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Supplemental information

**Extracellular electron transfer pathways to enhance
the electroactivity of modified *Escherichia coli***

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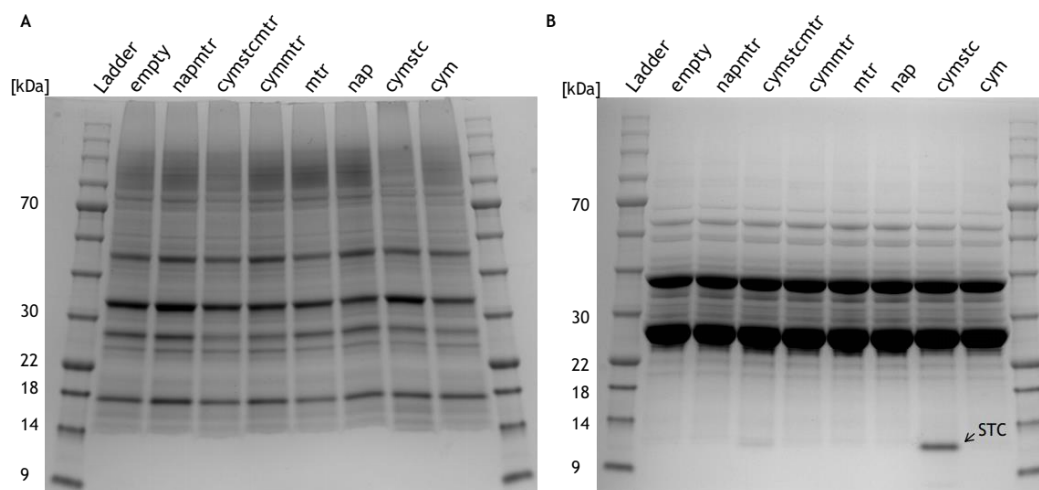


Figure S1. Coomassie brilliant blue G250 staining of SDS-PAGE gels. (A) Membrane and (B) periplasmic fractions stained after the enhanced chemiluminescence staining of the cytochromes (see Figure 1d-e in the main text).

Table S1. Overview of bacterial strains, plasmids and primers used in this study.

Strains, plasmids and primers	Characteristics	Source
Strains		
<i>E. coli</i> DH5α		
<i>E. coli</i> C43 (DE3)		
<i>S. oneidensis</i> MR-1		
Plasmids		
pEC86	CcmABCDEFGH expression, chloramphenicol resistance, tet promoter	[1]
pSB1ET2	empty backbone for cytochrome expression, kanamycin resistance, T7 promoter	[2]
pMO1	NapBC expression, kanamycin resistance, T7 promoter	This study
pMO1.1	NapBC and MtrCAB expression, kanamycin resistance, T7 promoter	This study
I5023	MtrCAB expression, kanamycin resistance, T7 promoter	[2]
pMO2	CymA and STC (<i>cctA</i> gene) expression, kanamycin resistance, T7 promoter	This study
pMO2.1	CymA, STC and MtrCAB expression, kanamycin resistance, T7 promoter	This study
I5049	CymA and MtrCAB expression, kanamycin resistance, T7 promoter	[3]
pMO2.2	CymA expression, kanamycin resistance, T7 promoter	This study
Primers		
1	5'- 3' gttttaacagggttattgcgaattctattccagcatccactaagt	
2	5'- 3' ctgcagcgccgctactagttagattagagttgttaactcatgctca	
3	5'- 3' agaagtaattgccgctgcaaGaattctattccagcatccactaagtc	
4	5'- 3' ctgcagcgccgctactagttag	
5	5'- 3' gttacaaactctaatactagaactagtagcgccgctgcag	
6	5'- 3' ggatgctggaaatagaattcgcaataaccctgttaaaaacctggc	
7	5'- 3' gttacaaactctaatactagaactagtagcgccgctgcag	
8	5'- 3' ggatgctggaaatagaattCttgcagcggaattactcttc	

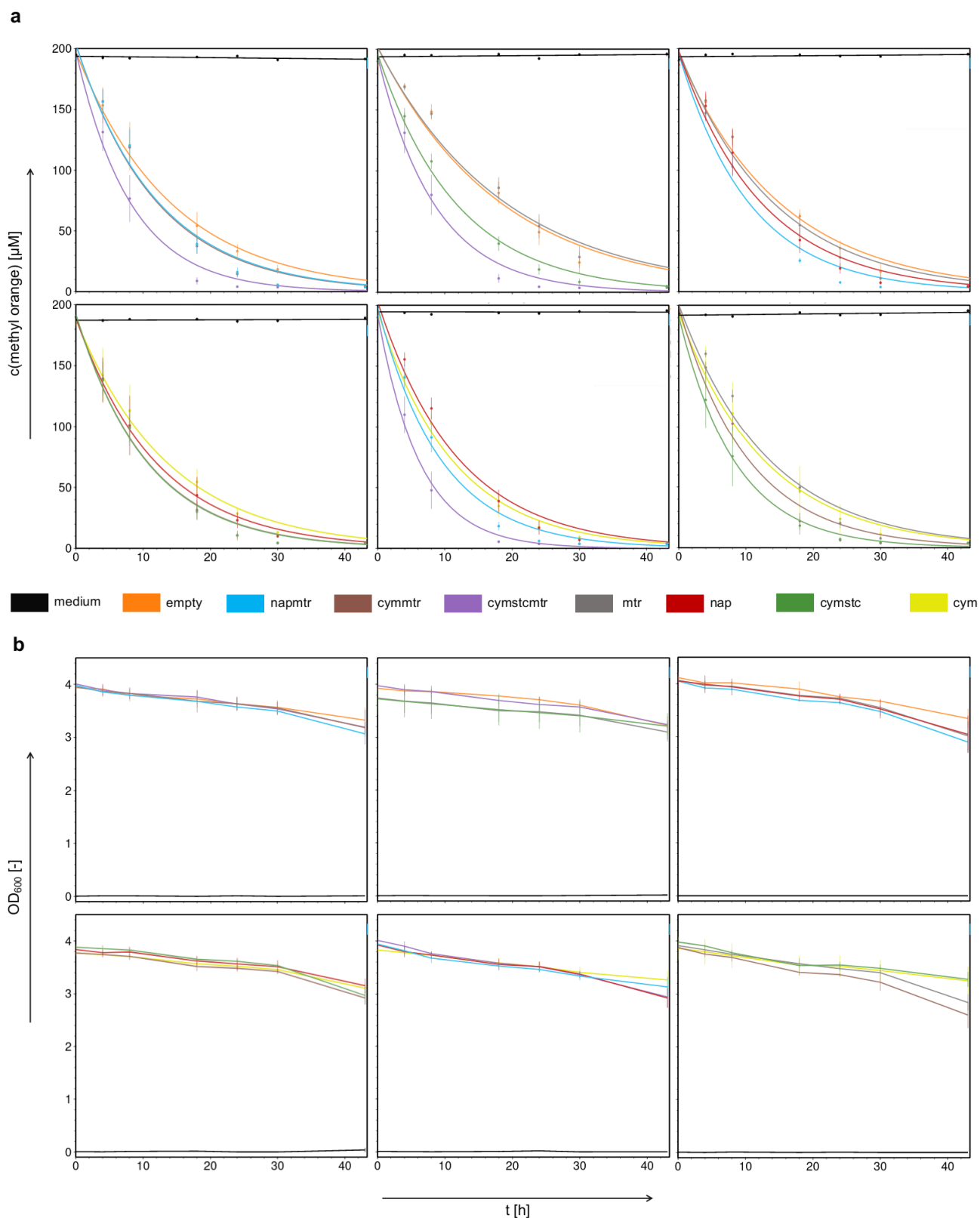


Figure S2. Methyl orange reduction assay. Individual replicate plots of (a) methyl orange concentration and (b) OD_{600} in assay tubes over 2 days. Experiments were carried out with four biological replicates per strain, and all strains were used in three separate experiments for a total of 12 replicates. Dots and bars represent average values and one standard deviation over four samples.

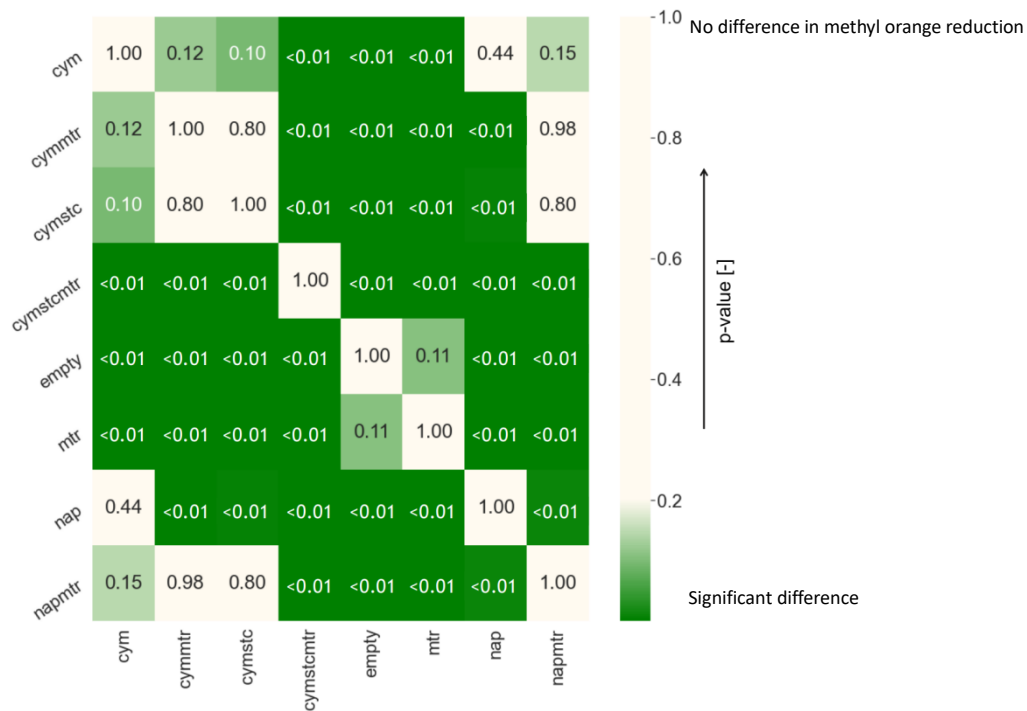


Figure S3. Comparative analysis of methyl orange reduction rates for different strains. P values obtained from two tailed two sample T-tests without assuming equal population variance (Welch's T-test) plotted against each strain pair. Smaller p-values (darker green) correspond to more significant differences between the strains. The analysis was performed on data shown in Figure 2b of the main text.

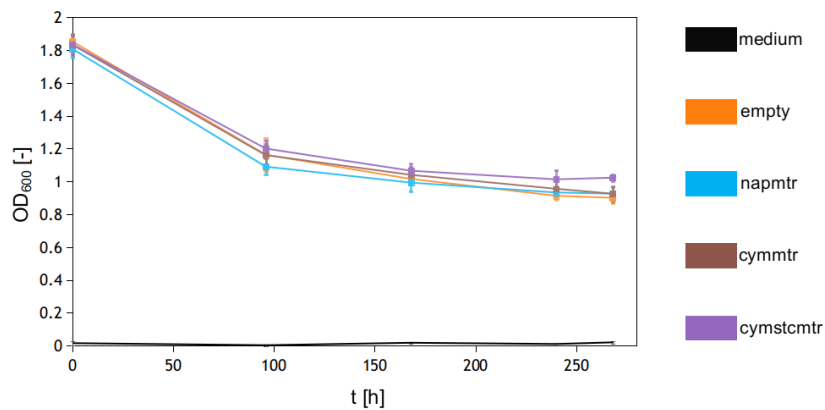


Figure S4. Measurements of the OD₆₀₀ over the course of 11 days during ferric citrate reduction assays. Data is shown as the mean of four biological replicates, with error bars indicating one standard deviation.

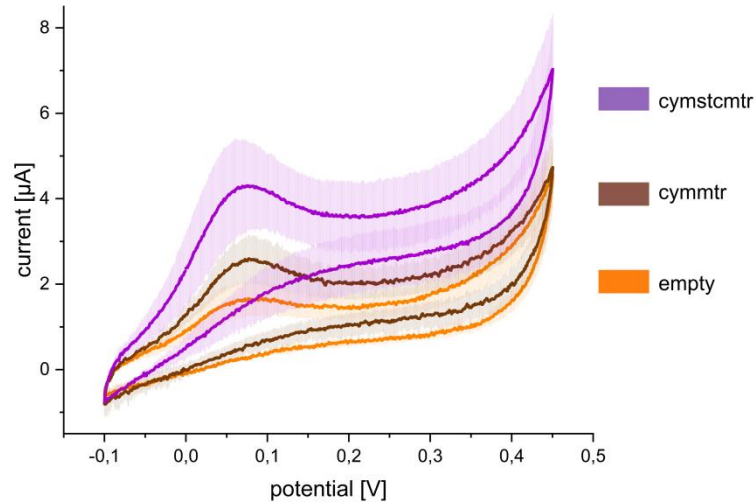


Figure S5. Cyclic voltammetry (CV) of different strains after chronoamperometry. CV measurements were performed at a scan rate of 2 mV/s, immediately after chronoamperometry (see main text, Figure 3). Data is shown for the last out of three cycles, with solid lines indicating the mean current and shaded areas indicating one standard deviation between three biological replicates per strain.

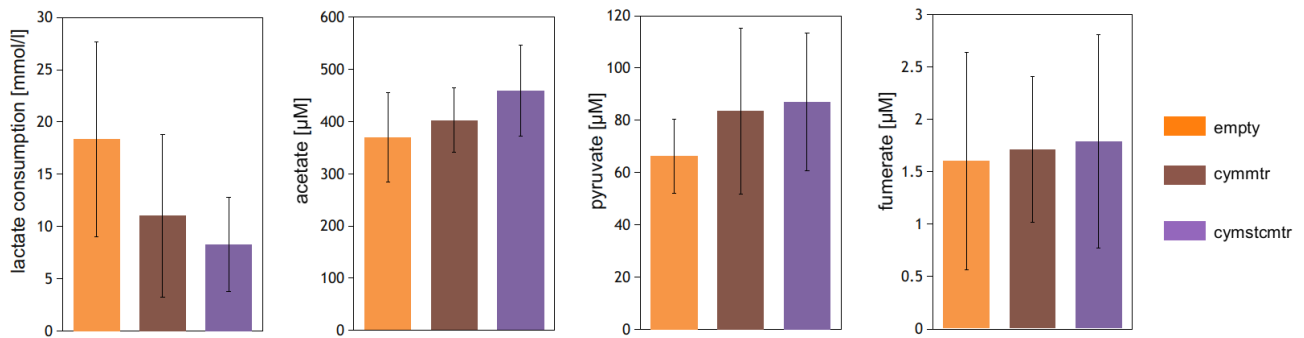


Figure S6. Lactate consumption and concentration of different fermentation products after chronoamperometric measurements in lactate-fueled electrochemical cells.

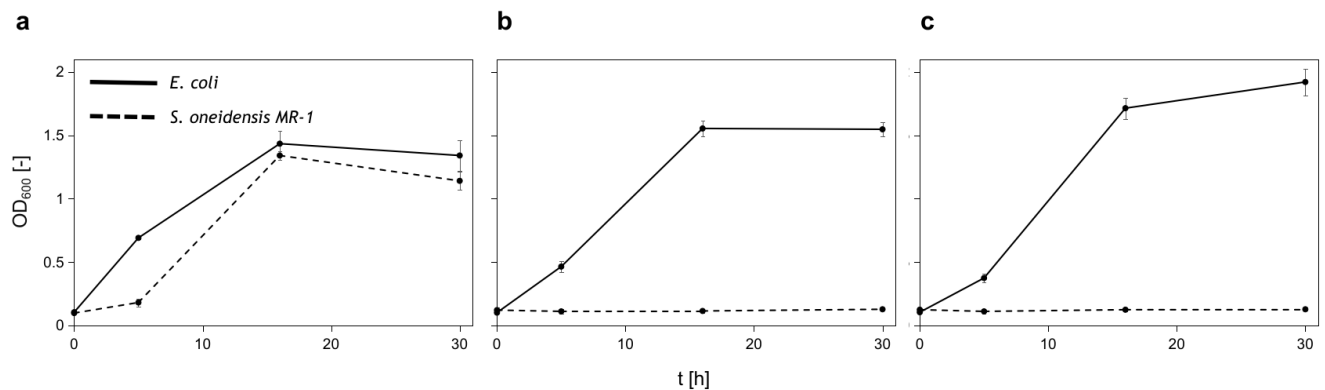


Figure S7. M9-medium supplemented with lactate (a), glycerol (b) and xylose (c) as growth substrates for *E. coli* and *S. oneidensis* MR-1. Bacterial growth over time was determined using OD₆₀₀ measurements from aerobic culture flasks kept under constant shaking at 30°C (*S. oneidensis* MR-1) and 37°C (*E. coli* C43(DE3)). Data is shown as the mean of three biological replicates, with error bars indicating one standard deviation.

Supplemental references

- [1] Arslan, E., Schulz, H., Zufferey, R., Künzler, P., and Thöny-Meyer, L. (1998). Overproduction of the *Bradyrhizobium japonicum* c-Type Cytochrome Subunits of the *cbb3* Oxidase in *Escherichia coli*. *Biochem Biophys Res Commun* 251, 744–747. 10.1006/bbrc.1998.9549.
- [2] Jensen, H.M., Albers, A.E., Malley, K.R., Londer, Y.Y., Cohen, B.E., Helms, B.A., Weigele, P., Groves, J.T., and Ajo-Franklin, C.M. (2010). Engineering of a synthetic electron conduit in living cells. *Proc Natl Acad Sci U S A* 107, 19213–19218. 10.1073/pnas.1009645107.
- [3] Jensen, H.M., TerAvest, M.A., Kokish, M.G., and Ajo-Franklin, C.M. (2016). CymA and Exogenous Flavins Improve Extracellular Electron Transfer and Couple It to Cell Growth in Mtr-Expressing *Escherichia coli*. *ACS Synth Biol* 5, 679–688. 10.1021/acssynbio.5b00279.