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Electron cryomicroscopy of single particles at subnanometer resolution

Wen Jiang and Steven J Ludtke

Electron cryomicroscopy and single-particle reconstruction have advanced substantially over the past two decades. There are now numerous examples of structures that have been solved using this technique to better than 10 Å resolution. At such resolutions, direct identification of α helices is possible and, often, β-sheet-containing regions can be identified. The most numerous subnanometer resolution structures are the icosahedral viruses, as higher resolution is easier to achieve with higher symmetry. Important non-icosahedral structures solved to subnanometer resolution include several ribosome structures, clathrin assemblies and, most recently, the Ca^{2+} release channel. There is now hope that, in the next few years, this technique will achieve resolutions approaching 4 Å, permitting a complete trace of the protein backbone without reference to a crystal structure.

Addresses
National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA

Corresponding author: Ludtke, Steven J (sludtke@bcm.tmc.edu)

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Introduction

The field of electron cryomicroscopy (cryo-EM) and, in particular, single-particle reconstruction has undergone dramatic growth in recent years (Figure 1). A combination of improvements in transmission electron microscope technology and rapid growth of computational capabilities has allowed this field to advance from achieving 30–40 Å resolution ten to fifteen years ago to now, when numerous structures at subnanometer resolution have been elucidated. At such resolutions, it is possible to directly determine the arrangement of secondary structure elements within three-dimensional reconstructions. As the field approaches 4–5 Å resolution, the goal of tracing the protein backbone solely from a single-particle reconstruction is on the verge of becoming a reality.

Single-particle reconstructions are generally divided into two categories: icosahedral particles and non-icosahedral particles. Typically, the resolution-limiting factor in single-particle reconstruction is the high noise levels present in the particle images due to dose limitations. As resolution increases, ever smaller doses are required to prevent radiation damage at the resolution of interest. In addition, specimen motion, beam coherence and other factors may limit the final resolution achievable for a given set of images. The most commonly studied icosahedral particles are large virus capsids, with masses sometimes exceeding 100 megadaltons. Combined with 60-fold symmetry, the effective signal to noise ratio of these particles is substantially higher than that of asymmetric particles, with masses typically measured in the hundreds of kilodaltons. In addition, the 60-fold symmetry reduces the number of particles required for a reconstruction at a given resolution by a factor of 60, permitting subnanometer resolution reconstructions to be performed using only a few thousand particles. Not surprisingly, the first structure solved at subnanometer resolution [1,2] was an icosahedral virus.

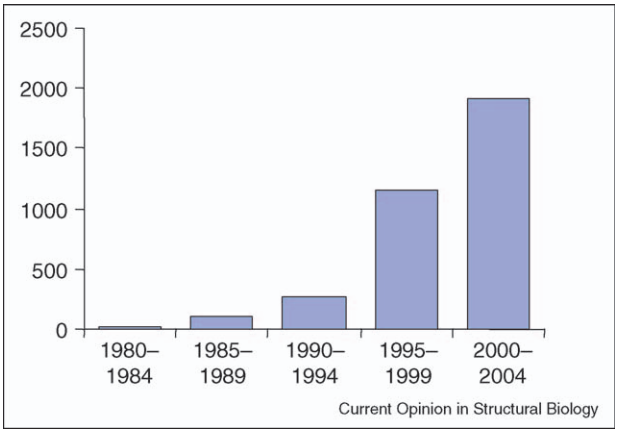
In this review, we survey subnanometer resolution reconstructions published to date. As these reconstructions are biologically diverse, the discussion is organized by the symmetry of the particle being discussed, starting with large icosahedral particles and gradually moving to lower symmetry particles.

Icosahedral viruses

Since achieving the subnanometer resolution milestone for the small hepatitis B virus core [1,2], the number of virus structures solved at this resolution has been steadily increasing. In fact, it is now possible to solve a virus structure from imaging to reconstruction and identification of secondary structure elements in well under a month. Although this throughput is still highly specimen dependent, it is beginning to transform cryo-EM into a routine laboratory technique, like cloning or gel filtration, for studying the structural and functional relationships of biological systems under various biochemical conditions or in various functional states.

Viruses from many different families have now been solved at subnanometer resolution: the small hepatitis B virus [1,2] and rhinovirus [3•] (~300 Å); the large adenovirus [4,5] (~930 Å) and herpes simplex virus [6] (~1250 Å); single-layered cytoplasmic polyhedrosis virus (CPV) [7]; multi-layered rice dwarf virus (RDV) [8] and reovirus [9••]; enveloped alphaviruses [10], dengue virus [11] and PM2 phage [12]; phages with a large non-icosahedral tail, such as

Figure 1



Growth of the cryo-EM field based on the number of publications identified using a Google Scholar search (with key words cryomicroscopy, cryoem and cryo-electron).

P22 [13••,14••] and ϕ29 [14••]; and complexes of rhinovirus with cellular receptors [3•]. From these structures, significant insights into viral assembly, maturation and evolution have been achieved.

Identification of viral protein folds

Structures determined at subnanometer resolution allow assignment of secondary structure elements within the reconstruction, which can then be used to establish protein folds. The hepatitis B virus core [1,2], herpes simplex virus capsid [6] and RDV capsid [8] are reconstructions for which secondary structure elements and protein folds were first proposed based on cryo-EM structures and later confirmed using X-ray crystallography [15,16•,17]. These successful cross-validations demonstrated that single-particle cryo-EM at subnanometer resolution provides accurate, biologically relevant structural information.

Evolutionary links among distinct viral families

The structure of the mature P22 phage at 9.5 Å resolution [13••] revealed, for the first time, that the coat protein of a short-tailed phage, P22, shares the same fold as that of a long-tailed phage, HK97, despite minimal sequence identity (Figure 2a,b). This led to the hypothesis that the major coat proteins of most of the tailed phages share the same protein fold and thus possibly a common ancestor. This concept is further supported by the recent publication of the structure of the ϕ29 phage capsid at 7.9 Å resolution [14••] (Figure 2c), the capsid structure of ε15 phage at 9.5 Å (W Jiang *et al.*, unpublished) and the crystal structure of isolated T4 vertex protein gp24 [18]. Surprisingly, the same fold has also been discovered in the floor domain of human herpes simplex virus (M Baker, W Jiang *et al.*, unpublished).

These findings are reshaping our understanding of the evolutionary relationships among virus families. The well-known jelly-roll fold shared by small viruses [19], the conserved major coat proteins of double-stranded (ds)DNA adenoviruses, PRD1 phage and archaeal *Sulfolobus* turreted icosahedral virus [20], and the inner 'T = 2' shell proteins of dsRNA reovirus [21], CPV [7], RDV [8], bluetongue virus [22] and L-A virus [23] together raise the possibility that all viruses might have evolved from a relatively small number of primordial progenitors.

Structural dynamics

A distinct advantage of single-particle analysis is the capability to solve the structures of macromolecules in different functional states. The structures of several viruses, such as herpes simplex virus [24] and HK97 phage [25], have been studied in various functional states at lower resolution. Structures representing the transition of the P22 phage from procapsid to mature phage have been solved to 8.5 Å and 9.5 Å resolution, respectively [13••]. These structures revealed the hinge motion of helices and sheets, and local refolding of one helix. These local conformational changes result in the large-scale expansion and angularization of the entire capsid, a signature of the maturation process of tailed phages.

Although not a virus, the E2 core of pyruvate dehydrogenase from *Bacillus stearothermophilus* has icosahedral symmetry. Several laboratories have studied this structure using both cryo-EM and X-ray crystallography. Two [26,27] have provided single-particle reconstructions at subnanometer resolution. One report [26] is of more technical than biological interest, as it was intended as a control for the development and evaluation of a new single-particle reconstruction methodology fundamentally different from that used for icosahedral virus particles.

Structures with lower symmetry

The number of lower symmetry particles solved at subnanometer resolution is much more limited and the solved structures are far more biologically diverse. We begin with the higher symmetry reconstructions and then consider progressively lower symmetries.

Clathrin can form assemblies with a variety of symmetries. The structure of the D6 hexagonal barrel form of clathrin was recently solved at ~12 Å, which was then extended to ~8 Å resolution [28••] through the use of additional subunit averaging beyond the D6 symmetry. α-Helical motifs can be clearly observed in the structure (Figure 3), making a convincing argument for true subnanometer resolution. This structure, combined with a moderately lower resolution reconstruction of mini-coats, provides some clues to how clathrin can form such diverse assemblies using fundamentally the same structural linkages.

GroEL (~800 kDa) represents an assembly that, despite the existence of several crystal structures, is still under active study using single-particle reconstruction. These