Overnight cultures in LB medium containing 100 µg/ml carbenicillin, 34 µg/ml chloramphenicol, 50 mM glucose were diluted 1000-fold into M9Y medium containing 50 mM glucose and grown with shaking at 37 °C to an A600 of about 0.8 (typically, 4–5 h). After cooling the cultures in ice, induction of gene expression was commenced by the addition of IPTG to 0.1 mM and shaking at 23 °C was continued overnight. Cells were collected by centrifugation (10 min, 9000*g*), resuspended in 20 mM Tris–HCl,pH 8.0, 20 mM NaCl, 2 mM EDTA and centrifuged as before. The cell pellet was resuspended in 50 mM sodium phosphate, pH 7.7, 300 mM NaCl, 10 mM imidazole, 14 mM 2-mercaptoethanol, 0.25%Tween 20, 0.1 mM PMSF (in a volume dependent on the method of cell disruption). Bacteria were lysed, e.g in a French press using two passes at 18,000 psi. The lysate was subjected to three 10 s bursts of sonication to reduce its viscosity, centrifuged at 10,000*g* for 30 min and the supernatant carefully removed from a partially loose pellet.

*NEW:*

*The protein extract was brought to 40% saturation at 0°C by the addition of solid ammonium sulphate (*[*http://www.science.smith.edu/departments/Biochem/Biochem\_353/Amsulfate.htm*](http://www.science.smith.edu/departments/Biochem/Biochem_353/Amsulfate.htm)*), while stirring, 10 min. After centrifugation for 10 min, 10.000 g, the clear supernatant was removed, its volume determined and brought to 60% saturation at 0°C by the addition of solid ammonium sulphate, while stirring, 10 min. The precipitate was collected by centrifugation, redissolved in 50 mM sodium phosphate, pH 7.7, 300 mM NaCl, 10 mM imidazole and dialyzed against the same, overnight at 4°C.*

Ni–NTA (Qiagen, Hilden, Germany) (0.1 ml suspension per gram wet weight cells) was collected by gentle centrifugation, the supernatant containing preservative discarded and the matrix resuspended in 50 mM sodium phosphate, pH 7.7, 300 mM NaCl, 10 mM imidazole. This suspension was added to the cleared cell lysate in a 50 ml screwcap tube and rotated end-over-end at 4 °C for 2 h. The sample was transferred to a 10 ml disposable chromatography column and the settled matrix washed extensively, at room temperature, in succession with 50 mM sodium phosphate, pH 7.7, 300 mM NaCl, 10 mM imidazole and 50 mM sodium phosphate, pH 7.7, 300 mM NaCl, 20 mM imidazole. The His-tagged protein was recovered by elution with 5 ml 50 mM sodium phosphate, pH 7.7, 300 mM NaCl, 250 mM imidazole. The enzyme was collected by addition of solid ammonium sulphate to 80% saturation, at 0 °C for 15 min, followed by centrifugation at 10,000*g* for 10 min. The precipitate was dissolved in 0.5–1 ml 100 mM Tris–HCl, pH 8.0, 2 mM DTT and dialysed against 50 mM Tris–HCl, pH 8.0, 1 mM DTT, 50% glycerol at 4 °C, overnight (QuixSep, Roth, Karlsruhe).The product was stored at -20 °C.

*Thrombin cleavage* Preparative thrombin cleavage of the expressed fusion proteins was performed in 20 mM Tris–HCl, pH 8.3, 150 mM NaCl for 4 h at room temperature, using an amount of thrombin (GE Healthcare, Munich, Germany) corresponding to 0.03% total protein (w/w). Following this treatment, the solution was made 20 mM with respect to imidazole and the trimmed synthetase recovered as the flow through after a 1 h Ni–NTA incubation, at 4 °C. The protein was recovered by ammonium sulphate precipitation and dialysis against 50 mM Tris–HCl, pH 8.0, 1 mM DTT, 50% glycerol at 4 °C, overnight (QuixSep, Roth, Karlsruhe).The product was stored at -20 °C.

Further purification, where necessary was performed by chromatography on a 1 ml SOURCE15Q column (Amersham), in 50 mM Tris, pH 7.6. The protein solution was diluted 1:5 with 50 mM Tris, pH 7.6 and applied to the column at 1ml/min. Substantial amounts of contaminating material are removed, as unbound species. The column is washed with buffer containing 80 mM NaCl and the enzyme recovered by elution at 300 mM NaCl. Protein is concentrated by dilution of the eluate to 50 mM NaCl with Tris buffer and reabsorption to a 1 ml column of DEAE cellulose in 50 mM Tris, pH 7.6. The enzyme is eluted by two consecutive 0.5 ml rinses with 1 M NaCl in buffer and dialysed against 50 mM Tris–HCl, pH 8.0, 1 mM DTT, 50% glycerol at 4 °C, overnight (QuixSep, Roth, Karlsruhe).