s7.m6.p1 **Investigations into the use of Dispersive-Mode Anomalous Scattering in Macromolecular Crystallography.** W. Shepard*[1], V. Favre-Nicolin[2], M.-L. Chesne[3], C. Abergel[4], S. Bos[2], L. Chantalat[3], R. Kahn[3], E. Lorenzo[2], F. Natali[5], T. Neisius[5], S. Pascarelli[5], J. Vicat[3] & J.-L. Hodeau[2]. *[1]LURE, Bât. 209d, B.P. 34, Centre Universitaire Paris-Sud, 91898 Orsay cedex, France; [2]Laboratoire de Cristallographie, CNRS, B.P. 166, 38042 Grenoble cedex 9, France; [3]IBS, 41 rue Jules Horowitz, 38027 Grenoble, France; [4]IBSM, 31 chemin Joseph Aiguier, 13402 Marseille cedex, France; [5]ESRF, B.P. 220, 38043 Grenoble cedex, France.*
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Simultaneous Multiple-wavelength Anomalous Diffraction (SMAD) and Dispersive Anomalous Fine Structure (DAFS) experiments employ dispersive X-ray optics to collect simultaneously the diffraction data of multiple X-ray wavelengths spanning an X-ray absorption edge either as several discrete wavelengths (SMAD) or as a continuous spectrum (DAFS). When applied to macromolecular crystallography, a SMAD experiment renders the possibility of recording all of the diffraction intensities of a MAD experiment on to a single image. This effectively allows Bragg reflections to be phased in a single exposure. SMAD and DAFS experiments have been carried out at the ESRF on beamline ID24 at different absorption edges on a variety of macromolecular crystals containing Fe, Zn, Se or Hg. The quality of the X-ray diffraction data in the SMAD case is equivalent to that collected on a monochromatic source, and the results show that the dispersive signal follows closely the expected trend of f' with respect to the wavelength. For DAFS diffraction images, a program has been written to integrate the wavelength "streaks" to produce XANES spectra for each reflection. The theoretical and technical aspects of both methods will be presented along with the results, and the potential applications to macromolecular crystallography will be discussed.

s7.m6.p2 **MAD phasing at long wavelengths**. J. Hauschild, G.P. Bourenkov, H.D. Bartunik, *Max-Planck Research Unit for Structural Molecular Biology, c/o DESY, Hamburg, Germany.*
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Anomalous phasing at long wavelengths > 2.0 Å may be used for the de-novo solution of protein structures. This is demonstrated by a test application of MAD phasing at the calcium K edge (3.07 Å) to bovine pancreatic trypsin (BPT). Diffraction data were measured from a shock-frozen BPT crystal at the wavelength 3.07 Å and at two remote wavelengths, 2.60 Å and 1.07 Å. All data were collected on the wiggler beamline BW6 at DORIS using a standard CCD detector (X-Ray Research). The data were corrected for absorption effects using a local scaling procedure. The locations of the Ca ion and the sulfur atoms in the structure were derived from the anomalous difference Patterson maps and used as start values in the calculation of MAD phases with SHARP[1]. Calculation of an initial electron density distribution at 3.5 Å resolution using these experimental phases and subsequent solvent flattening produced density maps of high contrast and quality.

The wavelength range 2.0 Å - 3.1 Å includes a number of absorption edges of potentially useful anomalous scatterers, in particular the K edge of Ca, the L3 edges of Xe (2.59 Å) and I (2.72 Å), and the M3 edge of U (2.88 Å). The combination of Xe derivatization techniques[2] using commercial equipment with the strong anomalous scattering in the vicinity of the L3 edge may provide a generally applicable method of phase determination.

Long-wavelength diffraction further may be used to enhance anomalous signals far from absorption edges. This is of particular interest for sulfur atoms (K edge at 5.02 Å). On the other hand, the anomalous contributions from remote absorption edges like S, P, or K, may complicate the interpretation of anomalous Patterson maps, in particular in the case of complex high-molecular weight structures. We investigated the feasibility of (SAD) phasing based on anomalous scattering from sulfur at wavelengths around 2.5 Å using diffraction data to 2.5 Å - 3.0 Å resolution.

The new possibilities of anomalous phasing at long wavelengths may also be exploited at low- and medium-energy synchotron radiation sources. For de-novo structure solution, experimental phases to = 3.5 Å resolution are desired. This is presently possible on BW6 for wavelengths < 3.1 Å. A further extension of the wavelength range to about 3.5 Å is of potential interest for a possible use of the U M5 edge[3]. Reaching sufficiently high resolution at such long wavelengths, however, is additionally complicated by the strong absorption in the sample and the resulting limitations to the thickness of crystals and their lifetime in the X-ray beam.

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