ANNEXURE
1. Chemicals & Strains

All the chemicals used in this work were of ultra pure grade and purchased from Sigma-Aldrich, St. Louis, MO-63103, USA. Growth media contents were from HiMedia laboratories, Mumbai, India. Ortho-phosphoric acid for Bradford reagent was purchased from Qualigens India Ltd., Mumbai, India.

Protein purification materials, columns, column materials and molecular biology kits were obtained from Amersham Biosciences (Part of GE healthcare), UK.

All the restriction enzymes were from New England biolabs (UK) Ltd., Herts, SG4 0TY, UK. T4 polynucleotide kinase was purchased from Amersham Biosciences (Part of GE healthcare), UK.

γ-32P ATP was purchased routinely from BRIT (Board of radiation and isotope technology), BARC, Mumbai, India.

T7 based expression vectors pET21d, pET23a, bacterial strain BL21 (DE3) and other strains to aid cloning and expression were from Novagen, EMD Biosciences Inc., Madison, WI-53719, USA while T5 promoter based expression vector pQE31 was from Qiagen GmbH, Hilden, Germany.

Centricons were purchased from Amicon, Beverly, MA-01915, USA. Hyperfilm-XP for autoradiograms was purchased from Amersham Biosciences (Part of GE healthcare), UK.

Primers were synthesized from Sigma-Genosys, The woodlands, TX-77380, USA. Lyophilized samples were dissolved in TE to make a 100 nM stock.

2. Reagents and Composition

All purification buffers, were properly filtered with 0.22 μm filters and degassed as per the guidelines of the column manufacturers.

A. Luria-Bertani (LB) media and YT Broth

LB: Tryptone 10g, Yeast extract 5g, NaCl 10g in 950 ml of deionized water. The contents are dissolved, made-up and sterilized by autoclaving for 20 min at 15 lb/in2.

YT: Tryptone 16g, Yeast extract 10g, NaCl 5g in 950 ml of deionized water. The contents are dissolved, made-up and sterilized by autoclaving for 20 min at 15 lb/in2.

For solid LB, 1.5 % agar is added before autoclaving.
B. Bradford Reagent

For 100 ml
Coomassie Brilliant Blue G 250 10 mg
85 % Ortho phosphoric acid 10 ml
Absolute ethanol 5 ml

10 mg G-250 was dissolved in 5 ml ethanol and then thoroughly mixed with 10 ml ortho-phosphoric acid. Volume was made up to 100 ml with TDW and solution was filtered through whatman filter no.1 and kept in brown bottles.

C. Tris Acetic acid EDTA (50X)

For 1000 ml
Tris 242 g
Acetic acid glacial 57.1 ml
0.5 M EDTA 100 ml

D. SDS PAGE (12%)

Resolving gel 5 ml
H₂O 1.6 ml
30 % Acrylamide 2.0 ml
1.5 M Tris, pH 8.8 1.3 ml
10 % SDS 0.05 ml
10 % APS 0.05 ml
TEMED 0.002 ml

Stacking gel 2 ml
H₂O 1.4 ml
30 % Acrylamide 0.33 ml
1.0 M Tris, pH 6.8 0.25 ml
10 % SDS 0.02 ml
10 % APS 0.02 ml
TEMED 0.002 ml

F. PAGE running buffer (1X)

Tris 3.02 g
Glycine 18.8 g
10 % SDS 10.0 ml

G. Native gel loading dye

Sucrose 40 % (w/v)
Bromophenol blue 0.25 % (w/v)
H. Antibiotics Stock

100 mg/ml and 50 mg/ml stock solutions for ampicillin and kanamycin were prepared in TDW and a 5 mg/ml tetracycline stock solution was prepared in ethanol and filtered with 0.22 µM syringe filters (Millipore, Massachusetts, USA).

I. IPTG & PMSF

1 M stock for isopropyl-beta-D-thiogalactopyranoside (IPTG) was prepared each time in TDW and filtered with 0.22 µM syringe filters and stored at -20°C. 200 mM stock of Phenyl methyl sulphonyl fluoride (PMSF) was made in absolute ethanol and stored at -20°C.