



# PeldorFit 2019

## User Manual

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# 1 Introduction

## 1.1 What is it for?

The program PeldorFit was developed for the analysis of orientation-selective Pulsed ELection-electron Double Resonance (PELDOR<sup>[1]</sup> or DEER<sup>[2]</sup>) data. PELDOR data is called orientation-selective if it depends on the relative orientation of the electron spin centers. Thus, orientation-selective PELDOR data encodes information about the distance and relative orientation between the electron spin centers. In order to extract this information, several PELDOR time traces are acquired with the pump and probe pulses applied to different spectral components of the electron spin centers and then analyzed all together. This analysis is the subject of PeldorFit.

The idea underlying PeldorFit is the fitting of PELDOR time traces using a geometric model of the spin system. The geometric model consists of a defined set of parameters, which describe the distance and relative orientation between the electron spin centers. A detailed description of these parameters is given in Chapter 1.2. The geometric parameters are essentially the fitting parameters of PeldorFit. In addition, the exchange coupling between the election spin centers and the pump pulse efficiency can be used as the fitting parameters. During the fitting process, the values of all fitting parameters are optimized by means of a genetic algorithm until they minimize the root-mean-square-deviation between the experimental and simulated PELDOR time traces. After the fitting, the precision of the optimized fitting parameters is evaluated by calculating error plots. More details about the optimization procedure can be found in Ref. [3].

## 1.2 Geometric models of a spin system

Since there is an infinite number of possible spin system geometries, it is impossible to describe all spin systems with a single geometric model. Therefore, PeldorFit is restricted to three particular cases:

- 1) a two-spin system with a unimodal distance and angle distributions (Model 1),
- 2) a two-spin system with a bimodal distance and angle distributions (Model 2),
- 3) a three-spin system with a unimodal distance and angle distributions (Model 3).

Each of these cases has its own model, which is described in the following.

### Model 1

The geometric arrangement used within model 1 is shown in Figure 1.1. It consists of two coordinate frames associated with the  $g$ -tensor axes of the two spin centers, named here spin A and spin B. Both spins are assumed to be point objects, i.e., spin delocalization is neglected. The reference coordinate system of the model is set to be coincident with the  $g$ -tensor axes of spin A. The orientation of the  $g$ -tensor axes of spin B is given by three Euler angles ( $\alpha, \beta, \gamma$ ) in accordance with the  $z$ - $x'$ - $z''$  convention. The vector connecting the two spins is described by three spherical coordinates: length  $r$ , polar angle  $\xi$ , and azimuthal angle  $\varphi$ . Thus, the relative orientation of the spins is described by six parameters ( $r, \xi, \varphi, \alpha, \beta, \gamma$ ). In order to account for conformational flexibility, all six parameters are allowed to have either a uniform distribution or a Gaussian distribution. In both cases, the distributions are described by two parameters, a mean value and a width (Figure 1.2). In the case of a Gaussian distribution, a standard deviation is used as the width parameter. Thus, in total 12 parameters are used to describe a non-rigid spin pair, ( $\langle r \rangle, \Delta r, \langle \xi \rangle, \Delta \xi, \langle \varphi \rangle, \Delta \varphi, \langle \alpha \rangle, \Delta \alpha, \langle \beta \rangle, \Delta \beta, \langle \gamma \rangle, \Delta \gamma$ ). Note that the model neglects the correlation between the values of individual geometric parameters. Strictly speaking, this assumption does not necessary hold for every molecular system. However, the correlation between individual geometric parameters is usually unknown and, therefore, is omitted in the PeldorFit analysis.

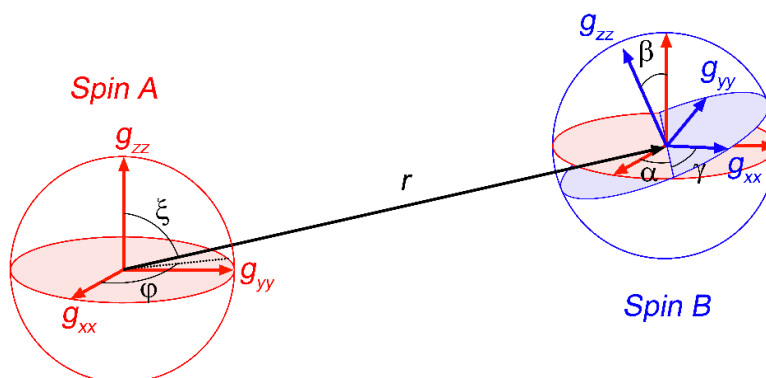
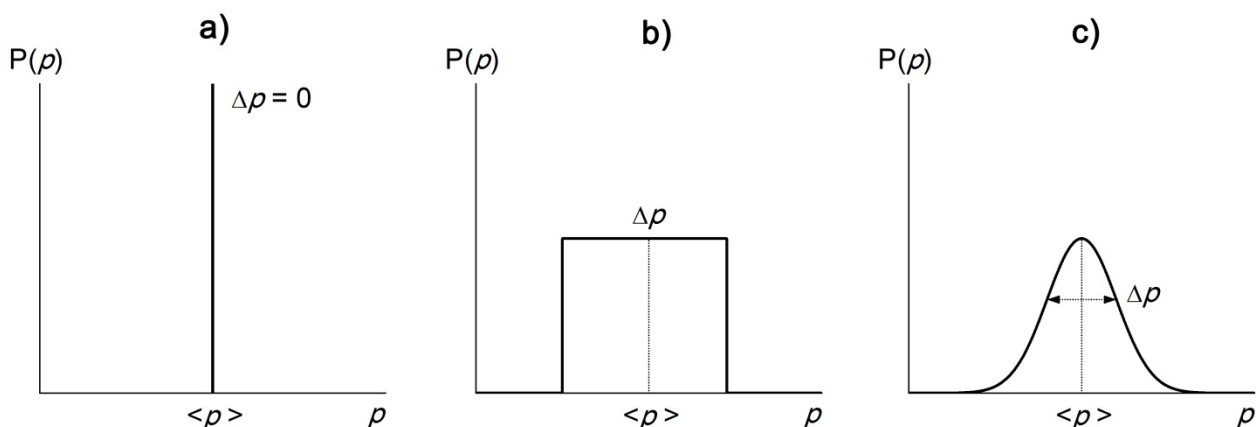


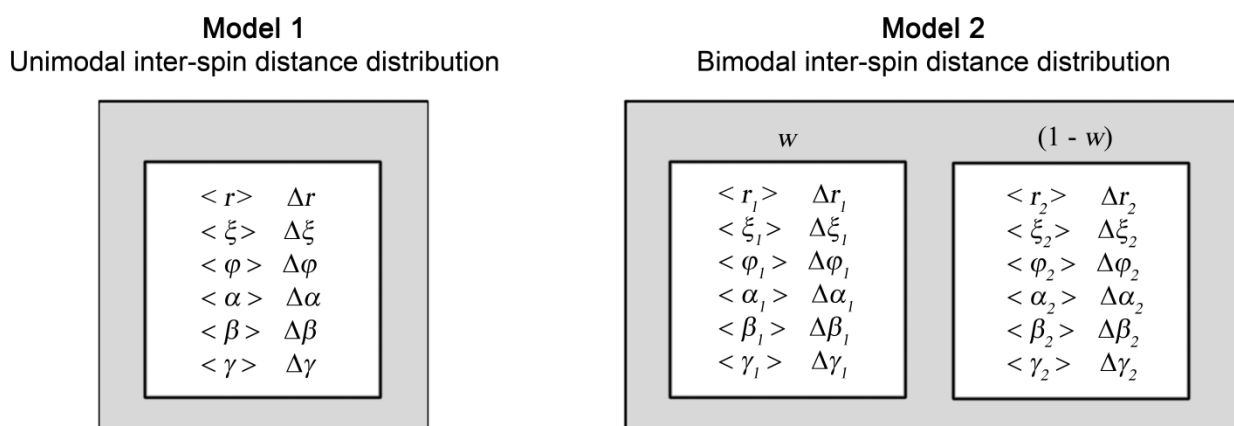
Figure 1.1. A PeldorFit model of a two-spin system.



**Figure 1.2.** The types of distributions used for the PeldorFit parameters. a) A single value  $\langle p \rangle$ . b) Uniform distribution with the mean value  $\langle p \rangle$  and the width  $\Delta p$ . c) Normal distribution with the mean value  $\langle p \rangle$  and the standard deviation  $\Delta p$ . Here,  $p$  denotes one of six geometric parameters ( $r, \xi, \varphi, \alpha, \beta, \gamma$ ).

### Model 2

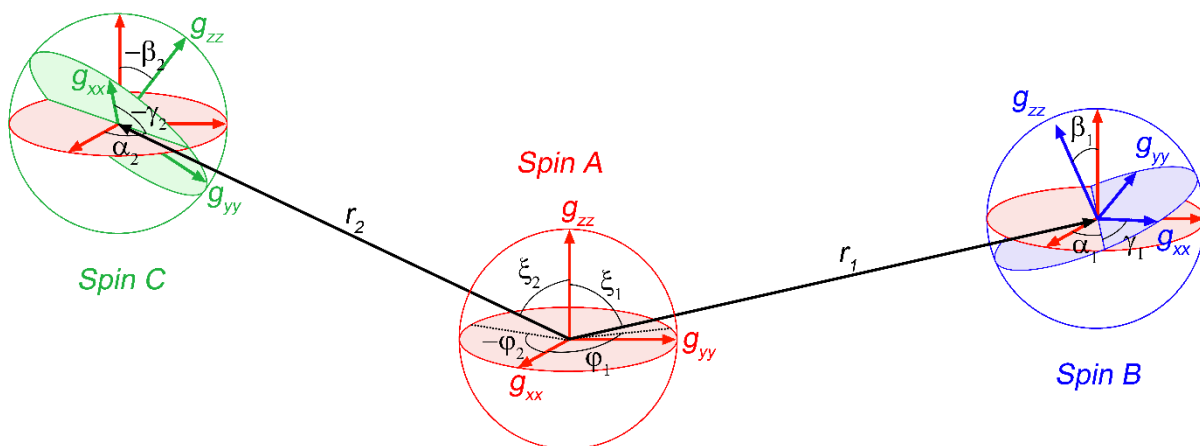
Model 2 is an extension to Model 1 in that it allows for a bimodal distribution of the distance and the angles. This model is useful if a spin-carrying molecule has two different conformations or if one of the spin centers, e.g. a spin label adopts preferentially two different conformations. In order to describe two different conformations of a spin pair, Model 1 needs to be extended by introducing a second set of geometric parameters  $r, \xi, \varphi, \alpha, \beta$ , and  $\gamma$ . Thus, Model 2 consist of twelve geometric parameters, ( $r_1, \xi_1, \varphi_1, \alpha_1, \beta_1, \gamma_1, r_2, \xi_2, \varphi_2, \alpha_2, \beta_2, \gamma_2$ ), and one additional parameter  $w$ , which describes the relative weight of both conformations (Figure 1.3). In complete analogy to the Model 1, each of the geometric parameters of Model 2 can have a single value, a uniform distribution, or a normal distribution (Figure 1.2). Thus, the total number of parameters can add up to 25.



**Figure 1.3.** The set of geometric parameters used by Model 1 and Model 2.

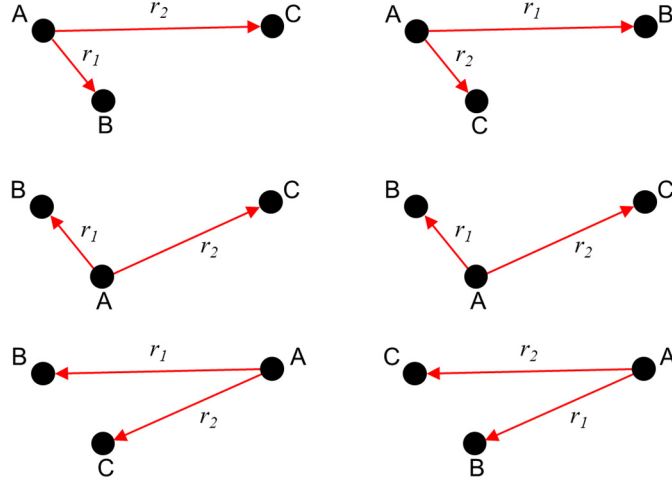
### Model 3

PELDOR studies are concerned not only with two-spin systems but also to systems with more than two spins. However, determination of distance and angular distributions from PELDOR data of multi-spin systems becomes increasingly difficult as the number of spins *per* molecule increase. Therefore, PeldorFit is restricted only to the case of three-spin systems. The geometric model for a three-spin system is depicted in Figure 1.4. This model uses the same set of parameters and assumptions as Model 1. The only difference to Model 1 is the third spin, called here spin C. The reference coordinate system of the model is set again to be coincident with the  $g$ -tensor axes of spin A. The position and orientation of spins B and C are described by two sets of parameters,  $(r_1, \xi_1, \varphi_1, \alpha_1, \beta_1, \gamma_1)$  and  $(r_2, \xi_2, \varphi_2, \alpha_2, \beta_2, \gamma_2)$ , respectively. As before, each of these parameters can have either a single value, a uniform distribution, or a normal distribution (Figure 1.2). As a result, the model includes up to 24 parameters.



**Figure 1.4.** A PeldorFit model of a three-spin system.

It is important to note that the values of the geometric parameters of Model 3 may depend on the assignment of spin centers to spins A, B, and C, respectively. This can be readily demonstrated on the example of three spin centers that are located at different corners of an irregular triangle. As can be seen from Figure 1.5, the same geometry of the three-spin system can be described in six different ways using the same geometric parameters (only  $r_1$  and  $r_2$  are shown). PeldorFit provides only one of those geometries, assuming that other geometries can be readily deduced when needed.



**Figure 1.5.** Different ways of assignment of three spin centers to spins A, B, and C, respectively.

### 1.3 Symmetry considerations

For simplicity, symmetry considerations will be discussed with regard to Model 1. Note that the same considerations are also valid for the more complex Models 2 and 3. Depending on the symmetry of the magnetic tensors ( $g$ - and  $A$ -tensors) of spins A and B, all five angular parameters ( $\xi$ ,  $\varphi$ ,  $\alpha$ ,  $\beta$ ,  $\gamma$ ) or only a subset of them are required to simulate PELDOR time traces. A summary of different spin center's symmetries and corresponding angular parameters is given in Table 1.1. For example, if the magnetic tensors of both spins are rhombic, the full set of angles is required. In contrast, three angles are sufficient if the magnetic tensors of both spins are axial. In this case, the angles  $\varphi$  and  $\gamma$  can be excluded from consideration. Thus, if the magnetic tensors of spins A and B are axial or isotropic, the number of fitting parameters in PeldorFit can be reduced.

**Table 1.1.** Number and ranges of the angular parameters  $\xi$ ,  $\varphi$ ,  $\alpha$ ,  $\beta$ , and  $\gamma$  required to simulate the PELDOR time traces in the cases of isotropic, axial, and rhombic spins A and B. Adapted from Ref. [3].

Spin A	Spin B	$\xi$	$\varphi$	$\alpha$	$\beta$	$\gamma$
axial	isotropic	$[0^\circ, 90^\circ]$	-	-	-	-
axial	axial	$[0^\circ, 90^\circ]$	-	$[0^\circ, 180^\circ]$	$[0^\circ, 90^\circ]$	-
rhombic	isotropic	$[0^\circ, 90^\circ]$	$[0^\circ, 180^\circ]$	-	-	-
rhombic	axial	$[0^\circ, 90^\circ]$	$[0^\circ, 180^\circ]$	$[0^\circ, 180^\circ]$	$[0^\circ, 90^\circ]$	-
rhombic	rhombic	$[0^\circ, 90^\circ]$	$[0^\circ, 180^\circ]$	$[0^\circ, 180^\circ]$	$[0^\circ, 90^\circ]$	$[0^\circ, 180^\circ]$

In addition, due to the inversion symmetry of the magnetic tensors, there is a number of relative orientations of spin centers, which provide identical PELDOR time traces. If each spin is considered as a point object, a  $180^\circ$  rotation of one of its  $g$ -tensor axes does not influence the shape of PELDOR time traces. In total 16 combinations of such axes rotations are possible for a two-spin system. Thus, each set of angles  $(\xi, \varphi, \alpha, \beta, \gamma)$  has 15 symmetry-related sets of angles, which cannot be distinguished by fitting PELDOR data. In order to account for this, PeldorFit calculates all 16 symmetry-related sets of angles in every fitting run. Finally, if both spins are spectroscopically identical, e.g., if they are both nitroxides, there is an additional uncertainty in assignment of these spins to spin A and spin B. Depending on which of the identical spins is assigned to spin A, two different sets of angles  $(\xi, \varphi, \alpha, \beta, \gamma)$  can be obtained. These sets of angles are however inter-convertible, when exchanging spin A with spin B and *vice versa*. Therefore, PeldorFit calculates only one of those sets.

#### 1.4 Technical information

PeldorFit is a console C++ application. The source code of the program can be found at

<https://github.com/dinarabdullin/PeldorFit2019>

The Linux and Windows executables of the program can be found at

<https://github.com/dinarabdullin/PeldorFit2019/releases>

The program is free of charge and can be distributed under GNU General Public License.

To speed up the calculations, it is recommended to run the program on a hardware with a large number of CPUs. All examples in this manual were calculated using a 64-core workstation from sys-Gen GmbH with 2.3 GHz processor frequency and 132 GB RAM.



## 2 Installation

Download the zip-archive “PeldorFit2019.zip” from the web page

<https://github.com/dinarabdullin/PeldorFit2019/releases>

and unzip it to a directory where the program will be stored. The archive contains two folders with the compiled executable files for Linux and Windows. That’s all!

## 3 Running the program

1. Open Terminal (Linux) or Command Prompt (Windows).
2. Go to the directory in which the PeldorFit executable file is stored:  
`cd ../PeldorFit2019/Linux` (for Linux)  
`cd ../PeldorFit2019/Windows` (for Windows)
3. Set the permission properties (only for Linux):  
`chmod 755 peldorfit`
4. Prepare a configuration file (Chapter 4) and run the program by the following command:  
`sh PeldorFit.sh ../config.cfg` (for Linux)  
`PeldorFit.exe ../config.cfg` (for Windows)

Examples for configuration files can be found in the [Examples](#) folder of the program. The description of these examples is given in Chapter 6.

## 4 Configuration file

A PeldorFit configuration file contains all input parameters of the program. It has a usual ASCII data format and a .cfg extension. The configuration file can be created and edited with common text editors, such as [Notepad++](#). Since the number of input parameters in a single configuration file is typically quite large, it is highly recommended to use the configuration files from [Examples](#) as a template to build your own configuration file. This will save time and reduce the probability of errors.

Each PeldorFit configuration file consists of five main parts:

- 1) experimental data and settings,
- 2) parameters of the spin system,
- 3) optimization parameters,
- 4) optimization settings,
- 5) output settings.

Each of these parts is described in detail below. Note that comment lines in the configuration file are indicated by symbols # or //. The comment lines, as well as empty lines, are ignored by PeldorFit. Here is an example of comment lines:

---

#----- PeldorFit2018 Configuration File -----

---

```
# Experimental parameters
#-----
// filename      : name of the data file
// detPiLength   : length of the detection pi-pulse (ns)
// detPiHalfLength: length of the detection pi/2-pulse (ns)
// pumpPiLength  : length of the pump pi-pulse (ns)
// detFreq       : detection frequency (GHz)
// pumpFreq      : pump frequency (GHz)
// magnField     : magnetic field (T)
```

---

## 4.1 Experimental data and settings

The first part of the configuration file specifies PELDOR data and corresponding experimental settings. This information is combined together in a list called [experimentals](#):

Taken from Examples/unimodal\_nitroxide\_nitroxide/bisnitroxide\_config.cfg

---

```
experimentals = (
    {filename = "Examples/unimodal_nitroxide_nitroxide/bisnitroxide_XX.dat";
    detPiLength = 14;
    detPiHalfLength = 7;
    pumpPiLength = 14;
    detFreq = 93.999600;
    pumpFreq = 93.930000;
    magnField = 3.3415; },

    {filename = "Examples/unimodal_nitroxide_nitroxide/bisnitroxide_YY.dat";
    detPiLength = 14;
    detPiHalfLength = 7;
    pumpPiLength = 14;
    detFreq = 93.999600;
    pumpFreq = 93.930000;
    magnField = 3.3443; },

    ...
);
```

---

In this list, information about each individual PELDOR experiment is enclosed in curly brackets. Within the curly brackets, the following parameters have to be defined:

<a href="#">filename</a>	A path to a data file which contains a background-subtracted PELDOR time trace. The first column of this file should contain time points in microseconds. The second column should contain the values of a signal normalized to 1.
<a href="#">detPiLength</a>	The length of a detection $\pi$ -pulse in [ns].
<a href="#">detPiHalfLength</a>	The length of a detection $\pi/2$ -pulse in [ns].
<a href="#">pumpPiLength</a>	The length of a pump pulse in [ns].
<a href="#">detFreq</a>	The frequency of detection pulses in [GHz].
<a href="#">pumpFreq</a>	The frequency of a pump pulse in [GHz].

**magnField**            The value of a magnetic field in [T].

Note that all commas and semicolons appearing in **experimentals** are important for reading out the configuration file by PeldorFit.

## 4.2 Parameters of the spin system

The second part of the configuration file specifies the spectroscopic parameters of the spin centers. First, one has to set the number of spins to either 2 or 3. In case of a three-spin system, one has to specify additionally whether the multi-spin effects<sup>[4,5]</sup> need to be taken into account or not. The multi-spin effects can be usually neglected when the modulation depths of all PELDOR time traces are low.<sup>[6]</sup>

*Taken from Examples/unimodal\_nitroxide\_nitroxide/bisnitroxide\_config.cfg*

---

```
nSpins = 2;  
multiSpinEffects = 0;
```

---

Here,

**nSpins**            A number of spin centers. It can be either 2 or 3.

**multiSpinEffects**    If set to 0, the multi-spin effects will be neglected.

                    If set to 1, the multi-spin effects will be taken into account for three-spin systems.

Next, the spectroscopic parameters of each of the spins are defined. For definiteness, the spins are denoted as **spinA**, **spinB**, and **spinC** (required only for three-spin systems):

*Taken from Examples/unimodal\_nitroxide\_nitroxide/bisnitroxide\_config.cfg*

---

```
spinA:  
{  
    g = [2.0104, 2.0073, 2.0033];  
    gStrain = [0.0004, 0.0003, 0.0001];  
    n = [1];  
    l = [1.0];  
    A = [8.0, 6.0, 96.0];  
    AStrain = [0.0, 0.0, 12.0];  
    lwpp = 22.4;  
};  
  
spinB:  
{  
    g = [2.0104, 2.0073, 2.0033];  
    gStrain = [0.0004, 0.0003, 0.0001];  
    n = [1];  
    l = [1.0];  
    A = [8.0, 6.0, 96.0];  
    AStrain = [0.0, 0.0, 12.0];  
    lwpp = 22.4;  
};
```

---

PeldorFit assumes that the resonance frequencies of spin centers are determined by a Zeeman interaction, a hyperfine interaction of electron spins and nuclear spins, and an inhomogeneous line broadening. Moreover, it is assumed that the  $g$  and hyperfine interaction ( $A$ ) tensors are collinear and, thus, have the same principal axes. The unresolved anisotropy of  $g$  and hyperfine interaction ( $A$ ) tensors can be taken into account via  $g$ -strain and  $A$ -strain, respectively. The naming of spectroscopic parameters is consistent with the program EasySpin ([www.easyspin.org](http://www.easyspin.org)):

- $g$**   $g$ -factor. It should always to contain 3 components:  
 $g = [g_{xx}, g_{yy}, g_{zz}]$  in the case of orthorhombic  $g$ -factor;  
 $g = [g_{\perp}, g_{\perp}, g_{\parallel}]$  in the case of axial  $g$ -factor;  
 $g = [g_{iso}, g_{iso}, g_{iso}]$  in the case of isotropic  $g$ -factor.
- $gStrain$**   $g$ -strain (the unresolved anisotropy of the  $g$ -factor). It should always to contain 3 components:  $gStrain = [\Delta g_{xx}, \Delta g_{yy}, \Delta g_{zz}]$ . If not needed, the notation  $gStrain = []$  should be used.
- $n$**  A number of equivalent magnetic nuclei coupled to electron spin. Up to two different types of equivalent nuclei can be used:  
 $n = []$  : no nuclear spins are coupled to the electron spin.  
 $n = [n_1]$  ( $n_1 > 0$ ) :  $n_1$  nuclear spins with  $I_1$  and  $A = [A_{1xx}, A_{1yy}, A_{1zz}]$  are coupled to the electron spin.  
 $n = [n_1, n_2]$  ( $n_1, n_2 > 0$ ) :  $n_1$  nuclear spins with  $I_1$  and  $A_1 = [A_{1xx}, A_{1yy}, A_{1zz}]$  and  $n_2$  nuclear spins with  $I_2$  and  $A_2 = [A_{2xx}, A_{2yy}, A_{2zz}]$  are coupled to the electron spin.
- $I$**  A nuclear quantum number(s) of a nucleus (nuclei) coupled to an electron spin. This parameter is connected to  $n$ :  
 $I = []$  if  $n = []$ ;  
 $I = [I_1]$  if  $n = [n_1]$   
 $I = [I_1, I_2]$  if  $n = [n_1, n_2]$
- $A$**   $A$ -tensor in [MHz]. It should consist of 0, 3 or 6 components in case of 0, 1 or 2 two types of equivalent nuclei, correspondingly. This parameter is connected to  $n$ :  
 $A = []$  if  $n = []$ ;  
 $A = [A_{1xx}, A_{1yy}, A_{1zz}]$  if  $n = [n_1]$   
 $A = [A_{1xx}, A_{1yy}, A_{1zz}, A_{2xx}, A_{2yy}, A_{2zz}]$  if  $n = [n_1, n_2]$
- $AStrain$**   $A$ -strain in [MHz] (the unresolved anisotropy of the  $A$ -tensor). It applies only to the first specified sort of nuclei and should contain 3 components:  $AStrain = [\Delta A_{1xx}, \Delta A_{1yy}, \Delta A_{1zz}]$ . If not needed, the notation  $AStrain = []$  should be used.
- $lwpp$**  A peak-to-peak linewidth in [MHz].

### 4.3 Optimization parameters

The third part of the configuration file specifies which geometric model of a spin system (see Chapter 1.2) will be used and which parameters of the chosen model will to be optimized. For this information, the configuration file contains a list called [parameters](#):

*Taken from Examples/unimodal\_nitroxide\_nitroxide/bisnitroxide\_config.cfg*

```
parameters = (  
  {opt = 1; mode = 1; range = [ 1.50, 2.50], value = 0.0}, // Parameter 1 : r1 mean  
  {opt = 1; mode = 1; range = [ 0.00, 0.10], value = 0.0}, // Parameter 2 : r1 width  
  {opt = 1; mode = 0; range = [ 0.0, 90.0], value = 0.0}, // Parameter 3 : xi1 mean  
  {opt = 1; mode = 0; range = [ 0.0, 90.0], value = 0.0}, // Parameter 4 : x1 width  
  {opt = 1; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 5 : phi1 mean  
  {opt = 1; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 6 : phi1 width  
  {opt = 1; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 7 : alpha1 mean  
  {opt = 1; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 8 : alpha1 width  
  {opt = 1; mode = 0; range = [ 0.0, 90.0], value = 0.0}, // Parameter 9 : beta1 mean  
  {opt = 1; mode = 0; range = [ 0.0, 90.0], value = 0.0}, // Parameter 10: beta1 width  
  {opt = 1; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 11: gamma1 mean  
  {opt = 1; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 12: gamma1 width  
  {opt = 0; mode = 1; range = [ 1.50, 2.50], value = 0.0}, // Parameter 13: r2 mean  
  {opt = 0; mode = 1; range = [ 0.00, 0.10], value = 0.0}, // Parameter 14: r2 width  
  {opt = 0; mode = 0; range = [ 0.0, 90.0], value = 0.0}, // Parameter 15: xi2 mean  
  {opt = 0; mode = 0; range = [ 0.0, 90.0], value = 0.0}, // Parameter 16: xi2 width  
  {opt = 0; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 17: phi2 mean  
  {opt = 0; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 18: phi2 width  
  {opt = 0; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 19: alpha2 mean  
  {opt = 0; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 20: alpha2 width  
  {opt = 0; mode = 0; range = [ 0.0, 90.0], value = 0.0}, // Parameter 21: beta2 mean  
  {opt = 0; mode = 0; range = [ 0.0, 90.0], value = 0.0}, // Parameter 22: beta2 width  
  {opt = 0; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 23: gamma2 mean  
  {opt = 0; mode = 0; range = [ 0.0, 180.0], value = 0.0}, // Parameter 24: gamma2 width  
  {opt = 0; mode = 0; range = [ 0.00, 1.00], value = 0.0}, // Parameter 25: ratio between r1 and r2  
  {opt = 0; mode = 0; range = [ 0.00, 5.00], value = 0.0}, // Parameter 26: J mean  
  {opt = 0; mode = 0; range = [ 0.00, 5.00], value = 0.0}, // Parameter 27: J width  
  {opt = 1; mode = 0; range = [ 0.85, 0.95], value = 0.0} // Parameter 28: scale factor for mod. depth  
);
```

In this list, each text line corresponds to one of the generalized parameters of all three available geometric models. For definiteness, all generalized parameters are numbered in a pre-defined manner and enter the [parameters](#) list in accordance to their serial number (the user cannot change the order). Table 4.1 lists the serial numbers of all generalized parameters and provides the description of each parameter.

Let's briefly go through the definitions of all parameters. The parameters no. 1-12 correspond to the mean values and distribution widths of  $r_1$ ,  $\xi_1$ ,  $\varphi_1$ ,  $\alpha_1$ ,  $\beta_1$ , and  $\gamma_1$ . These geometric parameters are used by all three geometric models of PeldorFit (Chapter 1.2). The parameters no. 13-24 correspond to the mean values and distribution widths of  $r_2$ ,  $\xi_2$ ,  $\varphi_2$ ,  $\alpha_2$ ,  $\beta_2$ , and  $\gamma_2$ . These parameters are relevant only for Models 2 and 3 (Chapter 1.2). The parameter no. 25 is needed only for Model 2 and describes the weight of the first conformation relative to the second conformation ( $w$  in Figure 1.3).



**Table 4.1.** The generalized parameters / optimization parameters of PeldorFit.

No. of parameter	Parameter description <sup>a</sup>	Units	Ranges	Modes <sup>b</sup>
1	$\langle r_1 \rangle$	nm	Any reasonable ranges inside [1.5, 8.0]	
2	$\Delta r_1$	nm	Any reasonable ranges inside [1.5, 8.0]	
3	$\langle \xi_1 \rangle$	degrees	[0, 90]	
4	$\Delta \xi_1$	degrees	[0, 90] for <b>mode = 0</b> [0, 30] for <b>mode = 1</b>	
5	$\langle \phi_1 \rangle$	degrees	[0, 180]	
6	$\Delta \phi_1$	degrees	[0, 180] for <b>mode = 0</b> [0, 60] for <b>mode = 1</b>	
7	$\langle \alpha_1 \rangle$	degrees	[0, 180]	
8	$\Delta \alpha_1$	degrees	[0, 180] for <b>mode = 0</b> [0, 60] for <b>mode = 1</b>	
9	$\langle \beta_1 \rangle$	degrees	[0, 90]	
10	$\Delta \beta_1$	degrees	[0, 90] for <b>mode = 0</b> [0, 30] for <b>mode = 1</b>	
11	$\langle \gamma_1 \rangle$	degrees	[0, 180]	
12	$\Delta \gamma_1$	degrees	[0, 180] for <b>mode = 1</b> [0, 60] for <b>mode = 2</b>	<b>mode = 0</b> : uniform distribution <b>mode = 1</b> : normal distribution
13	$\langle r_2 \rangle$	nm	Any reasonable ranges inside [1.5, 8.0]	
14	$\Delta r_2$	nm	Any ranges inside [1.5, 8.0]	
15	$\langle \xi_2 \rangle$	degrees	[0, 90]	
16	$\Delta \xi_2$	degrees	[0, 90] for <b>mode = 0</b> [0, 30] for <b>mode = 1</b>	
17	$\langle \phi_2 \rangle$	degrees	[0, 180]	
18	$\Delta \phi_2$	degrees	[0, 180] for <b>mode = 0</b> [0, 60] for <b>mode = 1</b>	
19	$\langle \alpha_2 \rangle$	degrees	[0, 180]	
20	$\Delta \alpha_2$	degrees	[0, 180] for <b>mode = 0</b> [0, 60] for <b>mode = 1</b>	
21	$\langle \beta_2 \rangle$	degrees	[0, 90]	
22	$\Delta \beta_2$	degrees	[0, 90] for <b>mode = 0</b> [0, 30] for <b>mode = 1</b>	
23	$\langle \gamma_2 \rangle$	degrees	[0, 180]	
24	$\Delta \gamma_2$	degrees	[0, 180] for <b>mode = 1</b> [0, 60] for <b>mode = 2</b>	
25	$w$		(0, 1)	
26	$\langle J \rangle$	MHz	Any reasonable ranges	<b>mode = 0</b> : uniform distribution
27	$\Delta J$	MHz	Any reasonable ranges	<b>mode = 1</b> : normal distribution
28	$\eta$		(0, 1]	<b>mode = 0</b> : $\eta$ is the same for all simulated PELDOR time traces <b>mode = 1</b> : $\eta$ is different for each PELDOR signal time traces

<sup>a</sup> For more details see Chapter 1.2.<sup>b</sup> The modes are relevant only if the parameter is optimized (**opt = 1**) or has a fixed value (**opt = 2**).

In addition to the geometric parameters, PeldorFit allows to account for exchange coupling between spin centers. Thus, the parameters no. 26 and 27 correspond to a mean value and a distribution width of an exchange coupling constant  $J$ . Note that  $J$  can have the same types of distribution as all geometric parameters of the model (see Figure 1.2). The last parameter with no. 28 is a scale factor for the modulation depth  $\eta$  ( $0 < \eta \leq 1$ ). This parameter is often very useful for reproducing experimental modulation depths of PELDOR time traces, because it allows taking into account imperfections of a pump pulse, incomplete spin labelling, etc.

Now let's return to the discussion of the [parameters](#) list. Each line of this list corresponds to one of the generalized parameters and contains four settings called [opt](#), [mode](#), [range](#), and [value](#). The most important setting is [opt](#), since it determines whether the corresponding parameter will be included into the final model or not:

- [opt](#)        If [opt](#) = 0, the parameter is excluded from the final model.
- If [opt](#) = 1, the parameter is included to the final model and will be optimized.
- If [opt](#) = 2, the parameter is included to the final model and will have a constant value (will not be optimized).

Using the [opt](#) setting, one can select the set of parameters that are relevant for PELDOR simulations and exclude all other parameters.

In the case of [opt](#) = 0, the values of other three settings ([mode](#), [range](#), and [value](#)) are irrelevant. The value of the corresponding parameter will be set automatically to 0.

In the case of [opt](#) = 1, the values of [mode](#) and [range](#) settings have to be specified in accordance to Table 4.1. For all geometric parameters and the  $J$  coupling constant, the statement [mode](#) = 0 sets the distribution type of the corresponding parameter to a uniform distribution, and the statement [mode](#) = 1 to a normal distribution. This definition differs, however, for the scale factor  $\eta$ : Here, [mode](#) = 0 means that a single value of  $\eta$  will be optimized for all PELDOR time traces, whereas in case of [mode](#) = 1 the value of  $\eta$  will be optimized for each PELDOR time trace separately. The [range](#) setting sets the lower and the upper bounds of the corresponding optimized parameter. The user is free to choose the bounds for each of the parameters. However, it is recommended to use the bounds given in Table 4.1.

Finally, in the case of [opt](#) = 2, the corresponding parameter will be set to a constant value, which is specified in [value](#). Before typing the value of the fixed parameter into the [value](#) field, make sure that this value is within the parameter's bounds given in Table 4.1.

## 4.4 Optimization settings

Optimization of a selected model and corresponding parameters is performed by a genetic algorithm. This algorithm has been shown to be very efficient when one deals with a large number of optimization parameters and needs to find a global minimum.<sup>[7-9]</sup> Importantly, a genetic algorithm has its own internal parameters, which determine its ability to find a global minimum. Optimal values of these parameters may vary depending on a particular PELDOR data set. Therefore, the configuration file allows user to set these values manually. However, it is highly recommended to use the internal parameters from the configuration files given in [Examples](#) (at least as starting values), because they were obtained after extensive tests of the genetic algorithm on several PELDOR data sets.

The intrinsic parameter of the genetic algorithm are stored in the list [genetic](#):

*Taken from Examples/unimodal\_nitroxide\_nitroxide/bisnitroxide\_config.cfg*

---

```
genetic:
{
    num_generations_max = 500;
    size_generation = 192;
    prob_crossover = 0.5;
    prob_mutation = 0.01;
    merit_function = 1;
    num_averages = 100000;
};
```

---

Here,

<a href="#">num_generations_max</a>	The total number of optimization steps (generations). This parameter determines the convergence of the genetic algorithm to a global minimum. The convergence can be monitored by plotting a goodness-of-fit vs. optimization step (see the output file <a href="#">score.dat</a> ). The values of a merit function have to be constant for the last 100 optimization steps. Otherwise, the value of <a href="#">num_generations_max</a> has to be increased.
<a href="#">size_generation</a>	The number of parameter sets (chromosomes) that are evaluated simultaneously by the genetic algorithm. A good practice is to set this parameter to 10 x (the number of fitting parameters).
<a href="#">prob_crossover</a>	The crossover rate.
<a href="#">prob_mutation</a>	The mutation rate. If the genetic algorithm converges but the fit to PELDOR time traces is not satisfactory, one can try to increase this parameter.

<code>merit_function</code>	<p>A merit function, which is used to judge how good the simulated PELDOR time traces fit to corresponding experimental PELDOR time traces (i.e., to calculate a goodness-of-fit).</p> <p>If <code>merit_function = 1</code>, the merit function is a <a href="#">root-mean-square-deviation</a> (RMSD).</p> <p>If <code>merit_function = 3</code>, the merit function is a <a href="#">Pearson product moment correlation coefficient</a> (PCC).</p> <p>If <code>merit_function = 2</code>, the merit function is RMSD/PCC.</p> <p>So far, all tests of PeldorFit were done using RMSD as a merit function.</p>
<code>num_averages</code>	<p>The number of Monte-Carlo averages used to simulate PELDOR signals. It determines how accurate the powder averaging will be done. (This value should not be lower than <math>10^5</math>.) This parameter has a strong effect on the duration of the optimization.</p>

## 4.5 Output settings

The last part of the configuration file specifies what information will be output by PeldorFit after the fitting is completed. The output settings are given in a list called `output`:

*Taken from Examples/unimodal\_nitroxide\_nitroxide/bisnitroxide\_config.cfg*

---

```
output:
{
    directory = "Examples/unimodal_nitroxide_nitroxide/Results/";
    record_spectrum = 0;
    record_score = 1;
    record_parameters = 1;
    record_fit = 1;
    record_symmetric_solutions = 1;
    record_error_plot = 1;
    error_plot_variables = ([1,2],[3,4],[5,6],[7,8],[9,10],[11,12]);
};
```

---

The `output` list contains the following settings:

<code>directory</code>	A path to a disk space where the output data will be stored.
<code>record_score</code>	If set to 1, a goodness-of-fit is recorded in dependence of the optimization step. Then, this dependence is saved into <a href="#">score.dat</a> .
<code>record_fit</code>	If set to 1, the experimental and simulated PELDOR time traces are saved into <a href="#">fit.dat</a> .
<code>record_parameters</code>	If set to 1, the optimized parameters of a geometric model are saved into <a href="#">parameters.dat</a> .

<a href="#">record_symmetric_solutions</a>	If set to 1, the symmetry-related sets of angular parameters (see Chapter 1.3) are saved into <a href="#">symmetric_parameters.dat</a> .
<a href="#">record_error_plot</a>	If set to 1, the goodness-of-fit is recorded in dependence of the fitting parameters specified in <a href="#">error_plot_variables</a> . Here, this dependence is denoted as an error plot, because it is usually depicted graphically and provides error estimates for optimized fitting parameters. Usually, each error plot is recorded just for one or two fitting parameters, whereas all other fitting parameters are set to their optimized values. All error plots are then saved into <a href="#">error_plot_X.dat</a> or <a href="#">errorplot_X_Y.dat</a> , where X and Y are replaced by the serial numbers of variables. For example, <a href="#">error_plot_1.dat</a> will contain the dependence of a goodness-of-fit on the parameter $\langle r_1 \rangle$ , and <a href="#">error_plot_3_4.dat</a> will contain the dependence of a goodness-of-fit on the parameters $\langle \xi_1 \rangle$ and $\Delta \xi_1$ .
<a href="#">error_plot_variables</a>	The variables of error plots. The variables of each individual error plot are given in square brackets. To specify the variables, the serial numbers of the generalized parameters have to be used (see Table 4.1). For example, a notation <a href="#">error_plot_variables = ([1], [3], [5])</a> means that three error plots will be recorded for the parameters $\langle r_1 \rangle$ , $\langle \xi_1 \rangle$ , and $\langle \phi_1 \rangle$ , respectively. The notation <a href="#">error_plot_variables = ([1, 2], [3, 4])</a> means that two error plots will be recorded for two pairs of parameters, $(\langle r_1 \rangle, \Delta r_1)$ and $(\langle \xi_1 \rangle, \Delta \xi_1)$ , respectively.
<a href="#">error_plot_size</a>	The number of points in a single error plot.
<a href="#">record_spectrum</a>	If set to 1, the EPR spectrum of a spin system is calculated using the parameters of <a href="#">SpinA</a> , <a href="#">SpinB</a> , and <a href="#">SpinC</a> (if included). The spectrum is computed in the frequency domain (in GHz), and the magnetic field is set to the <a href="#">magnField</a> value of the first experiment listed in <a href="#">experimentals</a> . After the calculation, the spectrum is saved into <a href="#">spectrum.dat</a> .

In order to switch off the recording of particular output data, the corresponding parameter should be set to 0.



Additionally, the program PeldorFit has an operation mode, which is called `error_plot_only`. This mode can be useful in cases, when the user has already done the fitting of PELDOR time traces and now just wants to use the previous results to generate the error plots for the optimized parameters. The `error_plot_only` mode allows to avoid repeating the optimization procedure (which is very time-consuming) and, instead, allows using the results of previous optimization to calculate the required error plots. The `error_plot_only` mode is activated as follows:

*Taken with some modifications from Examples/unimodal\_nitroxide\_nitroxide/bisnitroxide\_config.cfg*

---

```
error_plot_only:
{
    enable = 0;
    error_plot_variables = ([1,2]);
    error_plot_size = 1000;
    input_directory = "Examples/unimodal_nitroxide_nitroxide/Results/parameters.dat";
    output_directory = "Examples/unimodal_nitroxide_nitroxide/Results/";
};
```

---

where

<code>enable</code>	The switch, which inactivates the usual “optimization mode” and activates the “error plot only” mode: 0 = disable, 1 = enable
<code>error_plot_variables</code>	Same setting as <code>error_plot_variables</code> in the <code>output</code> list.
<code>error_plot_size</code>	Same setting as <code>error_plot_size</code> in the <code>output</code> list.
<code>input_directory</code>	The path to a disk space where a file with the optimized values of fitting parameters ( <code>parameters.dat</code> ) is stored.
<code>output_directory</code>	The path to a disk space where the output data will be stored.

## 5 Output data

Depending on the [output](#) settings of the configuration file, some or all of the following output files are generated by PeldorFit:

a) [score.dat](#)

This file stores the values of the merit function for each of the optimization steps (generations). The first column of the file lists to the numbers of optimization steps. The second column contains the corresponding values of a goodness-of-fit, e.g., RMSD values.

b) [fit.dat](#)

This file stores the best fits to the experimental PELDOR time traces. For each PELDOR time trace specified in the configuration file, PeldorFit writes three columns into the file. The first column corresponds to the time values in microseconds, the other two columns contain the values of the experimental signal and its fit, respectively.

c) [parameters.dat](#)

This file stores the optimized parameters of a geometric model. The first column of the file contains the names of the optimization parameters. The second column provides the optimized values of these parameters.

d) [symmetric\\_parameters.dat](#)

This file stores the 16 symmetry-related sets of optimized angular parameters. The first column of the file contains the names of the angular parameters. The second column contains the optimized values of the angular parameters, which are identical to the ones listed in [parameters.dat](#). The next 15 columns list the values of the symmetry-related angular parameters. Additionally, the RMSD values corresponding to all 16 sets of angular parameters are given in the last line of the file.

e) [errorplot\\_X.dat](#) or [errorplot\\_X\\_Y.dat](#) where X, Y = 1 - 28

This file stores an error plot for a single fitting parameter  $X$  (e.g.,  $\langle r_1 \rangle$ ) or for a pair of fitting parameter  $X$  and  $Y$  (e.g.,  $\langle r_1 \rangle$  and  $\Delta r_1$ ). Depending on the number of variables  $N$ , the first  $N$  columns of the file contain the values of the fitting parameters chosen for the error plot. The last column of the file contains the corresponding values of a goodness-of-fit, e.g., RMSD values.

f) [spectrum.dat](#)

This file stores the simulated ESR spectrum of the spin system. The first column of the file contains the values of the microwave frequency in gigahertz. The second column contains the corresponding intensities of the spectrum normalized to 1.

## 5.1 Plotting the output data by means of Python scripts

To enable graphical representation of the PeldorFit output data, common tools like Origin, Matlab, or matplotlib can be used. Here, several Python scripts were developed for this purpose:

- a) `plotScore.py` plots the data of `score.dat`;
- b) `plotFit.py` plots the data of `fit.dat`;
- c) `plotDistDistr.py` plots the distance distribution based on the data of `parameters.dat`;
- d) `plotErrorPlot.py` plots the data of `errorplot_X.dat` and `errorplot_X_Y.dat`.

In order to use these scripts, one needs to install [Python 2.7](#) and two additional Python libraries called [numpy](#) and [matplotlib](#) (all are free available). All scripts can be found in the PeldorFit directory `/PeldorFit2018/Visualization/`. To run these scripts:

1. Open Terminal (Linux) or Command Prompt (Windows).
2. Go to the directory in which the Python scripts are stored:

```
cd ../PeldorFit2019/Visualization
```

3. Set the permission properties (only for Linux):

```
chmod 755 [name of the script]
```

4. Run the script by the following command:

```
python [name of the script] [full path of the output file] [optional arguments]
```

In particular, the user has the following options:

[name of the script]	[full path of the output file]	[optional arguments]
<code>plotScore.py</code>	full path to <code>score.dat</code>	<code>--fontsize</code> (or <code>-fs</code> ): set the font size of the figure
<code>plotFit.py</code>	full path to <code>fit.dat</code>	<code>--fontsize</code> (or <code>-fs</code> ): set the font size of the figure
<code>plotDistDistr.py</code>	full path to <code>parameters.dat</code>	<code>--rmin</code> : set the lower bound of the distance <code>--rmax</code> : set the upper bound of the distance <code>--fontsize</code> (or <code>-fs</code> ): set the font size of the figure
<code>plotErrorPlot.py</code>	full path to <code>errorplot_X.dat</code> or <code>errorplot_X_Y.dat</code>	<code>--cmin</code> : set the lower bound of the colormap <code>--cmax</code> : set the upper bound of the colormap <code>--fontsize</code> (or <code>-fs</code> ): set the font size of the figure

## 6 Examples

This chapter provides examples for the analysis of orientation-selective PELDOR data-sets of three different model systems.

### 6.1 Two-spin system with a unimodal distance distribution

The first example corresponds to a W-band PELDOR data-set of a bis-nitroxide model compound **1**<sup>[10]</sup> (Figure 6.1). This data-set contains six PELDOR time traces, which were acquired using six different pump/detection positions across the nitroxide spectrum (Figure 6.1b). The details of the PELDOR experiments can be found in Ref. [10]. Now, let's apply PeldorFit to determine the distance distribution and the relative orientation of nitroxide spin centers in **1**.

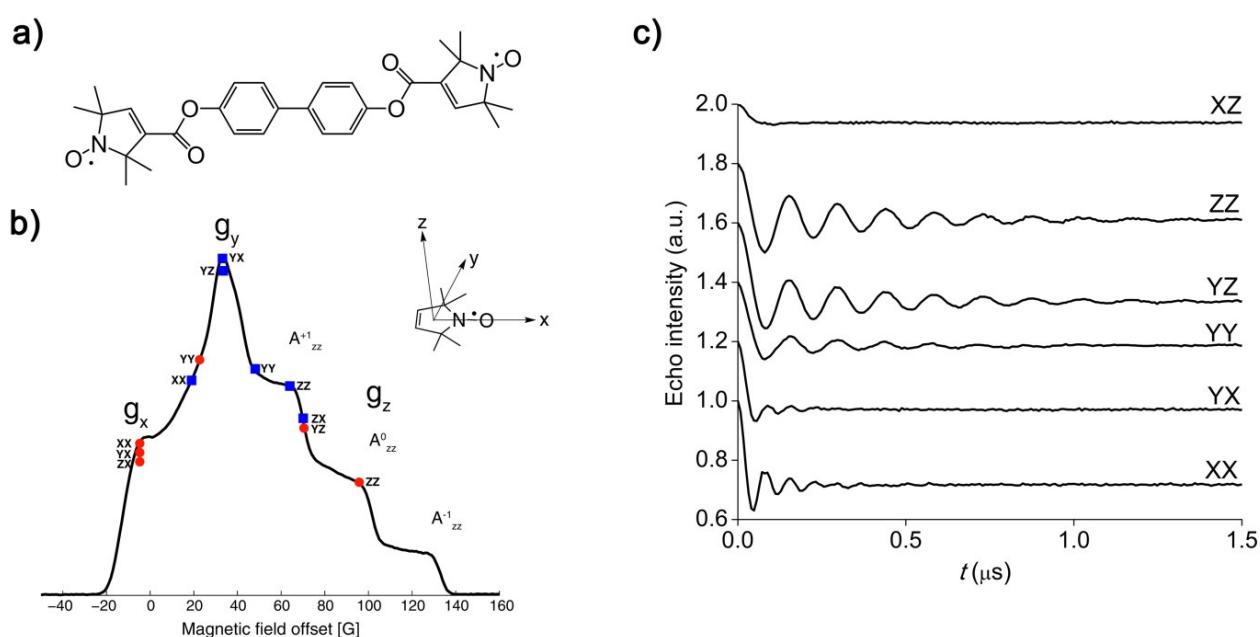
#### Step 1. Preparation of the configuration file

Before one runs the program, a configuration file with all input data of the program has to be prepared. The detailed description of how one creates such file and what information has to be provided is given in Chapter 4. Make sure that you have read that chapter! For **1**, the configuration file can be found in the following directories:

[/PeldorFit2019/Linux/Examples/unimodal\\_nitroxide\\_nitroxide/bisnitroxide\\_config.cfg](#) (Linux)

[/PeldorFit2019/Windows/Examples/unimodal\\_nitroxide\\_nitroxide/bisnitroxide\\_config.cfg](#) (Windows)

As follows from [bisnitroxide\\_config.cfg](#), the nitroxide spin centers of **1** are described by a two-spin model with a unimodal distance distribution (Model 1 in Chapter 1.2). As described in Chapter 1.2, this model consists of six geometric parameters  $r_1$ ,  $\xi_1$ ,  $\varphi_1$ ,  $\alpha_1$ ,  $\beta_1$ , and  $\gamma_1$ .



**Figure 6.1.** a) Lewis structure of bis-nitroxide model compound **1**. b) The echo-detected W-band EPR spectrum of **1**. Red dots and blue squares show the positions of the detection and pump pulses, respectively. The dots and squares are labelled with their respective PELDOR experiment. The inset shows a nitroxide and the relative orientation of the g-tensor indicated by arrows. c) The W-band PELDOR time traces of **1**. Adapted from Ref. [10].

The distribution of distance parameter  $r_1$  is set to a Gaussian distribution, whereas the distributions of all angular parameters,  $\xi_1$ ,  $\varphi_1$ ,  $\alpha_1$ ,  $\beta_1$ , and  $\gamma_1$ , are set to uniform distributions. Thus, the inter-nitroxide distance distribution is described by a mean distance  $\langle r_1 \rangle$  and its standard deviation  $\Delta r_1$ . Each of the angular distributions is also described by two values, a mean angle  $\langle a \rangle$  and a uniform width  $\Delta a$  ( $a = \xi_1, \varphi_1, \alpha_1, \beta_1$ , and  $\gamma_1$ ). In addition to the geometric parameters, the scaling factor for the modulation depth,  $\eta$ , is set to be a fitting parameter too. Since the PELDOR time traces of **1** were acquired on a cavity-free spectrometer and, therefore, the pump pulse efficiency was the same for all of them, a single value of  $\eta$  is used for all time traces.

### Step 2. Running the program

As soon as the configuration file is prepared, one can run PeldorFit. To do this, first open Terminal (Linux)/Command Prompt (Windows) and navigate to the directory

`cd ../PeldorFit2018/Linux` (for Linux) or

`cd ../PeldorFit2018/Windows` (for Windows).

Next, run the program by the following command:

`sh PeldorFit.sh Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg` (for Linux) or

`PeldorFit.exe Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg` (for Windows).

### Step 3. Operation of the program

Now the program is running. First, the program reads out the configuration file and pops up the message:

Loading input data from the config file...

Number of PELDOR signals is 6

Input data is loaded!

The last message appears only when the configuration file does not contain any syntactic mistakes. Next, the fitting of the PELDOR time traces begins. During the fitting, the parameters of the selected geometric model are optimized until the simulated PELDOR time traces provide the best fit to the experimental PELDOR time traces. The optimization is done by means of the genetic algorithm and proceeds through 500 iteration steps (see Chapter 4.4). Note that this procedure may take a long time (Running this example on a 64-core workstation resulted in the optimization time of 4.77 hours.). During the optimization process, the program pops up the following progress messages:

Optimization of the spin geometry via genetic algorithm... Please be patient!

Optimization step 1 / 500

...

Optimization step 500 / 500



In the end, the program saves the results of the fitting. The following messages appear:

Recording the goodness-of-fit vs optimization step... Done!

Recording the optimized values of fitting parameters... Done!

Recording the fits to the PELDOR signals... Done!

Recording the symmetry-related sets of fitting parameters... Done!

Recording the error plot... Done!

Finished! The optimization took 4.77 hours.

#### Step 4. The fitting results

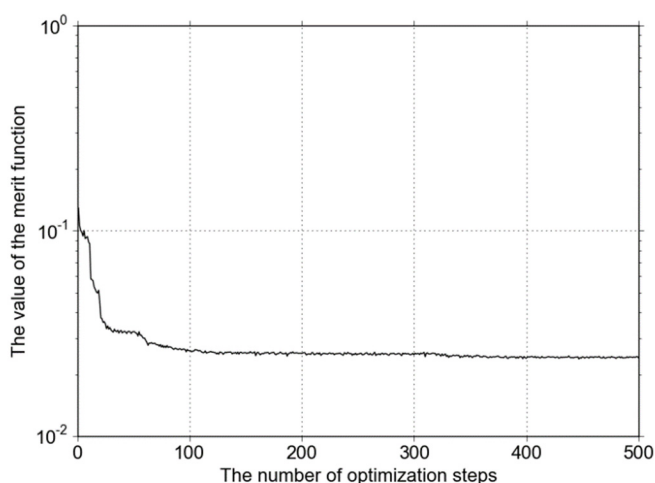
All results of the fitting are saved into the directory

[Examples/unimodal\\_nitroxide\\_nitroxide/Results/](#),

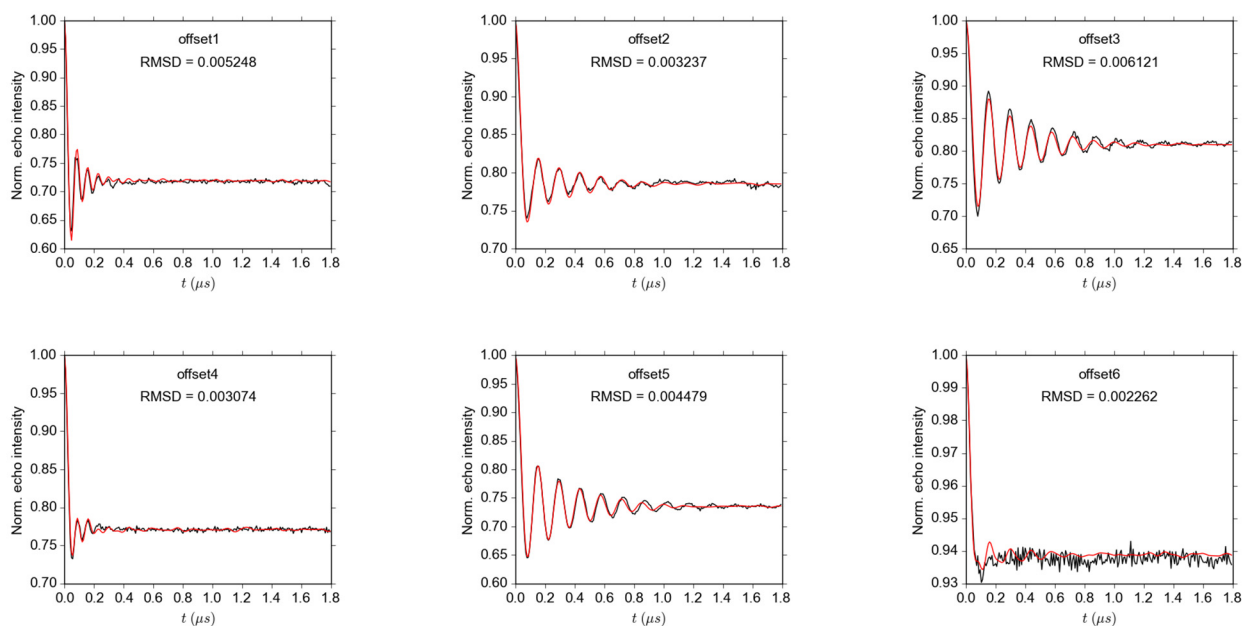
which is specified in the configuration file. The output files include

- [score.dat](#)
- [fit.dat](#)
- [parameters.dat](#)
- [symmetric\\_parameters.dat](#)
- [errorplot\\_1\\_2.dat](#), [errorplot\\_3\\_4.dat](#), [errorplot\\_5\\_6.dat](#), [errorplot\\_7\\_8.dat](#), [errorplot\\_9\\_10.dat](#),  
[errorplot\\_11\\_12.dat](#)

Let's briefly discuss the content of these files. First, it is important to make sure that the genetic algorithm has converged to the global minimum. This information is contained in the file called [score.dat](#). The content of this file is depicted in Figure 6.2. As can be seen, the RMSD between the experimental PELDOR time traces and their fits falls gradually down during the first 350 optimization steps and, after this, does not change anyhow significantly during the last 150 optimization steps. This shows that the global minimum was reached and that the optimal geometry of the spin system was found.



**Figure 6.2.** The goodness-of-fit, given by the RMSD between the experimental PELDOR time traces of **1** and their fits, is depicted in dependence of optimization step. Generated by means of [plotScore.py](#) (see Section 5.1).



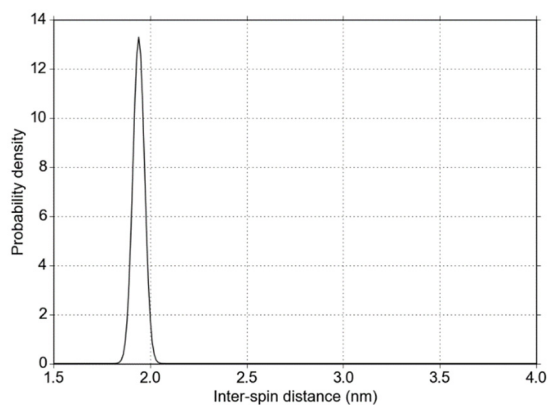
**Figure 6.3.** The PELDOR time traces of **1** (black lines) are overlaid with the corresponding fits (red lines). Generated by means of [plotFit.py](#) (see Section 5.1).

Although PeldorFit kept track of the goodness-of-fit by calculating the RMSD value, an additional check of the obtained fit by eye is advisable. The fit to the PELDOR time traces are stored in the file called [fit.dat](#). Figure 6.3 depicts the content of this file for **1**. As can be seen, the shapes of all PELDOR time traces is well reproduced by the corresponding fits. Thus, one can conclude that the optimized geometric model provides a good agreement with the PELDOR data.

Now, let's consider the optimized geometric model and, in particular, its parameters stored in the file called [parameters.dat](#). As was mentioned above, the parameters of the model include the mean values and the distributions widths of  $r_1$ ,  $\xi_1$ ,  $\phi_1$ ,  $\alpha_1$ ,  $\beta_1$ , and  $\gamma_1$ , as well as the scaling factor  $\eta$ . The optimized values of these parameters are listed in Table 6.1. The inter-nitroxide distance distribution corresponding to the optimized  $\langle r_1 \rangle$  and  $\Delta r_1$  is depicted in Figure 6.4.

**Table 6.1.** Optimized parameters of the geometric model of **1**.

Parameter	Value
$\langle r_1 \rangle$	1.94 nm
$\Delta r_1$ (standard deviation)	0.03 nm
$\langle \xi_1 \rangle$	90°
$\Delta \xi_1$ (uniform width)	12°
$\langle \phi_1 \rangle$	156°
$\Delta \xi_1$ (uniform width)	55°
$\langle \alpha_1 \rangle$	145°
$\Delta \alpha_1$ (uniform width)	33°
$\langle \beta_1 \rangle$	52°
$\Delta \beta_1$ (uniform width)	84°
$\langle \gamma_1 \rangle$	162°
$\Delta \gamma_1$ (uniform width)	50°
$\eta$	0.93



**Figure 6.4.** The inter-nitroxide distance distribution in **1**. Generated by means of [plotDistDistr.py](#) (see Section 5.1).

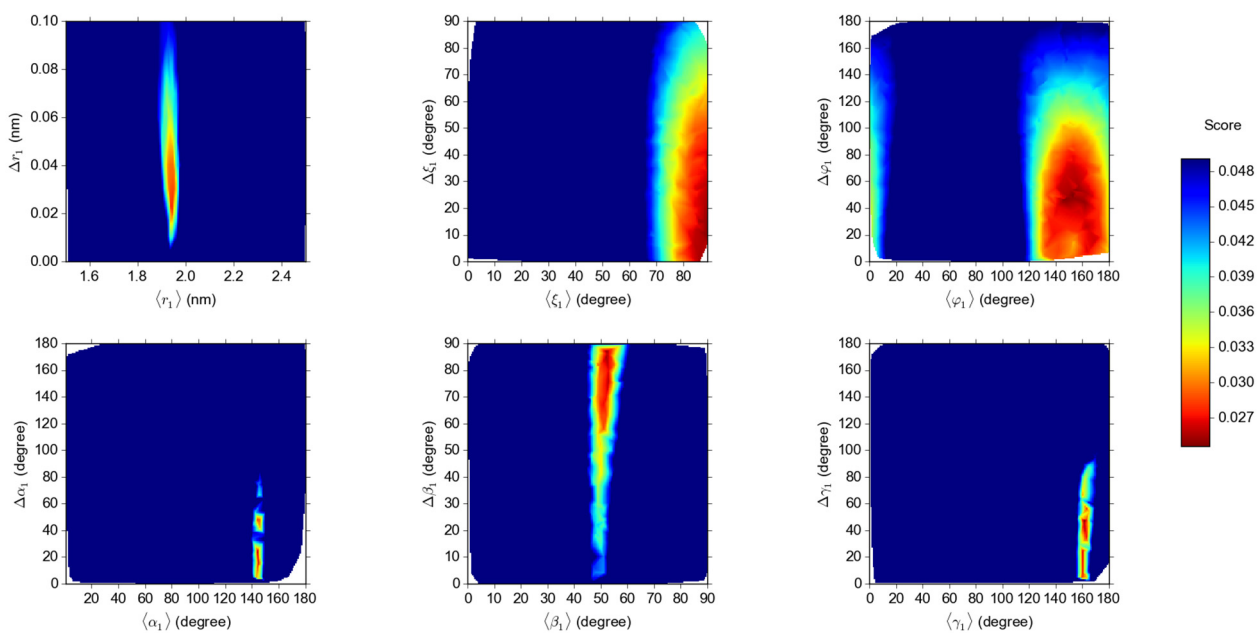
**Table 6.2.** Summary of the symmetry-related sets of angles for **1**.

Symmetric transformation <sup>[a]</sup>	$\langle \xi_1 \rangle, ^\circ$	$\langle \varphi_1 \rangle, ^\circ$	$\langle \alpha_1 \rangle, ^\circ$	$\langle \beta_1 \rangle, ^\circ$	$\langle \gamma_1 \rangle, ^\circ$
Fitting result	90	156	145	52	162
180° rotation about $g_{xx}^B$	90	156	325	128	18
180° rotation about $g_{yy}^B$	90	156	325	128	198
180° rotation about $g_{zz}^B$	90	156	145	52	342
180° rotation about $g_{xx}^A$	90	204	35	128	342
180° rotation about $g_{xx}^A$ and $g_{xx}^B$	90	204	215	52	198
180° rotation about $g_{xx}^A$ and $g_{yy}^B$	90	204	215	52	18
180° rotation about $g_{xx}^A$ and $g_{zz}^B$	90	204	35	128	162
180° rotation about $g_{yy}^A$	90	24	215	128	342
180° rotation about $g_{yy}^A$ and $g_{xx}^B$	90	24	35	52	198
180° rotation about $g_{yy}^A$ and $g_{yy}^B$	90	24	35	52	18
180° rotation about $g_{yy}^A$ and $g_{zz}^B$	90	24	215	128	162
180° rotation about $g_{zz}^A$	90	336	325	52	162
180° rotation about $g_{zz}^A$ and $g_{xx}^B$	90	336	145	128	18
180° rotation about $g_{zz}^A$ and $g_{yy}^B$	90	336	145	128	198
180° rotation about $g_{zz}^A$ and $g_{zz}^B$	90	336	325	52	342

<sup>[a]</sup>  $g_{xx}^A$ ,  $g_{yy}^A$ , and  $g_{zz}^A$  denote the principal components of the  $g$ -tensor of spin A;  $g_{xx}^B$ ,  $g_{yy}^B$ , and  $g_{zz}^B$  denote the principal components of the  $g$ -tensor of spin B.

As described in Chapter 1.3, the angles  $\langle \xi_1 \rangle$ ,  $\langle \varphi_1 \rangle$ ,  $\langle \alpha_1 \rangle$ ,  $\langle \beta_1 \rangle$ ,  $\langle \gamma_1 \rangle$  cannot be determined uniquely and have symmetry-related values. Therefore, PeldorFit calculates all 16 symmetry-related sets of angles and stores them in the file called [symmetric\\_parameters.dat](#). The content of this file is summarized in Table 6.2.

Finally, one has to quantify the precision of the optimized geometric parameters. Since PeldorFit deals with a large number of fitting parameters (13 in this case) and since they are interdependent, the error estimation is non-trivial. In PeldorFit, this task is approached by exploring the dependence of goodness-of-fit on a single fitting parameter or a pair of fitting parameters. While doing this, all other fitting parameters are set to their optimized values. The obtained dependences are called error plots, because they allow estimation of errors of individual parameters. In the present example, the error plots were recorded for six pairs of geometric parameters:  $\langle r_1 \rangle$  and  $\Delta r_1$  ([errorplot\\_1\\_2.dat](#)),  $\langle \xi_1 \rangle$  and  $\Delta \xi_1$  ([errorplot\\_3\\_4.dat](#)),  $\langle \varphi_1 \rangle$  and  $\Delta \varphi_1$  ([errorplot\\_5\\_6.dat](#)),  $\langle \alpha_1 \rangle$  and  $\Delta \alpha_1$  ([errorplot\\_7\\_8.dat](#)),  $\langle \beta_1 \rangle$  and  $\Delta \beta_1$  ([errorplot\\_9\\_10.dat](#)),  $\langle \gamma_1 \rangle$  and  $\Delta \gamma_1$  ([errorplot\\_11\\_12.dat](#)). All of them are depicted in Figure 6.5. All error plots show a single well-defined minimum. Interestingly, this minimum spans over different ranges for different geometric parameters. For example, the minima obtained for the angles  $\alpha$ ,  $\beta$ , and  $\gamma$  are sharper than the minima obtained for the angles  $\xi$  and  $\beta$ . Thus, all six geometric parameters are determined with slightly different precision. This result might be assigned to the different selectivity of the PELDOR data to individual angles.



**Figure 6.5.** The error plots obtained for **1**. Generated by means of `plotErrorPlot.py` (see Section 5.1).

## 6.2 Two-spin system with a bimodal distance distribution

The second example deals to the X-band PELDOR data-set of a nitroxide-labelled single cysteine mutant of azurin T21R1<sup>[11]</sup> (Figure 6.6). T21R1 contains two paramagnetic centers, the Cu<sup>2+</sup> ion and the nitroxide center. The Cu<sup>2+</sup> ion has a well-defined binding site in azurin and, therefore, its spin center has a fixed orientation with respect to the protein structure. The R1 side chain is intrinsically flexible and, in the given example, adopt two ensembles of conformations.<sup>[12,13]</sup> The most probable conformations of both ensembles are depicted in Figure 6.6a. Importantly, these two ensembles have different distances to the Cu<sup>2+</sup> center, giving rise to a bimodal inter-spin distance distribution.

The PELDOR time traces of T21R1 were recorded using four different frequency offsets. The pump pulse was always applied at the maximum of the nitroxide spectrum, whereas the detection pulses were set to be in resonance with different spectral components of Cu<sup>2+</sup> (Figure 6.6b) The details of the PELDOR experiments can be found in Ref. [11]. Now, let's apply PeldorFit to determine the distance distribution and the relative orientation of Cu<sup>2+</sup> and nitroxide spin centers in T21R1.

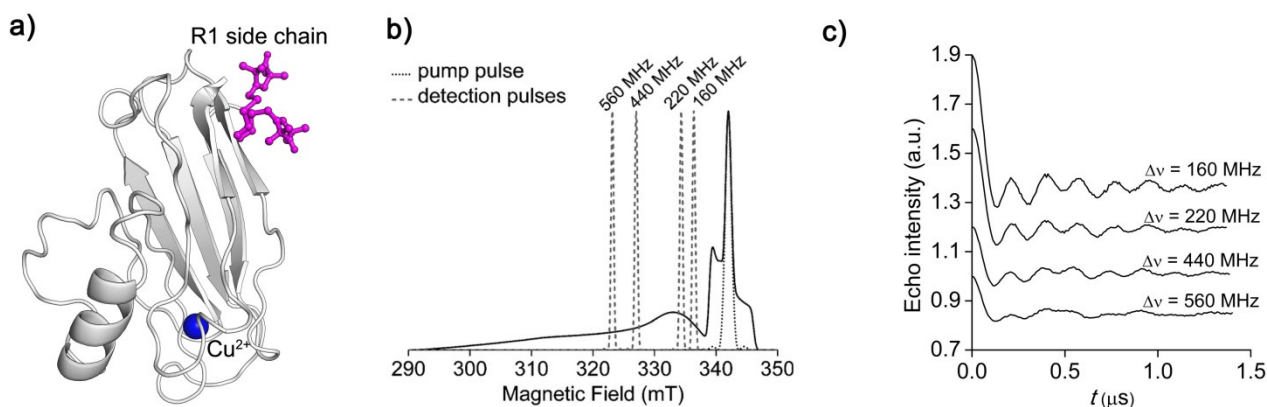
### Step 1. Preparation of the configuration file

The configuration file for T21R1 can be found in the directories:

[/PeldorFit2018/Linux/Examples/bimodal\\_copper\\_nitroxide/bimodal\\_config.cfg](#) (Linux)

[/PeldorFit2018/Windows/Examples/bimodal\\_copper\\_nitroxide/bimodal\\_config.cfg](#) (Windows)

To account for the two different conformations of the R1 side chain, a two-spin geometric model with a bimodal distance distribution (Model 2 in Chapter 1.2) is specified in the configuration file [bimodal\\_config.cfg](#). As described in Chapter 1.2, this model consists of two sets of geometric parameters ( $r_i, \xi_i, \varphi_i, \alpha_i, \beta_i, \gamma_i$ ), where  $i$  is 1 or 2. Each set of parameters corresponds to one of the R1 conformational ensembles. The relative weight of these ensemble is described by the



**Figure 6.6. a)** The crystal structure of the nitroxide-labelled azurin mutant T21R1 (PDB 4BWW). **b)** The echo-detected X-band EPR spectrum of T21R1 shown together with the simulated excitation profiles of the pump (dotted line) and the detection (dashed lines) pulses. **c)** The X-band PELDOR time traces of T21R1. Adapted from Ref. [11].



parameter  $w$ . The distance parameters  $r_i$  are set to have Gaussian distributions with mean values  $\langle r_i \rangle$  and standard deviations  $\Delta r_i$ . Due to the significant rigidity of the  $\text{Cu}^{2+}$  center and the R1 side chain in both conformational states, all angular parameters are assumed to have unique values and no distributions. Thus, only the mean angles but not their widths are included in the list of fitting parameters. Moreover, due to the axial symmetry of the  $\text{Cu}^{2+}$  g-tensor, the PELDOR time traces of T21R1 are insensitive to the angles  $\varphi_i$  (see Table 1.1). Therefore, both  $\varphi_i$  angles are excluded from consideration. Taken together, the final geometric model consists of the 13 parameters:  $\langle r_1 \rangle$ ,  $\Delta r_1$ ,  $\langle \xi_1 \rangle$ ,  $\langle \alpha_1 \rangle$ ,  $\langle \beta_1 \rangle$ ,  $\langle \gamma_1 \rangle$ ,  $\langle r_2 \rangle$ ,  $\Delta r_2$ ,  $\langle \xi_2 \rangle$ ,  $\langle \alpha_2 \rangle$ ,  $\langle \beta_2 \rangle$ ,  $\langle \gamma_2 \rangle$ , and  $w$ . In addition, the scaling factor for the modulation depth,  $\eta$ , is set to be a fitting parameter too. In contrast to the first example, the PELDOR time trace of T21R1 were acquired with different pump efficiencies and, therefore,  $\eta$  is optimized for each PELDOR time trace separately.

### Step 2. Running the program

To run PeldorFit, first open Terminal (Linux)/Command Prompt (Windows) and navigate into the directory

`cd ../PeldorFit2019/Linux` (for Linux) or

`cd ../PeldorFit2019/Windows` (for Windows).

Next, run the program by the following command:

`sh PeldorFit.sh Examples/bimodal_copper_nitroxide/bimodal_config.cfg` (for Linux) or

`PeldorFit.exe Examples/bimodal_copper_nitroxide/bimodal_config.cfg` (for Windows).

### Step 3. Operation of the program

Now the program is running. First, the program reads out the configuration file and pops up the message:

Loading input data from the config file...

Number of PELDOR signals is 4

Input data is loaded!

Next, the fitting of the PELDOR time traces begins. During the fitting, the parameters of the selected geometric model are optimized by the genetic algorithm. The optimization proceeds through 500 iteration steps. During the optimization, the program pops up the following progress messages:

Optimization of the spin geometry via genetic algorithm... Please be patient!

Optimization step 1 / 500

...

Optimization step 500 / 500

Finally, the program saves the results of the fitting. The following messages appear:

Recording the goodness-of-fit vs optimization step... Done!

Recording the optimized values of fitting parameters... Done!

Recording the fits to the PELDOR signals... Done!

Recording the symmetry-related sets of fitting parameters... Done!

Recording the error plot... Done!

Finished! The optimization took 1.30 hours.

#### Step 4. The fitting results

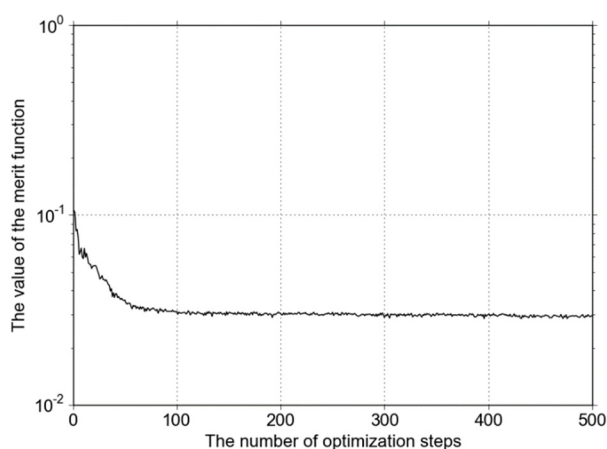
All results of the fitting are saved into the directory

[Examples/bimodal\\_copper\\_nitroxide/Results/](#),

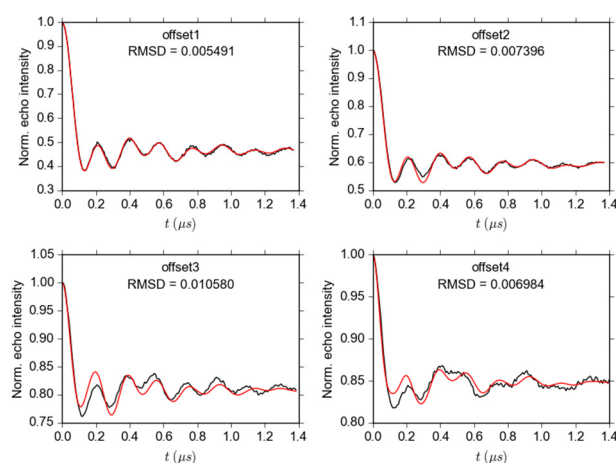
which is specified in the configuration file. The output files include

- [score.dat](#)
- [fit.dat](#)
- [parameters.dat](#)
- [symmetric\\_parameters.dat](#)
- [errorplot\\_1\\_2.dat](#), [errorplot\\_3.dat](#), [errorplot\\_7.dat](#), [errorplot\\_9.dat](#), [errorplot\\_11.dat](#),  
[errorplot\\_13\\_14.dat](#), [errorplot\\_15.dat](#), [errorplot\\_19.dat](#), [errorplot\\_21.dat](#), [errorplot\\_23.dat](#)

The content of the file [score.dat](#) is depicted in Figure 6.7. This figure reveals that the RMDS decreased during first 100 optimization steps and, then, reached a plateau during next 400 optimization steps. Based on this, one can be conclude that genetic algorithm has converged to the global minimum after 500 optimization steps. The content of the file [fit.dat](#) reveals that the good fit to the PELDOR data was obtained (Figure 6.8) and, consequently the optimized geometric model provides an adequate estimate of the relative orientation of  $\text{Cu}^{2+}$  and nitroxide spin centers. The parameters of this model, which are stored in the file [parameters.dat](#), are listed in Table 6.3. The distance distribution that corresponds to the parameters  $\langle r_1 \rangle$ ,  $\Delta r_1$ ,  $\langle r_2 \rangle$ ,  $\Delta r_2$  and  $w$  is depicted in Figure 6.9. As expected, it contains two well pronounced peaks which correspond to two different conformers of the R1 side chain.



**Figure 6.7.** The goodness-of-fit, given by the RMSD between the experimental PELDOR time traces of T21R1 and their fits, is depicted in dependence of



**Figure 6.8.** The PELDOR time traces of T21R1 (black lines) are overlaid with the corresponding fits (red

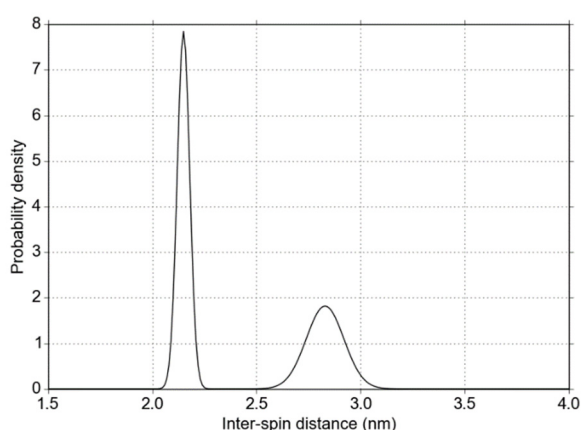
optimization step. Generated by means of [plotScore.py](#) (see Section 5.1).

**Table 6.3.** Optimized parameters of the geometric model of T21R1.

Parameter	Value
$\langle r_1 \rangle$	2.15 nm
$\Delta r_1$ (standard deviation)	0.03 nm
$\langle \xi_1 \rangle$	32°
$\langle \alpha_1 \rangle^*$	22°
$\langle \beta_1 \rangle$	2°
$\langle \gamma_1 \rangle^*$	87°
$\langle r_2 \rangle$	2.83 nm
$\Delta r_2$ (standard deviation)	0.09 nm
$\langle \xi_2 \rangle$	65°
$\langle \alpha_2 \rangle^*$	176°
$\langle \beta_2 \rangle$	3°
$\langle \gamma_2 \rangle^*$	107°
$w$	0.59

\* These parameters are poorly defined.

lines). Generated by means of [plotFit.py](#) (see Section 5.1).



**Figure 6.9.** The Cu<sup>2+</sup>-nitroxide distance distribution in T21R1. Generated by means of [plotDistDistr.py](#) (see Section 5.1).

The file [symmetric\\_parameters.dat](#) contains 16 symmetry-related sets of angular parameters. These are listed in Table 6.4.

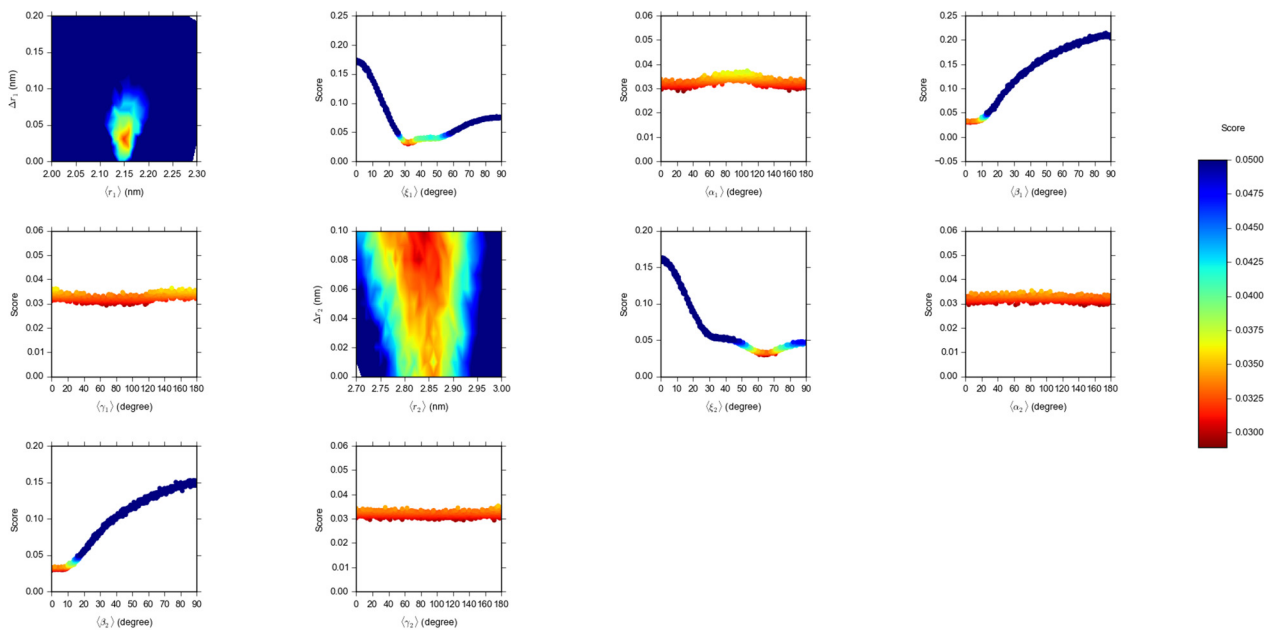
The last piece of information provided by PeldorFit is the so-called error plots. These plots reveal the dependence of the goodness-of-fit (RMDS) on the following parameters:  $\langle r_1 \rangle$  and  $\Delta r_1$  ([errorplot\\_1\\_2.dat](#)),  $\langle \xi_1 \rangle$  ([errorplot\\_3.dat](#)),  $\langle \alpha_1 \rangle$  ([errorplot\\_7.dat](#)),  $\langle \beta_1 \rangle$  ([errorplot\\_9.dat](#)),  $\langle \gamma_1 \rangle$  ([errorplot\\_11.dat](#)),  $\langle r_2 \rangle$  and  $\Delta r_2$  ([errorplot\\_13\\_14.dat](#)),  $\langle \xi_2 \rangle$  ([errorplot\\_15.dat](#)),  $\langle \alpha_2 \rangle$  ([errorplot\\_19.dat](#)),  $\langle \beta_2 \rangle$  ([errorplot\\_21.dat](#)),  $\langle \gamma_2 \rangle$  ([errorplot\\_23.dat](#)). All of them are shown in Figure 6.10. As can be seen, all distance parameters have a single well-defined minimum, i.e., they can be accurately determined from the PELDOR data of T21R1. To some extent, the same conclusion applies to the angles  $\langle \xi_i \rangle$  and  $\langle \beta_i \rangle$  ( $i = 1$  and  $2$ ). In contrast, the error plots for the angles  $\langle \alpha_i \rangle$  and  $\langle \gamma_i \rangle$  ( $i = 1$  and  $2$ ) do not show any defined minimum. Thus, these angles cannot be reliably determined from the present PELDOR data. The latter result can be well understood if one takes into account that the  $g$ -anisotropy of the nitroxide center is fairly low.

**Table 6.4.** Summary of the symmetry-related sets of angles for T21R1.

Symmetric transformation <sup>[a]</sup>	$\langle \xi_1 \rangle, ^\circ$	$\langle \phi_1 \rangle, ^\circ$	$\langle \alpha_1 \rangle, ^\circ$	$\langle \beta_1 \rangle, ^\circ$	$\langle \gamma_1 \rangle, ^\circ$
Fitting result	32	0	22	2	87
180° rotation about $g_{xx}^B$	32	0	202	178	93
180° rotation about $g_{yy}^B$	32	0	202	178	273
180° rotation about $g_{zz}^B$	32	0	22	2	267
180° rotation about $g_{xx}^A$	148	0	158	178	267
180° rotation about $g_{xx}^A$ and $g_{xx}^B$	148	0	338	2	273
180° rotation about $g_{xx}^A$ and $g_{yy}^B$	148	0	338	2	93
180° rotation about $g_{xx}^A$ and $g_{zz}^B$	148	0	158	178	87
180° rotation about $g_{yy}^A$	148	180	338	178	267
180° rotation about $g_{yy}^A$ and $g_{xx}^B$	148	180	158	2	273
180° rotation about $g_{yy}^A$ and $g_{yy}^B$	148	180	158	2	93
180° rotation about $g_{yy}^A$ and $g_{zz}^B$	148	180	338	178	87
180° rotation about $g_{zz}^A$	32	180	202	2	87
180° rotation about $g_{zz}^A$ and $g_{xx}^B$	32	180	22	178	93
180° rotation about $g_{zz}^A$ and $g_{yy}^B$	32	180	22	178	273
180° rotation about $g_{zz}^A$ and $g_{zz}^B$	32	180	202	2	267

Symmetric transformation <sup>[a]</sup>	$\langle \xi_2 \rangle, ^\circ$	$\langle \phi_2 \rangle, ^\circ$	$\langle \alpha_2 \rangle, ^\circ$	$\langle \beta_2 \rangle, ^\circ$	$\langle \gamma_2 \rangle, ^\circ$
Fitting result	65	0	176	3	107
180° rotation about $g_{xx}^B$	65	0	356	177	73
180° rotation about $g_{yy}^B$	65	0	356	177	253
180° rotation about $g_{zz}^B$	65	0	176	3	287
180° rotation about $g_{xx}^A$	115	0	4	177	287
180° rotation about $g_{xx}^A$ and $g_{xx}^B$	115	0	184	3	253
180° rotation about $g_{xx}^A$ and $g_{yy}^B$	115	0	184	3	73
180° rotation about $g_{xx}^A$ and $g_{zz}^B$	115	0	4	177	107
180° rotation about $g_{yy}^A$	115	180	184	177	287
180° rotation about $g_{yy}^A$ and $g_{xx}^B$	115	180	4	3	253
180° rotation about $g_{yy}^A$ and $g_{yy}^B$	115	180	4	3	73
180° rotation about $g_{yy}^A$ and $g_{zz}^B$	115	180	184	177	107
180° rotation about $g_{zz}^A$	65	180	356	3	107
180° rotation about $g_{zz}^A$ and $g_{xx}^B$	65	180	176	177	73
180° rotation about $g_{zz}^A$ and $g_{yy}^B$	65	180	176	177	253
180° rotation about $g_{zz}^A$ and $g_{zz}^B$	65	180	356	3	287

<sup>[a]</sup>  $g_{xx}^A$ ,  $g_{yy}^A$ , and  $g_{zz}^A$  denote the principal components of the  $g$ -tensor of spin A;  $g_{xx}^B$ ,  $g_{yy}^B$ , and  $g_{zz}^B$  denote the principal components of the  $g$ -tensor of spin B.



**Figure 6.10.** The error plots obtained for T21R1. Generated by means of `plotErrorPlot.py` (see Section 5.1).

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## 8 Afterword

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If you have any question about the program, I am opened for discussion: [abdullin@pc.uni-bonn.de](mailto:abdullin@pc.uni-bonn.de)