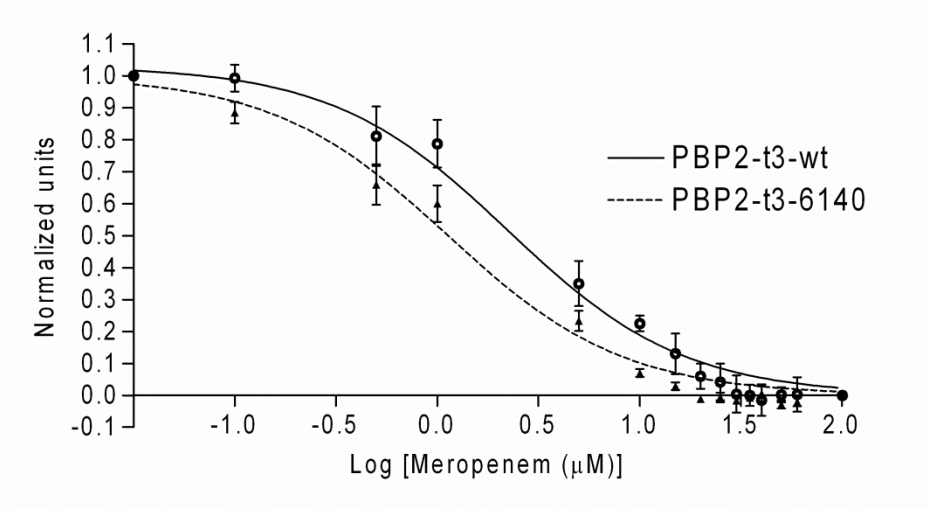
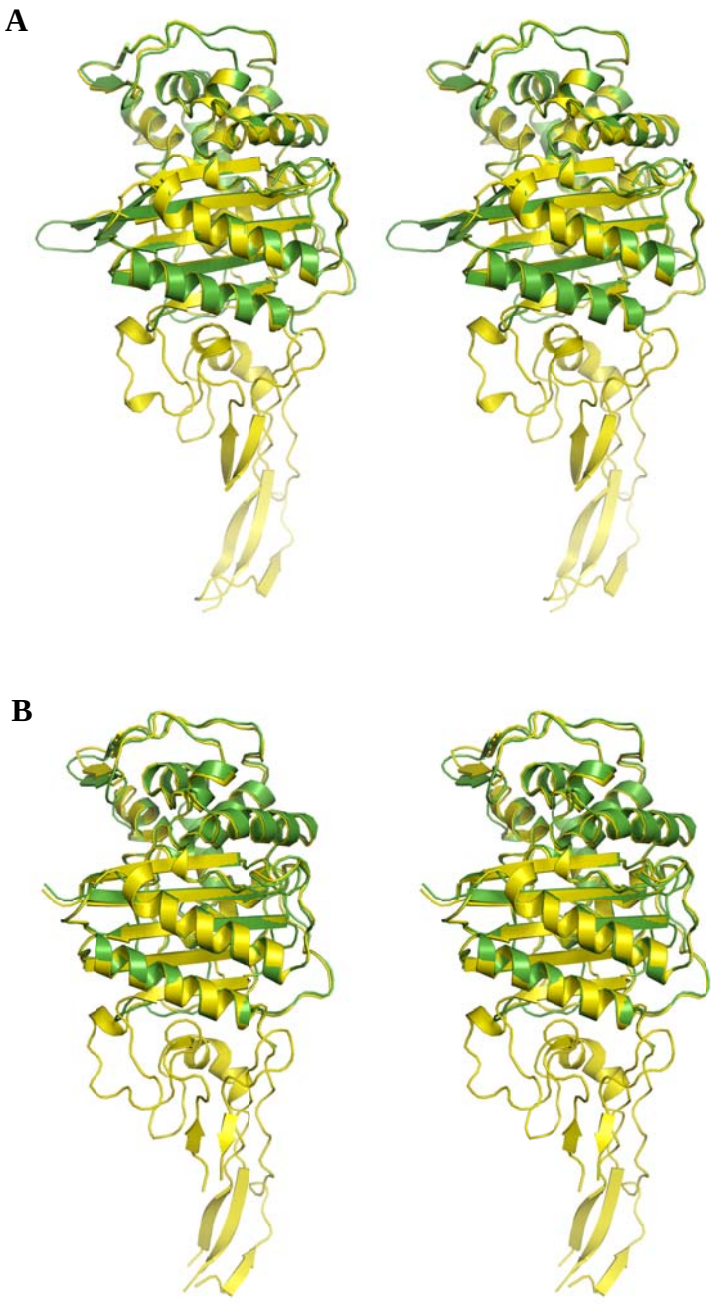
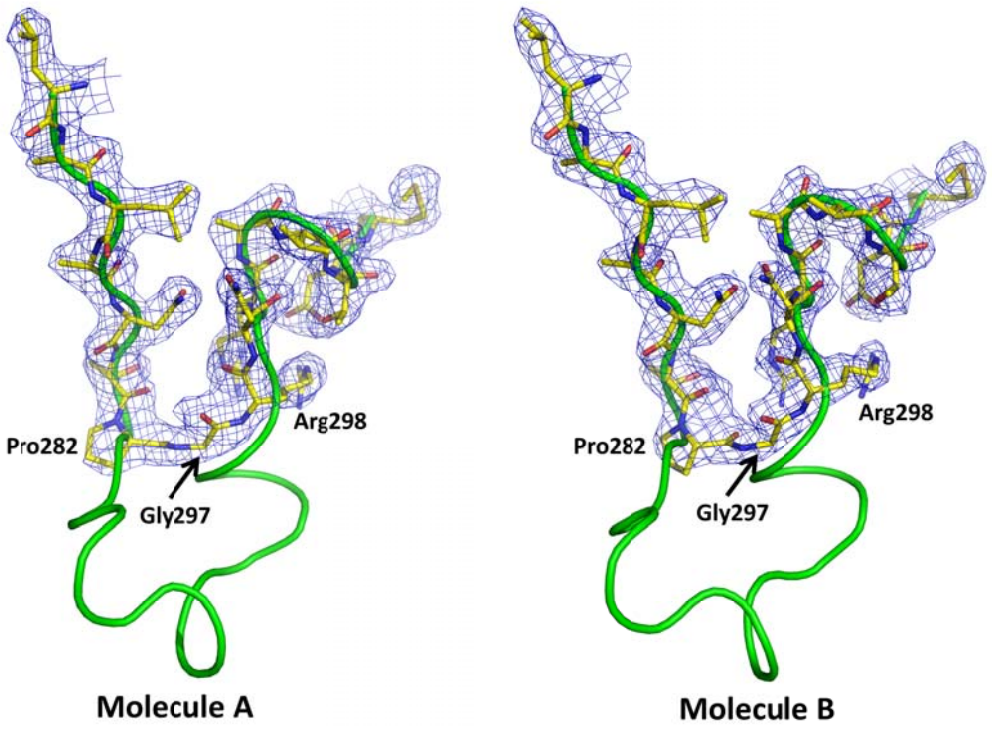
Supplementary Information



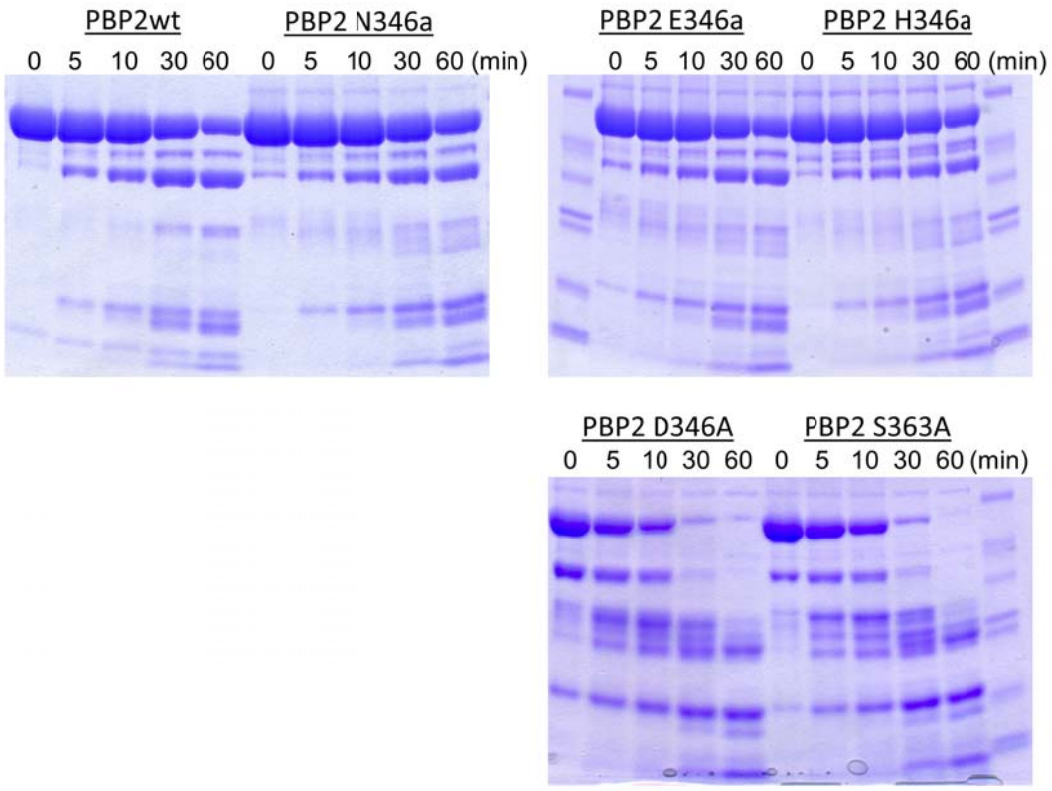
Supplemental Figure 1: Calculation of second-order rates constants for meropenem against truncated constructs of wild-type and 6140 PBP2. Data from three independent SDS-PAGE competition assay experiments (each in duplicate) were used to determine IC_{50} values for meropenem via curve fitting. The IC_{50} values are 2.30 μM (goodness of fit R^2 = 0.91) and 1.20 μM (R^2 = 0.96) for truncated wild-type PBP2 and PBP2-6140, respectively. The error bars are the standard error of the mean.



Supplemental Figure 2: Superimposition of wild-type (full-length) PBP2 vs PBP2-t3-6140. In this stereoview, the molecules are shown in cartoon format in which wild-type PBP2 is colored yellow and PBP2-t3-6140 is colored green. **A**, superimposition of molecule A of the respective asymmetric units and **B**, superimposition of molecule B of the respective asymmetric units.



Supplemental Figure 3: The 2|*Fo*|-|*Fc*| electron density of the "join" between Pro282 and Arg298 with intervening Gly linker for each molecule of the asymmetric unit of PBP2-t3-6140. Residues of PBP2-t3-6140 are shown as yellow bonds. The backbone of wild-type PBP2 (in green) is superimposed to show the region that was removed in the t3 construct, as well as the close overlap of the regions immediately preceding and following the join region in the two structures.



Supplemental Figure S4: Proteolytic susceptibilities of full-length PBP2 with different insertions at position 346a; namely, Asn, Glu and His. None of the insertion mutants increase the proteolytic susceptibility of PBP2 compared with the wild-type protein (PBP2wt). Also shown are the proteolytic susceptibilities of two mutants (D346A and S363A) that each disrupt a hydrogen bond between Asp346 and Ser363 of the SxN active-site motif and do increase the proteolytic susceptibility.