General summary

Coagulation factor V (FV) is a 330 kDa multi-domain protein which circulates in plasma at a concentration of 20 nM. When coagulation is initiated upon vascular injury, FV is activated by proteolytic removal of the central B domain by thrombin or activated factor X (FXa) and divides into a heavy chain (domains A1-A2) and a light chain (domains A3-C1-C2), bound by calcium-mediated interaction. Activated FV (FVa) acts as a cofactor for the serine protease FXa and together the two form the prothrombinase complex. This complex is responsible for a key procoagulant reaction, namely activation of prothrombin to thrombin that in turn results in effective acceleration of the clotting event. The cofactor role of FVa entails interacting with the membrane through its C domains, thereby increasing the affinity of FXa to the membrane, as well as positioning FXa protease domain appropriately to activate prothrombin. Activated protein C (APC) is a serine protease that inactivates FVa by cleaving off the A2 domain rendering it unable to act as a procoagulant cofactor.

Haemophilia is a genetic disorder characterised by a malfunctioning intrinsic tenase complex, ultimately resulting in poor thrombin formation and a subsequent prolonged coagulation. Stabilisation or protection of FVa in the prothrombinase complex could potentially have a beneficial effect by increasing thrombin formation leading to enhanced blood clotting in haemophilia patients. To explore this hypothesis *in vitro,* anti-FVa monoclonal antibodies (mAbs) and corresponding antigen binding fragments (fabs) were previously developed, and screened for their stabilising effects in haemophilia plasma.

The aim of this thesis was to apply a subset of these anti-FVa mAbs and fabs in studying FVa structure and function. Five mAbs were selected and epitope mapped by Hydrogen/Deuterium Exchange - Mass Spectrometry (HDX-MS). In Chapter 4, the structure of a single-chain FVa variant with a reduced B domain, FV-810, was investigated with characterisation techniques including HDX-MS and Negative Stain - Electron Microscopy (NS-EM). The F29 fab, which has been mapped to the A1 domain, was used to identify the domains in the NS-EM micrographs. In Chapter 5, membrane binding of FVa was examined by Surface Plasmon Resonance (SPR) and HDX-MS. The inherent avidity-inducing effect of two mAbs was evident in SPR and functional assays, and its potential effect in HDX-MS experiment is discussed. In Chapter 6, stabilising properties of the five anti-FVa mAbs and fabs were investigated in different *in vitro* assays, including in haemophilia A plasma and a simplified assay mimicking the coagulation in the presence and absence of APC. The A1-binding F29 mAb/fab had a protective effect on an APC cleavage site. F31 mAb/fab binding to the A3 domain had a stabilising effect on the activation process of FV to FVa, while elusive function of the C2-binding mAbs/fabs, F24 and F9, is discussed.