Department CMIC

Lecture 1 – FR1

POLITECNICO DI MILANO

Free-Radicals: Chemistry and Biology

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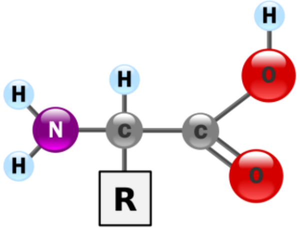
Dipartimento CMIC “Giulio Natta”

http://iscamap.chem.polimi.it/citterio/education/free-radical-chemistry/



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Lecture 1a

Current Status of Radicals Chemistry

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Interest for Radicals as Deduced from C.A.

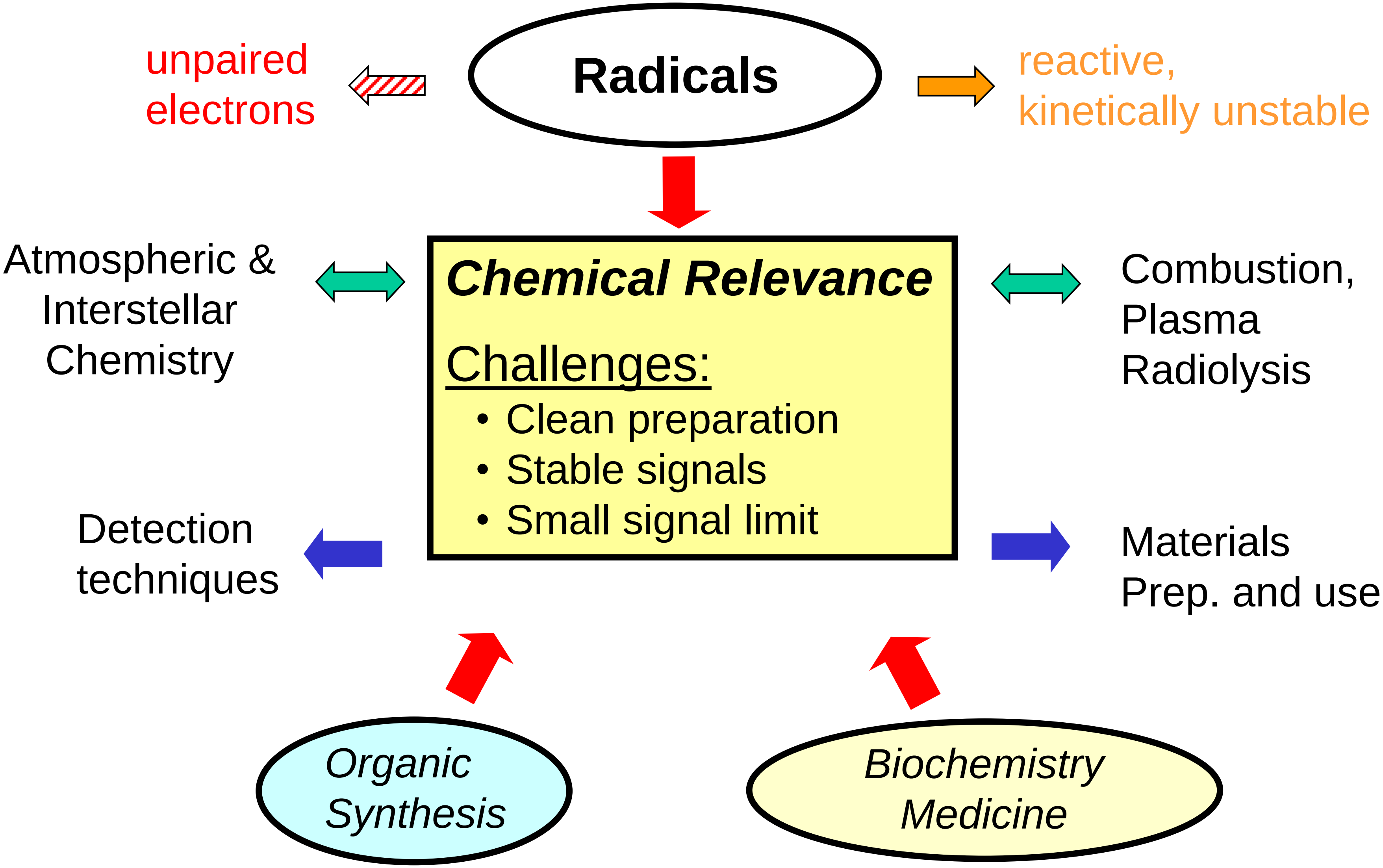
C.A. vol.	Years	Number of References	Mean number of references per year
66-75	1967-1971	10200	2040
76-85	1972-1976	11100	2250
86-95	1977-1981	12300	2460
96-105	1982-1986	14400	2880
106-125	1987-1991	18300	3660
126-145	1992-1996	23200	4640
146/147	1997	5400	5400
148/149	1998	6500	6500
150/151	1999	6800	6800
-	2006	10600	10600
-	2015	15700	15700

Representative Fields Interested in Radical Intermediates (C.A. 1997-1999)

Year	Organic Synthesis	Polymers	Material Sc.&Tc.
1997	580	1100	720
1998	630	860	910
1999	590	1040	950
2005	490	1150	1030
2015	430	1670	1400

Year	Environment	Combustion	Biology	Medicine
1997	270	150	410	1200
1998	300	130	520	1180
1999	320	160	590	1050

Chemical Relevance of Radicals



Radicals

unpaired electrons

reactive, kinetically unstable

Chemical Relevance

Challenges:

- Clean preparation
- Stable signals
- Small signal limit

Atmospheric & Interstellar Chemistry

Combustion, Plasma Radiolysis

Detection techniques

Materials Prep. and use

Organic Synthesis

Biochemistry Medicine

What is Just Known on Free-Radicals!

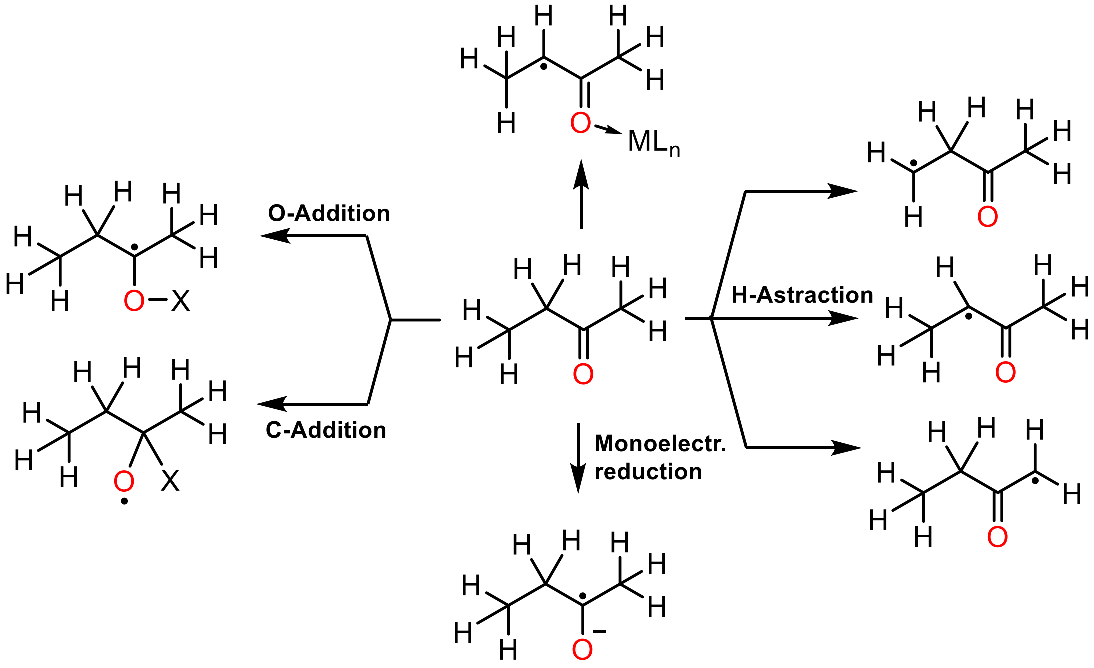
- Thermodynamic data and related correlations
- Kinetic data in gas phase and in solution and related correlations
- Reaction mechanisms of simple and complex reactions
- Sources of persistent and labile radicals centered to all relevant atoms: chemical, radiation, redox
- Structural features and theoretical interpretations
- Analysis by fast spectroscopic techniques and ESR
- Synthetic methodologies and strategies
- Control of stereochemistry in addition and atom transfer processes
- Role in atmosphere chemistry and ozone destruction
- Information on environmental concerns
- Basic information on radical involvements in biological systems
- Primary role of radicals in degenerative biological processes

Future Researches and Applications of Radicals

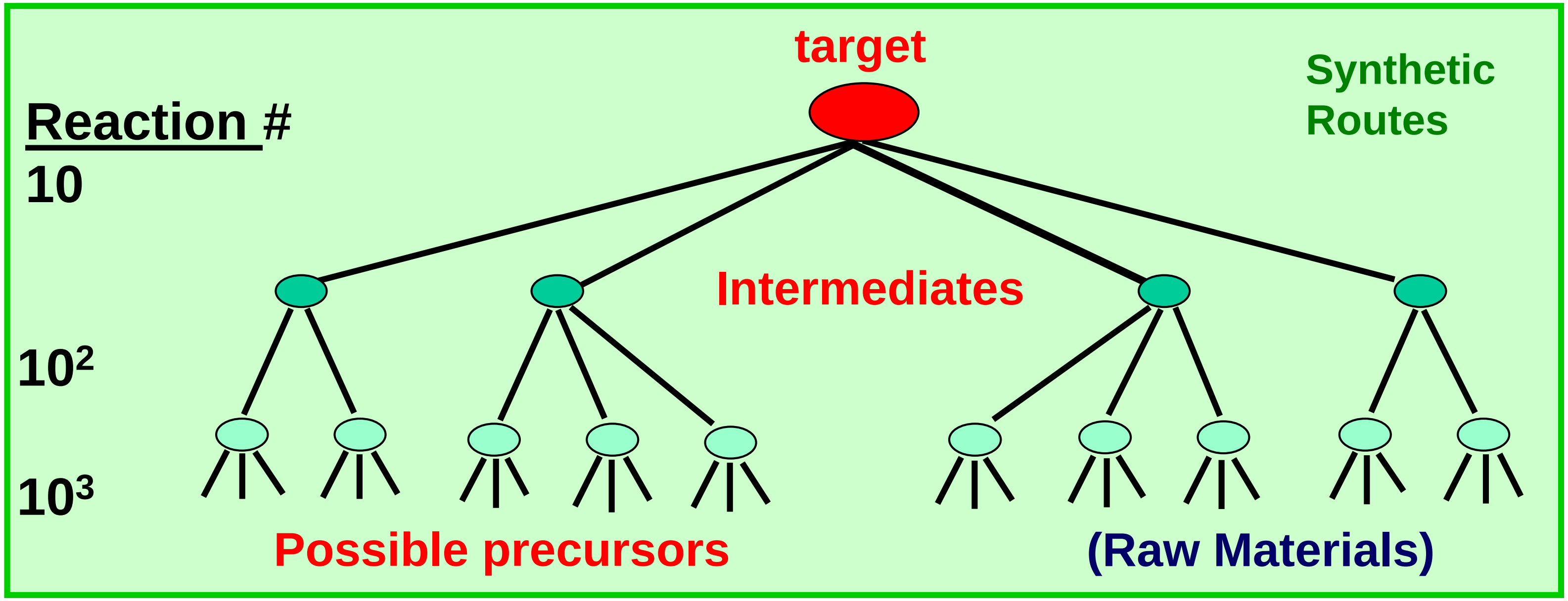
- Enzymatic radical reactions
- Role of metal coordination on reactivity and chemo-, regio- and enantio-selectivity
- Radicals in heterogeneous systems (kinetics and thermodynamics)
- Pollution control (formation and fate of radicals)
- Atmospheric radical chemistry
- Pyrolysis and flame control
- Role of radicals in activation of small stable molecules
- New radical sources and new synthetic sustainable applications
- Better control of radical polymerization
- Kinetic of radicals in confined media (nanostructures)

The Complexity of the Radicals Field

- All compounds (inorganic and organic) can be transformed in different paramagnetic species through several different methodologies. Therefore, the fields of Physics, Chemistry, Biology and Medicine of Radical Species is wide and difficult to cover in all detailed aspects.
- A simple example can explain the complexity of the field, starting from the diamagnetic compound methylethylketone (2-butanone)



Complexity of Chemical Space



Compounds 15 million

Known reactions several million

Possible Organic Compound with PM < 700 : 10^{36}

Lecture 1b

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What is a Radical

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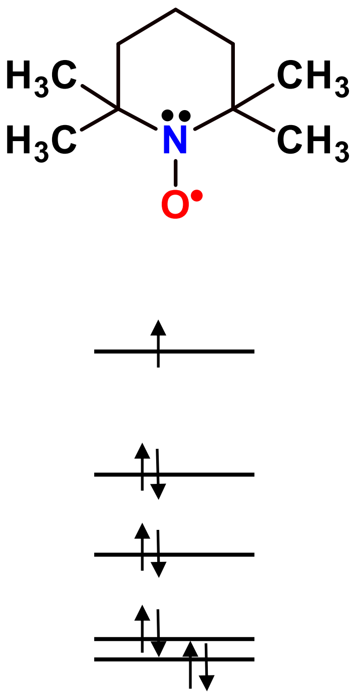
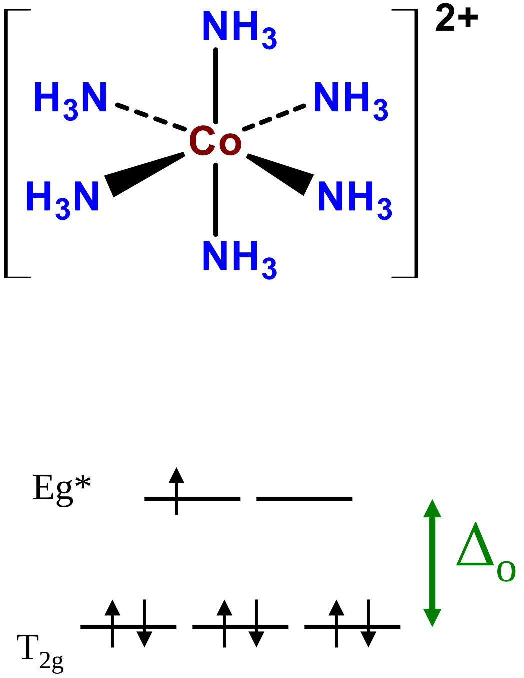
Definition of a Radical Specie

Each chemical specie (atom or organic, inorganic, organometallic molecule) possessing in the ground state orbitals with an unpaired number of electrons (e⁻).

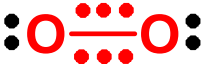
(1e⁻ : doublet state; 2e⁻ : triplet state 3e⁻ : quintet state**)**



TEMPO radical



Ion complex $[Co(NH_3)_6]^{2+}$

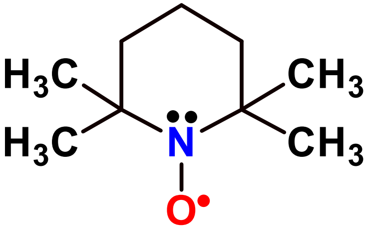


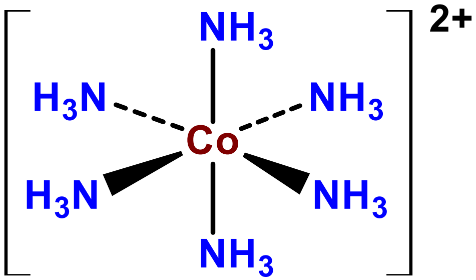
Oxygen Molecule

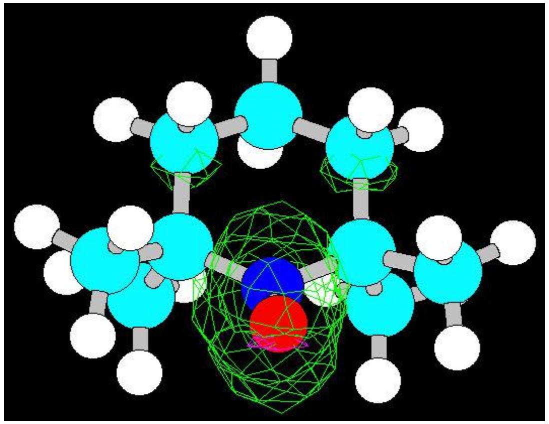
triplet state (2 e⁻)

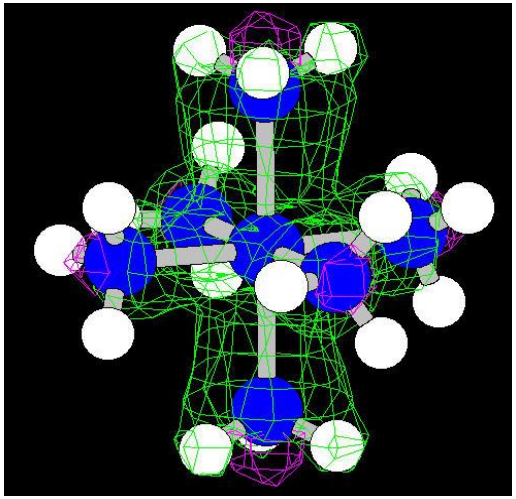
Spin Density of a Radical Centre

The spin density (fraction of electron) can be visualized by semi-empirical calculations such as UHF AM1 or measured experimentally by e.s.r. or by a magnetic equipment.









Some Classifications

Radical center: Atom of a paramagnetic specie carrying a relevant fraction of the electronic spin density (i.e. having the highest coefficient of the SOMO molecular orbital)

Examples **:**

Carbon centered free radicals: i.e. $\mathrm{H_3}\mathbf{C}^{\bullet}$, $\mathrm{(HO)H_2}\mathbf{C}^{\bullet}$, $\mathrm{H(O)}\mathbf{C}^{\bullet}$, $\mathrm{(^{-}O)H_2}\mathbf{C}^{\bullet}$

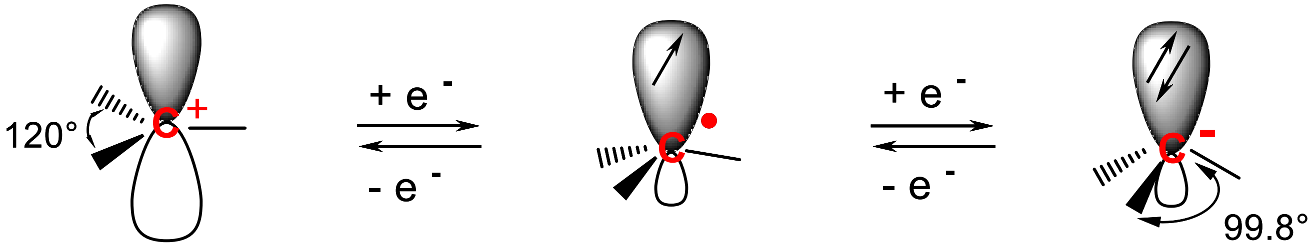
Oxygen centered free radicals: i.e. $\mathrm{H}\mathbf{O}^{\bullet}$, $\mathrm{CH_3}\mathbf{O}^{\bullet}$, $\mathrm{R_2N\text{-}}\mathbf{O}^{\bullet}$, $\mathrm{^{-}O\text{-}}\mathbf{O}^{\bullet}$

Nitrogen centered free radicals: i.e. $\mathrm{H_3}\mathbf{N}^{\bullet+}$, $\mathrm{(CH_3)_2}\mathbf{N}^{\bullet}$, $\mathrm{R_2N\text{-}(R)}\mathbf{N}^{\bullet}$,

Sulfur centered free radicals: i.e. $\mathrm{H}\mathbf{S}^{\bullet}$, $\mathrm{CH_3}\mathbf{S}^{\bullet}$, $\mathrm{R}\mathbf{S}^{\bullet+}\mathrm{SR}$

Metal centered free radicals: i.e. $\mathrm{R_3}\mathbf{Sn}^{\bullet}$, $\mathrm{R}\mathbf{Hg}^{\bullet}$,

Carbon Centered Free Radicals

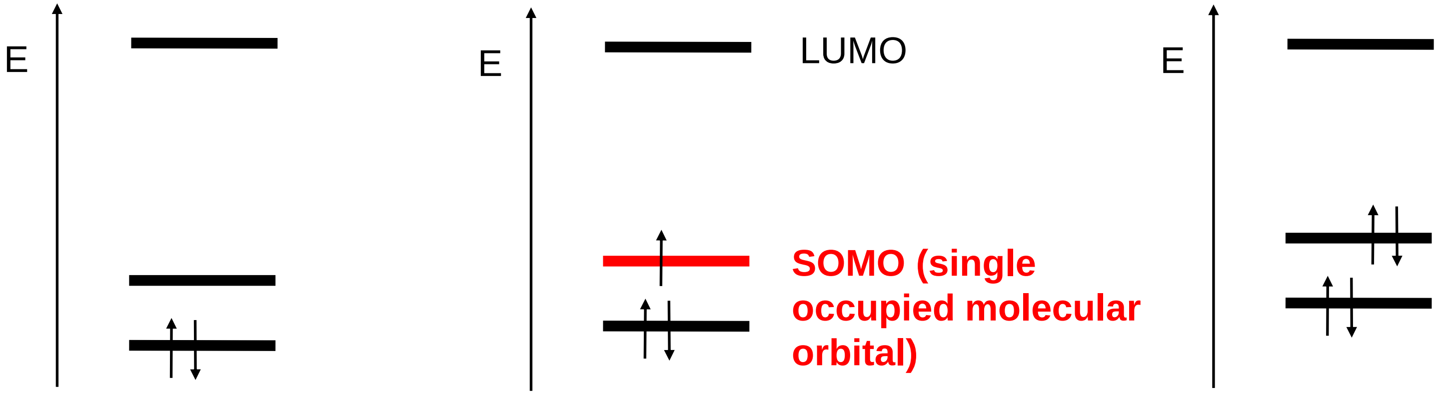


carbocation* *(planar)

carbon centered radical

carbanion (pyramidal)

i.e. **CH_3^+** **$CH_3^\bullet$** **CH_3^-**

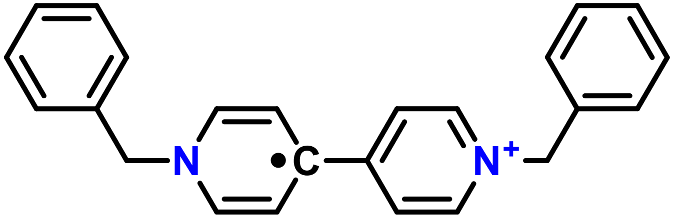


SOMO (single occupied molecular orbital)

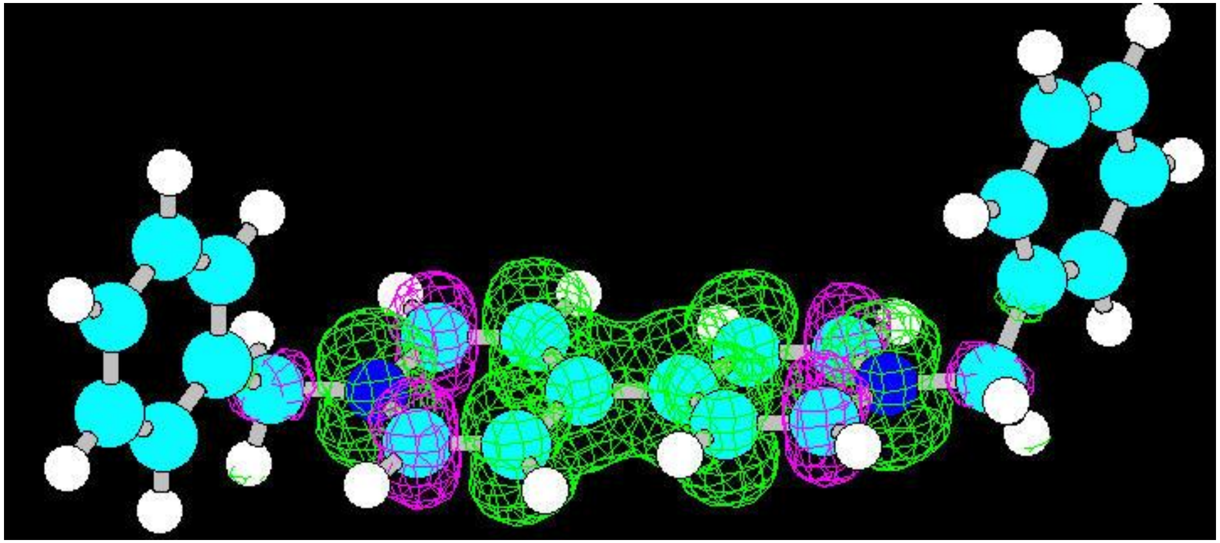
Delocalized Spin Density and Radical Centre

Odd **e⁻** species often have localized spin densities, but a single **e⁻** can also be delocalized over many centers, as in the viologen cation radical

N,N-dibenzyl-4,4’-bipiridinium
cation radical

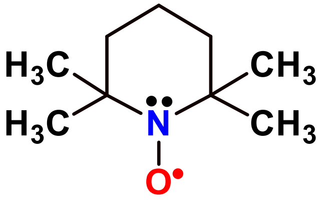


PM3-semiempirical calculation of the spin density



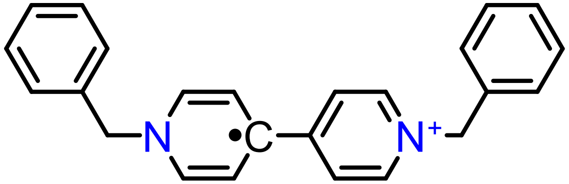
Type of Organic Compounds with Unpaired Electrons

1. Stable free radicals:



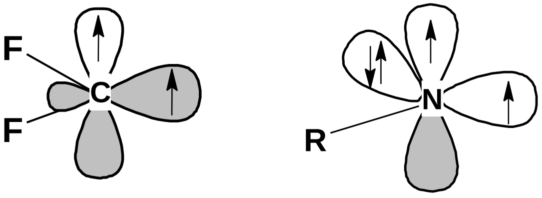
$t_{1/2}$ > 1 sec.

2. Radical cations or anions:

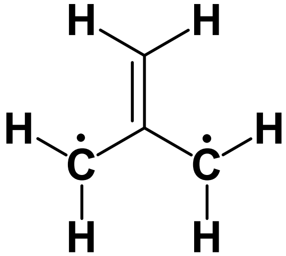


3. Organic diradicals with T ground state:

3a. Carbenes or nitrenes, single-centered (orthogonal orbitals)



3b. multi-centered radicals (non-orthogonal orbitals)



Time Classification of Radical Species

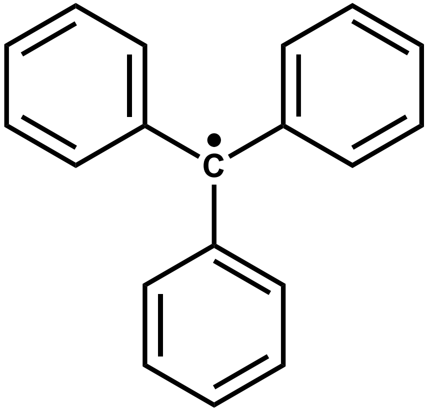
Persistent radical: $t_{1/2} >> 1$ s

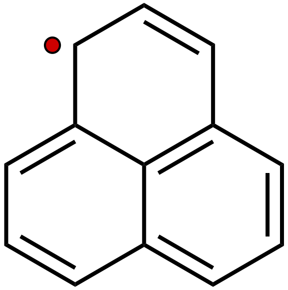
- Steric hindrance [$((CH_3)_3)C\cdot$]
- 3 electron bonds [NO, $^3O_2$]
- *d* or *f* metal ions and their complexes [Cu^{2+}, Fe^{2+}, etc.]

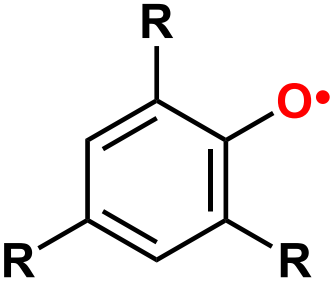
Labile radical: $t_{1/2} < 1$ s - Labile intermediates generated from organic, inorganic and organometallic precursors

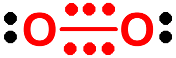
- Radiolysis (hν → matter)
- Thermolysis (weak bonds breaking, RO-OR)
- Redox Processes (mono-electronic Ox/Red)

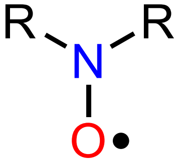
Persistent (Stable) Free Radicals (Examples)

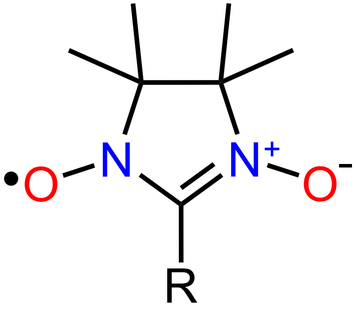


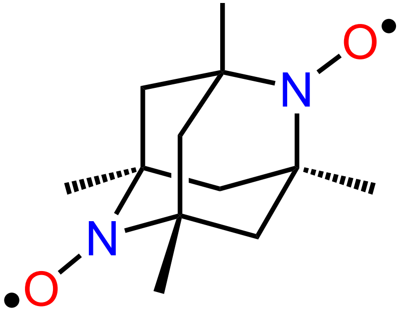


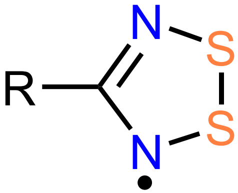


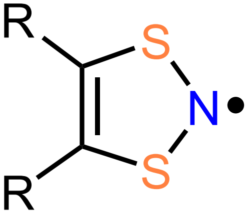


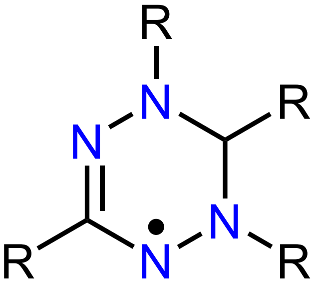


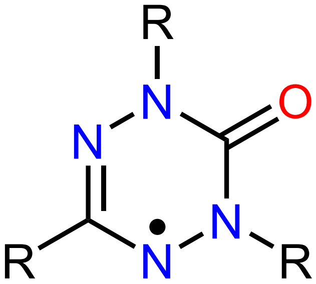






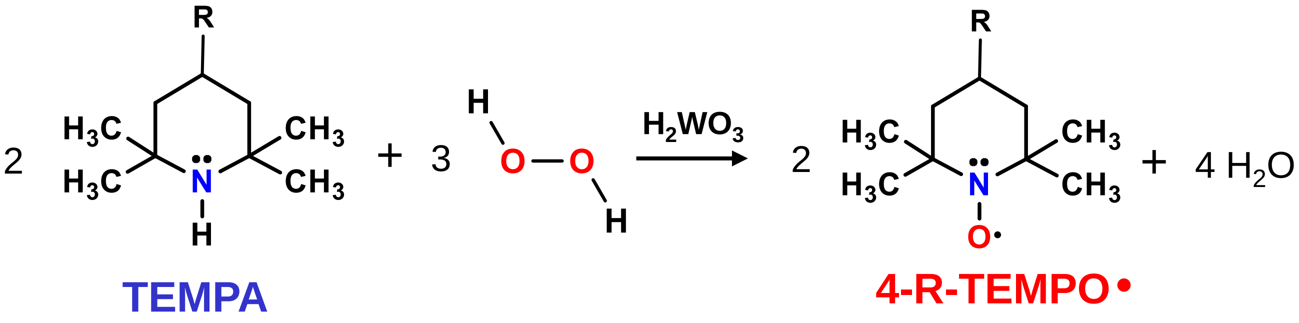






Preparation of a Persistent Radical

A) Source → persistent radical (only initiation)

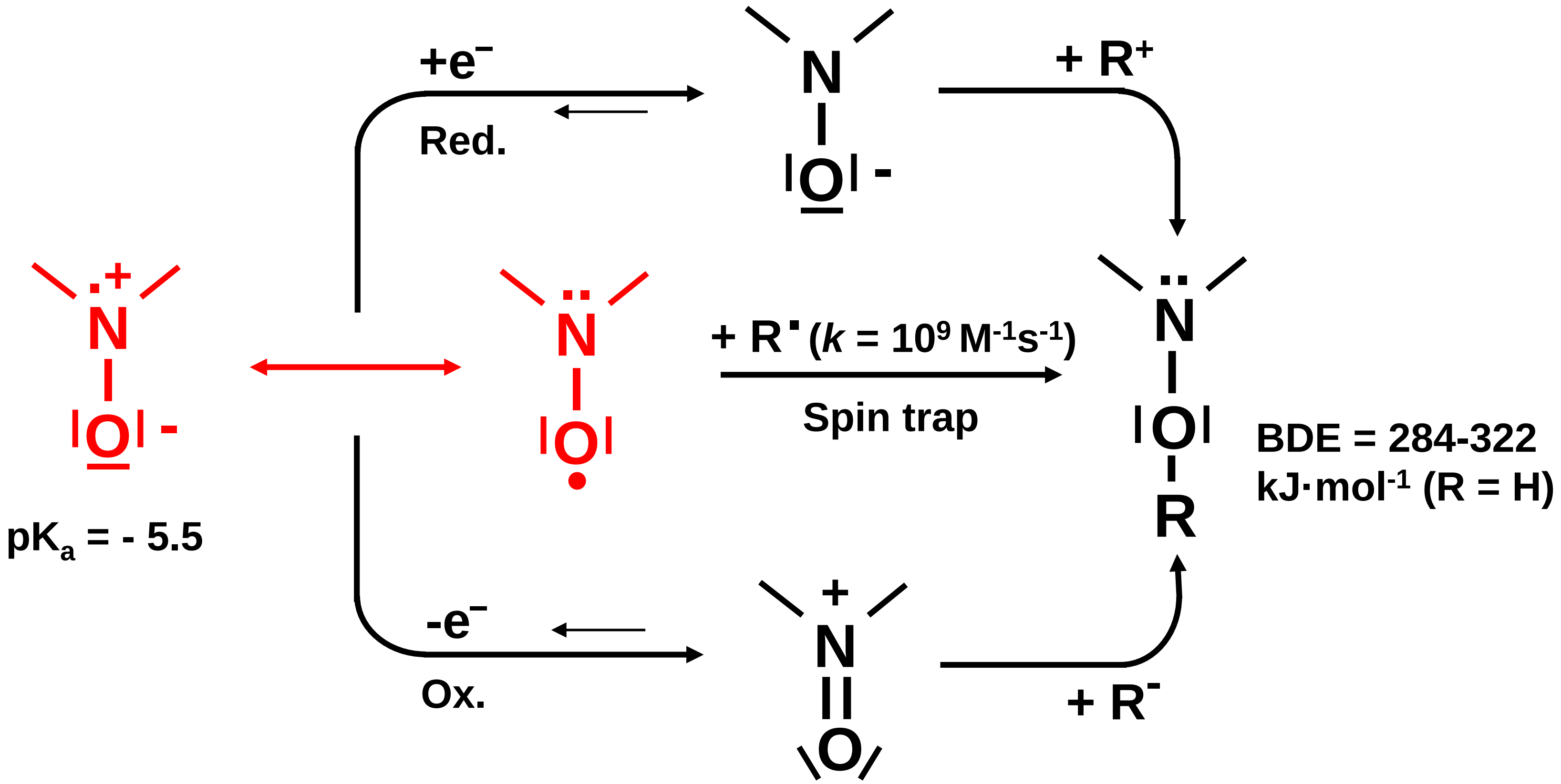


[E°$_{H_2O_2/H_2O}$ > E° $_{TEMPO/TEMPA}$]

Reaction conditions, batch process :

- **Solvent: water - pH 3-4 - addition of H_2WO_3 and stabilizing agent**
- **TMPA out phase, added once at the start**
- **T = 80-100°C, efficient stirring**
- **30-40 % H_2O_2 [1.05 molar amount] (added in order to keep a stationary concentration lower than 5 %)**
- **Yield** xx **: 86 % (R = H): 92% (R = OH)**

Nitroxide Radical Reactions



Nitroxide	**$E_{1/2}$**	**$E_{1/2}$ (V) (H_2O)**	**Nitroxide**	**$E_{1/2}$ (V)**
Di-t-Bu	0.187	0.657	4-O-TEMPO	0.301
TEMPO	0.203	0.728	Fremy Salt	0.901
4-OH-TEMPO	0.224	0.813	Succinimide-N-oxyl	1.112

Magnetic Moment of Radicals

Paramagnetism is directly related to the number of unpaired e⁻ :

$\mu^2 = 4s(s+1)$ where s = unpaired electron spin (1/2 for each unpaired e⁻)

One unpaired e^-, s = ½, and μ^2 = 4(1/2)(1/2 + 1) = 3, μ = 1.73 BM

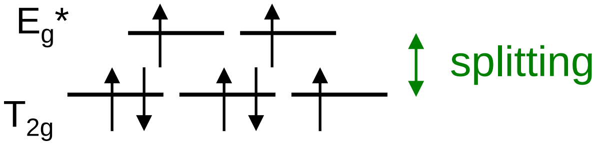
# UnP e⁻'s	Mag. Moment
1	1.73
2	2.83
3	3.87
4	4.90
5	5.92

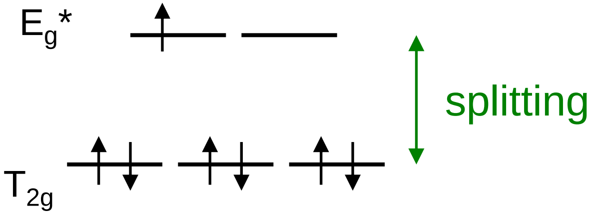
$[Co(H_2O)_6]^{+2}$ has a measured magnetic moment of 3.9.

$[Co(NH_3)_6]^{+2}$ has a magnetic moment of 1.7.

$[Co(H_2O)_6]^{+2}$ is a *high spin complex*
E_p > splitting

$[Co(NH_3)_6]^{+2}$ is a *low spin complex*
E_p < splitting



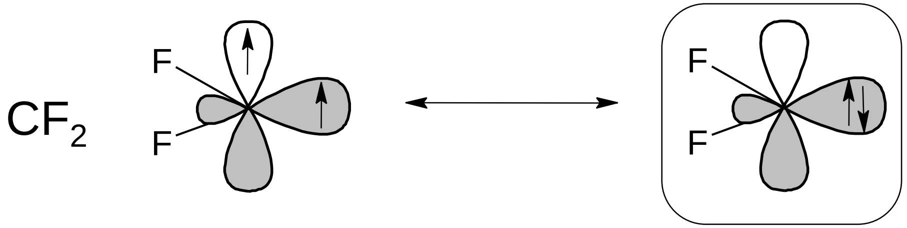


Carbenes: Singlet-Triplet States

Similar behaviour is observed in some organic molecules:

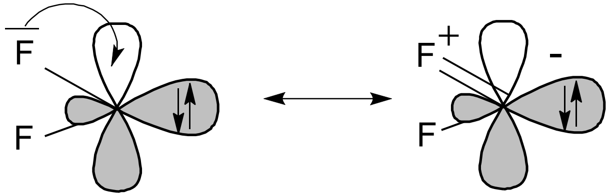
Carbenes exist as Singlets or Triplets depending on MO-splitting by a neighbouring atom.

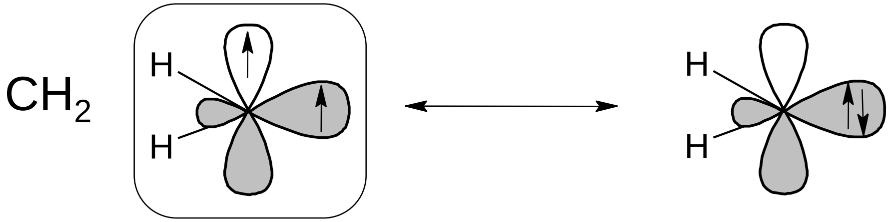
Qualitative resonance structure argument



-234.5 UHF T

-257.8 UHF S





No such resonance is possible

-203.5 UHF T

-161.9 UHF S
exp Δ = 38 kJ·mol^{-1}

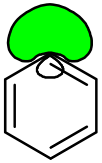
Sigma and Pi-Radicals

Radicals can be classified on the basis of the unpaired electron orbital symmetry :

1) **Sigma radicals** (σ)

The orbital has a positive lobe in a specific direction (analogous to a sigma bond)

Examples: Ph•



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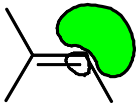
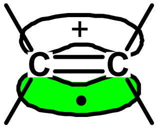


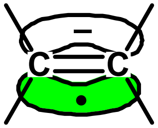
2) **Pi-radicals** (π)

The orbital has a π structure (analogous to a π bond)

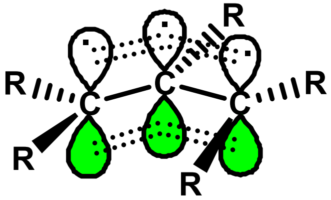
Examples: π-cation radical



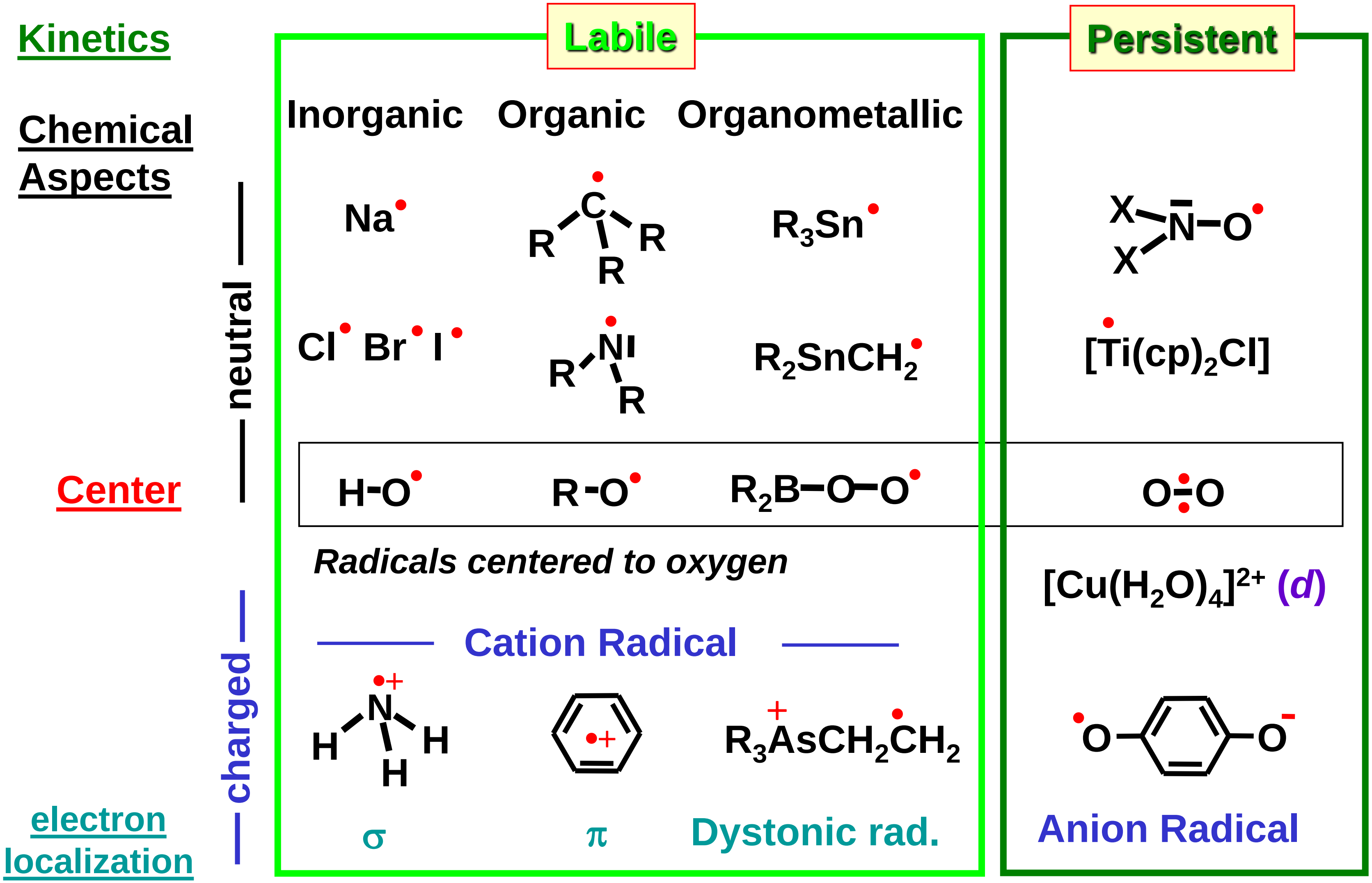
, π-anion radical



allyl (delocalized) radical



A General Classification of Radicals



Kinetics

Labile

Persistent

Chemical Aspects

Inorganic Organic Organometallic

neutral

$Na^{\bullet}$

$R_3Sn^{\bullet}$

$Cl^{\bullet}$ $Br^{\bullet}$ $I^{\bullet}$

$R_2SnCH_2^{\bullet}$

$^{\bullet}[Ti(cp)_2Cl]$

Center

$H-O^{\bullet}$

$R-O^{\bullet}$

$R_2B-O-O^{\bullet}$

Radicals centered to oxygen

$[Cu(H_2O)_4]^{2+}$ (*d*)

charged

Cation Radical

$R_3\overset{+}{A}sCH_2\overset{\bullet}{C}H_2$

electron localization

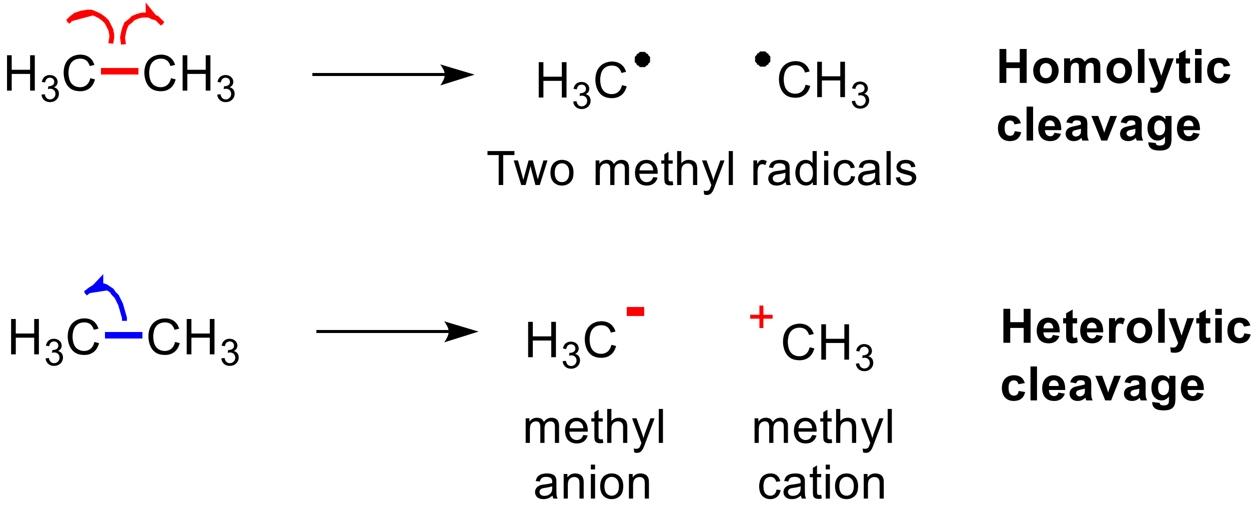
σ

π

Dystonic rad.

Anion Radical

Bond Cleavage as Source of Free Radicals



$H_3C{-}CH_3 \longrightarrow H_3C^{\bullet} \quad {}^{\bullet}CH_3$

Two methyl radicals

Homolytic cleavage

$H_3C{-}CH_3 \longrightarrow H_3C^{-} \quad {}^{+}CH_3$

methyl anion

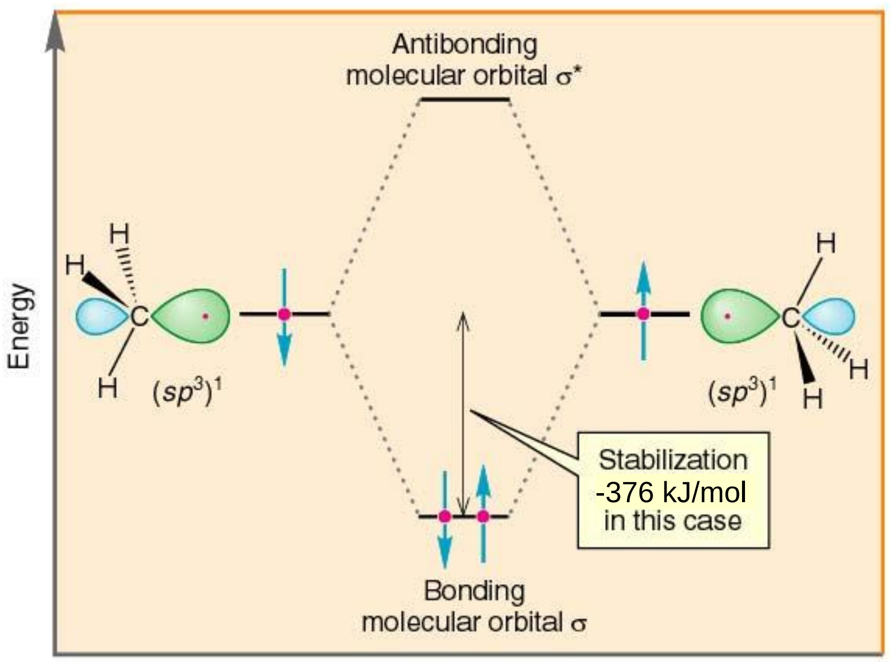
methyl cation

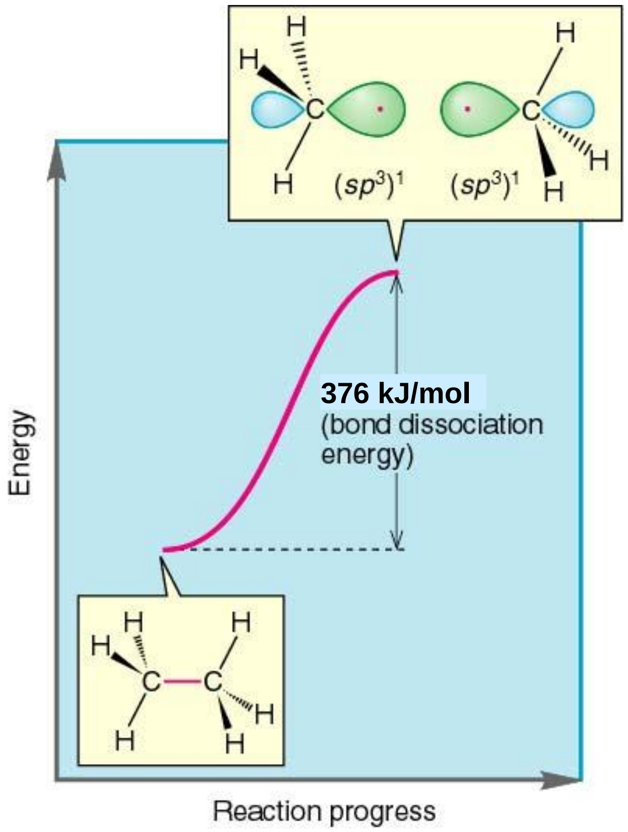
Heterolytic cleavage

- Homolytic cleavage of ethane leads to the corresponding methyl radicals
- **Note the ‘fish hook’ arrows!**
- Homolytic cleavage is energetically more favored than heterolytic cleavage in gas phase.

Radicals, Bonds and Homolytic Cleavage

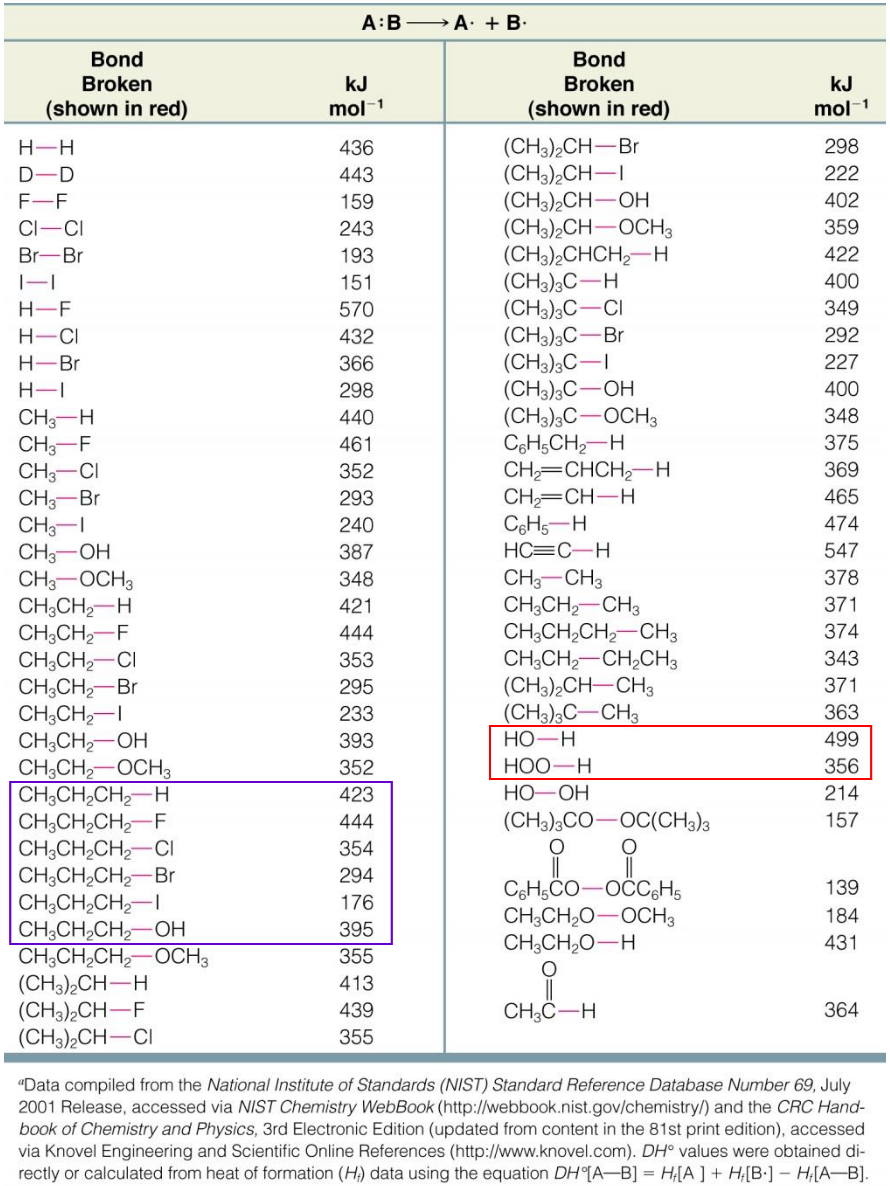
- **The combination of two radicals generally produce a dimer (formation of a sigma (σ) bond)**





- **Homolytic cleavage = Bond Dissociation Energy (BDE)**

Homolytic Bond Dissociation Energies



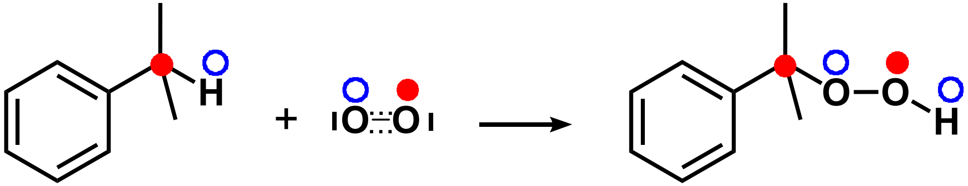
$A{:}B \longrightarrow A\cdot + B\cdot$

Bond Broken (shown in red)	kJ mol^{-1}	Bond Broken (shown in red)	kJ mol^{-1}
$H—H$	436	$(CH_3)_2CH—Br$	298
$D—D$	443	$(CH_3)_2CH—I$	222
$F—F$	159	$(CH_3)_2CH—OH$	402
$Cl—Cl$	243	$(CH_3)_2CH—OCH_3$	359
$Br—Br$	193	$(CH_3)_2CHCH_2—H$	422
$I—I$	151	$(CH_3)_3C—H$	400
$H—F$	570	$(CH_3)_3C—Cl$	349
$H—Cl$	432	$(CH_3)_3C—Br$	292
$H—Br$	366	$(CH_3)_3C—I$	227
$H—I$	298	$(CH_3)_3C—OH$	400
$CH_3—H$	440	$(CH_3)_3C—OCH_3$	348
$CH_3—F$	461	$C_6H_5CH_2—H$	375
$CH_3—Cl$	352	$CH_2{=}CHCH_2—H$	369
$CH_3—Br$	293	$CH_2{=}CH—H$	465
$CH_3—I$	240	$C_6H_5—H$	474
$CH_3—OH$	387	$HC{\equiv}C—H$	547
$CH_3—OCH_3$	348	$CH_3—CH_3$	378
$CH_3CH_2—H$	421	$CH_3CH_2—CH_3$	371
$CH_3CH_2—F$	444	$CH_3CH_2CH_2—CH_3$	374
$CH_3CH_2—Cl$	353	$CH_3CH_2—CH_2CH_3$	343
$CH_3CH_2—Br$	295	$(CH_3)_2CH—CH_3$	371
$CH_3CH_2—I$	233	$(CH_3)_3C—CH_3$	363
$CH_3CH_2—OH$	393	$HO—H$	499
$CH_3CH_2—OCH_3$	352	$HOO—H$	356
$CH_3CH_2CH_2—H$	423	$HO—OH$	214
$CH_3CH_2CH_2—F$	444	$(CH_3)_3CO—OC(CH_3)_3$	157
$CH_3CH_2CH_2—Cl$	354	$C_6H_5\overset{\overset{\displaystyle O}{\|}}{C}O—O\overset{\overset{\displaystyle O}{\|}}{C}C_6H_5$	139
$CH_3CH_2CH_2—Br$	294	$CH_3CH_2O—OCH_3$	184
$CH_3CH_2CH_2—I$	176	$CH_3CH_2O—H$	431
$CH_3CH_2CH_2—OH$	395	$CH_3\overset{\overset{\displaystyle O}{\|}}{C}—H$	364
$CH_3CH_2CH_2—OCH_3$	355		
$(CH_3)_2CH—H$	413		
$(CH_3)_2CH—F$	439		
$(CH_3)_2CH—Cl$	355		

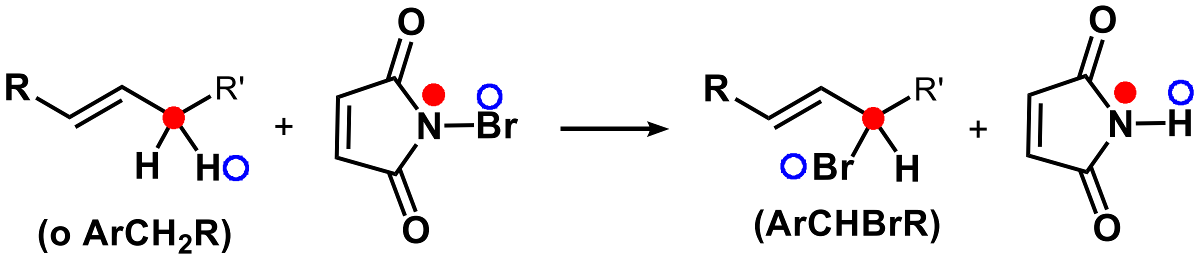
[a] Data compiled from the *National Institute of Standards (NIST) Standard Reference Database Number 69*, July 2001 Release, accessed via *NIST Chemistry WebBook* (http://webbook.nist.gov/chemistry/) and the *CRC Handbook of Chemistry and Physics*, 3rd Electronic Edition (updated from content in the 81st print edition), accessed via Knovel Engineering and Scientific Online References (http://www.knovel.com). $DH°$ values were obtained directly or calculated from heat of formation (H_f) data using the equation $DH°[A—B] = H_f[A\cdot] + H_f[B\cdot] - H_f[A—B]$.

Synthetic Applications of Reactions Involving Paramagnetic Intermediates

1) Autoxidation



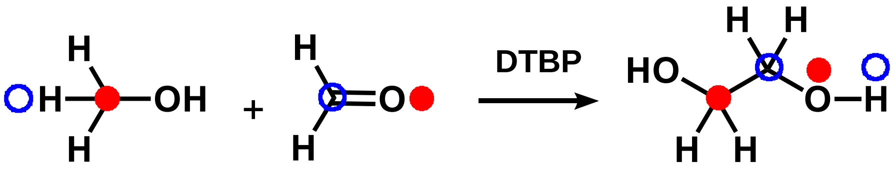
2) Halogenation



(o $ArCH_2R$)

($ArCHBrR$)

3) Addition (formation of C-C bonds)

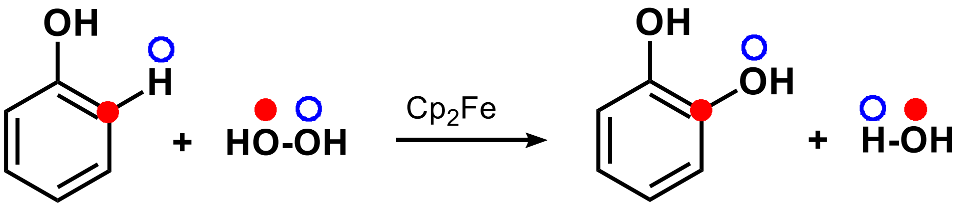


● Donor radical center

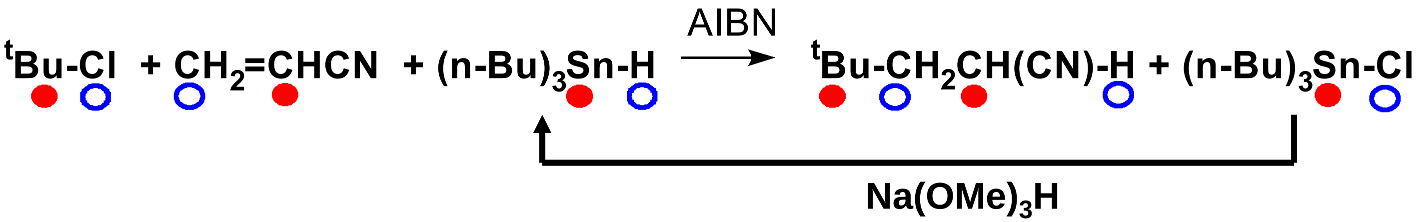
○ Acceptor radical center

Examples of Reactions Involving Paramagnetic Intermediates

4) Addition (formation of C-X bonds)

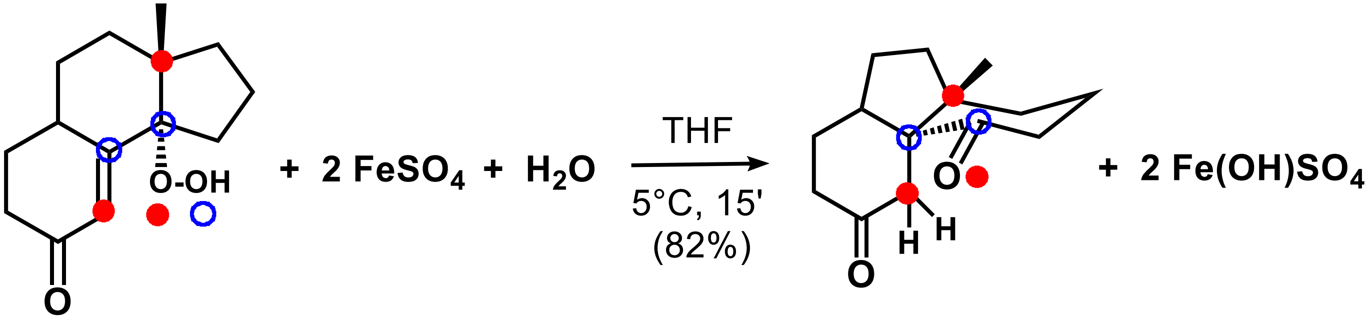


5) Reductive Addition (C-C and X-H)



$$^{t}\text{Bu-Cl} + \text{CH}_2\text{=CHCN} + (\text{n-Bu})_3\text{Sn-H} \xrightarrow{\text{AIBN}} {}^{t}\text{Bu-CH}_2\text{CH(CN)-H} + (\text{n-Bu})_3\text{Sn-Cl}$$

Na(OMe)$_3$H



Peculiarity of Radical Intermediates and Reactions

- High reactivity (k_p, k_t > 10^2; commonly moderate selectivity)
- Moderate o absent solvent effects
- A relevant number of kinetics data known
- Known dependence of reactivity/selectivity on molecular structure
- Known acid-base and redox equilibria at radical center (with extensive properties variations) and at other functional groups.
- Influence of charges on reactivity (dystonic, radical cation and anion)

- If labile, continuous initiation required (selective initiation)
- Compatible with all media (gas, liquid and solid phases)
- In chain processes, autocatalysis and inhibition are seldom active.
- If exothermic, explosions can occur through branching
- More than 10.000 reactions are known (mainly lab. experienced - some applied on industrial scale, i.e. O_2. Cl_2, NO_2, etc.)
- Can be designed starting from the knowledge on radical sources and reactivity of radical intermediates.

A Simplified View of Radical Reactions

$CH_3CH_2CH_2CH_2^{\bullet} + CH_3CH_2CH_2CH_2^{\bullet}$

$\xrightarrow[*]{R\text{-}H}$ $2\ CH_3CH_2CH_2CH_3$

Hydrogen abstraction

$\xrightarrow[*]{}$ $CH_3CH_2CH{=}CH_2 + CH_3CH_2CH_2CH_3$

Disproportionation

$\xrightarrow[*]{}$ $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$

Dimerization

$\xrightarrow[**]{}$ $CH_3CH_2^{\bullet} + CH{=}CH_2$

β-cleavage

* Bimolecular Reactions; ** Unimolecular Reactions

- These are important types of reactions that radicals can undergo

POLITECNICO DI MILANO

Lecture 1c

Free Radicals and Life

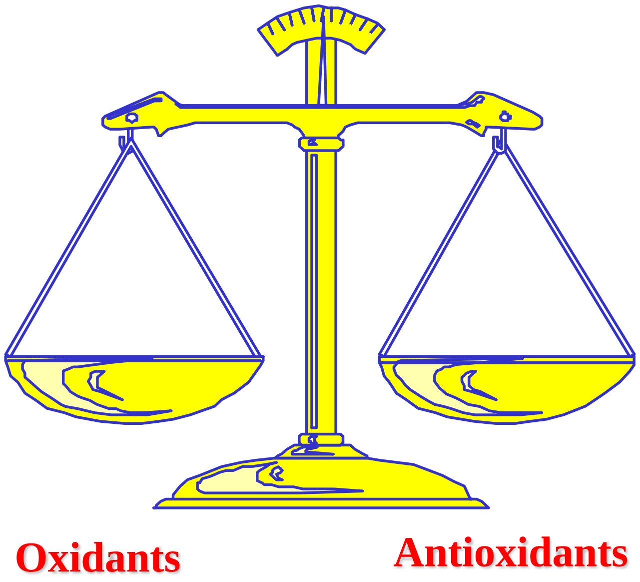
Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

Tissue Damage by Radicals

- strand breaks in DNA
  - may cause heritable mutations (in germ cells)
  - may induce cancer (in somatic cells)
- oxidation of polyunsaturated fatty acids in lipids
  - lipid peroxides are involved in atherogenesis
  - lipid peroxides break down to dialdehydes which modify proteins and nucleic acid bases
- oxidation of amino acids in proteins
  - different points of oxidation on C and other atoms (S, N, metals)
  - may lead to formation of antibodies against modified protein, and be a factor in auto-immune disease
  - oxidized amino acids may catalyze further formation of oxygen radicals
  - oxidized amino acids in LDL are involved in atherogenesis

Some Basic Beliefs of Oxidative Stress

- **Free radicals are produced in normal processes through the body’s use of oxygen**
- **Environmental pollutants and many drugs cause free radical production**
- **Free radicals can damage cell structure and function in chain reactions**
- **Antioxidants protect cells against free radicals by scavenging these species**



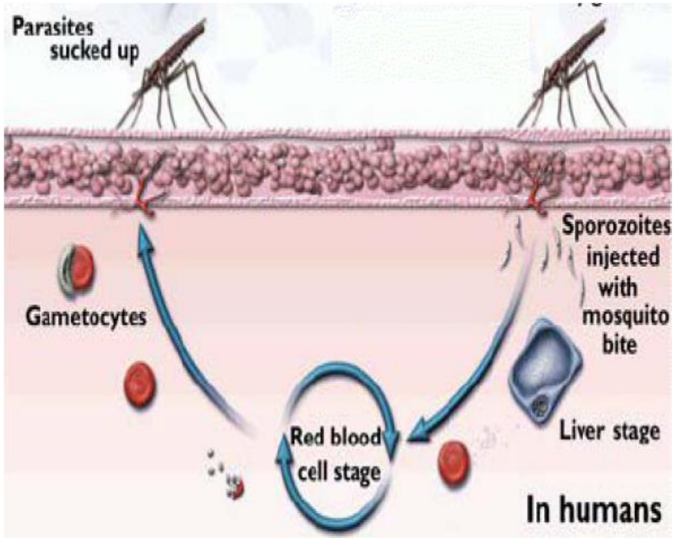
Free Radical Involvement in Pathophysiological Conditions

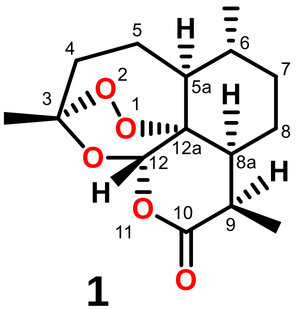
- Adriamycin cardiotoxicity
- AIDS
- Adult Respiratory Distress Syndrome
- Aging
- Alcoholism
- Alzheimer’s Disease
- Amyotrophic Lateral Sclerosis
- Atherosclerosis
- Diabetes
- Cancer
- Exercise
- Favism
- Iron Overload
- Myocardial Infarction
- Oxygen Toxicity
- Parkinson’s Disease
- Radiation Therapy
- Smoking
- Stroke
- Trauma

Drugs Based on Radical Reactions

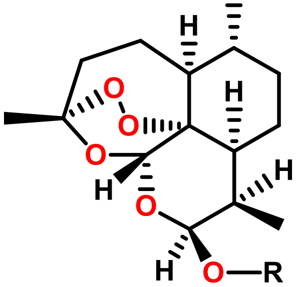
Structure of Antimalaria drugs:

Artemisinin derivatives

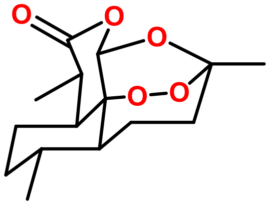




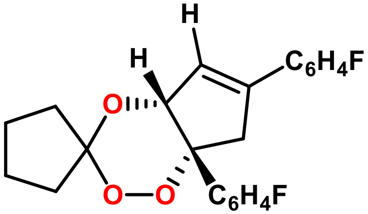
1



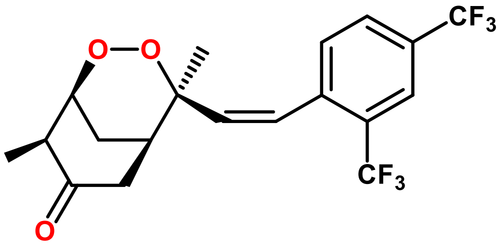
2a R = H Dihydroartemisinin
2b R = Me β-Artemether
2c R = Et Arteether
2d R = C(O)CH$_2$CH$_2$COONa Artesunate



1 Artemisin^a



3 BO7



4 Arteflene

Sources of Oxidative Stress

Exogenous

- PHOTOCHEMICAL AIR POLLUTANTS
  - nitrogen dioxide (NO_2)
  - sulfur dioxide (SO_2)
  - ozone (O_3)
- PESTICIDES
  - paraquat (herbicide)
  - Vacor (rodenticide)
- FOODS
  - sodium nitrite (food preservative)
  - fava beans
- DRUGS
  - sulfonamides (antimicrobials)
  - chloroquinones (antimalarials)
  - bleomycin (antitumor)
  - alloxan (insulin synthesis inhibitor)
- CHEMICALS
  - naphthalene (mothballs)
  - trinitrotoluene - TNT (explosive)

Endogenous

"REACTIVE OXYGEN SPECIES" (ROS)

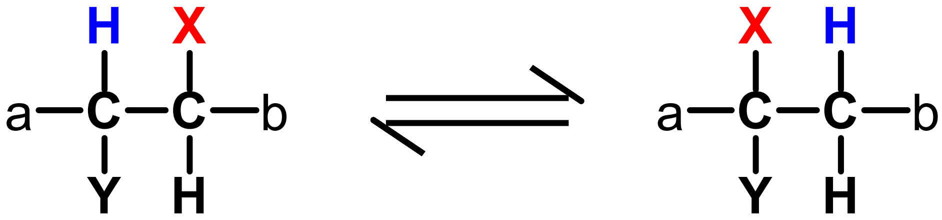
- superoxide radical $O_2^{\bullet -}$
- hydrogen peroxide H_2O_2
- hydroxyl radical $HO^{\bullet}$

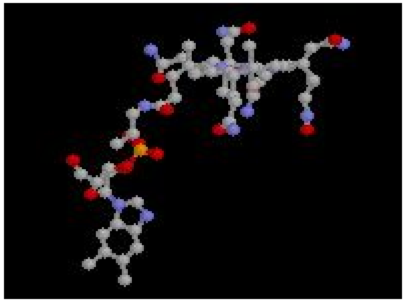
- ORGANIC HYDROPEROXIDES
  - lipid hydroperoxides (L-O-O-H)
  - other hydroperoxides (R-O-O-H)
- BIOACTIVATED FREE RADICALS ($R \rightarrow R^{\bullet}$)
  - carbon tetrachloride (hepatotoxic)
  - BaP radicals (carcinogenic)
  - many others

Examples of Radicals in Enzymatic Reactions

- Coenzyme B_{12}-dependent enzymatic reactions
- Ribonucleotide reductases (e.g. human enzyme and *Escherichia coli*)
- α-Lysine 2,3-aminomutase (‘poor man’s B_{12})
- Cytochrome P-450 dependent monooxygenases
- Penicillin biosynthesis
- Pyruvate formate lyase

Coenzyme B_{12}-dependent Enzymatic Rearrangements

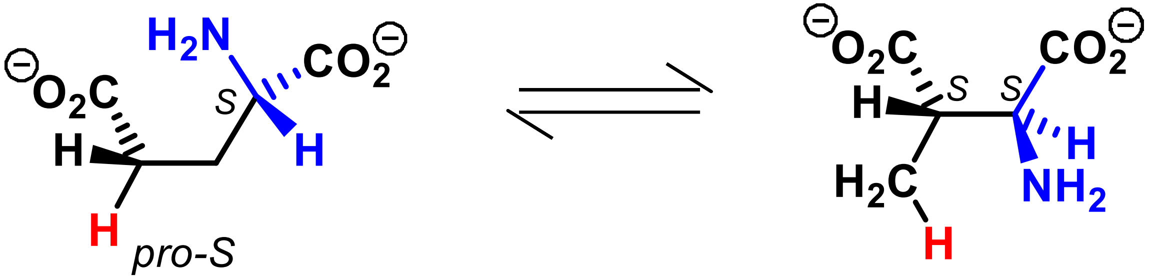




ENZYME	**a**	**b**	**X**	**Y**
Diol dehydratase	H or Me	H, Me, CF_3	OH	OH
Ethanolamine ammonia lyase	H	H or Me	NH_2	OH
Methylmalonyl CoA mutase	H	H	COSCoA	CO_2H
Glutamate mutase	H	H	$CH(NH_3^+)CO_2^-$	CO_2H
2-Methyleneglutarate mutase	H	H	$C(=CH_2)CO_2^-$	CO_2H

The Carbon Skeleton Mutases: Glutamate Mutase

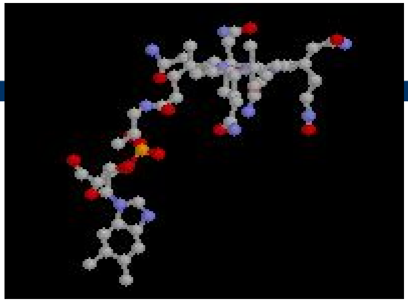
This enzyme was first isolated from the anaerobic bacterium *Clostridium tetanomorphum* and catalyses the rearrangement of glutamate to 3-methylaspartate:

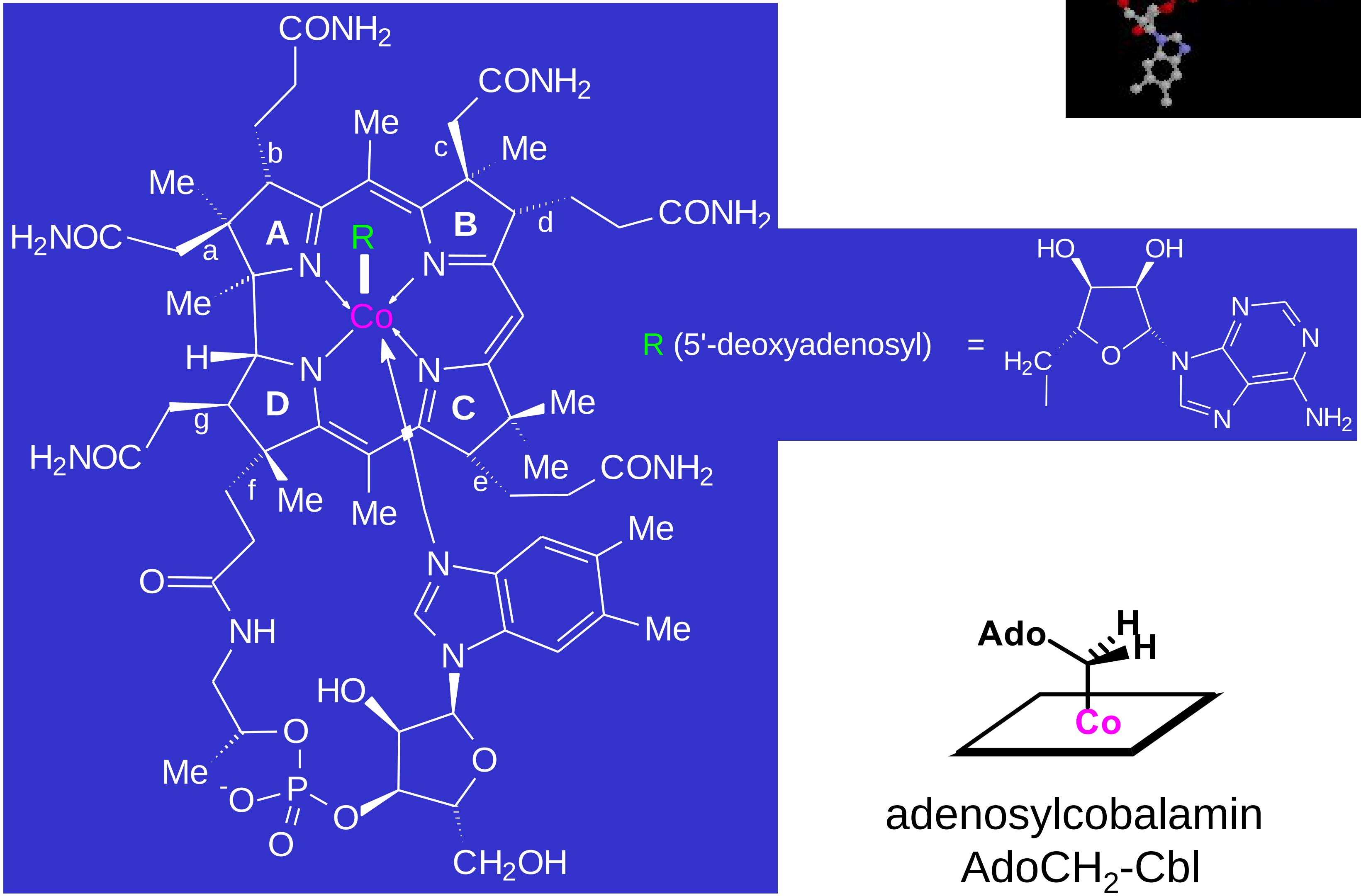


H. A. Barker found that the enzyme contained a light-sensitive, yellow-orange cofactor, which was subsequently identified as coenzyme B_{12}

(review: W Buckel and B T Golding, *Chem. Soc. Rev.*, 1996, **26**, 329-337)

Structure of Coenzyme B_{12}



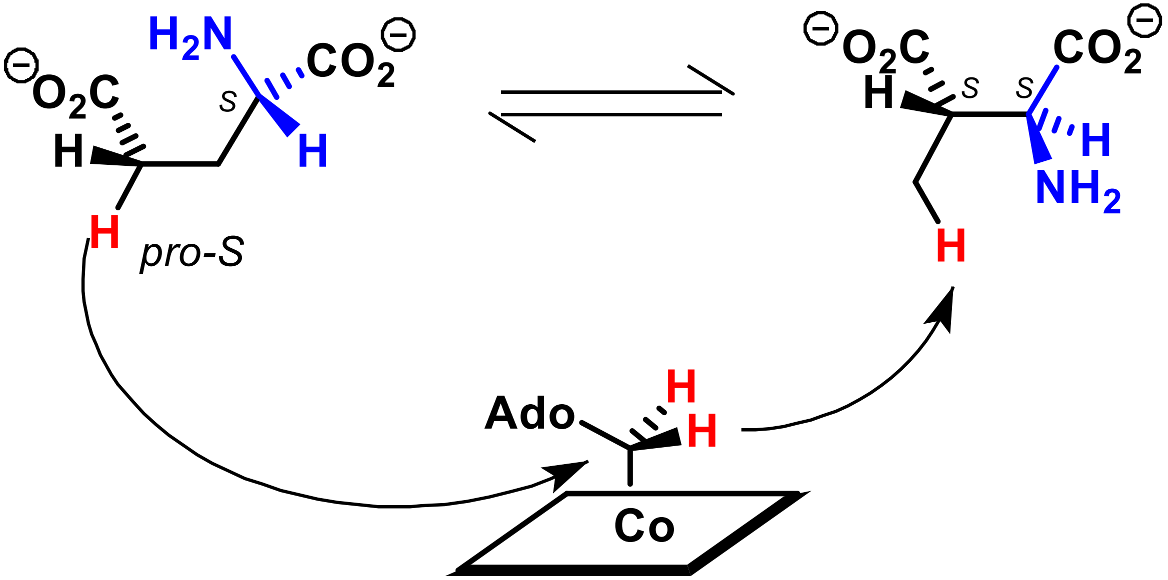


adenosylcobalamin

$AdoCH_2$-Cbl

Stereochemistry of Glutamate Mutase

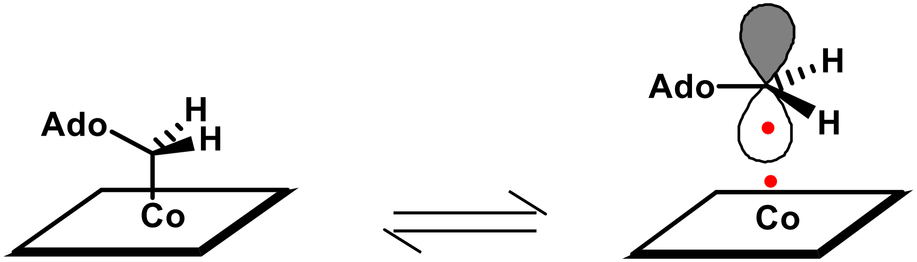
- $H_{pro\text{-}S}$ is abstracted from C-4 of glutamate.
- The abstracted H mixes with the 5'-methylene hydrogen of adenosyl-cobalamin.
- The glycinyl residue migrates to this C-4 with inversion of configuration



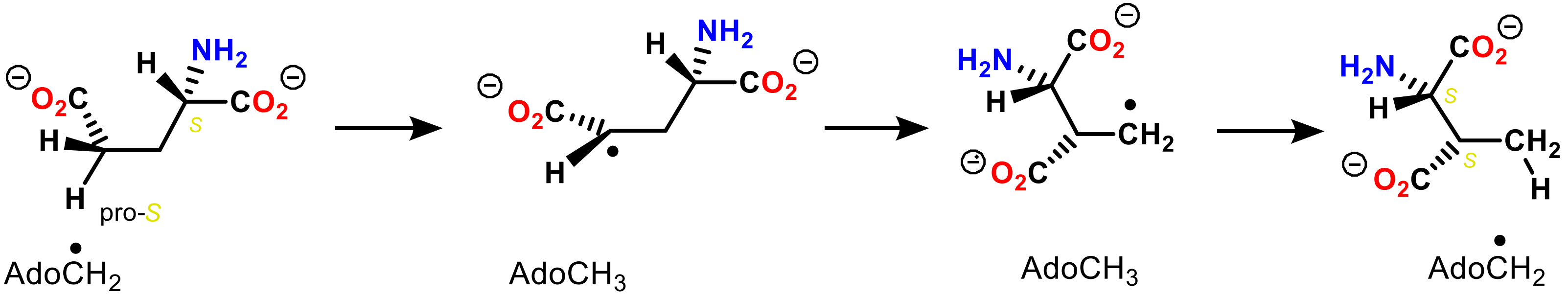
Adenosyl-cobalamin

Reaction Pathway for Glutamate Mutase

- Binding of the substrate to the enzyme-coenzyme complex triggers Co-C bond homolysis:

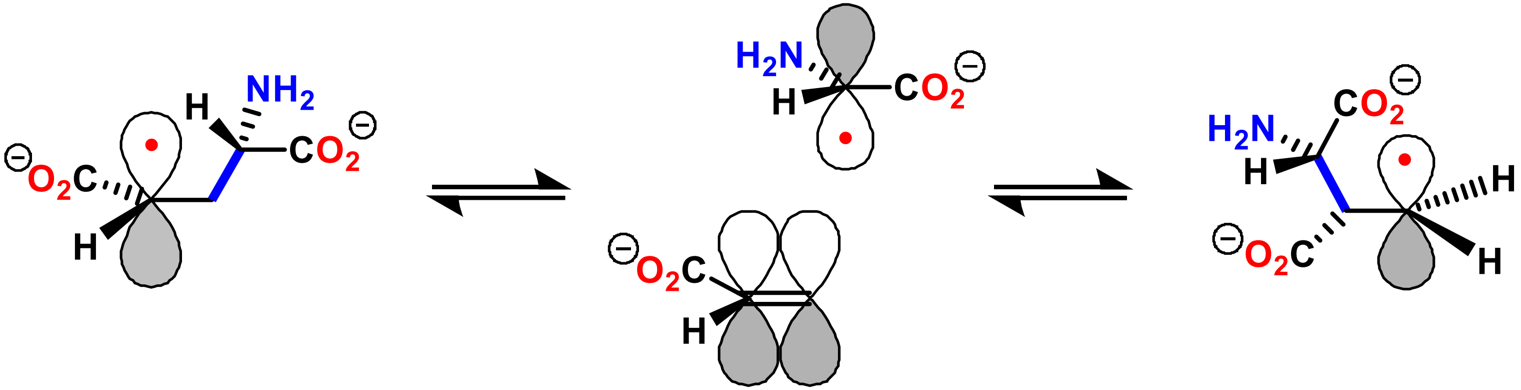


- The adenosyl radical initiates the reaction pathway by hydrogen atom abstraction from a substrate molecule:



Possible Rearrangement Mechanisms for Glutamate Mutase

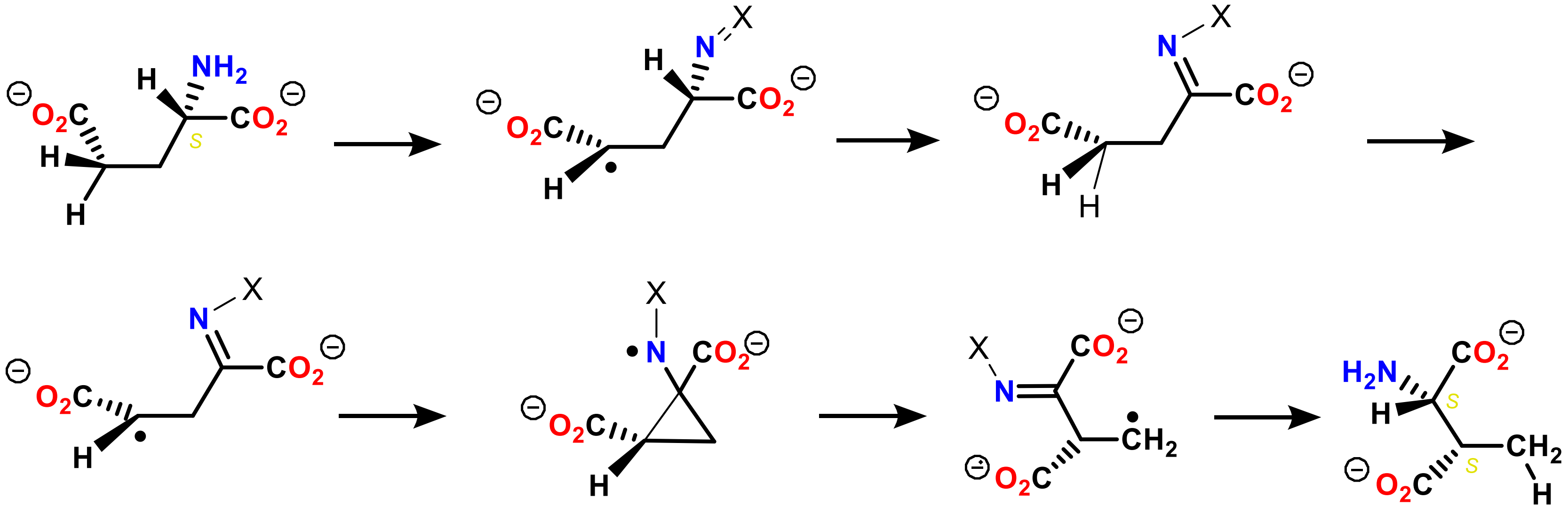
- Fragmentation-recombination pathway:



- Note that this mechanism has strict stereoelectronic requirements: the σ-bond undergoing cleavage must be properly aligned with the *p*-orbital of the 4-glutamyl radical.

Possible Rearrangement Mechanisms for Glutamate Mutase

- Addition-elimination *via* an intermediate imine:



- X contains a carbonyl group from the protein or a cofactor (e.g. pyridoxal)

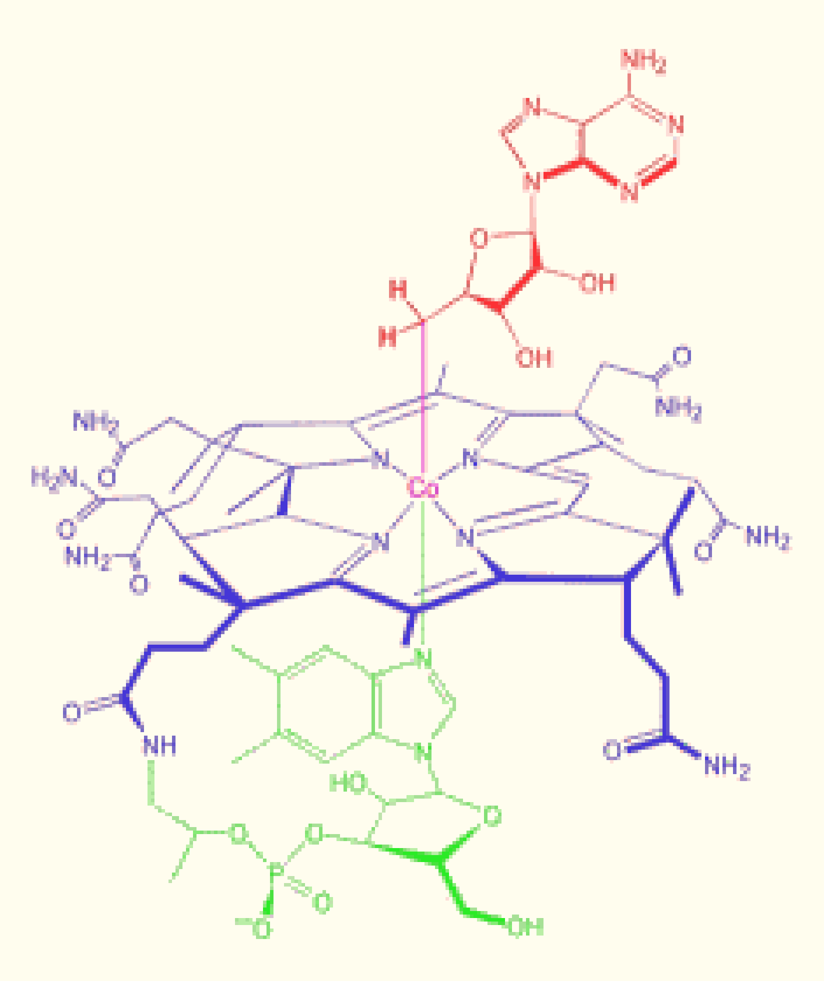
Tools for Elucidating the Mechanism of Coenzyme B_{12}-dependent Reactions

- Synthesis of substrate analogues, including isotopically labelled compounds.

- NMR and EPR studies of enzymatic reactions using substrate analogues.

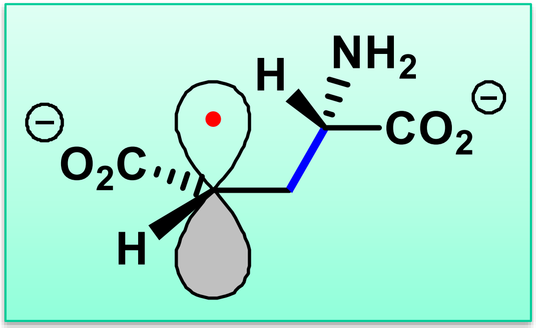
- Model studies.

- *Ab initio* calculations of reaction pathways

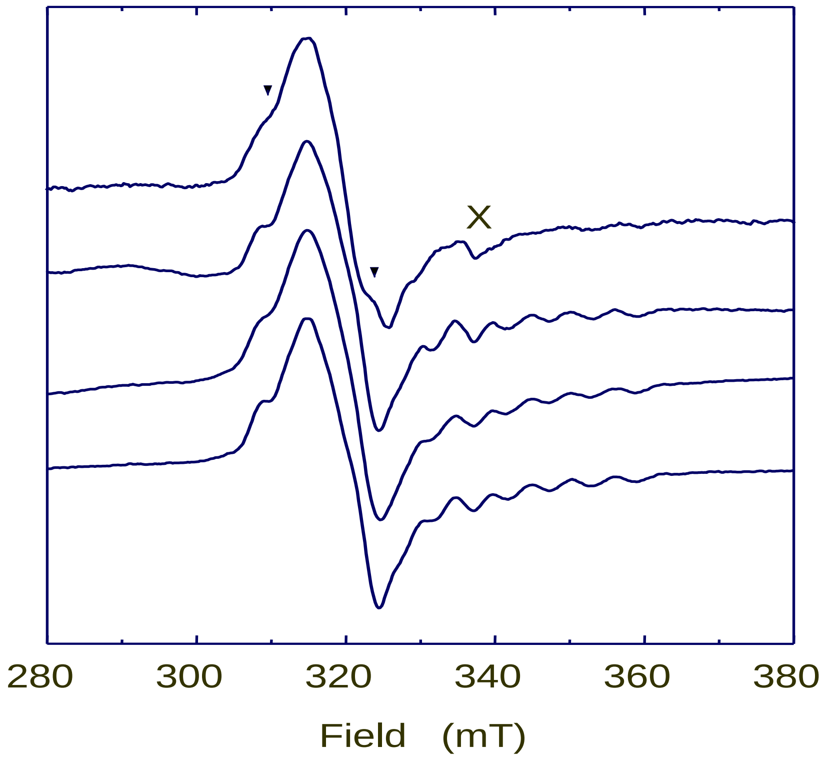


EPR Study of Glutamate Mutase

- Glutamates specifically labelled with ^{2}H, ^{13}C and ^{15}N were synthesised and experimented.
- Each compound was incubated with glutamate mutase + coenzyme B_{12} for ca. 20 s.
- The reaction mixtures were frozen in liquid N_2 and EPR spectra obtained.
- *These experiments identified the 4-glutamyl radical as an intermediate:*



EPR Study of Glutamate Mutase

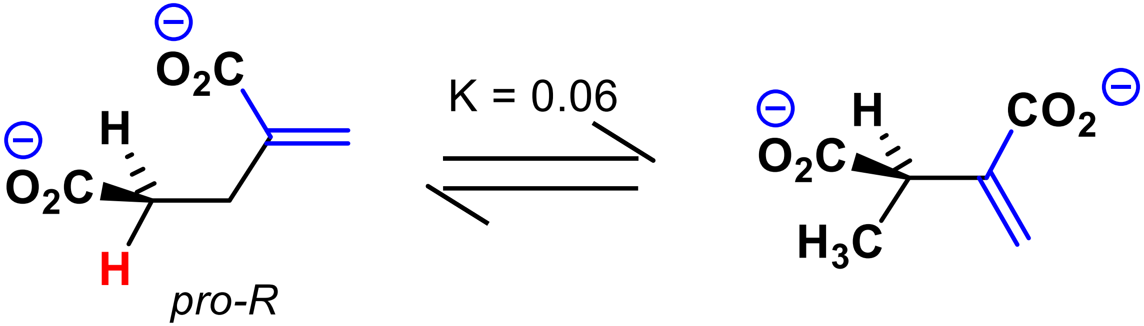


A) [4-^{13}C]-(*S*)-glutamate.
B) [3-^{13}C]-(*S*)-glutamate.
C) [2-^{13}C]-(*S*)-glutamate.
D) unlabelled (*S*)-glutamate.
(All spectra were recorded at 50 K)

EPR spectra of the radical species derived from incubating glutamate mutase and coenzyme B_{12} with ^{13}C-labelled (*S*)-glutamate.

2-Methyleneglutarate Mutase

- 2-Methyleneglutarate mutase from *Clostridium barkeri* catalyses the equilibration of 2-methyleneglutarate with (*R*)-3-methylitaconate:

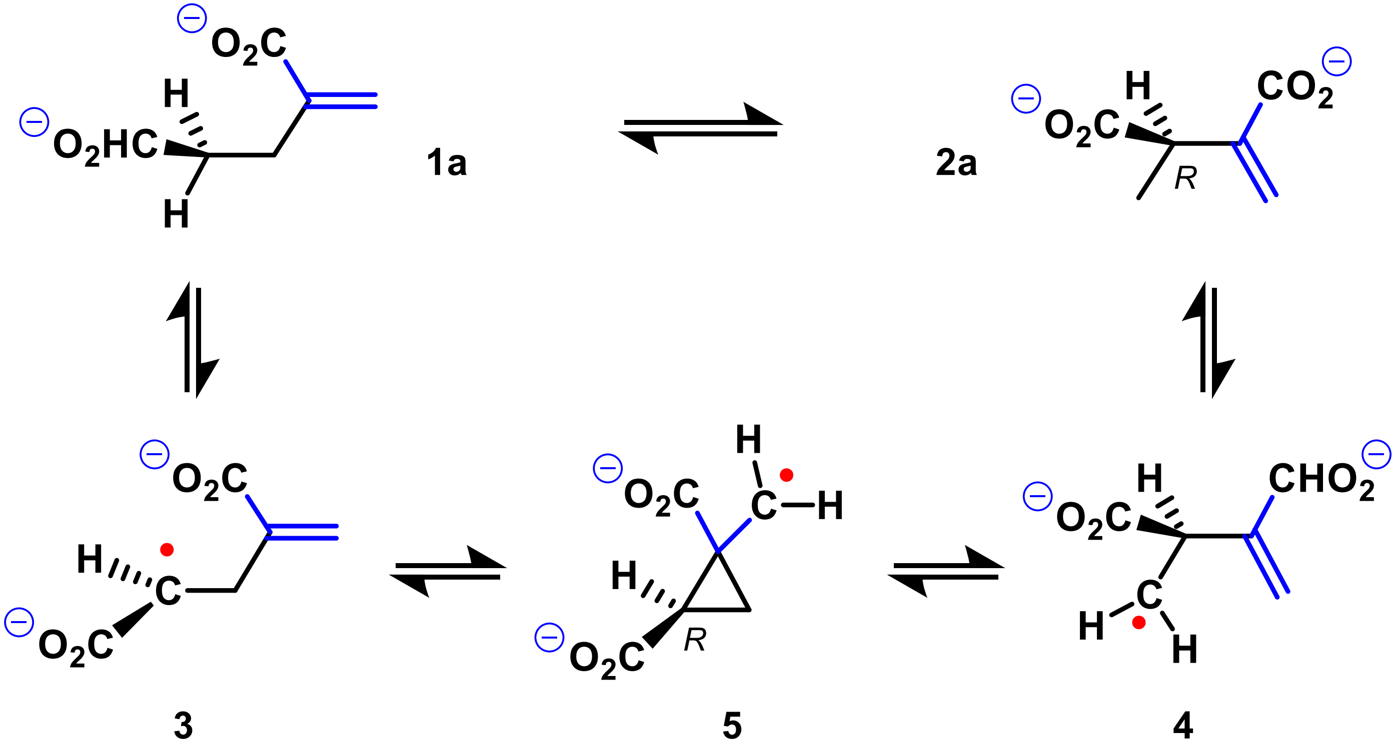


- The pink-orange enzyme is a homotetramer (300 kDa) containing AdoCH$_2$-Cbl.
- Removal of the coenzyme gives inactive apoenzyme, which can be re-activated by addition of AdoCH$_2$-Cbl.
- The active enzyme is susceptible to dioxygen, which converts bound AdoCH$_2$-Cbl into hydroxocobalamin.

C Michel, S P J Albracht, and W Buckel, *Eur J Biochem*, 1992, **205**, 767

Addition-elimination Mechanism for the Rearrangement

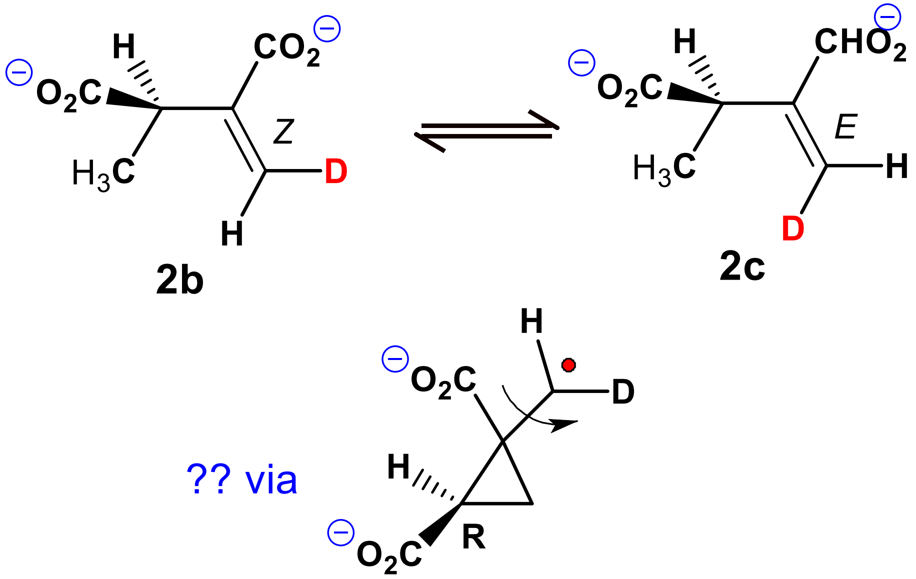
- Equilibration of 2-methyleneglutarate **1a** and (*R*)-3-methylitaconate **2a** and their corresponding radicals **3** and **4** *via* cyclopropylcarbinyl radical **5**:



Newcomb, M; Miranda, N. J. Am. Chem. Soc.2003, 125,4080.

Test of the Cyclopropylcarbinyl Mechanism

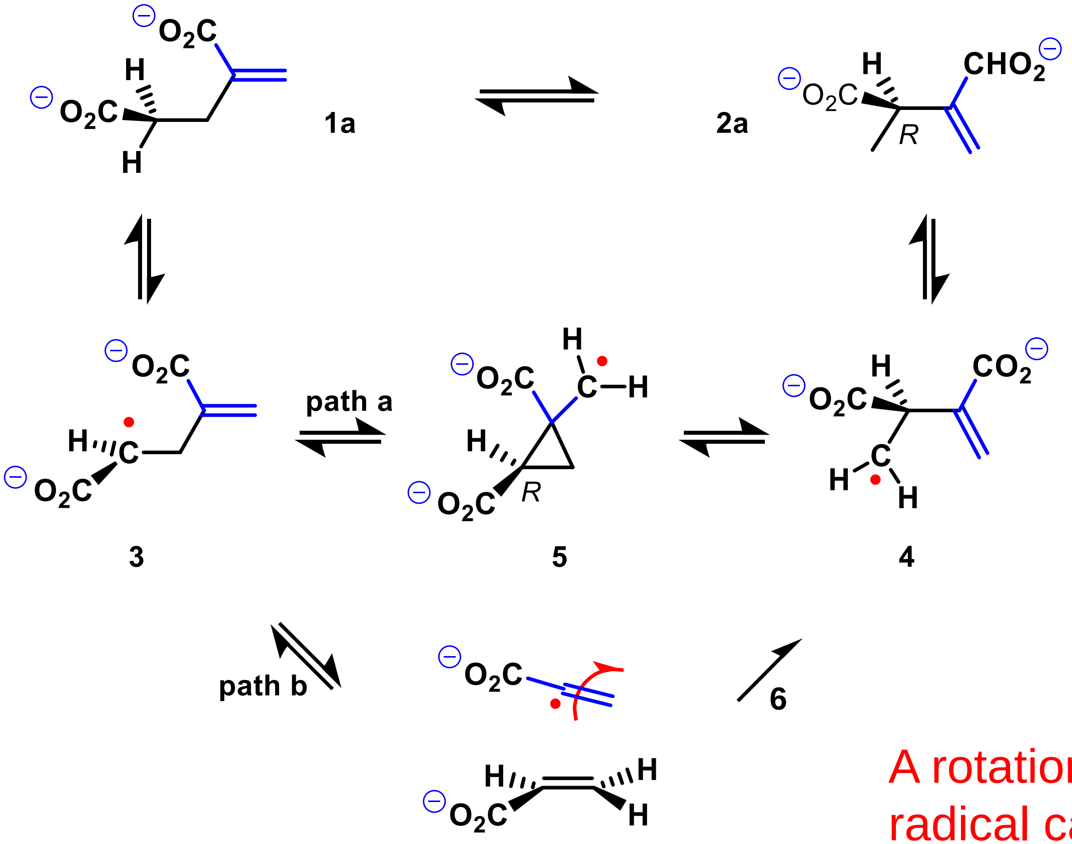
- If the energy barrier to rotation about the C-1/methylene bond in the cyclopropylcarbinyl radical is sufficiently low, then a stereospecifically deuterated 3-methylitaconate (say the *Z*-isomer **2b**) should equilibrate with its *E*-isomer **2c** when incubated with 2-methylene-glutarate mutase holoenzyme.



- *It does and also equilibrates with the corresponding E and Z isomers of 2-methyleneglutarate.*

Do These Results Prove the Cyclopropylcarbinyl Mechanism?

- Consider an alternative mechanism (‘fragmentation-recombination’) in which the substrate-derived radical **3** *fragments* to acrylate and the 2-acrylate radical **6** (path b).



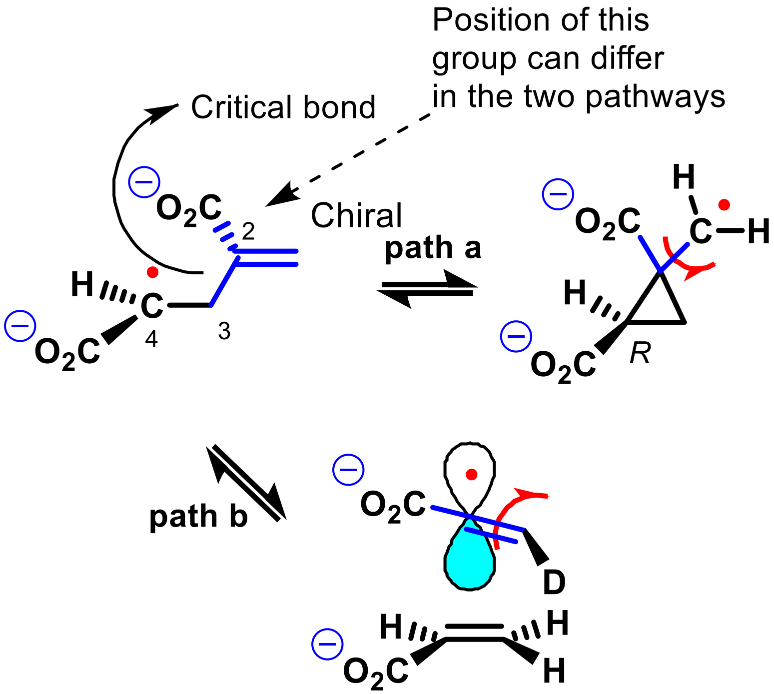
A rotation within the acrylate radical can explain the NMR results

Can The Two Mechanisms Be Distinguished?

- For conversion to the cyclopropylcarbinyl radical, the conformation shown is essential to achieve maximal overlap between the *p* orbitals at C-2 and C-4.

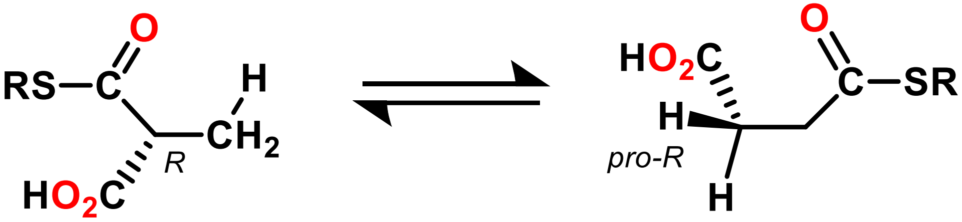
- For the fragmentation pathway, it suffices to achieve maximal overlap between the *p* orbital at C-4 and the critical C-2/C-3 σ-bond.

- The two alternative mechanisms can in principle be distinguished by the conformation of the substrate bound to the enzyme.



Methylmalonyl-CoA Mutase

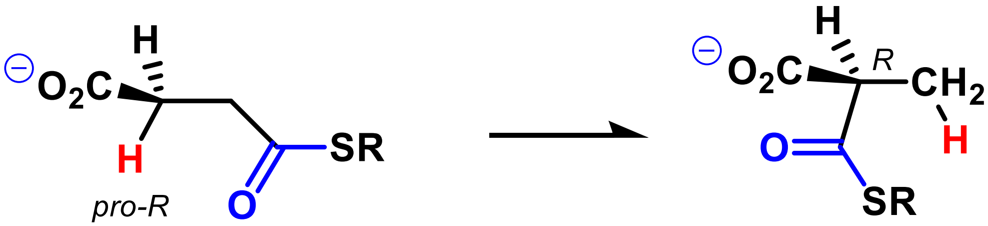
- This human enzyme converts the (*R*)-isomer of methylmalonyl-CoA to succinyl-CoA (RS = coenzyme A):



from propionate, a toxic product of the degradation of fats

enter Krebs cycle

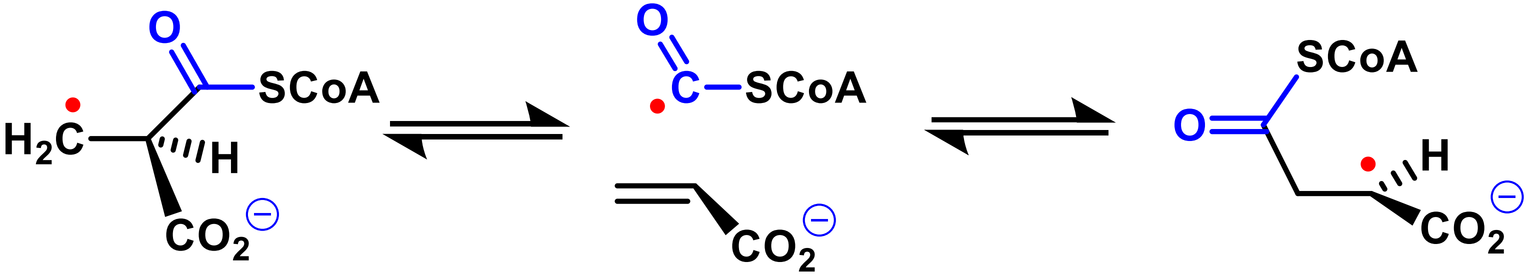
- In contrast to glutamate and 2-methyleneglutarate mutase, the migrating group (thioester residue) migrates with *retention* of configuration at the receiving locus:



Pathways for Methylmalonyl-CoA Mutase

- Consider three possible mechanisms for the interconversion of intermediate radicals, corresponding in structure to substrate and product:

Fragmentation-recombination:

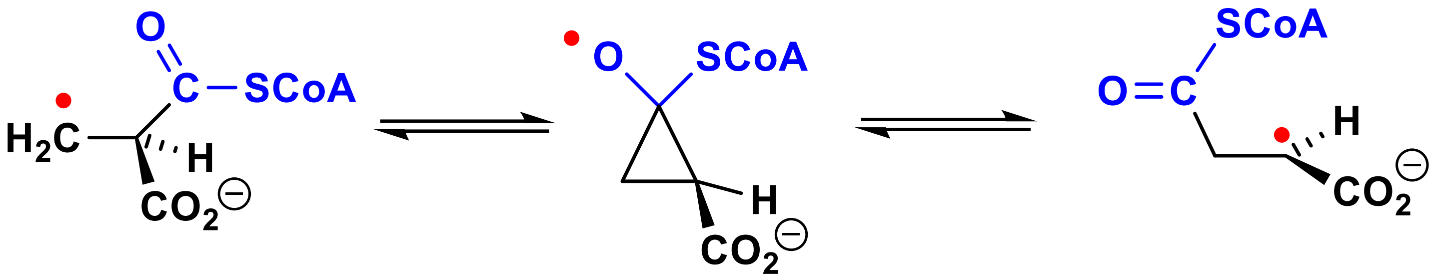


Radical corresponding to methylmalonyl-CoA

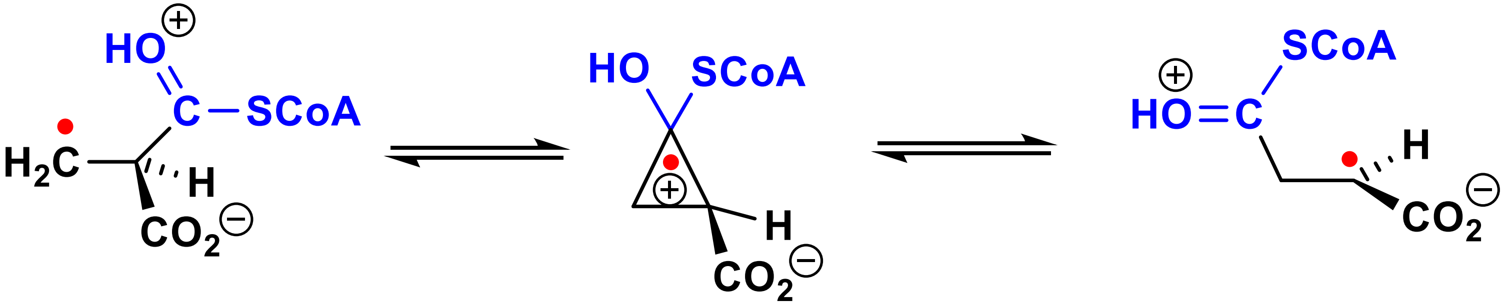
Radical corresponding to succinyl-CoA

Pathways for Methylmalonyl-CoA Mutase

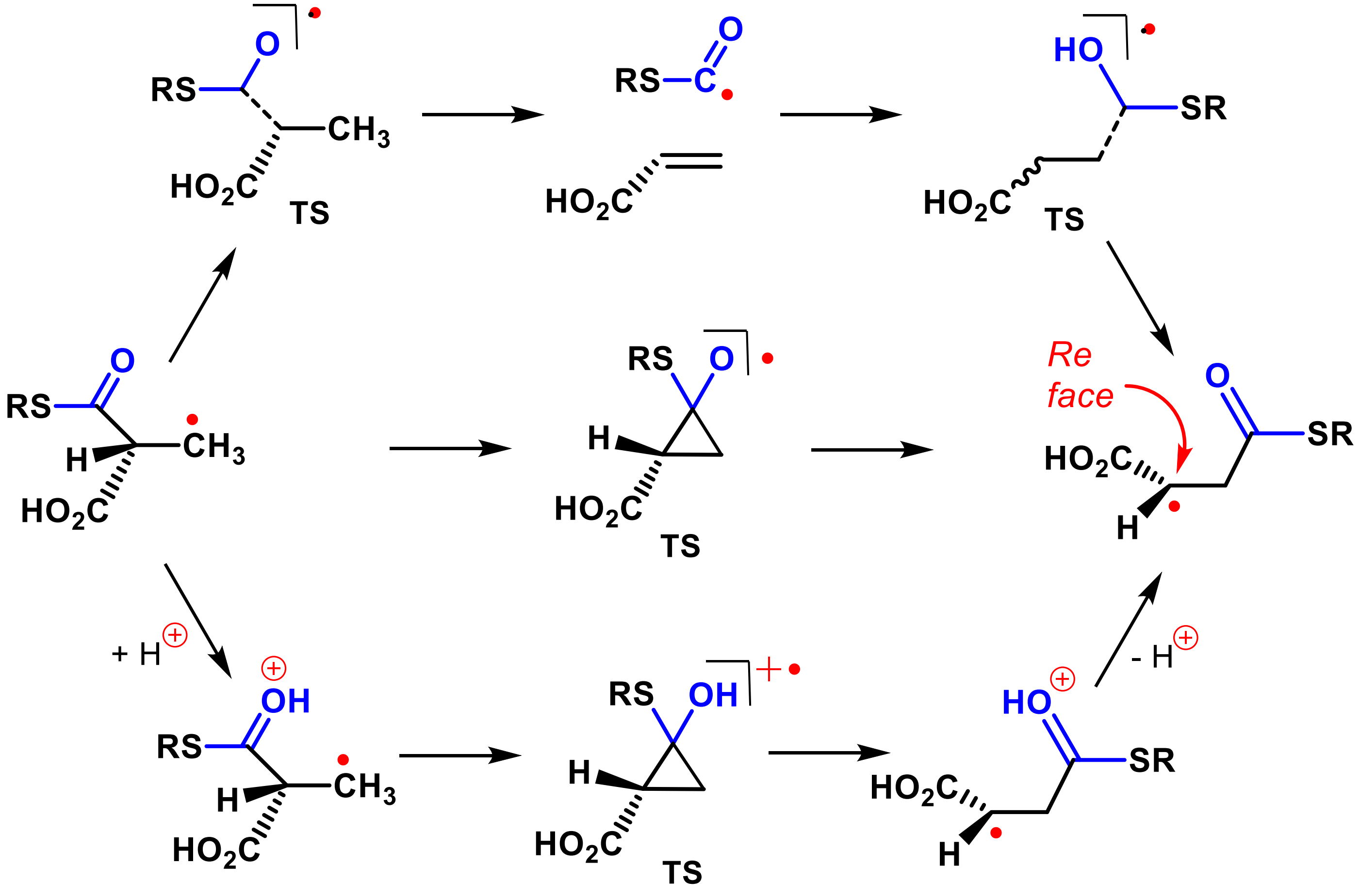
- *Addition-elimination:*



- *Addition-elimination after protonation:*



Mechanisms for the Rearrangement of the (R)-Methylmalonyl Radical to the Succinyl Radical



(RS = coenzyme A)

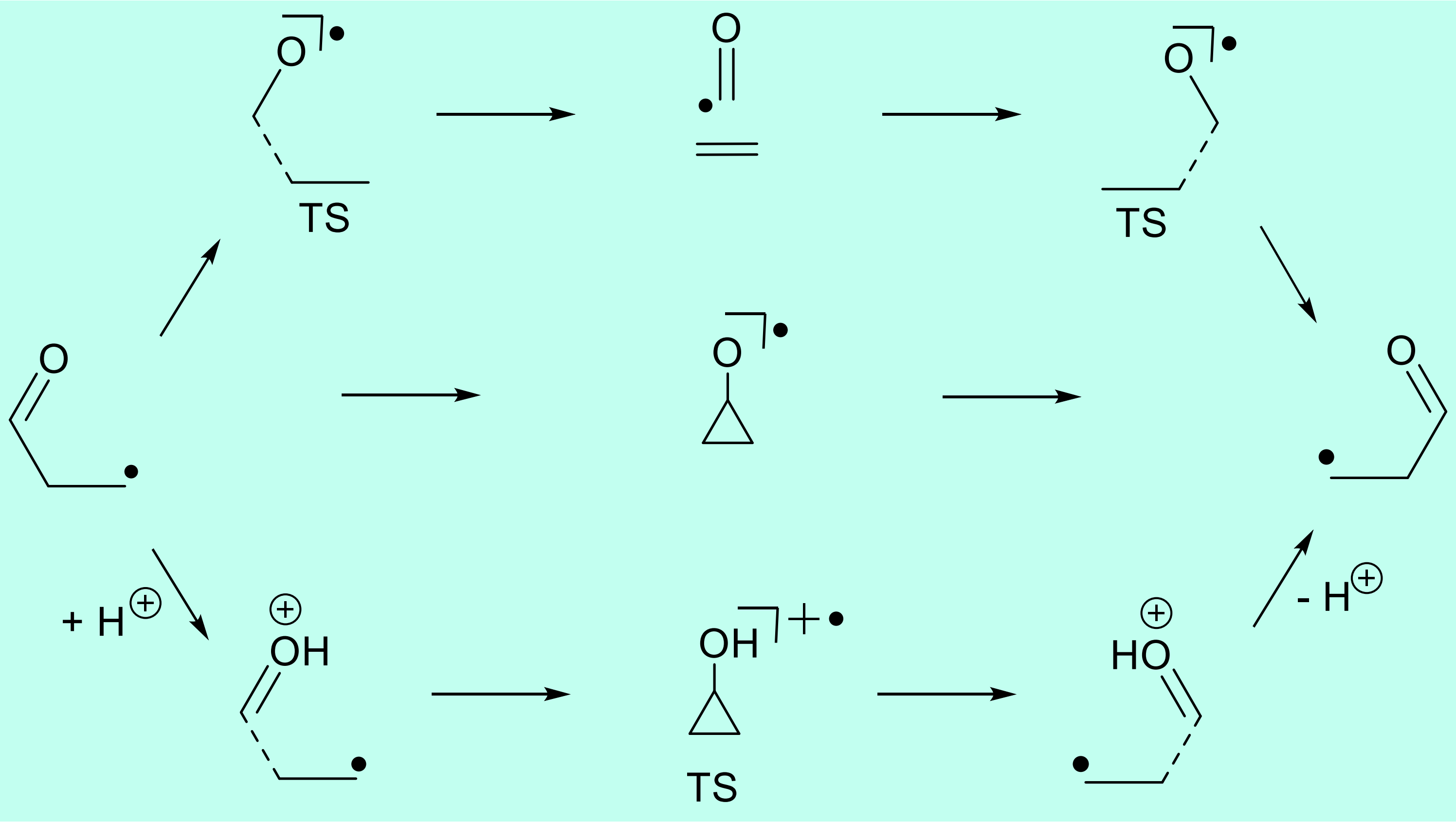
Calculation of Reaction Pathways

- *Ab initio* molecular orbital calculations were carried out on a model reaction, the degenerate rearrangement of the 3-propanal radical:

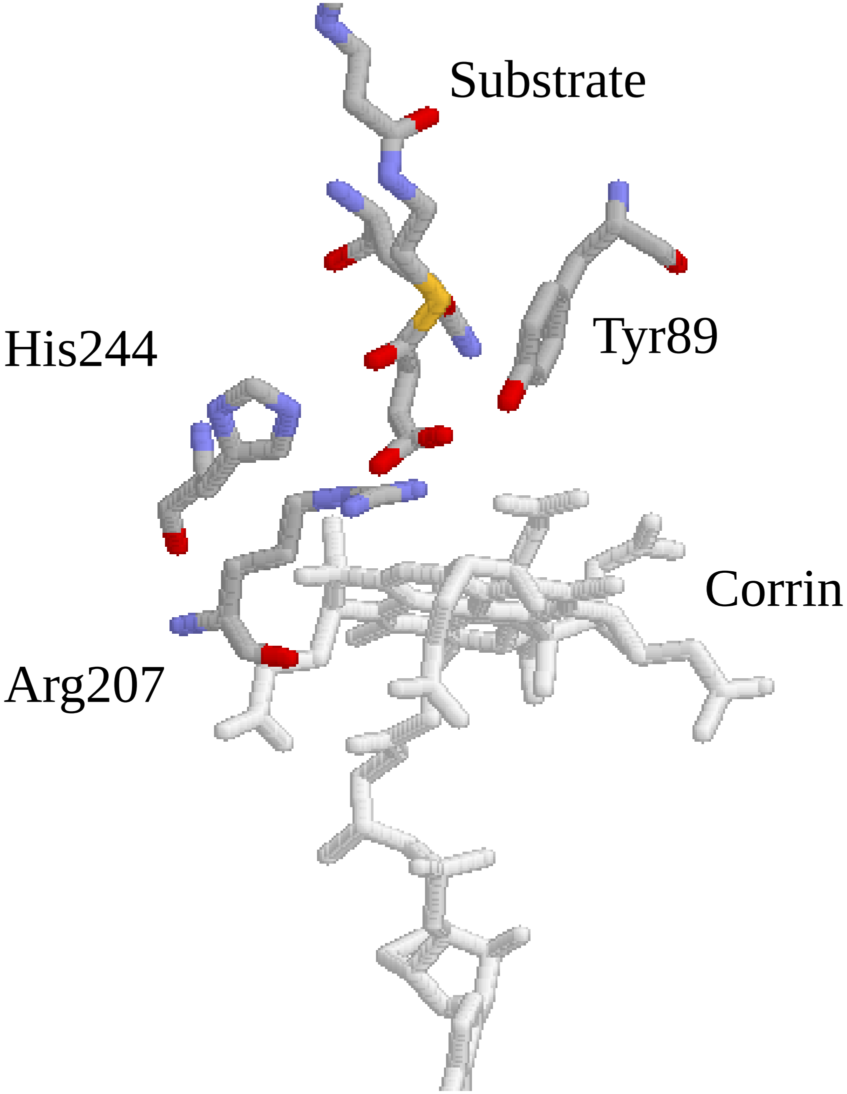
Pathway	$\Delta H^{\#}$ (kJ mol^{-1})
Fragmentation-recombination	96
Addition-elimination	47
Addition-elimination after protonation	10

D. M. Smith,, B. T. Golding, and L. Radom, *J. Am. Chem. Soc.*, 1999, **121**, 1037 and 1383

Possible Mechanisms for the Degenerate Rearrangement of the 3-Propanal Radical



Active Site of Methylmalonyl-CoA Mutase



Nearest histidine N - substrate C=O separation is 2.95 Å

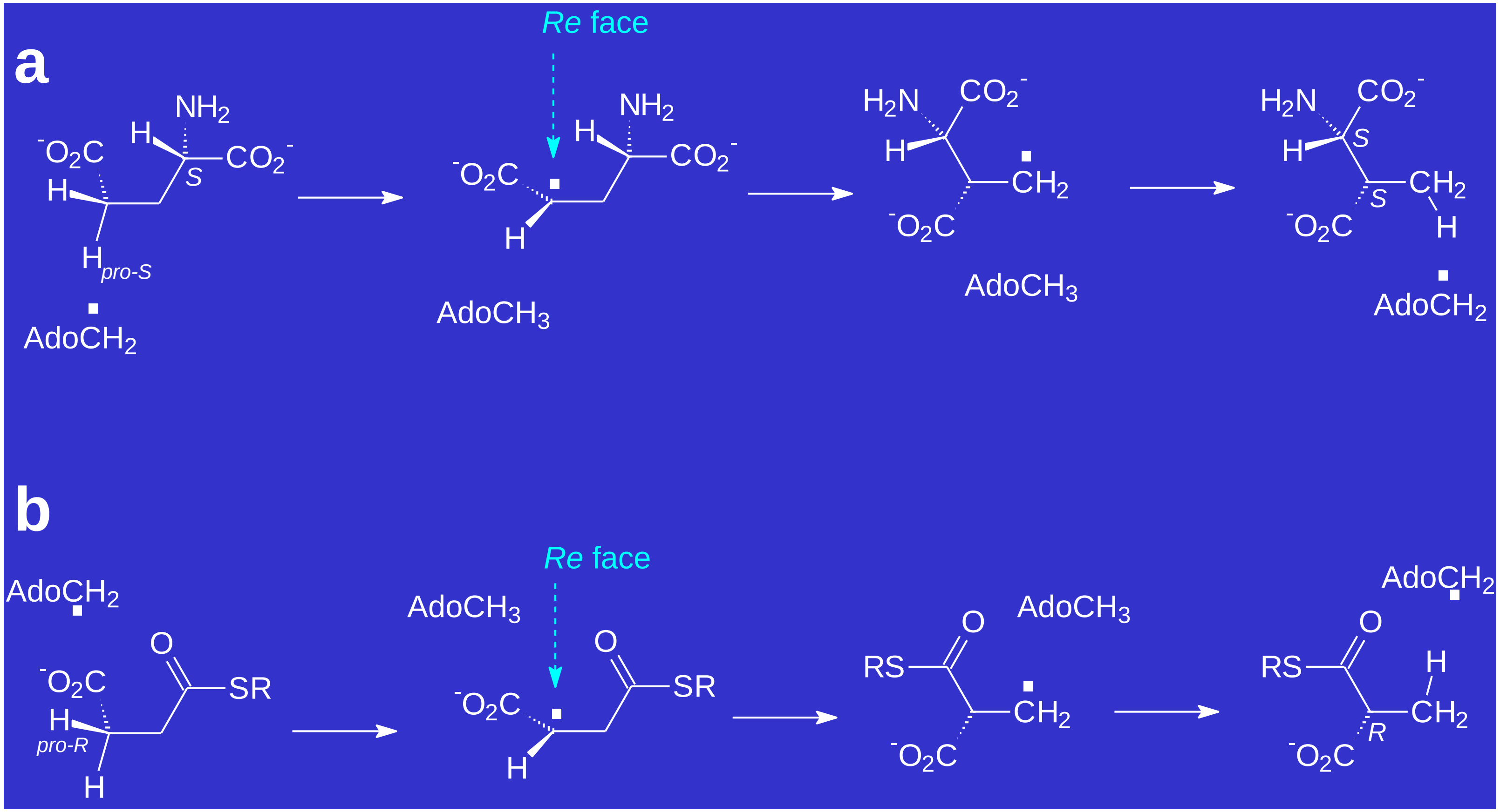
Cobalt - substrate *C*=O separation is 8.5 Å

(F Mancia and P R Evans, *Structure*, 1998, **6**, 711)

Possible Rationalisations

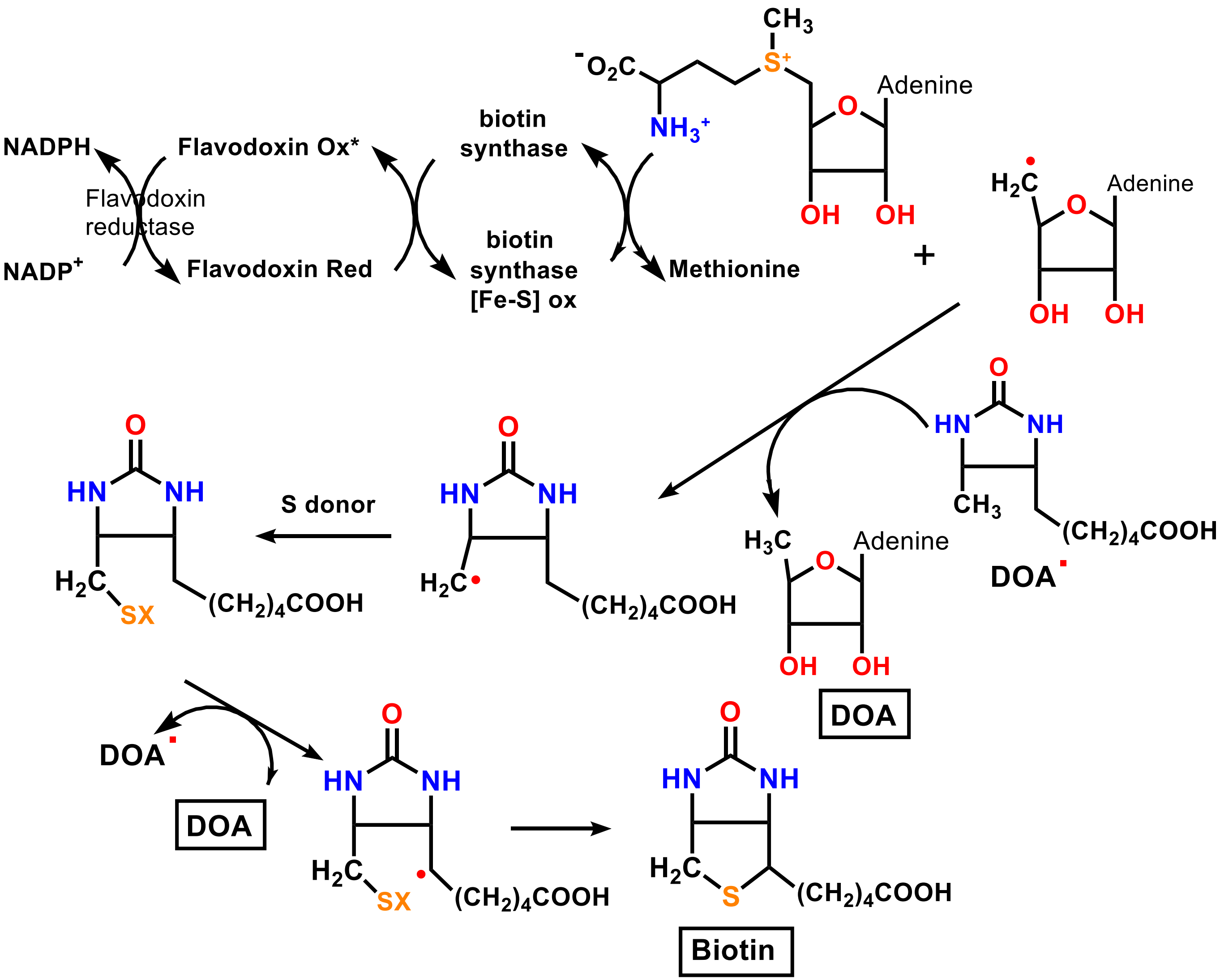
(a) the Inversion Pathway of Glutamate Mutase

(b) the Retention Pathway of Methylmalonyl-CoA Mutase



In path b, migration to the *Re* face may be blocked by deoxyadenosine.

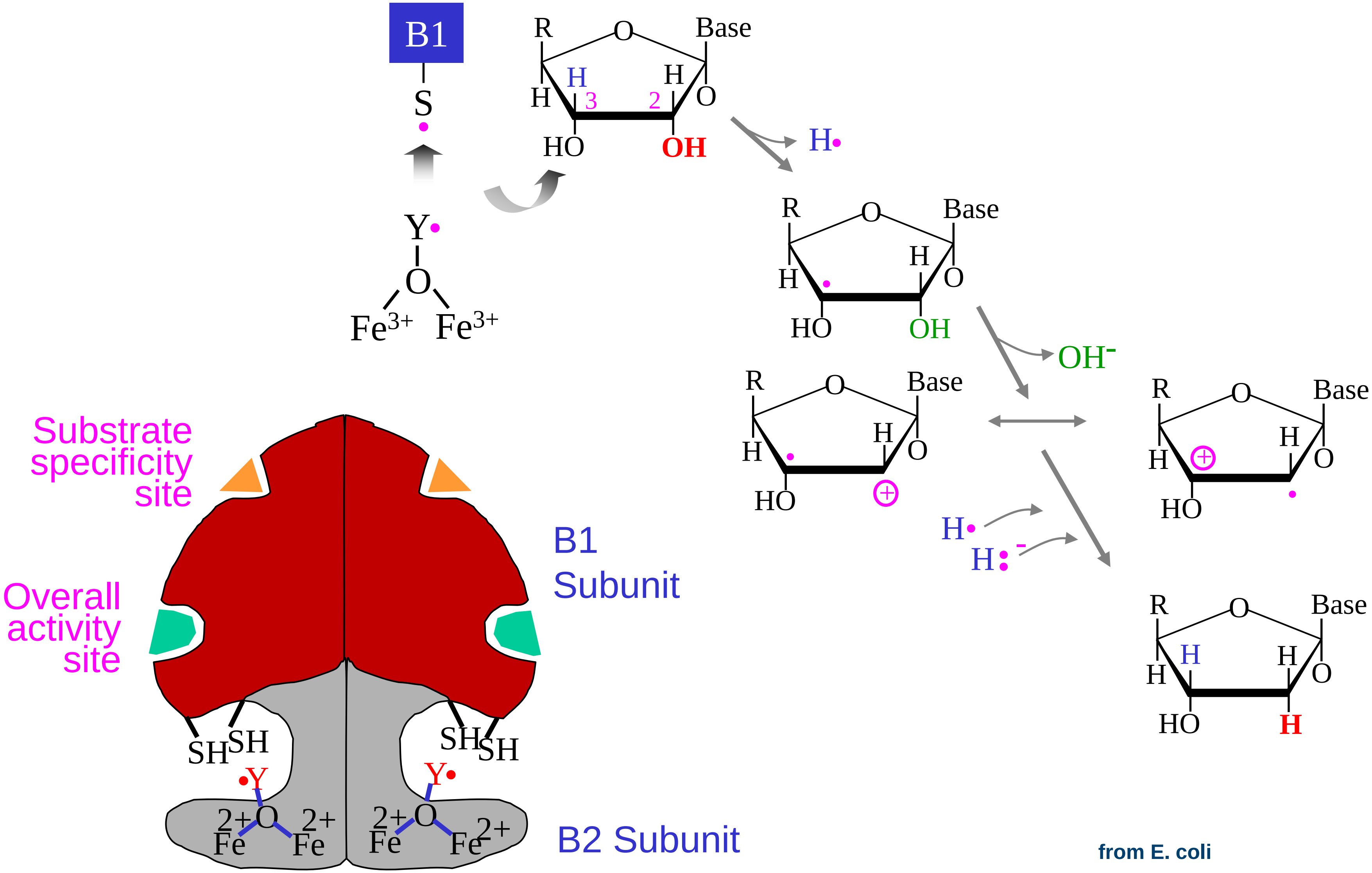
Role of S-Adenosylmethionine in the Synthesis of Biotin



Biological Radical Reactions Promoted by *S*-adenosylmethionine (AdoMet)

- Pyruvate–formate lyase (PFL), which converts pyruvate into acetyl CoA and formate;
- Anaerobic ribonucleotide reductase (ARR), which transforms nucleotides into deoxynucleotides;
- Lysine 2,3-aminomutase (LAM), which catalyses the isomerization of a- and b-lysine;
- Biotin synthase, which catalyses the synthesis of biotin

Ribonucleotide Reductase Radical Reaction



from E. coli