Supplementary information

A pH-dependent shift of redox cofactor specificity in a benzyl alcohol dehydrogenase of *Aromatoleum aromaticum* EbN1

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Methods

Calibration curves of HPLC-DAD

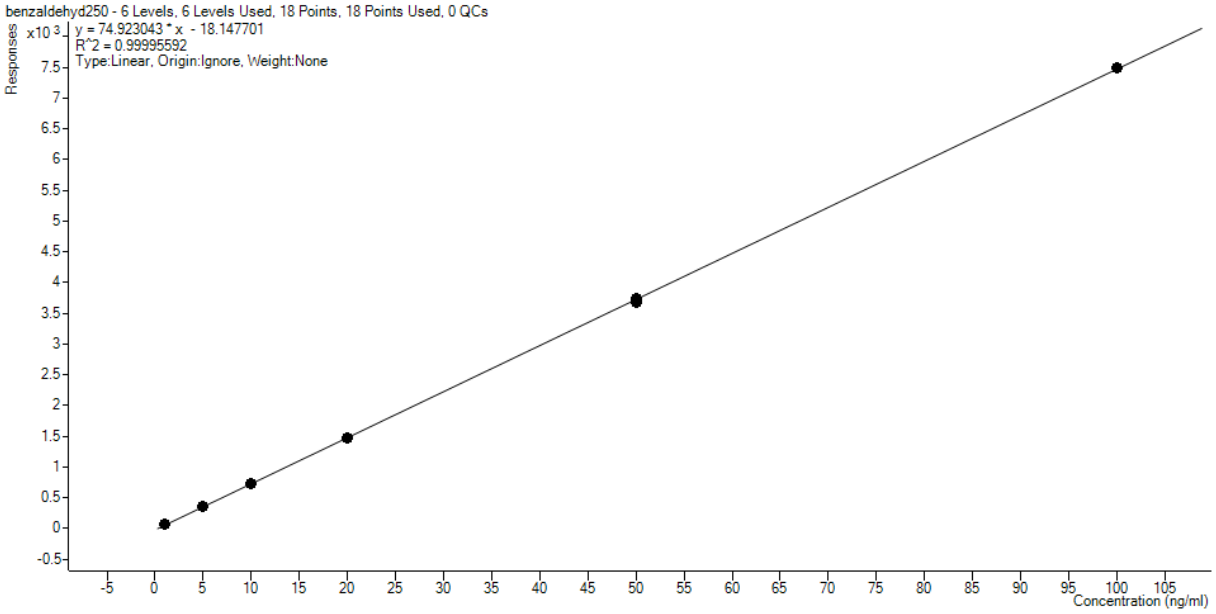


Fig. S1. Calibration curve for benzaldehyde (RT: 4.3 min); wavelength 250 nm.

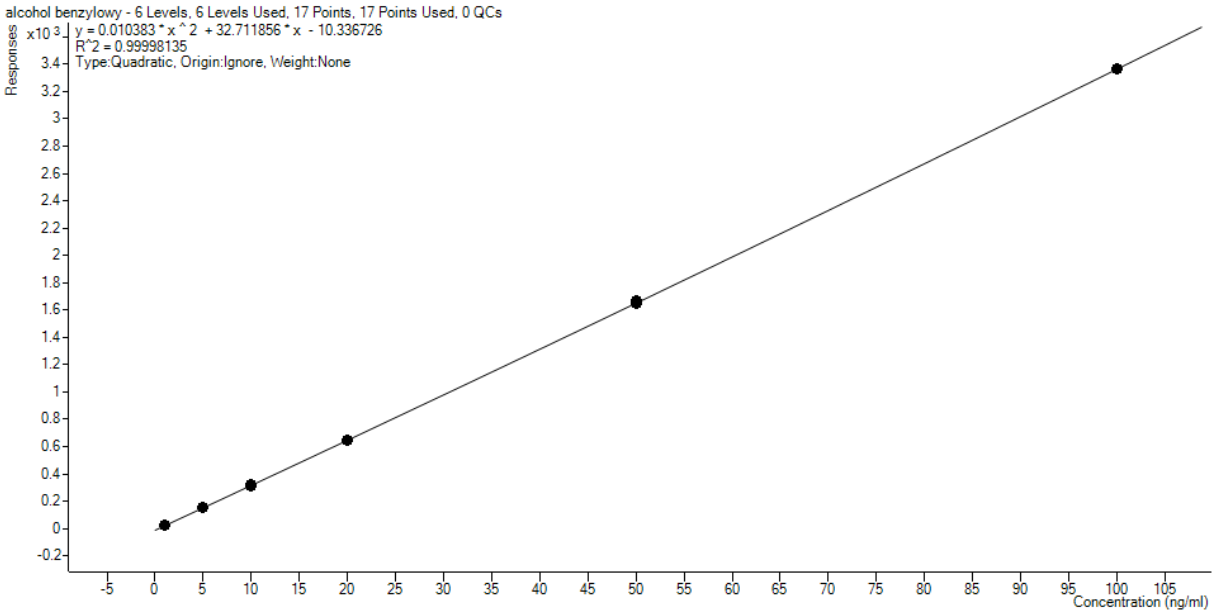


Fig. S2. Calibration curve for benzyl alcohol (RT: 1.9 min); wavelength 210 nm.

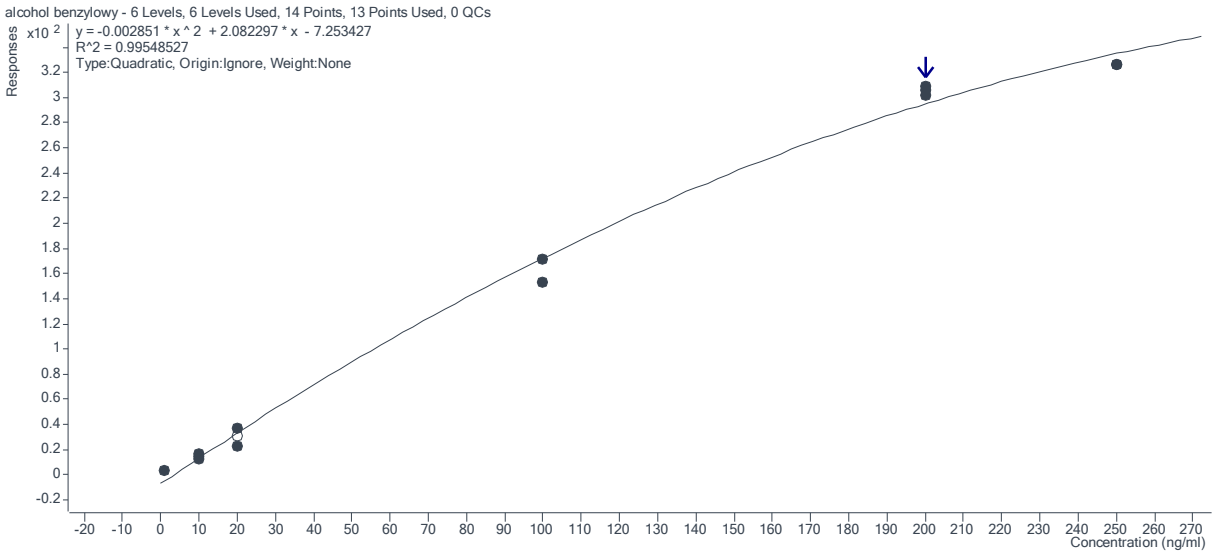


Fig. S3. Calibration curve for benzyl alcohol used in the analysis of cascade reaction (RT: 1.9 min); wavelength 210 nm.

Result

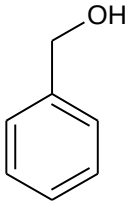
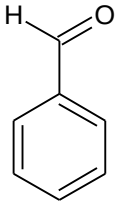
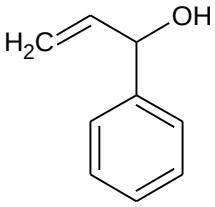
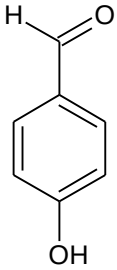
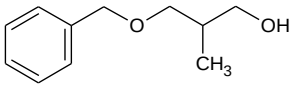
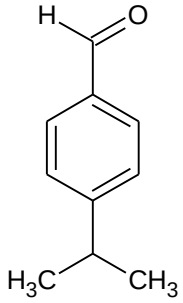
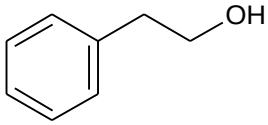
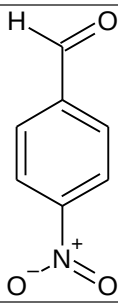
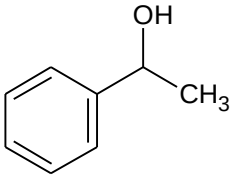
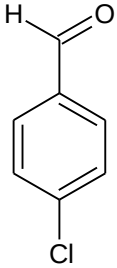
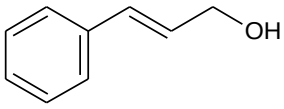
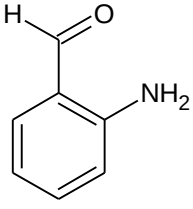
Element analysis

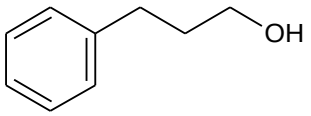
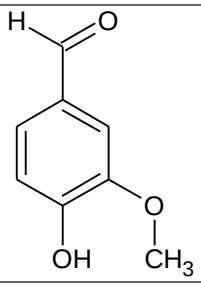
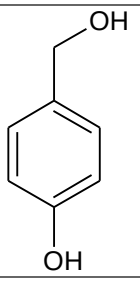
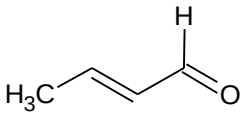
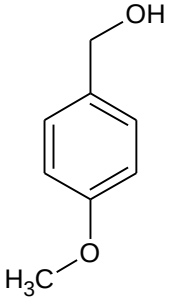
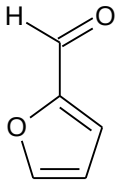
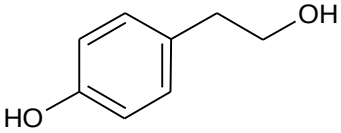
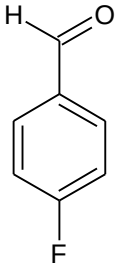
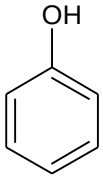
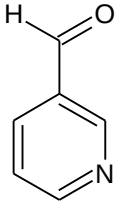
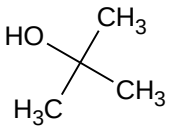
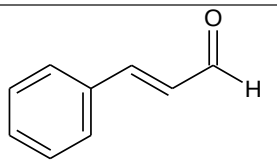
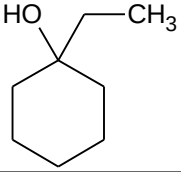
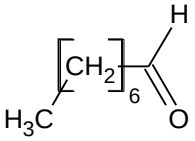
Table S1. Element analysis of BaDH. The element content is given as molar ratio per subunit after data correction for the empty buffer.

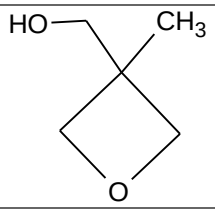
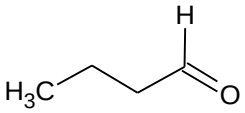
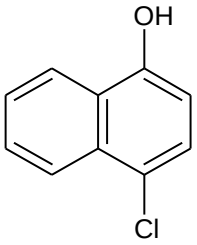
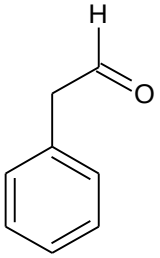
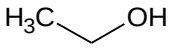
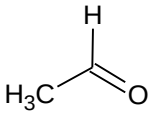
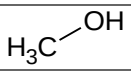
Element	Molar ratio
Mg	0.0015
P	0.221
Ca	< 0.02
Mn	< 0.001
Fe	0.370
Co	< 0.001
Ni	0.011
Cu	0.0018
Zn	1.34
Se	< 0.010
Mo	< 0.001
W	< 0.001

Substrates

Table S2.. Substrates used in the study: alcohols in NAD(P)$^+$ dependent oxidation and aldehydes in NAD(P)H dependent reduction

Alcohols		Aldehydes	
benzyl alcohol		benzaldehyde	
α-vinylbenzylalcohol		4-hydroxybenzaldehyde	
3-benzyloxy-2-methylpropan-1-ol		4-isopropylbenzaldehyde	
2-phenylethanol		4-nitrobenzaldehyde	
rac-1-phenylethanol		4-chlorobenzaldehyde	
3-phenyl-2-propen-1-ol		2-aminobenzaldehyde	

3-phenyl-1-propanol 	4-hydroxy-3-methoxybenzaldehyde 
4-hydroxybenzylalcohol 	(2*e*)-but-2-enal 
4-methoxybenzylalcohol 	furan-2-carbaldehyde 
2-(4-hydroxyphenyl)ethanol 	4-fluorobenzaldehyde 
phenol 	pyridine-3-carbaldehyde 
2-methylpropan-2-ol 	(2E)-3-phenylprop-2-enal 
1-ethylcyclohexanol 	octanal 

3-methyl-3-oxetane methanol		butanal	
4-chloro-1-naphthol		phenylacetaldehyde	
ethanol		acetaldehyde	
methanol			

pH models – data and fit details

Oxidation of benzyl alcohol with NADP+

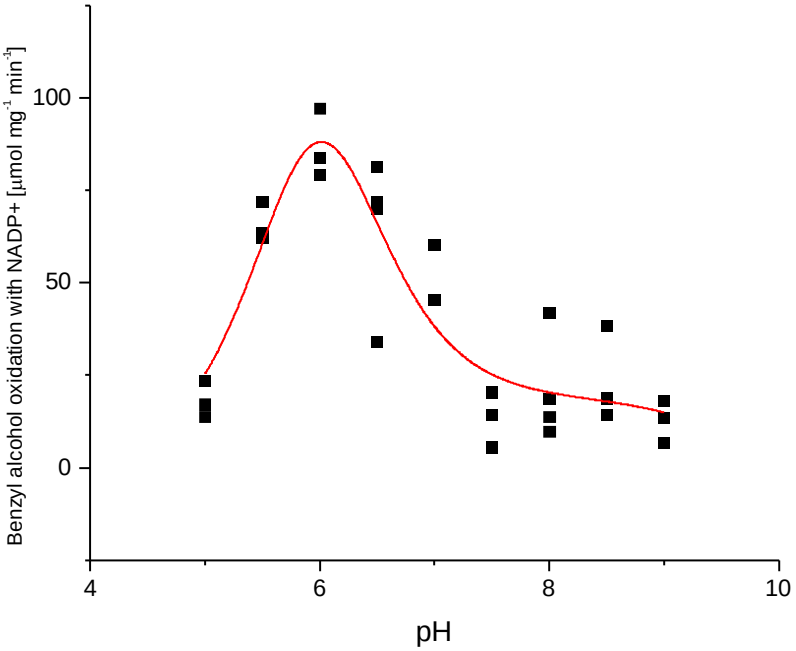


Fig. S4. pH dependence of benzyl alcohol oxidation with $NADP^+$ (black squares) with a 'bell-shaped' model fit (red line).

Table S3A. Statistical parameters non-linear regression of different models describing pH dependence of benzyl alcohol oxidation with $NADP^+$. Bold indicates the selected model.

	n	Degree of Freedom	Reduced χ^2	RSS	R^2	Adj. R^2
Bell Shaped*	**28**	**25**	**265.8054**	**6645.135**	**0.69884**	**0.67475**
Bell Shaped With Plateau	28	23	176.3341	4055.685	0.8162	0.78423
Plateau Shaped	28	24	517.3855	12417.25	0.43725	0.36691

*The bell-shaped model was selected over the bell-shaped with plateau model due to more statistically reliable parameters (i.e. non-zero errors of estimates)

Table S3B. Fit parameters for pH dependence of benzyl alcohol oxidation with $NADP^+$. LCL lower confidence limit, UCL upper confidence limit

Model	Parameter	Value	Standard Error	95% LCL	95% UCL
Bell Shaped	V^{max}_{lim}	113.03	23.65	64.32	161.73
	pK_a	5.44	0.23	4.97	5.92
	pK_b	6.84	0.23	6.37	7.32

Reduction of benzaldehyde with NADPH

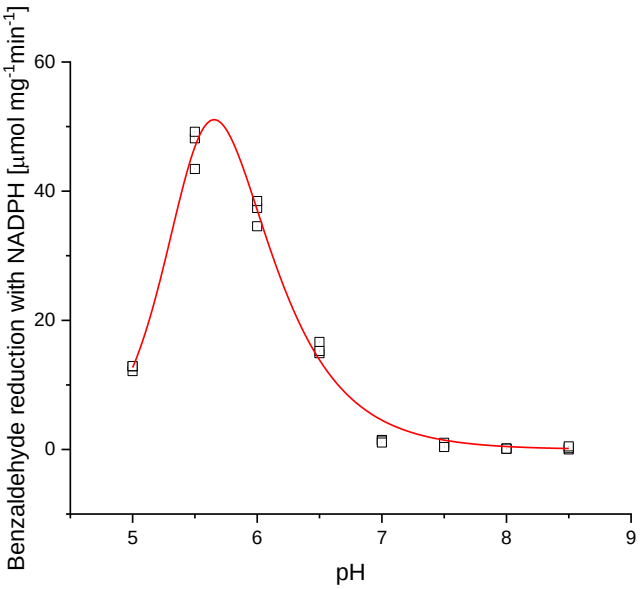


Fig. S5. pH dependence of benzaldehyde reduction with NADPH (squares) with a 'plateau-shaped' model fit (red line).

Table S4A. Statistical parameters non-linear regression of different models describing pH dependence of benzaldehyde reduction with NADPH. Bold indicates the selected model.

Model	n	Degree of Freedom	Reduced χ^2	RSS	R^2	Adj. R^2
Bell Shaped	24	21	121.76168	2556.99528	0.6359	0.60122
Bell Shaped With Plateau	**24**	**19**	**3.83221**	**72.81193**	**0.98963**	**0.98745**
Plateau Shaped	24	20	127.85222	2557.04438	0.63589	0.58128

Table S4B. Fit parameters for pH dependence of benzaldehyde reduction with NADPH. LCL lower confidence limit, UCL upper confidence limit.

Model	Parameter	Value	Standard Error	95% LCL	95% UCL
Bell Shaped With Plateau	V^{max}_{lim}	3.35	897.25	-1874.62	1881.32
	pK_a	5.50	19.85	-36.04	47.05
	pK_b	5.81	19.42	-34.83	46.45
	pK_c	5.13	10.25	-16.32	26.58
	α	102.13	29810.11	-62291.15	62495.41

Oxidation of benzyl alcohol with NAD^+

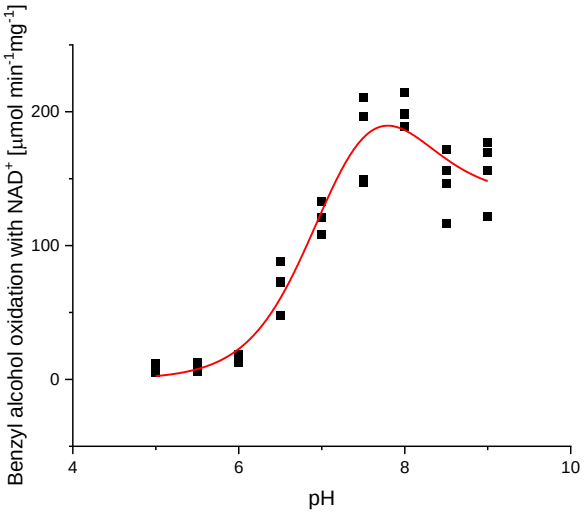


Fig. S6. pH dependence of benzyl alcohol oxidation with NAD^+ (black squares) with a 'plateau-shaped' model fit (red line).

Table S5A. Statistical parameters non-linear regression of different models describing pH dependence of benzyl alcohol oxidation with NAD^+. Bold indicates the selected model; RSS Residual Sum of Squares

Model	n	Degree of Freedom	Reduced χ^2	RSS	R^2	Adj. R^2
Bell Shaped	36	33	590.1	19473.68	0.89837	0.89221
Bell Shaped With Plateau	36	31	367.6	11394.24	0.94054	0.93286
Plateau Shaped	**36**	**32**	**356.1**	**11394.27**	**0.94054**	**0.93496**

Table S5B. Fit parameters for pH dependence of benzyl alcohol oxidation with NAD^+. LCL lower confidence limit, UCL upper confidence limit

Model	Parameter	Value	Standard Error	95% LCL	95% UCL
Plateau Shaped	$V^{max}_{plateau}$	250.52	37.85	173.42	327.61
	V^{max}_{lim}	137.39	16.28	104.23	170.55
	pK_a	7.00	0.14	6.71	7.29
	pK_b	8.03	0.44	7.13	8.93

Reduction of benzaldehyde with NADH

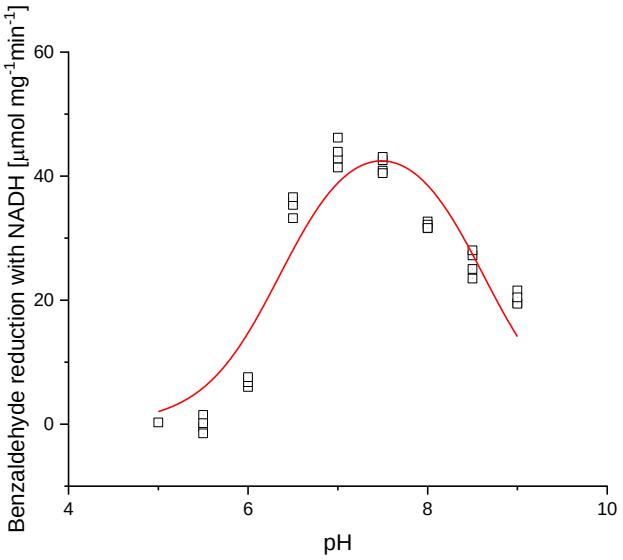


Fig. S7. pH dependence of benzaldehyde reduction with NADH (squares) with a 'plateau-shaped' model fit (red line).

Table S6A. Statistical parameters non-linear regression of different models describing pH dependence of benzaldehyde reduction with NADH. Bold indicates the selected model.

	n	Degree of Freedom	Reduced χ^2	RSS	R^2	Adj. R^2
Bell Shaped	**33**	**30**	**35.30**	**1058.92**	**0.86248**	**0.85331**
Bell Shaped With Plateau	33	28	12.21	341.86	0.9556	0.94926
Plateau Shaped	33	29	70.04	2031.22	0.73621	0.70893

*The values were corrected by the constant of -39.34 µmol mg^{-1} min^{-1}, i.e. minimal activity observed at pH 5.5 to enable convergence of the model which does not assume two plateaus. The second best 'bell-shaped' model was selected due to very high errors of estimates for V^{max}_{lim} in the bell-shaped with plateau model.

Table S6B. Fit parameters for pH dependence of benzaldehyde reduction with NADH. LCL lower confidence limit, UCL upper confidence limit

Model	Parameter	Value	Standard Error	95% LCL	95% UCL
Bell Shaped	V^{max}_{lim}	88.19*	3.43	41.84	55.87
	pK_a	6.36	0.11	6.14	6.59
	pK_b	8.61	0.11	8.38	8.84

Corrected back by the constant of 39.34 µmol mg^{-1} min^{-1}

Temperature dependency of benzyl alcohol oxidation with NAD^+

Table S7. Statistical parameters non-linear regression of Arrhenius model describing temperature dependence of benzyl alcohol oxidation with NAD^+.

Model	n	Degree of Freedom	A [U mg^{-1}]	E^a [J mol^{-1}]	R^2
Arrhenius	19	17	4.13 $x10^5$	2.35 $x10^4$	0.88

Equation: Arrhenius $k = A \cdot e^{\frac{-E^a}{R \cdot T}}$