In order to prevent excessive blood loss, the enzyme thrombin must be generated by the prothrombinase complex, a 1:1 stoichiometric complex of the serine protease factor Xa and its non-enzymatic cofactor factor Va assembled on the activated platelet surface. Two functionally-and structurally-distinct pools of Factor V, the procofactor of factor Va, exist in whole blood: the plasma-derived pool that is synthesized by the liver, and the platelet-derived pool, consisting of both an unactivated and activated cofactor. Data from our laboratory have shown that a non-dissociably-bound pool of platelet-derived factor Va is expressed on the surface of platelets activated with thrombin, and have suggested that it is bound through a GPI anchor at Ser^{692} of the heavy chain. This constitutes the first evidence of an endocytosed protein becoming GPI-anchored. The purpose of this project was to develop a protocol that would allow us to quantify the amount of GPI-anchored factor Va on the surface of thrombin-activated platelets. The GPI-anchored factor Va was detected by flow cytometry using a monoclonal α-factor V/Va heavy-chain antibody, biotinylated secondary antibody, and streptavidin conjugated to Alexa fluor 488. Due to the low binding affinity of the heavy-chain antibody that we used, we were unable to accurately quantify the amount of GPI-anchored factor Va on the surface; however, we have identified a new heavy chain antibody with a higher binding affinity that will be useful for this assay. We have also gained further insight into conditions to optimize the protocol, i.e. the appropriate platelet concentration for activation, conditions for platelet fixation, and inclusion of 1mM RGDS in all steps to prevent fibrin(ogen)-mediated platelet aggregation. By refining this protocol we will ultimately be able to quantify the expression of the GPI-anchored

factor Va on the surface of platelets over time, after activation with a variety of agonists.