Supplemental Data

Active Site-labeled Prothrombin Inhibits Prothrombinase *In Vitro* and Thrombosis *In Vivo*

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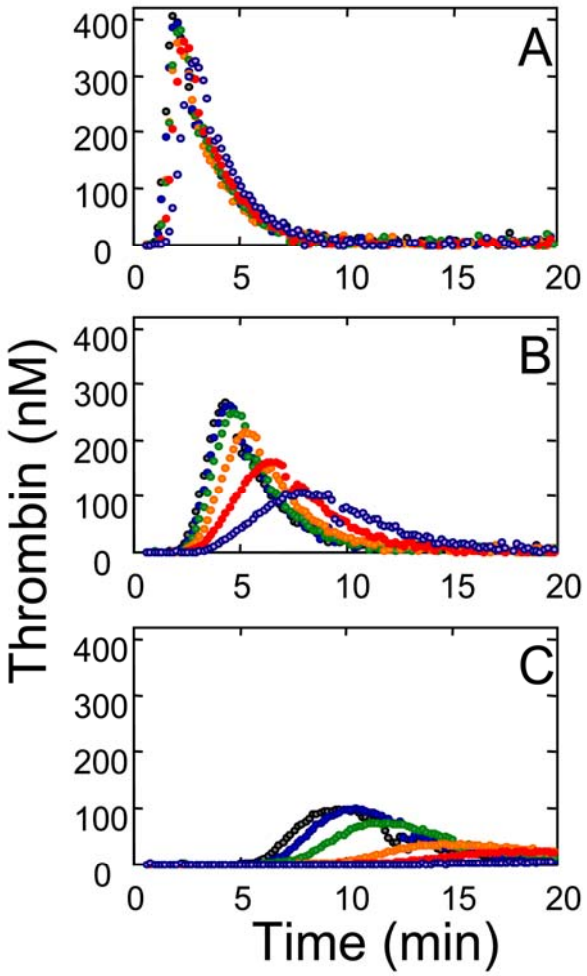


FIGURE 1S. Inhibition of thrombin generation in platelet-poor human plasma as a function of FPR-ProT at three different TF concentrations. Thrombin generation curves were measured in a total volume of 125 µl containing 80 µl platelet-poor human plasma by CAT as described under "Experimental Procedures". Thrombin generation was initiated by 40 pM TF (*A*), 7 pM TF (*B*) or 1.75 pM TF (*C*) in the presence of 30 µM PSPCPE vesicles, 40 µg/ml corn trypsin inhibitor, 16 mM $CaCl_2$, 0.34 mM fluorogenic thrombin substrate, and varying concentrations of FPR-ProT (µM): 0 (*gray*), 0.2 (*blue*), 0.4 (*green*), 0.6 (*orange*), 0.8 (*red*) and 1.0 (*violet*). All concentrations are final concentrations in the reaction mixtures.

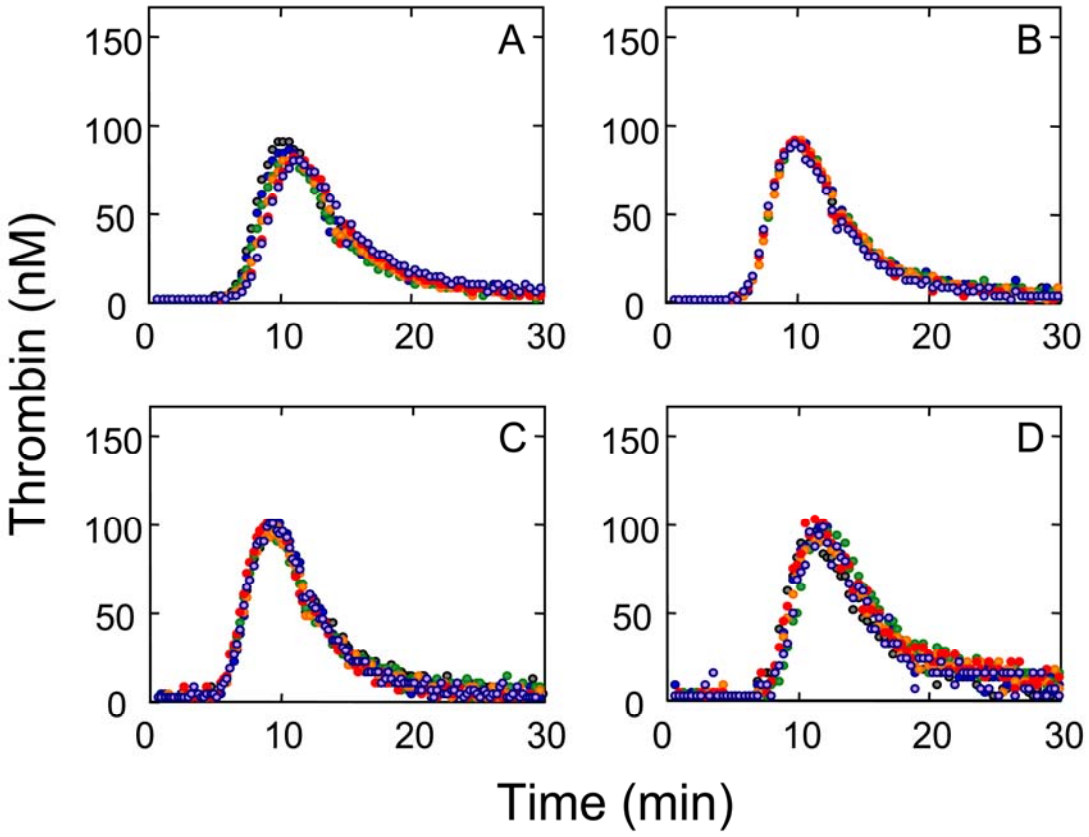


FIGURE 2S. Low TF-initiated thrombin generation in platelet-poor human plasma at varying concentrations of F1, F1.2, FPR-Pre 1, and FPR-thrombin. Thrombin generation curves were measured in a total volume of 125 μl containing 80 μl platelet-poor human plasma by CAT as described under "Experimental Procedures". Final concentrations were: 1.75 pM TF, 30 μM PSPCPE vesicles, 40 μg/ml corn trypsin inhibitor, 16 mM added $CaCl_2$, 0.34 mM substrate and 0 (*gray*), 0.2 (*blue*), 0.4 (*green*), 0.6 (*orange*), 0.8 (*red*) or 1.0 (*violet*) μM F1 (*A*), F1.2 (*B*), FPR-Pre 1 (*C*) or FPR-thrombin (*D*).

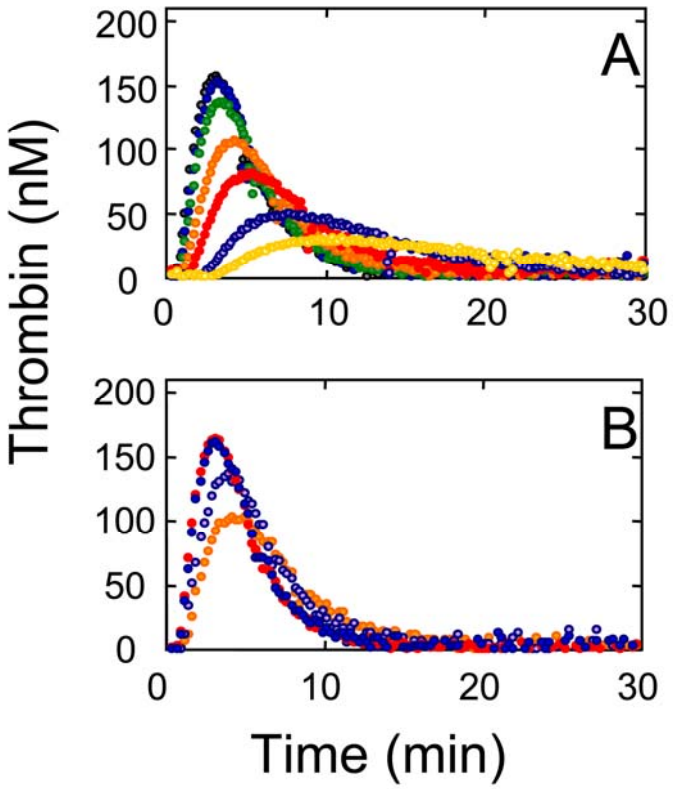


FIGURE 3S. Effect of FPR-ProT, F1, F1.2, and FPR-Pre 1 on factor Xa-initiated thrombin generation in platelet-poor human plasma. *A*. Thrombin generation curves were measured in a total volume of 125 µl containing 80 µl platelet-poor human plasma by CAT as described under "Experimental Procedures". Thrombin generation was initiated by addition of 0.7 nM human factor Xa in the presence of 30 µM PSPCPE vesicles, 40 µg/ml corn trypsin inhibitor , 16 mM $CaCl_2$, 0.34 mM substrate and 0 (*gray*), 0.2 (*blue*), 0.4 (*green*), 0.6 (*orange*), 0.8 (*red*), 1 (*violet*) or 1.2 (*yellow*) µM FPR-ProT. *B*. Thrombin generation in platelet-poor human plasma determined as in A, but with 1.2 µM of the following ProT derivatives: none (*red)*; F1 (*orange*); F1.2 (*violet*); FPR-Pre 1 (*blue*). All concentrations are final concentrations in the reaction mixtures.

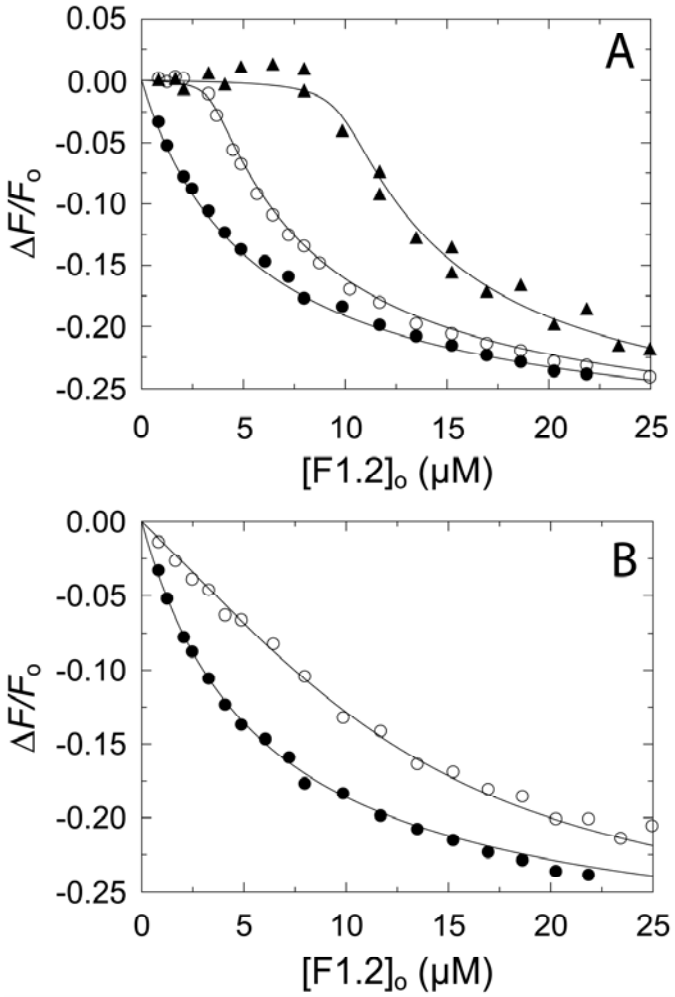


FIGURE 4S. Competitive equilibrium binding of F1.2 to [6F]FPR-thrombin and native Pre 2 or FPR-Pre 2. The fractional change in fluorescence ($\Delta F/F_o$) of 103 nM [6F]FPR-thrombin as a function of total F1.2 concentration ($[F1.2]_o$). *A.* in the absence (●) and presence of 3.5 (○) or 10 (▲) μM native Pre 2. *B.* as in *A* in the absence (●) or presence (○) of 10 μM FPR-Pre 2. The *lines* represent the simultaneous fits by the cubic competitive binding equation, with K_D for F1.2 binding to [6F]FPR-thrombin in *A* of 5.5 ± 0.5 μM and in *B* of 5.8 ± 0.5 μM, and K_D for F1.2 binding to native Pre 2 in *A* of 0.04 ± 0.03 μM, and for F1.2 binding to FPR-Pre 2 in *B* of 3.6 ± 0.6 μM. The stoichiometric factor was fixed at 1. Titrations were performed as described in "Experimental Procedures."

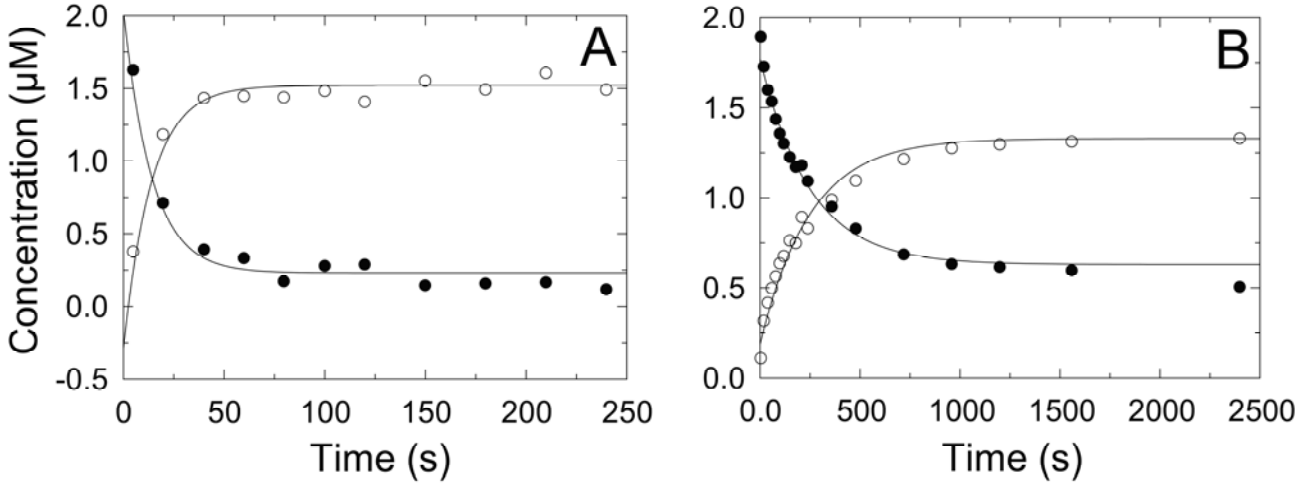
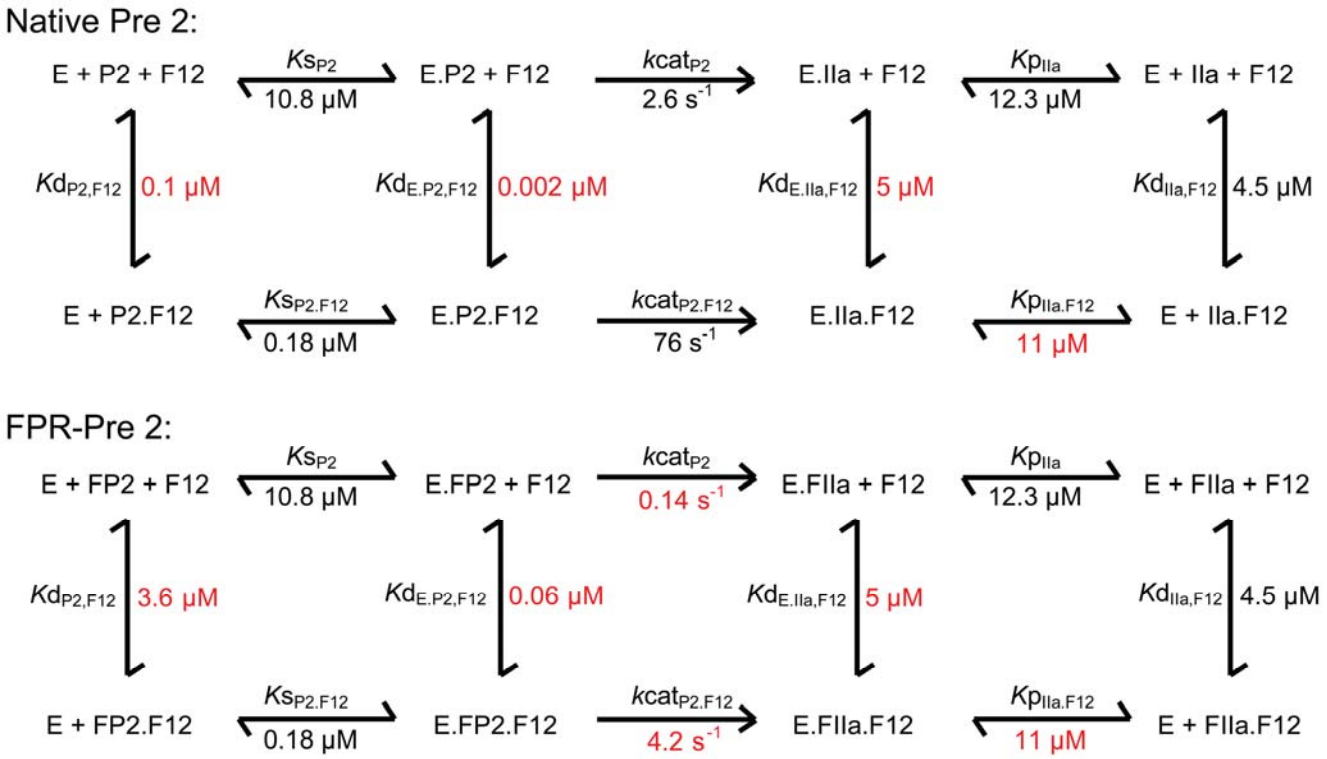


FIGURE 5S. Time-courses of cleavage of native Pre 2 or FPR-Pre 2 in the presence of F1.2 by prothrombinase. Concentration of Pre 2 (*A*) or FPR-Pre 2 (*B*) disappearance (●) and appearance of the reaction product, the thrombin B-chain (○). Reactions were performed at 37 °C with 2 μM Pre 2 or FPR-Pre 2 in the presence of 2 μM F1.2, 0.7 nM FXa, 50 nM FVa, 50 μM lipid vesicles (PSPCPE), and 60 μM DAPA. The *lines* represent the fits by single exponentials for both substrate depletion and product formation, with the parameters given in "Results". Reactions were performed and analyzed by SDS-gel electrophoresis and quantitative densitometry as described in "Experimental Procedures".



Scheme 1S

SCHEME 1S. Pathways of native Pre 2 and FPR-Pre 2 activation in the absence and presence of F1.2. Values in *black* are those of Kamath and Krishnaswamy (Ref. 59), and values in *red* are those supplied by the authors. Reprinted in modified form with permission.

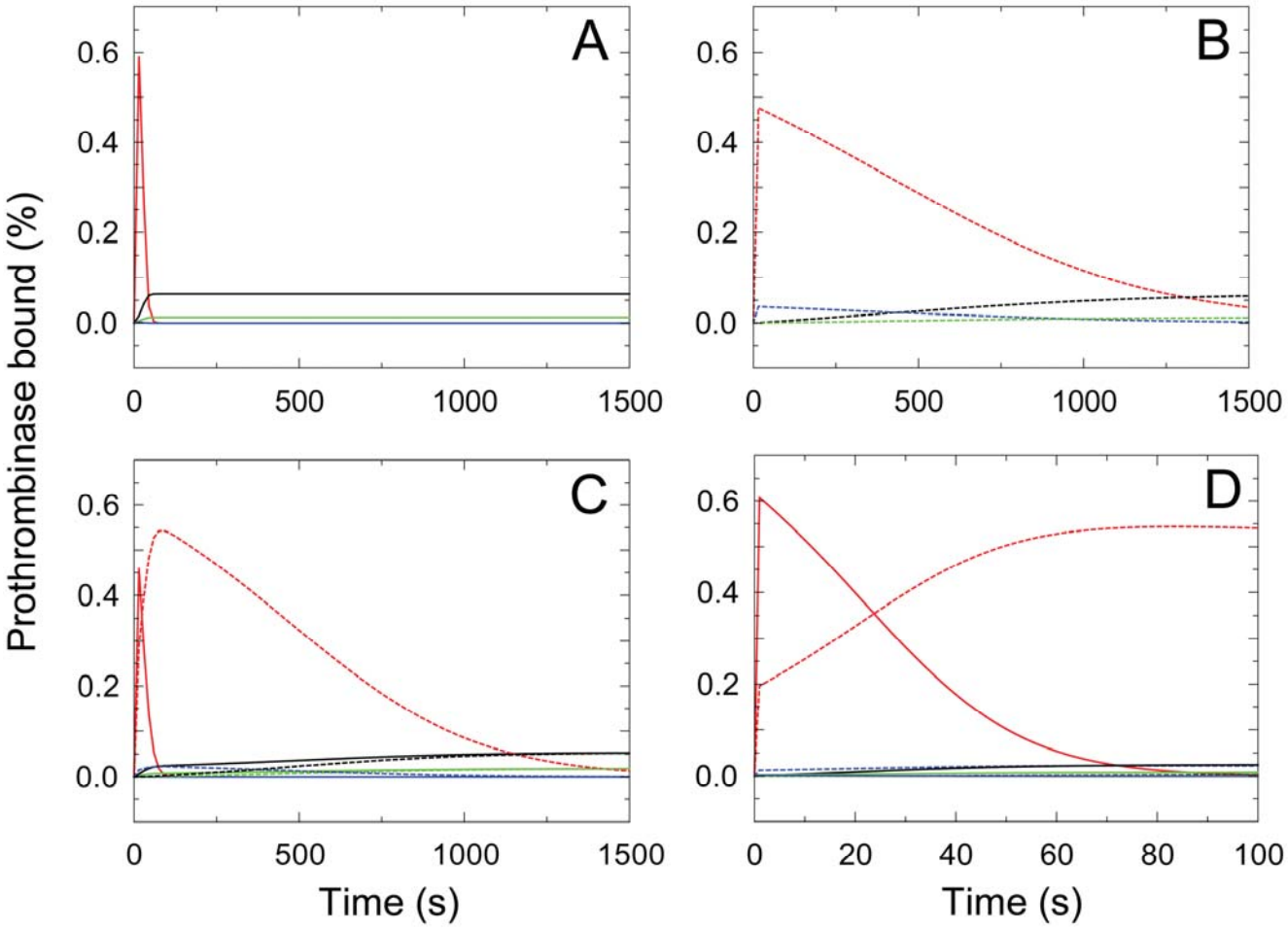


FIGURE 6S. Time-course simulations of the mechanism in Scheme 1S for reactions of Pre 2 and FPR-Pre 2 with 0.7 nM prothrombinase. *A.* Simulated time-courses for equimolar mixtures (1 μM) of native Pre 2 and F1.2, *B.* FPR-Pre 2 and F1.2, or *C.* 1 μM each of Pre 2 and FPR-Pre 2 with 2 μM F1.2. An expanded view of the initial 100 s of *C* is shown in *D*. Formation and disappearance of enzyme-bound complexes are represented as *solid lines* for species derived from native Pre 2 (Pre 2, *blue*; Pre 2·F1.2, *red*; thrombin, *black*; thrombin·F1.2, *green*), and as *dotted lines* for species derived from FPR-Pre 2 (FPR-Pre 2, *blue*; FPR-Pre 2·F1.2, *red*; FPR-thrombin, *black*; FPR-thrombin·F1.2, *green*). Simulations were done with KinTek Explorer (Refs. 64, 65), with bimolecular on-rate constants fixed at 10^9 M^{-1} s^{-1} and off-rate constants to give the rapid-equilibrium dissociation constants shown in Scheme 1S.

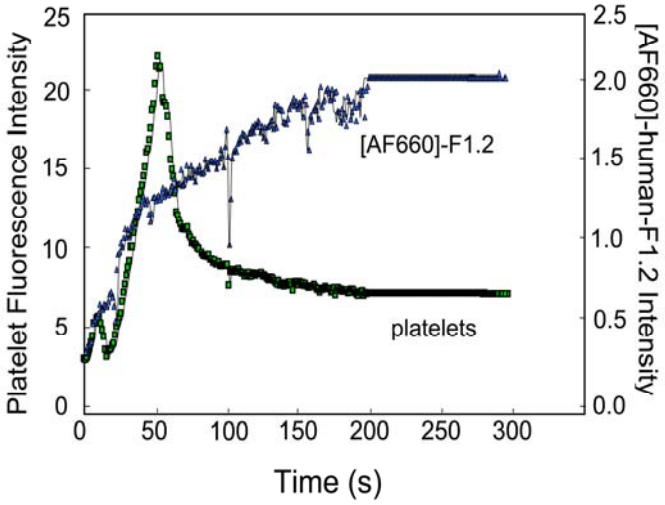


FIGURE 7S. Accumulation of platelets and [AF660]-F1.2 following laser-induced injury. Platelets (*green squares*) and [AF660]-F1.2 (*blue triangles*) were monitored for 5 min following laser injury. Notice platelet accumulation follows the reported triphasic pattern and is unaffected by the presence of [AF660]-F1.2.

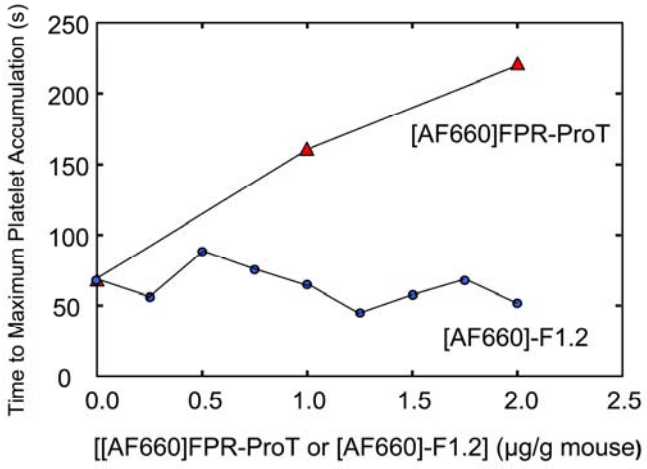


FIGURE 8S. Time to reach maximum platelet deposition following laser-induced injury in the presence of [AF660]FPR-ProT or F1.2. [AF660]-F1.2 or [AF660]FPR-ProT was titrated into animals prior to laser injury. Median time to achieve maximal platelet signal at each concentration of F1.2 (*blue circles*) and [AF660]FRP-ProT (*red triangles*). Notice the time to peak platelet accumulation increases with the dose of [AF660]FPR-ProT, but is minimally affected by increasing dosage of the Gla domain-containing F1.2.