Supplementary Material

Transhalogenation Catalysed by Haloalkane Dehalogenases Engineered to Stop Natural Pathway at Intermediate

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I. Conversion of 1-bromobutane in presence of chloride ions.

\textbf{A} \hspace{2cm} \textbf{B}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Supplementary_Figure_S1.png}
\caption{Conversion of 1-bromobutane in the presence of chloride ions. Conversion 1-bromobutane by the wild type DbeA (A) and DbjA (B) in the presence of large excess of chloride ions (1 M) monitored by GC-MS. Both wild type enzymes catalysed the formation of 1-butanol, whereas the transhalogenation product 1-chlorobutane was not detected.}
\end{figure}
II. Stopped-flow analysis of binding of halides to DbjA H280F

Supplementary Figure S2. Stopped-flow analysis of binding of halides to DbjA H280F. Fluorescence traces obtained upon mixing of DbjA H280F with chloride (A), bromide (B) and iodide (C).

Supplementary Figure S3. Binding of iodide to alkyl-enzyme intermediates of DbjA H280F. Dependence of the relative fluorescence on iodide concentration observed upon binding to alkyl-enzyme intermediates of DbjA H280F after reaction with 1,2-dibromoethane (A) and 1-chlorobutane (B). The solid lines represent the best fit by using extended Stern-Volmer equation.
III. Incubation of the DbjA H280F with different substrates in presence of iodide ions.

**Supplementary Table 1.** Formation of transhalogenation products from different substrates and iodide by the variant DbjA H280F.

<table>
<thead>
<tr>
<th>Time [hrs]</th>
<th>1-chlorobutane [mM]</th>
<th>1,2-dibromoethane [mM]</th>
<th>bis(2-chlorethyl)ether [mM]</th>
<th>2,3-dichloropropene [mM]</th>
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</table>

IV. Synthetic preparation of 2-chloro-3-iodo-1-propene

**Experimental Procedure**

Sodium iodide (30 g, 2 equivalents, 200 mmol) is mixed with acetone (70 ml) at room temperature, to this mixture 2,3-dichloroprop-1-ene (11.1 g, 1 equivalent, 100 mmol) is added and the resulting mixture is stirred at room temperature for 12 h. The resulting solution is gently concentrated on rotavap (40 °C bath temperature) to remove the acetone, then diluted with distilled water (150 ml). The lower heavy brownish organic phase containing a trace amount of elemental iodine was decolorized by shaking with dilute NaHSO₃ solution (15% solution, 100 ml). The resulting yellowish organic phase was dried over anhydrous Na₂SO₄ and filtered. The title product (13.35 g, 66% isolated yield) was obtained as yellow liquid with a pungent smell.

![Reaction Scheme](attachment:reaction_scheme.png)

**Supplementary Figure S4. Preparation of 2-chloro-3-iodo-1-propene.** The reaction of 2,3-dichloropropene with NaI in acetone (CF Plus Chemicals, Czech Republic).
Product Specification

Name: 2-chloro-3-iodo-1-propene
Formula: C₃H₄ClI
Formula Weight: 202.42 g/mol
Appearance (Form): liquid
Appearance (Turbidity): clear
Appearance (Colour): yellow
Stability: photolabile
Storage: in dark, 4 °C
Purity (NMR): 97-98%

Supplementary Figure S5. ¹H-NMR spectral characterisation of synthetized 2-chloro-3-iodo-1-propene. ¹H-NMR (300 MHz, CDCl₃, 25 °C) 4.11 (s, 2H), 5.34 (d, J = 1.6 Hz, 1H), δ 5.55 – 5.72 (poorly resolved d, 1H).
V. GC-MS analysis of the enzymatic transformation of 2,3-dichloro-1-propene to 2-chloro-3-iodo-1-propene

Supplementary Figure S6. Mass spectra of 2,3-dichloro-1-propene. The substrate of the enzymatic transformation, 2,3-dichloro-1-propene, analysed from the reaction mixture by using the gas chromatograph (Agilent 7890, USA) equipped with the capillary column ZB-5 (30m x 0.25mm x 0.25μm, Phenomenex) and connected with a mass spectrometer (Agilent 5975C, USA). The MassHunter Version B.07.00 and NIST Mass Spectral Search Programme for the NIST/EPA/NIH Mass Spectral Library 2.0 g used for mass spectra identification. The 2,3-dichloro-1-propene (D72603) was purchased from Sigma-Aldrich (Milwaukee, USA).
Supplementary Figure S7. Mass spectra of 2-chloro-3-iodo-1-propene. Comparison of the mass spectra of 2-chloro-3-iodo-1-propene produced by organic synthesis (A) and by enzymatic transformation of 2,3-dichloro-1-propene in the presence of iodide ions (B).
Supplementary Figure S8. Fragmentation patterns. The fragmentation patterns of 2,3-dichloro-1-propene (A) and 2-chloro-3-iodo-1-propene (B) and isotopic clusters for individual fragments (C) predicted by using Mass Frontier 1.0 software (HighChem, Slovak Republic).