

Coagulation or Inflammation; how does FXII choose its path?

Introduction: Factor XII triggers blood coagulation when blood comes into contact with negatively charged (bio)materials, such as plastics or glass. The physiological role of this mechanism is still unknown, but it is thought that it can drive host defense responses during tissue damage and infections. Besides triggering blood coagulation, Factor XII is responsible for the generation of the inflammatory peptide bradykinin. This peptide is an important inducer of pain and, more importantly, of vascular permeability. From research and clinical pathology it is clear that Factor XII plays a lead role in the development of thrombosis, a disease in which blood clots obstruct the vasculature (Renné et al. *J. Exp. Med.* 2004, Müller et al. *Cell* 2009). Independently, Factor XII-dependent bradykinin formation causes dangerous drops in blood pressure (hypotension) during severe infections and is responsible for angioedema attacks, which are hallmarked by dangerous swelling episodes of the upper airways, skin or gastro-intestinal tract.

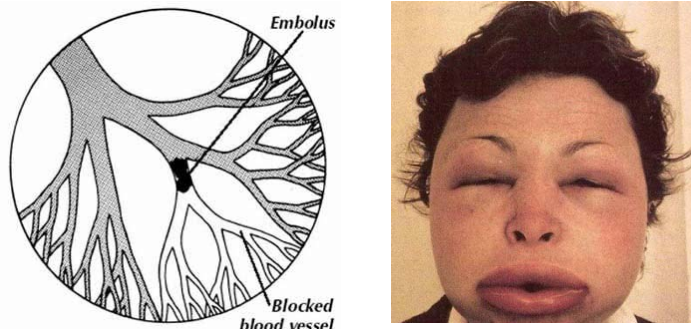


Figure 1. The two dangerous consequences of Factor XII activation. Thrombosis (left) and vascular leakage (right)

Several agonists are capable of activating Factor XII *in vivo*: some trigger both coagulation and inflammation, whereas others *only* trigger inflammation. At present, it is unclear by what mechanism Factor XII develops this apparent preference of each of its pathological pathways. This knowledge would be valuable for the successful targeting of activated Factor XII in diverse pathologies.

Hypothesis: It is currently thought that activated Factor XII is *one* enzyme with two diverging functions. The hypothesis of this research projects contradicts this model: there are two isoforms of Activated Factor XII, each with its own specific enzymatic activity. Identification of these isoforms will allow for the development of specific inhibitors against them, crippling the pathological roles of Factor XII in thrombosis and hypotension, respectively.

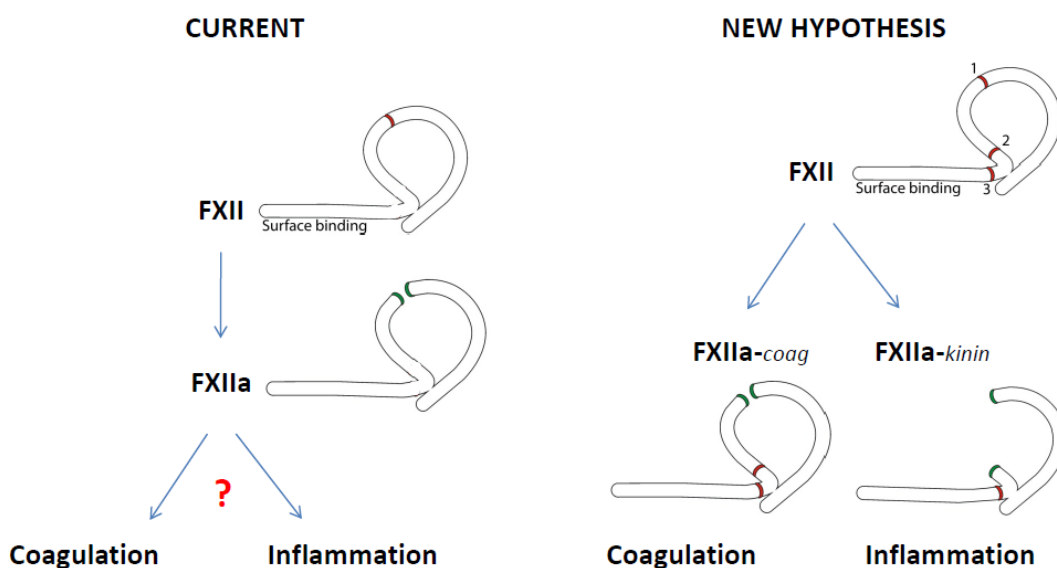
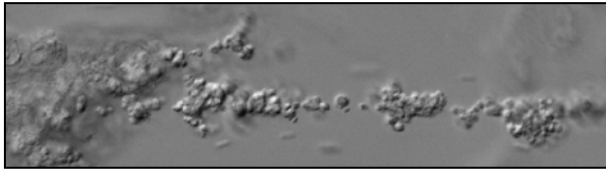


Figure 2. The old and new model for Factor XII activation. This new model takes into account that there are multiple cleavage sites within the protease domain, rather than one. Although the existence of these cleavage sites is commonly known, they have never been taken into functional consideration.

Aims:

1) To generate a set of mutants of factor XII. Several mutants of FXII will be prepared in which each of the cleavage sites (all argines) are inactivated (by replacement with alanines). These steps are performed in collaboration with the dept. of Crystallography at Utrecht University.



2) Characterization of these mutants in the context of clinical chemistry. What mutant(s) can support coagulation & thrombosis under flow (example left), and what mutant(s) can support activation of the kallikrein-kinin system?

3) Selection & generation of inhibiting nanobodies against the separate isoforms of activated Factor XII. Unlike other vertebrates, camelids produce antibodies with a single-chain variable region (VHH; see Figure 3). By immunizing Llama's with activated coagulation factors, we can generate VHH's against them. These isolated VHH's have the affinity of a conventional antibody, but only 10% of the size, allowing them to bind to epitopes which are unavailable to conventional antibodies. In preparation of this phase, Llama's have already been immunized with preparations of activated Factor XII and phage libraries will be ready for selections by the end of July 2011.

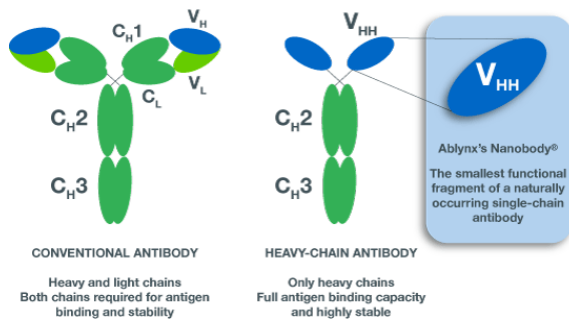


Figure 3. Nanobody generation.

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Techniques: Molecular Biology, Cell Culture, Recombinant Protein Expression, Clinical Chemistry, Real-time fluorescence microscopy, Surface Plasmon resonance analyses, Phage Display technology, Western Blotting, ELISA.

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