New and Notable

A New Understanding of Antibiotic Action via Solid-State NMR of Cells with Uniform Isotopic Labeling

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In this issue of the *Biophysical Journal*, Nygaard et al. (1) describe an exciting new solid-state NMR approach to probe the composition of whole bacterial cells and their cell-wall fractions. Use of uniform ^{13}C and ^{15}N labeling as well as magic-angle spinning and rotational-echo double-resonance (REDOR) selection of either Gly or non-Gly ^{13}C signals allows for NMR detection of many chemical functionalities including polysaccharide and protein (2–5). In my view, the most exciting result is the striking differences among unfiltered ^{13}C NMR spectra of whole bacterial cells grown with different antibiotics, as displayed in Fig. 7 in the article. Relative to control cells that were not treated with antibiotic, cells treated with fosfomycin antibiotic have a significantly smaller ratio of polysaccharide/protein ^{13}C signal intensities whereas cells treated with chloramphenicol have a significantly greater ratio. This correlates with the known inhibition of cell-wall synthesis by fosfomycin and protein synthesis by chloramphenicol. It is therefore likely that this new method can rapidly and quantitatively discern the general mechanism of action of a new antibiotic. This should be very useful in development and comparison of new antibiotics.

The article by Nygaard et al. (1) builds on earlier whole-cell solid-state NMR studies by Cegelski et al. (6) and Kim et al. (7) with more selective isotopic labeling. The binding modes and mechanisms of action of antibiotics such as oritavancin were elucidated in this work. Other applications of whole-cell solid-state NMR include quantitation and analysis of folding of recombinant proteins in inclusion bodies (8,9). The use of uniform labeling and REDOR filtering described in the article by Nygaard et al. (1) highlights the power of solid-state NMR to address important questions in complex biological materials such as bacterial cell walls. There are many problems in medicine and biotechnology for which these approaches should provide great insight with potential application to cell types other than bacteria.

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REFERENCES

1. Nygaard, R., J. A. H. Romaniuk, …, L. Cegelski. 2015. Spectral snapshots of bacterial cell-wall composition and the influence of antibiotics by whole-cell NMR. *Biophys. J.* 108:1380–1389.

2. Gullion, T., and J. Schaefer. 2011. Rotational-echo double-resonance NMR. *J. Magn. Reson.* 213:413–417.

3. Jaroniec, C. P., B. A. Tounge, …, R. G. Griffin. 2001. Frequency selective heteronuclear dipolar recoupling in rotating solids: accurate ^{13}C-^{15}N distance measurements in uniformly ^{13}C,^{15}N-labeled peptides. *J. Am. Chem. Soc.* 123:3507–3519.

4. Bodner, M. L., C. M. Gabrys, …, D. P. Weliky. 2004. Temperature dependence and resonance assignment of ^{13}C NMR spectra of selectively and uniformly labeled fusion peptides associated with membranes. *Magn. Reson. Chem.* 42:187–194.

5. Li, Y., B. J. Wylie, and C. M. Rienstra. 2006. Selective refocusing pulses in magic-angle spinning NMR: characterization and applications to multi-dimensional protein spectroscopy. *J. Magn. Reson.* 179:206–216.

6. Cegelski, L., D. Steuber, …, J. Schaefer. 2006. Conformational and quantitative characterization of oritavancin-peptidoglycan complexes in whole cells of *Staphylococcus aureus* by in vivo ^{13}C and ^{15}N labeling. *J. Mol. Biol.* 357:1253–1262.

7. Kim, S. J., L. Cegelski, …, J. Schaefer. 2008. Oritavancin exhibits dual mode of action to inhibit cell-wall biosynthesis in *Staphylococcus aureus*. *J. Mol. Biol.* 377:281–293.

8. Curtis-Fisk, J., R. M. Spencer, and D. P. Weliky. 2008. Native conformation at specific residues in recombinant inclusion body protein in whole cells determined with solid-state NMR spectroscopy. *J. Am. Chem. Soc.* 130:12568–12569.

9. Vogel, E. P., and D. P. Weliky. 2013. Quantitation of recombinant protein in whole cells and cell extracts via solid-state NMR spectroscopy. *Biochemistry.* 52:4285–4287.

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