discussion of the catalytic and allosteric mechanisms of this key regulatory enzyme, will be possible. Further interest to the science of crystal growth rises from the fact that two distinct crystal forms grow under identical conditions; this phenomenon will be explored in light of the molecular contacts.

PS04.08.08 CUBIC, TETRAGONAL AND ORTHORHOMBIC CRYSTAL FORMS OF HORSE SPLEEN APOFERRITIN. Geoffre, S., Gallois, B., Dautant, A., Granier, T., Langlois d'Estaintot, B., Michaux, M.A. & Précigoux, G., Unité de Biophysique Structurale, CNRS, Université de Bordeaux, 33405 Talence, France.

Ferritin is the universal iron storage protein utilized by most living cells to uptake and store iron, in a bio-available form , via redox mechanisms. X-ray structural studies show that haem is able to bind horse spleen apoferritin in a site similar to that observed in bacterioferritins with a stoichiometry 1:2.

In the crystal structures, and whatever the studied metalloporphyrin, the protoporphyrin IX is always observed free of metal. Beside that property, horse spleen apoferritin can be crystallized in three different space groups : cubic, F432 ; tetragonal, $P42_12$ and orthorhombic $P2_12_12$. The two later crystal forms were described some years ago (Harrison 1963, Hoy & al. 1974) but their crystal quality (6 Å resolution) could not allow a full structure investigation.

Thanks to a careful protein purification procedure, both horse spleen apoferritin crystals of tetragonal and orthorhombic forms, which diffract beyond 2.4 Å have been obtained. X-ray diffraction data were collected, on the three different crystal forms, with the LURE synchrotron radiation facilities.

We present a comparison of the structures of the three crystal forms : molecular packing and conformational differences will be discussed in relation with crystal symmetry differences.

MS04.08.09 THE MONOCLINIC CRYSTAL STRUCTURE FORM OF BACTERIOFERRITIN FROM *E. COLI*. Hospital, M.1, Dautant, A.1, Yariv, J.1, Précigoux, G.1, Kalb (Gilboa), A.J.2, Frolow, F.2 & Sweet, R.M.3 1Unité de Biophysique Structurale, CNRS, Université de Bordeaux, 33405 Talence, France. 2Departments of Structural Chemistry and Chemical Services, The Weizmann Institute of Science, Rehovot, Israel. 3Biology Department, Brookhaven National Laboratory, Upton, New York 11973-50000, USA.

The structure of a monoclinic, $P2_1$, crystal form of bacterioferritin from *E. coli* (cytochrome b1) was solved by molecular replacement and refined using as model the fundamental unit of this protein that consists of two protein subunits and a single haem. The haem is positioned in a special position on the two-fold axis of the dimer. The asymmetric unit of the monoclinic crystal consists of twelve such dimers and corresponds to the molecule of bacterioferritin (MW = 450 kD).

First, the orientation of the molecule has been successfully determined with a standard self-rotation followed by a locked self-rotation, then, the position in the unit cell, has been localized with the program AMoRe (Navaza, 1992). The model used was built from the coordinates of the tetragonal structure of cytochrome b1 (Frolow & al., 1994). Even at the 2.9 Å resolution, the following facts about this structure emerged. Thus it is confirmed that the haem is located at the interface of two subunits with as axial ligands the sulfur atoms of two symmetrically related Met52. Furthermore the presence of a di-metal center is observed in the inside of a four-helix bundle. The residues involved in the metal coordination spheres are four glutamates and two histidines. Both histidines ligands bind to the metals through their δ-nitrogen atoms.

The crystal packing corresponds to a situation halfway between the well known hexagonal closest packing and the orthorhombic all-face centred one.

PS04.08.10 CRYSTALLOGRAPHIC STUDIES ON THE ICOSAHEDRAL CORE OF THE PYRUVATE DEHYDROGENASE MULTIENZYME COMPLEX FROM *BACILLUS STEAROTHERMOPHILUS*. Tina Izard[a], Richard N. Perham[b], Arie de Kok[c], Wim G. J. Hol[a]. [a]Howard Hughes Medical Institute, Biomolecular Structure Center & Dept. Of Biological Structure, University of Washington, Box 357742, Seattle WA 98195-7742, USA, [b]Dept. of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK, [c]Dept. of Biochemistry, Agricultural University, Wageningen, The Netherlands

The bacterial and mammalian 2-oxoacid dehydrogenase multienzyme complex families catalyse the oxidative decarboxylation of 2-oxoacids (pyruvate, α–ketoglutarate and branched-chain 2-oxoacids) to produce the corresponding acyl-CoA and NADH. A well known member of the family is pyruvate dehydrogenase (PDH), occurring at the end of the glycolysis and providing the tricarboxylic acid cycle with acetyl-CoA. The architectural design of PDH is composed of a central core enzyme, dihydrolipoamide acetyltransferase (E2) with either octahedral (24-mer) or icosahedral (60-mer) symmetry, depending on the source of the enzyme. E2 binds the two peripheral enzymes, thiamin pyrophosphate (TPP) dependent decarboxylase (E1) and flavoenzyme lipoamide dehydrogenase (E3), leading to a molecular weight (M_r) of these systems of 5 to 10 million Da. In mammals and yeast, additional proteins are attached to the complex; the so-called protein X and a specific kinase and phosphatase. Deficiencies or malfunctioning of the complexes lead to severe pathological states such as numerous acidoses which are usually correlated with serious neurological dysfunctions.

The catalytic domain of E2 from *B. stearothermophilus* and *Enterococcus faecalis* PDH have been cloned, expressed in *E. coli* and purified. Of the former, crystals suitable for X-ray diffraction experiments grew within 10 days and diffract to about 4 Å resolution at cryo-temperatures. Here we describe the crystallisation of E2 from *B. stearothermophilus* and its preliminary analysis by X-ray crystallography.

PS04.08.11 MOLECULAR AGGREGATION OF THE NEUROPHYSINS. John P. Rose and Bi-Cheng Wang, Department of Biochemistry and Molecular Biology University of Georgia, Athens GA 30602, U.S.A.

Bovine neurophysin II has been crystallized in eight distinct crystal forms containing 1, 2, 3, 4, 6 and 12 molecules per asymmetric unit. The mode of molecular aggregation observed in the crystal structures may be paradigms of how the neurophysin-hormone complexes are packaged in the neurosecretory granules (NSG).

The neurophysins (NP) are a family of disulfide rich proteins responsible for the packaging and transport of the posterior pituitary hormones oxytocin (OT) and vasopressin (VP). Two closely related classes of neurophysins are known, one complexed with VP and the other with OT, this association reflecting the synthesis of each hormone and its associated NP via a common precursor.

During transport, the hormone is cleaved from its neurophysin carrier but remains associated with the protein as a non-covalent complex. The neurophysin-hormone complex is then stored in NSG until release into the blood stream. Within the NSG, the NP-hormone complex concentration can be as high as 1000 mg/ml. Although the mode of NP aggregation within the NSG is unknown, it has been postulated based on the high concentrations observed in the NSG that the complexes exist as dimers, higher aggregates, or even amorphous or crystalline precipitates thus the mode of NP association observed in the crystal structures may serve as a model for neurophysin packaging in the NSG's.

An analysis of the common modes of NP aggregation observed in the crystal structures will be presented.

Work supported by NIH grant GM-46828 and resources from the Pittsburgh Supercomputing Center.