Why Enzymes are Difficult to Simulate

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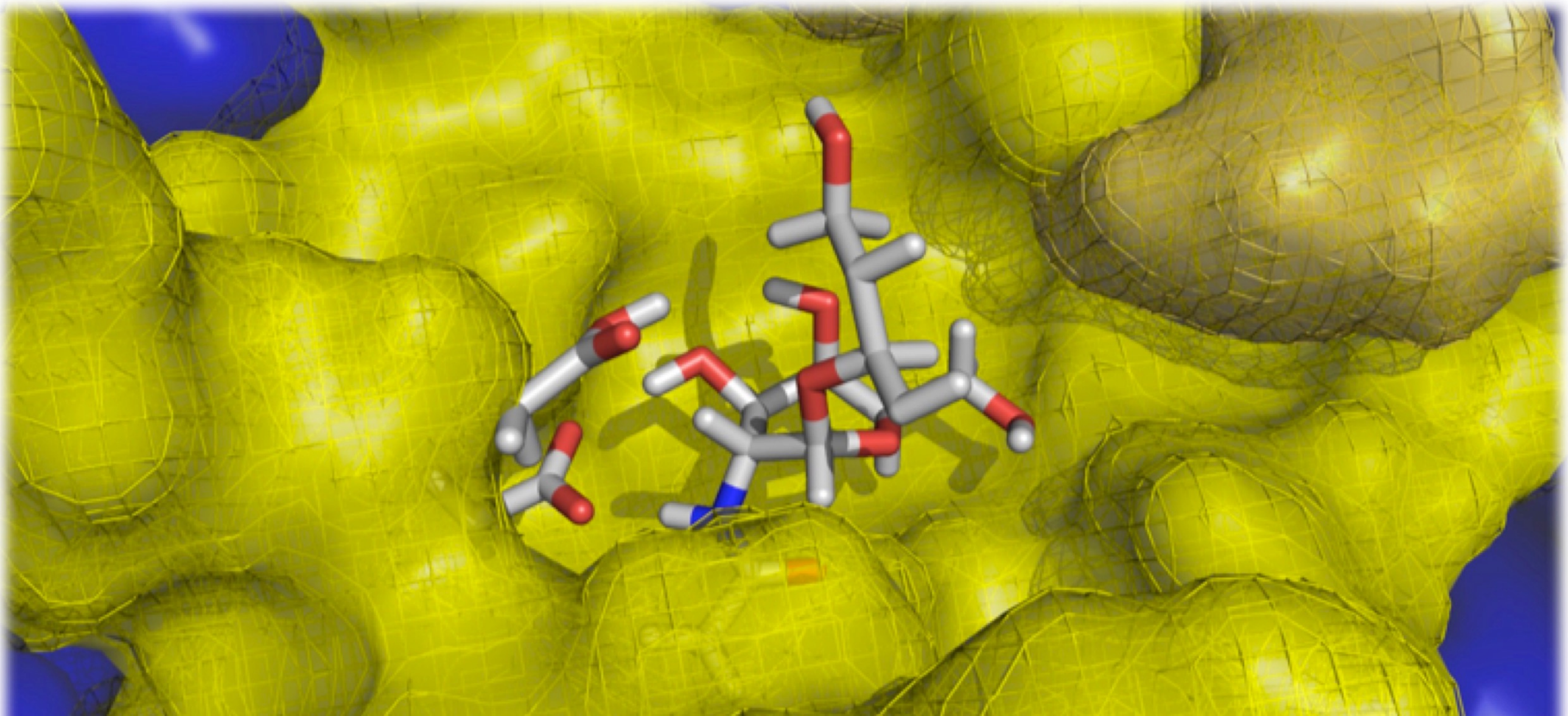

Outline

I. The Objective: to know the catalytic mechanism of an enzyme

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II. The Problem: Enzymes are molecules very difficult to simulate.

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I. The Objective: to know the catalytic mechanism of an enzyme

II. The Problem: Enzymes are molecules very difficult to simulate.

III. The Solutions: Strategies to simulate enzymes and calculate reaction mechanisms.

Objective

I. The Objective: to know the catalytic mechanism of an enzyme

Objective

The catalytic mechanism of an enzyme

Objective

The catalytic mechanism of an enzyme

✔ **E:S**

✔ **E:P**

Objective

The catalytic mechanism of an enzyme

✔ ? ? ? ? ? ? ✔

$$E{:}S \rightleftharpoons E{:}TS_1 \rightleftharpoons E{:}INT_1 \rightleftharpoons E{:}TS_2 \rightleftharpoons E{:}INT_2 \ \ldots \ E{:}INT_{n-1} \rightleftharpoons E{:}TS_n \rightleftharpoons E{:}P$$

Objective

The catalytic mechanism of an enzyme

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Hints about the chemical identity and structure of INTs & TSs:

Objective

The catalytic mechanism of an enzyme

✔ ? ? ? ? ? ? ✔

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Hints about the chemical identity and structure of INTs & TSs:

kinetics

Objective

The catalytic mechanism of an enzyme

✔ ? ? ? ? ? ? ✔

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Hints about the chemical identity and structure of INTs & TSs:

kinetics

mutagenesis

Objective

The catalytic mechanism of an enzyme

✔ ? ? ? ? ? ✔

$$E{:}S \rightleftharpoons E{:}TS_1 \rightleftharpoons E{:}INT_1 \rightleftharpoons E{:}TS_2 \rightleftharpoons E{:}INT_2 \ \ldots \ E{:}INT_{n-1} \rightleftharpoons E{:}TS_n \rightleftharpoons E{:}P$$

Hints about the chemical identity and structure of INTs & TSs:

- kinetics
- mutagenesis
- spectroscopy

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The catalytic mechanism of an enzyme

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Hints about the chemical identity and structure of INTs & TSs:

- kinetics
- mutagenesis
- spectroscopy
- S analogues

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The catalytic mechanism of an enzyme

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Hints about the chemical identity and structure of INTs & TSs:

- kinetics
- mutagenesis
- spectroscopy
- S analogues

Exp. data is generally not enough.
Many different mechanistic hypotheses MAY fit the data.

Objective

Why we need to know the chemical mechanism of an enzymatic reaction?

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Fundamental (bio)chemical knowledge

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To design drugs (40-50% are enzyme inhibitors)

Objective

Why we need to know the chemical mechanism of an enzymatic reaction?

Fundamental (bio)chemical knowledge

To design drugs (40-50% are enzyme inhibitors)

To design biocatalysts

The Problem

Why enzymes are so difficult ?

Why enzymes are so difficult?

1. Nature of energy contributions

Why enzymes are so difficult?

1. Nature of energy contributions
2. Spatial range of the atomic interactions

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2. Spatial range of the atomic interactions
3. Unprecedented chemistry

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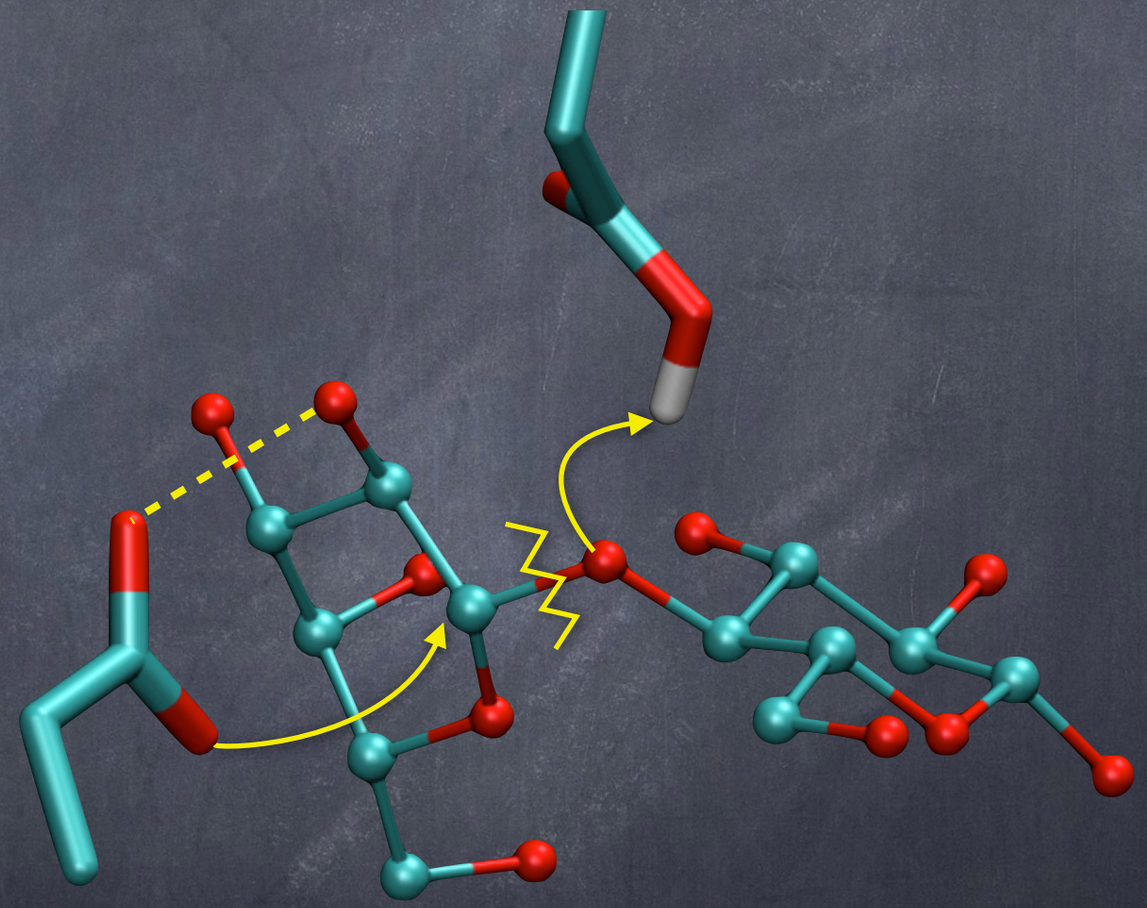
4. Enzyme flexibility

1. Nature of the energy contributions

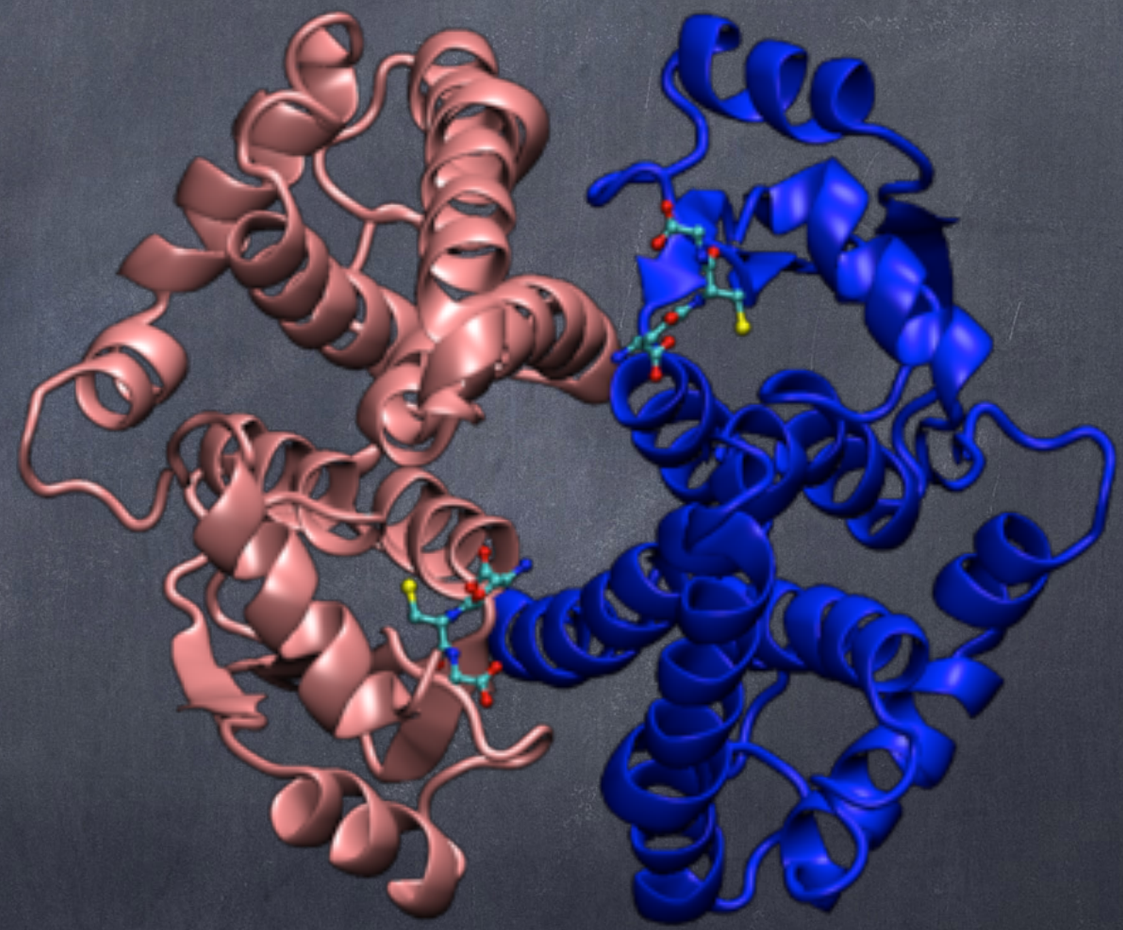
- Covalent

β-Galactosidase



1. Nature of the energy contributions

Glutathione Transferase



- Covalent
- Dipolar and ionic H-bonding

JPCB, 114, 12972, 2010.

JPCB, 114, 1690, 2010

CHEM EUR J, 14, 9591, 2008

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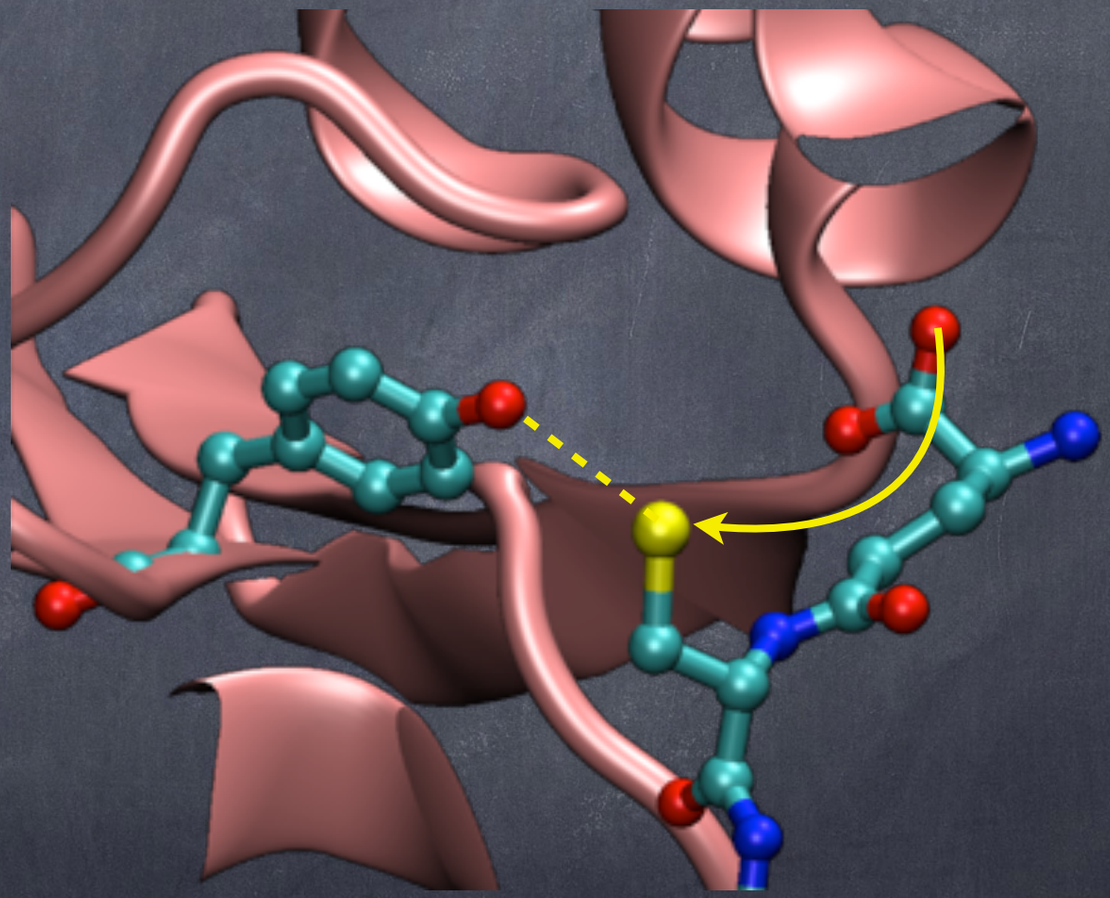

PT is the rate-limiting step

JPCB, 114, 12972, 2010.

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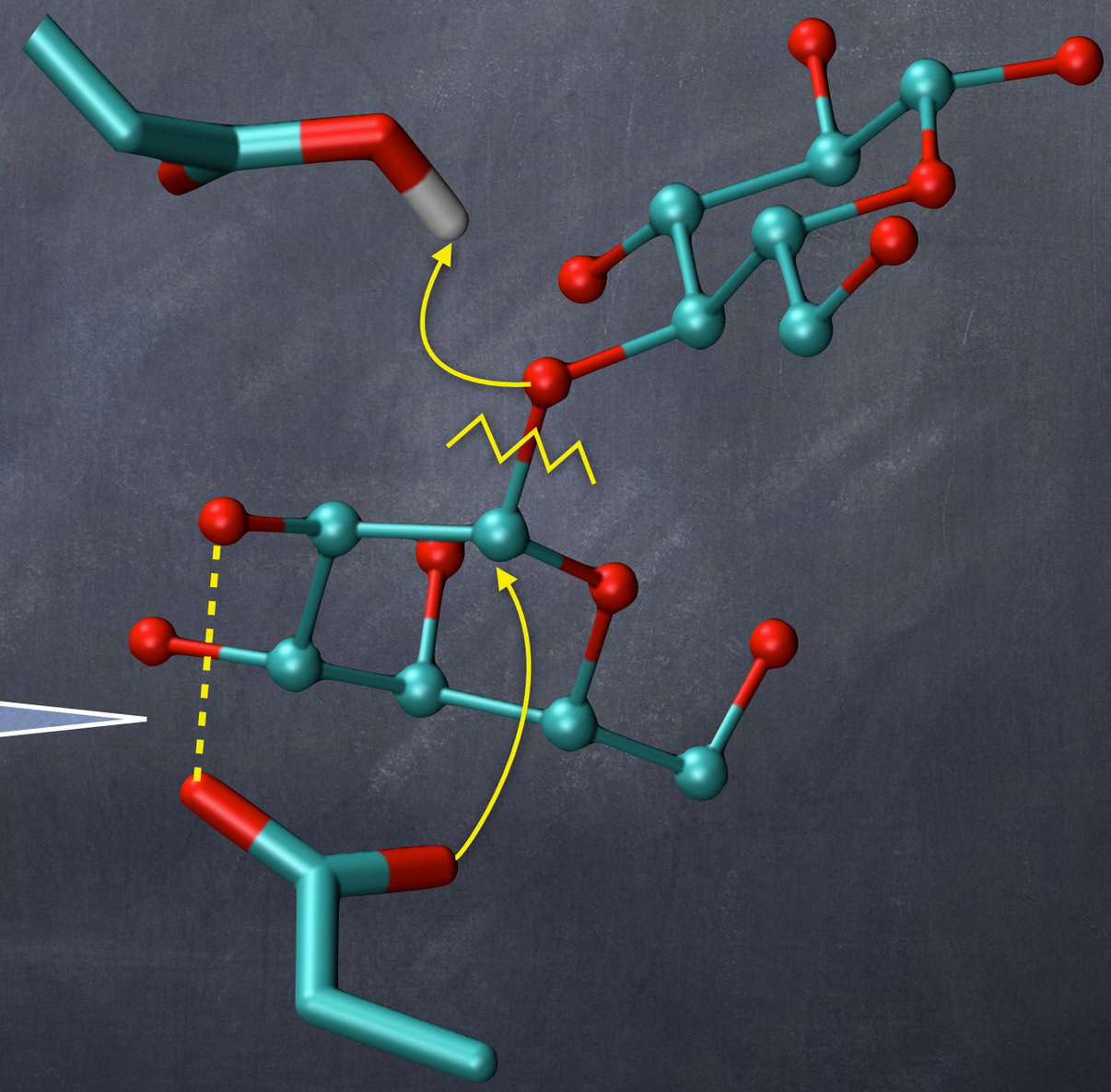
- Covalent
- Dipolar and ionic H-bonding

H bond increases Kcat by
4 orders of magnitude...

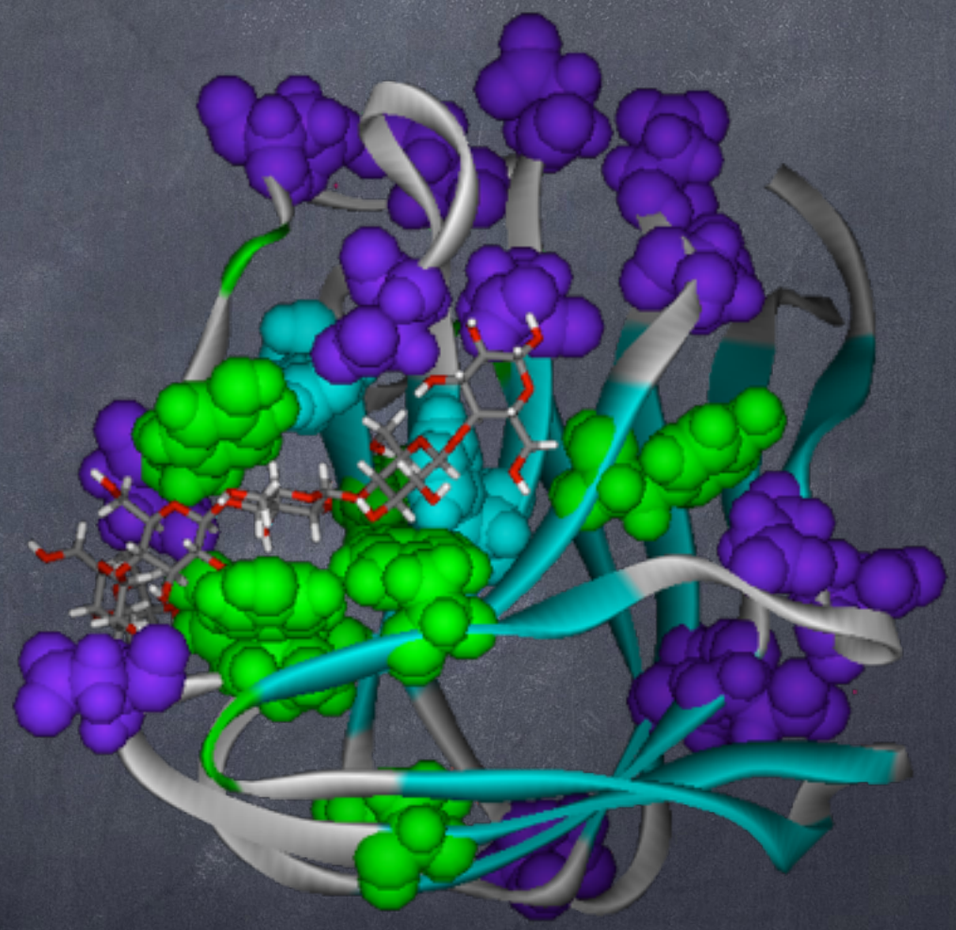
β-Galactosidase



1. Nature of the energy contributions

- Covalent
- Dipolar and ionic H-bonding
- Medium-range general dispersion

CMB-11



FEBS J., 275, 2524, 2008

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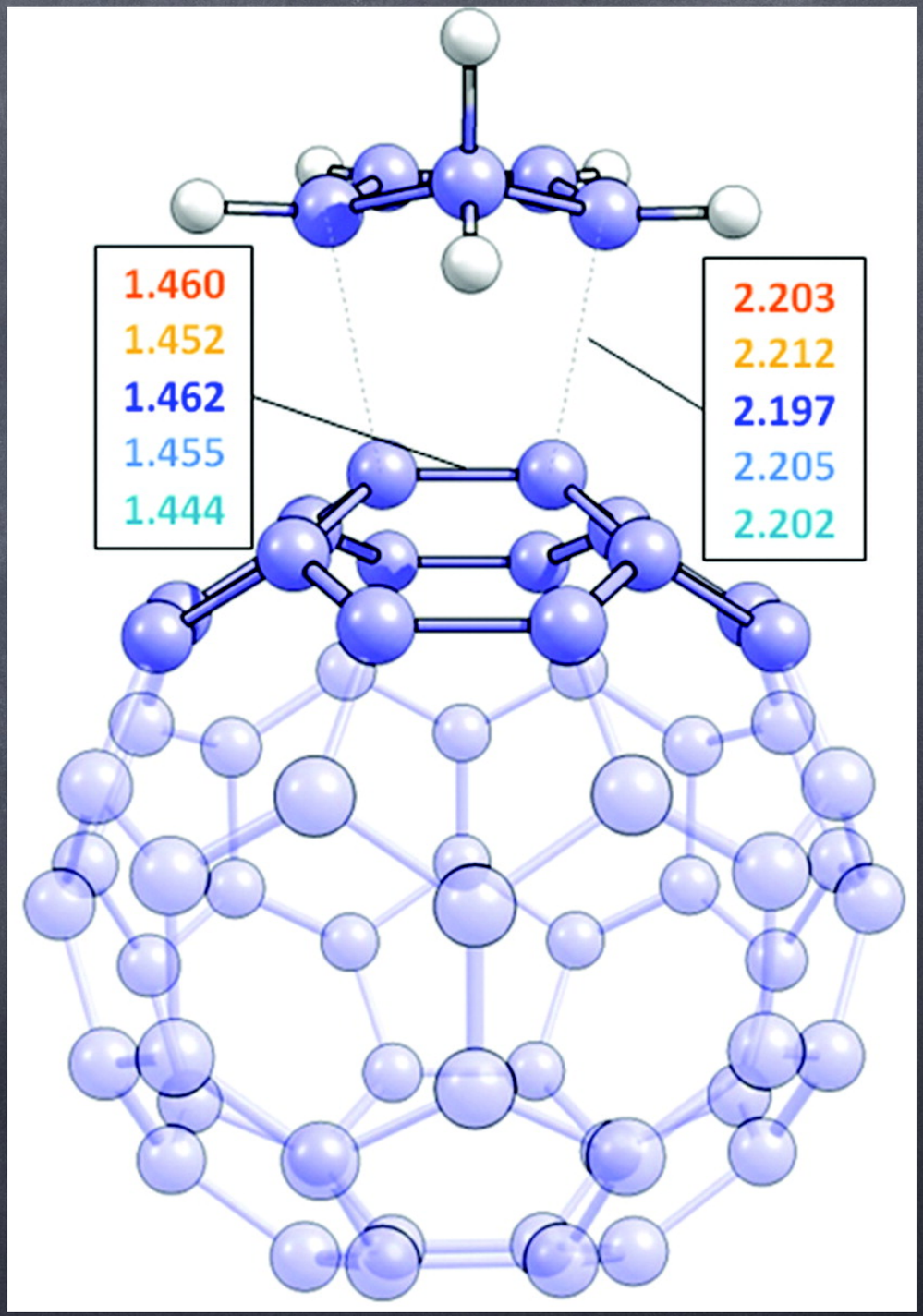

Published in: Sílvia Osuna; Marcel Swart; Miquel Solà; *J. Phys. Chem. A* **2011,** 115, 3491-3496.
DOI: 10.1021/jp1091575

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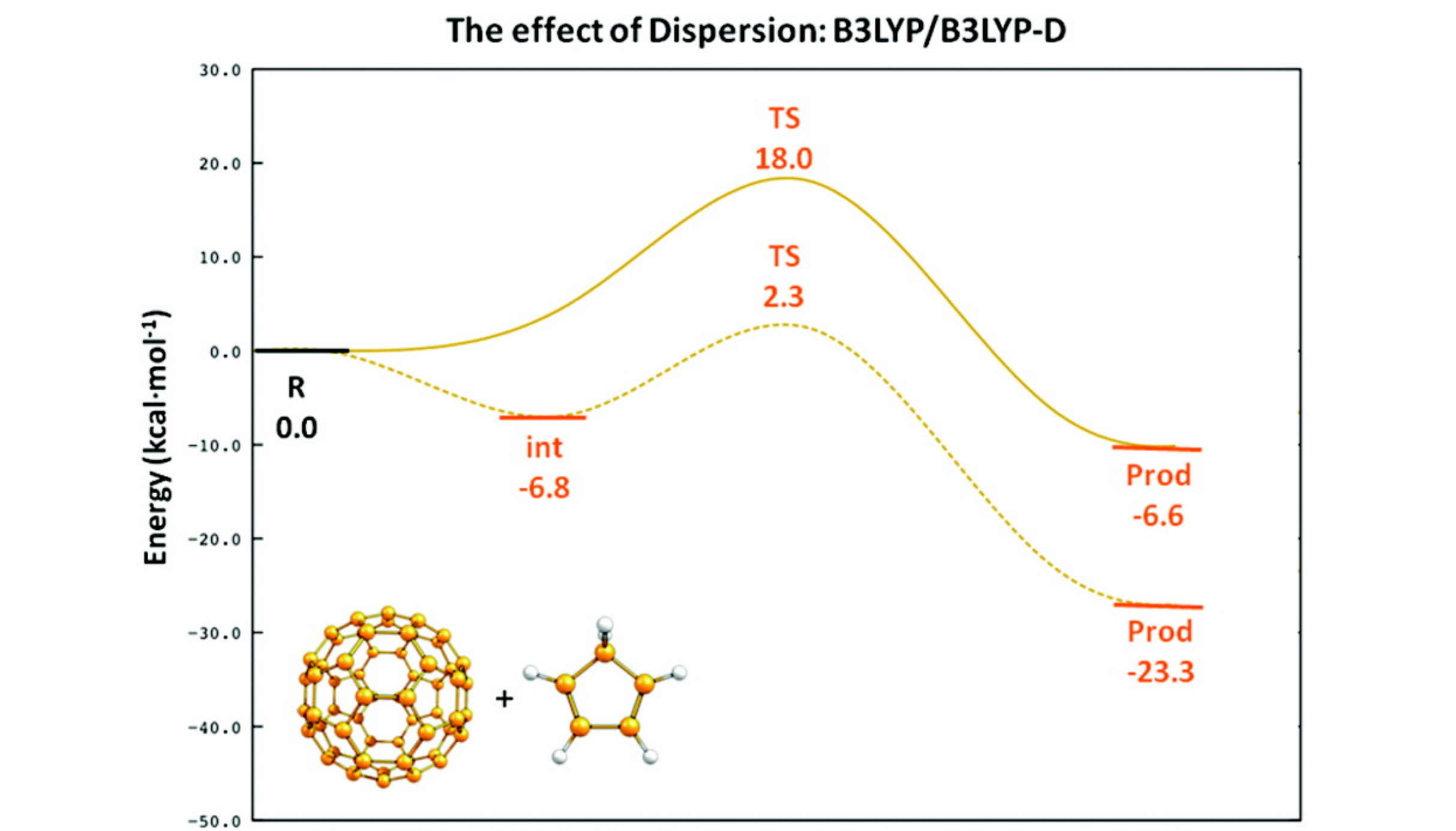

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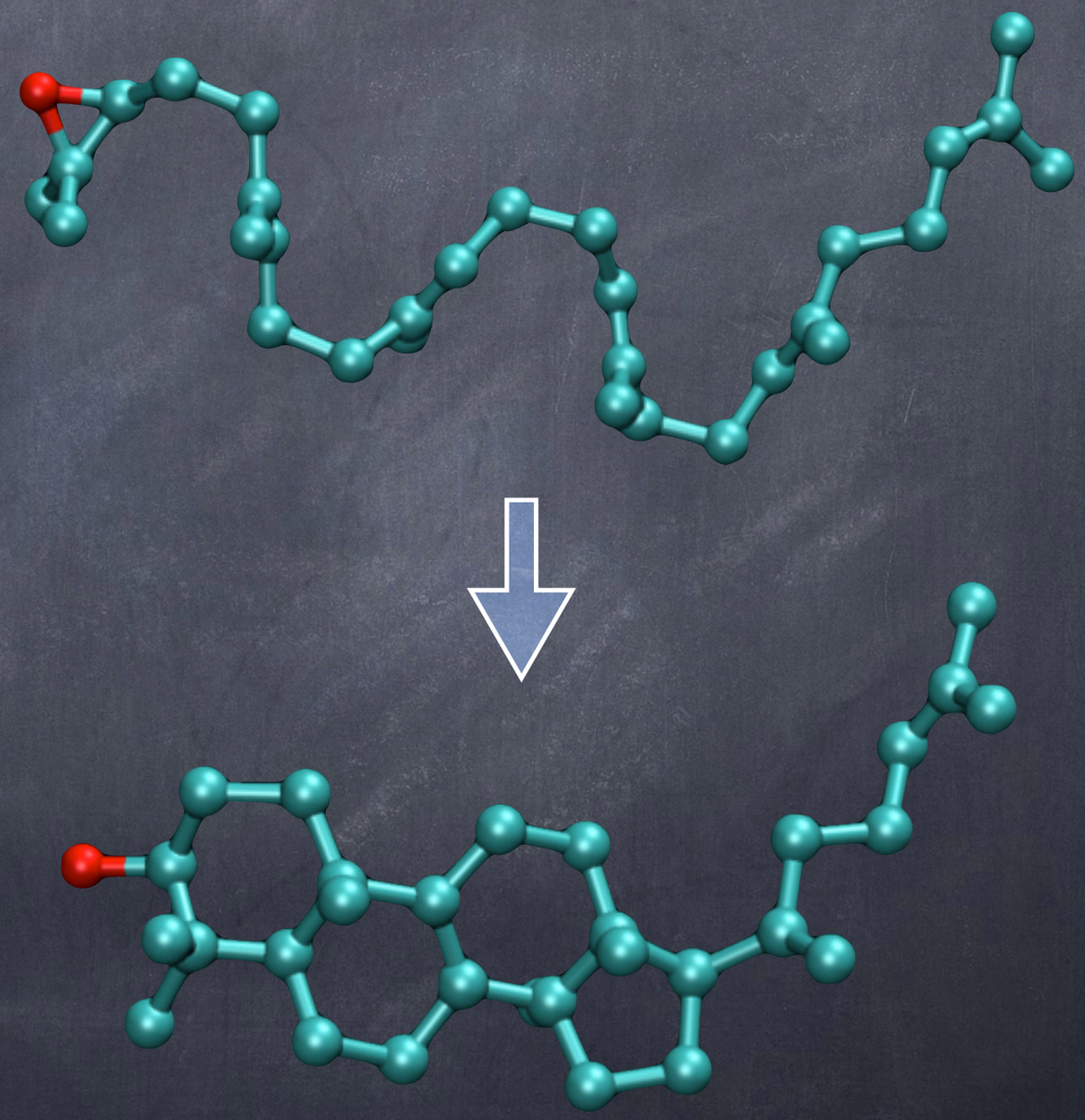
Oxidosqualene Cyclase

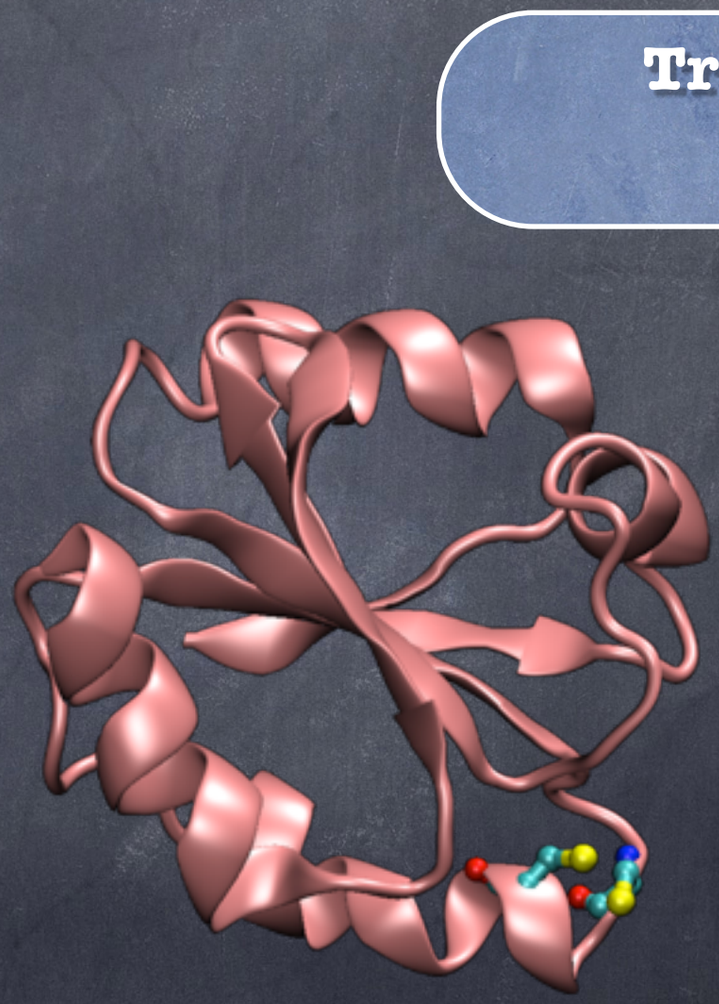


JCTC, 7, 2059, 2011.

1. Nature of the energy contributions

Thioredoxin Family

- Covalent
- Dipolar and ionic H-bonding
- Medium-range general dispersion
- π stacking, π-H-bonds, π-cation
- Medium & long-range coulomb



JCC 30, 710, 2009. JPCB, 110, 5758, 2006. JCC, 27, 966-975, 2006. JPCB, 112, 2511, 2008.

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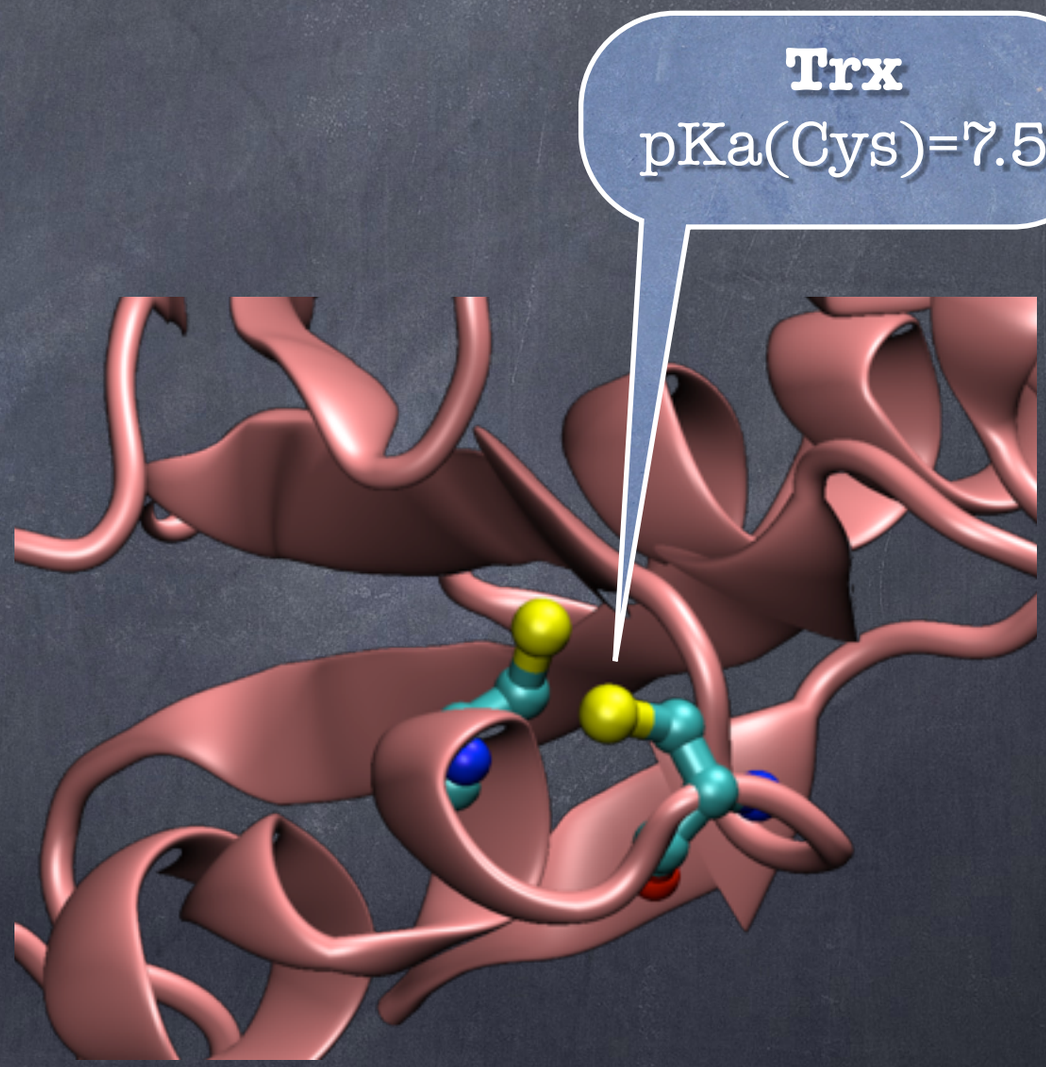

$pKa \propto \Delta E\ (S^- - SH)$

JCC 30, 710, 2009. JPCB, 110, 5758, 2006. JCC, 27, 966-975, 2006. JPCB, 112, 2511, 2008.

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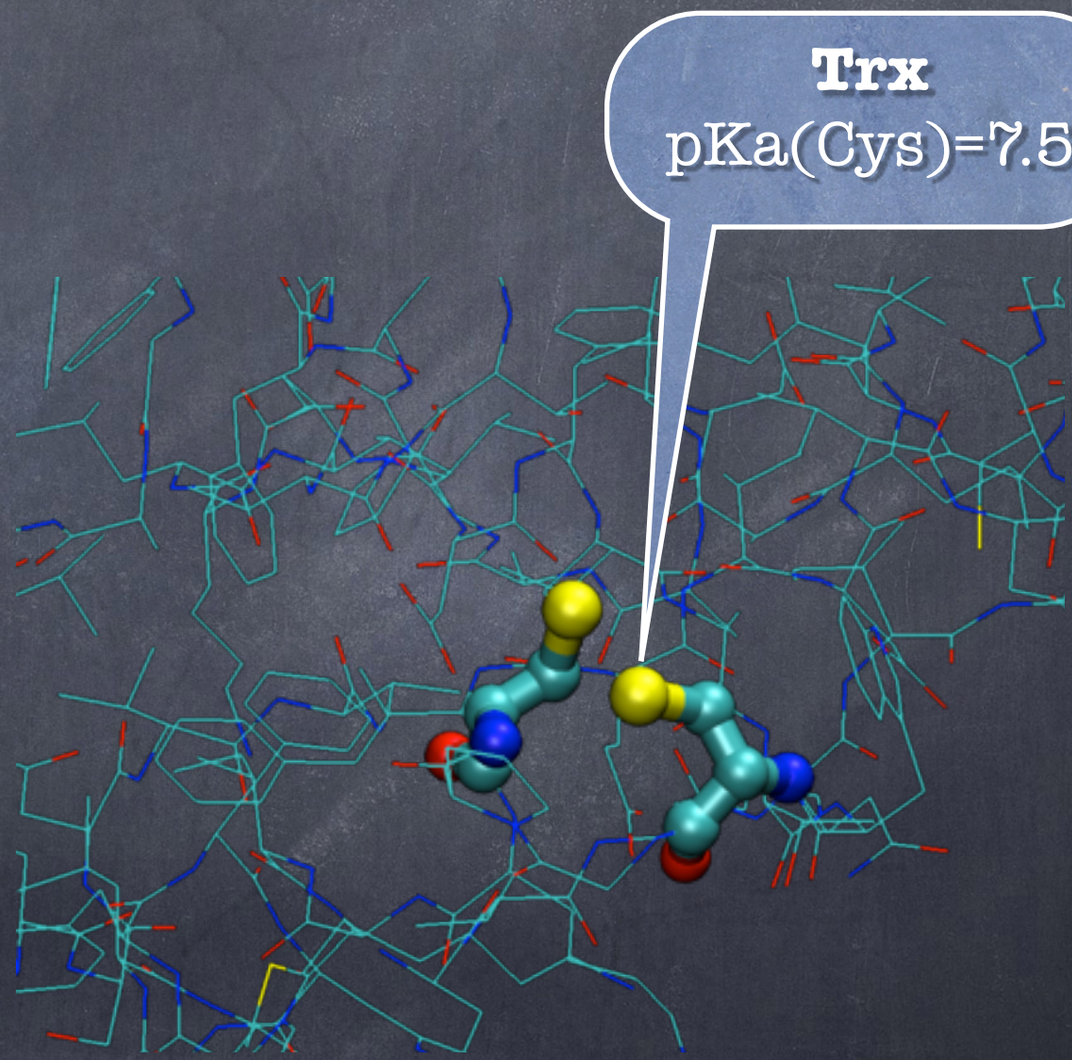

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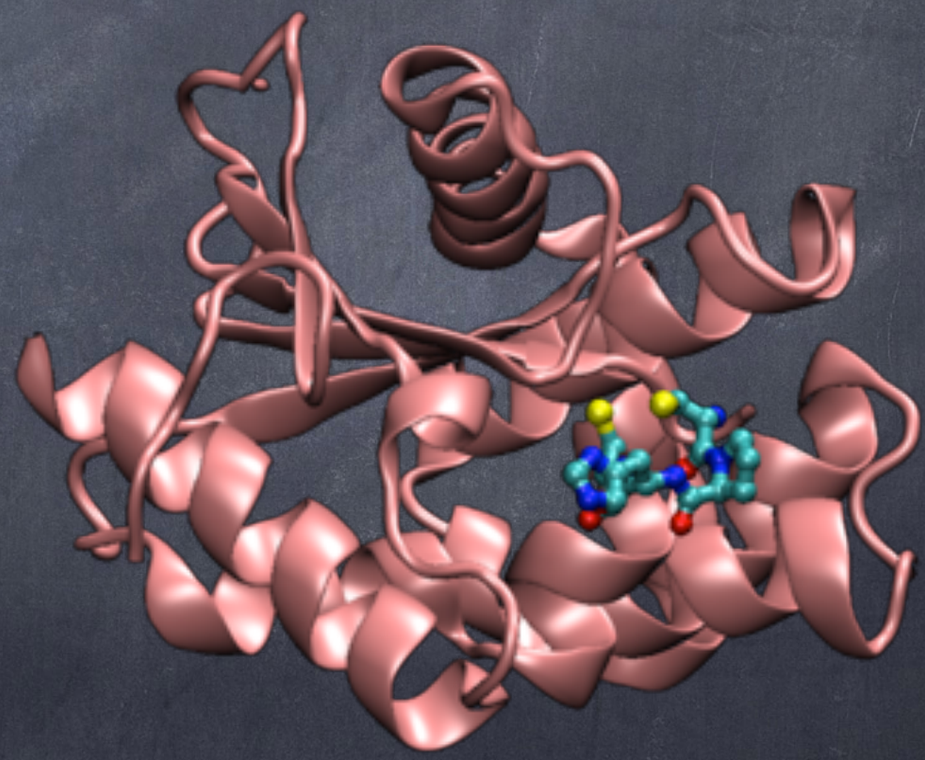
JCC 30, 710, 2009. JPCB, 110, 5758, 2006. JCC, 27, 966-975, 2006. JPCB, 112, 2511, 2008.

1. Nature of the energy contributions

Thioredoxin Family

DsbA

- Covalent
- Dipolar and ionic H-bonding
- Medium-range general dispersion
- π stacking, π-H-bonds, π-cation
- Medium & long-range coulomb



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JCC 30, 710, 2009. JPCB, 110, 5758, 2006. JCC, 27, 966-975, 2006. JPCB, 112, 2511, 2008.

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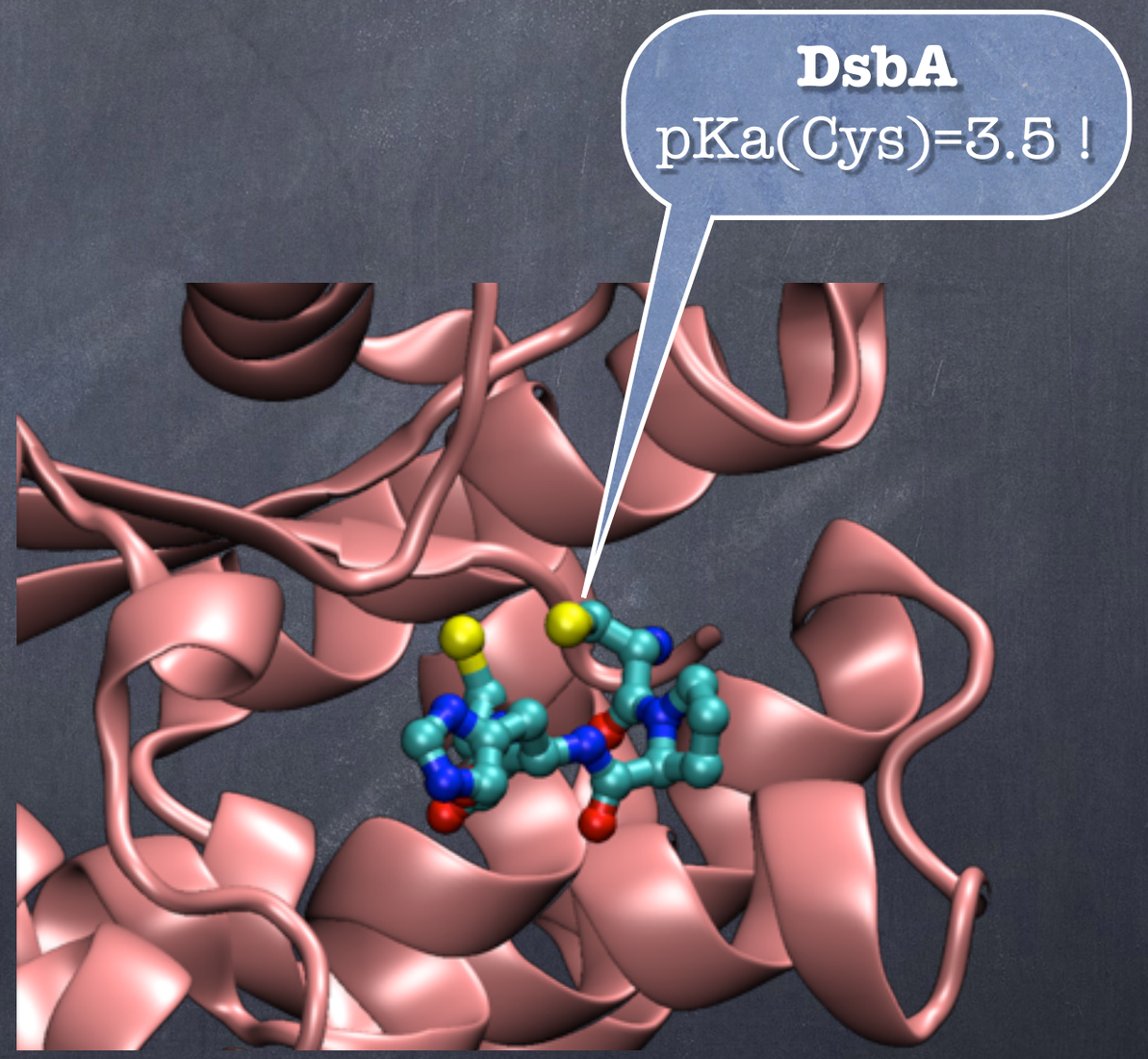
Thioredoxin Family



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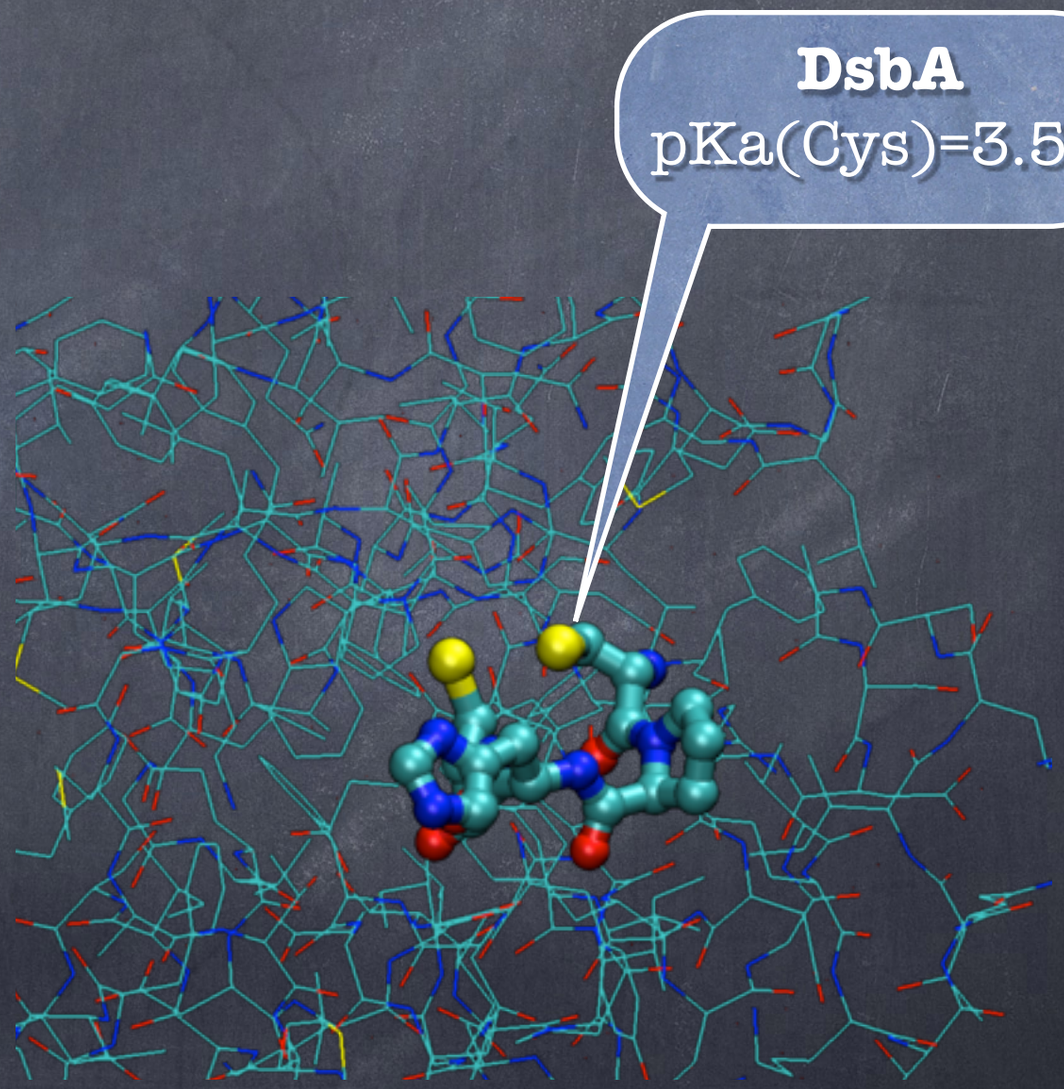

The active sites of Trx and DsbA are superimposable!

JCC 30, 710, 2009. JPCB, 110, 5758, 2006. JCC, 27, 966-975, 2006. JPCB, 112, 2511, 2008.

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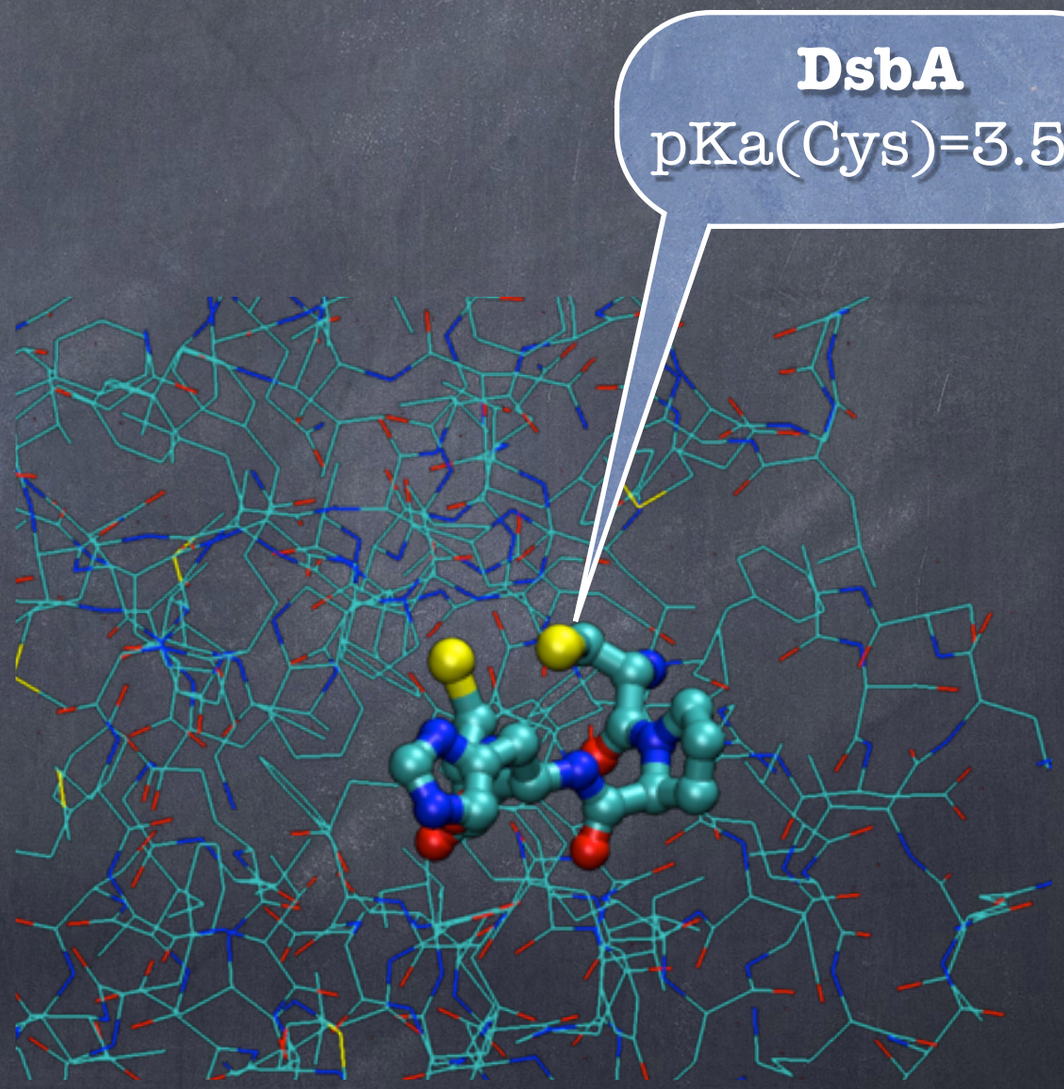

No 1st or 2nd shell interaction over stabilizes the thiolate.

JCC 30, 710, 2009. JPCB, 110, 5758, 2006. JCC, 27, 966-975, 2006. JPCB, 112, 2511, 2008.

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- Covalent
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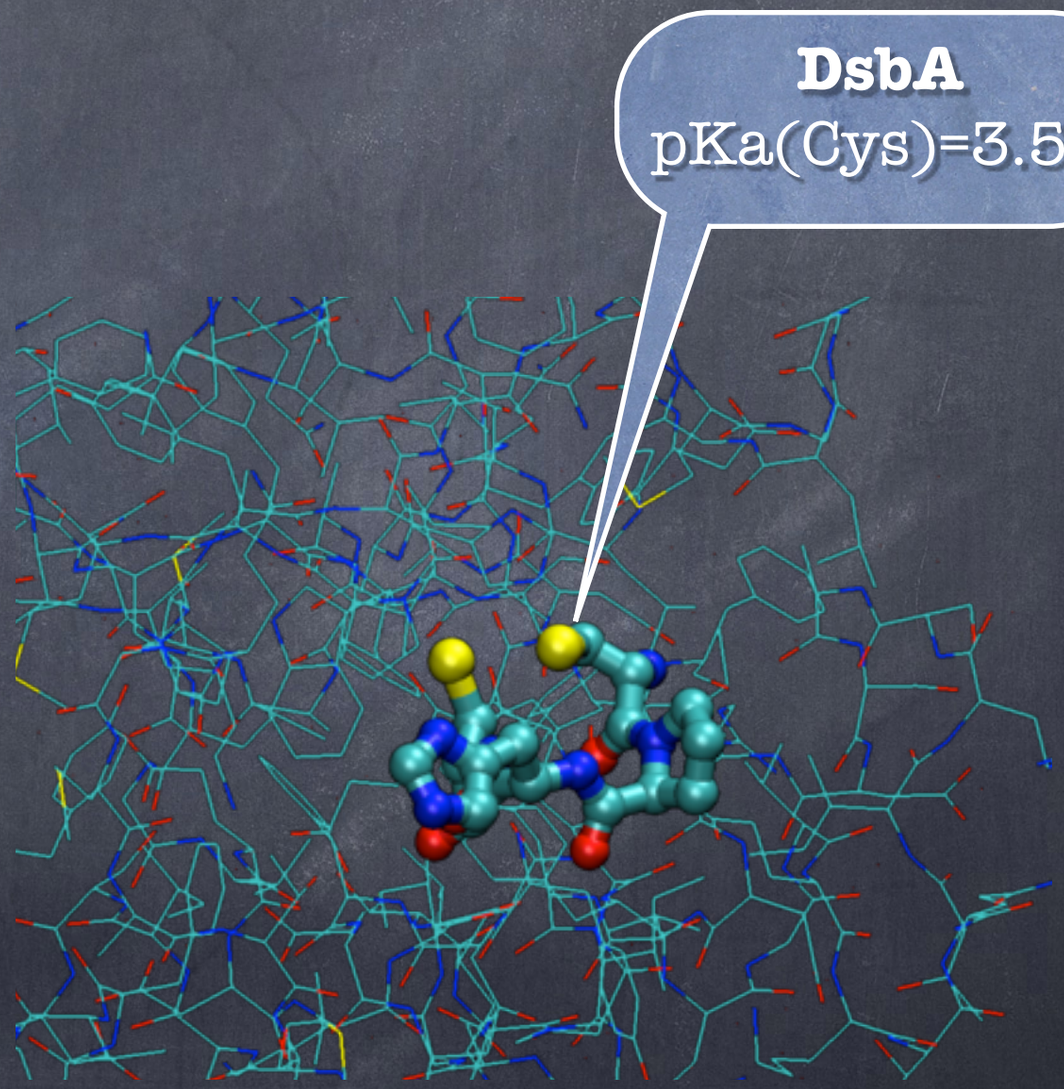
Thioredoxin Family



Long-range coulomb interactions make the difference!

JCC 30, 710, 2009. JPCB, 110, 5758, 2006. JCC, 27, 966-975, 2006. JPCB, 112, 2511, 2008.

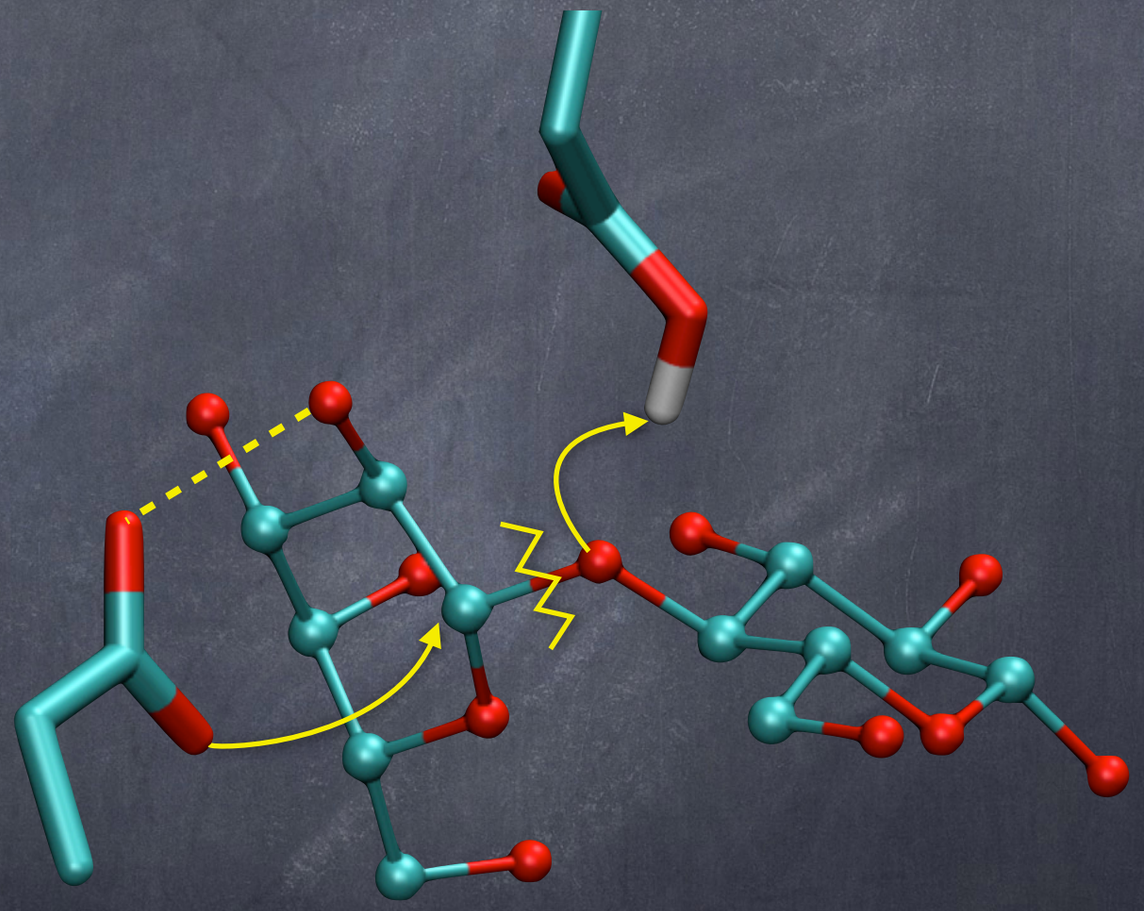
Why enzymes are so difficult?

1. Nature of energy contributions
2. Spatial range of the atomic interactions
3. Unprecedented chemistry
4. Enzyme flexibility

2.Spatial Range of the Atomic Interactions

- Covalent (< 3 Å)
- 1st shell interactions (< 4 Å)

β-Galactosidase

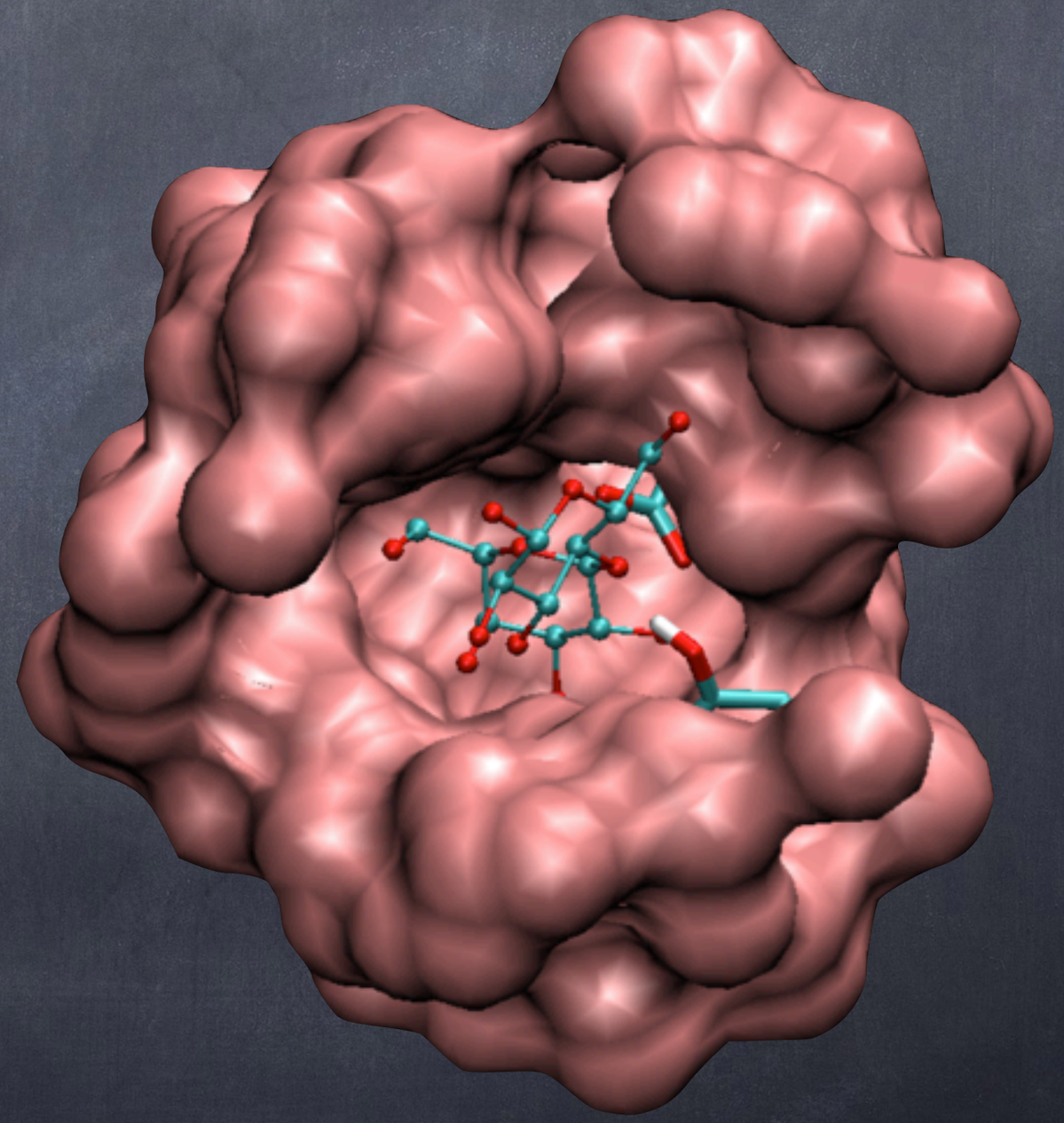


JCTC, 6, 421-433, 2010

2.Spatial Range of the Atomic Interactions

- Covalent (< 3 Å)
- 1st shell interactions (< 4 Å)
- 2nd shell medium range (<7 Å)

β-Galactosidase



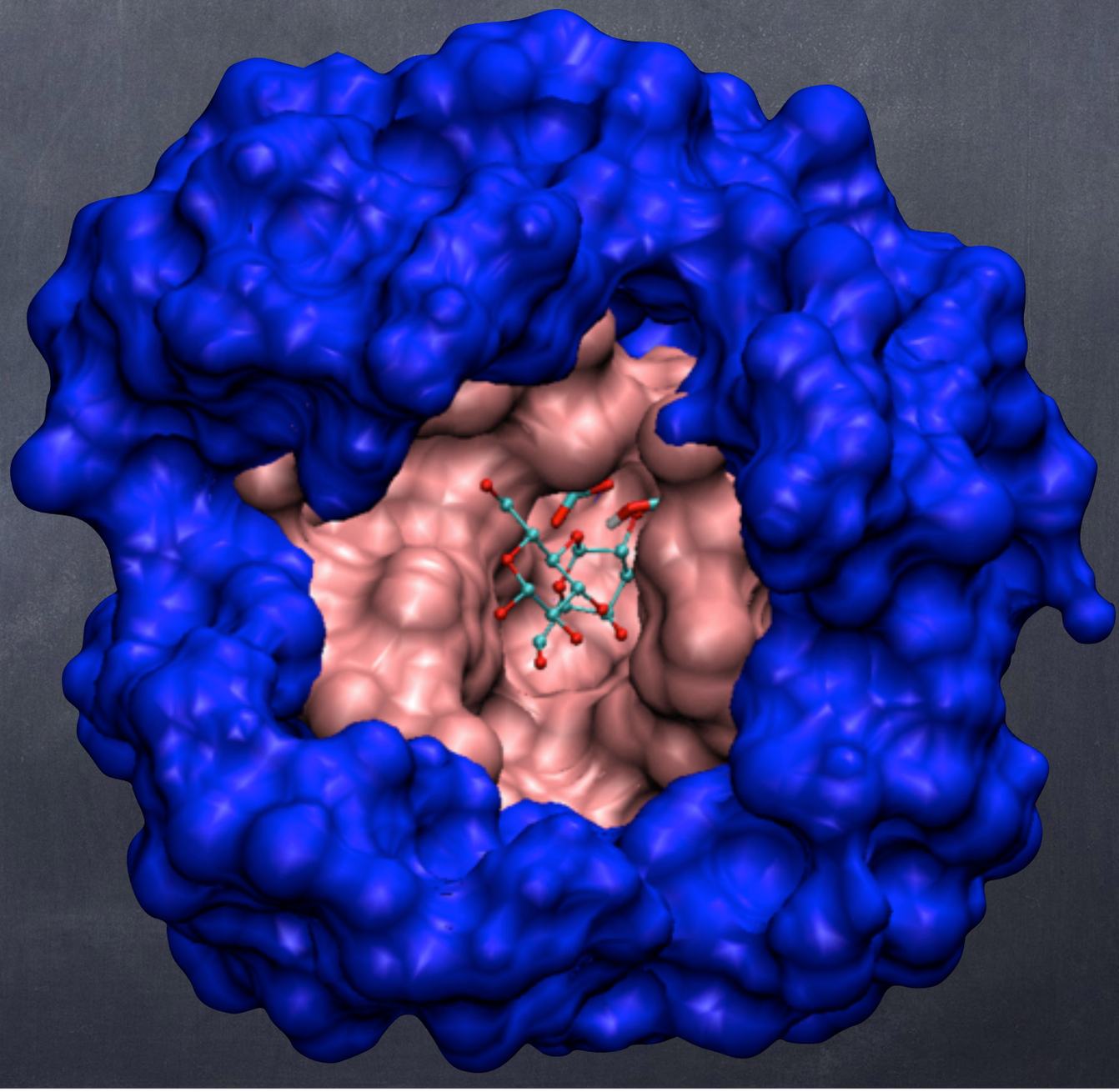
JCTC, 6, 421-433, 2010

2.Spatial Range of the Atomic Interactions

- Covalent (< 3 Å)
- 1st shell interactions (< 4 Å)
- 2nd shell medium range (<7 Å)
- Long range coulomb (7-20 Å)

dispersion	$\propto r^{-6}$
dipolar	$\propto r^{-3}$
ionic	$\propto r^{-1}$
but nº atoms	$\propto r^{+3}$!

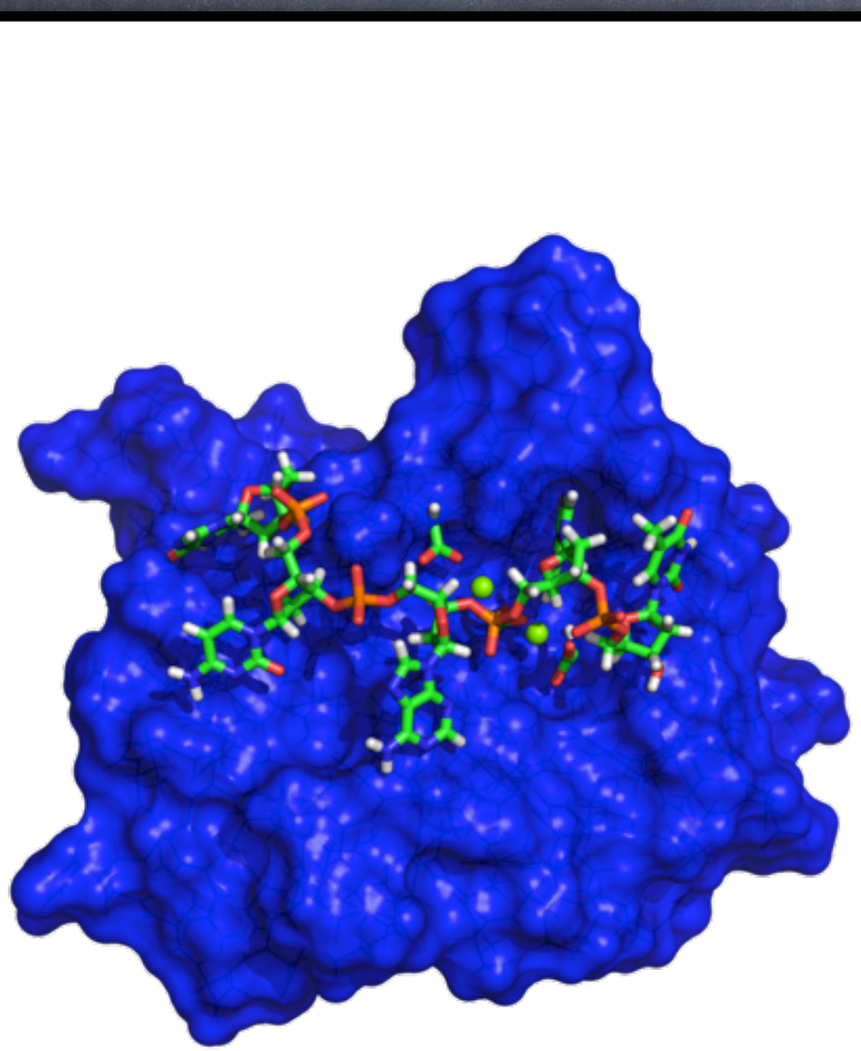
β-Galactosidase



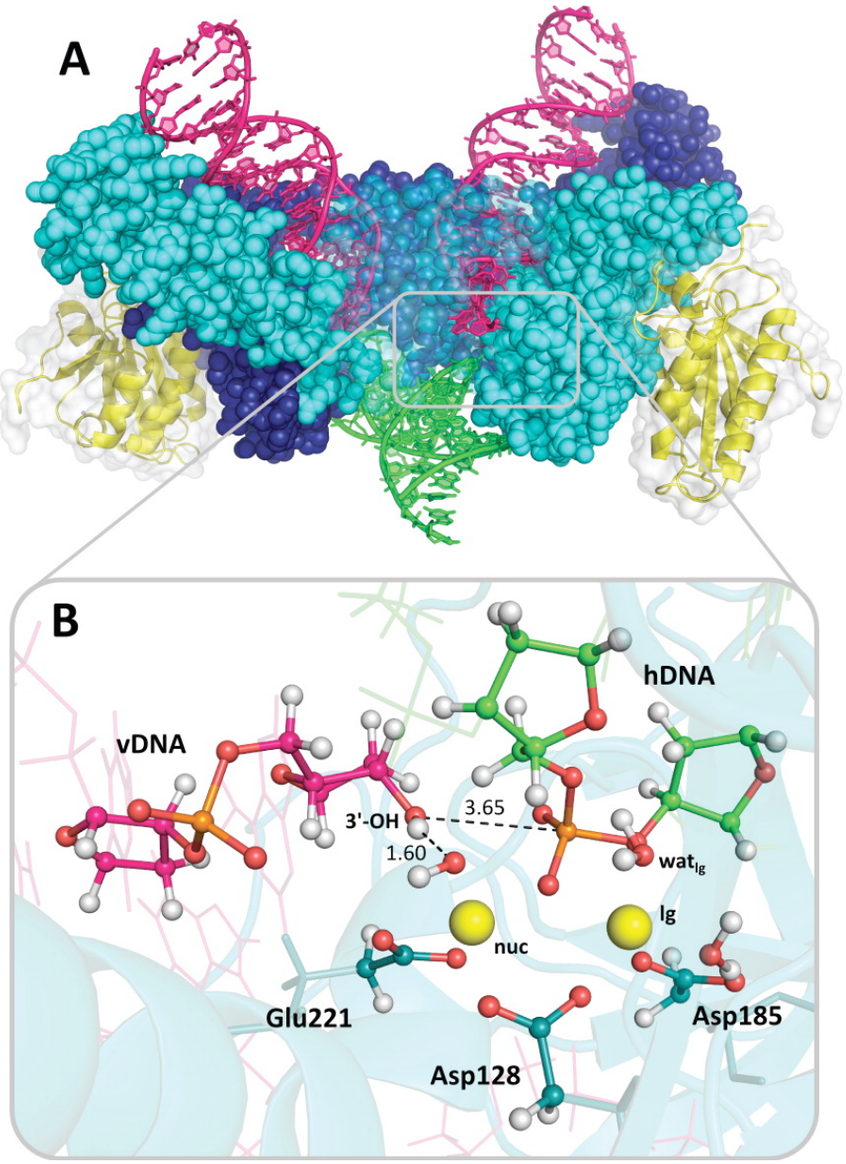
JCTC, 6, 421-433, 2010

2.Spatial Range of the Atomic Interactions

3’ End Processing
>2.500 atoms



Strand Transfer
>22.000 atoms



JACS, 134, 13436, 2012

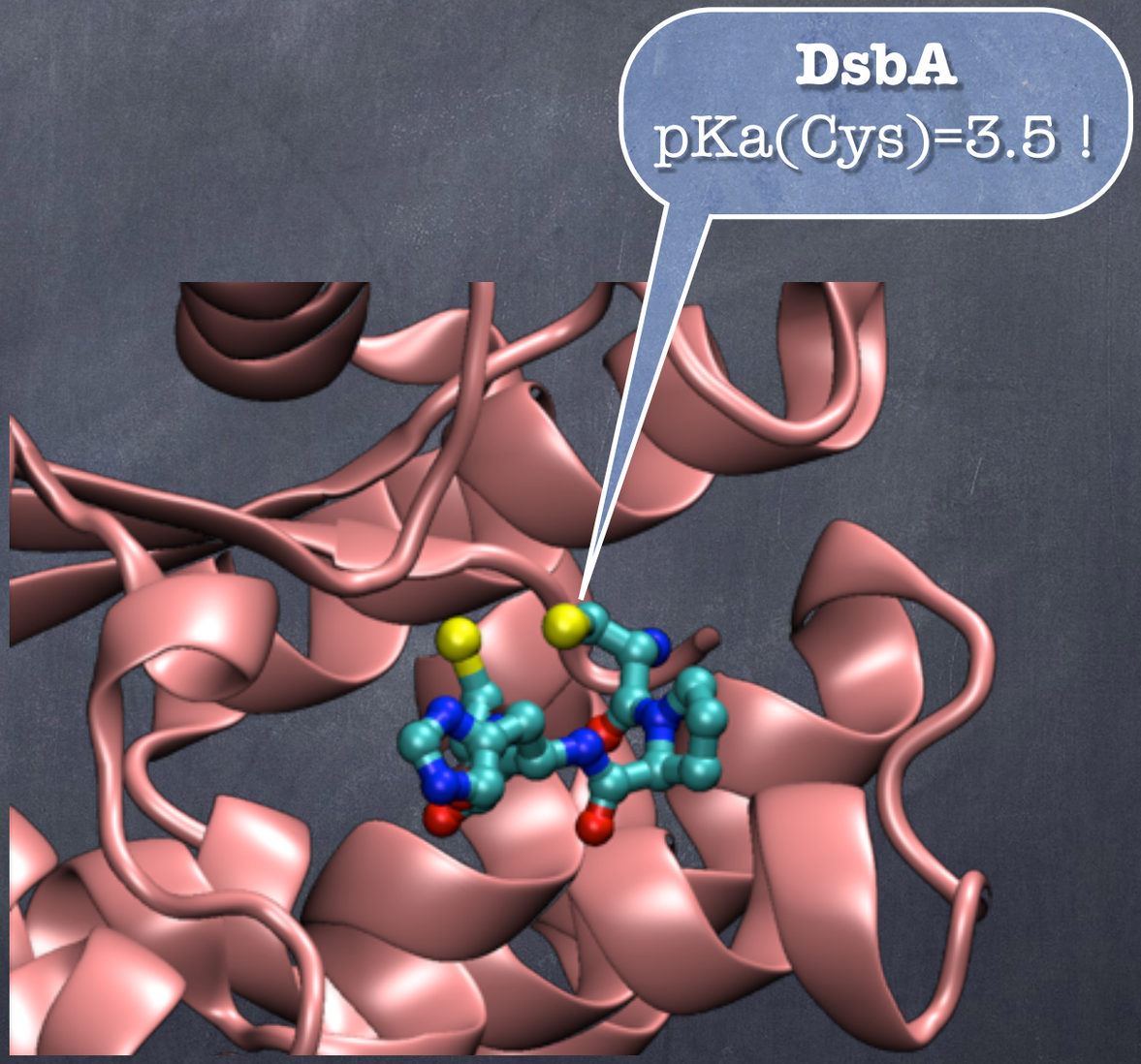
JCTC, 10, 5458, 2014

Why enzymes are so difficult?

1. Nature of energy contributions
2. Spatial range of the atomic interactions
3. Unprecedented chemistry
4. Enzyme flexibility

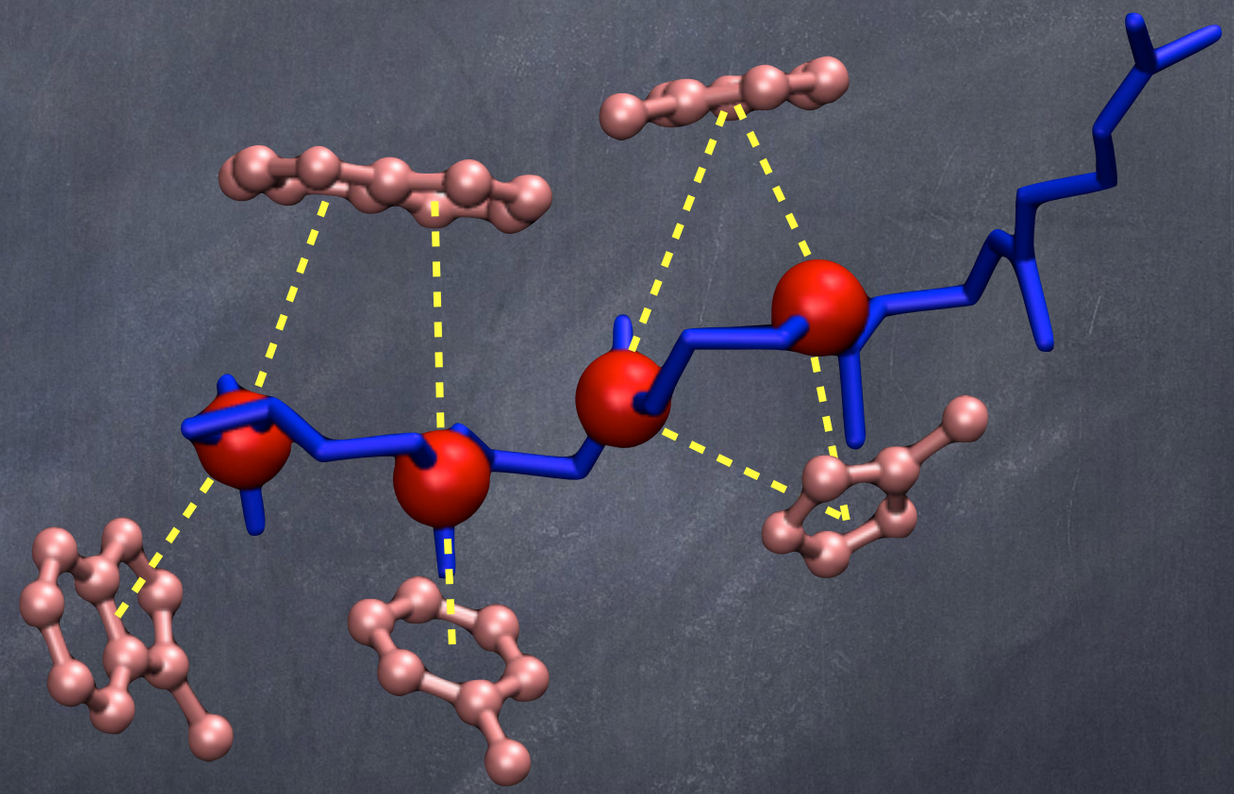
3. Unprecedented Chemistry

- Abnormal pKas



3. Unprecedented Chemistry

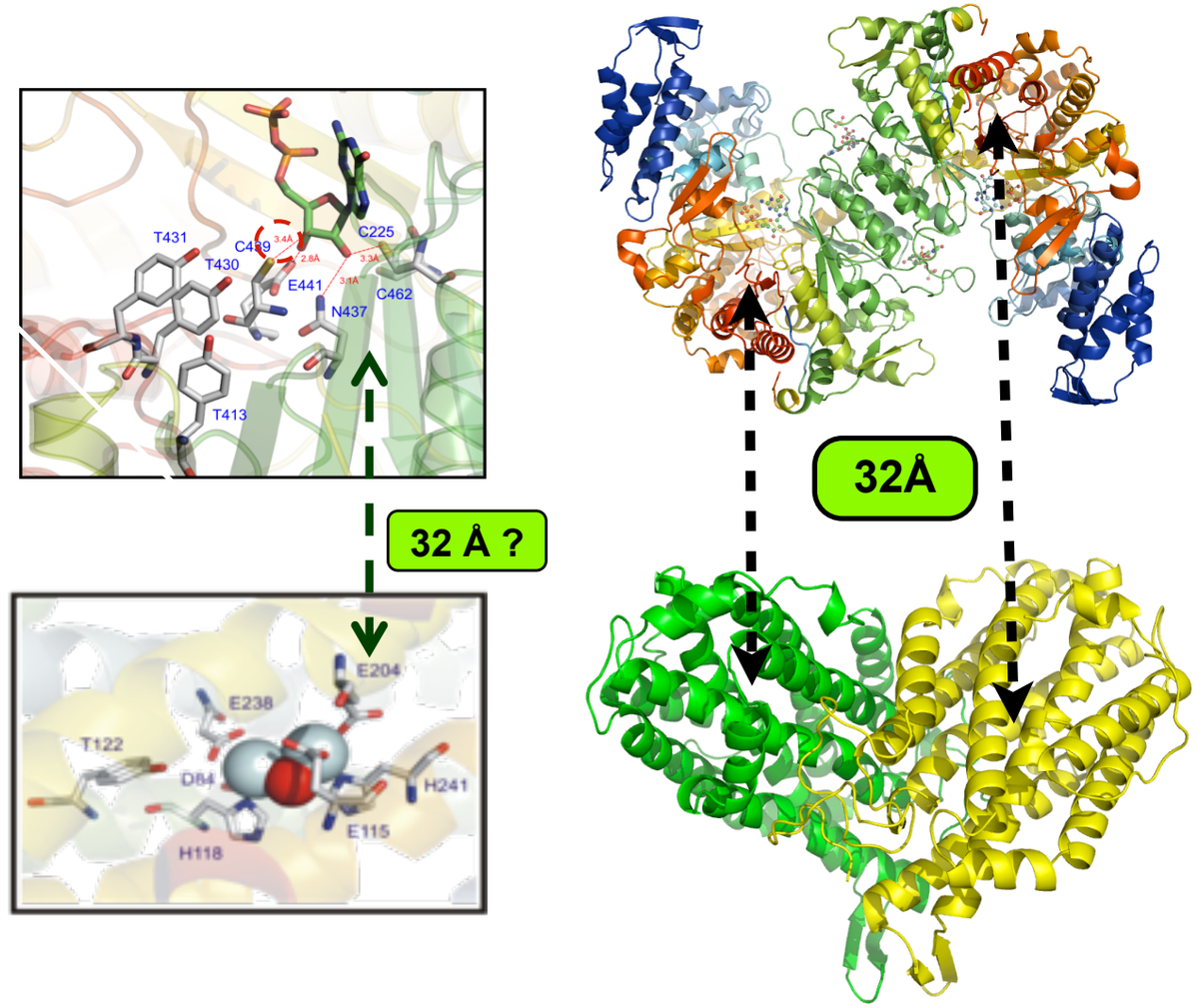
- Abnormal pKas
- Stable carbocations



3. Unprecedented Chemistry

- Abnormal pKas
- Stable carbocations
- Long-living radicals

Ribonucleotide Reductase



Dehydration through radical mechanism

JACS, 127, 5174, 2005

JCTC, 6, 2770, 2010

CHEMISTRY, 13, 8507, 2007

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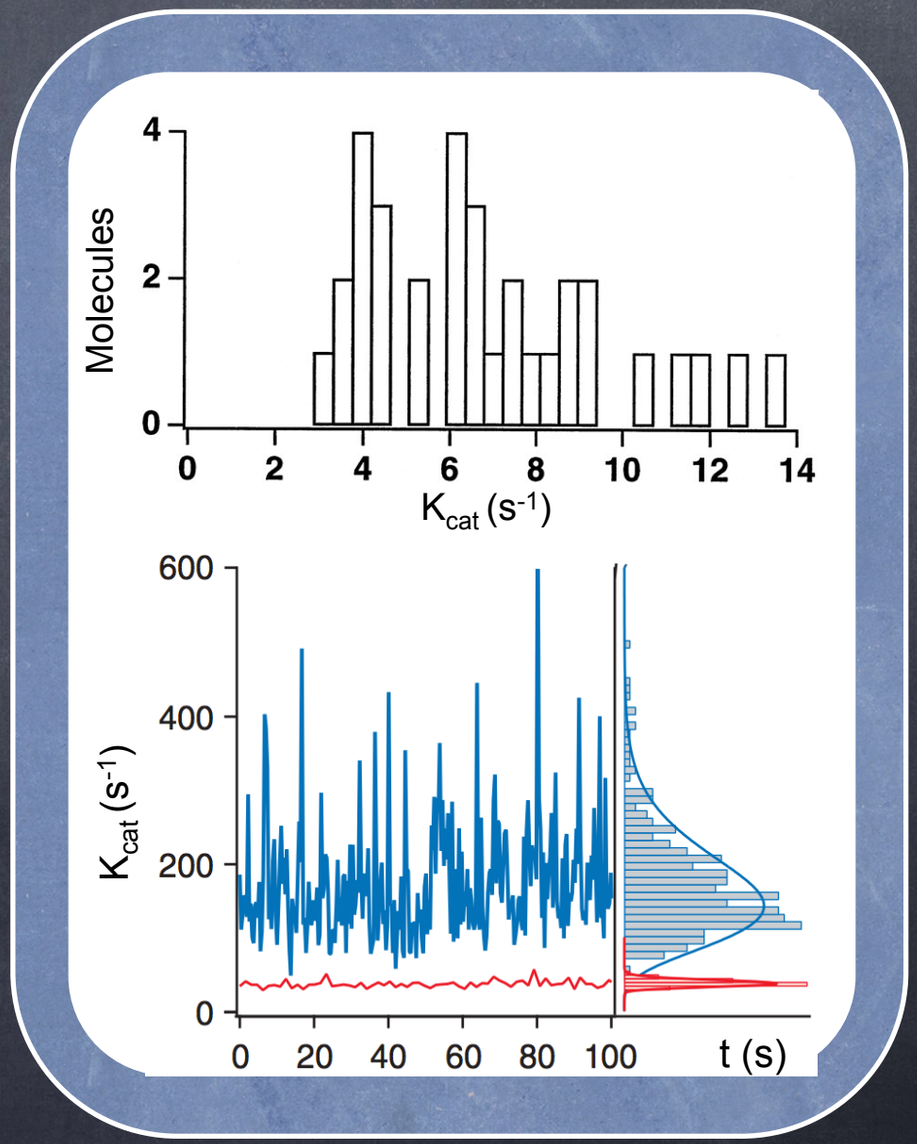
4. Enzyme flexibility

- Enzyme conformation affects binding pose and K_M
- Enzyme conformation affects K_{cat}

Static Flexibility:
In general < 1-2 kcal/mol
Possible artifact due to =1^{ry} structure
Averaged on XR structures

Dynamic Flexibility:
In general < 1-2 kcal/mol
Corr time >> MD can cover (µs-ms)
Averaged on XR structures



4. Enzyme flexibility

Movement	Timescale	Amplitude
Small Scale Movements		
bonds angles side chains	**fs-ps**	below 1 Å

Geom. Opt
Classical MD
Carr-Parrinello MD
QM/MM MD

4. Enzyme flexibility

Movement	Timescale	Amplitude
Small Scale Movements		
bonds angles side chains	**fs-ps**	below 1 Å
Medium Scale Movements		
rotamers loops extremities	**ns-µs**	1-10 Å

Geom. Opt
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4. Enzyme flexibility

Movement	Timescale	Amplitude
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Large Scale Movements		
Domains Subunits	**µs-ms**	5-10 Å

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Movement	Timescale	Amplitude
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Medium Scale Movements		
rotamers loops extremities	**ns-µs**	1-10 Å
Large Scale Movements		
Domains Subunits	**µs-ms**	5-10 Å
Global Movements		
Folding Association	**ms-h**	> 10 Å

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Classical MD
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QM/MM MD

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