Computer Modeling (Physical Chemistry) of Enzyme Catalysis, Metalloenzymes

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Outline

Physical Chemistry of Enzyme Catalysis

- (Enzymatic) Reaction Rate and Order
- Michaelis-Menten (and Enzyme) Kinetics

Metals in Enzymology (Theoretical Bioinorganic Chemistry)

- Stability Constants, Selectivity
- Spin-States in Biochemistry
- Crystal Field/Ligand Field Theories
- DFT vs. WFT Methods (Accuracy and Pitfalls)
- Relativistic Effects (Mild Introduction)

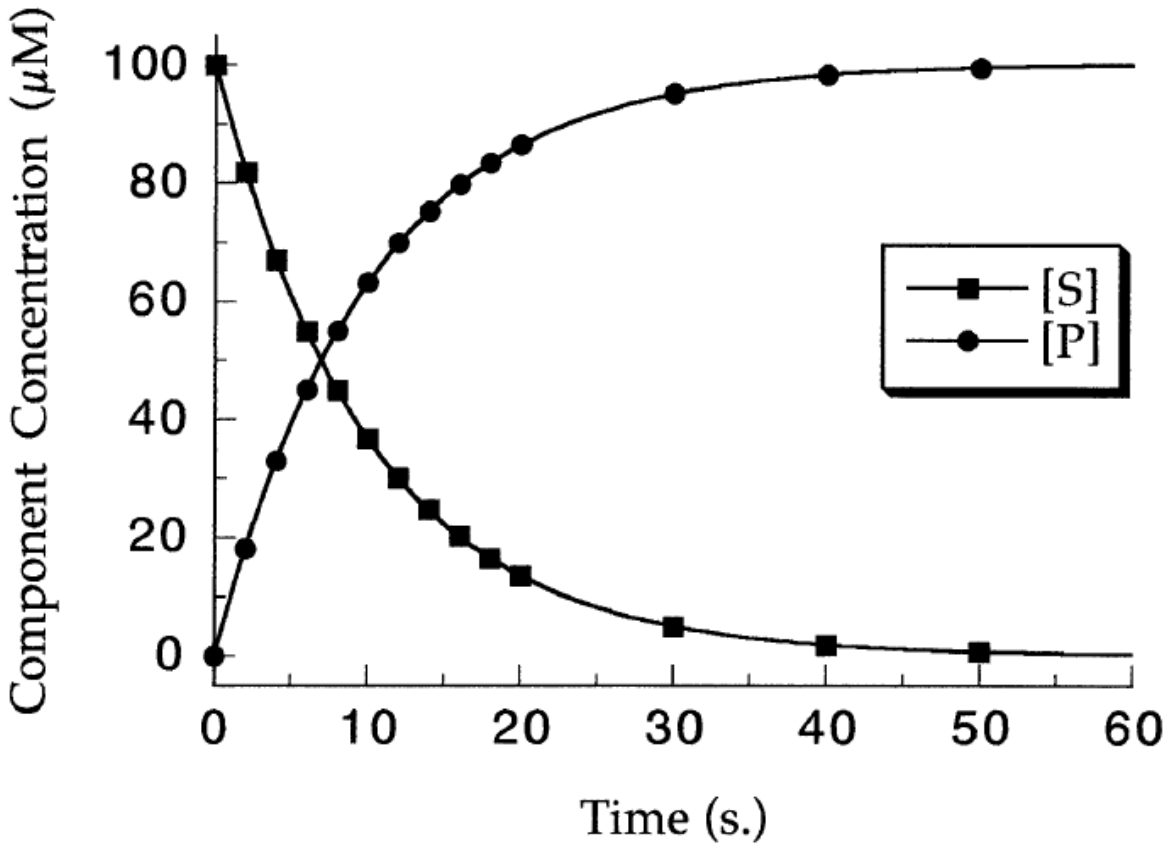


Figure 2.15 Progress curves of product development (circles) and substrate loss (squares) for a first-order reaction.

Chemical reaction: A + B + C + … → {P}

Typical rate equation: $v = k[A]^x[B]^y[C]^z\ldots$

x… reaction order with respect to **A**

x + *y* + *z* +… overall reaction order

Reaction order is not always equivalent to stoichiometry and can be determined only experimentally; allows to hypothesize reaction mechanism,

Knowledge of R.O. may suggest the rate-determining step (RDS) (= RLS)

Elementary Reaction: single reaction step, single transition state

Reaction order is then equivalent to stoichiometry

First order reactions

The reaction rate depends on a single reactant and the $x = 1$

Example: S_N1 reaction $ArN_2^+ + X^- \rightarrow ArX + N_2$, the rate equation is $v = k[ArN_2^+]$

Second order reactions

$x + y + \ldots = 2$
A + B + … → {P} can be *e.g.* $v = k[A]^2$ or $v = k[A][B]$

Example: $NO_2 + CO \rightarrow NO + CO_2$ is $v = k[NO_2]^2$

S_N2: $CH_3COOC_2H_5 + OH^- \rightarrow CH_3COO^- + C_2H_5OH$ is $v = k[CH_3COOC_2H_5][OH^-]$

Pseudo-first order reactions

If the concentration of one of the reactant stays constant $[B] = [B]_0$ (e.g. catalyst or excess concentration)

then $v = k[A][B] = k'[A]$

Determination of reaction order

Method of initial rates

$\ln v = \ln k + x \ln [A] + y \ln[B] + \ldots$

Integral Method

integrated rate law for a first-order reaction is

$\ln[A] = -kt + \ln[A]_0$

Method of Flooding

$v = k [A]^{\alpha}[B]^{\beta}$ in excess of reactant B becomes $v = k' [A]^{\alpha}$

(Henri-)Michaelis-Menten Kinetics

Leonor Michaelis
(1875-1949)

worked together in Berlin

Maud Leonora Menten
(1879-1960)

expanding on the work of Victor Henri who published the first successful mathematical model for describing enzyme kinetics in 1903

Michaelis, L.; Menten, M.L. (1913). "Die Kinetik der Invertinwirkung". *Biochem Z* **49**: 333–369; ***recent translation***: *Biochemistry*, **2011**, *50* (39), pp 8264–8269

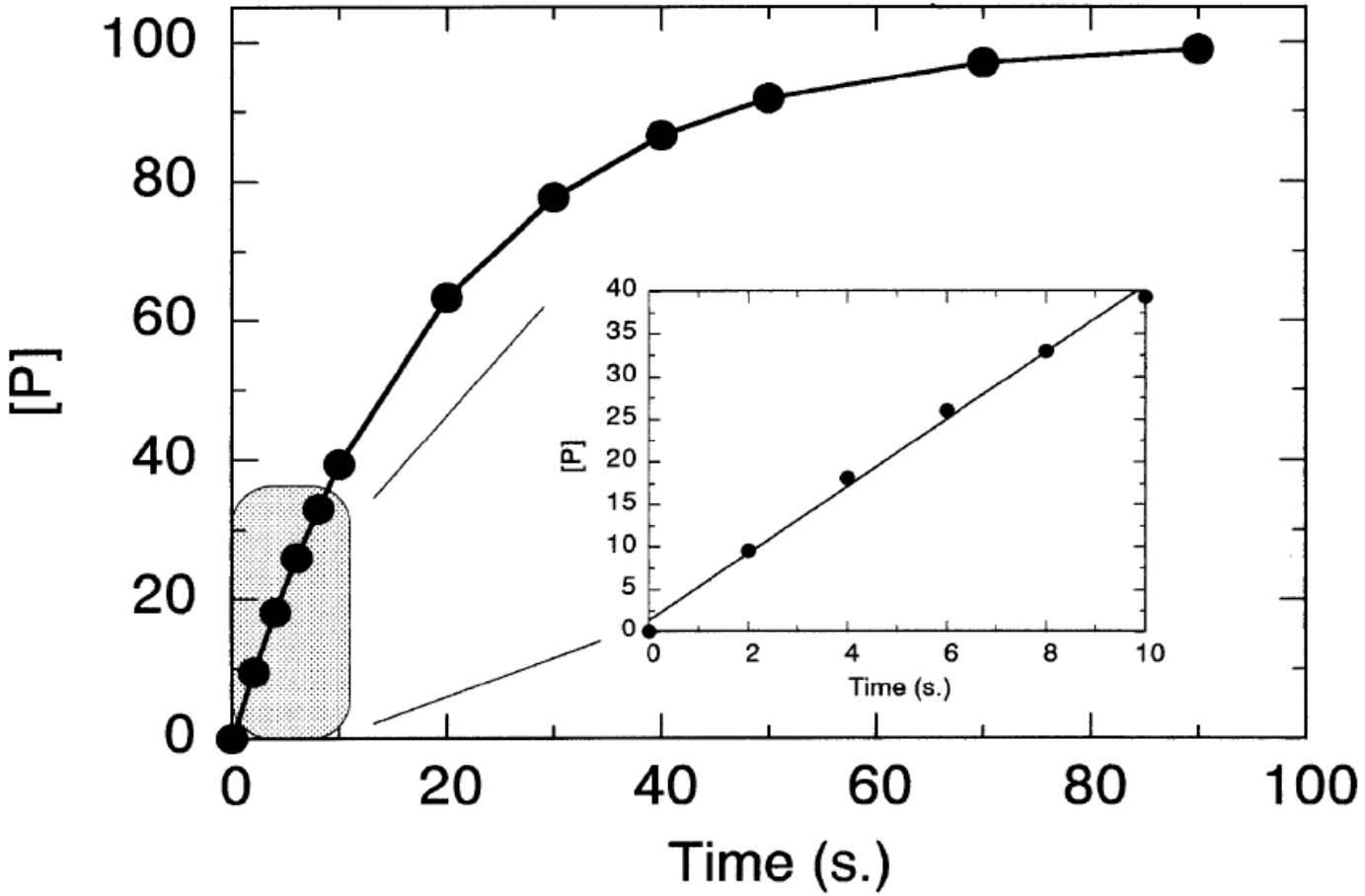


Figure 5.2 Reaction progress curve for the production of product during an enzyme-catalyzed reaction. Inset highlights the early time points at which the initial velocity can be determined from the slope of the linear plot of [P] versus time.

Substrate concentration

$$[\mathrm{S}] = [\mathrm{S}_0]e^{-kt}$$

$$v = -\frac{d[\mathrm{S}]}{dt} = \frac{d[\mathrm{P}]}{dt} = k[\mathrm{S}_0]e^{-kt}$$

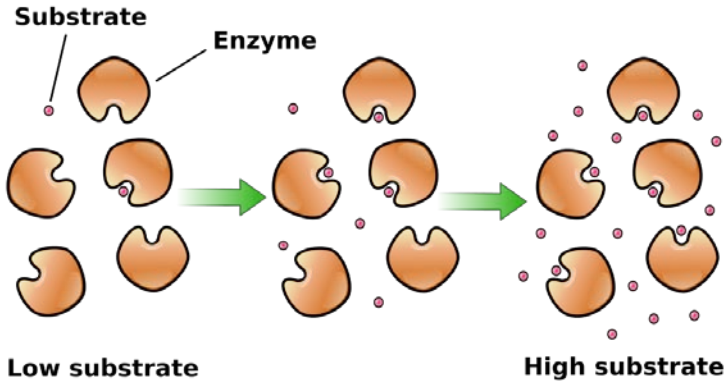
Initial velocity (~10% of substrate conversion)

$$v_0 = -\frac{\Delta[\mathrm{S}]}{\Delta t} = \frac{\Delta[\mathrm{P}]}{\Delta t}$$

Effect of substrate concentration on velocity

Brown (1902) – qualitative picture

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$



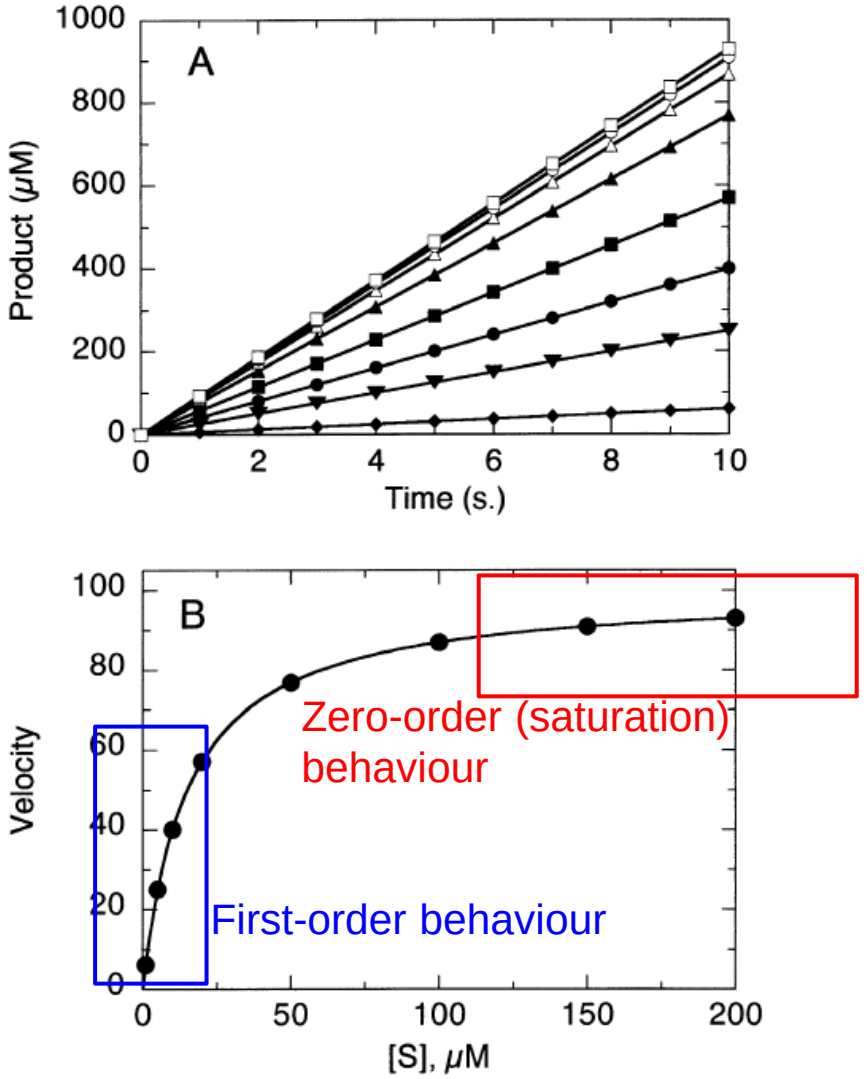


Figure 5.3 (A) Progress curves for a set of enzyme-catalyzed reactions with different starting concentrations of substrate [S]. (B) Plot of the reaction velocities, measured as the slopes of the lines from (A), as a function of [S].

THE RAPID EQUILIBRIUM MODEL OF ENZYME KINETICS

Henri (1903) and Michaelis & Menten (1913) put the Brown's model into mathematical framework

$$\mathrm{E} + \mathrm{S} \underset{K_S}{\rightleftharpoons} \mathrm{ES} \xrightarrow{k_{cat}} \mathrm{E} + \mathrm{P}$$

$$k_2 \ll k_{-1}$$

$$K_S = \frac{[\mathrm{E}]_f[\mathrm{S}]}{[\mathrm{ES}]}$$

Assuming $[\mathrm{S}] = [\mathrm{S}_f]$

$$[\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_S + [\mathrm{S}]}$$

$$v = k_{cat}[\mathrm{ES}]$$

$$v = \frac{k_{cat}[\mathrm{E}][\mathrm{S}]}{K_S + [\mathrm{S}]} \qquad V_{max} = k_{cat}[\mathrm{E}]$$

Original H-M-M equation

$$v = \frac{V_{max}[\mathrm{S}]}{K_S + [\mathrm{S}]} = \frac{V_{max}}{1 + \frac{K_S}{[\mathrm{S}]}}$$

However, this original approach is useful in single-turnover (rapid) reactions

THE STEADY STATE MODEL OF ENZYME KINETICS

Briggs and Haldane (1925)

Does not require $k_2 \ll k_{-1}$

Assumptions:

(1) In the initial stage $[\mathrm{E}] = [\mathrm{E}]_\mathrm{f} + [\mathrm{ES}]$

(2) $[\mathrm{S}] \gg [\mathrm{E}].$ $[\mathrm{S}]_\mathrm{f} \sim [\mathrm{S}]$

(3) In the initial stage, depletion of [S] is minimal and $\frac{d[\mathrm{ES}]}{dt} = 0$

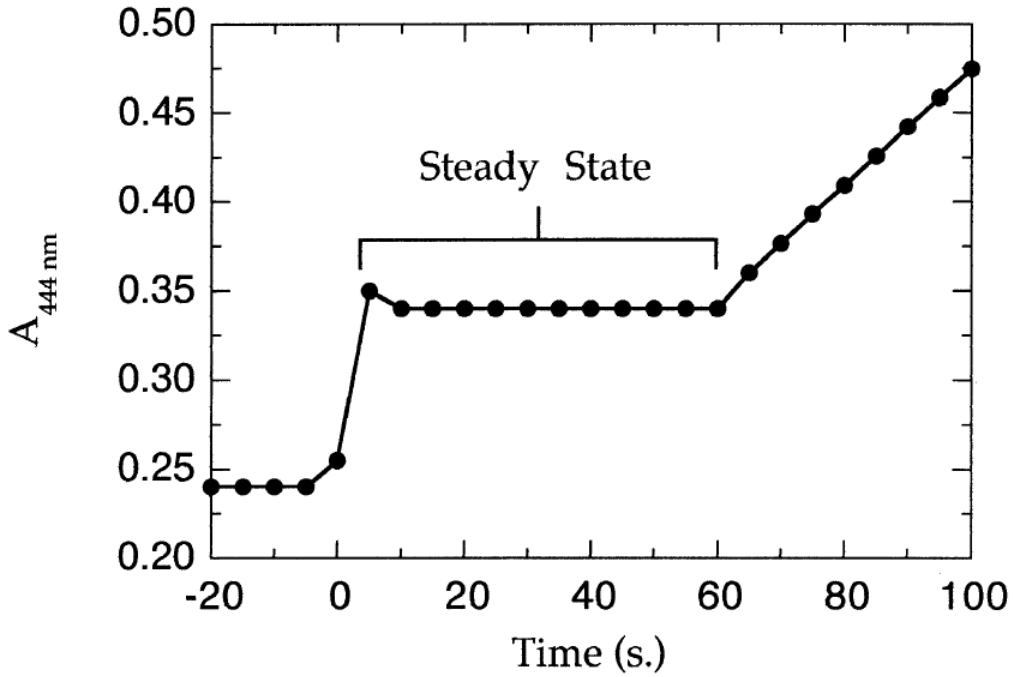


Figure 5.4 Development of the steady state for the reaction of cytochrome c oxidase with its substrates, cytochrome c and molecular oxygen. The absorbance at 444 nm reflects the ligation state of the active site heme cofactor of the enzyme. Prior to substrate addition (time < 0) the heme group is in the Fe^{3+} oxidation state and is ligated by a histidine group from the enzyme. Upon substrate addition, the active site heme iron is reduced to the Fe^{2+} state and rapidly reaches a steady state phase of substrate utilization in which the iron is ligated by some oxygen species. The steady state phase ends when a significant portion of the molecular oxygen in solution has been used up. At this point the heme iron remains reduced (Fe^{2+}) but is no longer bound to a ligand at its sixth coordination site; this heme species has a much larger extinction coefficient at 444 nm; hence the rapid increase in absorbance at this wavelength following the steady state phase. [Data adapted and redrawn from Copeland (1991).]

HMM Equation

$$v = k_2[\mathrm{ES}]$$

$$\frac{d[\mathrm{ES}]}{dt} = k_1[\mathrm{E}]_\mathrm{f}[\mathrm{S}]_\mathrm{f} \qquad \text{and} \qquad \frac{-d[\mathrm{ES}]}{dt} = (k_{-1} + k_2)[\mathrm{ES}]$$

$$k_1[\mathrm{E}]_\mathrm{f}[\mathrm{S}]_\mathrm{f} = (k_{-1} + k_2)[\mathrm{ES}]$$

$$[\mathrm{ES}] = \frac{[\mathrm{E}]_\mathrm{f}[\mathrm{S}]_\mathrm{f}}{K_\mathrm{m}} \qquad \textbf{defining} \qquad K_\mathrm{m} = \frac{k_{-1} + k_2}{k_1}$$

replace $[\mathrm{E}]_\mathrm{f}$ by $([\mathrm{E}] - [\mathrm{ES}])$

$$v = k_\mathrm{cat}[\mathrm{E}]\frac{[\mathrm{S}]}{[\mathrm{S}] + K_\mathrm{m}}$$

$$v = \frac{V_\mathrm{max}[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]} = \frac{V_\mathrm{max}}{1 + \dfrac{K_\mathrm{m}}{[\mathrm{S}]}}$$

The K_m – **Michaelis constant** - is the substrate concentration that provides a reaction velocity that is half of the maximal velocity obtained under saturating substrate conditions.

K_m is not equal to K_S in rapid equilibrium model, but can be used as the fairly good *relative* estimate of substrate binding affinity

The apparent unimolecular rate constant $\boldsymbol{k_{cat}}$ is also called *turnover number* and denotes the maximum number of enzymatic reactions catalysed per second. [s^{-1}]

In vivo, often [S] << K_m, (typically 0.1 – 1 K_M) the overall reaction may be limited by the diffusional rate of encounter of the free enzyme with substrate, which is defined by k_1. The rate constant for diffusional encounters between molecules like enzymes and substrates is typically in the range of 10^8—10^9 $M^{-1}s^{-1}$

⇒ **Catalytic perfection**

The **catalytic efficiency** of an enzyme is best defined by the ratio of the kinetic constants, k_{cat}/K_m

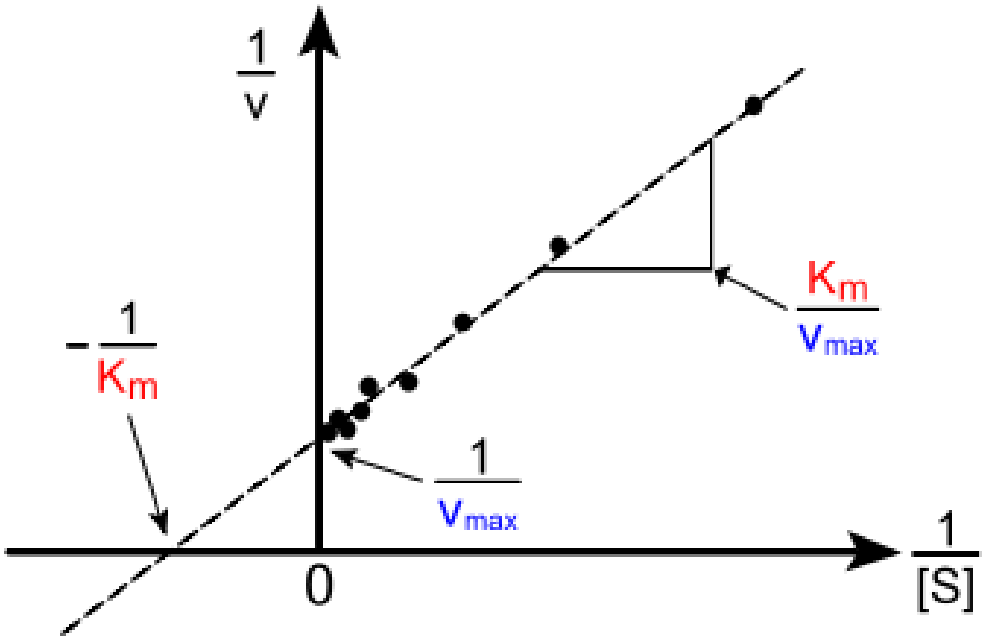
$$\Delta G_{ES^{\ddagger}} = -RT\ln\left(\frac{k_{cat}}{K_m}\right) + RT\ln\left(\frac{k_B T}{h}\right) \qquad \Delta\Delta G_{ES^{\ddagger}} = -RT\ln\left[\frac{(k_{cat}/K_m)^1}{(k_{cat}/K_m)^2}\right]$$

Linear plots of the Michaelis–Menten equation

The Lineweaver–Burk plot or double reciprocal plot is a common way of illustrating kinetic data.

Rearrange HHM to

$$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$



TRANSIENT STATE KINETIC MEASUREMENTS

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EX \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EP \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + P$$

pre—steady state kinetics

stopped-flow and rapid reaction quenching (RFQ)

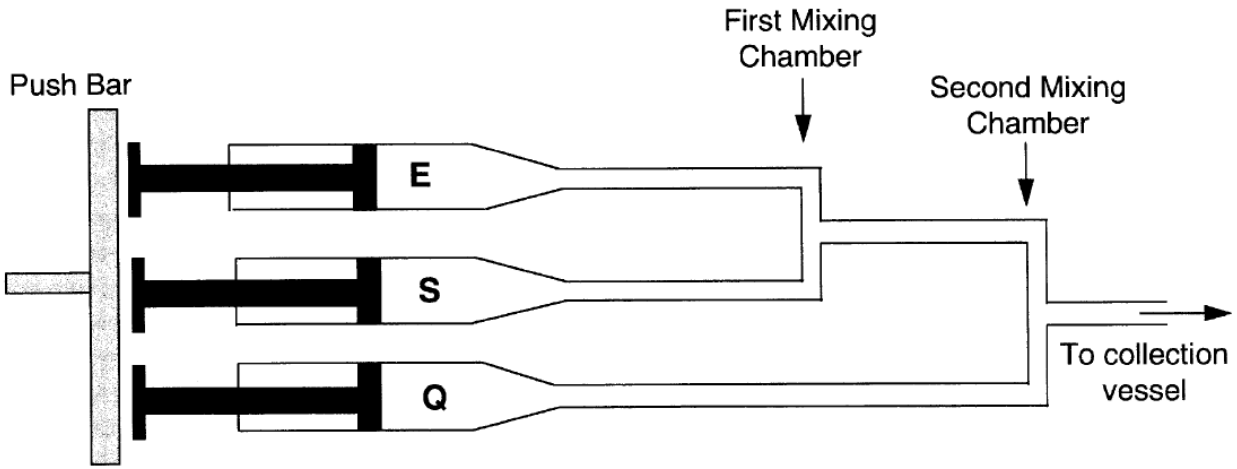
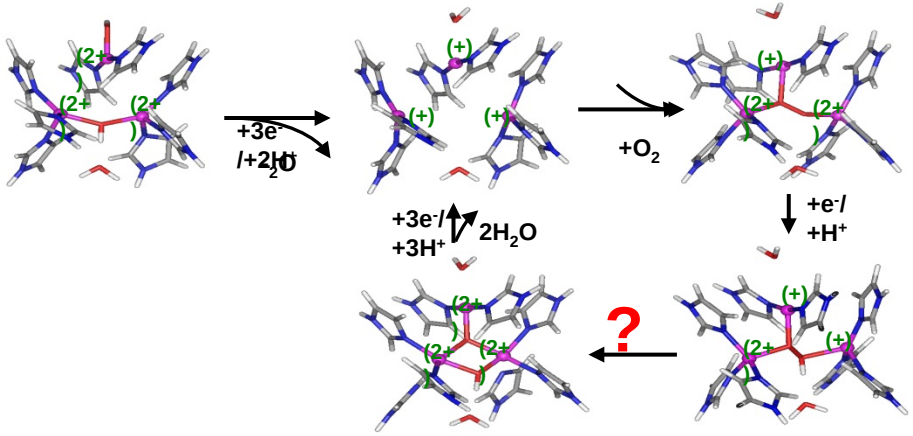
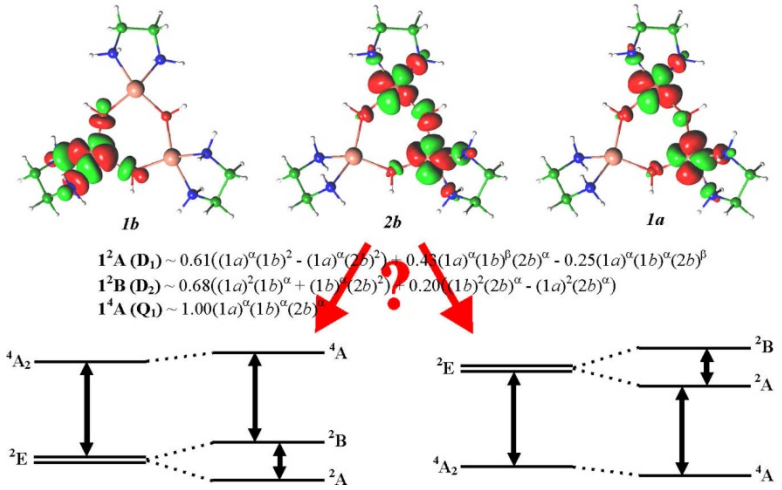
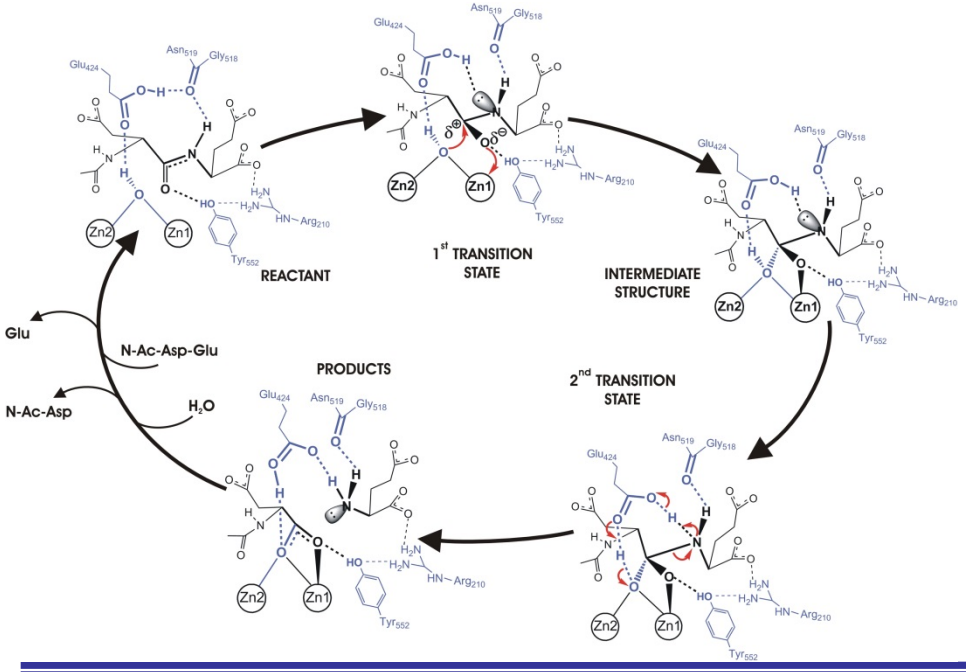


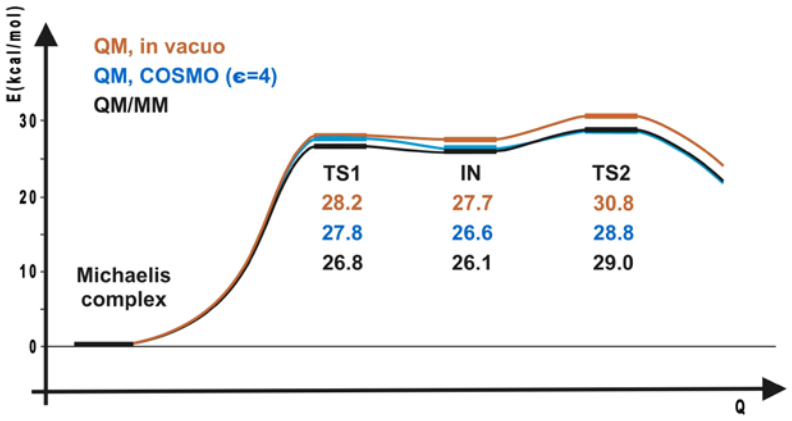
Figure 5.18 Schematic diagram of a typical rapid quench instrument for rapid kinetic measurements.

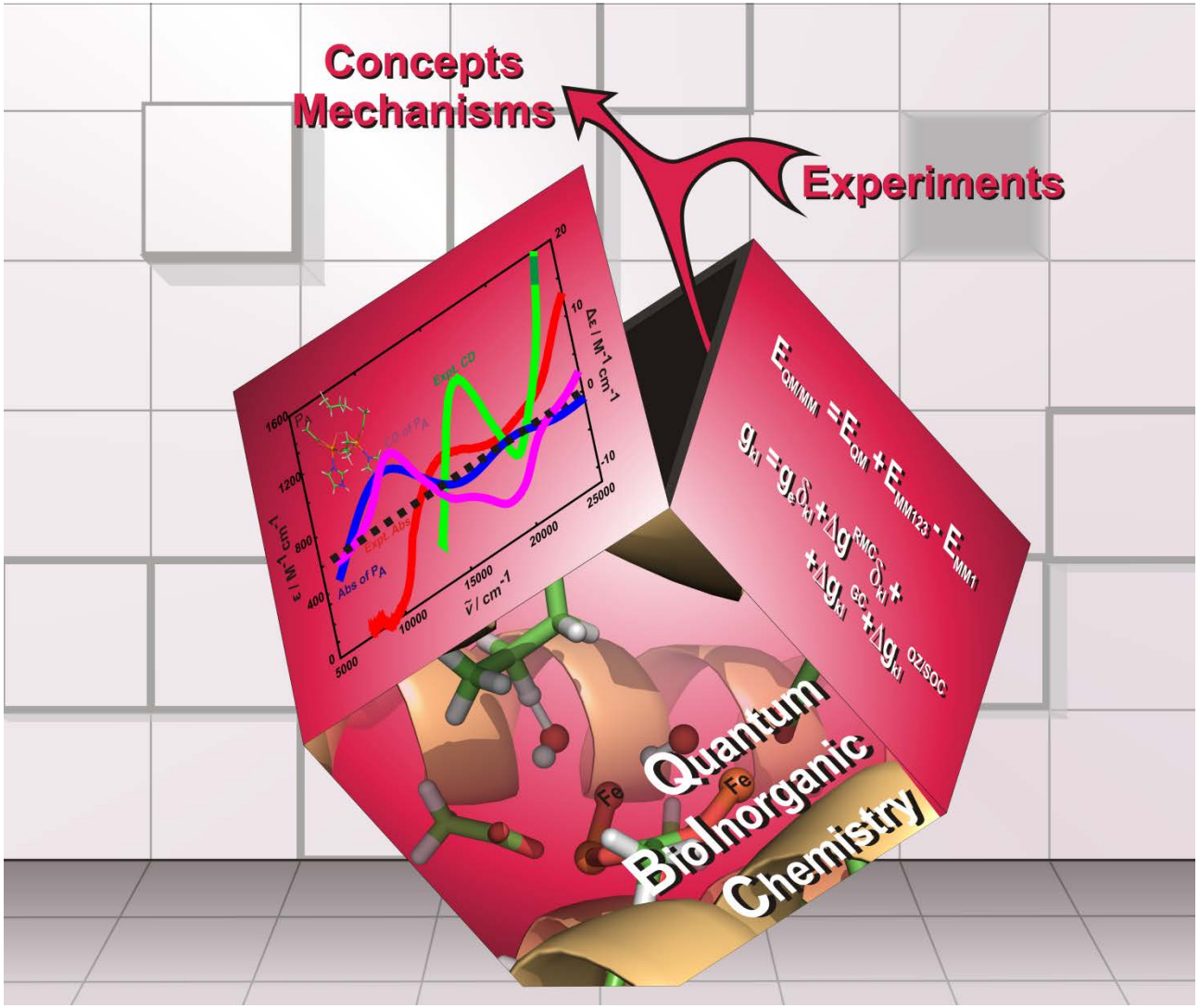




Theoretical Bioinorganic Chemistry







adapted from Rokob, T. A.; Srnec, M.; Rulíšek, L.: Theoretical Calculations of Physico-Chemical and Spectroscopic Properties of Bioinorganic Systems: Current Limits and Perspectives. *Dalton Trans.* **2012**, *41*, 5754-5768.

Protein Structure → Theoretical Model

full protein without conformational sampling

QM/MM

QM/MM/Exp (X-ray, EXAFS, NMR)

full protein with conformational sampling

QM/MD, QM/MM/FEP, QTCP

cluster model (active site only)

QM+solution (COSMO-RS, SMD, ...)

Calculations vs. Experiment

spectroscopic properties

Absorption, CD, MCD, EPR, IR, Raman, Mössbauer, NRVS,...

thermodynamic properties

reduction potentials, pK_a values, equilibrium constants

kinetic properties

rate constants, isotope effect

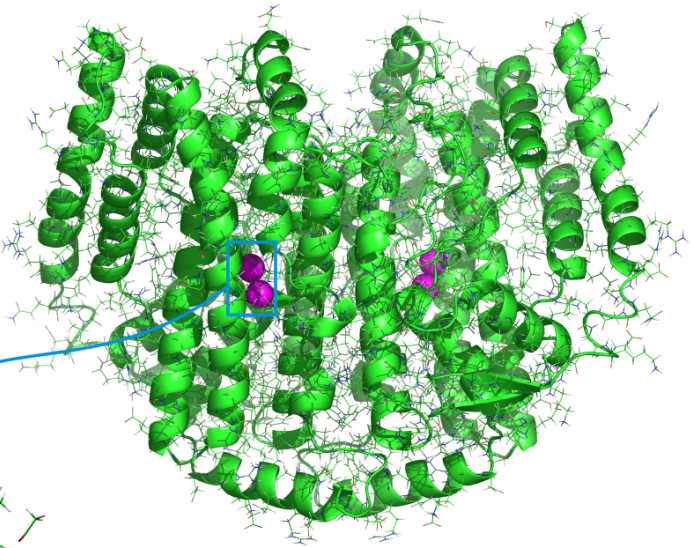
QM Methods

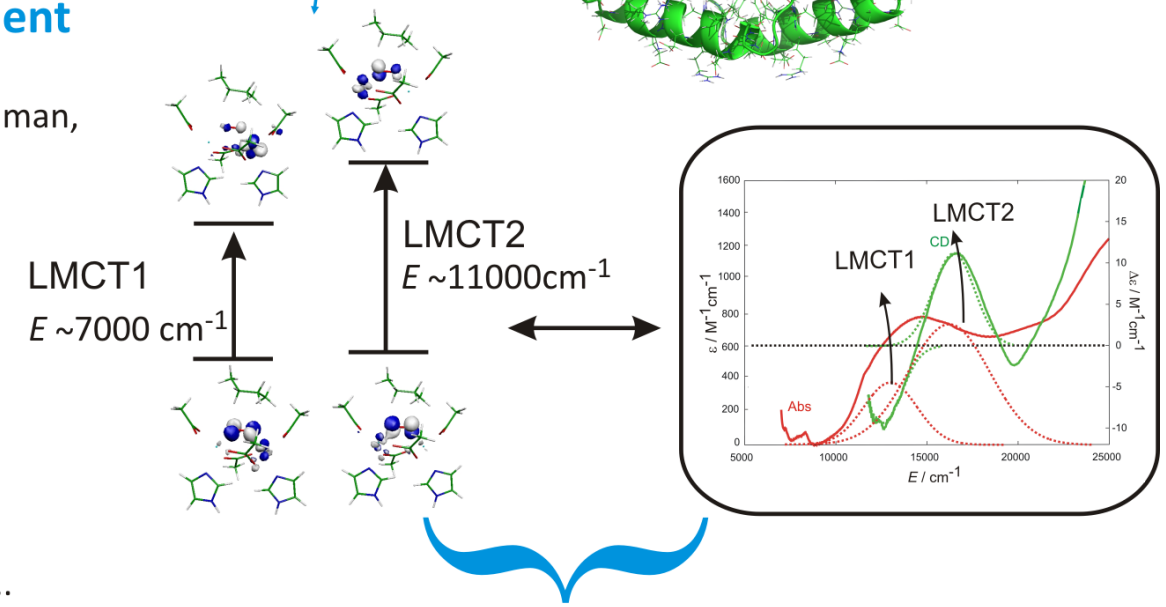
wave function methods

MR-SCF, MR-PT2, MRCI, DMRG, ...

density functional theory (DFT) methods

DFT, DFT+D, ...



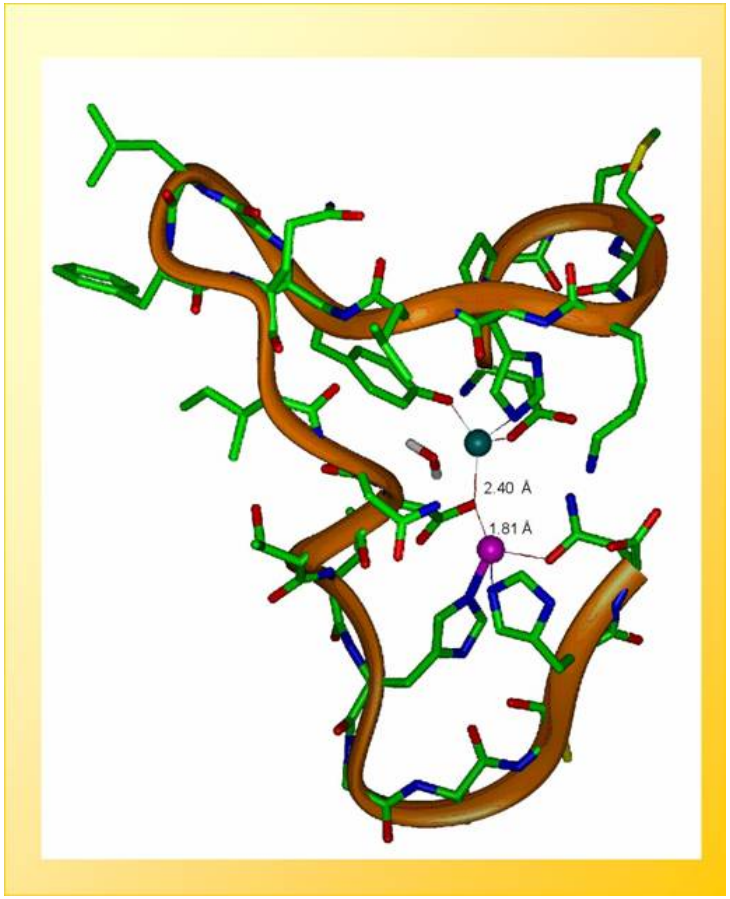


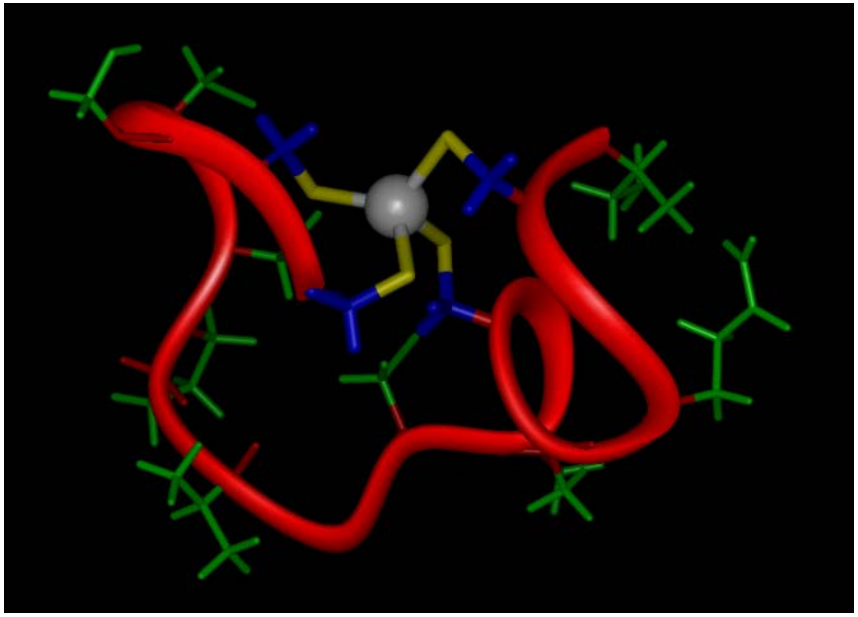
Concepts and Mechanisms

adapted from Rokob, T. A.; Srnec, M.; Rulíšek, L.: *Dalton Trans.* **2012**, *41*, 5754-5768.

Metal Ion Selectivity

Why Nature selected particular metal ion to perform particular task?





Non-equilibrium concentrations in cells

Cell	~	μM
Sea water	~	nM

- Metalloproteins
  - Uphill battle against Irving-Williams series

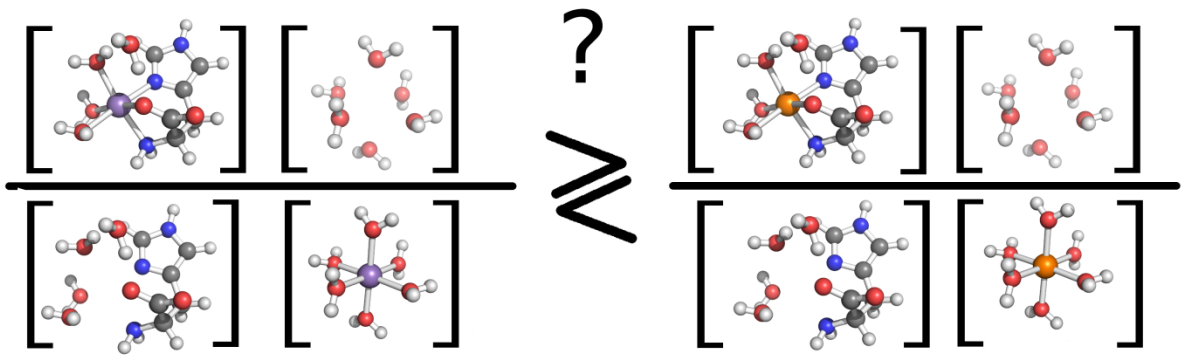
    Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II)

Thermodynamics of Metal Binding

$$K = \frac{[ML_n]}{[M][L]^n}$$

$$\Delta G = -RT \ln K$$

$$G = E_{el} + ZPE - RT\ln Q + G_{solv}$$

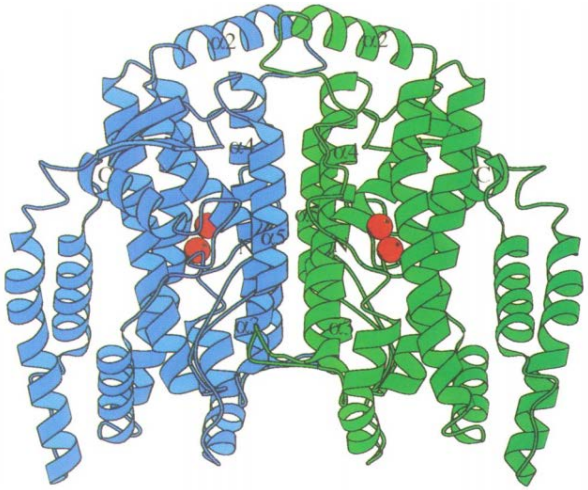


Modelling Metalloproteins

(crystal vs. ligand field theories, spin states in biochemistry, accuracy of QM methods, relativistic effects)

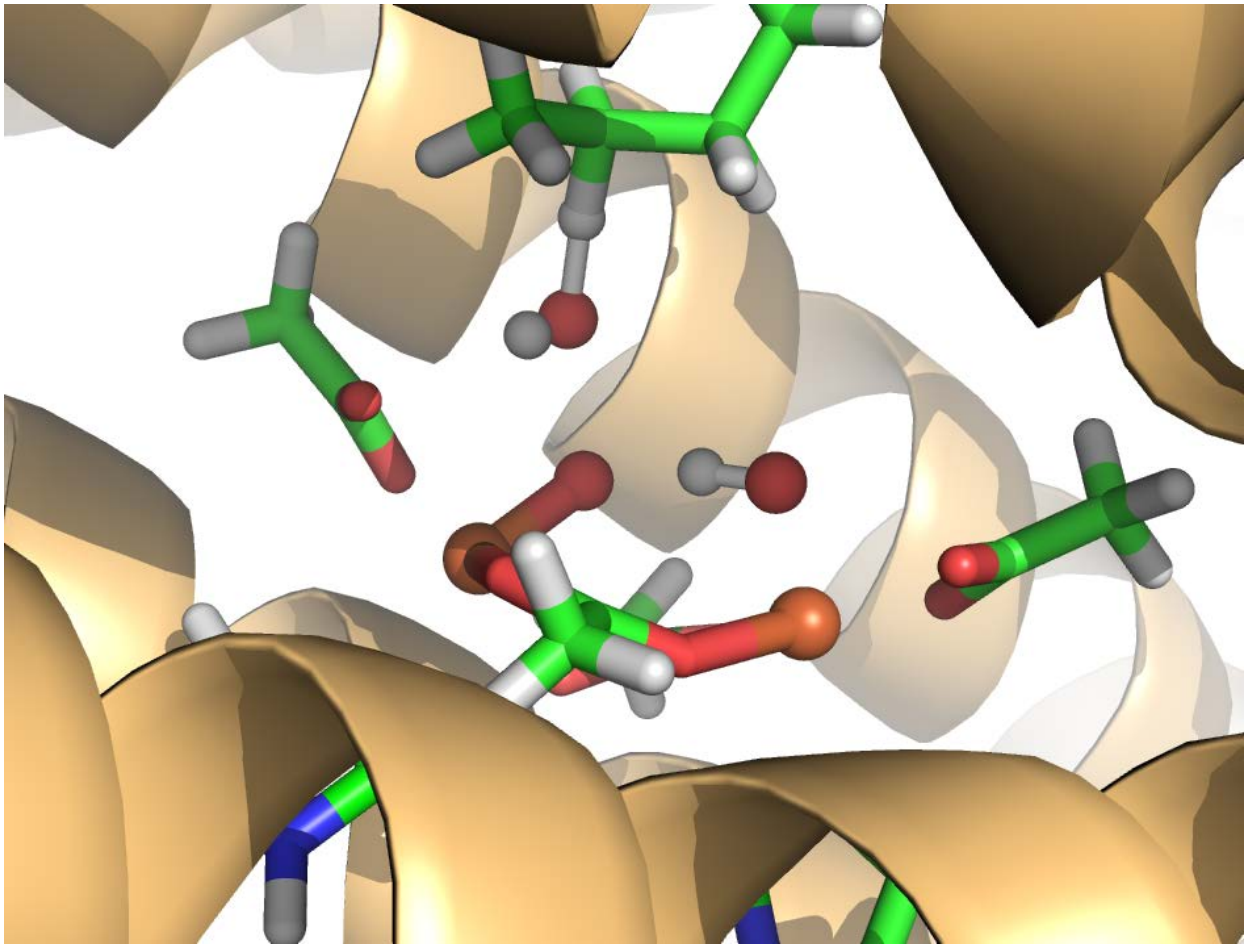
On the example of

Δ^9 Desaturase Reaction Mechanism



(Mystery of) Δ^9 Desaturase Reaction Mechanism

When you have eliminated all which is impossible, then whatever remains, however improbable, must be the truth



$NHFe_2$ Enzymes

Binuclear Non-Heme Iron Proteins

reaction type	representative enzyme	catalytic reaction
reversible dioxygen binding	hemerythrin	$[Fe^{II}Fe^{II}] \overset{+O_2}{\rightleftharpoons} [Fe^{III}Fe^{III}]{-}OOH$
hydroxylation[a]	methane monooxygenase	$[Fe^{II}Fe^{II}] + CH_4 \overset{+O_2}{\longrightarrow} [Fe^{III}Fe^{III}] + CH_3OH/H_2O$
1-e^- oxidation	ribonucleotide diphosphate reductase	$[Fe^{II}Fe^{II}] + Tyr \overset{+O_2}{\longrightarrow} [Fe^{III}Fe^{III}] + Tyr^{\bullet}$
desaturation	stearoyl–acyl carrier protein Δ^9-desaturase	$[Fe^{II}Fe^{II}] + \text{stearoyl ACP} \overset{+O_2}{\longrightarrow} [Fe^{III}Fe^{III}] + \text{oleoyl ACP}$
hydrolysis of phosphate ester	purple acid phosphatase	$[Fe^{II}Fe^{III}] + ROHPO_3 \overset{+H_2O}{\longrightarrow} [Fe^{II}Fe^{III}] + H_3PO_4$
NADH peroxidation	rubrerythrin	$[Fe^{II}Fe^{II}] + H_2O_2 \overset{+O_2}{\longrightarrow} [Fe^{III}Fe^{III}]$
ferroxidation	ferritin	$[Fe^{II}Fe^{II}] \overset{+O_2}{\longrightarrow} [Fe^{III}Fe^{III}]$

taken from: Solomon, E. I.; Brunold, T. C.; Davis, M. I.; Kemsley, J. N.; Lee, S. K.; Lehnert, N.; Neese, F.; Skulan, A. J.; Yang, Y. S.; Zhou, J. *Chem. Rev.* **2000,** *100*, 235-349.

Oxygen intermediates **P**, **P'**, **Q**, and **X** observed in ribonucleotide reductase (RR), Δ^9 desaturase (D9D) and methane monooxygenase (MMO), toluene/o-xylene momooxygenase, toluene 4-monooxygenase

S.J. Lippard, R. A. Friesner, E. I. Solomon, J. D. Lipscomb, L. Que, Jr., …

Δ^9 Desaturase

- one of the most important enzymes in the fatty-acid metabolism of plants
- catalyzes the oxidation of stearic acid

$$-CH_2–CH_2- + O_2 + 2e^- + 2\,H^+ \rightarrow -CH=CH- + 2\,H_2O$$

- the enzyme function is restored by a two-electron reduction mediated by ferredoxin

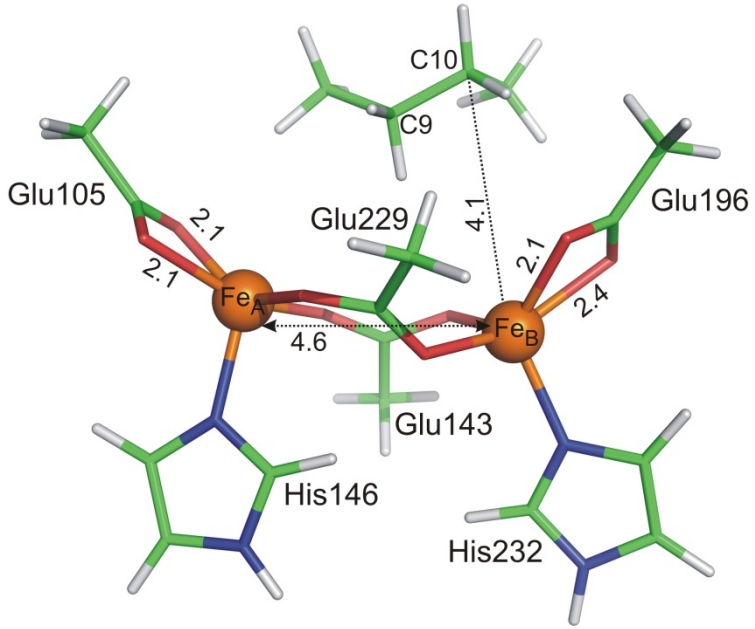
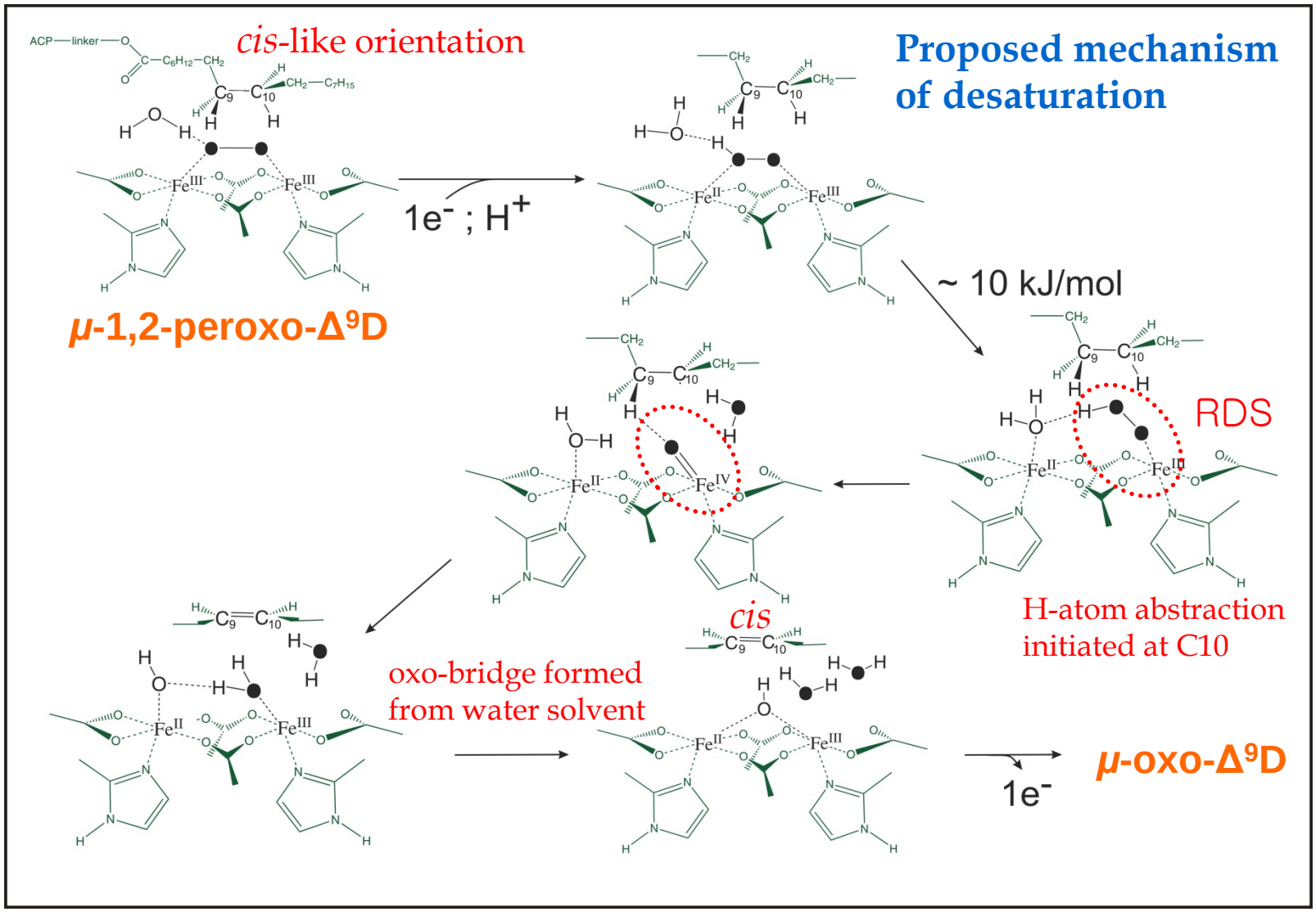


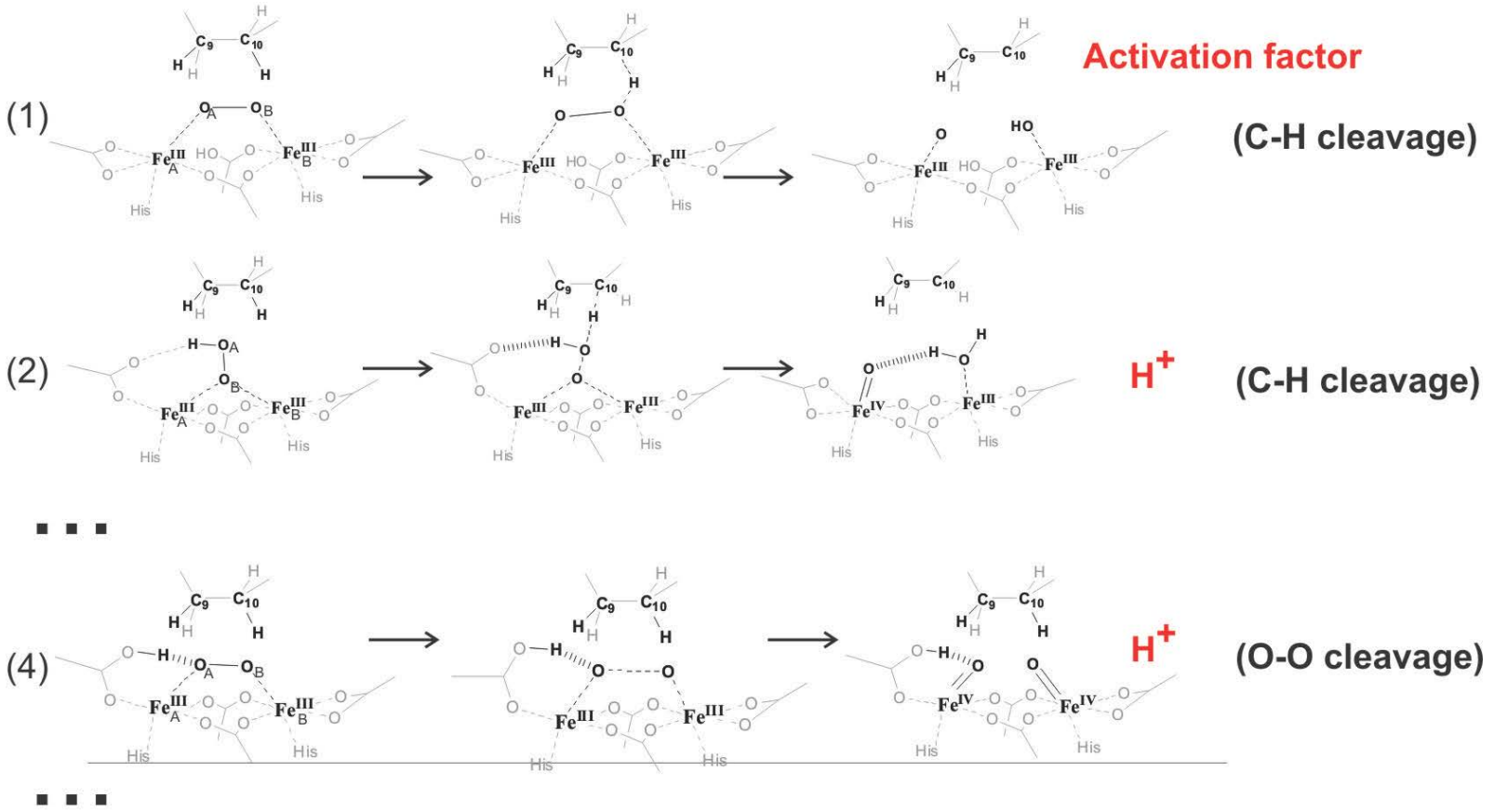
Figure: Quantum system for the reduced [Δ^9D…substrate] complex (distances in Å).

Reaction mechanism of Δ⁹-Desaturase *(working +e⁻ version)*

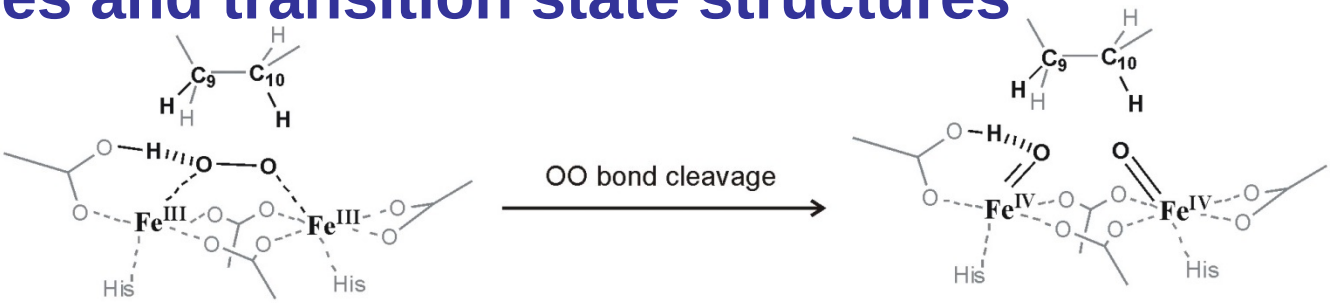


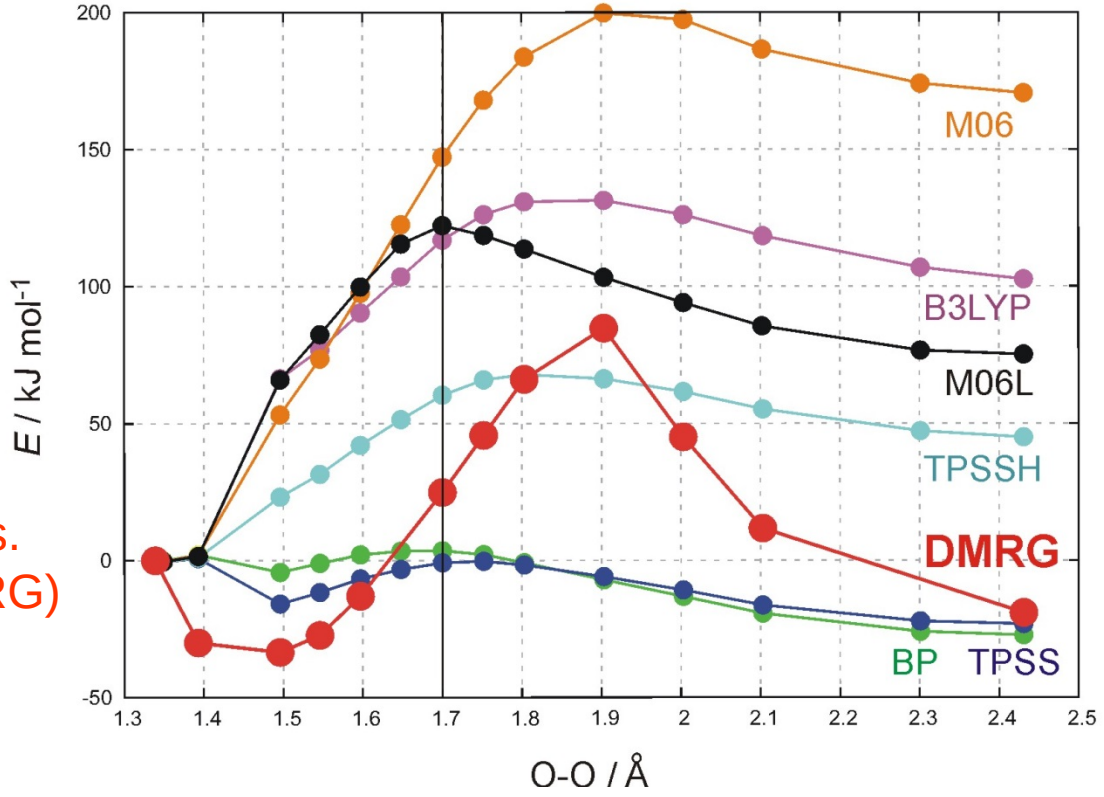
Reaction Pathways Studied

O-O vs. C-H cleavage; activation factors: -, H^+, H_2O

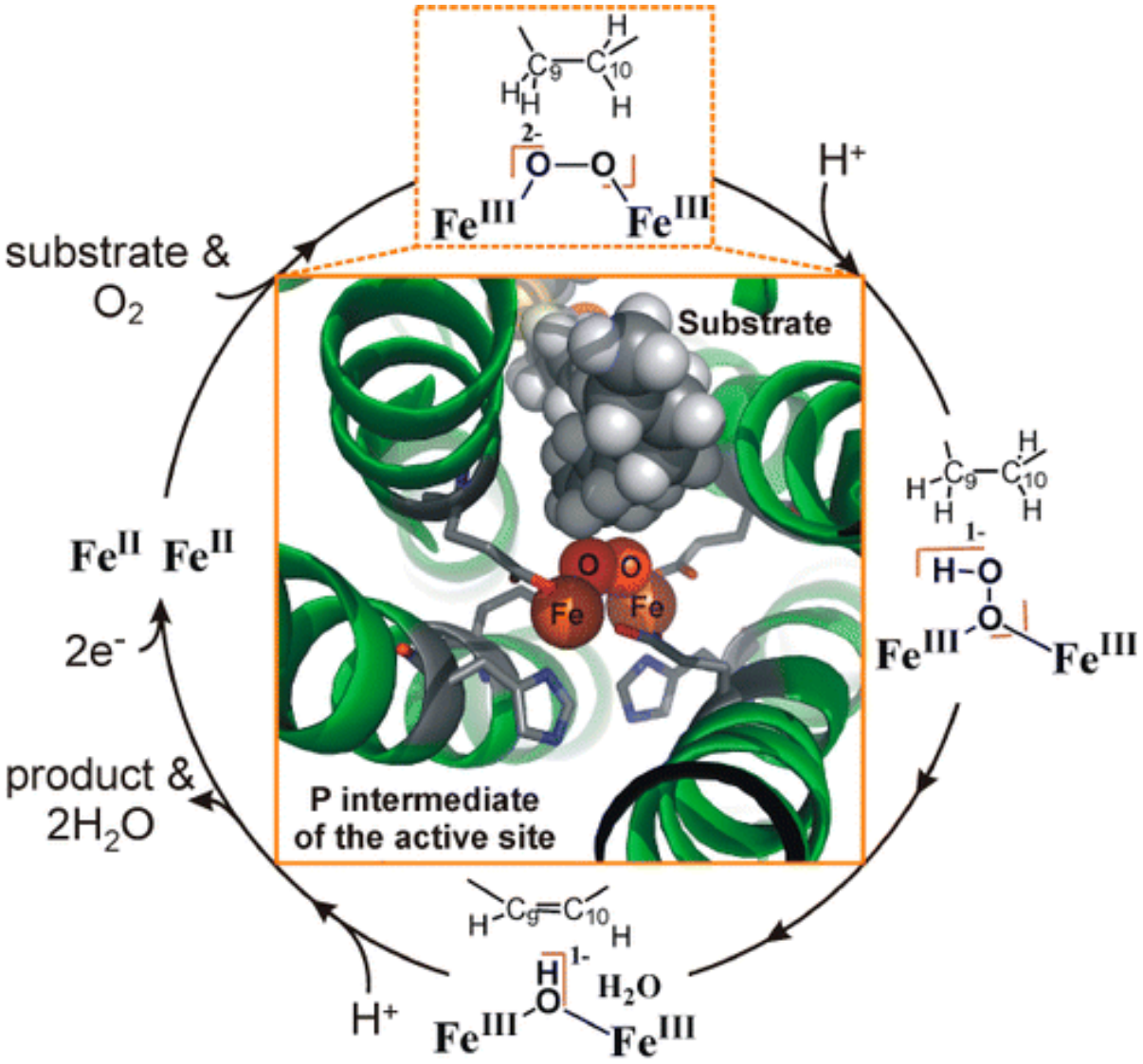


Difference between the DMRG-CASPT2 and DFT equilibrium geometries and transition state structures



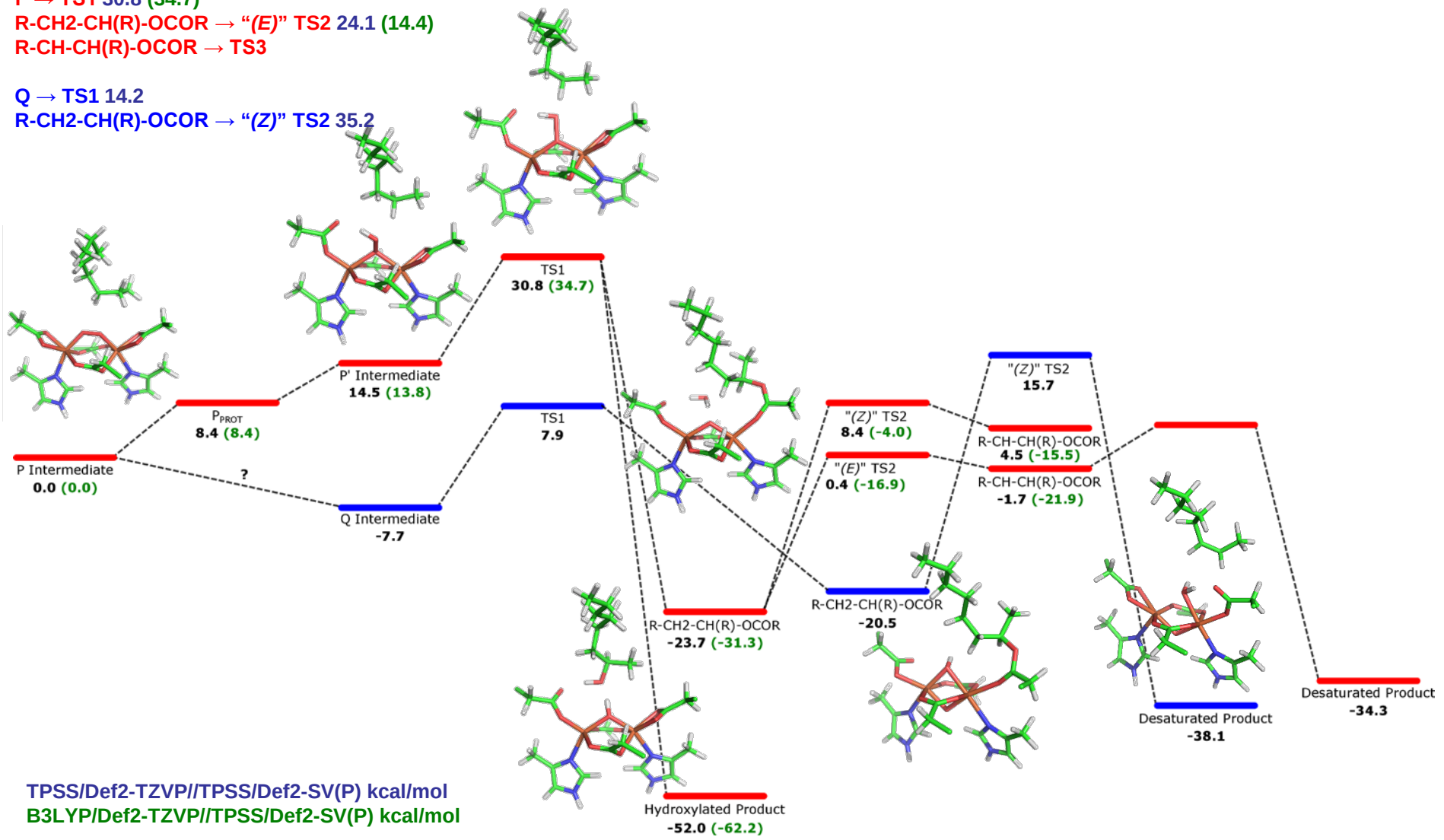


Peroxo (DFT) vs. superoxo- (DMRG) structures


substrate &
O_2
H^+
Substrate
P intermediate
of the active site
Fe^{II} Fe^{II}
2e⁻
product &
$2H_2O$
H^+
H_2O
Fe^{III}
Fe^{III}

P → TS1 30.8 (34.7)
R-CH2-CH(R)-OCOR → "*(E)*" TS2 24.1 (14.4)
R-CH-CH(R)-OCOR → TS3

Q → TS1 14.2
R-CH2-CH(R)-OCOR → "*(Z)*" TS2 35.2



TPSS/Def2-TZVP//TPSS/Def2-SV(P) kcal/mol
B3LYP/Def2-TZVP//TPSS/Def2-SV(P) kcal/mol

Towards ‘Functional Metallopetides’: En Route to Disentangle the Catalytic Power of Metalloproteins