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Technical Note

Rapid increase of near atomic resolution virus capsid structures determined by cryo-electron microscopy

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ABSTRACT

The recent technological advances in electron microscopes, detectors, as well as image processing and reconstruction software have brought single particle cryo-electron microscopy (cryo-EM) into prominence for determining structures of bio-molecules at near atomic resolution. This has been particularly true for virus capsids, ribosomes, and other large assemblies, which have been the ideal specimens for structural studies by cryo-EM approaches. An analysis of time series metadata of virus structures on the methods of structure determination, resolution of the structures, and size of the virus particles revealed a rapid increase in the virus structures determined by cryo-EM at near atomic resolution since 2010. In addition, the data highlight the median resolution (~3.0 Å) and size (~310.0 Å in diameter) of the virus particles determined by X-ray crystallography while no such limits exist for cryo-EM structures, which have a median diameter of 508 Å. Notably, cryo-EM virus structures in the last four years have a median resolution of 3.9 Å. Taken together with minimal sample requirements, not needing diffraction quality crystals, and being able to achieve similar resolutions of the crystal structures makes cryo-EM the method of choice for current and future virus capsid structure determinations.

1. Introduction

Although X-ray crystallography has been the method of choice for obtaining high resolution structural information for decades, cryo-EM has recently emerged as a good alternative for the structure determination of bio-molecules (Kuhlbrandt, 2014; Nogales, 2016), especially in the case of large assemblies like viruses and ribosomes ever since the determination of the first near atomic resolution cryo-EM structures (Amunts et al., 2015; Bai et al., 2013; Chen et al., 2009; Jiang and Tang, 2017; Yu et al., 2008). The recent improvements in achieving higher resolutions of bio-molecular structures by single particle cryo-EM that has been branded as "resolution-revolution", are made possible by a number of technological advances: 1) High-powered electron microscopes equipped with auto sample loaders (e.g., 300 kV Titan Krios (FEI)), 2) Superior direct electron detectors, such as the K2 Summit detector (Gatan Inc.), Falcon III (FEI) that improve upon the original Direct Electron detectors (Brilot et al., 2012) and are able to collect images as movies with short exposure times, 3) Software to collect (Glaeser et al., 2011; Suloway et al., 2005) and process the images (Grant and Grigorieff, 2015; Li et al., 2013; Zheng et al., 2017) as well as integrated pipelines to pick and classify particles (Lander et al., 2009; Tang et al., 2007) and perform subsequent image reconstructions (Ludtke et al., 1999; Lyumkis et al., 2013; Scheres, 2012; Yan et al., 2007), and 4) Requirement of only small amounts of samples without the need to produce diffraction quality crystals.

To highlight the impact of the above technological advances on the structure determination of spherical viruses in particular, we have analyzed data on the methods of structure determination, the resolution of the structures, and the size (diameter) of the particles since the late 1970s when the first virus crystal structures were determined (Abad-Zapatero et al., 1980; Harrison et al., 1978) and deposited in RCSB-PDB (Berman et al., 2000). We obtained the relevant information from VIPERdb (http://viperdb.scripps.edu), a repository and knowledge base of icosahedral virus structures (Carrillo-Tripp et al., 2009) represented in a single icosahedral convention and curated from the original entries deposited in RCSB-PDB. In addition to estimating the number of structures determined yearly by X-ray crystallography and cryo-EM, we have monitored the trends in improvements of resolution of the structures achieved by both methods over the years as well as the size (diameter) of the virus particles. Consideration was also given to the Electron Microscopy Data Bank (EMDB) (http://www.emdatabank.org/), which has come to existence in the early 2000s as a repository of

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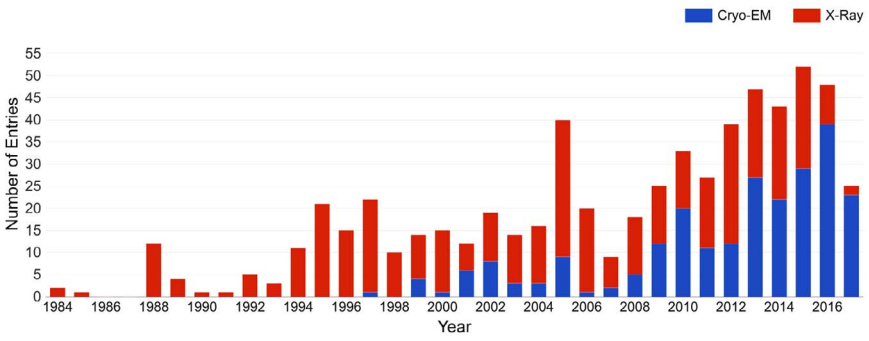

Fig. 1. Number of virus structures determined in each year by X-ray crystallography and cryo-electron microscopy (cryo-EM) based on data from VIPERdb/PDB. The full-length of the bars corresponds to the total number of virus structures (Y-axis) determined in each year (X-axis), while the length of the red and blue regions of the bars indicate the number of structures determined by X-ray crystallography and cryo-EM respectively. Of note, no virus structures were deposited in PDB in the years 1986 and 1987.

cryo-EM reconstructions (maps) (Lawson et al., 2016; Velankar et al., 2016). Even though the best model derived from the highest resolution cryo-EM map is deposited in PDB, researchers usually deposit multiple maps of varying resolutions from the same experiment under different identifiers in EMDB. Hence, the data from EMDB potentially skew the number of cryo-EM structures deposited each year and associated improvements in resolution. Because of this we chose to do our analysis based on the data from VIPERdb/PDB, which contains more representative X-ray and cryo-EM structures. However, the general trends in the improvement of resolution over the years from both databases are same (Fig. S1).

2. Rapid increase of near atomic resolution virus structures determined by cryo-EM since the year 2010

Fig. 1 shows the total number of virus structures determined each year since 1984 as well as the numbers partitioned according to the method of structure determination. With the exception of the years 1986 and 1987 when no virus structures were deposited, all virus structures deposited in the PDB between the years 1985–1996 were determined by X-ray crystallography. The first virus capsid (pseudo-atomic) model derived by docking the X-ray structures into cryo-EM reconstructions was deposited in 1997. The resolution of one of these reconstructions (PDB-ID: 1KVP) is 27.0 Å. Even though the first sub-nanometer (7.4 Å) cryo-EM structure of Hepatitis B virus (HBV) was reported in 1997 (Bottcher et al., 1997), which revealed the fold of the core protein of HBV de novo and represents a significant milestone in cryo-EM based structure determination, the highest resolutions of cryo-EM structures remained around 7 Å until 2008 (Table 1) when the first sub 4 Å structures were reported (see the next section). Between this time and 2010, the number of cryo-EM models deposited in PDB per year remained low (1–12). However, in 2010 there was a spike in the number of cryo-EM structures/models deposited (20). In fact, the majority of virus structures (20 out of 33) deposited that year were determined by cryo-EM. Furthermore, there has been a continual increase in the cryo-EM structures every year since 2006 and approximately 81% of the virus structures deposited in 2016 were determined by cryo-EM (Fig. 1). Remarkably, all the virus structures deposited so far in 2017, except for two, are cryo-EM structures (23 out of 25). This increase in the number of cryo-EM structures is on par with the increase in the total number of virus structures determined since 2007 (Fig. 1). On the other hand, the number of X-ray structures deposited each year since 2013 either remained the same or decreased (Fig. 1). This signifies the increase in application of cryo-EM methods for virus structure determinations.

3. Continued improvement in the resolution of cryo-EM virus structures since 2008

The first sub 4 Å cryo-EM structures determined were of a cytoplasmic polyhedrosis virus (diameter: ~630 Å) at 3.9 Å (Yu et al., 2008) and of a rotavirus (diameter: ~770 Å) at 3.8 Å resolution (Chen et al., 2009). Since then, the cumulative number of near atomic resolution cryo-EM virus structures has risen to 74 to date. The median resolution of cryo-EM structures has also improved from 7.1 Å in 2014 to 3.1 Å in 2017 with the previous highest resolution (2.79 Å in 2016) being surpassed this year (2.26 Å in 2017) (Table 1). While the cryo-EM structures show a greater variation in terms of resolution and the size of virus capsid (Figs. 2 and 3), the median resolution achieved by X-ray crystallography has remained rather constant (~3.0 Å) over the years (Fig. 2). Notably, the resolution of the majority of cryo-EM structures determined in the last 3 years is below 4 Å (Fig. 2 inset). The recent lower resolution cryo-EM structures primarily correspond to large virus structures and/or virus-receptor, virus-antibody complexes (Wang et al., 2013). Based on the trend line of resolution vs. time, the resolution of cryo-EM structures is poised to match the average resolution of 3.0 Å achieved by X-ray structures by the year 2020, if not sooner (Fig. 2).

4. Virion size is not a limitation for achieving higher resolution by cryo-EM

Even though the optimal size of virus particles studied by X-ray crystallography appears to be around 310 Å in diameter, no such limit exists for cryo-EM structures, which have a median diameter of 508 Å and some near atomic resolution structures with diameters exceeding 1000 Å (e.g., 1320 Å for Cytomegalovirus, PDB-ID: 5VKU) (Yu et al., 2017a) (Fig. 3). Based on the available data, the resolution achieved by cryo-EM appears to be mostly independent of particle size as opposed to X-ray crystal structures (Fig. 3 inset). In recent years, capsid structures determined by cryo-EM are also tending towards smaller sizes (diameters ≤ 300 Å). A possible explanation is that more of the structures in this range, previously determined by X-ray crystallography, are now being solved by cryo-EM perhaps due to ease of usage and similar resolutions achieved by the latter method. Alternatively, it is also possible that as more and more baseline structures are determined by X-ray crystallography, the focus shifts to virus-receptor or virus-antibody complexes for which cryo-EM is the preferred method due to difficulties in producing diffraction quality crystals of the complexes.

5. Conclusions

The recent technological advances that resulted in “resolution-revolution” of single particle cryo-EM are directly responsible for the increase in the near atomic resolution virus structures determined by cryo-EM. This method is attractive because it imposes no limitations on the size, symmetry, and complexity (e.g., virus-antibody complexes) of the particles and in most cases, is capable of achieving resolutions similar to X-ray crystallography. However, X-ray crystallographic methods may still hold the advantage in identifying the interactions between the proteins and solvent or small molecule compounds that are stabilized by the lattice forces, thereby providing greater details of virus-ligand complexes. Nonetheless, cryo-EM provides unique avenues for resolving the regions that are “blurred out” by the symmetry