A and eluted *via* a step gradient with 3 ml aliquots of buffer *A* at 0.1, 0.2, 0.3, 0.35, 0.4, 0.5 and 1.0 *M* NaCl concentrations. The fractions containing the protein were pooled and concentrated and subjected to gel filtration (AKTA Prime system, Pharmacia). The gel-filtration column (HiPrep 16/60 Sephacryl S-100 HR) was pre-equilibrated in buffer *B* [20 m*M* Tris–HCl pH 8.0, 10% glycerol, 100 m*M* NaCl, 1%(*v*/*v*) β-mercaptoethanol]. The mobile phase was run at a 1 ml min $^{-1}$ flow rate and the elution was collected in 3 ml fractions. All VP4 mutant constructs eluted from the gel-filtration column in a manner consistent with them being monomeric in solution. The fractions containing the purified protein were pooled and concentrated by centrifugal filtration (5 kDa cutoff; Millipore) for crystallization. The protein concentration was determined by measuring UV absorption at 280 nm. The extinction coefficient that was used for all protein constructs (9970 M^{-1} cm

constructs VP4(514–715,K674A) and VP4(514–716,K674A), respectively. Both constructs yielded multiple hits during initial crystallization screening and these conditions were optimized for crystal growth. The VP4(514–715,K674A) construct (203 residues, 21.6 kDa) crystallized under similar conditions to that of the self-cleaved form of VP4. A novel hit condition at room temperature, when optimized (Table 1; form 2), produced sizeable needle-shaped crystals (form 2; Fig. 1*b*