

Fig. S1. Western blot of His-tagged AlgBD59N. Mid-log phase PA14 Δ algB Δ kinB(+pH20T) and PA14 Δ algB Δ kinB(+pHisAlgBD59N) were induced for 4 hours with 0.2% arabinose, his-tagged proteins were enriched from whole cell lysates using HisPur Cobalt resin and 20ul eluate was separated by 10% SDS-PAGE, transferred to pvdf membrane, probed with a mouse anti-his antibody and detected with an HRP conjugated anti-mouse secondary antibody.

Supplemental methods

Cloning and expression of His-AlgB_{D59N}

AlgB_{D59N} was N-terminally his-tagged by amplifying algB_{D59N} from pAlgB_{D59N} using primers AC672 and NSC107. The resulting PCR product was digested with XbaI and HindIII and subcloned into pHERD20T. The resulting plasmid, pHisAlgBD59N, was introduced into PA14 $\triangle algB\Delta kinB$ to create PA14 $\triangle algB\Delta kinB$ (+pHisAlgBD59N). Five ml LB PA14 $\Delta a la B \Delta k in B$ (+pHisAlgBD59N) and PA14 $\Delta a la B \Delta k in B$ (+pH20T) cultures supplemented with 150ug/ml carbenicillin were grown overnight at 37°C, 250 rpm. The following morning they were subcultured 1:50 into 50 ml cultures and grown for 2 hours at 37°C, 250 rpm. At mid-log phase, 0.2% arabinose was added to the media to induce HisAlgBD59N expression. After a four hour induction, the cells were pelleted and the pellet frozen at -80C. Protein was isolated from the cell pellet using B-PER Bacterial protein extraction reagent (Thermo Scientific), including lysozyme, DNaseI and protease inhibitors (Roche Complete Mini, EDTA-free), according the manufacturer's instructions. We then enriched for His-tagged AlgBD59N by incubating half the lysate with HisPur Cobalt resin (Thermo Scientific) using a resin bed volume of 200ul according to the manufacturer's instructions. Following incubation of the lysate with the HisPur cobalt resin for 30 min., the resin was washed 6 times with wash buffer (50mM sodium phosphate, 300mM sodium chloride, and 10mM imidazole; pH7.4) and His-tagged proteins were eluted from the resin with 200ul elution buffer (50mM sodium phosphate, 300mM sodium chloride, and 150mM imidazole; pH7.4). The eluate (20ul) was separated using 10% SDS-PAGE, blotted to pvdf, blocked with 5% milk, and his-tagged proteins were detected using a mouse anti-His primary antibody (Invitrogen) and an HRP-conjugated antimouse secondary antibody (Cell Signaling) and visualized using chemiluminescence.