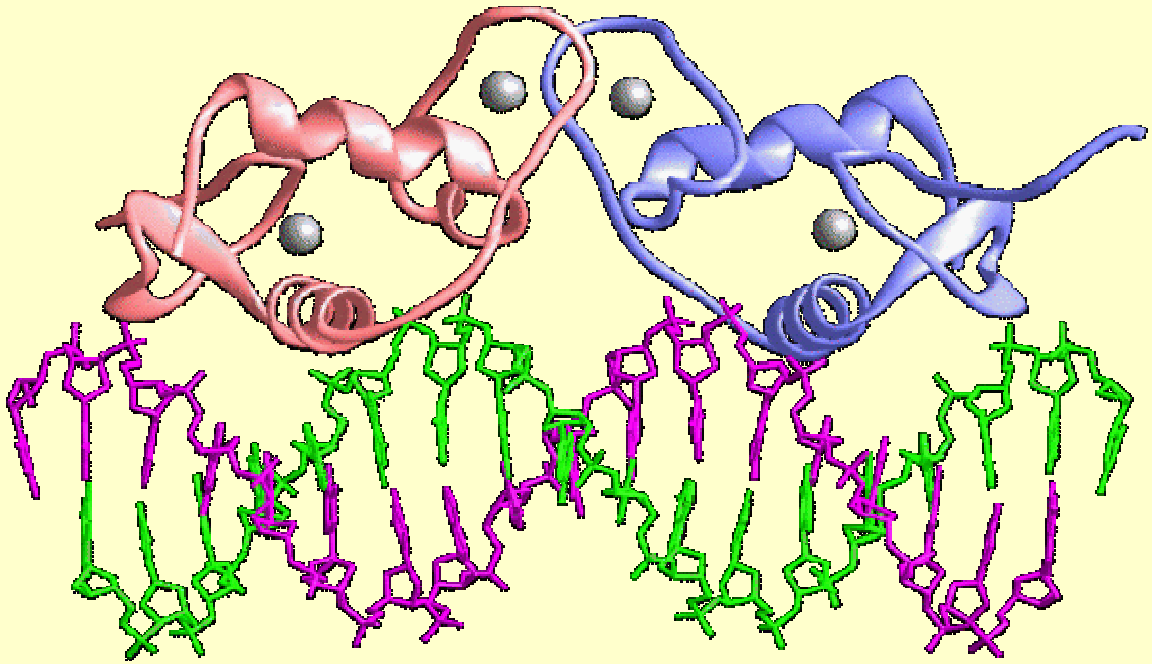
Embedding experimental and analytical approaches into year 2 bioscience lecture courses



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BIOL20141 Gene Expression

course outline

- Introduction to the course – including problem-based learning introduction
- Prokaryotic gene structure
- Gene expression in *E. coli*
- Chromatin structure
- Eukaryotic Gene expression
  - Gene expression and signalling in eukaryotes
  - Tissue-specific and developmental regulation - principles of differentiation.
- Post-transcriptional gene regulation:
  - RNA silencing
  - Splicing and processing
- Gene expression “problem” review
- The mechanism of eukaryotic protein synthesis
  - Global and mRNA specific regulation of protein synthesis
  - The mechanism and regulation of mRNA decay.
- Revision, review and course feedback

Self directed problem-based learning in gene expression BIOL20141

- Unit's predecessor was 100% fact based
- The problem solving exercise deals with concepts and material difficult to teach effectively in lectures
- Compels students to learn and problem solve independently

- General Aims
  - To promote understanding of experimental approaches to study of gene regulation
  - Data analysis and problem solving
  - Learning not teaching
  - To enhance learning of other taught material in the unit

Gene expression problem

core learning objectives for students

Aims

- To introduce analytical approaches used to study gene expression
- To enhance understanding of experimental approaches
- To develop numerical, interpretational and analytical problem-solving skills

General objectives

- By completion you should be able to
  - Understand principles of analytical approaches employed in the study of gene expression
  - interpret numerical data obtained from experiments
  - Qualitatively interpret experimental data in order to define signal transduction pathways and mechanisms of gene regulation

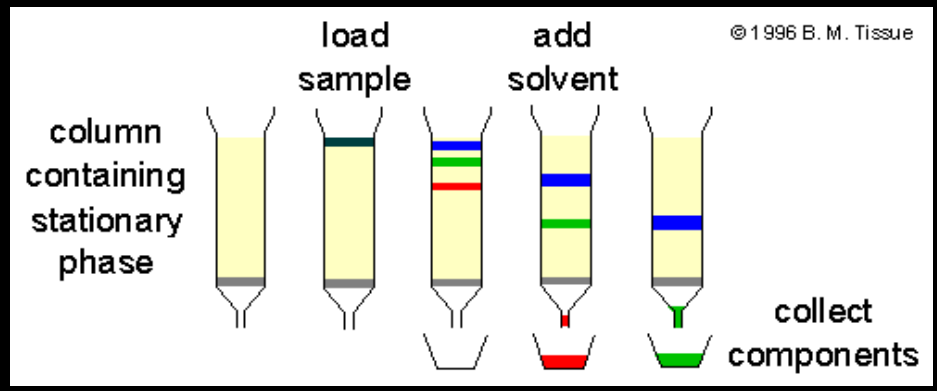
How we did it (late 90s-early 2000s)

- Students are given a paper problem outlining an experiment including data and approaches.
- Problem contains:
  - No explanations
  - Defined objectives and cues, comprehension questions
- Supported by:
  - Orientation in lectures 1 and 16
- Process
  - Students split into groups of ~10-12 (so ~12 groups)
  - Tasks such as compilation of presentations, on methodology, theory and solution to the problem are set
  - Discussion in a tutorial session chaired by a post-doc
- Issues
- Organisation is difficult and time consuming
- No formal assessment

PURIFICATION OF PROTEINS BY COLUMN CHROMATOGRAPHY

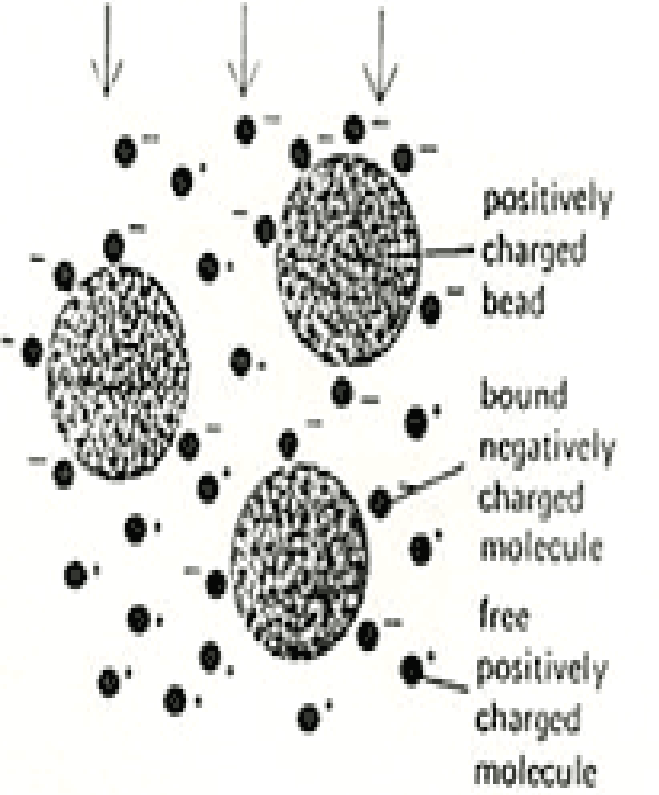
Proteins of interest are separated from cell components. Each purification step exploits different property of the protein. Used in both *analytical* and *preparative* applications

Column Chromatography:

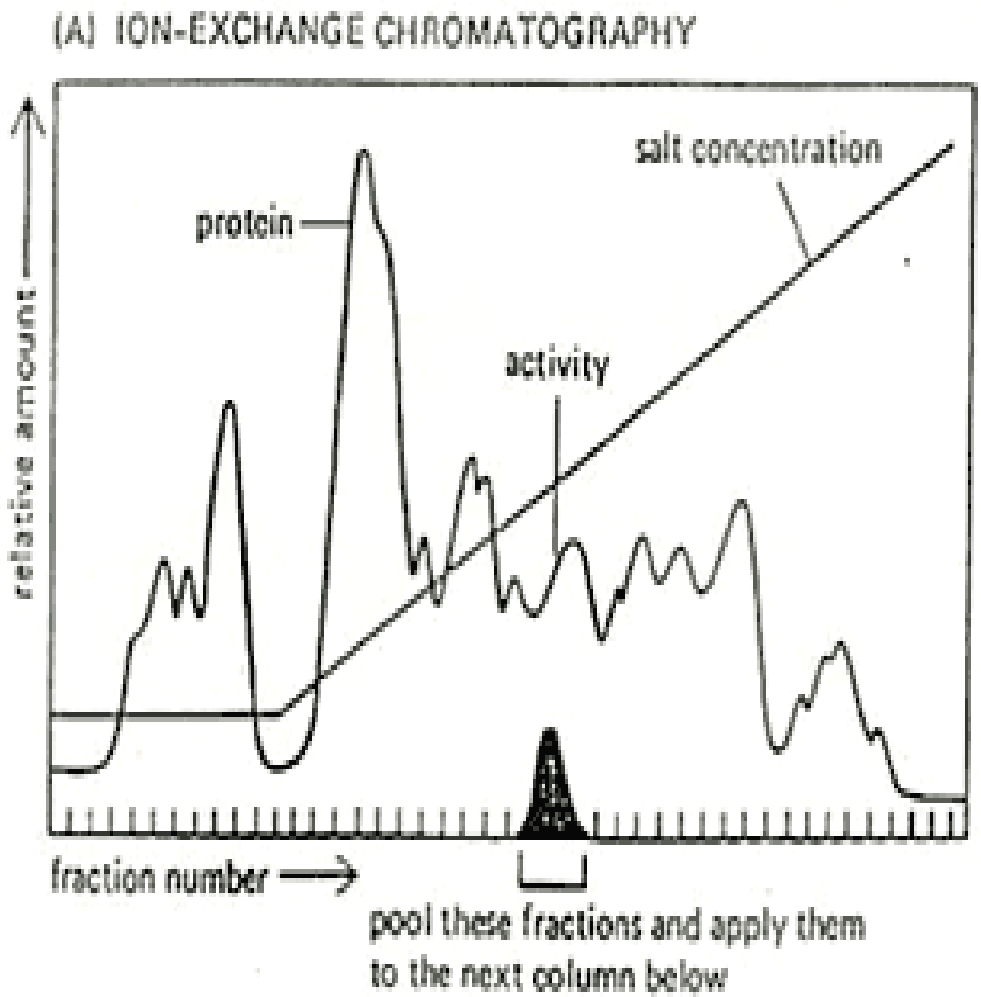


1) **Filled with insoluble material (cellulose or synthetic polymers)**
2) **Protein mixture applied, buffer flows continuously. Different proteins travel at different times and liquid emerging collected as fractions.**
3) **Analysed**

There are many different chromatography techniques:
a) Gel filtration chromatography
b) Ion-exchange chromatography
c) Hydrophobic chromatography
d) Affinity chromatography



(A) ION-EXCHANGE CHROMATOGRAPHY



In ion exchange chromatography, fractionation is based on electrostatic charge. The column is packed with small beads carrying either a positive or a negative charge (figure above). The extent to which a given protein species will bind to the column matrix is thus a function of the charge characteristics of the individual proteins.

Revision and introduction of eLearning 2004 on

- Students are given a paper problem outlining an experiment including data and approaches.
- Problem contains:
  - No explanations
  - Defined objectives and cues, comprehension questions
- Supported by:
  - Orientation in lectures 1, 16, 22
  - Web-based support
    - ePBL
      - References and resources
      - MCQs for self-assessment, including final problem questions
- Process
  - Progress through ePBL nodes over duration of course
  - Coordination of lectures and ePBL
  - Formal examination (a section of paper is now a data handling problem

Outline for BL2141 ePBL

Node 1 Protein purification and analysis
Chromatography – purification and analysis
gel electrophoresis (PAGE)
Western blotting

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Node 2 DNA protein interactions
Techniques and Principles
Dnase footprinting
EMSA
Methylation interference/protection

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Node 3 Reporter genes and promoter analysis
Reporters
Deletion analysis
Northern

↓

Node 4 Gene expression problem

↓

Node 5 Evaluation and reflection

1: "Protein Purification"

Available from: 28/09/2007 **to:** *open*

A wide variety of approaches are employed to elucidate the identity and function of protein transcription factors, such as the signal transduction pathways which regulate their activity. A key component of these investigations is the use of physical purification and separation techniques which permit us to purify a given transcription factor to homogeneity from a complex mixture of cellular proteins. The difficulty of doing this is compounded by the fact that most transcription factors are not abundant. The most significant breakthrough in this particular field was the development of sequence-specific DNA affinity chromatograpgy. Additionally chromatographic techiqus can be used to characterise transcription factors. SDS –polyacrylamide gel electrophoresis (SDS-PAGE) is a powerful separation technique which can be used to determine size and purity of polypeptides, and together with other information, their quaternary structure. It is also used to separate polypeptides prior to western blotting.

Aims of this node
- To introduce students to several key techniques used for protein sdeparation and characterisation
- To give students a general understanding of the physical principles and techniques which can be used to separate proteins on the basis of size, charge and affinity

Objectives
On completeion of this ePBL node, students should be able to
- Describe various methods of chromatographically separating proteins
- Understand principles underlying protein purification by column chromatography and SDS-PAGE

Keywords
- gel filtration
- ion exchange
- affinity
- size-charge ratio
- molecular sieving
- hydrophobic interaction
- SDS
- Electrophoresis
- Stokes radius

	Learning Tasks	Type	Location
1	Purification of proteins	Document	BL2141 course materials pages on SBS intranet
2	SDS-PAGE website	Website	www
3	LSM practical 5 - Protein purification	Practical	LSM handbook
4	Principles and techniques of practical biochemistry 5th edn. pp327-336	Document	JRULM (577.194W30)
5	Biochemistry 3rd edn (2000) matthews/van holde/ahern	Document	JRULM (577.1 M42) 5 copies; Joule Library Main Level U:612.015/MAT 8 copies; stopford
6	Biochemistry 4th edn. Stryer	Document	JRULM (577.1 S97)
7	Biochemistry 4th edn (1997) Zubay	Document	JRULM (577.1 Z17) ; Joule Library Main Level U:612.015/ZUB 5 copies
8	principles of protein purification pdf file	Document	www
9	Eukaryotic transcription factors. Latchman. (1991 academic press) pp27-31	Document	JRULM
10	gel filtration chromatography practical pdf file	Document	www
11	Protein methods 2nd edn. Bollag et al (1996)	Document	JRULM Blue zone ,Floor 2: 577.145/B45 1 copy ; Joule library Main Level U:547.75/BOL 1
12	Textbook of biochemistry with clinical correlations 5th edn ed. Devlin	Document	JRULM Blue zone ,Floor 2: 577.1/D65 7 copies; stopford library 577.1/D65 29copies
13	gel filtration chromatography web page	Website	www
14	gel filtration chromatography - quite concise ppt presentation	Website	www

Assessment MCQ: "Protein purification"

Assessment score of **100%** or above => Techniques for studying DNA-protein interactions

Begin Assessment

3. In SDS polyacrylamide gel electrophoresis, SDS (sodium dodecyl sulphate) is essential to

a) ◯ get rid of contaminating DNA and RNA so that only protein enters the gel

b) ◯ denature the protein and to ensure that all polypeptides have the same charge-mass ratio

c) ◯ Make sure proteins are clean and uncontaminated

d) ◯ break disulpide linkages to linearise polypeptides

4. Affinity chromatography can be used to separate proteins on the basis of:

a) ◯ specific interaction with other molecules

b) ◯ Net charge

c) ◯ Enzyme activity

d) ◯ molecular weight

5. Have a look at the table below. By completion of the table calculate which chromatographic step purifies the protein the most.

Fraction	Volume (ml)	Enzyme activity		protein		Protein Specific Activity (U/mg)	Purification (fold)	Yield (%)
		U	U/ml Total	mg	mg/ml			
Crude extract	1000	90000	90	8000	8.0	11.3	0	100
Ammonium sulphate precipitation	150	75000		1200		62.5	5.6	83.3
Heparin sepharose	20	26000		300				
DEAE-sephadex	30	9000		50				
affinity	3	5000		1.5				

Table showing puroification scheme for the enzyme lactate dehydogenase

a) ◯ heparin sepharose

b) ◯ ammonum sulphate precipitation

c) ◯ affinity

d) ◯ DEAE-sephadex

Submit Reset

Assessment of ePBL efficacy 2004-5

	no. students completed node
Node 1 protein purification	191
Node 2 DNA protein interactions	164
Node 3 reporter genes etc	134
Node 4 gene expression problem	91

	n	Problem section	Total	% students scoring higher in problem than rest of exam
Completed ePBL	91	57.8	52.5	59.8
Not completed ePBL	100	44.7	44.6	46.5
T			0.0025	

- ePBL optional but recommended

Assessment of ePBL efficacy 06-07

total			problem
	n	mean	mean
all ePBL	90	65.1 ± 13.7	68.0 ±22.9
<100% ePBL	63	58.9 ± 17.0	66.6 ± 23.5
T		0.009	0.34

- ePBL now assessed (up to 5% of total)

Changes 08-09

- Port ePBL to Blackboard
  - More versatile question formats
- Re-introduce student group work
  - ePosters and assessed discussion groups
  - Some peer assessment based on participation