Applications of Integration in Biomedical Science

by
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UCF EXCEL Applications of Calculus

Opinion Iclicker

Which of the following diseases/conditions do you think will have the most IMPACT on your generation?

A.) Cancer

B.) AIDS/HIV

C.) Tuberculosis

D.) Cardiovascular disease

E.) Autism

Opinion Iclicker

Which of the following diseases/conditions will be CURED in your lifetime?

A.) Cancer

B.) AIDS/HIV

C.) Tuberculosis

D.) Cardiovascular disease

E.) Autism

Calculus Topic: Defining area under the curve

Topic #1: Approximating rectangles

One possible method for estimating area under a given curve (or function) is the use of approximating rectangles

This is a simple method, but has limitations in its ability to accurately define the area

Approximating rectangles

- The use of this technique is inadequate to determine the area under a curve since it can overestimate and underestimate this area

- This section of the applications course will introduce you to concepts and methods in biomedical science that rely on calculus to determine the quantity of compounds and macromolecules

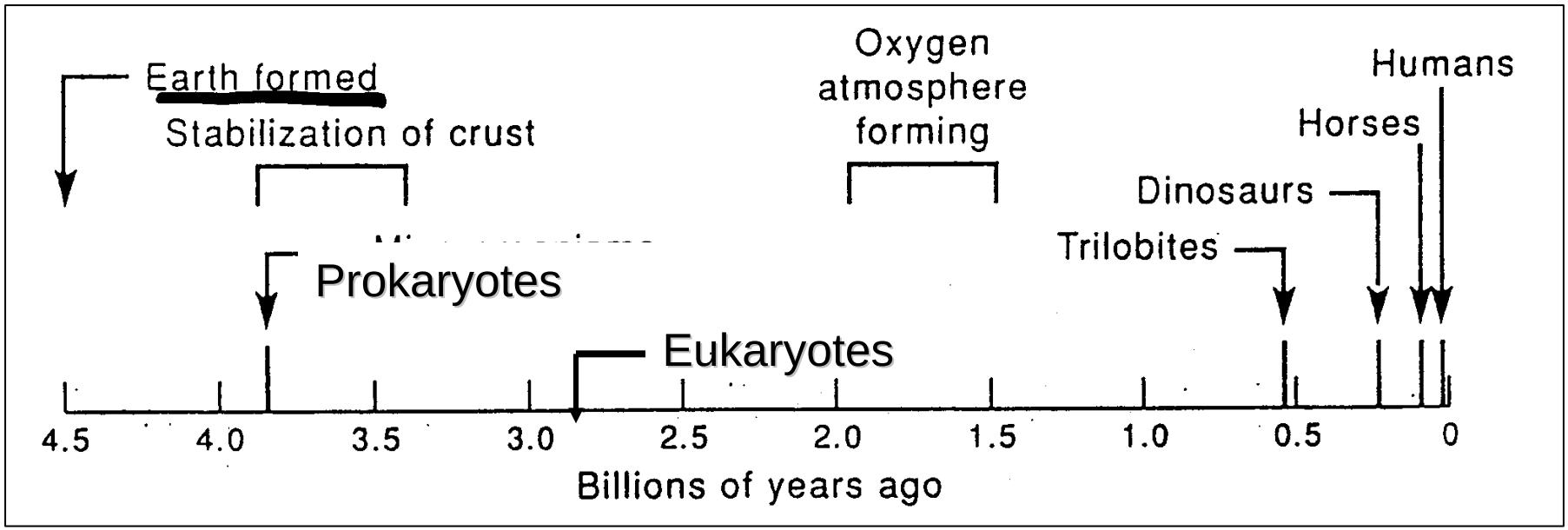
Applications of Integration in Biomedical Science

Some of the future courses (that you may take) that this will be relevant:

- MCB 3020 – General Microbiology
- BSC 3403C – Quantitative Biological Methods
- MCB 4414 – Microbial Metabolism
- BCH 4053 – Biochemistry I
- BCH 4054 – Biochemistry II

Life – Its existence on Earth

Time Line for Planet Earth



Prokaryotes

- involved in formation of the biosphere
- required for plant & animal survival

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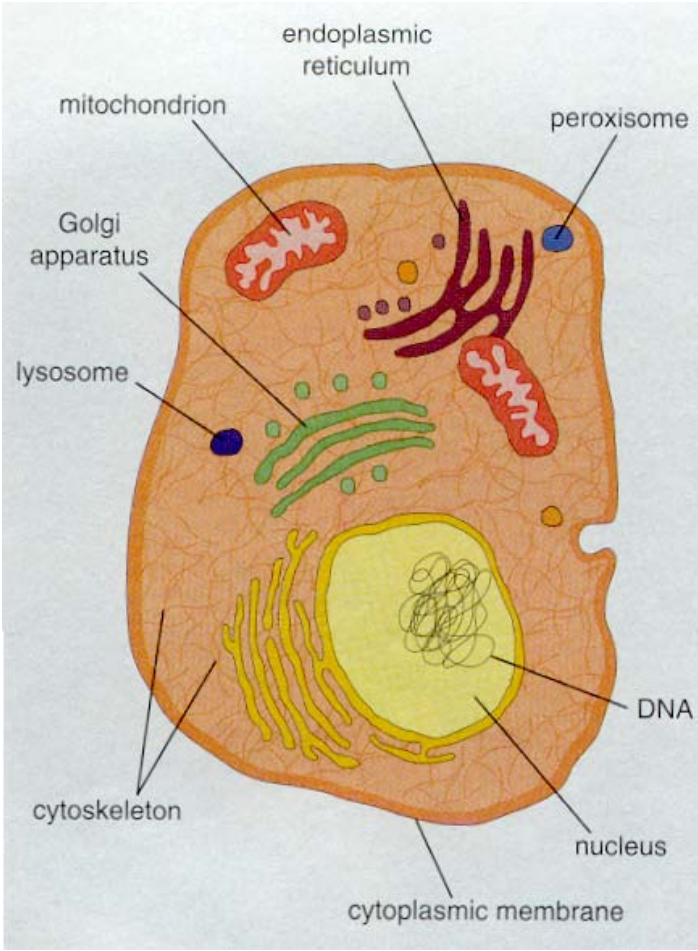
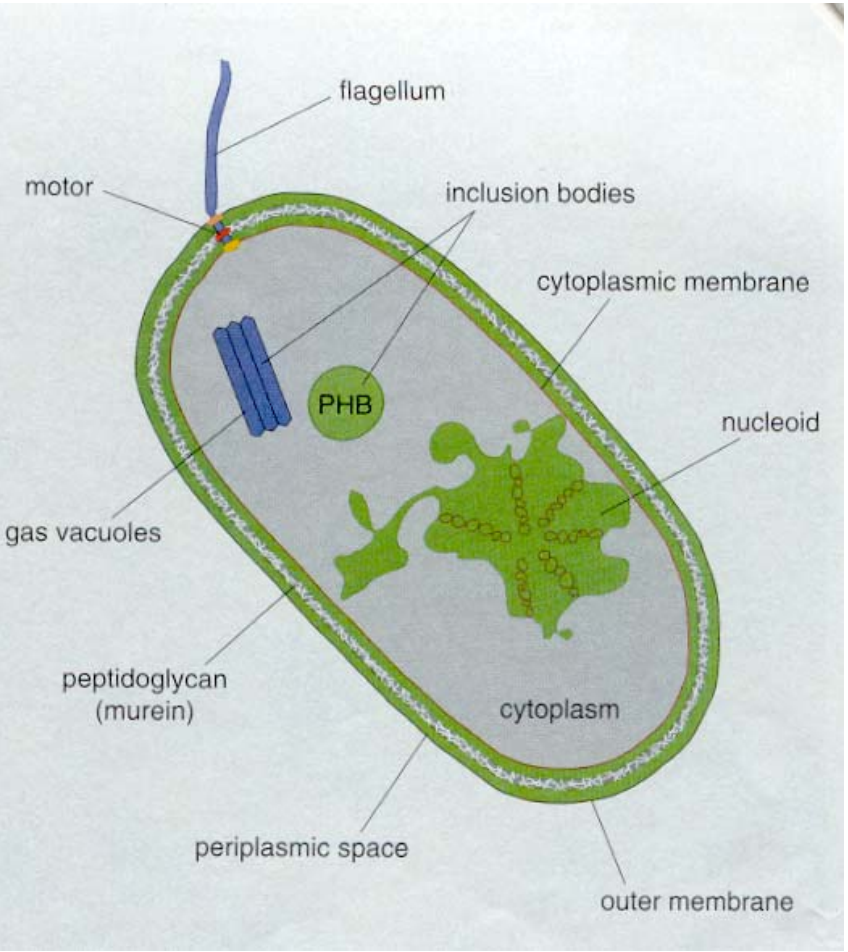
Life – Cellular level





eukaryotic cell **prokaryotic cell**

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What are cells made of (*E.coli*)?

CHNOPS:

Carbon

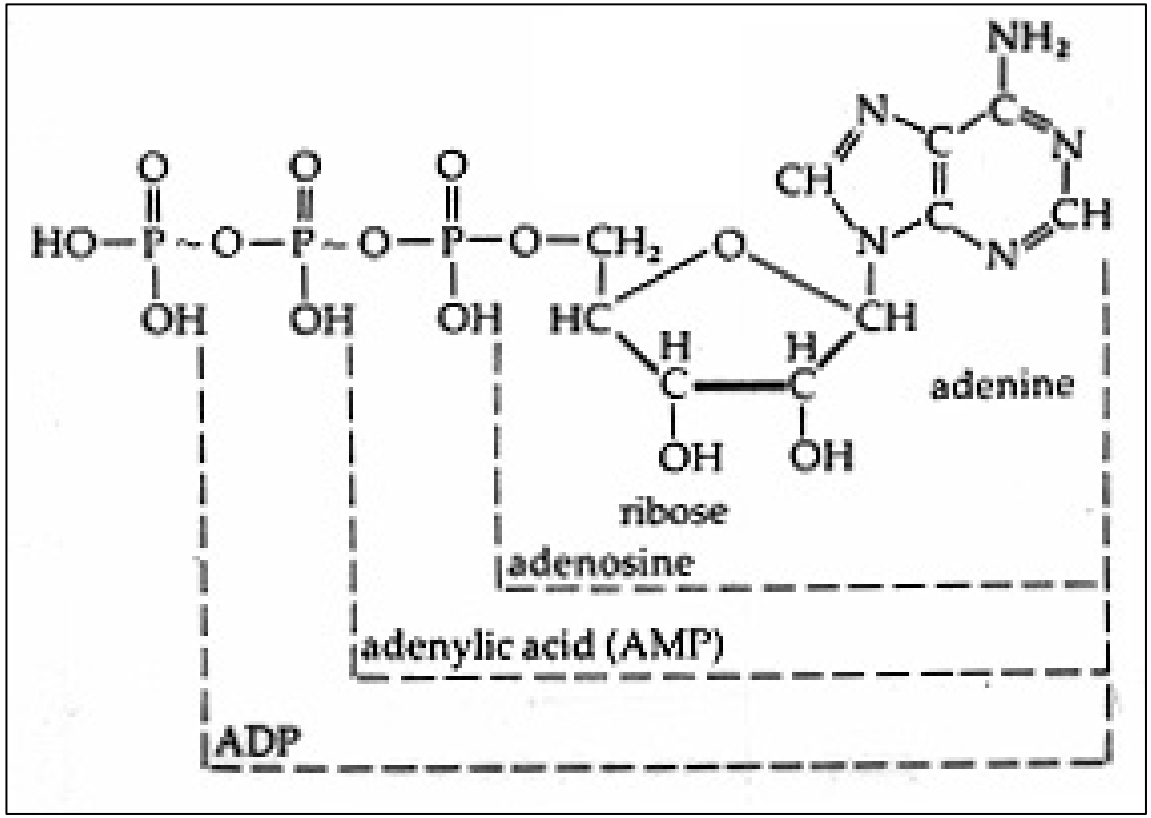
Hydrogen

Nitrogen

Oxygen

Phosphorus

Sulfur



Adenosine triphosphate - ATP

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Biological Macromolecules

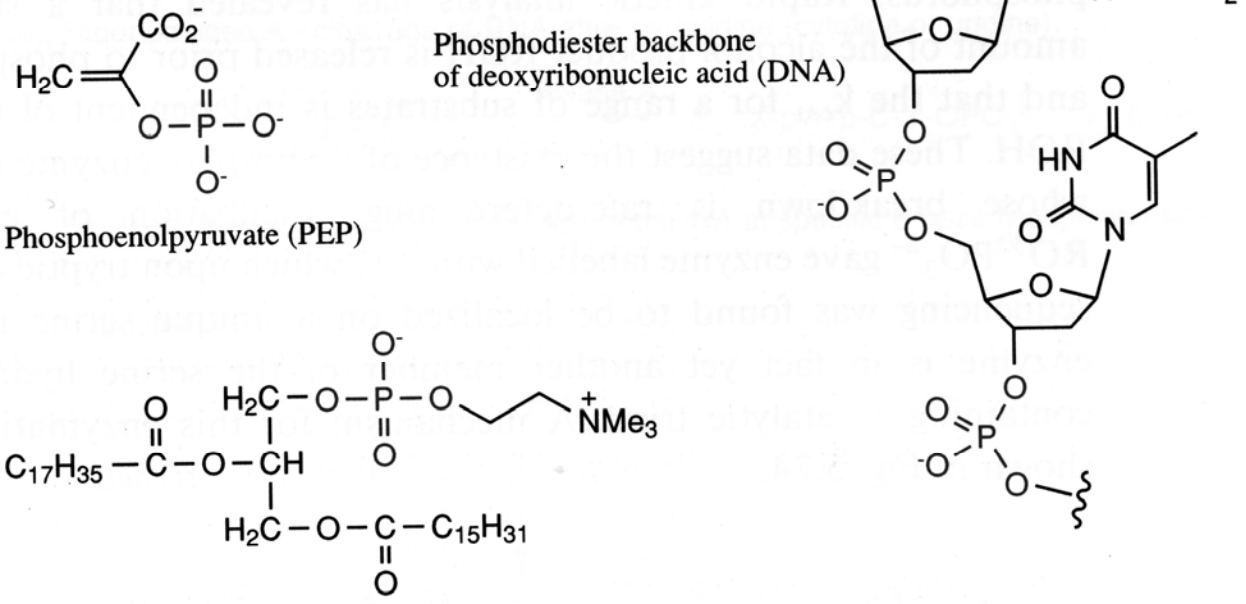


Fig. 5.22 Examples of biologically important phosphates.

Trace Elements

Human composition (complements of Dept. of Energy)

Dry weight %	
Carbon	61.7
Nitrogen	11.0
Oxygen	9.3
Hydrogen	5.7
Calcium	5.0
Phosphorus	3.3
Potassium	1.3
Sulfur	1.0
Chlorine	0.7
Sodium	0.7
Magnesium	0.3

Trace amounts of B, F, Si, V, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Sn, I.
There are some arguments as to the importance of other trace elements

Biological Cells – Complex mixtures

Basics:

DNA, RNA: Polymers of nucleic acids – encode proteins

Proteins: Polymers of amino acids – can be structural or act as enzymes

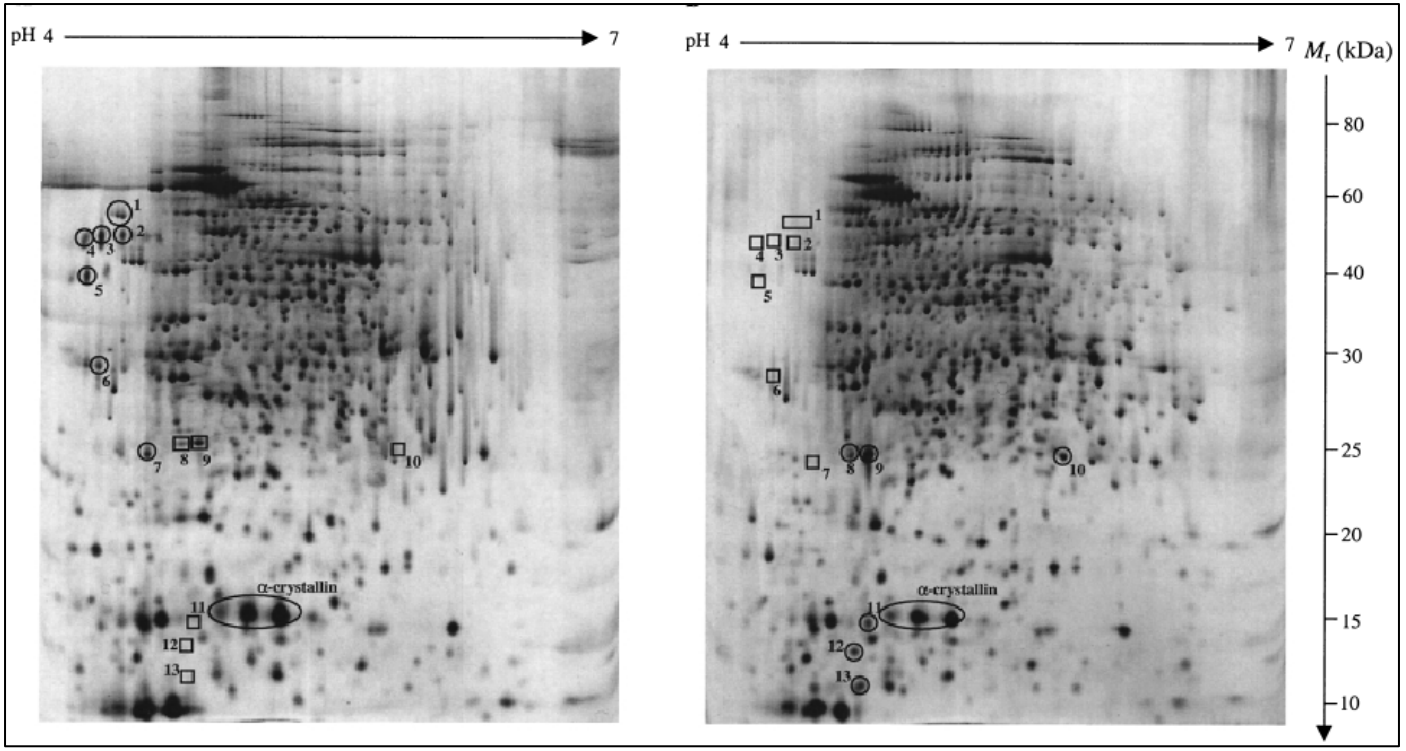
Lipids: Polymers of carbon – structural components of cell membranes

Biological Cells – Complex mixtures

- A given cell will have thousands of different proteins, RNA molecules and metabolites present under a particular growth condition
- Model system – *E. coli* – roughly 4400 genes!
- How do we define the 'role' of each individual protein (for example)?
- First we must purify this protein (or nucleic acid, or lipid) away from all other components, then study it in a test tube (*in vitro*)

2-dimensional gel electrophoresis

Proteome



2D-PAGE of *M. tuberculosis* proteins from log-phase growth (A) compared to starvation (B)

J.C. Betts *et al.* 2002 Molec. Microbiol. 43:717-731.

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Relevant examples - autism

- Recent reports on linkage of genetic mutation with autism spectrum disorder

March 2008 contactin 4:

http://www.ncbi.nlm.nih.gov/pubmed/18349135?dopt=Abstract

How do we know what a given protein does?

Relevant examples – cardiovascular disease

- Statin drugs – in the news
- These drugs target an enzyme, HMG-CoA reductase, the first dedicated step in cholesterol biosynthesis
- Cholesterol synthesis targeted by Vagelos and colleagues at Merck, beginning in 1970's

From target, to validation, testing, marketing and post-market studies – spans four decades!

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Relevant examples – cardiovascular disease

How did the discovery occur?

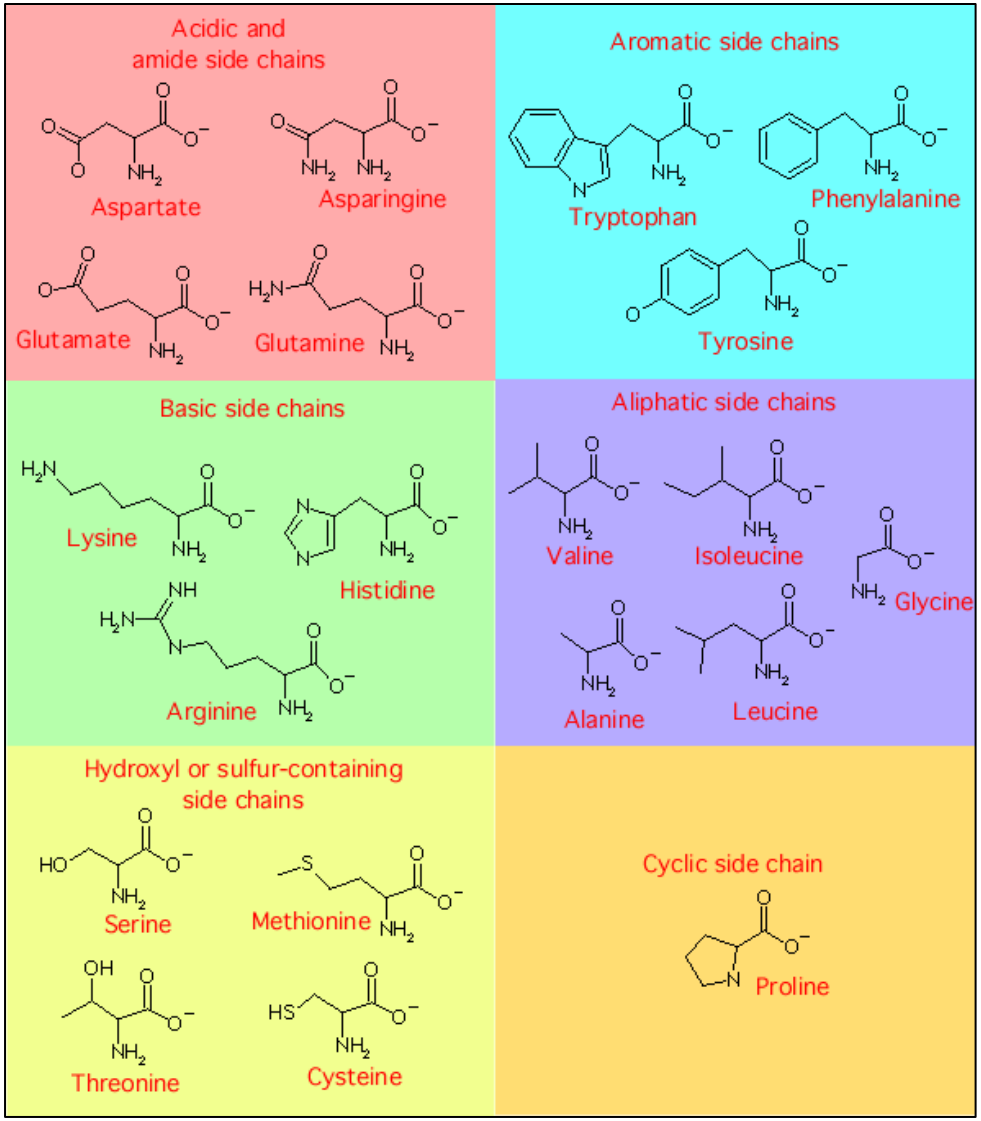
First had to elucidate the pathway for cholesterol synthesis (purify the protein catalysts)

Next, assays were developed to find novel inhibitors of enzymes (that could block cholesterol synthesis)

Once isolated, candidates had to be tested in cells, animals and eventually human clinical trials – prior to FDA approval

FIRST STEP – PURIFY THE PROTEIN!!!

Protein Purification



Proteins are polymers of amino acids

Protein sequence defines the chemical composition

Each protein has unique size, charge and shape

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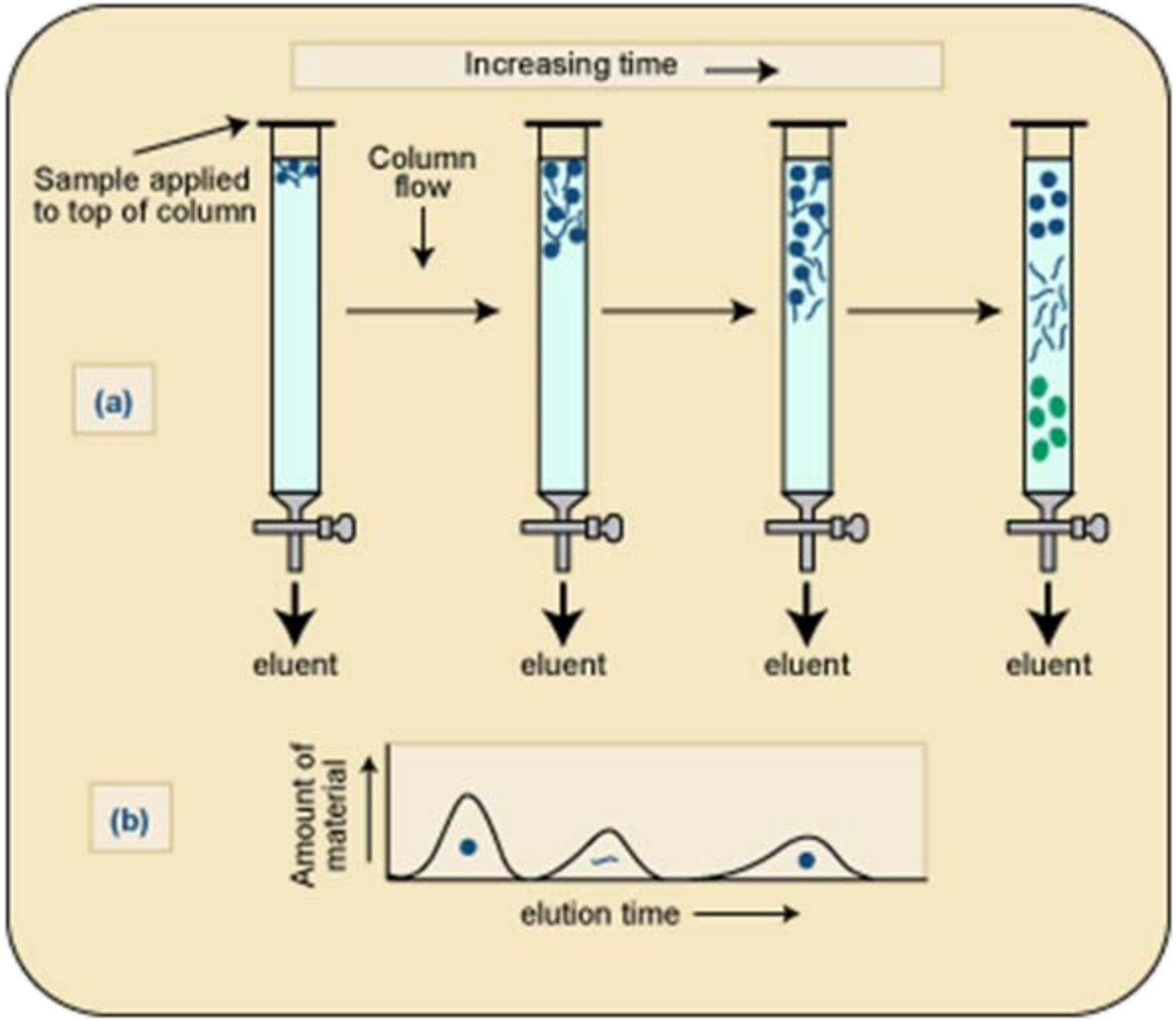
Chromatography – separation of mixtures

- Chromatography in general is the separation of compounds from mixtures using a **Solid phase** and a **mobile phase**
- Typically the solid phase is stationary, and held in place in a column
- The mobile phase (usually aqueous) moves through the solid phase and carries the sample

Chromatography – separation of mixtures



Samples separate from each other on the column due to differences in their unique properties:

1.) net charge
2.) hydrophobicity
3.) size
4.) specific affinity

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Chromatography – separation of mixtures

Types of chromatography used in protein purification:

1.) Ion Exchange

2.) Gel filtration

3.) Hydrophobic

4.) Affinity

Types of chromatography – Protein separations

1.) Ion exchange:

The solid phase has a strong or weak charged group (e.g. strong positive charge)

If a protein has a net negative charge (anionic), it will bind to a column that has a cationic group (positive charge). Each protein will have a slightly different net charge and thus mixtures of proteins can be separated based on net charge

Types of chromatography – Protein separations

2.) Gel filtration

Proteins will separate based on size, due to pores present in beads in the solid phase. The pores define the separation capabilities of the media (e.g. 30,000 MW to 3,000,000 MW)

Types of chromatography – Protein separations

3.) Hydrophobic Interaction Chromatography

Based on binding of hydrophobic amino acids (such as leucine, isoleucine) that are usually buried but occasionally present on the surface

Common groups on the stationary phase are phenyl groups or carbon chains

Types of chromatography – Protein separations

4.) Affinity Chromatography

Generally, proteins can be engineered to contain ‘tags’ at their ends that will bind to a certain group (e.g. metal). This tag is usually unique in the mixture and thus a ‘tagged’ protein can be purified quite readily from a cell extract using this procedure.

The use of protein tags has revolutionized the study of proteins in enzymes in the wake of the era of molecular biology and cloning.

How does this relate to Calculus???

To find and determine the quantity of a given protein, or other molecule of interest, we follow the elution of these molecules using a detector. This pattern is essentially a continuous function from one time period to the next as follows:

Samples eluting become a series of peaks that can be followed and quantified by area under the curve

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