Spring 2004 BCHS 3304 Final Exam Review-

1). The T➔R transition of hemoglobin upon binding of oxygen to the heme has been thoroughly investigated. On a thermodynamic level, this T➔R transition can be described as (primarily) an enthalpically driven process. Which of the following phenomena in the T➔R transition of hemoglobin is the likely enthalpic driving force?

a). Movement of the heme iron into the plane of the heme upon oxygen binding.
b). The binding of oxygen by the distal histidine (E7).
c). The exclusion of water from the oxygen-binding pocket.
d). The breaking of pre-existing and making of new C-terminal salt bridges at the □/□ interfaces.
e). The occlusion of the heme pocket by valine (E11).

□: **(d), the breaking of pre-existing and making of new C-terminal salt bridges at the □/□ interfaces is the enthalpic driving force for the T➔R transition in hemoglobin.**

2). At a pH more acidic than its isoelectric point, a protein will carry:

a). no ionic charge
b). a net positive charge
c). a net negative charge
d). a positive charge equal to the negative charge
e). I have no clue, where am I, who are all of these people

□**(b), at a pH more acidic than its isoelectric point, a protein will carry a net positive charge.**

3). Match the following protein with its appropriate characteristic.

a). Collagen
b). Chymotrypsin
c). □-Keratin
d). RNase A
e). Silk Fibroin
f). Creatine Kinase
g). Lysozyme
h). Prolyl Hydroxylase
i) Carbonic Anhydrase
j). Adenylate Kinase
k). IgG Antibody

I). 2 right-handed □-helices forming a left-handed coiled structure **(c)**
II). Left-handed proline helices **(a)**
III). oxonium intermediate **(g)**
IV). catalyzes 2 ADP ←➔ AMP + ATP **(j)**
V).stabilizes collagen structure using ascorbic acid **(h)**
VI). catalytic triad **(b)**
VII). pair of catalytic histidine residues **(d)**
VIII). solubilizes CO_2 as bicarbonate anion **(i)**
IX). antiparallel □-sheet structure comprised of primarily small, aliphatic residues **(e)**
X). maintains the muscle "energy reserve" **(f)**
XI). Sandwiched □-sheet structure with high-affinity ligand binding loops. **(k)**

4). Which of the following statements accurately describes the nature of a biologically active protein?

a). A biologically active protein is composed of a branching sequence of amphoteric, L-amino acids joined together by resonant amide bonds between neighboring residues, each exhibiting free rotation.

b). A biologically active protein is composed of a non-branching sequence of amphipathic, D-amino acids joined together by resonant amide bonds between neighboring residues, with each exhibiting no free rotation.

c). A biologically active protein is composed of a non-branching sequence of amphoteric, L-amino acids joined together by resonant amide bonds between neighboring residues, with each exhibiting no free rotation.

d). A biologically active protein is composed of a branching sequence of amphipathic, D-amino acids joined together by non-resonant amide bonds between neighboring residues, each exhibiting free rotation.

e). A biologically active proteins is composed of a non-branching sequence of amphoteric, L-amino acids joined together by non-resonant amide bonds between neighboring residues, with each exhibiting no free rotation.

: (c), a biologically active protein is composed of a non-branching sequence of amphoteric, L-amino acid joined together by resonant amide bonds between neighboring residues, with each exhibiting no free rotation.

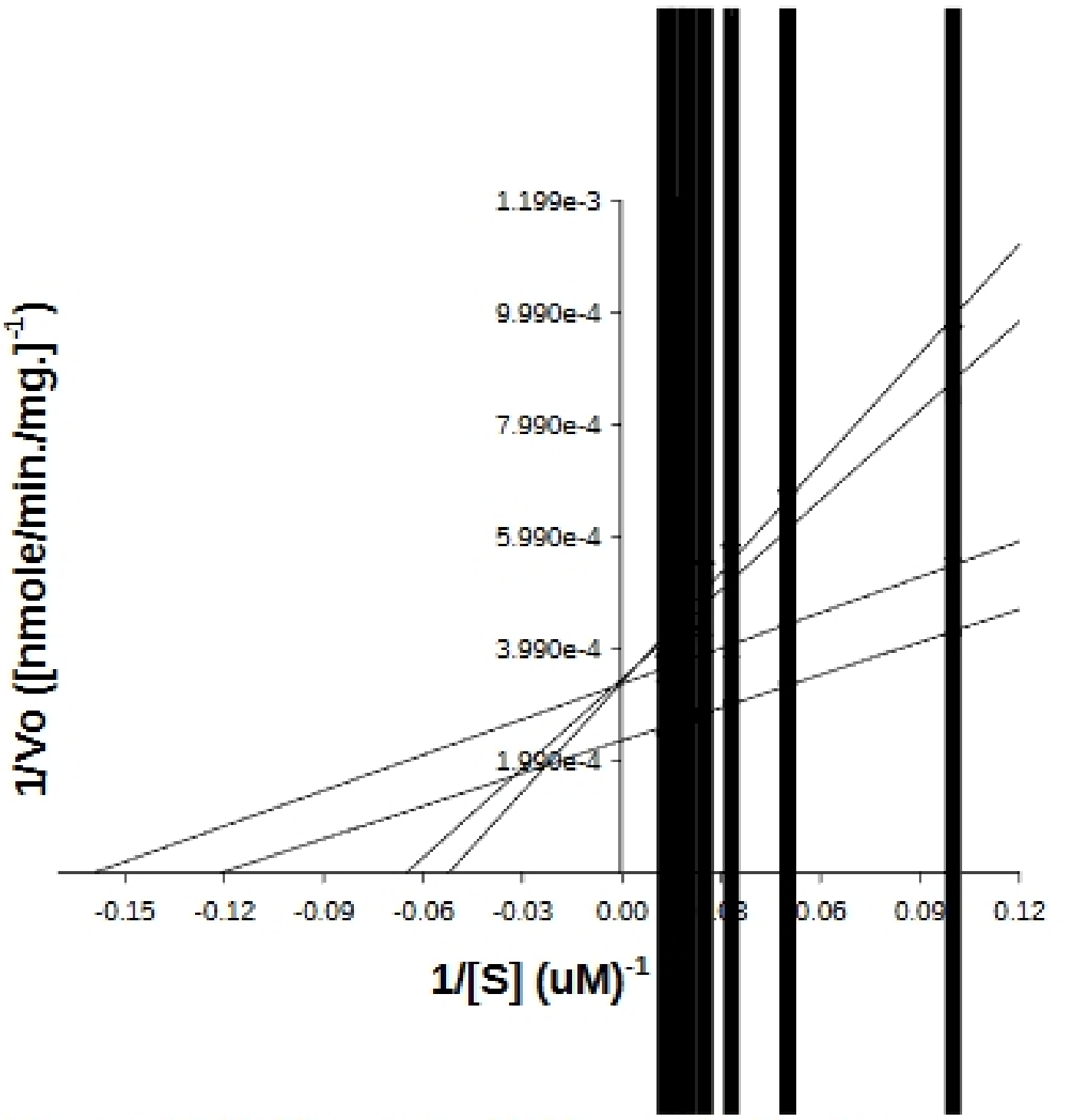
5). Consider the following proteins of the TCA cycle:

Protein-	Mass (kDa)-	pI-	Solubility Limit (% Salt)-
Pyruvate Dehydrogenase	1,100	8.3	25
Aconitase	15	5.0	35
-ketoglutarate Dehydrogenase	1,080	6.0	27
Succinyl-CoA Thiokinase	357	7.5	20
Fumarase	353	7.3	40
Malate Dehydrogenase	14	7.7	15

Outline a procedure to separate all of the enzymes of the TCA cycle from a crude mitochondrial homogenate, paying specific attention to separating Pyruvate Dehydrogenase from -ketoglutarate Dehydrogenase, Aconitase from Malate Dehydrogenase, and Succinyl-CoA Thiokinase from Fumarase in their native states, using affinity chromatography only as a last resort.

: Size exclusion chromatography could easily separate the six enzymes into three groups of two (Pyruvate dehydrogenase and -ketoglutarate dehydrogenase would elute first, Succinyl-CoA thiokinase and Fumarase would elute second, and Aconitase and Malate dehydrogenase would elute last). After Size exclusion, Ion exchange chromatography could be used to separate Pyruvate dehydrogenase from -ketoglutarate dehydrogenase and Aconitase from Malate Dehydrogenase. Salting In/Salting Out would be needed to separate Succinyl-CoA thiokinase and Fumarase. This separation scheme represents only one sequence of separation strategies that could be employed; others are possible.

6). Consider the following Lineweaver-Burk Plot:



●= no inhibitor. ▼= 300 nM inhibitor. ■ = 650 nM inhibitor. ◆= 900 nM inhibitor.

a). What type of inhibition is seen at low inhibitor concentrations?
b). What type of inhibition is seen at higher inhibitor concentrations?
c). Going from the absence of inhibitor to 300 nM inhibitor, is the apparent Km increasing or decreasing? Is the presence of the inhibitor making the substrate bind tighter or looser?
d). Going from 300 nM inhibitor to 650 nM and 900 nM inhibitor, is the apparent Km increasing or decreasing? Is the presence of the inhibitor making the substrate bind tighter or looser?
e). Going from the absence of inhibitor to the presence of inhibitor (300-900 nM inhibitor), is the apparent Vmax of the reaction increasing or decreasing?

: a). Uncompetitive inhibition. b). Competitive inhibition. c). Apparent Km is decreasing, and the substrate is binding tighter. d). Apparent Km is increasing and the substrate is binding looser. e). Apparent Vmax is decreasing.

7). Match the following reagent with its utility in protein primary structure determination.