Sequence Alignments

▶ Alignment of Protein Sequences
▶ Protein Structure Prediction and Structural Annotation of Proteomes

Sequential Proton Loss Electron Transfer (SPLET)

▶ Proton-Coupled Electron Transfer

SERRS

▶ Surface-Enhanced Raman Spectroscopy for Bioanalytics

SERS

▶ Cell Sensing with Raman Spectroscopy
▶ Surface-Enhanced Raman Spectroscopy for Bioanalytics

Seven-Transmembrane Domain Receptors

▶ Dynamics of Helix 8 in GPCR Function
▶ G Protein–Coupled Receptor Activation Based on X-ray Structural Studies
▶ Membrane Protein Structure
▶ Rhodopsin Activation Based on Solid-State NMR Spectroscopy
▶ Rhodopsin: Stability and Structural Organization in Membranes

SHG

▶ Second Harmonic Generation Microscopy (SHG)

SHIM

▶ Second Harmonic Generation Microscopy (SHG)

Sialidase

▶ Influenza Neuraminidase – Computational Studies

Side Chain Assignment

▶ Assignment of ^{19}F Resonances in Protein Solution State NMR Studies
▶ Protein NMR Resonances Assignment

Signal Conduction

▶ Bioelectricity, Ionic Basis of Membrane Potentials and Propagation of Voltage Signals

Signal Transduction at the Intracellular Surface of the Membrane-Integral G-protein–Coupled Receptors

▶ Dynamics of Helix 8 in GPCR Function
▶ G Protein–Coupled Receptor Activation Based on X-ray Structural Studies

Simulation of Spin-Label EPR Spectra

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Synonyms

Spectral simulations

Definition

Simulation of spin-label EPR spectra comprises a set of computational methods that allow extraction of magnetic and dynamic parameters such as rotational correlation times from EPR spectra by way of computing theoretical EPR spectra for given parameter sets employing various levels of spin physics and reorientational dynamics theory and using least-squares fitting algorithms to fit these spectra to the experimental ones.

Introduction

The EPR spectrum (▶ Spin-Labeling EPR of Proteins: Dynamics and Water Accessibility of Spin-Label Side Chains, ▶ EPR Spectroscopy: General Principles) of a nitroxide spin label attached to a biomolecule (▶ Chemistry of Spin Labeling, ▶ Spin Labeling of DNA and RNA, ▶ Spin-Labeling EPR of Proteins: Dynamics and Water Accessibility of Spin-Label Side Chains) is affected by the reorientational dynamics (▶ Spin-Labeling EPR of Proteins: Dynamics and Water Accessibility of Spin-Label Side Chains) of the nitroxide ring. The dynamics happens on three levels (see Fig. 1): (a) the spin label reorients *locally* with respect to the protein (or other biomolecule) it is attached to, (b) the protein rearranges *internally* via side chain and backbone motions, and (c) the protein tumbles *globally* with respect to the matrix, which is either an isotropic solution or an anisotropic environment like a lipid bilayer. The magnetic parameters of the spin label (▶ Spin-Labeling EPR of Proteins: Dynamics and Water Accessibility of Spin-Label Side Chains, ▶ EPR Spectroscopy: General Principles; effective g value and hyperfine coupling), determined by the orientation of the label relative to the external magnetic field, vary in time and lead to dynamic broadening effects in the EPR spectrum.

Information about the dynamic processes can be extracted from the EPR spectra by computational methods that simulate the spectra based on a model and fit the model to the experimental data. The appropriate theory level for spectral simulations depends on the timescale of the dynamics, summarily characterized by the rotational correlation time τ_c, compared to the timescale of the EPR experiment, which is about the inverse of the EPR spectral width
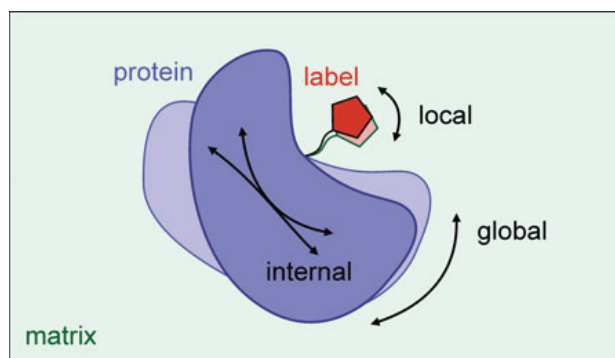
Simulation of Spin-Label EPR Spectra, Fig. 1 Local, internal, and global reorientational motions of nitroxide spin label and protein in a matrix
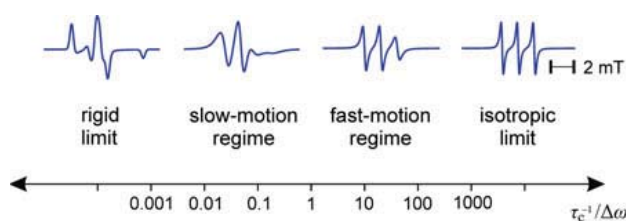
Simulation of Spin-Label EPR Spectra, Fig. 2 Motional regimes for nitroxide spin labels

$\Delta\omega$. Several motional regimes are distinguished: the rigid and the isotropic limits, and the slow-motion and fast-motion regimes (see Fig. 2).

Slow-Motion Regime

If the reorientational motion of the spin label is on a timescale similar to the EPR timescale, the EPR spectrum is broadened. Simulation methods for this regime differ in how the different levels of orientational dynamics are treated and can use either *deterministic* or *stochastic* models. Deterministic models are based on atomistic molecular dynamics (MD) simulations (▶ Molecular Dynamics Simulations of Carbohydrates) and are able to treat complex local and internal dynamics. Stochastic dynamics (SD) models describe the reorientational motion as diffusion of a single-particle rigid rotor and can account for simple local or global rotational dynamics. The most common stochastic model is Brownian rotational diffusion, a random walk in three-dimensional orientational space. The anisotropy of the reorientational rates

due to the nonspherical shape of the spin label is described by an anisotropic diffusion tensor whose orientation is tied to that of the nitroxide. For a free spin label in solution, the local environment is isotropic. On the other hand, it is generally anisotropic for a spin label bound to a protein or other biomolecule, resulting in preferential alignments and inaccessible orientations. The free energy of the label is a function of its orientation Ω and is described by an orientational potential $U(\Omega)$.

In SD models, the global dynamics of the protein can be treated at several levels. The MOMD (microscopic order macroscopic disorder) approach assumes the protein molecules in the sample are randomly oriented and immobile on the timescale of the local spin label dynamics, which is treated stochastically. The SRLS (slowly relaxing local structure) model allows for global stochastic dynamics of the protein in the matrix (Earle and Budil 2006).

Stochastic Liouville equation

Simple SD models such as Brownian diffusion in a restricting potential are best simulated by solving the stochastic Liouville equation (SLE) which describes the joint time evolution of both the quantum spin degrees of freedom and the classically treated label orientation, starting with transverse magnetization and an equilibrium orientational probability distribution of an ensemble of spin labels determined by $U(\Omega)$. A very efficient SLE solver was developed by Freed and coworkers (Earle and Budil 2006). The orientational probability distribution and the potential are expressed as linear combinations of rotational basis function. The computational effort scales with the number of basis functions, which increases with decreasing diffusion rate and increasing complexity of the potential. In principle, the SLE approach can be used to simulate a rigid-limit spectrum. However, the basis size required for achieving converged spectra is large, so that dedicated rigid-limit methods are preferred.

Trajectories

As a more general alternative to solving the SLE for a stochastic model, EPR spectra can be computed from sets of explicit orientational trajectories that describe the change in time of the orientation of the label. This method is applicable to trajectories obtained from both stochastic and deterministic models. A trajectory determines the time dependence of the magnetic parameters and therefore the EPR spectrum of a tumbling spin label. Given a trajectory, the time evolution of the magnetization following a 90° pulse is computed using spin density matrix or Bloch magnetization vector propagation (Sezer et al. 2008). From the resulting free induction decay (FID), the EPR spectrum is obtained by Fourier transformation. To generate a converged spectrum, FIDs of a set of trajectories generated from a range of possible initial orientations of the spin label have to be combined. The appropriate time resolution of the trajectories is determined by the spectral width via the Nyquist criterion. The length of the trajectory is determined by the required spectral resolution and has to be of the order of the transverse relaxation time T_2 (▶ Pulsed EPR: Principles and Examples of Applications to Hemeproteins) to yield accurate EPR spectra.

The computation of SD trajectories is fast, and the number and length of trajectories required for accurate spectral simulations are easily obtained. MD trajectories are computationally significantly more demanding. The expense of computing long MD trajectories can be avoided by relying on extrapolation (Oganesyan 2007), or by deriving an effective orientational potential from short MD trajectories and then using it to generate SD trajectories (Steinhoff and Hubbell 1996) or to solve the SLE (Budil et al. 2006). A single MD trajectory can be reused several times by rotation or resampling. In general, despite being much slower than the SLE approach, trajectory methods are superior in the complexity of dynamics than can be modeled. Global dynamics cannot rely on MD simulations, so stochastic models are used (DeSensi et al. 2008).

The methodology for simulating slow-motional EPR spectra of two coupled spin labels attached to the same macromolecule is not as developed as for single labels. There exist methods based on the SLE (Zerbetto et al. 2007) and on trajectories (Hustedt et al. 1997) for the simple case of a tumbling protein with two rigidly attached labels.

Rigid Limit

In the rigid limit, the spin labels are immobilized. Methods for the simulation of rigid-limit spectra of spin labels are identical to the ones used for transition

metal centers (see ▶ Simulation of Transition-Metal EPR Spectra). Perturbation theory methods based on the high-field approximation are usually adequate.

The solid-state spectrum of two coupled spin labels is broadened relative to the single-label spectrum by the distance-dependent dipolar coupling between the two nitroxides and can be simulated using either matrix diagonalization or, within the high-field approximation, perturbation theory (Hustedt et al. 1997). Spectra have to be integrated over nonuniform or partially correlated orientational distributions. A simplified decoupled method that convolves a single-nitroxide spectrum with a spectrum (Pake pattern) representing the dipolar coupling between the two nitroxides can be used to estimate distances between spin labels in doubly labeled systems (▶ Interspin Distance Determination by EPR; Steinhoff et al. 1997).

Fast-Motion Regime and Isotropic Limit

If the reorientational motion of a nitroxide spin label is fast compared to the EPR timescale, the EPR spectrum consists of three lines of different widths that depend on the details of the reorientational dynamics. Analytical expression based on Redfield perturbation theory can be used to simulate such spectra (Atherton 1993). The algorithms used for the slow-motion regime can be used as well. In the isotropic limit, where the motion is orders of magnitude faster than the EPR timescale, the Breit-Rabi equation affords an exact method without recurrence to perturbation theory approximations.

Cross-References

- ▶ Chemistry of Spin Labeling
- ▶ EPR Spectroscopy: General Principles
- ▶ Interspin Distance Determination by EPR
- ▶ Molecular Dynamics Simulations of Carbohydrates
- ▶ Pulsed EPR: Principles and Examples of Applications to Hemeproteins
- ▶ Simulation of Transition-Metal EPR Spectra
- ▶ Spin Labeling of DNA and RNA
- ▶ Spin-Labeling EPR of Proteins: Dynamics and Water Accessibility of Spin-Label Side Chains

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Simulation of Transition-Metal EPR Spectra

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Synonyms

Spectral simulations

Definition

Simulation of transition-metal EPR spectra comprises a set of computational methods that allow extraction of magnetic spin Hamiltonian parameters from one or a set of EPR spectra by way of computing theoretical EPR spectra for given parameter sets employing various levels of spin physics theory and using least-squares fitting algorithms to fit these spectra to the experimental ones.

Introduction

Solid-state EPR spectra of biological transition-metal centers and clusters contain a wealth of information on geometric and electronic structure. This information is extracted in two steps. First, the spectrum is simulated and fitted using a phenomenological spin Hamiltonian model (Weil and Bolton 2007; Mabbs and Collison 1992; Pilbrow 1990), yielding a set of magnetic and electronic parameters. In the second step, these parameters are interpreted and correlated to the geometric and electronic structure of the spin center using either analytical tools like ligand field theory or numerical quantum chemical calculations (Kaupp et al. 2004).

To simulate an EPR spectrum, a spin Hamiltonian model including number and types of spins and type and symmetry of the coupling tensors is chosen at the outset. The corresponding EPR spectrum is then computed as the sum of spectra from all the differently oriented spin centers in the sample, which in turn are sums over all transitions (energy level pairs). Each energy level pair from each spin center generally gives rise to a single resonance line with a certain *position*, *intensity, width,* and *shape*. This sum-over-lines approach for spectral simulations is valid in almost all situations encountered in practice, except for looping transitions, where the microwave quantum is able to bridge two energy levels for a set of orientations, but not for others (Gaffney and Silverstone 1998).

Line Positions

EPR line positions can be computed at two levels of theory, *perturbation theory* (PT) and *numerical matrix diagonalization* (ND). PT uses closed-form analytical solutions for the energy levels as a function of the magnetic field to compute the resonance fields (Weil and Bolton 2007). These expressions are valid under a set of assumptions, most importantly that the electron Zeeman interaction is much larger than any other interaction (high-field approximation). Often, the nuclear Zeeman and quadrupole interactions are neglected as well. PT approaches are very fast and the method of choice for simple systems containing one electron spin. Second-order methods are more accurate than first-order methods, and third-order methods are occasionally employed. PT methods have been implemented in many programs, but very few of them validate the assumptions for the particular input parameters. ND methods construct and numerically diagonalize the full spin Hamiltonian matrix of the system of coupled spins for several field values to obtain energy levels over a field range. The resulting energy level diagram is then used to locate resonance line positions. Computationally, ND methods are significantly more expensive than PT methods, but are accurate since no mathematical approximations or physical assumptions are involved.

Line Intensities

The intensity of an EPR absorption line is determined by several factors: (a) the excitation bandwidth factor, (b) the transition probability, (c) the $1/g$ factor, and (d) the polarization factor. (a) The excitation bandwidth factor describes to what degree a transition with energy difference ΔE is excited by the given microwave quantum v_{mw}. For continuous-wave EPR spectra, this is a simple delta function. (b) The transition probability is given by the squared matrix element of the transition operator and is computed from eigenvectors obtained either from PT or ND. The transition operator depends on the field configuration of the resonator. The common $\Delta m_S = \pm 1$ experiments are performed in resonators where the magnetic field component of the incident microwave is perpendicular to the static magnetic field. For detecting $\Delta m_S = 0$ transitions in integer-spin systems, resonators with a parallel configuration are employed and require a different operator. (c) The $1/g$ factor is a correction factor that accounts for the fact that EPR spectra are field sweeps rather than frequency sweeps. This factor is transition dependent and is the inverse of the field derivative of

the energy level separation, $(\mathrm{d}\Delta E/\mathrm{d}B)^{-1}$, which reduces to $1/g$ for a spin-1/2 system. (d) Lastly, the line intensity depends on the polarization, that is, population difference, between the two energy levels involved. Under thermal equilibrium, this is temperature dependent and given by the Boltzmann distribution. For a spin-1/2 system, the polarization factor can usually be neglected since it affects only the overall intensity, but not the shape of the spectrum. For high-spin systems with large zero-field splittings measured, its inclusion is essential for accurately modeling the relative intensities of different lines in low-temperature spectra.

Line Widths and Shapes

Individual resonance lines have nonzero widths for several reasons. (a) Lines show homogeneous lifetime broadening due to relaxation. The resulting line shape is Lorentzian. (b) Many splittings due to very small electron-nuclear and electron–electron couplings are unresolved in the EPR spectrum and give rise to a residual dipolar line broadening. In spectral simulations, this inhomogeneous broadening is summarily modeled by a Gaussian shape with orientation-dependent width. (c) Lines are also broadened due to structural heterogeneity between nominally identical spin centers in the sample. This gives rise to spin Hamiltonian parameter distributions called *strains* (g strain, D strain, etc.). In simulations, they are taken into account either by using analytical expressions and Gaussian shapes if they are narrow or by explicitly integrating over the distributions if they are wide. (d) The line width and shape is also affected by field modulation. Small-amplitude modulation is modeled by using derivatives of the line shapes, whereas overmodulation is simulated by convoluting the EPR absorption spectrum with a mathematical function describing the modulation.

Frozen Solutions and Crystals

EPR spectra of biological spin centers are most often acquired in frozen solutions. There, the orientations of the spin centers are random, and there is no preferential alignment in space. The EPR spectrum is the sum of the EPR spectra of all centers. Even though a typical sample contains 10^{14}–10^{17} differently oriented spin centers, only a small subset ($N = 10^2$–10^5) is used in a simulation. The computation timescales linearly with N, but simulated spectra converge to the true powder spectra only very slowly with increasing N. If N is too small, the spectrum shows spurious peaks due to the incomplete sampling of the orientational distribution. Methods for the choice of the N orientations range from Monte Carlo to sets that minimize the total repulsion energy between orientations. Except the worst ones, all methods perform roughly similarly. Interpolative methods that use spectral data computed for two close orientations to efficiently obtained spectral data for orientations in between are applied in several programs (Hanson et al. 2004; Stoll and Schweiger 2006). They substantially increase the convergence rate and allow N to be much smaller than in brute-force summation approaches.

Occasionally, protein single crystals are studied. Depending on the space group of the crystal, there might be up to 24 differently oriented protein sites in the unit cell. In addition, the protein might contain several symmetry-related spin centers. To compute a single-crystal EPR spectrum, spectra of all the unit cell sites and the spin centers have to be simulated and added.

Fitting

In order to fit a spin Hamiltonian model to an experimental EPR spectrum, least-squares fitting algorithms are used that iteratively simulate spectra starting from a set p of initial guess parameter values for the chosen spin Hamiltonian model and then adjust p until the deviation between the simulated spectrum $y_{\mathrm{sim}}(p)$ and the experimental spectrum y_{exp} is minimal. Methods differ in two respects: (a) in the way the error between simulation and experiment is computed, (b) in the algorithm that chooses successive parameter sets. (a) The error is always computed as the sum of the squared residuals between experiment and simulation. However, y_{sim} and y_{exp} are either used directly, or they are integrated, double integrated, or even Fourier transformed before the computation of the residuals. Integrals and double integrals tend to have larger convergence radii, whereas the Fourier transform can be advantageous for spectra with many narrow lines. (b) There is a vast gamut of available fitting

algorithms. They fall into two classes, *global* and *local*. Global methods such as Monte Carlo and genetic/evolutionary algorithms are able – at least in principle – to locate the global minimum. Local methods, such as the Simplex and Levenberg/Marquardt algorithms, can only find a minimum close to the starting set of parameters, but are more efficient. *Hybrid* methods that combine elements of both classes are becoming more popular due to their robustness.

Programs

Currently, the most widely adopted general software packages for EPR spectral simulations are Simfonia (implementing PT only, commercial), XSophe (ND only, commercial) (Hanson et al. 2004), and EasySpin (both PT and ND, noncommercial) (Stoll and Schweiger 2006). Many others are used, but are mostly tailored to specific applications.

Cross-References

▶ Simulation of Spin-Label EPR Spectra

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Simulations of IR and Raman Spectra

▶ Quantum Mechanical Simulations of Biopolymer Vibrational Spectra

Simultaneous Topography and Recognition Imaging (TREC)

▶ Atomic Force Microscopy (AFM) for Topography and Recognition Imaging at Single Molecule Level

Single Fluorophore Blinking

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Synonyms

Fluorescent intermittency; Photoblinking; Photoswitch

Definition

Light-emitting species, including organic dye molecules, fluorescent proteins and semiconductor particles, are prone to enter transient dark states, collectively referred to as "blinking" events. Depending on the nature of the fluorophore species, the illumination source and intensity, as well as the environmental conditions, blinking events can range from rare to frequent and last from microseconds to minutes. Blinking events are distinguished from photobleaching, the irreversible loss of fluorescence, by their temporary nature.

Temporary dark states can arise for a variety of reasons. In the case of organic fluorophores, transient dark states may occur due to reversible isomerization of the conjugated π orbital system, resulting in temporary formation of a low-fluorescent isomer and thus cycles of bright and dark states. Organic fluorophores may also undergo intersystem crossing, a low probability electronic transition to a triplet state, which is thought to arise from a "forbidden" inversion of an excited electron's magnetic dipole moment, or spin. Triplet