The overall under incorporation rate (UI) is calculated by taking the ratio of the area of peaks that correspond to under labeling to the total area of all isotopic peaks resulting from one glycan stucture. (as shown in heavy spectrum of Fig. 3) For example, in heavy spectrum of Fig. 3, the area of isotopic peak (labeld as $^{13}C_0{}^{15}N_0$, $^{13}C_0{}^{15}N_1$, $^{13}C_0{}^{15}N_2$, $^{13}C_1{}^{15}N_2$, $^{13}C_2{}^{15}N_2$, $^{13}C_3{}^{15}N_2$, $^{13}C_4{}^{15}N_2$) is A_i (i=1, 2, 3, 4, 5, 6, 7), so the overall under incorporation rate (UI) can be calculated as:

$$UI = \frac{\sum_{i=1}^{2} A_i}{\sum_{i=1}^{7} A_i}$$

For any glycan structure, if the number of nitrogens in the molecule is K and the number of isotopic peaks shown in the spectrum is M,

$$UI = \frac{\sum_{i=1}^{K} A_i}{\sum_{i=1}^{M} A_i}$$

And the incorporation rate for eack nitrogen can be calculated as:

$$Incorporation = \sqrt[K]{(1 - UI)}$$

The incorporation is 95% for the O-glycans we have shown for the quantifications.

To calculate the ratios of heavy to light glycan based on the spectrum of a mixture of two cell populations, we first generate the theoretical isotopic patterns for the light structure by a software called “emass” written by the Somerharju Lipid Group, University of Helsinki. (http://www.helsinki.fi/science/lipids/software.html) For example, in Fig. 6B, there are 7 isotopic peaks observed in the spectrum and the area of each peak is A_1 through A_7, repectively. Due to the peak overlapping and under incorporation, both of the light and heavy structures can contribute to the area of each peak. So if the actual area resulting from light structure is L_1 throgh L_7, from heavy structure is H_1 through H_7, then we have:

$L_1 + H_1 = A_1$ (1)

$L_2 + H_2 = A_2$ (2)

$L_3 + H_3 = A_3$ (3)

$L_4 + H_4 = A_4$ (4)

$L_5 + H_5 = A_5$ (5)

$L_6 + H_6 = A_6$ (6)

$L_7 + H_7 = A_7$ (7)

If the ratio of theoretical isotopic pattern for the 7 peaks from the light structure is $P_1(=1):P_2:P_3:P_4:P_5:P_6:P_7$, which we can generate by using the software “emass”, we can have:

$L_1 = L_1 * P_1 (P_1 = 1)$ (8)

$L_2 = L_1 * P_2$ (9)

$L_3 = L_1 * P_3$ (10)

$L_4 = L_1 * P_4$ (11)

$L_5 = L_1 * P_5$ (12)

$L_6 = L_1 * P_6$ (13)

$L_7 = L_1 * P_7$ (14)

By the definition of under incorporation rate we gave before,

$$UI = \frac{\sum_{i=1}^{3} Hi}{\sum_{i=1}^{7} Hi} \quad (15)$$

The value of UI can be determined based on the spectrum for heavy labeled glycans (not shown). Given that $H_i = A_i - L_i$, equaiton 15 can be rearranged, with a substitution of H_i into A_i and L_i according to eqution 1 – 14, to yield equation 16:

$$L_1 = \frac{A_1 + A_2 + A_3 - UI^* \sum_{i=1}^{7} A_i}{P_1 + P_2 + P_3 - UI^* \sum_{i=1}^{7} P_i} \quad (16)$$

As far as we know L_1, we can calculate L_i (i=2-7) and H_i (i=1-7) based on equations 1-14. The equation for Heavy/Light ratio will be:

$$\frac{\text{Amide-}^{15}\text{N-GLN}}{\text{Amide-}^{14}\text{N-Gln}} = \frac{\sum_{i=1}^{7} H_i}{\sum_{i=1}^{7} L_i} \quad (17)$$

For any glycan structure, if the number of nitrogens in the molecule is K and the number of isotopic peaks shown in the spectrum of light/heavy mixture is M, the equations 16 and 17 can be rewritten as

$$L_1 = \frac{\sum_{i=1}^{K} A_i - UI^* \sum_{i=1}^{M} A_i}{\sum_{i=1}^{K} P_i - UI^* \sum_{i=1}^{M} P_i} \quad (18)$$

$$\frac{\text{Amide-}^{15}\text{N-GLN}}{\text{Amide-}^{14}\text{N-Gln}} = \frac{\sum_{i=1}^{M} H_i}{\sum_{i=1}^{M} L_i} \quad (19)$$