

Why do some enzymes use only NADPH, while others use only NADH?

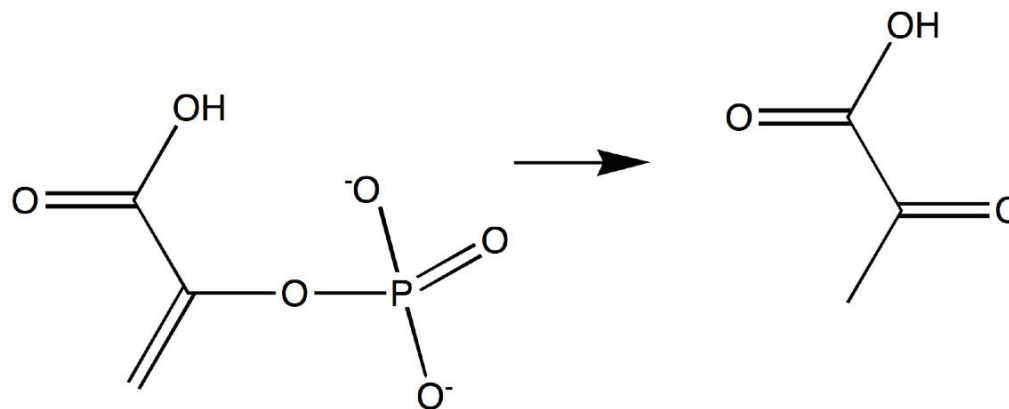
- 1) NADPH is used for oxidations, NADH for reductions
- 2) the levels of the NADH and NADPH pools can be different, allowing for differential regulation of processes utilizing one or the other.
- 3) species that are modified by NADH can only be “reverse-modified” by NAD⁺, while species modified by NADPH can only be “reverse-modified” by NADP⁺
- 4) NADPH reacts only with phosphorylated sugars, while NADH reacts with unphosphorylated sugars.
- 5) All enzymes that use NADH can also use NADPH



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- 5) All enzymes that use NADH can also use NADPH

Which of the following enzymes catalyzes this reaction?



1) aldolase

2) phosphoglycerate mutase

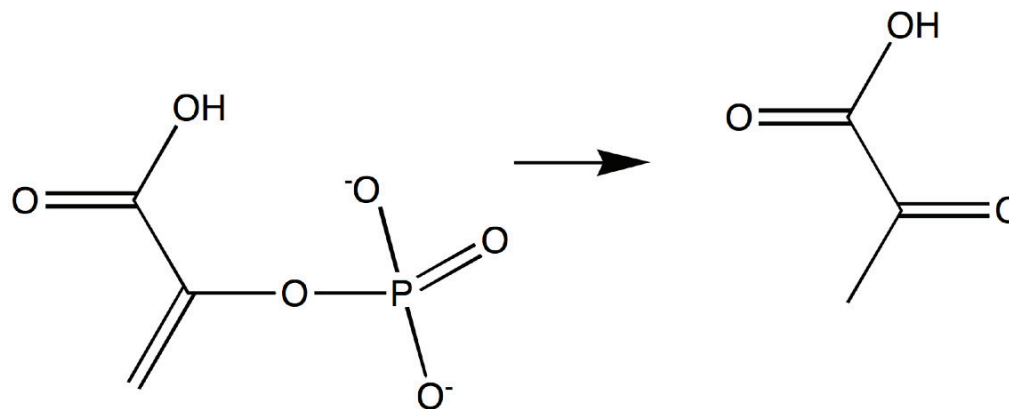
3) pyruvate kinase

4) phosphofructokinase

5) phosphohexose isomerase



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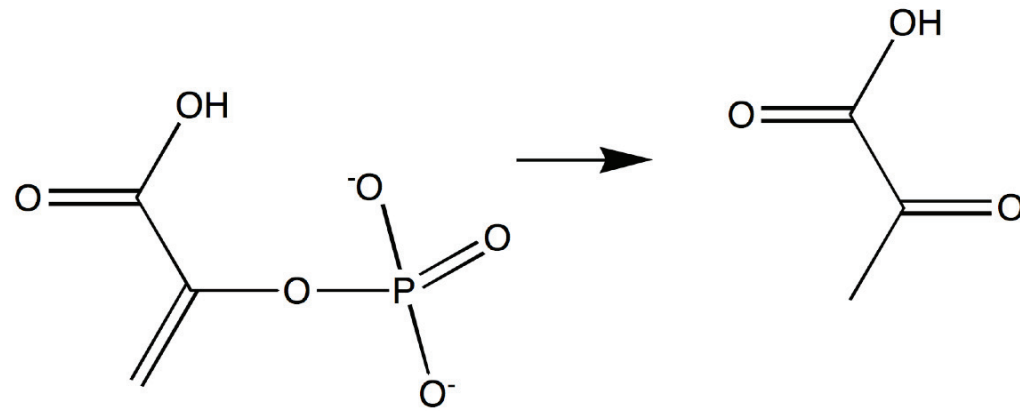
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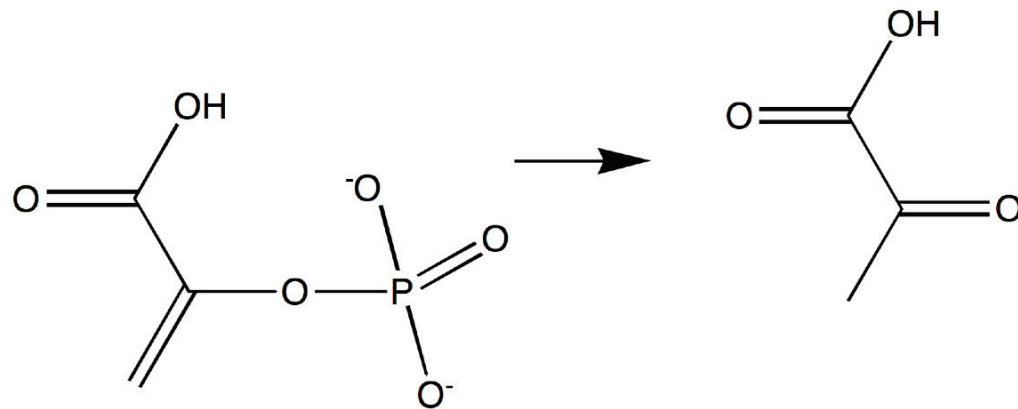
5) phosphohexose isomerase

2) phosphoglycerate mutase

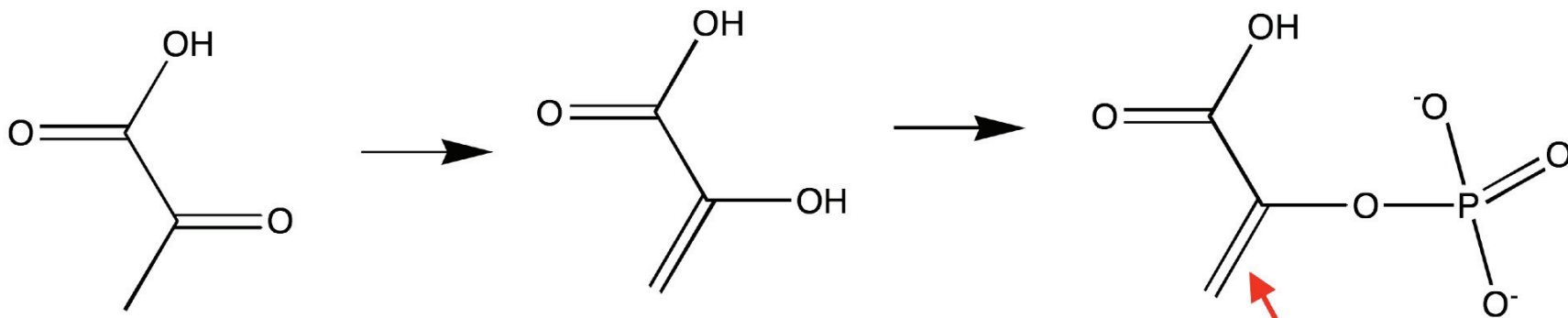
4) ~~phosphofructokinase~~



Look at this reaction in reverse. How do you phosphorylate a ketone??

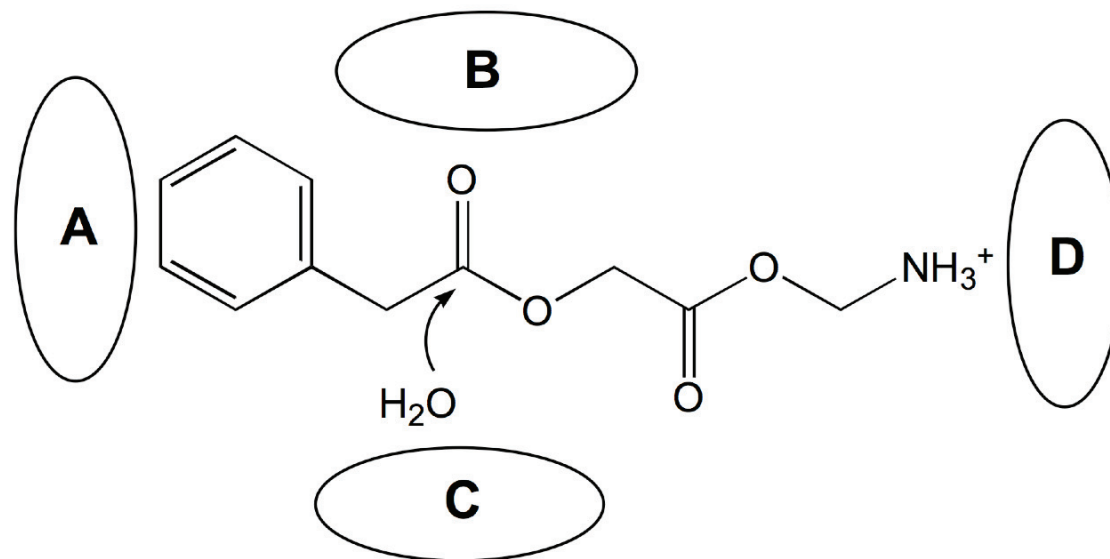


Look at this reaction in reverse. How do you phosphorylate a ketone??



Answer: keto-enol isomerization!

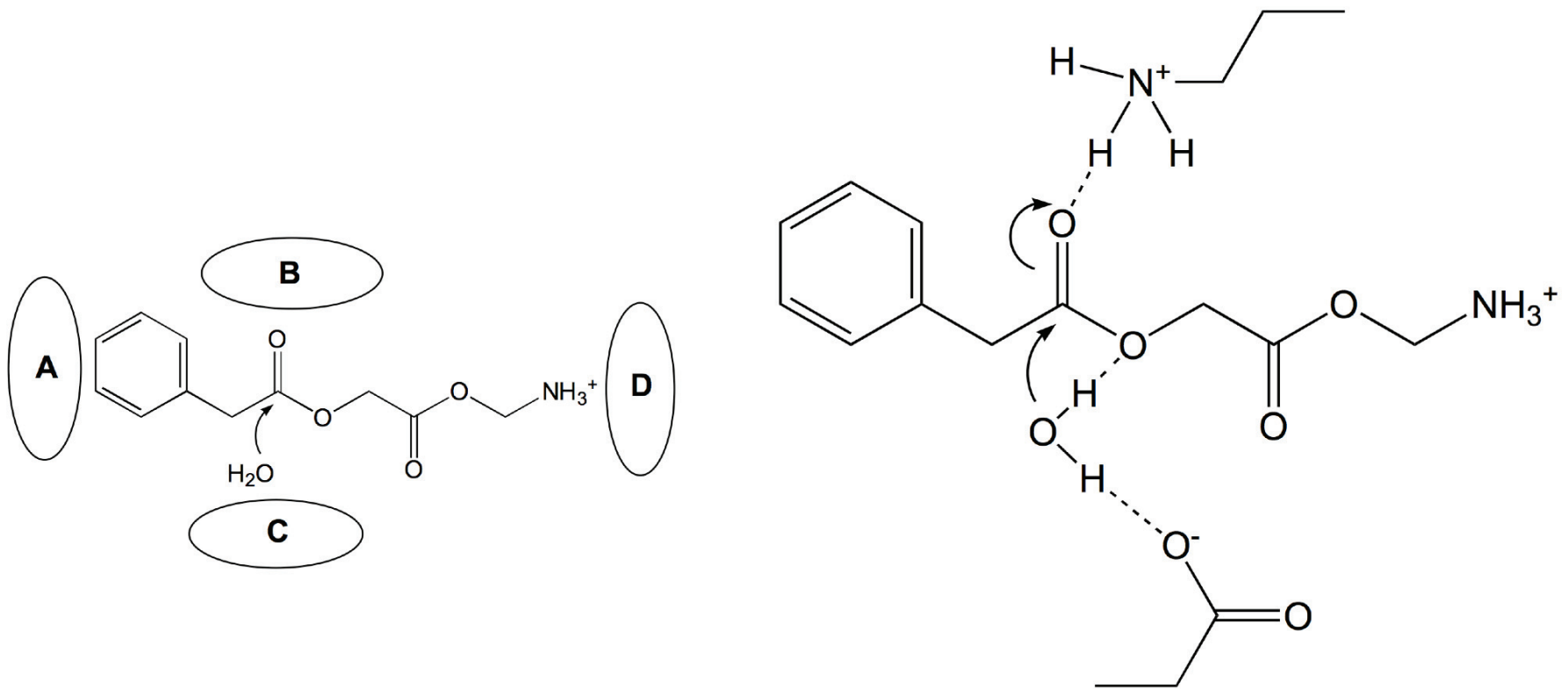
This was the clue!



Consider hydrolysis of the ester above
 Placement of which groups where will speed hydrolysis
 of the bound molecule?

- | | |
|--------------------------|--------------------------|
| 1) Phe at B and Glu at C | 2) Glu at B and Phe at C |
| 3) Asp at B and Lys at C | 4) Lys at B and Asp at C |
| 5) none of the above | |

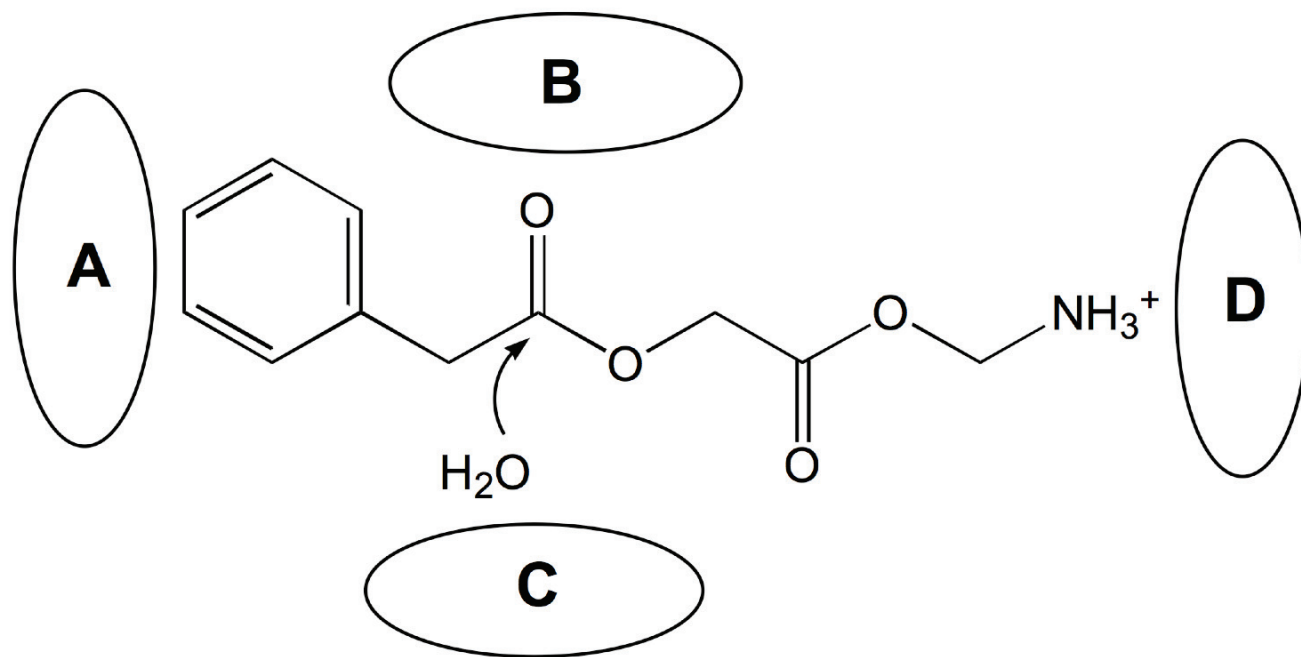




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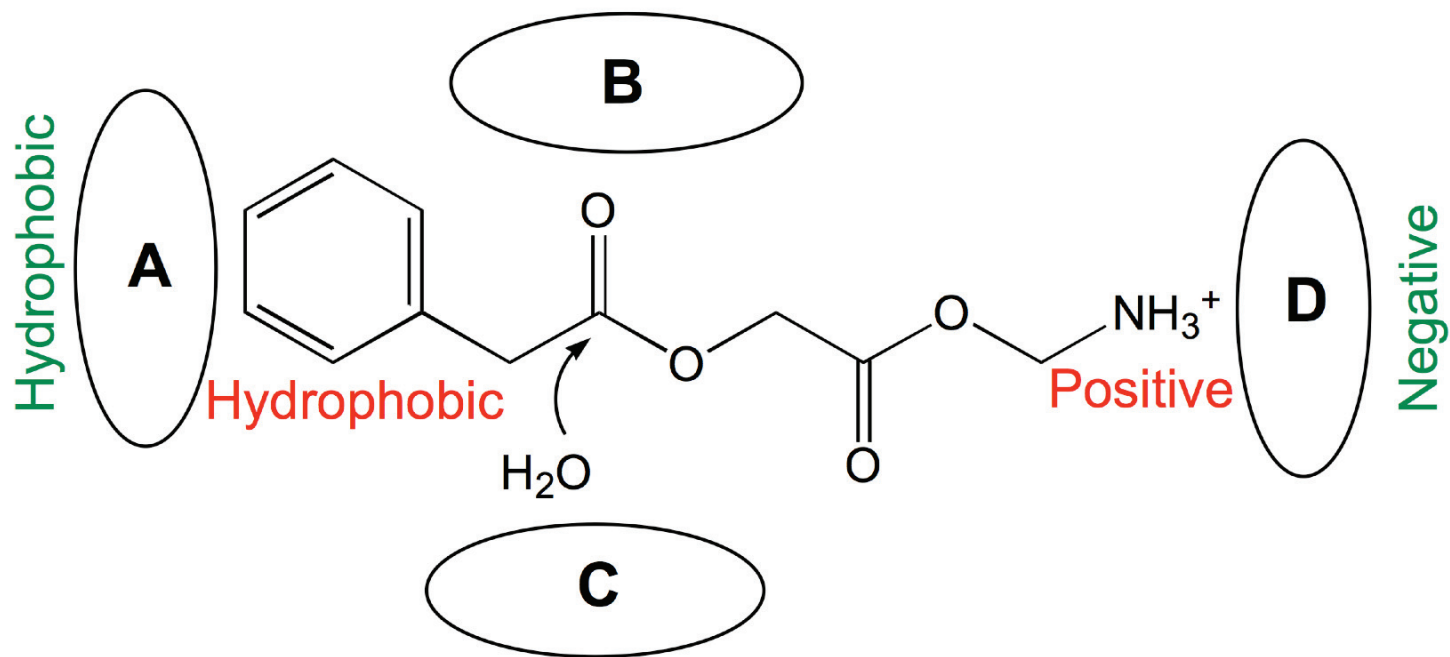


Consider hydrolysis of the ester above

Substrate specificity is best achieved by placement of which groups where?

- 1) Phe at B and Glu at C
- 2) Glu at A and Lys at D
- 3) Ile at A and Asp at D
- 4) Ser at B and His at C
- 5) none of the above

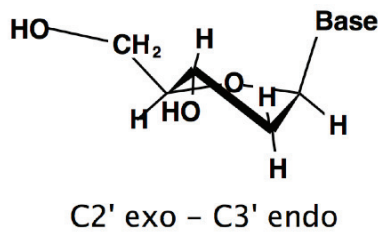
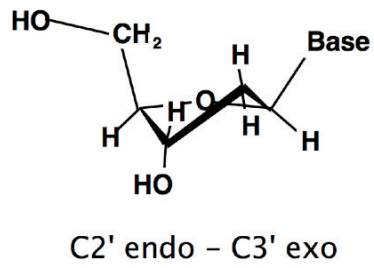




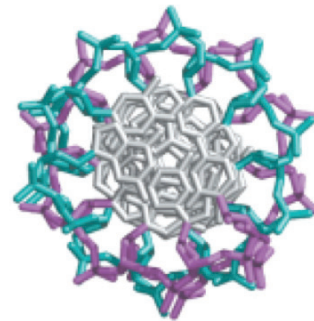
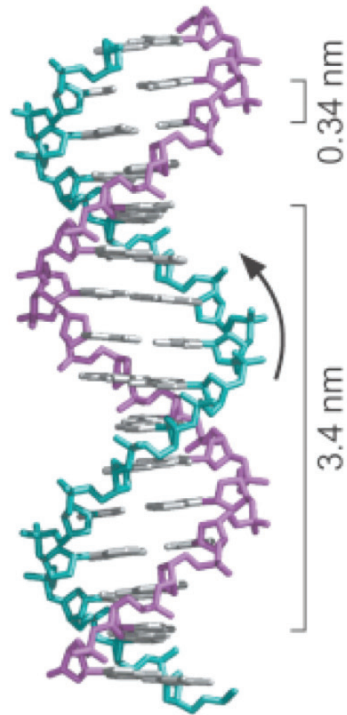
Consider hydrolysis of the ester above

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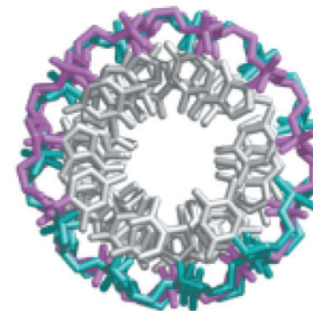
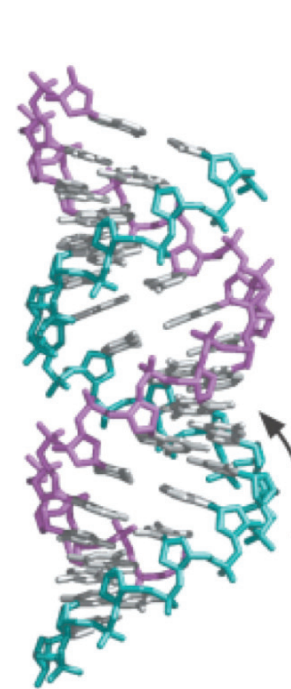
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- 3) Ile at A and Asp at D
- 4) Ser at B and His at C
- 5) none of the above



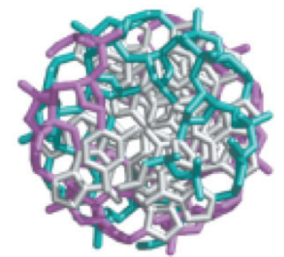
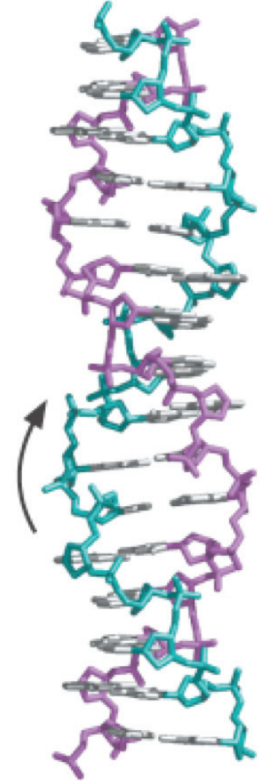
a B DNA



b A DNA

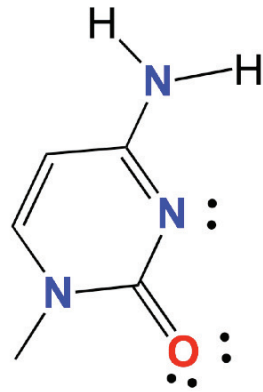


c Z DNA

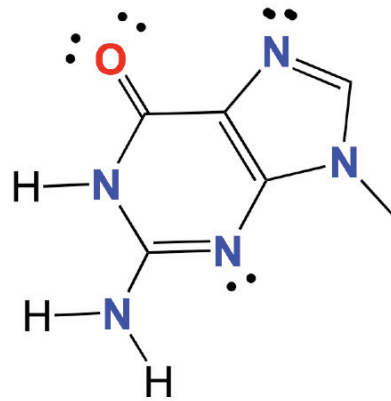


Why is Watson-Crick so good?

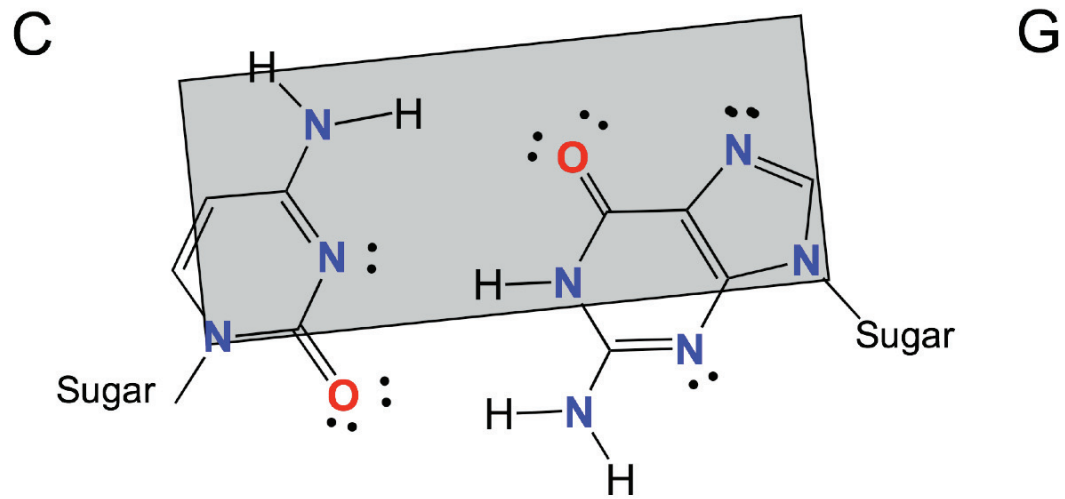
C



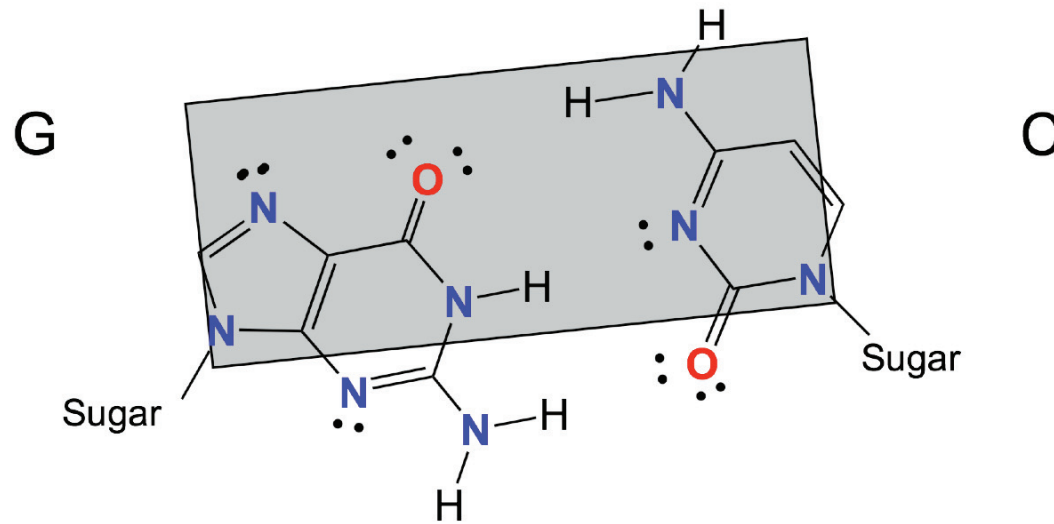
G



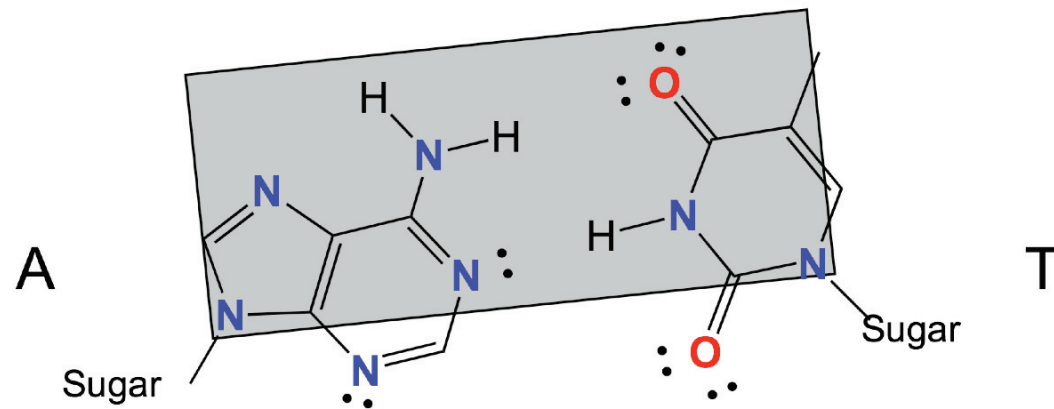
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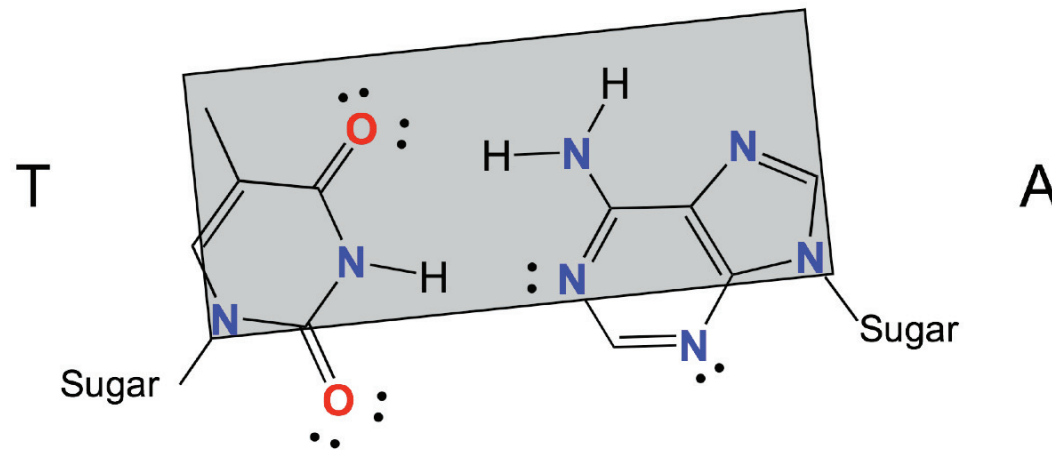
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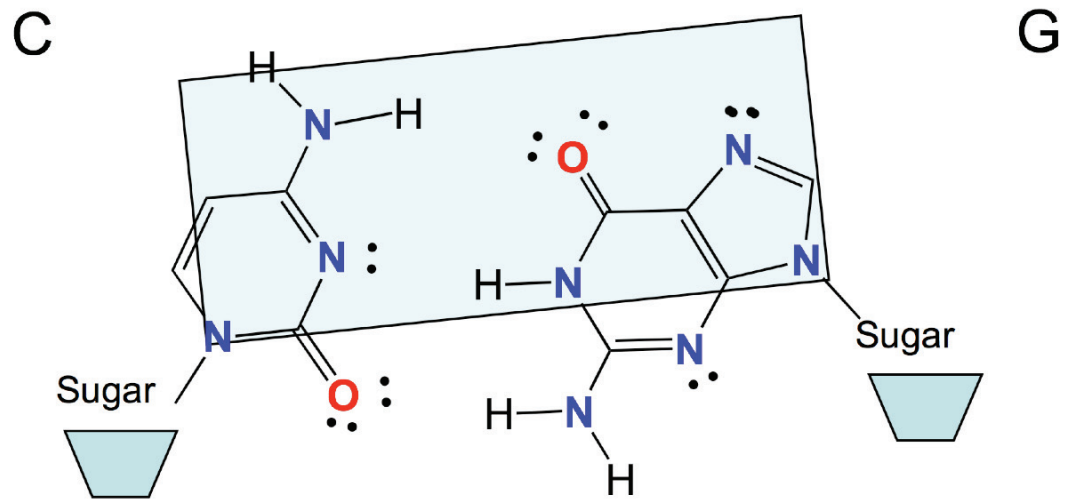
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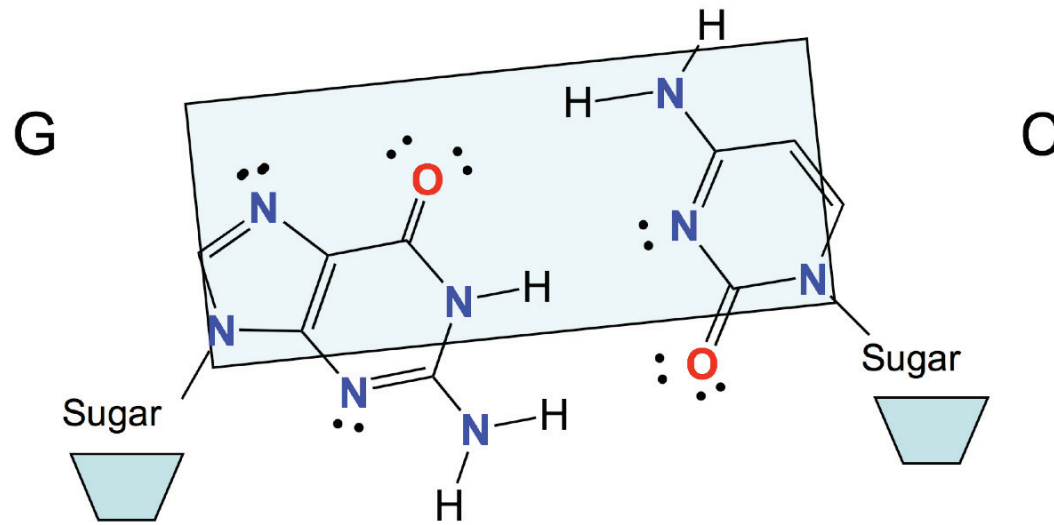
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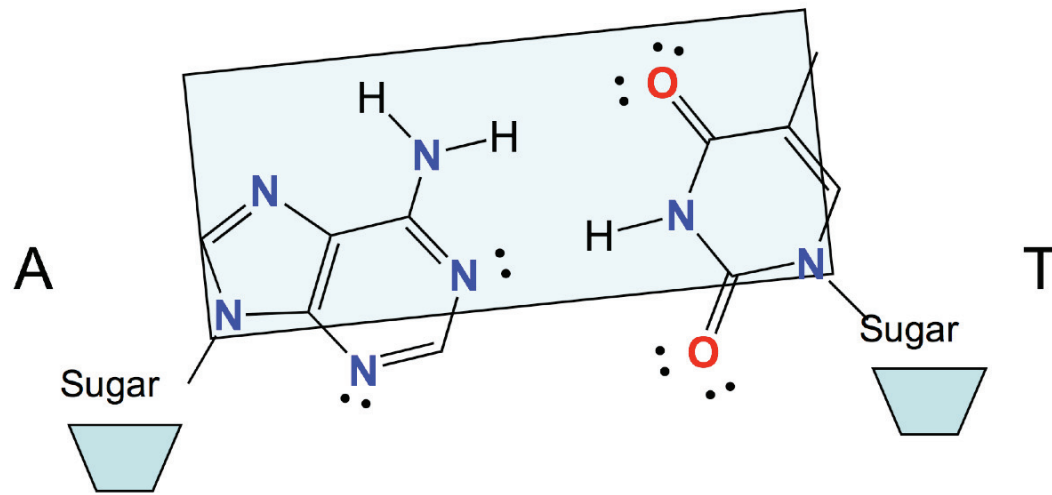
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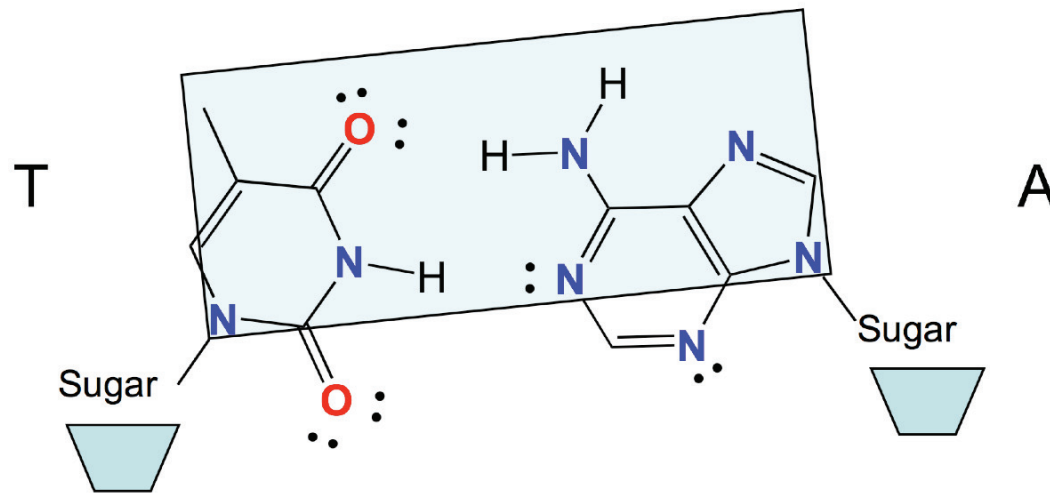
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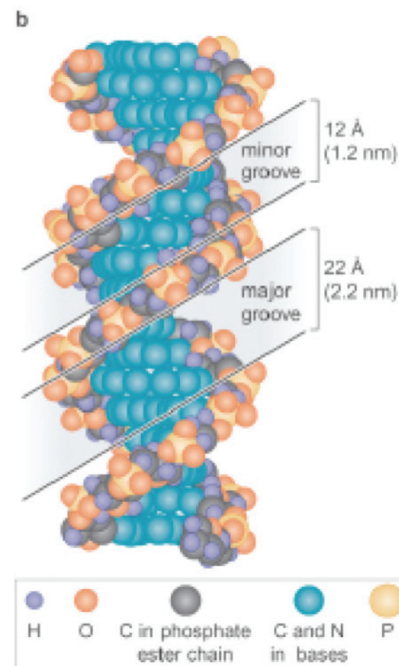
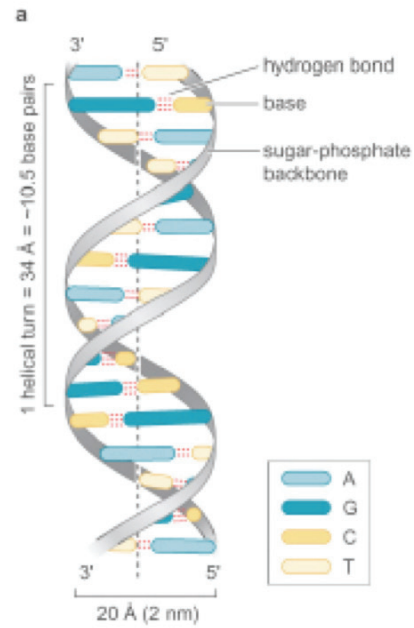
Why is Watson-Crick so good?



B-form in 3D

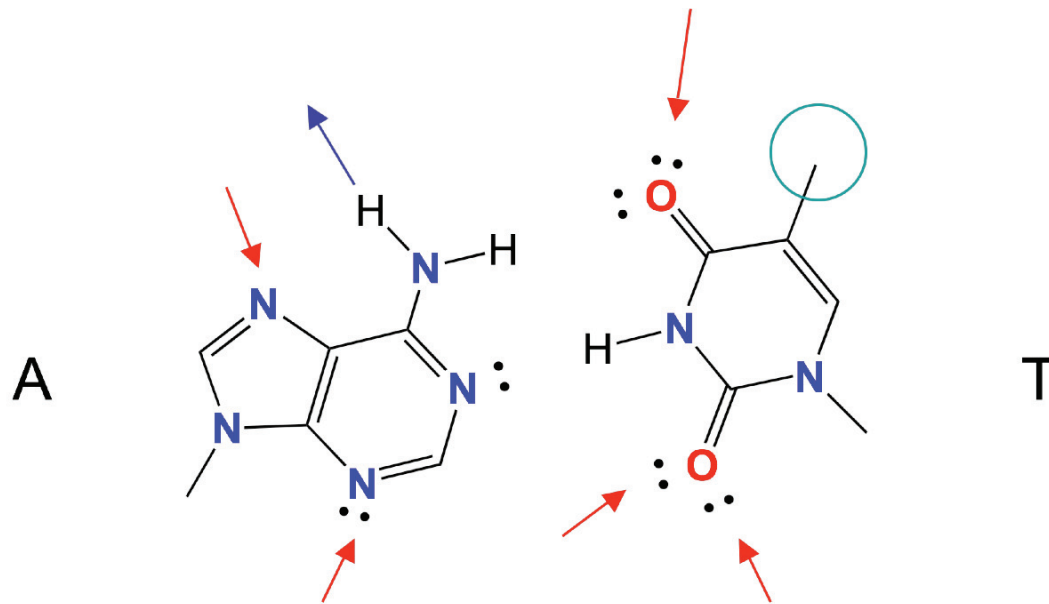
A-form in 3D

More in 3D



Why is the major groove so good?

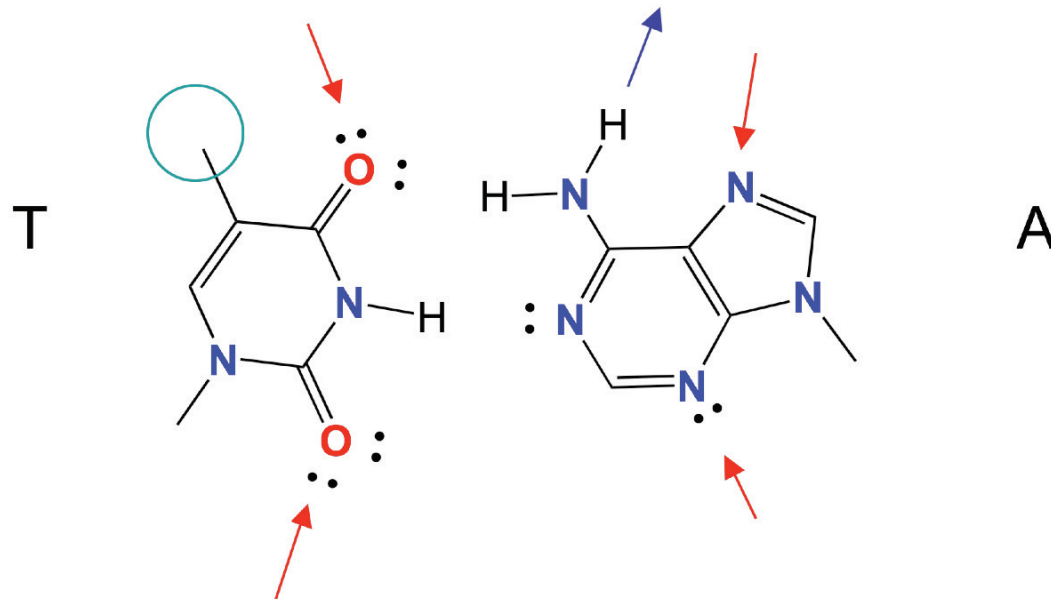
Major Groove



Minor Groove

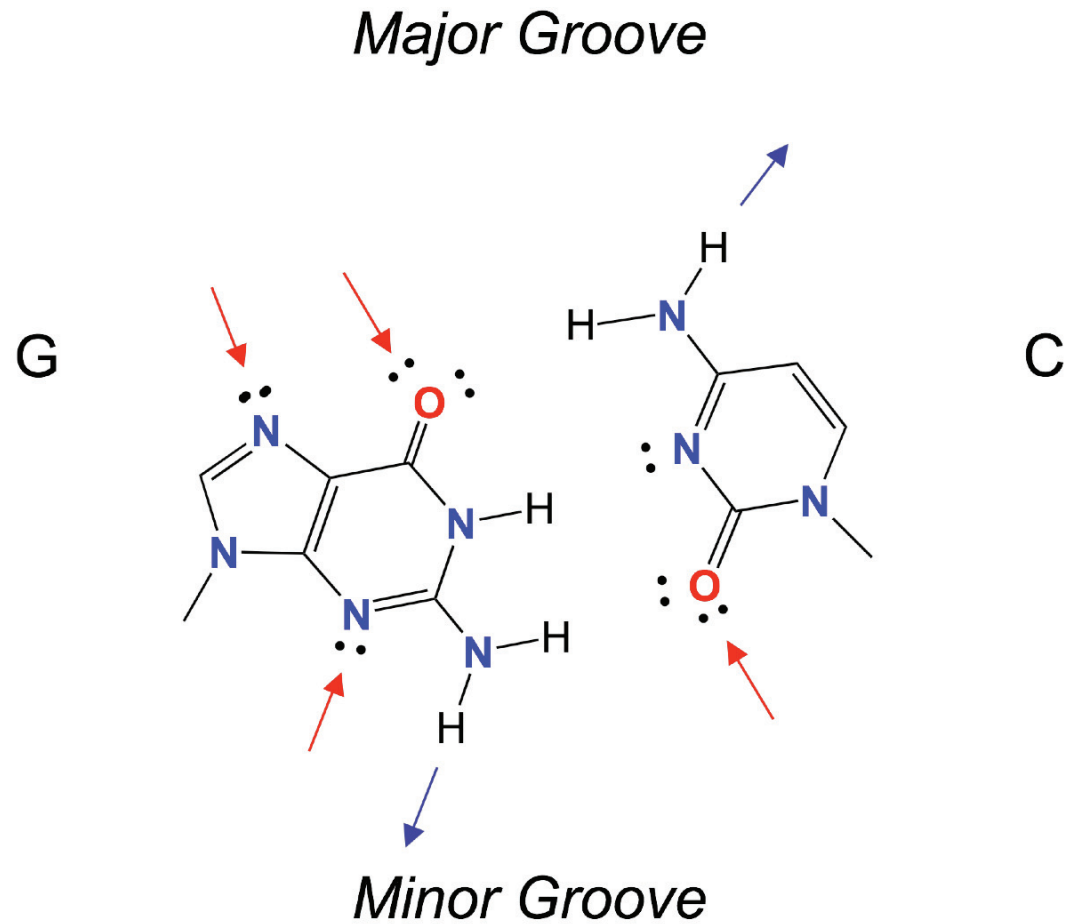
Why is the major groove so good?

Major Groove



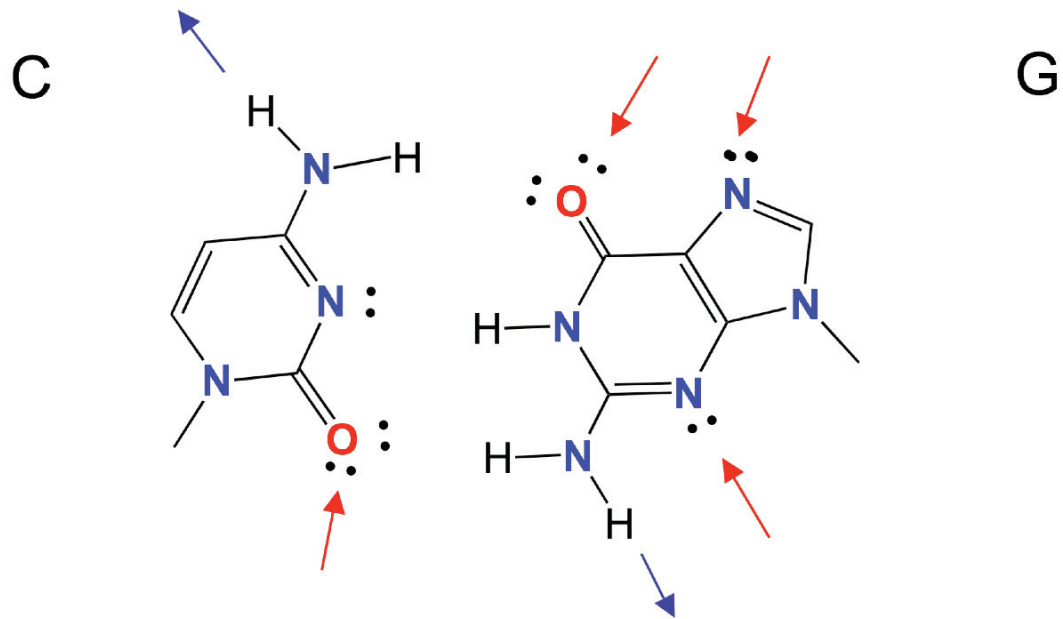
Minor Groove

Why is the major groove so good?



Why is the major groove so good?

Major Groove

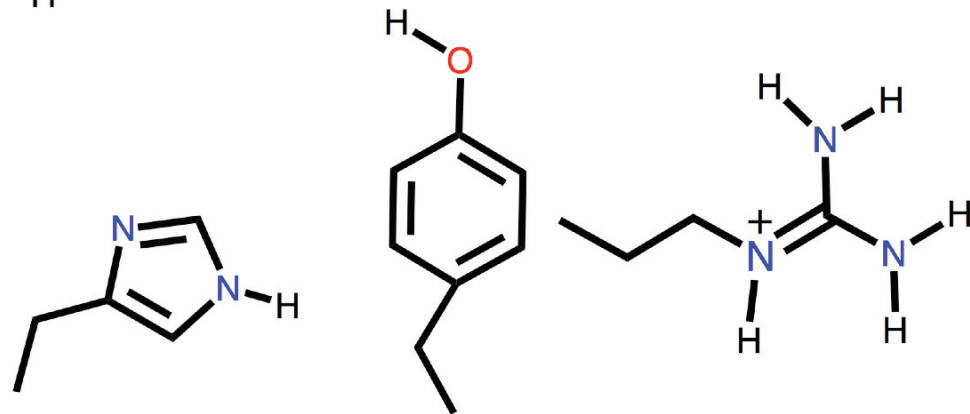
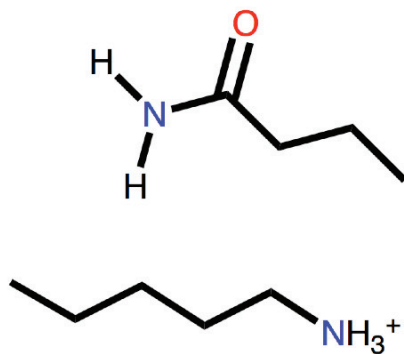
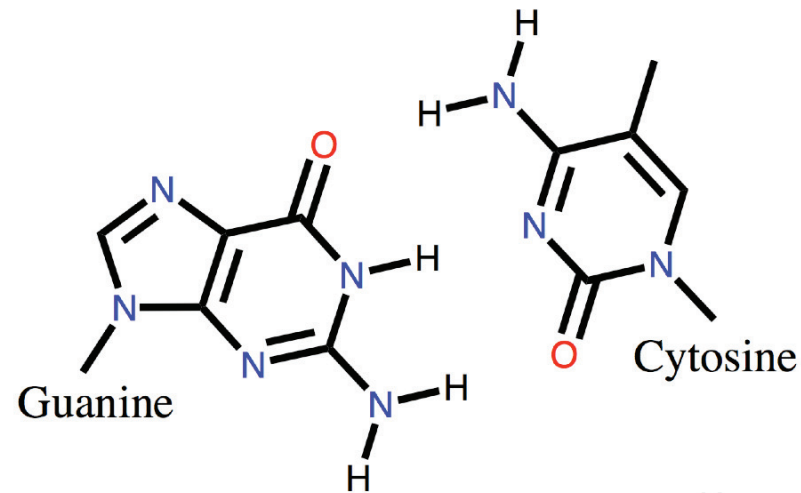


Minor Groove

Sequence-specific binding by proteins

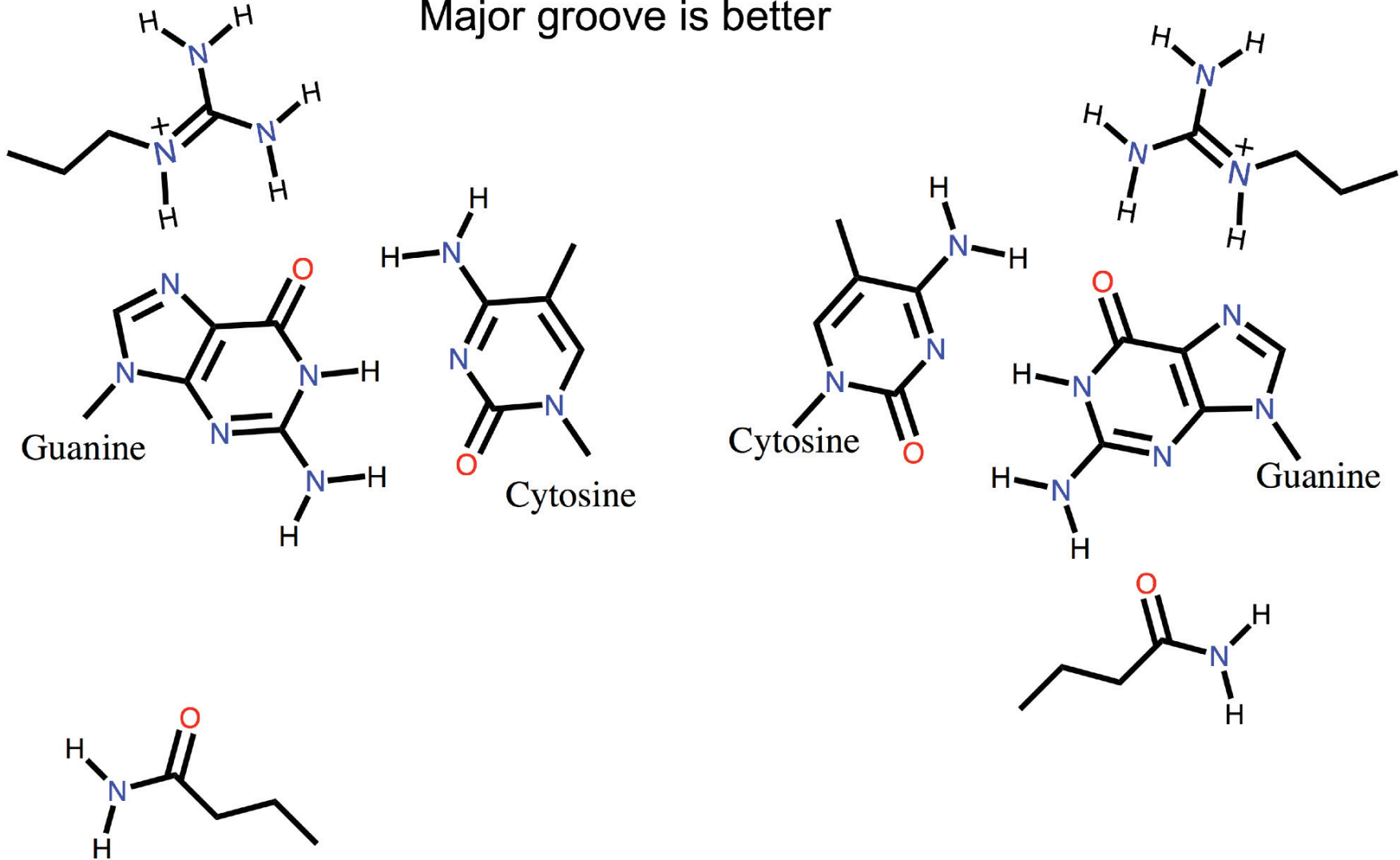
Find “two-fer” interactions

See an example



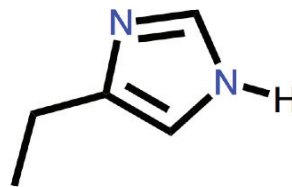
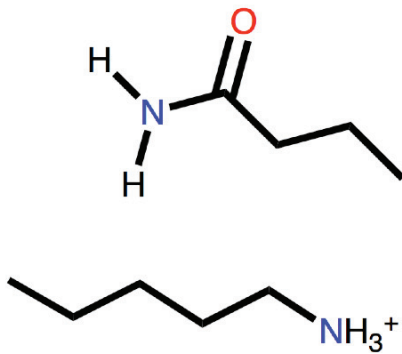
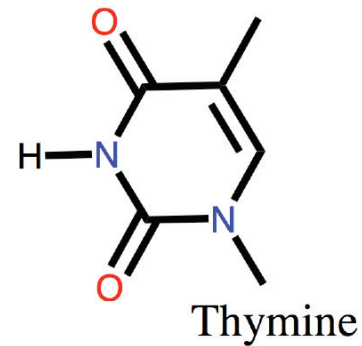
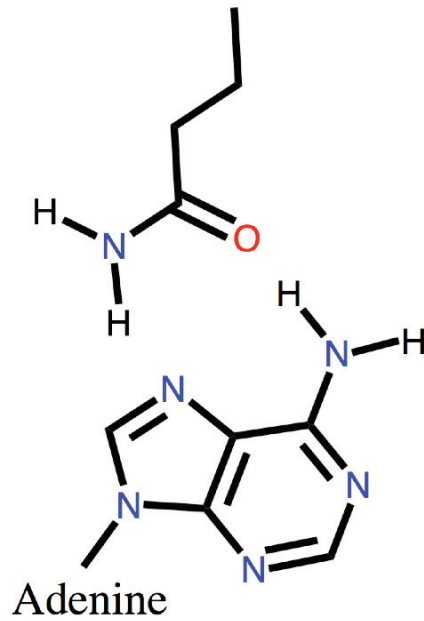
Sequence-specific binding by proteins

Major groove is better

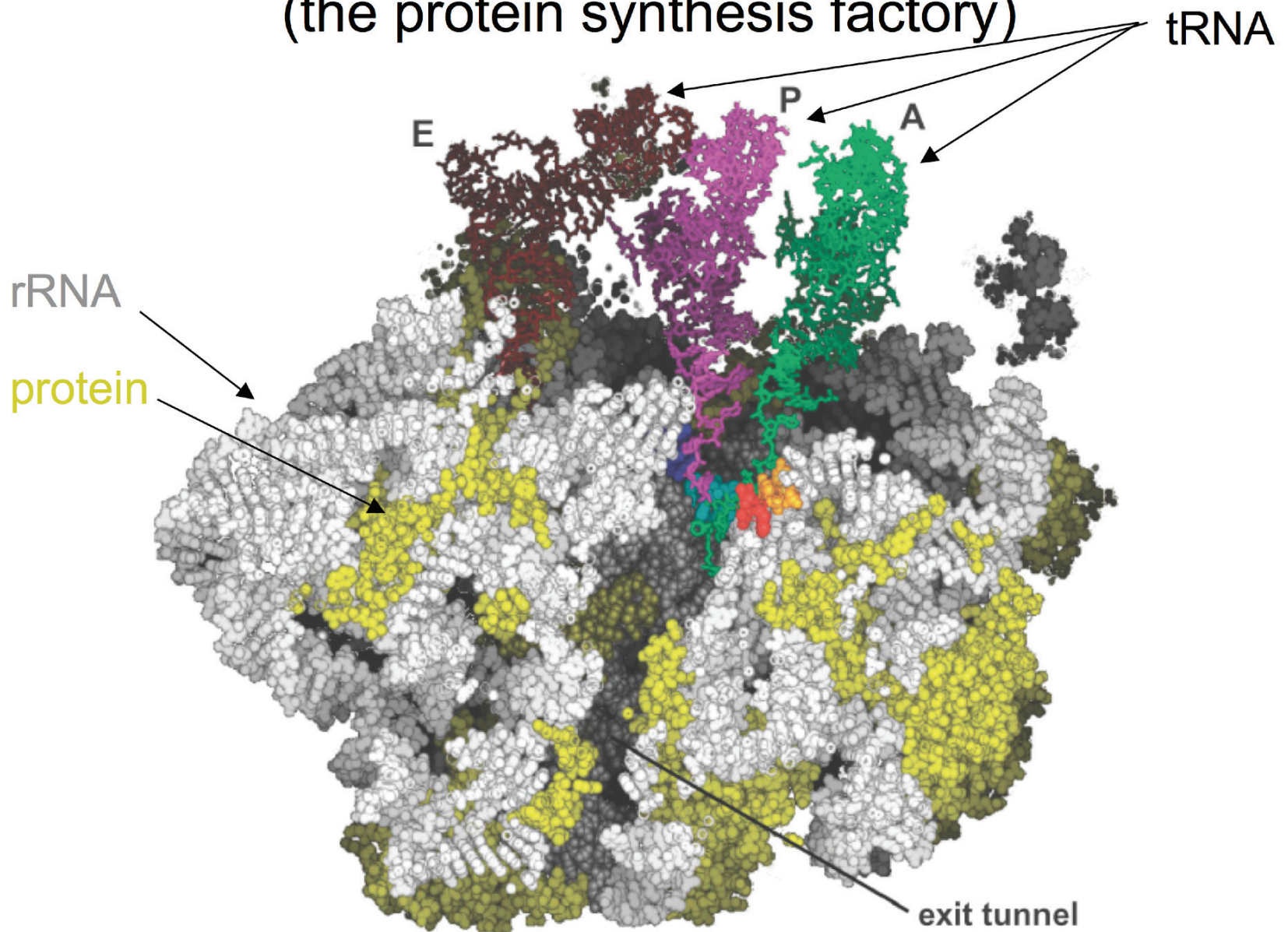


Sequence-specific binding by proteins

Find “two-fer” interactions



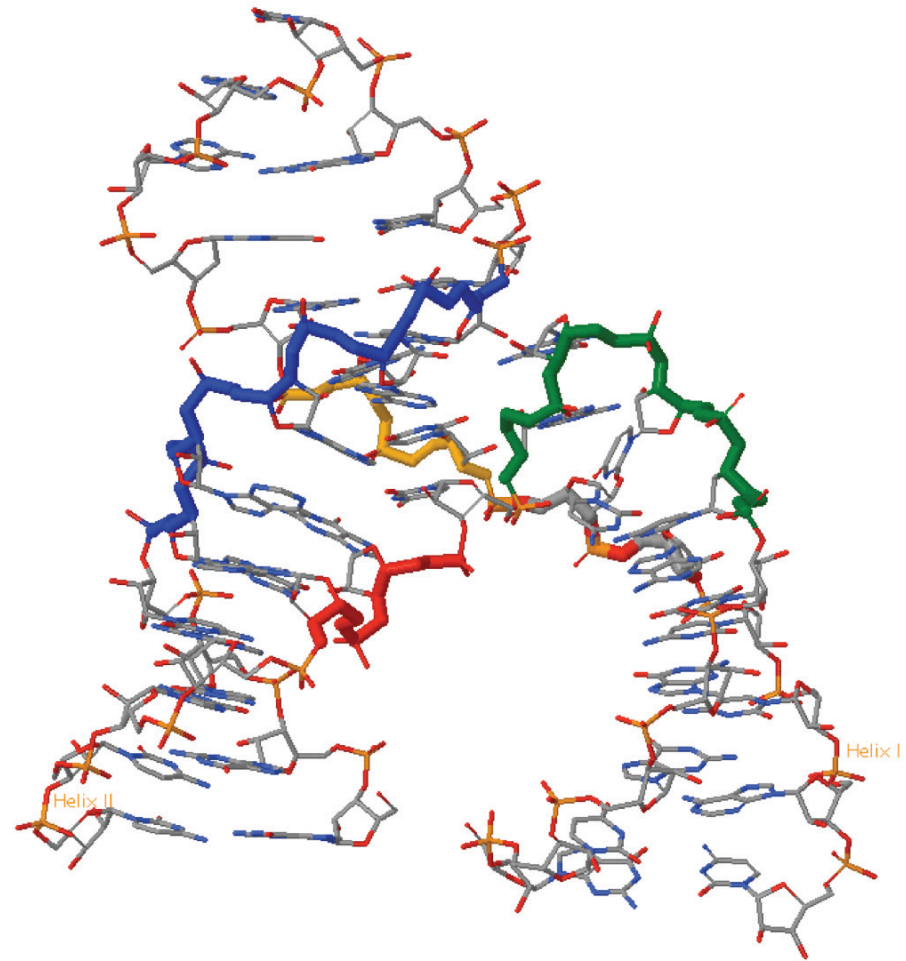
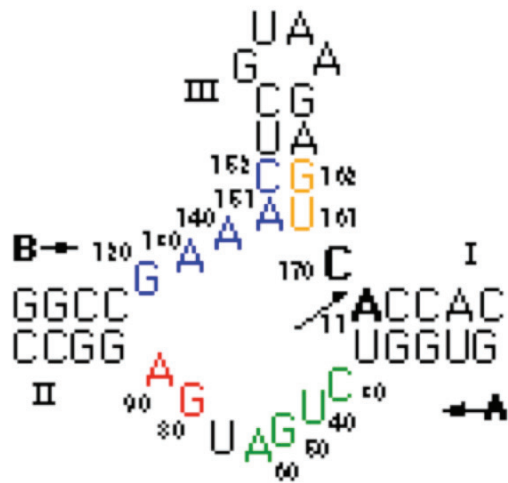
Ribosome (the protein synthesis factory)



RNA structures are diverse!!

More in 3D

- “Hammerhead” ribozyme
 - An “enzyme” made of RNA

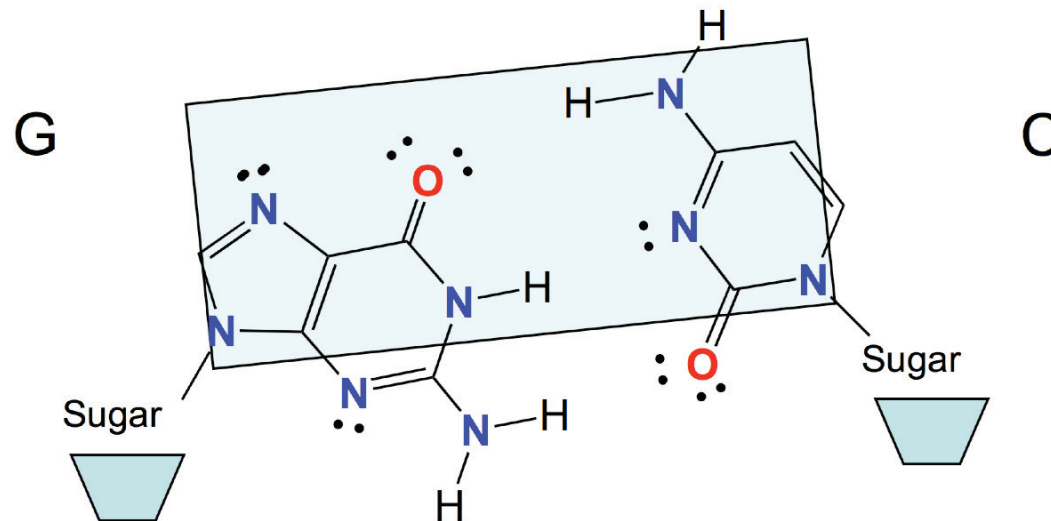


Classes of RNA

- mRNA - messenger RNA - encodes protein
- tRNA - transfer RNA - carries amino acids to the ribosome, recognizes a 3 base codon via Watson-Crick pairing
- rRNA - ribosomal RNA - forms the ribosome “enzyme” (with a little bit of protein here and there)
- snRNA - small nuclear RNA, a key part of small nuclear riboproteins - help to process pre-mRNA
- miRNA - micro RNA - 22 base RNAs that bind to other RNAs and regulate their expression
- siRNA - small interfering RNA - discovered by a UMass faculty member, who received the Nobel Prize in 2006 - 20-30 base RNAs that trigger a process that can selectively degrade (silence) target mRNAs. Effective for gene “knock-outs.”
- Riboswitches - elements within other RNAs that can bind to signal molecules, alter their conformation in response, and therefore control the target RNA

Copying DNA and RNA

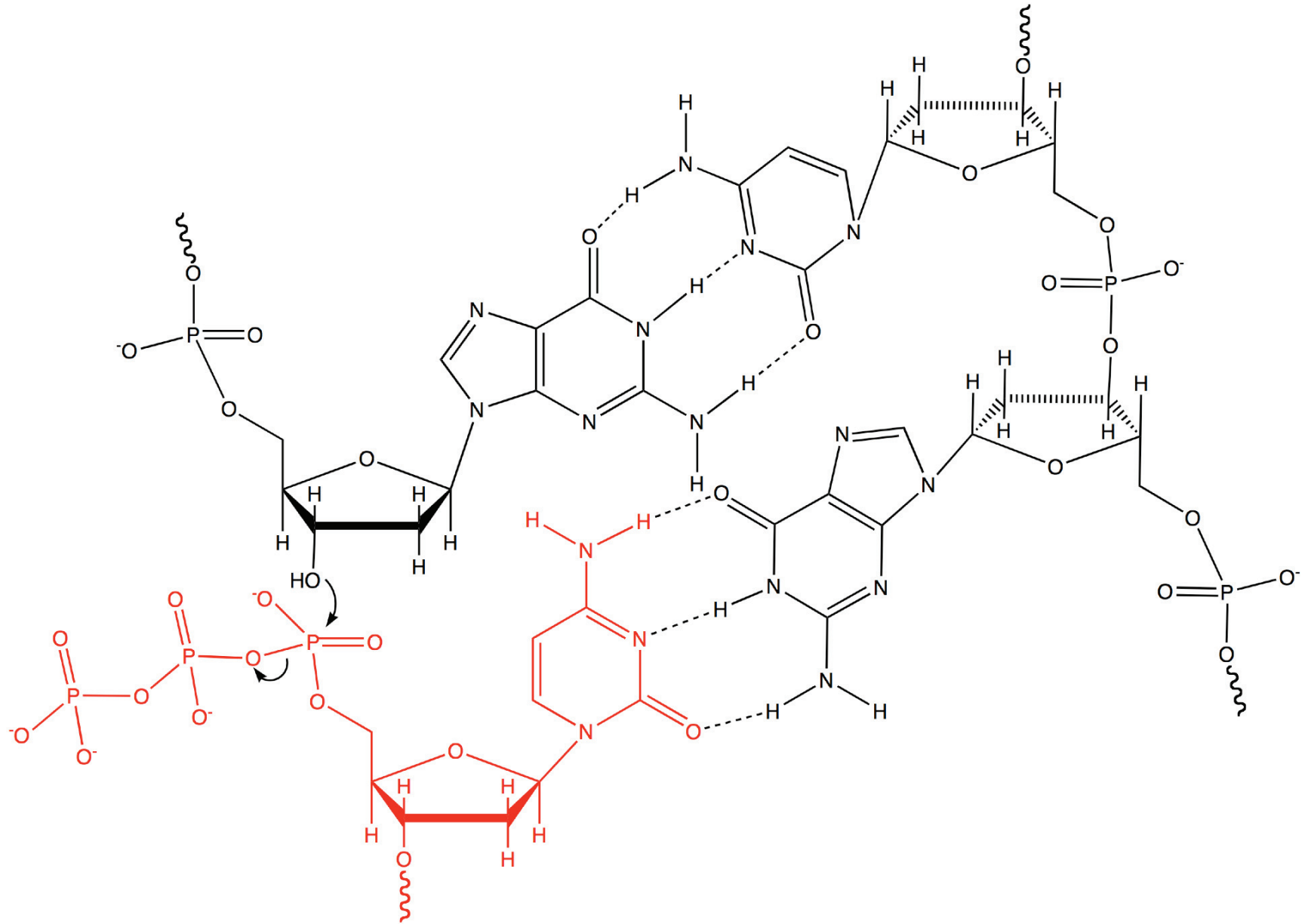
- RNA polymerase
 - makes (transient) RNA copies from a DNA template
 - When a protein (or RNA) is needed, RNA polymerase finds the appropriate region of the DNA and begins making multiple copies
- DNA polymerase
 - makes new (permanent) DNA copies from a DNA template
 - When a cell divides, DNA polymerase makes a complete copy of the entire DNA genome



Fidelity assured by isosteric nature of Watson-Crick base pairs

Copying DNA and RNA

- Nucleophilic attack by furanose hydroxyl on a phosphoric anhydride



PCR - Polymerase Chain Reaction

PCR - Polymerase Chain Reaction



↓ Heat, to melt DNA

The diagram illustrates the first step of a PCR cycle. It features two horizontal lines representing DNA strands: a top blue line and a bottom green line. A downward-pointing arrow is positioned between these lines, with the text 'Heat, to melt DNA' to its right. Below this, there is a gap, followed by another set of two horizontal lines (top blue, bottom green), representing the state of the DNA after the heating step.

PCR - Polymerase Chain Reaction

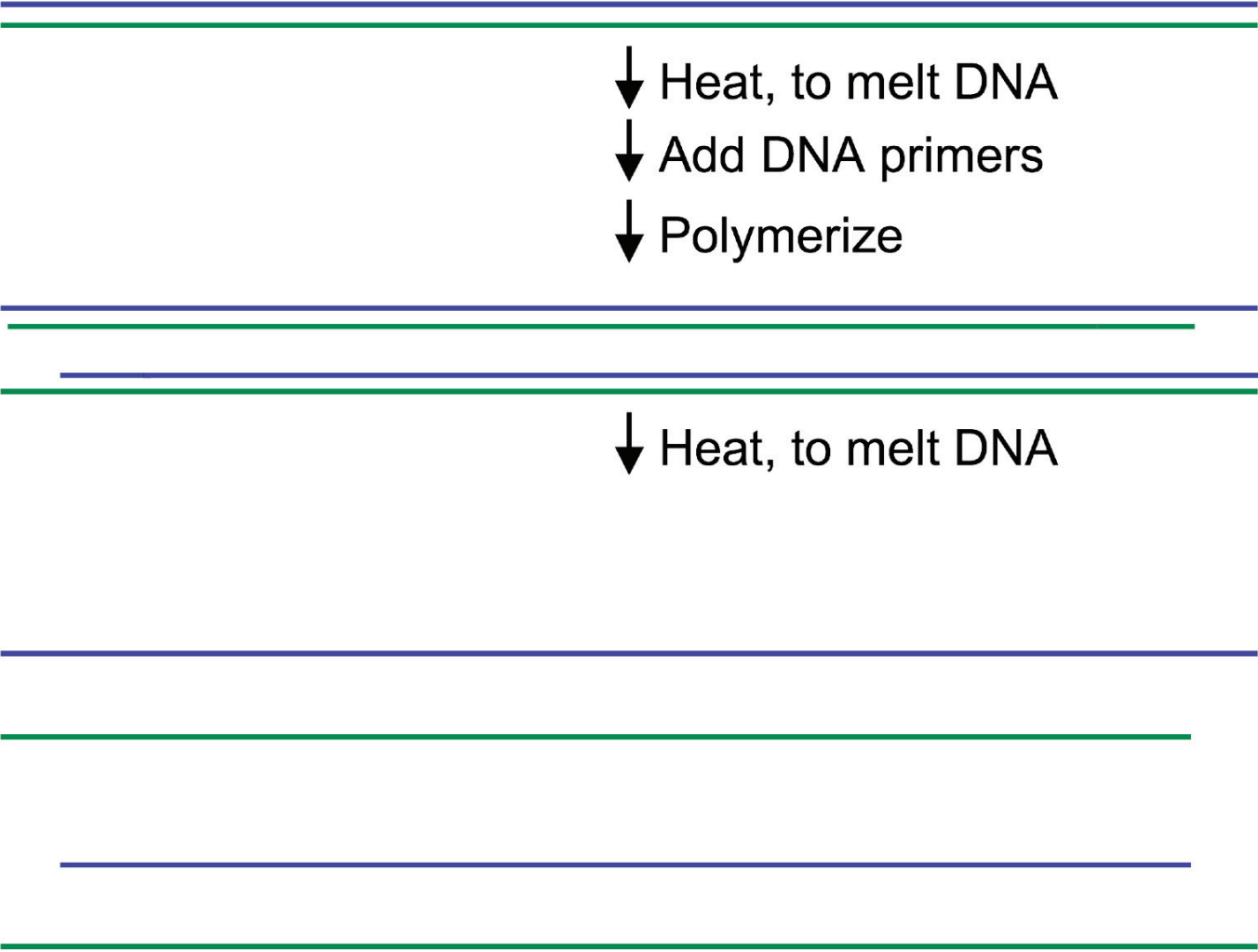
↓ Heat, to melt DNA
↓ Add DNA primers

— —

PCR - Polymerase Chain Reaction

-
-
- ↓ Heat, to melt DNA
 - ↓ Add DNA primers
 - ↓ Polymerize
-
-
-
-

PCR - Polymerase Chain Reaction

- 
- The diagram illustrates the PCR process using horizontal lines to represent DNA strands. The top section shows a double-stranded DNA molecule with a blue top strand and a green bottom strand. Below it, three steps are listed with downward arrows: 'Heat, to melt DNA', 'Add DNA primers', and 'Polymerize'. The second section shows the DNA strands separated, with a blue primer bound to the top strand and a green primer bound to the bottom strand. Below this, another 'Heat, to melt DNA' step is shown, with the strands separated again. The bottom section shows the final state with a blue primer bound to the top strand and a green primer bound to the bottom strand, with a gap between them indicating the site of polymerization.
- ↓ Heat, to melt DNA
 - ↓ Add DNA primers
 - ↓ Polymerize

↓ Heat, to melt DNA

PCR - Polymerase Chain Reaction

- ↓ Heat, to melt DNA
- ↓ Add DNA primers
- ↓ Polymerize

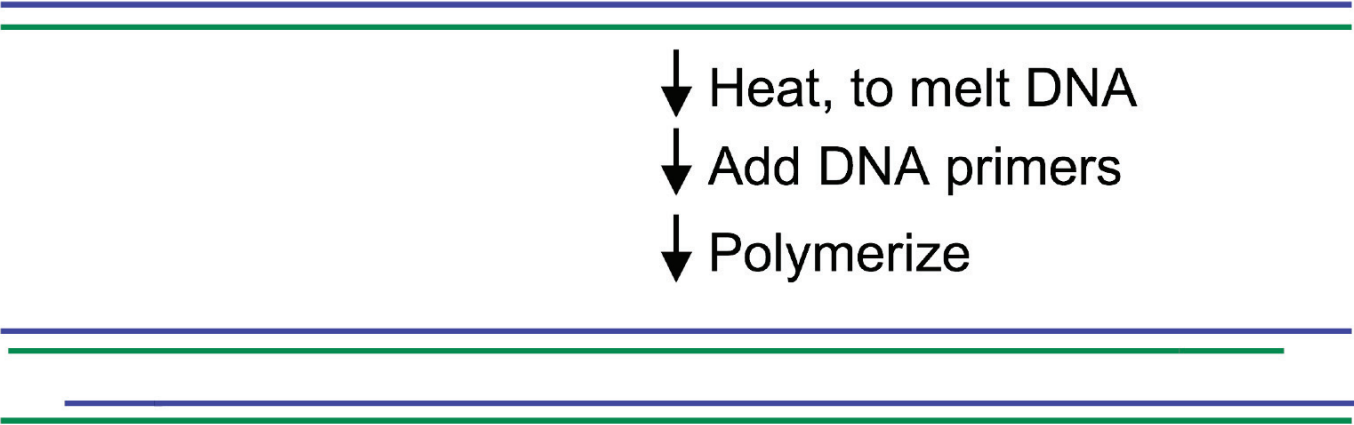
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PCR - Polymerase Chain Reaction

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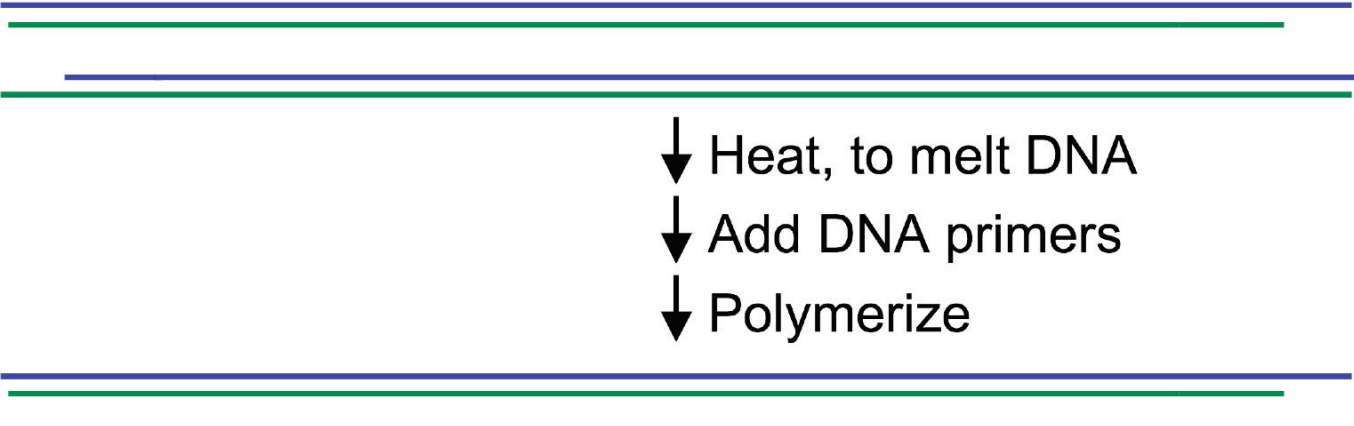
↓ Heat, to melt DNA
↓ Add DNA primers
↓ Polymerize

PCR - Polymerase Chain Reaction



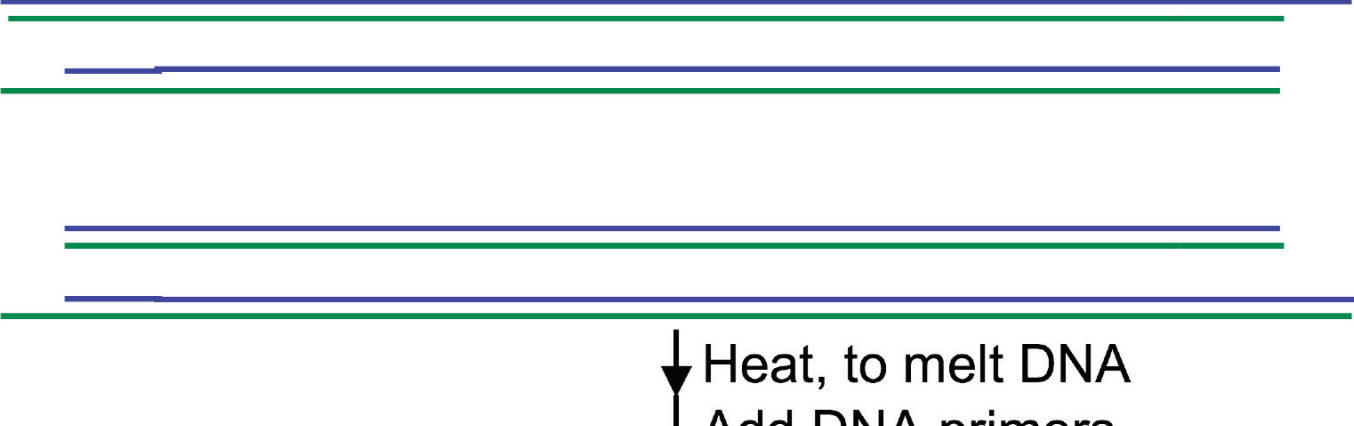
↓ Heat, to melt DNA
↓ Add DNA primers
↓ Polymerize

The diagram shows a pair of horizontal lines representing DNA. The top line is blue and the bottom line is green. A vertical arrow points down from the center of the lines, indicating the start of the first cycle.



↓ Heat, to melt DNA
↓ Add DNA primers
↓ Polymerize

The diagram shows two pairs of horizontal lines representing DNA. The top pair is blue and the bottom pair is green. A vertical arrow points down from the center of the lines, indicating the start of the second cycle.



↓ Heat, to melt DNA
↓ Add DNA primers
↓ Polymerize

The diagram shows four pairs of horizontal lines representing DNA. The top pair is blue and the bottom pair is green. A vertical arrow points down from the center of the lines, indicating the start of the third cycle.

PCR - Polymerase Chain Reaction

After 2 cycles, original DNA is amplified $2^2 = 4$ fold

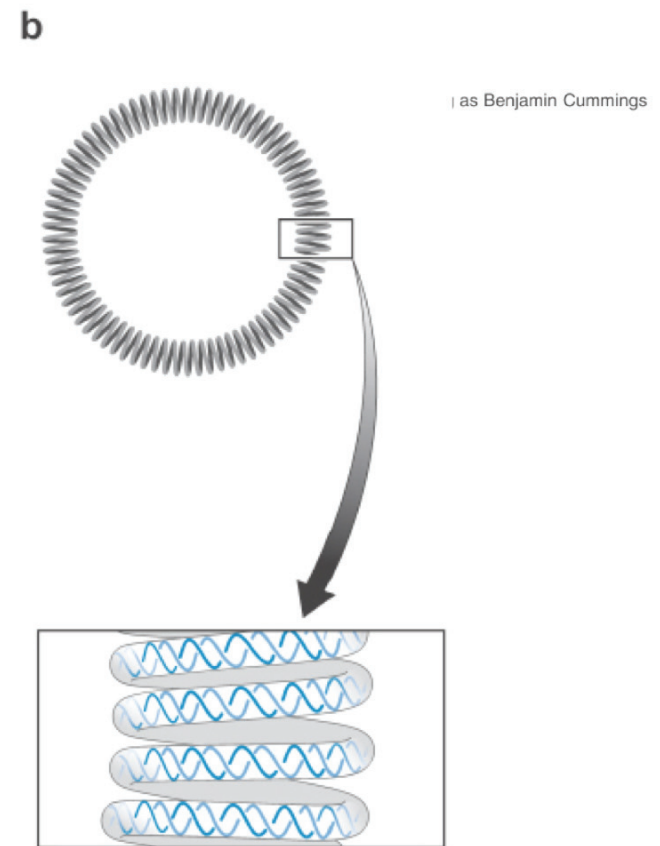
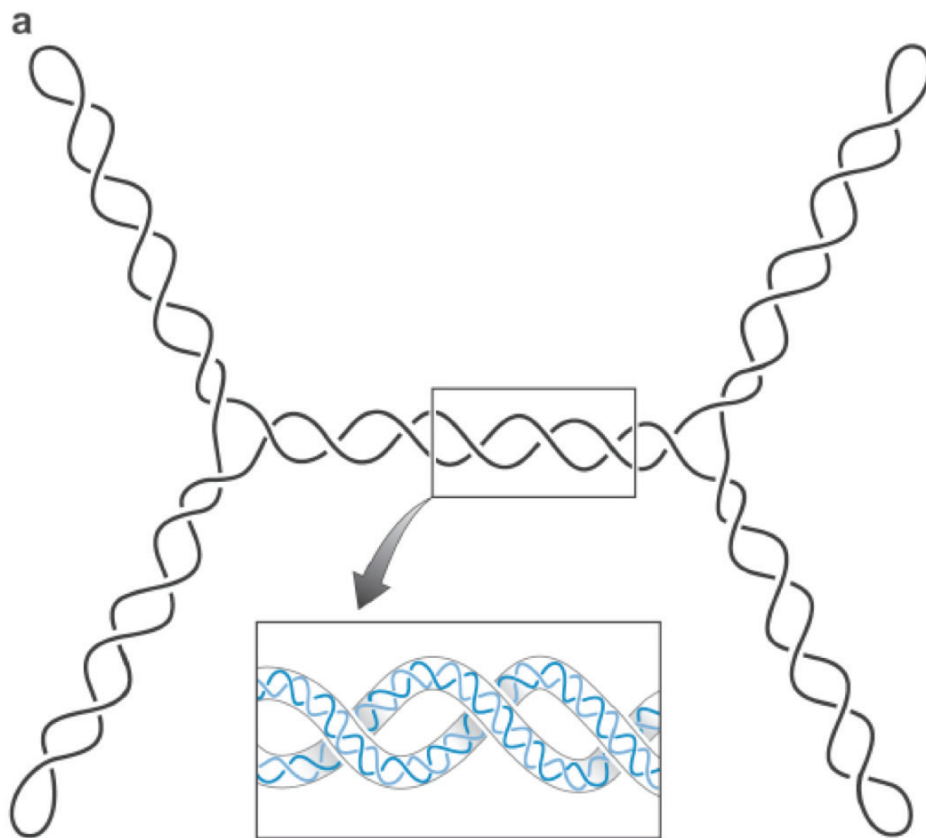
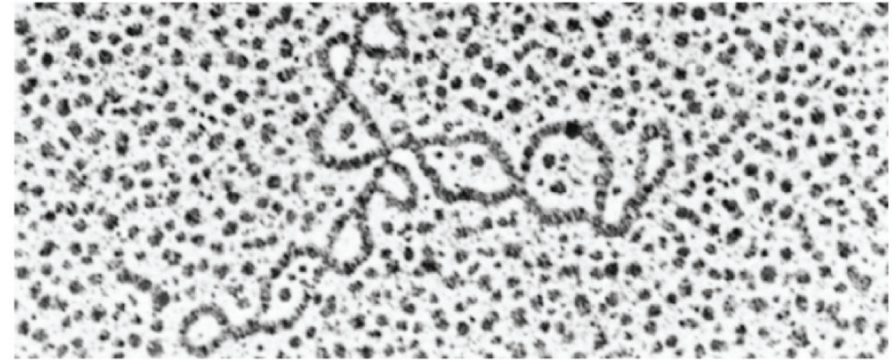
After 15 cycles, original DNA is amplified $2^{15} = 32,800$ fold

After 20 cycles, original DNA is amplified $2^{20} = 1,048,000$ fold

Applications:

- 1) Cloning DNA from small samples (old dinosaurs, etc)
- 2) Cloning, sequencing crime scene DNA
- 3) Combinatorial chemistry / genetics
- 4) many other uses

Supercoiling



Supercoiling

