MOLECULAR MODELLING AND DRUG DESIGNING
(BIOT 3231)

Time Allotted : 3 hrs **Full Marks : 70**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 5 (five) from Group B to E, taking at least one from each group.

Candidates are required to give answer in their own words as far as practicable.

Group – A
(Multiple Choice Type Questions)

1. Choose the correct alternative for the following: **10 × 1 = 10**

(i) Which of the following expressions is indicative of a Normal Mode analysis?
(a) $P_{canon}(T,E)$ proportional to $n(E)\ \omega_B(E)$ (b) $\delta f/\ \delta x_i=0$
(c) $U(T)=U_{trans}(T)+U_{rot}(T)+U_{vib}(T)+U_{vib}(0)$ (d) $\nu_i=\sqrt{\Delta_j}$

(ii) In computationally representing the binding of a ligand (as a possible drug) against a protein/neurotransmitter (as a target) for progression of the disease (pharmacological effects) which of the following biological events need to be considered?
(a) Cleavage of the substrate (b) Stabilization of a transition state
(c) Blockage of the protein's binding site (d) Any one or a combination of choices in (a), (b), (c)

(iii) Weak interactions in proteins that depend on conformations of a chain contribute towards the total conformational potential energy. Which of the following terms represent electrostatic interactions between charged atoms i and j on a protein?
(a) $CijR_{ij}^{-12}-D_{ij}R_{ij}^{-10}$ (b) $Q_iQ_j/€R_{ij}$ (c) $\Sigma_{angles}K_\theta(\theta-\theta_0)^2$ (d) $\Sigma_{bonds}K_r\ (r-r_0)^2$.

(iv) The binding of an anti-bacterial drug (e.g. trimethorphim) to an enzyme (e.g. DHFR) has been studied computationally to exploit the structural differences between bacterial and vertebrate DHFR. Energy minimization is a necessary step in this computational binding process. Which of the following choices represents a correct conclusion from that and similar computational studies?
(a) Conformation of trimethorphin was the same in the free state and when bound to DHFR
(b) Use of structures obtained from energy minimization calculations on isolated structures always leads to correct energy values
(c) The ligand-receptor interaction can lead to a conformation that is higher in energy than any of its energy structures
(d) None of the above.

(v) The term $\Sigma bondsKr(r - r_o)^2$ in an energy expression is indicative of
(a) torsion angle interaction (b) van dar Waals interactions
(c) hydrogen bonds (d) bond stretching.

(vi) Which of the following statements is true?
(a) Parallel combinatorial synthesis involves the synthesis of a large number of compounds using the same reaction sequence, where there is a mixture of compounds in each reaction vessel.
(b) Parallel combinatorial synthesis involves the synthesis of a large number of compounds using different reaction sequences, where there is a different, single compound formed in each reaction vessel.
(c) Mixed combinatorial synthesis involves the synthesis of a large number of compounds using a variety of different synthetic routes to produce a mixture of compounds in each reaction vessel
(d) A parallel combinatorial synthesis carried out in a specified number of vessels will produce less compounds than a mixed combinatorial synthesis.

(vii) Which one of the following choices represents an accurate definition/explanation of simulated annealing?
(a) It is a special case of "quenched" molecular dynamics simulation
(b) It is a special case of Monte Carlo simulations
(c) Temperature is gradually reduced during the simulation after the system is first heated
(d) All of the above.

(viii) Which of the following statements best describes structure-activity relationships (SAR)?
(a) The study of which functional groups are important to the chemical reactivity of the drug
(b) The study of the physicochemical properties that are important to the absorption of a drug into the blood supply
(c) The study of the structural features of a drug that are important to its biological activity
(d) The study of the structural features of a drug that are important to its chemical stability.

(ix) A 55-year-old woman with hypertension is to be treated with a thiazide diuretic. Thiazide A in a dose of 5mg produces the same decrease in blood pressure as 500 mg of thiazide B. which of the following statement best describe these results?
(a) Thiazide A has more efficacy than Thiazide B
(b) Thiazide A is about 100 times more potent than thiazide B
(c) Thiazide A has longer half life than Thiazide B
(d) Thiazide A has a wider therapeutic window than thiazide B.

(x) IC_{50} means
(a) concentration of a drug that is required for 50 percent inhibition in an assay
(b) concentration of a drug that is required for 50 percent activation in an assay
(c) 50 mg of a drug that is required for 50 percent inhibition in an assay
(d) 50 μg of a drug that is required for 50 percent activation in an assay.

Group–B

2. (a) Use a flowchart to represent the pipeline of a drug discovery process from identification of a gene to identification of a lead. Name four integrated advanced technologies that come into this process. What is the specific role of molecular modelling as a methodology in drug designing? Outline the role of either the Taylor series expansion OR a Lagrange multiplier in molecular modelling. Write out its full expression. *[(CO1,CO2)(Understand-apply/IOCQ)]*

(b) The following table shows a comparison of the use of the first derivative steepest descent and energy minimization procedure for the automated docking of the binding of the antibiotic netropsin to DNA.

	Initial refinement	(Av. Gradient <1kcalA^2)	Stringent minimization	Av.grad.<0.1kcalA^2)
Method	CPU time(s)	Number of Iterations	CPU time(s)	Number of iterations
Steepest Descent	67	98	1405	1893
Conjugate Gradients	149	213	257	357

Interpret the efficiency of the two minimization algorithms in the above-described binding process.
[(CO3)(Understand-analyze/HOCQ)]

(c) A molecule acetoxy cyclohexanol is being developed as a candidate drug against a receptor protein X that is implicated in the progression of a disease Z. Energy minimization is being performed as a precursor to developing this compound further as a lead. Answer the following procedural steps with respect to this operation (i) on what structures is the energy minimization being performed? (ii)name two parameters that are important for the minimization process mathematically (iii) what choices are generally considered routine as minimization algorithms? (iv) is it always applicable that the most energy minimized conformation is necessarily a bioactive one? Explain your answers. *[(CO6)(Apply/IOCQ)]*

4 + 4 + 4 =12

3. (a) (i) How does Molecular dynamics simulation(MDS) help in determination of the three dimensional structure of a protein? Illustrate using two techniques explaining how such simulations have been incorporated into the data analysis part of these two techniques. Your answer should preferably be in a flowchart form.
(ii) List the advantages of a molecular dynamics simulation over a Monte Carlo simulation.
[(CO1)(Understand-analyze/IOCQ)]

(b) (i) Use a table to differentiate between a Metropolis Monte Carlo (MMC) and Gibbs Ensemble Monte Carlo (GEMC) highlighting the implementation steps. What is the main application of GEMC? What are the advantages of GEMC over existing algorithms in this category?
(ii) Give one example of a nucleic acid simulation where a Monte Carlo simulation(MCS) and MDS have been combined over a long conformational sampling time. Explain how this simulation is ideally carried out simulation is carried out. *[(CO1)(Understand-analyze/IOCQ)]*

(4 + 2) + (3 + 3) = 12

Group – C

4. (a) What are the mathematical expressions for the bond length, bond angle and torsion angle variations (stretching, bending and rotational motions) in a MM based force field. Give the physico-chemical interpretation of these three expressions. *[(CO2)(understand-apply/LOCQ)]*

(b) Graphically represent the functional form of the potential energy curve for a bond (hint: Morse potential). Why is there a need for simpler expressions for bond stretching? Write out this simpler expression for bond stretching. For a molecule X-Y-Z, calculate the bond stretching energy using the following parameters: bond X-Y is stretched

to 2.4 Angstroms and bond Y-Z is stretched to 2.5A^0; assume the equilibrium bond distance, l_0, to be 1.8A^0 and the force constant (kcal mol^{-1} $A^{0\,-2}$) is 0.22. Assume the higher order form of the stretching potential.

[(CO2)(Understand-calculate/HOCQ)]

(c) (i) Write out the equation expressing the bending energy for an angle in a triatomic molecule X-Y-Z measuring deviations from the reference. Define all the parameters. How would this angle bending term change to incorporate cases of highly strained molecules? Explain the terms in the resultant equation.

[(CO2)(Explain-calculate/IOCQ)]

3 + 5 + 4 = 12

5. (a) Specify the physicochemical parameters that influence the characteristics of a drug. *[(CO3)(Remember/LOCQ)]*

(b) "The percent of neutral and percent of anionic form of acidic drug changes with change of pH from 3 to 11" How would you experimentally justify the above statement ?. *[(CO3) (justify/IOCQ)]*

(c) (i) Define the lipophilicity parameter for a drug. Itemize the biological and physicochemical properties of a drug that are affected by the lipophilicity parameter. How would you experimentally determine the variation in log D with the pH with the additional constraint that it is an amphoteric drug?

(ii) Calculate the log D value of a monoprotic acidic drug with the following parameters : pKa = 4.5, log P = 4.25, at pH = 6.5. *[(CO3)(Remember-calculate-analyse/IOCQ)]*

2 + 3 + (5 + 2) = 12

Group – D

6. (a) Two of the key steps in the process of drug discovery are selection/validation of a target and lead development/optimization. Using tables, itemize the methodology and tests needed for successful completion of these two steps. *[(CO3)(Remember-explain/IOCQ)]*

(b) As part of the pharmaceutical regulatory process, a therapeutic drug manufacturing entity is testing a set of drugs through three-set clinical trials. Data analysis was done after a full set of 50 drugs was passed through the three trials. Results indicate that the probability of failure was 56% in Phase 1; 34% in phase 2 and 45 % in Phase III. What was the total number of drugs that passed all three tests? Explain your answer. *[(CO4)(Calculate-explain/IOCQ)]*

6 + 6 = 12

7. (a) Based on two state receptor modelexplain the mechanism of action for an agonistand or an antagonist drug. *[(CO4) (Explain/IOCQ)]*

(b) Explain how you will determine the ED_{50} and LD_{50} of a new drug experimentally in mice? *[(CO4)(Understand/LOCQ)]*

(c) Mr Jones has zero kidney function and is undergoing hemodialysis 3 days per week while awaiting a kidney transplant. He takes metformin for type 2 diabetes mellitus and was previously stabilized (while his kidney function was adequate) at a dosage of 500 mg twice daily, given orally. The plasma concentration at this dosage with normal kidney function was found to be 1.4 mg/L. He has had 6 dialysis procedures and metformin toxicity is suspected. A blood sample now shows a metformin concentration of 4.2 mg/L. What was Mr Jones' clearance of metformin while his kidney function was normal? *[(CO6)(Analyze/HOCQ)]*

4 + (3 + 3) + 2 = 12

Group – E

8. (a) Enumerate four important general features of a molecular mechanics based force field. Write out the mathematical expression for a molecular mechanics based force field that represents the complete conformational energy for a protein. *[(CO5)(Understand-apply/LOCQ)]*

(b) What is the full form of the AMBER force field? For what molecules is the AMBER force field preferably used? What are the differences in AMBER force field compared to the generalized MM based one? In the Optimized potentials for Liquid Simulations (OPLS) force field, what are the combination rules applied for parameterization of non-bonded and repulsion interactions? What is the special characteristic of the OPLS model with respect to hydrogen bonding? *[(CO5)(Analyse/HOCQ)]*

(c) A scoring function is used to approximate the binding free energy for a ligand (L) binding to a receptor (P). Write the detailed expression for such a MM based scoring scheme defining all the terms. Graphically represent the Score vs Distance for such a"docking scoring" scheme. If such a scoring scheme approximates only part of the overall binding energy, how can the scoring function be improved? Answer in specific energy contribution terms. *[(CO5)-[Understand-explain/IOCQ)]*

3 + 5 + 4 = 12

9. (a) Use a flowchart to represent the structure based design of a HIV-I protease inhibitor starting with peptide-based diols. The flowchart should include key methodological (computer aided drug design-wise) and chemical modification steps. Define bioavailability and explain how in this case the potent inhibitor was structurally modified to improve upon its oral bioavailability. *[(CO6)(Remember-understand/IOCQ)]*

(b) Use principles of drug design to answer the following observation: Pharmaceutical regulation requires that N-acetylcysteine be added to the painkiller acetaminophen. What are the reasons? Your answer should include the metabolic profile of the drug. *[(CO6)(Principles-analyze/IOCQ)]*

(c) "Although structure-based drug design has proven to be quite effective in the discovery and development of new drugs, the inhibition of important enzymes leading to complex physiological responses has sometimes led to development of adverse side effects (ADRs)." Analyse this statement with the example of the two distinct forms of the enzyme cyclooxygenase and COX-2 inhibitor drugs that were developed. Your answer should also explain how essential concepts of computer aided drug design are explained through this example. *[(CO6)(Understand-Analyse/HOCQ)]*

4 + 3 + 5 = 12

Cognition Level	*LOCQ*	*IOCQ*	*HOCQ*
Percentage distribution	*14.6*	*63.5*	*21.9*

Course Outcome (CO):

After the completion of the course students will be able to

1. Understand the principles of molecular simulation techniques of Monte Carlo , molecular dynamics and energy minimization methods and their applications to studying equilibrium ad dynamic properties of biological macromolecules and their interactions
2. Understand principles of classical molecular mechanics and physicochemical properties to understand the interactions between potential drugs(small molecule ligands) and their targets (proteins, nucleic acids)
3. Understand the physicochemical basis and criteria necessary for application of molecular modelling principles to computer aided drug design
4. Application of pharmacokinetic and pharmacodynamic principles to the process of computer aided drug design
5. Understand the concepts and steps in molecular docking tools/algorithms and analyze the data obtained from them.
6. Applications of principles and concepts of molecular modelling and computer aided drug design to real life examples of drug discovery and development.

**LOCQ: Lower Order Cognitive Question; IOCQ: Intermediate Order Cognitive Question; HOCQ: Higher Order Cognitive Question.*