

**NIF-EN PROTEIN: ROLE AS A MOLYBDATE REDUCTASE IN THE BIOSYNTHESIS OF THE NITROGENASE IRON-MOLYBDENUM COFACTOR.** Namrata Singh<sup>1</sup>, Jirair Gevorkyan<sup>1</sup>, Alexander Angerhofer<sup>2</sup> and Robert Y. Igarashi<sup>1,\*</sup>, <sup>1</sup>Department of Chemistry, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, <sup>2</sup>Department of Chemistry, University of Florida, PO Box 117200, Gainesville, FL 32611

Nitrogenase is a metallo-enzyme that performs nitrogen fixation by reducing dinitrogen to ammonia using its Fe<sub>7</sub>MoS<sub>9</sub>C-*R*-homocitrate cluster (FeMo-co). Although it is well known that the biochemical synthesis of FeMo-co involves several enzymes; NifU, NifS, NifB, NifX, NifEN and NifY, the mechanistic understanding of the process is vague. NifEN is the enzyme where in an ATP and Fe protein dependent process, molybdate and homocitrate are incorporated to synthesize the FeMo-co. Molybdenum is provided to NifEN as Mo(VI)O<sub>4</sub><sup>2-</sup>, however in the FeMo-cofactor it exists as Mo(IV). Our studies confirm that the molybdate reduction is performed by NifEN. A molybdate dependent EPR signal ( $g = 2.00$ ) has been identified and assigned to the reduction of Mo(VI) to Mo(V). The molybdate dependent signal appears as an isotropic species and has a temperature and power profile distinct from that of the NifEN Fe-S cluster(s). Furthermore, inclusion of tungstate in the reaction diminishes the Mo-dependent signal.