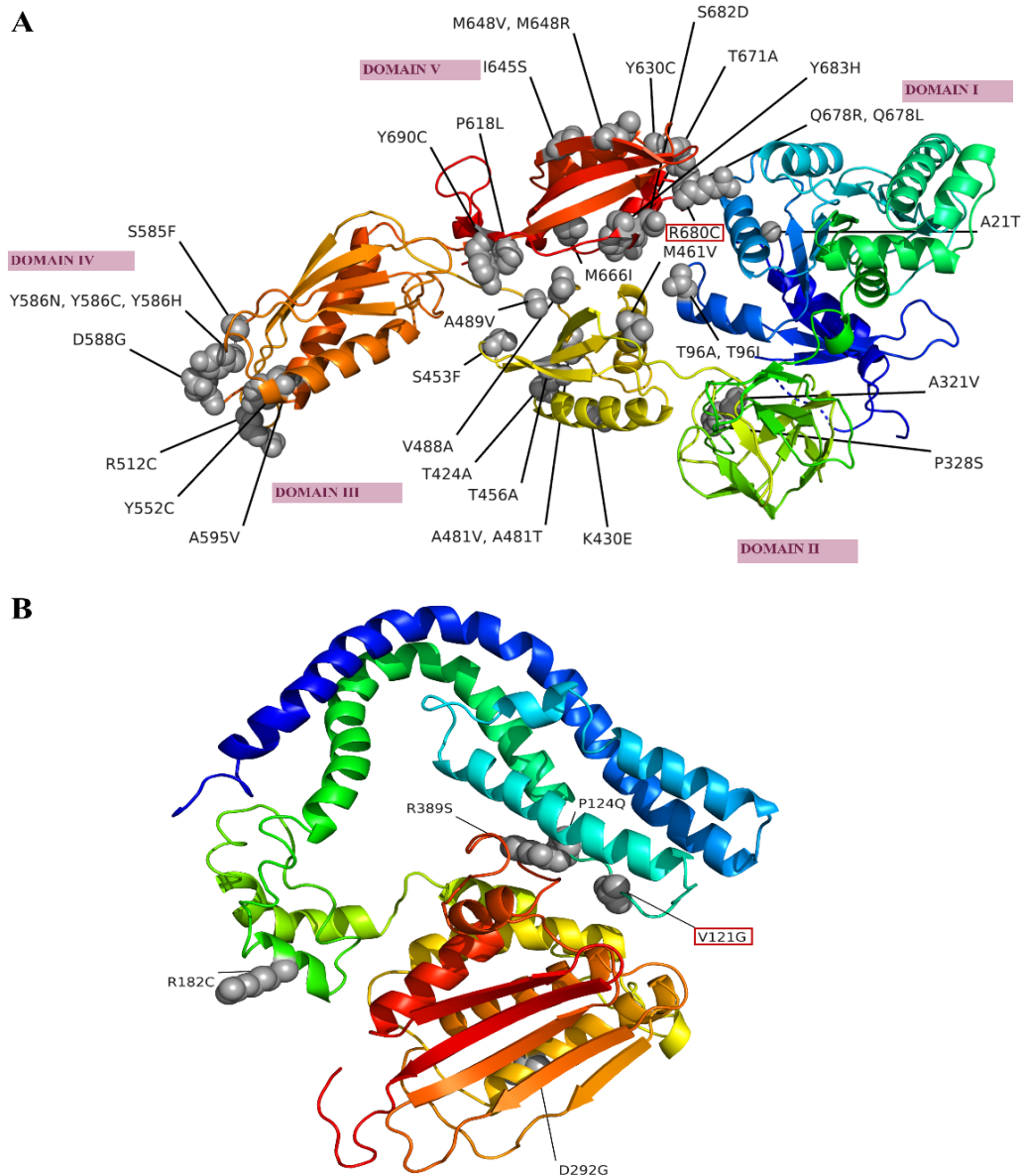
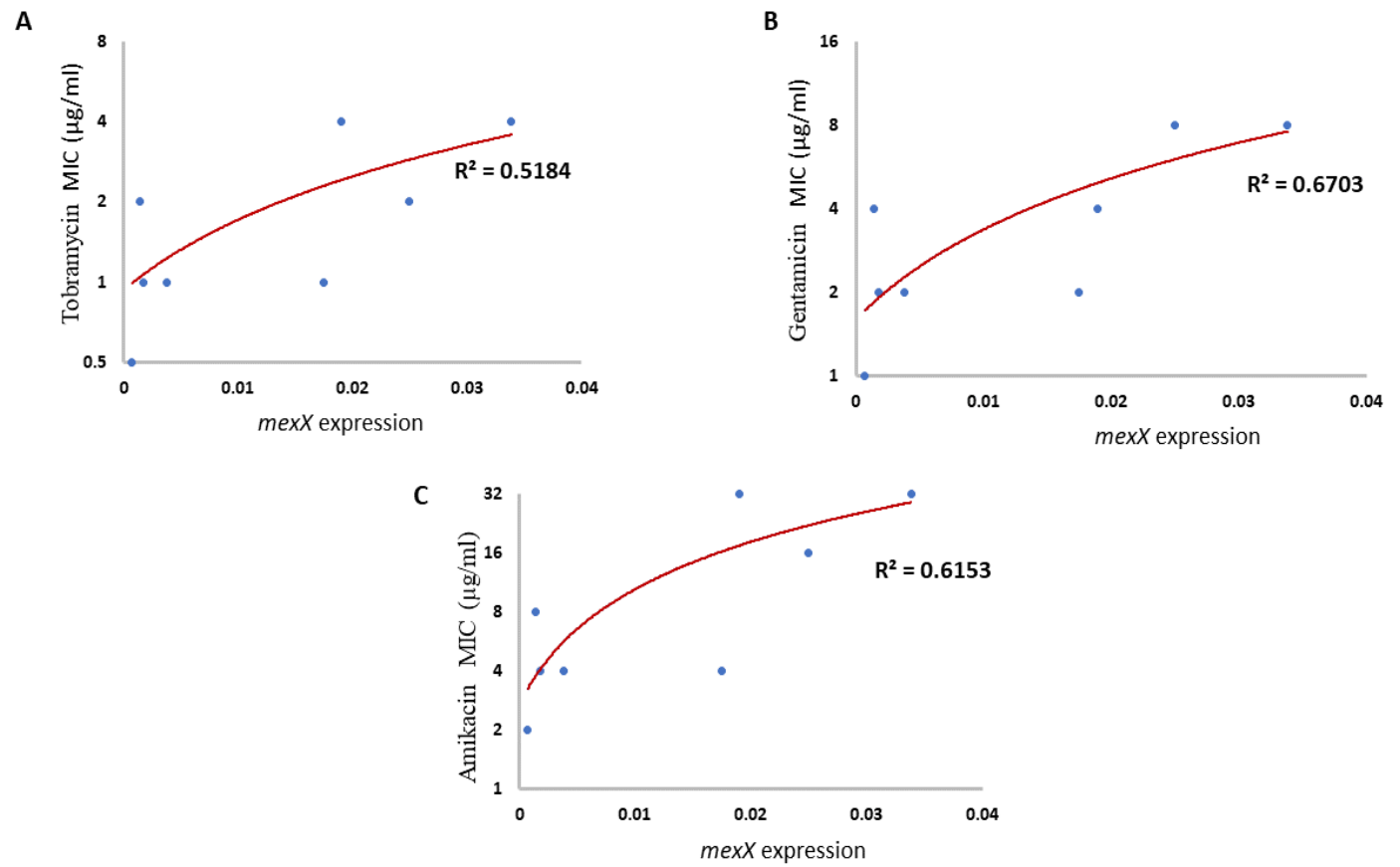


**Figure S1**



**Figure S1.** Mapping of frequent clinical variants in structural models of FusA1 and AmgS. Likely function-altering sequence variants present in two or more clinical isolates are shown with sidechains displayed in grey. **A.** Variants present in FusA1 (PDB Accession ID: 4FN5) and protein domains are represented. The location of the engineered residue (R680C) is shown. **B.** The partial structure of AmgS was predicted using Phyre2. The location of the engineered residue (V121G) is shown.

**Figure S2**



**Figure S2.** Correlation of aminoglycoside MICs with *mexX* expression. The MICs of engineered mutants were correlated with *mexX* expression and  $R^2$  values are shown in the graphs. (A) tobramycin, (B) gentamicin and (C) amikacin.