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Flash-cooling and Preliminary Low Temperature Neutron Diffraction Studies of the Crenarchaeal *Aeropyrum pernix* Flap Endonuclease-1 (FEN-1). S.J. Tomanicek¹, B. Shah², C.A. Schall², T.C. Mueser¹, B.L. Hanson¹, ¹Chemistry and ²Chemical Engineering, The Univ. of Toledo, Toledo, OH.

The flap endonuclease-1 (FEN-1) enzymes are structure-specific 5' to 3' DNA endonucleases that are members of the RAD2/RAD27 family of eukaryotic nucleases. FEN-1 enzymes are involved in the recognition and cleavage of flap DNA that is generated during the processing of Okazaki fragment primers during lagging-strand DNA synthesis and in processing strands displaced during DNA synthesis associated with repair. We have previously solved the X-ray structure of the native metal free *Aeropyrum pernix* (Ape) FEN-1 enzyme at 1.4 Å resolution. Low temperature neutron diffraction studies of Ape FEN-1 are aimed at examining the role of solvent in substrate recognition and the role of divalent metal ions in the catalytic mechanism of the FEN-1 enzymes. We have recently developed a method to reliably flash-cool large Ape FEN-1 crystals (0.3 mm³) with a mosaicity of approximately 0.2°. Following deuterium exchange of Ape FEN-1, crystals are grown using vapor diffusion experiments in the presence of a deuterated cryoprotectant. The crystals are then cryogenically preserved using a helium cryostat at 40K. Using one of our smallest crystals of Ape FEN-1 (0.064 mm³) we were able to attain diffraction at PCS.