

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI  
B.Sc. Biochemistry and Biotechnology  
Classes: BCHEM III and BIOL III  
First Semester Examination #1 2011/2012  
BCHEM 365 Biophysics  
September 2011

Sept. 29, 2011

Name: Prata Emmanuel Nsibor

Index of Program:

Time allowed: 2 hours

Index number

Questions 1-17: Circle the correct answer to the following questions 1 point each

1. The phenomenon of electrophoresis was observed for the first time by Thomas Swann
- (A) Oswald Avery in 1807  
(B) F. Reuss in 1828  
(C) Oswald Avery in 1828  
(D) F. Reuss in 1807

2. Electrophoresis is a technique used for Separating different types of molecules based on their patterns of movement in an electric field.

- (A) Separating  
(B) Detecting  
(C) Quantifying  
(D) Only (A) and (B)

3. Which of these statements is true?

- (A) Both proteins and nucleic acids possess positive and negative charges at any given pH. Under the influence of electrical field, they will migrate either to the cathode or to the anode.  
(B) Only proteins possess positive and negative charges at any given pH. Under the influence of electrical field, they will migrate either to the cathode or to the anode.  
(C) Only nucleic acids possess positive and negative charges at any given pH. Under the influence of electrical field, they will migrate either to the cathode or to the anode.  
(D) None of the above

4. An electrophoresis unit consists of  
(A) A power pack, gel slab, and gel comb  
(B) A plastic frame, power pack and buffer  
(C) A tank, plastic frame and gel comb  
(D) A power pack, tank, plastic frame and gel comb

5. The force, in newtons that drives a macromolecule with charge  $q$  toward an electrode, when placed in an electric field of potential difference  $V$  and separated by a distance  $d$ , is given by

6.  $F = qE$



- (B)  $qd$
- (C)  $dE$
- (D)  $qdE$

6. The velocity,  $v$ , of the charged macromolecule in Question 5, which encounters frictional resistance, is given by the equation

- (A)  $v = qE / f$
- (B)  $v = qf / E$
- (C)  $v = qdE / f$
- (D)  $v = qfE / d$

7. The electrophoretic mobility,  $\mu$ , of the charged macromolecule in Question 5 and 6 is given by

- (A)  $w/d$
- (B)  $w/Ed$
- (C)  $w/Eq$
- (D)  $w/E$

8. During electrophoresis, the current in the solution between the electrodes is conducted

- (A) mainly by the buffer ions, with a small proportion conducted by the sample ions
- (B) by buffer ions and sample ions equally
- (C) by only buffer ions
- (D) mainly by sample ions with a small proportion conducted by the buffer ions

9. The generation of heat in the medium during electrophoresis produces which of the following effects?

- (A) A decreased rate of diffusion of sample and buffer ions leading to broadening of the separated samples
- (B) The formation of convection currents, which leads to mixing of separated samples
- (C) A decrease of buffer viscosity and hence a sharpening of separated bands
- (D) All of the above

10. Electroendosmosis is a problem in electrophoresis caused by

- (A) heating fluctuations when power supply is not constant
- (B) presence of charged groups on the surface of the support medium
- (C) conduction of current by the buffer ions
- (D) the zone center migrating faster than the edges

11. For a good separation, electrophoresis grade agarose must have

- (A) many sulfate groups
- (B) few sulfate groups
- (C) moderate content of sulfate, carboxyl, and methoxyl groups
- (D) none of the above

12. In electroendosmosis the movement of electrolyte ions can be toward the

- (A) anode
- (B) cathode
- (C) zone center
- (D) no movement of electrolyte ions

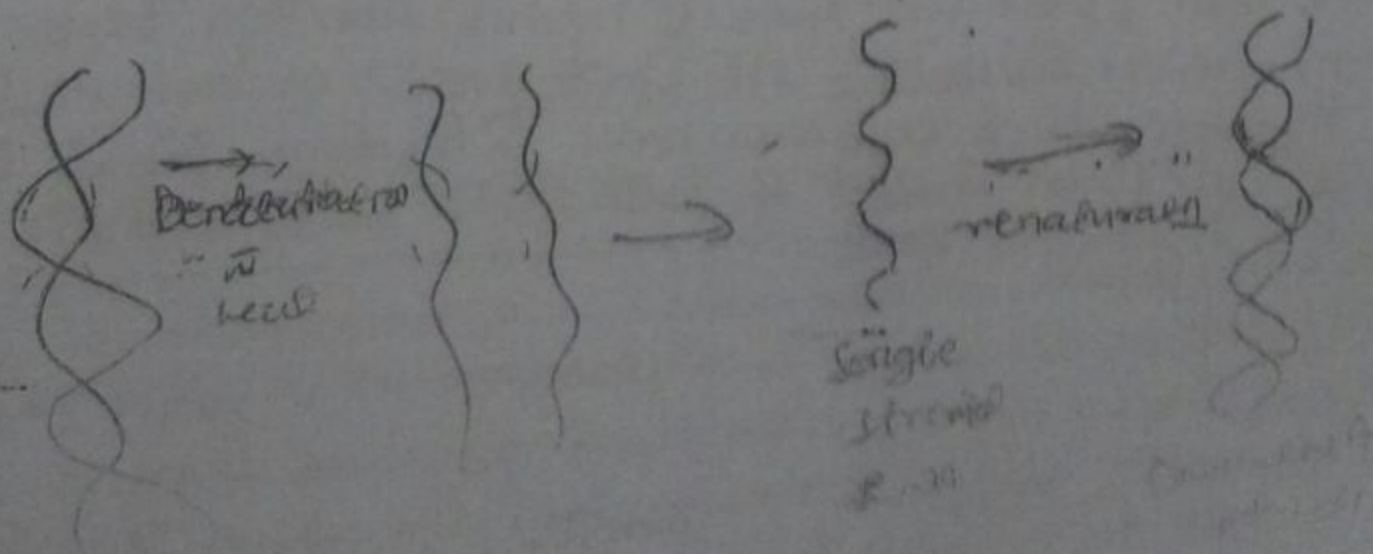


13. Which of these is true?  
 (A) Agarose is a linear polysaccharide made up of the basic repeating unit agarotriose  
 (B) Agarose is a branched polysaccharide made up of the basic repeating unit agarobiose  
 (C) Agarose is a linear polysaccharide made up of the basic repeating unit agarobiose  
 (D) Agarose is a branched polysaccharide made up of the basic repeating unit agarotriose
14. In what way does agarose support medium overcome convective currents during electrophoresis?  
 (A) The cross-linked structure  
 (B) The variable pore size  
 (C) both (A) and (B)  
 (D) None of the above
15. Gelling properties of agarose is attributed to  
 (A) both inter- and intramolecular hydrogen bonding within and between the long agarose chains  
 (B) cross-linking of agarose chains with sulfate groups  
 (C) both inter- and intramolecular methylene bridges within and between the long agarose chains  
 (D) All of the above
16. 1% agarose gel is good for the separation of  
 (A) only proteins  
 (B) only nucleic acids  
 (C) Either proteins or nucleic acids  
 (D) both proteins and nucleic acids
17. Which of these statements is true? (2 points)  
 (a) All proteins have a net negative charge  
 (b) All DNA have a net negative charge  
 (c) All proteins have net positive charge  
 (d) All DNA have neutral charge  
 (e) All of the above

#### Questions 18-24

Answer the following questions to the best of your knowledge

18. Use a drawing to illustrate the principle of nucleic acid hybridization (4 points)





- ☒ (c) Wilkins and Franklin
- (d) Franklin and Pauling

8. Which of these best represents base pairing in DNA?

- (a) Adenine with cytosine
- (b) Guanine with adenine
- (c) Thymine with guanine
- ☒ (d) Adenine with thymine

9. The G+C content of *M. phlei* is 70%, while that of yeast is 36%. Which of the following inferences can be made from this statement?

- (a) *M. phlei* DNA is expected to show a relatively weaker hyperchromic shift
- ☒ (b) *M. phlei* DNA is expected to show a relatively stronger hyperchromic shift
- (c) The rate of denaturation of *M. phlei* DNA at high pH will not differ from that of yeast
- (d) Rate of denaturation at high pH will be faster in *M. phlei* than in yeast DNA

10. Which of these bonds will produce a bulge in DNA structure?

- (a) Cytosine-guanine
- (b) Cytosine-cytosine
- ☒ (c) Guanine-guanine
- (d) Thymine-thymine



27 1/2  
48

Program Biochemistry  
Year of Program 3  
Name Opoku Michael Index number 3884009  
Time allowed: 1 hour

Fill in the blank spaces

1. Electrophoretic mobility is the rate of migration of a protein per unit of field strength.
2.  $cm^2/V\text{-sec}$  is the unit of Electrophoretic mobility
3. The intensity of staining and thickness of protein bands are indicative of their concentration x relative abundance
4. The position height of bands measured from the start point within their respective lanes indicates their c x relative sizes
5. Discontinuous gel electrophoresis systems are designed to improve resolution of a mixture of protein sample by the process of Stacking
6. Migration of the buffer front as it moves through the gel can be followed by the change in the refractive index between the regions containing the leading and trailing ions.
7. Blue Bump Native PAGE does not provide direct measurement of molecular weight, but can be used to estimate protein charge or subunit composition.
8. SDS makes all proteins have the same charge-to-mass ratio and identical electrophoretic mob
9. Ferguson plot showing same slope but different Y intercept means the native proteins have the same mass (Mr) but different charges
10. Ferguson plot having same Y intercept but different slopes means that the native proteins have the same charge but different masses
11. SDS PAGE overcomes Native PAGE effect on mobility by denaturation of the protein
12. Loading buffer for SDS-PAGE must contain glycerol to permit sample to sink down the well
13. Mercaptoethanol added to the sample buffer reduces any disulfide bridges in the protein that are holding together the protein tertiary structure.
14. By strongly binding to protein, SDS disrupts most non covalent bonds, thereby decreasing protein folding
15. Bromophenol blue is an organic insoluble tracking dye tank
16. Discontinuous buffer systems use different buffers for gel and electrode tanks and often two buffers within the gel, with a third buffer in the tank.
17. In discontinuous buffer system, the gel with low acrylamide percentage is the stacking gel



23. What do we mean by primary, secondary, tertiary and quaternary structures of proteins? (6 points)

- ① Primary structure of proteins is the basic sequence of amino acids linked together by a peptide bond
- ② Secondary structure of proteins is when the primary structure interacts with each other through hydrogen bonding. This is in the form of  $\alpha$  helix and  $\beta$  pleated sheets
- ③ Tertiary structure of proteins is when the individual polypeptide chains interact with each other through various bonds to form the biological function protein. And the folding of the protein results in the quaternary structure

24. *Drosophila melanogaster* has a double-stranded DNA with  $1.8 \times 10^8$  bp

(a) How many full double helical turns does this DNA contain? (3 points)

$$10.5 \text{ bp} = 1 \text{ helical turn}$$

$$\therefore 1.8 \times 10^8 \text{ bp} = \frac{1.8 \times 10^8 \text{ bp}}{10.5 \text{ bp}} \times 1 \text{ helical turn} = 1.7 \times 10^7 \text{ turns}$$

How long is the DNA in microns? (1 micron =  $10^4$  Å) (3 points)

$$\text{Spacing} = 3.4 \text{ Å} = 3.4 \times 10^{-10} \text{ m}$$

$$\therefore \text{length} = 1.8 \times 10^8 \times 3.4 \times 10^{-10} \text{ m} = 61,200 \text{ microns} / 17$$

(c) What is the molecular mass of this DNA? (3 points)

$$\begin{aligned} \text{Molecular mass} &= \text{base pairs} \times 660 \text{ D} \\ &= 1.8 \times 10^8 \text{ bp} \times 660 \text{ D/bp} \\ &= 1.188 \times 10^{11} \text{ D} \end{aligned}$$

Total points: 6

This exam is 10% of the overall grade for the course

Handwritten notes



18. In discontinuous buffer system, the ion common to both gels is Cl<sup>-</sup> and this is supplied by Tris-HCl buffer & Tris-HCl buffer.
19. The high pH in the resolving gel of discontinuous buffer systems is for destacking the proteins (ionization of glycine).
20. Acrylamide solution is allowed to polymerize in a gel cassette or plates.
21. To ensure a flat interface between the resolving and stacking gels in a discontinuous system we use Proteins & (water saturated n-butanol) w/ water & n-butanol.
22. Gels of 15% polyacrylamide are therefore useful for separating proteins in the molecular weight range of 10,000 to 100,000.
23. A plot of protein molecular weight against R<sub>f</sub> value is known as Laemmli plot or Ferguson plot.
24. A single band on SDS-PAGE means the sample is made of Pure protein or identical subunits (equal subunits).
25. Ampholytes are used to make the pH gradient in isoelectric focusing.

### Thought problems

26. You are all set to run your first SDS polyacrylamide-gel electrophoresis. You have boiled your samples of protein in SDS in the presence of mercaptoethanol and loaded them into the wells of a polyacrylamide gel. You are now ready to attach the electrodes. Does the positive electrode go to the top of the gel, where you loaded your proteins, or at the bottom of the gel?

→ at the bottom of the gel because, during the electrophoresis period, the charge samples that is the negatively charged ions are supposed to move down the gel to the positively charged electrode and as the proteins will turn to move upwards if the positive electrode is placed up. SDS - confers negative charges on protein uniformly.

27. For separation of proteins by two-dimensional polyacrylamide-gel electrophoresis, what are the two types of electrophoresis that are used in each dimension? Do you suppose it makes any difference which electrophoretic method is applied first? Why or why not?

① Isoelectric focusing (IEF)

② SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Yes, IEF should be done first because it requires the pI of the protein to separate them. However, the SDS-PAGE runs to denature the protein during electrophoresis and so when the SDS is added, the protein may not be a true pI. (22+ 10)



3 At high pH, in the basic medium, the two negatively charged phosphate groups repel each other leading to the breaking of the hydrogen bonds.

(4) Low salts have low ionic strength and low ionic strength repels the negative charge on the DNA causing the breaking of the bonds.

A graph to illustrate how temperature causes denaturation.

Double strand  
unwinds  
absorbs heat

At temperatures above  $85^{\circ}\text{C}$  the strands separate and this high temperature denatures the DNA.



21/10/11 Langmann

$\Delta = v/d$

J 2 E 4  
V 2 E 4  
 $u = \frac{V}{E} \frac{cm/s}{cm/V} = cm$   
Oct. 28, 2011

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

B.Sc. Biochemistry and Biotechnology  
Classes: BCHEM III and BIOL III  
First Semester Examination #2 2011/2012  
BCHEM 365 Biophysics  
October 2011

27 1/2  
48

Program Biochemistry

Year of Program 3

Name Opoku Michael

Index number 3884009

Time allowed: 1 hour

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15. Bromophenol blue is an organic inorganic tracking dye pink
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17. In discontinuous buffer system, the gel with low acrylamide percentage is the stacking gel



19. Name four factors that promote DNA denaturation. Explain how each affects the denaturation process. Use graphs where necessary. (10 points)

- (1) High temperature - At high temperature is above  $T_m$  the double strand DNA molecule begins to unwind leading to the separation of the strands. And  $T_m$  is the temperature at which half the DNA strands are melted.
- (2) Organic solvents like DMSO and formamide also denatures DNA
- (3) High pH
- (4) Low salt

20. Name three factors that bring about annealing of denatured DNA. Explain the mechanism of each. (6 points)

- (1) Concentration of DNA - the higher the concentration the greater the percentage reannealed.
- (2) Temperature - The best temperature for reannealing is about  $25^\circ\text{C}$  i.e. a lower temperature. When temperature is low, it allows for the diffusion of the strands to reanneal.
- (3) Time - The longer the time allowed for the reannealing process, the greater the amount that will anneal.
- (4)  $Cot - Cot$  curves measures the degree of annealing of the DNA.

21. Which of these DNA will have a higher melting temperature: AT-DNA and E. coli DNA? How do the GC contents vary? (4 points)

E. coli DNA will have a higher melting temperature than AT-DNA. Since the AT-DNA consists of only Adenine and Thymine which contains only 2 hydrogen bonds but the E. coli DNA consists of both AT bonds and GC bonds. GC has 3 hydrogen bonds.

22. What is hyperchromic shift? Explain its relevance in DNA denaturation. (4 points)

Hyperchromic shift is a phenomenon in which the DNA strands upon exposure to heat quenchers some of the absorption until it reaches the  $T_m$  where the double bond begins to break. It goes on the  $T_m$  of various DNA.



(b) How long is the DNA in microns? (1 micron =  $10^4$  Å) (3 points)

The spacing between base pairs is about 3.4 Å or  $3.4 \times 10^{-4}$  μm along the helical axis

So multiply the number of bp by  $3.4 \times 10^{-4}$  μm

$$1.8 \times 10^8 \text{ bp} \times 3.4 \times 10^{-4} \text{ μm/bp} = 6.12 \times 10^4 \text{ μm}$$

(c) What is the molecular mass of this DNA? (3 points)

One bp has a molecular mass of about 660 D, so multiply the number of bp by 660 D

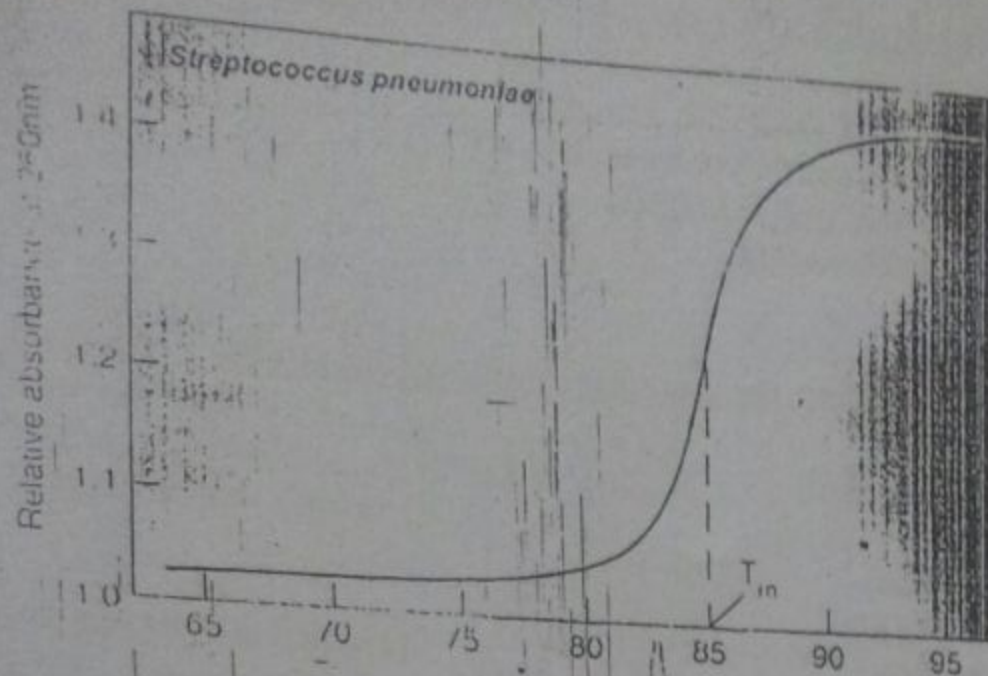
$$1.8 \times 10^8 \text{ bp} \times 660 \text{ D/bp} = 1.188 \times 10^8 \text{ D}$$

Total points: This exam is 10% of the overall grade for the course

80 x 5  
400

Antony Leite





### 3) Organic solvents

Organic solvents such as dimethyl sulfoxide (DMSO) and formamide : disrupt hydrogen bonds between DNA strands and promote denaturation

C) High pH: disrupt hydrogen bonds between DNA strands and promote denaturation

D) Low salt: removes ions that shield the negative charges on the 2 strands from each other

Name three factors that bring about annealing of denatured DNA. Explain the mechanism of each. (6 points)

Renaturation depends on

(A) Temperature

the best temperature for renaturation is about 25°C below the  $T_m$

This temperature is low enough because

- Does not promote denaturation

This temperature high enough to

- allow rapid diffusion of DNA molecules
- weaken transient bonding between the mismatched sequences and intrastrand base-pairing

Rapid cooling prevents renaturation

(B) DNA concentration

the higher the concentration, the more likely two complementary strands will encounter each other within a given time

The higher the concentration, the faster the annealing



*E. coli* DNA (relatively more complex, has genes of about 10,000) will have higher melting temperature. Relative GC content of 51% from Chargaff's data. AT-DNA (simple, highly repetitive, absence of single copy genes) has no GC content and is expected to have a low melting temperature.

21. What is hyperchromic shift? Explain its relevance in DNA denaturation. (4 points)

Nucleic acids absorb light at 260 nm because of the electrical nature of the bases. Close proximity of the bases in the 2 strands (double helix) quenches some of absorbance. When the 2 strands separate upon heating, quenching disappears and the absorbance increases by 30-40%. This is hyperchromic shift.  
Occurs when nucleic acids go from double stranded to single stranded structure

22. What do we mean by primary, secondary, tertiary and quaternary structures of proteins? (6 points)

The amino acid sequence of a protein is the primary structure

Interaction of the amino acids with their neighbors gives rise to secondary structure

$\alpha$ -helix: results from H-bonding among nearby amino acids

$\beta$ -pleated sheet: extended protein chains packed side by side and interact by H-bonding

The three dimensional shape of a polypeptide is its tertiary structure: involves twisting and folding of the chains to incorporate hydrophobic interactions, S-S linkages, van der Waals and electrostatic interactions. Give a bonus of 2 points student uses illustrations.

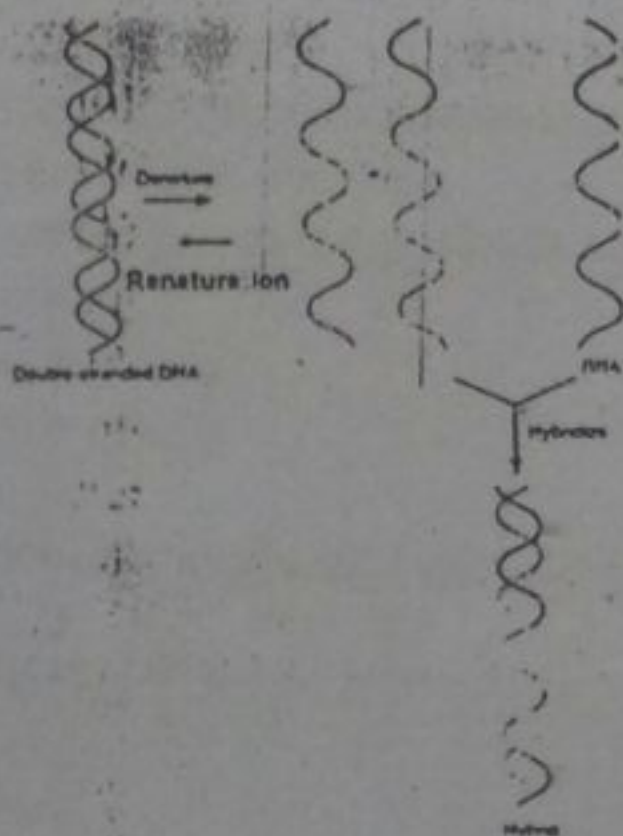
24. *Drosophila melanogaster* has a double-stranded DNA with  $1.8 \times 10^8$  bp. 2 points

(a) How many full double helical turns does this DNA contain? (3 points)

-One turn made of 10.5 bp. So divide the total number of bp by 10.5  
 $= 1.8 \times 10^8 \text{ bp} \times 1 \text{ helical turn} / 10.5 \text{ bp} = 1.714 \times 10^7 \text{ helical turns}$



## DNA hybridization



*Phenyl  
pH  
low salt  
organic solvents*

19. Name four factors that promote DNA denaturation. Explain how each affects the denaturation process. Use graphs where necessary. (10 points)

These conditions promote DNA denaturation

(A) High temperature

Heat DNA solution

the noncovalent forces holding the 2 strands together weaken and break

The two strands come apart in a process known as DNA denaturation/melting

The amount of strand separation or melting is measured by absorbance at 260 nm against temperature

Plot a graph of absorbance against temperature to follow DNA melting



- (X) Nuclides having the same number of neutrons (N). *Isotones*
- (XI) Isotope recommended to use as a label in new situations and combines the right balance between sensitivity and resolution. *P-32*
- (XII) A charge detector in which the potential is just large enough to attract most of the ions to the electrode before they encounter oppositely charged ions to neutralize themselves against. *ION*
- (XIII) An interaction of gamma radiation with matter in which a gamma ray uses part of its energy to eject an outer electron and the rest appears as a lower-energy gamma ray. *Com*
- (XIV) An interaction of gamma radiation with matter in which the gamma ray energy is converted into the mass and energy of an electron and a positron.
- (XV) A device used in slicing a specimen into ultra-thin sections required for examination with a microscope. *ultra*
- (XVI) A lens corrected for both flat field and colour aberration. *Plan lens*
- (XVII) Nuclides having the same atomic weight (A). *Isobars*
- (XVIII) Highest quality objective lens with chromatic corrections at three points and spherical corrections at two points; gives the best resolution and fewest optical defects. *Apochromat*
- (XIX) A type of objective lens that is corrected for chromatic aberration at two points, and spherical aberration at one point; simplest and least expensive. *Ach*
- (XX) Used in autoradiography to reduce exposure times and/or increase the sensitivity in the detection of radiolabeled samples.

# Answers

- A. Micrometer
- B. Compton Effect
- C. Strontium-89
- D. Pair production
- E. Cerenkov detector
- F. Proportional Chamber
- G. Isobars
- H. Isotones
- I. Achromat
- J. LINAC
- K. Isotopes
- L. Ionization Chamber
- M. Elutrap<sup>TM</sup>
- N. <sup>33</sup>P
- O. Technetium-99
- P. Plan lens
- Q. Intensifying screen
- R. Planachromat
- S. Apochromat
- T. Ultramicrotome

TURN OVER



KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,  
KUMASI  
COLLEGE OF SCIENCE  
FACULTY OF BIOSCIENCES

B. Sc. Biochemistry and Biotechnology First Semester Examination, 2006  
Third Year

BCHEM 365: BIOPHYSICS

November, 2006

THREE HOURS

Attempt question 1, and one other question from Section B.  
Answer both questions in the answer booklet provided.

Question 1. (50 Marks).

- (a) The passage below describes the Gram staining procedure. Write the missing word against the corresponding number in your answer booklet

"Gram-staining was first described by Christian Gram in 1884. In essence, the cells are stained with -1- and then treated with -2- solution as a -3-. The intensely stained cells are then washed with -4-. Gram-5- cells retain the stain, whereas gram-6- cells do not. To make the contrast between the two results obvious, the preparation is counterstained with a contrasting red dye (i.e. -7-), So that gram-8- cells are easily seen."

- (b) For each statement below numbered I to XX choose **only one** answer from the list A to T. Write the letter (A to T) of the answer against the number (I to XX) in your answer booklet.

- (I) A device for the extraction or elution of proteins after electrophoresis. *Electrophoresis*
- (II) A charge detector in which the potential is large enough for the ions to achieve significant kinetic energy and hence create a large number of ion-pairs. *Proposed*
- (III) A device in which charged particles are accelerated with multiple exposures to A.C. fields craftily arranged so that each time the particle is exposed to the field it is in the right phase to increase its energy. *15.3.0*
- (IV) Nuclides having the same atomic number (Z).
- (V) A charge detector that responds only to charged particles moving very close to the speed of light.
- (VI) A scale positioned on the microscope stage and focused at like a specimen; used to calibrate a microscope.
- (VII) Radioisotope most widely used in medicine.
- (VIII) Radioisotope used in many palliative medical procedures, usually to relieve pain.
- (IX) A lens corrected to yield flat fields rather than curved.





Program Biochemistry  
Year of Program 3  
Name Batu Emmanuel Nwaka Index Number 38798019  
Time allowed: 2 hours

Questions 1-17: Circle the correct answer to the following questions  
1. The phenomenon of electrophoresis was observed for the first time by

- (A) Oswald Avery in 1807
  - (B) F. Reuss in 1828
  - (C) Oswald Avery in 1828
  - ☒ (D) F. Reuss in 1807
- F. Reuss

2. Electrophoresis is a technique used for \_\_\_\_\_ different types of molecules based on their patterns of movement in an electric field.

- ☒ (A) Separating
- (B) Detecting
- (C) Quantifying
- (D) Only (A) and (B)

3. Which of these statements is true?

- ☒ (A) Both proteins and nucleic acids possess positive and negative charges at any given pH. Under the influence of electrical field, they will migrate either to the cathode or to the anode.
- ☒ (B) Only proteins possess positive and negative charges at any given pH. Under the influence of electrical field, they will migrate either to the cathode or to the anode.
- (C) Only nucleic acids possess positive and negative charges at any given pH. Under the influence of electrical field, they will migrate either to the cathode or to the anode.
- (D) None of the above

4. An electrophoresis unit consists of

- (A) A power pack, gel slab, and gel comb
- (B) A plastic frame, power pack and buffer
- ☒ (C) A tank, plastic frame and gel comb
- (D) A power pack, tank, plastic frame and gel comb

5. The force, in newtons that drives a macromolecule with charge  $q$  toward an electrode when placed in an electric field of potential difference  $E$  and separated by a distance  $d$  is given by

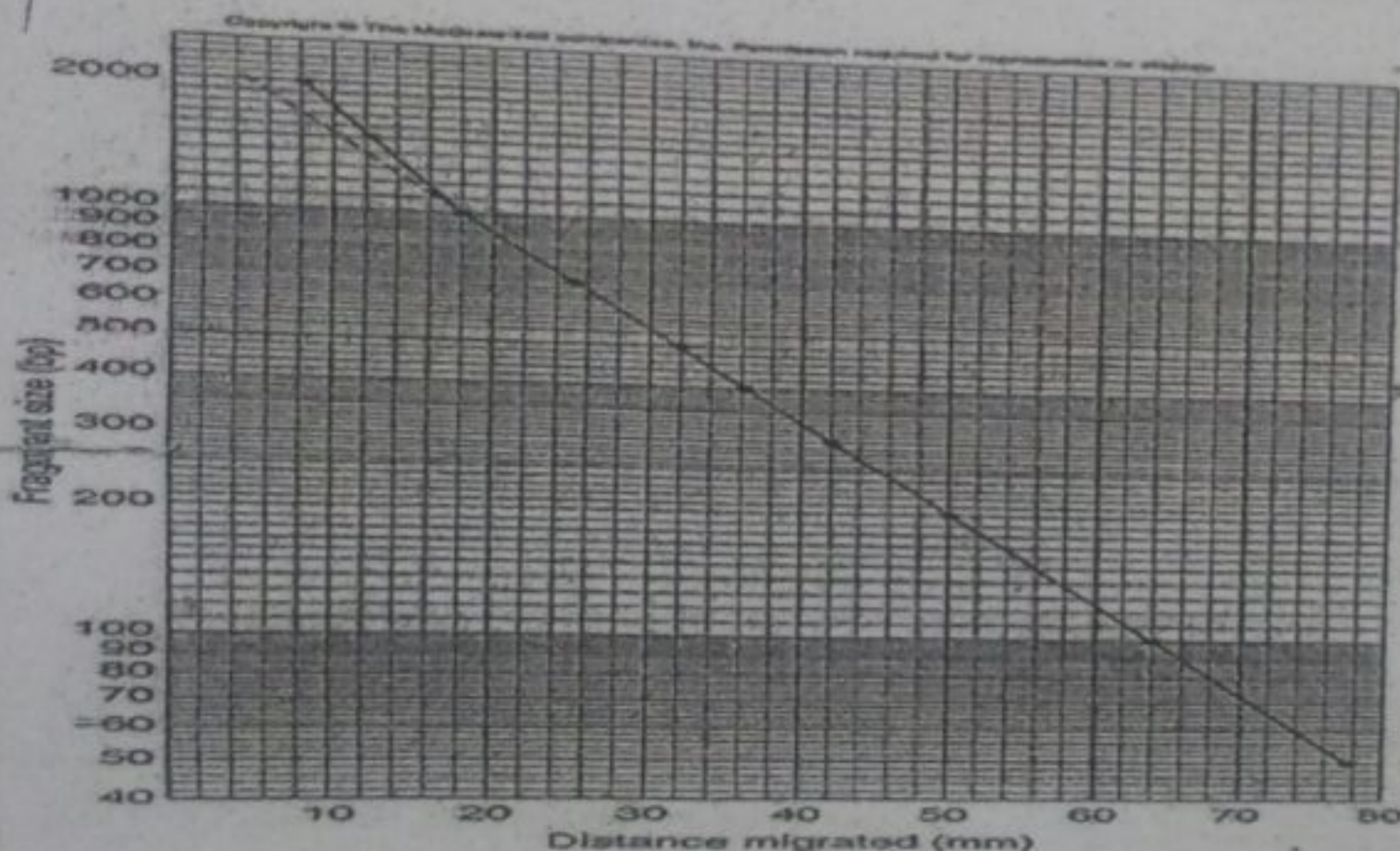
- ☒ (A)  $qEd$
- (B)  $qE$



Oct. 16, 2009

1. You have electrophoresed some DNA fragments on an agarose gel. Using the graph below answer the following

- What is the size of fragment that migrated 25 mm? *700 bp*
- How far did the 250 bp fragment migrate?
- One of the bands on your gel picture shows a 2.3 kb. Can you tell the distance migrated using this diagram? Explain. *No*
- Name and describe the method used for separating DNA fragments of up to several million bp like chromosomes *PAGE*



For a large DNA, the relationship between the log of fragment size and the distance migrated is linear.  
- large, double stranded DNA are fragments, running on a gel may produce more

PAGE, uses pulses of current through the gel. with longer pulses a few forward double and shorter pulses in the opposite a side way double

(b)

- Describe a DNA fingerprinting method using a minisatellite probe *RFLP*
- Describe a nonradioactive method for detecting a particular nucleic acid fragment in an electrophoretic gel

Total points: 45

This exam is 15% of the overall grade for the course



Total 15 points

Question 1

Avery and colleagues provided evidence that transforming substance was not protein but DNA by a combination of macromolecule elimination procedures and determination of the physical properties of the transforming material, while Griffith laid the foundation for identification of DNA as genetic material by demonstrating that present in the bacterial cell was a substance which could cause transformation of the phenotype of another cell. 2 points

Avery and colleagues: employed two approaches

(A) Used sequential elimination of macromolecules from the infected extract of the diseased mouse and studied effect of extract devoid of the macromolecules on transformation. 2 points

The following elimination methods were used:

- Removed protein with organic solvents: extract still transformed 1point
- Removed protein with enzymes: trypsin and chymotrypsin. No effect on transformation 1point
- Removed RNA with enzyme ribonuclease: no effect on transformation 1point
- These steps ruled out RNA and protein as transforming material 1point
- Removed DNA with enzyme DNase: destroyed transforming ability 1point

(B) Determined the physical properties of the transforming substance by use of four techniques:

5 points

1. Ultracentrifugation sedimented rapidly  
means transforming material has high molecular weight
2. Electrophoresis – showed high mobility (i.e., moved fast down the gel), and therefore has high charge-to-mass ratio
3. UV light absorption spectrum – maximum at 260 nm
4. Elemental chemical analysis – nitrogen to phosphorus ratio of 1.67 (expected for pure DNA)

Protein is however rich in nitrogen and low in phosphorus and would have a much higher N/P ratio

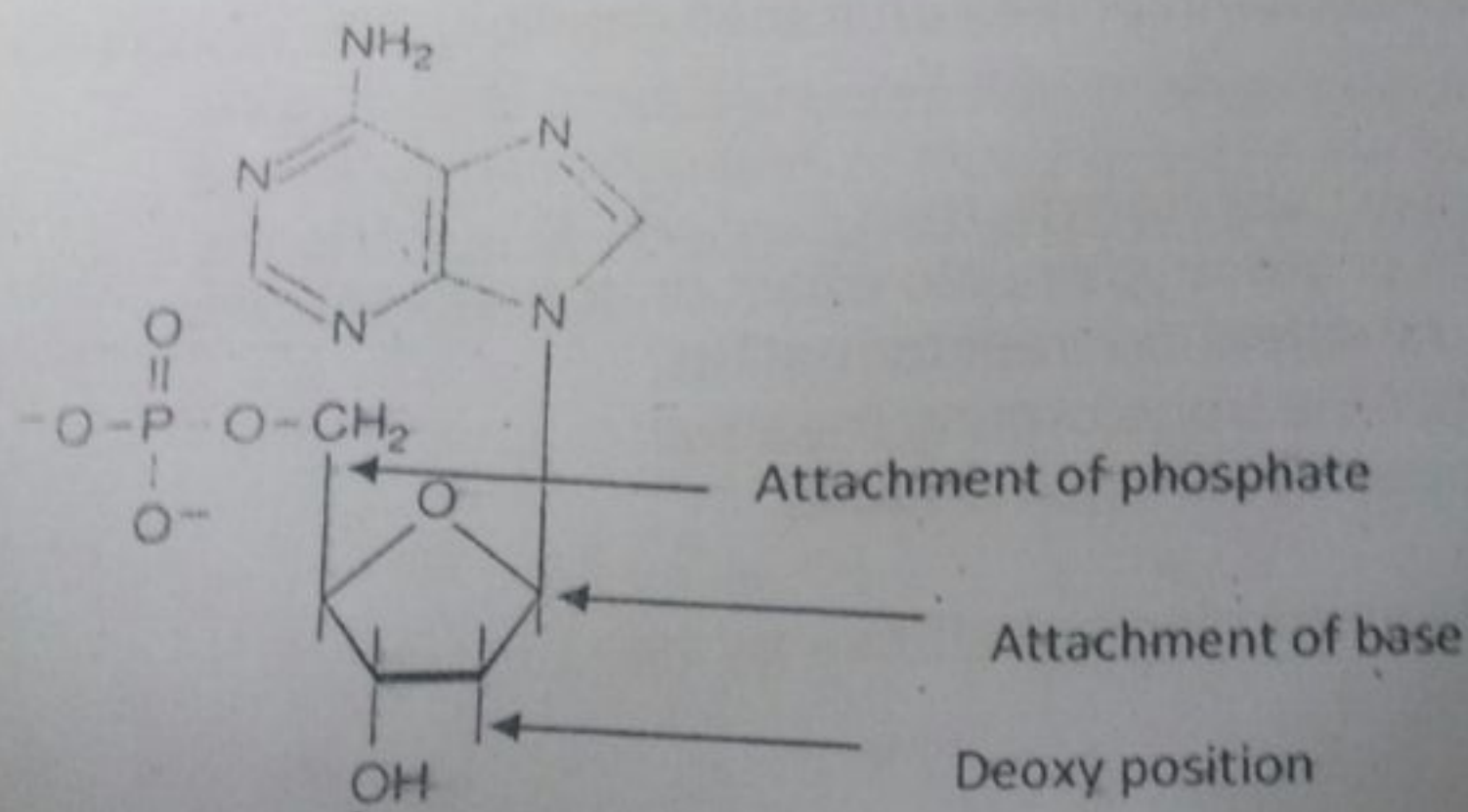
C) Did not infect any organism with extract from diseased mouse.



Concluded that because most of the labeled DNA with its  $^{32}\text{P}$  entered the infected cells while no  $^{35}\text{S}$  was found in the cell, the genes of this phage are made of DNA. 3 points

### Question 2

Structure of a deoxynucleoside monophosphate is presented below with the structure of sugar shown. Position of attachment of the base is 1' carbon of ribose and position of attachment of phosphate is shown by the arrow as 5' carbon. Deoxy position is also shown. 4 points



Deoxyadenosine-5'-monophosphate (dAMP)



...colleagues provided evidence that transforming substance was not protein but DNA by a combination of macromolecule elimination procedures and determination of the physical properties of the transforming material, while Hershey and Chase provided evidence that genes were made up of DNA using radiolabel technique. 2 points

Avery and colleagues: employed two approaches  
(A) Used sequential elimination of macromolecules from the infected extract of the diseased mouse and studied effect of extract devoid of the macromolecules on transformation. The following elimination methods were used:  
Removed protein with organic solvents: extract still transformed  
Removed protein with enzymes: trypsin and chymotrypsin: No effect on transformation  
Removed RNA with enzyme ribonuclease: no effect on transformation  
These steps ruled out RNA and protein as transforming material  
Removed DNA with enzyme DNase: destroyed transforming ability

Determined the physical properties of the transforming substance by use of four techniques: 2 points

Ultracentrifugation – sedimented rapidly  
means transforming material has high molecular weight  
Electrophoresis – showed high mobility (i.e., moved fast down the gel), and therefore has high charge-to-mass ratio  
light absorption spectrum – maximum at 260 nm  
Elemental chemical analysis – nitrogen to phosphorus ratio of 1.67 (expected for pure protein is however rich in nitrogen and low in phosphorus and would have a much higher

not infect any organism with extract from diseased mouse.

...sion: provided evidence that transforming substance was not a protein by means of light absorption spectrum at  $\lambda_{260}$ . Elemental analysis: N/P ratio of 1.67. 1 point

and Chase

...techniques such as radiolabeling of sulfur present in proteins and phosphorus in nucleic acids. Infected *E. coli* with bacteriophage T2 and examined the contents for which type of radioactive label it contained