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Title: Introducing HR- μ MAS NMR probe for μ g-scale samples

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NMR has already proven to be a tremendous spectroscopic tool in metabolomics. However, due to its low detection sensitivity, NMR analyses can be challenging especially for mass-limited samples. To overcome this issue, here we introduce a 'new' reliable methodology for profiling μ g/nL scale samples based on the Magic Angle Spinning (MAS) technique - High-Resolution μ MAS (HR- μ MAS). HR- μ MAS offers an outstanding opportunity for the detection of metabolites in μ g samples with good sensitivity and high spectral resolution, which could widen the existing NMR-based spectroscopy to metabolomic applications and pave the path to its utilization in numerous fields of study.

In comparison to the previous approach with the spinning μ -coil (HR-MACS) [1], HR- μ MAS [2] renders improvements in probe practicality, sample-preparation, data repeatability and in the flexibility of NMR experiments. Moreover, we also show the possibility of performing metabolic quantification using HR- μ MAS – important analytical component in metabolomics. The excellent NMR spectroscopic capabilities of HR- μ MAS shown here for NMR metabolic profiling on μ g/nL samples have demonstrated that HR- μ MAS could have an impact on future NMR-based metabolomics in biological applications.

[1] Wong, A., Li, X., Molin, L., Solari, F., Elena-Herrmann, B., Sakellariou, D. (2014) μ High Resolution-Magic-Angle Spinning NMR Spectroscopy for Metabolic Phenotyping of *Caenorhabditis elegans*. *Anal. Chem.*, 86, 6064–6070.

[2] Duong, N. T., Endo, Y., Nemoto, T., Kato, H., Bouzier-Sore, A.-K., Nishiyama, Y., & Wong, A. (2016). Evaluation of a high-resolution micro-sized magic angle spinning (HR μ MAS) probe for NMR-based metabolomic studies of nanoliter samples. *Anal. Methods*, 8(37), 6815–6820.

