Supercharging factor IXa for cryo-EM studies on the Xase complex

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Haematology comes in two favours: malignant and benign. Benign involves aspects related to the function and dysfunction of the blood itself, one of the most important of which is clotting (haemostasis). Too little clotting causes bleeding and too much or inappropriate clotting causes thrombosis. Although haemostasis is a mature field, exciting new discoveries are still being made and many fundamental issues are yet to be resolved. The advent of single particle cryo-electron microscopy makes it possible to address the fundamental mechanisms behind the regulation of haemostasis. My group focusses on the two homologous enzyme complexes at the end of the blood coagulation cascade, namely the intrinsic Xase ('tenase') and prothrombinase complexes. Each is composed of a serine protease and a large cofactor, and each complex has only single substrate. As the names suggest, Xase cleaves the zymogen factor (f) X to form the active protease fXa and prothrombinase cleaves the zymogen prothrombin to form thrombin. The components of the Xase complex were created through gene duplication events some 450 million years ago, followed by evolution of its new function. The importance of the Xase complex is illustrated by genetic deficiencies in either the cofactor fVIII or the protease fIX causing haemophilia A and B, respectively. Prothrombinase, composed of cofactor fVa and the protease fXa is the only enzyme that makes thrombin, so its importance is self-evident.

One of the important regulatory features of these two complexes is their dependence on 'activated' phospholipid membrane surfaces for assembly and function. This ensures that these enzyme complexes only form at sites of vascular damage. While this is a useful feature in limiting dissemination of blood coagulation, it does make structural biology studies a bit of a challenge. We overcame this challenge for prothrombinase by engineering a high-affinity variant of fXa that binds to fVa with a Kd in the nM range. This allowed us to solve the structures of prothrombinase in the absence of phospholipids with and without substrate, and this work is currently being completed. We plan to employ a similar approach to generate a high-affinity variant of fIXa to potentiate structural studies of the Xase complex. This work has only just begun and we have achieved a modest 10-fold increase in affinity. The rotation project would be to help design and create variants with nM affinity and to obtain preliminary maps of the Xase complex by cryo-EM. The PhD project would then continue to determine structures of the Xase complex with and without its substrate fX. The timing is excellent for a student interested in structural biology.