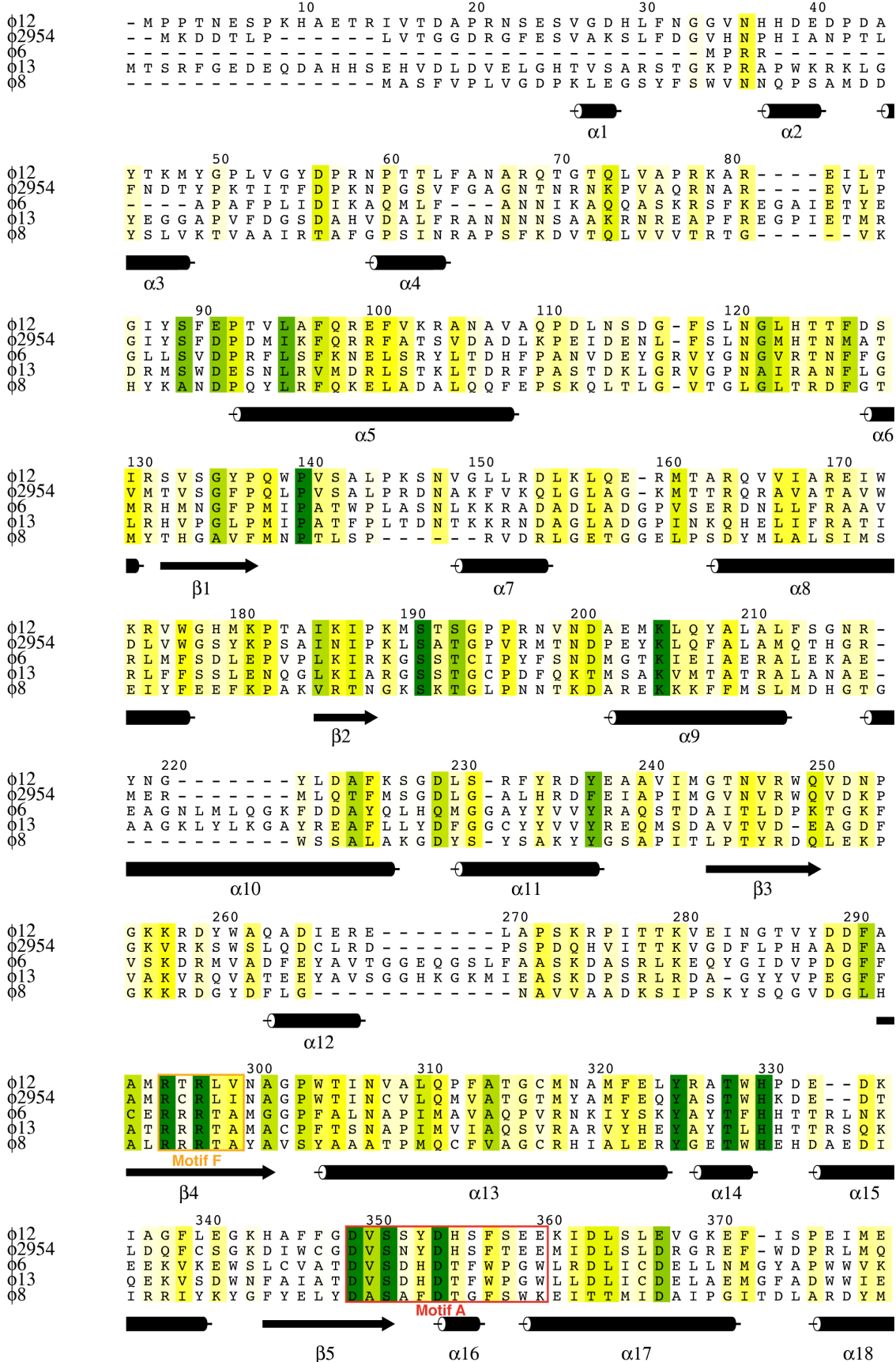


Supporting Information for:

Structure of the RNA-directed RNA Polymerase from the Cystovirus ϕ 12

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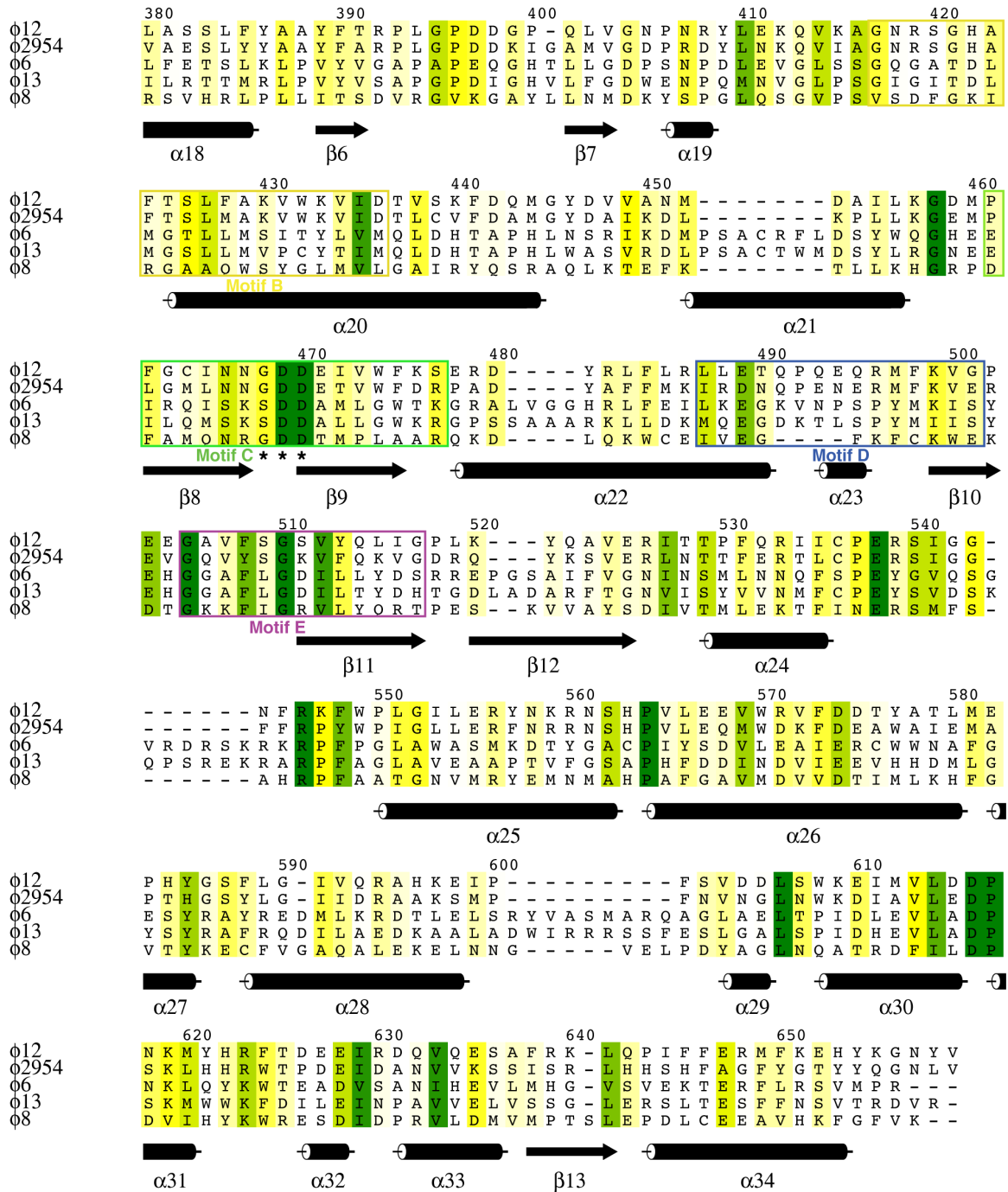


Fig. S1. Sequence alignment of the cystoviral P2 proteins. Sequence motifs that are conserved in viral RNA directed RNA polymerases are boxed and labeled – red (motif A), yellow (motif B), green (motif C), blue (motif D), magenta (motif E) and orange (motif F). The sequences of φ12 (NP_690823.1, note that we crystallized a M2A mutant), φ2954 (YP_002600763), φ6 (ABI120430.1), φ13 (NP_690817.1) and φ8 (NP_524561.1) are shown. Also indicated are the elements of secondary structure from φ12 P2 as determined by UCSF Chimera. The residues corresponding to the so-called catalytic triad in Motif C (GDD for φ12) are indicated by the ‘*’.

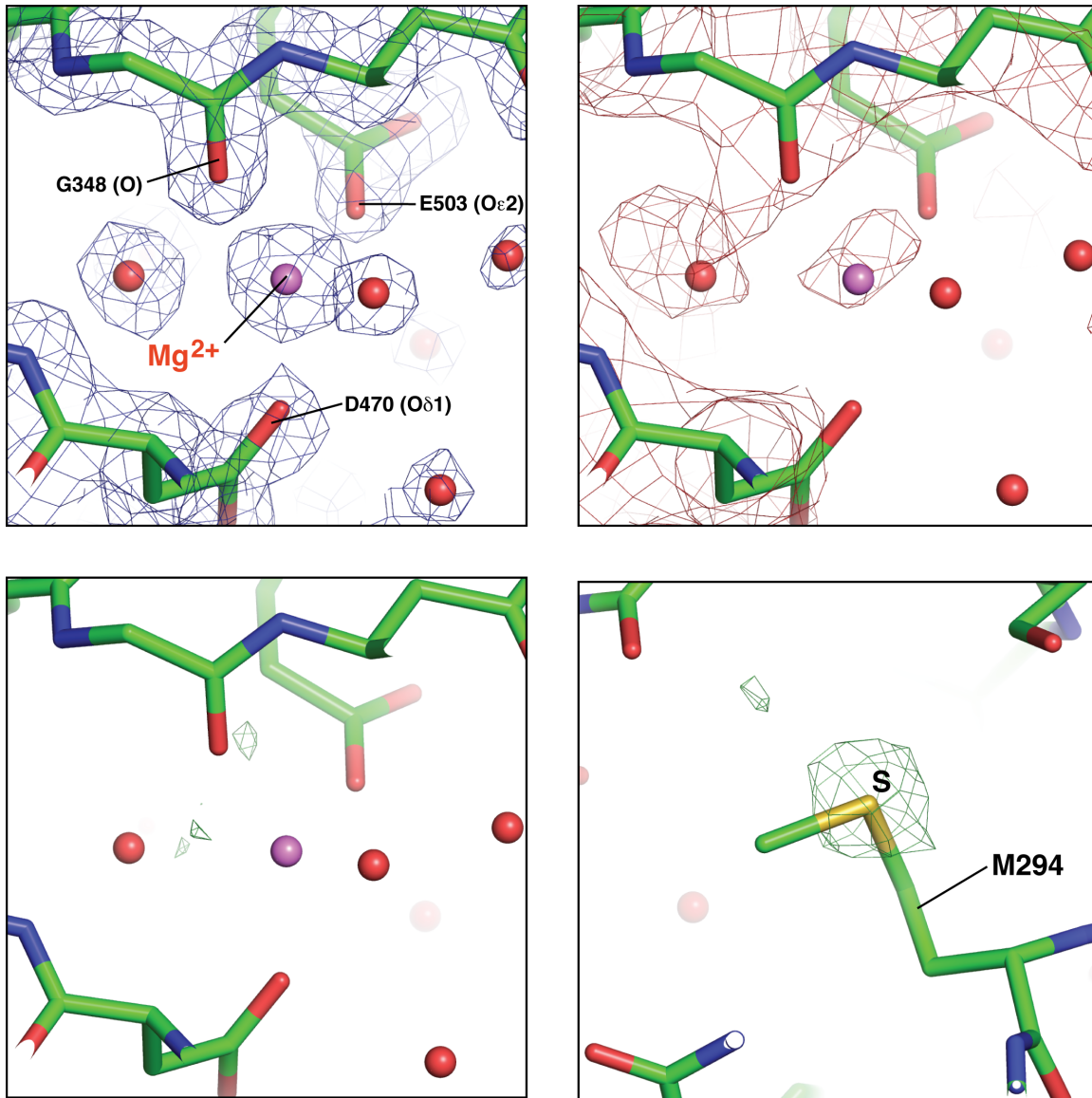


Figure S2. The top left panel depicts the sigmaA-weighted 2Fo-Fc map calculated from the final refined model (form A) with all the waters and metal ions included. Protein atoms that co-ordinate the metal ion are labeled. The top right panel depicts the original SAD map (at 2.35 Å) for Se-Met substituted P2 after solvent flattening contoured at 1σ . Presence of density at the Mg^{2+} ion position is clearly seen. The anomalous Fourier difference map calculated from the form A data (1.7 Å, contoured at 3σ), depicted in the bottom left panel, shows no density at the metal ion position. An intense peak at the metal ion position would be expected for a Mn^{2+} ion at the wavelength of data collection (0.98 Å). The bottom right panel depicts, as reference, the significant intensity seen in the same anomalous Fourier difference map for the sulfur position of M294.

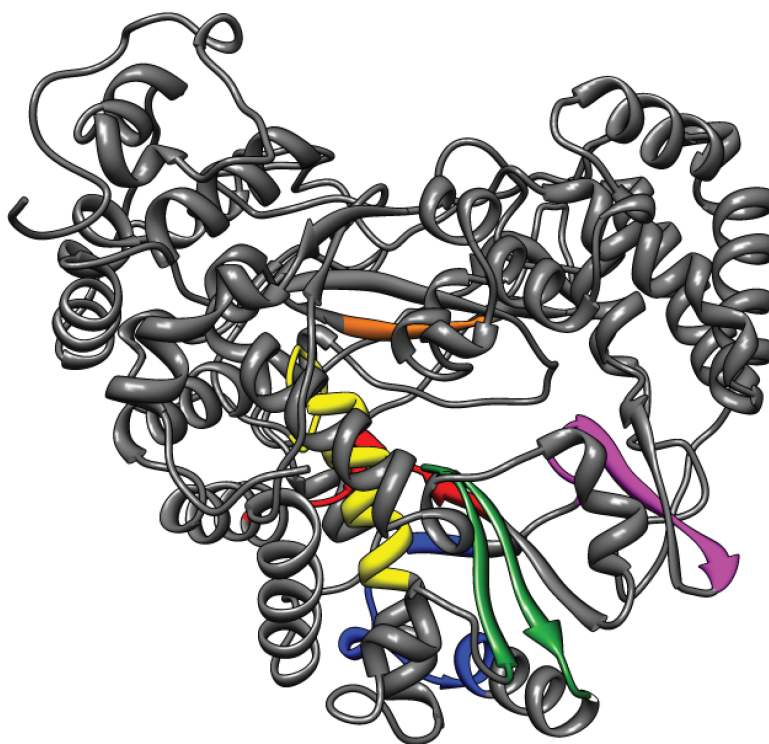


Fig. S3. The spatial location of the conserved RdRp sequence motifs is shown on the structure of ϕ 12 P2 – motif A (D349-E359; red), motif B (G417-D436; yellow), motif C (P461-S477; green), motif D (L487-V500; blue), motif E (G505-G517; magenta) and motif F (R295-V299; orange). Motifs A-E lie on the palm domain and motif F lies on the fingers domain.

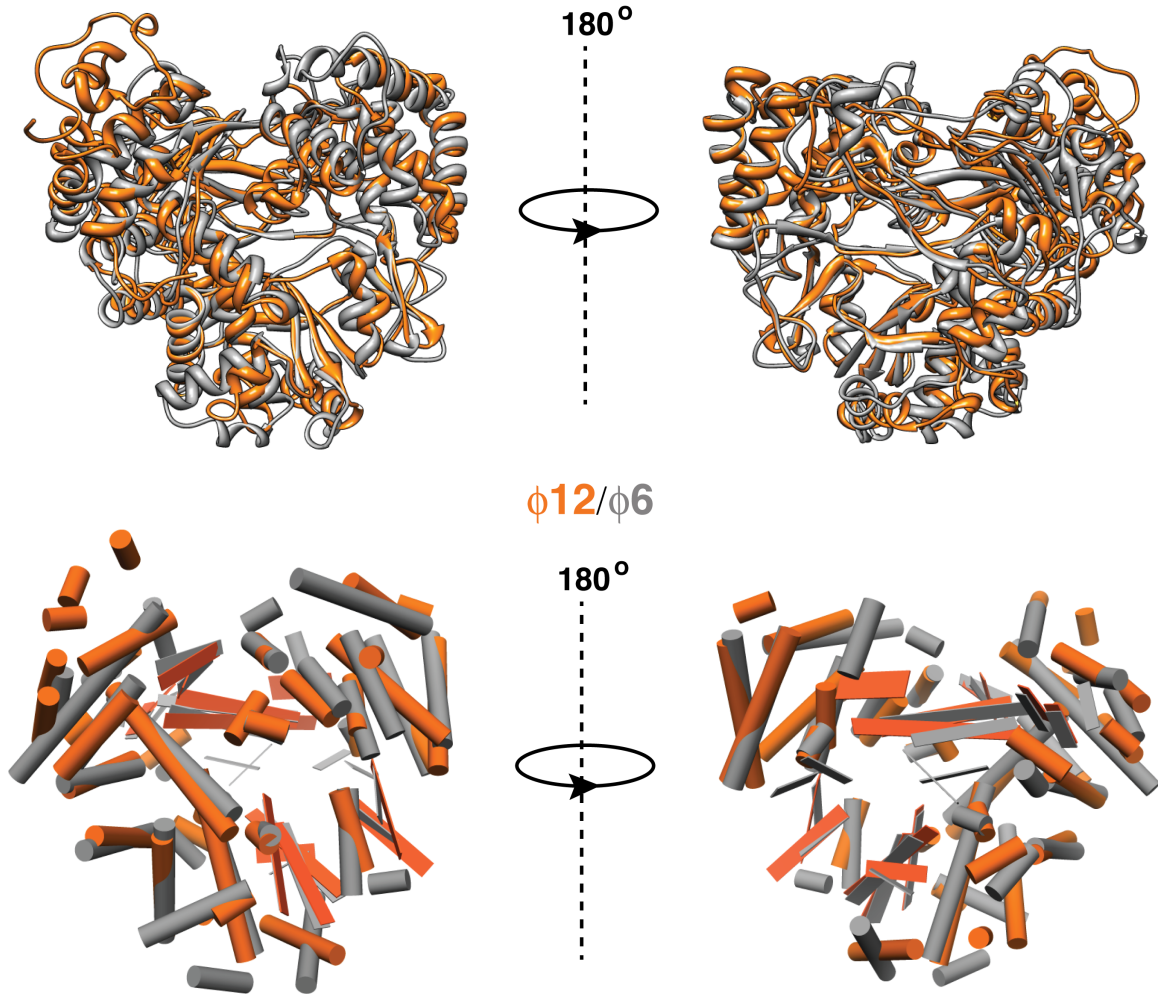


Fig. S4. Overlay of the structures of the P2 proteins from the $\phi 12$ (orange) and $\phi 6$ (PDB ID: 1HI8; grey) cystoviruses. The two structures are quite similar in spite of a sequence identity of only 18%. The RMSD over 338 atom pairs is 1.22 Å.

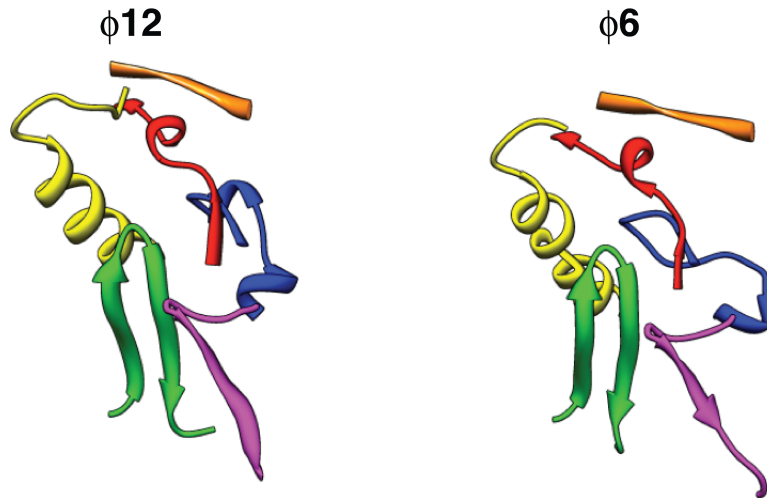


Fig. S5. Comparison of the spatial orientations of the conserved RdRp sequence motifs in the $\phi 12$ and $\phi 6$ cystoviruses. The coloring scheme is the same as in Fig. S3. $\phi 12$ P2 is shown on the left and $\phi 6$ P2 (PDB ID: 1HI8) is shown on the right. The largest differences are seen in motif D (blue).

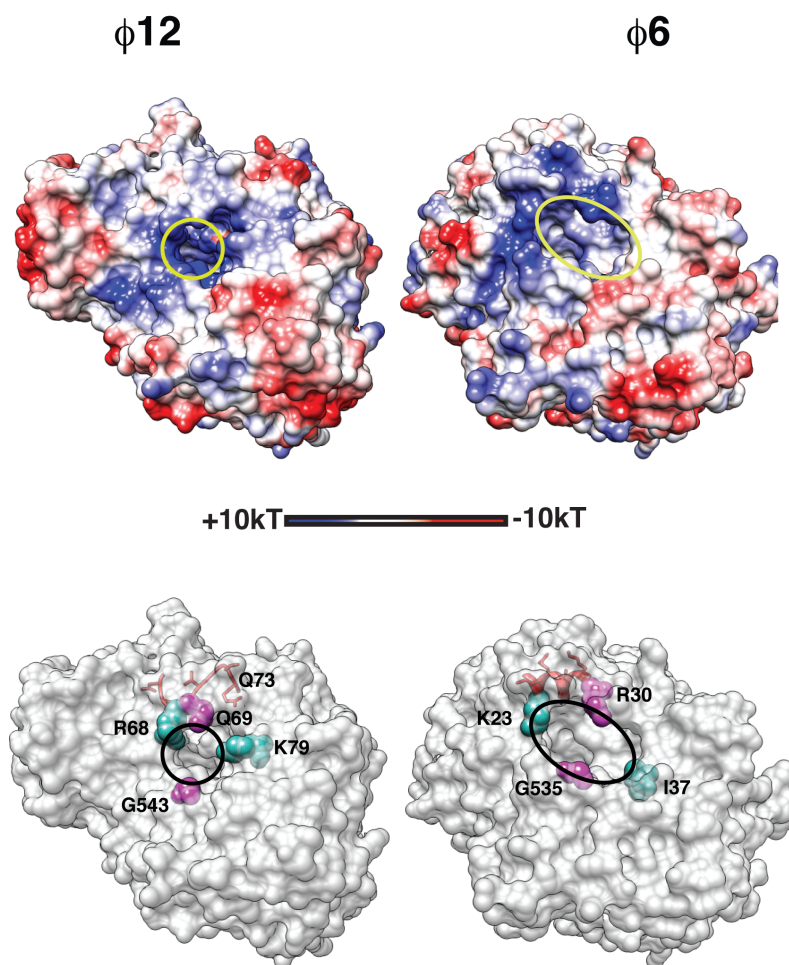


Figure S6. Comparison of the charge distribution near the template entry portals of $\phi 12$ (left) and $\phi 6$ (right) P2 is shown on the top panel. The size of the entrances to the portals is indicated by the yellow ovals. The bottom panel shows some key residues that line the entrance to the template tunnels of the $\phi 12$ (left) and $\phi 6$ (right) P2 proteins. The segment 66-73 (red) forms a flexible loop that partially occludes the entrance to the template tunnel in $\phi 12$ P2. The sidechains of R68, Q69 and Q73 point towards the mouth of the tunnel. In $\phi 6$ P2 (PDB ID: 1HI8, the corresponding region (22-29) is helical (red) and positioned away from the template tunnel entrance. The mouth of the template entry tunnel in $\phi 12$ P2 is roughly spherical with dimensions 12.0 Å, measured between Q69 (N ϵ 2) and G543 (C α) (both residues shown in magenta), by 11.9 Å, measured between R68 (C δ) and K79 (N ζ) (both residues shown in coral green). In case of $\phi 6$ P2, the entrance is more oval in shape with dimensions 13.1 Å, measured between R30 (N η 2) and G535 (C α) (both shown in magenta), by 20.9 Å, measured between K23 (C δ) and I37 (C α) (both residues shown in coral green). Both tunnels narrow as one approaches the catalytic site.