buffer (10 mM Tris (pH 8.0), 100 mM NaCl, 1 mM DTT, supplemented with 0.01% (v/v) Tween-20 and 0.2 mg ml^{-1} BSA), injected in triplicate (50 μL per min, 20°C) and binding data were collected at 2 Hz during the 12-30 second association and dissociation phases (Fig. 4a). The biosensor studies were complicated by the tendency of CHMP1B to bind non-specifically to the sensor chip, but this problem was minimized by keeping the CHMP1B analyte concentration below 20 μM, by subtracting the responses from a reference surface with GST alone, and in some cases by limiting the CHMP1B contact time to 12 seconds. All interactions reached equilibrium rapidly and dissociated within seconds during the dissociation phase. Dissociation constants were obtained by fitting the equilibrium responses to 1:1 binding models.

ESCRT-III assembly reactions. We performed assembly reactions as described below, and the resulting complexes were analyzed by TEM with negative staining.

$IST1_{NTD}$ was diluted from concentrated stocks in high salt buffer (350 mM NaCl, 50 mM Tris pH 7, 5% (v/v) glycerol, 1mM DTT) into equivalent buffers that contained either 100 mM NaCl or 350 mM NaCl. Assembly at 23 °C was followed in real time by light scattering at 330 nm (Fig. 6a).

CHMP1B was assembled by dialyzing the protein for 12 h into 30 mM NaCl, 10 mM Tris, pH 8.0, 1 mM DTT at a final protein concentration of 40 μM (4 ºC).

We co-assembled pure recombinant monomeric CHMP2A and CHMP3 proteins by incubating mixtures of the two proteins at 23 ºC for 30 min. Assembly conditions

Nature Structural & Molecular Biology: doi:10.1038/nsmb.1621