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Supporting Material

Dissociation of Bimolecular αIIbβ3-Fibrinogen Complex under a Constant Tensile Force

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Supplementary material
to the paper by Litvinov et al. "Dissociation of Bimolecular αIIbβ3-Fibrinogen Complex under a Constant Tensile Force"

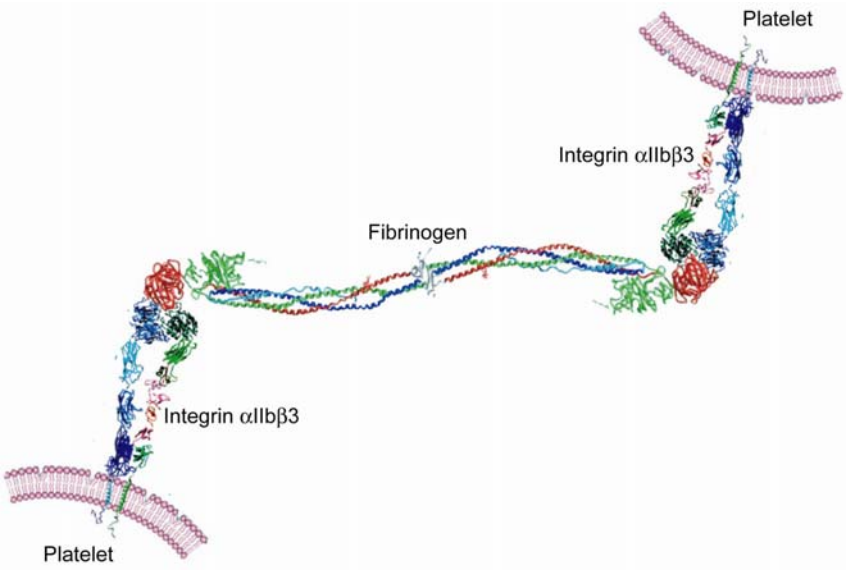

Figure S1. Cartoon of the integrin-fibrinogen interactions based on their crystal structures.

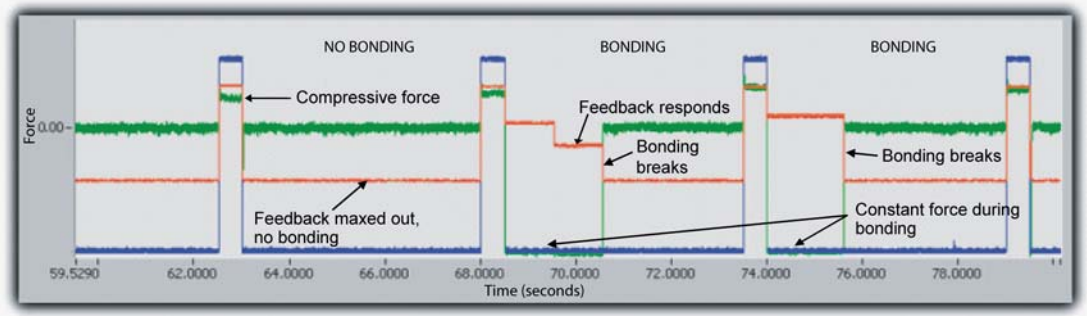

Figure S2. Data trace of αIIbβ3-fibrinogen interactions under a constant tensile force. The lines represent an actual force (green), a command signal (blue), and a feedback output (red). Pictured above are two separate bonding events, each with lifetimes of approximately 2 seconds. Notice how the feedback enables the system to maintain a constant force (green) during bonding.

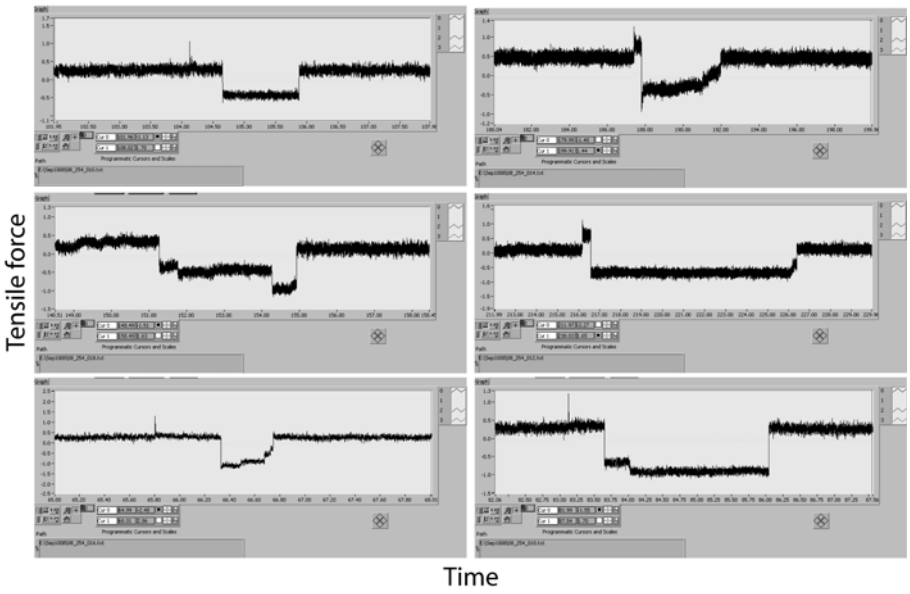

Figure S3. Different unbinding scenarios without tensile force clamp.

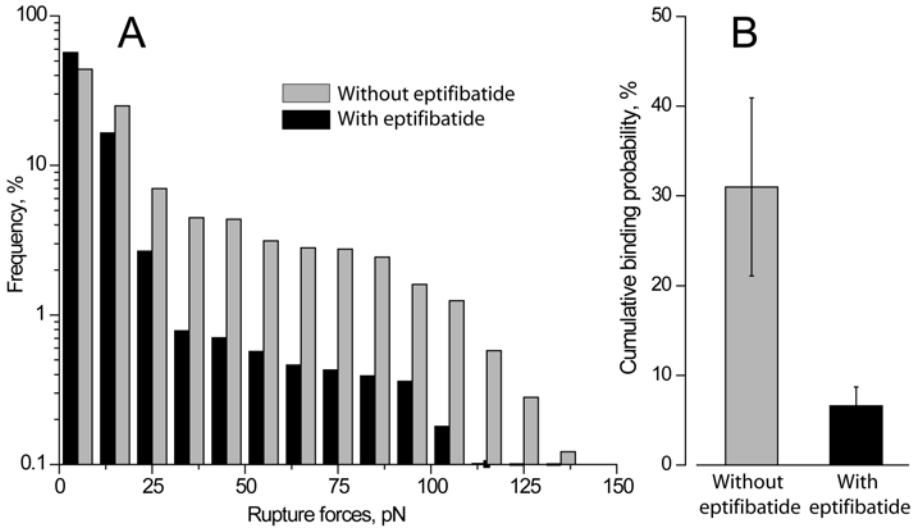

Figure S4. Testing the binding activity and specificity of the αIIbβ3 and fibrinogen preparations. (**A**) Rupture force spectra of the αIIbβ3-fibrinogen interactions in the absence and presence of 100 μg/ml eptifibatide, a specific αIIbβ3 antagonist. (**B**) Comparison of the cumulative binding probability for the αIIbβ3-fibrinogen interactions (forces >20 pN) derived from the histograms displayed in panel A.

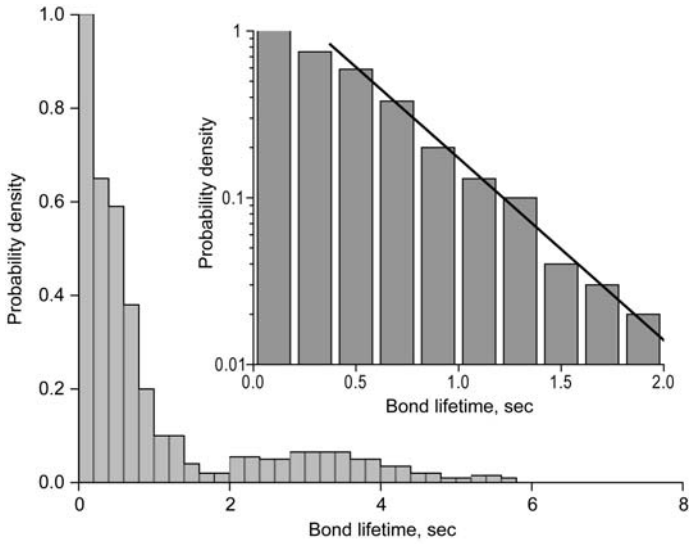

Figure S5. Histogram of bond lifetimes for the αIIbβ3-fibrinogen interactions measured under a constant tensile force. Inset – semi-logarithmic plot of the probability density distribution for the short interactions (<2s), showing the exponential function $p_u(t) = k_u \exp[-k_u t]$ to fit this portion of the histograms.

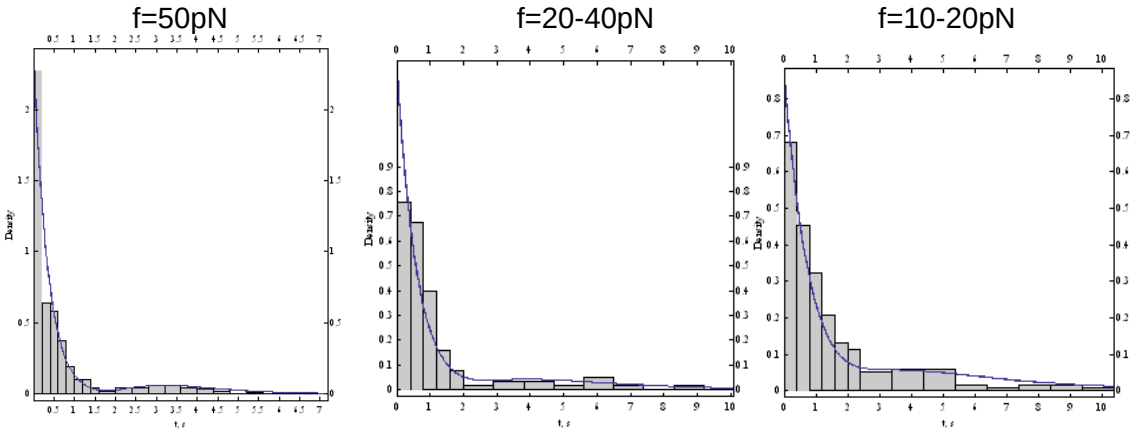

Figure S6. Histograms of bond lifetimes (bars) and fitting curves (solid line) obtained at different tensile forces.

Table S1

Control for non-specific interactions performed with various surface pairs other than integrin-fibronogen

Interacting surfaces	*n*	*Binding frequency*	*Bond lifetime range, s*
Plain-Plain	612	1.9%	0.008-0.061
Integrin-Ethanolamine	498	2.0%	0.008-0.093
Integrin-BSA	549	2.2%	0.008-0.480
BSA-Ethanolamine	621	0.9%	0.008-0.624
BSA-BSA	612	5.3%	0.008-0.371
BSA-Fibrinogen	622	1.3%	0.008-0.341
Ethanolamine-Ethanolamine	940	0.3%	0.008-0.038
Ethanolamine-BSA	531	1.1%	0.085-0.680
Ethanolamine-Fibrinogen	625	1.8%	0.011-0.554

Notes.

1. Characterization of the interacting control surfaces:

- non-activated uncoated silica pedestals against non-activated uncoated latex beads (plain-plain);
- glutaraldehyde-activated integrin-coated pedestals against EDAC-activated ethanolamine-coated beads (integrin-ethanolamine);
- glutaraldehyde-activated integrin-coated pedestals against EDAC-activated BSA-coated beads (integrin-BSA);
- glutaraldehyde-activated BSA-coated pedestals against EDAC-activated ethanolamine-coated beads (BSA-ethanolamine);
- glutaraldehyde-activated BSA-coated pedestals against EDAC-activated BSA-coated beads (BSA-BSA);
- glutaraldehyde-activated BSA-coated pedestals against EDAC-activated fibrinogen-coated beads (BSA-fibrinogen);
- glutaraldehyde-activated ethanolamine-coated pedestals against EDAC-activated ethanolamine-coated beads (ethanolamine- ethanolamine);
- glutaraldehyde-activated ethanolamine-coated pedestals pedestals against EDAC-activated BSA-coated beads (ethanolamine-BSA);
- glutaraldehyde-activated ethanolamine-coated pedestals against EDAC-activated fibrinogen-coated beads (ethanolamine-fibrinogen).

2. We also performed control inhibitory experiments in the presence of fibrinogen or αIIbβ3 in solution, but the results were not included in the paper for the following reasons. When free fibrinogen or αIIbβ3 were inserted into a chamber at nM to mM concentrations, the fibrinogen- and αIIbβ3-coated surfaces became even more reactive and stickier and displayed a complex multimodal bond lifetime distribution. It was impossible to discriminate between integrin-fibrinogen interactions, on the one hand, and fibrinogen-fibrinogen or αIIbβ3-αIIbβ3 interactions, on the other hand. Therefore, these traditional competitive inhibition experiments did not seem to work for fibrinogen and αIIbβ3 because of the large size of the molecules and their tendencies to oligomerize and adhere to artificial surfaces.