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.
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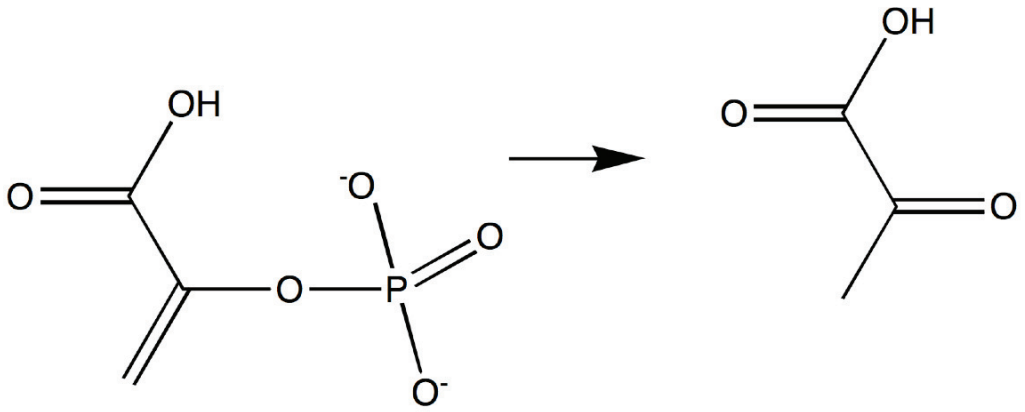

1) aldolase
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3) pyruvate kinase
4) phosphofructokinase
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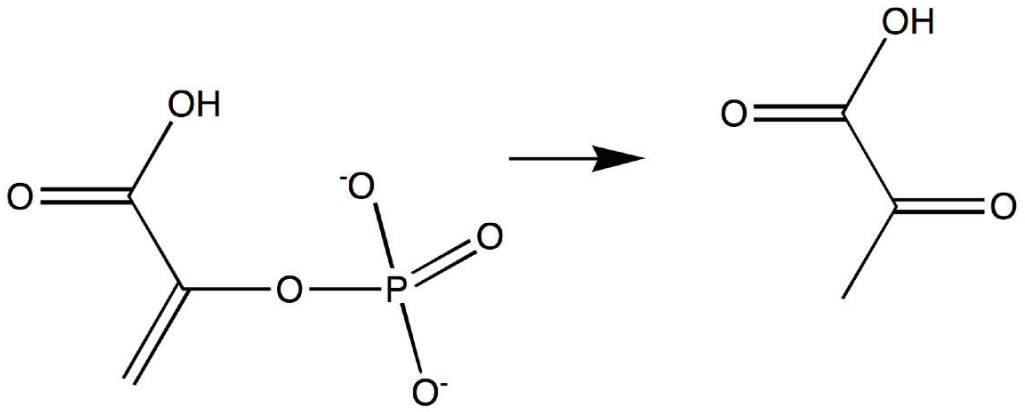

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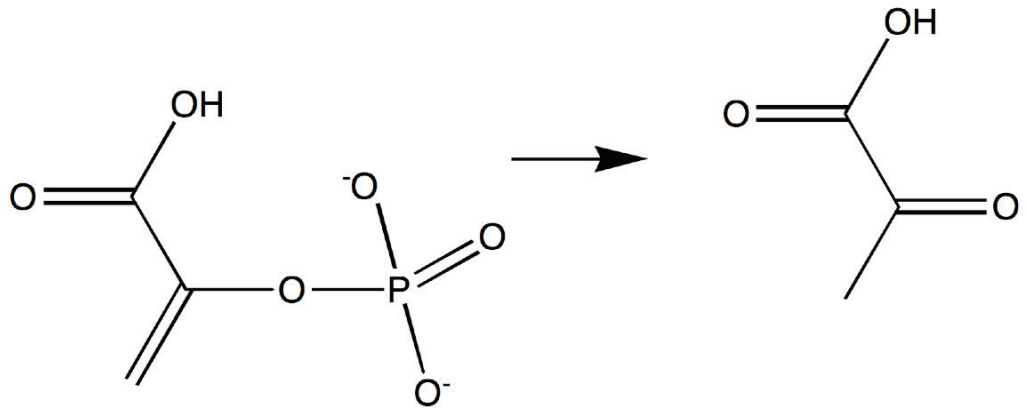
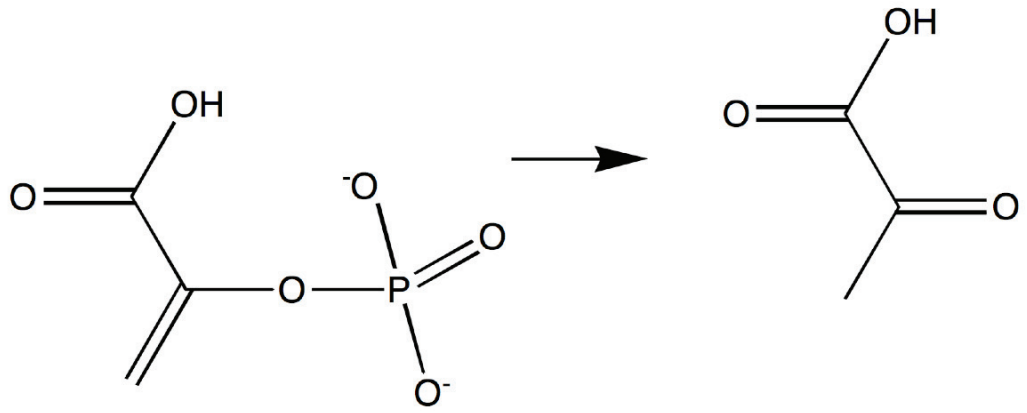


Look at this reaction in reverse. How do you phosphorylate a ketone??



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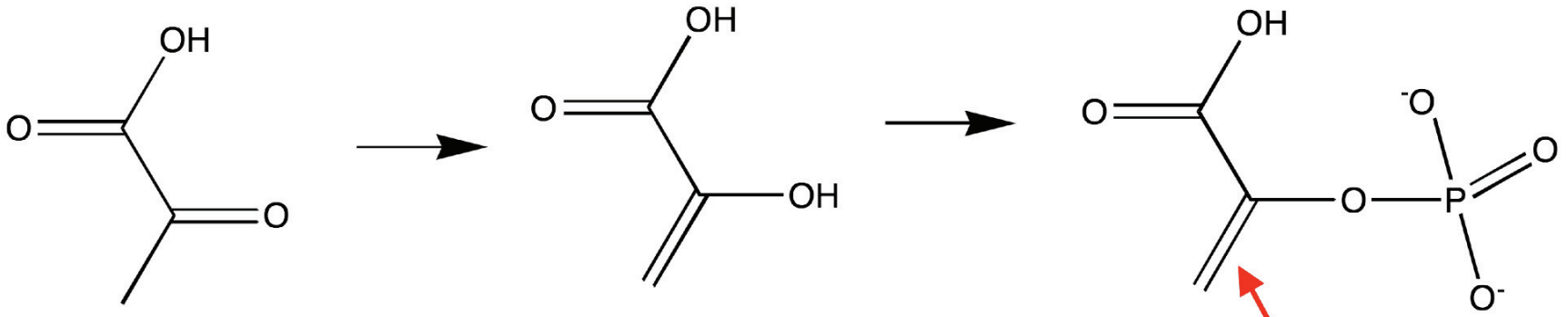
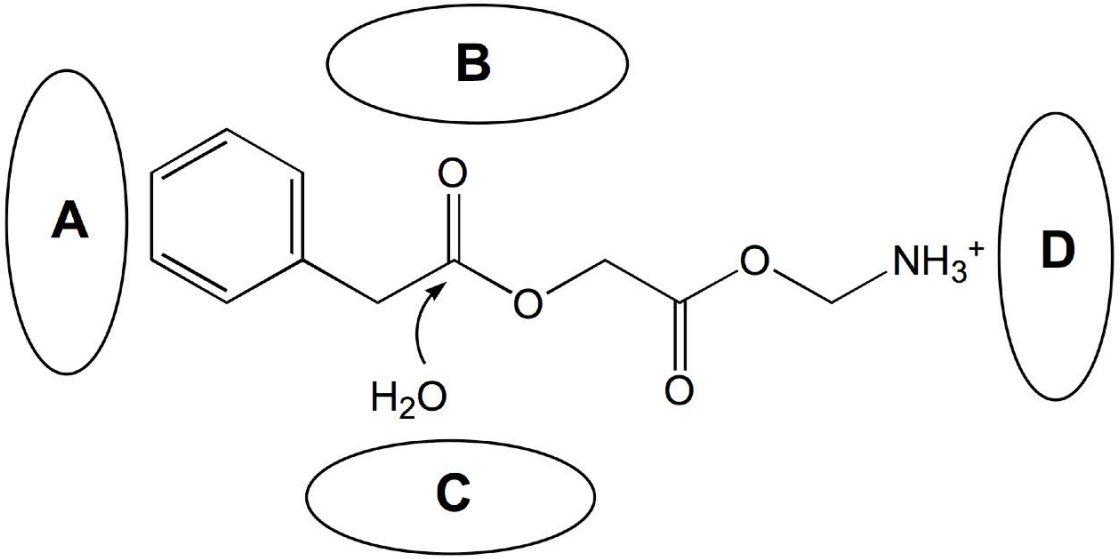


Answer: keto-enol isomerization!

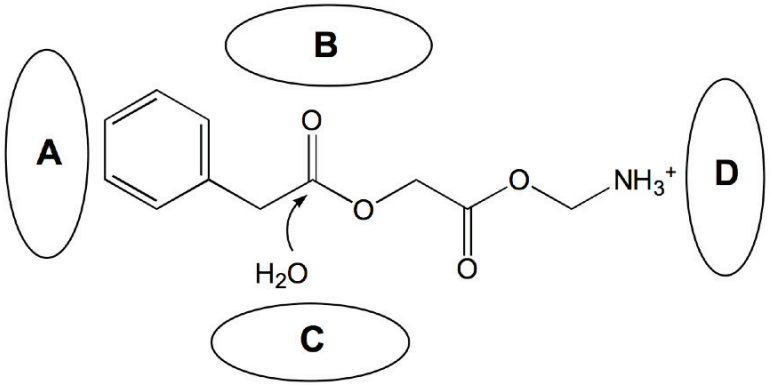
This was the clue!

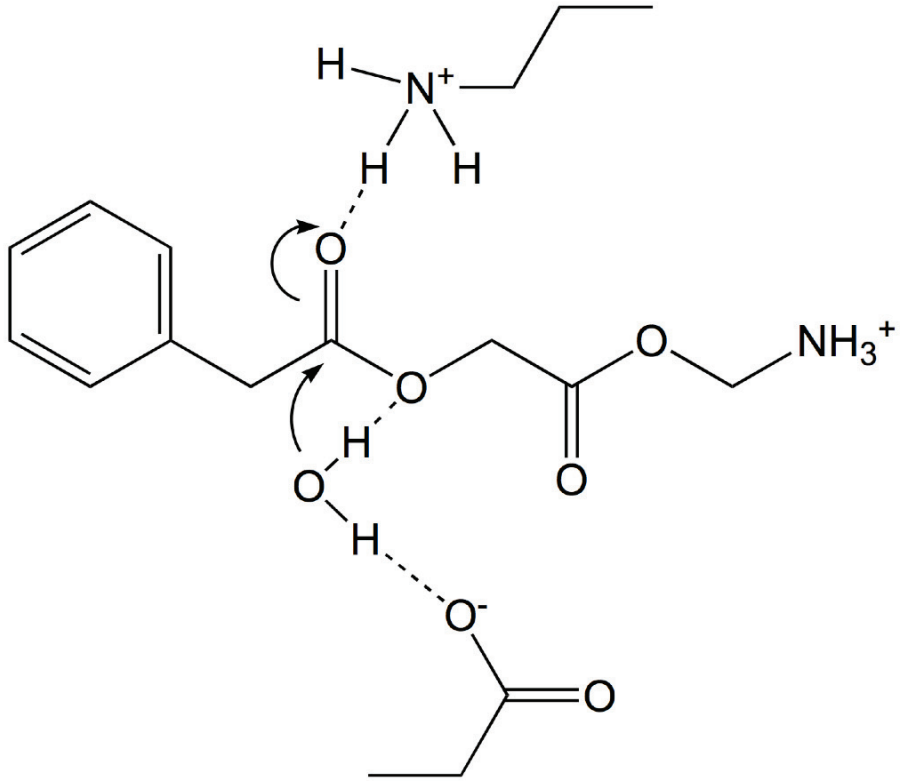


Consider hydrolysis of the ester above

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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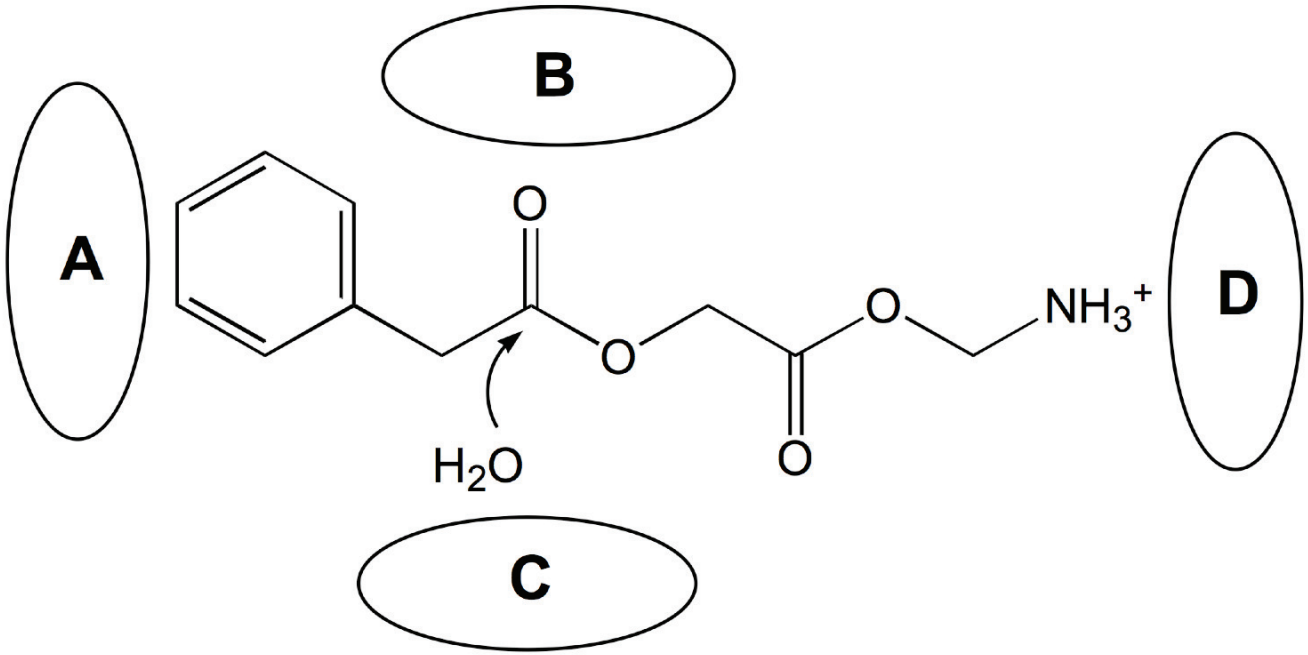

Consider hydrolysis of the ester above

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
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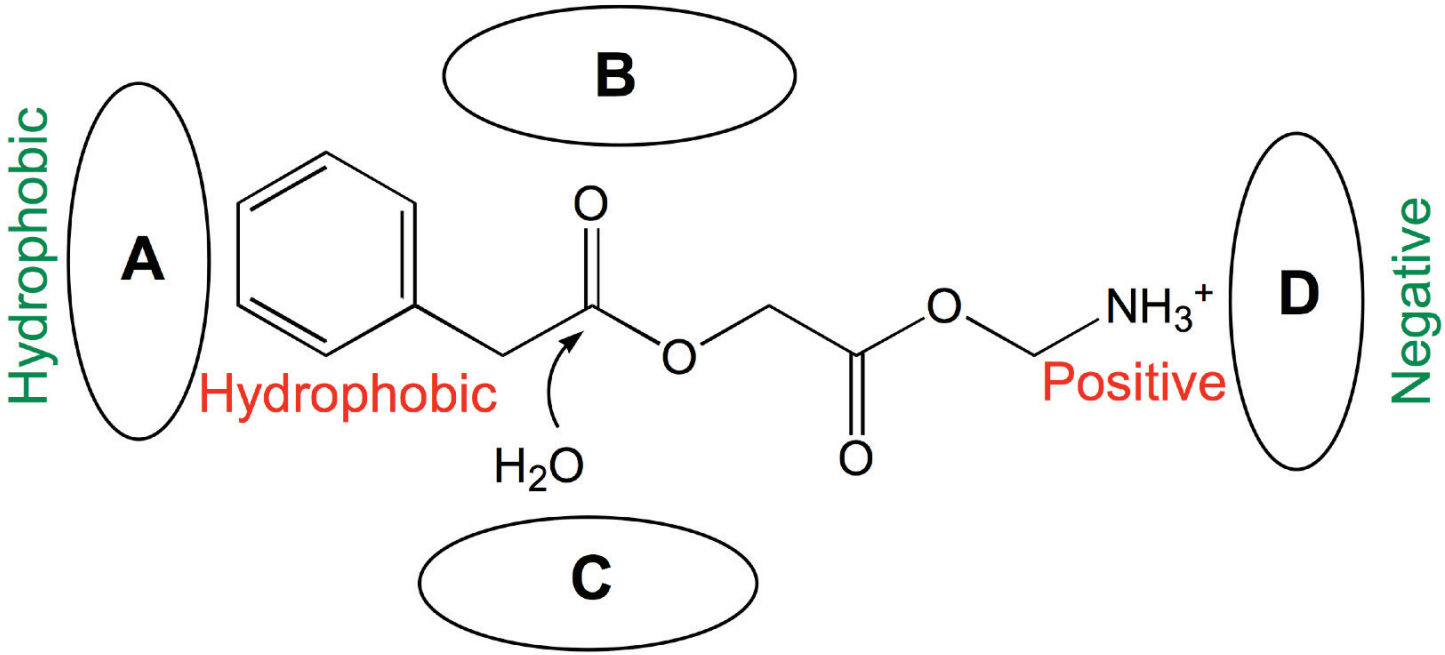

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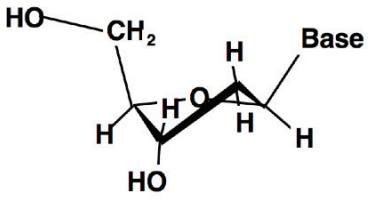
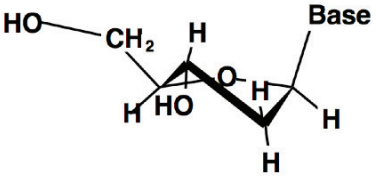


C2' endo – C3' exo



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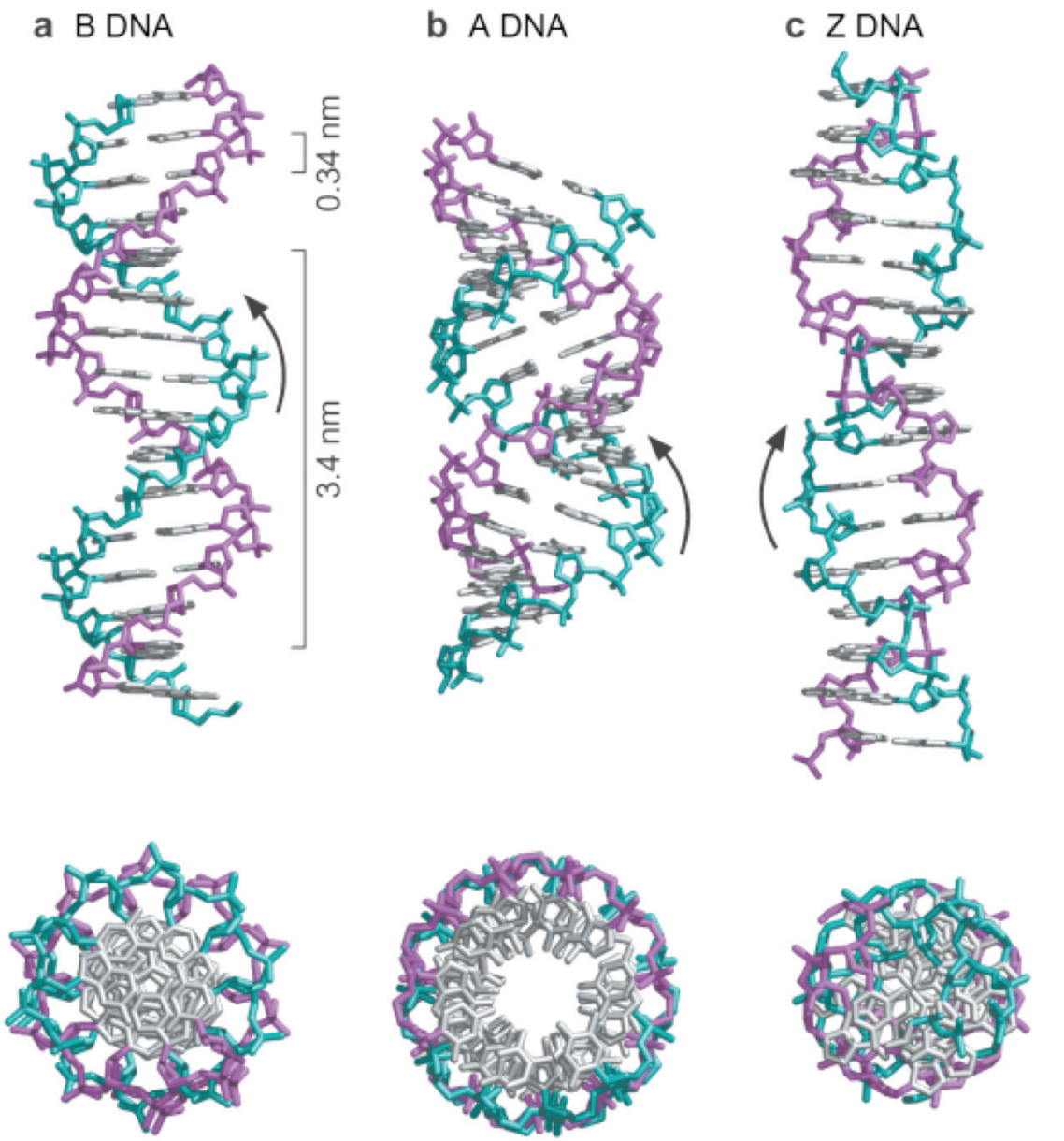
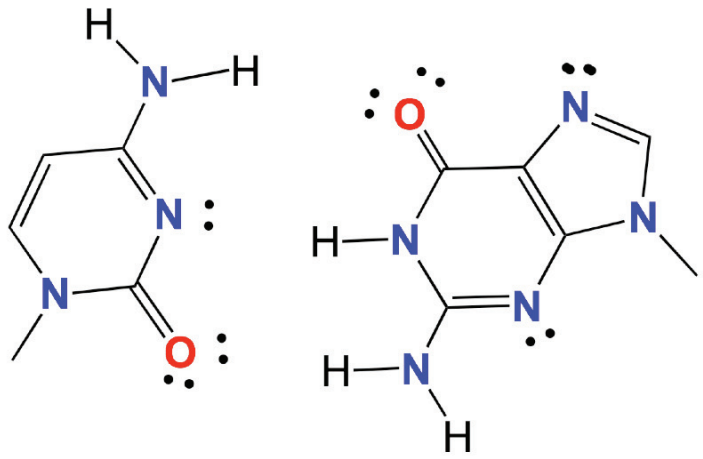


Why is Watson-Crick so good?

C



G

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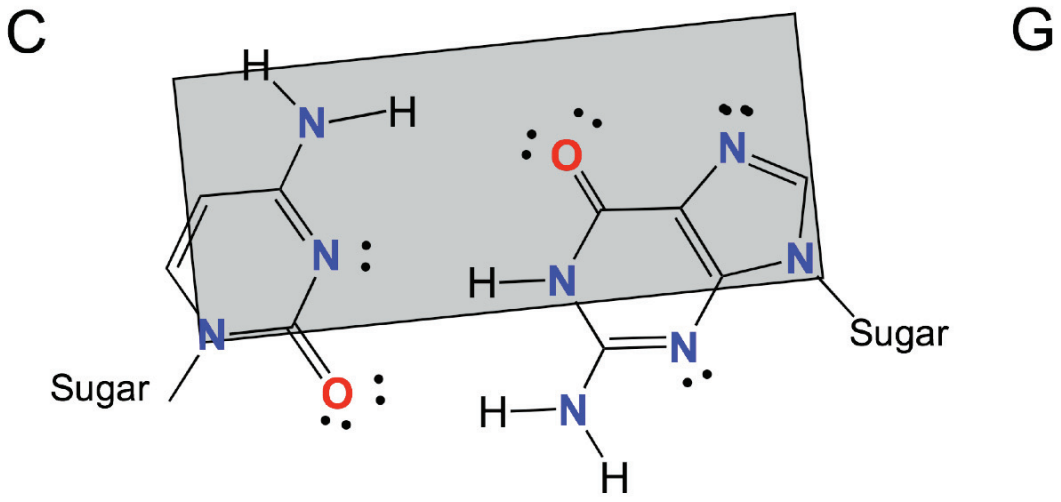

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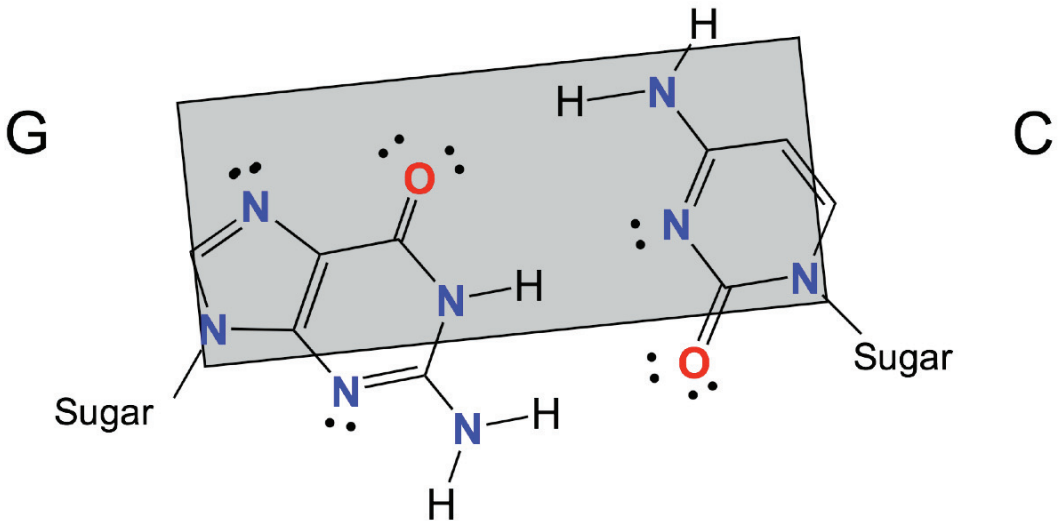

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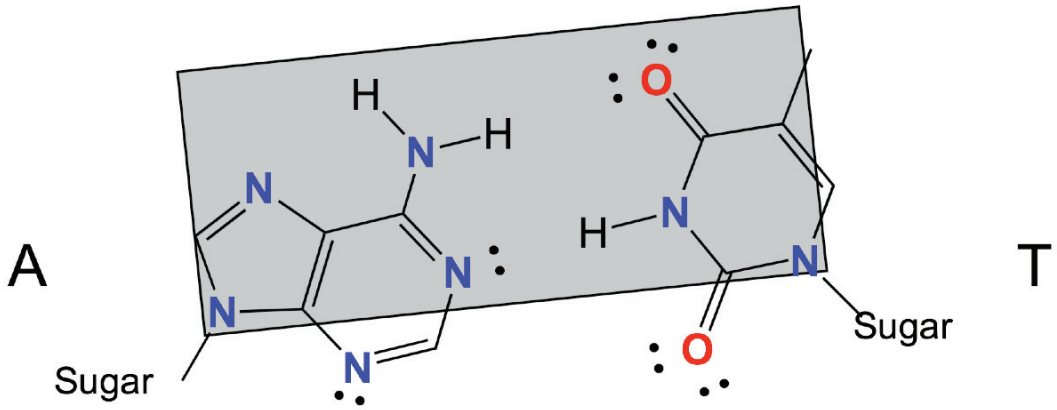

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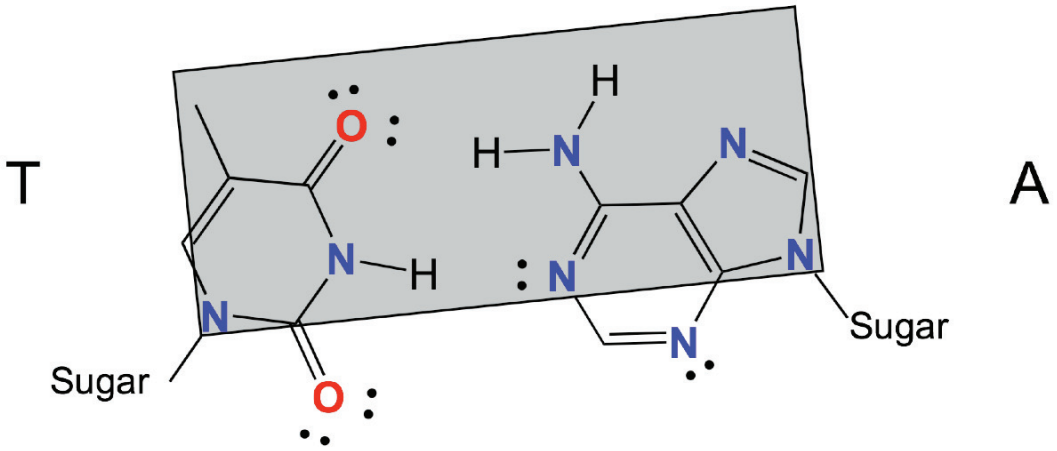

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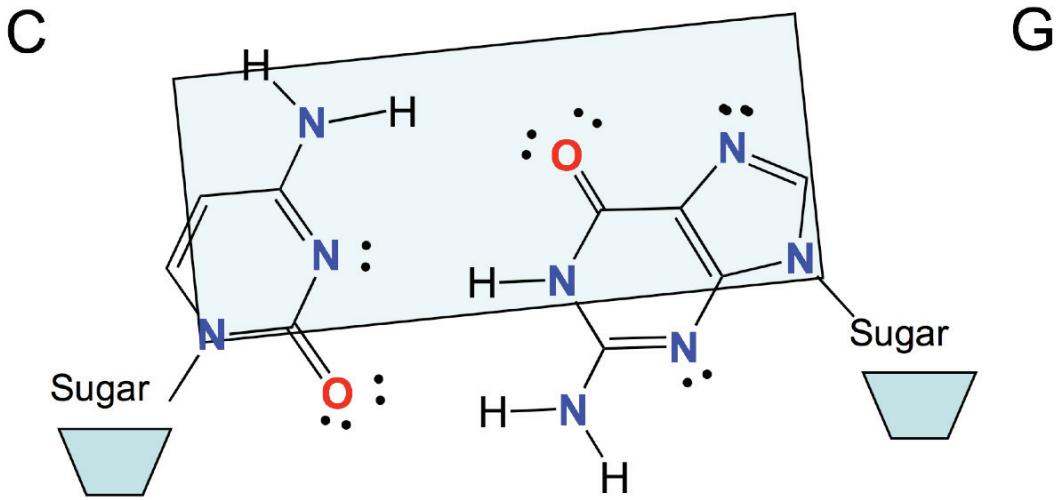

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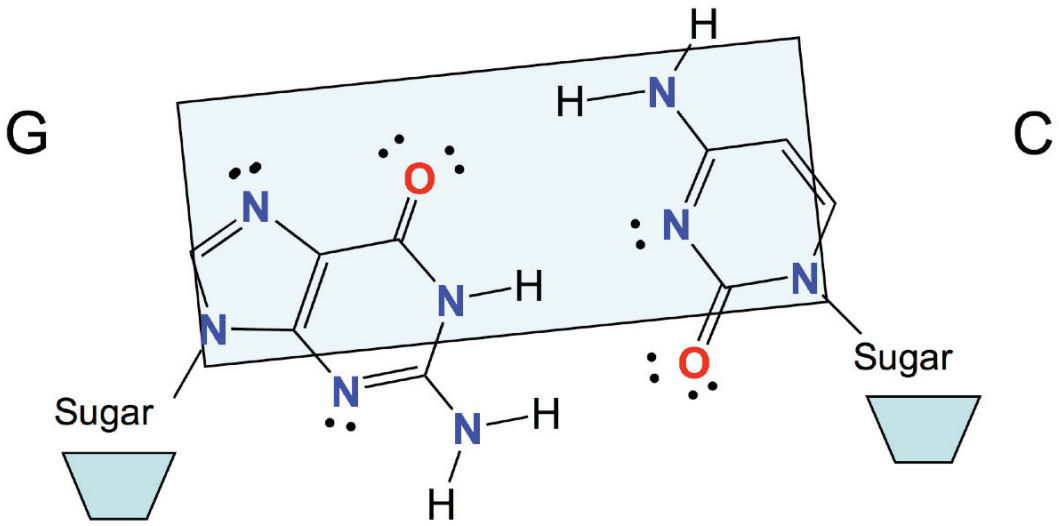

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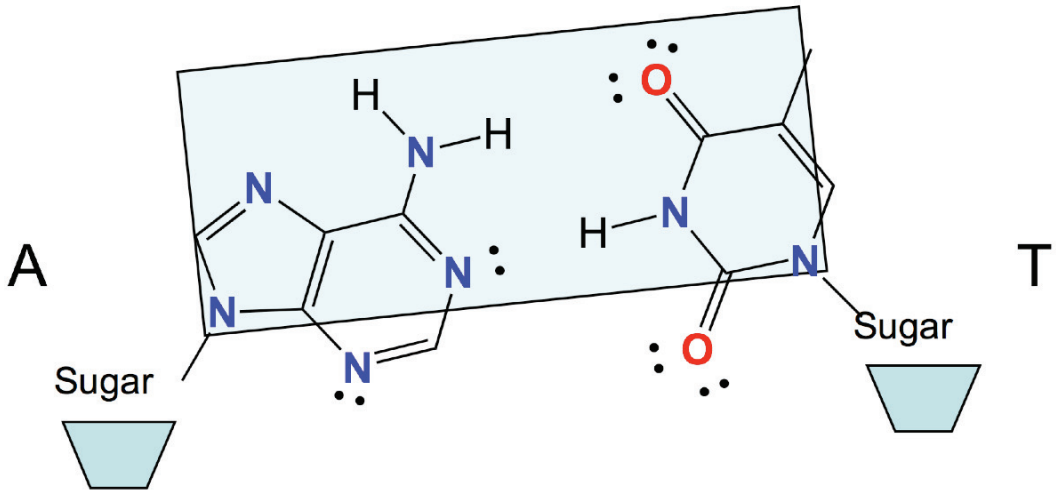

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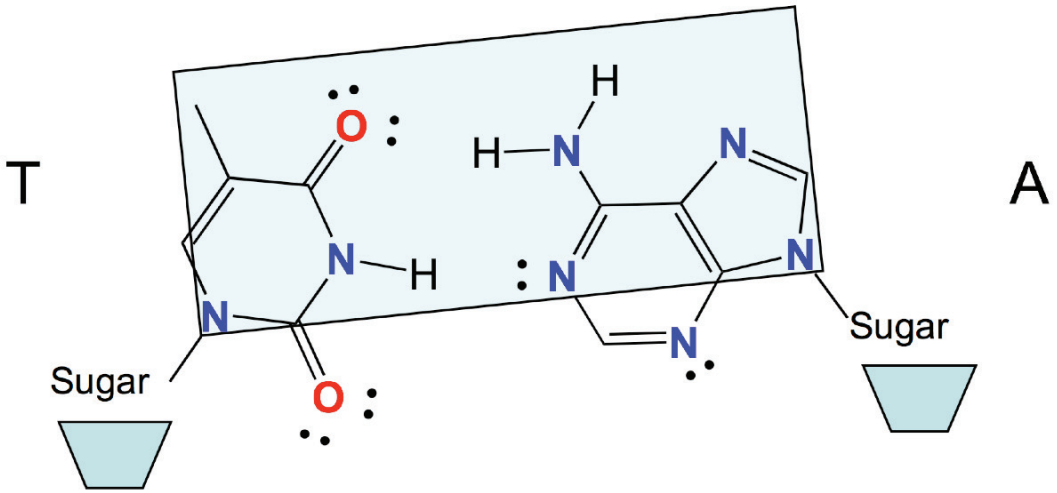
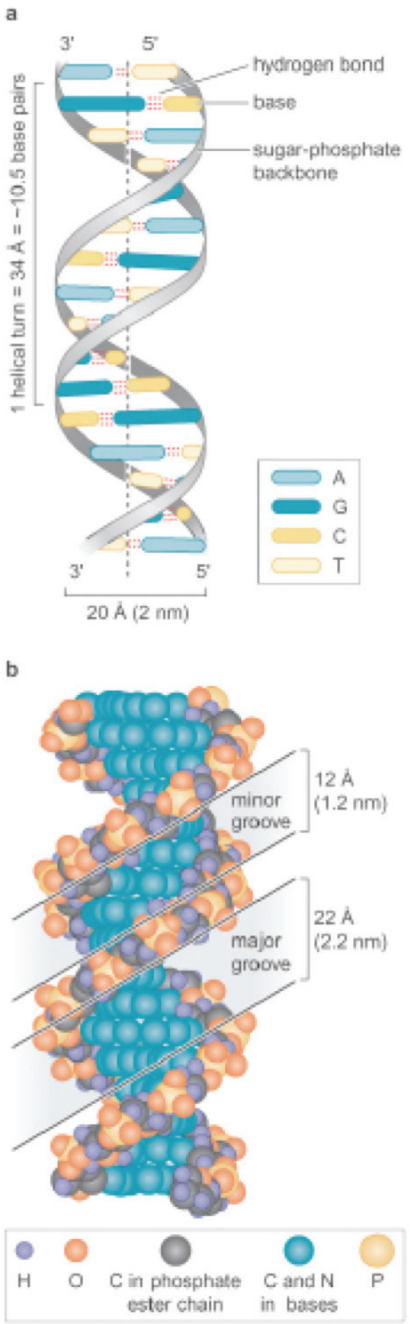


B-form in 3D

A-form in 3D

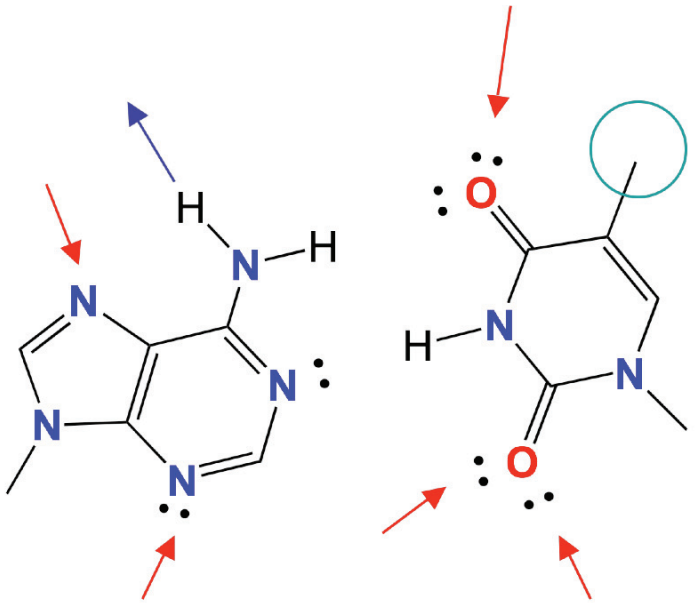
More in 3D



Why is the major groove so good?

Major Groove

A



T

Minor Groove

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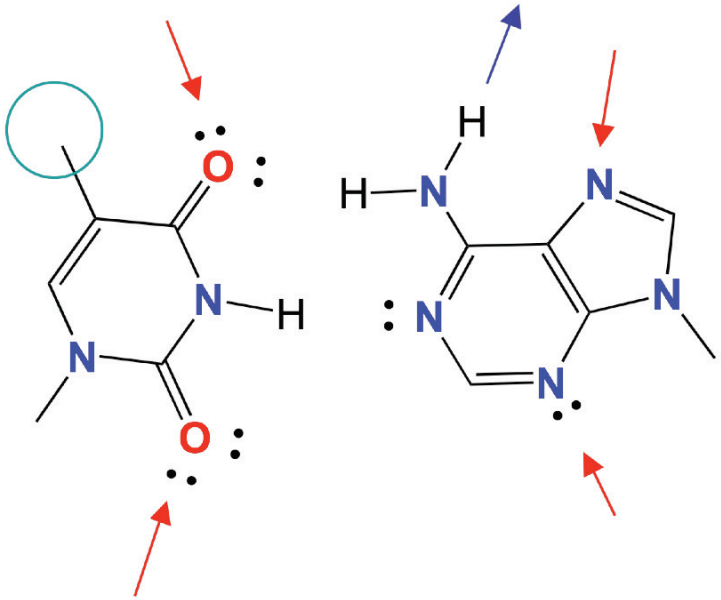
Major Groove

T



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Minor Groove

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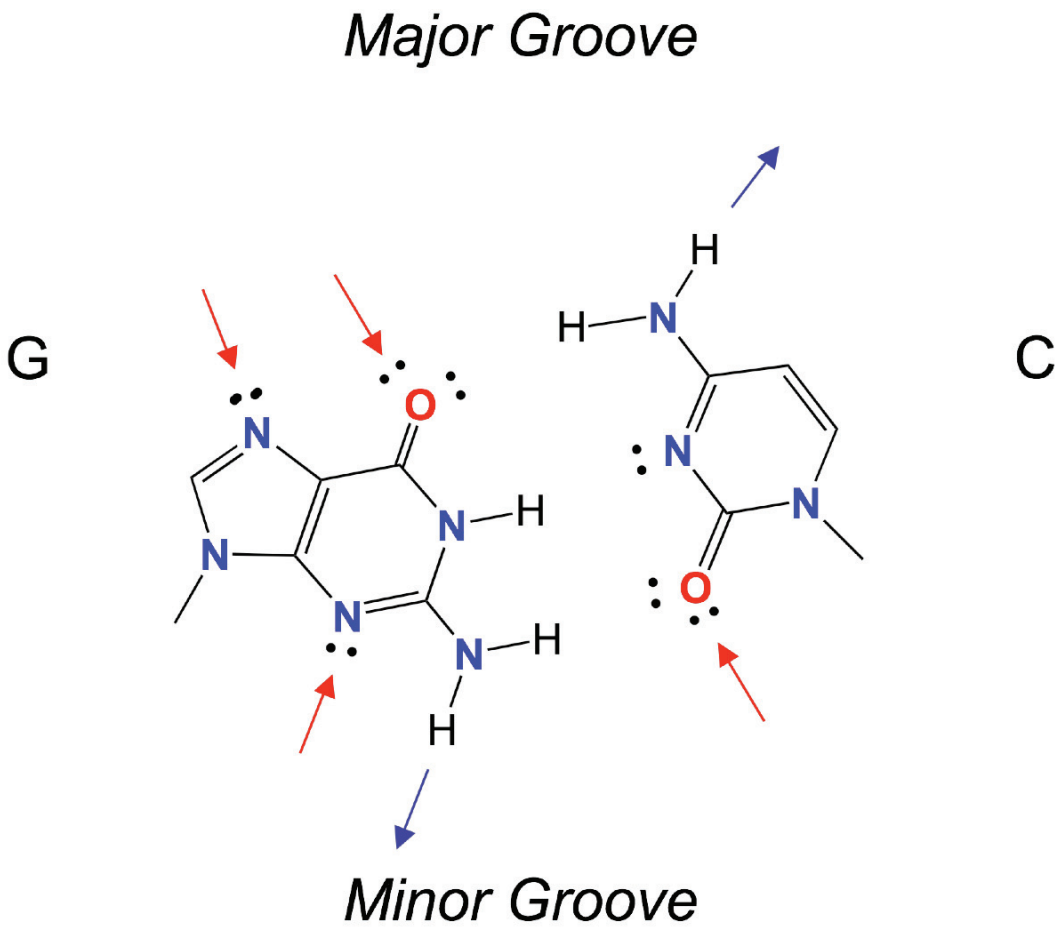

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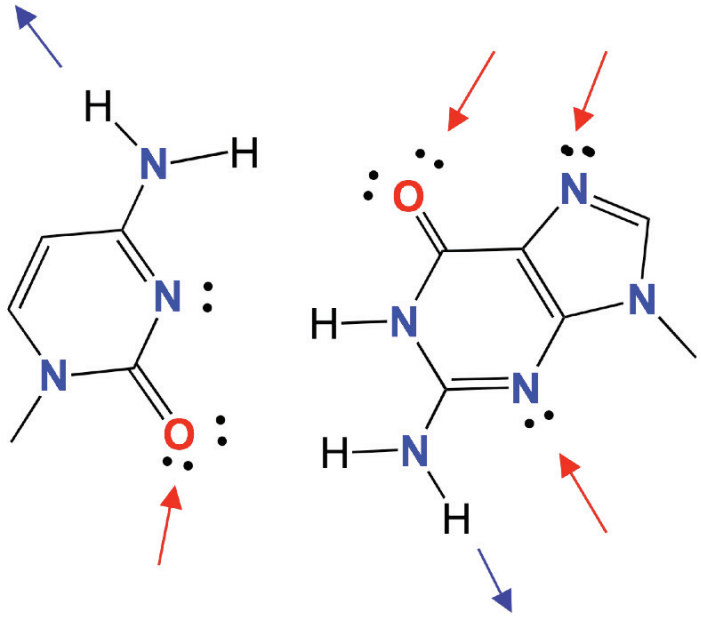


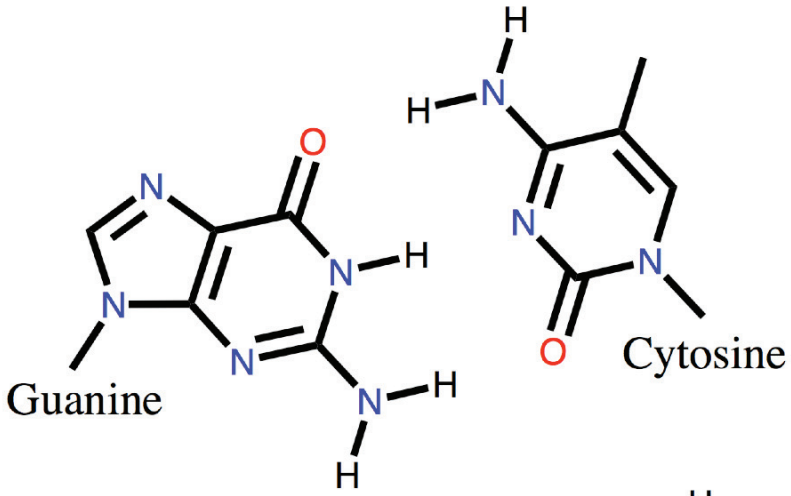
G

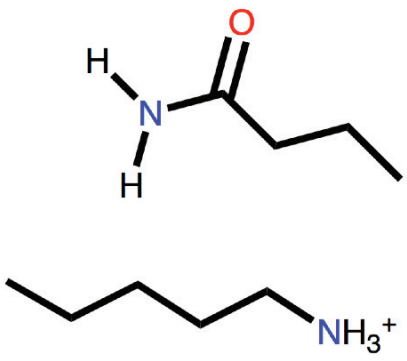
Minor Groove

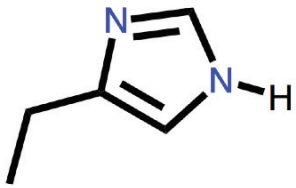
Sequence-specific binding by proteins

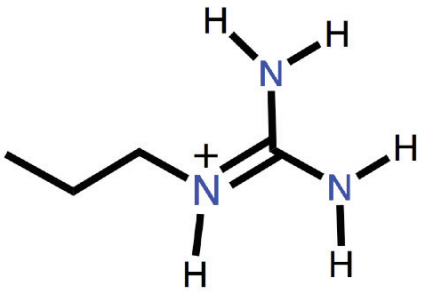
Find “two-fer” interactions

See an example



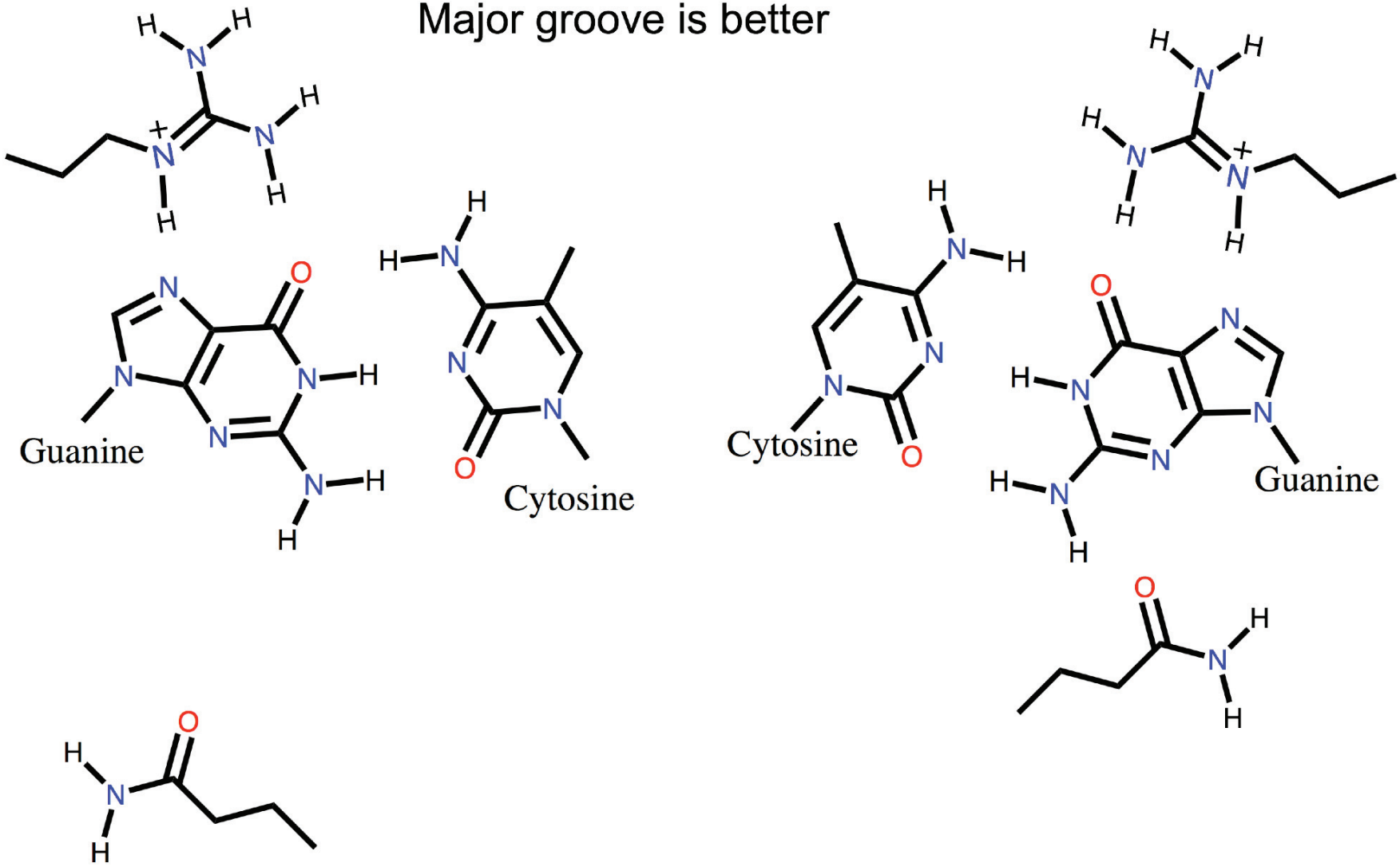






Sequence-specific binding by proteins

Major groove is better



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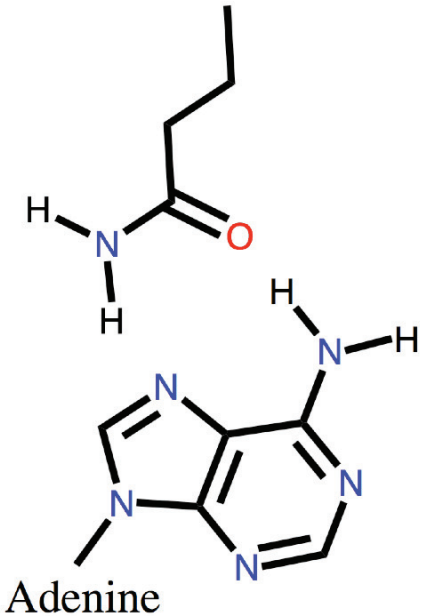
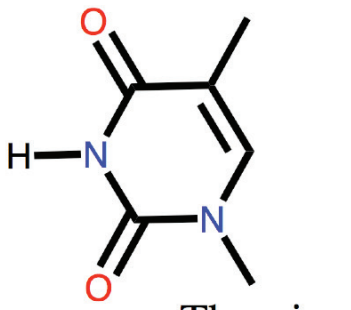
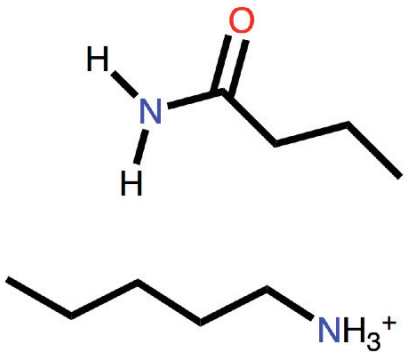
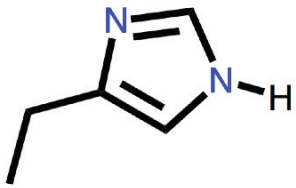


Adenine

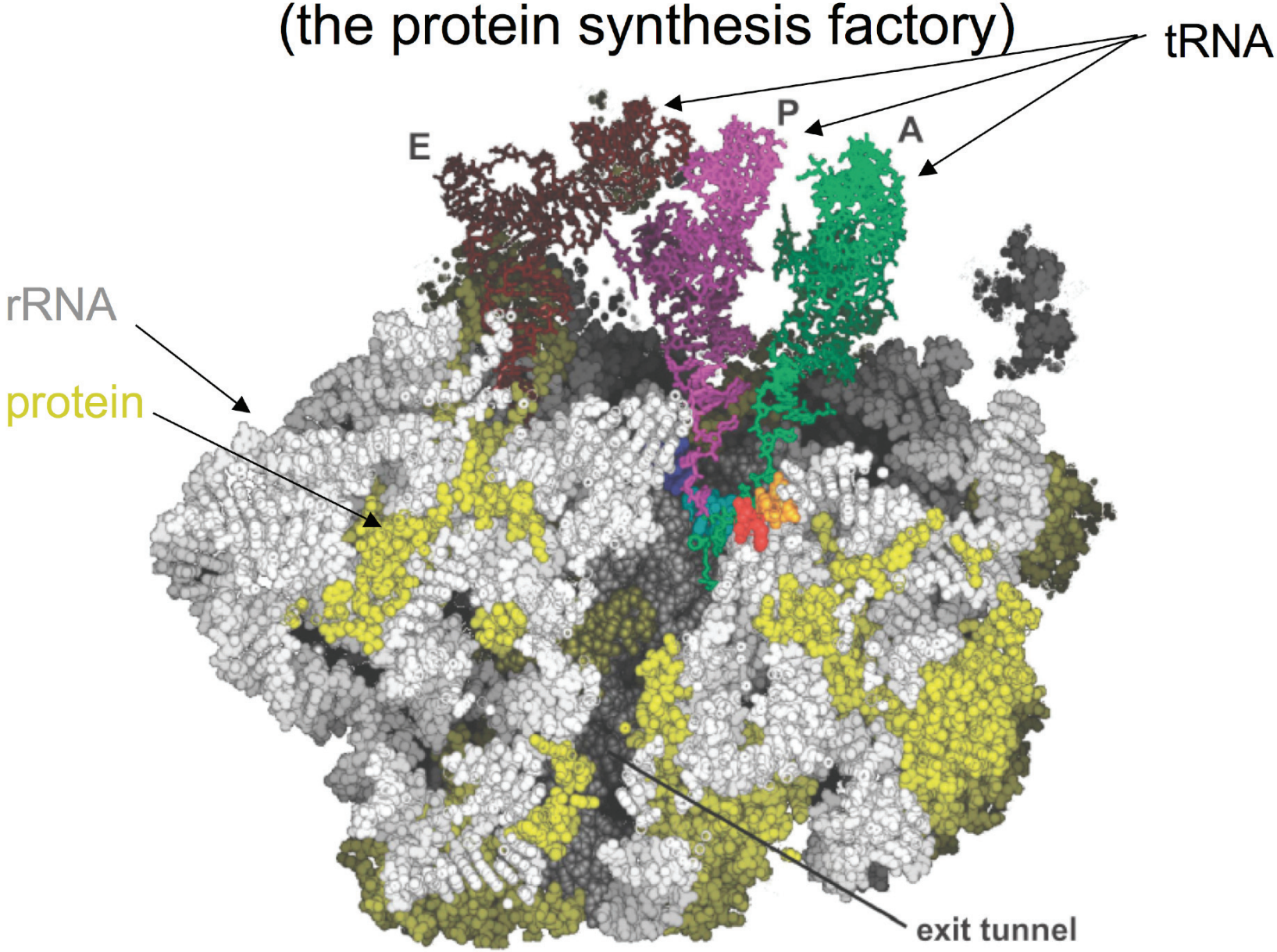


Thymine





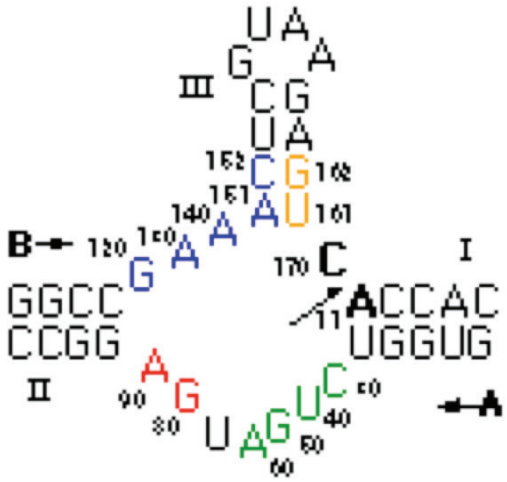
Ribosome
(the protein synthesis factory)

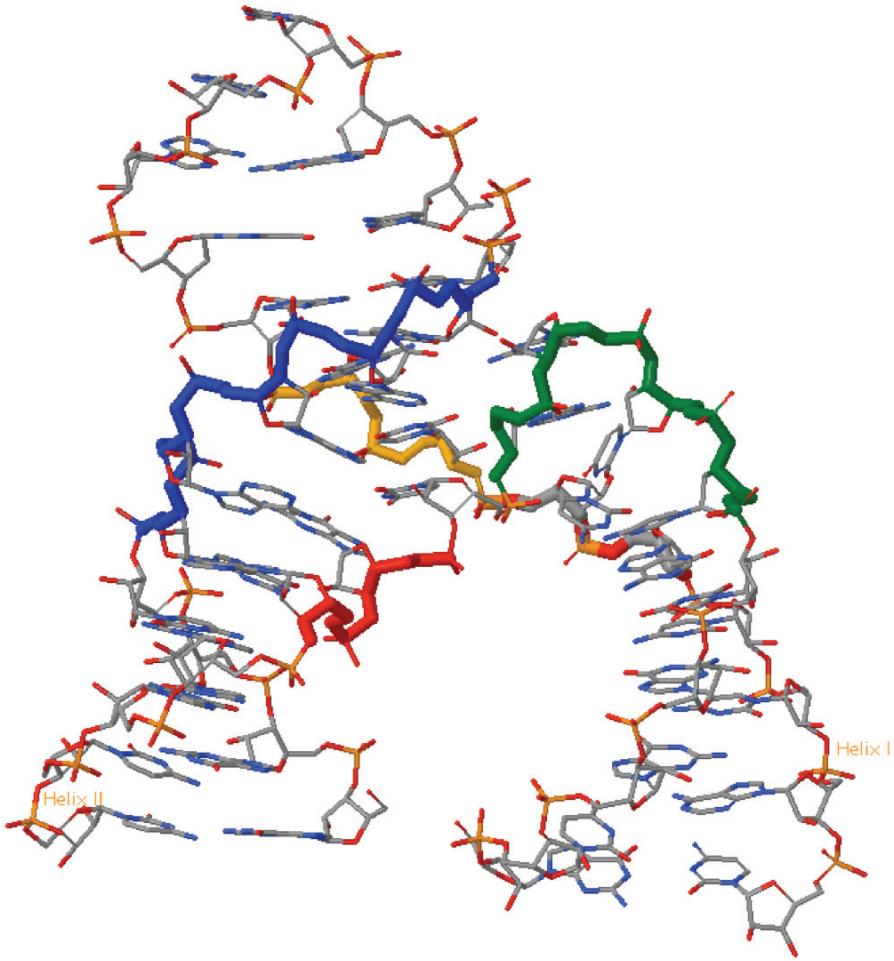


RNA structures are diverse!!

More in 3D

- "Hammerhead" ribozyme
  - An "enzyme" made of RNA





Classes of RNA

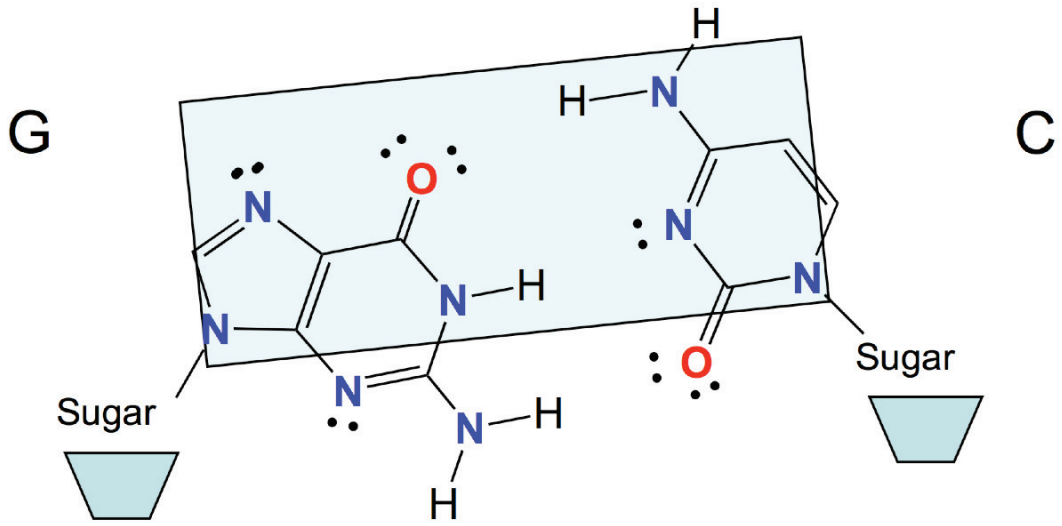
- mRNA - messenger RNA - encodes protein
- tRNA - transfer RNA - carries amino acids to the ribosome, recognizes a 3 base codon via Watson-Crick pairing
- rRNA - ribosomal RNA - forms the ribosome “enzyme” (with a little bit of protein here and there)
- snRNA - small nuclear RNA, a key part of small 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

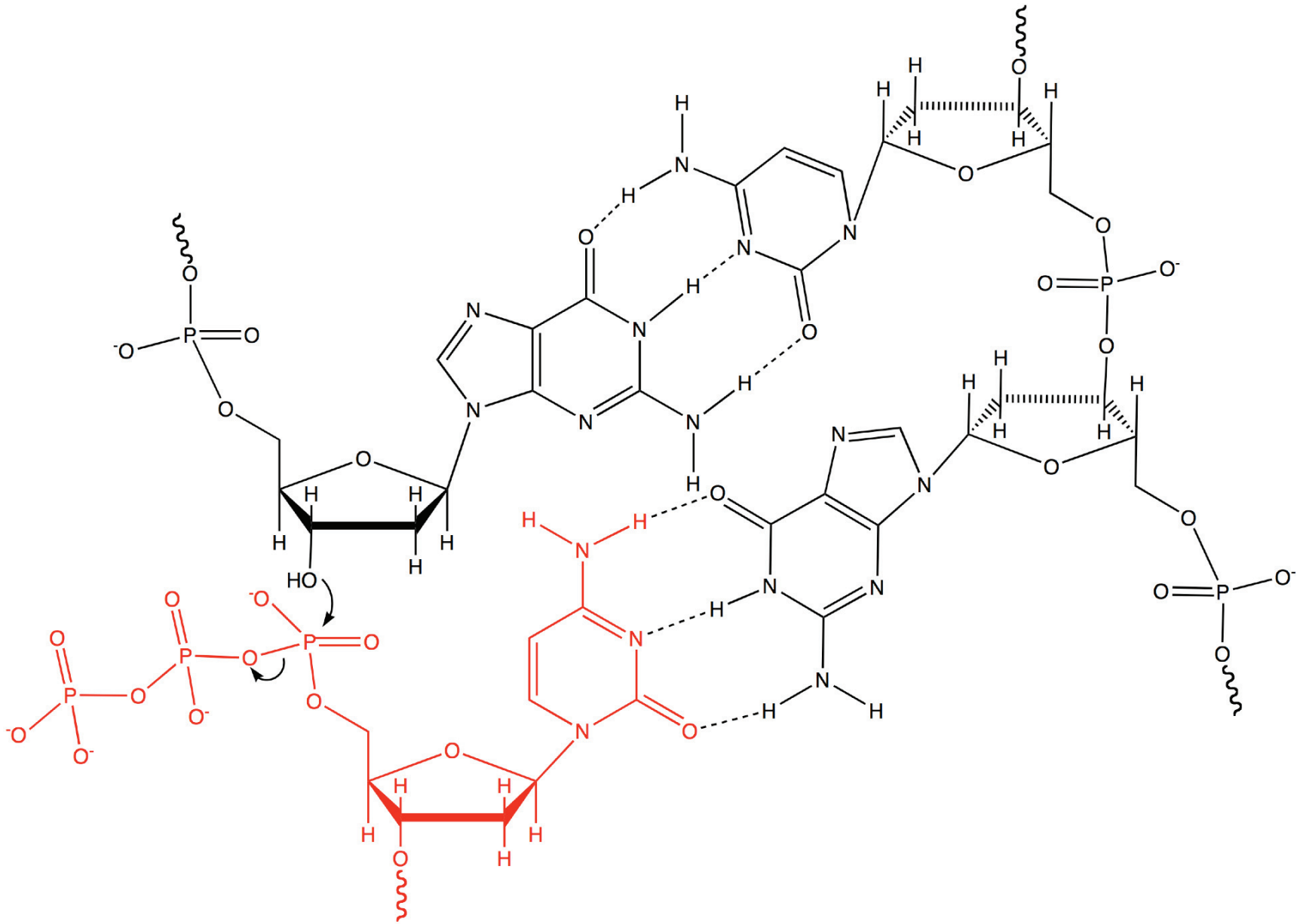
- DNA polymerase
  - makes new (permanent) DNA copies from a DNA template
  - When a cell divides, DNA polymerase makes a complete copy of the entire DNA genome



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

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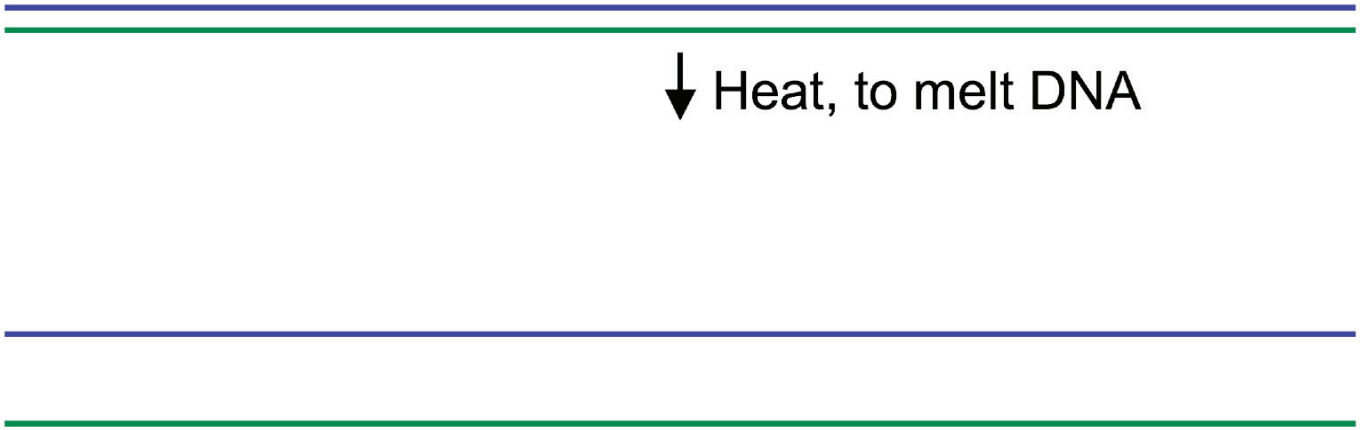
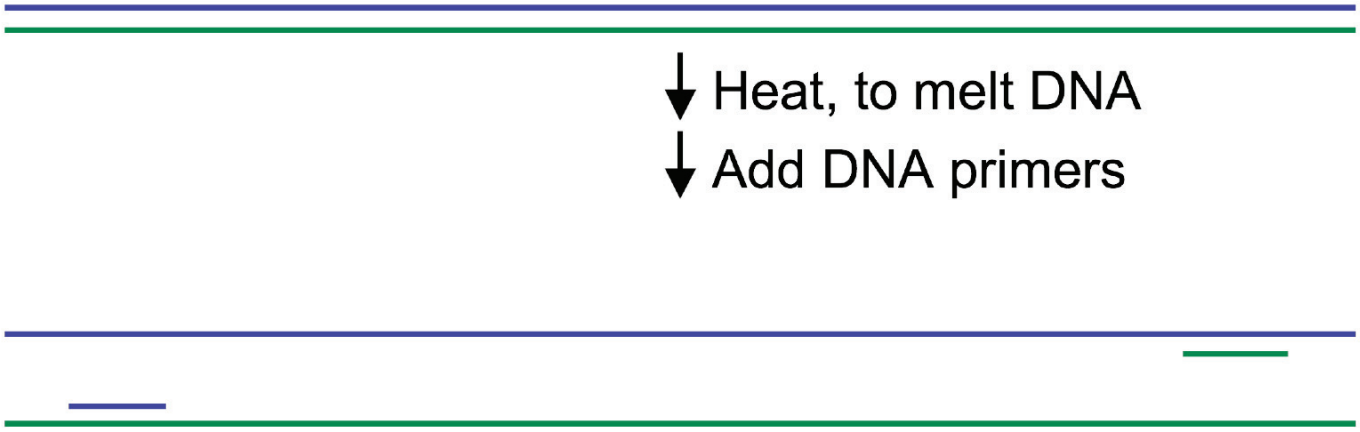


PCR - Polymerase Chain Reaction



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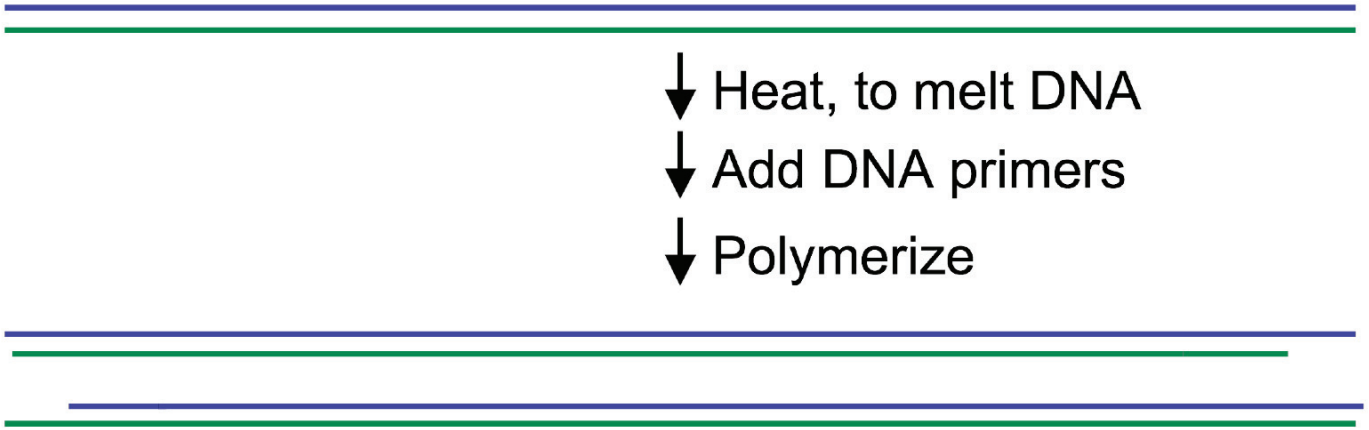
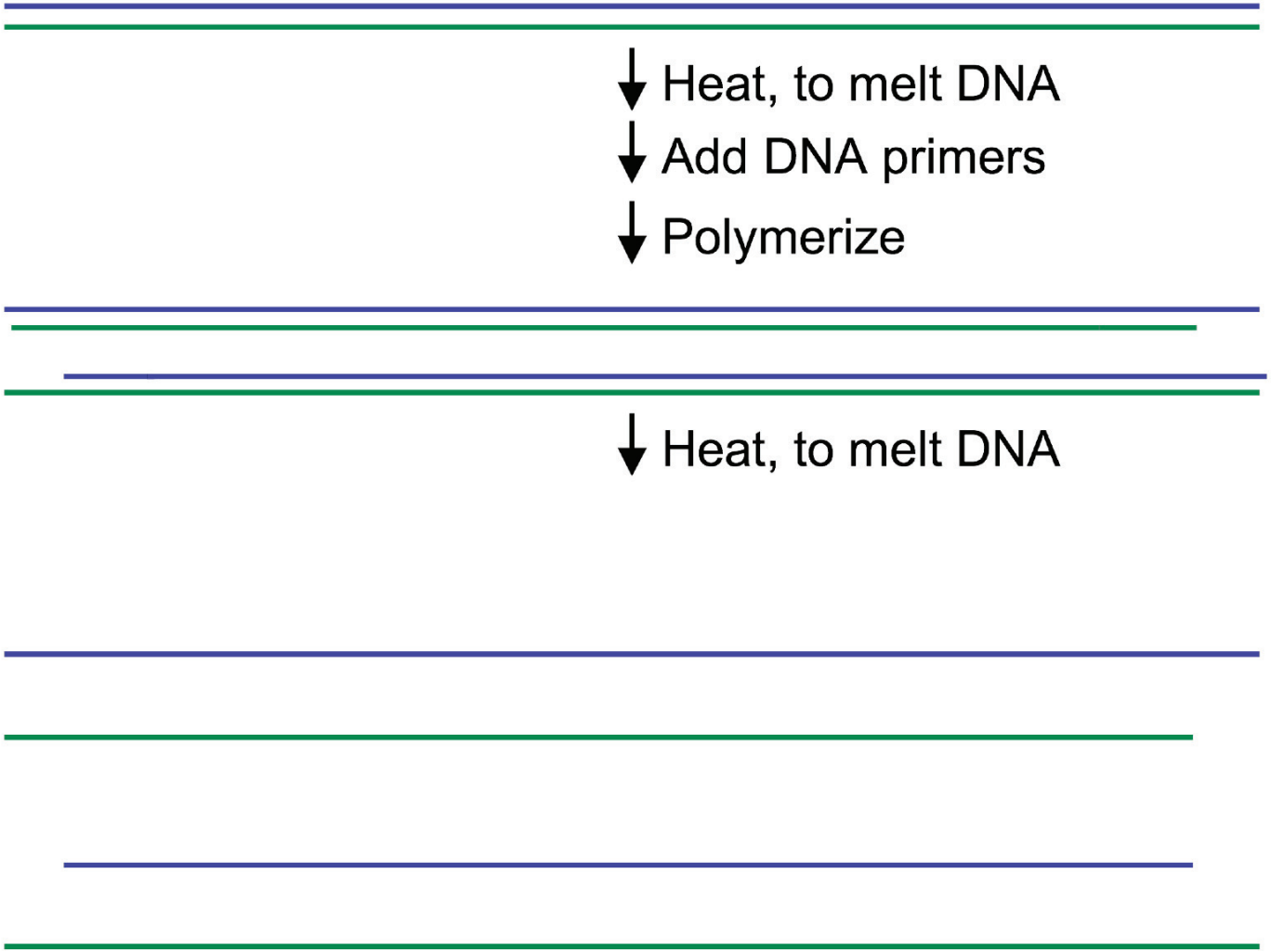


PCR - Polymerase Chain Reaction



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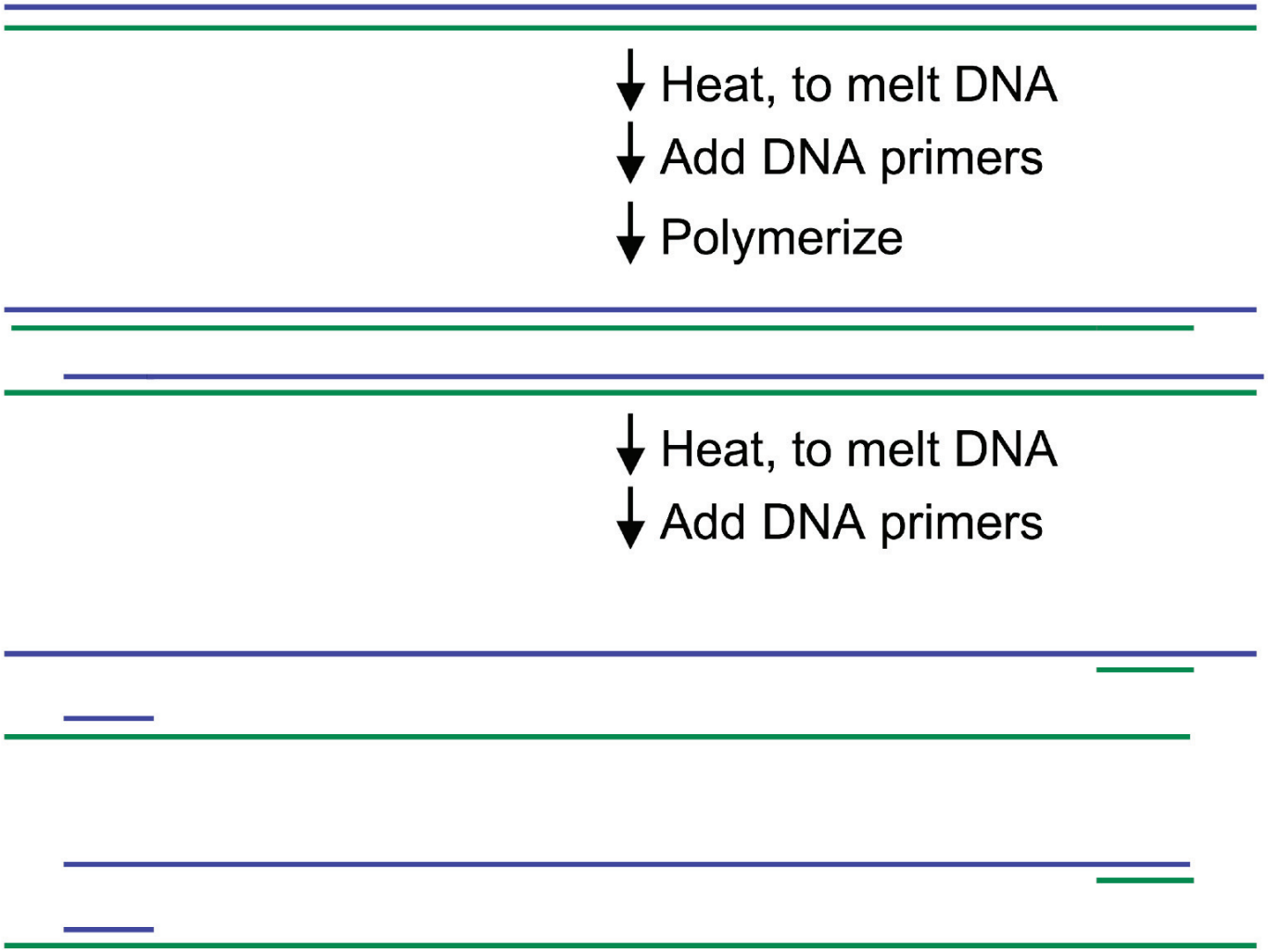
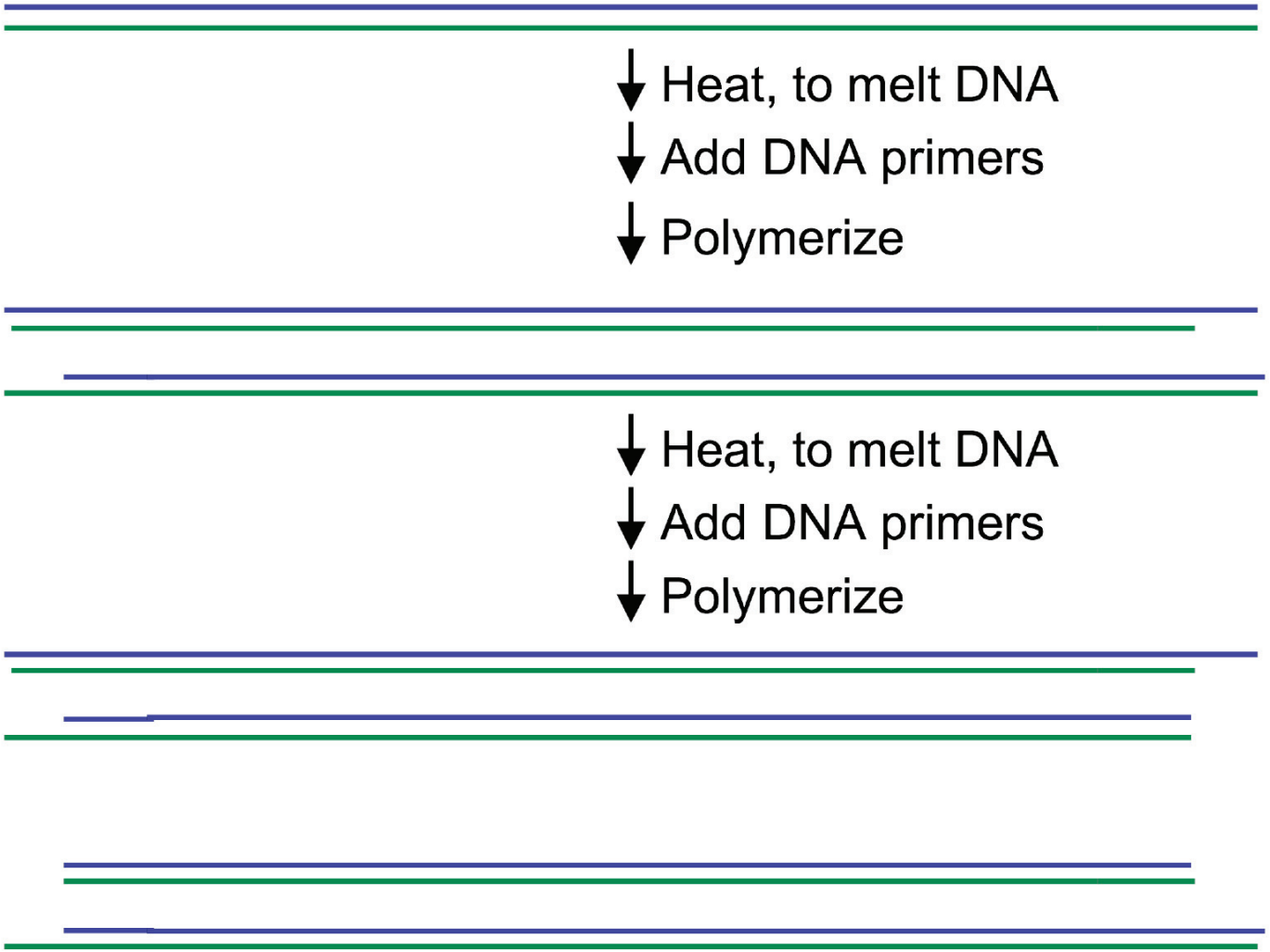
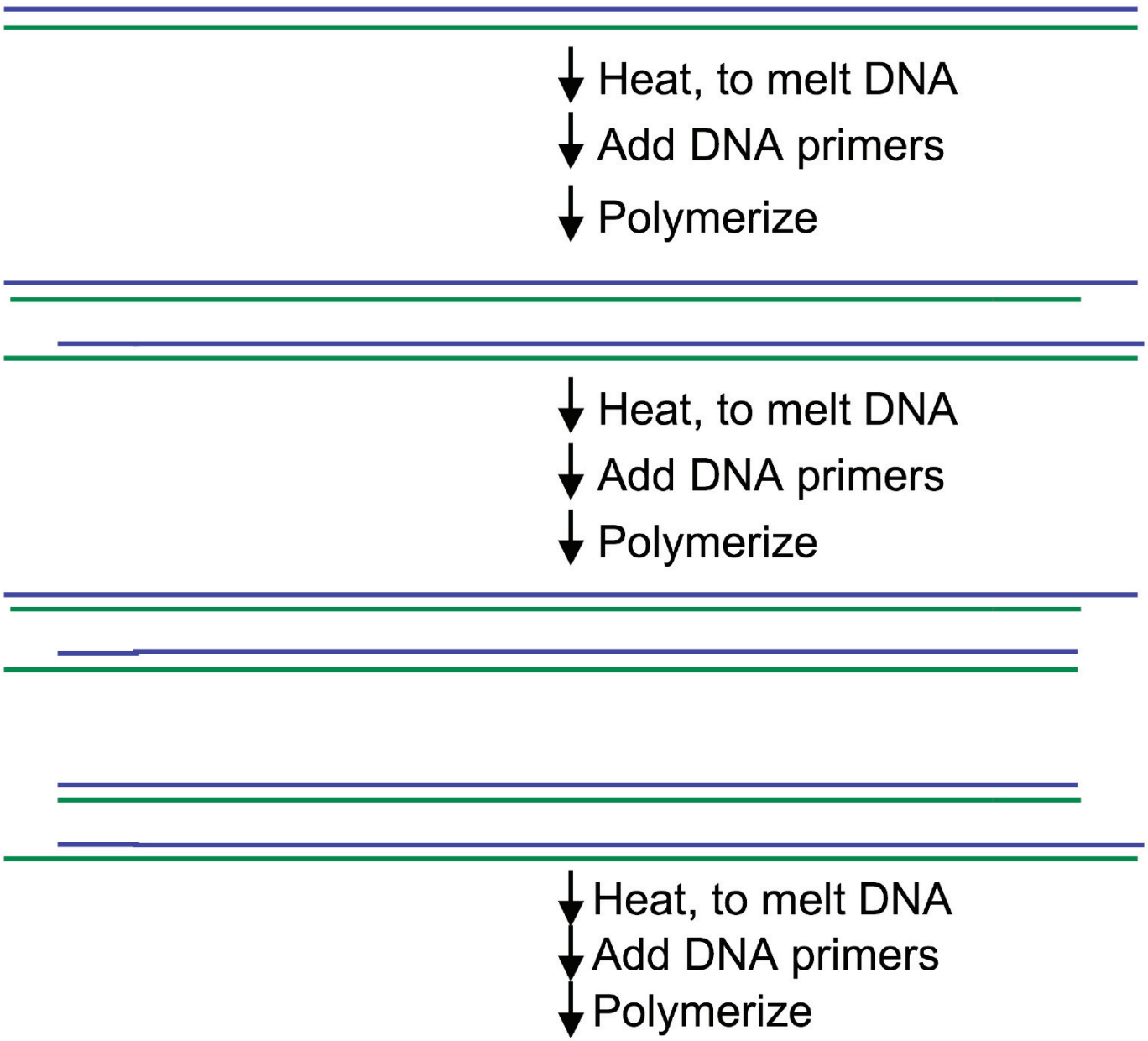


PCR - Polymerase Chain Reaction



PCR - Polymerase Chain Reaction



PCR - Polymerase Chain Reaction

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

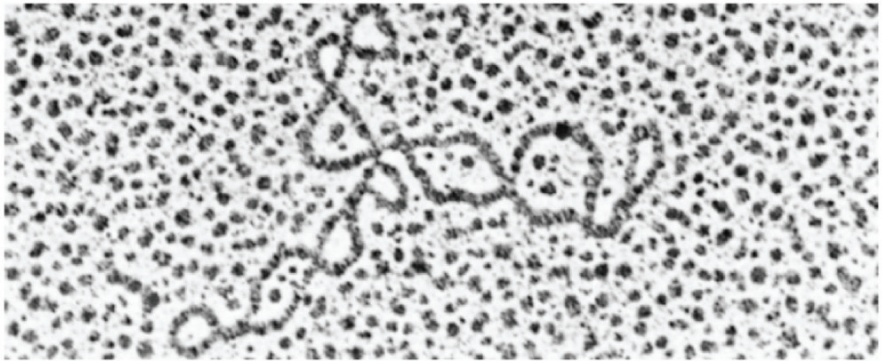
After 15 cycles, original DNA is amplified $2^{15} = 32{,}800$ fold

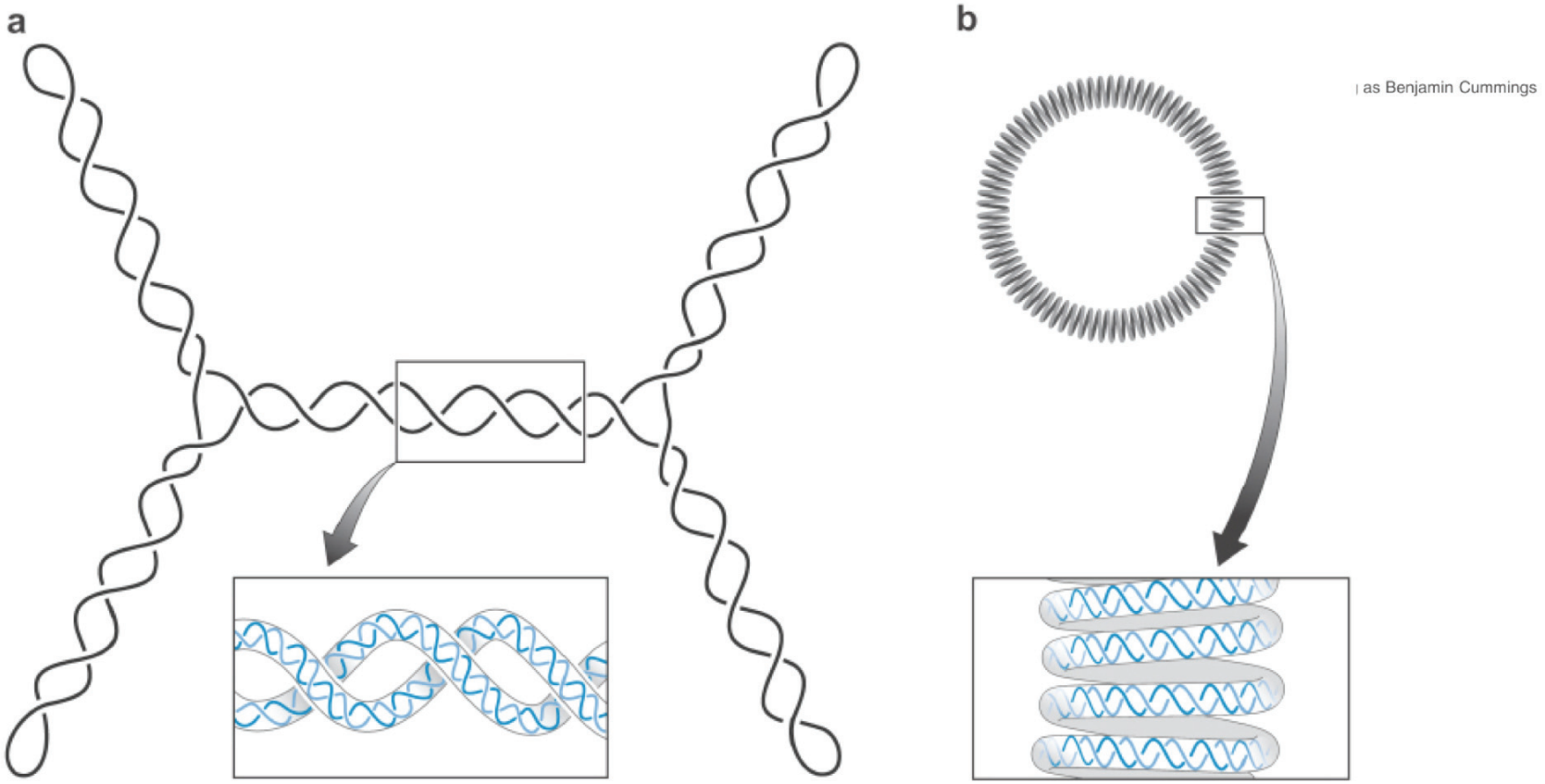
After 20 cycles, original DNA is amplified $2^{20} = 1{,}048{,}000$ fold

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