

## SUPPLEMENTAL DATA

### NOTECARIN D BINDS HUMAN FACTOR V AND FACTOR V<sub>A</sub> WITH HIGH AFFINITY IN THE ABSENCE OF MEMBRANES

Jennifer L. Newell-Caito, Malabika Laha, Anthony C. Tharp, Jonathan I. Creamer, Hong Xu, Ashoka A. Maddur, Guido Tans, and Paul E. Bock

### Signal Peptide

FXa -40 MGRPLHLVLLSASLACL LLLGESL FIFRREQANNILARVTR -1  
 NotD MAPQLLLCLILTF LWSLPEAESNVFLKSKVANRF LQRTKR

### Light Chain

FXa 1 ANSFLEEMKKGHLERECMEETCSYEEAREVFEDSKTNEFWNKYKDGQCETSPC 55  
 NotD SNSLFEEIRPGNIERECLEEKCSKEEAREVFEDNEKTETFWNVYVDGQCSSNPC

← Gla Domain →

FXa 56 QNQGCKKDGLGEYTCCLGEGEGKNCLEFTRKLCSLDNGDCDQFCHEEQNSVVC 110  
 NotD HYRGTCCKDGIGSYTCCLPNYEGKNCLEKVLVYQSCRVDNGNCWHFCRQVQSETQCS

← EGF1 Domain →      ← EGF2 Domain →

FXa 111 CARGYTLADNGKACIPTGYPGCKQTLER 139      Excised Peptide 140 RKR 142  
 NotD CAESYRLGVDGHSVAEGDES CGRN IKAR      NKR

#

### Activation Peptide

FXa 143 SVAQATSSSGEAPDSTITWPKPYDAADLDPTENPFDLLDFNQTPBERGDNNLTR 194  
 NotD -----EASLPDEFVQSQ--KATLLKKS DNP-----SPD-----IR

### Heavy Chain

FXa 16 IVGGQECCKDGECPWQALLINEENEGFCGGTILSEFYILTAACHLYQAKRFKVRVG 69  
 NotD IVNGMDCKLGECPWQAVLINEKGEVFCGGTILSP IHVLTAAHCINQTKSVSVIVG

← Exosite I →

FXa 70 DRNTEQEEGG--EAVHEVEVVIK-----HNRFTKETYDEDI AVLRLKTPIT 113  
 NotD EIDISRKETRRLLSVDKIYVHTKFEVPPNYYYVHQNFD R VAYDYDIAIIRMKTPIQ

← Exosite I →

FXa 114 FRMNVAPACLPERDWAESTLMTQKTGIVSGFGRTHEKGRQSTR LKMLEVPYVDRN 166  
 NotD FSENVVPA CLPTADFA NEVLMKQDSGIVSGFGRIRKEPTSN TLKVIITVPYVDRH

# \*a \*ab [ ]      R<sup>143</sup> - K<sup>156</sup>

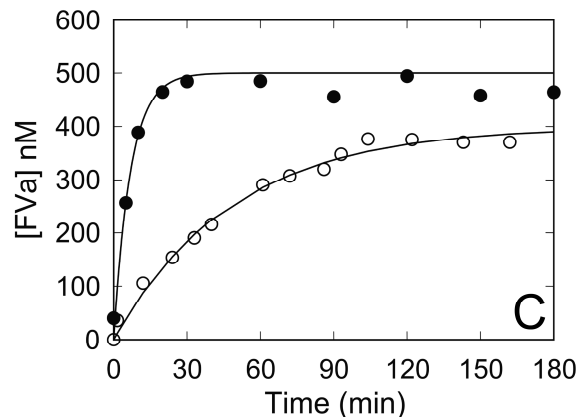
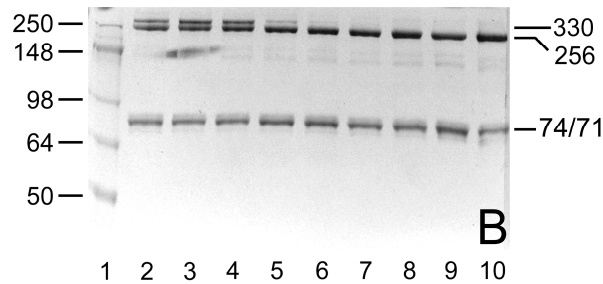
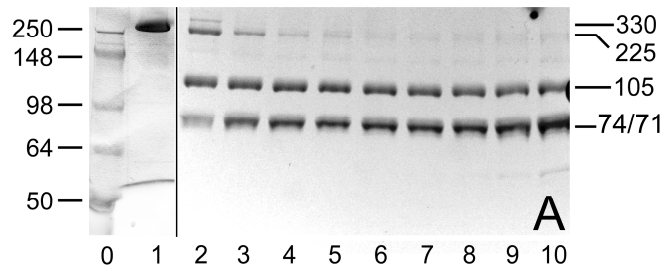
FXa 167 SCKLSSSFIITQNMFCAGYDTKQEDACQGDSGGPHVTRFKDTYFVTGIVSWGEGC 220  
 NotD TCMLSSDFRITQNMFCAGYDTLPQDACQGDSGGPHITAYRDTHFITGIISWGEGC

\*ab [ ]

FXa 221 ARKGKYG IYTKVTAFLKWIDRS MKTRGLPKAKSHAPEVITSSPLK 264  
 NotD ARKGKYG VYTKVSRFIPWIKKIMSLK-----

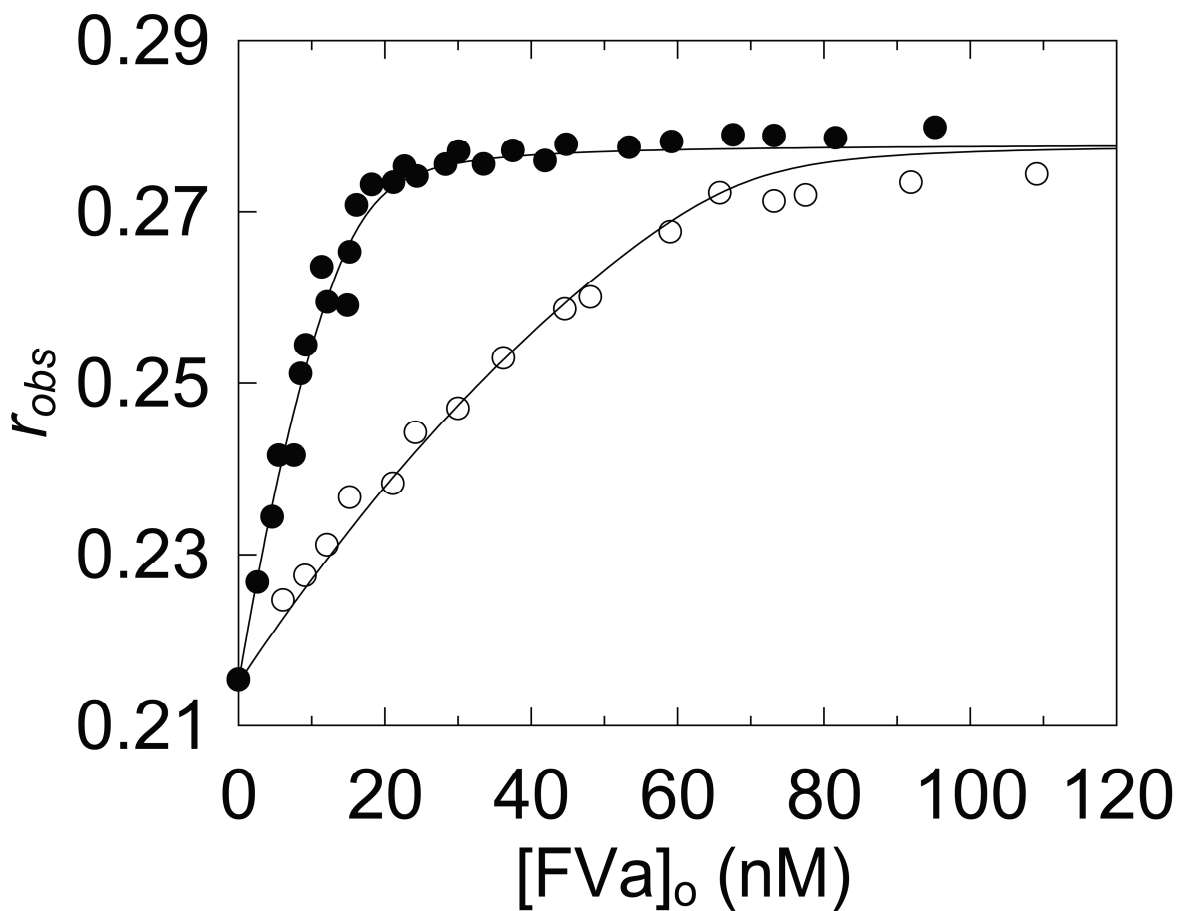
\*a +

**Figure 1S. Sequence alignment of FXa and NotD.** Sequence alignment of human  $\alpha$ -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<sup>16</sup>. 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<sup>140</sup>-R<sup>142</sup>, removed by an endopeptidase after FX secretion into plasma is shown (2). Conserved residues (*green*), identical residues (*blue*),  $\gamma$ -carboxylated glutamic acids (*red*), catalytic triad residues (*pink*), exosite II residues (*orange*), Tyr<sup>225</sup> (+), 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).

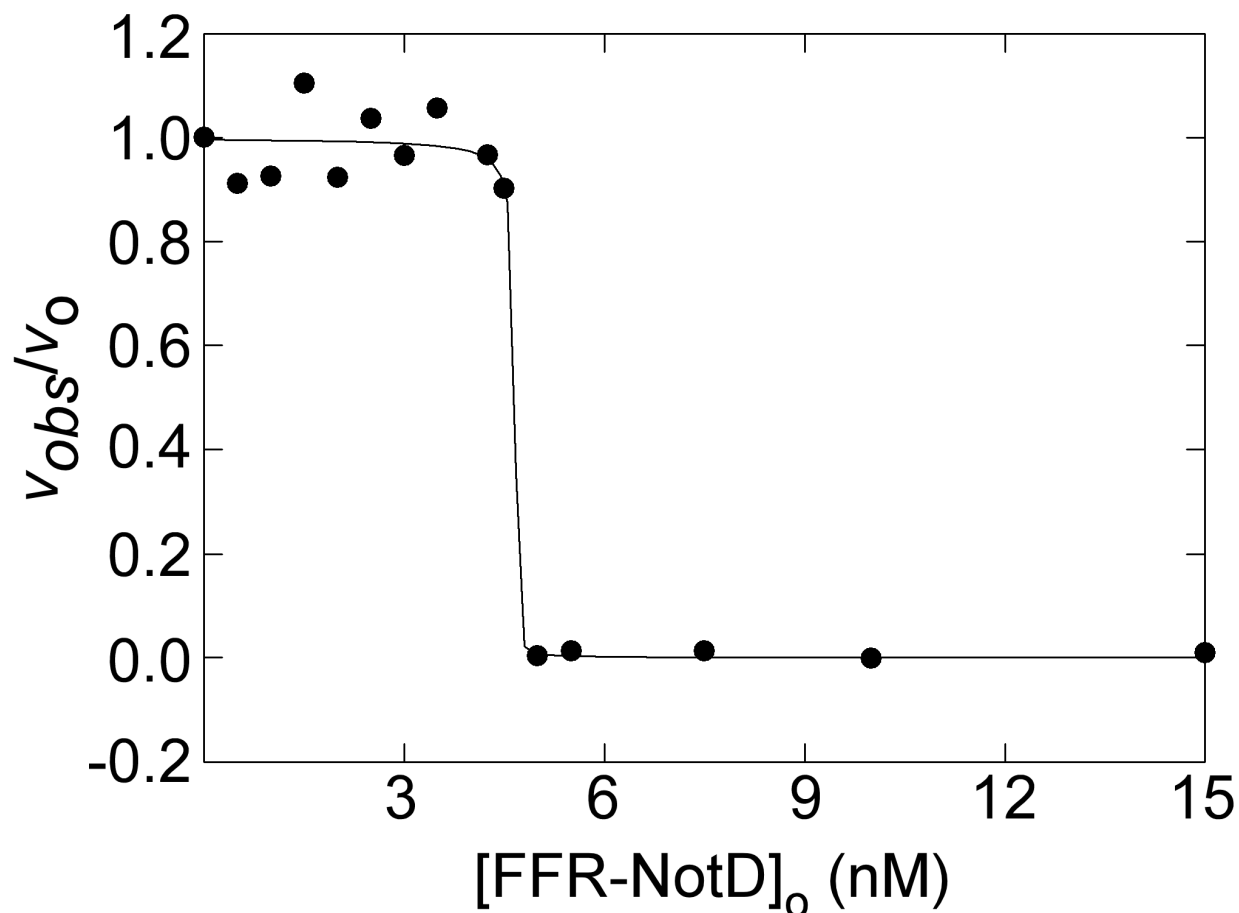


**Figure 2S. FV activation by thrombin or RVV-V.** A. FV (28.2  $\mu$ M) was incubated with thrombin (53 nM) at 37  $^{\circ}$ C. Aliquots were denatured under reducing conditions, 8  $\mu$ g of FV activation products was added to each lane and subjected to SDS-PAGE using 7.5% gels. FV used for these 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  $\mu$ M) 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  $\mu$ M CH<sub>3</sub>SO<sub>2</sub>-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 lines* 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  $\mu$ M 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 ( $v_{obs}/v_0$ ) of thrombin generation at 1 pM FXa and 5 nM FVa in the presence of 30  $\mu$ M PCPS LUVs as a function of total FFR-NotD concentration ( $[FFR-NotD]_0$ ). The solid 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  $1.07 \pm 0.05$  mol of FFR-NotD/mol of FVa. Reactions were performed and analyzed as described under “Experimental Procedures.”

## REFERENCES

1. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D. G. (2007) *Bioinformatics* **23**, 2947-2948
2. Leytus, S. P., Chung, D. W., Kisiel, W., Kurachi, K., and Davie, E. W. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3699-3702
3. Page, M. J., Macgillivray, R. T., and Di Cera, E. (2005) *J. Thromb. Haemost.* **3**, 2401-2408
4. Padmanabhan, K., Padmanabhan, K. P., Tulinsky, A., Park, C. H., Bode, W., Huber, R., Blankenship, D. T., Cardin, A. D., and Kisiel, W. (1993) *J. Mol. Biol.* **232**, 947-966
5. Rezaie, A. R., and Kittur, F. S. (2004) *J. Biol. Chem.* **279**, 48262-48269
6. Rezaie, A. R. (2000) *J. Biol. Chem.* **275**, 3320-3327
7. Yegneswaran, S., Mesters, R. M., and Griffin, J. H. (2003) *J. Biol. Chem.* **278**, 33312-33318
8. Rudolph, A. E., Porche-Sorbet, R., and Miletich, J. P. (2001) *J. Biol. Chem.* **276**, 5123-5128