Structural Biology Exercise #4:
Lysosomal α-mannosidase: several inherited ways to fall

Lysosomal α-mannosidase (LAM) is a protein, which breaks oligomeric mannose sugars released from degraded proteins, into single mannose sugars. Failure in LAM function results in a severe inherited disease, α-mannosidosis. Several inherited missense mutations are known, which result in α-mannosidosis in man, cat or cattle. We will now look at a few of these.
The structure of the bovine LAM is known from the bovine enzyme. The sequence of the bovine enzyme is 80% identical to the sequence of the human enzyme, so we can use the bovine LAM structure as such to look at the mutations in the human LAM.

1. On Your Marks...

Dowload the LAM structure and the paper from pdb
Open web browser and go to `http://www.pdb.org`
Find structure **`1o7d`** (lysosomal α mannosidase) and download it to your computer. Alternatively you can use the PyMOL PDB loader service–Plugin.

Copy of the paper describing this structure is on your stick, but you can also find the link to PubMed from the Protein Data Bank, at the page where you download the structure.

Open PyMOL
Find your α-mannosidase structure from `File/Open`
Save your PyMOL session from `File/Save session as/` and give it a name for ex. tutorial_LAM

2. Steady

What is lysosomal α-mannosidase? Peptides, domains, metal ions, disulfides and glycans.
In PyMOL, modify the display so that the molecule is easier to understand:

(H) Hide `everything`
(S) Show `cartoon`

(and if you prefer, from `Setting/Cartoon/Fancy helices → on`)

Rotate the molecule so that it is a bit easier to visualise; Lam is a big protein!
LAM in coded by a single gene, but the mature enzyme is proteolysed in several peptides. You can see the peptide ends on the surface of the protein.

(C) Colour `by chain`

This will colour the peptides individually, as defined in the pdb file.

If something went wrong, you can load the saved PyMOL session from the stick and select from the Command window `Scene/Recall/F1`

As you rotate the molecule, you notice that the native proteolytic cleavages do not follow the protein domain boundaries. This is very typical for post translational modifications on single gene

proteins; folding of the protein is completed before the proteolysis takes place, thus there is no reason why the proteolysis should be restricted by the fold.
The structure is however easier to understand if you colour it based on the domains. In the Command window:

`Display sequence`

Select the individual domains from the sequence and colour them with separate colours.
Remember to deselect `(sele)` after colouring, so that you do not combine it with the next domain.
There are 5 subunits:
α/β: residues `G51-P379`

`C (colour)` to any colour you like.

Don't forget to deselect (sele) before you continue!

3α: residues `Y380-G485`
β1: residues `L486-P584` **and** `P867-G874`
β2: residues `R603-A866`
β3: residues `P885-W1006`

In the end, for the whole structure, do

`(1o7d) → C/by element`

this way the amino acid side chains are easier to recognize later on.

When you have all five domains, it is good to save:

`Scene/Store/F2`
`File/Save Session`

If something went wrong, you can load the saved PyMOL session from the stick and select from the Command window `Scene/Recall/F2`

There are also other features, which might help you to understand the LAM structure:

Catalytic site
A metal ion defines the active site domain.
You can find it early in sequence. Select ZN

`(sele) → S/spheres`

The protein domain containing the Zn ion is the catalytic domain in the structure.

Glycosylation
LAM also contains several glycosylation sites. Some of these also visible in the crystal structure.
You can display these by

First select all `NAG, NDG, MAN` and `BMA` residues from the sequence

(they are in 3 separate places in seq view).

`S (show) sticks`

`C (colour)` to any colour you like

You can also rename the selection if you like.

The glycans do not appear attached to the protein. This is because we view the protein in the cartoon mode and individual residues are missing.

The glycosylation sites on the protein are listed in the paper describing the structure (or you could go back to the sequence or structure data bank to look for them).
Glycosylation sites on this structure are at

`N133, N367, N497, N645, N651, N692, N766 and N930`

Click on the black background to make sure that you do not have an active (sele) object.
Then select these Aspartic residues (N) from the `sequence` view.

```
(sele)→ S/sidechain/sticks
```

Now the glycans should appear attached to the protein structure.

A native glycosylation site typically contains 5-8 glycan residues. Not all of these are visible in the structure. This is typical for structures determined by X-ray crystallography, where most of the glycans are not visible due to their high mobility.

Disulfide bridges
The native LAM protein contains four complete disulfide bridges.

```
(1O7D)  → S/disulfides
```

In the structure, you only see two (one on the active site domain and one in the three helix bundle). This is because sometimes during the X-ray experiment, the X-rays destroy the protein sample and some atoms get blown off from the structure. This happens typically for cysteines or metal coordinating residues.
You can locate the remaining disulfides by finding the additional four cysteines from the sequence:

Select C268 and C273

```
(sele)→ S/side chain/sticks
(sele)→ A/ center
```

and
Select C493 and C501

```
(sele)→ S/side chain/sticks
(sele)→ A/ center
```

Save again:

```
Scene/Store/F3
File/Save Session
```

or find this state from the saved session your memory stick.

3. Go!

We will now look at the mutations which cause α-mannosidosis. In order to save your Scenes so far, you need to copy the (1O7D) object where you make the mutations.

(1O7D) → A/Duplicate object
(obj1) → A/rename selection
remove 'sele' with backspace and type Mutated <Enter> on the screen window.
Hide (1O7D) by clicking the grey object button black so that you do not mutate the wild type structure by accident.

Make the following disease associated mutations to (mutated).
For example:
C55F
means that cysteine 55 has mutated to phenylalanine.
Find C55 from the sequence and center on it
(sele) → A/center
(sele) → S/sidechain/sticks
Now you see the wild type residue in lysosomal α-mannosidase

Delesect (sele) by clicking on the black background.

Wizard/ mutagenesis
Select C55 from the sequence (Pymol will center on the residue)
On lower right,
change No mutation → PHE.
Select with the → -key, a rotamer
which seems to fit or is similar to the wild type. The red dots in the end of the mutated side chain indicate collision with the rest of the structure. You can adjust the slab (with the mouse scroll) so that the site is easier to view.
Click Apply This will make the mutation!
Then continue with the next mutation site or click Done.
This mutation is saved for you in the provided PyMol session as Scene F4

Here are some mutations to look at:
D74E
Aspartate to glutamate. This change is on the metal coordinating residue and the mutation will affect binding of the catalytic metal ion. Thus this mutation destroys LAM enzymatic activity and is an α-mannosidosis associated mutation.
(Prepared in Scene F5).

N413S
This is an example of a tolerated mutation. The mutations occurs in an area where the protein can adjust, and the wild type and new amino acid are similar in their size and polarity. This change occurs in normal population and is **not** α-mannosidosis associated.
(Prepared in Scene F6).

R750W
This is one of the oldest mutations in the LAM gene and occurs around the world, unlike many other mutations which are population or even family specific. The mutated 750Trp cannot form the same contacts as the wild type residue.
(Hint: Display the side chain R750 and select from A/find/polar contacts.)

(Prepared in `Scene F7`).

`G801D`

The Glycine amino acid has no side chain, thus changing it to any side chain containing residue is usually deleterious. In addition to the larger size, The Asp residue is also charged and does not fit to the hydrophobic pocket.
(Prepared in `Scene F8`).

`L956R`

Arginine mutations are common in disease associated mutations. This is due to the special properties of the side chain, size, charge and ability to form hydrogen bonds in many directions. Due to this, it also does not fit into just any place. Here is an example of an Arg inserting mutation. The mutation occurs on an interface of two domains, which is held together by hydrophobic contacts. An Arginine here is a trouble maker, proteins containing this mutation have difficulties in folding, and they are arrested at the ER.
(Prepared in `Scene F9`).

4. Finish!

The molecular analysis of the inherited mutations in α-mannosidase are published in

Kuokkanen, E., et al (2011) Molecular and cellular characterisation of novel α -mannosidosis mutations. *Human Molecular Genetics*, **20**, 2651-2661.

You have a copy of also this paper in your memory stick.