Hybrid Potential Simulations of Enzyme Catalysis

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Plan

- General Introduction
- Hybrid QC/MM Potentials
- Applications

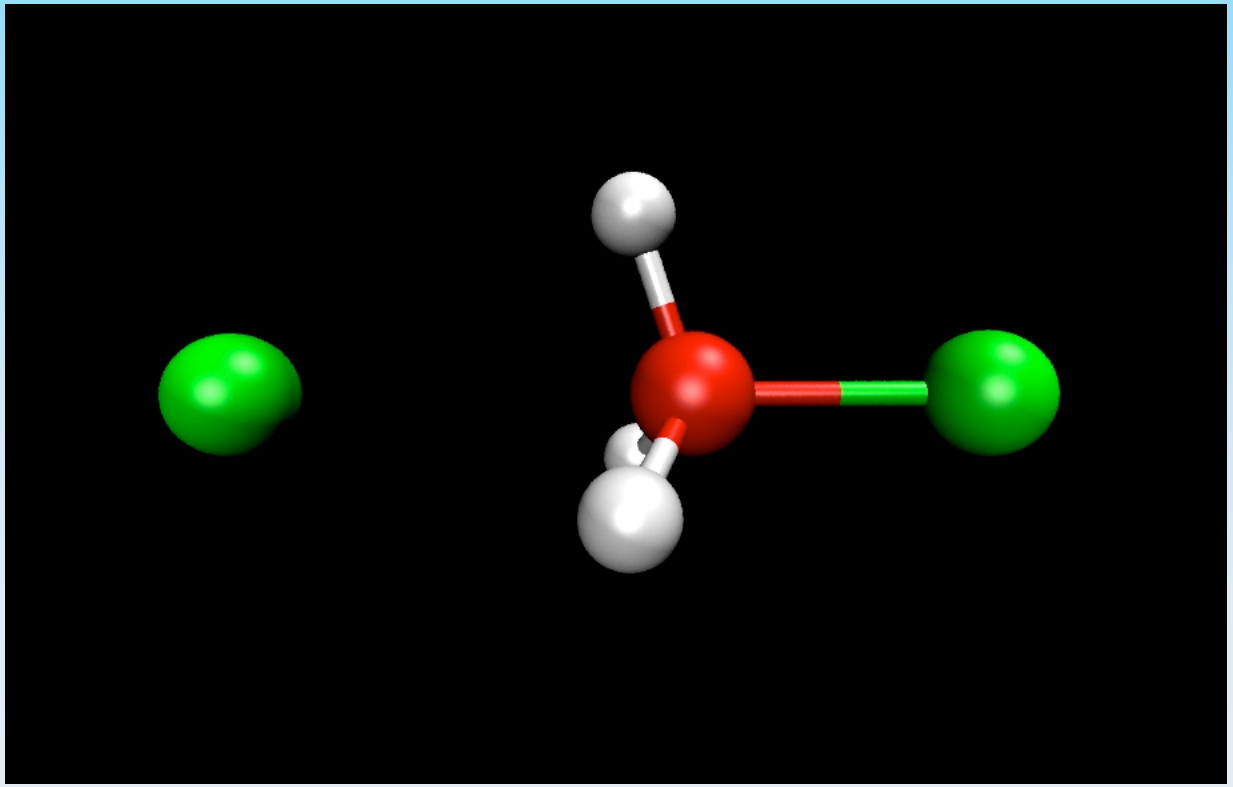
Acknowledgements

- All members of the LDM
- Guilherme Arantes
- Troy Wymore

- IBS (CEA/CNRS/UJF)
- European Union
- FAPESP
- PSC

Simple Chemical Reactions

$$Cl^- + CH_3Cl \longleftrightarrow ClCH_3 + Cl^-$$



Quantum Chemistry

Schrödinger Equation:

$$\hat{H}\Psi = E\Psi$$

Hamiltonian

Wavefunction

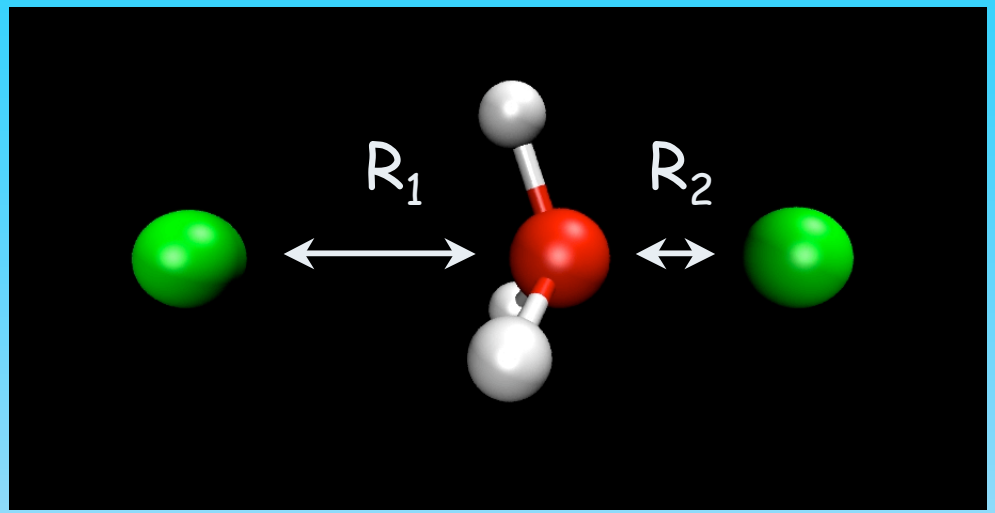
Potential Energy

Quantum Chemical Methods

- *Ab Initio*:
  - Density functional theory (DFT)
  - Molecular orbital (MO) methods; e.g. Hartree-Fock (HF)

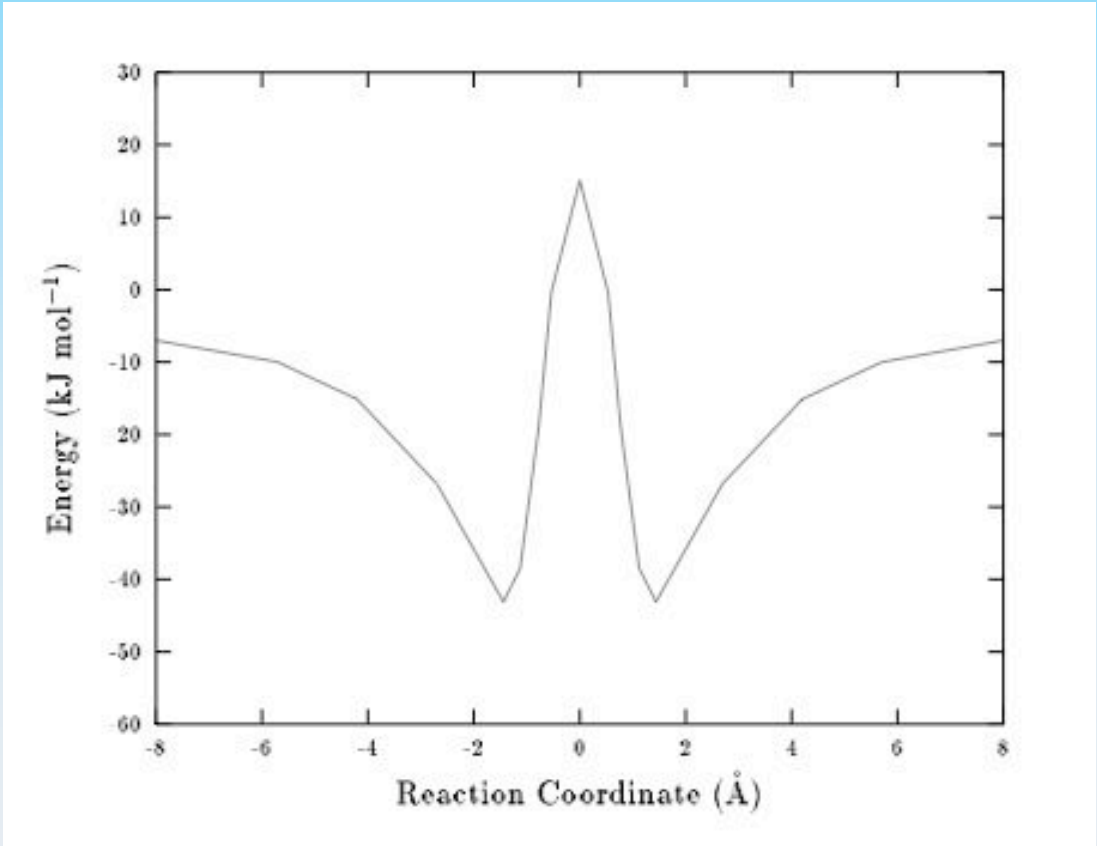
  Precise but slow.
- Semi-Empirical:
  - MO-based; e.g. AM1, MNDO, PM3

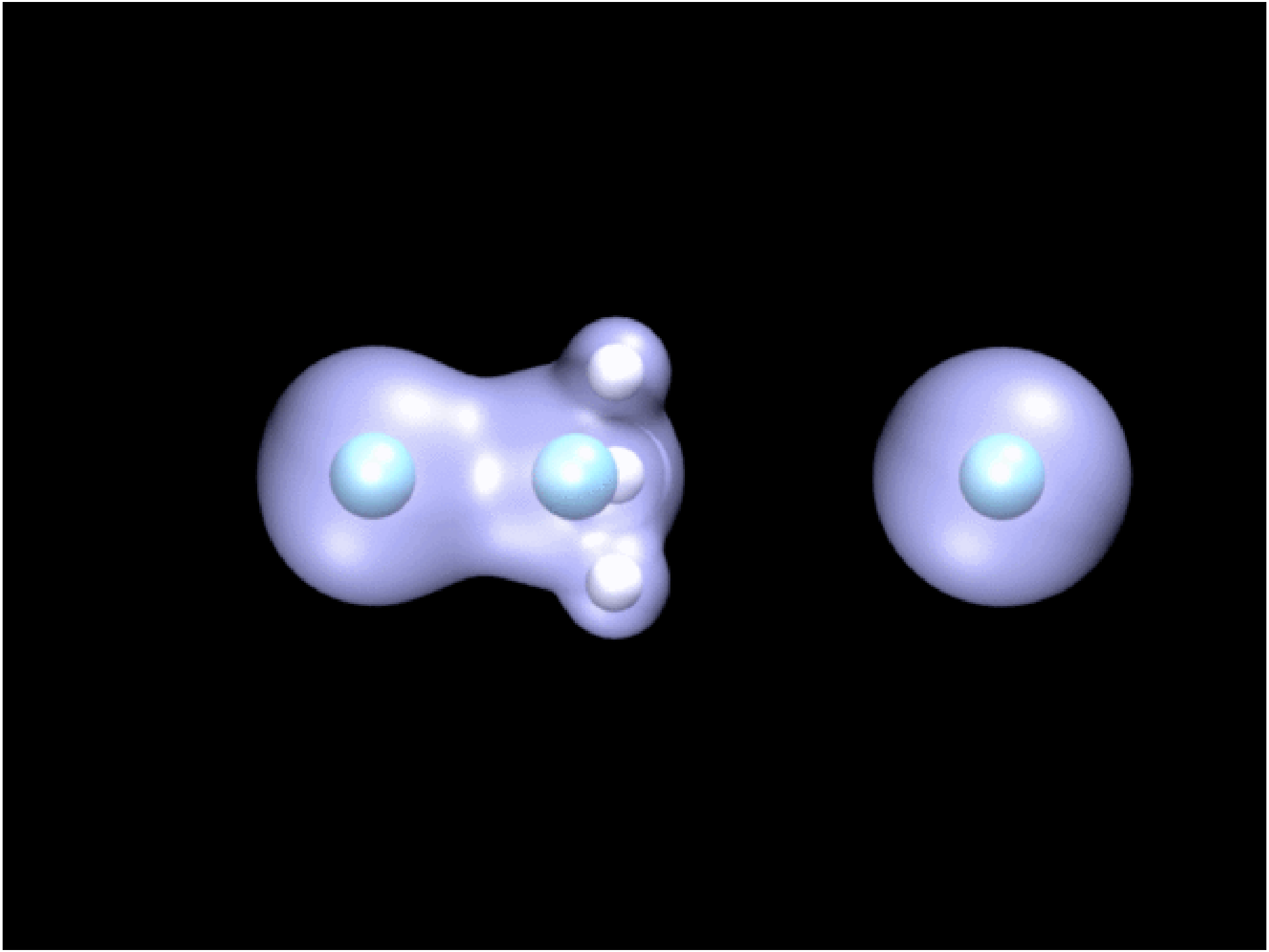
  Faster but more approximate.

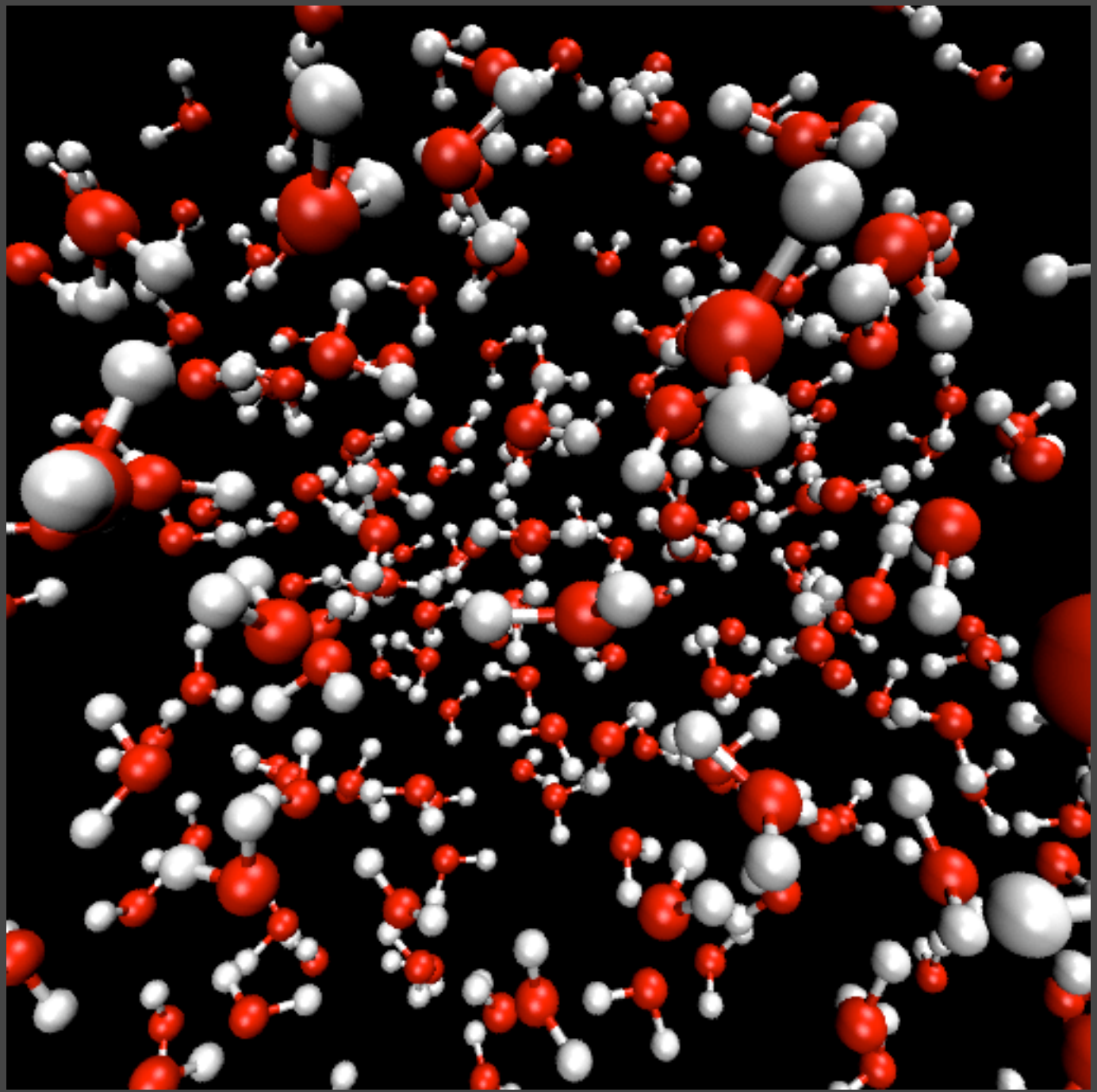


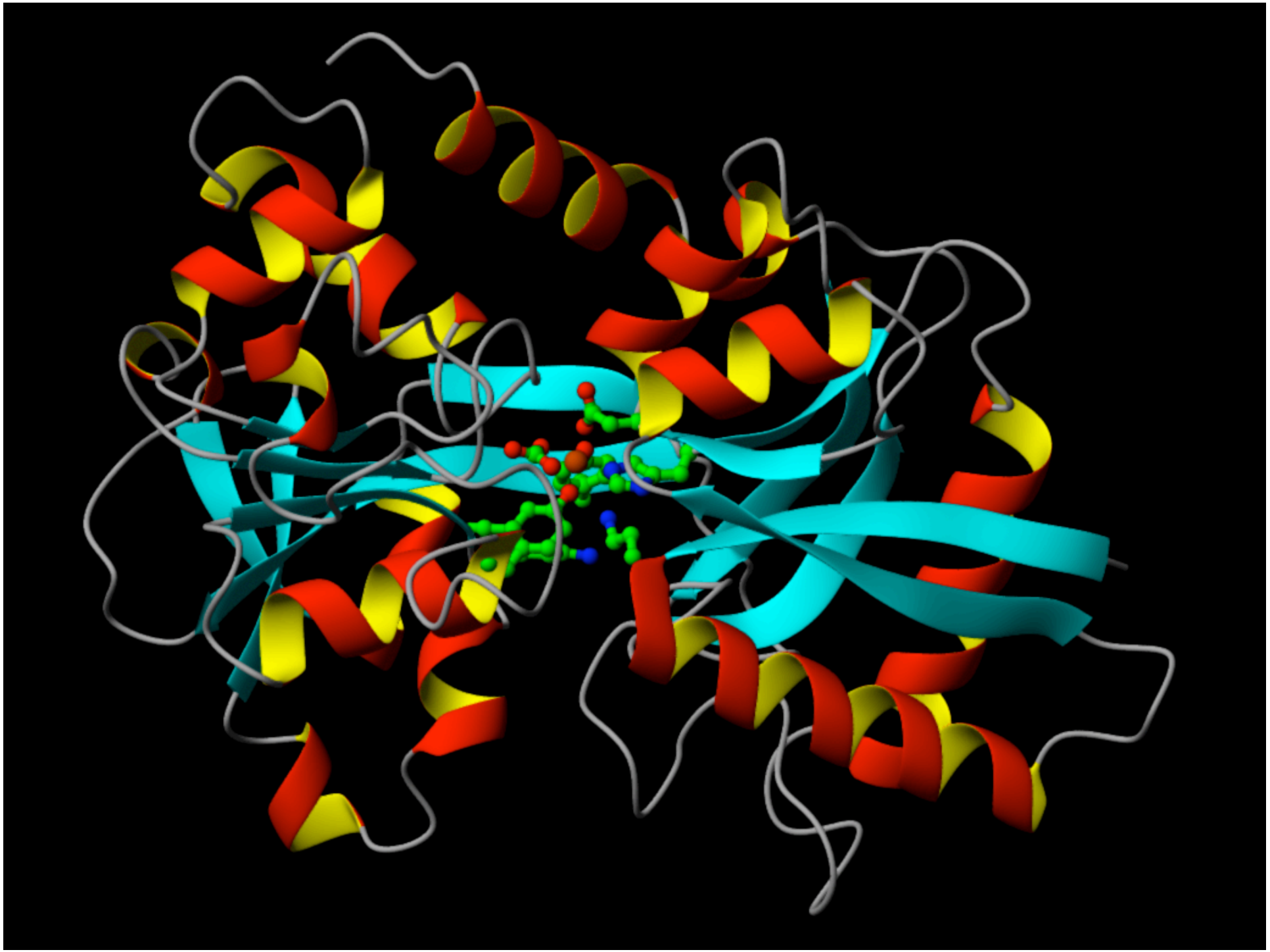
Reaction coordinate:
$R_1 - R_2$

Energy profile in vacuum.









Complex Chemical Reactions

Two problems linked to size:

- Calculation of the potential energy:

  Quantum chemical methods too expensive.

- Exploring the potential energy surface:

  Sampling of relevant conformations.

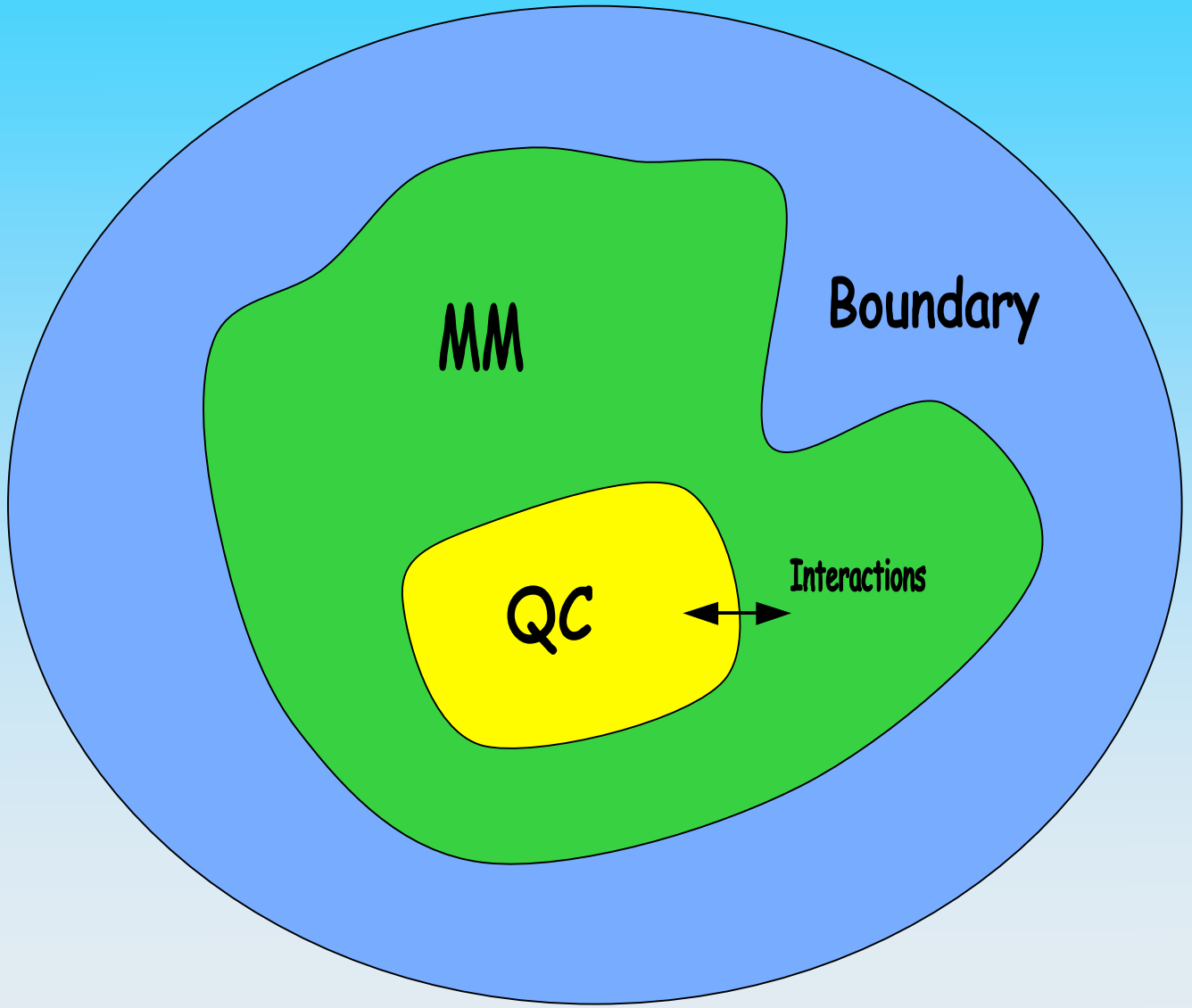
Hybrid Potentials

A combination of methods that treat different parts of a system at varying levels of precision.

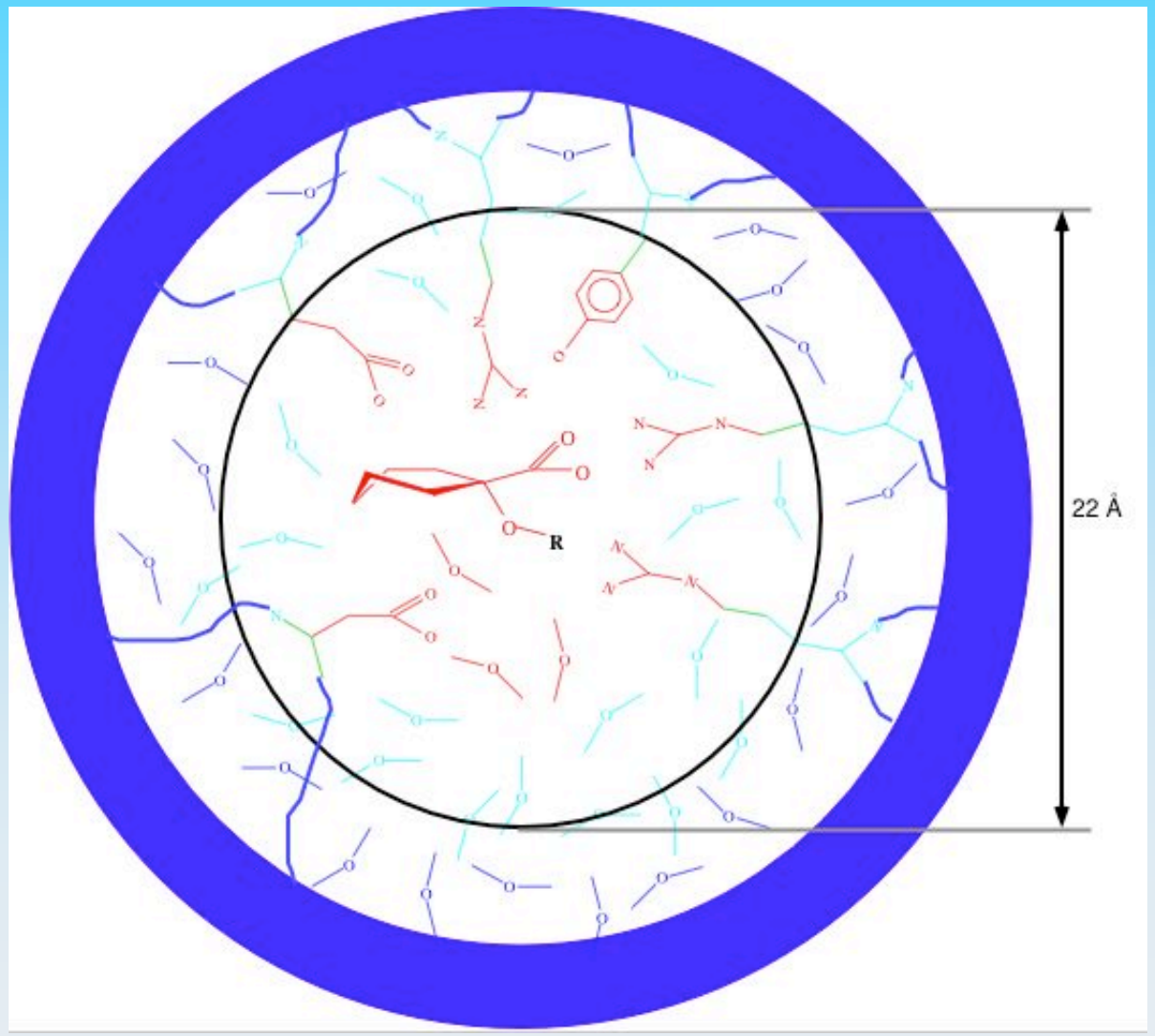
First QC/MM potentials conceived to study enzyme reactions:

A. Warshel and M. Levitt. *Theoretical Studies of Enzymatic Reactions: Dielectric Electrostatic and Steric Stabilization of the Carbonium Ion in the Reaction of Lysozyme*. J. Mol. Biol. 103, 227-249 (1976).

Dividing a System



An Enzyme Example



QC/MM Methods I

QC Potentials:

- *Ab initio* DFT and HF — Gaussian basis functions.
- Various semi-empirical methods.

MM Potentials:

- AMBER, CHARMM, OPLS-AA, UFF force fields.

Boundary:

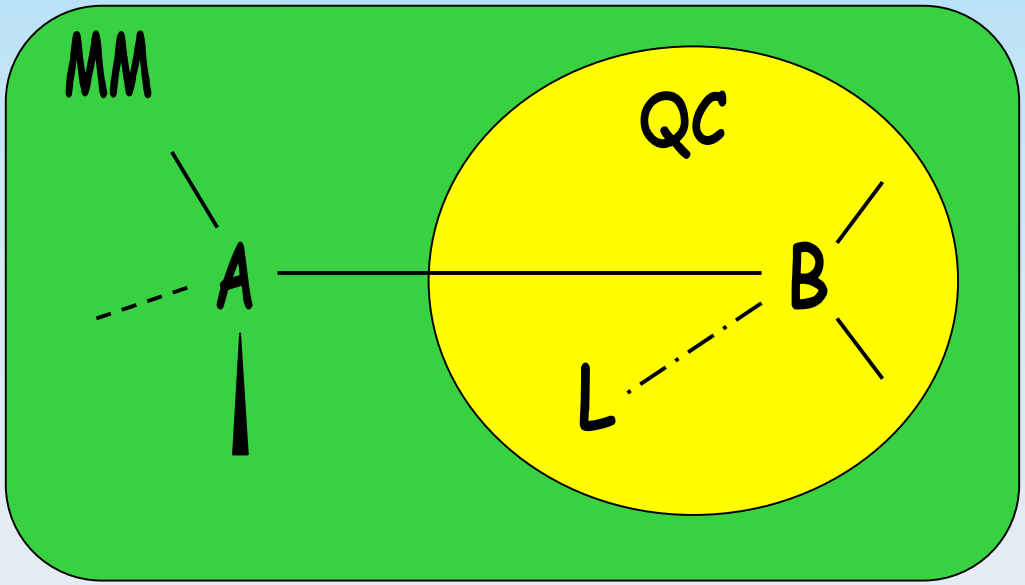
- Periodic boundary conditions.
- Truncated systems.

QC/MM Methods II

QC/MM Non-bonding Interactions:

- Electrostatic — full QC (e^- and n^+)/MM (point charge).
- Lennard-Jones.

QC/MM Covalent Interactions:



QC/MM Methods III

- Solve Hartree-Fock or Kohn-Sham equations to self-consistency each time an energy is calculated.

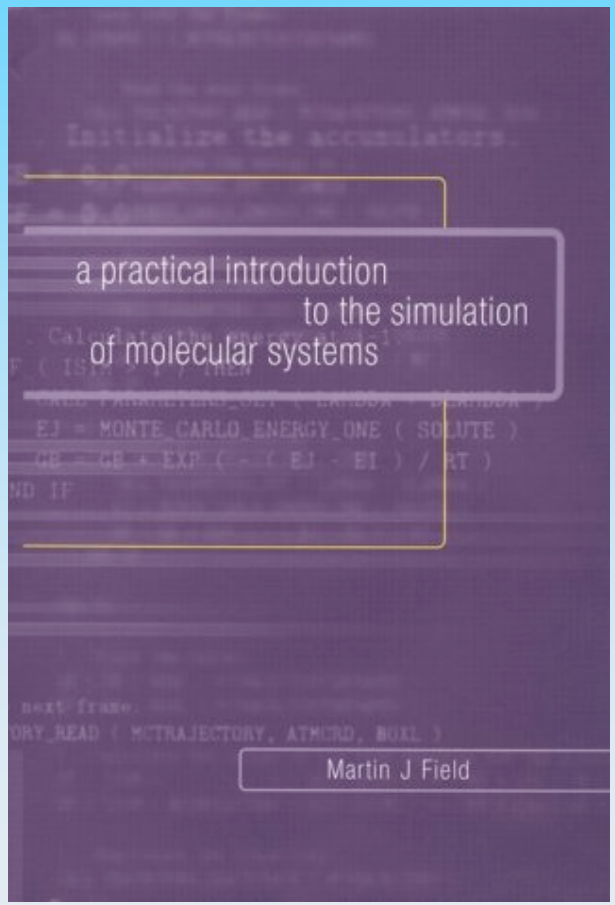
$$\hat{H}_{\text{Eff}}\Psi = E\Psi$$

- Use the energy and forces for:
  - Geometry optimization
  - Reaction path location
  - Molecular dynamics simulation
  - Free-energy determination

fDynamo

- M. J. Field. *A Practical Introduction to the Simulation of Molecular Systems*. Cambridge University Press, Cambridge, 1999.

- M. J. Field, M. Albe, C. Bret, F. Proust-de Martin and A. Thomas. *The Dynamo Library for Molecular Simulations using Hybrid Quantum Mechanical and Molecular Mechanical Potentials*. J. Comput. Chem. 21, 1088-1100 (2000).



fDynamo Characteristics

- Fortran 90/95.
- A library of modules - not a program.
- A variety of simulation approaches, including:
  - QC, MM and QC/MM potentials.
  - Geometry optimization.
  - Saddle point and reaction path finding.
  - Normal mode analysis.
  - MC and MD simulations.
  - Free-energy calculations.
- Serial and coarse-grained parallel versions.

Beyond fDynamo

Limitations of Fortran 90/95 include:

- Absence of many of the capabilities of modern computer languages (e.g. objects and inability to interact with the operating system).
- Lack of good open-source compilers.

Replacement approaches considered included C, C++, Eiffel, Fortran 2000, Java, Objective C, Python and Ruby.

pDynamo Characteristics

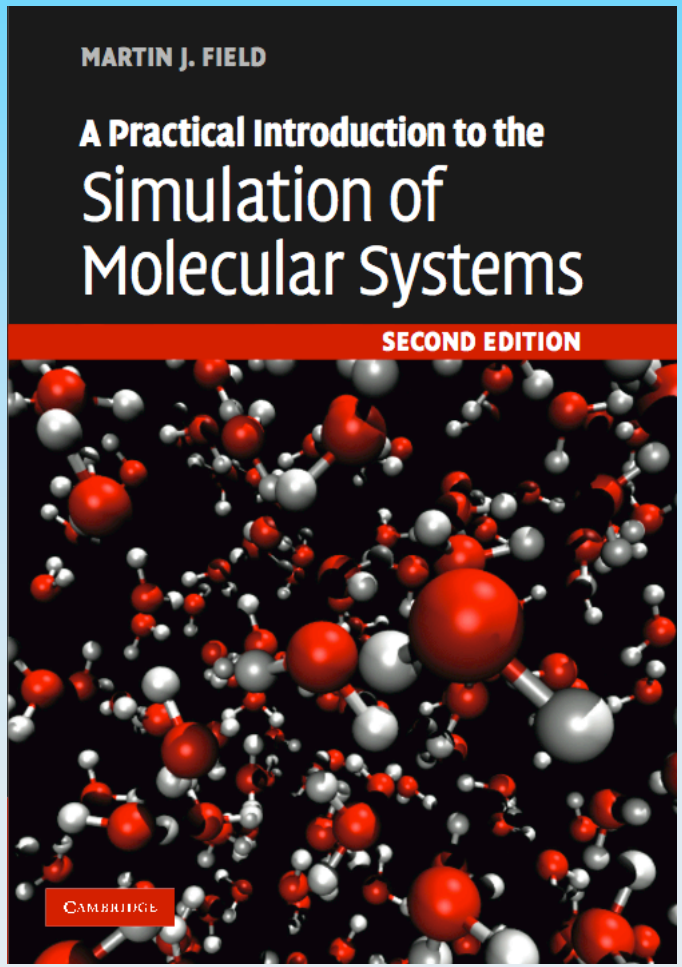
- Python/C:
  - Python for the majority of operations .
  - C for speed.
- A series of Python packages.
- Object-oriented structure.
- Equivalent functionality to fDynamo and more:
  - Arbitrary crystal symmetry.
  - Coupling to external programs (e.g. ORCA).
  - DFT methods.
  - More external file formats, ...

pDynamo

- M. J. Field. *A Practical Introduction to the Simulation of Molecular Systems*. Cambridge University Press, Cambridge, 2007 (2nd edition).
- M. J. Field. *The pDynamo Library for Molecular Simulations using Hybrid Quantum Mechanical and Molecular Mechanical Potentials*. J. Chem. Theo. Comput. 4, 1151-1161 (2008).

The library is available at:

http://www.pdynamo.org



Applications to Enzymes I

- Acetyl-coenzyme A synthase (Amara *et al.*, JACS '05).
- AIRase (Proust *et al.*, JACS '00).
- Aldehyde dehydrogenase (Wymore *et al.*, CBI '03).
- cAMP-dependent protein kinase (Díaz and Field, JACS '04).
- Chorismate mutase from *B. subtilis* (Martí *et al.*, JACS '01, Crehuet and Field, JPC '07).
- Fluorescent Proteins (Adam *et al.*, PNAS '08; Lelimousin *et al.*, Biochem. '09, JACS '10).
- HG(X)PRTase (Thomas *et al.*, JACS '02, '06; Crehuet *et al.*, JMGM '05).
- Hydrogenase from *D. gigas* (Amara *et al.*, JACS '99; Galván *et al.*, Proteins, '08).
- Influenza neuraminidase (Thomas *et al.*, JACS '99).
- PBPs (Oliva *et al.*, Proteins '03).
- PFOR (Amara *et al.*, Angew. Chem. Intl. Ed. '07).

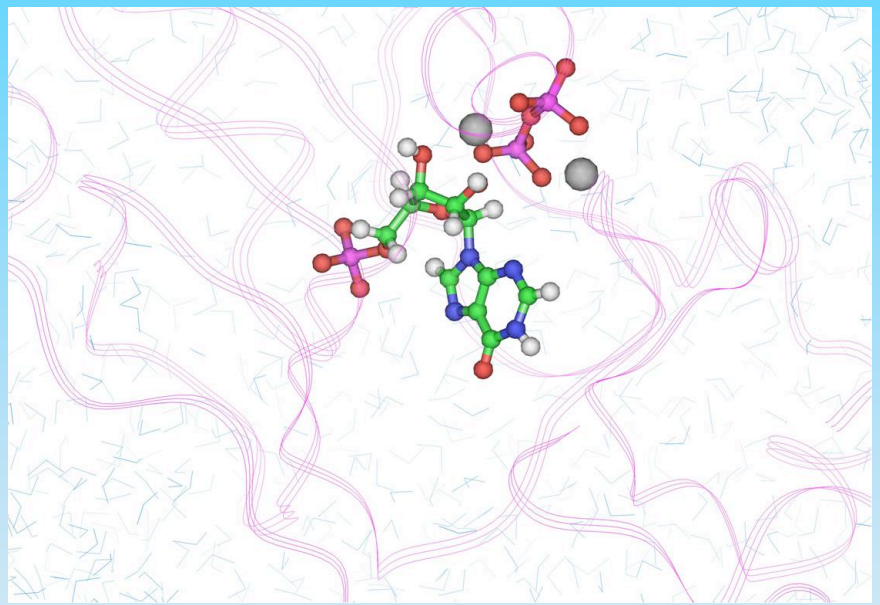
Applications to Enzymes II

- Hydrogenase from *D. gigas* (Amara *et al.*, JACS '99):
  - DFT/MM hybrid potential.
- Influenza neuraminidase (Thomas *et al.*, JACS '99):
  - QC/MM path-integral free-energy simulations.
- AIRase (Proust *et al.*, JACS '00):
  - *Ab initio* corrections to the semi-empirical QC/MM energies.
- Chorismate mutase from *B. subtilis* (Crehuet and Field, JPC '07):
  - TPS simulations.

Application to HGXPRTase I

• Malaria causes one million deaths per year in Africa alone.

• The causative agent, the protozoan *Plasmodium falciparum*, cannot synthesize purine nucleotides but scavenges its host's purine bases.



• Hypoxanthine-guanine-xanthine-phosphoribosyl-transferase (HGXPRTase) is a possible target for anti-malarial drugs.

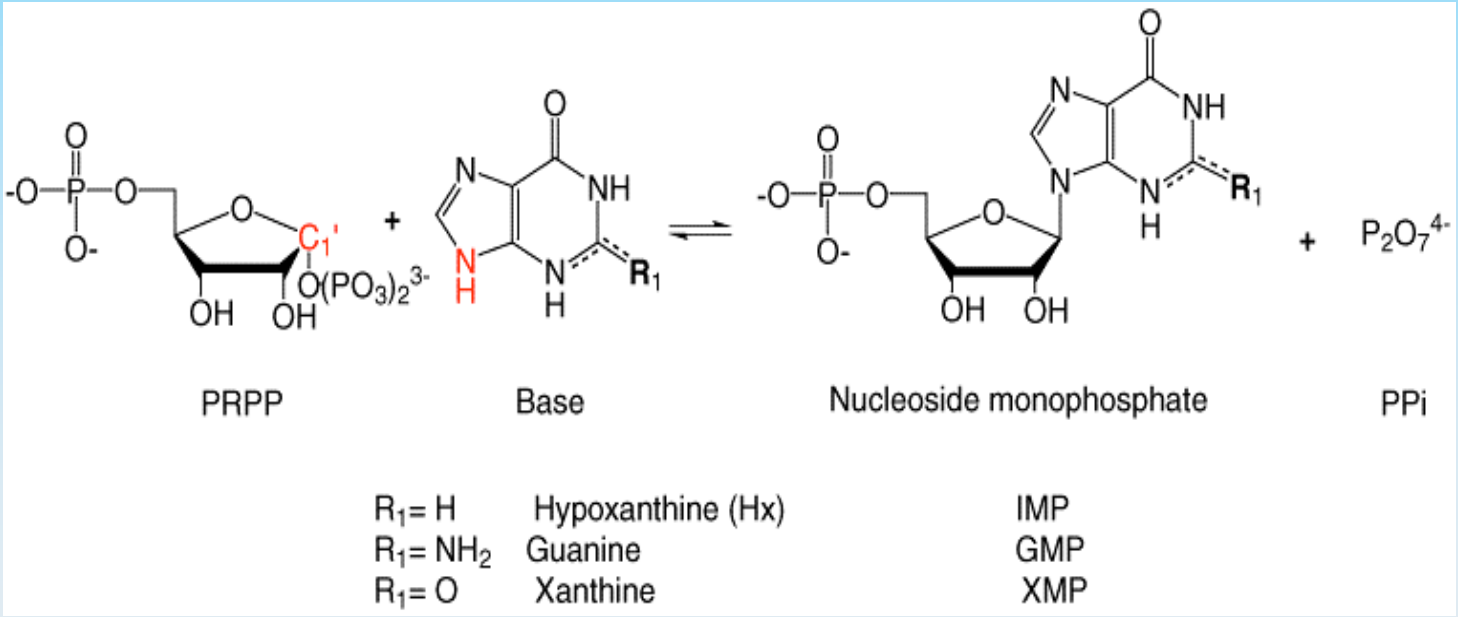
• Human HGPRTase has a much reduced affinity for xanthine.

Application to HGXPRTase II

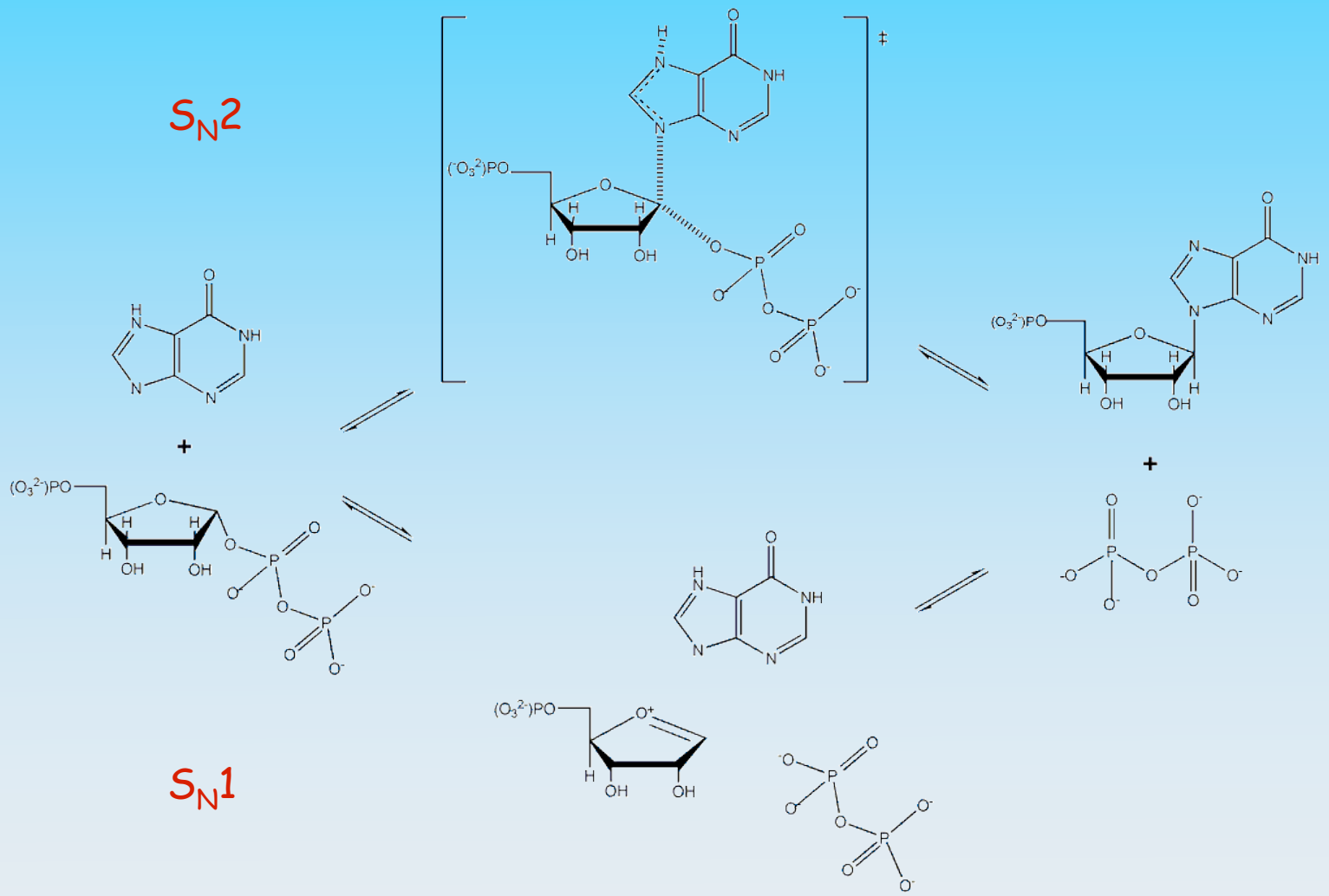
A. Thomas and M. J. Field (JACS '02)

R. Crehuet, A. Thomas and M. J. Field (JMGM '05)

A. Thomas and M. J. Field (JACS '06)



Application to HGXPRTase III



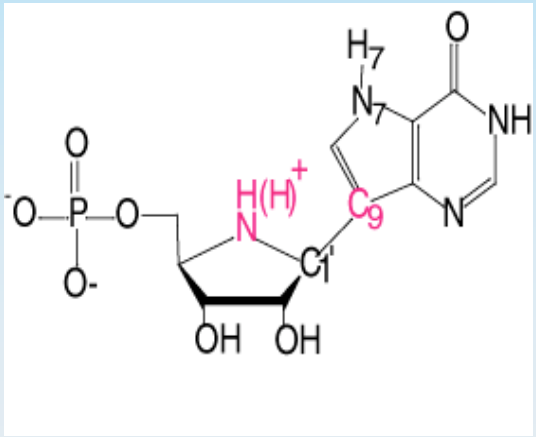
Application to HGXPRTase IV

S_N1 mechanism:

- Favoured by KIE measurements.

S_N2 mechanism:

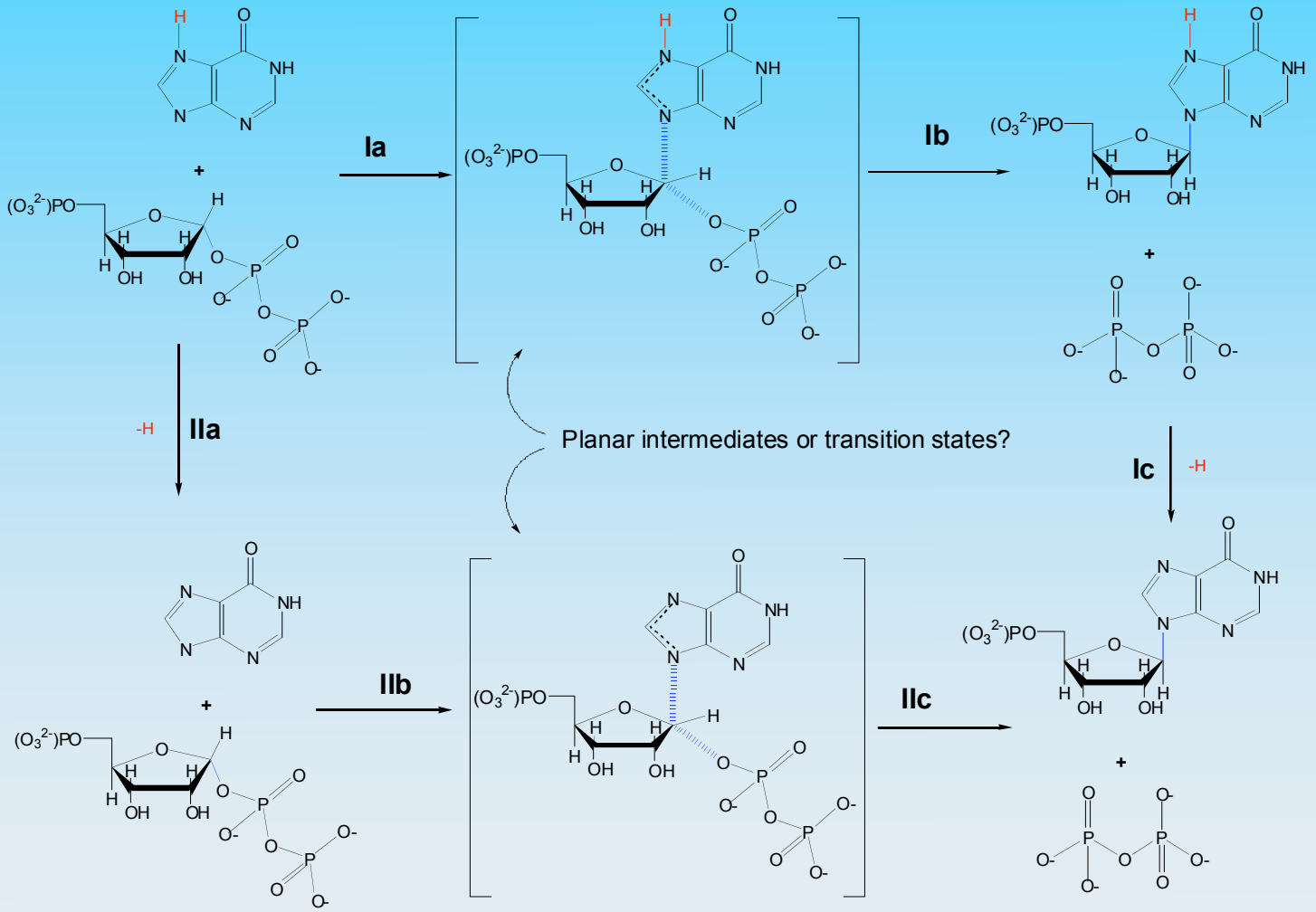
- X-ray crystallographic structure.



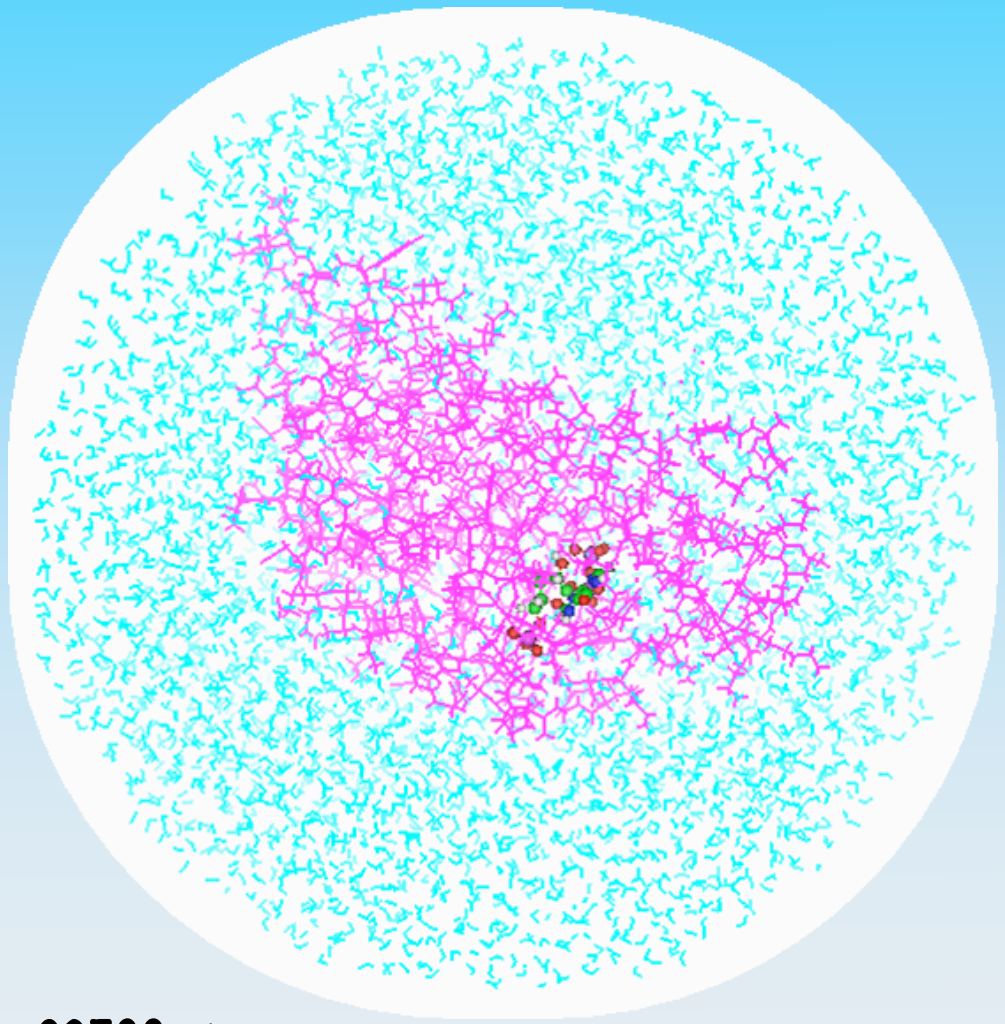
Immucillin HP —
transition state analog.

And what type of S_N1 or S_N2?
A_ND_N, D_NA_N, …

Application to HGXPRTase V

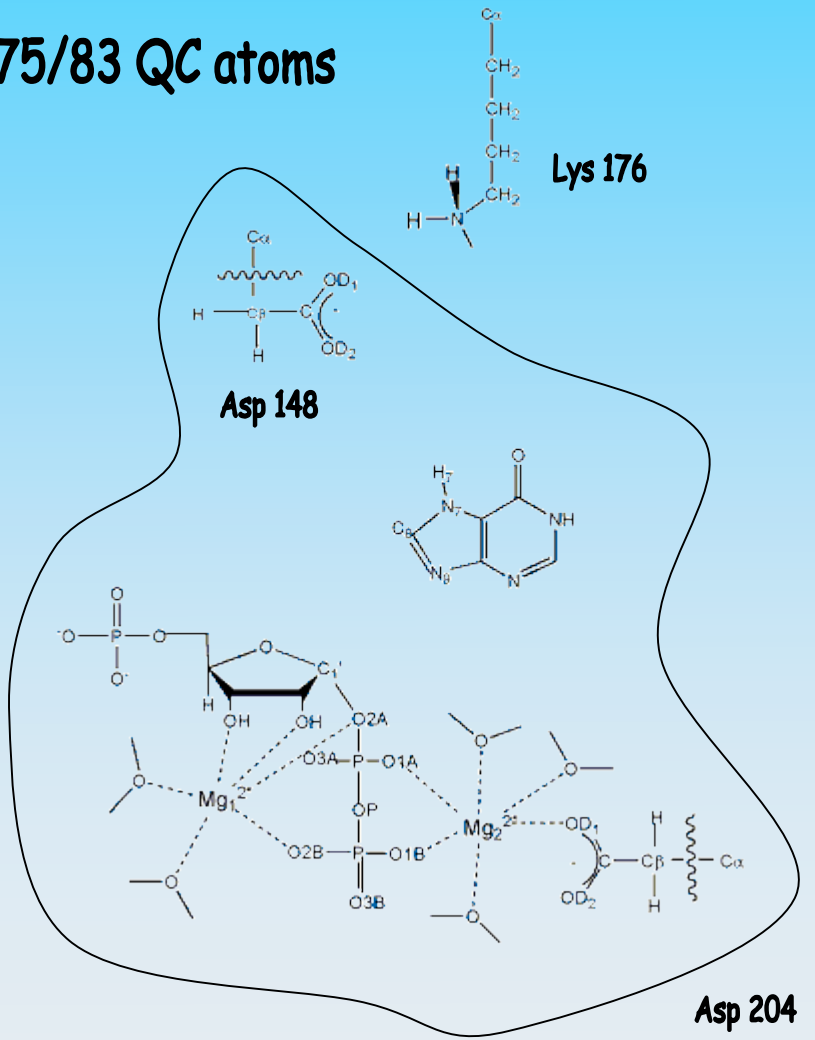


Application to HGXPRTase VI



~23500 atoms

75/83 QC atoms



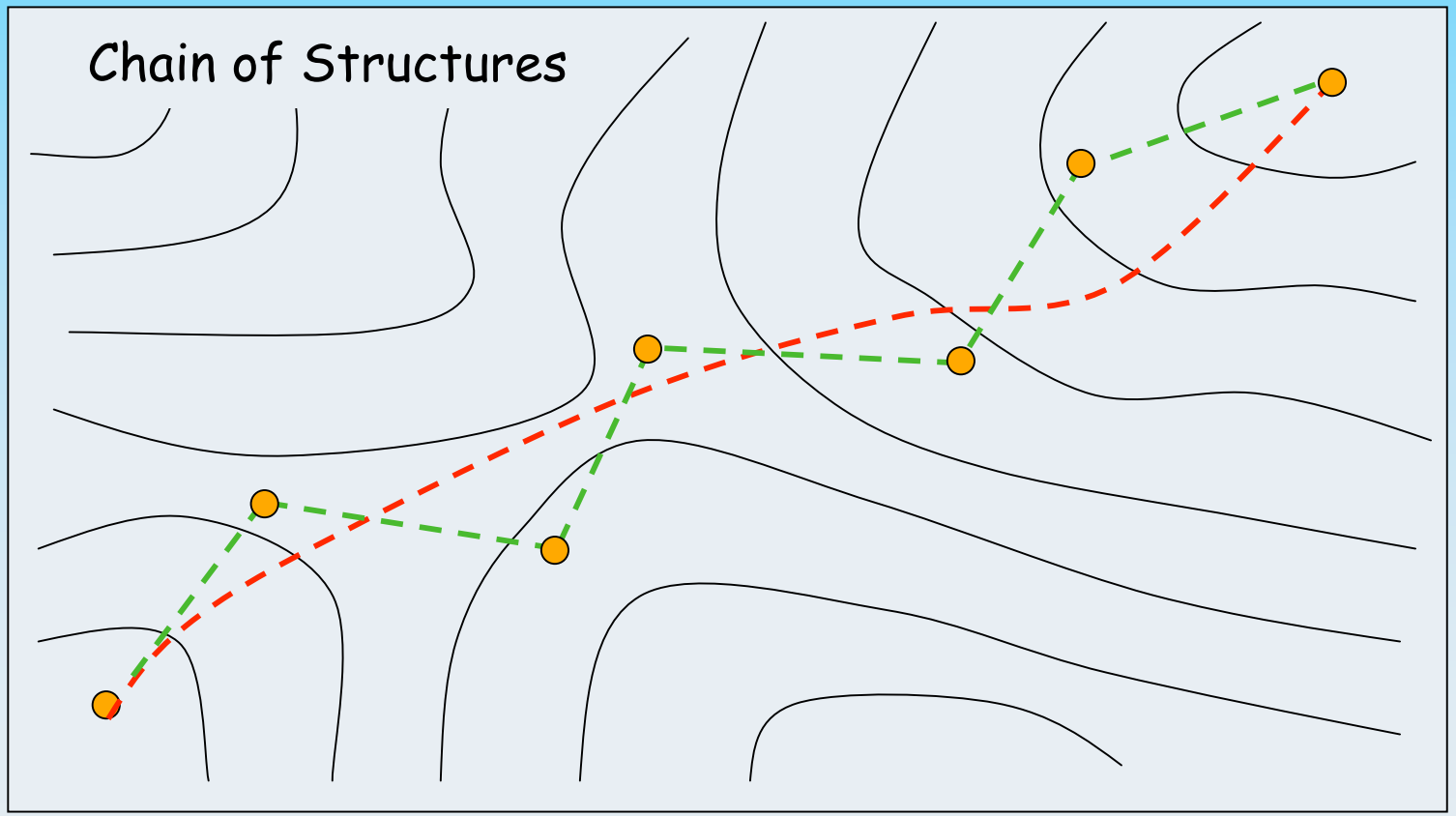
Application to HGXPRTase VII

A range of simulation approaches are necessary:

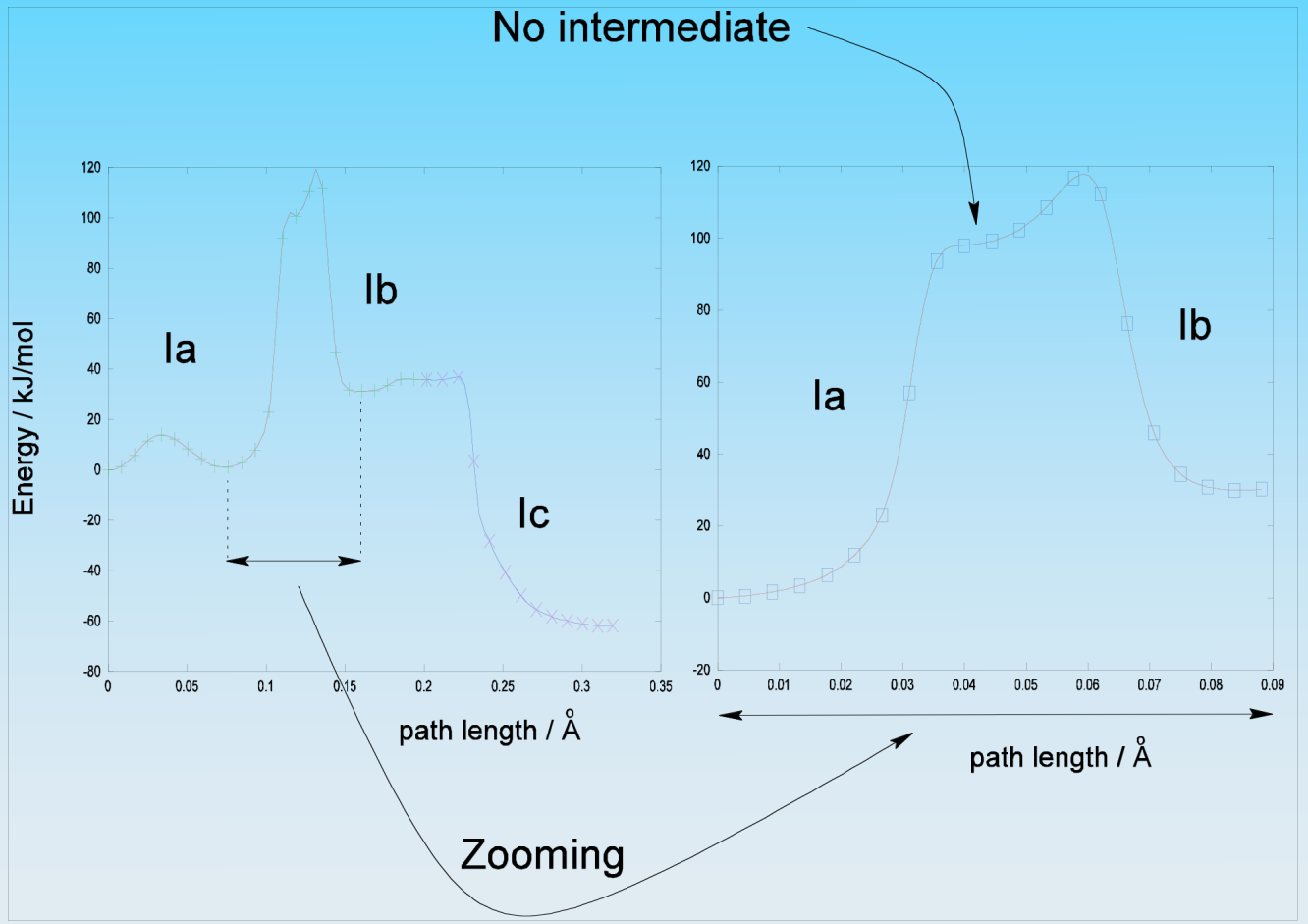
- Local approaches:
  - Critical point determination.
  - Reaction paths.
- Global approaches:
  - Molecular dynamics.
  - Free-energy calculations.

Nudged-Elastic-Band Method

Method due to H. Jónsson and co-workers.

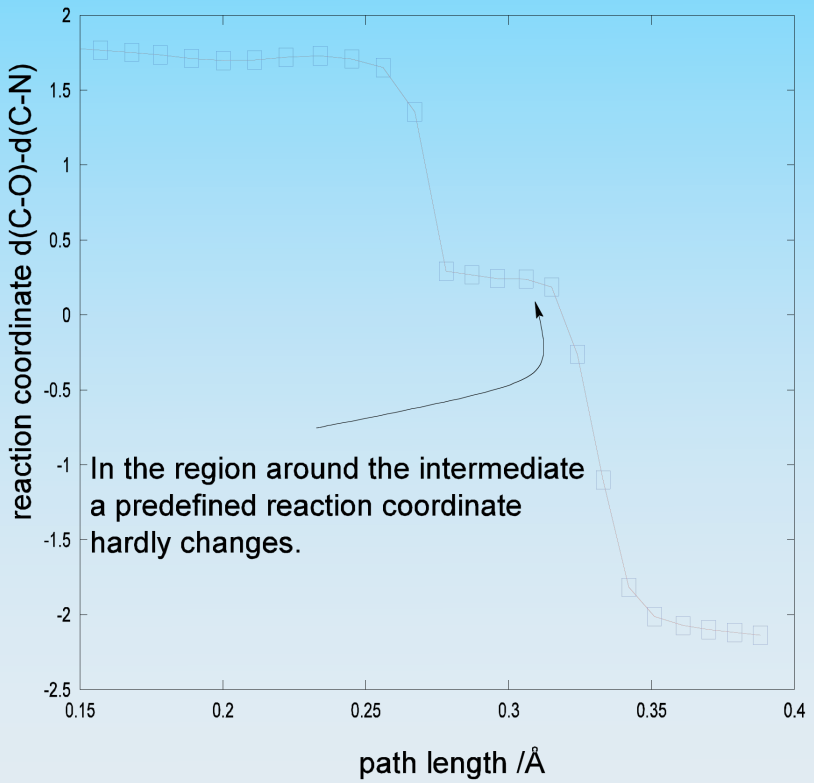


Nudged-Elastic-Band II

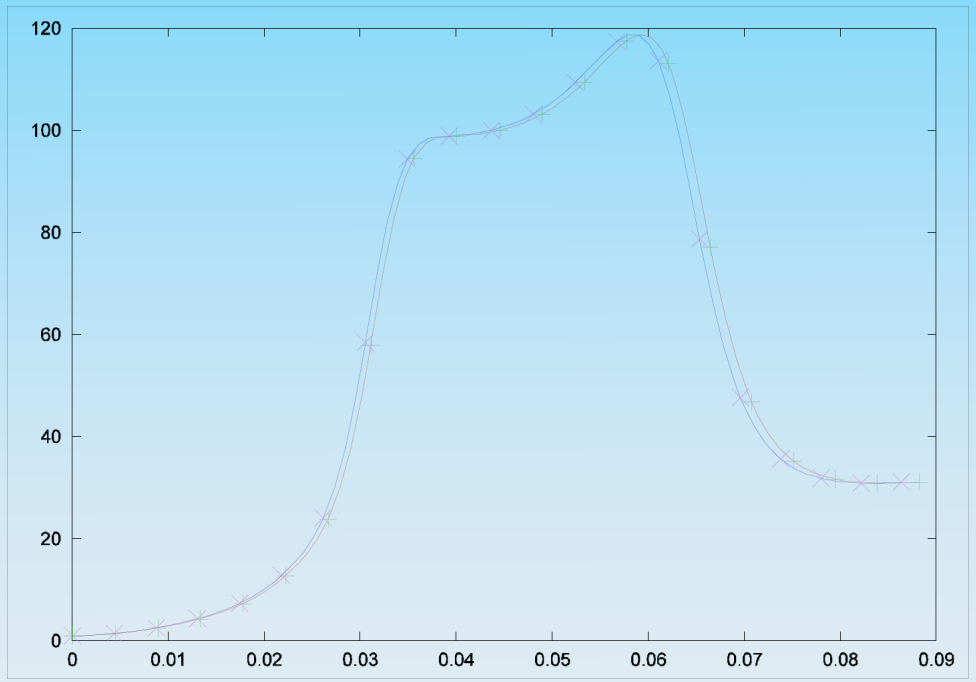


Nudged-Elastic-Band III

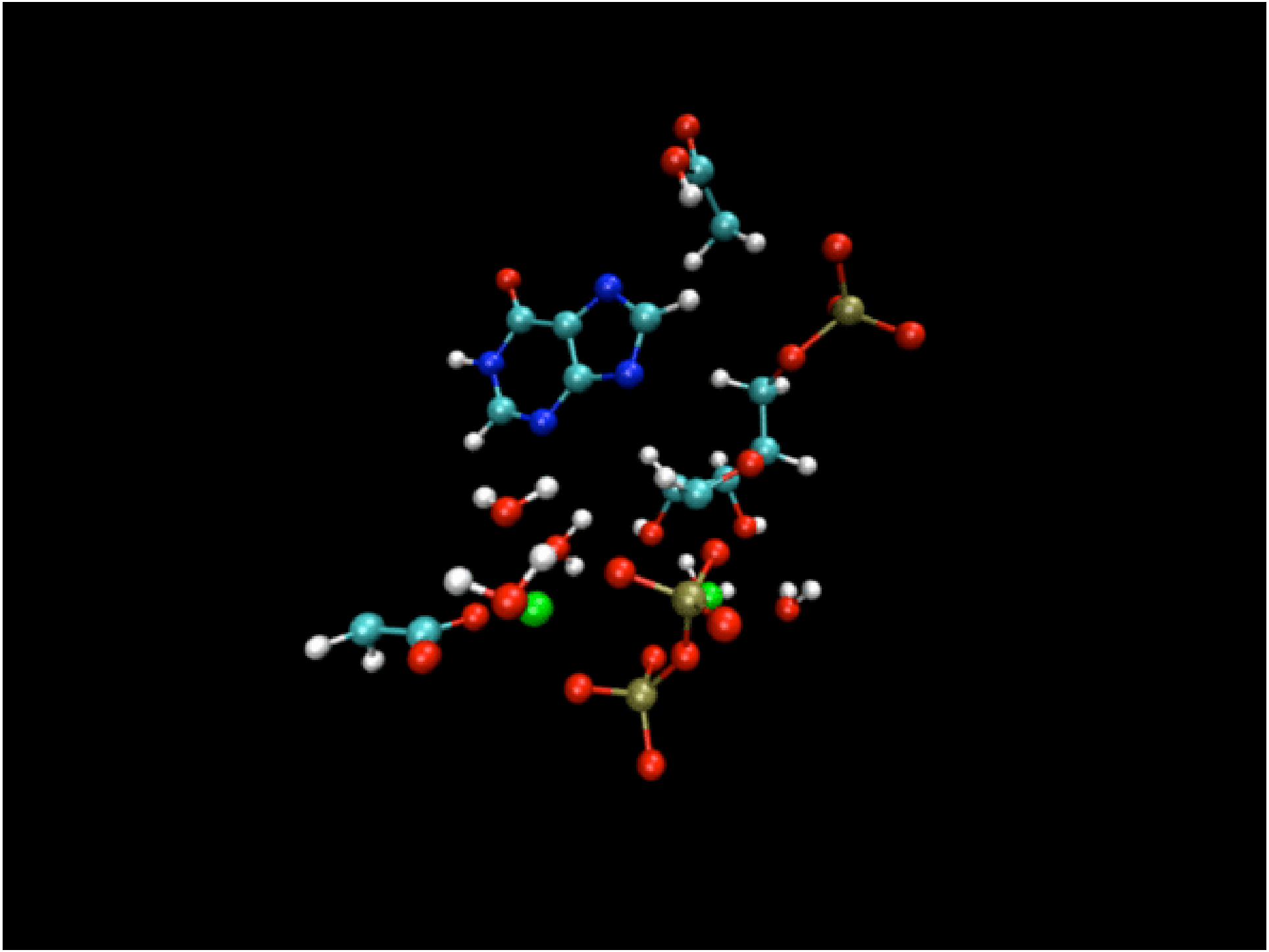
Reaction Coordinate Variables

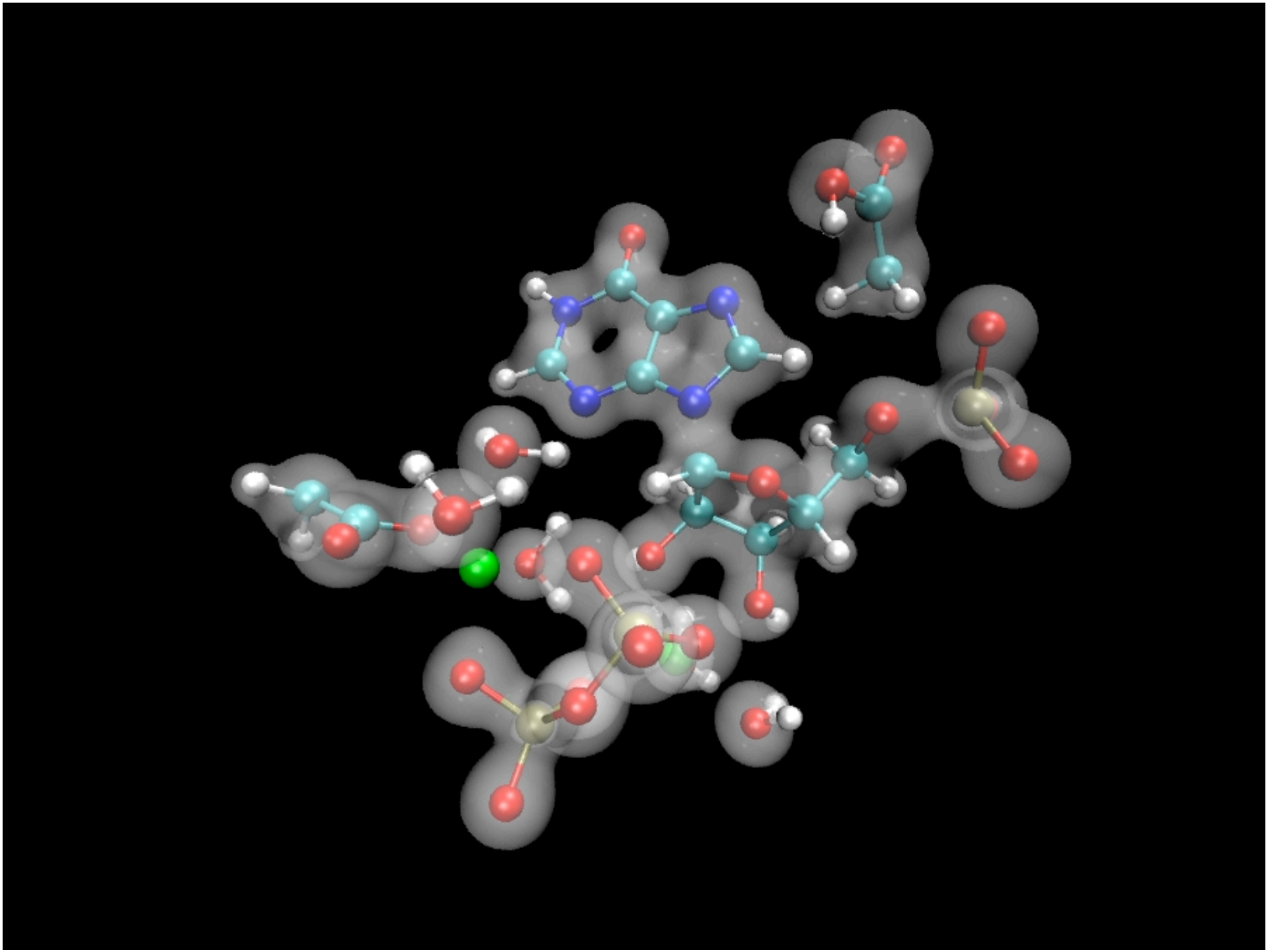


Temperature-Dependent Path



Red: 300 K ; blue: 0 K.





Free Energies

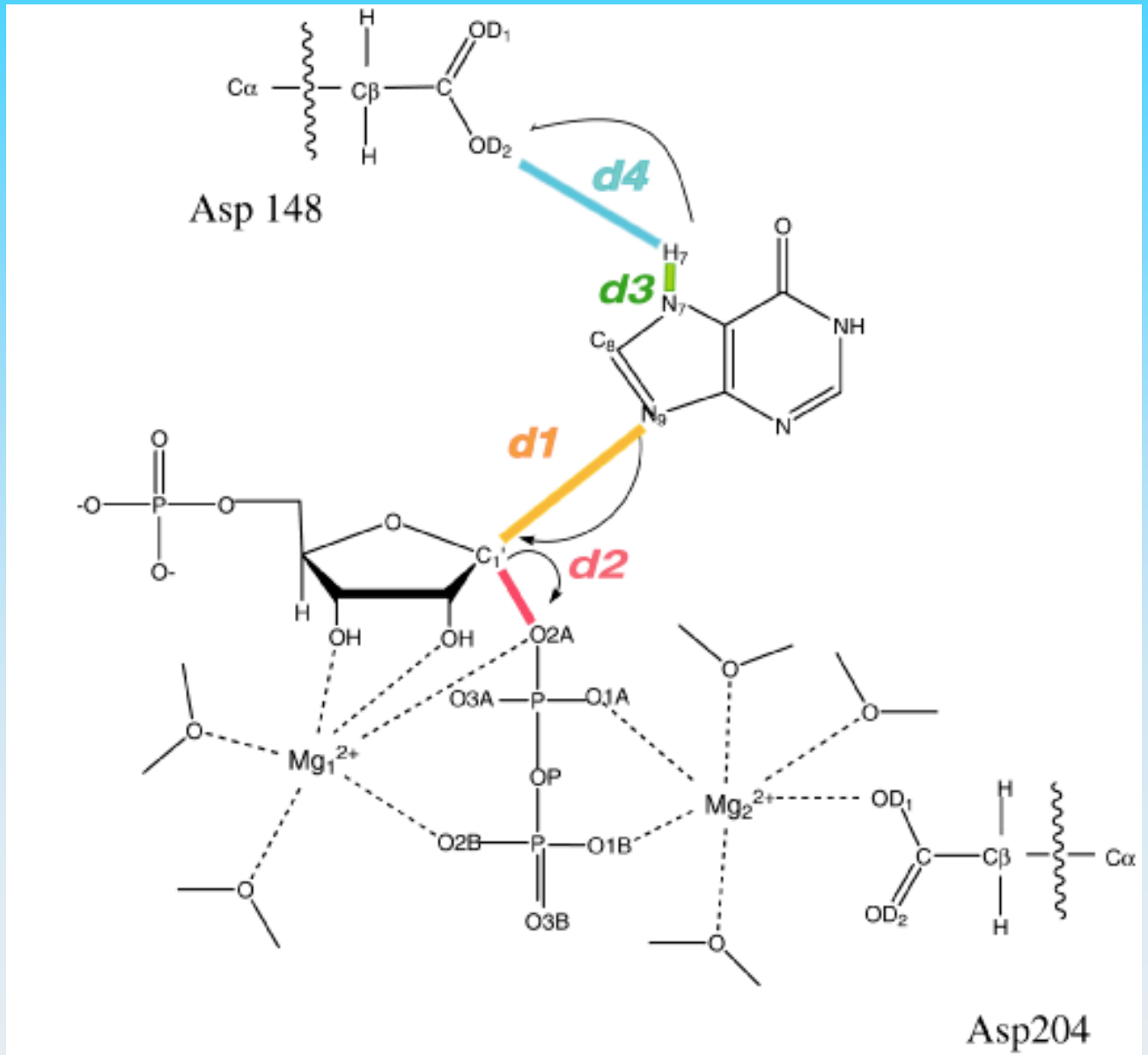
Thermodynamic average of a property χ:

$$\langle \chi \rangle = \int \chi(\Gamma)\rho(\Gamma)\mathrm{d}\Gamma$$

Potential of mean force:

$$\mathcal{W}(s) \propto -\mathrm{k_B T}\ \ln \int \exp(-\mathcal{V}(\Gamma, s)/\mathrm{k_B T})\,\mathrm{d}\Gamma$$

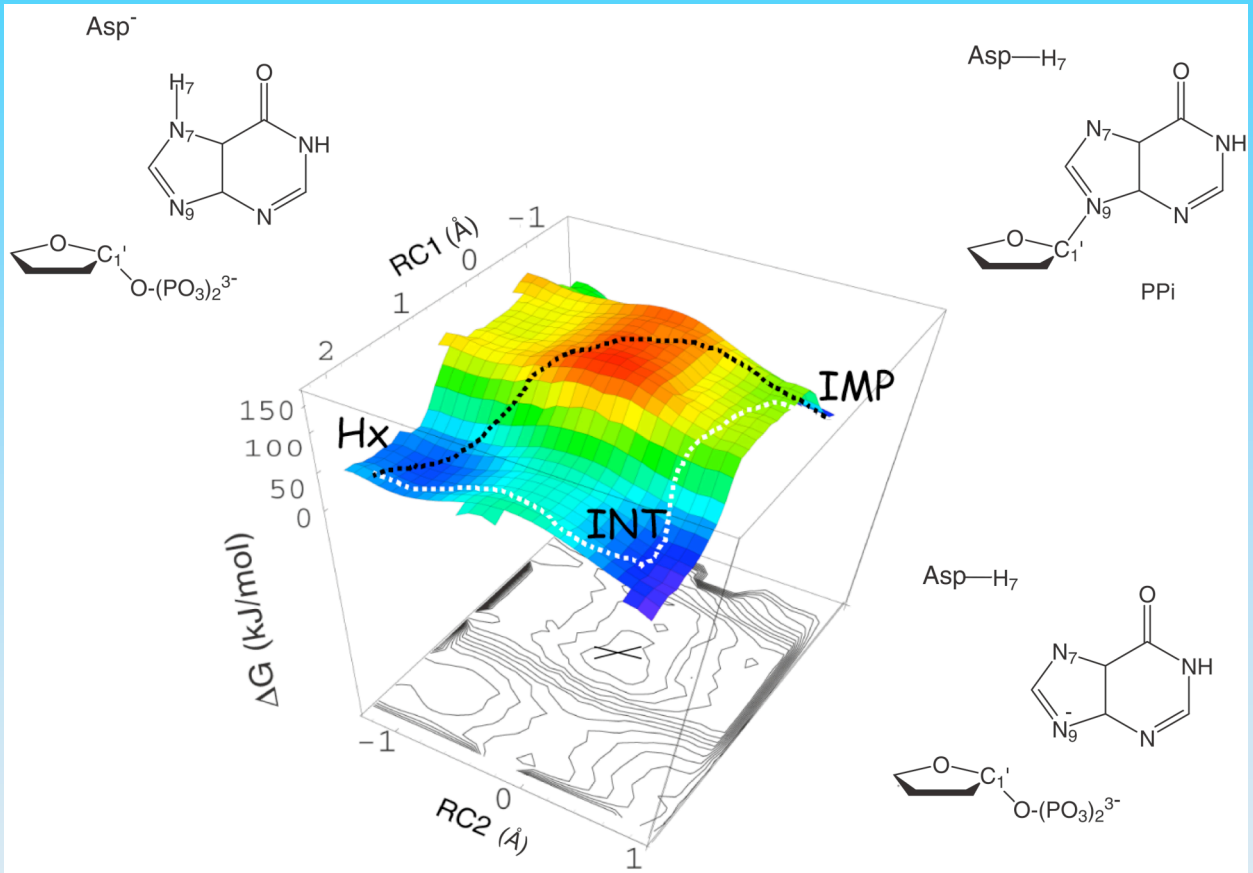
Application to HGXPRTase VIII



RC1 = d1 - d2

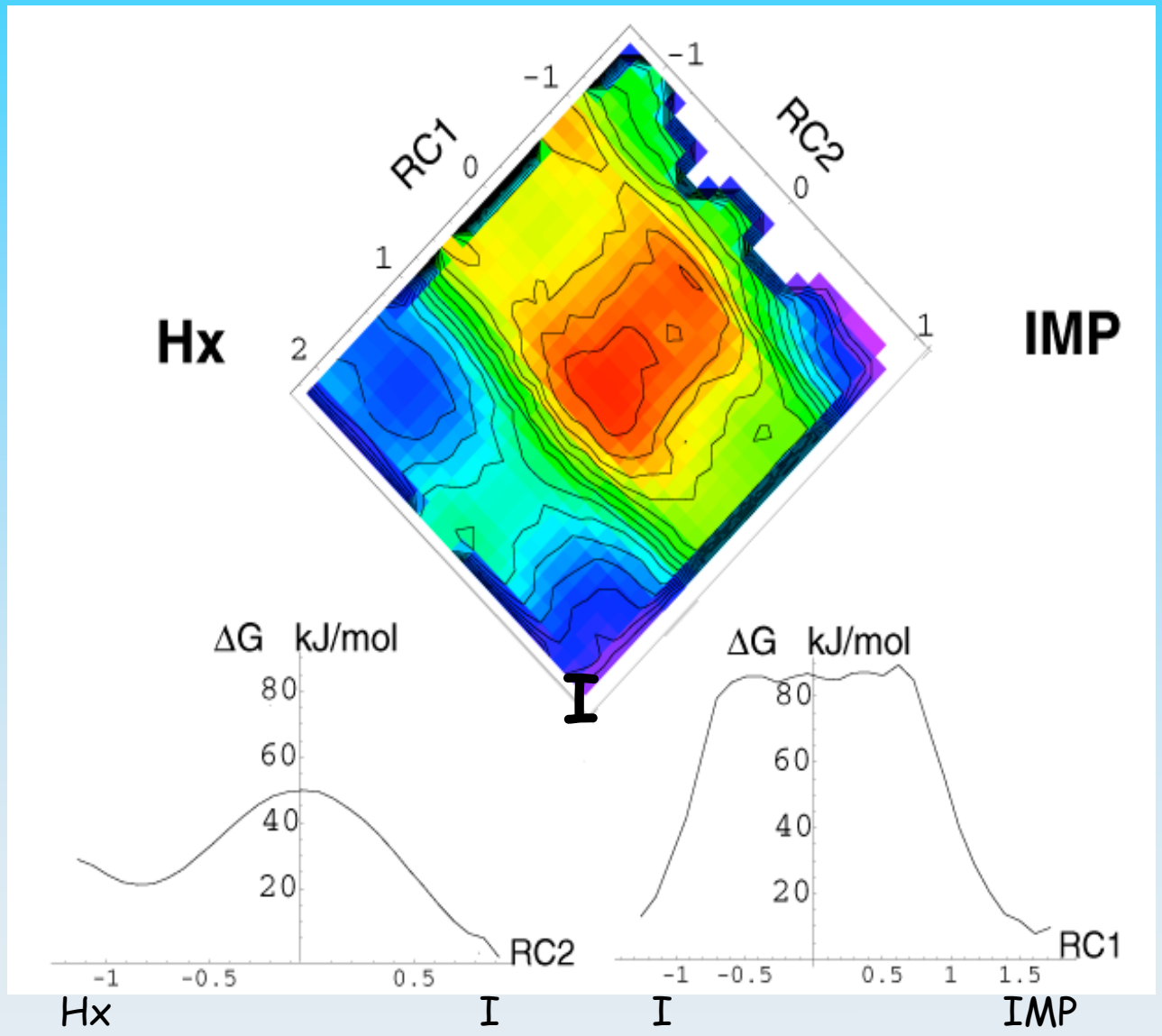
RC2 = d3 - d4

Application to HGXPRTase IX



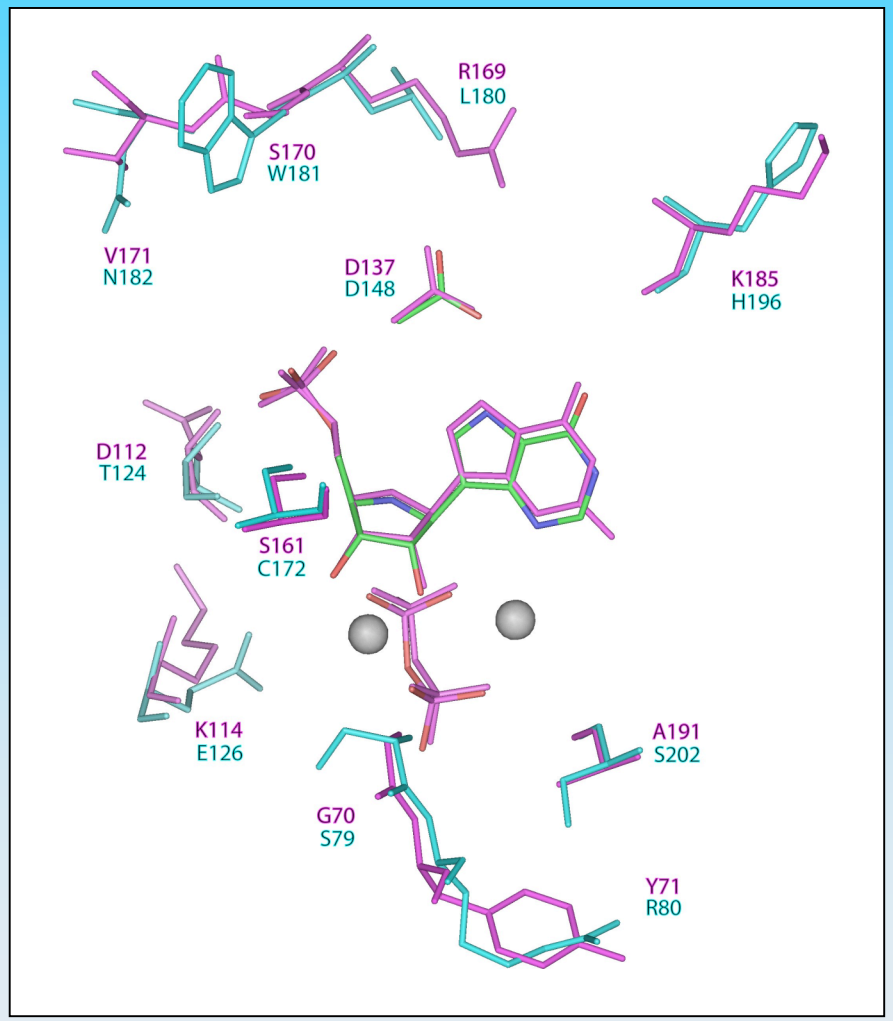
~ 20,000 hours of computer time.

Application to HGXPRTase X



Stepwise pathway favoured with a barrier of ~ 80 kJ mol^{-1}.

Application to HGXPRTase XI



Pf — blue ; human — magenta

- Comparison to the human enzyme — HGPRTase.
- Comparison of hypoxanthine and xanthine.

Application to HGXPRTase XII

- Overall mechanisms and energetics very similar.
- Proton transfer barriers higher in human enzyme.
- "Significant" differences in transition state structures:

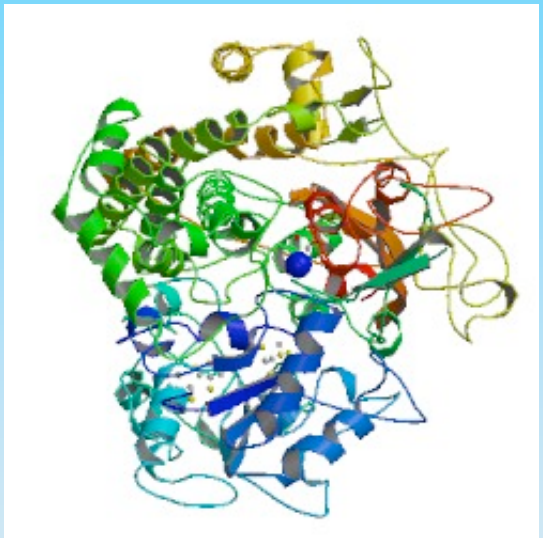
Distances (Å)	TS *Pf*	TS Human
C1'...N9	2.56 ± 0.08	1.82 ± 0.01
C1'...O2A	1.80 ± 0.22	2.29 ± 0.08

Perspectives - HG(X)PRTase

- Preferred mechanism:
  - Proton transfer
  - Glycosyl transfer
- Agreement with experimental data — barrier heights, KIEs.
- Ligand binding — affinities.

Proton Transfer Pathways in Hydrogenase I

I. Galván *et al.*, Proteins, '08
I. Galván and M. J. Field, J. Comput. Chem., '08



$$H_2 \Leftrightarrow 2H^+ + 2e^-$$

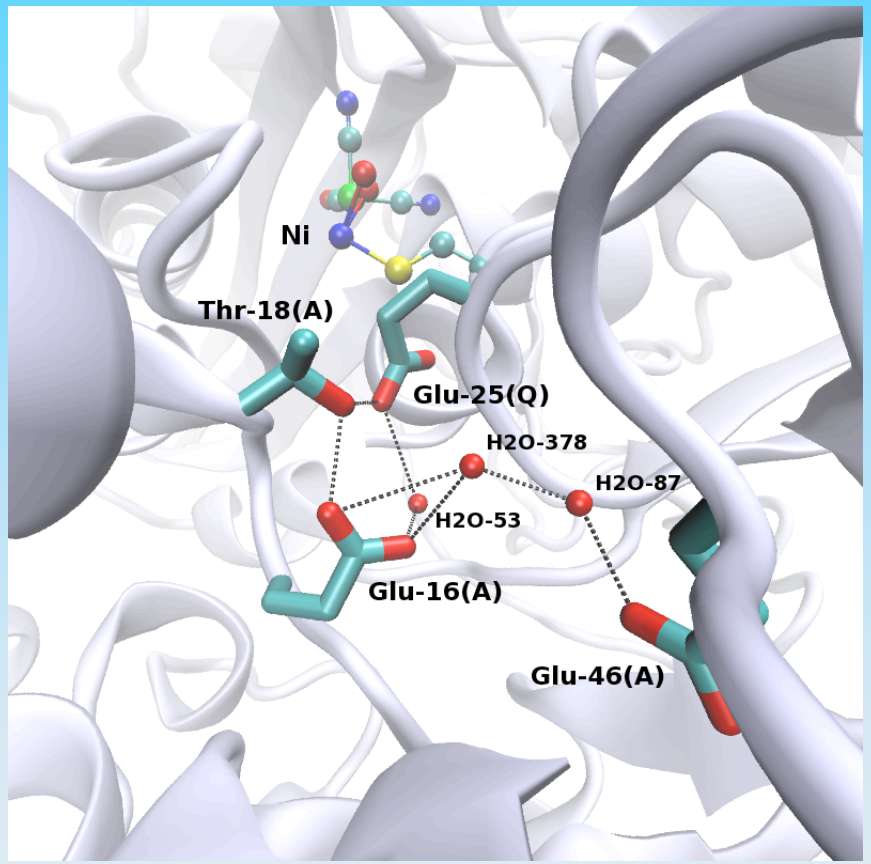
- Ni-Fe hydrogenase (A. Volbeda *et al.*, Nat. '95).
- Molecular channels (Y. Montet *et al.*, Nat. Struct. Biol. '97).
- Proton channels or pathways?

Proton Transfer Pathways in Hydrogenase II

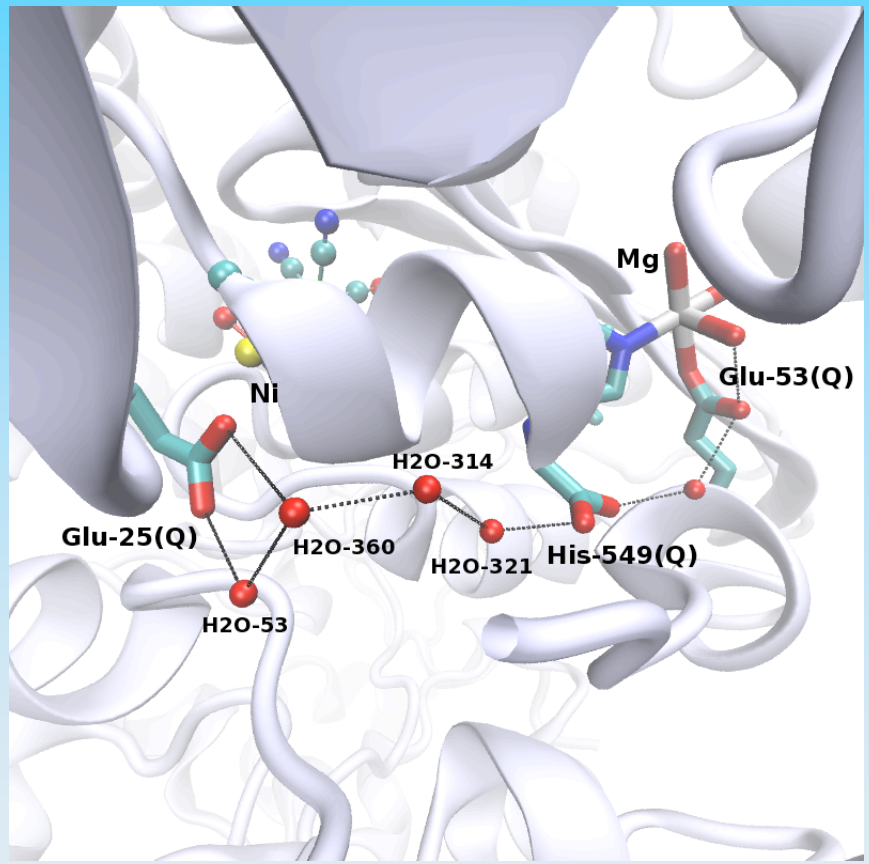
Strategy:

- Hypothesize possible pathways from X-ray structures.
- Identify individual proton transfer events.
- Evaluate partial NEB pathways.
- Piece together full profiles.

Proton Transfer Pathways in Hydrogenase III



$\Delta E^{\ddagger} \sim 40$ kJ mol^{-1}

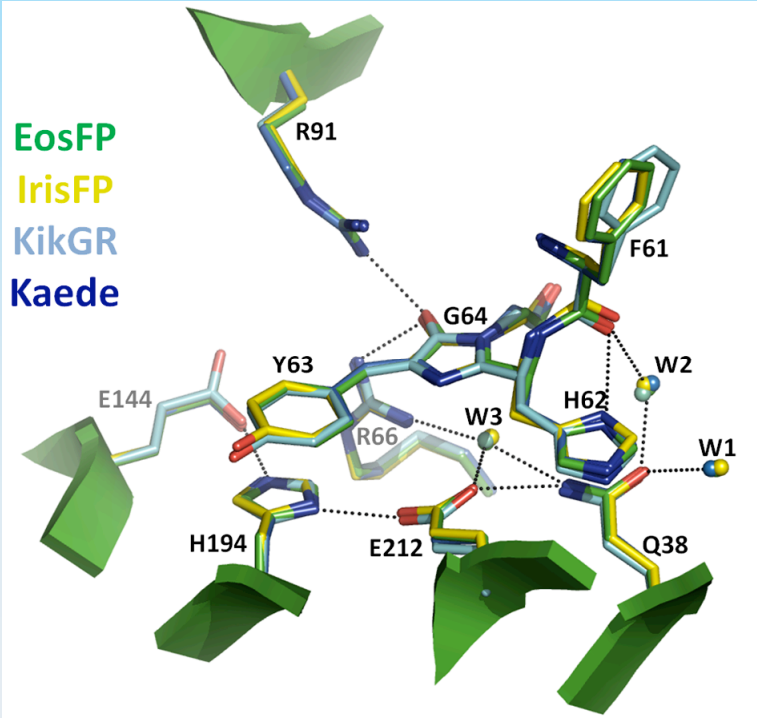


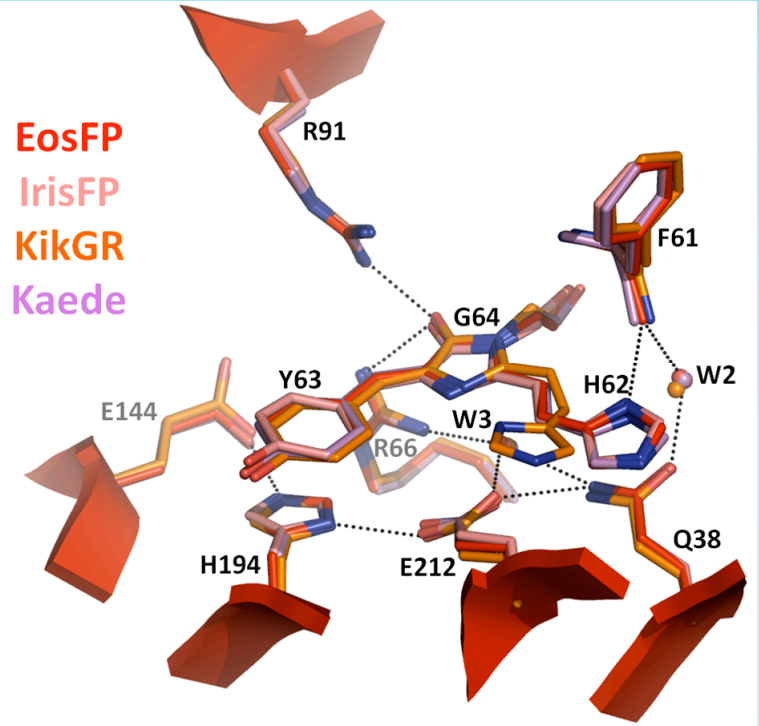
$\Delta E^{\ddagger} \sim 150$ kJ mol^{-1}

Fluorescent Proteins I

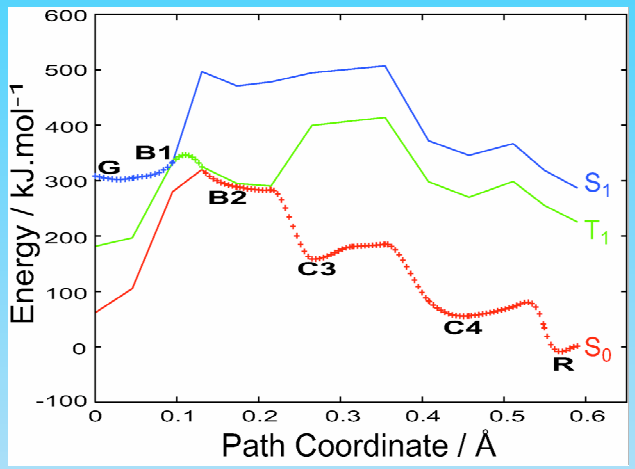
Mickael Lelimousin (in collaboration with
Virgile Adam, Dominique Bourgeois and Antoine Royant).

Irreversible transition between green and red emitting states
in photoconvertible FPs.

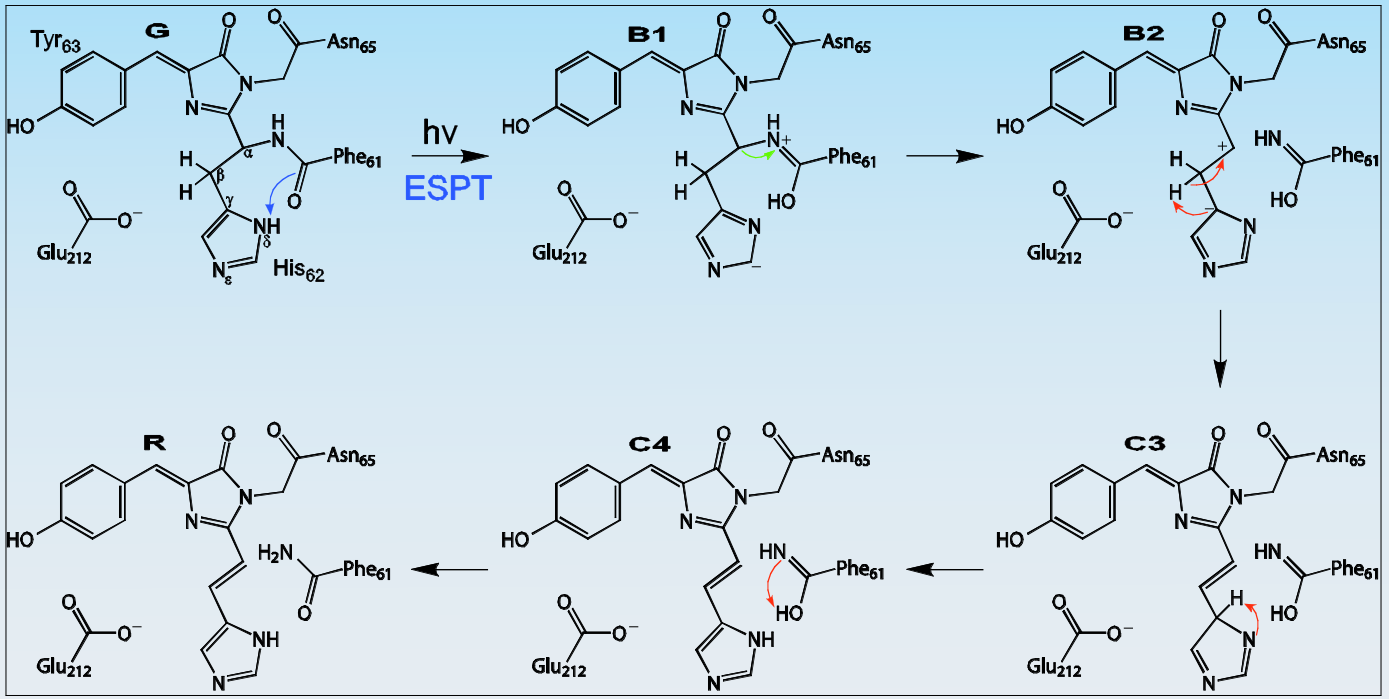




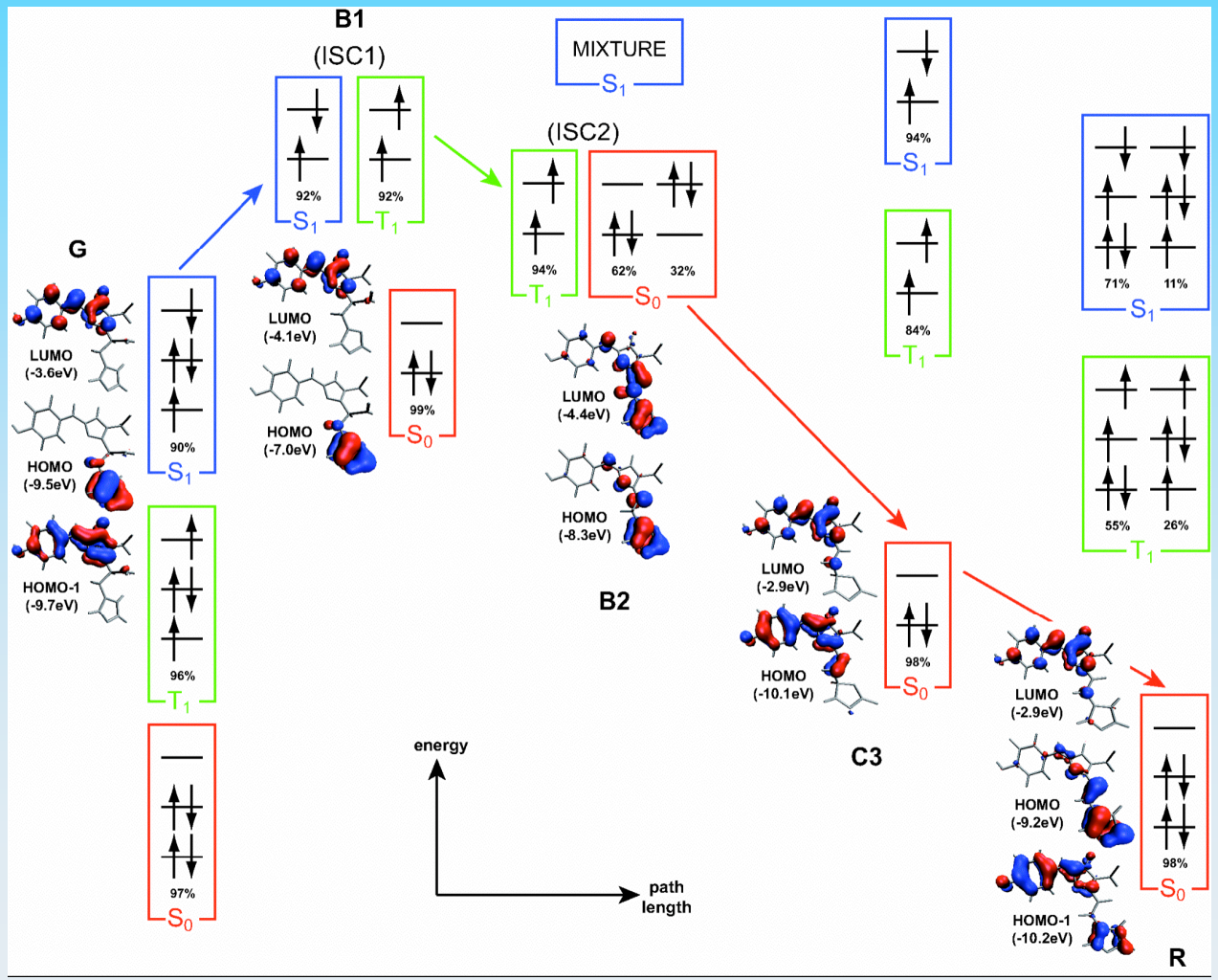
Fluorescent Proteins II



- Excited state NEB pathway calculations using a CI method.



Fluorescent Proteins III



Analysis and identification of factors important for photoconversion.