Chem 452 - Lecture 5 Catalytic Strategies Part 2

Question of the Day: What makes EcoRV a "kinky" enzyme, and how does this character trait help this enzyme to cleave DNA only at its cognate sequence?

Introduction

- Enzymes exhibit both catalytic power and specificity
- We will consider closely, four examples.

Introduction

- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic anhydrase (1ca2) 4.2.1.1
  - A Lyase, which adds water to CO_2.
- EcoRV (1rvb) 3.1.21.4
  - A Hydrolase, which cleave phosphodiester bonds in DNA
- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

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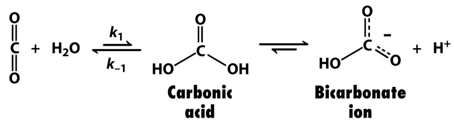
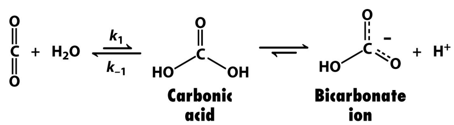
Carbonic Anhydrase

- CO_2 is a major waste produce of the catabolic (energy producing) metabolic pathways.
  - Transported out of the tissues as HCO_3^-.



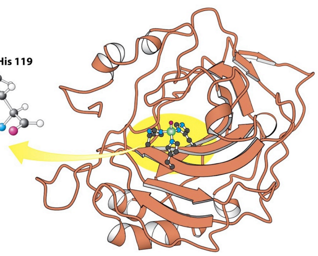
Carbonic Anhydrase

- While the uncatalyzed reaction is overall kinetically favorable, speed is of the essence.
  - Carbonic anhydrase is able to increase the catalytic rate constant to $k_{cat} = 10^6\ s^{-1}$!



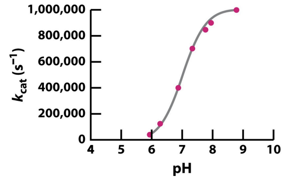
Carbonic Anhydrase

- The nucleophile in this reactions is OH^-
  - A Zn^{2+} ion is involved in generating the nucleophile



Carbonic Anhydrase

- The pH profile reveals a group that is involved in the catalysis, which has a pK_a of around 7



- Studies concluded that this was due to ionization of a water molecule.

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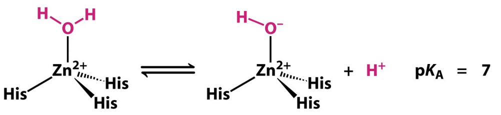

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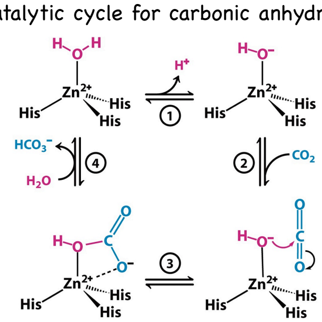
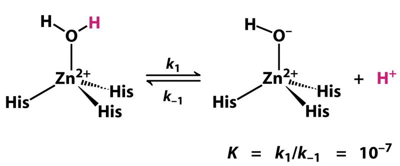
Cabonic Anhydrase

- The catalytic cycle for carbonic anhydrase



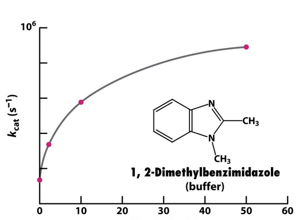
Carbonic Anhydrase

- The catalytic cycle for carbonic anhydrase
  - Because H^+ ions diffuse very rapidly ($k_{-1} \approx 10^{11}$ $M^{-1}s^{-1}$), $k_{cat} = K{\bullet}k_{-1} \approx 10^4$ s^{-1} (not 10^6 s^{-1}, as observed)



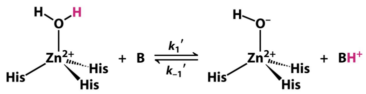
Carbonic Anhydrase

- Buffers can be shown to speed up the carbonic anhydrase reaction.



Carbonic Anhydrase

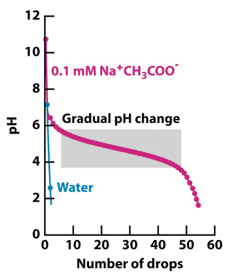
- Buffers can be shown to speed up the carbonic anhydrase reaction.
  - They help shift the reaction to the right.



$$K = k_1'/k_{-1}' \approx 1$$

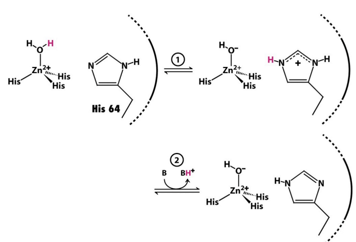
Carbonic Anhydrase

- Buffers provide a sink for the released hydrogen ions (H⁺).



Carbonic Anhydrase

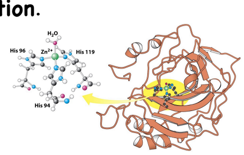
- His64 also helps mediate the the flow of H⁺ away from the active site and to the buffer.



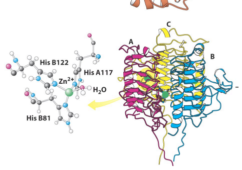
Carbonic Anhydrase

- Carbonic anhydrase also provides an example of **convergent evolution**.

α-carbonic anhydrase (Animals)



γ-carbonic anhydrase (Archean)



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EcoRV

- EcoRV is a **restriction endonuclease**.
  - It is a good model for demonstrating high substrate specificity.
  - The substrate is a specific sequence called the **cognate** sequence.
- EcoRV specifically cleaves DNA at the sequence **GATATC**
  - Like with many restriction endonucleases, the sequence for the complementary strand of the cognate sequence reads the same, but backwards.

Restrictions sites share this common property

EcoRV

- EcoRV is a **re** ... **ease**.
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- EcoRV specifi... t the sequence **GATATC**
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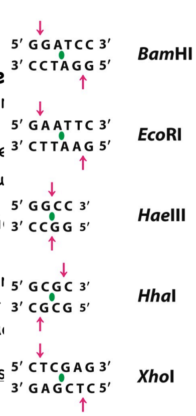
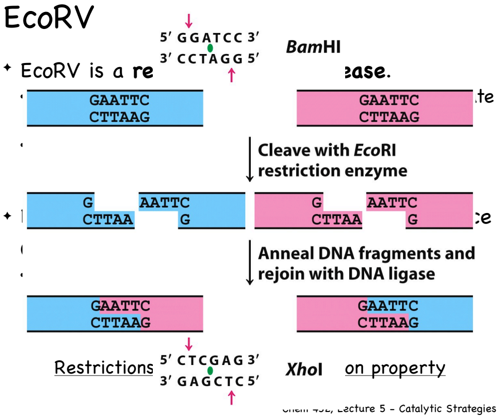
Restrictions ... on property



EcoRV



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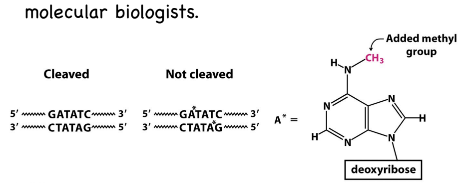
Restrictions sites share this common property

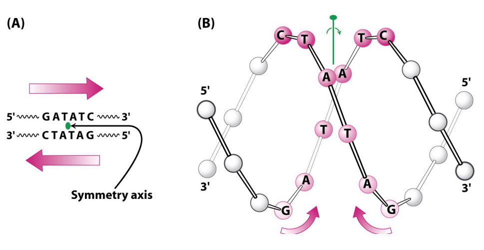
EcoRV

- EcoRV is a **restriction endonuclease**.
  - In conjunction with methylases, restriction endonucleases protect bacteria from viruses.
  - They have also become a powerful tool for molecular biologists.



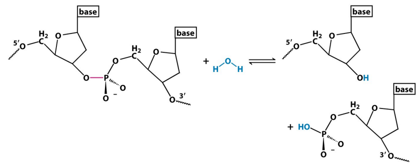
EcoRV

- Restriction sites have a 2-fold symmetry



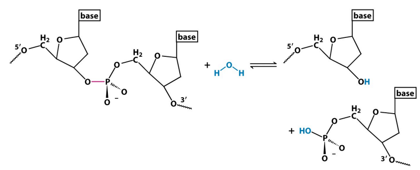
EcoRV

- Like chymotrypsin, the nuclease reaction is a hydrolase reaction.



EcoRV

- But does it also involve a covalently bound intermediate?



EcoRV

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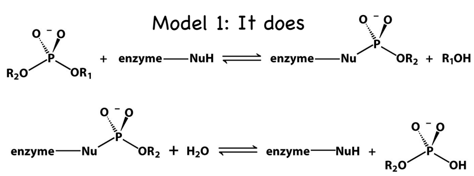
Model 1: It does

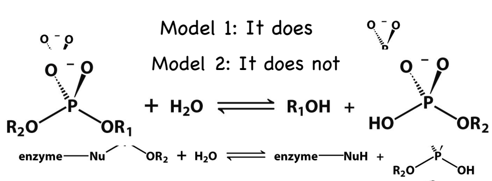


EcoRV

- But does it also involve a covalently bound intermediate?

Model 1: It does

Model 2: It does not



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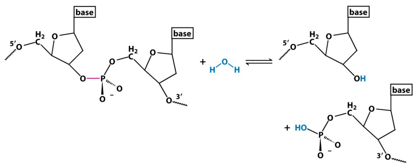
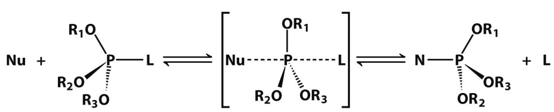


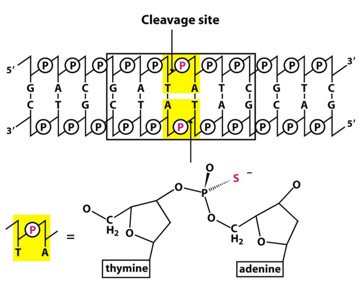
EcoRV

- Model 2 will invert the geometry about the phosphorous.
- Model 1 will not.



EcoRV

- A phosophorothionate label was used to answer this question.



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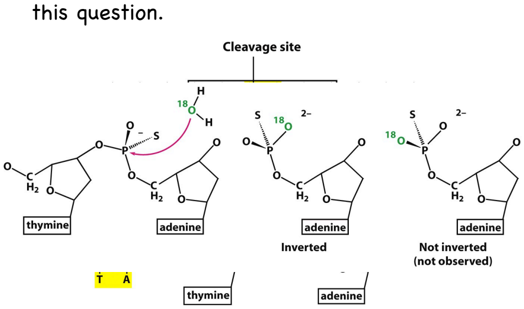
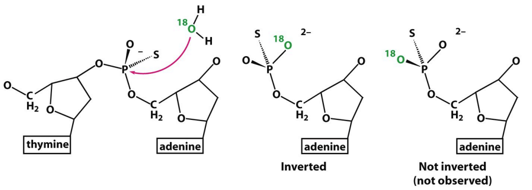


EcoRV

- And the answer is...
  - The symmetry is inverted
  - Therefore, Model 2 is the correct model.
    - The water reacts directly with the phosphate



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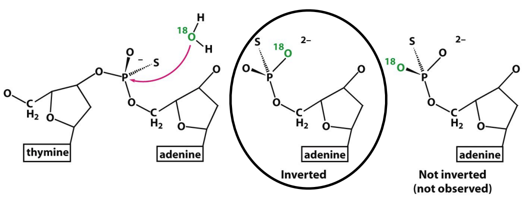

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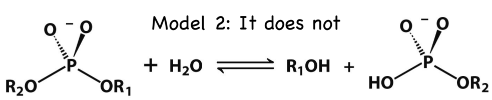
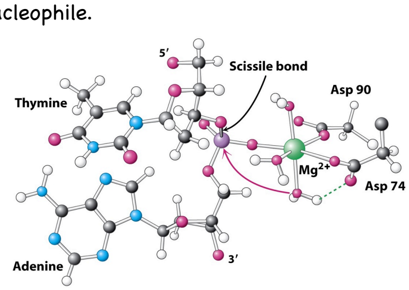


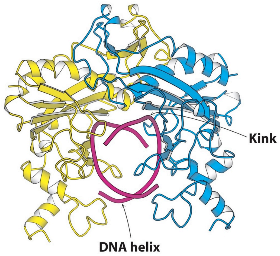
EcoRV

- Like carbonic anhydrase, a metal ion (Magnesium) is involved in generating the nucleophile.



EcoRV

- The active site is generated by a kinking of the DNA at the **cognate** site.



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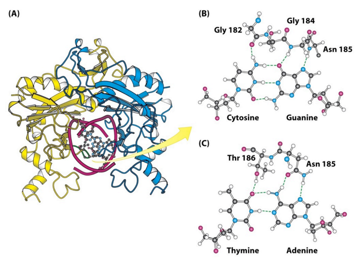

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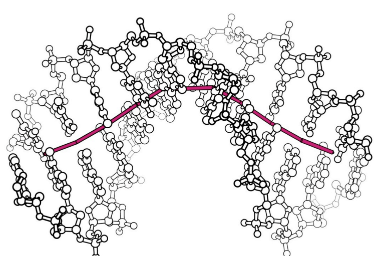

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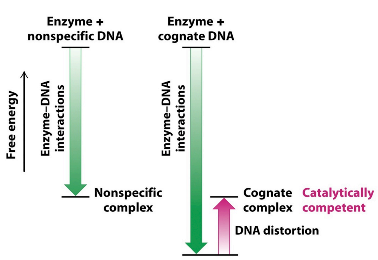

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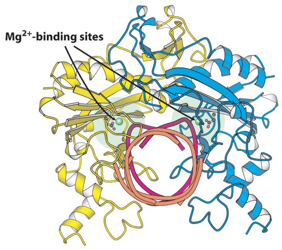

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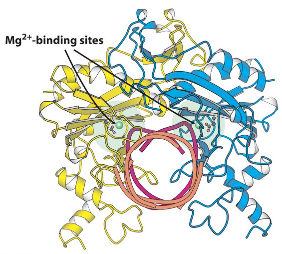
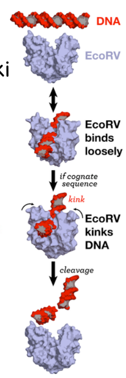
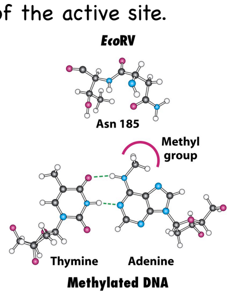




EcoRV

- The E. coli bacteria is protected from the EcoRV through a methylation tha blocks formation of the active site.



Next up

- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1 (Chapter 9)
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.
- Regulatory Strategies (Chapter 10)