**This will become the README file for the Github repository containing the MATLAB version of the isotopocule data corrections.**

**Size correction**

1. Open .xls file containing Isodat output. Note the two tabs at the bottom; one sheet contains raw data for each sample, the other contains raw data for the designated reference peak for each sample.
2. In the ‘sample’ sheet, bring all fragment data in line, then delete extra rows. Do the same on ‘standard’ sheet.
3. Copy the raw sample data from columns A-O in the raw data sheet into columns A-O in the correction template “00\_excel\_template.xlsx”). Copy the the ‘rR’ columns from the raw standard sheet (columns M, N, O) into columns Q, R, S in the correction template. Save with a new name that is specific to your dataset.
4. Check to make sure all references in columns Y, Z, and AA and AC, AD, and AE are correct. **Note that the 31R, 45R, and 46R in columns V-X are specific to the Casciotti lab’s N2O reference gas. Note also that the size correction in columns AC-AE is specific to the linearity of the Casciotti Lab Delta V.**

**Calibrating your instrument for scrambling**

*Open “N2OSPcalcs.m”. You will note that on line 33, two coefficients are defined to describe scrambling at the ion source. This is described in further detail in Frame and Casciotti, 2010 and Kelly et al. (in prep). Below is a brief summary of the method for the determination of these scrambling coefficients.*

1. Run two reference gases with known 15R-alpha and 15R-beta, prepared in the same format as samples(i.e., some amount of N2O reference gas injected into a bottle of seawater or DI water that has been pre-purged with He or N2 gas). Process and size-correct these data in the excel correction spreadsheet, as in steps 1-4 above.
2. Open “00\_excel\_template.xlsx”. Copy and paste the size-corrected 31R, 45R, 46R (columns AG, AH, and AI) from the size\_correction tab into the scrambling\_input tab, columns C-E.
3. Re-organize the size corrected data into pairs of reference materials by copy-pasting into columns H-M of the scrambling input template. The columns should be in the following order: 31R, 45R, 46R for reference #1, then 31R, 45R, 46R for reference #2.
4. Copy-paste columns H-M in the input template into their own .csv file, with no heading. Save the .csv file into the same directory as the Matlab scrambling scripts.
5. Open “constants.m.” Note that we specify a dictionary of reference materials in lines 28-32. If the reference materials used to calibrate scrambling are not in this dictionary, add them, following the format of the existing lines.
6. Open “automate\_gk\_setinputs.m”. Change line 21 to reflect your input filename. This should read something like:

inputfilename = 'example\_atm\_s2.csv';

1. Change line 26 to reflect what you would like the output filename to be. This should read something like:

outputfilename = 'example\_atm\_s2\_output.txt';

1. Change lines 30 and 31 to reflect the two reference materials you will use to calibrate scrambling. This should read something like:

ref1 = 'ATM';

ref2 = 'S2';

1. Run automate\_gk\_setinputs.m.
2. Open your output file and copy-paste the two columns back into the scrambling input template. The first column will be the gamma values and the second column will be the kappa values for each pair of samples. These should all be quite similar.

**Correcting raw Isodat files for isotopomers**

1. Copy and paste columns AG, AH, and AI (size corrected R’s) into a separate spreadsheet, making sure to ‘paste special’, ‘values’. This will become your matlab input file. Make sure your data columns are in the following order: 31R, 45R, 46R, then remove the headings. Save input file in .csv format, e.g. **example\_input.csv**, into the appropriate directory.
2. Open N2OSPcalcs.m in Matlab. Enter your input file name on line 21. It should read something like:

inputfilename = 'example\_input.csv';

1. Enter your desired output file name on line 27. This will overwrite any existing file with the same name, so be sure to change the output file name each time. It should read something like:

outputfilename = 'example\_output.txt';

1. Finally, modify line 33 to reflect the most appropriate scrambling coefficients for this set of samples (see below). It should read something like:

gamma\_kappa = [0.171 0.079];

1. Run N2OSPcalcs
2. Open your output file (**example\_output.txt** in this example). Output data contains columns in the following order: d15Nalpha, d15Nbeta, 15N site pref, d15Nbulk, d17O, d18O. Çopy and paste output data back into working spreadsheet in olive-highlighted cells (columns AK-AI)
3. Note that a scale decompression may be applied after the isotopomer calculation. This is calculated in the “scale-decompression” tab of the excel worksheet, and is applied in columns AU-AZ of the size\_correction tab.

**Calculating concentrations of 44N2O, 45N2O-alpha, 45N2O-beta, and 46N2O**

1. Calculate the amount (nmol) of 44N2O in your sample from the ratio of mass 44 peak area to N2O (nmol/Vs) for your instrument
2. Use the weight difference of the bottle pre- and post-analysis to determine the volume of sample run, and thus the concentration of 44N2O
3. We calculate the *concentrations* of 45N2O-alpha, 45N2O-beta, and 46N2O (for tracer experiments) from the delta value and associated 15/14 ratios