

# iMolTalk Workshop

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We will analyse the enzyme class of aspartic amino transferases (AAT), in details the SP entry **P00508** (AATM\_CHICK) and its corresponding structures: **7AAT** (apo, open form) and **1AMA** (holo, closed form).

## Summary of AATs:

- two isozymes (mitochondrial, cytosolic)
- around 400 amino acids long, homodimer
- bound cofactor: **PLP** (pyridoxal-5'-phosphate, derivative of vitamin B<sub>6</sub>)
- substrate binding causes conformational changes

## Tasks:

- Start with the Swiss-Prot entry **P00508** and read through it. The Feature record (FT) for “BINDING” points to residue 272.
- Find this residue in the SP sequence: what type? what environment?
- In a new browser window log on to <http://i.moltalk.org>
- Choose “secondary structure” assignment from the tools
  - Enter PDB code: 7AAT in the first field; press “Next>>”
  - Choose chain 'A'/#65; press “Next>>”
- Now find the SP residue 272 ('K', in sequence: ...SYA **K** NMG...) in the colored sequence
- What is the type of secondary structure this residue is in?
- To find the contacts this residue makes in the protein, simply click on it and choose the “contacts” button from the menu
- Another page is loaded with the atomic contacts of this residue (K258): distances with yellow background can indicate covalent bonds due to very short contact. This is the case for LYS258(atom NZ) in contact with PLP258(atom C4A).  
Congratulations, you just discovered the covalently bound cofactor!
- Click on PLP258 and choose the “contacts” button. A new page is loaded with the atomic contacts of the heterogeneous residue PLP. It shows that PLP makes a lot of hydrogen bonds, which could be analysed in more detail in a structure viewer. Interestingly, TYR70 from the other chain (B) in the dimer, as indicated by the yellow background of the chain2 column, makes a contact to PLP. This might be an important functional residue!
- At this stage, we might be interested in the quality of the structure. Is this what we see a confident result? At the top is some header saying “... in Chain: 'A' / 65”. Click on 'A' / 65 to get the menu for a chain and choose “Ramachandran plot”.
- In the Ramachandran plot we see a single outlier: S296. But 99.4% of all residues (not counting proline and glycine) are in the core and

Swiss-Prot  
P00508

Secondary  
Structure  
Assignment

Molecular  
Contacts

Ramachandran  
Plot

- allowed region, indicating an overall good quality of the structure.
- Click on 'A'/65 (the chain menu) and choose “C $\alpha$  distance map”.
- The distance map is limited to 400 residues width and height. So enter a clip region from: “-1” to: “399”. Finally the image loads. But it is difficult to see anything.
- At the top enter a new clip region from: “270” to: “310”. Highlight residue from: “296” to: “296”. Choose scale = 3.0. Click on “redisplay” to have a new distance map computed.
- Now S296 shows to be at the end of an  $\alpha$  helix in a tight turn. Click on the line of S296 and choose “contacts” from the residue menu.
- S296 does not make any clearly interesting contacts at the default distance threshold of 3.4 Angstroms. But we might find something at a larger distance. Choose “<<Prev” from the menu at right. And set the distance to 4.5 Angstroms. After pressing “Next >>”, in the new output many new contacts appear, including some to the second chain (B) and its cofactor PLP. An interpretation is difficult to make but together with reading the original publication of the structure it might become clearer. Possibilities: crystallisation artefact, close contacts on CB, conformational stress, ...
- Interface definition between the homodimers: go back to the main page and choose: “interface” from the tools.
- Enter PDB code: “7AAT”, next, choose chain A as first chain and chain B as second chain. Keep the distance threshold at 3.4 Angstroms.
- The report contains all hydrogen bonds and salt-bridges, as well as other unspecific contacts, between chain A and chain B. Here we find SER296 in chain B making a hydrogen bond to THR109 in chain A, but not the analog contact of SER296/A to THR109/B. The interface is built on dozens of hydrogen bonds and a couple of salt-bridges. In energetical terms this can be regarded as a very strong coupling of the two dimers.
- Look at the contacts of the heterogeneous group “AMA” in the structure “1AMA”. From there you may want to see the contacts of LYS258. The previously covalent bond of LYS258(NZ) to PLP(C4A) is broken and a new hydrogen bond from the NZ atom to the phosphate group of PLP is formed.

C $\alpha$  Distance Map

P-P Interface

Molecular Contacts

## Conclusions

From the above example you have learned how to find residues in a structure when the numbering differs from that in sequence databases.

Secondary structure assignment gave a first hint on the molecule's architecture.

By the use of the Ramachandran plot you have been able to judge the quality of the structure. It also gave a starting point for trying to find an interpretation of the “off” residue S296.

You have been looking at molecular contacts and the protein-protein interface of the two chains in the dimer to understand the molecular interactions that are necessary for the enzyme's function.

Good luck with your next analyses on <http://i.moltalk.org>