NATIONAL HIGH MAGNETIC FIELD LABORATORY

Initial SCH Bio Science

Supported by NSF DMR through the Magnet Lab and through the MRI Program

Also supported by an NIGMS National Resource Grant

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FLORIDA STATE UNIVERSITY

NIH NIGMS

NSF

SCH Operational Experience

- **SCH Operations**
  - **ramp up & ramp down** time: **~ 30 min each**
  - set up activities on Monday – often **2-3 hours of magnet time** available in late afternoon
  - Tuesday through Friday
    - **ramp up at 6:00 AM** – science staff must be in cell by 5:45 AM
    - **ramp down at ~3:30 PM**
    - **magnet trips** from field on average twice a week, sometime twice in a day – **costing 1.5 hours**
    - magnet needs to be ramped down **to mid field to change samples if no ferro shims used**. **If ferro shims used** ramp down to **zero field** to change samples.
    - **typically 5-7 hours at field per day**.
    - **Good week is 24-30 hours at field**

SCH 2018 Operations

- **SCH Operations**
  - **2018 Schedule:** the 52 weeks
    - **7 weeks** scheduled for **deep maintenance – no ops**
    - **5 weeks** for **additional maintenance**
    - **1 week** for Christmas
    - **8 weeks** scheduled **for CMP**
    - **31 weeks** scheduled **for NMR**
      - **4 weeks** lost to **emergency maintenance**
      - **2 weeks** were lost as individual days **for infrastructure**

  - **Operations at field during a good week**
    - **~ 2 hours: Monday**
    - **~ 5-7 hours: Tuesday – Friday**
    - **~ 26 hour of SCH NMR Operations for a average good week**
    - **650 hours at field for the year** (31-6 weeks) x 26) – assuming no additional lost weeks – we will be close to this number.
    - **650 hours is equivalent** to **less than 1 month of time on a supercon magnet.**

SCH Operational Costs are Considerable

- **SCH DC Power**
  - **14 MWatts to power the magnet**
  - **additional MWatts to run the chillers and pumps to cool the magnet**
  - **$17,000 for a week of SCH time**

- **Operational Staff for the power supplies, chillers, cryogenic system,**
  - **Operational Staff for the cryogenic system**
  - **a minimum of 3 personnel – fortunately shared with a second magnet during regular weekly operations**

- **Scientists/Engineers**
  - **A minimum of 2**
    - **more typically an average of 3**
    - **in the future this maybe more typically 2**
  - **The engineering effort away from SCH magnet continues in the the RF group**

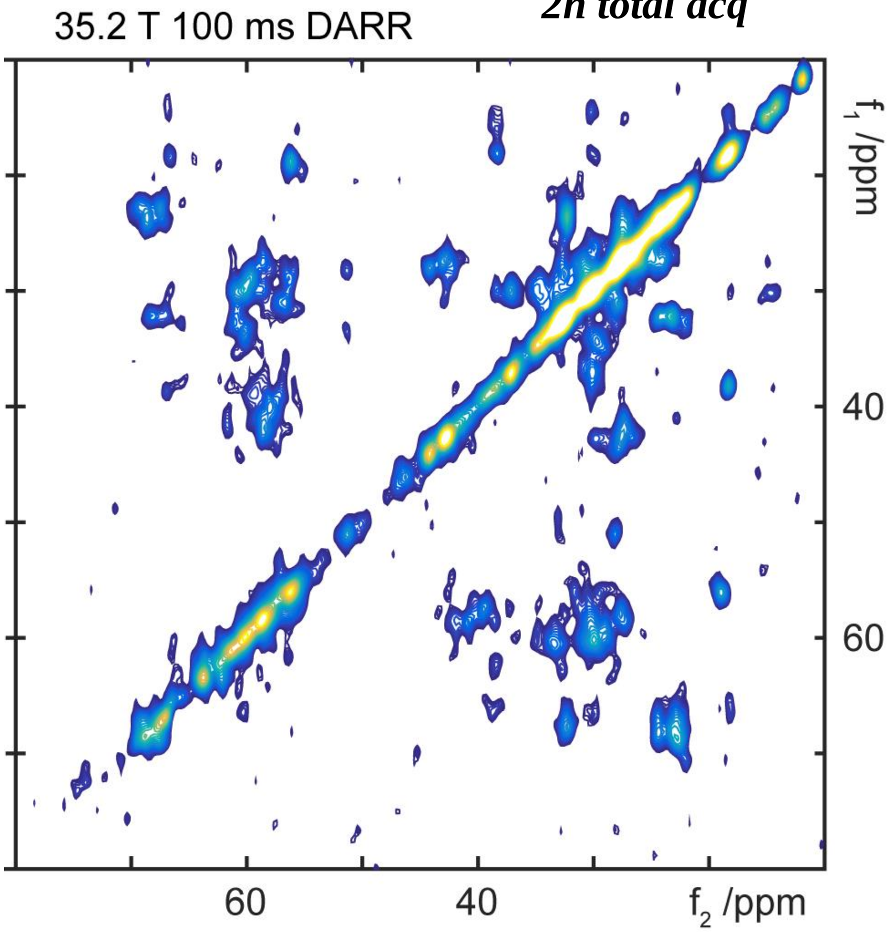
- **Power is approximately half of the operating cost**

- **Consequently, each hour is valuable and a great deal of thought and planning must go into each day of operation**

Droserasin 1 Plant Specific-Insert in Lipids

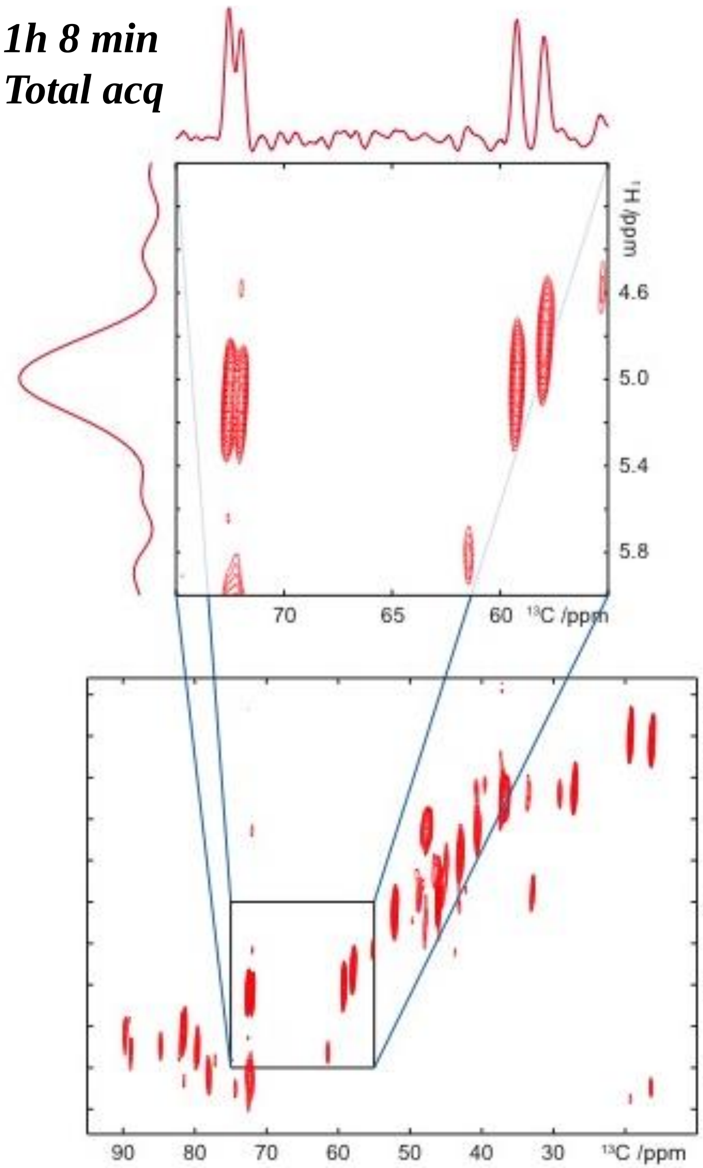
Data acquired in SCH at 1.5 GHz
^{1}H RF – 100 kHz
^{13}C RF – DARR 80kHz/ INEPT 50 kHz

2h total acq



^{1}H → ^{13}C INEPT of PSI in lipids
line widths: 0.4 ppm for both dimensions

1h 8 min
Total acq

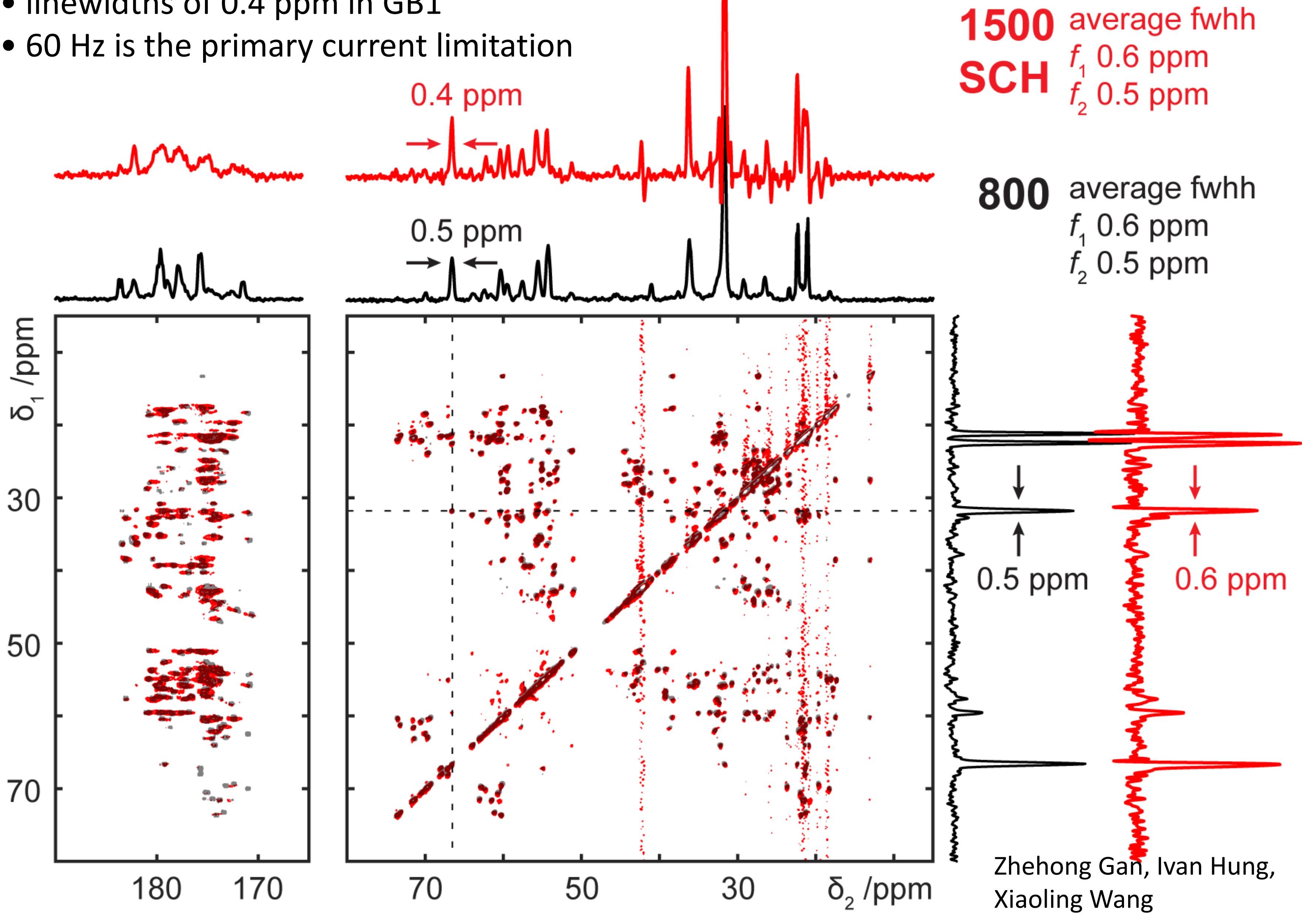


Rachel Martin, UC Irvine - shown with permission

Initial ^{13}C MAS Spectra in 2.0 mm MAS Triple Res Probe

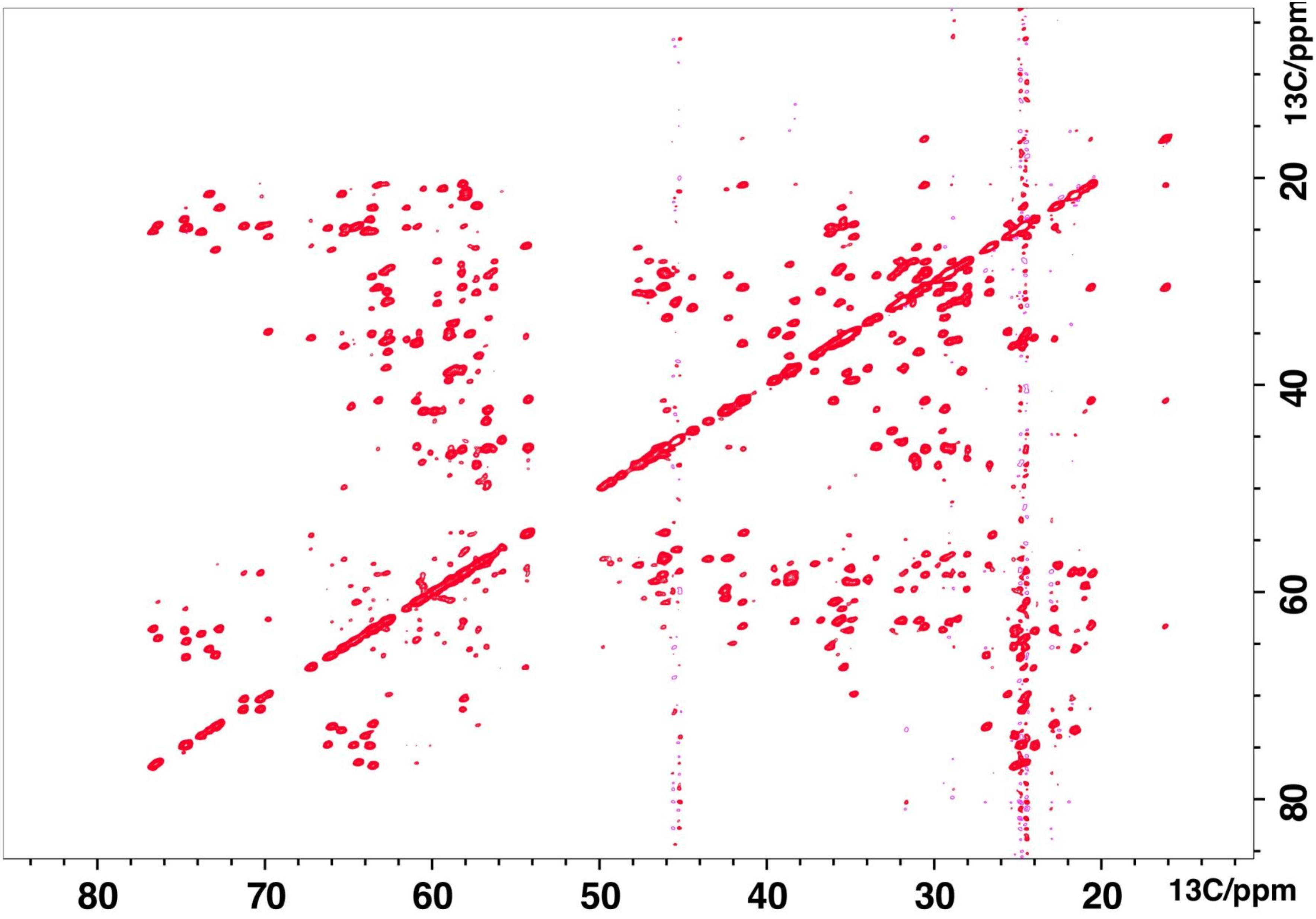
- Enhanced Sensitivity in the DARR spectrum
- linewidths of 0.4 ppm in GB1
- 60 Hz is the primary current limitation

24.4 kHz 2.0 mm MAS HXY Probe: 2 scans /t1 increment
91 min total acquisition



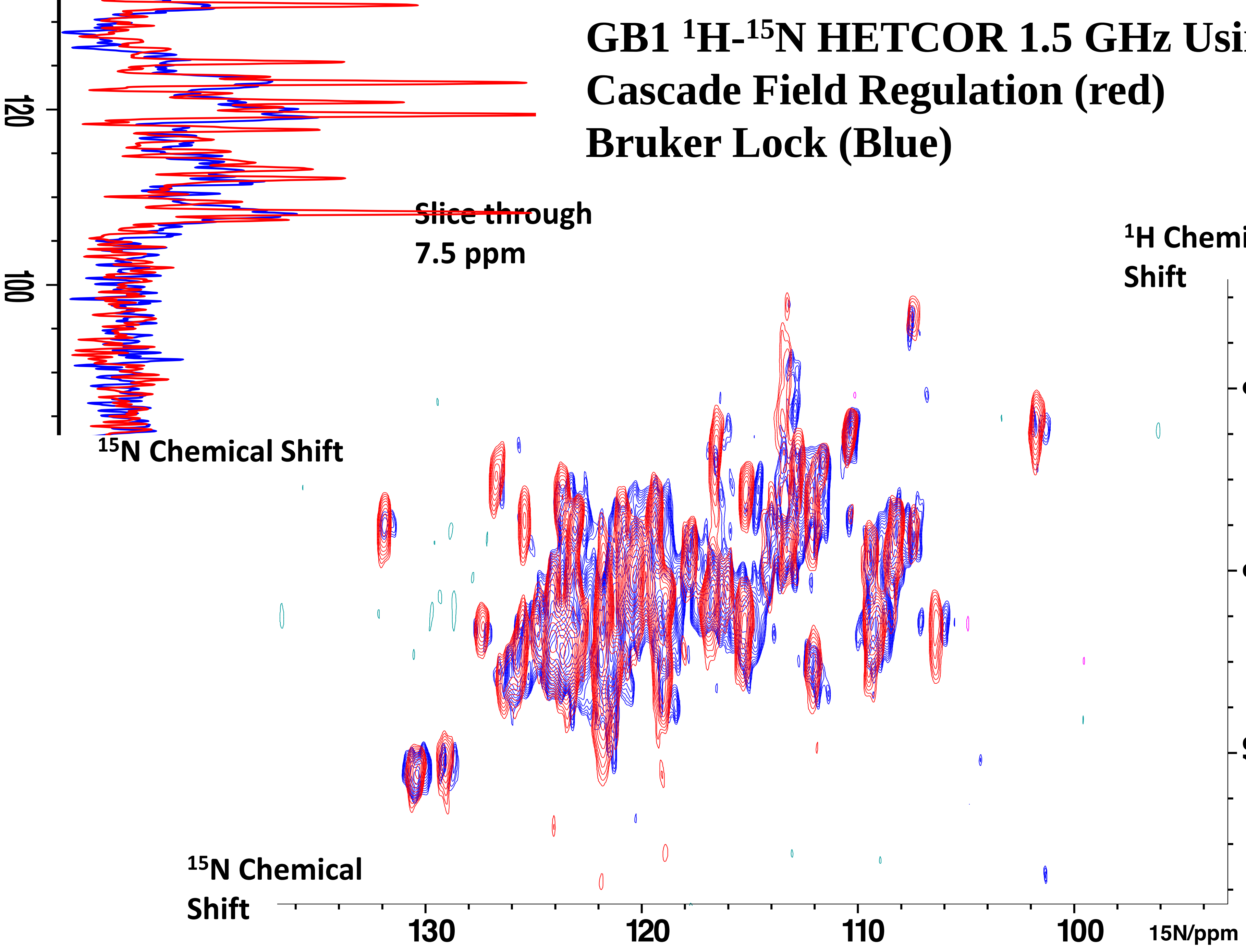
Zhehong Gan, Ivan Hung, Xiaoling Wang

GB1 250 ms DARR 1.5 GHz Cascade Field Regulation

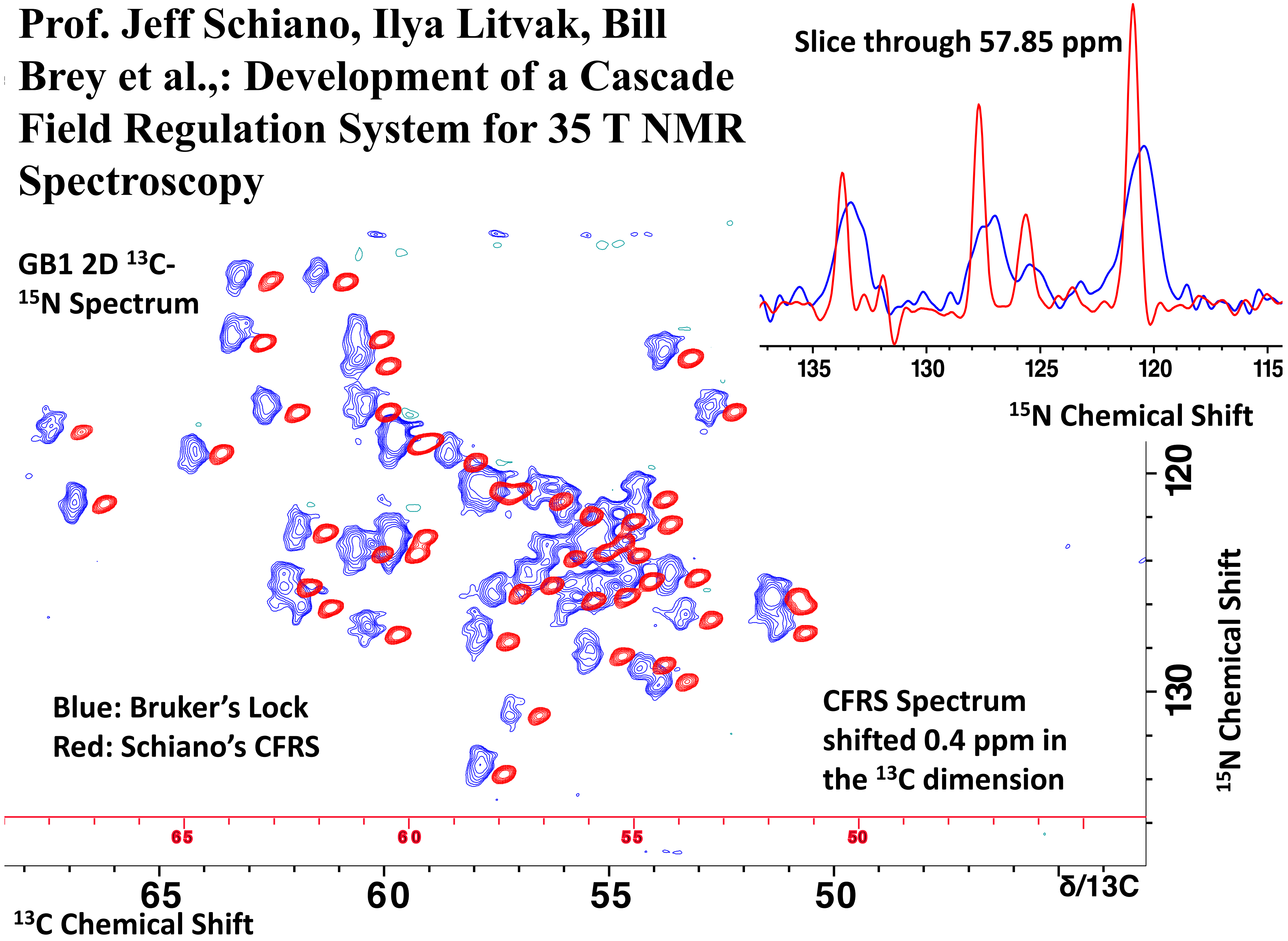


^{13}C Chemical Shift

GB1 ^{1}H-^{15}N HETCOR 1.5 GHz Using Cascade Field Regulation (red) Bruker Lock (Blue)



Prof. Jeff Schiano, Ilya Litvak, Bill Brey et al.,: Development of a Cascade Field Regulation System for 35 T NMR Spectroscopy



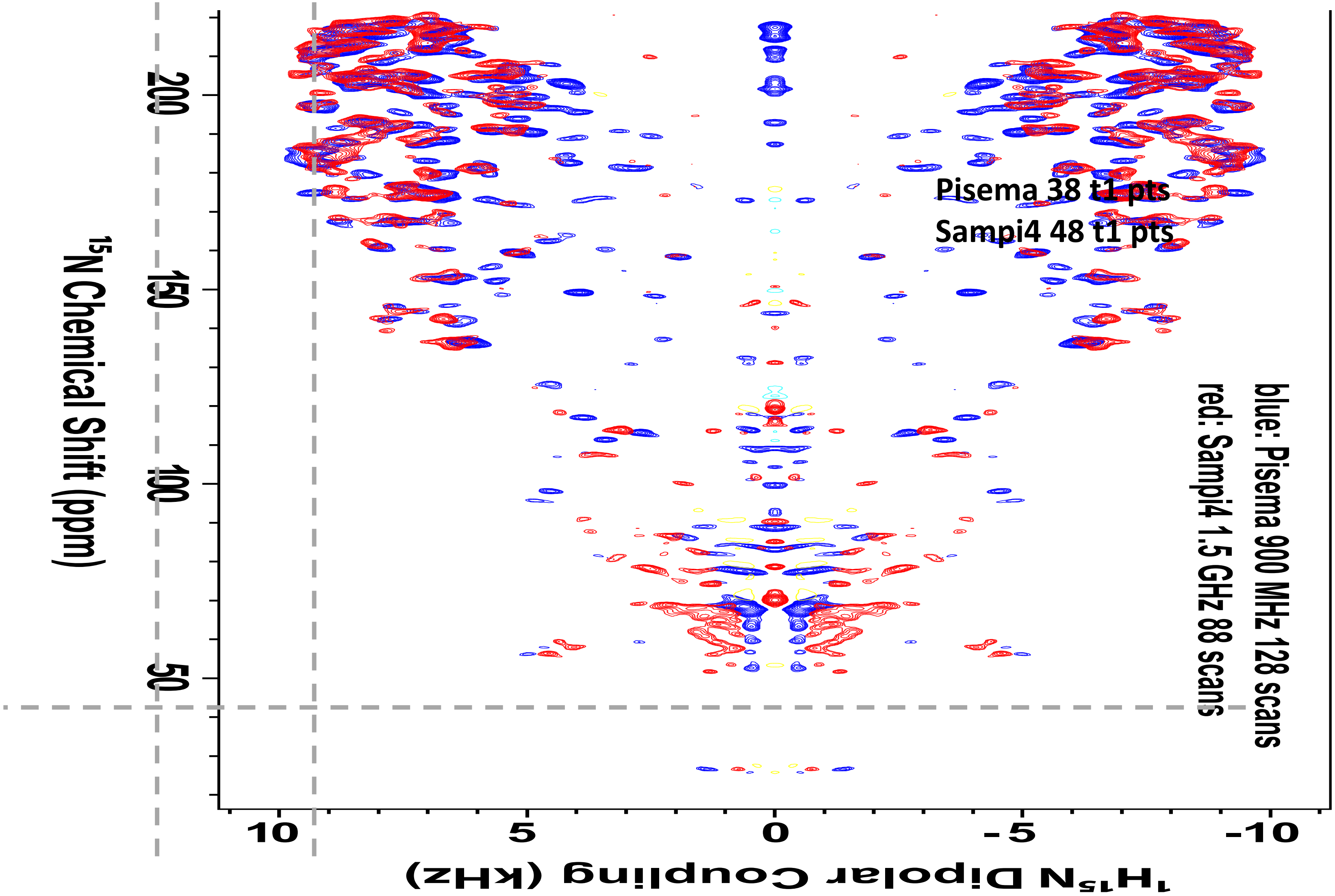
GB1 2D ^{13}C-^{15}N Spectrum

Slice through 57.85 ppm

Blue: Bruker's Lock
Red: Schiano's CFRS

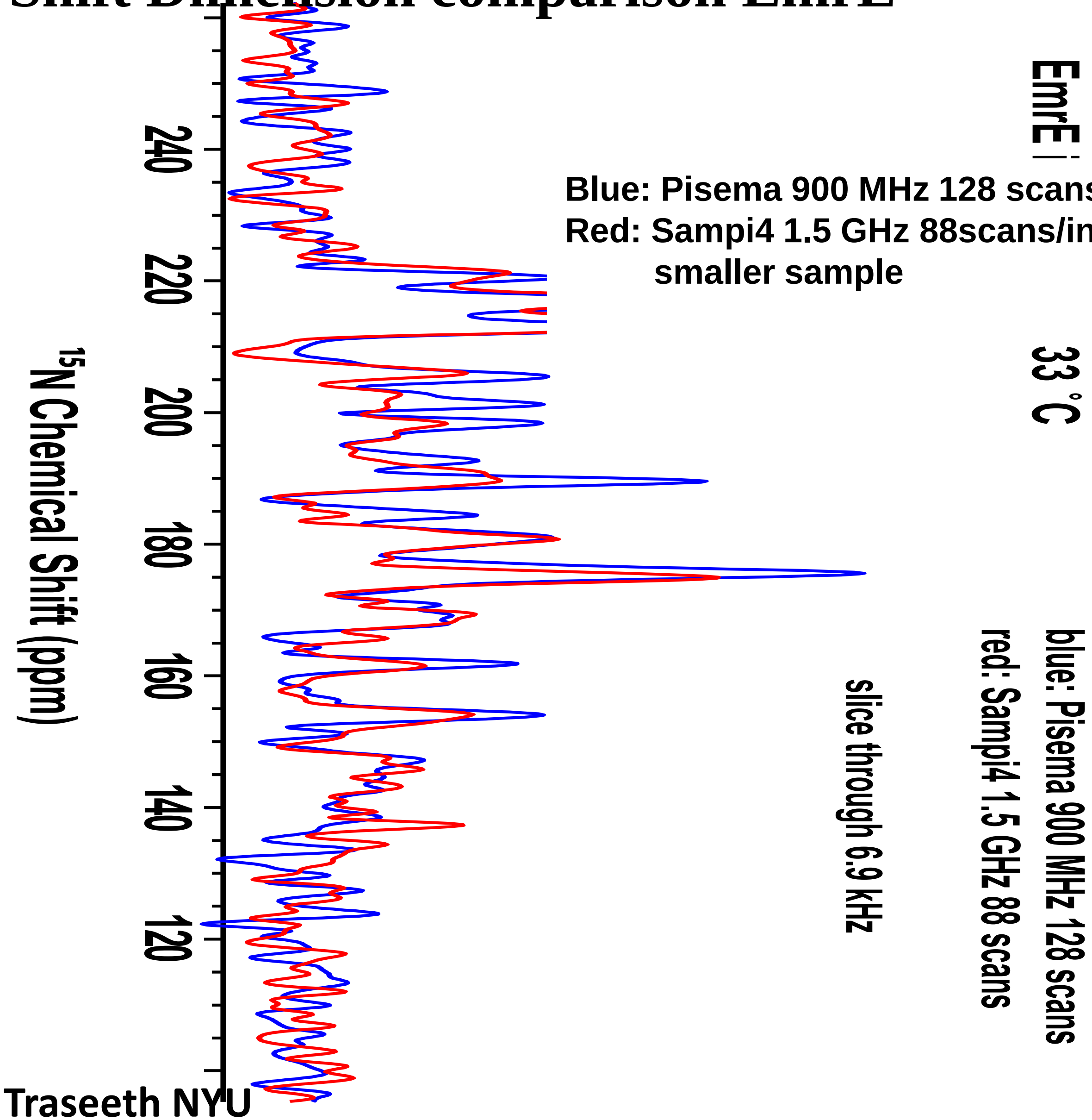
CFRS Spectrum shifted 0.4 ppm in the ^{13}C dimension

EmrE Aligned in Bicelles at 33°C



EmrE – Nate Traseeth NYU – shown with permission

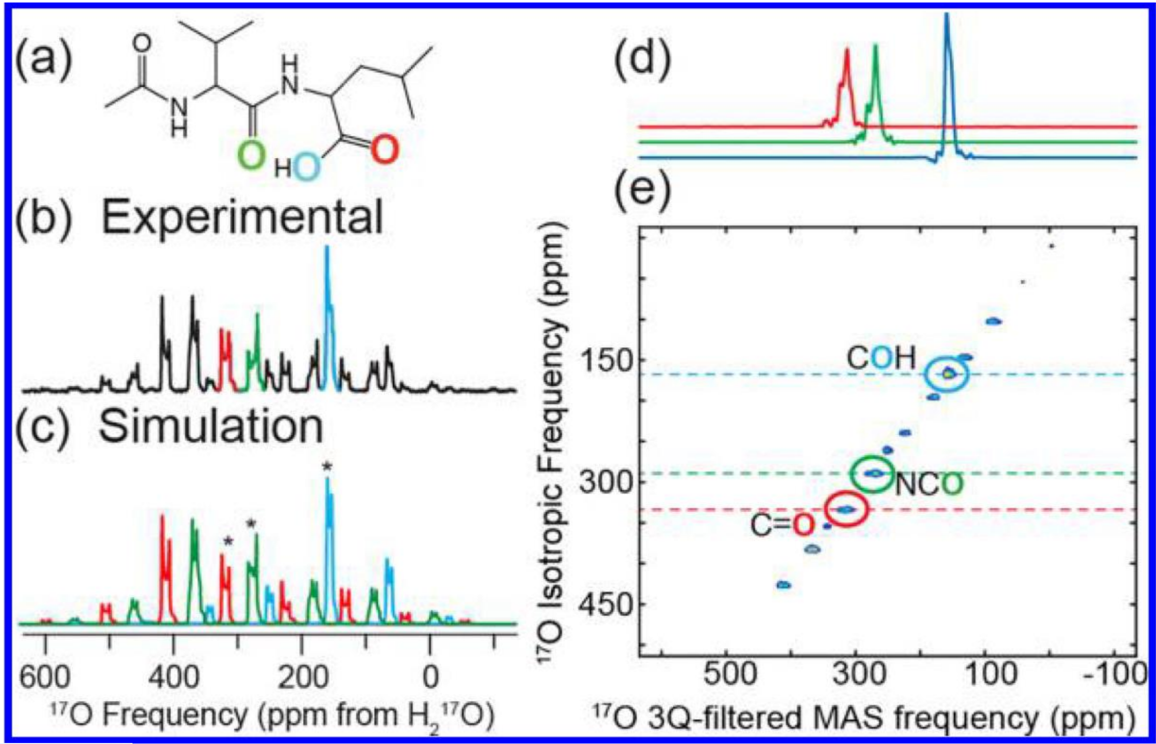
Chemical Shift Dimension comparison EmrE



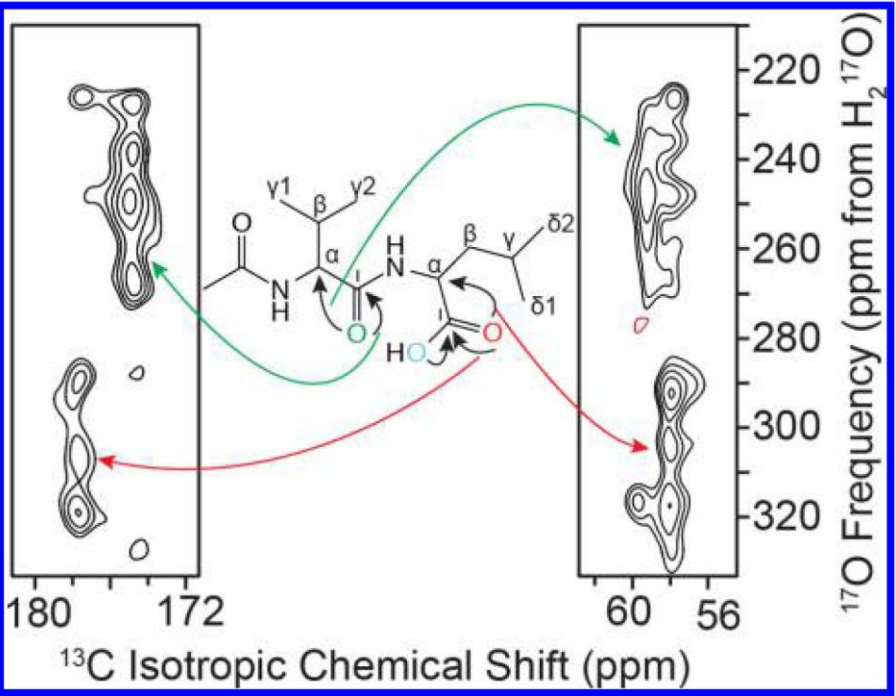
Blue: Pisema 900 MHz 128 scans/increment
Red: Sampi4 1.5 GHz 88scans/increment with smaller sample

EmrE – Nate Traseeth NYU with permission

Series Connected Hybrid Magnet – MAS Spectra of Uniform ^{13}C, ^{15}N and 70% ^{17}O N-Acetyl Val-Leu in 2.0 mm MAS HXY Probe



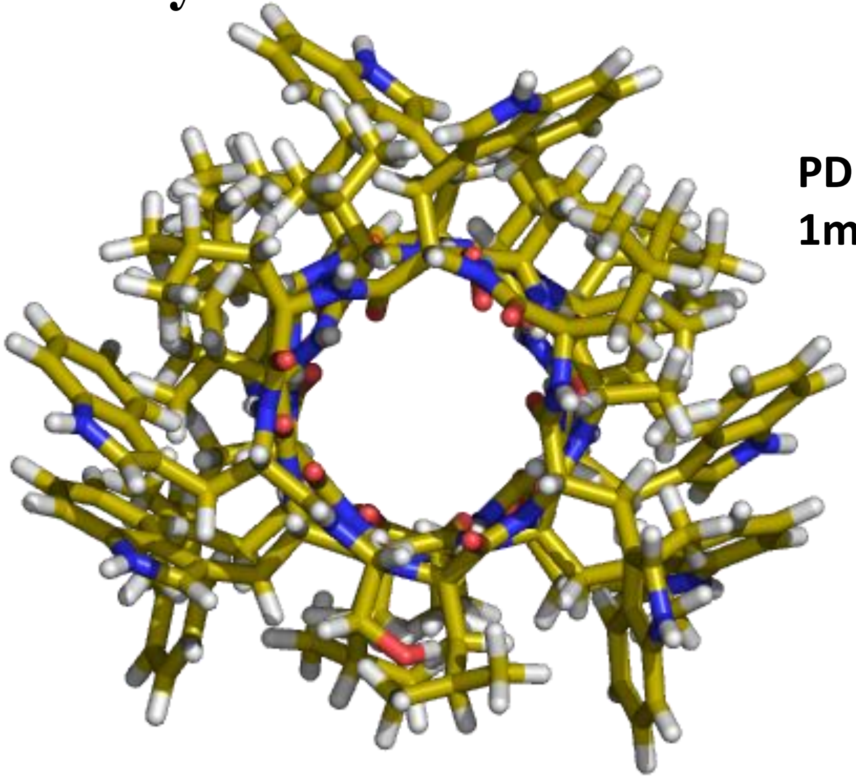
2D ^{13}C-^{17}O Spectra at 21T in a supercon Magnet - soon to be implemented at 35.2 T



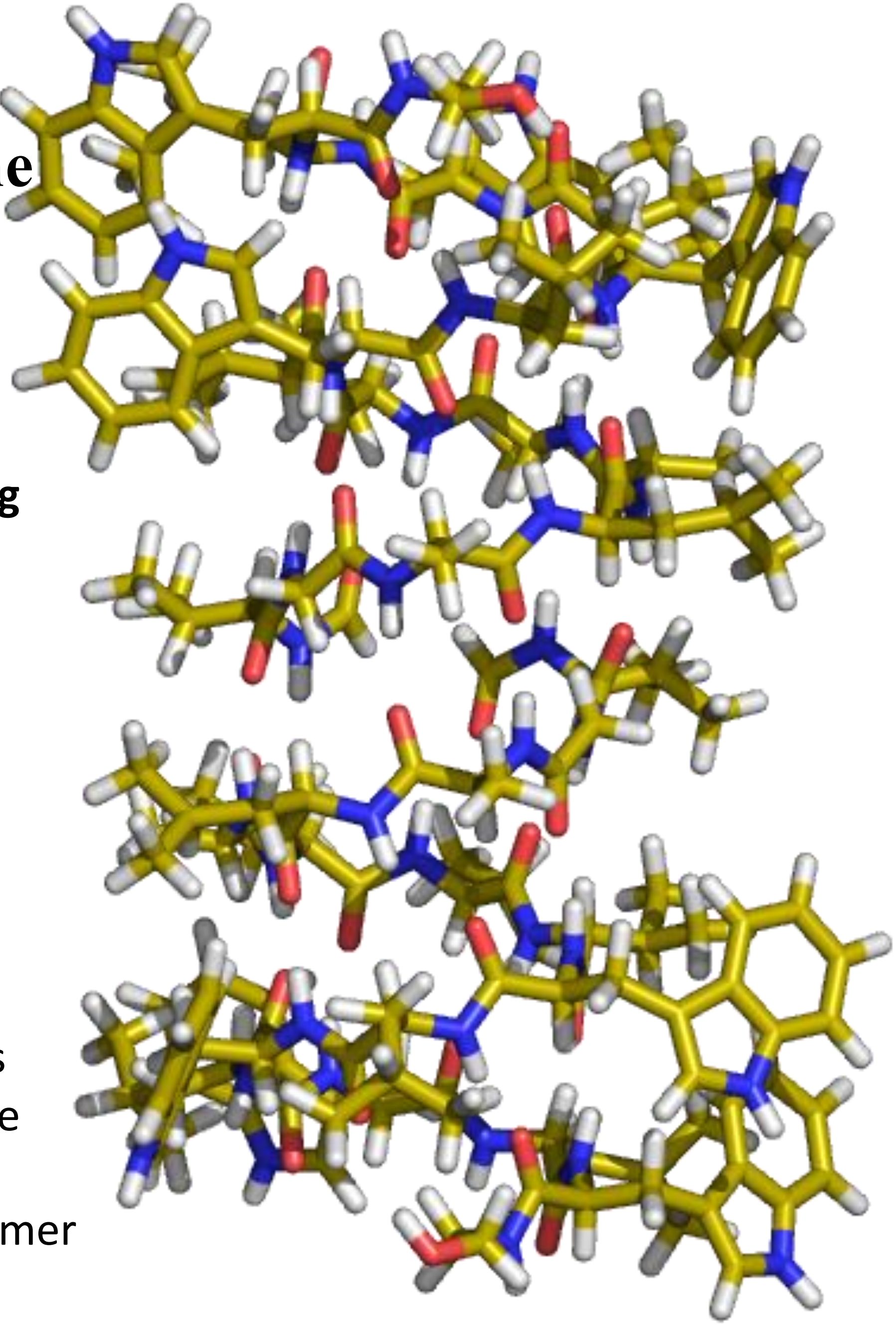
- 2D ^{17}O Triple Quantum MAS spectra at 35.2T and 19kHz spin rate.
- Quadrupolar interaction is not completely eliminated by MAS
- Signal averaging time is reduced by a factor of ~10 compared to 21 T

Keeler et al., (2017) JACS 139, 17953

Gramicidin A – The First All-Atom Transmembrane Structure to be Characterized in a Liquid Crystalline Lipid Bilayer Environment



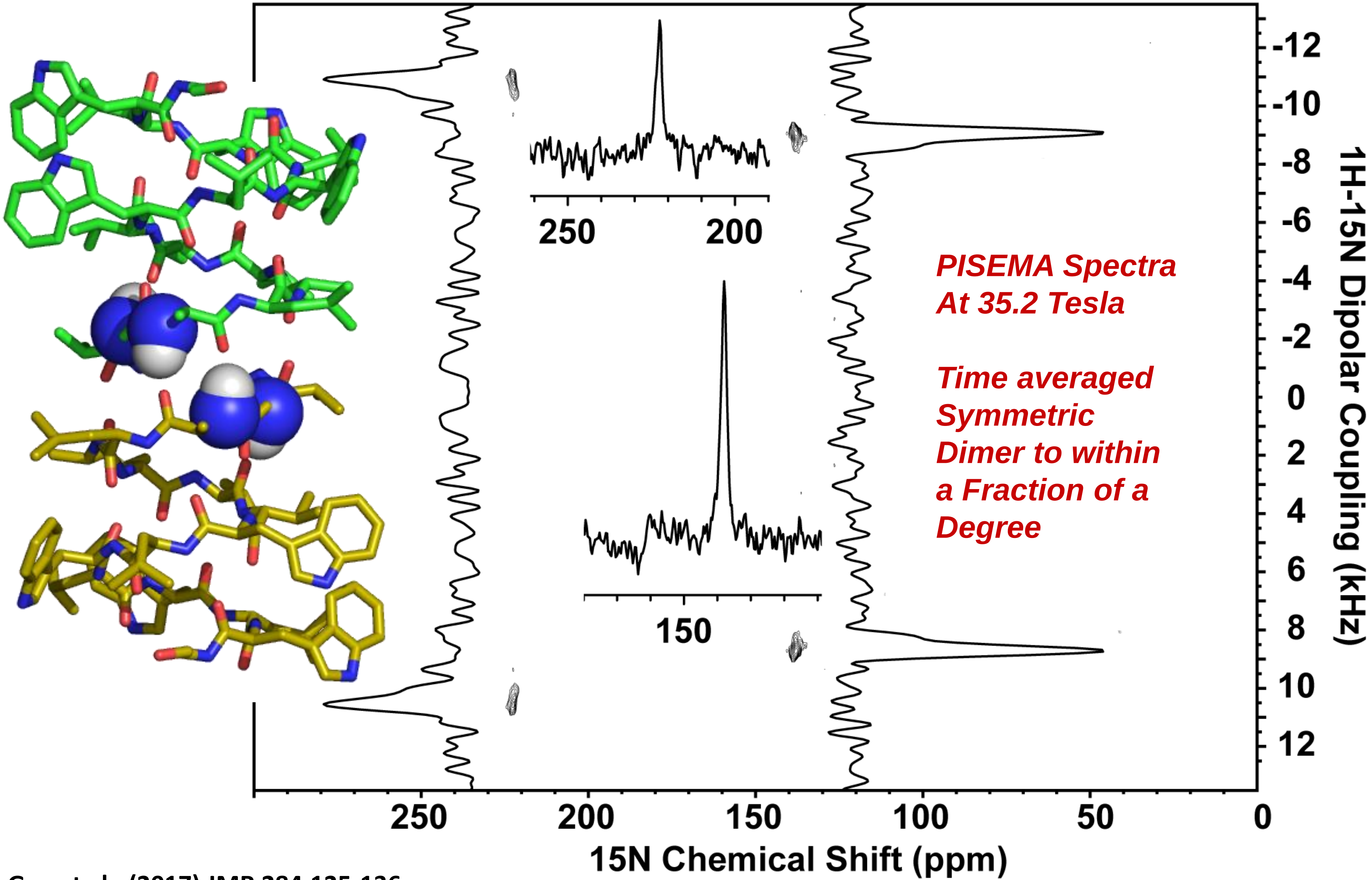
PDB 1mag



- An alternating sequence of L and D amino acids Forming a β-strand with all sidechains on one side forcing a helical structure
- All of the spectroscopy suggests a symmetric dimer

Ketchem et al., Science 1993; Ketchem et al., Structure, 1997

Enhanced Alignment of Gly_2, Ala_3 ^{15}N Labeled Gramicidin A in Liquid Crystalline Lipid Bilayers – Oriented Sample ssNMR:

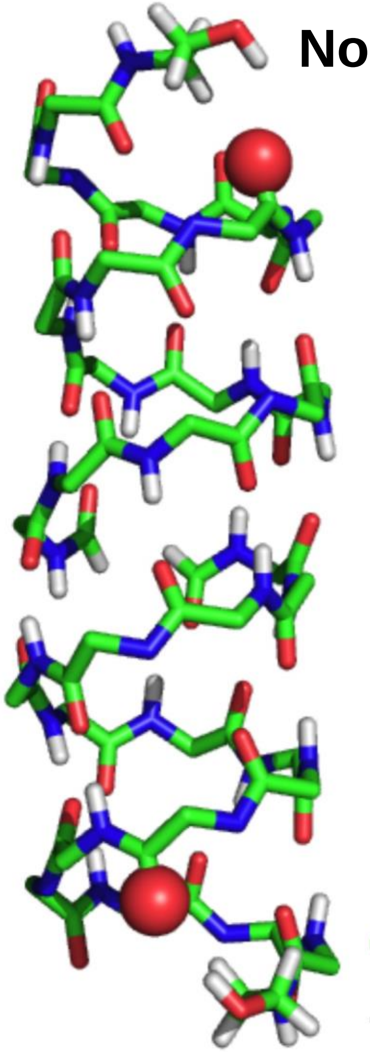


PISEMA Spectra At 35.2 Tesla

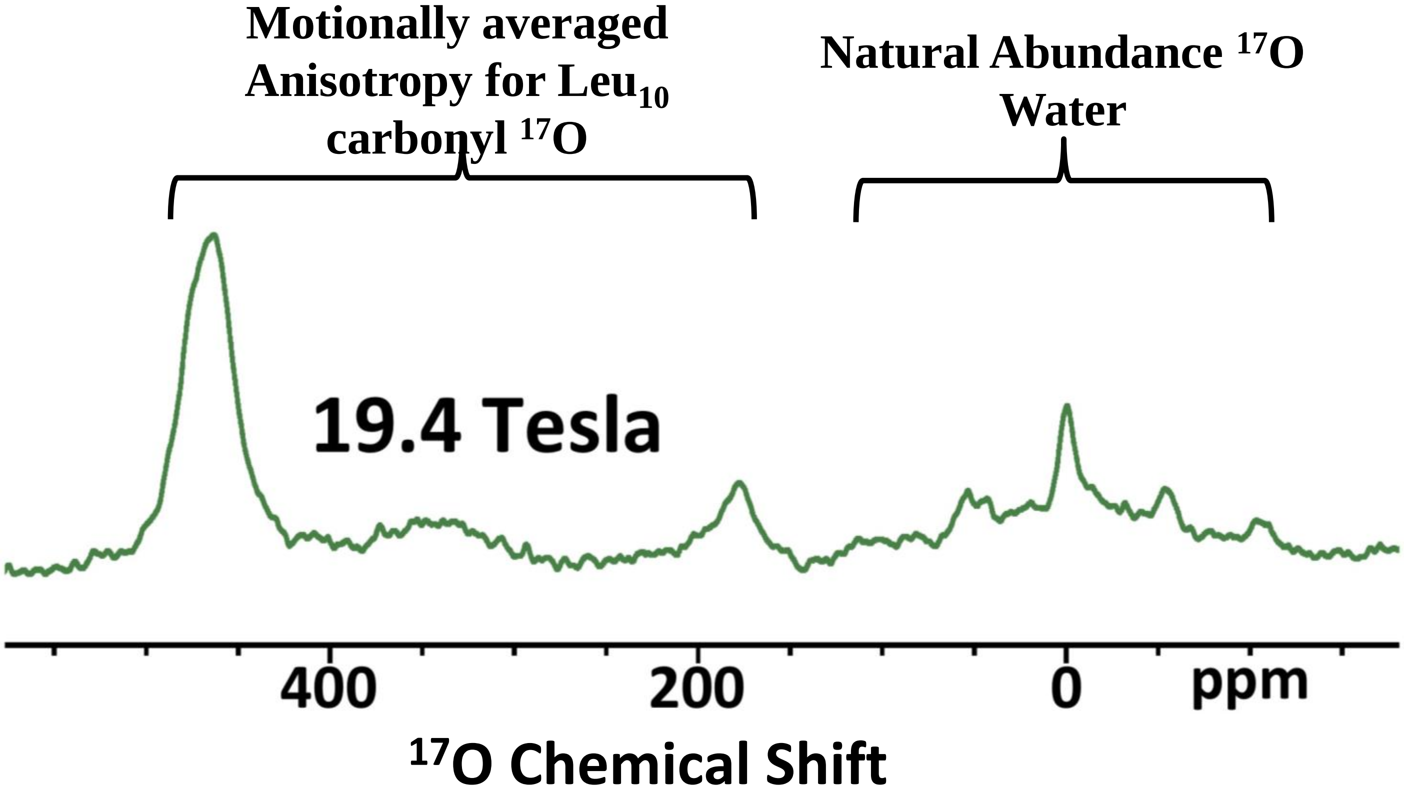
Time averaged Symmetric Dimer to within a Fraction of a Degree

Gan et al., (2017) JMR 284:125-136

Leu$_{10}$ ^{17}O Gramicidin A Aligned Parallel to DMPC Bilayer Normal and to Bo: OS ssNMR

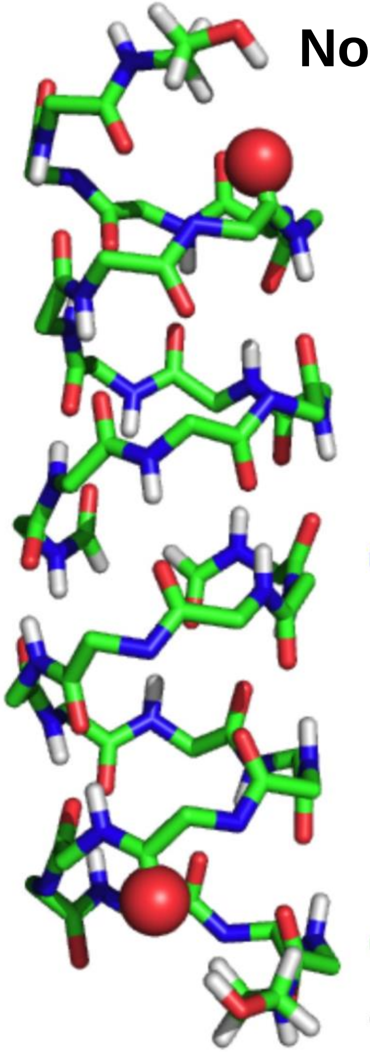


No ions

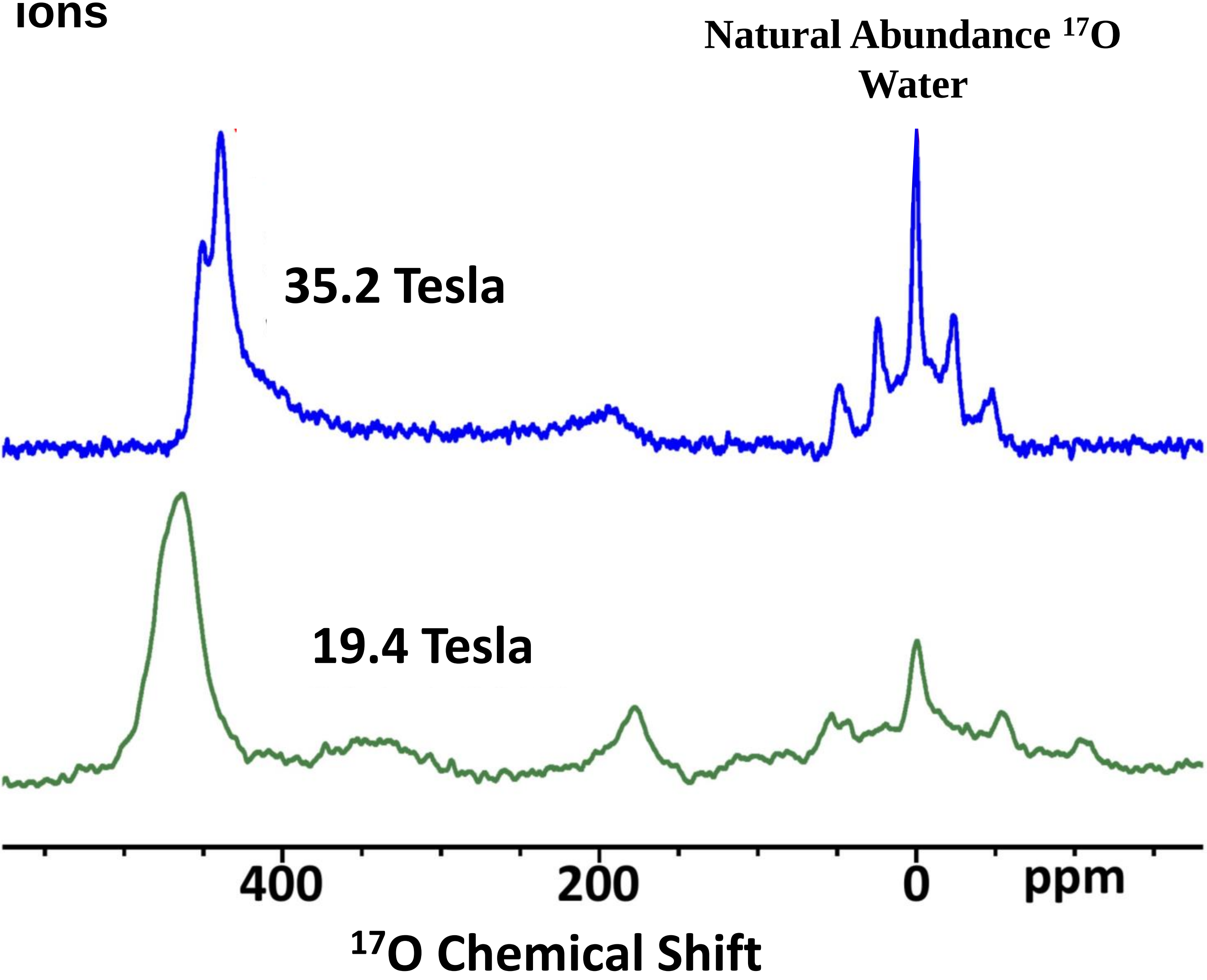


Eduard Chekmenev

Leu$_{10}$ ^{17}O Gramicidin A Aligned Parallel to DMPC Bilayer Normal: OS ssNMR

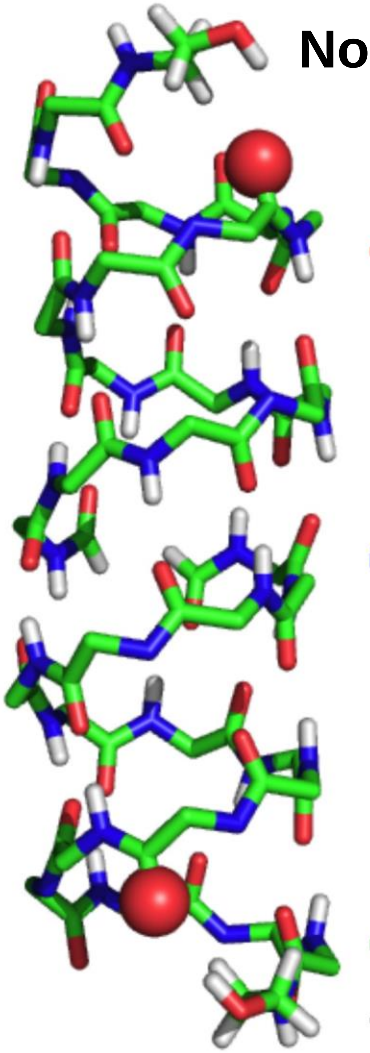


No ions

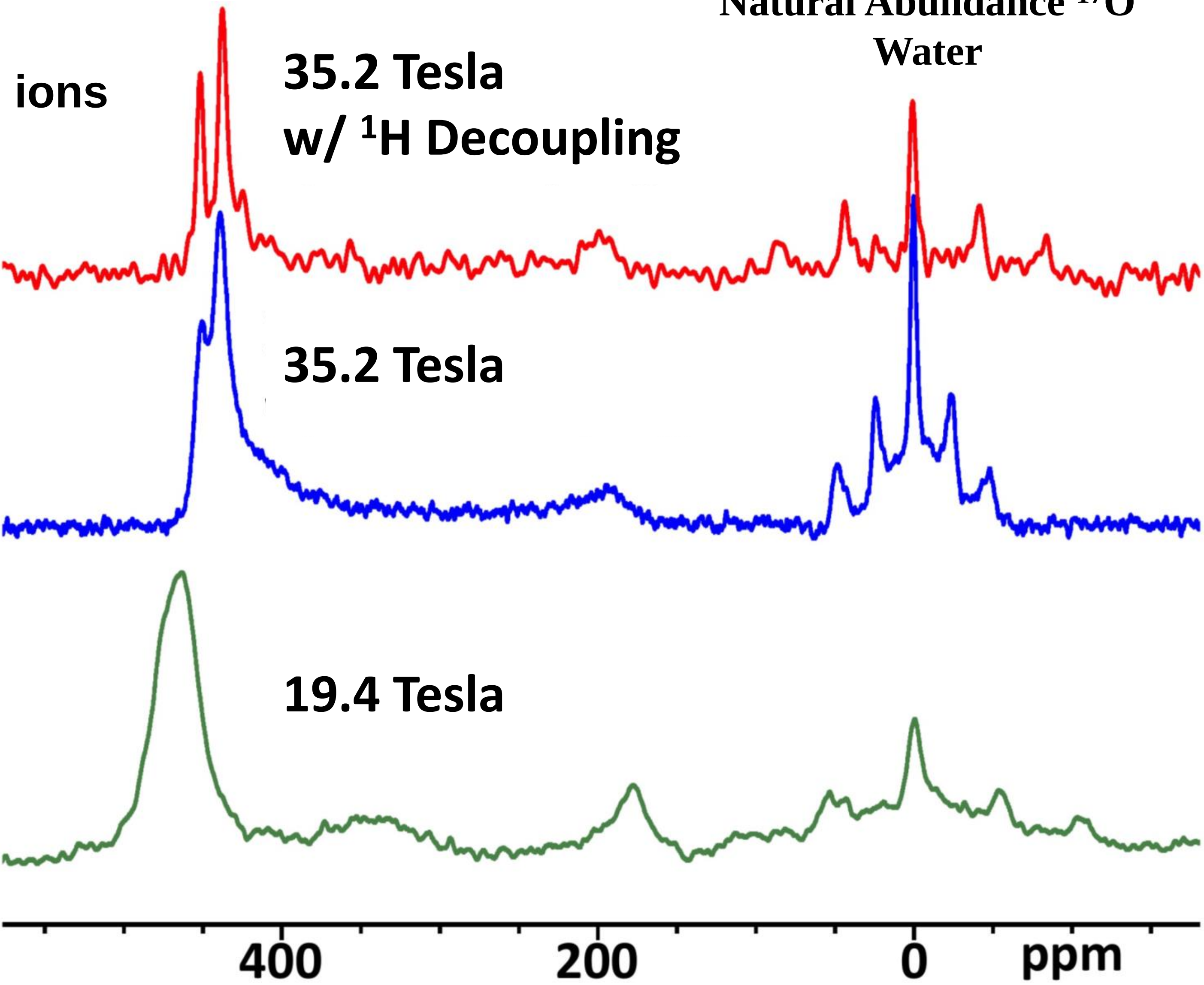


Joana Paulino,
Eduard Chekmenev

Leu_{10} ^{17}O Gramicidin A Aligned Parallel to DMPC Bilayer Normal: OS ssNMR



No ions



^{17}O Chemical Shift

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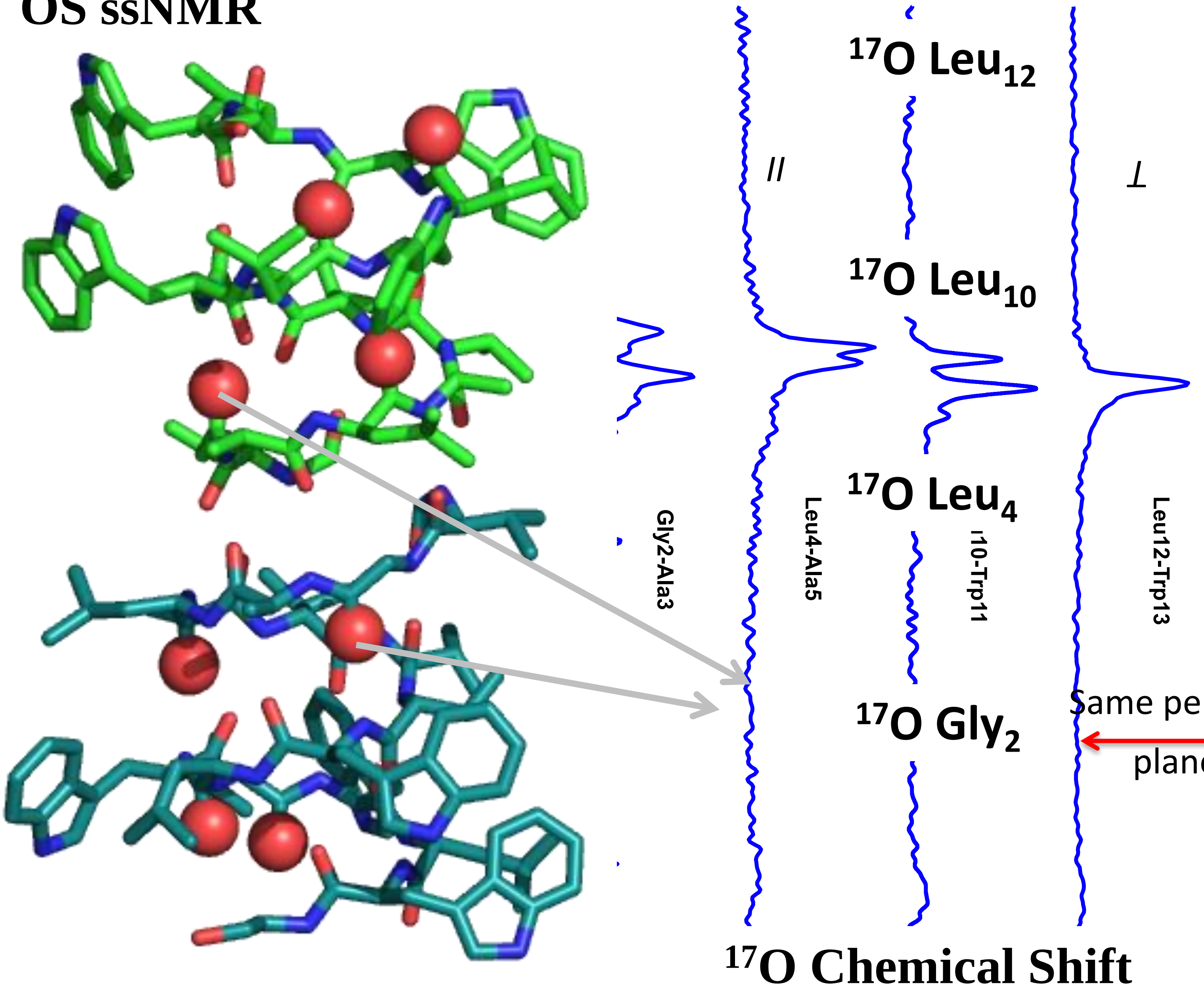
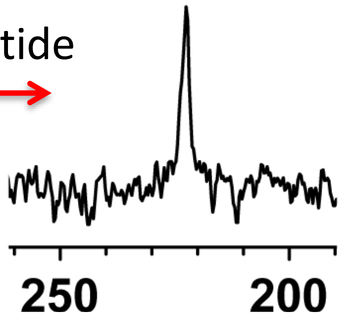
^{17}O Gramicidin A Aligned in Liquid Crystalline Lipid Bilayers: OS ssNMR



^{17}O Chemical Shift

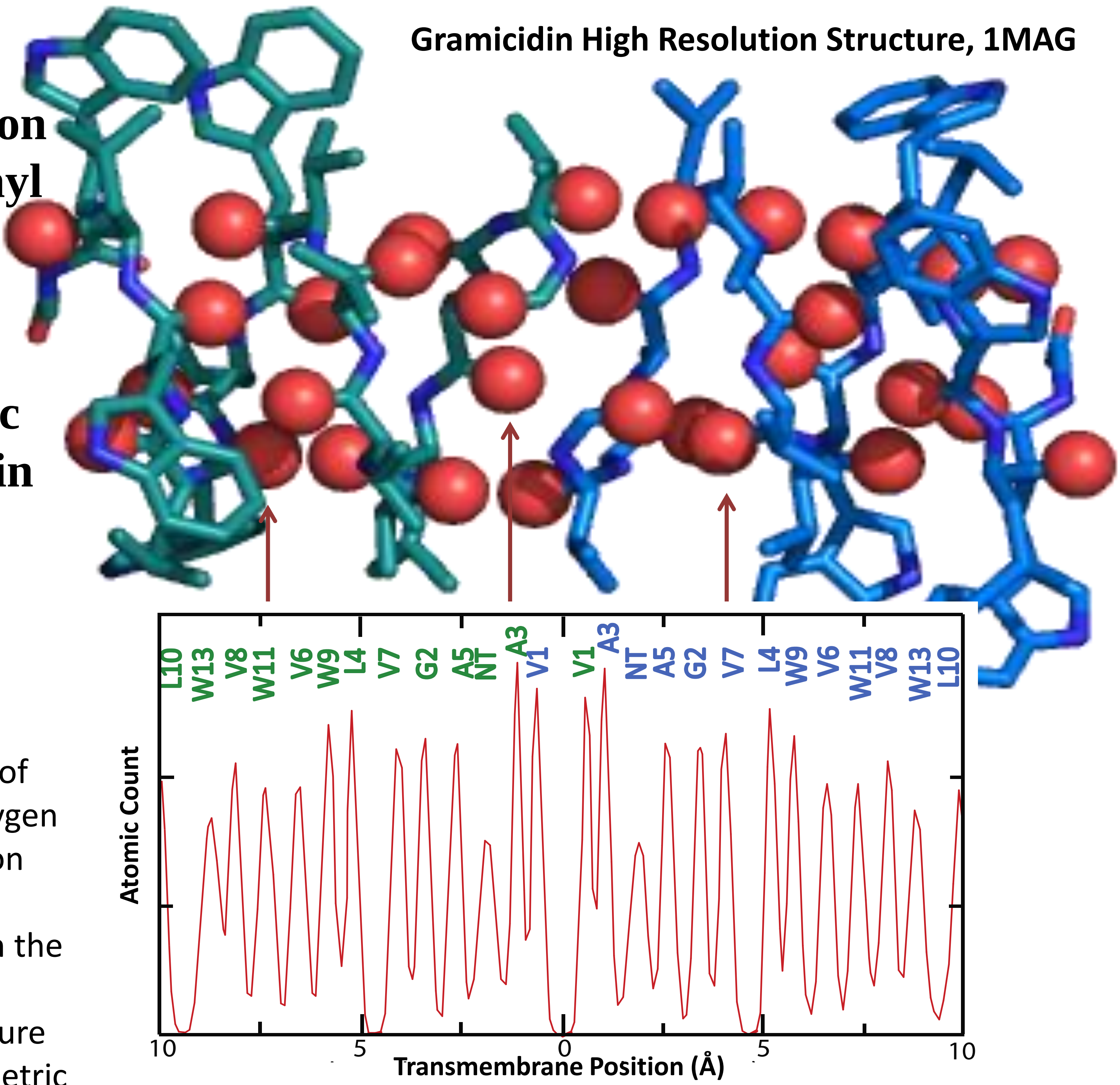
Same peptide plane

Keep this in mind



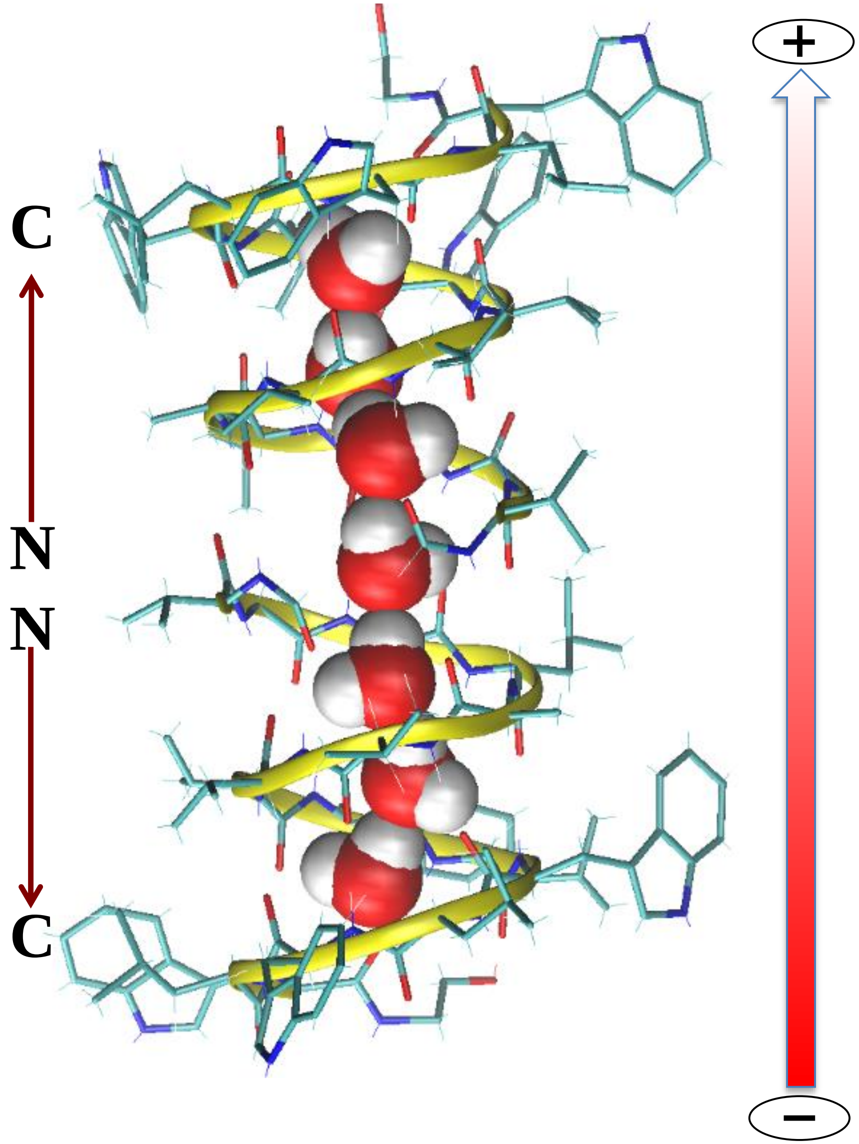
^{15}N Chemical Shift

Distribution of Carbonyl Oxygen Atoms in the Symmetric Gramicidin A Dimer

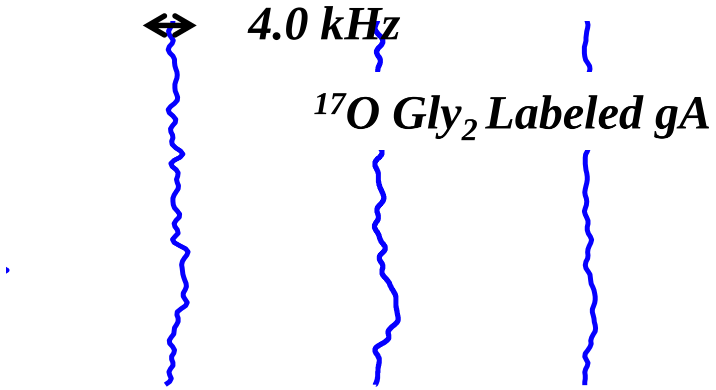

Gramicidin High Resolution Structure, 1MAG

Distribution of Carbonyl oxygen sites based on an MD simulation in the pore – very stable structure – very symmetric

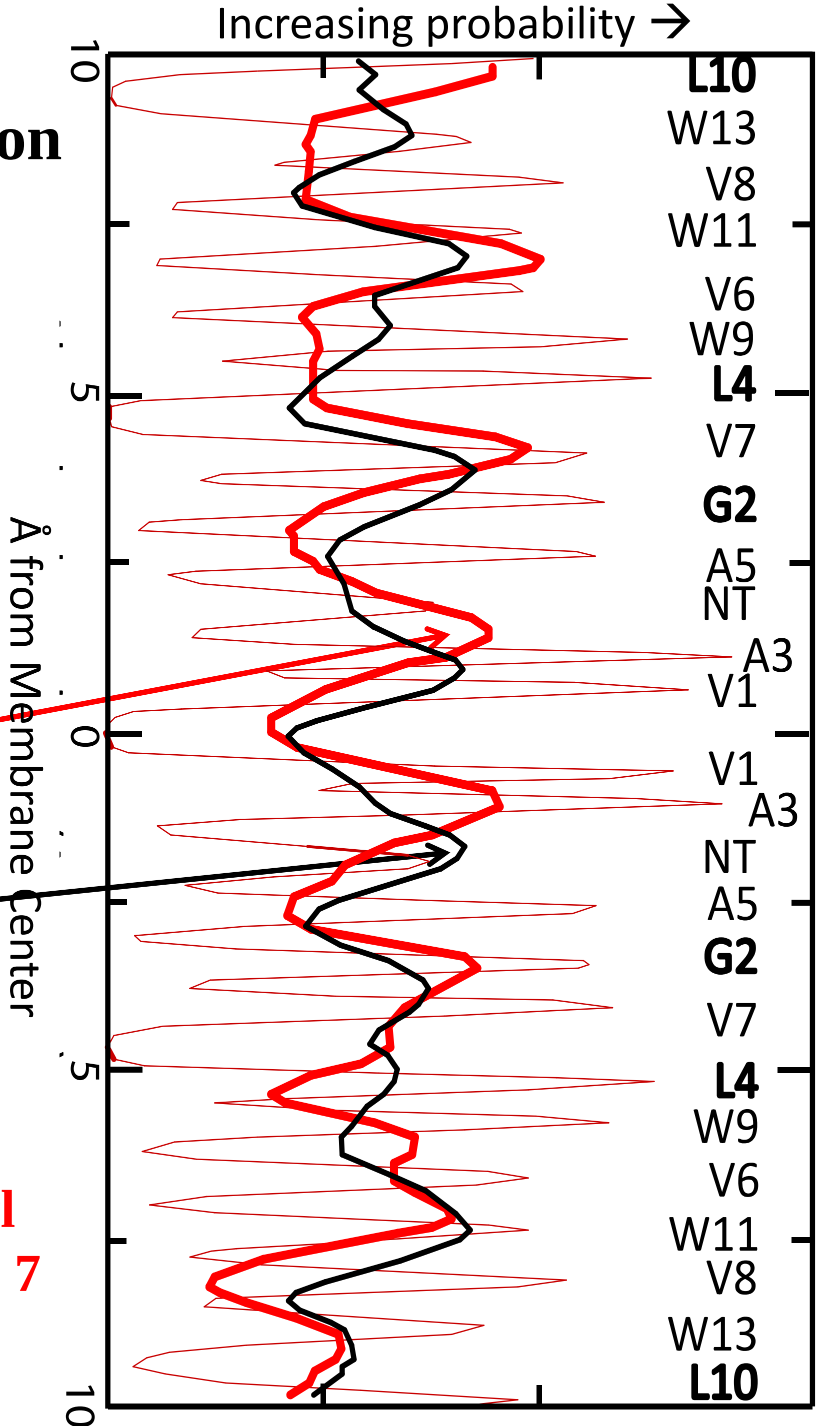
Waters in the Gramicidin Pore



- ***Gramicidin A - Single File Column of Water Molecules modeled by MD***
- ***7 or 8 Ordered Waters Molecules form Electric Dipole Moment***
- ***According to MD Simulations: Water Wire Reorients on the sub-ns Timescale***
- ***ssNMR resonances shows stability on sub-ms Timescale – 6 orders of magnitude difference***
- ***Is it the electric dipole of the water wire that induces 4.0 kHz shift?***



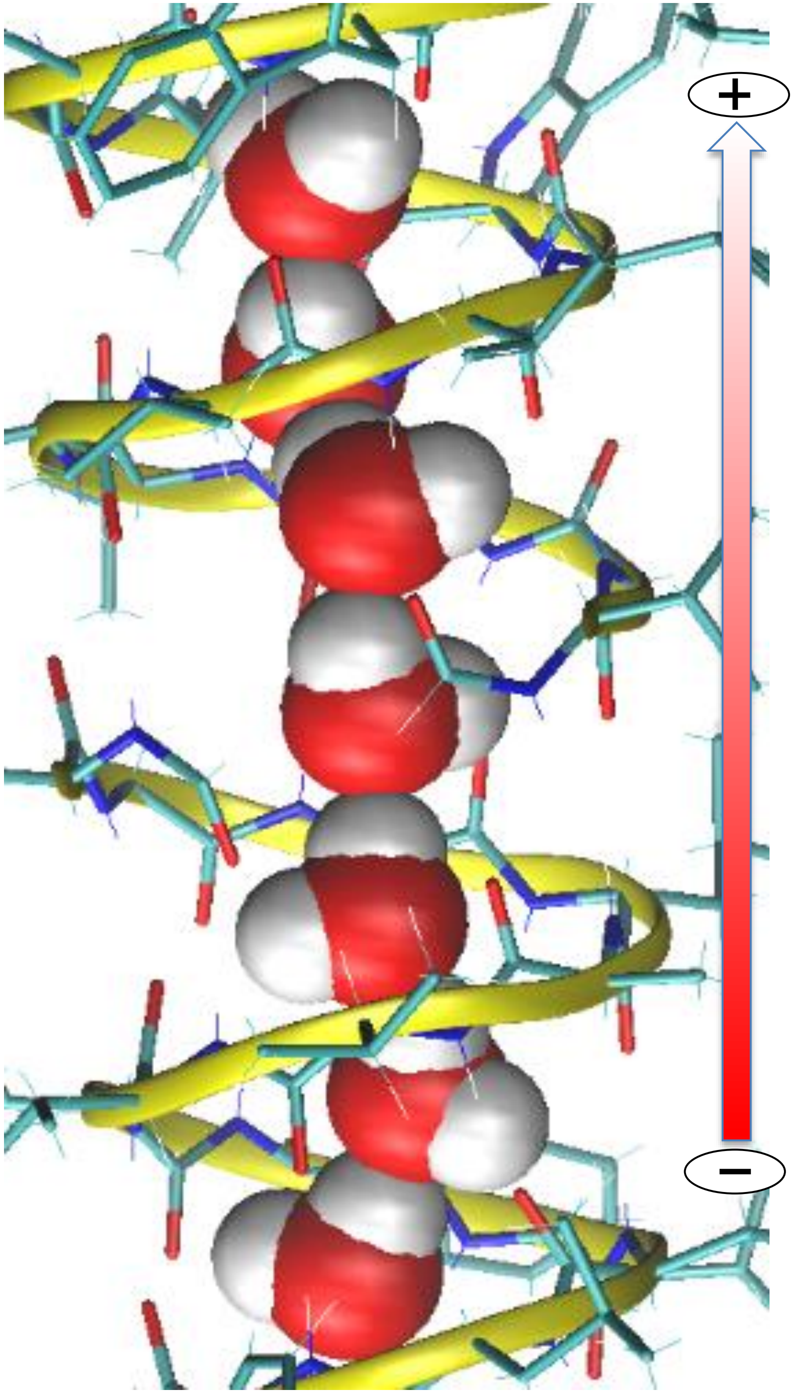
Statistical Distribution of Water Atoms Based on MD



Water Oxygens

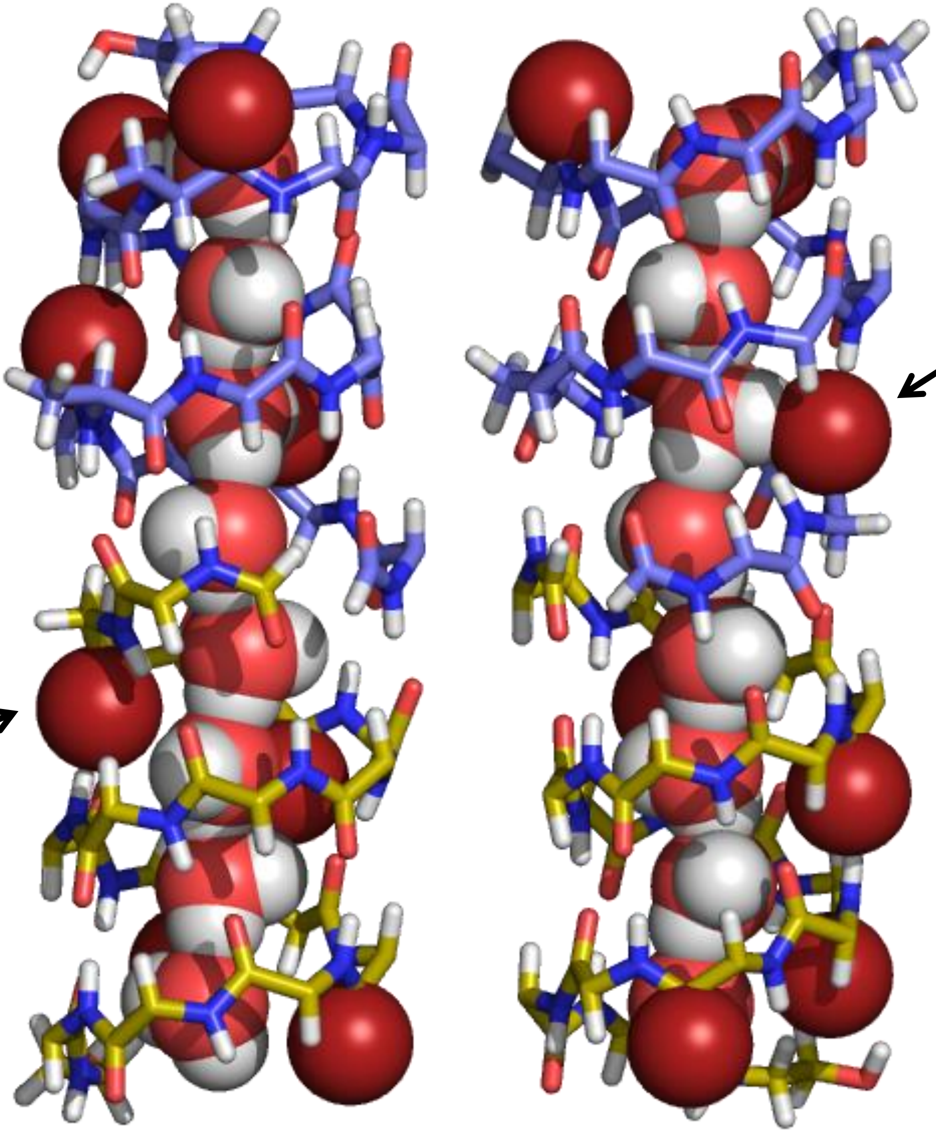
Both Water Hydrogens

26 Carbonyl Oxygens vs. 7 or 8 Waters



Selective Hydrogen Bonding Explains the Different Chemical Shifts

- Same MD Snapshot in two orientations showing one Gly2 carbonyl with an H-bond and the other without an H-bond



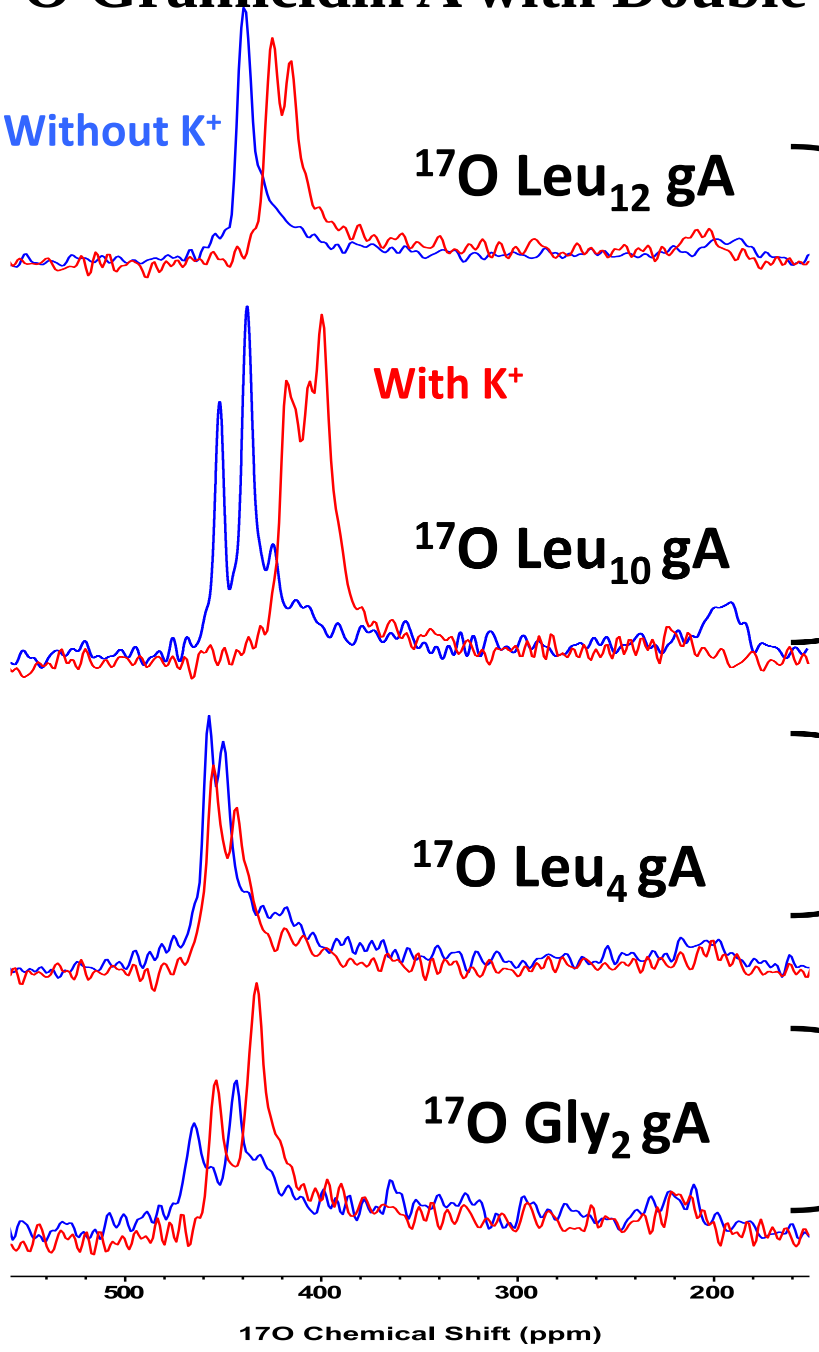
Gly2 Carbonyl Oxygen – Water H-bond

Gly2 Carbonyl Oxygen No H-bnd

Gramicidin A 1MAG

^{17}O Gramicidin A with Double Occupancy K$^+$

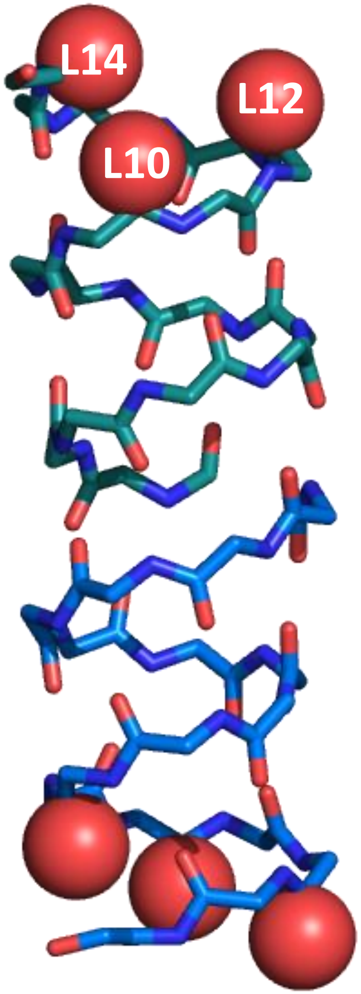
- **All data obtained at 35.2 T with ^{1}H decoupling**
- **K+ occupying both cation binding sites**



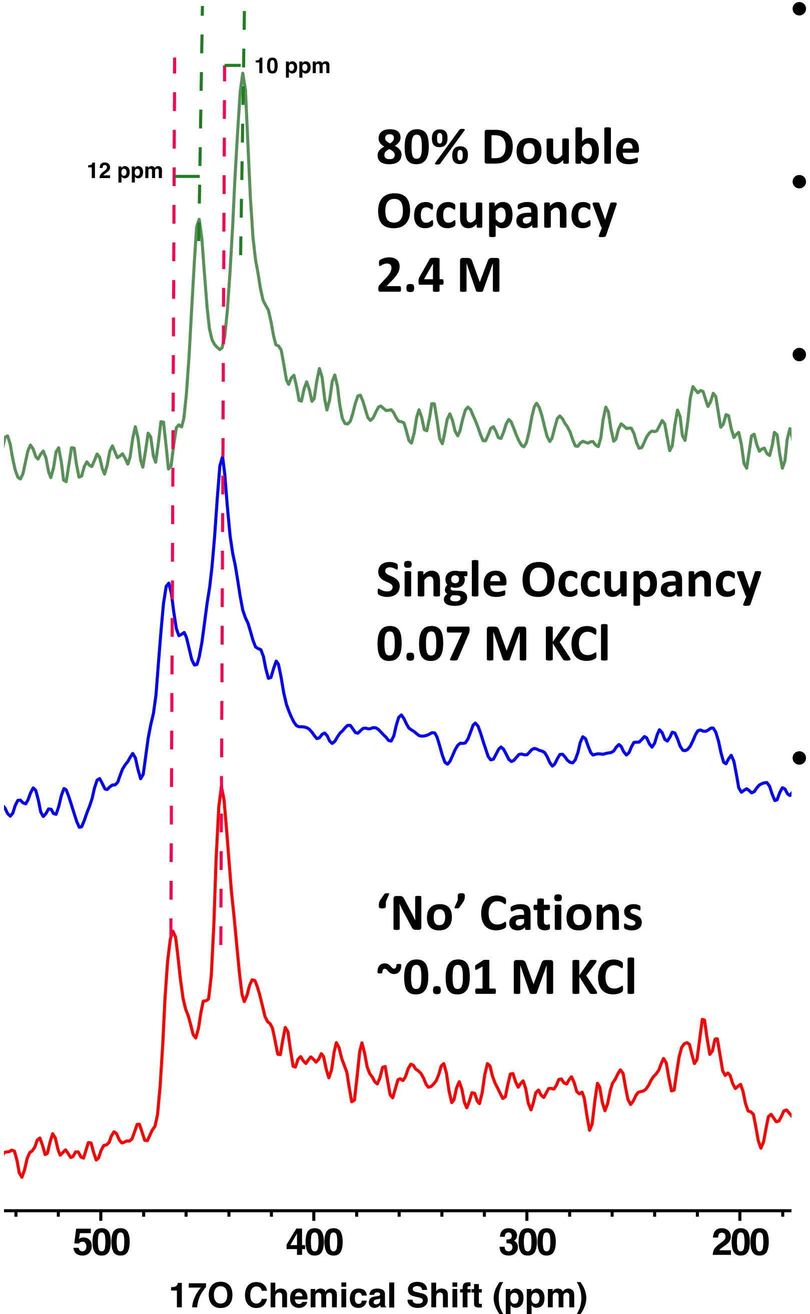
Both sites are part of the cation binding site

Leu$_4$ is far from the binding site

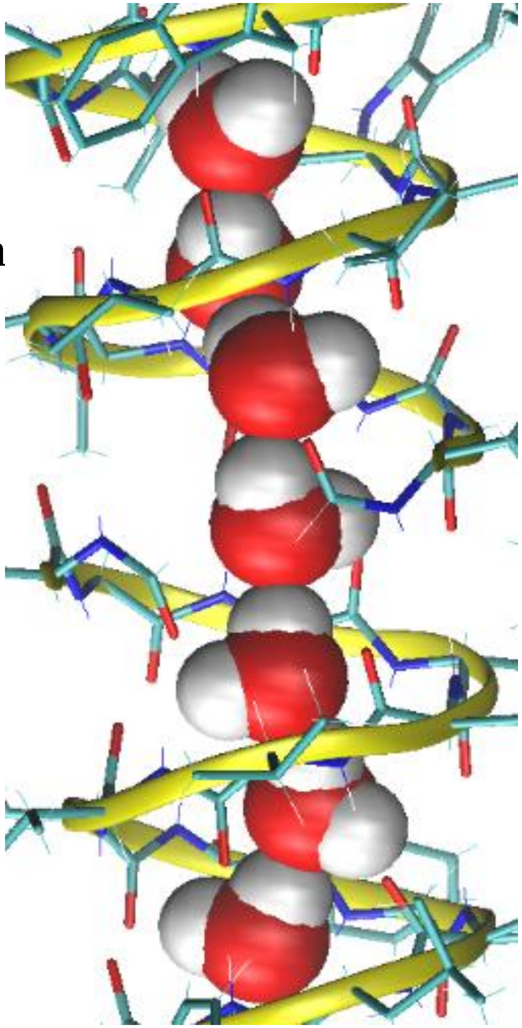
???



^{17}O Gly$_2$ gA without and with Single and Double K^+ Occupancy

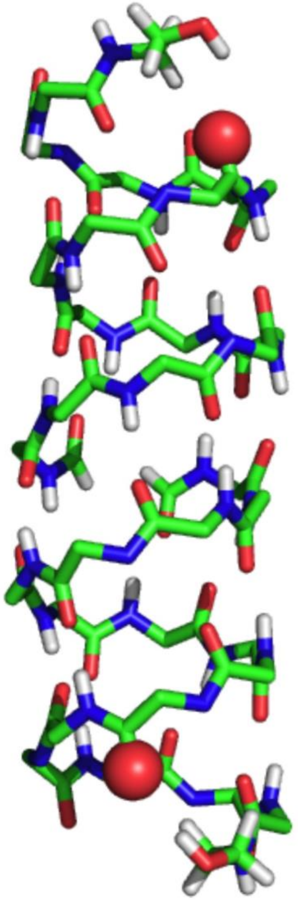


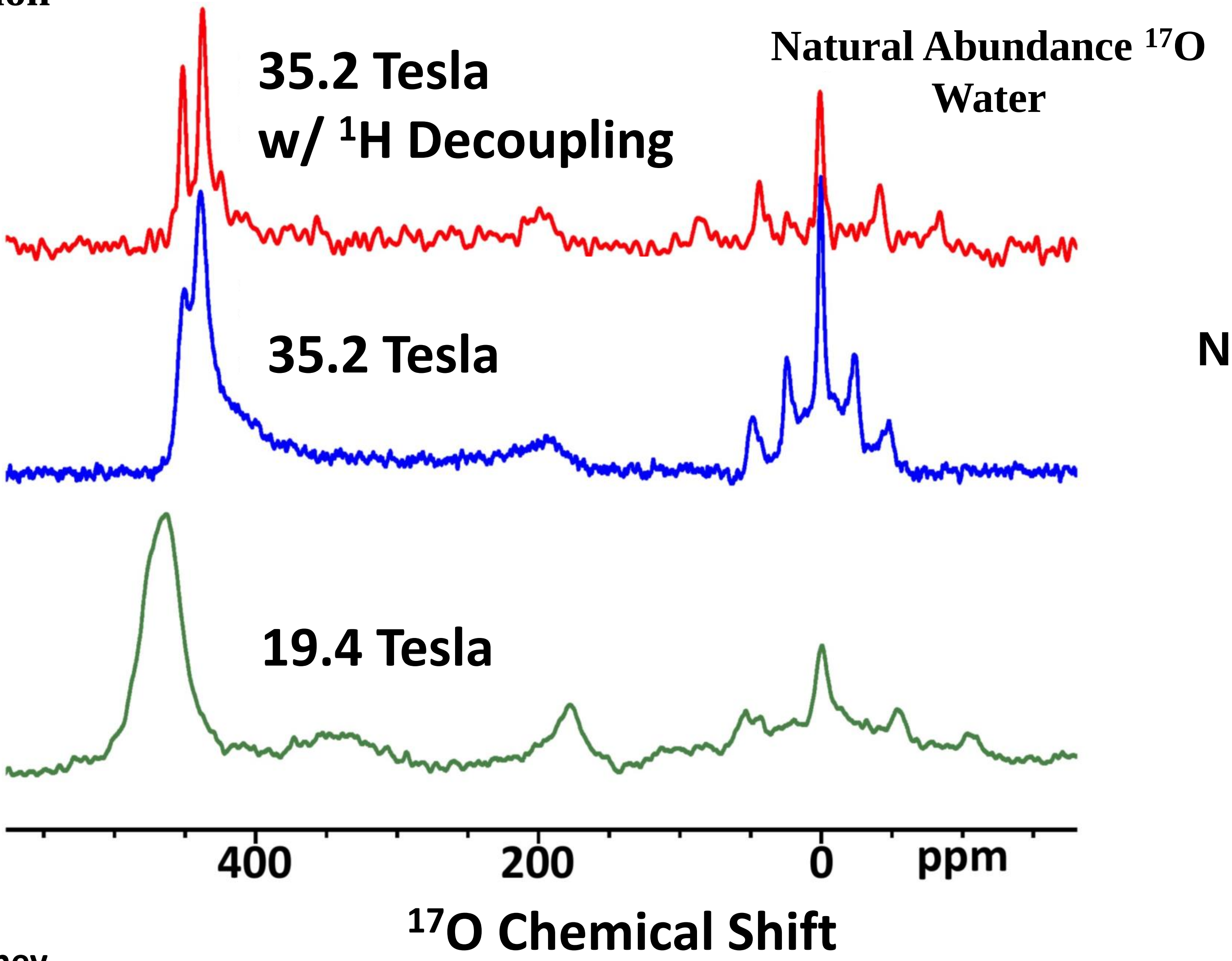
- Without cations the water dipole is intact from one side of the bilayer to the other.
- With single occupancy the water dipole is also stable.
- With double occupancy the water dipole is split inducing water flips near the gA-gA junction causing additional averaging.
- Importantly there is still the same number of waters.



Unique Chemistry Discovered by Ultra-High Field NMR:

- **How waters have unique interactions with gA breaking the dimeric symmetry**
- **How cations support that role and interfere with it as a function of concentration**
- **How ^{17}O spectroscopy at high fields can provide unique insights into biological function**





No ions

Joana Paulino,
Eduard Chekmenev

SCH NMR Users – so far in 2018

Nathaniel Traaseth – OS ssNMR EmrE membrane protein in bicelles - 1/18
Gang Wu – ^{17}O MAS ssNMR of organic solids - 6/18
Rob Schurko – ^{1}H-^{103}Rh MAS ssNMR of catalysts and model compounds - 4/18
Alex Nevzorov – OS ssNMR of Pf1 coat protein in bicelles - 1/18
Len Mueller – ^{17}O MAS ssNMR of Tryptophan synthase
Rachel Martin – MAS ssNMR of Droserasin – 4 & 9/18
Francessca Marassi – OS ssNMR of Y. pestis Ail - 1/18
Daniel Lee – MAS ssNMR metal oxide nanocrystals
Danielle Laurencin - MAS ssNMR of biomaterials - 4/18
Oliver Lafon – ^{71}Ga ssNMR of Ga2Se3 - 4 & 7/18
Hans Jakobsen – ^{95}Mo ssNMR of tetraoxoanions – 2/18
Yining Huang -^{17}O ssNMR of metal organic frameworks – 4 & 5/18
Sophia Hayes – ^{25}Mg of metal oxide thin films - 4/18
Oc Hee Hahn – ^{79}Br and ^{81}Br NMR of Perovskite crystals - 2/18
Robert Griffin – ^{17}O labeled water in amyloid forming peptide – 4 & 9/18
Cecil Dybowski – ^{67}Zn of ZnO-based pigments in paint films – 1 & 3/18
Myriam Cotten – OS ssNMR of metallopeptides bound to membrane surface - 3/18
Brad Chmelka – ^{23}Na, ^{27}Al, ^{35}Cl, ^{39}K, ^{71}Ga, ^{95}Mo, and ^{115}In in nanostructured solids - 6/18
Ed Chekmenev – ^{17}O gramicidin OS ssNMR (fill-in spectroscopy)
David Bryce – Quadrupolar spectroscopy of various organics and inorganics - 2/18

Conclusions:

- High Fields are going to be great
- There are the obvious advantages of dispersion, sensitivity, etc.
- Opening the periodic table by integrating many quadrupolar spectroscopy into our repertoire to solve important chemical questions
- The sensitivity of ^{17}O for characterizing the chemistry – not only for protein and nucleic acid studies, but for interactions with the macromolecular solubilizing environment.
- More spin ½ spectroscopy will be performed as the SCH magnet & spectrometer performance improves, but the focus will be on quadrupoles
- High Temperature Superconducting Materials and magnets are on their way – Mark Bird’s talk.

32T All superconducting Magnet reached full field December, 2017: 17T HTS component & 15T LTS component - Will be installed in our High B/T User Program 1/2019 at the NHMFL.