Multi-Frequency ESR at ACERT

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Molecular Dynamics by ESR

What is special about ESR, in particular spin-label ESR? (e.g. compared to NMR)

1. ESR is much more sensitive per spin (than NMR).
2. In time domain experiments ESR’s time-scale is nanoseconds. (NMR’s is milliseconds)
3. The spin-label spectrum is simple, & can focus on a limited number of spins.
4. ESR spectra change dramatically as the tumbling motion of the probe slows, thereby providing great sensitivity to local “fluidity”.
   (In NMR nearly complete averaging occurs, so only residual rotational effects are observed by $T_1$ & $T_2$.)
5. Multi-frequency ESR permits one to take “fast-snapshots” using very high-frequencies & “slow-snapshots” using lower frequencies to help unravel the complex dynamics of bio-systems.
6. Pulsed ESR methods enable one to distinguish homogeneous broadening reporting on dynamics vs. inhomogeneous broadening reporting on local structure.
**ESR Spectra in a Fluid**

PDT/Toluene at 250GHz

- **Motional Narrowing Regime**
- **Slow Motional Regime**
- **Rigid Limit**

![ESR Spectra Diagram](image)
Multi-Frequency ESR Simulation

A motional process that looks **fast** at lower frequencies....

Rotational Tumbling Time:
\[ \tau_R = 1.7 \times 10^{-9}s \]

For complex dynamics of proteins:
The **slow** overall & collective motions will show up best at **lower frequencies**.

Whereas
The **fast** motions will show up best at **higher frequencies**.

...will look **slow** at higher frequencies
Sensitivity to Anisotropic Motional Dynamics: High Frequency

Example: complexes of cyclodextrins with spin-labeled fatty acids
ESR Spectra of aqueous solutions of T4 Lysozyme spin-labeled at mutant site 72 at different frequencies & temperatures*
Ribbon model of T4 lysozyme showing R1 side chain at sites 72 (in middle of rigid 6 turn helix) & 131 (on more flexible $2\frac{1}{2}$ helix) with dynamic parameters of SRLS model indicated.

Spin labels R1 & R2

- R1: $Z = H$
- R2: $Z = CH_3$

Time scale

R1 internal motion restricted
The SRLS Model

MOMD Model: \( R_{\parallel}^0 \) and \( R_{\perp}^c \) → 0

Restricted Internal Motion

g-tensor frame

\( g_{xx}, g_{yy}, g_{zz} \)

\( \beta_{MG} \)

\( \Phi \)
Adding Atomistic Perspective to Mesoscopic (SLE) Approach *

- Model system: R1 linked to poly-Ala α-helix
- Conformational analysis ⇒ stable conformers
  ⇒ chain dynamics

No Free Parameters

EPR spectra of R1 in α-helix domain

- Overall protein reorientations
- Side chain dynamics

Assumption: Conformers with low barriers exhibit fast exchange. Conformers with high barriers exhibit no exchange.

Modified SLE:

\[
\frac{\partial \rho(\Omega_D,t)}{\partial t} = -i \mathcal{L}(\Omega_D) \rho(\Omega_D,t) - \left[T_2^{-1}(\Omega_D) + \Gamma(\Omega_D)\right] \rho(\Omega_D,t)
\]

\[\mathcal{L}(\Omega_D)\] Liouville superoperator with magnetic tensors partially averaged by chain dynamics

\[\Gamma(\Omega_D)\] Diffusion operator for overall protein tumbling

\[T_2^{-1}(\Omega_D)\] Linewidth contribution from chain dynamics (Redfield theory)

* F. Tombolato, A. Ferrarini, J.H. Freed
Molecular Dynamics Simulations: An Atomistic View *

Spin label dynamics

Experimental ESR spectrum

R1 Side-Chain containing nitrooxide moiety

Protein X-ray structure

Atomistic MD simulations

Calculated ESR spectrum

\[ \hat{H}(t) = \gamma_e \left( B \cdot G(t) \cdot \hat{S} + I \cdot A(t) \cdot \hat{S} \right) \]

* D. Sezer, J. H. Freed & B. Roux
Overall Assessment of Molecular Dynamics by Multi-Frequency ESR

- Multi-frequency approach supplies “snapshots” at different time-scales, but also provides “snapshots from different angles”: hf-tensor at lower frequencies; g-tensor at higher frequencies.

- SRLS is a “mesoscopic” approach with fewer fitting parameters than atomistic ones, but still requires the multi-frequency data to fit numerous parameters.

- The approach of Tombolato et al. adding “atomistic” detail to the SLE is a way to improve on the SRLS model. Its predictions are in reasonable agreement with the SRLS analysis of the multi-frequency spectra.
Overall Assessment of Molecular Dynamics by Multi-Frequency ESR

- **Tombolato et al.**: Side-chain motion of MTSSL is more complex than “mesoscopic” approach of SRLS.

- Fully atomistic MD study of Sezer et al. led to excellent fits to several multi-frequency spectra, but is more difficult to reconcile with SRLS analysis of extensive multi-frequency spectra.

- Sezer et al. provide explicit evidence that even successful fitting to extensive multi-frequency spectra is not in itself a guarantor of uniqueness of fit.

- We were not able to achieve a clear separation between MTSSL side-chain motions and local backbone motions.
One can, in general, fit an individual single frequency spectrum with somewhat higher quality of fit and perhaps fewer parameters than emerges in the fitting of spectra at several different frequencies. However, given the spectra at the different frequencies provide different perspectives on the molecular motions, and therefore taken together provide a fuller picture of the dynamics, then they should all be given significant weight.

A major achievement in this study is that consistently good fits are obtained at all frequencies with the same set of fitting parameters.
Formation & dissociation of head-to-head dimers by GAsI in DPPC (aligned samples)

Channel formation manifests as a break of Z-ordering.

Spin labeled gramicidin A in DPPC above the $L_\beta - P_\beta$ transition point starts to form channels.

.....due to the tilt of the nitroside moiety.

The conclusion, which could be made at 9 GHz only after simulations........

.....is very clear at 170 GHz.

$\Rightarrow$ the spectral intensity shifts from the XY- to the Z- region & back upon performing a cooling & heating cycle.

* hysteresis state, 15 min. after exposure at 310 K
§ equilibrium state, 24 h after exposure at 310 K
**170/240 GHz Bridge**

- **170GHz source:**
  - 40mW output power
  - 165-175GHz BW

- **240GHz source:**
  - 20mW output power
  - 235-245GHz BW

**Key Components:**
- Quasi-optical design
- Induction mode bridge
- Variable temperature
- Source
- Bridge
- Differential screw tuning drive
- Curved refocusing mirror
- Sample Stage
- Coupling mesh

**Graphs:**
- Output vs. Frequency
- Power Sweeps for Output
Schematic Diagrams of Quasi-Optical Bridges*

Reflection Bridge

Induction Bridge

**Stochastic Liouville Equation**

Assuming the “statistical independence” of the spin evolution and the molecular tumbling we may combine

the spin-density matrix, $\rho(t)$, and

the orientational distribution function, $P(\Omega,t)$, into

a combined spin and orientational distribution function, $\rho(\Omega,t)$, obeying:

$$\frac{\partial \rho(\Omega,t)}{\partial t} = -i[\hat{H}, \rho] - \Gamma_{\Omega} \rho(\Omega,t)$$

which is the **stochastic Liouville equation** (SLE).

Note, that we recover the normal density matrix by averaging $\rho(\Omega,t)$ over all $\Omega$:

$$\rho(t) = \langle \rho(\Omega,t) \rangle_{\Omega}$$

and we recover $P(\Omega,t)$ by setting the spin(s) $S, I = 0$. 
Basic Pulse Sequences in 2D-ESR

**COSY** pulses
coherence pathways

**2D-ELDOR** pulses
coherence pathways
**2D-ELDOR**

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**2D-ELDOR, A Powerful tool for Studying Membrane Dynamics Over Wide Temperature & Composition Ranges**

- Phases of Two Component System: DPPC/Chol
- Initial 2D-ELDOR Studies Show Phase Structure Changes in Plasma Membrane Vesicles (PMV) from RBL Cells upon Stimulation

- The spectra from an end-chain labeled lipid are distinctly different in the three different phases.
- The DPPC/Chol phase diagram determined by 2D-ELDOR is, in general, consistent with what was previously found.
- The ordering and dynamics are reliably obtained from the analysis of the 2D-ELDOR spectra.

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**2D-ELDOR provides better understanding of membrane phase structure in PMV**
WORKSHOP 2009 STRUCTURE AND DYNAMICS BY MULTI-FREQUENCY ESR/EPR

2D-ESR at 95GHz with High-Power Pulses

A Quasioptical, High Power Pulsed ESR Spectrometer at 95 GHz

Oriented CSL/DPPC membranes at 17°C

2D-ELDOR at 95 GHz
1 mM solution of TEMPONE in a mixture of water and glycerol.
The active sample volume was about 500 nl.
Spin-labeled Gramicidin A in Oriented Membrane (DPPC)

- Slow motional nitroxyl spectrum at 7°C.
- Orientation selection at 95 GHz (3.2 mm)
- $g_z$ parallel to membrane normal (z-ordered)
Spin-labeled Gramicidin A in Oriented Membrane

"2D-ELDOR (echo-like component) at 7C°"

Slow motional regime - coverage ~350 MHz

B₀ || n
Summary: 2D-ELDOR at cm and mm Waves

- It enables one to distinguish between homogeneous broadening (yielding dynamics) from inhomogeneous broadening (yielding ordering).

- It can provide dramatic distinctions between different membrane phases, and can discern subtle changes in biological membranes.

- Bulk and boundary lipids can be distinguished and characterized.

- New developments combining the advantages of 2D-ELDOR with the increased orientational resolution of high frequencies improves the power of ESR in studies of dynamics.

- Quasi-optical approach allows extrapolation of methodology to frequencies greater than 100 GHz.
Homogeneous $T_2$ for nitroxide vs. Rotational Diffusion Rate for Different Frequencies.
Computational challenges

The Lanczos Algorithm

Given the large basis sets generated by the SLE the time required to diagonalize the SLE matrix can become exorbitant.

- The Lanczos Algorithm (LA) leads to order-of-magnitude or even greater reductions in computation time by:
  - benefiting from the sparsity of the SLE matrix.
  - Projecting out the relevant sub-space.
  - Converting the SLE matrix to tri-diagonal form, which is then easily diagonalized.

- In addition, the LA provides an objective criterion to determine when a sufficient sub-space, $n_S$, has been generated. In modified form, the LA also provides an objective method for pruning the original basis set to go from $N \rightarrow N_{min}$

- Even after this laborious work is completed, the resulting basis sets are generally very large. This matter becomes even more critical in 2D-ELDOR experiments.
2D-FT-ESR

The two-pulse COSY experiment is described in the time-domain by

\[
S_{c \pm}(t_1, t_2) = \sum_{n,j} c_{nj} e^{-\Lambda_{\pm 1} j t_1} e^{-\Lambda_{-1} n t_2} e^{-\Delta(t_2 \pm t_1)^2}
\]

**Note:** \( \Lambda_{-1,i} = \Lambda_{+1,i}^* \)

- The \( \Lambda_{+1} \) are the complex eigenvalues of the SLE for ESR coherences. The real parts are the homogeneous, or \( T_2 \)-type decays; the imaginary parts are the resonant frequencies.
- The \( c_{nj} \) are coefficients determined by the eigenvectors of the SLE for the ESR coherences and by the starting vector \( |\varphi\rangle \).
- \( \Delta \) is the (Gaussian) inhomogeneous broadening.

These signals are normally viewed after a double FT:

\( t_1 \rightarrow \omega_1, t_2 \rightarrow \omega_2 \)
The **3-pulse 2D-ELDOR experiment** is described by:

\[
S_{c\pm}(t_1, T, t_2) = \sum_{n, j, m} c_{njm} e^{-\left(\Lambda_{+1}\right)_{j} t_1} e^{-\left(\Lambda_{0}\right)_{m} T} e^{-\left(\Lambda_{-1}\right)_{n} t_2} e^{-\Delta(t_2 \pm t_1)^2}
\]

Here we have also the eigenvalues \(\Lambda_0\) of the \(p_s = 0\) coherences. They are largely real and represent \(T_1\)-decay as well as spectral diffusion processes. The \(c_{njm}\) require computing the eigenvectors for these coherences.
The very high sensitivity to orientation and motion at 95 GHz requires even more accurate computations. This translates into much larger basis sets and Lanczos projections.

Unfortunately the Lanczos vectors lose their orthogonality (due to round-off errors) as these demands increase.

This made it impossible to simulate the 95 GHz slow-motional ESR spectra with existing algorithms.

A new method based on quasi-minimal residual (QMR) method combined with the Lanczos algorithm mitigates this problem, providing better accuracy for these eigenvectors and eigenvalues, and enabling simulation of these spectra.
Two-Dimensional Double Quantum Coherence: Sensitivity to Orientations for nitroxides at Ku, Ka, and W band

Simulation parameters:
\( B_0 = 12.5 \text{ kG}, \quad B_1 = 60 \text{ G}, \quad d = 25 \text{ MHz} \)

Euler angles defining the orientations of nitroxide magnetic frames, \((\alpha_1, \beta_1, \gamma_1; \alpha_2, \beta_2, \gamma_2)\): \((0^\circ, 45^\circ, 0^\circ; 90^\circ, 45^\circ, 90^\circ)\);
Rigid Limit 250 GHz Spectra of Labeled Lipids in DPPC Membranes and Plot of $g_{xx}$ vs. $A_{zz}$ Showing Polarity Gradient

- ○ - ‘pure’ DPPC dispersions; △ - GA/DPPC dispersions; ◊ - POPC dispersions.

The numbers correspond to the $n$-PC labels studied: 1 ⇒ 5-PC, 2 ⇒ 7-PC, 3 ⇒ 10-PC, 4 ⇒ 12-PC, 5 ⇒ 16-PC.
Complexes of quercetin and unsubstituted o-semiquinone anion-radical.

- Chemically generated o-semiquinone and quercetin radical solutions changed color from brown to dirty green due to complex generation.

- 240 GHz lineshape resembles the lineshape of a semiquinone radical in a semiquinone tar.

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o-semiquinone: \( g_{xx} = 2.00420, g_{yy} = 2.0034, g_{zz} = 2.00232 \)
complex: \( g_{xx} = 2.00565, g_{yy} = 2.0039, g_{zz} = 2.00231 \)
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