SUPPORTING INFORMATION

Asymmetric reduction of activated alkenes by pentaerythritol tetranitrate reductase: specificity and control of stereochemical outcome by reaction optimisation

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Supporting Figure S1. Steady-state kinetic data for the reduction of α,β-unsaturated activated alkenes with PETN reductase. Reactions (0.3 ml) were performed in buffer (50 mM KH_2PO_4/K_2HPO_4 pH 7.0) containing NADPH (100 µM), oxidizing substrate (2.5 µM to 50 mM), PETNR (12-2000 µM) and 5 % ethanol to solubilise the substrates. The reactions were followed continuously by monitoring NADPH oxidation at 340 nm for 2 minutes at 25°C. Reactions with oxidizing substrates that absorbed at 340 nm were monitored at 365 nm.

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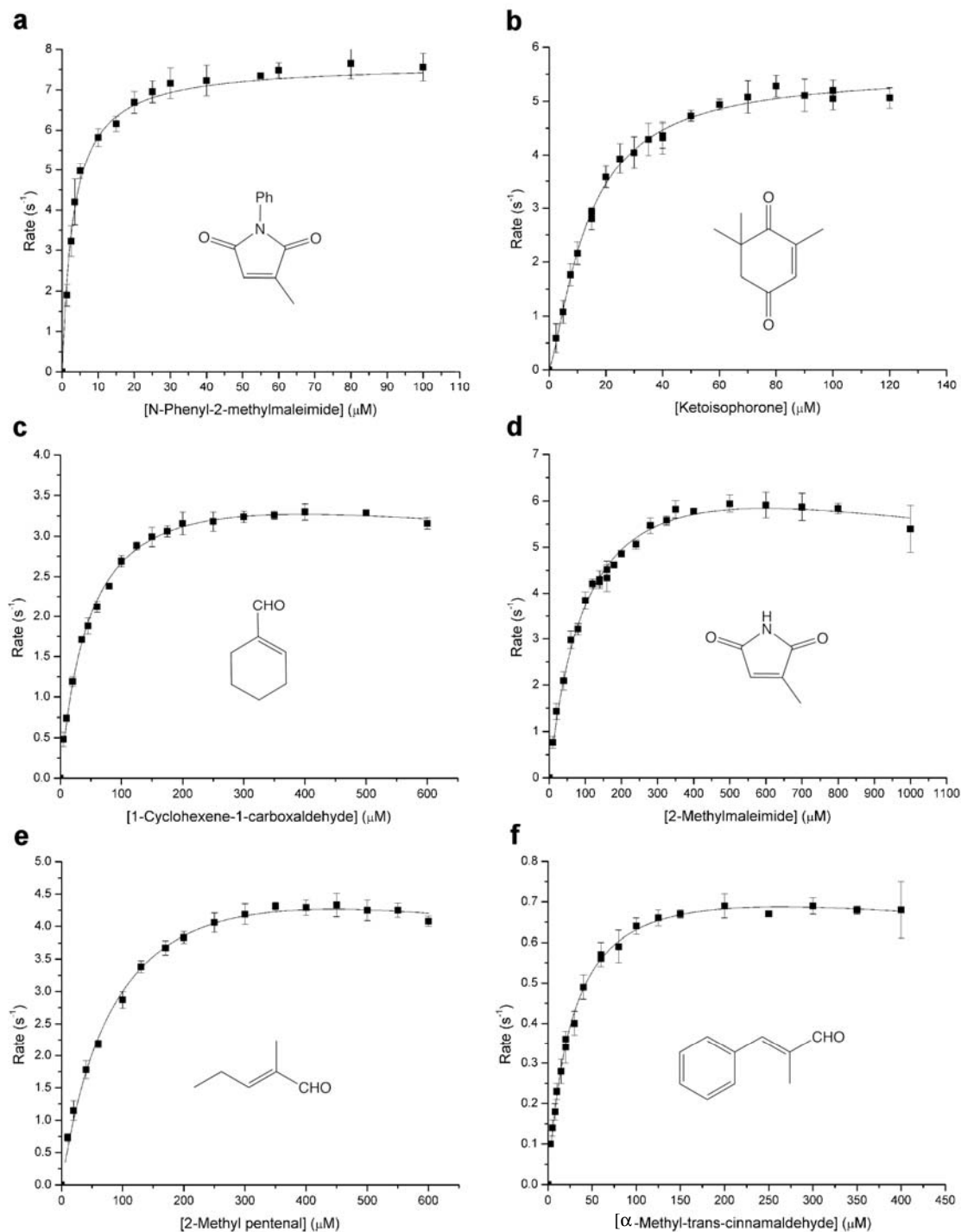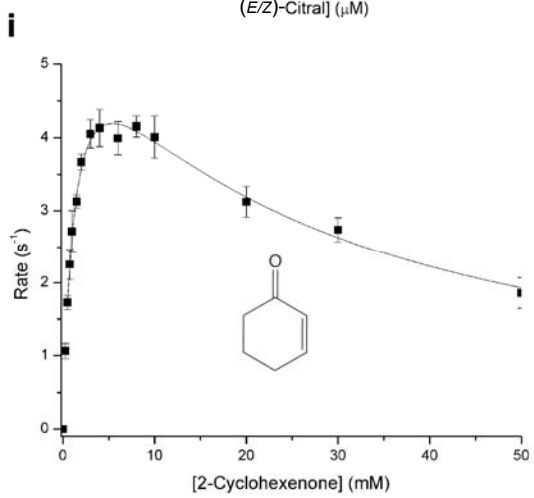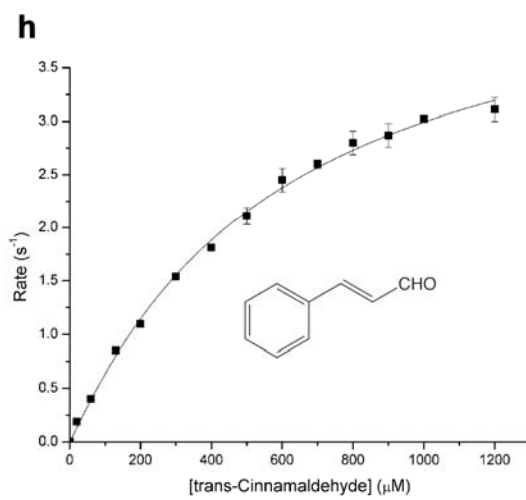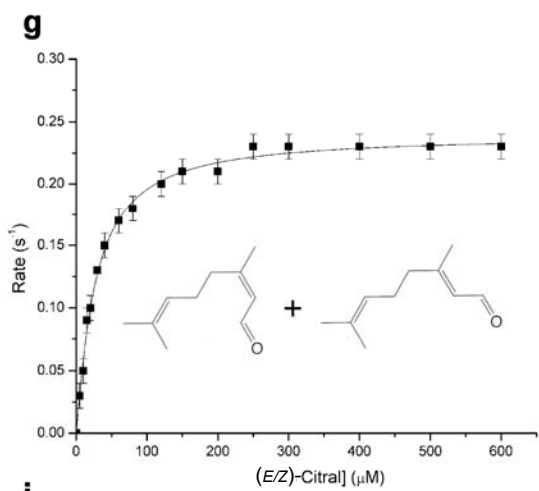
Supporting Figure S2. Models of the active site of isomers of (*E/Z*)-citral **11b**: (a): (*E*)-geranial ((*E*)-**11a**) and (b): (*Z*)-neral ((*Z*)-**11a**) into the active site of PETNR. The position of (2-CH) is derived from a superimposition of the 2-cylohexenone (**4a**)-PETNR structure onto the model (pdb code 1GVQ).[1] The modelling of (*E*)-geranial (Figure 1a) indicates significant clashes of the substrate with T26 residue. All residues, FMN and substrates were shown as atom coloured sticks with green, yellow and magenta carbons, respectively.
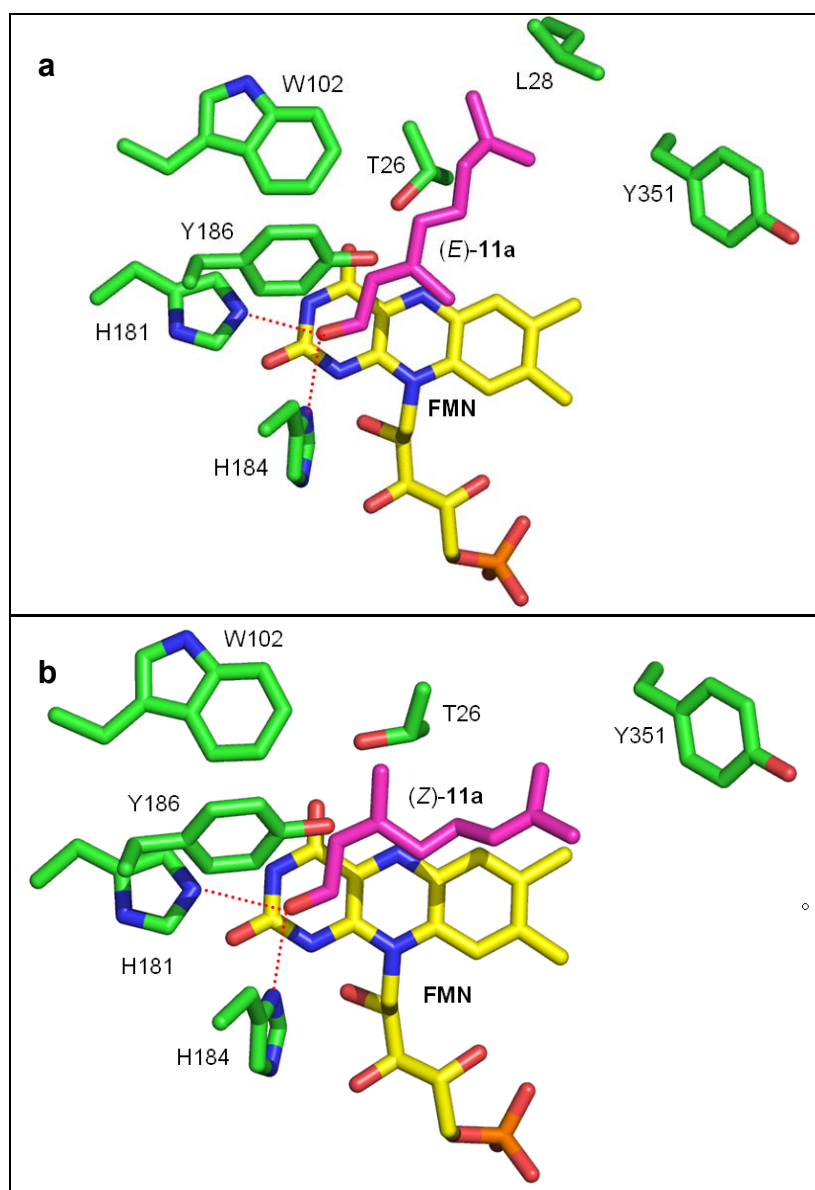
Supporting Figure S3. The effect of time on the reduction of ketoisopherone **3a** and product racemisation catalysed by PETNR (0.02 µM enzyme concentration): ■ - conversion; △ - ee; ○ - yield.
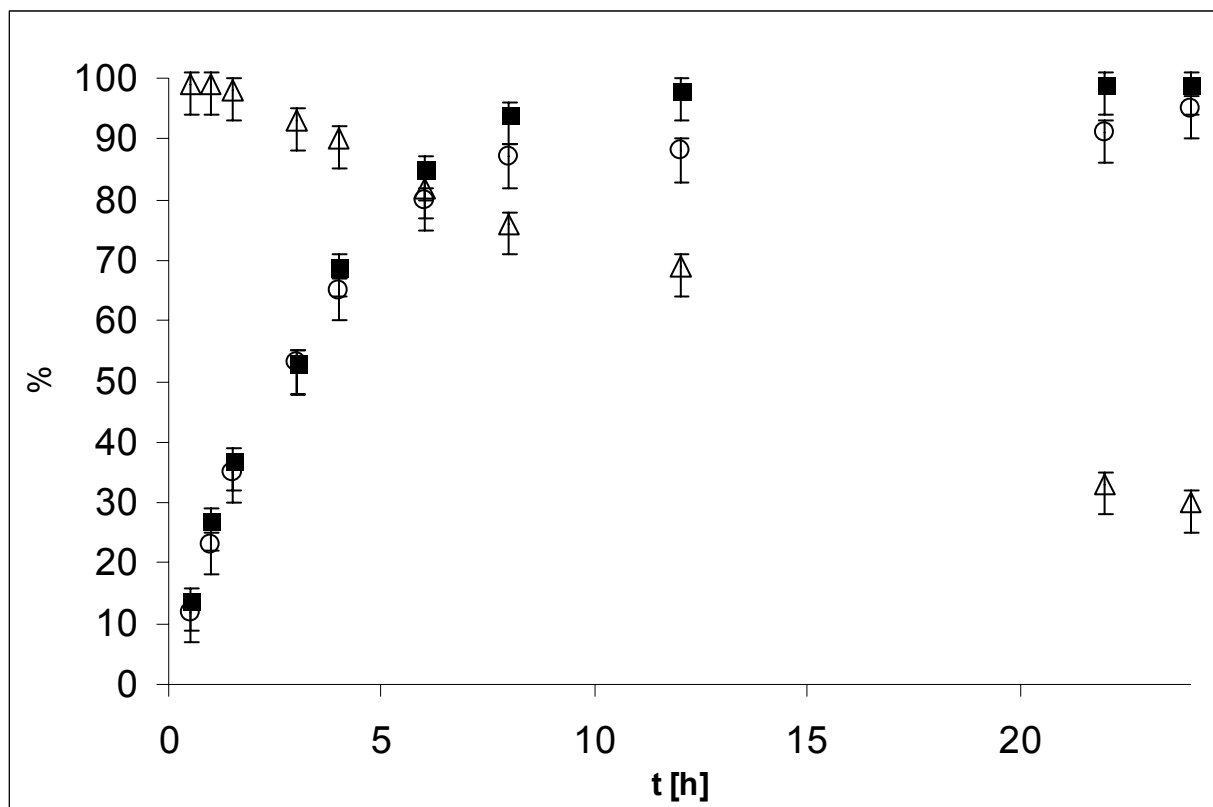
Table S1. The effect of time on the reduction of ketoisopherone **3a** and product racemisation catalysed by PETNR (2 µM enzyme concentration).[a]

Entry	Time (h)	Conv.[b] (%)	Yield[b] (%)	Ee[c] (%)
1	0.5	>99	98	97
2	1	>99	>99	95
3	1.5	>99	>99	93
4	2	>99	97	92
5	3	>99	97	87
6	4	>99	97	83
7	6	>99	97	72
8	8	>99	97	70
10	14	>99	97	48
12	24	>99	97	24

a) alkene **3a** (5 mM), PETNR (2µM), NADPH (6 mM) in phosphate buffer (50 mM, pH 7.0, 1 mL total volume), 30 °C at 130 rpm, 2h. Alkene **3a** was added as a DMF solution (2% final DMF solution);[b] by GC using DB-Wax column; [c] by GC using Chirasil-DEX-CB.

Supporting Figure S4. The effect of time on the reduction of ketoisopherone **3a** and product racemisation catalysed by PETNR (2 µM enzyme concentration): ■ - conversion; △ - ee; ○ - yield.
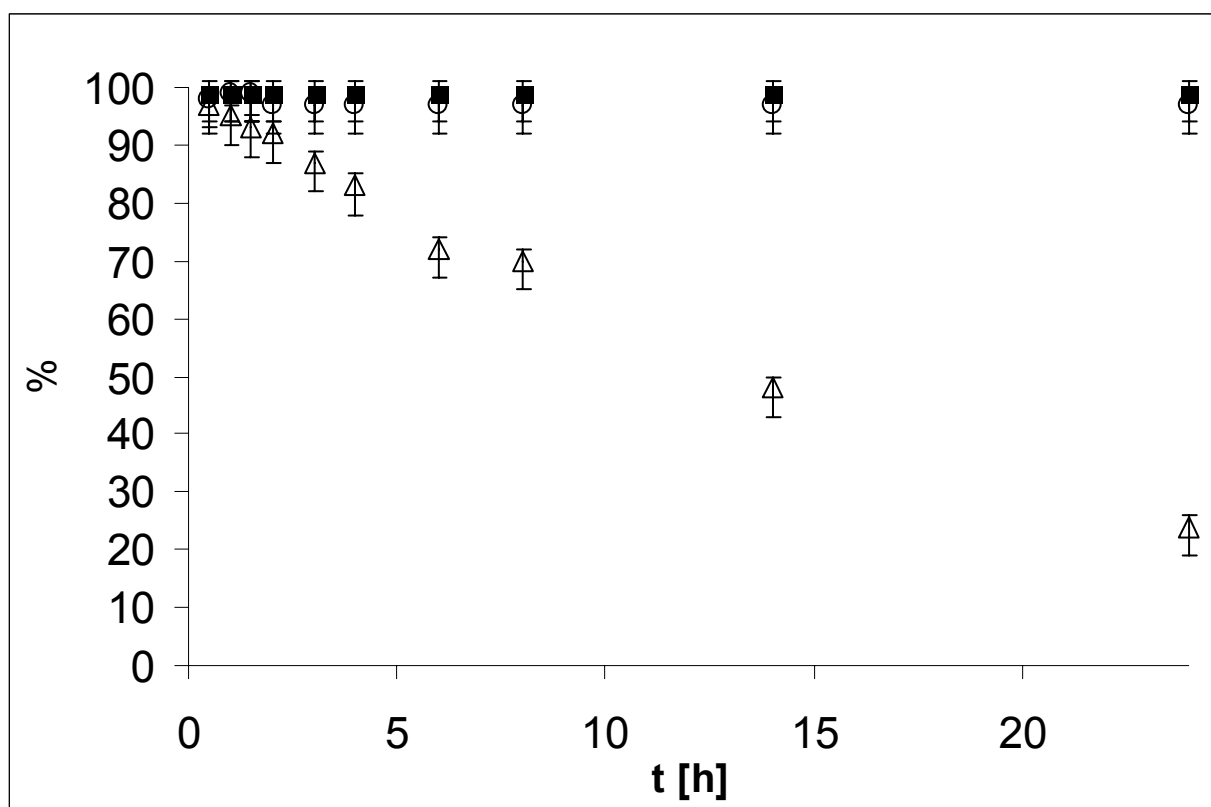
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[1]   H. Khan, R. J. Harris, T. Barna, D. H. Craig, N. C. Bruce, A. W. Munro, P. C. Moody, N. S. Scrutton, *J. Biol. Chem.* **2002**, *277*, 21906-21912.