Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Comprehensive assessment of measurement uncertainty in ^{13}C based metabolic flux experiments

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Additional files available under 10.1007/s00216-018-1017-7

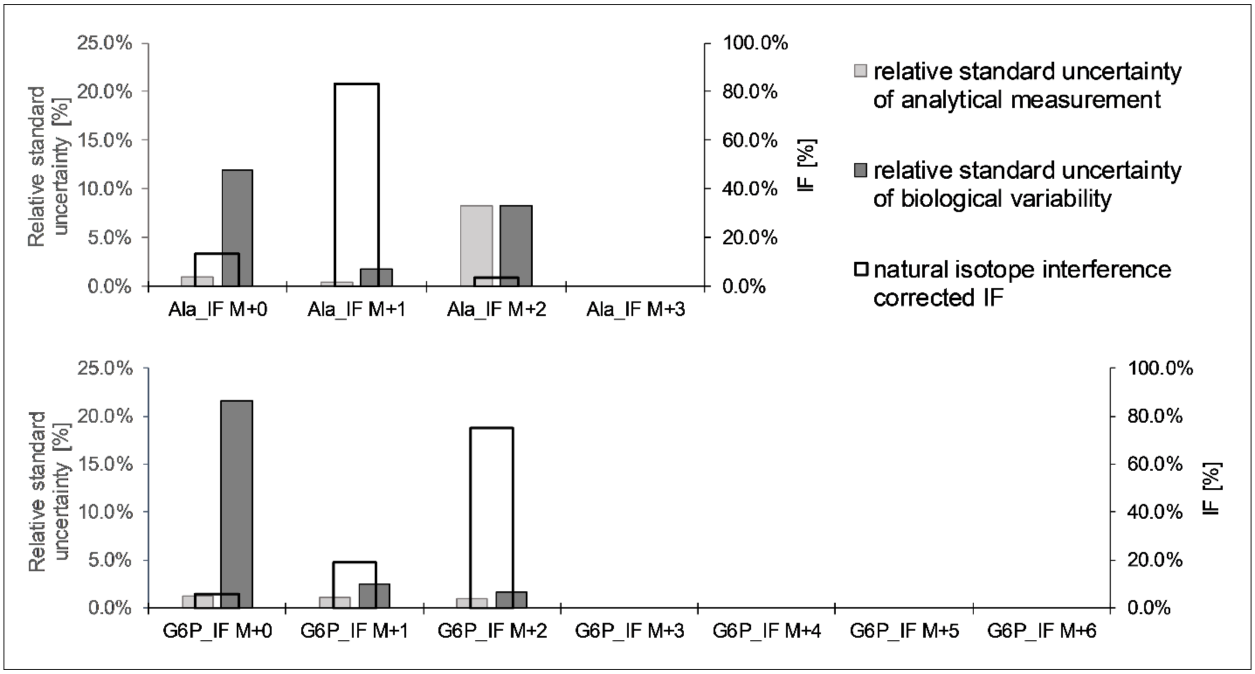

Fig. S1 Exemplary comparison of relative standard uncertainties originating either from the analytical measurement procedure or from the biological variability from glucose-6-Phosphate (G6P) and alanine (Ala). Apart from others, biological variability can be explained by differences in terms homogeneity of the studied cell population, environmental factors as well as metabolite specific turnover rates, making optimal quenching and sampling conditions central. For assessing the biological variability, the following model equation was used: $IF_{M+n\ corr} * f_{bio}$ (f_{bio}= biological factor (distribution = normal, $\bar{x}$= 1, s= specific)). For this purpose, the standard deviation of three biological replicates was assessed, subsequently corrected for the analytical uncertainty and applied as the variation s for the biological factor. To illustrate the dependence on the isotopologue fraction (IF)

Table S1 Equations for isotope interference correction of alanine (Ala) phosphoenolpyruvate (PEP), glyceraldehyde 3-phosphate (GAP), dihydroxyacetone phosphate (DHAP), 2-Phosphoglycerat (2PG), 3-Phosphoglycerat (3PG), erythrose-4-phosphate (E4P), ribulose-5-phosphate (Rl5P), ribose-5-phosphate (R5P), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P) and seduheptulose-7-phosphate (S7P) are given

Ala: C_3-backbone, to be corrected *sum formula*: C_9 Si_2

isotope inference corrected area of M+ *n* isotopologue	**Equation for correction**
A_0	A_0
$A_{1_{corr}}$	$A_1-(2b*A_0)-(6c*A_0)$
$A_{2_{corr}}$	$A_2-(b^2*A_0)-(2a*A_0)-(2b*A_{1_{corr}})-((6c*2b)*A_0)-(6c*A_{1_{corr}})-(15c^2*A_0)$
$A_{3_{corr}}$	$A_3-(b^2*A_{1_{corr}})-(2ab*A_0)-(2a*A_{1_{corr}})-(2b*A_2)-((6c*b^2)*A_0)-((6c*2a)*A_0)-((6c*2b)* A_{1_{corr}})-(6c*A_{2_{corr}})-((15c^2*2b)*A_0)-(15c^2* A_{1_{corr}})-(20c^3*A_0)$

PEP: C_3-backbone, to be corrected *sum formula*: C_{12} Si_3

isotope inference corrected area of M+ *n* isotopologue	**Equation for correction**
A_0	A_0
$A_{1_{corr}}$	$A_1-(3b*A_0)-(9c-A_0)$
$A_{2_{corr}}$	$A_2-(3b^2*A_0)-(3a-A_0)-(3b-A_{1_{corr}})-((9c*3b)*A_0)-(9c*A_{1_{corr}})-(36c^2*A_0)$
$A_{3_{corr}}$	$A_3-(b^3*A_0)-(6ab*A_0)-(3b^2*A_{1_{corr}})-(3a*A_{1_{corr}})-(3b*A_{2_{corr}})-((9c*3b^2)*A_0)-((9c*3a)*A_0)-((9c*3b)* A_{1_{corr}})-(9c*A_{2_{corr}})-((36c^2*3b)*A_0)-(36c^2*A_{1_{corr}})-(84c^3*A_0)$

GAP/DHAP: C_3-backbone, to be corrected *sum formula* C_{14} Si_3

isotope inference corrected area of M+ *n* isotopologue	**Equation for correction**
A_0	A_0
$A_{1_{corr}}$	$A_1-(3b*A_0)-(11c-A_0)$
$A_{2_{corr}}$	$A_2-(3b^2*A_0)-(3a*A_0)-(3b*A_{1_{corr}})-((11c*3b)*A_0)-(11c*A_{1_{corr}})-(55c^2*A_0)$
$A_{3_{corr}}$	$A_3-(b^3*A_0)-(6ab*A_0)-(3b^2*A_{1_{corr}})-(3a*A_{1_{corr}})-(3b*A_{2_{corr}})-((11c*3b^2)*A_0)-((11c*3a)*A_0)-((11c*3b)* A_{1_{corr}})-(11c*A_{2_{corr}})-((55c^2*3b)*A_0)-(55c^2*A_{1_{corr}})-(165c^3*A_0)$

2PG/3PG: C_3-backbone, to be corrected *sum formula*: $C_{15}\ Si_4$

isotope inference corrected area of M+ *n* isotopologue	**Equation for correction**
A_0	A_0
$A_{1_{corr}}$	$A_1-(4b*A_0)-(12c*A_0)$
$A_{2_{corr}}$	$A_2-(4a*A_0)-(6b^2*A_0)-(4b*A_{1_{corr}})-((12c*4b)*A_0)-(12c*A_{1_{corr}})-(66c^2*A_0)$
$A_{3_{corr}}$	$A_3-(12ab*A_0)-(4a*A_{1_{corr}})-(4b^3*A_0)-(6b^2*A_{1_{corr}})-(4b*A_{2_{corr}})-((12c*4a)*A_0)-((12c*6b^2)*A_0)-((12c*4b)*A_{1_{corr}})-(12c*A_{2_{corr}})-(66c^2*4b)*A_0)-(66c^2*A_{1_{corr}})-(220c^3*A_0)$

E4P: C_4-backbone, to be corrected *sum formula*: $C_{17}\ Si_4$

isotope inference corrected area of M+ *n* isotopologue	**Equation for correction**
A_0	A_0
$A_{1_{corr}}$	$A_1-(4b*A_0)-(13c*A_0)$
$A_{2_{corr}}$	$A_2-(4a*A_0)-(6b^2*A_0)-(4b*A_{1_{corr}})-((13c*4b)*A_0)-(13c*A_{1_{corr}})-(78c^2*A_0)$
$A_{3_{corr}}$	$A_3-(12ab*A_0)-(4a*A_{1_{corr}})-(4b^3*A_0)-(6b^2*A_{1_{corr}})-(4b*A_{2_{corr}})-((13c*4a)*A_0)-((13c*6b2)*A_0)-((13c*4b)*A_{1_{corr}})-(13c*A_{2_{corr}})-((78c^2*4b)*A_0)-(78c^2*A_{1_{corr}})-(286c^3*A_0)$
$A_{4_{corr}}$	$A_4-(b^4*A_0)-(12ab*A_{1_{corr}})-(12ab^2*A_0)-(6a^2*A_0)-(4a*A_{2_{corr}})-(4b^3-A_{1_{corr}})-(6b^2*A_{2_{corr}})-(4b-A_{3_{corr}})-((13c*12ab)*A_0)-((13c*4a)*A_{1_{corr}})-((13c*4b^3)*A_0)-((13c*6b^2)*A_{1_{corr}})-((13c*4b)*A_{2_{corr}})-(13c*A_{3_{corr}})-((78c^2*4a)*A_0)-((78c^2*6b^2)*A_0)-((78c^2*4b)*A_{1_{corr}})-(78c^2*A_{2_{corr}})-((286c^3*4b)*A_0)-(286c^3*A_{1_{corr}})-(715c^4*A_0)$

R5P/RI5P: C_5-backbone, to be corrected *sum formula*: C_{21} Si_5

isotope inference corrected area of M+ *n* isotopologue	**Equation for correction**
A_0	A_0
$A_{1_{corr}}$	$A_1-(5b*A_0)-(16c*A_0)$
$A_{2_{corr}}$	$A_2-(5a*A_0)-(10b^2*A_0)-(5b*A_{1_{corr}})-((16c*5b)*A_0)-(16c*A_{1_{corr}})-(120c^2*A_0)$
$A_{3_{corr}}$	$A_3-(20ab*A_0)-(5a*A_{1_{corr}})-(10b^3*A_0)-(10b^2*A_{1_{corr}})-(5b*A_{2_{corr}})-((16c*5a)*A_0)-((16c*10b^2)*A_0)-((16c*5b)*A_{1_{corr}})-(16c*A_{2_{corr}})-((120c^2*5b)*A_0)-(120c^2*A_{1_{corr}})-(560c^3*A_0)$
$A_{4_{corr}}$	$A_4-(20ab*A_{1_{corr}})-(30ab^2*A_0)-(10a^2*A_0)-(5a*A_{2_{corr}})-(5b^4*A_0)-(10b^3*A_{1_{corr}})-(10b^2*A_{2_{corr}})-(5b*A_{3_{corr}})-((16c*20ab)*A_0)-((16c*5a)*A_{1_{corr}})-((16c*10b^3)*A_0)-((16c*10b^2)*A_{1_{corr}})-((16c*5b)*A_{2_{corr}})-(16c*A_{3_{corr}})-((120c^2*5a)*A_0)-(120c^2*10b^2)*A_0)-((120c^2*5b)*A_{1_{corr}})-(120c^2*A_{2_{corr}})-((560c^3*5b)*A_0)-(560c^3*A_{1_{corr}})-(1820c^4*A_0)$
$A_{5_{corr}}$	$A_5-(b5*A_0)-(30a^2b*A_0)-(20ab*A_{2_{corr}})-(20ab^3*A_0)-(30ab^2*A_{1_{corr}})-(10a^2*A_{1_{corr}})-(5a*A_{3_{corr}})-(5b^4*A_{1_{corr}})-(10b^3*A_{2_{corr}})-(10b^2*A_{3_{corr}})-(5b*A_{4_{corr}})-((16c*20ab)*A_{1_{corr}})-((16c*30ab^2)*A_0)-((16c*10a^2)*A_0)-((16c*5a)*A_{2_{corr}})-((16c*5b^4)*A_0)-((16c*10b^3)*A_{1_{corr}})-((16c*10b^2)*A_{2_{corr}})-((16c*5b)*A_{3_{corr}})-(16c*A_{4_{corr}})-((120c^2*20ab)*A_0)-((120c^2*5a)*A_{1_{corr}})-((120c^2*10b^3)*A_0)-((120c^2*10b^2)*A_{1_{corr}})-((120c^2*5b)*A_{2_{corr}})-(120c^2*A_{3_{corr}})-((560c^3*5a)*A_0)-((560c^3*10b^2)*A_0)-((560c^3*5b)*A_{1_{corr}})-(560c^3*A_{2_{corr}})-((1820c^4*5b)*A_0)-(1820c^4*A_{1_{corr}})-(4368c^5*A_0)$

F6P/G6P: C_6-backbone, to be corrected *sum formula*: $C_{25}\ Si_6$

isotope inference corrected area of M+ *n* isotopologue	**Equation for correction**
A_0	A_0
$A_{1_{corr}}$	$A_1-(6b*A_0)-(19c*A_0)$
$A_{2_{corr}}$	$A_2-(6a*A_0)-(15b^2*A_0)-(6b*A_{1_{corr}})-((19c*6b)*A_0)-(19c*A_{1_{corr}})-(171c^2*A_0)$
$A_{3_{corr}}$	$A_3-(30ab*A_0)-(6a*A_{1_{corr}})-(20b^3*A_0)-(15b^2*A_{1_{corr}})-(6b*A_{2_{corr}})-((19c*6a)*A_0)-((19c*15b^2)*A_0)-((19c*6b)* A_{1_{corr}})-(19c*A_{2_{corr}})-((6b*171c^2)*A_0)-(171c^2*A_{1_{corr}})*(969c^3*A_0)$
$A_{4_{corr}}$	$A_4-(30ab*A_{1_{corr}})-(60ab^2*A_0)-(15a^2*A_0)-(6a*A_{2_{corr}})-(15b^4*A_0)-(20b^3*A_{1_{corr}})-(15b^2*A_{2_{corr}})-(6b*A_{3_{corr}})-((19c*30ab)*A_0)-((19c*6a)* A_{1_{corr}})-((19c*20b^3)*A_0)-((19c*15b^2)* A_{1_{corr}})-((19c*6b)* A_{2_{corr}})-(19c*A_{3_{corr}})-((171c^2*6a)*A_0)-((171c^2*15b^2)*A_0)-((171c^2*6b)* A_{1_{corr}})-(171c^2*A_{2_{corr}})-((969c^3*6b)*A_0)-(969c^3*A_{1_{corr}})-(3876c^4*A_0)$
$A_{5_{corr}}$	$A_5-(60a^2b*A_0)-(30ab*A_{2_{corr}})-(60ab^3*A_0)-(60ab^2*A_{1_{corr}})-(15a^2*A_{1_{corr}})-(6a*A_{3_{corr}})-(6b^5*A_0)-(15b^4*A_{1_{corr}})-(20b^3*A_{2_{corr}})-(15b^2*A_{3_{corr}})-(6b*A_{4_{corr}})-((19c*30ab)* A_{1_{corr}})-((19c*60ab^2)*A_0)-((19c*15a^2)*A_0)-((19c*6a)* A_{2_{corr}})-((19c*15b^4)*A_0)-((19c*20b^3)* A_{1_{corr}})-((19c*15b^2)* A_{2_{corr}})-((19c*6b)* A_{3_{corr}})-(19c*A_{4_{corr}})-((171c^2*30ab)*A_0)-((171c^2*6a)* A_{1_{corr}})-((171c^2*20b^3)*A_0)-((171c^2*15b^2)* A_{1_{corr}})-((171c^2*6b)* A_{2_{corr}})-(171c^2*A_{3_{corr}})-((969c^3*6a)*A_0)-((969c^3*15b^2)*A_0)-((969c^3*6b)* A_{1_{corr}})-(969c^3*A_{2_{corr}})-((3876c^4*6b)*A_0)-(3876c^4*A_{1_{corr}})-(11628c^5*A_0)$
$A_{6_{corr}}$	$A_6-(b^6*A_0)-(60a^2b*A_{1_{corr}})-(30ab*A_{3_{corr}})-(30ab^4*A_0)-(60ab^3*A_{1_{corr}})-(60ab^2*A_{2_{corr}})-(90a^2b^2*A_0)-(20a^3*A_0)-(15a^2*A_{2_{corr}})-(6a*A_{4_{corr}})-(6b^5*A_{1_{corr}})-(15b^4*A_{2_{corr}})-(20b^3*A_{3_{corr}})-(15b^2*A_{4_{corr}})-(6b*A_{5_{corr}})-((19c*60a^2b)*A_0)-((19c*30ab)* A_{2_{corr}})-((19c*60ab^3)*A_0)-((19c*60ab^2)* A_{1_{corr}})-((19c*15a^2)* A_{1_{corr}})-((19c*6a)* A_{3_{corr}})-((19c*6b^5)*A_0)-((19c*15b^4)* A_{1_{corr}})-((19c*20b^3)* A_{2_{corr}})-((19c*15b^2)* A_{3_{corr}})-((19c*6b)* A_{4_{corr}})-(19c*A_{5_{corr}})-((171c^2*30ab)* A_{1_{corr}})-((171c^2*60ab^2)*A_0)-((171c^2*15a^2)*A_0)-((171c^2*6a)* A_{2_{corr}})-((171c^2*15b^4)*A_0)-(171c^2*20b^3)* A_{1_{corr}})-((171c^2*15b^2)* A_{2_{corr}})-((171c^2*6b)* A_{3_{corr}})-(171c^2*A_{4_{corr}})-((969c^3*30ab)*A_0)-((969c^3*6a)* A_{1_{corr}})-((969c^3*20b^3)*A_0)-((969c^3*15b^2)* A_{1_{corr}})-((969c^3*6b)* A_{2_{corr}})-(969c^3*A_{3_{corr}})-((3876c^4*6a)*A_0)-((3876c^4*15b^2)*A_0)-((3876c^4*6b)* A_{1_{corr}})-(3876c^4*A_{2_{corr}})-((11628c^5*6b)A_0)-(11628c^5*A_{1_{corr}})-(27132c^6*A_0)$

S7P: C_7-backbone, to be corrected *sum formula*: $C_{29}\,Si_7$

isotope inference corrected area of M+ *n* isotopologue	**Equation for correction**
A_0	A_0
$A_{1_{corr}}$	$A_1-(7b*A_0)-(22c*A_0)$
$A_{2_{corr}}$	$A_2-(7a*A_{1_{corr}})-(21b^2*A_0)-(7b*A_{1_{corr}})-((22c*7b)*A_0)-(22c*A_{1_{corr}})-(231c^2*A_0)$
$A_{3_{corr}}$	$A_3-(42ab*A_0)-(7a*A_{1_{corr}})-(35b^3*A_0)-(21b^2*A_{1_{corr}})-(7b*A_{2_{corr}})-((22c*7a)*A_0)-((22c*21b^2)*A_0)-((22c*7b)* A_{1_{corr}})-(22c*A_{2_{corr}})-((231c^2*7b)*A_0)-((231c^2*A_{1_{corr}})-(1540c^3*A_0)$
$A_{4_{corr}}$	$A_4-(42ab*A_{1_{corr}})-(105ab^2*A_0)-(21a^2*A_0)-(7a*A_{2_{corr}})-(35b^4*A_0)-(35b^3*A_{1_{corr}})-(21b^2*A_{2_{corr}})-(7b*A_{3_{corr}})-((22c*42ab)*A_0)-((22c*7a)* A_{1_{corr}})-((22c*35b^3)*A_0)-((22c*21b^2)* A_{1_{corr}})-((22c*7b)* A_{2_{corr}})-(22c*A_{3_{corr}})-((231c^2*7a)*A_0)-((231c^2*21b^2)*A_0)-(231c^2*7b)* A_{1_{corr}})-(231c^2*A_{2_{corr}})-(1540c^3*7b)*A_0)-(1540c^3*A_{1_{corr}})-(7315c^4*A_0)$
$A_{5_{corr}}$	$A_5-(105a^2b*A_0)-(42ab*A_{2_{corr}})-(140ab^3*A_0)-(105ab^2*A_{1_{corr}})-(21a^2*A_{1_{corr}})-(7a*A_{3_{corr}})-(21b^5*A_0)-(35b^4*A_{1_{corr}})-(35b^3*A_{2_{corr}})-(21b^2*A_{3_{corr}})-(7b*A_{4_{corr}})-((22c*42ab)* A_{1_{corr}})-((22c*105ab^2)*A_0)-((22c*21a^2)*A_0)-((22c*7a)* A_{2_{corr}})-((22c*35c^4)*A_0)-((22c*35b^3)* A_{1_{corr}})-(22c*21b^2)* A_{2_{corr}})-((22c*7b)* A_{3_{corr}})-(22c*A_{4_{corr}})-((231c^2*42ab)*A_0)-((231c^2*7a)* A_{1_{corr}})-((231c^2*35b^3)*A_0)-((231c^2*21b^2)* A_{1_{corr}})-((231c^2*7b)* A_{2_{corr}})-(231c^2*A_{3_{corr}})-((1540c^3*7a)*A_0)-((1540c^3*21b^2)*A_0)-((1540c^3*7b)* A_{1_{corr}})-(1540c^3*A_{2_{corr}})-((7315c^4*7b)*A_0)-(7315c^4*A_{1_{corr}})-(26334c^5*A_0)$
$A_{6_{corr}}$	$A_6-(105a^2b*A_{1_{corr}})-(42ab*A_{3_{corr}})-(105ab^4*A_0)-(140ab^3*A_{1_{corr}})-(105ab^2*A_{2_{corr}})-(210a^2b^2*A_0)-(35a^3*A_0)-(21a^2*A_{2_{corr}})-(7a*A_{4_{corr}})-(7b^6*A_0)-(21b^5*A_{1_{corr}})-(35b^4*A_{2_{corr}})-(35b^3*A_{3_{corr}})-(21b^2*A_{4_{corr}})-(7b*A_{5_{corr}})-((22c*105a^2b)*A_0)-((22c*42ab)* A_{2_{corr}})-((22c*140ab^3)*A_0)-((22c*105ab^2)* A_{1_{corr}})-((22c*21a^2)* A_{1_{corr}})-((22c*7a)* A_{3_{corr}})-((22c*21b^5)*A_0)-((22c*35b^4)* A_{1_{corr}})-((22c*35b^3)* A_{2_{corr}})-((22c*21b^2)* A_{3_{corr}})-((22c*7b)* A_{4_{corr}})-(22c*A_{5_{corr}})-((231c^2*42ab)* A_{1_{corr}})-((231c^2*105ab^2)*A_0)-(231c^2*21a^2)*A_0)-(231c^2*7a)* A_{2_{corr}})-((231c^2*35b^4)*A_0)-((231c^2*35b^3)* A_{1_{corr}})-((231c^2*21b^2)* A_{2_{corr}})-((231c^2*7b)* A_{3_{corr}})-(231c^2*A_{4_{corr}})-((1540c^3*42ab)*A_0)-((1540c^3*7a)* A_{1_{corr}})-((1540c^3*35b^3)*A_0)-((1540c^3*21b^2)* A_{1_{corr}})-((1540c^3*7b)* A_{2_{corr}})-(1540c^3*A_{3_{corr}})-((7315c^4*7a)*A_0)-((7315c^4*21b^2)*A_0)-((7315c^4*7b)* A_{1_{corr}})-(7315c^4*A_{2_{corr}})-(26334c^5*7b)*A_0)-(26334c^5*A_{1_{corr}})-(74613c^6*A_0)$
$A_{7_{corr}}$	$A_7-(b^7*A_0)-(140a^3b*A_0)-(105a^2b*A_{2_{corr}})-(42ab*A_{4_{corr}})-(42ab^5*A_0)-(105ab^4*A_{1_{corr}})-(140ab^3*A_{2_{corr}})-(105ab^2*A_{3_{corr}})-(210a^2b^3*A_0)-(210a^2b^2*A_{1_{corr}})-(35a^3*A_{1_{corr}})-(21a^2*A_{3_{corr}})-(7a*A_{5_{corr}})-(7b^6*A_{1_{corr}})-(21b^5*A_{2_{corr}})-(35b^4*A_{3_{corr}})-(35b^3*A_{4_{corr}})-(21b^2*A_{5_{corr}})-(7b*A_{6_{corr}})-((22c*105a^2b)* A_{1_{corr}})-((22c*42ab)* A_{3_{corr}})-((22c*105ab^4)*A_0)-((22c*140ab^3)* A_{1_{corr}})-((22c*105ab^2)* A_{2_{corr}})-((22c*210a^2b^2)*A_0)-((22c*35a^3)*A_0)-((22c*21a^2)* A_{2_{corr}})-((22c*7a)* A_{4_{corr}})-((22c*7b^6)*A_0)-((22c*21b^5)* A_{1_{corr}})-((22c*35b^4)* A_{2_{corr}})-((22c*35b^3)* A_{3_{corr}})-((22c*21b^2)* A_{4_{corr}})-((22c*7b)* A_{5_{corr}})-(22c*A_{6_{corr}})-((231c^2*105a^2b)*A_0)-((231c^2*42ab)* A_{2_{corr}})-((231c^2*140ab^3)*A_0)-((231c^2*105ab^2)* A_{1_{corr}})-((231c^2*21a^2)* A_{1_{corr}})-((231c^2*7a)* A_{3_{corr}})-((231c^2*21b^5)*A_0)-((231c^2*35b^4)* A_{1_{corr}})-((231c^2*35b^3)* A_{2_{corr}})-((231c^2*21b^2)* A_{3_{corr}})-((231c^2*7b)* A_{4_{corr}})-(231c^2*A_{5_{corr}})-((1540c^3*42ab)* A_{1_{corr}})-((1540c^3*105ab^2)*A_0)-((1540c^3*21a^2)*A_0)-((1540c^3*7a)* A_{2_{corr}})-((1540c^3*35b^4)*A_0)-((1540c^3*35b3)* A_{1_{corr}})-((1540c^3*21b2)* A_{2_{corr}})-((1540c^3*7b)* A_{3_{corr}})-(1540c^3*A_{4_{corr}})-((7315c^4*42ab)*A_0)-((7315c^4*7a)* A_{1_{corr}})-((7315c^4*35b3)*A_0)-(7315c^4*21b^2)* A_{1_{corr}})-((7315c^4*7b)* A_{2_{corr}})-(7315c^4*A_{3_{corr}})-((26334c^5*7a)*A_0)-((26334c^5*21b^2)*A_0)-(26334c^5*7b)* A_{1_{corr}})-(26334c^5*A_{2_{corr}})-((74613c^6*7b)*A_0)- (74613c^6*A_{1_{corr}})- (170544c^7*A_0)$

Table S2 Isotopologue fractions of metabolites implemented into metabolic network model, including the Monte Carlo simulated

	Biological Replicate 1				**Biological Replicate 2**				**Biological Replicate 3**			
	Input areas (*before correction*)	**IF corrected %**	**IF corrected U**	**IF corrected rel.U**	Input areas *(before correction)*	**IF corrected %**	**IF corrected U**	**IF corrected rel.U**	Input areas *(before correction)*	**IF corrected %**	**IF corrected U**	**IF corrected rel.U**
PEP M	7187	**22.328**	**0.312**	**1.40%**	4070	**17.116**	**0.282**	**1.65%**	4337	**19.607**	**0.321**	**1.64%**
PEP M+1	26796	**77.672**	**0.312**	**0.40%**	20360	**81.348**	**0.406**	**0.50%**	18865	**80.393**	**0.321**	**0.40%**
PEP M+2	191	*-21.648*	*0.309*	*-1.43%*	5262	*-0.367*	*0.498*	*-135.46%*	220	*-21.579*	*0.321*	*-1.49%*
PEP M+3	124	*-9.990*	*0.092*	*-0.92%*	2917	**1.535**	**0.269**	**17.51%**	184	*-9.833*	*0.107*	*-1.09%*
GAP M	16889	**22.179**	**0.254**	**1.1%**	10200	**17.5**	**0.23**	**1.3%**	8485	**17.832**	**0.240**	**1.3%**
GAP M+1	59006	**71.467**	**0.495**	**0.7%**	47030	**75.8**	**0.52**	**0.7%**	37982	**74.983**	**0.534**	**0.7%**
GAP M+2	20733	**4.884**	**0.456**	**9.3%**	16298	**5.0**	**0.49**	**9.7%**	13614	**5.892**	**0.496**	**8.4%**
GAP M+3	9773	**1.469**	**0.183**	**12.4%**	7917	**1.7**	**0.20**	**11.9%**	6325	**1.293**	**0.213**	**16.5%**
2PG M	4838	**33.4**	**0.5085**	**1.5%**	1914	**11.4**	**0.2794**	**2.5%**	2181	**18.9**	**0.4297**	**2.3%**
2PG M+1	11267	**66.6**	**0.5085**	**0.8%**	15559	**88.6**	**0.2794**	**0.3%**	10029	**80.6**	**0.8006**	**1.0%**
2PG M+2	3270	*-5.8*	*0.5839*	*-10.1%*	2545	*-16.5*	*0.5298*	*-3.2%*	3560	**0.5**	**0.7539**	**141.6%**
2PG M+3	1886	*-0.6*	*0.3720*	*-58.4%*	1583	*-7.4*	*0.3088*	*-4.2%*	1815	*-0.1*	*0.4375*	*-775.0%*
DHAP M	58885	**26.4**	**0.26**	**1.0%**	36988	**22.1**	**0.2**	**1.0%**	30549	**20.5**	**0.22**	**1.1%**
DHAP M+1	171641	**69.7**	**0.46**	**0.7%**	133360	**73.5**	**0.5**	**0.6%**	118357	**73.9**	**0.48**	**0.6%**
DHAP M+2	58301	**3.7**	**0.42**	**11.4%**	45115	**4.0**	**0.4**	**11.0%**	41604	**5.2**	**0.45**	**8.7%**
DHAP M+3	24830	**0.2**	**0.14**	**64.1%**	19758	**0.4**	**0.1**	**38.7%**	18189	**0.5**	**0.15**	**31.5%**
3PG M	57203	**20.4**	**0.218**	**1.1%**	31769	**7.6**	**0.091**	**1.2%**	35850	**15.3**	**0.174**	**1.1%**
3PG M+1	242520	**79.6**	**0.218**	**0.3%**	397295	**92.4**	**0.091**	**0.1%**	210681	**84.7**	**0.174**	**0.2%**
3PG M+2	82359	*-0.9*	*0.521*	*-57.1%*	64116	*-16.8*	*0.496*	*-2.9%*	70005	*-1.2*	*0.549*	*-46.6%*
3PG M+3	36496	*-2.5*	*0.213*	*-8.6%*	29064	*-10.3*	*0.178*	*-1.7%*	32245	*-2.5*	*0.225*	*-9.2%*

	Biological Replicate 1				**Biological Replicate 2**				**Biological Replicate 3**			
	Input areas (*before correction*)	**IF corrected %**	**IF corrected U**	**IF corrected rel.U**	Input areas *(before correction)*	**IF corrected %**	**IF corrected U**	**IF corrected rel.U**	Input areas *(before correction)*	**IF corrected %**	**IF corrected U**	**IF corrected rel.U**
E4P M	0	**0.0**	**0.0000**	**-**	0	**0.0**	**0.0000**	**-**	0	**0.0**	**0.0000**	**-**
E4P M+1	1455	**100.0**	**0.0000**	**0.0%**	898	**71.5**	**2.0080**	**2.8%**	925	**100.0**	**0.0000**	**0.0%**
E4P M+2	459	*-2.9*	*1.8060*	*-63.3%*	667	**28.5**	**2.0080**	**7.0%**	276	*-4.5*	*2.1500*	*-47.7%*
E4P M+3	0	*-18.7*	*0.1787*	*-1.0%*	0	*-23.2*	*0.3901*	*-1.7%*	0	*-18.7*	*0.1788*	*-1.0%*
E4P M+4	0	*-4.4*	*0.0979*	*-2.2%*	0	*-8.5*	*0.2958*	*-3.5%*	0	*-4.4*	*0.0979*	*-2.2%*
R5P M	8298	**13.09**	**0.1883**	**1.4%**	6924	**9.99**	**0.1561**	**1.6%**	5106	**10.73**	**0.1778**	**1.7%**
R5P M+1	57540	**85.18**	**0.2624**	**0.3%**	63924	**87.97**	**0.7092**	**0.8%**	38603	**76.54**	**0.7125**	**0.9%**
R5P M+2	25087	*-0.09*	*0.7657*	*-828.7%*	27864	**0.12**	**0.7806**	**625.2%**	21403	**9.59**	**0.7218**	**7.5%**
R5P M+3	13721	*-0.73*	*0.3930*	*-53.9%*	15627	*-0.35*	*0.3048*	*-86.1%*	12976	**3.14**	**0.3742**	**11.9%**
R5P M+4	5211	**1.69**	**0.1953**	**11.5%**	5738	**1.59**	**0.1604**	**10.1%**	4371	*-0.41*	*0.2077*	*-50.6%*
R5P M+5	1756	**0.03**	**0.0881**	**269.1%**	2131	**0.32**	**0.0895**	**27.6%**	1291	*-0.58*	*0.1141*	*-19.5%*
Rl5P M	6800	**13.60**	**0.2081**	**1.5%**	4432	**8.95**	**0.1545**	**1.7%**	2647	**8.11**	**0.1659**	**2.0%**
Rl5P M+1	44736	**83.66**	**0.6909**	**0.8%**	46460	**90.02**	**0.2426**	**0.3%**	26403	**77.48**	**0.7535**	**1.0%**
Rl5P M+2	19641	**0.13**	**0.7665**	**573.3%**	19896	*-0.51*	*0.8104*	*-159.8%*	14918	**10.61**	**0.7518**	**7.1%**
Rl5P M+3	11495	**0.90**	**0.3307**	**36.6%**	10228	*-2.63*	*0.4064*	*-15.5%*	9263	**3.79**	**0.4192**	**11.1%**
Rl5P M+4	4099	**1.35**	**0.1867**	**13.8%**	3869	**1.03**	**0.2067**	**20.1%**	3012	*-0.90*	*0.2375*	*-26.3%*
Rl5P M+5	1577	**0.36**	**0.1066**	**29.8%**	1263	**0.01**	**0.0948**	**1488.1%**	748	*-1.25*	*0.1264*	*-10.1%*

	Biological Replicate 1				**Biological Replicate 2**				**Biological Replicate 3**			
	Input areas (*before correction*)	**IF corrected %**	**IF corrected U**	**IF corrected rel.U**	Input areas (*before correction*)	**IF corrected %**	**IF corrected U**	**IF corrected rel.U**	Input areas (*before correction*)	**IF corrected %**	**IF corrected U**	**IF corrected rel.U**
F6P M	12292	**9.8**	**0.1331**	**1.4%**	10408	**7.5**	**0.1105**	**1.5%**	8059	**6.8**	**0.1052**	**1.5%**
F6P M+1	49717	**34.6**	**0.3787**	**1.1%**	51064	**33.0**	**0.3791**	**1.1%**	43492	**33.3**	**0.3789**	**1.1%**
F6P M+2	93411	**53.6**	**0.4475**	**0.8%**	106413	**57.5**	**0.5808**	**1.0%**	89332	**56.4**	**0.5837**	**1.0%**
F6P M+3	47825	*-1.5*	*0.6353*	*-42.5%*	58596	**1.5**	**0.6699**	**46.0%**	50208	**2.2**	**0.6634**	**30.0%**
F6P M+4	25697	*-1.0*	*0.3584*	*-35.5%*	31496	*-0.5*	*0.2966*	*-59.8%*	28629	**1.0**	**0.3139**	**32.3%**
F6P M+5	11705	**2.0**	**0.1829**	**9.2%**	12003	**0.5**	**0.1641**	**32.5%**	10746	**0.3**	**0.1742**	**55.0%**
F6P M+6	3799	*-0.4*	*0.0769*	*-19.1%*	3521	*-0.4*	*0.0718*	*-17.0%*	3398	*-0.3*	*0.0839*	*-24.2%*
G6P M	135074	**7.5**	**0.0925**	**1.2%**	61876	**4.6**	**0.0589**	**1.3%**	56931	**4.7**	**0.0579**	**1.2%**
G6P M+1	423549	**19.7**	**0.2268**	**1.2%**	281296	**18.6**	**0.2176**	**1.2%**	250380	**18.2**	**0.2029**	**1.1%**
G6P M+2	1523656	**72.1**	**0.6795**	**0.9%**	1170308	**76.2**	**0.7093**	**0.9%**	1046916	**75.2**	**0.7001**	**0.9%**
G6P M+3	805286	**0.7**	**0.7525**	**103.8%**	616549	**0.5**	**0.7873**	**147.0%**	550890	**0.5**	**0.7785**	**158.4%**
G6P M+4	441285	*-1.6*	*0.2773*	*-17.4%*	340706	*-1.7*	*0.2868*	*-16.9%*	343251	**1.5**	**0.3063**	**20.4%**
G6P M+5	152261	*-0.6*	*0.1436*	*-25.3%*	116695	*-0.6*	*0.1476*	*-22.8%*	108089	*-1.1*	*0.1566*	*-14.4%*
G6P M+6	46143	*-0.5*	*0.0483*	*-9.7%*	36908	*-0.4*	*0.0507*	*-11.9%*	34085	*-0.8*	*0.0871*	*-10.7%*
S7P M	11269	**10.40**	**0.1578**	**1.5%**	7028	**7.41**	**0.1228**	**1.7%**	5353	**7.56**	**0.1336**	**1.8%**
S7P M+1	78733	**66.48**	**0.7571**	**1.1%**	69047	**68.38**	**0.7817**	**1.1%**	47172	**62.17**	**0.7401**	**1.2%**
S7P M+2	69363	**20.38**	**0.7962**	**3.9%**	62804	**22.64**	**0.8078**	**3.6%**	47476	**27.16**	**0.7703**	**2.8%**
S7P M+3	46702	**2.74**	**0.5387**	**19.6%**	41163	**1.40**	**0.5684**	**40.6%**	32009	**3.03**	**0.6073**	**20.0%**
S7P M+4	21976	*-0.53*	*0.3217*	*-60.4%*	19816	*-0.15*	*0.3330*	*-219.0%*	14852	*-1.86*	*0.3538*	*-19.0%*
S7P M+5	8984	*-0.14*	*0.1987*	*-141.9%*	7643	*-0.25*	*0.2029*	*-80.5%*	6108	*-0.67*	*0.2209*	*-33.1%*
S7P M+6	3007	*-0.06*	*0.0846*	*-153.4%*	2797	**0.17**	**0.0886**	**50.7%**	2182	*-0.11*	*0.1021*	*-93.7%*
S7P M+7	819	*-0.07*	*0.0379*	*-52.4%*	835	*-0.02*	*0.0480*	*-293.1%*	723	**0.08**	**0.0486**	**58.4%**

Table S3 Isotopologues from metabolites in the pentose phosphate pathway suggesting missing reactions in the model or measurement bias. The metabolites are expected to be part of a linear pathway and show the same labeling patterns, but differences of more than 10% are observed

		Average	SD
Glucose-6-Phosphate	G6P_M	3.0	1.2
	G6P_M1	17.6	0.7
	G6P_M2	77.8	0.9
	G6P_M3	1.6	2.8
	G6P_M4	0.0	0.0
	G6P_M5	0.0	0.0
	G6P_M6	0.0	0.0
6-phosphogluconate	6PGA_M	14.8	2.5
	6PGA_M1	20.9	2.8
	6PGA_M2	63.8	1.6
	6PGA_M3	0.2	0.4
	6PGA_M4	0.0	0.1
	6PGA_M5	0.1	0.2
	6PGA_M6	0.0	0.0

Table S4 Measured and simulated isotopologue fractions handling uncertainties using different strategies

	Measurement	Standard deviation (SD)	Simulated values using all measurements	Simulated values using measurements >2%	Simulated values using measurements > 2% and 3 fold SD increase to cover biological variance	Simulated values using measurements > 2% and 3-fold SD increase to cover biological variance except for F6P and G6P (M+2 andM+3)
GAP_M	0.192	0.021	0.157	0.157	0.157	0.154
GAP_M1	0.741	0.019	0.825	0.825	0.825	0.828
GAP_M2	0.053	0.004	0.018	0.018	0.018	0.018
GAP_M3	0.015	0.002	0.000			
DHAP_M	0.230	0.025	0.246	0.246	0.246	0.246
DHAP_M1	0.724	0.019	0.738	0.738	0.738	0.738
DHAP_M2	0.043	0.006	0.016	0.016	0.016	0.016
DHAP_M3	0.004	0.001	0.000			
E4P_M	0.000	0.152	0.041			
E4P_M1	0.905	0.134	0.929	0.929	0.929	0.932
E4P_M2	0.071	0.152	0.030	0.030	0.030	0.030
E4P_M3	0.000	0.021	0.000			
E4P_M4	0.000	0.019	0.000			
RI5P_M	0.102	0.024	0.038	0.038	0.038	0.037
RI5P_M1	0.837	0.051	0.922	0.922	0.922	0.922
RI5P_M2	0.034	0.051	0.040	0.040	0.040	0.040
RI5P_M3	0.007	0.026	0.001			
RI5P_M4	0.005	0.010	0.000			
RI5P_M5	0.000	0.007	0.000			
R5P_M	0.113	0.013	0.039	0.039	0.039	0.037
R5P_M1	0.832	0.049	0.920	0.921	0.921	0.922
R5P_M2	0.032	0.045	0.040	0.040	0.040	0.040
R5P_M3	0.007	0.017	0.001			
R5P_M4	0.010	0.010	0.000			
R5P_M5	0.000	0.004	0.000			
F6P_M	0.080	0.013	0.047			
F6P_M1	0.336	0.007	0.352	0.047	0.047	0.046
F6P_M2	0.558	0.016	0.575	0.352	0.352	0.344
F6P_M3	0.007	0.016	0.024	0.576	0.576	0.585
F6P_M4	0.000	0.008	0.000			
F6P_M5	0.009	0.007	0.000			
F6P_M6	0.000	0.000	0.000			
S7P_M	0.085	0.014	0.028	0.028	0.028	0.027
S7P_M1	0.657	0.026	0.669	0.669	0.668	0.669
S7P_M2	0.234	0.028	0.288	0.289	0.289	0.290
S7P_M3	0.024	0.007	0.014	0.014	0.014	0.015
S7P_M4	0.000	0.007	0.000			
S7P_M5	0.000	0.002	0.000			
S7P_M6	0.000	0.001	0.000			

S7P_M7	0.000	0.001	0.000			
G6P_M	0.030	0.012	0.022	0.022	0.022	0.022
G6P_M1	0.176	0.007	0.175	0.175	0.175	0.172
G6P_M2	0.778	0.009	0.769	0.769	0.769	0.772
G6P_M3	0.016	0.028	0.033			
G6P_M4	0.000	0.028	0.001			
G6P_M5	0.000	0.028	0.000			
G6P_M6	0.000	0.028	0.000			