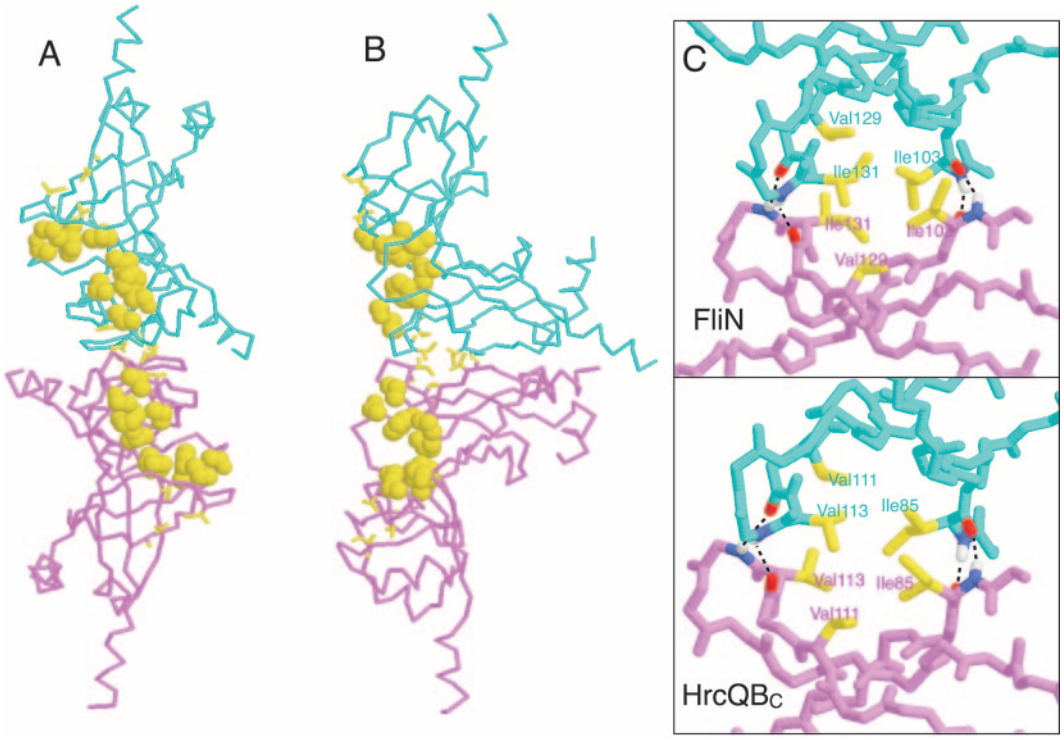

FIG. 9. Model of the FliN tetramer. (A) View along the twofold axis of the tetramer. The backbone of one dimer is turquoise, and the backbone of the other dimer is pink. Hydrophobic residues at the dimer-dimer interface (and also their dimer-related counterparts away from the interface) are yellow; residues of the hydrophobic patches are yellow and space filling. (B) View from a direction perpendicular to the tetramer twofold axis (from the right side of panel A). (C) Close-up of the dimer-dimer interface and comparison to the interface observed in the structure of the $HrcQB_C$ tetramer. The direction of view is similar to that in panel B. The yellow residues are conserved hydrophobic residues in both FliN and HrcQB. The dashed lines indicate hydrogen bonds between backbone atoms that are observed in the $HrcQB_C$ crystal structure and predicted in the FliN tetramer model.

tima FliN, corresponding to residues 82 and 83 in *E. coli* or *Salmonella*) and also in the neighborhood of the hydrophobic patch (positions 111, 127, and 128 in *T. maritima*) (see Fig. S3 in the supplemental material). One of the mutations near the hydrophobic patch (at position 111) appears to affect not only switching but also flagellar assembly, as shown by a reduction in the length of the flagellar hooks (43). The relative scarcity of motile but nonchemotactic mutants has been taken to indicate that FliN has only a small role in switching (27). Alternatively, FliN might play a critical role in switching, but with only a small part of the protein participating directly. The positions of the *che* mutations on the structure suggest that the dimer-dimer interface and the hydrophobic patch could have roles in switching. A site-directed mutation in the hydrophobic patch, described below, has a phenotype consistent with this.

In a study to identify important protonatable residues, Zhou et al. replaced several conserved acidic residues in FliN with alanine and found that none were critical for flagellar assembly or function. The positions mutated were positions 72, 76, 107, 112, 115, 127, 133, and 141 (*T. maritima* positions), most of which are on the sides of the saddle (the faces perpendicular to the hydrophobic patch) (see Fig. S3 in the supplemental material). Although none of the alanine replacements of acidic residues prevented FliN function, many of them resulted in an increase in the level of FliN needed for optimal swarming (82). This might indicate that these residues have a role in stabilizing the protein or facilitating its incorporation into the C ring. Glu127 is near the hydrophobic patch and might have other roles; replacement of this residue with lysine gives a nonchemotactic phenotype (27).

The surface hydrophobic patch is the most conspicuous feature of FliN. In the modeled FliN tetramer, the hydrophobic patches come together to form a ca. 50-Å-long hydrophobic cleft. This hydrophobic surface feature appears to have a function specific to FliN, because it is much smaller on $HrcQB_C$, which is otherwise fairly similar. Sequence alignments show that nonpolar character is well conserved in the residues that form the hydrophobic patch on FliN, and a Val-to-Asp mutation in the patch affected both motor switching (at 30°C) and flagellar assembly (at 38°C), so that swarming was prevented (Fig. 11). We cannot yet assign a particular function to the hydrophobic patch. The reduced flagellation and the CCW motor bias might indicate that FliN functions in both flagellar assembly and CW-CCW switching. While the switching defect is most dramatic, the hydrophobic patch is unlikely to function exclusively in switching because the residues that form it are conserved as hydrophobic residues even in *Aquifex aeolicus* and *Buchnera*, organisms that lack CheY and other proteins of the chemotactic signaling pathway (11, 64).

In the *fliN* mutant characterized by Vogler et al. (75), a temperature-sensitive defect in assembly was shown to result from a block in flagellar export. Further characterization of