

Figure 1. Assessment of FXII purity. (A) SDS-PAGE staining gel showing plasma FXII under non-reducing conditions (lane 1), under reducing conditions (lane 2), and FXIIa under reducing conditions (Lane 3). (B) Size exclusion chromatogram for 100 ug plasma FXII injected in 400 uL volume on a superdex 200 (10/300 GL) column. The small spikes indicate syringe pumps fillings. (C) SDS-PAGE staining gel showing recombinant FXII wildtype (Lane 1) and recombinant FXII S544A mutant (Lane 2) purified from transfected HEK293 cell cultures. L: Protein Ladder; mAU: milli-absorbance unit; KDa: Kilo-Dalton; and MPa: mega Pascal.

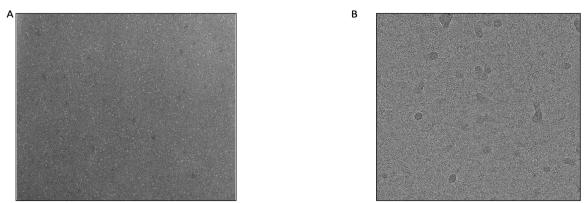


Figure 2. Representative micrographs for plasma FXII using electron microscope. (A) A representative negative stain micrograph for plasma FXII (0.003 mg/mL) taken using JEOL 1200 EX II 120 KV TEM (40K magnification). (B) A representative cryo-Em movie for plasma FXII (0.2 mg/mL) taken using 300 KV Krios titan Cryo-TEM.

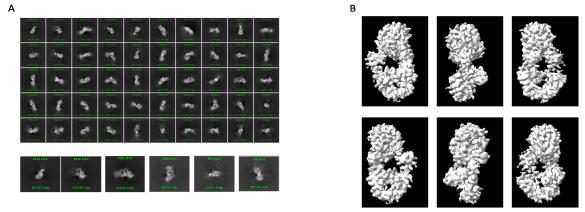


Figure 3. Single particle analysis of plasma FXII cryo-Em data. (A) Selected 2D classes for plasma FXII. Most 2D classes are glossy and lacking details (top panel) while few classes show better alignment of particles (bottom panel). (B) Initial plasma FXII 3D model. Presented are 6 views with 60° rotation along the y-axis. Plasma FXII was plunge frozen at 0.2 mg/mL and data was collected for 12 hours on Krios 300 KV Cryo-TEM. Collected movie frames were processed for single particle construction using cryoSPARC v3.2 to obtain initial model.