

Pathway Tools Tutorial Protein complexes

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.

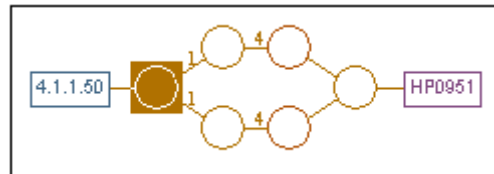


Figure 1 adenosylmethionine decarboxylase

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 decarboxylase proenzyme	HP0951-MONOMER
2	alpha subunit cleavage product	adenosylmethionine decarboxylase alpha subunit cleavage product	
3	beta subunit cleavage product	adenosylmethionine decarboxylase beta subunit cleavage product	
4	alpha subunit tetramer	adenosylmethionine decarboxylase alpha subunit tetramer	not required
5	beta subunit tetramer	adenosylmethionine decarboxylase beta subunit tetramer	not required
6	adenosylmethionine decarboxylase	adenosylmethionine 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.
3. 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).

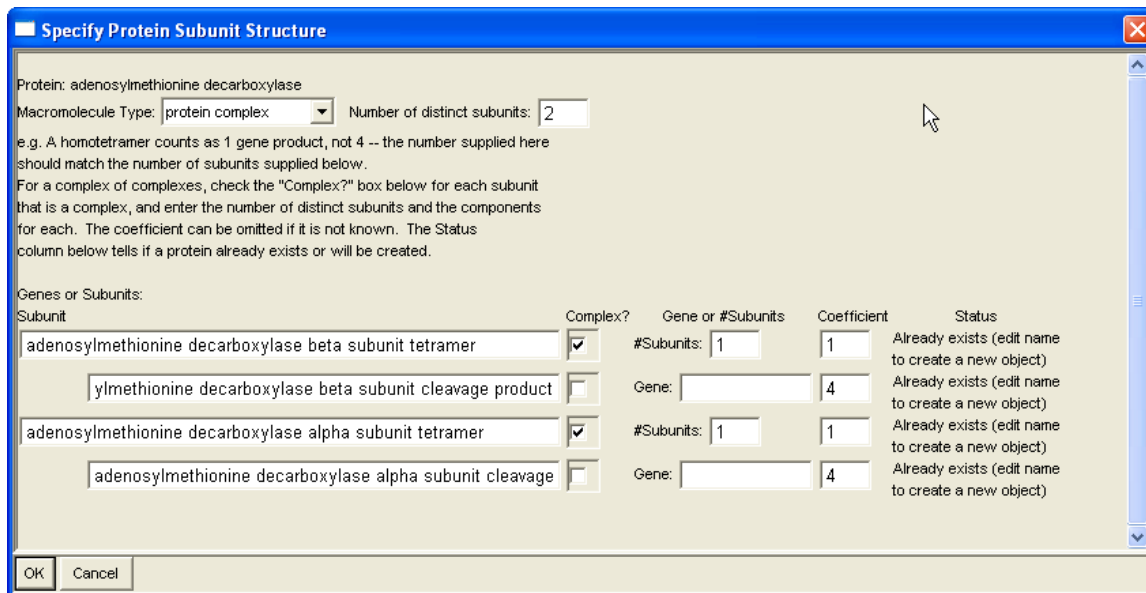


Figure 2

5. 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!