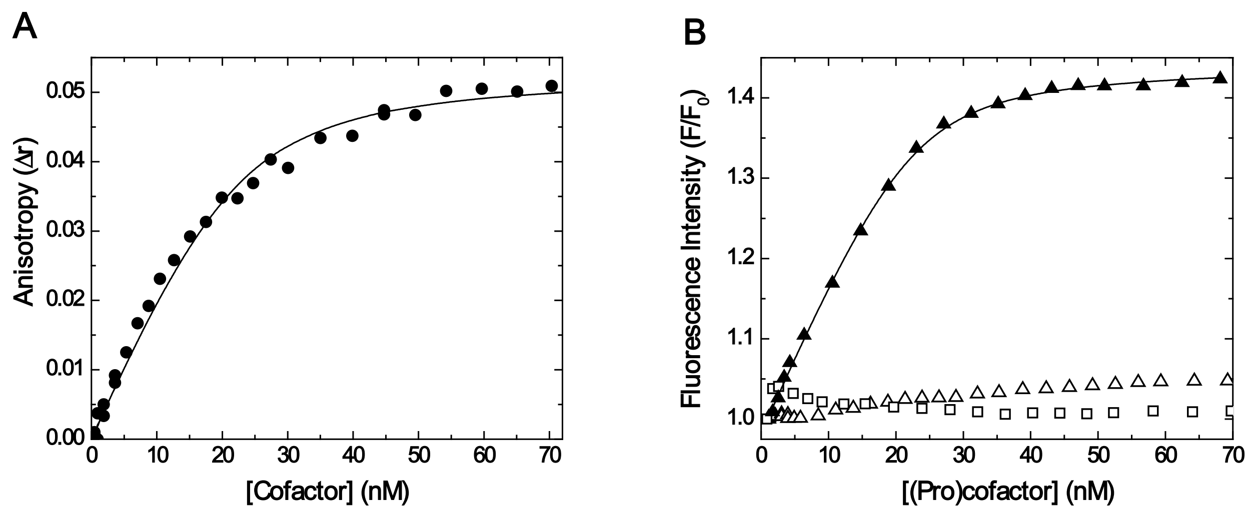
Mettine H.A. Bos and Rodney M. Camire
A Bipartite Autoinhibitory Region within the B-domain Suppresses Function in Factor V

SUPPLEMENTAL DATA



Supplemental Figure S1. Direct binding measurements: FV-B152 and FV-B104. Reaction mixtures containing 20 nM OG_{488}-FXa and 50 μM PCPS were titrated with increasing concentrations of FV/Va variants. The change in fluorescence anisotropy (Δr; panel A) or intensity (F/F_0; panel B) was measured and analyzed as described in "Experimental Procedures". The lines are drawn following analysis to independent, non-interacting sites. The data are representative of 2 similar experiments. Symbols are as follows: ***panel A***, FV-B152 (●); ***panel B***, FV-810 (▲), FV-B104 (△), and PD-FV (□). The fitted dissociation constants (K_d) for the variants are as follows: ***panel A:*** FV-B152, 2.6 ± 0.4 nM; ***panel B***: FV-810, 1.6 ± 0.1 nM; FV-B104 and PD-FV, not able to determine values.

A

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                  1493                                              1537        1545
                  |                                                 |           |
H. sapiens        TDYIEIIPKEEVQSS----EDD-Y-AEIDYVPYDDPYKTDVRTNINSSRDPDNIAAW--YL--R
P. troglodytes    TDYIEIIPKEEVQSS----EDD-Y-AEIDYVPYDDPYKTDVRTNINSSRDPDNIAAW--YL--R
P. pygmaeus       TDYIEIIPKEEVQSS----EDD-Y-AEIDYVPYDDPYKTDVRTNINSSRNPDNIAAW--YL--R
O. cuniculus      GDYIEIIPRQEVQ-S---EEDD--SSEIDYVSYDDPYKTDLRTDINSSRNPDSIAAW--YL-LR
D. ordii          GDYIEIIPREDYEGS----EDD-Y-AELYYVNYDNPYETDTRADTTSSRNPDNIAAW--YL--R
C. jacchus        TDYIEIIPREEVQSS----EDD-YT-ETDYVSYDDPYKTDVRTNINASRNPDNIAAW--YL--R
B. taurus         GDYIEIIPRQKEESS----EED-Y-GEFEFVAYNDPYQTDLRTDINSSRNPDNIAAW--YL--R
S. scrofa         GDYVEIIPRQQEENS----EED-Y-VKIDYVEYDDPYQTDVRTDINSSRNPDNIAAW--YL--R
A. melanoleuca    GDYIEIIPRQKEESS----EE--YTAEIDYVAYDDPYQTDTRTDINSSRNPDNIAAW--YL--R
C. familiaris     GDYIEIIPRQKEESS----EE--YSAEIGYVTYDDPYQTDTRTDINSSRNPDNIAAW--YL--R
M. lucifugus      GEYIEITPREKQQSS----EEDVY--EIVTVTFNNPYQTDIRTDINSSRNPDRIAAS--YL--R
L. africana       GDYVEIIPRQEFHSS----EED--SVEIDYVRYDDPYQTDIRTDINSCRNPDSIAAW--YL--R
E. caballus       GDYVEIIPRQEFHSS----EED--SVEIDYVRYDDPYQTDIRTDINSCRNPDSIAAW--YL--R
M. musculus       GDDVEIVPSEEPERI----DED-Y-AEDDFVTYNDPYRTDTRTDVNSSRNPDTIAAW--YL--R
R. norvegicus     GDDVEMIPSEELESI----DED-Y-PEDDYVTYNDPYKTDTRANVNSSRNPDTIAAW--YL--R
M. domestica      GDYIEYDPGQDVKKS-EDEDDDDY-----YVHYDDPYQTDRRTDTSSLRNPDNIAAW--YL--R
S. harrisii       GGYVEYIPGQAVKDSDDDDDDDDYDFEATFVPYDDPYKTDRRTDTSSLRNPDNIAAW--YL--R
O. anatinus       GDYIEYVPGPEIQNS----DED--LAMIQYVAYDNPYENDFRANPYTLRNPDTIASW--YI--R
G. gallus         GNY-EYTSGEYYTEDTSGDEYEYY-----YVSFDDPYMTDPKLNVNEQRNPDNIAE--HYL--R
M. gallopavo      GNY-EYTSGEYYSEGTSGDEYEYY-----YVSFDDPYMTDPKLNVNEQRNPENIAE--HYL--R
A. carolinensis   GDYQEYIIDDTDDEDSTSDSFE-Y----QMVHYDNPYTMDSRLDTSAARNPDDIAKS--YL--R
X. tropicalis     GMRPQIIIGLPGLDEGDYVELNVDEIDEDVHIKKVEYEELYKTEAQEYTNPDKIIQQ--YL--R
D. rerio          FNDYELYIP-KQDQEADFDGLLDHPEEYEYVEYKDP------SKTADVQALDATSQ--HLL--K
T. rubripes       FSDYELYLPGDEPDHLDVDQRNVKANEYEYVNYKDP-----RSNE-DAKNL-HLHQTRKYLENR
T. nigroviridis   FSDYELYLPGDEPDRLGSDEQNVKANEYEYVSYKDP-----SSDE-DAKNF-HLDQTNRLKQNA
O. latipes        FSDYQLYLPGKEPDHLEINKQDVTANEYEYVMFKDP------SDHKDIMNMNLDEQTKYYLELS
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B

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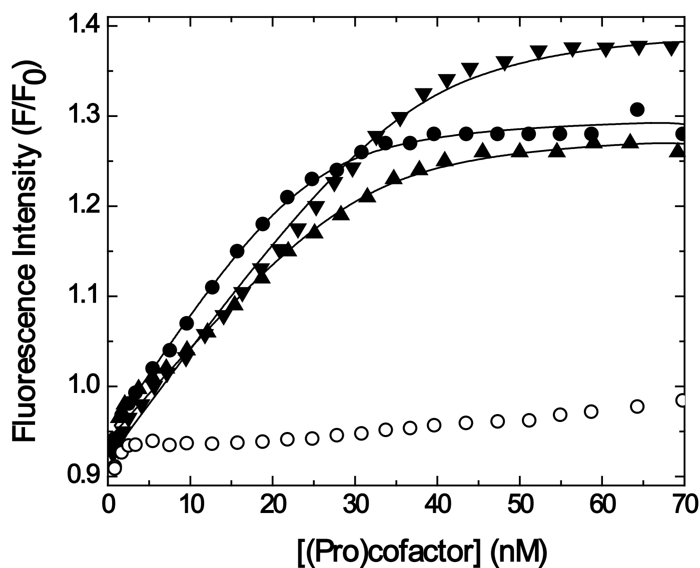
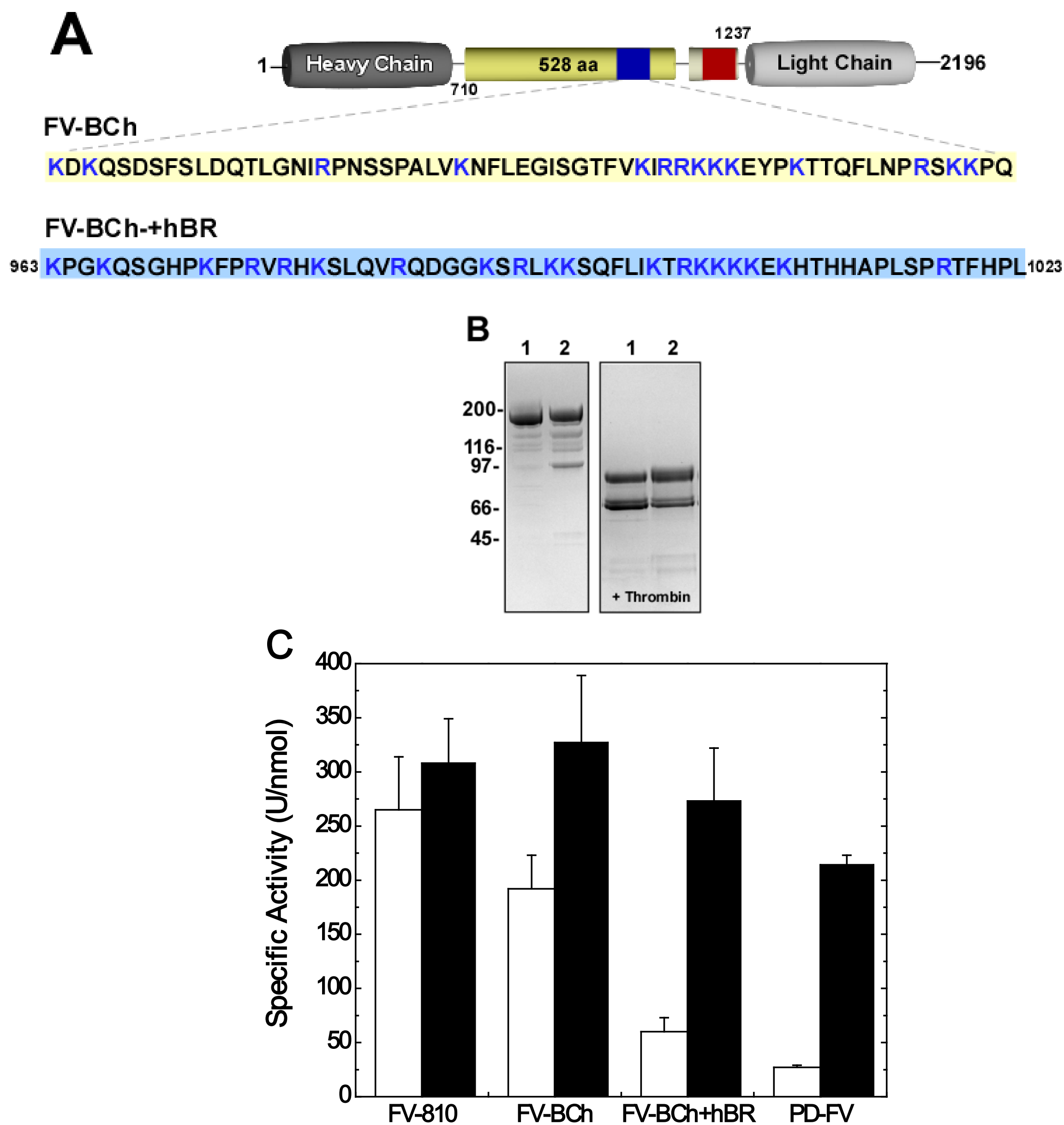
                 964                                                                              1008                        1018
H. sapiens       K--PGK---QSG-HPKFPRV---RHKSL-QV-R------Q--DG-GKSRLKKSQFLIKTRKKKKE---KHTHH-A------PLSPR
P. troglodytes   K--LGK---QSG-HPKFPRV---RHKSL-QV-R------Q--DG-GKSRLKKSQFLIKTRKKK-E---KHTHH-A------PLSPR
P. pygmaeus      K--PGK---QSG-HPKFPRV---RHKSL-QV-R------Q--DG-GKSRLKKSQFLIKTRKKKKE---KHTHH-A------PLSPR
O. cuniculus     K--PRK---HSG-H-TFPGF---KHTSL----RAE---Q---GGGNRRPKKSPFSIKTRKKKKE-E-KLVHY-V------PLSPR
D. ordii         ------R---P-N-H--LT-PM--RRK-F-QV-K------Q---RGGNSGLMRGQFLIRTRKKK------KLTQH-V------PLSPR
C. jacchus       K--PGK---PSG-HPEFPRV---RHKIL-QV-R------Q--DG-GNSGLKKSHLLIKTRKKRKE---KQTHHT-------PLSPR
B. taurus        KNSHGK---QSG-HPTF-LVT--RRKPL-QD-R------Q--DR-RNSRLKEGLPLIRTRRKKKE-E-KPAYH-V------PLSPR
S. scrofa        K--HGK---QRG-HPIF--VT--RHKLL-QE-R------Q--DE-GNSILKKGRFFIRTRRKKKE--RKPVHH-V------PLSPR
A. melanoleuca   K--HRKL----SG-HPKFSGI---RNKPL-QV-K------Q--DG-GNSGLKKIPFFIKTRKKKEE---KFAHHTA-------LSPR
C. familiaris    K--HRK---QSG-YPKFSGV---RNKTL-QV-K------W--DR-GNGGLKKSLFLIKTRKKKKE-Q-KLAHP-I------PLSPR
M. lucifugus     K--HG---EQ-GGHPKFPGI---RHKFL-QT-RP--------G-GSSRVKNSTFLIRTRKR-------KPAHHT-------PLSPR
L. africana      K--HGK---QTG-HPGFSGV---RHKPL-QV-R------Q--DG-KSHGLKKTSFYIKTRKKKKE--GKLPTH-V------LMSPR
E. caballus      -----K---QGD-HPKFSGI---RHKSP-QV-R------Q--DG-GNRGLKKSPFLIKTRKKKKE-E-KLAHH-V------PQSPR
M. musculus      R----K---PSD-LPTFSGV---GHKSP-HV-R------Q--EE-ENSGFQKRQLFIRTRKKKK--NKKLALHS-------PLSPR
R. norvegicus    ----GK---PSN-LPTFSRF---RHKSP-HV-R------Q--EE-ESGDFKKRQLFIRTRKKKK--NRKFALHS-------PLSPR
M. domestica     -----------------------RQKPL-KIGK---------NT-ANGRFQKSSFLIKTRKRKRI-N-KSVHN-V------PMSPR
S. harrisii      ------------------------HKPL-KIGK---------NR-ENGRFRKSSVLIKTRKRKRV---KLADN--L-----LMSPR
O. anatinus      R--QGR---PVS-HQKFA---EHRQKIK-RA-RPKFLNQGADG-ESTPIRPAMTFIKTRRKK-I-D-KSASHFA-------MSPR
G. gallus        K-H-------KDKQSDSFSLDQ-----TLGNI-RPNSSP--A-LVKNFLEGISGTFVKIRRKKKEY-PKTTQ-F--------LNPR
M. gallopavo     K-HHMK---KDGGFSHLKG--E-KHKGN-H-KRPPLKRK--EGNKNDTLSTSGTFVKIRRKKKEY-PKTTQ-F--------LNPR
A. carolinensis  K-KNKK-AYKENSLPEL-GQAEINNK-N-KV-----LNEQ---RYENS--S-SGTFIKIRRK--E---KPT-NS--------LTPR
X. tropicalis    K-TQ---EEQNAKKPTK-------RY--F-QV-RPI--------RYRTS--SNE-TRMLTRRKKRLGNVNGSLSNDTGNDGVFSPR
D. rerio         KYVKDKSA-ANSNKPK----IE-KEK-K-KVY-------Q---RVKPK--K--GYGMKTKKSK-DY--KPQPRSS------FSPR
T. rubripes      K----K---YFEMSPQTN------KKKTR-KVNRPH-RPQ----------K--GHGMKTKRRK-EY--KPQPRSGL----PLSPR
T. nigroviridis  -----K---YLEKSPKT--QQ---KKMK-KVTVPH-RPQ----------K--GHGMKTKRRK-DYEPK-A-KSGL----PFSPR
O. latipes       K----K---YFEKETLTTTPPP--KKTK-KV-V--LK-Q------KPK--K--GHGMKTRRKKV-Y--KPQERSDL----PLSPR
```

Supplemental Figure S2. Alignment of FV AR and BR B-domain segments. The conserved acidic region (AR; *panel A*; residues 1492-1538) or basic region (BR; *panel B*; residues 963-1008) sequences derived from the central B-domain of human FV were aligned using a modified Clustal W algorithm (AlignX Module; Invitrogen Corporation, Carlsbad, CA, USA) to corresponding regions from several select vertebrates. In both panels, the alignments were extended to include thrombin cleavage sites at Arg^{1545} (panel A) and Arg^{1018} (panel B). Highlighted residues (yellow, red, or blue) represent identical or highly homologous amino acids at a particular location. Red highlighted residues are also acidic and blue are basic.



Supplemental Figure S3. Direct binding measurements: FV-B-ptex, FV-B-Fish, FV-B-Ch, and FV-B-Ch^{+hBR}. Reaction mixtures containing 30 nM OG_{488}-FXa and 50 μM PCPS were titrated with increasing concentrations of FV/Va variants. The change in fluorescence intensity (F/F_0) was measured and analyzed as described in "Experimental Procedures". The lines are drawn following analysis to independent, non-interacting sites. The data are representative of 2 similar experiments. Symbols are as follows: FV-B-ptex (▼), FV-B-Fish (▲), FV-B-Ch (●), and FV-B-Ch^{+hBR} (○). The fitted dissociation constants (K_d) for the variants are as follows: FV-B-ptex 1.5 ± 0.2 nM; FV-B-Fish 1.8 ± 0.8 nM; FV-B-Ch 1.9 ± 0.3 nM; and FV-B-Ch^{+hBR} not able to determine a value.



Supplemental Figure S4. Characterization of FV-B-Ch Derivatives. *Panel A.* The human heavy chain (A1-A2) and light chain (A3-C1-C2) are connected via the B domain derived from *G. gallus* (*dark yellow bar*), which comprises 528 amino acids and spans residues 710-1237 in **FV-BCh**. The *blue box* represents the BR and its sequence is expanded. In **FV-B-Ch^{+hBR}**, only the human BR (963-1023) was exchanged for the chicken BR; the remaining B-domain sequence was derived from chicken. *Panel B.* Purified proteins (5 μg/lane) prior to (*left*) and after treatment with thrombin (*right*) were subjected to SDS-PAGE under reducing conditions and visualized by staining with Coomassie Brilliant Blue R-250. *Lane 1*, FV-B-Ch; *lane 2*, FV-B-Ch^{+hBR}. The apparent molecular weights of the standards are indicated. *Panels C.* The specific clotting activity of FV-810, FV-B-Ch, FV-B-Ch^{+hBR}, and PD-FV before (*white bars*) or after treatment with thrombin (*black bars*) was determined by a FV-specific PT-based clotting assay as described in "Experimental Procedures". The data are the means ± S.D. of 3-8 similar experiments. FV-810 and PD-FV are for reference and are from Fig. 3A.