Supplementary Material

to

Identification of a chemoreceptor for C2- and C3-carboxylic acids

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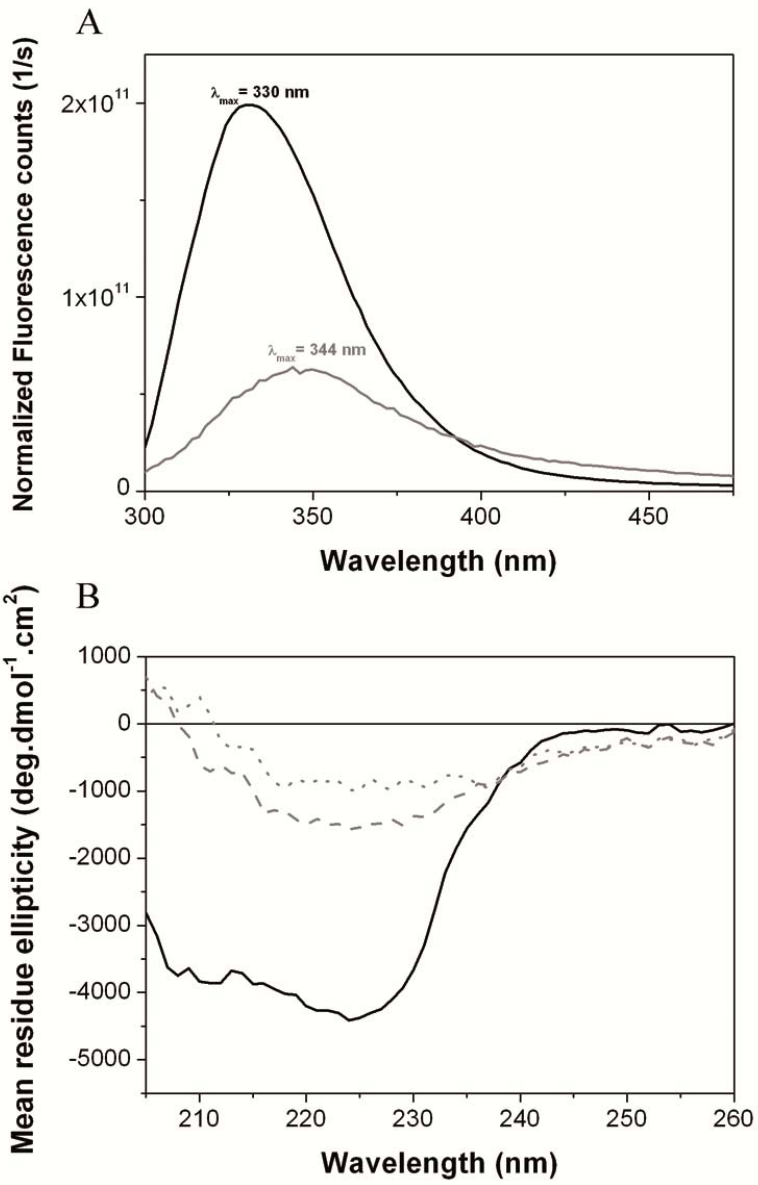
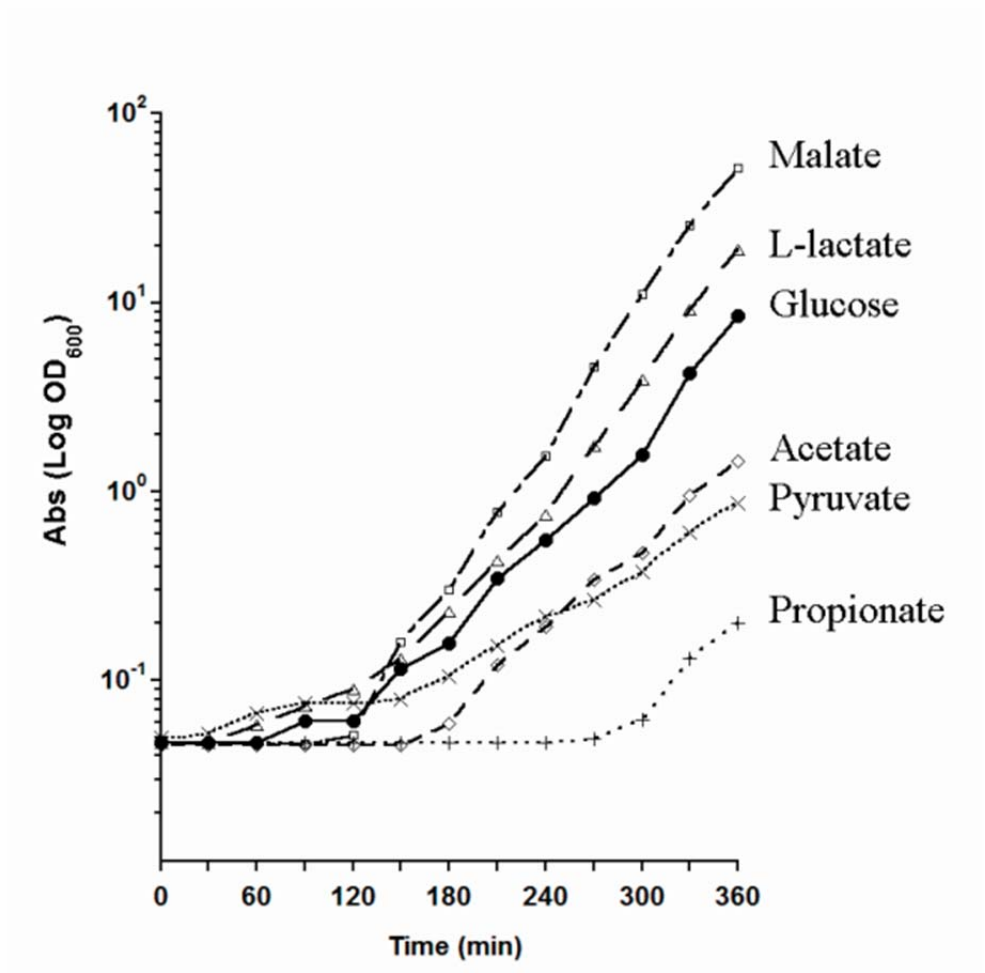
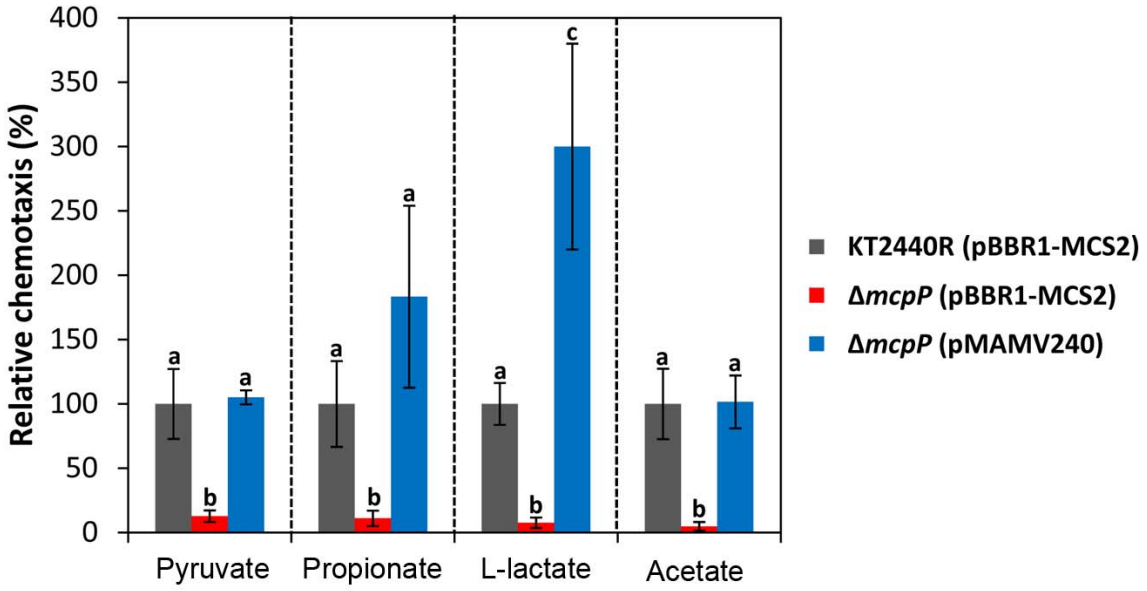


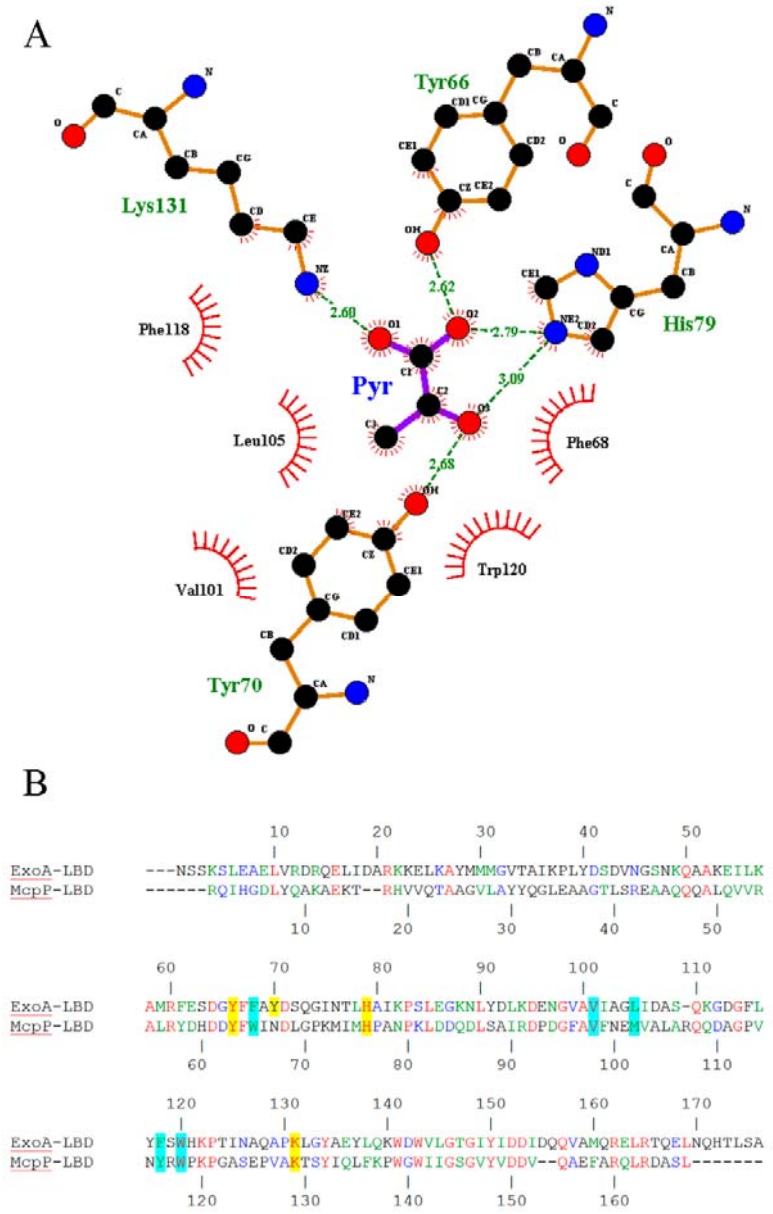
Supp. Figure 1) Analysis of NbaY-LBD by intrinsic tryptophan fluorescence and circular dichroism (CD) spectroscopy. A) Tryptophan fluorescence emission spectrum of 4 µM NbaY-LBD in polybuffer (black line) and in the presence of 3 M guanidine hydrochloride (grey line). The wavelengths of maximal fluorescence are indicated. B) Far UV CD spectra of 16.4 µM NbaY-LBD at 25ºC (black line), 50ºC (dashed grey line) and 85ºC (dotted grey line).



Supp. Figure 2) Growth curves of *P. putida* KT2440R in the presence of different compounds as carbon source. Assays were conducted in M9 minimal medium supplemented with 10 mM of each compound. Assays were conducted with the four McpP ligands and glucose and malate as references. Shown are representative curves. The doubling times calculated form three different experiments were as follows: Malate 33 ± 2.51 min, L-Lactate 36.47 ± 3.03 min, Glucose 40.76 ± 2.13 min, Acetate 46.47 ± 2.81 min, Pyruvate 89 ± 8.53 min, Propionate 46.2 ± 2.03 min.



Supp. Fig. 3) Genetic complementation of *P. putida* KT2440R Δ*mcpP* using quantitative capillary chemotaxis assays. Sodium pyruvate, sodium propionate, sodium L-lactate and sodium acetate were used at 0.5 mM, the concentration at which optimal chemotaxis was observed. Data were corrected with the number of cells that migrated into the capillaries containing buffer and are expressed as the percentage of bacteria in the capillaries relative to the wild type strain. Data are means and standard deviations from at least three independent experiments. Data were statistically analyzed using the one-way analysis of variance. Bars with the same letter are not significantly different (P-value < 0.5), whereas different letters indicate statistically significant differences.



Supp. Fig. 4) Conservation of amino acids involved in pyruvate recognition of CACHE sensor domains. A) Amino acids involved in pyruvate binding in the LBD of an uncharacterized chemoreceptor of *Vibrio parahaemolyticus* (pdb ID 4EXO). The figure was generated using LigPlot+ (1). (B) Alignment of the McpP-LBD and 4ExoA-LBD sequences. Amino acids in red are identical, in green highly similar and in blue weakly similar. Amino acids involved in pyruvate binding in the 4EXO structure as well as their conserved counterparts in McpP-LBD are shaded in yellow (hydrogen bonds) and cyan (hydrophobic interactions). The sequence alignment was done using the CLUSTALW algorithm (2) of the NPSA server (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html) using a gap opening penalty of 10 and a gap extension penalty of 0.1.

Supp. Fig. 5) NbaY-LBD homology model generated using the Phyre2 (3) server. Eighty nine percent of residues were modelled with a confidence above 90 %.

Supp. **Table 1) Compounds used for microcalorimetric binding studies to NbaY-LBD and McpP (PP2861)-LBD**. Compounds that bound are underlined and the corresponding thermodynamic data are shown in Table 2.

McpS (PP2861)-LBD	NbaY-LBD
Benzoates	**Benzoates**
Benzoate	2-Nitrobenzoate
2-Nitrobenzoate	3-Nitrobenzoate
3-Nitrobenzoate	4-Nitrobenzoate
4-Nitrobenzoate	Sodium Benzoate
3,5-Dinitrobenzoate	**Monocarboxylic acids**
Dicarboxylic acids	Pyruvate
Malate	L-Lactate
Succinate	Propionate
Fumarate	Acetate
Malonate	**Other aromatic compounds**
Monocarboxylic acids	Naphthalene
Glyoxylate	Benzene
<u>Acetate</u>	Toluene
<u>Pyruvate</u>	2-Nitrotoluene
<u>Propionate</u>	**Other compounds**
<u>L-Lactate</u>	Urea
D-Lactate	
Phosphoenolpyruvate	
Butyrate	
Other C3 compounds	
Acetone	
1-Propanol	
2-Propanol	
Amino acids	
L-Alanine	
L-Glutamate	
Tricarboxylic acids	
Citrate	

References

1. **Laskowski RA, Swindells MB.** 2011. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J. Chem. Inf. Model. **51:**2778-2786.
2. **Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG.** 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25:**4876-4882.
3. **Kelley LA, Sternberg MJ.** 2009. Protein structure prediction on the Web: a case study using the Phyre server. Nat.Pprotoc. **4:**363-371.