COMMENT AND REPLY

Reply to the Comment on:

"An Efficient Method to Calculate the Aggregated Isotopic Distribution and Exact Center-Masses" by Jürgen Claesen, Piotr Dittwald, Tomasz Burzykowski, Dirk Valkenborg, *J. Am. Soc. Mass Spectrom.* **23, 753–763 (2012)**

We would like to thank Professor Böcker for pointing out the omission of the SIRIUS software in the comparisons presented in our paper [1]. Indeed, we did miss this method as it was not published in a mass-spectrometry-related journal, but as a manuscript in the Gene Expression section of the Bioinformatics journal. We do appreciate that Professor Böcker supplemented our paper by reporting the results of a comparison of SIRIUS and our method. It is reassuring that the results indicate a comparable performance of SIRIUS and BRAIN in terms of accuracy and running times.

The very small differences in the theoretical average mass of molecules (6), (8), (9) and (10), pointed out by Professor Böcker, are due to a wrongly reported Table 1 in our article [1]. We mistakenly assumed that the NIST [2] values used by IsoDalton [3] were identical to the IUPAC 1997 standard (Table 1). However, this confusion did not influence the comparison between BRAIN and the other methods reported in our paper, as all the abundances and masses in our comparison were changed to the values used by IsoDalton [3].

In fact, the reported differences in average mass illustrate the sensitivity of the isotopic distribution calculations to the used mass and abundance probability values of the elemental isotopes. Even for very small differences in mass between NIST and IUPAC1997, the isotopic distribution and mass-centers of large molecules (heavier than 50 kDa, say) are influenced. For larger fluctuations of the masses and/or abundances of elemental isotopes, the effect on the isotopic distribution is even more pronounced and, in some cases, becomes troubling, as illustrated in Tables 2 and 3. The tables

Table 1. Masses and Abundances of NIST Used by IsoDalton and the IUPAC1997 Standard. Differences are Indicated in Bold

Isotope	Mass (ma/u) NIST	Abundance (%) NIST	Mass (ma/u) IUPAC1997	Abundance (%) IUPAC1997
1H	1.0078250321	99.9885	1.0078250321	99.9885
2H	2.0141017780	0.0115	2.0141017780	0.0115
12C	12	98.93	12	98.93
13C	13.0033548378	1.07	13.0033548378	1.07
14N	14.0030740052	99.632	14.0030740052	99.632
15N	15.0001088984	0.368	15.0001088984	0.368
16O	15.9949146	99.757	15.9949146	99.757
17O	16.9991315	0.038	16.9991312	0.038
18O	17.9991604	0.205	17.9991603	0.205
32S	31.97207069	94.93	31.97207070	94.93
33S	32.97145850	0.76	32.97145843	0.76
34S	33.96786683	4.29	33.96786665	4.29
36S	35.96708088	0.02	35.96708062	0.02

Table 2. Differences Between the Theoretical Mono-Isotopic Mass According to Isotopic Masses and Abundances Used by BRAIN and IsoDalton and by the Other Algorithms

	BRAIN and IsoDalton (NIST)	IUPAC1997	IsoPro	Mercury and Emass	NeutronCluster
1	1045.534515	0.000000	0.000107	0.000063	0.000110
2	5729.600867	0.000000	0.000601	0.000335	0.000618
3	11616.849350	0.000000	0.001227	0.000711	0.001259
4	16812.954775	0.000000	0.001853	0.001064	0.001902
5	45387.007033	0.000000	0.004910	0.002760	0.005049
6	66389.862474	−0.000001	0.007203	0.004035	0.007402
7	112823.879546	−0.000001	0.012161	0.006926	0.012493
8	186386.799265	0.000000	0.019265	0.011225	0.019812
9	398470.366994	−0.000002	0.043930	0.023847	0.045118
10	533403.475090	−0.000001	0.058168	0.033063	0.059703

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Table 3. Differences Between the Theoretical Average Masses According to Isotopic Masses and Abundances Used by BRAIN and IsoDalton and by the Other Algorithms

	BRAIN and IsoDalton (NIST)	IUPAC1997	IsoPro	Mercury and Emass	NeutronCluster
1	1046.181107	0.000000	−0.009008	−0.020371	−0.103529
2	5733.510759	0.000000	−0.035371	−0.095561	−0.544411
3	11624.448751	0.000000	−0.075947	−0.203052	−1.102731
4	16823.321352	0.000000	−0.112699	−0.300445	−1.561747
5	45415.679370	0.000000	−0.306794	−0.804546	−4.256819
6	66432.455561	0.000001	−0.431371	−1.151801	−6.207399
7	112895.125932	0.000000	−0.769039	−2.011219	−10.617362
8	186506.052594	0.000001	−1.353590	−3.423114	−17.977714
9	398722.972484	0.000002	−2.691820	−6.911665	−37.143630
10	533735.214651	0.000002	−3.696823	−9.568879	−50.007462

show the differences between the theoretical mono-isotopic masses (Equation 24 in [1]) and theoretical average masses (Equation 25 in [1]) of 10 molecules [1] based upon the default isotope information used by BRAIN [1] and IsoDalton [3] (NIST), the IUPAC1997 standard [4], IsoPro [5], Mercury [6] and Emass [7], and NeutronCluster [8] (Table S1 in [1]).

The differences between the theoretical average masses of molecule (10) vary from 0.000002 Da (IUPAC1997) to 50.007462 Da (isotope information of NeutronCluster), which is no longer ignorable. For extremely large molecules, this will be also the case for the mono-isotopic masses.

By this sensitivity analysis, we can conclude that deviations of the elemental isotope abundance values and/or isotope masses do have a large influence on the average mass of the molecule.

Finally, we also acknowledge that the molecular formula of molecule (7), reported in Table 2 of our article, is missing 40 sulfur atoms. However, the correct atomic composition was used in our calculations.

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References

1. Claesen, J., Dittwald, P., Valkenborg, D., Burzykowski, T.: An efficient method to calculate the aggregated isotopic distribution and exact center-masses. *J. Am. Soc. Mass Spectrom.* **23**(4), 753–756 (2012)
2. NIST Isotope data.
3. Snider, R.K.: Efficient calculation of exact mass isotopic distributions. *J. Am. Soc. Mass Spectrom.* **18**, 1511–1515 (2007)
4. Rosman, K.J.R., Taylor, P.D.P.: Isotopic compositions of the elements 1997. *Pure Appl. Chem.* **70**(1), 217–235 (1998)
5. Senko, M.W.: IsoPro computer program 3.0.
6. Rockwood, A.L., Van Orden, S.L.: Ultrahigh-speed calculation of isotope distributions. *Anal. Chem.* **68**, 2027–2030 (1996)
7. Rockwood, A.L., Haimi, P.: Efficient calculation of accurate masses of isotopic peaks. *J. Am. Soc. Mass Spectrom.* **17**, 415–419 (2006)
8. Olson, M., Yergey, A.: Calculation of the isotope cluster for polypeptides by probability grouping. *J. Am. Soc. Mass Spectrom.* **20**, 295–302 (2009)