

Macromolecular Interactions. Ligand-binding Problem Set

Robert Stroud

Use PYMOL. The scripts I provide on the website contain adequate commands for the problem set and will help you through it until you learn how to code in PYMOL.

PDB Files:

ts.pdb (PDB ID: 2TSC)	For all questions except #6
posttrans.pdb (PDB ID: 1BID)	For question #6

1. Active site: Thymidylate Synthase (TS) is a dimer of identical subunits, each of 264 amino acids in length. Locate the ligands bound at one of the active centers. Are both monomers necessary for formation of each active site?

2. Structural role: Four conserved Arginine side chains coordinate to bind phosphate of dUMP. Two arginines can be mutated to a wide variety of other amino acids without much change in function (or structure). One affects cofactor binding affinity when mutated. One other Arg can not be mutated at all without loss of function; crystal structures show that these mutants lead to a change in protein structure. Suggest which of these four arginines would be most critical to binding cofactor.

3. Role of proline in helix: It is often suggested that proline acts as a 'helix breaker', since its nitrogen is unavailable as a hydrogen bond donor. Look for a proline in an alpha helix. Yes, it does remove one hydrogen bond donor, but besides this, does it break the helix?

4. Drug Resistance: TS is a target for anticancer drugs, including fluoro-uracyl (FdUMP). In patients treated with FdUMP a drug resistant mutation occurs in which conserved Tyr 4 is altered to Histidine 'Y4H'. Since Tyr 4 is not at the active site in contact with dUMP (FdUMP binds at the exact same site as dUMP) can you suggest why this mutant could produce resistance to FdUMP?

5. Unique sheet - sheet packing: Normally when one looks in from the side of any beta sheet and perpendicular to the individual strands, each successive strand of the sheet is rotated anticlockwise as you progress through the strands from near to far. This is true of parallel strands, or antiparallel stranded beta sheets. The carbonyl oxygens of successive amino acids lie in the plane of the sheet and alternate between pointing left, and pointing right.

Identify the region of beta sheet in the monomer A, and in monomer B. Back to back, these sheets encode the subunit - subunit interface. This interface is extremely unusual in that, when you look at the sheets from the direction perpendicular to the sheets, the

strand direction in the back sheet is rotated clockwise with respect to that in the front sheet, the opposite of almost all other such interfaces. This produces an almost unique 'Beta - kink' in the sheet within each monomer, around the amino acids Gly 31, Gly 204, Arg 166, and this in turn produces the cup-shaped 'floor' of the active site. The kink can be seen as where the successive carbonyls in several strands cease to alternate from one side to the other, in sync with each other. Can you suggest, in terms of the usual shape of a twisted beta sheet, why this contra rotation causes this distortion? Hint; Think of this as packing of two sheets which have a sort of propeller twist. Imagine placing two such sheets against each other so that they fit together. Now twist the bottom sheet clockwise until they start to run into one another..

6. Post translational modification: In most species of TS there are more residues before residue 1 in the sequence, and the equivalent of E. coli residue #1 is always an acidic glutamate side chain, that interacts with totally conserved threonines 46, 47. (listed as 47, 48 in the 'posttrans.pdb' data set due to the N-terminal modification being labeled as #1.. It is a bookkeeping error- so look for threonines numbered 47, and 48) Mutation of both 46, 47, linked closely to cofactor binding leads to complete loss of activity. In E.coli TS the N terminal residue #1 is methionine. The N-terminal becomes modified by addition of CO₂, to make a carbamate, to preserve the interaction. How is this rather chemically unstable modification stabilized in the protein structure, and how does it fulfil the role of the glutamate normally present at position #1?

7. Catalytic water: A group is required to abstract the hydrogen from 5C of dUMP - yet all residues have been mutated singly without total loss of function. Can you see what might be the catalytic base?

8. External salt bridge conserved, important: The G H helices (residues 93-98, and 102-122) abut each other in the structure, with a loop in between them that is external. What role does the totally conserved sequence - DQ- at 110, 111 play that could account for this?

9. A Critical interaction. Asn 177 is totally conserved in TS, and serves as a specificity encoding side chain for the Uridine base of dUMP, making hydrogen bonds acceptor to N3, donor to O4. Less obviously, Gln 165 is conserved. Suggest why that might be important?