This is **PPM 3.0** version for running multiple PDB files, different from one implemented in PPM web server. It works in Linux environment. The distribution includes:

1. Source code files (.f) and **Makefile**
2. Amino acid residue library, **res.lib**
3. Examples of input files (**1membrane.inp** and **2membranes.inp**). Please upload test pdb files for these examples (1ucu, 6jnf, 5v5s and 5o66) to the same directory.

Compilation of executable. You will need **gfortran** compiler (use “make” command- see **Makefile**). The example of Makefile uses “fortran-9”. You may replace it to fortan version existing in your system (typically in directory ./usr/bin). Makefile will produce an executable named “immers”. There could be multiple warnings during compilation. Do not pay attention as far as the executable “immers” has been generated.

Running: immers<**1membrane.inp**>output\_file

The executable, library (**res.lib)** and all input and coordinate (.pdb) files are assumed to be located in the same working directory

**Input**

This PPM version allows two different types of input files: for single and multiple membranes (**1membrane.inp** and **2membranes.inp**, respectively). The number in the first line in each file (1 or 2) denotes the type of input.

**1membrane.inp** . In each line:

0 or 1 -“do not use” or “use” heteroatoms in the input PDB file, respectively (solvent molecules are always excluded).

MOM - type of membrane (see list of 3-letter codes for membranes below)

“in” or “out” means topology of N-terminus of first subunit included in the corresponding input PDB file

With this option, for every input pdb file, the program will selected automatically the flat or curved membrane boundaries, whichever had the lower calculated transfer energy.

**2membranes.inp** (see the example):

no – heteroatoms are not included

5v5s.pdb

2 – number of independent membranes of independent sections of the same membrane

GnO – type of first membrane (see list of 3-letter codes for membranes below)

Planar – if the membrane is prededfined to be only flat (use “Curved” if the possibility of formation of curved membrane needs to be tested)

in – topology

A,B,C – list of subunits located in the first membrane.

And so on.

**Output includes the following files:**

1. **Output .pdb coordinate files** with calculated membrane boundaries (e.g. **1abcout.pdb** if the name of input pdb file, as included in 1membrane.inp and 2membranes.inp, was 1abc.pdb).

2. Files named “**datapar1**, “**datapar2**” and “**datasub1**”. They include tables of calculated parameters (hydrophobic thickness/immersion depth, tilt angles, etc.) for the set of input PDB files.

**datapar1** includes parameters of proteins that were predefined or automatically assigned as having flat hydrophobic membrane boundaries. For example,

1gzm.pdb ;31.8; 1.6; 11; 0; -77.1;

means that hydrophobic thickness/depth is 31.8+-1.6 A, tilt angle is 11+-0 degrees, and transfer energy is -77.1. kcal/mol

Several output lines are included for each protein with several membranes.

**datapar2** provides parameters of proteins that were automatically assigned as having curved hydrophobic membrane boundaries. For example,

6b8h.pdb ;29.4; 80.; 2;-159.4;-152.4;

means that hydrophobic thickness/depth is 29.4 Å, the calculated radius of curvature id 80 Å, tilt angle is 2֯, transfer energy in curved membrane is -159.4 kcal/mol, and transfer energy in flat membrane would be -152.4 kcal/mol.

**datasub1** includes only proteins automatically identified as transmembrane by PPM. For example,

1gzm.pdb ;A;11; 1( 35- 60), 2( 72- 99), 3( 109- 133), 4( 151- 173), 5( 202- 224), 6( 253- 277), 7( 286- 308)

provides name of subunit, calculated tilt angle of the subunit and its TM segments

3. Several supplementary working files may be generated by the program in the same directory.

Please do not pay attention to “floating-point exceptions” message during execution.

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| **Membrane type** | **code** |
| Plasma membrane (mammalian) | PMm |
| Plasma membrane (plants) | PMp |
| Plasma membrane (Fungi) | PMf |
| ER (fungi) | Erf |
| ER (mammalian) | ERm |
| Golgi membrane | GOL |
| Lysosome membrane | LYS |
| Endosome membrane | END |
| Vacuole membrane | VAC |
| Outer mithochondrial membrane | MOM |
| Inner mitochondrial membrane | MIM |
| Thylakoid membrane (plants) | THp |
| Thylakoid membrane (bacteria) | THb |
| Gram-negative bacteria outer membrane | GnO |
| Gram-negative bacteria inner membrane | GnI |
| Gram-positive bacteria inner membrane | GpI |
| Archaebacteria cell membrane | ARC |
| Undefined membrane | empty space |
| DLPC (diC12:0 PC) bilayer | LPC |
| DMPC (diC14:0 PC) bilayer | MPC |
| DOPC (diC18:1∆9c PC) bilayer | OPC |
| DEuPC (diC22:1∆13c PC) bilayer | EPC |
| DPC(C12PC) micelle | MIC |

The capitalization (the lower or upper case) of letters is important.