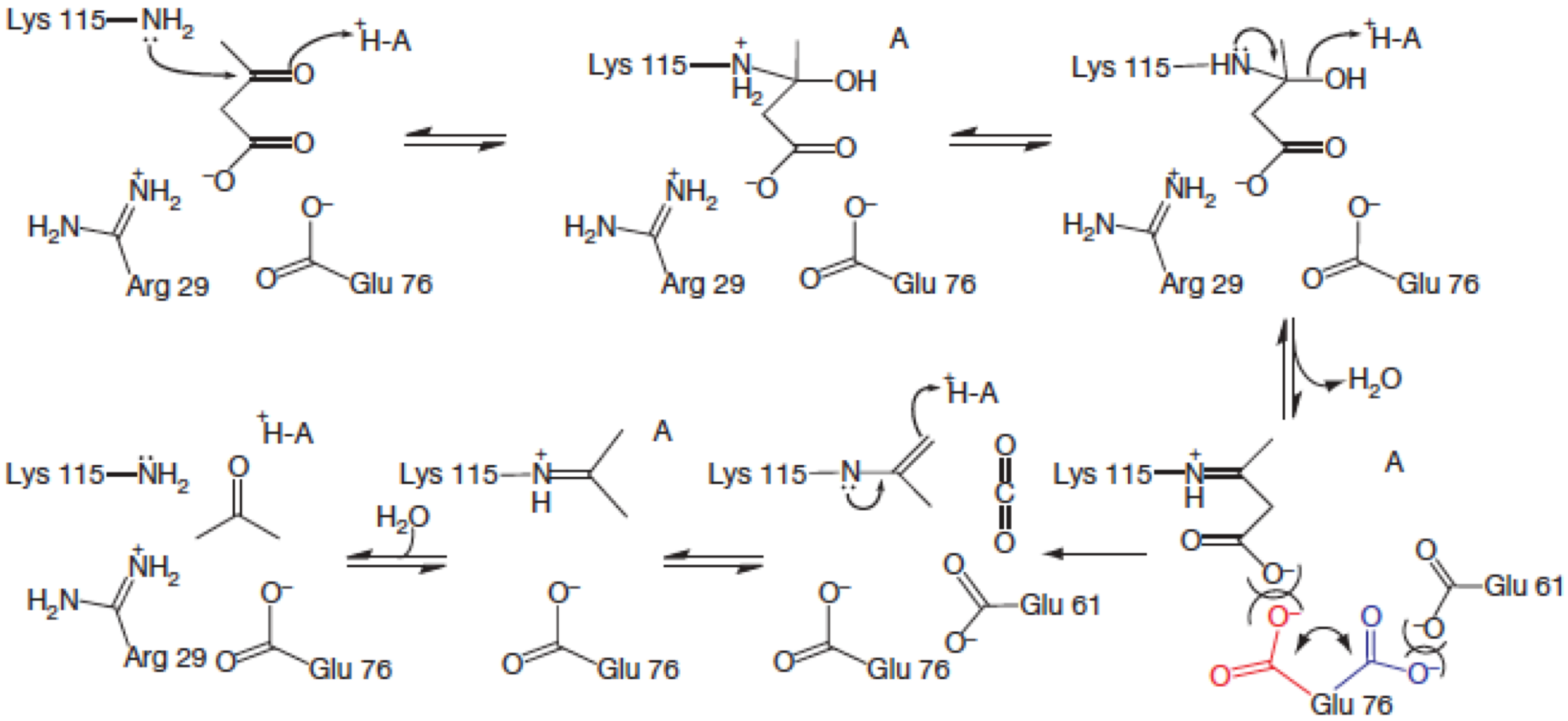
Malonyl-CoA Decarboxylase



Hexanoyl-CoA (substrate analog)

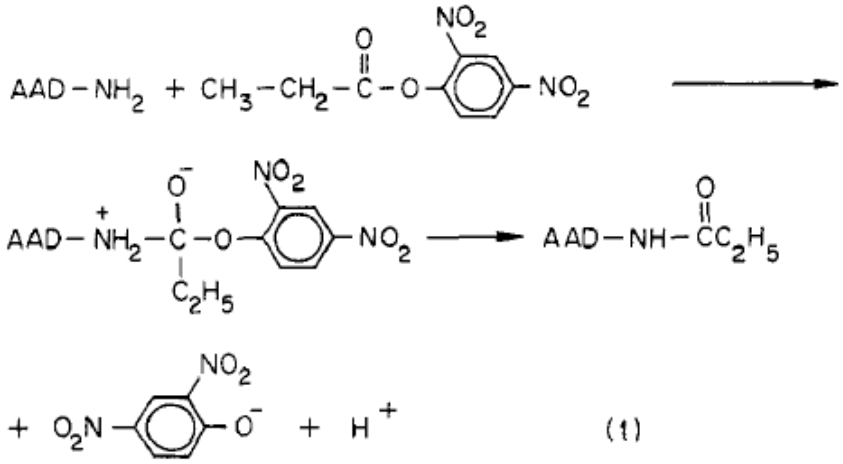
Ferrer et al. (1999) *Nat. Struct. Biol.* **6**, 775

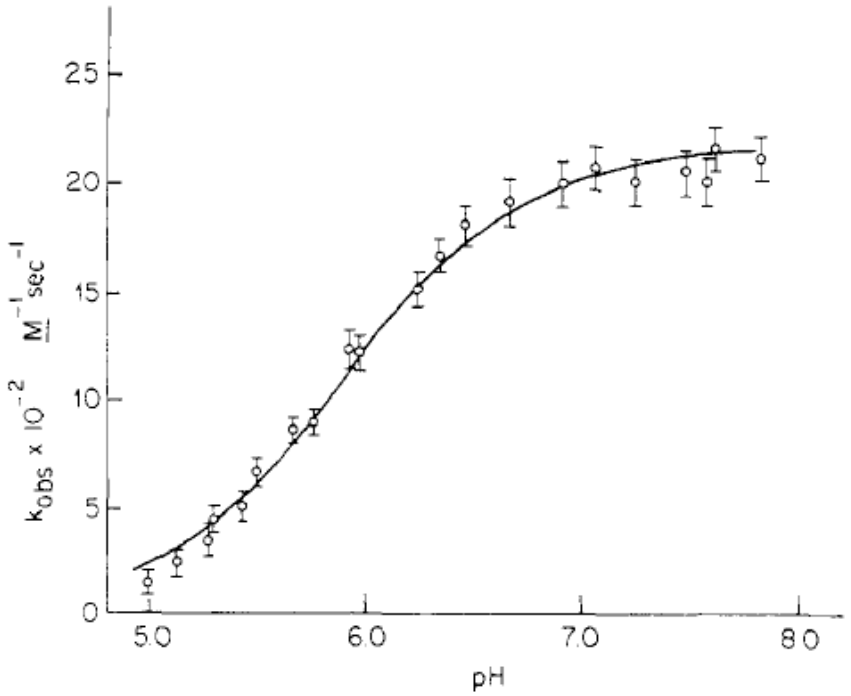
Proposed AADC Mechanism



Vol 459|21 May 2009|doi:10.1038/nature07938

Acetoacetate Decarboxylase





p*K* of the Lysine Amino Group at the Active Site of Acetoacetate Decarboxylase*

Donald E. Schmidt, Jr.,† and F. H. Westheimer‡

Acetoacetate Decarboxylase

Biochemistry **1996,** *35,* 41−46

Mechanism of the Reaction Catalyzed by Acetoacetate Decarboxylase. Importance of Lysine 116 in Determining the pK_a of Active-Site Lysine 115[†]

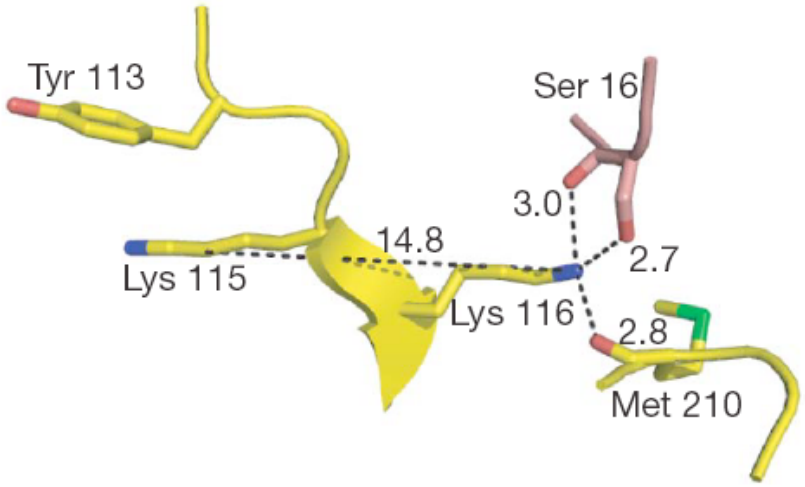
Lane A. Highbarger and John A. Gerlt*,[‡]

Table 1: Kinetic Constants for Wild Type (WT), Mutant, and Aminoethylated Samples of AAD at pH 5.95 and 25 °C[a]

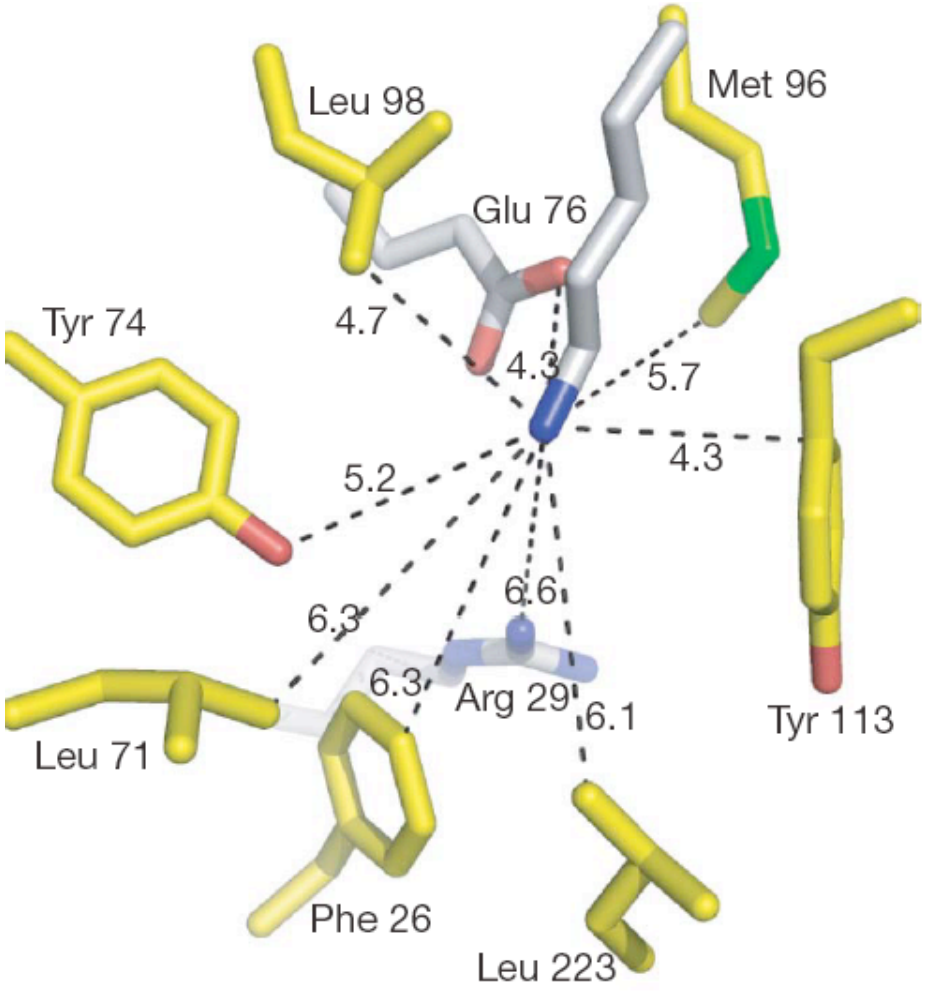
enzyme	k_{cat} (s^{-1})	K_m (mM)	pK_a
WT	1560 ± 25	8.2 ± 0.8	6.40 ± 0.02
K115C	0	NA[b]	
K115Q	0	NA[b]	
K115C-EA	119 ± 20	11.6 ± 1.2	
WT-EA	740 ± 25	7.2 ± 0.8	
K116C	38 ± 6	8.4 ± 1.4	> 9.2
K116N	30 ± 6	10.0 ± 2.4	> 9.2
K116R	302 ± 15	14.7 ± 1.6	6.27 ±
K116C-EA	410 ± 20	8.0 ± 0.4	6.17 ± 0.10

[a] Rates were determined from three independent trials using three substrate concentrations higher and three lower than the K_m. The rates for each substrate concentration were determined in duplicate. Kinetic constants were calculated from three independent determinations of k_{cat} and K_m. [b] Not applicable.

Acetoacetate Decarboxylase



K116 is not near K115



Non-polar environment around K115

Vol 459|21 May 2009|doi:10.1038/nature07938

Oxaloacetate Decarboxylase