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Electronic Supplementary Information (ESI) for:

A biocatalytic redox isomerisation

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1) Experimental details

Chemicals & Enzymes

All chemicals were purchased from Sigma- Aldrich in the highest purity available and used without further purification.

TADH was produced according to a previously published procedure. [1] *E. coli* BL21 (DE3) harboring pASZ2 (pET 11a derivative containing the gene for TADH) was cultivated in Overnight Express ™ Autoinduction System (Novagene) at 37°C for 24h. The cell pellet was resuspended in buffer and incubated at 80°C for 20 minutes. The supernatant after ultracentrifugation contained more than 90% pure TADH (as estimated from SDS page gel analysis, Figure S1) and was used as is as biocatalyst.

*Ts*ER was produced according to a previously published procedure.[2] *E. coli* BL21 (DE3) harboring a pET22b(+) containing the gene for TsER was cultivated in minimal medium at 30°C. At an OD_{600} of 0.6 protein expression was induced by addition of 0.1 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside) and cultivation was continued overnight. Similar to TADH, *Ts*ER was purified from the resuspended cells by heat treatment yielding >90% pure enzyme (as estimated from SDS page gel analysis, Figure S1)

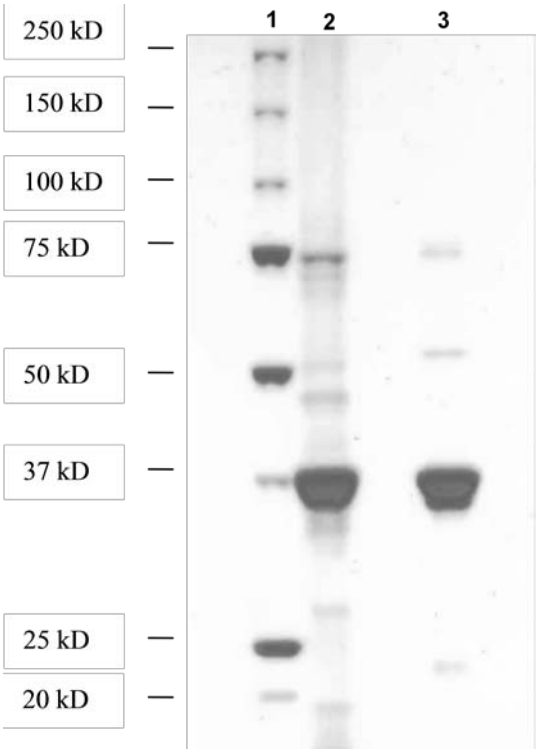

Figure S1. Estimation of the purity of heat-purified TADH (lane 2) and *Ts*ER (lane 3).

General procedure

A typical redox isomerisation reaction was performed on 10 mL scale at 40°C. At intervals, samples were withdrawn and extracted with two volume equivalents ethyl acetate (containing 5 mM dodecane as internal standard) and analyzed by (chiral) GC. All concentrations were determined according to calibrations using authentic standards.
Unless stated otherwise, the reaction conditions were as follows (Table S1).

Table S1. Representative reaction conditions.

Component	Concentration
MOPS buffer (pH8)	50 mM
$CaCl_2$	5 mM
TADH	2.4 μM
TsER	0.56 μM
NAD^+	0.5 mM
Cyclohexenol	10 mM

Analytical Methods

Enzyme concentrations were estimated using the Pierce 660 nm protein assay (Thermo scientific) according to the manufacturer's specifications.

Gas chromatography was conducted using a Shimadzu GC-17A with AOC-201 automatic injector, column: CP wax 52 CB (50 m × 0.53 mm, film tickness 2.0 μm). For chiral GC, a chiraldex G-TA column (50 m ×0.25 mm, thickness 0.12 μm) was used.
A typical GC chromatogram is shown in Figure S2.

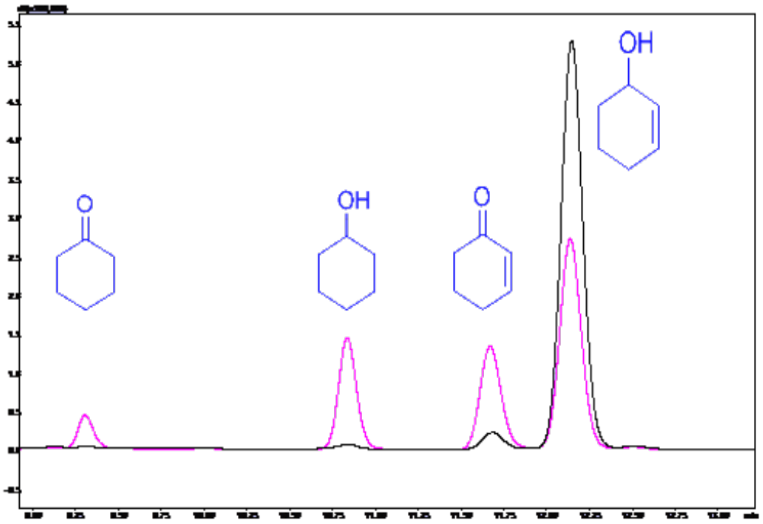

Figure S2. A typical chromatogram of a redoxisomerisation reaction. Black: sample taken immediately after start of the reaction, pink: sample taken after 4h reaction time. Please note that the starting material already contained trace amounts of cyclohexanone; this was taken into account by subtracting this value from the cyclohexanone concentrations determined in the reaction.

2) Variation of [*Ts*ER] at fixed [TADH]

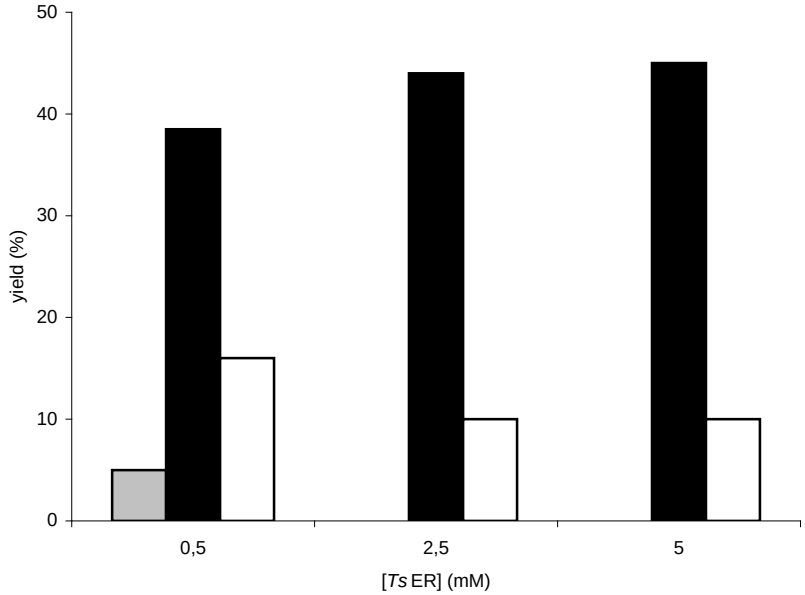

Figure S 3 Effect of increasing [*Ts*ER] (at a fixed concentration of TADH of 2.4 μM) on the product distribution.. ■ cyclohexanone, ⊡ 2-cyclohexen-1-one**,** □ cyclohexanol. The reaction mixtures were analysed after 6h.

3) Reagent evaporation

As mentioned in the manuscript, the current experimental setup suffered from significant mass balance problems, which we attribute to evaporation into the gas phase. Figure S4 shows the evaporation rates for the reagents determined under reaction conditions in the absence of catalysts.
Optimization of the experimental setup is currently underway in our laboratory.

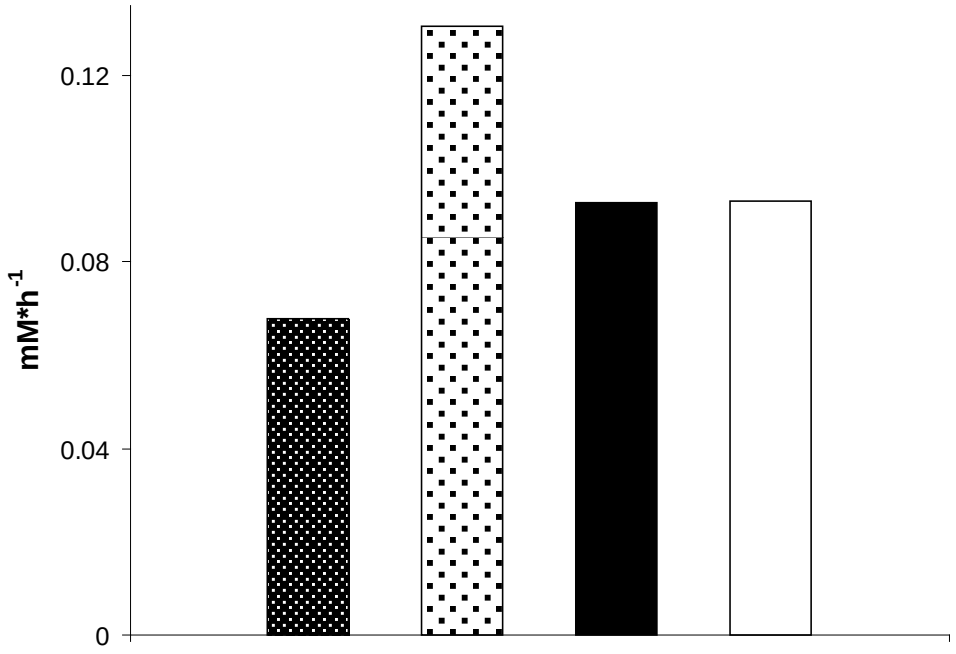

Figure S 4 evaporation rates of 2-cyclohexen-1-ol ▩, 2-cyclohexen-1-one ⊡, cyclohexanone ■ and cyclohexanol □ measured over 4 hrs.

4) Confirmation of the kinetic resolution of rac-cyclohexenol

The significant drop in cyclohexenol oxidation rate after approx. 50% conversion was attributed to a stereopreference of TADH. To validate this assumption, the TADH-catalysed oxidation of cyclohexanol was followed by chiral GC (Figure S5), confirming that indeed one enantiomer is oxidized preferentially.

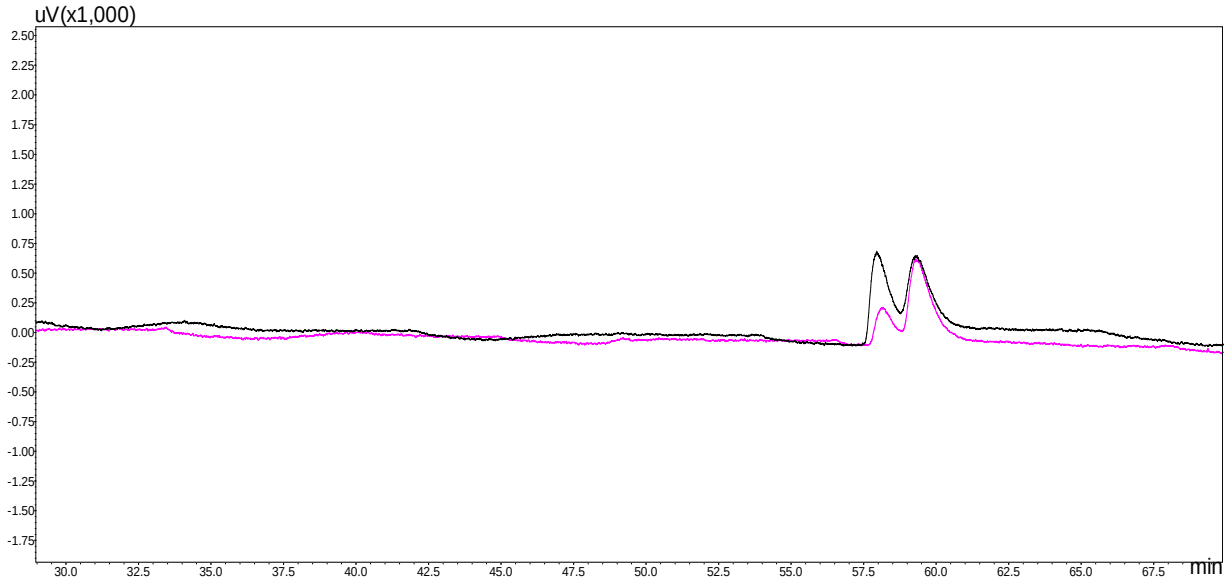

Figure S 5. Chiral analysis of 2-cyclohexen-1-ol oxidation catalyzed by TADH. Black: sample withdrawn at to; pink: sample after 15h; conditions: [TADH] = 2.25 μM, [2-cyclohexen-1-ol]= 10 mM, [NAD^+]= 0.5 mM, pH= 8, T= 40º C.

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5) Mechanism for the formation of phenol (based on the NAD-independent) disproportionation mechanism

As reported earlier[3, 4] enoate reductases are also capable of the so-called cyclohexenone disproportionation (Figure S7) explaining the formation of trace amounts of phenol in some reactions.

Figure S7: Schematic mechanism of disproportionative phenol formation.

1. V. Höllrigl, F. Hollmann, A. Kleeb, K. Buehler and A. Schmid, *Appl. Microbiol. Biotechnol.*, 2008, **81**, 263-273.
2. D. J. Opperman, L. A. Piater and E. van Heerden, *J. Bacteriol.*, 2008, **190**, 3076-3082.
3. A. D. N. Vaz, S. Chakraborty and V. Massey, *Biochem.*, 1995, **34**, 4246-4256.
4. C. Stueckler, T. C. Reiter, N. Baudendistel and K. Faber, *Tetrahedron*, 2010, **66**, 663-667.