herent flexibility of protein molecules.

But does the behaviour of protein molecules inside a crystal have any relevance to the dynamic properties of protein molecules in solution? Petsko and Ringe⁹ have reviewed this question and the evidence (which includes studies of enzymic catalysis and of hydrogen-deuterium exchange in the crystal) suggests that the answer is often yes. The reason is that typically 50 per cent of the volume of a protein crystal consists of wide expanses of the solvent of crystallization (usually bulk water or an aqueous salt solution), with relatively few actual contacts between protein molecules. Although crystal contacts can have a damping effort on the atomic displacements¹⁰, it is frequently found that large-scale conformational variability can be accommodated within protein crystals. In the crystals of immunoglobulin IgG, for example¹¹, the entire F₂ domain (a region of relative molecular mass 50,000) is disordered and cannot be seen in the electron-density maps. Such cases of disorder must be related to the inherent flexibilities of the protein molecules concerned, which in turn may be related to their function.

In the past few years, the use of high-intensity synchrotron radiation for crystallographic data collection has shown that many protein crystals exhibit diffuse scattering maxima. The study of these potentially very informative adjuncts to diffraction patterns is only just beginning. Nevertheless, it is to be hoped that before long, analysis of X-ray diffuse scattering from such crystals will reveal much more about cooperative motions and conformational variability in protein molecules. □

1. Karplus, M. & McCammon, J.A. *A. Rev. Biochem.* **53**, 263-300 (1983).
2. Frauenfelder, H., Petsko, G.A. & Tsernoglou, D. *Nature* **280**, 558-563 (1979).
3. Artymiuk, P.J. *et al. Nature* **280**, 563-568 (1979).
4. Doucet, J. & Benoit, J.P. *Nature* **325**, 643-646 (1987).
5. Guinier, A. (translated by Lorrain, P. & Lorrain, D. S.-M.) *X-ray Diffraction in Crystals, Imperfect Crystals and Amorphous Bodies* (Freeman, San Francisco, 1963).
6. Phillips, G.N. Jr, Fillers, J.P. & Cohen, C. *Biophys. J.* **32**, 485-502 (1980).
7. Helliwell, J.R. *et al. Biochem. Soc. Trans.* **14**, 653-655 (1986).
8. Bauer, K., Jagodzinski, H., Dörner, B. & Grimm, H. *Phys. Stat. Sol.* **B48**, 437-443 (1971).
9. Petsko, G.A. & Ringe, D. *A. Rev. Biophys. Bioengng.* **13**, 331-371 (1984).
10. Finzel, B.C. & Salemme, F.R. *Nature* **315**, 686-688 (1985).
11. Colman, P.M., Deisenhofer, J., Huber, R. & Palm, W. *J. molec. Biol.* **100**, 257-282 (1976).

Peter Artymiuk is in the Department of Biochemistry, The University of Sheffield, Sheffield S10 2TN, UK.

Reinhold Benesch (1919 – 1986)

SOME people are said to be accident-prone. Reinhold Benesch, who died in New York on 30 December 1986, was discovery-prone. His first piece of research was a contribution to the British war effort. When asked to develop a way of packing eggs for the troops more tightly, he fed hens with sulfanilamide, which inhibited the enzyme carbonic anhydrase that is essential for calcification of their eggshells; the shell-less 'floppy' eggs could be packed like matchboxes. Benesch never told me whether the army adopted his invention. Once he sent me a reprint of a paper entitled *Homos and Heteros Among the Hemos* inscribed "With cordial greeting R'B". The paper was about homo- and hetero-oligomers of haemoglobin and the signature symbolized the 'heterodimer' Ruth and Reinhold Benesch. They got married in England in 1946, emigrated to the United States in 1947 and for the past 26 years worked together at the Columbia College of Physicians and Surgeons. They became the joint authors of 125 papers, all but 13 on haemoglobin. Ruth's contribution to them is inseparable from Reinhold's.

Oxygen equilibrium curves of purified human haemoglobin solutions differ from those of haemolysates. This made the Cambridge physiologist Joseph Barcroft suspect as early as 1921 the presence of a "third substance ... which forms an integral part of the oxygen haemoglobin complex". Years later, it also made his pupil F.J.W. Roughton dismiss my X-ray work on pure haemoglobin crystals as physiologically irrelevant. In 1967 the Benesch

discovered that Barcroft's third substance is 2,3-diphosphoglycerate (DPG), long known to be present in erythrocytes in equimolar proportion to haemoglobin. That discovery opened a new era in the physiology of respiratory carriage. Some of the best among the ensuing flood of papers are those by the Benesch themselves. They demonstrated that one mole of DPG combines with one mole of deoxy-, but not with oxyhaemoglobin and that DPG lowers the oxygen affinity in a physiologically advantageous way. They determined the thermodynamics of DPG binding; its influence on the Bohr effect and its interactions with other solutes; they also foreshadowed its location in the central cavity between the β -chains which A. Arnone later determined by X-ray analysis. Subsequent work by several investigators indicated that DPG also binds weakly to oxyhaemoglobin and that its more powerful analogue inositol hexaphosphate (IHP) binds to oxyhaemoglobin quite strongly. Judging by chemical evidence, DPG and IHP appeared to bind to the same residues as in deoxyhaemoglobin, even though the cleft where they bind is closed in oxyhaemoglobin. I tried to solve that paradox by X-ray analysis, but drew a blank. The Benesch invented an ingenious method for measuring the dissociation constants of weakly bound ligands and used it to demonstrate that DPG and ATP, and by implication also IHP, bind, not to oxyhaemoglobin tetramers, but to $\alpha\beta$ -dimers, where the residues that are tucked away in the cleft of the tetramer are exposed. The satisfying

answer to this long-standing puzzle forms the subject of the Benesch's last, and perhaps most elegant, joint paper.

On several other occasions their work brought light where there had been darkness. The oxygen equilibrium curves of human haemoglobin measured by different workers used to be inconsistent, because their solutions contained variable amounts of DPG and they used anionic buffers which bound to deoxyhaemoglobin and reduced the oxygen affinity in varying degrees. The Benesch developed methods of stripping away the DPG and introduced bis Tris buffer which has a pK of 6.5 and is mostly unchanged at physiological pH, so that it is not bound by deoxyhaemoglobin. Only since then have oxygen equilibria become reproducible. They started their first work on haemoglobin in response to reports that its SH groups contribute to the Bohr effect. The Benesch disproved this, even though they found that the SH reagent N-ethylmaleimide halves the alkaline Bohr effect. That discovery led me to examine the N-ethylmaleimide derivative by X-ray analysis, which revealed that this reagent inhibits formation of a salt bridge formed by the carboxy-terminal histidines of the β -chains and thus led to the first interpretation of a physiological function of haemoglobin on a stereochemical basis. The paper on homos and heteros among the hemos demonstrated that tetramers of β - and γ -chains, which undergo no change in quaternary structure, bind oxygen non-cooperatively, thus proving that a heterodimer undergoing a structural transition is essential for cooperativity.

On loss of oxygen, sickle-cell haemoglobin polymerizes into long fibres. Knowledge of the contacts between the molecules in the fibres would facilitate the design of anti-sickling drugs. The Benesch explored these contacts by making hybrids of β -chains carrying the sickle-cell mutation with a variety of mutant α -chains. They found their effects to be highly specific: some mutations had no influence on the solubility of deoxyhaemoglobin S; some raised it; one lowered it and changed the fibre geometry. The results enabled them to map out a hitherto unrecognized contact area. Recently they discovered that folate, in its poly- γ -glutamate complex, is carried in the blood by haemoglobin to which it binds at the same site as DPG.

Reinhold Benesch was a first-class biochemist and also an accomplished wit and comedian who brought laughter to the dreariest of scientific meetings. His inventiveness and originality shone in his papers and in discussion; his wide literary knowledge and *joie de vivre* made him a delightful companion on non-scientific occasions.

Max Perutz

Max Perutz is at the MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, UK.