**Computationally Generating Phosphatidylserine Formulas**

To compensate for the incompleteness of existing lipid databases, a computational approach was employed to generate a near-exhaustive set of chemically feasible phosphatidylserine lipids. As this list was to be used exclusively for MS1-based annotations our algorithm did not generate or consider in any way the structures of the generated lipids but instead generated only the molecular formula, their corresponding neutral masses, and the ‘[M-H+e]’ adduct masses for every computationally generated lipid. For mass calculations every atom was assumed to be the most abundant stable isotope for that element and the masses were computed using the python mpmath library. Isotope masses and electron masses were taken from NIST.

Our algorithm uses the molecular formula for the PS headgroup as a base and generates potential lipids by simulating the addition of -CH2- to the side chains as well as the removal of 2 H to form a double bond. The number of added -CH2- groups is bounded by the *m/z* range of the MS1 acquisitions assuming a charge of z=-1 (i.e., 1000 amu) while the number of double bonds possible is bounded by the number of added -CH2- groups. The headgroup formula was modified accordingly to generate LPS, PS O-, and LPS O- variants of the PS lipids. All generated formulas were compared against an in-lab target list of known PS lipids and variants to ensure the accuracy of our generated formulas.

**Computational PS Formula Search**

The full feature table was annotated using the computationally generated PS lipids as follows. First, an *m/z-*only search is performed for the M-H+e adduct of every computationally generated lipid. A 5-ppm cutoff is used for this search. Second, if a match is found (hereafter referred to as the m+13C0 match) , the m+13C isotopologues of this formula are then generated in increasing order of 13C count (e.g., m+13C1 then m+13C2, etc.). These isotopologues are then searched against the feature list using their *m/z* values. Any possible matches are then further filtered to eliminate matches that did not co-elute with the m+13C0 feature. A slight buffer equal to rtime minus rtime\_left\_base on the left and rtime\_right\_base minus rtime on the right ensures that minor retention time shifting will not result in lost assignments. Isotopologues are also checked to ensure that in at least 90% of samples, the more heavily isotoped isotopologue is less intense than the previously identified isotopologue (i.e., the intensity of m+13C2 must be less than m+13C1 in at least 90% of samples). This intensity check minimizes confusion between m+13Cn isotopologues and various degrees of unsaturation between lipids when n is even. This isotopologue search continues until no such match is found. Since less abundant lipids will have fewer detectable isotopologues, using the number of observed isotopologues to measure the certainty of an assignment is not straightforward; however, this number of detected isotopologues provides subjective circumstantial evidence that an assignment is plausible.