Using Mutability Landscapes To Guide Enzyme Thermostabilization

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Supporting Figures

Figure S1. Chiral GC chromatograms of the γ-nitroaldehyde products obtained by enzymatic acetaldehyde addition to *trans*-β-nitrostyrene. Racemic standard (A), product obtained using 4-OT wild type (B), product obtained using 4-OT variant A33D (C), product obtained using 4-OT variant R11I (D) and product obtained using 4-OT variant R11Y (E).
Figure S2. Chiral GC chromatograms of the γ-nitroaldehyde products obtained by enzymatic acetaldehyde addition to trans-β-nitrostyrene. Racemic standard (A), product obtained using 4-OT variant R11I/M45Y/F50A (B), product obtained using 4-OT variant R11Y/M45Y/F50A (C), and product obtained using 4-OT variant A33D/M45Y/F50A (D).
Figure S3. SDS-PAGE gels of purified enzymes. 4-OT wild-type (A), 4-OT variant A33D (B), 4-OT variant R11I (C), and 4-OT variant R11Y (D).
Supporting Table

**Table S1.** Relative activity, enantioselectivity and stability of 4-OT variants.

| Catalyst       | Relative activity | e.r.      | Abs. conf. | $T_m$ (°C) |
|----------------|-------------------|-----------|------------|-------------|
| WT             | 1                 | 97:3      | $S$        | 74          |
| A33D           | 2.3               | >99:1     | $S$        | 78          |
| R11I           | 0.7               | 95:5      | $S$        | 94          |
| R11Y           | 0.4               | 98:2      | $S$        | 92          |
| R11I/A33D      | 0.2               | 99:1      | $S$        | 60          |
| R11L/A33D      | 0.2               | 97:2      | $S$        | 64          |
| R11Y/A33D      | 0.2               | 99:1      | $S$        | 65          |
| R11F/A33D      | 0.2               | >99:1     | $S$        | 76          |
| R11Q/A33D      | 0.5               | >99:1     | $S$        | 51          |
| M45Y/F50A      | 1.3               | 6:94      | $R$        | 57          |
| A33D/M45Y/F50A | 3.5               | 8:92      | $R$        | 84          |
| R11I/M45Y/F50A | 3.1               | 5:95      | $R$        | 96          |
| R11Y/M45Y/F50A | 2.2               | 6:94      | $R$        | 92          |