

P2Y₁₂ or P2Y₁ inhibitors reduce platelet deposition in a microfluidic model of thrombosis while apyrase lacks efficacy under flow conditions.

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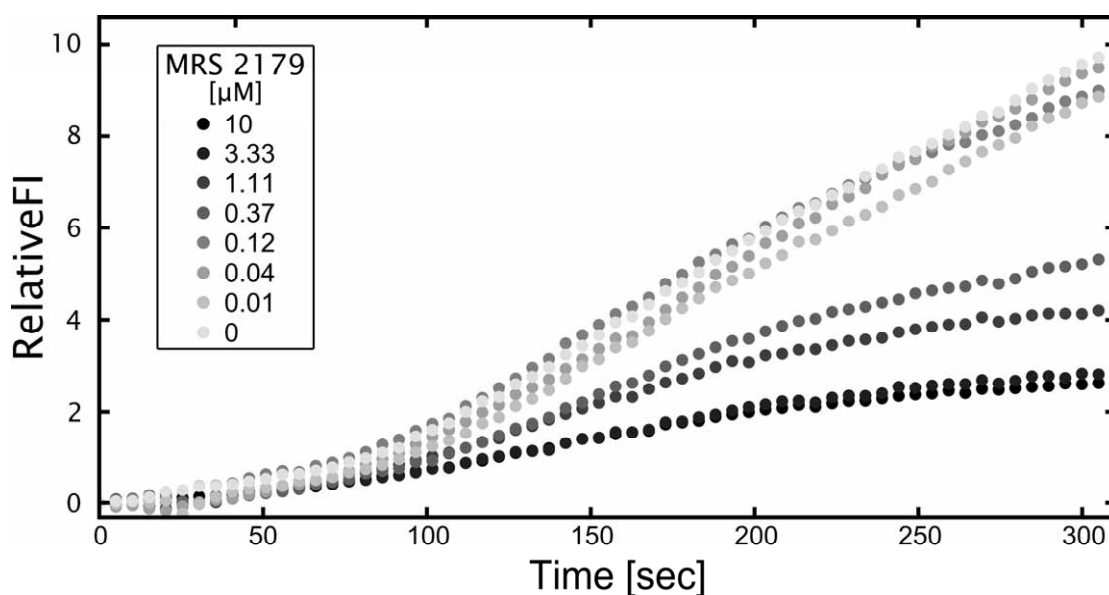


Fig. S1: Kinetic traces of fluorescent platelet accumulation with time with different concentrations of inhibitor. Dynamics of platelet accumulation on collagen display dose-dependence behavior that was shown as micrographs in Figure 3 at 0, 60, 120, and 300 seconds at varying concentrations of MRS 2179 under both CTI and PPACK anticoagulation. It is important to note that the initial adhesion events within the first 60 to 90 seconds are essentially unaffected by inhibitor concentration, however subsequent platelet layers are prevented from activating at the higher inhibitor concentrations, leading to a plateau in fluorescence intensity, while the uninhibited samples continue with a net increase in bound platelets.

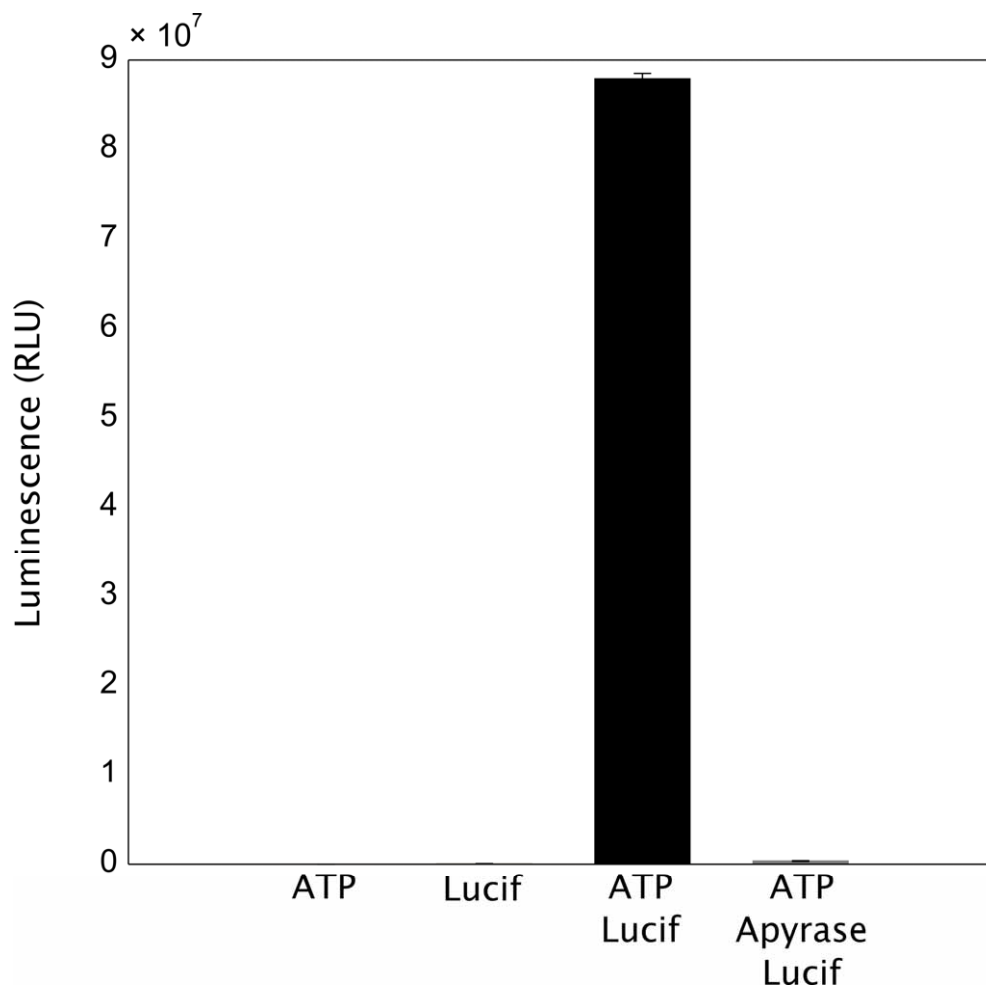


Fig. S2: Apyrase activity verification. Verification of the apyrase activity was performed by incubating 0.5 U/mL apyrase with 5 μ M ATP for 10 minutes prior to addition of luciferase reagent (Cell Titer-Glo, Promega Corporation, Madison, WI) and measured on a luminescent plate reader (EnVision, PerkinElmer, Waltham, MA). Controls of ATP in buffer, luciferase reagent in buffer, and ATP with luciferase reagent without apyrase are shown for comparison ($n = 3$, $p < 0.01$ between ATP with luciferase and ATP preincubated with apyrase and luciferase).

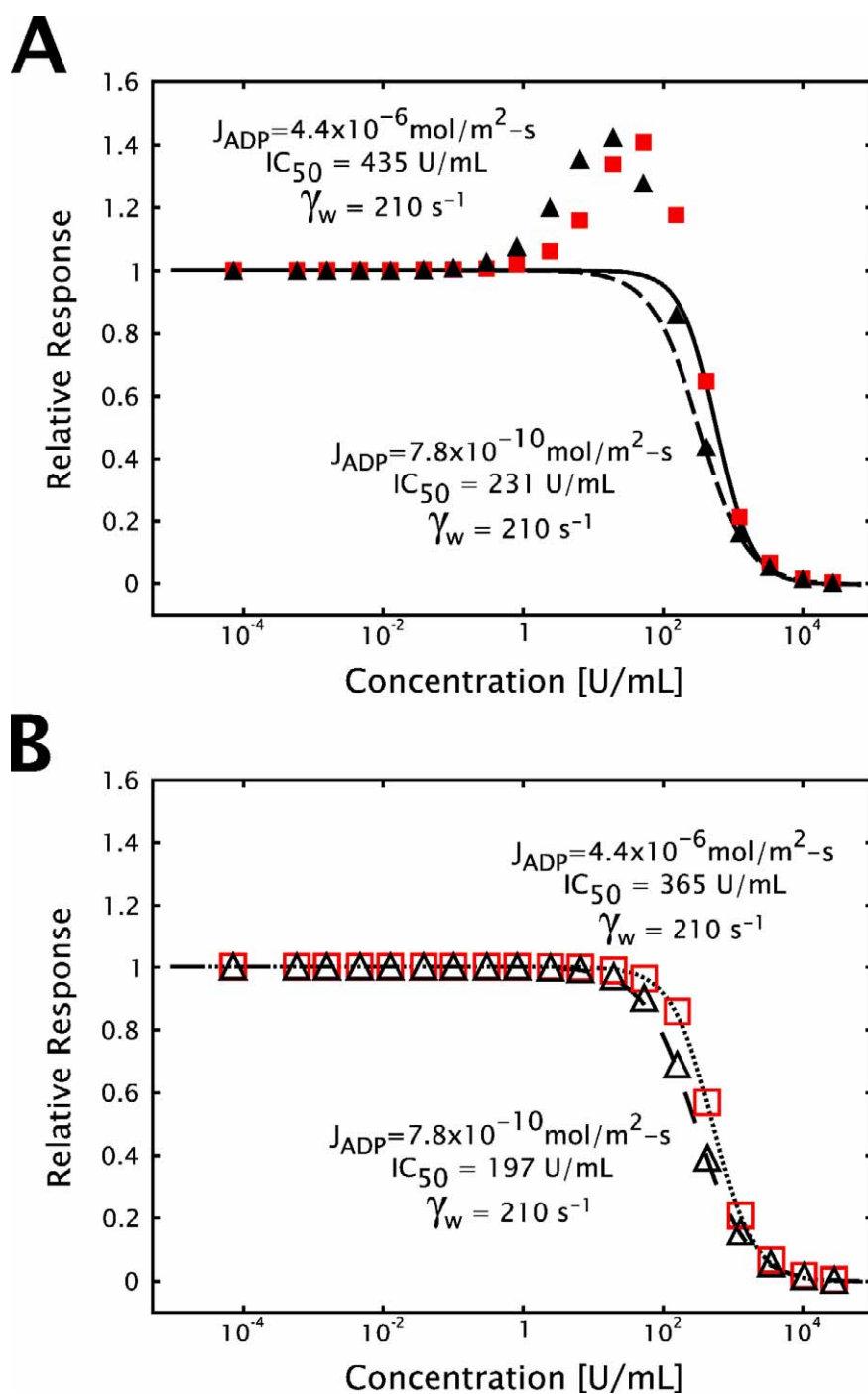


Fig. S3: Flux insensitivity of open model system. Since estimates of ADP flux from platelets accumulating at a growing thrombus vary in the literature over four orders of magnitude, the numerical simulation of the open system was run at two values (high, from Neeves, LOC, 2008, $4.4 \times 10^{-6} \text{ mol/m}^2\text{-s}$ shown in red squares, and low, Badimon, Circulation, 1988, $7.8 \times 10^{-10} \text{ mol/m}^2\text{-s}$ shown in black triangles) with unequal (A, closed symbols) and equal (B, open symbols) kinetic rates for ATP and ADP hydrolysis. The slight shifts in calculated IC_{50} s indicate that the behavior is insensitive to the flux at the surface.

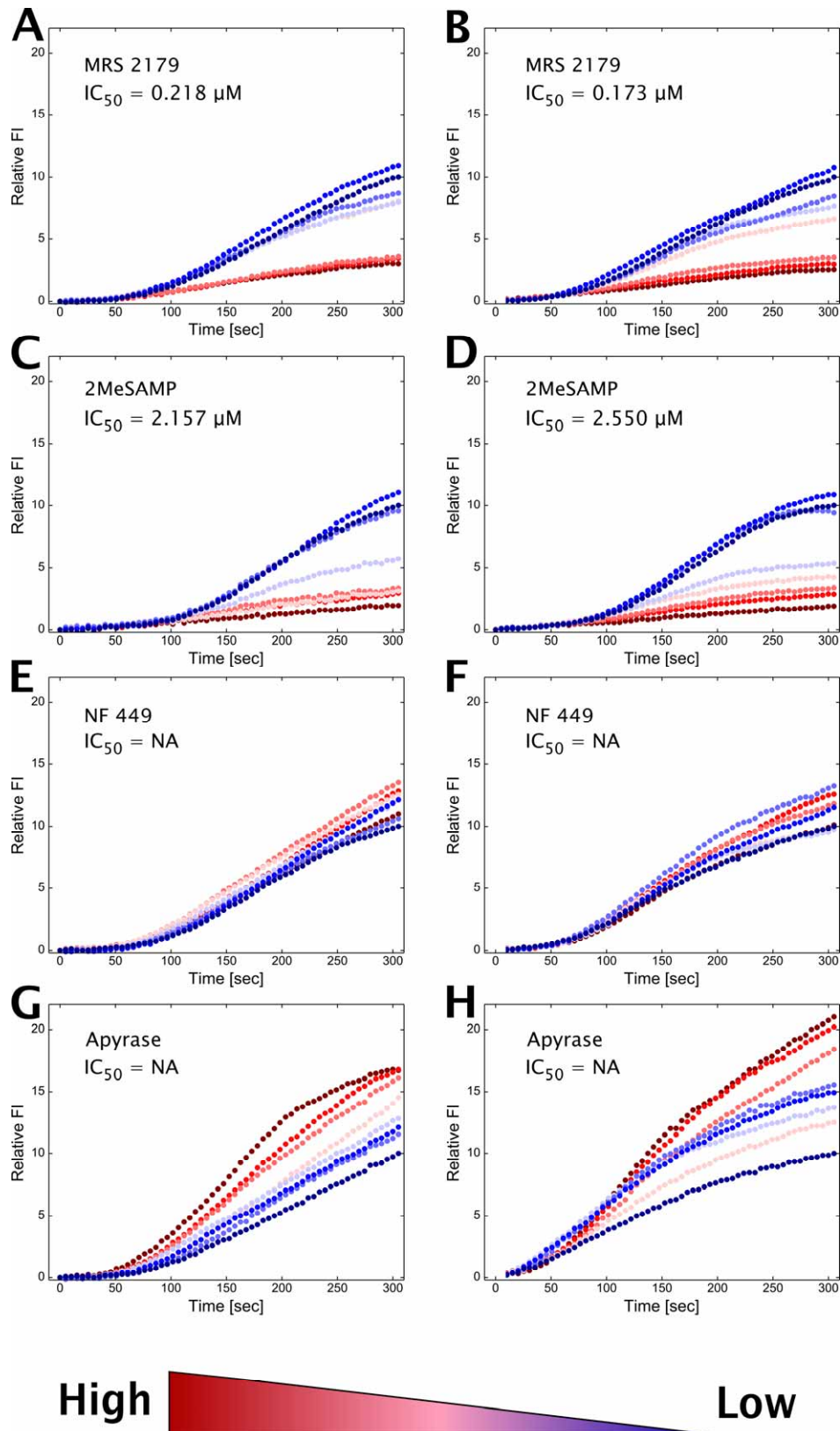


Fig. S4: Kinetic traces of platelet deposition for each inhibitor. Representative kinetic traces of each inhibitor without (A, C, E, G) or with (B, D, F, H) thrombin inhibition ($100 \mu\text{M}$ PPACK) are shown. IC_{50} curves were calculated using the

intensity at 300 sec for MRS 2179 (0 – 10 μ M), 2-MeSAMP (0 – 200 μ M), NF 449 (0 – 10 μ M) and apyrase (0 – 100 U/mL).