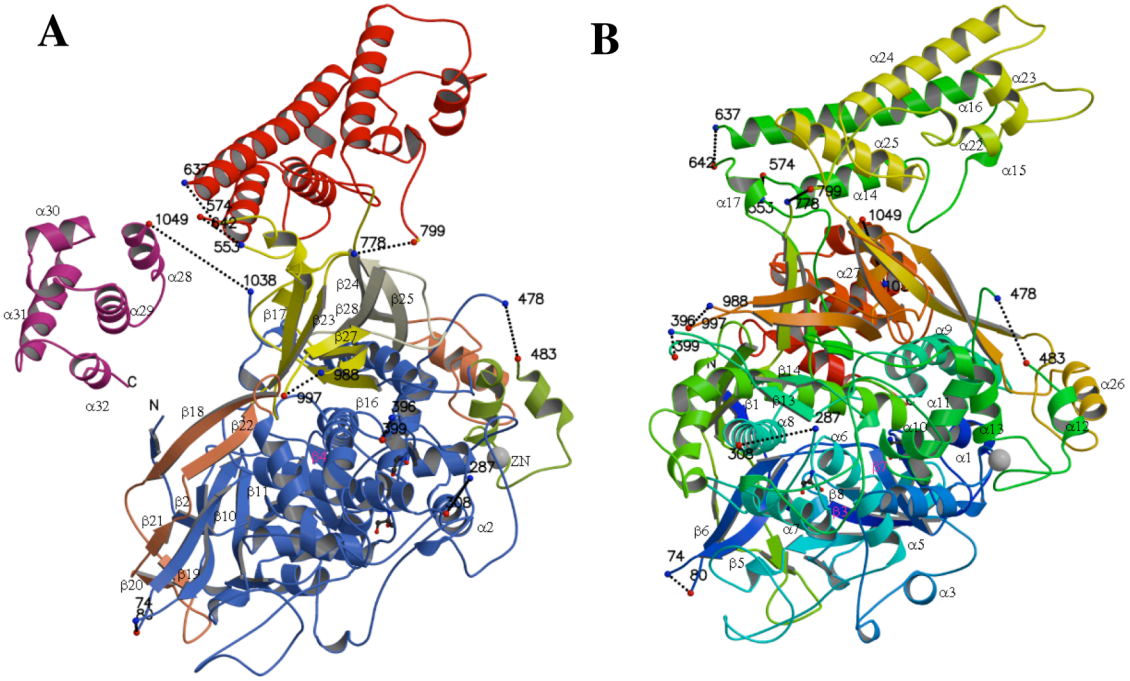
All organisms including animal viruses use specific proteins to bind single-stranded DNA (ssDNA), rapidly in a non-sequence specific, flexible and co-operative manner during the DNA replication process. The crystal structure of a 60 residue C-terminal deletion construct of ICP8, the major single-stranded DNA-binding protein from herpes simplex virus -1 (HSV-1) was determined at 3.0 Å resolution. The structure reveals a novel fold, consisting of a large N-terminal domain (residues 9–1038) and a small C-terminal domain (residues 1049-1129). On the basis of the structure and the nearest-neighbor interactions in the crystal, a model describing the site of ssDNA binding and explaining the basis for co-operative binding is presented. This model agrees with the beaded morphology observed in electron micrographs.



Structure of ICP8 (**A**). Overall view of the ICP8 structure. Dotted lines represent disordered regions with blue and red balls signifying the N-and C-terminal ends of the disordered regions. (Sequence information using the same color code is given Fig. 4). The shoulder region is colored blue; the zinc binding region is green and the part of the polypeptide chain linking the neck and shoulders as a single folding unit is orange. The neck is colored in yellow (front) and grey (back). The head is in red and the C-terminal helical domain in purple. (**B**) The structure rotated 60° along x-axis relative to Fig. 1A. The blue to red color gradient follows from N to C-terminus. In this orientation, the C-terminal domain is behind the neck.

Both SeMet and MMA-containing ICP8ΔCcc crystals were cryo-protected by brief soaks in 20% glycerol buffered at pH 6.3 before cryo-cooling in liquid nitrogen. Multi-wavelength anomalous dispersion (MAD) data from crystals of SeMet- ICP8ΔCcc were collected at 100 K using synchrotron radiation at the 17-ID IMCA-CAT beamline of the Advanced Photon Source (Argonne) at three/two different wavelengths around the selenium absorption edge. A full diffraction data set was collected for the MMA-derivative at 100 K, using the BW7B beam line of EMBL Hamburg Outstation. The diffraction data were processed using the HKL programme package (24). Data collected at the ESRF on this project were not useful in phasing and the highest resolution diffracting crystal was used for refinement.

The structure was solved by the MAD method (25). Initially from a first SeMet containing crystal (CRYST-1), F_A values were obtained using XPREP (Bruker-AXS Inc.) to 4.0 Å enabling the selenium substructure to be solved (50 out of 56 seleniums) using the program SHELXD (26). Phases were then obtained to 4.0 Å from the two wavelength MAD data. The phases were extended to 3.2 Å by using density modification procedures and two-fold non-crystallographic symmetry (NCS) averaging (27). 55% of model was built using a semi automatic procedure with the programs MAID (28), RESOLVE (29) and O (30). Later, phases were extended to 3.0 Å using data from another crystal