Supplementary material

Expression and purification of recombinant AfsA-GST

To produce AfsA-GST fusion protein *afsA* was cloned to the pGEX-4T1 plasmid (GE Healthcare) as follows. The *afsA* sequence was amplified by PCR with B2682 genomic DNA as a template using the following primers: *afsA*-F 5'-*GAATTC*ATGCCCGAAGCAGCAGTCTTGATCG-3' (with an EcoRI site shown by italics) and *afsA*-R 5'-*GCGGCCGC*TCAGCCGTGGGCCCGGGGGCCGGAC-3' (with a NotI site shown by italics). The blunt-end PCR product was cloned to the pJET1.2 plasmid (Thermo Fisher Scientific). The EcoRI-NotI fragment was excised from pJET1.2-*afsA* and cloned between the EcoRI and NotI sites of pGEX-4T1, resulting in pGEX-4T1-*afsA*, which was transformed to *E. coli* BL21.

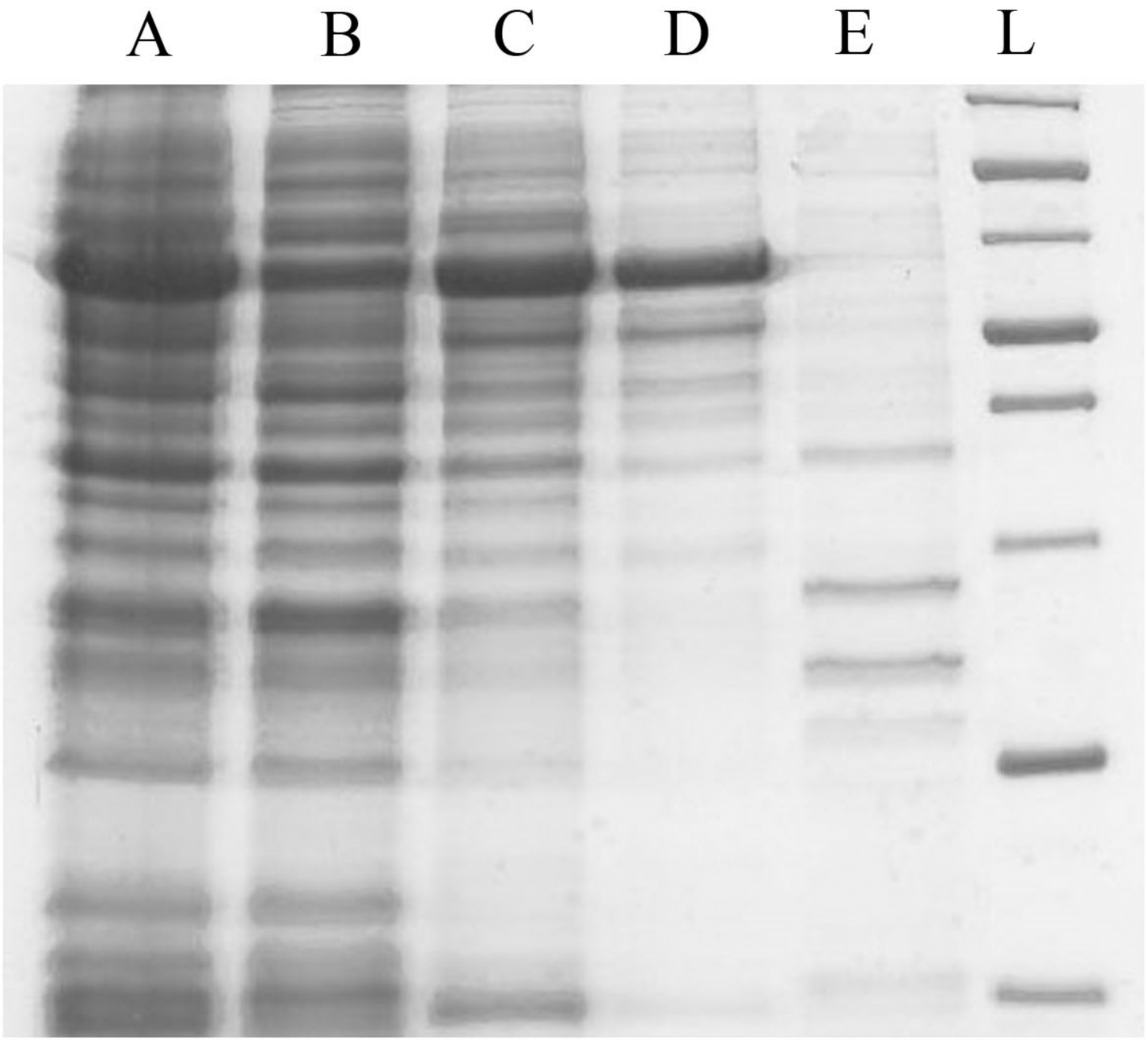
During the production of AfsA-GST this *E. coli* strain was incubated in Luria Broth (LB) medium supplemented with 100 µg ml^{-1} ampicillin (30 °C; 250 rpm). Fusion protein expression was induced by 0.1 mM IPTG. Cells harvested by centrifugation were washed and suspended in PBS buffer. Cells were disrupted by sonication. Expression yielded low amount of fusion protein in the soluble fraction due to the formation of insoluble inclusion bodies (Supplemetaly Figure). In order to dissolve the inclusion bodies the pellet obtained from sonication was solubilized with 5M urea [Harper and Speicher, 2011]. To refold the denatured AfsA-GST urea was removed by successive dialysis [Harper and Speicher, 2011]. To purify the recombinant AfsA-GST protein Gluthatione GST-bind resin (Millipore) was used by following the manufactural instructions. GST-fusion protein bound to the resin was either eluted directly by 10 mM reduced gluthatione containing elution buffer or was cleaved on the resin by thrombin. Each of the steps were monitored by SDS-PAGE (Supplemetary Figure).

Reference

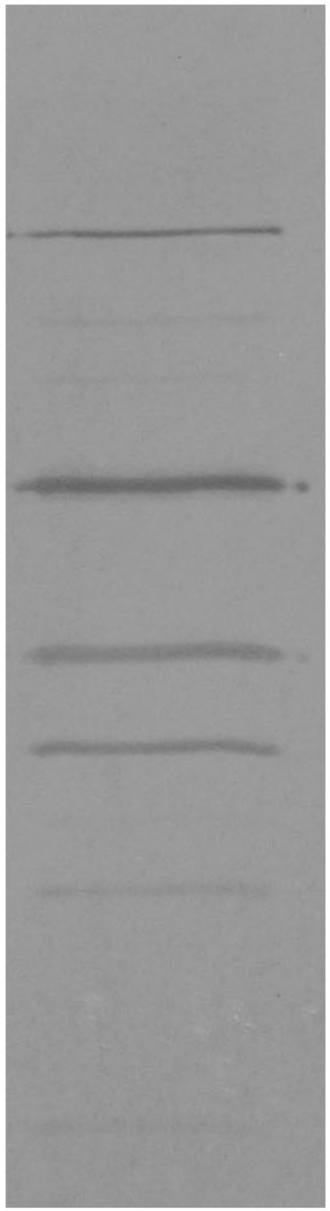
Harper S, Speicher DW: Purification of proteins fused to glutathione S-transferase. Methods Mol Biol 2011;681:259-80.

Supplementary Figure Legend

Purification of recombinant AfsA (Panel A) and its detection by antibody (Panel B). Panel A: The AfsA-GST fusion protein was purified from the culture of *E. coli* BL21 harboring pGEX-4T1-*afsA* with Gluthatione GST-bind resin and was cleaved by thrombin. Symbols: A: sediment after sonication, B: supernatant after sonication, C: sediment after urea treatment, D: purified AfsA-GST fusion protein, E: AfsA and GST proteins after the cleavage of the fusion protein by thrombin, L: protein molecular weight ladder. Purified AfsA-GST and AfsA proteins were used for the immunization of rabbits. Panel B: The purified rabbit polyclonal antibodies proved to be active against the recombinant AfsA-GST and AfsA proteins by Western blot. Note that the AfsA protein (36 kDa) contains a thrombin cleavage site that results the smaller fragment (28 kDa).



Panel A



Panel B