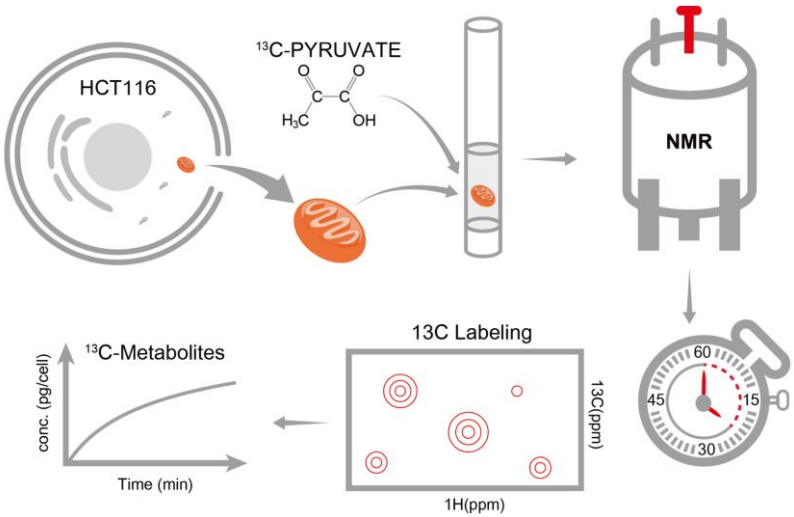
# Real-time Live Metabolomics with 2D Heteronuclear NMR in Hierarchical Biological Systems

[1]Wen Jun Xu, [2]He Wen, [1]Han Sun Kim, [3]Yoon-Joo Ko, [4]Seung-Mo Dong,[4]In-Sun Park, [5]Jong In Yook & [1]Sunghyouk Park*

[1]Natural Product Research Institute, College of Pharmacy, Seoul National University, Gwanak-gu, 151-742 Seoul, Korea; [2]Department of Biochemistry and Molecular Biology, Shenzhen University School of Medicine, 518060 Shenzhen, China; [3]National Center for Inter-University Research Facilities, Seoul National University, Gwanak-gu, 151-742 Seoul, Korea; [4]Department of Anatomy, College of Medicine, Inha University, Nam-gu, 402-751 Incheon, Korea; and [5]Department of Oral Pathology, Oral Cancer Research Institute, College of Dentistry, Yonsei University, Seodaemun-gu, 120-752 Seoul, Korea

Recent studies point out the link between altered metabolism and various diseases, but metabolic monitoring of biological systems have mostly been performed at fixed time points on extracted material. As NMR is a non-destructive technique, we applied heteronuclear 2D NMR to living or functional biological systems. At the protein level, we present a triple resonance NMR-based approach for specific detection of glutaminase activity using stable-isotope labeled glutamine in live cells. Compared to conventional methods involving coupled enzyme assays, the proposed approach is direct because it detects the presence of the H−N−CO amide spin system. The approach was applied to investigating the effects of a glutaminase inhibitor and the inhibitory effects of glucose on glutamine metabolism in live cells. The approach can be extended to other enzymes targeting carbon bonds using HCACO. At the organelle level, we monitored the mitochondrial metabolism in real time. We used 13C pyruvate as a tracer to monitor its metabolism in isolated and functional human mitochondria. We found acetyl phosphate synthesis from pyruvate in live human mitochondria which has been largely forgotten since its first description about 70 years ago in bacteria. The kinetic profile of acetyl phosphate formation was biphasic, and its transient nature suggested its role as a metabolic intermediate. The method also allowed for the estimation of pyruvate dehydrogenase (PDH) enzyme activity through monitoring of the acetylCoA formation, independent of competing cytosolic metabolism. We also detected lactate production from pyruvate in live mitochondria which is modulated by p53, a well-known tumor suppressor. Although lactate production has been known only in cytosol, the mitochondrial formation was confirmed with the recovery of lactate formation upon treatment of specific inhibitor of mitochondrial pyruvate carrier followed by a membrane permeabililzer. At the cell level, the live metabolomic method allowed for metabolomic differentiation between cancer and normal cells on the basis of time dependent changes in metabolite concentrations. Cancer cells were found to have large in- and out-flux of pyruvate as well as increased net production of alanine and acetate. The method also enabled evaluation of the metabolic effects of galloflavin whose anticancer effects have been attributed to its specific inhibition of lactate dehydrogenase. Our approach revealed previously unknown functional targets of galloflavin, which were further confirmed at the protein levels. How the live metabolomic method can be expanded to other hierarchical biological systems will be also presented.




1) Wen H, et al., , Angew Chem Int Ed Engl. 54(18):5374-7 (2015)
2) Lee S, et al., ACS Chem Biol. 11(11):3140-3145 (2016)
3) Jin X, et al , Angew Chem Int Ed Engl. 55(28):7939-42(2016)
4) Xu WJ, et al, Proc. Natl. Acad. Aci. U.S.A., 115(16), 4152-4157 (2018)
5) Bezawork-Geleta et al., Nat Comm., 9(1), 2221 (2018)4, 541 (2015).