Structural characterization of interaction between human Ubiquitin Specific Protease 7 and Immediate Early Protein ICP0 of Herpes Simplex Virus-1

Alexandra K. Pozhidaeva1, Kareem N. Mohni2, Sirano Dhe-Paganon3, Cheryl H. Arrowsmith4, Sandra K. Weller1, Dmitry M. Korzhnev1 and Irina Bezsonova1,†

1Department of Molecular Biology and Biophysics, University of Connecticut Health, Farmington, CT 06030, USA
2Department of Biochemistry, Vanderbilt University Medical Center, Nashville, TN 37232, USA
3Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02215, USA
4Structural Genomics Consortium, University of Toronto, Toronto, Ontario M5G 1L7, Canada

†To whom correspondence should be addressed: Irina Bezsonova, Department of Molecular Biology and Biophysics, University of Connecticut Health Center, 263 Farmington Ave, Farmington, CT 06030, USA; Tel.: +1(860) 679 2769; Fax: +1(860) 679 3408; e-mail: bezsonova@uchc.edu

Keywords: deubiquitination, USP7, Herpesvirus, ICP0, NMR, protein structure, protein – protein interaction

Background: Herpesvirus infection is dependent upon the viral E3 ligase ICP0 and its interaction with the human deubiquitinating enzyme USP7.

Results: The interaction between USP7 and ICP0 was structurally characterized using solution NMR.

Conclusion: ICP0 is recognized by ubiquitin-like domains 1 and 2 (UBL12) of the USP7.

Significance: Details of the USP7 – ICP0 interaction provide new insights into function of the USP7 ubiquitin-like domains.

ABSTRACT
Human ubiquitin-specific protease 7 (USP7) is a deubiquitinating enzyme that prevents protein degradation by removing polyubiquitin chains from its substrates. It regulates stability of a number of human transcription factors and tumor suppressors and plays a critical role in the development of several types of cancer, including prostate and small cell lung cancer. In addition, human USP7 is targeted by several viruses of the Herpesviridae family and is required for effective Herpesvirus infection. The USP7 C-terminal region (C-USP7) contains five Ubiquitin-Like domains (UBL1-5) that interact with several USP7 substrates. Although structures of the USP7 C-terminus bound to its substrates could provide vital information for understanding USP7 substrate specificity, no such data has been available to date.

In this work we have demonstrated that the USP7 UBL domains can be studied in isolation by solution NMR spectroscopy and have determined the structure of the UBL1 domain. Furthermore, we have employed NMR and viral plaque assays to probe the interaction between the C-USP7 and HSV-1 immediate-early protein ICP0 (infected cell protein 0), which is essential for efficient lytic infection and virus reactivation from latency. We have shown that depletion of the USP7 in HFF-1 cells negatively affects the efficiency of HSV-1 lytic infection. We have also found that USP7 directly binds ICP0 via its C-terminal UBL1-2 domains and mapped the USP7 binding site for ICP0. This study, therefore, represents a first step toward understanding the molecular mechanism of C-USP7 specificity toward its substrates and may provide the basis for future development of novel anti-viral and anti-cancer therapies.

Conjugation of ubiquitin to target proteins is important in multiple cellular processes, including signaling cascades, proteasomal degradation and protein localization. Mono- and polyubiquitination can be reversed by deubiquitinating enzymes
(DUBs), which cleave the isopeptide bond between ubiquitin and a substrate. Ubiquitin-specific protease 7 (USP7), also known as Herpesvirus-associated ubiquitin-specific protease (HAUSP), is a 135 kDa cysteine isopeptidase (1). Misregulation of the USP7 was linked to multiple human pathologies, including prostate and non-small cell lung cancers (2,3).

A number of USP7 substrates have been identified, and USP7 knockdown was shown to affect cellular levels of at least 36 proteins in LS174T colon carcinoma cells (4). Among a variety of substrates, the USP7 deubiquitates both p53 tumor suppressor and its main regulator E3 ligase Hdm2 (human double minute 2) and thus plays a central role in maintaining an appropriate p53 level in the cell (5-8). Other important targets of USP7 include several transcription factors such as FOX(O)4, PTEN, NF-kB and HLTF; DNA replication and repair proteins such as UbE2E1, claspin and MCM-BP; and proteins involved in epigenetic control of gene expression such as DNMT1 and PRC1 (2,9-15).

In addition to cellular substrates, USP7 is known to interact with proteins from all three subfamilies of the Herpesviridae family of viruses. Specifically, it interacts with immediate early protein ICP0 from Herpes Simplex Virus-1 (HSV-1), which is required for efficient lytic infection and reactivation of latent and quiescent viral genomes (16). USP7 also binds two proteins from Kaposi’s sarcoma-associated herpes virus (KSHV) LANA and vIRF4 (17,18). LANA is the major viral protein expressed in all forms of KSHV-associated malignancies, and vIRF4 is the key player in the switch from latency to lytic reactivation (17,18). USP7 also interacts with the EBNA1 protein from Epstein-Barr virus (EBV), which competes with the p53 transcription factor for USP7 binding (19,20). Thus, USP7 binding to EBNA1 results in p53 destabilization and blockage of the DNA damage response caused by EBV infection (19,20). Finally, USP7 binds the UL35 protein from cytomegalovirus essential for viral replication and particle formation (21). In addition to Herpesvirus targets, USP7 was recently shown to interact with at least one adenoviral protein, E1B-55K, a component of the E1B-55K/E4-orf6 E3 ligase complex that degrades a wide range of intracellular proteins. Downregulation or specific inhibition of USP7 destabilizes E1B-55K and negatively affects viral replication (22,23). Due to the central role USP7 plays in viral infection and cancer development pathways, it represents an attractive target for the design of new anti-viral and anti-cancer therapies. Although knowledge of molecular mechanisms of USP7 specificity towards its diverse substrates is essential for drug development, to date these mechanisms remain poorly understood.

Structurally, USP7 consists of an N-terminal substrate-binding TRAF-like domain followed by a ubiquitin-binding catalytic domain and a large 64 kDa C-terminal region (C-USP7, residues 557-1102) that contains five ubiquitin-like domains (UBL 1-5) shown in Figure 1A (19,24,25). USP7 complexes with several substrates have been previously characterized, including peptides derived from p53, Hdm2, HdmX, UbE2E1, MCM-BP, vIRF4 and EBNA1 (7,14,15,17,20,26). Remarkably, all these substrates share a common USP7-binding P/AxxS motif and interact with the same site located in the TRAF-like domain. On the other hand, the function of the USP7 C-terminal UBL domains remains largely unknown. Interestingly, UBL domains were found in at least 16 members of the USP family. All of them share a characteristic β-grasp ubiquitin fold, although their sequence similarity to ubiquitin may be very limited. Although a few of these UBLs have been characterized, the functions of others, including UBLs of the USP9, USP24, USP34, USP47 and USP48 remain elusive (27). The ICP0 E3 ligase from HSV-1 is the first USP7 substrate shown to interact exclusively with C-USP7, suggesting that the UBL domains may function as a platform for substrate binding. Recent studies revealed that the USP7 C-terminal region contains additional binding sites for p53 and Hdm2, as well as for the transcription factor FOX(O)4, which interacts exclusively with the C-terminus (9,28). However, at this time no structural data is available for any of these C-USP7 complexes.

Here, we report a structural study of the C-USP7 – substrate interaction. With the use of NMR spectroscopy we have shown that individual USP7 UBL domains and/or two-domain constructs are stable in isolation and can be studied independently in solution. Furthermore, we have determined solution NMR structure of the USP7 UBL1 domain and demonstrated that it is nearly identical to the crystal structure of the UBL1 in the context of the intact C-terminus (25). Finally, we have characterized the interaction between C-USP7 and the ICP0 protein from HSV-1 virus and mapped the ICP0 binding interface on the surface of the UBL1-UCL2 domains. Our work represents the first structural study of the USP - substrate recognition mediated by its UBL domains. Since many of the USPs contain multiple UBLs (27), it
is likely that substrate recognition by the UBL domains may prove to be a common feature shared among a class of UBL-containing USPs.

**EXPERIMENTAL PROCEDURES**

**In vitro studies:**

Protein expression and purification. Sequences encoding UBL1 (residues 537-664), UBL12 (residues 535-775), UBL3 (residues 775-888) and UBL45 (residues 884-1102) domains of the human USP7 were sub-cloned into pET28a-LIC Vector (Structural Genomics Consortium). All proteins were purified following the same protocol. Plasmids containing recombinant proteins with the N-terminal Hist-tag were transformed into *Escherichia coli* BL21 (DE3) strain. Proteins were expressed in M9 minimal medium containing 20 mM NaH$_2$PO$_4$, 10 mM imidazole, 1 mM PMSF, pH 7.4, lysed by sonication and centrifuged at 15,000 rpm for 45 min. The supernatant was applied to a Ni$_2$O-based M9 medium using uniformly $^{13}$C/$^1$H-glucose as the sole carbon source. Transformed cells were grown at 37°C to an OD$_{600}$ of 0.6-0.8. Protein expression was induced by adding 1 mM IPTG followed by incubation overnight at 20°C. Cells were harvested, resuspended in buffer containing 20 mM NaH$_2$PO$_4$, 250 mM NaCl, 10 mM imidazole, 1 mM PMSF, pH 7.4, lysed by sonication and centrifuged at 15,000 rpm for 45 min. The supernatant was applied to a Ni-NTA column and proteins were eluted with a buffer containing 20 mM NaH$_2$PO$_4$, 250 mM NaCl, 250 mM imidazole, pH 7.4. Following thrombin or TEV digestion to remove the Hist-tag, proteins were additionally purified using either HiLoad Superdex 75 (for UBL1, UBL3) or HiLoad Superdex 200 (for UBL12, UBL45) columns (GE Healthcare). Final samples used for NMR experiments contained 0.8-1.2 mM of purified protein, 20 mM NaH$_2$PO$_4$, 250 mM NaCl, 2 mM DTT, pH 7.0. The $^{15}$N/$^{13}$C-labeled UBL1 sample additionally contained 0.5 mM PMSF, 1 mM benzamidine and 1 mM TCEP.

NMR spectroscopy and structure calculation. 1H-$^{15}$N HSQC spectra for all C-USP7 constructs were recorded on 800 MHz ($^1$H) Agilent VNMRS spectrometer at 25°C. All experiments for the UBL1 structure calculation were acquired on 600 MHz and 800 MHz ($^1$H) Bruker Avance spectrometers at 25°C. The data were processed with NMRPipe (29) and analyzed with Sparky (30) and CARA (31). The backbone and side-chain resonance assignments of the uniformly $^{15}$N/$^{13}$C-labeled UBL1 domain were performed using standard 2D $^1$H-$^{15}$N HSQC, $^1$H-$^1$C HSQC and 3D HNCA, HNCO, HBHA(CO)NH, CBCA(CO)NH, HC(C)H- and (H)CCH-TOSY experiments (32). Sequence specific resonance assignment of the UBL1 was done using ABACUS software (33,34). Nearly complete UBL1 NMR resonance assignment was obtained with 93.3% of all expected chemical shifts assigned. NMR distance restraints were obtained from 3D $^{15}$N- and $^{13}$C-edited NOE-HSQC experiments (32). H-bond restraints were added on the basis of NOE analysis. Backbone dihedral $\phi$ and $\psi$ angle restraints were derived from the analysis of N, HN, HA, Ca, CB and CO chemical shifts using TALOS+ (35). Structures were calculated using CYANA (36). A total of 200 structures of UBL1 were calculated and 20 structures with the lowest energy were selected and refined using CNS (37).

The backbone resonance assignments of the $^{15}$N/$^{13}$C-labeled deuterated UBL12 construct in its free and ICP0-bound forms were performed using 2D $^1$H-$^{15}$N TROSY and TROSY-based 3D HNCA, HNCOA, HNCAO, HN(1)CAB and $^{15}$N-edited NOE-HSQC (32) recorded on 800 MHz ($^1$H) Agilent VNMRS spectrometer at 25°C. The data were processed with NMRPipe and analyzed with CcpNmr Analysis (38).

**NMR Studies of Protein Dynamics.** The backbone $^{15}$N relaxation experiments for the $^{15}$N-labeled UBL1 were performed at 25°C on 500 MHz ($^1$H) Agilent VNMRS spectrometer as described elsewhere (39,40). All experiments were performed on a C576S UBL1 mutant generated using QuikChange Kit (Agilent) to avoid slow cysteine-induced dimerization. $^{15}$N $R_1$ and $R_1^p$ relaxation rates were measured from a series of eight spectra recorded at different relaxation delays. Relaxation rates were extracted from exponential fits of peak intensities. $^{15}$N $R_2$ were obtained from longitudinal $R_1$ and rotating-frame $R_1^p$ rates (41) measured at a spin-lock field strength of 1592.4 Hz. $^{15}$N ($^1$H) NOE experiment was carried out in an interleaved manner by recording 2D $^{15}$N-$^1$H spectra with and without $^1$H saturation period of 2.5 s using inter-scan delay of 8 s. Heteronuclear NOE values were calculated as ratios of peak intensities in corresponding 2D $^1$H–$^{15}$N correlation spectra. Errors in NOE values were calculated based on peak intensities and the measured spectral noise levels.

**NMR binding experiments.** ICP0 binding to individual USP7 UBL domains was assessed by a series of titration experiments monitored by recording 800 MHz ($^1$H) $^1$H-$^{15}$N HSQC spectra of the domains. Peptide GPRKCARTRH corresponding to the ICP0 residues 617-627 was
custom synthesized (GenScript). The titrations were performed by gradual addition of the unlabelled peptide to ~0.5 mM 15N-labeled UBL domain samples (up to 1:10 protein to peptide ratio). Both, the wild-type UBL1 and C576S mutant constructs were tested, providing the identical results. Frequency (chemical shift) perturbations were calculated for each amino acid residue as $\Delta \omega = (\Delta \omega_\text{H}^2 + \Delta \omega_\text{N}^2)^{1/2}$, where $\Delta \omega_\text{H}$ and $\Delta \omega_\text{N}$ are 15N and 1H frequency differences between ICP0-bound and free states, respectively. The obtained $\Delta \omega$ values were mapped onto the surface of the crystal structure of the UBL12 tandem (PDB ID: 2YLM) (25).

Isothermal Titration Calorimetry (ITC). The affinity of USP7 UBL12 – ICP0 interaction was determined by ITC. Measurements were performed with a Nano ITC instrument (TA Instruments) in a buffer containing 20 mM NaH2PO4, 100 mM NaCl, 2 mM β-mercaptoethanol, pH 7 at 25°C. ICP0 peptide at a concentration of 686 μM was titrated into 85 μM UBL12. A total of 20 injections were made with 300 second time intervals in between. ITC data were analyzed with NanoAnalyze software (TA Instruments). Data fitting was performed using an “independent” model after correcting for the heat produced by ICP0 dilution.

In vivo studies:

Cell lines and Viruses. Vero and HFF-1 cells were purchased from the ATCC. HFF-1 cells were maintained in DMEM supplemented with 10% fetal bovine serum and used between passages 5 and 10. Vero cells were maintained in DMEM supplemented with 5% fetal bovine serum. Strain 17+ was used as wild type HSV-1. Virus in 1863 is derived from strain 17+ and was obtained from Chris Preston (MRC Virology Unit, Glasgow, Scotland). This virus contains the tk gene under the control of the HCMV promoter/enhancer inserted in the tk gene.

Growth yield of HSV-1 on shUSP7 cells. Lentivirus generation and virus infection were done as previously described (42). Briefly, the pLKO.1 system was used to package lentiviruses and deliver shRNA into target cells. HFF-1 cells were infected with lentiviruses expressing shRNA against GFP (GCAAGCUGACCCUGAGUUCA) or USP7 targeting sequence (GGCAACCTTTACAGTCTACT) and selected with 2 μg/mL Puromycin. 72 hours post-lentiviral infection cells were infected with HSV-1 (in1863) at multiplicities of infection (MOI) of 0.1 PFU/cell. Progeny virus was collected at 24 hours post infection and titers were determined on Vero cells by staining with crystal violet or X-gal for β-galactosidase-positive plaques as previously described (42).

Western blot analysis was used to visualize USP7 and ICP0. Ku70 was used as a loading control. After lentiviral transfection cells were infected with HSV-1 at an MOI of 2 PFU/cell. Four hours post infection cells in 35mm dishes were lysed in 2X SDS sample buffer (4% SDS, 20% glycerol, 100mM Tris pH 6.8, 100mM DTT, 10% β-Mercaptoethanol, 1X protease inhibitor cocktail (Roche), and 0.1% bromophenol blue) and boiled for 5 minutes. Proteins were resolved by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked for 1 hour in 5% non-fat dry milk dissolved in TBST. Primary antibodies were diluted in blocking solution and incubated overnight at 4°C. Primary antibodies used include monoclonal mouse ICP0 (5H7) (1:1,000; Abcam), polyclonal rabbit USP7 (H200) (1:1,000; Santa Cruz), and monoclonal mouse Ku70 (Ab-4) (1:5,000; NeoMarker).

RESULTS

Isolated USP7 UBL domains can be studied independently in solution – The full length USP7 as well as the C-USP7 have long represented a challenge for structural studies because of their rapid degradation during recombinant expression in E. coli (43). As a consequence, difficulties in C-USP7 production hampered investigation of its structure and function. The recent crystal structure of the isolated C-USP7 produced in insect cells (25) revealed the domain organization of the C-USP7 and confirmed that it consists of five ubiquitin-like domains. In order to produce stable constructs of the C-USP7 suitable for studies in solution we have tested bacterial expression of all individual UBL domains and their combinations in E. coli. We found that the isolated UBL1 and UBL3 express well and result in stable soluble proteins. In contrast, UBL2, UBL4 and UBL5 are unstable and degrade rapidly when expressed on their own. However, the two-domain combinations UBL12 and UBL45 express well and are stable and soluble. These results are in agreement with the C-USP7 crystal structure, which shows extensive interactions between UBL1 and UBL2 as well as between UBL4 and UBL5. $^{1}H$-$^{15}N$ HSQC NMR spectra of UBL1, UBL12, UBL3 and UBL45 are shown in Figure 1B. High quality of spectra and good chemical shift dispersion suggest that isolated UBL domains and/or their combinations described above represent
Solution structure of the USP7 UBL1 domain.

Figure 2 shows solution NMR structure of the USP7 UBL1 domain (residues 537-664). According to secondary structure prediction obtained from the backbone $^1$H, $^{15}$N and $^{13}$C NMR chemical shifts using TALOS+ program (35), UBL1 adopts a characteristic ubiquitin-like $\beta$-grasp fold formed by $\beta_1$-$\beta_2$-$\alpha_3$-$3_{10}$-$\beta_4$-$\alpha_5$-$\alpha_3$-$\beta_4$ elements (Fig. 2A, top panel). The bottom panel on Figure 2A shows order parameters $S^2$ for the backbone amide groups of the domain predicted from NMR chemical shifts using Random Coil Index (RCI) approach (44). High $S^2$ values of 0.8 – 0.9, indicative of limited backbone flexibility, were obtained for a majority of the residues. The exceptions are a linker connecting the first two helices with the first $\beta$-strands of the $\beta$-grasp fold and two large loops: loop I and loop II. The final ensemble of the 20 lowest energy structures (Fig. 2B) displays root mean square deviation (rmsd) of 1.96 ± 0.44 Å for the backbone and 2.70 ± 0.38 Å for all heavy atoms (see Table 1 for structural statistics). As expected, the overall structure of the domain is well defined in the NMR ensemble with the exception of the two loops. (Fig. 2B). The structure consists of a long kinked N-terminal $\alpha$-helix (helices $\alpha_1$ and $\alpha_2$) followed by a $\beta$-grasp fold (Fig. 2C, D). The $\alpha_3$-helix is packed against an anti-parallel $\beta$-sheet ($\beta_2$-$\beta_1$-$\beta_3$-$\beta_4$-$\beta_4$) that forms a barrel-like structure (Fig. 2D). In this barrel, the $\beta_3$-strand is very short and is stabilized by a single hydrogen bond between the backbone amide group of Leu 638 and carboxyl of Leu 622 from the $\beta_4$-strand. The N-terminal helix $\alpha_1$ does not belong to the ubiquitin-like fold and, in the context of the full length USP7, connects C-UBL1 with the catalytic domain of the enzyme.

Although the sequence similarity of the USP7 UBL1 to ubiquitin is only 21%, it closely resembles a ubiquitin fold (PDB ID: 1UBQ) with the backbone rmsd between the UBL1 and ubiquitin of 2.4 Å for regular secondary structure elements. Significant differences, however, are observed in the loop regions. Thus, the UBL1 loop I that connects the $\beta_1$- and $\beta_2$-strands (residues 571-591) as well as loop II between the $\beta_3$- and $\beta_4$-strands (residues 625-637) are notably longer than those of ubiquitin. The hydrophobic patch equivalent of the ubiquitin Ile44 patch (45) is also present in UBL1 and is centered around Trp623 (Fig. 2D).

Figure 2E shows an overlay of the solution (red) and crystal (blue; PDB ID: 2YLM) structures of the UBL1 domain. The UBL1 NMR structure is in good agreement with the crystal structures of the entire C-UBL1 (PDB ID: 2YLM) and the UBL12 tandem (PDB ID: 4PYZ) with the backbone rmsd between NMR and crystal structures of 2.40 Å and 2.35 Å, respectively. The most significant difference is in the loop conformations. The long loop II connecting the $\beta_3$- and $\beta_4$-strands is poorly defined in the solution structure with only short and medium-range ($|i-j|<5$) NOEs observed in this region (Fig. 2F). This is in contrast to the crystal structure where residues 625-635 form type-I $\beta$-hairpin. The position of the hairpin is stabilized by interaction with the UBL2 domain, including inter-domain salt bridges formed by residues Arg628 and Asp764, as well as Arg634 and Glu737. Similarly, position of the loop I connecting $\beta_1$- and $\beta_2$-strands (residues 571-591) is poorly defined in our solution structure, while in the crystal structure it is stabilized by several inter-domain hydrogen bonds with the UBL2 residues. Additionally, in the crystal structure, relative position of the two long loops is constrained by hydrogen bond between the backbone CN group of Lys633 (loop II) and OD2 group of Asp582 (loop I), while in the NMR ensemble the loops sample multiple conformations. Overall, the observed differences between the two structures may result from additional domains present in the X-ray structure and the fact that NMR is sensitive to protein dynamics in solution.

Backbone dynamics of the USP7 UBL1 domain –

To confirm flexible conformations of the loops we have investigated the extent of conformational dynamics of the UBL1 domain using the backbone $^{15}$N NMR relaxation measurements, including $^{15}$N R$_1$ and R$_2$ rates and heteronuclear $^{15}$N-$^1$H NOE values (39-41). These data report on Brownian rotational diffusion of a protein and its conformational flexibility on the picosecond to nanosecond time scales. $^{15}$N R$_1$ and R$_2$ relaxation rates and $^{15}$N-$^1$H NOEs suggest that the UBL1 is a rigid molecule with increased sub-nanosecond time-scale flexibility of the two long UBL1 loops, as evidenced by a decrease in $^{15}$N-$^1$H NOE values, lower R$_2$ rate and elevated R$_1$ rates for amino acid residues located in the loops I and II (Fig. 3). Additionally, relaxation rates and NOEs suggest that the N-terminal $\alpha$-helix that does not belong to the $\beta$-grasp fold is also highly dynamic on the sub-nanosecond timescale.

USP7 is required for the efficient HSV-1 infection in HFF-1 cells – As a first step toward understand-
ing the mechanism of substrate recognition by the C-USP7, we have characterized its interaction with the ICP0 E3 ligase from HSV-1. ICP0 was the first discovered and remains the best studied substrate of the USP7 that interacts exclusively with the enzyme’s C-terminus (19). ICP0 is a 775 amino acid residue long immediate early protein that is expressed immediately after infection. Although ICP0 doesn’t interact with DNA directly, it acts as a promiscuous activator for all three temporally regulated kinetic classes of viral genes (reviewed in (46)). It was previously shown that the ICP0 self-ubiquitination promotes its own degradation; however, the formation of a complex with cellular USP7 stabilizes ICP0 and rescues it from proteasomal degradation (47,48). Mutations that abolish the USP7 binding lead to ICP0 destabilization during HSV-1 infection in U2O2 cells (48). Moreover, treatment of HeLa cells with siRNA against USP7 leads to a defect in ICP0 accumulation and reduced expression of many ICP0 target genes, including ICP4 and UL42 (49). Furthermore, ICP0 interaction with USP7 causes migration of the latter from nucleus to cytoplasm where it inhibits NF-kB dependent inflammatory cytokine response to protect HSV-1 from innate immunity (50).

Altogether, these data suggest that USP7 not only plays an essential role in regulation of ICP0 levels in the infected cell, but also is required for establishment of successful HSV-1 infection. This makes USP7/ICP0 binding interface an attractive target for the development of antiviral therapeutics. Therefore, we have explored the effect of USP7 knockdown on the efficiency of HSV-1 infection. Although ICP0 is not essential for virus replication in cultured cells, ICP0-null mutant viruses grow poorly at low MOI. Because this effect is cell type dependent, we have utilized limited-passage human diploid fibroblasts since they are sensitive to the presence of ICP0 (reviewed in (46)). HFF-1 cells were infected with lentiviruses containing USP7 compared to cells expressing shRNA against GFP. These results suggest that the presence of USP7 in the cell and its interaction with ICP0 are essential for establishment of successful lytic infection at low MOI in HFF-1 cells.

The first two USP7 UBL domains (UBL12) are responsible for interaction with ICP0 – The minimal ICP0 region required for interaction with USP7 was previously defined by R. Everett et al. as residues 619-626 (47). This short peptide contains several Lysine and Arginine residues, which make it highly positively charged. On the other hand, the region of USP7 responsible for interaction with ICP0 was roughly defined using limited proteolysis to reside within residues 599-801 (19). This region contains a part of UBL1, the entire UBL2 and a part of the UBL3 domains. To determine the domain(s) of C-USP7 responsible for interaction with ICP0 we have performed a series of NMR binding experiments. Isolated $^{15}$N-labeled UBL1, UBL12 and UBL3 domains of the USP7 were gradually titrated with the unlabeled ICP0 peptide (residues 617-GPRKCARKTRH-627) and binding-induced changes in peak positions in $^1$H.$^{15}$N HSQC spectra of the domains were monitored. As seen in Figure 5B and C, no peak shifts were observed for the UBL1 and UBL3, indicating that these domains alone are not sufficient for interaction with the ICP0 peptide. In contrast, the addition of ICP0 peptide to the $^{15}$N UBL12 caused progressive changes in the positions and intensities of a number of peaks in its $^1$H.$^{15}$N HSQC spectrum indicative of specific ICP0 binding (Fig. 5A).

In the course of titration with ICP0, the peaks in the NMR spectrum of UBL12 do not move gradually, but rather disappear and then show up in the new positions in the spectrum, indicative of slow on the NMR time-scale binding process. Therefore, to measure UBL12 – ICP0 binding affinity and determine thermodynamic parameters for this interaction we have employed isothermal titration calorimetry (ITC) (Fig. 5D). The results indicate that UBL12 binds ICP0 in 1:1 stoichiometry. The best fit of the titration curve was obtained with an independent site model with dissociation constant ($K_D$) of $14.6 \pm 1.3 \mu M$ and enthalpy ($\Delta H$) of $-24.5 \pm 0.7$ kcal/mol, resulting in entropy ($\Delta S$) of $-60.2$ cal/(mol K) and Gibbs Free energy ($\Delta G$) of $-6.6$ kcal/mol at 25°C. High $\Delta H$ value suggests that reaction is enthalpy-driven with primary contributions to the complex stabilization likely resulting from electrostatic interactions and/or hydrogen bonds.
Mapping of UBL12-ICP0 binding site – As seen in Figure 5A, a large number of peaks have shifted in 1H-15N HSQC spectrum of the USP7 UBL12 as a result of ICP0 peptide binding. Due to slow on the NMR time scale binding, the backbone resonances of the UBL12 in complex with ICP0 cannot be assigned by tracing peak shifts in the UBL12 spectrum during the titration. Therefore, to quantify ICP0-induced changes in peak positions and thus identify the UBL12 amino acid residues responsible for ICP0 recognition, we have assigned the backbone resonances of the free and ICP0-bound forms of the UBL12.

Figure 6A summarizes the extent of the UBL12 chemical shift assignments. Circles below the amino acid sequence correspond to assigned peaks in 1H-15N HSQC spectra of the free and ICP0-bound UBL12 constructs. UBL1 and UBL2 portions of UBL12 are colored in blue and green, respectively. A total of 74% of expected NMR resonances of free UBL12 and 82% resonances of ICP0-bound UBL12 have been assigned. The remaining peaks were broadened beyond detection. The residues with missing assignments in either free or ICP0-bound UBL12 are marked by stars and arrows above the amino acid sequence. Most of these residues are missing in the spectrum of free UBL12 and appear only after ICP0 binding. Such a behavior points to changes in the UBL12 conformation and dynamics as well as changes in chemical environment of the nuclei on the interaction interface associated with the substrate recognition.

ICP0 binding led to substantial frequency (chemical shift) changes for the vast majority of the UBL12 amide resonances (Fig. 5A, 6F) with largest perturbations (>150 Hz) observed for the residues Gly631, Leu638, Val685, Leu699, Ile709, Ser710, Ser752, Leu757 and Asp758. The complex formation noticeably improved the quality of the spectrum, suggesting that ICP0 binding stabilizes the UBL12 tandem (Fig. 5A, bottom panel). As a result, a number of intense peaks appeared in the UBL12 spectrum upon ICP0 binding. While the backbone amide resonances for 171 out of 230 non-proline residues were observed for the free protein, 25 additional peaks were assigned for the complex (Fig. 6A). The vast majority of these new peaks correspond to residues located either on the UBL1/UBL2 interface or in its close proximity (Glu572, Phe575, His578, Gln579, Asn581, Asp582, Ile620, Gln626, Arg628, Asn630, Thr632, Glu663, Thr664, Val665, Met686, Asn700, Tyr701, Tyr706 and Val738) (Fig. 6D). In addition, 8 peaks in 1H-15N HSQC spectrum of the UBL12 disappeared upon ICP0 binding, pointing to changes in the UBL12 conformation and/or dynamics caused by the complex formation. These peaks correspond to residues Asp680, Lys681, Asp682, Gly763, Asp764 and Ile765 located on the UBL1/UBL2 interface and residues Glu759 and Leu760 located in the loop between β1- and β2-strands of the UBL2.

Overall, mapping binding-induced frequency (chemical shift) changes onto the UBL12 structure (25) identified residues sensitive to ICP0 binding in both UBL1 and UBL2 domains. The regions of the UBL1 and UBL2 with NMR peaks affected by ICP0 addition are shown on the protein surface in Figures 6C and D. The UBL1 residues exhibiting pronounced frequency (chemical shift) changes are mainly located in the loops I and II. The residues of UBL2 affected by ICP0 binding are located in the two distinct regions. The first region includes a hydrophobic patch formed by residues in strands β1, β2, β3 and β8 of the UBL2 β-sheet. This face of the UBL2 domain makes extensive contacts with the UBL1, which protect this patch from solvent exposure (Fig. 6D). The second region is solvent exposed and includes residues from the two loops: the UBL2 loop connecting β2-strand with the following α-helix, and the loop connecting β4 and β5 strands (Fig. 6C and D). These loops form a negatively charged cluster on the UBL2 surface, which provides a binding interface for the positively charged ICP0 peptide.

DISCUSSION

Owing to USP7 involvement in maintenance of several important tumor suppressors and key viral proteins, it has emerged as a potential therapeutic target for treatment of cancer and infections caused by DNA viruses (51). Therefore, the mechanisms of USP7 specificity toward its diverse substrates are of significant interest.

While functions of the USP7 N-terminal TRAF and catalytic domains were previously characterized, the functions of its UBL domains remain largely unknown. Roughly, UBLs within the USP family of proteins can be divided into three groups: N-terminal, C-terminal or embedded in the USP catalytic core (52). The USP7 UBL domains belong to the third group of the C-terminal UBLs. Previous studies revealed that UBLs of USP7 enhance its enzymatic activity, since even small truncations of the extreme C-terminus dramatically decrease the USP7 catalytic activity (25,28). Furthermore, it was recently shown that the UBL domains can function as a binding platform for several USP7 substrates.
However, the precise location of the C-USP7 substrate binding sites remained unknown (9,19,28).

USP7 is unique within the USP family, since, unlike other family members, it contains a tandem array of the five UBL domains. As a consequence of large size of the USP7 C-terminus, it is challenging to study all five tandem UBL domains together. Here we show that this complex system can be simplified by the isolation of stable individual UBL domains and/or constructs containing two consecutive UBL domains. Our NMR data reveal that these constructs can be studied in isolation in solution, providing new insights into the C-USP7 structure, dynamics and substrate specificity. Although there are two available X-ray crystal structures of the C-USP7 (PDB IDs: 2YLM, 4PYZ), these structures provide little information about protein dynamics. In this study we focused on the UBL1 domain and UBL12 tandem. We have: (i) determined three-dimensional NMR structure of the UBL1 domain and characterized its dynamic properties, (ii) narrowed down the ICP0-binding region of the USP7 to the UBL12 domains, and (iii) obtained high-resolution NMR mapping of the USP7 binding site for ICP0.

Once inside the cell, HSV-1 encounters a hostile environment created by host proteins that are intrinsically antiviral. HSV-1 encodes proteins such as ICP0 intended to inactivate cellular antiviral mechanisms (46). The interaction between ICP0 and USP7 is essential to prevent ICP0 degradation during the infection, and in this study, we have structurally and functionally characterized this interaction. As a first step, we have investigated the effect of USP7 ablation on the efficiency of HSV-1 lytic infection. We utilized shRNA to generate USP7 knockdown cells and demonstrated that in cells lacking USP7 virus growth is severely reduced. These results suggest that the USP7-ICP0 interaction is important for the establishment of effective lytic HSV-1 infection in HFF-1 cells.

Next, we used solution NMR to structurally characterize the ICP0 – USP7 interaction, and have shown that USP7 binds ICP0 via its first two C-terminal UBL domains, providing evidence that the USP7 UBL domains function as a substrate interacting platform. The ICP0 peptide used in this study was previously identified as a minimal USP7-binding region of ICP0 (47). This peptide is composed of eleven amino acid residues, six of which are positively charged and three are polar (Fig. 7B). Thus, of the two USP7 regions affected by ICP0 binding, the one located at the UBL2 β-sheet is unlikely to directly interact with ICP0 because it consists of mainly hydrophobic residues buried on the UBL1/UBL2 interface. On the other hand, the second region encompassing the UBL2 loops, one between the β2-strand and the following α-helix and the other between the β3 and β5 strands, has negatively charged surface and is more likely to directly bind positively charged ICP0 peptide. High enthalpy of complex formation (-24.5 ± 0.7 kcal/mol) measured by ITC provides further evidence of the electrostatic nature of UBL12 – ICP0 interaction.

NMR chemical shift mapping revealed that the binding of the two proteins is likely mediated by electrostatic interactions. This is in agreement with previous mutational analysis of ICP0, which identified Arg619, Lys620, Lys624 and Arg626 as residues critical for interaction with the USP7 (47) (Fig. 7A). Interestingly, although the UBL1 domain alone does not interact with ICP0 (Fig. 5B), it exhibits extensive binding-induced chemical shift changes in the context of the UBL12 construct (Fig. 6F). Notably, UBL1 residues involved in ICP0 binding belong to the two long flexible loops located in the close proximity of the UBL1 - UBL2 interface, supporting the notion that these two domains may function in tandem. The presence of UBL2 may constrain conformation of the UBL1 flexible loops, as seen in crystal structures of the C-USP7 UBL domains. Overall, our data suggests that UBL12 may undergo micro- to millisecond opening/closing events, resulting in the observed line broadening and/or disappearance of peaks on the interface of UBL1 and UBL2 domains. As evident from chemical shift mapping, most of the UBL12 binding site for ICP0 is located on the UBL2 surface with an additional participation of residues from loop II of the UBL1. We speculate that the substrate binding may lock the two domains in a closed conformation, thus, limiting micro- to millisecond conformational sampling and quenching exchange contributions to transverse relaxation. This model could explain chemical shifts changes and line narrowing on the inter-domain interface observed upon ICP0 binding.

The HSV-1 ICP0 has never been structurally characterized, although it was shown to contain an N-terminal RING domain similar to one from an immediate-early protein of equine herpes virus. (53,54). The USP7-binding motif of ICP0 is located within the molecule’s C-terminus (residues 619-626). We used Jpred (55) to predict the
secondary structure of the HSV-1 ICP0 and found that its C-terminal region is predicted to contain a structured domain with α-α-β-β-α-β-β arrangement (Fig. 7A). Furthermore, we have compared the HSV-1 ICP0 protein with its orthologues from other Alphaherpesvirinae subfamily viruses using SIB Blast followed by multiple sequence alignment in ClustalW (56) and conservation score calculation using ConSurf server (57,58) and found that the ICP0 sequence has two highly conserved regions. The first region includes the N-terminal RING domain characteristic of E3 ubiquitin ligases, while the second contains a yet uncharacterized C-terminal domain (CTD). Figure 7A shows a multiple sequence alignment of the ICP0 CTD. Remarkably, the USP7 binding motif (residues 619-626) is one of the best conserved regions within the ICP0 C-terminus and it is predicted to form an α-helix. According to the helix wheel projection, this helix is amphipathic (positively charged on one side and neutral on the other), which may facilitate its interaction with a negatively charged cluster on the UBL2 surface (Fig. 7B). Other predicted secondary structure elements of the ICP0 CTD are also conserved, supporting the notion that ICP0-like proteins harbor a structured CTD in addition to the N-terminal RING domain. High amino acid conservation in the ICP0 region involved in USP7 binding implies that ICP0 interaction with the USP7 is not unique to HSV-1 but may represent a common feature shared by other members of the Herpesvirus family.

The fact that the five non-homologous USP7 UBL domains can interact with multiple USP7 targets suggests that the UBL domains serve as a versatile binding platform and endow the enzyme with specificity to its diverse substrates. However, until now, none of the C-USP7 substrate binding sites has been characterized at high resolution. In this work, for the first time, we have structurally and functionally characterized the interaction of the C-USP7 with its first identified target – HSV-1 ICP0 protein. Our results, along with future studies of other C-USP7 substrate-binding sites, may unravel determinants of the USP7 specificity towards its substrates and provide a structural basis for the future development of new therapeutic strategies to prevent lifelong HSV-1 infection and virus expansion among human population.

Acknowledgments
We thank Dr. Alexander Lemak for his help with UBL12 chemical shift assignment and Khiem Nguyen and Dr. Olga Vinogradova for assistance with ITC experiment. Also we thank Dr. Yulia Pustovalova and Dr. Mariana Quezado for helpful discussions. This work was supported by Charles H. Hood Foundation Child Health Research Award and State of Connecticut Department of Public Health Biomedical Research Grant to I.B. Chemical shift assignments of UBL1 have been deposited to the Biological Magnetic Resonance Data Bank (BMRB) under accession number # 16789. 20 lowest energy structures of the USP7 UBL1 domain were deposited to Protein Data Bank (PDB) under the code # 2KVR.

Conflict of interest
The authors declare that they have no conflicts of interest with the contents of this article.

Authors contributions
AKP performed all experiments and data analysis associated with USP7-ICP0 binding and wrote a manuscript. KHM and SKW performed and analyzed in vivo experiment shown in Figure 4. IB, SDP and CHA contributed to NMR structure determination of UBL1 domain. AKP and DMK performed and analyzed NMR dynamics experiments. All authors reviewed the results and approved the final version of the manuscript.
REFERENCES
Structural characterization of USP7 – ICP0 interaction


42. Mohni, K. N., Livingston, C. M., Cortez, D., and Weller, S. K. (2010) ATR and ATRIP are recruited to herpes simplex virus type 1 replication compartments even though ATR signaling is disabled. J. Virol. 84, 12152-12164
47. Everett, R. D., Meredith, M., and Orr, A. (1999) The ability of herpes simplex virus type 1 immediate-early protein Vmw110 to bind to a ubiquitin-specific protease contributes to its roles in the activation of gene expression and stimulation of virus replication. J. Virol. 73, 417-426
Structural characterization of USP7 – ICP0 interaction

FIGURE LEGENDS

Figure 1. Isolated USP7 UBL domains can be studied independently in solution. A. Schematic representation of the domain arrangement of the human USP7 protein. B. 2D $^1$H-$^{15}$N HSQC spectra of the UBL1 (blue), UBL12 (green), UBL3 (black) and UBL45 (red) domains.

Figure 2. Solution NMR structure of the USP7 UBL1 (residues 537-664). A. Top panel shows TALOS+ derived probabilities of α-helix and β-sheet formation, marked as P(α) and P(β), respectively. Bottom panel shows RCI-based backbone amide order parameters, S$^2$. B. The backbone representation of the ensemble of 20 NMR lowest-energy structures of the USP7 UBL1. C. Amino acid sequence of the USP7 UBL1 domain with cartoon representation of its secondary structure elements shown at the top. Helices are colored in red, β-strands are shown in green. D. Ribbon representation of the UBL1 structure with secondary structure elements and loops I and II labeled. E. Superposition of solution (red) and crystal (blue; PDB ID: 2YLM) structures of the UBL1. F. NOE connectivity plot of UBL1. Flexible loops I and II are labeled and shown in grey.

Figure 3. The backbone dynamics of the UBL1 from $^{15}$N relaxation. A. $^{15}$N R$_1$, R$_2$ relaxation rates and $^{15}$N($^1$H) heteronuclear NOE values shown as a function of the UBL1 residue number. Secondary structure elements are shown at the bottom. B. Mapping of heteronuclear 1-NOE values onto the UBL1 structure. 1-NOE values are mapped as a color gradient (blue to magenta) and ribbon radius. Dynamic regions correspond to intense magenta color and thicker ribbon. All experimental relaxation data were acquired at 500 MHz ($^1$H) and 25 °C.

Figure 4. USP7 is required for successful establishment of HSV-1 lytic infection. HFF-1 cells were infected with lentiviruses expressing shRNA against USP7 (shUSP7) in order to downregulate cellular USP7. shRNA against GFP (shGFP) was used as a control. A-B. After selection, knockdown cells were infected with HSV-1 at an MOI of 0.1 PFU/cell. Progeny virus was collected at 24 hours post infection and titers were determined on Vero cells by staining with either crystal violet (A) or X-gal for β-galactosidase-positive plaques (B). In B, values correspond to the average of three independent experiments and errorbars correspond to one standard deviation. C. Efficiency of USP7 knockdown and ICP0 stability were tested by Western blot analysis. USP7 knockdown cells were infected with HSV-1 at an MOI of 2 PFU/cell and cell lysates were prepared at 4 hours post infection and subjected to Western blot analysis for ICP0 and USP7. Ku70 serves as a loading control.

Figure 5. NMR and ITC analysis of the USP7 UBL domain interactions with the ICP0$_{617-627}$ peptide. Overlay of $^1$H-$^{15}$N HSQC spectra of the UBL12 (A), UBL1 (B), and UBL3 (C) domains before (red) and after addition of the unlabeled ICP0 peptide (residues 617-627) to a final protein:peptide ratio of 1:10 (blue). In A, the signals of UBL12 with chemical shift perturbations over 100 Hz are labeled. Among these, the peaks corresponding to residues located on the ICP0-binding interface are labeled with asterisks. The bottom panel shows changes in line shapes for selected UBL12 peaks upon ICP0 binding. D. ITC profiles for UBL12 binding to ICP0. Left panel shows heat change upon ligand addition; right panel shows an integrated ITC isotherm and its best fit to an independent site model.

Figure 6. Analysis of ICP0 binding to the USP7 UBL12 tandem. A. Completeness of the backbone assignment for the free and ICP0-bound UBL12. Here and later UBL1 and UBL2 domains are colored in shades of blue and green, respectively. Circles below the protein sequence represent amino acid residues with assigned NMR resonances in $^1$H-$^{15}$N HSQC spectra of the free (light circles) and ICP0-bound UBL12 (dark circles). Open spaces indicate residues with unassigned amide peaks. Asterisks indicate UBL12 residues with assigned amide peaks in the bound state only, while arrows indicate residues with assigned amide peaks in the free state only. B. Ribbon representation of the UBL12 (pdb ID: 2YLM) (25). C. Mapping of the frequency differences between the free and ICP0-bound UBL12 $\Delta\omega=(\Delta\omega_\alpha^2+\Delta\omega_\beta^2)^{1/2}$ on the UBL12 surface. Color gradient corresponds to $\Delta\omega$ values, with larger changes shown by more intense color. D. Mapping of the UBL2 residues located on the UBL1/UBL2 interface and affected by ICP0 binding. Same as in C (top view) but rotated about z-axis by 90°; for clarity, UBL1 is shown as orange.
Structural characterization of USP7 – ICP0 interaction

ribbon. E. Close-up view of the UBL12 ICP0-binding interface. F. Per-residue amide frequency differences between the free and ICP0-bound states of the UBL12 (Δω, Hz) as a function of residue number. Residues with peaks missing in one of the two spectra (free or bound) are shown as bars with the maximum Δω of 650 Hz to indicate that these residues are sensitive to ICP0 binding. Prolines and residues with missing amide group assignment in both spectra are shown as empty spaces. Secondary structure elements are shown at the top.

Figure 7. C-terminal region of or the ICP0 may contain structured domain. A. Sequence alignment of the HSV-1 ICP0 (residues 604-775) and a number of its viral orthologues performed in ClustalW (56). Viruses are Human Herpesvirus 1 (HSV1), Chimpanzee alpha-1 herpesvirus (ChHV), Human Herpesvirus 2 (HSV2), Cercopithecine Herpesvirus 1 (CeHV1), Cercopithecine Herpesvirus 16 (CeHSV16), Macropodid Herpesvirus 1 (MaHV1) and Saimiriine Herpesvirus 1 (SHV1). Residues are colored according to conservation scores calculated using ConSurf server (57, 58). Secondary structure elements predicted in Jpred (55) for the HSV-1 ICP0 are shown at the top. Arrows above the alignment indicate residues critical for USP7 binding identified in previous mutagenesis analysis (47). B. HSV-1 ICP0 peptide (residues 617-627) shown in a helical wheel projection created using EMBOSS Pepwheel program. Hydrophobic residues are shown as grey spheres, polar residues as diamonds and basic residues as blue spheres.
### Table 1. NMR structure determination statistics

<table>
<thead>
<tr>
<th>Summary of restraints</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NOE distance restraints</td>
<td></td>
</tr>
<tr>
<td>Short range (</td>
<td>i-j</td>
</tr>
<tr>
<td>Medium range (1&lt;</td>
<td>i-j</td>
</tr>
<tr>
<td>Long range (</td>
<td>i-j</td>
</tr>
<tr>
<td>Total</td>
<td>3041</td>
</tr>
<tr>
<td>Dihedral angles (φ and ψ)</td>
<td>187</td>
</tr>
<tr>
<td>Hydrogen bonds</td>
<td>32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deviation from experimental restraints</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NOE (Å)</td>
<td>0.022±0.003</td>
</tr>
<tr>
<td>Dihedral restraints (degrees)</td>
<td>1.757±0.123</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deviation from idealized geometry</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonds (Å)</td>
<td>0.019</td>
</tr>
<tr>
<td>Angles (degrees)</td>
<td>1.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ramachandran plot statistics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Most favored regions</td>
<td>77.2%</td>
</tr>
<tr>
<td>Additionally allowed regions</td>
<td>21.8%</td>
</tr>
<tr>
<td>Generously allowed regions</td>
<td>0.8%</td>
</tr>
<tr>
<td>Disallowed regions</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RMSD from mean structure (Å)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All residues</td>
<td></td>
</tr>
<tr>
<td>Backbone atoms</td>
<td>1.96±0.44</td>
</tr>
<tr>
<td>Heavy atoms</td>
<td>2.70±0.38</td>
</tr>
<tr>
<td>Residues in ordered regions¹</td>
<td></td>
</tr>
<tr>
<td>Backbone atoms</td>
<td>0.62±0.09</td>
</tr>
<tr>
<td>Heavy atoms</td>
<td>1.25±0.14</td>
</tr>
</tbody>
</table>

¹ The following secondary structure elements were included as ordered regions in the RMSD calculation: 539-548, 551-554, 556-570, 592-595, 602-613, 617-619, 621-624, 649-652, 658-663.
Figure 2

A

Loop I

Loop II

B

N

C

D

E

F

NMR  X-RAY

Loop I

Loop II

Trp623

α2

α3

β2

β3

β4

β5

α1

α2

α3

α4

α5

PQQLVERLQEEKRIEAQKRKRQEAEHLTMQVQIVAEDEQFCGHQGNDMYDEEKVKYTFFKLKN

SLAEFVQLLSQTMGFPQDQIRLWMQARSGKPRAMLNEADGNKTMLIEQSLNENPWTTFLK

L

Loop I

Loop II

P(α)

P(β)

S²

540 550 560 570 580 590 600 610 620 630 640 650 660

540 550 560 570 580 590 600 610 620 630 640 650 660

540 550 560 570 580 590 600 610 620 630 640 650 660
Figure 3

A

B

Loop I

Loop II

Loop I

Loop II
Figure 4

A

B

C

Virus Yield (Log10)

10^{-5} \text{ virus dilution}

10^{-4} \text{ virus dilution}

shGFP

shUSP7

ICP0

USP7

Ku70

Figure 4

A

B

C

Virus Yield (Log10)

10^{-5} \text{ virus dilution}

10^{-4} \text{ virus dilution}

shGFP

shUSP7

ICP0

USP7

Ku70
Figure 7

**A**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV1</td>
<td>604 RSSLGPRPA--EQGPRKCVRKTRHDAE---GA-PPAPGPTRYLPISGVSSVVALAPYLNKTVTGDCLPVLDMETGDIGAYVVLVGRDCHLARALADAEPQWARRSRLPEPAPGR</td>
</tr>
<tr>
<td>ChHV</td>
<td>674 RSSLGPRPA--EQGPRKCVRKTHDAD---RA-PPAPGPTRYLPVAGLSSVVALAPYLNKTLTGDCLPVLDTDTGAIGAYVVLVGRDCHLARALADAEPQWARRSRLPEPAPGR</td>
</tr>
<tr>
<td>HSV2</td>
<td>647 RSSLGPRPA--EQGPRKCVRKTRHDAE---GA-PPAPGPTRYLPISGVSSVVALAPYLNKTVTGDCLPVLDMETGDIGAYVVLVGRDCHLARALADAEPQWARRSRLPEPAPGR</td>
</tr>
<tr>
<td>CeHV1</td>
<td>532 RSSLGPRPA--EQGPRKCVRKTRHDAE---GA-PPAPGPTRYLPISGVSSVVALAPYLNKTVTGDCLPVLDMETGDIGAYVVLVGRDCHLARALADAEPQWARRSRLPEPAPGR</td>
</tr>
<tr>
<td>CeHV16</td>
<td>405 RSSLGPRPA--EQGPRKCVRKTRHDAE---GA-PPAPGPTRYLPISGVSSVVALAPYLNKTVTGDCLPVLDMETGDIGAYVVLVGRDCHLARALADAEPQWARRSRLPEPAPGR</td>
</tr>
<tr>
<td>MaHV1</td>
<td>381 RSSLGPRPA--EQGPRKCVRKTRHDAE---GA-PPAPGPTRYLPISGVSSVVALAPYLNKTVTGDCLPVLDMETGDIGAYVVLVGRDCHLARALADAEPQWARRSRLPEPAPGR</td>
</tr>
<tr>
<td>SaHV1</td>
<td>538 RSSLGPRPA--EQGPRKCVRKTRHDAE---GA-PPAPGPTRYLPISGVSSVVALAPYLNKTVTGDCLPVLDMETGDIGAYVVLVGRDCHLARALADAEPQWARRSRLPEPAPGR</td>
</tr>
</tbody>
</table>

**B**

Conservation score
Structural Characterization of Interaction between Human Ubiquitin Specific Protease 7 and Immediate Early Protein ICP0 of Herpes Simplex Virus-1
Alexandra K. Pozhidaeva, Kareem N. Mohni, Sirano Dhe-Paganon, Cheryl H. Arrowsmith, Sandra K. Weller, Dmitry M. Korzhnev and Irina Bezsonova

J. Biol. Chem. published online July 29, 2015

Access the most updated version of this article at doi: 10.1074/jbc.M115.664805

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts