Supplemental File 2. IEMM flowchart: Pseudo-code of algorithm for deamidation quantification using isotopic envelope modeling method is listed below.

```
For each peptide containing N or Q reported by SEQUEST in a SCX fraction

    numberOfPeptideForms = count(unModifiedForm+number of deamidations in the
    peptide)

    baseMass = m/z(unmodified form of peptide)

    basePeptide = unmodified form of peptide

    For each charge state detected for the peptide

        generateIonChromatogram(baseMass, z)
    End

    Display ion chromatograms for all charge states, wait for user to select integration
    retention time bounds

    For each charge state (z) detected for the peptide

        averagedMassSpectrum = generateAveragedMassSpectrum(retentionTimeBounds,
                               baseMass, z)

        IEMM = guessAnIEMM(basePeptide, z, averagedMassSpectrum)

        optimizedIEMM = optimizeGuessedIEMM(IEMM, averagedMassSpectrum)

        Generate predicted mass spectrum using optimizedIEMM and superimpose on
        averagedMassSpectrum
    End
End

Procedure generateIonChromatogram(baseMass, z)

    lowMass = baseMass; highMass = baseMass+numberOfPeptideForms/z;

    Generate a compound ion chromatogram (CIC) with masses between lowMass and
    highMass and smooth it by applying Savitzky-Golay filter (3rd degree, 9 seconds window
    width, 2 cycles)
End Procedure

Procedure generateAveragedMassSpectrum(retentionTimeBounds,baseMass, z)

    Average MS scans between retentionTimeBounds

    lowMass = baseMass–5.0; highMass = baseMass+numberOfPeptideForms/z+5.0;

    Display averaged mass spectrum between lowMass and highMass

    Return generated mass spectrum
End Procedure
```

Procedure guessAnIEMM(basePeptide, z, averagedMassSpectrum)

Generate predicted isotopic envelopes (PIEs) for all peptide forms (7 isotopic peaks each)

Set fittingInterval as lowestMassInAllPIEs-2.0 and highestMassInAllPIEs+2.0

Smooth peaks in averagedMassSpectrum by applying Savitzky-Golay filter (3^{rd} degree, 0.09 Da window width, 2 cycles) and find peaks above 5% of the baseline intensity

Match predicted isotopic peak positions to averagedMassSpectrum using centroided mass values

Determine peak position parameter ($\overline{X}$) by solving equation 2 using top 2 most intense matched peaks of predicted and measured isotopic envelope

Determine peak width parameter (σ) by solving equation 4 using top 2 most intense matched peaks in measured isotopic envelope

For each peptide form (j)

Determine monoisotopic peak intensity from averagedMassSpectrum

Subtract intensity contribution due to the isotopic peaks of prior forms and set the resultant intensity divided by 4 as I_j

End

determineBackground(averagedMassSpectrum, fittingInterval)

Generate a composite IEMM function (shown in eq. 1) for all peptide forms using initial parameters

Return generated composite IEMM

End Procedure

Procedure determineBackground(averagedMassSpectrum, fittingInterval)

Slice the averagedMassSpectrum between fittingInterval into 0.5 Dalton wide bins

Collect lowest intensity valley in each bin and fit a cubic polynomial through the set

End Procedure

Procedure optimizeGuessedIEMM(IEMM, averagedMassSpectrum)

Formulate a least squares function between IEMM and raw averagedMassSpectrum within fittingInterval (see FCNBase data structure in Minuit documentation provided in the Methods section of the manuscript)

Lock higher order background parameters of the IEMM

optimizedIEMMParameters = performLeastSquaresMinimization (leastSquaresFunction, IEMMParameters)

```
        Release the lock on higher order background parameters and lock peak parameters
        in the optimizedIEMMParameters

        finalIEMMParameters = performLeastSquaresMinimization (leastSquaresFunction,
                                                               optimizedIEMMParameters)

        Return optimized IEMM (finalIEMMParameters)
End Procedure


Procedure performLeastSquaresMinimization(leastSquaresFunction, IEMMParameters)

        // Start comment block
                Please see the Minuit documentation referenced in the Methods section
                of the manuscript for details on MnStrategy, MnFunctionParameters,
                and MnMiGrad data structures
        // End comment block

        Set the minimization strategy to medium using MnStrategy data structure of Minuit

        Set IEMMParameters as the parameters to be optimized using MnFunctionParameters

        Call the MnMiGrad function minimizer using the leastSquaresFunction and the
        IEMMParameters

        Return optimized IEMMParameters determined by the function minimizer
End Procedure
```