library using 200 ng DNA was selected based on its retention of function rate: 40% of clones had activity not less than 40% less than wild type. This library had an error rate of 1.8 missense mutations per gene among sequenced hits. DNA shuffling was performed to recombine twelve activating mutations, which were distributed over two gBlocks (block 1: W2R, F35S, M123V, N166D, M233V, and T292S; block 2: E17G, 168V, M144T, L182F, F274S, and T312A). These two gBlocks and the wild-type gBlock served as template for DNA shuffling following a modified protocol of Stemmer (41). The resulting library was cloned into pET22(b)+ between restriction sites NdeI and XhoI in frame with the C-terminal his-tag for expression in E. coli BL21 E. cloni EXPRESS cells.

Due to low recombination frequency of the DNA shuffling library, a recombination library of mutations found at residues M123, M144, N166, L182, M233, F274, and T292 (generation 2) was constructed using SOE PCR (42). The mutagenesis primers encoded the desired mutations and also the corresponding wild-type sequences. Because the distances between some of the target sites would have resulted in very short fragments, we built the library in two steps. During the first step, we generated five fragments encompassing residues M123, N166, M233, and T292 using Phusion polymerase according to the manufacturer’s recommendations. The fragments were then DpnI-digested, gel-purified, and used as template for the subsequent assembly PCR using the flanking primers only. The assembly PCR product served as template for a second round of PCRs generating the fragments carrying the remaining mutations, followed by the same procedure as described above. The final assembly product was cloned into pET22(b)+ between restriction sites NdeI and XhoI in frame with the C-terminal his-tag for expression in E. coli BL21 E. cloni EXPRESS cells.

**High-Throughput Screening.** For high-throughput expression, BL21 E. cloni EXPRESS cells carrying pTRpB wild-type and variant plasmids were grown in 96-well deep-well plates in 300 μL TBamp at 37 °C and 80% humidity with shaking at 250 rpm overnight. TBamp expression cultures (630 μL) were inoculated with 20 μL of the overnight cultures and continued to grow at 37 °C and 80% humidity with shaking at 250 rpm for 3 h. Expression was induced with the addition of IPTG to a final concentration of 1 mM to cold-shocked (20 min on ice-water bath) cultures. The expression continued for another 20 h at 20 °C with shaking at 250 rpm. Cells were then centrifuged at 4,000 × g for 10 min and frozen at −20 °C overnight. For screening, cells were allowed to thaw at room temperature and then lysed in lysis buffer (400 μL per well of 200 mM phosphate buffer, pH 8, with 1 mg/mL lysozyme, 40 μM PLP, and ∼0.05 mg/mL DNase I) for 1 h at 37 °C. After centrifugation at 5,000 × g for 20 min, a 160-μL aliquot of the lysate was transferred into PCR plates (USA Scientific), heat-treated for 1 h at 75 °C, and then spun again at 1,000 × g and 4 °C for 30 min. After the addition of 160-μL assay buffer (200 mM phosphate buffer, 20 mM indole, 0.75 mM L-serine, pH 8) to 40 μL of cleared, heat-treated lysates in UV-transparent assay plates (Evergreen Scientific), formation of L-tryptophan took place at 75 °C for up to 1 h (generation 1:1 h; generations 2 and 3: 3.6 min). The reactions were then quenched in an ice-water bath, and the amount of L-tryptophan formed was recorded at 290 nm with a plate reader (Tecan Infinite M200).

**Kinetics.** pTRpB activity (kcat) was measured by monitoring tryptophan formation in a UV1800 Shimadzu spectrophotometer (Shimadzu) at 75 °C over 1 min at 290 nm using ΔA290 = 1.89 mM−1 cm−1 (15). The assay buffer contained 200 mM potassium phosphate,
pH 8, and 5 μM PLP. Michaelis–Menten constants (Kₘ) for indole were determined using a concentration range from 0.4 to 0.015 mM indole with the concentration of l-serine fixed at 25 mM. Kₘ values for l-serine were measured with a concentration range from 50 to 0.5 mM l-serine with the concentration of indole fixed at 250 μM. Data were fit using an in-house nonlinear least-squares algorithm to the Michaelis–Menten equation implemented in MATLAB.

UV-Vis Spectroscopy. Spectra were collected between 550 and 250 nm on a UV1800 Shimadzu spectrophotometer using 20 μM of enzyme in 200 mM potassium phosphate (pH 8.0) in a quartz cuvette. Samples were incubated at 75 °C for >3 min to ensure a stable temperature was reached. Stage 1 of the reaction was initiated by addition of 20 mM l-serine, and the spectra were measured in <10 s to limit production of pyruvate from deamination of l-serine, which absorbs at 320 nm.

Substrate Selectivity. Purified enzyme was used to measure the relative rate of PTrpS and PTrpB. NCAA production compared to 6. To determine the temperature initiated by addition of 20 mM l-serine, and the spectra were measured in <10 s to limit production of pyruvate from deamination of l-serine, which absorbs at 320 nm.

Thermostability Measurements. To determine the temperature where 50% of enzymes are rendered inactive (T₅₀), 5 μM of PTrpB wild type and 1 μM of its variants were incubated at varying temperature ranges (wild type: 80–99 °C; variants 75–95 °C) for 1 h. After quenching on ice, the residual activity was measured as described above.

Crystallography. Freshly purified PTrpB (described above) was buffer exchanged against 20 mM Hepes (pH 7.85) with 5% glycerol and frozen at −80 °C until further use. Sparse matrix screening was performed using an Art Robbins Gryphon Nano crystallization robot using 0.2 μL of 11 mg/mL PTrpB with 40 μM PLP and 0.2 μL of well solution. Crystals were found in a solution of 25% PEG3350 and 0.1 M NaHepes (pH 7.5) after 3 d. Crystals were then routinely grown as sitting drops against a 1-mL reservoir of 15–25% PEG3350 and 0.1 M NaHepes (pH 7.85) with mother liquor comprised of 1.5 μL of 8.0 mg/mL PTrpB and 1.5 μL of well solution. Ligand-bound structures were determined by soaking crystals of PTrpB with 15 mM L-tryptophan or 100 mM l-serine for 2 min. Crystals were cryoprotected through oil immersion in Fomblin Y (Sigma) and flash-frozen in liquid N₂ until diffraction. Diffraction data were collected remotely at the Stanford Synchrotron Radiation Laboratories on beamline 12-2. Crystals routinely diffracted at or below 2.0 Å, and the data were integrated and scaled using XDS (43) and AIMLESS (44). A resolution cutoff of 0.3 applied along the strongest axis of diffraction (44, 45). These data contributed to model quality as judged by R_free in the final bin <0.4. Structures were solved using molecular replacement with PHASER, as implemented in CCP4 (46, 47). The search model comprised a single monomer of PTrpB (PDB ID code 1V8Z) (21) subjected to 10 cycles of geometric idealization in Refmac5 and removal of all ligands. Model-building was performed in Coot (48) beginning with data processed at 2.4 Å, followed by subsequent inclusion of increasingly higher-resolution shells of data with relaxed geometric constraints. This procedure was particularly important for the structures of l-tryptophan- and l-serine-bound PTrpB, which contained a large rigid body motion of the COMM domain. Refinement was performed using REFMAC5 (49). The MolProbity server was used to identify rotamer flips and to identify clashes (50). After the protein, ligand, and solvent atoms were built, TLS operators were added to refinement, which resulted in substantial improvements in R_free for the models. Crystallographic and refinement statistics are reported in Table S3.

A similar procedure was used for the crystal structure of PTrpS, with a protein concentration of 4.0 mg/mL. Sparse matrix screening identified crystals grew in 20% PEG 3350 and 0.2 M Na citrate. Crystals were routinely grown in 24-well format with 16–20% PEG 3350 and 0.15–0.2 M Na citrate. Cryoprotection was achieved by the addition of 25% glycerol and flash-frozen in liquid N₂. Crystals regularly diffracted to ~3.0 Å, and a single crystal was found that diffracted to 2.76 Å. A molecular replacement search model was made using an oβ-dimer of PTrpS (PDB ID code 1WDW) (22). Model-building proceeded as described above. Data in the final bin were weak, but contributed to model quality as measured by R_free < 0.4 in the final bin. Coordinates are deposited in the Protein Data Bank with ID codes 5E0K (PTrpS), 5DVZ (PTrpB), 5DW0 (Ser-bound PTrpB), and 5DW3 (Trp-bound PTrpB).

Identification of Nonnatural Amino Acid Products. The identities of the amino acid products were confirmed by 1H NMR and LRMS. Proton NMR spectra were recorded on a Varian 300-MHz or Bruker 400-MHz spectrometer. Proton chemical shifts are reported in parts per million (δ) relative to tetramethylsilane and calibrated using the residual solvent resonance (DMSO, δ 2.50 ppm; CD₃OD, δ 3.31 ppm; D₂O, δ 4.79 ppm). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), doublet of doublets (dd), doublet of doublets of doublets (ddd), triplet (t), triplet of doublets (td)], coupling constants [Hz], integration). Fluorine NMR spectra were recorded on a 300-MHz (282 MHz) spectrometer without proton decoupling. Fluorine chemical shifts are reported in ppm relative to FCCl₃ (δ 0.00 ppm) and were calibrated automatically by the spectrometer using the solvent deuterium lock signal. Low-resolution mass spectra were obtained using an Agilent 1290 UHPLC-LCMS. The optical purity of the products was estimated by derivatization with FDNP-alanamide as described below.

Reaction with heat-treated lysate. All reactions were conducted using PTrpB, which was prepared by heat treatment as described in SI Materials and Methods, Cloning, Expression, and Purification of PTrpA and PTrpB. The protein was used as a solution in potassium phosphate buffer (50 mM, pH 8) and was found to have a concentration of 134 μM, as determined by specific activity (Table 1). Serine and PLP were used as aqueous solutions (500 mM and 15 mM, respectively).

A 6-mL crimp vial was charged sequentially with the indole analog (50 μmol), dimethyl sulfoxide (50 μL), serum (100 μL), and PLP (3.5 μL). The resulting suspension was diluted with 772 μL of potassium phosphate buffer (200 mM, pH 8). Finally, the enzyme solution was added, and the vial was sealed and immersed in an oil bath that had been equilibrated to 75 °C. After 12 h, the reaction mixture was allowed to cool to room temperature, and then purified directly on C-18 silica (20 mL column volume) with 0–50% methanol/H₂O.

Determination of optical purity. The amino acids were used as 0.2-M solutions in 1 N aqueous (aq.) HCl. FDNP-alanamide was used as a solution in acetone (33 mM). In a 2-mL vial, the amino acid (2.5 μL, 0.50 μmol) was diluted in 1 mL NaHCO₃ (67 μL). FDNP-alanamide (30 μL, 1 μmol) was added, and then the vial was immersed in an oil bath that had been equilibrated to 40 °C. After 12 h, the reaction mixture was allowed to cool to room temperature, and then diluted with 1:1 water/acetonitrile (900 μL). The resulting solution was subjected to centrifugation (20,000 × g, 4 °C, 5 min). The supernatant was analyzed directly by LC-MS. Each amino acid was identified by LC-MS.
acid was derivatized with both racemic and enantiopure FDNP-alamamide for comparison. All amino acid products were enantiopure. Absolute stereochemistry was inferred by analogy to l-tryptophan.

\[ \text{H NMR (400 MHz, CD}_2\text{OD)} \delta 7.60 (d, J = 7.1 \text{ Hz, 1H}), 7.26 (d, J = 7.4 \text{ Hz, 1H}), 7.06-6.97 (m, 2H), 3.83 (dd, J = 9.9, 4.2 \text{ Hz, 1H}), 3.26 (ABX, J_{AX} = 9.9 \text{ Hz, J}_{BX} = 4.2 \text{ Hz, J}_{AB} = 15.2 \text{ Hz, v}_{AB} = 164.0 \text{ Hz, 2H}), 2.42 (s, 3H). \text{LRMS (ESI) (m/z)} \]

for \([\text{M+H}^+\text{]} C_{13}H_{15}N_2O_4 \text{ requires 219.1, observed 219.1.} \]

\[ \text{H NMR (300 MHz, DMSO-d}_6) \delta 1.40 (s, 1H), 7.23 (s, 1H), 7.18 (d, J = 8.1 \text{ Hz, 1H}), 7.00 (td, J = 7.9, 5.2 \text{ Hz, 1H}), 6.69 (dd, J = 11.5, 7.8, 0.8 \text{ Hz, 1H}), 3.52-3.36 (m, 2H), 2.90 (dd, J = 15.8, 11.1, 1H). \text{\textsuperscript{19}F NMR (282 MHz, DMSO-d}_6) \delta -124.26 (dd, J_{F-H} = 11.3, 5.2 \text{ Hz}). \text{LRMS (ESI) (m/z) for [M+H]^+] C_{11}H_{12}F_2N_2O_2 requires 223.1, observed 223.1.} \]

\[ \text{H NMR (400 MHz, CD}_2\text{OD)} \delta 7.91 (d, J = 1.8 \text{ Hz, 1H}), 7.29 (d, J = 8.5 \text{ Hz, 1H}), 7.23 (s, 1H), 7.21 (dd, J = 8.6, 1.9 \text{ Hz, 1H}), 3.82 (dd, J = 9.1, 4.1, 1H), 3.29 (ABX, J_{AX} = 9.1 \text{ Hz, J}_{BX} = 4.2 \text{ Hz, J}_{AB} = 15.2 \text{ Hz, v}_{AB} = 124.0 \text{ Hz, 2H}). \text{LRMS (ESI) (m/z) for [M+H]^+] C_{12}H_{12}BrN_2O_2 requires 283.0 and 285.0.} \]

As the HCl salt: \[ \text{H NMR (400 MHz, D}_2\text{O)} \delta 8.16 (s, 1H), 7.84 (d, J = 8.2 \text{ Hz, 1H}), 7.62 (d, J = 8.6 \text{ Hz, 1H}), 7.52 (t, J = 7.7 \text{ Hz, 1H}), 7.25 (t, J = 7.5 \text{ Hz, 1H}), 4.99 (ABX, J_{AX} = 4.1 \text{ Hz, J}_{BX} = 6.0 \text{ Hz, J}_{AB} = 15.6 \text{ Hz, v}_{AB} = 44.0 \text{ Hz, 2H}), 4.54 (dd, J = 6.0, 4.1 \text{ Hz, 1H}). \text{LRMS (ESI) (m/z) for [M+H]^+] C_{13}H_{12}BrN_2O_2 requires 206.1, observed 206.1.} \]

As the HCl salt: \[ \text{H NMR (400 MHz, D}_2\text{O)} \delta 7.22 (d, J = 7.3 \text{ Hz, 1H}), 7.16 (t, J = 7.7 \text{ Hz, 1H}), 6.83 (t, J = 7.4 \text{ Hz, 1H}), 6.70 (d, J = 7.9 \text{ Hz, 1H}), 4.25 (q, J = 6.0 \text{ Hz, 1H}), 3.70-3.56 (m, 2H), 3.46 (q, J = 8.3 \text{ Hz, 1H}), 3.37 (q, J = 8.4 \text{ Hz, 1H}), 3.00 (t, J = 8.2 \text{ Hz, 2H}). \text{LRMS (ESI) (m/z) for [M+H]^+] C_{12}H_{12}N_2O_2 requires 207.1, observed 207.1.} \]

\[ \text{H NMR (400 MHz, CD}_2\text{OD)} \delta 10.48 (d, J = 2.2 \text{ Hz, 1H}), 7.30 (d, J = 8.5 \text{ Hz, 1H}), 6.95 (d, J = 2.2 \text{ Hz, 1H}), 6.69 (d, J = 2.1 \text{ Hz, 1H}), 6.51 (dd, J = 8.5, 2.1 \text{ Hz, 1H}), 3.38 (dd, J = 9.2, 3.6 \text{ Hz, 1H}), 3.02 (ABX, J_{AX} = 9.1 \text{ Hz, J}_{BX} = 3.8 \text{ Hz, J}_{AB} = 15.0 \text{ Hz, v}_{AB} = 155.5 \text{ Hz, 2H}). \text{LRMS (ESI) (m/z) for [M+H]^+] C_{11}H_{13}N_2O_3 requires 221.1, observed 221.1.} \]

\[ \text{H NMR (300 MHz, D}_2\text{O)} \delta 8.16 (dd, J = 5.0, 1.4 \text{ Hz, 1H}), 8.06 (dd, J = 7.9, 1.5 \text{ Hz, 1H}), 7.31 (s, 1H), 7.13 (dd, J = 7.9, 4.9 \text{ Hz, 1H}), 3.96 (dd, J = 7.3, 5.1 \text{ Hz, 1H}), 3.30 (ABX, J_{AX} = 7.3 \text{ Hz, J}_{BX} = 5.2 \text{ Hz, J}_{AB} = 15.4 \text{ Hz, v}_{AB} = 33.0 \text{ Hz, 2H}). \text{LRMS (ESI) (m/z) for [M+H]^+] C_{10}H_{12}N_2O_2 requires 206.1, observed 206.1.} \]

Fig. S1. Distribution of activating mutations identified through random mutagenesis and screening. (A) Positions on PfTrpB where activating mutations were discovered are displayed as spheres. The mutations are W22R, G4C, V11A, E17G, K20E, E23V, F355, N355, Y41C, I68V, M123V, I127S, M144T, N150T, N166D, Y178C, H180R, Y181C, L182P, M233V, M233I, F274S, F274L, D284G, T292S, T321A, and T323A. Mutations located in the COMM domain are colored blue, those within 5 Å of PfTrpA (red) are cyan, those present in PfTrpB\textsuperscript{101} are yellow, and the remainder are gray. (B) Positions on PfTrpB where activating mutations were discovered using PfTrpB\textsuperscript{101} as the parent for random mutagenesis. The mutations are G4D, E5K, Y10H, P12L, E13G, E21V, L39Q, K67I, L146V, D220E, N267S, G272D, and D284E. Color scheme is the same as A; yellow spheres are mutations present in the final variant PfTrpB\textsuperscript{102}. 

Buller et al. www.pnas.org/cgi/content/short/1516401112
Fig. S2. UV-vis absorption spectra for native and engineered TrpB. A, D, and E are the same as in Fig. 2. PLP absorption spectra for each enzyme are shown in black and share \( \lambda_{\text{max}} = 412 \text{ nm} \), characteristic of E(Ain). Addition of 20 mM L-Ser causes a red-shift to 420 nm (red), consistent with E(Aex) formation for PfTrpB and PfTrpB4G9 (B), which also has an absorbance band at \( \sim 320 \text{ nm} \) that we attribute to formation of pyruvate through serine deaminase activity. L-Ser addition to PfTrpS, PfTrpB4D11 (C), and PfTrpB0B2 causes a shift in \( \lambda_{\text{max}} \) to 350 nm and a broad shoulder extending to 550 nm. The 350-nm absorption is attributed to the E(A-A), and the shoulder indicates a mixed population of E(Aex) and E(A-A). All spectra collected with 20 \( \mu \text{M} \) enzyme. To limit the amount of pyruvate production, spectra were taken as quickly as possible (<10 s) after addition of L-Ser.

Fig. S3. Structural comparison between StTrpS and PfTrpB in E(Ain) and E(Aex) forms. (A) Alignment of StTrpB (green; PDB ID code 1BKS) and PfTrpB (light blue) in their substrate-free [E(Ain)] forms yields an rmsd of 0.5 Å. Small differences exist in the structures near the \( \alpha \)-subunit (red) binding site, and the COMM domain of PfTrpB (dark blue) is in a slightly more open conformation than for StTrpB. (B) Alignment of StTrpB (green; PDB ID code 2CLL) and PfTrpB (light blue) with L-serine covalently bound as the external aldimine, E(Aex), yields an rmsd of 0.5 Å.

Fig. S4. Interactions between PfTrpA and PfTrpB that are disrupted in PfTrpB0B2. (A) Hydrogen bond between H275\( ^{\beta} \) (gray) and D43\( ^{\alpha} \) (red) in the gate-open conformation is adjacent F274\( ^{\beta} \) (yellow), which is mutated to Ser in the PfTrpB4D11 and PfTrpB0B2 enzymes. (B) A salt bridge (dashes) between R148\( ^{\alpha} \) (red) and E17\( ^{\beta} \) (yellow) is removed with the E17G mutation present in PfTrpB4D11 and PfTrpB0B2. P12\( ^{\beta} \) is located along the indole tunnel (C) between the subunits. Steric constraints indicate that the P12L mutation of PfTrpB0B2 would disrupt this tunnel, which might contribute to the large drop in Trp synthase activity of PfTrpB0B2.
Fig. S5. Hypothetical reaction coordinate diagram to illustrate inversion of TrpA effector activation to inhibition. Native enzyme, such as TrpB (blue), with multiple reaction transition states. Effector addition or introduction of mutations (green) increases the rate of the reaction by lowering the free energy of the rate-limiting step (RLS), while simultaneously raising the energy of a different transition state thereby generating a new RLS. The same changes applied a second time (red), as might occur with PfTrpA addition to the engineered TrpB, can lead to a free-energy barrier higher than for the native enzyme and a reduction in overall reaction rate.

Fig. S6. SDS/PAGE of PfTrpA, PfTrpB, and PfTrpS. 1, PfTrpA; 2, PfTrpB; 3–5, aliquots from 1.5-mL fractions from PfTrpA pull-down using His-tagged PfTrpB; 4–20% gradient, SDS/PAGE. Molecular weights from ColorPlus Prestained Protein Ladder (New England Biosystems) listed on the right.

Table S1. Comparison of TrpB thermophilic enzymes

<table>
<thead>
<tr>
<th>Host</th>
<th>$k_{cat}$, s$^{-1}$</th>
<th>$K_M$, mM l-Ser</th>
<th>$K_M$, μM indole</th>
<th>$k_{cat}$ change with TrpA</th>
<th>Thermal stability indicators</th>
<th>Ref(s).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermotoga maritima</td>
<td>4.2</td>
<td>110</td>
<td>40</td>
<td>2.4</td>
<td>Kinetics measured at 80 °C</td>
<td>18</td>
</tr>
<tr>
<td>Thermococcus kodakarenis</td>
<td>1.04 ± 0.03</td>
<td>n/a</td>
<td>63 ± 5</td>
<td>3.3</td>
<td>5% activity after 1 h at 80 °C</td>
<td>19</td>
</tr>
<tr>
<td>Pyrococcus furiosus</td>
<td>0.31 ± 0.02</td>
<td>1.2 ± 0.1</td>
<td>77 ± 12</td>
<td>3.2</td>
<td>$T_{50} = 94.7 ± 1.2$</td>
<td>20–22</td>
</tr>
</tbody>
</table>

Values for PfTrpB are those reported here; the references supply similar data measured by a different group. n/a, not available.

Table S2. Biochemical characterization of native and engineered enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Mutations</th>
<th>$k_{cat}$, s$^{-1}$</th>
<th>$K_M$, mM l-Ser</th>
<th>$K_M$, μM indole</th>
<th>$k_{cat}/K_M$, mM$^{-1}$·s$^{-1}$ (Ind)</th>
<th>$k_{cat}$ change with TrpA</th>
<th>$T_{50}$ °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PfTrpS</td>
<td>–</td>
<td>1.0 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>20 ± 2</td>
<td>50</td>
<td>–</td>
<td>&gt;95</td>
</tr>
<tr>
<td>PfTrpB</td>
<td>–</td>
<td>0.31 ± 0.02</td>
<td>1.2 ± 0.1</td>
<td>77 ± 12</td>
<td>4</td>
<td>3.2</td>
<td>95.1 ± 1.3</td>
</tr>
<tr>
<td>PfTrpB$^{2G9}$</td>
<td>T292S</td>
<td>1.1 ± 0.2</td>
<td>0.84 ± 0.04</td>
<td>14 ± 3</td>
<td>78</td>
<td>0.34</td>
<td>94.7 ± 1.2</td>
</tr>
<tr>
<td>PfTrpB$^{D11}$</td>
<td>E17G, I28V, F274S, T292S, T321A</td>
<td>2.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>11 ± 2</td>
<td>200</td>
<td>0.3</td>
<td>83.6 ± 2.4</td>
</tr>
<tr>
<td>PfTrpB$^{P2B2}$</td>
<td>P12L, E17G, I28V, F274S, T292S, T321A</td>
<td>2.9 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>8.7 ± 1.8</td>
<td>330</td>
<td>0.04</td>
<td>87.3 ± 1.3</td>
</tr>
</tbody>
</table>

Assay parameters are described in SI Materials and Methods.
<table>
<thead>
<tr>
<th>Protein</th>
<th>PDB ID code</th>
<th>Ligand</th>
<th>Space group</th>
<th>Cell dimensions, Å</th>
<th>Cell angles</th>
<th>Data collection</th>
<th>Refinement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a,b,c = 87.1, 111.9, 160.8</td>
<td>α = β = γ = 90°</td>
<td>Wavelength, Å</td>
<td>Total no. of reflections</td>
</tr>
<tr>
<td>PfTrpB</td>
<td>5DVZ</td>
<td>None</td>
<td>P2₁2₁2₁</td>
<td>87.1, 111.9, 160.8</td>
<td>90°</td>
<td>0.9795</td>
<td>161,910</td>
</tr>
<tr>
<td></td>
<td>5DW0</td>
<td>l-Serine</td>
<td>P2₁2₁2₁</td>
<td>84.2, 109.1, 160.8</td>
<td>90°</td>
<td>0.9795</td>
<td>11,976</td>
</tr>
<tr>
<td></td>
<td>5DW3</td>
<td>l-Tryptophan</td>
<td>P2₁2₁2₁</td>
<td>83.7, 108.9, 160.1</td>
<td>90°</td>
<td>0.9795</td>
<td>11,976</td>
</tr>
<tr>
<td></td>
<td>5E0K</td>
<td>None</td>
<td>P2₁2₁2₁</td>
<td>87.7, 225.0, 296.2</td>
<td>90°</td>
<td>0.9795</td>
<td>11,976</td>
</tr>
</tbody>
</table>

**Data collection**
- **Wavelength, Å**: 0.9795
- **Beamline**: SSRL 12.2
- **Resolution, Å**: 40 - 1.69
- **No. observations**: 865,034
- **Completeness (%)**: 99.6 (99.9)
- **Rmerge (%)**: 0.050 (0.899)
- **CC1/2**: 0.995 (0.439)
- **I/σI**: 7.3 (0.6)
- **Redundancy**: 4.9 (4.9)

**Refinement**
- **Total no. of reflections**: 161,910
- **Total no. of atoms**: 12,278
- **Final bin (Å)**: (1.73–1.69)
- **Rwork (%)**: 20.2 (39.1)
- **Ramachandran plot favored, %**: 96.8
- **Allowed, %**: 100
- **Outliers, %**: 0

Values in parentheses are for the highest-resolution shell. $R_{merge}$ is $\Sigma I_o - l/\Sigma I_o$, where $I_o$ is the intensity of an individual reflection, and $l$ is the mean intensity for multiply recorded reflections; $R_{work}$ is $\Sigma (|F_o| - |F_c|)/|F_o|$, where $|F_o|$ is an observed amplitude and $|F_c|$ a calculated amplitude; $R_{free}$ is the same statistic calculated over a 5% subset of the data that has not been included.