The rapid growth of antimicrobial resistance is a pressing public health concern that requires the discovery of novel antibiotics. Recently, antimicrobial peptides that inhibit undrugged bacterial pathways have been identified leading many to believe that synthetic antimicrobial peptides may serve to combat antimicrobial resistance. Our research seeks to understand the molecular mechanism outlining the antimicrobial peptide, Pdi1, which binds the bacterial ribosome. To investigate the Pdi1-ribosome interaction, we developed a biolayer interferometry pipeline to measure binding rates and affinity. Peptides were synthesized via solid-phase peptide synthesis and N-terminally biotinylated with biotin-PEG_{24}-NHS ester. Biotinylated peptides were suspended on streptavidin coated biosensors and exposed to increasing concentrations of purified ribosome to measure association and dissociation. Our binding data indicates that Pdi1 binds the bacterial ribosome with a picomolar binding affinity and demonstrates a remarkably slow off-rate. Additional studies of Pdi1 peptides containing alanine substitutions revealed that replacement of residues 14, 18, and 19 resulted in significantly decreased binding response. Our results suggest that these residues make necessary contacts with the ribosome and make significant contributions to Pdi1 binding affinity. These data serve as a foundation for future peptide-based structure-activity relationship campaigns and the development of potent Pdi1 analogues.