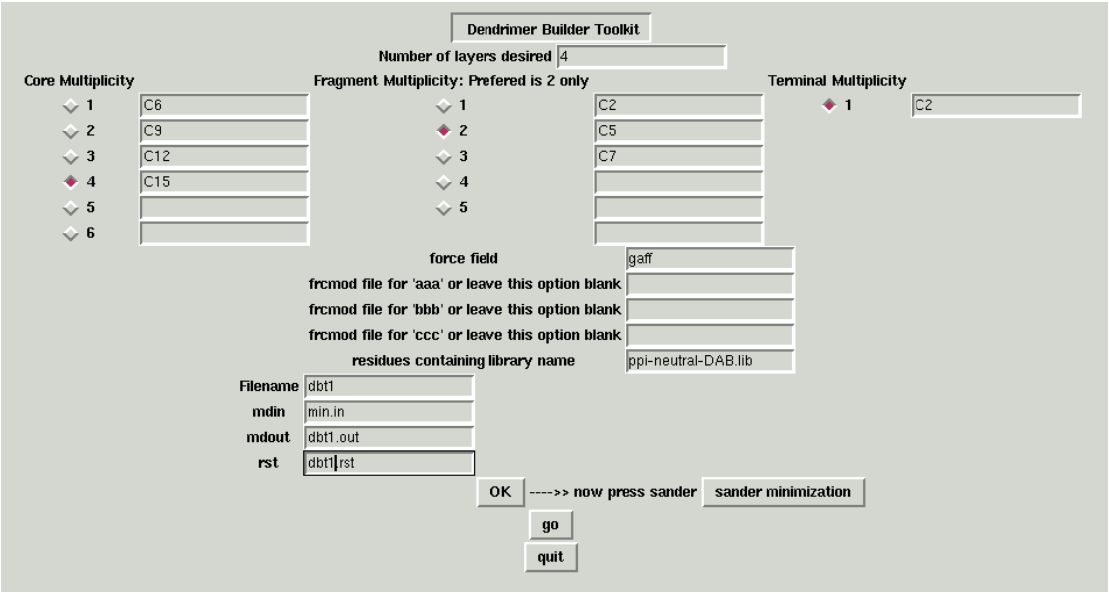
Tutorial for building G5 PPI dendrimer (DAB core) at neutral pH (2/3 protonation)

1) Open terminal and type: **perl dbtv1_2.pl** (see image). Press enter key (linux by default most cases has perl but install PERL-Tk before using perl dbtv1_2.pl). It should open GUI like this:



Note: Here residue designations in the ppi-neu.lib are as follows:

'aaa' – core residue (non-protonated)

'bbb' – repeating fragment (protonated)

'ccc' – terminal group (protonated)

'ddd' – repeating fragment 'bbb' (non-protonated)

2) Type entry in **Number of layers desired**: here put **4** (for G5 PPI). This number will run loops in such a way that alternative layers of 'bbb' and 'ddd' will be put around core 'aaa' and finally terminals 'ccc' will join the 'ddd'.

3) Core Multiplicity is number of arms of central core ('aaa' residue in

ppi-neu.lib): set it **4** by clicking between 1 to 6 buttons. Then, write the name of joining atoms that you want to join with 'bbb' or 'ddd'. Only one atom name in each box, here it is **C6**, **C9**, **C12** and **C15**. (For your own residues and library the name of joining atoms can be seen in ***xleap*** --> **loadoff your.lib** --> **edit aaa** --> **display names** or also from your.ac file while preparing residues. In this tutorial, you can see the atom name by loading ppi-neu.lib in *xleap*).

4) Fragment Multiplicity is number of arms minus one i.e. n-1. Set it **2** in this case (3 arms - 1) and write atom names. One atom name in each box, here it is **C2**, **C5**, **C7**. The name of joining atoms can be seen in ***xleap*** **--> loadoff ppi-neu.lib** --> **edit bbb or edit ddd**--> **display names** or also from your.ac file while preparing residues.

Important: Please note, the atom which becomes joining head atom is written last. Here it is **C7**.

5) Terminal Multiplicity is by default set to **1** only. User cannot change it. Write the name of joining atom (here it is **C2**), which is going to attach with repeating fragment atom ('ddd' residue). The name of joining atoms can be seen in ***xleap*** --> **loadoff ppi-neu.lib** --> **edit ccc** --> **display names** or also from your.ac file while preparing residues.

6) Write the name of **force field** file, here it is **gaff**.

7) Write the **name of frcmod** file for residues 'aaa', 'bbb', 'ccc' and 'ddd', if any, otherwise keep it blank. In this tutorial it is not desired.

8) Write residue containing **library name**. Here **ppi-neu.lib**.

9) Now PRESS "**go**" BUTTON (above the "quit" button).

Next step (10) is different in auto dbtv2.pl

10) Now user should be able see some files named dbt1, dbt2, dbt3, dbt4 and dbt_term. Only one dbt_term will be formed, but number of dbt files will depend upon the number of layers desired. These contain the set of instructions (*tleap* / *xleap* commands only), which defines the manner of joining the residues. The initial dbt file (dbt1) contains information about the joining of central core 'aaa' with single first layer of 'bbb' or 'ddd' residues. Each dbt files gives information about the single sequential layers of residues ('bbb' or 'ddd'). The dbt_term contains information for the joining of terminal 'ccc' residues. For lower generations, one can see lesser number of commands/instructions in dbt files, but for higher generation there will be many number of lines (*e.g.* try to put Six number of layers and look for dbt6 file).

NOTE: Before proceeding further, replace 'bbb' with 'ddd' in the generated dbt2 and dbt4 files so as to obtain alternative layers of 'bbb' and 'ddd' repeating fragments in the final G5 PPI dendrimer. This change will help to obtain PPI dendrimer configuration with 2/3 protonation, where terminal and alternative layers are protonated (to mimic neutral pH conditions reported in experiments).

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Do not press "go" button, otherwise it will overwrite dbt2 and dbt4 files and all the modification done above have to repeat.

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11) Write **file name** dbt1 (generated from step 9), **mdin file** (input file name for minimization, given by user), **mdout** (output file name), **rst** (in the space provided in text boxes.

12) PRESS "**OK**" button and then press "**sander minimization**" button.

Wait till minimization is done and when it is over at bottom of your terminal a message will be displayed "**File name dbt1 JOB DONE**".

Now onward, 11th and 12th step will repeat; just change the name of files. The last file will be dbt_term (for joining terminal residues).

13) Change **file name** as dbt2 and rest of mdin, mdout and rst names depend upon user. Repeat step 12. Similarly use dbt3 as a file name and repeat step 12. Then use dbt4 and repeat step 12 and finally, use dbt_term as file name and repeat step 12.

14) PRESS "**quit**" button. You can see USER.mol2 and load in *xleap*.

```
source leaprc.gaff
loadoff ppi-neu.lib
G5 = loadmol2 USER.mol2
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15) In USER.mol2 check number of atoms and the overall charge on the dendrimer. Total atoms should be 1466 and charge should be +84 for G5 PPI (DAB core) at neutral pH.