On the Activation of Integrin αIIbβ3: Outside-in and Inside-out Pathways

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Supporting Material

Supporting Methods

Comparison of IMC interactions in integrin versus integrin-talin systems

With the end state of the system from the integrin-talin binding, twelve simulations were run with the following procedure. The final system coordinates were minimized to re-randomize the initial velocities and then equilibrated for 5 ns. Next, 5 ns of steered molecular dynamics (SMD) simulations were run with the following parameters: k = 0.05 kcal/mol/Å^2 (i.e. the dummy spring placed between the dummy atom moving with a constant velocity and the steered atom) and velocity = 10 Å/ns. The spring constant and pulling velocity were reduced to allow for potential conformational changes to occur that would affect the necessary force required to maintain the pulling of the dummy atom. The atom being pulled on is the carbon-α of R995 (the αIIb subunit's contribution to the Inner Membrane Clasp, or IMC). Furthermore, the other two residues on the $\beta 3$ subunit (D723 and E726) of the IMC were fixed.

With integrin αIIbβ3 alone, the same steps were taken excluding talin and the steps needed to bind talin to integrin. Twelve simulations were run first to minimize and equilibrate for 5 ns. Next, the same pulled and fixed residues were used to conduct steered molecular dynamics for 5 ns.

Analysis of results between integrin versus integrin-talin systems

For the first step in the binding of integrin and talin, visual inspection and energy plots were used to assess the tightness of the binding. For the comparison of integrin to integrin-talin systems, besides qualitative analyses, three parameters were used to compare those results quantitatively: the force and work required to break the IMC, the distances of the IMC residues prior to the pulling/fixing simulations, and the initial energies of the IMC residues prior to the pulling/fixing simulations.

To calculate the force required to break the IMC, the van der Waals (VdW), Electrostatic, and total non-bonded (VdW + Electrostatic) energies were calculated between the $\beta 3$ subunit residues D723/E726 and the αIIb subunit residue R995 throughout the entire simulation. The force corresponding to when the energy of interaction is consistently above 10 kcal/mol is defined as the force required to break the IMC. The work to separate the IMC was calculated using the scalar projection of the force vector to the velocity of the pulled atom (R995 carbon-α), integrated over the time required to separate a particular distance. The initial energy of the IMC prior to the pulling/fixing simulation was also recorded from that data with 100 ps window-averaging. The initial distance was also calculated as the average distance between the carbon-α atoms of D723-R995 and E726-R995 from the entire distances of these atoms throughout the

simulation with 100 ps window-averaging. In addition, the interaction energies between K320 on talin and D723 on the integrin β3 were calculated.

Finally, a permutation two-sample t-test was used to check the statistical significance of the differences in those parameters between the two conditions, integrin-talin or integrin alone, with a sample size of 12 values in each condition. A permutation t-test follows a standard unpaired two sample t-test with the following equation:

$$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \quad (1)$$

where $\overline{x_i}$ = mean of the values of the i^{th} condition
s_i = standard deviation of the values of the i^{th} condition
n_i = number of values in the i^{th} condition ($n_1 = n_2 = 12$ in our case),

except that a permutation t-test does not require the assumption of a Gaussian distribution to determine the p-value for significance (34). In our permutation test, we generated the distribution of t-values for every possible condition switch arrangement. Then, we examined if the actual t-value is greater than 95% of the permuted t-values of this distribution for a one-tailed test, or the absolute t-value is greater than 95% of the absolute permuted t-values for a two-tailed test. We performed a one-tailed test because we hypothesized that our parameters would shift in one direction, which with reference to the integrin-only condition, would be towards a lower IMC binding energy (i.e. larger distance, less force to separate) for the integrin-talin condition.

Supporting Tables

	α-Subunit	β-Subunit
RGD1 (Near β-propeller-βA Interface)	E48	P170
RGD2	S46, Q47, E48, R153, E157	P170
RGD3	D232, D628, D817, R897	D127, E312, N313, K600, K611, K650, E671

Table S1: Several binding sites for RGD peptides on integrin were found. Most binding sites were near the βA-βTD pocket.

	cyto-β3	NPLY	W739-NPLY	A710-NPLY	NPLY-T762
1. Interaction Energy	-0.81, 0.0049**	-0.62, 0.0537	-0.77, 0.0092**	-0.45, 0.1869	-0.82, 0.0038**
2. R734-E1006 Distance	-0.70, 0.0251*	-0.54, 0.1076	-0.67, 0.0330*	-0.26, 0.4671	-0.65, 0.0425*

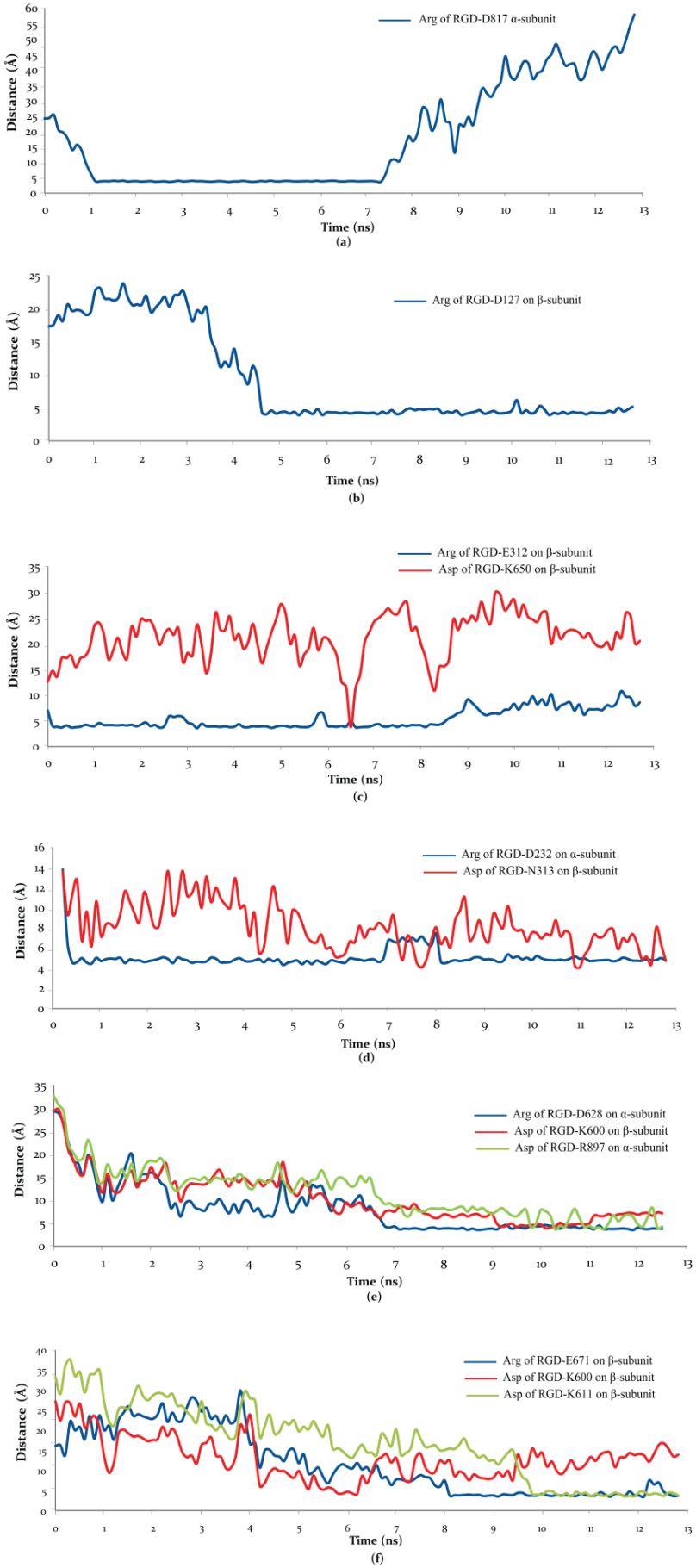
Table S2: Correlational values (presented in the format of "rho, p-value") using Spearman's rho of the distance between the centers of mass with selected regions of the integrin β3-subunit and the talin-1 F3 domain to: (1) the energy of interaction between R734 and E1006; (2) the distance between most terminal carbons on R734 and E1006. This table shows that using energy of interaction to quantify the additional interaction of R734-E1006 produces a more stable and significant result than does distance. A comparison of these correlational values suggests that talin binding to the membrane-distal region of the β3 tail (NPLY-T762) is most important in weakening the additional interaction. (* = $p < 0.05$, ** = $p<0.01$).

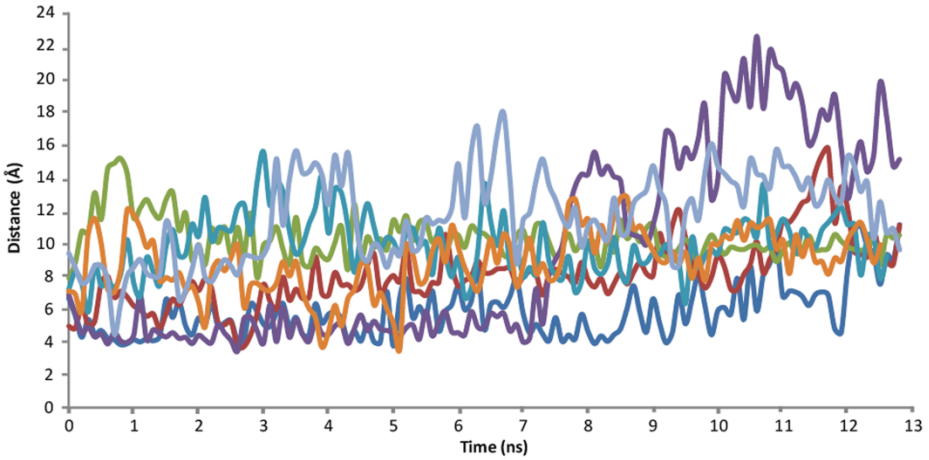
		1. αIIb-β3 extracellular domains	2. βTD-βA domains
Centers of Mass Distance		-0.12, 0.75	-0.26, 0.47
Interaction energy	VdW	0.41, 0.24	0.26, 0.47
	Electrostatic	0.05, 0.88	-0.21, 0.56
	Total Non-bond	0.05, 0.88	-0.27, 0.45

Table S3: Correlational values (presented in the format of "rho, p-value") using Spearman's rho of the distance between the center of mass with the cytoplasmic domain of the integrin β3 subunit and the talin-1 F3 domain to the center of mass distance or interaction energies of (1) αIIb and β3 extracellular domains and (2) βTD and βA domains. This table shows that there are no significant changes to the extracellular domains of integrin with relation to talin binding to integrin.

Supporting Figures

Figure S1: Distance between interacting residues of integrin with the RGD peptide for RGD-included runs. (a) and (b) represent the type1 interaction with only one permanent bond between the Arg of the RGD peptide and integrin. (c) and (d) are corresponding to the type2 interaction, wherein the Arg of the RGD interacts permanently with integrin while the Asp of the RGD peptide interacts only temporarily with another single residue of integrin. (e) and (f) show the cases where the Arg of the RGD permanently bound to a residue of integrin and the side chain of the Asp of the RGD switched back and forth between two other residues of integrin, which is called type3 binding.





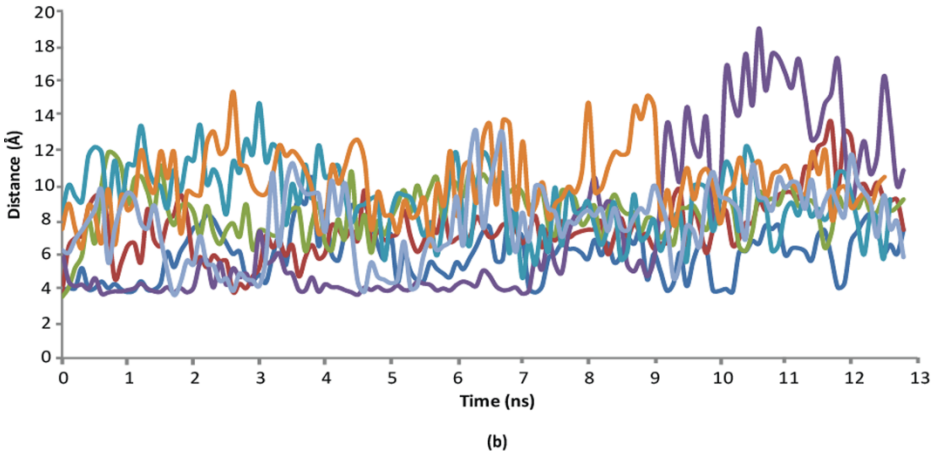


Figure S2: Disruption of the key interaction group K350-S673/674 detaches the βTD from the βA domain. (a) Distance between S673 and K350 as a function of the simulation time for six RGD-included as well as one non-RGD run (light blue). The simulation that led to βA-βTD detachment is shown in purple. The interaction is disrupted at ~7.5ns (b) Distance between S674 and K350 as a function of the simulation time for six RGD-included as well as one non-RGD run (light blue). The simulation that led to βA-βTD detachment is shown in purple.

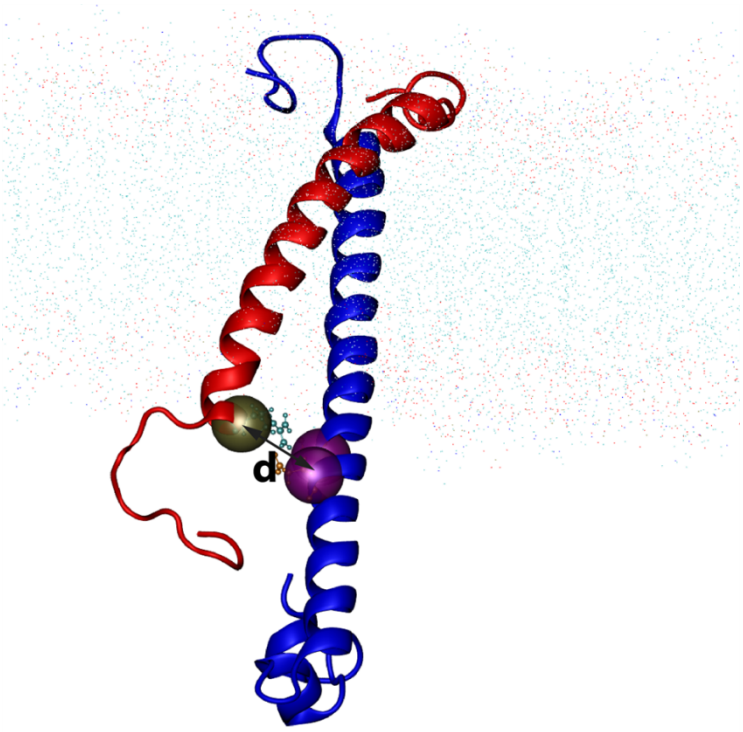


Figure S3: The tan atom is the pulled atom, and the two purple atoms are the fixed ones. This is the same for integrin-talin or integrin-only systems. Distance "d" is the distance measured between the IMC residues.