

Supplementary Information

Protein Interactions in the Murine Cytomegalovirus Capsid

Revealed by CryoEM

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Running title: Murine cytomegalovirus capsid structure

Key words: cytomegalovirus; herpes simplex virus type 1; cryo electron microscopy; three-dimensional; major capsid protein.

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Legends of Supplemental Figures:

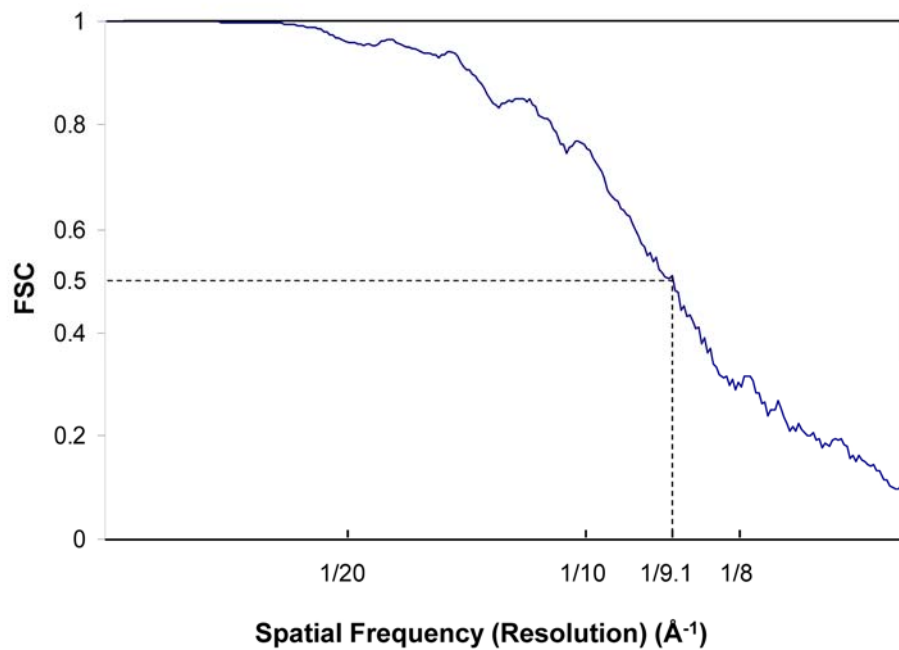


Fig. S1

Figure S1. Fourier shell correlation (FSC) curve. The estimated resolution of the MCMV capsid is 9.1 Å at FSC = 0.5.

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HSV-1: 484 ANPYGAYVAAP-AGPAADMQQFLNawgQRLAHGRVVRWVAEGQMTPEQFMQPDNANLALE 542
HMM : aellgleverepelanlarrlrefye...akaeapvtdnaakaelttedflkpenelLr1E
MCMV : 478 RALCRREFVREHMAHATRRLVHFYQ---ARIDPRTANEAKHDFSTKEFAKVDNYLLFTE 534

HSV-1: 543 LHPAFDFDFVGVADVELPggdvppagpgEIQATWRVVNGNLPLALCPAAFRDARGLELGV- 601
HMM : lhPlFDffvaqedvelp.....avratpRiivGniPlpLapadFreargkqleea
MCMV : 535 LHPFFDFCFHTENGQV-----RPLCTPRIMVGNLPEALAPADFHDLRAKQALEL 583

HSV-1: 602 -GRHAM--APATIAAVRGAFDDRNYPAVFYLLQAAIHGSEHVFCALARLVVQCITSYWNN 658
HMM : aklakle.seatvevvqetledpnYPelfYliealiHGseeaFlalarlvaqcinsywen
MCMV : 584 TKVRAPEgHEATLQVLRASLTDHQYPELFYLIESLIHGDPAAFETGIELVTRCVNNYWRQ 643

HSV-1: 659 TRCAAFVNDYSLVSIVVVTYLG-GDLPEECMAVYRDLVAHVEALAQLVDDFTLtgPELGGQ 717
HMM : sgrlaFvnsfemvklIathlgdgelpeevlavYrklIsevralkrlvskltl.neqlgee
MCMV : 644 RGLLAFANSYDMVRLIATRLGDGAVVPAAYTHYRNLLSITRFVARTCELTGL-NGRLCDE 702

HSV-1: 718 AQAELNHLMRDPALLPPLVWDCDALMRRAAldrhRDCRVSAAGHD-PVYAAACNVATADF 776
HMM : sleelvnallDpallpPlvydcalkree...rnkvkaggeelkaaeaeernaevsf
MCMV : 703 PLLAYVSALHDPRLWPPFVQA---LPRNA---NLVRVVADDVPLDAAHIEERNPGTSD 754

HSV-1: 777 N-RNDGQLLHNTQARAA--DAADDRPHRGADWTVHHKIYYYVMVPAFSRGRCTAGVRFD 833
HMM : verlenlIaheravvddrreadedeprdeeeelvleKifYyvvlPaltngrvCgmGvdIk
MCMV : 755 VARMIAMDQAEPLFVDARRTSD-----EEMVAQKVYYLCLVPAVLNNHACGAGLNLK 806

HSV-1: 834 RVYATL---QNMVPE---IAPGEECPSPvtdpahplhpaNLVANTVAMFHNGRVV-V 886
HMM : nvlItlfyneavvvpd...evaeaaield.....nllaetlndllhnseva.v
MCMV : 807 HLLVKLFYTKFFLTADpdsLTAGEEALTNn-----PLLAALVRDVATDENVTaN 855

HSV-1: 887 DGPAMLTlQVLAHNMAERTTALCSAAPDAGANTASTT-NMRFIDGALHAGILLMAPQHL 945
HMM : dadalreLqelvlInvaertkaleveaalDaaqrtaate.nfrvldgvLynGlllmaipkr
MCMV : 856 QAA--EELFHLVAHVPEAQAQMLEIRAALDPAQRHGAPSaGFESLQHVLYNGFCMTTPVKL 913

HSV-1: 946 DHTIQnGDYFYPLPVHALFAGAdhvANAPNFPPALRDLRSRQVPL-----VPPALGA 997
HMM : drtva.eeyfypvPvhklyadp...avaatlnaeirelleelps.qRndggFpvpPalaa
MCMV : 914 L-----QEYLTVIPFHRFYSDP---GLAATANHDIRVFLNDFPQyQRCDGGFPLSPIFAH 965

HSV-1: 998 NYFSSIRQPVVQHVRESAAGENALTyALMAGYFKISPVALHHLKLTGLH 1045
HMM : eyyewhrsPvakYaecaatlsls.aLlamyfKlsPvalilqlkklH
MCMV : 966 EYHHWHRTPFSCYSAACAHTLESVL-TLAIMHHKMSPVSIAAALSRMGLH 1013

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Fig. S2

Figure S2. Sequence Alignment of MCPud of HSV-1 and MCMV

Alignment of sequence between the MCP upper domain of MCMV with HSV-1, obtained from the Pfam Sequence Search server (Finn et al., 2010) (<http://pfam.sanger.ac.uk/search>).

Only the results corresponding to residues of HSV-1 MCPud (aa 484 to 1045) are shown.

The section which is discussed in Figure 2 is highlighted in red.

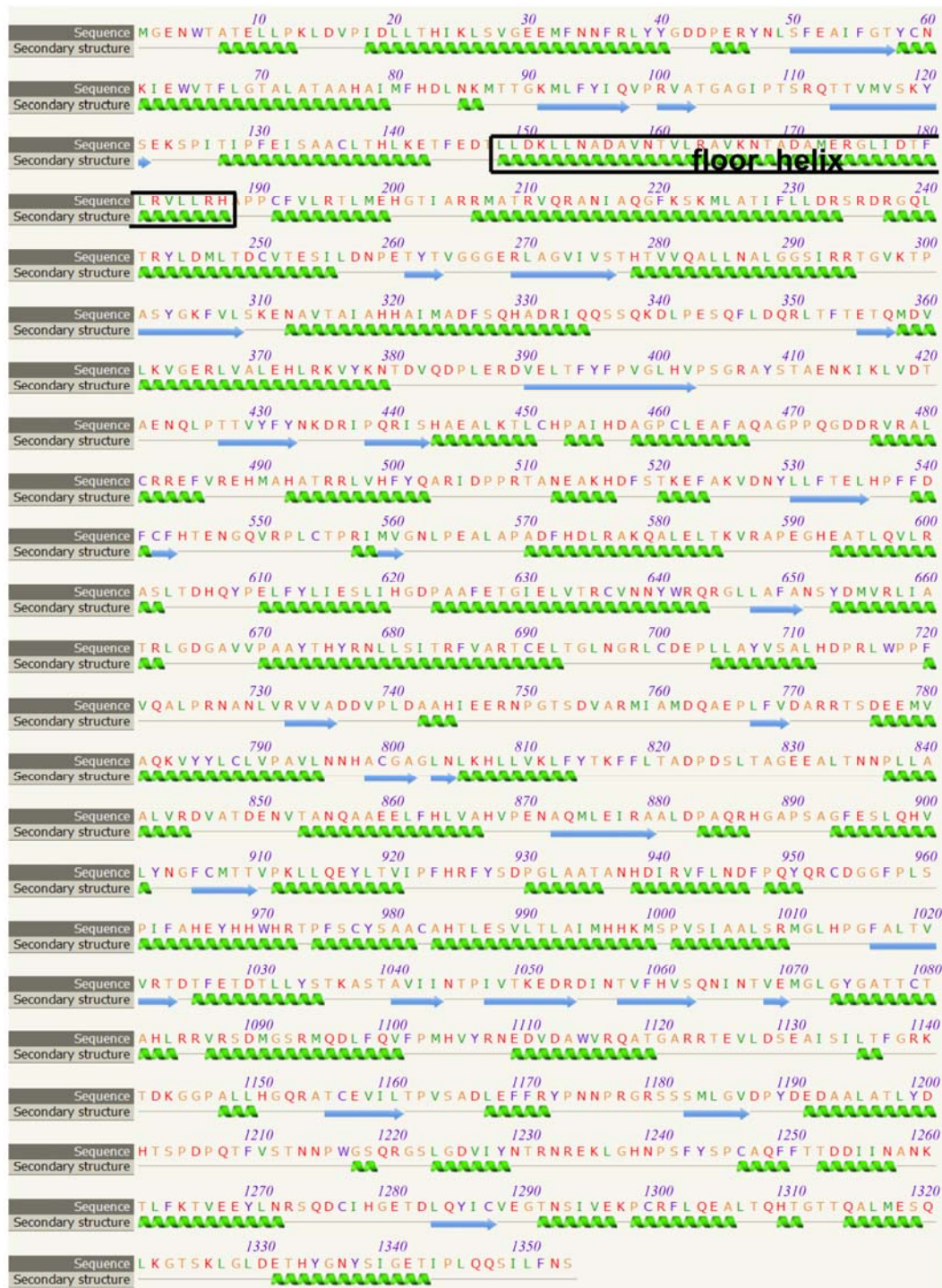


Fig. S3

Figure S3. Secondary structures of MCMV MCP. The prediction was carried out by three different prediction servers (Jpred Secondary Structure Prediction Server (Cole et al., 2008), PsiPred Protein Structure Prediction Server (Buchan et al., 2010) and Phyre² Protein Fold Recognition Server) and only the results from Phyre² Protein Fold Recognition Server (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>) are shown. Green helical ribbon: helix; blue arrow: β strand; grey line: loop.

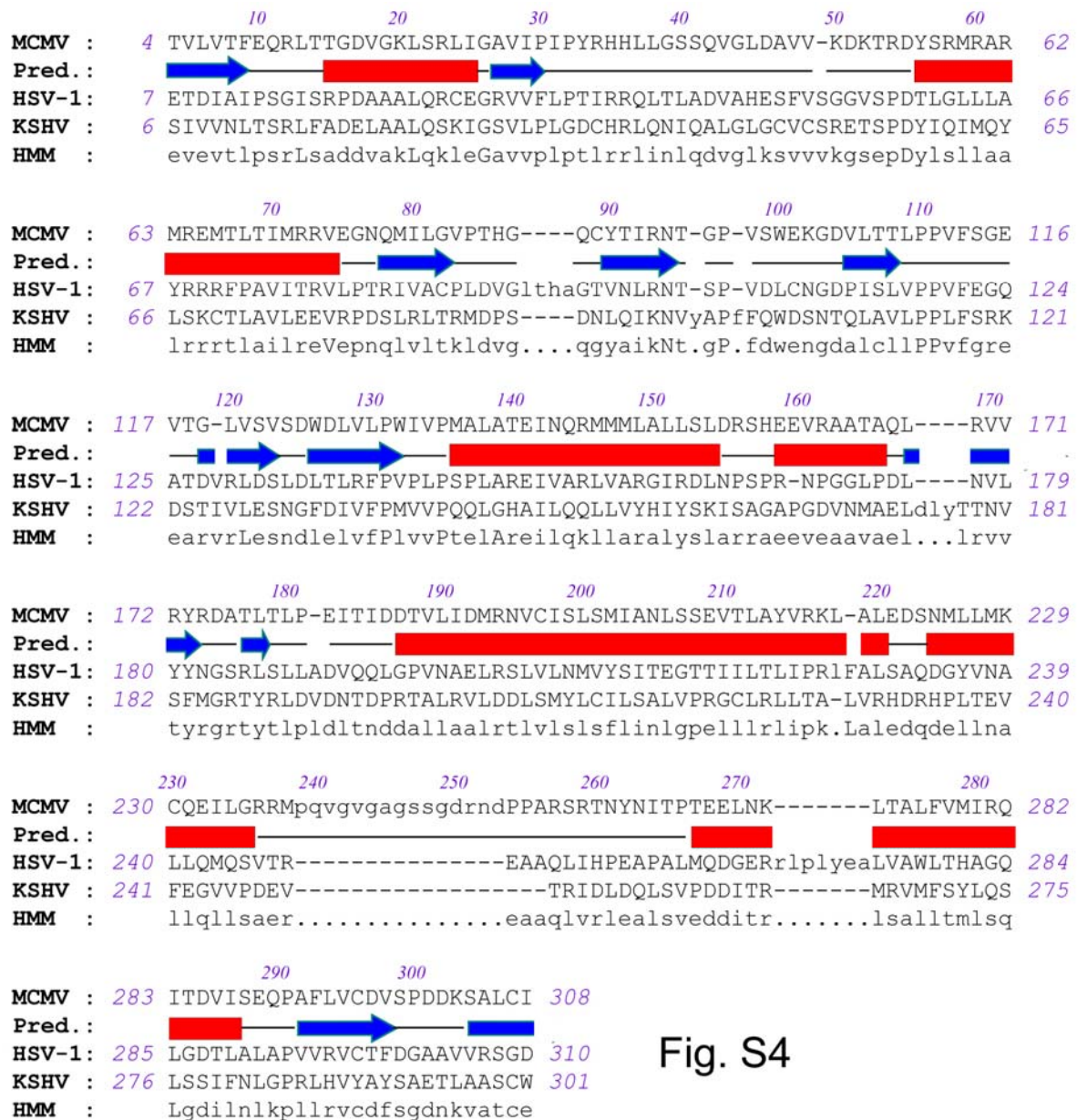


Fig. S4

Figure S4. Multi-sequence alignment of TRI-2 protein homologs across three herpesvirus subfamilies: MCMV M85, HSV-1 VP23, KSHV ORF26 and secondary structure prediction of MCMV TRI-2. Secondary structure prediction of MCMV TRI-2 by the PsiPred Protein Structure Prediction Server (Buchan et al., 2010) (<http://bioinf.cs.ucl.ac.uk/psipred/>) and are depicted in the second row. Red box: helix; blue arrow: β strand; black line: loop. The Hidden Markov Model (HMM) of the alignment generated by the Pfam server is shown in the last row. Numbers indicate amino-acid residual numbers in each sequence. Dashed lines in the sequences (dotted lines in the HMM model) represent deletions.

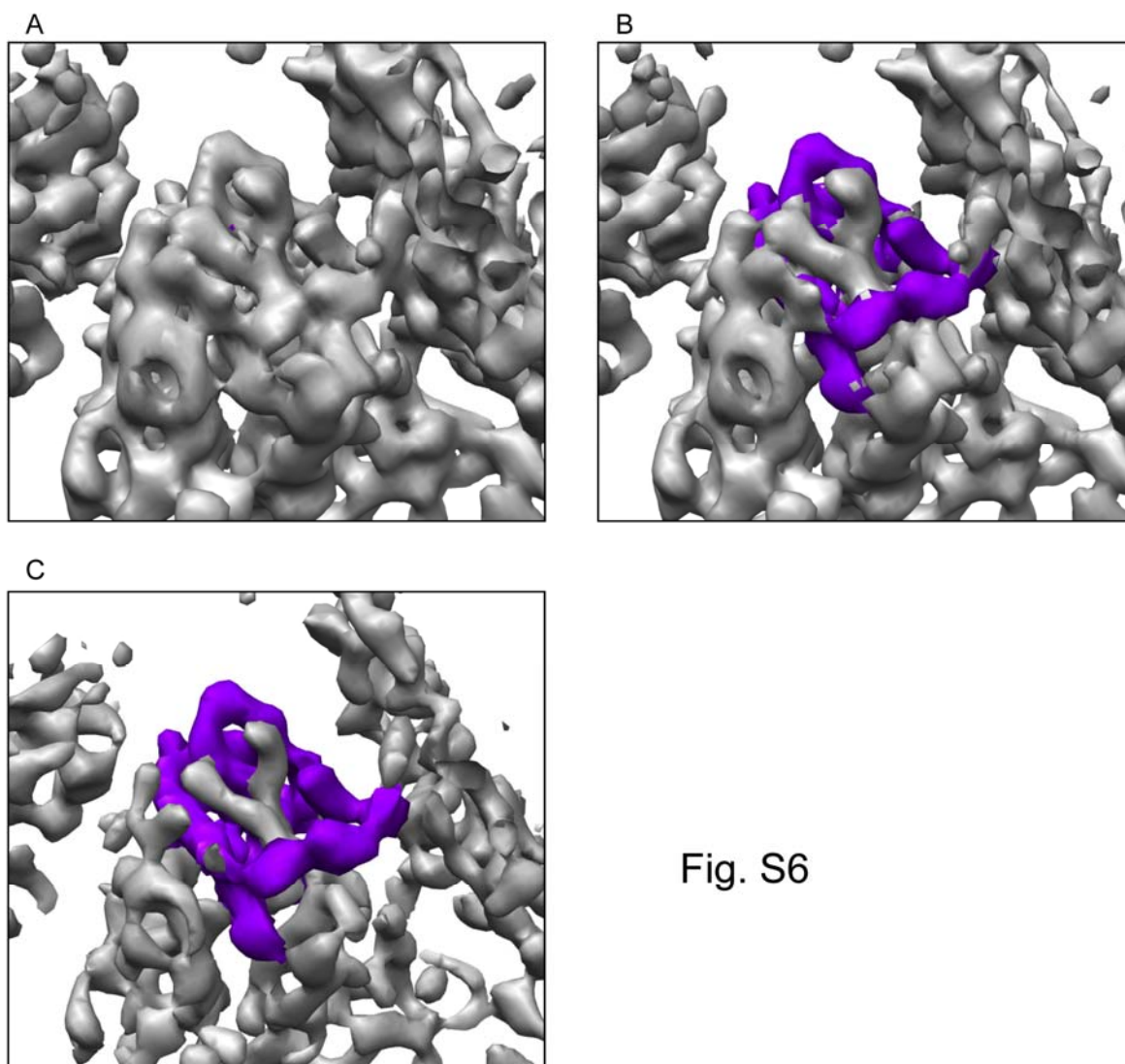


Fig. S6

Figure S6. Estimation of molecular boundaries by varying density threshold for display. The averaged triplex density map was shown at three different density threshold values of 3.0 (A), 3.4 (B) and 4.5 (C). The densities corresponding to TRI-2a (purple) begin to be resolved from the rest of densities when the threshold density is above 3.4. This threshold-based estimation was used in conjunction with other available constraints to establish the final boundaries (see text).

Legends for Supplementary Movies:

Movie 1: MCMV Capsid Map from 3-fold View.

Radially color-shaded surface representation of the MCMV capsid reconstruction at ~9.1 Å resolution. One half of the full map is rotated, revealing hexons, pentons, triplexes arranged according to T=16 icosahedral symmetry on the capsid.

Movie 2: Fitting of cryoEM density of MCMV MCP with atomic models of HSV-1 MCPud and with the pseudo-atomic model of MCMV MCPud.

The cryoEM density map is shown in semitransparent gray, then the X-ray model of HSV-1 MCPud (ribbon with red helices) is superimposed, followed by the pseudo-atomic model of the MCMV MCPud (ribbon with purple helices).

Movie 3: Structure of triplex heterotrimer. Related to Fig. 4.

A triplex heterotrimer, is shown first, followed by separation of its three subunits, TRI-1 (magenta), TRI-2a (green), TRI-2b (yellow) individually, to reveal interactions among these subunits.

Movie 4: Interaction between triplex and its adjacent hexons. Related to Fig 5.

A triplex and its three associated hexons are shown together to reveal interactions between triplex heterotrimer and MCP.