

ALAN E. LEVITON STUDENT RESEARCH AWARD REPORT

Photo courtesy, Michelle A. Berny.



*Thrombin Interactions with Clots Formed under Shear Flow: The Role of  $\gamma'$ -Fibrinogen*

Michelle A. Berny

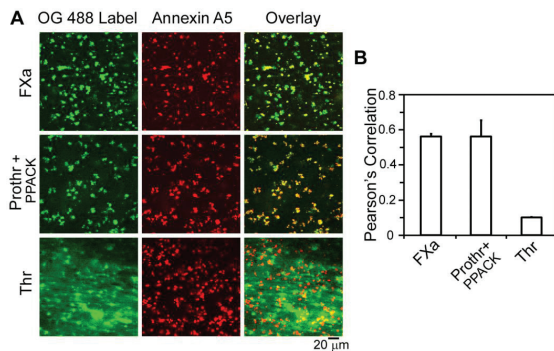
Department of Biomedical Engineering

Oregon Health and Science University, Portland, OR

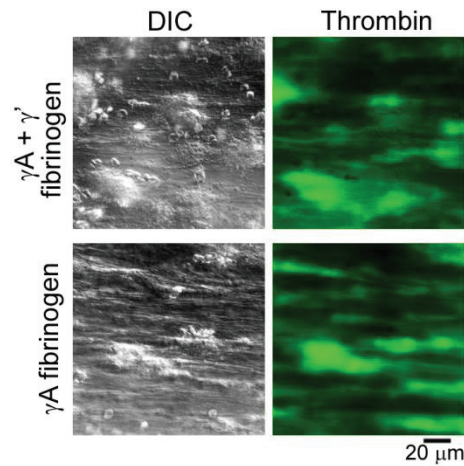
Michelle Berny is the recipient of the 2010 AAAS, Pacific Division Alan E. Leviton Student Research Award. Owen J. T. McCarty was her advisor.

To examine the mechanisms of thrombin binding to blood clots, clots were formed in vitro by co-perfusion of reconstituted blood (platelets, red blood cells, and fibrinogen-deficient plasma supplemented with  $\gamma$ A- or  $\gamma'$ -fibrinogen) with  $Ca^{2+}/Mg^{2+}$ /tissue factor into capillary tubes. The role of  $\gamma'$ -fibrinogen, containing the high affinity thrombin-binding site, in thrombin-clot binding was investigated by forming clots in the absence or presence of  $\gamma'$ -fibrinogen, then post-labeling clots with fluorescently labeled (OG488) thrombin. As indicated in Figure 1, OG488-thrombin bound to clots formed with and without  $\gamma'$ -fibrinogen, suggesting that the low affinity thrombin-binding sites on fibrinogen may play an important role in thrombin-clot interactions.

Thrombin is generated from its precursor, prothrombin, by factor Xa (FXa) mediated cleavage. To further elucidate thrombin-clot interactions, we investigated the localization of proteins involved in the generation of thrombin. Clots were formed by co-perfusion of sodium citrate anticoagulated human blood with  $Ca^{2+}/Mg^{2+}$ /tissue factor into capillary tubes. Following formation, clots were labeled with OG488-labeled coagulation factors in combination with AF647-labeled annexin A5, which binds with high affinity to exposed phosphatidylserine (PS) on the surface of highly activated platelets. Importantly, PS has been shown to provide  $Ca^{2+}$ -mediated binding sites for coagulation factors to assemble. In confirmation of this, our data show that the labeling of OG488-FXa on clots substantially overlapped with PS-exposing platelets (Figure 2). To evaluate this quantitatively, the Pearson's overlap coefficient, a value from -1 to +1 which ranks the pixel overlap of two fluorescent colors, was determined. Quantitatively, the overlap between annexin A5 and FXa was confirmed by a high Pearson's overlap coefficient,  $R_r$ , be-



**Figure 2.** Localization of factor Xa (FXa) and pro(thrombin) on blood clots. Clots were formed by co-perfusion of blood with  $Ca^{2+}/Mg^{2+}$ /tissue factor and labeled with annexin A5 (red) or OG488-labeled coagulation factors (green). A, Fluorescent images of FXa, prothrombin (Prothr), thrombin (Thr), and annexin A5 binding. B, Pearson's correlation coefficient showing overlap between PS-exposing platelets and green labels. Mean $\pm$ SEM.



**Figure 1.** Clots formed in the absence or presence of  $\gamma'$ -fibrinogen support thrombin binding. Fibrinogen deficient plasma was supplemented with  $\gamma$ A- and  $\gamma'$ -fibrinogen (top) or  $\gamma$ A-fibrinogen (bottom) and added to washed platelets and red blood cells to make reconstituted blood. Reconstituted blood was co-perfused with  $Ca^{2+}/Mg^{2+}$ /tissue factor into capillary tubes. Formed clots were post-labeled with OG488-thrombin and imaged with differential interference contrast (DIC) and fluorescence microscopy.

tween labels of 0.56. In parallel, when post-labeling was performed with prothrombin, strong overlap between prothrombin and PS-exposing

platelets was observed, resulting in a Pearson's correlation of 0.56 (Figure 2). In marked contrast, OG488-thrombin distributed over clots and fibrin fibers, with a low overlap ( $R_r = 0.10$ ) with annexin A5 (Figure 2).

Together, these results point to an initial binding of prothrombin to PS-exposing platelets, after which it redistributes to the platelet-fibrin thrombus once cleaved into thrombin.

In December 2010, I defended my graduate thesis, earning my Ph.D. in Biomedical Engineering. My studies on the mechanisms of thrombin-clot interactions, supported by the AAAS, were integral to my thesis.