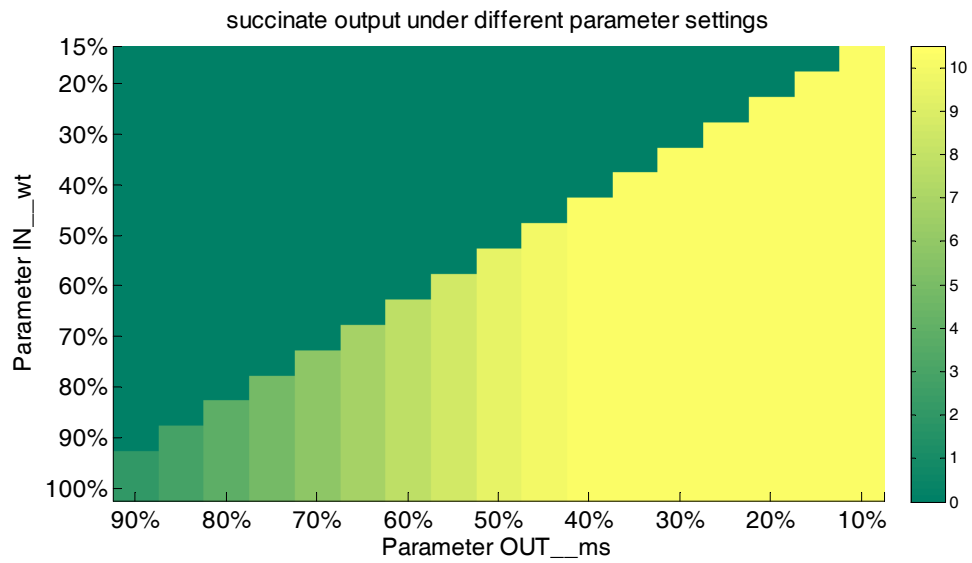
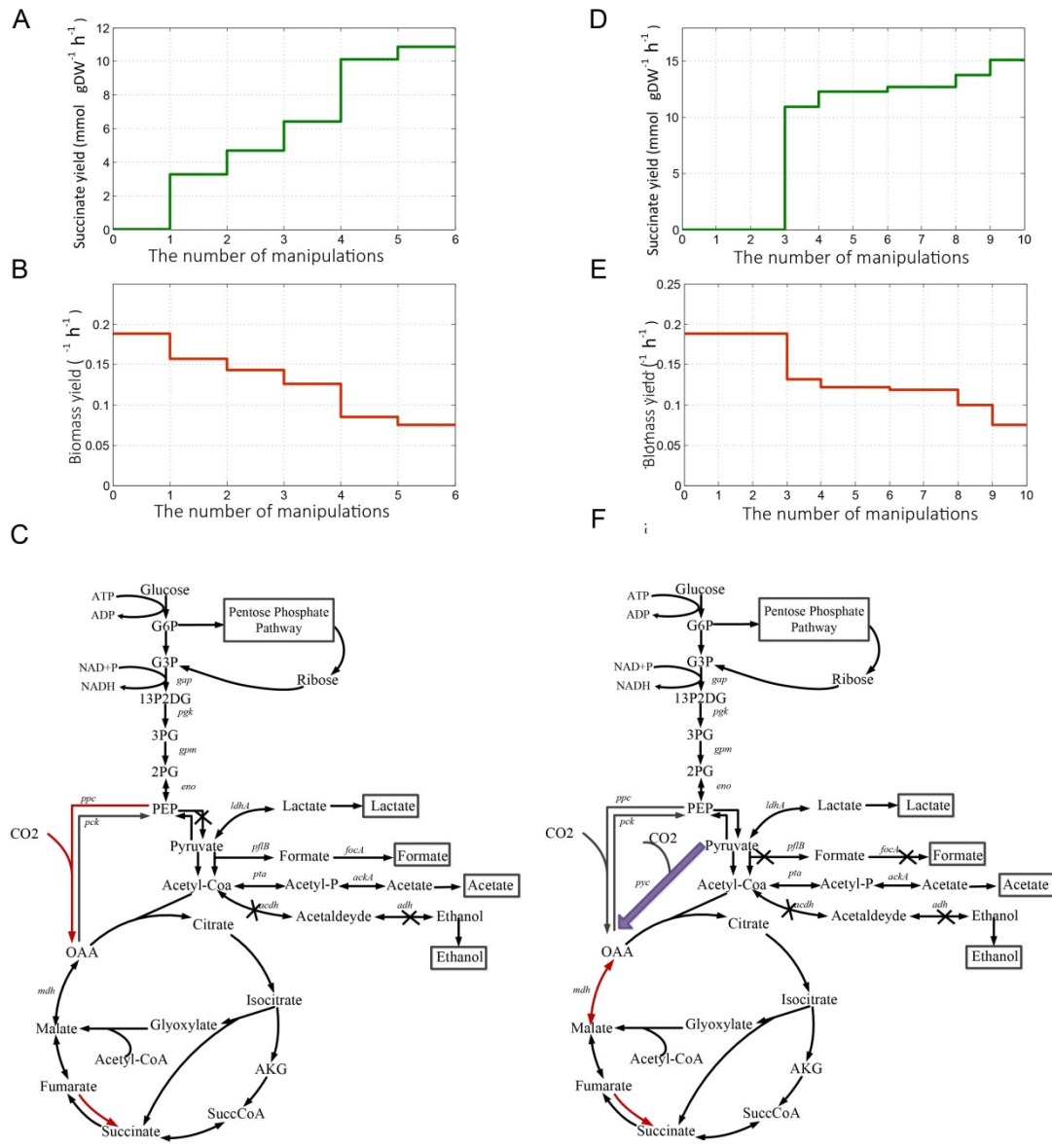


## Supplementary



Supplementary Figure S1. Succinate output in the predicted manipulations under different ( $IN\_wt$ ,  $OUT\_ms$ ) parameter settings. There are 170 settings when  $IN\_wt=[100\%, 95\%, 90\%, 85\%, 80\%, 75\%, 70\%, 65\%, 60\%, 55\%, 50\%, 45\%, 40\%, 35\%, 30\%, 25\%, 20\%, 15\%]$ ;  $OUT\_ms=[90\%, 85\%, 80\%, 75\%, 70\%, 65\%, 60\%, 55\%, 50\%, 45\%, 40\%, 35\%, 30\%, 25\%, 20\%, 15\%, 10\%]$ . The unit for the succinate production is  $\text{mmol gDW}^{-1} \text{h}^{-1}$ .



Supplementary Figure S2. The optimization procedure of succinate production by OP-Synthetic in *E. coli* iAF1260 model. Without the addition of gene *pyc*: (A) Succinate yield after every step. (B) Biomass yield after every step. (C) The optimization strategy shown in iAF1260 *E. coli* map; With the addition of gene *pyc*: (D) Succinate yield after every step. (E) Biomass yield after every step. (F) The optimization strategy shown in iAF1260 *E. coli* map. The signal × represents gene deletion. Red lines represent gene up regulation. The purple line represents the addition of the heterologous gene *pyc* which needs overexpression.

Supplementary Table S1. Setting of exchange reactions by OP-Synthetic

	Succinate overproduction	Succinate overproduction with gene <i>pyc</i>	Neuac overproduction
Export reactions that input or output value is unlimited	'EX_succ(e)' 'EX_h2o(e)' 'EX_co2(e)' 'EX_nh4(e)'	'EX_succ(e)' 'EX_h2o(e)' 'EX_co2(e)' 'EX_nh4(e)'	'EX_ca2(e)' 'EX_cl(e)' 'EX_cobalt2(e)' 'EX_cu2(e)' 'EX_mg2(e)' 'EX_mn2(e)' 'EX_mobd(e)' 'EX_zn2(e)' 'EX_k(e)' 'EX_o2(e)' 'EX_nh4(e)' 'EX_so4(e)' 'EX_nh4(e)' 'EX_etoh(e)' 'EX_h2o(e)'

(Note that the reaction names are consistent with the *E. coli* iAF1260 metabolic model).

Supplementary Table S2. The succinate optimization strategy under different (*IN\_wt*, *OUT\_ms*) parameter settings in core *E.coli* metabolic network.

<i>(IN_wt</i> , <i>OUT_ms</i> )	Optimization strategy (comparison with (90%, 40%))
(90%,40%)	ALCD2x- ETOHt2r- EX_etoh(e) (×), ACALD (×), PYK (×), FUM (↑), MDH (↑), PPC (↑), ACONTb-ACONTa-CS(↓)
<b><i>OUT_ms</i>=10%</b>	
(15%,10%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(20%,10%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(25%,10%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(30%,10%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(35%,10%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(40%,10%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(45%,10%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(50%,10%)	-PPC(↑),-ACONTb-ACONTa-CS(↓)
(55%,10%)	-ACONTb-ACONTa-CS(↓)
(60%,10%)	-ACONTb-ACONTa-CS(↓)
(65%,10%)	-ACONTb-ACONTa-CS(↓)
(70%,10%)	-ACONTb-ACONTa-CS(↓)
(75%,10%)	-ACONTb-ACONTa-CS(↓)
(80%,10%)	Same
(85%,10%)	-PPC (↑)
(90%,10%)	Same
(95%,10%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓)
(100%,10%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), +ATPS4r(↓)
<b><i>OUT_ms</i>=15%</b>	
(20%,15%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(25%,15%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(30%,15%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(35%,15%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(40%,15%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(45%,15%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(50%,15%)	-PPC(↑), -ACONTb-ACONTa-CS(↓)
(55%,15%)	-ACONTb-ACONTa-CS(↓)
(60%,15%)	-ACONTb-ACONTa-CS(↓)
(65%,15%)	-ACONTb-ACONTa-CS(↓)
(70%,15%)	-ACONTb-ACONTa-CS(↓)
(75%,15%)	-ACONTb-ACONTa-CS(↓)
(80%,15%)	Same
(85%,15%)	Same

(90%,15%)	Same
(95%,15%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓)
(100%,15%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)
<b>OUT_ms =20%</b>	
(25%,20%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(30%,20%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(35%,20%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(40%,20%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(45%,20%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(50%,20%)	- PPC(↑), -ACONTb-ACONTa-CS(↓)
(55%,20%)	-ACONTb-ACONTa-CS(↓)
(60%,20%)	- ACONTb-ACONTa-CS(↓)
(65%,20%)	- ACONTb-ACONTa-CS(↓)
(70%,20%)	- ACONTb-ACONTa-CS(↓)
(75%,20%)	-ACONTb-ACONTa-CS(↓)
(80%,20%)	Same
(85%,20%)	Same
(90%,20%)	Same
(95%,20%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓)
(100%,20%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)
<b>OUT_ms =25%</b>	
(30%, 25%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(35%, 25%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(40%, 25%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(45%, 25%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(50%, 25%)	-PPC(↑), -ACONTb-ACONTa-CS(↓)
(55%, 25%)	-ACONTb-ACONTa-CS(↓)
(60%, 25%)	-ACONTb-ACONTa-CS(↓)
(65%, 25%)	-ACONTb-ACONTa-CS(↓), + ACCOAC(↓)
(70%, 25%)	-ACONTb-ACONTa-CS(↓), + ACCOAC(↓)
(75%, 25%)	-ACONTb-ACONTa-CS(↓), + ACCOAC(↓)
(80%, 25%)	Same
(85%, 25%)	Same
(90%, 25%)	Same
(95%, 25%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓)
(100%, 25%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)
<b>OUT_ms =30%</b>	
(35%, 30%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(40%, 30%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(45%, 30%)	-PPC(↑), -ACONTb-ACONTa-CS(↓)
(50%, 30%)	-ACONTb-ACONTa-CS(↓)

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(55%, 30%)	-ACONTb-ACONTa-CS(↓)
(60%, 30%)	-ACONTb-ACONTa-CS(↓)
(65%, 30%)	-ACONTb-ACONTa-CS(↓)
(70%, 30%)	Same
(75%, 30%)	Same
(80%, 30%)	Same
(85%, 30%)	Same
(90%, 30%)	Same
(95%, 30%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓)
(100%, 30%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)

#### ***OUT\_ms =35%***

(40%, 35%)	- FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(45%, 35%)	-PPC(↑), -ACONTb-ACONTa-CS(↓)
(50%, 35%)	-ACONTb-ACONTa-CS(↓)
(55%, 35%)	-ACONTb-ACONTa-CS(↓)
(60%, 35%)	-ACONTb-ACONTa-CS(↓)
(65%, 35%)	-ACONTb-ACONTa-CS(↓)
(70%, 35%)	-ACONTb-ACONTa-CS(↓)
(75%, 35%)	Same
(80%, 35%)	Same
(85%, 35%)	-MDH(↑)
(90%, 35%)	Same
(95%, 35%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓)
(100%, 35%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)

#### ***OUT\_ms =40%***

(45%, 40%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(50%, 40%)	-PPC(↑), -ACONTb-ACONTa-CS(↓)
(55%, 40%)	-ACONTb-ACONTa-CS(↓)
(60%, 40%)	-ACONTb-ACONTa-CS(↓)
(65%, 40%)	-ACONTb-ACONTa-CS(↓)
(70%, 40%)	-ACONTb-ACONTa-CS(↓)
(75%, 40%)	-ACONTb-ACONTa-CS(↓)
(80%, 40%)	Same
(85%, 40%)	-MDH(↑)
(90%, 40%)	Same
(95%, 40%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓)
(100%, 40%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)

#### ***OUT\_ms =45%***

(50%, 45%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓)
(55%, 45%)	-ACONTb-ACONTa-CS(↓)
(60%, 45%)	-ACONTb-ACONTa-CS(↓)

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(65%, 45%)	-ACONTb-ACONTa-CS(↓)
(70%, 45%)	-ACONTb-ACONTa-CS(↓)
(75%, 45%)	-ACONTb-ACONTa-CS(↓)
(80%, 45%)	Same
(85%, 45%)	Same
(90%, 45%)	Same
(95%, 45%)	+FORti-EX_for(e) (↓), +PFL(↓)
(100%, 45%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)

#### **OUT\_ms =50%**

(55%, 50%)	-PPC(↑), -ACONTb-ACONTa-CS(↓)
(60%, 50%)	-ACONTb-ACONTa-CS(↓)
(65%, 50%)	-ACONTb-ACONTa-CS(↓)
(70%, 50%)	-ACONTb-ACONTa-CS(↓)
(75%, 50%)	-ACONTb-ACONTa-CS(↓)
(80%, 50%)	-ACONTb-ACONTa-CS(↓)
(85%, 50%)	Same
(90%, 50%)	Same
(95%, 50%)	+FORti-EX_for(e) (↓), +PFL(↓)
(100%, 50%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)

#### **OUT\_ms =55%**

(60%, 55%)	-ACONTb-ACONTa-CS(↓), -ALCD2x- ETOHt2r- EX_etoh(e) (×)
(65%, 55%)	-ACONTb-ACONTa-CS(↓)
(70%, 55%)	-ACONTb-ACONTa-CS(↓)
(75%, 55%)	-ACONTb-ACONTa-CS(↓)
(80%, 55%)	-ACONTb-ACONTa-CS(↓)
(85%, 55%)	-ACONTb-ACONTa-CS(↓)
(90%, 55%)	-ACONTb-ACONTa-CS(↓)
(95%, 55%)	Same
(100%, 55%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)

#### **OUT\_ms =60%**

(65%, 60%)	-FUM(↑), -MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓), - ALCD2x- ETOHt2r- EX_etoh(e) (×)
(70%, 60%)	-ACONTb-ACONTa-CS(↓), -ALCD2x- ETOHt2r- EX_etoh(e) (×)
(75%, 60%)	-ACONTb-ACONTa-CS(↓)
(80%, 60%)	-ACONTb-ACONTa-CS(↓)
(85%, 60%)	-ACONTb-ACONTa-CS(↓)
(90%, 60%)	-ACONTb-ACONTa-CS(↓)
(95%, 60%)	-ACONTb-ACONTa-CS(↓)
(100%, 60%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), + ATPS4r(↓)

#### **OUT\_ms =65%**

(70%, 65%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓), -ALCD2x-ETOHt2r-EX_etoh(e) (×)
(75%, 65%)	-ACONTb-ACONTa-CS(↓), -ALCD2x-ETOHt2r-EX_etoh(e) (×)
(80%, 65%)	-ACONTb-ACONTa-CS(↓)
(85%, 65%)	-ACONTb-ACONTa-CS(↓)
(90%, 65%)	-ACONTb-ACONTa-CS(↓)
(95%, 65%)	-ACONTb-ACONTa-CS(↓)
(100%, 65%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), +ATPS4r(↓)
<b>OUT_ms =70%</b>	
(75%, 70%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓), -ALCD2x-ETOHt2r-EX_etoh(e) (×)
(80%, 70%)	-ACONTb-ACONTa-CS(↓)
(85%, 70%)	-ACONTb-ACONTa-CS(↓)
(90%, 70%)	-ACONTb-ACONTa-CS(↓)
(95%, 70%)	-ACONTb-ACONTa-CS(↓)
(100%, 70%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), +ATPS4r(↓)
<b>OUT_ms =75%</b>	
(80%, 75%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓), -ALCD2x-ETOHt2r-EX_etoh(e) (×)
(85%, 75%)	-ACONTb-ACONTa-CS(↓), -MDH(↑)
(90%, 75%)	-ACONTb-ACONTa-CS(↓)
(95%, 75%)	-ACONTb-ACONTa-CS(↓)
(100%, 75%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), +ATPS4r(↓), -PPC(↑)
<b>OUT_ms =80%</b>	
(85%, 80%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓), -ALCD2x-ETOHt2r-EX_etoh(e) (×)
(90%, 80%)	-ACONTb-ACONTa-CS(↓)
(95%, 80%)	-ACONTb-ACONTa-CS(↓)
(100%, 80%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), +ATPS4r(↓), -PPC(↑)
<b>OUT_ms =85%</b>	
(90%, 85%)	-MDH(↑), -PPC(↑), -ACONTb-ACONTa-CS(↓), -ALCD2x-ETOHt2r-EX_etoh(e)(×)
(95%, 85%)	-ACONTb-ACONTa-CS(↓)
(100%, 85%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), +ATPS4r(↓), -PPC(↑)
<b>OUT_ms =90%</b>	
(95%, 90%)	-ACONTb-ACONTa-CS(↓)
(100%, 90%)	+FORti-EX_for(e) (↓), +PFL(↓), +RPI(↓), +THD2(↓), +GLUDy(↓), +ATPS4r(↓), -PPC(↑)

Comparison of results of different parameter settings to (IN\_wt, OUT\_ms)= (90%, 40%). ‘+’

indicates added manipulations, '-' indicates deleted manipulations. (Note that the reaction names are consistent with the *E. coli* iAF1260 metabolic model).

Supplementary Table S3. The succinate optimization strategy under different (*IN\_wt*, *OUT\_ms*) parameter settings using iAF1260 metabolic network.

<b>(<i>IN_wt</i>, <i>OUT_ms</i>)</b>	<b>Optimization strategy (comparison with (90%, 40%))</b>
<b>(90%,40%)</b>	ALCD2x- ETOHt2r- EX_etoh(e) (×) , ACALD (×) , PYK (×) , FRD2(↑), PPC(↑)
<b><i>OUT_ms</i> =10%</b>	
<b>(15%,10%)</b>	- FRD2(↑), -PPC(↑)
<b>(20%,10%)</b>	- FRD2(↑), -PPC(↑)
<b>(25%,10%)</b>	- FRD2(↑), -PPC(↑)
<b>(30%,10%)</b>	- FRD2(↑), -PPC(↑)
<b>(35%,10%)</b>	- FRD2(↑), -PPC(↑)
<b>(40%,10%)</b>	- FRD2(↑), -PPC(↑)
<b>(45%,10%)</b>	-PPC(↑)
<b>(50%,10%)</b>	- FRD2(↑)
<b>(55%,10%)</b>	Same
<b>(60%,10%)</b>	Same
<b>(65%,10%)</b>	+MDH(↑)
<b>(70%,10%)</b>	+MDH(↑)
<b>(75%,10%)</b>	Same
<b>(80%,10%)</b>	Same
<b>(85%,10%)</b>	-PPC(↑)
<b>(90%,10%)</b>	Same
<b>(95%,10%)</b>	+ACONTb-ACONTa-CS(↓)
<b>(100%,10%)</b>	+ACONTb-ACONTa-CS(↓),+FORti-EX_for(e) (↓), +PFL(↓), +PGCD(↓), +PGI(↓), +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP_L(↓)
<b><i>OUT_ms</i> =15%</b>	
<b>(20%,15%)</b>	- FRD2(↑), -PPC(↑)
<b>(25%,15%)</b>	- FRD2(↑), -PPC(↑)
<b>(30%,15%)</b>	- FRD2(↑), -PPC(↑)
<b>(35%,15%)</b>	- FRD2(↑)
<b>(40%,15%)</b>	- FRD2(↑)
<b>(45%,15%)</b>	Same
<b>(50%,15%)</b>	- FRD2(↑)
<b>(55%,15%)</b>	+MDH(↑)
<b>(60%,15%)</b>	Same
<b>(65%,15%)</b>	+MDH(↑)
<b>(70%,15%)</b>	+MDH(↑)
<b>(75%,15%)</b>	Same
<b>(80%,15%)</b>	Same

(85%,15%)	Same
(90%,15%)	Same
(95%,15%)	+ACONTb-ACONTa-CS(↓)
(100%,15%)	+ACONTb-ACONTa-CS(↓),+FORti-EX_for(e) (↓), +PFL(↓), +PGCD(↓), +PGI(↓), +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP_L(↓)

#### **OUT\_ms =20%**

(25%,20%)	- FRD2(↑), -PPC(↑)
(30%,20%)	- FRD2(↑), -PPC(↑)
(35%,20%)	- FRD2(↑)
(40%,20%)	- FRD2(↑)
(45%,20%)	Same
(50%,20%)	- FRD2(↑)
(55%,20%)	Same
(60%,20%)	Same
(65%,20%)	+MDH(↑)
(70%,20%)	+MDH(↑)
(75%,20%)	Same
(80%,20%)	Same
(85%,20%)	Same
(90%,20%)	Same
(95%,20%)	+ACONTb-ACONTa-CS(↓)
(100%,20%)	+ACONTb-ACONTa-CS(↓),+FORti-EX_for(e) (↓), +PFL(↓), +PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑) +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP_L(↓)

#### **OUT\_ms =25%**

(30%, 25%)	-FRD2(↑), -PPC(↑)
(35%, 25%)	-FRD2(↑), -PPC(↑)
(40%, 25%)	-FRD2(↑), -PPC(↑)
(45%, 25%)	+PFL (×) , +EX_for(e)-FORtex(e) (×)
(50%, 25%)	+PFL (×) , +EX_for(e)-FORtex(e) (×)
(55%, 25%)	Same
(60%, 25%)	Same
(65%, 25%)	+ MDH(↑)
(70%, 25%)	+ MDH(↑)
(75%, 25%)	+PFL (×) ,+ EX_for(e)-FORtex(e) (×)
(80%, 25%)	Same
(85%, 25%)	Same
(90%, 25%)	Same
(95%, 25%)	+ACONTb-ACONTa-CS(↓)
(100%, 25%)	+ACONTb-ACONTa-CS(↓),+FORti-EX_for(e) (↓), +PFL(↓), +PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑) +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP_L(↓)

#### **OUT\_ms =30%**

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(35%, 30%)	-FRD2(↑), -PPC(↑)
(40%, 30%)	-FRD2(↑), -PPC(↑)
(45%, 30%)	Same
(50%, 30%)	+ MDH (↑)
(55%, 30%)	- PPC (↑)
(60%, 30%)	Same
(65%, 30%)	+ MDH (↑)
(70%, 30%)	+ MDH (↑)
(75%, 30%)	Same
(80%, 30%)	Same
(85%, 30%)	Same
(90%, 30%)	Same
(95%, 30%)	+ACONTb-ACONTa-CS(↓)
(100%, 30%)	+ACONTb-ACONTa-CS(↓),+FORti-EX_for(e) (↓), +PFL(↓), +PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑) +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP_L(↓)

#### **OUT\_ms=35%**

(40%, 35%)	-FRD2(↑), -PPC(↑)
(45%, 35%)	Same
(50%, 35%)	+ MDH (↑)
(55%, 35%)	- PPC (↑)
(60%, 35%)	Same
(65%, 35%)	+ MDH (↑)
(70%, 35%)	+ MDH (↑)
(75%, 35%)	Same
(80%, 35%)	Same
(85%, 35%)	Same
(90%, 35%)	Same
(95%, 35%)	+ACONTb-ACONTa-CS(↓)
(100%, 35%)	+ACONTb-ACONTa-CS(↓),+FORti-EX_for(e) (↓), +PFL(↓), +PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑) +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP_L(↓)

#### **OUT\_ms=40%**

(45%, 40%)	- PPC (↑)
(50%, 40%)	-FRD2 (↑) , + MDH (↑)
(55%, 40%)	- PPC (↑)
(60%, 40%)	Same
(65%, 40%)	+ MDH (↑)
(70%, 40%)	+ MDH (↑)
(75%, 40%)	Same
(80%, 40%)	Same
(85%, 40%)	Same
(90%, 40%)	Same
(95%, 40%)	+ACONTb-ACONTa-CS(↓)

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(100%, 40%) +ACONTb-ACONTa-CS(↓),+FORti-EX\_for(e) (↓), +PFL(↓),  
+PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑)  
+GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP\_L(↓)

**OUT\_ms =45%**

(50%, 45%) -FRD2 (↑)  
(55%, 45%) - PPC (↑)  
(60%, 45%) Same  
(65%, 45%) + MDH (↑)  
(70%, 45%) + MDH (↑)  
(75%, 45%) Same  
(80%, 45%) Same  
(85%, 45%) Same  
(90%, 45%) Same  
(95%, 45%) Same  
(100%, 45%) +ACONTb-ACONTa-CS(↓),+FORti-EX\_for(e) (↓), +PFL(↓),  
+PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑)  
+GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP\_L(↓)

**OUT\_ms =50%**

(55%, 50%) -PPC(↑)  
(60%, 50%) Same  
(65%, 50%) Same  
(70%, 50%) + MDH (↑)  
(75%, 50%) Same  
(80%, 50%) Same  
(85%, 50%) Same  
(90%, 50%) Same  
(95%, 50%) Same  
(100%, 50%) +ACONTb-ACONTa-CS(↓),+FORti-EX\_for(e) (↓), +PFL(↓),  
+PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑)  
+GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP\_L(↓)

**OUT\_ms =55%**

(60%, 55%) -ALCD2x- ETOHt2r- EX\_etoh(e) (×) , -PYK (×)  
(65%, 55%) -ALCD2x- ETOHt2r- EX\_etoh(e) (×) , -PYK (×) , + MDH (↑)  
(70%, 55%) -PYK (×) , + MDH (↑)  
(75%, 55%) -PYK (×)  
(80%, 55%) -PYK (×)  
(85%, 55%) -ALCD2x- ETOHt2r- EX\_etoh(e) (×)  
(90%, 55%) - PYK (×)  
(95%, 55%) - PYK (×)  
(100%, 55%) +ACONTb-ACONTa-CS(↓),+FORti-EX\_for(e) (↓), +PFL(↓),  
+PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑)  
+GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP\_L(↓)

**OUT\_ms =60%**

(65%, 60%) + MDH (↑) , -ALCD2x- ETOHt2r- EX\_etoh(e) (×) , -PYK (×)

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(70%, 60%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -PYK (x) , +MDH (↑)  
 (75%, 60%) -PYK (x)  
 (80%, 60%) -PYK (x)  
 (85%, 60%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -PYK (x)  
 (90%, 60%) -PYK (x)  
 (95%, 60%) -PYK (x)  
 (100%, 60%) +ACONTb-ACONTa-CS(↓),+FORti-EX\_for(e) (↓), +PFL(↓),  
 +PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑)  
 +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP\_L(↓)

**OUT\_ms =65%**

(70%, 65%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -ACALD (x) , -PYK (x)  
 (75%, 65%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -ACALD (x) , -PYK (x)  
 (80%, 65%) -PYK (x)  
 (85%, 65%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -ACALD (x)  
 (90%, 65%) -PYK (x)  
 (95%, 65%) -PYK (x)  
 (100%, 65%) +ACONTb-ACONTa-CS(↓),+FORti-EX\_for(e) (↓), +PFL(↓),  
 +PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑)  
 +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP\_L(↓)

**OUT\_ms =70%**

(75%, 70%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -ACALD (x) , -PYK (x)  
 (80%, 70%) -PYK (x)  
 (85%, 70%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -ACALD (x) , -PYK (x)  
 (90%, 70%) -PYK (x)  
 (95%, 70%) -PYK (x)  
 (100%, 70%) +ACONTb-ACONTa-CS(↓),+FORti-EX\_for(e) (↓), +PFL(↓),  
 +PGCD(↓), +PGI(↓), -FRD2(↑),  
 +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP\_L(↓)

**OUT\_ms =75%**

(80%, 75%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -ACALD (x) , -PYK (x)  
 (85%, 75%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -ACALD (x) , -PYK (x)  
 (90%, 75%) -PYK (x)  
 (95%, 75%) -PYK (x) , -PPC(↑)  
 (100%, 75%) +ACONTb-ACONTa-CS(↓),+FORti-EX\_for(e) (↓), +PFL(↓),  
 +PGCD(↓), +PGI(↓), -FRD2(↑), -PPC(↑)  
 +GLUDy(↓),+PPKr(↓),+PSERT(↓),+PSP\_L(↓)

**OUT\_ms =80%**

(85%, 80%) -ALCD2x- ETOHt2r- EX\_etoh(e) (x) , -ACALD (x) , -PYK (x) ,  
 -FRD2(↑), -PPC(↑)  
 (90%, 80%) -PYK (x) , -PPC(↑)  
 (95%, 80%) -PYK (x) , -PPC(↑)  
 (100%, 80%) +FORti-EX\_for(e) (↓), +PFL(↓),-FRD2(↑), +GLUDy(↓),  
 +PPKr(↓)

**OUT\_ms =85%**

<b>(90%, 85%)</b>	-ALCD2x- ETOHt2r- EX_etoh(e) (×) , -ACALD (×) , -PYK (×) , -FRD2(↑), -PPC(↑)
<b>(95%, 85%)</b>	-PYK (×) , -PPC(↑)
<b>(100%, 85%)</b>	+FORti-EX_for(e) (↓), +PFL(↓), -FRD2(↑), -PPC(↑) +GLUDy(↓), +PPK <sub>r</sub> (↓)
<b><i>OUT_ms</i> =90%</b>	
<b>(95%, 90%)</b>	-PYK (×) , -PPC(↑)
<b>(100%, 90%)</b>	+FORti-EX_for(e) (↓), +PFL(↓), -FRD2(↑), -PPC(↑) +GLUDy(↓)

Comparison of results of different parameter settings to (*IN\_wt*, *OUT\_ms*)=(90%, 40%). ‘+’ indicates added manipulations, ‘-’ indicates deleted manipulations. (Note that the reaction names are consistent with the *E. coli* iAF1260 metabolic model).

We chose *IN\_wt* from 15% to 100% and *OUT\_ms* from 10% to 90% both with 5% increase at each step. We have applied OP-Synthetic on all the 170 parameter combinations when *OUT\_ms*<*IN\_wt* on succinate overproduction both in core *E.coli* model and iAF1260 genome-scale model. The optimization strategies and the object yields under different parameter settings are presented in Fig S1, Table S2, and Table S3. Following is the conclusion:

- (A) Compared with the results gotten by (*IN\_wt*, *OUT\_ms*)= (90%, 40%), 53 out of the 170 parameter settings can get the same optimization strategies in succinate overproduction using genome-scale model (Table S3). Almost all of the other parameter settings can get the results within 2 different manipulations.
- (B) In some parameter settings, like (*IN\_wt*, *OUT\_ms*)= (75%, 70%), (*IN\_wt*, *OUT\_ms*)= (80%, 75%) in Table S3, the ALCD2x- ETOHt2r- EX\_etoh(e) (×) , ACALD (×) , PYK (×) can’t be found. The reason is that the reaction range between the wild type and the theoretical manipulated type is too close, since the difference between *IN\_wt* and *OUT\_ms* is too small. On the contrary, when (*IN\_wt*, *OUT\_ms*)= (95%, 10%), OP-Synthetic will predict more manipulations, because of a big difference between *IN\_wt* and *OUT\_ms*.
- (C) When *IN\_wt*=100%, more manipulations are found, since  $I_{bound}$  interval is smaller, more reaction will exceed  $I_{bound}$ .
- (D). A lower *OUT\_ms* tends to get a higher objective yield in theory.