on adjacent helices A and D (Fig. 1B). Homologous residues in a similar structural setting are proposed for GM-CSF and other cytokines that also bind to receptors of the hematopoietic supergroup (*18*).

There remain other computational approaches to gauging the correctness of the structure with existing coordinates (*12*, *19*). However, there now appears to be sufficient evidence to merit a reappraisal of the IL-2 x-ray fold (*3*). As in other cases (*20*), the utility of the new model lies in simulating further experiments and a full refinement of the IL-2 structure.

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Response: In answer to the comment by Bazan, we have completed a refinement of a molecular model of human recombinant interleukin-2 (IL-2) in which Cys^{125} is replaced with alanine ([Ala^{125}]IL-2) (*1*). The x-ray crystallographic data have a resolution of 2.5 Å and were collected by diffractometer on triclinic crystals, as previously reported (*2*). We used the program FRAGLE (DuPont version 6.6dup) (*3*), which allows one to replace manually built segments of a model with molecular fragments of refined protein structures, to build models of the two independent molecules in the unit cell. We used the program XPLOR (*4*) to refine molecular models with simulated annealing and restrained least-squares positional refinement. Phase combination between phases computed from a molecular model and "solvent-flattened" phases (that is, model-independent phases computed from a solvent-flattened multiple isomorphous replacement map) was done as previously described (*5*).

The following evidence supports the correctness of our current model. (i) The refinement statistics are consistent with a correct structure. (ii) The folding topology is consistent with that of GM-CSF (*6*) and IL-4 (*7*). (iii) Residues that are widely separated in the primary sequence of mouse IL-2 have been identified by exhaustive substitution mutagenesis with binding to either the p55 or the p70 receptor subunit. In our current model, these residues map to two contiguous patches on the surface of the human [Ala^{125}]IL-2 structure (*8*).

Historically, methods such as chemical modification in crystals have been used to identify specific amino acids or amino acid types, which serve as "brass tacks" to pin down the sequence of a protein model into a relatively noisy map (*9*). In the case of IL-2, Cys^{125} was identified correctly (*2*) as a site at which mercurials bind in crystals of IL-2, but do not bind in crystals of [Ala^{125}]IL-2. Also, crystals were iodinated in an attempt to identify tyrosines and place the reminder of the sequence in register in the maps. The major site of iodination in the crystal was assumed incorrectly to correspond to the

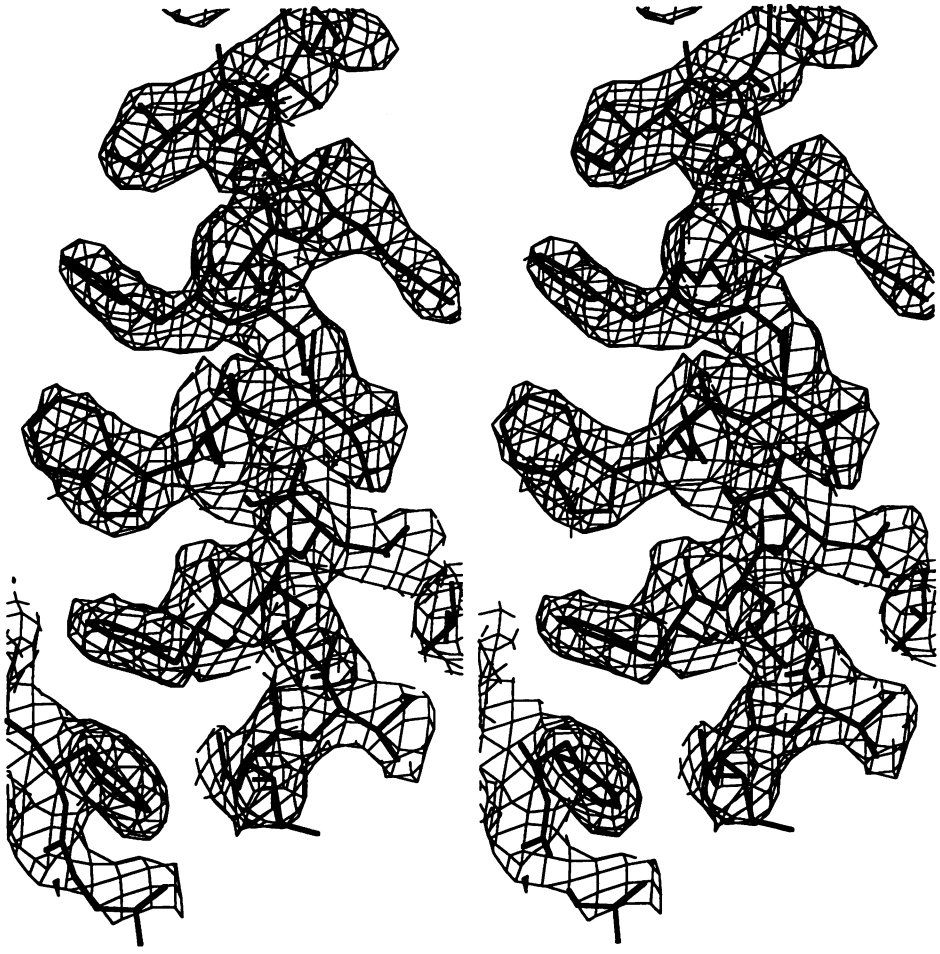

Fig. 1. Stereogram of a portion of the final $(2F_o - F_c)\exp(i\alpha)$ map; phases computed as described in (*1*).

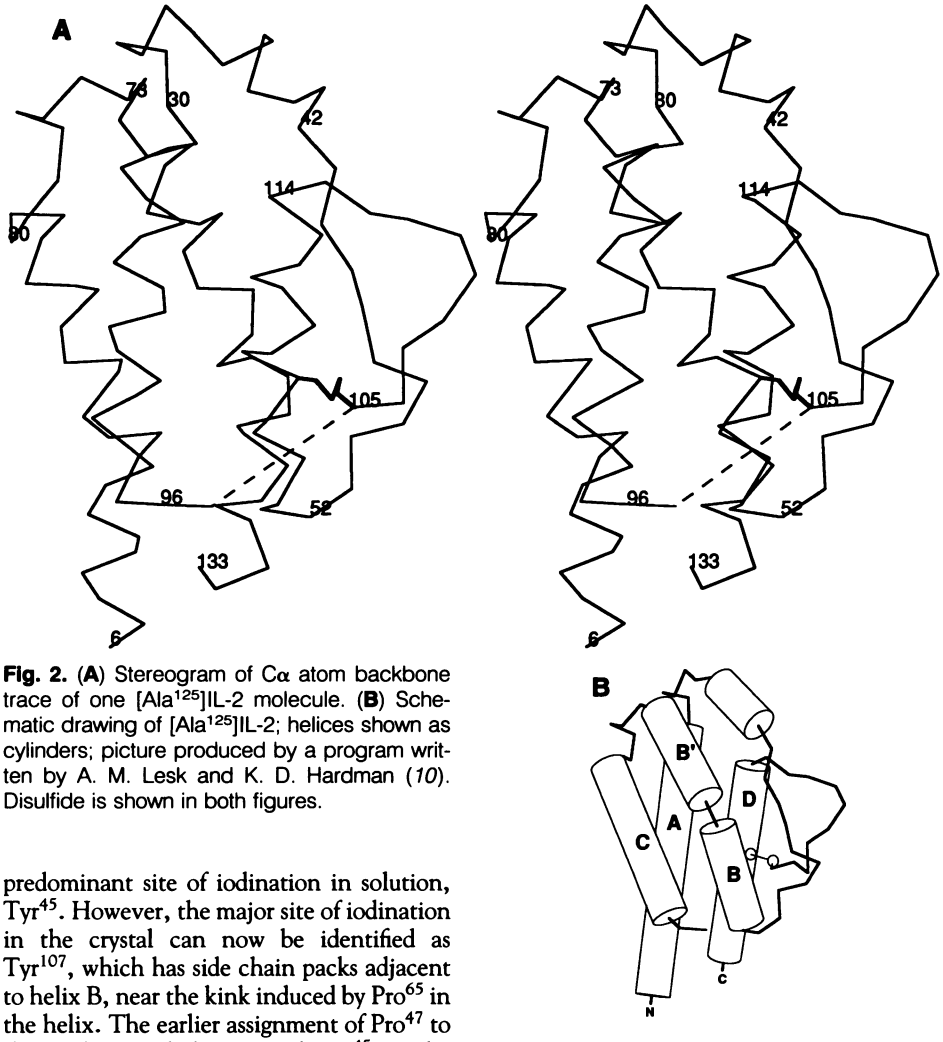

Fig. 2. (**A**) Stereogram of Cα atom backbone trace of one [Ala^{125}]IL-2 molecule. (**B**) Schematic drawing of [Ala^{125}]IL-2; helices shown as cylinders; picture produced by a program written by A. M. Lesk and K. D. Hardman (*10*). Disulfide is shown in both figures.

predominant site of iodination in solution, Tyr^{45}. However, the major site of iodination in the crystal can now be identified as Tyr^{107}, which has side chain packs adjacent to helix B, near the kink induced by Pro^{65} in the helix. The earlier assignment of Pro^{47} to the proline in helix B, and Tyr^{45} to the residue whose side chain was adjacent to the iodine site, led to our misinterpretation of the connectivity of the IL-2 molecule.

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1. D. B. McKay *et al.*, in preparation; Refinement was initiated with a minimal molecular model. In the first cycle, the model included the α-helices of the four-helix bundle for each molecule, as well as an additional short helical segment for one of the molecules, for a total of 189 amino acid residues out of a possible 266; no connections were built between helices. The human IL-2 sequence was incorporated for the 15-residue COOH-terminal helix of each molecule, in which the position of Ala^{125}/Cys^{125} had been unambiguously identified in (*2*). For the remainder of the model, side chains that appeared to fill the density of the solvent-flattened map were used without reference to the IL-2 sequence. After one cycle of refinement and phase combination, it became clear in the resulting phase-combined $2F_o - F_c$ maps that the connection leading into the COOH-terminal helix had been incorrectly traced in (*2*). Correcting this error allowed us to build a model of an additional 26 residues. After the second cycle of refinement and phase combination, the placement of the IL-2 sequence in the model became apparent. The first two cycles of refinement included 5982 reflections between 8.0 and 2.7 Å resolution. For the map calculation, solvent-flattened phases were used between 40.0 and 6.0 Å, combined phases between 6.0 and 3.0 Å, and model phases between 3.0 and 2.7 Å. Once the placement of the sequence was clear, the resolution was extended to 2.5 Å and included 7275 reflections. Solvent-flattened phases were used between 40.0 and 6.0 Å, combined phases between 6.0 and 3.5 Å, and model phases between 3.5 and 2.5 Å (Fig. 1). Subsequent cycles of refinement have yielded models that include residues 6 to 97 and 105 to 133 for each of the two molecules in the unit cell. The final *R* factor was 0.202, with root-mean-square (rms) deviations from ideal geometry of 0.21 Å for bonds and 4.0° for angles. Essentially all of the peptide dihedral angles lie within allowed regions on a Ramachandran diagram. The first five amino acids could not be resolved. Also, although there is density in the maps that generally appears to connect residue 97 to residue 105 in both molecules, we could not trace a peptide with an unambiguous conformation for this connection at any stage of the refinement; hence residues 98 to 104 have been omitted in the final model. No solvent molecules have been incorporated. Throughout the model-building and refinement, the two molecules in the unit cell were built independently. After refinement, when the atoms of the polypeptide backbone of one molecule were superimposed on the second, the rms distance between corresponding atomic positions was 0.51 Å. Coordinates have been deposited in the Brookhaven Protein Data Bank.
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11. I thank K. Flaherty for assistance in the computations during refinement and G. Zurawski *et al.* (*8*) for permission to reference their data before publication. IL-2 and [Ala^{125}]IL-2 protein on which crystallographic data were collected was originally provided by Amgen Biologicals (Thousand Oaks, CA), as acknowledged previously (*2*).

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Response

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