



Figure 2-Figure Supplement 1: Schematic representation of isotopic spectral analysis (ISA) adapted from Kelleher et al (Kelleher and Masterson, 1992). The fractional abundance of each sterol peak in an unlabeled and labeled sample can be used to calculate the rate of newly synthesized sterols. In the unlabeled sample, the fraction abundance of M=0 is 0.74 (we will call this $M0_n$); for the labeled sample, it is 0.23 ($M0_t$). If we sample a sterol as the pool is turning over, the fractional abundance of M=0 ($M0_m$) will decrease from 0.74 to 0.23. The corresponding values of $M2_m$ will increase from 0.04 to 0.29. Using this data, the fraction of molecules that are newly synthesized (g) can be estimated using isotopic spectral analysis (ISA). Using ISA, the value of $M0_m$ has two components: 1) $M0_n$ times the fraction of molecules not labeled ($1-g$). 2) $M0_t$ times the fraction of molecules newly synthesized (g). These two values can be summed to get the value of $M0_m$. This same analysis can be done for any of the peaks. Then, by solving for g , the fraction of molecules newly synthesized can be determined based on $M0_m$, $M0_n$, and $M0_t$. $M0_m$ is the measured value that is obtained from MS. $M0_n$ is determined from an unlabeled sample or from a natural abundance calculator. $M0_t$ can be determined from samples exposed to label long enough to ensure turnover of the entire pool, or using mass isotopomer distribution analysis (MIDA) (Hellerstein and Neese, 1999). The biosynthetic rate was calculated by fitting the relationship between g and time to a first-order kinetic model. The rate constant was multiplied by the concentration, yielding the biosynthetic rate, as demonstrated in Figure 2B.