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NMR and x-ray studies show large movements within proteins

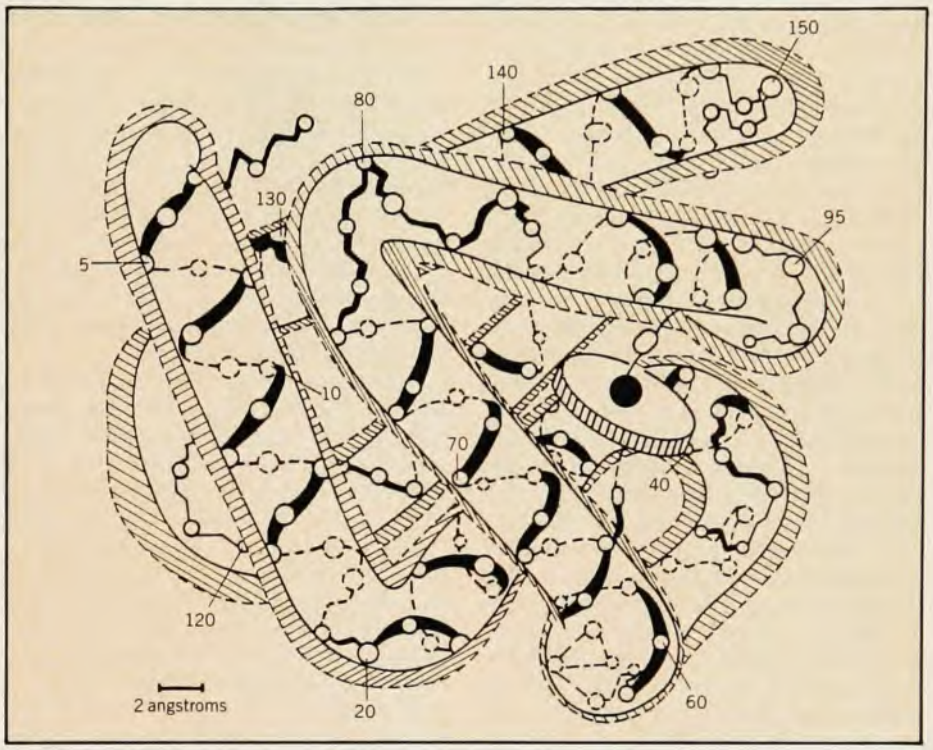
X-ray crystallography provides information on the average structure of protein molecules. The images of atoms have always been broadened, but until the last few years this broadening was generally attributed to inaccuracies in the structure determination, lattice disorder and purely thermal vibration. Most crystallographers were skeptical about interpreting the temperature factors as structural motion. A typical small globular protein has a molecular weight of 20 000 a.m.u. and a diameter of 30–40 Å.

Recent theoretical and experimental studies of muscle protein myoglobin suggest that portions of the molecule can experience large mean square displacements. A similar effect has been found in lysozyme. Extremely precise nmr studies of a small protein—bovine pancreatic trypsin inhibitor—have explored the dynamics on a nanosecond time scale. Measurements[1] done at Stanford University on fluorescent depolarization in bovine serum albumen and azurin indicate significant motion on a subnanosecond time scale.

Intramolecular motions have also been suggested by the trapping of a solvent (such as water) inside a molecule, earlier nmr studies that show portions of the molecule rotating or vibrating with respect to each other, fluorescence quenching and relaxation, and isotope exchange.

One example of the biological importance of such motions is the binding of oxygen to the muscle protein, myoglobin, which stores oxygen until it is needed for oxidation. The binding site is buried inside the molecule and is only accessible to oxygen if the protein is flexible.

The motions cover an enormous time range—from seconds to nanoseconds—depending on the process.



Backbone (mainchain) structure of myoglobin. The solid lines indicate the static structure as given in *Atlas of Protein Sequence and Structure* (M. O. Dayhoff, ed.), 1972. Circles denote the backbone positions; some sequence numbers are given. The shaded area gives the region reached by conformational substates with a 99% probability. (From reference 2.)

Background. In 1955 K. Linderstrøm-Lang (Carlsberg Laboratory, in Copenhagen, Denmark) studied the mechanism of hydrogen and deuterium exchange in proteins. His work suggested that large-scale fluctuations, which occur very rarely (some only once in many months at room temperature), would open the interior of the protein to a solvent. Several years ago William Lipscomb (Harvard University), in studying the protein carboxypepsidase, found that it can trap water inside itself. Studies indicate that protein crystals contain substantial amounts of water—up to values greater than a half. Lipscomb notes that Lyle Jensen (University of Washington), who has concentrated on the small protein rubridoxin, has provided evidence that much of this water is ordered. Jensen and his colleagues have shown that not only the water structure but also the atomic motion can be modeled.

In 1975 Brian Sykes (then at Harvard) and independently Kurt Wüthrich and Gerhard Wagner (ETH, Zurich), using nmr, found that the tyrosine aromatic rings in bovine pancreatic trypsin inhibitor (PTI) rotate through 180 deg at a rapid rate—1000/sec. At the same time I. D. Campbell, C. M. Dobson and R. J. P. Williams (Oxford) observed the same effects in lysozyme. (PTI and lysozyme are valuable proteins to study because they are relatively small—58 amino-acid residues, very stable, and have an accurately determined x-ray structure.)

Martin Karplus and Bruce Gelin (Harvard) interpreted these data as due to flipping of the protein side chains and showed that it was possible to estimate the rate of reorientation from a knowledge of the structure of the protein. At Oxford University Williams and his collaborators showed that the rate of ring flips in proteins varied from protein to protein and made correlations between the flips and the internal structure of the enzyme. By now many groups have seen ring flips—in lysozyme, cytochrome-C, ribonuclease, snake toxins and other proteins.

X-ray studies. Recently Hans Frauenfelder (University of Illinois), Gregory A. Petsko (MIT) and Demetrius Tsernoglou (Wayne State University School of Medicine) have done x-ray diffraction[2] in myoglobin at four different temperatures, from 220 K to 300 K. For x-ray scattering, the mean square displacement is in first approximation given by the sum of the contributions from conformational

substates, vibration, lattice disorder, and rotation and translation of the entire protein. The data suggest that rotation and translation can be neglected. The contribution from lattice disorder can be estimated by comparing x-ray and Mossbauer values for the iron atom in myoglobin. Fritz Parak in Munich had already measured the Mossbauer effect on myoglobin crystals so that Frauenfelder, Petsko and Tsernoglou could then obtain the sum of conformational and vibrational mean square displacement for all 1261 nonhydrogen atoms of myoglobin. If these displacements were entirely due to vibrations, they should be small, typically less than 0.02 Å^2, and vary linearly with absolute temperature. The observed displacements, however, behave very differently. Some atoms, particularly those close to the pocket where the oxygen binds to the heme iron, have very small displacements that increase more rapidly than expected with increasing temperature. Many atoms on the outside show mean square displacements as large as 0.3 Å^2 that are temperature independent. Both of these types of displacement cannot be easily explained by vibrations and support the existence of conformational substates. Thus it appears that myoglobin resembles a solid at its center but behaves like a semi-liquid near the surface. Some atoms may move as much as a few angstroms and create channels large enough for oxygen to enter and leave the protein.

A related experiment, reported in the same issue of *Nature* as the paper by Frauenfelder, Petsko and Tsernoglou, has been done by P. J. Artymiuk, C. C. F. Blake, D. E. P. Grace, S. J. Oatley, David C. Phillips and M. J. E. Sternberg (Oxford University). They studied[3] two varieties of the enzyme, lysozyme, whose structure was solved by Phillips in 1964.

Phillips and his collaborators studied the motion parameters of both hen-egg and human lysozyme and found that the patterns of atomic displacements derived from independent crystallographic refinement are "broadly similar." They believe "it is reasonable to allocate some part of the observed displacement to molecular motion. However, there must be further investigation of methods, such as the analysis of the temperature dependence of the displacements, that may help in apportioning the relative contributions of static disorder and motion to the observed displacements."

NMR studies. Unlike the x-ray measurements just discussed, which give information on spatial variation, nmr measurements in solution provide information on temporal variation. One can study rate processes in the 10^{-8}–10^{-11}-sec range, such as tumbling of proteins in solution, rotational motion around single bonds and peripheral motion of aliphatic amino acids. For these studies, the popular technique is longitudinal spin relaxation.

Wüthrich and his collaborators at ETH have been doing studies[4] in the range 10^{-5}–1 sec (mostly in PTI). They observe internal aromatic ring flips by identifying an individual ring, measuring the rotation rate and determining the activation energy from the temperature dependence. By dissolving the protein in D_2O, they cause amide protons to be replaced by D in seconds or minutes. When the deuteron exchange occurs, a resonance disappears from the spectrum. The group also measured the temperature of the transition of the protein from globular to denatured form. Wüthrich told us one would like to know if there are any internal fluctuation modes (under physiological conditions) far from the transition temperature, and are the modes related to the folding/unfolding transition? His partial answer is that there is a correlation between amide proton exchange and the stability of the protein, but there are no correlations between ring mobility and denaturation.

At the University of Indiana, Frank R. N. Gurd and his collaborators have been doing nmr studies of myoglobin, interpreting the data in terms of motion, thus complementing the work of Frauenfelder and his collaborators. Unlike x-ray measurements, nmr is capable of seeing relatively rare events. Thus, Gurd and his collaborators observe[5] side-chain ring flips, which cause atoms to move about 2 Å. And he sees wobbling along the long axis of a component of roughly 2 Å.

In contrast, the largest displacement found by Frauenfelder and his collaborators is about 0.5 Å. The different values arise presumably because the nmr method is only sensitive to large fluctuations, whereas the x-ray method sees an average. Gurd compares the picture one gets from x rays as a view of an entire town on a foggy day and the picture one gets from nmr as a view of a few specific main streets on a clear day.

Now Michael Rothgeb, formerly at Indiana and now at Illinois, along with Eric Oldfield, is studying myoglobin in the crystalline state, rather than the usual water solution. He puts deuterium markers inside the protein and is finding motional behavior inside. Using the deuterium nmr technique in a solid, motions on a time scale of 10^{-5}–10^{-11} sec may be studied. Rothgeb and Oldfield very recently reported at a conference in England that because of the large magnetic susceptibility of the myoglobin molecule, they can produce microcrystals that are essentially perfectly ordered by intense magnetic fields (85 kG). Even though myoglobin is in powdered form, because of this magnetic ordering, the experimenters can obtain pseudo single-crystal nmr spectra and thus infer the spatial organization of a variety of groups in the myoglobin and in principle other proteins. Once analysis of the data is complete, the results should be directly comparable with the x-ray crystallographic studies of Frauenfelder and his collaborators.

Molecular-dynamics calculations. At Harvard, Karplus and his collaborators have been doing molecular-dynamics calculations on proteins. In this approach one solves simultaneously the classical equations of motion for all the atoms of the molecule for a suitable time period and then one analyzes the atomic trajectories. Karplus had done such a calculation for molecules with three or four atoms as long as 16 years ago. So the concept was available but he didn't feel it was worth attempting large molecules until the calculation could be checked against experiment. The ring-flipping experiments on PTI encouraged Karplus to try to explain it. He, J. Andrew McCammon and Bruce R. Gelin published[6] such a calculation for PTI. An empirical potential function for the interaction between all of the heavy atoms in the protein is used. The energy of the protein is expressed as the sum of the bond stretching terms, the angle-bending terms, the torsional angle terms (all for groups bonded together), and terms for groups that are not bonded—Lennard-Jones terms, hydrogen bonds and electrostatic terms. The parameters of the various terms are obtained from experimental data for small peptides.

From the energy function the force on each atom is found by differentiation. They integrate the classical equations of motion and adjust the kinetic energies of all the protein atoms over a period of 20–30 picosec so as to equilibrate the system at a given temperature. After the system has been equilibrated, they integrate for an additional period of 20–100 picosec to obtain the magnitude and time development of the fluctuations. The group's 1977 paper gives a picture of what the temperature factors would be if they arose entirely from fluctuations. Their calculation gives exact short-time results for an approximate protein model, which does not have the molecule inside a crystal. The experiments, on the other hand, deal with real protein molecules in crystals.

Very recently Karplus and Sundaramoorthi Swaminathan (Harvard) have done a molecular-dynamic calculation for myoglobin. They find approximately the same atom displacements as those found by Frauenfelder, Petsko and Tsernoglou. Karplus and Swaminathan find a mean square displacement for backbone atoms of about 0.3 Å^2. Frauenfelder and his collaborators find about 0.45 Å^2, which they then correct by subtracting about 0.2 Å^2 for lattice disorder in the crystal. Now Petsko and collaborators are planning to use the Harvard calculated mean square

displacements in the x-ray refinement to see how much the results differ from those obtained when the mean square displacements derived from x-ray diffraction are used. —GBL

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"Double quasar" could be gravity effect

Earlier this year observations of a close pair of visible astronomical sources previously identified with a quasar candidate from low-resolution radio data appeared to indicate that the pair is actually a single object doubled by the effect of a "gravitational lens." More recent radio observations make some difficulties for the hypothesis, but do not necessarily rule it out. The exact nature of the object(s) is thus still uncertain.

The original observations were part of a survey to identify optical counterparts of quasar candidates found with the Jodrell Bank (University of Manchester) radio telescope. One of the objects, 0957 + 561, in Ursa Major, was most probably associated with a close pair of blue stellar objects. To confirm the identification, Dennis Walsh (Jodrell Bank), Robert F. Carswell (Cambridge University) and Ray J. Weymann (University of Arizona) measured[1] the spectra of the two sources using the 2.1-m telescope at Kitt Peak and the 2.3-m University of Arizona telescope in March. Not only do the two objects both have spectra characteristic of quasars, the two spectra are substantially the same: Key emission and absorption lines appear in both spectra with similar intensities and widths, and the red shifts inferred from the spectra are the same to within the experimental uncertainties of a few hundred kilometers per second.

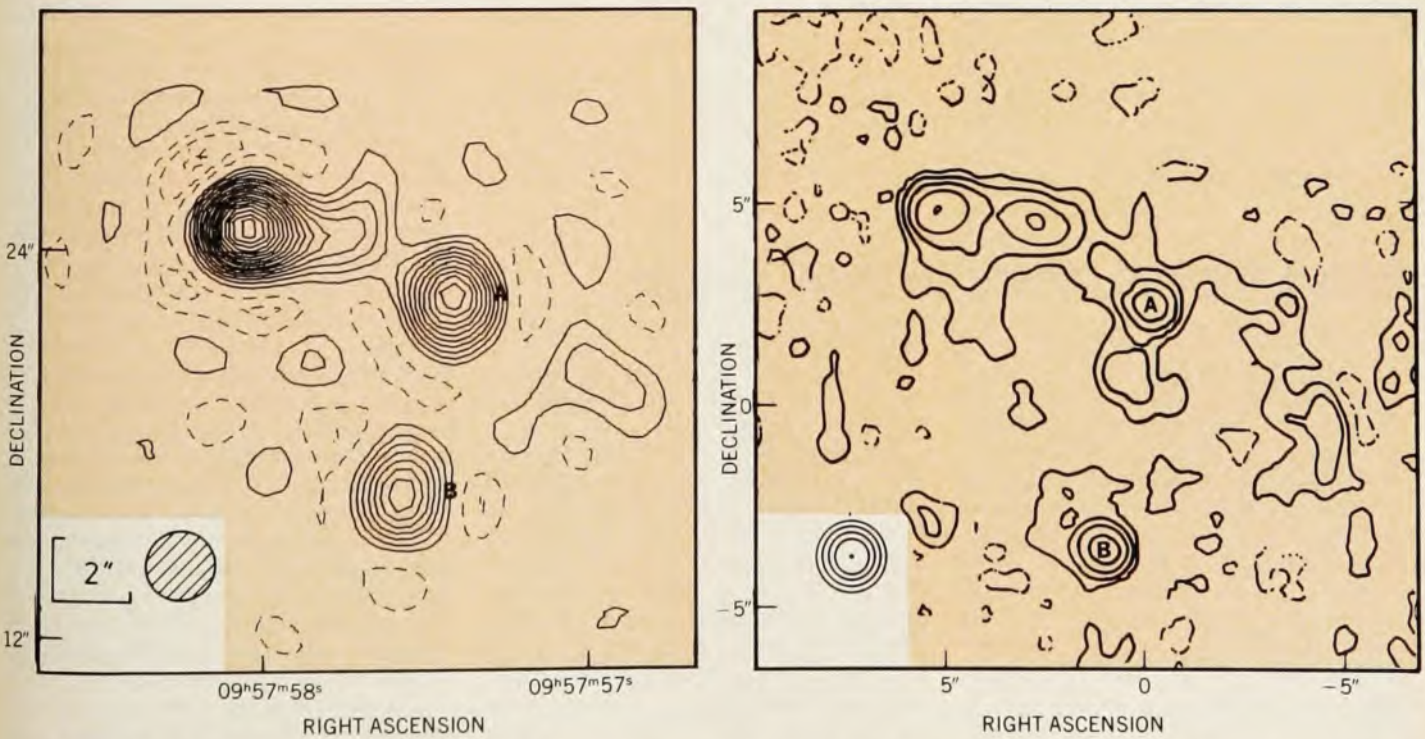
At this time Weymann was also helping with the testing of the Multiple Mirror Telescope on nearby Mt. Hopkins, and QSO 0957 + 561 seemed a suitable test object. Conditions were not entirely ideal—the MMT active optics were not implemented, for example, so the secondary mirrors had to be aligned by hand.

Weymann, Walsh and Carswell, together with their MMT colleagues Frederic Chaffee Jr, Marc Davis, and Nathaniel Carleton, discuss the spectra, which are among the first scientific results from the MMT, in a letter to be published in the *Astrophysical Journal*.[2] The observations confirm the remarkable similarity of the two spectra and show a striking coincidence of the two red shifts—they are still the same to within the significantly smaller experimental errors (15 km/sec—compared with overall red shifts of 10^5 km/sec).

There are differences in the two objects, however. The northerly of the two (designated A) is brighter and somewhat bluer by about 0.3 magnitude. But the difference in color, for example, can be due to different absorption along the two light paths rather than by differences in the sources.

Gravity lens. The spectra of quasars are generally very different, so that having two quasars not only close together but also with very similar spectra seemed too much of a coincidence. Accordingly, Walsh, Carswell and Weymann suggested that they might be dealing with a single object. A double image could be explained if there were a single, massive, and relatively dark object near the line of sight that acts as a "gravity lens" to bend the light from the source. Depending on the shape of the lens and its position relative to the object, one can see the original object magnified, split into two or turned into a ring. One can get a similar effect from the base of a wine glass, Carleton told us. (One has to break the bowl off to see the full range of effects.) The effect of the lens could account for the observed magnitude difference between A and B.

The idea that gravitational fields can act as lenses by bending light is as old as general relativity. In the 1930's the late



Radio maps of the twin quasar 0957 + 561 at 6 cm. The sources labelled A and B are those that have tentatively been identified with the optical sources. On the left is the map obtained with the Cambridge 5-km telescope in May; that on the right is derived from observations made in June with the Very Large Array in New Mexico, whose effective aperture is near 40 km. The two insets show the image of a pure point source.