SUPPLEMENTARY INFORMATION

Conformational landscape of integrin α4β1 and its recognition of VCAM and conformation-stabilizing small molecules

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Supplementary Table 1. Cryo-EM data collection and refinement statistics.

Ligand	**α4β1 apo bent** (EMD-73431)	**α4β1 apo less bent** (EMD-73432)	**α4β1 apo headpiece** (EMD-73433) (PDB 9YST)	**α4β1–12G10–VCAMD1D3** (EMD-73434) (PDB 9YSU)	**α4β1–12G10–VCAMD1D3 core** (EMD-73435) (PDB 9YSV)
Data collection and processing					
Facility	**Harvard Cryo-EM Center for Structural Biology**				
Microscope	Titan Krios			Titan Krios	
Detector	Falcon 4i			Falcon 4i	
Magnification	165,000			165,000	
Voltage (kV)	300			300	
Electron exposure (e–/Å^2)	50			50	
Defocus range (μm)	-0.7 to -2.2			-0.7 to -2.2	
Pixel size (Å)	0.736			0.736	
Symmetry imposed	C1			C1	
Initial curated particle images (no.)	589,924			568,842	
Final particle images (no.)	50,810	68,872	343,751	106,786	195.804
Map resolution (Å)	3.1	3.2	2.2	2.4	2.2
FSC threshold	0.143	0.143	0.143	0.143	0.143
Refinement					
Initial model used (PDB code)			4IRZ, AlphaFold3	4IRZ, 7NWL, 9YST	9YSU
Model resolution (Å)			0.5	0.5	0.5
FSC threshold					
Model resolution (Å)			2.5	2.7	2.4
Map sharpening *B* factor (Å^2)			61.0	59.8	59.1
Model composition					
Non-hydrogen atoms			8397	11769	8102
Protein residues			1026	1458	994
Ligands			5 Ca^{2+}	5 Ca^{2+}	5 Ca^{2+}
			1 Mg^{2+}	1 Mg^{2+}	1 Mg^{2+}
			11 NAG	13 NAG	10 NAG
			1 BMA	1 BMA	1 BMA
B factors (Å^2)					
Protein (min/max/mean)			63.20/248.59/122.57	63.72/330.36/149.03	56.83/194.15/93.27
Ligand (min/max/mean)			88.41/193.43/152.55	96.66/222.74/176.24	80.23/191.46/152.68
R.m.s. deviations					
Bond lengths (Å)			0.003	0.003	0.003
Bond angles (°)			0.451	0.454	0.488
Validation					
MolProbity score			1.26	1.21	0.94
Clashscore			2.95	1.99	0.91
Poor rotamers (%)			0.68	0.56	0.48
Ramachandran plot					
Favored (%)			96.96	96.34	97.05
Allowed (%)			3.04	3.66	2.95
Disallowed (%)			0	0	0

Supplementary Table 1 (continuation). Cryo-EM data collection and refinement statistics.

Ligand	**α4β1–12G10–VCAMD4D6** (EMD-73436) (PDB 9YSW)	**α4β1–12G10–VCAMD4D6 core** (EMD-73437) (PDB 9YSX)	**α4β1–12G10** (EMD-73438) (PDB 9YSY)	**α4β1–12G10 core** (EMD-73440) (PDB 9YSZ)
Data collection and processing				
Facility	Harvard Cryo-EM Center for Structural Biology			
Microscope	Titan Krios		Titan Krios	
Detector	Falcon 4i		Falcon 4i	
Magnification	165,000		165,000	
Voltage (kV)	300		300	
Electron exposure (e–/Å^2)	50		50	
Defocus range (μm)	-0.7 to -2.2		-0.7 to -2.2	
Pixel size (Å)	0.736		0.736	
Symmetry imposed	C1		C1	
Initial curated particle images (no.)	298,831		362,110	
Final particle images (no.)	55,382	120,254	203,316	203,316
Map resolution (Å)	2.7	2.5	2.4	2.3
FSC threshold	0.143	0.143	0.143	0.143
Refinement				
Initial model used (PDB code)	9YSU, AlphaFold3	9YSW	9YSU	9YSU
Model resolution (Å) FSC threshold	0.5	0.5	0.5	0.5
Model resolution range (Å)	3.0	2.7	2.7	2.5
Map sharpening *B* factor (Å^2)	59.1	69.6	60.1	65.0
Model composition				
Non-hydrogen atoms	11,540	8,105	10,214	7,391
Protein residues	1,459	994	1261	904
Ligands	5 Ca^{2+} 1 Mg^{2+} 16 NAG 1 BMA	5 Ca^{2+} 1 Mg^{2+} 11 NAG 1 BMA	5 Ca^{2+} 1 Mg^{2+} 13 NAG 1 BMA	5 Ca^{2+} 1 Mg^{2+} 10 NAG 1 BMA
B factors (Å^2)				
Protein	77.67/336.34/155.06	78.06/201.91/116.79	79.00/348.01/153.50	65.04/184.74/101.47
Ligand	103.53/154.49/115.07	110.10/215.60/174.07	116.13/145.63/119.00	104.73/136.42/105.48
R.m.s. deviations				
Bond lengths (Å)	0.002	0.004	0.003	0.004
Bond angles (°)	0.460	0.487	0.458	0.487
Validation				
MolProbity score	1.25	1.16	1.16	1.01
Clashscore	2.60	1.82	2.10	1.22
Poor rotamers (%)	1.11	0.71	0.65	0.79
Ramachandran plot				
Favored (%)	96.96	96.65	96.97	96.99
Allowed (%)	3.04	3.35	3.03	3.01
Disallowed (%)	0	0	0	0

Supplementary Table 2. X-ray data collection and refinement statistics.

Ligand	**α4β1 *apo* Mg/Ca (PDB 9YOC)**	**α4β1 Bio-1211 Mg/Ca (PDB 9YOB)**	**α4β1 Bio-1211 Mn/Ca (PDB 9YOA)**	**α4β1 Firategrast Mg/Ca (PDB 9YO3)**	**α4β1 Firategrast Mn/Ca (PDB 9YO2)**
Beamline	ESRF ID30B	EMBL P13	EMBL P13	EMBL P13	EMBL P13
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell (a, b, c) (Å)	83.94, 109.65, 130.86	84.17, 109.80, 131.28	84.46, 109.64, 131.19	82.88, 109.39, 124.72	82.81, 110.49, 124.41
(α, β, γ) (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Wavelength (Å)	0.873128	0.97626	0.97624	0.97626	0.97626
Resolution (Å)	72.64-2.90/3.00-2.90[a]	35.56-3.00/3.10-3.00[a]	35.51-2.75/2.84-2.75[a]	62.36-2.15/2.22-2.15[a]	45.95-1.95/2.02-1.95[a]
Number of reflections (total/unique)	257,149/34,662	228,561/30,310	276,247/36,481	468,840/62,298	620,156/83,906
Completeness (%)	99.64/99.55[a]	99.69/99.51[a]	99.77/99.78[a]	99.48/98.81[a]	99.48/98.99[a]
$I/\sigma(I)$	5.3/0.6[a]	5.9/0.4[a]	5.9/0.3[a]	8.3/0.8[a]	8.1/0.7[a]
{R_{merge}[b]} or $CC_{1/2}$[c]	{0.22/2.83[a]}	{0.247/5.725[a]}	{0.19/0.2[a]}	{0.14/2.31[a]}	{0.131/2.687[a]}
R_{work}/R_{free}[d]	0.270/0.301	0.250/0.289	0.269/0.288	0.217/0.245	0.206/0.249
RMSD: Bond (Å)	0.004	0.009	0.008	0.004	0.010
Angle (°)	0.64	0.70	0.62	0.63	0.78
Ramachandran plot[e]	95.69/4.31/0.0	96.7/3.23/0.0	97.18/2.72/0.0	98.05/1.76/0.20	97.17/2.83/0
Molecules/ asymmetric unit	1	1	1	1	1
Conformational state[f] (molecule 1)	State 1	State 1	State 5	State 3	State 5
MolProbity score	1.20	1.13	1.11	0.81	1.15
Clash score	1.59	1.85	1.98	1.05	2.23

Supplementary Table 2 (continuation). X-ray data collection and refinement statistics.

Ligand	**α4β1 DS-13d Mg/Ca (PDB 9YO9)**	**α4β1 DS-13d Mn/Ca (PDB 9YO8)**	**α4β1 DS-13g Mg/Ca (PDB 9YO7)**	**α4β1 DS-13g Mn/Ca (PDB 9YO6)**	**α4β1 MR-128 Mg/Ca (PDB 9YO5)**	**α4β1 MR-128 Mn/Ca (PDB 9YO4)**
Beamline	EMBL P13	EMBL P13	EMBL P13	EMBL P13	ESRF ID30B	ESRF ID30B
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell (a, b, c) (Å)	83.47, 109.60, 125.03	83.24, 110.17, 123.71	83.35, 109.40, 125.26	83.46, 109.65, 125.37	83.75, 110.35, 126.60	83.53, 109.90, 125.58
(α, β, γ) (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Wavelength (Å)	0.97626	0.97624	0.97624	0.873128	0.873128	0.873128
Resolution (Å)	29.50-2.00/2.07-2.00[a]	38.93-2.53/2.62-2.53[a]	27.78-2.11/2.16-2.11[a]	82.52-2.22/2.29-2.22[a]	69.84-2.55/2.64-2.55[a]	69.55-2.22/2.29-2.22[a]
Number of reflections (total/unique)	684,033/90,962	292,706/38,645	502,938/66,495	436,922/57,581	292,438/39,005	433,650/57,881
Completeness (%)	99.85/99.90[a]	99.75/99.60[a]	99.82/99.79[a]	99.50/99.06[a]	99.94/99.87[a]	99.78/99.21[a]
I/σ(I)	7.7/0.8[a]	5.4/0.4[a]	8.8/0.8[a]	7.9/1.0[a]	7.9/1.0[a]	7.0/0.7[a]
{R_{merge}[b]} or $CC_{1/2}$[c]	{0.148/2.722[a]}	{0.235/4.513[a]}	{0.143/2.692[a]}	{0.223/2.420[a]}	{0.223/2.420[a]}	{0.161/2.712[a]}
R_{work}/R_{free}[d]	0.199/0.226	0.223/0.263	0.218/0.248	0.232/0.258	0.226/0.254	0.240/0.262
RMSD: Bond (Å)	0.007	0.005	0.003	0.003	0.004	0.009
Angle (°)	0.64	0.62	0.60	0.56	0.63	0.65
Ramachandran plot[e]	96.40/3.60/0.0	96.22/3.78/0.0	96.27/1.59/1.03	96.10/3.80/0.1	96.72/3.32/0.0	96.29/3.71/0.0
Molecules/ asymmetric unit	1	1	1	1	1	1
Conformational state[f] (molecule 1)	State 1	State 5	State 1	State 1	State 1	State 1
MolProbity score	1.09	1.03	1.32	1.10	1.03	0.97
Clash score	1.29	0.93	2.74	1.18	1.16	0.68

[a] Numbers correspond to the last resolution shell.

[b] $R_{merge} = \sum_h\sum_i|I_i(h) - \langle I(h)\rangle| / \sum_h\sum_i|I_i(h)$, where $I_i(h)$ and $\langle I(h)\rangle$ are the ith and mean measurement of the intensity of reflection *h*.

[c] $CC_{1/2}$ = correlation coefficient between two random half datasets[72].

No I/σ cutoff was applied.

[d] $R_{work} = \sum_h||F_{obs}(h)| - |F_{calc}(h)|| / \sum_h|F_{obs}(h)|$, where $F_{obs}(h)$ and $F_{calc}(h)$ are the observed and calculated structure factors, respectively; R_{free} is the R value obtained for a test set of reflections consisting of a randomly selected 1 % subset of data excluded from refinement.

[e] Residues in favorable, allowed, and outlier regions of the Ramachandran plot as reported by MolProbity.

[f] Conformational states were assigned by superimposing the βI domain of the structure of interest with 8 discrete crystallographic snapshots observed (Lin, K. et al. “Selective, tight-binding inhibitors of integrin α4β1 that inhibit allergic airway responses”. J. Med. Chem. 42, 920–934 [1999])., with state 1 being the closed, unliganded control, and state 8 being the fully open, liganded state. There are 6 intermediate states in between states 1 and 8.

Mg/Ca: 1 mM $MgCl_2$, 1 mM $CaCl_2$

Mn/Ca: 2 mM $MnCl_2$, 0.1 mM $CaCl_2$

Supplementary Table 3. VCAM mutant impact on cell spreading.

Variant	Max % spread WT	EC_{50} (μg/mL) WT	Max % spread mutant	EC_{50} (μg/mL) mutant	Fold change Mutant / WT
R36A	72	10	70	9	1
R36E	70	8	50	29	0.28
Q38E	76	4	44	40	0.1
Q38K	76	4	36	>50	<0.08
Q38G	76	4	74	2	2
Q38L	75	4	79	1	4
Q38F	77	10	78	16	0.62
I39K	77	8	38	>50	<0.16
I39R	77	9	45	41	0.22
I39A	69	8	21	>50	<0.16
I39V	69	8	55	8	1
D40E	76	4	51	15	0.25
P42G	69	9	56	9	1
L43A	82	7	19	>50	<0.14
L43K	82	7	4	>50	<0.14

In Figure 9 of Newham, P., et al. (1997). "α4 integrin binding interfaces on VCAM-1 and MAdCAM-1. Integrin binding footprints identify accessory binding sites that play a role in integrin specificity." J Biol Chem 272(31): 19429–19440, horizontal lines were drawn at half-maximal spreading in each panel to determine EC_{50} values.

Supplementary Table 4. WaterMap statistics.

Structure	No. of waters in the ligand binding pocket (within 5 Å of Bio 1211)	No. of high-energy waters ($\Delta G > 2.0$ kcal/mol) in the ligand binding pocket	No. of high-energy waters ($\Delta G > 2.0$ kcal/mol) located in DU pocket
apo α4β1 (PDB 9YST)	111	10	6
α4β1–12G10 (PDB 9YSY)	120	12	6
α4β1–VCAM D1D3 (PDB 9YSU)	123	9	6
apo α4β1 (PDB 9YOC)	114	10	6