

# **Extra-Homework Problem Set**

**=> Will not be graded, but might be a good idea for self-study**

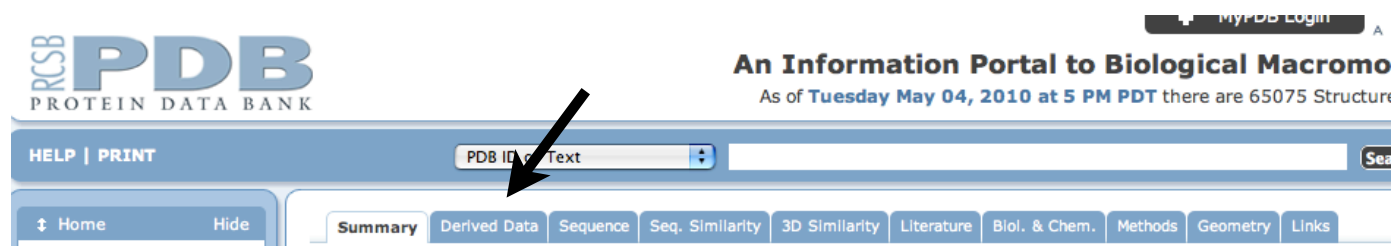
**=> Solutions are posted at the end of the problem set**

Your adviser asks you to find out about a so far unpublished protein structure of an enzyme called **Ferredoxin-NADP reductase** (s)he is very interested in. At a conference, (s)he heard that this new structure, which was solved at close to 1 Angstrom, was already deposited in the PDB, but does not recall the PDB code or the organism it was cloned from.

=> Search for the structure in the PDB using the information above and download the PDB file.

=> What **ligands** were found in the structure? Where could the smaller ligand have come from?

You decide to explore the **domain architecture** of this enzyme now. Go to the „**Derived data**“ tab on top.



Look at the **Pfam classification**:

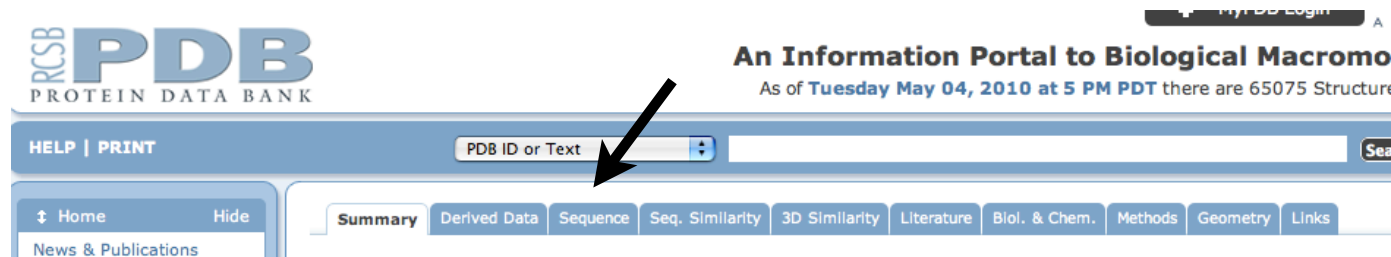
=> How many domains are assigned to this enzyme by Pfam?

=> What kind of functions do they fulfill?

(HINT: You can learn about the Pfam-assigned function for a particular domain by clicking on the little bent arrows next to the Pfam accession numbers. On the linked pages, you will find InterPro entry descriptions).

Now you want to learn which sequence segments correspond to which domain / where the **domain boundaries** are.

=> Go to the „Sequence“ tab.



=> You see the amino acid sequence with assigned secondary structure elements (by the DSSP algorithm) and domain annotation, per default according to the **Domain Parser (DP)** algorithm.

Domain Parser assigns **domain boundaries based on structural aspects** derived from the 3D-structure \*).

[You can mouse over the secondary structure elements and domain annotations to get explanations for the symbols.]

#### Chain Display

Chain A (polymer 1) [help] [fasta] [text/markup]

Description Ferredoxin--NADP reductase  
Chain Type polypeptide(L)  
UniProtKB reference **Q41736**  
Length 311 residues  
DP domain assignment **3LO8A0** 302 residues  
[hide] [reference]  
DSSP secondary structure 27% helical (12 helices; 85 residues)  
[hide] [reference] 27% beta sheet (18 strands; 84 residues)

More annotations

Select

Currently displayed: SEQRES sequence. [display external (UniProtKB) sequence]

Sequence Details



=> How many domains (comprising how many residues) does Domain Parser assign to this protein?

\*) The underlying assumption is that residue-residue contacts are denser within a domain than between domains.

You want to find out whether the domain annotation will be different if the **assignment is based on sequence similarity** rather than structure:

=> Add a **Pfam** annotation from the pull-down menu. The Pfam (i.e. sequence-based) annotation will appear underneath the DP annotation for comparison.

=> Are there differences between the assigned domains / domain boundaries by Pfam and DP?

Chain A (polymer 1) [help] [fasta] [text/markup]

Description Ferredoxin--NADP reductase  
Chain Type polypeptide(L)  
UniProtKB reference Q41736  
Length 311 residues  
DP domain assignment 3LO8A0 302 residues  
[hide] [reference]

More annotations

✓ Select

domain assignment

PDP

domain assignment from sequence

Pfam

InterPro

secondary structure

DSSP

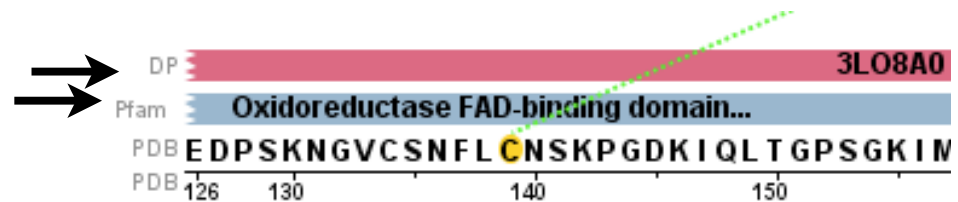
Stride

Author-approved

PDB

YWE GQSYGVIPPGENPKKPGAPQNVRLYSIA

Looking at the Pfam domain annotations, what can you conclude regarding the molecular function of the enzyme?



=> For this exercise, we stick with the Pfam annotation.

Make a note of the residue-identifiers at the Pfam-assigned domain boundaries  
i.e. residue numbers and 3-letter code residue names.  
(You will need this for data visualization).

Since there is no export option available, also a screenshot of the current setup is helpful.

If the multi-annotated display is confusing for you, you might want to hide one or more annotations.

You now want to **visualize** the domains assigned by Pfam on the 3D structure of the reductase, e.g. using the PDB-provided Jmol-application:

=> open a Jmol window for the reductase (3LO8)

=> in the Jmol script command line, select the before noted domain boundaries in the following format:

```
select resno >= ## and resno <= ##
```

e.g.:

```
select resno = 131    a single residue
```

```
select resno = 217, resno = 1426    two residues
```

```
select resno >= 195 and resno <= 277    a range of residue serial numbers
```

and analogously for selection of atoms:

```
select atomno = 131    a single atom
```

```
select atomno = 217, atomno = 1426    two atoms
```

```
select atomno >= 195 and atomno <= 277    a range of atom serial numbers
```

### **Please remember:**

You need to select an object first, then you can choose its style, then via scheme its color or the type of representation (e.g. cartoon or sticks).

Your result might look somewhat like this:

FADbinding domain in orange,  
NADP+ binding domain in cyan \*)

!! In addition, it might be a VERY good idea to review the various Jmol options to select, stylize, mark and measure residues, atoms or bonds in the Jmol pulldown menus we looked into during the computer lab Jmol tutorial on lecture day 5 (April 13th) .....



A good resource for Jmol commands, although sometime a bit cryptic:  
<http://chemapps.stolaf.edu/pe/protexpl/htm/seleccmd.htm>

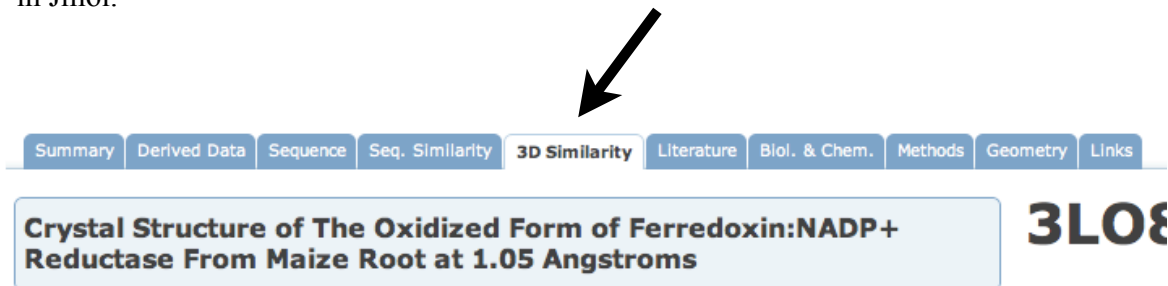
\*) The remaining grey areas in the structure graphic represent those parts of the sequence, that had not been annotated to a particular domain by Pfam. Still, when looking at the fold in 3D, it becomes clear that the purely sequence-based assignment of domain boundaries (such as in Pfam) is not perfect !

=> Finally, you are interested whether there are also **structural homologs** (e.g. similarities in 3D-structure, not only in sequence) of your enzyme existing in the PDB.

=> Conveniently, the the structure-comparison algorithm FATCAT is built into the PDB, so you only need to click on the 3D-similarity tab on top.

A number of proteins with similar structures / folds as your molecule of interest, represented by their PDB codes, are listed.

To view individual C<sub>alpha</sub> backbone comparisons, click on the respective "view" sign and a 3D structure superposition appears in Jmol.



The screenshot shows the PDB entry page for 3LO8. A black arrow points to the "3D Similarity" tab in the top navigation bar. Below the tabs, the title "Crystal Structure of The Oxidized Form of Ferredoxin:NADP+ Reductase From Maize Root at 1.05 Angstroms" is displayed next to the PDB ID "3LO8".

### Structural Similarities for the Entities in PDB 3LO8

The following structural similarities have been found using the jFATCAT-rigid algorithm. In order to reduce the number of clustering has been applied and a representative chain is taken from each cluster.

#### Entity #1: Chains: A

**Description:** Ferredoxin--NADP reductase polypeptide(L)

**Length:** 311

##### 3LO8.A (chain 1) vs. representatives of other sequence clusters (chain 2)

Rank	Results	Chain 2	Title	P-value	Score	Rmsd	Len1	Len2	%Sim1	%Sim2
1	<a href="#">view</a>	2B5Q.A	Ferredoxin--NADP	0.0	752.04	1.51	302	292	93	97

**Update**

The data are up to



# Solutions

PDB code and source:	3LO8
Ligands	<ul style="list-style-type: none"><li>- Sodium ion (probably a (crystallization) buffer component</li><li>- FAD</li></ul> <p>=&gt; HINT: Ligand structures can be viewed by mousing over the ligand's name identifier.</p>
Pfam classification	<ul style="list-style-type: none"><li>- NAD_binding_1,    Oxidoreductase NAD-binding domain</li><li>- FAD_binding_6    Oxidoreductase FAD-binding domain</li></ul>
Domain Parser	assigned only one domain over (almost) the entire fold, comprising 302 residues
Pfam	<p>assigned 2 domains: one FAD-binding domain, residues Glu79 - Leu158 and one NADP<sup>+</sup>-binding domain, residues Met170 - Asp285</p> <p>=&gt; This enzyme is predicted to have electron-transfer function, involving the cofactors FAD in one and NADP(H) in the other domain.</p>