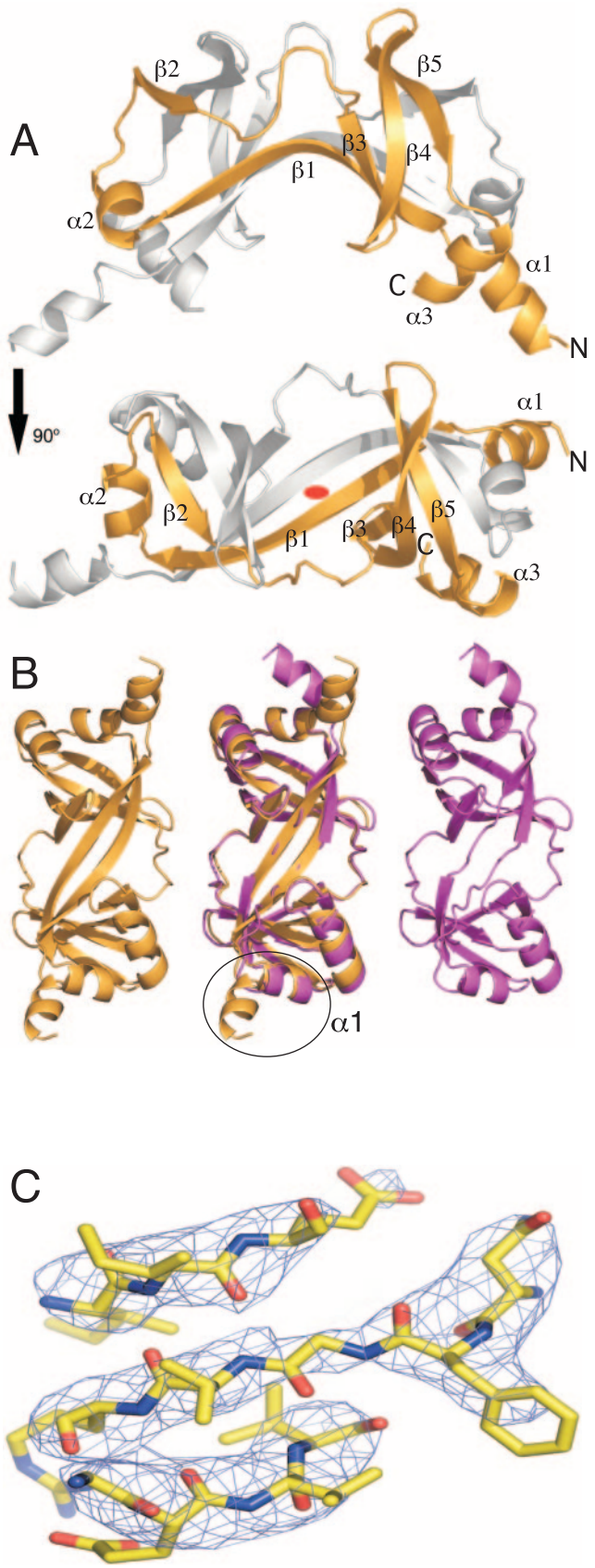

FIG. 3. (A) Two views of the FliN dimer, with one chain gold and the other white. Beta strands are labeled β1 to β5, and alpha helices are labeled α1 to α3. The dimer twofold axis is vertical in the top diagram, and the view in the lower diagram is along the twofold axis, from the top of the dimer as viewed in the top diagram. (B) FliN structure solved in this study (left), 1O6A FliN structure (right), and overlay of the two structures (middle). The view is along the twofold dimer axis, but from the direction opposite that in the lower diagram in panel A. The major difference is in the position of helix α1, as indicated. (C) Simulated annealing omit map computed by using CNS (10), with the following residues omitted: residues 103 to 105 (top strand), 126 to 129 (bottom strand), and 134 to 138 (middle strand). NCS restraints were not applied during the simulated annealing refinement. The Fo − Fc map is contoured at 3.5× RMSD.

similarly purified and crystallized, and data were collected at three wavelengths near the selenium edge, at a resolution of 3.4 Å. Anomalous scattering from selenium was detectable, but the quality of the data was not sufficient to allow phase determination. Phases for the best 3.4-Å data set (Table 1) were eventually obtained by molecular replacement using coordinates for a *T. maritima* FliN structure determined by the Joint Center for Structural Genomics and released to the Protein Data Bank in November 2003 (accession code 1O6A). The FliN molecule is a dimer. Simulated-annealing omit maps gave interpretable electron density for residues 68 to 152 of chain A and residues 68 to 154 of chain B. Forty-five residues at the N terminus of each chain were not visible, presumably because they were less ordered. The structure was rebuilt and refined at a 3.4-Å resolution to an *R* factor (R_{free}) of 22.4% (28.6%) (Table 2), with good geometry. The resulting structure is very similar to the structure of 1O6A overall, with a root mean square deviation (RMSD) of 0.9 Å for main-chain atoms in the central portion of the molecule (residues 80 to 150). Like the present structure, 1O6A did not show clear electron density for parts N terminal of residue 68.

The FliN dimer is roughly the shape of a saddle, and the overall dimensions are 60 by 35 by 30 Å (Fig. 3). The two chains in the crystallographic asymmetric unit are similarly folded (RMSD for main-chain atoms, 0.4 Å) and are related by an approximate twofold axis. Each chain contains three α helices and five β strands. The two chains intertwine to form two β barrels near the middle of the molecule, each containing strands from both subunits. The subunits are held together by extensive β-strand interactions, chiefly between the β1 strands of the two chains and between β2 of one chain and β5 of the other. Each end of the molecule is capped by a group of three helices, α2 from one subunit and α1 and α3 from the other. Helices α2 and α3 are packed against each other and against the rest of the molecule. Helix α1 is directed away from the main body of the molecule and is positioned differently in our structure than in 1O6A, implying that its location is influenced by crystal packing forces (Fig. 3B).

Conserved hydrophobic residues are found in the buried core of the protein and also in a patch on the protein surface. Conservation of buried hydrophobic residues is not unusual and indicates that the various FliN homologs shown in Fig. 4 have similar folds. Several conserved hydrophobic side chains (primarily Leu85, Thr110, Val128, Val130, and Phe135) form a patch on the protein surface that is centered on the twofold axis (Fig. 5). These hydrophobic residues do not appear to have critical structural roles, and so their conservation might