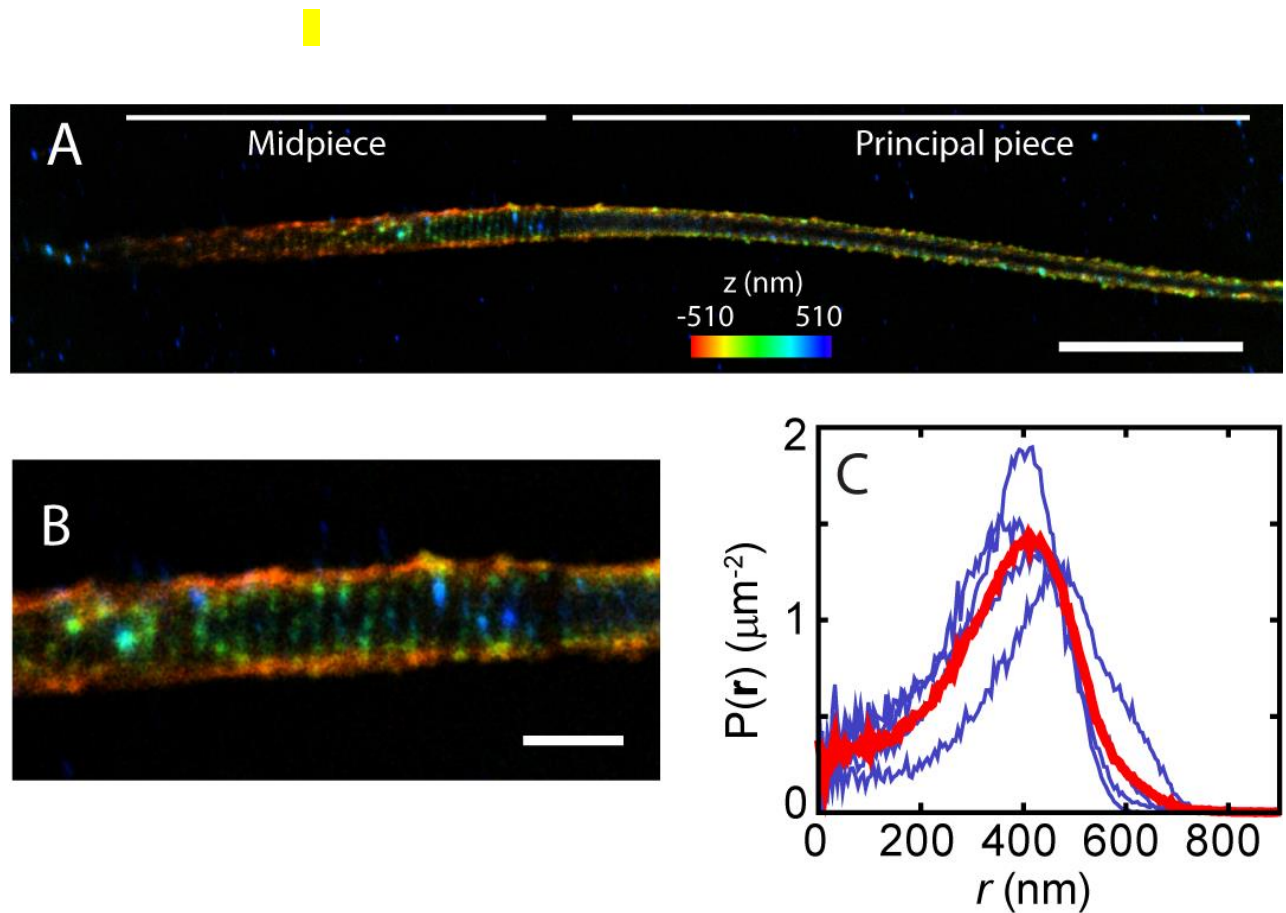
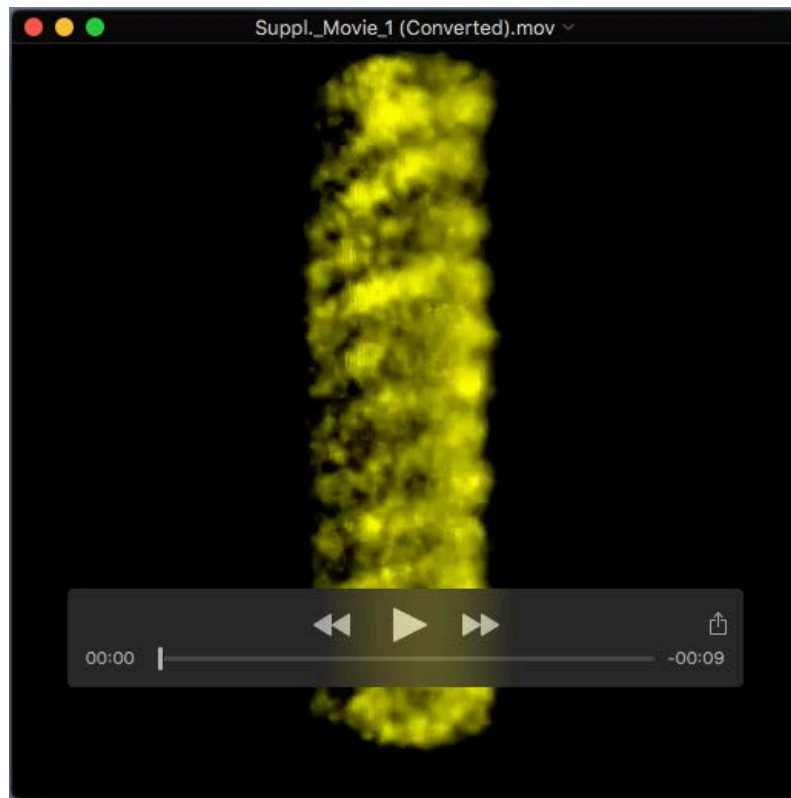


Supplemental Figure 1: Validation of antibodies by Western blotting. Sperm protein lysates were separated by SDS-PAGE and proteins were detected with the indicated specific antibodies. **(A)** Proteins were separated in 10 % gels, transfer to PVDF membranes and protein detection was done with anti-adducin specific antibody (cat. ab51130). Left panel corresponds to incubation with anti-adducin antibody alone, and right panel corresponds to incubation with anti-adducin antibody in the presence of 200 times excess of blocking peptide (BP). Membranes were stripped and sequentially probed with anti-tubulin antibody for loading control. **(B)** Proteins were separated in 7 % gels, transfer to PVDF membranes and protein detection was done with anti-spectrin specific antibody (cat. s-1515). Images are representative of three independent experiments.



Supplemental Figure 2: Superresolution localization of spectrin in the sperm midpiece obtained with an alternative antibody. (A) STORM reconstruction of spectrin in the sperm flagellum where the structure in both the midpiece and principal piece are observed. (B) Zoom of a section of the midpiece of panel A. (C) Radial distribution of spectrin localization in the midpiece. Blue lines are distributions from four individual cells and the thick red line is their average. The scale bar in panel A is 5 μm , and in panel B is 1 μm . Anti-spectrin antibody used was purchased from Sigma (cat. s-1390).



Movie 1: 3D STORM reconstruction of actin labeled with phalloidin-AF647 in the midpiece of the sperm flagellum.