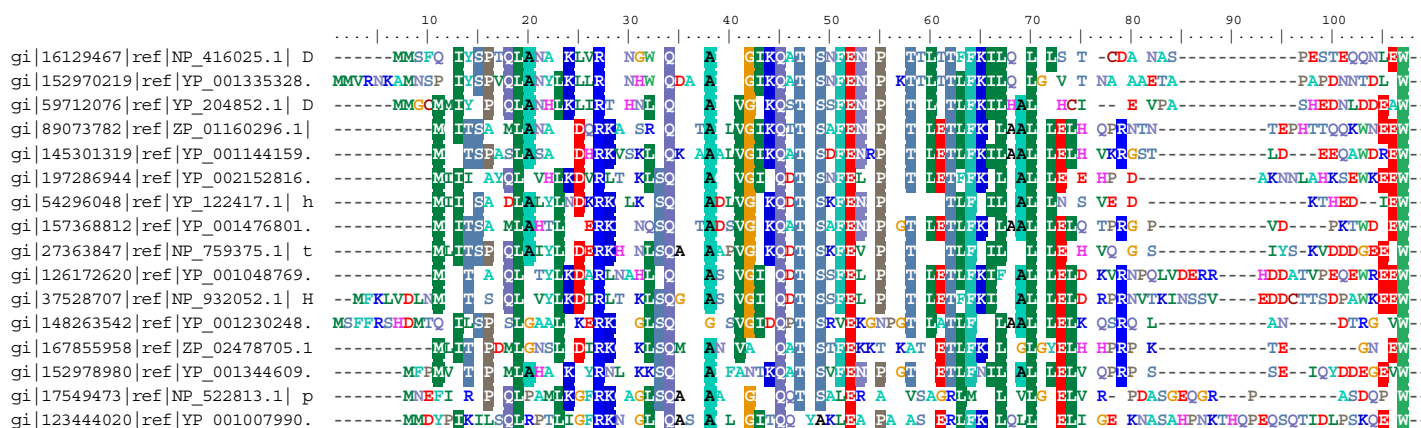


Supplementary materials



Supplementary Figure 1. *hipB* sequence alignment.

Cloning

We amplified the ~1600-bp *hipBA* mini-operon from DH5 α *E. coli* genomic DNA by means of PCR. The amplicon was designed with a His-tag coding sequence (at the C-terminus of *hipA*) and a BamHI/BspHI restriction sites (underlined) at the 3' and 5' ends, respectively. To speed up the process we split the long reverse primer into two overlapping parts. The following primers were used:

F-1: TAGTAGTTCATGATGAGCTTTCAGAAGATCTATAGCCCA
 R-1: ATGGTGATGGTGATGCTTACTACCGTATTCTCGGCTTAACCG
 R-2: CTACTAGGATCCCTATTAGTGATGGTGATGGTGATGCTTACTACC

Primers F-1 and R-1 were used in the first 50ul PCR which was allowed to run for ten cycles. A 1ul aliquot of the first reaction was used as a template for the second reaction (twenty-two cycles) with primers F-1 and R-2. Both reactions were set up with the annealing temperature of 54°C and other parameters as recommended by the polymerase manufacturer (PfuUltra, Stratagene). The single PCR product was gel-purified, digested with BspHI/BamHI, and ligated with the pET28a(+) vector that was digested with NcoI/BamHI. Competent DH5 α *E. coli* (Invitrogen) were transformed with the ligation products and plated on Kanamycin-LB-Agar. Five colonies were picked and the presence of the insert in all of them was verified by restriction digest (XbaI + BamHI). We submitted plasmid DNA for sequencing and proceeded with expression studies immediately, in the interest of expediency. All five sequences were found to be correct.

Small-scale expression and purification

We transformed BL21(DE3) (Stratagene) *E. coli* competent cells with the *hipBA* expression vector and grew individual colonies in 5-ml aliquots of the following media: LB, YT, TB. Protein expression was initiated by the addition of IPTG to 0.5 mM at 20°C and 37°C for 16 and 6 hours, respectively. We also tried expressing the complex in the auto-inducing “Magic Medium” (Invitrogen) which was used according to manufacturers’ instructions. The cells were harvested by centrifugation and lysed in a cocktail of 3:1 v/v mix of B-PER and Y-PER lysis reagents (Pierce), augmented with 0.1 mg/ml hen egg lysozyme and bovine pancreatic DNase-I (Sigma). Lysates were clarified by centrifugation and the supernatants were loaded on 50- μ l His-SELECT (Sigma) resin in 1-ml miniature plastic columns (Bio-Rad). The resin was washed

several times with 1ml aliquots of buffer A (250 mM NaCl, 25 mM TRIS pH 8.0, 10 mM imidazole), then eluted with 80ul of buffer B (250 mM NaCl, 25 mM TRIS pH 8.0, 200 mM imidazole). The presence of hipB and hipA proteins in all eluates was confirmed by SDS-PAGE and mass-spectroscopy. Based on the intensity of gel bands and the expected molecular weights of the two proteins we estimate that the two components of the complex were present in approximately 1:1 molar ratio, which is consistent with the previously published results (Ref). Expression in Magic Medium and TB at 37°C resulted in the highest yield of the complex.

Large-scale expression and purification

Biomass from four liters of the auto-inducing medium grown at 37°C overnight was used for large-scale purification of hipBA. The cells were lysed in the same fashion as described in the previous section. All cell lysis and purification steps were performed at 4°C. Clarified lysate was loaded on a 20-ml column packed with His-SELECT (Sigma) resin, washed with buffer A until A_{280} stabilized, then eluted with a gradient of 0-100% of buffer B over five column volumes. Collected eluate fractions were analyzed by SDS-PAGE and the protein-containing fractions were pooled. We intended to use ion exchange as the second step of purification (even though hipBA was essentially pure after the first step) however the complex precipitated when the ionic strength was lowered below 13 mS/cm. Therefore, initially we further purified the complex using size exclusion chromatography Sephadex S-200 in buffer C (250 mM NaCl, 25 mM TRIS pH 8.0, 0.1 mM TCEP). In later experiments we established that size exclusion does not improve the quality of the crystals and therefore this step was omitted. The yield of purified protein was 90 mg based on an A_{280} value taken in 6M guanidine.

The hipBA complex was buffer-exchanged into buffer C and concentrated to 9 mg/ml via ultrafiltration. An aliquot of protein solution was flash-frozen in liquid nitrogen, stored for an hour at -80°C and then thawed. Unfortunately, most of the protein precipitated upon thawing; therefore we stored the rest of the protein at 4°C where the protein appears to be stable and reproducibly crystallizable for at least one month.