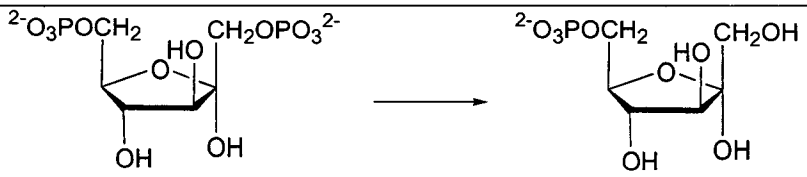
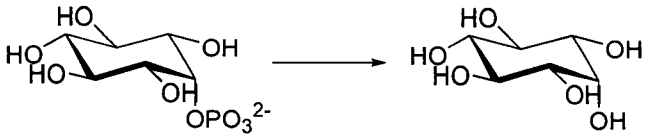
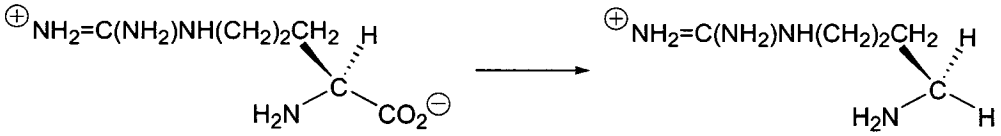
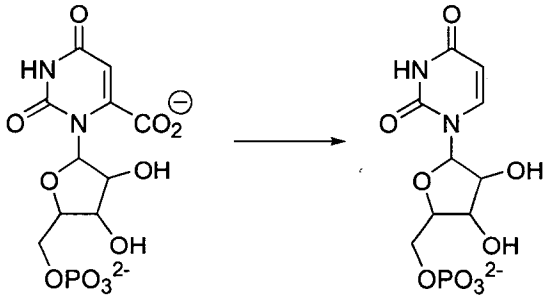
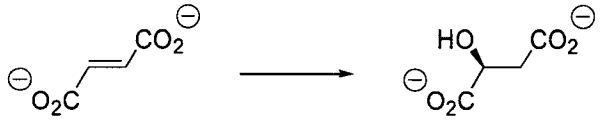
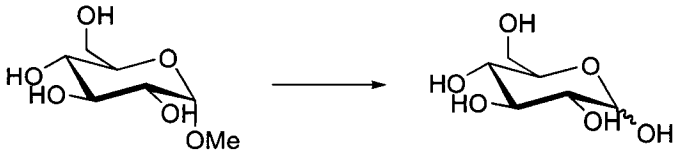
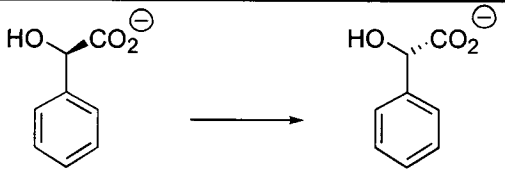
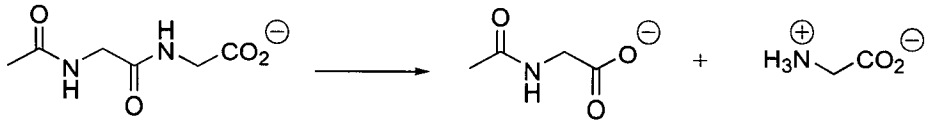
Table S1. Reactions catalyzed by the 24 Wolfenden enzymes.

Enzyme	Reaction
FBP	
PPA	Phosphorylase A+ 4 H_2O ⟶ 2 Phosphorylase B+ 4 Phosphate
IMP	
ADC	
ODC	
FUM	
SPA	
STN	DNA or RNA ⟶ Hydrolyzed DNA or RNA

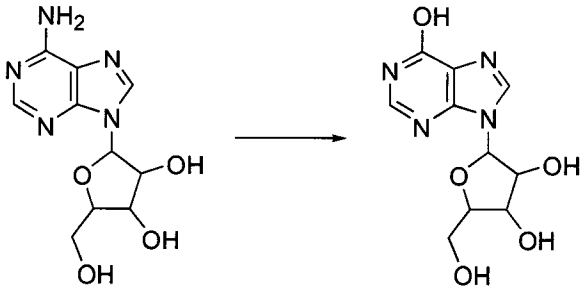
MRA



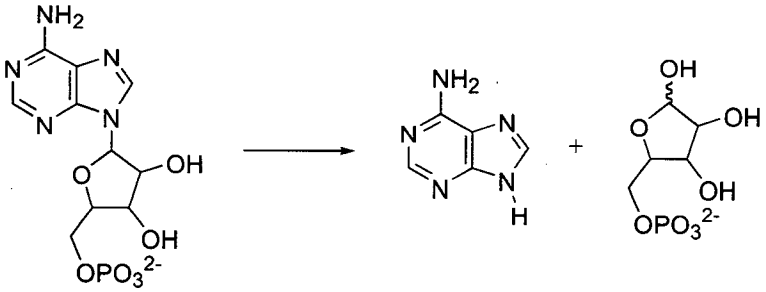
CPB



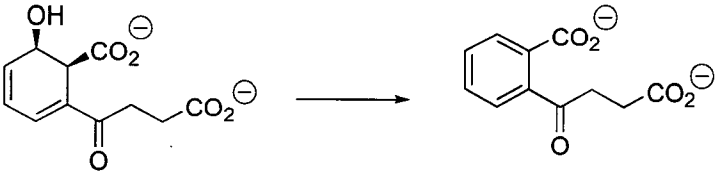
ADA



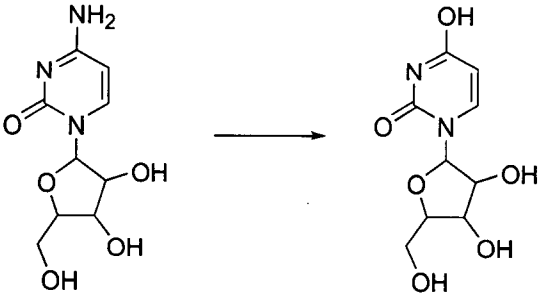
AMN

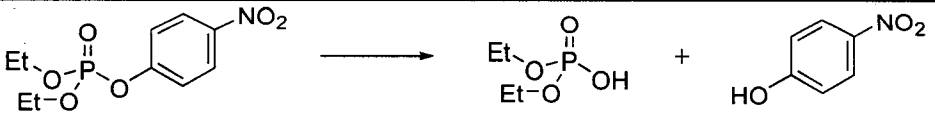


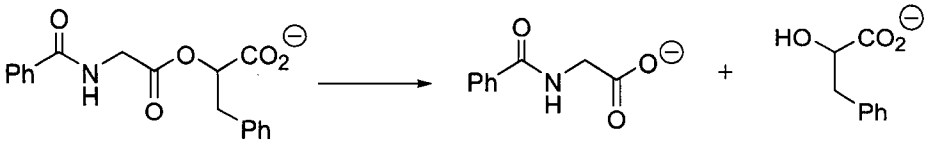
OSBS

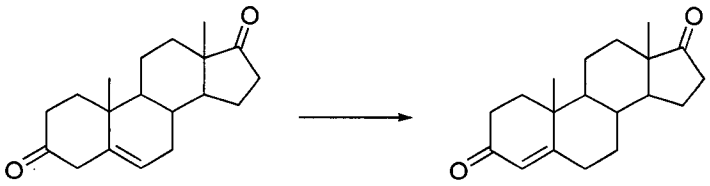


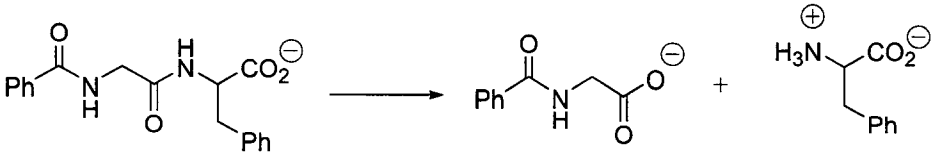
CDA

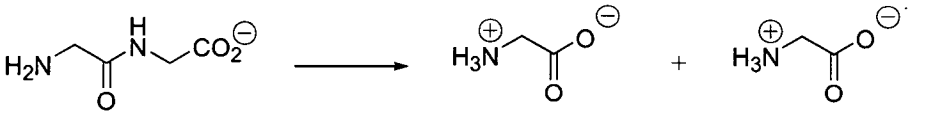


PTS 

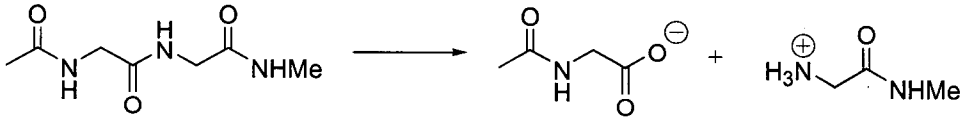
CPA(O) 

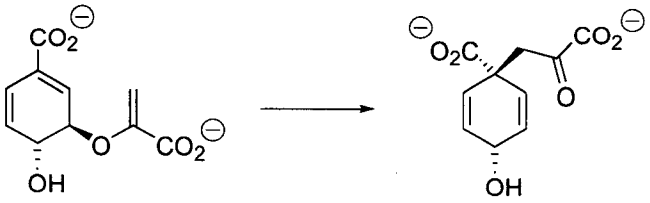
KSI 

CPA(N) 

ATD 

TIM 

ACE 

CMU 

CAN $CO_2 + H_2O \longrightarrow H_2CO_3$

CYC Suc-Ala-Ala-*cis*-Pro-Phe-*p*NA ⟶ Suc-Ala-Ala-*trans*-Pro-Phe-*p*NA

Table S2. Literatures cited for Table 1 in the text.

Enzyme	Literature
FBP	Kelley, N.; Giroux, E. L.; Lu, G.; Kantrowitz, E. R. Glutamic acid residue 98 is critical for catalysis in pig kidney fructose-1,6-bisphosphatase. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 848–852.
PPA	Taddei, N. et al. Aspartic-129 is an essential residue in the catalytic mechanism of the Low M(R) phosphotyrosine protein phosphatase. *FEBS Lett.* **1994**, *350*, 328–332.
IMP	Miller, D. J.; Beaton, M. W.; Wilkie, J.; Gani, D. The 6-OH group of D-inositol 1-phosphate serves as an H-bond donor in the catalytic hydrolysis of the phosphate ester by inositol monsphosphatase. *ChemBioChem* **2000**, *1*, 262–271.
ADC	Hanfrey, C.; Sommer, S.; Mayer, M. J.; Burtin, D.; Michael, A. J. Arabidopsis polyamine biosynthesis: absence of ornithine decarboxylase and the mechanism of arginine decarboxylase activity. *Plant Journal* **2001**, *27*, 551–560.
ODC	Houk, K. N.; Lee, J. K.; Tantillo, D. J.; Bahmanyar, S.; Hietbrink, B. N. Crystal structures of orotidine monophosphate decarboxylase: Does the structure reveal the mechanism of nature's most proficient enzyme? *ChemBioChem* **2001**, *2*, 113–118.
FUM	Rose, I. A.; Warms, J. V. B.; Kuo, D. J. Proton-transfer in catalysis by fumarase. *Biochemistry* **1992**, *31*, 9993–9999.
SPA	Sinnott, M. *Comprehensive Biological Catalysis: A Mechanistic Reference*; Academic Press: San Diego, 1998.
STN	Weber, D. J.; Meeker, A. K.; Mildvan, A. S. Interactions of the acid and base catalysts on staphylococcal nuclease as studied in a

	double mutant. *Biochemistry* **1991**, *30*, 6103–6114.
MRA	Schafer, S. L. et al. Mechanism of the reaction catalyzed by mandelate racemase: Structure and mechanistic properties of the D270N mutant. *Biochemistry* **1996**, *35*, 5662–5669.
CPB	Sinnott, M. *Comprehensive Biological Catalysis: A Mechanistic Reference*; Academic Press: San Diego, 1998.
ADA	Wilson, D. K.; Quiocho, F. A. A pre-transition-state mimic of an enzyme — X-Ray structure of adenosine-deaminase with bound 1-deazaadenosine and Zinc-activated water. *Biochemistry* **1993**, *32*, 1689–1694.
AMN	Sinnott, M. *Comprehensive Biological Catalysis: A Mechanistic Reference*; Academic Press: San Diego, 1998.
OSBS	Babbitt, P. C. et al. The enolase superfamily: A general strategy for enzyme-catalyzed abstraction of the alpha-protons of carboxylic acids. *Biochemistry* **1996**, *35*, 16489–16501.
CDA	Xiang, S.; Short, S. A.; Wolfenden, R.; Carter, C. W. Jr. The structure of the cytidine deaminase product complex provides evidence for efficient proton transfer and ground-state destabilization. *Biochemistry* **1997**, *36*, 4768–4774.
PTS	Lewis, V. E.; Donarski, W. J.; Wild, J. R.; Raushel, F. M. Mechanism and stereochemical course at phosphorus of the reaction catalyzed by a bacterial phosphotriesterase. *Biochemistry* **1988**, *27*, 1591–1597.
CPA(O)	Alvarez-Santos, S.; Gonzalez-Lafont, A.; Lluch, J. M.; Oliva, B.; Aviles, F. X. Theoretical study of the role of arginine 127 in the water-promoted mechanism of peptide cleavage by carboxypeptidase A. *New J. Chem.* **1998**, *22*, 319–325.

KSI	Park, H.; Merz, K. M. Jr. Molecular dynamics and quantum chemical studies on the catalytic mechanism of Delta(5)-3-ketosteroid isomerase: The catalytic diad versus the cooperative hydrogen bond mechanism. *J. Am. Chem. Soc.* **2003**, *125*, 901–911.
CPA(N)	Sinnott, M. *Comprehensive Biological Catalysis: A Mechanistic Reference*; Academic Press: San Diego, 1998.
ATD	Silvaggi, N. R.; Anderson, J. W.; Brinsmade, S. R.; Pratt, R. F.; Kelly, J. A. The crystal structure of phosphonate-inhibited D-Ala–D-Ala peptidase reveals an analogue of a tetrahedral transition state. *Biochemistry* **2003**, *42*, 1199–1208.
TIM	Sinnott, M. *Comprehensive Biological Catalysis: A Mechanistic Reference*; Academic Press: San Diego, 1998.
ACE	Riordan, F.; Harper, J. W.; Martin, M. The catalytic mechanism of angiotensin converting enzyme and related zinc enzymes. *J. Cardiovasc. Pharmacol.* **1986**, *8*, S29–S34.
CMU	Lee, Y. S.; Worthington, S. E.; Krauss, M.; Brooks, B. R. Reaction mechanism of chorismate mutase studied by the combined potentials of quantum mechanics and molecular mechanics. *J. Phys. Chem. B* **2002**, *106*, 12059–12065.
CAN	Mauksch, M.; Brauer, M.; Weston, J.; Anders, E. New insights into the mechanistic details of the carbonic anhydrase cycle as derived from the model system $[(NH_3)_3Zn(OH)]^+/CO_2$: How does the H_2O/HCO_3^- replacement step occur? *ChemBioChem* **2001**, *2*, 190–198.
CYC	Fanghänel, J. Enzymatic catalysis of the peptidyl-prolyl bond rotation: Are transition state formation and enzyme dynamics directly linked? *Angew. Chem., Int. Ed.* **2003**, *42*, 490–492.

Table S3. Uncatalyzed reaction rates reported by Wolfenden.

Hydrolase	FBP	PPA	IMP	SPA	STN
k_{uncat} (s^{-1})	2.0×10^{-20}	2.0×10^{-20}	2.0×10^{-20}	1.9×10^{-15}	1.7×10^{-13}
Hydrolase	CPB	ADA	AMN	CDA	PTS
k_{uncat} (s^{-1})	1.8×10^{-11}	1.8×10^{-10}	1.0×10^{-11}	3.2×10^{-10}	7.5×10^{-9}
Hydrolase	CPA(O)	CPA(N)	ATD	ACE	CAN
k_{uncat} (s^{-1})	2.7×10^{-10}	3.0×10^{-9}	1.5×10^{-9}	1.1×10^{-9}	1.3×10^{-1}
Isomerase	MRA	KSI	TIM	CMU	CYC
k_{uncat} (s^{-1})	3.0×10^{-13}	1.7×10^{-7}	4.3×10^{-6}	2.6×10^{-5}	2.8×10^{-2}
Lyase	ADC	ODC	FUM	OSBS	
k_{uncat} (s^{-1})	2.0×10^{-17}	2.8×10^{-16}	1.1×10^{-14}	1.6×10^{-10}	

Table S4. Intrinsic k_{uncat} abstracted from the 24 Wolfenden enzymes.

Enzyme	Intrinsic k_{uncat} (s^{-1})	Reaction type, functional group, transformation
FBP+PPA+IMP	2.0×10^{-20}	Hydrolyze phosphoric monoester
ADC+ODC*	1.5×10^{-16}	Decarboxylate
FUM	1.1×10^{-14}	Hydrolyze C=C
SPA	1.9×10^{-15}	Hydrolyze O–glycosyl
STN	1.7×10^{-13}	Hydrolyze DNA or RNA
MRA	3.0×10^{-13}	Racemize acids and derivatives
CPB+CPA+ATD+ACE*	1.4×10^{-9}	Hydrolyze peptide bonds
ADA+CDA*	2.5×10^{-10}	Hydrolyze C–N (not peptide bonds)
AMN	1.0×10^{-11}	Hydrolyze N-glycosyl
OSBS	1.6×10^{-10}	Cleave C–O
PTS	7.5×10^{-9}	Hydrolyze phosphoric triester
CPA	2.7×10^{-10}	Hydrolyze carboxylic ester bond
KSI	1.7×10^{-7}	Transpose C=C
TIM	4.3×10^{-6}	Interconvert aldehyde and ketone
CMU	2.6×10^{-5}	Claisen rearrangement
CAN	1.3×10^{-1}	Hydration of CO_2
CYC	2.8×10^{-2}	*Cis–trans* proline isomerization

*Mean value is taken as intrinsic k_{uncat}.

Table S5. PDB codes for the X-ray crystal structure of enzyme–substrate complexes and ΔSASA calculated with Grasp*.

PDB code	ΔSASA (Å^2)	PDB code	ΔSASA (Å^2)	PDB code	ΔSASA (Å^2)
1A05	208	1H61	341	1TYP	176
1AWB	266	1H62	310	1UAE	577
1BK0	442	1H7F	286	1VTK	338
1DWP	121	1H7G	326	2VTK	277
1F80	452	1H7H	312	1XYB	218
1FAG	331	1HCB	116	2AK3	292
1FC4	171	1IMA	257	2MBR	357
1GDG	236	1IMB	271	3CEV	214
1GEX	237	1JAY	605	7DFR	400
1GEY	359	1LBF	374	9RUB	320
1GN8	415	1NGS	234	1QJC	358
1PBE	175				

*Nicolls, A., Sharp, K. A., Honig, B., Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins Struct. Funct. Genet,* **11**, 281–296 (1991). **For the 34 complexes, ΔSASA = 305±111 Å^2.