

EXPLORING ICOSAHEDRAL VIRUS STRUCTURES WITH VIPER

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Abstract | Virus structures are megadalton nucleoprotein complexes with an exceptional variety of protein–protein and protein–nucleic-acid interactions. Three-dimensional crystal structures of over 70 virus capsids, from more than 20 families and 30 different genera of viruses, have been solved to near-atomic resolution. The enormous amount of information contained in these structures is difficult to access, even for scientists trained in structural biology. Virus Particle Explorer (VIPER) is a web-based catalogue of structural information that describes the icosahedral virus particles. In addition to high-resolution crystal structures, VIPER has expanded to include virus structures obtained by cryo-electron microscopy (EM) techniques. The VIPER database is a powerful resource for virologists, microbiologists, virus crystallographers and EM researchers. This review describes how to use VIPER, using several examples to show the power of this resource for research and educational purposes.

ASYMMETRIC UNIT

The asymmetric unit of an icosahedral virus structure is defined as the smallest part of the structure from which the complete structure of the virus can be built using a specific set of 60 rotational matrices that describe the 5:3:2 symmetry of the virus particle.

A wealth of virus-structure information that is useful to virologists can be derived from the coordinates of high-resolution icosahedral virus structures deposited in the Protein Data Bank (PDB)¹. However, it can be a challenging task — even for experienced structural biologists — to obtain information such as protein–protein interactions that requires multiple ASYMMETRIC UNITS of the symmetric particles. This need led to the creation of Virus Particle Explorer (VIPER)², a web-based information centre that placed all the virus-subunit coordinates deposited in the PDB into a single standard orientation. This enabled easy comparisons among the structures and facilitated the subsequent development of visualization and computational tools to deal with icosahedral nucleoprotein particles. The visualizations and derived structural information for each virus are organized to help students and researchers understand the details of virus structures.

A first-time visitor to VIPER is led through the details of the various aspects of the site by clicking the ‘First Time Users’ link available from every page of the website. One can enter the world of viruses with the display of a gallery of user-selected individual

virus structures or a family of virus structures by visiting the ‘Visual VIPER’ tool from the ‘Utilities/Tools’ section of the VIPER main menu. A researcher can use the same tool to compare these viruses at various levels of protein-subunit organization in their capsid shells. Similarly, a beginner in structural virology can use the tool ‘Icosahedral Server’ as an informative means for building paper models of quasi-equivalent virus capsids. The same location provides an explanation of the geometric principles of quasi-equivalence theory and its application to larger virus capsids.

After characterizing a new virus and assigning it to a family based on genome-sequence similarity, an investigator can use VIPER to determine whether structural similarities exist with related viruses in the database. For example, a virologist working on the biological aspects of a new picornavirus can gather information on all the related structures belonging to the *Picornaviridae* from VIPER by first visiting the ‘Name Index’ page and listing the viruses by family, or by using the search tool ‘Find a Virus’ to access the structures of interest. The user can then visually compare this same set of structures with the ‘Visual VIPER’ tool to study

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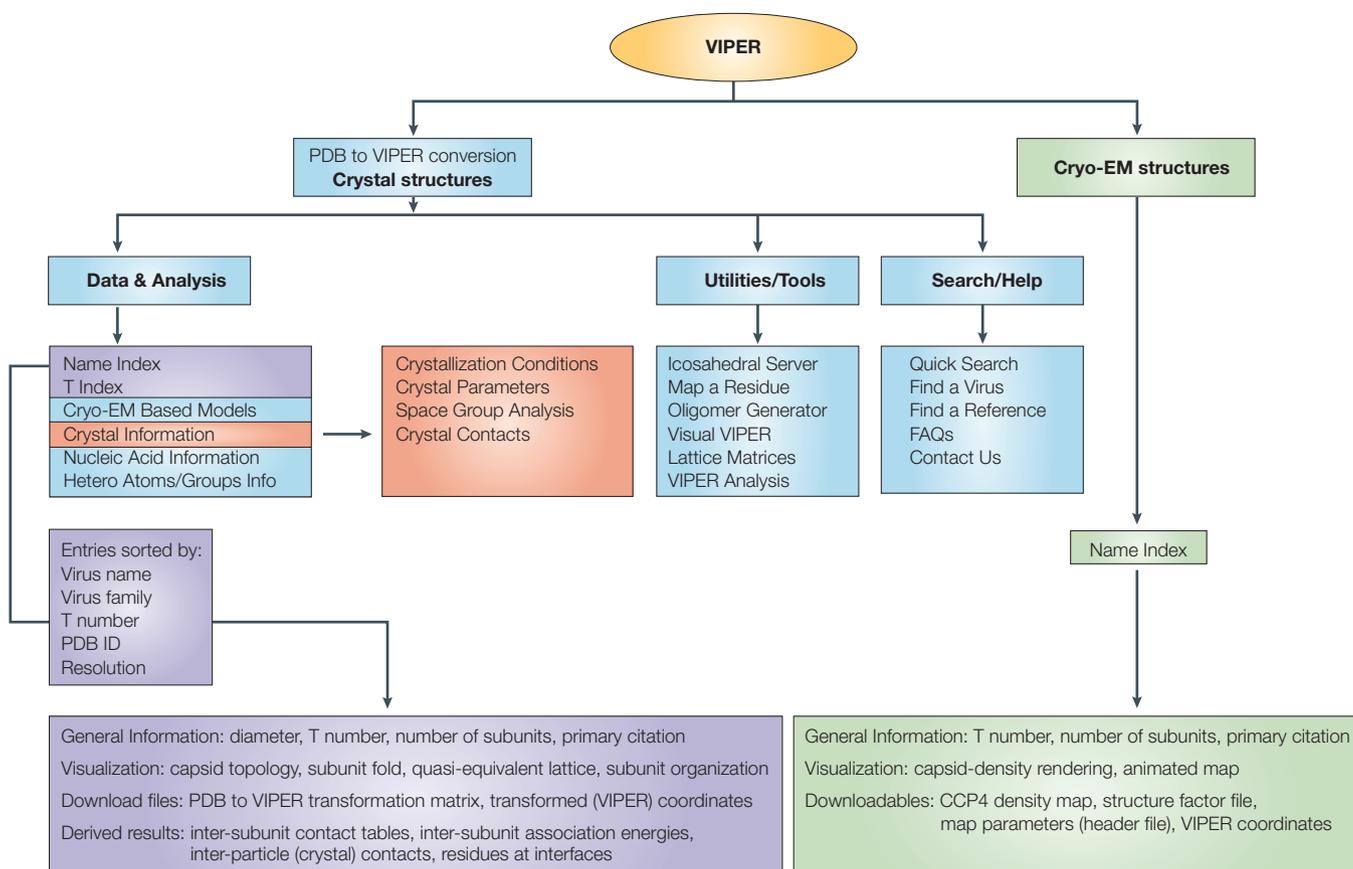


Figure 1 | **Flow chart of the VIPER website organization.** The primary data held in VIPER are the atomic coordinates of virus structures obtained by X-ray crystallography. The low–medium-resolution virus structures obtained by cryo-electron microscopy (cryo-EM) and three-dimensional reconstruction techniques are currently being introduced into VIPER. CCP4, collaborative computational project number 4; PDB, Protein Data Bank; T number, triangulation number.

the topological features, details of the subunit fold and CAPSOMERES. Crystallization conditions for related viruses can be found under the ‘Crystal Information’ page, which can provide a starting point for the user to design crystallization protocols for the new virus. If a study of the assembly/disassembly properties of this virus is being addressed using mutational analysis and if a reasonable sequence alignment with related viruses exists, then the location of residues suitable for mutation can be determined with the tool ‘Map a Residue’. Further, these residues could be analysed for their contribution to the stability of specific interfaces by examining the association-energy calculations presented for each of the related structures. If coordinates for the new virus can be obtained by homology modelling, using sequence alignment with a closely related known structure in VIPER, a possible structure for the whole virus particle can be generated by uploading the modelled coordinates into the ‘OLIGOMER Generator’ tool of VIPER. Indeed, structural virologists and crystallographers can submit the coordinates of newly solved virus structures for VIPER analysis through a web-based form that leads to creation of an individual web page for the virus, with links to visualizations and derived information. A page generated for a user is accessible only to the user.

In addition to analysing the existing high-resolution crystal structures, VIPER now includes a selected set of low–medium-resolution virus structures obtained using cryo-electron microscopy (cryo-EM) techniques. This doubles the number of structures in VIPER and includes structures of viruses that are too large to crystallize, particles of which the morphology changes at different pH values or ionic strength, structures of intermediates in the pathway of virus assembly, as well as viruses complexed with receptors, antibodies or chemically attached proteins (BOX 1).

VIPER provides a unique opportunity to compare virus structures obtained by two independent experimental techniques — cryo-EM and crystallography. While structures obtained by crystallographic methods have details at atomic resolution, there are limitations to the size of the virus particles that can be studied by this method, and to the homogeneity and amounts of virus samples that are required for crystallization. By contrast, recent advances in obtaining and analysing cryo-EM data on virus structures has not only been possible at sub-nanometer resolutions, but also provides the necessary information to obtain a range of virus structures represented by different populations present in non-homogeneous virus samples.

CAPSOMERES

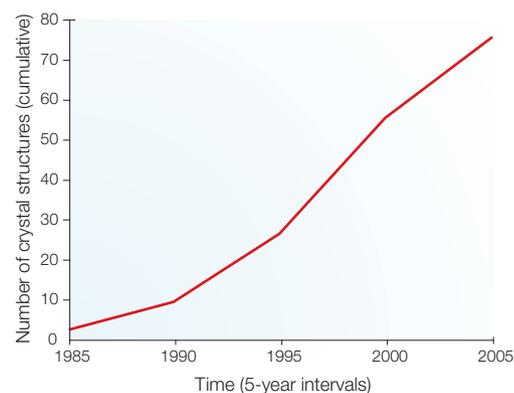
The obvious surface features of the virus particle, which are observable in an electron-microscopy-reconstructed density.

OLIGOMER

The representation of the protein subunit on the viral capsid as dimer, trimer, pentamer, hexamer and so on.

Box 1 | Database architecture

VIPER was recently upgraded from a static HTML site to a dynamically driven site in which all the pages are created 'on the fly' as users browse the site. All of the data presented in VIPER is stored in an underlying relational database using MySQL, which is retrieved and presented using server-side web scripting tools such as PERL (practical extraction and report language), hypertext preprocessor (PHP) and JAVA. The database currently holds 62 unique virus structures — not including the structures of serotypes, mutants and viruses complexed with small ligands — from 21 families and 30 different genera of viruses, all described in the single virus orientation (see the table). The growth of the field of structural virology is clear when examining the graph showing 75 of the crystal structure entries in VIPER (see the figure). Further renovation of the VIPER site will soon incorporate a more extensive scope of the viral structures found in the Protein Data Bank, more than doubling the number of unique entries contained in VIPER. The structural data, such as atomic coordinates, and derived results, such as inter-subunit residue-residue contacts, that are currently present as text files on the web server will also be stored in the relational database, which is expected to increase the usefulness of VIPER.



Database	Total entries	Crystal structure entries	Models based on EM maps	Cryo-EM structures
VIPER	86	62 (unique entries)*, 13 (derived entries)†	6 (unique entries)*, 5 (derived entries)†	N/A
VIPER EMDb	41	N/A	N/A	25 (unique entries)*, 16 (derived entries)†

*Unique entries are the main virus entries of VIPER that exclude derived structures. †Structures such as those of serotypes, mutants and viruses complexed with small ligands. EM, electron microscopy; N/A, not applicable, VIPER, Virus Particle Explorer; EMDb, cryo-EM database.

VIPER provides a wealth of material beyond these few examples — figures, derived results, transformed coordinates, density maps — and in the following sections a detailed description of this tool is presented, together with descriptions of the individual virus pages, utilities, tools, calculations and other features.

VIPER organization and standards

There are currently four main features of the VIPER website: the structural data and analyses, the crystallographic information, the utilities that are provided by VIPER, and a set of search functions and help sections (FIG. 1; BOX 1). A complete list of the structures found in the data section of VIPER can be accessed in several ways, including using a sorted list of the virus names; using a sorted list of virus families; by virus-particle symmetry, which is described using T (TRIANGULATION) NUMBERS; or by displaying the resolution of the different viral crystal structures. The viruses listed are all linked to individual entry pages that detail specific information for each virus as well as links to useful tools and downloadable files. Most of the structure files in the database are obtained from the PDB¹. PDB coordinates are transformed and stored in a single standard orientation of the virus particle (FIG. 2), enabling the user to readily compare structures and to automate computations.

The standard particle orientation used in VIPER aligns mutually perpendicular two-fold axes of an ICOSAHEDRON with the three orthogonal axes of a Cartesian coordinate system. Icosahedral five-fold and

three-fold axes lie in the X-Z plane, so that the order of axes going from Z to X is Z(2), 3, 5, X(2). Likewise, a standard icosahedral asymmetric unit (one of 60 for an icosahedron) is always used to store the unique coordinate set, so that oligomeric structures can be readily generated through 'point and click' by the user.

Individual VIPER entry pages

Structural data for each virus can be found on the individual VIPER entry page (FIG. 3), together with links to crystal-related information and features that allow the user to explore the structures. Data available for each virus structure include a resume (or summary) of the structure, downloadable files, derived results, visualization and a list of related structures.

The resume describes the level of resolution of the virus structure, the virus family name, the T number, the number of subunits that form the virus capsid, the diameter of the particle, together with links to the PDB, [Swiss-Prot](#) sequence database³ and [Entrez PubMed](#) descriptions and abstracts.

The transformed structural coordinates in the VIPER standard orientation and the asymmetric unit can be downloaded from the individual virus pages, together with the matrix that was used to convert these coordinates from PDB to VIPER convention. The transformed coordinates are useful for users that are carrying out analyses that are beyond the scope of VIPER, or for users that wish to use graphical features in display programs that are not supported by VIPER.

T (TRIANGULATION) NUMBER

The theoretical basis for the structure of isometric viruses was described by Caspar and Klug with their concept of identical elements in quasi-equivalent environments. They defined all possible polyhedra in terms of structure units. The icosahedron itself has 20 equilateral triangular facets, and therefore 20 T structure units, in which T is the triangulation number given by the rule $T = Pf$, in which P can be any number of the series 1, 3, 7, 13, 19, 21, 31 and so on ($= h^2 + hk + k^2$, for all pairs of integers, h and k having no common factor) and f is any integer.

ICOSAHEDRON

A polyhedron comprising 20 equilateral triangular facets and 12 vertices that has rotational symmetry described as 5:3:2 symmetry. There are six 5-fold axes of symmetry passing through the vertices, ten 3-fold axes extending through each face and fifteen 2-fold axes passing through the edges of an icosahedron. The VIPER database only holds data for icosahedral virus structures.

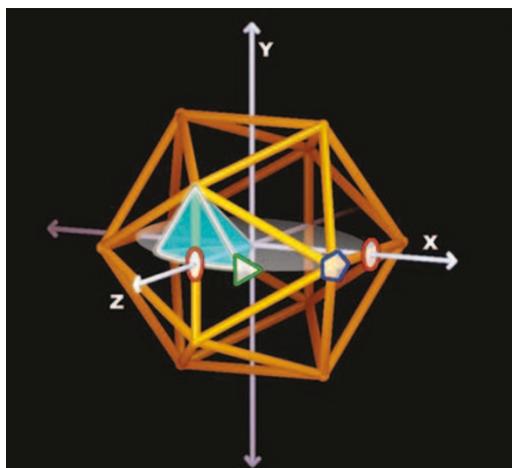


Figure 2 | Standard orientation of virus particles in the VIPER database. The three mutually perpendicular two-fold axes of the virus particles (represented as an icosahedral cage) coincide with the Cartesian X, Y and Z axes, and the particle icosahedral five-fold and three-fold axes lie in the X-Z plane in the following order: Z(2), 3, 5, X(2), as shown by an oval (two-fold), a triangle (three-fold) and a pentagon (five-fold). The standard asymmetric unit for storing the unique coordinates is defined by the wedge bordered by three planes. The first is the Z-X plane. The second intersects the origin, the five-fold axis closest to Z in the Z-Y plane and the three-fold axis between Z and X. The third intersects the origin, the same five-fold axis as plane 2 and the three-fold axis between Z and -X. The volume defined is shaded with these planes in the figure.

Derived results that a user might generate include protein–protein (inter-subunit) and protein–nucleic-acid interactions that are displayed in the form of contact tables. Residue–residue contacts are classified as hydrophobic–hydrophobic, polar–polar, polar–hydrophobic or basic–acidic. Quantitative descriptions of interactions for each unique interface, calculated using the molecular mechanics program **CHARMM**⁴, are stored separately. Inter-subunit association energies (calculated based on the atomic buried surface areas and solvation parameters⁵) and buried surface areas for the unique interfaces, along with the residues contributing the most stability to the interfaces, allow users to assess the relative strengths of viral interfaces. These data can be used to help the user to hypothesize about assembly pathways in viruses⁶.

Visualization

Owing to the complexity of virus capsid structures, their graphical visualization is an important aspect of VIPER and is achieved through both static and interactive representations. The use of these graphical interfaces assumes no prior experience with molecular display programs, and they are organized so that the user can generate publication-quality figures directly from the site. A series of static representations of the entire virus capsid are presented in the upper left-hand corner of the images section on an individual virus entry page (FIG. 3). With the aid of the molecular modelling system **Chimera**⁷, the arrangement of the discrete virus subunits and their interactions in the

capsid are clearly delineated through a surface-rendered representation, which can be viewed at different levels of resolution. The particle can also be visualized as a cutaway, which exposes the inside of the capsid. Last, the complete particle can be viewed with a single asymmetric subunit represented as a ribbon drawing that is superimposed onto the particle. A **GRASP**⁸ (graphical representation and analysis of structural properties) rendering of the capsid is also shown, in which colours are depth-cued along a monochromatic spectrum. Other static images include schematic representations of virus-particle geometry, subunit structure and subunit organization.

Among the interactive representations available on VIPER is a PDB viewer that displays a C- α TRACE of the viral asymmetric unit coloured by chain, which uses the java-based program **WebMol**⁹ (developed by D. Walther). The user can rotate, translate and zoom in on the virus molecule, provided that their web browser is java-enabled. Double-clicking on the WebMol thumbnail launches a more extensive version of the viewer, with additional graphical features and tools. The WebMol thumbnail will load automatically when a user visits an individual virus page, but the user has the ability to disable this feature by selecting the ‘Disable WebMol Displays’ button. This prevents the automatic loading of the asymmetric unit thumbnails, allowing speedier browsing of VIPER. To enable the WebMol thumbnails, the user should simply click on the ‘Enable WebMol Displays’ button. VIPER provides links to another java-based program, **QuickPDB** (developed by I. Shindyalov and P. Bourne). This launches a window with a C- α trace line rendering of the asymmetric subunit that is interactive and is dynamically tied to the subunit sequence. Visualization from individual virus pages can also be done using NCBI’s **Cn3D** web plug-in¹⁰ and MDL Chemscape’s **Chime** plug-in. Additional capsid-visualization tools are discussed in the Utilities/Tools section.

Crystal information

The crystal information pages have several roles. They provide the details of crystal growth conditions for all of the VIPER entries, enabling the user to examine the chemical environment in which the structure was determined. These data can help crystallographers to devise starting conditions to crystallize viruses for the first time. The information contained in these pages was extracted from the published literature and it is displayed together with the relevant Entrez PubMed entry. The ‘Crystal Info’ page contains crystallographic information such as unit cell parameters, space group, Z value (number of virus particles per unit cell), R-factor (a measure of the level of disagreement between observed and calculated structure factors) and the resolution (in Å) of the virus structures. These values, in conjunction with the information presented in the ‘Space Group’ pages (which contain statistics on the frequency distribution of virus structures in various packing configurations), can be useful in

C- α TRACING

A simplified representation of the tertiary structure of the subunit, in which a C- α atom represents each residue and the subsequent C- α atoms are connected by a line.

PREV **Black Beetle Virus** **NEXT**

Resume: <ul style="list-style-type: none"> PDB-ID: 2BBV Resolution: 2.80 Å Swiss-Prot: P04329 Family: <i>Nodaviridae</i> Genus: <i>Alphanodavirus</i> T Number: 3 # of Subunits: 180 Diameter (Å): Ave: 324 Max: 346 <ul style="list-style-type: none"> Crystal Information Primary citation References 	Computations: <ul style="list-style-type: none"> Quasi-Equivalence Contact Tables Association Energies Residues at the interfaces How were these computed? 	Download files: <ul style="list-style-type: none"> PDB Header PDB to Viper matrix VIPER Coordinates Construct an Oligomer of this Virus Map a residue on this Virus
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Click on the following images to enlarge:
[click here for information about these graphics](#)

Figure 3 | **Individual VIPER entry page for black beetle virus.** This page shows the resume, computational results, download files and visual representations for the virus structure. Derived results organized by the quasi-equivalent surface lattice of the virus allow for straightforward comparisons of quasi-equivalent interfaces. These include contact tables that list residue–residue interactions at protein interfaces classified by interaction types (for example, polar–polar, salt bridges, hydrophobic–polar and so on), association energies and buried surface areas for the protein–protein and protein–nucleic-acid (if present) interfaces, along with a list of residues that contribute significantly to the association energies of interfaces. Visualization of the virus structure is at various levels of organization, including the topology of the virus particle, a schematic diagram showing virus-particle quasi-symmetry, the tertiary structure of the subunit and the subunit organization of the viral capsid. The complete virus particle can be visualized at low, medium or high resolution, in addition to a cutaway inside view. Dynamic viewing of the asymmetric unit of a virus particle can be achieved with the tools WebMol and QuickPDB. Figures and data presented at VIPER are free for downloading and for use in publications or presentations, provided the citation to the VIPER publication² is given.

CAGE
The geometric representation of the icosahedral symmetry of a virus particle.

VRML
Virtual reality modelling language used in three-dimensional viewing of the tertiary structure of subunits in the VIPER tool 'Map a Residue'.

assessing the possible space group of virus crystals obtained by a user, which might be ambiguous at an early stage of study. More importantly, the space-group assignment for a given virus crystal structure not only shows which regions of virus particles interact and how these interactions are propagated in the crystal lattice, resulting in the crystal formation, but it has also been observed to represent biologically active regions on viral surfaces in some virus structures¹¹. The 'Crystal Contacts' page uses a program written by

P.N. and J.E.J.¹¹ to specify residues that are involved in inter-particle protein–protein contacts in the crystal lattice, and the data are displayed both graphically and numerically as buried surface area resulting from crystal contacts. During the crystallization of mutant viruses under similar crystallization conditions as the wild-type virus, crystal-contact information can help to determine whether the mutant is likely to crystallize isomorphously to the wild type. These pages can be accessed from the main VIPER menu, but the data for a particular virus can be accessed from the individual virus web pages.

Genome structure

Some virus particles, such as **flock house virus**, contain ordered portions of their genome. These nucleic-acid regions obey the icosahedral symmetry of the capsid, and coordinate models can be determined. VIPER has annotated and displayed the nucleic-acid structure (see 'Nucleic Acid Information' section under the main menu item 'Data') for those viruses that have ordered regions of the genome in their crystal structures. A stereo view of the CAGES that are based on the surface lattices with the ordered portions of nucleic acid superimposed on them is provided for each of the viruses listed. In addition, there is a summary of the 'Hetero Atoms/Groups', which describes water molecules, nucleotides, chemical compounds and metal ions that are associated with the virus crystal structures.

Utilities/tools

This section is designed to help understand and analyse virus structures in a user-friendly environment, for beginners as well as experienced researchers. VIPER tools include 'Icosahedral Server', 'Map a Residue', the 'Oligomer Generator' and 'Visual VIPER'.

The 'Icosahedral Server' (developed by C. Qu) helps users to understand icosahedral symmetry and the rules of quasi-equivalence^{12,13} that dictate virus-particle geometry. The surface lattice symmetry of a virus particle can be described with a T number ($T=h^2+hk+k^2$, in which h and k are integers). T numbers indicate the number of subunits in the icosahedral asymmetric unit, and the corresponding surface lattices can be examined with three-dimensional paper models made by folding templates downloaded from the 'Icosahedral Server' web page.

The VIPER tool 'Map a residue' helps the user to identify the location(s) of any user-specified residue(s) in a structure (FIG. 4). The result is an image of an asymmetric subunit in the ribbon rendering of the structure, with specified residue(s) highlighted as purple CPK (Corey–Pauling–Koltun) models. This also links to a view of the image in three dimensions with a VRML (virtual reality modelling language) plug-in. This tool is particularly useful for virologists that are undertaking mutational analyses of a virus, as shown in the example presented in FIG. 4. The residues of interest can be analysed for their participation in inter-particle contacts in the crystal by looking at

Map a Residue



This tool will display a given residue on the chosen Virus Subunit. The resulting image will be a ribbon rendering of the asymmetric subunit, with the desired residue given as a CPK representation colored purple.

(if the entered residue is not found within the subunit, the result will only display the asymmetric subunit)

• Pick a Virus/Capsid

- Bacteriophage PRD1 SUS607 mutant model (T=pT25) - 1gw8
- Bacteriophage PRD1 Wt Model (T=pT25) - 1hb9
- Bacteriophage PRD1 Wt Model (T=pT25) - 1gw7
- Bacteriophage Qb (T=3) - 1qbe
- Bean Pod Mottle Virus (T=pT3) - 1bmv
- Black Beetle Virus (T=3) - 2bbv**
- Bluetongue Virus (T=13) - 2btv
- Bovine Enterovirus (T=pT3) - 1bev
- Brome Mosaic Virus (T=3) - 1js9
- Canine Parvovirus (CPV) Empty (T=1) - 2cas

• Enter Residue Numbers of Interest:

Warning: chain ids should be specified, if and when present along with the residue numbers (e.g. a265, b280, c310). We suggest that you refer to the Transformed coordinates in the VIPER page of the entry for this information

A207

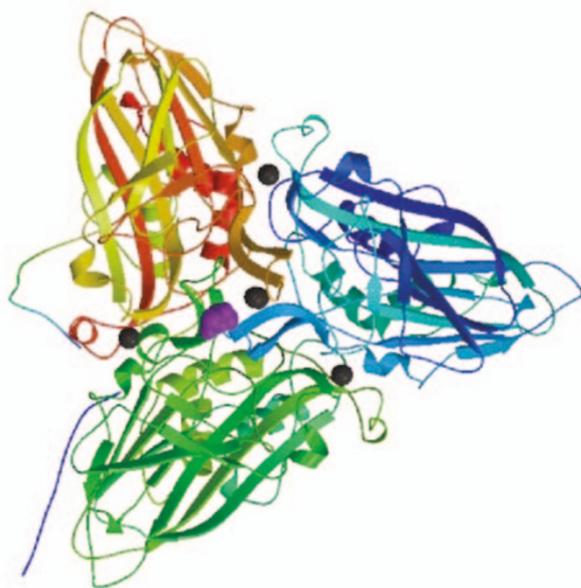


Figure 4 | The ‘Map a Residue’ tool of VIPER. This tool can be used to identify the location(s) of the chosen residue(s). The user-specified residue A207 of black beetle virus is displayed as a purple CPK (Corey–Pauling–Koltun) model on the tertiary structure of the A subunit of the virus. The crystal contacts page for this virus shows that the residue A207 participates in the inter-particle contacts in the crystals. It is therefore possible that any mutation to this residue could potentially interfere in forming the virus crystals in the space group ($P4_232$) of the original entry.

the buried-residue list provided for many of the virus structures in the VIPER database. If the residues are present at the inter-subunit contacts, their contribution to the specific protein interface can be found in the association-energies section of the individual virus web pages.

The ‘Oligomer Generator’ is a frequently used tool in the VIPER utilities. It allows users to create a coordinate file of a specific viral oligomer (FIG. 5). It is also possible to upload the coordinates of an asymmetric unit of a virus that is not in the VIPER database for personal use, provided that the coordinates conform to the VIPER convention and that the T number is known. This might be useful for examining preliminary structural information as it becomes available to the user. The ‘VIPER Analysis’ utility provides a web-based deposition form for structural analyses of user-supplied virus coordinates. These ‘personal’ VIPER pages are secure and are not accessible to other site users.

Users are shown a labelled cage that corresponds to the data input for the virus of their choice, from which they specify the asymmetric units to be used in the construction of the oligomer. The option of quickly constructing a 1/4, 1/3, 1/2, 2/3 or whole capsid is also provided through the use of a simple pull-down menu. The ‘Oligomer Generator’ calculates and displays the coordinates for the given portion or the whole virus capsid structure in a format that can be downloaded, as well as providing the matrices used for the calculation of the oligomer. Symmetry matrices listed under ‘Utilities/Tools’ provide the sixty matrices that generate the icosahedral virus particle in the VIPER standard orientation. The ‘Oligomer Generator’ tool uses these matrices to generate parts/whole of the virus particle. If the option was selected, a moveable thumbnail rendering of the final oligomer is shown (see FIG. 5, in which a pentamer of the A subunits of **black beetle virus** is generated). The tool requires the user to provide an e-mail address, so that the URL for downloading the generated coordinates can be sent to the user.

‘Visual VIPER’ is a tool that can be used to compare viral properties such as topology, subunit structure, capsomer organization and inter-particle (crystal) contact regions among a set of viruses (FIG. 6). Although all of the viruses in the database are listed individually, they can also be grouped by family or individual selection to enable the user to quickly create a custom gallery of viral properties. This tool has frequently been used by virologists to generate figures that are useful for teaching purposes.

Search functions

Locating a particular virus entry or group of viruses from a list can be tedious. For this reason, VIPER provides a search function for quick retrieval of virus information. In addition to the virus-entry pull-down menus and a PDB ID query box at the top of every page, VIPER contains a more refined search engine that can locate viruses based on user-defined characteristics. The ‘Find a Virus’ page allows the user to enter a set of criteria by which to search the database, as specific or broad as necessary, resulting in a list of entries that fit the search conditions. Search parameters include virus name, family, genus, resolution, T number, space group, crystal system and model

Oligomer Generator

[Click here for Detailed Instructions](#)

(1) Pick a capsid from the VIPER database:

Bacteriophage PRD1 Wt Model (T=pT25)
 Bacteriophage PRD1 Wt Model (T=pT25)
 Bacteriophage Qb (T=3)
 Bean Pod Mottle Virus (T=pT3)
Black Beetle Virus (T=3)
 Bluetongue Virus (T=13)
 Bovine Enterovirus (T=pT3)
 Brome Mosaic Virus (T=3)

-OR- upload your own PDB
(WARNING: coordinates must be in the VIPER convention)

(2) Would you like to generate complete/part(s) of the particle?

(if you have chosen to generate full/part of particle jump to steps (5) & (6) and click on **SUBMIT**)

(3) Select the T number of your virus/capsid.
(Select T=1 for pT2, pT3, pT25, pT169 structures)

(4) Select the SUBUNIT and MATRIX identifiers
(based on the numbers in the adjacent DIAGRAM e.g. C1,B6,C7,B8,C9,B5 OR simply 1, 2, 3, etc.)

(5) Use only the C-alpha atoms?
 Yes No

(6) Display graphical preview?
 Yes No

(7) Enter your e-mail address: (Mandatory)
* You will receive an e-mail describing how to access the results. We respect your privacy and will NOT share your address with any third party.*

Figure 5 | The ‘Oligomer Generator’ tool of VIPER. This example shows the generation of a pentamer formed by the A subunits of black beetle virus using the ‘Oligomer Generator’ tool of VIPER. Each virus structure includes a schematic cage diagram of the arrangement of subunits in the capsid that is displayed and can be used in specifying the portion(s) or whole of the viral capsid that the user seeks to produce. The user is notified by e-mail with a link to download the generated coordinates.

type (X-ray or cryo-EM). Although the user is not required to enter a search value for all the fields, any of the fields can be used in conjunction with another, creating a query that suits the needs of the user. In this manner, the user can create a customized list of viruses that share a common set of traits for analysis.

The EM database

The VIPER database recently expanded and now includes virus structures that have been obtained by cryo-EM and three-dimensional reconstruction techniques in addition to the existing X-ray crystal structures of viruses. Cryo-EM is an important technique that is used to examine large macromolecular structures, often at sub-nanometer resolution. Virus particles have frequently been the subject of cryo-EM studies because their symmetry helps the structure determination. Such structures provide morphological detail for viruses. Moreover, fitting atomic-resolution models to the cryo-EM density maps to generate a pseudo-atomic-resolution structure often increases their utility. Cryo-EM structures and pseudo-atomic models are particularly useful tools with which to follow changes in virus particles during their maturation or during interactions with the cellular receptor.

Currently, the VIPER cryo-EM database (**VIPER EMBD**) holds electron densities for 25 unique virus structures — excluding structures of serotypes, mutants, viruses grown from different expression systems, full and empty forms of virus or assembly intermediates — from 15 different virus families (see the table in BOX 1). These EM density maps, obtained from four different laboratories, do not conform to a single orientation or to a common map format. The orientation and map formats were standardized when the maps were submitted to the VIPER database. The CCP4 (collaborative computational project number 4) electron-density map format¹⁴, transparent to many computer platforms and easily converted to the different map formats used by the EM community, was selected for use as the VIPER standard. The orientation of virus particles described by these maps is the same as those of the crystal-structure coordinates (in VIPER) as indicated in FIG. 2. Individual virus web pages were created similar to the X-ray structures of VIPER. Each of these entry pages provides a summary of the virus name and family, T number, number of subunits and map resolution (FIG. 7). Downloads include a CCP4 format electron-density map along with a text file named Header, containing the map descriptors, number of grid points along the three axes of the map, the pixel size, the resolution of the structure and the transformations done on the original map while converting it to the VIPER conventions, among other properties. A text file containing the structure factors computed using the electron density can also be downloaded so that a user can generate a density map with their own Fourier synthesis program. A set of coordinates of any models that have been fitted into the density map, such as virus antibody or virus receptor complexes, can also be downloaded. Links are provided to the primary citations of these structures in PubMed and also for closely related structures that are available in the cryo-EM and X-ray databases of VIPER. The visualization programs Chimera⁷ and PyMol¹⁵ are used in the depth-cued surface rendering and animations of the density maps. Existing tools, including ‘Visual VIPER’, can be used to compare the cryo-EM and X-ray structures.

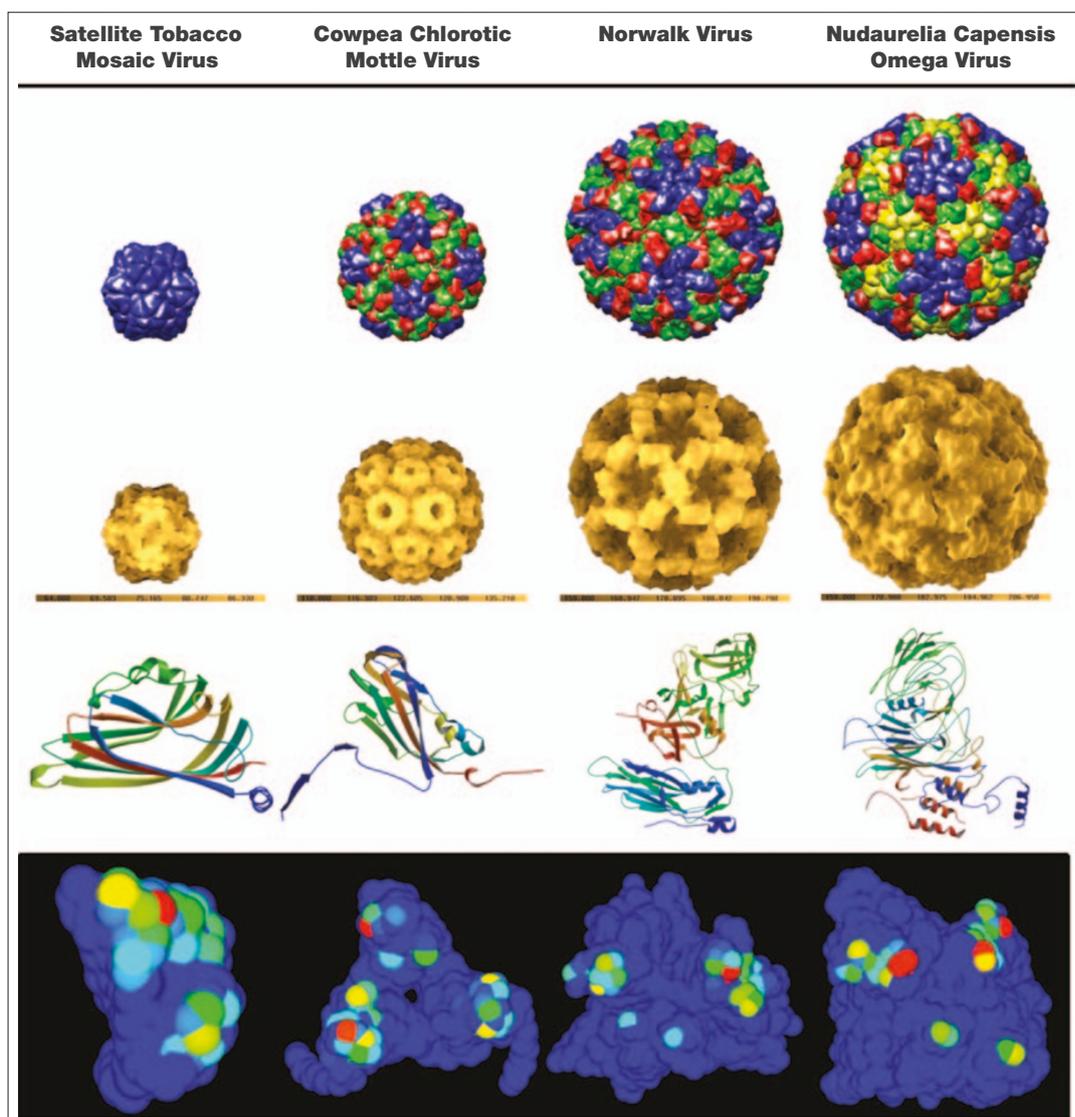


Figure 6 | **The 'Visual VIPER' tool.** This figure reveals the comparative features that are available in VIPER using the tool 'Visual VIPER'. The viruses shown (left to right) are viruses with triangulation number (T)=1 (satellite tobacco mosaic virus), $T=3$ (cowpea chlorotic mottle virus), $T=3$ (Norwalk virus) and $T=4$ (Nudaurelia capensis ω virus). From top to bottom are shown a 5-Å-resolution rendering of the subunit coordinates, a radial rendering view of the particle, the ribbon representation of the subunit, and inter-particle contacts in crystals mapped onto the asymmetric unit of the virus particle.

The EM database includes a deposition form for uploading new structures that includes all of the data that are needed to create the virus page and the various downloadable pages. Our long-term goal is to have all published cryo-EM virus structures deposited in VIPER and to have newly determined cryo-EM virus structures released in VIPER simultaneously with publication. Tools that will tie the EM and X-ray databases more closely are currently under development.

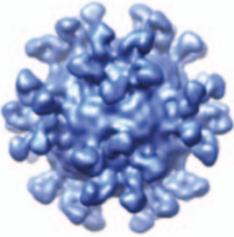
Summary

All of the virus atomic coordinates in VIPER conform to a specific standard icosahedral orientation that allows easy global comparisons among viruses and calculation of derived information. Each viral capsid

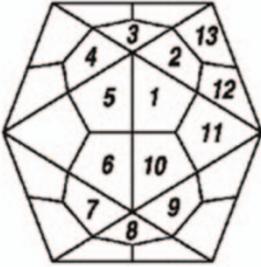
structure in the VIPER database is represented pictorially and with an extensive computational analysis highlighting inter-subunit residue contacts, binding energies, quasi-equivalence and proposed assembly pathways. VIPER also provides a series of web-based tools that allow users to visualize and explore the data provided, and to generate a coordinate file of the entire viral capsid or a specified portion of it. The user can visually compare specific properties among a chosen set of virus structures and can identify the location of specified residues on the capsid. VIPER is currently expanding its scope to include the electron-density maps that are available for low-medium-resolution virus structures obtained by cryo-EM techniques that will complement the existing high-resolution crystal structures in the database.

Human Rhino Virus 14 (Fab1)

<p>Resume:</p> <ul style="list-style-type: none"> • VIPER-ID: em_1hrc • Resolution: 29 Å • Source: • Family: Picornaviridae • T Number: 1 • # of Subunits: 60 • Primary citation 	<p>Download files:</p> <p>Header Density Map (CCP4 Format) Structure factors (Plain Text Format) VIPER Model Coordinates</p>	<p>Related Structures:</p> <p>EM entries Human Rhino Virus 14 (D1 D2 ICAM; em_1hrc) Human Rhino Virus 16 (em_2hrc) Human Rhino Virus 16 (D1 D2 ICAM; em_2hrc) X-Ray entries Human Rhinovirus 14 (4fvy) Human Rhinovirus 15 (1aym) Human Rhinovirus 2 (1fnp) Human Rhinovirus 1A (1rtal) Human Rhinovirus 3 (1fhu)</p>
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Rendered surface



T = 1 Lattice

<p>Visualization:</p> <p>Animated GIF</p>	<p>Related Links:</p> <p>EMAN SPIDER Image Science's em2em</p>
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Figure 7 | **Sample page for an electron-microscopy entry in VIPER.** Shown here is the web page for Fab1 attached human rhinovirus 14. It includes a resume for the structure; download files such as a CCP4 format electron density map together with a header file containing map parameters, a flat text file of structure factors for reconstructing the density and file containing the coordinates modelled into the density; links to related X-ray and cryo-electron-microscopy structures; figures showing density rendering and a schematic cage diagram representing the virus-particle symmetry; and an animated figure showing the density map in three dimensions.

The VIPER database, which houses visualization and analysis tools for virus structural data obtained using two different experimental techniques, will be a powerful centre for virologists, microbiologists, crystallographers and EM researchers.

VIPER – the future

The VIPER database is entering a new phase with the inclusion of residue-level and atomic-level details that will enable users to explore structures in more depth. This includes organization of all the information (primary and derived) in a MySQL database format for interrogation by more sophisticated queries than can currently be used. Such a database allows the examination of multiple parameters simultaneously for comparative purposes. For example, the relationship between the presence of proline residues adjacent to protein–protein interactions of high affinity was shown by searching for the strongest protein–protein interactions in buried surfaces areas, followed by a search of all amino acids within 5 residues on either side of the point of interaction. Prolines were present at a significantly higher frequency than other amino acids. It was more straightforward to carry out this search for all of the viruses in the database with the MySQL format than would have been possible with earlier formats.

VIPER will continue to be a central web resource for virus-structure information obtained using X-ray crystallography and cryo-EM techniques, annotated with visual and derived results, and containing tools to search, construct and compare structures. The database is updated weekly and new features are added as requested by users and when a need is identified. Therefore, this excellent community resource should benefit a wide range of users.

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Competing interests statement

The authors declare no competing financial interests.

Author's note

Any information on the VIPER website can be freely downloaded and simply requires the citation of the original paper: Reddy, V.S., Natarajan, P., Okerberg, B., Li, K., Damodaran, K.V., Morton, R.T., Brooks, C.L. III & Johnson, J.E. Virus Particle Explorer (VIPER), a website for virus capsid structures and their computational analyses. *J. Virol.* **75**, 11943–11947 (2001).

Online links

DATABASES

The following terms in this article are linked online to:
Entrez: <http://www.ncbi.nlm.nih.gov/Entrez>
 black beetle virus | flock house virus

FURTHER INFORMATION

Jack Johnson's laboratory: <http://lav.scripps.edu>
VIPER: <http://mmts.scripps.edu/viper>
CHARMM: <http://www.charmm.org>
Chime: <http://www.mdl.com/products/framework/chime>
Chimera: <http://www.cgl.ucsf.edu/chimera>
CN3D: <http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>
Entrez-PubMed: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>
GRASP: <http://trantor.bioc.columbia.edu/grasp>
PDB: <http://www.rcsb.org/pdb>
PyMol: <http://pymol.sourceforge.net>
QuickPDB: <http://pdobos.sdsc.edu/applet/QuickPDB.html>
SwissProt: <http://www.expasy.ch>
VIPER EMBD: http://mmts.scripps.edu/viper/em_index.php
WebMol: <http://www.cmpfarm.ucsf.edu/~walther/webmol.html>
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