Supporting Information

Protein Z-dependent protease inhibitor (ZPI) is a physiologically significant inhibitor of prothrombinase function

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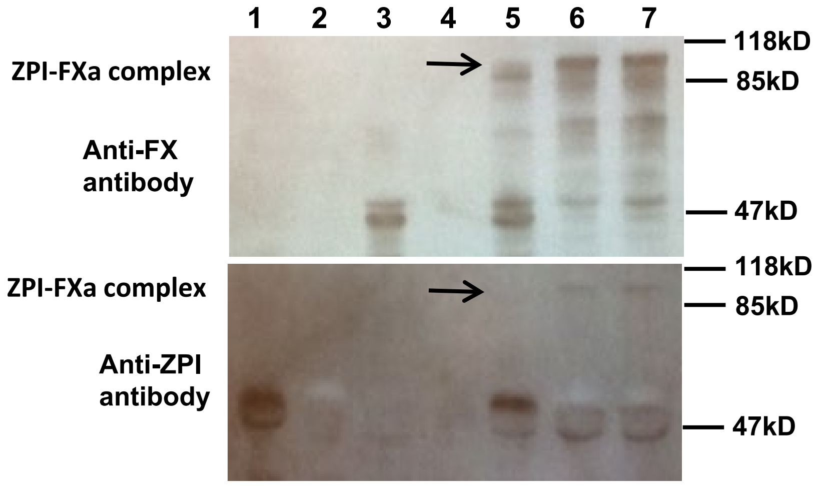
Supplementary Figure S1

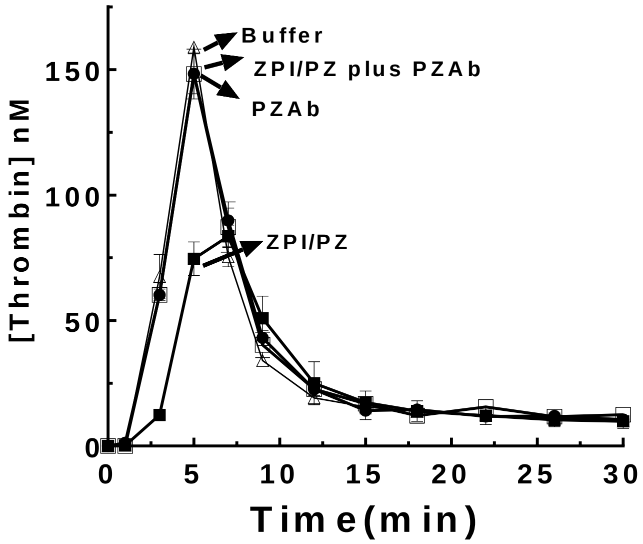


ZPI	+	-	-	-	+	+	+
PZ	-	+	-	-	-	+	+
FXa	-	-	+	-	+	+	+
FVa	-	-	-	+	-	-	+

Supplementary Figure S1.

Immunoblotting analysis of ZPI/PZ inhibition of membrane-associated free or prothrombinase-bound FXa
Reactions of 200nM ZPI and 50nM FXa were done +/- 200nM PZ and +/- 100nM FVa as indicated in Tris buffer pH 7.4, 25°C, in the presence of 25 µM PS/PC, 5 mM $CaCl_2$. Reactions were initiated with FXa or FXa/FVa complex and quenched after 10 mins with 400 µM EGR-chloromethylyketone. Samples were denatured in pH 2.0 SDS buffer by heating at 100°C for 5 mins, and then adjusted to neutral pH and 30 µl samples analyzed by 10% SDS-PAGE. Protein bands were transferred to PVDF membranes, and then detected either with anti-factor Xa (Enzyme Research Laboratories) or anti-ZPI (Abnova) antibodies. Multiple bands in lanes 5,6,7 of the upper gel below the main ZPI-FXa complex band reflect complex degradation. The weak signal for the ZPI-FXa complex bands in the lower gel result from the relatively poor reactivity of the anti-ZPI antibody with conformationally altered ZPI in the ZPI-FXa complex.

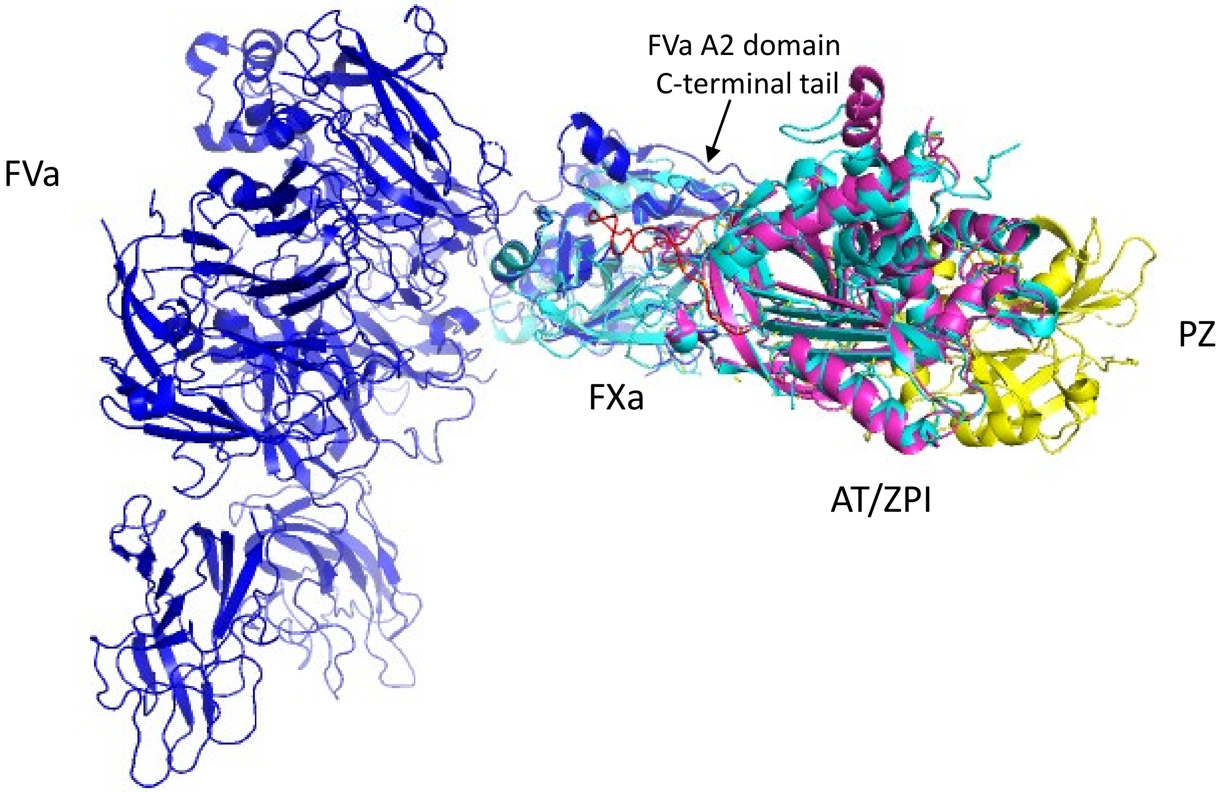
Supplementary Figure S2



Supplementary Figure S2.

PZAB does not inhibit thrombin generation in PZ-deficient plasma Thrombin generation in PZ-deficient plasma was measured at pH 7.4, 37^{o}C as in Fig. 8 with or without the addition of: i) buffer (Δ), ii) PZAb (160 mg/ml) (□), iii) ZPI/PZ (25 nM each) (■), or iv) ZPI/PZ plus PZAb (●) as indicated. Data were derived from three independent measurements ± SD.

Supplementary Figure S3



Supplementary Figure S3.
Model of ZPI-PZ complex interaction with FXa bound to FVa in prothrombinase: The model was made by aligning the structures of prothrombinase from P. Textalis snake venom (blue; pdb:4BXS) with the human antithrombin (AT)-FXa Michaelis complex (cyan; pdb: 2GD4) and the ZPI-PZ complex (ZPI in magenta and PZ in yellow; pdb: 3F1S). The reactive loop of antithrombin (in red) is bound in the active-site of FXa in a manner that places both antithrombin and the ZPI-PZ complex in an orthogonal orientation relative to FVa and thus minimally interferes with the interaction of FXa with FVa in prothrombinase except for the A2 domain C-terminal peptide interaction of FVa with the heparin binding exosite of FXa. The model was prepared using PyMol software v. 2.2.3.

Supplementary Table S1

Supplementary Table S1. Parameters derived from thrombin generation assays in normal human plasma with TF, FXa and FXIIa triggers

	TF, 5pM			**TF, 2pM**			**TF, 0.6pM**		
	+PZAb	-	+ZPI-PZ	+PZAb	-	+ZPI-PZ	+PZAb	-	+ZPI-PZ
LT	1.7±0	1.7±0	1.9±0.2	3.1±0.1*	3.4±0.1	3.7±0.1*	4.9±0.2*	6.6±0.2	7.7±0.1*
TP	249.0±6.0*	172.0±7.0	101.0±9.0*	146.0±4.0*	79.0±0.3	39.0±0.7*	64.6±8.5*	42.4±0.3	18.8±1.2*
TTP	3.7±0.1*	4.1±0.1	4.4±0.1*	6.1±0.1*	6.4±0.1	6.7±0.1*	9.2±0.2*	9.7±0.1	11.1±0.2*
ETP	984.0±14.0*	901.0±41.0	782.0±44.0*	979.0±68.0*	718.0±3.0	435.0±25.0*	701.0±64.0*	502.0±52.0	204.0±6.0*

	FXa, 0.7nM			**FXa, 0.23nM**			**FXa, 0.03nM**		
	+PZAb	-	+ZPI-PZ	+PZAb	-	+ZPI-PZ	+PZAb	-	+ZPI-PZ
LT	1.0±0,1	1.0±0.1	1.0±0.1	1.3±0.1	1.3±0.1	1.3±0.1	4.5±0.2	4.6±0.2	4.7±0.3
TP	218.5±0.8*	169.7±1.4	106.6±1.0*	118.5±5.4*	75.3±0.6	45.8±0.3*	31.8±0.6*	24.2±0.6	14.7±0.2*
TTP	3.0±0.0.1	3.0±0.1	3.0±0.1	4.0±0.1	3.7±0.3	3.6±0.2	9.6±0.2	9.5±0.1	9.7±0.2
ETP	849.0±14.4*	796.7±23.6	701.5±47.5*	788.0±7.4*	656.7±23.9	546.5±0.5*	555.7±16.8*	459.0±51.2	173.5±26.5*

	FXIIa, 18nM			**FXIIa, 3nM**			**FXIIa, 0.8nM**		
	+PZAb	-	+ZPI-PZ	+PZAb	-	+ZPI-PZ	+PZAb	-	+ZPI-PZ
LT	8.0±0.2*	9.0±0.2	9.5±0.1*	9.8±0.5*	10.7±0.4	12.2±0.2*	11.3±0.1*	12.6±0.1	13.5±0.1*
TP	196.0±4.1	198.0±2.2	153.1±6.2*	101.8±5.4	97.8±9.1	65.4±5.4*	88.3±1.0*	77.3±0.5	49.6±2.5*
TTP	10.3±0.1*	11.3±0.4	12.2±0.1*	13.0±0.1*	14.3±0.2	15.3±0.3*	15.3±0.1*	16.0±0.2	16.6±0.1*
ETP	928.3±49.6	913.7±15.6	848.0±24.0*	795.0±35.3	770.7±62.6	496.5±39.5*	672.2±17.5*	576.9±38.0	374.9±8.5*

*_P_<0.05 vs. no PZAb or ZPI-PZ. LT, lag time, min. TP,thrombin peak,nM. TTP, time to thrombin peak, min. ETP, endogenous thrombin potential, nM thrombin x min.