SUPPEMENTARY MATERIAL

# Noncanonical Proteolytic Activation of Human Prothrombin by Subtilisin from *Bacillus subtilis* may Shift the Procoagulant-Anticoagulant Equilibrium Toward Thrombosis

**Giulia Pontarollo[#], Laura Acquasaliente[#], Daniele Peterle, Ilaria Artusi and Vincenzo De Filippis**

From the Department of Pharmaceutical and Pharmacological Sciences, University of Padua, via Marzolo, 5, Padua, 35131 Italy

[#]These authors equally contributed to this work

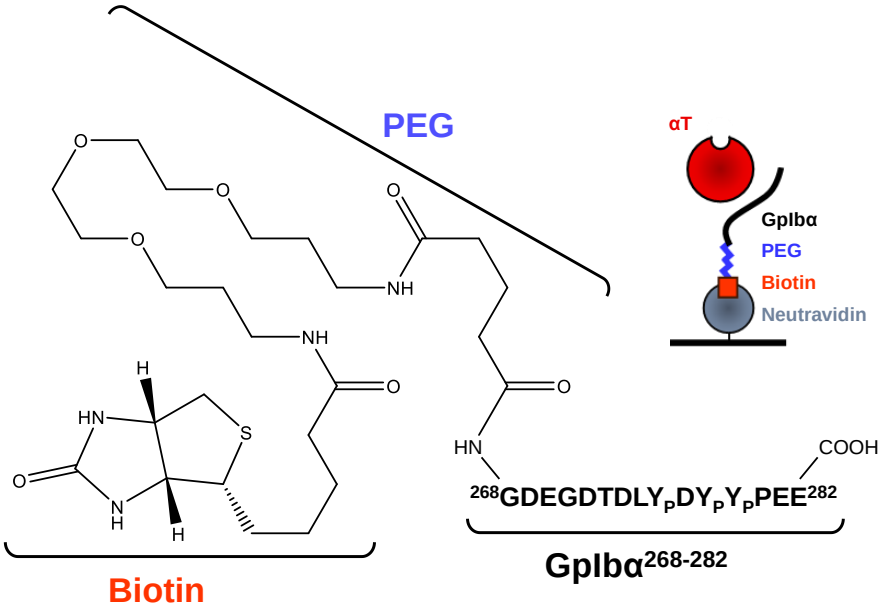


**Figure S1. Structure of the bivalent reagent biotinyl-PEG-[GpIbα(268-282)].**

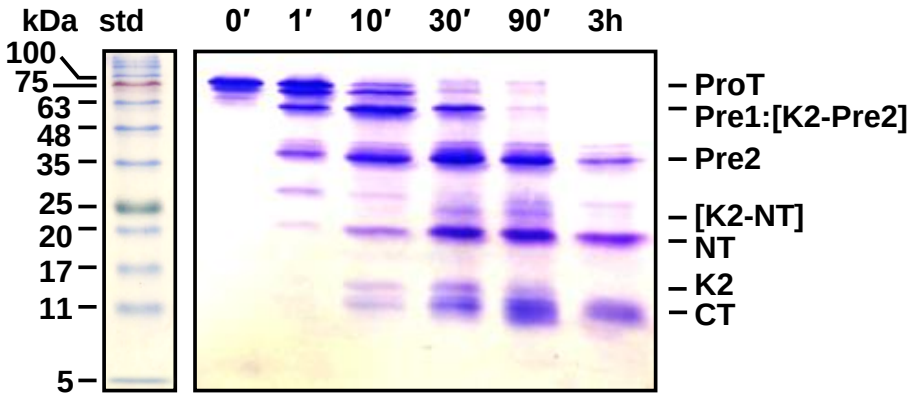


**Figure S2**. **Time course analysis of the proteolysis reaction of ProT by subtilisin.** Non-reducing electrophoretic analysis of the time-course reaction of ProT (0.1 mg/ml) with subtilisin (0.05 μg/ml) at 37°C. At time points, aliquots (100 μl, 10 μg) of the proteolysis mixtures were precipitated with cold trichloroacetic acid (TCA), analyzed by SDS-PAGE (4-14% acrylamide) and Coomassie stained. Std: molecular weight protein standards. The identity of the major bands are indicated: Pre1, Pre2, K2-NT, NT, K2, and CT. At variance with the SDS-PAGE analysis of Fig. 2A (nonreducing 12%-acrylamide), after 10-30 min reaction, the K2 and CT bands are resolved in the nonreducing 14%-acrylamide gel. At longer reaction times, the K2 band progressively disappears, due to further proteolysis, whereas CT remains stable in the time range explored.

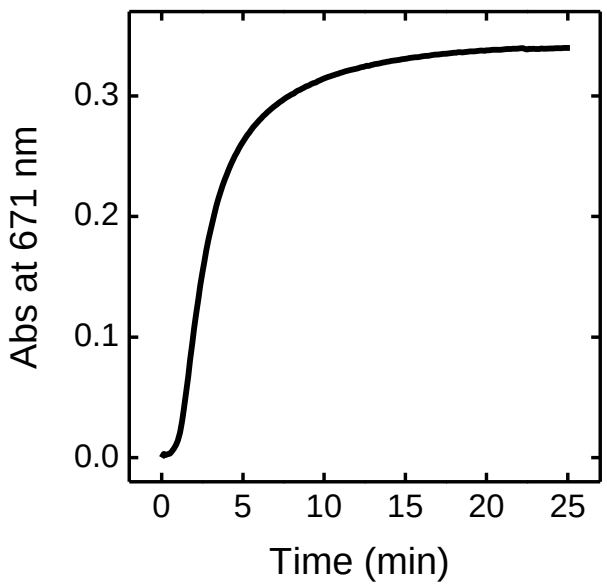


**Figure S3. Fibrin generation induced by ecarin.** The turbidimetric analysis was performed on 1:2 diluted human plasma at 37°C adding to a human fibrinogen solution (440 nM, 800 μl) ecarin (2.5 nM, 0.25 UI) and recording the absorbance increase of the solution at 671 nm. The characteristic parameters obtained from the ecarin clotting curve are reported in Fig. 10B of the main text.