

## Exercise 4: Creating protein complexes that have several levels

• Please only modify the *H. pylori* database.

Many bacterial adenosylmethionine decarboxylases are first synthesized as a single polypeptide (the pro-enzyme) that self-cleaves into two smaller polypeptides (alpha and beta). Each cleavage product forms a homotetramer, and the two tetramers bind together forming a heterooctamer, which is the functional enzyme.



Figure 1 adenosylmethionine decarboxylase

For the sake of the exercise, we will pretend that the enzyme exists in *H. pylori* (it most likely doesn't), and that it is encoded by HP0951 (which is definitely not correct).

The protein objects that are required are as follows:

(protein 1) - the gene product (proenzyme)

(protein 2) - the alpha subunit – a modified form of (protein 1)

(protein 3) - the beta subunit – a modified form of (protein 1)

(protein 4) - the alpha subunit tetramer – a complex of (protein 2)

(protein 5) - the beta subunit tetramer – a complex of (protein 3)

(protein 6) - the final octamer – a complex of (protein 4) and (protein 5)

These protein objects are described in Table 1 in the next page.

|   | Protein                          | Common Name  | Frame ID           |
|---|----------------------------------|--|--------------------|
| 1 | gene product                     | adenosylmethionine<br>decarboxylase<br>proenzyme                         | HP0951-<br>MONOMER |
| 2 | alpha subunit cleavage product   | adenosylmethionine<br>decarboxylase alpha<br>subunit cleavage<br>product |                    |
| 3 | beta subunit cleavage product    | adenosylmethionine<br>decarboxylase beta<br>subunit cleavage<br>product  |                    |
| 4 | alpha subunit tetramer           | adenosylmethionine<br>decarboxylase alpha<br>subunit tetramer            | not required       |
| 5 | beta subunit tetramer            | adenosylmethionine<br>decarboxylase beta<br>subunit tetramer             | not required       |
| 6 | adenosylmethionine decarboxylase | adenosylmethionine<br>decarboxylase                                      |                    |

## Table 1

- 1. Open the gene HP0951 in the H. pylori PGDB
- 2. Rename the gene product (HP0951-MONOMER) to "adenosylmethionine decarboxylase proenzyme" via the Protein Editor or Synonym Editor.
- Create (protein 2): Open HP0951-MONOMER in the Protein Editor, and click on the "Modified Forms" tab. Click on "Create New Modified Form" and provide the common name (from Table 1). The frame ID of the new protein appears in the title of the window – copy it to Table 1. Click OK, and repeat, creating (protein 3).
- 4. Create a new protein (Protein → new). Select "protein complex", and type "2" for "number of distinct subunits". Type the names of proteins 4 and 5 into the "subunit" name fields, and click the "Complex?" checkbox next to each of them. Type the frame IDs of proteins 2 and 3 for the subunits of protein 4 and 5, respectively (the system will convert the frame ID to the full name as soon as you click out of the text field). Now enter the coefficients 1 for the upper level, and 4 for the lower level (see figure 2).

| Specify Protein Subunit Structure  |                                      |  |
|--|--------------------------------------|--|
| Protein: adenosylmethionine decarboxylase<br>Macromolecule Type: protein complex  Number of distinct subunits: 2<br>e.g. A homotetramer counts as 1 gene product, not 4 the number supplied here<br>should match the number of subunits supplied below.<br>For a complex of complexes, check the "Complex?" box below for each subunit<br>that is a complex, and enter the number of distinct subunits and the components<br>for each. The coefficient can be omitted if it is not known. The Status<br>column below tells if a protein already exists or will be created. |                                      | ₽  |
| Genes or Subunits:<br>Subunit  | Complex? Gene or #Subunits Coefficie | nt Status  |
| adenosylmethionine decarboxylase beta subunit tetramer   | #Subunits: 1                         | Already exists (edit name                            |
| ylmethionine decarboxylase beta subunit cleavage product   | Gene: 4                              | Already exists (edit name<br>to create a new object) |
| adenosylmethionine decarboxylase alpha subunit tetramer  | ▼ #Subunits: 1 1                     | Already exists (edit name                            |
| adenosylmethionine decarboxylase alpha subunit cleavage  | Gene: 4                              | Already exists (edit name<br>to create a new object) |
|  |                                      | ■ 11113 & HOH ONJOON                                 |
| OK Cancel  |                                      |  |

Figure 2

- Click OK, and the Protein Editor will open up. Type the common name of protein 6 for the new complex. The frame ID of the complex appears in the window title – copy it to Table 1.
- 6. Click OK to close the Protein Editor. Your proteins should look like Figure 1, lacking the reaction.
- 7. From the Reaction menu, select "Search by EC#". Type "4.1.1.50" and click OK.
- 8. When the reaction page shows up, right click on the object handle, and select Edit → Create/Add Enzyme. When the "Choose Protein" window appears, type the frame ID of protein (6) and click OK. When the protein Editor opens up, select the "Enzymatic Activity" tab and type "adenosylmethionine decarboxylase". Click OK, and admire your work!