

Supplementary Material for

Title: Teaching an old pET new tricks: Tuning of inclusion body formation and properties by a mixed feed system in *E.coli*

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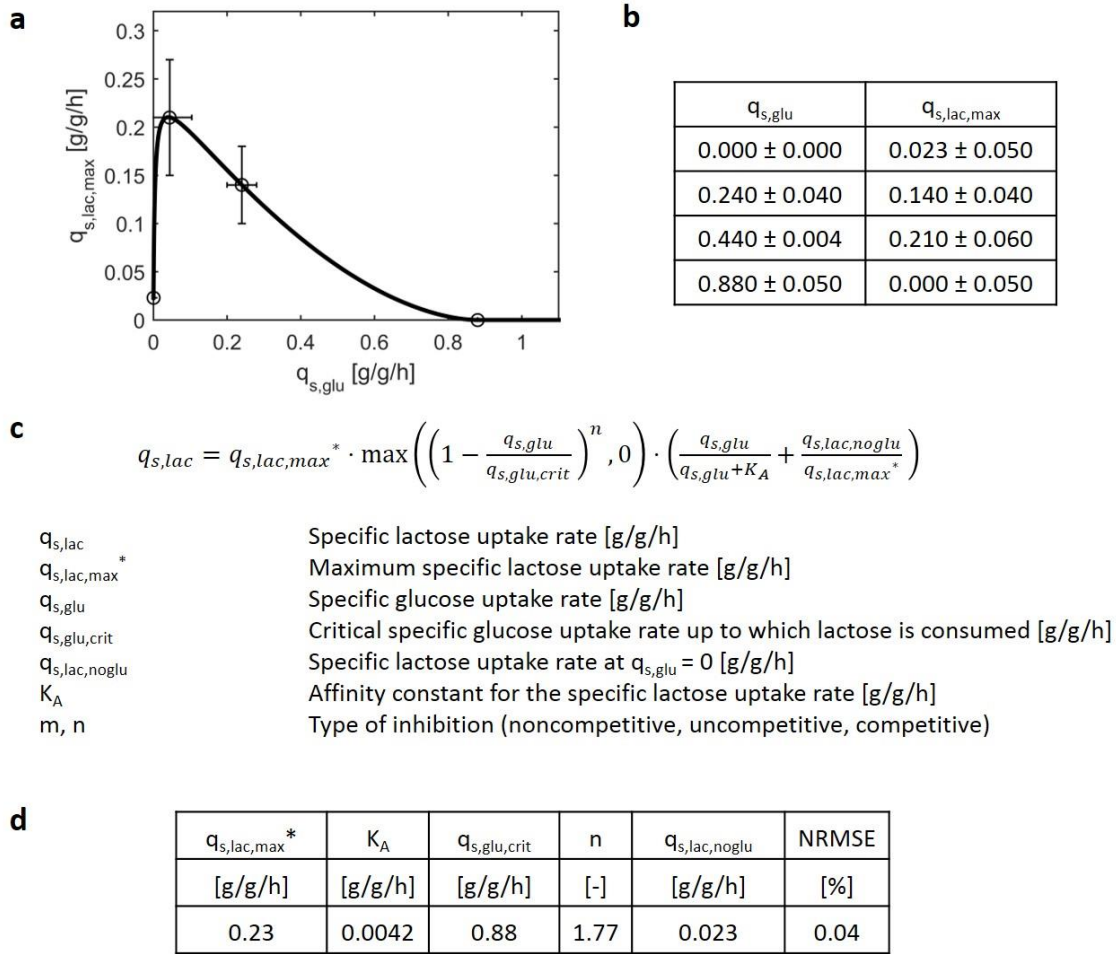
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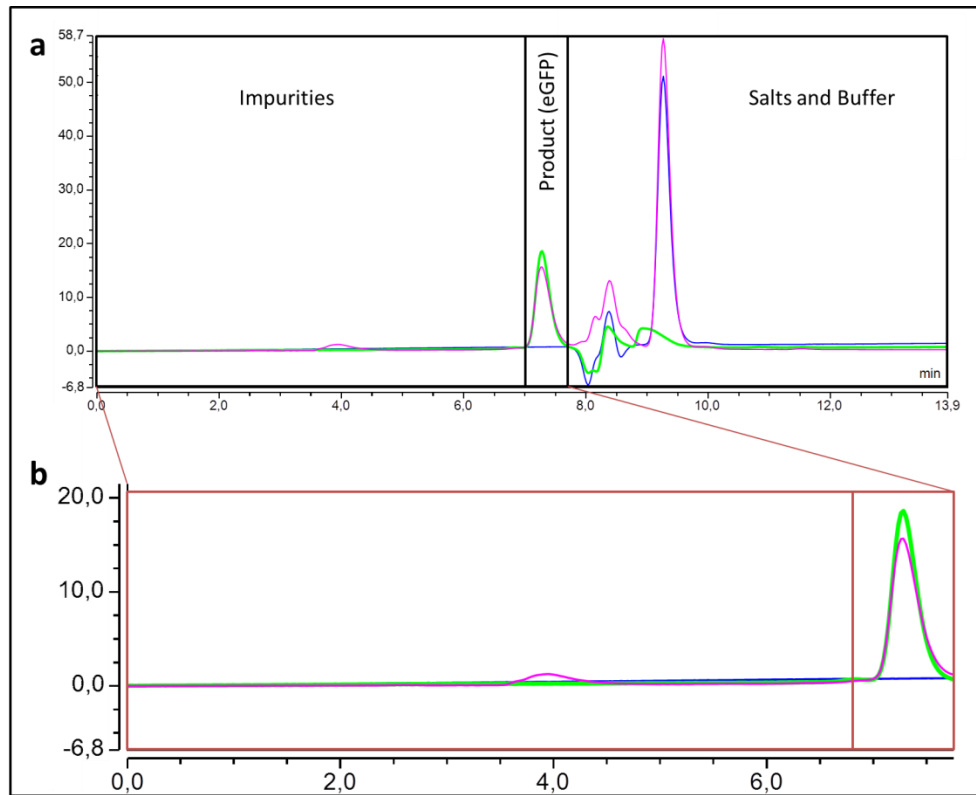
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Supplementary Fig. S1. Maximum specific uptake rate of lactose ($q_{s,lac,max}$) as a function of the specific uptake rate of glucose ($q_{s,glu}$) for the BL21(DE3) strain expressing GFP. **(a)** Data points (open circles) were obtained from several batch and fed-batch cultivations and fitted by the mechanistic model displayed in (c). **(b)** Experimentally evaluated values of $q_{s,lac,max}$ at certain $q_{s,glu}$ for the BL21(DE3) strain expressing GFP. **(c)** Equation of mechanistic model describing $q_{s,lac,max}$ as a function of $q_{s,glu}$ **(d)** Fitted model parameters to the equation displayed in (c) for the BL21(DE3) strain expressing GFP, normalized root mean square error (NRMSE) indicates error between fitted curve and experimental data points. Data taken from our previous study (Wurm DJ, Veiter L, Ulonska S, Eggenreich B, Herwig C, Spadiut O (2016) The E. coli pET expression system revisited-mechanistic correlation between glucose and lactose uptake Appl Microbiol Biotechnol 100:8721-8729 doi:10.1007/s00253-016-7620-7)



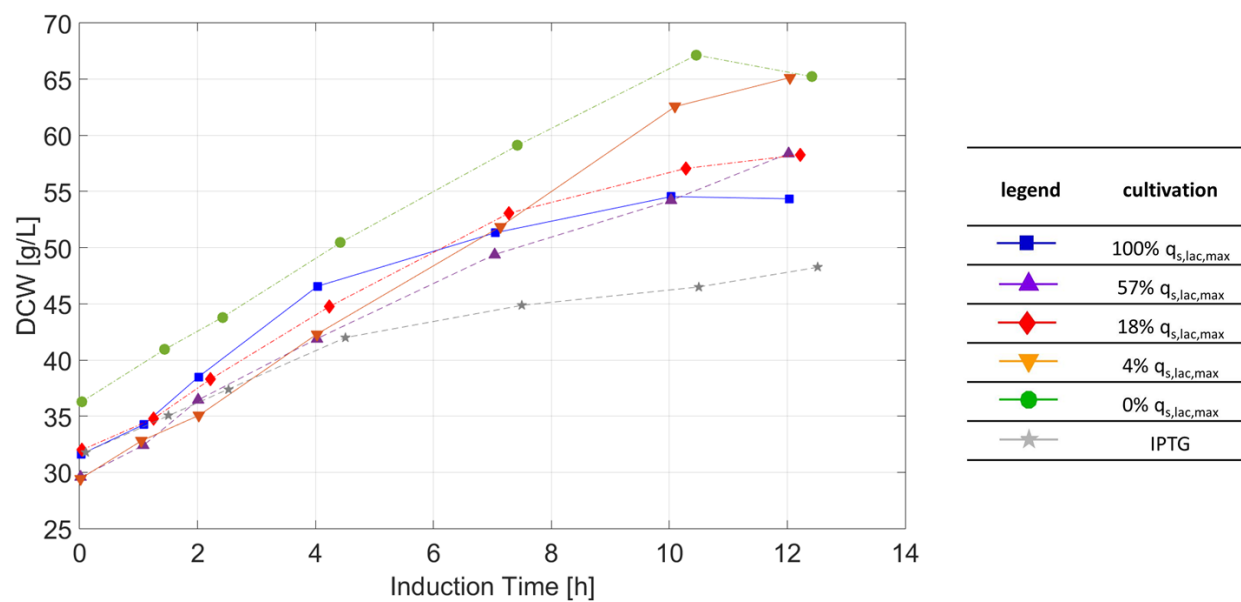
Supplementary Fig. S2. Exemplary Impurity monitoring data showing a Size Exclusion Chromatogram of solubilized and refolded product. Purity is evaluated by dividing product peak by total protein (Impurities + Product). Green, purified Green Fluorescent Protein. Blue, background (buffer). Pink, product sample. **a** whole chromatogram. **b** zoom of chromatogram.

Supplementary Table S1. Specific product titers of inclusion bodies **a**, soluble product **b** and total product **c**

a)	Specific inclusion body titer					
induction time [h]	100% $q_{s,lac,max}$ [mg/g]	57% $q_{s,lac,max}$ [mg/g]	18% $q_{s,lac,max}$ [mg/g]	4% $q_{s,lac,max}$ [mg/g]	IPTG [mg/g]	no inducer [mg/g]
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	55.2 ± 6.2	31.7 ± 3.6	22.2 ± 2.5	26.2 ± 2.9	32.3 ± 3.6	0.0 ± 0.0
12	106.1 ± 11.9	97.0 ± 10.9	59.1 ± 6.6	41.4 ± 4.7	75.7 ± 8.5	0.0 ± 0.0

b)	Specific soluble product titer					
induction time [h]	100% $q_{s,lac,max}$ [mg/g]	57% $q_{s,lac,max}$ [mg/g]	18% $q_{s,lac,max}$ [mg/g]	4% $q_{s,lac,max}$ [mg/g]	IPTG [mg/g]	no inducer [mg/g]
0	0.8 ± 0.1	3.4 ± 0.4	0.5 ± 0.1	7.6 ± 0.9	0.8 ± 0.1	0.0 ± 0.0
1	2.4 ± 0.3	5.3 ± 0.6	3.3 ± 0.4	4.9 ± 0.6	27.2 ± 3.1	0.0 ± 0.0
2	34.6 ± 4.0	20.6 ± 2.4	16.2 ± 1.9	12.1 ± 1.4	40.1 ± 4.6	0.0 ± 0.0
4	86.0 ± 9.9	55.7 ± 6.4	60.0 ± 6.9	30.7 ± 3.5	91.8 ± 10.6	0.0 ± 0.0
12	96.2 ± 11.1	142.0 ± 16.3	148.6 ± 17.1	175.4 ± 20.2	134.2 ± 15.4	0.0 ± 0.0

c)	Specific total product titer					
induction time [h]	100% $q_{s,lac,max}$ [mg/g]	57% $q_{s,lac,max}$ [mg/g]	18% $q_{s,lac,max}$ [mg/g]	4% $q_{s,lac,max}$ [mg/g]	IPTG [mg/g]	no inducer [mg/g]
0	0.8 ± 0.1	3.4 ± 0.5	0.5 ± 0.1	7.6 ± 1.0	0.8 ± 0.1	0.0 ± 0.0
1	2.4 ± 0.3	5.3 ± 0.7	3.3 ± 0.5	4.9 ± 0.7	27.2 ± 3.7	0.0 ± 0.0
2	34.6 ± 4.7	20.6 ± 2.8	16.2 ± 2.2	12.1 ± 1.7	40.1 ± 5.5	0.0 ± 0.0
4	141.2 ± 19.3	87.4 ± 12.0	82.2 ± 11.3	56.9 ± 7.8	124.1 ± 17.0	0.0 ± 0.0
12	202.3 ± 27.7	239.0 ± 32.7	207.7 ± 28.5	216.7 ± 29.7	209.9 ± 28.8	0.0 ± 0.0



Supplementary Fig. S3. Dry cell weight concentration (DCW) as a function of induction time for all cultivations. Error of triplicates was below 3%.