Enzyme Catalyzed C-S Bond Forming Reactions

Florian P. Seebeck
→ seebeck.chemie.unibas.ch/en/home/
→ @SeebeckLab

Lecture plan:

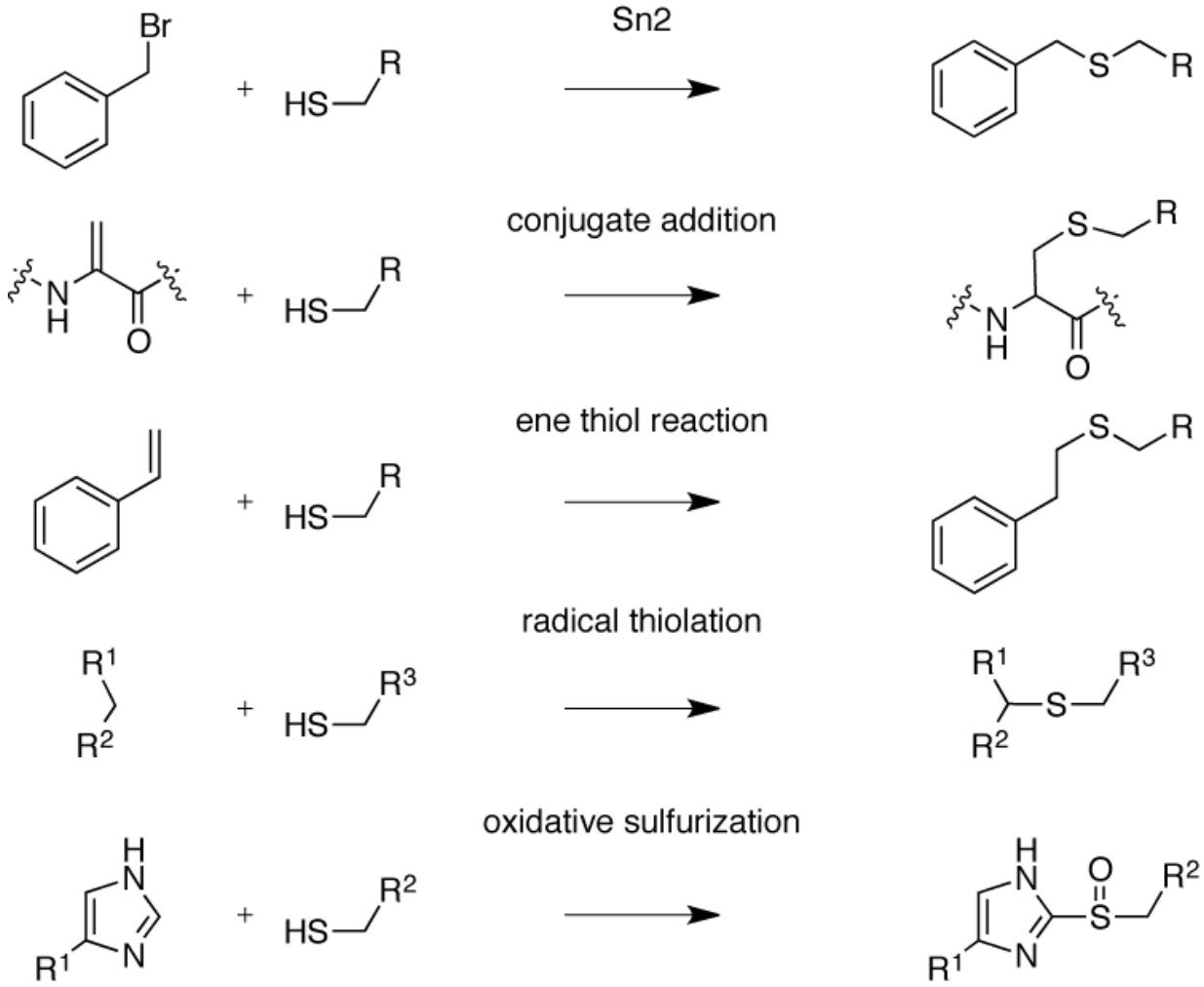
part I: acid/base catalyzed C-S bond formation

part II: metal catalyzed C-S bond formation

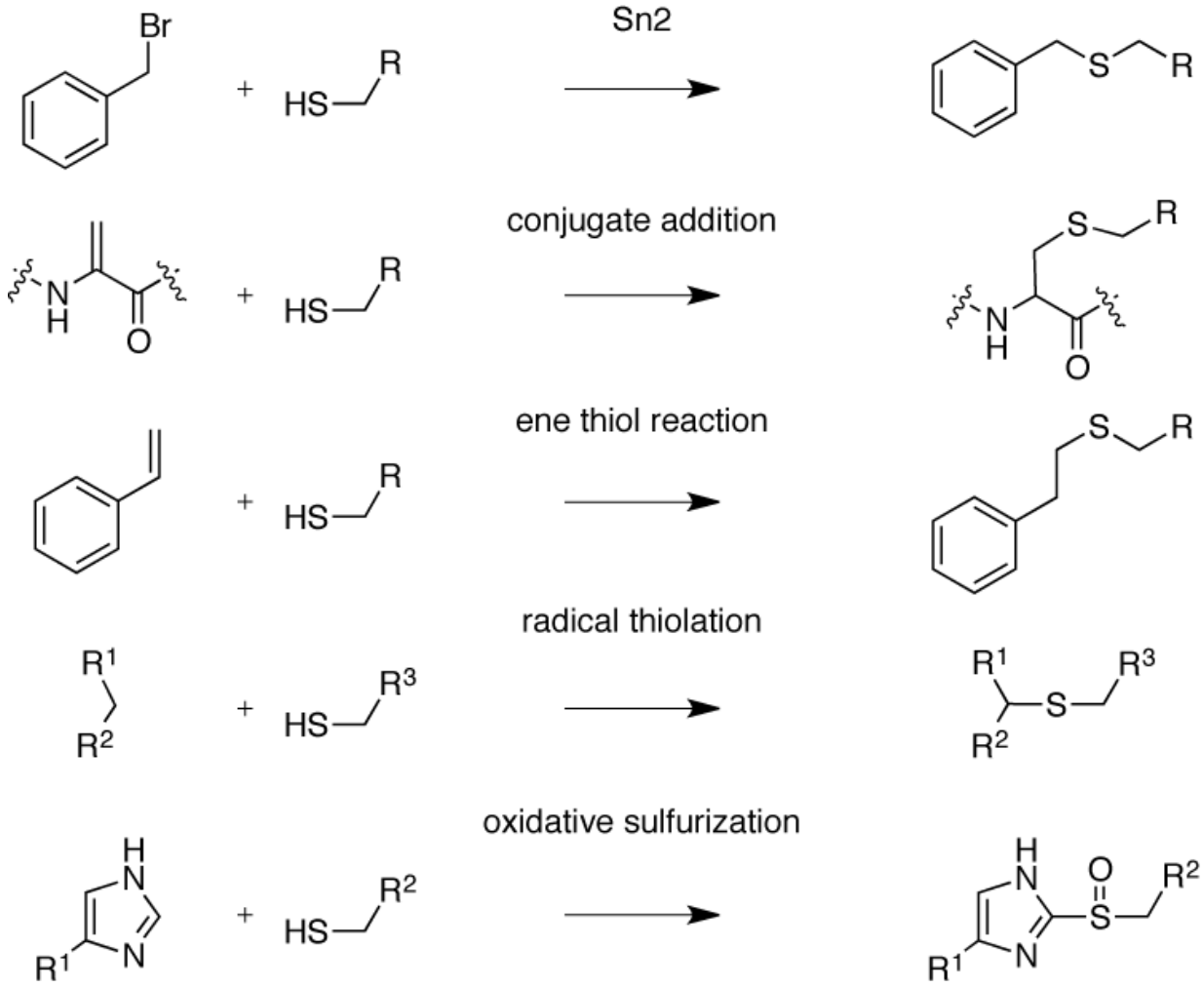
part III: metal mediated radical C-S bond formation

Part I: acid/base catalyzed C-S bond formation

C-S bond forming chemistry



C-S bond forming chemistry & enzymology



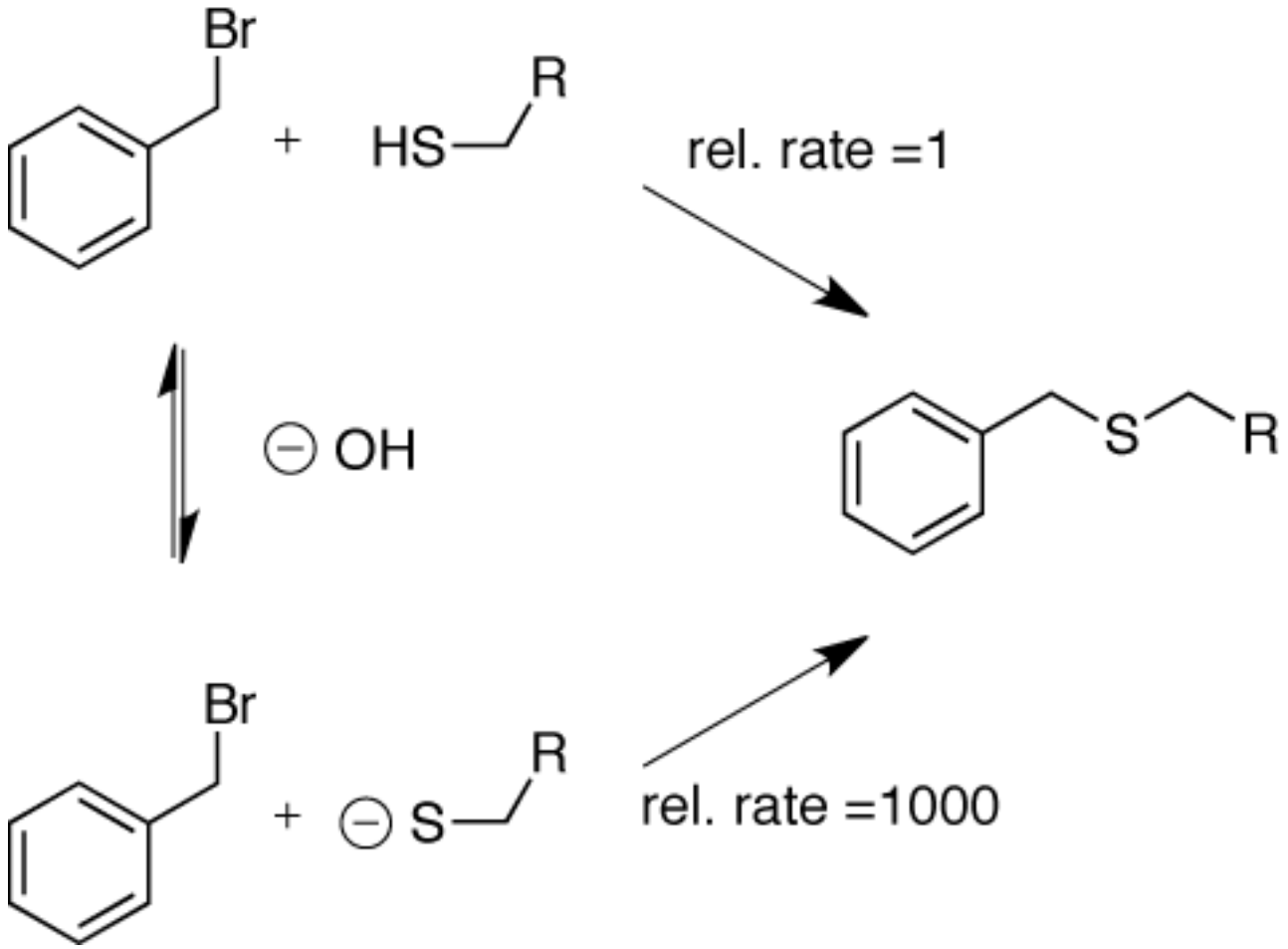
GST
Glutathione S-transferase

LanC
lantibiotic cyclase

BioB
biotin synthase

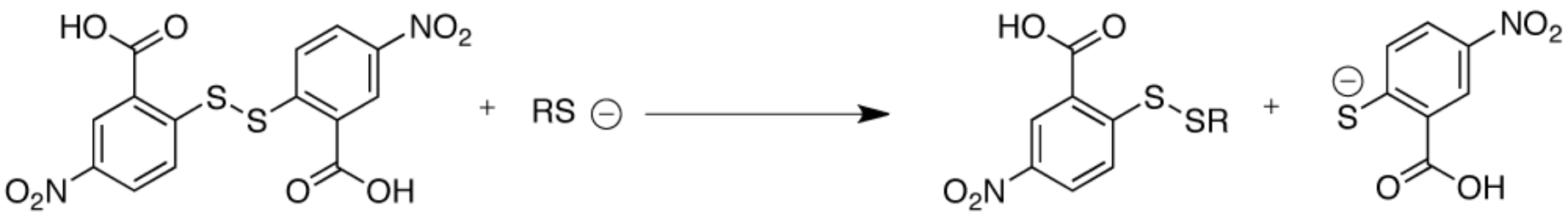
EgtB & OvoA
sulfoxide synthases

Specific base catalysis



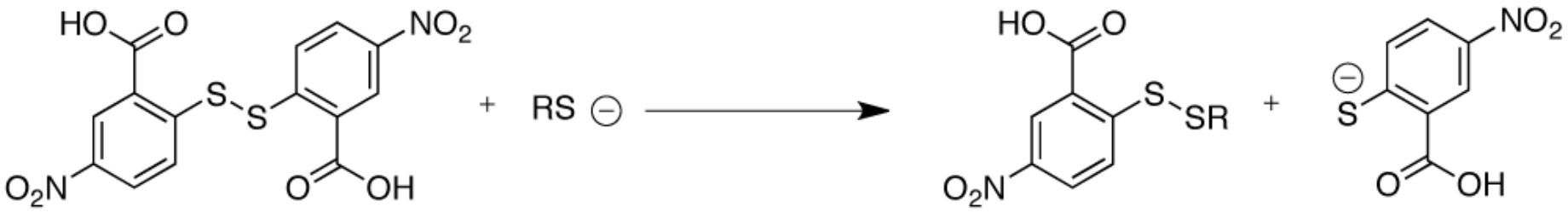
→ Is the p*K*a of a thiolate a good metric to estimate the reactivity in substitution reactions?

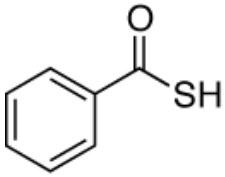
Rates of thiol-disulfide interchange reactions



Whitesides 1976

Rates of thiol-disulfide interchange reactions





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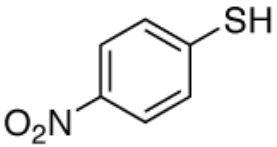
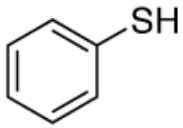


6.6



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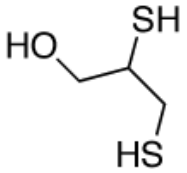

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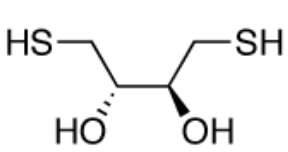

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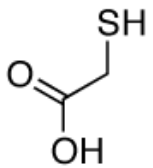

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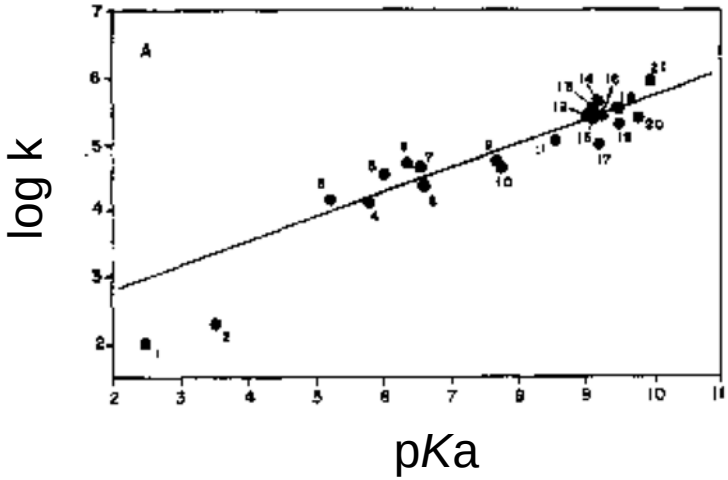
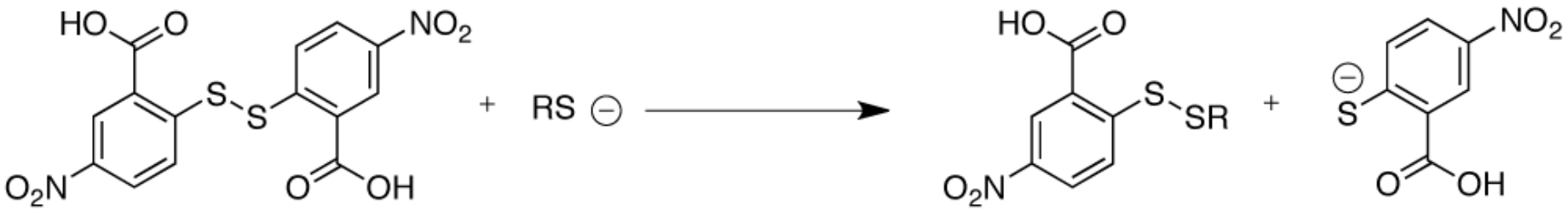
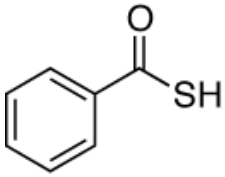


Rates of thiol-disulfide interchange reactions





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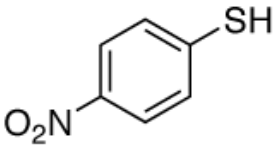

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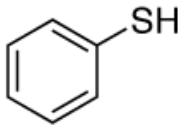

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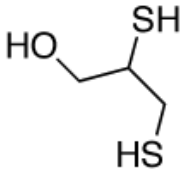
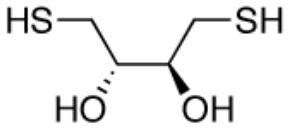


8.6



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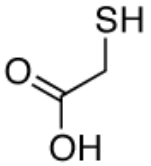

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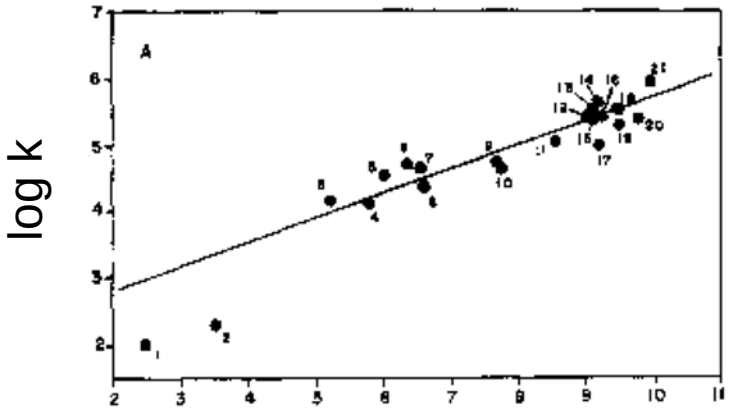
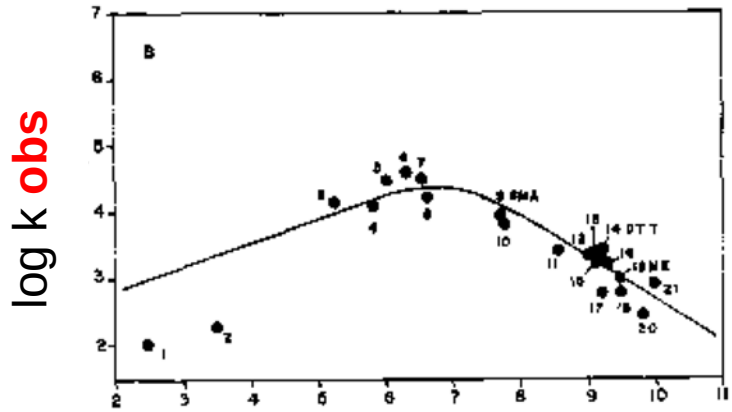
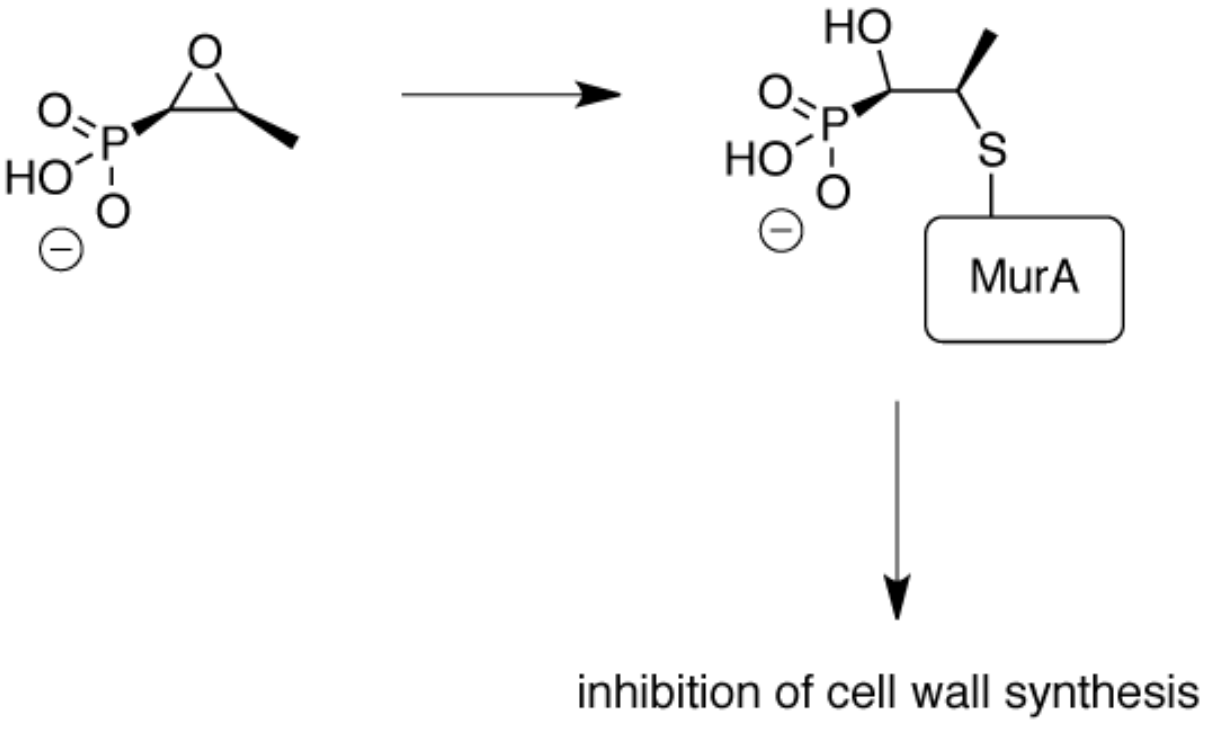




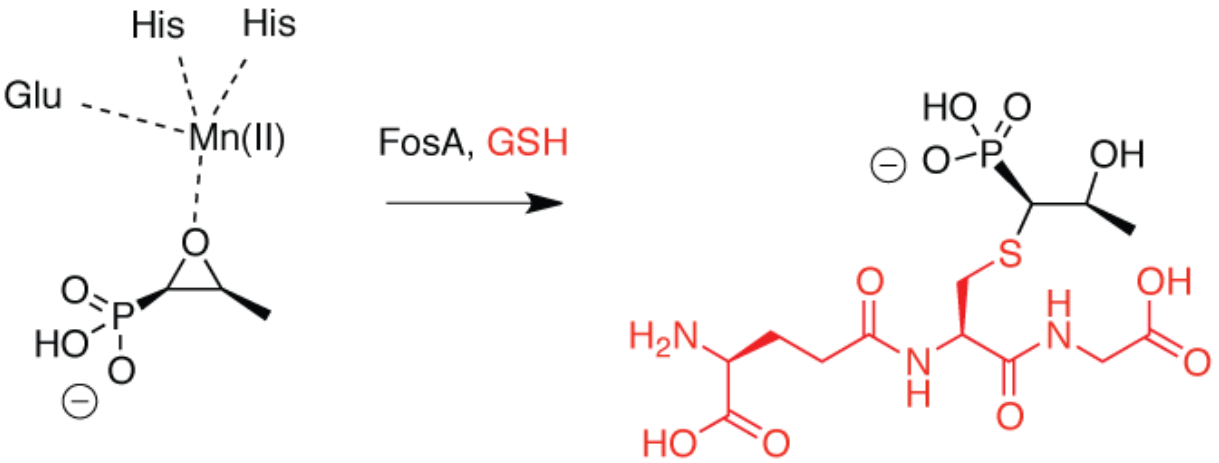
p*K*a

Whitesides 1976

Lewis acid catalysis in fosfomycine resistance enzyme FosA

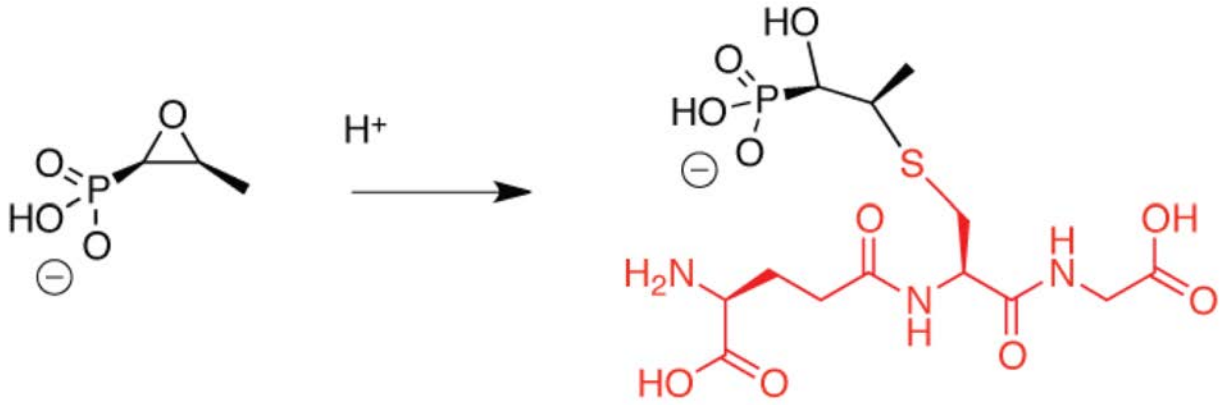


Lewis acid catalysis in fosfomycine resistance enzyme FosA



k_{cat}= 450 s^{-1}

k_{cat}/K_M = 10^7 $M^{-1}s^{-1}$



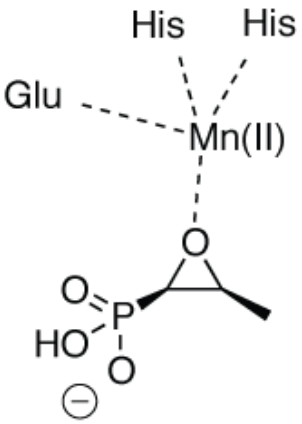
k_{uncat} = 10^{-8} s^{-1}

Catalytic proficiency
= (k_{cat}/K_M) k_{uncat} = 10^{15} M^{-1}

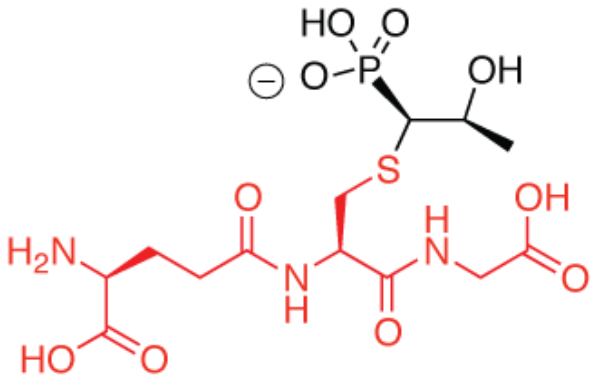
$\Delta\Delta G^{\ddagger}$ = 21 kcal/mol

Miller 2002

Lewis acid catalysis in fosfomycine resistance enzyme FosA



FosA, GSH



k_{cat}= 450 s^{-1}

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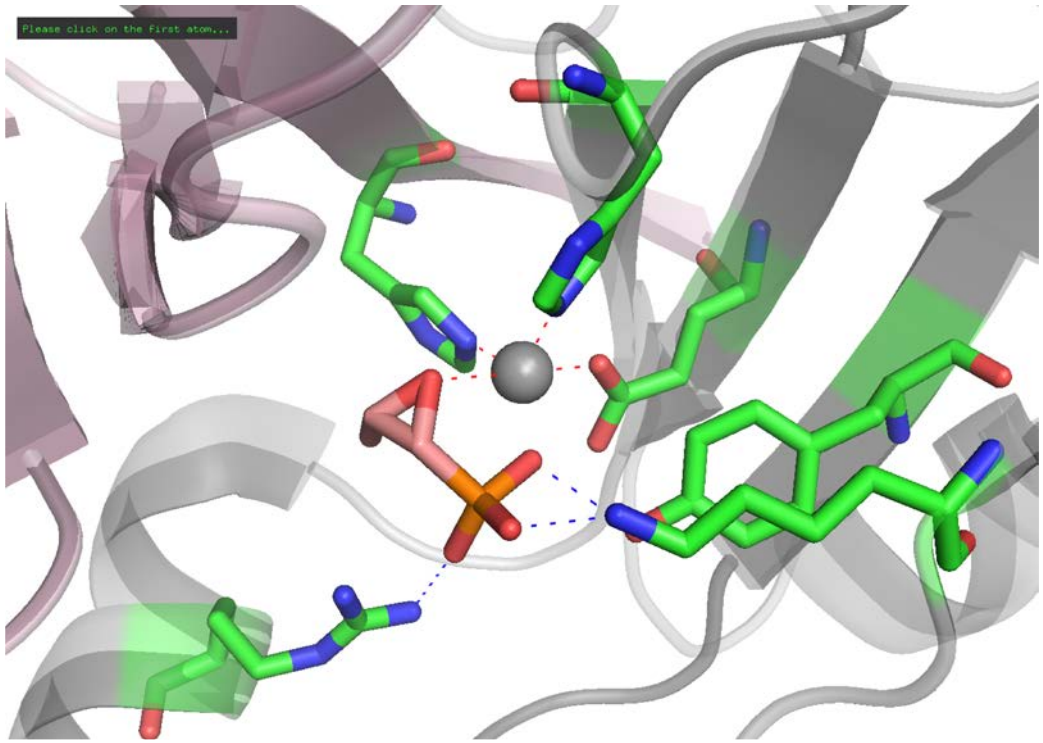
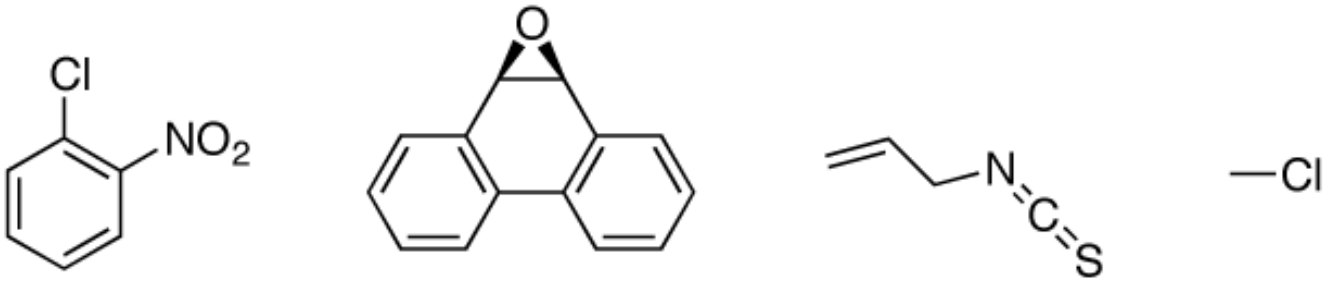
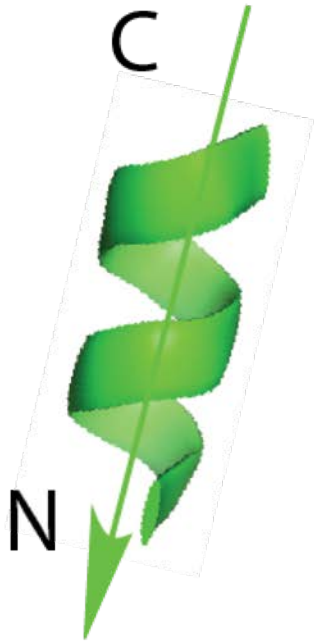


Rife 2002

Glutathione S-transferases: jack of all traits



General bases dependent glutathione S-transferase A1-1

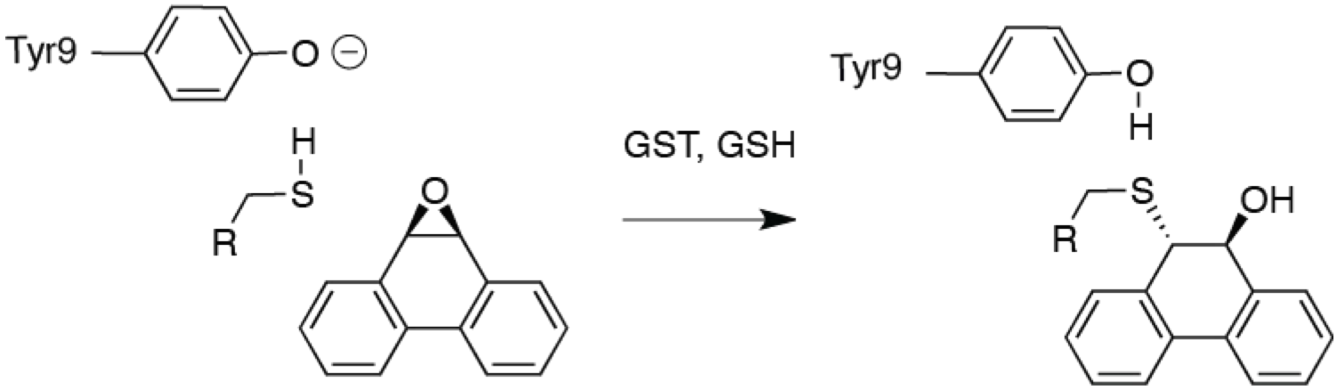


pKa_{tyr} in solution = 11

pKa_{tyr} in GST = 8.8

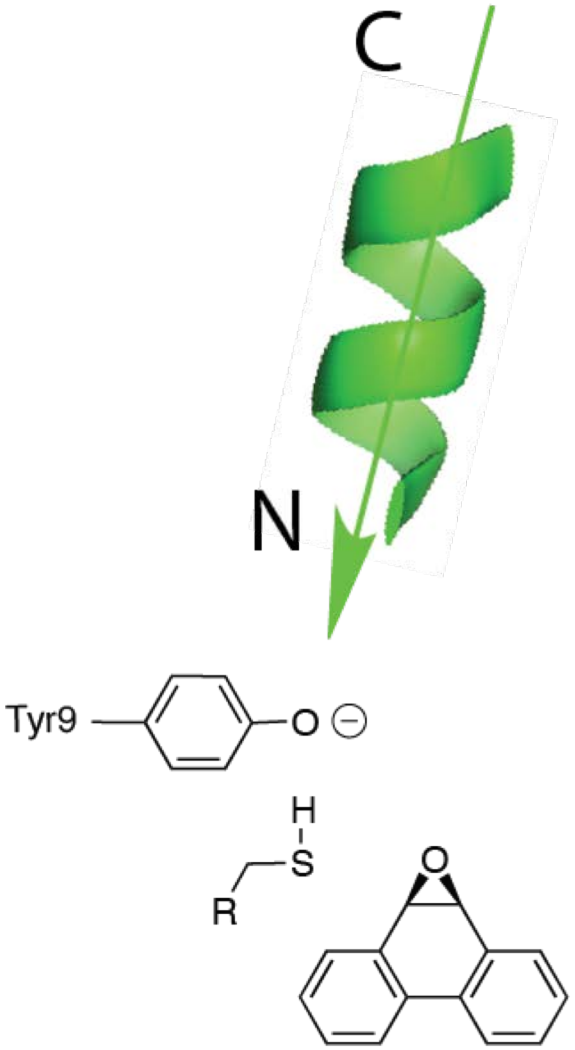
pKa suppression of 2 pH units
$\Delta G = RT\ln(100) = 2.7$ kcal/mol

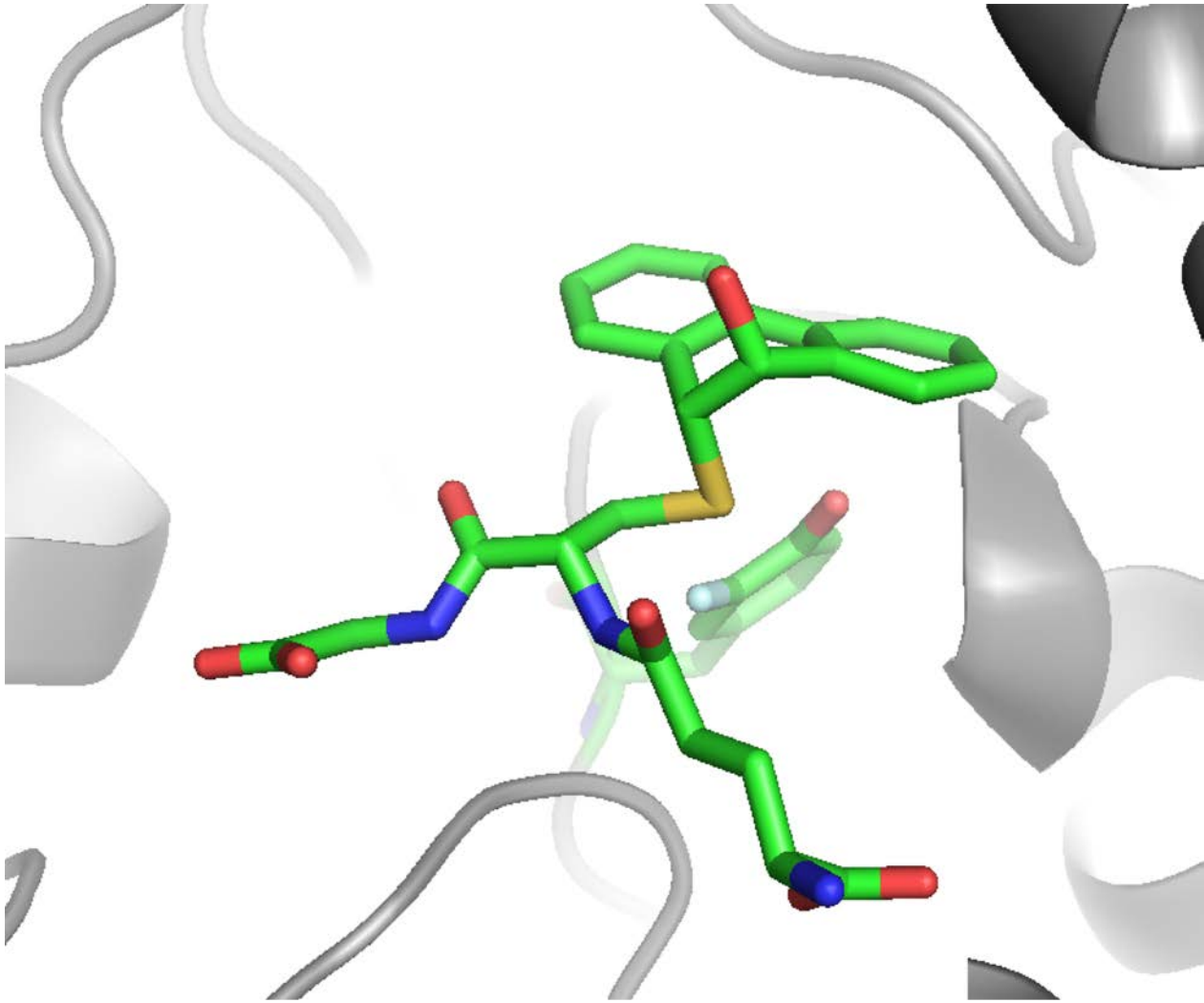
R = gas constant ≈ 2 kcal/K/mol; T = 300 K



Atkins 1993

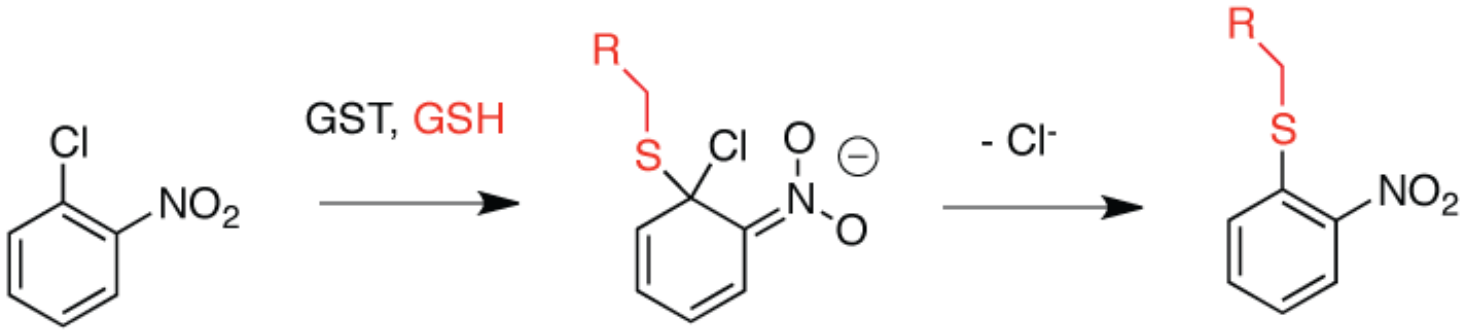
General bases dependent glutathione S-transferase A1-1





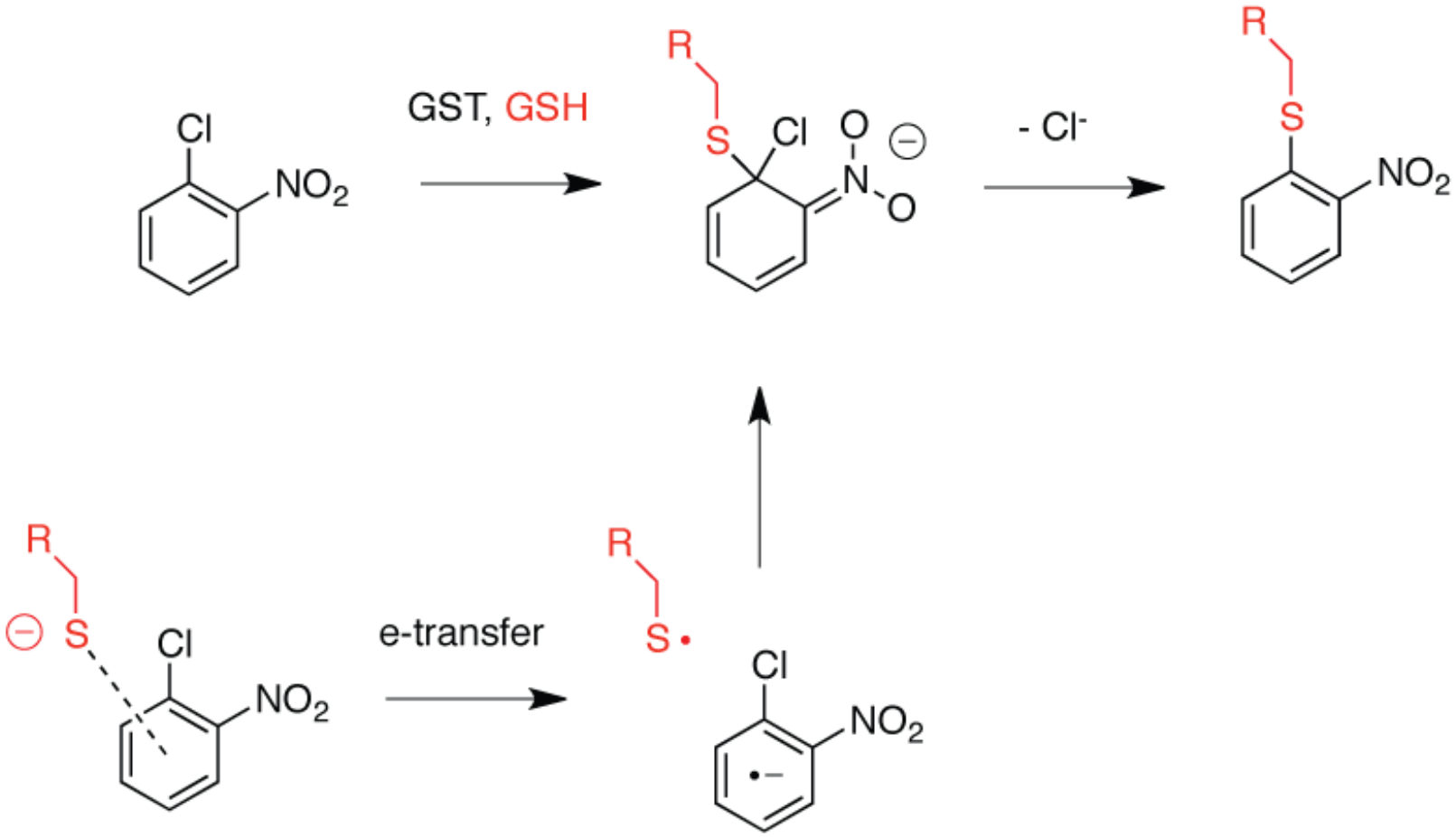
Atkins 1993

Nucleophilic aromatic substitution by GST



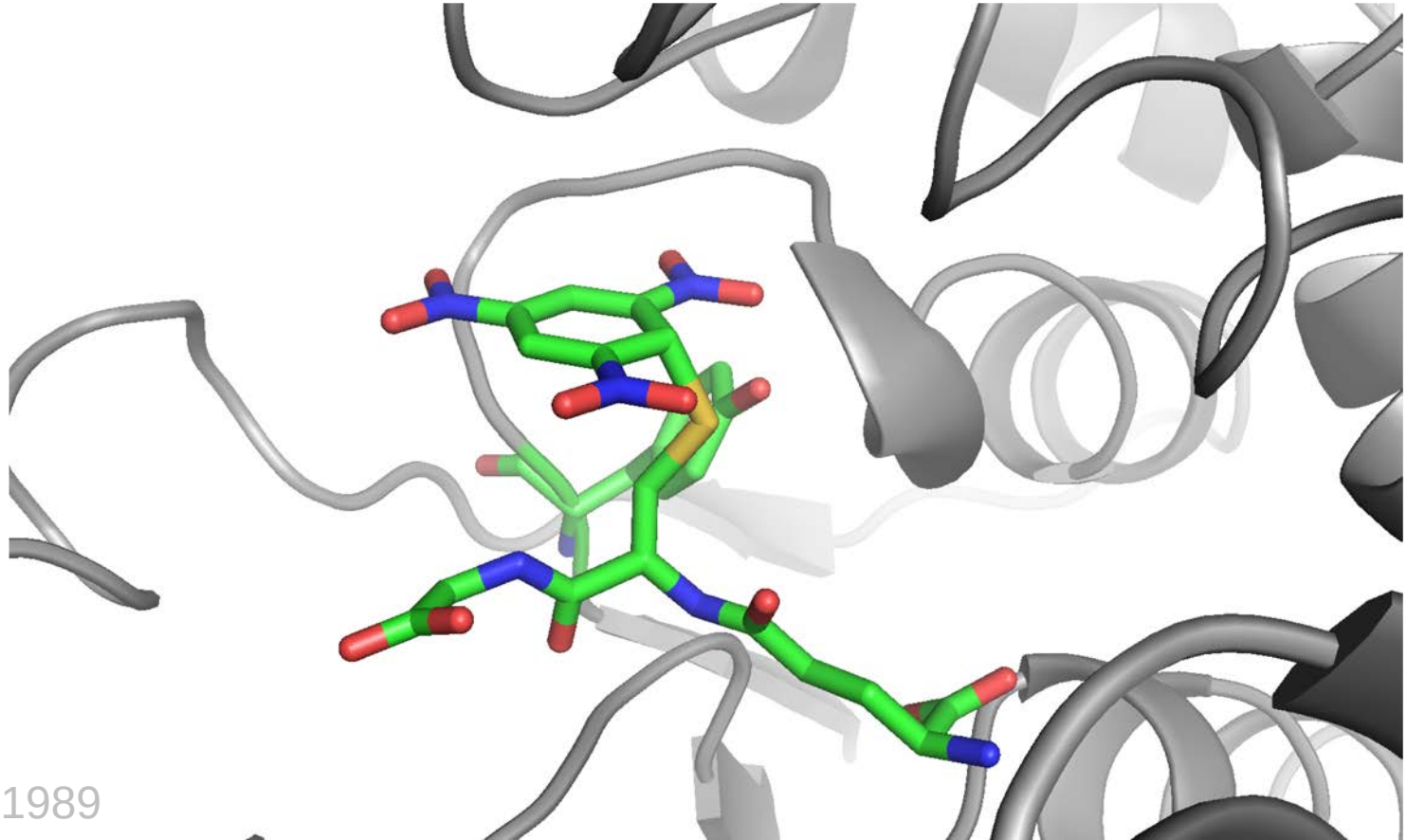
Atkins 1993, Parsons1996

Nucleophilic aromatic substitution by GST



Possible non-enzymatic mechanism

Nucleophilic aromatic substitution by GST



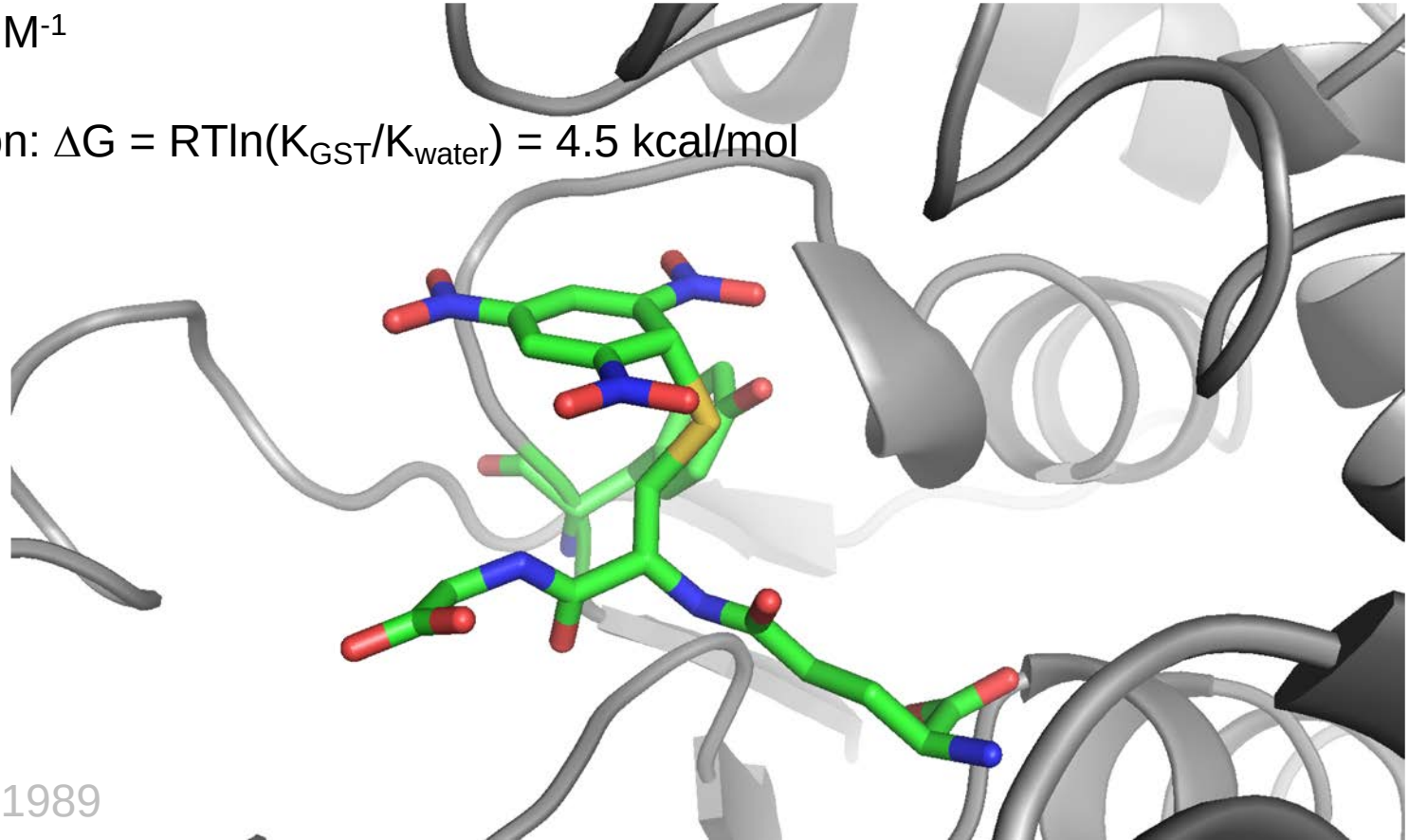
Graminski 1989

Nucleophilic aromatic substitution by GST

K_{GST} = 50000 M^{-1}

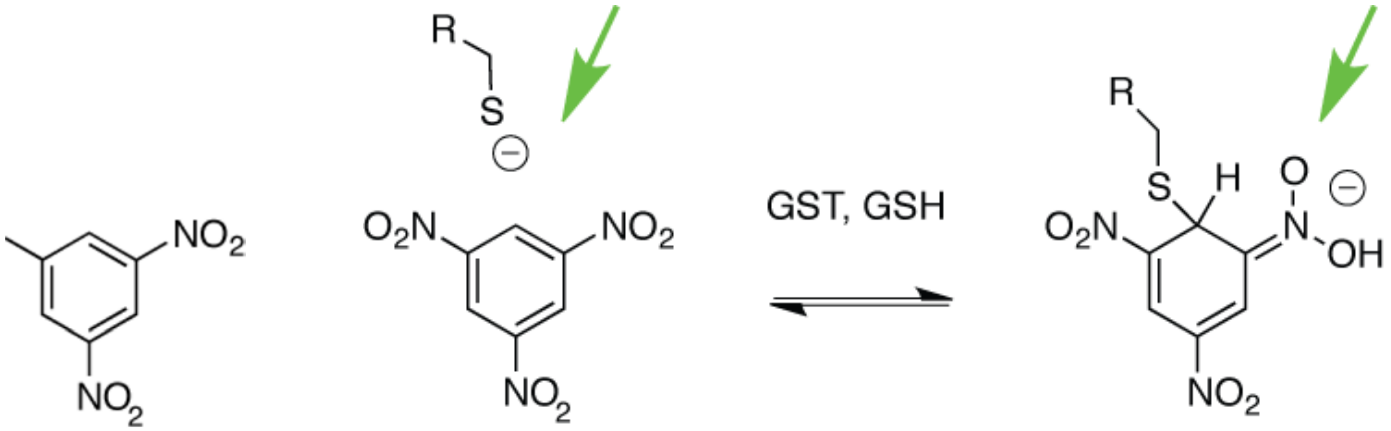
K_{water} = 28 M^{-1}

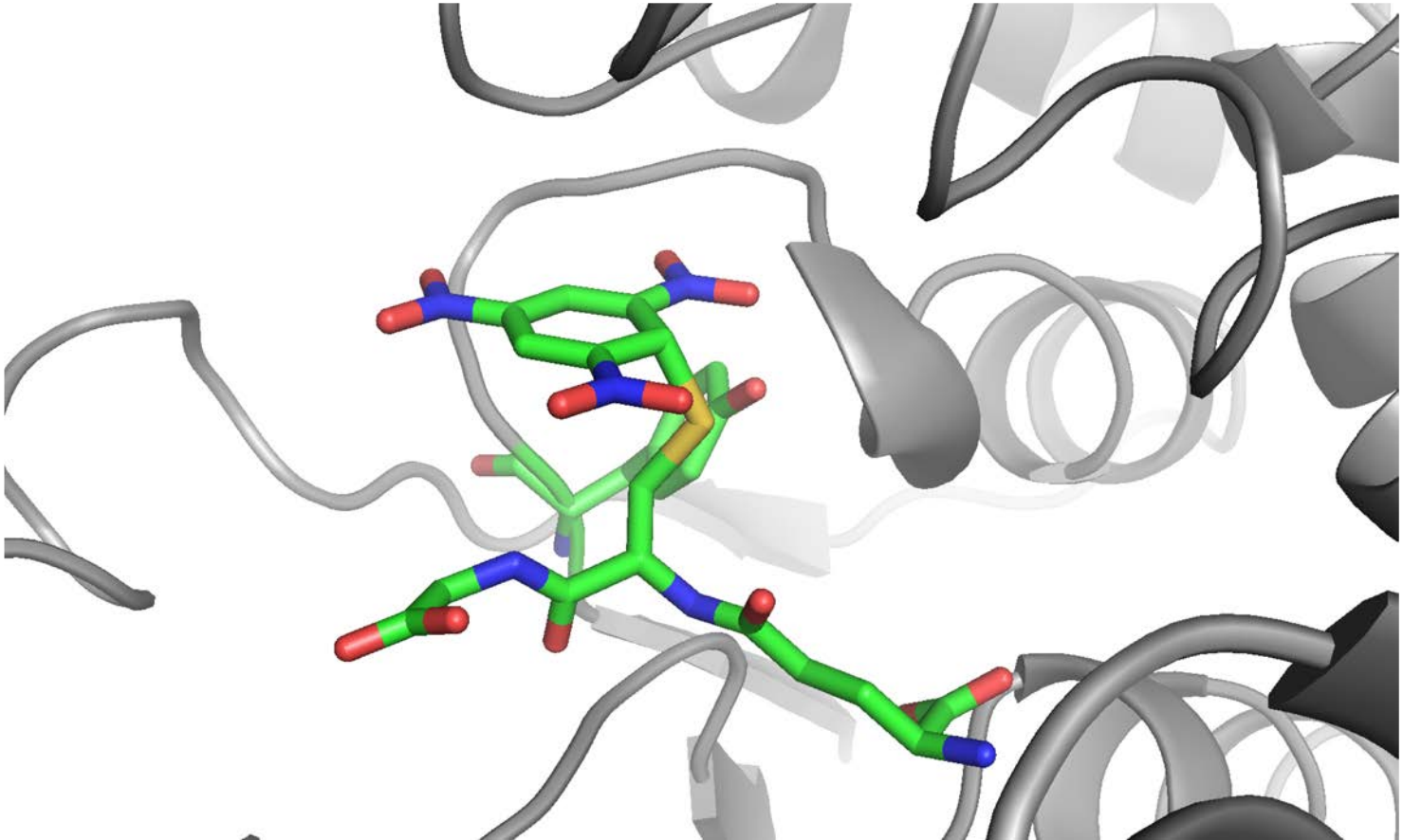
Stabilization: $\Delta G = RT\ln(K_{GST}/K_{water}) = 4.5$ kcal/mol



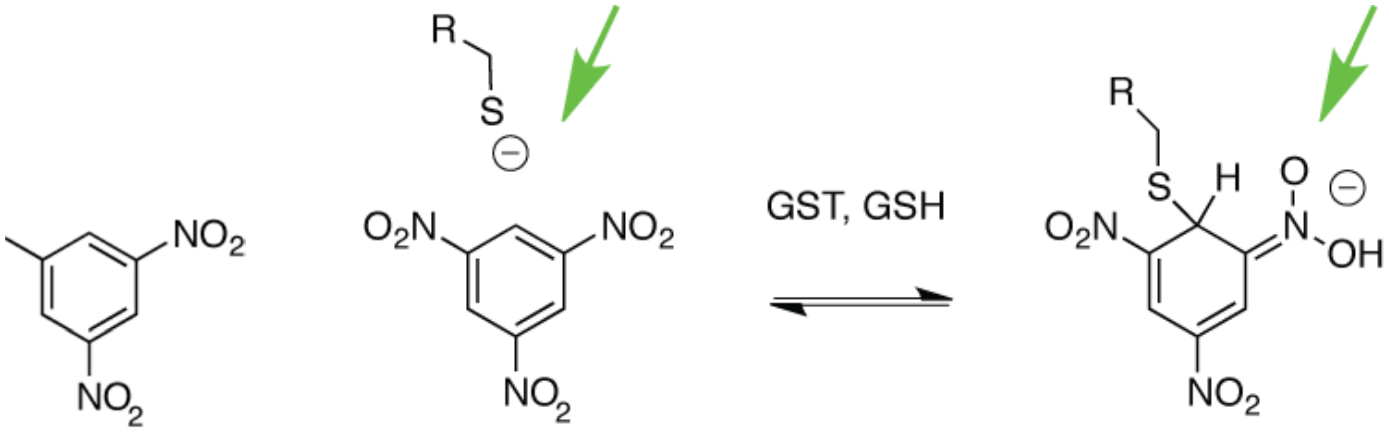
Graminski 1989

Nucleophilic aromatic substitution by GST

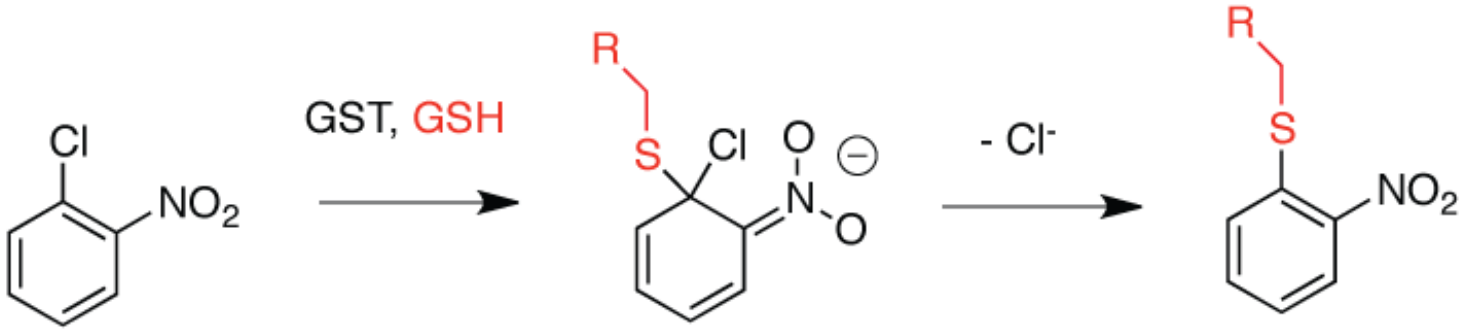




Nucleophilic aromatic substitution by GST

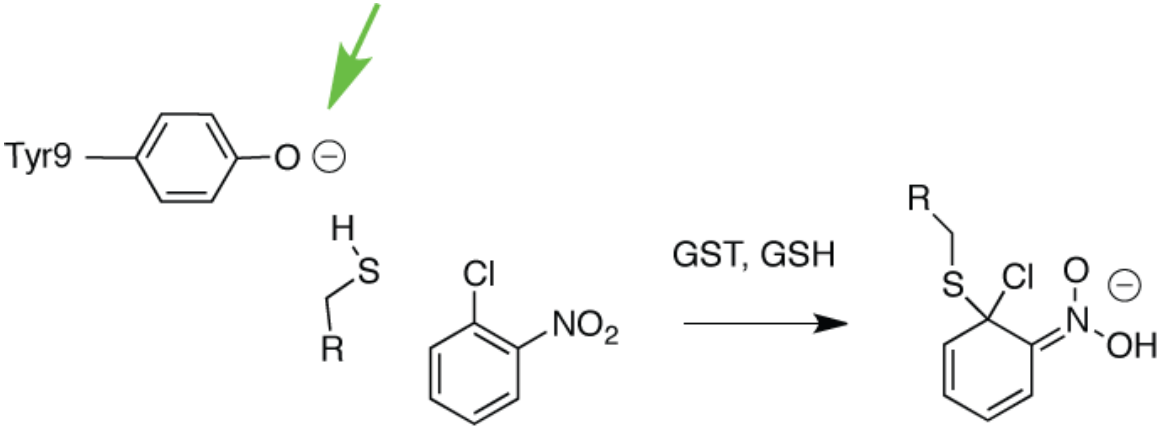


K_i > 1.5 mM K_i = 50 uM

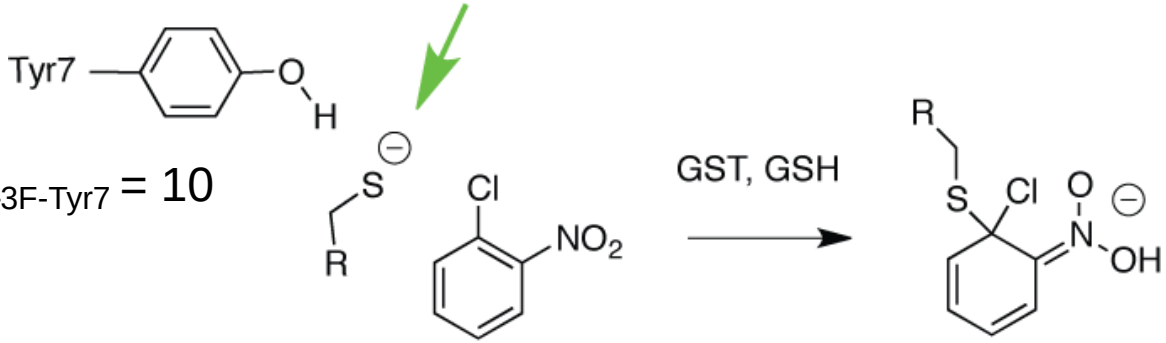


Distinction between base and electrostatic catalysis by GST

Atkins 1993



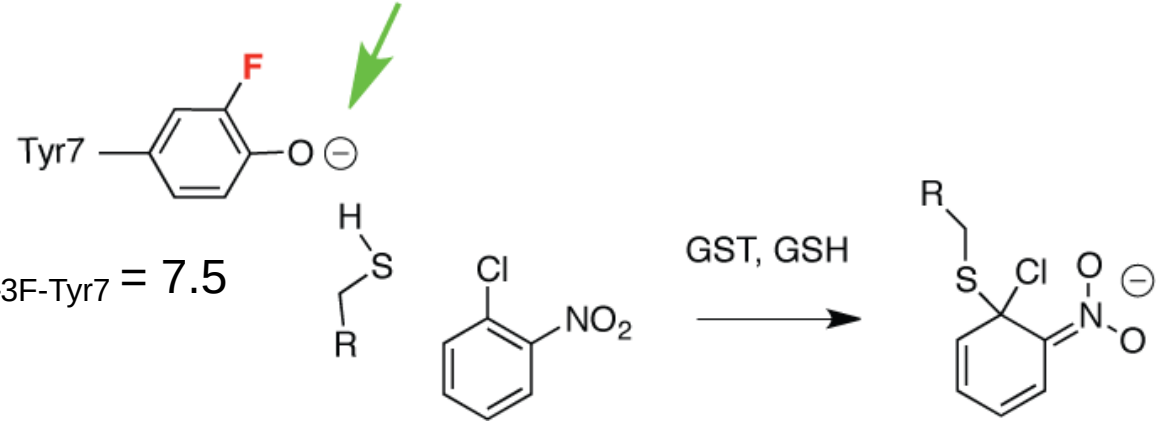
Parsons1996



$pKa_{3F\text{-}Tyr7} = 10$

Solvent isotope effect in k_{cat}/K_M

0.9 ± 0.1

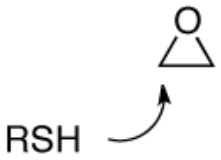


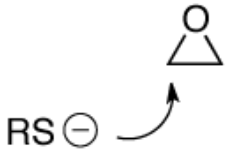
$pKa_{3F\text{-}Tyr7} = 7.5$

0.5 ± 0.2

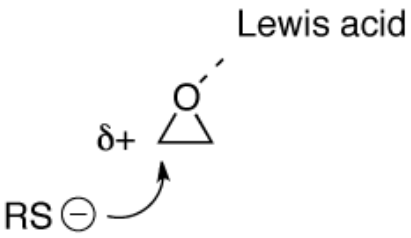
Review – Part I

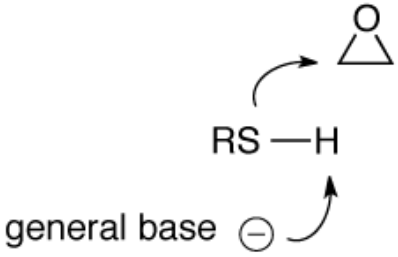
slow

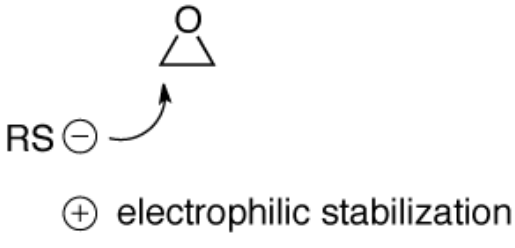


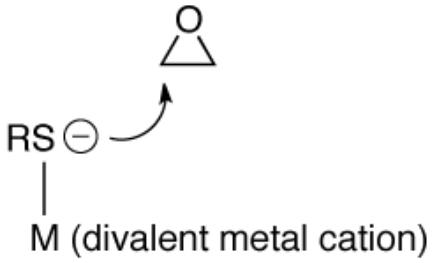


slow at physiological pH



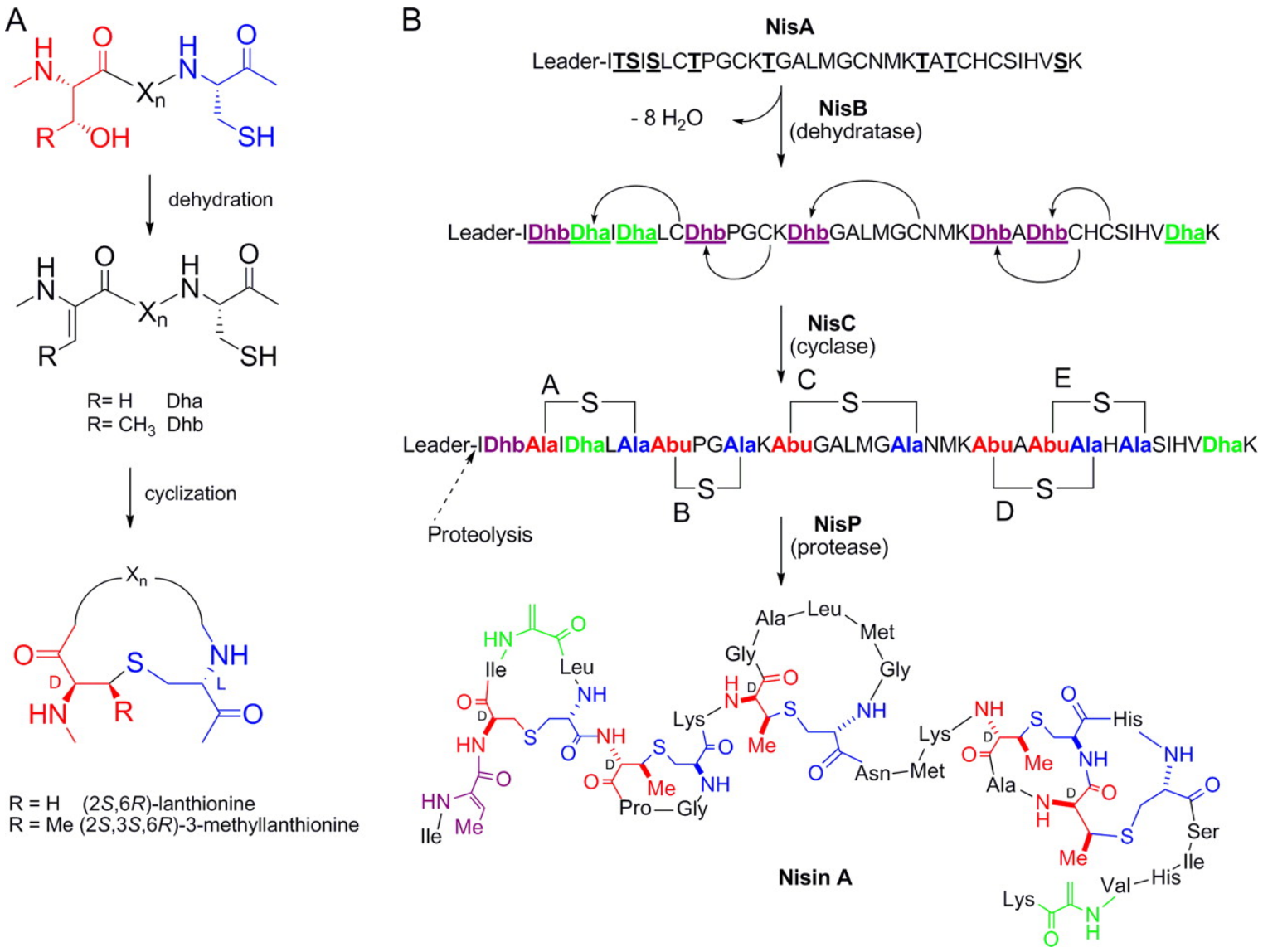






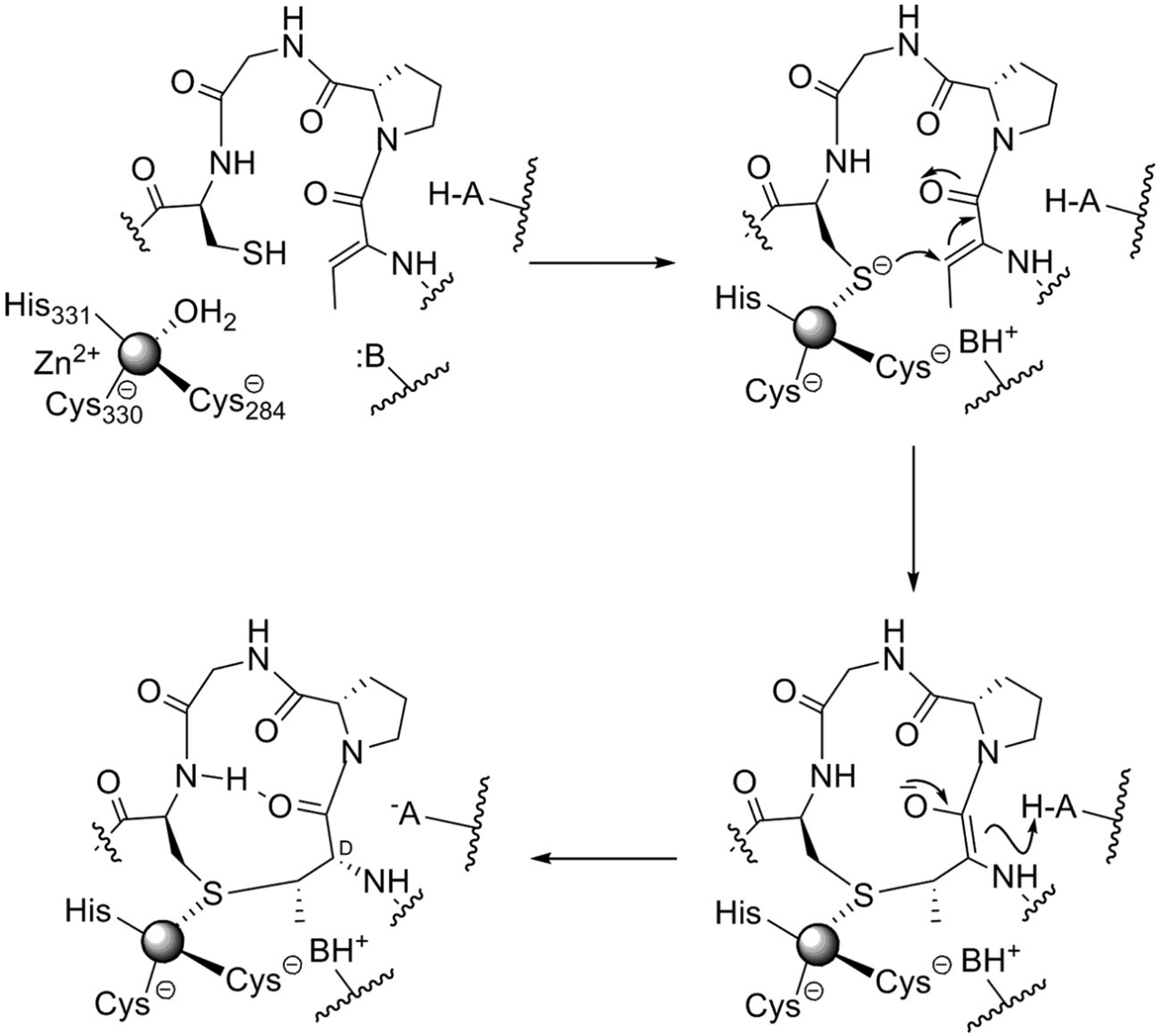
Part II: metal mediated C-S bond formation

Nisin cyclase: metal catalyzed C-S bond foramtion



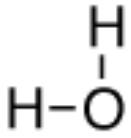
Li 2007

Nisin cyclase: conjugate addition



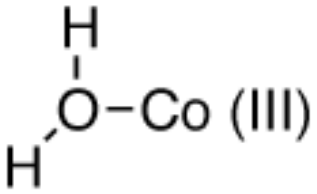
Li 2007, Li 2006

Are divalent transition metals fat protons?



$pKa_{H2O} = 15.7$

$[^{-}OH]_{pH7} = 100\ nM$

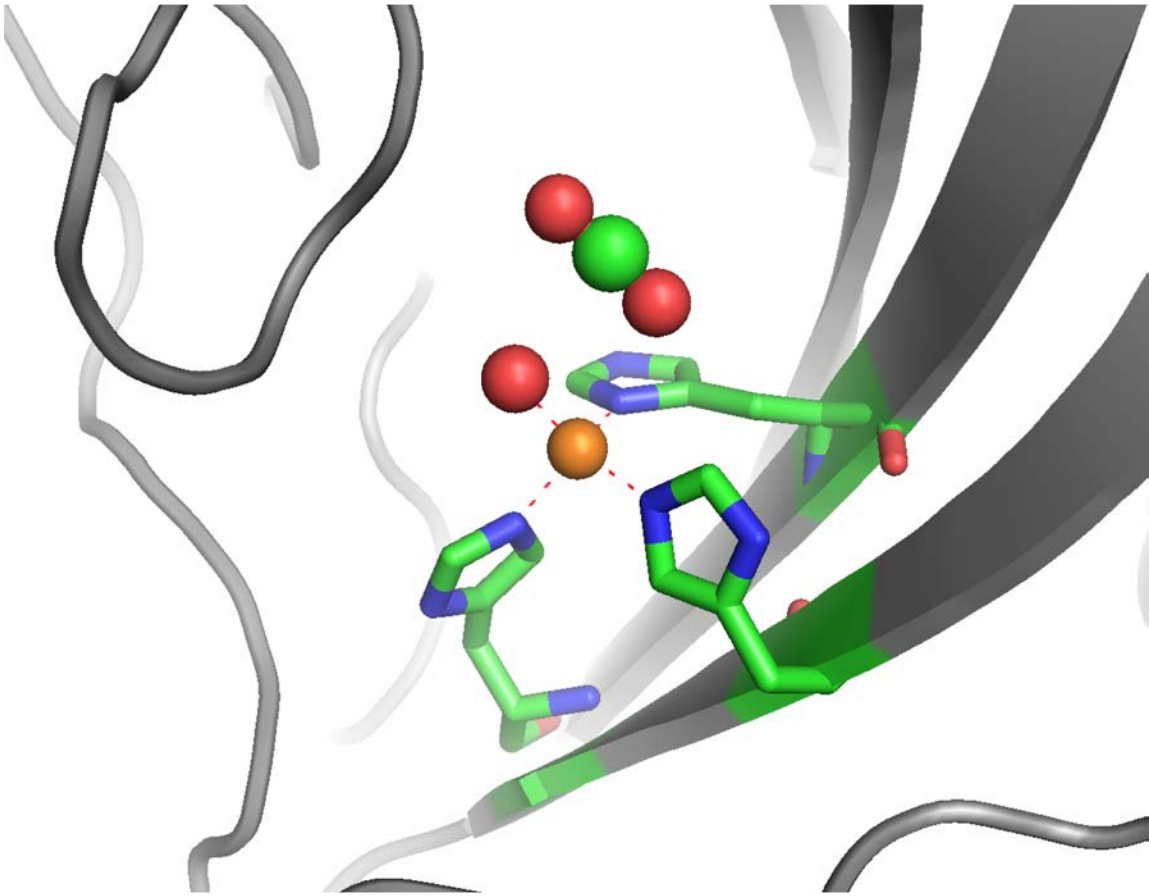


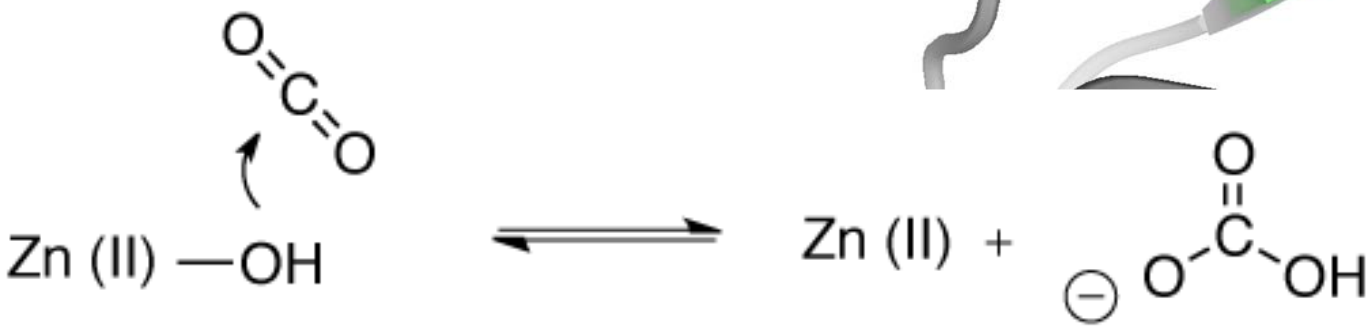
pKa = 6.5

10^{9}-fold increased acidity
40-fold reduced nucleophilicity

Fersht 1999

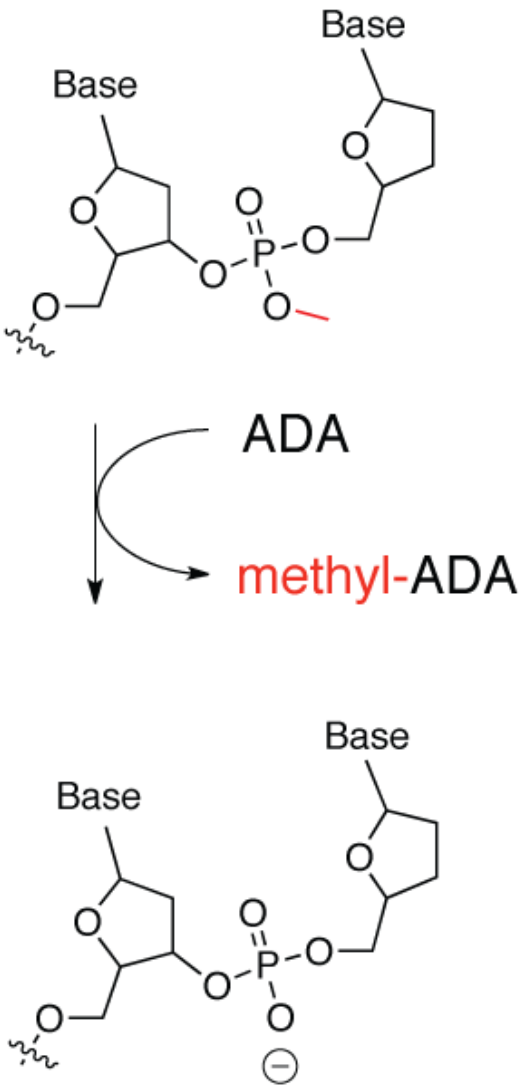
Nucleophile activation in carbonic anhydrase

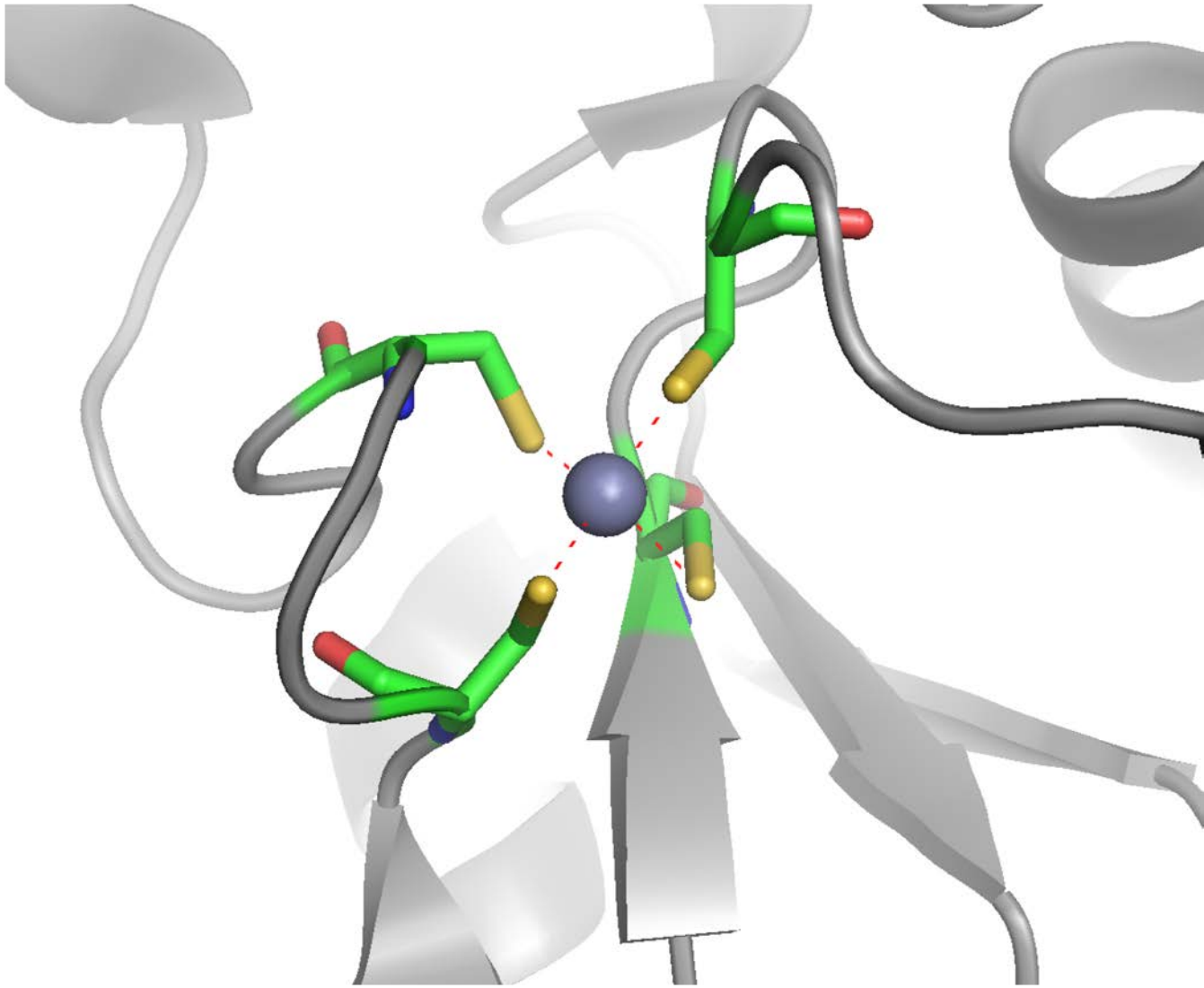




2VVA, Sjoblom 2009

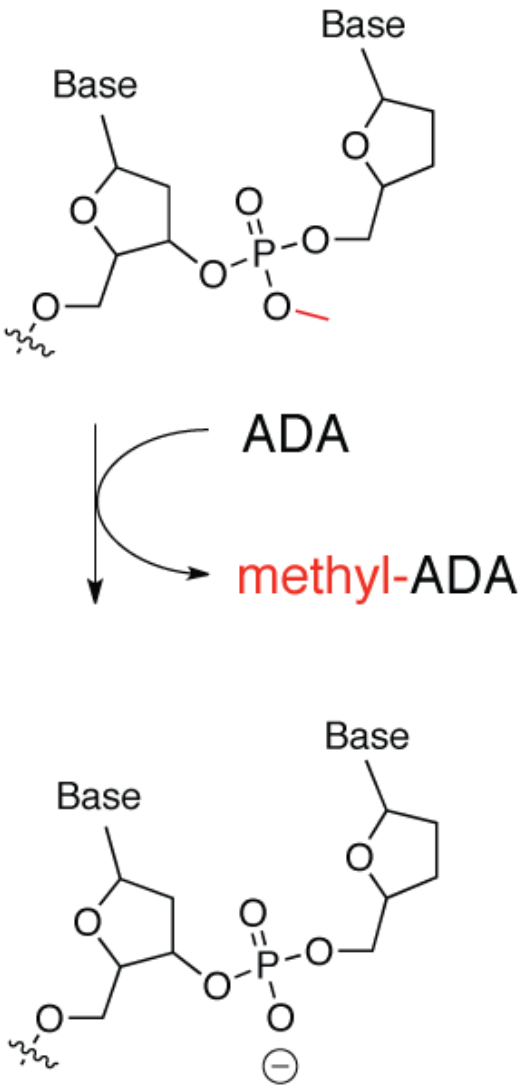
Sacrificial O^6 -alkyl guanine transferase

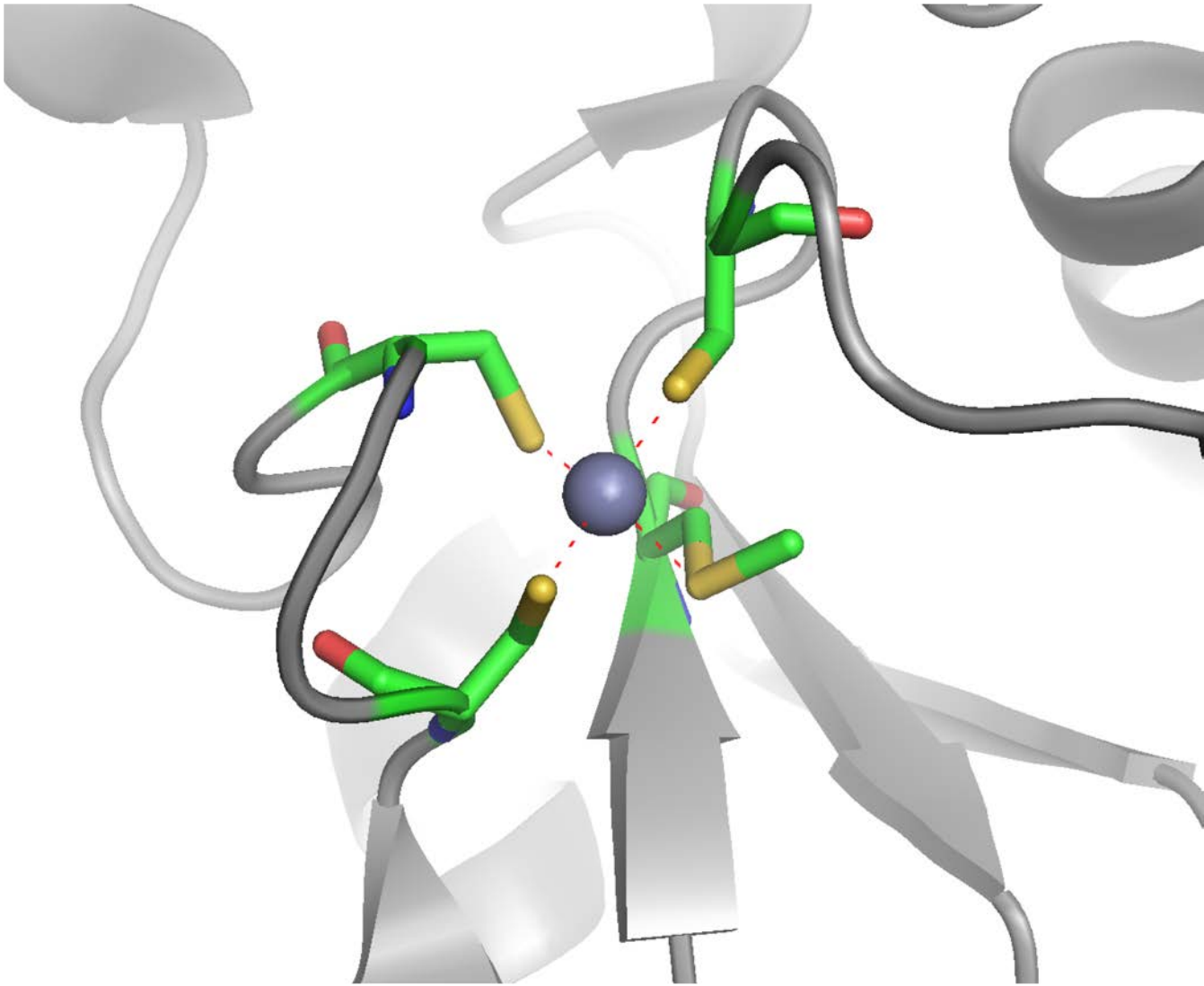




1U8B, He 2005

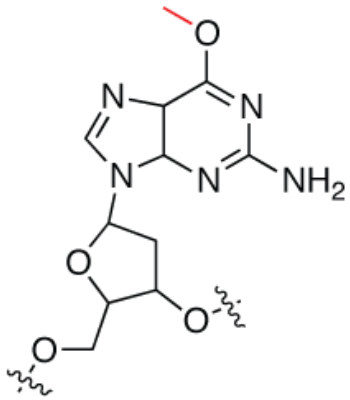
Sacrificial O^6 -alkyl guanine transferase





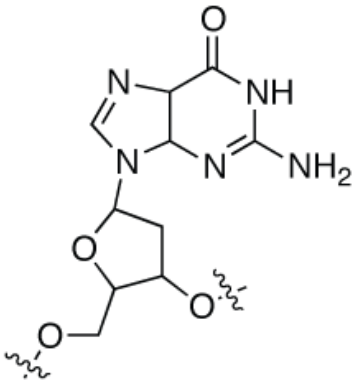
He 2005

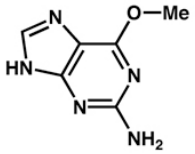
Example: Sacrificial O^6 -alkyl guanine transferase



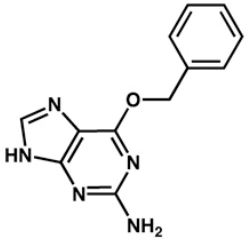
ADA

methyl-ADA

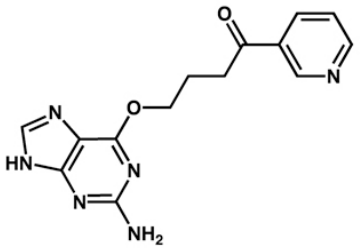




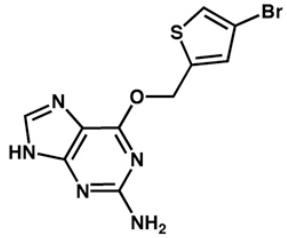
O^6-mG



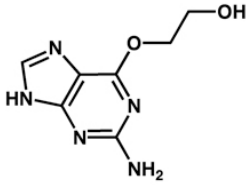
O^6-bG



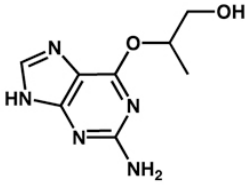
O^6-pobG



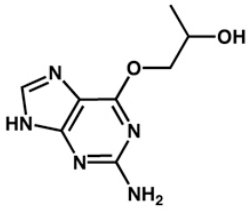
O^6-btG



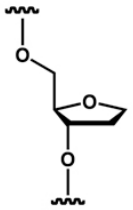
O^6-HOEtG



O^6-1hpG



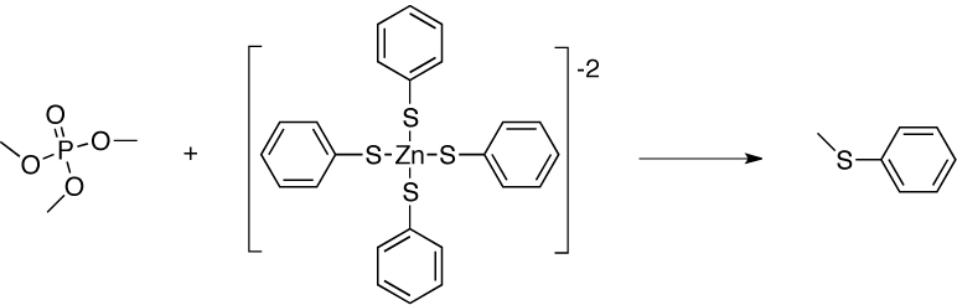
O^6-2hpG



abasic site

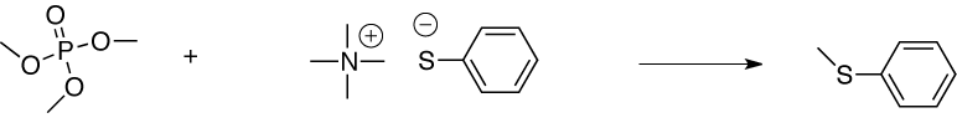
He 2005

Model alkyl transfers: zinc fingers

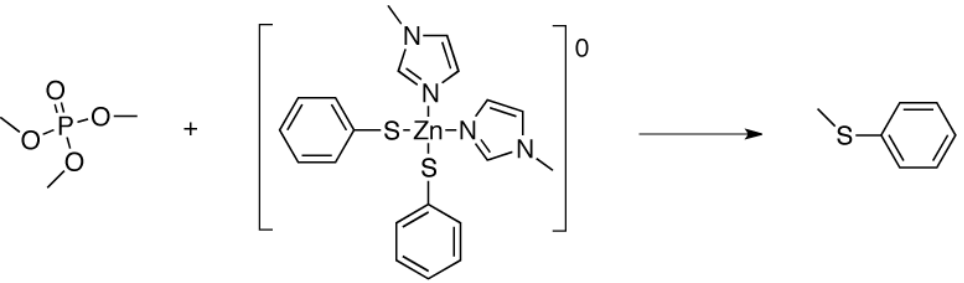


DMSO, 24°C, pseudo 1st order

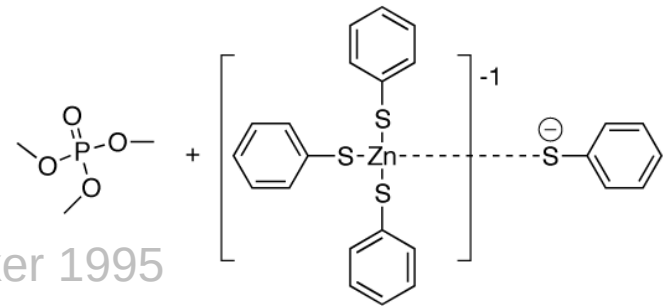
$k = 0.8 \times 10^{-4}\ s^{-1}$



$k = 1.1 \times 10^{-4}\ s^{-1}$



$K < 5 \times 10^{-5}\ s^{-1}$

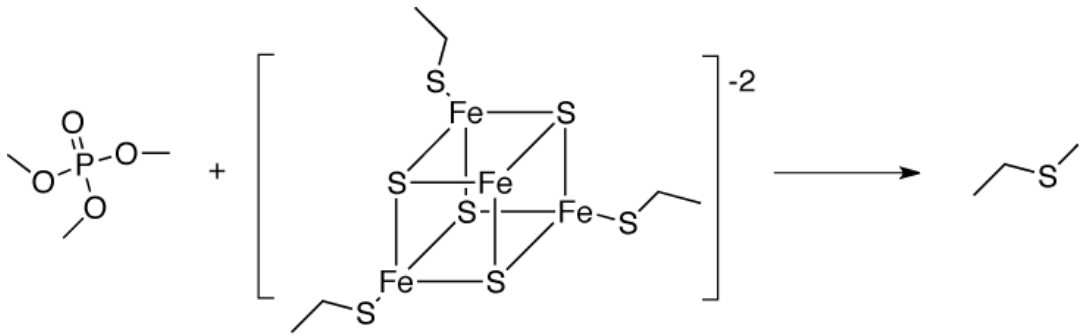


Dissociative mechanism

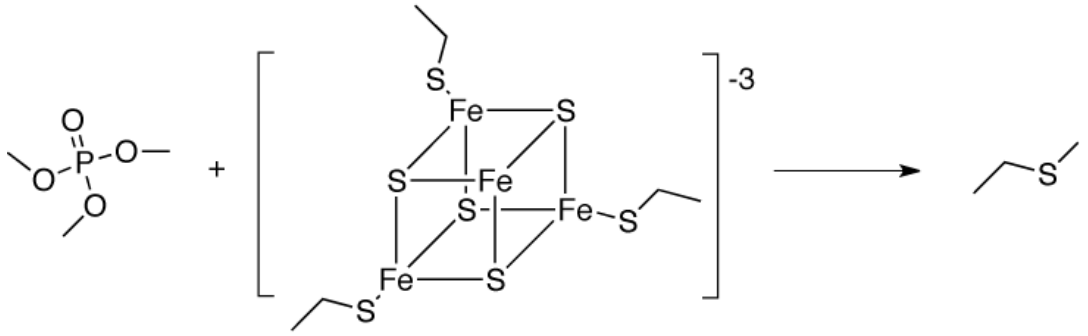
Wilker 1995

Model alkyl transfers: iron sulfur clusters

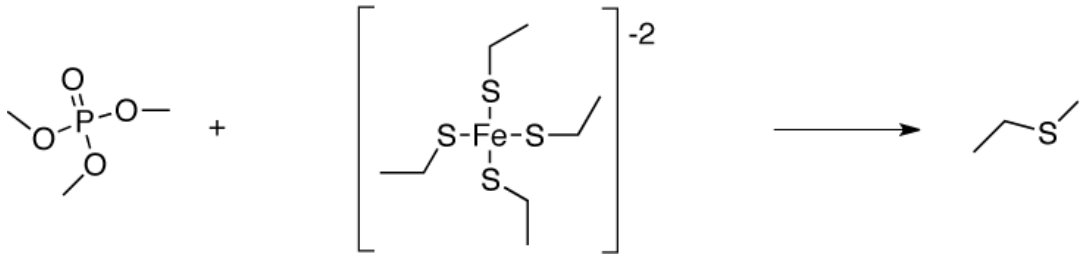
DMSO, 24°C, pseudo 1st order



$k = 10^{-7}\ s^{-1}$



$k = 10^{-5}\ s^{-1}$



$k = 10^{-3}\ s^{-1}$

Wilker 1999

Model alkyl transfers: iron sulfur clusters

→ charge of the complex

→ charge delocalization lowers nucleophilicity

→ metal enhanced nucleophilicity is not observed

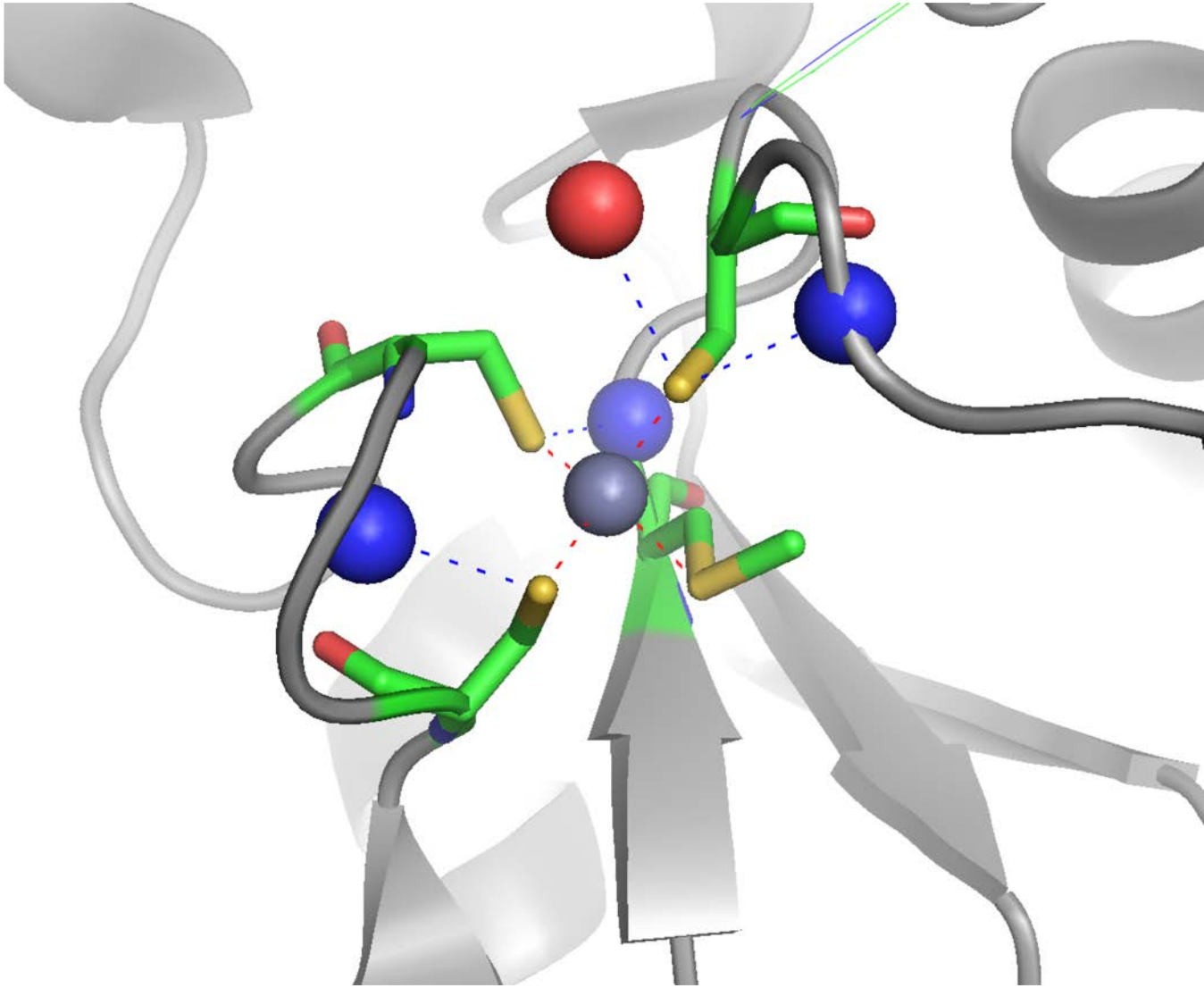
→ $Zn(SR)_4^{-2}$ alkylation may proceed via dissociative mechanism

→ Zinc fingers and Fe/S clusters can catalyze S-alkylation

Q:
→ specificity for one S-ligand
→ nucleophilicity of mixed sphere Zn sites
→ Second sphere influences

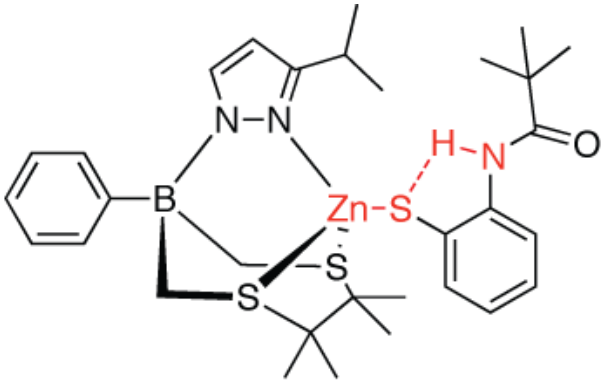
Wilker 1999

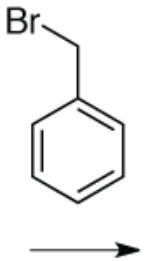
Sacrificial O^6 -alkyl guanine transferase

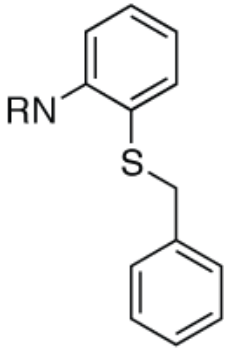


He 2005

Influence of the secondary sphere







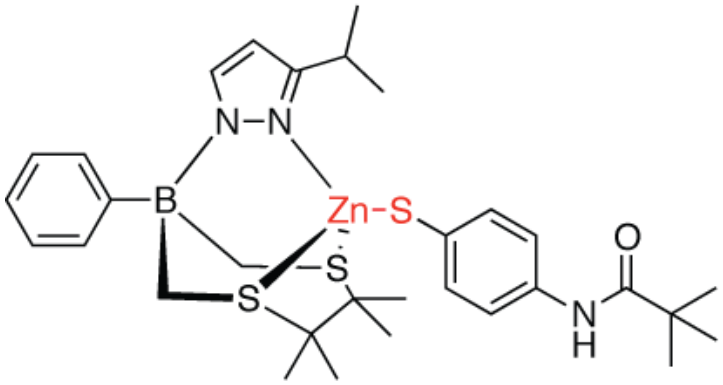
Toluene, 60°C, SN2

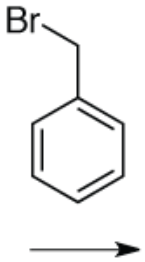
k_{ortho} = 1.3 x 10^4 $M^{-1}s^{-1}$

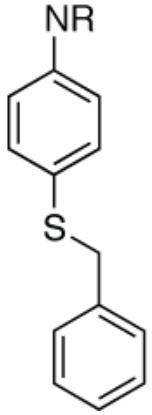
k_{rel} = 1

$k_{ortho,deutero}$ = 4.0 x 10^4 $M^{-1}s^{-1}$

k_{rel} = 3







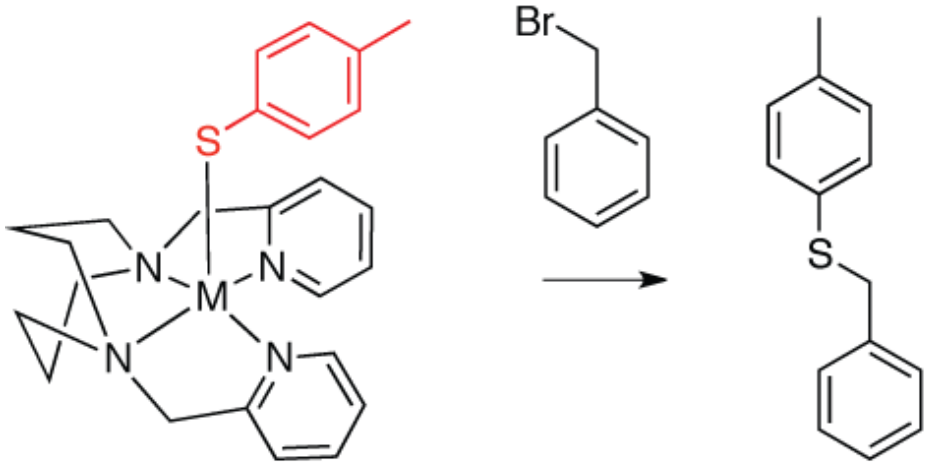
k_{para} = 4.4 x 10^5 $M^{-1}s^{-1}$

k_{rel} > 30

k_{Cd} is 17 fold larger than k_{Zn} → ionic radius (Zn) = 75 pm
ionic radius (Cd) = 95 pm

Chiou 2003

Influence of the metal



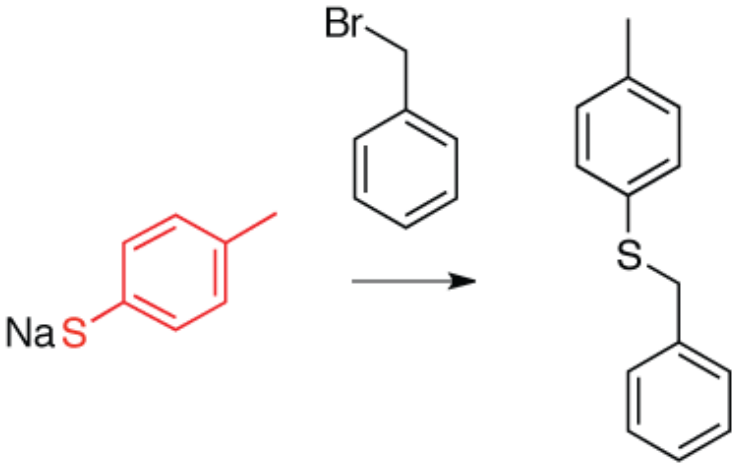
Acetonitrile, 30°C

k_{Fe} = 5 x 10^{-3} $M^{-1}s^{-1}$

k_{Co} = 3 x 10^{-3} $M^{-1}s^{-1}$

k_{Ni} = 3 x 10^{-2} $M^{-1}s^{-1}$

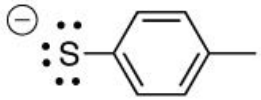
k_{Zn} = 2 x 10^{-2} $M^{-1}s^{-1}$



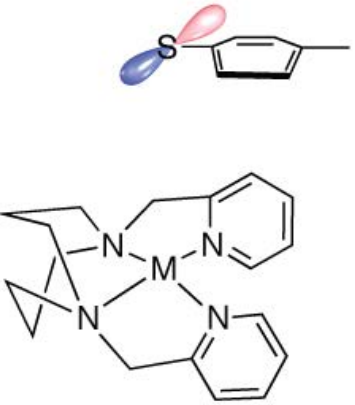
k_{Na} = >> 10^{-2} $M^{-1}s^{-1}$

Fox 2004

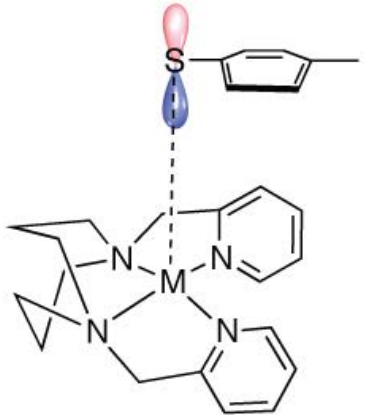
Influence of the metal

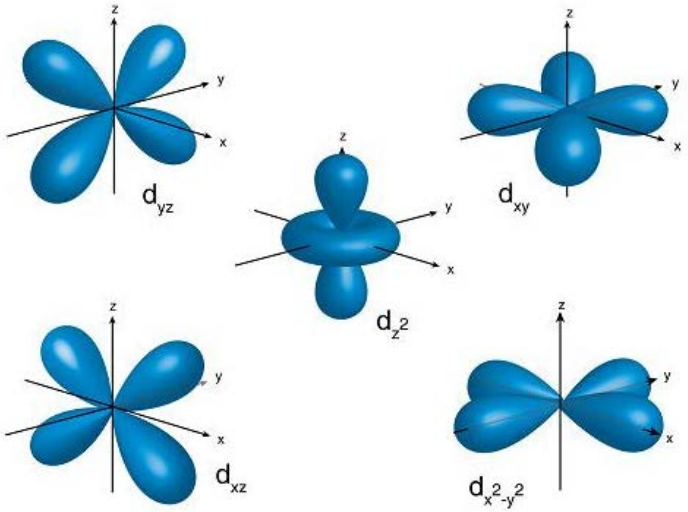


$\pi_{in\ plane\ (ip)}$



$\pi_{orthogonal\ (op)}$

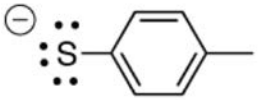




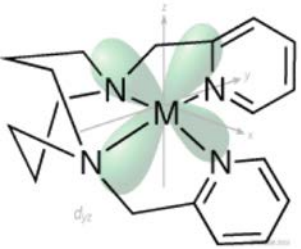
- π_{op} forms with the metal d_{z2} orbital a σ-bond
- π_{ip} may engage stabilizing or destabilizing interactions with the d_{yz} orbital

Fox 2004

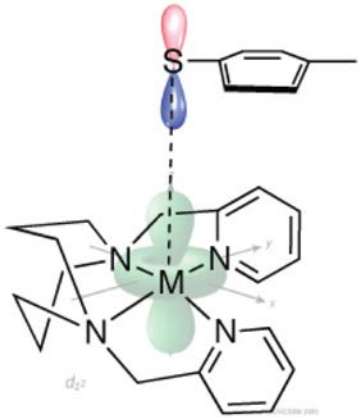
Influence of the metal

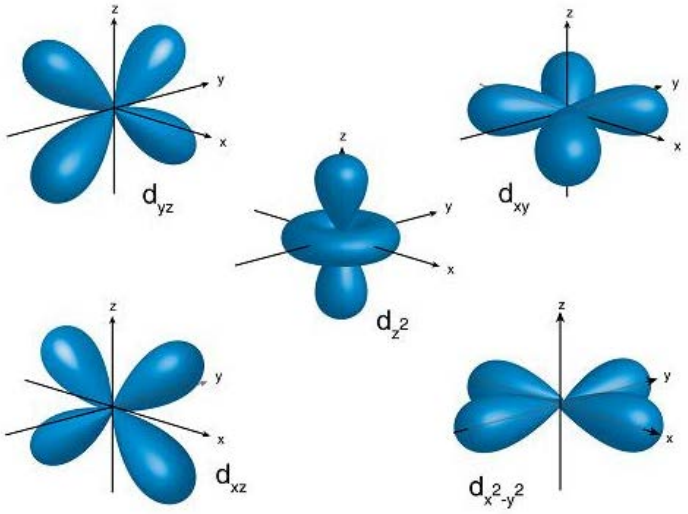


$\pi_{in\ plane\ (ip)}$



$\pi_{orthogonal\ (op)}$

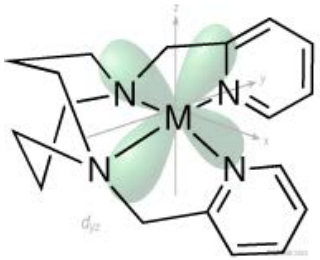


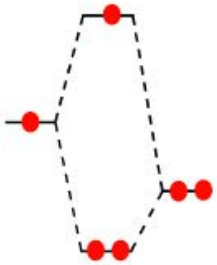


- π_{op} forms with the metal d_{z2} orbital a σ-bond
- π_{ip} may engage stabilizing or destabilizing interactions with the d_{yz} orbital

Fox 2004

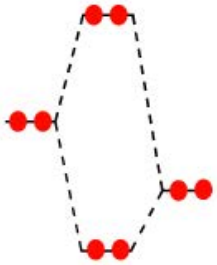
Influence of the metal





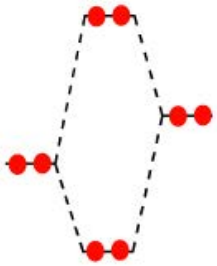
π_{ip} lone pair stabilized

→ partial e-transfer to the metal, reduced nucleophilicity



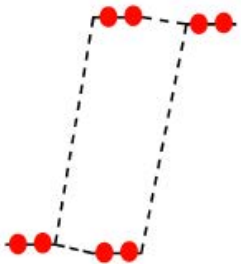
π_{ip} lone pair stabilized (filled/filled interaction)

→ no e-transfer



π_{ip} lone pair destabilized (filled/filled interaction

→ increased nucleophilicity

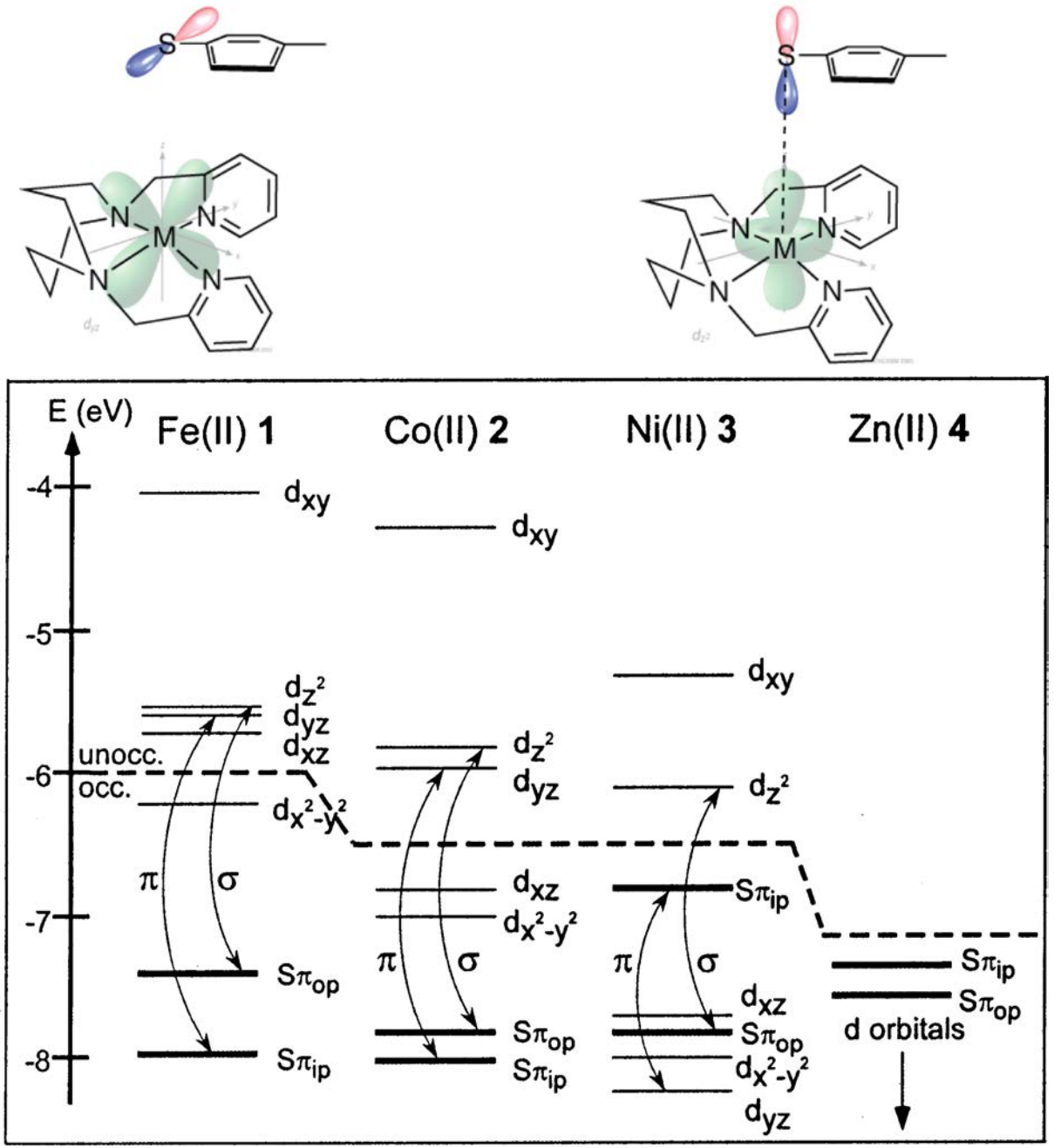


fully ionic bonding character, covalency is lost

→ increased nucleophilicity

Fox 2004

DFT calculated molecular orbital energy-level diagrams



Fox 2004

Three electronic effects influence the nucleophilicity of thiolate complexes of first row divalent transition metals

19 K Potassium 39.098	20 Ca Calcium 40.078	21 Sc Scandium 44.956	22 Ti Titanium 47.88	23 V Vanadium 50.942	24 Cr Chromium 51.996	25 Mn Manganese 54.938	26 Fe Iron 55.933	27 Co Cobalt 58.933	28 Ni Nickel 58.693	29 Cu Copper 63.546	30 Zn Zinc 65.39	31 Ga Gallium 69.732	32 Ge Germanium 72.61	33 As Arsenic 74.922	34 Se Selenium 78.09	35 Br Bromine 79.904	36 Kr Krypton 84.80
37 Rb Rubidium 84.468	38 Sr Strontium 87.62	39 Y Yttrium 88.906	40 Zr Zirconium 91.224	41 Nb Niobium 92.906	42 Mo Molybdenum 95.94	43 Tc Technetium 98.907	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.906	46 Pd Palladium 106.42	47 Ag Silver 107.868	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.71	51 Sb Antimony 121.760	52 Te Tellurium 127.6	53 I Iodine 126.904	54 Xe Xenon 131.29

- **d-orbitals decrease in energy relative to the thiolate MOs** (contraction due to increasing effective nuclear charge)

- **filled/filled or filled/half filled interactions** modulate ligand based HOMO

- **Spin densities** play no role in this system

→ **Zn (II) is ideal for alkylation reactions:**

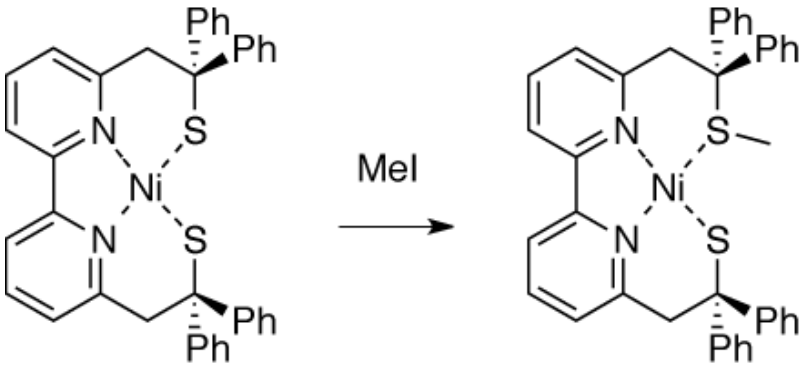
A) forms nucleophilic thiolate complexes

B) low affinity for the neutral thioether product (product inhibition)

Fox 2004

Comparison between Ni (II) and Zn (II)

- Much fewer proteins depend on Ni (II) than on Zn (II)
- Ni (II) is highly toxic

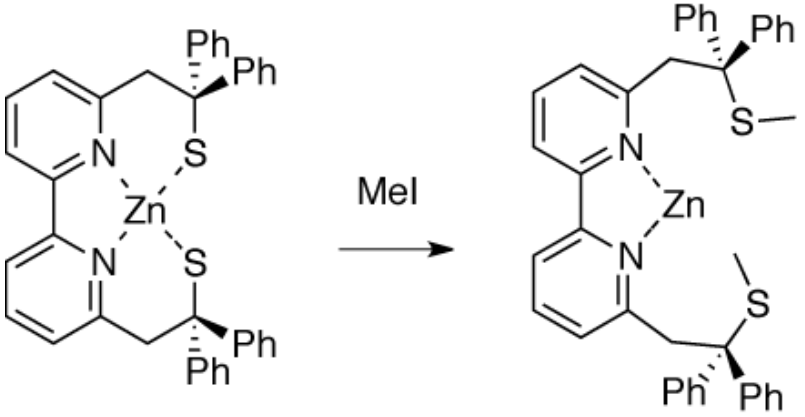


Chloroform, 28°C

$k_{1,Ni}$ = 2 x 10^{-2} $M^{-1}s^{-1}$

$k_{2,Ni}$ << $k_{1,Ni}$

→ reduced nucleophilicity, steric hindrance



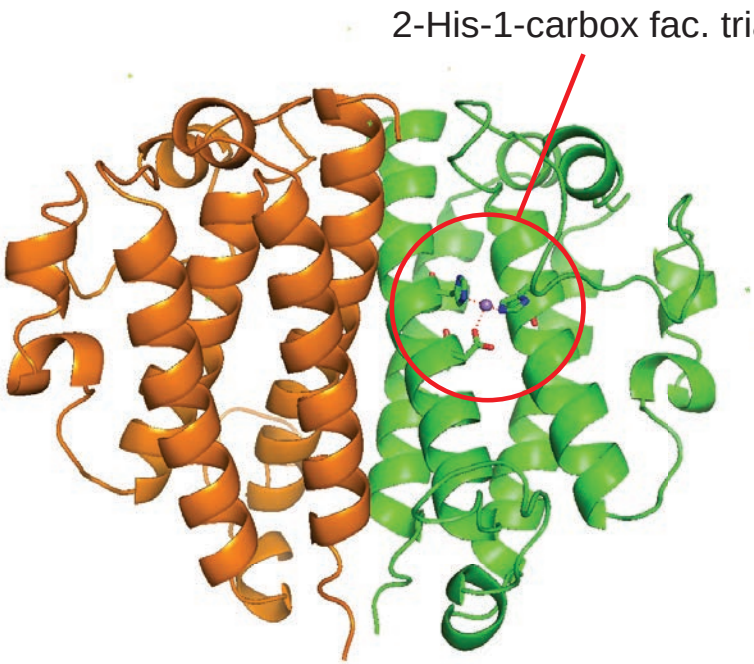
$k_{1,\ Zn}$ = 3 x 10^{-3} $M^{-1}s^{-1}$

$k_{2,\ Zn} > k_{1,\ Zn}$

Calculated bond strengths: Ni – $S_{thiolate}$ ≥ Ni - $S_{thioether}$ ≥ Zn - $S_{thiolate}$ >> Zn - $S_{thioether}$

Ni – $S_{thiolate}$ and Ni - $S_{thioether}$ are covalent; Zn - $S_{thiolate}$ is ionic

DinB_2: a new class of zinc-dependent GSTs



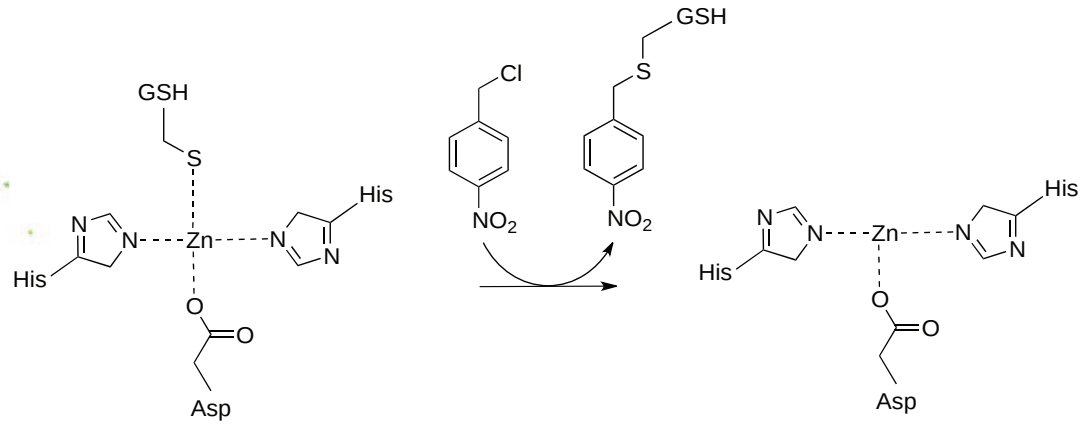
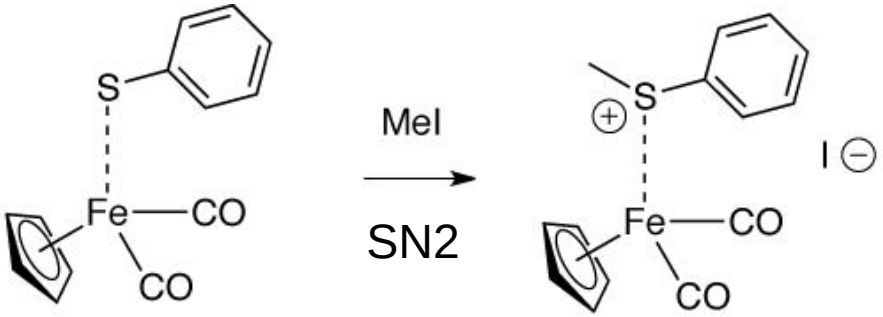


Table 1: Summary of the Michaelis-Menten parameters with different metals.

Metal	K_M [μM]	k_{cat} [min^{-1}]	k_{cat}/K_M [$min^{-1}M^{-1}$]	R-sq
Zn	1.6 ± 0.3	0.56 ± 0.01	350000	0.91
Co	26 ± 5	0.59 ± 0.03	23000	0.96
Mn	4.1 ± 1	0.25 ± 0.01	61000	0.91
Ni	6526 ± 1836	21.5 ± 6	3300	0.99
Fe	1480 ± 735	1.62 ± 0. 3	1090	0.84

Destabilizing dπ – pπ orbital interactions in iron (II)-thiolate compexes



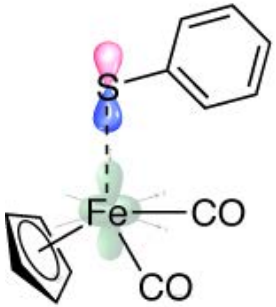
Acetone, 20°C

k_{methyl} = 2 x 10^{-3} $M^{-1}s^{-1}$

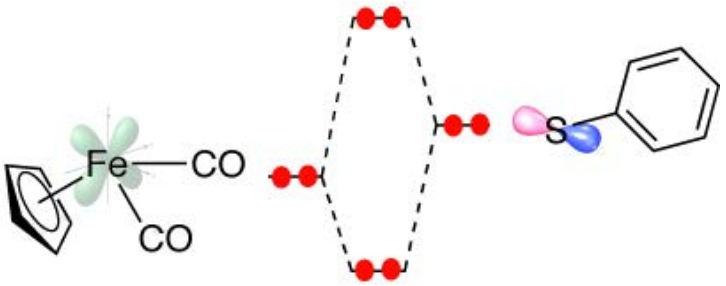
k_{ethyl} = 9 x 10^{-5} $M^{-1}s^{-1}$

$k_{isopropyl}$ << k_{ethyl}

- pseudooctahedral d^6 complex
- CO: π-acceptor ligands, thiolate: π-donor ligand



d_{z2} and 3_{py} form a σ-bond



d_{z2} and 3_{px} form a π- and π* orbitals

→ π* is the sulfur based HOMO
→ Homo is destabilized by 1.3 eV (23 kcal/mol)

Ashby 1988

Destabilizing dπ – pπ orbital interactions in iron (II)-thiolate compexes

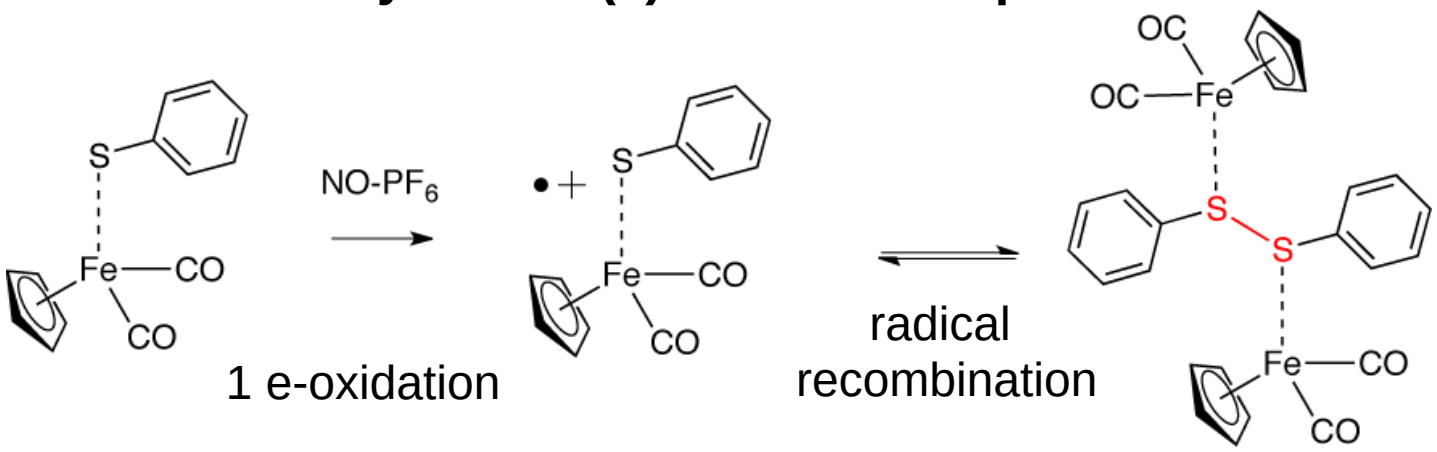
→ Homo is destabilized by 1.3 eV (23 kcal/mol)

(gas phase photoelectron spectroscopy & computation)

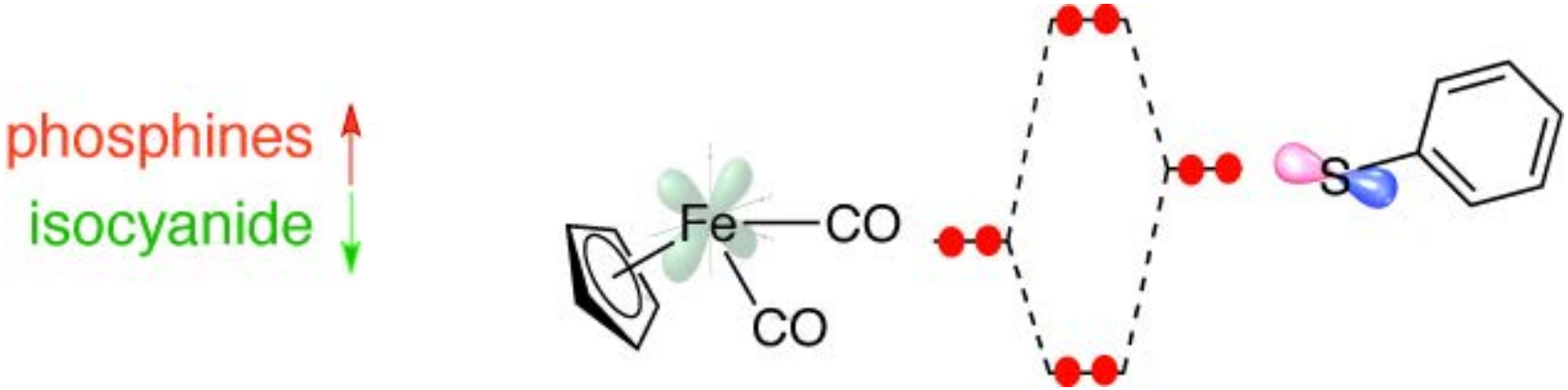
→ S-nucelophilicity is enhanced due to dπ-pπ filled-filled interactions

Ashby 1988

Redox chemistry in iron (II)-thiolate compexes

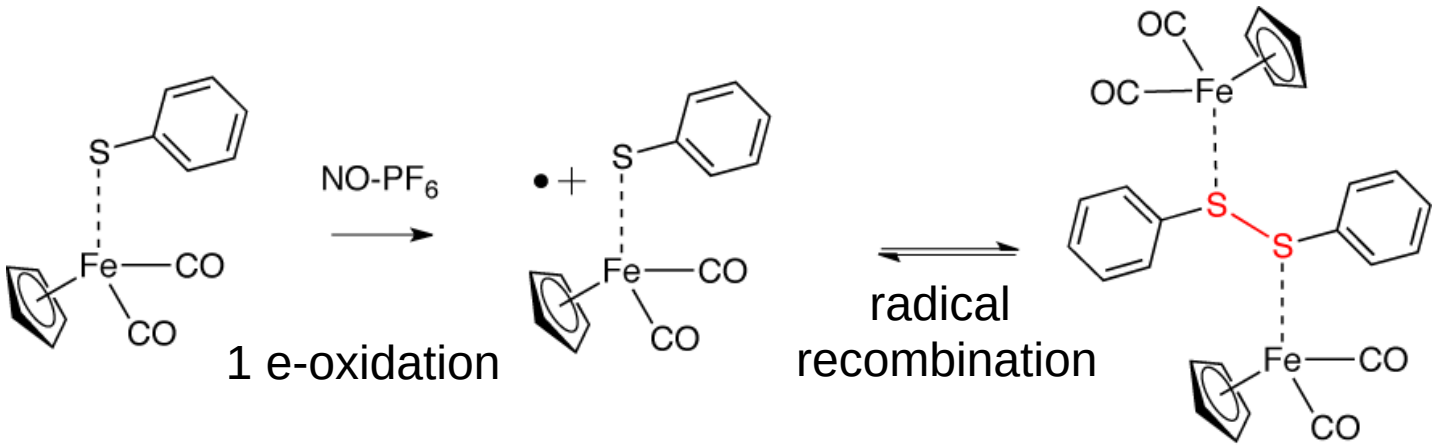


Phosphine ligands disfavor, isocyanide ligands favor disulfide bond formation

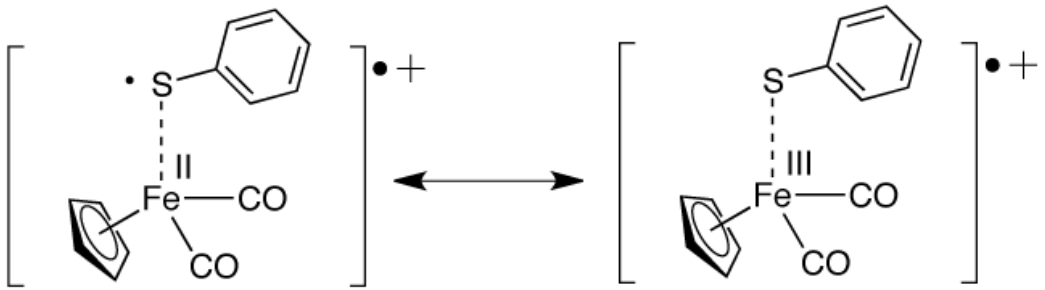


Ashby 1988

Redox chemistry in iron (II)-thiolate compexes



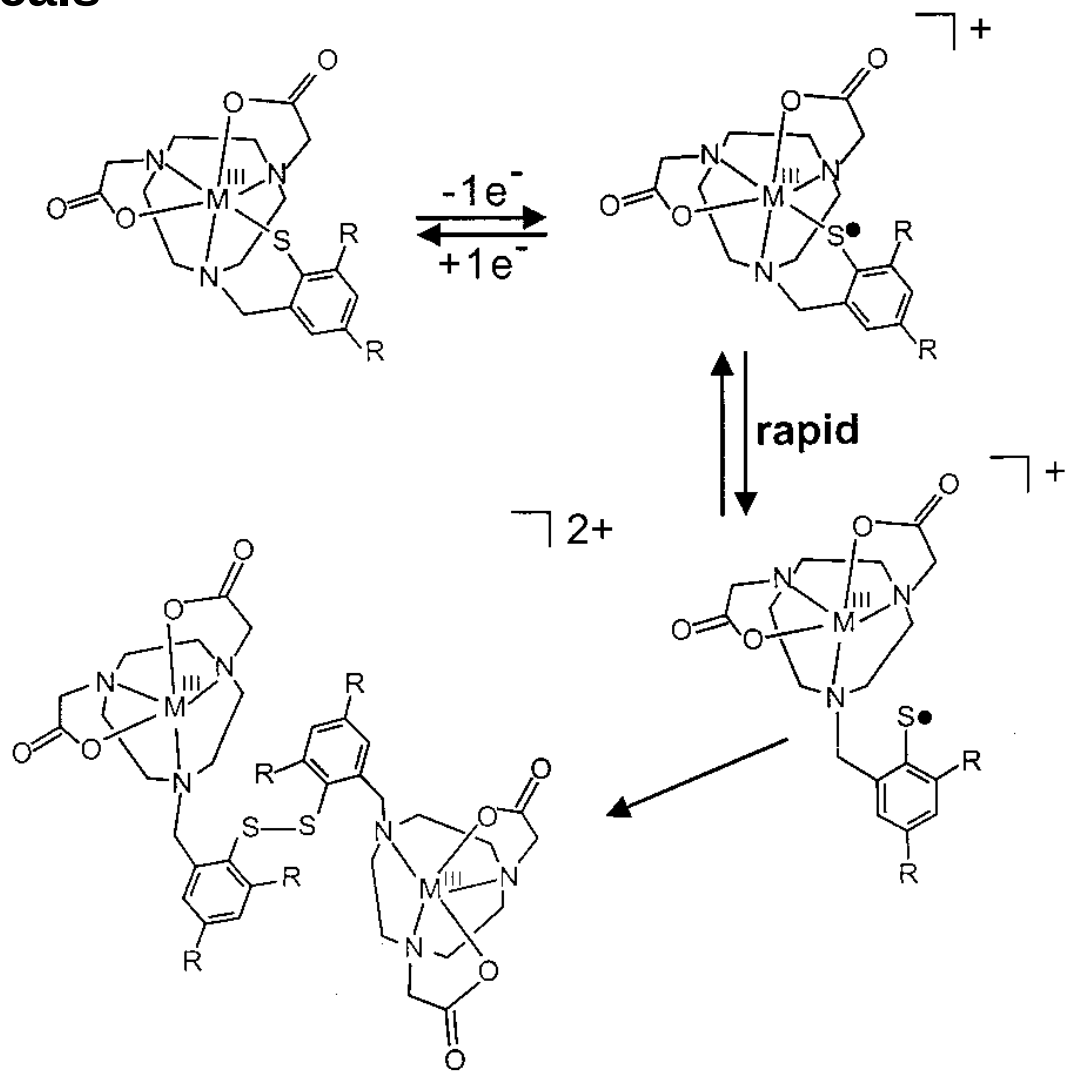
Phosphine ligands disfavor, isocyanide ligands favor disulfide bond formation



→ the SUMO (Singly Occupied Molecular Orbital) has predominantly S-character

Ashby 1988

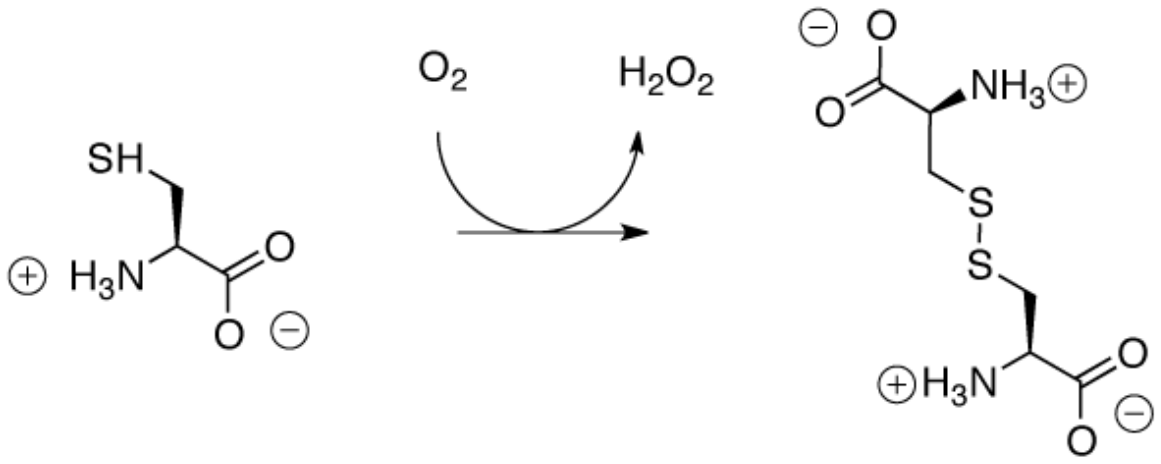
One electron oxidation of iron (III)-SR complexes produce sulfur-based radicals



$[M^{III}L^{2}]$ M=Ga, Fe, Co; R=H

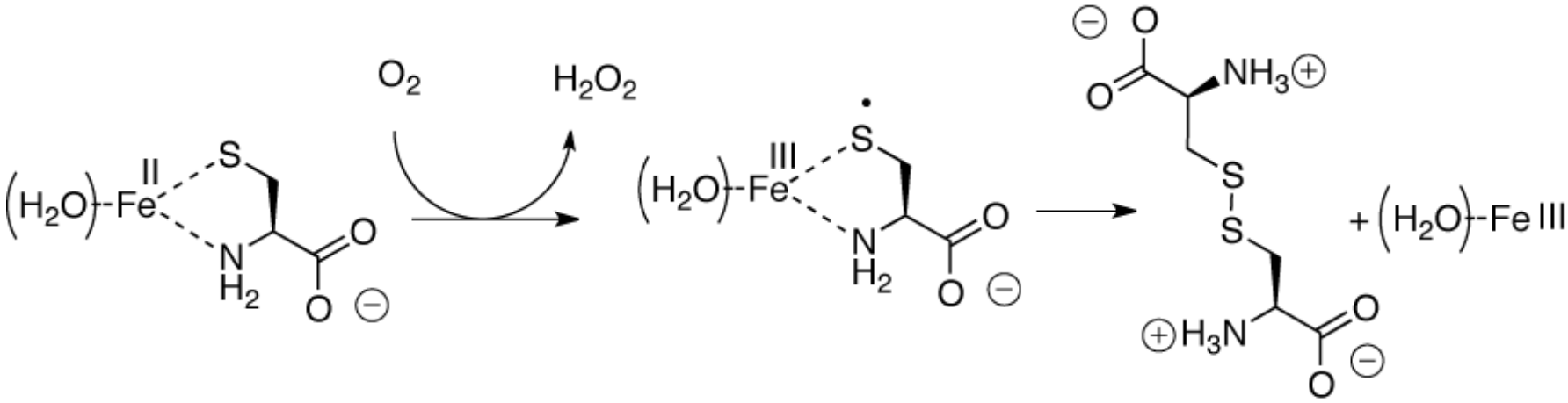
Kimura 2001

Auotooxidation of cysteine in metal-free aquous solutions

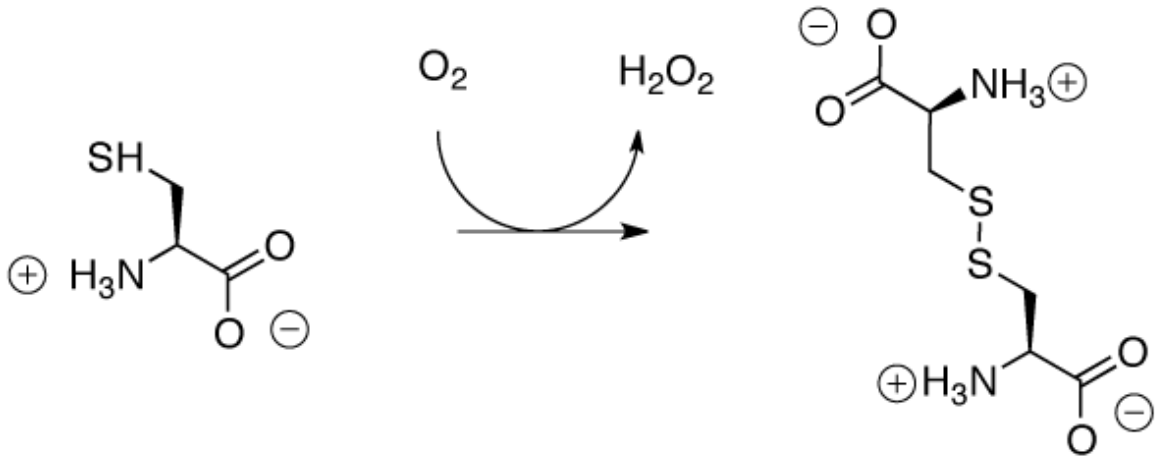


Metal-free oxidation of cysteine does not happen!

Autooxidation of cysteine in ~~metal-free~~ aquous solutions

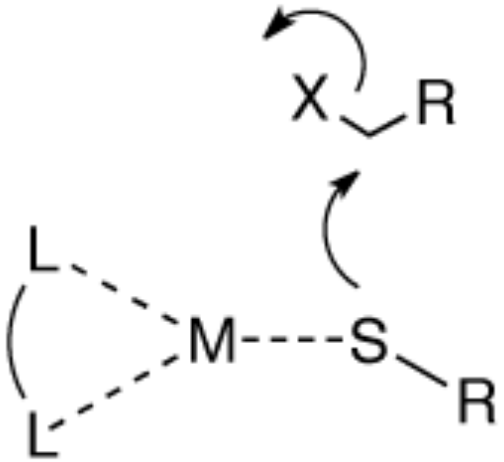


A possible mechanism for iron (II) catalyzed cysteine “autooxidation”



Metal-free oxidation of cysteine does not happen!

Review – Part II

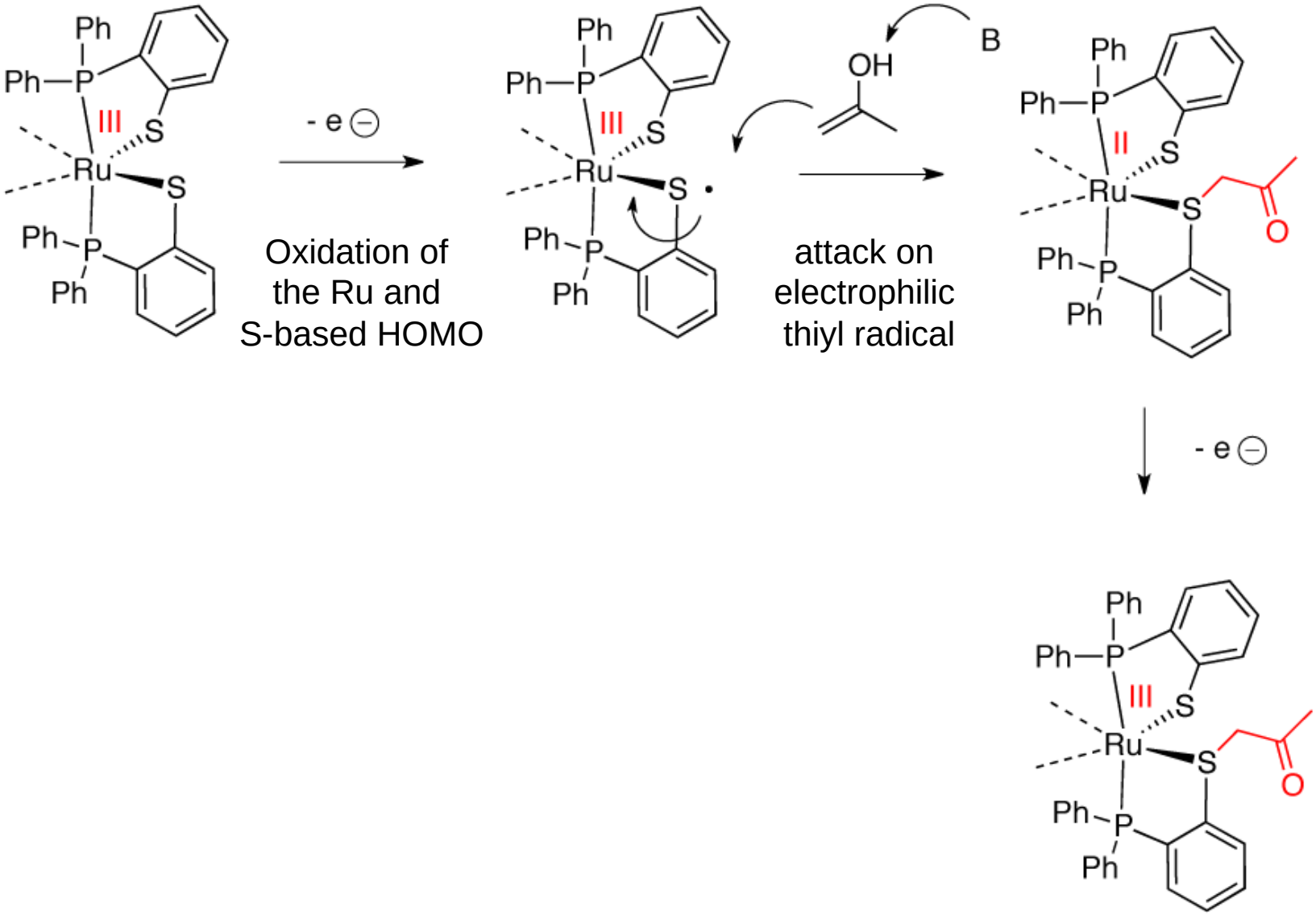


- metal enhanced S-nucleophilicity is not observed compared to sodium thiolates
- Zn (II)-SR bonds are highly ionic → activation through charge concentration
- Ni (II)-SR bonds are significantly covalent → activation through orbital interactions
- Fe (II)-SR complexes with strong p-acceptor ligands also destabilize the S-HOMO
- DinB_2 proteins present a new class of Zn-dependent GSTs
- Redox active metal centers may induce thiyl-radical chemistry

Part III: metal mediated radical C-S bond formation

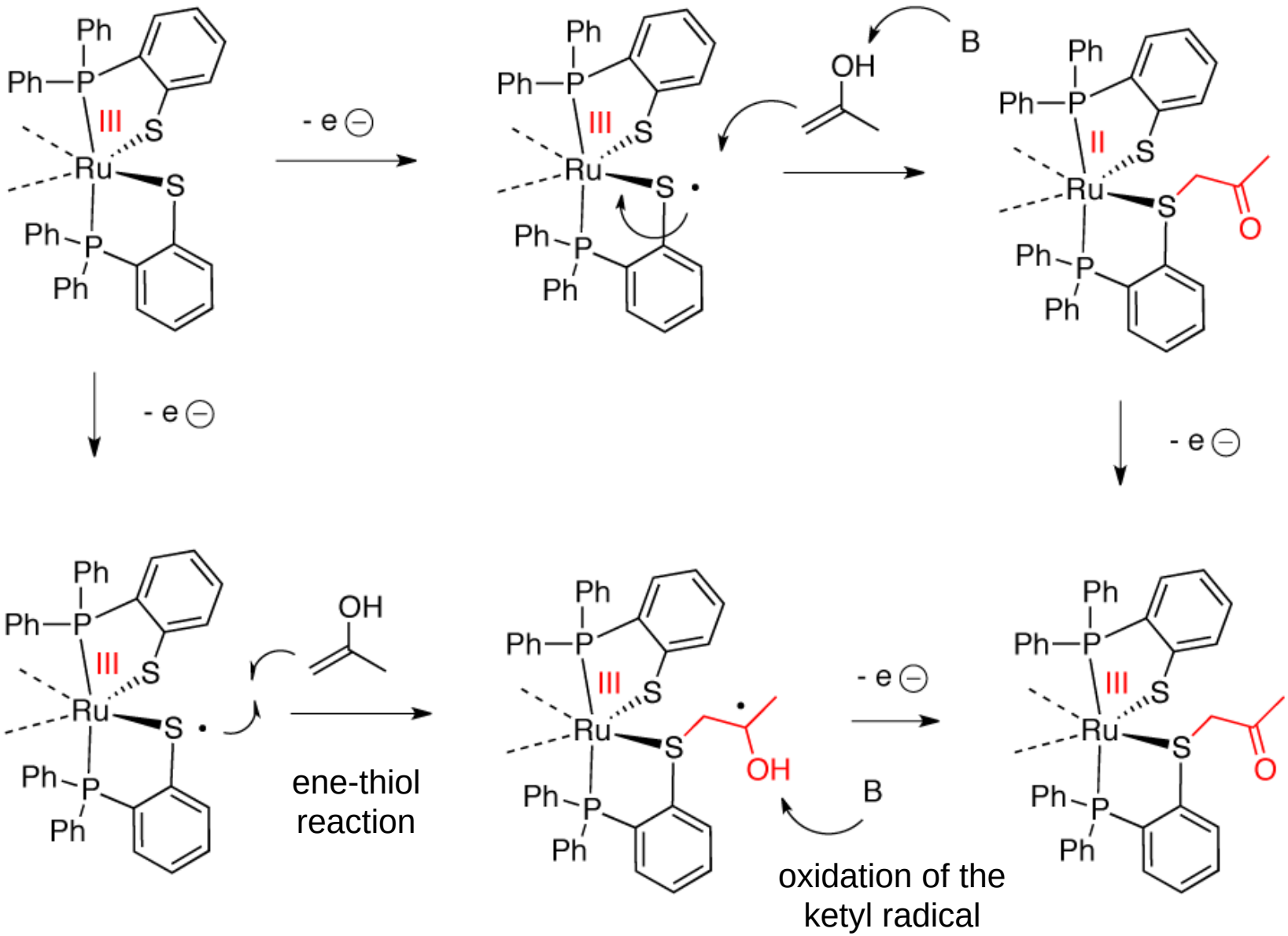
3

Metal mediated sulfur radical chemistry



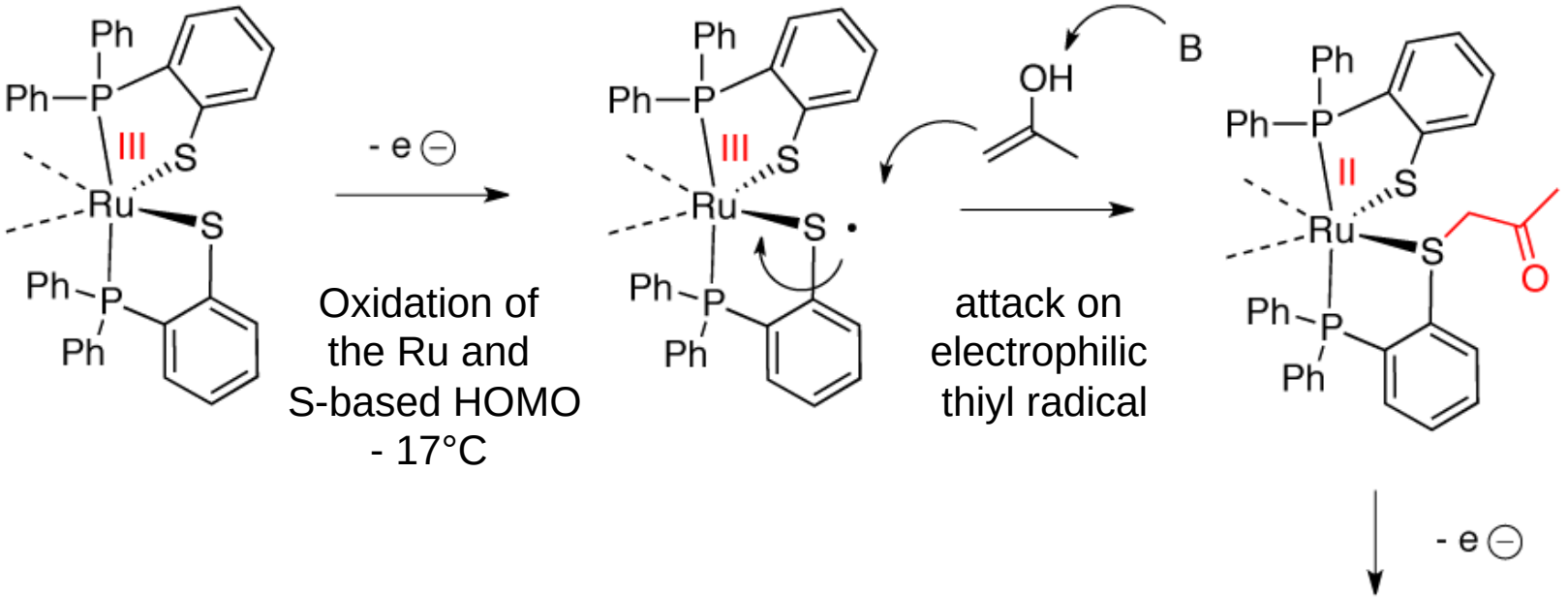
Poturovic 2005

Metal mediated sulfur radical chemistry



Poturovic 2005

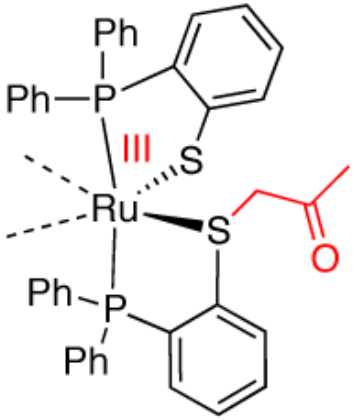
Metal mediated sulfur radical chemistry



Non—enolizable solvents (acetonitril, THF) do not react → homolytic C-H cleavage is not important

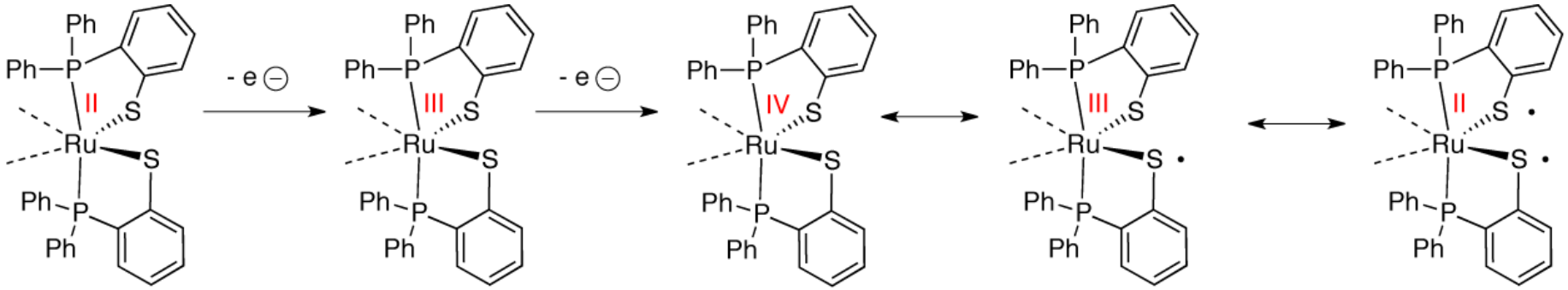
No change of the Ru oxidation state

Two step (e + e) oxidation

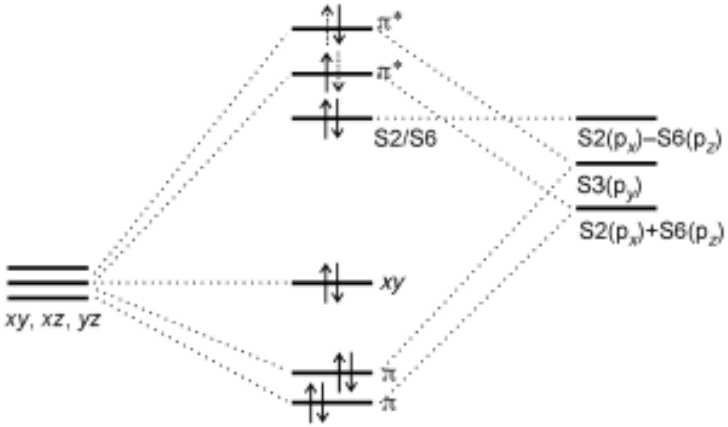


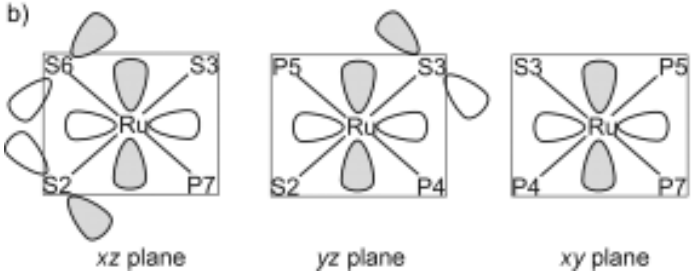
Poturovic 2005

Metal mediated sulfur radical chemistry

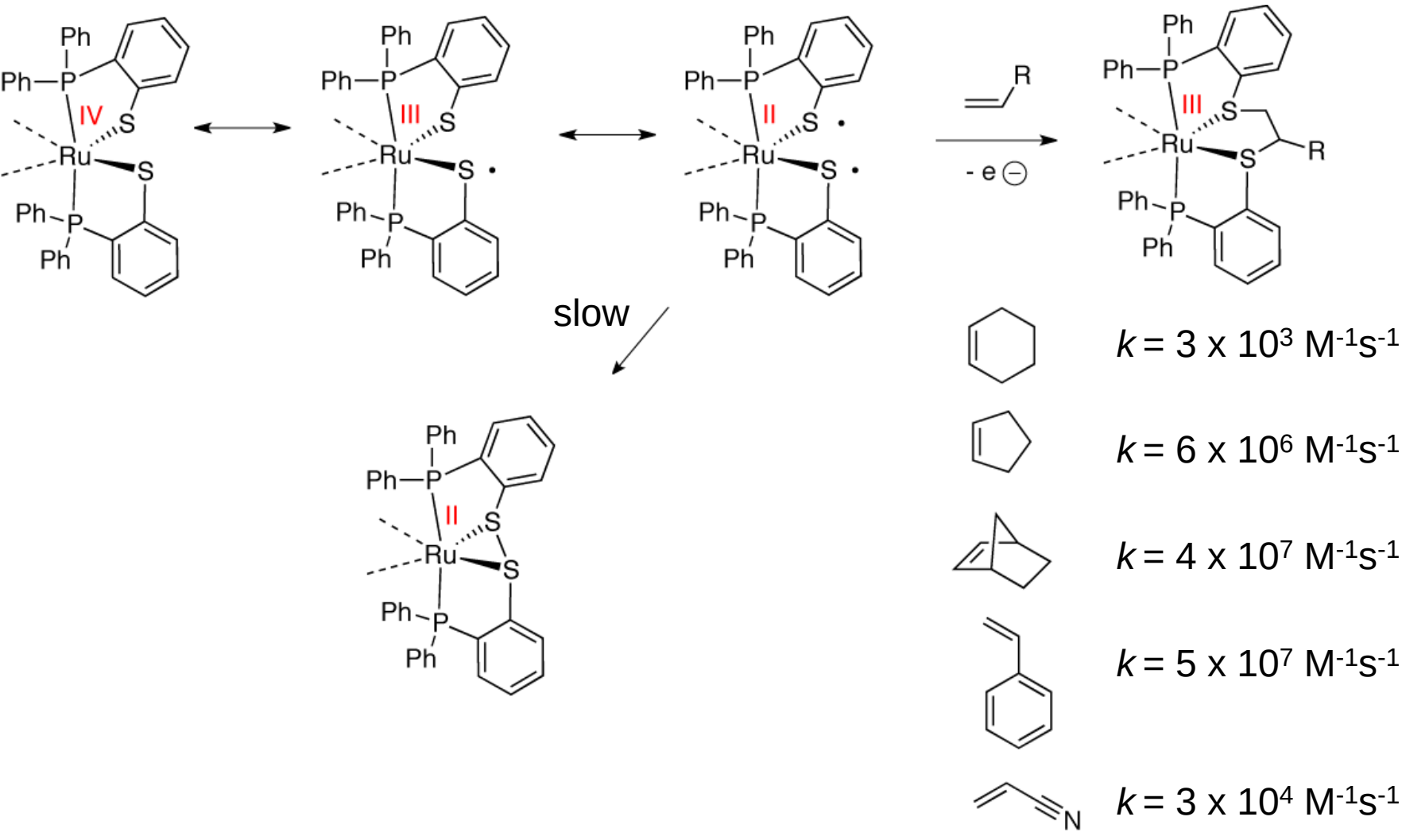


HOMO & HOMO-1 have
π^* character with significant
Ru and S character
(DFT calculation)

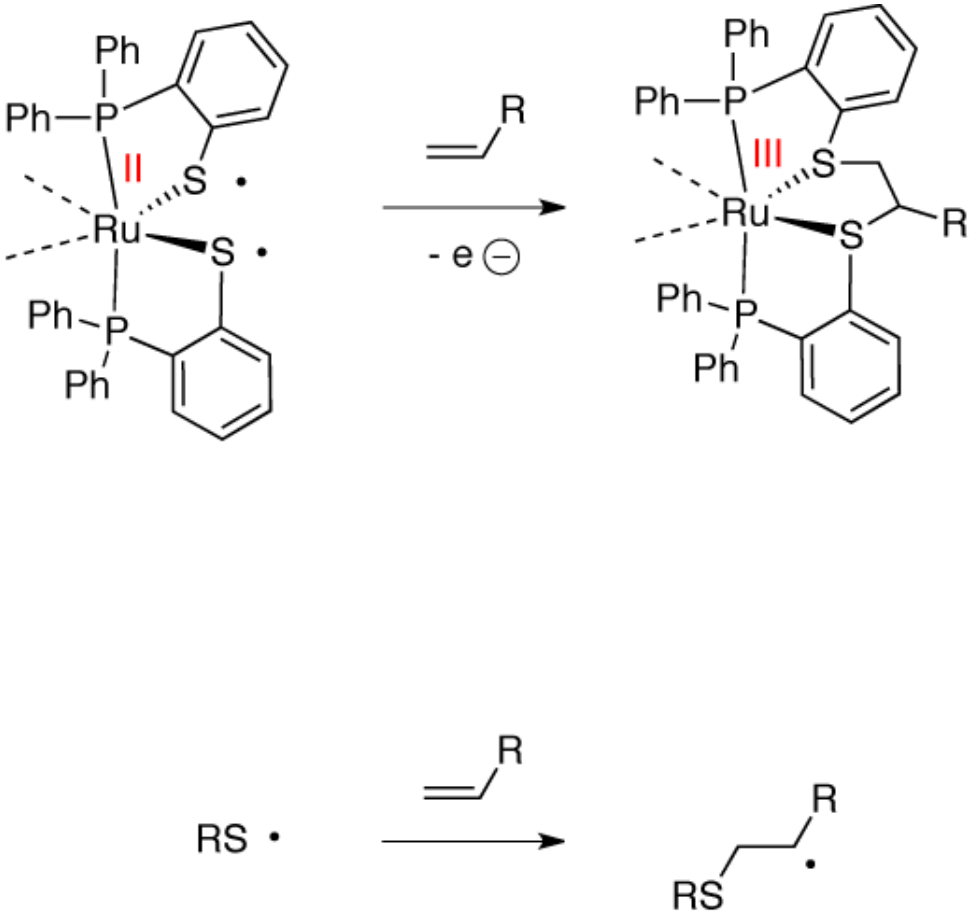




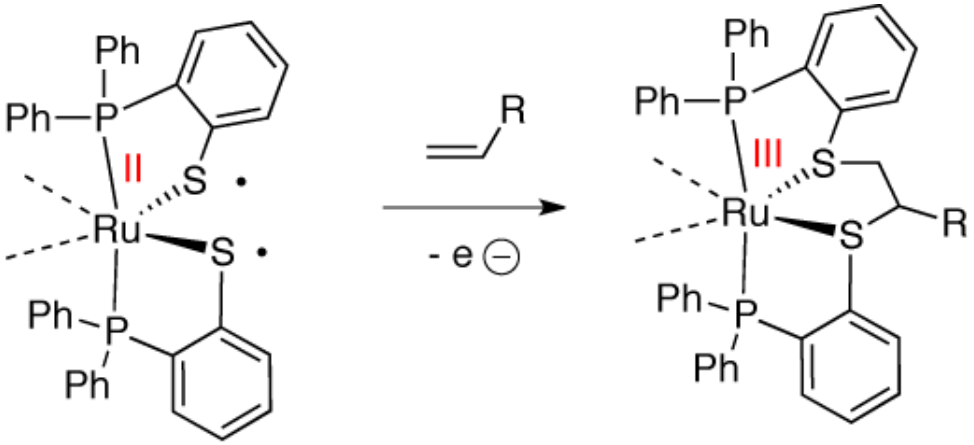
Metal mediated sulfur radical chemistry



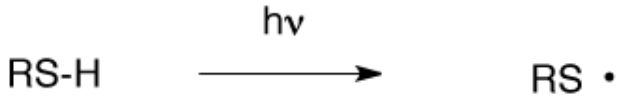
The ene-thiol reaction



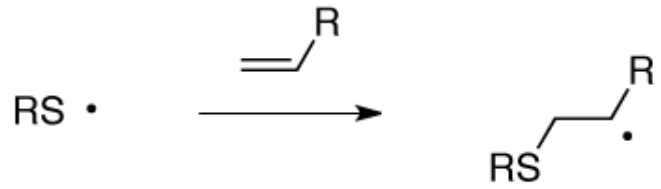
The ene-thiol reaction



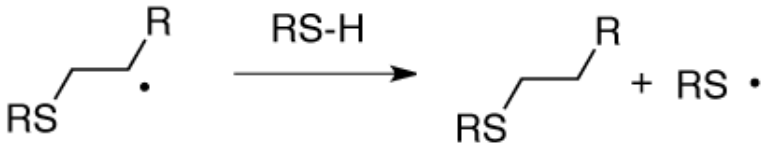
initiation



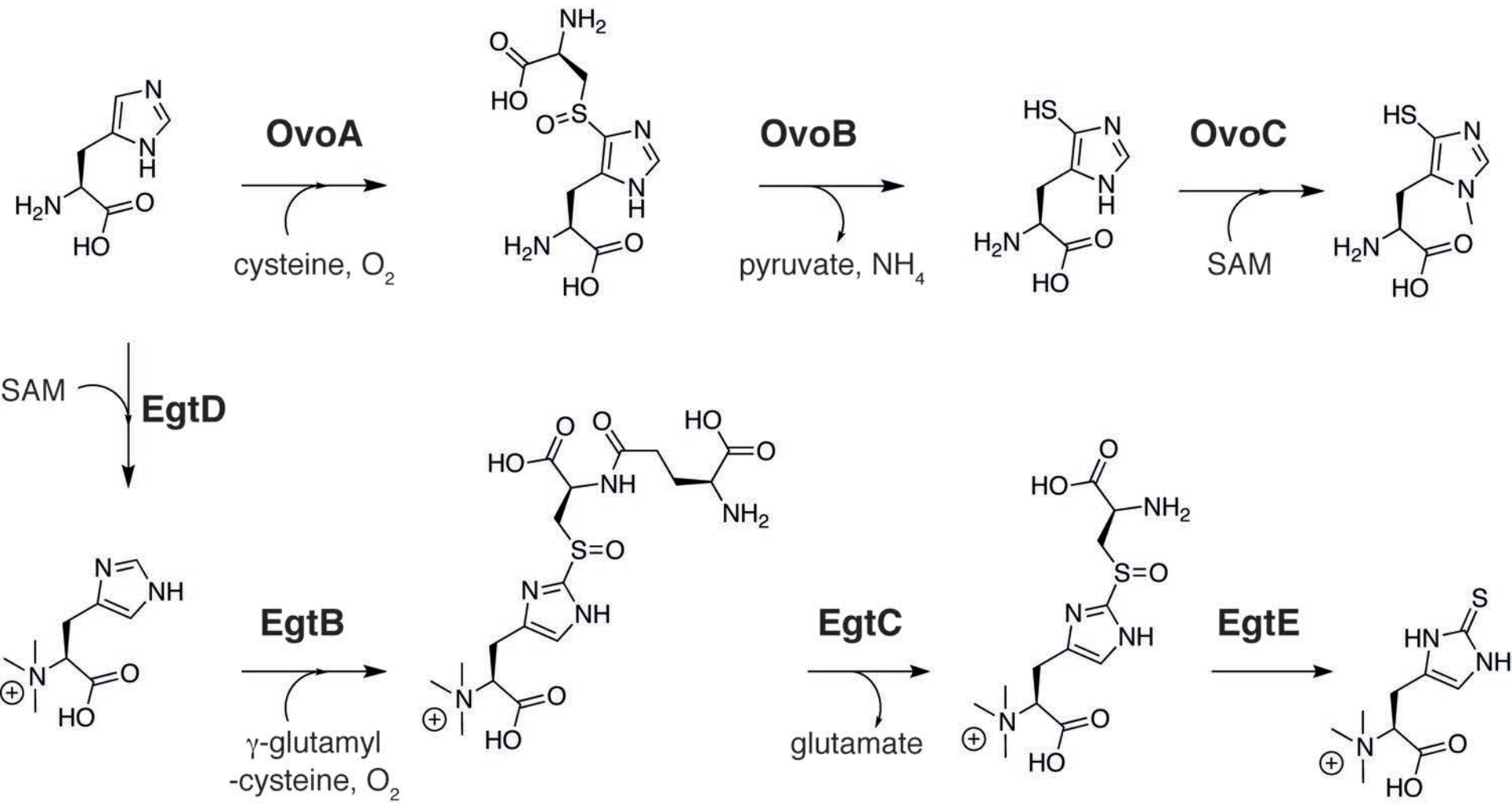
propagation 1



propagation 2

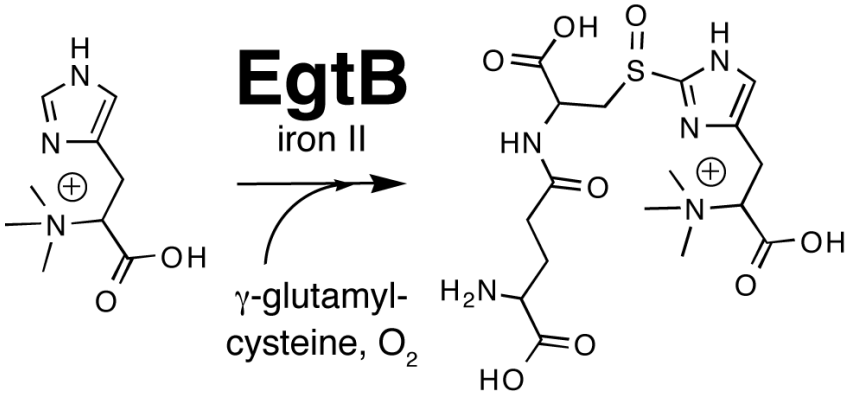


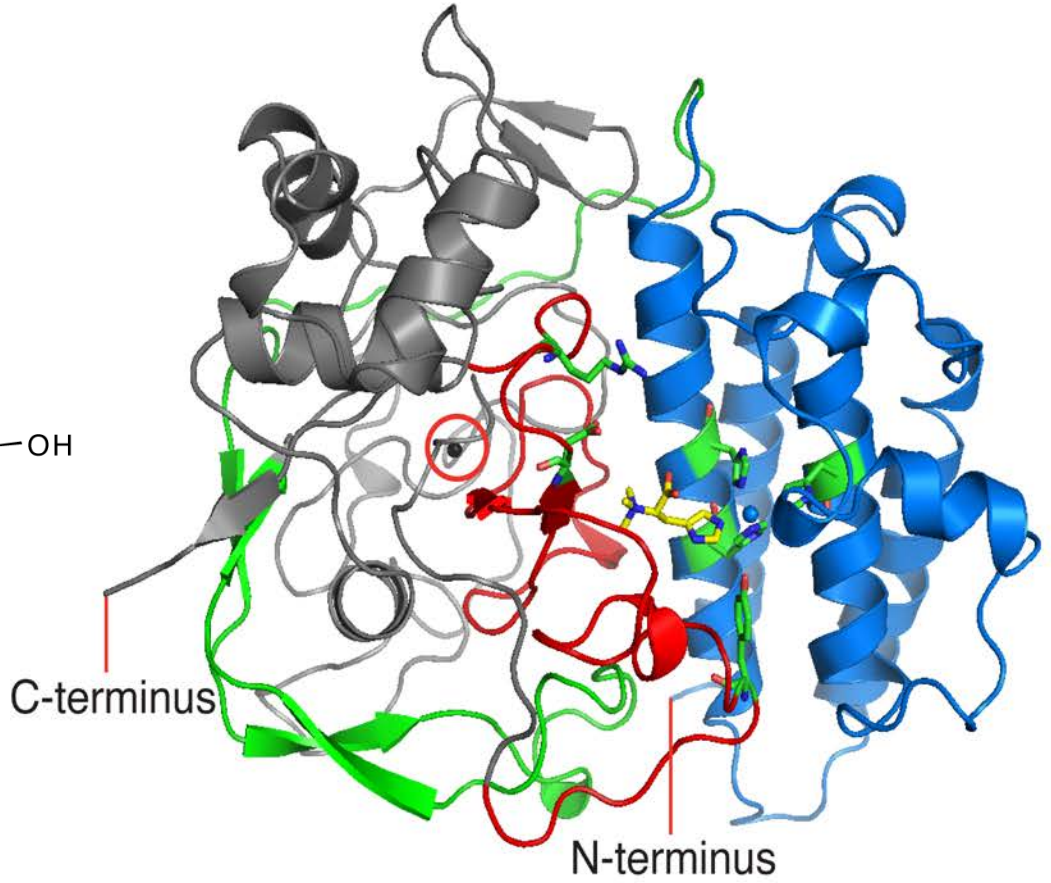
Biosynthesis of thiohistidines



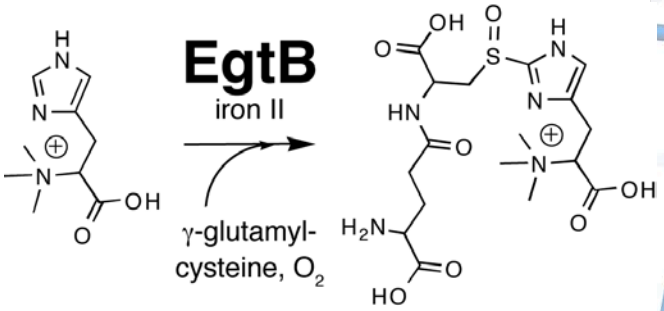
Seebeck 2010, Braunshausen 2011

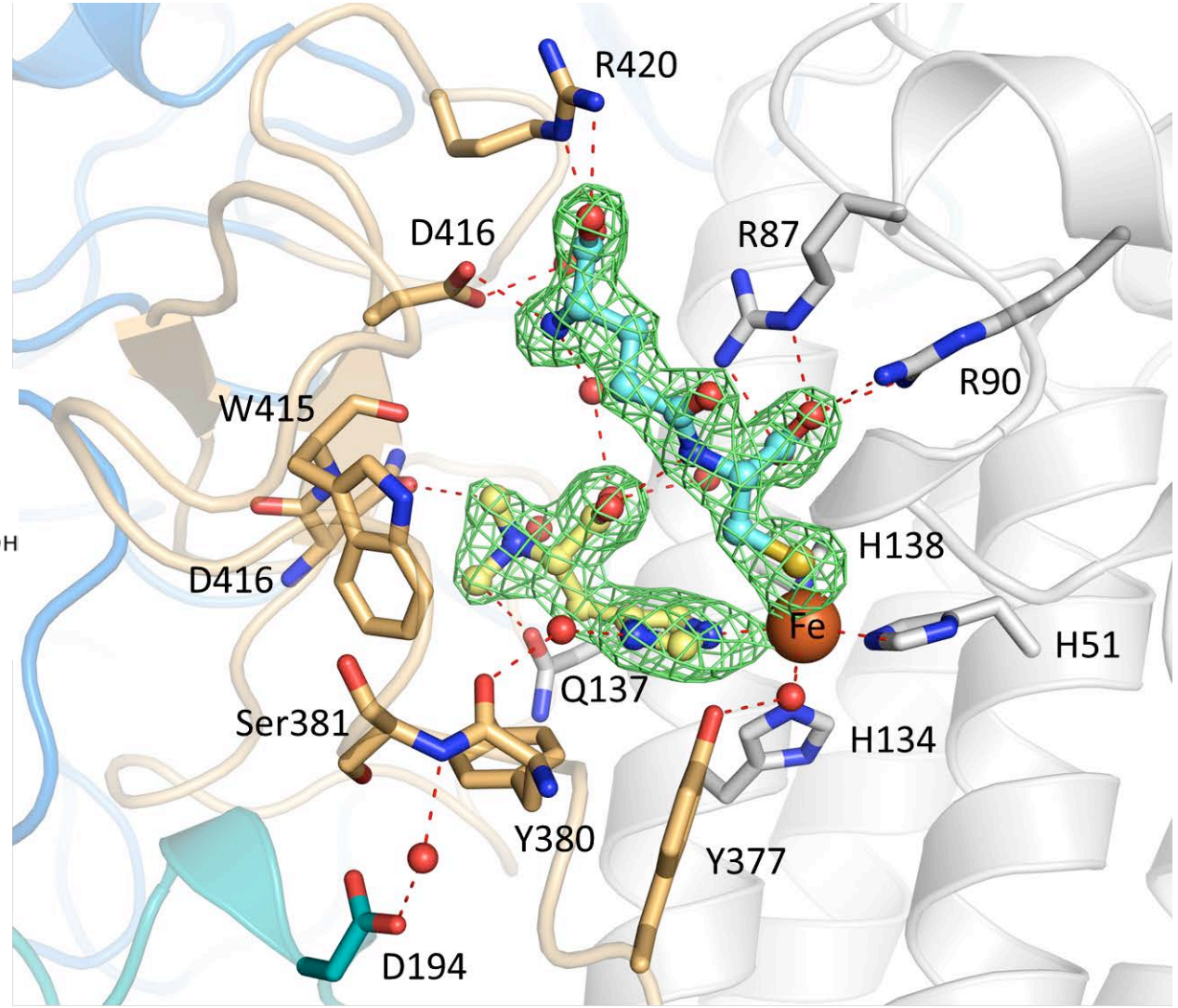
The sulfoxide synthase EgtB



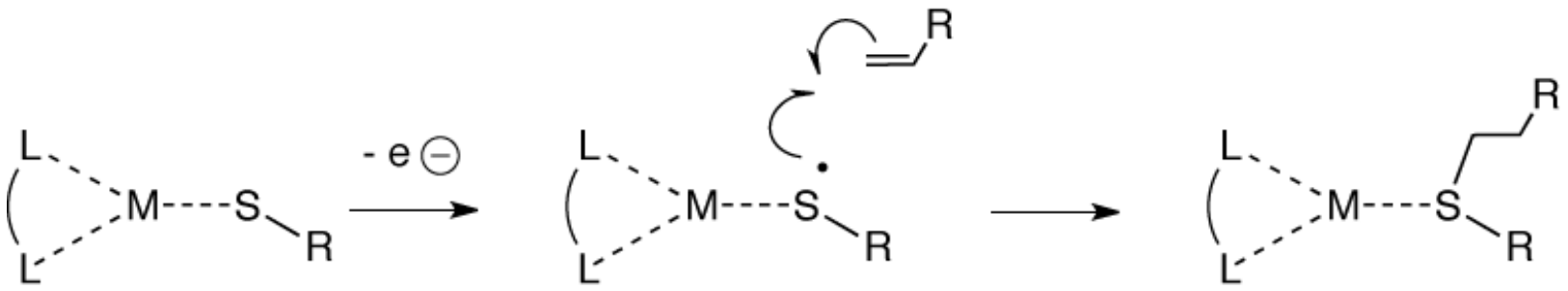


EgtB in complex with trimethylhistidine and iron



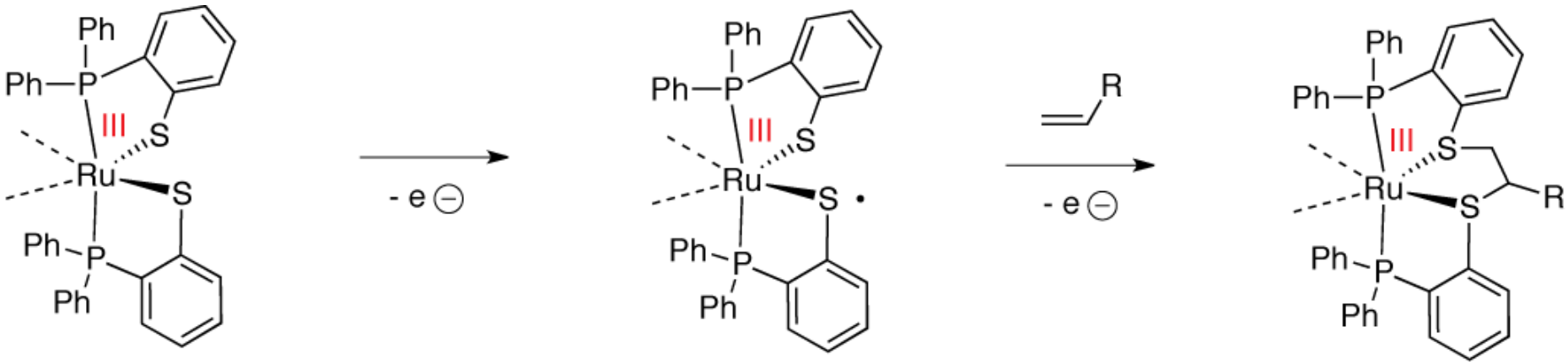


Model chemistry: oxidative sulfur transfer

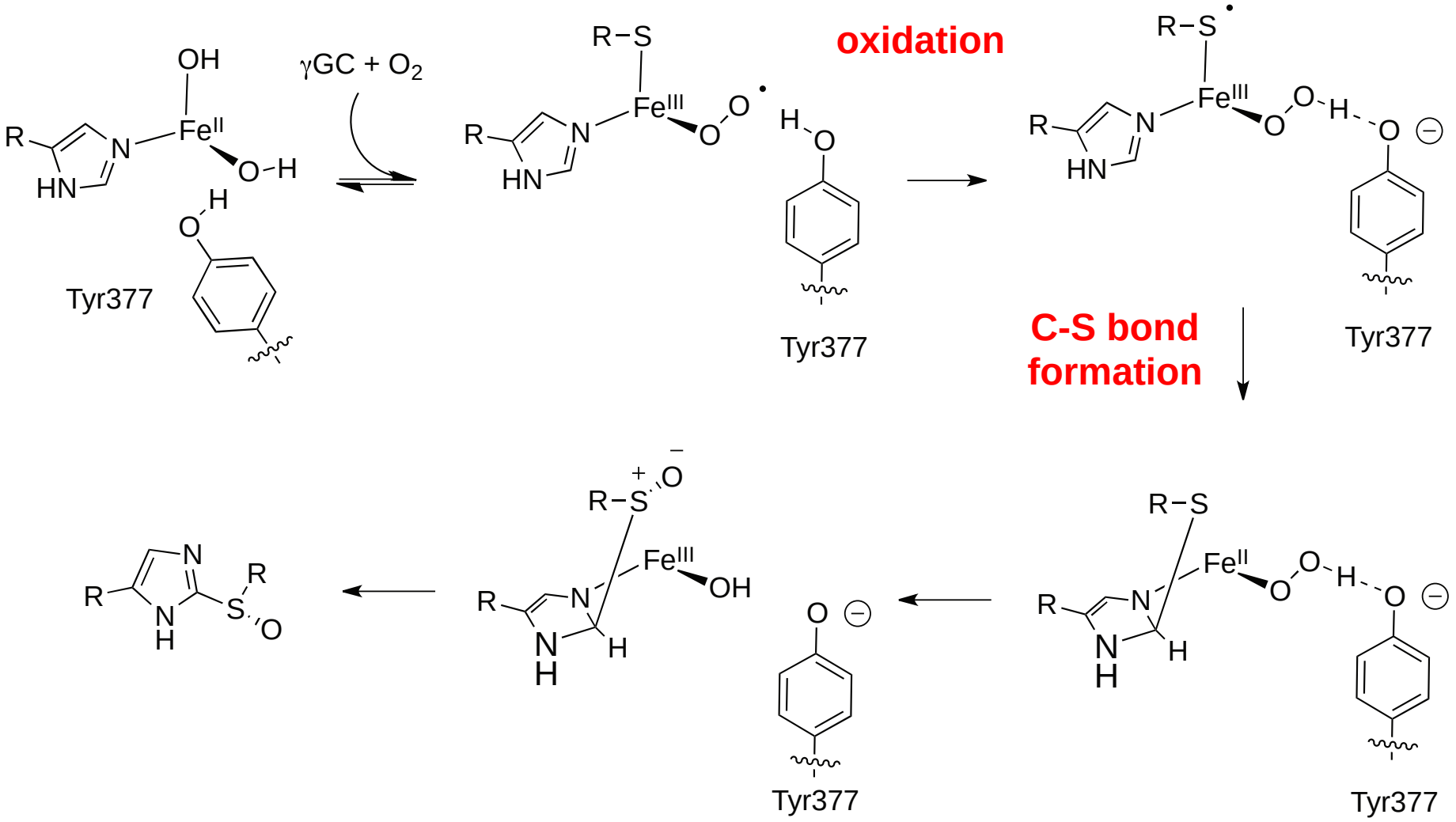


oxidation

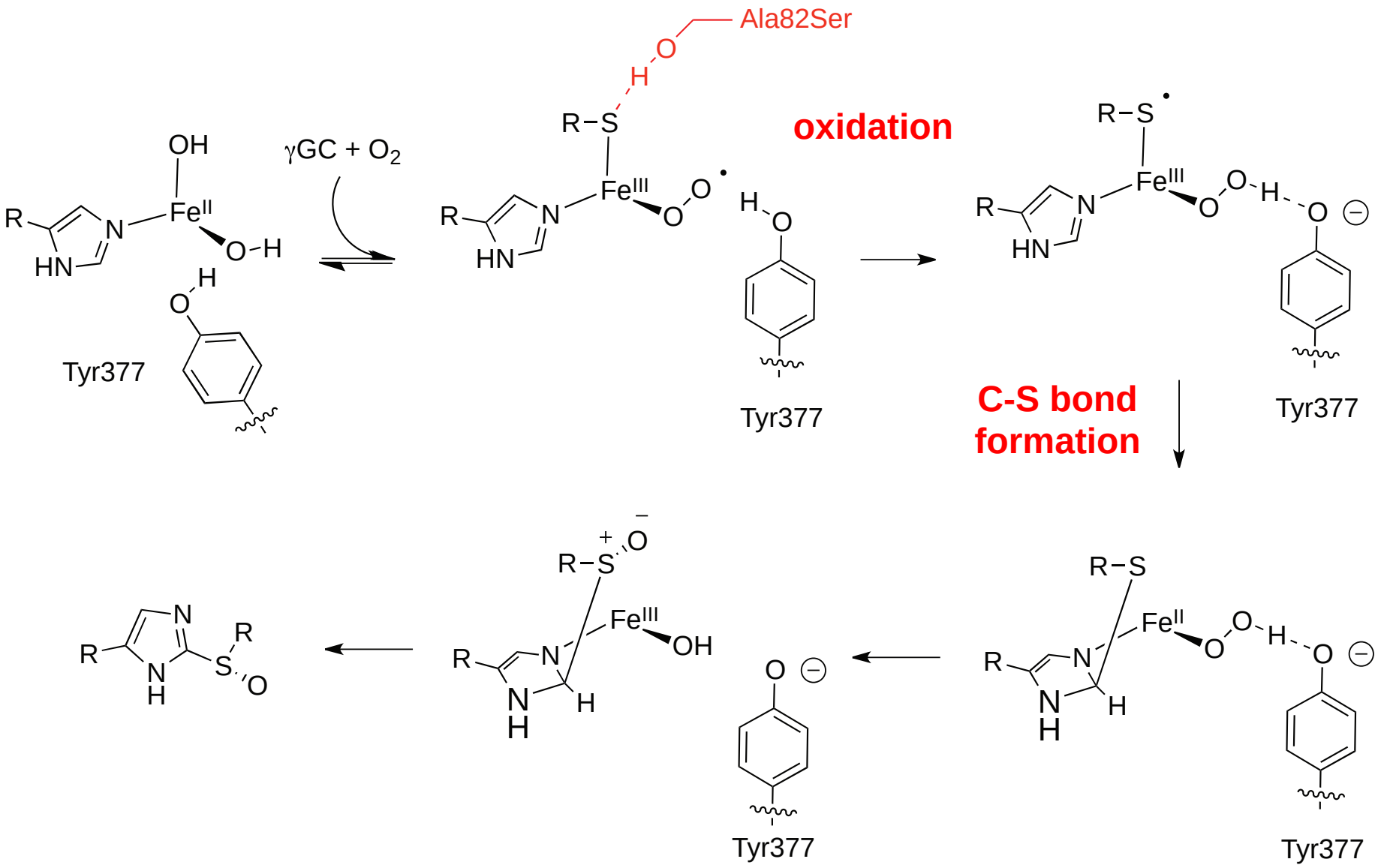
C-S bond formation



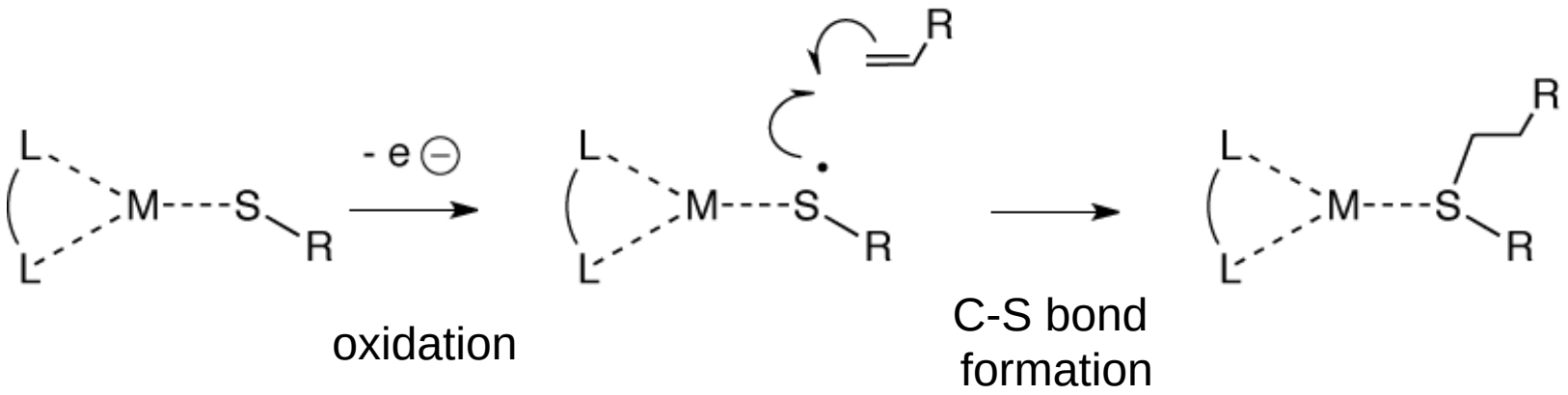
A possible mechanism for EgtB



Project 1: modulating the electron density on the substrate



Review – Part III



→ oxidative sulfur transfer

→ Ru-based complexes generate stabilized thiyl radicals

→ thiyl radicals can attack a broad range of alkenes

→ prediction of reaction rates depends on several parameters

→ mixing of charge transfer & triplet configuration are important

Questions ?