enable more robust virtual screening and lead optimization[10] during drug design by allowing explicit incorporation of conformational heterogeneity and uncertainty into the screening and optimization technologies. Analysis of ensembles may provide insights into why a particular lead is not binding with the expected specificity or how the spatial limits might be defined in fragment-based lead identification and optimization. In sum, we hope that our discussion of the many benefits of representing crystallographic structures by an ensemble of conformations will encourage its adoption within the crystallographic community.

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Berman, Henrick, Nakamura & Arnold respond:

The Worldwide Protein Data Bank (WWPDB; www.wwpdb.org) maintains a comprehensive archive of macromolecular structural data representing the foundations of our molecular understanding of the processes of life. At the end of 2005, the PDB contained three-dimensional coordinates for nearly 35,000 structures determined experimentally by crystallography, NMR and cryo-EM. A confluence of advances in molecular biology, protein chemistry, genomics and proteomics, as well as in X-ray and NMR technology, has led to an explosion of the number of structures available. Improvements in the quality of experimental data collection, structure solution and structure-refinement techniques and a generally enhanced understanding of biological structure and dynamics have led to an increasing appreciation that macromolecules exist in a continuum of conformations in the crystalline state as well as in solution.

The WWPDB welcomes the suggestion by Furnham *et al.* of improving the representation of uncertainty and heterogeneity in crystallographic data by the deposition of ensemble structures. The description of structural heterogeneity is an issue that the annotation staff of the WWPDB deals with on a daily basis. Examples of heterogeneity in the PDB archive range from localized disorder associated with multiple conformations of side chains or of bound ligands to ensembles of complete protein models.

The WWPDB is committed to providing accurate representations of crystallographic structural data, including associated uncertainties, and to delivering these data in a form that is accessible to existing software tools. As the complexity of the representation of structural data increases, software compatibility becomes a larger issue. We agree that at the level of deposition, only minor procedural modifications would be required. Users of the PDB already handle NMR ensembles through software specific to such model sets. Analogous new options are required for the PDB to make crystallographic ensembles available for different purposes. The WWPDB staff has been considering modes by which depositors of crystallographic ensembles could designate a 'best' model, as Furnham *et al.* describe, that either is the one fitting best to the experimental data or is a single structure representing the composite or consensus properties of the ensemble.

In our experience, there is considerable diversity in the ways that crystallographic, simulation and visualization applications handle multiple models. For example, downstream software applications need to take into account that for some crystallographic model sets, all atoms must be considered in structure factor and electron density map calculations (and that there may be connected subsets of partially occupied portions of the structure), whereas for other sets each model is independent and has its own associated refinement statistics. Domain analysis software could use the first or 'best' model, whereas active site analysis, docking procedures, molecular simulations and other modeling procedures would need to process all relevant atom sets in turn for a thorough analysis. We encourage software developers producing and using structural data to work together to arrive at a consensus approach for handling multi-model data. The WWPDB will enthusiastically contribute to this activity, rapidly adopt any resulting standard and coordinate with crystallographers, software developers, end users and the International Union of Crystallography in considering and developing optimal implementations for the scientific community. In this way, we will continue to be able to most accurately represent a molecular view of the structure and dynamics of living cells, and the PDB will continue to be a central resource for the efficient design of new drugs, vaccines and biomaterials for diverse applications in medicine and life sciences.

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