Examples of Studies on Enzymatic Reactivity

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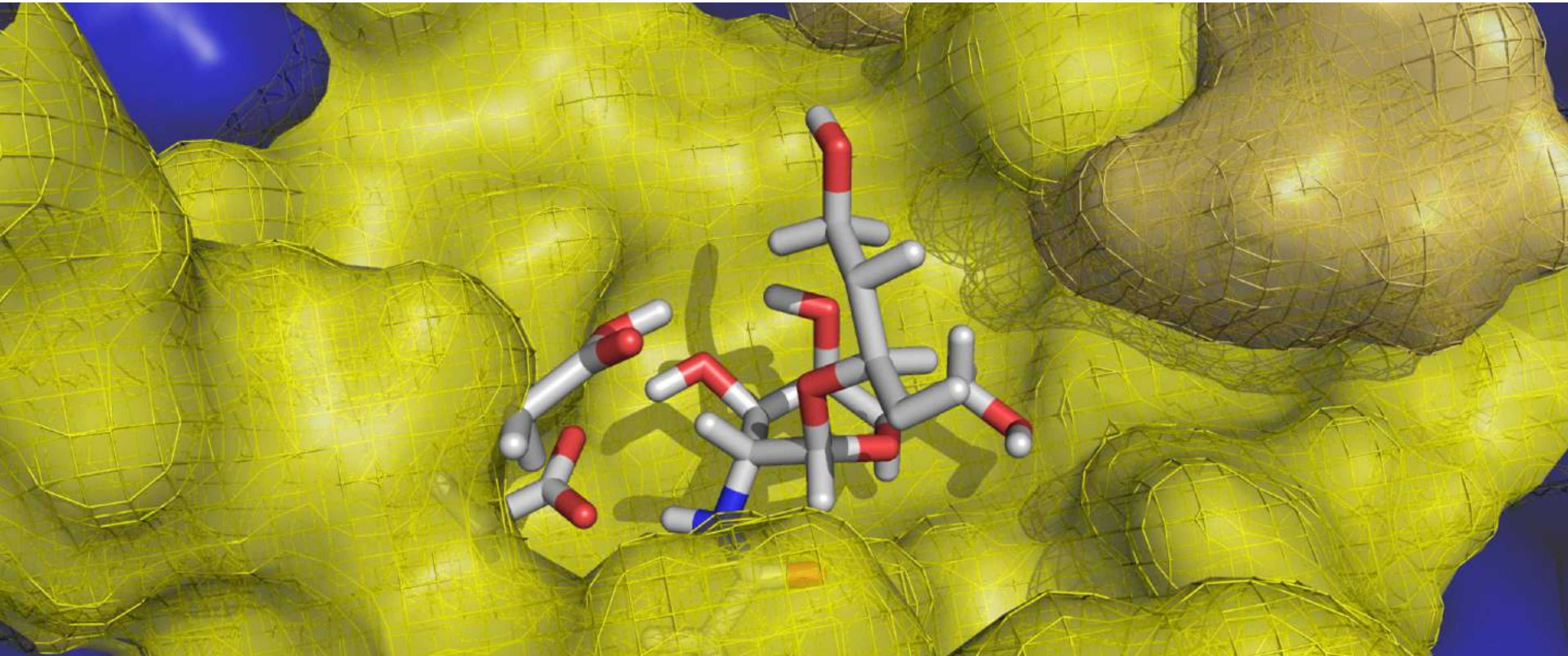
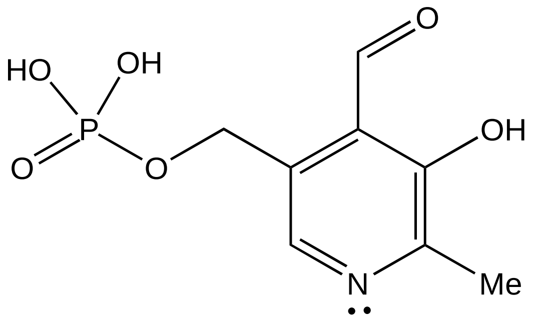
Department of Chemistry and Biochemistry
University of Porto
Portugal



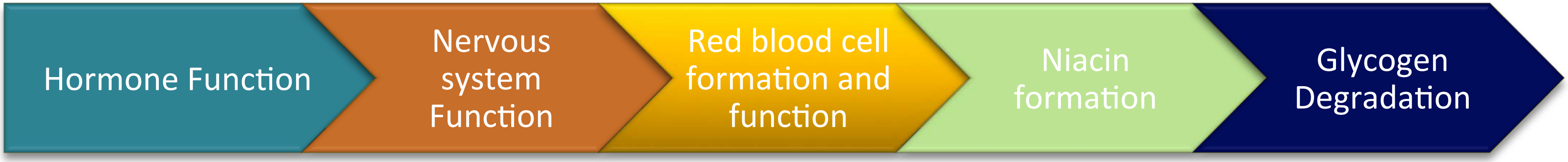
EXAMPLE: MECHANISM OF ACTIVATION OF PLP-DEPENDENT ENZYMES

PLP-Dependent Enzymes

PLP is a ***cofactor*** *that plays a vital role in human physiology and has associated over 3% of all enzymes, comprising over 140 different enzymatic activities.*



Pyridoxal Phosphate (PLP)



Hormone Function

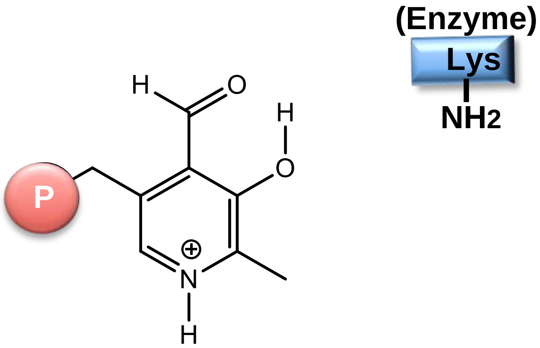
Nervous system Function

Red blood cell formation and function

Niacin formation

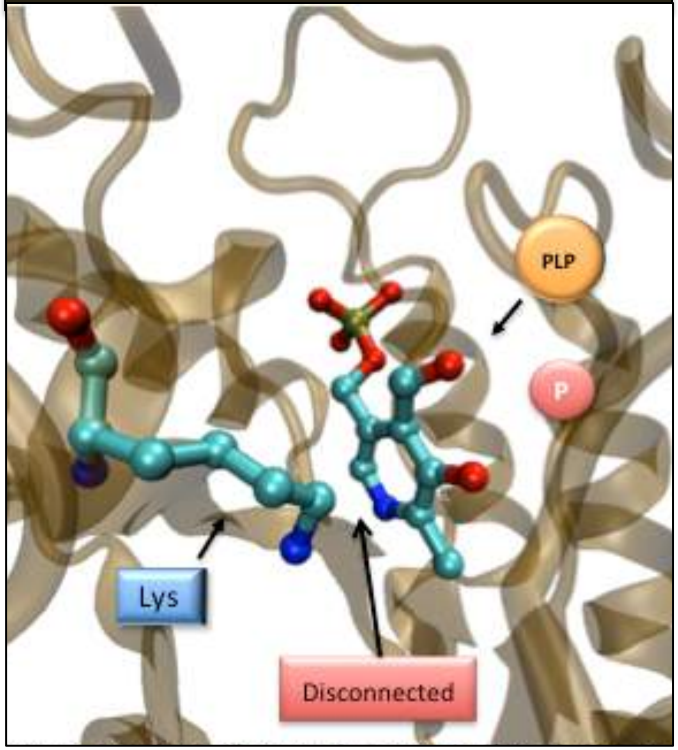
Glycogen Degradation

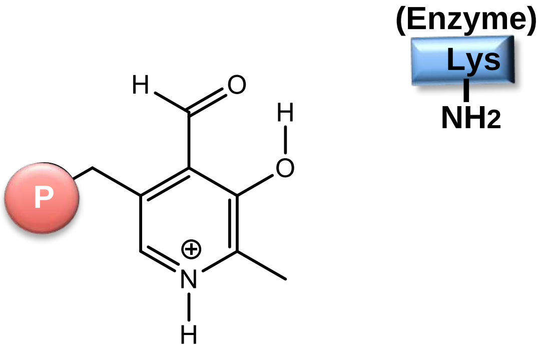
PLP-dependent enzymes



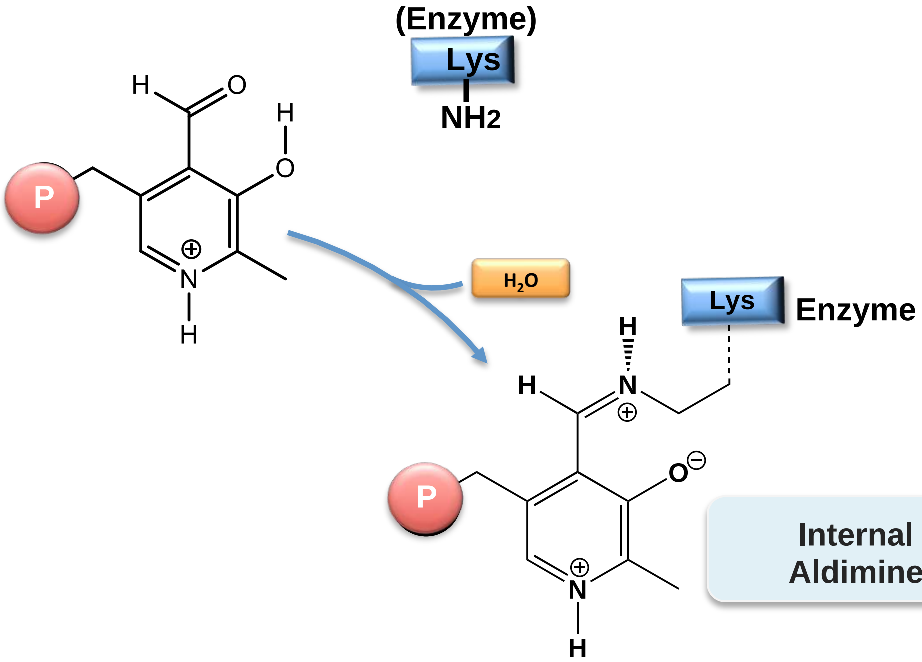
PLP-dependent enzymes

PDB **2OO0** – Initial State





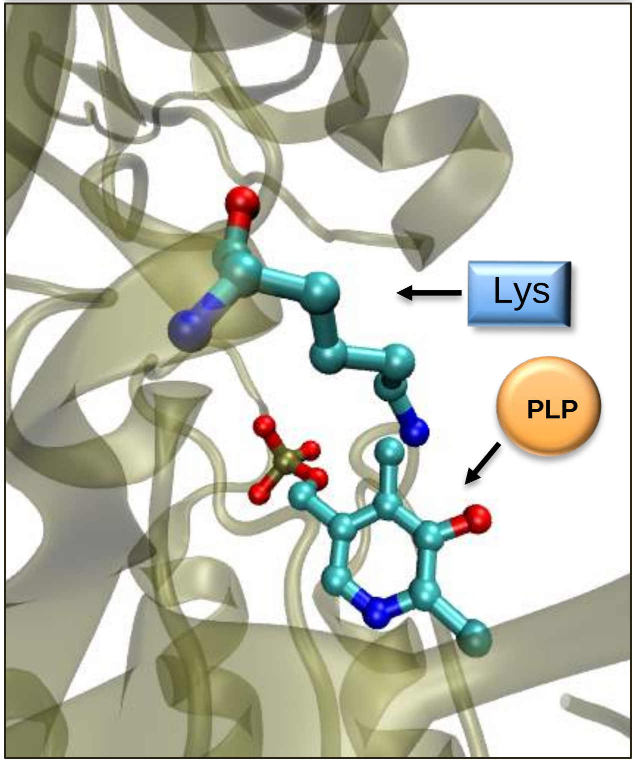
PLP-dependent enzymes



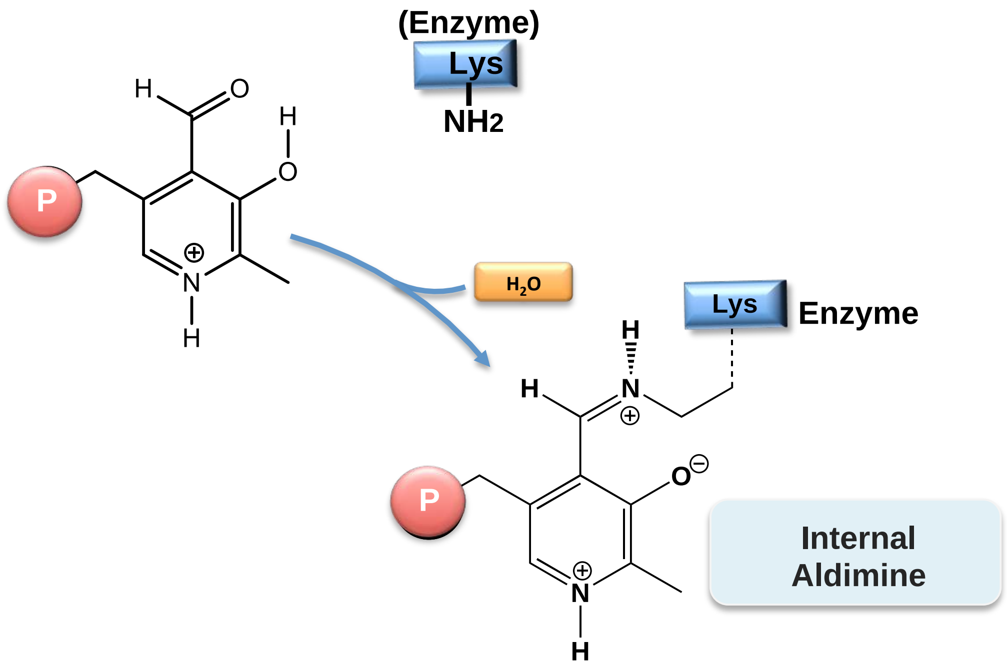
Internal Aldimine

PLP-dependent enzymes

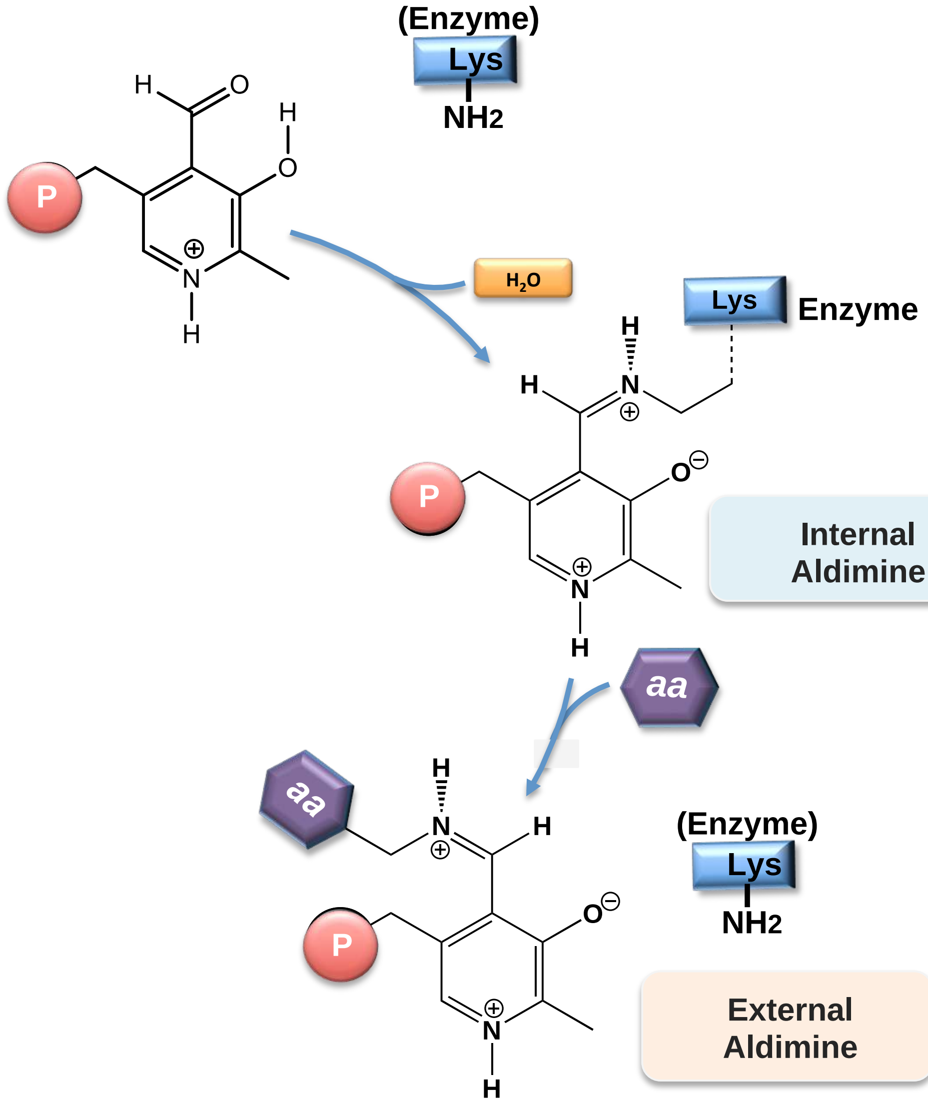
PDB: **7ODC** – Internal Aldimine



Counts, K. G.; Wong, I.; Oliveira, M. A. *Biochemistry* **2007**, *46*, 379-386.



PLP-dependent enzymes

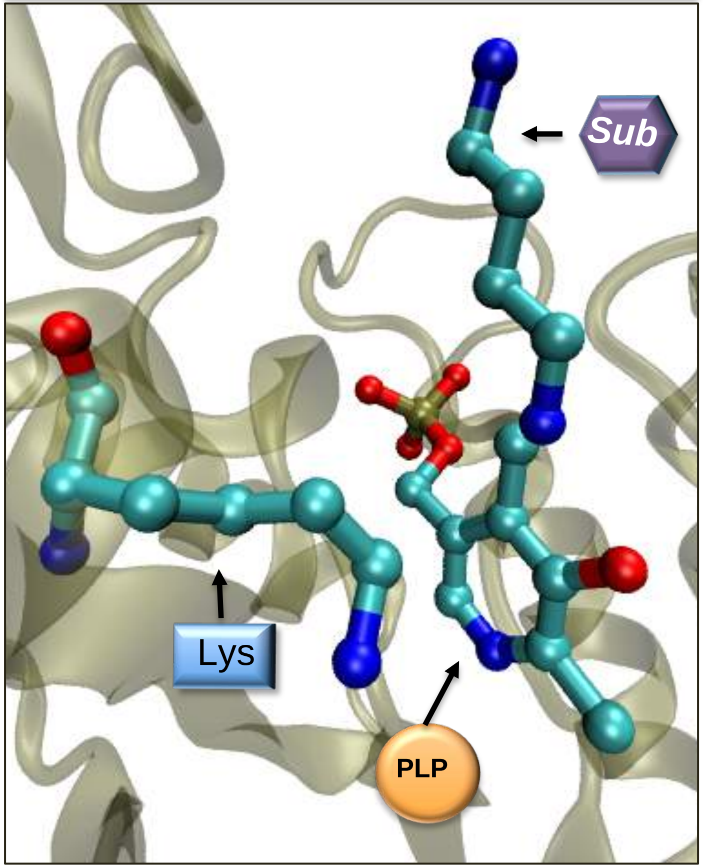


Internal Aldimine

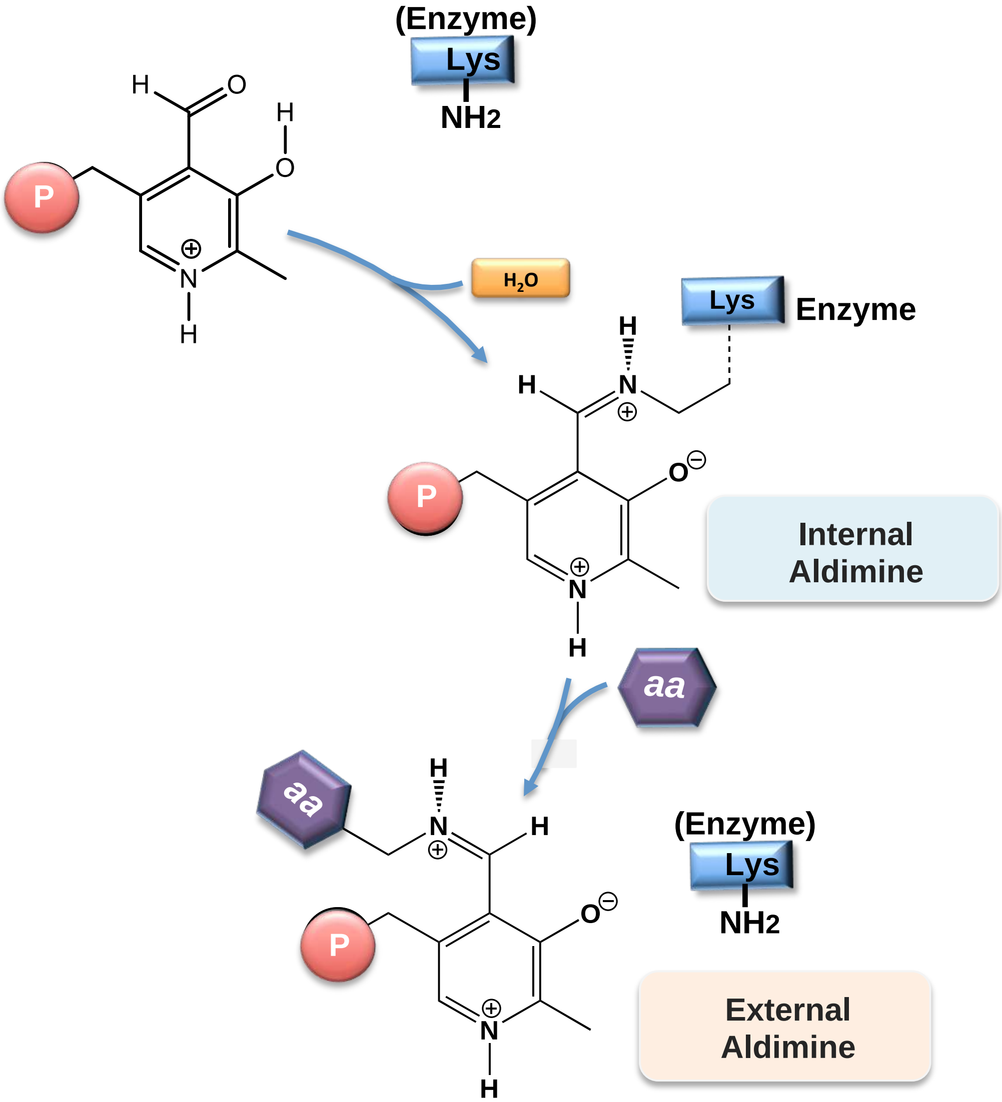
External Aldimine

PLP-dependent enzymes

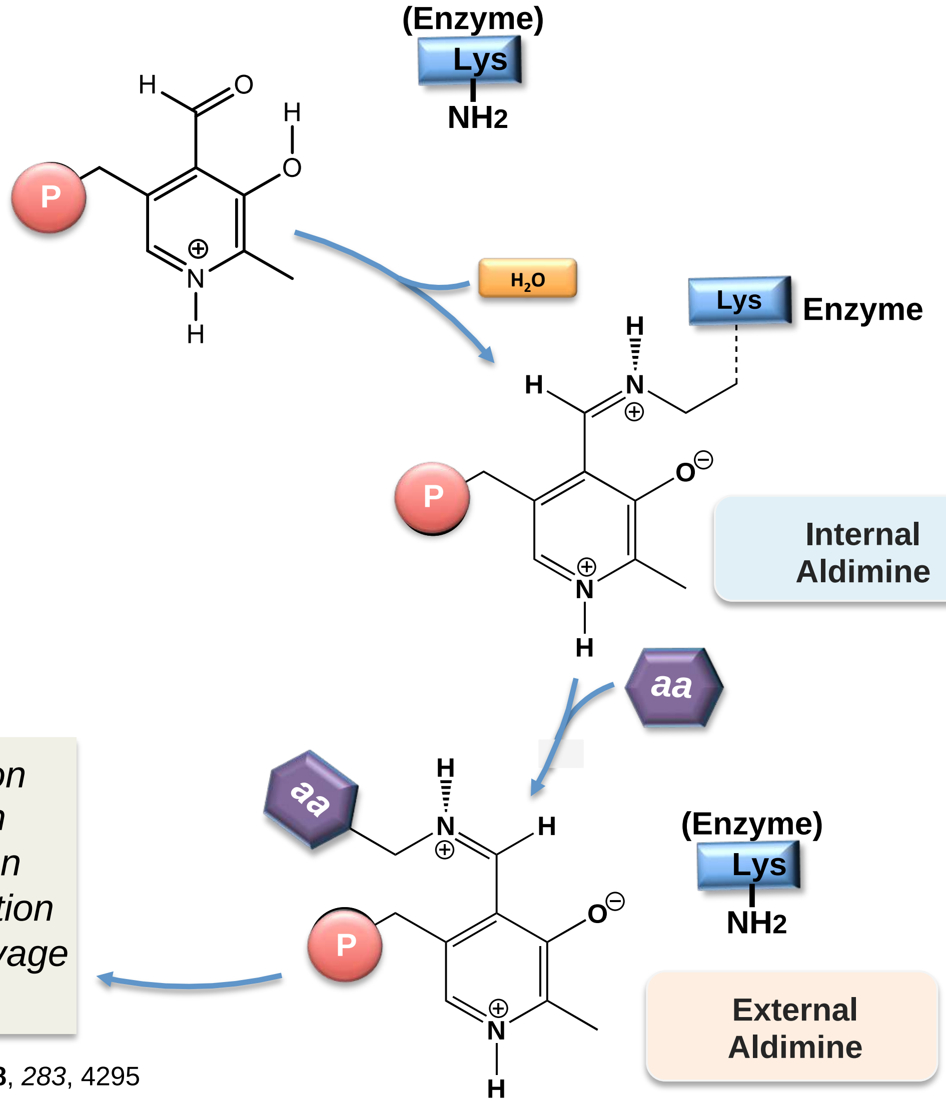
PDB: **1F3T** – External Aldimine



Jackson, L. K.; Brooks, H. B.; Osterman, A. L.; Goldsimith, E.; Phillips, M. *Biochemistry* **2000**, *39*, 11247–11257.



PLP-dependent enzymes



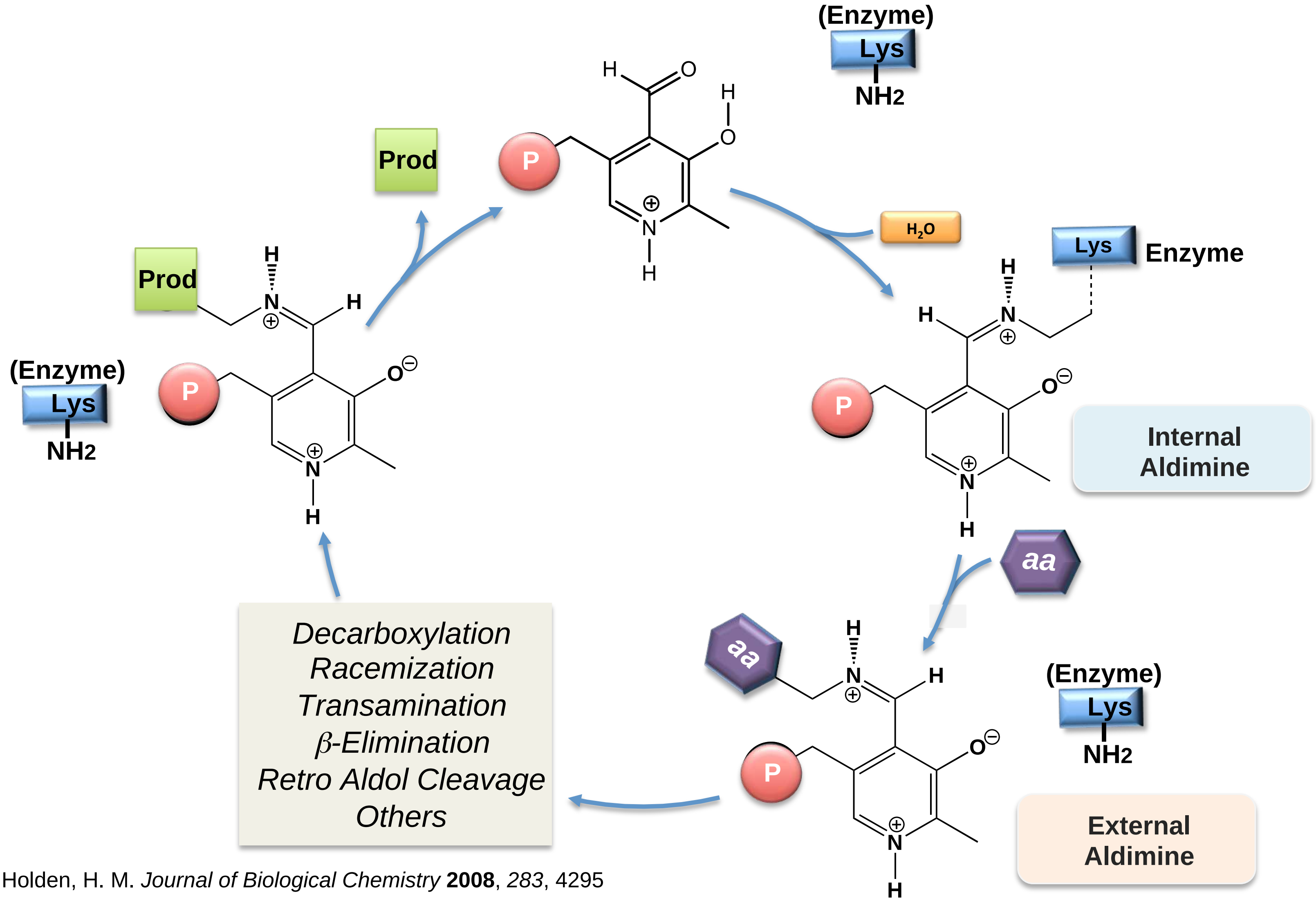
Internal Aldimine

External Aldimine

Decarboxylation
Racemization
Transamination
β and γ- Elimination
Retro Aldol Cleavage
Other

Cook, P. D.; Holden, H. M. *Journal of Biological Chemistry* **2008**, *283*, 4295-4303

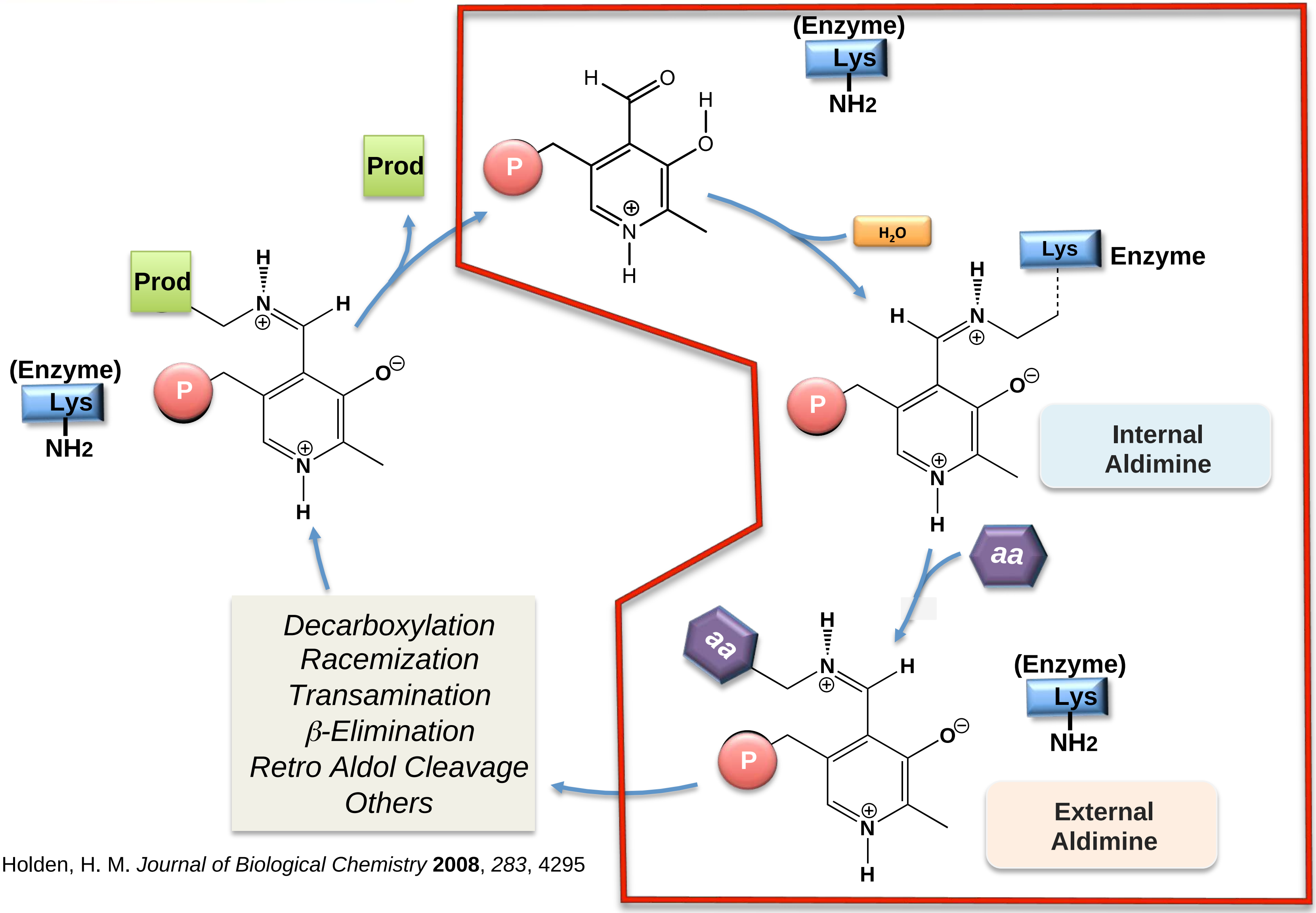
PLP-dependent enzymes



Decarboxylation
Racemization
Transamination
β-Elimination
Retro Aldol Cleavage
Others

Cook, P. D.; Holden, H. M. *Journal of Biological Chemistry* **2008**, *283*, 4295 -4303

PLP-dependent enzymes

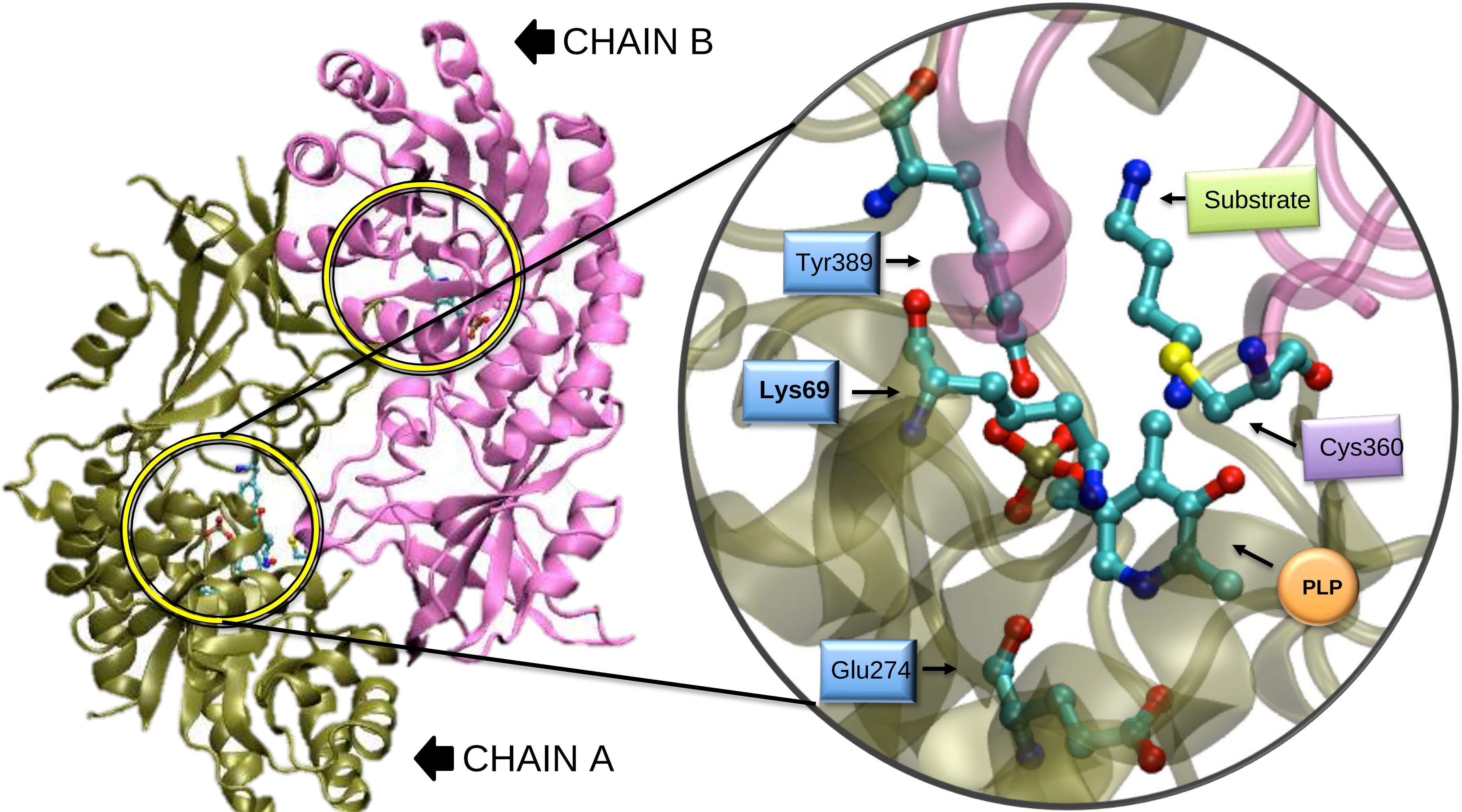


Decarboxylation
Racemization
Transamination
β-Elimination
Retro Aldol Cleavage
Others

Cook, P. D.; Holden, H. M. *Journal of Biological Chemistry* **2008**, *283*, 4295-4303

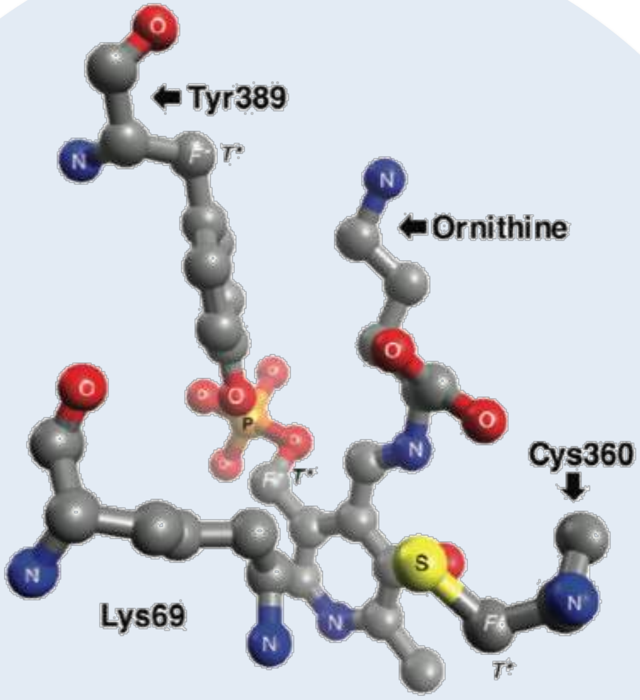
COMPUTATIONAL DETAILS

Computational Details



Dufe, V.; Ingner, D.; Heby, O.; Khomutov, A.; Persson, L.; Al-Karadaghi, S. *Biochem J* **2007**, *405*, 261-268. - PDB entry 2OO0.

Cluster Model



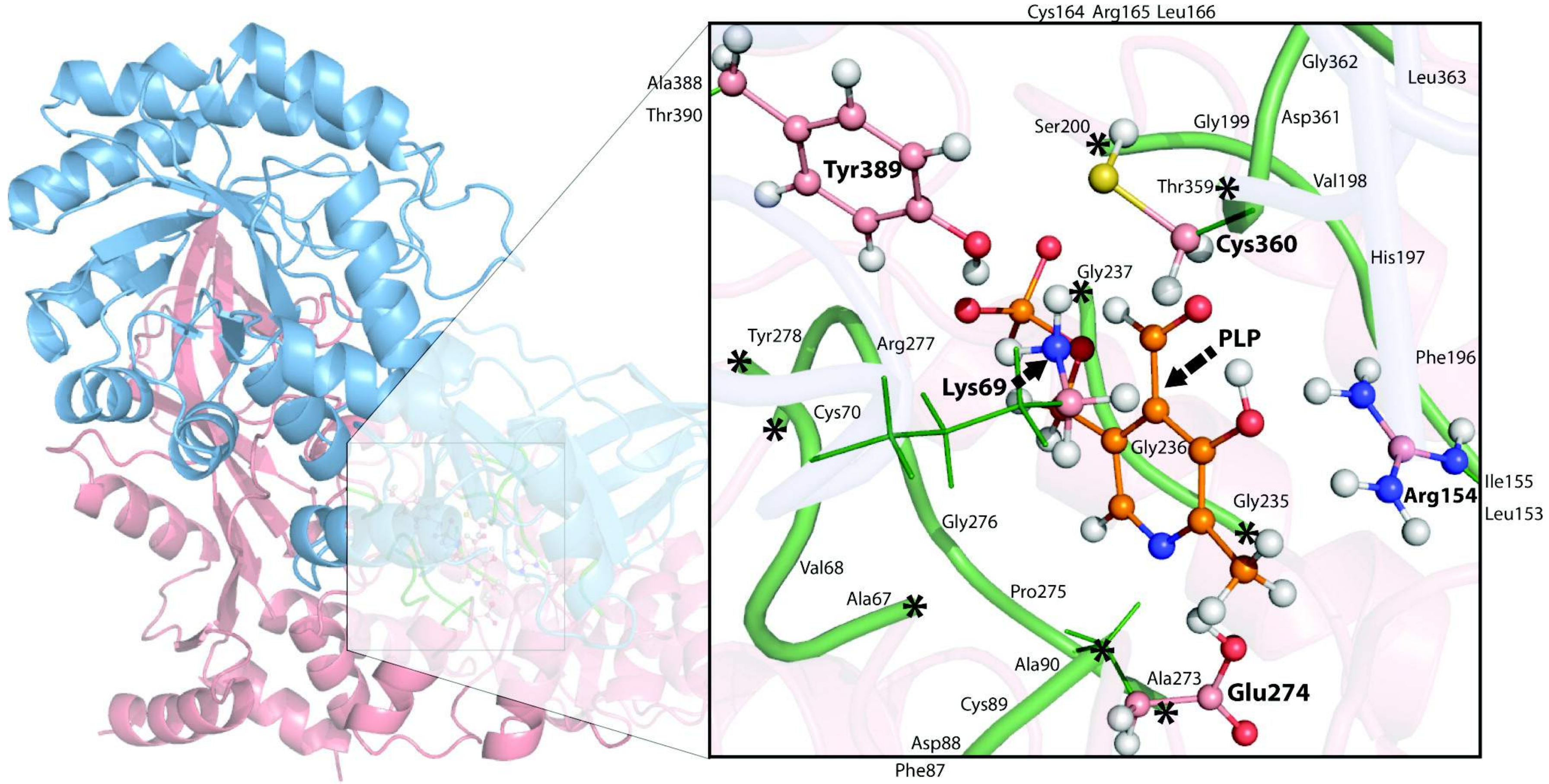
Ornithine Decarboxylase

CLUSTER MODEL =
Substrate +
First shell of residues (at least).
Full QM, 100-200 atoms

Theoretical Level –DFT (108 atoms)	
Geometry	M06 / 6-31G(d) & B3LYP/6-31G(d)
Energy	M06/6-311++G(3df,3pd) & M06-2X/6-311++G(3df,3pd) & B3LYP/6-311++G(3df,3pd) & IEFPCM (ε=4)

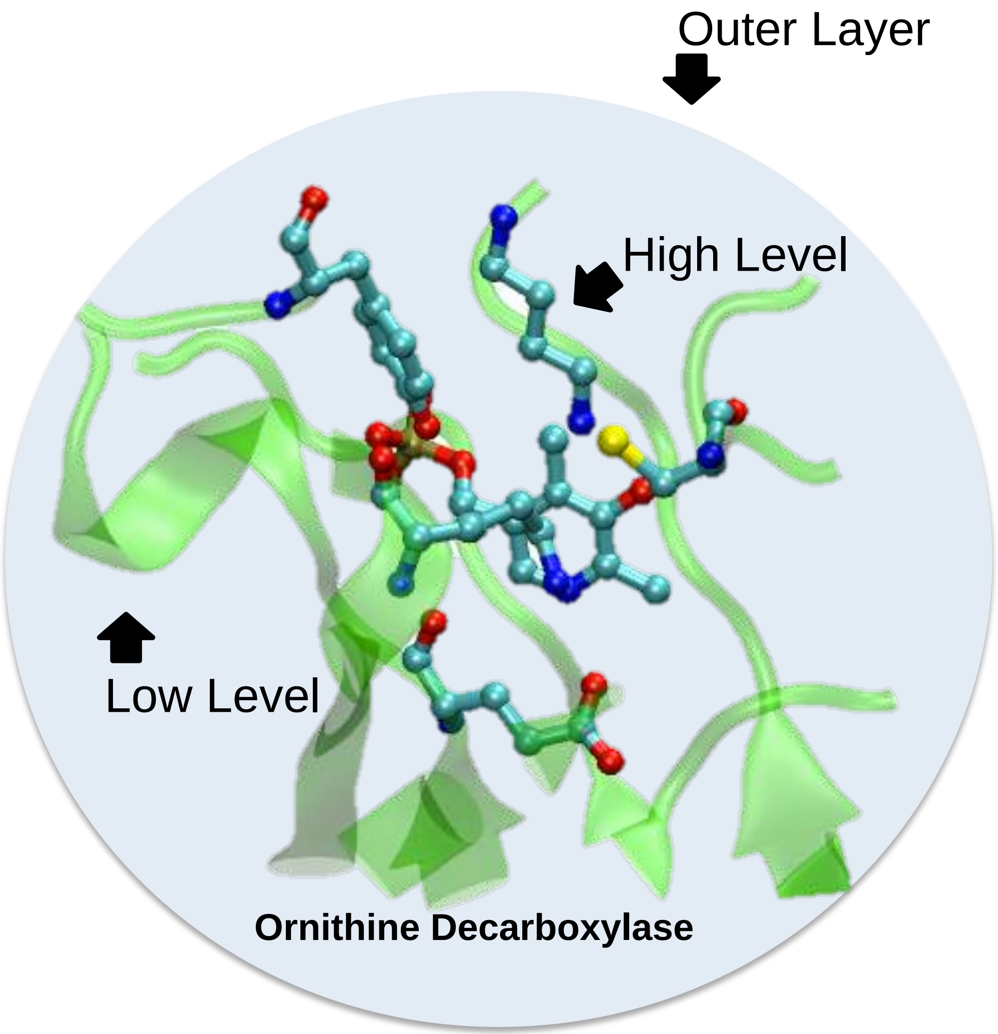
J Am Chem Soc, 129, 1378, 2007 J Chem Theory Comput, 7, 1177, 2011 Chemistry, 15, 4243, 2009

QM/QM Model



J Phys Chem B, 114, 12972, 2010. J Chem Theory Comput, 6, 2770, 2010. Acc Chem Res, 41, 689, 2008

QM/QM Model



QM/QM MODEL

8-10 Å around substrate.

Two-level QM for geometry.

One-level QM for Energy.

SINGLE POINT ENERGY

Layer	Theoretical Level
High Level	M06 / 6-311++G(3df,2pd)
Low Level	B3LYP/ 6-31G(d) + IEFPCM (e=4)

GEOMETRY OPTIMIZATION

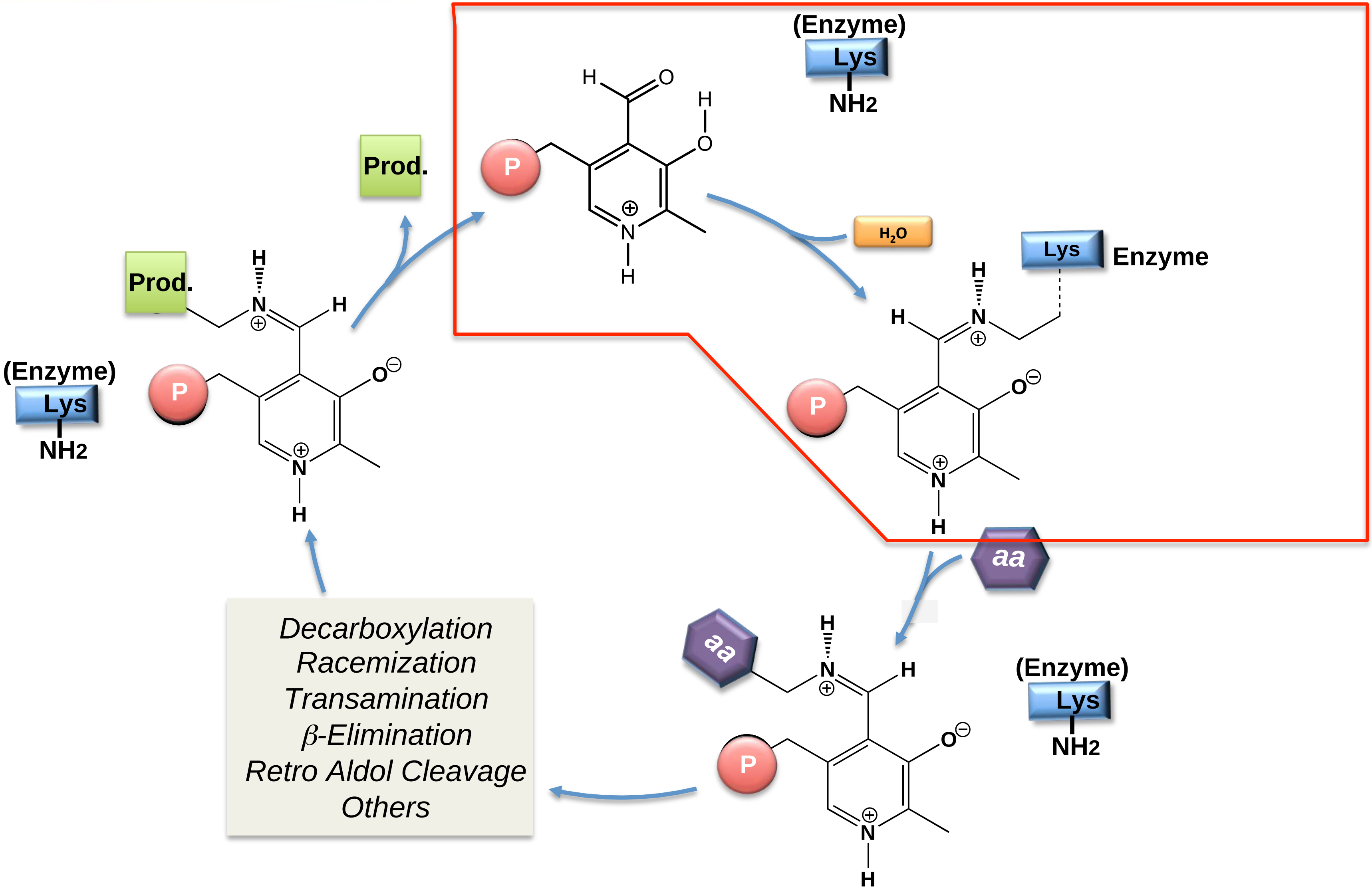
Layer	Theoretical Level	Nº atoms
High Level	B3LYP/ 6-31G(d)	66
Low Level	AM1	604

J Phys Chem B, 114, 12972, 2010. J Chem Theory Comput, 6, 2770, 2010. Acc Chem Res, 41, 689, 2008

RESULTS

EXAMPLE 1: COMMON REACTION MECHANISM FOR PLP-DEPENDENT ENZYMES

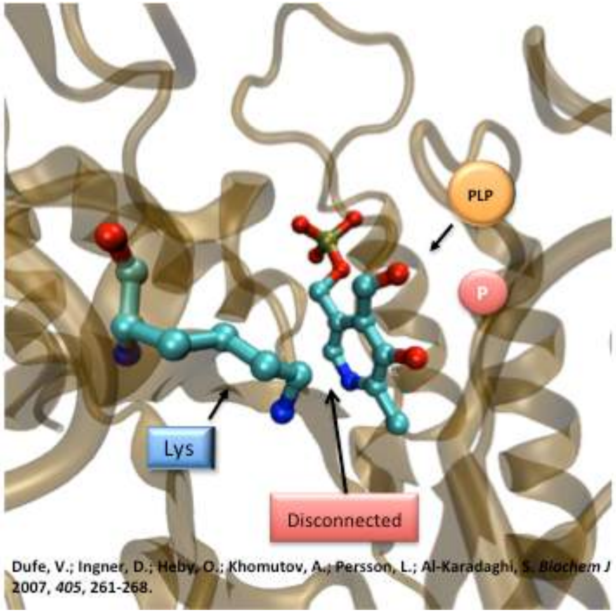
Formation of the Internal Aldimine



Decarboxylation
Racemization
Transamination
β-Elimination
Retro Aldol Cleavage
Others

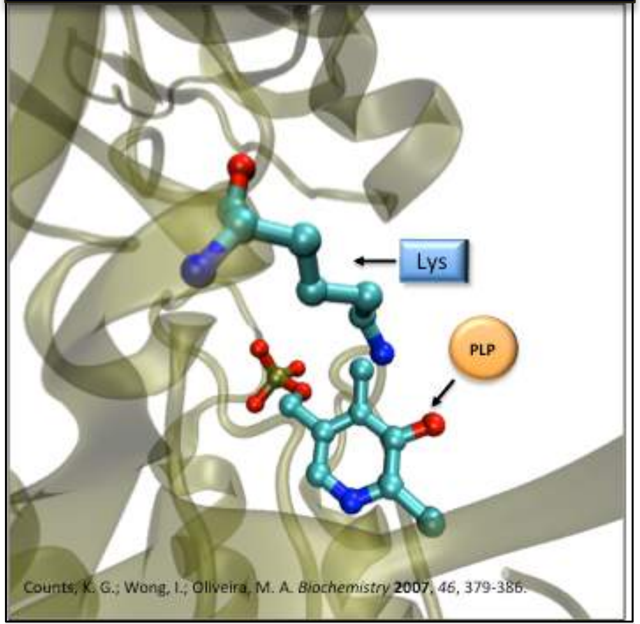
Formation of the Internal Aldimine

PDB **2OO0** – Initial State



Dufe, V.; Ingner, D.; Heby, O.; Khomutov, A.; Persson, L.; Al-Karadaghi, S. *Biochem J* **2007**, *405*, 261-268.

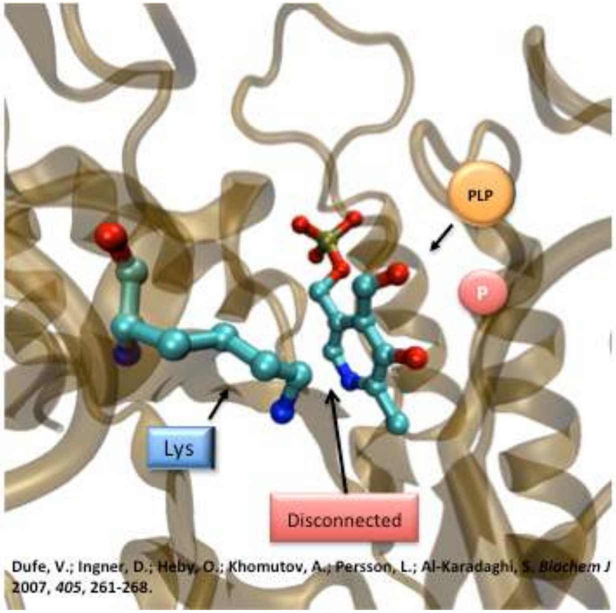
PDB: **7ODC** – Internal Aldimine



Counts, K. G.; Wong, I.; Oliveira, M. A. *Biochemistry* **2007**, *46*, 379-386.

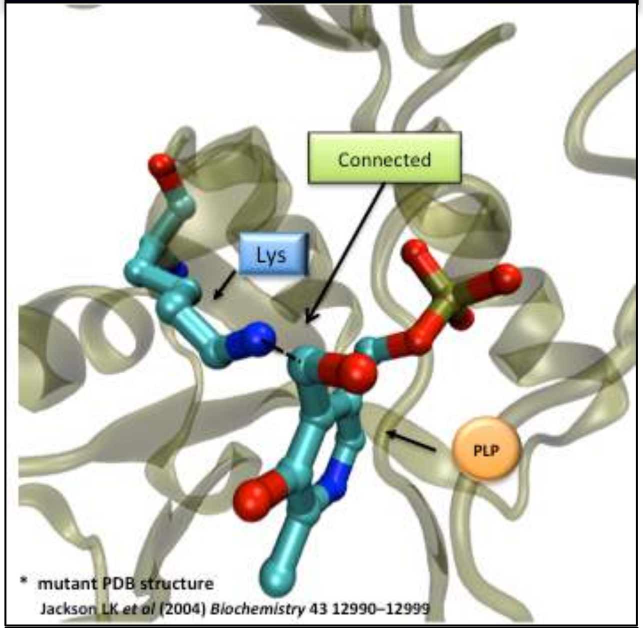
Formation of the Internal Aldimine

PDB **2OO0** – Initial State



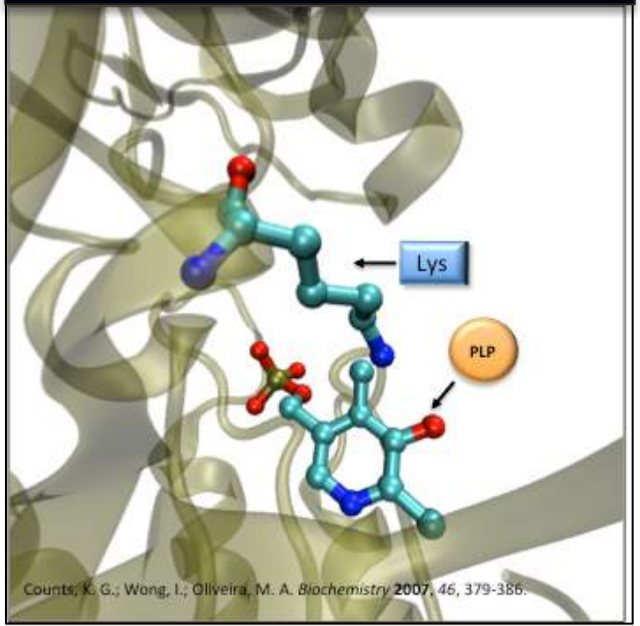
Dufe, V.; Ingner, D.; Heby, O.; Khomutov, A.; Persson, L.; Al-Karadaghi, S. *Biochem J* 2007, *405*, 261-268.

PDB: **1SZR** – Carbinolamine intermediate



* mutant PDB structure
Jackson LK *et al* (2004) *Biochemistry* 43 12990–12999

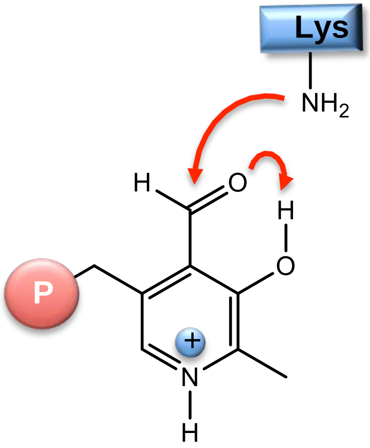
PDB: **7ODC** – Internal Aldimine



Counts, K. G.; Wong, I.; Oliveira, M. A. *Biochemistry* 2007, *46*, 379-386.

Formation of the Internal Aldimine

Imine formation

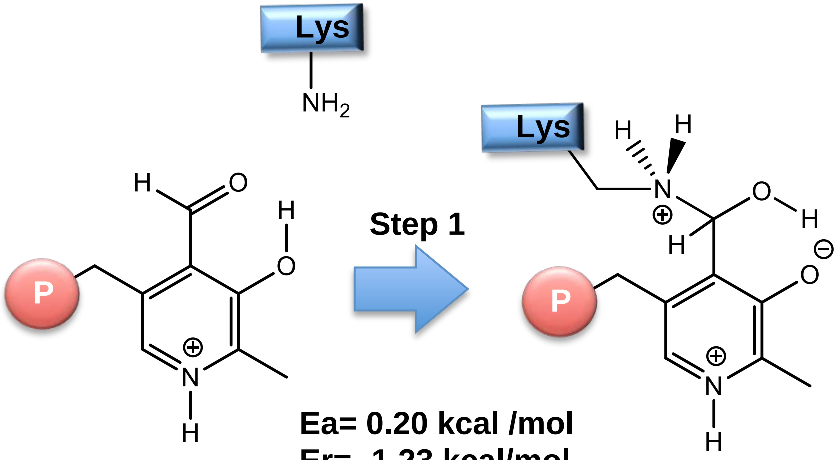


Inactive enzyme

J Am Chem Soc 2011, 133, 15496

Formation of the Internal Aldimine

Imine formation

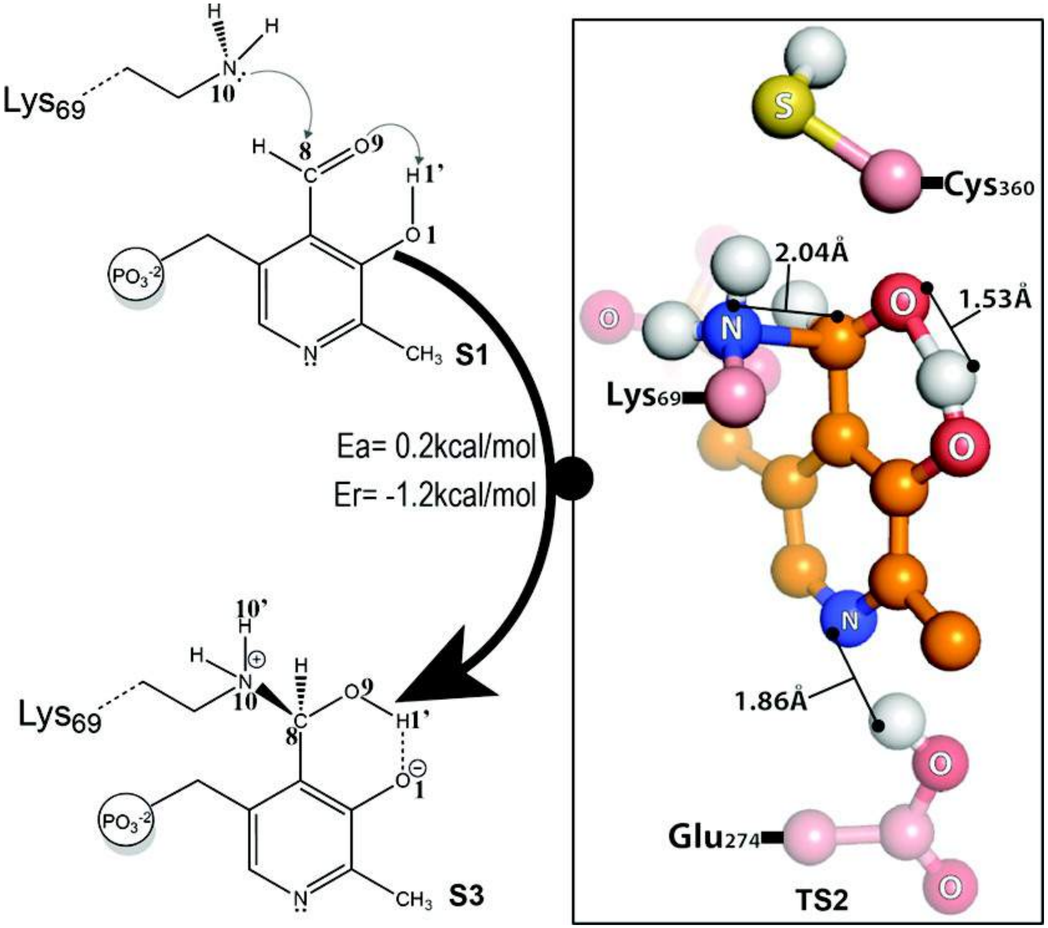


Ea= 0.20 kcal /mol
Er= -1.23 kcal/mol

Inactive enzyme

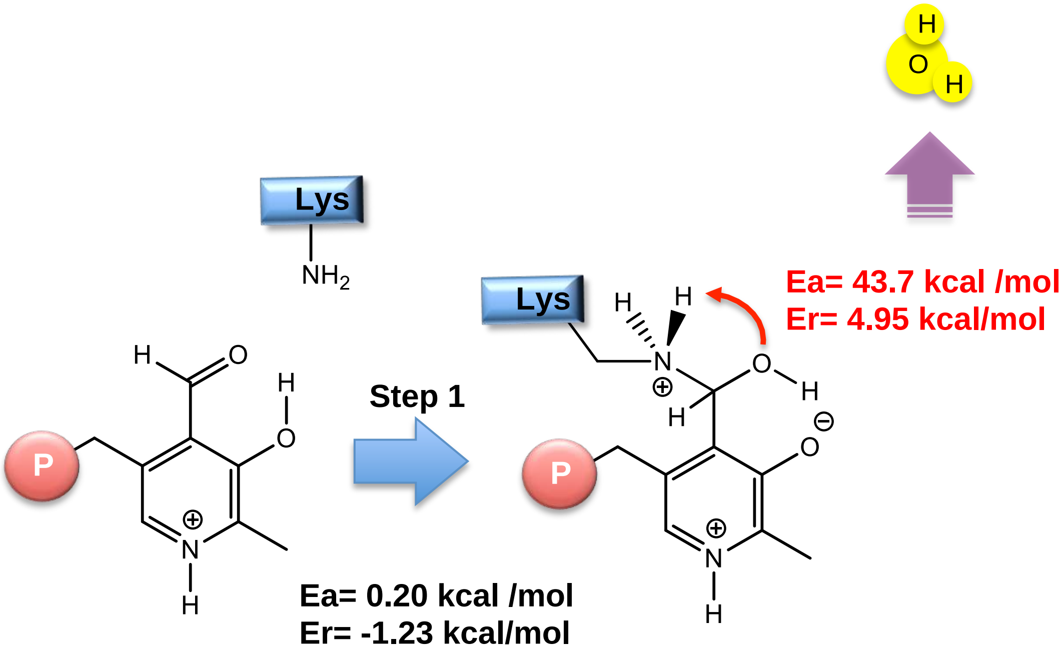
Formation of the Internal Aldimine

Imine formation



Formation of the Internal Aldimine

Imine formation



Step 1

Ea= 0.20 kcal /mol
Er= -1.23 kcal/mol

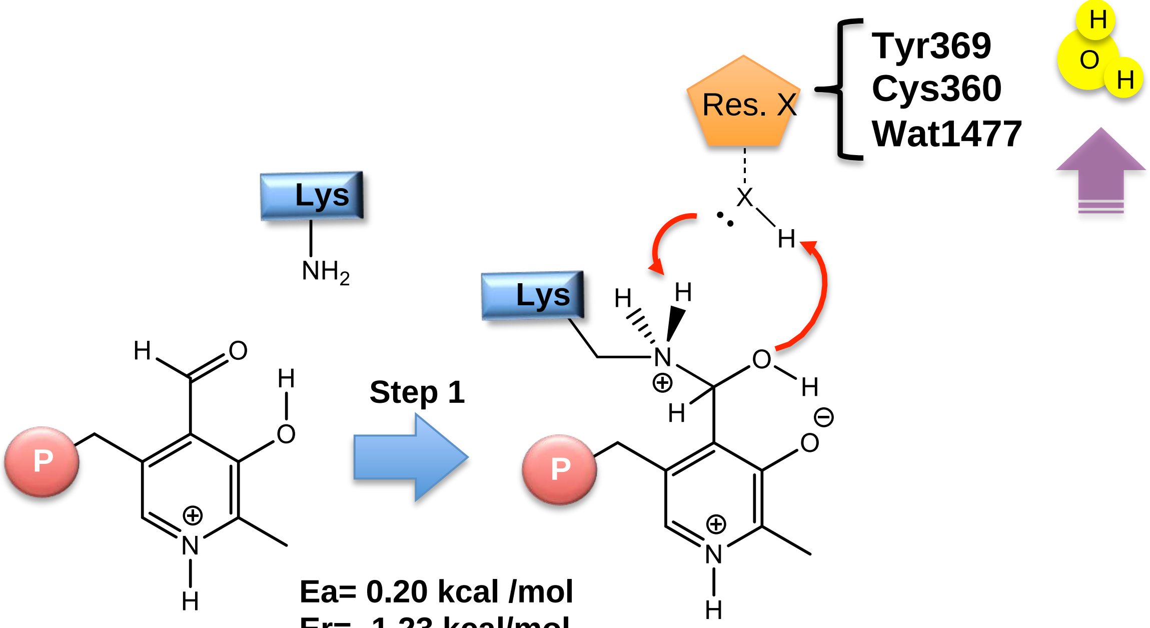
Ea= 43.7 kcal /mol
Er= 4.95 kcal/mol

Inactive enzyme

PLP bonded to Lys69

Formation of the Internal Aldimine

Imine formation



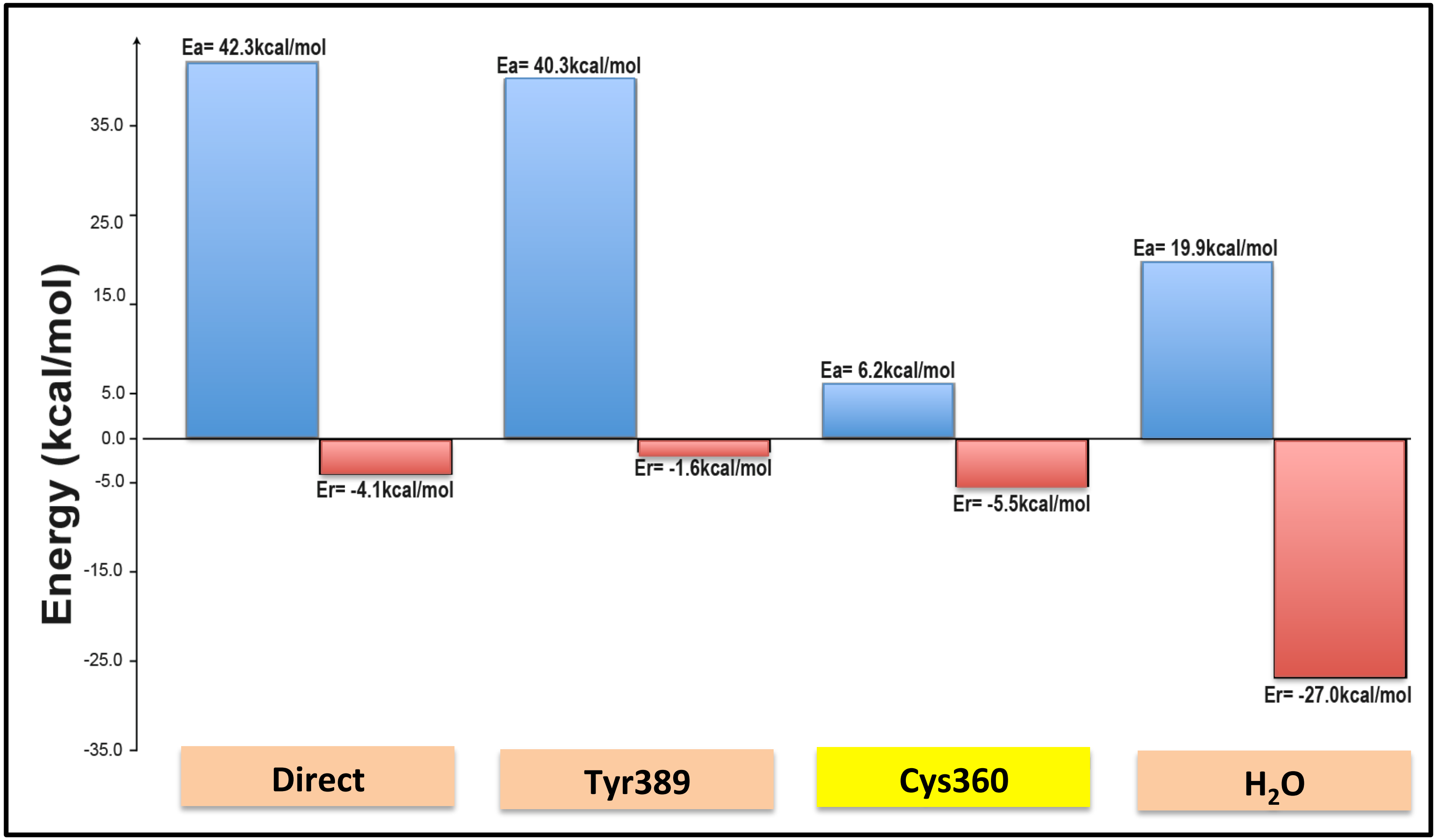
Ea= 0.20 kcal /mol
Er= -1.23 kcal/mol

Inactive enzyme

PLP bonded to Lys69

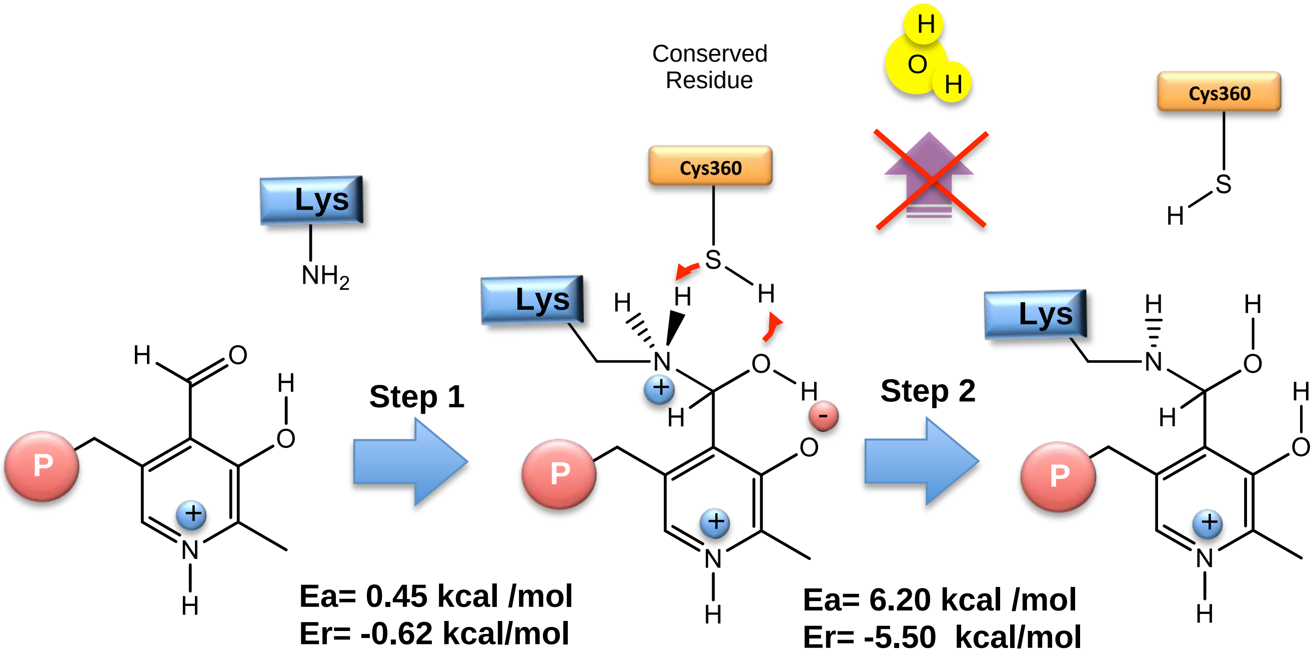
Formation of the Internal Aldimine

Imine formation



Formation of the Internal Aldimine

Imine formation



Conserved Residue

Step 1

Ea= 0.45 kcal /mol
Er= -0.62 kcal/mol

Step 2

Ea= 6.20 kcal /mol
Er= -5.50 kcal/mol

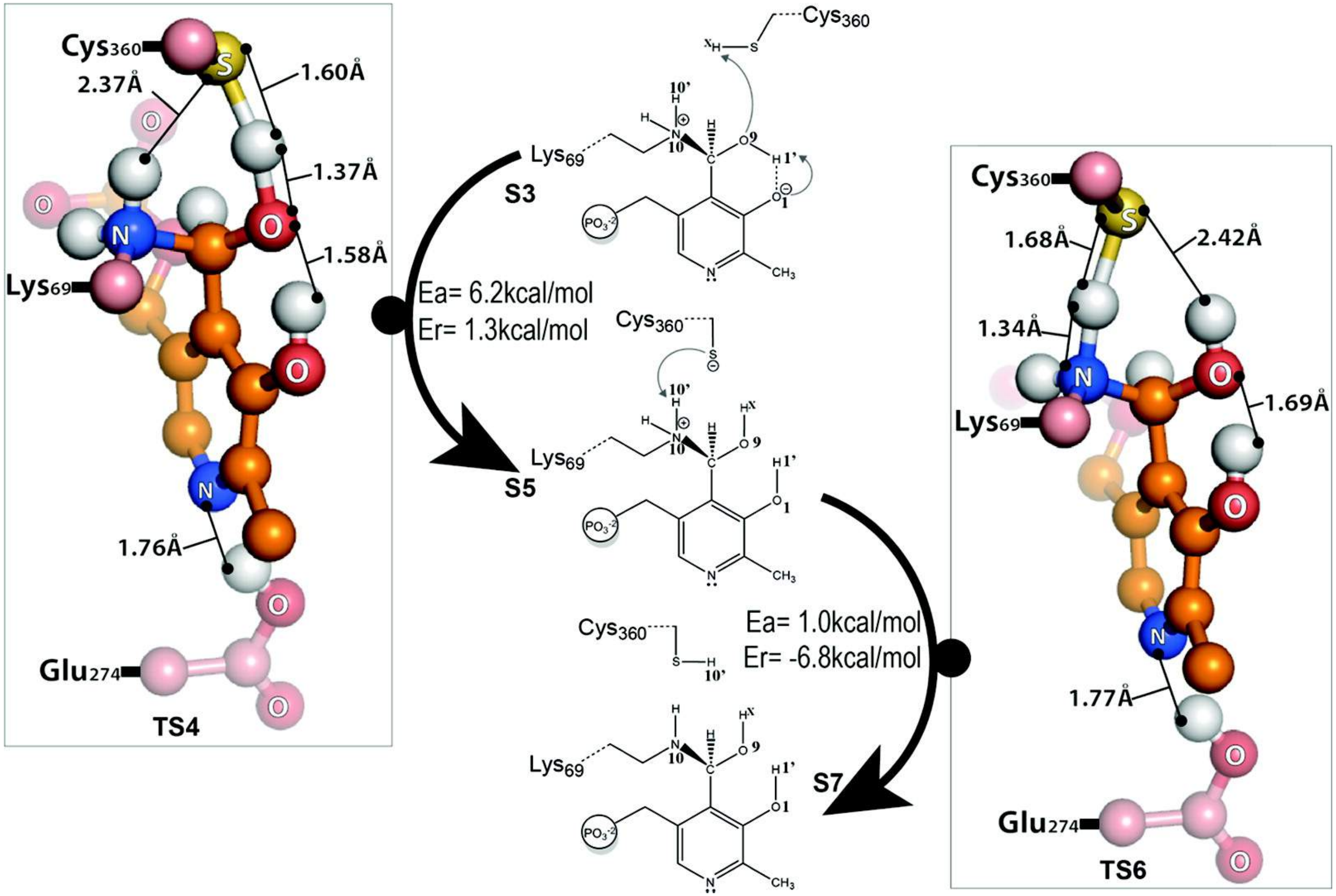
Inactive enzyme

PLP bound to Lys69

Carbinolamine intermediate

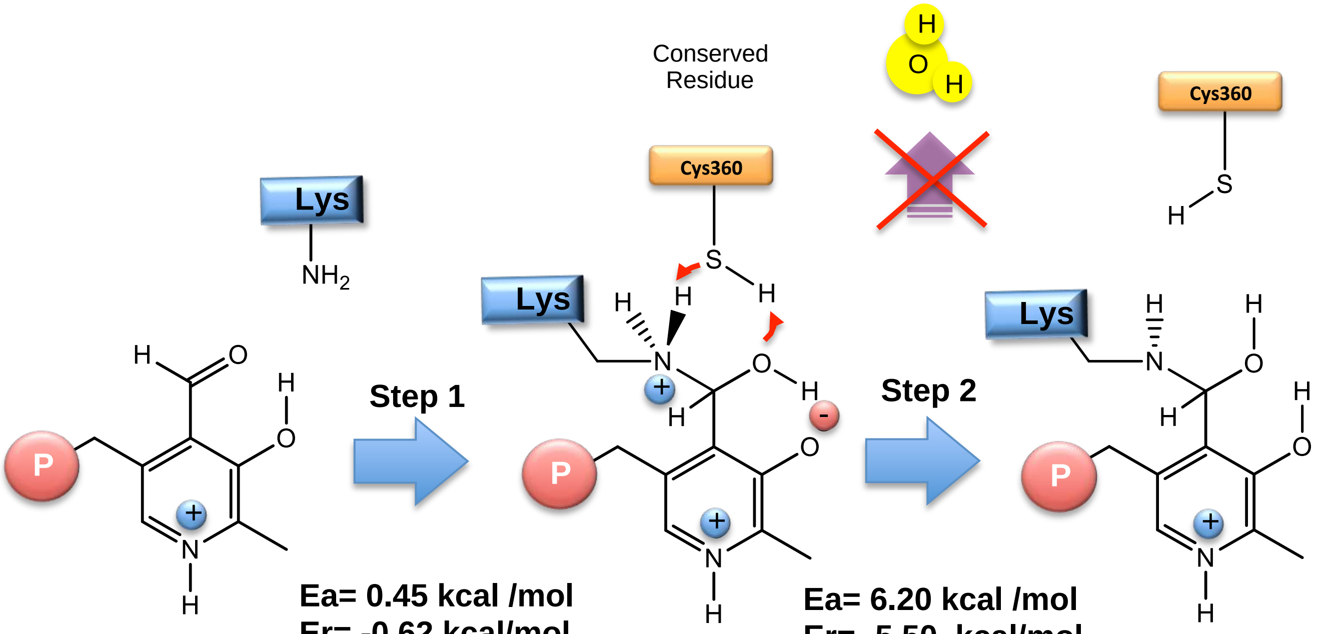
Formation of the Internal Aldimine

Imine formation



Formation of the Internal Aldimine

Imine formation



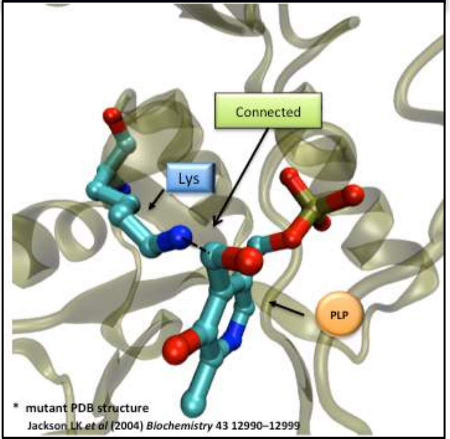
Step 1

Ea= 0.45 kcal /mol
Er= -0.62 kcal/mol

Step 2

Ea= 6.20 kcal /mol
Er= -5.50 kcal/mol

PDB: **1SZR** – Carbinolamine intermediate



* mutant PDB structure
Jackson LK *et al* (2004) *Biochemistry* 43 12990–12999

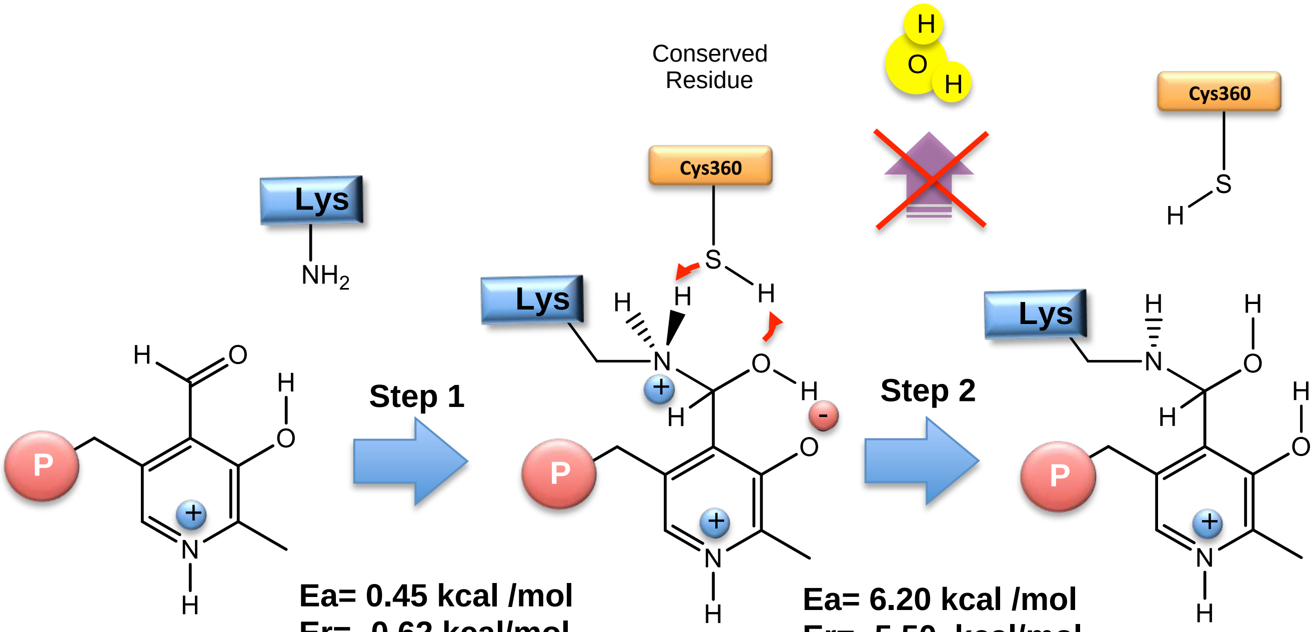
Inactive enzyme

PLP bound to Lys69

Carbinolamine intermediate

Formation of the Internal Aldimine

Imine formation



Ea= 0.45 kcal /mol
Er= -0.62 kcal/mol

Ea= 6.20 kcal /mol
Er= -5.50 kcal/mol

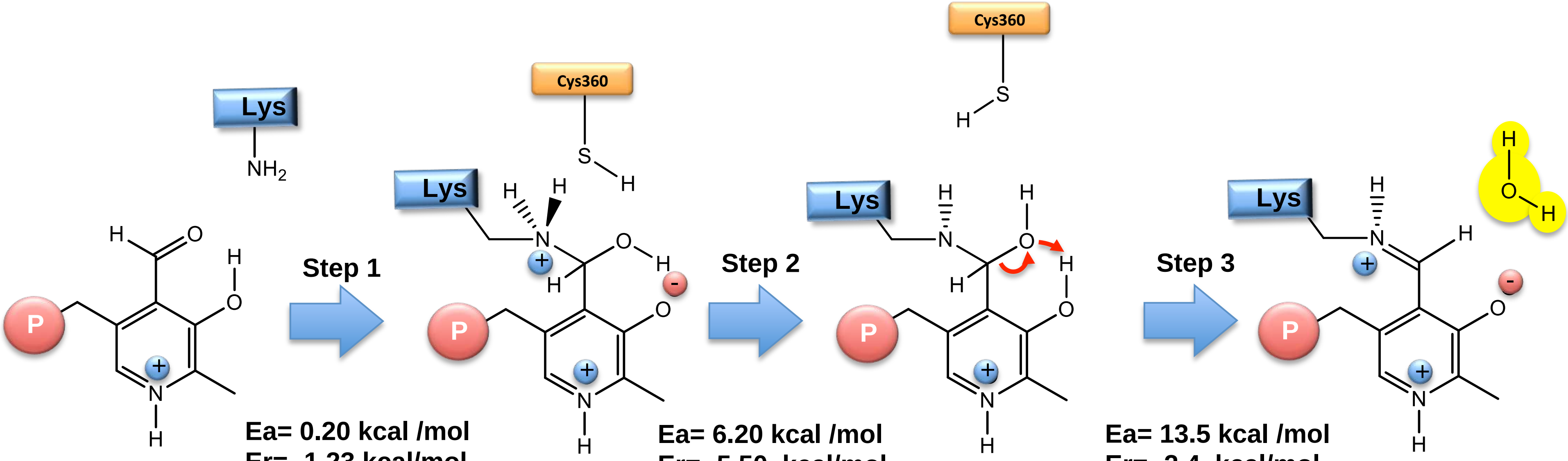
Inactive enzyme

PLP bound to Lys69

Carbinolamine intermediate

Formation of the Internal Aldimine

Imine formation



Step 1

Ea= 0.20 kcal /mol
Er= -1.23 kcal/mol

Step 2

Ea= 6.20 kcal /mol
Er= -5.50 kcal/mol

Step 3

Ea= 13.5 kcal /mol
Er= -2.4 kcal/mol

Inactive enzyme

PLP bound to Lys69

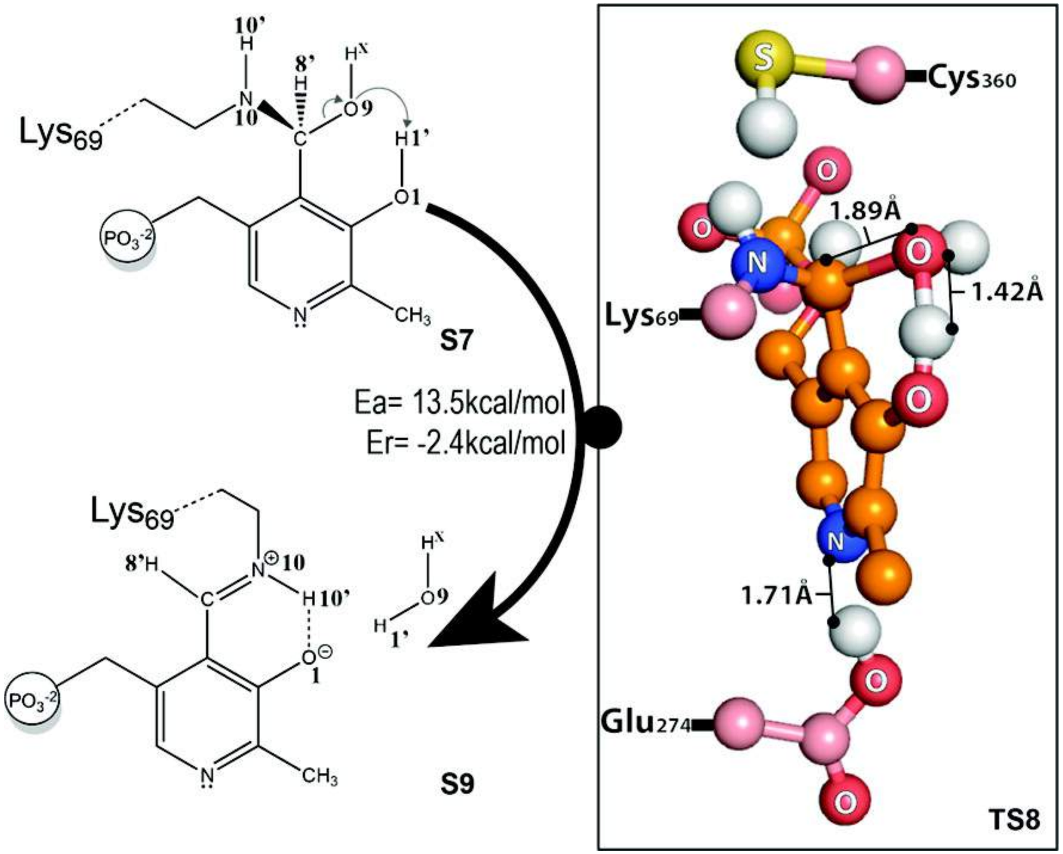
Carbinolamine intermediate

Internal Aldimine

J Am Chem Soc 2011, 133, 15496

Formation of the Internal Aldimine

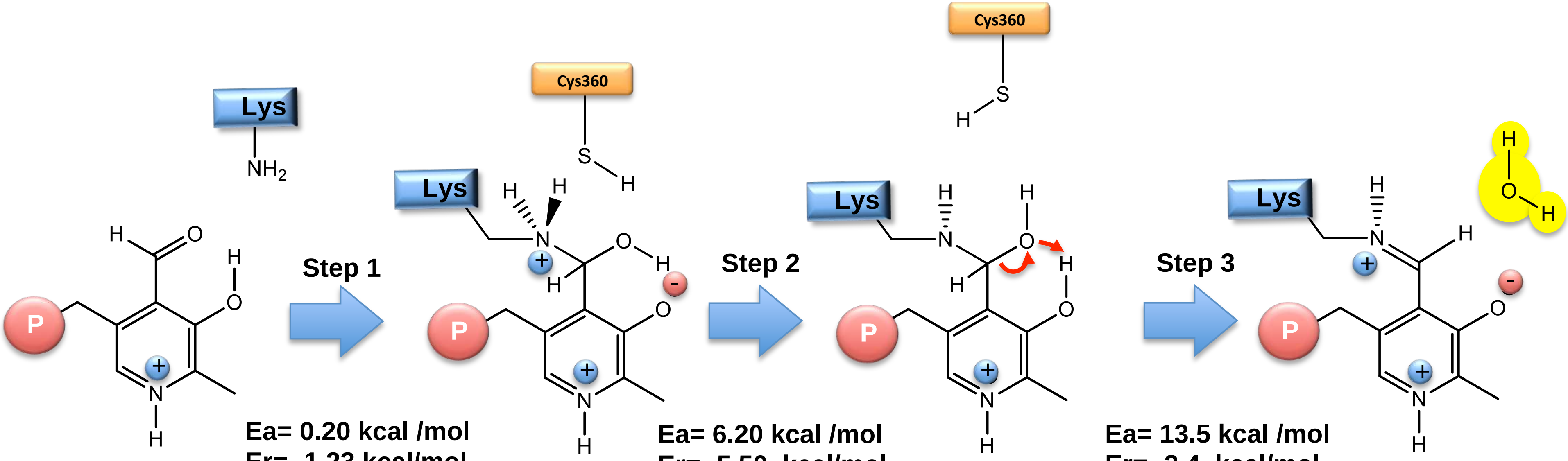
Imine formation



J Am Chem Soc 2011, 133, 15496

Formation of the Internal Aldimine

Formation of the Internal Aldimine complete



Ea= 0.20 kcal /mol
Er= -1.23 kcal/mol

Ea= 6.20 kcal /mol
Er= -5.50 kcal/mol

Ea= 13.5 kcal /mol
Er= -2.4 kcal/mol

Inactive enzyme

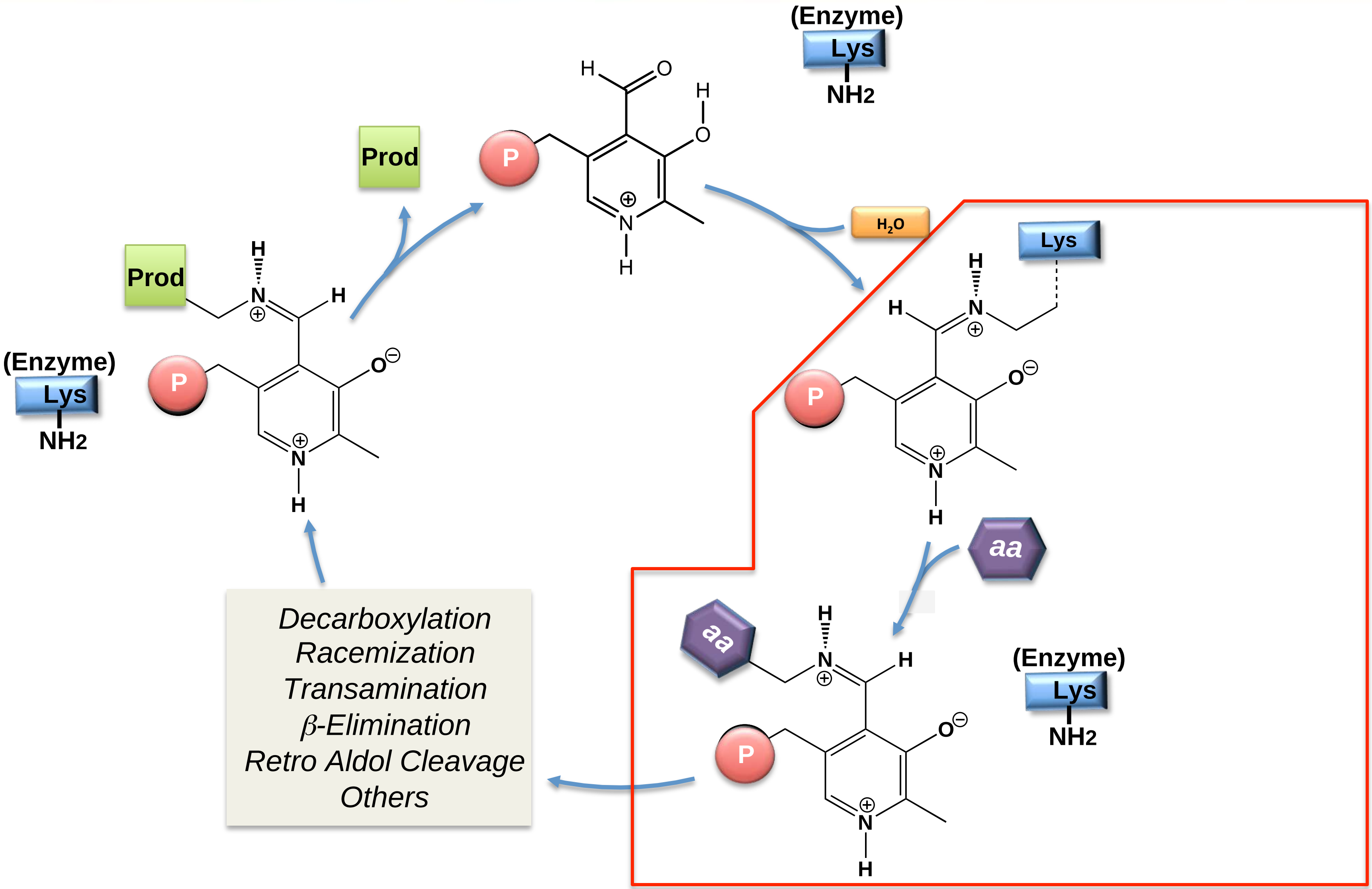
PLP bound to Lys69

Carbinolamine intermediate

Internal Aldimine

J Am Chem Soc 2011, 133, 15496

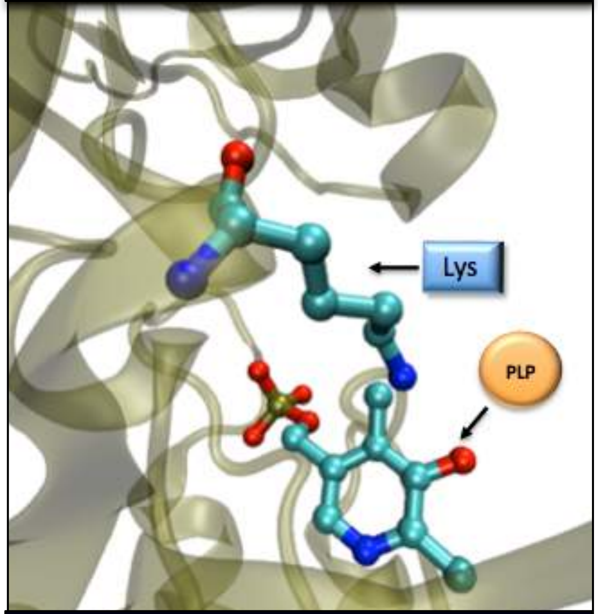
Transimination



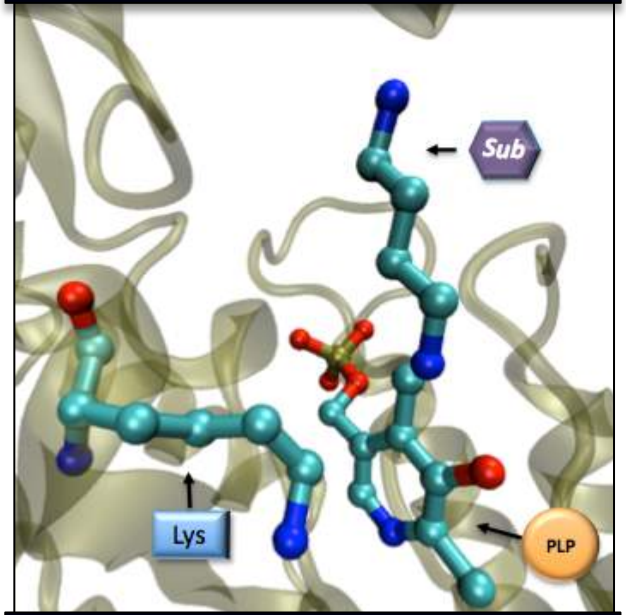
Decarboxylation
Racemization
Transamination
β-Elimination
Retro Aldol Cleavage
Others

Transimination

7ODC-Internal Aldimine

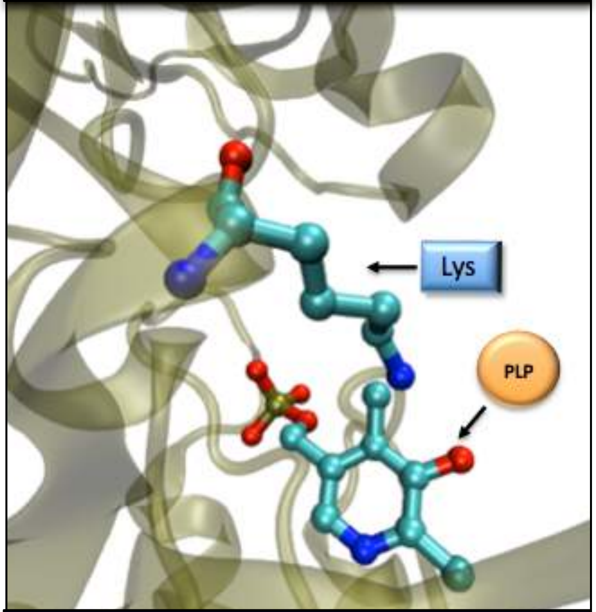


1F3T-External Aldimine

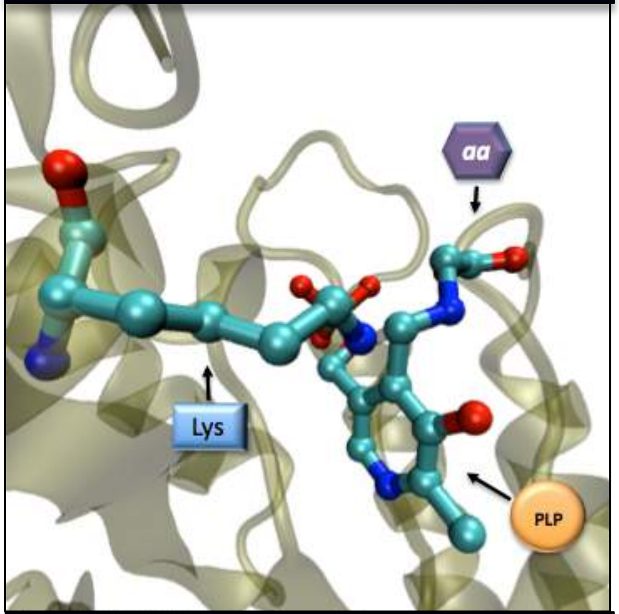


Transimination

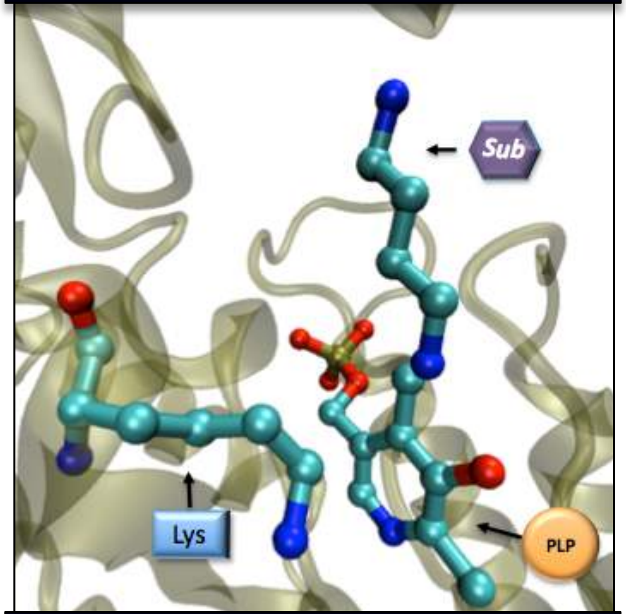
7ODC-Internal Aldimine



1SRZ-Gemdiamine Intermediate

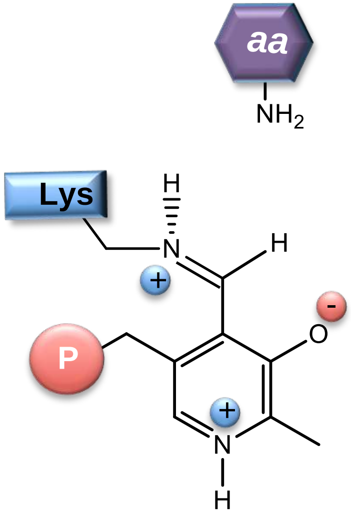


1F3T-External Aldimine



Transimination

Transimination reaction

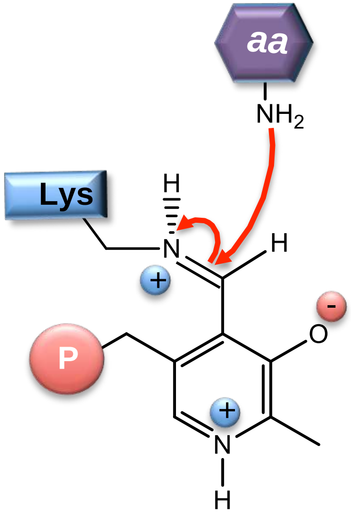


Internal Aldimine

J Chem Theory Comput 2011 7, 1356-1368

Transimination

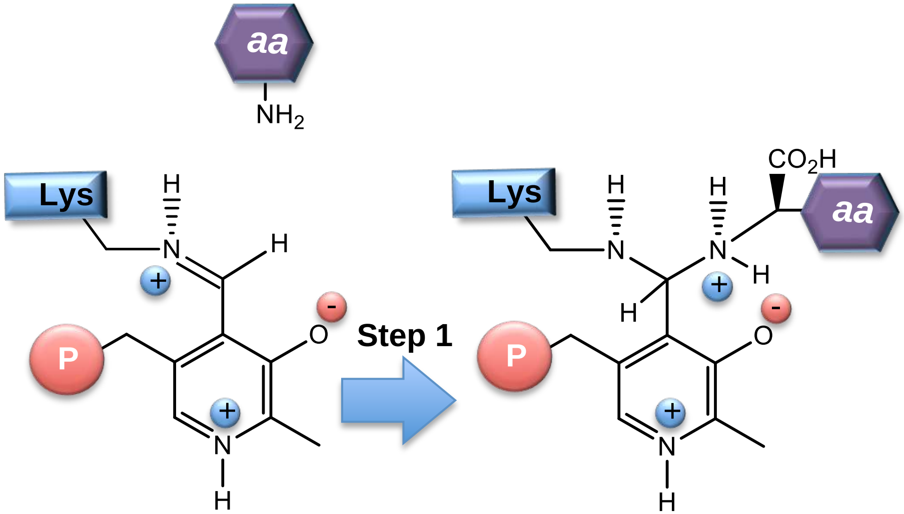
Transimination reaction



Internal Aldimine

Transimination

Transimination reaction



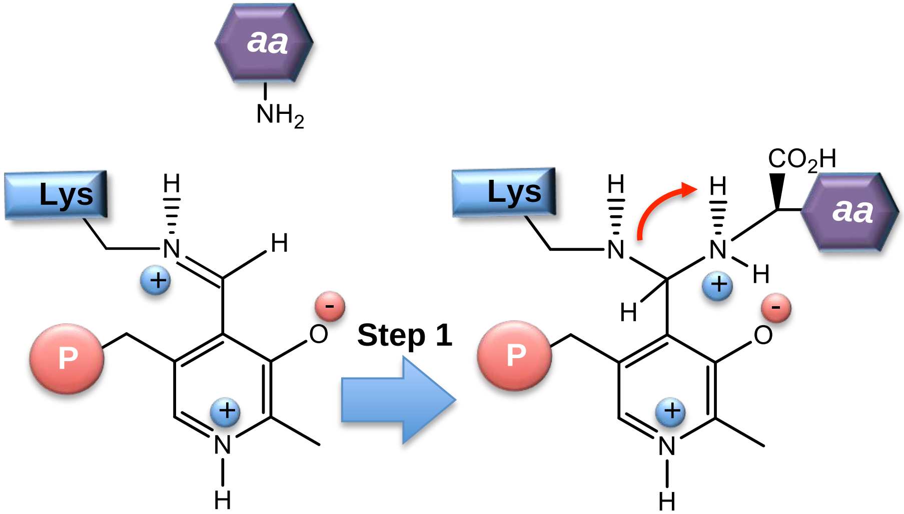
Ea= 10.8 kcal /mol
Er= -5.7 kcal/mol

Internal Aldimine

Gemdiamine Intermediate

Transimination

Transimination reaction



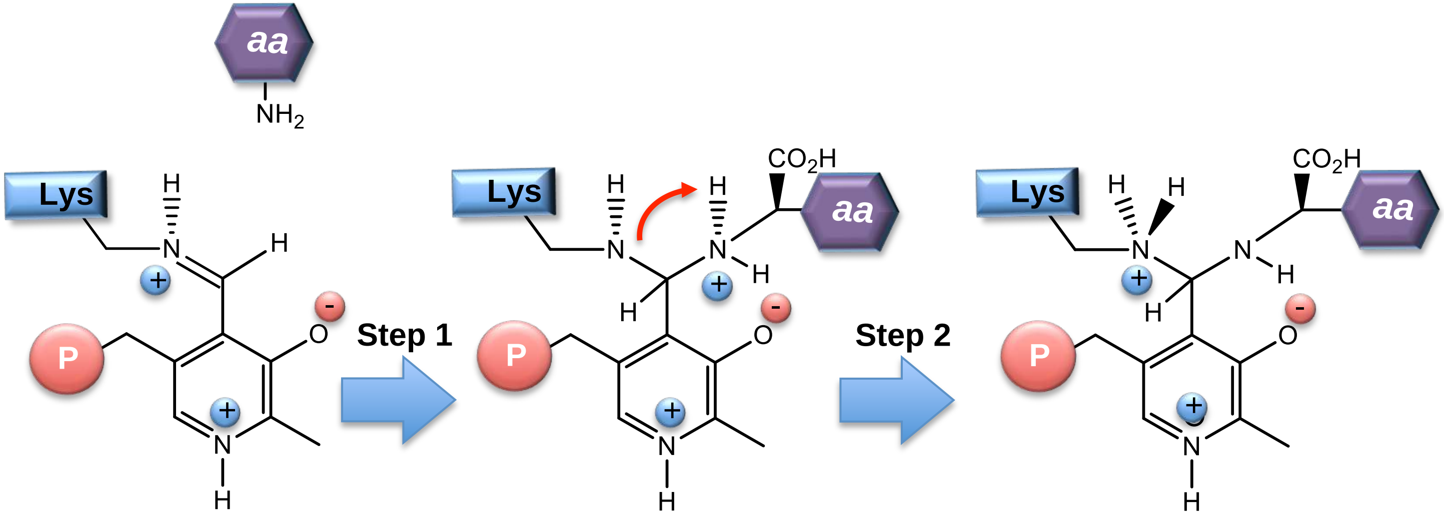
Ea= 10.8 kcal /mol
Er= -5.7 kcal/mol

Internal Aldimine

Gemdiamine Intermediate

Transimination

Transimination reaction



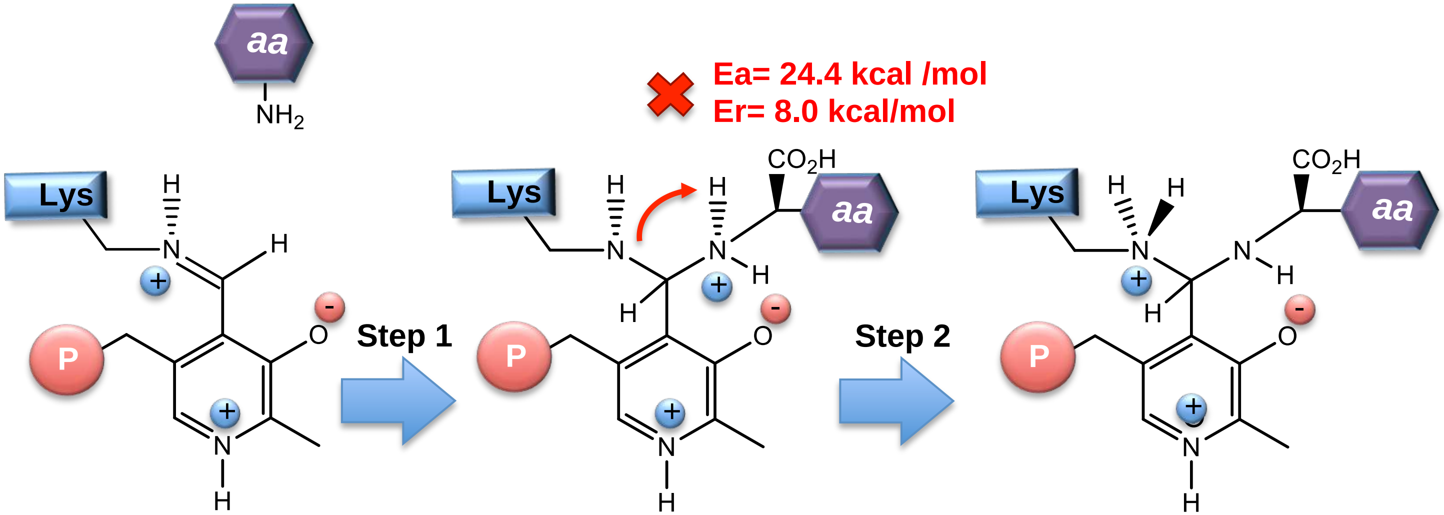
Ea= 10.8 kcal /mol
Er= -5.7 kcal/mol

Internal Aldimine

Gemdiamine Intermediate

Transimination

Transimination reaction



Ea= 24.4 kcal /mol
Er= 8.0 kcal/mol

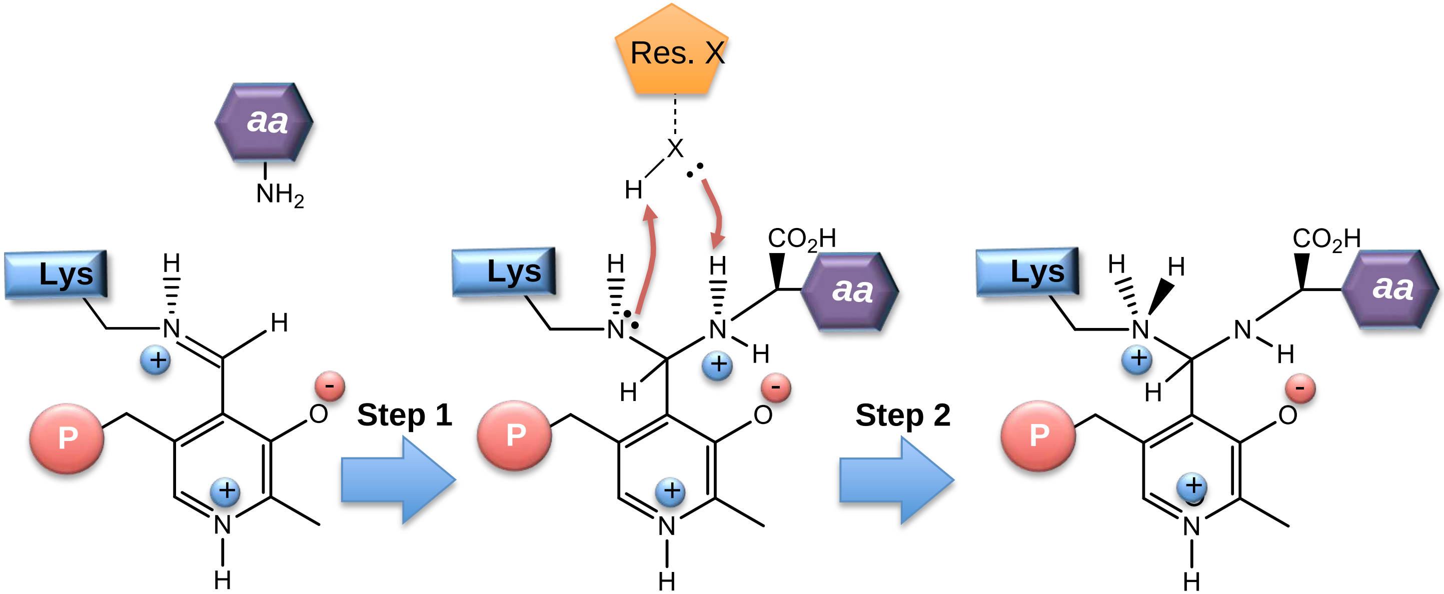
Ea= 10.8 kcal /mol
Er= -5.7 kcal/mol

Internal Aldimine

Gemdiamine Intermediate

Transimination

Transimination reaction



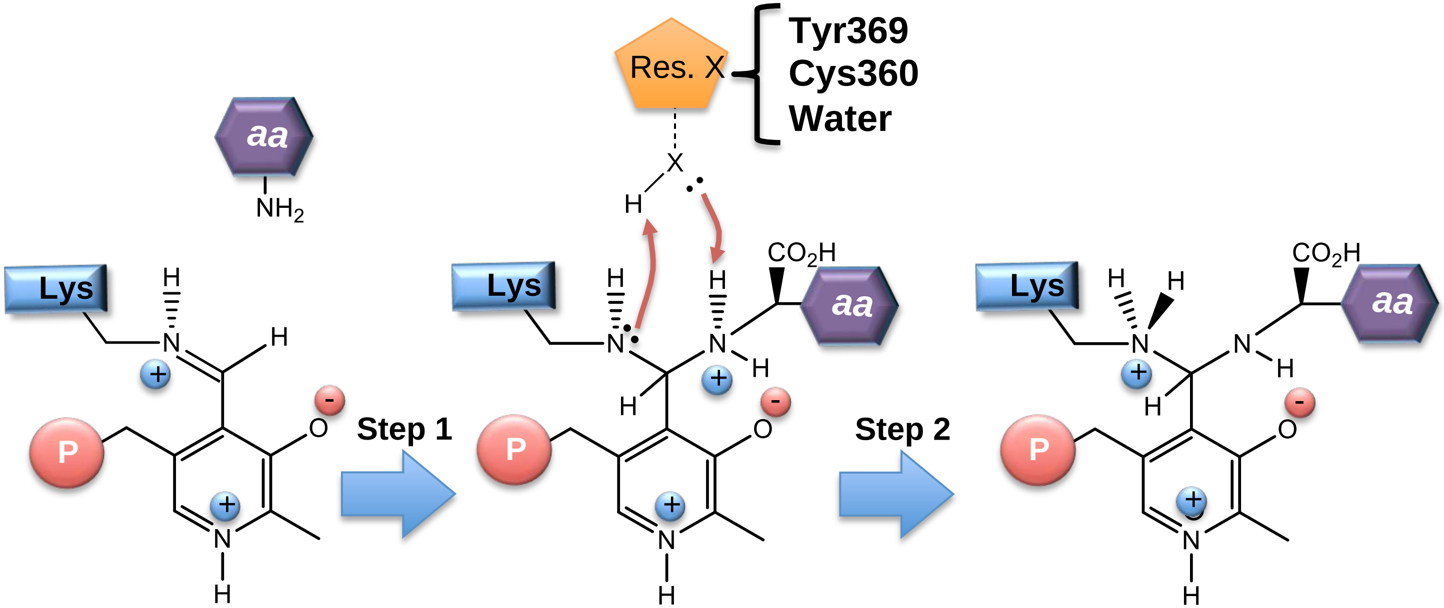
Ea= 10.8 kcal /mol
Er= -5.7 kcal/mol

Internal Aldimine

Gemdiamine Intermediate

Transimination

Transimination reaction



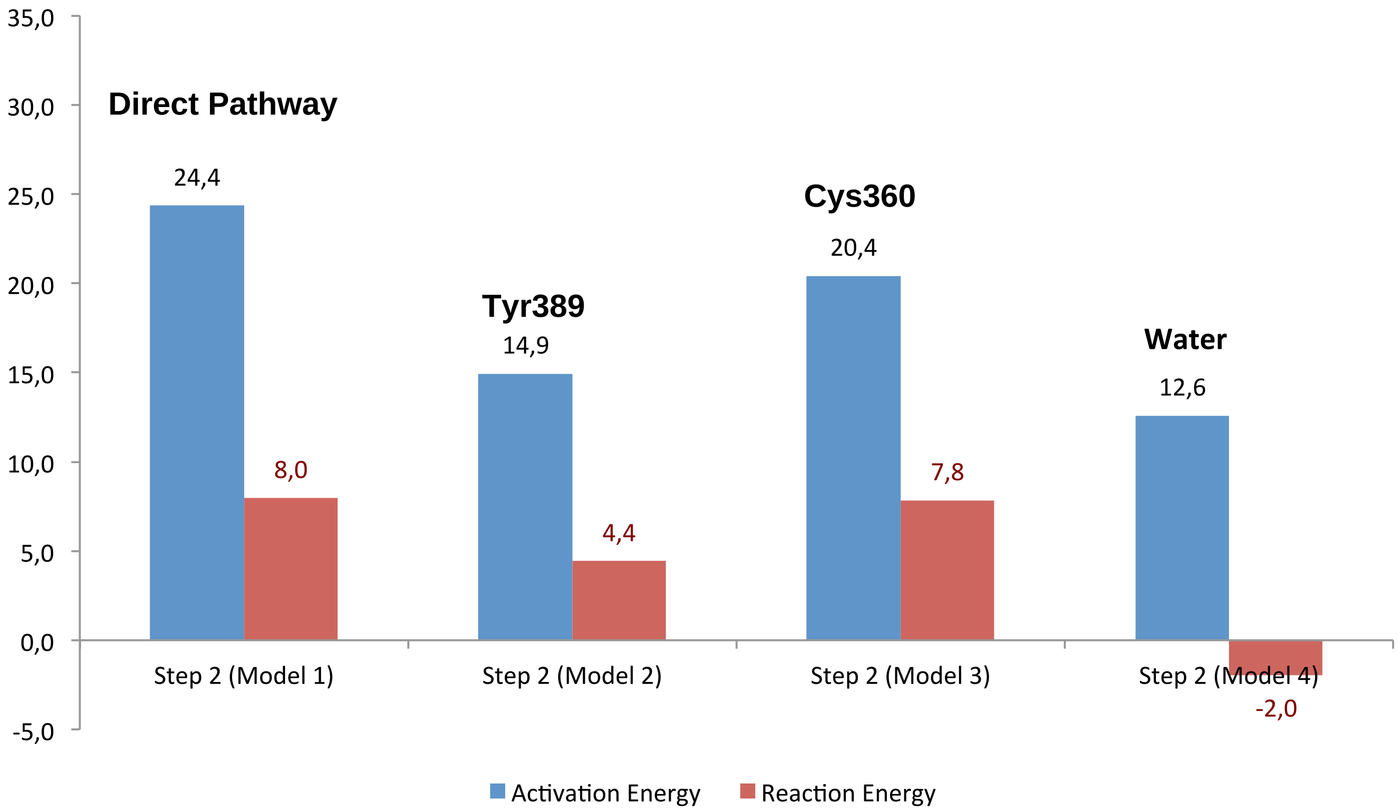
Ea= 10.8 kcal /mol
Er= -5.7 kcal/mol

Internal Aldimine

Gemdiamine Intermediate

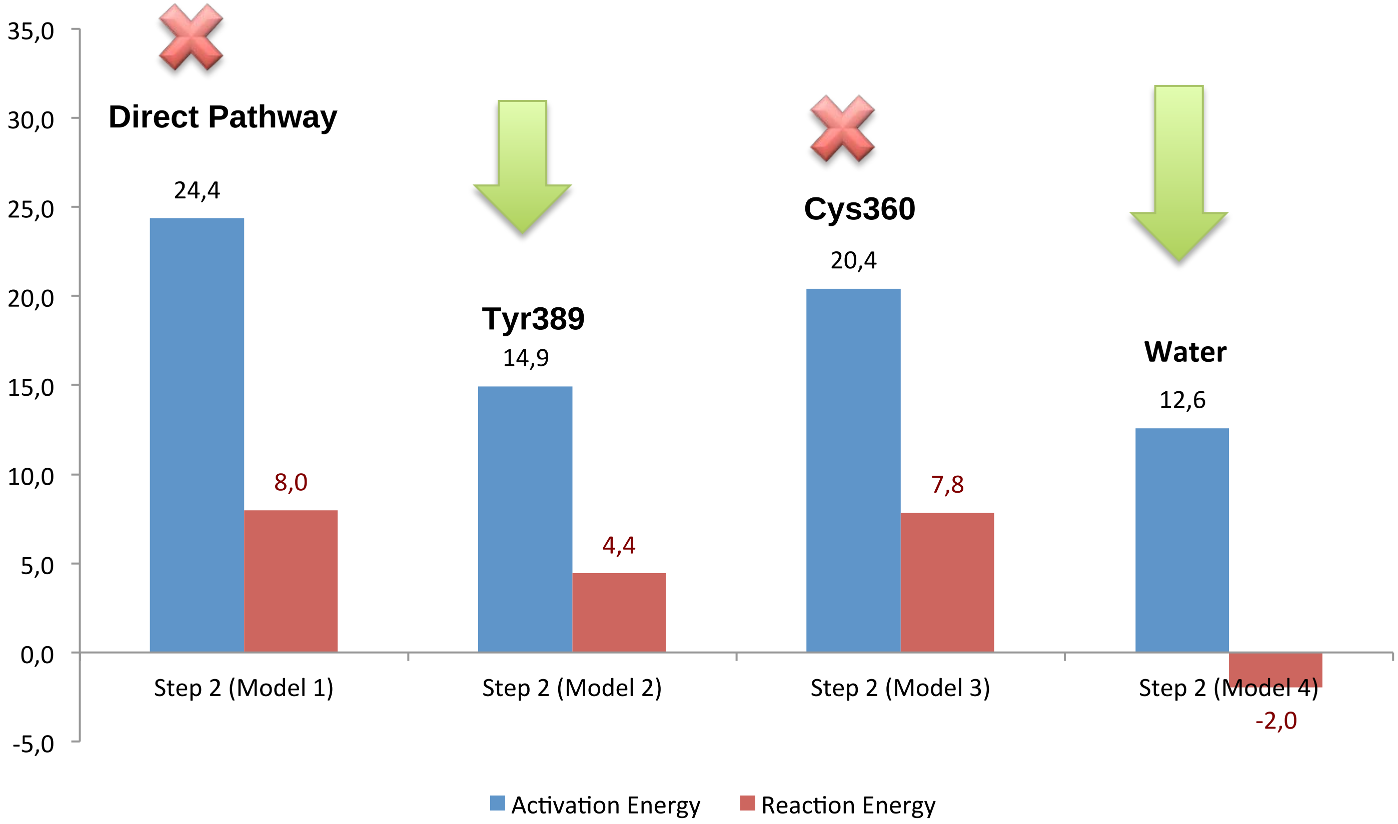
Transimination

Transimination reaction – Step 2



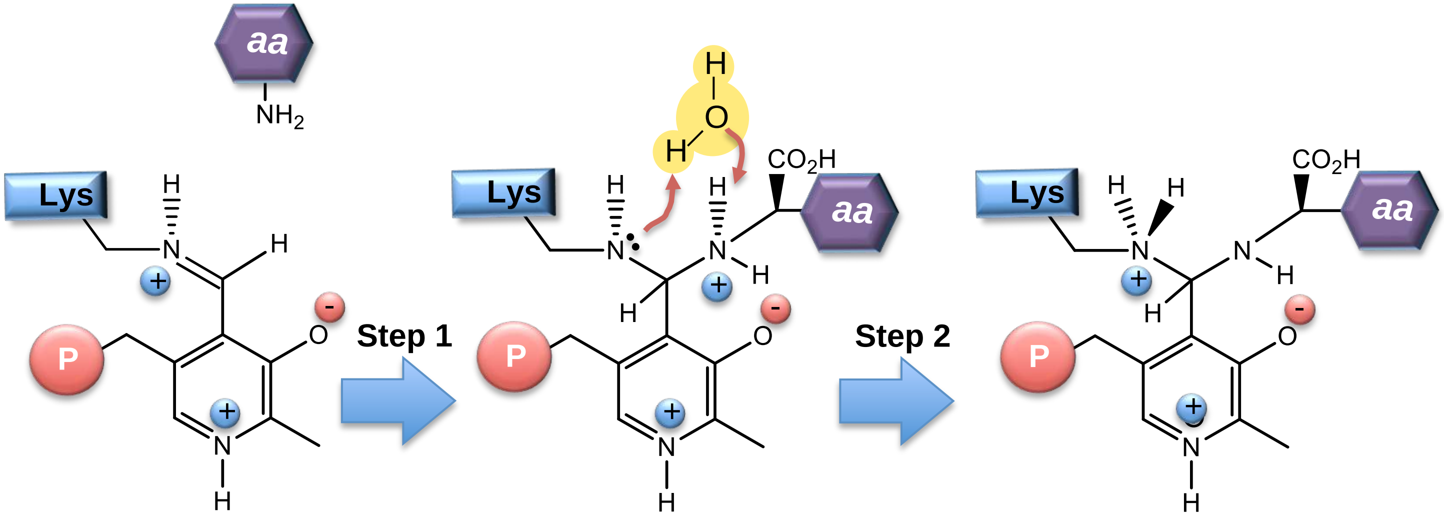
Transimination

Transimination reaction – Step 2



Transimination

Transimination reaction



Ea= 10.8 kcal /mol
Er= -5.7 kcal/mol

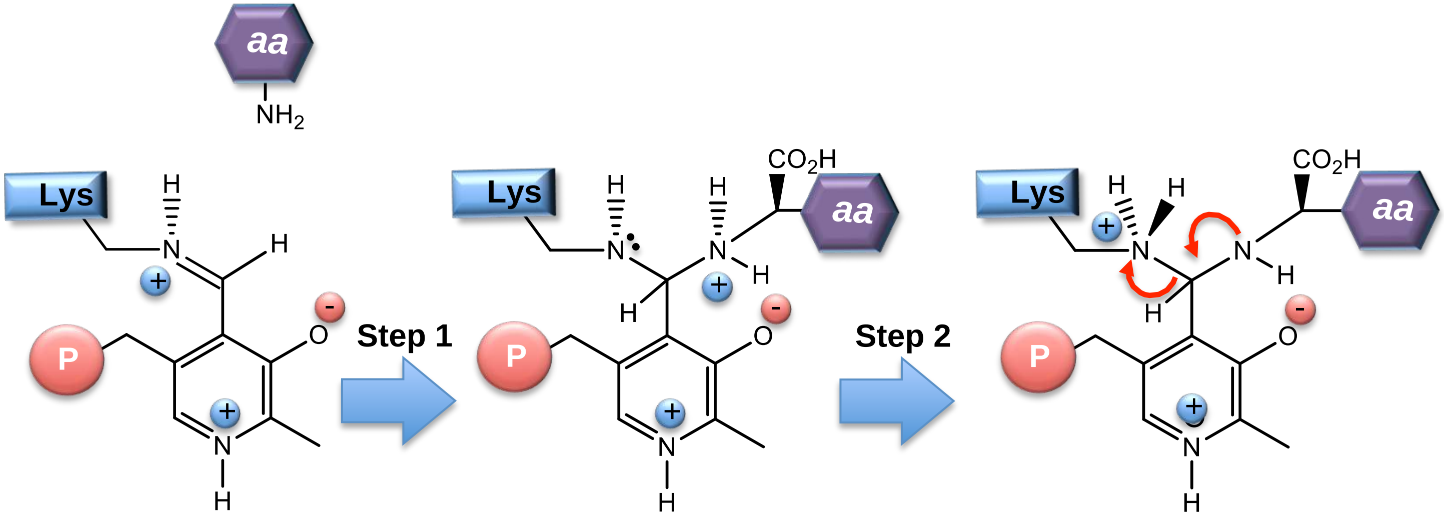
Ea= 12.6 kcal /mol
Er= -2.0 kcal/mol

Internal Aldimine

Gemdiamine Intermediate

Transimination

Transimination reaction



Ea= 10.8 kcal /mol
Er= -5.7 kcal/mol

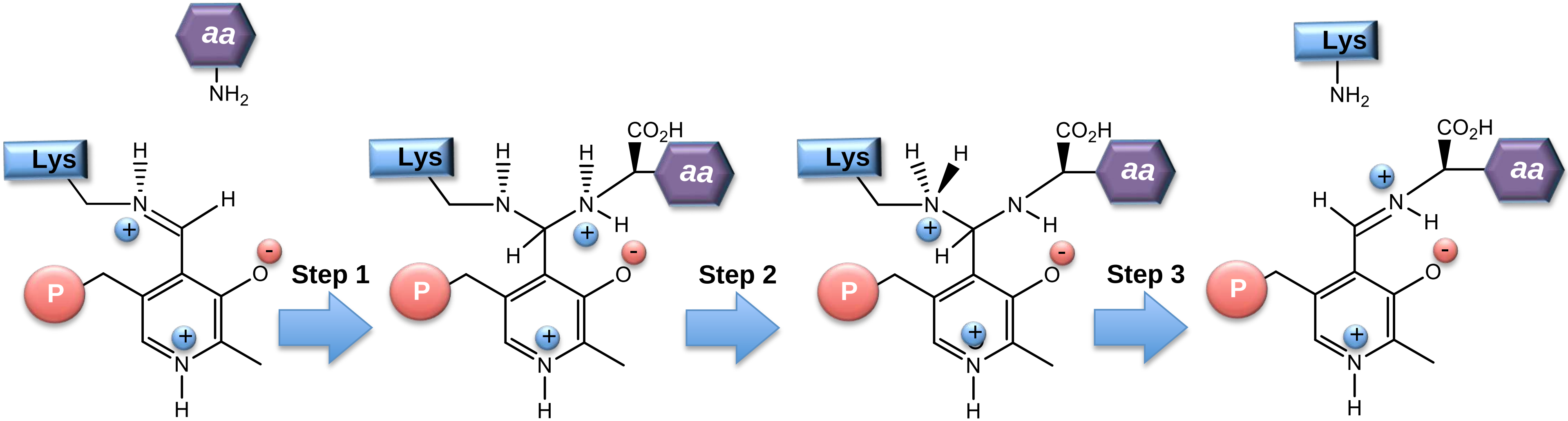
Ea= 12.6 kcal /mol
Er= -2.0 kcal/mol

Internal Aldimine

Gemdiamine Intermediate

Transimination

Transimination reaction



Ea= 10.8 kcal /mol
Er= -5.7 kcal/mol

Ea= 12.6 kcal /mol
Er= -2.0 kcal/mol

Ea= 2.2 kcal /mol
Er= -4.3 kcal/mol

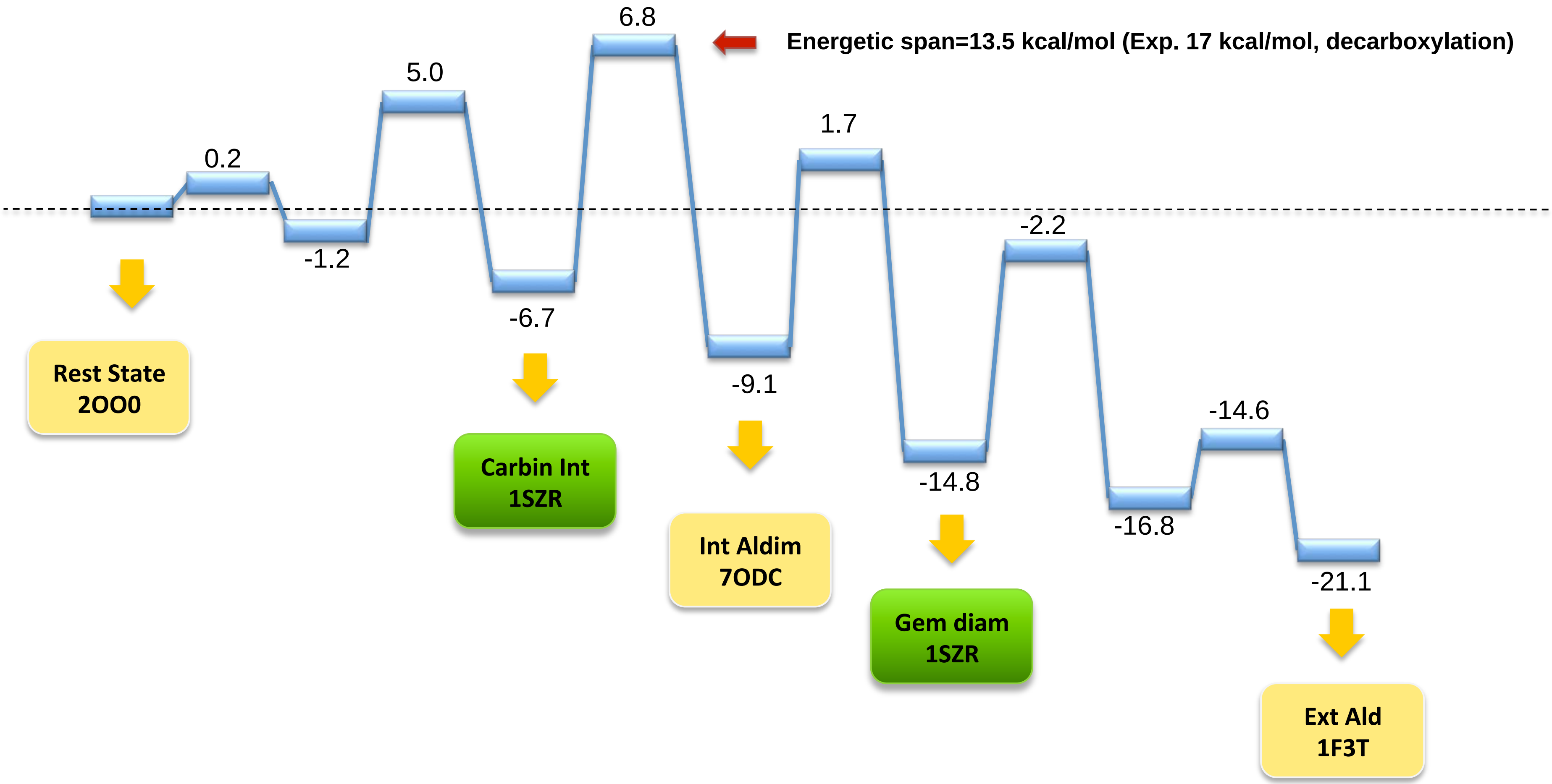
Internal Aldimine

Gemdiamine Intermediate

External Aldimine

J Chem Theory Comput 2011 7, 1356-1368

Complete Potential Energy Profile



Energetic span=13.5 kcal/mol (Exp. 17 kcal/mol, decarboxylation)

Rest State
2OO0

Carbin Int
1SZR

Int Aldim
7ODC

Gem diam
1SZR

Ext Ald
1F3T

J Am Chem Soc 2011, 133, 15496 J Chem Theory Comput 2011 7, 1356

Conclusions

- This study provides for the first time an **atomic level portrait** of the formation of the imine intermediate and the transimination reaction in a enzymatic environment.

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- The mechanism should be **common to all PLP dependent enzymes** that have amino acids as substrates.

J Am Chem Soc 2011, 133, 15496 J Chem Theory Comput 2011 7, 1356-1368

Conclusions

This study provides for the first time **an atomic level portrait** of the formation of the imine intermediate and the transimination reaction in a enzymatic environment.

The mechanism should be **common to all PLP dependent enzymes** that have amino acids as substrates.

The results explain many unrelated experimental results :

- The conserved Cys360 is required for the activation of the enzymes
- The transimination reaction is favored by Tyr389 or by a conserved water molecule.
- The role of a hypothetical conserved water molecule has been revealed

J Am Chem Soc 2011, 133, 15496

J Chem Theory Comput 2011 7, 1356-1368

EXAMPLE 2: HYDROLYSIS OF GLYCOSIDIC BONDS BY β-GAL

β-galactosidase

Catalyses both the hydrolysis and transglycosylation of polyssacharides

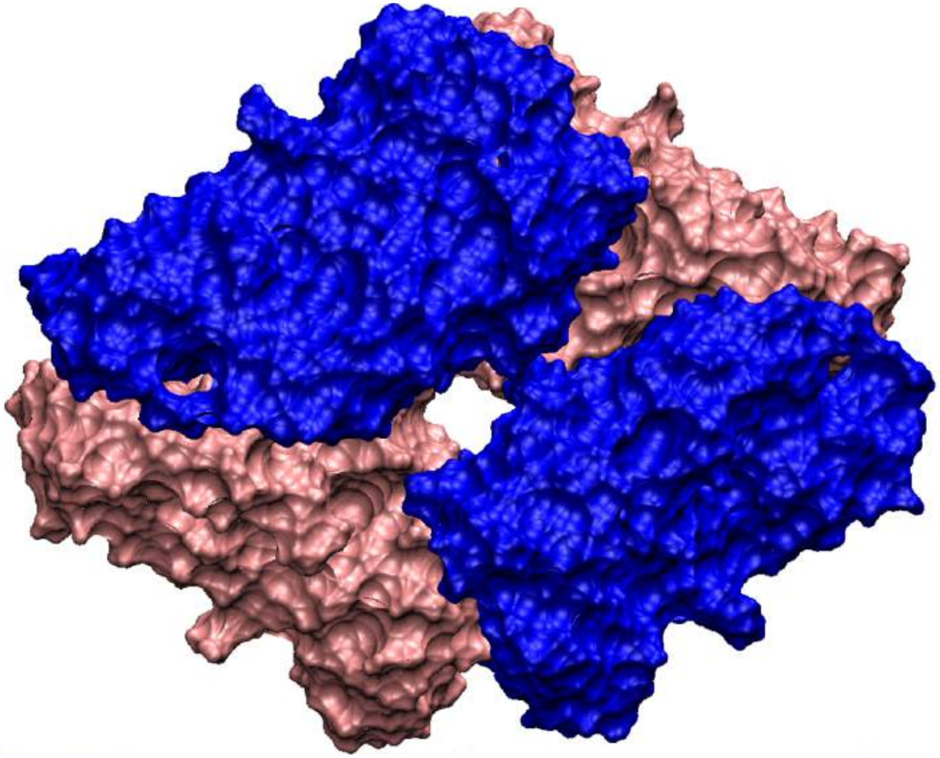
Marked preference for lactose as

substrate

Allolactose is the major transglycosylation product

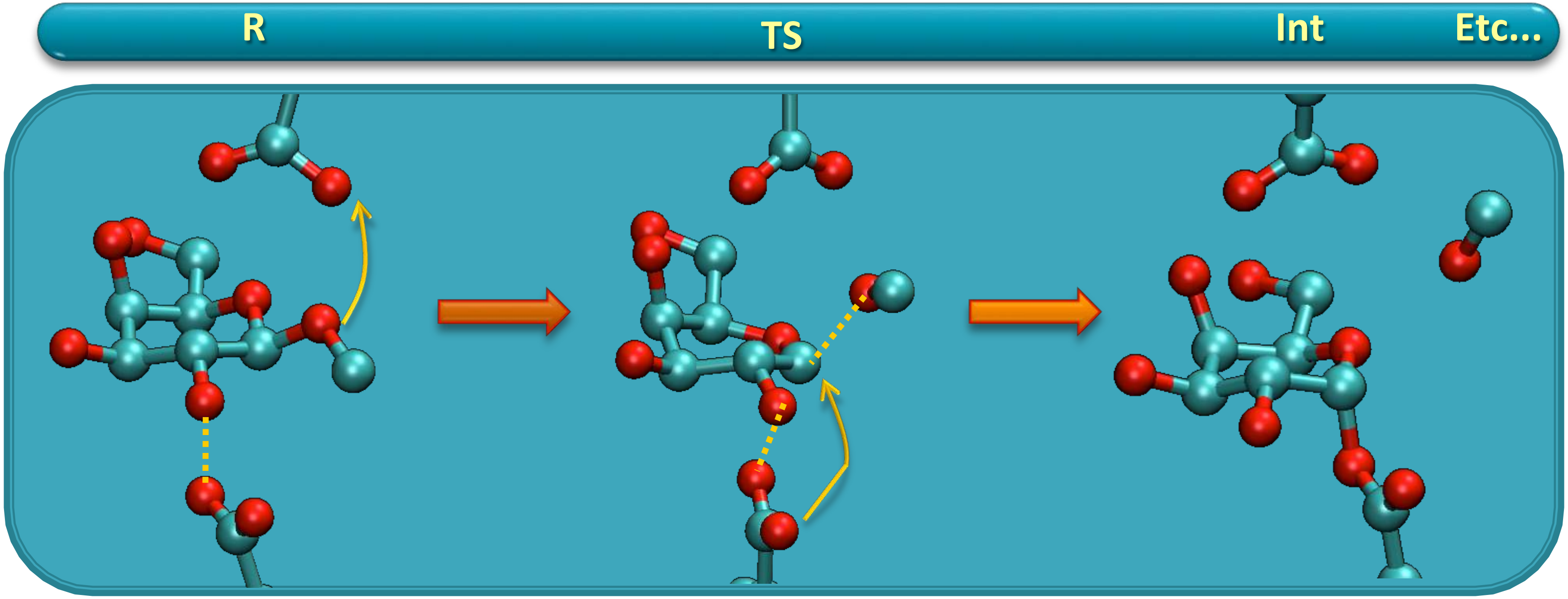
Great interest for the food and cosmetic industries

OBJECTIVE: Understand the catalytic mech and transglyc stereospecificity



COMPUTATIONAL DETAILS

Hydrolysis of glycosidic bonds– The Hamiltonian

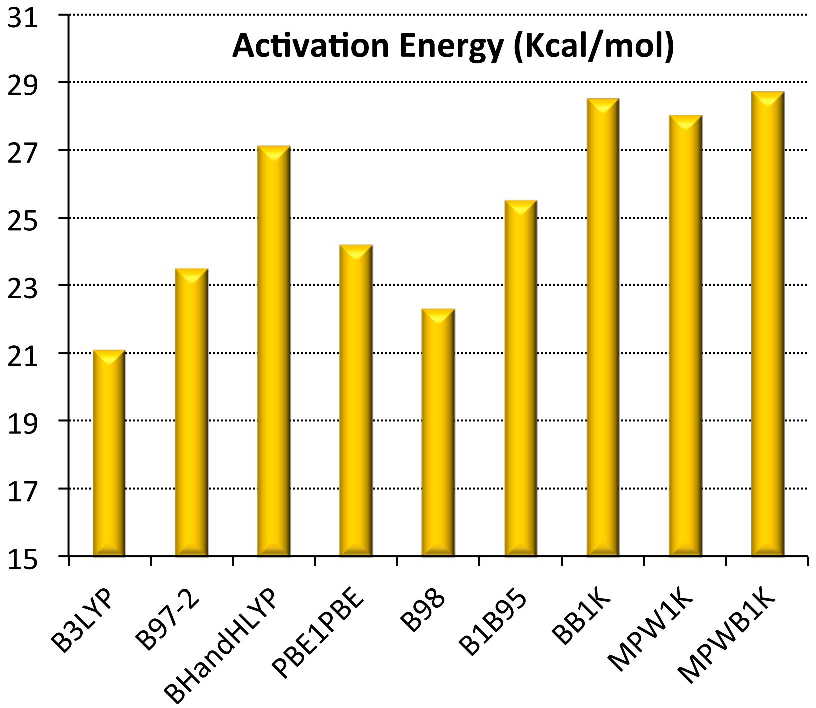


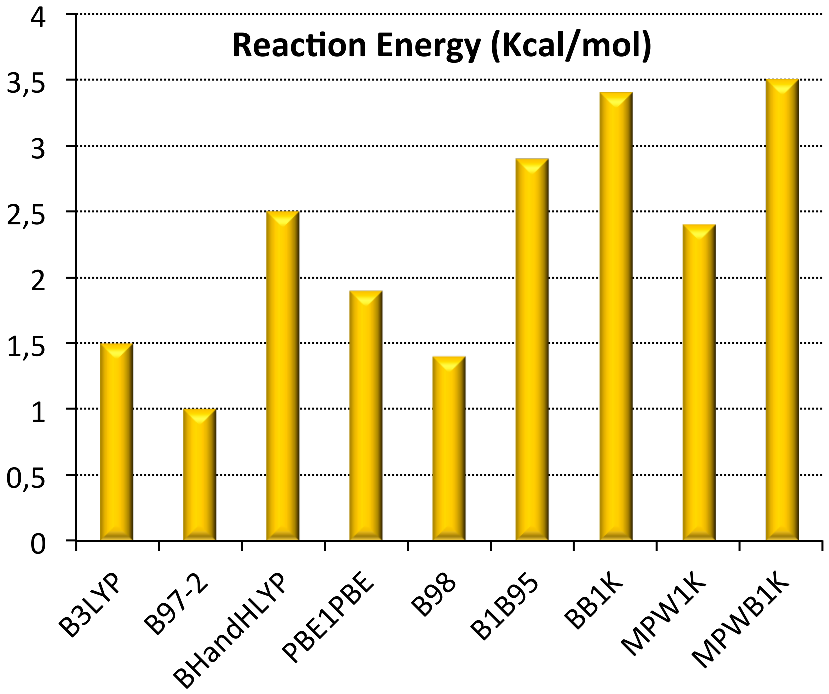
Small Model used to compare DFT functionals using the 6-311++G(2d,2p) basis set

Small Model used to benchmark selected DFT functionals against CCSD(T)/CBS calculations

Benchmarking of DFT Functionals

Very large differences (over 7 Kcal/mol) between E_{act} of different “reliable” DFT functionals





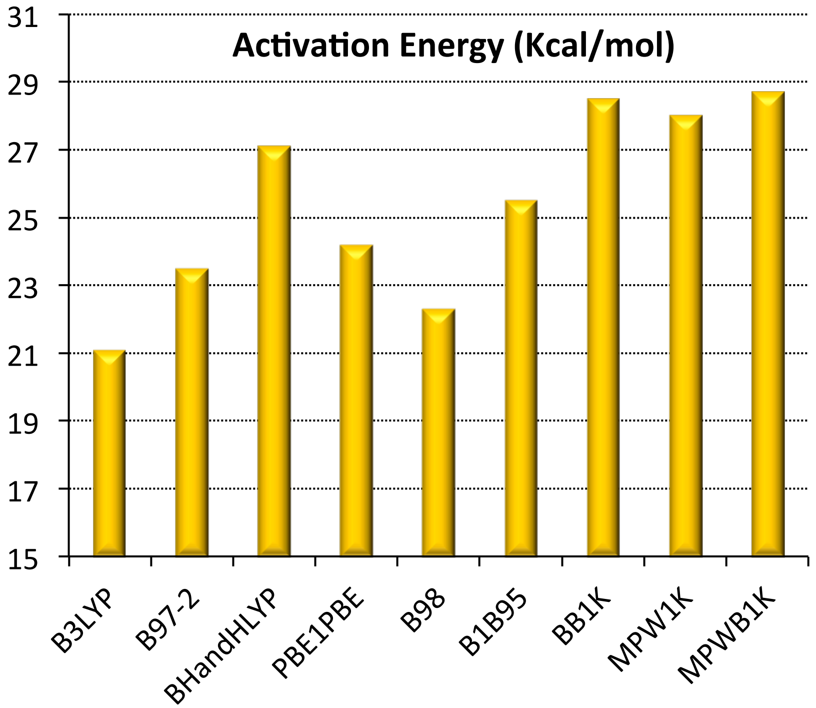
J Comp Chem, 29, 2565, 2008

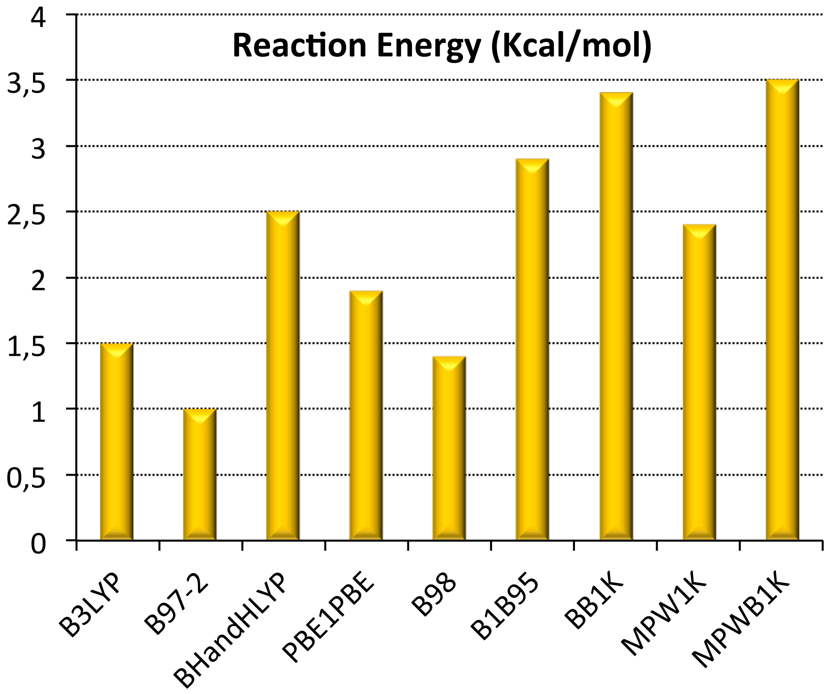
Theor Chem Acc, 119, 119, 2011

Benchmarking of DFT Functionals

Very large differences (over 7 Kcal/mol) between E_{act} of different "reliable" DFT functionals

Small differences (lower than 2.5 kcal/mol) in E_R





J Comp Chem, 29, 2565, 2008

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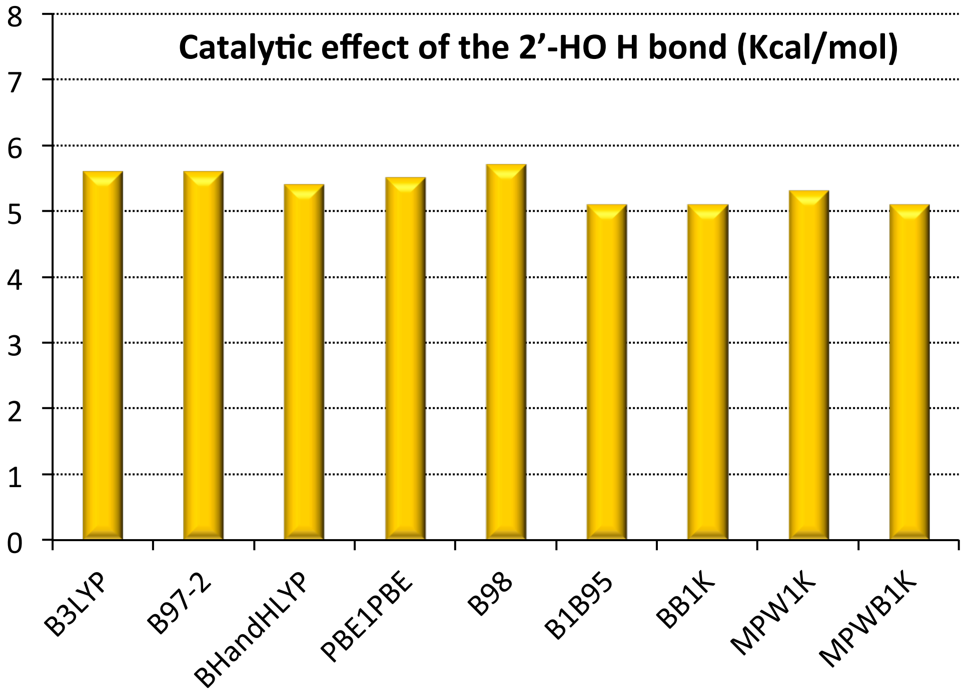
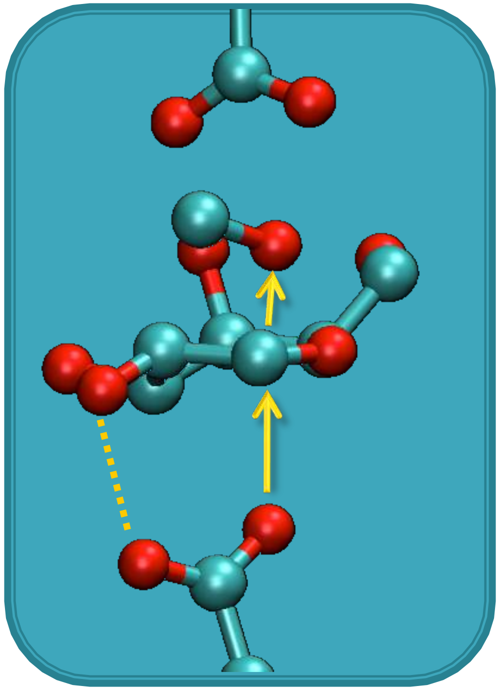
Benchmarking of DFT Functionals

Very large differences (over 7 Kcal/mol) between E_{act} of different "reliable" DFT functionals

Small differences (lower than 2.5 kcal/mol) in E_R

Catalytic effect (H-bond) very similar when measured with different functionals (< 0.6 Kcal/mol)



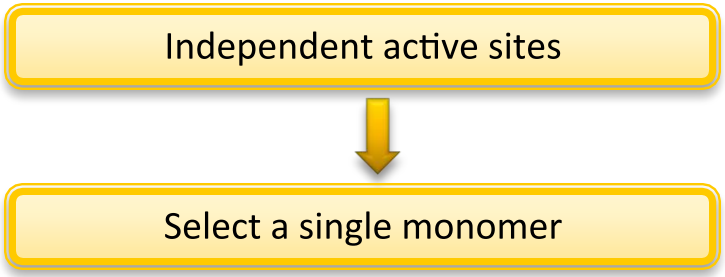


J Comp Chem, 29, 2565, 2008

Theor Chem Acc, 119, 119, 2011

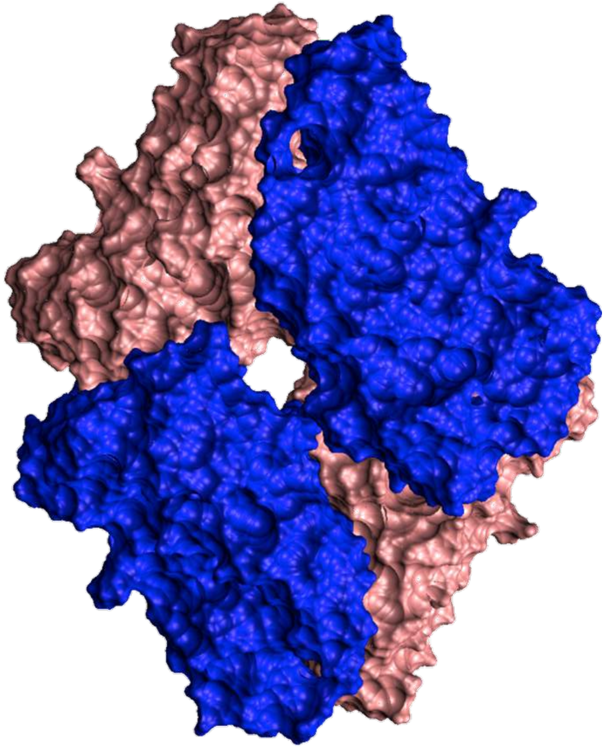
LARGE QM/MM MODEL

Initial Structure



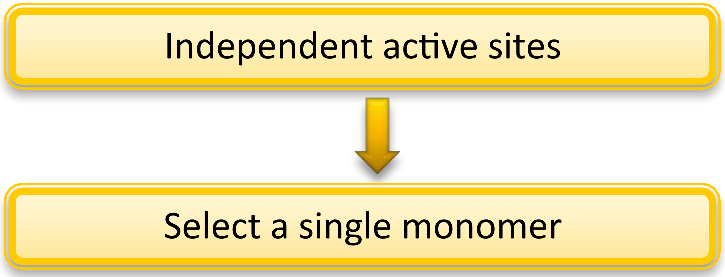
Independent active sites

Select a single monomer



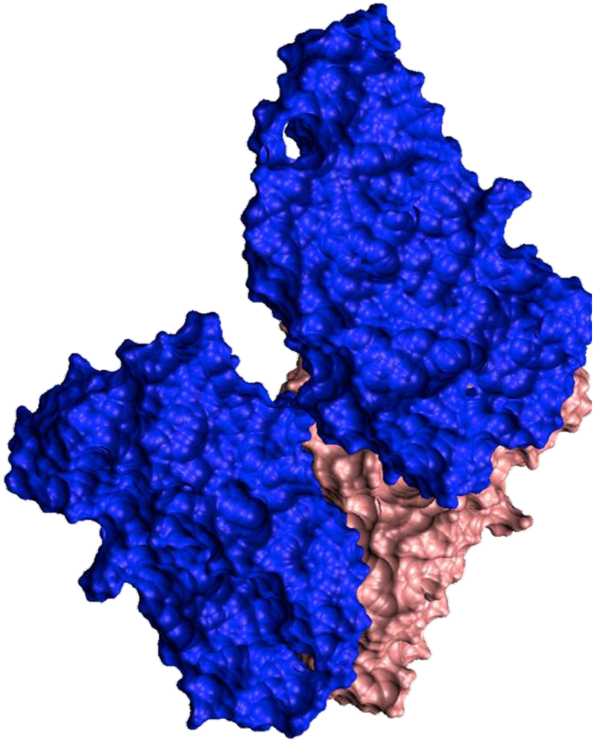
J Chem Theory Comput, 6, 421, 2010

Initial Structure



Independent active sites

Select a single monomer

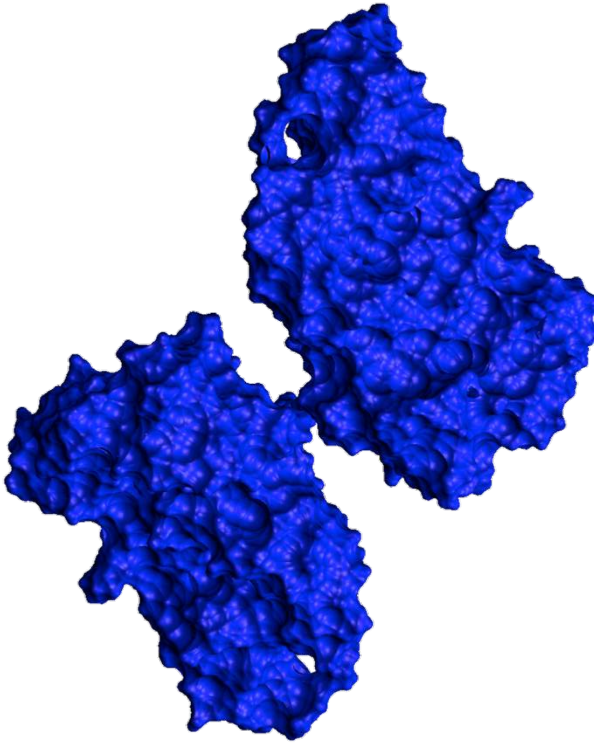


J Chem Theory Comput, 6, 421, 2010

Initial Structure

Independent active sites

Select a single monomer

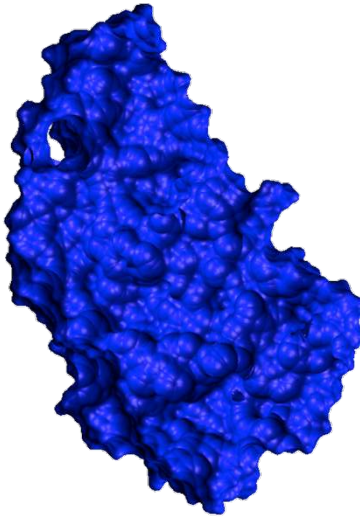


J Chem Theory Comput, 6, 421, 2010

Initial Structure

Independent active sites

Select a single monomer



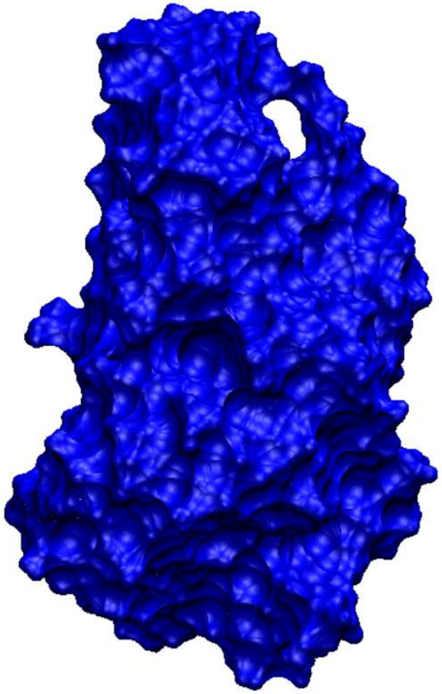
J Chem Theory Comput, 6, 421, 2010

Initial Structure

Independent active sites

↓

Select a single monomer

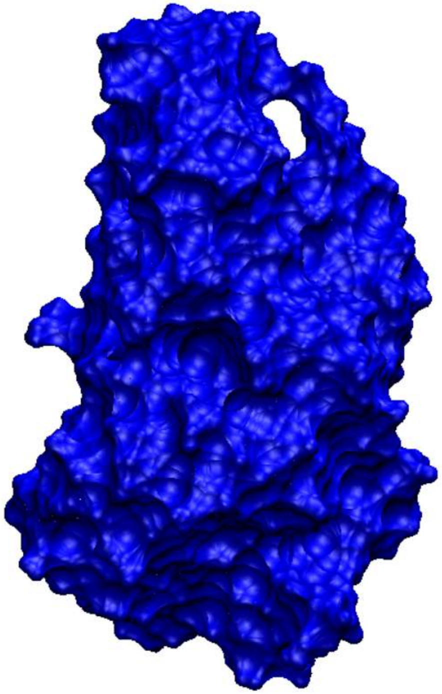


J Chem Theory Comput, 6, 421, 2010

Initial Structure

Independent active sites

Select a single monomer



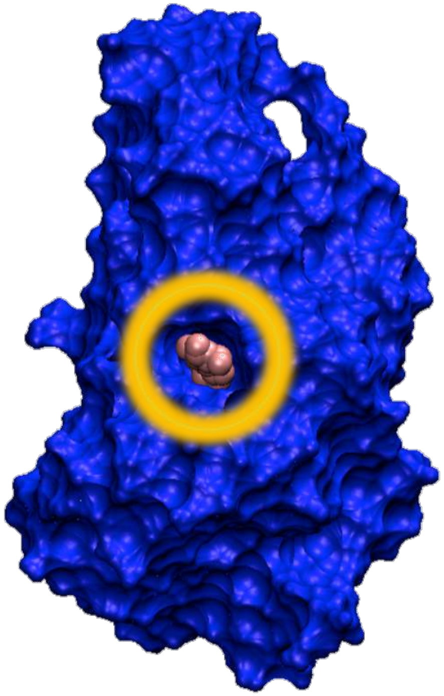
X-ray structures have substrate analogs in the “shallow mode”

J Chem Theory Comput, 6, 421, 2010

Initial Structure

Independent active sites

Select a single monomer

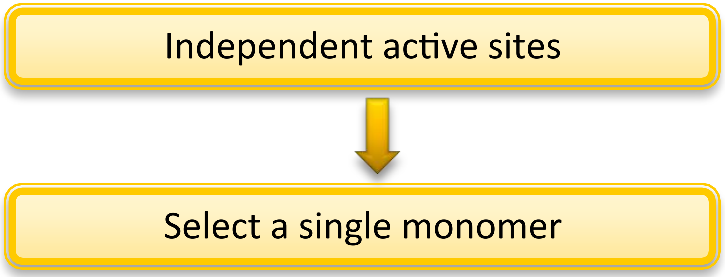


X-ray structures have substrate analogs in the "shallow mode"

Dock substrate in the "deep mode"

J Chem Theory Comput, 6, 421, 2010

Initial Structure



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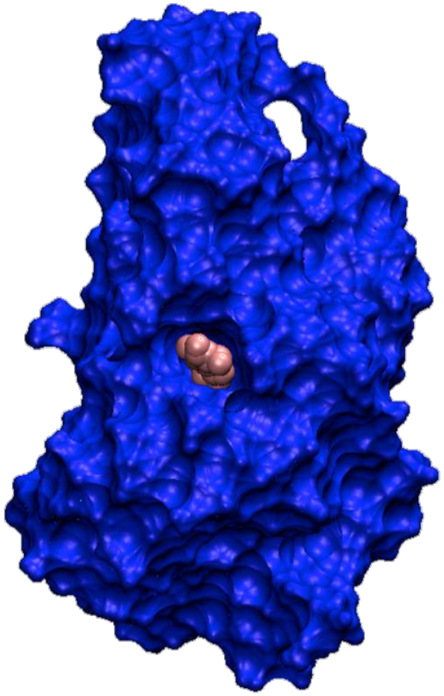
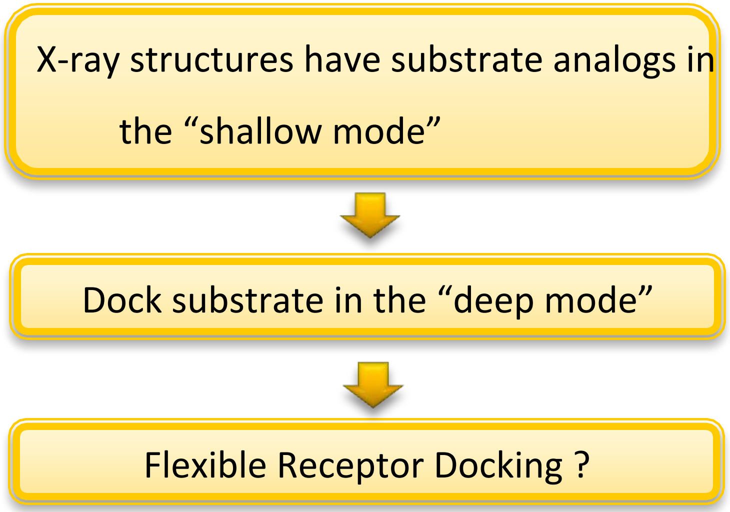




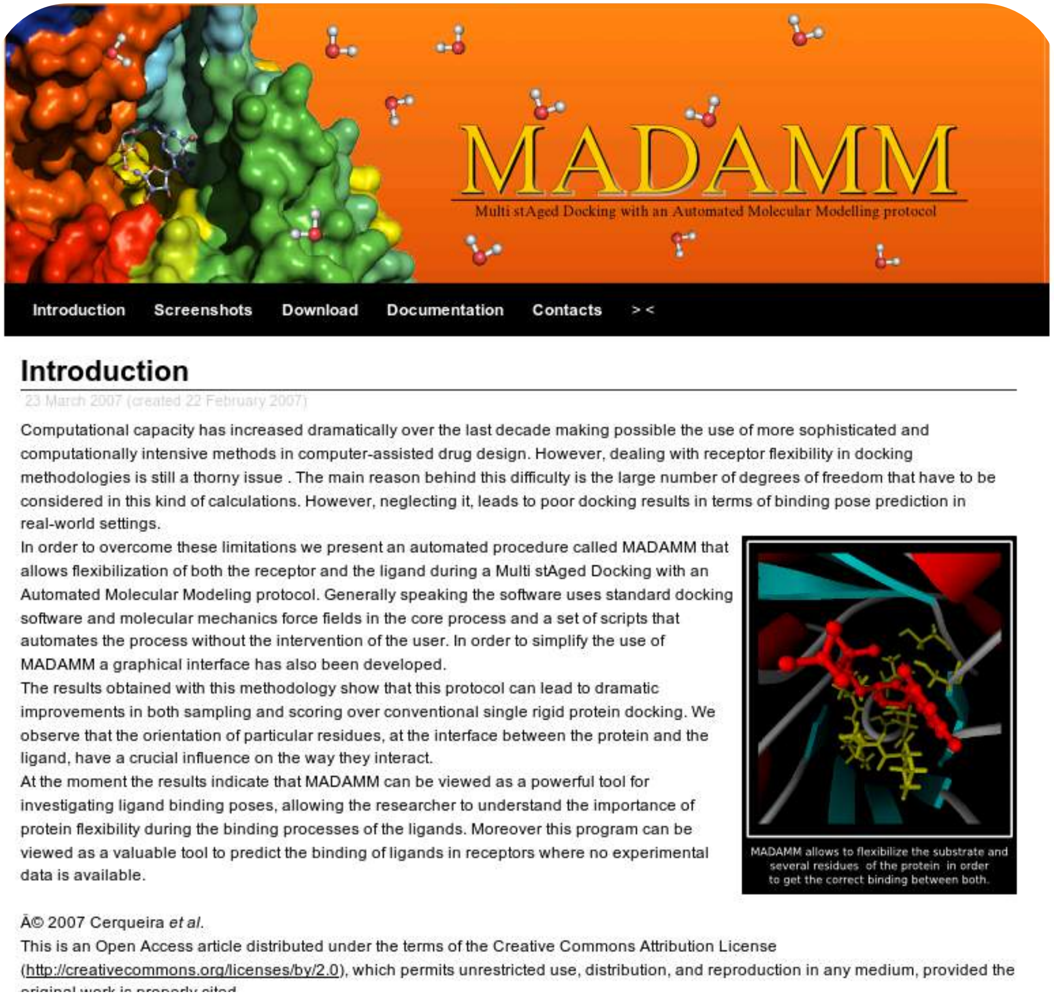
X-ray structures have substrate analogs in the “shallow mode”

Dock substrate in the “deep mode”

Flexible Receptor Docking ?

J Chem Theory Comput, 6, 421, 2010

MADAMM - Flexible Docking



MADAMM

Multi stAged Docking with an Automated Molecular Modelling protocol

Introduction Screenshots Download Documentation Contacts > <

Introduction

23 March 2007 (created 22 February 2007)

Computational capacity has increased dramatically over the last decade making possible the use of more sophisticated and computationally intensive methods in computer-assisted drug design. However, dealing with receptor flexibility in docking methodologies is still a thorny issue . The main reason behind this difficulty is the large number of degrees of freedom that have to be considered in this kind of calculations. However, neglecting it, leads to poor docking results in terms of binding pose prediction in real-world settings.

In order to overcome these limitations we present an automated procedure called MADAMM that allows flexibilization of both the receptor and the ligand during a Multi stAged Docking with an Automated Molecular Modeling protocol. Generally speaking the software uses standard docking software and molecular mechanics force fields in the core process and a set of scripts that automates the process without the intervention of the user. In order to simplify the use of MADAMM a graphical interface has also been developed.

The results obtained with this methodology show that this protocol can lead to dramatic improvements in both sampling and scoring over conventional single rigid protein docking. We observe that the orientation of particular residues, at the interface between the protein and the ligand, have a crucial influence on the way they interact.

At the moment the results indicate that MADAMM can be viewed as a powerful tool for investigating ligand binding poses, allowing the researcher to understand the importance of protein flexibility during the binding processes of the ligands. Moreover this program can be viewed as a valuable tool to predict the binding of ligands in receptors where no experimental data is available.

MADAMM allows to flexibilize the substrate and several residues of the protein in order to get the correct binding between both.

Â© 2007 Cerqueira *et al.*

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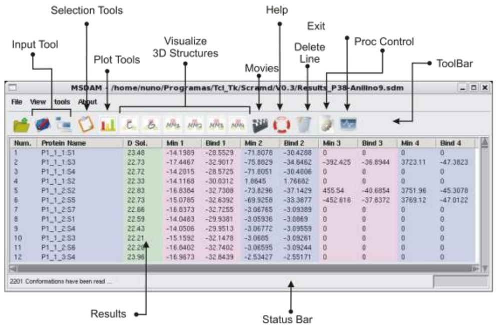
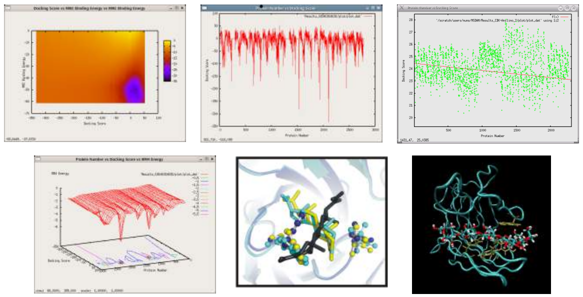
A **M**ULTI
ST**A**GED
DOCKING WITH
AN **A**UTOMATED
MOLECULAR
MODELING PROTOCOL



MSDAM - /home/nuno/Programas/Tcl_Tk/Scramd/V0.3/Results_P38-Anilino9.sdm

Num.	Protein Name	D Sol.	Min 1	Bind 1	Min 2	Bind 2	Min 3	Bind 3	Min 4	Bind 4
1	P1_1_1:S1	23.48	-14.1989	-28.5529	-71.8076	-30.4288	0	0	0	0
2	P1_1_1:S3	22.73	-17.4467	-32.9017	-75.8829	-34.6462	-392.425	-36.8944	3723.11	-47.3823
3	P1_1_1:S4	22.72	-14.2015	-28.5725	-71.8051	-30.4006	0	0	0	0
4	P1_1_1:S2	22.33	-14.1168	-30.0312	1.8645	1.76682	0	0	0	0
5	P1_1_2:S2	22.83	-16.8384	-32.7308	-73.8298	-37.1429	455.54	-40.6854	3751.96	-45.3078
6	P1_1_2:S5	22.73	-15.0765	-32.6392	-69.9258	-33.3877	-452.616	-37.8372	3769.12	-47.0122
7	P1_1_2:S7	22.66	-16.8373	-32.7255	-3.06765	-3.09389	0	0	0	0
8	P1_1_2:S1	22.59	-14.0483	-29.9381	-3.05936	-3.0869	0	0	0	0
9	P1_1_2:S4	22.43	-14.0506	-29.9513	-3.06772	-3.09559	0	0	0	0
10	P1_1_2:S3	22.21	-15.1592	-32.1478	-3.0685	-3.09261	0	0	0	0
11	P1_1_2:S6	22.20	-16.8402	-32.7402	-3.06595	-3.09244	0	0	0	0
12	P1_1_3:S4	23.96	-16.9673	-32.8439	-2.53427	-2.55171	0	0	0	0

2201 Conformations have been read ...

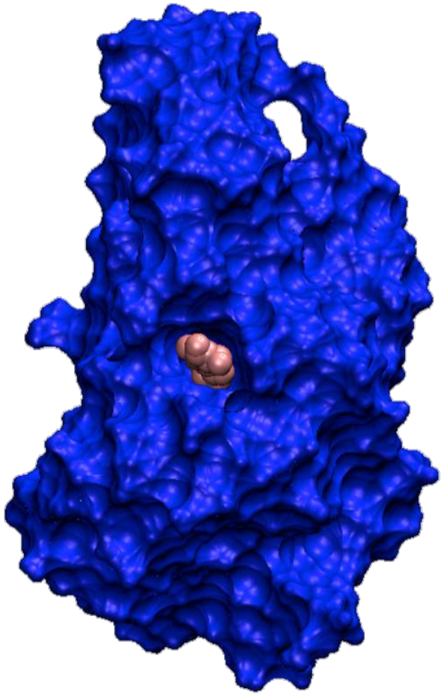


Proteins, 74, 192, 2009.

Initial Structure

Independent active sites

Select a single monomer



X-ray structures have substrate analogs in the “shallow mode”

Dock substrate in the “deep mode”

Flexible Receptor Docking ?

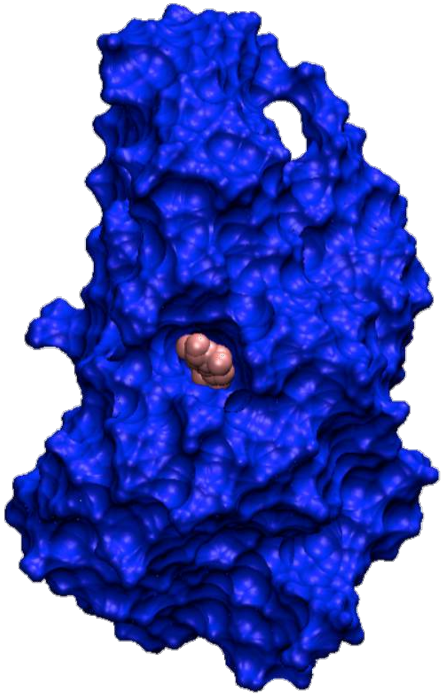
Unbound and Inhibitor bound structures highly similar

J Chem Theory Comput, 6, 421, 2010

Initial Structure

Independent active sites

Select a single monomer



X-ray structures have substrate analogs in the "shallow mode"

Dock substrate in the "deep mode"

Flexible Receptor Docking ?

Unbound and Inhibitor bound structures highly similar

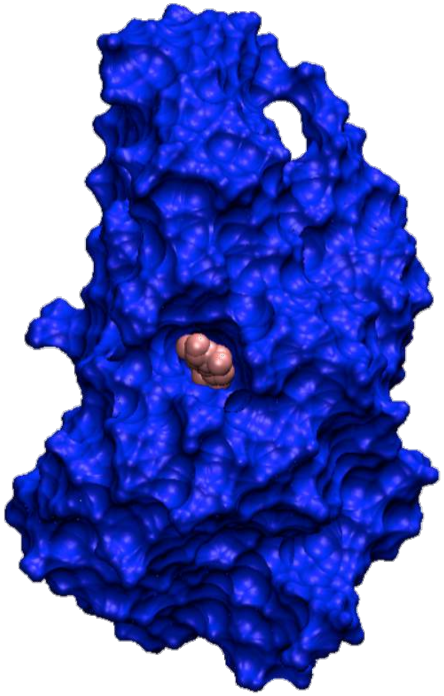
Biochemical constraints satisfied within the unbound structure

J Chem Theory Comput, 6, 421, 2010

Initial Structure

Independent active sites

Select a single monomer



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Flexible Receptor Docking ?

Unbound and Inhibitor bound structures highly similar

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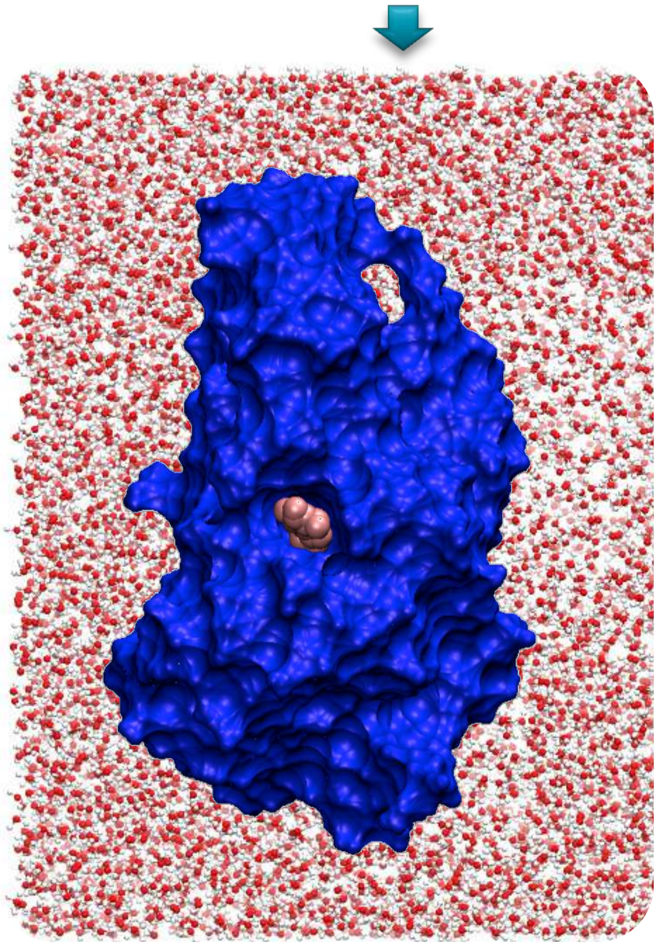
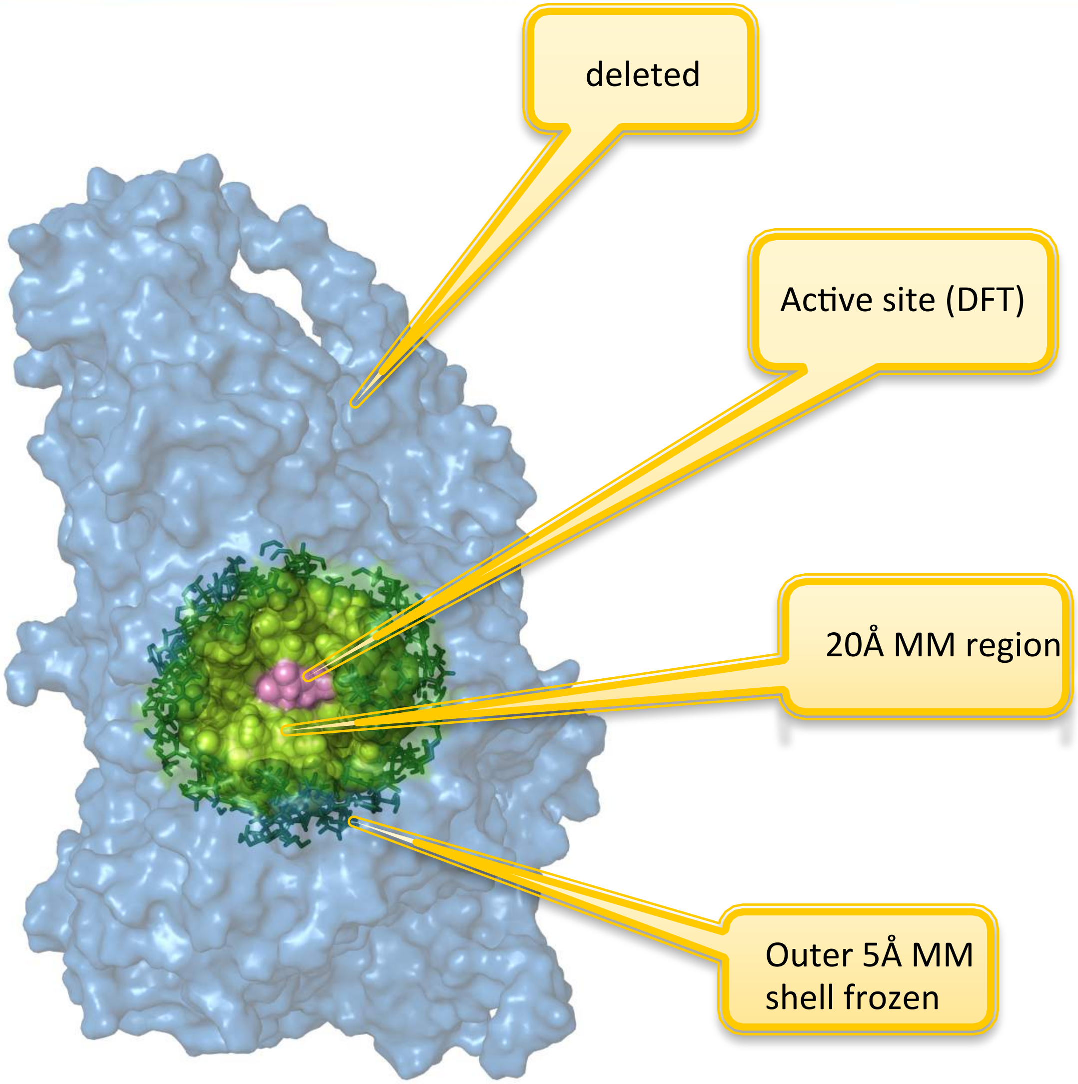
Rigid Receptor Docking .

J Chem Theory Comput, 6, 421, 2010

Initial Structure

Solvation + CM MD simulation





deleted

Active site (DFT)

20Å MM region

Outer 5Å MM shell frozen

J Chem Theory Comput, 6, 421, 2010

J Phys Chem B, 115, 14752, 2011.

Initial Structure

Final energies – QM/MM

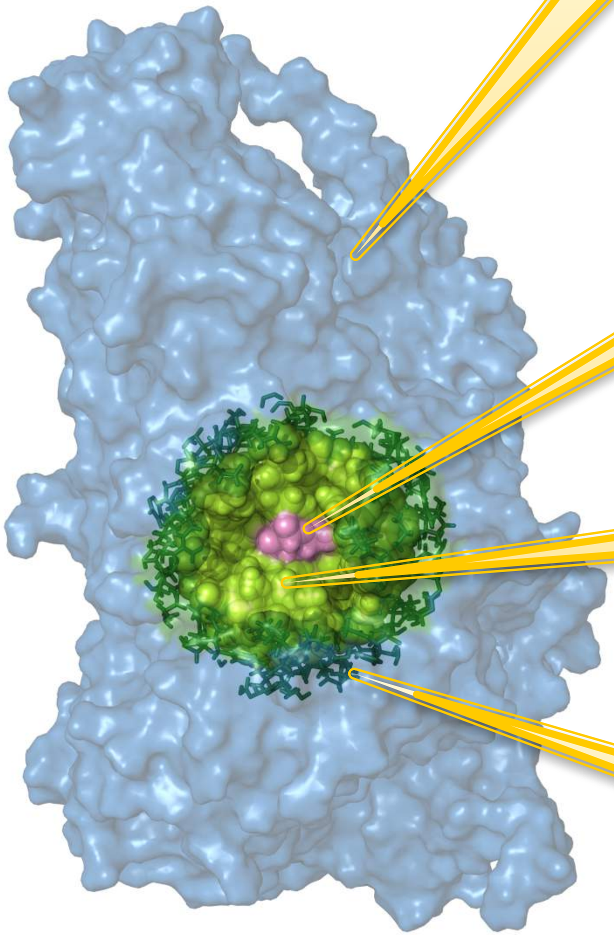
BB1K/6-311++G(2d,2p) ≈ 170 atoms

Amber FF ≈ 2700 atoms

Geom – QM/MM

B3LYP/6-31G(d) ≈ 50 atoms

Amber FF ≈ 2700 atoms



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J Chem Theory Comput, 6, 421, 2010

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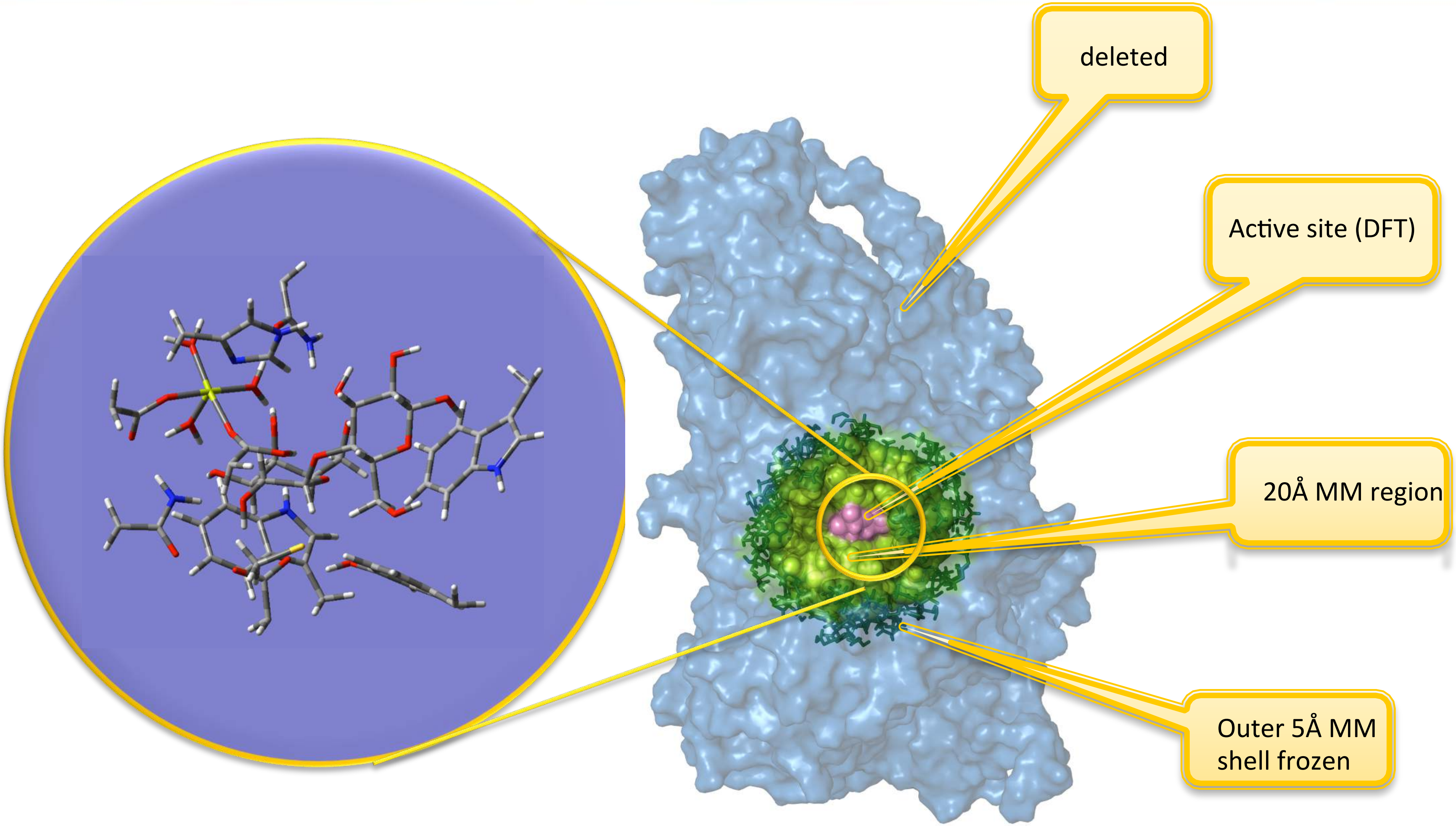
Initial Structure



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Active site (DFT)

20Å MM region

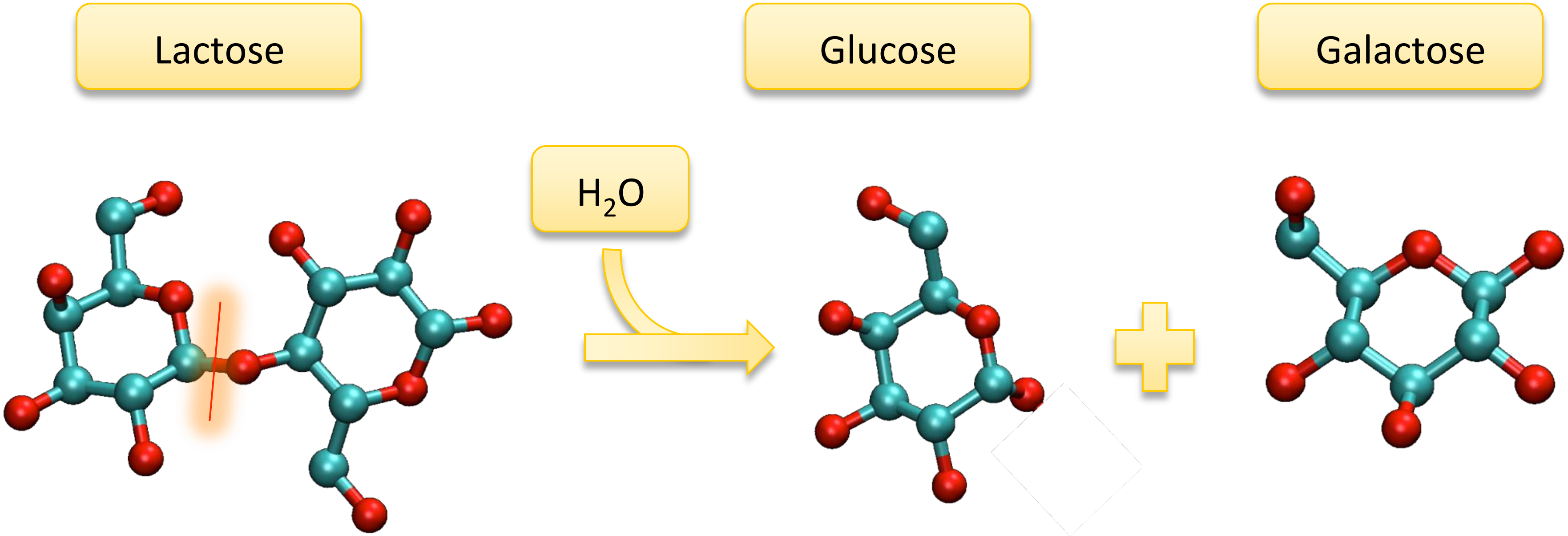
Outer 5Å MM shell frozen

J Chem Theory Comput, 6, 421, 2010

J Phys Chem B, 115, 14752, 2011.

RESULTS

Hydrolysis



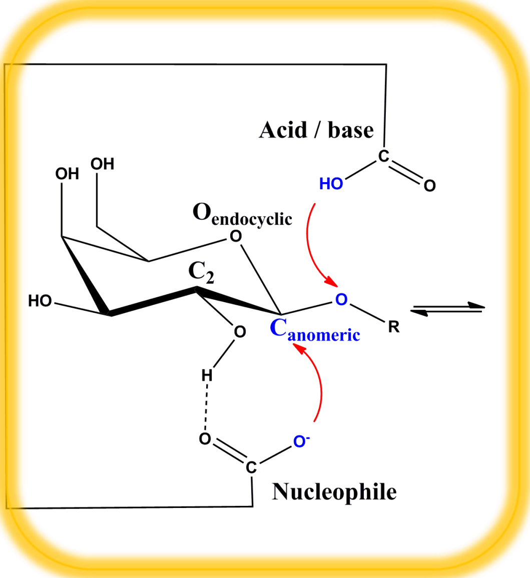
K_{cat} (water) = 10^{-15} s^{-1}, 37 kcal/mol, $t_{1/2}$=22 Myears

K_{cat}(β-gal)= 10^{2} s^{-1}, 15 kcal/mol $t_{1/2}$= 0,01 s

Theor Chem Acc, 122, 283, 2009

Hydrolysis

Reactants

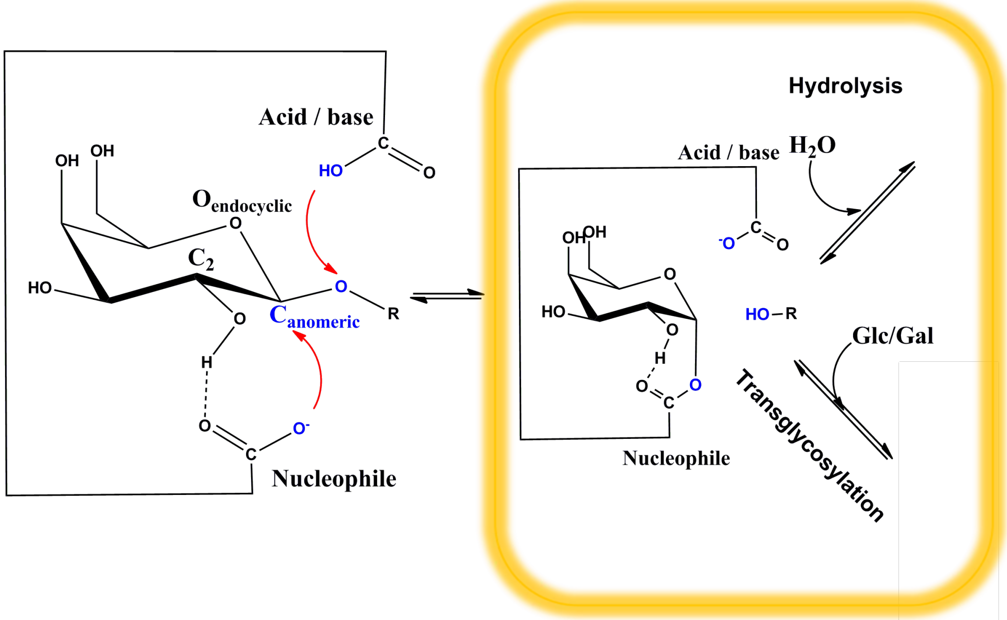


J Chem Theory Comput, 6, 421, 2010

Hydrolysis

Reactants

Covalent Intermediate



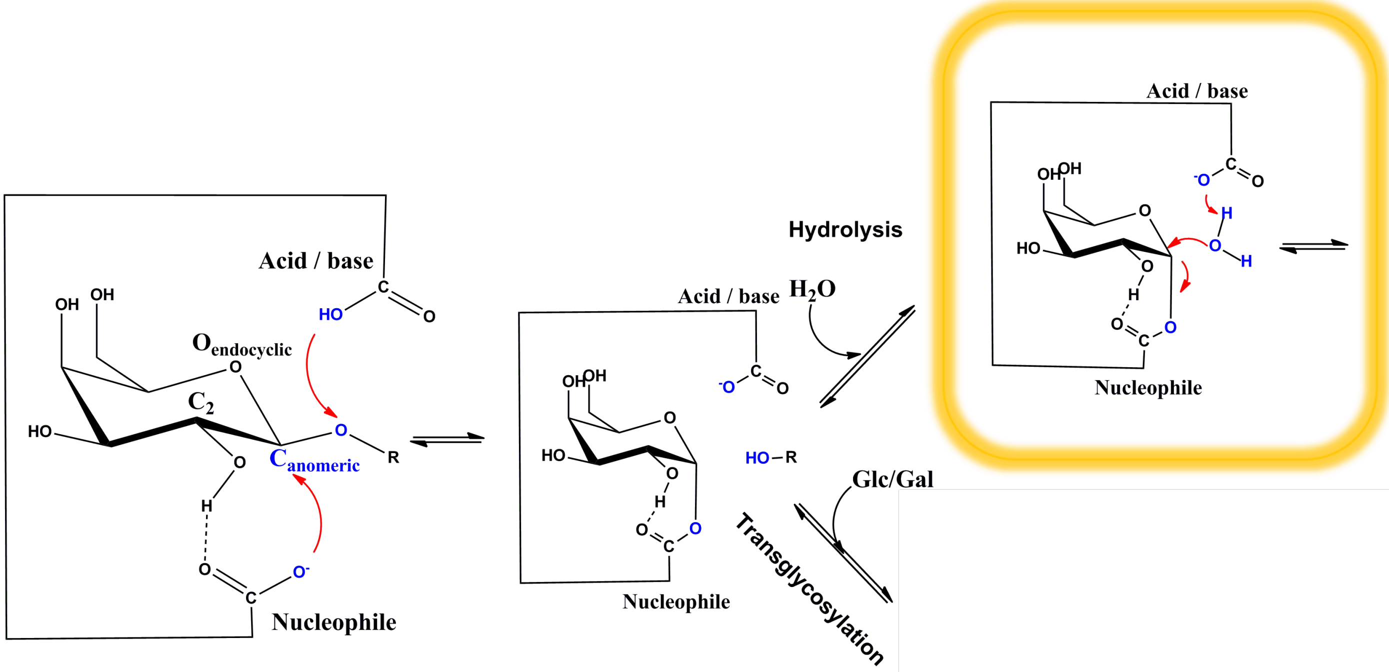
J Chem Theory Comput, 6, 421, 2010

Hydrolysis

Reactants

Covalent Intermediate

Water Attack



J Chem Theory Comput, 6, 421, 2010

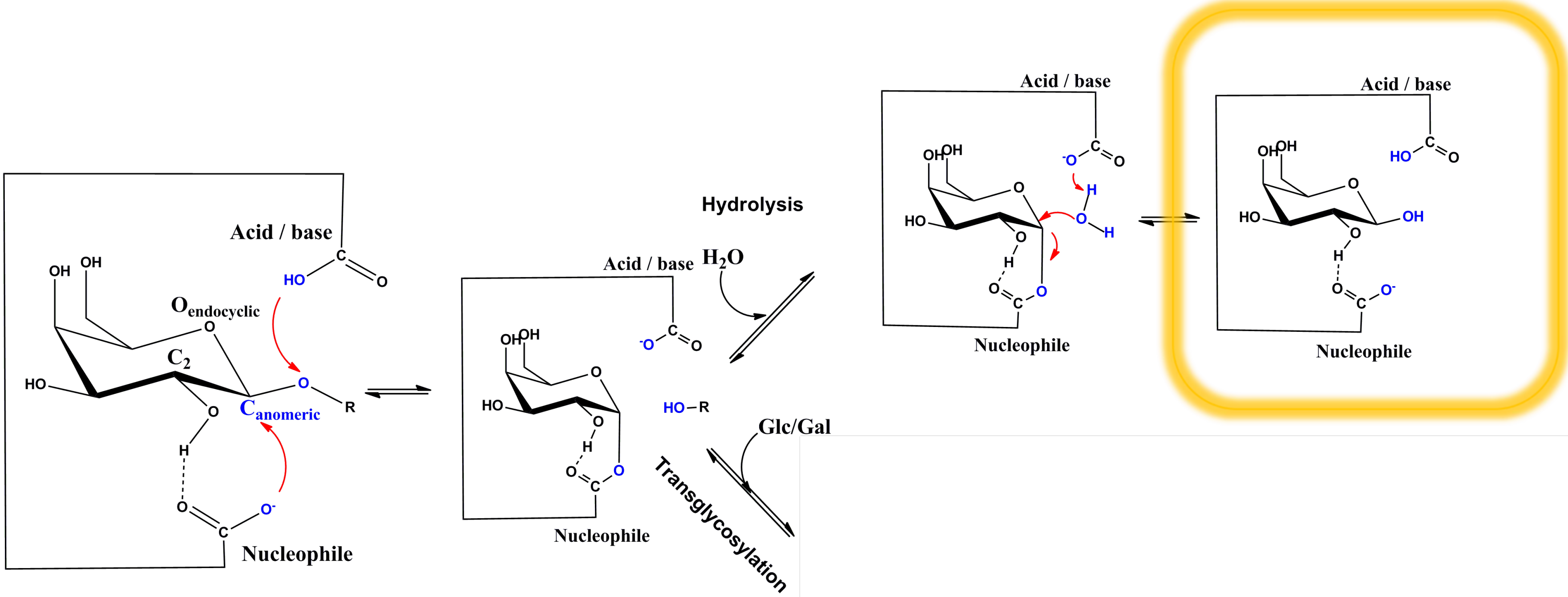
Hydrolysis

Reactants

Covalent Intermediate

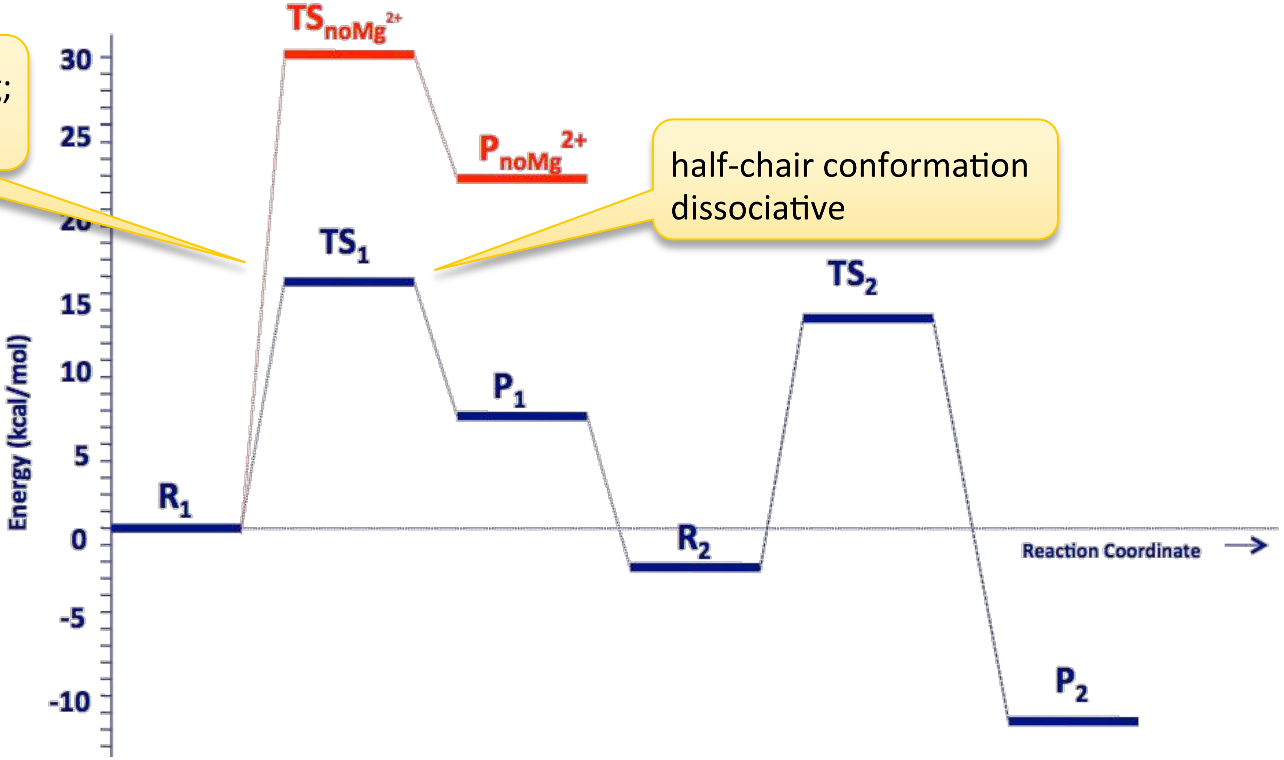
Water Attack

Products



J Chem Theory Comput, 6, 421, 2010

Hydrolysis



Exp: rate limiting;
Ea=15 Kcal/mol;

half-chair conformation
dissociative

Reactants

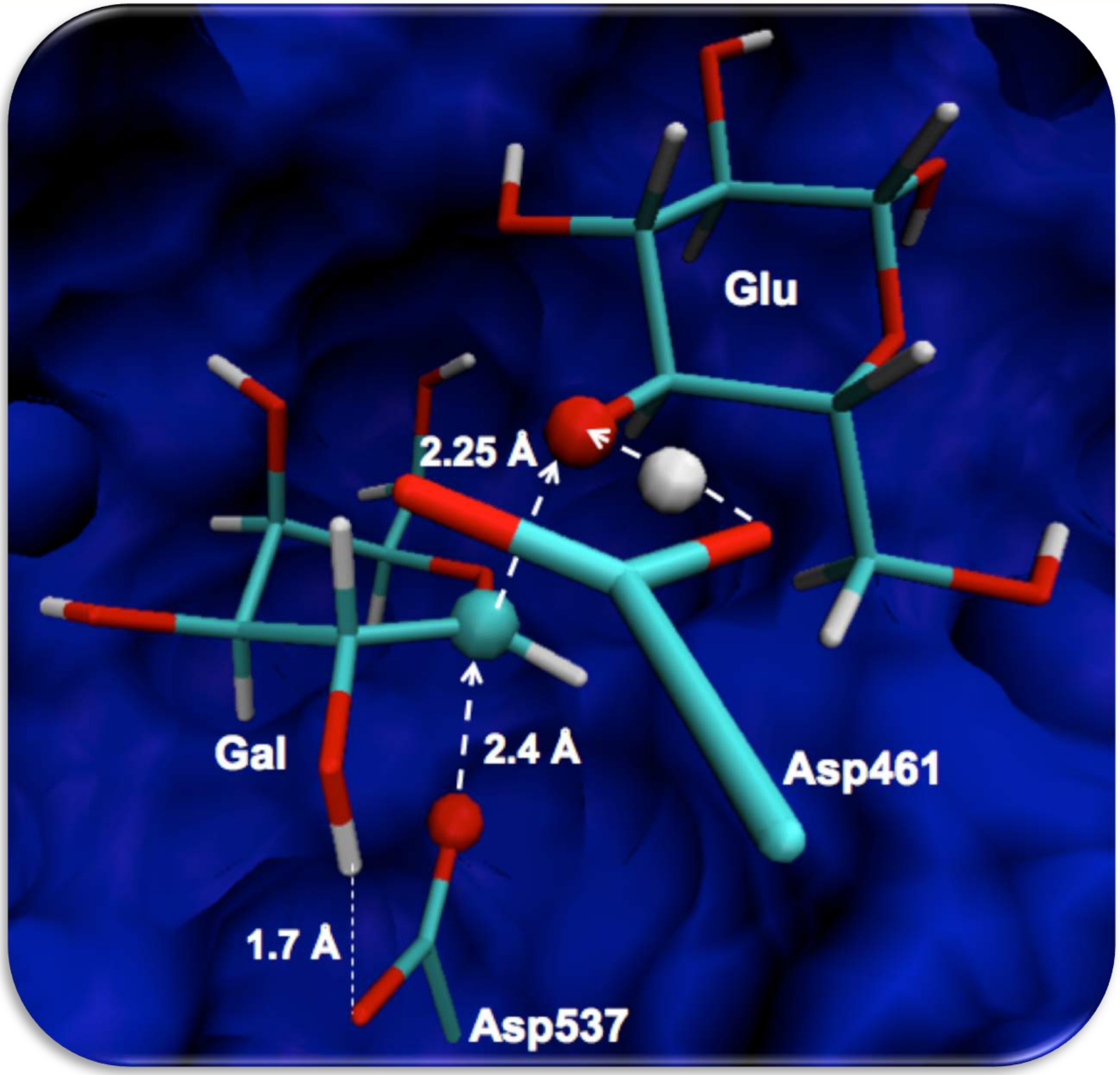
Covalent Intermediate

Water Attack

Products

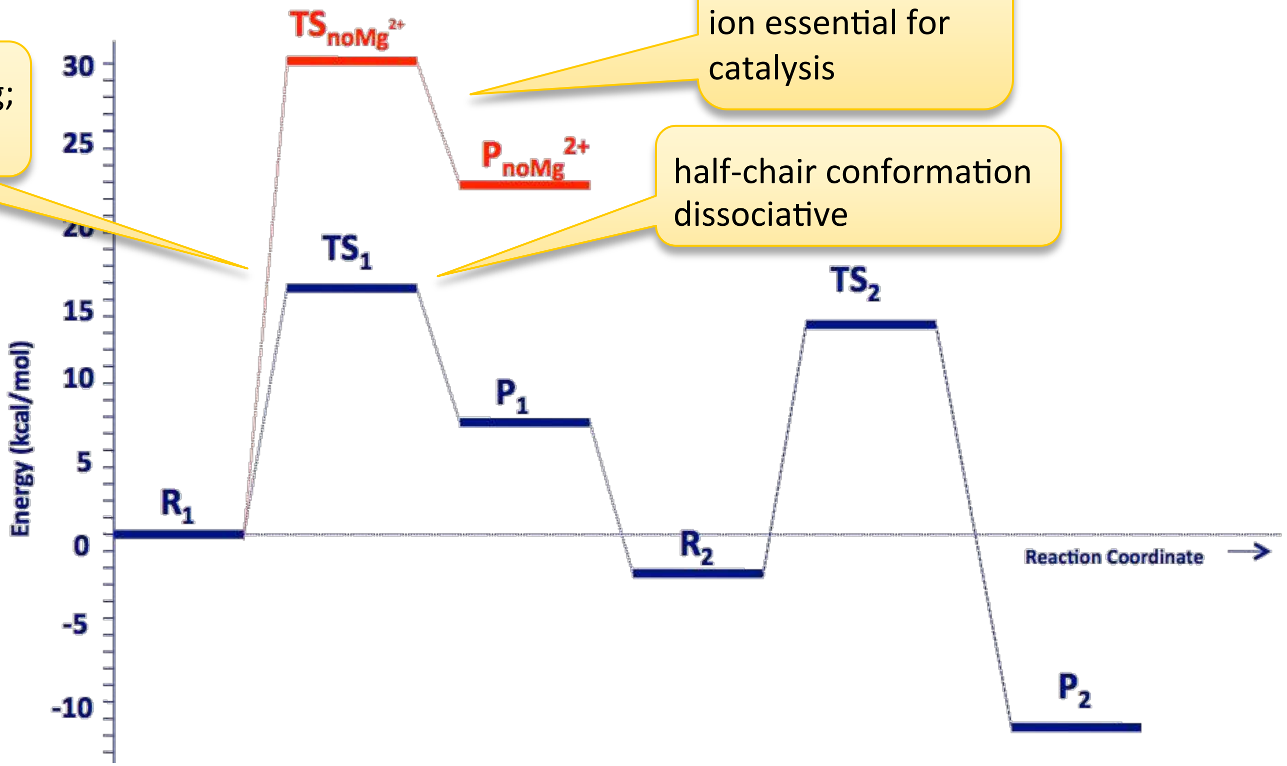
J. Chem. Theory Comput, 6, 421, 2010.

Hydrolysis



J. Chem. Theory Comput, 6, 421, 2010.

Hydrolysis



Exp: rate limiting;
Ea=15 Kcal/mol;

Active site Mg^{2+} ion essential for catalysis

half-chair conformation dissociative

Reactants

Covalent Intermediate

Water Attack

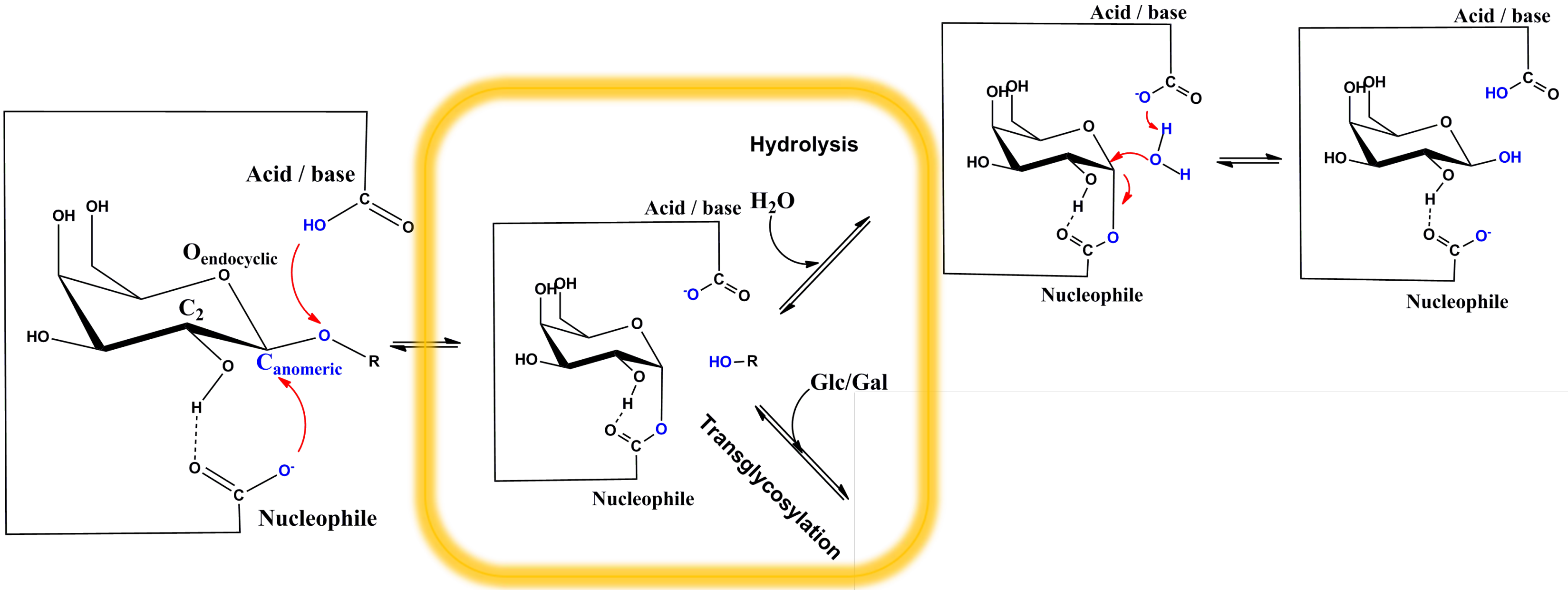
Products

J. Chem. Theory Comput, 6, 421, 2010.

Transglycosylation

Reactants

Covalent Intermediate



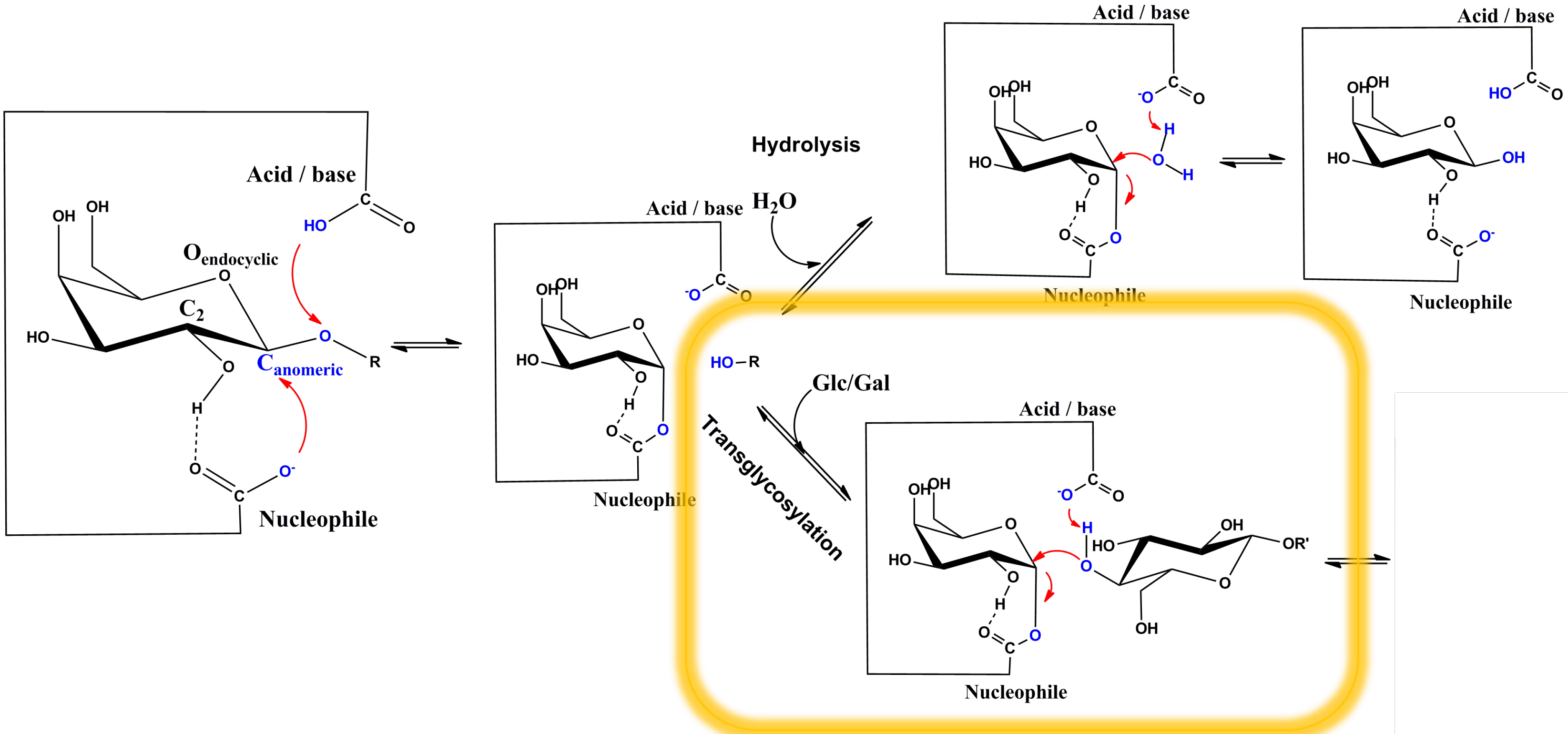
J Chem Theory Comput, 6, 421, 2010

Transglycosylation

Reactants

Covalent Intermediate

Sugar Attack



J Chem Theory Comput, 6, 421, 2010

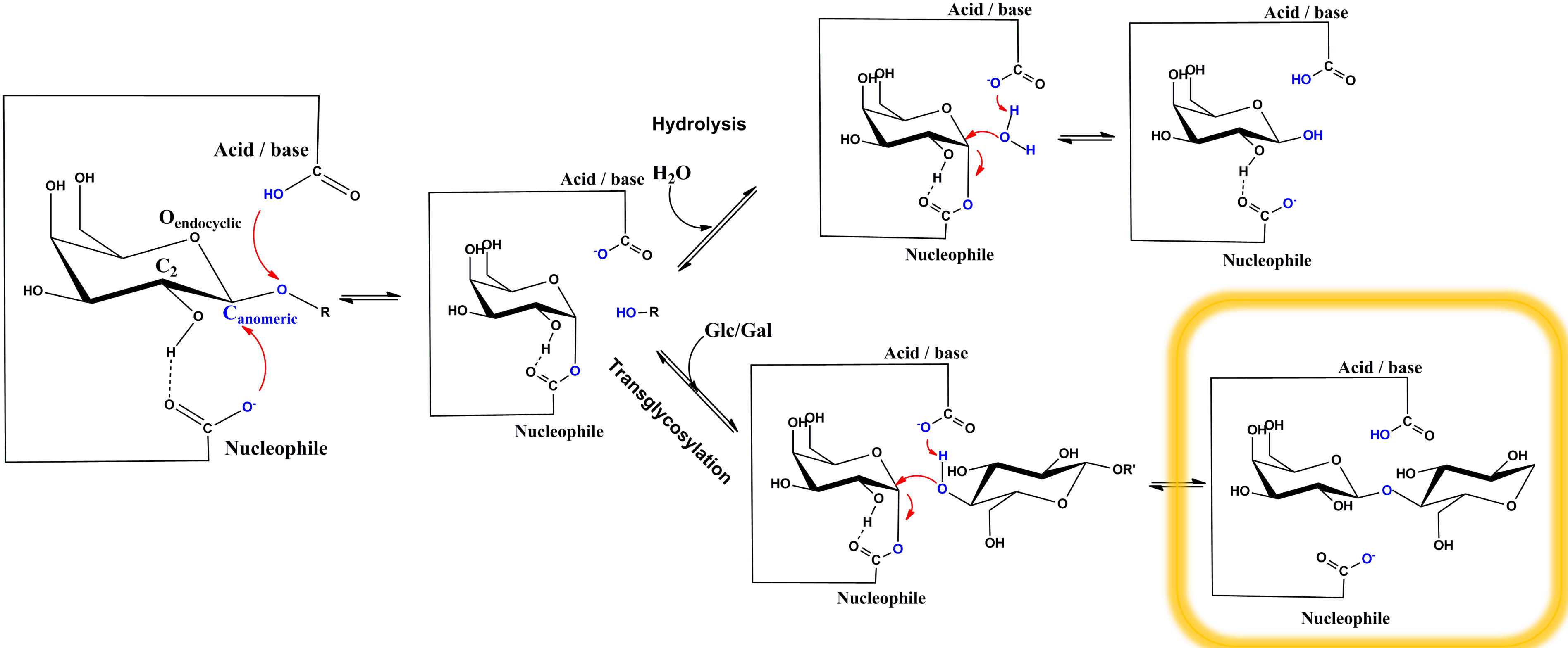
Transglycosylation

Reactants

Covalent Intermediate

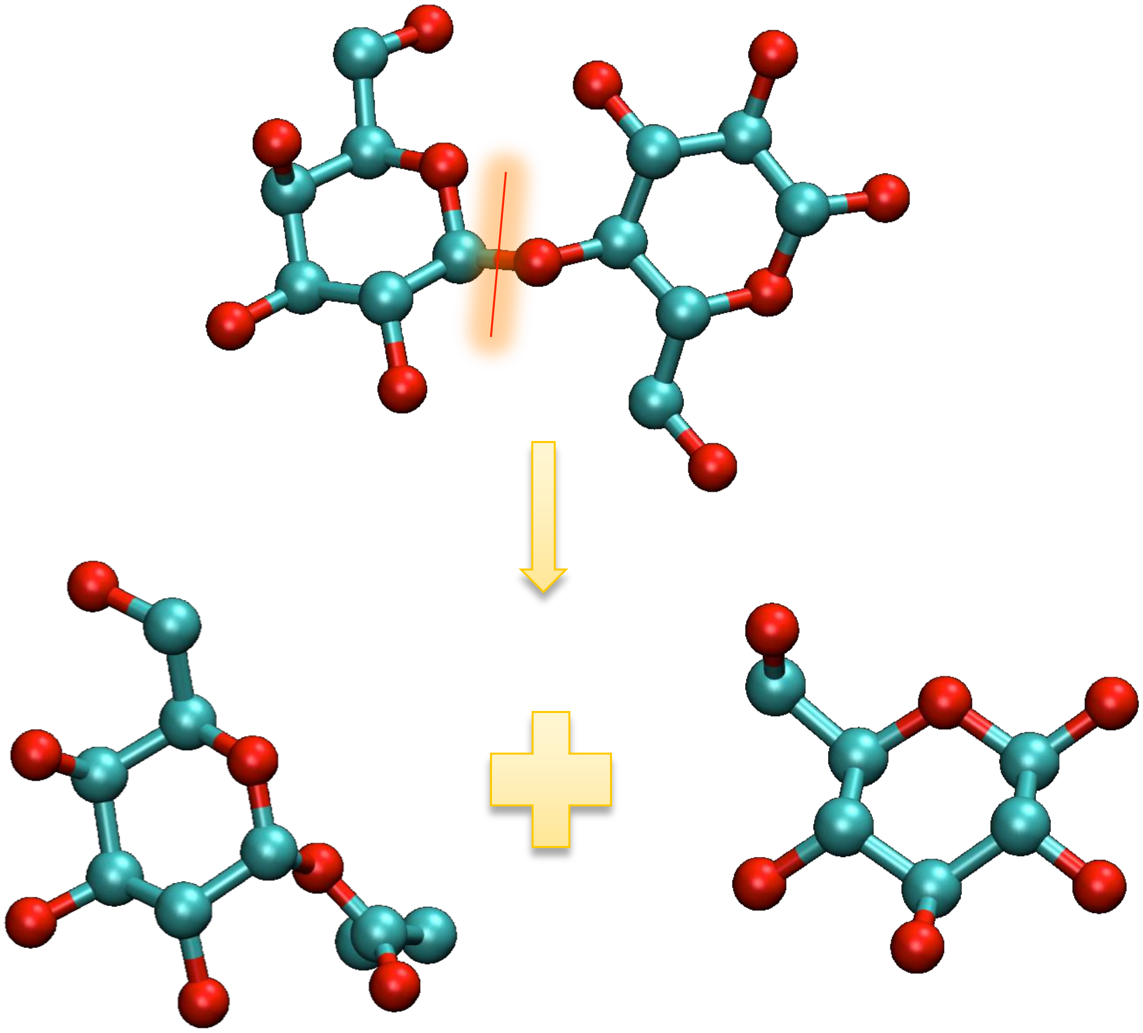
Sugar Attack

Products



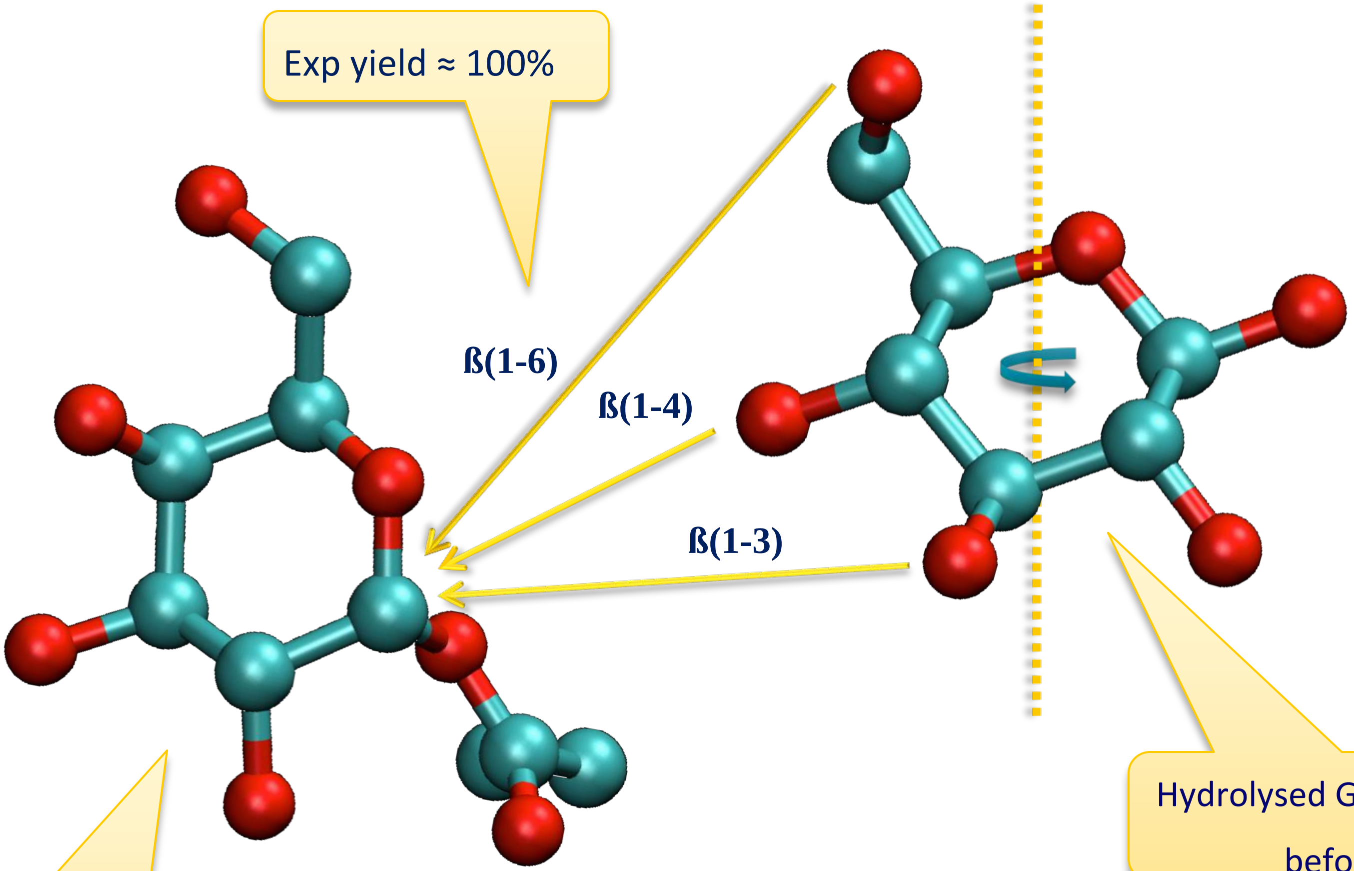
J Chem Theory Comput, 6, 421, 2010

Transglycosylation



Theor Chem Acc, 122, 283, 2009

Transglycosylation



Exp yield ≈ 100%

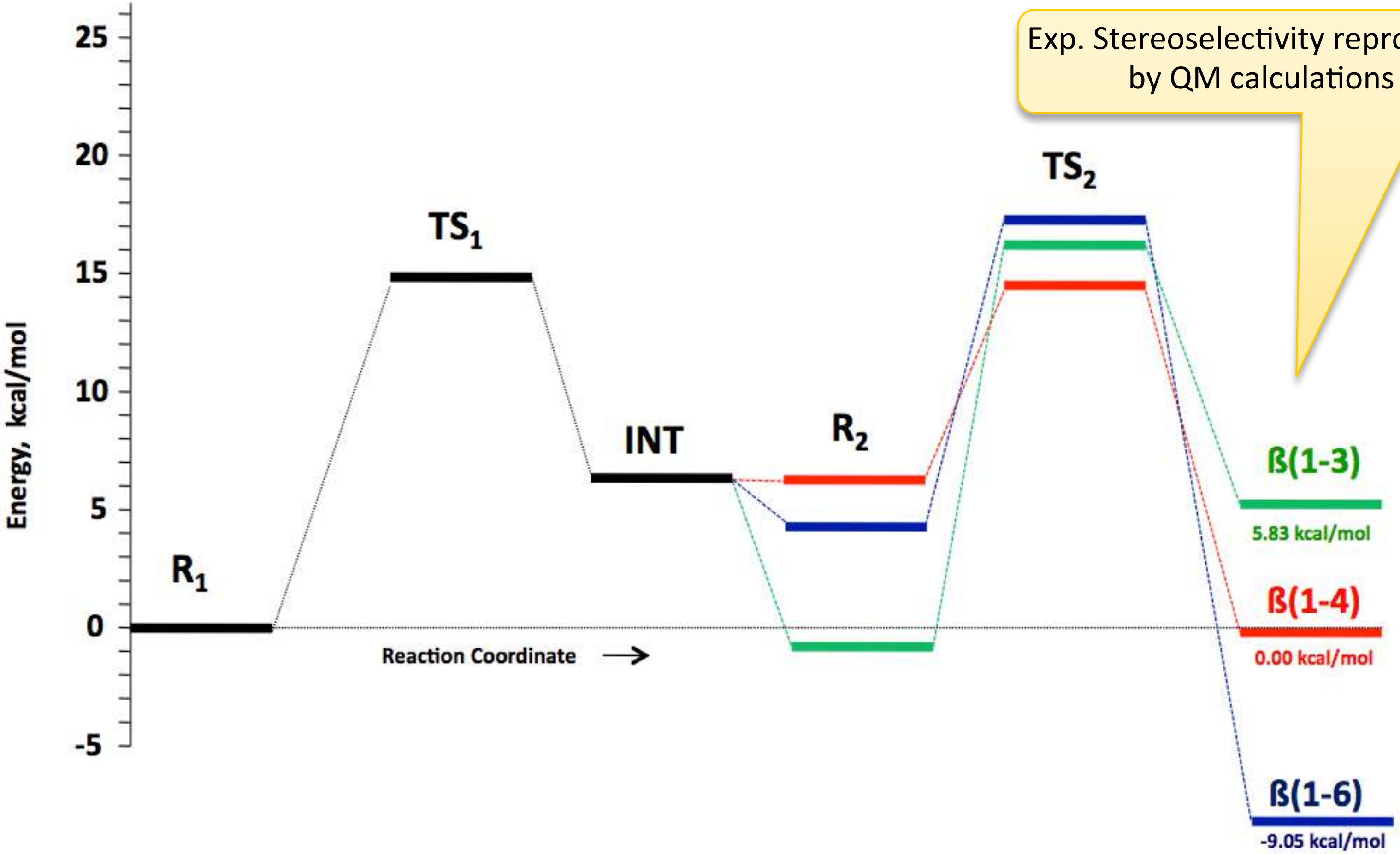
Galactose-Enzyme Covalent Intermediate

Hydrolysed Glu may attack Gal

before dissociation

Theor Chem Acc, 122, 283, 2009

Transglycosylation



Exp. Stereoselectivity reproduced by QM calculations

Reactants

Covalent Intermediate

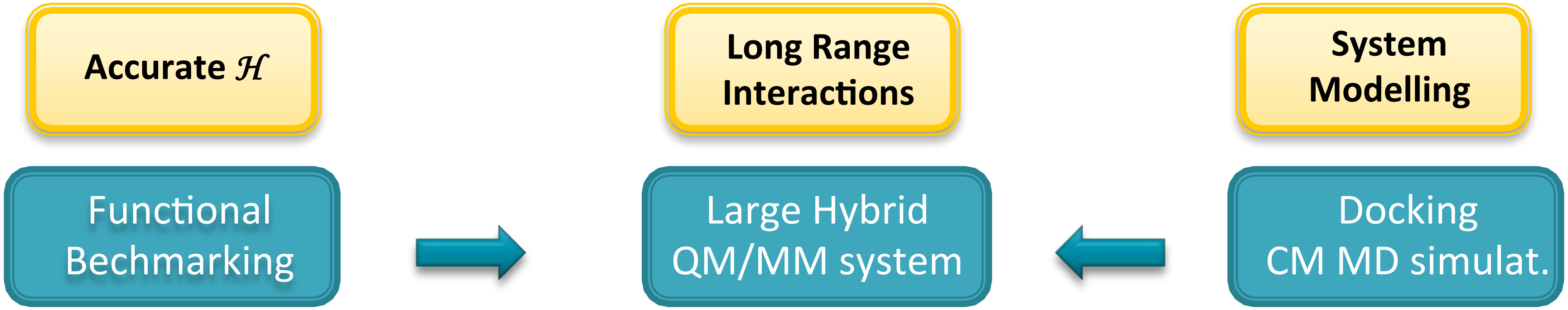
Sugar Rotation

Sugar Attack

Products

J. Chem. Theory Comput, 6, 421, 2010.

Flux of Information



Accurate $\mathcal{H}$

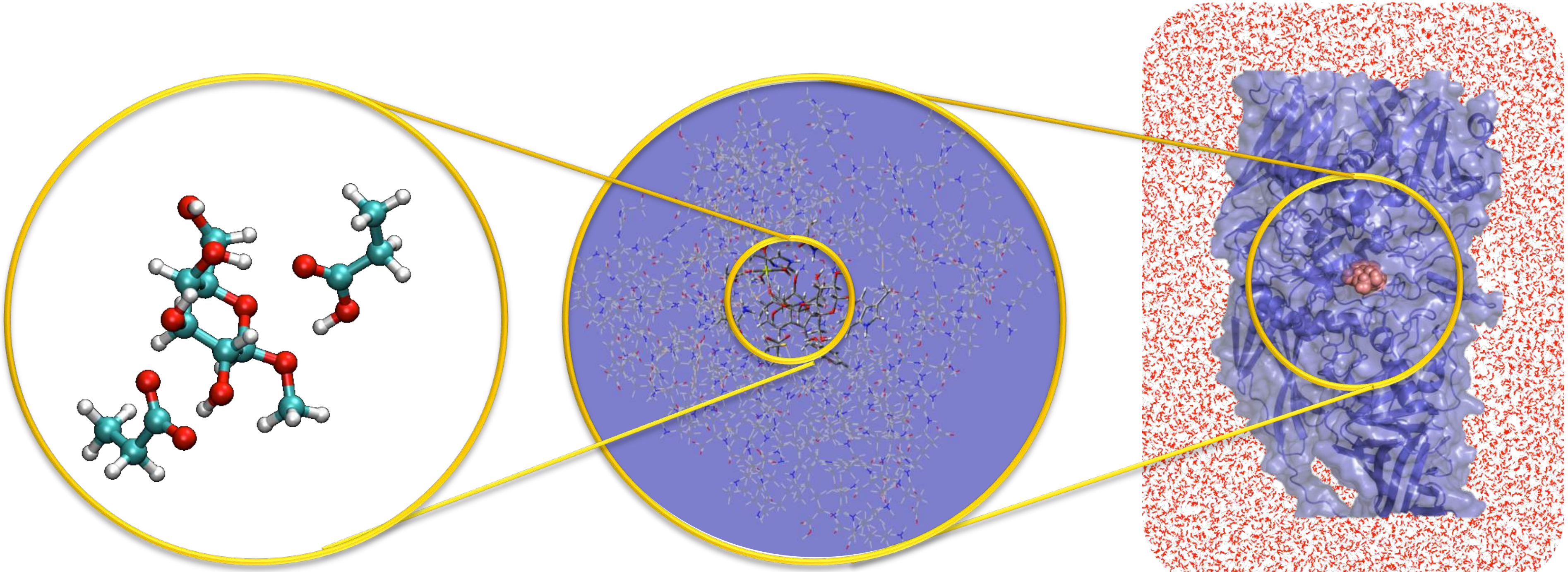
Functional Bechmarking

Long Range Interactions

Large Hybrid QM/MM system

System Modelling

Docking CM MD simulat.



J. Chem. Theory Comput, 6, 421, 2010

J. Phys. Chem. B, 115, 14752, 2011.

Conclusions

Computational enzymology can give atomic-level insight into reaction mechanisms

It can be used both to rationalize experimental results and to predict phenomena difficult/inaccessible to experiments

Further methodologic developments are needed to, e.g., include sampling over multiple enzyme conformations in the simulations.

Examples of Studies on Enzymatic Reactivity

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