
Ribonucleotide Reductases

Literature

Reichard, Stubbe…

Review articles

Stubbe, J.; Van Der Donk, W. A. *Chem. Biol.* **1995**, *2*, 793.

Jordan, A.; Reichard, P. *Annu. Rev. Biochem* **1998**, *67*, 71.

Kolberg, M.; Strand, K. R.; Graff, P.; Kristoffer Andersson, K. *Biochim. Biophys. Acta* **2004**, *1699*, 1.

Nordlund, P.; Reichard, P. *Annu. Rev. Biochem* **2006**, *75*, 681.

Holmgren, A.; Sengupta, R. *Free Radical Biol. Med.* **2010**, *49*, 1617.

(Some) seminal articles

Isolation:

Reichard P, Baldesten A, Rutberg L. *J. Biol. Chem.* **1961**, *236*, 1150.

Radical enzyme:

Ehrenberg A, Reichard P. *J. Biol. Chem.* **1972**, *247*, 3485.

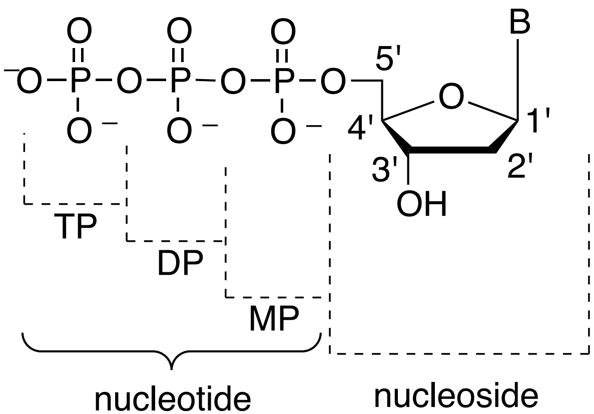
Reichard, P.; Ehrenberg, A. *Science* **1983**, *221*, 514

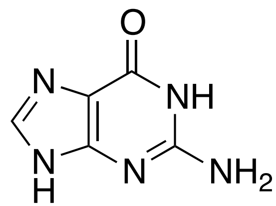
X-ray structures:

Uhlin, U.; Eklund, H. *Nature* **1994**, *370*, 533.

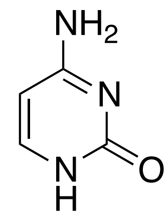
Nordlund, P.; Eklund, H. *J. Mol. Biol.* **1993**, *232*, 123.

Nucleobases

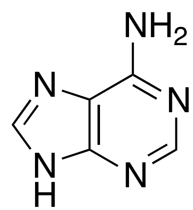




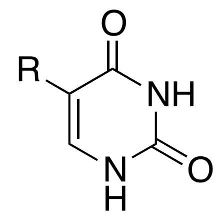
Guanine



Cytosine

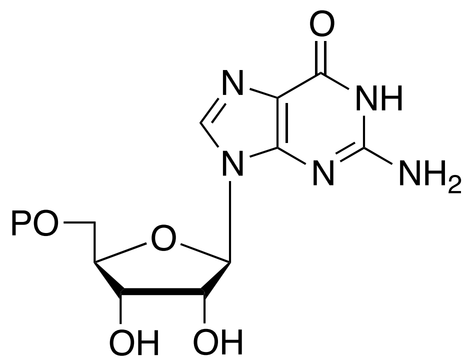


Adenine

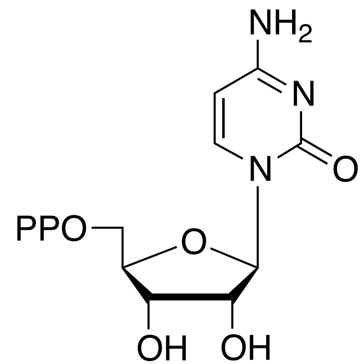


Thymine (R= CH_3)
Uracil (R= H)

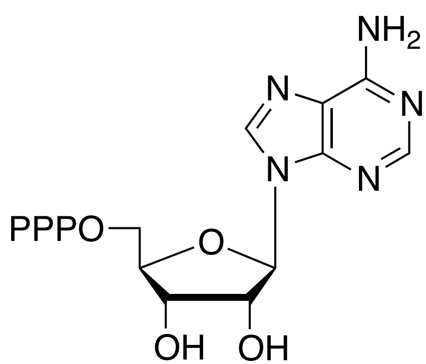
Nucleotides



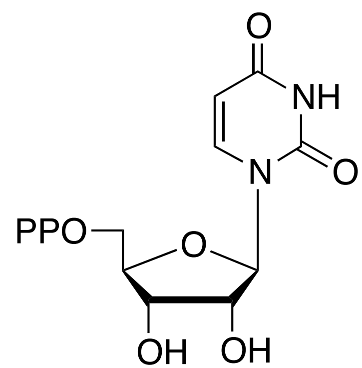
Guanosine monophosphate
(GMP)



Cytidine diphosphate
(CDP)

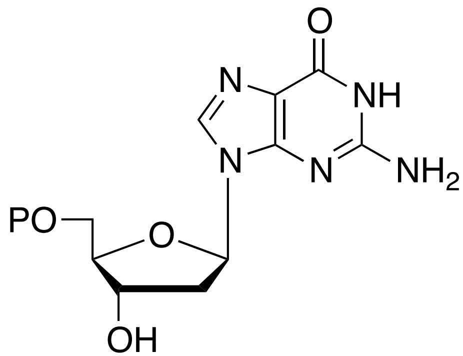


Adenosine triphosphate
(ATP)

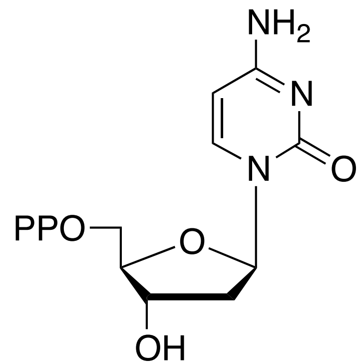


Uridine diphosphate
(UDP)

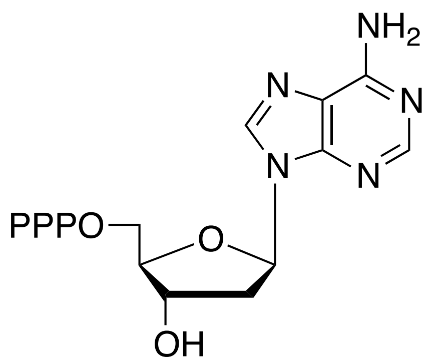
Nucleotides



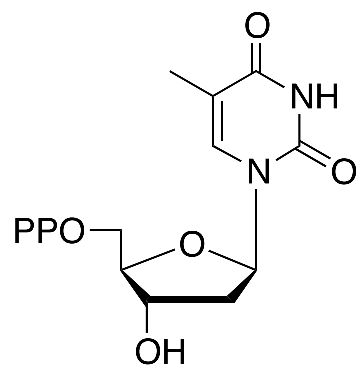
Deoxyguanosine monophosphate
(dGMP)



Deoxycytidine diphosphate
(dCDP)

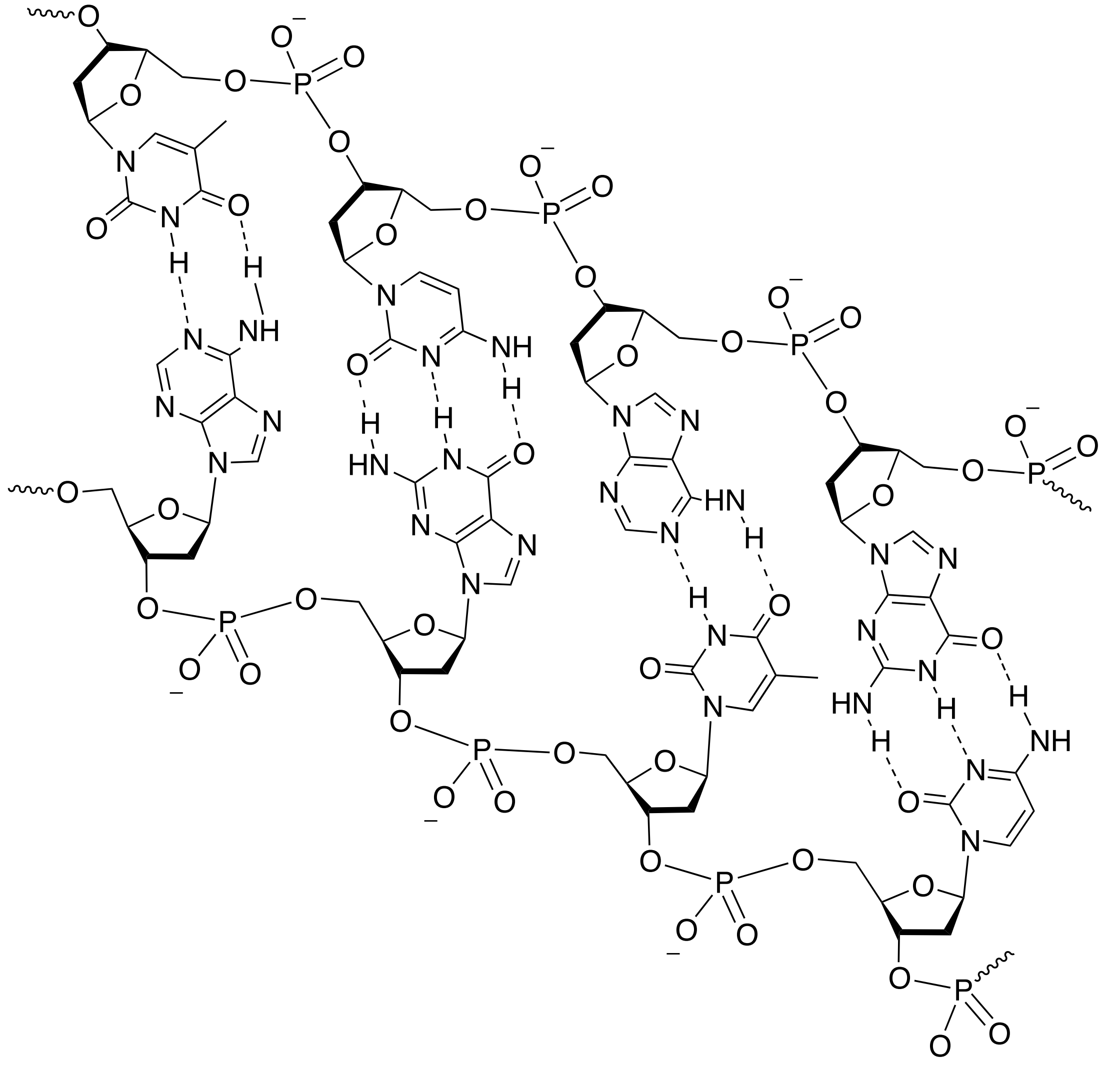


Deoxyadenosine triphosphate
(dATP)

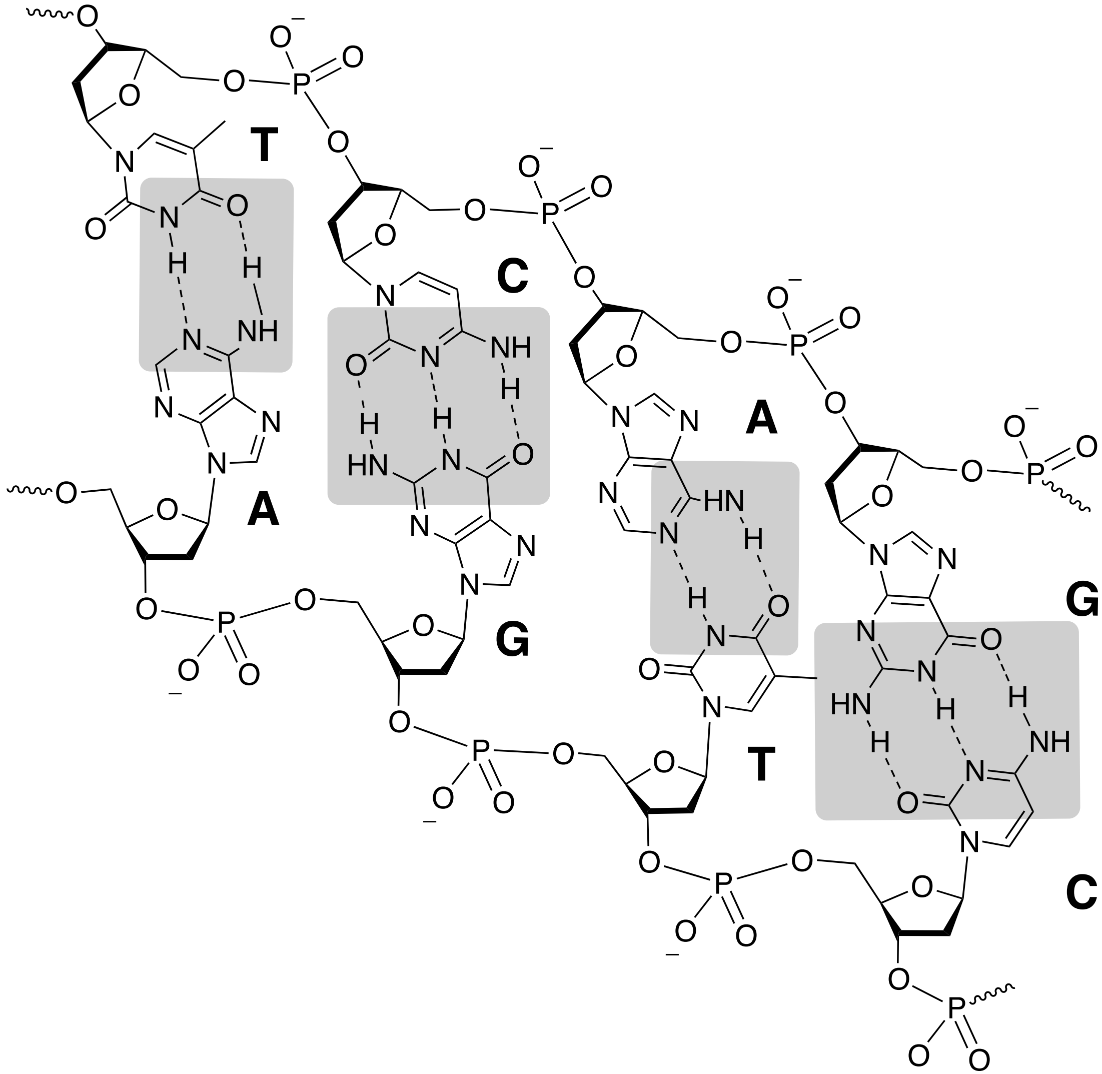


Deoxythymidine diphosphate
(dTDP)

Double Strand DNA

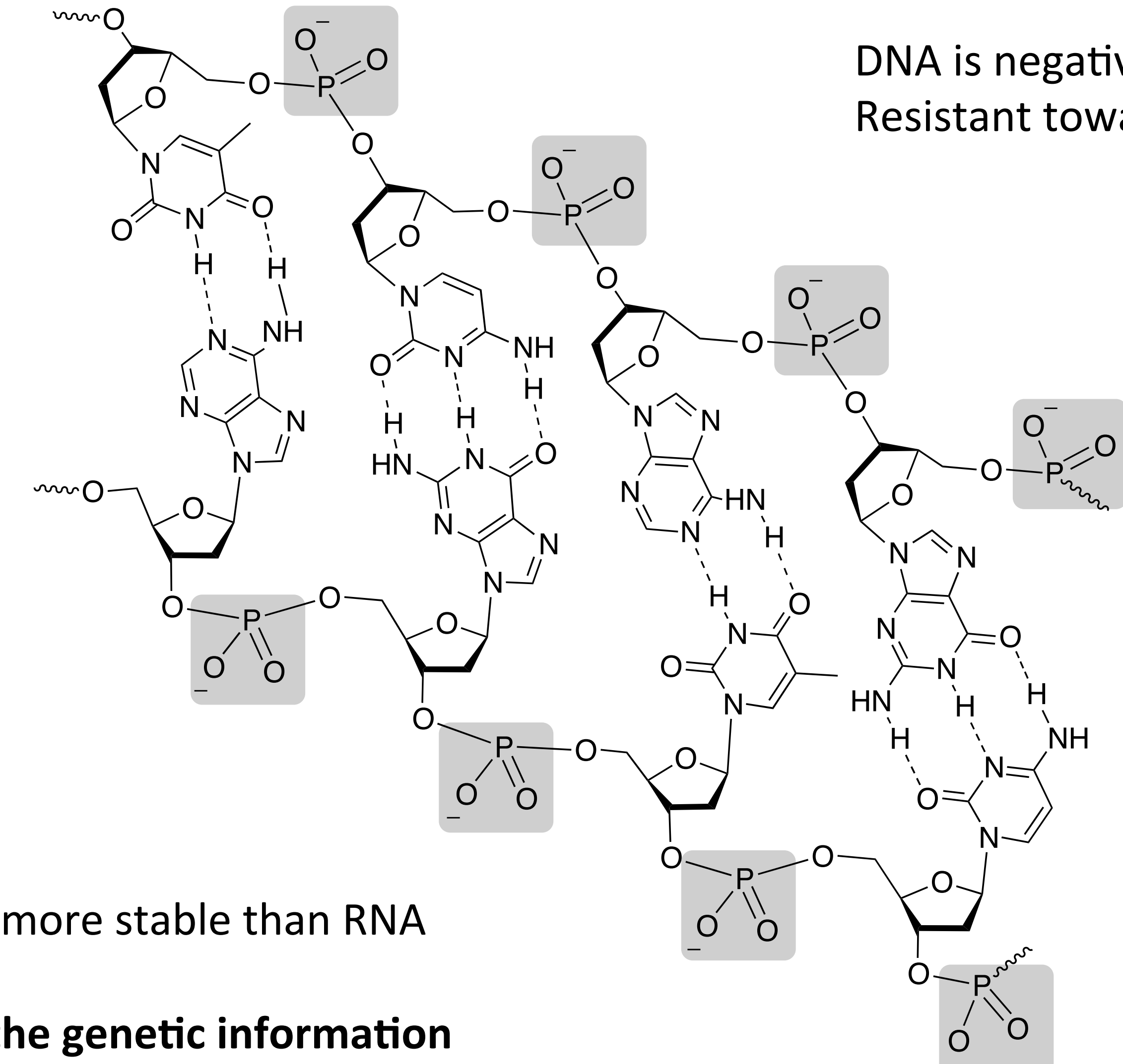


Double Strand DNA



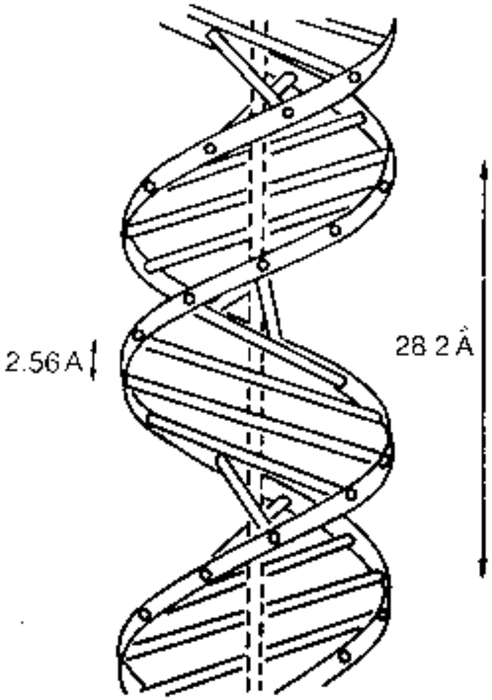
Double Strand DNA

DNA is negatively charged (pH 7)
Resistant toward basic hydrolysis

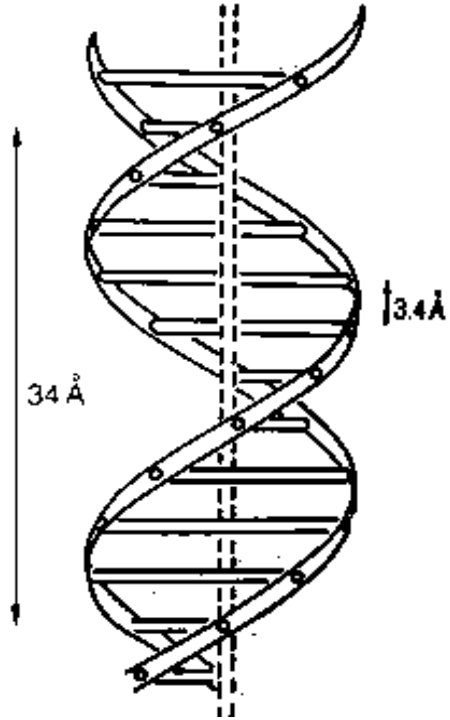


DNA About 100 fold more stable than RNA toward hydrolysis
→ **Conservation of the genetic information**

Nucleotides



A-form



B-form

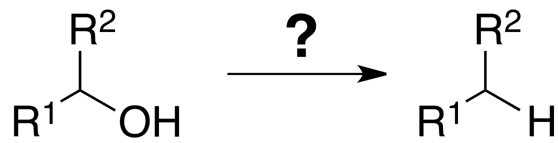
4-OH group lock the conformation of the ribose

RNA: only A-form helix

DNA: generally B-form helix (A-form possible)

DNA duplex (slightly) less stable than RNA. DNA more flexible

Deoxygenation Reactions



<u>Chemically</u>

1) Barton McCombie
2) MsCl, $LiEt_3BH$
3) DEAD, PPh_3, $H_2N–NHSO_2(2-(NO_2)C_6H_4)$
   J. Am. Chem. Soc. **1997,** *119,* 8572-8573

….

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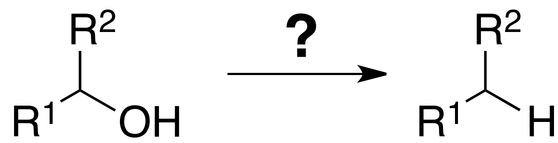

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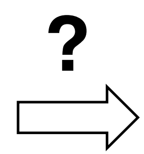
….

<u>Under physiological conditions?</u>

deoxynucleotide 

Ribonucleotide Reductases (RNRs)

1961 Isolation from *E. Coli* of an enzyme that catalyses the transformation of nucleosides diphosphates (NDP) into deoxynucleosides diphosphates (dNDP)

1972 The enzyme contains a stable tyrosyl radical bounded to a dinuclear iron center (still present after the two weeks required for purification)
→ **First radical enzyme**.

Other RNRs were isolated: three classes

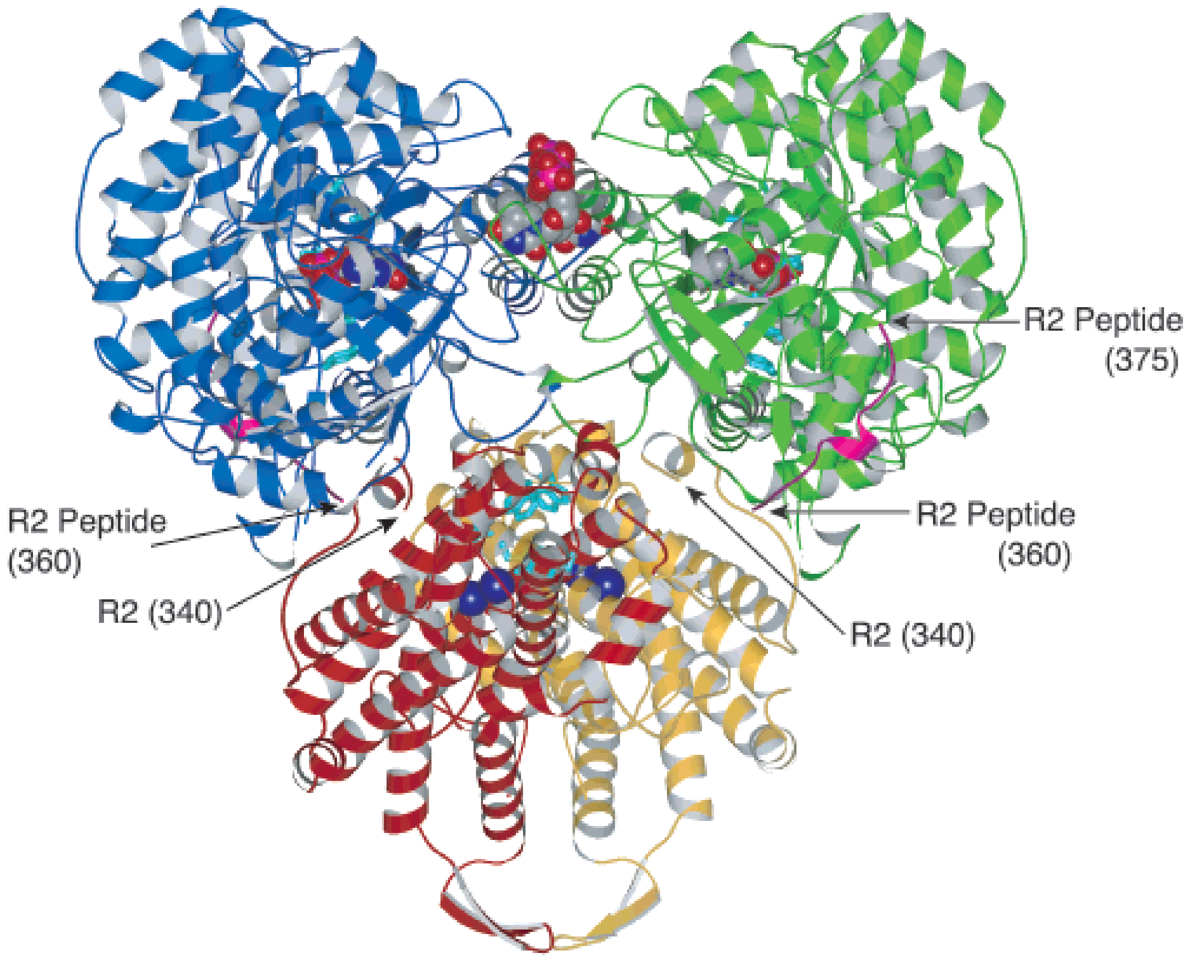
Class I RNR from *E. coli* is the most studied

(class I in all eukariotes, *e.g.* mammal cells)

Composed of two homodimeric subunits ($\alpha_2\beta_2$ or R1 and R2)
X-ray structures for each subunits were obtained

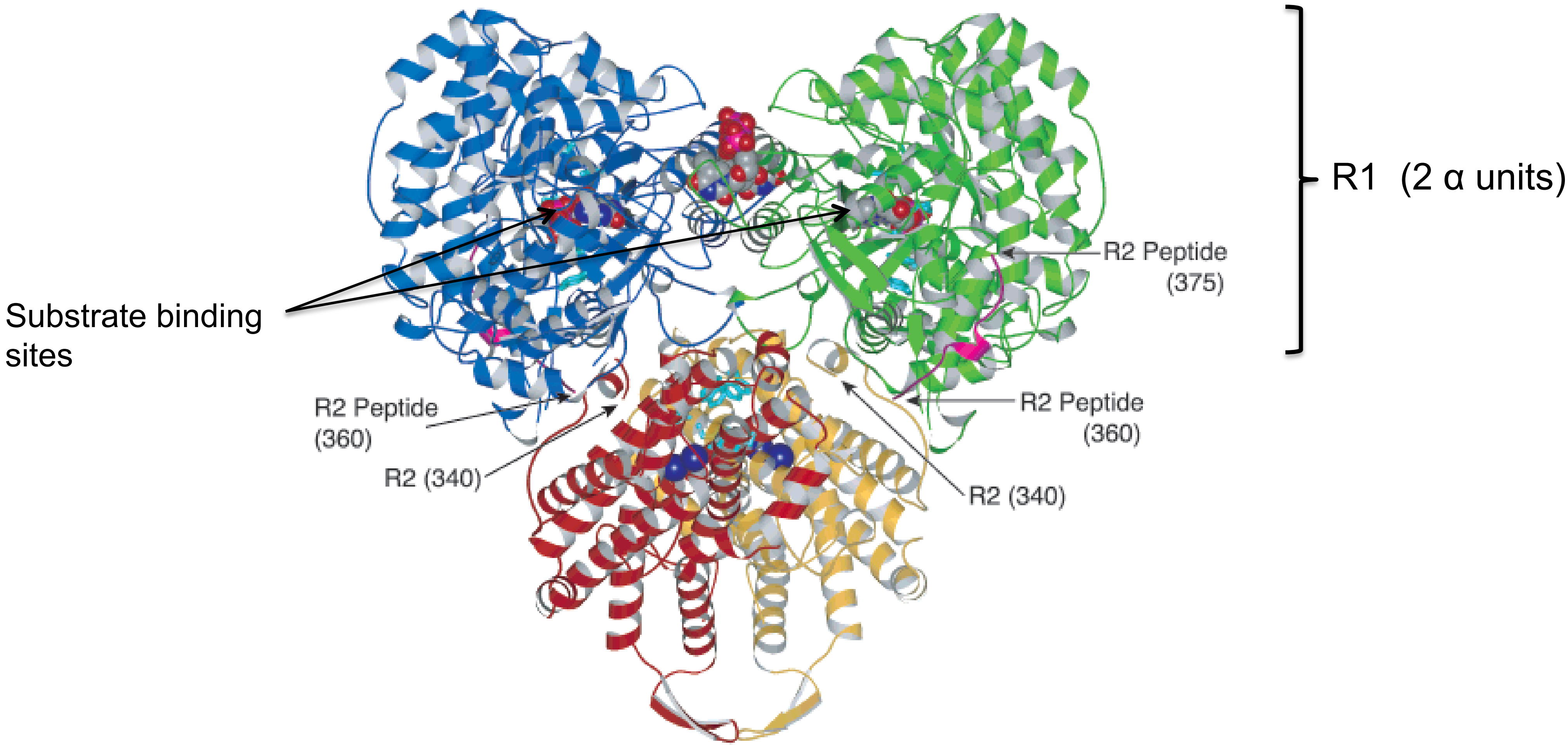
Strictly aerobic: oxygen dependent formation of the tyrosyl radical

E. Coli's RNR Quaternary Structure



Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y. *Chem. Rev.* **2003**, *103*, 2167.

E. Coli RNR Quaternary Structure



Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y. *Chem. Rev.* **2003**, *103*, 2167.

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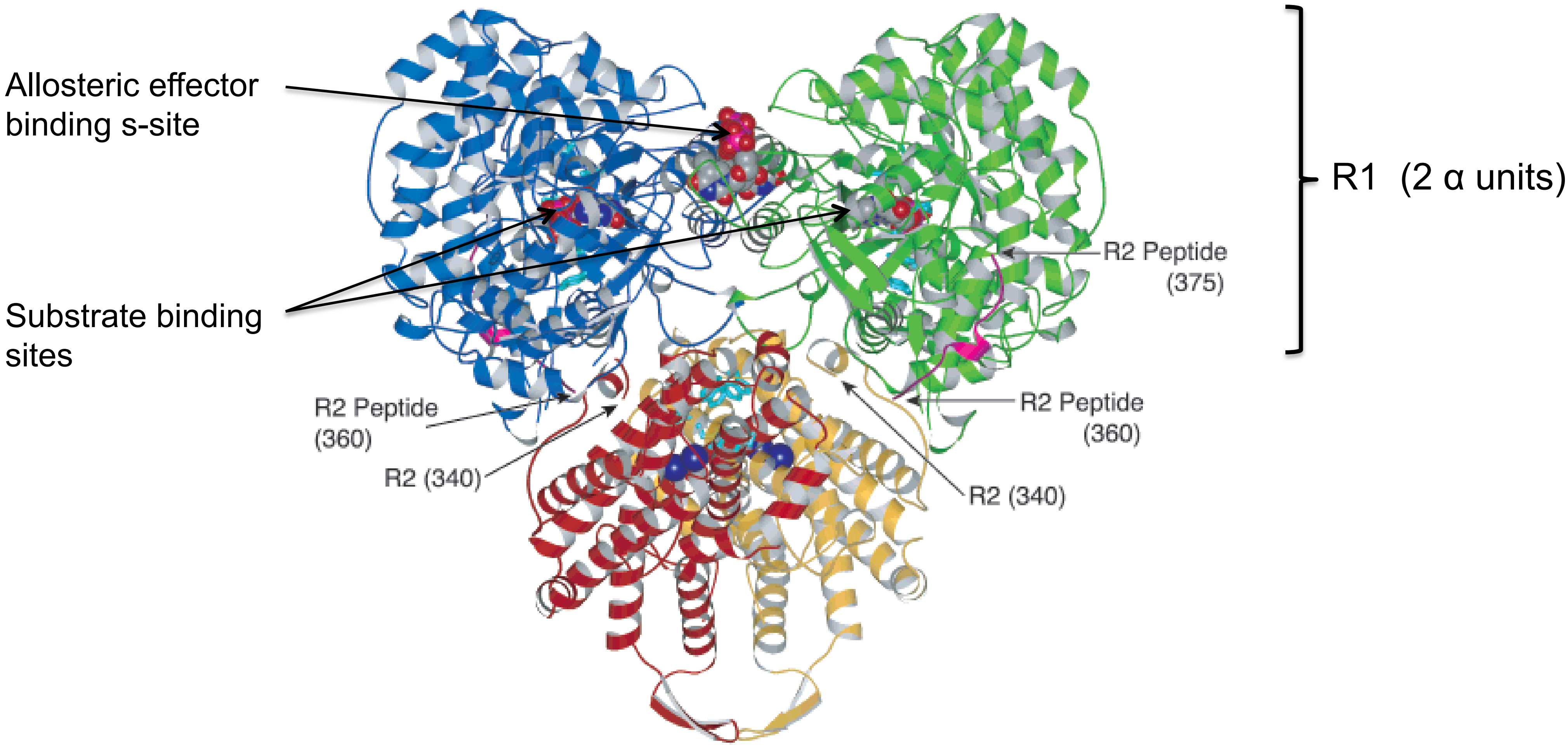


Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y. *Chem. Rev.* **2003**, *103*, 2167.

Allosteric Effectors

<u>General:</u> molecule that binds an enzyme and modifies its activity (not the substrate)

<u>For RNRs</u>

s-site: The allosteric specificity-site can bind ATP, dATP, dTTP, and dGTP
Modifies the structure of the protein thereby the substrate's affinity

ATP and dATP stimulate the reduction of CDP and UDP
dTTP and dGTP stimulate GDP and ADP reduction, respectively

A → C (U)
T → G
G → A

a-site: activity-site
ATP binding stimulates the activity
dATP inhibits the activity (may disturb the long range PCET)

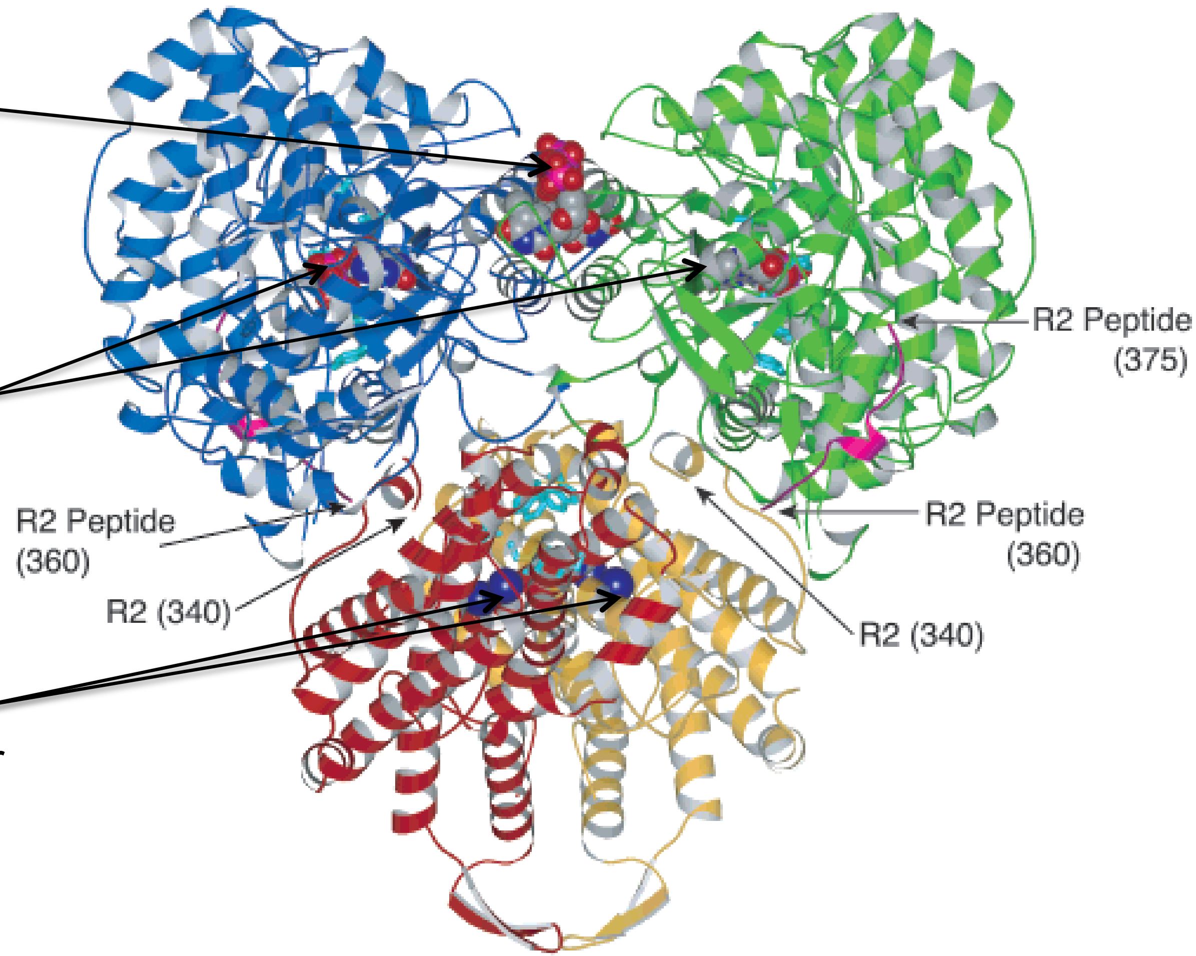
Nordlund, P.; Reichard, P. *Annu. Rev. Biochem* **2006**, *75*, 681.

E. Coli RNR Quaternary Structure

Allosteric effector binding s-site

Substrate binding sites

Tyrosyl radical dinuclear iron center



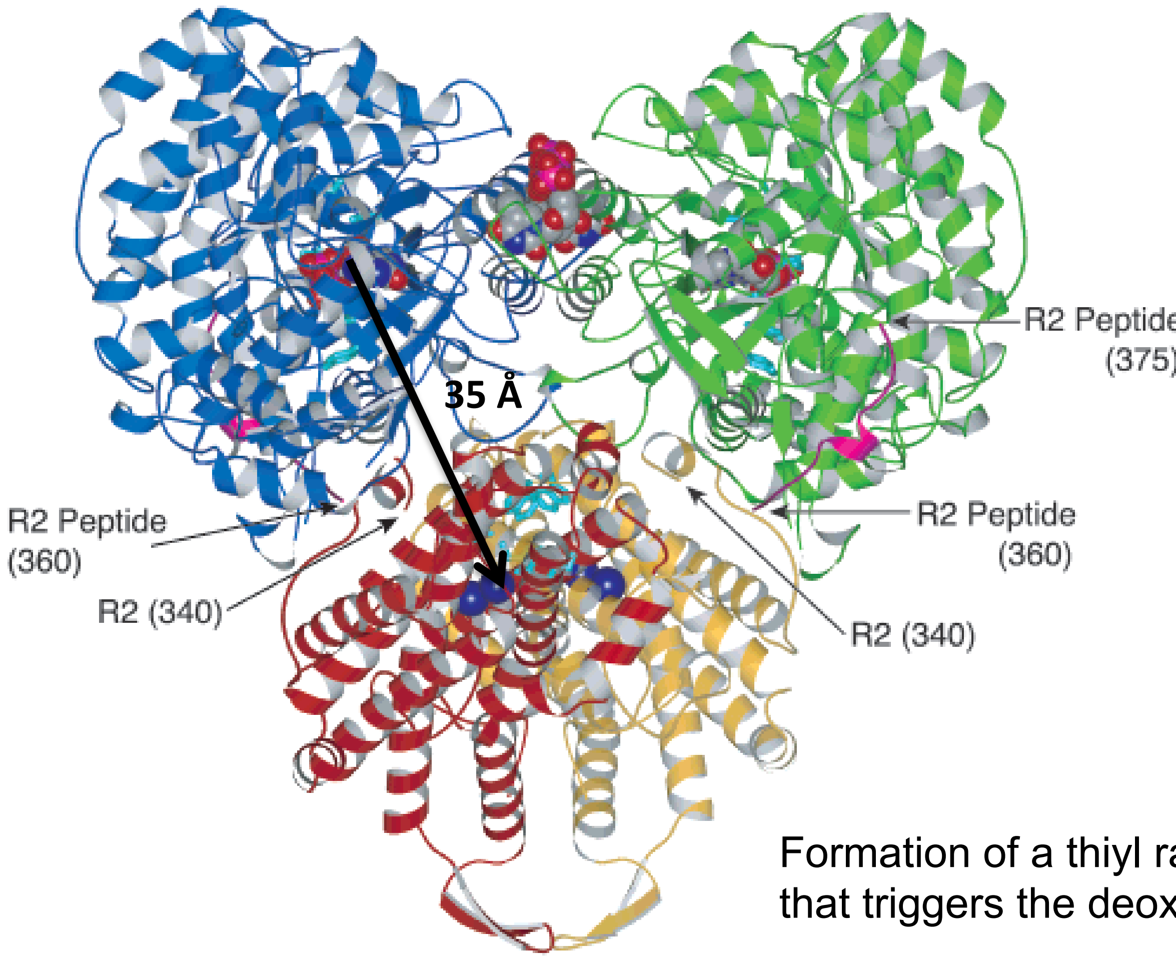
R1 (2 α units)

R2 (2 β units)

Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y. *Chem. Rev.* **2003**, *103*, 2167.

Initiation: Long Range PCET

Proton coupled electron transfer (PCET) from a cysteine S–H bond (R1) to the tyrosyl radical (R2)



Formation of a thiyl radical in the active site that triggers the deoxygenation process

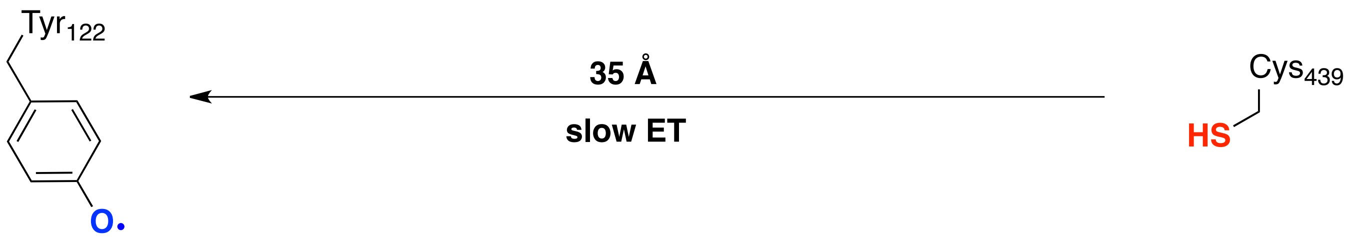
Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y. *Chem. Rev.* **2003**, *103*, 2167.

How does an Electron Flies Over 35 Å?

Rate of electron superexchange (or tunneling) can be described by Marcus theory.

$k_{ET} = k_0 – \exp(\beta d)$
Over 35 Å, $k_{ET} < 1\ s^{-1}$

β: transmission coefficient ($Å^{-1}$)
d: distance between donor and acceptor (Å)



Cordes, M.; Giese, B. *Chem. Soc. Rev.* **2009**, *38*, 892.
Gray, H. B.; Winkler, J. R. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3534.

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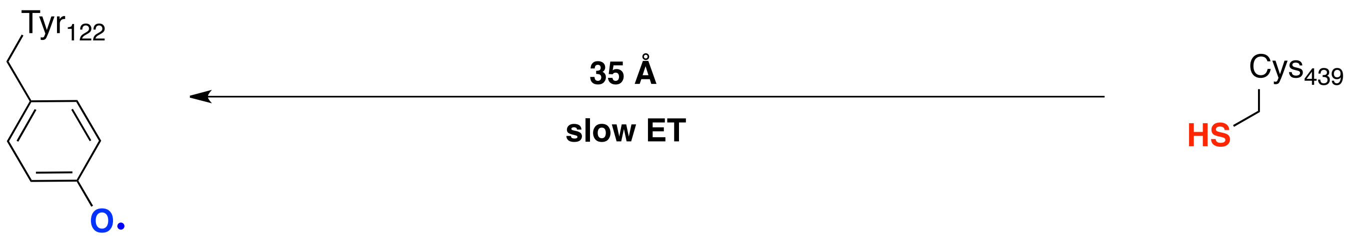
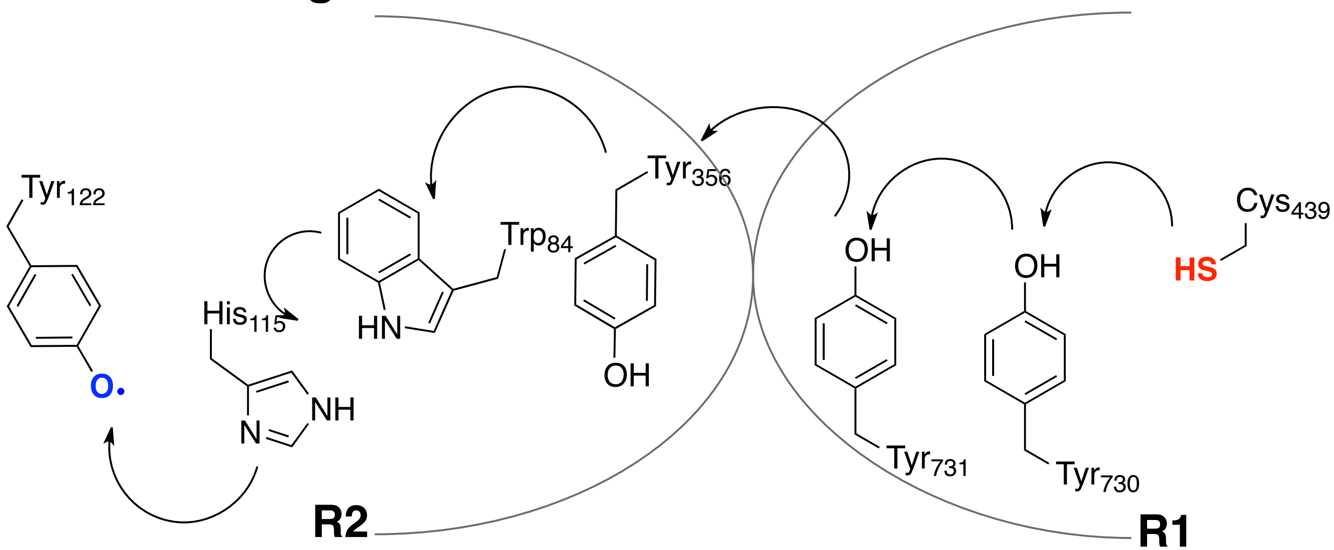


Hopping mechanism through amino acids aromatic side chains



Cordes, M.; Giese, B. *Chem. Soc. Rev.* **2009**, *38*, 892.
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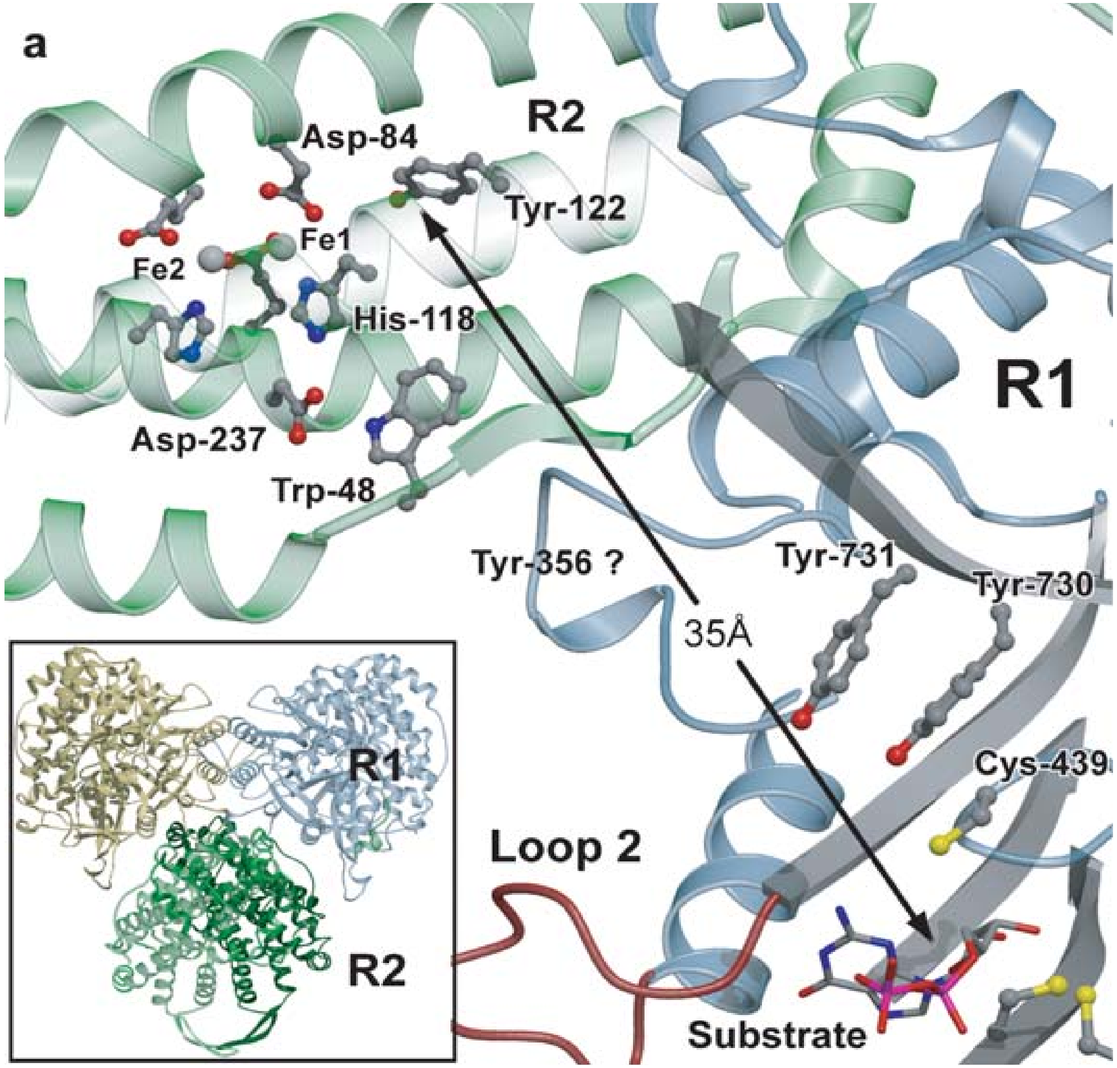
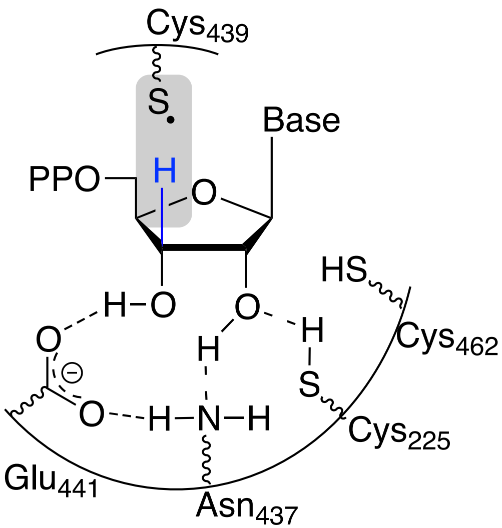


Nordlund, P.; Reichard, P. *Annu. Rev. Biochem* **2006**, *75*, 681.

Mechanism of the Deoxygenation



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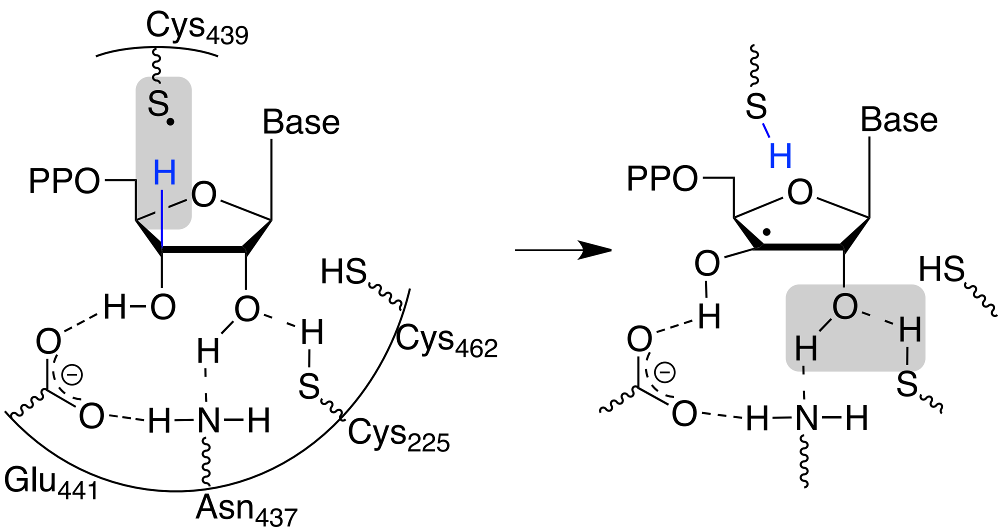
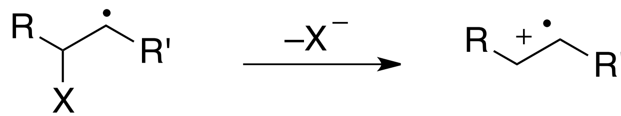


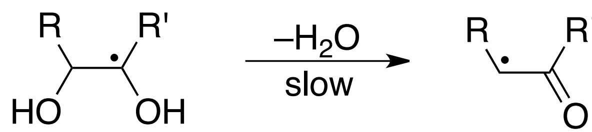
BDE C–H > BDE S–H

→ Endothermic reaction (thermodynamic equilibrium on the side of the S centred radical)

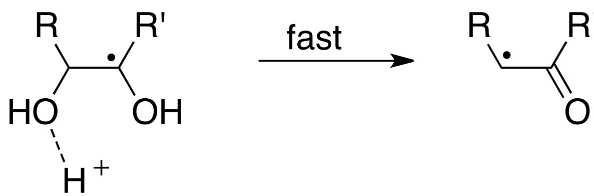
Fragmentation

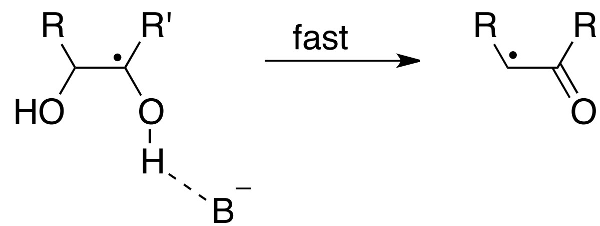


X =O_2CR, OP(O)$(OR)_2$



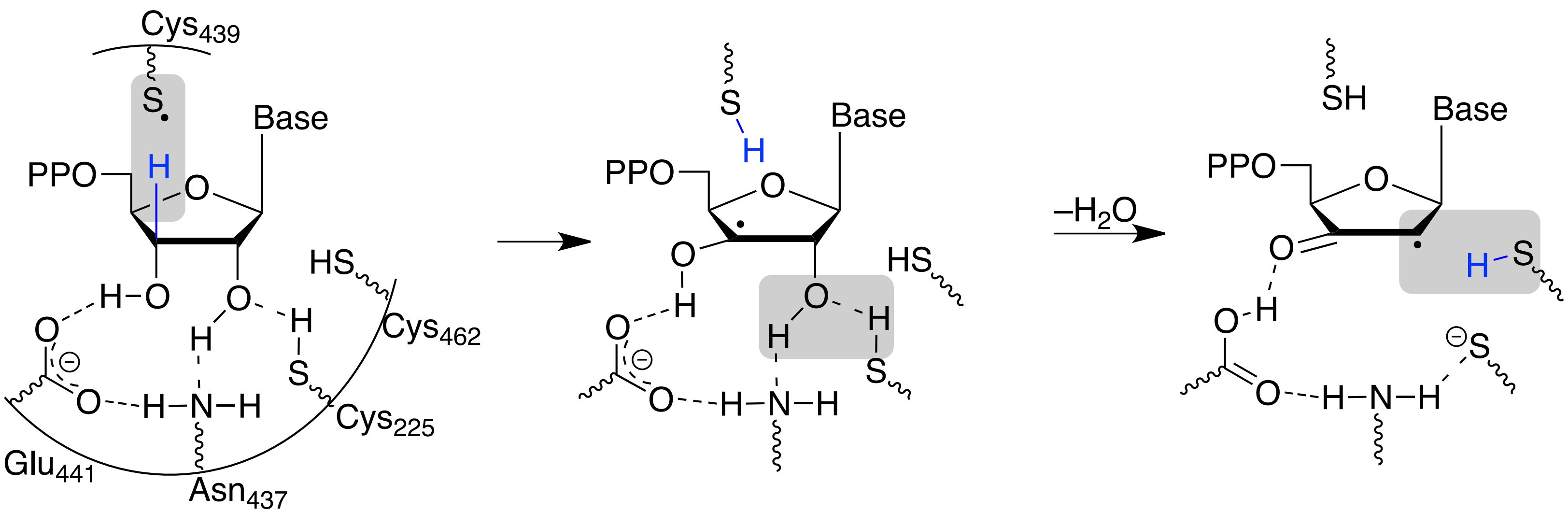
Acid or base catalysis favors the elimination





Beckwith, A. L. J.; Crich, D.; Duggan, P. J.; Yao, Q.; *Chem. Rev*. **1997**, *97*, 3273.
Lenz, R.; Giese, B. *J. Am. Chem. Soc.* **1997**, *119*, 2784.

Mechanism of the Deoxygenation

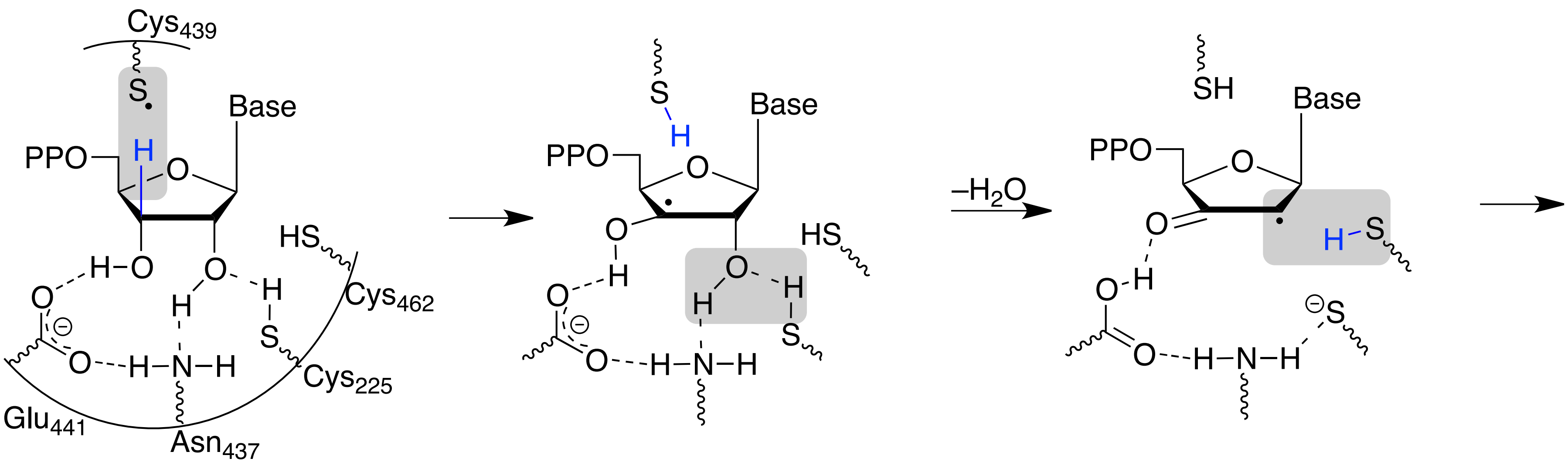


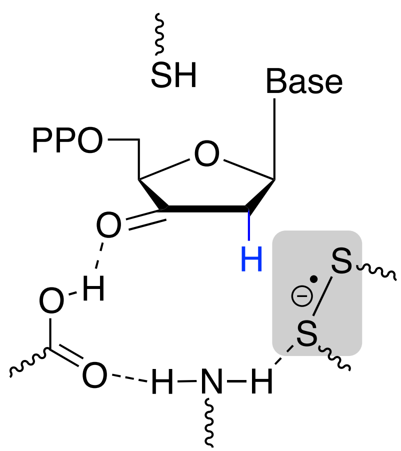
BDE C–H > BDE S–H

→ Endothermic reaction (thermodynamic equilibrium on the side of the S-centred radical)

→ Process driven by the rapid (irreversible) elimination of H_2O

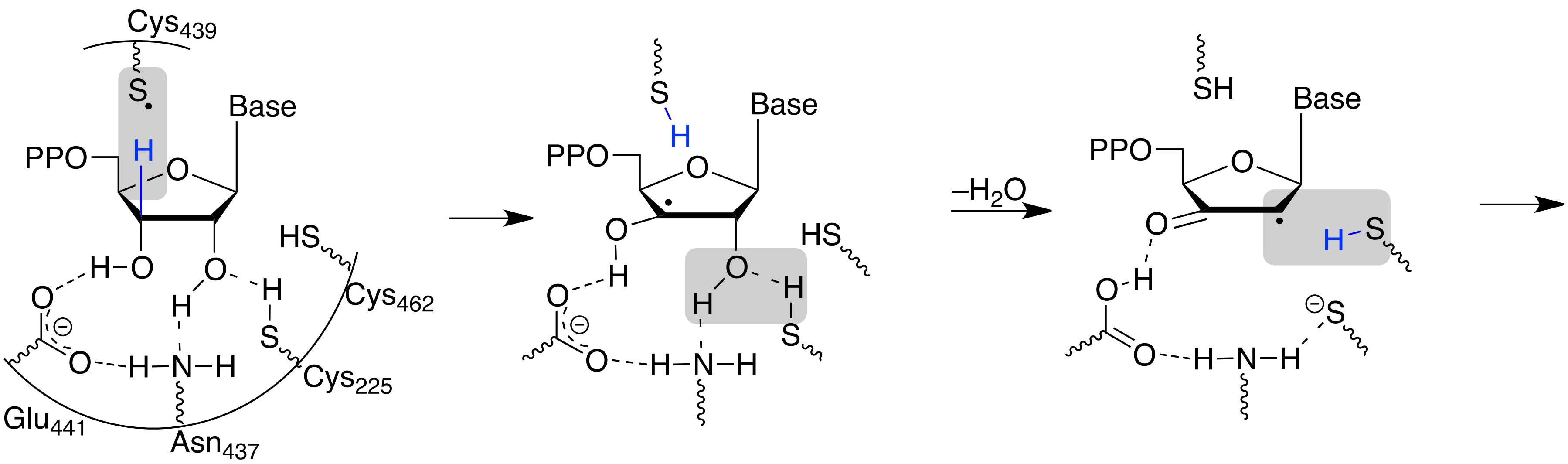
Mechanism of the Deoxygenation

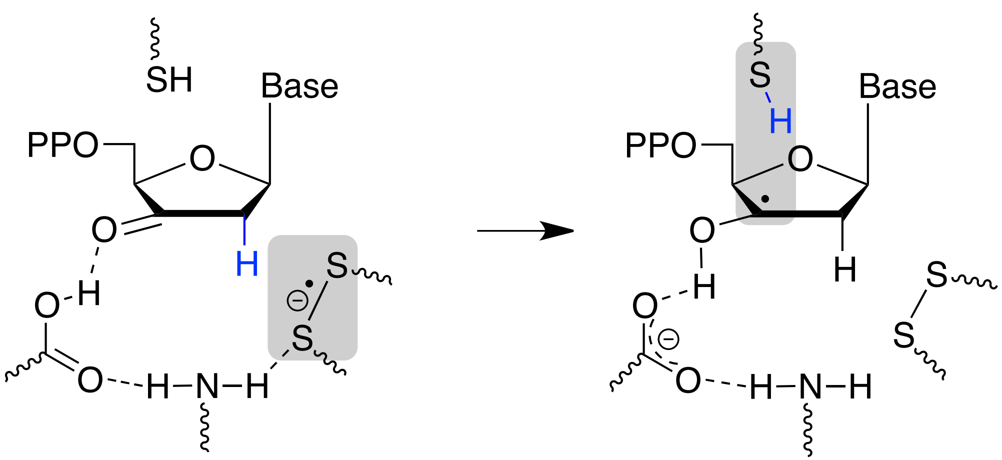




Protonation of C=O by Glu 441 increases its oxidation potential.

Mechanism of the Deoxygenation

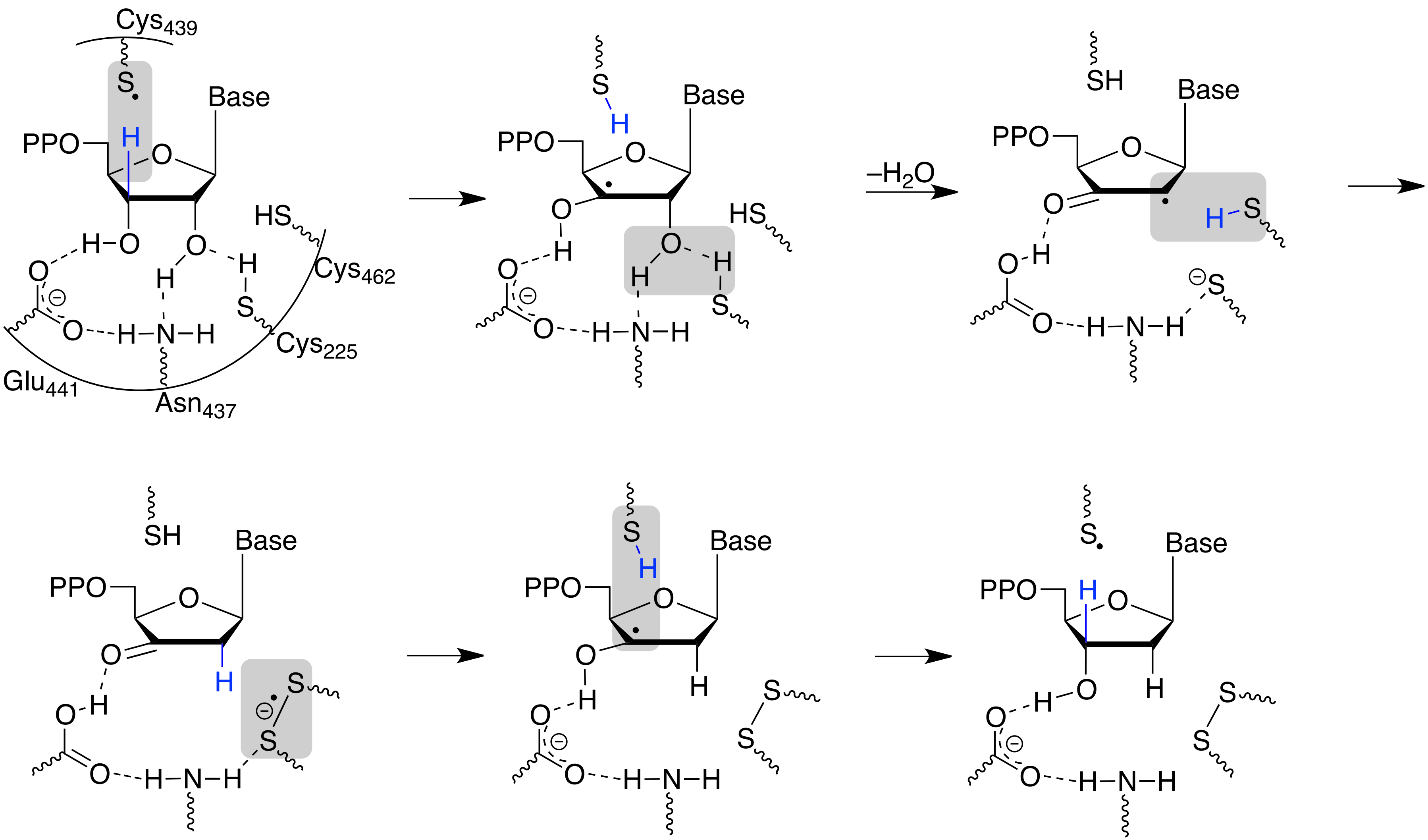




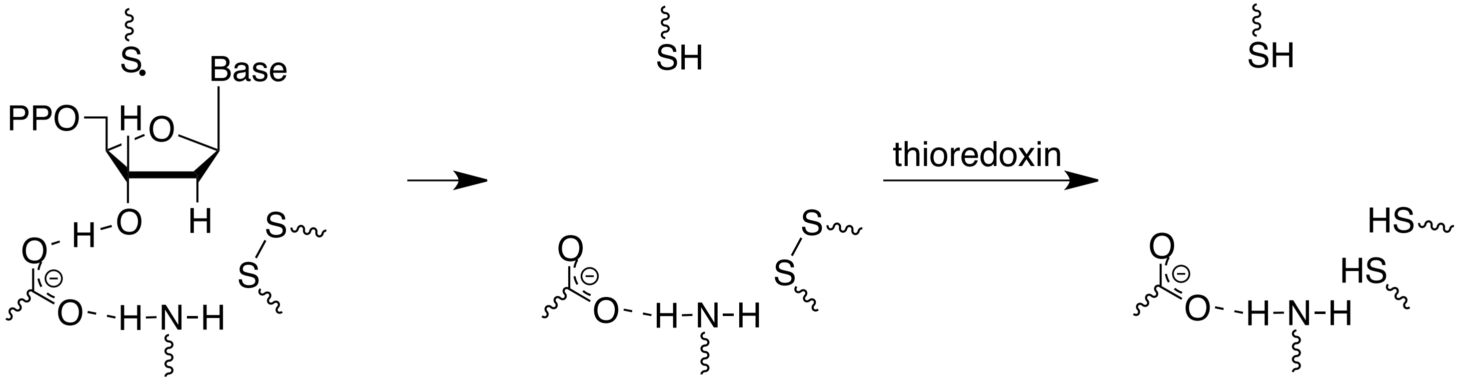
Protonation of C=O by Glu 441 increases its oxidation potential.

PCET through H-bond (role of H_2O molecule?)

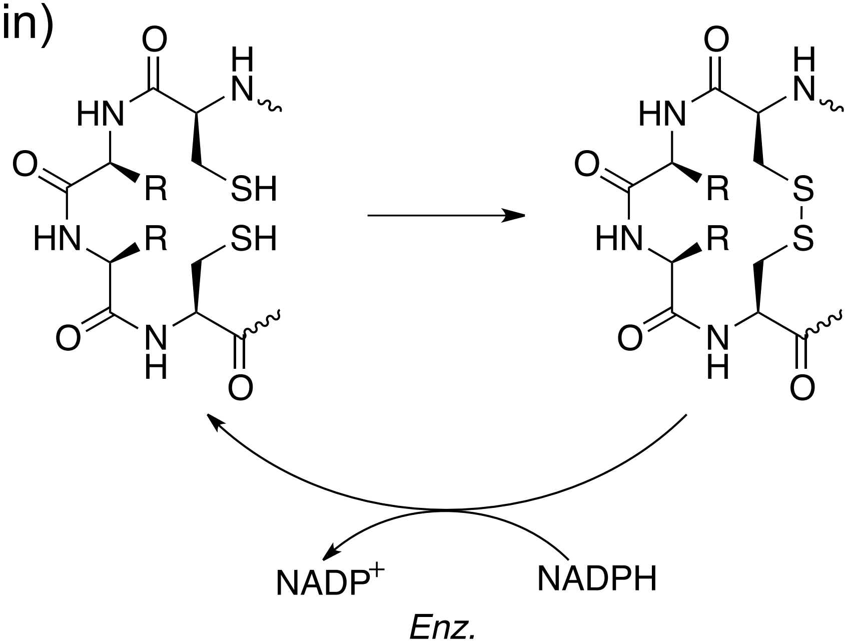
Mechanism of the Deoxygenation



Reduction of the Disulfide Bridge



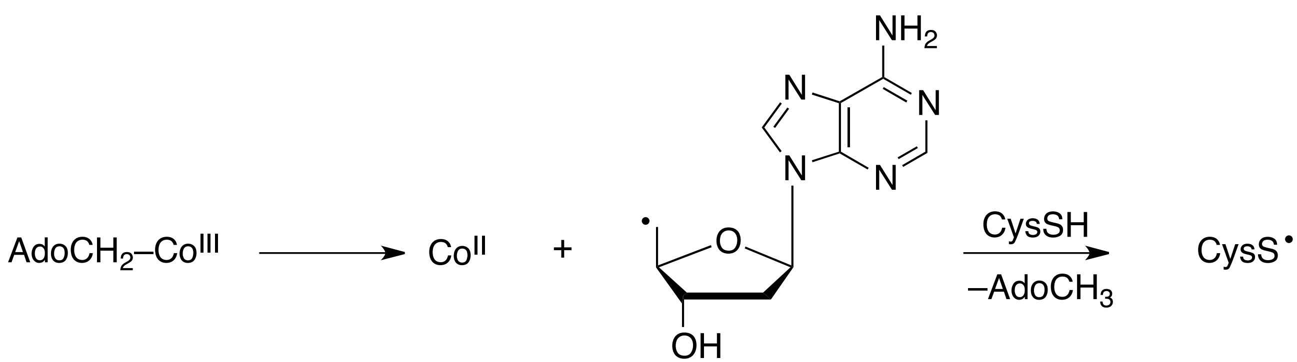
Thioredoxin (12 kDa protein)



Class II RNRs

Aerobic or anaerobic enzyme, one subunit
Mechanism similar to class I RNRs
(same amino acid residues in the active site, thioredoxin as external reductant)

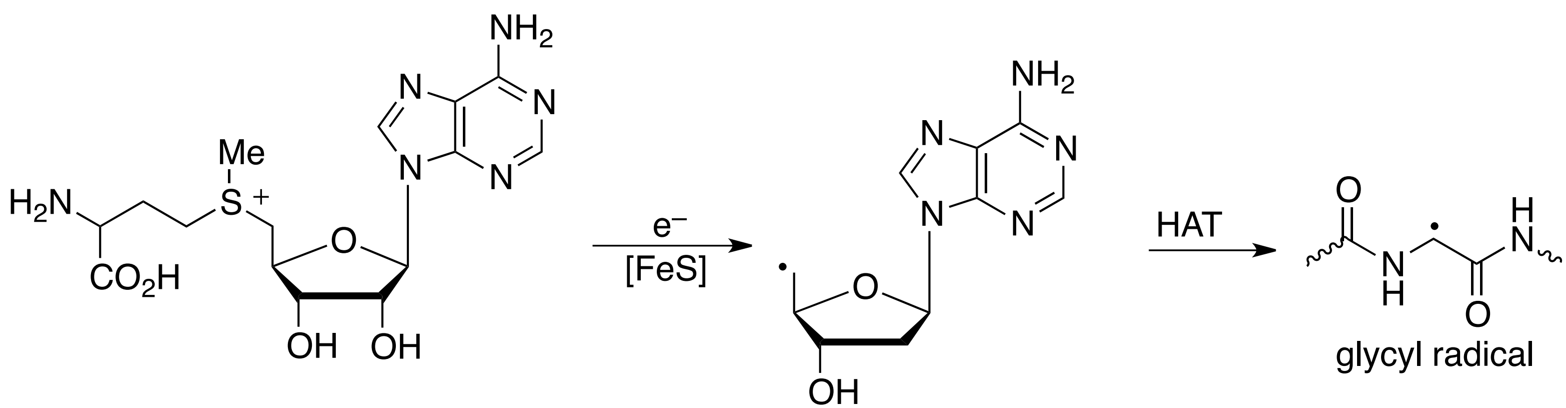
Initiation by a cobalamin B12 neighbouring the active site

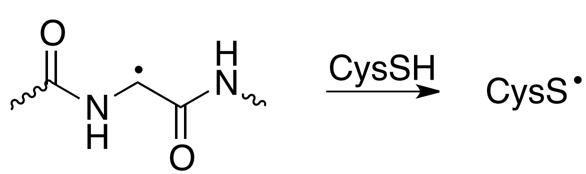


Class III RNRs

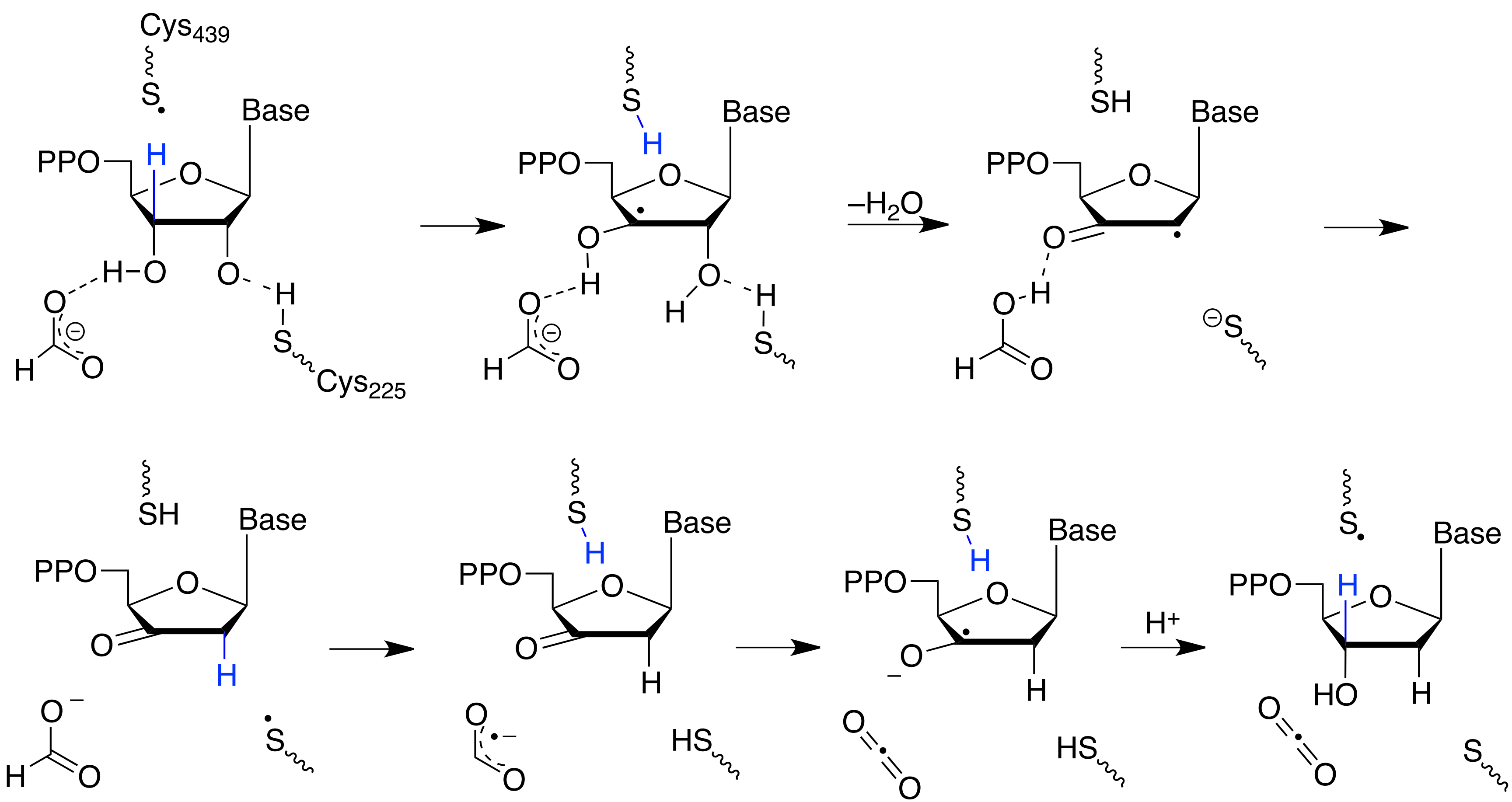
Anaerobic enzyme (2 dimeric subunits, idem class I), the less studied of the family
Also reduce NTP
A formate molecule is the final reductant

Iniation *via* iron sulfur cluster and SAM





Class III RNRs Mechanism



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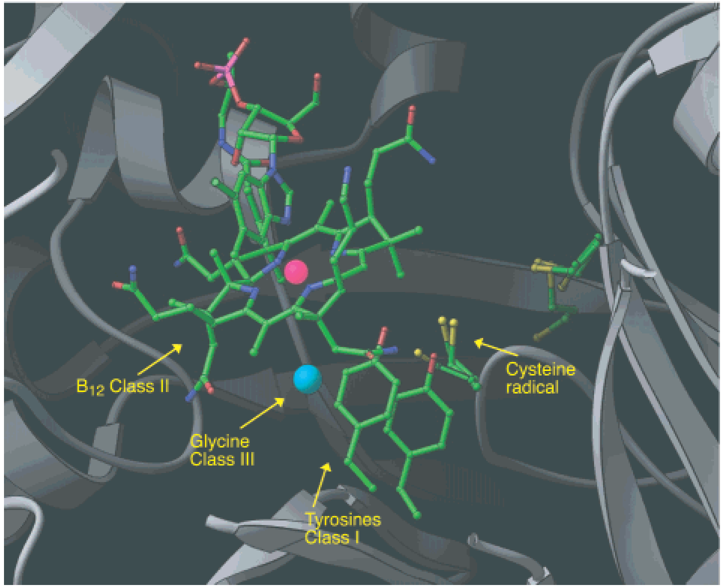
A Common RNR Ancestor?

Poor level of homology in the primary structures of the three classes

Related mechanisms of regulation and deoxygenation
For the three classes: imilar positionment of the first cysteine and of the initiator in the active site



Class III RNRs may be seen as an archaic version of the enzyme (present in *archaebacteria*
➞ Importance for the hypothetised transition from a RNA world to DNA

Literature

Reichard, Stubbe…

Review articles
Stubbe, J.; Van Der Donk, W. A. *Chem. Biol.* **1995**, *2*, 793.
Jordan, A.; Reichard, P. *Annu. Rev. Biochem* **1998**, *67*, 71.
Kolberg, M.; Strand, K. R.; Graff, P.; Kristoffer Andersson, K. *Biochim. Biophys. Acta* **2004**, *1699*, 1.
Nordlund, P.; Reichard, P. *Annu. Rev. Biochem* **2006**, *75*, 681.
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(Some) seminal articles
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X-ray structures:
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Nordlund, P.; Eklund, H. *J. Mol. Biol.* **1993**, *232*, 123.