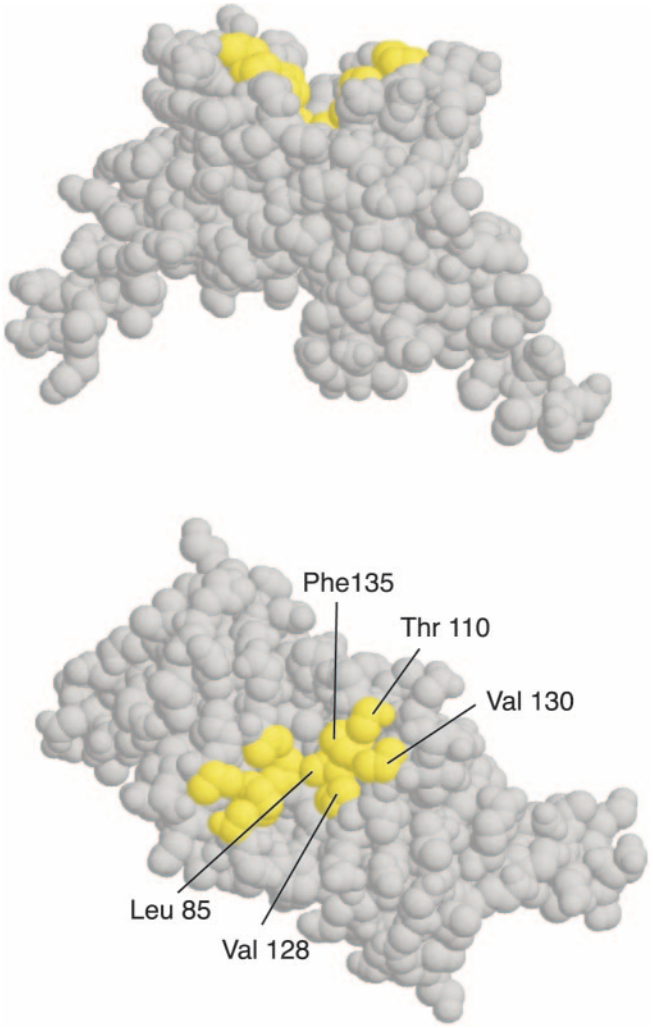

FIG. 5. Two surface representations of the FliN dimer, with the surface hydrophobic patch highlighted in yellow. Residues contributing to the patch are indicated. The views are similar to those in Fig. 3, except that they are rotated by approximately 20° about the twofold axis to reveal the concave shape of the hydrophobic patch (in the upper diagram).

8). The FliN/FliM ratio estimated in this way was 3.2:1. A similar experiment in which silver staining was used gave a FliN/FliM ratio of 4.2:1 (data not shown). The measured FliN/FliM ratio is thus consistent with the subunit composition $FliM_1$-$FliN_4$ and rules out the composition $FliM_2$-$FliN_2$.

The FliM-FliN complex behaved like a single species in velocity-sedimentation experiments, with a sedimentation coefficient of 5.3 S (Fig. 7). When we used the mass determined by sedimentation equilibrium and hydration like that assumed for isolated FliN, the calculated shape factor was ~1.1, which indicates that the shape of the FliM-FliN complex is less eccentric than the shape of FliN alone.

Comparison to HrcQB: a model for the FliN tetramer. HrcQB is a paralog of FliN that functions in the type III secretion apparatus of the phytopathogen *Pseudomonas syringae* (3, 16, 26). HrcQB and FliN show significant sequence similarity (see Fig. S1 in the supplemental material), and the recently reported crystal structure of the C-terminal domain of HrcQB ($HrcQB_C$) has a fold very similar to that of FliN (16) (PDB accession code 109Y). Like FliN, the subunits of $HrcQB_C$ intertwine to form dimers, but the $HrcQB_C$ dimers are further associated into tetramers in the crystal. The association between $HrcQB_C$ dimers buries more than 1,200 $Å^2$ of surface and is stabilized by hydrophobic interactions, hydrogen bonds between backbone segments in an antiparallel β-strand arrangement, and hydrogen bonds between side chains. The sedimentation experiments with FliN showed that it also forms tetramers, either by itself (the *E. coli* protein) or in a complex with FliM (the *T. maritima* proteins). The large shape factor of the FliN tetramer suggests that it has an elongated shape, as does the $HrcQB_C$ tetramer. The dimer-dimer interface in $HrcQB_C$ is formed in part from the hydrophobic residues Ile85, Val111, and Val113 (the residue numbers are the numbers for the full $HrcQB_C$ sequence; the numbers used in PDB entry 109Y are 44 lower). Hydrophobic character is conserved at the corresponding positions in FliN (Ile103, Val129, and Ile131 in *T. maritima* FliN) (Fig. 4). These correspondences suggest that the FliN tetramer may have a subunit arrangement similar to that of $HrcQB_C$. Accordingly, we used the $HrcQB_C$ structure as a guide in docking two FliN dimers together to form a model for the tetramer.

As noted above, the dimer-dimer interface in $HrcQB_C$ is stabilized by four hydrogen bonds between backbone atoms, as well as by hydrophobic interactions. The initial FliN tetramer model was constructed by manually aligning the dimers to bring together the backbone hydrogen bonding groups (Val130 O to Asp132′ N and Ile103 O to Ile103′ N, and their symmetry-related counterparts). The structure was then energy minimized by using a utility in Swiss-PDB viewer (22). Following energy minimization, the four backbone H bonds were retained, and more than 1,600 $Å^2$ of surface was buried, including the hydrophobic residues Ile103, Val129, and Ile131 (Fig. 9). The dimer-dimer interface of the energy-minimized structure was also stabilized by hydrogen bonds between the side chains of residues Glu105, Asp132, Arg138, and Glu105. The sequence alignment shows that these residues retain hydrogen bond donor or acceptor potential in most FliN proteins, and the corresponding residues in $HrcQB_C$ (Glu87, Glu114, and Gln120) contribute most of the interdimer hydrogen bonds in that molecule. Thus, while the details should be considered speculative, the model shows that the main features of the dimer-dimer interface in $HrcQB_C$ can be reproduced with FliN. The modeled FliN tetramer is elongated, and the approximate dimensions are 110 by 40 by 35 Å.

Functional importance of the hydrophobic patch. The hydrophobic patches on the dimers (Fig. 5) come together in the tetramer to form a hydrophobic cleft that is approximately 50 Å long (Fig. 10B). Although FliN and HrcQB have similar folds overall, there are clear differences in the segment that frames the hydrophobic patch (residues 107 to 114 in *T. maritima* FliN) (Fig. 10A). The conformation of this segment appears to be well determined by the crystal structures, because it is the same in our structure as in 1O6A, which involved different crystallization conditions and a different space group. The 107 to 114 segments in the FliN dimer are farther apart than the corresponding segments in $HrcQB_C$, and together with the more hydrophobic character of residues 110 and 130