Supplementary material to EA5036 Away from the edge II: In-house Se-SAS phasing with chromium radiation

Preparation of Protein

For the preparation of recombinant protein, the *C. thermocellum* JW20 682 gene was processed using with Gateway Cloning Technology (Invitrogen). Thus, genomic DNA of the bacterium served as a template for amplification of the gene by polymerase chain reaction (PCR). Primers were designed to amplify the whole open-reading frame and optimized using Expression Primer 3.0 software (PREMIER Biosoft International). The sense (5`-GGGGACAAGTTTGTA-CAAAAAAGCAGGCTGTATGGTATGGGCTATCAGAGGTG-3`) and anti-sense (5`-GGGGACCACTTTGTACAAGAAAGCTGGGTATTAAGTCAAATCCGGCCTTAACA-3`) primers were flanked with the attB1 and attB2 recombination sites (underlined), respectively. For PCR, the AccuPrime Pfx Super Mix (Invitrogen) was used. The following temperature protocol was applied during the reaction: 95°C for 5 min, then 35 cycles of 95°C for 15 s, 60°C for 30 s, 68°C for 30 s. The entry clone was created by BP recombination reaction using the PCR product and the pDONR-221 entry vector (Invitrogen). The reaction mixture was incubated at 25°C for 24 hours, the reaction was stopped by proteinase K treatment, and the product was used to transform TOP10 competent cells (Invitrogen). A single colony was isolated and plasmid DNA was purified using the QIAprep Spin Miniprep Kit (Qiagen). The correct insert was verified by sequencing and then used in the LR reaction with destination vector pET15G (received from Ming Luo at The University of Alabama at Birmingham) to create the expression clone. The reaction was incubated for 24 hrs at 25°C and treated with proteinase K. BL21(DE3) cells (Invitrogen) were used to transform the destination clone DNA. The expressed protein was soluble at 37°C and contained an N-terminal 6xHis tag followed by a thrombin-recognition site and an attB1 recombination site.

Cells containing the expression vector with the gene encoding Cth-682 were used to inoculate 1 L of modified auto-inducing medium (Studier personal communication) containing 100 µg/mL ampicillin and 125 mg/L selenomethionine. The mixture was shaken for 20 hrs at 37°C. Cells were harvested by centrifugation (12000 x g, 15 min). The cell pellet was resuspended in lysis buffer (100 mM NaCl, 20 mM Hepes, 5 mM PMSF, 5 mM 2-mercaptoethanol, pH 7.6). Sonication was used for cell lysis. Cell lysate was then submitted to heat treatment at 65°C for 1h in a water bath followed by centrifugation (27000 x g, 30 min) to remove cell debris. The supernatant was loaded onto a Ni affinity column (HiTrap 5 ml chelating HP; Amersham Biosciences) previously equilibrated with buffer A (200 mM NaCl, 20 mM imidazole, 20 mM HEPES, pH 7.6) and eluted with a linear gradient from 0 to 500 mM imidazole. Peak fractions

were analyzed by SDS-PAGE. Collected protein fractions were concentrated to 3mL and applied to a gel filtration column (superdex 75 HiLoad 16/60; Amersham Biosciences). Proteins were eluted with 100 mM NaCl, 20 mM HEPES, PH 7.6. After SDS-PAGE examination, the Cth-682 was concentrated to 55 mg/mL (based on extinction at 280 nm wavelength).