SUPPLEMENTAL DATA

Molecular dynamics simulations of forced unbending of integrin $\alpha_V \beta_3$

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Supplemental Methods

Building complete ectodomain models of unliganded and liganded integrin $\alpha_V \beta_3$

The first crystal structure of unliganded integrin $\alpha_V \beta_3$ ectodomain (PDB code 1U8C (Xiong et al., 2004)) did not resolve the α_V EE' loop (residue 839-867), the β_3 PSI-hybrid linker (residue 51-53), and the β_3 EGF1 and EGF2 domains (residue 435-522). The EE' loop contains the proteolytic site after Arg860 that generates the heavy and light chains in matured α_V (Suzuki et al., 1987). Because this loop is flexible and does not interact with other domains, we left it out in our simulations. To add the missing EGF1 and EGF2 domains, we used the crystal structure of a β_2 fragment (PDB code 2P28 (Shi et al., 2007)) as a template because β_2 and β_3 have ~41% sequence identity and ~54% similarity in the region containing the PSI, EGF1, and EGF2 domains (Fig. S1A). The hybrid and EGF3 domains in the 1U8C structure was first aligned to the corresponding domains in the 2P28 structure (Fig. S1B). MODELLER (Sali and Blundell, 1993) was used to build the PSI, EGF1, and EGF2 domains with the hybrid and EGF3 domains fixed. PSI was taken into account to avoid clashes between PSI and EGF1 and assure a longdistance disulfide bond between PSI and EGF1 (Cys13-Cys435). The β_3 fragment model so obtained includes the PSI, hybrid, EGF1, EGF2, and EGF3 domains, which had similar quality as the β_2 template according to the discrete optimized protein energy (DOPE) (Fig. S1F).

Because the β_2 template is in an extended conformation, our initial β_3 model was also extended. To fit this β_3 model into the bent $\alpha_V\beta_3$ structure, we performed a TMD simulation

without solvation using AMBER8 (Case et al., 2004). The extended model was first equilibrated for 1 ns (Fig. S1E). The hybrid domain in the β_3 model was then forced to move towards the hybrid domain of the bent $\alpha_V\beta_3$ with a force constant of 0.01 kcal mol⁻¹ Å⁻² with the EGF3 domain constrained. In the first ns of the bending simulation, the β_3 model gradually bent over (Fig. S1C) and the RMSD relative to the bent structure for all the heavy atoms of the PSI, hybrid, and EGF3 domains decreased from ~100 Å to ~9 Å. The PSI, hybrid, and EGF3 domains were next forced to move towards the corresponding domains in the bent $\alpha_V\beta_3$ with a force constant of 0.1 kcal mol⁻¹ Å⁻². Finally, the force constant was increased to 1 kcal mol⁻¹ Å⁻², yielding the final RMSD of ~1 Å. The bent β_3 model so obtained had no major conflicts with the α_V subunit in the 1U8C structure (Fig. S1D). The PSI-hybrid linker (residue 51-53) and the EGF1 and EGF2 domains (residue 435-522) from the bent β_3 model were added to the original 1U8C structure to generate a complete model of the unliganded integrin $\alpha_V\beta_3$ ectodomain (i.e. U1).

The crystal structure of integrin $\alpha_V\beta_3$ ectodomain liganded with a cyclic-RGD ligand (PDB code 1L5G (Xiong et al., 2002)) did not resolve the α_V EE' loop (residue 839-867), the β_3 PSI domain (residue 1-54), and the β_3 EGF1 and EGF2 domains (residue 435-531). Because the 1L5G structure is nearly identical to the 1U8C structure except for regions near the ligand-binding site at the β A domain, we simply added the PSI domain (residue 1-56) and the EGF1, EGF2, and part of EGF3 domains (residue 435-548) from the bent β_3 model to the 1L5G structure to obtain a complete model for the liganded integrin $\alpha_V\beta_3$ ectodomain (i.e. L1).

Supplemental Figure Legends

Figure S1. Building complete ectodomain models of integrin $\alpha_V \beta_3$. A. Amino acid sequence alignment between β_2 and β_3 in the region containing the PSI, EGF1, and EGF2 domains with ClustalW2 (Larkin et al., 2007). B. Alignment of the template and the homology model. On the left, the hybrid and EGF3 domains (blue) from the crystal structure of integrin $\alpha_V \beta_3$ (PDB code 1U8C) were aligned to the template (gray) of the β_2 fragment (PDB code 2P28) before homology modeling. On the right, the final homology model (red) was compared to the template. C. Targeting the extended β_3 model (red) to the bent β_3 structure (gray) by TMD simulation. Snapshots were taken at indicated times. D. The final bent β_3 model (red) in the bent $\alpha_V \beta_3$ structure (α_V , yellow; β_3 , gray). E. RMSD relative to the bent structure for all heavy atoms of the PSI, hybrid, and EGF3 domains during the TMD simulation. F. Comparison of the DOPE score of the homology model with the template.

Figure S2. Comparison of the homology model of the EGF1 and EGF2 domains with the crystal structure. On the left and middle, structures were aligned on C α atoms of other parts of β_3 than the EGF1 and EGF2 domain. On the right, the EGF1 and EGF2 domains were aligned on their own C α atoms. The homology model is in red and the crystal structure (PDB code 3IJE) is in blue.

Figure S3. Changes in headpiece-tailpiece interactions interactions at the major force peaks. Buried SASAs (upper row, left ordinate) and numbers of H-bonds (lower row, left ordinate) of hybrid (blue), βA (cyan), EGF4 (red), and βTD (magenta) domains as well as pulling force (gray, both rows, right ordinate) were plotted vs. simulation time for the U1 SMD 2 (left column) and U1 SMD 3 (right column). Some of the curves were obscured due to overlapping.

Figure S4. H-bonds at the hybrid/EGF4 interface of U2. The starting structures at the hybrid/EGF4 interface of U2 are shown. Residues involved in H-bonds are shown as sticks. H-bonds are indicated by dashed lines. The hybrid and EGF4 domains are colored in orange and tan, respectively.

Figure S5. Changes in headpiece-tailpiece interactions during the unbending of the liganded $\alpha_V\beta_3$. Buried SASAs (colored, left ordinate) of indicated domains were plotted vs. simulation time along with pulling force (gray, right ordinate) in L1 SMD 1 and L2 SMD.

Figure S6. Amino acid sequence alignment of the α_V subunit. The sequences were aligned across species (A) or human α family members (B) by using ClustalW2 (Larkin et al., 2007). The sequences were retrieved from UniProt Knowledgebase (UniProtKB) (Jain et al., 2009). Conserved mutations at residue 457 and the α -genu metal ion site are highlighted. "*" indicates identical residues, ":" indicates conserved substitutions, and "." indicates semi-conserved substitutions.

Supplemental Movie Legends

Movie S1. Unbending of unliganded integrin $\alpha_V \beta_3$ in U1 SMD 1. The COM of the C α atoms of the central β sheet of the βA domain was pulled and the COM of the C α atoms of the βTD

was constrained. The running simulation time is indicated. The α_V subunit was in cyan and the β_3 subunit was in pink. The same color coding was used in all movies.

Movie S2. Unbending of unliganded integrin $\alpha_V \beta_3$ in U2 SMD. The COM of the C α atoms of the central β sheet of the βA domain was pulled and the COM of the C α atoms of the βTD was constrained.

Movie S3. Rebending of partially-extended unliganded integrin $\alpha_V \beta_3$ in U1 free MD 1. The simulation started from the partially-extended structure that was selected from U1 SMD 1. Nothing was constrained during the simulation. The bent structure is in gray for comparison.

Movie S4. Relaxation of fully-extended unliganded integrin $\alpha_V\beta_3$ in U1 free MD 2. The simulation started from the fully-extended structure that was selected from U1 SMD 1. Nothing was constrained during the simulation.

Movie S5. Unbending of liganded integrin $\alpha_V \beta_3$ in L1 SMD 1. The COM of C α atoms of the RGD ligand (green spheres) was pulled and the COM of the C α atoms of the β TD was constrained.

Movie S6. Unbending of liganded integrin $\alpha_V \beta_3$ in L2 SMD. The COM of the C α atoms of the RGD ligand (green spheres) was pulled and the COM of the C α atoms of the β TD was constrained.

Supplemental Reference

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Supplemental Figure S1 Click here to download high resolution image







Supplemental Figure S4 Click here to download high resolution image





Supplemental Figure S6 Click here to download high resolution image

A

Residue 457

tr10425981042598_XENLA	ENGYPOLLVGAFGADKAILYRARPVITVTSLLEVNPTILNPESKTCILP- 483	
tr BIWAV5 BIWAV5_XENTR	ENGYPOLLVGAFGADKAILYRARPVITVTSLLEVNPTILNPESKTCSLP- 361	
tr D2SYX8 D2SYX8_CAMDR	ENGYPDLIVGAFGVDRAVLYRARPVITVNAGLEVYPSILNODNKTCPLPG 495	
tr A2RQD8 A2RQD8 PIG	KNGYPDLIVGAFGVDRAVLYRARPVITVNAGLEVYPSILNODNKTCPLPG 494	
SDIPSO7461ITAV BOVIN	KNGYPDLIVGAFGVDRAVLYRARPVITVNAGLEVYPSILNOFNKTCPLPG 495	
spip067561ITAV HUMAN	ENGYPOLIVGAFGVDRAILYRARPVITVNAGLEVYPSILNODNETCSLPG 495	
SDIP43406 ITAV MODSE	ENGYPOLVVGAFGVDRAVLYRARPVVTVNAGLEVYPSILNOLNKICPLPG 495	
SDIP260081ITAV CEICK	KNGYPDLIVGAFGVDTAVLYRARPVIRVNAALEVNPTILNEENKACSLA- 482	
triB3D103183D103 DANKE	ONGYPDLIVGAFGADKAILYRARPVISVNTTLDISPOILNEFOKSCTLPG 491	
	a nanu matal lon elte	
	a-genu metai ion site	
tr[042598]042598_XENLA	KSTADSSGLLPILNQFTPANITKQAHILLDCGEDNICKPSLKLSVESEQK 633	
tr BIWAVS BIWAVS_XENTR	KSTADSSGLLPILNQFTPTNITKQAHILLDCGEDNICKPS1KLSVESEQK 511	
tr D25YX8 D25YX8_CAMDR	RTAADATGLQP1LNQFTPANVSRQAH1LLDCGEDNVCKPKLEVSVDSDQK 645	
tr A2RQD8 A2RQD8_PIG	TTAADVTGLQPILNQFTPANISRQAHILLDCGEDNVCKPKLEVSVDSDQK 644	
sp[P80746]ITAV_BOVIN	RTAADATGLOPILNOPTPANVSROAHILLOCGEDNVCKPKLEVSVDSDQK 645	
spiP06756 ITAV HUMAN	RTAADTTGLOPILNOFTPANISROAHILLDCGEDNVCKPKLEVSVDSDQK 645	
sp P43406 ITAV_MOUSE	RTAADATGLOPILNOFTPANVSROAHILLDCGEDNVCKPKLEVSVNSDOK 645	
sp P26008 ITAV_CHICK	KTAVDATGLEPILNQFIPANMSRQAHILLDCGEDNICXPKLEVSVRSDQK 631	
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KSAADKTGLLPILDQSAPTNYTKQAHILLDCGEDNICKPDLKLSVVSDQN 640

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tr|B3DJQ3|B3DJQ5_DANRE

Residue 457

013349 ITAD_HUMAN	LRSLPVLKVGVAMRFSPVEVAKAVYRCWEEKP-SALEAGDATVCLTIQKSSLDQLG-DI	669
P20702 ITAX_BUMAN	LRTEPVLWVGV5MQFIPABIPESAFECERQVV-SEQTLVQSNICLVIDKR5KNLLGSEDL	671
P11215 ITAM_BUMAN	LRSQPVLRVKAIMEFNPREVARNVFECNDQVV-KGKBAGBVRVCLHVQKSTRDRLREGQ1	670
P20701 ITAL_HUMAN	LSSRPVVDMVTLMSFSPARIPVHEVECSYSTSNKMKEGVNITICPQIKSLIPQPQGRL	667
P56199 1TA1_BUMAN	FWSRDVAVVKVTMNFEPNKVNTOKKNCH-MEGKETVCINATVCFDVKLK-SKEDTIY	716
P17301 1TA2_EUMAN	LWSQSIADVAIEASFTPEKITUVNKNAQ	692
075578 TA10_HUMAN	LSSRPIVELTPSLEVTPOAISVVDRDCK-REGOBAVCLTAALCFOVTSR-TPGRWDE	694
09UKX5 ITA11_HUMAN	LWSRPVVQINASLHPEPSKINIPERDCK-RSGRDATCLAAFLCPTPIFL-APHFQTT	687
PO6756 ITAV_EUMAN	YRARPVITVNAGLEVYPSILNODNKTCSLPGTALKVSCPNVRFCLKADGKG	519
P\$370811TA8_EUMAN	YRARPVVTVDAQLLLHPMIINLTNKTCQVPDSMTSAACPSLRVCASVTGQS	534
PO864811TA5_EUMAN	YRGRPIVSASASLTIPPAMFNPLERSCSLEG-NPVACINLSFCLNASGKE-VAD-	538
PO8514 ITA2B_HUMAN	YRAQPVVKASVQLLVQDS-1NPAVKSCVLPQTKTPVSCFNIQMCVGATGBN	531
P23229 1TA6_BUMAN	PRSEPVINIQKTITVTPNEIDURDKTACGAPSGICLQVKSCPEY-TANPA-GYNP	553
013683 ITA7_HUMAN	FRARPILHVSHEVSIAPRSIDIE PNCAGGHS-VCVDLRVCFSY-IAVPS-SYSP	564
P26006 1TA3_BUMAN	LRARPVINIVHETLVP-RPAVLDPALCTATS-CVQVELCPAVNQSAGNPNYRR	510
P13612 ITA4_BUMAN	LRTEPVVIVDASLSHPESVNETKFDCVENGWPSVCIDLTLCFSYKGKEVPG	517
Q13797 1TA9_EUMAN	LRARPVITVDVSIFLPGSINITAPQCEDGQQPVNCLNVTTCFSFBGKEVPE	507
P38570 ITAE_HUMAN	FRSRPVVRLEVSMAPTPSALPIGFNGVVNVRLCPEISSVTTASESG	721
CONTRACTOR (CONTRACTOR)	and the second of the second	

a-genu metal ion site

Q13349 1TAD_BUMAN	COODGLC COLGVTLSFSG-	LQTLTVGSSLBLNVI	802
P20702 ITAX_BUMAN	CGADHICDONLGISFSFPG-		804
P11215 ITAM HUMAN	CONDNICODDLSITFSFMS		803
P20701 ITAL_BUMAN	CGEDKKCEANLRVSFSPAR-		804
P5619911TA1_BUMAN	CONKEKCI SDLSLEVATT-EKD	-LLIVRSQNDKFNVS	842
P1730111TA2 EUMAN	CGEDGLCI SDLVLDVRQI PAAQEQ		826
07557811TA10_HUMAN	CGPDNELVTDLVLQVNMD1RGSRK-		827
Q9UEX5 ITA11_HUMAN	INFORREVPOLVLDARSDLPTAMEYCORVLRKPAQDCSAYT	LSFDTTVFIIESTRQEVAVE	841
P06756 ITAV_BUMAN	CGEDNVCKPKLEVSVDSDQKK-	ITIGDDNPLTL1	658
P5370811TA8_EUMAN	EGEDNECVPDLKLSARPDKRQ		673
P0864811TA5_EUMAN	CGEDNICYPDLOLEVFGEONE-		677
P08514]1TA28_HUMAN	CGEDDVCVPQLQLTASVTGSP-	-LLVGADNVLELQ	665
P2322911TA6_HUMAN	CGDDNVCNSNLKLEYK-PCTREGNQDKPSYLPIQKG-		714
Q13683 ITA7_BUMAN	CGEDKICOSNLQLVBARFCTRVSDT-EFQPLPNDVDGT-	-TALFALSGOPVIGLE	732
P26006 ITA3_BUMAN	CGPDNKCESNLOMRAAFVSEQQQKLSRLQYS-	RDVRKLLLS	654
P13612 ITA4_EUMAN	FCABENCSADLQVSAKIGFLKPHEN		666
Q13797 1TA9_EUMAN	NCRSEDCAADLQLQGKLLLSSMDEK-	-TEYLALGAVENISLN	658
P38570 ITAE_BUMAN	EKNELF CVABLQLATTVSQQE	LVVGLTKELTLN	855
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