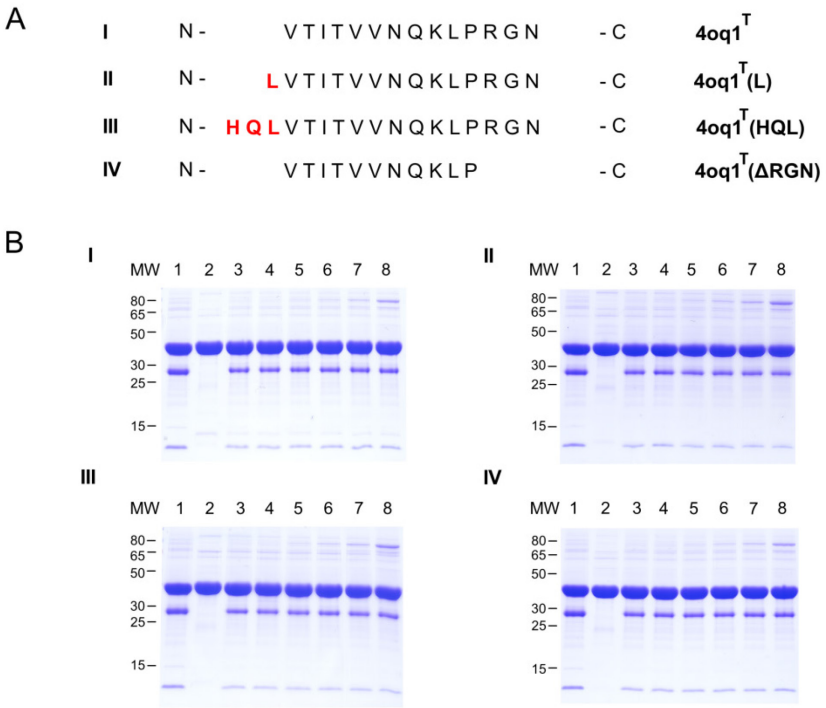


**S8 Fig: Rates of isopeptide bond formation of 4oq1$^{T}$-MBP variants on the minute scale.** (A) Sequence alignment of different 4oq1$^{T}$-MBP variants (I: 4oq1$^{T}$ wildtype (residues V246-N259), II: N-terminal L245 extension of 4oq1$^{T}$, III: N-terminal H243-Q244-L245 extension of 4oq1$^{T}$, IV: C-terminal R257-G258-N259 truncation of 4oq1$^{T}$). (B) Comparative covalent intermolecular bond formation assay between different 4oq1$^{T}$-MBP variants and mCherry-4oq1$^{C}$ (0min-30min). Purified 4oq1$^{T}$-MBP variants and mCherry-4oq1$^{C}$ were mixed each at 15 µM (final conc.) for 30min at 25°C with shaking at 500 rpm before boiling (10min, 95°C) and SDS-PAGE with Coomassie staining. Interaction I: mCherry-4oq1$^{C}$ + 4oq1$^{T}$ (wildtype), interaction II: mCherry-4oq1$^{C}$ + 4oq1$^{T}$ (L), interaction III: mCherry-4oq1$^{C}$ + 4oq1$^{T}$ (HQL), interaction IV: mCherry-4oq1$^{C}$ + 4oq1$^{T}$ (ΔRGN). (lane 1: mCherry-catcher input (30µM), lane 2: tag input (30µM), lane 3: 0min, lane 4: 1min, lane 5: 3min, lane 6: 5min, lane 7: 10min, lane 8: 30min). Same volume was loaded on the gel. MW stands for molecular weight (kDa).