Supporting Information

Nan et al. 10.1073/pnas.1219982110

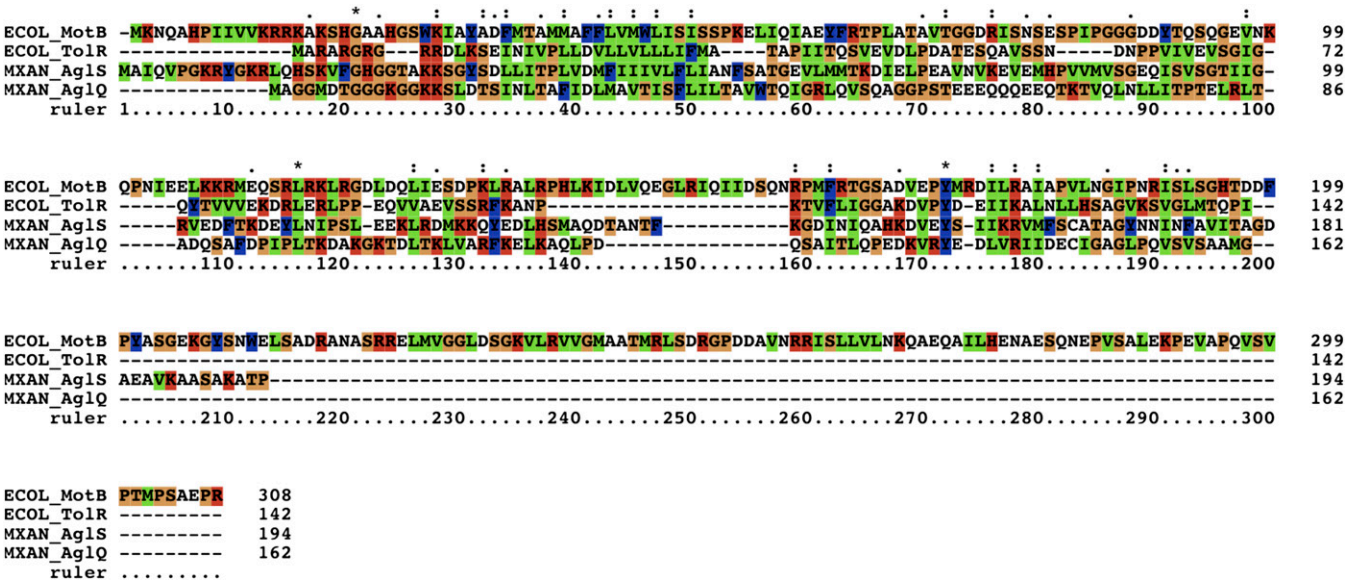


Fig. S1. Sequence alignment of *Myxococcus xanthus* MotB homologs AglS and AglQ with *Escherichia coli* MotB and TolR. Both AglS and AglQ lack the C-terminal peptidoglycan attachment motif of MotB, making the motor complexes free to move in the membrane.

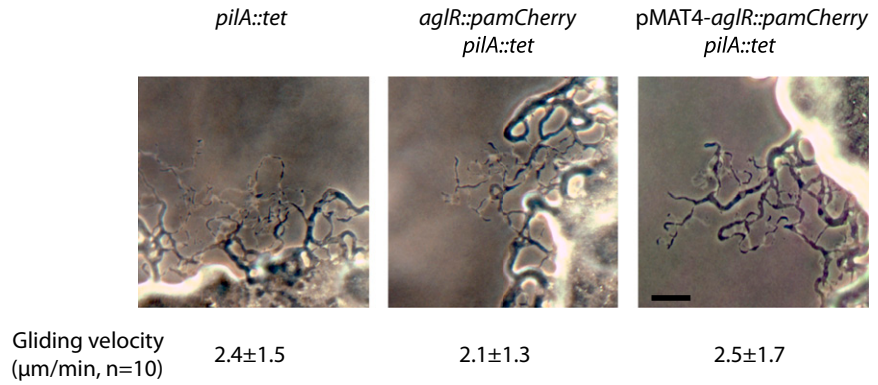


Fig. S2. Expressing AglR-pamCherry as the sole (*Center*) or an extra (*Right*) source of AglR does not cause any defect in gliding motility comparing to the wild type (*left*). *M. xanthus* cells were grown on casitone yeast extract (CYE) plates containing 1.5% (wt/vol) agar. To solely display gliding motility, the type IV pili powered twitching motility was eliminated by an insertion into the *pilA* gene, which encodes pilin, the building block of pilus. (Scale bar, 100 μm.)

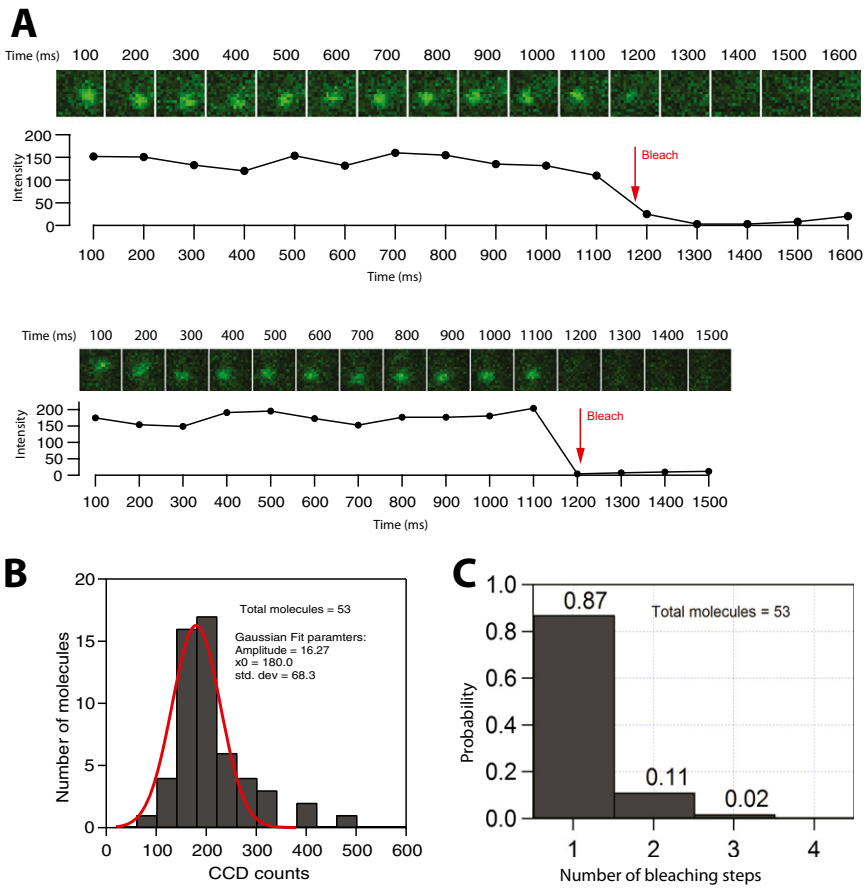
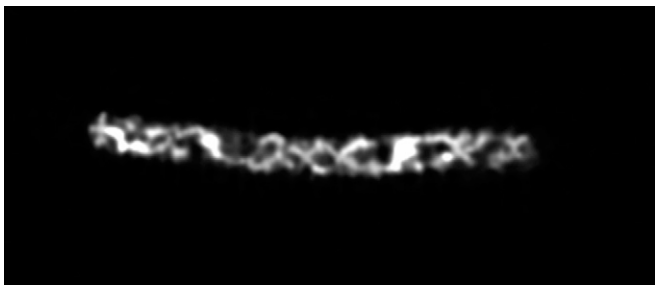


Fig. S3. Bleaching analysis confirms that most fluorescent spots tracked represented single molecules of AglR-pamCherry. (*A*) Two examples of the bleaching behavior of typical AglR-mCherry single molecules are shown. Each fluorescence spot was bleached in a one-step manner. (*B*) Gaussian fitting of the fluorescence intensity of 53 spots. (*C*) Among 53 spots analyzed, 46 (87%) spots were bleached in a one-step manner. Only six (11%) spots were bleached in two steps and one (2%) spot in three steps. The spots that required multiple steps of bleaching were excluded from tracking and data analysis because they usually showed colliding or splitting behaviors during the process of imaging.

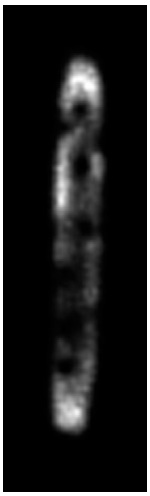


Movie S1. Z-stack images of two fixed cells expressing AglR-pamCherry (Fig. 2), obtained with structured illumination microscopy (SIM).

Movie S1

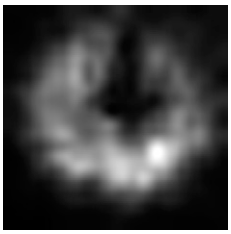
Movie S2. Z-stack images of two fixed cells expressing AglR-pamCherry (Fig. 2), obtained with SIM.

Movie S2



Movie S3. Lateral view of the rotational motion of AglR-pamCherry helix. To avoid possible artifacts caused by the unevenness of agar surface, *aglR::pamCherry pilA::tet* cells were suspended in 1% (wt/vol) methylcellulose solution. Images were captured in 2-s time resolution, and the image sequence was played with the speed of six frames per second (12 × real time).

Movie S3

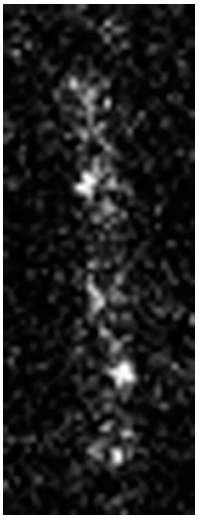


Movie S4. Polar view of the rotational motion of the AglR-pamCherry macrostructure when *aglR::pamCherry pilA::tet* cells are suspended in 1% (wt/vol) methylcellulose solution. The helix rotates 1,180° in 20 s, indicating a rotation speed of ~9.83 rpm. Images were taken at 2-s intervals and played with the speed of six frames per second (12 × real time). Movies of polar view were used to estimate the rotation speed of the AglR-pamCherry macrostructure.

Movie S4

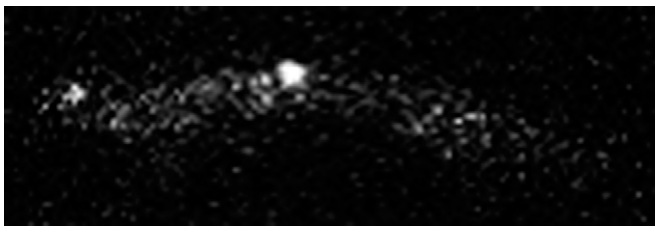
Movie S5. AglR-decorated macrostructure rotated as cells moved on 1.5% (wt/vol) agar surface. Images of a *aglR::pamCherry pilA::tet* cell were captured at 2-s intervals on the Olympus DeltaVision microscope with a Rhodamine filter. The movie was obtained by processing the series of images collected with QuickTime Pro software and played with the speed of six frames per second (12 × real time).

Movie S5



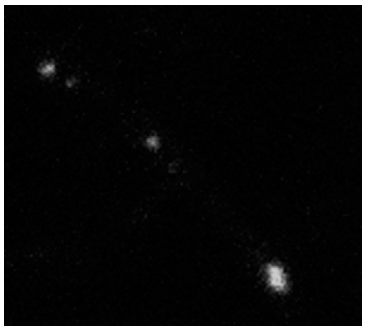
Movie S6. The typical behavior of AglR-pamCherry molecules observed by photoactivatable localization microscopy (PALM; Fig. 3*A*). Two molecules were imaged at 200-ms intervals in two different cells, and the movie was played with the speed of 10 frames per second (2 × real time). The trajectories of AglR molecules are typically projected into zigzag traces in 3D, suggesting a 3D rotational motion along helical trajectories.

Movie S6



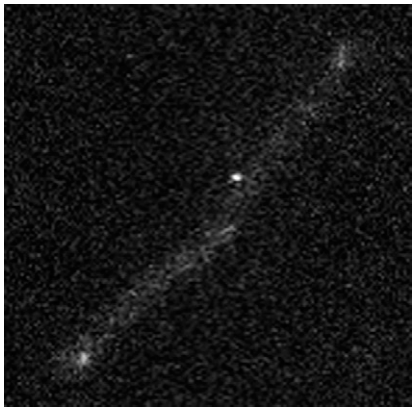
Movie S7. The typical behavior of AglR-pamCherry molecules observed by PALM (Fig. 3*A*). Two molecules were imaged at 200-ms intervals in two different cells, and the movie was played with the speed of 10 frames per second (2 × real time). The trajectories of AglR molecules are typically projected into zigzag traces in 3D, suggesting a 3D rotational motion along helical trajectories.

Movie S7



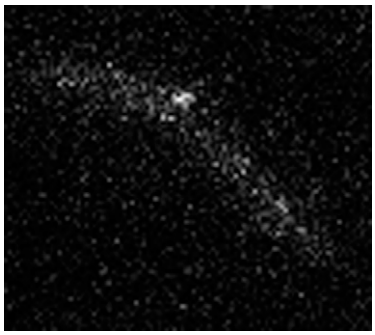
Movie S8. MXAN_6483, another *E. coli* MotA homolog in *M. xanthus*, does not show any rotational motion, indicating that the observed movement of AglR is a specific motility-related behavior. The MXAN_6483 molecule was imaged at 100-ms intervals, and the movie was played with the speed of 10 frames per second (real time).

Movie S8



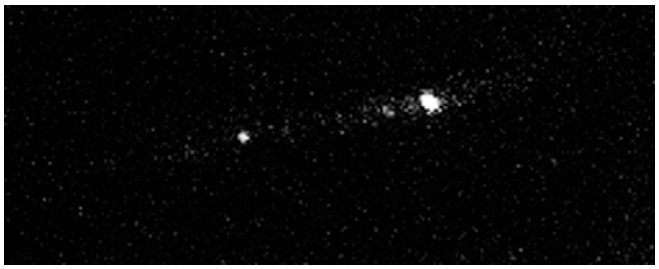
Movie S9. The typical behavior of AglR-pamCherry molecules observed by PALM, combined with total internal reflection fluorecence microscopy (Fig. 3*B*). Two molecules were imaged at 100-ms intervals in two different cells, and the movie was played with the speed of 10 frames per second (real time). In the movie, only half (180°) of the cylindrical cell surfaces were imaged, which allows fast imaging with high signal-to-noise ratio. Due to geometrical projection, AglR-pamCherry molecules usually display maximum V_{2D} at the centers of the projected cell surfaces and minimum V_{2D} at the cell borders.

Movie S9



Movie S10. The typical behavior of AglR-pamCherry molecules observed by PALM, combined with TIRFM (Fig. 3*B*). Two molecules were imaged at 100-ms intervals in two different cells, and the movie was played with the speed of 10 frames per second (real time). In the movie, only half (180°) of the cylindrical cell surfaces were imaged, which allows fast imaging with high signal-to-noise ratio. Due to geometrical projection, AglR-pamCherry molecules usually display maximum V_{2D} at the centers of the projected cell surfaces and minimum V_{2D} at the cell borders.

Movie S10

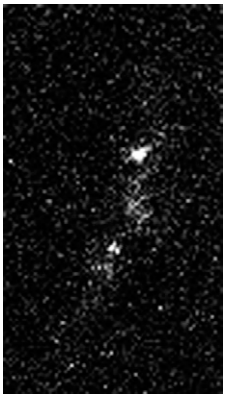


Movie S11. Two AglR-pamcherry molecules rotate in opposite directions in the same cell, suggesting that the rotation observed with PALM is not a result of the rotation of cell bodies (Fig. 3*C*). Two molecules were imaged at 100-ms intervals in each cell, and the movie was played with the speed of 10 frames per second (real time).

Movie S11

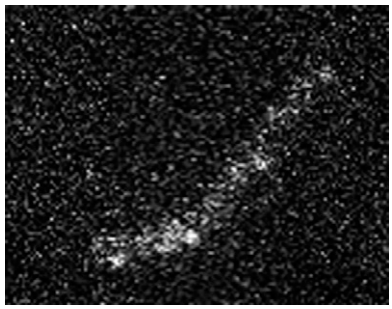
Movie S12. AglR molecules slow down near the center of cell surfaces, the sites cells contact substratum during gliding. An example is shown in this movie (also see Fig. 4*D*). The AglR molecule was imaged at 100-ms intervals, and the movie was played with the speed of 10 frames per second (real time).

Movie S12



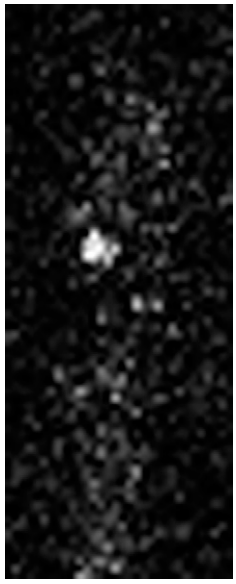
Movie S13. We embedded cells in 1.5% agar to mimic an extreme condition in which cells contact with gliding substratum with their whole surfaces. In this condition, less than 5% AglR molecules showed significant motion (>800 nm/s; Fig. 5 *B* and *C*), indicating that almost all of the motors slowed down. This result further proved our hypothesis that motors slow down at the cell–substratum contact sites. The AglR molecule was imaged at 100-ms intervals, and the movie was played with the speed of 10 frames per second (real time).

Movie S13



Movie S14. The abnormal behavior of AglR in the *agmU* deletion mutant (Fig. 7 *A–C*). AglR molecules move with much lower velocity, and many molecules were observed moving in linear trajectories with frequent pauses and reversals. The AglR molecule was imaged at 100-ms intervals, and the movie was played with the speed of 10 frames per second (real time).

Movie S14



Movie S15. The abnormal behavior of AglR in the *aglZ* deletion mutant (Fig. 7 *D–F*). AglR molecules move actively, with the maximum V_{2D} even faster than in the WT. However, AglR molecule movement seems to be undirected and slows down at random positions. The AglR molecule was imaged at 100-ms intervals, and the movie was played with the speed of 10 frames per second (real time).

Movie S15