cellPACK: a virtual mesoscope to model and visualize structural systems biology

Graham T Johnson1–3, Ludovic Autin1, Mostafa Al-Alusi1, David S Goodsell1, Michel F Sanner4 & Arthur J Olson1

CellPACK assembles computational models of the biological mesoscale, an intermediate scale (10–100 nm) between molecular and cellular biology scales. CellPACK’s modular architecture unites existing and novel packing algorithms to generate, visualize and analyze comprehensive three-dimensional models of complex biological environments that integrate data from multiple experimental systems biology and structural biology sources. CellPACK is available as open-source code, with tools for validation of models and with ‘recipes’ and models for five biological systems: blood plasma, cytoplasm, synaptic vesicles, HIV and a mycoplasma cell. We have applied CellPACK to model distributions of HIV envelope protein to test several hypotheses for consistency with experimental observations. Biologists, educators and outreach specialists can interact with CellPACK models, develop new recipes and perform packing experiments through scripting and graphical user interfaces at http://cellPack.org/.

The biological mesoscale lies between the cellular and molecular scales at lengths of about 10–100 nm. Except in special cases, atomic details of the mesoscale are generally too small to resolve by microscopy and too large and heterogeneous to determine with methods such as X-ray crystallography and NMR spectroscopy. Methods of cellular tomography provide the most detailed experimental view of this level, with many successes in the localization of larger macromolecules (such as ribosomes) within whole cells1–2. However, an atomic-resolution view of this intermediate scale—in spite of its utility in hypothesis generation, science communication and simulation—remains sparsely modeled and visualized compared to larger and smaller scales of study3–5.

No methods currently exist to observe the mesoscale in atomic-resolution detail; however, many sources of data may be used to synthesize a view of this level. For the past 20 years, we have taken a semi-quantitative approach to integrate these diverse data into a coherent model, creating artistic depictions of cellular environments (Fig. 1a) based on recipes consisting of ultrastructural data from light and electron microscopy, atomic structures from X-ray crystallography and NMR spectroscopy, and biochemical data on concentrations and interactions6–7. More recently, we and other groups8 have developed computational approaches that automate the steps of this semi-quantitative approach to extend the results from two-dimensional (2D) paintings into 3D models that can be explored, animated, simulated, analyzed and easily edited and updated. This process involves two conceptual steps: gathering data to create a recipe for the model and using this recipe to build a virtual model.

As the field of structural biology is advancing quickly, methods to automate the first step of the pipeline, the generation of a recipe based on available data, are essential. This is a challenging goal given the heterogeneous nature of the data, but we have developed automated methods for several key steps. For example, we have created tools to integrate bioinformatics data from sources such as Stanford’s WholeCellViz9 and atomic structures from the Protein Data Bank. Because much of the data that our approaches use require manual curation, we have begun projects to implement these recipes in a way that allows community experts to update and improve the recipes across all scales of detail, to extrapolate predictions where reasonable and to vote with confidence values for all contributing parameters and resulting assemblies.

Given a molecular recipe, the construction of a quantitative 3D mesoscale model requires solving a nontrivial loose-packing problem. In biological systems, this includes packing soluble, membranous and fibrous components with proper localizations and biologically relevant interactions. Packing problems are a popular topic of study in mathematics, engineering and biology. The nonbiological methods are typically limited to simple components such as boxes and spheres10,11 or to providing one non-interacting packing-type solution at a time, such as surface-packing, volumetric-packing or tree-branching algorithms12,13, which can contribute only partial solutions toward recreating the organic complexity of a mesoscale model such as HIV (Fig. 1a,c). Other common nonbiological methods pack non-discrete components that can expand to fill space or contract to avoid overlaps14 or rely on macroscale gravitational forces15,16. Physicists and engineers have applied modified molecular dynamics methods to pack spherical and cylindrical granules17,18; however, molecular dynamics methods operate on a scale too small and computationally expensive to provide a solution for mesoscale packing. Biological constraint modelers such as the integrative modeling platform (IMP)19, procedural and analytical modeling approaches such as Molecular Silverware20,21 and relaxation approaches such

1Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, California, USA. 2Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, California, USA. 3California Institute for Quantitative Biosciences (QB3), University of California, San Francisco, San Francisco, California, USA. Correspondence should be addressed to A.J.O. (olson@scripps.edu) or G.T.J. (graham@grahamj.com).

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as Brownian dynamics modeling\textsuperscript{22,23} are currently able to build large-scale models. However, they are designed to function locally and do not model ultrastructural features, such as organelle membranes, or fibrous molecules, such as actin. For modeling large cellular subjects (up to and beyond tens of micrometers), a hybrid method is needed that combines local methods for populating defined spaces with multiscale methods that integrate ultrastructure and infrastructure while tracking and satisfying all input constraints. Such a framework must further recapitulate the complex interplay of randomness and specific interaction that lies at the heart of biology.

As the data available for biological systems at the molecular level increase in size and complexity, the creation of structurally integrative models of such systems has become a substantial bottleneck in the process of simulation and analysis. An important goal for this work is to automate the creation of these complex mesoscale models so that large numbers of models that are statistically consistent with the known structural and compositional characteristics of a system can be easily generated.

To achieve this goal, we have developed two computational toolkits, autoPACK and cellPACK, for modeling mesoscale content (Fig. 1b,c). autoPACK is a generalized algorithm that provides a heuristic solution to the irregular loose-packing problem and simultaneously integrates regular and procedural packing solutions. autoPACK can be used, for example, to fill an architectural engineering model with concrete aggregate in preparation for earthquake simulations, or it can fill an artery with blood cells at appropriate densities to generate a histological model for a medical illustration. cellPACK is built on autoPACK, optimizing the method for biological data and generating probabilistic 3D models of large sections of cells that can contain dozens to millions of molecules. cellPACK additionally provides tools to store, visualize, analyze and interact with the results to make mesoscale models and mesoscale modeling accessible to a variety of audiences.

To view models, install the software, learn from tutorials or participate in evaluating, editing or initiating models, visit http://cellPACK.org/. The version of cellPACK used for this paper is archived in the Supplementary Software. The latest open-source code and custom scripts used for this study are available at https://github.com/gj210/autoPACK/.

**Figure 1** cellPACK creates 3D models of the cellular mesoscale. (a) A hand-drawn painting of HIV showing three complex packing types—volumetric, surface and procedural (fibrous)—that must interoperate in a mesoscale modeler. (b) autoPACK is a generalized packing algorithm that positions collections of objects (ingredients) into, onto, or outside of volumes to satisfy provided constraints. It operates multiple types of packing algorithms on a single unified model using an efficient global tracking grid. (c) cellPACK is a biological extension of autoPACK optimized to pack molecular structures and other data types into biological volumes. In this image, cellPACK generates an editable model of HIV by packing from a recipe of molecular ingredients\textsuperscript{25} into the ultrastructure of an HIV envelope surface.

**RESULTS**

**autoPACK**

autoPACK is a generalized packing algorithm that defines a desired volume and stochastically packs it with objects, called ingredients, according to a recipe (Fig. 1b). It uses an ‘omniscient’ grid to discretize and describe a volume, which allows multiple types of modular packing algorithms to interoperate on the same model. Ingredients of arbitrary shape are placed into the allowable 3D space with minimal overlap and with random distribution. Constraints and agent behaviors may be associated globally or with specific ingredients to add specific modes of interaction. A grid-based approach is used to generate models quickly while tracking global parameters; however, the packing algorithms explore continuous space within each grid voxel to prevent lattice regularities. In tests, autoPACK could combine several complex packing algorithms to integrate three different major localization modes—volumetric, surface and procedural—into unified models.

The current release of autoPACK can pack ingredient objects of any size and shape into a unified model scene with complete randomness, up to and beyond biological concentrations, without requiring scene-specific adjustments (Supplementary Fig. 1). autoPACK accomplishes this by sorting the ingredients in order of packing difficulty and weighting the more difficult ingredients with higher priorities, favoring them to be picked and positioned first. Procedural ingredients (for example, fibrous or branching growth) are seeded into the volume and typically expand early in the fill, and larger surface ingredients typically deposit earlier because they are more difficult to pack than globular soluble ingredients. This prioritized approach is similar to packing a moving truck: it is easy to pack a truck densely by stacking small boxes around previously loaded large furniture, but it is difficult to position a 12-seat dining room table into a truck jumbled with fragile knickknacks.

**cellPACK**

cellPACK is a biology-specific extension of autoPACK that consists of a database of mesoscale recipes (such as cytoplasm or synaptic vesicles) and examples of models for each recipe. cellPACK extends the general packing capabilities of autoPACK with numerous modules for cell- and molecule-specific packing. cellPACK also includes optional protocols and links to
third-party algorithms that provide component solutions to the global packing problem.

autoPACK uses a cooking metaphor, building a model scene from a recipe by placing ingredients into containers defined by surface meshes. Ingredients (typically molecules in cellPACK) are stochastically packed into containers (typically organelle ultrastructure output from surface-segmented tomograms as 3D polygonal meshes) up to densities provided by organelle-specific recipes. Each ingredient comes with associated properties, including a structural representation (such as a molecular surface or volume occupancy shell) and behaviors, such as a particular collision-avoidance method or a list of binding partners, that will modify the ingredient's global and local packing. In the resultant model, each ingredient retains a connection to various other forms of data to enable deeper analysis, preparation for systems integration or large-scale simulations, or modification of the ingredient representations. The algorithm and data structures function recursively across scales to enable organelle recipes to be combined into larger models at the cellular- and tissue-scale levels. For example: container ingredients, enable organelle recipes to be combined into larger models at the cellular- and tissue-scale levels. For example: container ingredients, once placed, can be subsequently packed with nested recipes of ingredients specific to that container's surface and interior.

cellPACK provides scripting and graphical user interfaces (GUIs) to access viewing, analysis and modeling modules. An online database enables invited users to critique and modify recipes and ingredients while maintaining version histories. Viewing software was built with open-source GUI plug-ins using uPy24, our ubiquitous Python application programming interface (API), that enable any uPy-enabled host to load existing models or run cellPACK interactively. Current hosts include molecular viewers such as the Python Molecular Viewer (PMV)25 and professional animation software such as Cinema 4D (Maxon), Maya (Autodesk) and Blender. The molecular viewer UCSF Chimera26 now includes an autoPACK result file reader to load and analyze cellPACK scenes. These uPy and Chimera plug-ins generate representations of ingredients positioned into model scenes and allow for interaction and analysis using cellPACK analysis tools coupled with functions native to each host.

Interacting with cellPACK and cellPACK results
cellPACK can run as a standalone Python algorithm or as a plug-in to a variety of uPy-hosting software packages. With uPy, a single uPy GUI can function across multiple 3D viewers with no redundant programming. The cellPACK GUI is organized in a user-friendly format to expose the more complex options to advanced users as needed (Supplementary Fig. 2a). As detailed in the Online Methods, a novice user is initially presented with a View tab that enables him or her to load cellPACK models directly from the prototype cellPACK database. The user can switch to the Pack tab to modify and pack models using existing recipes from the database or local files and can export the modified recipes and models. Users can also switch to a Build tab to construct recipe files and ingredient components from scratch. In all of the tabs, a user can choose from simple, intermediate, advanced or debugger versions of the GUI. The more advanced options expose more parameter widgets in the GUI.

Audiences of varying levels of modeling expertise and interests can interact with cellPACK software or with cellPACK results through the project website. The site offers visual representations of models as static images, animated movies and interactive 2D and 3D multiscale viewers (Supplementary Fig. 2b), and visitors may add comments to critique models and suggest improvements. To create the types of online representations shown on the website, users can download and install the cellPACK software to visualize and modify cellPACK ingredients and recipes, perform analysis and generate visual output. As it is an open-source project, we provide both the source code and an API for easy scripting, and we encourage users to modify and upload an a: all software components to participate in development.

cellPACK analysis tools
Our early prototypes of autoPACK underscored the need for effective analysis tools to quantify the success of different packing algorithms. autoPACK currently offers several basic analysis tools for assessing the positional and orientational randomness of placement in packing (Fig. 2 and Supplementary Fig. 3). Position and orientation histograms are used to highlight variations about a mean uniform distribution (frequency) line for randomly distributed objects or about a target frequency curve for gradient affected objects. Box plots can reveal any global skewing across orthogonal selections from within a fill volume. Size-dependent available volume graphs quantify the distribution of negative spaces to reveal pore networks accessible to objects of different sizes.

We used these tools, for instance, to quantify edge effects in the packing algorithm when applied to large, homogeneous fields of spheres of diverse size (Fig. 2a). The first method, using random ingredient selection, shows a clear bias as quantified in the void-pore network, the box plot of distribution statistics and the graph of occupancies binned along the two axes. The second method, which uses the current default packing modes of autoPACK, shows a more uniform distribution by all of these analysis methods and fills models up to and beyond the most common biological densities. Similar graphs validate the construction of a scene with an artificial gradient of concentration and several ingredients designed with shapes that present packing challenges commonly found in biological ingredients (Fig. 2b). Two difficult ingredients (a multipiece concave ‘C’ shape and a procedurally grown flexible fiber) pack randomly among a red convex polarized object that is given two intentional packing biases: a distribution27–29 and an orientation30,31 preference for the lower left corner of the 2D fill area. Nearest-neighbor, distance-to-center and point-of-closest-contact data can be exported to analyze mixing or interaction biases, but we have not yet implemented formal tools to output these graphs for interactive quantification of molecular interactions within cellPACK scenes. Decoupling the goals and constraints of packing from the packing algorithms will further ease recipe analysis, algorithm verification and algorithm comparison; these are important topics for future work.

Biological application and validation
To test and validate the functionality of cellPACK, we have chosen four systems of increasing complexity and for which extensive biophysical and ultrastructural data are available. We chose blood plasma (Fig. 3) as a simple initial case to test the packing algorithm in the absence of ultrastructural compartmentation. The molecular composition of blood plasma is structurally and biochemically well described, and it has a relatively low protein density (~6% occupancy by volume), about
one-fifth that of prokaryotic cytoplasm. Plasma is largely homoge-
neous but still has numerous fibrous components, so it is an
effective biological test for the combination of rigid body and
procedural filling. Most of the ingredients were treated as rigid
bodies based on atomic structures, and von Willebrand factor is modeled with a procedural snake-growth algorithm.
The user can adjust the ‘waviness’ and kinking of this structure,
guided by low-resolution structures from electron microscopy.
This 1,000 nm × 1,000 nm × 50 nm volume of blood plasma
contains 28,755 ingredient instances that cellPACK positioned in
116 s using a typical laptop computer. Readers can interactively
explore and comment on the full square micrometer of blood
plasma online at http://gigapan.org/gigapans/85568.

Synaptic vesicles were chosen as a system that requires pack-
ing of soluble and membrane-bound molecules in the context of
a known ultrastructure. A large multidisciplinary collaboration
determined the molecular contents of an average synaptic vesicle
and modeled its structure. We converted their modeled protein
structures and quantification statistics into cellPACK parameters
to model a number of stochastic variations of this synaptic vesicle.

Figure 2 | Analysis tools quantify randomness and packing bias. (a) The two indicated methods for packing are evaluated with autoPACK analysis tools.
(b) Three complex ingredients with different packing types can interact while recapitulating the intended distribution functions in this 2D packing.
All graphs show data averaged over 1,000 independent runs chosen to have an error bound ≤5%. Histogram bar heights indicate the average frequency per bin
with error bars equal to the margin of error at 95% confidence intervals (1.96 × s.e.m.). Supplementary Figure 3 provides larger graphs with values labeled.
recipe at a variety of sizes (Fig. 4). In addition, we used the same recipe to create a molecular model of a larger volume of densely packed vesicle surfaces segmented from an electron tomogram\textsuperscript{34}. Despite the complexity of this task, even the massive surface proteins such as the vesicular ATPase (vATPase) were able to interdigitate with neighboring vesicles and pack together at the high observed per-vesicle concentrations. The 100-nm cube of interdigitating synaptic vesicles (Fig. 4c) was created in less than 1 h on a standard laptop computer, with most of that time spent packing the lipids into the bilayers. Simpler models—for example, those that model a single synaptic vesicle with only the surface proteins represented (as in Fig. 4a,b)—typically require \textasciitilde 25 s to fully pack. Note that vesicles in Figure 4c were modeled as spheres—a common technique for hand-segmenting in tomography. These estimating spheres generate a more constrained negative space in which to pack a high density of surface proteins than do carefully traced vesicles, but cellPACK can pack the reduced tight negative space that results outside of the mathematical spheres. In this particular fill, a probabilistic soft collision was applied that rarely allows proteins to penetrate neighboring bilayers. This option is turned off by default, but we applied it here to highlight cellPACK’s flexible ability to control interaction across scales, from proteins to organelles in this case.

The modular nature of cellPACK is designed to promote the easy combination of recipes to create unified, hierarchical models and to enable experts to contribute to the whole recipe or recipe components within their field of expertise. This capability was tested using two hybrid models generated from integrated recipes (Fig. 5). The model of HIV in blood plasma combines seven different recipes into a single model, ranging from the outermost recipe for blood plasma (Fig. 3), to a recipe for the distribution of envelope glycoprotein\textsuperscript{35} (Env), to recipes for host proteins and two model options for the cone-shaped capsid\textsuperscript{36,37}. The Mycoplasma mycoides model packs DNA into a surface container using a fibrous-extension packing mode that is constrained by probabilistic placement of DNA-binding proteins that stochastically generate particular bend angles. The cytoplasm and surface recipes pack simultaneously but have lower priorities. Lipopolysaccharides grow from the surface with a fibrous packing mode.

cellPACK as a research tool

We have used cellPACK to simulate experimental results from fluorescence microscopy, similarly to the approach described by Gardner et al.\textsuperscript{38}, to test six competing hypotheses for distribution of the Env glycoprotein in immature HIV-1 virions (Fig. 6). The models seek to reconcile two conflicting experimental results: super-resolution stimulated emission depletion (STED) fluorescence microscopy results\textsuperscript{39} have been interpreted to support a random distribution of Env on the surface of the virion; however, the matrix protein (Ma) region of the Gag polyprotein inside the

\textbf{Figure 3} | cellPACK model of a 1,000 nm × 1,000 nm × 50 nm volume of human blood plasma based on a mesoscale recipe containing information for the most abundant macromolecules. Magnification and scales are indicated. In the top half and inset, each ingredient has a unique color. The bottom half and key, colored by source of the structural data (Data Type) used for each ingredient, indicate the variation in resolution and uncertainty.
membrane, which may interact with Env, has an asymmetric distribution in cryo-electron microscopy studies. Models were created using cellPACK with exploratory variations in Env and MA molecular interaction recipe parameters leading to whole virus models with different emergent structural properties, and fluorescence images were simulated from these models (Online Methods). As in the experimental study, these fluorescence images were classified by the number of foci, and these values were compared with the experimental distribution of foci. On the basis of our simulated results, a model of a completely random distribution of Env gives substantially fewer single foci than is observed by fluorescence microscopy in immature virions, indicating that the asymmetric distribution of Gag in immature virions may be causing at least some measure of asymmetry in Env distribution. As expected, models with clustered Env are clearly not consistent with observations. The results, however, are not sensitive enough to distinguish between several models of interaction between Env and Gag, although three models of specific interaction (R-InMa, closeMa and closeMa-Env) show better fit to data than the model of passive incorporation (R-offMa). Within the observed ranges of Env quantification, the number of Env molecules packed into a virion does not affect the foci count in any of the models (Supplementary Fig. 4), which agrees with the original observations. However, the distribution statistics are dependent on the technique used to distinguish the number of foci (Supplementary Fig. 5 and Online Methods) in each STED image, and we chose a method designed to match the manual counting method used for the published experimental work.

**DISCUSSION**

The goal of cellPACK is to create a framework that integrates multiple types of data across multiple scales into comprehensive spatial models for analysis, communication and simulation. On one level, cellPACK models integrate data and theory to produce editable community models, allowing an iterative crowdsourcing process to refine the current understanding and to impel hypothesis-driven research and collaboration. The models can be used as starting points for mesoscale algorithms such as Brownian dynamics simulations and could provide foundations for whole-cell dynamics projects such as the 3D virtual cell (http://3dvcell.org/). On another level, cellPACK offers immediate applications for peer communication, education and outreach, and the concurrent development of our uPy and embedded PMV (ePMV) software (ref. 42 and http://biochem.web.utah.edu/iwasa/projects/HIV.html) provides easy access to these tools for users from diverse backgrounds. The modular open-source architecture allows for growth and adaptation as new computational approaches and theories of mesoscale biology emerge.

**Figure 5** | cellPACK can integrate recipes into unified hybrid models that may be iteratively refined by content experts and systems-biology database improvements. (a) A model of HIV in blood plasma shows seven different recipes (each with a different color-coding) unified into a single model. (b) A complete model of the HIV life cycle generated as part of the autoPACK Visualization Challenge (http://autopack.cg.org; ref. 43) using the cellPACK software in conjunction with molecular modeling packages. Image reproduced with permission from J. Klusáková. (c) Our first model of a whole cell, M. mycoides, integrates recipes with surface, fibrous and volumetric ingredients into a single comprehensive model.

**Figure 6** | cellPACK models may be used to simulate and interpret results from fluorescence microscopy. We constructed a cellPACK recipe for six hypotheses for the interaction of HIV-1 Env and Gag in immature virions. (a) Three cellPACK HIV models with Env fluorophores are superimposed over their corresponding simulated fluorescence images. (b) Samples of simulated fluorescence images from each of the six the models. (c) An automated foci-counting algorithm was used to analyze the 3,000 images generated for each model, with the sample number chosen to have an error bound <5%. $\chi^2$ analysis with two degrees of freedom, performed to duplicate the significance assessment reported in the original experiment, shows that several models with specific interaction between Gag and Env are consistent with the observed distribution for the immature PR(−) virion, whereas as a completely random distribution (R-noMa) and a model that clusters Env (closeEnv) do not. WT, wild type; conditions described in the Online Methods.
METHODS
Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
G.T.J. conceived of autoPACK and cellPACK and designed the original code, wrote the core code, designed user interfaces and edited interface code, conducted experiments and analyzed results. L.A. wrote core code, designed and wrote the interface code, wrote analysis code, and designed and conducted HIV experiments and analyses. M.A.-A. co-designed and wrote prototype autoPACK code. D.S.G. co-conceived of and guided cellPACK design and designed HIV experiments. M.F.S. transposed the prototype autoPACK code to Python with G.T.J., added cellPACK packing and grid preparation features, and structured and cleaned the first drafts of the current core code. A.J.O. guided code and experiment design and implementation.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Overview. cellPACK is a toolkit that generates probabilistic 3D models based on a list of ingredients and a set of constraints, which may include 3D structures of biomolecules, 3D representation of membranes and compartments, and procedural description of fibrous components such as DNA or polysaccharides. The GUIs to cellPACK facilitate end-user interactions, and public project software repositories facilitate open-source development.

To view models, install the software, learn from tutorials or participate in evaluating, editing, or initiating models, please visit http://cellPACK.org/. The open-source code and custom scripts used for this study are available at https://github.com/gj210/autoPACK/. Images, movies, and online 3D viewers are at http://cellPACK.org/recipes/.

Data structure. cellPACK operates as a stand-alone Python algorithm that takes inputs from recipe files to produce result files, but most end users currently access cellPACK through several GUIs. End users may work with cellPACK on many levels: to view the resulting model of a previous packing; to load and modify an existing recipe in order to produce a new packing result; or to construct recipes, ingredients, and their components from scratch. In each case, the user interacts with a consistent cellPACK data structure, allowing easy transition between these levels to work throughout any nested recipe hierarchy and allowing cellPACK to operate across all data scales.

We have defined a generalized referential hybrid model format (HMF) file that is used throughout cellPACK’s prototype database to store recipe and ingredient data. cellPACK generates and reads the generalized hybrid model file (HMF), a flexible referential file type that can adapt to handle multiple (and future) data types while minimizing data redundancy. cellPACK generates and reads XML and JSON versions of HMF. The cellPACK algorithm uses recipe scripts, HMF files, or recipe GUI parameters to assemble input data from a variety of formats and produces a collection of HMF output files in a directory that includes a primary recipe file with pointers to any nested recipe or ingredient files, an autopACK result file (.apr) for each container, intermediate construction files and analysis files. An ingredient file (actually a specific variation of the more general recipe file type) contains data or reference pointers to data in public databanks that allow the hosting software to reconstruct meshes (such as ultrastructural organelles or protein representations) for use in modeling, viewing, or analysis. Nested interactions and general HMF flexibility allow a recipe to become an ingredient in a higher-level recipe to minimize redundancy when working across multiscale data. We described autopACK with ‘recipes’ and ‘ingredients’ for semantic clarification and convenience, but from a programming perspective, autopACK uses only the concept of ‘recipe’ as its single universal data structure. An ingredient is a more specific recipe type, defined as a recipe that includes an associated structure (or for procedural ingredients, a potential structure) and in the simplest terms can be considered “an object to pack.” An .apr file contains a list of ingredient IDs with positions and orientations as described in the online documentation (http://www.autopack.org/documentation/-apr-autopack-result-file-anatomy/) as well as a pointer back to the recipe that created the .apr. By default, the structural and other data files that the HMF files ultimately reference get downloaded into a cache directory system that reconstructs the needed portion of the online database on the fly during an interactive modeling or viewing session. The software can also access the ingredients directly from local or cloud storage via browser loading or via custom file paths to known sources.

As described in Supplementary Figure 2, the following levels of data structure interaction are visually separated into separate tabbed panels of the default cellPACK GUI.

Interaction level 1: view the resulting model of a previous packing. At the top (and simplest) level of interaction with the data structure, a user can visualize a model that resulted from any stored previous packing via the autopACK result file (.apr). The .apr file points to the main recipe that produced the result, followed by a list of component IDs and matrices (organized by any subrecipes) needed to rebuild the scene (see http://www.autopack.org/documentation/-apr-autopack-result-file-anatomy/). To visualize this model, cellPACK reconstructs the scene components (container and ingredient representations and packing surfaces) as described in the “cellPACK host scene preparation...” section below. It then generates, organizes, positions and orients one instance (a computationally efficient copy) for every ingredient ID per matrix line listed in the .apr file. Last, cellPACK produces a corresponding GUI as shown in Supplementary Figure 2, all in one step. The user may additionally load analysis files associated with the .apr, such as arrays that describe the distance relationships within the volume. The bottom-level ingredient recipes do not typically point to a default .apr file, but a properly constructed primary or mid-level recipe file includes a pointer to a default .apr file that any user can exactly recreate by packing the default recipe.

The input and output HMF files can be accessed directly for visualization and analysis or for other uses such as Brownian dynamics simulation. For example, we provide uPy plug-ins that construct cellPACK scenes into a host’s native GUI, where 3D models (coarse molecular surfaces by default) represent each ingredient. To enable standard laptop computers to handle the bulky data of a typical cellPACK model, uPy constructs cellPACK scenes into hosts with efficient sensible organizations that are native to each system, taking advantage of hardware instancing and other host optimizations that reduce data redundancy and other computational bottlenecks where available.

Displaying cellPACK results in uPy-enabled hosts provides access to the capabilities of each host for interacting with and analyzing the model. With the View tab, entire organelles or individual ingredients can be hidden, deleted, added, colored, etc. Representations of molecular ingredients can be swapped and altered. In conjunction with native host functions, distances can be calculated, or more sophisticated energetic potentials can be displayed as 3D heat maps. Points of closest contact can be illuminated, and simulations can be initiated or animations generated with a variety of techniques such as those described in our embedded Python Molecular Viewer (ePMV) paper41.

Interaction level 2: modify an existing recipe to produce a new packing result. At this deeper interaction level, a user can work directly with the recipe file (i.e., the same ‘primary recipe’ file and all nested recipe files that the resulting .apr file will point back to when produced) to reproduce a packing result or modify parameters in the collected recipe(s) to produce a new packing result. When this recipe is run, it will produce only an .apr file by default, but the user may also select to write out a collection of grid arrays and other files useful in analysis. Depending on the host software used
to perform the packing, cellPACK can also store a representative image or movie of the packing result (useful for automated packing while exploring parameter space). The primary recipe file can be a bottom-level recipe that sets parameters directly upon container and ingredient Python objects, or it can refer to more complex nested recipes that it unites (as in the HIV model in Fig. 5a), with the option to override particular subrecipe ingredients or parameters (Supplementary Fig. 6). HMF flexibility allows a primary recipe to combine subrecipe pointers with container and ingredient Python objects. The result of a new packing run can be written to an .apr and visualized from the View tab or visualized immediately from memory using the same process described in “Data structure level 1” above.

As demonstrated in Figure 2, randomness and biases from different packing methods can be quantified with built-in analysis tools such as the planar distribution histograms (generated with matplotlib, http://matplotlib.org/) and box-and-whisker plots. Position and orientation histograms are used to highlight variations about a mean uniform distribution (frequency) line for randomly distributed objects or about a target frequency curve for gradient-affected objects. When packing with the default benchmark goal of a uniform random distribution, spikes in the planar distribution histogram averaged over multiple runs can indicate problems in the algorithm or recipe data that warrant further manual investigation. Box-and-whisker plots can reveal at a glance any global skewing across orthogonal selections from within a fill volume.

Size-dependent available-volume graphs mathematically track the distribution of negative spaces, revealing what scales of protein-sized molecules could diffuse through these ‘pore networks’. Variations of these void-pore networks can be used to calculate permeability or as a starting point to simulate microscale flows (especially useful in macroscale engineering applications via the more generalized autoPACK software, e.g., ref. 44).

Interaction Level 3: construct recipes, ingredients, and components from scratch. Whether nested in other recipes or a single flat (bottom-level) recipe, a recipe structure eventually points to containers and ingredients that it assembles and can optionally modify or override with parameters. Containers and ingredients become Python objects when cellPACK operates upon them as inputs, and a user can choose to generate them directly as recipe lists using HMF keywords in either XML or Json formats (inline or with file pointers to subrecipes) or parametrically with a Python script. The uPy cellPACK GUI provides a Build tab to assist with recipe production. The current version enables a user to load a variety of data representations (for example, the surface model of a Protein Data Bank file (PDB) generated with ePMV or any molecular viewer) in order to produce an ingredient-level recipe file: for example, a container with an associated recipe of ingredients to pack, or an ingredient with associated structure and collisionTree files.

The cellPACK GUI working in uPy-supported hosts also allows users to create recipes (that use only cellPACK defaults) on the fly by dragging and dropping ingredient and container geometries into provided scene groups (see a demonstration at http://www.youtube.com/watch?v=S94GUmFQ7yM). cellPACK recognizes the new ingredients placed and generates the default ingredient parameters by type. cellPACK then allows the user to modify these parameters through the standard Pack tab GUI. Recipes can be exported from the GUI or .apr files created by clicking a “Pack” button.

cellPACK host preparation for result visualization and interactive packing. To generate a cellPACK model, a user provides an environment volume, a selection volume to pack, and at least one recipe of ingredients. The environment volume may be an empty box or divided into containers that compartmentalize the space. A selection volume defines some subsection of the environment volume as the volume to be packed and is independent of container boundaries—the inset rectangle of Supplementary Figure 7a (labeled “User selection” in Supplementary Fig. 7b) is an example of a selection volume used for packing. Environment volumes, selection volumes, containers, recipes, ingredients and parameters that define how these packing components interact can be declared in scripts, imported from other file types, created live in the visualization hosting software, or modified live through a cellPACK GUI. When a cellPACK recipe is run to produce a new packing result, the result is both stored in memory and written to an .apr file along with optional analysis files. To visualize the result of a previous packing from a file (via the View tab) or a current packing from memory (via a new Packing run from within the Pack tab), cellPACK first generates a representation of each unique geometry that will be represented in the final model to prepare the software. These single geometric models get efficiently instanced during visualization. Certain types of packing modules can optionally use this ‘visualization root’ geometry directly in combination with capabilities native to the hosting visualization software to provide novel packing options, such as native collision detection or physics engines.

Definition of scene components. Selection volumes define the volume to pack. A selection volume can be defined mathematically in a script, manually as an interactive selection in a 3D viewport, or automatically set equal to the environment container described in the next section. A selection volume is always orthogonal to the origin of the scene but may be set to any scale on the x, y, and z axes, including scales equal to 0 to produce a point, a line, or 2D plane selections. Selections can have names and be looped in a Python packing script: for example, a user could build and name multiple cubic objects in his visualization host software and then loop through these objects as selection boundaries to pack each in succession (or in parallel with multiprocessing) to fill a larger volume in a checkerboard fashion. With scripting, a polygonal object can be used to make complex 3D selection shapes.

Environment and ultrastructure containers. Similarly to the selection volume, cellPACK requires at least one container, which is defined by default as the environment selection box. The environment container may be defined mathematically in a script, manually as an interactive selection in a 3D viewport, or automatically as a bounding box of a set of polygonal data (containers): for example, a model of organelles exported from a segmented tomogram with surface meshes centered on the bilayers.

When visualizing a result or performing an interactive packing, cellPACK first analyzes the prepared cellPACK scene in the 3D host to store any existing objects and the names of any annotated objects found. If the declared or live selection volume intersects with any found container objects and a container’s annotation name matches a recipe name, then cellPACK associates that

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Supplementary Fig. 7e

With a recipe established, in order to produce Supplementary Fig. 8, we begin by placing all ingredients within the selection volume before the cellPACK environments.

Currently, containers may be imported or referenced as polyhedral meshes (vrml, collada, fbx, .c4d, .maya, .max, blend, or .si file types) or as files of vertices and faces. The environment container may contain a recipe as well, and the environment box is typically used to bound what cellPACK would otherwise consider to be an infinite ‘external’ recipe.

Recipes. A minimal recipe HMF file contains a list of ingredients (ingredients are programmatically just subrecipes) and a recipe name (often associated with a container name) to pack. A more typical recipe file may also include parameter overrides for particular ingredients; pointers to other recipe files that the code recursively integrates into the model; containers or pointers to container files; a selection box or selection name (to pick up interactive selections) in which to bound ingredient packing; a subrecipe group that combine the ingredients of multiple subrecipes associated with each container; container or global environment modification parameters such as prohibited regions of packing or preferential packing-gradient modifications; or overrides to default global packing parameters, result file paths, etc.

More sophisticated recipes can be scripted in Python to enable script-level modification of cellPACK functions: for example, to loop through multiple packings, to generate multiple ingredients with just a few lines of code by iterating parameter values in loops, or to write out custom analysis files.

Ingredients. An ingredient is a specialized recipe type that has a geometric representation associated with it (this can be a single mesh or an entire packing result, for example), most simply defined as an object to pack. A minimal ingredient HMF file contains an ingredient type, a name, a collisionTree list or collisionTree file pointer, and a representation or representation file pointer. Collision trees are used for efficient collision detection using the default jitter packing module—one of the local packing modules available in cellPACK—and to update the packing grid with all other packing modules. A more typical ingredient file may also include ingredient parameter default overrides (for example, PriorityWeight, packing mode, rotation boundaries); interaction partners or interaction partner file pointers; data references (for example, PDB or EMD files used to generate the representation including pointers to custom PDB files stored in the cellPACK database, as well as nonspatial data); or alternate parameter triggers used by the code for logic operations (for example, to alter parameters mathematically if packing in particular environments).

Currently, cellPACK recognizes a variety of ingredient types. These include: (i) ingredients that have been manually or computationally placed within the selection volume before the cellPACK run; (ii) soluble rigid-body ingredients that will be packed within given volumes; (iii) membrane-spanning ingredients (including lipids) that orient normal and with defined directionality to a membrane surface with variable axial rotation and precession; (iv) custom procedural ingredients that build an extended structure from smaller primitives, such as actin filaments and DNA; and (v) procedural fiber ingredients that extend variable-length wavy, kinked, and branched curves through the volume. In addition, HMF files allow an existing recipe or recipe result .apr file to be used as a nested-recipe ingredient. For example, to pack a cellPACK model of HIV into blood plasma, the HIV HMF recipe or result can be treated as an ingredient for efficient referential reconstruction into the scene using the same methods but recursively described in “Data structure” above. If treated as a result, the entire HIV model represented by a provided .apr file would pack as a single rigid body, but if treated as a recipe, the HIV envelope container would pack first into the serum before each of the HIV subrecipe ingredients could pack into it, according to their global priority, while interacting with the blood plasma components as they simultaneously pack.

Producing a new packing result. Step 1: build a grid (or load an existing grid). With a recipe established, in order to produce a new result, cellPACK first organizes all of the containers and ingredients declared in the recipe (and recursively through any nested recipes) to create master lists. cellPACK uses these lists to subdivide the environment container into a grid that associates multiple types of data with the spatial data of the packing scene. The grid describes the space and tracks changes efficiently, for example, by enabling algebraic identification of neighboring points and previously placed ingredients for distance evaluations, collision tests, or agent interactions between objects.

To create the grid (Supplementary Fig. 8), cellPACK sorts the list of all ingredients by the ‘packing radius’ parameter (by default: the radius of gyration of a molecule) and uses the smallest packing radius to establish a cubic grid with a diagonal spacing equal to that smallest packing radius (Supplementary Fig. 7c,d). By default, the grid fills the selection box, but it can optionally be expanded to fill the environment box to reduce any boundary issues, to prepare for multiple consecutive packings in the same packing scene, or to incorporate grid-weighting effects from nearby containers or previous ingredients that are not already contained by the selection box.

After building the master grid, cellPACK uses the list of containers to compartmentalize the grid (Supplementary Fig. 7e). cellPACK uses a signed-distance function to assign each point in the grid to an appropriate container and to specify whether it is inside or outside of its surface. The system acts recursively, so surfaces can be nested in other surfaces, and the points inside of one surface and outside of a deeper surface remain associated with the recipe for the more superficial surface. For example, HIV in situ nests from superficial to deep as (i) host blood plasma (environment recipe bound by the environment box); (ii) HIV envelope (surface recipe); (iii) HIV matrix (volume recipe); (iv) HIV capsid (surface recipe with no ingredients, only a container representation); (v) HIV genome region (volume recipe). See more recipe interaction details in Supplementary Figure 6.

If there are particularly small molecules (such as lipids or ions), rather than refining the entire master grid volume for one subset of very small ingredients, separate subdivisions are assigned and
associated with the master grid. These finely subdivided grids are then associated with lookup tables in the master grid for fast sorting. For example, surface polygons with sides longer than the master grid spacing are subdivided to make fine surface points for lipid packing and tracking, allowing them to pack tightly in a continuous membrane. At the same time, the master grid is only as fine as it needs to be to allow all of the macromolecular ingredients to explore continuous space while avoiding collisions.

The grid is thus compartmentalized, and each discrete voxel in space is associated with a container and its accompanying recipe of ingredients. In the final grid-preparation step, cellPACK weights the grid with any optional container-level packing bias functions provided in the recipe (Supplementary Fig. 7f). During packing (detailed below), the signed-distance relationships along with the nearest neighbors, weighting, and other data stored with each grid point get updated to provide efficient lookup tables rather than recalculating relationships redundantly during packing.

To calculate the total number of each ingredient instance that should be placed into each compartment’s surface or interior, the area for each container surface and the volume of each compartment (including the exterior compartment) is calculated. Each ingredient’s molarity or surface density is then multiplied by the organelle volume or surface and modified by any special localization gradient values that may affect local concentrations within a volume or surface. As noted in Figure 4, logic or mathematical operators can modify the total number of ingredients to recalculate observed values. To generate a whole number for the number of instances that will be packed, the numberOfInstances parameter value calculated from the concentration gets rounded up or down probabilistically. For example, if the raw number-of-Instances calculates to 14.73 molecules for the container, there is a 73% chance that the number will round up to 15, otherwise it gets rounded down to 14. This ensures that for volumes and surfaces with very low concentrations, there will still be a finite chance to include one instance of the ingredient.

Step 2: ingredient packing priority (order is important). Prior to packing, the master list of ingredients is sorted by a PriorityWeight ingredient parameter that reflects the difficulty of packing. Early studies revealed that larger, longer, and more branched ingredients are harder to pack homogeneously than smaller globular ingredients as a volume gets crowded. We developed a simple default definition of priority weighting that gives more difficult ingredients a higher probability to pack earlier in the process:

\[
\text{PriorityWeight} = (\text{Surface Area}/\text{Volume}) \times \text{Encompassing Radius}^2 \times C
\]

where the surface area and volume are calculated by MSMS, the encompassing radius is the maximal extent of the molecule from its packing center, and the constant \( C \) is set to 1 by default. The \( C \) term allows an ingredient or recipe to bias the PriorityWeight of an ingredient or to tailor a \( C \) value across multiple packing results until desired densities are reached. Users can completely override the PriorityWeight of each ingredient with values coded into the ingredient file or broadly in the recipe file, or they can override the default PriorityWeight function with a recipe script. Ingredients with user-defined negative PriorityWeights will pack first, in succession, starting from the ingredient with the most negative packing priority. Ingredients with a packing priority of 0 will have the packing priority calculated by the default PriorityWeight equation shown above, which will then be considered with ingredients that have user-defined positive PriorityWeights. An manually overridden positive PriorityWeighted ingredients will be sorted with the calculated PriorityWeighted ingredients and will then pack in a random probability-biased order. For example, in the synaptic vesicle model (Fig. 4), all of the bulky vesicular ATPase proteins were given negative PriorityWeights to ensure that they were given space to pack within the vesicles. Large positive PriorityWeights were used to ‘encourage’ the lipids to pack as the last of the surface ingredients but well before the small glutamates attempt to pack into the matrix of the vesicle. Without these empirically adjusted overrides, all ingredients would pack in a random but weighted order by default.

Step 3: pack and track. With all of the recipe and ingredient data connected to the spatial data of the containers via the grid, cellPACK uses the local packing method declared in each ingredient file—for example, an agent packing spring method described in the “Procedural packing” section below is detailed in Supplementary Figure 9—to place recipe ingredients onto and into their appropriate containers (Supplementary Fig. 7h, right). Regardless of the local packing method, when a suitable place for an ingredient is found, an instance of the ingredient gets deposited and the grid is updated with knowledge of the ingredient and any pertinent data linked in from the ingredient’s file. Distances to the ingredient surfaces are updated and stored in the neighboring grid points, and the ingredient identification is added to any points where the newly deposited ingredient becomes a new closest ingredient. When an ingredient gets picked, cellPACK’s ingredient algorithm searches through a list of grid-point distances (that may be weighted globally or locally by other factors) and can pick only a point with a large enough minimum distance to accommodate the ingredient’s packing radius. The algorithm for a typical randomly packing ingredient will analyze the current density of the fill and, if sparse, will pack on any random point rather than running the expensive sorting calculation. This calculation makes the filling more efficient later when the fill becomes denser and the sorting gets triggered appropriately. The filling loop continues until there are no more ingredients to add or until there are no more points available with radii large enough to accommodate more ingredients.

Procedural packing (branching fibers or crystals). Procedural ingredients grow according to rules defined in their ingredient file (Supplementary Fig. 9). When a procedural ingredient is added, it can grow up to its maximum length immediately or get deposited as a procedural ‘seed’ for later extension (Supplementary Fig. 10). At any time in the fill, a live seed can be called upon (initiated by an agent, random probability, or immediately upon its placement) to grow, pause, or die. As each unit is added, the total length or other property of the ingredient is evaluated and a probability is calculated to either continue or terminate the growth. Growth can also be paused, returning into the main loop to continue packing other ingredients elsewhere but leaving the end of this fiber or crystal as a dormant seed that could continue to grow once triggered. As the ingredient is extended, other types of seeds can be added to create branches. For example, in the construction of an actin cytoskeleton, a unit of actin with Arp2/3 could be added, creating a branch that could be extended at any time.
Preferential packing: gradients, interactomes, agents, density clustering. By default, cellPACK places ingredients randomly on, in, or around polyhedral surfaces, creating a homogeneous distribution. If desired, global and local controls can be applied to generate preferential and even deterministic deposition. While placing objects, a variety of probability logic–driven and agent placement systems can be applied to recapitulate current data or theories of localization or interaction in the model. This may be specified at a global or local level. For instance, a Ran-GTP ingredient can be globally biased to pick a point closer to the center of a dividing cell or closer to the cell's chromatin or on a local level; an ingredient with agent behaviors can bear a list of binding partners that affect how it packs.

If not offered in the core code, special traits can be programmed into recipe scripts. For example, to match the Takamori paper's observations when constructing the synaptic vesicle recipe (Fig. 4), we found it necessary to implement a method for ‘quantization’ effects: experimental observations from Takamori et al. had revealed that, perhaps through some unknown sorting mechanism, every observed vesicle included at least one vATPase regardless of the vesicle's diameter. Within a cellPACK Python recipe script, the default probability of placement for the vATPase ingredient was modified with a simple logic operation run after the number of vATPases was calculated for a given vesicle (if vATPaseCount >= 0: vATPaseCount = 1) to ensure that at least one of these types of low-copy-number ingredients were included in each vesicle.

In the current release of cellPACK, we have implemented a few local agent methods. When an agent picks a location in which to position itself, it gets a list of neighbors in that area, scans the list for known binding partners, and probabilistically determines whether it should be paired with any of them. When an agent decides to pair, one of several approaches can be used to position the new ingredient adjacent to an existing interaction partner. As demonstrated in the lipid packing of HIV in Figure 5a, the closePacking algorithm weights the points surrounding the existing partner(s) and attempts to pack within this list of points; as demonstrated in the hexagonal packing of Gag-Ca in Figure 6, binding transformations can be used to constrain growth from a selected interaction partner edge; or as demonstrated with the DNA-binding protein HU in Figure 5c, binding transformations can be constrained along a spline or surface, etc.; and a nonspecific tight binding method generates a spring(s) to pair the two binding surfaces of the ingredients and relaxes the spring-connected system with appropriate collision detection that includes all neighboring geometry or atoms. This type of biased interaction can assist with the construction of larger cellular environments, for instance, to guide the interaction of DNA-binding proteins with DNA. Another approach modifies the weighting of the grid that surrounds particular container or ingredient surfaces to increase or decrease the probability of packing for any agent ingredient parameterized to recognize those particular grid biases. Less specifically, an ingredient can pack against itself or other ingredients using an optional closePacking method that biases the grid to encourage ingredients to pack tightly against previously packed ingredients. This approach serves well to pack lipid bilayers up to their correct densities from large libraries of single or patch lipid ingredients we have isolated from molecular dynamics–resolved lipid PDB files.

A recent experimental integration of the LipidWrapper software via a postprocessing protocol has provided a faster and more robust solution to pack lipids into bilayers. We are now working with the LipidWrapper programmers to integrate this as a new optional packing module specifically designed to pack lipids as patches onto surfaces directly in cellPACK with standard priority weighting and all other cellPACK features. The integration of third-party packages such as LipidWrapper for packing molecular dynamics–solved lipid patches into ultrastructure surfaces (http://nbcr.ucsd.edu/data/sw/hosted/lipidwrapper/)—and several previously integrated packages including panda3D with Bullet and Open Dynamics Engine physics engine support in Python (http://www.panda3d.org/), RAPID for triangle intersection detection (http://gamma.cs.unc.edu/OBB/), and SciPy with KDTree to quickly collect neighboring molecules or polygons (http://www.scipy.org/)—demonstrates how the modular framework of cellPACK can expand to interoperate an extensive variety additional of packing and analysis algorithms via Python as the project continues to grow.

cellPACK models and simulated fluorescence microscopy. We tested six models for distribution of HIV-1 envelope glycoprotein (Env) for consistency with recent observations from fluorescence nanoscopy (FN). In the FN experiments, Env distribution was visualized with fluorescent antibodies, and virions were manually classified into three categories: particles with a single focus of fluorescence (presumed to be one cluster of Env), particles with two foci, and particles with three or more foci. For an immature virion (the PR(–) phenotype, missing the protease essential for maturation), the study authors observed that 28% of the virions had a single focus, 24% had two foci, and 48% had three or more foci.

cellPACK generated ten models for the distribution of the Gag protein in the immature virion to be consistent with observations reported from cryo-electron tomography (Gag-CET). Gag is observed to form hexamers, which assemble to form an irregular array that covers roughly two-thirds of the inner surface of the membrane. Our Gag models are generated with a cellPACK neighbor-growth packing module, which in this case extends hexagonal tiles from any existing hexagonal tile edges as described in the “Preferential packing” section above. An open hexagon edge is selected randomly, and a tile is hinged on that edge to an angle that is perpendicular to the packing surface. If this new hexagon does not collide with an existing tile, the position is accepted. Because it is impossible to perfectly cover a curved shape using regular hexagons, this tiling introduces similar defects as observed in the tomography models. The hexagon radii of 55 Å match the size provided in the tomography models, and the tiling is programmed to stop when the coverage reaches 60% of the packing surface's surface area to match to observed coverage by Gag. The script is provided at http://mgldev.scripps.edu/projects/autoPACK/web/scripts/testTiling.py.
cellPACK projected the Gag hexagons onto the 150-nm-diameter envelope packing surface of HIV-1_0.1.6 and positioned Ma hexamers to be consistent with the Gag-CET observations.

An ingredient for Env is modeled from the trimer generated from PDB entry 4NCO with the b12 Fab fragment from PDB entry 2NY7, currently stored as HIV1_Env_4nco_0_1_3 in the cellPACK database. No structural information is available for the entire C-terminal domain\(^{49-51}\), so it is approximated as a sphere of 450 amino acids, with a radius of 2.4 nm.

The Env model is placed randomly within the constraints of the six competing hypotheses represented in six recipes with hypothesis labels from Figure 6: R-noMa places Env randomly on a uniform virion membrane as a control; R-offMa places Env in any empty space with no Gag hexagon (i.e., onto the 40% empty region of the envelope surface); R-InMa places Env in the center of any Gag hexagon (i.e., restricted to the 60% Gag covered region); closeMa places Env next to any of the Gag hexagons to simulate a 100% Env binding to Gag, but with some occlusion by the Env C-terminal tail; closeMa-Env places Env next to any of the Gag or next to any existing Env with a 50% chance to choose either; closeEnv places Env next to any existing Env.

For each hypothesis, cellPACK performed 100 models of Env distribution on each of the ten Gag virion models. Each model included 7 ± 5 Env trimers, as a control; R-offMa places Env in any empty space with no Gag hexagon (i.e., onto the 40% empty region of the envelope surface); R-InMa places Env in the center of any Gag hexagon (i.e., restricted to the 60% Gag covered region); closeMa places Env next to any of the Gag hexagons to simulate a 100% Env binding to Gag, but with some occlusion by the Env C-terminal tail; closeMa-Env places Env next to any of the Gag or next to any existing Env with a 50% chance to choose either; closeEnv places Env next to any existing Env.

For each hypothesis, cellPACK performed 100 models of Env positions are computed using standard cellPACK recipes with the constraints provided for each hypothesis.

The resultant models are viewed from three orthogonal directions, and fluorescence images are simulated using a Gaussian filter that approximates the point spread function, which assumes a 400-Å resolution of the STED setup. The 3D coordinates of the fluorophores are interpolated within a 3D grid with 100 Å grid-point spacing. The interpolation to the camera resolution is done by incrementing the closest grid point to each Env position. The grid is then convoluted with a 3D Gaussian function with HWFM = 400.0 Å to produce a simulated fluorescence image.

The convoluted grid is summed individually on the three axes and interpolated to the proper resolution of 1 pixel = 200 Å. The 2D grid is then displayed using an offset ‘hot’ color map.

Images are automatically separated into the three categories used in experimental FN work (single, two, and multiple foci) to generate the statistics for each model, for comparison with Figure 3b from the FN paper\(^{39}\). Before the counting, the images are filtered by removing background values below a certain threshold and are then subject to a feature-detection algorithm provided by the package Scipy\(^{52}\). This approach returns the number of connected pixels forming a blob. In order to make the distinction between the three categories, we look at the size of each of these features, assuming a small blob represents a unique foci, whereas a large, diffuse one can represent multiple foci (Supplementary Fig. 4).

Supplementary Figure 5 describes the sensitivity of the three categories to the parameters of this counting algorithm, which was used to provide a more objective and controlled measure than manual human counting.

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