Electron Paramagnetic Resonance (EPR) Spectroscopy for Bionanomaterial Measurements

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AFOSR Biophysics Program Review

CNST Center for Nanoscale Science & Technology

NIST National Institute of Standards and Technology • U.S. Department of Commerce

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Mission: To promote U.S. innovation and industrial competitiveness by advancing *measurement science, standards,* and *technology* in ways that enhance economic security and improve our quality of life.

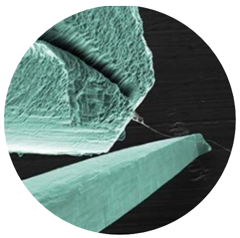

MML

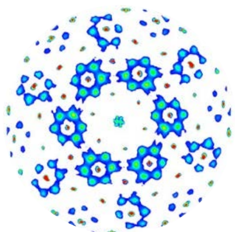

PML


EL


ITL


CTL


CNST


NCNR

Metrology Laboratories

Driving innovation through measurement science and standards

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Accelerating the adoption and deployment of advanced technology solutions

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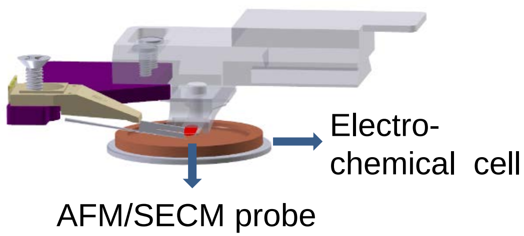
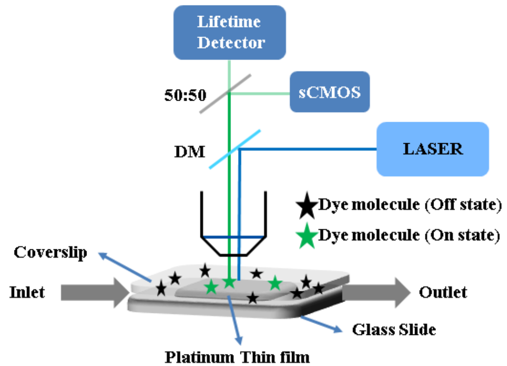
Providing world class, unique, and cutting-edge research facilities

Szalai Lab Overview

Past…

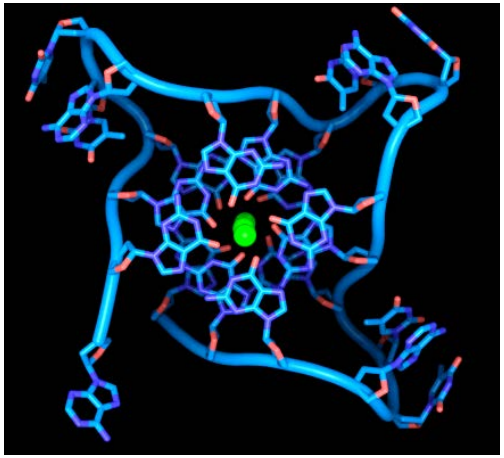




Detect catalytically active regions of nanostructured solid-liquid interfaces

Extend super-resolution fluorescence and scanning electrochemical-atomic force microscopy to catalysts

current…

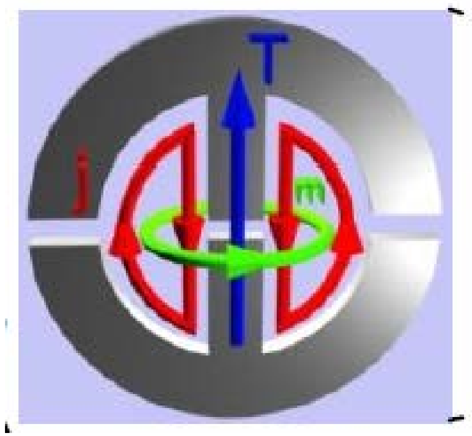


www.quadruplex.org

Improve interspin distance measurements in bionanomaterials

Implement new nanoscale architectures alongside instrumentation to improve sensitivity and applicability

and future.



Basharin *et al Phys. Rev. B*, **2017** *95* 035104

Push sensitivity limits of induction-detection EPR spectroscopy

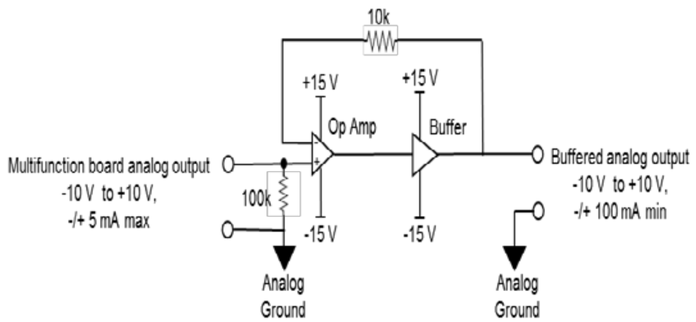
Fabricate customizable metamaterial microresonators for volume-limited samples (thin-film interfaces, biological samples)

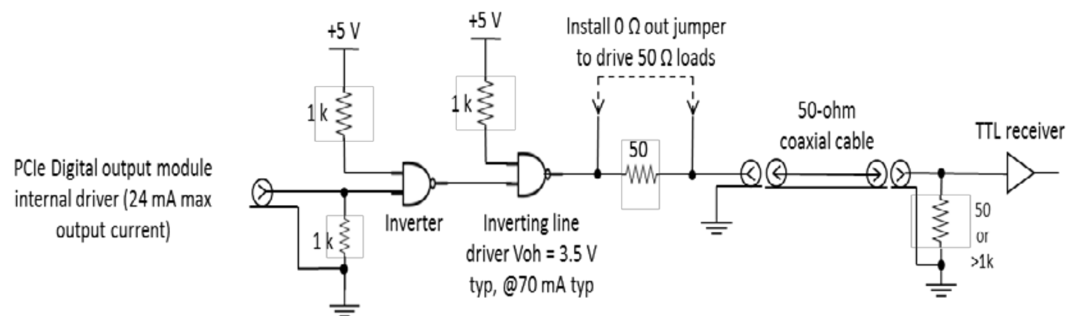
Outline

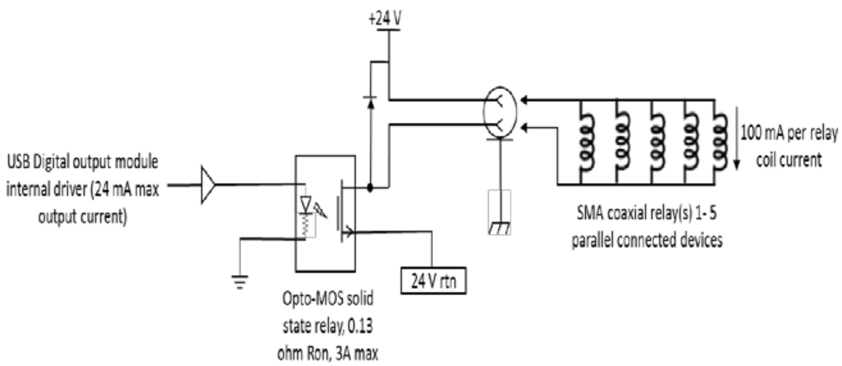
I. Biomacromolecular structure determination of quadruplex DNA nanomaterials using pulsed EPR spectroscopy

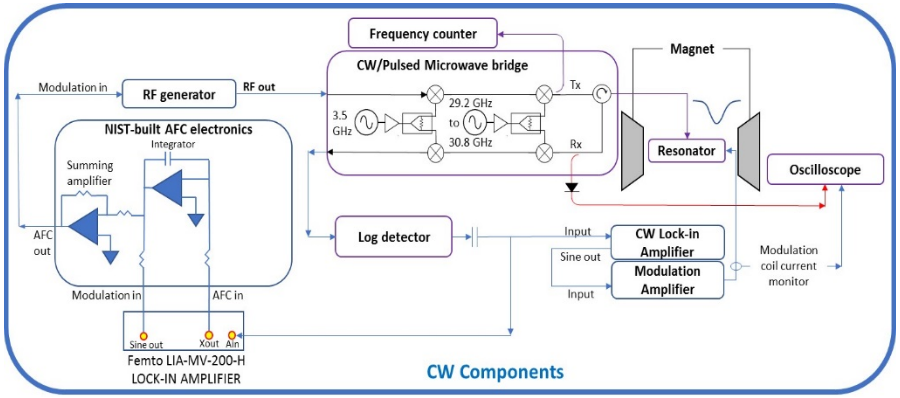
II. Design, capability and future of Szalai Lab EPR instrumentation

This talk is dedicated to Alan H. Band (1959 – 4/10/2018): friend, colleague, and electrical engineer extraordinaire.

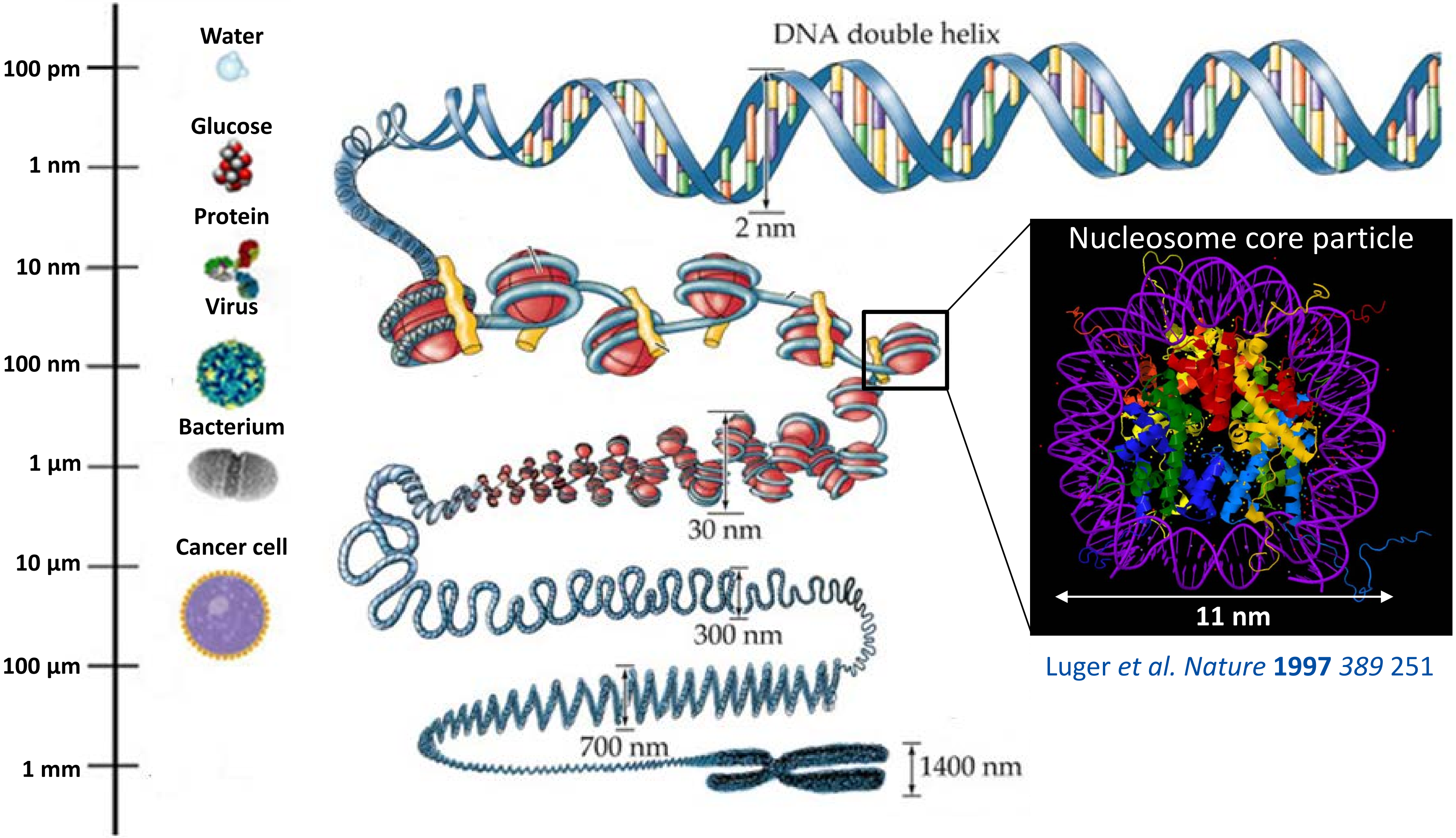








Distance scales of biological systems

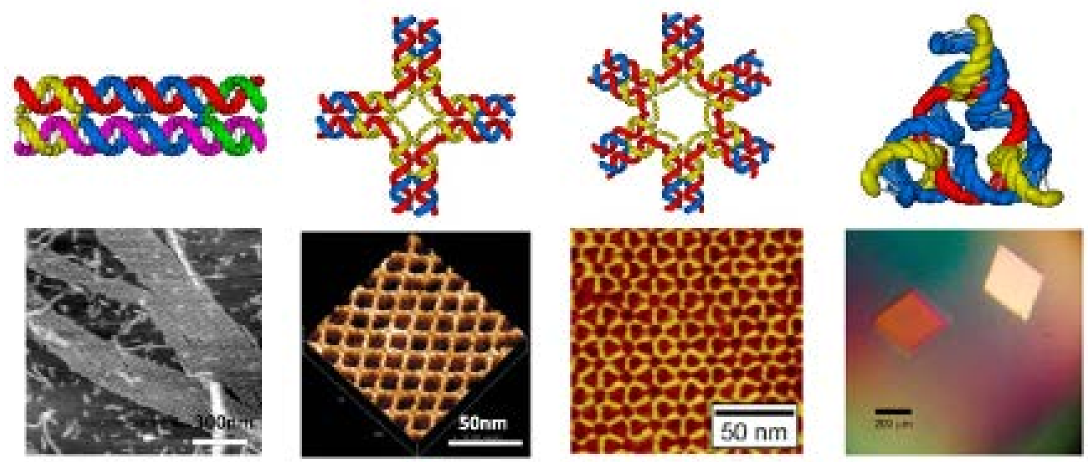


Luger *et al. Nature* **1997** *389* 251

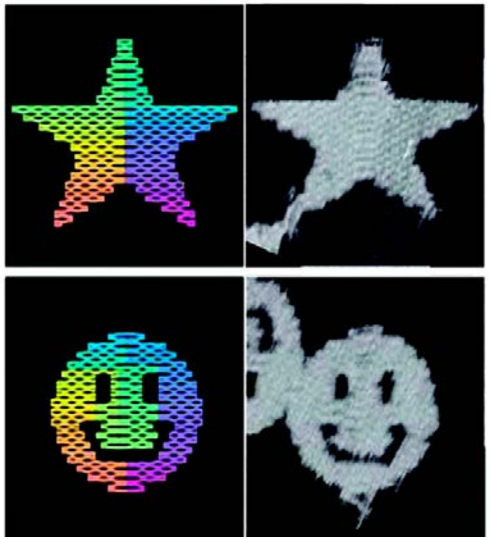
http://nano.cancer.gov/learn/understanding/

DNA nanotechnology is duplex-centric

- DNA strand association is controllable and predictable
- Molecular-level structure of duplex DNA is known
- Duplex DNA is stiff; single-strands are flexible
- Synthesis and modification of DNA is facile
- DNA is biocompatible



Zhang et al. *J. Am. Chem. Soc.* **2014** *ASAP*

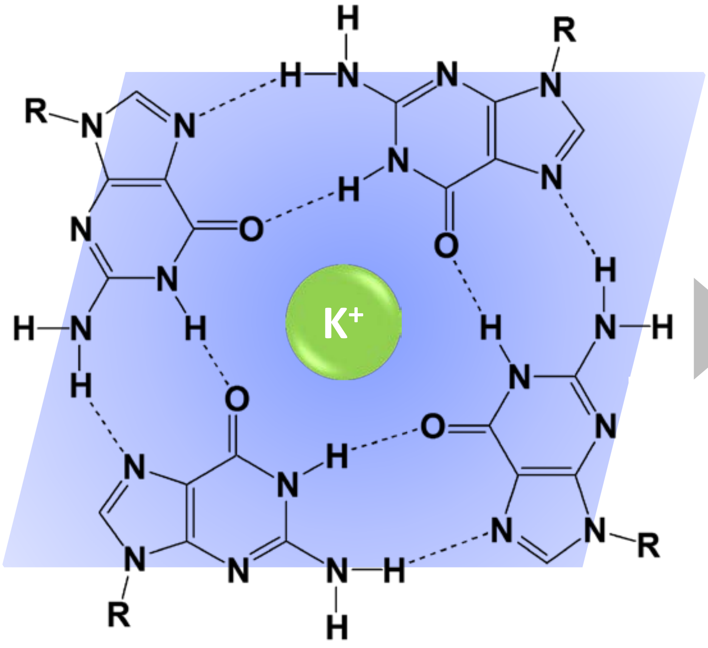


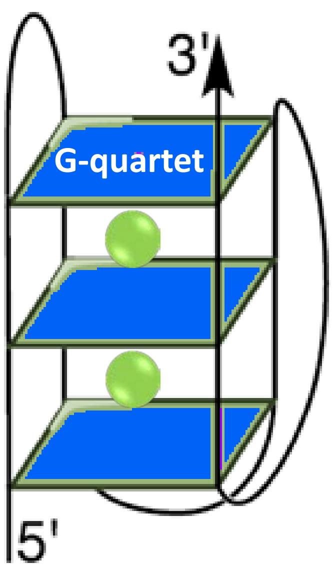
Rothemund *Nature* **2006** *440* 296

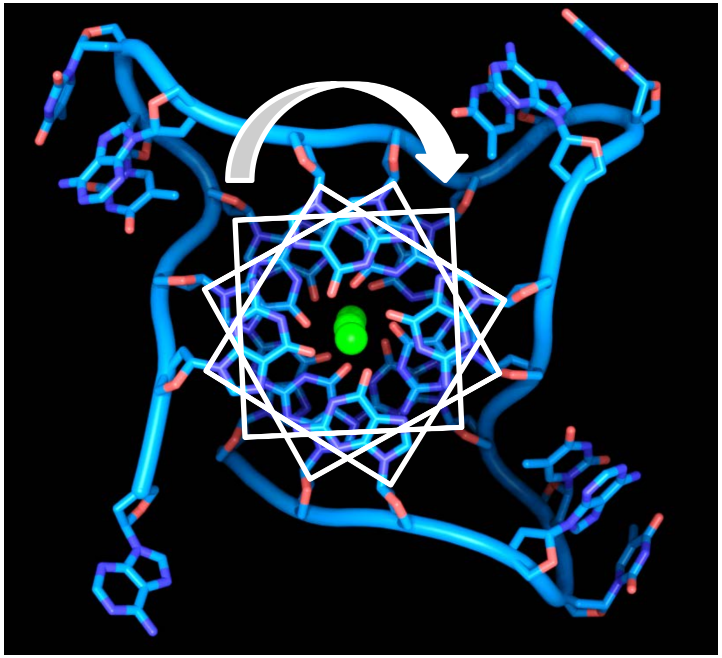
Non-canonical nucleic acid structures are uncommon in DNA nanotechnology

Stacks of guanine quartets make G-quadruplexes

- Guanine quadruplexes (GQs) form with K^+, Na^+, or NH_4^+
- Tracts of contiguous guanines are required for GQ formation
- G-rich DNA sequences have been identified in the human genome







http://www.quadruplex.org/

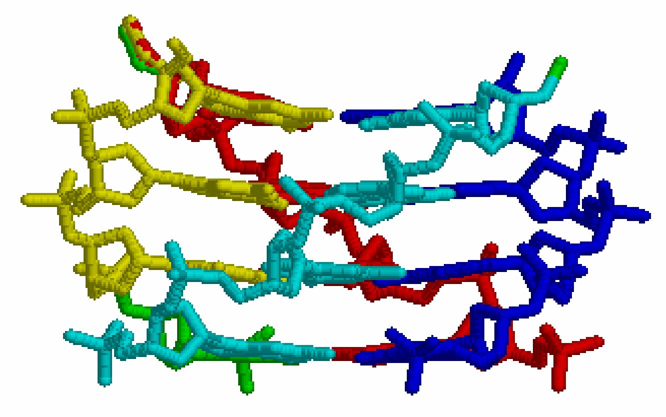
GQs contain variable numbers of G quartets and have helical pitch

GQ building blocks can be systematically varied

- Start with small, tetramolecular GQs

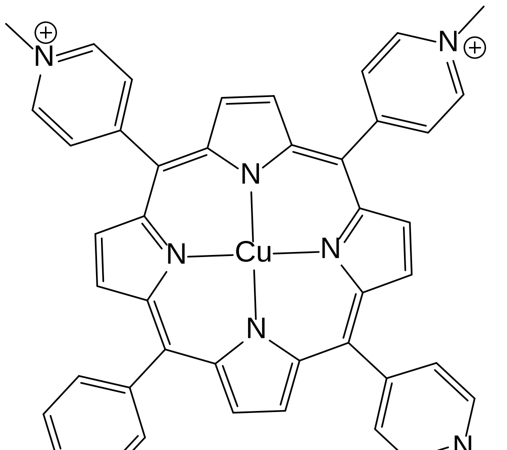
Abbreviation	Sequence
G4	T_4GGGGT_4
G6	$T_4GGGGGGT_4$
G8	$T_4GGGGGGGGT_4$
G10	$T_4GGGGGGGGGGT_4$

XRD tetramolecular GQ



Phillips et al. *J. Mol. Biol.* **1997** 273 171

- Add a ligand with a unique measurement "handle"



CuTMpyP4

≈1 nm

Donohue & Szalai *Phys. Chem. Chem. Phys* **2016** *18* 15447
Sabharwal et al. *FEBS J.* **2014** *281* 1726
Mendez & Szalai, *Nanoscale Res. Lett.* **2013** *8* 210
Mendez & Szalai *Biopolymers* **2009** *91* 841
Evans et al. *J. Biol. Inorg. Chem.* **2007** *12* 1235
Keating & Szalai *Biochemistry* **2004**, *43*, 15891

Our GQ:CuTMPyP4 samples are well-characterized

- Tetramolecular GQs of increasing length bind CuTMpyP4
- GQs with or without CuTMpyP4 fold properly
- GQs with CuTMpyP4 can be purified
- GQs bind 2 CuTMpyP4

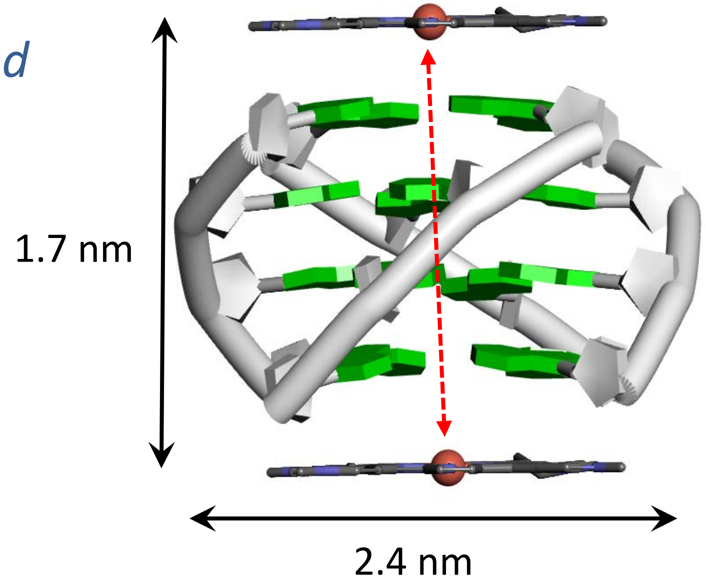
Where do the 2 CuTMpyP4 molecules bind?

Figure by M. Donohue

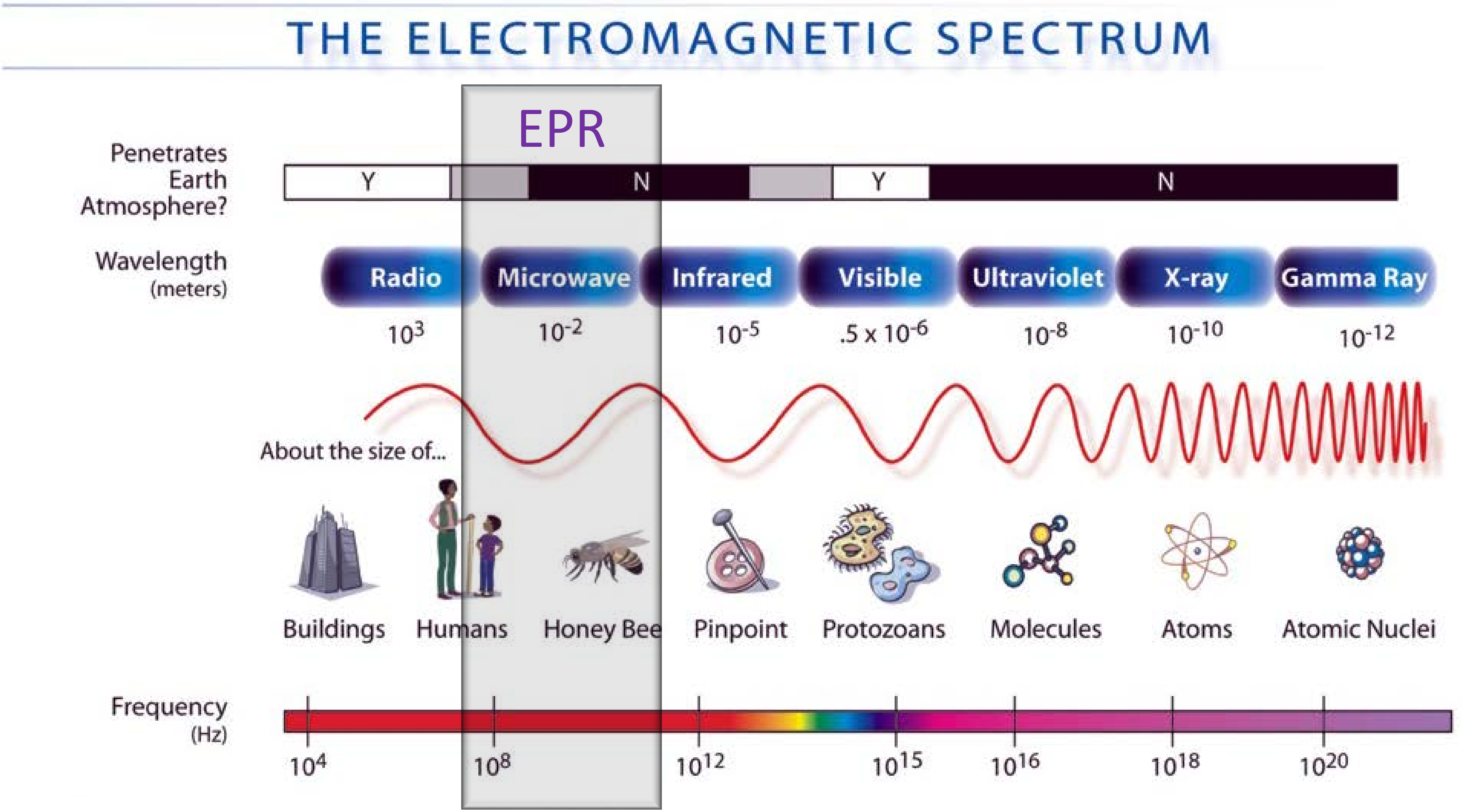
→ *We hypothesize that CuTMpyP4 binds above and below G quartet stacks in our constructs.*

→ *Predict that as GQ length increases, Cu-Cu distance should increase.*

Test this hypothesis by measuring the inter-Cu(II) distance using pulsed Electron Paramagnetic Resonance (EPR) spectroscopy.



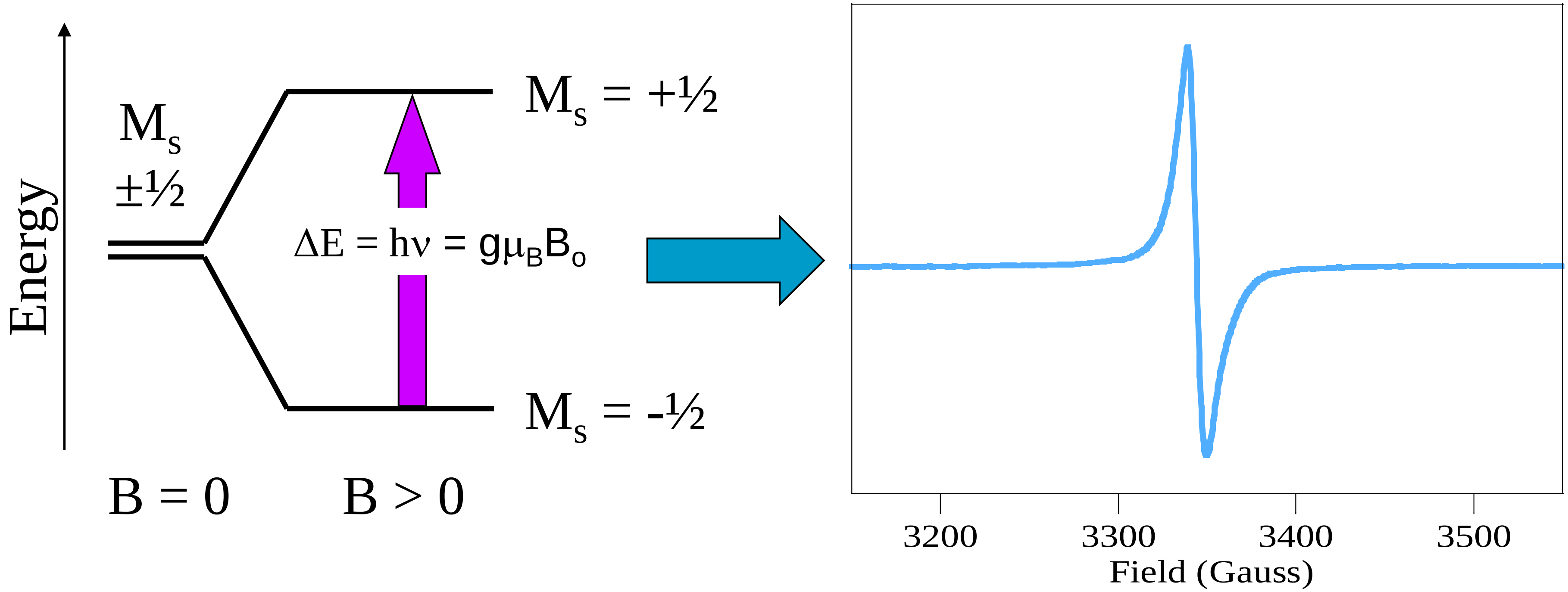
Electron paramagnetic spectroscopy uses microwaves



http://www.ilibrarian.net/science/electromagnetic_spectrum.jpg

Electron energy levels split in a magnetic field

Paramagnets can be probed by electron paramagnetic resonance (EPR) spectroscopy to reveal their properties in solids, liquids, and gases.



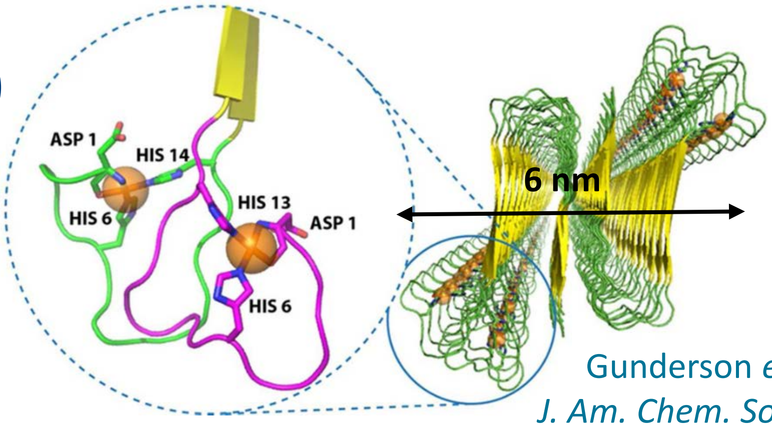
EPR spectroscopy flips electron spins (absorption of microwave energy) in an applied magnetic field

The scope of inductively-detected EPR is broad

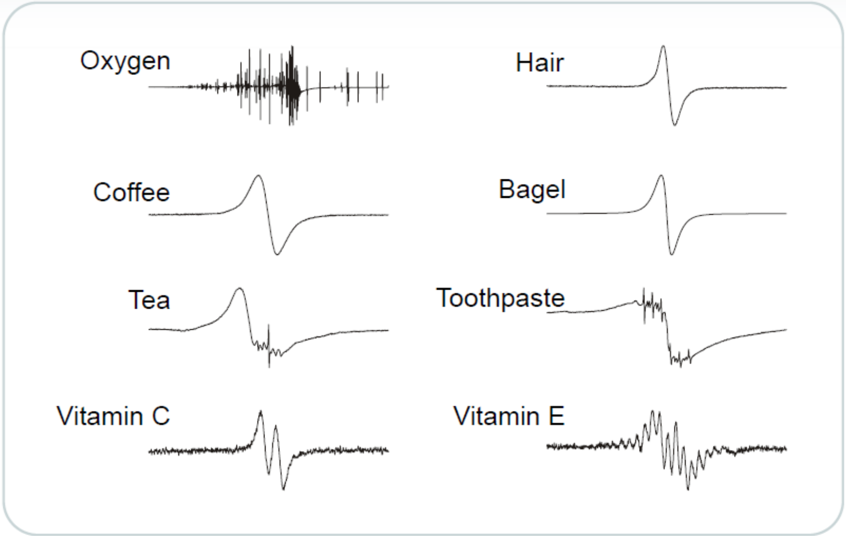
Materials
- Polymers
- Biomaterials (proteins, NAs, lipids)
- Semiconductors
- Glasses/ceramics
- Magnetic materials
- Pharmaceuticals

Information
- Dopant identity
- Point defects
- Oxidation state/electronic config.
- Site symmetry
- Exchange and dipolar interactions
- Concentration/identity of radicals

Pulsed EPR spectroscopy reveals details of Cu^{2+} binding to amyloid fibrils



Gunderson *et al.*
J. Am. Chem. Soc. **2012** 134 18330

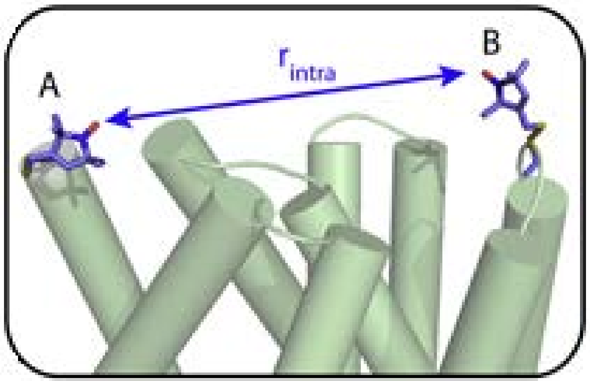


https://www.cif.iastate.edu/sites/default/files/uploads/EPR/ModernEPRapps.pdf

We use the EPR analog of FRET to measure distances

Pulsed ELectron-electron DOuble Resonance (PELDOR) also known as Double Electron-Electron Resonance (DEER)

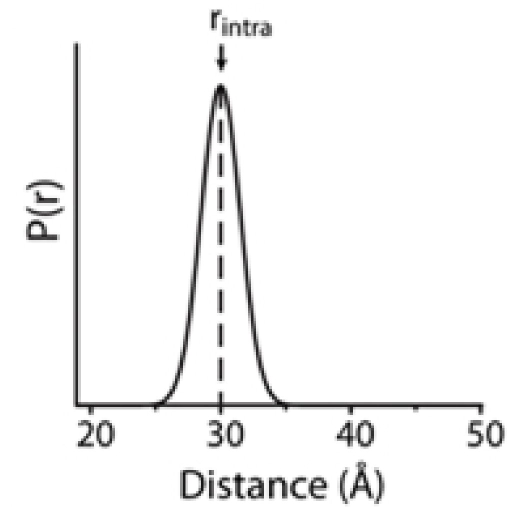
Biomolecule with two spin probes



http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3224804/

PELDOR/DEER

Distance between spins



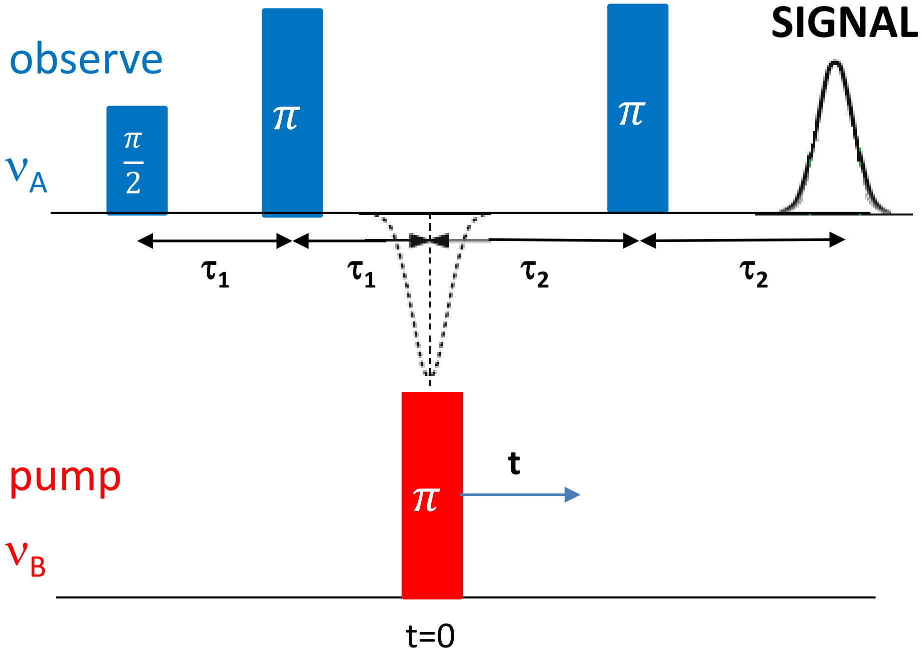
Measurement information:

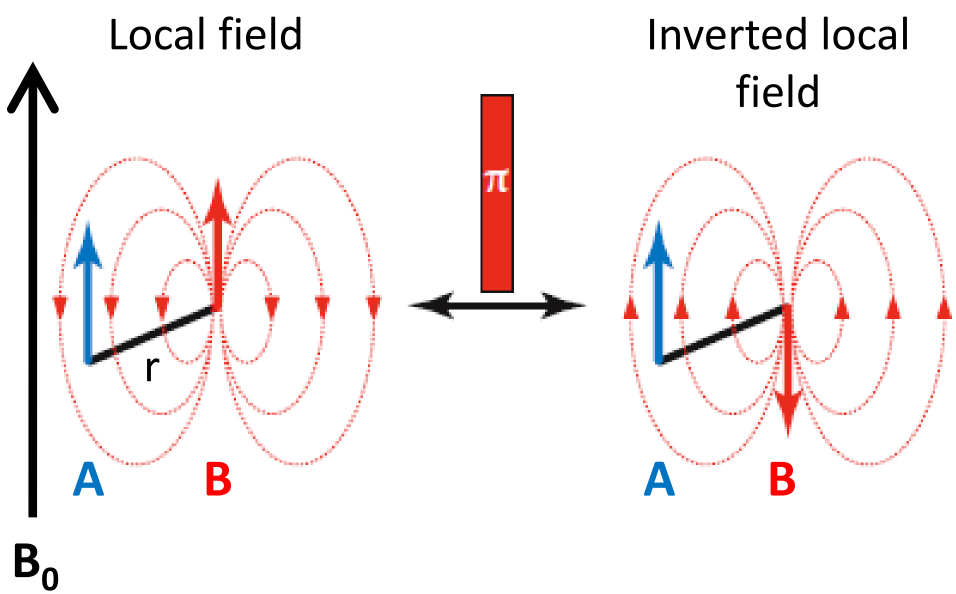
- Accessible distance range is 2.0 nm to 8.0 nm
- Uses two identical probes (FRET requires two different probes)
- Detects conformational changes of biomacromolecules – e.g. motor proteins
- Ideal for difficult to characterize biomacromolecules - e.g. membrane proteins
- Requires about the same amount of material as FRET measurements
- Carried out in solid state (frozen samples)

PELDOR (aka DEER) is a pump-probe experiment

- Induce & monitor spin echo from A spins
- Modulate echo intensity of A spins by inverting B spins

Molecule with two nitroxide spin labels



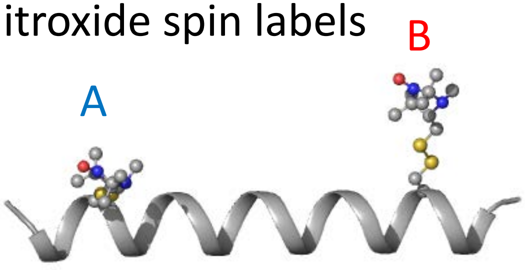


Jeschke, *Annu. Rev. Phys. Chem* **2012** *63* 419

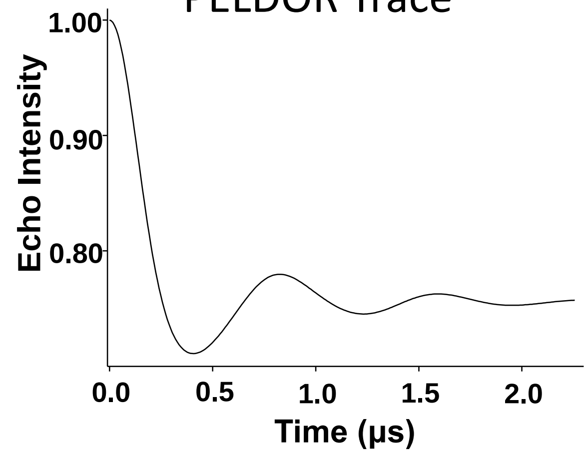
Modulation of the echo intensity requires inverting as many B spins as possible

Spins with narrow spectral extent are easy to probe

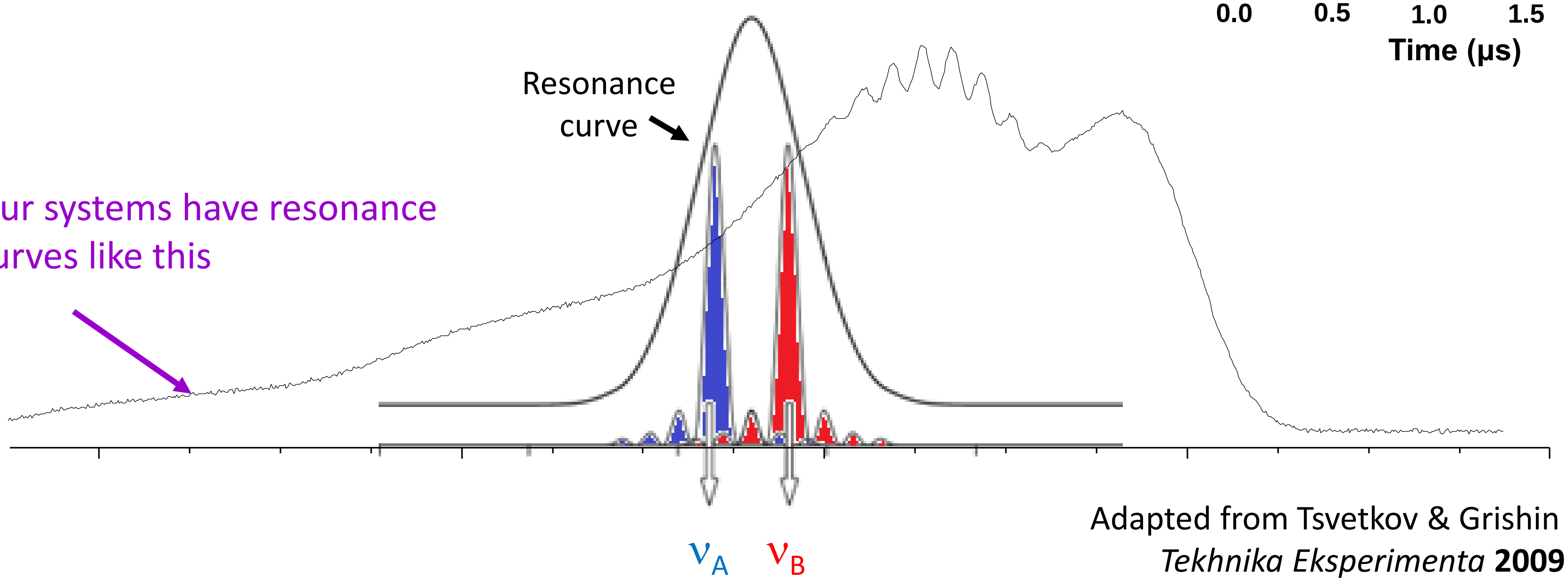
Molecule with two nitroxide spin labels



Apply PELDOR pulse sequence



Our systems have resonance curves like this

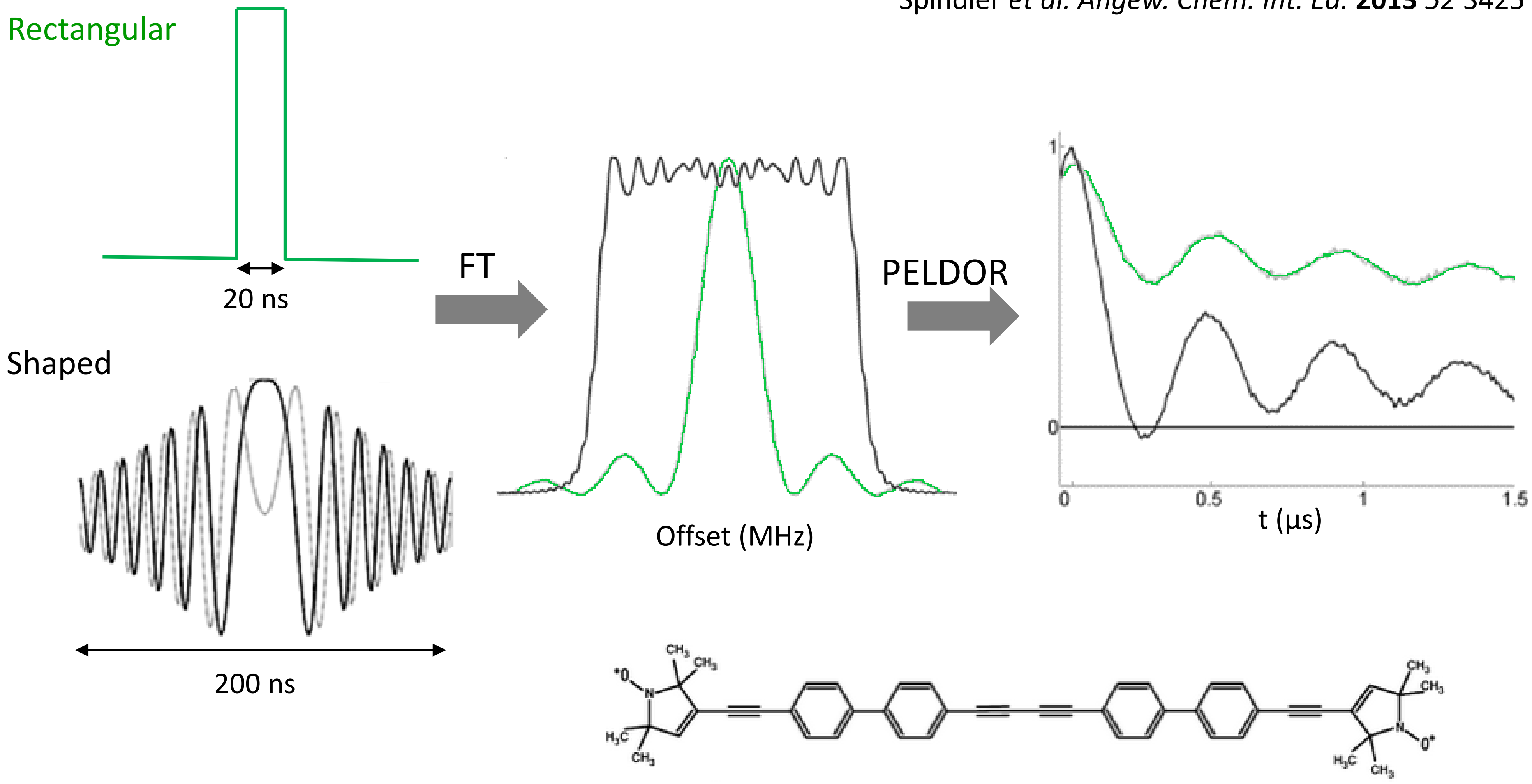


Adapted from Tsvetkov & Grishin *Pribory i Tekhnika Eksperimenta* **2009** *5* 5

Spins with large spectral extent require wider excitation bandwidth

Shaped pulses increase excitation bandwidth

Spindler *et al. Angew. Chem. Int. Ed.* **2013** *52* 3425



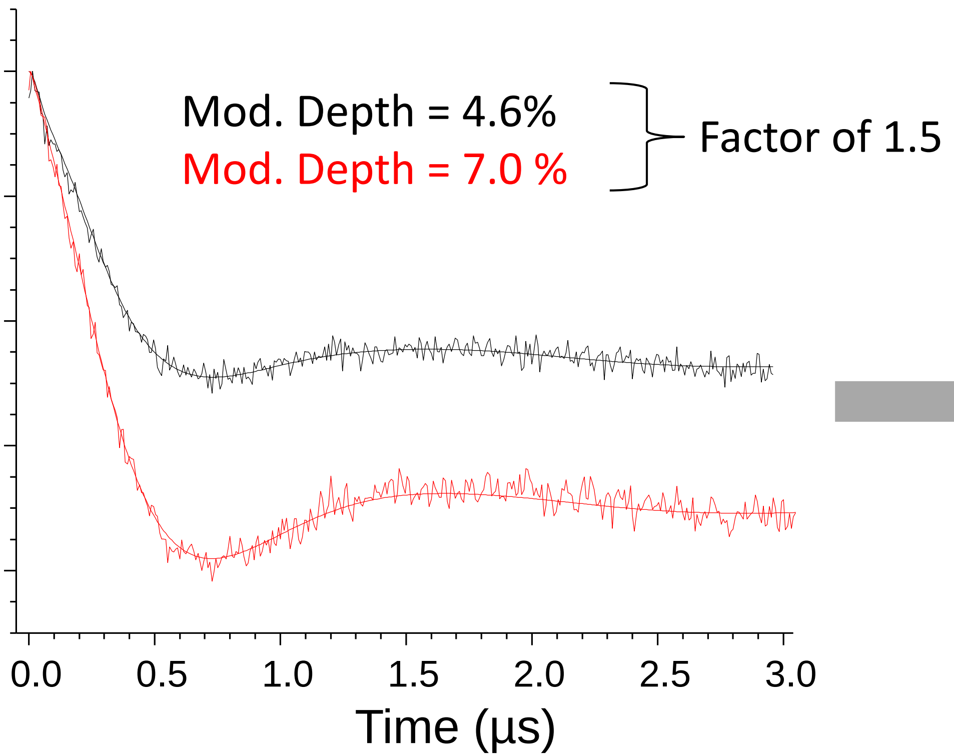
For narrow lineshapes, pulse shaping improves the PELDOR measurement

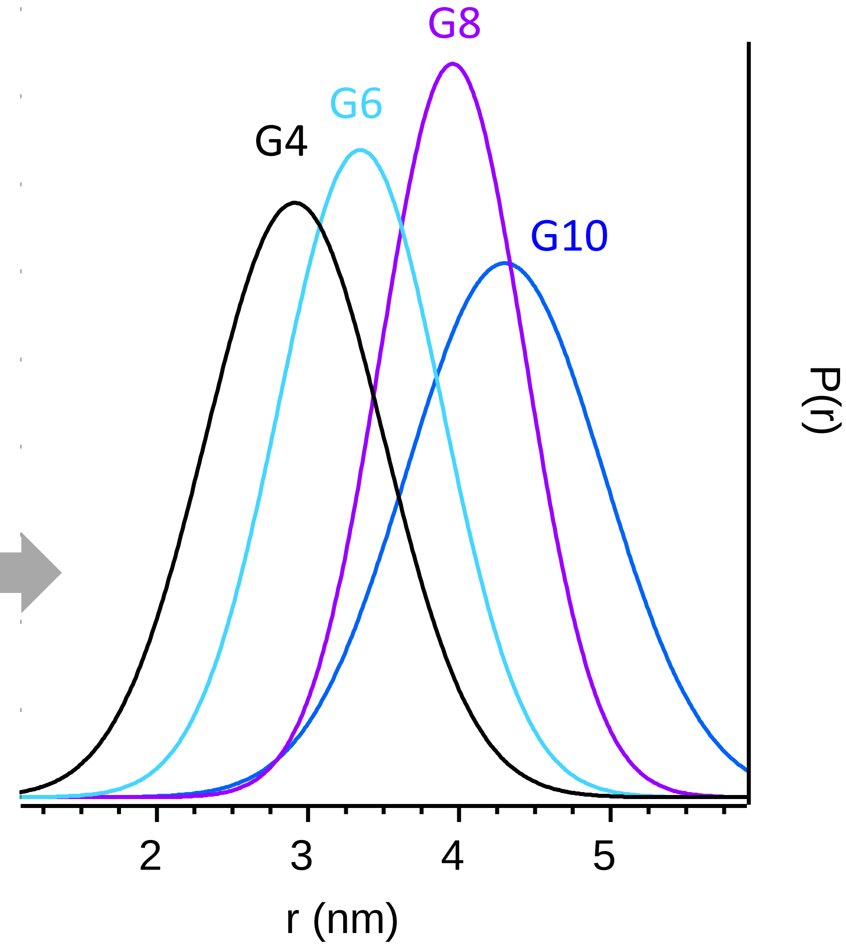
Our modulation depth improves with shaped pulses

Matt's data

— 26 ns rectangular (39 MHz)

— 200 ns shaped (150 MHz)





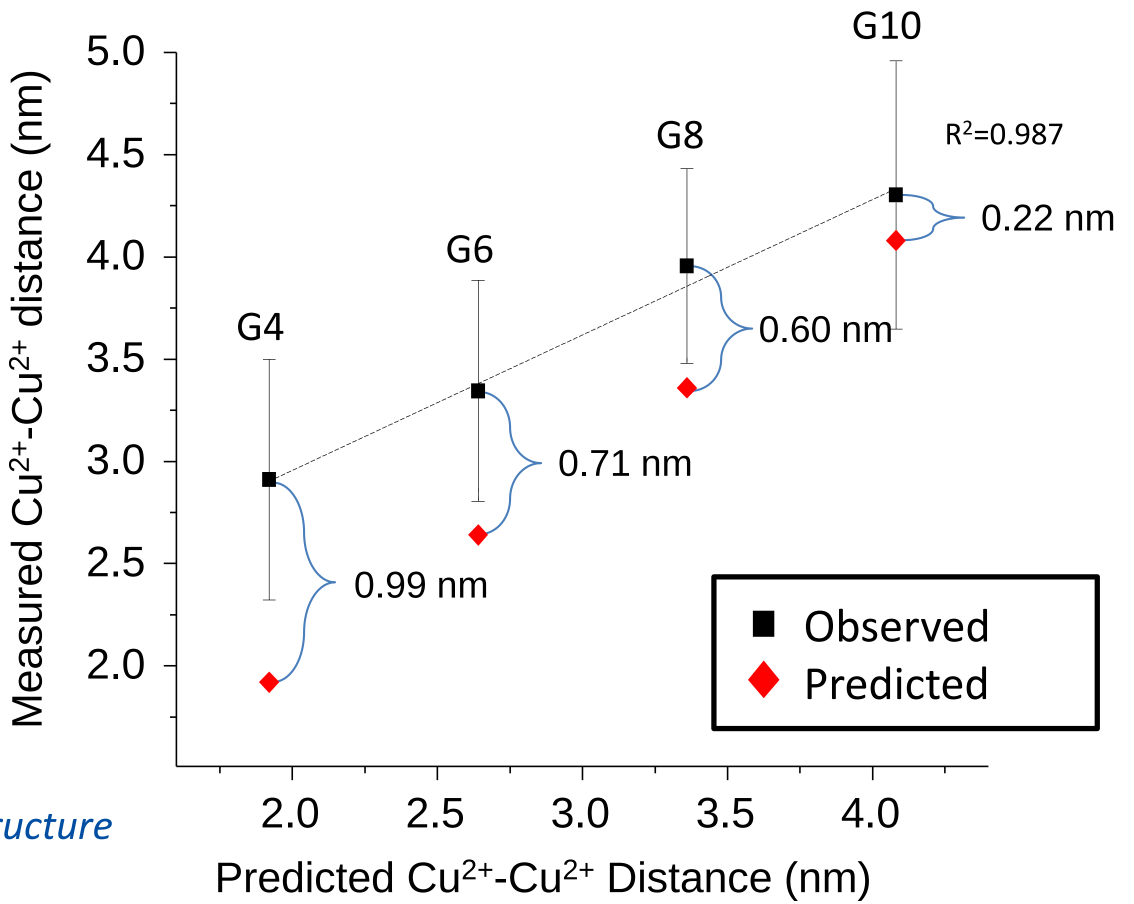
Fitting algorithm: Stein *et al. Methods in Enzym*. **2015** *563* 531

Shaped (sech/tanh) pump pulse improves PELDOR measurements for our system

Cu-Cu distance increases as GQ length increases

Hypothesis: As GQ length increases the Cu-Cu distance should increase if a CuTMpyP4 molecule binds at each end of the G quartet stacks.

Quadruplex	Cu^{2+}-Cu^{2+} (nm)
G4	2.91 ± 0.59
G6	3.35 ± 0.54
G8	3.96 ± 0.48
G10	4.30 ± 0.65



<u>Predicted distances based on:</u>

G-G = 0.36 nm

Phillips et al. *J. Mol. Biol.* **1997** 273 171

G-TMpyP4 = 0.42 nm

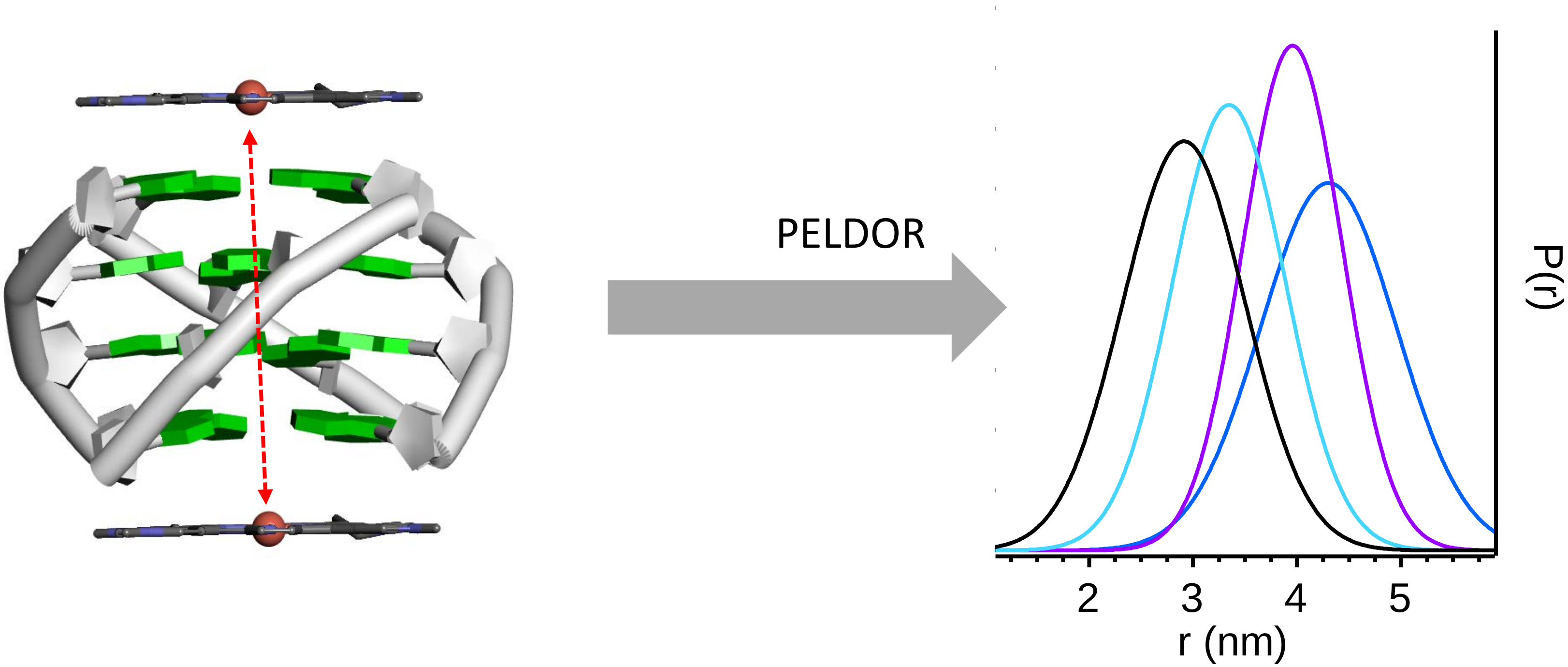
Phan *et al. Nat. Chem. Biol.* **2005** ***1*** 167-173

Stretching the entire quadruplex–ligand structure is most physically realistic.
It also does not invoke an enthalpic penalty due to increasing the ligand-GQ distance.

Donohue & Szalai *Phys. Chem. Chem. Phys* **2016** *18* 15447

Summary of GQs as a measurement platform

- Shaped pump pulse improves DEER of CuTMpyP4
- Cu-Cu distances support end-stacking of CuTMpyP4 on GQs
- Helical expansion/compression likely at play in this system



Donohue & Szalai *Phys. Chem. Chem. Phys* **2016** *18* 15447

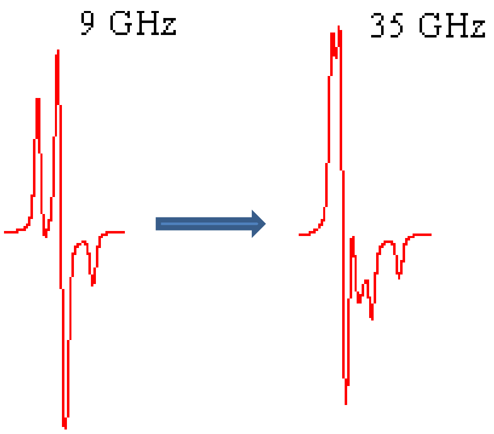
Outline

I. Biomacromolecular structure determination of quadruplex DNA nanomaterials using pulsed EPR spectroscopy

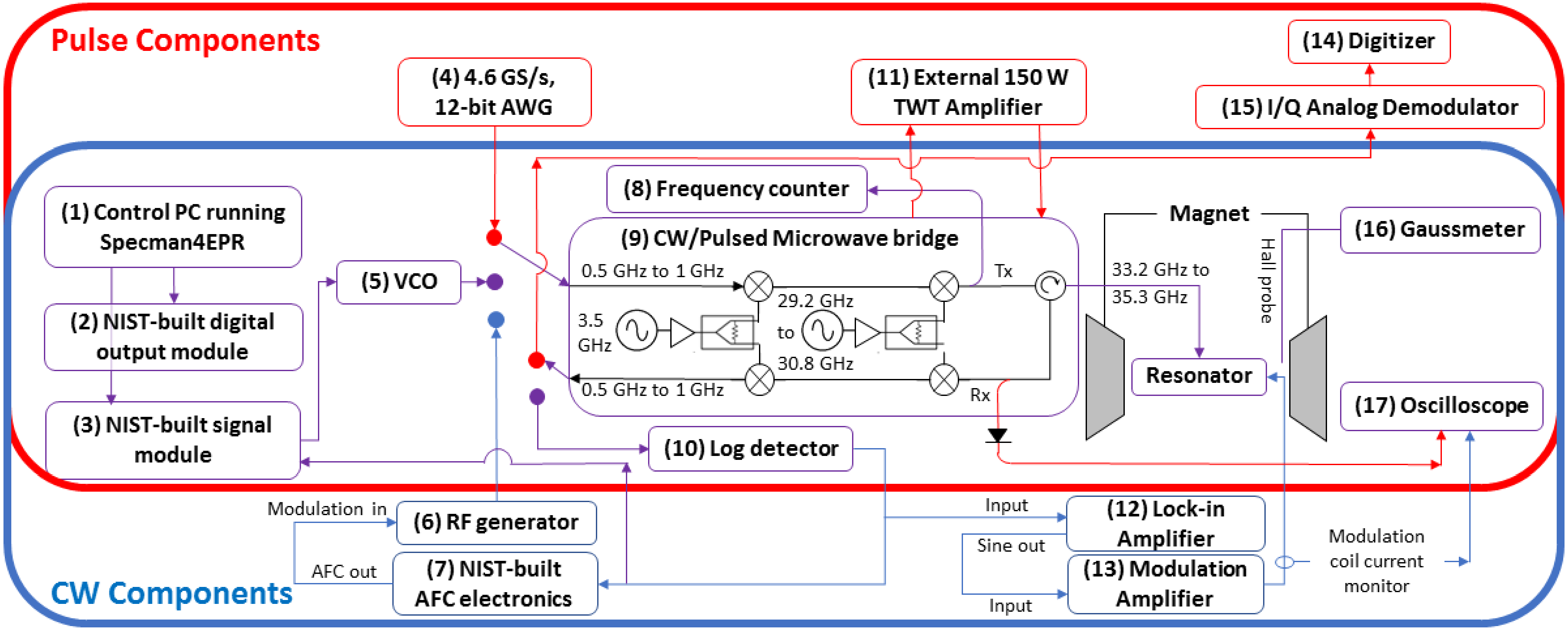
II. Design, capability and future of Szalai lab EPR instrumentation

Spectral resolution increases at higher frequencies

$$\hat{H} = \underbrace{\beta_e S \cdot g \cdot B_0}_{\text{Field dependent}} + \underbrace{S \cdot D \cdot S + S \cdot A \cdot I}_{\text{Field independent}}$$



Our 34 GHz EPR spectrometer system is versatile



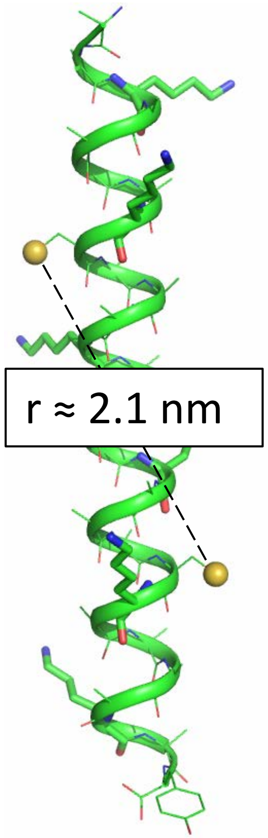
Features:

- Compatible with off-the-shelf excitation sources (AWG, RF generator, VCO).
- Compatible with commercially-available detection devices (high speed digitizer, external demodulator, RF envelope detector)
- Single shot echo S/N values above 30

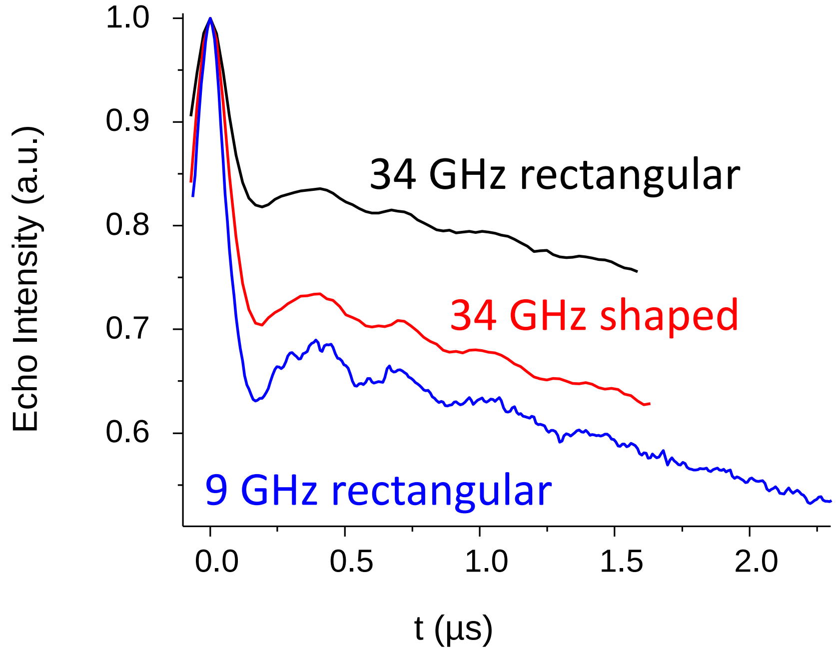
“Integration of a Versatile Bridge Concept in a 34 GHz Pulsed/CW EPR Spectrometer”
A. Band, M. Donohue, B. Epel, S. Madhu, V. Szalai *J. Magn. Reson.* **2018** *288* 28-36

NIST 34 GHz spectrometer cuts acquisition time

PolyA_9,22

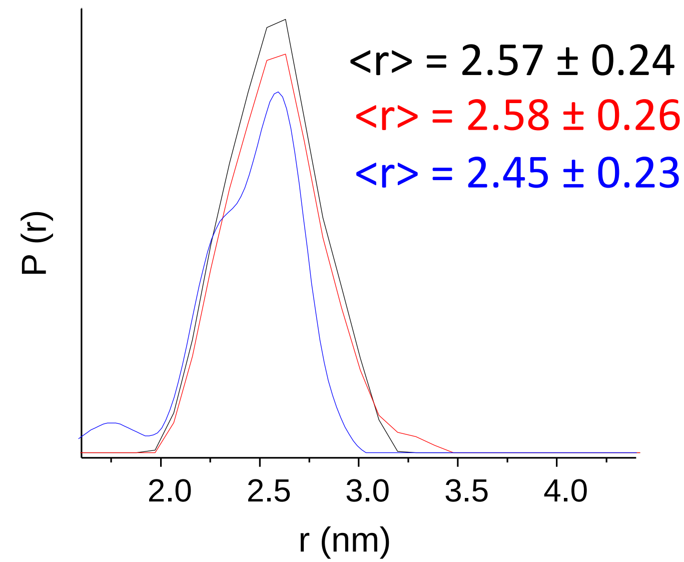


Raw data



S/N improvement @ Q-band

Ensemble interspin distance



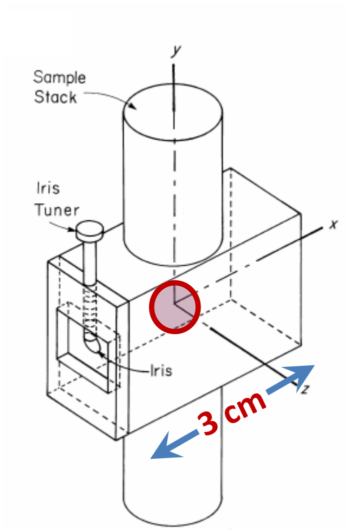
All distances within error

Our 34 GHz spectrometer increases S/N & cuts data acquisition time by 20-fold

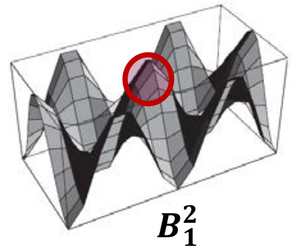
Integration of a Versatile Bridge Concept in a 34 GHz Pulsed/CW EPR Spectrometer
A. Band, M. Donohue, B. Epel, S. Madhu, V. Szalai *J. Magn. Reson.* **2018** *288* 28-36

Conventional inductive-detection EPR has limitations

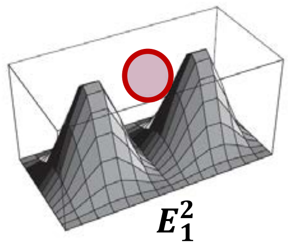
Bulk samples are no problem, but… some samples are volume-limited.



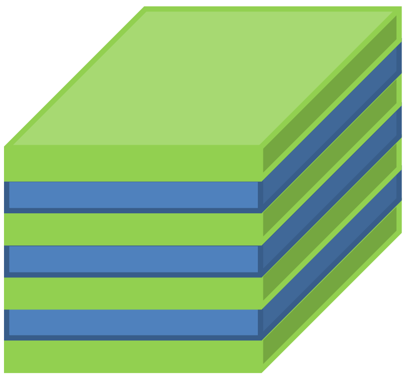
$\mathrm{TE_{102}}$ cavity



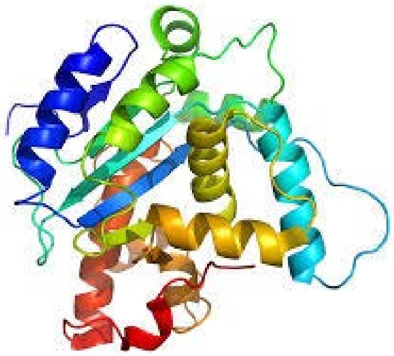
B_1^2



E_1^2



Thin-film superlattices



Biomacromolecules

Active volume ≈ 10 µL to 100 µL

Sample volume < 1 µL

Resonator sensitivity depends on

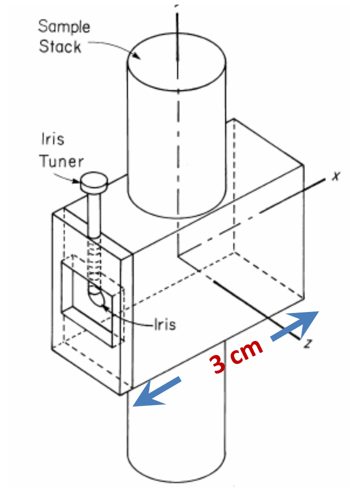
$$Fill\ factor = \frac{V_{sample}}{V_{active}}$$

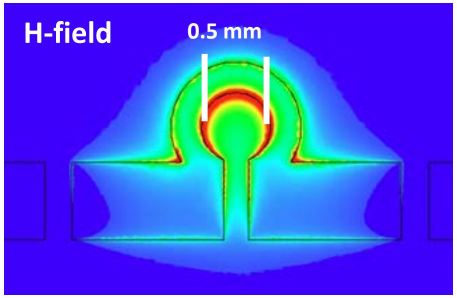
Conventional cavity resonators are not suitable for volume-limited samples

Microresonators can help solve the sensitivity problem

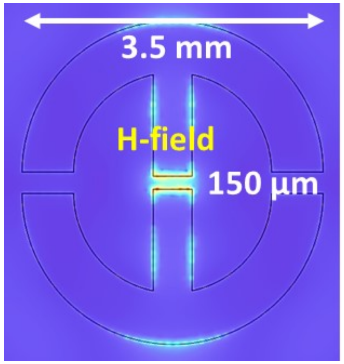
Resonator performance depends on -

- active volume
- fill factor
- Q-factor





Narcowicz *et al J. Magn. Reson* **2005** *175* 275

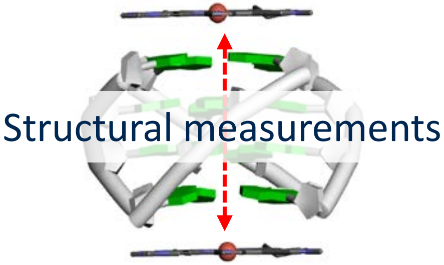


	Cavity	Planar loop-gap	Inverse anapole*
Detectable spins	$10^{10} / \sqrt{Hz}$	$10^{8} / \sqrt{Hz}$	$10^{7} / \sqrt{Hz}$
Concentration sensitivity	nM	µM	sub-µM
Active Volume	10,0000 nL	1 nL	< 1 nL
Fill factor	< 0.0001	Approaches 1	Approaches 1
Q-factor	10^3	< 50	10^2 - 10^3
P-to-B_1 conversion (G/$\sqrt{W}$)	1	15	100

*Calculated on the basis of simulations

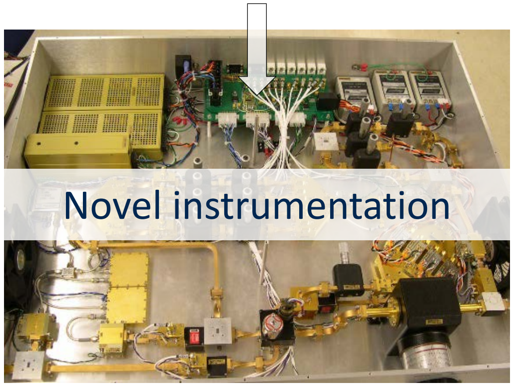
The take away messages…

1. EPR spectroscopy uses paramagnetic centers to provide information on a wide variety of (bio)nanomaterial systems.



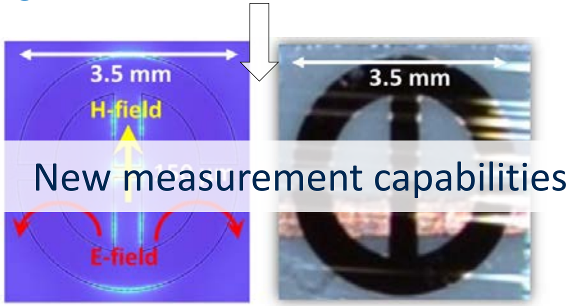
Donohue & Szalai *Phys. Chem. Chem. Phys* **2016** *18* 15447

2. NIST EPR spectrometers implement pulse shaping that improves distance measurements in soft nanomaterials and diversifies spin probe choice.



Band, Donohue, Epel, Madhu & Szalai *J. Magn. Reson.* **2017** revisions submitted

3. Our current instrumentation development is focused on achieving higher sensitivity with smaller sample volumes for our research partners.



Abhyankar, Agrawal, McMichael & Szalai **2017** and beyond.

Thanks to those who do the work!

- GQ assembly & characterization measurements:
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  - Alan Band
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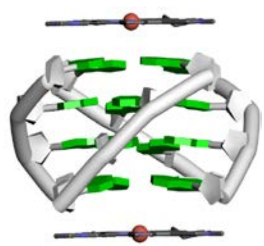
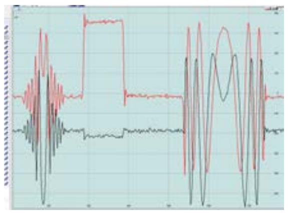
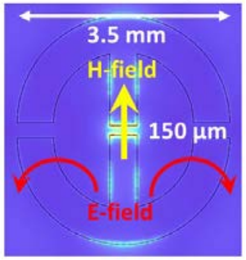
  - Dr. Nandita Abhyankar
  - Dr. Robert McMichael
  - Dr. Amit Agrawal
  - Dr. Jan Obrzut
  - Dr. Caglar Emiroglu





Dr. Matthew Donohue

"Inverse" anapole



Dr. Nandita Abhyankar

My cyberEPR experts:
Andrei A. @ U of AZ
Alexey S. @ PSU
Raanan C. @ Technion
George C. & Ed R. @ Mulheim an der Ruhr
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$$$
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Thank you!