Deuterated Lipids Methods Summary

For full details, see the published article. Briefly: 30 mL culture of E coli in Enfors minimal media (deuterated or otherwise), collected by centrifugation at 4k x g. E. coli was adapted to deuterated media over multiple passages. Lipids were extracted from E coli cell pellets following the Matyash protocol with slight variations described in the manuscript supplemental. Cell lysis was performed with bead beating or ultrasonication. Extraction solvents were in the ratio 10:3:25 MTBE:methanol:water. An 87 min reversed phase chromatography method was employed which used the following solvents:

Mobile phase A: 60% acetonitrile, 40% water (4 g/L ammonium acetate)

Mobile phase B: 90% isopropanol, 10% acetonitrile (4 g/L ammonium acetate).

100 um ID column 1.7 um C18 Kinetex (100 A) stationary phase. LTQ-Orbitrap Pro was used. 30k resolution MS1 scans followed by 10 DDA scans with 30 NCE CID. This was the same for both negative and positive polarities. Lipid peaks areas were generated by manual integration in MS-DIAL. For fitting to generate pD values, isotopolouge abundance was unit normalized to the highest abundance isotopolouge. Manual ID was performed based on fragmentation patterns and chromatographic characteristics with MS-DIAL as a QC check. Lipids were identified at the lipid species level, summing the carbons of individual tails.