



**Figure 1. The complex of factors IXa and VIII/VIIIa formed on phosphatidylserine-containing membranes.** (A) SDS-PAGE of FVIII, which is a heterodimer of heavy (A1-A2-B domains) and light chains (A3-C1-C2), and FIXa, which is a disulfide-bridged heavy (catalytic domain) and light chains (membrane-binding Gla-domain and EGF1/2 domains). (B) Fluorescent anisotropy changes of the active-site labeled FIXa upon addition of FVIII in the presence of vesicles (PC:PS 75:25) reveal 1:1 binding with nanomolar affinity. (C) Cryo-EM structure of FVIII at 200  $\mu$ M CHAPS. The 2D classes show a triangle geometry in the organization of A1, A2, A3 domains. The C1, C2 domains bind to the membrane. (D) Screening of UltrAuFoil grids with FIXa at 1.5  $\mu$ M (left) and FIXa-FVIII-vesicles at 5 $\mu$ M:0.3 $\mu$ M:0.1 $\mu$ M (right). The inset shows 2D classes of vesicle-bound FVIII with or without the density attributed to FIXa. (E) Screening of NiTi grids with 0.3 $\mu$ M FVIII activated by 0.06 $\mu$ M thrombin, alone (left) or in the presence of other components of the complex (right).