

## CLAIMS

1. A method of determining a concentration of a target glycopeptide analyte in a sample, the method comprising:
  - measuring an abundance for a non-glycosylated endogenous peptide (NGEP) of a sample;
  - determining a corrected NGEP abundance for the NGEP using an external standard;
  - measuring an internal standard abundance for an internal standard having a known internal standard concentration;
  - determining an NGEP concentration for the NGEP as a function of the corrected NGEP abundance, the known internal standard concentration, and the internal standard abundance;
  - determining a normalized abundance of a target glycopeptide analyte of the sample as a function of a measured abundance of the target glycopeptide analyte and the measured abundance of the NGEP on the same protein as the target glycoprotein analyte; and
  - determining a target glycopeptide analyte concentration as a function of the NGEP concentration and the normalized abundance.
2. The method of claim 1, wherein the NGEP concentration comprises a product of a ratio of the corrected NGEP abundance and the internal standard abundance and the known internal standard concentration.
3. The method of any one of claims 1-2, wherein the normalized abundance is determined when one or two glycans are identified at a target glycosylation site on the protein.
4. The method of any one of claims 1-3, wherein:
  - the target glycopeptide analyte concentration is a product of the NGEP concentration and the normalized abundance; and
  - the normalized abundance is the quotient of the first measured abundance and the second measured abundance.

5. A method for preparing samples for a liquid chromatography/mass spectrometry (LC-MS) sample run and normalizing abundance data of a plurality of sample runs, the method comprising:

preparing run samples for the plurality of sample runs for LC-MS analysis, the preparing comprising:

generating a first set of run samples, each of the first set of run samples including an external standard; and

generating a second set of run samples, each of the second set of run samples including at least two target glycopeptide analytes and an internal standard;

combining at least two run samples of the first set of run samples to create a pooled standard;

analyzing each run sample according to a run order, wherein the run order specifies a relative order of analysis for the run samples of the sample run; and

normalizing the raw abundance data of the plurality of sample runs using the pooled standard of each sample run as a reference.

6. The method of claim 5, wherein a first run sample of the first set of run samples occupies an adjacent position in the run order to a second run sample of the second set of run samples.

7. The method of claim 5, wherein a run sample of the first set of run samples occupies a position in the run order before all of the run samples of the second set of run samples.

8. The method of claim 5, wherein a run sample of the first set of run samples occupies a position in the run order after all of the run samples of the second set of run samples.

9. The method of claim 5, wherein a run sample of the first set of run samples occupies one position for at least every five positions in the run order.

10. The method of claim 5, wherein the run order comprises a single run sample of the first set of run samples positioned at least every fifteen positions in the run order.

11. The method of claim 5, wherein the run order comprises a single run sample of the first set of run samples followed by a range of between two and twenty-nine run samples of the second set of run samples.

12. The method of claim 5, wherein the run order comprises a single run sample of the first set of run samples followed by a range of between four and fourteen run samples of the second set of run samples.

13. The method of any one of claims 5-12, further comprising:  
positioning at least one run sample comprising BSA before all the run samples of the first set of run samples and the second set of run samples in the run order.

14. The method of any one of claims 5-13, further comprising:  
positioning at least one run sample comprising BSA after all the run samples of the first set of run samples and the second set of run samples in the run order; and  
positioning at least one blank run sample before a run sample of the first set of run samples or the second set of run samples in the run order.

15. The method of any one of claims 5-14, wherein the step of preparing further comprises:  
enzymatically digesting glycoprotein structures of the run samples to generate the external standard of each run sample of the first set of run samples and the internal standard and the at least two target glycopeptide analytes of each run sample of the second set of run samples.

16. The method of claim 15, further comprising:  
normalizing raw abundance data generated from each of the at least two target glycopeptide analytes of a same run sample of the second set of run samples using the internal standards.

17. The method of any one of claims 5-16, wherein the external standard comprises a non-glycosylated endogenous peptide (NGEP).

18. The method of any one of claims 5-17, wherein:  
each of the at least two target glycopeptide analytes has a corresponding internal standard for a reference;

the internal standard of at least one run sample of the second set of run samples is a surrogate; and

a source of the external standard includes serum.

19. The method of any one of claims 5-18, wherein an external standard of the first set of run samples includes at least one pooled plasma digest when a run sample in the second set of runs samples includes a patient plasma sample.

20. The method of claim 19, wherein the patient plasma sample includes a target analyte.

21. The method of any one of claims 5-20, wherein the concentration of the external standard and the pooled standard is unknown prior to analyzing.