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Virus Assembly and Maturation: Auto-regulation Through Allosteric Molecular Switches

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Abstract

We generalize the concept of allostery from the traditional non active-site control of enzymes to virus maturation. Virtually all animal viruses transition from a procapsid, non infectious state, to a mature infectious state. The procapsid contains an encoded chemical program that is executed following an environmental cue. We developed an exceptionally accessible virus system for the study of the activators of maturation and the down stream consequences that result in particle stability and infectivity. *Nudaurelia Capensis* Omegavirus (N ω V) is a T=4 icosahedral virus that undergoes a dramatic maturation in which the 490Å spherical procapsid condenses to a 400Å icosahedral shaped capsid with associated specific auto-proteolysis and stabilization. Employing X-ray crystallography, time resolved electron cryo-microscopy and hydrogen/deuterium exchange as well as biochemistry it was possible to define the mechanisms of allosteric communication among the 4 quasi-equivalent subunits in the icosahedral asymmetric unit. These gene products undergo proteolysis at different rates, dependent on quaternary structure environment, while particle stability is conferred globally following only a few local subunit transitions. We show that there is a close similarity between the concepts of tensegrity, associated with geodesic domes and mechanical engineering, and allostery, associated with biochemical control mechanisms.

Keywords

virus maturation; time resolved cryoEM; virus structure; virus dynamics; auto-proteolysis; hydrogen/deuterium exchange

Introduction

Virus capsids are highly organized protein oligomers composed of one or more gene products. Minimalistic viruses build their capsids from multiple copies of a single gene

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product yet they perform all the functions required for the virus life cycle i.e.: assemble in the correct conformation; specifically package and protect their genetic material; mediate host interaction and cell invasion; release the genome in the cell environment for replication and translation. This task list describes particles that are highly multifunctional protein oligomers. Displaying the appropriate function at the proper time requires auto-regulation in the capsid quaternary-structure conformation.

Viruses capsids fit well into the classical definition of allosteric systems; i.e. they are oligomers involving several identical units; they possess at least one axis of symmetry; and they undergo quaternary structure changes when cued by regulators distant from the region that directly effects function ^{1; 2}. Only recently, however, have virus capsid properties been understood from an allosteric perspective. This is mainly due to the methodological challenges that are associated with the biophysical study of mega-dalton protein complexes.

Allosteric regulation is a critical part of the self-assembly properties of capsid-proteins. Purified preparations of subunits from hepatitis B virus (HBV) ³, bacteriophage MS2 ⁴ and a variety of plant viruses spontaneously assemble in vitro into capsid-like structures that are comparable to virions ⁵. Allosteric regulation of capsid protein assembly is important to avoid kinetic traps and associated aberrant particle formation. It is possible to alter such allosteric control, in vitro, by changing ionic strength, pH, or temperature resulting in aberrant particle assembly. Studies with HBV ⁶ and MS2 revealed that RNA ⁷ can also act as an allosteric regulator by binding assembly-competent capsid dimers and driving coordinated association.

Enveloped viruses display specific mechanisms to control the activation of fusion peptides that allow these viruses to enter a host. If we think of the fusion peptide as the “active site”, they are generally hidden and inhibited by the quaternary structure of an oligomeric assembly. Influenza virus hemagglutinin is an excellent example ⁸⁹. Its fusion peptide is concealed and inhibited in the base of the stalk by the trimeric interactions of the hemagglutinin subunits. The hemagglutinin chains are cleaved at a single site by a cellular protease, making the entire assembly metastable. Under the low pH of endosomes the trimer is displaced from the local energy minimum and the quaternary and tertiary structures of the molecule undergo large-scale reorganizations that refolds portions of the subunits and places the fusion peptide at a totally exposed position ready for interaction with the endosomal membrane. Initial inhibition of exposure is achieved by the folding and assembly of the hemagglutinin subunits under conditions that favor concealing the fusion peptide ⁹. This inhibition happens only once and cannot be achieved again after the protein has refolded at low pH. Zlotnick and Mukhopadhyay recently reviewed both the themes briefly described above ⁵.

Here, we examine the role of allostery on virus maturation. Maturation is the transition from a non-infectious particle that contains all of the viral constituents, to an infectious virion. It is virtually universal in the life cycles of animal viruses and presents a target for antiviral development, as evidenced by the protease inhibitors that prevent maturation of Human Immunodeficiency Virus ¹⁰.

Allosteric contacts between subunits control viral maturation

Virus precursor particles, provirions, undergo a maturation step that involves one or more modifications leading to infectivity. Such modifications include proteolytic cleavages promoted by cellular or viral encoded proteases (e.g. HIV and influenza virus) or activation of an autocatalytic reaction of the capsid-protein, independent of host or viral encoded proteases (e.g. Picorna- Noda- Reo- and Tetraviruses) ¹¹. This endopeptidase activity is not

a typical enzyme in that each active site functions once and releases a peptide, usually with membrane lytic properties, that remains non-covalently associated with the capsid.

A detailed description of the mechanism of cleavage is still missing for some virus groups, however, the endopeptidase activity is well described for the nodaviruses, which are positive ssRNA, T=3 viruses, formed by 180 copies of the capsid protein. Key glutamate and asparagine residues in the noda Flock House Virus (FHV) are required for cleavage as confirmed by site directed mutagenesis^{12; 13}. The role of these residues is consistent with the formation a cyclic imide intermediate that is hydrolyzed to cleave following the asparagine residue¹⁴. Assembly competent D75N and N363T mutant subunits were unable to undergo auto-cleavage, however, the resultant particles were otherwise closely similar to virions¹⁵.

Studies with FHV showed that virus purified shortly after infection was only partially cleaved, and that maturation can proceed in vitro. The cleavage is slow, requiring several hours for completion, with an unusual kinetic pattern that is not explained by a first-order rate law, as would be expected for an intra-molecular reaction. Indeed, the cleavage rate slowed more rapidly than predicted, a clear indication that an allosteric mechanism affected the maturation process^{13; 15; 16}.

While significant insights into maturation were obtained from the study of FHV, a quantitative description of the capsid cleavage kinetics and the associated allosteric mechanism required a system where maturation could be precisely initiated in vitro. *Nudaurelia Capensis* Omegavirus (N ω V) is a tetravirus with a positive ssRNA encapsidated in T=4 particles, formed by 240 copies of a single gene product¹⁷ (Fig. 1). The T=4 N ω V has a cleavage site that is closely similar to the T=3 nodaviruses and a demonstrated comparable mechanism (Fig. 1b)^{18; 19; 20}. However, differently from FHV, Virus-like Particles (VLP) of N ω V, made by expressing the 70 kD capsid protein in a baculovirus system, can be purified in a metastable procapsid state. The procapsid packages cellular RNA²¹ and, when purified at pH 7.6, is a round and porous particle with a diameter of 490 Å. This procapsid state places all the subunits in nearly equivalent environments with dimeric interactions apparent on the VLP surface. When the pH is dropped to 5.0 the procapsid condenses into a 410 Å, faceted, icosahedral-shaped particle, identical to the structure of wild type N ω V virions (Fig. 1a). Maturation differentiates subunit contacts into strikingly different, but quasi-equivalent, interactions with neighbors (Fig. 1c)²².

The large conformational change that occurs at pH 5 initiates the autocatalytic reaction at asparagine 570. The cleavage results in the capsid protein beta (residues 1 -570) and the γ peptide (residues 571-644) that has lytic activity in the presence of phospholipid liposomes²³. Procapsid contraction is fast, occurring in 100 milli seconds after the pH is changed to 5 as demonstrated by stop-flow and time resolved SAX analysis^{24; 25}. On the other hand, cleavage is a slow process, displaying the same kind of bizarre kinetic profile (Fig. 1a) observed in FHV that is refractory to interpretation by standard kinetic models²⁶.

The γ peptides remain non-covalently associated with the mature capsid and assume different tertiary structures in each of the asymmetric units. A-subunits form capsid pentamers while those labeled B, C and D form pleated quasi-hexamers with 2-fold icosahedral symmetry (Fig. 1c). The quasi 6-fold axes relate equivalent subunit interfacial surfaces, but these surfaces display different interactions with their neighbors. The CD and DB interfaces are closely similar and “flat” (the dihedral angle between the mean planes of the subunits is 180°), while the BC interfaces at the edges of the icosahedron are “bent” (the dihedral angle is 138°). The difference in these interactions is due to the presence (flat) or absence (bent) of extended helices corresponding to residues 624 to 638 that are part of the

C-terminal γ peptides of the C and D subunits (Fig. 1c). These “switch helices” are related by a quasi-2-fold axis and function as a chock that prevents the hinge-bending that occurs at the BC interface. The BC and AA interfaces at the edges of the icosahedron have the same dihedral angle and are also related by quasi 2-fold symmetry. Residues 598-644 of the γ peptides (that include the polypeptide forming the chock at the flat contact) are not visible in subunits A and B allowing the BC and AA interface to bend about a molecular hinge and form the bent contact. Maturation initiation in vitro and slow cleavage kinetics make NwV an ideal model to identify a mechanism of allosteric regulation in its maturation. We propose that allosteric effects are responsible for activation of the endopeptidase activity because all components of the autocatalytic site are present in each subunit and the large conformational change from procapsids to capsids does not significantly alter the tertiary structure and active sites²². Moreover, the cleavage site is structurally identical in all 4 asymmetric subunits, however, the unusual kinetics suggests differential rates for the reaction. We posit that the change in inter-subunit contacts during the large conformational change indirectly drive activation of the autocatalytic site and that the unique quasi-equivalent interactions between subunits results in different rates of cleavage.

The crucial importance of the allosteric contacts in the capsid is also evidenced by the fact that acidic pH is only necessary to drive the capsid transition but is not required for the autocatalytic reaction as partially cleaved particles can complete maturation if the pH is raised to 7.0²⁶. Low pH induces the protonation of negatively charged residues that dominate the subunit contacts, neutralizing repulsive charges and allowing the procapsid to collapse into the compact capsid state. The procapsid size can be precisely controlled by titrating the pH; the lower the pH, the more compact the capsid. Below pH 6.0 the capsid reaches a conformation that activates cleavage^{25; 26}. The conformation transition is reversible until ~10% of the subunits are cleaved. Cleavage of more than 10% of the subunits locks the particle in the compact state, even if the pH is raised to 7²⁷. Retention of the mature capsid morphology with limited cleavage allows proteolysis to continue even at neutral pH.

The dramatic effect of partial cleavage on the reversibility of the particle morphology is a profound example of allostery. Indeed changing 24 or fewer subunits alters the global behavior of the remaining 216 subunits.

Capsid dynamics and virus maturation – emerging structural tools reveal mechanisms for allostery in the NwV system

Allostery is defined as a functional change of a protein caused by the binding of a ligand to a region that is not at the active site. In 1965, Monod, Wyman and Changeux already proposed that the observed functional effects were probably caused by the displacement of the equilibrium between preexisting conformational states². Characterizing these displacements and their functional consequences has been challenging but with major progress in the last two decades. NMR is arguably the most powerful approach to study dynamics for proteins less than 60kD, providing at the same time detailed structural information as well as parameters describing protein structure fluctuations²⁸. Mega Dalton protein complexes such as viruses are far beyond the maximum size for NMR analysis but are amenable to study by X-Ray crystallography and Cryo-Electron Microscopy. X-ray crystallography is intrinsically biased towards highly stable structures, since they are more likely to form crystals. Therefore, crystal structures usually report minimal energy configurations and are, for example, highly informative when comparing ligand bound and unbound conformations. Regardless of their position on the energy landscape, such structures provide valuable, near-atomic information, for interpreting data generated by lower resolution methods that are sensitive to macromolecular dynamics. Cryo-EM images

offer a comprehensive view of the continuum of macromolecular conformations at the moment of vitrification in liquid ethane, but methods to extract this dynamic information are just being developed^{29; 30}. Next, we describe how Cryo-EM experiments, coupled with the X-ray structure of mature N ω V, allowed us to examine the dynamic properties of individual subunits during N ω V maturation (Fig 2).

The cleavage kinetics of N ω V is slow, making it possible to isolate partially mature particles and determine their structures by Cryo-EM. Structures were determined at 3 minutes, 30 minutes and 4 hours after maturation was initiated by lowering the pH from 7.6 to 5. Kinetic analyses showed that the proportion of subunits cleaved at these time points is 10%, 50% and 80%, respectively (see Fig 1a). At pH 5 the particle size does not change during the maturation process allowing comparison of the active sites at specific time points by difference cryo-EM density maps ρ (fully mature 100% cleaved) $-\rho$ (partially cleaved). The precise location of the cleavage site for each subunit was located using the 2.8 Å X-ray structure of mature N ω V. We assume that if subunit cleavage has not occurred the active site is not totally formed and will differ from those in the fully-cleaved particle³¹.

At 3 minutes, the active sites of subunits adjacent to the 5-fold axes (A subunit) and 3 fold axes (D subunit) were closely similar to the completely mature capsid (Fig 2a). On the other hand, highly significant differences were seen at the active sites of the B and C subunits. Therefore, most, if not all cleavage observed at 3 minutes occurred in the A and D subunits. At 30 minutes, A and D showed no difference from the mature particle and the B subunit showed a significant decrease in the difference density, indicating that this subunit now contributed to cleavage. Only at 4 hours, did the C subunit show significant reduction in difference density making it the last to contribute to the cleavage and complete maturation. These results unequivocally show that the unusual kinetics of the cleavage derives from the T=4 quasi-equivalence of N ω V resulting in the subunit- specific rates of maturation³¹ (Fig. 2a).

Each difference map described above provides dynamic information based on two data sets, a time resolved data set and the fully mature equilibrium data set. We recently developed an approach that provides comparable information from just one time resolved data set²⁹. The method uses a maximum likelihood algorithm that explicitly takes into account the continuous variability from one instance to another instance of the particles in the data set. The result describes the heterogeneity of the particle as a variance plotted at every voxel of the reconstructed density. We posit that active sites in the process of forming will have higher variance than those already formed. When the average variance was computed in volumes at the active sites, the subunit variance differentiated in the same way as observed in the difference maps with subunits A and D showing close to half the variance of B and C at the 3 minute and 30 minute time points. At 4 hours the difference in variance among the active sites was negligible (Fig. 2b). This data also revealed that not only the active site is stabilized with cleavage, but also the whole protein shell becomes more stable, as can be seen by heat maps of the capsid surface in Fig. 2b²⁹.

These data indicates that cleavage is also important for the stabilization of the subunits contacts of the capsid. Large conformational change of a mutant that does not cleave (N570T) is reversible, with the particle contracting normally at pH 5 but expanding again when the pH is raised to 7.6²⁷. Sub nanometer cryoEM studies of the non-cleavable mutant in the compact state shows that the helices at the flat contact are virtually invisible in the density while they are clearly visible in the density of the fully mature particle with all subunits cleaved. This implies that these helices remain dynamic prior to cleavage and that cleavage allows their irreversible, immobilization at the flat contact with the consequent loss of overall particle dynamics³². Since cleavage of only 10% of the subunits stops

reversibility, there must be a level of allosteric interaction that propagates through the particle within the first 3 minutes of maturation.

Additional evidence for subunit intercommunication comes from the phenotypes observed with another mutation. The substitution E73Q, results in particles with aberrant cleavage kinetics. A and D cleave normally, while B and C require six times longer than normal to cleave³¹. Residue E73 is far from the active site and participates in interactions with residues from an adjacent subunit (Fig. 1b). A salt bridge, disrupted by the E73Q mutation in the D subunit, affects the position of the switch helix that stabilizes the flat contact of the capsids. As A and D cleavage kinetics are normal, the stabilization of these contacts must be important to modulate the cleavage of B and C, specifically³¹. The mutations described and their consequence suggest that the allosteric behavior characterized to date, due to quasi-equivalence, is just a sample of the remarkable control exerted by a single gene product when it resides in different quaternary structure environments.

Far greater detail is potentially available with time-resolved Hydrogen/Deuterium exchange analysis coupled with mass spectrometry (HDX). HDX occurs in the context of the assembled particle, regardless of its maturation state. Moreover, the particle is permeable to deuterium so the exchange depends only on the conformation of a particular region of polypeptide³³. In general, polypeptide regions with secondary structure exchange deuterium much slower than those in an extended conformation. At specified time points lowering the pH to 3 quenches the exchange. The particles are disassembled and digested with pepsin, maintaining pH 3, generating polypeptides of 3 to 15 amino acids. These are separated chromatographically by size and their masses determined. The mass, coupled with the sequence, allows assignment of polypeptides to their location in the primary structure of the protein. The change in mass with the time of exposure to deuterium allows the percent of protons exchanged for deuterons to be calculated.

The results can map changes in secondary structure that occur during maturation. If, for example, a region of polypeptide changed from an extended state to a helical state during maturation, this would be reported in the rates of HD exchange for the same polypeptide in the procapsid and capsid. Greater detail can be achieved in quasi-equivalent capsids as the same polypeptide may have different conformations in different quasi-equivalent subunit positions. This is reported by a bimodal distribution of HD exchange where there are different rates of exchange that depend on the different conformations of the same polypeptide. The potential for the method is demonstrated by analysis of residues 588-595 of the N ω V gamma peptide. The peptide in the procapsid showed a single population in HDX that exchanged approximately 30% of its protons, while the same peptide from capsids revealed a bimodal distribution, indicating one class of peptide readily exchanged 80% of its protons while the other exchanged virtually no protons (Fig 3). Analysis of the available structural data allows these results to be rationalized. Tang et al³², on the basis of an 8Å resolution cryoEM reconstruction of N ω V procapsid, proposed that the region corresponding to the switch helices in all the subunits had roughly the same helical structure since all the dimer contacts were closely similar. At the resolution of their study, this would include residues 588-595. The crystal structure of the capsid shows that this peptide corresponds to a region that is structurally different at subunits A/B and C/D.

Subunits A and B display a well formed helix in this region while subunits C and D are in an extended conformation in this region, clearly explaining the difference in exchange rates for the two classes of polypeptide (Fig. 3c). Given this indication of sensitivity to changes during maturation as well as quasi-equivalent differences, we believe that N ω V will prove to be a good model system for conducting high-resolution HDX experiments to measure polypeptide conformations before, during, and after maturation.

Functional Significance of Allosteric Regulation of Autocatalysis

In this section we show how the coordinated maturation pathway of NωV have important implications for virus function and infectivity. The γ peptides covalently liberated by the cleavage reaction have subunit specific folds (Fig. 1c). The γ peptides of subunits A and B are partially ordered with only residues 571-595 visible as a strait alpha-helix in the electron density. The γ peptides associated with subunit A form pentameric helical bundles that are separated from the outside of the particle only by loops of the shell-forming viral jelly roll (Fig. 1c). On the other hand, γ peptides from subunits C and D are nearly fully ordered (571-641) with residues 624-638 forming the critical helices that support the flat contact. With A and D cleaving early in maturation, the pentameric helical bundles are liberated rapidly and one of the two helices at the flat contact is fully positioned quickly³¹.

Like all non-enveloped animal viruses, NωV harbors membrane lytic activity required for cell entry and this activity is conferred by γ peptides²³. By correlating the membrane lytic activity of partially mature NωV capsids with the extent of cleavage, we could attribute the lytic effect to a subset of gamma peptides that is generated within the first minutes of maturation. As D- γ position is inappropriate for release, γ coming from the pentamers (A-subunits) must account for the lytic activity.

Therefore, rapid initial cleavage of A subunits assures infectivity immediately after maturation onset. Rapid cleavage of D subunits assures particle stability at pH values above 7²³. Note that the Maximum-likelihood cryo-EM reconstructions revealed that mature particles contain a highly dynamic region at the center of the five-folds, despite the overall stabilization caused by auto-proteolysis (Fig 2b). It appears that all of the essential functions of autocatalysis are performed in the first 30 minutes in spite of the slow cleavage of B and C subunits that require many hours.

Tensegrity vs Allostery

Over the last years, virus capsids have been regarded as highly efficient nanocontainers that could be designed to deliver therapeutically relevant molecules inside cells. In order to develop functional nanoparticles from viral proteins or engineered materials, it became important to understand biomechanical forces that govern virus assembly and stability. Experimental data derived from nanoindentation measurements by atomic force microscopy, have been showing that concepts borrowed from macroscopic materials science are relevant to describe the physical properties of viral shells³⁴.

The concept of tensegrity was previously applied to discuss the decreased rate of cleavage. This is a contraction coined by Buckminster Fuller of the terms “tensional -integrity”. It emphasizes that the force placed at a particular location on a geodesic dome or icosahedral virus particle is distributed throughout the particle^{15; 35}.

In this formalism, the autocatalytic sites, prior to cleavage, are under local strain, but the structure is globally stabilized by the quaternary organization of the capsid. Following cleavage, the local strain is relieved, and the reduction of stress is distributed around the particle, slowing the next reaction proportionately. Tensegrity in these terms is very similar to allostery. We contend that the descriptions above provide a detailed chemical mechanism for the generic force description normally associated with mechanical engineering. Thus the two concepts are closely similar, if not identical.

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Highlights

How is virus maturation influenced by quasi-equivalence?

Subunit interfaces affect the formation of active sites for auto-proteolysis.

Different active sites are next to quasi-equivalent interfaces, affecting cleavage.

The biological roles of liberated peptides reflect the rate that they are produced.

Figure 1.

Quasi-symmetric contacts as allosteric regulators of NωV autocatalysis (a) NωV assembles as procapsids that do not present any autocatalytic activity. The procapsid conformation is stable at pH 7.6 but acidification drives the transition from the procapsid state (490 Å diameter) towards the compact capsid state (410 Å diameter). The change in quaternary structure alters the subunit contacts triggering the autocleavage reaction of protein α, producing the main capsid protein β (residues 1-571) and the γ peptide (571-644) that remains non-covalently associated to the capsid. Even though the conformational transition from procapsid to capsid is fast (ms) the autoproteolytic reaction is slow taking days to completion. The plot was derived from SDS-PAGE analysis of a maturation time-course. Note the unusual kinetics, with fast and a slow components. (b) An illustration of the tertiary structure of one of the subunits of NωV showing the location of the cleavage site between residues N570 and W571. The zoom-in view shows that the cleavage site is similar for all the 4 asymmetric units of NωV capsid (shown in blue-A, red-B, green-C and yellow-D) indicating that allosteric factors must modulate cleavage onset and kinetics. (c) Arrangement of the four asymmetric units in NωV T=4 mature capsid. A-subunits form pentamers, while B, C and D form pleated hexamers. Thick black lines represent bent contacts (~138°) and dashed lines highlight the flat contacts of the capsid. Black crosses indicate the location of the cleavage site inside the capsid. An inside view of the capsid is shown on the right, highlighting the differences in the gamma peptide structure for each of the subunits. γ peptides are depicted with strong colors and N570 is shown as spheres. The flat interfaces of the capsid are stabilized by the c-terminal helix of γ peptide coming from C and D subunits, shown as rods. Note that the residues forming the switch helix in C and D are not ordered in A and B.

Figure 2.

Subunit-specific dynamic properties of NωV maturation intermediates (a) Time resolved analysis of NωV maturation by Cryo-EM difference density maps 10 Å around the cleavage site of the four quasi-equivalent positions after lowering the pH from 7.6 to 5.0³¹. Increased dynamics in the partially cleaved capsid generates extra densities (shown as a mesh) that are not observed in the mature capsid. This analysis revealed that maturation is clearly asymmetric. A and D subunits cleavage sites are the first to be stabilized, accounting for 50% of the subunits cleaved after 30 minutes in pH 5.0 (see cleavage kinetic in figure 1 a). B and C take longer to get in position and form the slow component of the kinetic curve. (b) Same data sets were used to develop and test a new technique to extract dynamic information from Cryo-EM reconstructions²⁹. The structure of capsids at each maturation state were solved de novo using a maximum likelihood algorithm that explicitly takes into account the continuous variability of the particles present in the data set. The result describes the heterogeneity of the particle as a variance plotted at every voxel of the reconstructed density. The graph show that the variance computed for the same region shown in (a) are in close agreement with the difference density maps. This new methodological approach allowed the analysis of the whole particle dynamics during maturation, as shown by the surface coloring of the capsids. The cut-way view of the mature capsid (3 days) was colored with a different heat-map scale to reveal the relatively high dynamic region in the center of the capsid pentamers.

Figure 3.

Hydrogen-deuterium exchange coupled to mass-spectrometry as tool for mapping regions with distinctive dynamic behavior during maturation. N ω V capsids and procapsids were analysed with a time-course of deuterium uptake followed by pepsin digestion and mass-spectrometry analysis. Each proton that is exchanged for a deuterium results in a mass increase of 1 amu. (a) The isotope patterns for the segment KSEATTSV (588-595) that is part of γ -peptide, present a monomodal distribution in procapsids and bimodal distribution in capsids. (b) Deuterium uptake curves for the same peptide. While the capsid present two distinct populations with a fast and slow exchange rate, the procapsid exchange at an intermediary rate. (c) Structural environment for the analyzed peptide at the capsid crystal-structure. The same sequence is structured either as an strait alpha-helix in A/B (blue/red, respectively) or as a flexible loop in C/D (green/yellow, respectively). Note that part of the loop is not ordered in the crystal structure for C-subunit.