2010 Fall Semester
Introduction to Nanotechnology (IA)–Midterm Exam Answer Keys

Please choose only 5 out of the 7 questions listed below to answer. There is no extra point for answering more than 5 questions.

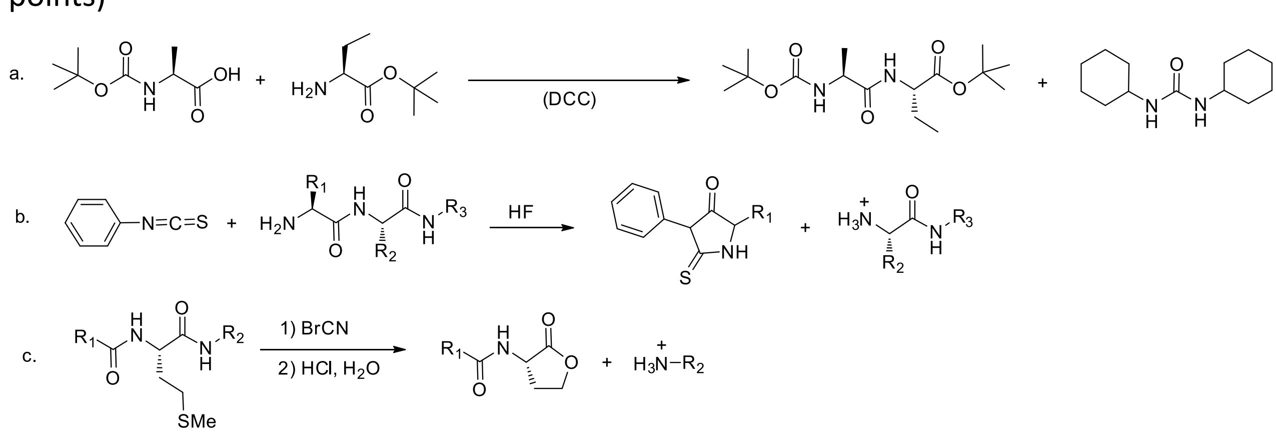
1. (A) Please write down 3 differences between Prokaryotes and Eukaryotes. (**6** points)

   (B) Please write down 4 basic structures (or organelles) of a mammalian cell and describe functions, respectively. (**8** points)

   (C) Give 3 examples of applying nanotechnology to study cell biology. (**6** points)

2. (A) Draw the steric structure of tripeptide RGD. (**6** points)

   (B) Provide the reaction mechanisms of the following reactions. (**2** points for each, total **6** points)



(C) Explain the following: (**2** points for each, total **8** points)

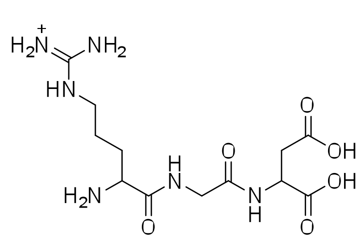
a. Isoelectric point (pI) of a protein.

b. Ion-exchange chromatography.

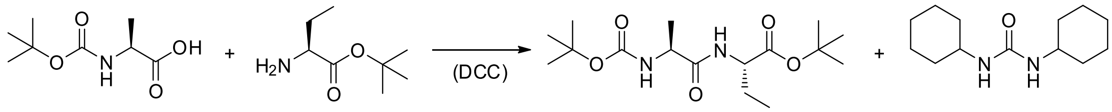
c. Quaternary structure of a protein.

d. α-helix of the polypeptide.

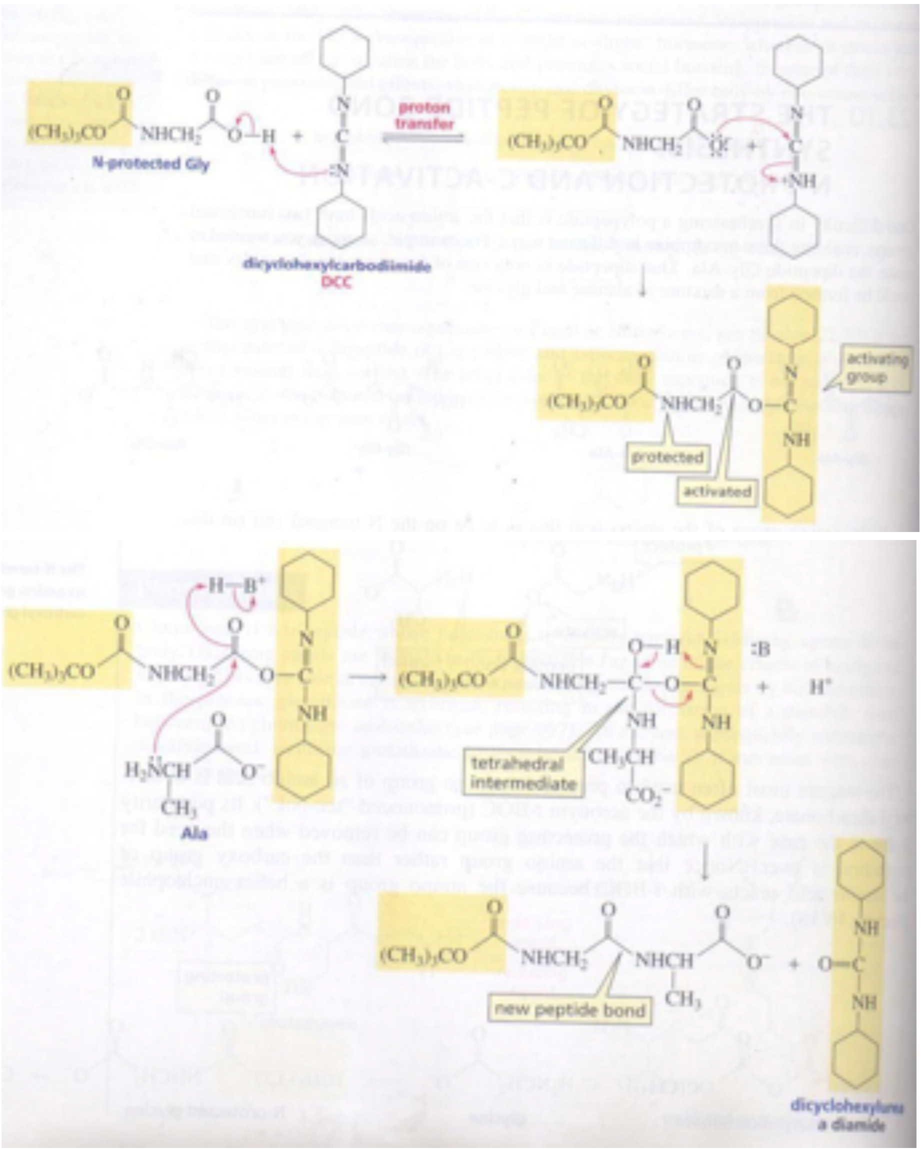
(A)



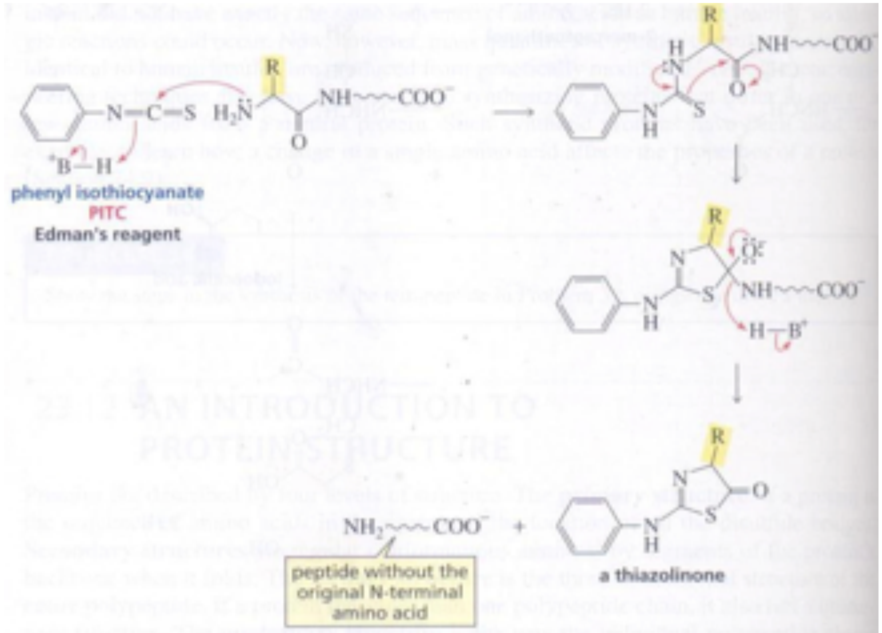
(B)


(DCC)

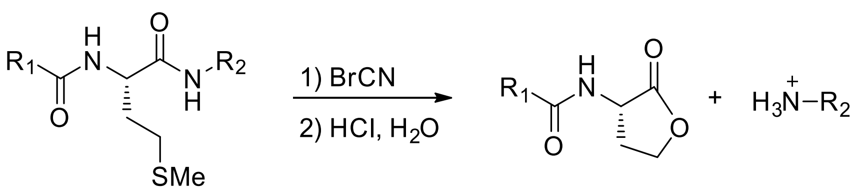
a.

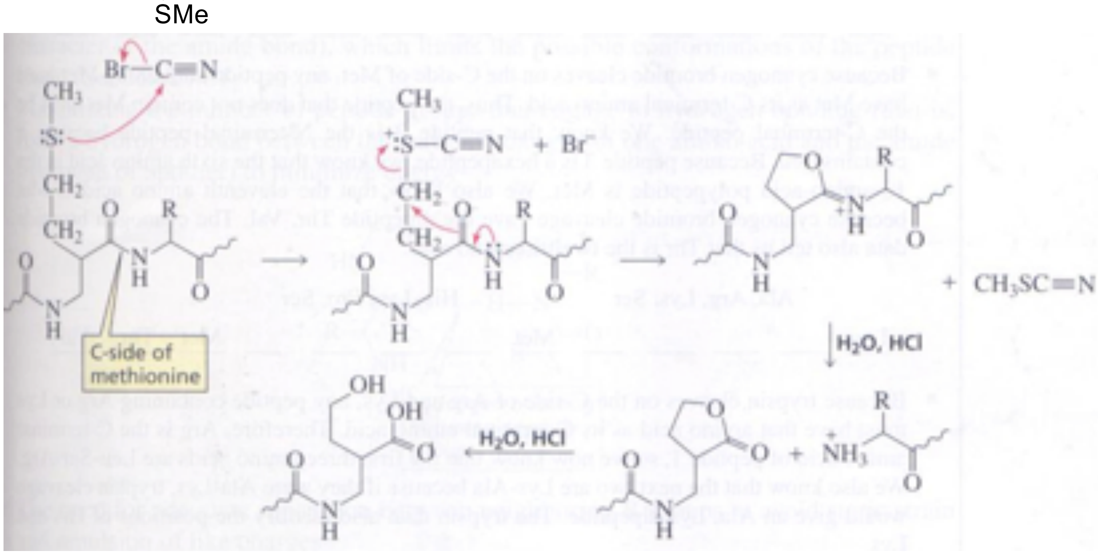

N-protected Gly
proton transfer
dicyclohexylcarbodiimide
DCC
activating group
protected
activated
tetrahedral intermediate
Ala
new peptide bond
a diamide

b.



c.





(C)

1、 Isoelectric point (pI) of a protein

The isoelectric point (pI), sometimes abbreviated to IEP, is the pH at which a particular molecule or surface carries no net electrical charge.

Amino acids which make up proteins may be positive, negative, neutral or polar in nature, and together give a protein its overall charge. At a pH below their pI, proteins carry a net positive charge; above their pI they carry a net negative charge.

2、 Ion-exchange chromatography

Ion-exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their charge. Ion exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge.

3、 Quaternary structure of a protein

Quaternary structure is the arrangement of multiple folded protein or coiling protein molecules in a multi-subunit complex.

4、 α-helix of the polypeptide

A common motif in the secondary structure of proteins, the alpha helix (α-helix) is a right-handed coiled or spiral conformation, in which every backbone N-H group donates a hydrogen bond to the backbone C=O group of the amino acid four residues earlier ($i + 4 \rightarrow i$ hydrogen bonding).

3. (A) "*Proteomics*" is a new research field; please briefly describe its definition. (**5** points)

   (B) Mass spectrometry has become one of the core technologies for proteomics.

   (i) What does mass spectrometer measure? (**2** points)

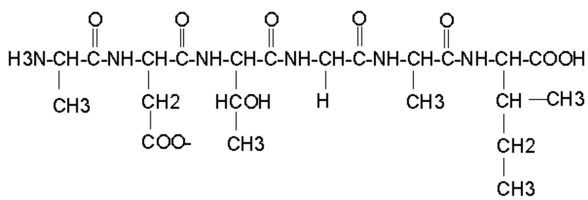
   (ii) Please describe a method that converts an analyte into "ion". (**3** points)

   Please use the following example to show (C) ***Peptide Mass Fingerprinting*** (D) ***Tandem mass spectrometry*** for protein identification.

   (C) You can use the following protein sequence as an example. (**5** points)

   MVYIIAEIGC NHNGDINLAK KMVDVAVSCG VDAVKFQTFK AEKLISKFAP KAEYQKATTG TADSQLEMTK RLELSFEEYL EMRDYAISKG VETFSTPFDE ESLEFLISTD MPIYKIPSGE ITNLPYLEKI GKQQKKVILS TGMAVMEEIH QAVNILRQNG TTDISILHCT TEYPTPYPSL

   (D) How can we use the fragmentation from CID to derive the sequence? You can use the following peptide sequence to predict the fragment. (**5** points)



   (E) For the identification of phosphorylated protein in complex sample, such as cell or tissue, enrichment/isolation is still required before mass spectrometry analysis. Please explain why? (Bonus problem: **3** points)

(A) Qualitative(l point) and Quantitation(l point) analysis of all the protein in the organism (3point).

(B) (i) mass to charge ratio, *m/z* (2point)

(ii) ESI or MALDI or EI or FAB (write full name and explain) (3point)

(C) (2 point) Digestion: generate a unique set of peptides

(l point) Mass analysis of the peptides

(2 point) Database search

(D) (2 point) CID Full name

(2 point) draw the position of y and b ions

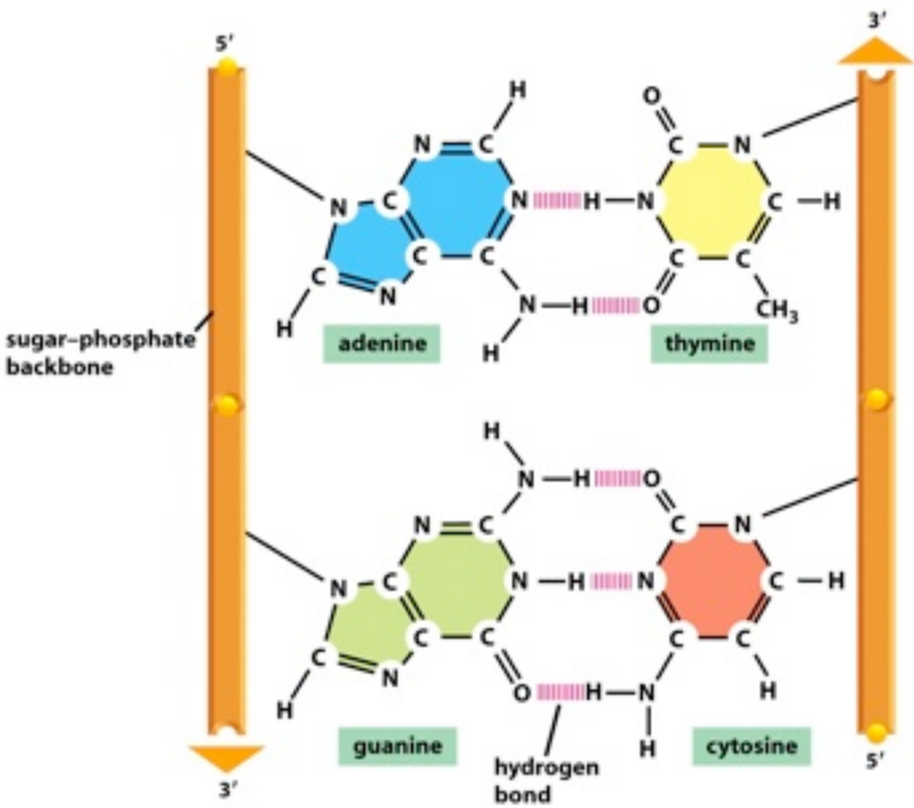
(l point) match the fragment to peptide sequence

(E) Low abundance or phosphopeptides are negatively charged

(Any one of the answers gets 3 point)

4. (A) A double-stranded DNA has a structure like the one below. Please indicate the four bases (**A**-adenine, **C**-cytosine, **T**-thymine, **G**-guanine) in the boxes below. (**8** points)

   (B) Watson-Crick base pairing tells us there are hydrogen bonds between nucleic acid bases. Please indicate where the hydrogen bonds are. (**5** points)



(C) Polymerase chain reaction (PCR) is a powerful way for DNA amplification. If a PCR cycle has 90% of efficiency, how many cycles it takes to reach 10^6-fold amplification? (**7** points)

$(2x0.9)^n \geq 10^6$, $n \log(1.8) \geq 6$, $n = 24$ (i.e. > 23.5).

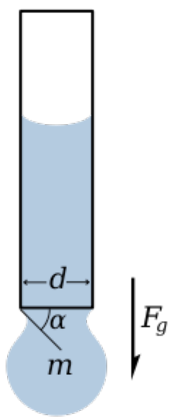
5. Please describe the therapeutic implications of identifying cancer stem cells in Cancer Patients? (**20** points)

Most therapies (chemotherapy and radiation) target rapidly proliferating, non-tumorigenic cells and spare the relatively quiescent cancer stem cells. Moreover, it has been shown that multidrug resistance genes such as ABC transporters are expressed in CSCs. Therefore, conventional therapeutics may not necessarily be able to act specifically on CSCs. Moreover, Cancer stem cells have greater invasive and migratory properties and can home to specific tissue niches. Since CSCs are believed to mediate cancer metastasis or cancer relapse after chemotherapy, therefore, to develop an efficient therapeutics that can specifically target cancer stem cells would be able to prevent cancer metastasis or to kill this chemotherapy-refractory population.

6. (A) Describe qualitatively why there is a tendency for surface tension to minimize the surface area of a liquid drop. (**4** points)

As the molecules exposed to surface has less ~~to~~ interaction with others, it has excess free energy, which ~~can~~ multiplyed by all surface mol. gives surface ~~area~~ energy (tension)
To minimize total free energy droplets tends to take shape with min energy

(B) Pendant drop technique is commonly used to determine the surface tension of a liquid (see figure below). Determine the surface tension γ if the diameter of the tube d = 5 mm, the contact angle α = 30°, and the mass of the droplet m = 0.1 gram. (**8** points)



$F_s = \pi d \gamma \sin\alpha$

$F_g = mg$

$\pi d \gamma \sin\alpha = mg$

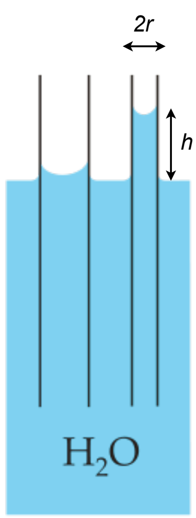
$\gamma = \dfrac{mg}{\pi d \sin\alpha}$

$= \dfrac{10^{-4} \times 9.8}{3.14 \times 5\times10^{-3} \sin 30}$

$= \dfrac{9.8\times10^{-4}}{3.14\times5\times10^{-3}\times0.5} = 1.25\times10^{-1}$ N/m $= 0.125$ N/m ✓

$m = 0.1$ gram $= 10^{-4}$ kg
$d = 5$ mm $= 5\cdot10^{-3}$ m
$g = 9.8$ N/kg

(C) Due to capillary action, derive the height, h, of a capillary column can be expressed by the following, where γ is the liquid-air surface tension, θ the contact angle, ρ the density of liquid, g local gravitational field strength, and r radius of tube. (**8** points)



$$h = \frac{2\gamma\cos\theta}{\rho g r}$$

由 Surface tension force = weight.

$2\pi r \gamma \cos\theta = \pi r^2 h \cdot \rho g$.

$h = \dfrac{2\gamma\cos\theta}{\rho g r}$ ✓

7. (A) Describe what is an entropic barrier of a biomolecule? (**5** points)

   A confined space which is less than the effective size of a molecule (such as radius of gyration) could reduce the degree of freedom (or configurations) of a molecule and hence increase the free energy. It is thus not energetically favorable for a molecule to be in without external force. The interface between a non or less confined space (high entropy state) and a more confined space (low entropy state) is regarded as an entropic barrier for a molecule.

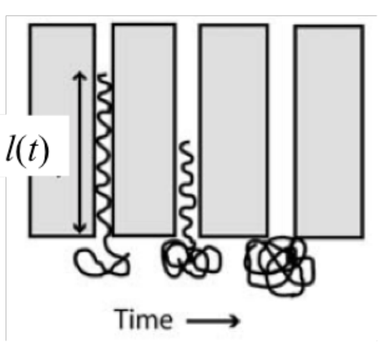
   (B) Give an example of using entropic barrier for a real application. (**5** points)

   Use an array of entropic barriers for free-solution DNA separation/fractionation.

   (C) The following figure illustrates the extraction of a relaxed DNA strand from a nanochannel. The entropic force driving the extraction is due to the difference in configuration space on either side of the nanochannel/microchannel interface. This process of self-extraction, which only occurs for molecules straddling the interface, is called a recoil process. In this scenario, derive the following relation:

$$l(t) = [2f(t_f - t)/\xi]^{1/2},$$

   where $l(t)$ is the insertion length in the nanochannel, f the entropic force, ξ is the hydrodynamic drag per unit extended length in the nanochannel, and t_f is the time of complete extraction. (**10** points)



entropic force is balanced with viscous drag force $= \xi l v$, $v = \frac{dl}{dt}$

$$\xi l \frac{dl}{dt} = f$$

$$\int_L^0 l\,dl = \frac{f}{\xi} \int_t^{t_f} dt$$

$$-\frac{l^2}{2} = \frac{f}{\xi}(t - t_f)$$

$$l = \sqrt{\frac{2f}{\xi}(t_f - t)}$$ ✓

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