Published in final edited form as:

J Phys Chem B. 2017 October 12; 121(40): 9331–9335. doi:10.1021/acs.jpcc.7b06736.

Whole Ribosome NMR: Dipolar Couplings and Contributions to Whole Cells

Rie Nygaard^{1,2,3}, Joseph A. H. Romaniuk^{1,2}, David M. Rice^{1,4}, and Lynette Cegelski^{1,*}

¹Stanford University, Department of Chemistry, 380 Roth Way, Stanford CA 94305

Abstract

Solid-state NMR is a powerful tool for quantifying chemical composition and structure in complex assemblies and even whole cells. We employed N{P} REDOR NMR to obtain atomic-level distance propensities in intact ¹⁵N-labeled *E. coli* ribosomes. The experimental REDOR dephasing of shift-resolved lysyl amine nitrogens by phosphorous was comparable to that expected from a calculation of N-P distances involving the lysines included in the crystal structure coordinates. Among the nitrogen contributions to the REDOR spectra, the strongest dephasing emerged from the dipolar couplings to phosphorous involving nitrogen peaks ascribed primarily to rRNA and the weakest dephasing arose from protein amide nitrogens. This approach is applicable to any macromolecular system and provides quantitative comparisons of distance proximities between shift-resolved nuclei of one type and heteronuclear dephasing spins. Enhanced molecular specificity could be achieved through the use of spectroscopic filters or specific labeling. Furthermore, ribosome ¹³C and ¹⁵N CPMAS spectra were compared with those of whole cells from which the ribosomes were isolated. Whole-cell signatures of ribosomes were identified and should be of value in comparing overall cellular ribosome content in whole-cell samples.

Keywords

CPMAS; REDOR; solid-state NMR; ribosomes; whole-cell NMR

Introduction

The ribosome is the ultimate cellular machine. Ribosomes are made up of ribosomal RNA (rRNA) and over 50 proteins in bacteria, organized into large and small subunits, to translate genetic instructions into proteins. Tremendous contributions over decades have transformed early electron microscopy images and Plaster of Paris models of the glove-like assembly¹ into high-resolution atomic-level coordinates through X-ray crystal structure determinations and, more recently, through cryo-electron microscopy (EM). High-resolution structures are now available for many ribosomes purified from bacteria, including *Haloarcula marismortui* (the first 50S subunit structure published in 2000),² *Thermus thermophilus*,³ *E. coli*,⁴ *B. subtilis*,⁵ and *Mycobacterium smegmatis*.⁶ The first eukaryotic ribosome was determined for

*Corresponding author: Contact information: cegelski@stanford.edu; telephone: 650-725-3527; fax: 650-723-4817.

²These authors contributed equally to this work.

³Current affiliation: Columbia University, Department of Physiology and Cellular Biophysics

⁴Current affiliation: University of California Merced, School of Natural Sciences

the yeast, *Saccharomyces cerevisiae*, in 2010.⁷ Since then, cryo-EM structures with 2.5–5 Å resolution have been reported for the 80S ribosomes from human,⁸ *Drosophila*,⁹ *Plasmodium falciparum*,¹⁰ and *Trypanosoma cruzi*,¹¹ and for the mammalian mitochondrial and spinach chloroplast ribosomes.^{12–13}

These structures have yielded invaluable insight into ribosome function and the modes of action of ribosome targeting antibiotics. New ribosome structures continue to be determined from other species and domains of life, revealing major similarities, but also structural differences.^{14,15} Differences in ribosome structures across kingdoms, for example, enable the use of ribosome-targeting antimicrobials to arrest bacterial protein synthesis while not significantly affecting human ribosome function. Computational studies have sought to examine and understand the statistical propensity of various amino acids involved in protein-RNA contacts lending structural integrity to ribosomes and other protein-RNA complexes, often by examining dozens of available and relevant crystal structures of certain types.^{16,17} Yet, within available structures, electron density can be missing or imprecise near protein sidechains.

We demonstrate that important atomic-level distance propensities can be obtained for intact ribosomes using Rotational-echo Double Resonance (REDOR) solid-state NMR.¹⁸ REDOR NMR is uniquely suited to determining individual and site-specific internuclear distances within biological assemblies as well as measuring the collective proximities between a certain type of nucleus distributed over many sites and dephasing spins to capture propensities of contacts or distance distributions.¹⁹ For example, a 1.1 ms C{N} REDOR experiment can identify all carbons in a sample that are bonded to N and provides the percent of carbons at a specified chemical shift in that one-bond contact distance. Longer distances are measured and evaluated by increasing the REDOR evolution time for the experiment. The approach does not require crystalline material and REDOR measurements have been made in purified proteins and enzymes, in bacterial whole cells and even in intact plant leaves.¹⁹ Atomic-level detail for DNA packaging in Bacteriophage T4 capsids employed N{P} REDOR to describe the electrostatic interactions and mechanisms for DNA charge balance in capsid packaging.²⁰ Furthermore, we have been developing approaches to identify and compare spectral signatures of specific biomolecules in the context of intact whole cells.^{21–24} For example, we were able to distinguish whether bacteria were treated with a cell-wall targeting antibiotic (fosfomycin) or a protein synthesis inhibitor targeting the ribosome (chloramphenicol) by inspecting the natural abundance ¹³C NMR spectra of antibiotic-treated whole cells.²²

Here, we introduce a REDOR approach to evaluate the total collection of lysine-RNA proximities in intact ribosomes from *E. coli*. We performed N{P} REDOR NMR on uniformly ¹⁵N-labeled ribosomes to measure the spectral dephasing of shift-resolved lysyl amines by ribosomal RNA (rRNA) phosphates and report on the collective dipolar couplings between [e-¹⁵N]Lys and ³¹P nuclei. Results are compared with expectations from the available proximities in the X-ray crystal structure (Figure 1A). REDOR dephasing of other nitrogens in the system, including rRNA and protein amides, provide internal comparisons for relative distance propensities. Additionally, with approximately 20,000 ribosomes per bacterium, ribosomes can account for up to one fourth of the dry mass of the cell.²⁵ We

compared ^{13}C and ^{15}N NMR spectra of ribosomes with whole-cell spectra and identified spectral signatures of ribosomes that could be valuable in comparing ribosome content even in unperturbed whole-cell samples.

Materials and Methods

Ribosome and whole-cell sample preparation

Both whole cell and ribosome samples for NMR analysis were extracted from *E. coli* grown in M9 minimal media with ^{15}N (^{15}N enrichment) and/or ^{13}C ($^{13}\text{C}_6$ glucose (99% ^{13}C enrichment)) to yield uniformly ^{15}N -labeled ribosomes or ^{13}C and ^{15}N -labeled whole cell samples. To ensure complete labeling, a culture of *E. coli* MRE-600 was started in LB broth and grown overnight, diluted 1:100 in M9 medium and grown to mid-exponential phase, and then further diluted 1:50 in M9 medium to prepare another overnight culture. This overnight culture was diluted 1:500, grown to an optical density of 0.60 at 600 nm, and harvested by centrifugation at 10,000 g for 4 minutes at 4°C, and washed twice in ice-cold 5mM HEPES, pH 7.4. For whole-cell samples, these cell pellets were frozen with liquid nitrogen, and lyophilized. For purification of ribosomes, the cell pellets were resuspended in 20 mL of lysis buffer (70 mM KCl, 10 mM MgCl_2 , 10 mM Tris-HCl pH 7.4, PMSF and DNase (10 $\mu\text{g}/\text{mL}$)), and disrupted with 0.1 μm glass beads for three cycles of one minute disruption and one minute cooling on ice. The lysed cells were washed with 5mM HEPES, centrifuged for collection, and filtered prior to loading onto an 8-mL Monolith column45 (CIMmultusTM QA-8 Advanced Composite Column (Quaternary amine)) from BIA Separations for purification by FPLC.²⁶ A pure ribosome sample was obtained with elution at 450–550 mM NH_4Cl . The ribosome fractions were combined, concentrated, and exchanged into lyophilization buffer (5 mM HEPES, 0.5% PEG-8000, and 30 mM trehalose). The purification resulted in greater than 10 mg ribosomes per liter of bacterial cell culture.

Solid-state NMR and REDOR

Solid-state CPMAS²⁷ and $\text{N}\{\text{P}\}$ REDOR NMR were employed in this study. REDOR NMR enables the measurement of heteronuclear dipolar couplings between heteronuclear spin pairs.¹⁸ The dipole-dipole coupling between heteronuclei within a magnetic field is dependent upon both spatial and spin coordinates. Magic-angle spinning averages over the spin coordinates and suppresses the dipolar interactions in a coherent manner. The REDOR measurement utilizes the application of rotor-synchronized radiofrequency pulses to operate exclusively on the spin coordinates and interferes with the complete suppression of the dipolar coupling by magic-angle spinning. This recoupling, or reintroduction of the dipolar coupling, enables the measurement of dipolar couplings and hence distances. The $^{15}\text{N}\{^{31}\text{P}\}$ REDOR dephasing of the lysyl peak is reported as the experimental dephasing (S/S_0) for two REDOR evolution times (4.48 and 16 ms). The S value was calculated from the difference in peak heights of the lysyl peak in the S_0 and S spectra. Experimental uncertainties are represented with error bars. Error bars were calculated by measuring the standard deviations of the noise in a signal free region of the spectra (–200 to –400 ppm) and propagating the error through the S and S/S_0 calculations.²⁸ Specifically, the error/

uncertainty of S is the $\sqrt{[\text{error in } S_0]^2 + [\text{error in } S]^2}$. NMR data were processed in vnmrj 4.0 and using custom scripts in Matlab.

All NMR experiments were performed in an 89 mm bore 11.7 T magnet (Agilent Technologies, Danbury CT) using a home-built four-frequency HPCN transmission line probe (^1H 500.92 MHz, ^{31}P 202.78 MHz, ^{13}C 125.96 MHz and ^{15}N 50.76 MHz) with a four channel DD2 console (Agilent Technologies), employing high-power linear Transmitter/Receiver (T/R) line switching on the ^{13}C channel and a passive diode preamp on the ^{15}N channel. Samples were spun at 8000 Hz or 7143 Hz in thin-wall 5mm zirconia rotors and maintained at -5°C with an FTS Chiller (FTS Thermal Products, SP Scientific, Warminster, PA) supplying nitrogen at -25°C . Field strengths for ^{13}C and ^{15}N cross polarization were all 50 kHz with a 10% ^1H linear ramp centered at 57 kHz. The echo-detection and REDOR π pulses were all 10 μs (for ^{15}N and ^{31}P) and SPINAL ^1H decoupling was applied at 72 kHz during acquisition and at 100 kHz during REDOR periods. The CPMAS mixing time was 1.5 ms and the recycle time was 3.0 s for all experiments. ^{13}C chemical shifts were referenced to tetramethylsilane as 0 ppm using a solid adamantane sample at 38.5 ppm. The ^{15}N chemical shift scale was referenced to ammonia at 0 ppm where solid L-[amide- ^{15}N]Asn appears at 114.5 ppm. Referencing to ammonia at 0 ppm is a change from some previous uses of solid NH_4SO_4 for which L-[amide- ^{15}N]Asn would be reported as 89.1 ppm. The 4.4 ms (32 T_r) evolution time N{P} REDOR measurement was performed with 7143 Hz magic angle spinning and the 16 ms (128 T_r) evolution time N{P} REDOR measurement was performed with 8000 Hz magic angle spinning.

Results

Biochemical Characterization of purified Ribosomes

Isotopically labeled ribosomes were purified from *E. coli* MRE600, the strain from which ribosomes were purified for the crystal structure determination reported in 2005.⁴ Specifically, ribosomes were isolated from bacteria harvested at OD_{600} of 0.6; purified using a Monolith chromatography column; and analyzed by UV-Vis spectrophotometry and protein gel electrophoresis. The 280 nm to 260 nm absorbance ratio was greater than 1.8, consistent with the RNA-to-protein ratio for an intact ribosome sample. The purified ribosome preparation was also compared with a commercially available *E. coli* ribosome standard (New England Biolabs 70S ribosomes) by protein gel electrophoresis and confirmed the integrity of the ribosomes in having the correct protein profile (Fig. 1A).

REDOR for Ribosomes

N{P} REDOR was employed to determine the experimental dephasing of ribosomal nitrogens by phosphates in rRNA using uniformly ^{15}N -labeled ribosomes. Lysine sidechain nitrogens are resolved from other amino acid sidechain nitrogens and RNA nitrogens, whereas histidine and arginine sidechains overlap with RNA nitrogens (Figure 1B). Thus, we sought a quantitative analysis of lysine dephasing by phosphorous and considered dephasing of other nitrogens more holistically for potential comparative power in considering all nitrogens in the sample. We performed N-P REDOR NMR with evolution times of 4.4 and 16 ms. The 16-ms REDOR spectra yielded significant dephasing of

nitrogen peaks and immediately revealed a wide range of average dipolar couplings between different types of nitrogens and rRNA in the ribosome. The lysine nitrogens exhibited 28% dephasing and will be considered further below in comparison with expectations from the crystal structure. RNA-only contributions dominate the spectrum between 140–180 ppm. This region also contains contributions from histidine nitrogens, but these are expected to be small based on CPMAS spectral intensities for other uniformly ^{15}N -labeled proteins.²⁹ More specifically, adenosine and guanosine position 9 nitrogens appear at 170 ppm and serve as the attachment point to the sugar ring with its monophosphate. As expected, dephasing of this peak was strong (35%) and should serve as an internal ruler for maximal dephasing in ribosome samples. In contrast, the protein amide peak exhibited the weakest dephasing by ^{31}P , with a S/S_0 of 6%. This minimal dephasing is consistent with the protein backbone amide nitrogens being more distant, on average, from phosphates than rRNA nitrogens. Dephasing of lysyl sidechain nitrogens was strong and exhibited a S/S_0 of 28%. We took the opportunity to consider the lysine dephasing in a more detailed manner in the context of ribosome structure.

E. coli ribosomes typically contain 55 proteins (22 in the small subunit and 33 in the large subunit) and three distinct rRNA strands (5S and 23S rRNA in the large subunit and the 16S rRNA in the small subunit).²⁵ The total protein content includes 685 lysines. The crystal structure of the *E. coli* ribosome⁴ included modeled density for 558 lysines. The difference from the total number of ribosome lysines is attributed to two sources: (i) 43 fewer lysines were included because the ribosomes used for crystallography were depleted of the S1 protein, and (ii) additional missing lysines were attributed to flexible proteins that were not included in the structure.⁴ The proximities of all 558 lysines annotated in the crystal structure to phosphates were binned in 0.5 Å increments, resulting in the distance distribution presented in Figure 1C. The expected $N\{P\}$ REDOR S/S_0 curve was calculated for the total collection of ribosomal lysine sidechain nitrogens with the proximities to ^{31}P nuclei as binned in the histogram (Figure 1D). Simulated REDOR curves for single ^{15}N - ^{31}P distances are provided for comparison to illustrate how collections of dipolar couplings result in an appropriately weighted REDOR curve. Strong dipolar couplings (short distances) contribute to strong early dephasing and the rapid rise in the S/S_0 curve, whereas weaker dipolar couplings require longer evolution times in order to contribute to REDOR dephasing. Ultimately, all 558 lysines would be dephased by phosphorous in the sample, with the REDOR curve reaching a maximum plateau of 1.0.

The calculated curve predicted 30% dephasing for the lysyl nitrogen dephasing by phosphorous after 16 ms of evolution time (Figure 1D). We observed 28% dephasing, close to what was predicted from the X-ray structure. Technically, the observed dephasing of 28% is just under 30% and is consistent with the set of lysines not reported in the crystal structure as being farther away from phosphorous. An additional REDOR experiment was performed with a 4.4 ms evolution time and yielded 4.6% dephasing (Figure 1D). REDOR NMR captured the distance distribution between all the lysyl amine nitrogens and phosphates in purified ribosomes, without the need for crystalline preparations, and was comparable to the calculation based on the crystal structure. This approach could be employed to examine possible differences in the propensity of contacts among other prokaryotic and eukaryotic ribosomes. Furthermore, specific labeling with strategically selected ^{15}N -labeled amino

acids could be employed to measure distance distributions between residues of interest and phosphates without overlapping contributions from other ribosome nitrogens. Such an approach would enable broad profiling and determination of the statistical propensity for sidechains of arginines or histidines, for example, to be proximate to rRNA.

In addition to the specific analysis of N-P dipolar couplings, the one-dimensional REDOR spectra revealed the rich spectral profile of ribosomes that differs markedly from protein NMR and even whole-cell NMR spectra, due to the unique RNA and protein composition of the ribosome. Thus, we took the opportunity to directly compare the ^{15}N and also ^{13}C NMR spectra of purified ribosomes with whole cells from which the ribosomes were purified.

NMR Comparisons of Nitrogen and Carbon Content in Ribosomes and Whole Cells

The N{P} REDOR full-echo S_0 spectrum revealed the unique nature of the ribosome nitrogen composition (Figure 1B). Nucleic acid ^{15}N peaks contribute significantly to the total ^{15}N spectral intensity of ribosomes and are salient features in a ribosome ^{15}N spectrum, readily distinguishing it from a typical protein or whole-cell NMR spectrum. Uniformly ^{15}N -labeled ribosomes and whole cells were first compared by ^{15}N cross-polarization magic-angle spinning (CPMAS) NMR. The two ^{15}N NMR spectra were normalized to the peptide backbone peak centered at 120 ppm to permit inspection of the relative content of RNA and nitrogen-containing sidechains with respect to the protein amide content (Figure 2A, left). The much higher RNA-to-protein ratio in purified ribosomes is reflected in the CPMAS spectra, noting the major rRNA chemical shifts at 70–90 ppm and 147, 160, and 170 ppm. The lysyl amine nitrogen also exhibits a more downfield chemical shift than the average whole-cell amine peak (36-ppm in ribosomes), perhaps due to the propensity for lysine-phosphate hydrogen bonding in ribosomes. A comparison is also provided in which the ribosome and whole-cell spectra are scaled to match intensities in the salient rRNA peaks near 150 and 170 ppm (Figure 2A, right). Based on this scaling and taking into account sample masses, the ribosome spectrum accounts for approximately 25% of the integrated area of the whole-cell spectrum, consistent with the expected ribosome content in whole cells. Thus, comparisons of whole-cell ^{15}N NMR spectra may be of value in comparing ribosome status, by evaluating intensity changes in the 140–180 ppm peaks.

^{13}C CPMAS spectra of purified *E. coli* ribosomes and whole cells also revealed clear differences consistent with the compositional differences between the two systems. In whole cells, the carbonyl peak near 175 ppm is often used as the salient reference peak and the lipid peak near 30 ppm is also usually an intense peak in the spectrum. All the other carbon contributions in the cell are spread out over many different biomolecules, yielding broad spectral contributions through carbon chemical shifts between 10–180 ppm. In ribosomes, however, a unique ^{13}C spectrum is obtained and the rRNA sugar peaks, which are detectable by ^{13}C NMR, are notably taller than the ribosome carbonyl peak. Collectively, these data emphasize how solid-state NMR can capture chemical composition noninvasively in intact systems and suggest that ^{15}N and ^{13}C whole-cell NMR should be of value in evaluating ribosome levels in intact cells.

Conclusions

Solid-state NMR can be recruited in many different ways to examine chemical composition and architecture in complex assemblies, regardless of size or complexity or potentially unknown components present. One can define and quantify the total collection of carbons and nitrogens at specified chemical shifts and, even more specifically, through spectral filters and specific labeling to resolve overlapping chemical shift contributions. Interest in quantification is a particular motivation for relying on one-dimensional NMR methods, including REDOR which enables quantitative accounting of dipolar couplings in a system. Here, we introduced a solid-state NMR approach to characterize chemical composition and intermolecular interactions in intact ribosomes purified from *E. coli*, without requiring any degradative or invasive analysis. We used this system as a model system, believing that the crystal structure coordinates should largely represent the relevant functional state for most of the ribosome. Dipolar couplings between lysyl amine nitrogens and rRNA phosphates were measured experimentally with REDOR NMR and were comparable to calculations derived from the X-ray structure coordinates. The REDOR measurements, however, did not require optimizations to prepare crystalline material; were performed on lyophilized ribosomes; and were not hindered by flexibility that could be present in solution and not captured in crystals. In addition, we took the opportunity to compare ribosome ^{13}C and ^{15}N CPMAS NMR spectra with whole-cell spectra and identified salient ribosome signatures that should be of future value in quantifying ribosome content or mapping interactions with ribosome-binding compounds in the context of intact whole cells.

Acknowledgments

This material is based upon work supported by the National Science Foundation CAREER Award 1453247 (L.C.) and the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R01GM117278 (L.C.). R.N. was supported the Lundbeck Foundation Postdoctoral Fellowship.

REFERENCES CITED

1. Lake JA. Ribosome Structure Determined by Electron Microscopy of Escherichia coli Small Subunits, Large Subunits and Monomeric Ribosomes. *J Mol Biol.* 1976; 105:131–139. [PubMed: 792456]
2. Ban N, Nissen P, Hansen J, Moore P, Steitz T. The Complete Atomic Structure of the Large Ribosomal Subunit at 2.4 Å Resolution. *Science.* 2000; 289:905–920. [PubMed: 10937989]
3. Wimberly BT, Brodersen DE, Clemons WM Jr, Morgan-Warren RJ, Carter AP, Vonnrhein C, Hartsch T, Ramakrishnan V. Structure of the 30S Ribosomal Subunit. *Nature.* 2000; 407:327–339. [PubMed: 11014182]
4. Schuwirth BS, Borovinskaya MA, Hau CW, Zhang W, Vila-Sanjurjo A, Holton JM, Cate JHD. Structures of the Bacterial Ribosome at 3.5 Angstrom Resolution. *Science.* 2005; 310:827–834. [PubMed: 16272117]
5. Beckert B, Abdelshahid M, Schafer H, Steichen W, Arenz S, Berninghausen O, Bechmann R, Bange G, Turgay K, Wilson DN. Structure of the Bacillus subtilis hibernating 100S ribosome reveals the basis for 70S dimerization. *EMBO J.* 2017; doi: 10.15252/embj.201696189
6. Hentschel J, Burnside C, Mignot I, Leibundgut M, Boehringer D, Ban N. The Complete Structure of the Mycobacterium smegmatis 70S Ribosome. *Cell Rep.* 2017; 20:149–160. [PubMed: 28683309]
7. Ben-Shem A, Jenner L, Yusupova G, Yusupov M. Crystal Structure of the Eukaryotic Ribosome. *Science.* 2010; 330:1203–1209. [PubMed: 21109664]
8. Khatter H, Myasnikov AG, Natchiar SK, Klaholz BP. Structure of the Human 80S Ribosome. *Nature.* 2015; 520:640–645. [PubMed: 25901680]

9. Anger AM, Armache JP, Berninghausen O, Habeck M, Subklewe M, Wilson DN, Bechmann R. Structures of the Human and Drosophila 80S Ribosome. *Nature*. 2013; 497:80–85. [PubMed: 23636399]
10. Wong W, Bai XC, Brown A, Fernandez IS, Hanssen E, Condrón M, Tan YH, Baum J, Scheres SH. Cryo-EM Structure of the Plasmodium falciparum 80S Ribosome Bound to the Anti-protozoan Drug Emetine. *eLife*. 2014; doi: 10.7554/eLife.03080
11. Liu Z, Gutierrez-Vargas C, Wei J, Grassucci RA, Sun M, Espina N, Madison-Antenucci S, Tong L, Frank J. Determination of the Ribosome Structure to a Resolution of 2.5 Å by Single-Particle Cryo-EM. *Protein Sci*. 2017; 26:82–92. [PubMed: 27750394]
12. Greber BJ, Boehringer D, Leibundgut M, Bieri P, Leitner A, Schmitz N, Aebersold R, Ban N. The Complete Structure of the Large Subunit of the Mammalian Mitochondrial Ribosome. *Nature*. 2014; 515:283–286. [PubMed: 25271403]
13. Bieri P, Leibundgut M, Saurer M, Boehringer D, Ban N. The Complete Structure of the Chloroplast 70S Ribosome in Complex with Translation Factor pY. *EMBO J*. 2017; 36:475–486. [PubMed: 28007896]
14. Noller HF. RNA Structure: Reading the Ribosome. *Science*. 2005; 309:1508–1514. [PubMed: 16141058]
15. Moore PB. Structural Biology. A Ribosomal Coup: E. coli at Last! *Science*. 2005; 310:793–795. [PubMed: 16272105]
16. Bahadur RP, Zacharias M, Janin J. Dissecting Protein-RNA Recognition Sites. *Nucleic Acids Res*. 2008; 36:2705–2716. [PubMed: 18353859]
17. Ellis JJ, Broom M, Jones S. Protein-RNA Interactions: Structural Analysis and Functional Classes. *Proteins*. 2007; 66:903–911. [PubMed: 17186525]
18. Gullion T, Schaefer J. Rotational-Echo Double-Resonance NMR. *J Magn Reson*. 2009; 213:413–417.
19. Cegelski L. REDOR NMR for Drug Discovery. *Bioorg Med Chem Lett*. 2013; 23:5767–5775. [PubMed: 24035486]
20. Yu TY, Schaefer J. REDOR NMR Characterization of DNA Packaging in Bacteriophage T4. *J Mol Biol*. 2008; 382:1031–1042. [PubMed: 18703073]
21. Romaniuk JAH, Cegelski L. Bacterial Cell Wall Composition and the Influence of Antibiotics by Cell-Wall and Whole-Cell NMR. *Phil Trans R Soc B*. 2015; doi: 10.1098/rstb.2015.0024
22. Nygaard R, Romaniuk JAH, Rice DM, Cegelski L. Spectral Snapshots of Bacterial Cell-Wall Composition and the Influence of Antibiotics by Whole-Cell NMR. *Biophys J*. 2015; 108:1380–1389. [PubMed: 25809251]
23. Rice DM, Romaniuk JAH, Cegelski L. Frequency-Selective REDOR and Spin-Diffusion Relays in Uniformly Labeled Whole Cells. *Solid State Nucl Magn Reson*. 2015; 72:132–139. [PubMed: 26493462]
24. Zhou X, Cegelski L. Nutrient-Dependent Structural Changes in *S. aureus* Peptidoglycan Revealed by Solid-State NMR Spectroscopy. *Biochem*. 2012; 51:8143–8153. [PubMed: 22974326]
25. Nierhaus KH. Structure, Assembly, and Function of Ribosomes. *Curr Top Microbiol Immunol*. 1982; 97:81–155. [PubMed: 7035074]
26. Trauner A, Bennett MH, Williams HD. Isolation of Bacterial Ribosomes with Monolith Chromatography. *PLoS ONE* [Online]. 2011; 6:2. [accessed June 30, 2017] <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0016273>.
27. Schaefer J, Stejskal EO. C-13 Nuclear Magnetic-Resonance of Polymers Spinning at Magic Angle. *J Am Chem Soc*. 1976; 98:1031–1032.
28. Kovacs FA, Fowler DJ, Gallagher GJ, Thompson LK. A Practical Guide for Solid-state NMR Distance Measurements in Proteins. *Concepts Magn Reson*. 2007; 30A:21–39.
29. McDowell LM, Schmidt A, Cohen ER, Studelska DR, Schaefer J. Structural Constraints on the Ternary Complex of 5-enolpyruvylshikimate-3-phosphate Synthase from Rotational-Echo Double-Resonance NMR. *J Mol Biol*. 1996; 256:160–171. [PubMed: 8609607]

Figure 1. Whole Ribosome NMR

(A) View of the ribosome crystal structure (PDB 4V4Q),⁴ highlighting lysines in cyan and ϵ -nitrogen atoms of all the lysines as cyan spheres. SDS-PAGE characterization verified integrity of the uniformly ^{15}N -labeled ribosome preparation, with lanes: M (markers), 1 (Ribosome standard, New England Biolabs), 2 (^{15}N -labeled ribosomes), 3 (1:10 dilution of ^{15}N -labeled ribosomes). (B) N{P} REDOR spectra obtained for a 16 ms evolution time reveals percent dephasing of nitrogens near to phosphorous. (C) Distribution of lysyl amine to phosphorous contacts for lysines present in the ribosome crystal structure. (D) REDOR S/S_0 values for 4.48 ms and 16 ms evolution times are 0.05 ± 0.03 and 0.263 ± 0.009 , respectively, and fit well to the calculated REDOR curve corresponding to the distribution of binned distances from panel C (solid black). Representative single distance REDOR curves are provided for comparison (dashed).

Figure 2. Whole Ribosome and Whole Cell NMR Comparison

(A) ^{15}N CPMAS spectral overlays of purified ribosomes and the whole cells from which they were isolated (normalized to the nitrogen peptide peak at 120 ppm) demonstrate the dramatic differences in the nitrogen chemical composition of ribosomes versus that of whole cells (left). Scaling the ribosome spectrum to approximately match the whole-cell 147 and 170 peaks, given that the ribosome nitrogen peaks should be the dominant contributors in these regions, yielded a ribosome spectrum with 25% the spectral area of the whole-cell spectrum (right). (B) A ^{13}C CPMAS spectral overlay of ribosomes and whole cells (normalized by the carbonyl peak height at 176 ppm) similarly reflects the dramatic carbon compositional differences, noting the enhanced ribosome intensity in aromatic and sugar carbons with respect to the carbonyl peak.