User Manual

In order to run the program, the user should provide the GNOM (ATSAS suite) output file, and the electron or scattering length densities of the molecule, hydration layer and solvent in e/A^3 or 10^-10 cm^-2. Scattering data should be provided in (A^-1) units. During the run and at each temperature step, the program writes on the disk the current system configuration in two pdb files (model, hydration layer), and a log text file. After the program has finished an additional ASCII file containing the experimental data and the fit is written on the disk. All output filenames begin with the project name provided by the user. Output pdb files can be visualized with standard programs like pymol and may also serve as input to the analysis tools provided by the ATSAS suite.

By default the program runs in Dialog mode. However a parameter ASCII file can be provided as command line argument, containing user input in exactly the same order as asked in the Dialog mode. Each parameter should be placed in a newline in the input file.

During input, the user is asked to specify a loosness penalty weight that is related to the final compactness of the structure. The default value is a safe choice, but the parameter can be relaxed at will.

Running the program in Fast/Slow or Expert mode will affect the size of the beads and concequently their overall number during the annealing procedure. If the annealing takes too long, consider to increase the size of the beads.

The parameter 'knots' defines the number of the points of the curve that are fitted during the simulated annealing procedure.

The annealing schedule parameter affects the speed of the temperature decrease at each annealing step (Temp=anneal. schedule x Previous Temp).

Trials per bead at each temperature is set by default equal to 100. Larger values can be set in advanced mode.

In a successful run the goodness of fit Rf should be less than 10^-1 and loosness (loose) should be less than 0.02.

Note that if qmax is larger than about 0.2-0.25 A^-1 then by default the program attempts to subtract an appropriate small constant from the experimental data in order to force q^-4 Porod behavior at higher q. In order to compare results with

the program DAMMIN, the same constant may be subtracted for an identical considered q-range.

By setting the electron density of the hydration layer equal to zero, the program runs without hydration beads.

Example Program Dialog

```
 ************************************************************
**
 *** Ab-Initio low-resolution shape determination
of         ***
 *** hydrated biological molecules from SAXS/SANS
data       ***
 *** DENFERT version 2.0.0, June
2015                          ***
 *** Alexandros Koutsioubas (a) & Javier Perez
(b)           ***
 *** (a) JCNS outstation at MLZ, Forschungszentrum
Jülich    ***
 *** (b) Beamline SWING, Synchrotron
SOLEIL                   ***
 *** please reference: J. Appl. Cryst. (2013) 46,
1884       ***
 ************************************************************
**

run with command line argument -help for brief usage
instructions

FAQ

[F] fast, [S] slow, [A] advanced mode?.............
A
------ Choose run mode. Speed of executation is affected by
the number of dummy atoms.
[X] SAXS data or [N] SANS data?..................... X
------ Choose x-ray or neutron scattering mode.
Project name.......................................
Lysozyme
------ All output files will begin with the given project name
GNOM output filename...............................
gnolyz.out
------ output filename (with extension) of the program GNOM
(ATSAS suite)
Qmax (default=0.25A^1).............................
0.25
------ Maximum wave vector to be considered by the algorithm
Maximum Diameter - Dmax =   50.0 A .................
50
------ Maximum diameter of the molecule (Default value taken
```

```
from GNOM file)
Particle's electron density
(default=0.44e/A^3).........0.44
------ Particle electron density (default value for protein
molecules)
Solvent/buffer electron density (default=0.334e/A^3)..
0.334
------ Buffer electron density (default value for pure water)
Hydration layer contrast (default=0.03e/A^3).........
0.03
------ Hydration layer contrast (default values ~10% higher
than for bulk water)
Calculating contribution of internal inhomogeneities... Please
wait...
Constant to subtract from SAXS data = 0.359E-01 ...... 0.359E-
01
------ Constant Subtraction in order to obtain the equivalent
"shape curve"
Number of knots (default= 20).........................
20
------ Number of number of the points of the curve that are
fitted during the simulatedannealing procedure.
Dummy atom radius
(default=1.795A).....................1.795
------ Packing radius of dummy atoms (should be kept < 4.5A if
possible)
Initial Annealing Temperature (default=0.001).........
0.001
------ Temperature of the first annealing step
Annealing Schedule (default=0.90).....................
0.90
------ at each step T is the equal to the previous T times the
schedule factor
Penalty weight (default=0.600E-
02).................0.006
------ Penalty weight for particle's compactness
Trials per bead at each temperature (default=100) ....
100
------ Max trials per annealing step (increase for even better
convergence)


  Generating initial model...
 Parameters of Simulated Annealing run
 -------------------------------------
 Project name : Lysozyme
 Gnom SAXS Input file : gnolyz.out

 Solvent/buffer electron density (e/A^3) :       0.334
 Hydration layer contract (e/A^3) :              0.030
 Particle electron density (e/A^3) :             0.440
```

```
 Qmax (A^-1) :                                        0.250
 Maximum Diameter of the particle (A) :                50.0
 Bead packing radius (A) :                            1.795
 Number of experimental points :                        90
 Number of knots :                                      20
 Subtracted Constant (SAXS):                     0.359E-01
 Initial Annealing Temperature :                 0.100E-03
 Annealing Schedule :                                0.950
 Trials per bead at each temperature step :            100
 looseness penalty :                             0.600E-02
```

------ At the start of each run, a summary of parameter values is displayed

```
   Total number of beads of the initial model: 1505
 Initial scattering curve calculation...
 Rf^2 of the initial configuration: 0.108E+00

 Start of simulated annealing procedure:
 Initial annealing temperature: 0.100E-03
 ------------------------------------------------------------
---------
 Temp=0.100E-03 | Rf^2 =0.775E-01| Rf^2 + penalties =0.778E-01
| loose=0.407E-01
 success=   296/   714 | #beads=  713(   702) | Rg=  15.56A |
Volume =  23342A^3
 acceptance ratio high... skipping to lower annealing
temperature...
 ------------------------------------------------------------
---------
```

------ At each annealing step the model's parameters related the score function are diplayed together with the particle's volume and radius of gyration. Also the number of succesfull bead reconfigurations is given.

----- At the end of the annealing process, chi against the experimental curve is given and a file containing the final fit is generated.

Example of running the program in non-dialog mode

In order to run the simulated annealing session that is presented above without passing through the dialog procedure, a parameter file should be passed to the program as argument.

```
./denfert_linux64 <parameter filename>
```

the parameter file should have the following syntax

```
A
X
Lyz_test
gnolyz.out
0.25


0.44
0.334
0.027

1.8
0.001
0.90

100
```

an empty line in the input file means that the default value should be used.