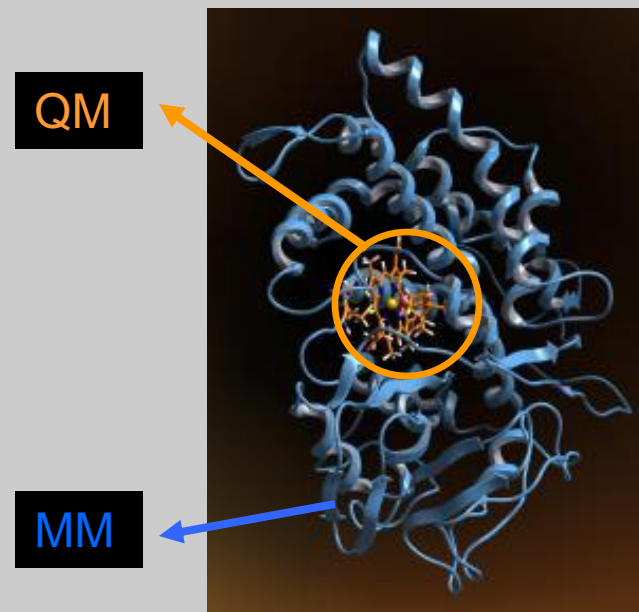


QM/MM: Understanding Enzyme Function and Reactivity. Methods and Applications

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NOTUR , Tromsø, 3. June 2008

Why use QM/MM methods?

- Enzymes, proteins are big systems that can not currently be handled by pure QM methods.
- QM methods are required to model reactivity (bond breaking, forming etc..). These phenomena generally take place in a limited and well defined region of the enzyme.
- MM are efficient methods to model molecules (conformational change, dynamics, electrostatics etc....).
- **QSite** allows to study large systems in shorter time by combining QM (**Jaguar**) and MM (**Impact**) methods. Special techniques have been implemented to run faster and to efficiently handle metal containing compounds (metalloproteins).



➤ **Methods**

- QM techniques.
- How to treat interfaces in QM/MM?

➤ **Applications**

- Methane Monooxygenase: reaction mechanism. What is the role of the protein?
- Cytochrome P450: Role of Propionates
- Accuracy of QM/MM methods.

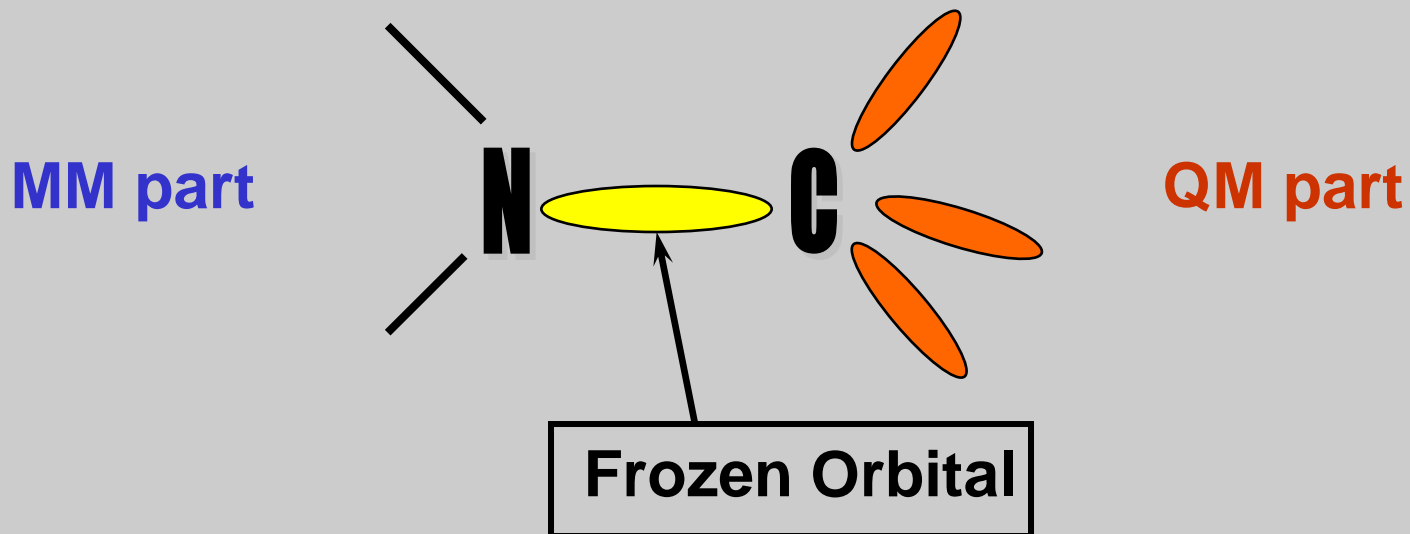
QM Methods: General Considerations

- QSite relies on Jaguar and thus takes advantage of all techniques developed for Jaguar.
 - DFT (B3LYP, M05, M06, etc),
HF
LMP2 only for single point calculations.
 - UDFT and broken symmetry approach possible.
 - Pseudospectral Methods: Fast Algorithms to compute the 2 electron integrals.
 - Special algorithms to improve the initial guess for metal containing compounds.

QM/MM Methods: Frozen Orbital Interface

- Modelling the interface between the QM and the MM regions is a difficult task.

First approach: the frozen orbital method.

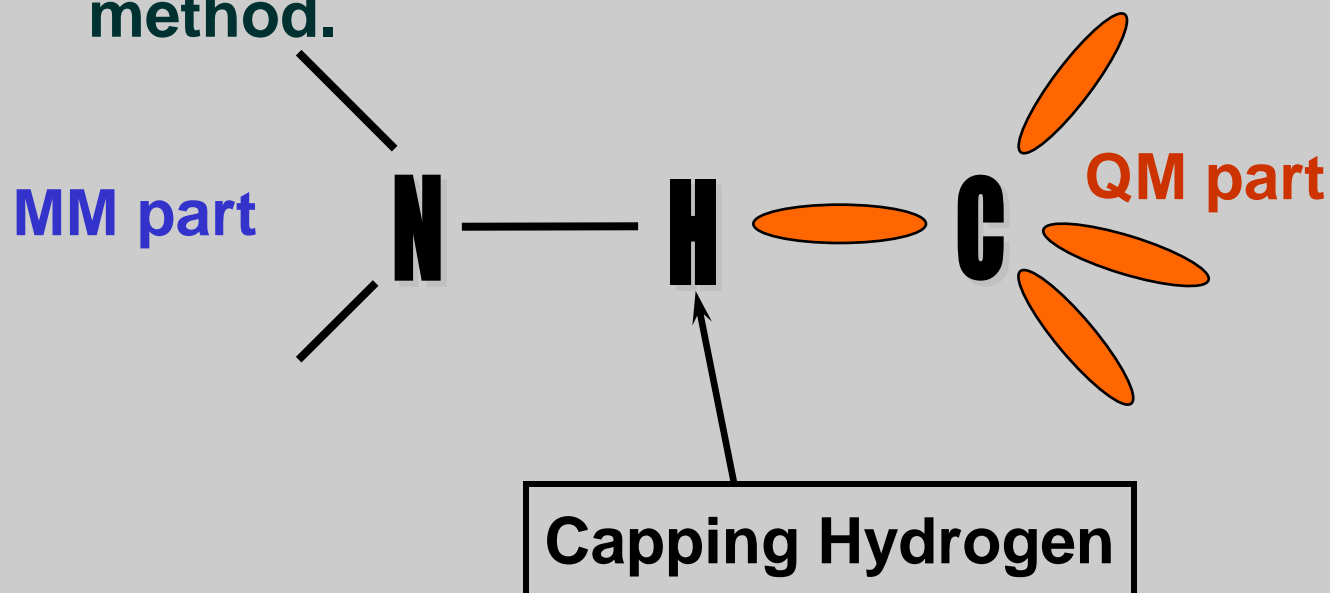


QM/MM Methods: Validations of the Frozen Orbital Interface

- **Relative energetics of dipeptide side chain rotamers**
 - Accurate QM energies computed at LMP2/cc-pVTZ (-f) level
 - ~200 rotamer states for all 20 amino acids
 - Average errors 0.5-1.0 kcal/mol (comparable to force field)
- **Hydrogen bonded dimers**
 - ~200 dimers – functional groups representing amino acid backbone, side chains
 - Average deviation vs LMP2 binding energies ~0.5-1 kcal/mol
- **Deprotonation tests – compared with full QM calculations**
 - Average error ~0.5 kcal/mol (out of ~200-400 kcal/mol)
- **Systematically increase size of MM region, number of QM/MM cuts**
 - Extensive tests for dioxygen binding in hemerythrin
 - Average errors ~0.5-1.0 kcal/mol

QSite: The Hydrogen Cap (Link-Atom) Method

- Frozen orbitals are accurate but need to be parametrized. Only available for amino acids in proteins.
- Another Method: the Hydrogen capped (link-atom) method.

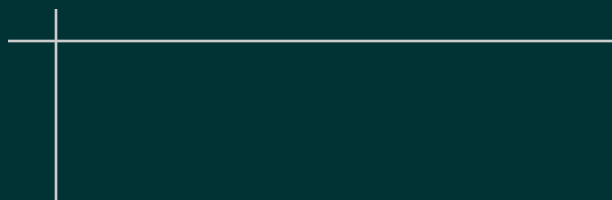


QSite: The Hydrogen Cap (Link-Atom) Method

- **Treatment of the electrostatic at the QM/MM boundary for the Hydrogen cap method requires to be adjusted:**
 - **It can be useful to use a smooth charge distribution on the MM atoms next to the QM region.**
 - **A point charge with modified parameter can also be used on this MM atom.**

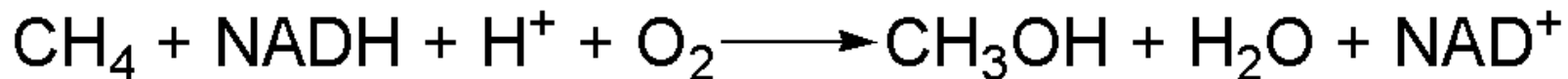


QM/MM: Applications to Study Reactivity: Methane Monooxygenase



Applications: MMO reactivity.

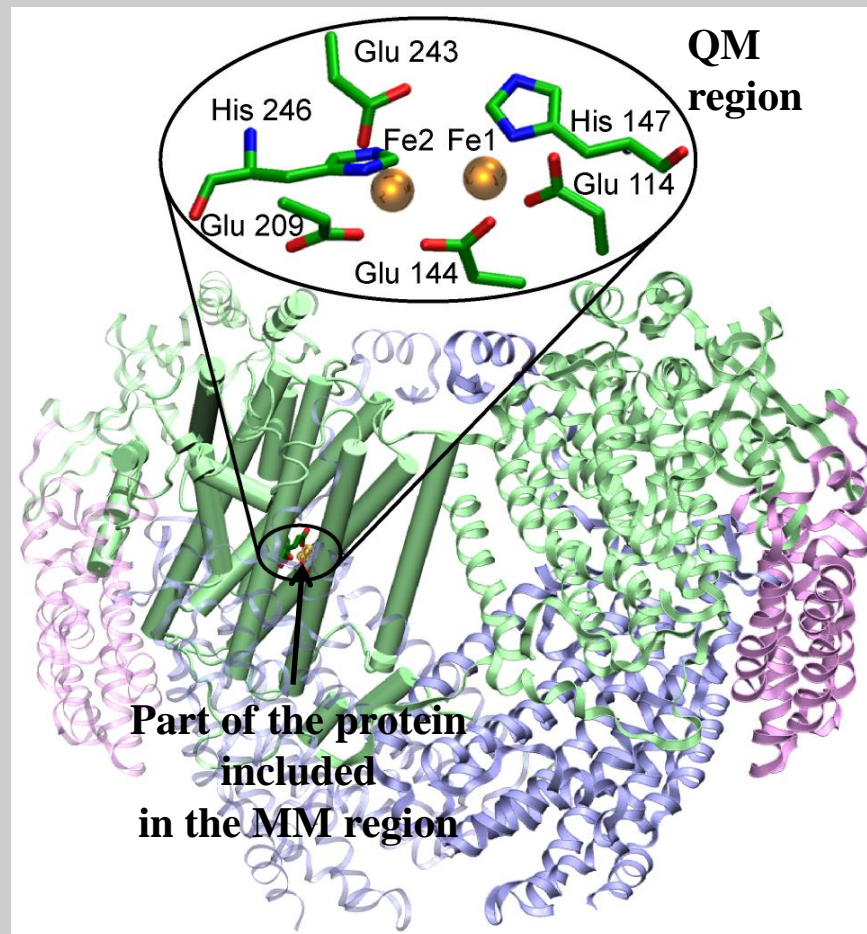
- Multicomponent enzymatic complex that catalyses the conversion of CH_4 into CH_3OH .



- ✓ Reductase component (MMOR): e^- transfer from NADH.
 - ✓ MMOB: required for efficient catalysis.
 - ✓ MMOH: Hydroxylase catalyses the oxydation at a carboxylate-bridged dinuclear iron center.
-
- Study of the intermediates in catalytic cycle of Methane Monooxygenase.

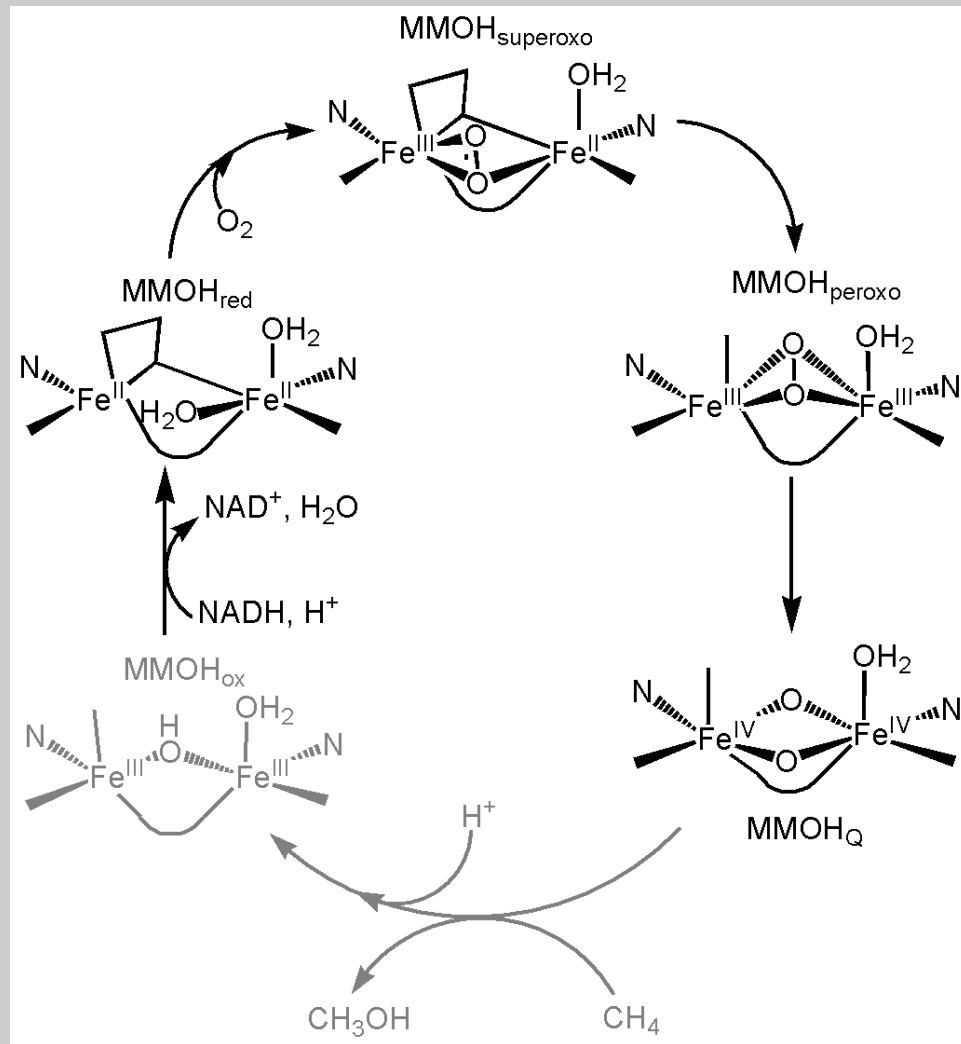
Applications: MMO reactivity.

- ✓ MM part: sphere of 35Å
- ✓ QM: ~ 150 atoms.
- ✓ Geometry: lacvp**.
- ✓ Single point calculation: Lacv3p**, cc-pVTZ(-f).
- ✓ Antiferromagnetic coupling of the diiron center when required.



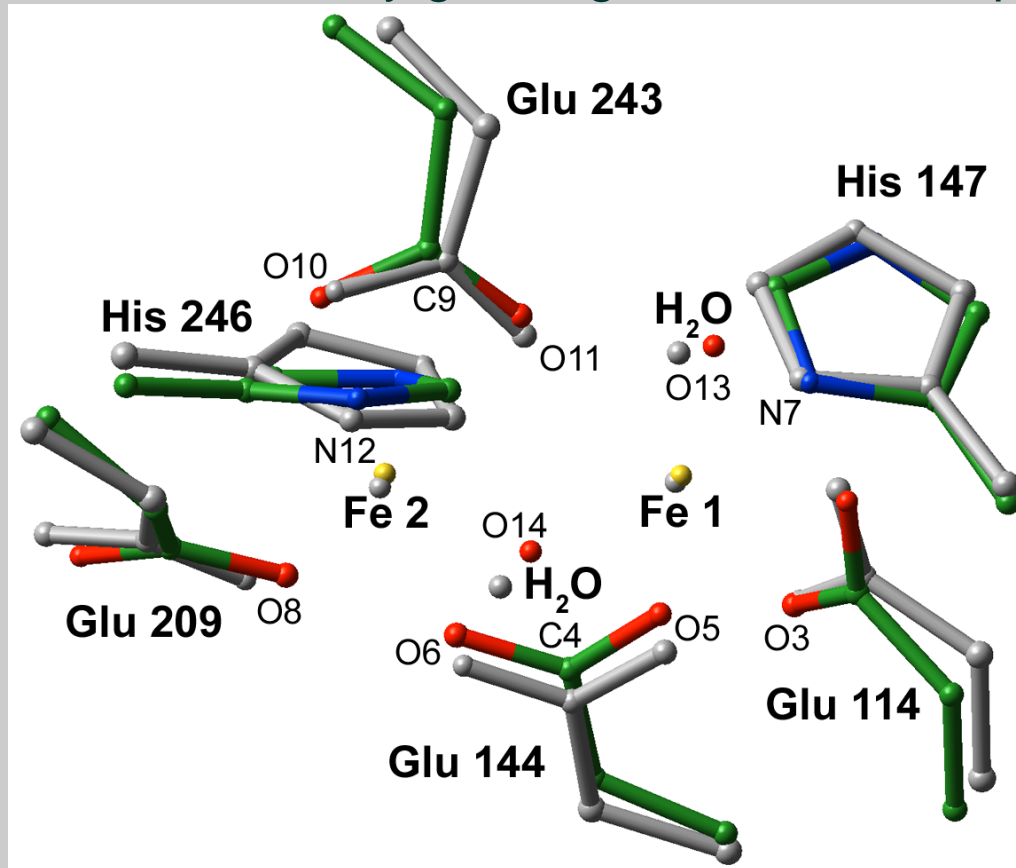
Applications: MMO reactivity.

- Intermediates studied along the catalytic cycle.



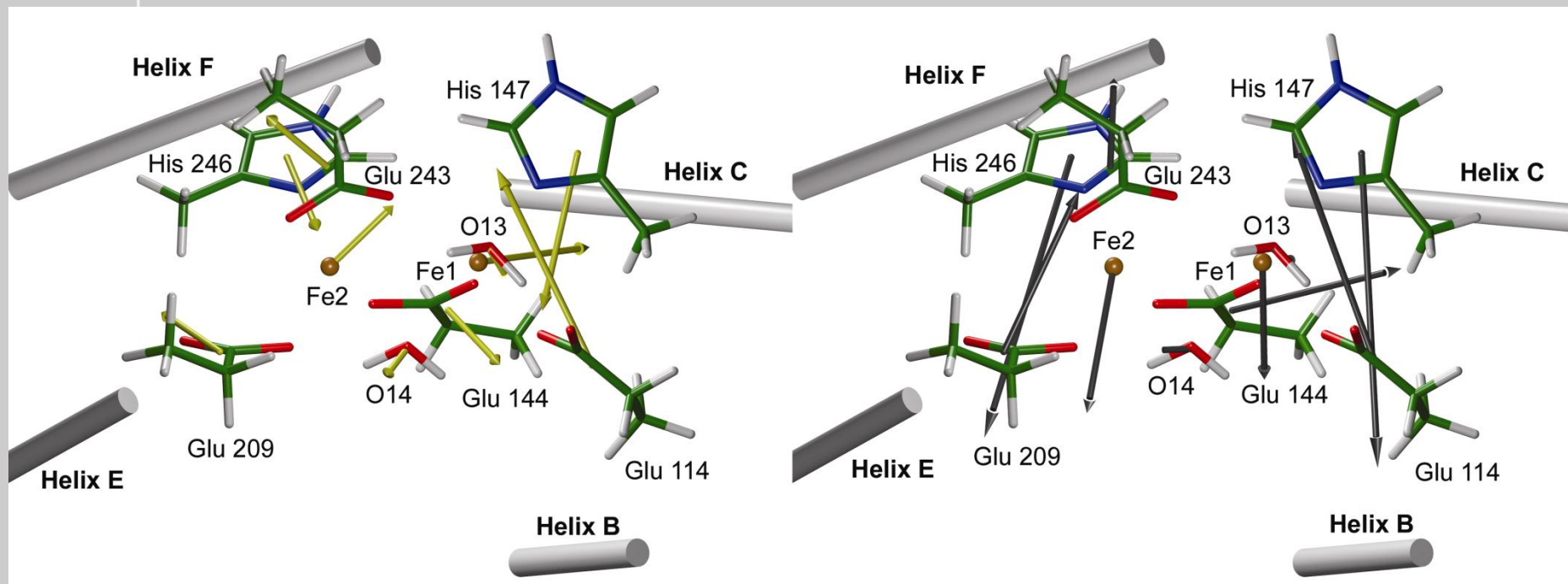
Applications: MMO reactivity.

- QM/MM Model in very good agreement with experiment.



- Fe-Fe distance is in very good agreement with experiment (RMSD 0.33 Å whereas a large discrepancy was observed with QM models).

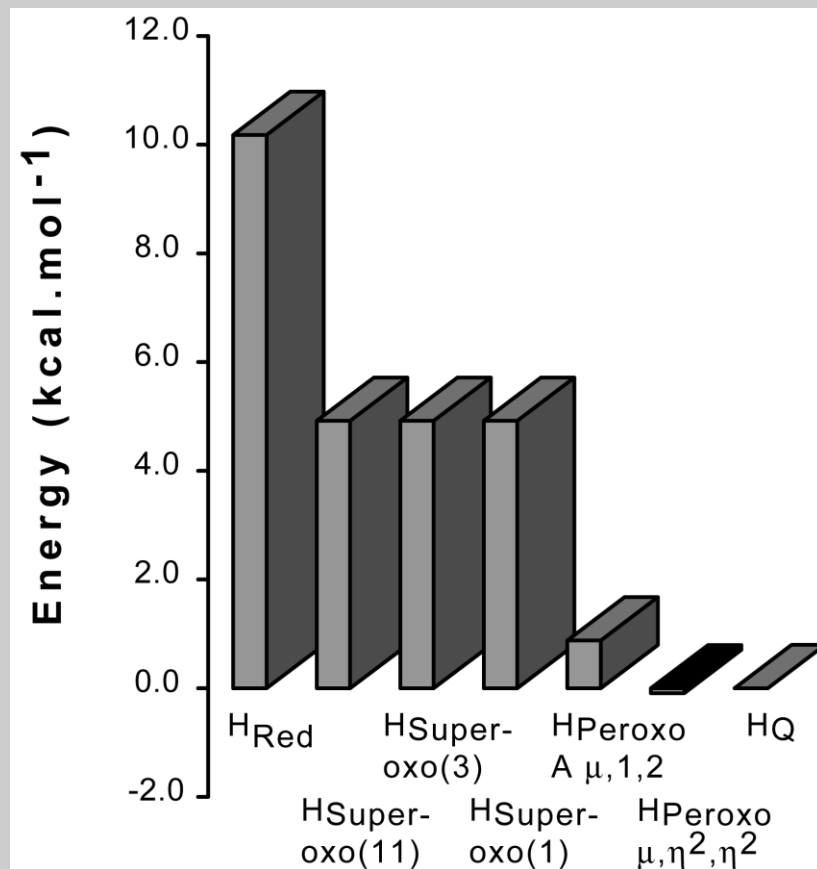
Applications: MMO reactivity.



- Forces of the protein are acting on the active site.
- These forces seem to compress the active site and can be explained by the reduced Fe-Fe distance observed in the QM/MM calculations and in the experiment.

Applications: MMO reactivity.

Species	$S_z(S_z+1)$	QMMMM	QM	Difference
H _{red}	9	3.29	3.64	0.35
H _{red-no-wat}	9	3.38	3.76	0.38
H _{superoxo}	11	3.44	3.79	0.35
H _{superoxo}	3	3.41	3.65	0.24
H _{superoxo}	1	3.46	3.51	0.05
H _{peroxo} (μ - η^2 , η^2)	1	3.48	3.61	0.13
H _{peroxo} (μ - η^2 , η^1)	1	3.27	3.36	0.09
H _{peroxo} (A μ 12)*	1	3.47	3.57	0.10
H _{peroxo} (S μ 12)*	1	3.64	4.02	0.38
H _Q *	1	2.84	2.70	-0.14



- The protein matrix decreases the Fe-Fe distance for all the intermediates along the path.

Applications: Substrate hydroxylation by MMO

- **6 substrates studied, 5 of which have been investigated experimentally by Lippard and coworkers**
 - Methane – large KIE, so H atom abstraction is rate determining
 - Ethane – KIE=1, so H atom abstraction is not rate determining
 - Hypothesis: barrier to substrate diffusion into protein is rate limiting step for ethane
 - Consistent with calculations
 - Estimated barrier height is 16 kcal/mol
 - Calculated free energy barrier for ethane reaction is 12 kcal/mol
 - Experimental and calculated methane barriers are larger than 16 kcal/mol, so this is consistent as well

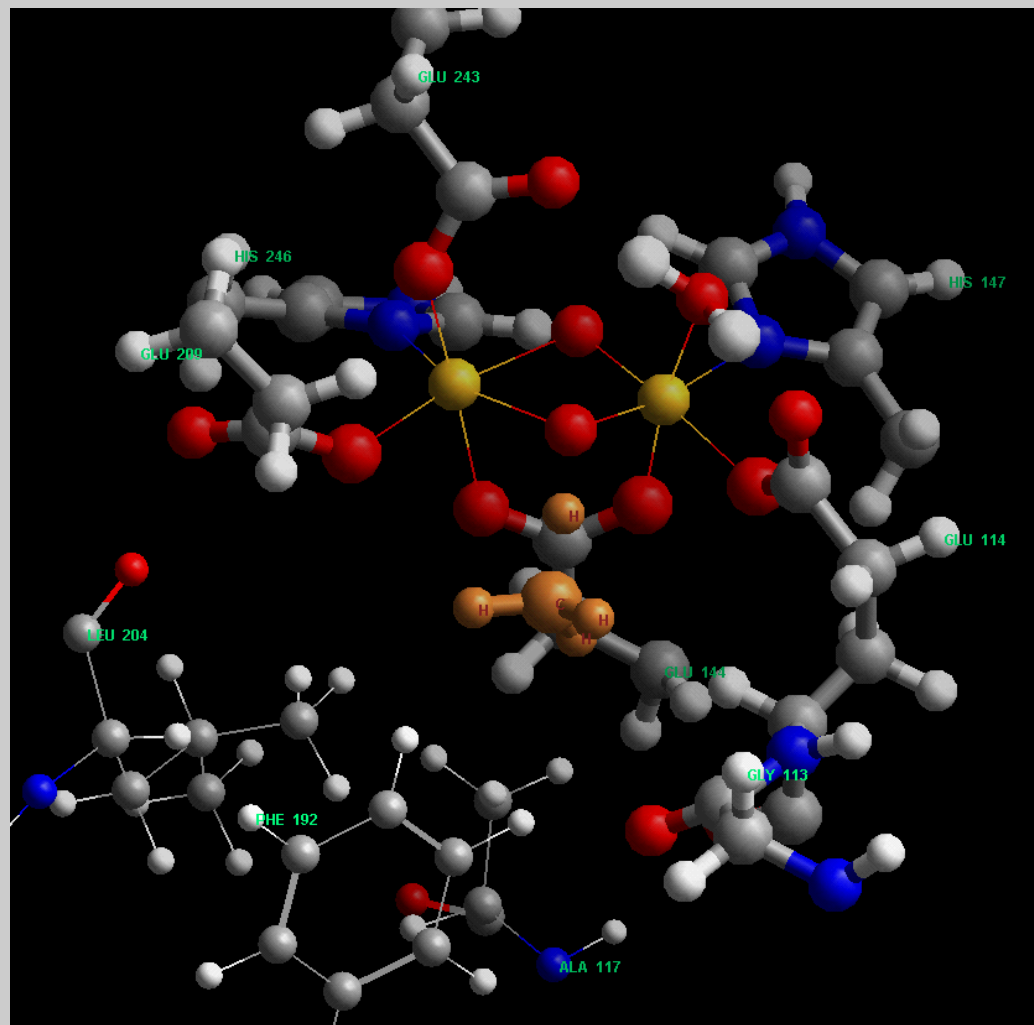
Applications: Substrate hydroxylation by MMO

Study of
transition states
for the
Hydroxylation:

CH_4 substrate

H-abstraction
transition state

Then hydrogen
rebound.

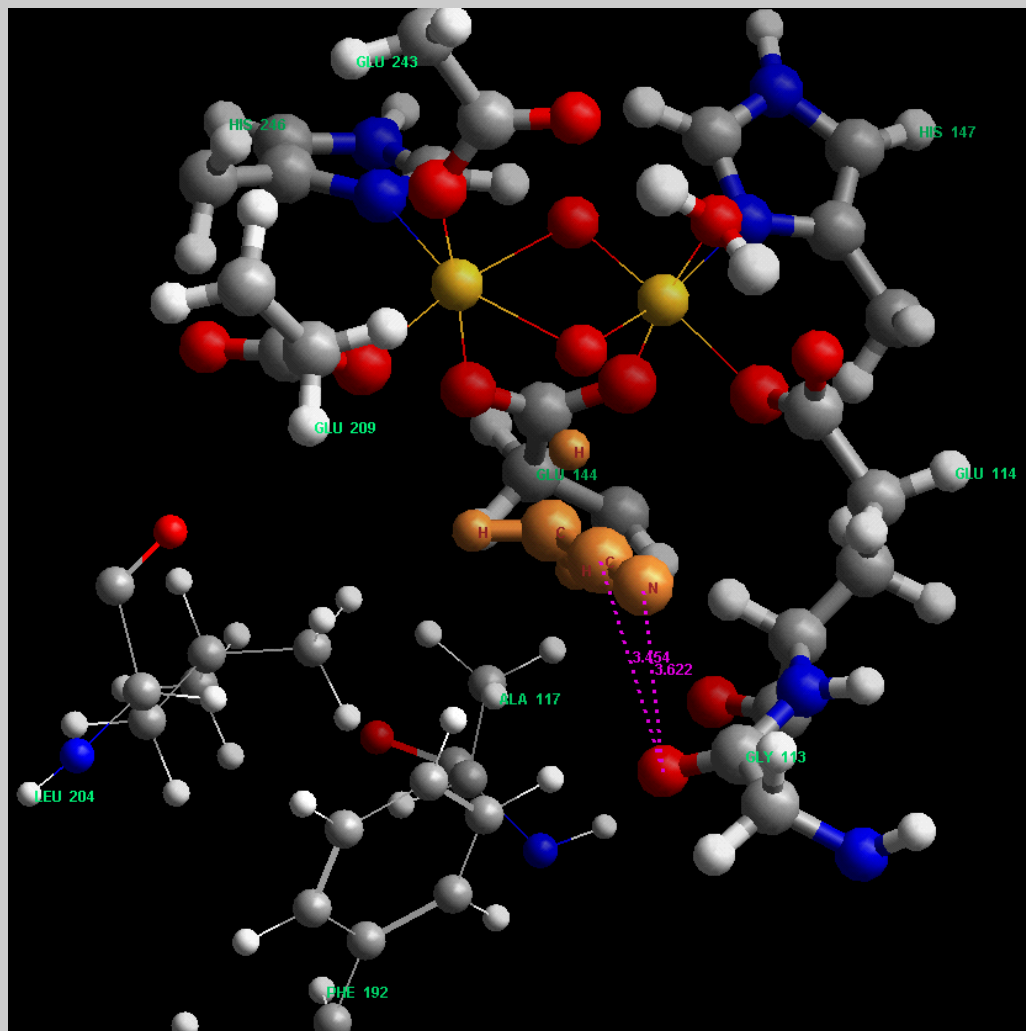


Applications: Substrate hydroxylation by MMO

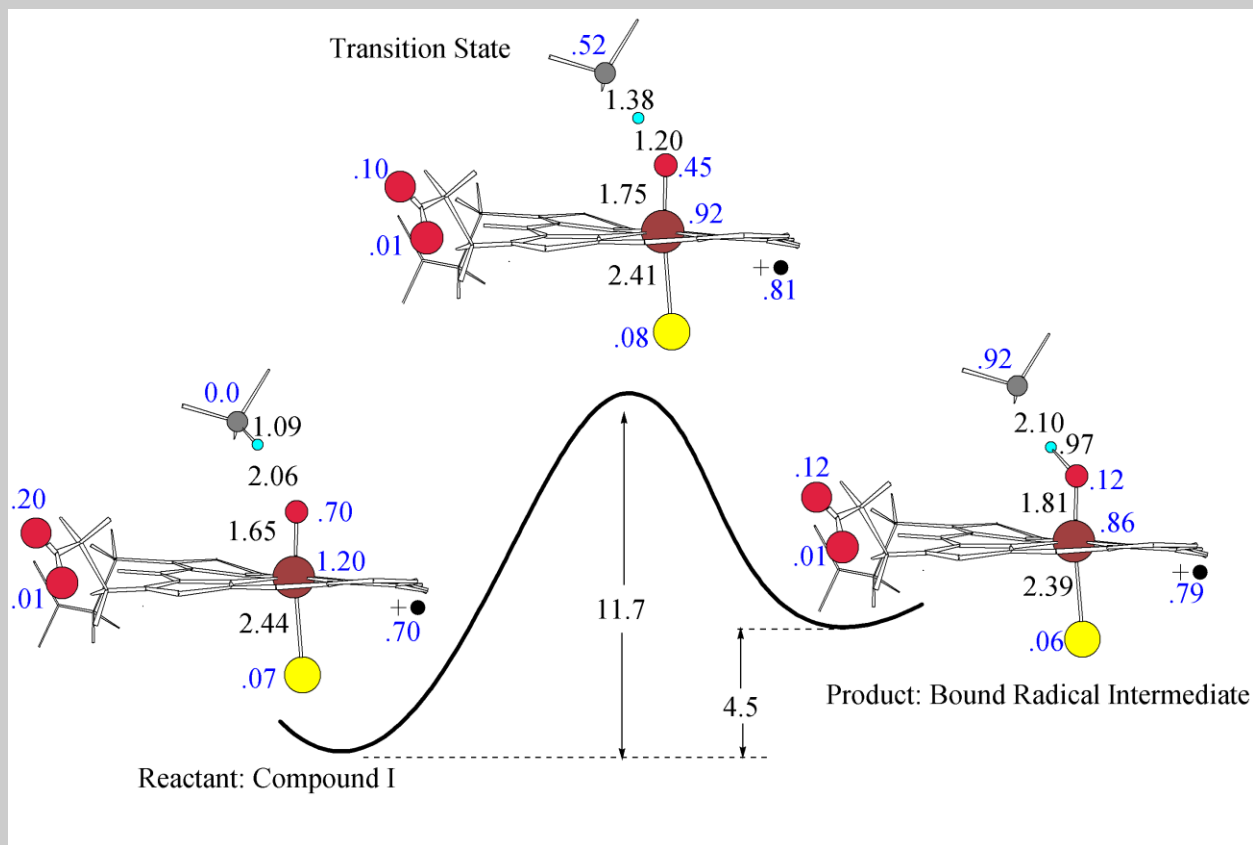
Hydroxylation:

CH_3CN
substrate

H-abstraction
transition state



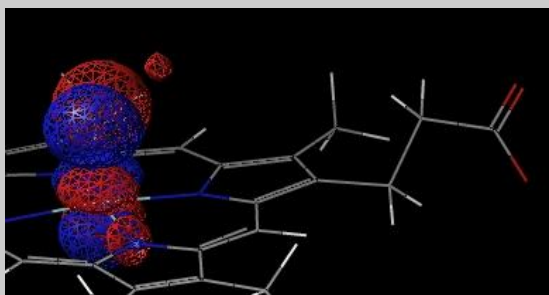
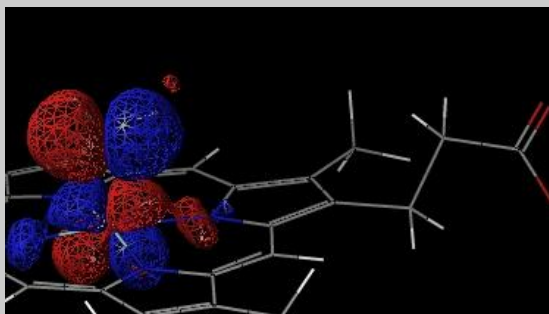
Applications: Cytochromes P450



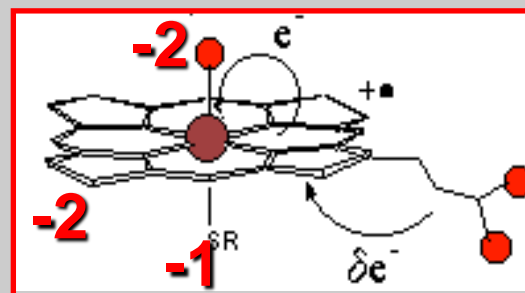
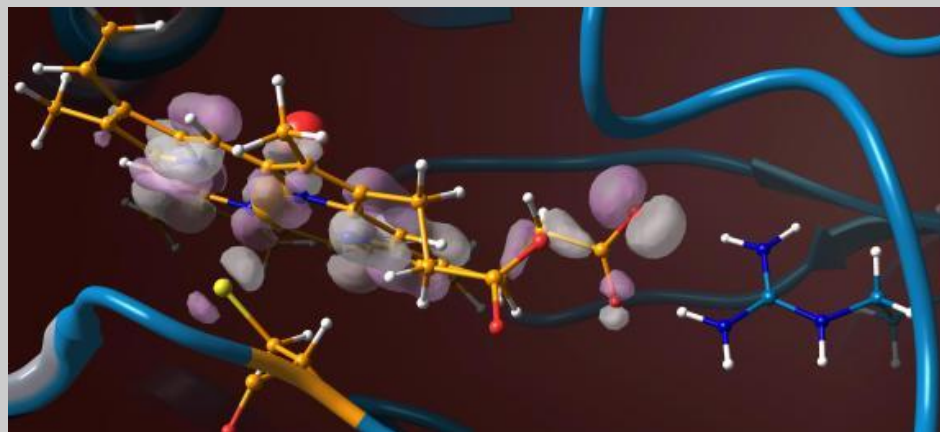
Low hydrogen abstraction barrier and spin delocalization on the propionates.

Applications: Cytochromes P450

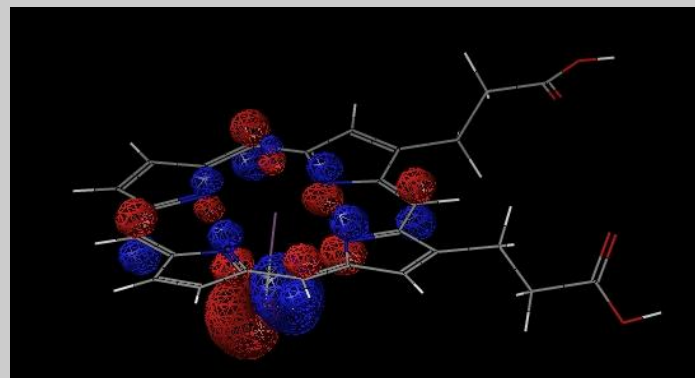
Two unpaired electrons in compound I in the Iron-oxo moiety



Delocalized third unpaired electron

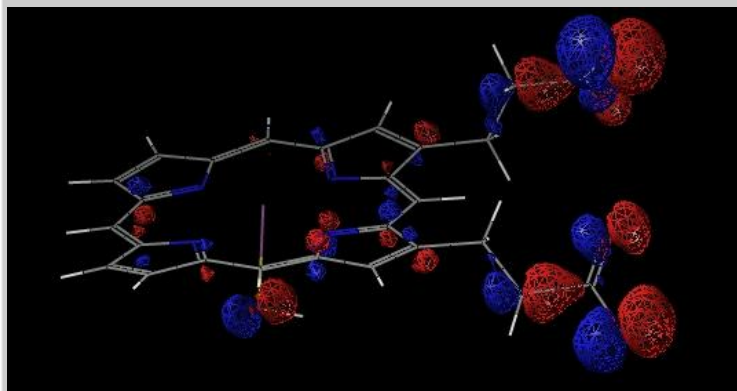
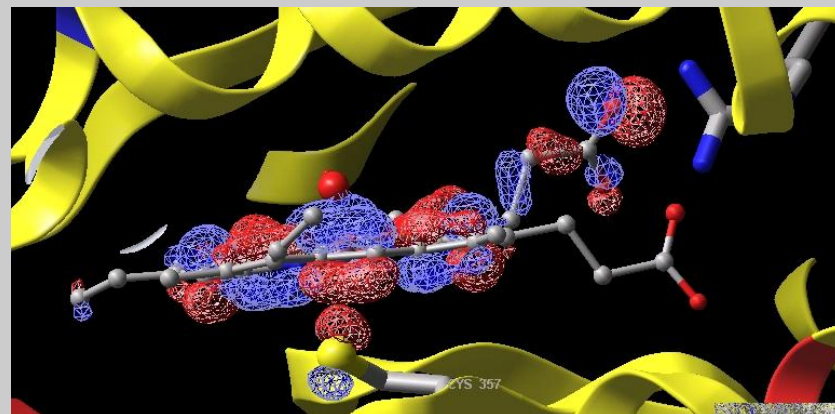


Applications: Cytochromes P450



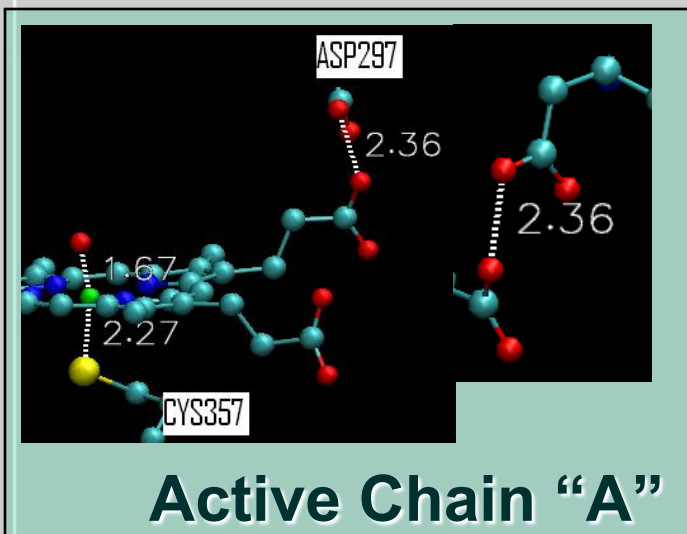
→ **Perfect Screening**

**Intermediate,
“protein screening”**



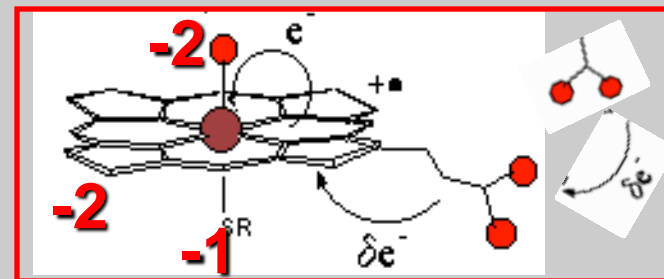
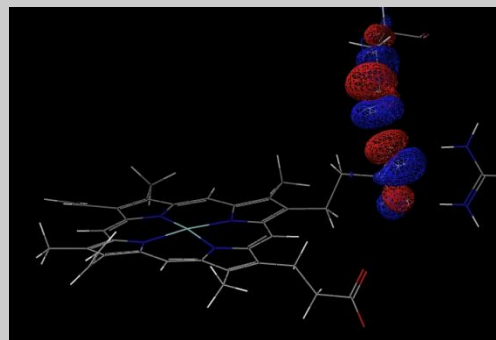
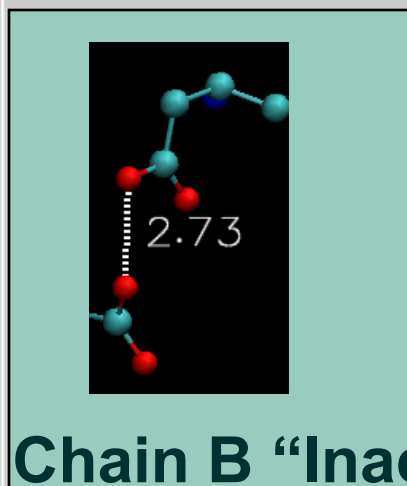
→ **Non-screening**

Applications: Cytochromes P450



	QM/MM		MM	
	COO ⁻	COOH	COO ⁻	COOH
O-O distance	2.32	2.8	3.3	2.78

QM/MM minimizations where the Hem, Cys357, camphor, Asp297 and Arg299 are treated at the quantum level of theory



QM/MM Methods: Accuracy

➤ First principles calculation of reaction rates in enzymes is challenging.

- Issues about intrinsic DFT accuracy for barrier heights.
- Issues about accurate treatment of metals by DFT (low lying energy states, energies, relativistic effects, SO coupling).
- Must properly position substrate – highly nontrivial particularly without crystal structures – induced fit effects on protein can be critical. Need to sample different conformations.
- Calculations of entropy are very difficult.
- Nevertheless some progress has been made – most reactions (in MMO and other systems) work reasonably well.

QM/MM Methods: Accuracy

Summary of Thermochemical Results for Enzymatic Reactions
(data in kcal/mol)

Enzyme	Reaction	Calc.	Expt
MMO/acetone	hydrox.	13.5	13.9
MMO/nitrometh.	hydrox.	18.2	16.2
MMO/methane	hydrox *	18.6	15.4
Hr	O ₂ binding	5.3	7.2
TIM	proton transfer	14.1	13
Beta-lactamase	hydrolysis	14.3	14.3

Gherman et al. JACS, 127, 1025, 2005;

Guallar et al., J. Mol. Biol., 337, 227, 2004

Wirstam et al., JACS, 125, 3980, 2003

Gherman et al. JACS, 126, 7652, 2004

QM/MM Applications in Drug Design

- QM/MM methods are computationally expensive: can not be applied in high throughput screening.
Computers are faster and faster.
- Place to improve accuracy of the methods.
Active development of new functionals in DFT.
- Polarization effects are generally neglected but they need to be taken into account in certain cases.
- Full protein description + *ab initio* modelling of the active/binding site. Good for cases where force fields failed to accurately represent the system: systems with transition metal for instance.

QM/MM: Future Developments

- **Looking into more problematic cases for QPLD:**
 - QM treatment of metal ions: currently under study. Promising results were obtained.
- **Improved binding energy predictions**
 - Coupling QPLD and XP Glide.
 - Using QM charges in the MM part of the MM/GBSA calculations improves results.
 - Script to calculate binding energies from QM/MM calculations.
- **Combining QPLD and Induced Fit Docking (IFD)**
 - QM/MM charges can be used through the charges specified in the maestro file.
 - Improved results can be obtained using these charges in case of a study on P450 CYP3A4 (Reynolds *et. al.* ACS meeting Aug. 2007).

Acknowledgements:

Rich Friesner Steve Lippard

Woody Sherman

Victor Guallar Ben Gherman

Other Schrödinger Applications
Scientists and Developers

The logo for Schrödinger, featuring the word "SCHRÖDINGER" in a bold, dark green, sans-serif font. The letter "Ö" is stylized with two red dots above it, representing the umlaut. The logo is centered on a white background.