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Protein-Protein Interactions: Crystal Structures of Methotrexate-linked Dimers in Complex with *E. coli* Dihydrofolate Reductase. Vivian Cody, Kim Chisum, Jim Pace, Hauptman-Woodward Medical Research Institute, 73 High St., Buffalo, NY 14203, C.R. Wagner, J.C.T. Carlson, Univ. of Minnesota, Minneapolis, MN, 55455.

To validate the role of ligand conformation in induced protein dimerization, methotrexate (MTX) dimers with γ -glutamate flexible linkers were synthesized that showed selectivity in the dimerization of *E. coli* dihydrofolate reductase (DHFR), but not mouse DHFR. Crystal structures are reported for binary complexes with either C9 or C12-linked MTX dimers (MTX₂-C9 or MTX₂-C12) and ecDHFR. All structures crystallized in space group P6₁ with two enzyme complexes in the asymmetric unit. Data were collected to 1.8 Å for the MTX₂-C12 complex, and synchrotron data collected to 1.6 Å for the MTX₂-C9 complex. These data revealed density for MTX in both enzyme active sites, but no clear density for the C9 or C12 linked bridge of the MTX dimers. Although there is density for a partially occupied linked MTX that follows the enzyme surface in two orientations in the MTX₂-C9 complex, there is little indication of density across the dimer interface for the MTX₂-C12 complex. These data suggest that the dimerization model for ecDHFR is more complex than previously reported and that dimerization enhancers with less flexible linkers are needed in order to clearly delineate the linker geometry. Supported in part by GM-51670 (VC).