Transformed centrifugation at 10,000×g and resuspended in

Transformed bacteria with CPG2 and TAT-CPG2 constructs weregrown in the LB medium containing 100 µg/ml ampicillin at 37 ? C to reach an optical density of0. 6 at 600 nm. ExpressionM1 of the fusion proteins was induced at 0. 5 mM and 1 mM of IPTG. Cellswere grown at either 37 ? C or 28 ? C for 4 h.

To increase the level of soluble TAT-CPG2, the induction temperature and IPTG concentration for proteinexpression were optimized. Experiments were carried out using0. 5 and 1 mM IPTGand the inductionprocess was performed at 28 ? C and 37 ? C. Purificationof CPG2 and TAT-CPG2 fusion proteins were carried out under native and denaturingconditions by the batch method of Qiagen. For purification of recombinantproteins under native condition the bacterial cellswere harvested by centrifugation at 10, 000×g and resuspended in binding buffer(50 mM NaH2PO4, 300 mM NaCl, 10 mM imidazole, and 1 mM PMSF at pH 8). Lysozyme was added at a concentration of 0. 1 mg/ml andincubated on ice for 30 min.

Cells were then disrupted by sonication (6 ×10 sbursts at 200–300 W with a 10 s cooling period in-between)on ice. The bacterial lysates were centrifuged (10, 000×g at 4 °C for 30 min), andthe supernatants were added to a 50% Ni-NTA resin pre-equilibrated with bindingbuffer and mixed gently by shaking (200 rpm on a rotary shaker) at 4 °C for 60min. The lysate–Ni-NTA mixture was loaded on an emptyPD10 column. The column was washed twice with 4 ml wash buffer (50 mM NaH2PO4, 300 mM NaCl, 20 mM imidazole, pH 8). The CPG2 and TAT-CPG2 fusion proteins wereeluted with elution buffer (50 mM NaH2PO4, 300 mM NaCl, 250 mM imidazole, pH 8). Eluted proteinswere desalted on a PD10 desalting column. For purification under denaturing

condition, pelletsfrom purification under native condition were resuspended in buffer B (100 mM NaH2PO4, 10 mM Tris-HCl, 8 Murea, pH 8) and stirred for 60 min at room temperature. The mixture was thencentrifuged at 10, 000×g for 30 min at room temperature to pellet the cellulardebris.

Supernatant was mixed with 50% Ni-NTA resin and gently shaked (200 rpmon a rotary shaker) at room temperature for 60 min. The lysate–Ni-NTA mixture wasadded to a column and washed twice with 4 ml buffer C (100 mM NaH2PO4, 10 mM Tris-HCl, 8 M urea, pH 6. 3). The recombinant proteins were eluted 4 timeswith 0.

5 ml buffer D (100 mM NaH2PO4, 10 mM Tris-HCl, 8 Murea, pH 5. 9), followed by 4 times elution with 0. 5 ml buffer E (100 mM NaH2PO4, 10 mM Tris-H, 8 M urea, pH 4. 5). Monomers generally elute in buffer D, whilemultimers and aggregates elute in buffer E. The eluted proteins were desaltedby PD10 desalting column. The purified fusion proteins were verified bySDS/PAGE, coomassie brilliant blue staining and western blot analysis withanti-His6-peroxidase antibody (1: 500; Roche).

The protein concentrations wereestimated by the Bradford method (Bradford, 1976). M1?????? ???