



Figure 1. Protein samples, binding assessment, and preliminary cryo-EM data of the liposome-assembled intrinsic Xase complex of coagulation factors VIII and IXa. (A) SDS-PAGE of non-reduced and reduced samples of FVIII and FIXa. FVIII represents a heterodimer of the heavy (A1-A2-B domains) and light chains (A3-C1-C2 domains). The B domain is heterogeneously processed by intracellular proteases, which, however, does not preclude single-particle cryo-EM analysis (see panel C). FIXa contains disulfide-bridged heavy (catalytic domain) and light chains (membrane-binding Gla-domain and EGF domains). (B) Fluorescent anisotropy changes of the active-site labeled FIXa upon addition of FVIII in the presence of liposomes (PC:PS 75:25) reveal a stoichiometric 1:1 binding with nanomolar affinity. (C) Cryo-EM structure of FVIII resolved in the presence of 200 μ M CHAPS. The 2D classes show a triangle geometry in the spatial organization of A1, A2, and A3 domains. The C1 and C2 domains represent ‘legs’ that bind to the membrane surface. (D) Preliminary cryo-EM screening of FVIII and liposomes in the presence of 200 μ M CHAPS. This image represents the sample of interest. (E) Previously resolved cryo-EM structure of liposome-bound factor Va, which serves as a cofactor protein in another coagulation complex, called prothrombinase.