

## Key

### Problem 1

From these data, calculate  $k_{\text{cat}}$  and  $K_m$  of Botox-A for SFP.

$$K_m = 1.5 \times 10^{-5} \text{ M (15 } \mu\text{M)} \quad +2 \text{ points}$$

$$k_{\text{cat}} = 860 \text{ s}^{-1} \quad +2 \text{ points}$$

What is the specificity constant?

$$k_{\text{cat}}/K_m = 5.7 - 5.8 \times 10^7 \text{ M}^{-1}\text{s}^{-1} \quad +1 \text{ points}$$

Comment on the efficiency of the Botox-A – is it a “catalytically perfect” enzyme?

Close, but not quite there +1 points

What sort of inhibitor is BTAI-27: **uncompetitive** +3 points

What is the  $K_i$  and/or  $K_i'$  of BTAI-27?

$$K_i' = 5.2 - 5.3 \times 10^{-8} \text{ M (53 nM)} \quad +2 \text{ points}$$

Based on these, do you think BTAI-27 will be a good therapeutic agent?  
(Briefly explain why or why not.)

It would not be ideal, for either (or both) of 2 reasons:

- 1) You'd prefer a competitive inhibitor, rather than waiting for the toxin to have already bound the substrate before the inhibitor can act. (Just binding it might inactivate it, but at least inactivation would not be catalytic.)
- 2) The  $K_i'$  is somewhat high. In order to completely inhibit Botox, you'd want to have  $\sim 0.5 \mu\text{M}$  in the body (i.e.  $10 \times K_i'$ ), which would be about  $35 \mu\text{moles}$ , for a 100 kg body (assuming 70% water). If the MW of the compound were 1000 Da, someone would need 35 mg dispersed through the body.)

+1 points

+12 points for an analysis done correctly & clearly

For numbers:

Full credit for being within 20% of the numbers above.

-1/2 if not, but within 1 order of magnitude.

Count off 1/2 point for each order of magnitude off. (But do not go below zero.)

-50% for no units or wrong units

## Problem 2

Which mechanism are these data most consistent with?

a) "Ping-Pong" mechanism +3 points

Give the reasons for your conclusion. +2 points

Because you get a set of parallel lines when you plot  $1/v$  vs.  $1/[S]$  (using either substrate, while keeping the other substrate constant).

Determine the following parameters for phosphorylase kinase:

$K_m$  and/or  $K_d$  for Trx(ox):  $K_m = 2.5 \times 10^{-5} \text{ M}$  (25  $\mu\text{M}$ ) +2 points

$K_m$  and/or  $K_d$  for NADPH:  $K_m = 8.8 \times 10^{-4} \text{ M}$  (0.88 mM) +2 points

$k_{\text{cat}}$  of the enzyme :  $k_{\text{cat}} = 465 \text{ s}^{-1}$  (460-470  $\text{s}^{-1}$ ) +2 points

It is known that this enzyme uses a covalently bound flavin to transfer electrons from NADPH to Trx, but has no other cofactors. Based on your analysis, answer the following questions:

1. Do you think that the sites where NADPH and Trx bind are distinct or overlapping?

They are likely to be overlapping, which would explain why a Ping-Pong mechanism would have to operate. (i.e. First NADPH would reduce the flavin, and  $\text{NADP}^+$  would leave. Then Tr(ox) would bind near the flavin and get reduced by it before leaving as Tr(red), concomitantly returning the enzyme back to its original state (oxidized flavin).)

+1 points

2. If you added NADPH alone to thioredoxin reductase, what would happen to the flavin?

It would reduce the flavin.

+1 points

+11 points for an analysis done correctly & clearly

For numbers:

Full credit for being within 20% of the numbers above.

-1/2 if not, but within 1 order of magnitude.

Count off 1/2 point for each order of magnitude off. (But do not go below zero.)

-50% for no units or wrong units

### Problem 3

+8 pts to follow directions and fill in the information at top of form (+2 pts each).

+5 pts for each description and +6 pts for the compare/contrast.

Penalties for not following directions:

-4 if species not sufficiently different

-6 if enzymes discussed in class

### Problem 4

a) Briefly describe the symmetry relations in ATCase ( $C_2$ ,  $C_3$ ,  $D_2$ , etc) – how would you classify each of these: **+1 point each  $\Rightarrow$  3 pts total**

a. The  $C_3$  catalytic trimer (e.g. 1EKX):  $C_3$

b. The  $R_2$  regulatory dimers (e.g. 3AT1):  $C_2$

c. The entire  $(C_3)_2(R_2)_3$  dodecamer (e.g. 1Q95):  $D_3$

b) Briefly describe the tertiary structure of each subunit (R & C).

What kind of domains does it have (e.g. what class)? **+2.5 pts each  $\Rightarrow$  5 pts total**

**C subunit:** Could be considered a tight association of 2 sub-domains, each of which is a parallel  $\beta$ -sheet of 4 or 5 strands made of  $\beta$ - $\alpha$ - $\beta$  motifs. Thus, whether you consider it 1 domain or 2 (and arguments could be made for either), they are all  $\alpha/\beta$ . It starts with a short  $\beta$ -strand that could be considered 5<sup>th</sup> strand of the N-terminal  $\beta$ -sheet, followed by a loop and then an  $\alpha$ -helix before starting the N-terminal  $\beta$ -sheet [ $\beta(2)$ - $\alpha$ - $\beta(1)$ - $\alpha$ - $\beta(3)$ - $\alpha$ - $\beta(4)$ ], to a long  $\alpha$ -helix that starts the C-terminal sub-domain [ $\beta(3)$ - $\alpha$ - $\beta(2)$ - $\alpha$ - $\beta(1)$ - $\alpha$ - $\beta(4)$ ]-long loop with several short helices- $\beta(5)$ - $\alpha$ (short)- $\beta(6?)$ ] to a long helix that runs through middle of protein and interacts with the 2 N-terminal  $\alpha$ -helices. Note: numbering of strands is left to right, viewed with strands running up, as seen from “outside” the structure. In both cases, the last strand is *very short* (and some might not even consider it part of the sheet).

**R subunit:** Again, made of 2 domains (although, in this case, they are more obviously separate domains) with  $\beta$ -sheets at the core of each. This time they are anti-parallel sheets made mostly of  $\beta$ -hairpins (but the N-terminal sheet has 2  $\beta$ - $\alpha$ - $\beta$  motifs). At the N-terminus is a short loop to domain 1 [ $\beta(3)$ - $\alpha$ - $\beta(1)$ - $\beta(2)$ - $\alpha$ - $\beta(4)$ - $\beta(5)$ ], followed by a long loop to domain 2 [ $\beta(1)$  to a long loop including short helix to  $\beta(2)$ - $\beta(3)$ - $\beta(4)$  to a short helix].

**1.5 pts each for just getting # of domains and class of domains right**

c) Briefly describe the dimerization interface seen in the R2 regulatory dimer. **+1 pt**

The N-terminal domains interface by H-bonding at the edge of the sheets (strand 5 of each sheet) to make one continuous 10-stranded anti-parallel  $\beta$ -sheet. (Also the first  $\alpha$ -helix of each N-terminal domain interact, with Phe27 from each subunit making contact.)

- d) Examine the active site in the R state (1Q95). Identify 7 residues that interact with the analog and list them in the table below, along with the kind of interaction you think it makes with the substrate (e.g. H-bond donor, H-bond acceptor, ionic interaction, van der Waals, etc.). Examine those same residues in the T state (5AT1) and indicate whether you think their conformation/position is similar or different in the T state.

| Residue (amino acid & #)       | Likely role   | Similar or different in T state? |
|--------------------------------|---|----------------------------------|
| Ser 52                         | Sidechain -OH is possible H-bond donor to $P_i$ (2.9 Å)   | Similar                          |
| Thr 53                         | Peptide N is possible H-bond donor to $P_i$   | Similar                          |
| Arg 54                         | Charge-charge interaction with $P_i$ and possible H-bond donor too (2.8 Å)  | Somewhat similar/diff            |
| Thr 55                         | Sidechain -OH is possible H-bond donor to $P_i$ (2.7 Å)   | Similar                          |
| Arg 105                        | Charge-charge interaction with $\alpha$ -CO <sub>2</sub> <sup>-</sup> and $P_i$ (3.0 and 2.7 Å); possible H-bond donor to $P_i$ (2.7 Å) and keto carbonyl (3.0 Å) | Similar                          |
| His 134                        | H-bond donor to carbonyl O of PALA (2.8Å distance and geometry OK)  | Similar                          |
| Arg 167                        | Charge-charge interaction with $\alpha$ -CO <sub>2</sub> <sup>-</sup> and possible H-bond donor too (2.8 Å)   | Similar                          |
| Arg 229                        | Charge-charge interaction with $\beta$ -CO <sub>2</sub> <sup>-</sup> and possible H-bond donor too (2.9 Å)  | Somewhat different               |
| Gln 231                        | Possible H-bond donor from sidechain amide N to $\beta$ -CO <sub>2</sub> <sup>-</sup> (2.8 Å)   | Different                        |
| Arg 234                        | (weak) charge-charge interaction with $P_i$ (5.5 Å)   | Different                        |
| Lys 84 (from adjacent subunit) | Charge-charge interaction with $P_i$ (and $\beta$ -CO <sub>2</sub> <sup>-</sup> ) and possible H-bond donor to $P_i$ (2.8 Å)                                      | Different                        |
| Ser 80 (from adjacent subunit) | Possible H-bond donor to $P_i$ , but might be too far away in this structure (3.1 Å)  | Different                        |

+1 pt for each amino acid residue (1/2 for identity & 1/2 for role in T&R)  $\Rightarrow$  7 pts total

List of shame: 109, 114, Ser131, Gln137, Cys138, Thr168, Ser252, Pro226, Leu267, Pro268

- e) Which amino acids are important for binding the effector (CTP)? Identify 5 amino acids involved in binding CTP. For each, indicate how it interacts with CTP. Then examine the same residue in the ATP-bound structure and indicate if it is playing the same role with ATP (if different, describe how it interacts with ATP, *if* it does).

| Residue (amino acid & #)               | Likely role in binding CTP   | Similar or different role with ATP?   |
|--|--|---|
| Lys 94                                 | Charge-charge interaction with $\alpha$ -P <sub>i</sub> and possible H-bond donor too (2.7 Å)                                    | Slightly different orientation, but could still do both (and interacts with different P <sub>i</sub> )  |
| Lys 60                                 | Possible H-bond donor (or acceptor, if deprotonated) to ribose 2'-OH (2.5 Å), as well as donor to carbonyl O of cytosine (2.7 Å) | Orientation changes and farther from ribose – can no longer donate strong H-bond. Also farther from adenine cyclic N, but might be able to donate H-bond to it. |
| Ile 12                                 | Carbonyl O of peptide is possible H-bond acceptor from exocyclic amine of cytosine (3.0 Å)                                       | Too far to accept H-bond from exocyclic amine of adenine (3.4 Å)  |
| Ala 11, Ile 12, Val 17, Ile 86, Tyr 89 | van der Waals contact with cytidine (Tyr89 <i>too far</i> to H-bond well to cytosine -NH <sub>2</sub> )                          | Pretty much the same role   |
| Val 91                                 | van der Waals contact with $\alpha$ -P <sub>i</sub>  | Similar (but less contact?)   |
| Asp 19                                 | Possible H-bond acceptor (or donor, if protonated) from ribose 3'-OH (3.0 Å)   | Somewhat different (in fact, closer)  |
|  |  |   |
| Lys 56                                 | (very weak) ionic interaction with $\gamma$ -P <sub>i</sub> (6.7 Å)  | Similar   |
| Glu 90                                 | (very weak) repulsive ionic interaction with $\alpha$ -P <sub>i</sub> (6.4 Å)  | Pretty similar ( <i>still far!</i> )  |

**List of shame:** Val 9 (NOT possible H-bond acceptor from ribose 2'-OH (geometry all wrong)), Asn84 (too far to H-bond), Glu10, His20 (too far! >6.5 Å), 44, 45, Glu90 (too far), Cys109 (too far), Cys114 (too far)

+1 pt for each amino acid residue (1/2 for identity & 1/2 for role with CTP/ATP) ⇒ 5 pts total

- f) Based on your analysis above, suggest a mutation that would make the enzyme more resistant to CTP (i.e. decrease affinity for CTP), but that would have less effect upon binding ATP. Just tell me the residue (amino acid name and number) and what you would change it to. Sketch 2 sets of curves below for how you would expect your mutant

to behave compared to the wild-type version of ATCase. In each, plot activity vs. [Asp] with (1) no additions, (2) + 0.5 mM CTP, (3) + 0.5 mM CTP + 1 mM ATP.

OK, this is harder than I thought. Changing Lys 60 to something that could not H-bond (like Leu or Arg) would be a good one to try. Assuming that you get one, the basic change is that the curve with CTP will not shift as much to the right.

+1 pt for a mutation suggestion, +1 for each graph  $\Rightarrow$  3 pts total

### **Problem 5**

If you did the problem, then you already know.

(And if you didn't, maybe you don't deserve to know...)