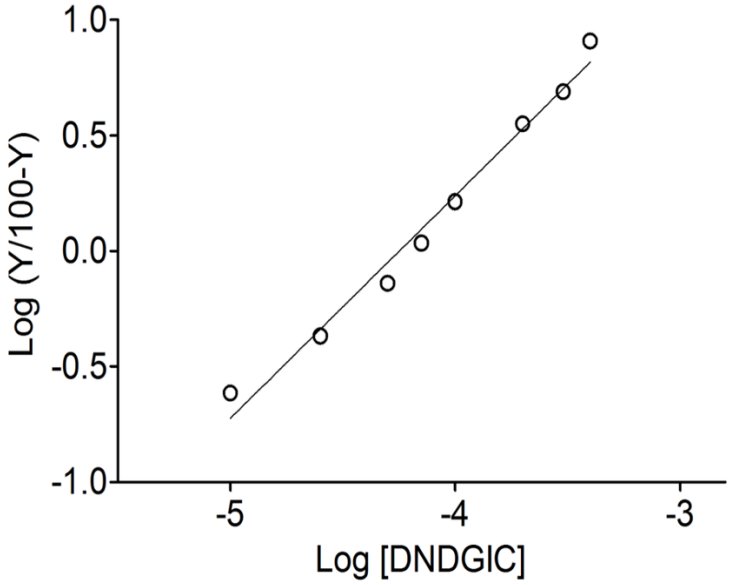
Name: _____ Final Exam

The following exam references work from:

Bocedi *et al.* (2016). Evolution of Negative Cooperativity in Glutathione Transferase Enabled Preservation of Enzyme Function. *Journal of Biological Chemistry* [In Press].

1. The authors perform assays in solutions at 0.1 mM Potassium phosphate (pH 7.4); 20 mM glutathione; and 2 mM nitrosoglutathione. List the masses of each constituent (neglecting water) that would be needed to prepare 1 L of a 10x stock solution at:
    - 1 mM Potassium phosphate (pH 7.4; pKa 7.21) [KH_2PO_4 (136 g/mole) and K_2HPO_4 (174 g/mole)]
    - 200 mM glutathione [$C_{10}H_{17}N_3O_6S$ (307 g/mole)]
    - 20 mM nitrosoglutathione [$C_{10}H_{16}N_4O_7S$ (336 g/mole)]

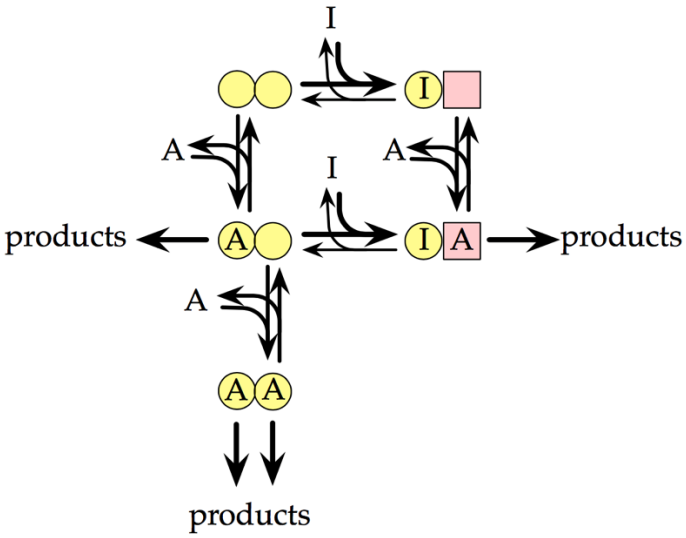
2. The authors find that the evolutionarily oldest GSTs functioned in a non-cooperative manner with respect to binding of the dinitrosyl-diglutathionyl-iron complex (DNDGIC). They reported data for one member of the Cysteine sub-family (*S.* paucimobilis):



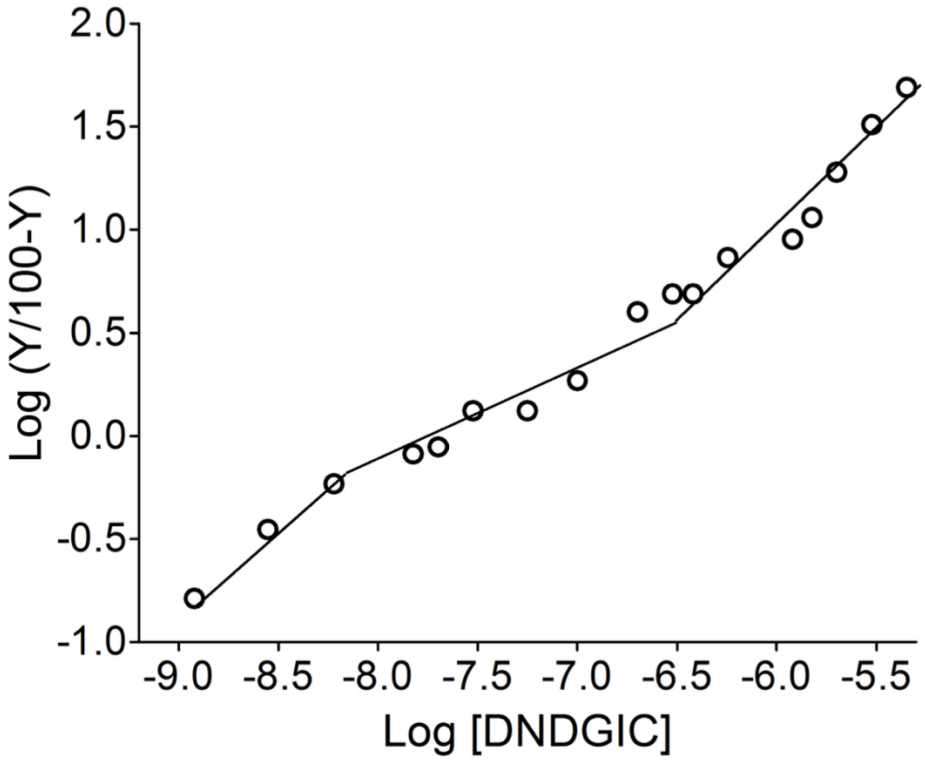
a. Quantitatively explain why it was appropriate for the authors to conclude that the enzyme from this Cysteine sub-family behaves non-cooperatively.

b. Determine the equilibrium dissociation constant for DNDGIC with this Cysteine sub-family GST.

c. Redraw (don't just draw on top of) the mechanism provided by the authors to represent the non-cooperative mechanism of the Cysteine sub-family GSTs. In the original mechanism, A is the substrate, and I is the inhibitor.



3. The authors find that some of the evolutionarily newer GSTs found in humans functioned in a cooperative manner with respect to binding of the dinitrosyl-diglutathionyl-iron complex (DNDGIC). They reported data for one member of the Tyrosine sub-family (*H. sapiens* GSTP1-1):



a. At low [DNDGIC], what observation in the actual Hill graph supports that GSTP1-1 acts non-cooperatively in a T-state?

b. Determine the dissociation binding constant for DNDGIC from the T-state.

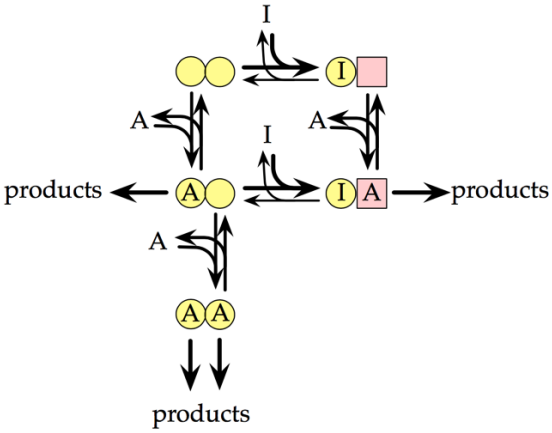
c. At high [DNDGIC], what observation in the actual Hill graph supports that GSTP1-1 acts non-cooperatively in an R-state?

d. Determine the dissociation binding constant for DNDGIC from the R-state.

e. Compare your dissociation equilibrium constants to the T- and R- states. Does DNDGIC bind more tightly to the T-state or the R-state?

f. If a solution contains 50 T-state enzymes and 50 R-state enzyme when no DNDGIC is bound (100 total enzymes), predict how many T-state and R-state enzymes will exist when one DNDGIC is bound.

g. Redraw (don't just draw on top of) the mechanism provided by the authors to represent the MWC mechanism of the Tyrosine sub-family GSTs. In the original mechanism, A is the substrate, and I is the inhibitor.



4. Besides kinetic investigations, the authors analyze previously determined structural information for the Tyrosine and Cysteine sub-families of GSTs to consider structural factors affecting negative cooperativity. Throughout the paper, the authors present a sequential model of cooperative inhibitor binding where the binding of inhibitor to one subunit induces a structural change in the adjacent subunit to form a structurally asymmetric dimer. For the exam, you have considered the MWC model where the dimer remains symmetrical but exists in two conformations (T-state and R-state). The following text is from the paper. In your opinion, does it support the sequential or MWC model for inhibitor binding (you can select only one… it cannot be both)?

   Underline the parts of the passage that support your decision.

Structural requirements for negative cooperativity in GSTs — A classical structural explanation for negative or positive cooperativity is that one subunit, once it has bound a specific ligand, modifies the structure of the adjacent free subunit. The subunit interfaces, as they appear from the X-ray structures of many dimeric GSTs, help us to identify the structural requirements for the observed negative cooperativity. The two adjacent monomers display three types of interactions: polar contacts, hydrogen bonds and hydrophobic interactions. For the mammalian Tyr-GSTs the combination of mutational, kinetic, and structural studies provides strong evidence for the structural basis of cooperativity, in particular for GSTP1-1. By analogy, but with less experimental evidence, we suggest similar structural requirements for negative cooperativity in other GSTs below. In the Tyr-GSTs a typical hydrophobic contact, important for inter-subunit communication, is the "lock-and-key" motif. This structural trait is formed by an aromatic residue (key residue) from domain I in one subunit wedged into a hydrophobic pocket formed by helices 4 and 5 in domain II of the other subunit (lock apparatus). The lock-and-key motif is a common feature of Pi, Mu, and Alpha class GSTs where the key residue is either phenylalanine or tyrosine (Tyr49 or Tyr50 with Met1 residue in *h*GSTP1-1) buried in a hydrophobic pocket formed by Met91, Val92, Gly95, Pro128, Phe129, and Leu132 of the second subunit chain. Mutagenesis has been used to investigate the importance of the key residue for dimerization, stability and cooperativity found in GSTP1-1, GSTA1-1, and GSTM1-1. The *S. hematobium* GST, which also shows a relevant negative cooperativity ($n_H = 0.65$) lacks this specific motif but shows an alternative insertion of an aromatic residue (Tyr92) into a hydrophobic cavity of the adjacent subunit formed by Lys80 and Met85 but also surrounded by the Arg76 and Tyr77. In the Ser- GSTs, a lock-and-key motif is always present, but different from that reported in mammalian Alpha/Mu/Pi classes. A striking characteristic of this motif involving the 'key' residue is that it not only inserts into a hydrophobic pocket of the neighboring subunit, but also itself acts as part of the "lock" for the other subunit "key". In addition, the "key" residues from both subunits hook around each other in an aromatic pi–pi interaction, through slightly offset aromatic ring stacking, generating a "clasp" in the middle of the subunit interface. The "clasp" motif is formed by an aromatic residue of Phe104 of one subunit and the Arg67, His100, Leu103, and Val107 of the second subunit and *vice versa* occur among the Phe104 of the second subunit and the same four residues of the first subunit. The "clasp" motif appears like two hands interlocked with the two Phe104 residues in the central portion. The human GSTT2-2 lacks this peculiar motif but, similar to the *S. hematobium* GST, an alternative aromatic residue (Tyr73) is inserted in a hydrophobic cavity of the adjacent subunit formed by Leu89 and Ala93 possibly acting as an ancillary transmission device for cooperativity. In the Cys-GSTs no similar inter-subunit connections are observed, in full agreement with the absence of any

kinetic or binding cooperativity.

5. For the kinetic analysis proposed in the paper, the substrate binding step completely equilibrates before any ES is turned over to product (this is the rapid equilibrium limit).:

$$E + S \overset{K_1}{\Leftrightarrow} ES \overset{k_2}{\rightarrow} E + P$$

A. Write a rate equation for the appearance of product in terms of the concentration of ES and rate constant(s).

$$v_0 = \frac{d[P]}{dt} =$$

B. Define the equilibrium <u>association</u> constant K_1 in terms of the concentrations of E, S, and ES.

$$K_1 =$$

C. Rearrange your expression for K_1 to isolate the concentration of ES.

$$[ES] =$$

D. Write a mass balance equation for the total concentration of enzyme.

$$[E]_T =$$

E. Rearrange your mass balance equation to isolate the concentration of free enzyme.

$$[E] =$$

F. Substitute your expression for the concentration of free enzyme into your expression for the concentration of ES (part C).

$$[ES] =$$

G. Rearrange your expression to isolate the concentration of ES.

$$[ES] =$$

H. Substitute your expression for the concentration of ES into your original rate equation (part A).

$$v_0 = \frac{d[P]}{dt} =$$

I. What is the limit of your rate equation as substrate concentration becomes very high?

$$V_{max} =$$

J. Incorporate V_{max} into your rate equation.

$$v_0 = \frac{d[P]}{dt} =$$

K. Divide the numerator and detonator by K_1. This should look similar to the MM equation result.

$$v_0 = \frac{d[P]}{dt} =$$

6. The authors describe the chemical properties required for a Tyr residue of Tyr-GSTs to act in the communication between adjacent subunits:

"In the Tyr-GSTs a typical hydrophobic contact, important for inter-subunit communication, is the "lock-and-key" motif. This structural trait is formed by an aromatic residue (key residue) from domain I in one subunit wedged into a hydrophobic pocket formed by helices 4 and 5 in domain II of the other subunit."

The authors describe the chemical properties required for the same Tyr residue of Tyr-GSTs to act in the inhibition by DNDGIC:

"Crystal structure of dinitrosyl-glutathionyl iron complex (DNDGIC) bound to GSTP1-1 (one GSH is replaced by a Tyr residue which completes the coordination shell of the iron ion with its -OH group)."

a. What amino acid could you mutate the Tyr residue to that would preserved the cooperativity but eliminated the DNDGIC inhibition?

b. What amino acid could you mutate the Tyr residue to that would preserved the DNDGIC inhibition but eliminated the cooperativity?