Inservice:

Proteins & Enzymes

The University of Texas Southwestern Medical Center at Dallas
Saturday, Dec. 2, 2006
Meeting Room NG3.202
9:00 a.m. – 3:00 p.m.

Program

9:15-10:30	Joel Goodman	NG3.202	Intro to Proteins and Enzymes
10:30-11:00	David Russell	NG3.202	A Case of Mistaken Identity
11:15-11:45	David Russell	L5.262	Visit to the Russell lab
12:00-12:45	Lunch	NG3.202	
12:50-1:30	Chad Brautigam	NB10.606	Introduction to Protein Structure
1:30-2:30	Joel Goodman	NG3.202	Measuring Alkaline Phosphatase Activity
2:30-3:00	Joel Goodman	NG3.202	Discussion: Proteins & Enzymes in the Classroom

Introduction to Proteins and Enzymes

- Basics of protein structure and composition
- The life of a protein
- Enzymes
  - Theory of enzyme function
  - Not all enzymes are proteins / not all proteins are enzymes
  - Enzyme REGULATION
- Setting up an enzyme assay
  - Buffer, cofactors, substrate, enzyme

Why are Proteins Important?

- Major class of catalysts in the cells; responsible for metabolism
- Proteins interconvert energy
- Proteins permit selective import and export of molecules from cells and organelles
- Proteins allow movement
- Proteins provide structural support for the organism and individual cells

What is a protein?

- Linear chain of amino acids
  - Amino acid: NH_3 - $C_\alpha X$ - COOH
  - 20 naturally occurring ones; essential and nonessential
- Contains 3 or 4 hierarchies of structure
  - Primary structure: sequence
  - Secondary structure: alpha helix, beta strand, turn, [random coil]
  - Tertiary structure: beta barrel, ARM repeats, helix-loop helix, etc.
  - Sometimes contains quaternary structure (protein-protein interactions)

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Properties of Amino Acids

Name (Residue)	3-letter code	Single code	Relative abundance (%) E.C.	MW	pK	VdW volume(Å^3)	Charged, Polar, Hydrophobic
Alanine	ALA	A	13.0	71		67	H
Arginine	ARG	R	5.3	157	12.5	148	C+
Asparagine	ASN	N	9.9	114		96	P
Aspartate	ASP	D	9.9	114	3.9	91	C-
Cysteine	CYS	C	1.8	103		86	P
Glutamate	GLU	E	10.8	128	4.3	109	C-
Glutamine	GLN	Q	10.8	128		114	P
Glycine	GLY	G	7.8	57		48	-
Histidine	HIS	H	0.7	137	6.0	118	P,C+
Isoleucine	ILE	I	4.4	113		124	H
Leucine	LEU	L	7.8	113		124	H
Lysine	LYS	K	7.0	129	10.5	135	C+
Methionine	MET	M	3.8	131		124	H
Phenylalanine	PHE	F	3.3	147		135	H
Proline	PRO	P	4.6	97		90	H
Serine	SER	S	6.0	87		73	P
Threonine	THR	T	4.6	101		93	P
Tryptophan	TRP	W	1.0	186		163	P
Tyrosine	TYR	Y	2.2	163	10.1	141	P
Valine	VAL	V	6.0	99		105	H

http://dwb.unl.edu/Teacher/NSF/C10/C10Links/www.ccp14.ac.uk/ccp/web-mirrors/llnlrupp/Xray/tutorial/protein_structure.htm

More on Primary Structure

Amino Acid

QuickTime™ and a
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Peptide Bond Formation (a condensation reaction)

QuickTime™ and a
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Peptide Bond Formation (a condensation reaction)

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Peptide Bond Formed

QuickTime™ and a
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http://dwb.unl.edu/Teacher/NSF/C10/C10Links/www.ccp14.ac.uk/ccp/web-mirrors/llnlrupp/Xray/tutorial/protein_structure.htm

ϕ φ, and Hydrogen Bonds, Dictate Secondary Structure

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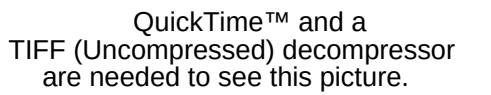
http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/protein.htm

An Alpha Helix



http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/protein.htm

Three representations of an alpha helix

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Ball and stick

Backbone

Ribbon, or
Linguini diagram

http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/protein.htm

Beta strands are connected by hydrogen bonds

http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/protein.htm

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Alpha helix

Two beta strands

Beta Strains Make Pleated Sheets

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http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/protein.htm

Helices and Strands Can Make Folds

A Simple Example of Tertiary Structure

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are needed to see this picture.

EF hand in Calmodulin (helix turn helix)

http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/protein.htm

Other Common Folds

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AB barrel

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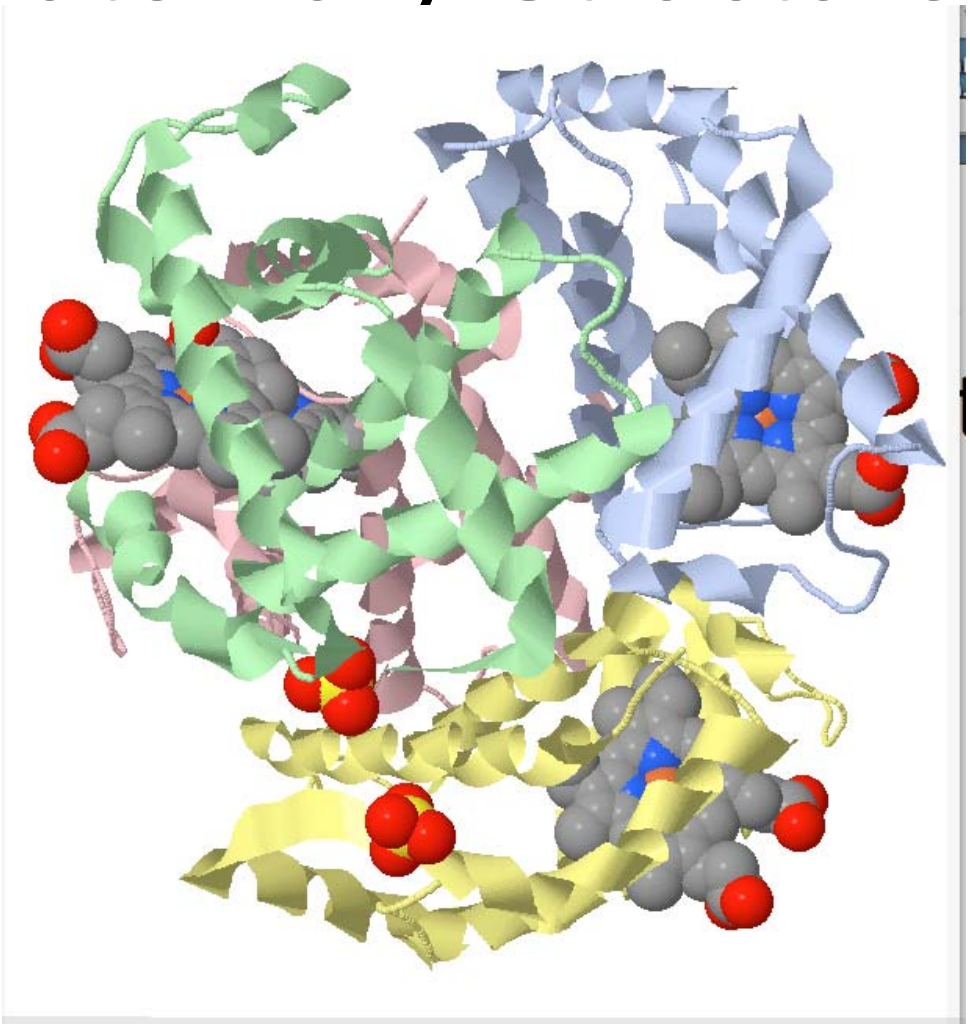
are needed to see this picture.

Beta barrel

http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/glycolysis.htm#tim

Polypeptides Can Associate to Form Quaternary Structures

Hemaglobin
(deoxy form)



http://molvis.sdsc.edu/fgij/fg.htm?mol=2hhd

You can observe proteins dynamically in three dimensions

http://molvis.sdsc.edu/fgij/index.htm

Jmol website

The Life of a Protein

- Synthesis
- Folding
- Targeting
- Function
- Death

Proteins are Polymerized on Ribosomes

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are needed to see this picture.

http://www.elmhurst.edu/~chm/vchembook/584proteinsyn.html

Protein Elongation

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are needed to see this picture.

http://www.elmhurst.edu/~chm/vchembook/584proteinsyn.html

Protein Termination

QuickTime™ and a
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are needed to see this picture.

http://www.elmhurst.edu/~chm/vchembook/584proteinsyn.html

Protein Synthesis Web Animation

http://www.johnkyrk.com/DNAtranslation.html

Evolution of proteins

- Some pre-RNA polymer is believed to have come first; RNA evolved from that
- RNA can function as enzymes
- RNA became the template for DNA
- Amino acids are attracted to codons
- RNA catalyzed condensation of amino acids
- Ribosomes evolved to increase efficiency and fidelity

Folding of proteins

- Structure of proteins in inherent in primary sequence (shown first for ribonuclease by Anfinson)
- Proteins fold to reach their lowest energy
- But cytoplasm is full of molecules; proteins would self-fold with difficulty
- Chaperones bind hydrophobic surfaces and protect proteins during folding
- Misfolding results in aggregation (Alzeimer's disease, prion disease)

Folding Occurs to Reach Lowest Energy

QuickTime™ and a
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are needed to see this picture.

- http://cnx.org/content/m11467/latest/

Protein Targeting

- About half of proteins are destined for other locations in the cell.
- They have “targeting sequences” to get them there.
- Many accessory proteins are required for targeting.

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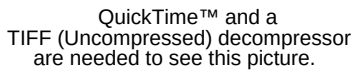
http://www.windows.ucar.edu/earth/Life/cell_intro.html

Mitochondrial import, 2006



Rehling and Pfanner (2004) Nat Rev MCB

Protein Death

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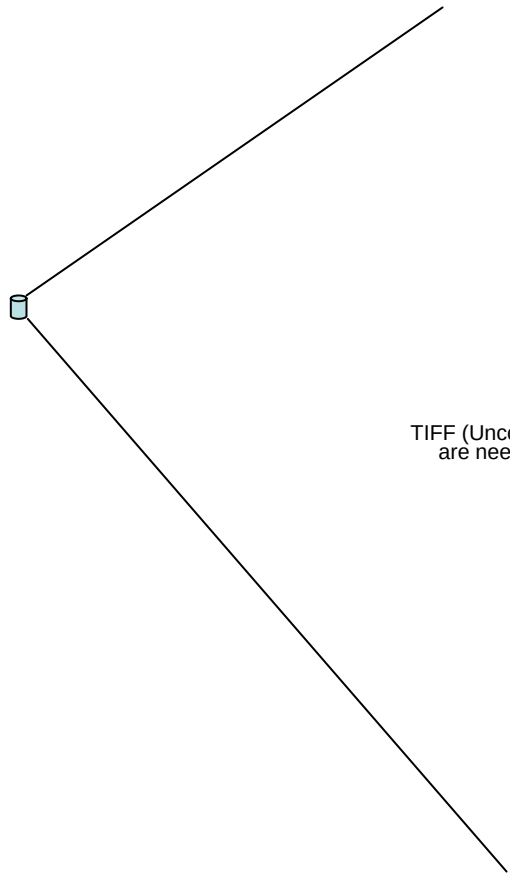
are needed to see this picture.



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Death in the Lysosome

Death in the Proteosome

http://cellbio.utmb.edu/cellbio/lysosome.htm

http://en.wikipedia.org/wiki/Proteasome

Enzymes

What is an Enzyme?

- A protein molecule produced by living organisms that catalyses chemical reactions of other substances without itself being destroyed or altered upon completion of the reactions.
- Sometimes require cofactors (often metals or vitamin derivatives)
- Six types of reactions: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.

- Ribozymes: RNA-based enzymes

http://cancerweb.ncl.ac.uk/cgi-bin/omd?query=enzyme&action=Search+OMD

Consider a Phosphatase

- Function: Desphosphorylation of proteins and nucleotides
- Reaction:

$$R\text{-}O\text{-}PO_3 + H_2O \rightarrow R\text{-}OH + PO_4H$$

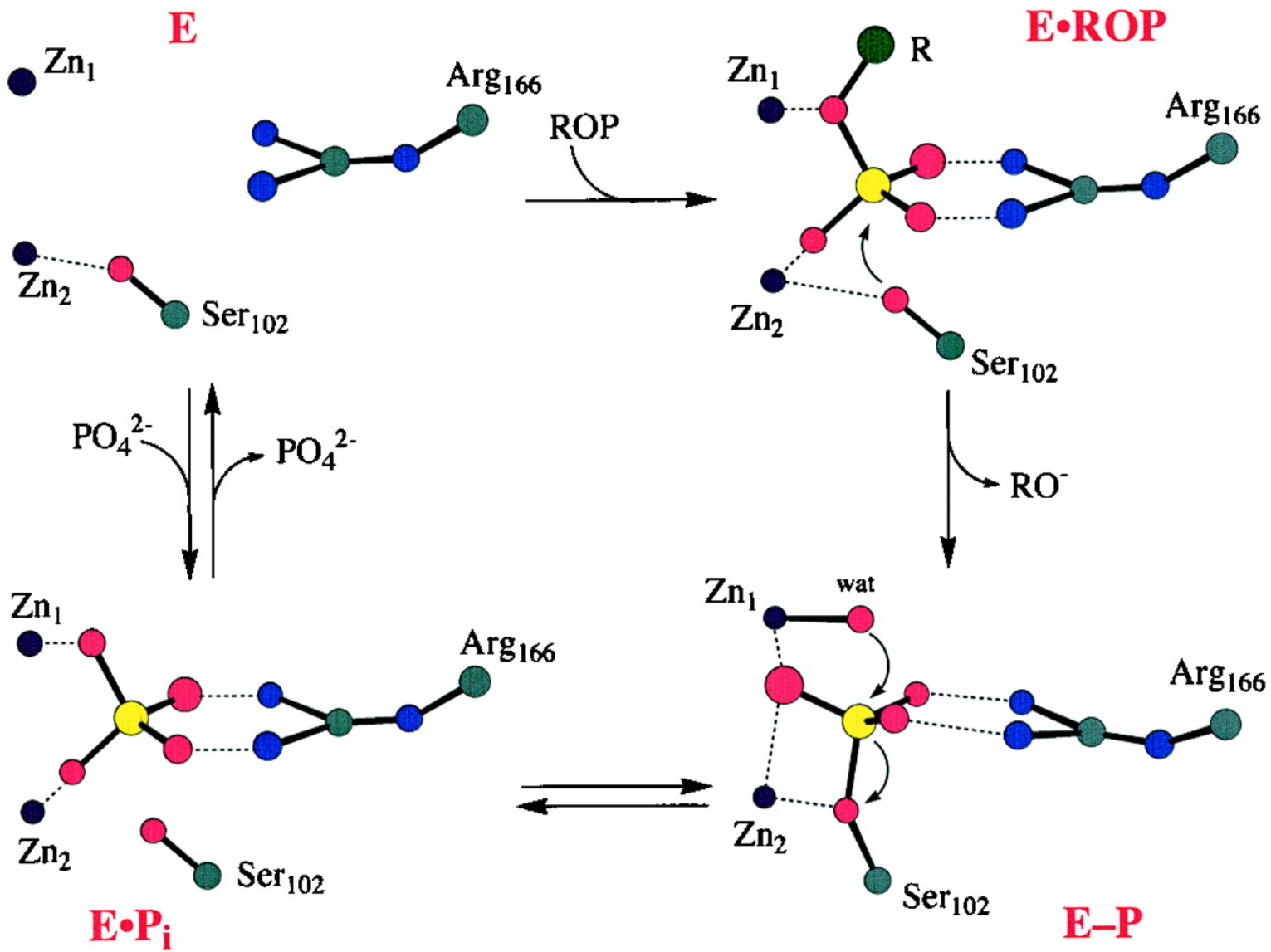
J Biol Chem, Vol. 274, Issue 13, 8351-8354, March 26, 1999

COMMUNICATION

A Model of the Transition State in the Alkaline Phosphatase Reaction*

Kathleen M. Holtz, Boguslaw Stec, and Evan R. Kantrowi

Bacterial Alkaline Phosphatase



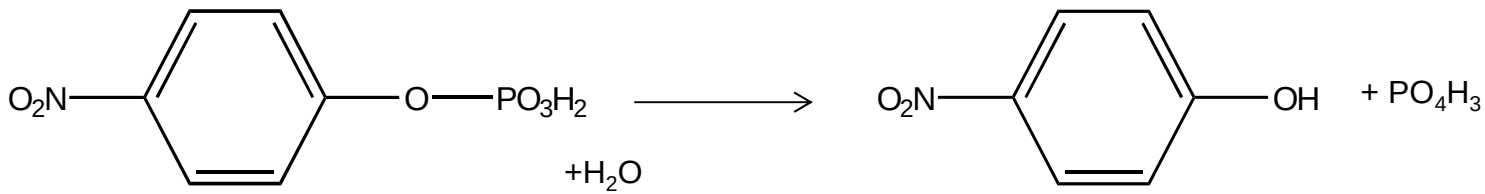
http://www.jbc.org/cgi/content/full/274/13/8351

Why study enzyme kinetics?

- Elucidation of enzyme mechanisms
  - Dependence on cofactors, pH, etc.
- Role of enzymes in pathways
  - Rate-limiting step?
- Discovery of true substrates and products
- Inhibition by drugs

Use of a “Model Substrate” to Study AP

- Para-nitrophenyl phosphate (*p*NPP)
- Hydrolyzed to para-nitrophenol



Clear

Yellow

What we shall do?

- Divide up into 7 groups
- Each group will perform a different reaction:

1) Does zinc stimulate?
2) Is magnesium required?
3) Does the reaction stop completely on ice?
4) What happens with no substrate?
5) What if you lower the pH?
6) What if you add phosphate?
7) THE GOLD STANDARD