High risk human papillomaviruses (HPVs) are non-enveloped small DNA viruses of great medical importance. Among the large group of HPVs known by now, sexually transmitted genital HPVs can cause cervical cancer. Efficient infection by HPV16 and HPV18 pseudovirions requires interactions of particles with cell-surface receptor heparan sulfate oligosaccharide. To understand the virus-receptor interactions for HPV infection, we determined the crystal structures of HPV16 and HPV18 capsids bound to the oligosaccharide receptor fragment using oligomeric heparin. The HPV-heparin structures revealed multiple binding sites for the highly negatively charged oligosaccharide fragment on the capsid surface, which is different from previously reported virus-receptor interactions in which a single type of binding pocket is present for a particular receptor. We performed structure-guided mutagenesis to generate mutant viruses, and cell binding and infectivity assays demonstrated the functional role of viral residues involved in heparin binding. These results provide a basis for understanding virus-heparan sulfate receptor interactions critical for HPV infection and for the potential development of inhibitors against HPV infection.

Human papillomaviruses (HPVs) are non-enveloped small DNA viruses of great medical importance. Among the large group of HPVs known by now, sexually transmitted genital high risk HPV types are the cause for the development of a variety of epithelial tumors, especially cervical carcinoma (1). Cervical cancer is the second leading cause of death among female cancer patients worldwide. HPV16 and HPV18 stand out, as they are causally linked to >70% of cervical cancer cases (2).

HPV particles consist of 72 pentamers of the major capsid protein L1, which forms the virus outer shell and encapsidates the viral DNA (3, 4). The minor capsid protein L2 is present at up to 72 copies and is hidden inside the capsid with exception of a small N-terminal section (5, 6). Efficient infection by HPV16 and HPV18 pseudovirions requires the interactions of the L1 protein with extracellular matrix (ECM)- and cell surface-resident heparan sulfate receptor in vitro (7–9) as well as in vivo models (10). Homologs of heparan sulfate polysaccharide or heparin, secreted by mast cells, can inhibit HPV infection (7–9).

Cell-surface heparan sulfates are linear and highly negatively charged oligosaccharides that are covalently linked to proteins. They can serve as the attachment receptors for several important human virus pathogens (7, 11, 12). Despite considerable efforts, the interactions between HPV and the heparan sulfate oligosaccharides that initiate infection are poorly understood. Here, we determined the co-crystal structure of HPV16 and HPV18 capsids bound to oligomeric heparin. We found that the highly negatively charged heparin fragment binds to multiple locations on the capsid surface mainly through charge-charge interactions. On the basis of the structure, we generated mutant virus to disrupt the interactions with heparin. ECM and cell binding assays combined with infectivity measurements showed that substitution of key HPV residues involved in binding the oligosaccharide receptor decreased virus binding to the ECM and cell surface and/or reduced viral infectivity. The results of the mutational and functional analyses provide evidence supporting the biological relevance of the molecular interactions of the viral capsid with the heparin fragment observed in the crystal structure.

EXPERIMENTAL PROCEDURES

Expression and Purification of HPV18 and HPV16 L1 Capsids—The assembly-deficient mutants of HPV16 L1 and HPV18 L1 were cloned and purified as described by Bishop et al. (13, 14). Briefly, the L1 proteins were expressed as GST-L1 fusion proteins in Escherichia coli using 0.2 mM isopropyl-β-D-thiogalactopyranoside induction overnight at room temperature. After cell lysis by sonication in lysis buffer (50 mM Tris-HCl (pH 8.0), 0.2 M NaCl, 1 mM DTT, 1 mM EDTA, and 10 mM PMSF), urea (ultrapure grade) was slowly added to the lysate to a final concentration of 3.0 M. The mixture was incubated at room temperature for 1 h with gentle shaking and then dialyzed against three changes of buffer over an 18-h
period at 4 °C. After centrifugation at 25,000 x g for 75 min, the supernatant was passed through a glutathione affinity column to bind GST-L1 fusion proteins. After cleaving L1 from the GST fusion protein by thrombin, the free L1 pentamer was purified by Superdex 200 (60/16 column) size-exclusion chromatography.

Preparation of Size-defined Heparin Oligosaccharides—The purified L1 pentamers of HPV16 and HPV18 were used for co-crystallization with size-defined heparin oligosaccharides of 8 and 10 monosaccharide units (8- and 10-mers, respectively). The size-defined oligosaccharides were prepared from bovine lung heparin by partial deamination at pH 1.5 for 3 h on ice, followed by reduction with NaBH₄ for 2 h at room temperature and separation on a P-10 column essentially as described (15). Heparin oligosaccharides were quantified by colorimetric determination of hexuronic acid using the method of meta-hydroxydiphenyl method with glucuronic acid as a standard and converted by an arbitrary factor of 3 to saccharide mass (16).

Crystallization and Data Collection—Purified L1 pentamers of HPV16 and HPV18 were concentrated to 8–10 mg/ml in buffer containing 20 mM Tris (pH 8.0), 50 mM NaCl, and 1 mM DTT. L1 pentamers and purified heparin oligosaccharides were mixed at a 1:10 molar ratio. The mixtures were incubated for 30 min to 1 h at 18 °C before setting up crystallization trays. Crystallization trials were given using NeXtal crystallization screens. Diffraction quality crystals were obtained by the hanging-drop vapor diffusion method, followed by incubation at 18 °C for 10–21 days in the presence of only the 10-mer heparin oligosaccharide for both HPV16 and HPV18 capsids under the crystallization conditions tested. Crystals of HPV16 and HPV18 capsids bound to the heparin oligosaccharide were obtained under different buffer conditions. HPV16 crystals were obtained in buffer containing 30% PEG 400, and 0.1 mM MES at pH 6.5. HPV18 crystals were obtained in buffer containing 20% PEG 3350 and 0.2 mM potassium acetate. Diffraction data sets were collected at beamline 8.2.2 of the Advanced Light Source (Lawrence Berkeley National Laboratory), with 2.8 Å resolution for HPV16 and 3.4 Å resolution for HPV18. The data were processed using HKL2000 (see Table 1).

Structure Determination and Refinement—Both structures were solved by molecular replacement using MOLREP of the CCP4 suite (17). The assembly-deficient pentamers of HPV16 and HPV18 (14) were used as a corresponding search model, yielding one pentamer for the HPV16 L1-heparin complex and three pentamers for the HPV18 L1-heparin complex in the asymmetric unit with space groups P2₁ and P1, respectively. The structures were refined with alternative cycles of model building and refinement using CNS (18) and program O (19). The electron density maps were well featured because of the powerful phase improvement method of non-crystallographic symmetry averaging (5-fold for the HPV16-heparin complex and 15-fold for the HPV18-heparin complex) used at the early stages of the refinement and model building of the protein part to improve the phases. The heparin oligosaccharides were built into the electron density calculated from the improved L1 model. The heparin coordinate (Protein Data Bank code 2AXM (20)) from the data base was used as the basis for the model building. A final round of refinement resulted in good statistics, as shown in Table 1.

HPV16 Mutant Virus Generation and Cell Binding/Infectivity Assays—Site-directed mutagenesis was performed using PfuUltra hotstart polymerase (Stratagene). Introduction of mutations was confirmed by sequencing. Pseudoviruses harboring pEGFP were generated using the 293TT cell line as described (21). Particles were characterized by L1- and L2-specific Western blotting, by real-time PCR, and by ELISA using anti-HPV16 virus-like particle (VLP) polyclonal and monoclonal antisera. For infection assays, identical amounts of particles were used, yielding up to 30% infection rates for the WT (1 x 10⁷ pseudoviral genome equivalents/well). Where indicated, heparin chains (>15 kDa) or size-defined fragments were added to WT HPV16 pseudovirus prior to infection of 293TT cells. Infection of 293TT cells was monitored by flow cytometry 72 h post-infection. Infection of HaCaT cells was measured by visually counting GFP-expressing cells 72 h post-infection. Infectivity data given were based on at least four different virus preparations with three replicates each.

For cell and ECM binding assays, HaCaT cells were grown on coverslips for 2 days. For ECM binding assays, cells were completely dislodged and removed by EDTA (0.5 mM in Dulbecco’s PBS). The ECM deposited onto the glass slide was incubated with rabbit anti-laminin 5 antibody (Abcam ab14509) for 30 min at 37 °C. Preincubation with anti-laminin 5 antiserum was used to prevent binding to laminin 5. In addition, it was used to identify ECM depositions for the subsequent quantitative analysis. Pseudovirions (1 x 10⁷ pseudoviral genome equivalents/well) were added in complete DMEM. After 1 h of binding at 37 °C, unbound particles were washed out with PBS, and HPV16-specific monoclonal antibody H16.56E was used to detect bound pseudovirions. For cell binding assays, cells were incubated with anti-laminin 5 antiserum, and HPV16 pseudovirions were subsequently added for 1 h at 4 °C. Samples were fixed with 4% paraformaldehyde for 15 min at room temperature, and primary antibody was detected with Alexa Fluor-labeled goat secondary antibody (Invitrogen) and fluorescently labeled phalloidin. Images were captured by confocal microscopy (Zeiss 510 laser scanning confocal microscope). Within individual experiments, the same microscope settings and exposure times were used. For quantification of pixel number and area size, the LSM server software provided with the confocal microscope was used. The ability of particles to bind to the ECM was expressed as the number of pixels of capsid-specific signal in the area normalized to laminin 5. Binding to the cell was measured based on projections of three z-stacks for one sample and expressed as the number of pixels of capsid-specific signal normalized to phalloidin staining. The average region of interest analyzed was not significantly different among groups. Statistical significance was calculated by Student’s t test.

Heparin ELISA—Heparin (50 μg/ml) dissolved in double-distilled H₂O was coated onto heparin glycosaminoglycan binding plates (Iduron, Manchester, United Kingdom) overnight in the dark at room temperature, washed, and blocked.
with PBS and 1% BSA. Equal amounts of the indicated particles diluted in PBS and 1% BSA were added and incubated for 1 h at 37 °C. After extensive washing, rabbit polyclonal antisera K75 (1:5000) was added for 1 h at 37 °C. Bound antibody was subsequently detected using horseradish peroxidase-coupled goat anti-rabbit secondary antisera (Jackson ImmunoResearch Laboratories) and tetramethylbenzidine as substrate. The reaction was stopped with 1N HCl, and absorbance was measured at 405 nm.

RESULTS

Structure Determination of HPV Capsids Bound to Heparin Oligosaccharides—HPV VLPs or pseudovirions can be assembled from pentameric L1 capsids in vivo or in vitro (22–24). Pentameric L1 and VLPs have similar surface epitopes as virions and are recognized by all known neutralizing monoclonal antibodies (13, 14, 25). Crystal structural studies showed that the surface structural features of the pentameric L1 units are identical to those of the pentamers in the assembled small VLPs (14, 23). These structural similarities led us to use the pentamer for co-crystallization with a size-defined heparin oligosaccharide to study HPV-receptor interactions presented in this work.

We determined the crystal structures of HPV16 and HPV18 L1 capsids in complex with heparin oligosaccharides at resolutions of 2.8 Å for HPV16 (Fig. 1, a–c, and Table 1) and 3.4 Å for HPV18 (Fig. 1, d–f, and Table 1). The pentameric L1 capsids of HPV16 and HPV18 in complex with heparin oligosaccharide have the same surface loop conformation as heparin-free capsids (14, 23). Most of the heparin fragments built in the model were shorter than the full 10-sugar residues used for the crystallization. This is possibly due to the flexibility of

FIGURE 1. Overall structures of HPV L1 capsid binding to heparin oligosaccharides. a–c, structure of the HPV16 L1 pentamer in complex with heparin fragments. d–f, structure of the HPV18 L1 pentamer in complex with heparin fragments. When the L1 capsids are drawn as surface (a, b, d, and e), the heparin molecule is drawn as a stick model. When L1 is shown as a ribbon (c and f), the heparin oligosaccharides are shown as surface. The electrostatics of the HPV16 and HPV18 L1 capsid surface are color-coded as follows: red, negative charge; blue, positive charge; and white, neutral. The heparin binding on the L1 capsid surface appears to be asymmetric in a and d, which is attributed to different types of binding sites and also to the fact that some of the binding sites are blocked by another pentamer through crystal packing.
the heparin chain such that only those residues interacting with the HPV capsid have defined electron density and the rest of the fragment is invisible. All of the heparin molecules were found to bind only to the outer surface of the pentameric L1 capsid in both HPV types, even though the interior sides are also accessible for heparin molecules.

Overall Structural Feature of Receptor Binding—One striking feature observed in these co-crystal structures is that there are multiple sites with different sets of residues on the surface of L1 capsids to bind the heparin fragment for both HPV16 (Fig. 1, a–c) and HPV18 (Fig. 1, d–f). All of the bound heparin fragments are located primarily on the top outer rim or the lateral surface (side wall) of the viral capsid for both viruses (Fig. 1, a–f). On the side wall, extended heparin oligosaccharide chains bind to the L1 capsids from the top to the base of the pentamer (Fig. 1, c and f). The structures show no heparin molecule binding near the top surface area around the pentameric central hole for both HPV16 (Fig. 1a) and HPV18 (Fig. 1d), where highly negatively charged surface areas are located.

Detailed Interactions between Viral Capsids and Heparin Oligosaccharides—For both HPV16 and HPV18, all heparin-binding sites are conformational, consisting of residues scattered on more than one surface loop and more than one subunit on the primary sequences. They come together in the three-dimensional structure to form the binding patches. The surface loops of the pentameric L1 capsid participating in binding heparin are colored in Fig. 2a, all of which are on the outer rim of the pentamer, away from the pentameric hole. For HPV16, the four different types of binding modes for the heparin oligosaccharides are shown in Fig. 2 (b–e), with two located on the top rim (b and c) and two on the side wall (d and e) of the pentameric capsid. For the two binding sites on

![Figure 2](image-url)
the top rim of the capsid, one is composed of residues from FG/HI-loops of two neighboring monomers (Lys-278, Thr-266, Asn-285, and Lys-361) (Fig. 2b), and the other is composed of residues from FG/HI/BC-loops of two monomers (Lys-356, Thr-358, Thr-266, Lys-54, and Asn-56) (Fig. 2c). For the two types of binding sites on the side wall, the heparin fragments bind almost vertically (Fig. 2, d and e). Both start on the BC-loop (Asn-57 and Lys-59) and reach down to the base of the pentamer, where they interact with residues on the α4-loop (Lys-443 or Asn-450/Lys-452). The residues participating in binding the heparin fragment are either positively charged lysines (Lys-54, Lys-59, Lys-278, Lys-356, and Lys-361) that are conserved between HPV16 and HPV18 or the less conserved polar residues Asn, Gln, and Thr (such as Asn-57, Gln-194, and Thr-358). Through these positively charged lysines or other polar residues, the viral capsid forms charge-charge interactions and polar interactions (hydrogen bonds) with the negatively charged heparin molecule.

The overall heparin-binding mode of the HPV18 capsid is similar to that of HPV16, involving the same surface loops as shown in Fig. 2a, even though the residues may differ. Four different types of binding sites for the heparin oligosaccharides are located on similar locations on the surface of the viral capsid (Fig. 2a–d). Two of the binding sites are located on the top rim, interacting with residues either from FG/HI-loops (Fig. 3a) or from HI/EF-loops coming from two neighboring subunits (Fig. 3b). The two types of heparin-binding sites, located on the side wall of the pentameric capsid, interact with EF- or BC/EF-loops on the top part of the capsid and with the α4-loop (Lys-439–Lys-453) near the bottom of the capsid (Fig. 3, c and d). Similar to HPV16, the residues of HPV18 interacting with the heparin fragment are also charged residues and polar residues on these surface loops (Fig. 1g).

**Role of Heparin-binding Amino Acids in Viral Infectivity**—To verify the biological relevance of the interactions of the viral capsid with heparin observed in the crystal structure, we generated virus mutants with Ala mutations of the key residues of HPV16 L1 involved in heparin binding. We used these mutant viruses to examine ECM binding, cell binding, and infectivity (Table 2). For these studies, we utilized the pseudovirus system, which is well accepted for studying infectious entry of HPV. It uses 293TT cells harboring high copy numbers of a marker plasmid to express capsid proteins, which self-assemble and encapsidate the plasmid (21).
of conformational neutralizing epitopes (data not shown). Compared with the WT, all mutant viruses showed significantly reduced infectivity to various extents in both cell lines used (Table 2). However, only mutants with exchanges at Lys-278 and Lys-361 had strongly reduced binding to ECM (Fig. 4a and Table 2) and cells (Fig. 4b and Table 2). Cell but not ECM binding was consistently reduced for Asn-57/Lys-59/Lys-442/Lys-443, even though it did not reach significance. Similar results were obtained using a heparin-based ELISA. The Lys-278/Lys-361 mutant particles showed only residual binding, whereas WT and the other mutant particles bound efficiently (Fig. 5a). These data support the crystal structure that shows heparin binding by Lys-278/Lys-361 on the very accessible top surface of the L1 pentamer. Surprisingly, WT HPV16 pseudoviral infection was not inhibited by heparin oligomers used for the crystallization studies even when present at high concentrations, in contrast to high molecular weight heparin (Fig. 5b). However, we have previously demonstrated that oligomeric heparin binds to HPV16 VLPs (12). Taken together, the results suggest that the affinity of the short oligomeric heparin binding to pseudovirions is not high enough to compete with the binding to the long chain ECM- and cell surface-resident heparan sulfate. This is in line with our unpublished observations pointing to the existence of an extended interaction of long heparin chains with all three binding sites accompanied by potential conformational changes of the L1 capsid protein.5 The results of the mutational and functional analyses provide evidence supporting the biological relevance of the molecular interactions of the viral capsid with the heparin fragment observed in the crystal structure.

DISCUSSION

HPV16 and HPV18 use heparan sulfate chains as primary receptors for attachment to the cell surface to initiate subsequent infection. We used x-ray crystallography, mutagenesis, and functional assays to understand the virus-receptor interactions that are important for the infection of these tumor viruses.

The co-crystal structures of the two HPV capsids revealed that heparin oligosaccharides bind to the virus surface mainly through charge-charge and polar interactions. Perhaps the

|          | ECM binding | Cell binding | Infectivity |
|----------|-------------|--------------|-------------|
|          | 293TT       | HaCaT        |             |
| WT       | 100 (±19.8) | 100 (±39.4)  | 100 (±20.0) |
| Lys-278/Lys-361 | 4.3 (±2.0)* | 3.9 (±2.7)*  | 0.3 (±0.3)* |
| Lys-54/Lys-356   | 87.5 (±19.0)| 137.1 (±42.7)| 13.1 (±5.3)*|
| Lys-54/Lys-278/Lys-361/Lys-356 | 0.6 (±0.2)* | 9.8 (±4.0)*  | 0.1 (±0.2)* |
| Asn-57/Lys-59/Lys-442/Lys-443 | 121.6 (±31.8)| 54.7 (±13.4)| 5.9 (±1.0)* |

*p < 0.0001.

FIGURE 4. ECM and cell binding of mutant HPV16. a, mutant virus binding to the ECM. Red, laminin 5; green, viral capsid. b, mutant virus binding to HaCaT cells. Red, actin; green, viral capsid. Projections of three confocal sections are displayed. The color of the channels was changed using Adobe Photoshop CS2 software. The five mutants are listed on the corresponding panels. The results are summarized in Table 2. ctrl, no-virus control.
Interactions of HPV Capsids with Cell-surface Receptor

and HPV16 Lys-443/Lys-452 in the helix α4-loop region (Fig. 1g).

Interestingly, despite the multiple site-binding mode, no heparin molecule binds to the large open area around the pentameric center of L1 capsids of both HPV types (Fig. 1, a and d). The surface electrostatics of the capsids clearly indicates that this large area for both HPV types has a high density of negative charge (negative charge colored in red in Fig. 1, a and d). This highly negatively charged area should exert repulsion to the negatively charged heparin oligosaccharides. Interestingly, we noticed that this predominantly negatively charged area around the pentameric center is also a conserved feature among other HPV types with known structures (HPV35 and HPV11) (14), suggesting that this area should not bind a heparan sulfate receptor for other HPV types and may be required for other conserved functions for HPVs, such as for binding other cellular factors during viral infection. A comparison between the L1 capsid structures with and without heparin binding revealed no obvious conformational change for both HPV16 and HPV18 (data not shown), except for the slight movements of the surface loops and the residues directly involved in oligosaccharide binding. Previous indirect evidence for L1 particle conformational changes following cell attachment was reported (30). Although this issue would require further investigation, one possible explanation for this discrepancy is that the longer high molecular weight heparin and/or additional cellular factors may be required for the possible induction of certain changes in L1 particle conformation.

Our structure-guided mutagenesis suggests that Lys-278–Lys-361 on the top of the pentamer probably serve as the primary heparan sulfate-mediated ECM and cell attachment site. Even though the other two sites on the side of the pentamer do not seem to be essential for primary attachment, they are important for infectious entry. The side wall location of these two sites on the pentamer may mean that their interaction with heparin occurs after the primary attachment on the top, which is consistent with our unpublished observations that suggest their contribution as an extended binding site.5 There are some discrepancies between some of the binding and infectivity data presented here and the data in our initial characterization of HPV16 mutants that included lysine residues at positions 278, 356, and 361 (12). In our initial studies, we used pseudovirus particles that were not fully matured. In contrast to mature virions, immature HPV16 pseudoviruses treated with furin convertase do not require heparan sulfate for infection (31, 32). The presence of immature particles in the preparations used previously by Knappe et al. (12) may explain the residual infectivity observed in that study. In addition, cell binding assays were performed differently using COS-7 cells and Western blotting instead of HaCaT cells and immunofluorescence to measure particle binding.

In summary, the crystal structures of the complexes of HPV16 and HPV18 capsids with the heparin fragment reveal that heparin oligosaccharides bind to multiple sites located on the readily accessible virus surface areas on the top rim and side walls of the pentameric capsids. Both HPV types interact with the highly negatively charged heparin fragment through
Interactions of HPV Capsids with Cell-surface Receptor

its positively charged and polar residues, which come from two or three subunits to form conformational binding sites. Mutational and functional studies of the mutant virus based on the virus-receptor structures support the biological role of the residues involved in heparin binding for viral infectivity.

Acknowledgments—We thank the staff at synchrotron beamlines 4.2.2, 5.0.2, 8.2.2, and 8.3.1 in the Advanced Light Source at the Lawrence Berkeley National Laboratory for assistance in data collection.

REFERENCES
1. zur Hausen, H. (2009) Virology 384, 260–265
2. Bosch, F. X., Manos, M. M., Muñoz, N., Sherman, M., Jansen, A. M., Pető, I., Schiffman, M. H., Moreno, V., Kurman, R., and Shah, K. V. (1995) J. Natl. Cancer Inst. 87, 796–802
3. Baker, T. S., Newcomb, W. W., Olson, N. H., Cowsert, L. M., Olson, C., and Brown, J. C. (1991) Biophys. J. 60, 1445–1456
4. Kirnbauer, R., Taub, J., Greenstone, H., Roden, R., Dürst, M., Gissmann, L., Lowy, D. R., and Schiller, J. T. (1993) J. Virol. 67, 6929–6936
5. Buck, C. B., Cheng, N., Thompson, C. D., Lowy, D. R., Steven, A. C., Schiller, J. T., and Trus, B. L. (2008) J. Virol. 82, 5190–5197
6. Richards, R. M., Lowy, D. R., Schiller, J. T., and Day, P. M. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 1522–1527
7. Giroglou, T., Florin, L., Schäfer, F., Streeck, R. E., and Sapp, M. (2001) J. Virol. 75, 1565–1570
8. Christensen, N. D., Reed, C. A., Culp, T. D., Hermont, P. L., Howett, M. K., Anderson, R. A., and Zaneveld, L. J. (2001) Antimicrob. Agents Chemother. 45, 3427–3432
9. Buck, C. B., Thompson, C. D., Roberts, J. N., Müller, M., Lowy, D. R., and Schiller, J. T. (2006) PLoS Pathog. 2, e69
10. Johnson, K. M., Kines, R. C., Roberts, J. N., Lowy, D. R., Schiller, J. T., and Day, P. M. (2009) J. Virol. 83, 2067–2074
11. Lindahl, U., Lidholt, K., Spillmann, D., and Kjellén, L. (1994) Thromb. Res. 75, 1–32
12. Knappe, M., Bodevin, S., Selinka, H. C., Spillmann, D., Streeck, R. E., Chen, X. S., Lindahl, U., and Sapp, M. (2007) J. Biol. Chem. 282, 27913–27922
13. Bishop, B., Dasgupta, J., and Chen, X. S. (2007) Virology 14. Bishop, B., Dasgupta, J., Klein, M., Garcea, R. L., Christensen, N. D., Zhao, R., and Chen, X. S. (2007) J. Biol. Chem. 282, 31803–31811
15. Spillmann, D., Witt, D., and Lindahl, U. (1998) J. Biol. Chem. 273, 15487–15493
16. Blumenkrantz, N., and Asboe-Hansen, G. (1973) Anal. Biochem. 54, 484–489
17. Lebedev, A. A., Vagin, A. A., and Murshudov, G. N. (2008) Acta Crystallogr. 64, 33–39
18. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. 54, 905–921
19. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. A 47, 110–119
20. DiGabriele, A. D., Lax, I., Chen, D. I., Svahn, C. M., Jaye, M., Schlessinger, J., and Hendrickson, W. A. (1998) Nature 393, 812–817
21. Buck, C. B., Pastrana, D. V., Lowy, D. R., and Schiller, J. T. (2004) J. Virol. 78, 751–757
22. Hagensee, M. E., Yaegashi, N., and Galloway, D. A. (1993) J. Virol. 67, 315–322
23. Chen, X. S., Garcea, R. L., Goldberg, I., Casini, G., and Harrison, S. C. (2000) Mol. Cell 5, 557–567
24. Conway, M. J., and Meyers, C. (2009) J. Dent. Res. 88, 307–317
25. Selinka, H. C., Florin, L., Patel, H. D., Freitag, K., Schmidtke, M., Makarov, V. A., and Sapp, M. (2007) J. Virol. 81, 10970–10980
26. Stehle, T., Yan, Y., Benjamin, T. L., and Harrison, S. C. (1994) Nature 369, 160–163
27. Roelvink, P. W., Mi Lee, G., Einfeld, D. A., Koveshi, I., and Wickham, T. J. (1999) Science 286, 1568–1571
28. Rommel, O., Dillner, J., Fligge, C., Bergsdorf, C., Wang, X., Selinka, H. C., and Sapp, M. (2005) J. Med. Virol. 75, 114–121
29. Selinka, H. C., Giroglou, T., Nowak, T., Christensen, N. D., and Sapp, M. (2003) J. Virol. 77, 12961–12967
30. Day, P. M., Lowy, D. R., and Schiller, J. T. (2008) J. Virol. 82, 12565–12568
31. Buck, C. B., Thompson, C. D., Pang, Y. Y., Lowy, D. R., and Schiller, J. T. (2005) J. Virol. 79, 2839–2846