

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

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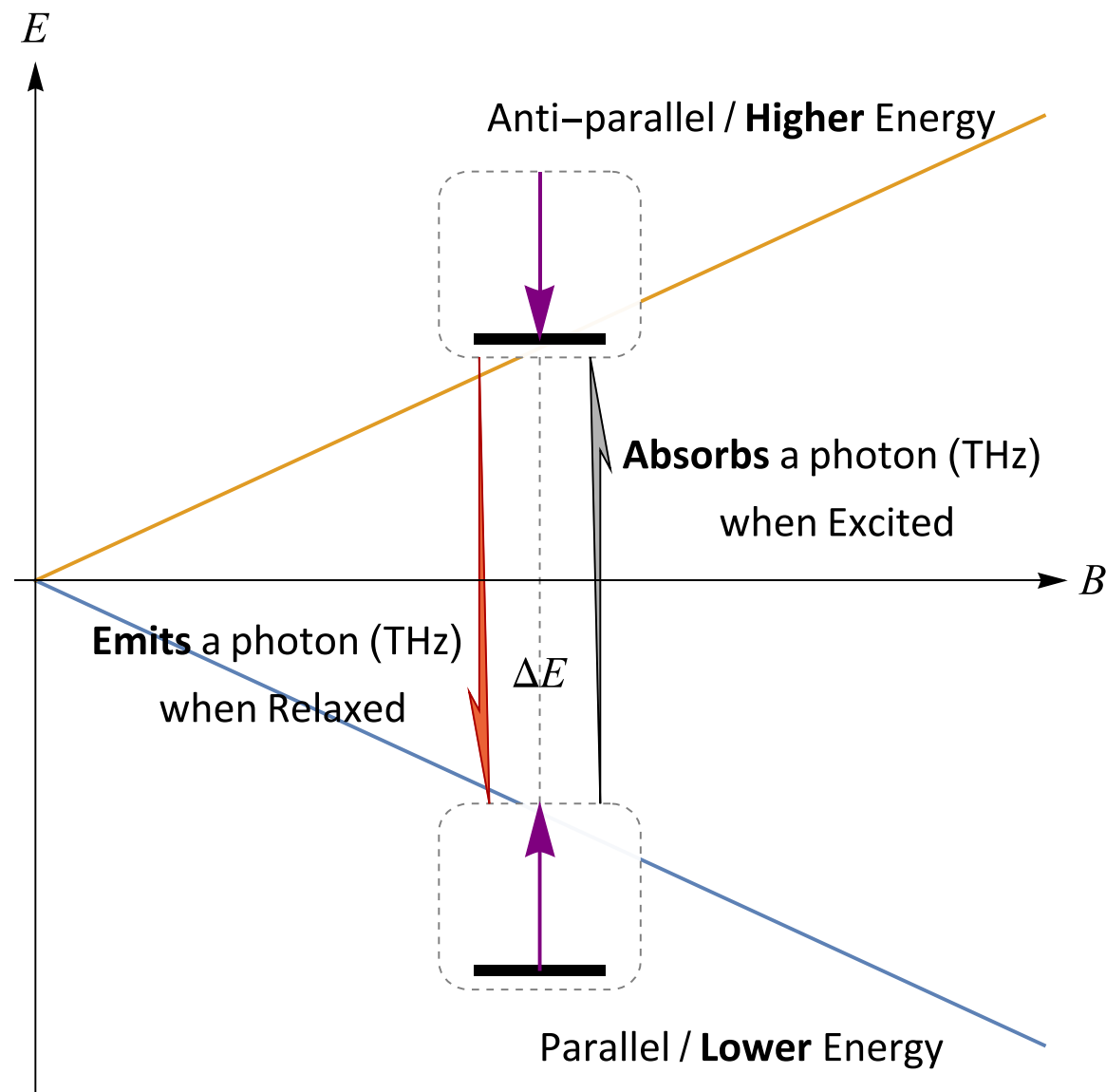
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Mentors: Prof. Mark S. Sherwin, Dr. Marzieh Kavand

# 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.

➤ **FIG 1:** Zeeman Splitting Diagram.



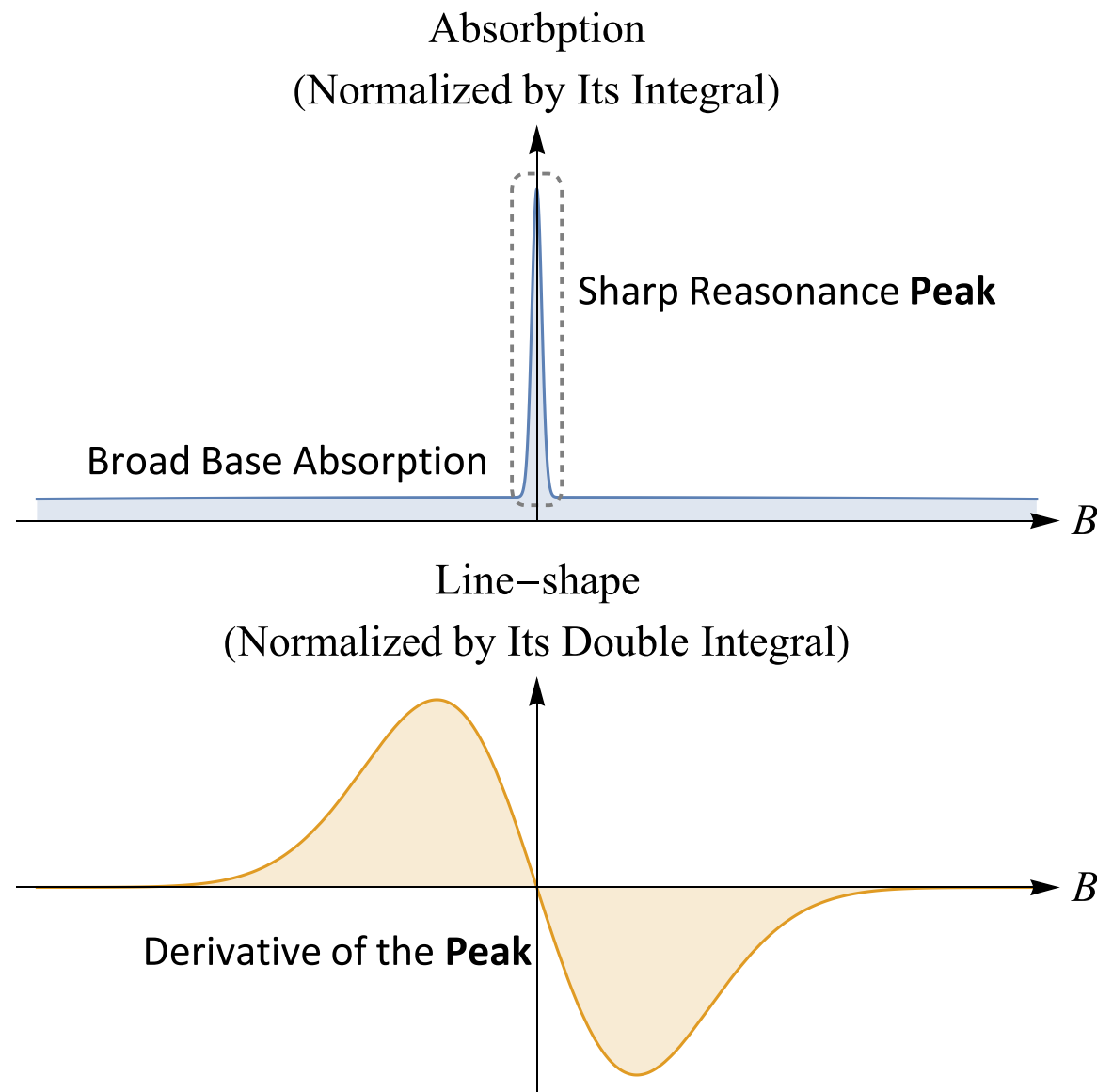
# What is CW-EPR?

- **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.

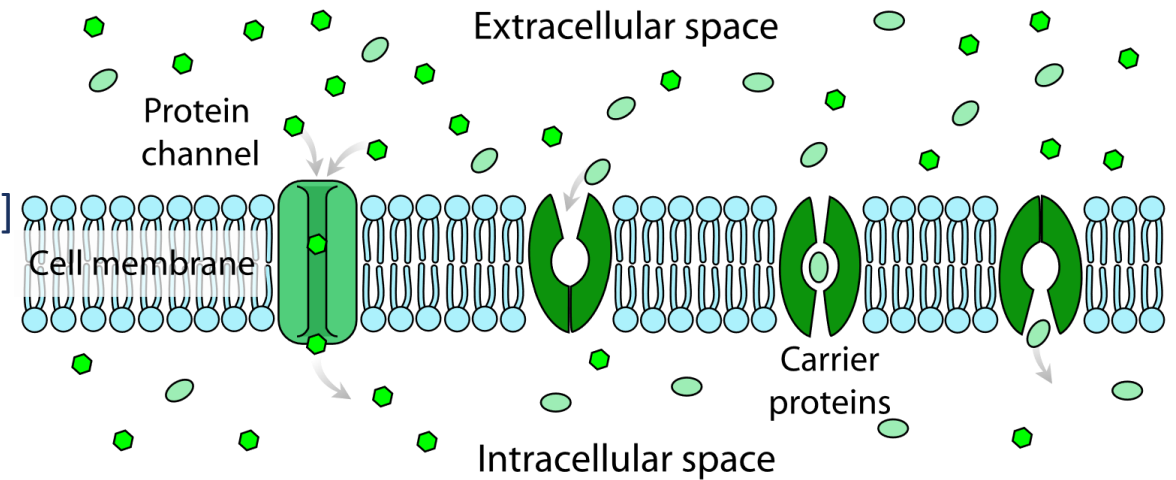
➤ **Bottom:** Line-  
shape. Zoomed in  
derivative of the  
absorption peak.



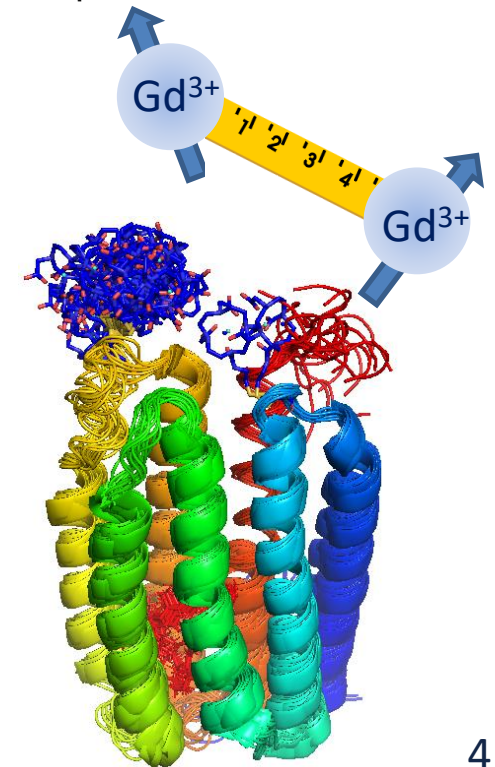
# CW-EPR in Protein Studies: Make a Movie!

- 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.

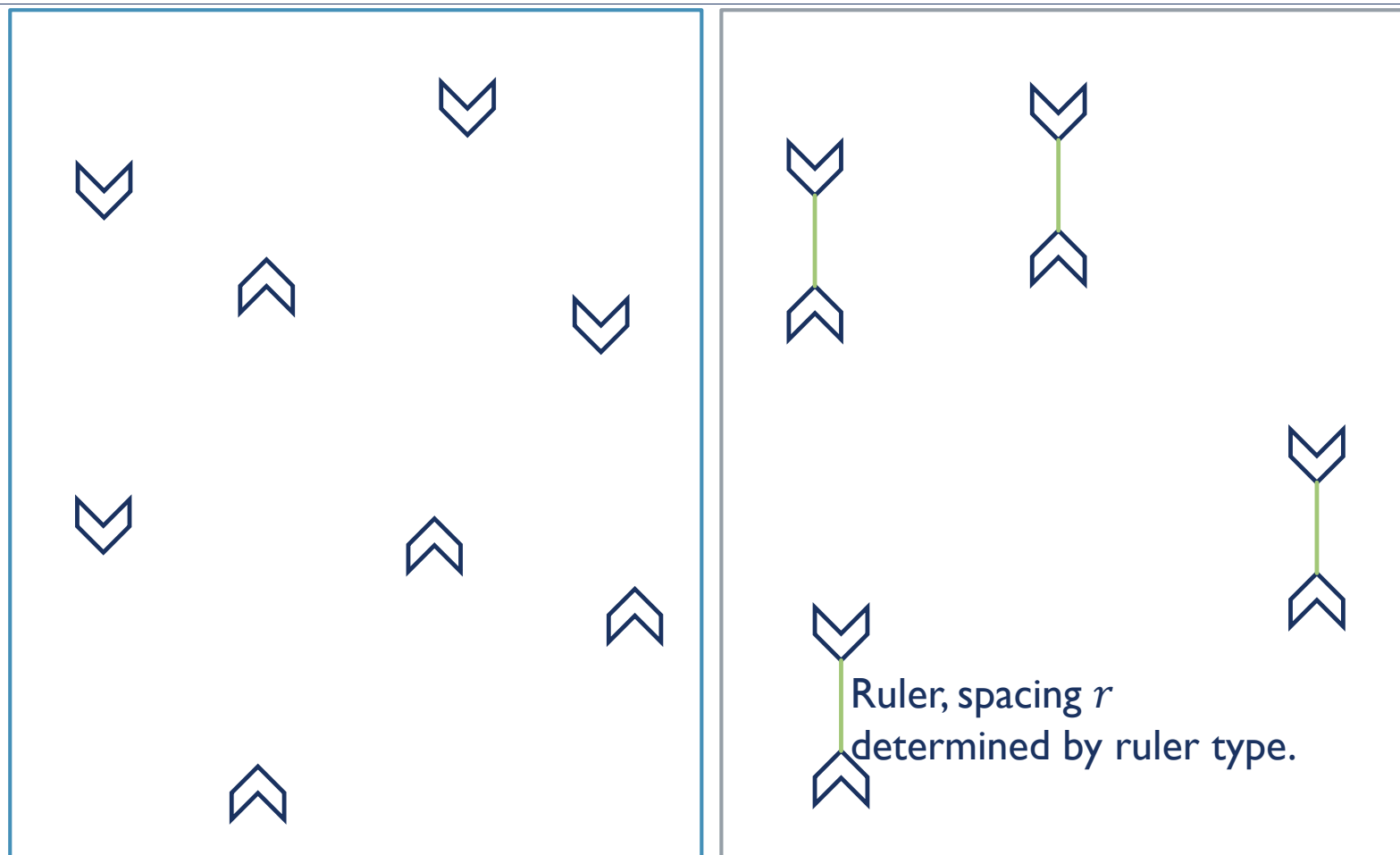
## Step 3

### 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).

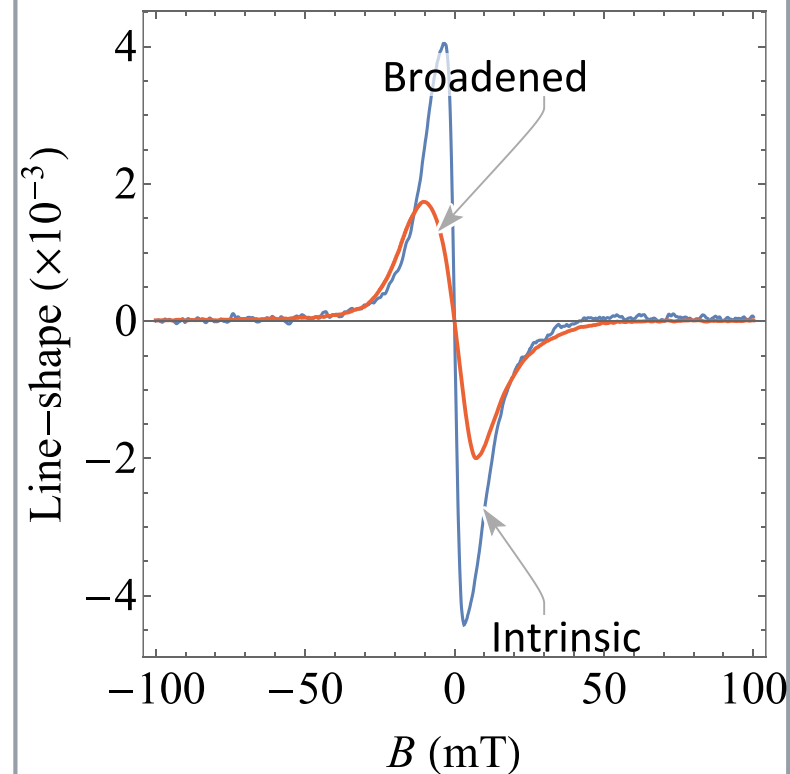
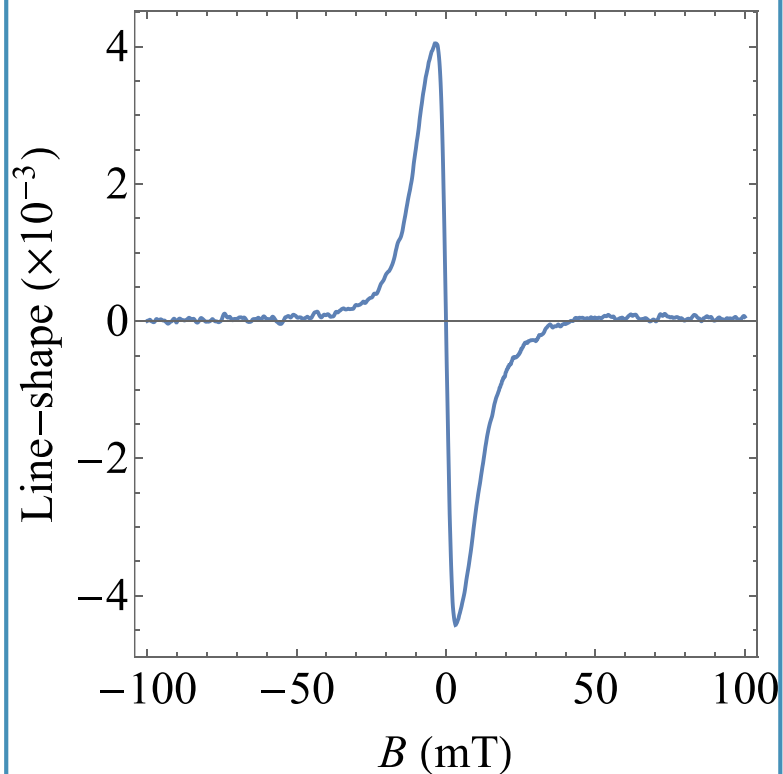


➤ **FIG 4:** Labeling of rulers.  
**LEFT:** Free labels; **RIGHT:** Labels at fixed separations.

(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:  $1_1$  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:

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

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

$$\hat{H}_{SS} = \hat{\mathbf{S}}_1 \cdot T \cdot \hat{\mathbf{S}}_2.$$

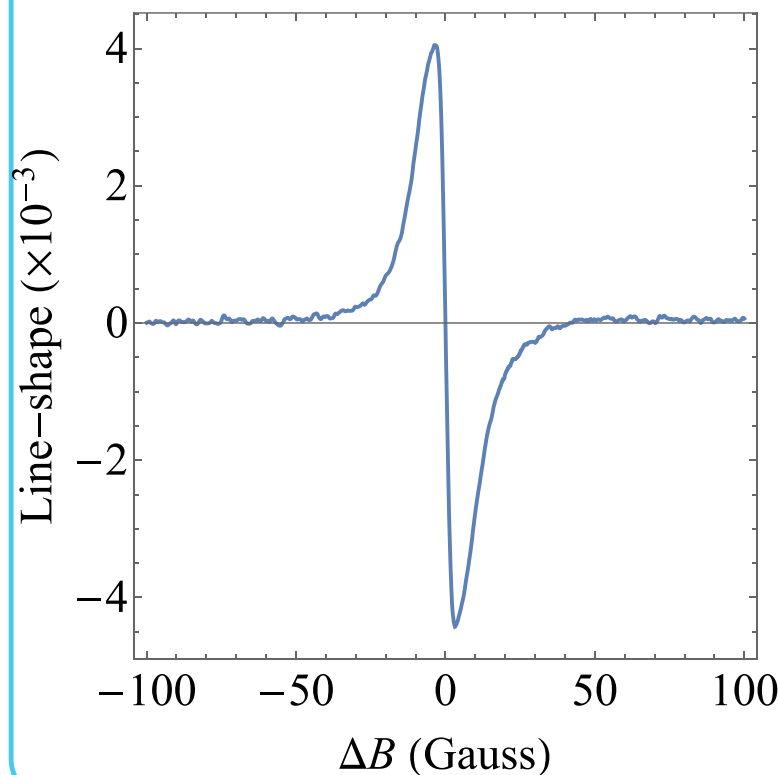
- It provides a **significant dipolar broadening** determined by **spin-spin distance**, which can be analyzed as a **Pake convolution broadening**.
- It dominates with high magnetic field.



## Step 2: Pake Convolutional Broadening (Fixed $r$ )

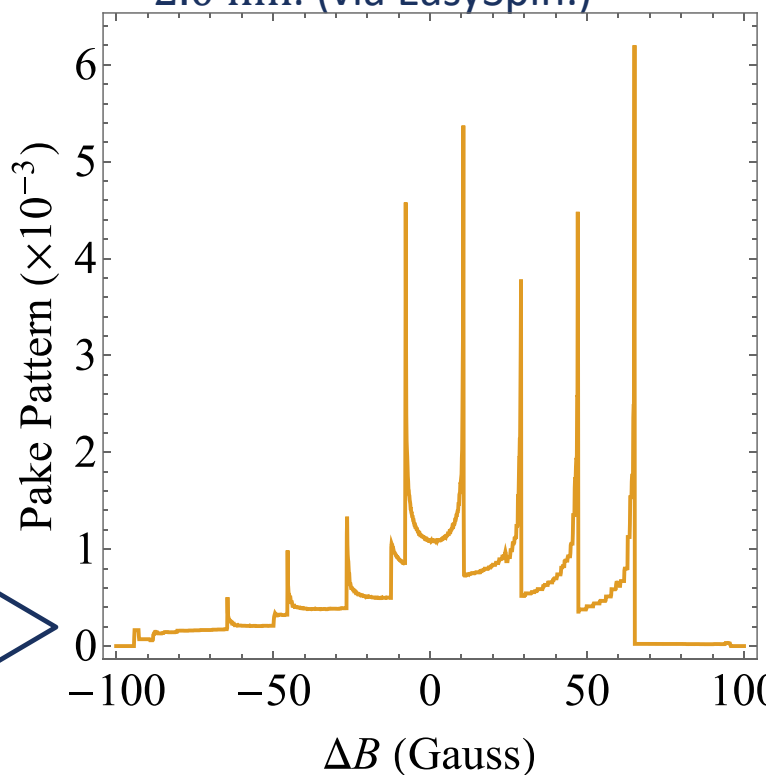
### 1. Intrinsic Line-shape

FIG 6a: Intrinsic Line-shapes.



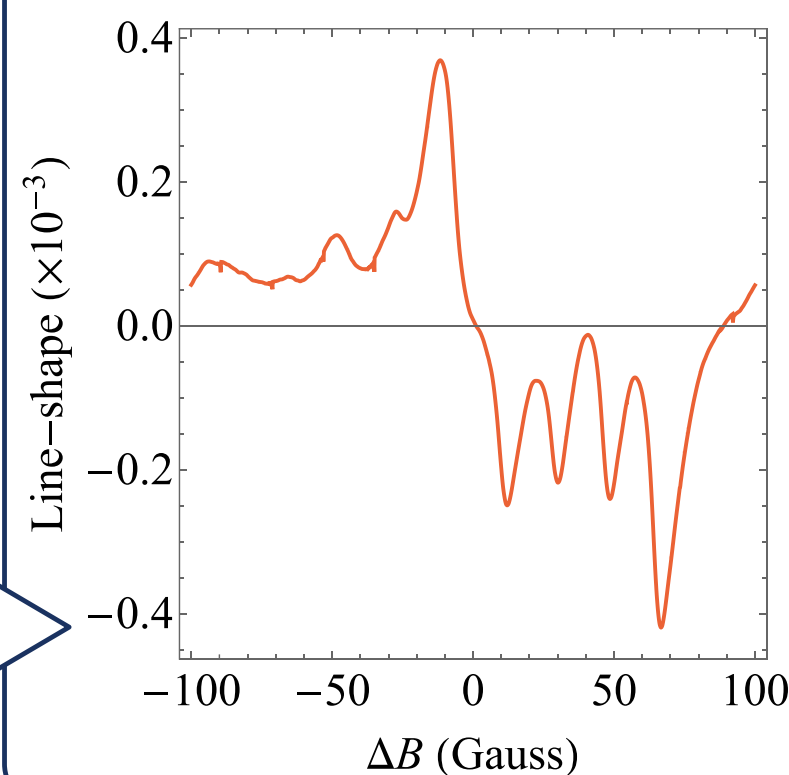
### 2. Pake pattern

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



### 3. Convolution

FIG 6c: Simulated broadening.

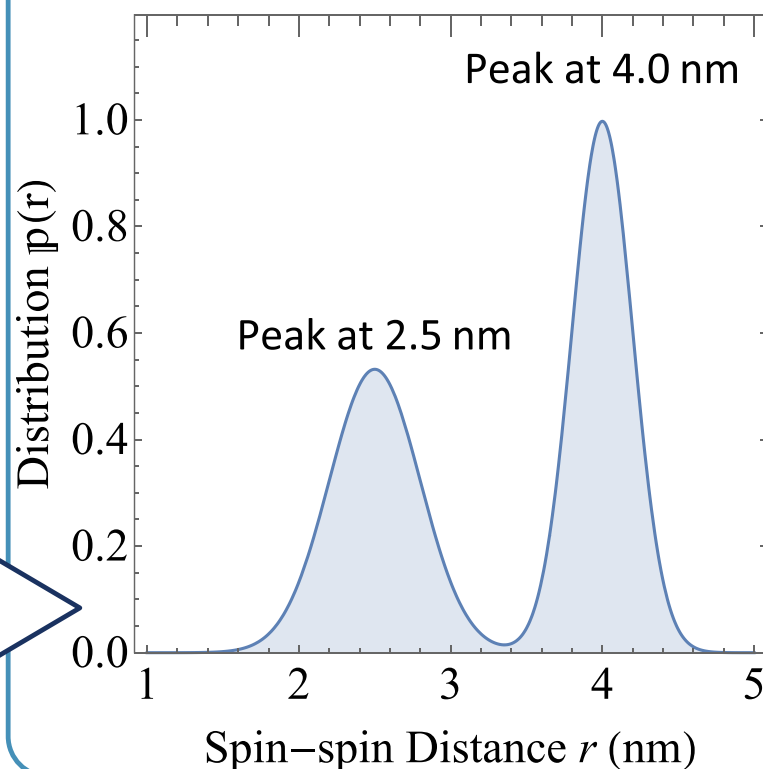


## Step 2: Pake Convolutional Broadening ( $r$ Distribution)

- 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.

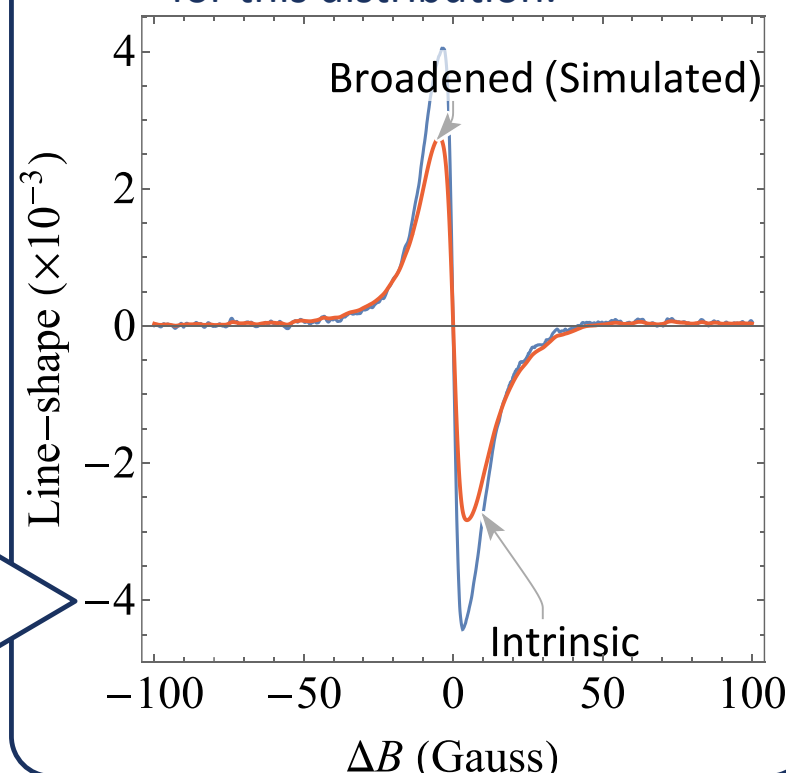
### 4. Distribution

FIG 7a: Combined Gaussian distribution of  $r$ .



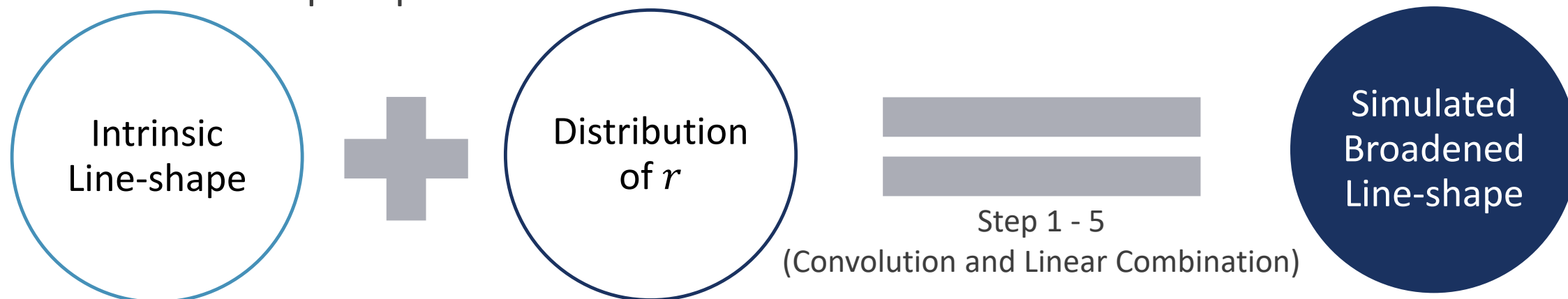
### 5. Linear Combination

FIG 7b: Simulated broadening for this distribution.



## Step 2 Fit Function and Fit Process (CWdipFit)

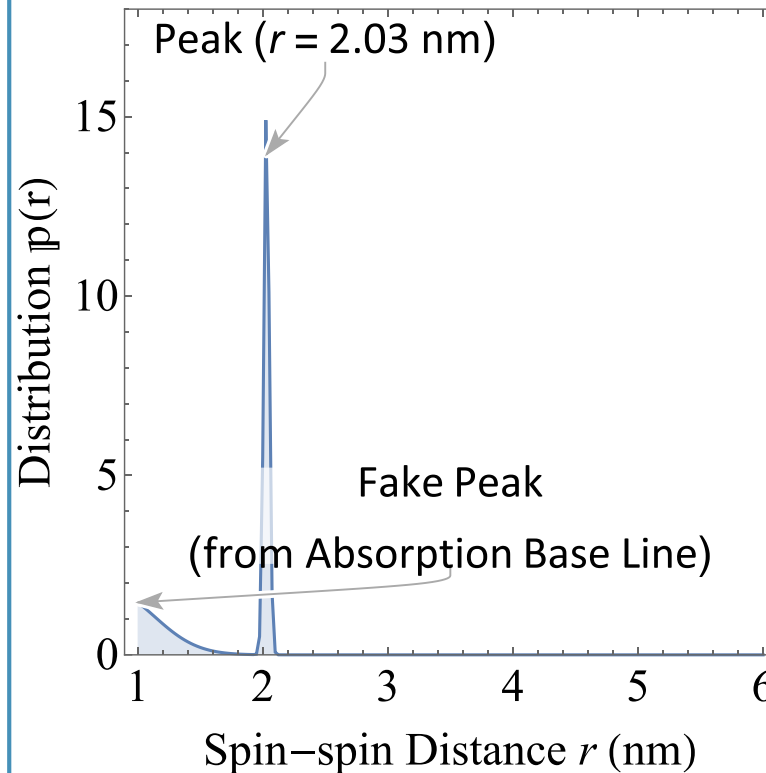
- In other words, we create a **fit function** that can simulate broadening with any distribution of spin-spin distance  $r$ .



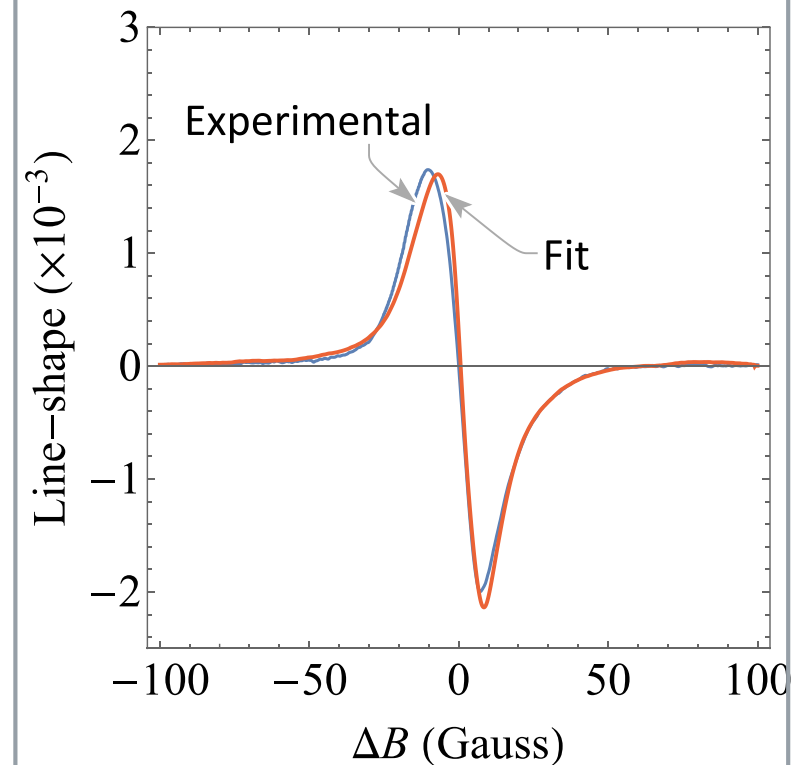
- Fit the function with Simplex / direct-search method to experimentally observed line-shape to get distribution of  $r$ .
- This method is indeed the mechanism of package **CWdipFit**, which we modified and now reverse-engineered for this usage.

## Result: Distance Measurement with $1_1$ Ruler

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



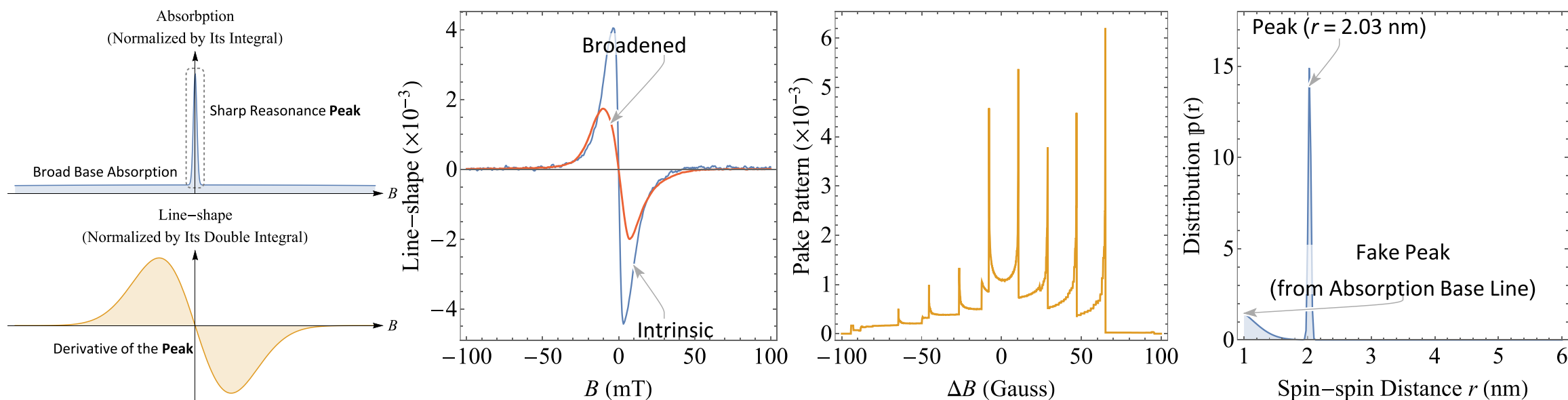
➤ **FIG 8a:** Distribution of spin-spin distance  $r$  from the fit.

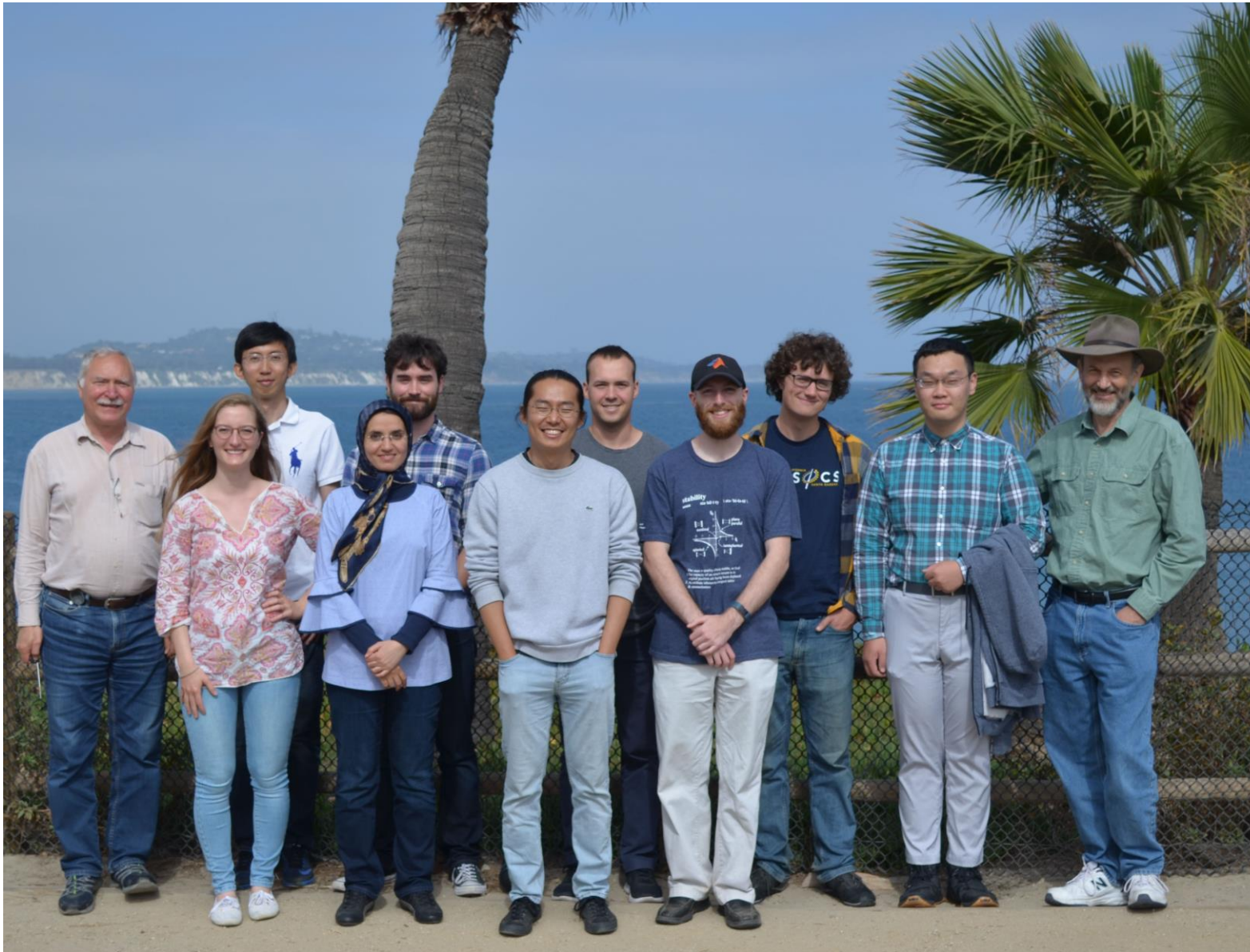


➤ **FIG 8b:** Fit result compared to experimental line-shape.

# Conclusion

- Distance measurement from CW-EPR line-shape analysis of Gd(III) labels is a reliable method of probing structures.
- Distances **up to  $\sim 3.4$  nm** are proved to be measurable, with temperature up to **above 200 K**.





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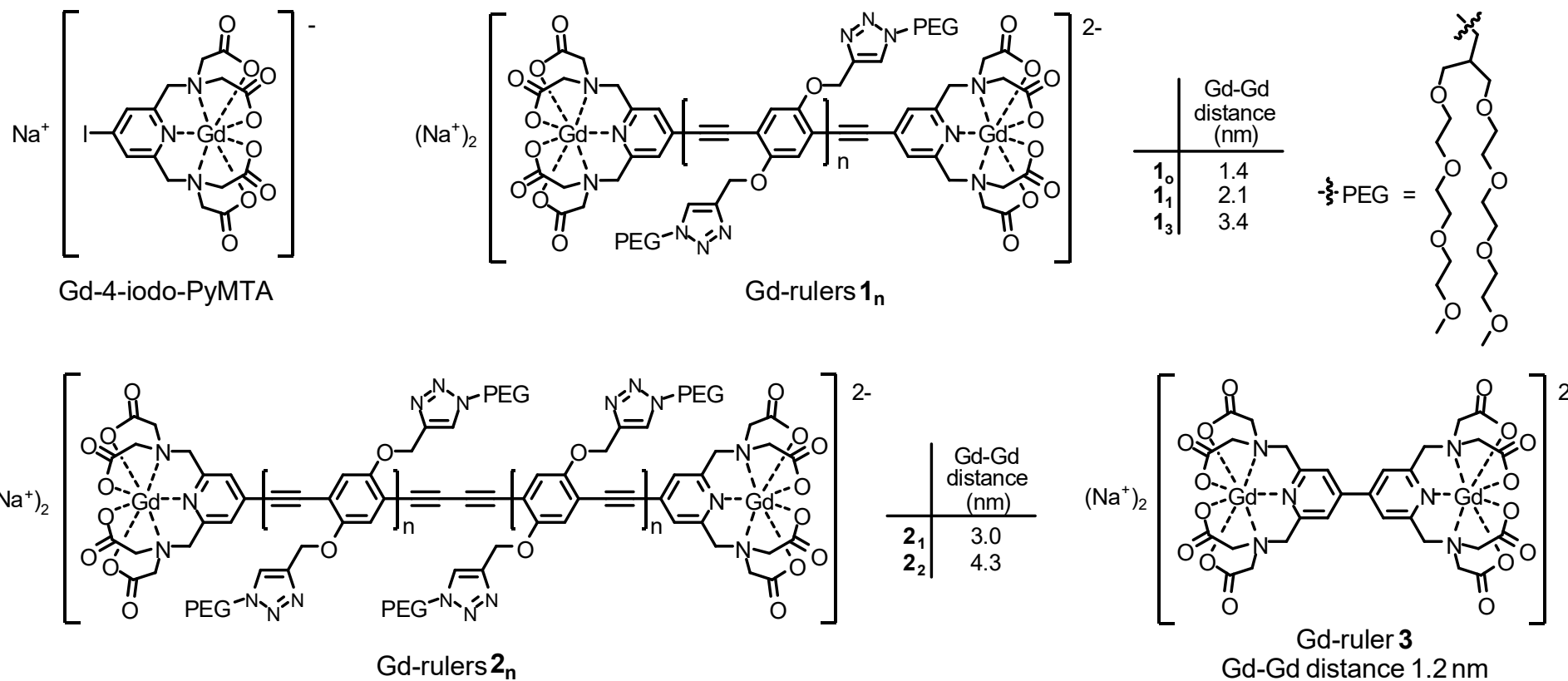
# Acknowledgement

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. (N)

[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,

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

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

- $\hat{H}_{ZFS}$  is for zero field splitting (unique to spins higher than 7/2 ),

$$\hat{H}_{ZFS} = \sum_{n=1}^2 \hat{\mathbf{S}}_n \cdot \mathbf{D}_n \cdot \hat{\mathbf{S}}_n.$$

- $\hat{H}_{HF}$  is for hyperfine couplings,

$$\hat{H}_{HF} = \sum_{n=1}^2 \hat{\mathbf{S}}_n \cdot \mathbf{A}_n \cdot \hat{\mathbf{I}}_n.$$



## Extra Slides: Experimental Equipments

- **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-moded 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 D<sub>2</sub>O and glycerol-d<sub>8</sub> used as the matrix for the EPR experiments at 30 K.

[Credit: Jessica Clayton *et al.*, *Phys.Chem.Chem.Phys.*, 2017, **19**, 5127]

# Extra Slides

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[Gag Credit: Dr. Eric Mefford]