

Maximum entropy and population heterogeneity in continuous cell cultures meet experimental data, preliminary results

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1 Materials and Method

1.1 Model framework

The model framework used is detaily explained in (Fernandez-de Cossio-Diaz, Leon, & Mulet, 2017) and (Fernandez-de Cossio-Diaz & Mulet, 2019).

1.2 Experimental data

Experimental data was taken from (Rath, 2017). In this work the author performed 6 continuous cultures, (A, B, C, D, E, F), with the cell line AGE1.HN.AA1. The parental line AGE1.HN was established by the company ProBioGen (ProBioGen AG, Berlin, Germany) from a tissue sample of a human brain. All culture's feed mediums were based on the standard 42-Max-UB-medium, which is serum-free and was specially developed for the AGE1.HN cell line *Table 1*. The experiments were run under various conditions, differing mainly in the dilution rate (D) and the feed medium composition of glucose (GLC), glutamine (GLN) and galactose ($GAL/GALC$) *Table 2*.

For each experiment, a steady-state condition was reached, ($A, B, C, D, E, F01$), and several observables was reported *Tables 3 – 4*. Particularly relevant for this work was the growth rate (μ), D , the viable cell density (Xv) and the medium concentration (s) and derived uptake rate (u) for a set of metabolites (GLC , lactose (LAC), GLN , ammonium (NH_4), GAL , pyruvate (PYR), glutamate (GLU), alanine (ALA), asparagine (ASP)). A unit conversion was required to make experimental data and our model compatible. For this propose the only external data needed was the cell mass density. It was used $0.25 \text{ pgDW} / \mu\text{m}^3$ (Niklas, Schröder, Sandig, Noll, & Heinzle, 2011). ;

2 Results

2.1 Low concentrations of phosphatidylethanolamine

Because phosphatidylethanolamine isn't a reported component of the 42-Max-UB-medium, *Tabla 1*, and it can be a carbon source for the GEMs, its concentration was set first to the lowest value possible. For Recon3D it was fixed to 0.1mM, a handpicked value, and for CHO it wasn't present in the feed medium at all.

Flux balance analysis with molecular crowding (FBA) was performed similarly to (Fernandez-de Cossio-Diaz & Mulet, 2019). Plots of μ and Xv as a function of ξ are shown in *Figure 1*. Both networks failed to reach the experimental values for both observables. In the case of Xv , in all the explored ξ range (from 1 to 1000 gDW hr/ L) the value was underestimated. This result may have several causes. One could be that the GEM have a "too expensive" biomass equation. We mean that one or more biomass required precursors might be overestimated. However, this reason seems hard to sustain because we modified Recon3D biomass equation with the reported anabolic biomass demand for AGE1.HN.AA1 (Niklas, Priesnitz, Rose, Sandig, & Heinzle, 2013). We use the reported anabolic demand of proteins, lipids, DNA, RNA and carbohydrates to fix the total demand of this same groups in the original Recon3D biomass equation and the changes wasn't too big. For CHO we kept the biomass equation unmodified. Nonetheless, a modification in the biomass equation would be an elegant way to solve the problem. Other plausible cause could be a big difference between the models frameworks. But, both works model the chemostat in an equivalent fashion, as can be seen when comparing equations 1 with 3.25 and 2 with 3.28 and 3.29 from (Fernandez-de Cossio-Diaz et al., 2017) and (Rath, 2017) respectively.

Moreover, other parameter than can affect Xv is the bleeding coefficient, ϕ , defined in perfusion systems as the fraction of cells that escape from the culture through a cell-retention device (Fernandez-de Cossio-Diaz et al., 2017). Because, (Rath, 2017) doesn't report the use of any retention devise in the cultures this parameter was taken as 1.0, but lower values will cause to increment the predicted value of Xv by the model. Finally, it needs to be taken into consideration that we are using GEMs that are not directed curated for the working cell line. This is, by far, the most difficult factor to solve, because the curing of a genome scale metabolic network is a very laborious labor.

On other hand, correlations of experimental and modeled uptakes, *Figure 2*, was performed. The graphs shows better results for the uptakes of *GLC* and *GLN*, and worse correlations for uptakes such as *PYR*, *NH4* and *LAC* for both GEMs. In general, CHO had better correlations than Recon3D. This could be, maybe, explained because Recon3D is a general GEM, allowing the model to access to all the possible genome repertory present in humans, a fact that is not accurate for a cell in the *in vivo* scenario. Additionally, CHO was curated for a defined cell line, representing a more restricted and realistic network. Anyway, it is remarkable that the models reproduce uptakes like *GLC* and *GLN* regarding

all the previous consideration.

2.2 High concentrations of phosphatidylethanolamine

Two main reasons lead us to consider a less realistic scenario, in which the feed medium concentration of phosphatidylethanolamine are set to a high value, 20 mM (handpicked value), for both GEMs.

The first reason was the previous FBA results.

the This result was unexpected, due to the similarities between chemostat

3 References

Fernandez-de Cossio-Diaz, J., Leon, K., & Mulet, R. (2017). Characterizing steady states of genome-scale metabolic networks in continuous cell cultures. *PLoS Computational Biology*, 13(11), 1–22. doi: 10.1371/journal.pcbi.1005835

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Niklas, J., Priesnitz, C., Rose, T., Sandig, V., & Heinzle, E. (2013). Metabolism and metabolic burden by a 1 -antitrypsin production. *Metabolic Engineering*, 16, 103–114. Retrieved from <http://dx.doi.org/10.1016/j.ymben.2013.01.002> doi: 10.1016/j.ymben.2013.01.002

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Rath, A. (2017). *Characterisation of cell growth, metabolism and recombinant protein production during transient and steady state conditions for the human cell line AGE1.HN-AAT* (Doctoral dissertation). Retrieved from https://pure.mpg.de/pubman/item/item_2508673_4

4 Tables and Figures

Substance	Value	Dimension	Analytical method
Pluronic	1.0	g/L	as stated by Xell
NaHCO ₃	2.1	g/L	as stated by Xell
Osmolality	290.0	mOsm/kg	FPDO ^a
pH value	7.4	-	pH meter
GALC	0.5	g/L	AEC ^b
GLC	5.5	g/L	Bioprofile
AMM	0.3	mM	Bioprofile
LAC	0.0	g/L	Bioprofile
PYR	2.9	mM	AEC
GLU	636.9	μM	AEC
ALA	437.1	μM	AEC
ARG	1588.2	μM	AEC
ASN	920.4	μM	AEC
ASP	2197.9	μM	AEC
CYS	963.1	μM	AEC
GLY	1196.0	μM	AEC
HIS	642.7	μM	AEC
ILE	1744.9	μM	AEC
LEU	1893.2	μM	AEC
LYS	1256.0	μM	AEC
MET	601.3	μM	AEC
PHE	1039.4	μM	AEC
PRO	1040.5	μM	AEC
SER	3027.4	μM	AEC
THR	1502.4	μM	AEC
TRP	383.8	μM	AEC
TYR	1109.7	μM	AEC
VAL	1811.9	μM	AEC

a: freezing point depression osmometer (FPDO); b: anion exchanger chromatography (AEC);

Table 1: Measured medium composition of the 42-MAX-UB standard medium. Extracted from (Rath, 2017)

Exp. ID	<i>DR</i> (1/h)	Preculture (passage no.)	<i>GLC</i> (mM)	<i>GLN</i> (mM)	<i>GALC</i> (mM)
A	0.0140	7	10	5	3
B	0.0120	7	10	5	3
C	0.0100	5	10	5	3
D	0.0150	8	10	2	3
E	0.0133	4	8	2	3
F01	0.0150	10	10	5	0

Table 2: The dilution rates, preculture ages and the 42-Max-UB-medium modified components concentrations used in (Rath, 2017) for the 6 steady states. Table adapted from (Rath, 2017)

Variable	Exp. A		Exp. B		Exp. C		Exp. D		Exp. E	
	Average \pm SD	Rel. SD (%)	Average \pm SD	Rel. SD (%)	Average \pm SD	Rel. SD (%)	Average \pm SD	Rel. SD (%)	Average \pm SD	Rel. SD (%)
Setpoints:										
<i>DR</i> (h)	0.0140	-	0.0120	-	0.0100	-	0.0150	-	0.0133	-
GLC feed conc. (mM)	10.0	-	10.0	-	10.0	-	10.0	-	8.0	-
GLN feed conc. (mM)	5.0	-	5.0	-	2.0	-	2.0	-	2.0	-
GALC feed conc. (mM)	3.0	-	3.0	-	3.0	-	3.0	-	3.0	-
GLC/GLN ratio (mol/mol)	2	-	2	-	2	-	5	-	4	-
<i>X_V</i> (E6 cells/mL)	2.490 \pm 0.100	4.0	2.745 \pm 0.118	4.3	2.735 \pm 0.056	2.0	1.489 \pm 0.043	2.9	0.998 \pm 0.025	2.5
<i>X_D</i> (E6 cells/mL)	0.210 \pm 0.032	15.2	0.291 \pm 0.021	7.4	0.378 \pm 0.047	12.5	0.086 \pm 0.006	6.5	0.075 \pm 0.014	18.5
<i>CV_V</i> (μ L/mL)	6.56 \pm 0.48	7.4	7.19 \pm 0.67	9.4	6.23 \pm 0.21	3.4	4.33 \pm 0.27	6.3	3.40 \pm 0.17	4.9
μ (1/h)	0.0152 \pm 0.0002	1.2	0.0133 \pm 0.0001	1.1	0.0114 \pm 0.0002	1.4	0.0159 \pm 0.0001	0.6	0.0143 \pm 0.0002	1.4
<i>CD</i> (μ m)	17.1 \pm 0.25	1.5	17.2 \pm 0.19	1.1	16.3 \pm 0.24	1.5	17.7 \pm 0.30	1.5	18.7 \pm 0.20	1.1
GLC (mM)	0.0 \pm 0.00	-	0.0 \pm 0.00	-	0.0 \pm 0.00	-	0.0 \pm 0.00	-	0.0 \pm 0.00	-
LAC (mM)	2.1 \pm 1.52	-	0.5 \pm 0.02	3.5	0.6 \pm 0.04	7.3	12.5 \pm 0.60	5.0	12.7 \pm 0.40	3.0
GLN (mM)	0.8 \pm 0.08	10.0	5.5 \pm 0.19	3.5	5.9 \pm 0.09	1.5	0.5 \pm 0.10	11.1	0.5 \pm 0.10	13.7
AMM (mM)	3.4 \pm 0.22	6.7	1.9 \pm 0.06	2.9	1.7 \pm 0.02	0.9	1.5 \pm 0.03	2.1	1.6 \pm 0.02	1.6
GALC (mM)	1.9 \pm 0.08	4.5	1.9 \pm 0.06	2.9	1.7 \pm 0.02	0.9	2.1 \pm 0.10	2.5	2.4 \pm 0.10	3.2
PYR (mM)	0.2 \pm 0.00	1.8	0.1 \pm 0.00	3.2	0.1 \pm 0.00	3.2	0.3 \pm 0.04	12.6	1.1 \pm 0.10	5.3
GLU (mM)	1.2 \pm 0.08	6.2	1.3 \pm 0.01	0.5	1.0 \pm 0.05	5.0	0.7 \pm 0.10	12.9	0.8 \pm 0.10	12.8
ALA (mM)	1.4 \pm 0.09	6.4	0.2 \pm 0.03	13.8	0.1 \pm 0.01	14.2	0.3 \pm 0.02	8.9	1.0 \pm 0.05	5.2
ASP (mM)	1.6 \pm 0.06	3.9	1.5 \pm 0.05	3.2	1.3 \pm 0.02	1.2	2.4 \pm 0.17	7.1	2.8 \pm 0.30	10.8
ALAT (mg/L)	57.9 \pm 3.03	5.2	81.0 \pm 9.21	11.4	72.1 \pm 1.09	1.5	50.9 \pm 1.50	2.9	44.6 \pm 2.80	6.2
<i>Y_{CvV/glc}</i> (μ L/mmol)	711 \pm 47	6.6	827 \pm 77	9.4	753 \pm 17	2.3	482 \pm 30	6.3	518 \pm 18	3.5
<i>Y_{CvV/gln}</i> (μ L/mmol)	1679 \pm 140	8.3	1770 \pm 145	8.2	1601 \pm 52	3.3	3617 \pm 215	6.0	2837 \pm 201	7.1
<i>Y_{lac/glc}</i> (mol/mol)	0.2 \pm 0.15	-	0.0 \pm 0.01	-	0.0 \pm 0.00	-	1.2 \pm 0.10	5.3	1.8 \pm 0.10	3.2
<i>Y_{amm/glc}</i> (mol/mol)	0.8 \pm 0.05	6.6	1.2 \pm 0.04	3.6	1.3 \pm 0.03	1.9	1.0 \pm 0.05	4.9	1.1 \pm 0.10	4.8
<i>Y_{ala/gln}</i> (mol/mol)	0.2 \pm 0.02	9.5	0.0 \pm 0.00	-	0.0 \pm 0.00	-	0.0 \pm 0.00	-	0.6 \pm 0.04	7.9
<i>q_{GLC}</i> (mmol/g/L h) ^a	21.43 \pm 1.56	7.3	16.17 \pm 1.76	10.9	15.13 \pm 0.51	3.4	33.05 \pm 2.17	6.6	27.64 \pm 1.33	4.8
<i>q_{LAC}</i> (mmol/g/L h) ^a	-4.43 \pm 3.05	-	0.00 \pm 0.00	-	0.00 \pm 0.00	-	-41.23 \pm 4.13	10.0	-49.68 \pm 1.30	2.6
<i>q_{GLN}</i> (mmol/g/L h) ^a	9.10 \pm 0.82	9.0	7.55 \pm 0.72	9.5	7.12 \pm 0.31	4.3	4.40 \pm 0.28	6.4	5.07 \pm 0.41	8.1
<i>q_{AMM}</i> (mmol/g/L h) ^a	-7.19 \pm 0.67	9.3	-9.21 \pm 0.97	10.5	-9.55 \pm 0.30	3.2	-4.48 \pm 0.35	7.8	-5.66 \pm 0.33	5.9
<i>q_{PYR}</i> (mmol/g/L h) ^a	1.94 \pm 0.21	10.8	1.55 \pm 0.22	14.3	2.46 \pm 1.26	51.1	1.92 \pm 0.24	12.3	1.61 \pm 0.37	23.3
<i>q_{PYR}</i> (mmol/g/L h) ^a	5.82 \pm 0.52	8.9	4.70 \pm 0.57	12.1	4.48 \pm 0.11	2.4	9.10 \pm 0.64	7.1	7.04 \pm 0.55	7.8
<i>q_{GLU}</i> (mmol/g/L h) ^a	-1.31 \pm 0.18	14.0	-1.05 \pm 0.10	9.4	-0.52 \pm 0.09	17.9	-0.71 \pm 0.31	43.5	-0.88 \pm 0.38	43.8
<i>q_{ALA}</i> (mmol/g/L h) ^a	-2.14 \pm 0.27	12.8	0.39 \pm 0.03	6.7	0.54 \pm 0.03	5.9	0.10 \pm 0.12	127.8	-2.81 \pm 0.26	9.4
<i>q_{ASP}</i> (mmol/g/L h) ^a	1.19 \pm 0.19	15.9	1.20 \pm 0.10	8.1	1.49 \pm 0.05	3.5	-0.41 \pm 0.62	150.7	-2.16 \pm 1.15	53.1
<i>q_{ALAT}</i> (mg/cell d) ^a	-7.87 \pm 0.19	2.4	-8.54 \pm 1.34	15.7	-6.33 \pm 0.19	3.0	-12.31 \pm 0.60	4.9	-14.29 \pm 1.08	7.5

^a: Substrate uptake is indicated by a positive rate, whereas a negative value indicates a production rate.

Table 3: Steady-state values reported by (Rath, 2017) for different parameters from continuous cultivations, varying GLC and GLN feed concentrations and with 3 mM GAL. Table taken from (Rath, 2017)

Variable	Exp. F01	
	Average \pm SD	Rel. SD (%)
Setpoints:		
<i>DR</i> (1/h)	0.0150	-
GLC feed conc. (mM)	10	-
GLN feed conc. (mM)	5	-
GALC feed conc. (mM)	0	-
GLC/GLN ratio (mol/mol)	2	-
X_V (E6 cells/mL)	1.701 ± 0.023	1.3
X_D (E6 cells/mL)	0.081 ± 0.004	5.1
CV_V (μ L/mL)	4.54 ± 0.24	5.2
μ (1/h)	0.0156 ± 0.0000	0.2
CD (μ m)	17.2 ± 0.33	1.9
GLC (mM)	0.0 ± 0.00	-
LAC (mM)	7.8 ± 0.64	8.2
GLN (mM)	1.9 ± 0.18	9.3
AMM (mM)	3.4 ± 0.17	5.1
PYR (mM)	0.4 ± 0.02	6.6
GLU (mM)	1.0 ± 0.05	5.2
ALA (mM)	0.9 ± 0.07	8.2
ASP (mM)	1.9 ± 0.21	10.8
AIAT (mg/L)	44.0 ± 1.71	3.9
$Y_{CVV/glc}$ (μ L/mmol)	626 ± 25	4.0
$Y_{CVV/gln}$ (μ L/mmol)	1865 ± 201	10.8
$Y_{lac/glc}$ (mol/mol)	0.9 ± 0.07	8.2
$Y_{amm/gln}$ (mol/mol)	1.3 ± 0.04	3.1
$Y_{ala/gln}$ (mol/mol)	0.2 ± 0.02	8.5
q_{GLC} (nmol/ μ L h) ^a	24.9 ± 1.08	4.3
q_{LAC} (nmol/ μ L h) ^a	-22.6 ± 1.27	5.6
q_{GLN} (nmol/ μ L h) ^a	8.4 ± 1.07	12.7
q_{AMM} (nmol/ μ L h) ^a	-10.9 ± 1.13	10.3
q_{PYR} (nmol/ μ L h) ^a	4.5 ± 0.31	6.9
q_{GLU} (nmol/ μ L h) ^a	-0.7 ± 0.15	20.4
q_{ALA} (nmol/ μ L h) ^a	-1.99 ± 0.33	16.3
q_{ASP} (nmol/ μ L h) ^a	1.65 ± 0.64	39.0
q_{AIAT} (pg/cell d) ^a	-9.2 ± 0.25	2.7

^a: Substrate uptake is indicated by a positive rate, whereas a negative value indicates a production rate.

Table 4: Steady-state values reported by (Rath, 2017) of different parameters from continuous cultivations, varying GLC and GLN feed concentrations and without GAL. Table taken from (Rath, 2017)

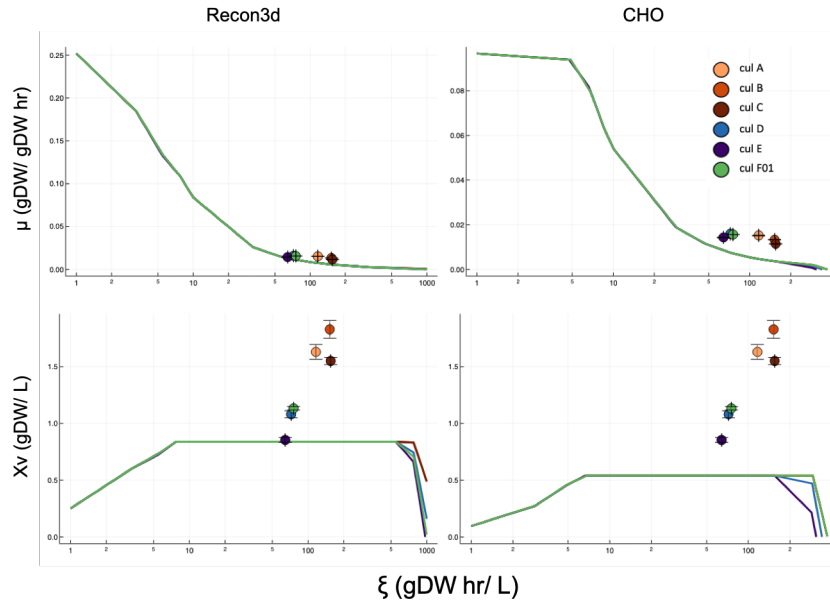


Figure 1: FBAwMC results showing the growth rate, μ , and the viable cell density, X_v , dependence of ξ for the six steady states. The solid lines represent the model predictions and the colored points show the experimental results. The model data was obtained for feed mediums with low (Recon3D) and zero (CHO) concentration of phosphatidylethanolamine

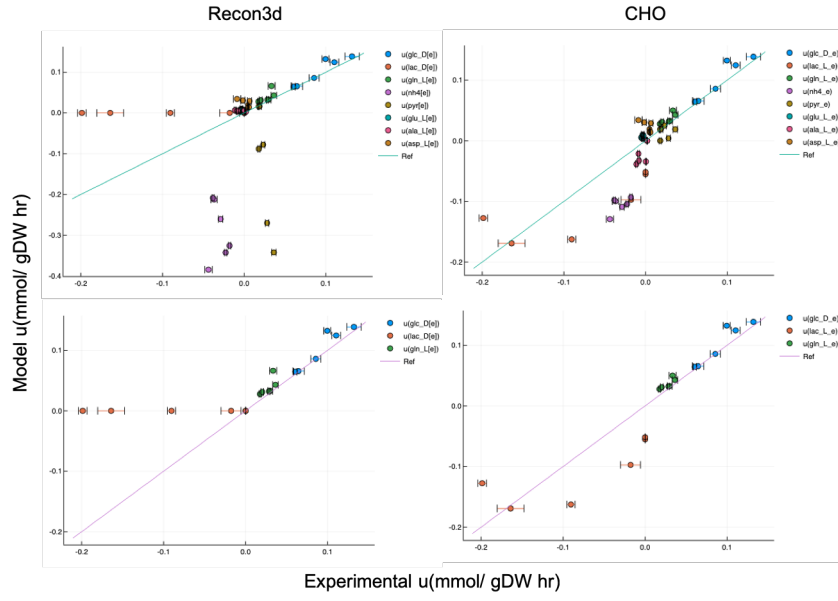


Figure 2: Correlations of all the experimental uptakes, upper graphs, and a selected subset, inferior graphs, respect to the predicted value from FBAwMC with low (Recon3D) and zero (CHO) concentration of phosphatidylethanolamine.

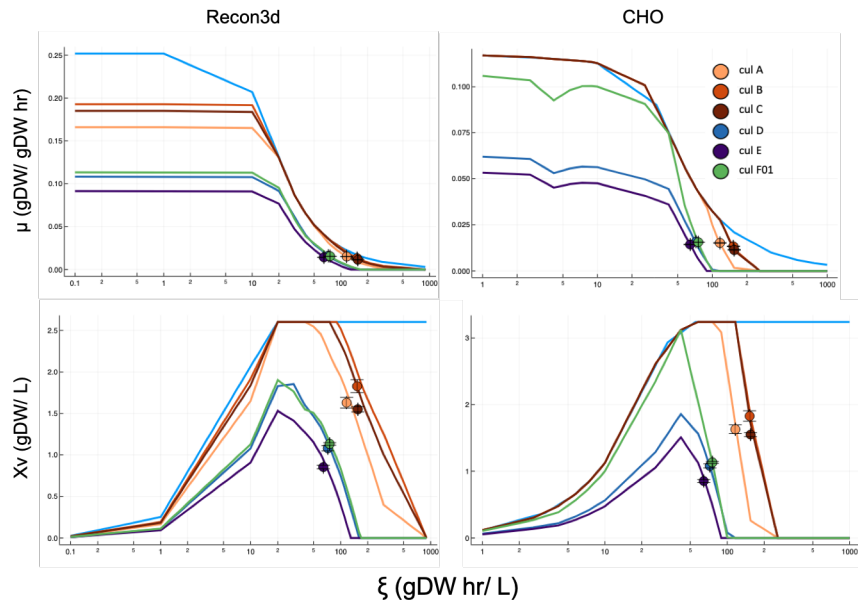


Figure 3: EP and FBA results showing the growth rate and the viable cell density dependence of ξ for the six culture conditions. The solid lines represent the model predictions and the color points show the experimental results. FBA results are shown as the solid light blue line.

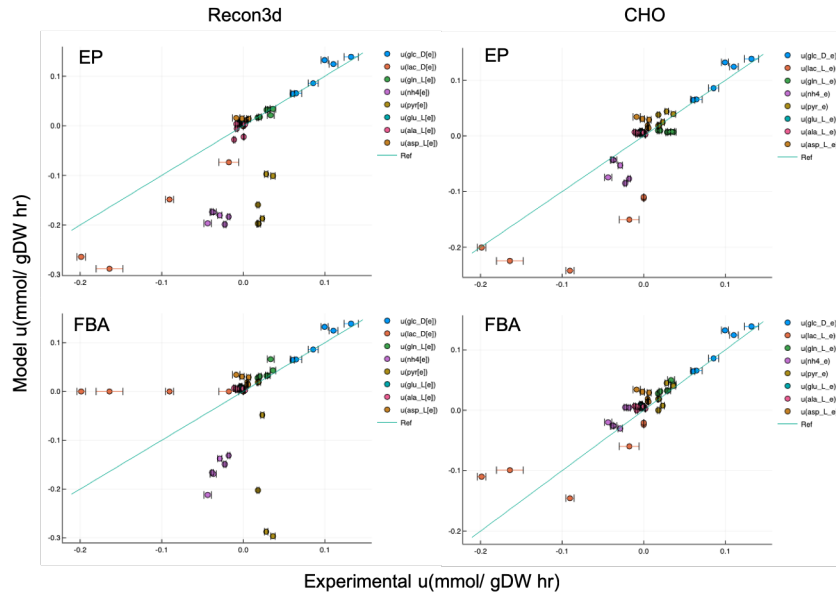


Figure 4: Correlations of all the experimental uptakes compared with the predicted value from EP and FBA for GEMs with high concentration of phosphatidylethanolamine.

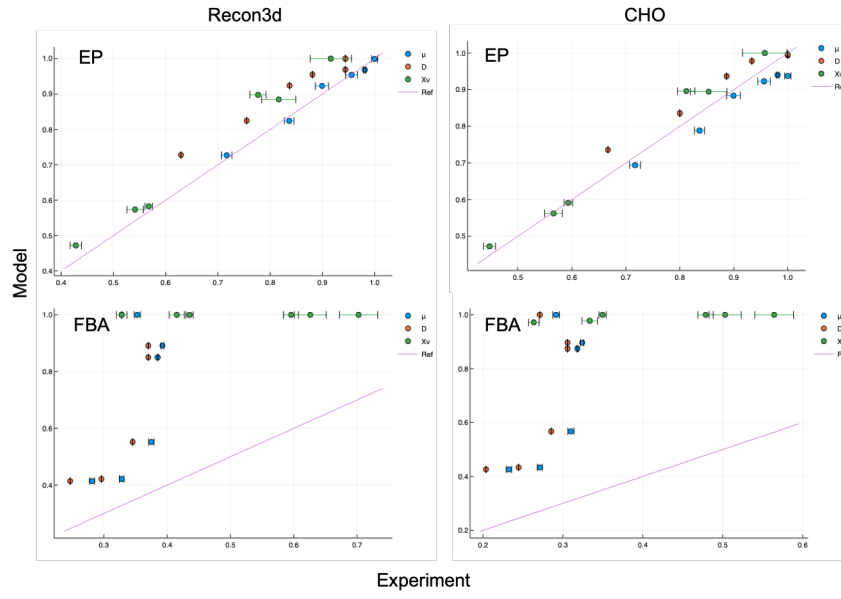


Figure 5: Normalized correlations of the experimental μ , D and Xv compared with the predicted value from EP and FBA for GEMs with high concentration of phosphatidylethanolamine.