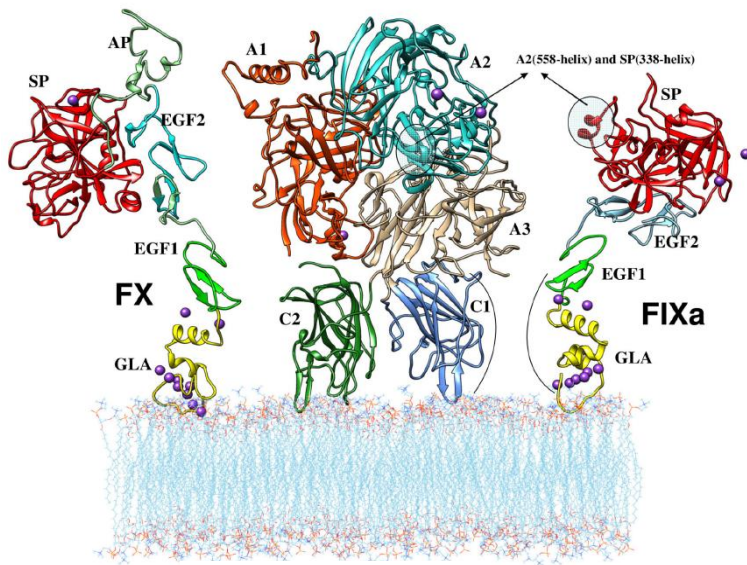


Unraveling Factor VIII and FIX activation

General Background

Coagulation Factor VIII (FVIII) and coagulation Factor IX (FIX) are essential plasma proteins of the clotting cascade.



From Venkateswarlu, D. 2014

Their deficiency or defectiveness lead to the bleeding disorders Hemophilia A (concerning FVIII) and B (in case of FIX). FVIII performs its role as a cofactor for activated factor IX (FIXa) in the so-called tenase complex during the proteolytic conversion of factor X (FX) into activated FX (FXa). FVIII in plasma circulates in a complex with its carrier protein von Willebrand factor (vWF), which protects FVIII from rapid clearance from the circulation. Proteolytic activation of FVIII into activated FVIII (FVIIIa) by thrombin results in the dissociation of the FVIII-vWF complex. FVIII activation, besides the release from vWF, implies conformational rearrangements that promote the exposure of FIXa and FX binding sites. Nonetheless, the affinity for procoagulant phosphatidylserine-

containing membranes increases upon activation.

Although major progresses in the biochemical characterization of FVIII and FIX have been made, the precise detail of its activation and the dynamic conformational changes assisting this transition are still largely unknown.

Aim

In this project we aim to unravel the dynamic rearrangements at the basis of FVIII and FIX activation with a quantitative mass spectrometry approach. The detailed understanding of FVIII and FIX activation will be of great importance for the treatment of the life-threatening bleeding disorders Hemophilia A and B.

Techniques

- Site-directed mutagenesis
- Culturing of human cell line
- Protein purification and characterization
- Kinetic assays
- Footprinting-Mass Spectrometry

Duration: At least 6 months. Master students interested in biochemistry and molecular biology who are looking for a challenging and interesting internship are encouraged to contact Nadia Freato. E-mail address: n.freato@sanquin.nl phone number: 020-5121286