Assignment 6 (22 pts) Biochemistry 5721 AU22 To be submitted as a single PDF upload at http://carmen.osu.edu. 1. For the binding reaction between a protein P and its ligand L … (a) (2 pts) Derive an expression for the fractional saturation Y of binding sites on the protein as a function of the equilibrium dissociation constant KD and the free ligand concentration [L] (b) (2 pts) Plot Y versus [L] from [L] = 0 to L=10KD (c) (2 pts) Use mass balance and derive an expression for the fractional saturation of binding sites on the protein Y as a function of the equilibrium dissociation constant KD and the total ligand and protein concentrations [Ltot], [Ptot] (d) (3 pts) If , plot Y versus [Ltot] from 0 to when ... i. (micromolar = 10-6 M) ii. (nanomolar = 10-9 M) Is a 1000-fold excess of ligand over protein sufficient to saturate the sites (>95% bound) in each case? (e) (3 pts) If at 25°C , and the binding reaction releases 147 kJ/mol (i.e., ΔH is negative), what will be the dissociation constant at 37°C? Will inhibiting the protein be harder or easier to at 310K compared to at 295K? Show your work. 2. Genomic DNA sequences are detected by hybridization with a labeled DNA probe that is complementary to the target sequence and forms a double strand. (a) (2 pts) For the equilibrium (probe + target → double strand) express K, the equilibrium constant for the association of the probe with the target, in terms of cprobe, the total probe concentration, ctarget, the total concentration of the target sequence, and Y, the fraction of target hybridized to the probe to form a double strand. Assume that the probe is present in large excess over target. (b) (2 pts) If at 25°C, pH 7, 1 M NaCl, the probe binds its target with a ΔG° of -40 kJ/mol, and ΔH = -267 kJ/mol, find the melting temperature of the probe from the target, defined as the temperature at which half of the target is hybridized to the probe. Assume that cprobe = 10-4 M and ctarget = 10-8 M, and that ΔH is temperature-independent. (c) (2 pts) In practice, annealing reactions are carried out in much lower salt concentrations. A simple, common correction is Tm([Na+ ]) = Tm(1M) + 12log[Na+ ]. What are the Tm values in [Na+ ] = 0.5, 0.05 and 0.005 M? Why does [Na+ ] alter the Tm? (d) (4 pts) Does the melting temperature as defined in part (c) go up, down, or remain the same when the following changes are made? Give reasons for your answers. i. The probe concentration is doubled ii. The target concentration is doubled iii. The probe contains a single base mismatch iv. The salt concentration is decreased.