Orientational resolution of spin labels in multi-frequency studies - High Field ESR makes a difference

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G-factor resolution

\[ \Delta H_0 = \frac{h \nu \left( \frac{1}{g_1} - \frac{1}{g_2} \right)}{\mu_B} \approx \frac{h \nu \Delta g}{4 \mu_B} \]

For spin labels: orientational resolution.
Orientation of magnetic X, Y, Z axes in the magnetic field

Macroscopically ordered systems
Orientation of the reporter nitroxide in the matrix (monocrystal, aligned membrane etc.)
Orientation of the reporter group in the molecular frame

Macroscopically disordered systems
Anisotropic motion
Relative orientation of magnetic and diffusion axes

Molecular structure, conformation, molecular dynamics, thermodynamics
Sensitivity to all modes of molecular motion

Advantages of different time scale in multifrequency studies

HFHF ESR

Visual clarity.

NB: no complicated simulations in this talk, very simple models
**Example: a nitroxide radical**

\[ g_x = 2.0091, \quad g_y = 2.0061, \quad g_z = 2.0023, \quad A_x = 6.2, \quad A_y = 6.3, \quad A_z = 33.6 \]

The full extent of the **9 GHz spectrum** at the rigid limit is determined by the *largest of the principal values of the hyperfine splitting*, that is \( A_{zz} \).

\[ \Delta H_0 = \frac{h \nu \Delta g}{4 \mu_B} \approx 11 \text{ G} \]

\[ 2A_{zz} \approx 67 \text{ G} \]
At High Field ESR the g-factor overwhelms the hyperfine splitting. Different orientations of the radical resonate in different field.

\[ \Delta H_0 = \frac{h \nu \Delta g}{4 \mu_B} \approx 210 \text{ G} \]
In partially oriented systems (e.g. aligned membranes) changing the orientation in the magnetic field $H_0$ manifests itself at HFHF ESR in shifting spectral intensity between X-, Y- and Z- regions. X-, Y- or Z- (or intermediate) ordering can be inferred by simple inspection.


250 GHz

$\psi = 0^\circ$

$\psi = 90^\circ$

$170 \text{ GHz}$

$0^0$

$45^0$

$90^0$

MOMD
X-band: no substantial field shift of spectral intensity with orientation.

*Extensive spectral simulations* are often needed to infer the orientation of the magnetic axes relative to the membrane normal.
High-field ESR on aligned membranes

**X-Band:** one can get ESR spectra corresponding to different orientation of the membrane normal relative to the magnetic field direction merely by rotating the sample in the resonator.

**High frequency ESR** requires very thin (<100 μm) flat samples with $H_0$ directed perpendicular to the sample in order to minimize dielectric losses.

1997: **Fabry–Perot shunt resonator** (Barnes and Freed) to accommodate a thin sample with its flat surface perpendicular to the optical axis of the incident FIR beam.
**Microtome technique on aligned membranes**

B. Dzikovski, K. Earle, S. Pachtchenko, and J. H. Freed  
*J. Magn. Reson.*, 2006, **179**, 273

**ISDU** aligned DPPC membrane  
sample is 300 μm thick.

*Microtome sample, microscopic view*

80 μm slices cut of the sample are utilized in high-field ESR

0.4% CSL in aligned DPPC membrane

170GHz  
25°C

\[ H_0, \text{ G} \]

\[ 0^0, 45^0, 90^0 \]
Formation and dissociation of head-to-head dimers by spin-labeled gramicidin A in DPPC

Channel formation manifests as a break of \textit{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 nitrooxide moiety.

\textit{The conclusion}, which could be made at 9 GHz only after simulations........

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

The mass gravity of the spectra \textbf{drifts from the XY- to Z- region} and back upon the cooling and then heating cycle.

We attribute the considerable hysteresis time in the cycle to a slow dissociation of the channel form in the $L_\beta$ phase.

aligned membrane: $0^\circ$ orientation

9.6 GHz

170 GHz

\textsuperscript{*} \textit{hysteresis state}, 15 min. after exposure at 310 K

\textsuperscript{*} \textit{equilibrium state}, 24 h after exposure at 310 K
For a macroscopically disordered sample the orientation of the nitroxide moiety manifests itself only as a result of anisotropic molecular motion about the principal axis of the molecular frame.

A system with no macroscopic ordering (e.g. membrane vesicles).

Near rigid limit, the spectrum converges to a “powder” spectrum, not sensitive to molecular structure, only magnetic tensors of the nitroxide.
Inclusion compounds of spin labeled molecules with cyclodextrins

Cyclodextrines (CDs) are cyclic oligomers of D-glucopyranose.

β-CD
7 glucose units

γ-CD
8 glucose units


Z -rotation

Z -rotation with a diffusion tilt angle

X -rotation
Z-rotation vs. slow motion

9.4GHz

170 GHz

Averaging effective tensor components

\( \frac{g_{xx} + g_{yy}}{2}, \frac{g_{xx} + g_{yy}}{2}, g_{zz} \)

\( \frac{A_{xx} + A_{yy}}{2}, \frac{A_{xx} + A_{yy}}{2}, A_{zz} \)

Averaging real tensor components

\( g_{xx}, g_{yy}, g_{zz}, A_{xx}, A_{yy}, A_{zz} \)
Because of the axial anisotropy of the hyperfine tensor for nitroxides and low g-factor resolution at X-band, HF ESR has much better sensitivity to Z-rotation. HF ESR is needed to determine the rate and activation energy for this motion.

\[ R_{\|} = 1.7 \cdot 10^8 \text{s}^{-1}; \Delta E = 6.3 \text{Kcal} \cdot \text{mol}^{-1} \]
Diffusion tilt angle

16-sasl in both $\beta$- and $\gamma$-CD:

fast anisotropic rotation ($R_\parallel / R_\perp \sim 5 \cdot 10^8 / 5 \cdot 10^7$) about Z-axis with the diffusion tilt angle $\beta_d = 38^0$ for $\beta$-CD and $32^0$ for $\gamma$-CD.
Dramatic change in the spectrum within a ~ 40K interval for both CDs:

What happens? Change in conformation or cessation of motion?

X-band cannot answer the question
240 GHz

New component with $\beta_d$

Effects of Z-rotation

But HFHF can…
Fast nearly ideal X-rotation: derivatives of TEMPO in β-CD

The rotation about X-axis is very fast, $R_\parallel = 10^9 \text{ s}^{-1}$ at 260K, $10^{-8}\text{s}^{-1}$ at 150K. Molecular motion is observable down to 50K (!)
Even though X-band sensitive to X-rotation….

it gives a *slower snapshot of molecular motion* and requires the FIM model with partial averaging of magnetic tensors to simulate all spectral features.

At 240 GHz Brownian diffusion works OK

9.4GHz  240GHz

FIM simulation  

NLSL best fits (dots)

ΔE=1.7Kcal·mol⁻¹
TEMPO-derivatives in $\gamma$-CD vs. $\beta$-CD

Similar to $\beta$-CD, in $\gamma$-CD TEMPO-derivatives undergo very fast X-rotation, at 260K \( R_|| > 2 \cdot 10^9 \text{s}^{-1} \). Unlike $\beta$-CD, the motion abruptly stops below 200K. Between 200-230K two components (immobile and X-rotating) are discernible both at X-band and HF.

![Graphs showing TEMPO-derivatives at 9.4GHz and 240GHz](image)
While X-band spectra are ambiguous, simulations at HFHF for γ-CD clearly require the \textit{MOMD model} and \textit{asymmetric potential}. For β-CD the model of simple axial rotation works well.

\textbf{β-CD:} perfect hub/axis match

\textbf{γ-CD:} the cavity is larger. The rotating molecule is located on its side causing anisotropic effects in axial rotation.

\[
R_{\perp} = 2 \cdot 10^7 \text{s}^{-1}; \quad R_{\parallel} = 2 \cdot 10^9 \text{s}^{-1}, \quad \text{C}_{20}/\text{C}_{22}/\text{C}_{40} = 2.0/4.4/4.4
\]
Another kind of orientation: relative orientation of $A$- and $g$- frames.

If $g$- and $A$- frames are not collinear, these “$A_{zz}$” are different

In $\beta$-CD shows ~ 1G difference yielding a ~ $10^0$ tilt

In single crystals of TEMPOL the tilt has been reported $8.5^0$

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