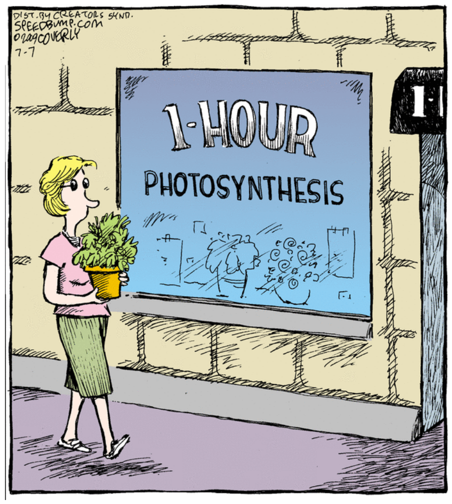
November 23, 2011 Physics 131 Prof. E. F. Redish

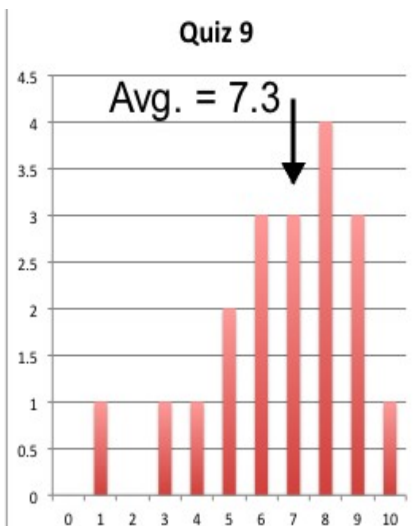
- **Theme Music:**
  Cannonball Adderly
  Work Song
- **Cartoon:**
  DaveCoverley
  Speed Bump



11/23/11 Physics 131 **1**

Quiz 9

	9.1	9.2	9.3
A	32%	26%	68%
B	95%	37%	5%
C	47%	37%	5%
D		21%	84%
E		95%	
F		5%	



11/23/11 Physics 131 **2**

Guest Lecture: Prof. A. LaPorta

RNA Polymerase, the Ribosome and the Work-Energy theorem

Enzymes that process DNA or RNA (for example, RNA polymerase, DNA polymerase, the Ribosome) must move along the substrate as they perform their function. Sometimes the DNA or RNA molecule being processed forms a structure that must be disrupted before the enzyme can move forward. This poses a physical barrier that these enzymes must overcome, and it is interesting to consider how much force the enzyme must generate or how much work it must do in order to proceed.

Using an optical trapping apparatus, we can apply a force to a DNA or RNA molecule and measure the resulting displacement. This allows us to measure the force necessary to disrupt a structure and the amount of work that is done during the disruption process. I will consider two structures, an RNA hairpin that terminates transcription by RNA polymerase and an RNA pseudoknot that causes a ribosomal frame shift, and discuss how force and work measurements give us insight into genetic processes.

11/23/11 Physics 131 3

Work and Molecular Biology

Work and Molecular Biology

$$W = \int \vec{F} \cdot d\vec{s}$$

Work occurs when a force is exerted over a distance.

$$W = \Delta KE$$
$$= \tfrac{1}{2} m v_f^{\,2} - \tfrac{1}{2} m v_i^{\,2}$$

Doing work on a free particle causes a change in velocity – kinetic energy.

Work and Molecular Biology

$$W = \int \vec{F} \cdot d\vec{s}$$

Work occurs when a force is exerted over a distance.

$$W = \Delta PE$$

Doing work against a conservative force will create potential energy.

$$= \tfrac{1}{2} k x_f^{\,2} - \tfrac{1}{2} k x_i^{\,2}$$ (compress a spring)

$$= mg\left(h_f - h_i\right)$$ (lift a mass against gravity)

Work and Molecular Biology

Calling the potential energy $U(x)$

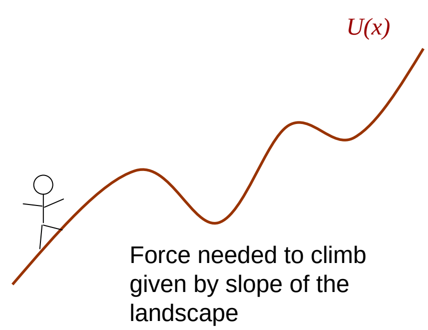
$$U(x) = -\int_0^x F dx'$$

This tells us how to calculate $U(x)$ if we know F.

If we need to calculate F from $U(x)$, we can take a derivative.

$$\frac{d}{dx} U(x) = -\frac{d}{dx}\left[\int_0^x F dx'\right]$$

$$-\frac{dU}{dx} = F$$



Work and Molecular Biology

$$U(x) = -\int_0^x F dx'$$

$$F = -\frac{dU}{dx}$$

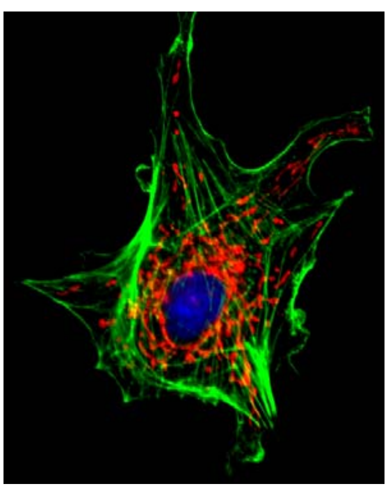
Using an optical trap we can

•Measure how much work an individual molecule does

•Measure the potential energy function of a molecule

•Measure the force necessary to drive a chemical reaction.

The Cell as Information Processor



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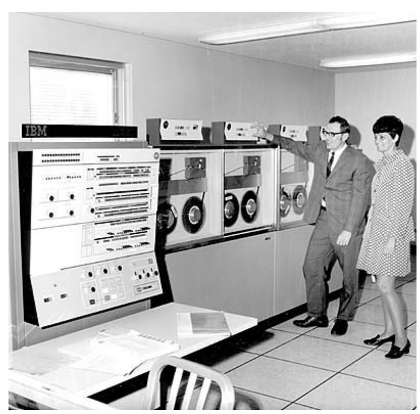
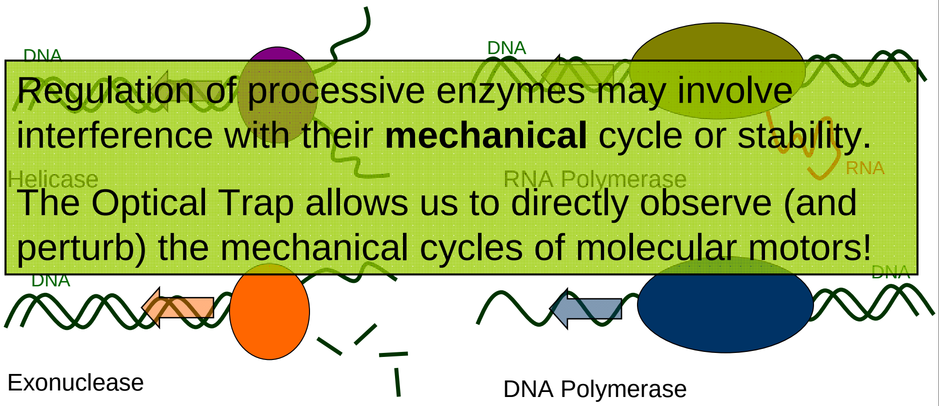


Information in cells is processed by *Processive Enzymes*

Enzymes are molecular motors, which convert energy released by NTP hydrolysis to mechanical work as they translocate along the substrate

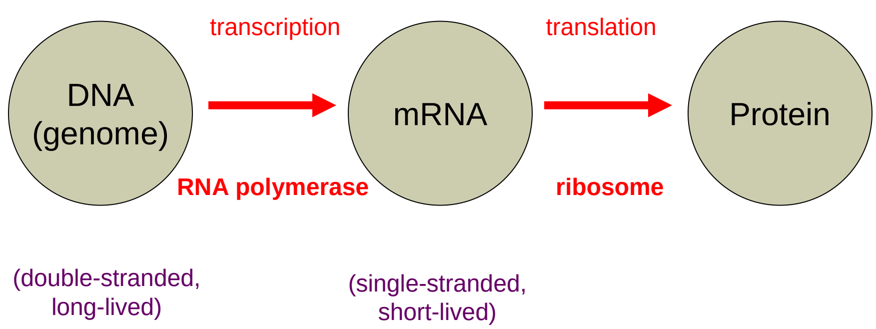


Regulation of processive enzymes may involve interference with their **mechanical** cycle or stability.

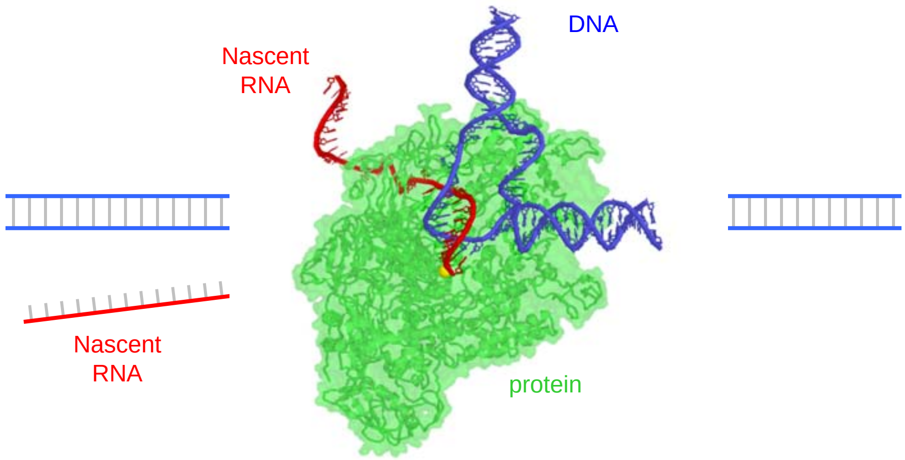
The Optical Trap allows us to directly observe (and perturb) the mechanical cycles of molecular motors!

The Cell as Information Processor

(prokaryotic organisms)

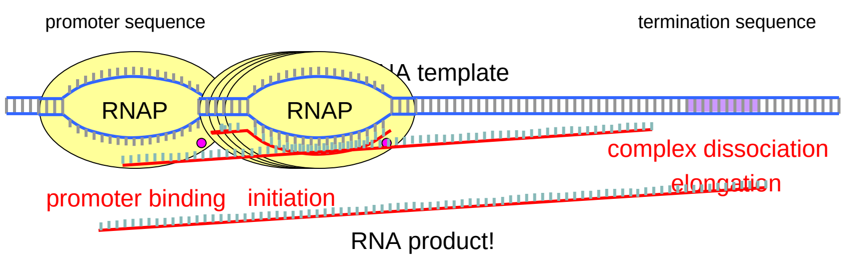


Structure of *E. coli* RNA Polymerase Elongation Complex

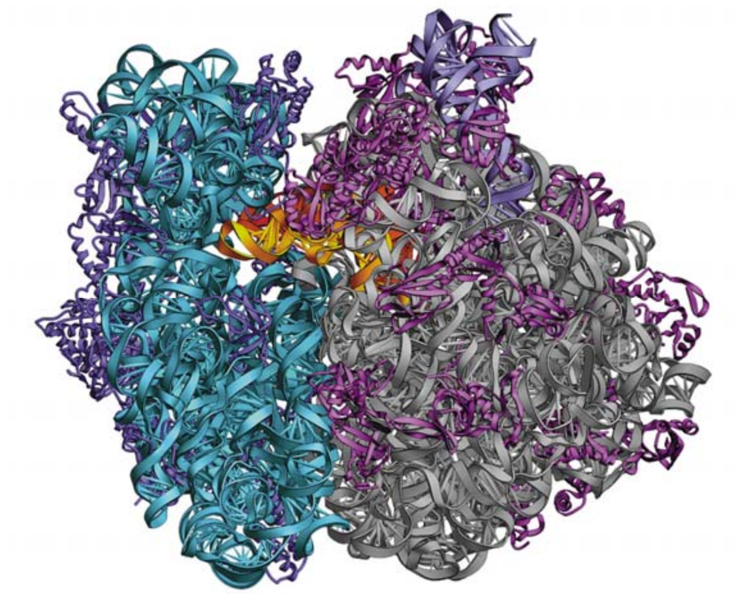


Darst lab structure

Lifecycle of RNA Polymerase Activity



Structure of the Ribosome (70 S)



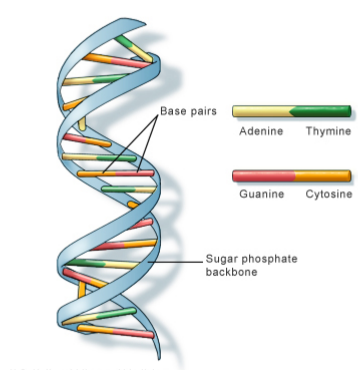
Contrast of DNA and RNA

RNA is typically single-stranded in the cell, bases are exposed.

DNA is typically double-stranded, bases are concealed in the interior of the structure.

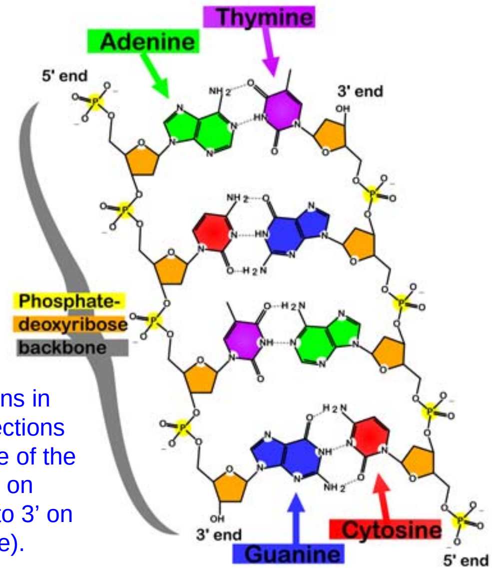
The double helix is extremely rigid compared with a single-stranded polymer.

Interesting structures tend to form in RNA being synthesized or used as a template for protein synthesis.



double-stranded DNA

Base-pairing

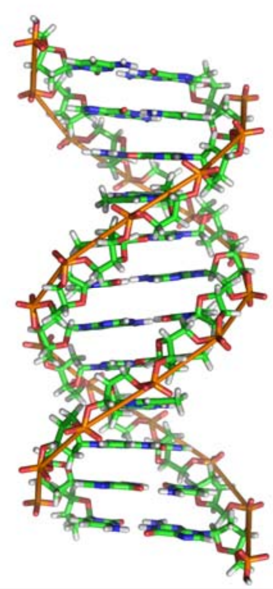


Base-pairing occurs due to hydrogen bonding of the bases (3 hydrogen bonds for a C-G pair, 2 hydrogen bonds for an A-T or A-U pair).

Backbone runs in opposite directions on either side of the helix (3' to 5' on one side, 5' to 3' on the other side).

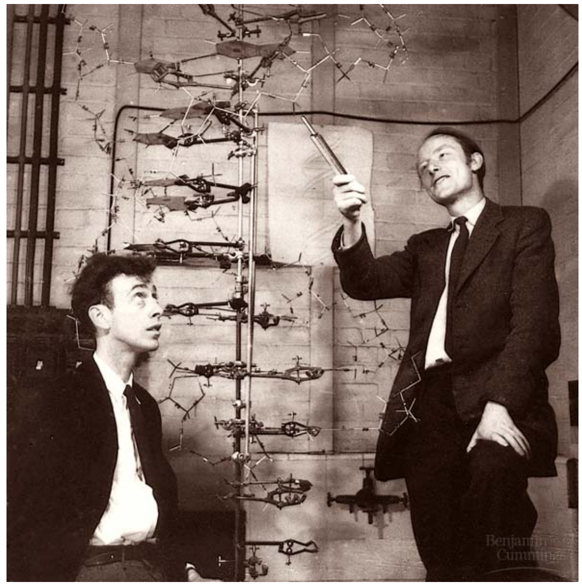
Base-pairing

Double Stranded DNA structure

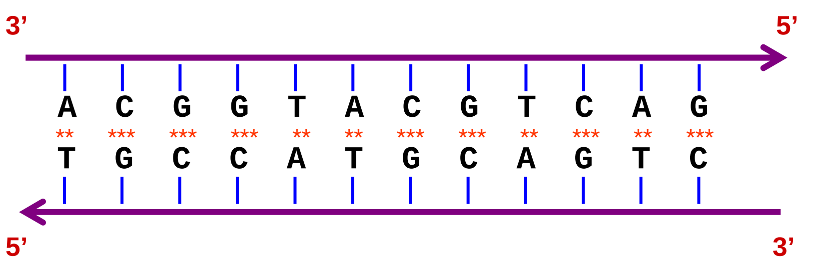


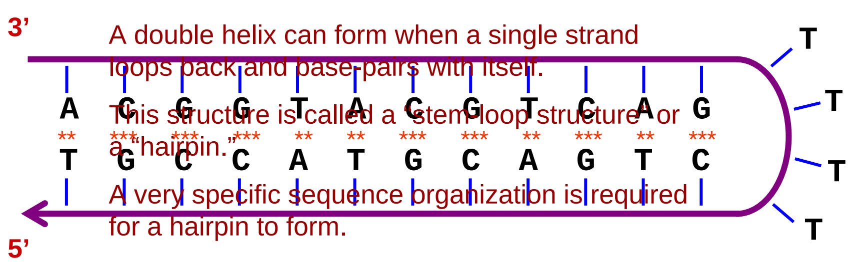
Base-pairing

Double Stranded DNA structure



Base-pairing



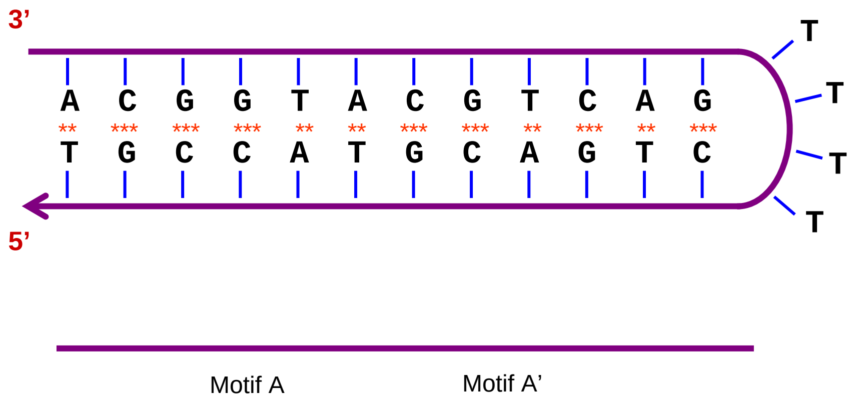


A double helix can form when a single strand loops back and base-pairs with itself.

This structure is called a “stem-loop structure” or a “hairpin.”

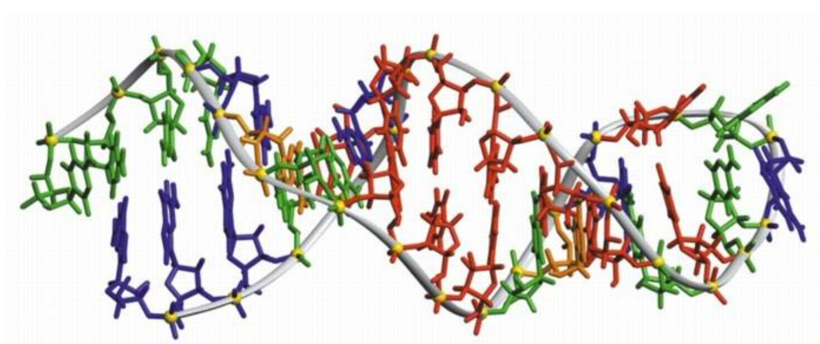
A very specific sequence organization is required for a hairpin to form.

Hairpin

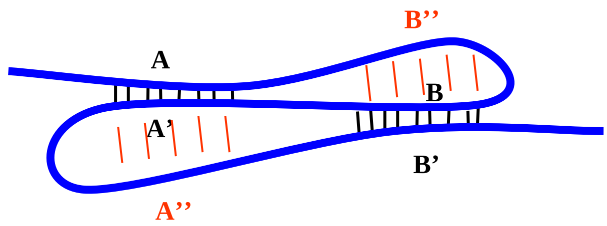


The sequence structure required is called a palindrome. It consists of a sequence motif A, followed by the sequence motif A’, which is the retro-grade complement.

Hairpin



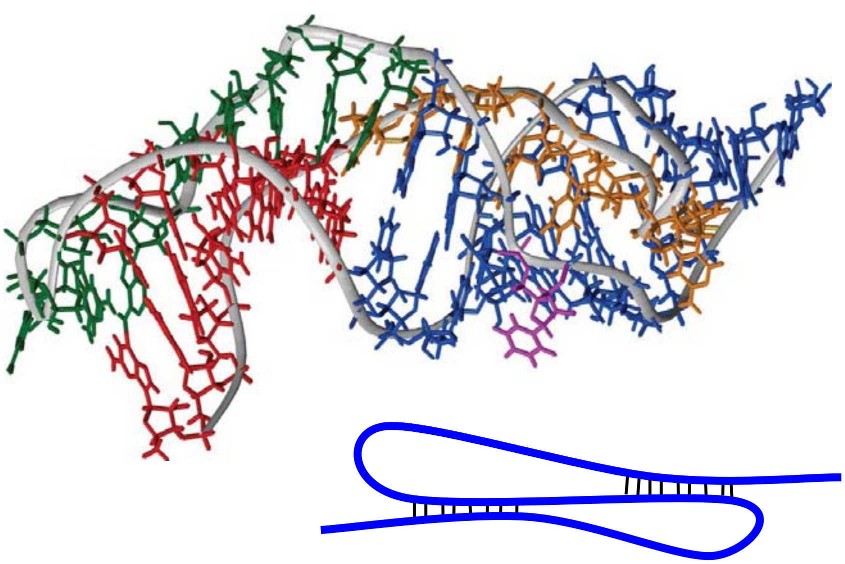
Another structure - Pseudoknot



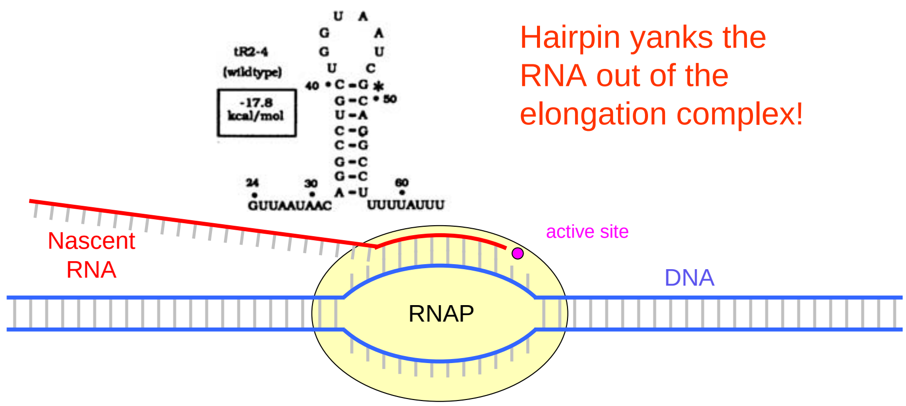
Motif A   Motif B’’   Motif B   Motif A’   Motif A’’   Motif B

A pseudoknot is two interleaved hairpins. Added complexity comes from the fact that the “loops” actually interact to form DNA triplex.

Another structure - Pseudoknot



A Terminating Sequence

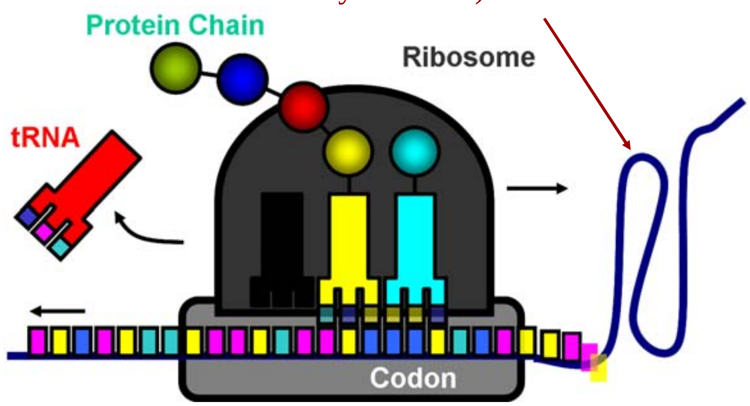


Hairpin yanks the RNA out of the elongation complex!

Darst lab structure

Structure of the Ribosome (70 S)

Formation of Pseudoknot results in -1 frame shift, premature termination of translation. (Truncated protein is synthesized)

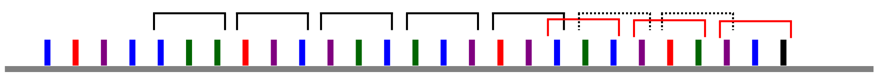


Frame Shift

Pseudoknot yanks the RNA out of the ribosome's mouth, causing the RNA to move left, or equivalently the ribosome to move right.

After this frame shift, the ribosome recognizes 3-base codons shifted backwards by 1 base (-1 frameshift).

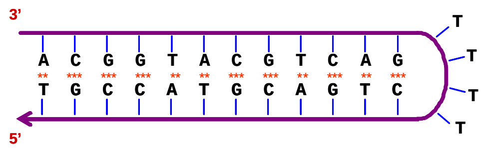
In the new reading frame, the ribosome encounters a stop codon, resulting in synthesis of a truncated protein.

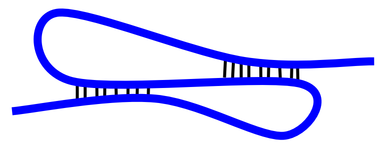


Pseudoknot or Hairpin

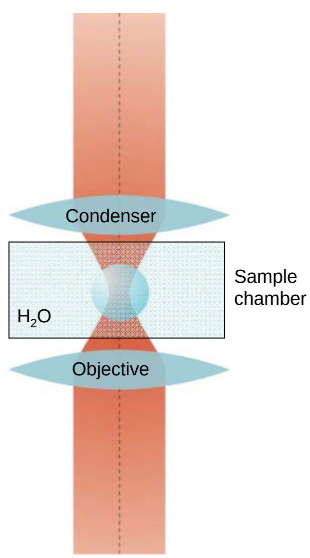
- How much energy is necessary to disrupt a pseudoknot or hairpin?
- Does RNA polymerase have the ability to resist the terminating hairpin?
- Does the ribosome have enough energy to disrupt the pseudoknot?

Question – What would energy as a function of extension look like?



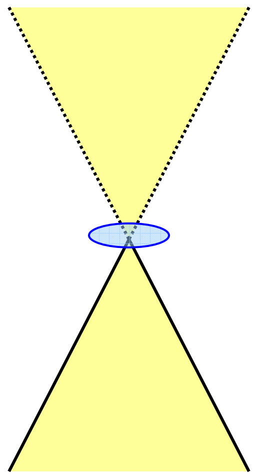


What is an optical trap?



Particles with optical density greater than water are drawn to the focal volume of the laser beam

Another view:
the particle is a lens which may refract the trapping beam

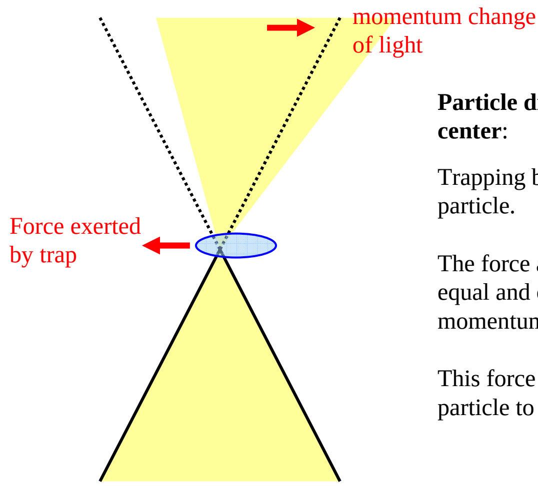


Particle at trap center:

Trapping beam passes through without change.

No force

Another view:
the particle is a lens which may refract the trapping beam



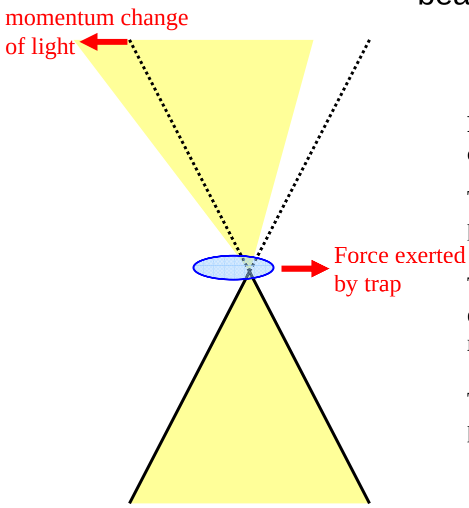
Particle displaced from trap center:

Trapping beam is deflected by the particle.

The force acting on the particle is equal and opposite to the change in momentum of the trapping beam.

This force tends to restore the particle to the center of the trap.

Another view:
the particle is a lens which may refract the trapping beam



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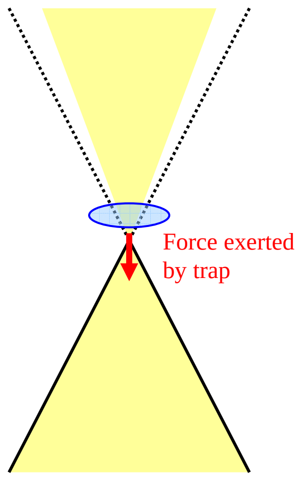


Particle above trap center:

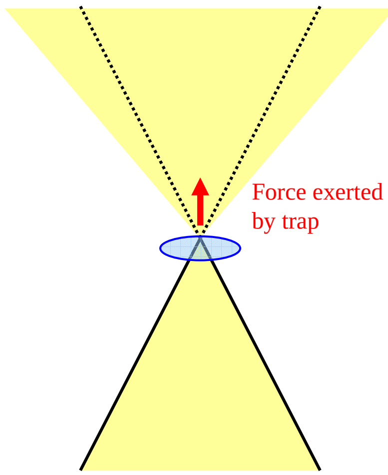
Trapping beam becomes more columated.

Foreward momentum of the beam increases, so force pushes particle in the opposite direction.

Particle is pushed towards trap center

Another view:

the particle is a lens which may refract the trapping beam



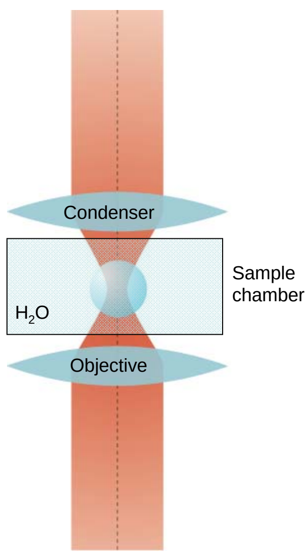
Particle below trap center:

Trapping beam becomes less columated.

Foreward momentum of the beam decreases, so force pushes particle in the opposite direction.

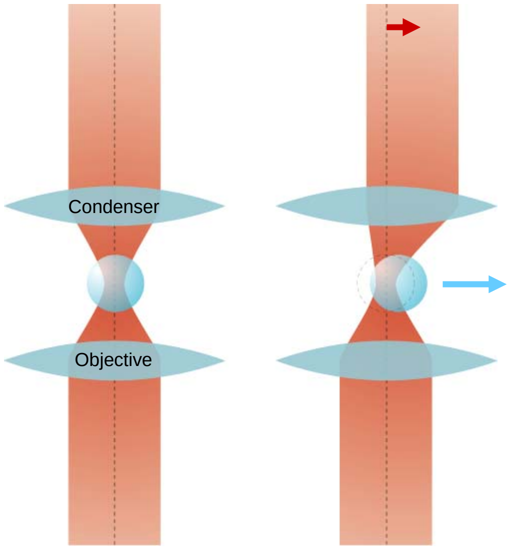
Particle is pushed towards trap center

What is an optical trap?



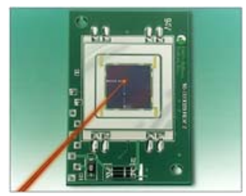
Particles with optical density greater than water are drawn to the focal volume of the laser beam

We measure the deflection of the laser beam



The position of the bead relative to the laser focus is proportional to the bending angle.

We measure this angle by calculating the center of intensity of the laser beam at the back focal plane of the condenser with a position sensitive detector.



Pacific Silicon Sensors, Inc.

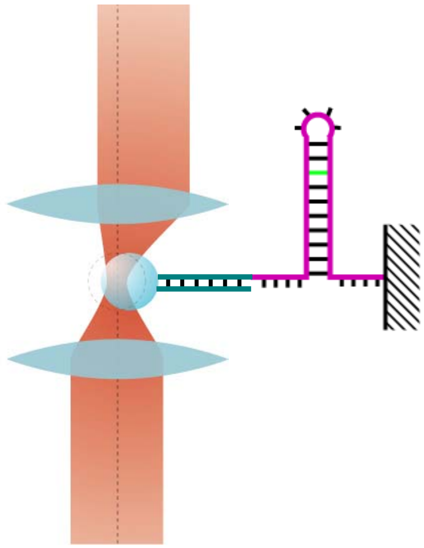
Question – How much force can an laser beam generate?

We measure the force as we pull the structure open.

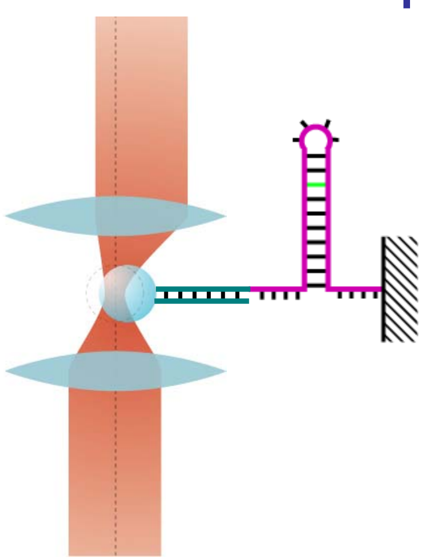
Using

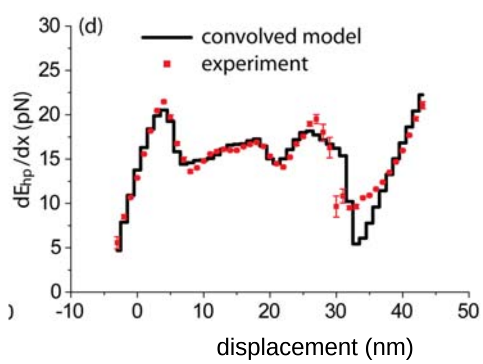
$$F = -\frac{dU}{dx}$$

We can determine the slope of the energy landscape.



We measure the force as we pull the hairpin open.





We measure the force as we pull the pseudoknot open.