

KEY

Friday Questions

1. According to the authors, "Ser 187 in ColD does not interact directly with the sugar substrate, whereas Asn 185 in GDP-perosamine synthase participates in a hydrogen bond with the ring oxygen of the hexose moiety." Call up PDB entry 3DR7 which is GDP-PS complexed with 3-deoxyperosamine.

- (a) Give the distance between the atoms that are interacting as described above. *4.88 Å (It's not visible in x-ray)*
- (b) Is the distance you report the length of the hydrogen bond? If not, why not? *No, it is the N → O distance*

2. Look at the focused view of the active site that you created for Wednesday Question 4c for these questions. If something you need to see was cut off, go back to 3GR9.

(a) According to the authors, "The side chain carboxylate of α -ketoglutarate forms a salt bridge with the guanidinium group of Arg 250 whereas its α -carboxylate interacts with the guanidinium group of Arg 331.

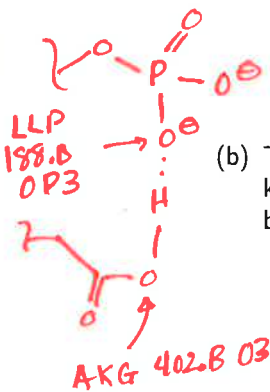
i. Draw one of these salt-bridges showing all atoms including hydrogen at realistic bond angles. Be sure your answer is consistent with the *pH*.

ii. Is this really a salt bridge? *Yes, but there is clearly a hydrogen bonding component as well*

(b) The authors continue after the sentence above: "Note that one of the carboxylate oxygens of the α -ketoglutarate is located within ~ 2.3 Å of a PLP phosphoryl oxygen, thus suggesting that a proton is shared between these two atoms."

i. Draw a picture of what they are talking about, showing full functional groups and all protons that should be present given the structure and *pH*. Be sure to label the exact atoms that the authors are talking about, including their Chimera labels, and show the shared hydrogen.

ii. Give the exact distance they refer to when they say " ~ 2.3 Å." *2.235 Å*



3. The authors say "The ability to perform a dehydration reaction most likely requires changes in residues not immediately surrounding the sugar substrate. Indeed, dehydratase activity requires the activation of a water molecule to attack the aminomannoseen-PLP intermediate, which is not required for aminotransferase activity." Call up structure 3B8X.

- (a) Find the aminomannoseen-PLP in a diagram in the paper. *Scheme 2 last full structure in left column*
- (b) Structure 3B8X contains a "sugar" at the active site.

i. Draw out the structure of the sugar portion of the "sugar" at the active site, including stereochemistry, plus, the aldimine or ketimine that is present. *on back*

ii. Is it an aldimine or ketimine? Give some evidence to support your answer.

(c) The "sugar" is not the aminomannoseen, but it is analogous to it.

i. Give the identifiers of the carbons in the "sugar" which correspond to the enamine carbons in the aminomannoseen. *G4M 500.A C4G G4M 500.A C3G also on back*

ii. Is there (circle Yes/No) a water molecule positioned for the attack on the aldimine (give the functional group being attacked)?

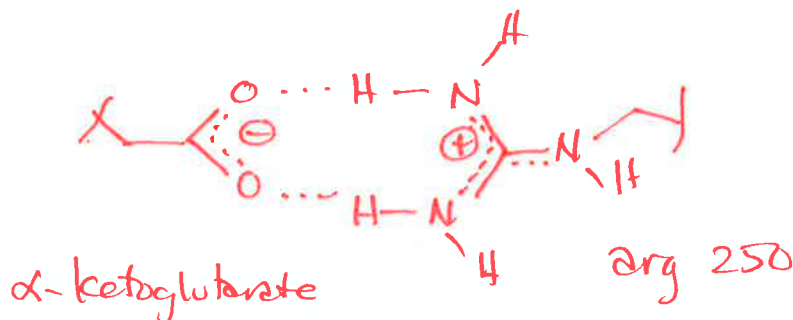
iii. What is the identifier of the water if it is present? *NP 756*

iv. What is the identifier of the atom to be attacked? *G4M 500.A C4A*

v. 3B8X is a H188N ColD mutant How far is the closest atom of N to the atom to be attacked?

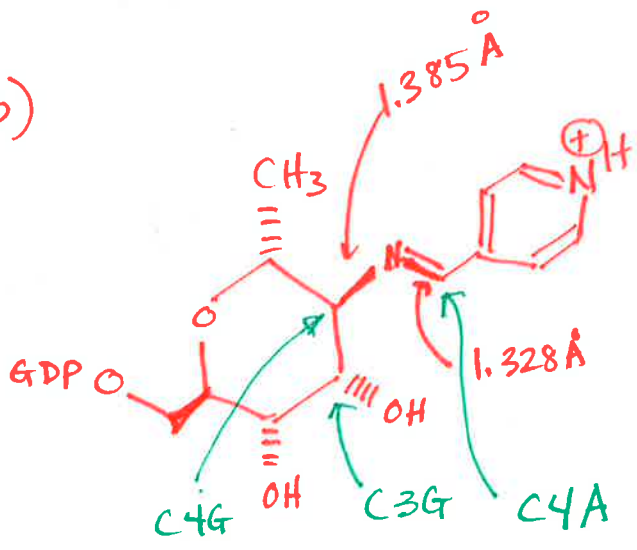
The 2 chains are slightly different see over

2(a)(i)



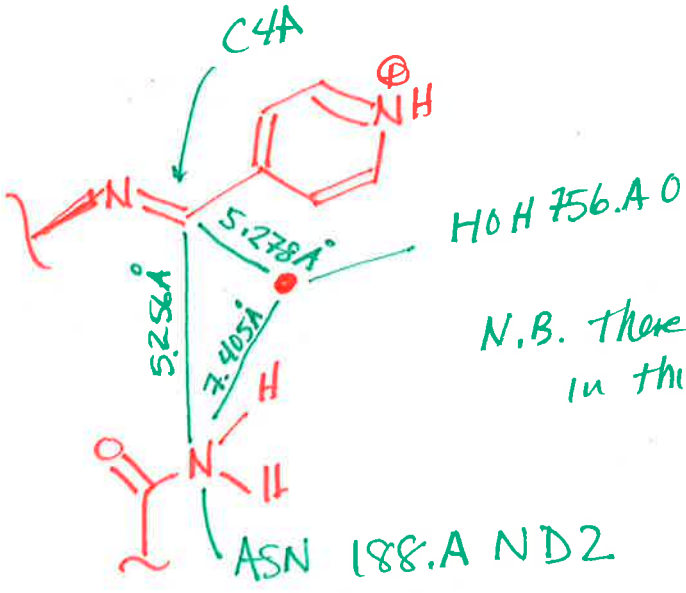
see over

3 (b)



looks like an
aldimine

3 (c)



N.B. there is no H₂O molecule
in this location on chain B