01.X–1 NEUTRON DIFFRACTION AS A COMPLEMENTORY TOOL TO STUDY DYNAMICS OF PROTEINS. By Lennart Sjölin, Dept of Inorganic Chemistry, Chalmers University of Technology, S-412 96 Göteborg, Sweden.

The classic view of proteins has been static in character because of the dominat role of the information provided by X-ray crystallography for these very complex systems. Since neutron-diffraction can distinguish between deuterium and hydrogen, exchange of e.g. amide protons can easily be studied with this method, a feature providing crystallographers with a tool to study dynamics. It has been shown in the study of BPTI (Wlodawer, this volume) that exchange of amide protons in the crystalline phase very closely resemble results obtained in solution with the 2D-NMR technique (Wagner and Wuthrich, J.Mol.Biol.160, 343, 1982). In addition, Kuwajima and Baldwin (J.Mol.Biol., 169,281,1983) have measured exchange rates for amide protons constituting the S-peptide of Rnase -S, and the results confirm our findings from neutrondiffraction maps. An analysis of temperature factors calculated as regional averages show a clear correlation between regions with low average temperature factors and amide protection. Further, it is a well known fact that the temperature factors are reliable as detailed measures of internal mobility.
It is the aim to compare results from various structural methods as 2D-NMR and crystallography with proton exchange studies by the means of neutron diffraction in the frame of dynamics.

01.X–2 THE D_2O STRUCTURE OF CRAMBIN BY NEUTRON DIFFRACTION AT 1.5 Å. Martha M. Teeter, Department of Chemistry, Boston University, Boston, MA 02215 USA and Anthony A. Kossiakoff, Genentech, Inc., South San Francisco, California, 94080 USA.

Crambin is a small plant protein (MW = 4700) with no known function. However, crystals of it diffract to 0.88 A (Teeter and Hendrickson, J. Mol. Biol. (1979) 127, 219-224). Neutron diffraction data for a crambin crystal (1.4 mm^3) soaked in D_2O for several weeks was collected at the High Flux Beam Reactor, Brookhaven National Laboratory, 73% of the data to 1.5 A and 43% of that to 1.2 A has been obtained and refined using Konnert/Hendrickson restrained least squares refinement against the 0.945 A X-ray model as a starting point.

Other proteins studied by neutron diffraction have shown a definite tendency for amide protons in α-helices to exchange except when they lie at intramolecular contact points. In crambin, no such pattern appears despite the high solvent accessibility of these protons (crambin is so small that it has little protected interior.) The D_2O neutron structure reveals that 53% of crambin's amide protons were unexchanged in this experiment.

Crambin crystals contain about 80% ordered solvent molecules consisting primarily of water. Despite the fact that the protein was crystallized from 60% ethanol, only 2 ethanol molecules had been located in the X-ray studies. The deuteriums associated with the water structure can be divided into three distinct classes: well-ordered, two-fold disordered and four-fold disordered. The arrangement of deuterium atoms in the solvent will be discussed.

Finally, an analysis of the hydrogen-bonding geometry for the protein and solvent and the protein secondary structure characteristics will be presented.

01.X–3 INVESTIGATION OF HYDROGEN EXCHANGE IN PROTEIN CRYSTALS BY NEUTRON DIFFRACTION By Alexander Wlodawer, National Bureau of Standards, Washington, DC 20234, and National Institutes of Health, Bethesda, Maryland 20205, U. S. A.

Hydrogen exchange was studied using the technique of neutron diffraction in two proteins: a complex of RNase A with an inhibitor uridine vanadate and in the bovine pancreatic trypsin inhibitor. Seventeen amides were found to be protected from exchange in a crystal of RNase A soaked for three months in a deuterated solution containing uridine vanadate, a strong competitive inhibitor, while three additional amides were partially exchanged. These results are very similar to the pattern of exchange found in the native RNase. Only three amides reported as fully protected in the native enzyme were found not to be protected in the complex, while five partially protected amides were also not protected. Two amides which were not protected in native RNase appear to be protected in the complex. I interpret these results as an indication of the reliability of neutron measurements of hydrogen exchange, as well as a proof of remarkably small (if any) differences between the dynamic behavior of the native and complexed RNase.
A comparison of results obtained with neutrons and NMR was made possible by the study of hydrogen exchange in BPTI. I found 11 protected amides after 3 months of soaking of a crystal in a solution containing 30% PEG 20,000 at pH 8.2. Nine of these amides participate in β sheet structure, one makes a hydrogen bond to a side chain and one is protected by an interaction with a tyrosine ring. Protected amides include all but one (Asn44) 'slow' amides as determined by 2D-NMR (Wagner and Wüthrich, J. Mol. Biol. 160, 343, 1982). Thus the agreement between the results obtained with these two methods is excellent.

01.1–1 STRUCTURE ANALYSIS OF F_1-PART OF PROTON TRANSLOCATING ATPase AT 3.5 Å RESOLUTION.
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Crystals of F_1-portion of proton translocating ATPase from rat liver mitochondria diffract up to 3.5 Å resolution. A 9 Å resolution structure has been obtained and work is underway to complete the structure at high resolution. Data to 3.5 Å resolution were collected photographically for the native and three heavy atom derivatives by oscillation methods (Space group = R32). For this, synchrotron radiation was employed (λ = 1.57 A), (Cornell High Energy Synchrotron Source (CHESS), Cornell University, Ithaca, New York).

The oscillation films were scanned with the help of Evans & Sutherland vector graphics and an optronics scanner. The scanned data sets were processed and reduced to the final data set using the post refinement procedures (Winkler, Schutt and Harrison, Acta Cryst. (1979) A35, 901). These reduced data are being used to obtain the structure of the F_1-ATPase at high resolution.