Archives of Biochemistry and Biophysics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Review

Transmission electron microscopy and the molecular structure of icosahedral viruses

Carmen San Martín

Department of Macromolecular Structure and NanoBioMedicine Initiative, Centro Nacional de Biotecnología (CNB-CSIC), Darwin 3, 28049 Madrid, Spain

ARTICLE INFO

Article history:
Received 30 April 2015
and in revised form 1 June 2015
Available online xxxx

Keywords:
Virus structure
Virus assembly
Three-dimensional reconstruction
Cryo-electron microscopy
Cryo-electron tomography
Icosahedral symmetry
Symmetry mismatch

ABSTRACT

The field of structural virology developed in parallel with methodological advances in X-ray crystallography and cryo-electron microscopy. At the end of the 1970s, crystallography yielded the first high resolution structure of an icosahedral virus, the $T = 3$ tomato bushy stunt virus at 2.9 Å. It took longer to reach near-atomic resolution in three-dimensional virus maps derived from electron microscopy data, but this was finally achieved, with the solution of complex icosahedral capsids such as the $T = 25$ human adenovirus at ~3.5 Å. Both techniques now work hand-in-hand to determine those aspects of virus assembly and biology that remain unclear. This review examines the trajectory followed by EM imaging techniques in showing the molecular structure of icosahedral viruses, from the first two-dimensional negative staining images of capsids to the latest sophisticated techniques that provide high resolution three-dimensional data, or snapshots of the conformational changes necessary to complete the infectious cycle.

From the humble 2D beginnings to the first subnanometer 3D maps

The study of highly symmetrical virus capsids has historically been at the forefront of methodological advances in structural biology. For example, early X-ray diffraction analyses of high-concentration solutions of small icosahedral plant viruses required methodological modifications to deal with the (then) extremely large periodicities of hundreds of Ångstroms [1,2]. Conversely, these first observations suggesting some periodicity in the specimens paved the way for the prediction by Watson and Crick that viruses would have polyhedral capsids to compensate for the limited coding capacity of their genomes [3]. X-ray diffraction studies on tomato bushy stunt virus (TBSV)[1] crystals proved the prediction by showing diffraction patterns reflecting icosahedral symmetry [4]. Twenty years later, protein crystallography had advanced sufficiently [5] to actually show the TBSV coat protein fold at 5.5 Å, illustrating how Caspar and Klug's quasi-equivalence theory [6] is in fact fulfilled in a $T = 3$ particle [7]. Crystallography of TBSV also yielded the first virus structure solved at "atomic" (2.9 Å) resolution [8].

Soon after the Crick and Watson prediction, negative staining electron microscopy (EM) images proved well suited to show not only the general shape and size, but also the number and organization of capsomers in complex icosahedral viruses such as adenovirus (AdV) (Fig. 1A) [9]. These studies showed direct proof of the Caspar and Klug predictions of larger triangulation numbers that could not be tackled by crystallography at the time, but were limited to observation of two-dimensional projections of the three-dimensional objects. By virtue of the large depth of focus of the transmission electron microscope, the images contain information on all planes of a typical virus specimen. At the end of the 1960s, De Rosier and Klug showed that the three-dimensional information could be recovered from these projections using newly developed computational techniques based on the central section theorem [10]. The group soon adapted these techniques to icosahedral objects and produced the first three-dimensional virus maps (again including TBSV) from negative staining EM images [11]. Negative staining was not the ideal technique for a faithful representation of the molecular structure, however, as specimens were known to be deformed by flattening, and the stain delineated only the outer surfaces of the viral particle.

E-mail address: carmen@cnb.csic.es

[1] *Abbreviations used:* TBSV, tomato bushy stunt virus; EM, electron microscopy; AdV, adenovirus; cryo-EM, cryo-electron microscopy; CTF, contrast transfer function; FEG, field emission guns; HBVc, hepatitis B virus core; BTV, bluetongue virus; SFV, Semliki Forest virus; NCωV, nudaurelia capensis omega virus; CCMV, cowpea chlorotic mottle virus; DENV, dengue virus; HRV-2, human rhinovirus type 2; FMDV, foot and mouth disease virus; CPV, cytoplasmic polyhedrosis virus; BMV, brome mosaic virus; GCRV, grass carp reovirus; cryo-ET, cryo-electron tomography; STIV, *Sulfolobus* turreted icosahedral virus.

http://dx.doi.org/10.1016/j.abb.2015.06.001
0003-9861/

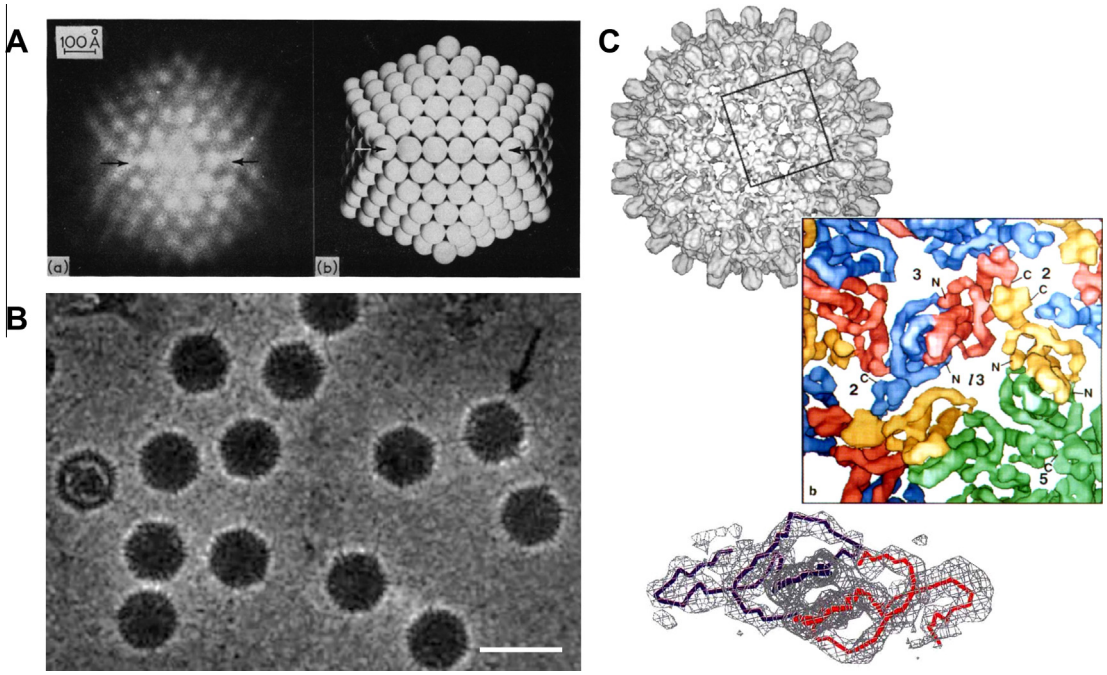

Fig. 1. From the 2D beginnings to the first subnanometer 3D maps. (A) One of the first EM images of a virus, showing negatively stained human adenovirus (left) and the derived model for capsomer distribution (right). Arrows indicate the 5-fold capsomers. Reproduced from [9], with permission. (B) Part of one of the first cryo-EM images of viruses, showing frozen hydrated human adenovirus particles. The image was taken with a 100 kV electron microscope at 8 μm underfocus. The arrow points to a particle with the receptor binding fibers visible. The bar represents 100 nm. Modified from [12], with permission. (C) One of the first virus cryo-EM maps at subnanometer resolution: HBVc at 7.4 Å. Top: surface rendering of the complete $T = 4$, 360 Å-diameter particle, calculated from ~6000 projections. A zoom in the area indicated by a black rectangle is shown in the center panel. Numbers indicate the positions of the icosahedral 2-, 3- and 5-fold axes. Colors represent the four independent copies of the HBVc protein in the asymmetric unit. Bottom, the density for a single HBVc protein dimer is shown as viewed from outside the shell, with the two modeled chains in red and purple. Modified from [20], with permission.

In the late 1980s, cryo-electron microscopy (cryo-EM) came to solve these limitations, providing the first stunning images of viruses (including AdV, phage T4, and Semliki Forest Virus) in a frozen-hydrated state (Fig. 1B) [12]. In cryo-EM, the biological macromolecules are vitrified in solution by rapid immersion in liquid ethane, and kept at very low temperatures (near −180 °C) throughout storage, transfer to the microscope and imaging. In this way, the samples conserve their water content and molecular structure. The images obtained are a direct function of sample density, not of the staining agent cast, so that the 2D projections now also bear information on internal features of the particle. The first cryo-EM reconstructions of icosahedral viruses used images taken in microscopes with thermionic electron sources working at ~100 kV; they averaged tens of particles without correcting for the effects of the microscope contrast transfer function (CTF), and achieved resolutions in the 20–40 Å range. Many examples of these early works can be found in a previous comprehensive review [13]. This resolution range was enough to reveal the shape and packing arrangement of capsomers in many virus families such as the $T = 4$ alphaviruses [14], the multishelled rotavirus [15], or the $T = 25$ AdV [16]; it was also enough to allow the first glimpses of the large conformational changes concomitant with maturation and genome packaging in bacteriophage and herpesviruses [17–19].

The qualitative leap to high resolution came with the popularization of field emission guns (FEG) as electron sources in the late 1990s. The electron beam generated by thermionic electron guns has low spatial coherence, reflected as a rapid decay in signal amplitude beyond 20 Å resolution. FEG, on the other hand, produce highly coherent electron beams, and consequently the images contain useful information all the way to high frequencies. Development of CTF correction algorithms was then critical to achieve the full potential of the much improved data. The evolution of the software and hardware needed for aligning and averaging hundreds or thousands of particles was also a key factor for the take-off of cryo-EM as a high resolution technique. The breakthrough arrived with the publication of two reports on the hepatitis B virus core (HBVc) at subnanometer resolution (7.4 and 9 Å) [20,21], little more than a decade after the first cryo-EM images of viruses had been obtained (Fig. 1C). At resolutions better than 10 Å, the four α-helices that make up the HBVc capsid protein were resolved, but the actual pathway of the polypeptide chain still held uncertainties. Heavy-metal and peptide mapping studies helped to remove these uncertainties by localizing the termini of the polypeptide chain [22,23].

Making more of the maps with hybrid methods

Even as cryo-EM data collection and processing were improving, it was realized that more information could be extracted from moderate resolution maps by combining them with high resolution structures of their components [24]. Many viruses present particularly challenging problems for crystallography, not only because of their large size and complex capsid composition, but also because of the difficulties involved in obtaining the large particle concentrations necessary for crystallization, or because flexible capsid components hinder production of highly ordered crystals [25]. In contrast, individual viral components might be more amenable to overexpression and crystallization; their atomic structures give a very detailed picture of each capsid component, but do not provide information on their mutual relationship in the virion. On the other hand, it soon became straightforward with cryo-EM to obtain pictures of complete virions, although at lower resolution than crystallography maps.

These two sources of 3D information (atomic structure of individual components, and medium resolution of complete viral particles) can be combined by fitting the known crystal structures to