Supplementary data

Figure 1. Ability of various hTS mutants to support growth of thymidylate synthase deficient TX61- *Escherichia coli* cells. Increase in the optical density, measured with a green filter, (expressed as units of the Klett-Summerson colorimeter, where 1 Klett units = 4 x 10^6 cells) of the culture in mimimal medium in the absence of thymidine, except for TX61+ (100 μM added). TX61- and TX61+ correspond to the growth of untransformed cells. The remainder are transformed with the pTS080 plasmid carrying human thymidylate synthase, either wild-type (hTS) or containing the indicated mutation (expressed as single-letter amino acid changes).

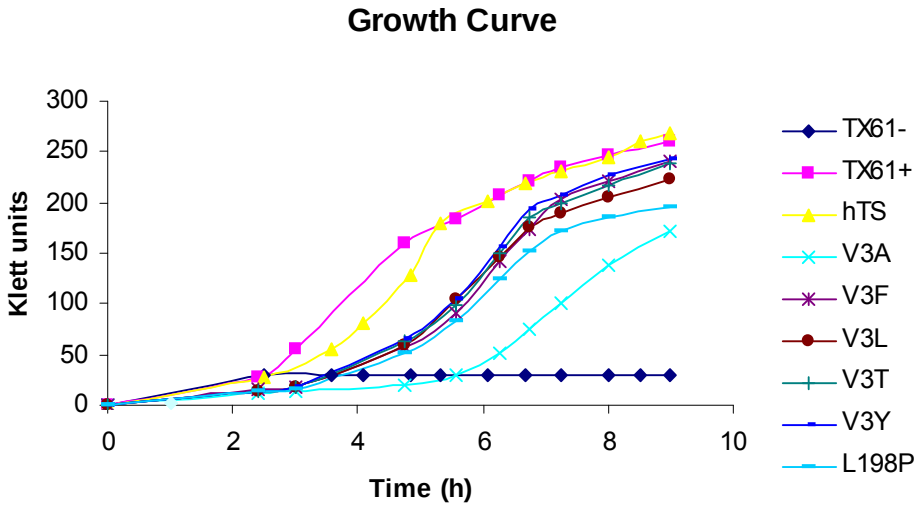

Figure 2. Protein levels of hTS wild type and variants at Val3. After 16 hours of growth the cells in 5 ml cultures were harvested by centrifugation, sonicated in 1 ml buffer A (50 mM Tris-base, 1 mM EDTA and 14 mM β-ME), centrifuged again to remove debris, and 20 µg supernatant protein was used for 12%SDS-PAGE followed by Coomassie staining and western blotting. Blots were probed with a murine anti-human TS monoclonal antibody (D3B31). Lane 1: purified recombinant hTS; lane 2: BlueRanger Prestained Protein Marker (Pierce Biotech, Rockford, IL); lane 3: untransformed TX61^{-} cells; lane 4: wt hTS; lane 5: V3A; lane 6: V3F; lane 7: V3L; lane 8: V3T; lane 9: V3Y. A: Western blot analysis of total cell extracts from exponentially growing transformed and untransformed TX61- cells. B: Total proteins analyzed by SDS-PAGE stained with Coomassie blue.

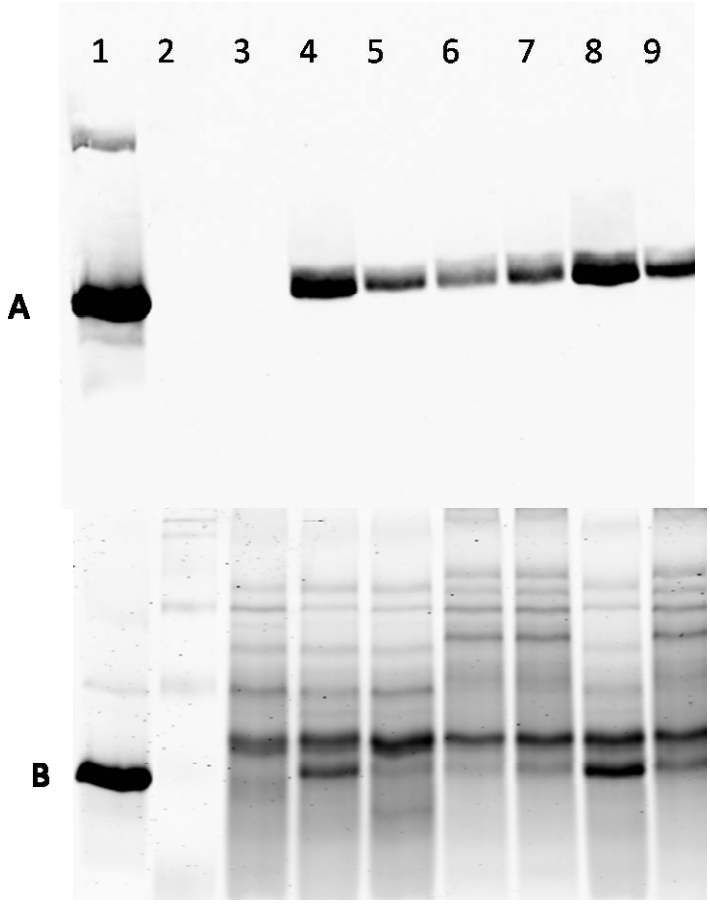

Figure 3. Stereo view of the polyalanine fragment from V3F high salt structure.

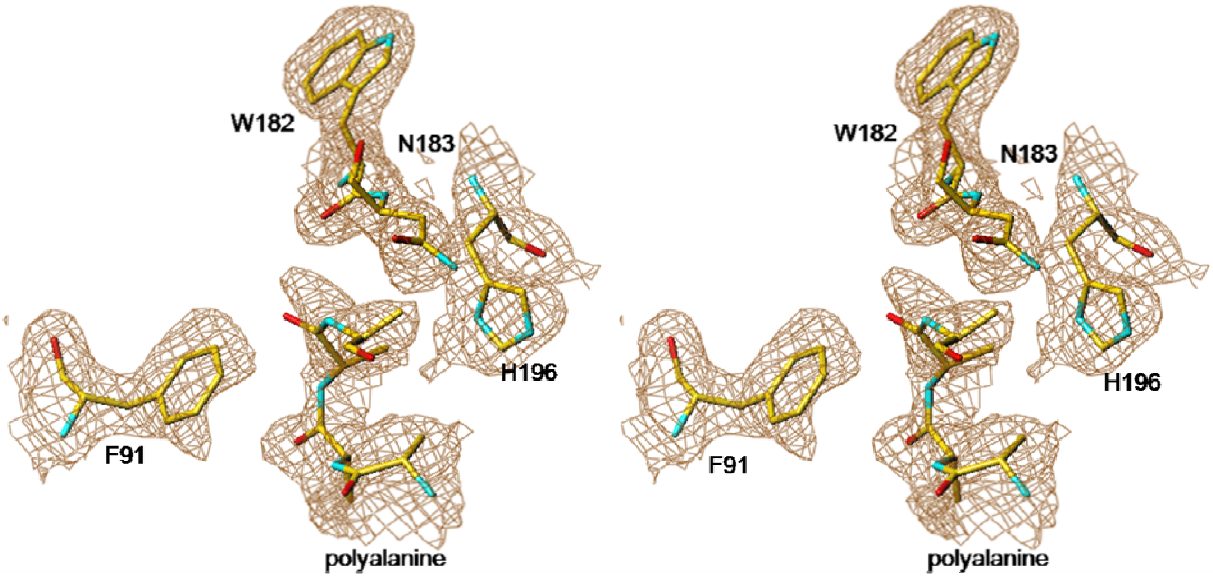

Figure 4. Enzyme velocity as a function of dUMP concentration.