Ultra-fast MAD Data Collection on the 19ID Beamline at the Advanced Photon Source*

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Synchrotron radiation from the Structural Biology Center's (SBC) undulator beamline (ID19) was used to collect a four-wavelength MAD experiment at 100 K. The experiment was completed to 2.25-Å resolution in 23 minutes on a single crystal of a 16-kDa protein containing three SeMet residues. The SeMet-labeled protein from a thermophile was produced in the *E. coli* over-expression system that was supplemented with SeMet under conditions known to suppress methionine biosynthesis in the bacteria resulting in a 93% incorporation of SeMet into the protein. The protein was purified in two steps and it crystallized readily in the orthorhombic space group C2221 with unit cell parameters a = 62.6 Å, b = 64.8 Å, c = 74.3 Å. At each wavelength, in a single pass, 60 two-degree oscillation images were collected in 345 seconds using a mosaic 3x3 CCD detector.[1] Data were processed with HKL2000.[2] Two of the three selenium sites were located automatically using the program RSPS in the CCP4 suite.[3] These sites were refined with MLPHARE[3] which gave an overall FOM of 0.72 for data between 10-2.25 Å. The protein model was built automatically with the program wARP[4] which was used first to extend phases to 1.7 Å, and then automatically trace a complete main chain of the protein with polyalanine/serine. The program also automatically built 58% of the side-chain residues of the protein. This ultra rapid MAD data collection procedure has already been successfully executed at the SBC 19ID beamline for two other larger protein structures (MW > 55kDA). Thus, this method in combination with new automated procedures for model building such as wARP provides an experienced crystallographer with the opportunity to solve a protein structure in one working day.

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*This work was supported by the US Department of Energy, Office of Biological and Environmental Research, under Contract W-31-109-ENG-38.

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[3]CCP4 (1994) Acta Cryst. D50, 760-763.
[4]Perrakis A., et al., (1997) Acta Cryst. D53, 448-455.