

FRETnps Tools Documentation

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Starting FRETnpSTools

Run `FRETnpSTools.m` from MATLAB or run the executable `FRETnpSTools.exe`. The main window enables to start different subprograms (Figure 1).

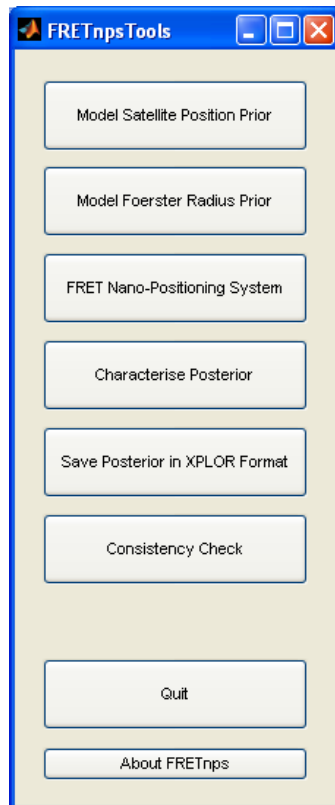


Figure 1 FRETnpSTools main window

- *Model Satellite Position Prior*
Calculate the volume accessible to a fluorescent dye which is attached to a macromolecule via a flexible linker. The volume is approximated with a linear combination of isotropic 3d-Gaussians.
- *Model Foerster Radius Prior*
Determines the Förster radius prior from the bulk fluorescence anisotropies of donor and acceptor dye and the isotropic dynamically averaged Förster radius ($\kappa^2 = 2/3$). A Monte-Carlo approach is used, and the simulated prior is approximated with a linear combination of image-charge like kernels.
- *FRET Nano-Positioning System*
Calculates the posterior probability density of the antenna dye molecule position from measured FRET efficiencies, Förster radii and satellite dye positions.
- *Characterise Posterior*
Summarises the antenna dye molecule position posterior in a few numbers: average position, standard deviations in principal directions and maximum position.
- *Save Posterior in XPLOR Format*
Convert a posterior density saved in binary format to a XPLOR density.
- *Consistency Check*
Analyse whether FRET measurements are consistent.

Notes:

- Several `FRETnpSTools` subprograms use the standard `MATLAB` output window for text output. If the executable was started, text output will be written into a shell window.
- All spatial information displayed or entered into the panels has the units of Å which is used in the PDB files.

Modelling Satellite Positions

After clicking on the *Model Satellite Position Prior* button in the FRETnpSTools main window the subprogram ModelSatellitePosition is started. In the ModelSatellitePosition window (Figure 2) one can model the prior information about the satellite dye molecule, given the background information – in this case the structure of the macro-molecule the satellite dye is attached to, the attachment point of the linker, length and diameter of the linker, and diameter of the satellite dye molecule.

In order to calculate the accessible volume perform the following steps

- 1) Load a Protein Data Bank (.pdb) file by clicking on the file menu (...) button in the *macromolecular structure* panel.
- 2) Specify the coordinates and the name of the atom to which the is attached (*attachment point* panel). Alternatively the atom name can be replaced by its Van der Waals radius.
- 3) Specify the length and diameter of the linker in the *linker* panel.
- 4) Enter the approximate satellite dye diameter in the *dye* panel. The satellite dye is treated as a sphere, and the linker is attached to its surface.
- 5) Click the *calculate density* button.

In the calculation it is assumed that the satellite dye molecule could be anywhere inside the accessible volume, therefore the prior is constant inside this volume and zero outside. The result of the calculation is a density evaluated on a 3-dimensional rectangular grid. The grid spacing is set to 1/5 of the smallest size of linker length, linker diameter and dye diameter.

The algorithm starts with all lattice points where a sphere with the same diameter as the linker can be put without clashing with the macro-molecule. Additionally, in order to allow the linker to leave the point of attachment, we allow all points that lie inside a sphere with the diameter of the linker centred at the attachment point of the linker to the macromolecule. From these lattice points the algorithm chooses those that can be connected to the lattice point located closest to the attachment point by a line with length smaller than the linker length + half of the dye diameter. However, the line must connect neighbouring (and accessible) lattice points only. Neighbouring lattice points are all points within third order neighbourhood. Finally, only the lattice points remain selected where a sphere with diameter equal to the diameter of the dye can be placed without causing a clash with the macromolecule. All lattice points that have survived the selection steps are given equal probability.

The calculation is finished after the accessible volume is displayed in the *position prior density* panel (Figure 2). The graph can be rotated with the mouse.

Optionally one can save the raw density in XPLOR format by clicking on the *save density as XPLOR file* button. The file extension is .posprior.raw.xplor. Please use density level 1 for displaying the 50% isosurface in a molecular viewing program.

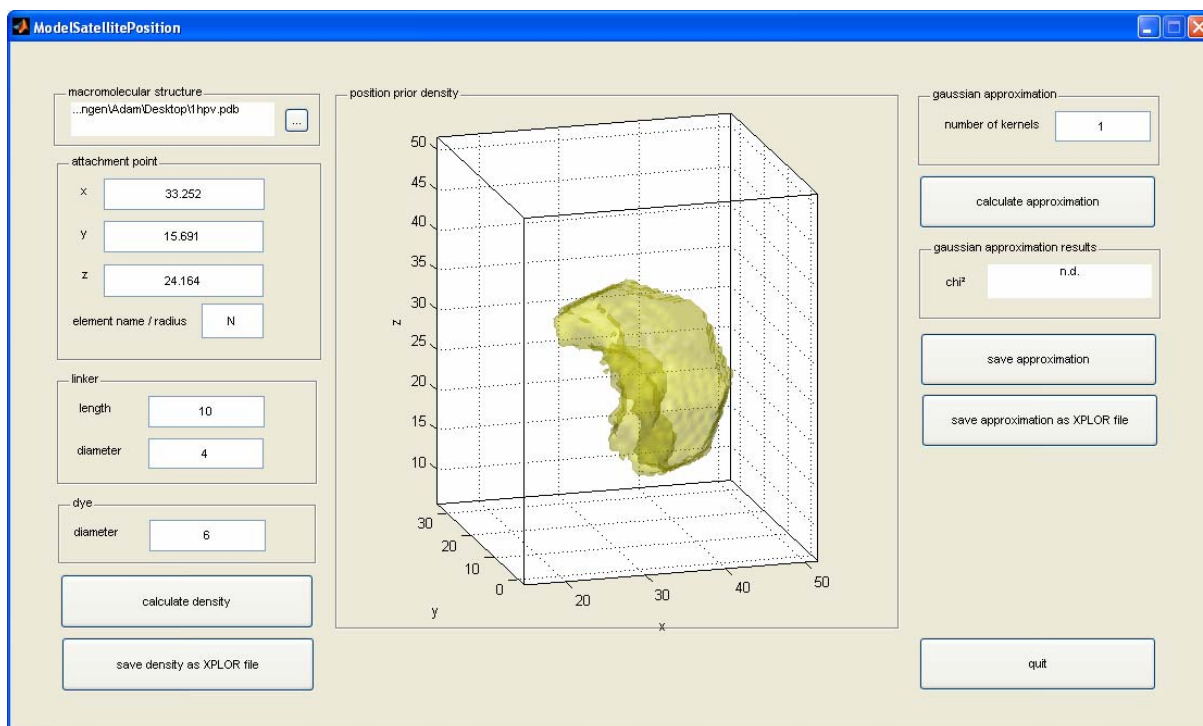


Figure 2 ModelSatellitePosition window with calculated raw density

In order to use the computed accessible volume in the current version of the NPS calculation it has to be approximated by a linear combination of isotropic Gaussians. Because of calculation speed issues all kernels have the identical width but different amplitude. The number of Kernels is set in the *gaussian approximation* panel. The more kernels are used the better the approximation will be, but also the time for calculating the approximation will increase (and of course also the time to calculate the posterior using the FRETnps subprogram).

The calculation of the approximation is started by clicking on the *calculate approximation* button.

After the calculation is finished, the χ^2 per grid voxel is displayed in the *gaussian approximation results* panel. The smaller χ^2 the better the approximation of the raw density. In the *position prior density* panel the calculated approximation is displayed as an isosurface at 50% of its maximum (Figure 3).

One can save the approximation by clicking the *save approximation* button. In this case a file is written that contains the approximation results, namely, the positions, amplitudes and widths of the Gaussian kernels. This file (extension `.posprior.mat`) can be read with the FRETnps subprogram.

Optionally one can save the approximated density in XPLOR format by clicking on the *save approximation as XPLOR file* button. See Table 1 for the saved confidence levels.

The *quit* button closes the ModelSatellitePosition window.

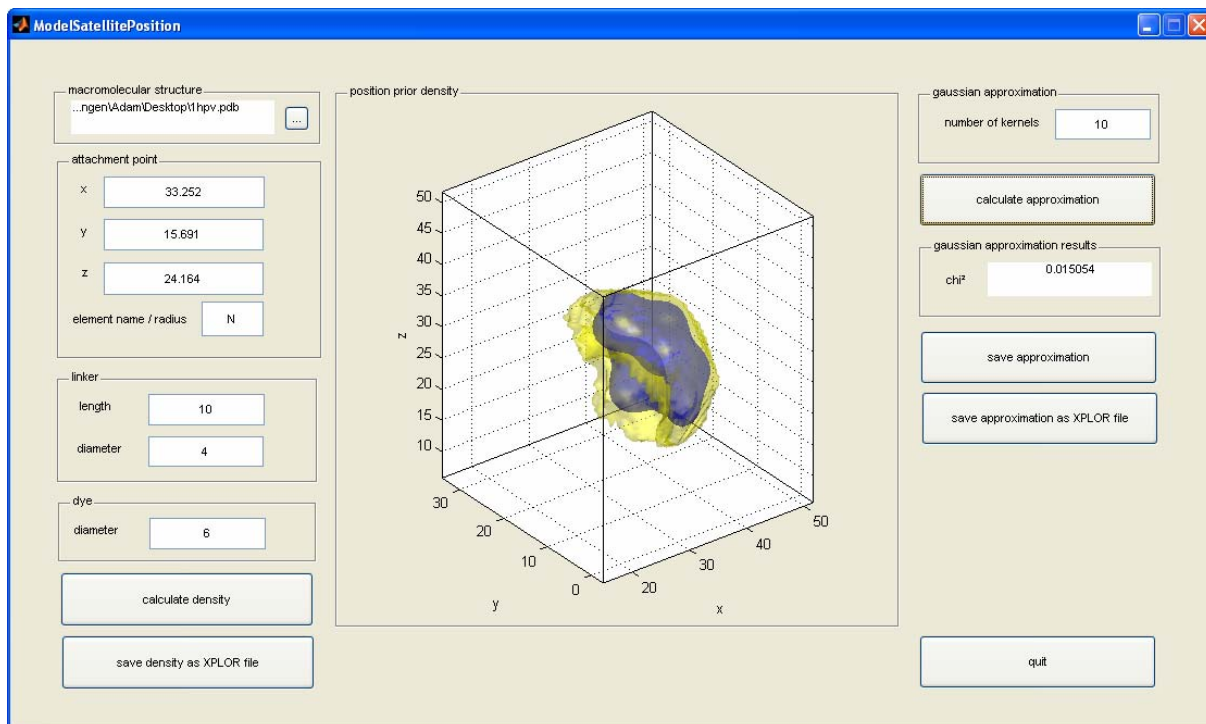


Figure 3 ModelSatellitePosition window with raw density and its Gaussian approximation

Modelling The Förster Radius

By clicking on the *Model Foerster Radius* button in the FRETnpSTools main window the ModelFoersterradiusPrior subprogram window is opened (Figure 4).

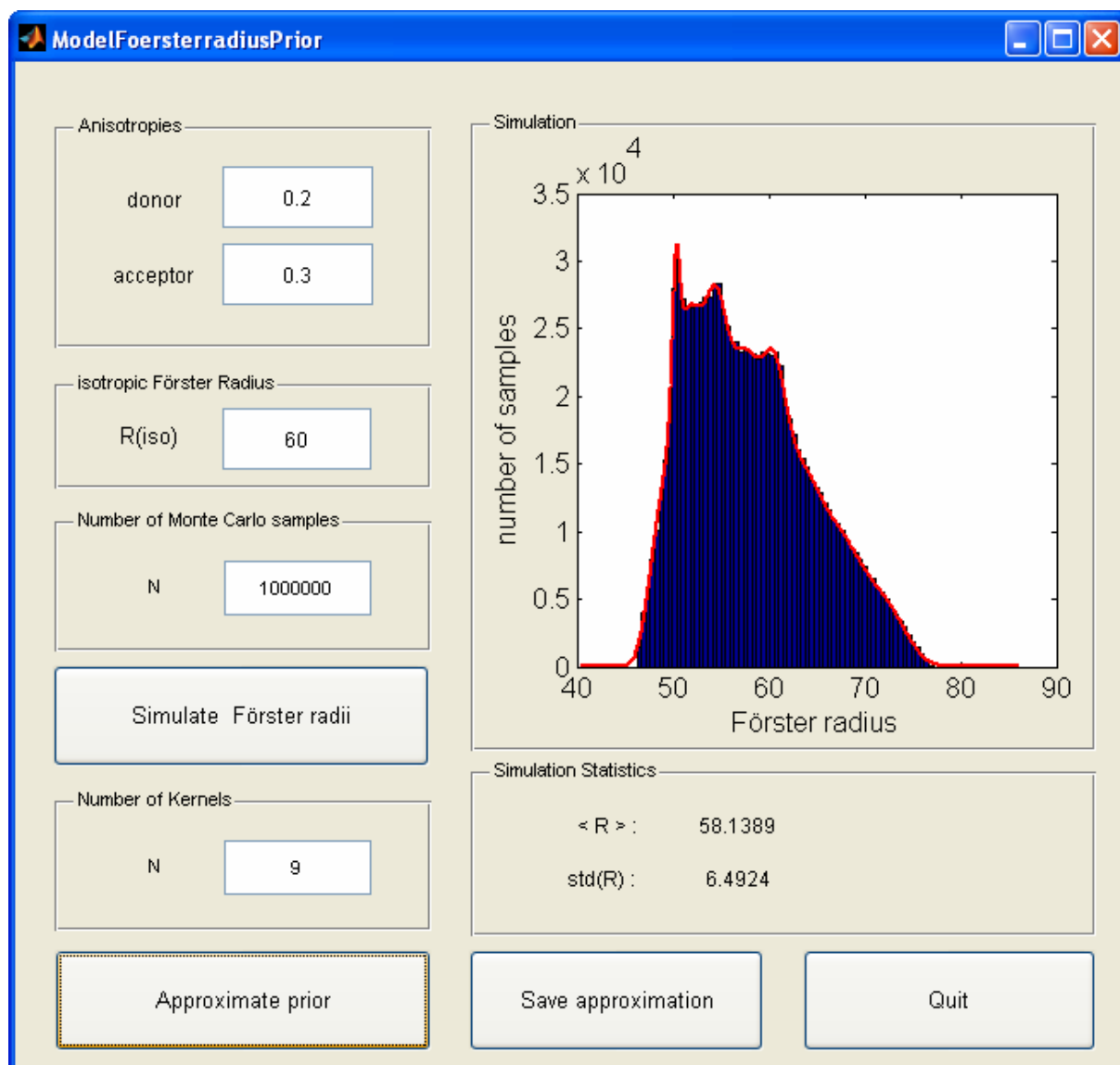


Figure 4 ModelFoersterradiusPrior window

The program simulates possible values of the Förster radius given the anisotropies of donor and acceptor dye and the Förster Radius in the isotropic ($\kappa^2 = 2/3$) case. It is assumed, that the average orientation of the transition dipoles of the dyes is not known.

The anisotropies, the isotropic Förster radius and the number of simulations can be typed into the panels *Anisotropies*, *isotropic Förster Radius* and *Number of Monte Carlo samples*, respectively. The button *Simulate Förster radii* starts the simulation.

In the *Number of Kernels* panel one sets the number of image-charge like kernels used in the approximation, and the *Approximate prior* button starts the fit algorithm.

The result of the simulation is displayed in the *Simulation* panel. The histogram depicts the simulated values of the Förster radius; the red line shows the approximation result.

The values for average and standard deviation of the simulated probability density are printed in the *Simulation Statistics* panel.

One can save the approximation by pressing the *Save approximation* button. In this case a file is written that contains the approximation results, namely, the positions, amplitudes and widths of the image-charge like kernels. This file (extension `.frdprior.mat`) can be read with the `FRETnps` subprogram.

FRET Nano-Positioning System

The FRETnps program is started from the FRETnpsTools main window by clicking on the *FRET Nano-Positioning System* button. The FRETnps window (Figure 5) consists of several elements:

- The *Measured Data* panel is used to enter/edit information about the FRET measurement, Förster radius and satellite position.
- The *Add*, *Overwrite* and *Remove* buttons are used to manipulate the currently edited FRET measurement in the *Measurements in use* list. Clicking on measurements in this list displays it in the *Measured Data* panel.
- The >>>> and <<<< buttons transfer measurements between the *Measurements in use* and *excluded Measurements* lists. In that way measurements can be excluded from and re-included into the analysis.
- In the *Spatial Prior* panel prior information about the antenna dye molecule position is entered.
- Data from all FRET measurements contained in the lists, as well as the spatial prior settings and the grid information can be saved / loaded with the *Save Measurements / Load Measurements* buttons. If a posterior calculation has been performed, the saved file also contains the calculated radial profiles.
- In the *Grid* panel the position of the box for evaluation of the antenna dye position density is defined, as well as the spacing between neighbouring voxels.
- The *Spatial Prior* panel contains information about the steric constraints of the antenna dye molecule.
- By clicking on the *Calculate Posterior* button FRETnps starts to calculate the position of the antenna dye molecule. The results are displayed in the *Posterior* and *Results* panels. One can change the displayed probability density iso-surface by entering a different value in the *(iso-surface PD)/(maximum PD)* edit box. The entered value sets the ratio of the iso-surface probability density and the maximum probability density.
- The *Save Posterior* button enables saving the marginalized posterior for further analysis. The *Save Confidence* button exports the posterior into a XPLOR density file that can be loaded by molecule viewing programs.
- Exit the FRETnps program pressing the *Quit* button.

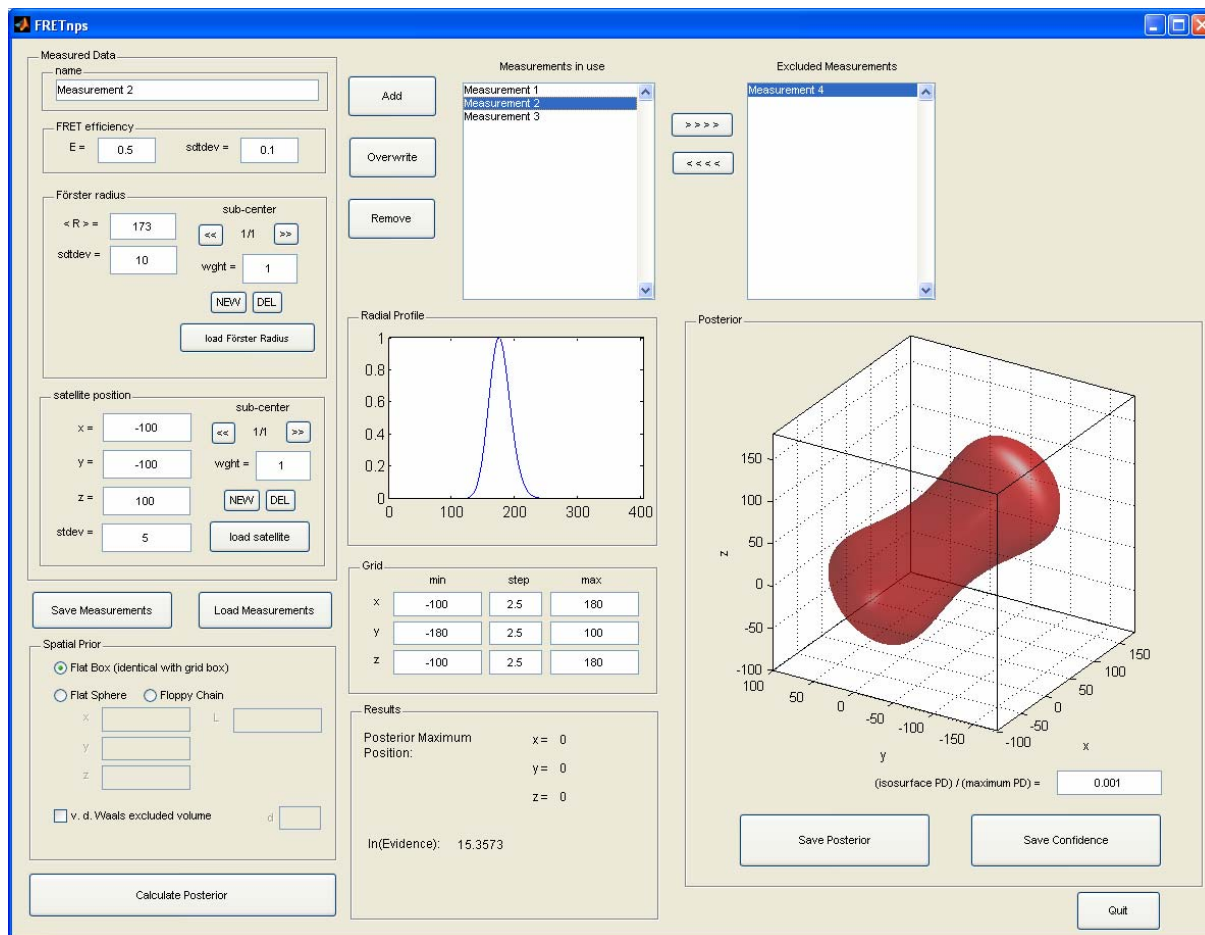


Figure 5 FRETnps window

Input of measured data

Measured data is entered into various edit boxes in the *Measured Data* panel:

- 1) Write the name of the measurement into the *name* subpanel.
- 2) Enter the measured FRET efficiency and its standard error into the *FRET efficiency* subpanel. Use values between 0 and 1, % units will lead to an error.
- 3) There are two ways of entering information about the Förster radius:
 - a. Click on the *load Förster radius* button in the *Förster radius* subpanel and select the file that contains a previously stored approximation (see *Save approximation* button in the *ModelFoersterRadiusPrior* subprogram).
 - b. Enter the positions of the Förster radius and its uncertainty manually. If more than one value is feasible, specify them by clicking on the *NEW* button in the *Förster radius* subpanel. Each sub-center can be also deleted using the *DEL* button. The << and >> buttons enable scrolling through the different feasible sub-center values. Note: You always have to specify the weight of the sub-center in the *wght* edit box and the standard deviation in the *stdv* edit box.
- 4) There are two ways of entering information about the satellite dye position:
 - a. Click on the *load satellite* button in the *satellite position* subpanel and select the file that contains a previously stored approximation (see *save approximation* button in the *ModelSatellitePosition* subprogram).

- b. Enter the positions of the satellite and its uncertainty manually. If more than one position is feasible, specify them by clicking on the *NEW* button in the satellite position subpanel. Each position can be also deleted using the *DEL* button. The << and >> buttons enable scrolling through the different feasible satellite positions. Note: You have always to specify the weight of the sub-satellite in the *wght* edit box.

5) Add the measurement to the *Measurements in use* list by clicking the *Add* button.

Repeat steps 1-5 for any new measurement.

In order to change some values select a measurement in the *Measurements in use* list (the data for this particular measurement will be displayed in the *Measured Data* panel) and edit the data. Accept the changes by clicking on the *Overwrite* button.

You can delete a measurement by selecting it in the *Measurements in use* list and pressing the *Remove* button.

If you just want to exclude a measurement from the analysis you can transfer it into the *excluded Measurements* list by pressing the >>>> button. The <<<< button does the reverse and transfers a measurement selected in the *excluded Measurements* list back into the *Measurements in use* list.

Specifying the evaluation grid

The probability density of the antenna dye position is evaluated on a 3-dimensional grid. In the *Grid* panel the extent of the grid and its spacing is set. You should make sure that the grid size is large enough not to cut substantially away probability density. Also the spacing should be fine enough such that the main maximum of the posterior is sampled often enough.

Specifying the Prior for the antenna dye position

In the *Spatial Prior* panel several constraints on the antenna dye molecule position can be set, that are used to calculate the prior probability density:

- 1) **Flat Box:** There is no further constraint besides the region (=box) given by the grid position and size. The prior is constant within this region, and 0 in the exterior of the box.
- 2) **Flat Sphere:** Additionally to the box the antenna dye position has to be closer than the distance L to a point (x,y,z) . Enter distance and position in the edit boxes in the *Spatial Prior* panel.
- 3) **Excluded Volume:** Besides the flat box or sphere the position of the antenna dye molecule can be excluded from the interior of a molecule saved in PDB format. Check the *v. d. Waals excluded volume* checkbox to enable this constraint and load a PDB file. Also the Van der Waals diameter of the antenna dye molecule has to be specified in the edit box d .
- 4) **Floppy Chain:** If the antenna dye is attached to the molecule of interest with a linker of known length, one can constraint it to be in the volume accessible to this chain. For this the *v. d. Waals excluded volume* checkbox must be checked. The attachment point of the linker is set in the x , y , z edit boxes, the length in the L edit box, the Van der Waals diameter of the chain is set in the d edit box.

Saving the input data

With the *Save Measurements* / *Load Measurements* buttons you can save / load all data contained in the *Measurements in use* and *Excluded Measurements* lists, as well as the data in the *Spatial Prior* and *Grid* panels, and – if calculated – the radial profiles. The files have the extension `.meas.mat`.

Calculating the antenna dye position & saving the results

The antenna dye position density is calculated by pressing the *Calculate Posterior* button. During calculation the radial profiles are calculated first, then the marginalized posterior density is evaluated at the grid points, finally the antenna dye position prior is applied. After finishing the calculation the posterior is displayed graphically in the *Posterior* panel. The plot can be rotated with the mouse. With a right-click on the plot one can choose between several views (x-y, x-z and y-z) from a context menu. This makes determining the approximate position in 3D easier.

You can browse through the radial profiles of the measurements in the *Measurements in use* list. The profile of the selected measurement will be displayed in the *Radial Profile* panel.

The natural logarithm of the evidence, i.e. the posterior integrated over all model parameters, is displayed in the *Results* panel. Note that the given value is normalized by the constant probability density of the antenna dye position prior. This ensures that the evidence is a measure for the overlap of the fuzzy spheres only and independent of the size of the antenna dye position prior.

The position of the grid point with highest probability density is also displayed in the *Results* panel.

All calculated results can be saved with the *Save Posterior* button. The saved files have the extension `.post.mat`.

With the *Save Confidence* button one (i) calculates confidence levels for the antenna dye position density, (ii) transforms the density so that the calculated confidence levels correspond to fixed values (Table 1), (iii) and saves it as XPLOR file (extension `.cd.xplor`).

The XPLOR files can be loaded into a molecular viewing program such as Pymol. In Pymol, first load the respective pdb file. Then select *load object* and select the appropriate XPLOR file. Select *actions, mesh* (or *surface*) and the desired *level* according to the values given in Table 1 (e.g. 1 for displaying the 68% confidence level). Note that displaying other values like the ones given in Table 1 is not meaningful.

Table 1 confidence levels and XPLOR density levels

confidence level	1	0.9545	0.68269	0.38292	0.1	0.05	0.01	0
XPLOR density value	0	0.5	1	2	3	4	5	6

The FRETnp program is exited with the *Quit* button.

Utility tools for processing posterior information

In this section some useful programs are explained that can be accessed from the FRETnpsTools main window.

Displaying mean and variance of the antenna dye position

After clicking on the *Posterior Mean & Variance* button you can load a posterior file (extension `.post.mat`) and calculate the average antenna dye position. The standard deviation of the antenna dye position is displayed for the three principal directions, and these directions are given using two angles: theta is the angle between the positive z-axis and the principal direction; phi is the angle between the positive x-axis and the projection of the principal direction on the x-y-plane.

Converting .post.mat files into XPLOR format

By clicking on Save Posterior in XPLOR format one can load a posterior file (extension `.post.mat`) and convert it into XPLOR format (extension `.cd.xplor`). See description for the *Save Confidence* button in the FRETnps program.

Checking the consistency of measurements

One should always check whether all FRET measurements are consistent with each other. By pushing the *Check Consistency* button a utility program is started that loads a posterior file (extension `.post.mat`) and displays two kinds of radial profiles, observed from the center of mass of each satellite position prior (called “measurement origin”).

One kind is the average radial profile of the posterior density (*Posterior Profile*); the average is taken over all directions from the measurement origin, all directions are treated with equal weight. The other kind of radial profile is the average radial profile of all fuzzy spheres of one measurement in the direction of the posterior density (*Measurement Profile*); here the averaging is twofold:

- i) The directional average of the radial profile of one fuzzy sphere (generated by one sub-satellite) from the measurement origin is calculated, the weight for a particular direction is given by the amount of posterior probability that lies in this direction.
- ii) The average of the radial profiles from i) over the different sub-satellites of a FRET measurement. In this case the weights for the individual sub-satellites are used.

If most radial profiles are overlapping well (this is subjective) then all measurements are consistent with each other. Otherwise, if some or all radial profiles are overlapping badly, the measurements are inconsistent. Note that also the profiles of excluded measurements are calculated. In this way you can check the influence of specific FRET measurements on the analysis result.