1. (25 pts) Insulin is an important hormone produced by the pancreas that allows cells to process glucose. Diabetes mellitus, affecting 16 million people in the U.S., is a condition in which insulin production or utilization is impaired. Current treatment for diabetes often involves subcutaneous injection of insulin.

a) Access the RCSB Protein Data Bank at http://www.pdb.org/pdb. Perform a search on insulin and access the human insulin two disulfide model to answer the following:

i) What is the primary chemical structure for insulin?

ii) What is the secondary structure of insulin?

iii) From the Ramachandran plot analysis, what is the range of psi, phi values for most of the amino acid residues in insulin?

b) Could insulin be readily radiolabeled with ^{125}I for detecting its adsorption? Explain.

Numerous research efforts underway are investigating controlled release approaches that would eliminate the need for daily insulin injections. Such strategies typically involve exposure of insulin to synthetic material surfaces. To investigate the effects of adsorption on the secondary structure of insulin, Mollmann et al. (*Eur. J. Pharm. Sci.* **2006**, *27*, 194) performed circular dichroism(CD) measurements on insulin in phosphate buffered saline (PBS) solution and adsorbed to the surface of colloidal Teflon particles 215 nm in diameter and stabilized in water with a small fraction of negatively charged sulfate groups on their surface. The CD data are shown in the figure below.

c) What is the effect of adsorption on the secondary structure of insulin?

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See Fig. 2 in Mollmann, Susanne H., Lene Jorgensen, Jens T. Bukrinsky, Ulla Elofsson, Willem Norde, and Sven Frokjaer. "Interfacial Adsorption of Insulin: Conformational Changes and Reversibility of Adsorption." *Eur J Pharm Sci* 27 (2006): 194-204

d) From the CD data provided, calculate the fractional α helical content for insulin in solution and adsorbed to the Teflon particles.

e) Why might changes in the secondary structure of insulin be important to consider in developing controlled delivery strategies?

Mollmann et al. further studied the adsorption of FITC-labeled insulin to determine if the fluorescent dye changed the adsorption kinetics. For this experiment, mixtures of FITC-labeled and unlabeled insulin were prepared in different ratios to reach the same total insulin concentration (a concentration that corresponded to near monolayer coverage). They found that the fluorescence intensity increased linearly with the fraction of FITC-labeled insulin in solution, and concluded from this result that FITC-labeled and unlabeled insulin have comparable affinities for the Teflon beads.

f) Do you agree with their conclusion? Develop a model to describe the fractional coverage (ν_F) for the FITC-labeled component of the mixed insulin solution, based on a modified Langmuir model. Under what conditions does ν_F scale linearly with the fraction of FITC-labeled insulin [P_F]?

2. (20 pts) Prosthetic vascular grafts are commonly employed in the treatment of atherosclerosis, as a means to bypass occluded blood vessels. Read through the text on pp. 479-483 (1st ed.: pp. 420-422) to assist in answering the questions below.

a) What materials are currently used in commercial vascular grafts?

b) What is the most common reason for failure of synthetic vascular grafts of small diameter?

c) Describe the expected synthetic/biological material interface at the interior and exterior surfaces of a vascular graft many months post-implantation.

Fibrinogen is known to play an important role in clot formation. Makogonenko et al. (*Biochemistry* **2002**, *41*, 7907) investigated the interaction of adsorbed fibrinogen with fibronectin, employing surface plasmon resonance (SPR).   The figure below shows fibronectin adsorption data at 22 °C on a gold surface coated with fibrinogen (dashed line, 100 nM fibronectin), and the same surface after subsequently being treated with thrombin (solid lines, 0.25 – 3.0 nM fibronectin).

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See Fig. 2(a) in Makogonenko, E., G. Tsurupa, K. Ingham, and L. Medved. "Interaction of Fibrin(ogen) with Fibronectin: Further Characterization and Localization of the Fibronectin-Binding Site." *Biochemistry* 41 (2002): 7907-7913.

d) What do the units of SPR response indicate?

e) Explain why fibrinogen binds fibronectin only after exposure to thrombin.

f) From the inset of k_{obs} data obtained from the fits to the SPR data for thrombin-exposed fibrinogen, estimate the value of the dissociation constant K_D.

g) Based on the K_D value you obtained for (f), would you expect fibronectin to bind to adsorbed fibrinogen *in vivo*? Explain your answer, stating any assumptions you make.

h) What role does adsorbed fibronectin have in the body's response to an implanted biomaterial?