

Small angle x-ray scattering of the intrinsic tenase complex bound to lipid nanodisc provides insight into intermolecular contacts between factors VIIIa/IXa

Kenneth Childers¹

¹*Western Washington University*

childek2@wwu.edu

The intrinsic tenase (Xase) complex, formed by factor (f)VIIIa and fIXa, binds to platelet surfaces and catalyzes the activation of factor X, stimulating thrombin production in the blood coagulation cascade. Structural organization of the lipid-bound Xase complex remains largely unknown, hindering our understanding of the structural underpinnings that guide Xase complex assembly. Here, we aimed to characterize the Xase complex bound to a lipid nanodisc using bio-layer interferometry and small angle X-ray scattering. Using immobilized lipid nanodiscs, we measured binding rates and nanomolar affinities for Xase complex proteins. An ab initio molecular envelope of the nanodisc-bound Xase complex allowed us to computationally model fVIIIa and fIXa docked onto a flexible lipid membrane and identify protein-protein interactions. Our results highlight multiple points of contact between fVIIIa and fIXa, including a novel interaction at the fVIIIa A1-A3 domain interface. Our results also shed light on how binding to fIXa prevents complete dissociation of the fVIIIa A2 domain in solution. Lastly, we identified hemophilia A/B-related mutations with varying severities at the fVIIIa/fIXa interface that may regulate Xase complex assembly. Together, our results provide an updated model of the lipid-bound Xase complex and structural insight into mutations at the dimer interface that may disrupt or stabilize the enzyme complex.