EFEPR

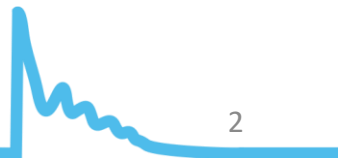# Relaxation–Induced Dipolar Modulation Enhancement (RIDME)
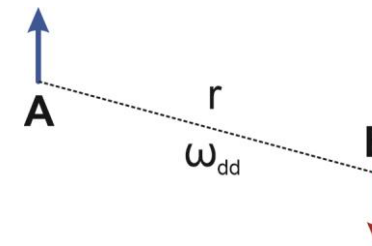
Tutorial

Sergei Kuzin
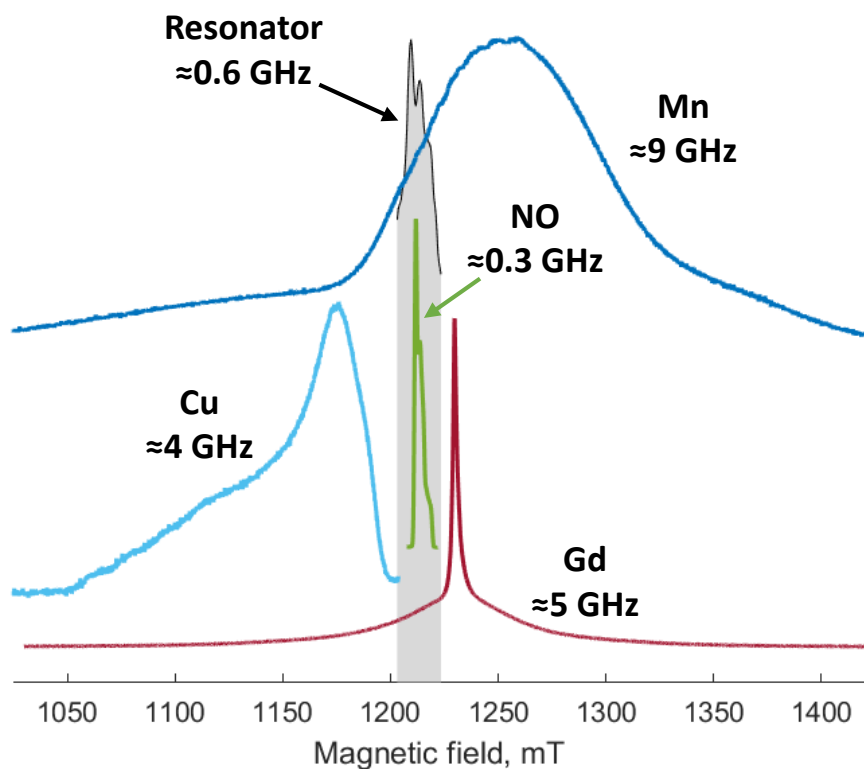
ETH Zurich

06.09.2023

Plan for today

- Short introduction of RIDME: why, when and how

- In-real-time setup for a copper-nitroxide biradical at Q band

- Example of data analysis using DeerLab

- Q&A, extra remarks, advanced topics (if time permits)
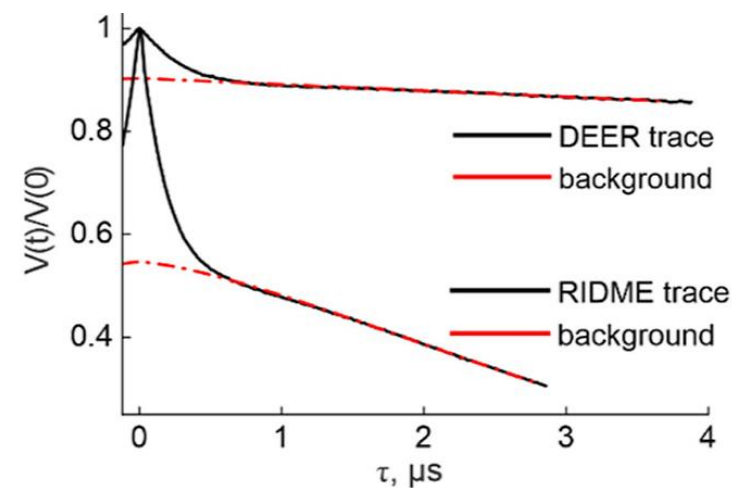
Motivation

Let's measure… DEER?..

Resonator ≈0.6 GHz

Mn ≈9 GHz

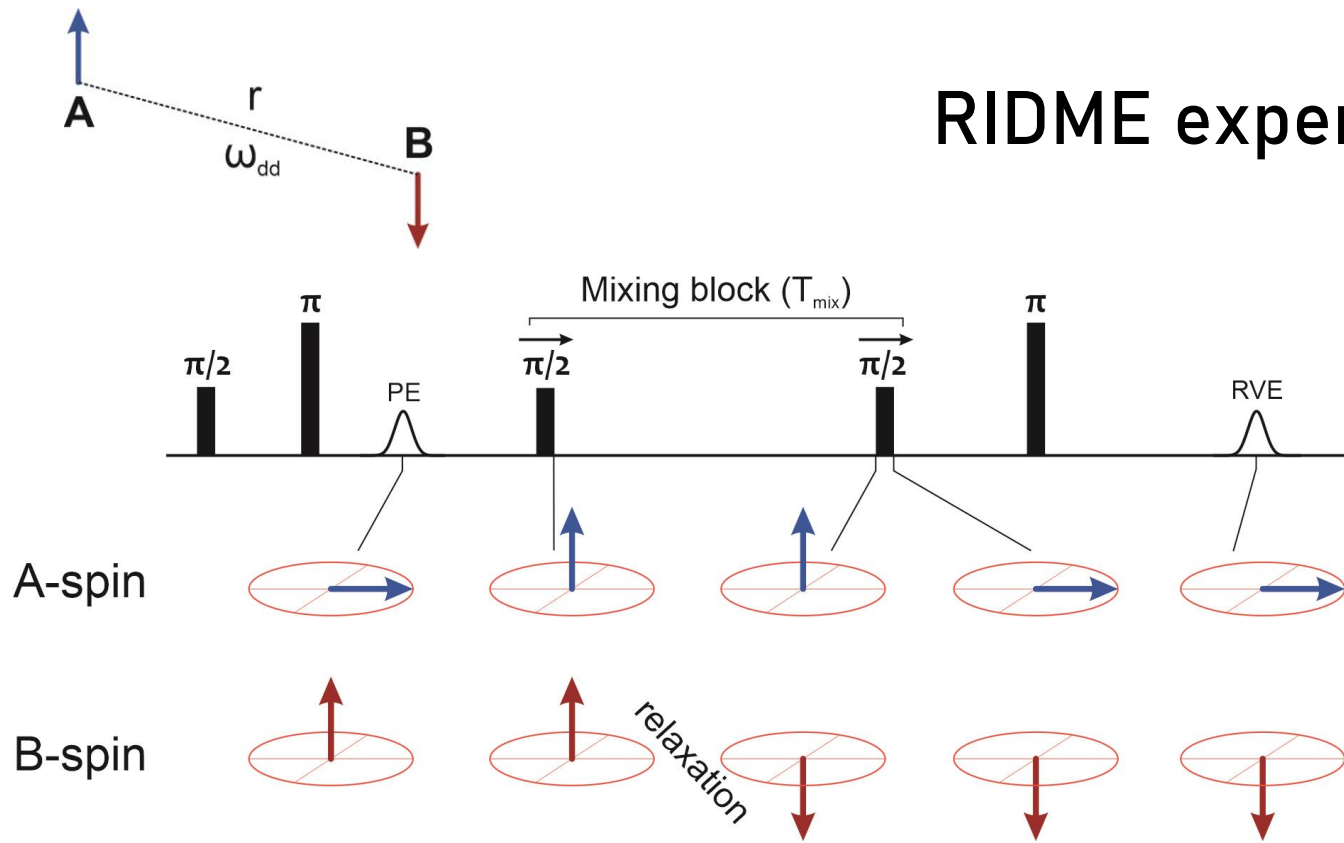**NO ≈0.3 GHz**

Cu ≈4 GHz

Gd ≈5 GHz

Q band ($\nu_{mw} = 34 - 36\,\text{GHz}$)

Double-frequency experiments perform poorly when

- One of the centers is of large spectral width

- The two centers have substantially separated EPR spectra

- One of the centers relaxes fast [10.1016/j.jmr.2022.107327]

RIDME experiment

Mixing block (T$_{mix}$)

A-spin

B-spin

relaxation

B-spins relax during the mixing block

All species presented in the B's EPR spectrum relax

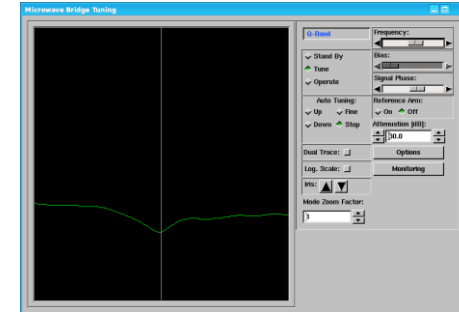|		B-spin (relaxing)			
		Organic	Cu^{2+}	Gd^{3+}, Mn^{2+}	$LS\text{-}Fe^{3+}$
A-spin (observer)	Organic	-	+	+	±
	Cu^{2+}	-	+	+	+
	Gd^{3+}, Mn^{2+}	-	-	+	+
	$LS\text{-}Fe^{3+}$	-	-	-	±

Optimal conditions:

- Hetero-spin case: $T_1(A) > T_1(B)$
- Homo-spin case: $T_1 / T_2 \approx 5$ [a]

[a] 10.1039/C6CP04884F

Some practical hints
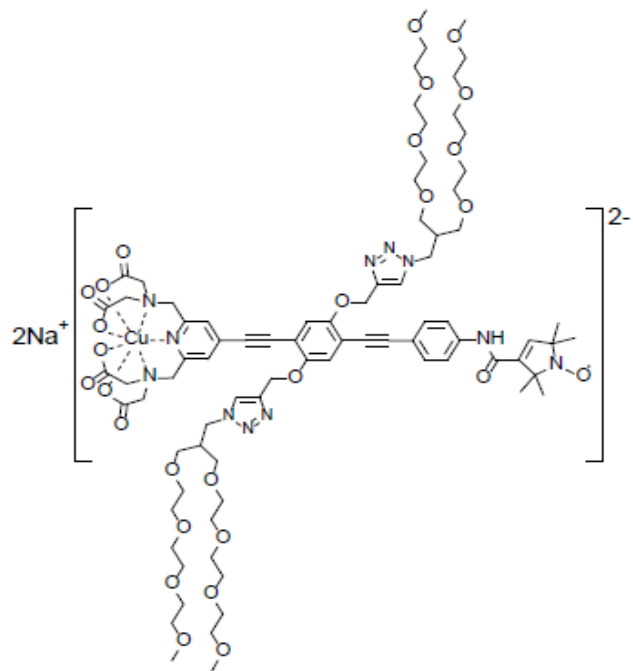
- Single–frequency experiment » center of the mode is OK

- The unique pulse power can be used ($t_\pi = 2 \cdot t_{\pi/2}$) as long as $\omega_{dd}[\text{MHz}] \cdot t_\pi[\text{ns}] \ll 1000$
  - Important! The maximum of the trace (zero time) is then expected at $t = d_1 + t_\pi - t_{\pi/2}$

- Modulation depth depends on mixing time approximately as $\lambda \approx \frac{2S_B}{2S_B+1} \cdot \left(1 - \exp\left(-\frac{T_{1,B}}{T_{\text{mix}}}\right)\right)$. However, at longer mixing times the background gets steeper.

Rule of thumb for temperature choice in RIDME

		B-spin (relaxing)			
		Organic	Cu^{2+}	Gd^{3+}, Mn^{2+}	$LS\text{-}Fe^{3+}$
A-spin (observer)	Organic	-	~40 K	~30 K	~10 K
	Cu^{2+}	-	~20 K	~20 K	~10 K
	Gd^{3+}, Mn^{2+}	-	-	~20 K	~10 K
	$LS\text{-}Fe^{3+}$	-	-	-	~5 K

Auxiliary measurements

EDFS Q band

T$_1$

NO

x10

Cu

1050   1100   1150   1200

Magnetic field, mT

① Echo–detected
Field Sweep

Demonstration sample
~ 50 µM of [CuPyMTA]–NO ruler
Solvent: H$_2$O + H$_8$-Gly
Q band

② T$_1$-relaxation

(A–spin → srt)
(B–spin → T$_{mix}$)

③ T$_2$-relaxation
(A–spin → trace length)

3.8 ms
2.2 ms
1.4 ms

22.4 µs
9.4 µs
5.3 µs

T_1, µs

● NO
● Cu

30   35   40   45   50

T, K

6

Measurement set-up

Pulse sequence: p0 | d_1 | 2*p0 | d_1 | PE | t | p0 | d_4 | p0 | d_2-t | 2*p0 | d_2 | RVE

$\pi/2$ | π | $\pi/2$ | $\pi/2$ | π

$\pm x$ | $\pm x, \pm y$ | $\pm x, \pm y$

2.5 nm
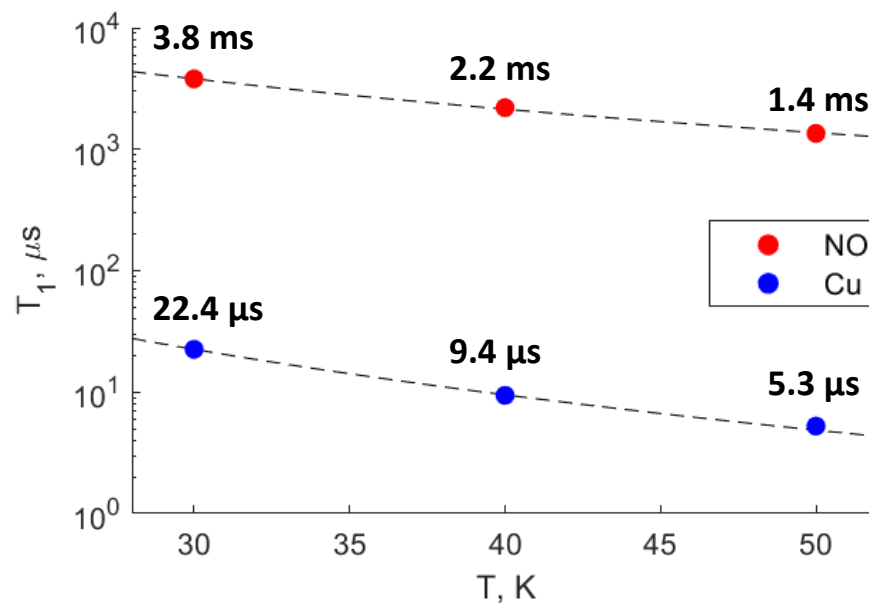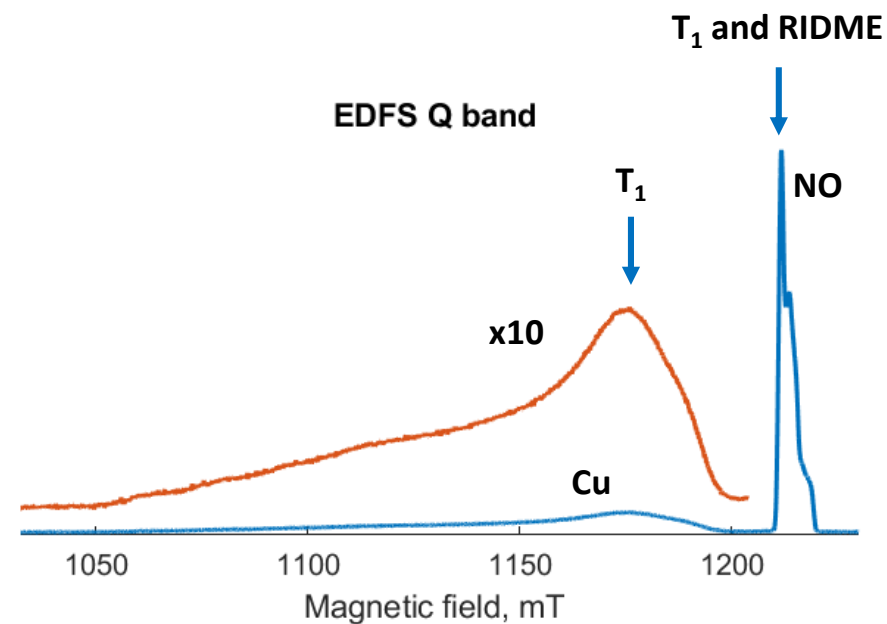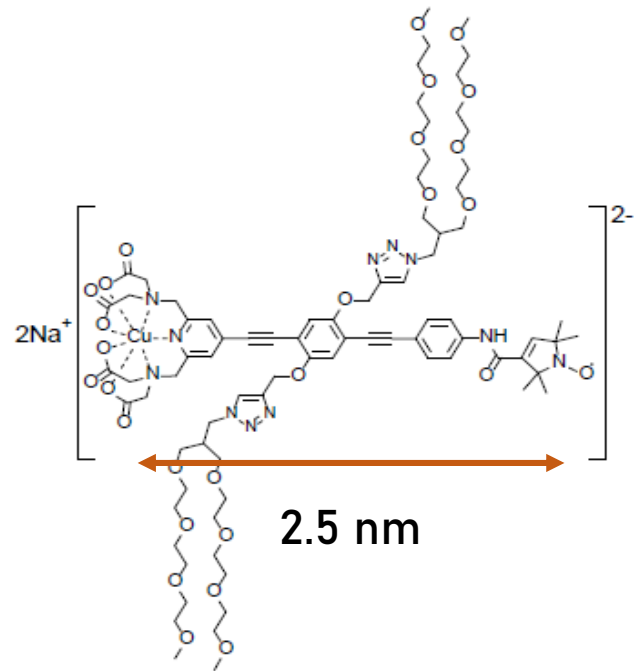
Demonstration sample
~ 50 µM of [CuPyMTA]–NO ruler
Solvent: H_2O + H_8-Gly
Q band
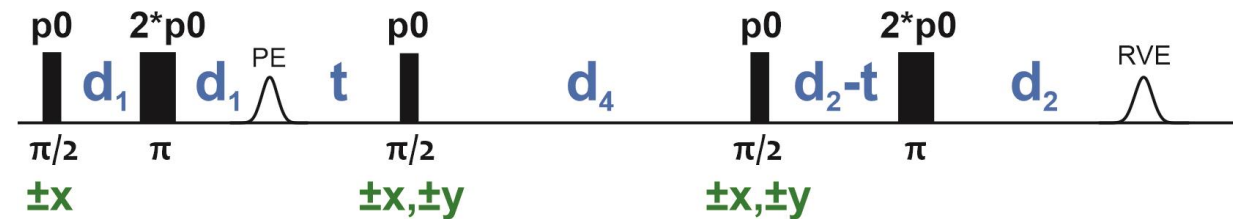
Estimation

$$\omega_{dd} \approx \frac{52.04\ \text{MHz} \cdot \text{nm}^3}{2.5^3\ \text{nm}^3} = 3.3\ \text{MHz}$$

$$T_{dd} \approx \frac{1}{3.3\ \text{MHz}} = 300\ \text{ns}$$

$$T_1(\text{Cu}) \approx 9\ \mu s$$

Conclusion

$$t_{\pi/2} = 12\ \text{ns}, t_\pi = 24\ \text{ns}$$

$$d_2 \sim 5 T_{dd} \approx 1.5\ \mu s$$

$$\Delta\tau \sim \frac{T_{dd}}{20} \approx 8\ \text{ns}$$

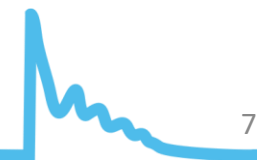$$\text{sx} = \frac{d_1 + d_2 - 2d_3}{\Delta\tau} = 168$$

$$T_{\text{mix}} = 32\ \mu s$$

PulseSPEL

p0	12 ns
p1	24 ns
d1	400 ns
d2	1.5 us
d3	280 ns
d4	32 us
d30	8 ns
h	20
n	1
(d31)	16 ns
(m)	8

Measurement time = $8 \cdot m \cdot \text{sx} \cdot h \cdot \text{srt} \cdot n$

Our measurement time = $8 \cdot 1 \cdot 200 \cdot 20 \cdot 0.012\ [\text{s}] \cdot 1 = 384\ \text{s}$

Data analysis with DeerLab

Evidence of exchange coupling
10.1039/D0CP03105D

Mostly, the same workflow as for 4p-DEER

```python
# load necessary packages

t,V = dl.deerload("...") # load data
Vexp = dl.correctphase(V)
Vexp /= np.max(Vexp)

t += d3 # zero-time correction. The maximum of
# the pathway 1 must mean t = d1

r = np.arange(1.5, 4, 0.02) # nm, distance axis

myridme = dl.ex_ridme(0.4, 1.5, [1], 0.024)
Vmodel = dl.dipolarmodel(t, r, experiment=myridme,
                         Bmodel=dl.bg_strexp)

results = dl.fit(Vmodel, Vexp) # data fitting
print(results)

# plotting
```
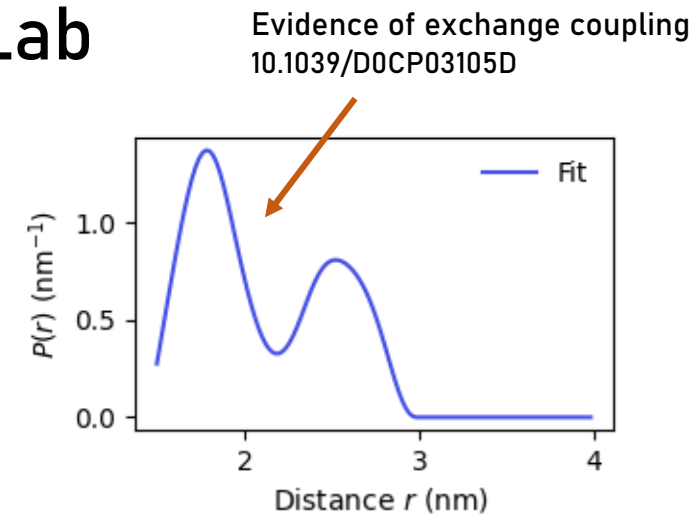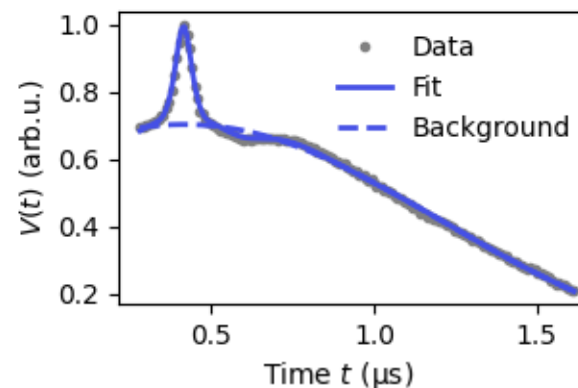


```
Model parameters:

=========== ======= ... ====== ===================
 Parameter   Value  ...  Unit   Description
=========== ======= ... ====== ===================
   mod       0.294  ...         Modulation depth
  reftime    0.413  ...   µs    Refocusing time
   decay     0.838  ...  µs⁻¹   Decay rate
  stretch    2.049  ...         Stretch factor
=========== ======= ... ====== ===================
```
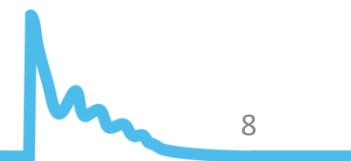
More DeerLab & RIDME examples: github.com/JeschkeLab/DeerLabWorkshop2023/

(1) basic analysis of a RIDME trace with built-in tools
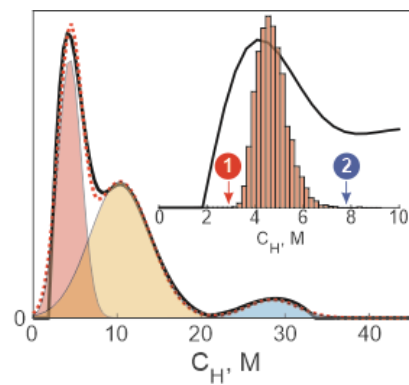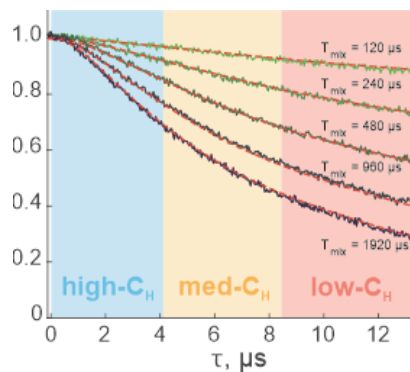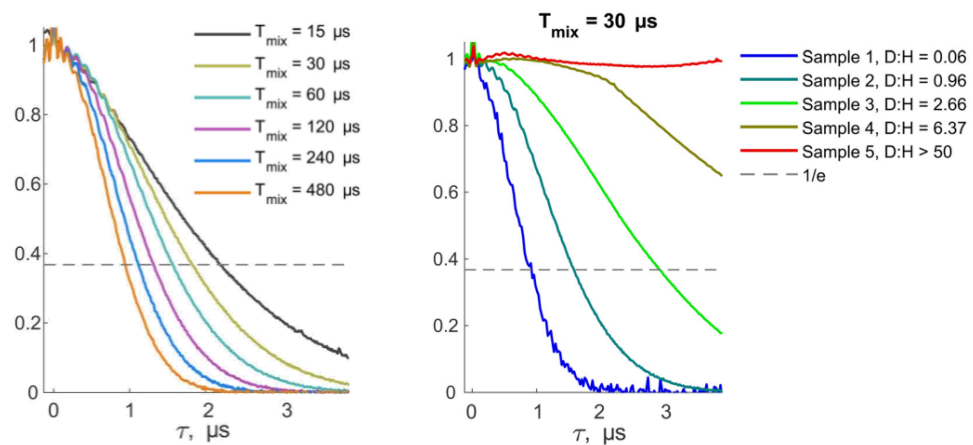
(2) modulation depth vs. mixing time

(3) analysis of a RIDME trace with a customized background function

(4) analysis of a RIDME trace with dipolar overtones
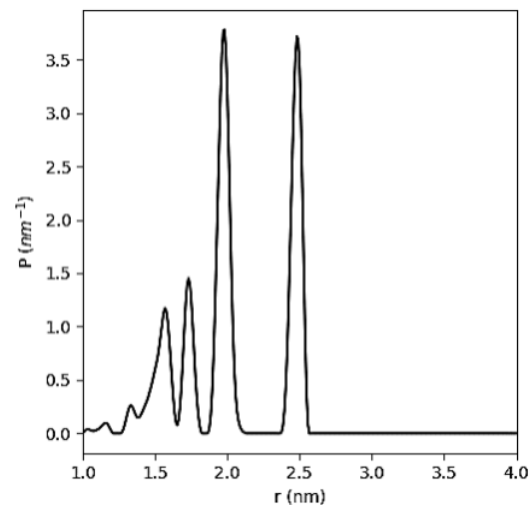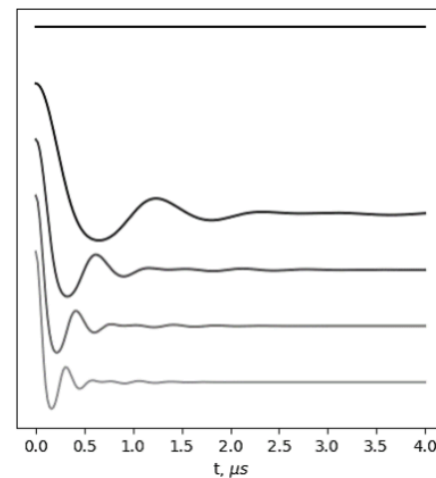
Advanced topics

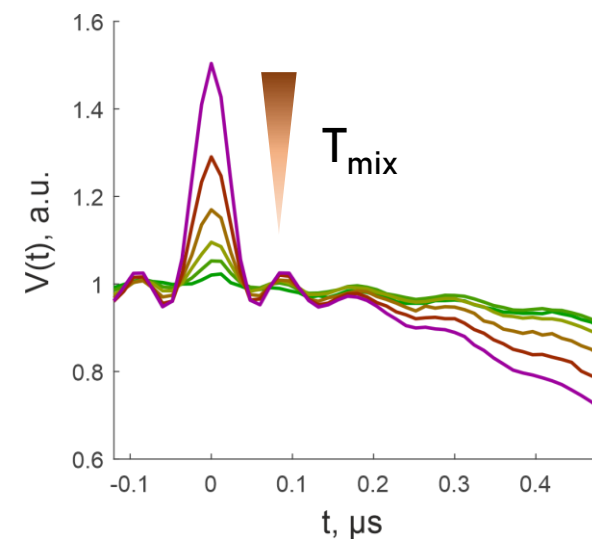| Shape of the background | Dipolar overtones | Zero-time artifact |

Further reading

- **Original papers and Overviews**

  Kulik et al. (**2001**)   10.1016/S0009-2614(01)00721-7

  Milikisyants et. al. (**2009**)   10.1016/j.jmr.2009.08.008

  Astashkin (**2010**)   10.1016/bs.mie.2015.06.031

- **Intermolecular background**

  Keller, Yulikov et al. (**2019**)   10.1039/C8CP07815G

- **ESEEM averaging**

  Keller, Yulikov et al. (**2016**)   10.1016/j.jmr.2016.09.016

- **Spectral diffusion in RIDME background**

  Kuzin, Jeschke, Yulikov (**2022**)   10.1039/D2CP03039J

- **Dipolar overtones in high-spin systems**

  Keller et al. (**2017**)   10.1039/C7CP01524K

  Azarkh et al. (**2022**)   10.1016/j.jmr.2022.107217

- **Applications**

  Wort et al. (**2019**)   10.1002/ange.201904848

  Yang et al. (**2018**)   10.1021/acs.jpclett.8b02663

  Azarkh et al. (**2019**)   10.1021/acs.jpclett.9b00340

  Akhmetzyanov et al. (**2016**)   10.1039/C6CP05239H

- **Advances and variations**

  Abdullin et al. (**2021**)   10.1007/s00723-021-01326-1

  Wort et al. (**2023**)   10.1016/j.jmr.2023.107460

  Kuzhelev, Fedin, Bagryanskaya et al. (**2018**)   10.1039/C8CP01093E

RIDME & DeerLab