

AnisoDipFit

User Manual

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1 Introduction

1.1 What is it for?

Pulsed electron paramagnetic resonance dipolar spectroscopy (PDS)^[1,2] offers several techniques for measuring the dipolar coupling between electron spin centers. The PDS signal is acquired in the form of a time trace, which is modulated by dipolar frequencies. These frequencies can be obtained from the time trace by applying Fourier transform. For disordered samples, Fourier transform yields a distribution of dipolar frequencies, known also as dipolar spectrum. Importantly, dipolar spectra encode information about the distance between electron spin centers and, in some cases, also their relative orientation. In order to extract this spatial information, specialized algorithms of data analysis are required. In the case of the $S = 1/2$ centers with isotropic g -factors of 2.0023, the PDS data analysis can be done by means of the program DeerAnalysis.^[3] This program is also applicable to $S = 1/2$ centers, which have a small g -anisotropy (difference in g -values smaller than 0.1), such as nitroxide radicals. However, if the g -anisotropy of electron spin centers is significant, DeerAnalysis cannot be applied and alternative algorithms of data analysis are required.

The program AnisoDipFit was developed for the analysis of PDS signals that correspond to spin systems consisting of one isotropic and one anisotropic $S = 1/2$ centers. The mathematical background of this analysis is described in Refs. ^[4-6]. AnisoDipFit supports two operation modes: the simulation mode and the fitting mode. In the simulation mode, the PDS signal is calculated using the pre-defined geometric model of a spin system (see Chapter 1.2) and the spectroscopic parameters of spin centers. In the fitting mode, the geometry of a spin system (see Chapter 1.2) is optimized until the simulated PDS signal provides the best fit to the experimental PDS signal. This optimization procedure is done by means of a genetic algorithm.^[5]

The manual is organized as follows. In the rest of Chapter 1, the geometric model of AnisoDipFit and the technical information about the program are provided. Chapters 2 and 3 describe how one can download and run AnisoDipFit with user data, respectively. Chapter 4 provides a comprehensive description of the AnisoDipFit configuration file. The output of the program is described in Chapter 5. Finally, Chapter 6 provides several examples of using AnisoDipFit for the simulation and fitting of PDS signals.

1.2 Geometric model 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, the AnisoDipFit model of a spin system is limited by the following assumptions:

- 1) The spin system consists of two well-localized electron spin centers denoted as spin A and spin B.

- 2) Both electron spin centers have an effective spin $S = 1/2$. Spin A has an isotropic or almost isotropic g -tensor, whereas spin B has an anisotropic g -factor.
- 3) The reference coordinate system of the model is set to be coincident with the g -tensor axes of spin B. A vector that connects spin A with spin B is described by three spherical coordinates: a length r , a polar angle ξ , and an azimuthal angle φ (Figure 1.1).

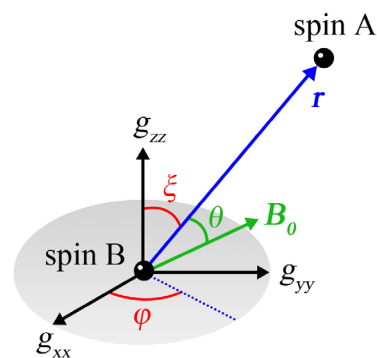


Figure 1.1. The DipFit model of a spin system.

- 4) In order to account for the conformational flexibility of the spin system, all three geometric parameters, namely r , ξ , and φ , are allowed to have either a uniform distribution or a normal distribution. In both cases, the distributions are described by two parameters, a mean value and a width (Figure 1.2). In the case of the normal distribution, the standard deviation is used as the width parameter.
- 5) Since the PDS data do not provide enough information about potential correlations between the geometric parameters r , ξ , and φ , the geometric model is simplified by assuming the correlations to be zero.

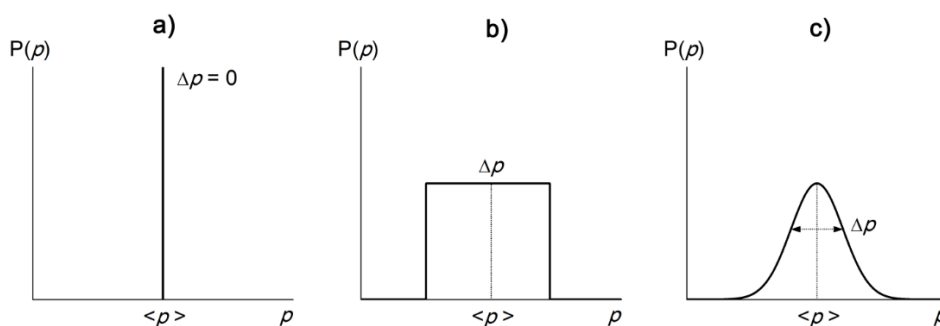


Figure 1.2. The distribution types used for the AnisoDipFit parameter $p \in (r, \xi, \varphi)$. **a)** A single value $\langle p \rangle$. The width Δp equals to 0. **b)** Uniform distribution with the mean value $\langle p \rangle$ and the width Δp . **c)** Normal distribution with the mean value $\langle p \rangle$ and the standard deviation Δp .

1.3 Technical information

AnisoDipFit is a console application written in Python3. The source code of the program can be found at

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

The Linux and Windows executables of the program can be downloaded from

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

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

All examples in this manual were tested 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 with the AnisoDipFit executables at

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

and unzip it into the directory where the program will be stored. That's all!

3 Running the program

- 1) Open Terminal (Linux) or Command Prompt (Windows).
- 2) Navigate into the directory with the AnisoDipFit executable:
`cd ../AnisoDipFit`
- 3) Set the permission properties (only for Linux):
`chmod 755 AnisoDipFit`
- 4) Prepare the configuration file (see Chapter 4) and run the program by the following command:
`sh AnisoDipFit.sh [path to the configuration file]` (for Linux)
`AnisoDipFit.exe [path to the configuration file]` (for Windows)

4 Configuration file

The AnisoDipFit configuration file contains all input parameters of the program. It has the usual ASCII data format and the *.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 the “examples” folder as a template to build your own configuration file. This will save time and reduce the probability of errors.

In general, the AnisoDipFit configuration file can contain up to eight information blocks:

- 1) operation mode,
- 2) experimental data and settings,
- 3) parameters of the spin system,
- 4) simulation settings,
- 5) fitting settings,
- 6) error analysis settings,
- 7) calculation settings,
- 8) output settings.

The information blocks 1-3 and 7-8 are mandatory. The information blocks 4-6 are optional and need to be specified only if the corresponding mode was selected. Each of these information blocks is described in detail below.

Note that comment lines in the configuration file are indicated by symbol # in the beginning of the line. Comment lines, as well as empty lines, are ignored by AnisoDipFit at execution.

4.1 Operation mode

As mentioned in Chapter 1, AnisoDipFit has two main operation modes: simulation and fitting. In the simulation mode, the PDS signal is calculated using the pre-defined geometric model of a spin system (Chapter 4.4). In the fitting mode, the geometric model of a spin system is optimized until the simulated PDS signal provides the best fit to the experimental one (Chapter 4.5). After the fitting, the precision of an optimized geometric model and its parameters is estimated through the error analysis. In addition, AnisoDipFit has an operation mode called “error analysis” mode. In this mode, the results of the fitting, which was done in the past, can be used to re-run or to extend the initial error analysis (Chapter 4.6).

The operation mode of AnisoDipFit is specified in the following line:

```
mode = 1;
```

(Source: *AnisoDipFit/examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg*)

Description:

`mode = 0` Activates the simulation mode.

`mode = 1` Activates the fitting mode.

`mode = 2` Activates the error analysis mode.

4.2 Experimental data

AnisoDipFit can be used for analysis of two types of experimental data, the background-free PDS time traces or the PDS spectra. The file paths to these data can be provided in the following way:

```
path_spectrum = "";  
path_timetrace = "examples/example02_ls_iron(III)_trityl/timetrace.dat";
```

(Source: *AnisoDipFit/examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg*)

Note that all files, which contain the experimental data, need to have the ASCII data format. If one wants to simulate/fit only the PDS time trace, `path_spectrum` has to be initialized by an empty string: `path_spectrum = ""`. If one wants to simulate/fit only the PDS spectrum, `path_timetrace` has to be initialized by an empty string: `path_timetrace = ""`. For the fitting and error analysis modes, at least one of the data types and the corresponding file path has to be provided. For the simulation mode, there is no need to specify any experimental data and, therefore, both file paths can be left empty. In cases, when the experimental data is still provided, the results of the simulation will be displayed together with the experimental data.

4.3 Parameters of the spin system

A spin system that is considered in AnisoDipFit consists of two $S = 1/2$ centers denoted as `spinA` and `spinB` (Chapter 1.2). Currently, `spinA` can have only an isotropic or almost isotropic g -tensor, whereas the g -tensor of `spinB` can also be anisotropic. The principal g -factors of both spins can be given as follows:

```
# trityl
spinA:
{
  type = "isotropic";
  g = [2.0032, 2.0032, 2.0032];
};

# low-spin iron(III)
spinB:
{
  type = "anisotropic";
  g = [1.56, 2.28, 2.91];
};
```

(Source: *AnisoDipFit/examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg*)

Description:

type The type of an electron spin center: “isotropic” or “anisotropic”.

g g-factor. It should always contain 3 components: $\mathbf{g} = [g_{xx}, g_{yy}, g_{zz}]$ in the case of an orthorhombic g-factor, $\mathbf{g} = [g_{\perp}, g_{\perp}, g_{\parallel}]$ in the case of an axial g-factor, $\mathbf{g} = [g_{iso}, g_{iso}, g_{iso}]$ in the case of an isotropic g-factor.

4.4 Simulation settings

These settings are relevant only for the simulation mode but can be omitted for the fitting and error analysis modes.

First, the type of simulated data is provided in the list called [simulation_modes](#):

```
simulation_modes:
{
  spc = 0;
  timetrace = 0;
  spc_vs_theta = 0;
  spc_vs_xi = 1;
  spc_vs_phi = 0;
  spc_vs_temp = 0;
};
```

(Source: *AnisoDipFit/examples/example01_ls_iron(III)_nitroxide/config_ex01_1.cfg*)

Description:

spc If set to 1, the dipolar spectrum will be simulated. If no experimental spectrum was defined (see [path_spectrum](#)), the frequency ranges will be set automatically.

timetrace If set to 1, the dipolar time trace will be simulated. If no experimental time trace was defined (see [path_timetrace](#)), the time ranges will be set automatically and the modulation depth will be set to [mod_depth](#).

spc_vs_theta If set to 1, the dipolar spectrum will be simulated in dependence of the angle θ between the inter-spin vector \vec{r} and the direction of the applied magnetic field \vec{B}_0 (Figure 1.1).

spc_vs_xi If set to 1, the dipolar spectrum will be simulated in dependence of the angle ζ between the inter-spin vector \vec{r} and the g_{zz} -axis of spin B (Figure 1.1).

<code>spc_vs_phi</code>	If set to 1, the dipolar spectrum will be simulated in dependence of the angle φ between the projection of the inter-spin vector \vec{r} on the xy -plane of spin B and the g_{xx} -axis of spin B (Figure 1.1).
<code>spc_vs_temp</code>	If set to 1, the dipolar spectrum will be simulated in dependence of temperature. This dependence is relevant only for RIDME data acquired at liquid helium temperatures. ^[5]

In order to switch off unwanted simulations, the corresponding setting should be set to 0.

Next, the parameters of the simulation(s) need to be specified in the list `simulation_parameters`:

```
simulation_parameters:
{
  r_mean = 2.50;
  r_width = 0.00;
  xi_mean = 0.0;
  xi_width = 0.0;
  phi_mean = 0.0;
  phi_width = 0.0;
  temp = 300.0;
}
```

(Source: *AnisoDipFit/examples/example01_ls_iron(III)_nitroxide/config_ex01_1.cfg*)

Description:

<code>r_mean</code>	The mean value $\langle r \rangle$ of the inter-spin distance distribution $P(r)$. Given in [nm].
<code>r_width</code>	The width Δr of the inter-spin distance distribution $P(r)$ (Figure 1.2). Given in [nm]. The shape of $P(r)$ is specified in the <code>calculation_settings</code> list (Chapter 7).
<code>xi_mean</code>	The mean value $\langle \xi \rangle$ of the angular distribution $P(\xi)$. Given in [degree].
<code>xi_width</code>	The width $\Delta \xi$ of the angular distribution $P(\xi)$ (Figure 1.2). Given in [degree]. The shape of $P(\xi)$ is specified in the <code>calculation_settings</code> list (Chapter 7).
<code>phi_mean</code>	The mean value $\langle \varphi \rangle$ of the angular distribution $P(\varphi)$. Given in [degree].
<code>phi_width</code>	The width $\Delta \varphi$ of the angular distribution $P(\varphi)$ (Figure 1.2). Given in [degree]. The shape of $P(\varphi)$ is specified in the <code>calculation_settings</code> list (Chapter 7).
<code>temp</code>	The temperature of the PDS experiment in [K]. Do not set this parameter to 0! Default value is 300 K.

Finally, several simulation settings have to be provided in the list `simulation_settings`:

```
simulation_settings:
{
  theta_ranges = [0.0, 90.0, 91];
  xi_ranges = [0.0, 90.0, 10];
  phi_ranges = [0.0, 90.0, 91];
  temp_ranges = [1.5, 300.0, 91];
  mod_depth = 0.5;
  faxis_normalized = 1;
  plot_3d = 0;
}
```

(Source: *AnisoDipFit/examples/example01_ls_iron(III)_nitroxide/config_ex01_1.cfg*)

Description:

<code>theta_ranges</code>	Defines the θ axis for the <code>spc_vs_theta</code> simulation. This axis is defined by three numbers: <code>theta_ranges</code> = [θ_{min} , θ_{max} , N_θ], where θ_{min} and θ_{max} are the minimal and maximal values of θ , respectively, and N_θ is the number of evenly spaced samples in the interval [θ_{min} , θ_{max}].
<code>xi_ranges</code>	Defines the ξ axis for the <code>spc_vs_xi</code> simulation. This axis is defined by three numbers: <code>xi_ranges</code> = [ξ_{min} , ξ_{max} , N_ξ], where ξ_{min} and ξ_{max} are the minimal and maximal values of ξ , respectively, and N_ξ is the number of evenly spaced samples in the interval [ξ_{min} , ξ_{max}].
<code>phi_ranges</code>	Defines the φ axis for the <code>spc_vs_phi</code> simulation. This axis is defined by three numbers: <code>phi_ranges</code> = [φ_{min} , φ_{max} , N_φ], where φ_{min} and φ_{max} are the minimal and maximal values of φ , respectively, and N_φ is the number of evenly spaced samples in the interval [φ_{min} , φ_{max}].
<code>temp_ranges</code>	Defines the temperature axis (T axis) for the <code>spc_vs_temp</code> simulation. This axis is defined by three numbers: <code>temp_ranges</code> = [T_{min} , T_{max} , N_T], where T_{min} and T_{max} are the minimal and maximal values of T , respectively, and N_T is the number of evenly spaced samples in the interval [T_{min} , T_{max}].
<code>mod_depth</code>	The modulation depth of the simulated time trace. It needs to be given when the simulation of the time trace is activated (<code>timetrace</code> = 1) and no experimental time trace was defined (<code>path_timetrace</code> = "").
<code>faxis_normalized</code>	If set to 1, the frequency axis of the simulated dipolar spectra will be normalized by the dipolar coupling constant of an isotropic spin pair: $\nu_0 = \frac{\mu_0}{4\pi} \frac{\beta_e^2 g_e^2}{r^3},$ where μ_0 is the vacuum permeability, β_e is the Bohr magneton, g_e is the g -factor of free electron, and r is set to the value of <code>r_mean</code> in the <code>simulation_parameters</code> list.
<code>plot_3d</code>	If set to 1, the results of the <code>spc_vs_xi</code> , <code>spc_vs_phi</code> , and <code>spc_vs_temp</code> simulations will be depicted as 3-dimensional plots.

4.5 Fitting settings

These settings are relevant for the fitting and error analysis modes but can be omitted for the simulation mode.

A list with all possible fitting parameters of AnisoDipFit is called `fitting_parameters` and has the following structure:

```
fitting_parameters:
{
  r_mean :    opt = 1;    range = [2.00, 3.00]; value = 0.0  };
  r_width :   opt = 1;    range = [0.00, 0.10]; value = 0.0  };
  xi_mean :   opt = 1;    range = [ 0.0, 90.0]; value = 0.0  };
  xi_width :  opt = 1;    range = [ 0.0, 30.0]; value = 0.0  };
  phi_mean :  opt = 1;    range = [ 0.0, 90.0]; value = 0.0  };
  phi_width : opt = 1;    range = [ 0.0, 30.0]; value = 0.0  };
  temp :      opt = 0;    range = [ 9.0, 11.0]; value = 10.0 };
};
```

(Source: *AnisoDipFit/examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg*)

In this list, each text line corresponds to one of the fitting parameters (Table 4.1) and contains three settings called `opt`, `range`, and `value`. The most important setting is `opt`, since it determines whether the corresponding parameter will be included into the fitting or not:

`opt` If `opt = 0`, the parameter is excluded from the fitting.

 If `opt = 1`, the parameter is included into the fitting.

In the case of `opt = 1`, the ranges for the corresponding parameter have to be specified in the `range` field. The appropriate ranges of all fitting parameters are given Table 4.1. In the case of `opt = 0`, the corresponding parameter will be set to a constant value, which has to be provided in the `value` field. The units of the `range` and `value` settings for each of the fitting parameters are given in Table 4.1.

Table 4.1. The fitting parameters of AnisoDipFit.

Parameter keyword	Parameter description ^[a]	Ranges	Units
<code>r_mean</code>	The mean value $\langle r \rangle$ of the inter-spin distance distribution $P(r)$	Any reasonable ranges	nm
<code>r_width</code>	The width Δr of the inter-spin distance distribution $P(r)$	Any reasonable ranges	nm
<code>xi_mean</code>	The mean value $\langle \zeta \rangle$ of the angular distribution $P(\zeta)$	[0, 90]	degree
<code>xi_width</code>	The width $\Delta \zeta$ of the angular distribution $P(\zeta)$	[0, 90]	degree
<code>phi_mean</code>	The mean value $\langle \varphi \rangle$ of the angular distribution $P(\varphi)$	[0, 90]	degree
<code>phi_width</code>	The width $\Delta \varphi$ of the angular distribution $P(\varphi)$	[0, 90]	degree
<code>temp</code> ^[b]	The temperature of the PDS experiment	Any reasonable ranges	K

^[a] The type of the distributions $P(r)$, $P(\zeta)$, and $P(\varphi)$ is specified in the `calculation_settings` list (Chapter 7).

^[b] This parameter is relevant only for the RIDME data acquired at liquid helium temperatures.^[5]

Another important aspect of the fitting is an optimization algorithm. Currently, AnisoDipFit allows using only one optimization algorithm, namely the 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.^[7–10] Importantly, the 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 PDS data set. Therefore, the configuration file allows users to set these parameters manually. However, it is highly recommended to use the genetic algorithm’s parameters from the configuration files given in the “examples” folder, because these parameters were obtained after extensive tests of the genetic algorithm on numerous PDS data sets.

The parameters of the optimization algorithm are provided in the list [fitting_settings](#):

```
fitting_settings:
{
  fitted_data = "timetrace";
  display_graphics = 0;
  method = "genetic";
  num_generations = 500;
  size_generation = 128;
  prob_crossover = 0.5;
  prob_mutation = 0.01;
};
```

(Source: *AnisoDipFit/examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg*)

Description:

fitted_data	The type of the fitted data: “timetrace” or “spectrum”. If fitted_data = “timetrace”, the file path of the experimental PDS time trace has to be specified in path_timetrace . If fitted_data = “spectrum”, the file path of the experimental PDS spectrum has to be specified in path_spectrum .
display_graphics	If set to 1, the results of fitting will be displayed after each optimization step. In order to fasten the fitting procedure, display_graphics is recommended to be set to 0.
method	The type of the optimization algorithm. Currently, only the genetic algorithm can be chosen: method = “genetic”.
num_generations	The total number of optimization steps (generations). ^[10]
size_generation	The number of parameter sets (chromosomes) that are evaluated simultaneously by the genetic algorithm. ^[10] A good practice is to set this parameter to 10 x (the number of fitting parameters).
prob_crossover	The crossover rate. ^[10]
prob_mutation	The mutation rate. ^[10]

4.6 Error analysis settings

These settings are relevant for the fitting and error analysis modes but can be omitted for the simulation mode.

The fitting of PDS data by AnisoDipFit yields the optimized values of fitting parameters, whose precision is unknown. To fill this gap, AnisoDipFit performs an error analysis. Since the parameter space is quite large (up to 7 fitting parameters) and the fitting itself is very time-consuming (hours), usual error analyses, such as bootstrap^[11] and Bayesian analysis^[12], cannot be done within a reasonable time. Therefore, the error analysis is done in a less general way. First, χ^2 is recorded in dependence of $\langle r \rangle$ and Δr , $\langle \zeta \rangle$ and $\Delta \zeta$, $\langle \varphi \rangle$ and $\Delta \varphi$, and T . While recording each of these dependences, the remaining fitting parameters are set to their optimized values. For example, when χ^2 is recorded as a function of $\langle r \rangle$ and Δr , the values of $\langle \zeta \rangle$, $\langle \varphi \rangle$, $\Delta \zeta$, $\Delta \varphi$, and T are fixed at the corresponding optimized values (or at the user-defined values, if some of them were not optimized). Determination of parameters' errors from the obtained dependencies relies on the assumption that the contributions to χ^2 from $P(r)$, $P(\zeta)$, $P(\varphi)$, and T are uncorrelated, at least, near the global minimum. This assumption was confirmed in the previous study,^[6] but in general, it allows to estimate only the lower bound of parameters' uncertainty. Prior to the error estimation, each two-dimensional dependence, e.g. $\chi^2(\langle r \rangle, \Delta r)$, is converted into two one-dimensional dependencies that are optimized with respect to the first or second parameter of the corresponding two-dimensional dependence, e.g. $\chi^2(\langle r \rangle)$ and $\chi^2(\Delta r)$. The obtained one-dimensional dependencies are then used to determine parameter ranges, in which the deviation of the χ^2 values from the minimal χ^2 is less than $\Delta \chi^2$. The threshold $\Delta \chi^2$ is build up out of two contributions. The first contribution, $\Delta \chi_{ci}^2$, takes into account errors related to the noise in the experimental data and possible discrepancies between the actual spin system and its geometric model. $\Delta \chi_{ci}^2$ is calculated using the user-defined confidence level, e.g. a 3σ confidence level. If one assumes that the measurement errors are distributed normally, $n\sigma$ confidence level corresponds to $\Delta \chi_{ci}^2 = n^2$.^[11] Thus, for the 3σ confidence level one obtains $\Delta \chi_{ci}^2 = 9$. The second contribution, $\Delta \chi_{ne}^2$, takes into account the numerical error, which is mostly determined by the accuracy of the Monte-Carlo integration. The value of $\Delta \chi_{ne}^2$ is estimated by calculating χ^2 for the 10^4 identical sets of optimized fitting parameters and, subsequently, finding the difference between the maximal and minimal values of χ^2 . Lastly, the determined uncertainty ranges are converted into the errors of the fitting parameters by calculating the largest deviation of each parameter from its optimized value within the corresponding uncertainty ranges.

The settings of the error analysis have to be specified in the list called `error_analysis`:

```
error_analysis:
{
  variables = (("r_mean", "r_width"), ("xi_mean", "xi_width"), ("phi_mean", "phi_width"));
  Ns = 10000;
  confidence_interval = 3;
  path_optimized_parameters = "";
};
```

(Source: *AnisoDipFit/examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg*)

Description:

variables The list of fitting parameters used for the error analysis. If no error analysis is wanted, this list should be left empty: `variables = ()`. Otherwise, the variables of each separate error calculation should be enclosed in round brackets, e.g., `variables = (("r_mean", "r_width"), ("xi_mean", "xi_width"), ("temp"))`. In the given example, the following three dependences will be recorded: 1) the two-dimensional dependence of χ^2 on $\langle r \rangle$ and Δr , 2) the two-dimensional dependence of χ^2 on $\langle \zeta \rangle$ and $\Delta \zeta$, and 3) the one-dimensional dependence of χ^2 on temperature. Note that the names of fitting parameters are given in accordance to their keywords (column 1 in Table 4.1) While recording the dependence of χ^2 on the selected fitting parameter(s), the latter one(s) will be randomly set to **Ns** different values within the ranges specified in the **fitting_parameters** list.

Ns The number of points in each validation data set.

confidence_interval The confidence interval of fitting parameters in σ units. If one assumes that the measurement errors are distributed normally and the fitting model is linear in its parameters, the 1σ , 2σ , 3σ confidence intervals include the true value of the fitting parameter with the probabilities of 68 %, 95 %, and 99.7 %, respectively.

As mentioned in Chapter 4.1, AnisoDipFit has an additional operation mode called “error analysis” mode. The idea behind this mode is the same as for the error analysis described above, but in contrast to the fitting mode, the error analysis can be done post factum, i.e., using the results of a fitting that was completed in the past. In order to activate this mode, one has to set `mode = 2` (Chapter 4.1) and provide the path to the file `parameters.dat`, which stores the optimized values of the fitting parameters (Chapter 5.2). The latter file path has to be given in the `path_optimized_parameters` field of the `error_analysis` list:

```
error_analysis:
{
    variables = (("xi_mean", "phi_mean"), ("r_width", "xi_mean"), ("r_width", "xi_width"),
                ("r_width", "phi_mean"), ("r_width", "phi_width"));
    Ns = 10000;
    confidence_interval = 3;
    path_optimized_parameters = "examples/example02_ls_iron(III)_trityl/for_validation/parameters.dat"
};
```

(Source: AnisoDipFit/examples/example02_ls_iron(III)_trityl/config_ex02_2.cfg)

4.7 Calculation settings

Calculation settings, which are relevant for all three operation modes, have to be provided in the list `calculation_settings`:

```
calculation_settings:
{
  Ns = 1000000;
  r_distr = "normal";
  xi_distr = "normal";
  phi_distr = "normal";
  fmin = 0;
  fmax = 0;
  tmin = 0;
  tmax = 0;
  g_selectivity = 0;
  magnetic_field = 0;
  noise_std = 0;
};
```

(Source: *AnisoDipFit/examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg*)

Description:

<code>Ns</code>	The number of Monte-Carlo samples used in numerical integration. It determines how accurately the PDS time trace or the PDS spectra will be simulated. ^[6] Do not set this value below 10^6 !
<code>r_distr</code>	The shape of the inter-spin distance distribution $P(r)$: “uniform” or “normal” (Figure 1.2).
<code>xi_distr</code>	The shape of the angular distribution $P(\xi)$: “uniform” or “normal” (Figure 1.2).
<code>phi_distr</code>	The shape of the angular distribution $P(\varphi)$: “uniform” or “normal” (Figure 1.2).
<code>fmin</code>	The minimal frequency of the simulated spectrum. Given in [MHz].
<code>fmax</code>	The maximal frequency of the simulated spectrum. Given in [MHz].
<code>tmin</code>	The minimal time value of the simulated time trace. Given in [μ s].
<code>tmax</code>	The maximal time value of the simulated time trace. Given in [μ s].
<code>g_selectivity</code>	If set to 1, the difference in spin polarization for the different g-values of spin B will be taken into account. ^[5] This parameter is relevant only for the RIDME data acquired at liquid helium temperatures.
<code>magnetic_field</code>	The value of the applied magnetic field used in the PDS experiment. Given in [T]. This parameters has to be provided only if <code>g_selectivity = 1</code> .
<code>noise_std</code>	The standard deviation of noise in the experimental data. When it is unknown, set <code>noise_std</code> to 0.

4.8 Output settings

In the last part of the configuration file, output settings are provided in the list called `output`:

```
output:
{
  directory = "";
  save_data = 1;
  save_figures = 1;
};
```

(Source: *AnisoDipFit/examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg*)

Description:

<code>directory</code>	The output directory of AnisoDipFit. AnisoDipFit will create a new folder inside this directory and name it in accordance to the current date and time. All simulation/fitting/error analysis results will then be saved into this folder. When left empty, i.e. <code>directory = ""</code> , the directory of the configuration file will be used as the output directory.
<code>save_data</code>	The data obtained from simulation/fitting/error analysis will be saved into DAT files (Chapter 5).
<code>save_figures</code>	The graphical data obtained from simulation/fitting/error analysis will be saved into PNG files (Chapter 5).

5 Output data

The output data of AnisoDipFit is stored in two forms: numerical and graphical. The numerical data is saved as DAT files, whereas the graphical data as PNG files (see `output`). The detailed description of all output files is given below.

5.1 Simulation output

Depending on the simulation (Chapter 4.4) and output (Chapter 4.8) settings of the configuration file, the following output files will be created:

- `spc.dat`
 - Data:* a simulated dipolar spectrum.
 - Content:* column 1 – frequency values in [MHz];
column 2 – a simulated spectrum;
column 3 – an experimental spectrum (optional).
- `timetrace.dat`
 - Data:* a simulated dipolar time trace.
 - Content:* column 1 – time points in [μ s];
column 2 – a simulated time trace;
column 3 – an experimental time trace (optional).

- [spc_vs_X.dat](#), where **X** = [theta](#), [xi](#), [phi](#), or [T](#)
Data: dipolar spectra simulated for the different values of parameter **X**.
Content: column 1 – frequency values in [MHz];
next columns – a spectrum which is simulated with the **X** value given in the first row of the column.
- [spc.png](#)
Content: the picture of a simulated dipolar spectrum.
- [timetrace.png](#)
Content: the picture of a simulated dipolar time trace.
- [spc_vs_X.png](#), where **X** = [theta](#), [xi](#), [phi](#), or [T](#)
Content: the picture of dipolar spectra simulated for the different values of parameter **X**.

5.2 Fitting output

Depending on the fitting (Chapter 4.5), validation (Chapter 4.6), and output (Chapter 4.8) settings of the configuration file, the following output files will be created:

- [score.dat](#)
Data: the goodness of fit (χ^2) in dependence of optimization step.
Content: column 1 – optimization steps;
column 2 – χ^2 values.
- [fit.dat](#)
Data: a fit to an experimental PDS time trace or an experimental PDS spectrum.
Content: If [fitted_data](#) = “[spectrum](#)”:
column 1 – frequency values in [MHz];
column 2 – an experimental PDS spectrum;
column 3 – a fit.
If [fitted_data](#) = “[timetrace](#)”:
column 1 – time points in [μ s];
column 2 – an experimental PDS time trace;
column 3 – a fit.
- [parameters.dat](#)
Data: the optimized values of fitting parameters.
Content: column 1 – the names and the units of fitting parameters;
column 2 – the values of fitting parameters;
column 3 – information about activated (“Y”) and deactivated (“N”) fitting parameters (identical to the [opt](#) setting of the [fitting_parameters](#) list);
column 4 – the errors of fitting parameters;

- [parameter_errors-X.dat](#) or [parameter_errors-X-Y.dat](#), where **X** and **Y** are the keywords used for the fitting parameters (see column 1 in Table 4.1)
Data: the goodness of fit (χ^2) in dependence of fitting parameter(s) **X** (and **Y**)
Content: For one fitting parameter **X**:
column 1 – **X** values;
column 2 – χ^2 values;
For two fitting parameter **X** and **Y**:
column 1 – **X** values;
column 2 – **Y** values;
column 3 – χ^2 values.
- [score.png](#)
Content: the picture of the goodness of fit (χ^2) in dependence of optimization step.
- [fit.png](#)
Content: the picture of a fit to an experimental data.
- [parameter_errors.png](#)
Content: the picture of the goodness of fit (χ^2) in dependence of the fitting parameters specified in the [error_analysis](#) list.
- [confidence_intervals.png](#)
Content: the picture of the goodness of fit (χ^2) in dependence of single fitting parameters specified in the [error_analysis](#) list.

6 Examples

This chapter provides several examples of how one can use AnisoDipFit to simulate and to fit the PDS data.

6.1 Simulation of the dipolar spectra of a low-spin Fe^{3+} -organic radical spin system

The first example deals with the spectral simulations for a spin system consisting of a low-spin Fe^{3+} (ls-Fe^{3+}) and an organic radical. The principal g -values of the ls-Fe^{3+} are set to $g_{1xx} = 1.56$, $g_{1yy} = 2.28$, and $g_{1zz} = 2.91$. The organic radical is assumed to have an isotropic g -factor which is equal to $g_e = 2.0023$. The distance between both spin centers is fixed at 2.50 nm, whereas the values of the ζ and φ angles are varied in the range $[0^\circ, 90^\circ]$ with steps of 10° and 30° , respectively. Thus, the angular dependence of the dipolar spectrum will be explored.

The workflow of this simulation includes the following steps:

- 1) *Prepare the configuration file.* The detailed information on how one creates such a configuration file is given in Chapter 4. The ready-to-use configuration files can be found in the following directory:

[/AnisoDipFit/examples/example01_ls_iron\(III\)_nitroxide](#)

This directory contains four configuration files [config_ex01_i.cfg](#), where $i = 1, 2, 3$, and 4. Each of these configuration files sets up the spectral simulation in dependence of ζ (the [spc_vs_xi](#) simulation mode), while setting φ to one of four different values: $\varphi = 0^\circ$ ($i = 1$), $\varphi = 30^\circ$ ($i = 2$), $\varphi = 60^\circ$ ($i = 3$), and $\varphi = 90^\circ$ ($i = 4$). Note that the [faxis_normalized](#) parameter is set to 1 in all four configuration files, meaning that the frequency axis of the simulated spectra will be normalized by the dipolar coupling constant ν_0 of an isotropic spin pair with the same inter-spin distance as mentioned above.

- 2) *Run the program.* First, open Terminal (Linux) or Command Prompt (Windows) and navigate to the directory:

[cd ../AnisoDipFit](#)

If needed, set the permission properties (only for Linux):

[chmod 755 AnisoDipFit](#)

Finally, run the program by the following command:

[sh AnisoDipFit.sh examples/example01_ls_iron\(III\)_nitroxide/config_ex01_i.cfg](#) (for Linux)

[AnisoDipFit.exe examples/example01_ls_iron\(III\)_nitroxide/config_ex01_i.cfg](#) (for Windows)

- 3) *Monitor the progress status.* During the program operation, the following messages should appear in Terminal or Command Prompt:

[Reading out the configuration file... \[DONE\]](#)

[Starting the simulation...](#)

[Running the pre-calculations... \[DONE\]](#)

[Calculating the dipolar spectrum vs xi... 100% \[DONE\]](#)

[The simulation is finished](#)

Saving the simulation results into the directory:

.../AnisoDipFit/examples/example01_ls_iron(III)_nitroxide/2020-01-01_00-00/ [DONE]

- 4) *Explore the simulation results.* During the simulation, a new folder will be created inside the directory /AnisoDipFit/examples/example01_ls_iron(III)_nitroxide and the results of simulations will be saved in that folder. In the given example, the program will create two output files, [spc_vs_xi.dat](#) and [spc_vs_xi.png](#). The content of [spc_vs_xi.png](#) will pop up on the screen after the simulation.

Dipolar spectra, obtained from all four simulations, are shown in Figure 6.1. This figure reveals a prominent deviation of the simulated spectra from the Pake doublet.^[13] Whereas the Pake doublet has two characteristic singularities, referred to as perpendicular ($\theta = 90^\circ$) and parallel ($\theta = 0^\circ$) components, all calculated spectra here, display three singularities instead. In analogy to the Pake doublet, these singularities can be subdivided into two perpendicular components, which correspond to $\theta = 90^\circ$, and one parallel component, which corresponds to $\theta = 0^\circ$. Moreover, the frequencies, at which the singularities appear in Figure 6.1, do not have a fixed ratio, as in the case of the Pake doublet, but depend on the principal g -values of the ls-Fe^{3+} center and the angles ζ and φ . This dependence can be readily explained on example of four spectra corresponding to the angular combinations $(\zeta, \varphi) = (0^\circ, 0^\circ)$, $(0^\circ, 90^\circ)$, $(90^\circ, 0^\circ)$ and $(90^\circ, 90^\circ)$. For the angular combination $(0^\circ, 0^\circ)$, the inter-spin vector \vec{r} is collinear to the g_{zz} -axis of the ls-Fe^{3+} g -tensor. Consequently, the parallel component of the spectrum is scaled by g_{zz} , yielding a singularity at $2(g_{zz}/g_e)\nu_0 \approx 2.91 \nu_0$. Then, the other two components of the ls-Fe^{3+} g -tensor give rise to two perpendicular components, which appear at $(g_{xx}/g_e)\nu_0 \approx 0.78 \nu_0$ and $(g_{yy}/g_e)\nu_0 \approx 1.14 \nu_0$. The same assignment of singularities also holds for the spin pair geometry with $(\zeta, \varphi) = (0^\circ, 90^\circ)$, because the shape of the dipolar spectrum does not depend on the φ angle as soon as the ζ angle equals 0° . For the angular combination $(90^\circ, 0^\circ)$, \vec{r} is aligned along the g_{xx} -axis of the ls-Fe^{3+} g -tensor. Thus, the parallel component of the spectrum is scaled by g_{xx} and appears at components $2(g_{xx}/g_e)\nu_0 \approx 1.56 \nu_0$, whereas two perpendicular components are scaled by g_{yy} and g_{zz} and appear at $(g_{yy}/g_e)\nu_0 \approx 1.14 \nu_0$ and $(g_{zz}/g_e)\nu_0 \approx 1.45 \nu_0$, respectively. Finally, the angular combination $(90^\circ, 90^\circ)$ corresponds to the case where \vec{r} is collinear to the g_{yy} -axis of the ls-Fe^{3+} g -tensor. In this case, the parallel component of the spectrum is determined by the value of g_{yy} , which yields the singularity at $2(g_{yy}/g_e)\nu_0 \approx 2.28 \nu_0$, whereas the perpendicular components of the spectrum are scaled by g_{xx} and g_{zz} and appear at $(g_{xx}/g_e)\nu_0 \approx 0.78 \nu_0$ and $(g_{zz}/g_e)\nu_0 \approx 1.45 \nu_0$.

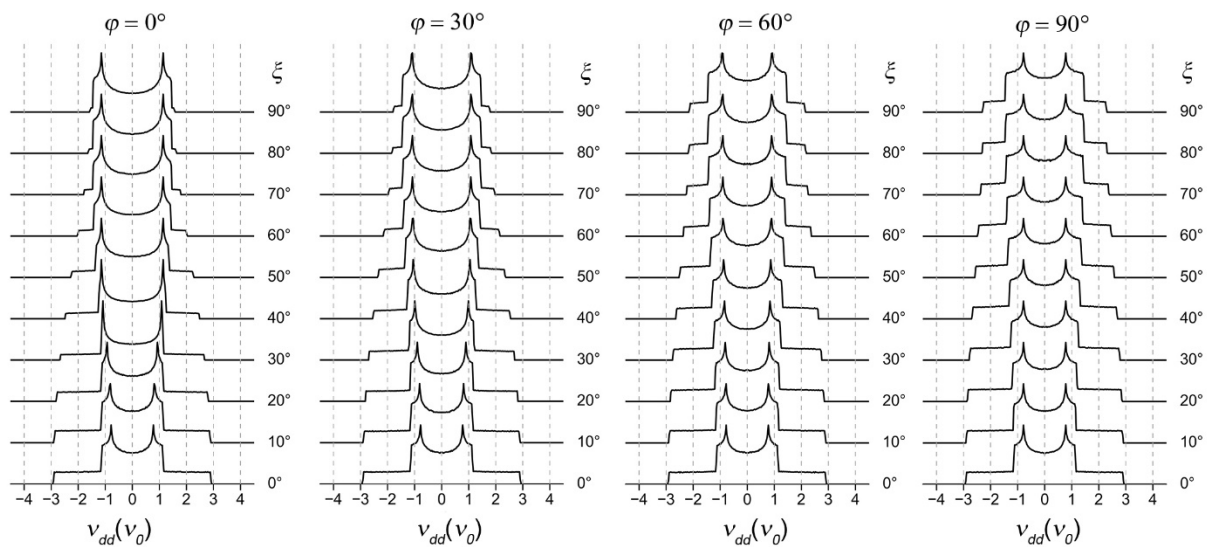


Figure 6.1. The angular dependence of the dipolar spectrum, which corresponds to the spin system consisting of an isotropic organic radical with $g = g_e$ and an anisotropic ls-Fe³⁺ with $g = [1.56, 2.28, 2.91]$. The angles ζ and φ are defined in Figure 1.1.

6.2 Fitting of the PDS time trace of a low-spin Fe^{3+} -trityl spin system

In the second example, the fitting and error analysis modes of AnisoDipFit will be explored. The PDS data, which is used for this example, was acquired on the model compound **1**^[6] (Figure 6.2). This compound contains two spatially separated electron spin centers, the trityl radical and the low-spin Fe^{3+} (ls-Fe^{3+}). The former spin center has an almost isotropic g -factor of 2.0032, whereas the latter one shows a pronounced g -anisotropy with three principal components $g_{xx} = 1.56$, $g_{yy} = 2.28$, and $g_{zz} = 2.91$. The PDS measurements on **1** were done using the pulse sequence relaxation induced dipolar modulation enhancement (RIDME)^[14,15] and yielded the background-free time trace shown in Figure 6.3b. Here, this time trace will be fitted using the parameters of $P(r)$, $P(\zeta)$, and $P(\varphi)$ as fitting parameters.

The workflow of this fitting includes the following steps:

- 1) *Prepare the configuration file.* The detailed information on how to create such a configuration file is given in Chapter 4. The ready-to-use configuration file [config_ex02_1.cfg](#) can be found in the following directory:

[/AnisoDipFit/examples/example02_ls_iron\(III\)_trityl](#)

As follows from this configuration file, the RIDME time trace of **1** is going to be fitted (see [fitted_data](#)). The mean values and the widths of the distributions $P(r)$, $P(\zeta)$, and $P(\varphi)$ are set to be the fitting parameters (see [fitting_parameters](#)). All three distributions are approximated by normal distributions (see [calculation_settings](#)) and, therefore, their widths are given by standard deviations. The genetic algorithm is set to be the fitting method. Its internal parameters are provided in [fitting_settings](#). Among them, the total number of optimization steps is set to 500. For [error analysis](#), the recording of the following data sets is activated: 1) the two-dimensional dependence of χ^2 on $\langle r \rangle$ and Δr , 2) the two-dimensional dependence of χ^2 on $\langle \zeta \rangle$ and $\Delta \zeta$, and 3) the two-dimensional dependence of χ^2 on $\langle \varphi \rangle$ and $\Delta \varphi$. Each of these data sets will contain 10^4 data points (see [Ns](#)). The errors of the fitting parameters will be determined at the 3σ level (see [confidence_interval](#)).

- 2) *Run the program.* First, open Terminal (Linux) or Command Prompt (Windows) and navigate to the directory:

[cd ../AnisoDipFit](#)

If needed, set the permission properties (only for Linux):

[chmod 755 AnisoDipFit](#)

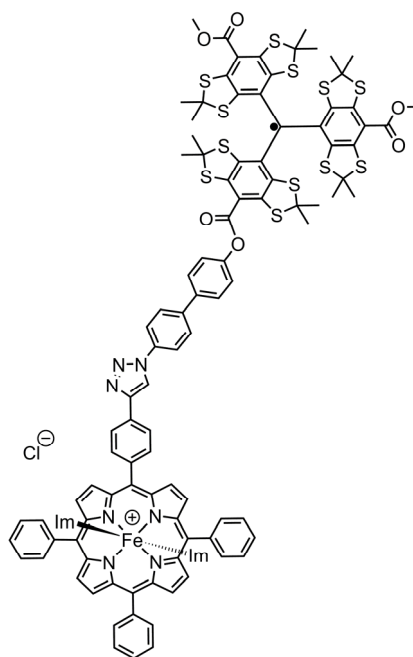


Figure 6.2. The chemical structure of the model compound **1**.

Finally, run the program by the following command:

`sh AnisoDipFit.sh examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg` (for Linux)

`AnisoDipFit.exe examples/example02_ls_iron(III)_trityl/config_ex02_1.cfg` (for Windows)

- 3) *Monitor the progress status.* During the program operation, the following messages should appear in Terminal or Command Prompt:

```
Reading out the configuration file... [DONE]
Starting the fitting...
Optimization step 500 / 500: chi2 = 0.002799
The fitting is finished. Total duration: 17:26:27.807848
Saving the fitting results into the directory:
.../AnisoDipFit/examples/example02_ls_iron(III)_trityl/2020-01-01_00-00/ [DONE]
Plotting the fitting results... [DONE]
Starting the error analysis...
Calculating chi2 in dependence of fitting parameters ...
Numerical error = 0.000283
Minimal RMSD = 0.002971
RMSD threshold = 0.003566
Calculating the RMSD in dependence of fitting parameters ...
Calculation step 3 / 3
Calculating the errors of fitting parameters ...
Numerical error (chi2) = 53.877103
Score threshold (chi2) = 62.877103
The error analysis is finished. Total duration: 9:29:43.359587
Saving the results of the error analysis into the directory:
.../AnisoDipFit/examples/example02_ls_iron(III)_trityl/2020-01-01_00-00/ [DONE]
Plotting the results of the error analysis... [DONE]
Optimized fitting parameters:
```

Parameter	Value	Optimized	Precision (+/-)
r mean (nm)	2.637	Y	0.012
r width (nm)	0.058	Y	0.029
xi mean (deg)	85.505	Y	20.764
xi width (deg)	22.960	Y	27.073
phi mean (deg)	84.853	Y	34.421
phi mean (deg)	50.985	Y	31.559
temperature (K)	10.000	N	nan

Here, a few comments have to be made. First, the calculation time strongly depends on the used hardware (Chapter 1.3). Second, the optimized values of the fitting parameters might slightly differ between single fitting trials. Importantly, this deviation is below the error of the fitting parameters (see below).

- 4) *Explore the fitting results.* During the fitting, a new folder will be created inside the directory `/AnisoDipFit/examples/example02_ls_iron(III)_trityl` and the results of the fitting will be saved in that folder. The output files include `score.dat`, `fit.dat`, `parameters.dat`, `parameter_errors-r_mean-r_width.dat`, `parameter_errors-xi_mean-xi_width.dat`, `parameter_errors-phi_mean-phi_width.dat`, and the corresponding graphical files (see Chapter 5.2).

Next, the results of the fitting will be briefly discussed. First, it is important to make sure that the genetic algorithm has converged to the global minimum. This information can be obtained from the output files [score.dat](#) and [score.png](#), which contain the dependence of χ^2 on the optimization step. In the present fitting, the χ^2 fell gradually down during the first 270 optimization steps and, after this, did not change significantly during the last 230 optimization steps (Figure 6.3a). This reveals that the minimum of the optimization problem was reached. Since the genetic algorithm is capable to find the global minimum even for optimization problems with several local minima, the obtained minimum is most likely global. This is supported by fact that a good fit to the RIDME data was indeed obtained. The fit is stored in the output files [fit.dat](#) and [fit.png](#) and depicted here in Figure 6.3b. The corresponding optimized values of the fitting parameters are stored in the output file [parameters.dat](#). The content of this file is listed in Figure 6.3c. In order to estimate the errors of these parameters, the following data was recorded: 1) the two-dimensional dependence of χ^2 on $\langle r \rangle$ and Δr ([parameter_errors-r_mean-r_width.dat](#)), 2) the two-dimensional dependence of χ^2 on $\langle \zeta \rangle$ and $\Delta \zeta$ ([parameter_errors-xi_mean-xi_width.dat](#)), and 3) the two-dimensional dependence of χ^2 on $\langle \varphi \rangle$ and $\Delta \varphi$ ([parameter_errors-phi_mean-phi_width.dat](#)). The picture of these data sets is stored in the output file [parameter_errors.png](#) and shown here in Figure 6.3d. In this figure, the uncertainty ranges of the fitting parameters are depicted in dark red, and the optimized values of the fitting parameters are depicted by white dots. As expected, all dots appear within the uncertainty ranges mentioned above, meaning that the optimized values of the fitting parameters lay within the corresponding uncertainty ranges. In order to determine the errors of fitting parameters, all three two-dimensional dependencies from Figure 6.3c were transformed into one-dimensional dependencies, which contain the dependence of χ^2 on each single fitting parameter. The one-dimensional dependencies are stored in the output file [confidence_intervals.png](#), whose content is depicted in Figure 6.3e. As can be seen, all one-dimensional dependencies reveal a single, well-defined minimum. The χ^2 threshold, which was used to determine the uncertainty intervals of the fitting parameters, is depicted by the black dashed line. The errors of individual fitting parameters, which were derived from the uncertainty intervals, are listed Figure 6.3c. The errors of the mean inter-spin distance $\langle r \rangle$ and its standard deviation Δr are ± 0.02 nm and ± 0.03 nm, respectively. The errors of the mean angles $\langle \zeta \rangle$ and $\langle \varphi \rangle$ equal to $\pm 21^\circ$ and $\pm 34^\circ$, respectively. The corresponding standard deviations $\Delta \zeta$ and $\Delta \varphi$ have similar errors, which equal to $\pm 27^\circ$ and $\pm 32^\circ$, respectively. Taken all that, one can conclude that the AnisoDipFit analysis of the RIDME time trace of **1** allowed determining the distance distribution $P(r)$ with a sub-angstrom precision and the angular distributions $P(\zeta)$ and $P(\varphi)$ with a moderate precision of $\sim 30^\circ$. Note that the precision of the angular parameters strongly depends on how anisotropic the g -factor of [spin B](#) is (in the given example, spin B is the ls-Fe^{3+}). It will be shown in Section 6.4 that the precision of the angular parameters can be significantly increased if spin B has a larger g -anisotropy than the ls-Fe^{3+} .

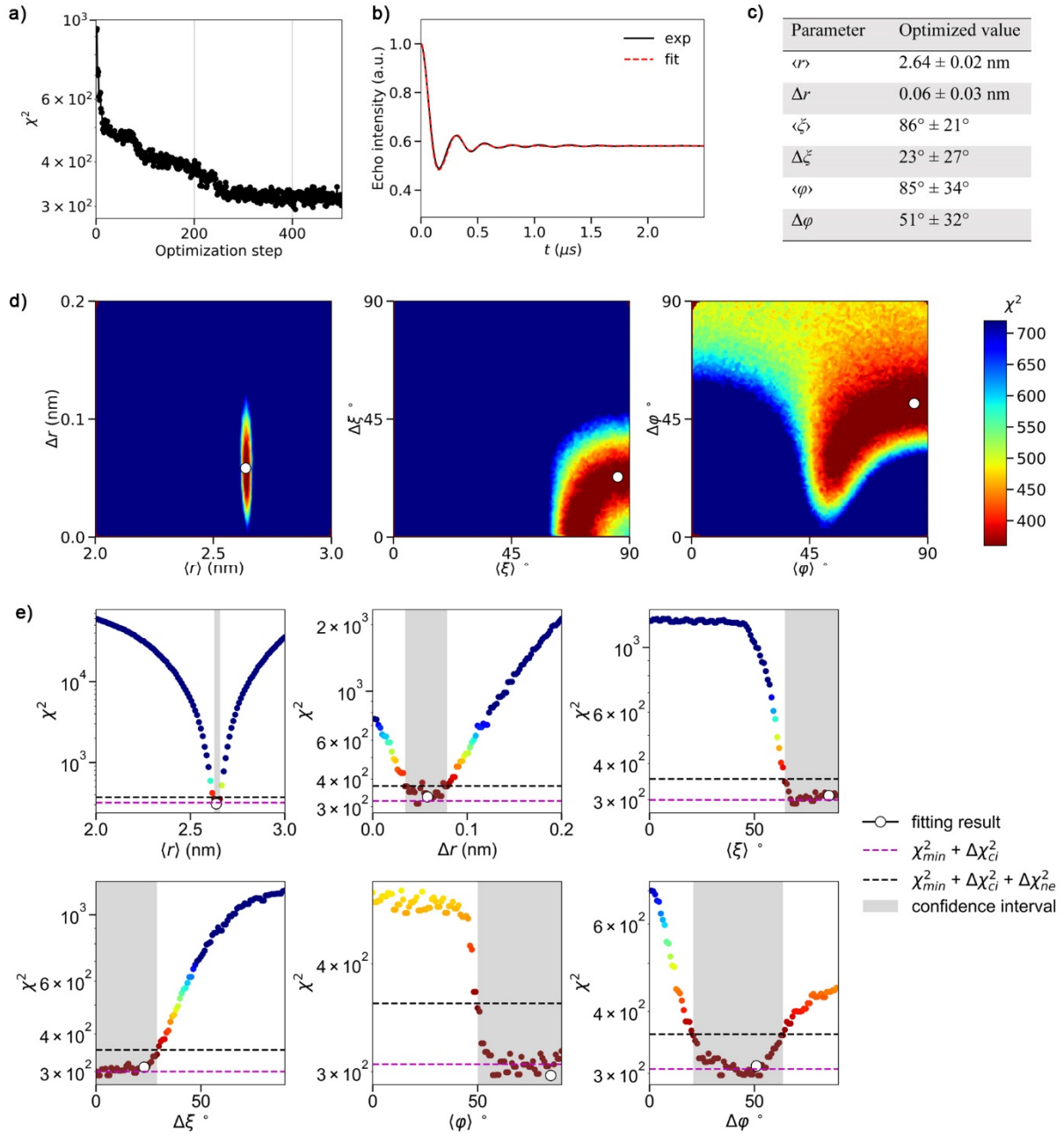


Figure 6.3. Fitting the RIDME time trace of **1** by AnisoDipFit. **a)** The dependence of χ^2 on the optimization step. **b)** The RIDME time trace (black line) is overlaid with the corresponding fit (red line). **c)** The optimized values of the fitting parameters. **d)** The dependence of χ^2 on different pairs of fitting parameters. Dark red regions correspond to the parameters' uncertainty intervals, which lie below the χ^2 threshold. The optimized values of the fitting parameters are depicted by white dots. **e)** The dependence of χ^2 on single fitting parameters. The χ^2 threshold is depicted by the black dashed line. This threshold consists of two contributions, $\Delta \chi^2_{ci}$, which is determined at the 3σ confidence level, and $\Delta \chi^2_{ne}$, which takes into account the numerical error. The uncertainty ranges of the fitting parameters are shown as gray intervals.

In the second part of this example, the results of the fitting described above will be used to test the error analysis mode of AnisoDipFit. As was mentioned in Chapter 4.6, the idea behind this mode is identical to the already mentioned error analysis. The only difference is that this analysis will be done post factum, i.e., using the results of the fitting, which was completed in the past. In order to run AnisoDipFit in the error analysis mode, one has to do the following steps:

- 1') *Prepare the new configuration file.* The ready-to-use configuration file [config_ex02_2.cfg](#) can be found in the following directory:

[/AnisoDipFit/examples/example02_ls_iron\(III\)_trityl](#)

The configuration file [config_ex02_2.cfg](#) is based on the configuration file [config_ex02_1.cfg](#) used for the fitting. However, several changes were made to the latter file. First, the [operation mode](#) was changed from “fitting” to “error analysis”. Second, the path to the existing [parameter.dat](#) file was specified in [path_optimized_parameters](#). Third, the recording of five new data sets is activated: 1) the two-dimensional dependence of χ^2 on $\langle\zeta\rangle$ and $\langle\varphi\rangle$, 2) the two-dimensional dependence of χ^2 on Δr and $\langle\zeta\rangle$, 3) the two-dimensional dependence of χ^2 on Δr and $\Delta\zeta$, 4) the two-dimensional dependence of χ^2 on Δr and $\langle\varphi\rangle$, 5) the two-dimensional dependence of χ^2 on Δr and $\Delta\varphi$. Again, each of these data sets will contain 10^4 data points (see [Ns](#)) and the errors of the fitting parameters will be determined at the 3σ level (see [confidence_interval](#)).

- 2') *Run the program.* First, open Terminal (Linux) or Command Prompt (Windows) and navigate to the directory:

[cd ../AnisoDipFit](#)

If needed, set the permission properties (only for Linux):

[chmod 755 AnisoDipFit](#)

Finally, run the program by the following command:

[sh AnisoDipFit.sh examples/example02_ls_iron\(III\)_trityl/config_ex02_2.cfg](#) (for Linux)

[AnisoDipFit.exe examples/example02_ls_iron\(III\)_trityl/config_ex02_2.cfg](#) (for Windows)

- 3') During the program operation, the following messages should appear in Terminal or Command Prompt:

[Reading out the configuration file... \[DONE\]](#)

[Starting the error analysis...](#)

[Calculating chi2 in dependence of fitting parameters ...](#)

[Calculation step 5 / 5](#)

[Calculating the errors of fitting parameters ...](#)

[Numerical error \(chi2\) = 63.304005](#)

[Score threshold \(chi2\) = 72.304005](#)

[The error analysis is finished. Total duration: 13:09:51.910827](#)

[Saving the results of the error analysis into the directory:](#)

[../AnisoDipFit/examples/example02_ls_iron\(III\)_trityl/2020-01-01_00-00/ \[DONE\]](#)

[Plotting the results of the error analysis... \[DONE\]](#)

[Optimized fitting parameters:](#)

Parameter	Value	Optimized	Precision (+/-)
r mean (nm)	2.637	Y	nan
r width (nm)	0.058	Y	0.031
xi mean (deg)	85.505	Y	16.408
xi width (deg)	22.960	Y	11.462
phi mean (deg)	84.853	Y	30.393
phi mean (deg)	50.985	Y	22.675
temperature (K)	10.000	N	nan

- 4') *Explore the validation results.* During the validation, a new folder will be created in the directory `/AnisoDipFit/examples/example02_ls_iron(III)_trityl` and the results of validation will be saved in that folder. The output files include `parameter_errors-xi_mean-phi_mean.dat`, `parameter_errors-r_width-xi_mean.dat`, `parameter_errors-r_width-xi_width.dat`, `parameter_errors-r_width-phi_mean.dat`, `parameter_errors-r_width-phi_width.dat`, and the corresponding graphical files `parameter_errors.png` and `confidence_intervals.png`.

The content of `parameter_errors.png` is depicted in Figure 6.4. As can be seen, the minima of all χ^2 plots appear at the same values of the fitting parameters as the minima in Figure 6.3c. Thus, the results of two validations are consistent with each other.

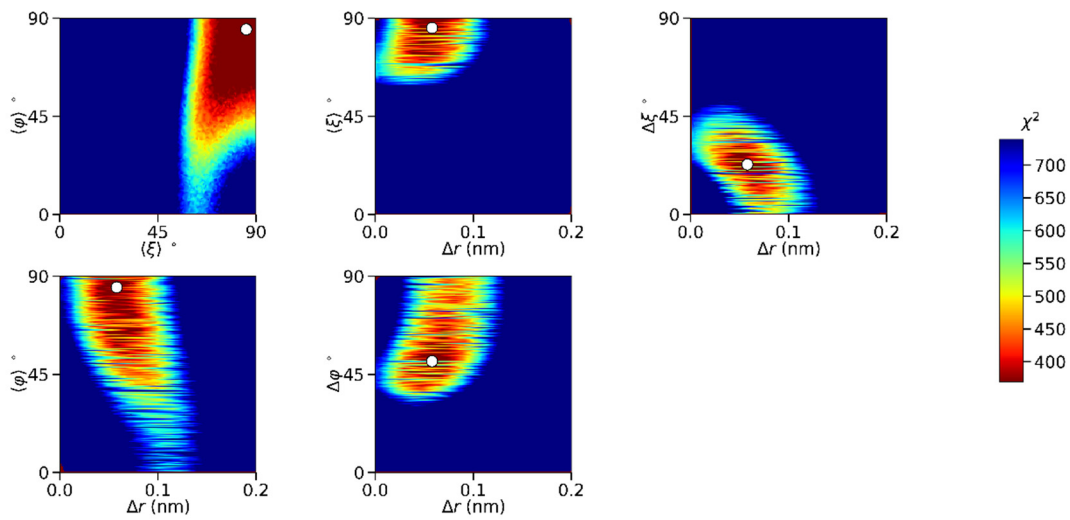


Figure 6.4. The dependence of χ^2 on different pairs of fitting parameters. Dark red regions correspond to the parameters' uncertainty intervals, which lie below the χ^2 threshold. The optimized values of the fitting parameters are depicted by white dots.

6.3 Simulation of the dipolar spectra of a high-spin Fe^{3+} -organic radical spin system

In the next example, the simulation mode of AnisoDipFit is used once again. The spectral simulations are done for a spin system consisting of a high-spin Fe^{3+} (hs-Fe^{3+}) and an organic radical. In contrast to the ls-Fe^{3+} considered in Chapter 6.1, the hs-Fe^{3+} has $S = 5/2$ and, therefore, does not satisfy the requirements of AnisoDipFit (Chapter 1.2). However, if the zero-field splitting (ZFS) of the hs-Fe^{3+} significantly exceeds its Zeeman energy and the thermal energy, the hs-Fe^{3+} can be considered an effective $S = 1/2$ center,^[5] which makes AnisoDipFit still applicable. Thus, only this particular case of a large zero-field splitting is considered here. The g -factor of the hs-Fe^{3+} is set to be axial with the principal components $g_{xx} = g_{yy} = 6.0$ (also denoted as g_{\perp}) and $g_{zz} = 2.0$ (also denoted as g_{\parallel}). The g -factor of the organic radical is assumed to be isotropic and equal to $g_e = 2.0023$. In analogy to the example of Chapter 6.1, the inter-spin distance is fixed to 2.50 nm. Due to the axial symmetry of the hs-Fe^{3+} center, the angle φ has no effect on the dipolar spectrum and, therefore, is set to 0° . In contrast, the angle ζ is expected to have an effect on the dipolar spectrum and will be varied in the range $[0^\circ, 90^\circ]$ with a constant step of 0.25° .

Additionally, the dipolar spectrum will be simulated in dependence of the angle θ between the inter-spin vector \vec{r} and the direction of the applied magnetic field \vec{B}_0 . The simulation will be done for two particular cases, $\zeta = 0^\circ$ and $\zeta = 90^\circ$. The idea behind these simulations will become clear later.

The workflow of this fitting includes the following steps:

- 1) *Prepare the configuration file.* The detailed information on how one creates such a configuration file is given in Chapter 4. The ready-to-use configuration files can be found in the following directory:

[/AnisoDipFit/examples/example03_hs_iron\(III\)_nitroxide](#)

This directory contains three configuration files [config_ex03_i.cfg](#), where $i = 1, 2$, and 3 . The configuration file [config_ex03_1.cfg](#) initializes the spectral simulations in dependence of ζ , whereas the configuration files [config_ex03_2.cfg](#) and [config_ex03_3.cfg](#) initialize the spectral simulations in dependence of θ and set ζ to 0° and 90° , respectively. Note that the [faxis_normalized](#) parameter is set to 1 in all three configuration files, meaning that the frequency axis of the simulated spectra will be normalized by the dipolar coupling constant ν_0 of an isotropic spin pair with the same inter-spin distance as mentioned above.

- 2) *Run the program.* First, open Terminal (Linux) or Command Prompt (Windows) and navigate to the directory:

[cd ../AnisoDipFit](#)

If needed, set the permission properties (only for Linux):

[chmod 755 AnisoDipFit](#)

Finally, run the program by the following command:

[sh AnisoDipFit.sh examples/example03_hs_iron\(III\)_nitroxide/config_ex03_i.cfg](#) (for Linux)

[AnisoDipFit.exe examples/example03_hs_iron\(III\)_nitroxide/config_ex03_i.cfg](#) (for Windows)

- 3) *Monitor the progress status.* During the program operation, the following messages should appear in Terminal or Command Prompt:

For `config_ex03_1.cfg`:

```
Reading out the configuration file... [DONE]
Starting the simulation...
Running the pre-calculations... [DONE]
Calculating the dipolar spectrum vs xi... 100% [DONE]
The simulation is finished
Saving the simulation results into the directory:
.../AnisoDipFit/examples/example03_hs_iron(III)_nitroxide/2020-01-01_00-00/ [DONE]
```

For `config_ex03_2.cfg` and `config_ex03_3.cfg`:

```
Reading out the configuration file... [DONE]
Starting the simulation...
Running the pre-calculations... [DONE]
Calculating the dipolar spectrum vs theta... 100% [DONE]
The simulation is finished
Saving the simulation results into the directory:
.../AnisoDipFit/examples/example03_hs_iron(III)_nitroxide/2020-01-01_00-00/ [DONE]
```

- 4) *Explore the simulation results.* During the simulation, a new folder will be created within the directory `/AnisoDipFit/examples/example03_hs_iron(III)_nitroxide` and the results of the simulations will be saved in that folder. For the simulation based on `config_ex03_1.cfg`, the folder will contain the files `spc_vs_xi.dat` and `spc_vs_xi.png`. The content of `spc_vs_xi.png` will pop up on the screen after the simulation (Figure 6.5a). For the simulations based on `config_ex03_2.cfg` and `config_ex03_3.cfg`, the folder will contain the files `spc_vs_theta.dat` and `spc_vs_theta.png`. Again, the content of the graphical file will appear on the screen (Figure 6.5b-c).

As can be seen in Figure 6.5a, the simulated spectra have prominent differences to the Pake doublet.^[13] Firstly, they are significantly broader than the Pake doublet. This stems from the fact that the dipolar coupling frequencies are proportional to the effective g -factors of the hs-Fe^{3+} , which are 3 times larger than g_e for two out of three canonical orientations. Secondly, the shapes of the simulated spectra differ from the shape of the usual Pake doublet and, as expected, depend strongly on the angle ζ . In order to provide a deeper insight into the obtained shapes, the dipolar spectrum is plotted as a function of θ for two particular cases, $\zeta = 0^\circ$ and $\zeta = 90^\circ$. In the case of $\zeta = 90^\circ$ (Figure 6.5b), the parallel component ($\theta = 90^\circ$) of the spectrum scales with g_{yy} of the hs-Fe^{3+} , which is about 3 times larger than g_e . Consequently, the parallel component appears in the spectrum at $\pm 6\nu_0$ and not at $\pm 2\nu_0$ as for the Pake doublet. The perpendicular component ($\theta = 0^\circ$) of the spectrum scales with the g -values of the hs-Fe^{3+} ranging from $g_{zz} \approx g_e$ to $g_{xx} \approx 3g_e$. This gives rise to two features in the spectrum at $\pm\nu_0$ and $\pm 3\nu_0$, respectively. In the case of $\zeta = 0^\circ$ (Figure 6.5c), the perpendicular component ($\theta = 90^\circ$) of the spectrum scales with g_{xx} and g_{yy} of the hs-Fe^{3+} . Since $g_{xx} = g_{yy} \approx 3g_e$, the perpendicular component of the spectrum appears at $\pm 3\nu_0$. The parallel component ($\theta = 0^\circ$) of this spectrum scales with g_{zz} of the hs-Fe^{3+} and, thus,

should appear at $\pm 2\nu_0$. Since the parallel component has a smaller probability and appears at lower frequencies than the perpendicular component, it does not yield any prominent features in the spectrum.

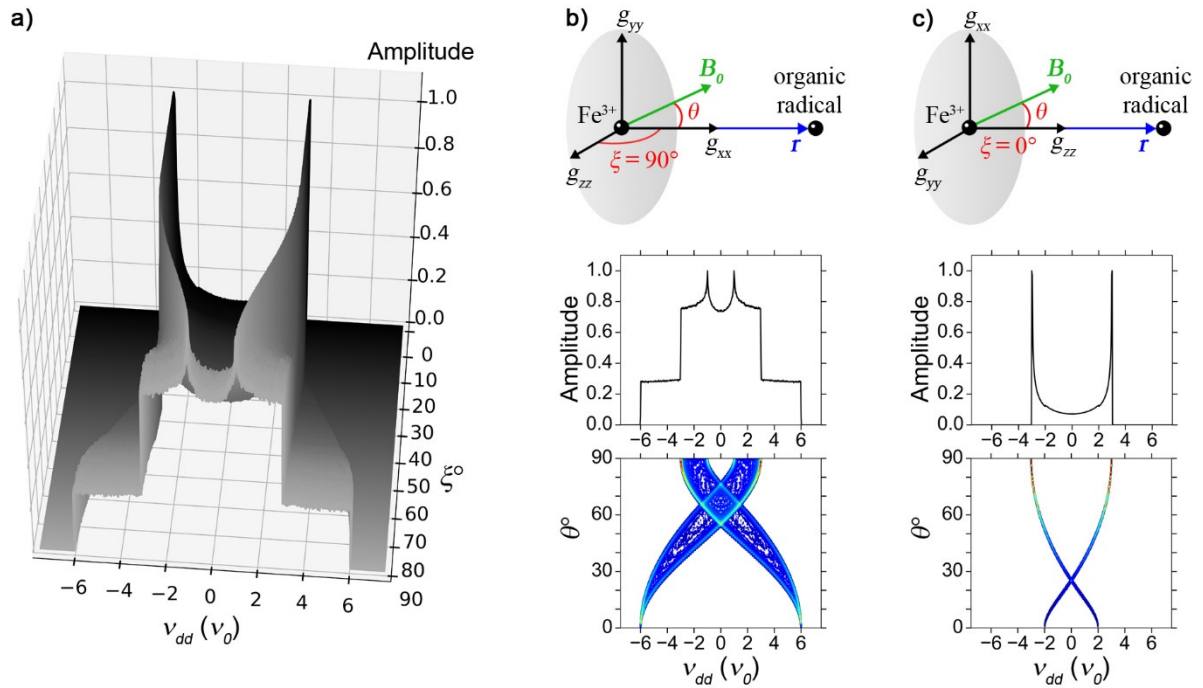


Figure 6.5. a) The angular dependence of the dipolar spectrum that corresponds to the spin system consisting of an isotropic organic radical with $g_{iso} = g_e$ and an anisotropic hs-Fe^{3+} with $g_{aniso} = [6.0, 6.0, 2.0]$. The angle ξ is defined in Figure 1.1. For the particular cases of the b) $\xi = 0^\circ$ and c) $\xi = 90^\circ$, the dipolar spectrum is also shown in dependence of the angle θ . Top: The geometric model of the spin system with the fixed ξ angle. Middle: The corresponding dipolar spectrum. Bottom: The corresponding dipolar spectrum plotted against θ .

6.4 Fitting of the dipolar spectrum of a high-spin Fe^{3+} -nitroxide spin system

In the last example, AnisoDipFit is applied to the fitting of a RIDME spectrum acquired on the MTSL-labeled mutant Q8R1 of the heme protein met-myoglobin (Figure 6.6).^[5] This met-myoglobin mutant contains two spatially separated electron spin centers, the nitroxide and the high-spin Fe^{3+} (hs- Fe^{3+}). The hs- Fe^{3+} has the large axial ZFS ($D \sim 9.26 \text{ cm}^{-1}$, $E = 0.0023 \text{ cm}^{-1}$),^[17] which allows considering this ion as an effective $S = 1/2$ center at Q-band and at the temperatures below 3 K.^[5] The g -factor of this center is very anisotropic and has the principal components $g_{xx} = 5.93$, $g_{yy} = 5.94$, and $g_{zz} = 2.00$. In contrast, the g -anisotropy of the nitroxide center is so small that it can be neglected in the data analysis by setting all g -values to g_e . Since the g -factor of the hs- Fe^{3+} is almost axial, the RIDME spectrum of Q8R1 does not depend

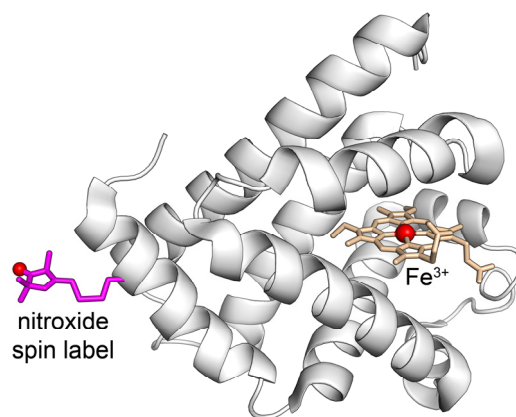


Figure 6.6. The structural model of the met-myoglobin mutant Q8R1. This model is based on the crystal structure of met-myoglobin (PDB-ID 1wla)^[18], which is shown in white. The heme group and the Fe^{3+} ion are depicted as light orange sticks and a red sphere, respectively. A single conformer of the nitroxide spin label, attached at the position Q8, is depicted by magenta sticks. The corresponding oxygen atom is shown as a red sphere.

on the angle φ . Therefore, this angle will be excluded from the fitting and fixed at a constant value of 0° . Thus, the fitting of the RIDME spectrum will be done using the parameters of only two distributions, $P(r)$ and $P(\zeta)$, as fitting parameters. In addition, the temperature of the RIDME experiment will be used as a fitting parameter. This has the following reasons: 1) If the Zeeman energy of the hs- Fe^{3+} becomes comparable to the thermal energy, the corresponding RIDME spectrum becomes temperature dependent (for explanation see Ref. ^[5]). The RIDME measurements on Q8R1 apply to this case. 2) The error of the experimentally measured temperature was above the precision required for accurate spectral simulations.

The workflow of this fitting includes the following steps:

- 1) *Prepare the configuration file.* The detailed information on how one creates such a configuration file is given in Chapter 4. The ready-to-use configuration file [config_ex04.cfg](#) can be found in the following directory:

[/AnisoDipFit/examples/example04_hs_iron\(III\)_nitroxide](#)

As follows from this configuration file, the data, which is going to be fitted (see [fitted_data](#)), is the RIDME spectrum of Q8R1 (see [path_spectrum](#)). The mean values and the widths of the distributions $P(r)$ and $P(\zeta)$, as well as the temperature, are selected to be the fitting parameters (see [fitting_parameters](#)). The distributions $P(r)$ and $P(\zeta)$ are approximated by normal distributions (see [calculation_settings](#)) and, therefore, their widths are given as standard deviations. The genetic algorithm is set to be the fitting method. Its internal parameters are provided in [fitting_settings](#). Among them, the total number of optimization steps is set to 500. For [error_analysis](#), the recording

of the following data sets is activated: 1) the two-dimensional dependence of χ^2 on $\langle r \rangle$ and Δr , 2) the two-dimensional dependence of χ^2 on $\langle \xi \rangle$ and $\Delta \xi$, and 3) the two-dimensional dependence of χ^2 on the temperature. Each of these data sets will contain 10^4 data points (see [Ns](#)). The errors of the fitting parameters will be determined at the 3σ level (see [confidence_interval](#)).

- 2) *Run the program.* First, open Terminal (Linux) or Command Prompt (Windows) and navigate to the directory:

```
cd ../AnisoDipFit
```

If needed, set the permission properties (only for Linux):

```
chmod 755 AnisoDipFit
```

Finally, run the program by the following command:

```
sh AnisoDipFit.sh examples/example04_hs_iron(III)_nitroxide/config_ex04.cfg (for Linux)
```

```
AnisoDipFit.exe examples/example04_hs_iron(III)_nitroxide/config_ex04.cfg (for Windows)
```

- 3) *Monitor the progress status.* During the program operation, the following messages should appear in Terminal or Command Prompt:

```
Reading out the configuration file... [DONE]
```

```
Starting the fitting...
```

```
Optimization step 500 / 500: chi2 = 0.130504
```

```
The fitting is finished. Total duration: 16:39:59.540339
```

```
Saving the fitting results into the directory:
```

```
../AnisoDipFit/examples/example04_hs_iron(III)_nitroxide/2020-01-01_00-00/ [DONE]
```

```
Plotting the fitting results... [DONE]
```

```
Starting the error analysis...
```

```
Calculating chi2 in dependence of fitting parameters ...
```

```
Calculation step 3 / 3
```

```
Calculating the errors of fitting parameters ...
```

```
Numerical error (chi2) = 24.844337
```

```
Score threshold (chi2) = 33.844337
```

```
The error analysis is finished. Total duration: 9:16:26.985827
```

```
Saving the results of the error analysis into the directory:
```

```
../AnisoDipFit/examples/example04_hs_iron(III)_nitroxide/2020-01-01_00-00/ [DONE]
```

```
Plotting the results of the error analysis... [DONE]
```

```
Optimized fitting parameters:
```

Parameter	Value	Optimized	Precision (+/-)
r mean (nm)	2.765	Y	0.016
r width (nm)	0.088	Y	0.025
xi mean (deg)	79.304	Y	1.134
xi width (deg)	0.273	Y	1.126
phi mean (deg)	0.000	N	nan
phi mean (deg)	0.000	N	nan
temperature (K)	2.184	Y	0.207

Here, a few comments have to be made. First, the calculation time strongly depends on the PC used (Chapter 1.3). Second, the optimized fitting parameters might slightly differ between single fitting trials. Importantly, this deviation is below the error of the optimized parameters (see below).

- 4) *Explore the fitting results.* During the fitting, a new folder will be created within the directory [/AnisoDipFit/examples/example04_hs_iron\(III\)_nitroxide](#) and the results of fitting will be saved in that folder. The output files include [score.dat](#), [fit.dat](#), [parameters.dat](#), [parameter_errors-r_mean-r_width.dat](#), [parameter_errors-xi_mean-xi_width.dat](#), [parameter_errors-temp.dat](#), and the corresponding graphical files (see Chapter 5.2).

Next, the results of the fitting will be briefly discussed. The discussion will begin by exploring the content of the files [score.dat](#) and [score.png](#). These files contain the dependence of χ^2 on the optimization step, which is depicted in Figure 6.7a. This figure reveals that χ^2 decreased during first 50 optimization steps and, then, reached a plateau during the next 450 optimization steps. Based on this, one can conclude that the genetic algorithm has converged to the minimum. The fit to the RIDME spectrum, which corresponds to the obtained minimum, is stored in the output files [fit.dat](#) and [fit.png](#) and depicted in Figure 6.7b. As can be seen, the shape of the experimental RIDME spectrum is well reproduced by the obtained fit, revealing the minimum that was found by the genetic algorithm is a global minimum. The corresponding optimized values of the fitting parameters are stored in the output file [parameters.dat](#), whose content is listed in Figure 6.7c. In order to estimate the errors of these parameters, the following data was recorded: 1) the two-dimensional dependence of χ^2 on $\langle r \rangle$ and Δr ([parameter_errors-r_mean-r_width.dat](#)), 2) the two-dimensional dependence of χ^2 on $\langle \xi \rangle$ and $\Delta \xi$ ([parameter_errors-xi_mean-xi_width.dat](#)), and 3) the one-dimensional dependence of χ^2 on the temperature ([parameter_errors-temp.dat](#)). The picture of these data sets is stored in the output file [parameter_errors.png](#) and shown here in Figure 6.7d. In this figure, the uncertainty ranges of the fitting parameters are depicted in dark red, and the optimized values of the fitting parameters are depicted by white dots. As expected, all dots appear within the uncertainty ranges mentioned above, meaning that the optimized values of the fitting parameters lay within the corresponding uncertainty ranges. In order to determine the errors of fitting parameters, all three two-dimensional dependencies from Figure 6.7d were transformed into one-dimensional dependencies, which contain the dependence of χ^2 on each single fitting parameter. The one-dimensional dependencies are stored in the output file [confidence_intervals.png](#), whose content is depicted in Figure 6.7e. As can be seen, all one-dimensional dependencies reveal a single, well-defined minimum. The χ^2 threshold, which was used to determine the uncertainty intervals of the fitting parameters, is depicted by the black dashed line. The errors of individual fitting parameters, which were derived from the uncertainty intervals, are listed Figure 6.7c. The errors of the mean inter-spin distance $\langle r \rangle$ and its standard deviation Δr are ± 0.02 nm and ± 0.03 nm, respectively. The error of both angular parameters, $\langle \xi \rangle$ and $\Delta \xi$, is $\pm 1^\circ$. Thus, both distributions, $P(r)$ and $P(\xi)$, could be extracted from the RIDME spectrum of Q8R1 with a very high precision. The optimized value of the temperature equals to 2.2 ± 0.2 K. This value deviates from the experimental temperature (3 K)^[5] by 1.0 ± 0.3 K. The obtained deviation is in agreement with the reported experimental error of the temperature measurement (~ 1 K).^[5]

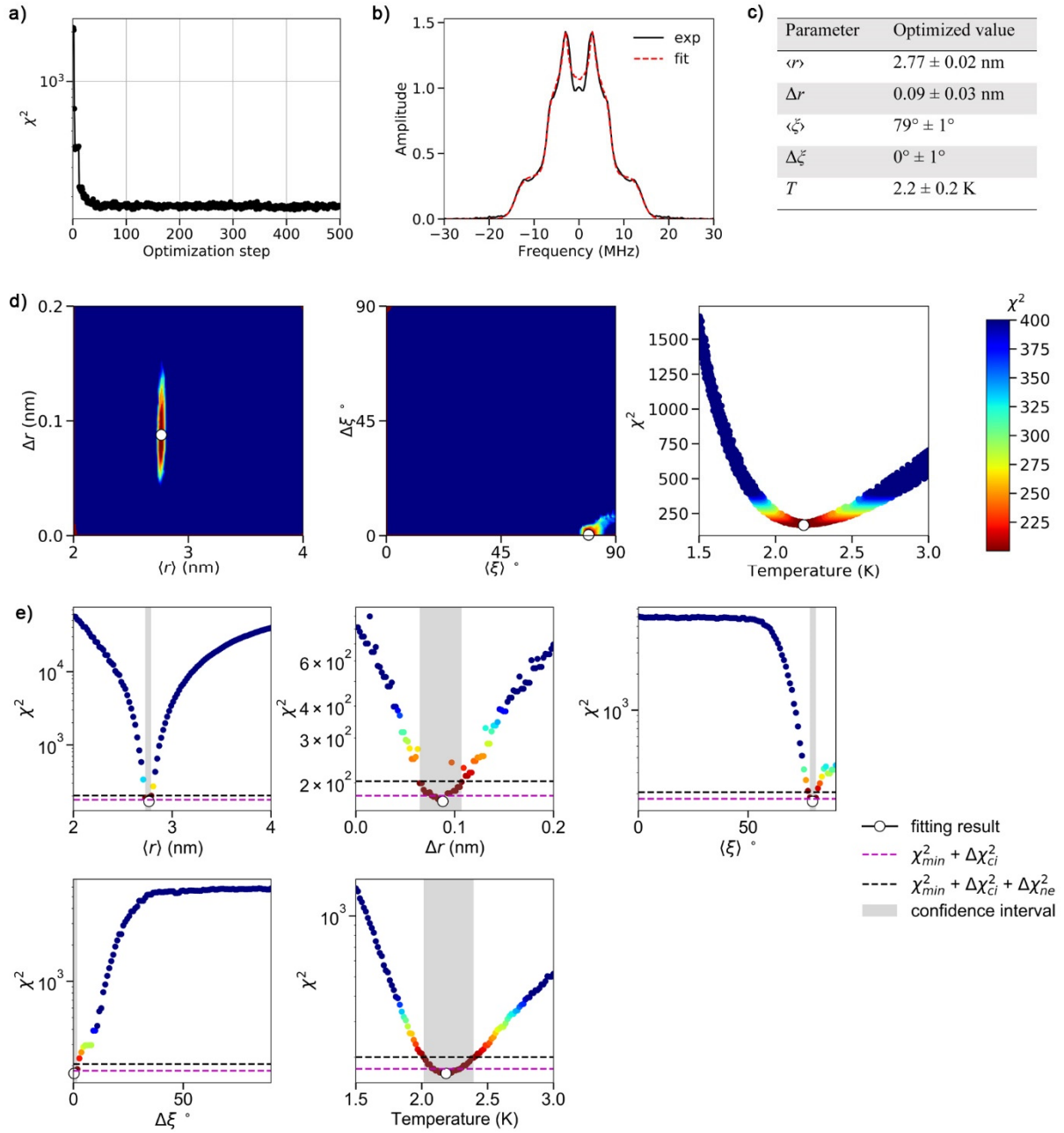


Figure 6.7. Fitting the RIDME spectrum of Q8R1 by AnisoDipFit. **a)** The dependence of χ^2 on the optimization step. **b)** The RIDME spectrum (black line) is overlaid with the corresponding fit (red line). **c)** The optimized values of the fitting parameters. **d)** The dependence of χ^2 on different pairs of fitting parameters. Dark red regions correspond to the parameters' uncertainty intervals, which lie below the χ^2 threshold. The optimized values of the fitting parameters are depicted by white dots. **e)** The dependence of χ^2 on single fitting parameters. The χ^2 threshold is depicted by the black dashed line. This threshold consists of two contributions, $\Delta\chi_{cl}^2$, which is determined at the 3σ confidence level, and $\Delta\chi_{ne}^2$, which takes into account the numerical error. The uncertainty ranges of the fitting parameters are shown as gray intervals.

7 References

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