s7.m6.o1 **Anomalous Diffraction with Soft X-ray Synchrotron Radiation.** P. Carpentier[a], C. Berthet-Colominas[b], M. Capitan[c,d] , M.-L. Chesne[a], E. Fanchon[a], R. Kahn[a], S. Lequien[c,e], H. Stuhrmann[a,f], D. Thiaudière[c,g], J. Vicat[a], P. Zielinski[h], *[a] Institut de Biologie Structurale CEA/ CNRS F-38027 Grenoble France, [b] EMBL Outstation Grenoble, [c] ESRF Grenoble, [d] Universitad Autonoma Madrid Spain, [e] CEA Saclay France, [f] GKSS Geesthacht Germany, [g] LURE, Orsay, France, [h] Nuclear Physics, Krakow, Poland*
Keywords: MAD, DAFS, soft X-rays

X-ray diffraction at wavelengths beyond 3Å (at energies below 4 keV) opens new possibilities for the use of anomalous dispersion in MAD and DAFS methods. With the X-ray K-absorption edges, Ca, K, Cl, S, P, and Si appear as newcomers. Huge magnetic resonant scattering is observed at $L_{II/III}$ edges of transition elements and $M_{IV/V}$ edges of heavy elements. Heavy elements, like U and Th, show an unusually strong anomalous dispersion which is characterized by 110 electron units.

Anomalous diffraction with soft X-rays has been used in the study of a broad variety of materials, like polymers [1], liquid crystals [2], protein crystals [3], ribosomes [4], and ferroelectrics [5]. The various experimental methods which have been used at different places to cope with the special technical requirements of soft X-ray diffraction will be discussed.

First feasibility studies of soft X-ray diffraction were done recently at the beamline ID1 of ESRF, Grenoble. The MAD method was used to study of an uranium derivative of asparaginyl-tRNA synthetase at wavelengths near the U M_V edge. The preliminary data show the presence of the dispersion due to uranium. In another experiment, DAFS from the ferroelectric $(CH_3NH_3)_5Bi_2Cl_{11}$ has been measured at 30 wavelengths near the K-edge of chlorine (4.4Å) [5]. These experiments show that soft X-ray diffraction at ID1 of ESRF is feasible for materials and also for proteins. The potential of ID1 in soft X-ray diffraction which remains to be exploited is considerable.

[1] Mardalen J., Riekel, C., Müller H. (1994), J. Appl. Cryst. 27: 192-195
[2] Mach, P., Pindak, R., Levelut.A.-M., Barois, P., Nguyen, H.T., Baltes, H., Hird, M., Toyne, K., Seed, A., Goodby, J.W., Huang, C.C., & Furenlid,L., (1999) Phys. Rev. E 60, 6797-6802
[3] Stuhrmann S., Braunwarth W., Doose R., Dauvergne F., Gabriel A., Knöchel A., Marmotti M., Stuhrmann H.B., Thomas J., Trame C., Lehmann M.S. (1997) J. Synchrotron Rad. 4, 298-310
[4] Stuhrmann S., Hütsch M., Trame C., Thomas J., & Stuhrmann H.B. (1995) J. Synchrotron Rad. 2, 83-86
[5] Kahn R., Carpentier P., Berthet-Colominas,C., Capi-tan M., Chesne M.-L., Fanchon E., Lequien S., Thiau-dière D., Vicat J., Zielinski P., (2000) J. S. R. in press

s7.m6.o2 **A combined anomalous scattering and direct methods approach to solve apocrustacyanin proteins (C_1 and A_1).** *A. Olczak[1], M. Cianci[1], J. Raftery[1], P.J. Rizkallah[2], D. Moorcroft[1], E.J. Gordon[3], S. McSweeney[4], N.E. Chayen[5], P. Zagalsky[6] and J.R. Helliwell[1]. *[1]Chemistry Dept, University of Manchester. [2]Daresbury Laboratory. [3]IBS, Grenoble. [4]ESRF and EMBL, Grenoble. [5]Imperial College, London. [6]Royal Holloway and Bedford New College, London.*
Keywords: disulphides; softer X-rays; ultra-high resolution

Crustacyanin is a multimacromolecular protein plus carotenoid astaxanthin complex responsible for the blue coloration of the carapace of the lobster Homarus gammarus. The proteins provide a structural basis for the spectral shift of the carotenoid in its complexed state. In the History of this protein crystal system the crystal structure determinations have proved problematic through lack of a suitable molecular replacement model from within the lipocalin family, lack of good heavy atom derivatives (including xenon) and lack of a seleno methionine variant. A new approach is being attempted involving joint anomalous scattering and 'direct methods'. In Manchester tests involving concanavalin A combining the Mn K edge anomalous differences from ELETTRA with ultra-high resolution data from CHESS indicate that knowledge of the Mn and Ca sites alone have a mean phase error of 76º (to 1.0 Å resolution)[1,2]. For apo C_1 and apo A_1 their disulphides' partial structure is akin to the Mn and Ca situation in concanavalin A. Hence a search is underway for the cysteine sulphur atoms in the six putative disulphide bridges in the asymmetric unit based on data recorded at 2.0 Å (softer X-ray) wavelength from apo A_1 and at 1.488 Å wavelength from apo C_1 on the SRS. In house, in parallel, we have undertaken a very high redundancy (17 fold) 1.8 Å data collection from lysozyme at CuK_α, following the Dauter et al[3] sulphurs' approach with NSLS data to 1.5 Å, to form test data for locating S-S bridges and phasing with strong data to 3.0 Å (akin to apo C_1 and A_1). These data are promising and yield the Patterson anomalous peaks for lysozyme disulphides (peak heights vary to some extent according to atom B factors). This confirms the possibility of identifying sulphur atoms from their anomalous signal. In another step in this approach towards the solving of the crustacyanin structures ultra-high (0.94 Å) resolution data have also been recorded on the Quadriga end station undulator beamline at ESRF from a frozen apo C_1 crystal. A jigsaw of the tools to solve this difficult system is falling into place and can be of general applicability.

[1] N.S. Hunter, T.J. Gleichmann, M. Helliwell, L. Barba, E. Busetto, A. Lausi, H.J. Price and J.R. Helliwell "Crystallographic MAD Phasing Strategies Explored Using ELETTRA Sincrotrone Mn K-Edge Data to 2.1 Å and Use of CHESS Establishes the Diffraction Resolution Limit as 0.92 Å for the Protein Mn, Ca Concanavalin A", Croatica Chemica Acta, (1999), 72: 673-684
[2] N.S. Hunter, PhD Thesis, (2000), University of Manchester
[3] Z. Dauter, M. Dauter, E. de la Fortelle, G. Bricogne, G.M. Sheldrick "Can Anomalous Signal of Sulfur Become a Tool for Solving Protein Crystal Structures", J. Mol. Biol., (1999), 289: 83-92.