Supplementary Material for

Assessing and Resolving Model Misspecifications in Metabolic Flux Analysis

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Table S1. Metabolic reactions and exchange fluxes in the Chinese hamster ovary metabolic model (adapted from [16,25]).

#	Formula									
1	Glc	→	G6P							
2	G6P	→	2 DHAP							
3	DHAP	→	Pyr							
4	G6P	→	R5P							
5	Pyr	→	Lac							
6	mAcCoA	→	AcOH							
7	Mal	→	Pyr							
8	mPyr	→	mAcCoA							
9	mAcCoA	+	mOxal	→	mCit					
10	mCit	→	mαKG							
11	mαKG	→	mSucCoA							
12	mSucCoA	→	mSuc							
13	mSuc	→	mMal							
14	mMal	→	mOxal							
15	Oxal	→	Mal							
16	Glu	+	Pyr	→	αKG	+	Ala			
17	mGlu	→	Amm	+	mαKG					
18	Glu	+	Amm	→	Gln					
19	Glu	+	Oxal	→	Asp	+	αKG			
20	Glu	→	Pgl							
21	Ser	→	Pyr	+	Amm					
22	Ser	→	Gly	+	NMFH4					
23	Amm	+	NMFH4	→	Gly					
24	Thr	→	Gly	+	mAcCoA					
25	Cys	+	αKG	→	Glu	+	Pyr			
26	His	→	Glu	+	NMFH4	+	2 Amm			
27	Arg	+	αKG	→	2 Glu					
28	Pro	→	Glu							
29	Asn	→	Amm	+	Asp					
30	Ile	+	αKG	→	mSucCoA	+	Glu		+	mAcCoA
31	Leu	+	αKG	+	mSucCoA	→	2 mAcCoA	+	mSuc	+	Glu
32	Lys	+	αKG	→	Glu	+	2 mAcCoA	+	Amm		
33	Met	+	Ser	→	mSucCoA	+	Cys	+	Amm	+	NMFH4
34	Phe	→	Tyr							
35	Trp	→	2 mAcCoA	+	Ala	+	Amm			
36	Tyr	+	αKG	+	mSucCoA	→	Glu	+	Mal	+	2 mAcCoA + mSuc
37	Val	+	αKG	→	mSucCoA	+	Glu			

38	G6P	→	Carb							
39	9 mCit	+	9 Mal	→	OA	+	9 mMal	+	9 Oxal	
40	1.9 Gln	+	1.3 Asp	+	0.5 Gly	+	1.3 NMFH4	+	R5P	→ 1.9 Glu
		+	0.8 Mal	+	DNA					
41	2.091 Gln	+	1.194 Asp	+	0.489 Gly	+	0.978 NMFH4	+	R5P	→ 2.091 Glu
		+	0.683 Mal	+	RNA					
42	0.095 Ala	+	0.048 Asp	+	0.039 Asn	+	0.063 Arg	+	0.028 Cys	+ 0.052 Gln
		+	0.064 Glu	+	0.078 Gly	+	0.022 His	+	0.052 Ile	+ 0.088 Leu
		+	0.089 Lys	+	0.02 Met	+	0.021 Phe	+	0.028 Pro	+ 0.057 Ser
		+	0.061 Thr	+	0.006 Trp	+	0.02 Tyr	+	0.059 Val	→ Prot
43	mMal	+	αKG	→	mαKG	+	Mal			
44	mMal	+	Cit	→	mCit	+	Mal			
45	Glu	→	mGlu							
46	Pyr	→	mPyr							
47	FoOH	→	NMFH4							

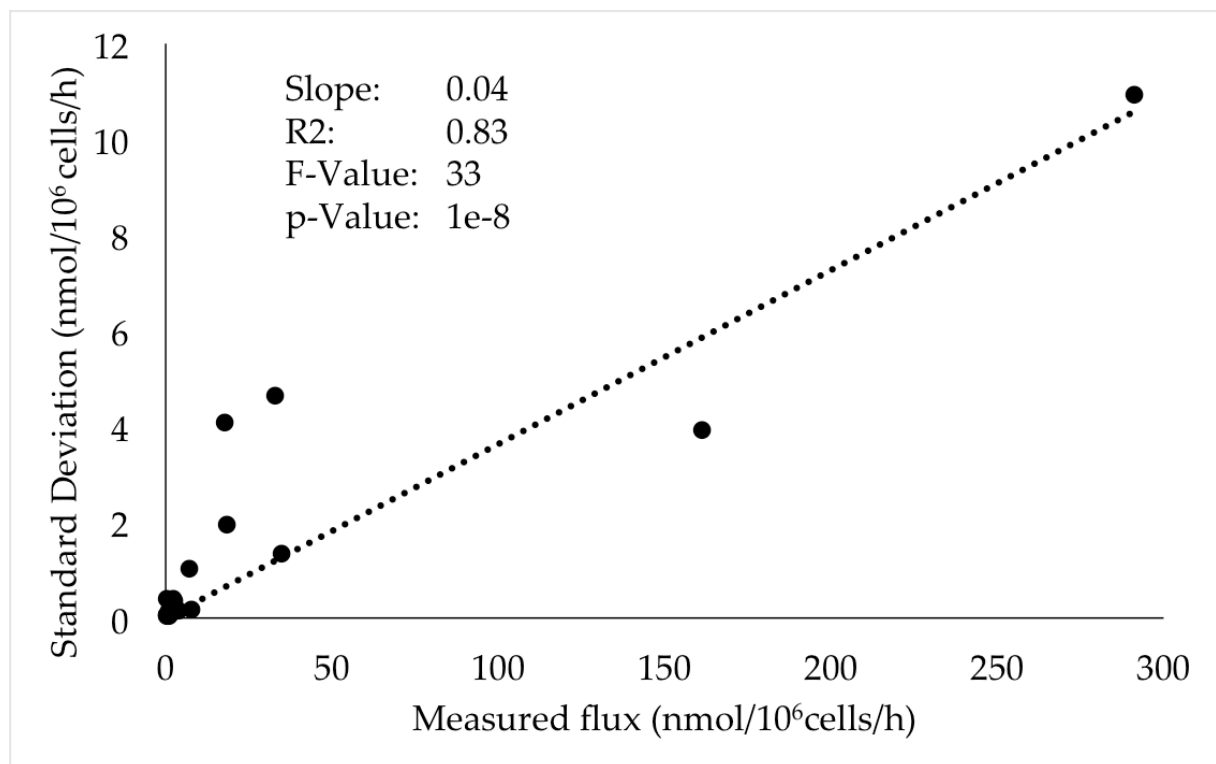| Exchange Fluxes				Measured	Standard deviation
	→	Ala		-34.9	1.34
	→	2 Cys		0.33	0.06
	→	Gly		-2.39	0.22
	→	Ser		2.48	0.37
	→	Thr		1.59	0.17
	→	Arg		2.38	0.39
	→	Glu		-0.34	0.41
	→	Gln		7.08	1.02
	→	His		0.97	0.15
	→	Pro		1.93	0.15
	→	Asp		2.04	0.13
	→	Asn		2.24	0.20
	→	Ile		1.41	0.11
	→	Leu		2.61	0.25
	→	Lys		1.69	0.12
	→	Met		0.86	0.05
	→	Phe		1.15	0.08
	→	Trp		0.32	0.07
	→	Tyr		1.05	0.07
	→	Val		2.18	0.13
	→	Lac		-291	10.9
	→	Glc		161	3.93
	→	Amm		-17.8	4.08
	→	Carb		-2.14	0.27
	→	OA		-1.37	0.17
	→	DNA		-0.31	0.04
	→	RNA		-0.93	0.11
	→	Prot		-32.9	4.64
	→	Ala	+ Gln	18.3	1.96
	→	FoOH		-7.75	0.19
	→	AcOH		-1.07	0.06
	→	Pyr		-2.83	0.17
	→	Cit		-1.61	0.11
	→	Pgl		-4.00	0.17

Figure S1 Coefficient of variation analysis of the measured exchange fluxes in CHO cell culture data [16].

Table S2. Intracellular flux estimates (flux distribution) of the CHO cell culture.

Reaction number	$\hat{v}_{I,GLS}\left(\frac{nmol}{1e6cells\,h}\right)$
1	161.2
2	157.8
3	315.5
4	1.2
5	291.2
6	1.1
7	19.6
8	15.0
9	12.5
10	-1.5
11	17.8
12	17.4
13	18.1
14	12.5
15	12.3
16	19.3
17	-0.2
18	-21.5
19	0.0
20	4.0
21	-6.8
22	7.2
23	-1.3
24	-0.4
25	0.0
26	0.3
27	0.3
28	1.0
29	1.0
30	-0.3
31	-0.2
32	-1.2
33	0.2
34	0.5
35	0.1
36	0.9
37	0.3
38	2.1
39	1.4
40	0.3
41	0.9
42	32.3
43	19.5
44	-1.6
45	-0.2
46	15.0
47	-7.8

Reaction number	$\hat{v}_{I,GLS}\left(\frac{nmol}{1e6cells\,h}\right)$

Table S3 Case study II: Other misspecification tests using the F-test (values represent rates)

m	n_{v_I}	n_{v_E}	n_{v_0}	CoV	TP	FN	FP	TN
100	60	45	2	0.01	0.93	0.07	0.10	0.90
				0.05	0.89	0.11	0.11	0.89
				0.1	0.88	0.12	0.10	0.90
				0.2	0.82	0.19	0.09	0.91
			5	0.01	1.00	0.00	0.12	0.88
				0.05	1.00	0.00	0.11	0.89
				0.1	0.99	0.01	0.12	0.88
				0.2	0.99	0.01	0.12	0.89
			10	0.01	1.00	0.00	0.14	0.86
				0.05	1.00	0.00	0.12	0.88
				0.1	1.00	0.00	0.13	0.88
				0.2	0.99	0.01	0.12	0.88

m	n_{v_I}	n_{v_E}	n_{v_0}	CoV	TP	FN	FP	TN
100	60	55	2	0.01	0.97	0.03	0.11	0.90
				0.05	0.95	0.05	0.10	0.90
				0.1	0.91	0.09	0.09	0.91
				0.2	0.83	0.17	0.10	0.90
			5	0.01	1.00	0.00	0.10	0.90
				0.05	1.00	0.00	0.11	0.89
				0.1	0.99	0.01	0.08	0.92
				0.2	0.98	0.02	0.08	0.92
			10	0.01	1.00	0.00	0.13	0.87
				0.05	1.00	0.00	0.11	0.89
				0.1	1.00	0.00	0.11	0.89
				0.2	1.00	0.00	0.11	0.89

m	n_{v_I}	n_{v_E}	n_{v_0}	CoV	TP	FN	FP	TN
100	55	55	2	0.01	0.84	0.16	0.10	0.90
				0.05	0.83	0.17	0.11	0.90
				0.1	0.80	0.20	0.09	0.91
				0.2	0.72	0.28	0.10	0.90
			5	0.01	0.98	0.02	0.13	0.87
				0.05	0.98	0.02	0.14	0.86
				0.1	0.97	0.04	0.13	0.87
				0.2	0.93	0.07	0.11	0.89
			10	0.01	1.00	0.00	0.14	0.86
				0.05	1.00	0.00	0.13	0.87
				0.1	1.00	0.00	0.14	0.86
				0.2	0.99	0.01	0.13	0.87

m	n_{v_I}	n_{v_E}	n_{v_0}	CoV	TP	FN	FP	TN
100	65	45	2	0.01	0.95	0.05	0.10	0.90
				0.05	0.93	0.07	0.11	0.90
				0.1	0.90	0.11	0.10	0.90
				0.2	0.81	0.19	0.08	0.92
			5	0.01	1.00	0.00	0.12	0.88
				0.05	1.00	0.00	0.11	0.90
				0.1	1.00	0.00	0.10	0.90
				0.2	0.98	0.02	0.10	0.90
			10	0.01	1.00	0.00	0.09	0.91
				0.05	1.00	0.00	0.12	0.88

$n_{species}$	n_{vI}	n_{vE}	n_{vOmit}	CoV	TP	FN	FP	TN
				0.1	1.00	0.00	0.11	0.89
				0.2	1.00	0.00	0.10	0.90
$n_{species}$	n_{vI}	n_{vE}	n_{vOmit}	CoV	TP	FN	FP	TN
50	30	25	2	0.01	0.86	0.14	0.11	0.89
				0.05	0.82	0.18	0.10	0.90
				0.1	0.75	0.25	0.09	0.91
				0.2	0.69	0.31	0.09	0.91
			5	0.01	0.99	0.01	0.10	0.90
				0.05	0.98	0.02	0.10	0.90
				0.1	0.97	0.03	0.10	0.90
				0.2	0.92	0.08	0.11	0.89
			10	0.01	1.00	0.00	0.10	0.90
				0.05	1.00	0.00	0.09	0.91
				0.1	1.00	0.00	0.09	0.91
				0.2	0.99	0.02	0.11	0.90
m	n_{v_I}	n_{v_E}	n_{v_O}	CoV	TP	FN	FP	TN
200	120	100	2	0.01	0.76	0.24	0.11	0.89
				0.05	0.73	0.27	0.10	0.90
				0.1	0.67	0.33	0.07	0.93
				0.2	0.58	0.42	0.10	0.90
			5	0.01	0.97	0.03	0.16	0.84
				0.05	0.95	0.05	0.11	0.89
				0.1	0.94	0.07	0.13	0.87
				0.2	0.88	0.12	0.13	0.88
			10	0.01	1.00	0.00	0.15	0.85
				0.05	0.99	0.01	0.16	0.84
				0.1	1.00	0.01	0.13	0.87
				0.2	0.98	0.02	0.15	0.85
			20	0.01	1.00	0.00	0.14	0.86
				0.05	1.00	0.00	0.14	0.86
				0.1	1.00	0.00	0.15	0.86
				0.2	1.00	0.00	0.14	0.86

Table S4 Case study II: Performance of RESET tests on homoscedastic data (values represent rates).

m	n_{v_I}	n_{v_E}	n_{v_O}	CoV[a]	RESET test (p = 1)				RESET test (p = 2)			
					TP	FN	FP	TN	TP	FN	FP	TN
100	**60**	**50**	**2**	**0.01**	0.09	0.91	0.05	0.95	0.21	0.79	0.05	0.95
				0.05	0.08	0.92	0.04	0.96	0.19	0.81	0.05	0.95
				0.1	0.08	0.92	0.05	0.95	0.20	0.80	0.06	0.94
				0.2	0.07	0.93	0.04	0.96	0.17	0.83	0.05	0.95
			5	**0.01**	0.09	0.91	0.04	0.96	0.29	0.71	0.05	0.95
				0.05	0.10	0.90	0.04	0.96	0.31	0.69	0.04	0.96
				0.1	0.11	0.89	0.06	0.94	0.30	0.71	0.05	0.95
				0.2	0.10	0.90	0.04	0.96	0.30	0.70	0.04	0.96
			10	**0.01**	0.12	0.88	0.06	0.94	0.41	0.59	0.05	0.95
				0.05	0.11	0.89	0.05	0.96	0.42	0.58	0.04	0.96
				0.1	0.11	0.89	0.05	0.95	0.41	0.59	0.06	0.94
				0.2	0.13	0.87	0.06	0.94	0.43	0.57	0.05	0.95

[a] *In silico* were generated in the same manner as in Case Study II, but using independent and identically distributed Gaussian noise with a constant variance (i.e. homoscedastic noise) at the specified CoV.

Table S5 Case study III: Iterative procedure for resolving model misspecification in the CHO model (k = 2).

k	n_{extra}	n_{omit}	Number of remaining reactions[a]	
			Extra reactions	Omitted reactions
2	5	5	0	0
	8	8	0	0

[a]The number of remaining reactions corresponds to the average over 100 generations of the stoichiometric matrix $S_{I,true}$, of the median number across 100 in silico data simulations.