Data Analysis in Gd(III) Spin Labeled CW-EPR Distance Measurements for Proteins

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What is CW-EPR?

- **EPR**: Electron paramagnetic resonance.
  - Manipulating electron spins in strong and even magnetic field with Terahertz E&M waves.
  - Just a bit of like NMR but for electrons.

![Zeeman Splitting Diagram](image)

- **FIG 1**: Zeeman Splitting Diagram.
What is CW-EPR?

- **CW**: Continuous Wave.
  - Input “continuous” THz radiation of constant frequency and magnitude.
  - Sweep background magnetic field to get absorption pattern.

- **Line-shape**:
  - Derivative of peak in this pattern is usually called Line-shape.

- **FIG 2: CW-EPR Line-shape (Simplified Demonstration)**
  - **TOP**: Absorption pattern.
  - **Bottom**: Line-shape. Zoomed in derivative of the absorption peak.

- Absorption (Normalized by Its Integral)
  - Sharp Resonance Peak
  - Broad Base Absorption

- Line–shape (Normalized by Its Double Integral)
  - Derivative of the Peak
CW-EPR in Protein Studies: Make a Movie!

- By CW-EPR, we can observe structures of proteins in near natural environments:
  - Non-invasive;
  - Aquatic;
  - Near room temperature (> 200 K tested).

- FIG 3a: Some Membrane Proteins. [Credit: Wikipedia]

- FIG 3b: The protein we are study now (photo-activated proton pump). [Credit: Dr. Jessica Clayton]
CW-EPR in Protein Studies: Basic Steps

- A prove-of-concept test for Step 1 and 2 was done with ruler molecules.

**Step 1**

CW-EPR Line-shapes
Line-shapes proteins are measured, after Gd(III) spin labels attached.

**Step 2**

Distance between Labels
Contrast and analysis of the line-shapes with **CWdipFit** gives us the distance between spins.

**Step 3**

Reconstruct the Structure
From these distance data, live structures of proteins can be reconstructed.
Step 1: Labeling Rulers

- The labels are either free, or separated at fixed distances by ruler molecules.
- **Ruler molecules**: Rigid molecules that can separate spin labels at given distances.
- The labels are complex of Gd(III) ion (spin 7/2).

**FIG 4**: Labeling of rulers.

**LEFT**: Free labels; **RIGHT**: Labels at fixed separations.

(Only for demonstration, NOT real labels)
Step 1: Line-shapes

- The line-shape get **broadened** with introduction of second label.

[FIG 5]: Line-shapes from labels.

**LEFT**: Intrinsic line-shape for free labels;
**RIGHT**: Broadened line-shape for labels separated by rulers.

[Data Credit: $I_1$ ruler by Dr. Jessica Clayton at 30 K]
Step 2: Why it Broadens?

- From Zeeman splitting, we know part of Hamiltonian for our 2 Gd(III) spin system as:

\[ \hat{H}_Z = \sum_{n=1}^{2} \mu_B g \mathbf{B} \cdot \hat{S}_n. \]

- As the Gd(III) labels interact with each other, we need Hamiltonian for spin-spin (dipolar) interactions.

\[ \hat{H}_{SS} = \hat{S}_1 \cdot T \cdot \hat{S}_2. \]

- It provides a significant dipolar broadening determined by spin-spin distance, which can be analyzed as a Pake convolution broadening.

- It dominates with high magnetic field.
Step 2: Pake Convolutional Broadening (Fixed $r$)

1. Intrinsic Line-shape

**FIG 6a**: Intrinsic Line-shapes.

2. Pake pattern

**FIG 6b**: Pake pattern at $r = 2.0$ nm. (via EasySpin.)

3. Convolution

**FIG 6c**: Simulated broadening.
Step 2: Pake Convolutional Broadening ($r$ Distribution)

- In reality, distance are not single fixed values, but a **distribution**.
- We suppose distribution in form of several Gaussians combined.
- The combined line-shape are **linear combination** of line-shapes of different $r$ weighted by the distribution.

**4. Distribution**

**FIG 7a:** Combined Gaussian distribution of $r$.  
- Peak at 4.0 nm
- Peak at 2.5 nm

**5. Linear Combination**

**FIG 7b:** Simulated broadening for this distribution.
Step 2 Fit Function and Fit Process (CWDipFit)

- In other words, we create a **fit function** that can simulate broadening with any distribution of spin-spin distance $r$.

  - Fit the function with Simplex / direct-search method to experimentally observed line-shape to get distribution of $r$.

  - This is method is indeed the mechanism of package **CWDipFit**, which we modified and now reverse-engineered for this usage.
Result: Distance Measurement with $1_1$ Ruler

- For our $1_1$ ruler:
  - Theoretical distance between spin labels is: \(2.1\ \text{nm}\).
  - With our fitting with CWdipFit, we measure the distance as: \((2.03 \pm 0.05)\ \text{nm}\).

➢ FIG 8a: Distribution of spin-spin distance \(r\) from the fit.

➢ FIG 8b: Fit result compared to experimental line-shape.

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2018 UCSB Undergrad Summer Research Symposium
Conclusion

- Distance measurement from CW-EPR line-shape analysis of Gd(III) labels is a reliable method of probing structures.

- Distances **up to ~ 3.4 nm** are proved to be measurable, with temperature up to **above 200 K**.
Acknowledgement

Special Thanks for Dr. Marzieh Kavand and Blake Wilson for help and suggestions!
Extra Slide: Ruler and Label

- **FIG 9**: Structures of ruler molecules and labels. [Credit: Jessica Clayton et al., *Phys.Chem.Chem.Phys.*, 2017, **19**, 5127]
Extra Slides: Full Hamiltonian

Full Hamiltonian is given as:

$$\hat{H} = \hat{H}_Z + \hat{H}_{SS} + \hat{H}_{ZFS} + \hat{H}_{HF}.$$  

Here,

- $\hat{H}_Z$ is for Zeeman splitting,
  $$\hat{H}_Z = \sum_{n=1}^{2} \mu_B g_B B \cdot \hat{S}_n.$$  

- $\hat{H}_{SS}$ is for spin-spin (dipolar) interaction,
  $$\hat{H}_{SS} = \hat{S}_1 \cdot T \cdot \hat{S}_2.$$  

- $\hat{H}_{ZFS}$ is for zero field splitting (unique to spins higher than 7/2),
  $$\hat{H}_{ZFS} = \sum_{n=1}^{2} \hat{S}_n \cdot D_n \cdot \hat{S}_n.$$  

- $\hat{H}_{HF}$ is for hyperfine couplings,
  $$\hat{H}_{HF} = \sum_{n=1}^{2} \hat{S}_n \cdot A_n \cdot \hat{I}_n.$$
**THz Source:** A solid-state source, which multiplies a 15 GHz synthesizer 16× to achieve an output frequency of 240 GHz, produces CW power of 50 mW.

**Sample Holder:** Samples of 8–10 mL volume were placed into a Teflon sample cup. The sample was backed by a mirror and mounted within a modulation coil at the end of an over-modeled waveguide (Thomas Keating Ltd). This assembly was loaded into a continuous flow cryostat (Janis Research Company) mounted in the room temperature bore of the magnet.

**Solution:** Glass transition of a 60:40 (v:v) mixture of D2O and glycerol-d8 used as the matrix for the EPR experiments at 30 K.

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[Gag Credit: Dr. Eric Mefford]