SUPPLEMENTAL DATA

NOTECARIN D BINDS HUMAN FACTOR V AND FACTOR V_A WITH HIGH AFFINITY IN THE ABSENCE OF MEMBRANES

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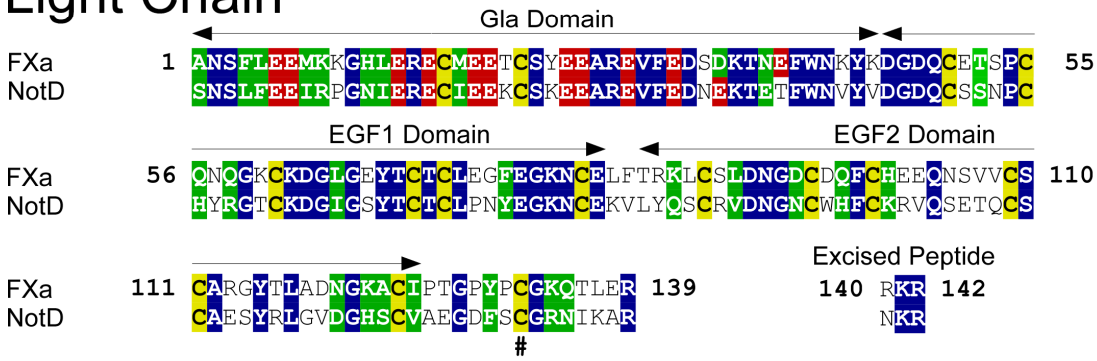
Signal Peptide

Light Chain



Activation Peptide

Heavy Chain

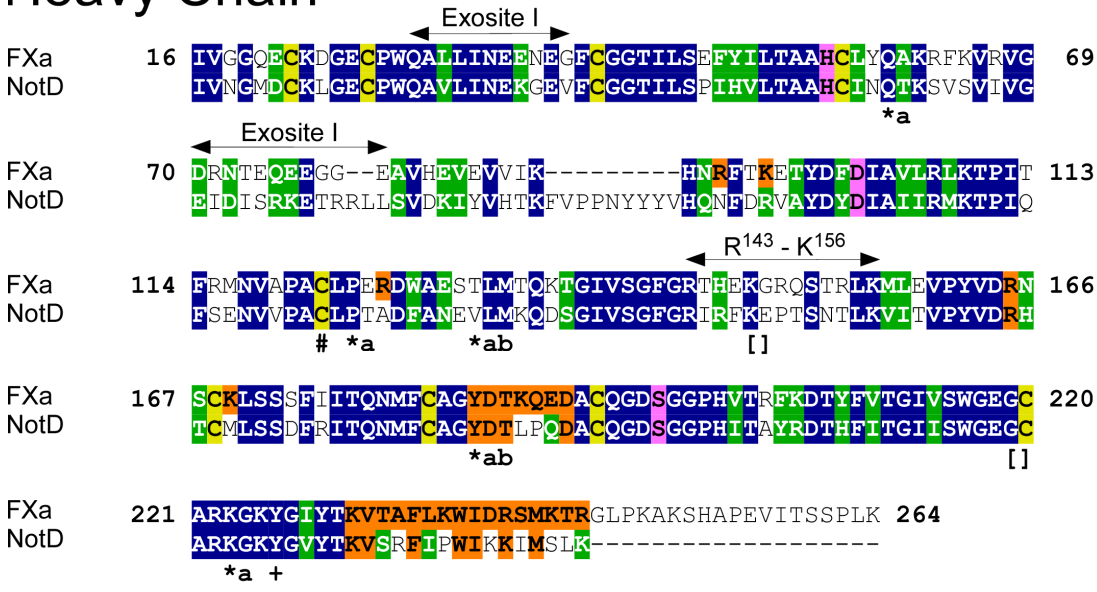


Figure 1S. Sequence alignment of FXa and NotD. Sequence alignment of human α-FXa [NP_00495.1] and NotD [AAZ14091] from *Notechis scutatus* was performed using the Clustal 2.0.10 multiple sequence alignment algorithm (1). Amino acids are numbered sequentially for the light chain through the activation peptide. Heavy chain is labeled using chymotrypsinogen numbering from Ile^{16}. The "*a(b)" under the sequence denotes an amino acid(s) insertion and the brackets "[ ]" identifies deletions in the FX gene as compared to chymotrypsinogen. The excised tripeptide, R^{140}-R^{142}, removed by an endopeptidase after FX secretion into plasma is shown (2). Conserved residues (*green*), identical residues (*blue*), γ-carboxylated glutamic acids (*red*), catalytic triad residues (*pink*), exosite II residues (*orange*), Tyr^{225} (+), cysteine residues that form a disulfide bridge linking heavy and light chain (#), and cysteine residues responsible for intramolecular disulfide bond formation (*yellow-green*) are shown (3-8).

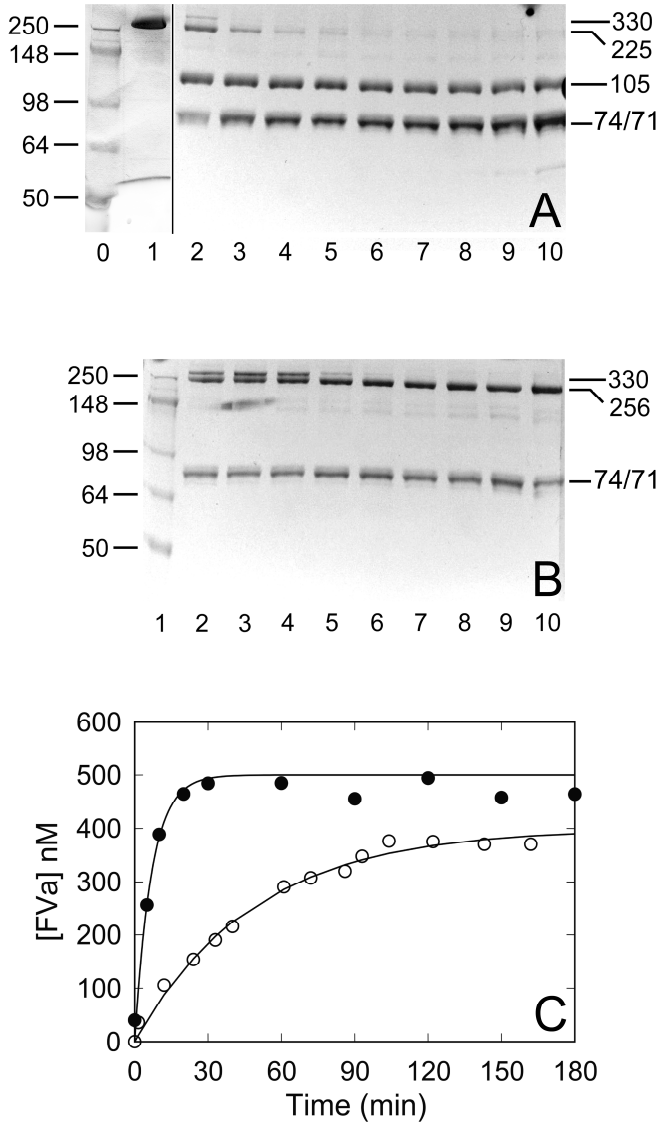


Figure 2S. FV activation by thrombin or RVV-V. *A*. FV (28.2 μM) was incubated with thrombin (53 nM) at 37 °C. Aliquots were denatured under reducing conditions, 8 μg of FV activation products was added to each *lane* and subjected to SDS-PAGE using 7.5% gels. FV used for these experiments run on a different, identical gel in the absence of thrombin is shown in *lane* 1. Reaction times (min): *lane* 2 (0.5), 3 (5), 4 (15), 5 (30), 6 (45), 7 (60), 8 (90), 9 (120), and 10 (180). *B*. FV (28.2 μM) incubated with RVV-V

(55 nM) as in *A*. Molecular mass markers are shown in *lane* 0 in *A*. and *lane* 1 in *B*. in kDa. *C*. Progress curves of 500 nM FV activation by 0.2 nM thrombin (●) or RVV-V (○). The concentration of FVa formed (*[FVa]*) was determined from a stoichiometric titration of the fractional change in the initial velocity (v_{obs}-v_o/v_o) of 200 μM CH_3SO_2-LGR-*p*NA hydrolysis by 6 nM NotD as a function of total concentration of thrombin generated FVa. Reactions were performed and analyzed as described under "Experimental Procedures." The *solid line*s represent the nonlinear least-squares fits by a single exponential. Activity assays were performed and analyzed as described under "Experimental Procedures."

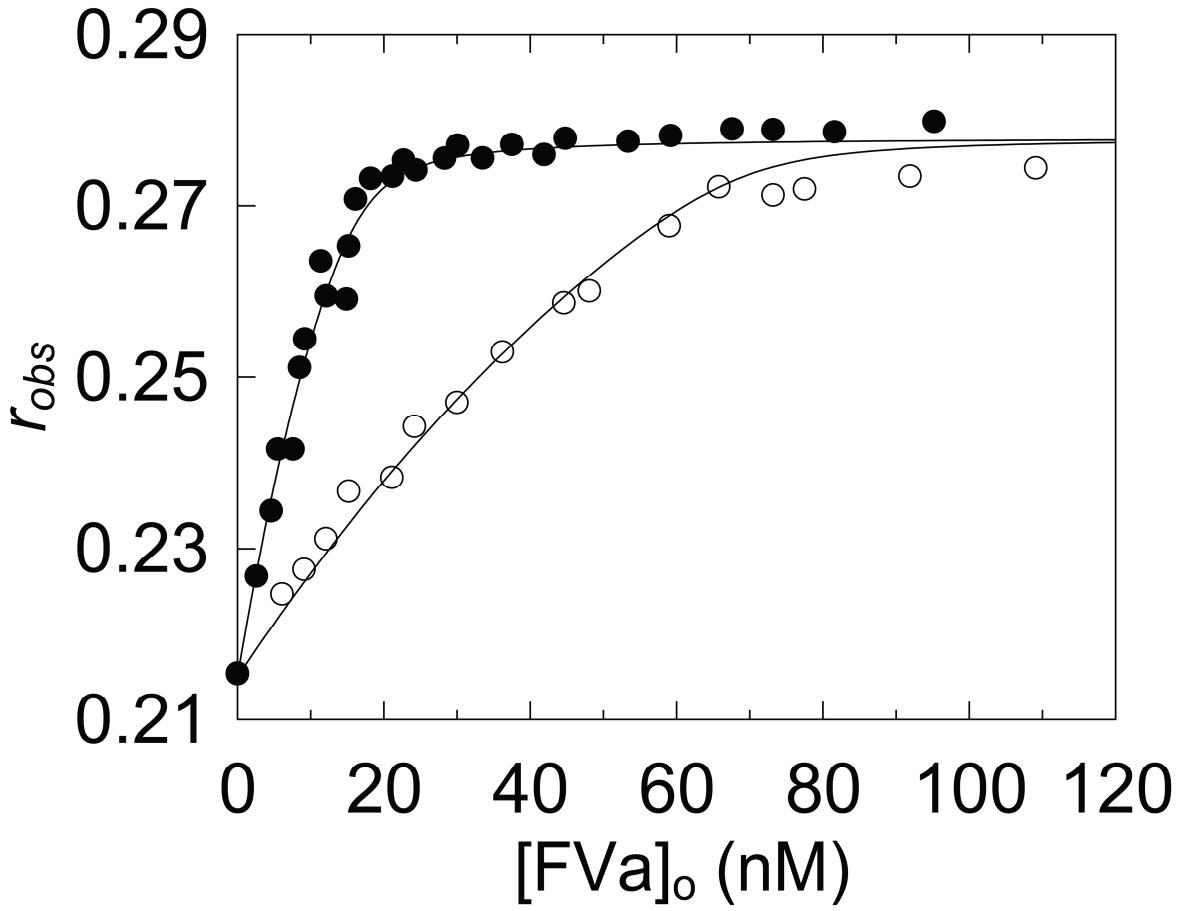


Figure 3S. Binding of FVa to [OG]EGR-FXa. Observed anisotropy (r_{obs}) of 12 (●) or 55 nM (○) [OG]EGR-FXa in the presence of 50 μM PCPSPE SUVs as a function of total concentration of FVa ($[FVa]_o$). The *solid lines* represent the fit by the quadratic binding equation with the parameters given in Table 1. Anisotropy titrations were performed and analyzed as described under "Experimental Procedures."

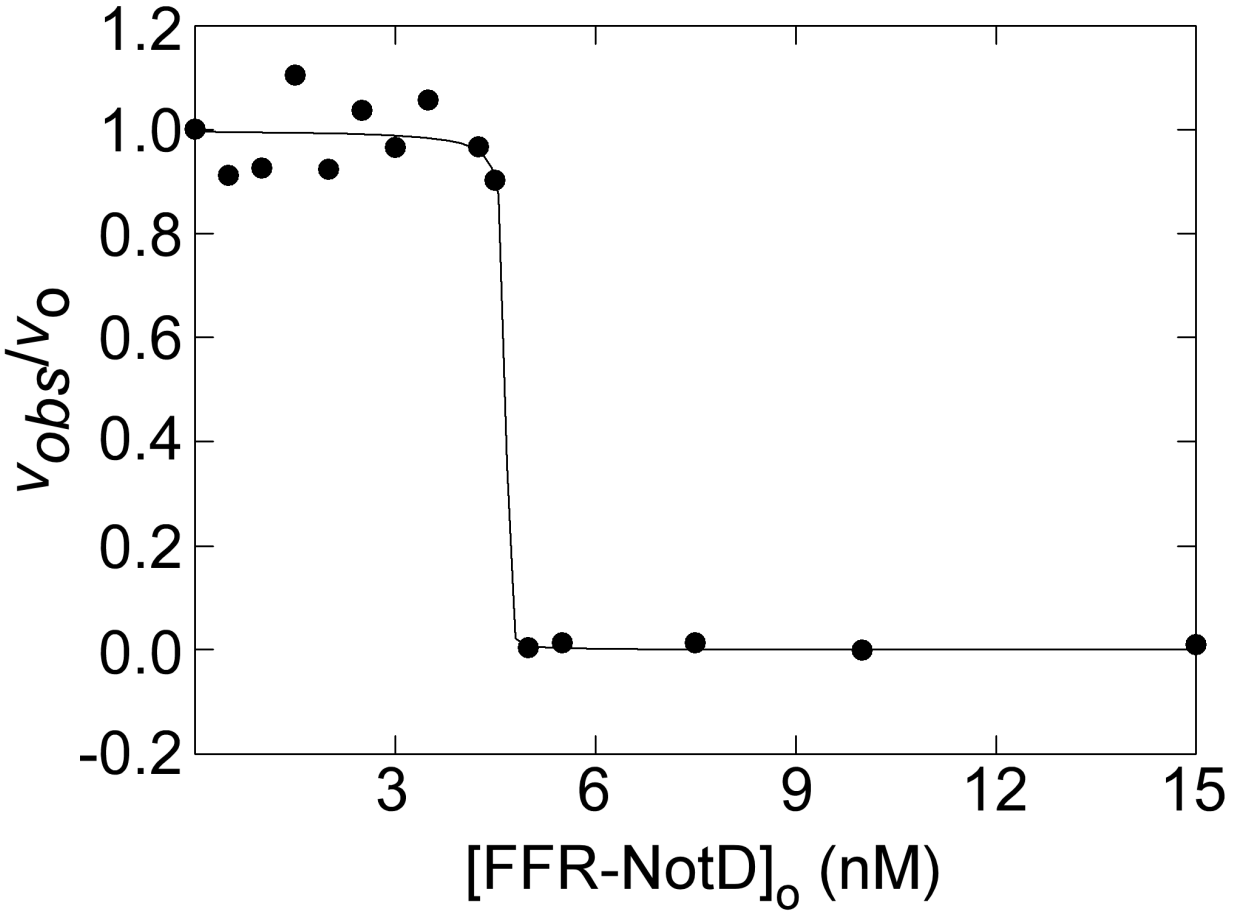


Figure 4S. Inhibition by FFR-NotD of ProT activation by prothrombinase. Fractional change in initial velocity (v_{obs}/v_o) of thrombin generation at 1 pM FXa and 5 nM FVa in the presence of 30 μM PCPS LUVs as a function of total FFR-NotD concentration ($[FFR\text{-}NotD]_o$). The s*olid line* represents the nonlinear least-squares fit of the cubic competitive binding equation for competitive binding of FXa and FFR-NotD to FVa with the stoichiometric factor of 1.07 ± 0.05 mol of FFR-NotD/mol of FVa. Reactions were performed and analyzed as described under "Experimental Procedures."

REFERENCES

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