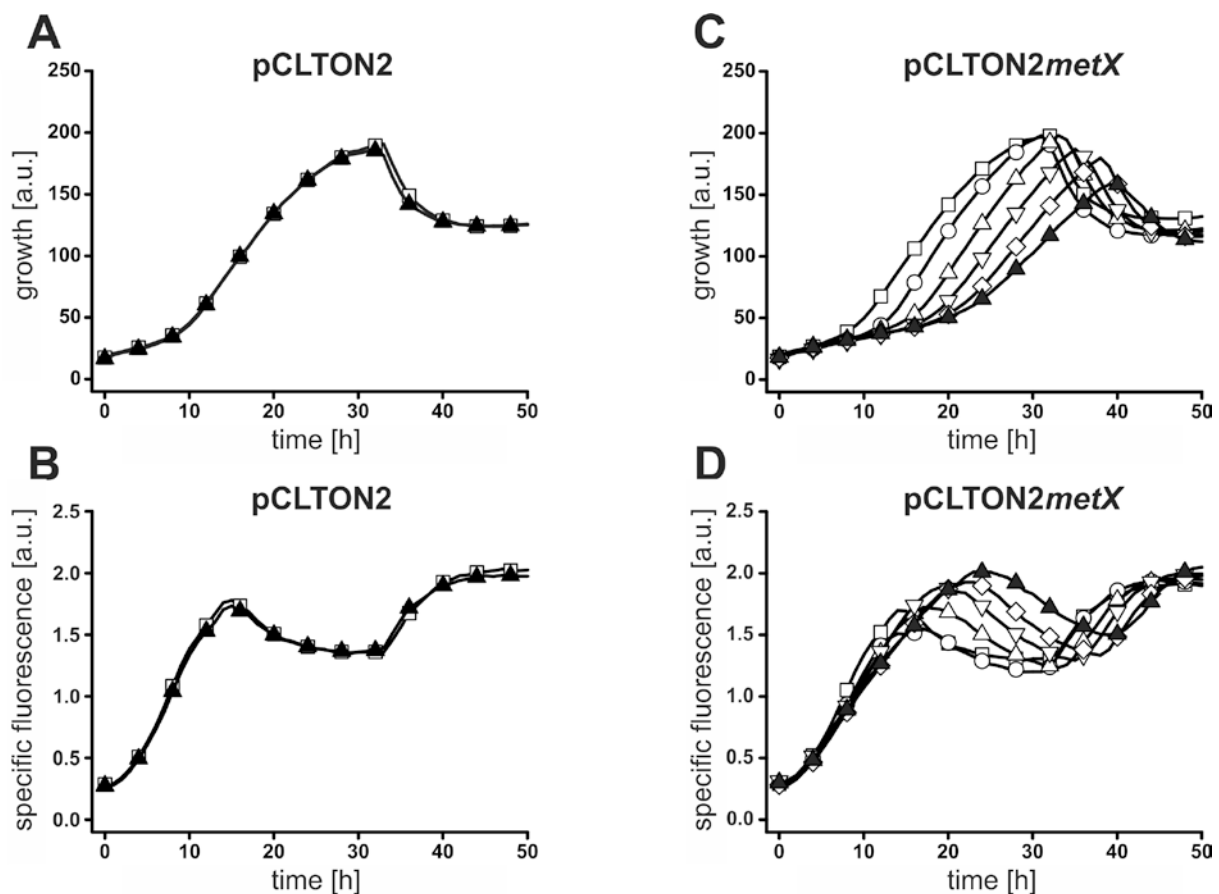


## Imbalances in sulfur assimilation and synthesis of sulfur-containing amino acids: Visualization at the single-cell level

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**FIG S1** Culture fluorescence of strain KK42 due to *metX* expression and pSenOAS1. (A) Growth of strain KK42 pSenOAS1 pCLTON2 ± 150 ng ml<sup>-1</sup> anhydrotetracycline (atc). (B) Fluorescence of cultures shown in A. (C) Growth of strain KK42 pSenOAS1 pCLTON2*metX* at different atc concentrations. (D) Fluorescence of cultures shown in C. The atc concentrations used in C and D (in ng ml<sup>-1</sup>) were as follows: 0, □; 10, ○; 25, △; 50, ▽; 100, ◇; 150, ▲. For the control in A and B only the response to the extreme concentrations of 0 (□) and 150 (▲) ng ml<sup>-1</sup> are shown.

## Molecular work

The primers used in this study are listed in Table S1. Plasmids were constructed in *E. coli* DH5 $\alpha$ MCR from PCR-generated fragments by using *C. glutamicum* ATCC 13032 DNA as a template. *E. coli* was transformed by the RbCl<sub>2</sub> method and *C. glutamicum* via electroporation (4). Homologous recombination and selection for gene deletion in *C. glutamicum* was done as described (3). Inserts in constructed plasmids were sequenced, and all transformants analyzed by plasmid and PCR analysis, respectively.

The pCLTON vector used throughout the work was pCLTON2. To construct pCLTON2 both pEKEx3 and pCLTON1 were PstI/MunI digested, and the 4.6kb-backbone of pEKEx3 ligated with the 3.5kb-tet-regulator cassette of pCLTON1 (1, 2). For construction of pSenOAS1, *cysR* was amplified using primers *cysR*-for and *cysR*-rev-Sall. The promoter region of NCgl1289 was amplified using primers NCgl1289 -for and NCgl1289 -rev-RBS-NdeI. The resulting PCR fragments were used in an overlap extension PCR with *cysR*-rev-Sall and NCgl1289 -rev-RBS-NdeI as primers. The resulting sensor cassette was digested with Sall and NdeI as was plasmid pSenLys to cut out the lysine sensor cassette. The pSenLys backbone was then ligated to the sensor cassette, resulting in pSenOAS1. The construction of pSenOAS3 was done in the same fashion by using *cysI*-for and *cysI*-rev-RBS-NdeI to amplify the promoter region of *cysI*. For construction of pCLTON2metX, *metX* was amplified using primers *metX*-for and *metX*-rev. The resulting PCR product was digested with SmaI and PstI and ligated into SmaI/PstI-digested pCLTON2.

**TABLE S** Primers used for constructions

Primer	Sequence	Features
<i>cysR</i> -for	5'-GACGTCGACTTAGGGTACGAGAGTAAGTG-3'	-
<i>cysR</i> -rev-Sall	5'-GCAGGTTGGACGGTATTATG-3'	Sall
NCgl1289 -for	5'-CATAATACCGTCCAACCTGCCACTACACCACAGGCTTC-3'	-
NCgl1289 -rev-RBS-NdeI	5'-CATATGATATCTCCTTCTTAAAGTTCAGCTTTGTGCAGTGGAAAGTAGGGCTTAG-3'	RBS, NdeI
<i>cysI</i> -for	5'-CATAATACCGTCCAACCTGCATATTCACGGTGAACCTAAC-3'	-
<i>cysI</i> -rev-RBS-NdeI	5'-CATATGATATCTCCTTCTTAAAGTTCAGGGTCTTCTTGCTTAATTCCTCG-3'	RBS, NdeI
<i>metX</i> -for	5'-GTACCTGCAGAAGGAGATATAGATATGCCACCTCGCGCCTCAG-3'	SmaI
<i>metX</i> -rev	5'-GCGCCGGGTTATTAGATGTAGAACTCGATGTAGG-3'	PstI

## REFERENCES

1. Hoffelder M, Raasch K, van Ooyen J, Eggeling L. 2010. The E2 domain of OdhA of *Corynebacterium glutamicum* has succinyltransferase activity dependent on lipoyl residues of the acetyltransferase AceF. *J Bacteriol.* **192**:5203-5211.
2. Lausberg F, Chattopadhyay AR, Heyer A, Eggeling L, Freudl R. 2012. A tetracycline inducible expression vector for *Corynebacterium glutamicum* allowing tightly regulable gene expression. *Plasmid* **68**:142-147.
3. Schäfer A, Tauch A, Jäger W, Kalinowski J, Thierbach G, Pühler A. 1994. Small Mobilizable Multipurpose Cloning Vectors Derived from the *Escherichia coli* Plasmids Pk18 and Pk19 - Selection of Defined Deletions in the Chromosome of *Corynebacterium glutamicum*. *Gene* **145**:69-73.
4. Tauch A, Kirchner O, Löffler B, Gotker S, Pühler A, Kalinowski J. 2002. Efficient electrotransformation of *Corynebacterium diphtheriae* with a mini-replicon derived from the *Corynebacterium glutamicum* plasmid pGA1. *Current Microbiology* **45**:362-367.