The Catalytic Mechanism of an Enzymatic Reaction

Pedro Alexandrino Fernandes,

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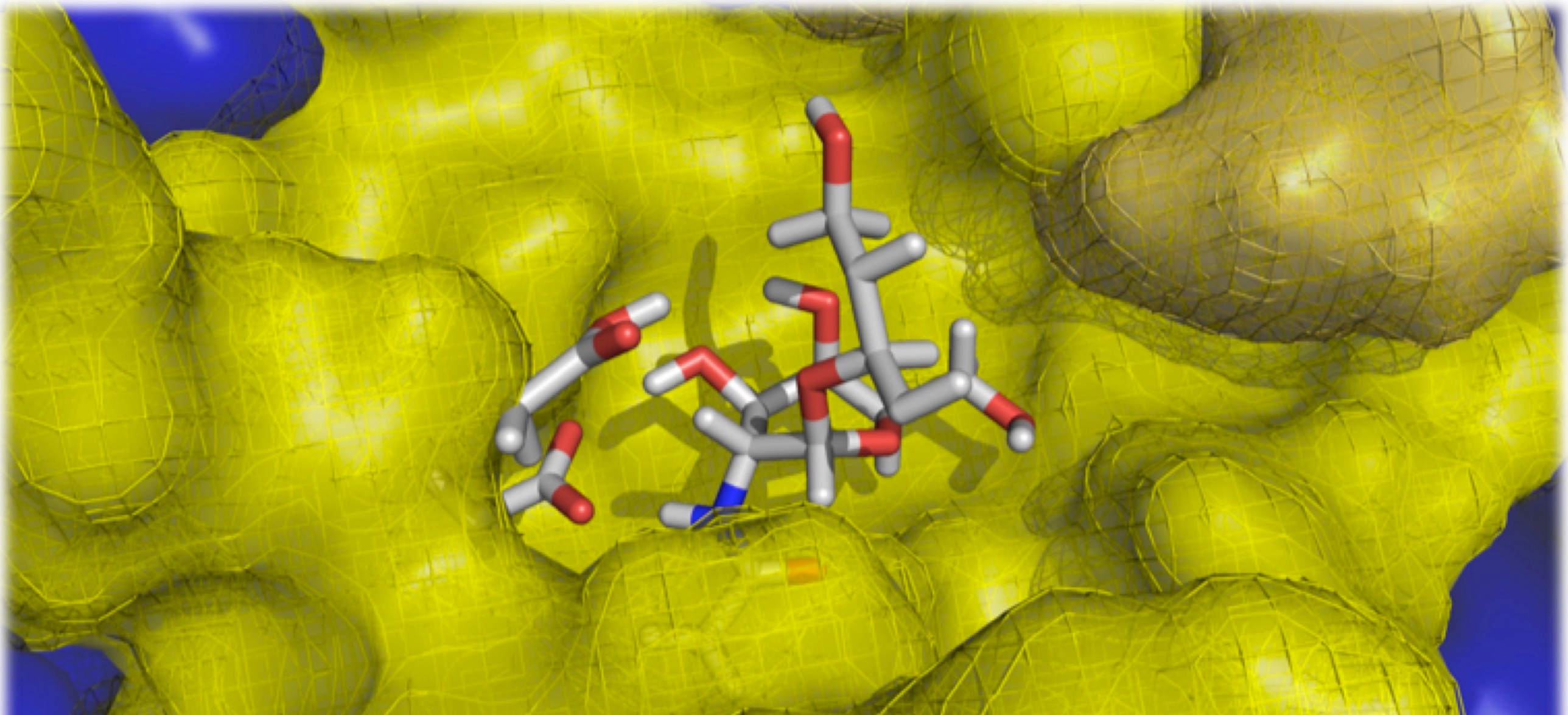
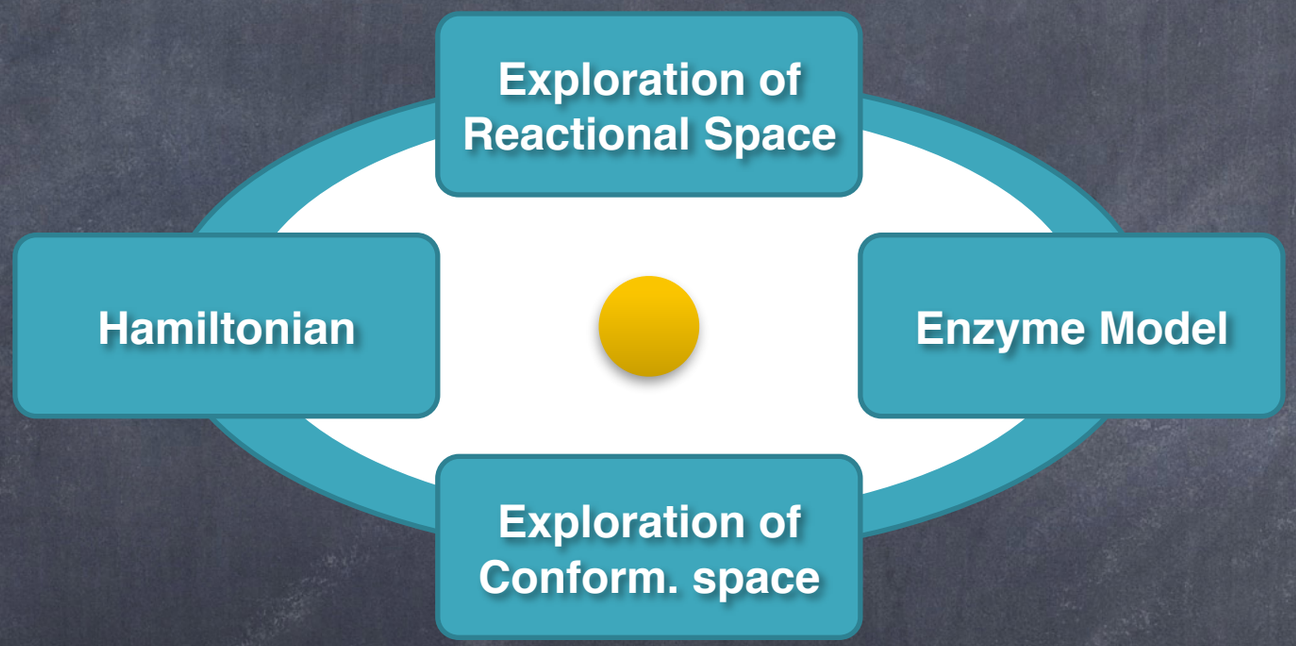


Why enzymes are so difficult?

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

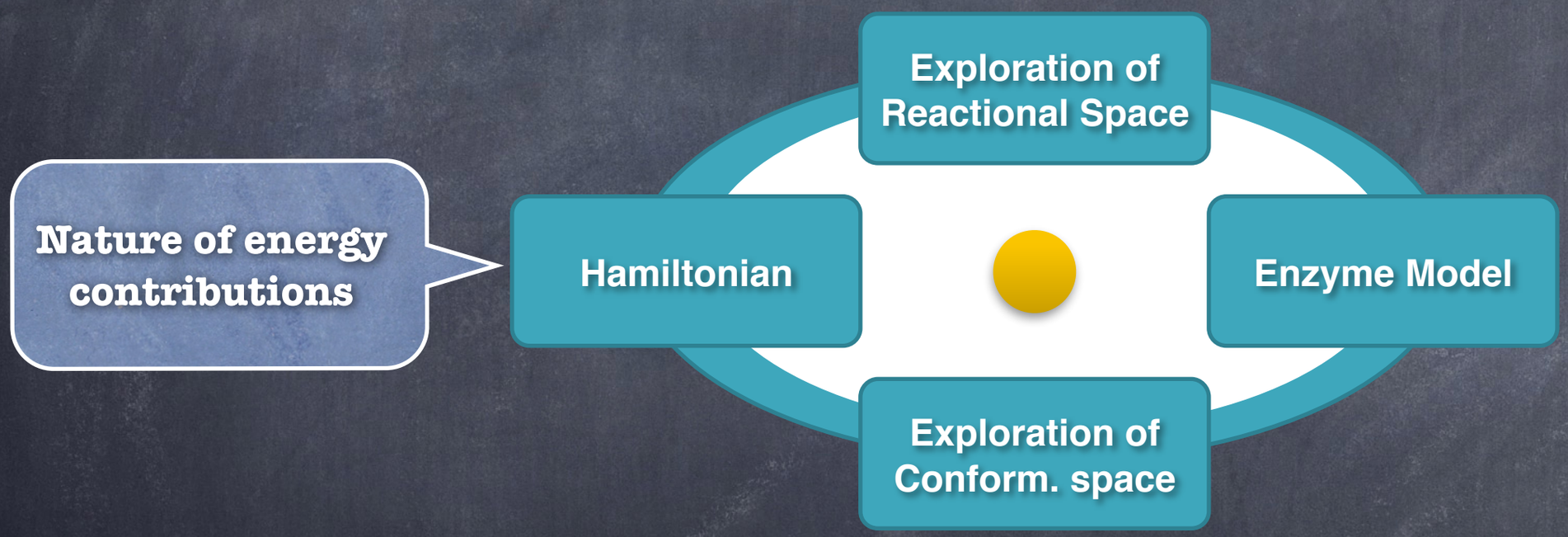
Solutions for the problem



Acc Chem Res, 2008, 689

PCCP, 2012, 12431

Solutions for the problem



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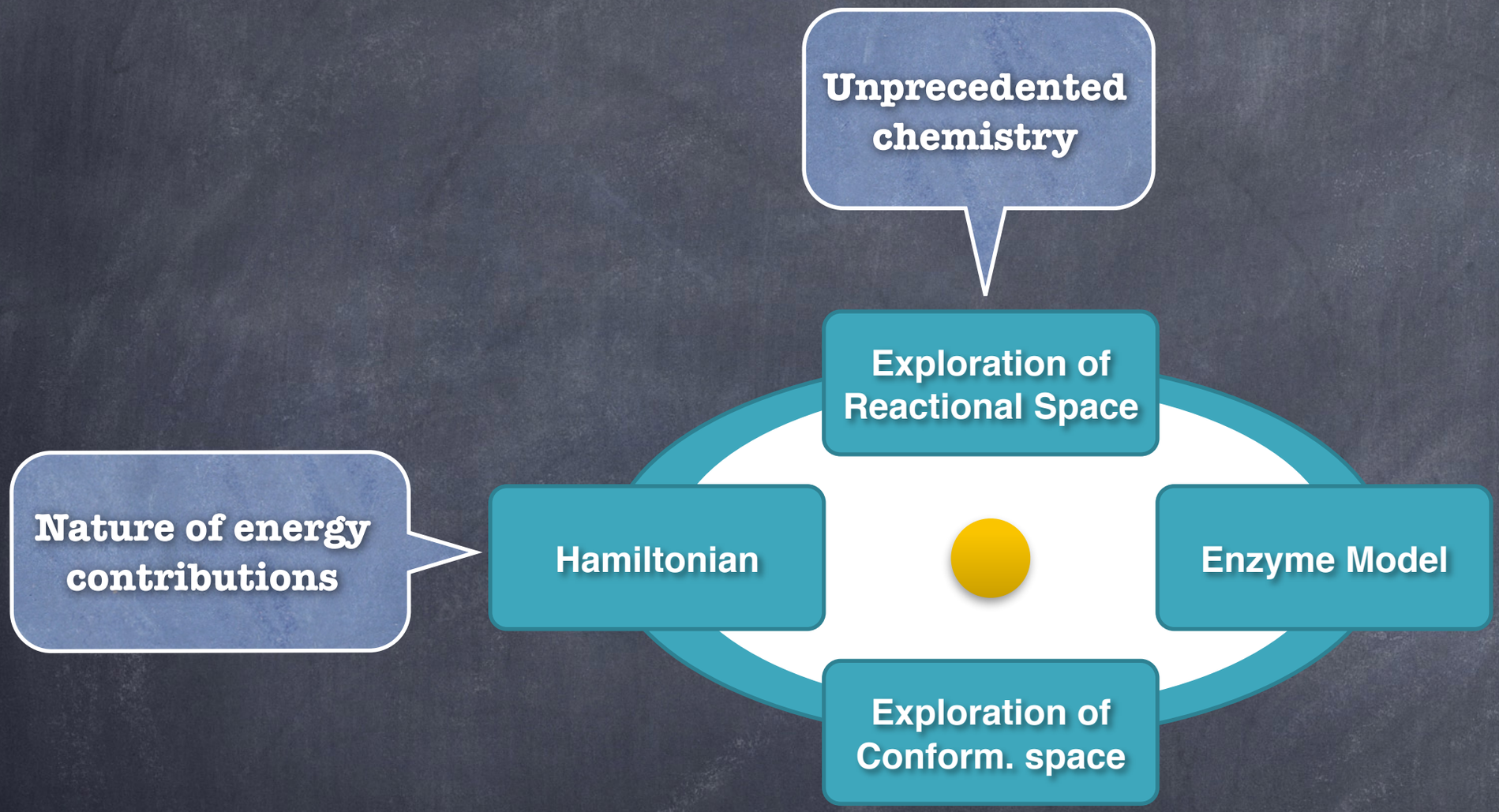
Solutions for the problem



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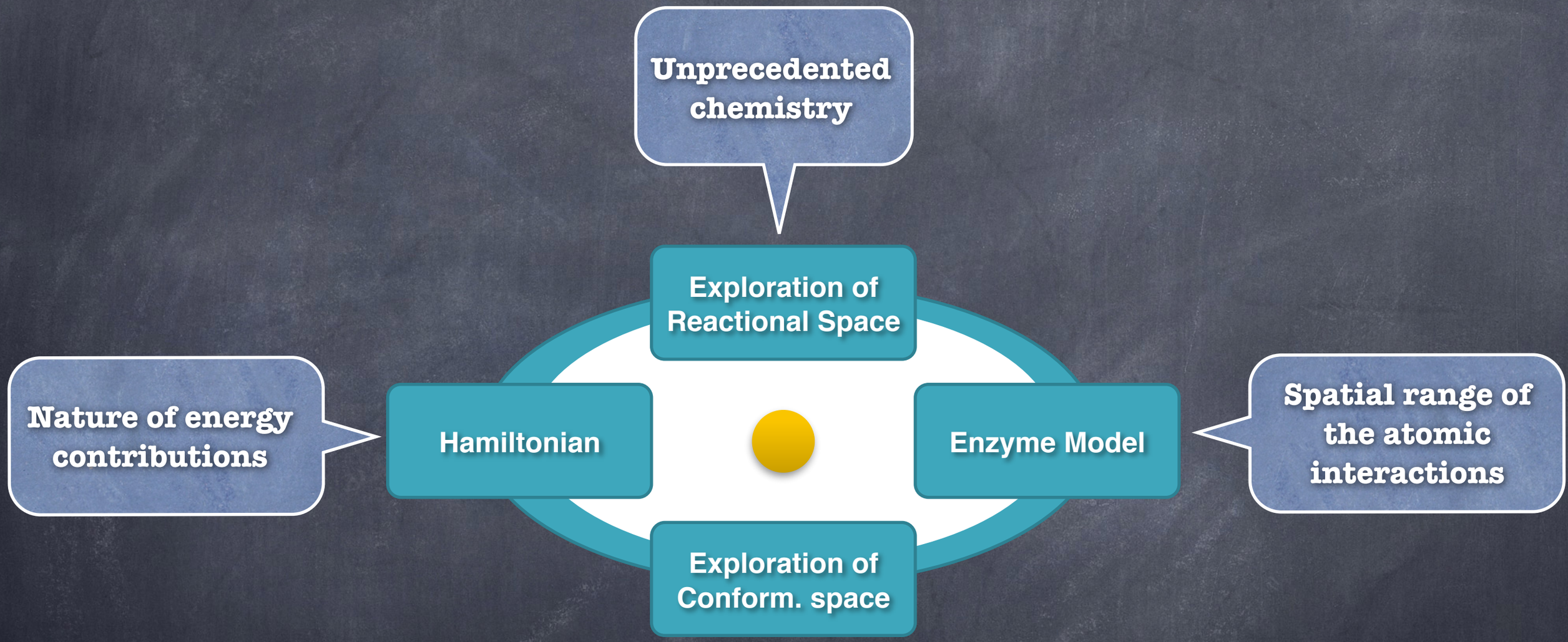
Solutions for the problem



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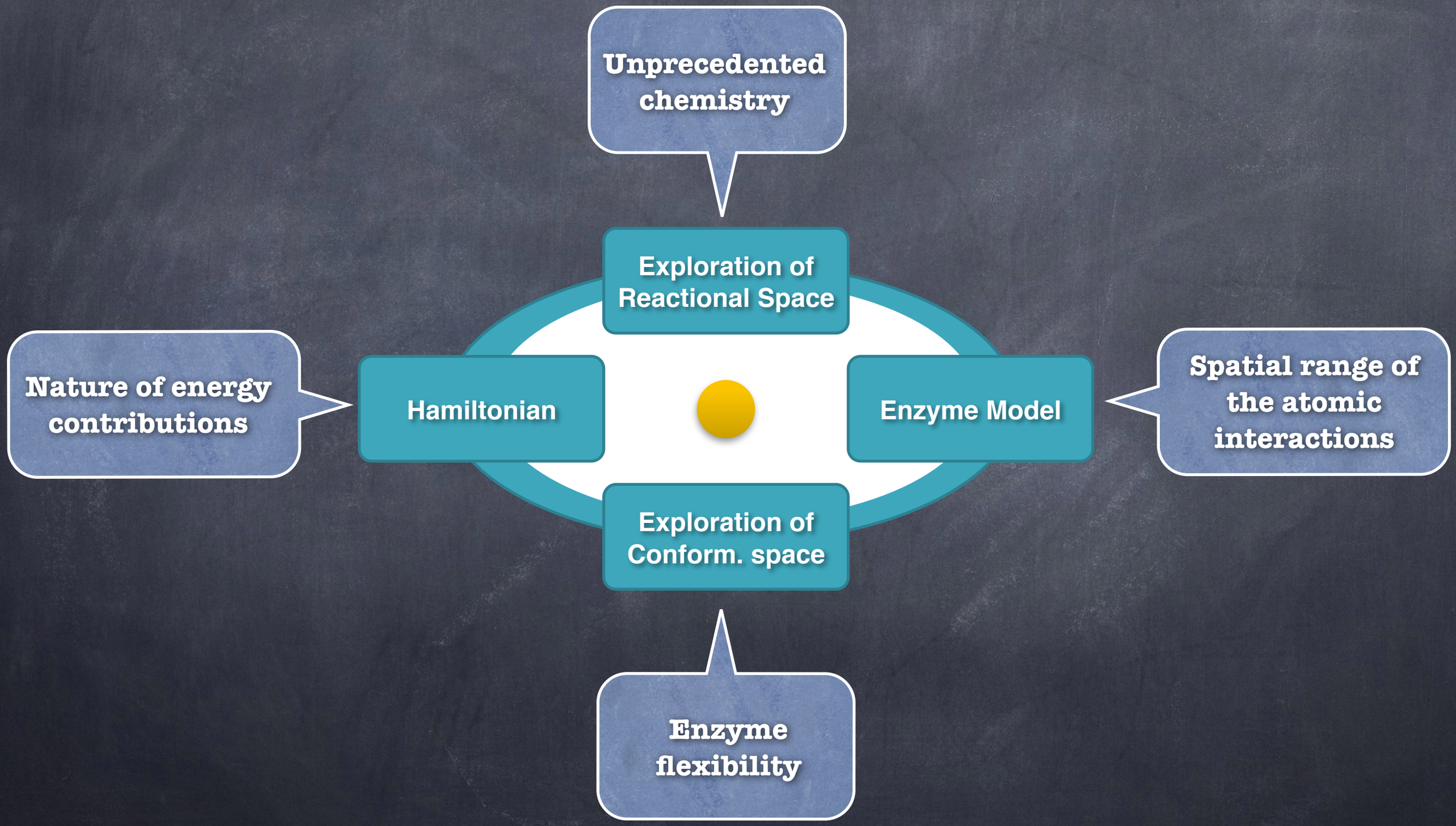
Solutions for the problem



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PCCP, 2012, 12431

Solutions for the problem

Unprecedented chemistry

Exploration of Reactional Space

Nature of energy contributions

Hamiltonian

Enzyme Model

Spatial range of the atomic interactions

Exploration of Conform. space

Enzyme flexibility

Acc Chem Res, 2008, 689

PCCP, 2012, 12431

The Hamiltonian

1. Nature of energy contributions

The **adequate Hamiltonian** must simultaneously describe well a wide range of interactions

The Hamiltonian

Size of the QM region usually 100-500 atoms

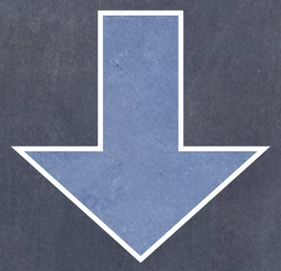
The Hamiltonian

Size of the QM region usually 100-500 atoms

DFT is the only practical QM theoretical level

The Hamiltonian

Size of the QM region usually 100-500 atoms



DFT is the only practical QM theoretical level

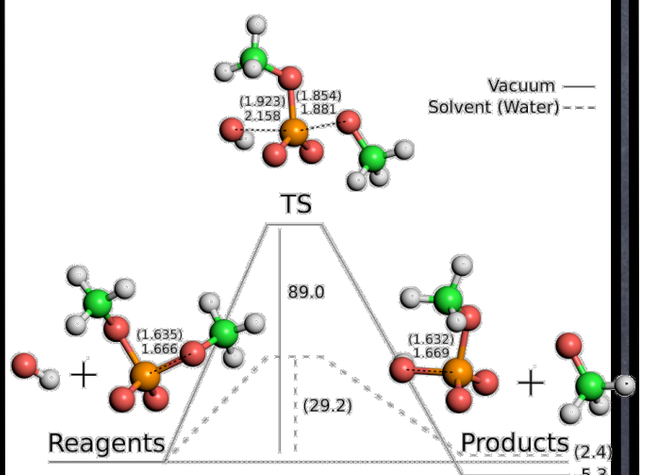
Problem: Functional performance is case-dependent

Benchmarking Density Functionals

Benchmarking of the DFT functionals before embarking in QM/MM calculations is highly advisable

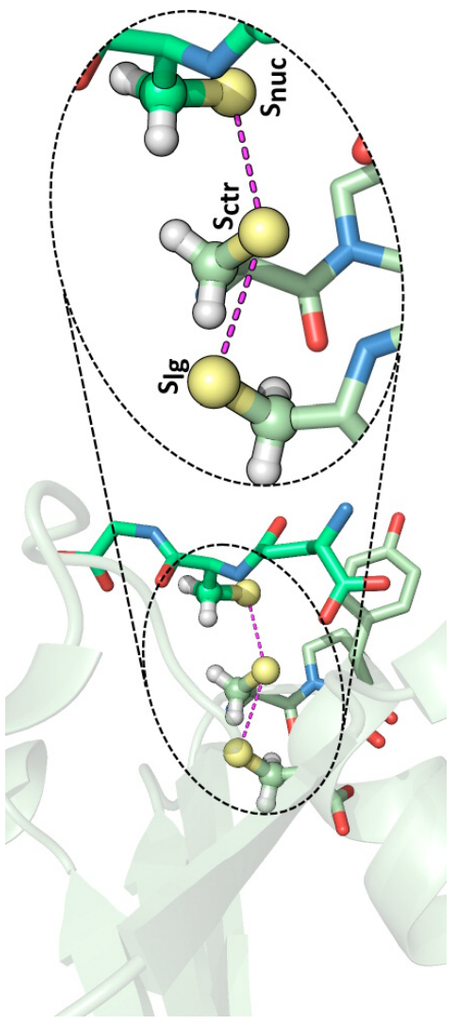
Benchmarking Density Functionals

Specific heavy atom transfer



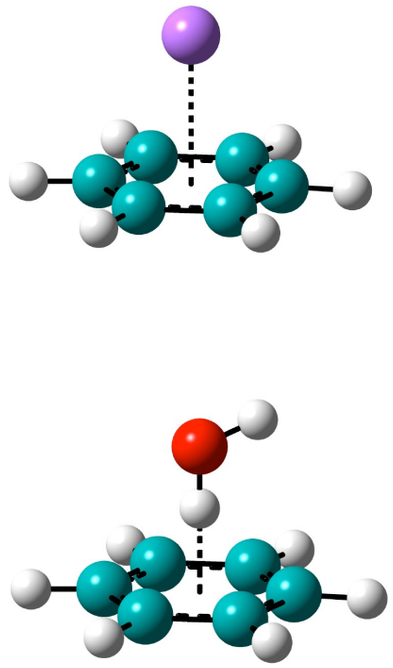
JCTC. 2010, 6, 2281

Specific heavy atom transfer



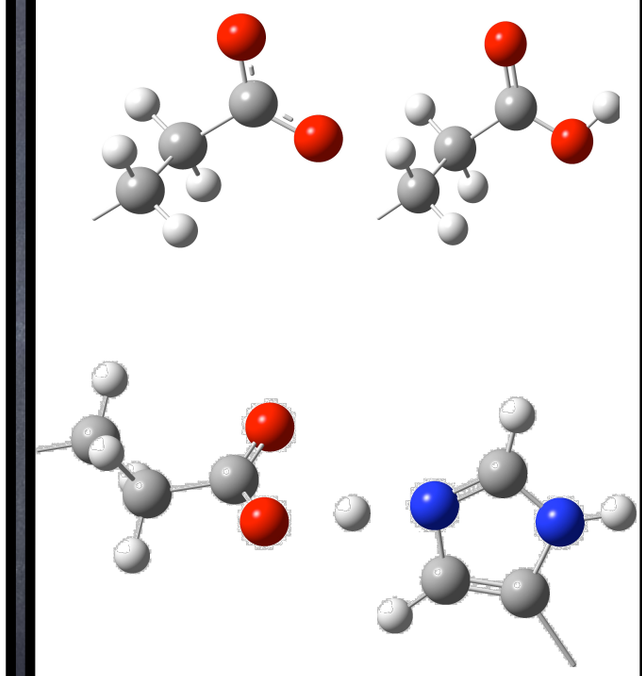
JCTC. 2014, 10, 4842

Specific interactions



JCTC. 2011, 7, 2059

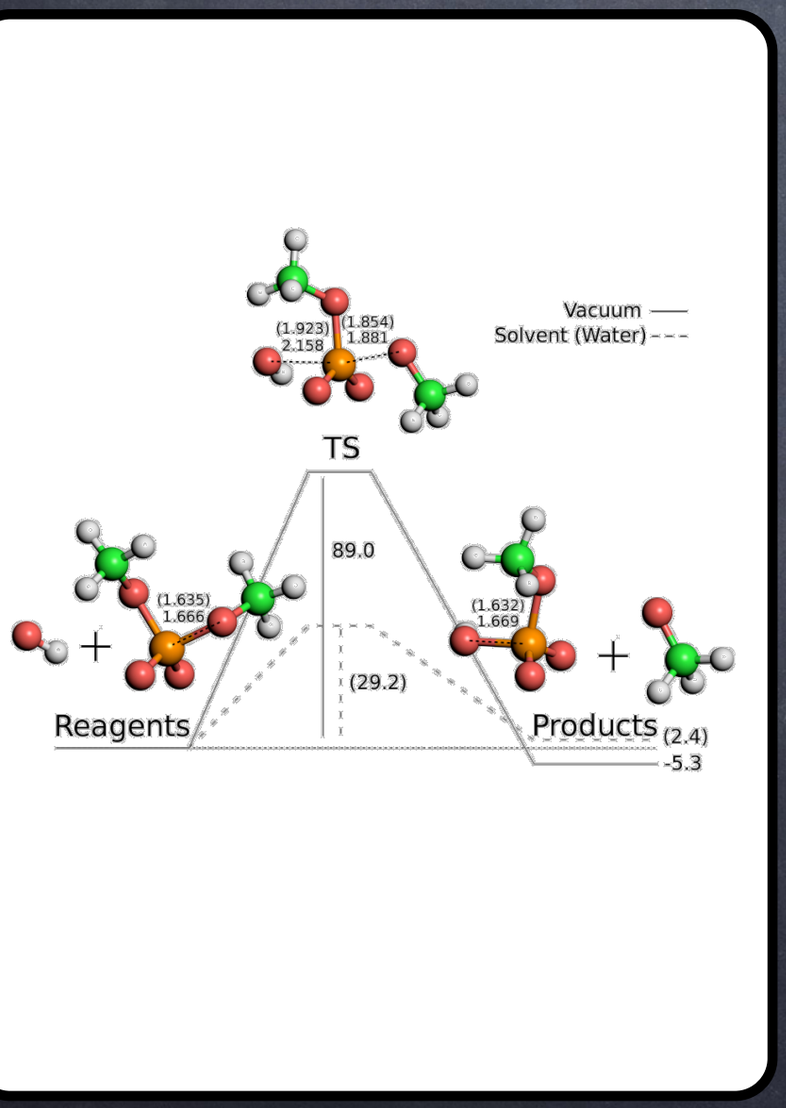
PA an PT of/between **residues**



JCTC. 2011, 7, 3898

Benchmarking Density Functionals

Specific heavy atom transfer



EXAMPLE:

Hydrolysis of phosphodiester bonds.

JCTC. 2010, 6, 2281

Benchmarking Density functionals

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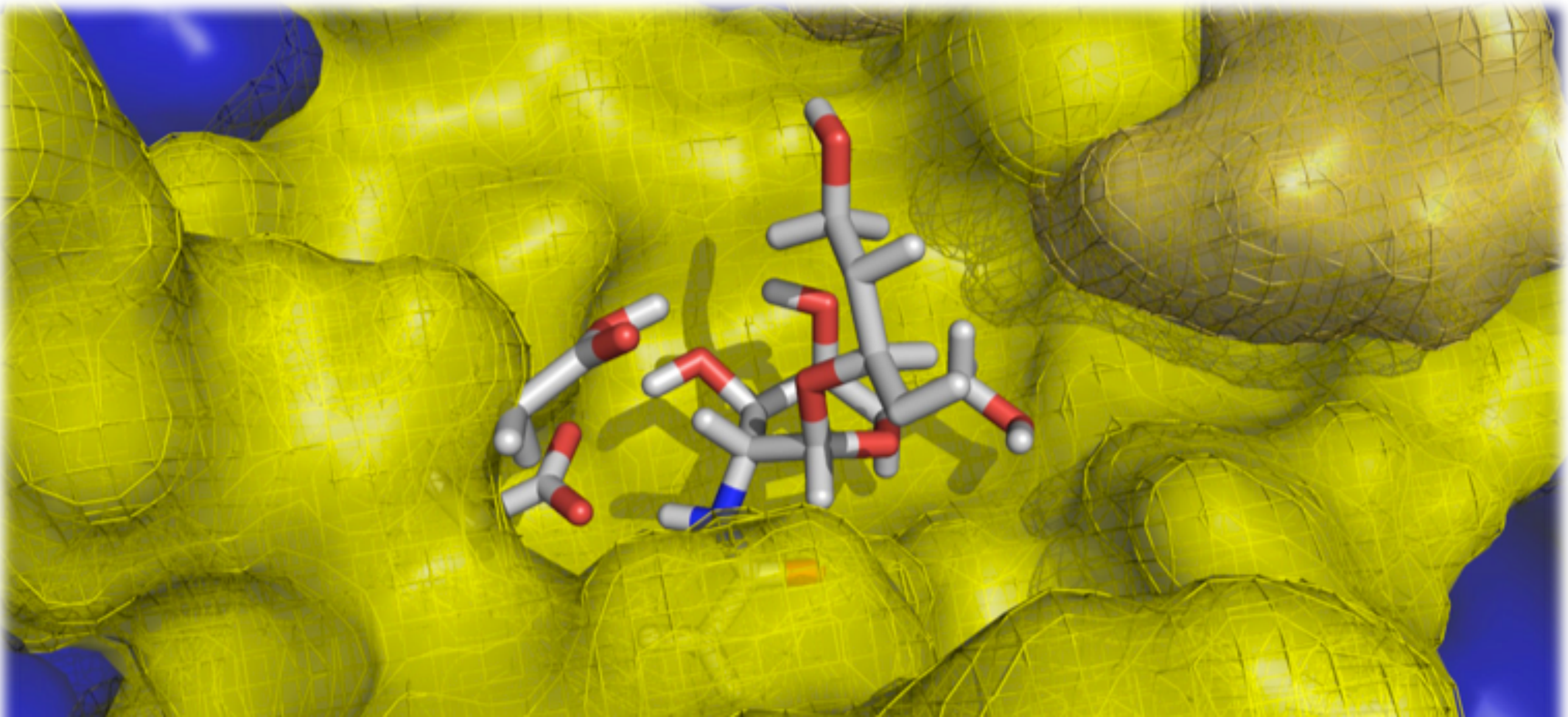
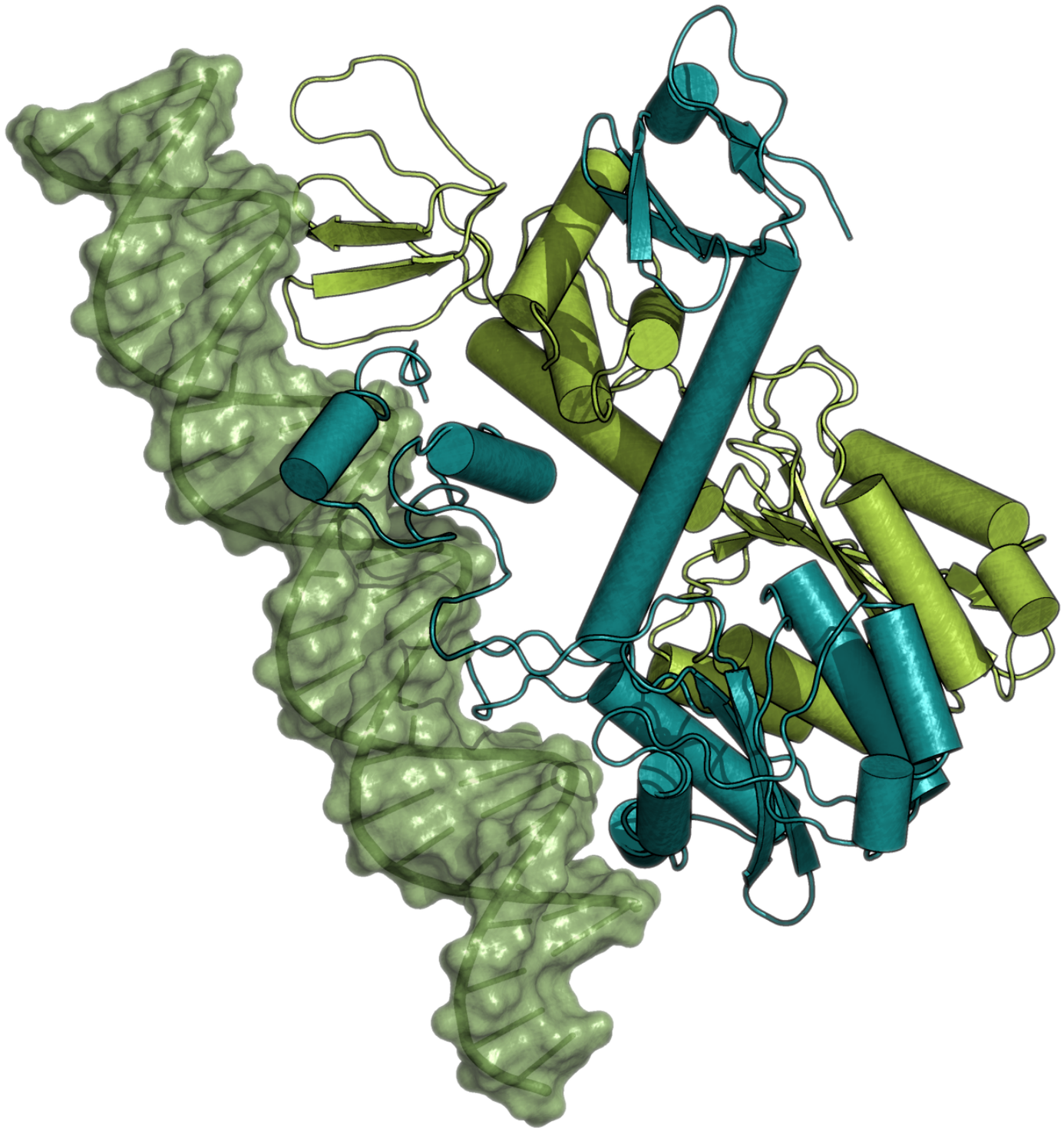


HIV-1 Integrase



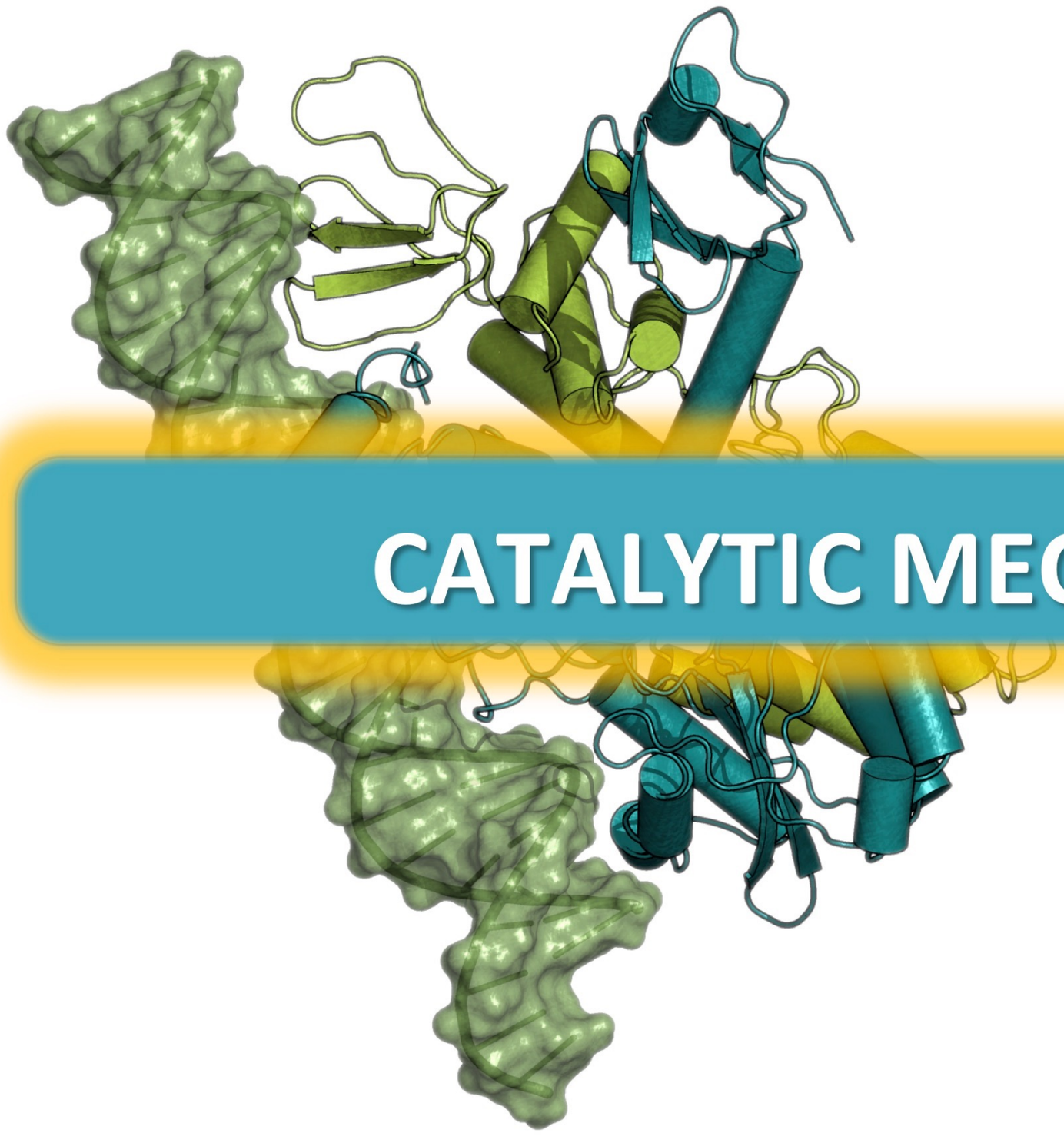
Integrates HIV-1 viral DNA into the human DNA.

Important for the treatment of HIV-1 Infection.

Drug discoveryfor Integrase extremely slow.

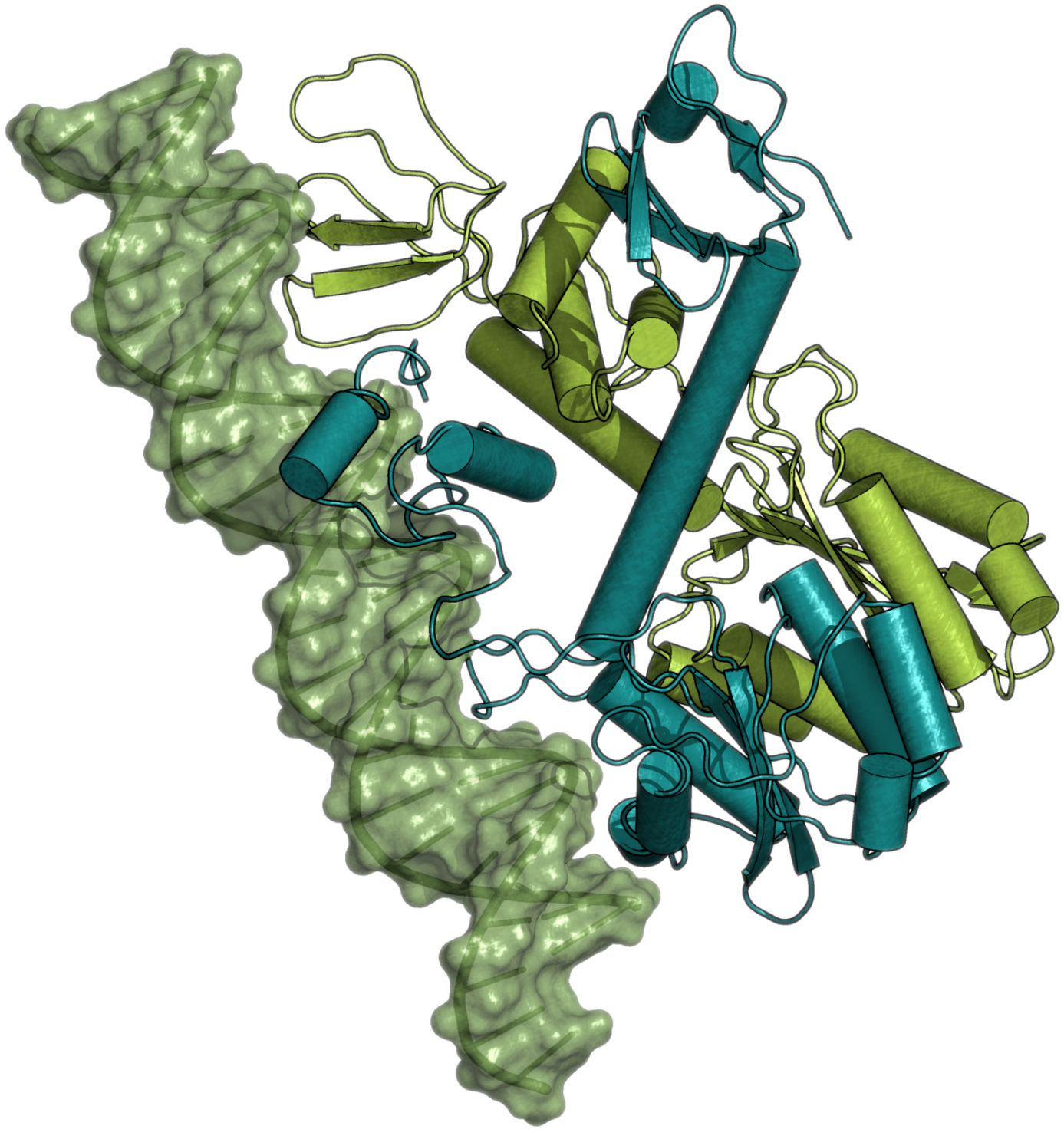
Lack of knowledge about structure and mechanism

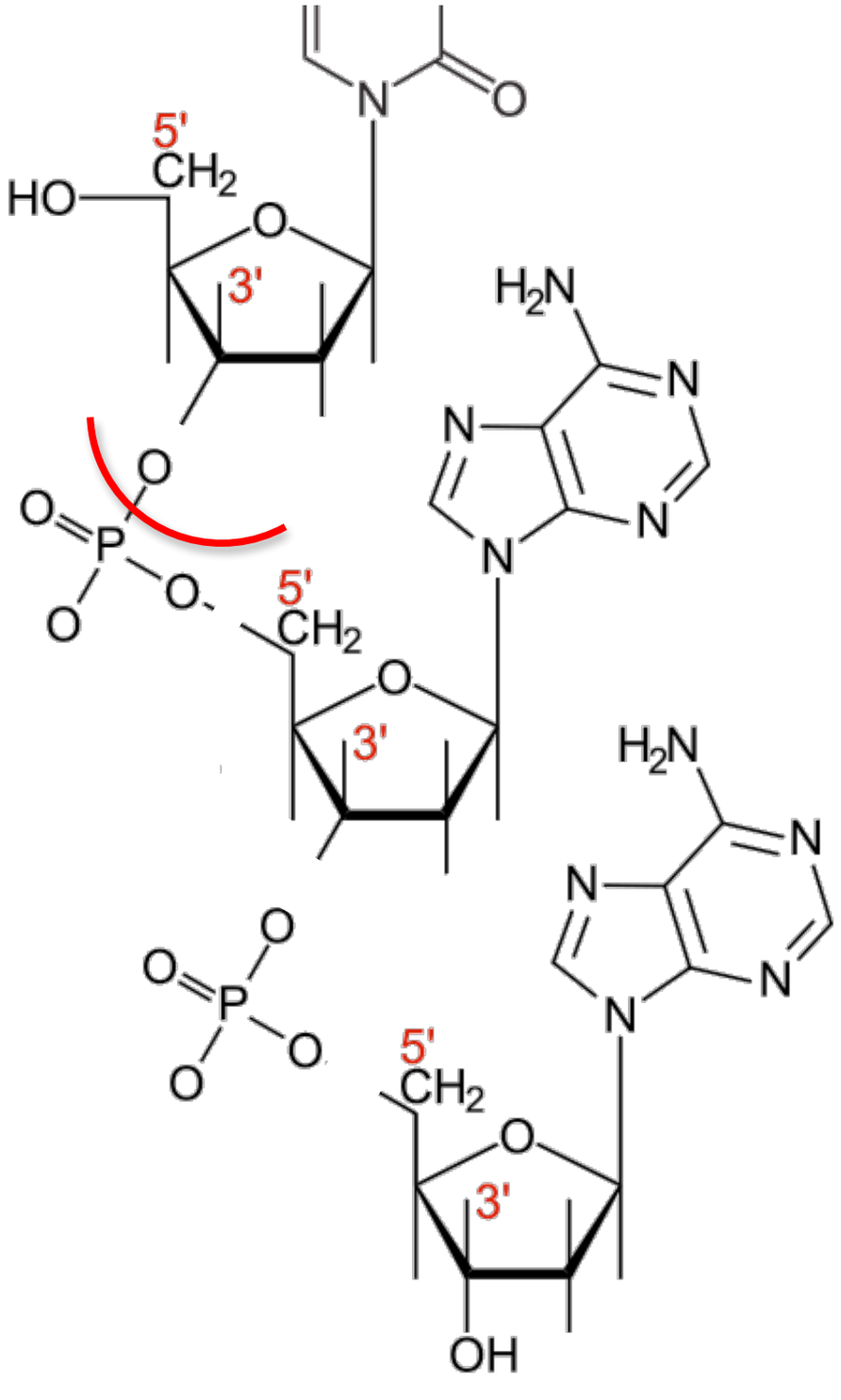
HIV-1 Integrase



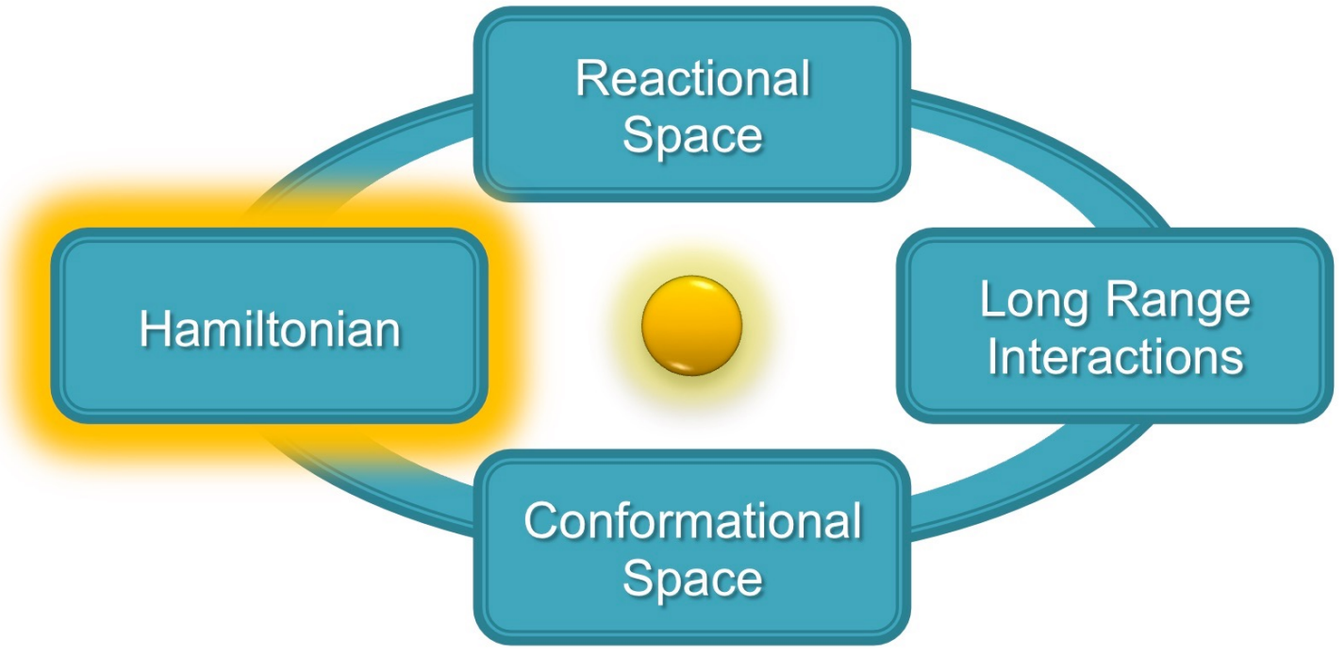
CATALYTIC MECHANISM?

HIV-1 Integrase

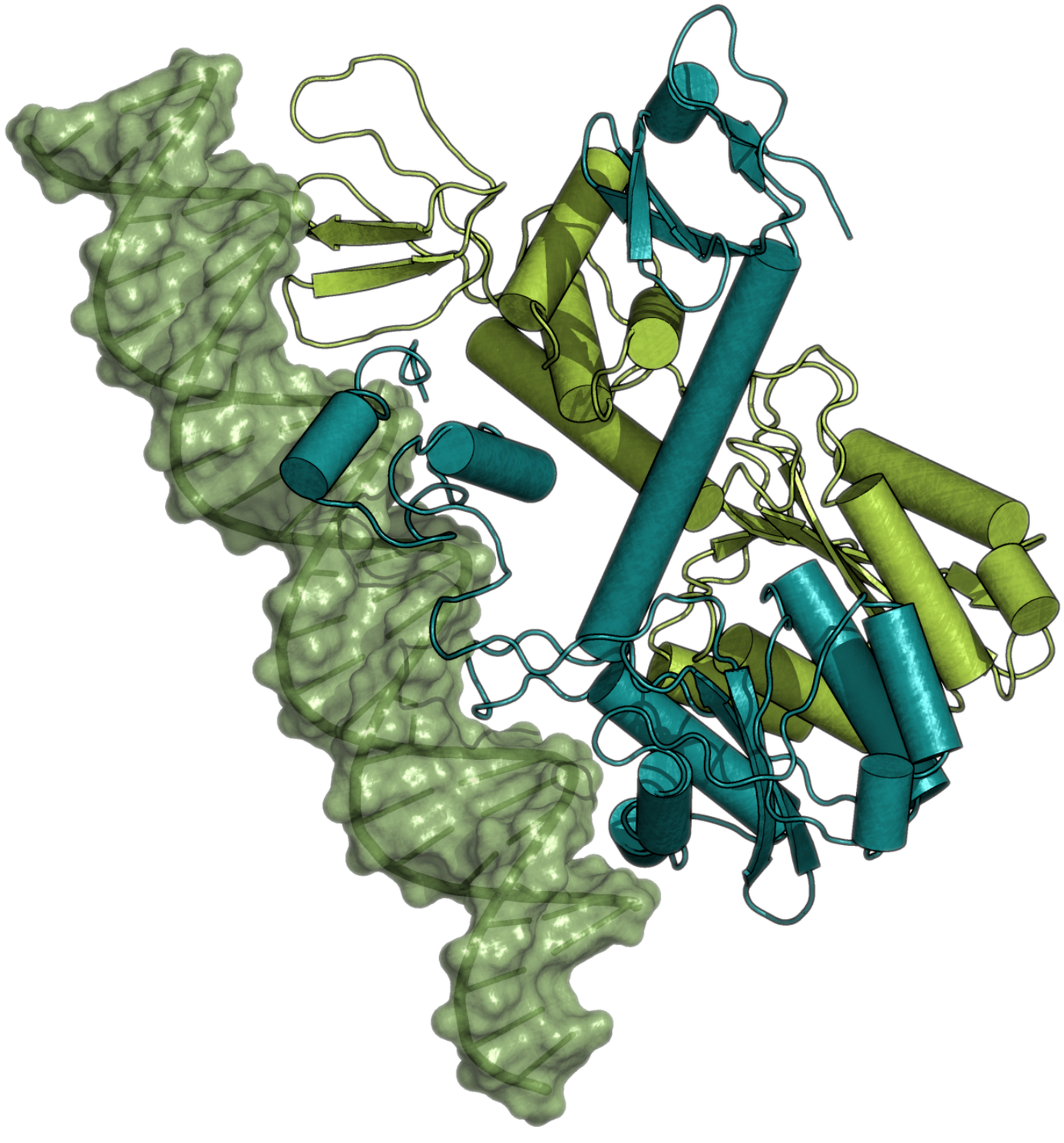


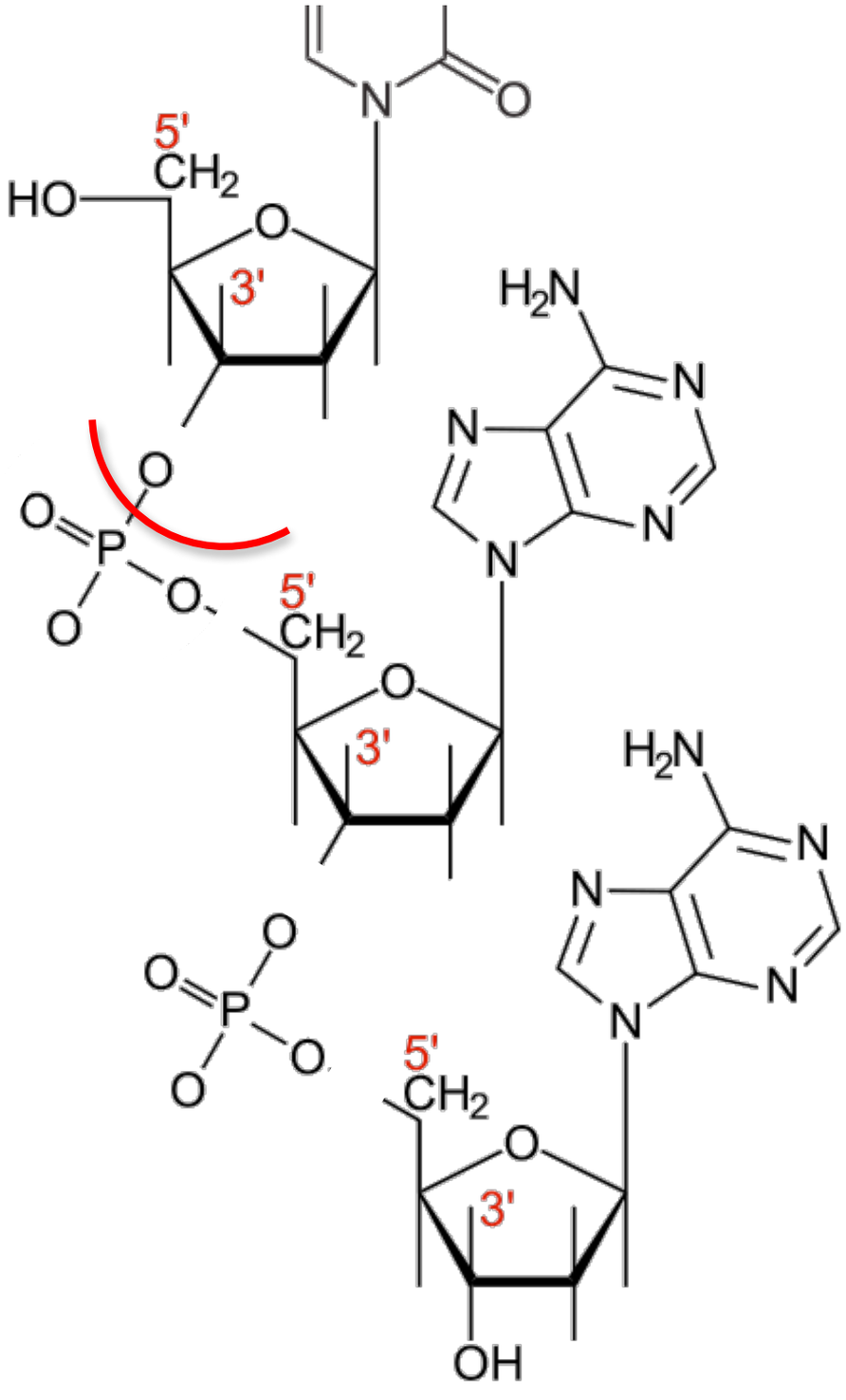


The Hamiltonian

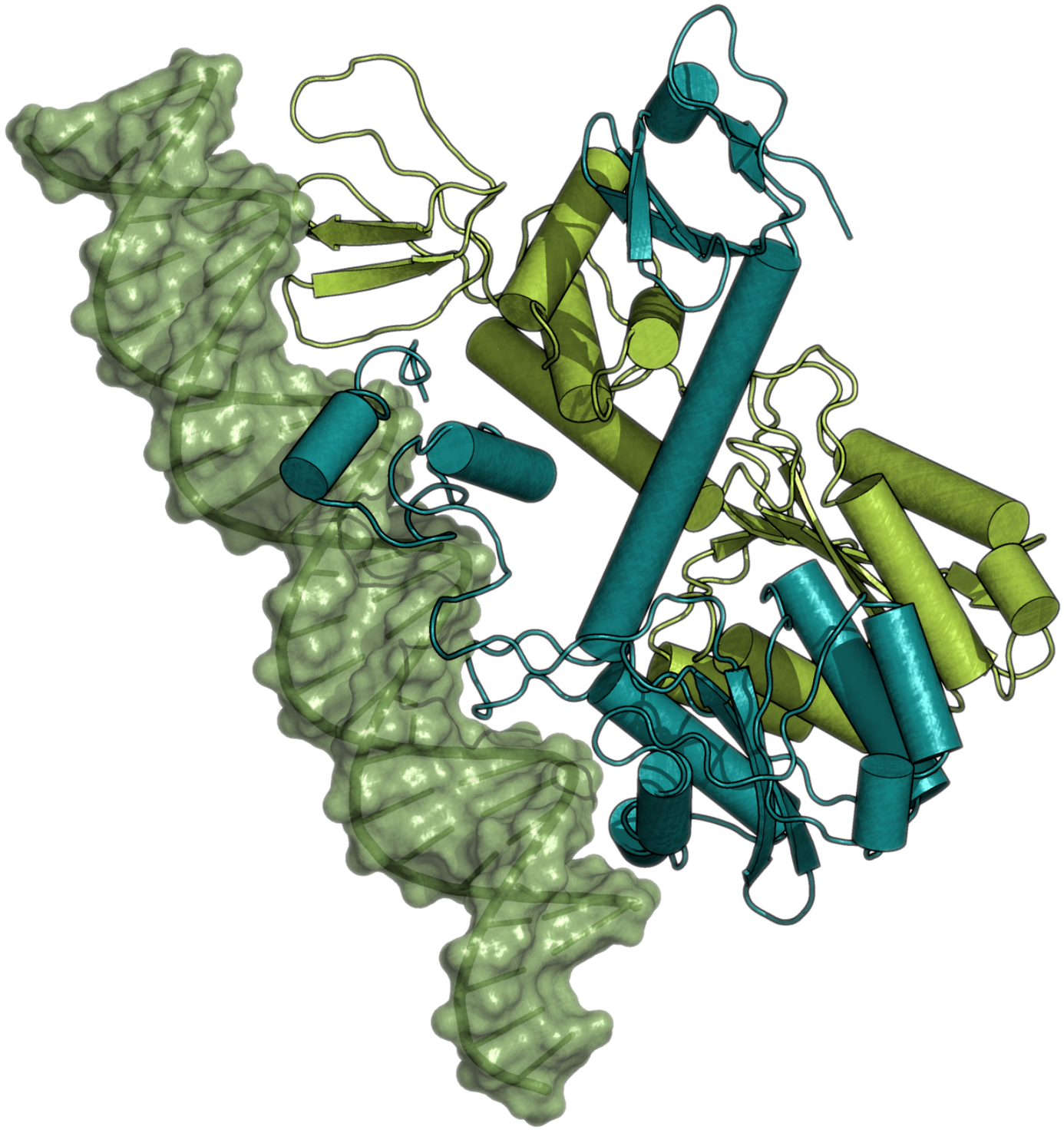


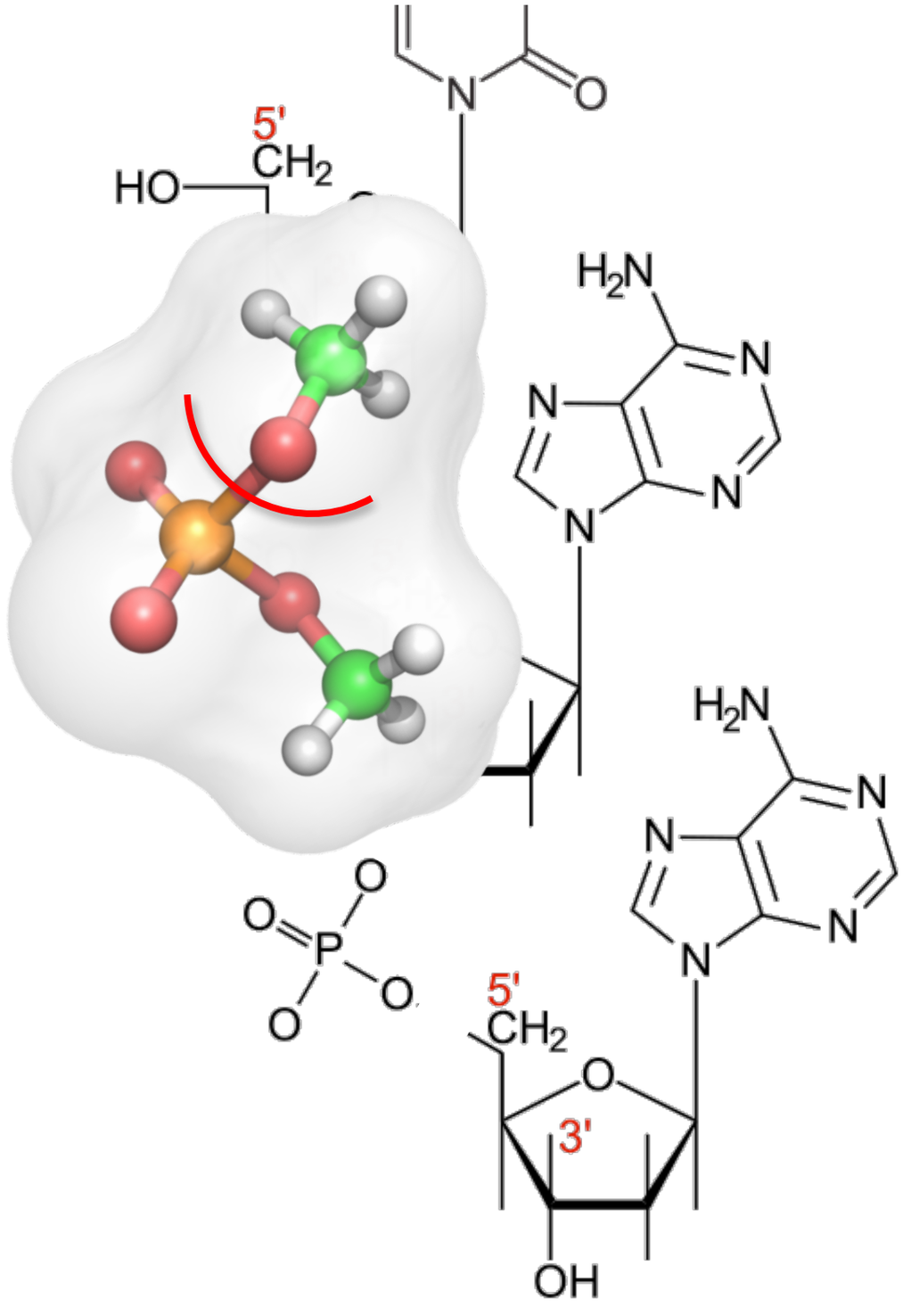
The Hamiltonian



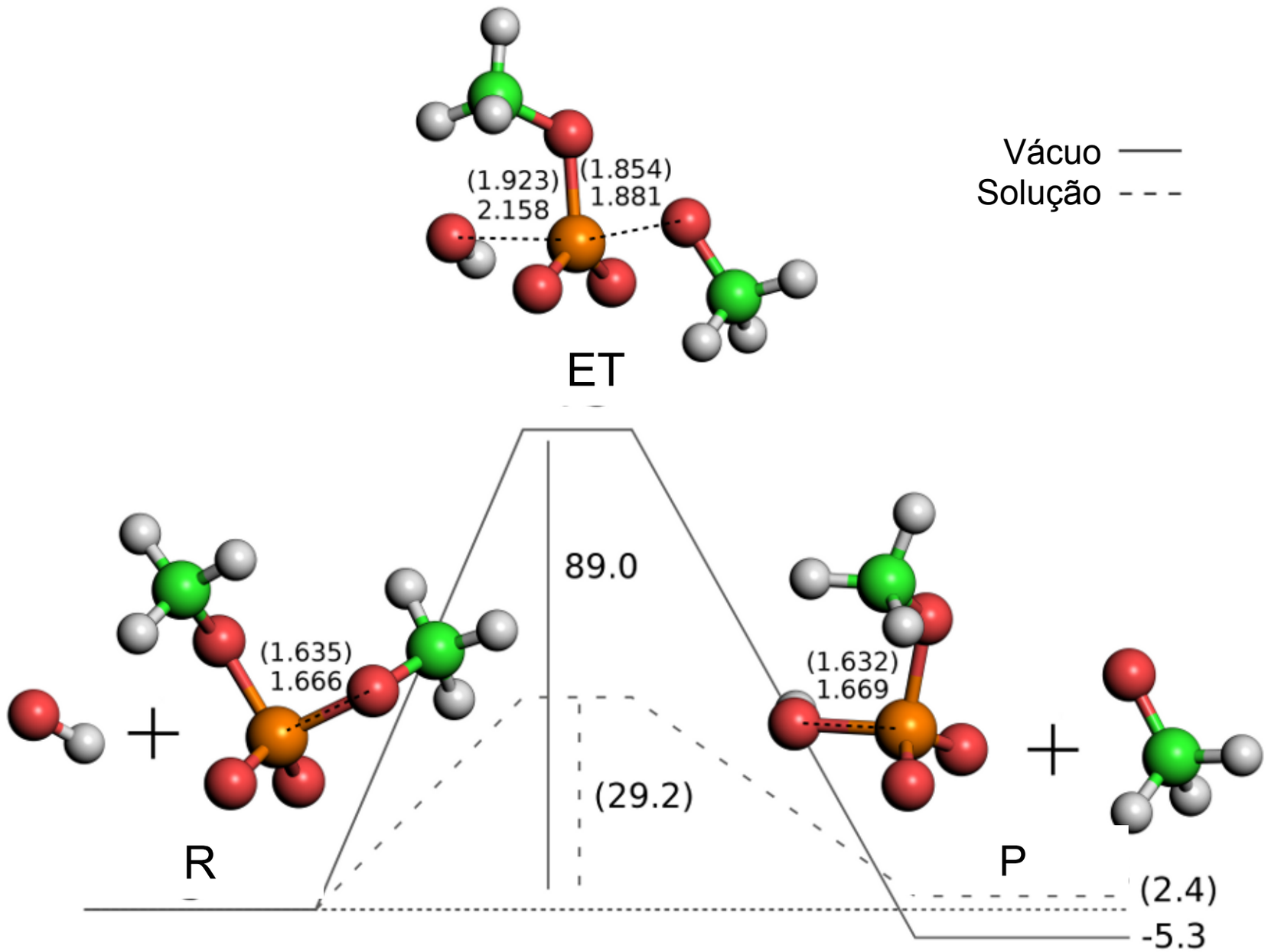


Accuracy of Density Functionals?



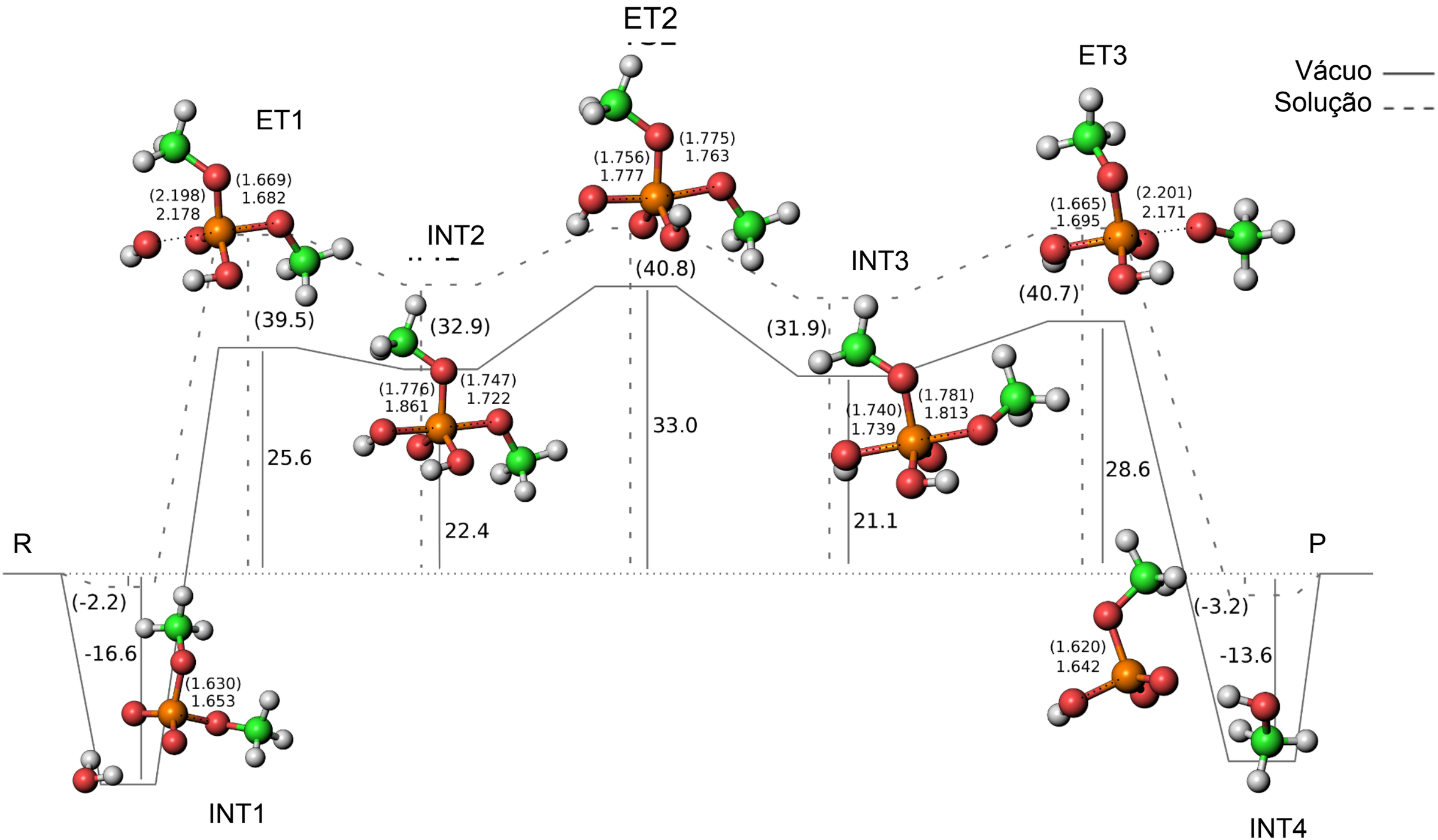


Hydrolysis of Phosphodiester Bonds by HO^-.



B3LYP/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p)

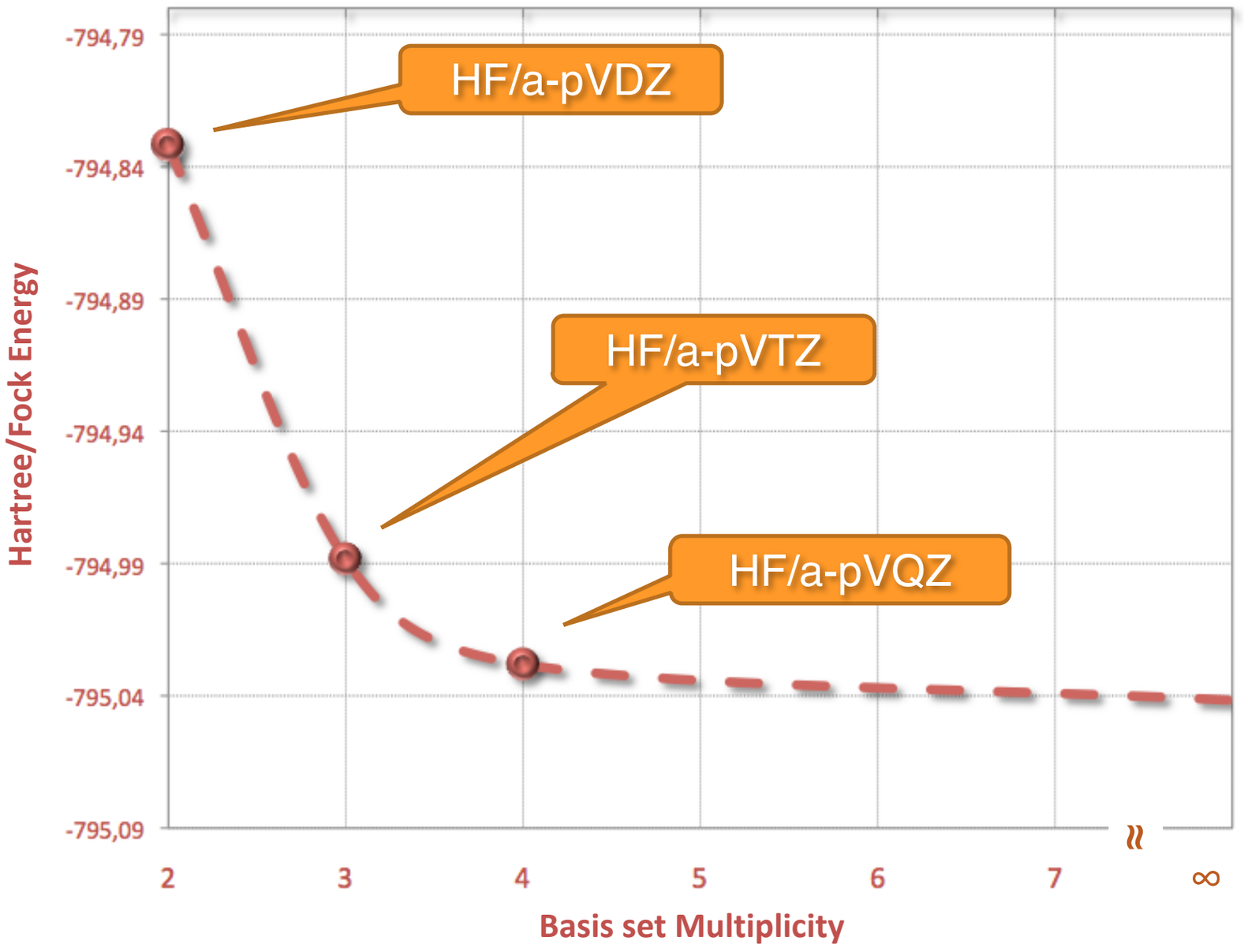
Hydrolysis of Phosphodiester Bonds by H_2O.



B3LYP/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p)

Reference PES: CCSD(T)/CBS.

$$E_{\infty}^{TOT} = E_{\infty}^{HF} + E_{\infty}^{corr}, \quad E_{\infty}^{HF} = E_{X}^{HF} + a \cdot \exp(-bX), \quad E_{\infty}^{corr} = \frac{X^3}{X^3 - (X-1)^3} E_{X}^{corr} - \frac{X^3}{X^3 - (X-1)^3} E_{(X-1)}^{corr}$$



Halkier Method

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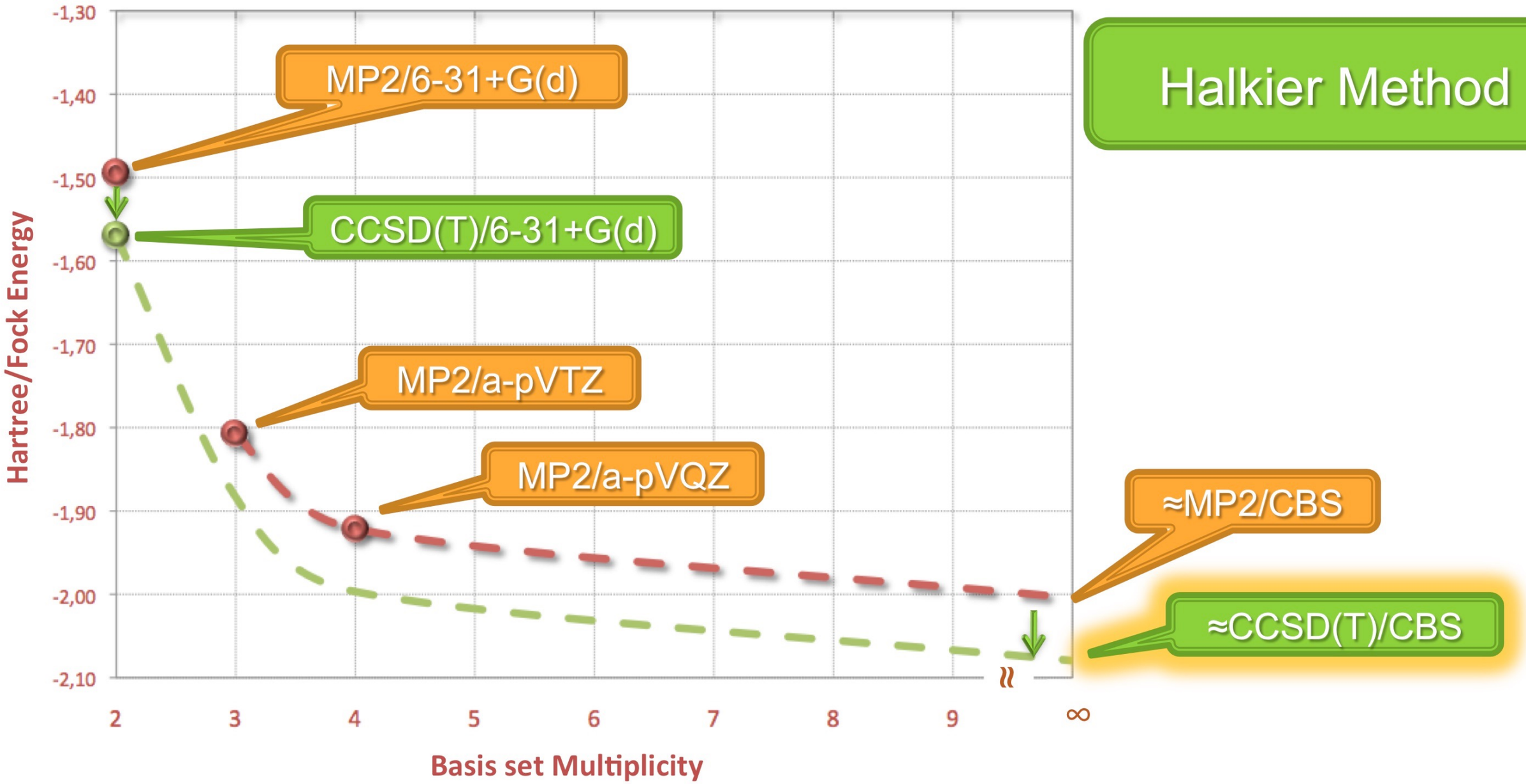
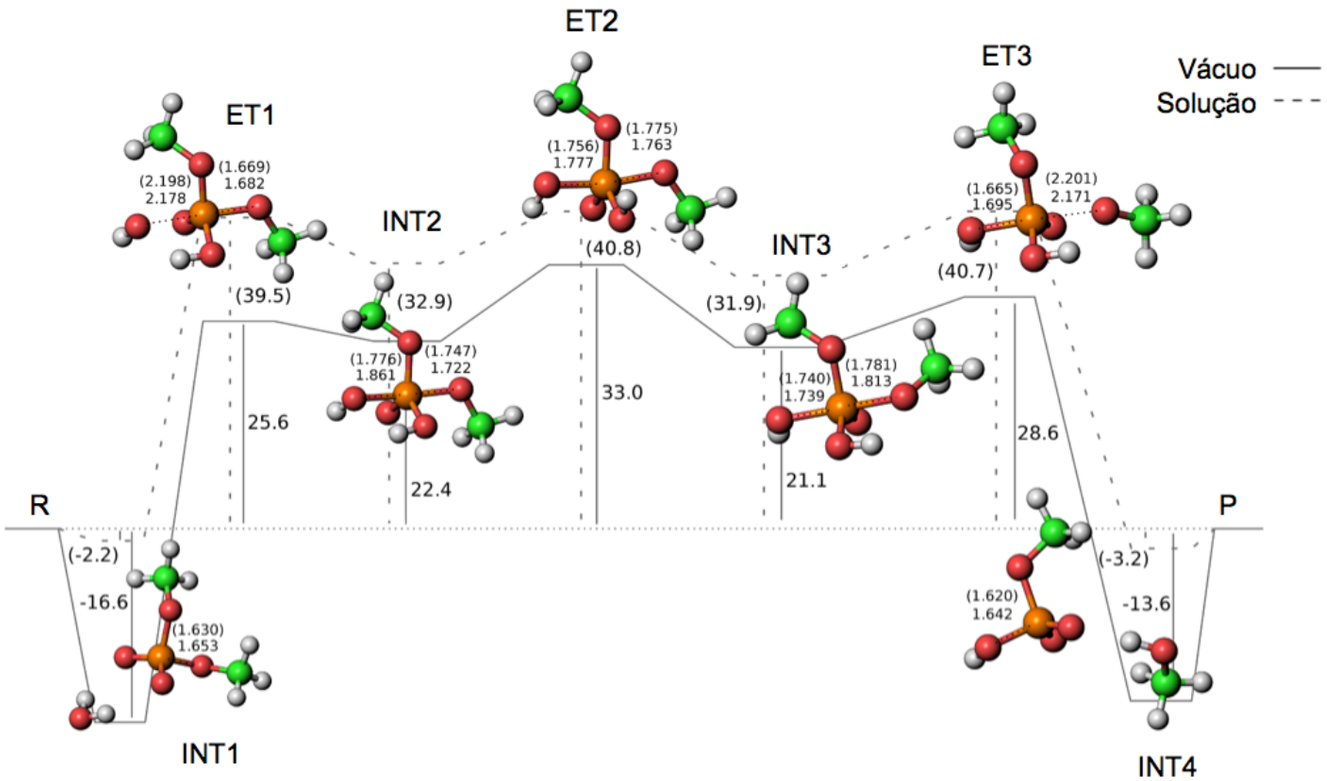
Halkier Method

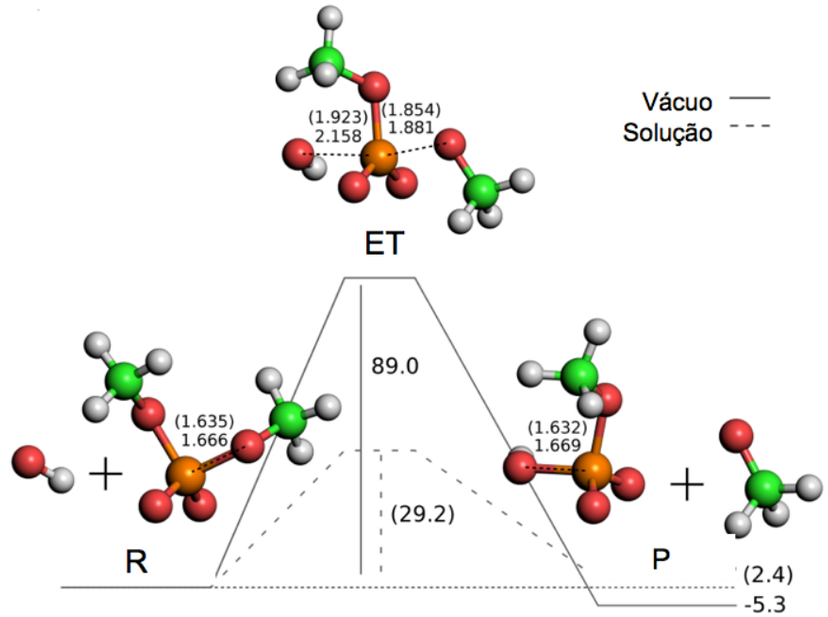


Reference PES: CCSD(T)/CBS.

Hydrolysis by H_20



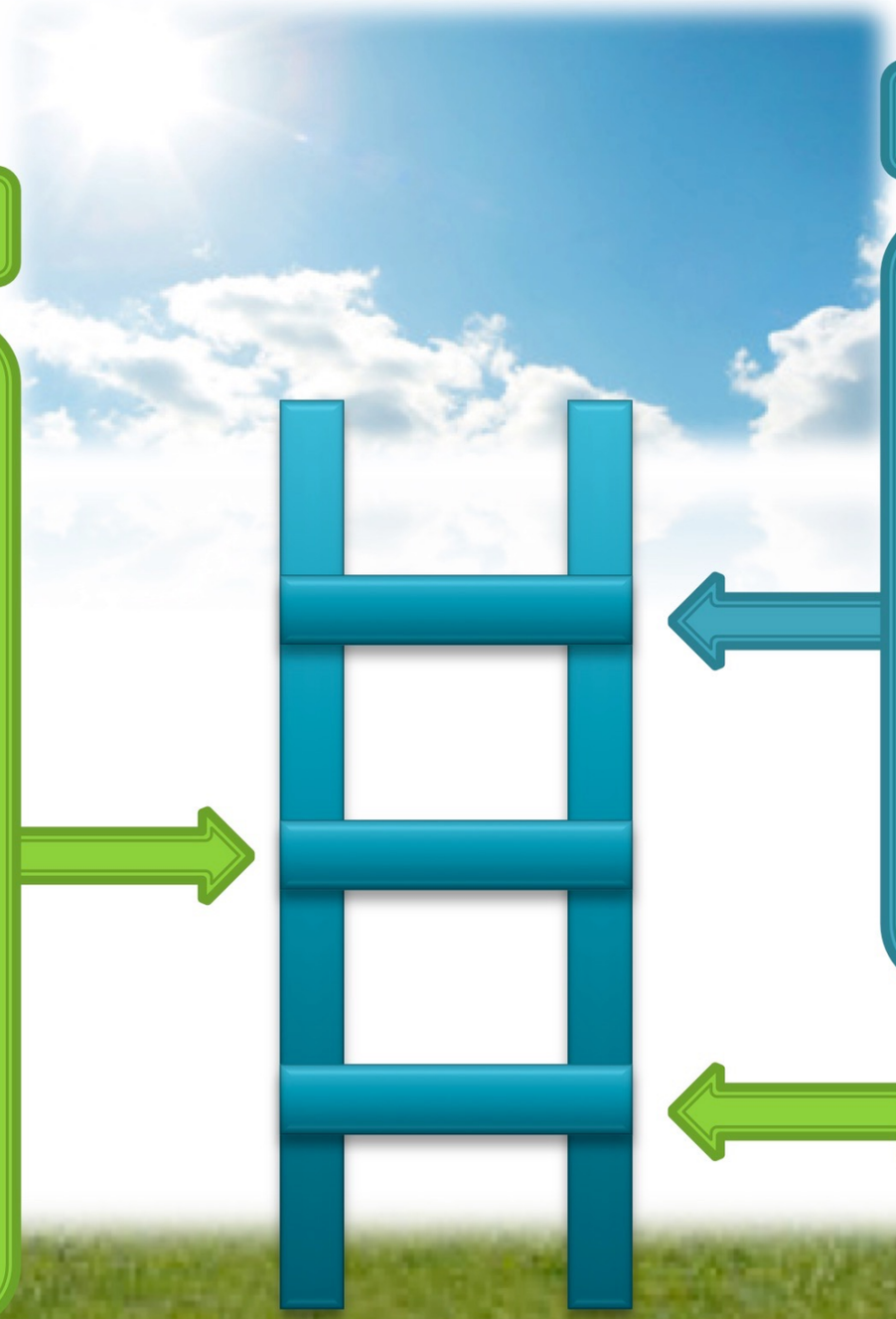
Hydrolysis by HO^-



ΔG^*(calculated) = 38,0 kcal·mol^{-1}
ΔG^*(exp.) ≈ 38,5 kcal·mol^{-1}

ΔG^*(calculated) = 34,7 kcal·mol^{-1}
ΔG^*(exp.) ≈ 35 kcal·mol^{-1}

Performance of 50 Density Funcionals



GGA

BLYP
BP86
BPBE
BPW91
G96LYP
HCTH93
HCTH147
HCTH407
MPWLYP
MPWLYP1W
MPWPW91
OLYP
PBE1W
PBELYP1W
PBEPBE
XLYP

H-GGA

B1LYP
B3LYP
B3P86
B3PW91
B97-1
B97-2
B98
BhandH
BhandHLYP
MPW1K
MPW1N
MPW1PW91
MPW1S
MPW3LYP
O3LYP
PBE1PBE

M-GGA

BB95
M06 - L
MPWB95
TPSSLYP1W
TPSSTPSS
VSXC

HM-GGA

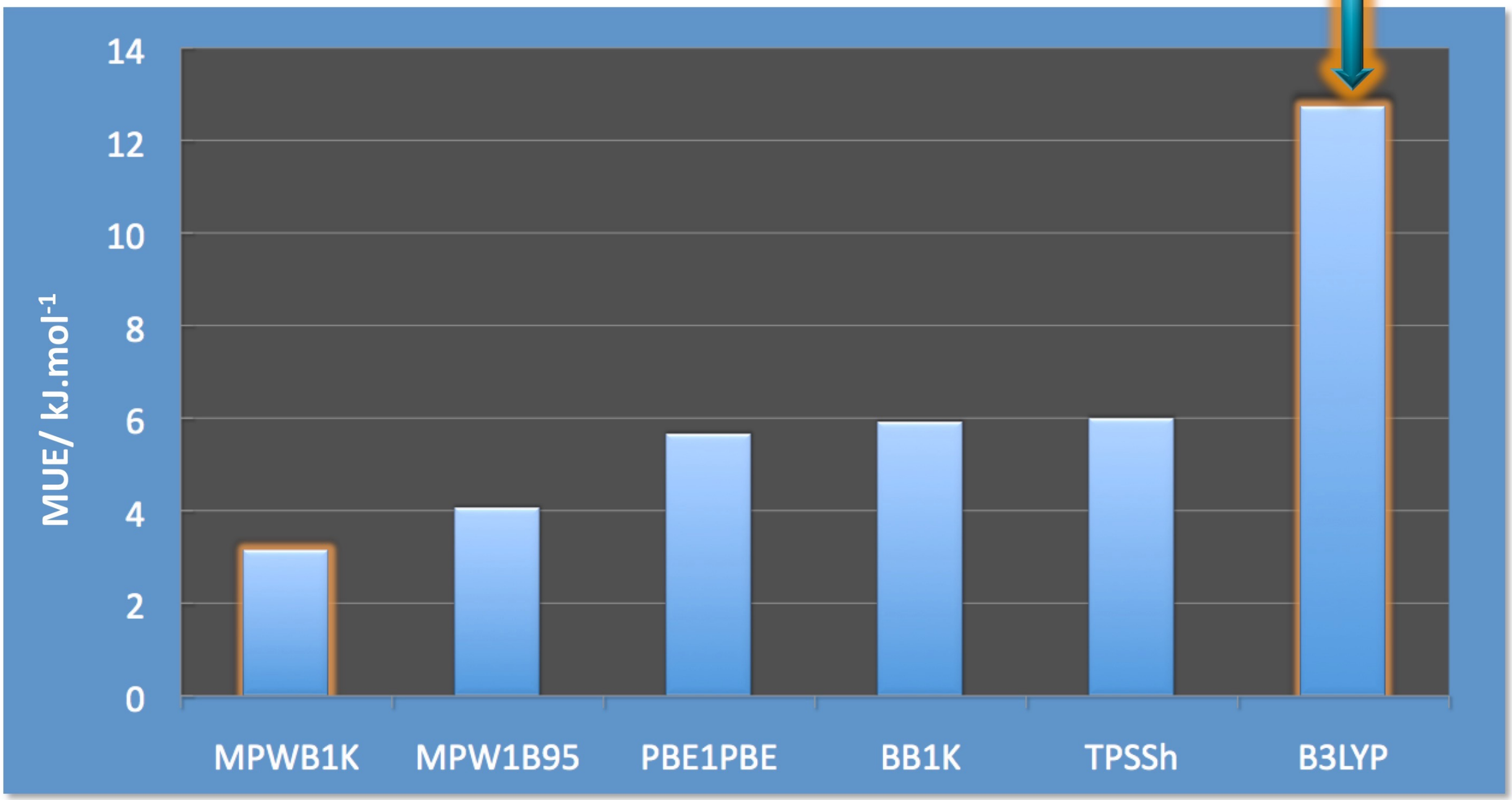
B1B95
BB1K
M06
M06 - 2X
M06 - HF
MPW1B95
MPW1KCIS
MPWB1K
MPWKCIS1K
PBE1KCIS
TPSS1KCIS
TPSSh

LSDA

SVWN3
SVWN5

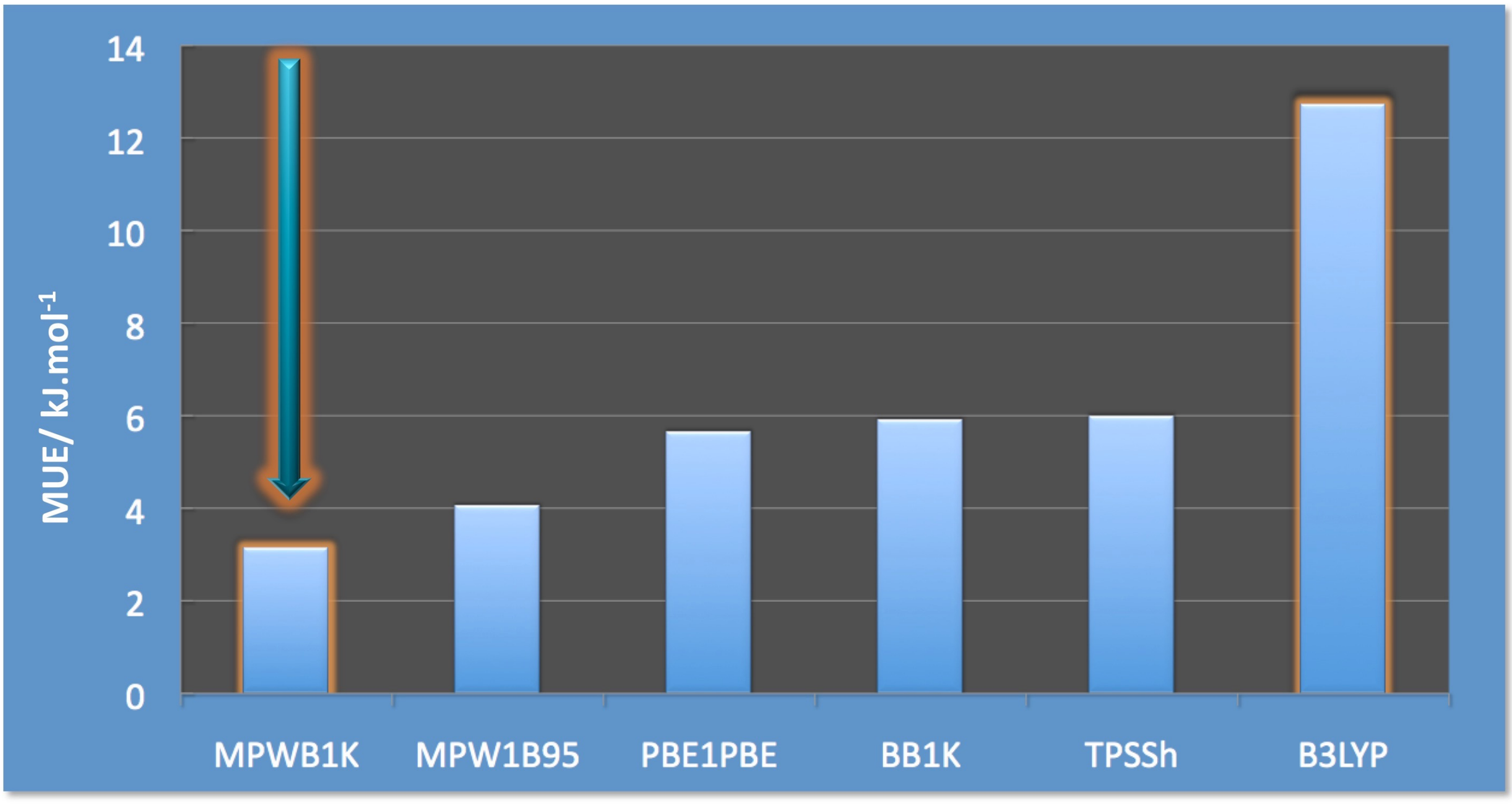
Performance of 50 Density Funcionals

B3LYP: TSs=2,9 kcal/mol; Minima=3,1 kcal/mol; Average=3,0 kcal/mol



Performance of 50 Density Funcionals

MPWB1K: TSs=0,3 kcal/mol; Minima=1,0 kcal/mol; Average=0,7 kcal/mol



Conclusion

- At the end one knows the expectable accuracy of each density functional.

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- One may choose to use **B3LYP** knowing that the barriers and reaction energies will be overestimated by about 3kcal/mol.

Conclusion

- At the end one knows the expectable accuracy of each density functional.

- One may choose to use **B3LYP** knowing that the barriers and reaction energies will be overestimated by about 3kcal/mol.

- One may choose to use MPWB1K knowing that the barriers and reaction energies will be very close to the ones of CCSD(T)/CBS

Conclusions

Further Reading

JPCA, 2007, 111, 10439
JCTC, 2010, 6, 2281
JCTC, 2011, 7, 2059
JCTC, 2011, 7, 3898
JCTC, 10, 4842

Benchmarking Density functionals

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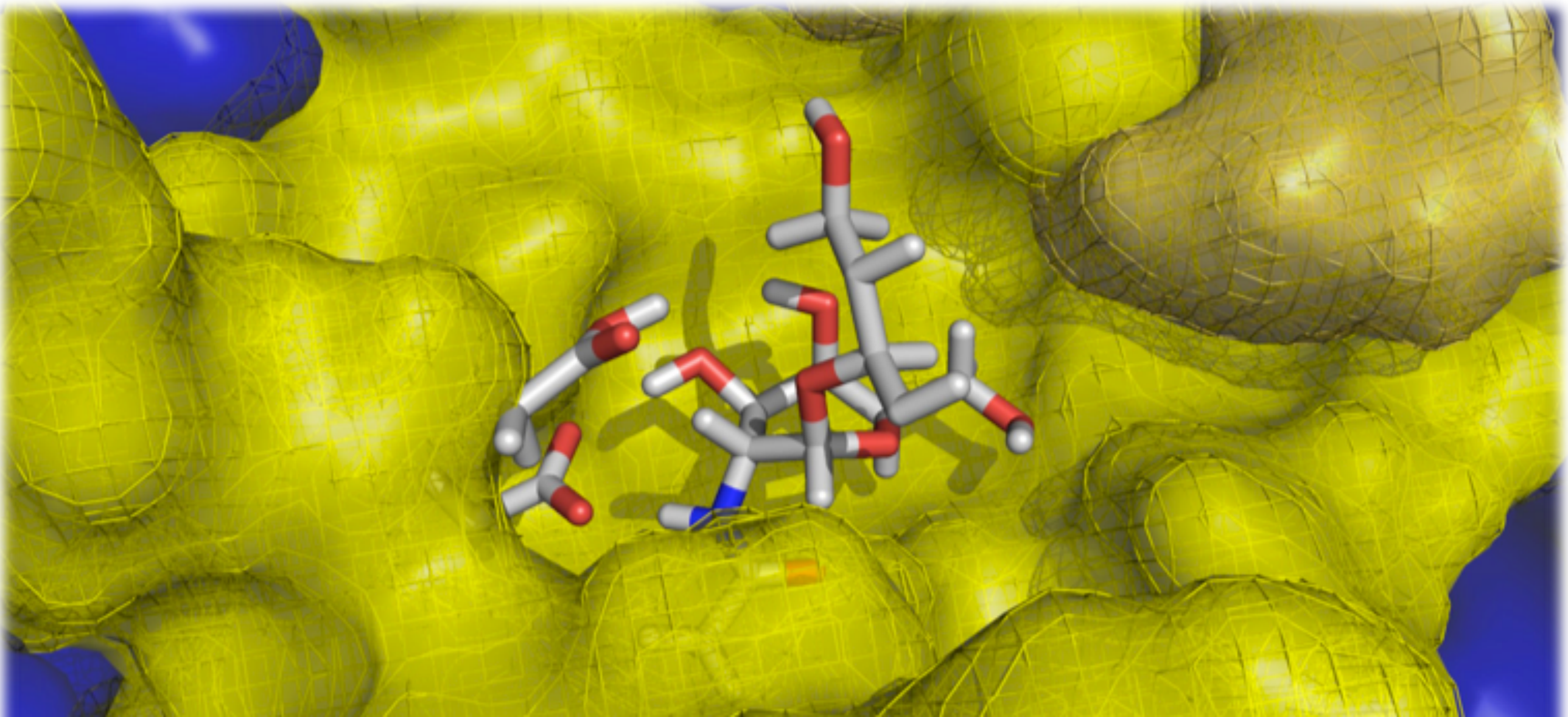

Benchmarking Density Functionals

What about the QM/MM interaction?

JCTC, 6, 2770, 2010.

Benchmarking Density Functionals

What about the QM/MM interaction?

Mechanical Embedding

Electrostatic Embedding

JCTC, 6, 2770, 2010.

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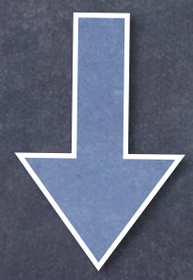

LJ Dispersion
Classical electrostatics

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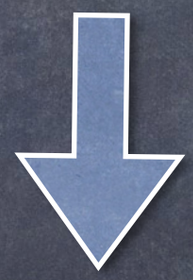

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How reliable are the "QM" point charges?

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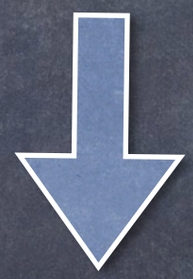

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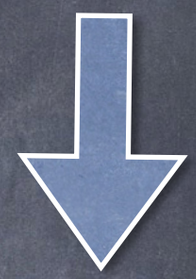

LJ Dispersion
MM charges interact with $\rho_e(r)$

JCTC, 6, 2770, 2010.

Benchmarking Density Functionals

What about the QM/MM interaction?

Mechanical Embedding

LJ Dispersion
Classical electrostatics

How reliable are the "QM" point charges?

Electrostatic Embedding

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MM charges interact with $\rho_e(r)$

>>Time consuming!

JCTC, 6, 2770, 2010.

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Unprecedented chemistry

Exploration of Reactional Space

Nature of energy contributions

Hamiltonian

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Spatial range of the atomic interactions

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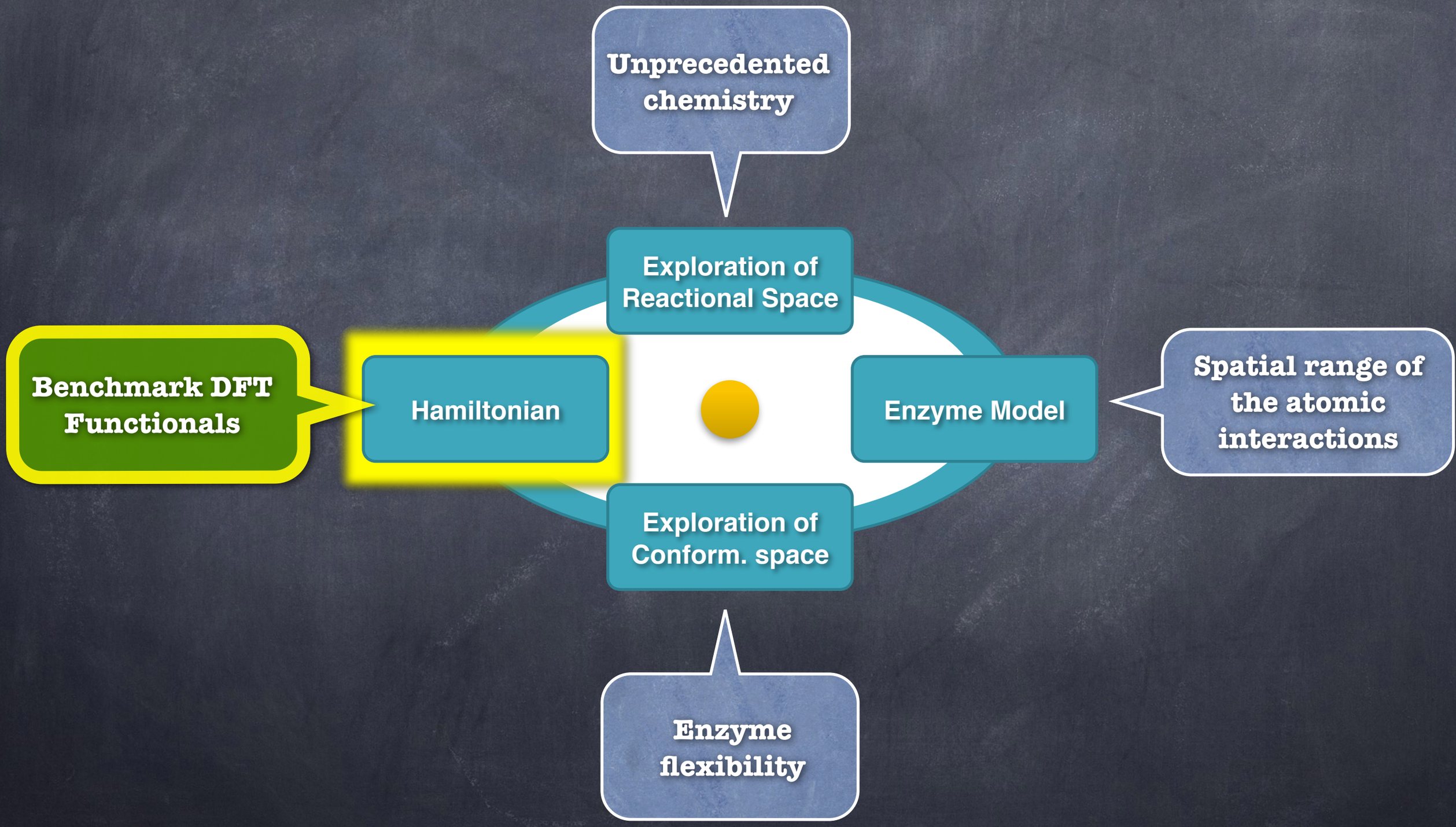

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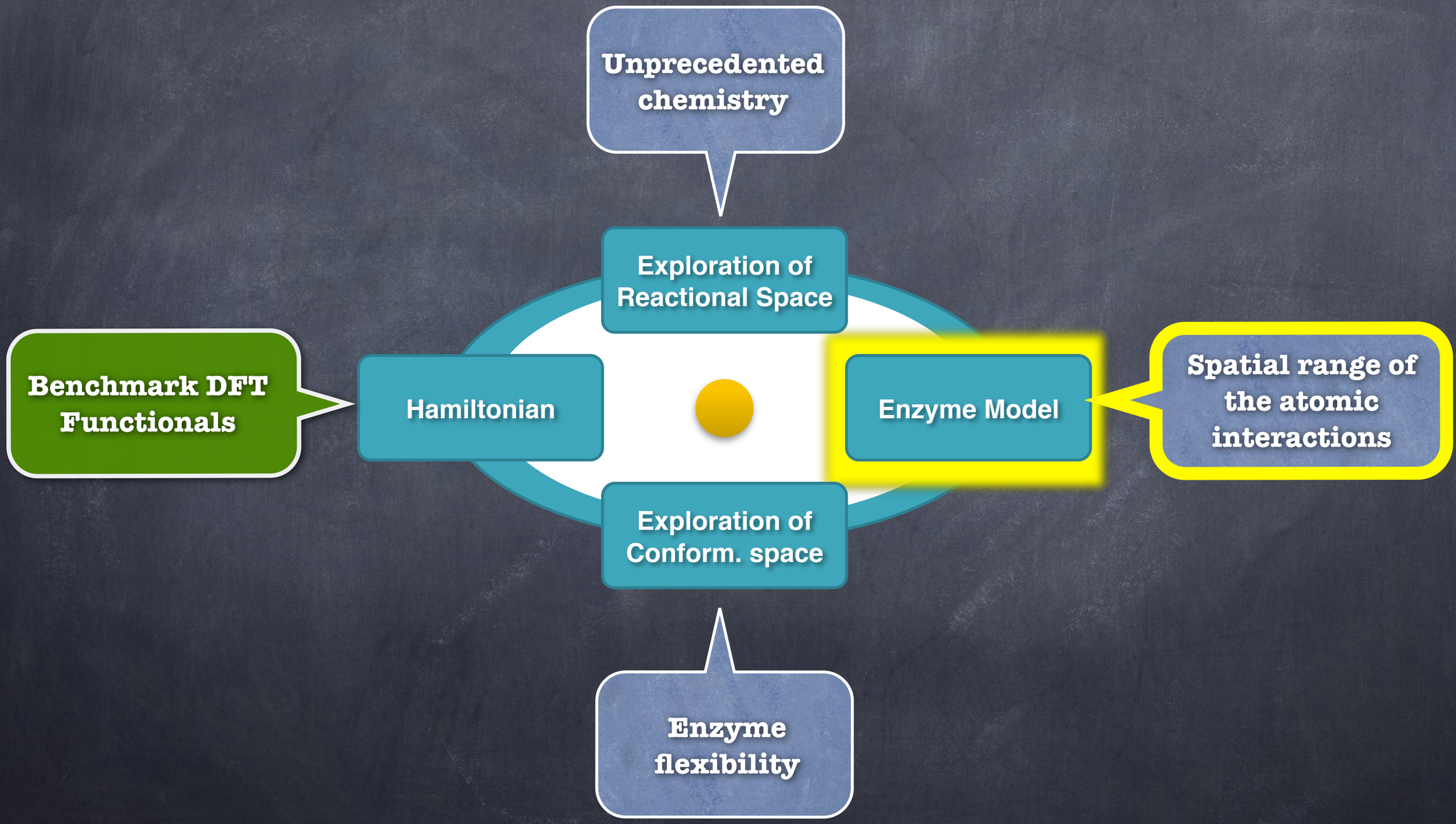

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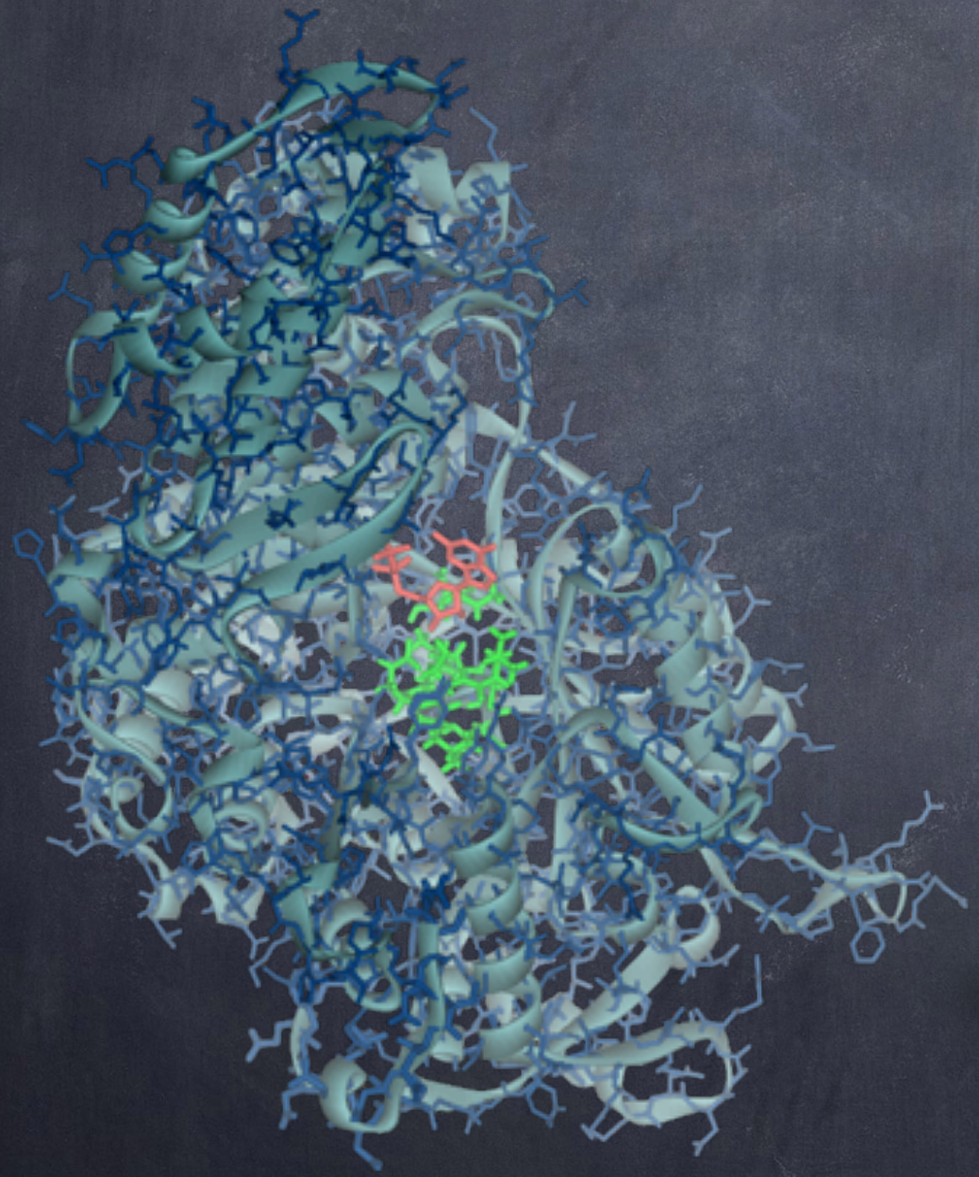
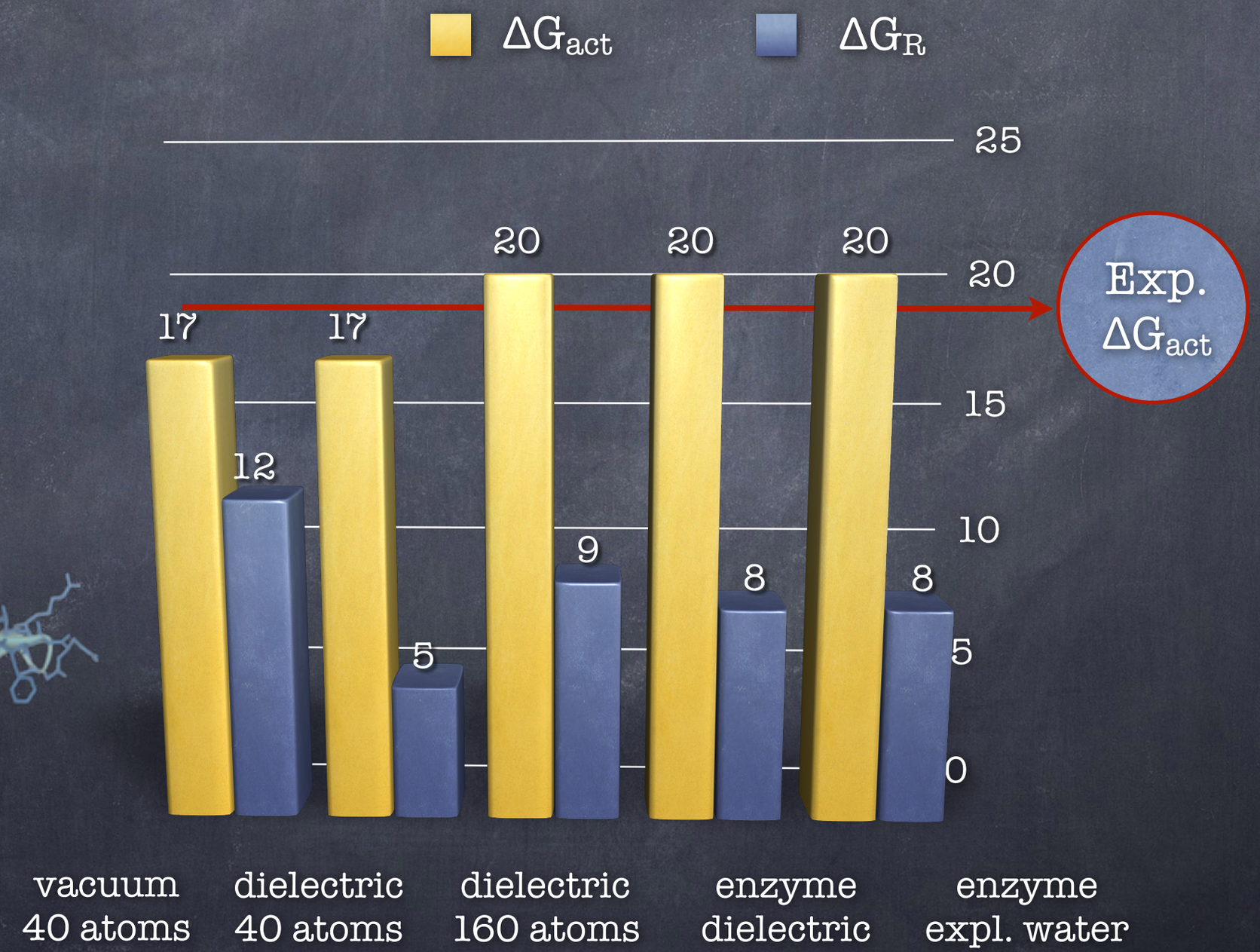
The Molecular Models of Enzymes

Ribonucleotide Reductase

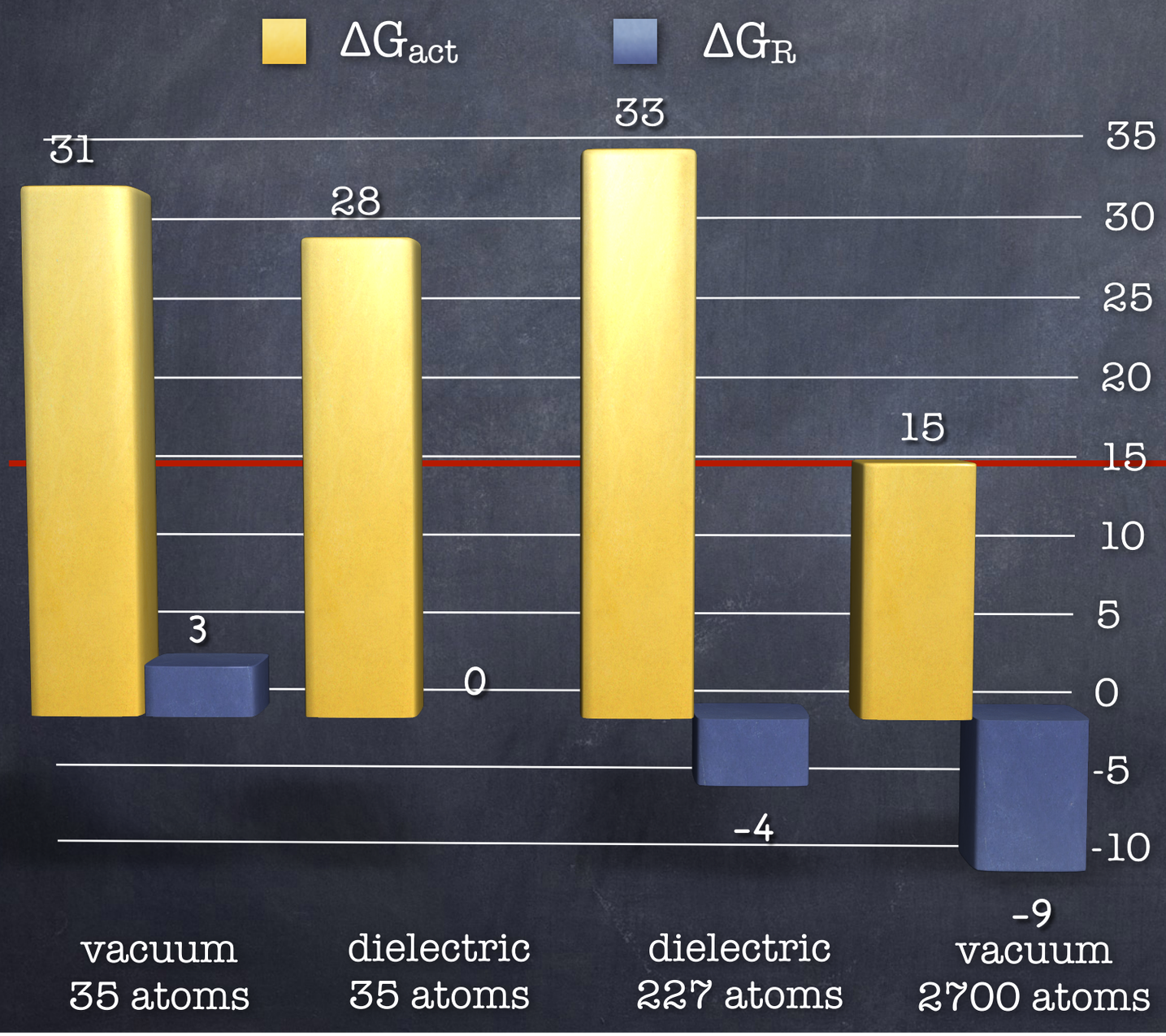


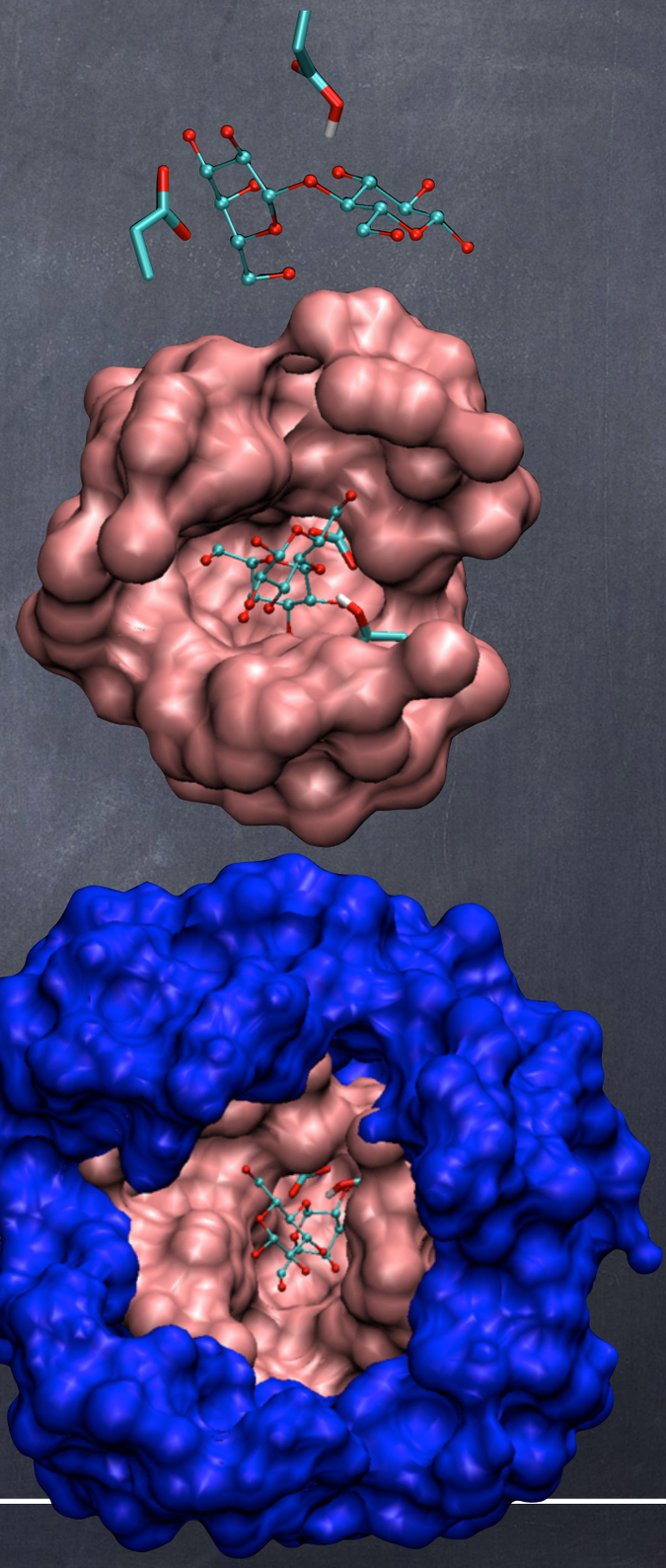


BIOPHYS J., 90, 2109, 2006

The Molecular Models of Enzymes

β-Galactosidase

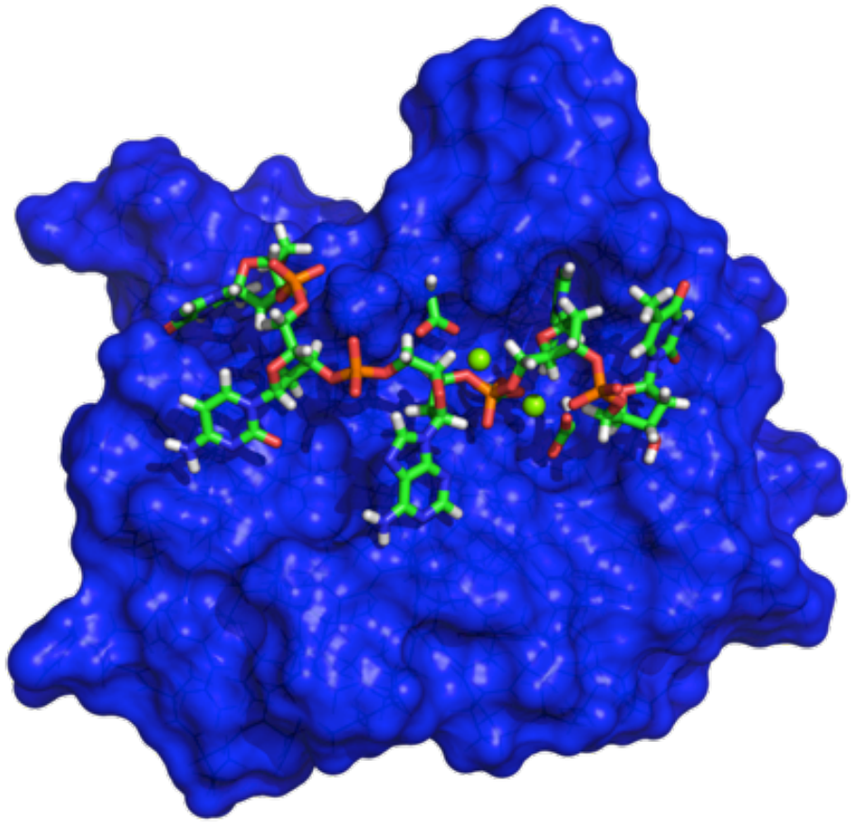




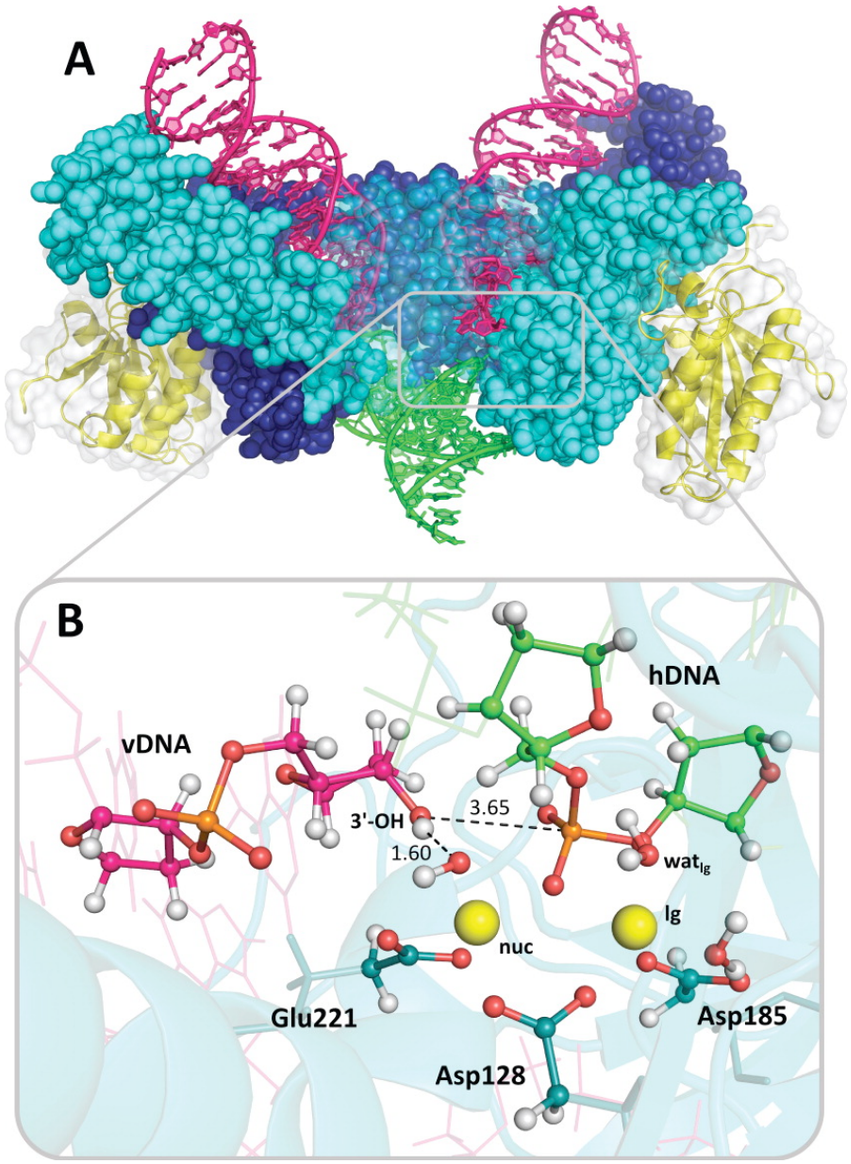
JCTC, 6, 421-433, 2010

The Molecular Models of Enzymes

3’ End Processing
>2.500 atoms



Strand Transfer
>22.000 atoms



JACS, 134, 13436, 2012

JCTC, 10, 5458, 2014

Solutions for the problem

Unprecedented chemistry

Exploration of Reactional Space

Benchmark DFT Functionals

Hamiltonian

Enzyme Model

Elect. Embedd QM/MM, >200 QM atoms

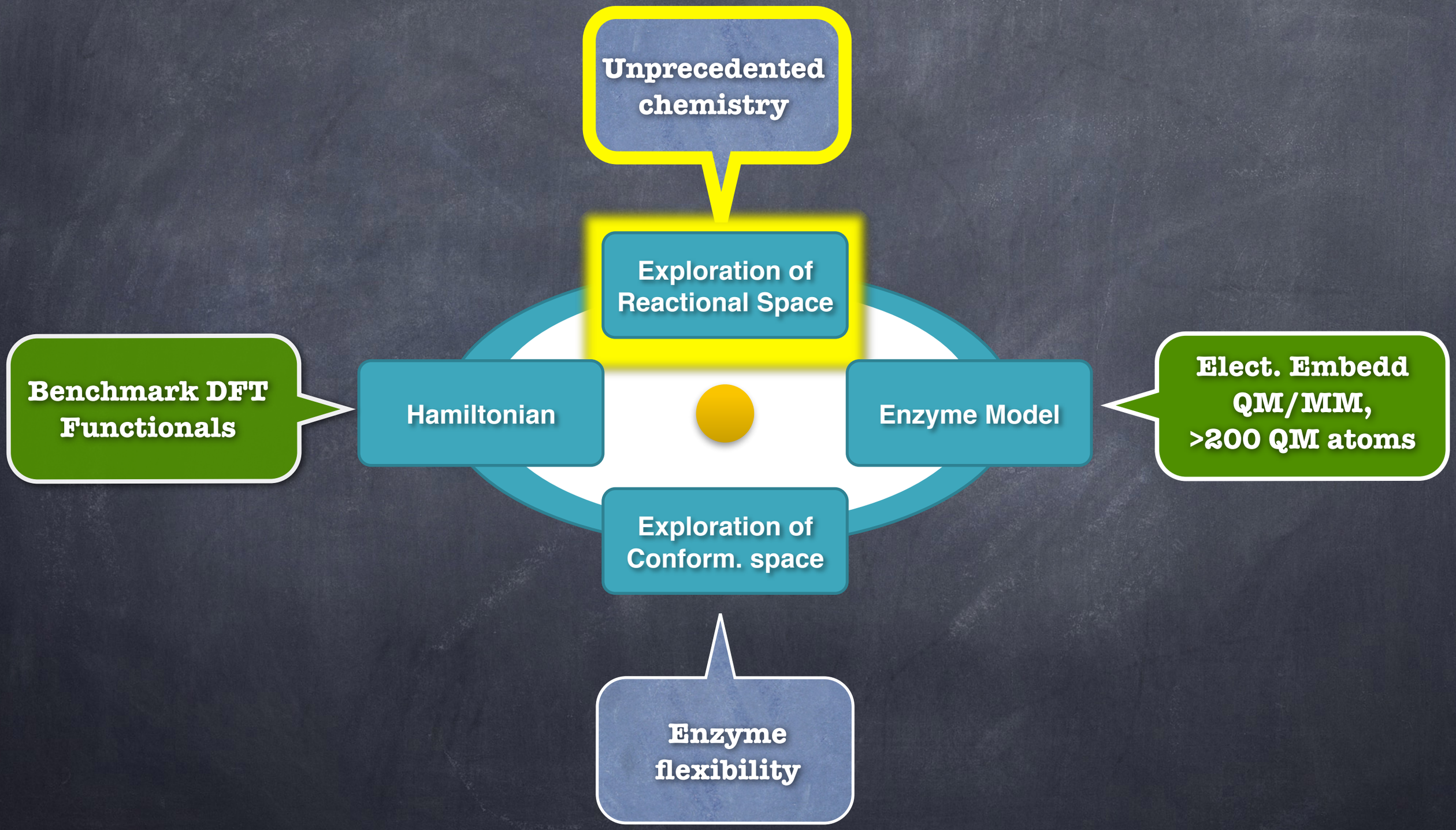
Exploration of Conform. space

Enzyme flexibility

Acc Chem Res, 2008, 689

PCCP, 2012, 12431

Solutions for the problem

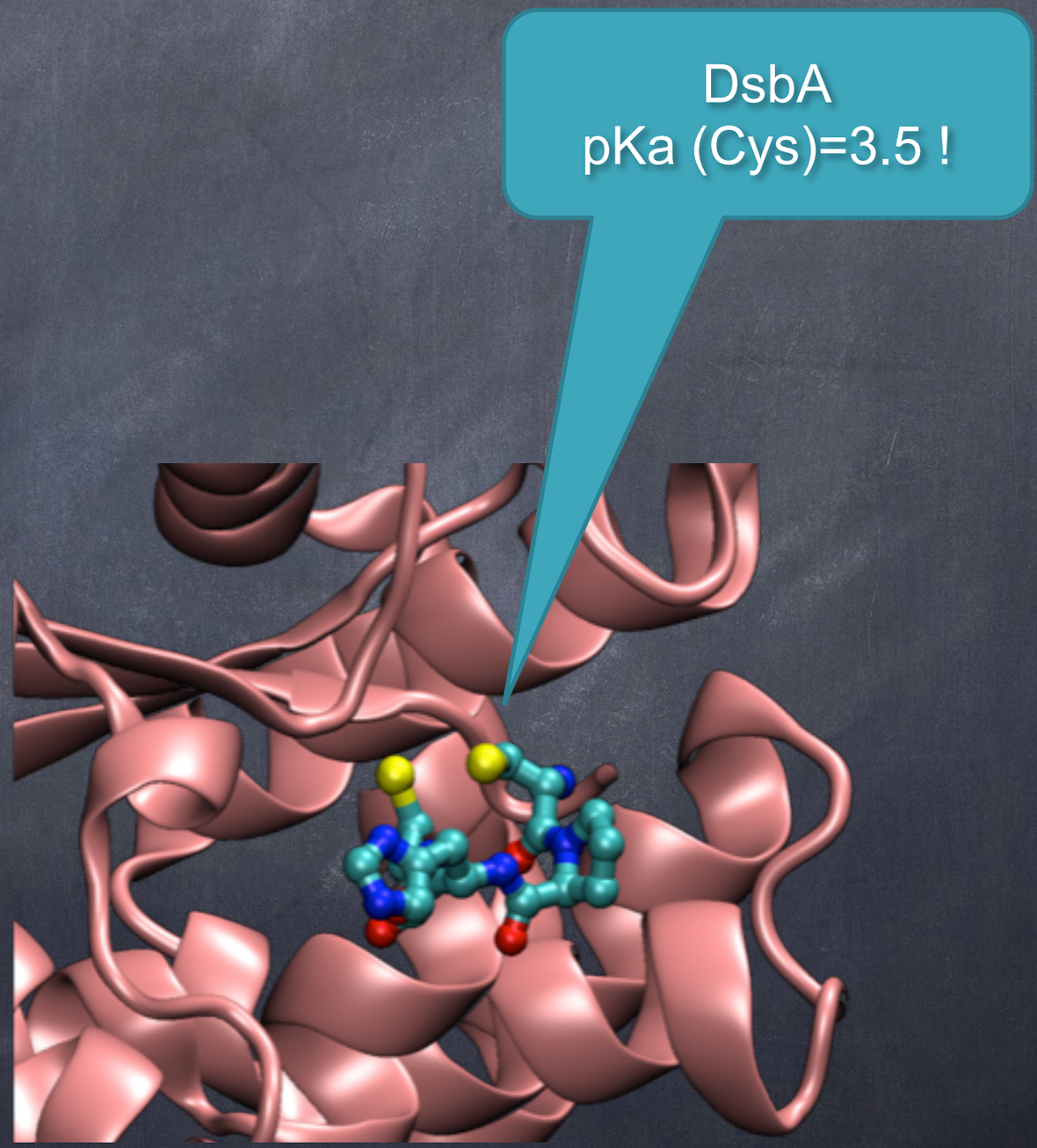


Acc Chem Res, 2008, 689

PCCP, 2012, 12431

Unprecedented Chemistry

- Abnormal pKas

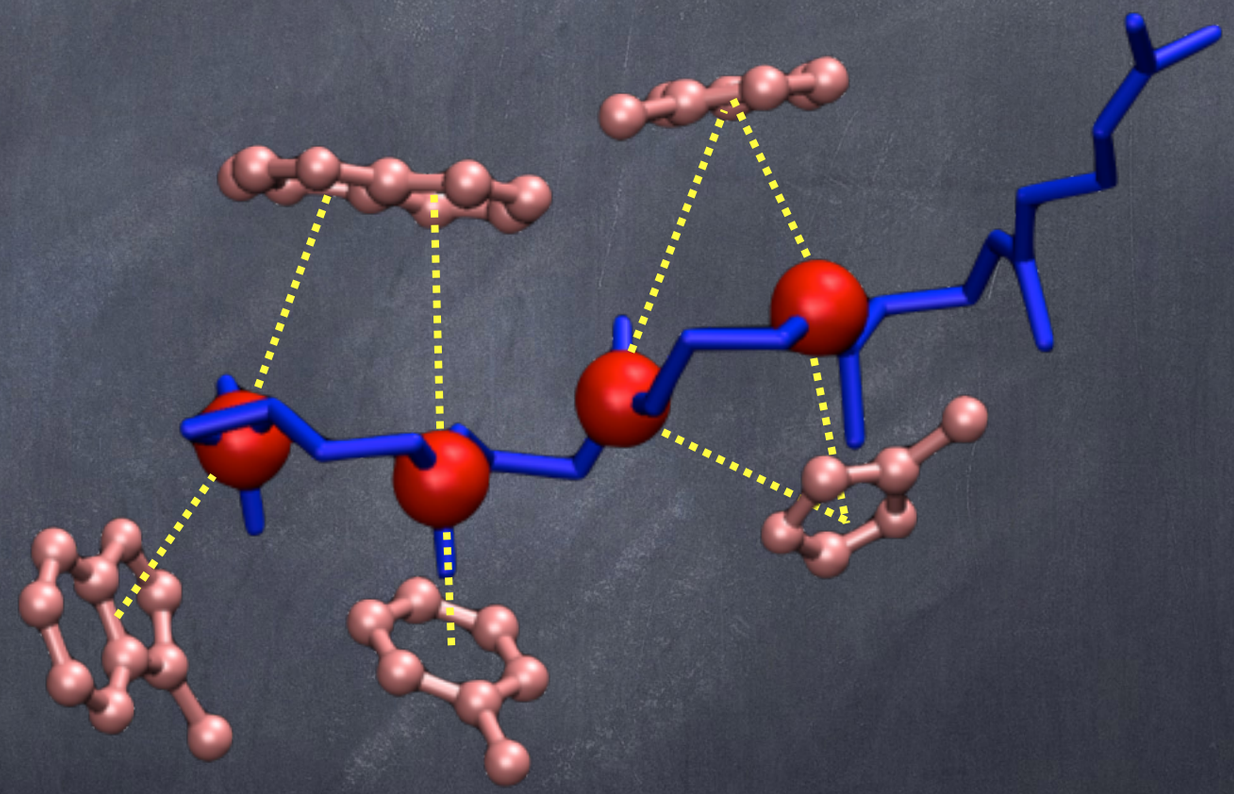


JCC 30, 710, 2009 JPCB, 110, 5758, 2006 JCC, 27, 966-975, 2006 JPCB, 112, 2511, 2008

Unprecedented Chemistry

- Abnormal pKas
- Stable carbocations

Oxidosqualene cyclase



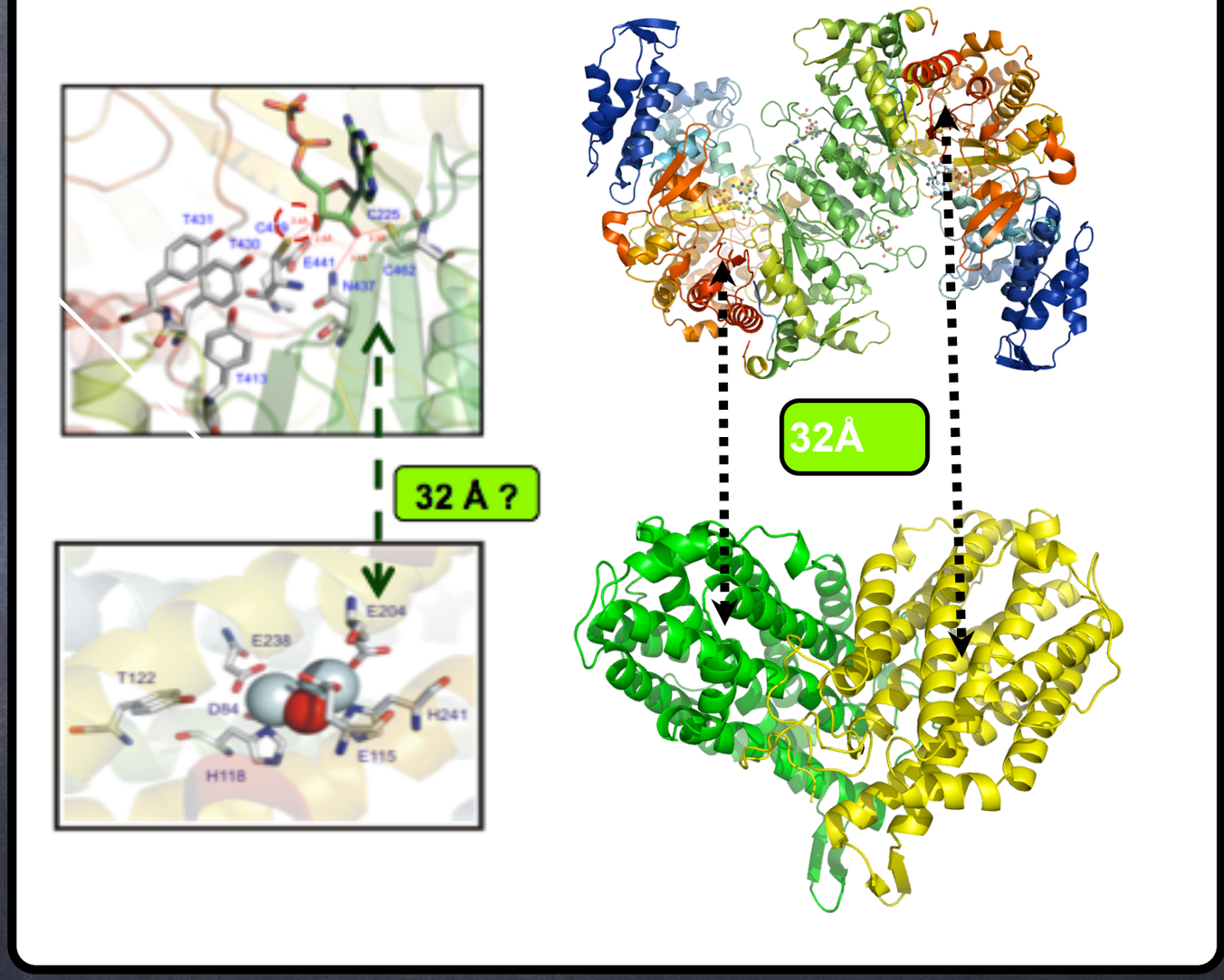
- π-cation interactions stabilize rate-limiting steps

JCTC, 7, 2059, 2011.

Unprecedented Chemistry

- Abnormal pKas
- Stable carbocations
- Long living aliphatic radicals

Ribonucleotide Reductase



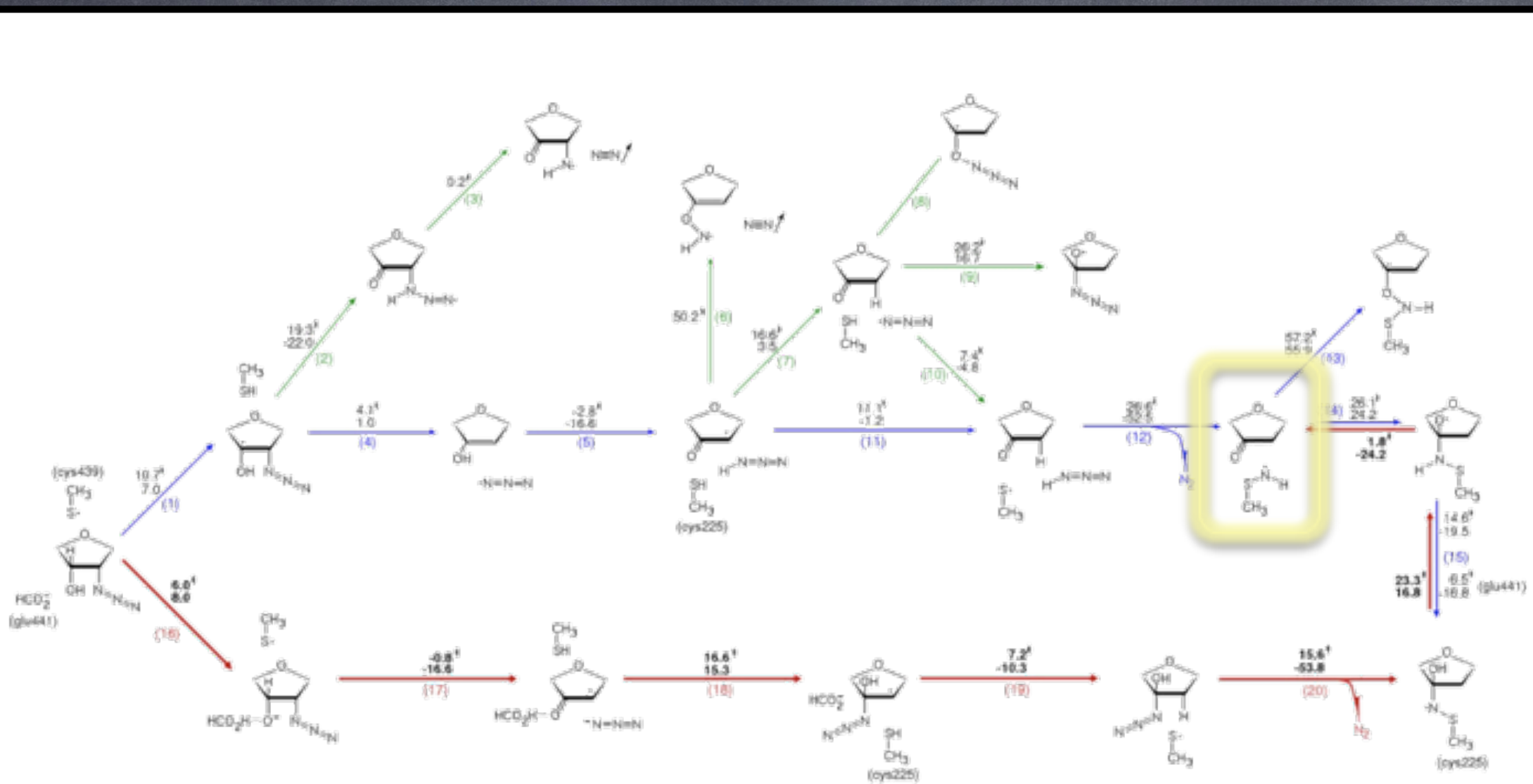
Dehydration through radical mechanism

JACS, 127, 5174, 2005

JCTC, 6, 2770, 2010

Chemistry, 13, 8507, 2007

The diversity of the Chemical Space



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JCTC, 6, 2770, 2010

CHEMISTRY, 13, 8507, 2007

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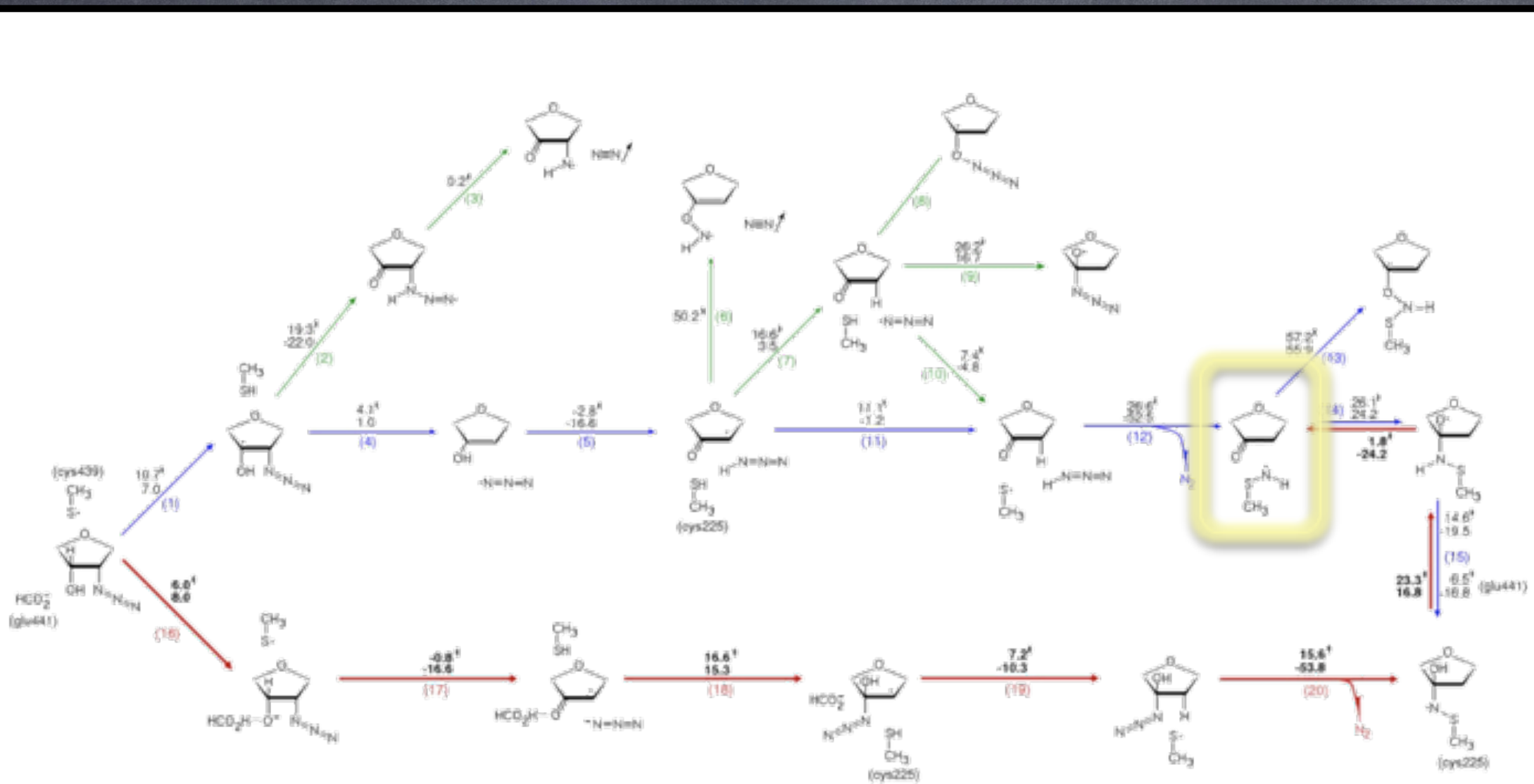

Systematic testing of mechanistic hypotheses

JACS, 127, 5174, 2005

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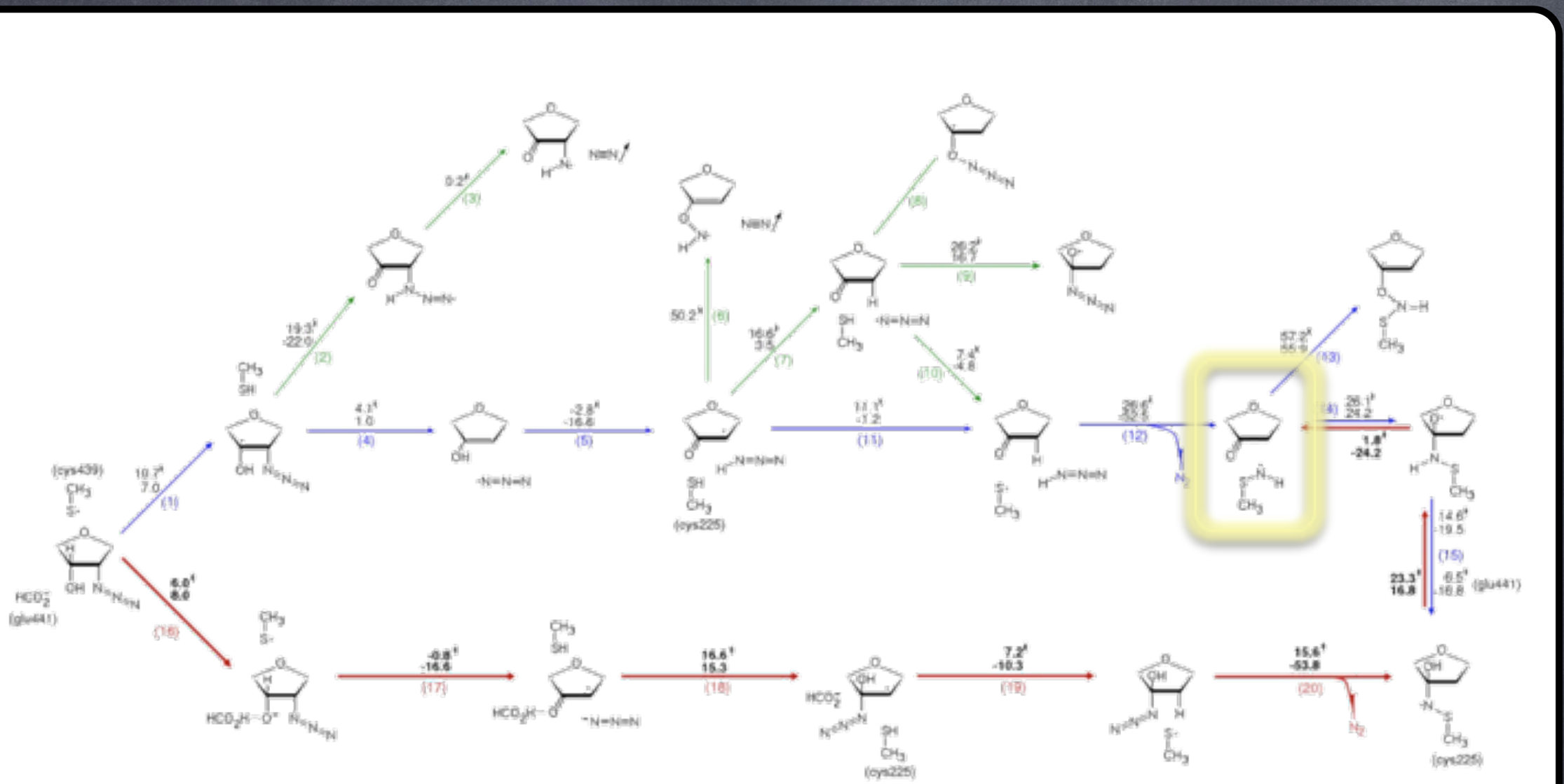
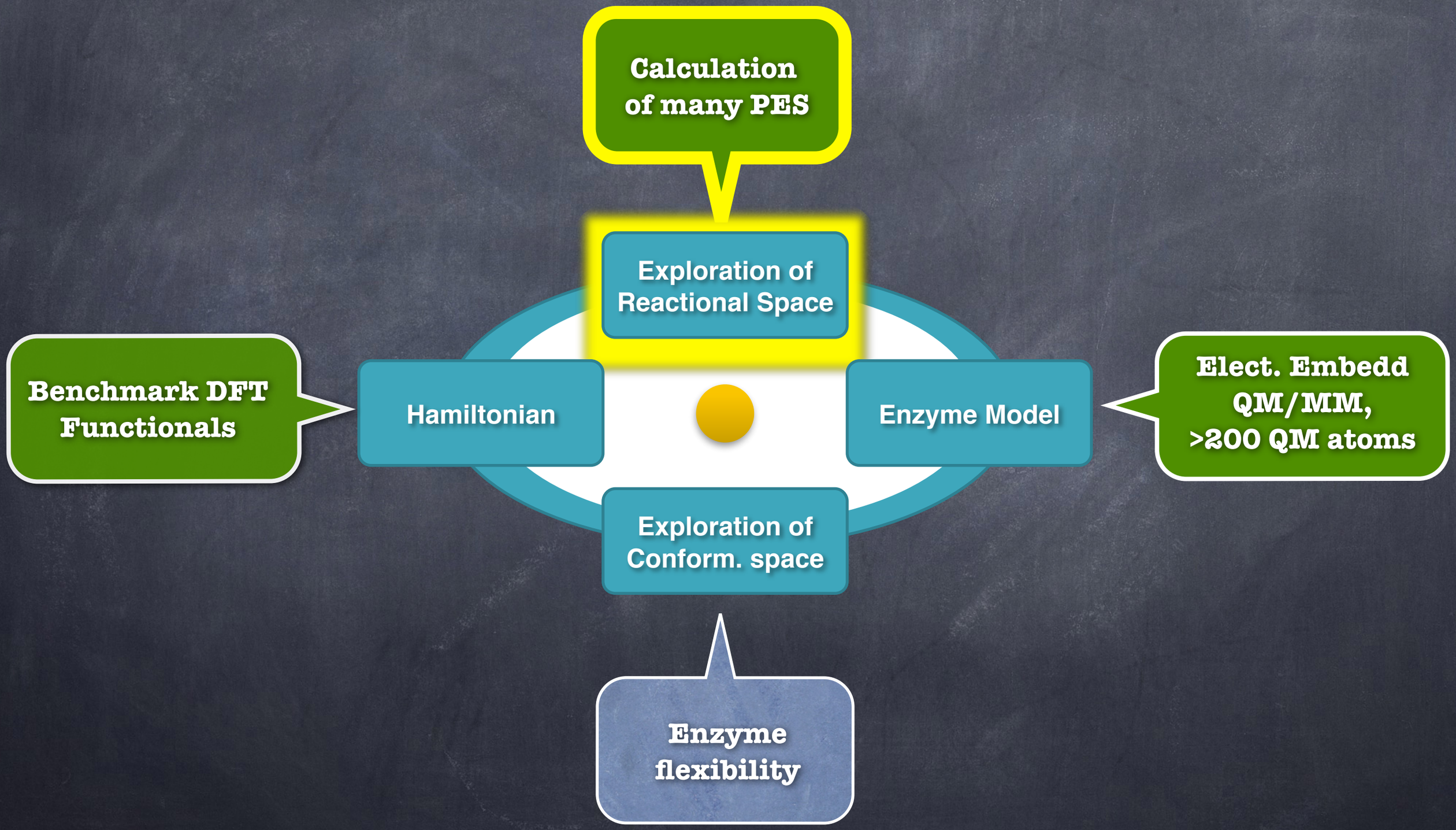


Systematic testing of mechanistic hypotheses

Cross-check with all available exp data

JACS, 127, 5174, 2005 JCTC, 6, 2770, 2010 CHEMISTRY, 13, 8507, 2007

Solutions for the problem



Acc Chem Res, 2008, 689

PCCP, 2012, 12431

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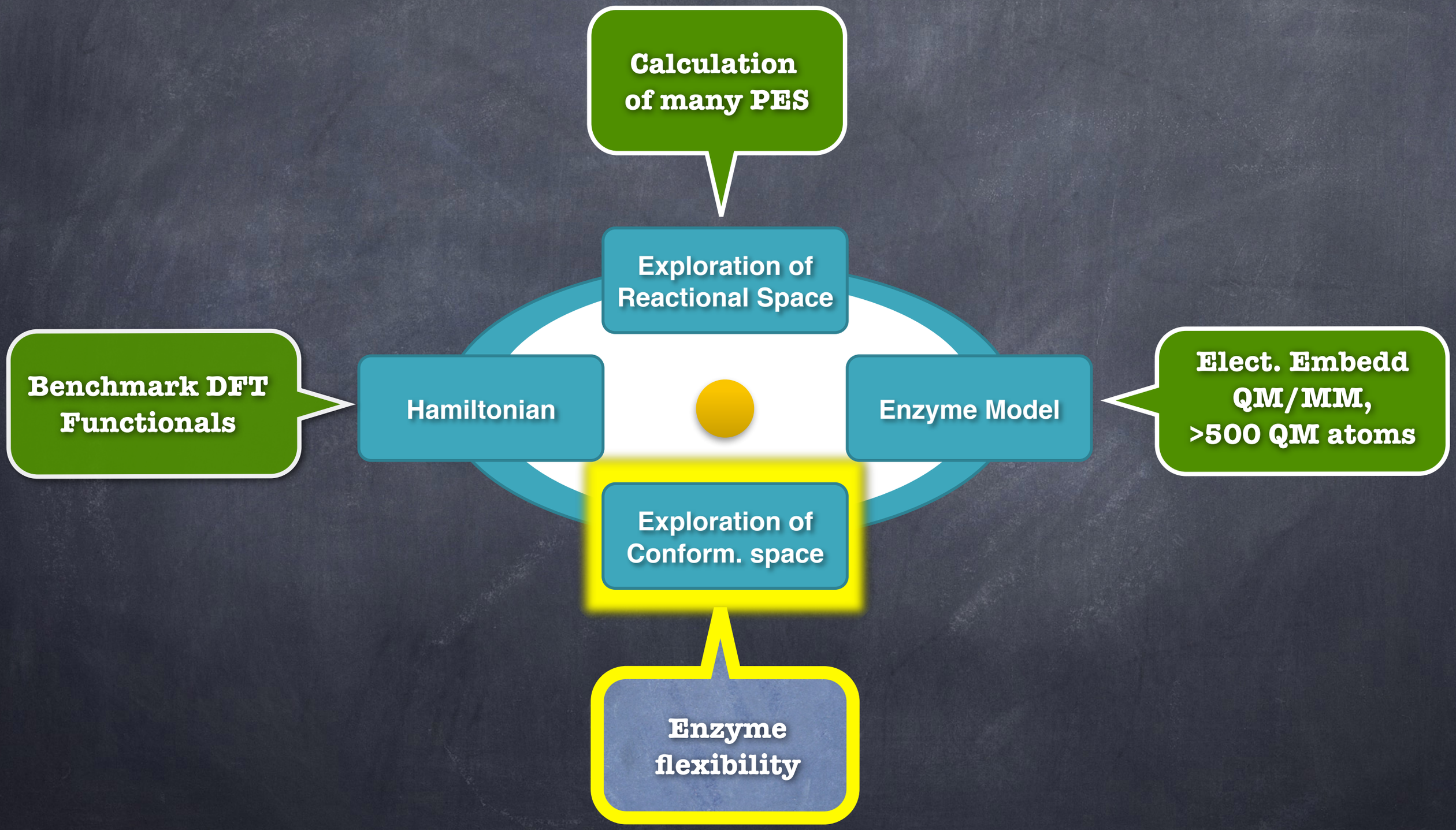

Acc Chem Res, 2008, 689

PCCP, 2012, 12431

Enzyme conformations

Two ≠ problems

Enzyme conformations

Two ≠ problems

Generating relevant uncorrelated conformations

Enzyme conformations

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Opt=CPMD=QMMM MD
CMD better

Generating relevant uncorrelated conformations

Other methods may unfold the enzyme

Enzyme conformations

Two ≠ problems

Opt=CPMD=QMMM MD
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Generating relevant
uncorrelated conformations

Other methods may
unfold the enzyme

Averaging conformations
or
checking conformation effect

Enzyme conformations

Two ≠ problems

Generating relevant uncorrelated conformations

Opt=CPMD=QMMM MD
CMD better

Other methods may unfold the enzyme

Averaging conformations
or
checking conformation effect

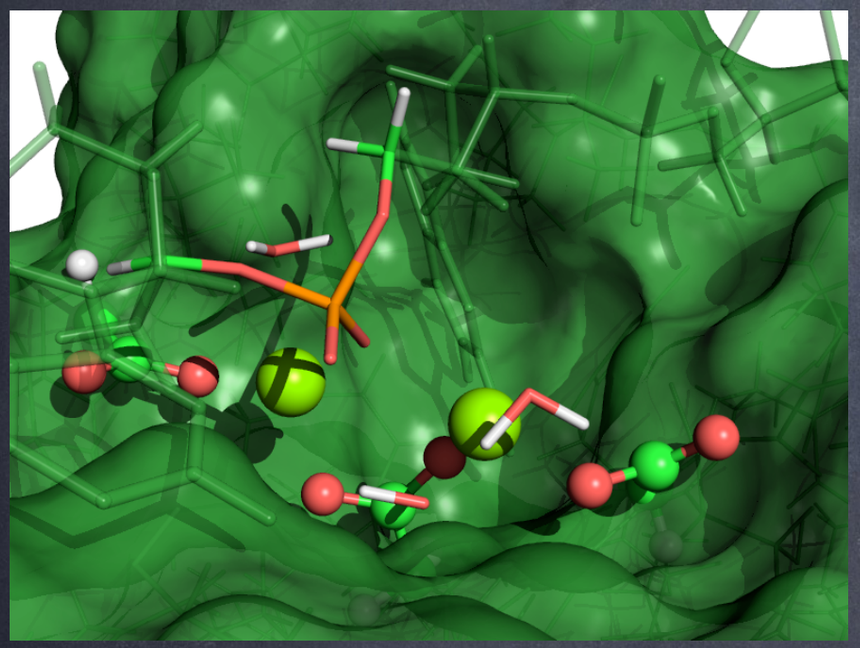
ab initio/DFT
only checks effects

SE methods
average within their accuracy

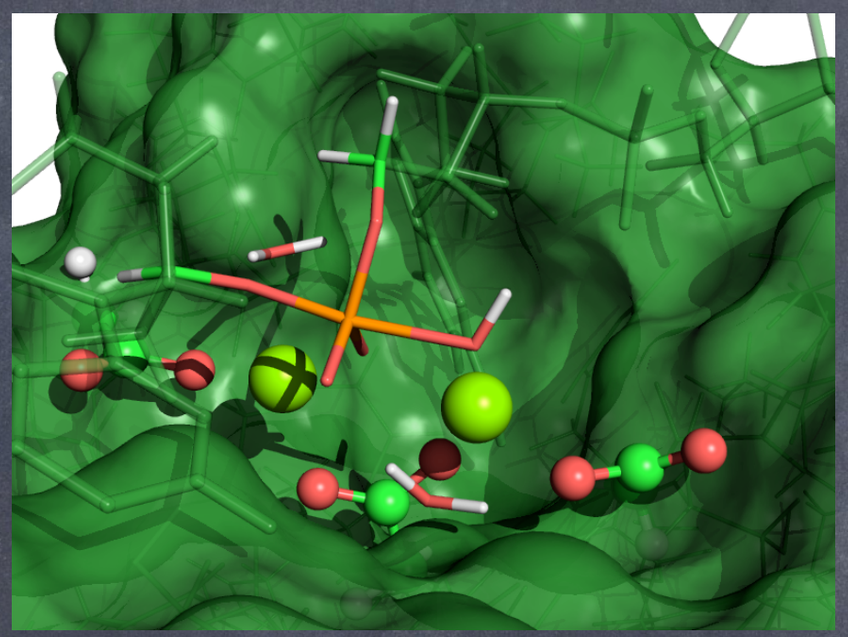
Enzyme conformations

Hydrolysis of phosphodiester bonds

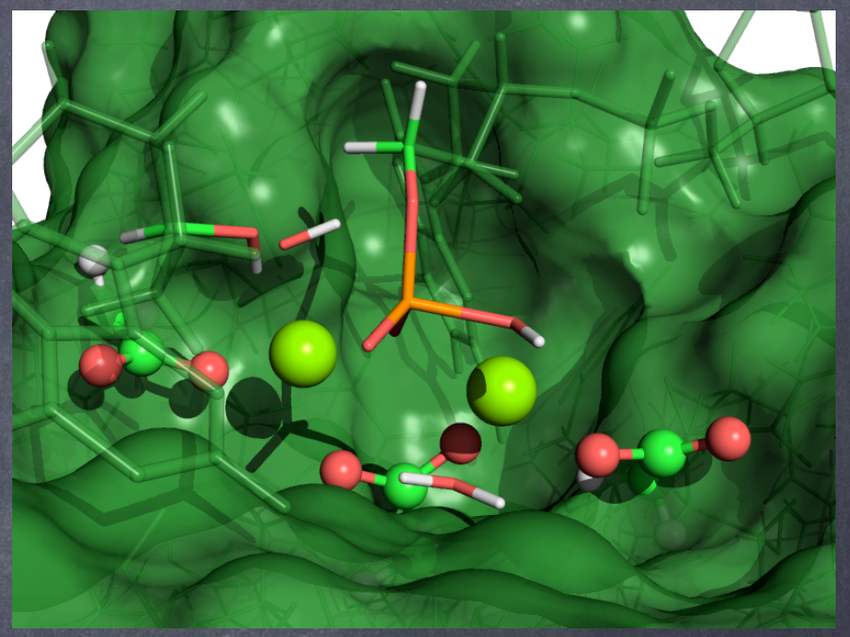
Reactants

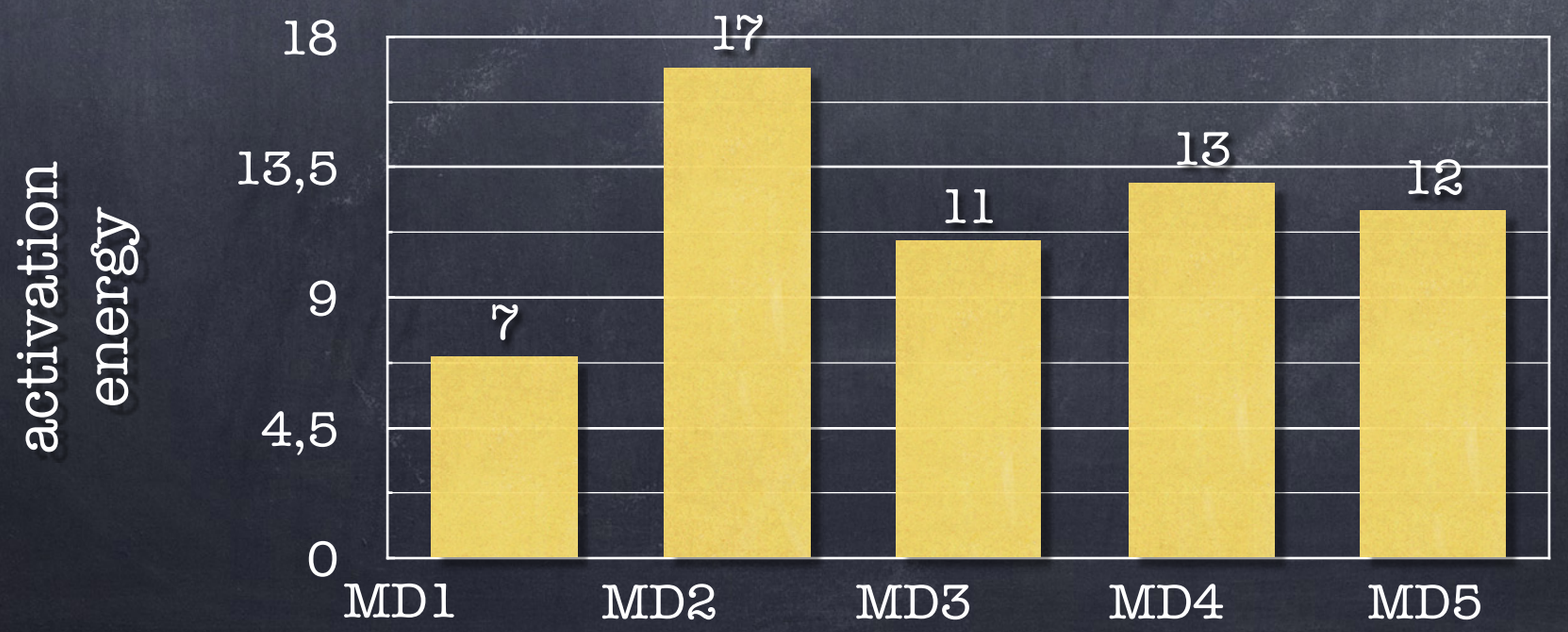


TS



Products



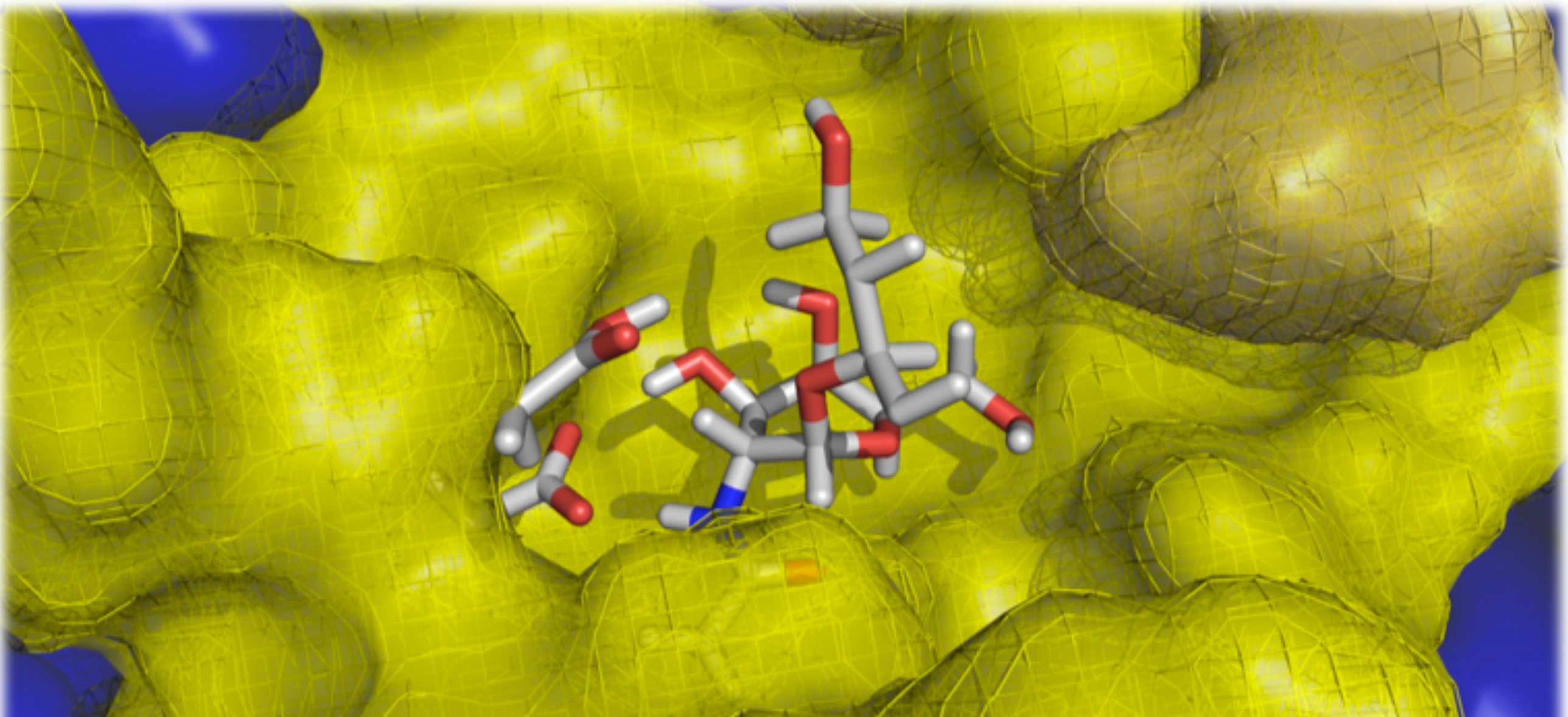


Influence of Enzyme Conformation on Kinetics

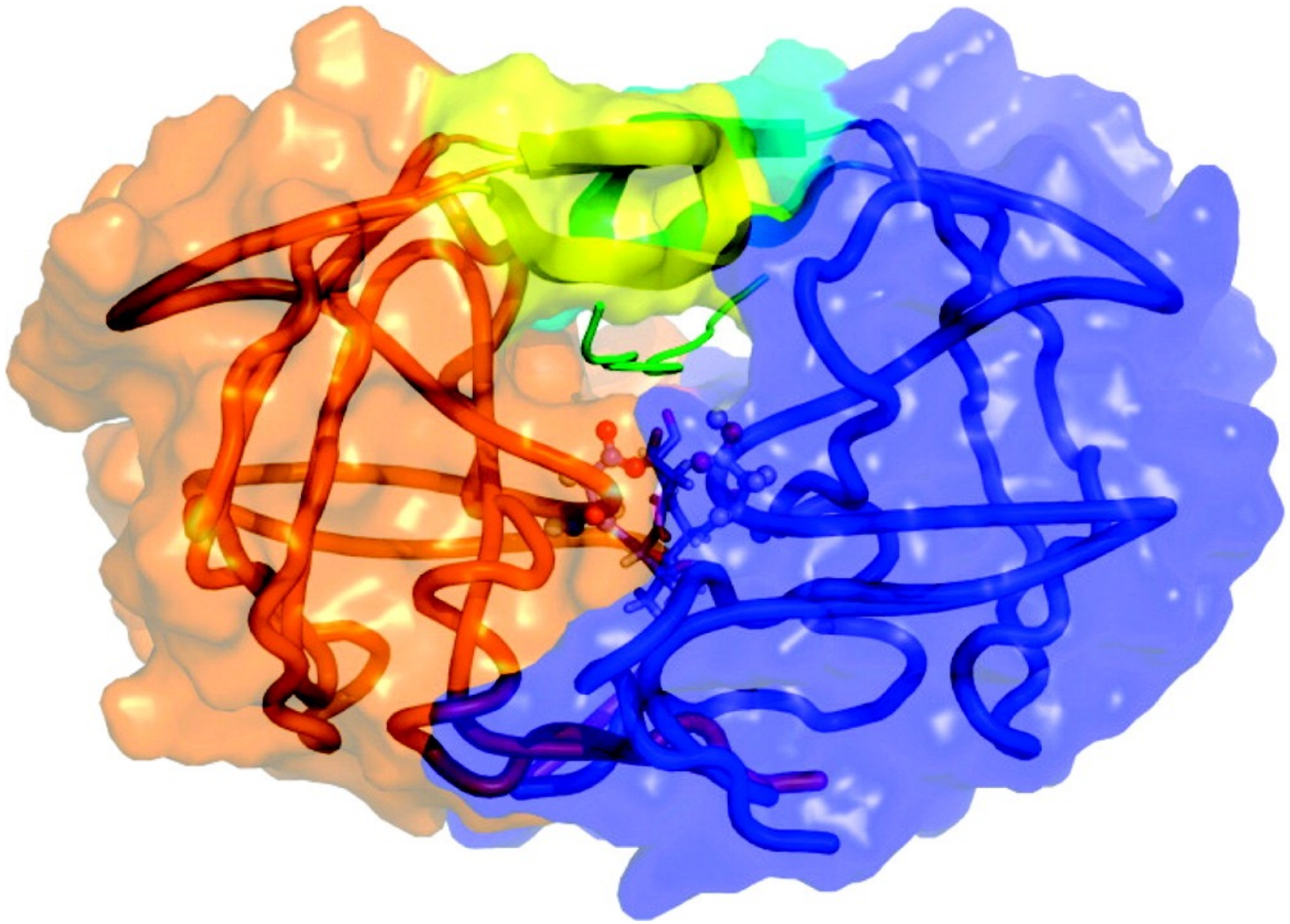
Pedro Alexandrino Fernandes,

Dep. Chemistry & Biochemistry, University of Porto, Portugal

pedro.fernandes@fc.up.pt



Example: HIV-1 Protease



Example: HIV-1 Protease

QM/MM model.
QM layer: 30 atoms.
MM layer: 3232 atoms.
Electrostatic embedding in all calculations.

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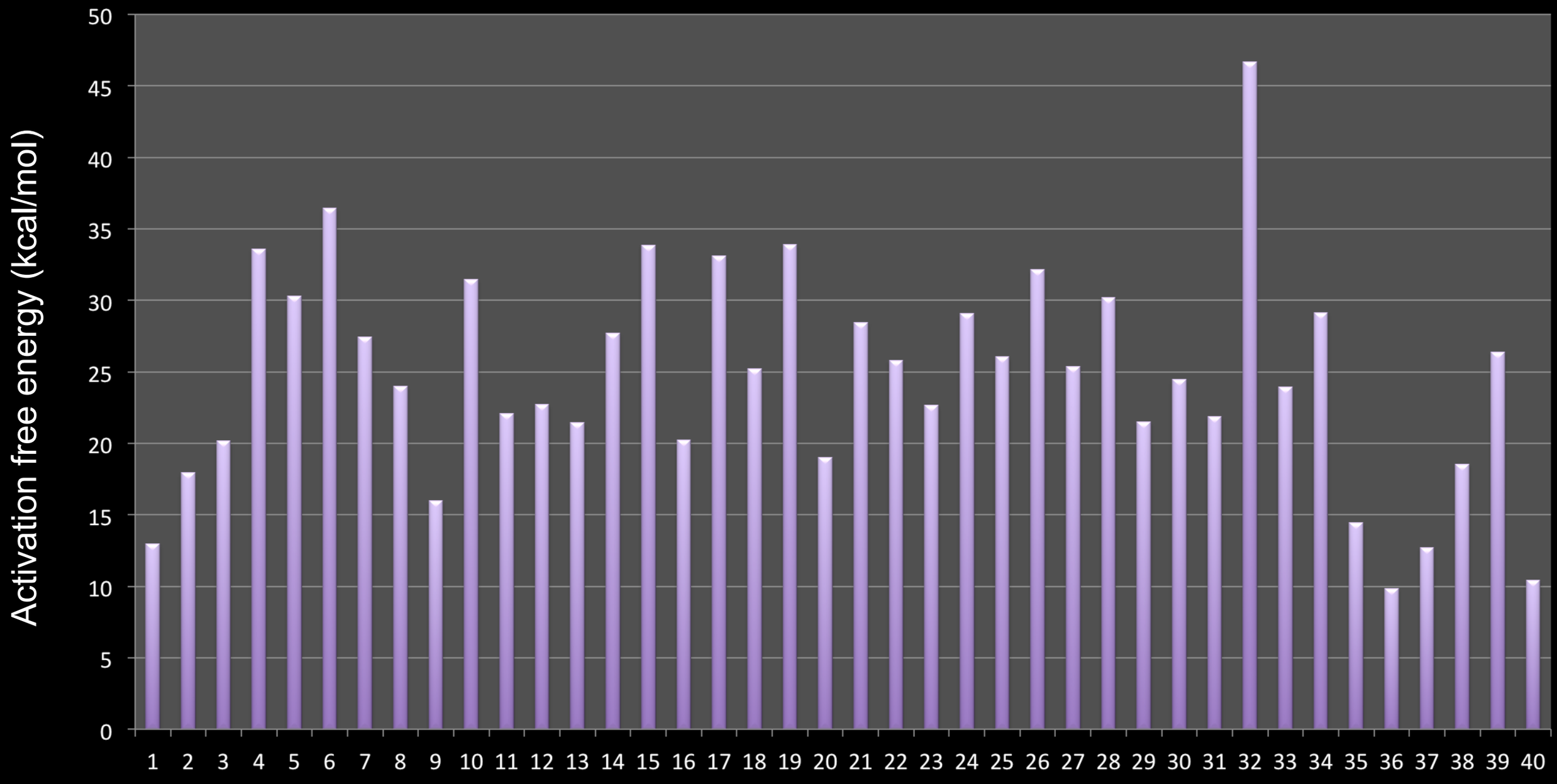
QM/MM model.
QM layer: 30 atoms.
MM layer: 3232 atoms.
Electrostatic embedding in all calculations.

Extract coordinates every 2 ns of several MD simulations.
Optimize the reactants state.
Scan the distance between the nucleophile and the amide carbon.
Optimize the maximum to a TS, without any constraints.
Frequency calculation.
IRC to identify the reactants.
Optimize the reactants again.

Influence of Enzyme Conformation on Kinetics

Activation free energy for 40 different enzyme conformations



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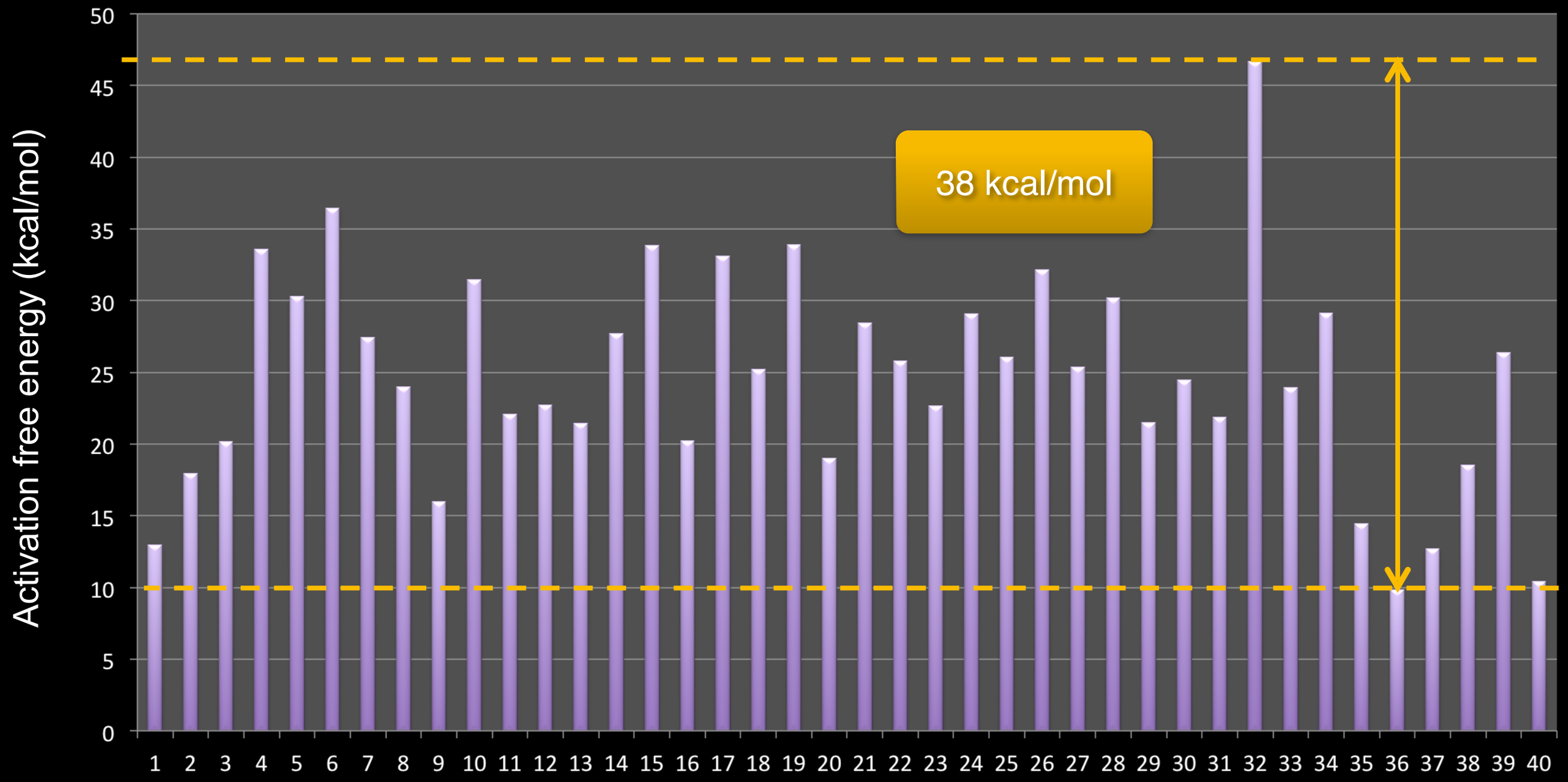
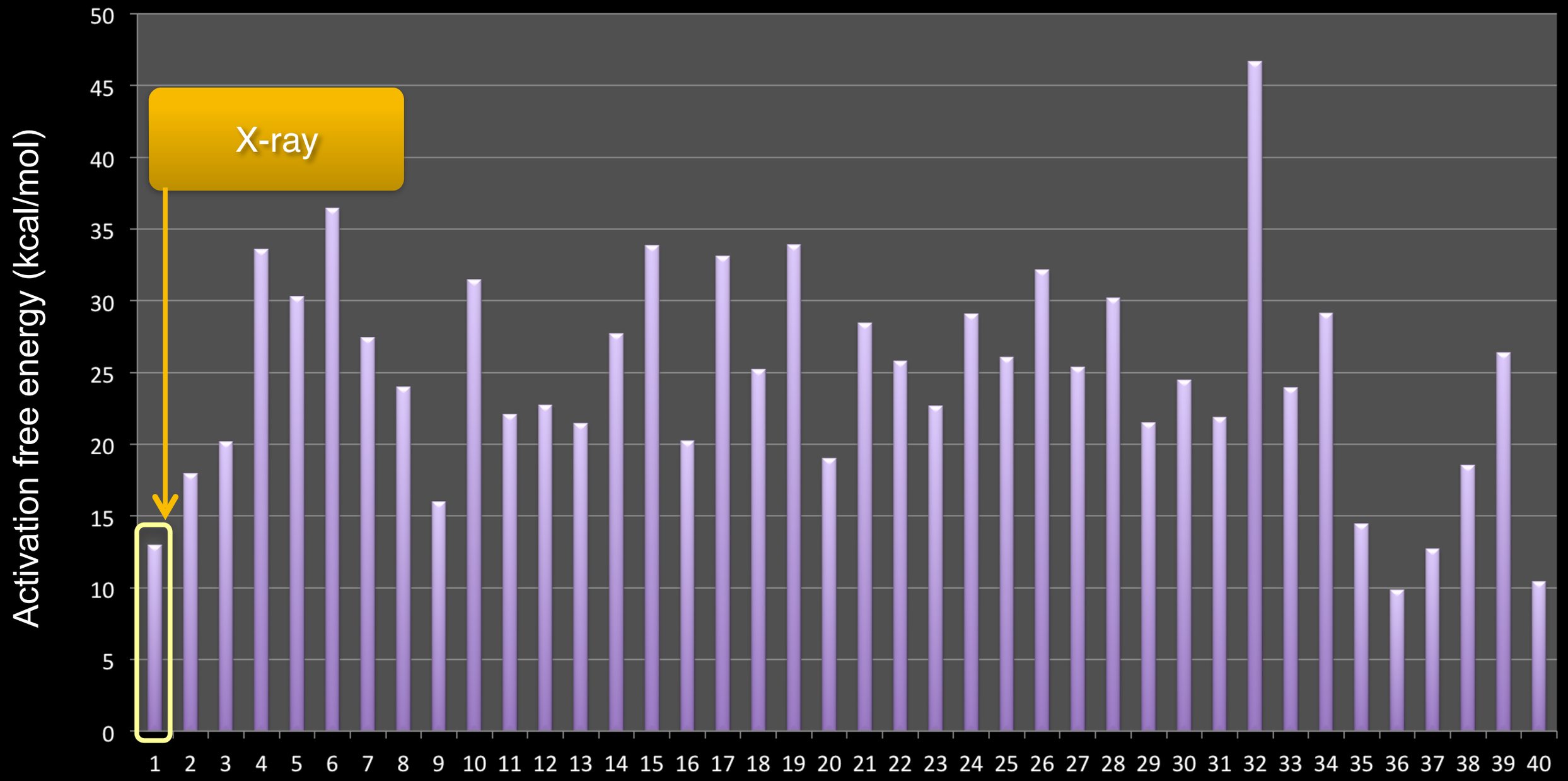


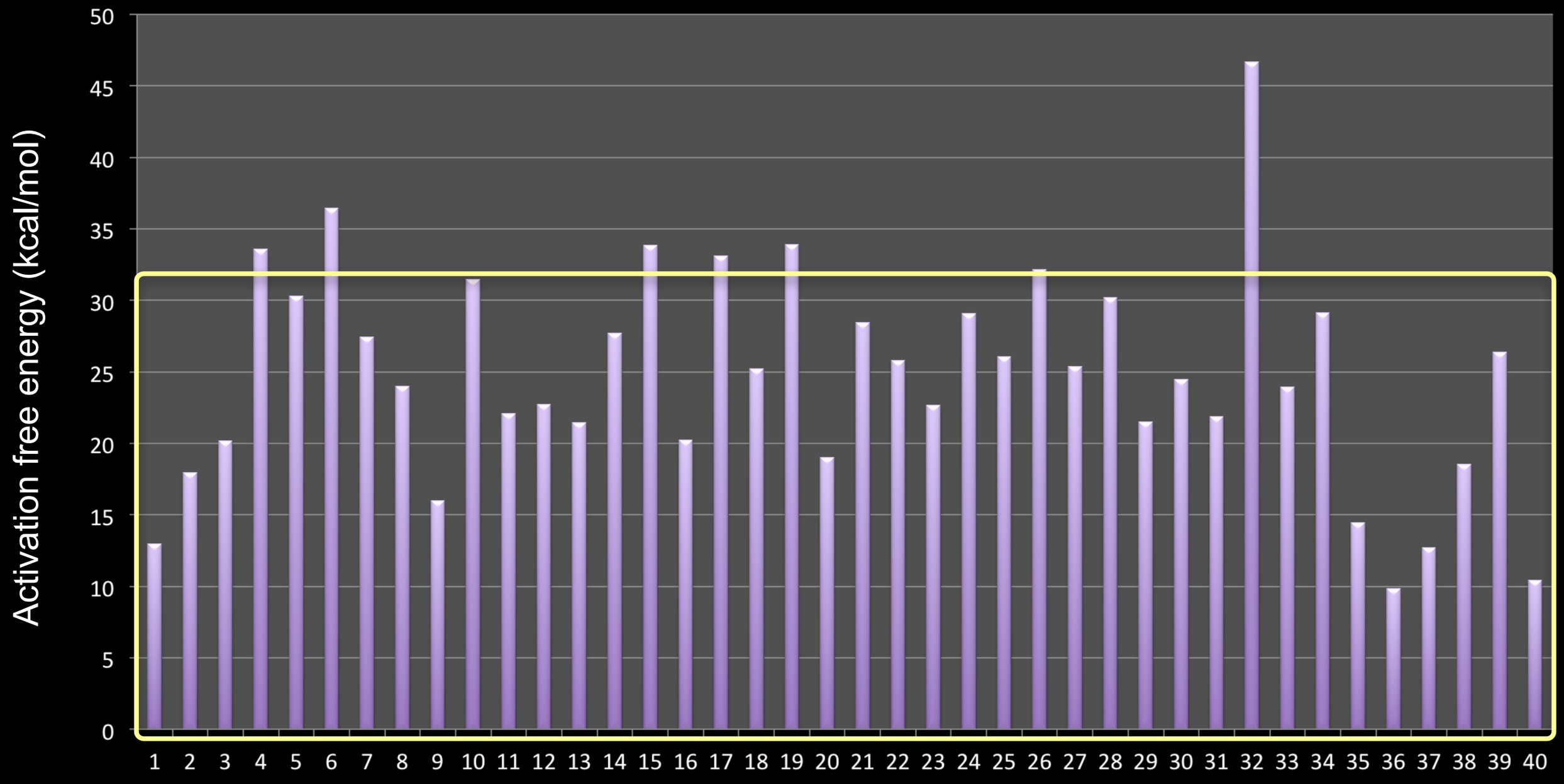
Influence of Enzyme Conformation on Kinetics

$\Delta G_{X\text{-}ray}$=13.4 kcal/mol <ΔG>=9 kcal/mol



Influence of Enzyme Conformation on Kinetics

ΔG > ~34 kcal/mol obtained with a different chemical mechanism



Why ΔG_{act} fluctuates so much?

Origin of the fluctuations?

Why ΔG_{act} fluctuates so much?

Origin of the fluctuations?

Differences at the active site ?

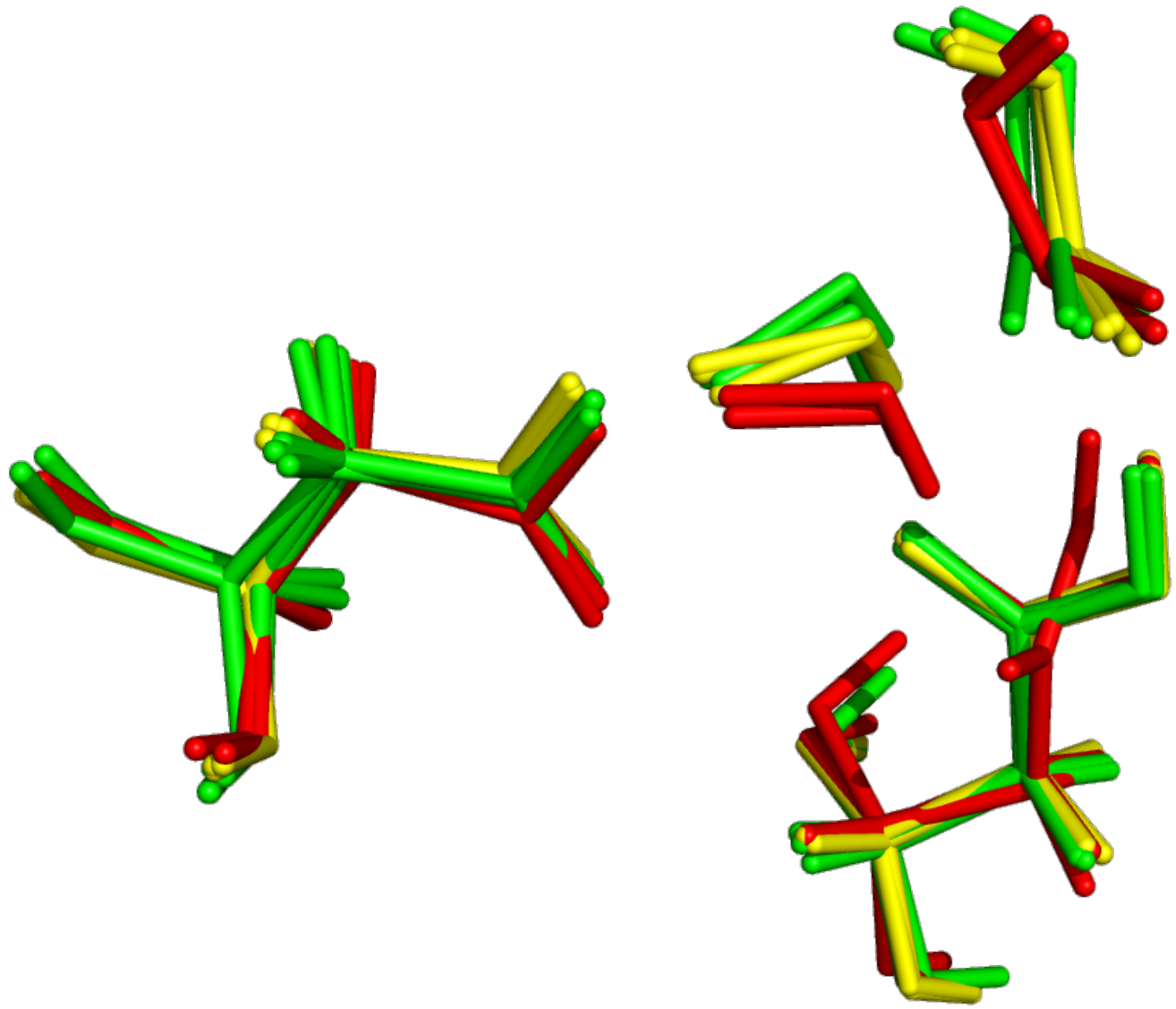
Why ΔG_{act} fluctuates so much?

Origin of the fluctuations?

Differences at the active site ?

Differences in protein folding?

Why ΔG_{act} fluctuates so much?

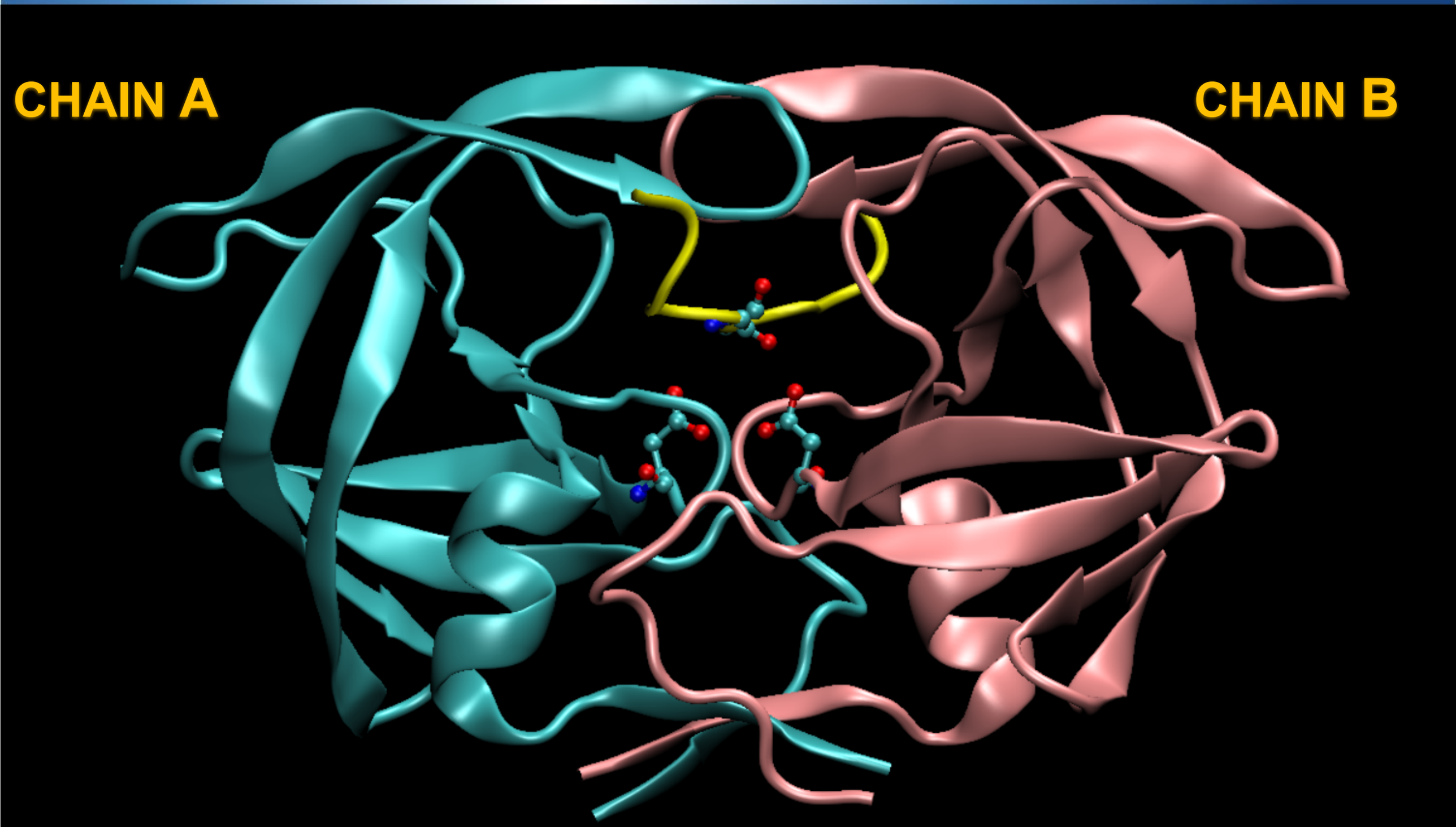


Active site
ΔG_{act}> 20 kcal/mol

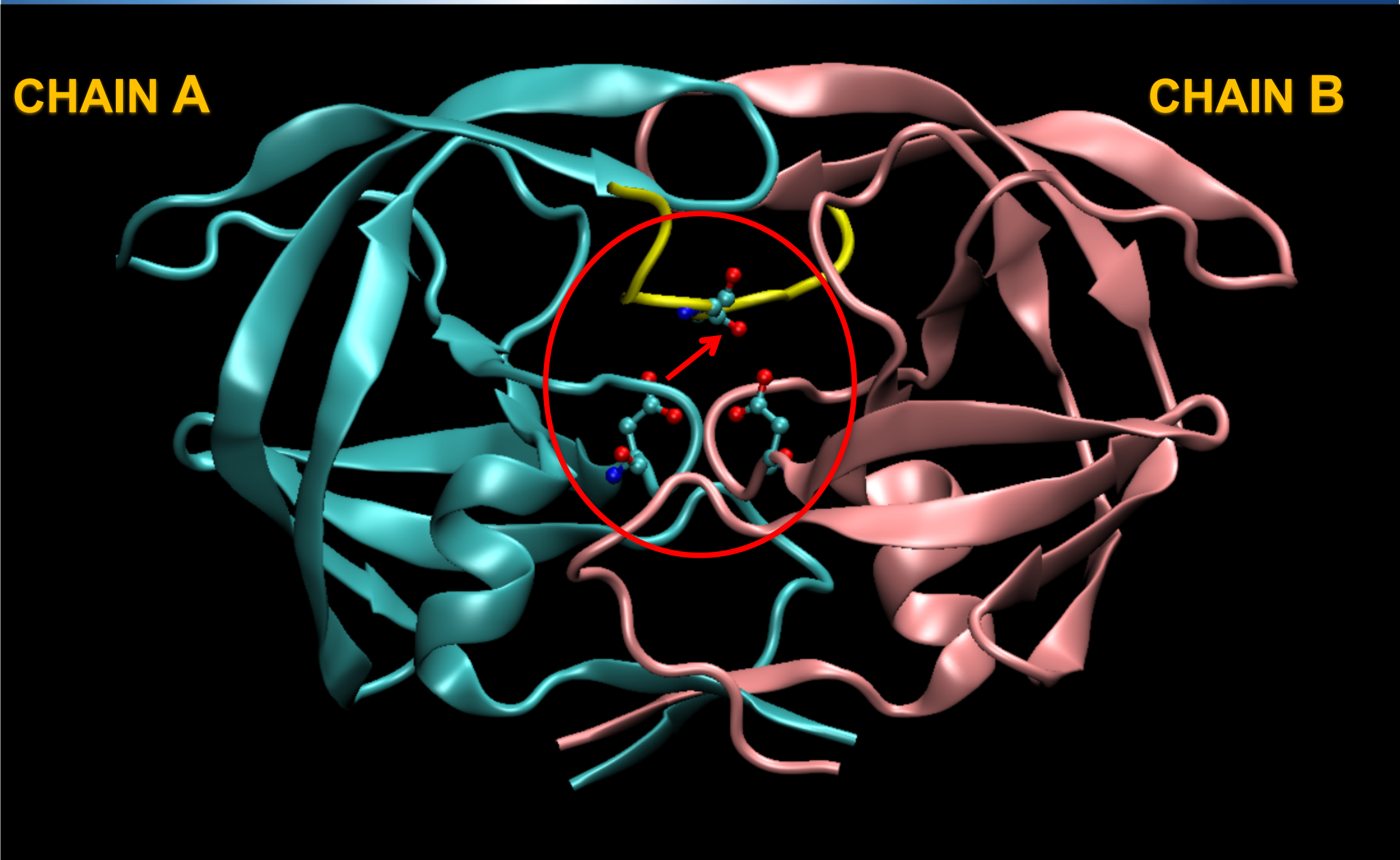
Active site
ΔG_{act} =15-20 kcal/mol

Active site
ΔG_{act}<15 kcal/mol

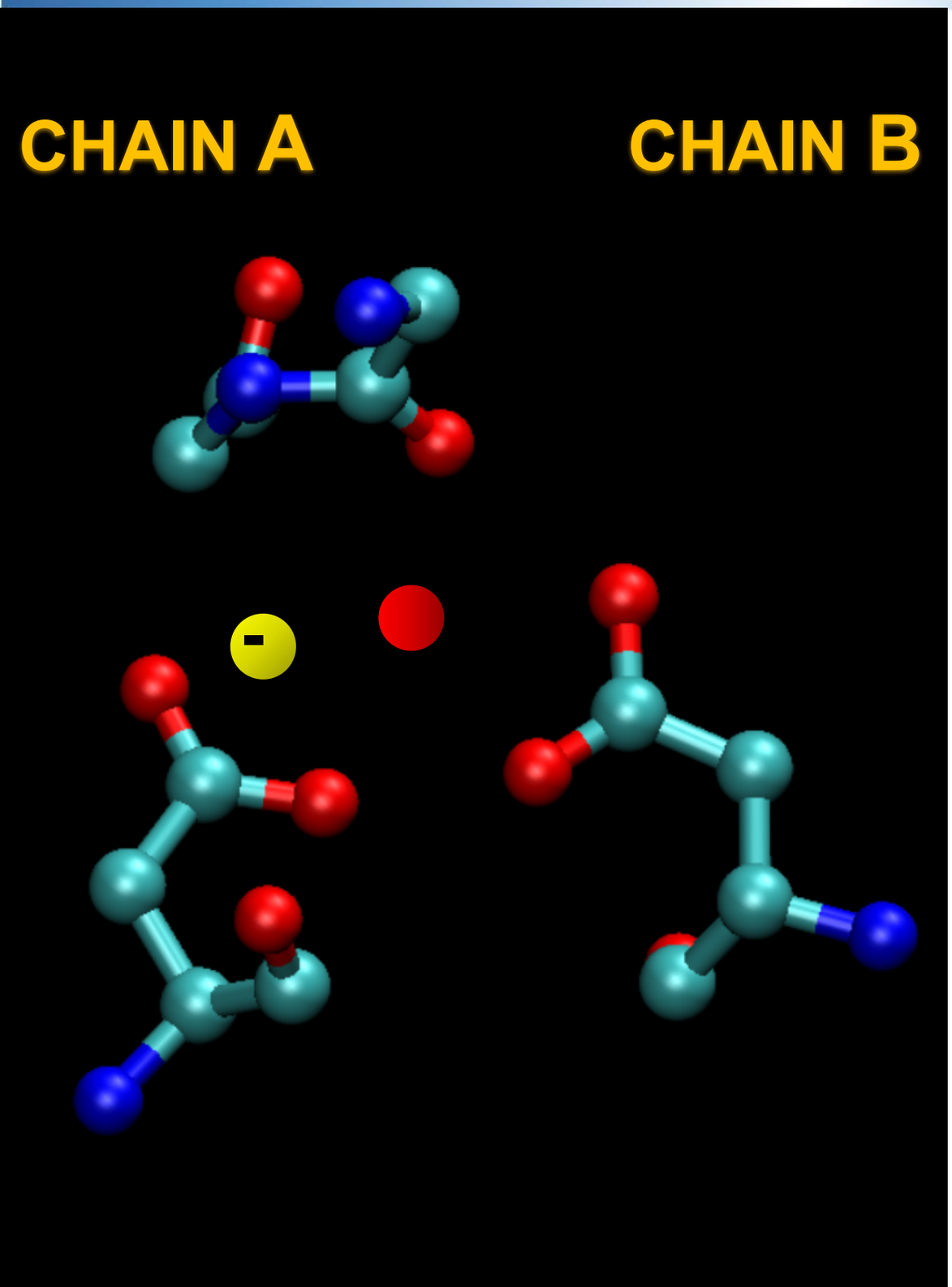
Influence of long-range interactions

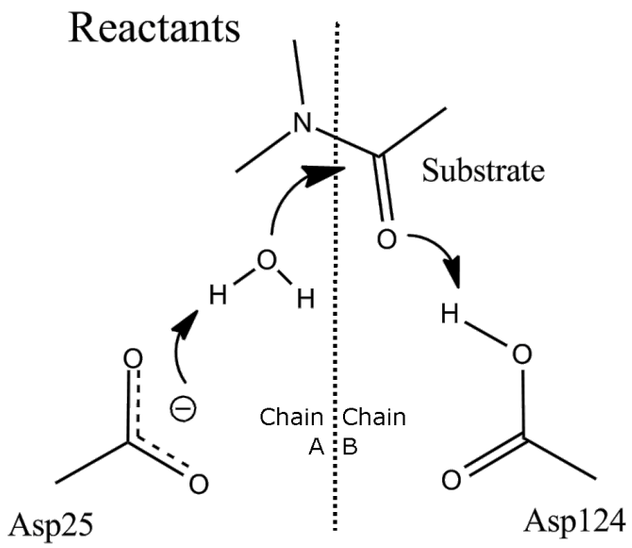


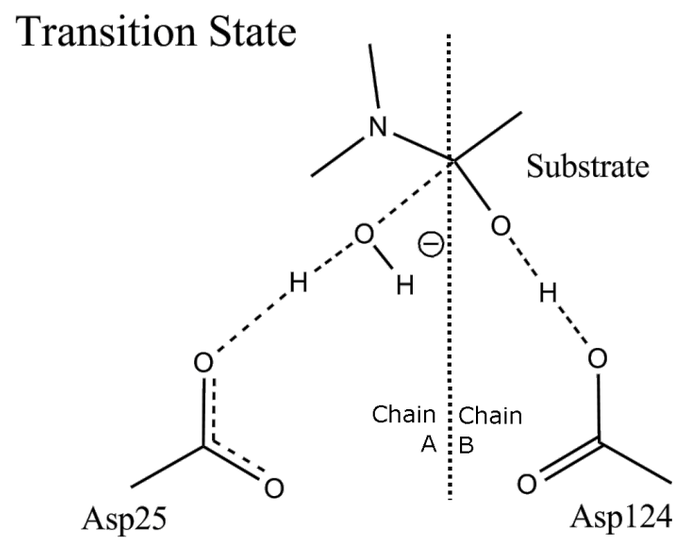
Influence of long-range interactions



Redistribution of electronic density between the R and the TS.







During the reaction mechanism significant charge density migrates from Asp25 of chain A to the substrate (direction A→B)

Influence of each residue for ΔG_{act}

A set of new calculations was done. In each one a residue was deleted.

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We generated 196 mutants, each with a different deletion.

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The energy of the reactants and TS recaculated for each mutant.

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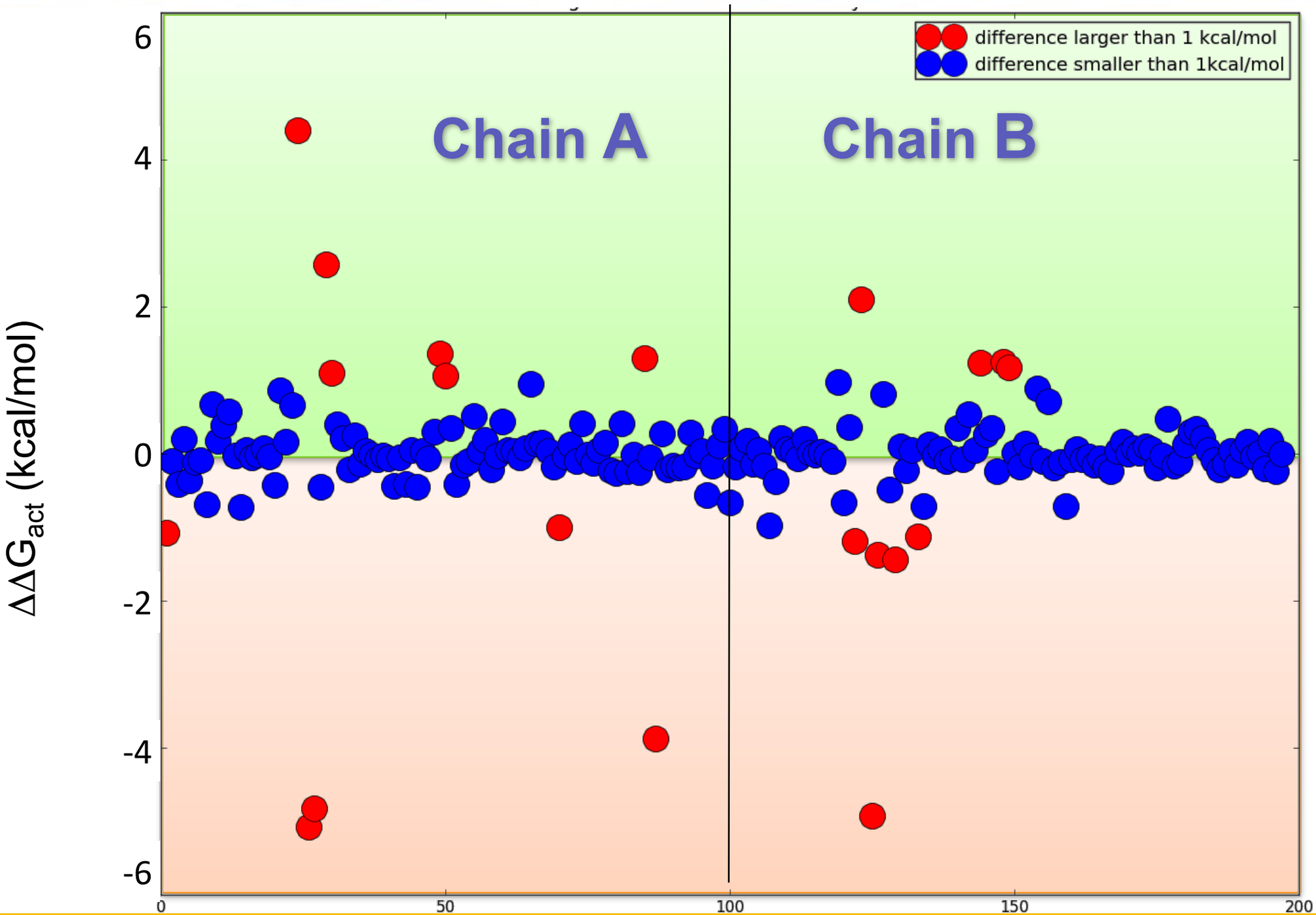
The energy of the reactants and TS recaculated for each mutant.

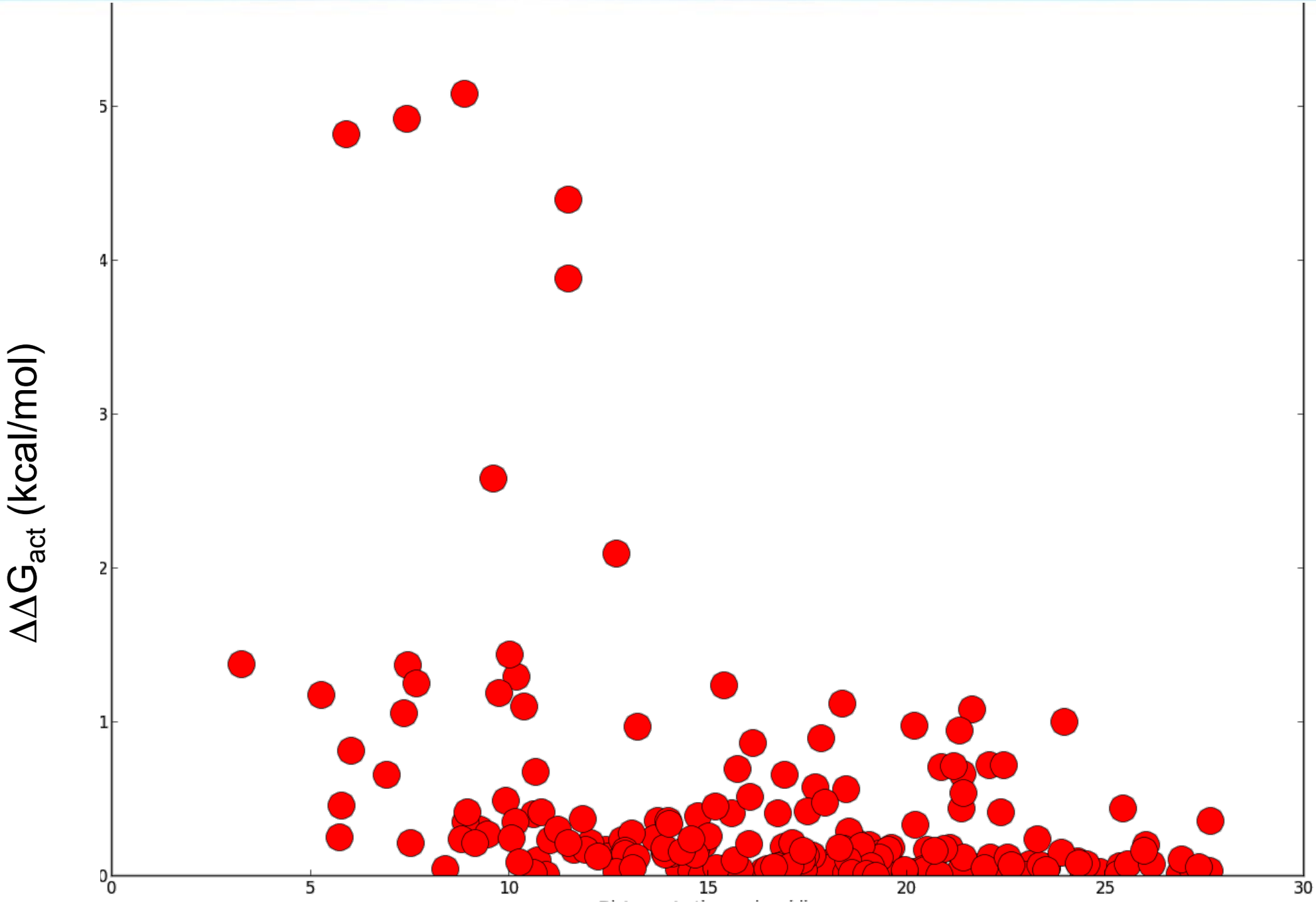
At the end we will have done 196x40x2= 15.680 QM/MM calculations.

Influence of each residue for ΔG_{act}



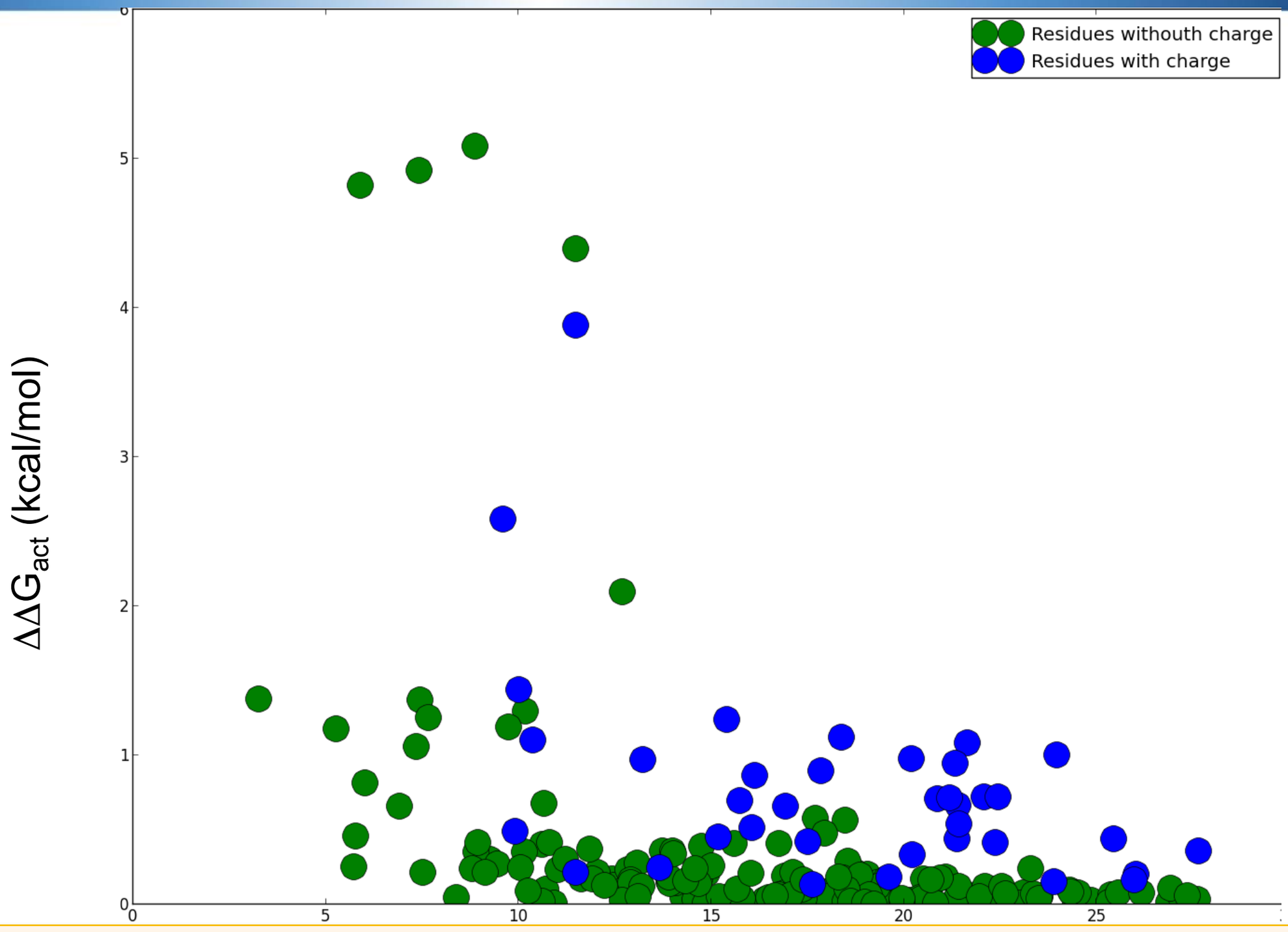
$\Delta\Delta G_{act}$ by residue (Larger than or smaller than 1 kcal/mol)

Influence of each residue for ΔG_{act}



$|\Delta\Delta G_{act}|$ *vs.* distance to the nucleophile

Influence of each residue for ΔG_{act}



$|\Delta\Delta G_{act}|$ *vs.* distance to the nucleophile, discriminating charged residues

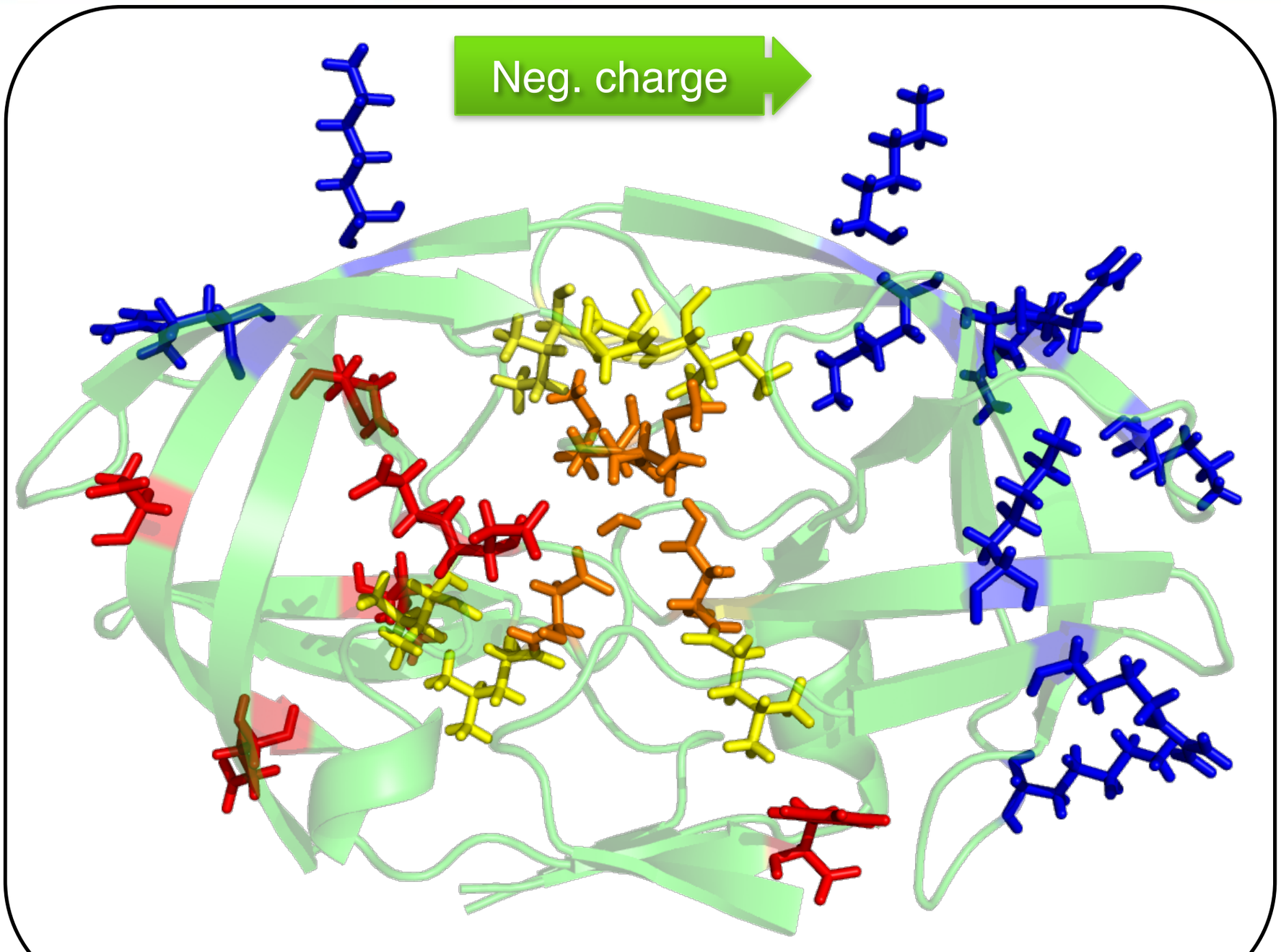
Influence of each residue for ΔG_{act}

Positive residues

Negative residues

Neutral residues

Asp25, H_2O, S



Residues with $\Delta\Delta G > 0.5$ kcal/mol.

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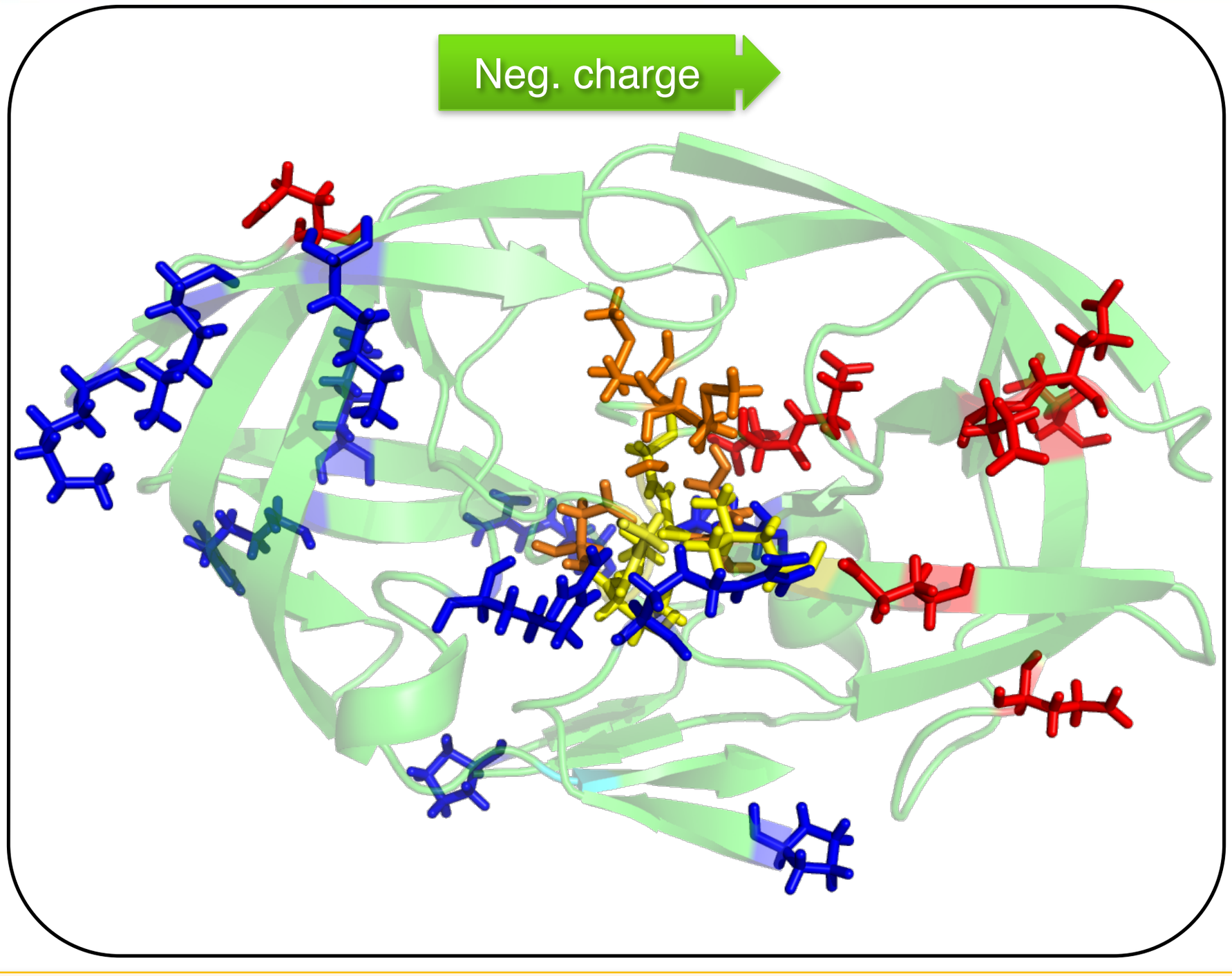

Residues with $\Delta\Delta G < -0.5$ kcal/mol.

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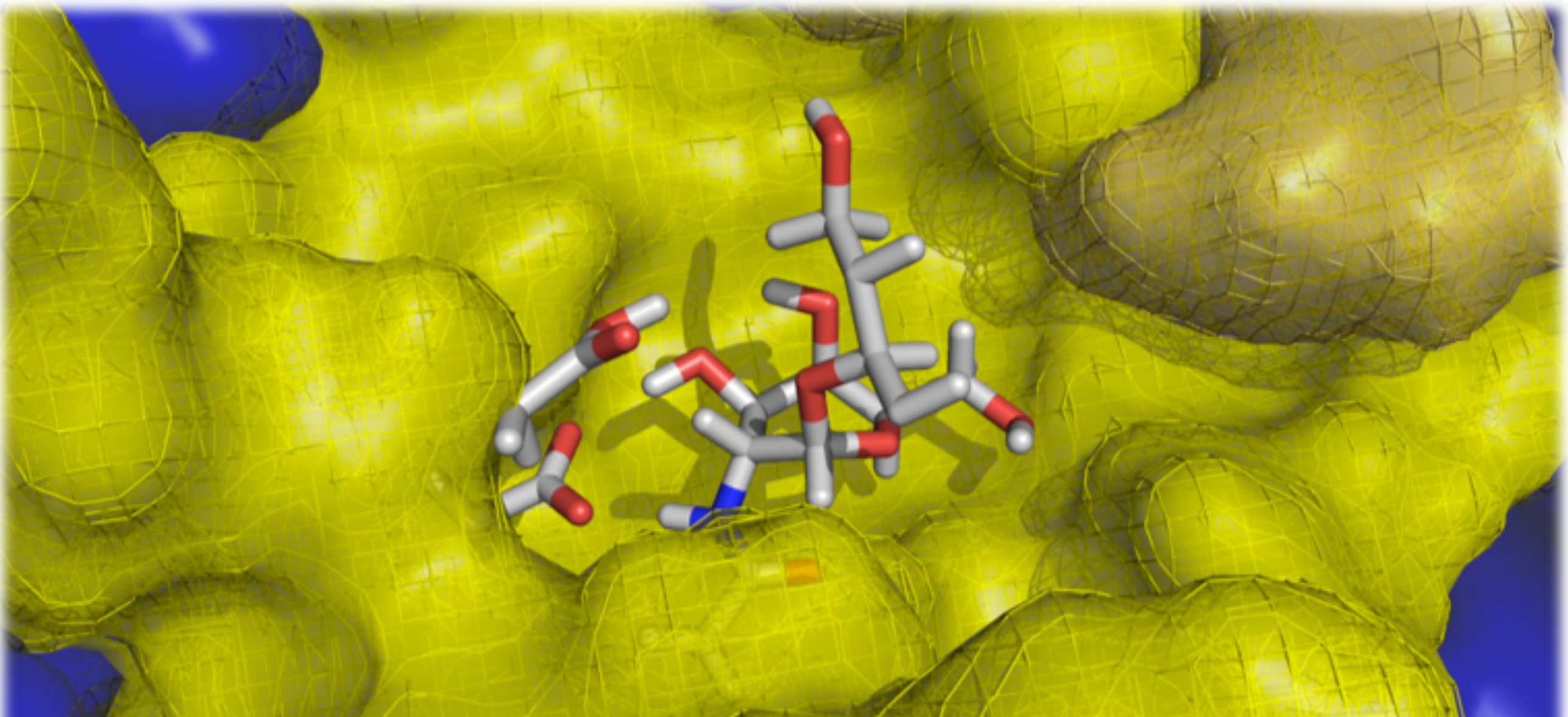

Conclusions

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The theoretical method should describe all these interactions with consistent accuracy.

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Methods to check and sample enzyme flexibility should be further developed. It is important to include conformational changes in the timescale of the enzyme kinetics, instead of just short scale fluctuations around the starting folding.

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Methods to check and sample enzyme flexibility should be further developed. It is important to include conformational changes in the timescale of the enzyme kinetics, instead of just short scale fluctuations around the starting folding.

In short, the CONSISTENCY in the correctness of the treatment of all these aspects is the key for accurate studies of enzyme catalysis.

Enzyme Catalysis

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