Pigino et al., http://www.jcb.org/cgi/content/full/jcb.200905103/DC1

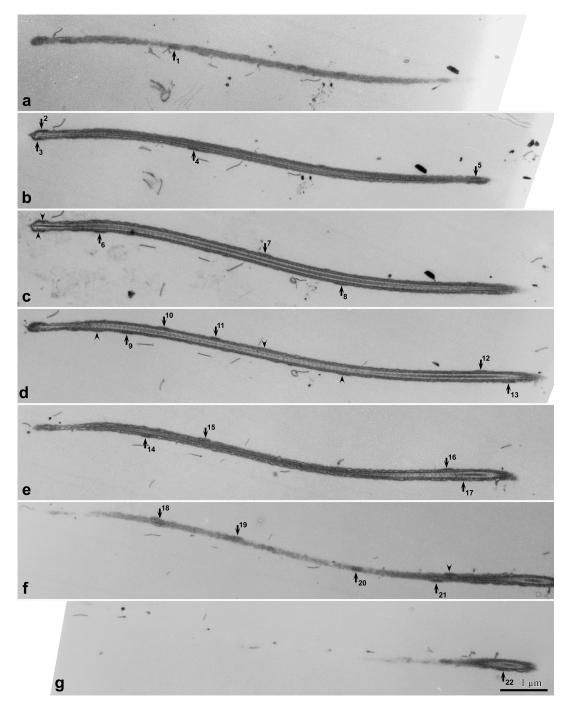


Figure S1. Analysis of the number of IFT trains per flagellum using serial section TEM. Consecutive longitudinal serial sections (a–g, section thickness of  $\sim$ 40 nm) show 10 µm of a flagellum (almost its entire length). IFT trains are labeled by arrows and numbered. IFT trains visible in two consecutive sections are labeled by an arrow in the first section they appear in and by an arrowhead in the following section (IFT trains 2, 3, 6, 7, 8, and 16). In this flagellum (cell 4, flagellum No. 1), 22 IFT trains were detected.

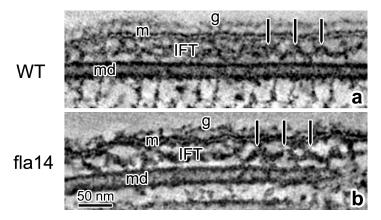


Figure S2. Anterograde IFT trains in WT and fla14 flagella are structurally similar. These longitudinal, virtual sections through a tomogram of a WT (a) or fla14 (b) flagellum illustrate the striking similarity in the wavy pattern (black lines) seen in long IFT trains in flagella of these two cell types. g, glycocalyx; m, flagellar membrane; md, microtubular doublets.



Figure S3. This schematic cross section of a *C. reinhardtii* flagellum shows the organization of an anterograde IFT train with its links to both the flagellar axoneme and membrane, and the hypothetical positions of its motors and cargo.

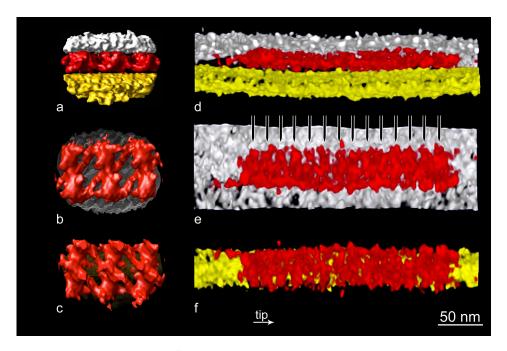
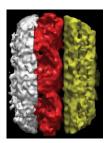


Figure S4. Anterograde and retrograde IFT trains have different 3D structures. Figs. 7 and 10 have been combined in this figure for a direct comparison of the 3D surface-rendering models of long (a–c) and short (d–f) IFT trains.



Video 1. "Virtual flight" along the z axis of a tomographic reconstruction obtained from a flagellum of the fla14 C. reinhardtii mutant. The total number of frames in the video is 150 and the frame rate is 30 frames per second. Fig. 5 a shows a view of the tomogram from this video.



Video 2. **Surface-rendering model of three anterograde IFT units from fla14.** The model has been reconstructed by electron tomography and 3D particle averaging. The total number of frames in the video is 612 and the frame rate of the final video is 25 frames per second. White, membrane; red, IFT particles; yellow, MT doublet. Fig. 7 shows views of the surface rendering from this video.

Table S1. Number of IFT trains per flagellum as determined by serial section standard EM

Cell	Flagellum No. 1	Flagellum No. 2
Cell No. 1	22 IFT trains	13 IFT trains
Cell No. 2	12 IFT trains	16 IFT trains
Cell No. 3	15 IFT trains	ND°
Cell No. 4	22 IFT trains	27 IFT trains

<sup>&</sup>quot;Not determined. The number of IFT trains could not be determined because the flagellum was not sectioned longitudinally, but showed a wavy configuration, going in and out of the sectioning plane.