

Properly carried out, high-resolution X-ray diffraction data collection followed by careful least-squares refinement can give the spatial distribution of the high-frequency mean-square displacements in a protein. These displacements reflect both individual atomic fluctuations in hard variables (bond lengths and bond angles) and collective motions involving soft variables (torsion angles, nonbonded interactions). Blundell and associates have undertaken precisely this study on avian pancreatic polypeptide, measuring data beyond 1 Å resolution and refining the structure by small-molecule procedures, including full anisotropic thermal ellipsoids in the model. Lower frequency, large amplitude motions and rapid but improbable motions are not quantifiable, but they may lead to such complete disorder that their existence can at least be inferred from the absence of interpretable electron density for some sections of the structure. Significant reduction in on cooling establishes that proteins are mobile even in the crystalline state, and that static disorder is not the dominant contributor to the individual mean square displacements. The only published procedure for restrained anisotropic protein refinement implements restraints based on bond directions²⁹; this approach is invalidated by the result from molecular dynamics that collective motions destroy such a correlation. Unfortunately, refinement of unrestrained anisotropic thermal ellipsoids requires six parameters for each atom instead of a single isotropic B parameter, and even 1.5 Å resolution data do not provide enough overdeterminacy. Certainly some small, well-diffracting proteins should be amenable to many of the sophisticated small-molecule analyses, as they yield X-ray data to resolutions comparable to simple organic structures.⁸⁹ X-Ray diffraction can provide atomic-resolution information about the initial and final states of a process such as ligand binding or triggered conformational change, but the pathway is much more difficult to unravel. Interior residues are more rigid than groups on the surface, and structural constraints are reflected in restricted motion even for surface residues. Disordered regions in electron density maps are no longer automatically taken as signs of errors in structure determination. Ringe has shown that a bulky ligand can be used to prevent the return of a channel to its resting state after binding, allowing the residues that move to form the channel to be identified. The most important type of analysis that awaits is anisotropic B factor refinement, which would give the principal directions of motion added to the .amplitude information now obtained