

Supplementary Materials for

Crystal structure of bovine herpesvirus 1 glycoprotein D bound to nectin-1 reveals the basis for its low-affinity binding to the receptor

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Fig. S1

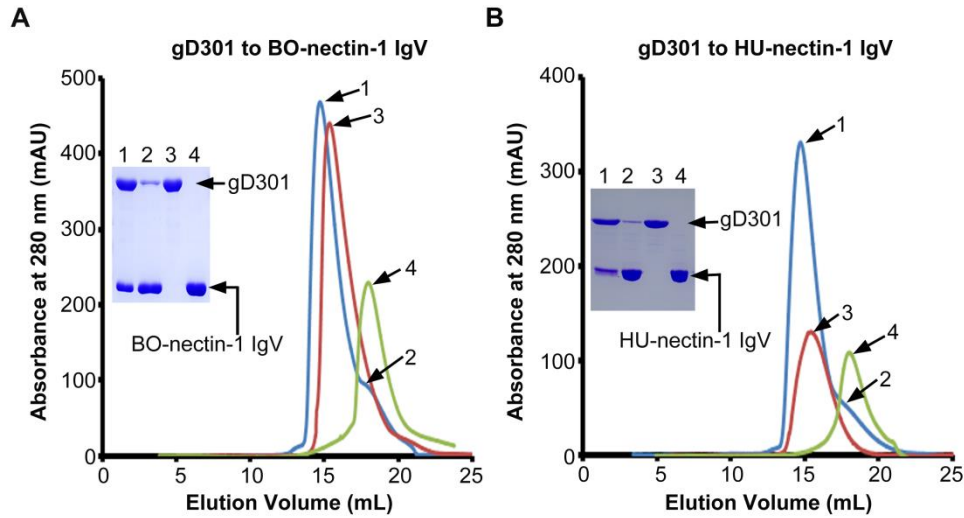


Fig. S1. Interactions between BHV-1 gD301 and nectin-1 IgV analyzed by analytical gel filtration chromatography. The gD301 protein, the IgV domain proteins of BO- and HU-nectin-1 and the gD/nectin-1 mixtures are individually loaded onto a calibrated Superdex 200 Increase 10/300 GL (GE health) column. The overlaid chromatographs and the SDS-PAGE migration profiles of the pooled samples are shown. **(A)** BHV-1 gD301 with BO-nectin-1 IgV. Lane 1: the complex of gD301 bound to BO-nectin-1 IgV; lane 2: excessive BO-nectin-1 IgV; lane 3: gD301 alone; lane 4: BO-nectin-1 IgV alone. **(B)** BHV-1 gD301 with HU-nectin-1 IgV. Lane 1: the complex of gD301 bound to HU-nectin-1 IgV; lane 2: excessive HU-nectin-1 IgV; lane 3: gD301 alone; lane 4: HU-nectin-1 IgV alone.

Fig. S2

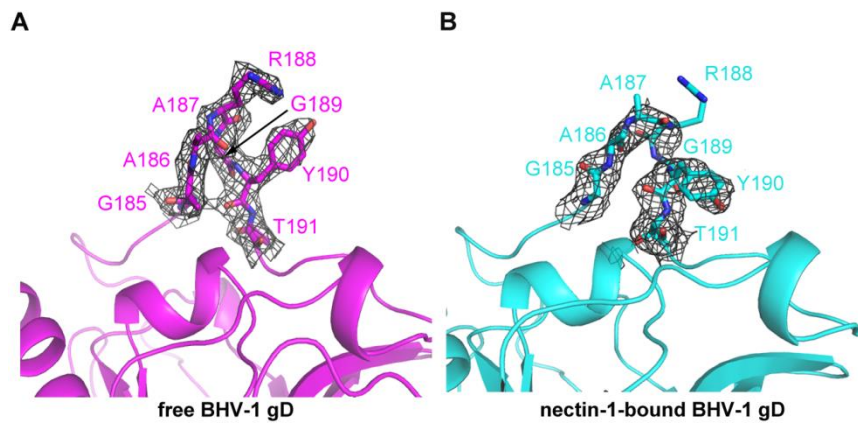


Fig. S2. Electron densities depicted for residues G185-T191 of the $G/\alpha 2$ inter-loop in the free (panel **A**) and nectin-1-bound (panel **B**) gD structures. The densities are contoured at 1.0σ by using the $2 |F_o| - |F_c|$ map.

Fig. S3

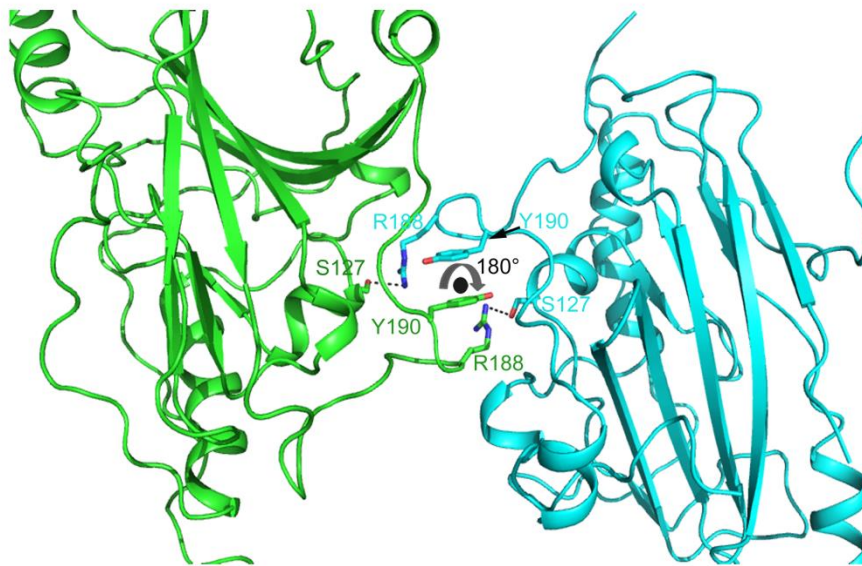


Fig. S3. The amino acid interactions stabilizing the “stretched” conformation for the $G/\alpha 2$ inter-loop in the free BHV-1 gD structure. Those residues referred to in the text are shown and labeled. Dashed lines indicate H-bonds. The two-fold symmetry axis is highlighted as a black dot.

Fig. S4

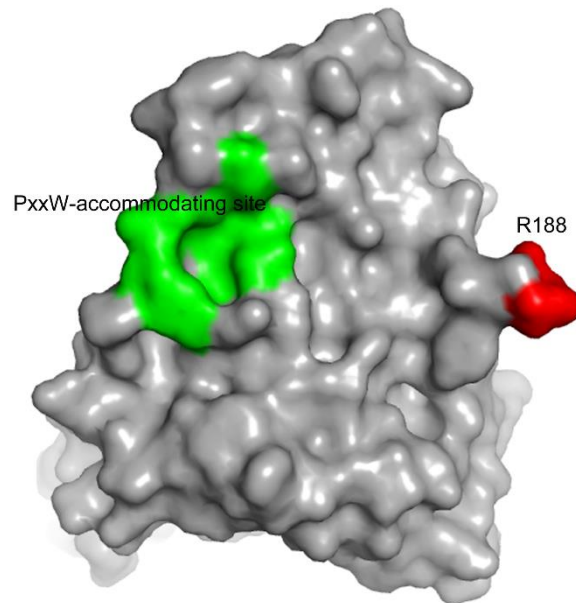


Fig. S4. A surface representation showing the potential PxxW-accommodating site in BHV-1 gD. The site, which is deduced based on the previously reported dimeric structure of HSV-1 gD (PDB code: 2C36), are highlighted in green. Residue R188 that interferes with the nectin-1 binding is highlighted in red. Clearly shown is that R188 is far away from the potential PxxW-accommodating site in BHV-1 gD.

Table S1. Data collection and refinement statistics.

	gD301	gD301/Bo-nectin-1
Data collection		
Space group	C2	C2
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	116.27, 104.39, 98.77	180.87, 64.23, 102.93
α , β , γ (°)	90.00, 112.50, 90.00	90.00, 91.78, 90.00
Wavelength (Å)	1.03645	0.97892
Resolution (Å) ^a	50.00-2.50 (2.59-2.50)	50.00-2.20 (2.28-2.20)
R _{sys} or R _{merge} ^{a,b}	0.110 (0.573)	0.069 (0.783)
I/ σ I ^a	21.11 (2.86)	23.90 (1.86)
Completeness (%) ^a	99.8 (98.4)	98.1 (97.0)
Redundancy ^a	6.4 (5.4)	6.0 (5.7)
Refinement		
Resolution (Å)	37.43-2.50	45.20-2.20
No. reflections	37566	
R _{work} /R _{free} ^c	0.2063/ 0.2459	0.1996/0.2359
No. of atoms		
Protein	5883	5680
Ligand/ion	84	56
Water	69	199
B-factors		
Protein	37.1	61.8
Ligand/ion	65.6	84.9
Water	31.7	56.4
R.m.s. deviations		
Bond lengths (Å)	0.013	0.011
Bond angles (°)	1.140	1.350
Ramachandran plot ^d		
Favored region (%)	95.63	96.56
Allowed region (%)	4.37	3.44
Outlier region (%)	0	0
PDB code	6LS9	6LSA

^a Values for the resolution of outmost shell are given in parentheses.

^b $R_{\text{merge}} = \frac{\sum_i \sum_{\text{hkl}} |I_i - \langle I \rangle|}{\sum_i \sum_{\text{hkl}} I_i}$, in which I_i is the observed intensity, and $\langle I \rangle$ is the average intensity from multiple measurements.

^c R_{free} is the R factor for a subset (5%) of reflections, which doesn't participate in the refinement.

^d Ramachandran plots were calculated by PROCHECK.

Table S2. Statistics on the surface areas buried in different alphaherpesviral gD ligands and in the nectin-1 receptor.

Complex Buried area/ Å ²	HSV-1 gD/HU- nectin-1	HSV-2 gD/HU- nectin-1	PRV gD/SW- nectin-1	BHV-1 gD/BO- nectin-1
gD	908.2	938.4	1118.1	966.4
nectin-1	879.2	971.9	1170.9	950.1
Total buried surface area	1787.4	1910.3	2289	1916.5

The buried surface areas are calculated based on the complex structures of HSV-1 gD bound to HU-nectin-1 (PDB code: 3SKU), HSV-2 gD bound to HU-nectin-1 (PDB code: 4MYW), PRV gD bound to SW-nectin-1 (PDB code: 5X5W) and BHV-1 gD bound to BO-nectin-1 (reported in this study), respectively.

Table S3. Residues in BHV-1 gD interfacing with the nectin-1 receptor.

gD in the BHV-1-gD/BO-nectin-1 complex	nectin-1 in the BHV-1-gD/BO-nectin-1 complex
P15 (N-loop)	F129, P130 (7 contacts)
A16 (N-loop)	F129 (4 contacts)
Y17 (N-loop)	K61, F129 (24 contacts)
P18 (N-loop)	F129 (2 contacts)
E26 (N-loop)	M85 (1 contact)
W28 (N-loop)	Q64, N82, M85 (26 contacts)
H29 (N-loop)	I80, N82, M85, G86, V87, S88 (29 contacts)
S127 (IgV-like core)	E125 (1 contact)
Y190 (C-extension)	G73, S74, K75 (35 contacts)
A194 (C-extension)	S74 (3 contact)
L209 (C-extension)	N77, I80, S88, L90 (17 contacts)
T210 (C-extension)	Q76, N77, L90 (24 contacts)
Y211 (C-extension)	K75, Q76, N77 (6 contacts)
L212 (C-extension)	N77 (4 contacts)
T213 (C-extension)	Q64 (1 contact)
Q214 (C-extension)	Q64, I80 (5 contacts)
Y215 (C-extension)	N133 (3 contacts)
Y216 (C-extension)	T63, A127, T128, F129 (29 contacts)
A220 (C-extension)	F129 (5 contacts)
A223 (C-extension)	F129 (2 contacts)
I224 (C-extension)	T128, F129, P130 (16 contacts)
Y227 (C-extension)	P130 (7 contacts)

The amino acids of BHV-1 gD located within a distance of 4.5 Å from the bound nectin-1 receptor are selected and listed in the left column. The gD domains to which these residues belong are shown in parentheses. Reciprocally, the amino acids interacting with each of the gD interface residue are listed in the right column with the number of the inter-molecular contacts summarized in parentheses.

Table S4. Comparison of the nectin-1 residues contacting BHV-1 gD with those interfacing with PRV, HSV-1 and -2 gDs.

Nectin-1 in the BHV-1-gD/BO-nectin-1 complex	Nectin-1 in the PRV-gD/SW-nectin-1 complex	Nectin-1 in the HSV1-gD/HUnectin-1 complex	Nectin-1 in the HSV2-gD/HU-nectin-1 complex
—	—	S59 (1 contact)	S59 (2 contacts)
K61 (2 contacts)	K61 (8 contacts)	K61 (10 contacts)	K61 (8 contacts)
T63 (12 contacts)	T63 (10 contacts)	T63 (15 contacts)	T63 (5 contacts)
Q64 (9 contacts)	Q64 (20 contacts)	Q64 (19 contacts)	Q64 (13 contacts)
—	T66 (1 contact)	T66 (1 contact)	T66 (3 contact)
—	Q68 (1 contact)	Q68 (6 contacts)	Q68 (12 contacts)
—	A70 (1 contact)	—	—
G73 (9 contacts)	G73 (5 contacts)	—	G73 (4 contacts)
S74 (8 contacts)	S74 (11 contacts)	—	S74 (3 contacts)
K75 (22 contacts)	K75 (24 contacts)	K75 (7 contacts)	K75 (13 contacts)
Q76 (12 contacts)	Q76 (15 contacts)	Q76 (10 contacts)	Q76 (14 contacts)
N77 (21 contacts)	N77 (29 contacts)	N77 (35 contacts)	N77 (42 contacts)
I80 (8 contacts)	I80 (11 contacts)	I80 (8 contacts)	I80 (11 contacts)
—	Y81 (1 contact)	Y81 (1 contact)	Y81 (3 contacts)
N82 (9 contacts)	N82 (5 contacts)	N82 (6 contacts)	N82 (4 contacts)
M85 (17 contacts)	M85 (20 contacts)	M85 (32 contacts)	M85 (33 contacts)
G86 (5 contacts)	G86 (4 contacts)	G86 (2 contacts)	G86 (5 contacts)
V87 (6 contacts)	V87 (4 contacts)	—	V87 (3 contacts)
S88 (12 contacts)	S88 (8 contacts)	S88 (10 contacts)	S88 (15 contacts)
L90 (13 contacts)	L90 (10 contacts)	L90 (19 contacts)	L90 (13 contacts)
—	A91 (9 contacts)	—	—
—	P92 (2 contacts)	—	—
—	I123 (4 contacts)	—	—
E125 (1 contact)	E125 (10 contacts)	E125 (5 contacts)	E125 (6 contacts)
A127 (4 contacts)	A127 (8 contacts)	A127 (4 contacts)	A127 (1 contact)
T128 (9 contacts)	T128 (8 contacts)	—	T128 (2 contacts)
F129 (52 contacts)	F129 (45 contacts)	F129 (21 contacts)	F129 (32 contacts)
P130 (17 contacts)	P130 (22 contacts)	P130 (42 contacts)	P130 (18 contacts)
—	—	T131 (9 contacts)	T131 (2 contacts)
—	G132 (2 contacts)	—	—
N133 (3 contacts)	N133 (15 contacts)	N133 (8 contacts)	N133 (5 contacts)
—	E135 (8 contacts)	—	—
—	Q137 (1 contact)	—	—

The nectin-1 amino acids located within a distance of 4.5 Å from the bound ligands (BHV-1 gD based on the complex structure reported in the current study, PRV, HSV-1 and -2 gDs according to

previously reported complex structures of PDB codes 5X5W, 3U82 and 4MYW, respectively) were selected, and then listed in a pairwise manner. The number of vdw contacts that each interface residues contribute is summarized in parenthesis. Those amino acids utilized to contact all the four viral ligands are highlighted in boldface.