Molecular recognition

Important: DNA hybridization is *specific*

Example: mix these three pieces of ssDNA together. What happens?

1
CTAACGCG
2
TTTACCCG

\[ -\text{AATCCCGGATTGCATTAACGACCTTC}\]

1 hybridizes to complementary location within 3. 2 does not hybridize because it is not complementary.

\[ \text{CTAACGCG} \]
\[ -\text{AATCCCGGATTGCATTAACGACCTTC}\]

“molecular recognition”
Bonds vs. intermolecular interactions

Atoms within a molecule (or monomers within a polymer) are attached to each other by strong, covalent (chemical) bonds.

- Bonds are not broken or formed easily, typically an enzyme is needed (like using a tool: scissors or soldering iron).

Two molecules, or two parts of the same molecule, are attracted to each other by forces that are significant, but 10-100 times weaker than true bonds. Self-assembly is due to these forces.

- self-assembled structures are not permanently attached, they can be pulled apart by heating, for example.

Protein structure

Sequence of amino acids in a protein is called the *primary structure*
Proteins self-assemble into complicated shapes (tertiary structures).

The *binding pocket* (active site) in an *enzyme* is shaped specifically to recognize and hold a *substrate* molecule in such a way as to facilitate a chemical reaction.

**Important concept:** structure ↔ function

*Molecular recognition* w/ proteins:
- an enzyme recognizes a particular substrate
- an antibody recognizes a particular antigen

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**Molecular Diagnostics**

Detecting Pathogens using Molecular Recognition
Conventional diagnostics

Pathogen (virus, bacteria, fungi, parasites) detection and identification is critical for proper treatment.

- Direct observation under microscope
  - slow
  - requires high skill level
  - low sensitivity
  - cannot distinguish between similar organisms

- Growth in culture followed by mouse inoculation
  - slow and expensive
  - some pathogens cannot be grown in culture
  - uses animal

Molecular diagnostic techniques exploit molecular recognition

Immunological procedures: Use antibodies that recognize specific antigens associated with a pathogen.

DNA probe: Prepare DNA probes that bind specifically to pathogen DNA.
Immunological Diagnostic Procedures

Based on the specific binding between an antibody and a target site (e.g. an antigen molecule) associated with a pathogenic microorganism or a particular type of cell associated with disease.

Some infectious diseases with specific markers:
- Chlamydia
- Hepatitis B
- Herpes
- HIV
- Rubella
- Anthrax

Tumor markers:
- carcinoembryonic antigen
- prostate-specific antigen
- interleukin-2 receptor

A marker is a characteristic molecule (or part of a molecule) on the surface of a cell or virus that can be recognized by an antibody.

Typical immuno-assay

- Bind sample to a solid support.
- Add a marker-specific antibody that is labeled by a fluorescent particle.
- Wash to remove any unbound antibody.
- The presence of fluorescent light from the sample indicates that antibodies bound to the target antigen.
DNA probe diagnostic systems

• Requires that a characteristic DNA sequence is known. This is called the target DNA. The procedure aims to determine if this DNA is present in a sample.

• The target DNA can be distinctive to a particular pathogen, or it could represent a genetic defect associated with a disease (e.g. Alzheimer’s, MS …).

DNA probe procedure

• Prepare a fluorescently- or radioactively-labeled DNA probe that is complementary to the target DNA.

• Expose source DNA to the labeled probe. Wash away excess unbound probe.

• Check for presence of label.
Genetic Engineering

Learning to use another organism’s machines

Whose machines do we want to use?

Bacteria
e.g. *E. Coli*

- no cell nucleus
- circular DNA molecules (plasmids) common
- DNA readily inserted and exchanged
Eukaryote cells used in biotechnology

- chromosomes contained in cell nucleus
- a variety of *organelles*

- Yeast: single cell organism
  *Saccharomyces cerevisiae*

- Established cell lines from plants/animals in culture, e.g. Chinese hamster ovary (CHO) cells