

W0097

Neutron Diffraction Structure of *E. coli* Dihydrofolate Reductase in Complex with the Chemotherapeutic Methotrexate at 2.2Å Resolution. Brad Bennett^{*}, Paul Langan[†], Leighton Coates[†], Marat Mustyakimov[†], Benno Schoenborn[†], Elizabeth Howell^{*}, Chris Dealwis^{*‡}, ^{*}Dept. of Biochemistry, Cellular and Molecular Biology, Univ. of Tennessee, Knoxville, TN 37996, [†]Los Alamos National Laboratory, Biosciences Div., Los Alamos, NM 87545.

The role hydrogen atoms play in biochemical processes cannot be overstated, yet they are difficult to visualize by X-ray crystallography. Neutron crystallography has a proven track record in locating hydrogen, but limited neutron fluxes and accessibility to reactor sources have made it impractical. Spallation neutron sources provide a new arena for protein crystallography, as higher fluxes and time-of-flight measurements enhance data collection efficiency. Here we report a 2.2Å resolution neutron structure and a 1.0Å ultrahigh resolution X-ray (UHRX) structure of *E. coli* Dihydrofolate Reductase (DHFR) in complex with methotrexate (MTX), a chemotherapeutic agent. Neutron data were collected on a 0.25mm³ D₂O-soaked crystal at the Protein Crystallography Station (PCS) at the spallation source operated by Los Alamos Neutron Scattering Center (LANSCE). This study provides an example of using spallation neutrons to identify protonation states *directly* in macromolecules from nuclear density maps. In particular, the neutron structure reveals the N1 atom of MTX is protonated, and thus charged, when MTX is bound to DHFR. In contrast, the UHRX structure does not directly identify the protonation state of either MTX or the active site Asp27 residue. However, results from full matrix refinement of this structure show that the Asp27 carboxylate bond lengths are equivalent, indicating the Asp27 is charged when MTX is bound. Taken together, these results clarify a long-standing controversy, revealing that the Asp27•MTX interaction is ionic in nature. Additionally, the neutron maps show that nearly 2/3 of amide backbone hydrogens in DHFR have been exchanged for deuterium.