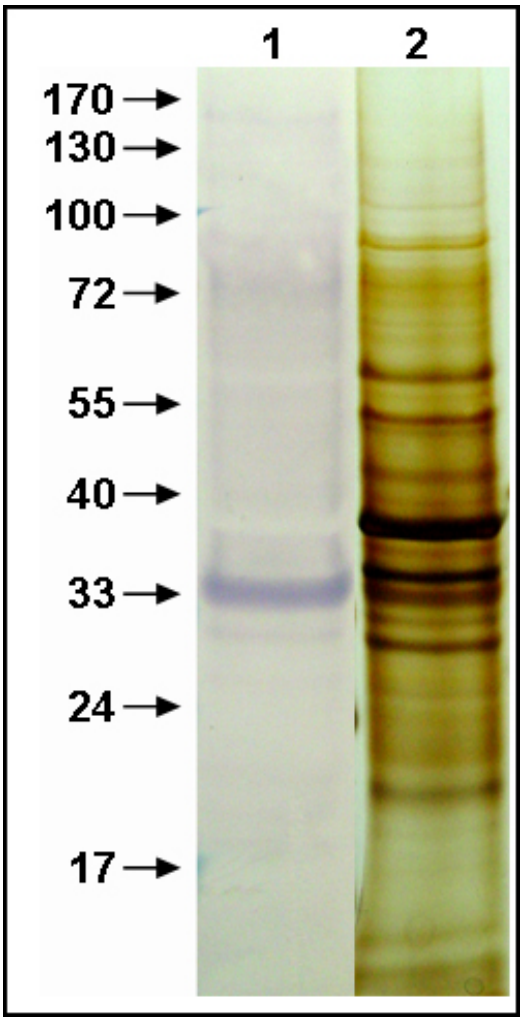

Figure S1: Immunolabeling of Iho670. Western blot (left) and conventional SDS-PAGE (10 % separation gel, silver staining) of a fiber containing fraction. The solution contained several other proteins as can be seen after silver staining. Immunological detection of Iho670 on the Western blot was performed with a 1° AB rabbit-α-Iho670 (dilution 1:500.000) and a 2° AB goat-α-rabbit (linked to alkaline phosphatase, dilution 1:25.000).

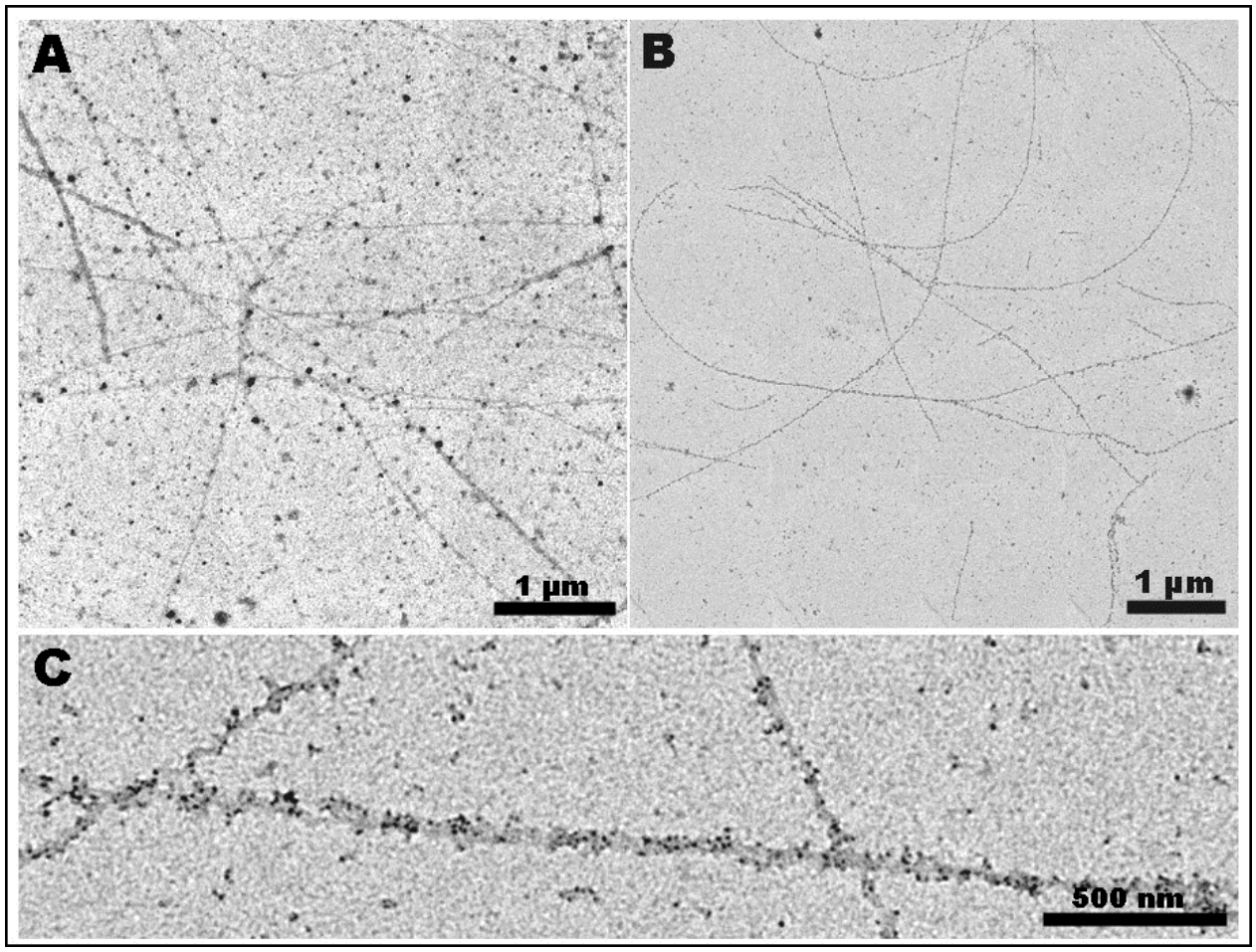

Figure S2: Immunolabeling of Iho670 on gold grids after heat denaturation. (A) Immunogold labeling of filaments from *I. hospitalis* cells grown on carbon-coated gold grids with 1° AB rabbit-α-Iho670 (dilution 1:100) and a 2° AB goat-α-rabbit + ultra small gold (dilution 1:50) including silver anhancement (6 min). (B) Immunogold labeling of filaments from cells grown on carbon-coated gold grids after a heat denaturation step (also see Meyer, 2010), 1° AB rabbit-α-Iho670 (dilution 1:10) and 2° AB goat-α-rabbit + 6 nm gold (dilution 1:5) (C) The selected area in (B) at higher magnification illustrates antibody binding over the whole filament. Negative staining. Scale bars: 1 µm (A, B), 500 nm (C).

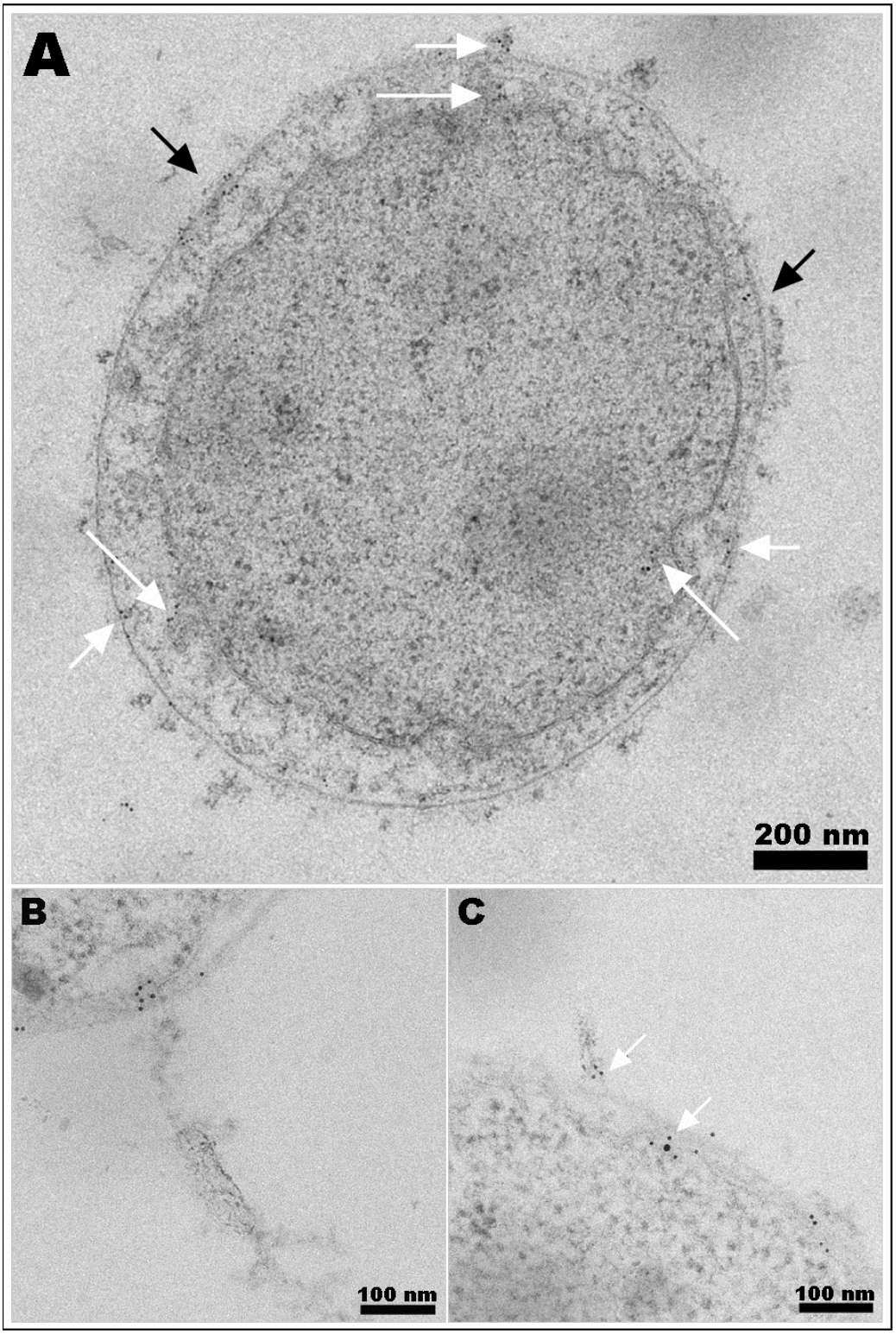

Figure S3: Immunogold localization of Iho668. Localization of the protein Iho668 on ultrathin sections of *I. hospitalis*. As a primary antibody, an antiserum rabbit-α-Iho668rek against the recombinant protein was used (dilution 1:50). The 2° AB consisted of goat-α-rabbit + 6 nm gold (dilution 1:10). In this experiment, Iho668 could be localized in both cellular membranes with a majority of gold particles and therefore proteins in the outermost cellular membrane. The crowding of gold particles at selected areas indicates clustering of the protein (white arrows), often at areas where filamentous structures which may represent fibers, pass both membranes (B, C). Scale bars: 200 nm (A), 100 nm (B, C).

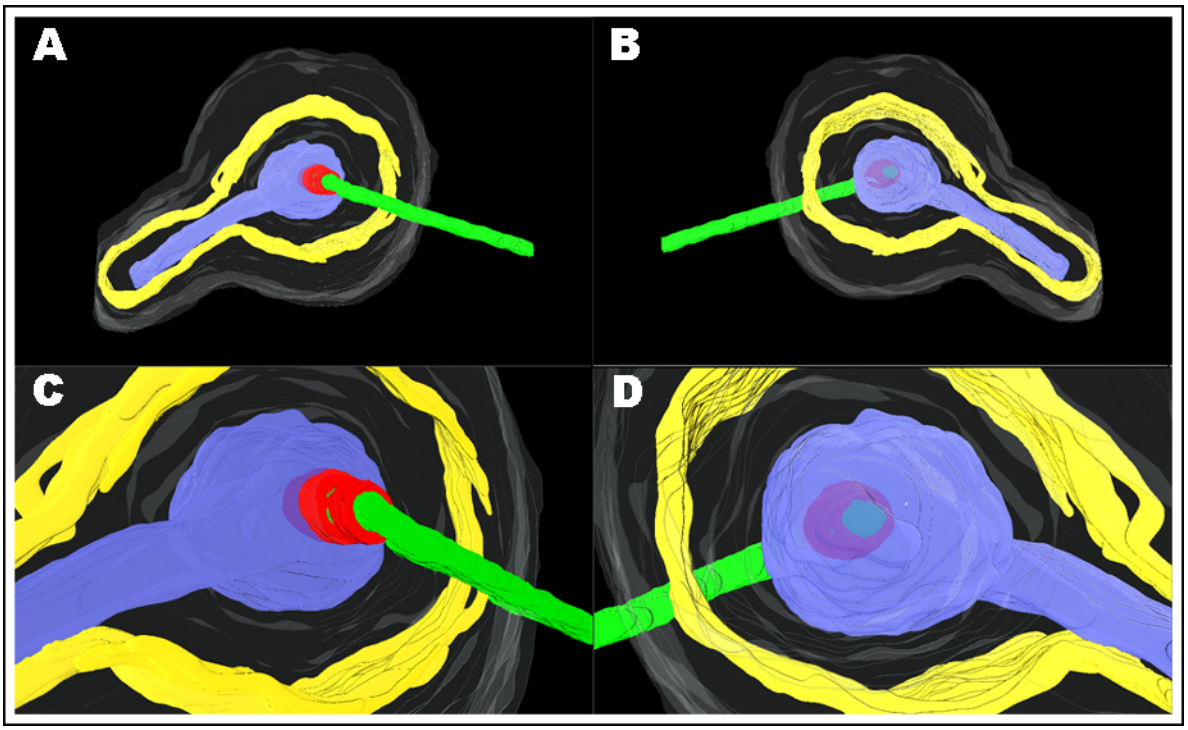

Figure S4: Model of a fiber-associated complex of *I. hospitalis* using Amira. Illustration of the arrangement in a fiber-associated complex by generating a SurfaceView model with Amira. (A+B) Bottom view, (C+D) top view. Blue, spherical structure; green, fiber; grey, outermost cellular membrane; red, tube encircling fiber; yellow, outline of cytoplasmic membrane.