Introduction: *Pseudomonas aeruginosa* uses a population-dependent communication mechanism called quorum sensing (QS) to regulate gene expression. QS controls a wide range of biological processes, including virulence factor production, biofilm formation and antibiotic resistance. The QS threshold and response in *P. aeruginosa* is defined by anti-activators, such as QteE (quorum threshold expression element) and QsrO (quorum sensing repressive ORF).

Objectives: Here, we sought out to determine the three-dimensional structures of QteE and QsrO to elucidate their mechanisms of action. To reach our goal, we first established an experimental strategy for the production of soluble, correctly-folded QS anti-activators.

Material and Methods: QteE was cloned into the following vectors: pET-RP1B (His$_6$), pETM30-MBP (His$_6$-MBP), pETM30-GST (His$_6$-GST), pET-32a (Trx), and pET-22b (pelB). QsrO was cloned in pET-RP1B, pETM30-MBP, pETM30-GST, pET-32a, and pSTEF-HGB1 (His$_6$-GB1). Protein expression was evaluated by SDS PAGE.

Results and Discussion: All QteE constructs showed insoluble expression in *Escherichia coli* BL21 (DE3) cells at 37°C and 18°C. QteE contains 5 cysteine residues in its primary sequence. We then hypothesized that QteE may be stabilized by disulfide bonds and subcloned it in the pET-22b vector, which directs recombinant protein expression to the periplasmic space. Periplasmic expression significantly increased QteE solubility. In addition, the gene encoding QsrO was cloned into the pET-RP1B plasmid and expressed as an N-terminal fusion protein to the His$_6$ tag. The fusion construct expressed insolubly in *E. coli* BL21 (DE3) cells at both temperatures. We then subcloned QsrO in a set of plasmids harboring different expression/purification tags, including His$_6$-MBP, His$_6$-GST, His$_6$-GB1, Trx, and a C-terminal His$_6$ tag. Heterologous expression of these different fusion proteins is currently being investigated to determine a condition that yields soluble QsrO.

Conclusion: The production of sufficient amounts of soluble, correctly-folded recombinant QteE and QsrO will enable their structural determination by Nuclear Magnetic Resonance, shedding light into the molecular basis of QS inhibition in *P. aeruginosa*. 