Prediction of crucial interaction for icosahedral capsid self-assembly by configuration space atlasing using EASAL
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Abstract
Knowing the assembly-crucial interaction of virus capsid help us understand its assembly process. Validating assembly-crucial interaction via mutagenesis experiments in wet lab is usually very slow. We predict the assembly crucial interactions by measuring the entropy change of the capsid assembly system before and after the removal of each interaction. The entropy-change is measured by analysis of the viral capsid configuration space on two levels. On the interface level the entropy of the small interface assembly system is approximated by the sampling of the configurational space using the recently reported geometric method called EASAL (efficient atlasing and sampling of assembly landscapes). On the capsid level the method statistically learns how to combine the crucialness of interactions for assembly of each interface (obtained using EASAL) with crucialness of each interface for assembly of capsid (obtained from capsid structure and symmetry) to predict crucialness of interactions for capsid assembly. The learning algorithm uses - for training - a fraction of the mutagenesis-based experimental data about assembly-crucialness of interactions. The remainder of the mutagenesis data is used to validate.

1 Introduction
Viral shell self-assembly is a critical part of the viral life-cycle. Understanding the process of assembly illuminates the pathophysiology of infectious disease and allows us to target assembly process with drugs and vaccines. Entropy is the driving force of such superamolecular assembly process. However the high dimension of the configuration space of viral capsid make it difficult to calculate the entropy directly. This paper decompose the analysis of the viral capsid configuration space into two levels. For the small interface assembly system the configuration space is sampled directly and together with the symmetry of the capsid they measure the entropy of the big capsid assembly system.

1.1 Previous Work, Scope and Motivation
Like most other supramolecular assemblies that occur widely in nature, viral shell self-assembly is an extremely robust, rapid and spontaneous process, which make it very difficult to understand. Spontaneity makes it difficult to control in vitro, rapidity makes it difficult to get snapshots, and robustness makes it difficult to isolate crucial combinations of assembly-driving interactions. Most available experimental data on supramolecular assembly are labor and resource-intensive, making blind alleys extremely expensive. This generates a strong motivation to develop effective mathematical and computational models for supramolecular assembly that can inform further experimentation.

At the nanoscale level, supramolecular assembly is affected by the configurational entropy of small assemblies at inter-monomeric interfaces, driven by weak forces and non-covalent binding. The exact
computation of configurational considered a notoriously difficult problem in chemical theory and computational chemistry. All the methods reply on effectively compute volumes of configuration space regions \[1,2\], which usually can only be achieved by sampling. Monte Carlo and Molecular Dynamics can only be claimed as uniform sampling in the limit, when they “run for sufficiently long, or starting from sufficiently many initial configurations.” Methods based on the principal component analyses of the covariance matrices from a trajectory of samples in internal coordinates generally overestimate the volumes of configuration space regions with high geometric or topological complexity. Ab initio methods such as \[3\] based on geometric algebras are used to give bounds or to approximate configurational entropy without relying on Monte Carlo or Molecular Dynamics sampling. However, it is not clear how to extend them beyond restricted assembly systems such as a chain or loop of rigid molecular components each consisting of at most 3 atoms, where each component is noncovalently bound to the each neighboring component at exactly 2 sites.

For larger, microscale assemblies, they are treated as being recursively assembled as an interface assembly system, from small number of stable intermediate subassemblies \[4\]. The assembly model \[4\] based on the “local-rules” theory \[6–9\] compute the combinatorial entropy considering both the number of different assembly way and the microscale kinetics at each assembly stage. But it relies crucially on the simplified representation of the monomers and their geometric interactions.

The goal of this paper is to combine the nanoscale configurational entropy and the microscale combinatorial entropy to analyze the virus capsid assembly, by taking advantage of the recently reported geometric sampling method called EASAL (efficient atlasing and sampling of assembly landscapes) and the symmetry of the capsid. Then the entropy can be used to predict the assembly-crucial interactions.

2 Method for predicting assembly-crucial interactions

To understand the virus assembly process, we are interested in those crucial interactions whose removal disrupts the assembly. In the wet lab, one can mutate a specific residue to disable all interactions between this residue and others. The effect of the mutation on assembly efficacy was measured by the concentration of successfully assembled viral shells via cryo-electron microscopy. This approach is called mutagensis analysis. It is a very slow experiment. Testing less than 100 residues on a single virus takes more than 2 years.

Since entropy is closely related to the stability of and probability of the successful assembly, the removal of a crucial interaction should have substantial effect on the entropy. To predict the crucial

![Figure 1. Protein Capsid of Adeno-Associated Virus.](a)
interactions, we measure the change of the system entropy when removing certain interactions by sampling the configuration space.

For small systems, it is possible to sample the configuration space and evaluate the entropy directly. But for the virus capsid assembly system consisting of more than 60 monomers, the configuration space is too big to be sampled directly because of its high dimension. Instead we break the whole assembly into small interface assemblies recursively, and sample the configuration space of each sub-assembly separately.

2.1 Background: Sampling of configuration space using EASAL

2.1.1 Interface assembly system

A *interface assembly system* consists of (i) a small number $k$ (at most 10) rigid molecular motifs, each specified as the positions of the atom centers in a 3 dimensional local coordinate system; (ii) a potential energy function whose terms include sterics as Hard-Sphere potentials, weak inter-atomic interactions as Lennard Jones potentials, and implicit solvent terms [10]. A *interface assembly configuration* lives in a $6(k-1)$ dimensional cartesian space representing the rotations and translations of the local coordinate systems of $k-1$ of the rigid molecular motifs with respect to one fixed rigid motif. EASAL (Efficient Atlasing and Search of Assembly Landscapes) is our new suite of algorithms that completely maps configuration spaces with a high dimensionality and geometric complexity while providing provable guarantees. It leverages the following features.

2.1.2 Active geometric constraint regions

For the type of potential energy function described above, the geometry and topology of potential energy basins - that are of interest for an entropy computation - can be completely partitioned into *active geometric constraint regions*. I.e, regions of the configuration space that satisfy (bounds on) sets of inter-residue distances or angles (a restricted class of semi-algebraic sets). EASAL’s operation is based entirely on these constraints. The choice of geometric constraints depends on the potential energy function and partition into the approximately constant potential energy regions of the configuration space. Then each region is uniquely labeled by a set of geometric constraints that are explicitly active in that region.

2.1.3 Thom-Whitney Stratification, Convexification and Atlas

A classical way to describe a partition into active geometric constraint regions is as a topological complex using the so-called Thom-Whitney stratification of semi-algebraic sets [11]. Intuitively, the configuration space is partitioned into strata. Each stratum consists of active geometric constraint regions of the same effective dimension. The “children” of a given region are 1 lower dimensional regions obtained when one additional geometric constraint is satisfied or active. The zero dimensional regions thus consists of rigid configurations. In [12], we have shown that assembly configuration spaces can be *atlased*, i.e., they have convexifiable active constraint regions using so-called *Cayley*, or distance parameters, following a new theory developed by some of the authors [13][14], who also showed that cartesian configurations that correspond to a Cayley configuration can be computed efficiently using an optimally parametrized algebraic system [15].

2.2 Background: Entropy

The free energy landscape is crucial to understand the stability of the supra-molecular conformations and the transition between them. The free energy $F$ of a single energy well system is given by:

$$ F = -k_B T \ln Q $$
where $k_B$ is the Boltzmann’s constant and $T$ is the absolute temperature. And the partition function $Q$ is a integral over the energy well $w$:

$$Q = \int_w e^{-\frac{E(x)}{k_B T}} \, dx$$

The entropy $S$ is related to both free energy $K$ and average energy $\langle E \rangle$:

$$S = \frac{-F + \langle E \rangle}{T}$$

Over a region $C$ of constant Energy $E_c$ and volume $V_c$, the entropy is merely a function of volume:

$$S_c = k_B \ln V_c$$  \hfill (1)$$

where $V_c = \int_C dx$.

In a system of multiply energy wells $w_i$, each of which has constant energy $E_i$, the partition function of each energy well $Q_i$ can be expressed as a weighted sum of the energy wells’ volume $V_i$:

$$Q_i = \int_{w_i} dx \cdot e^{-\frac{E_i}{k_B T}} = V_i \cdot e^{-\frac{E_i}{k_B T}}$$ \hfill (2)$$

The normalized partition function $p_i$ is the probability of finding the system in the energy well $w_i$:

$$p_i = \frac{Q_i}{\sum_i Q_i}$$ \hfill (3)$$

2.3 EASAL based approximation entropy-change to predict interface assembly-crucial interactions

For an interface assembly system, the energy of the configuration is determined by the number of active constraints: the potential energy is the sum of energy terms of each constraint and when a constraint gets activated, its corresponding potential energy term will be at minimum. When sampling using EASAL, the active constraint regions of lower dimension have a higher number of active constraints, hence their energy level is also lower. Specifically, zero dimensional active constraint regions are local minima on the energy landscapes and they locate around the center of energy wells. These energy wells represent states that the system may end up with during assembly. Among these energy wells one one corresponds to the successfully assembly state. Its center is the “true realization” that represents the successful assembly.

The stability of the system and the likelihood of the successful assembly are related to two properties of the energy landscape, both of which can be approximated by the output of EASAL:

**Volume of low energy regions** The volume of the energy well can be approximated by the number of distinct Cartesian realizations. Given a Cartesian space threshold distance $\varepsilon$, two realizations will be counted as two distinct ones if and only if their distance is larger than $\varepsilon$. Assuming the EASAL samples are dense enough in Cartesian space, the volume of an active constraint region can be approximated by the count of distinct realizations. In what follows, we refer the count of distinct realizations in all the 0-d active constraint regions as “distinct copies”.

**Normalized partition function** As shown in in Eq. (2), the partition function of the energy well is the weighted sum of volumes. While the volume can be approximated by the count of distinct realizations, the weight is the Boltzmann factor calculated using the energy level that is determined by the number of active constraints. For systems whose potential energy has $N$ Lenard Jones terms, each of which has
active energy level $E_l$ and non-active energy level $E_h$, the potential energy of a realization with $N_a$ active constraints is:

$$E = N_a E_l + (N - N_a) E_h = N E_h - N_a (E_h - E_l)$$

its corresponding Boltzmann factor is:

$$e^{-\frac{E}{kT}} = e^{-\frac{N E_h + N_a (E_h - E_l)}{kT}} = C \cdot (e^{\frac{E_h - E_l}{kT}})^{N_a}$$

where $C = e^{-\frac{N E_h}{kT}}$ is a constant of the system and will be canceled out when calculating the normalized partition function. So the equivalent weight is:

$$w(N_a) = (e^{\frac{E_h - E_l}{kT}})^{N_a}$$

We use the typical Lennard Jones potential depth for Argon atoms that $E_h - E_l = 0.997 \text{kJ/Mol}$\cite{10}:

$$w(N_a) \approx 1.5^{N_a}$$

With the volume and weight calculated, the normalized partition function is computed using Eq. (2) and (3):

$$Q_{true}^* = \frac{\sum_{\mathbf{x} \in R_{true}^*} w(N_a(\mathbf{x}))}{\sum_{\mathbf{x} \in R_0^*} w(N_a(\mathbf{x}))}$$

where $R_{true}^*$ is the set of distinct realizations within the energy well of “true realization” and $R_0^*$ is the set of distinct realization of all 0-d active constraint regions. In what follows, we refer to $Q_{true}^*$ as “weighted ratio”.

These two criteria “distinct copies” and “weighted ratio” measures the change of system entropy when removing an interaction. The interactions are removed one at a time and we predict the crucial ones based on the change of the two criteria.

2.4 Statistical model for combinatorial entropy

We describes how different combinations of interface assemblies can lead to the capsid assembly by path: the minimal set of interfaces that are led to successful assembly. Fig. 2 shows a successful assembled T=3 virus capsid generated by a path and an incomplete capsid generated by a set of interfaces that is not a path. The crucialness of an interface is related to which path it participates, its impact on each path and the probability that the assembly will take these paths.

Enumerating all the paths  A simple graph algorithm can be used to check whether a set of interface types is a path. We represent the capsid as a graph $G = (V, E)$ where every vertex $v \in V$ represents a monomer and every edge $e \in E$ represents an interface. A mark of edge $M(e) = I$ is defined by the type of each interface $I$. For a set of interfaces type $\{I_i\}$, if the graph $G' = (V, E')$ where $E' = \{e | M(e) \in \{I_i\}\}$ is connected, than $\{I_i\}$ is a path. Given the limited number of interface types, we can find all paths by enumerating and checking all interface type sets.

Statistical model  Given the crucialness of interactions within each interface and all the possible paths of the successful assembly, we use a statistical model to measure the impact of removing each interaction on the whole capsid assembly. The model consists of the following parts:

- For each interface $I$, the probability of breaking the interface when dropping an interaction $r$ is $P_r^I$. This is a measure by the two criteria “distinct copies” $c_{true}$ and “weighted ratio” $r_{true}$. A simple linear model is used to describe the relation:

$$P_r^I = sigmoid(a_I \cdot c_{true} + b_I \cdot r_{true} + c_I)$$
The probability of breaking a path when dropping an interaction can be approximated via the equation:

$$C^r_p = 1 - \prod_{I \in p} (1 - w_I \cdot P^r_I)$$  \hspace{2cm} (4)$$

where $w_I \in [0, 1]$ is the weight to describe the importance of an interface within a path. When $w_I = 0$, the corresponding term in Eq. (4) will vanish. And the interaction with bigger $P^r_I$ will have bigger chance of breaking the path.

The overall impact on the whole capsid assembly is measured by an impact function. It is the sum of the probability over all the paths.

$$H(r) = \sum_p C^r_p$$

**Determining the model parameters**  In the above model, $a_I$, $b_I$, $c_I$ and $w_I$ are all unknown parameters. We decide their value via training. For a given partial order over the interactions $T = \{(r_i, r_j) : r_1 \text{ has bigger impact on the capsid assembly than } r_2\}$, the impact function should preserve such partial order that, for a pair of residues with known order $(r_1, r_2) \in T$, their impact functions should satisfy $H(r_1) > H(r_2)$. We design a loss function:

$$L = \sum_{(r_i, r_j) \in T} \text{sigmoid}(H(r_i) - H(r_j))$$

When the impact function satisfy the partial order, the loss function will be minimal. So the parameters can be determined by:

$$\arg \min_{a_I, b_I, c_I, w_i} L$$
2.5 Data and Validation Methodology

We run our analysis on both T=1 (AAV2 and MVM) and T=3 viruses (BMV) and verify the result with mutagenesis experiment.

**Input of EASAL**  For the analysis of the interface assembly system, the 3D structure of monomers and the “true realization” of each interface are extracted from the known X-ray structure of virus coat protein [17][18]. The monomers are treated as rigid bodies with no internal deformation. The potential energy of an interface assembly includes the hard sphere potential between all atoms with the Van der Waal’s radius set to 1.2 Å. Although theoretically the potential energy should include the Lennard Jones potential of all atom pairs, we believe that only the set of atom pairs that are close enough to interact and also conserved in related viruses could have major contribution to the entropy. We refer these 10-20 interactions as the candidate interactions of each interface. Both the monomer 3d structures and candidate interactions are input of the EASAL algorithm for sampling the configuration space.

**Mutagenesis analysis result**  Mutagenesis analysis results are used to validate our prediction of crucial interactions. In a mutagenesis experiment, a mutation on a residue will prevent all its atoms from establishing interaction with others. This is equivalent to removing all interactions of a residue from the assembly system. The residues are classified by the yield of successfully assembled capsid compared to wild type after the mutation: 100% means the mutation has no affect on the assembly and the residue will be marked as non-crucial; 0% means the assembly is completely disrupted, the residue will be marked as crucial.

To validate our prediction with the mutagenesis analysis result, we analyze the effect of mutating a specific residue by sampling the configuration space after removing not one, but all the candidate interactions that have one end on the residue. We directly compare the prediction with the mutagenesis result without considering the configurational entropy. This comparison is reasonable especially for T=1 virus since the total number of different paths is small.

**Training set for learning the statistic model**  To take into account the combinatorial entropy of the whole capsid assembly we use the statistical model from Section 2.4. A training set of partial order over residues is needed to determine the unknown parameters. This could be directly extracted from the mutagenesis result by comparing the capsid yield after mutating two residues. The ordered pairs of residues with defined partial order are split into two parts, one serves as the training set to determine the parameters of the model, and the other will be used to evaluate how well the impact function preserve the partial order.

3 Results

3.1 Prediction based on configuration space analysis of single interface

Fig. 3 shows the change of each interface’s configuration space measured by the two criteria of EASAL’s output. Both criteria are scaled so that the value corresponds to the wild type system are 1.0. Every residue is marked by the mutagensis result indicating whether it is crucial.

For most of the interfaces, there are not enough results from wet lab that can be used to evaluate our prediction. But for those interfaces who do have enough data, for example the pentamer of AAV, the 2d plot shows a good separation between the crucial and the non-crucial residues excluding few outliers. Overall the crucial residues have relatively low weighted ratio and high distinct copies.
Adding extra interactions  To overcome the problem of insufficient lab result, and also to remove possible bias introduced when picking the candidate interactions, we add 2 more candidate interactions to the system that are not picked at the first place hence are not likely to be crucial, and do the analysis again. The result for the T=1 viruses are shown in Figure 4. The convex hull of crucial residues are marked as blue region and the extra residues are marked as yellow. Overall the added residues have higher weighted ratio than the crucial residues and similar distinct copies.

Note concerning the left out interface  As shown in the figures, some interfaces are left out: trimer interfaces for T=1, T=3 virus and hexamer interfaces for T=3 virus have no predictions. For trimer interfaces, we could not obtain useful crucialness rankings due to heavy influence of steric caused by interdigitation. This tallied with the fact that mutagenesis of the trimer interface interactions could not disrupt assembly. We do not believe that assembly of the capsid is sensitive to any of the trimer interactions. And for the hexamer interface of BMV, most of the residues cross-link to the RNA hence have no effect on assembly. We conjecture that the assembly proceeds primarily by dimeric and pentameric interface interactions. Trimers interdigitate and contribute to stability of the capsid after the assembly is complete.

3.2 Prediction based on statistic model

The Fig. 5 shows the residues ranked by the impact function calculated using the statistical model for AAV, MVM and BMV. The residue listed higher in the table have bigger impact function value. Mutagenesis result are used to mark all the residues by color coding. Blue means the residue is crucial while red means non-crucial. White means no mutation has been done on the residue. Our model
Figure 4. 2D plot of residue crucialness for each interface with extra interactions

successfully preserve the overall partial order of the residues.

Note about the ranking for MVM For MVM there is no “non-crucial” residues in the mutagenesis result, hence no partial order can be extracted from it directly to train the model. To overcome this problem, we manually label several “non-crucial” residues for each interface that diverge most from those known “crucial” residues in terms of the selected criteria.

3.3 Computational Time and Resources

All the analysis are done using the C++ implementation of EASAL. To test on a 3.2 Ghz i5-2500K CPU with 8GB memory. The program runs 1.5 hours to sample the configuration space of the dimer interface assembly system of AAV. It uniformly sample the Cayley space, covering 21.6 million Cayley configurations and 173 million Cartesian realizations. Among them there are 941 thousand realizations are feasible without collision.

4 Discussion

Our crucial interaction prediction based on configuration space sampling and configurational entropy calculation classify the residues by their crucialness within a interface system. And for the whole capsid assembly, the statistical model ranks the residues based on the prediction for each interface and the combinatorial entropy of the big system. The prediction is validated by the mutagenesis analysis result and methods are shown to be effective and efficient.

There are several observations we made during the development of the method that may lead to future work.
Figure 5. Rank of residues generated by the statistical model. The residue listed higher in the table have bigger impact function value. Mutagenesis result are used to mark all the residues by color coding. Blue means the residue is crucial while red means non-crucial. White means no mutation has been done on the residue. A) AAV. B) MVM. C) BMV.

Extra interactions The candidate interactions that serve as the input of EASAL are hand picked and pre-screened. Some interactions are excluded because they are not likely to be crucial based some prior experience and some are excluded since no mutation on that residue is possible for now. This could potentially introduce bias in that the picked interaction are already likely more crucial than the others. In addition, since other non-crucial interactions also contribute to the potential energy, ignoring them will change the energy landscape. For better analysis, an extended set of candidate interactions should be used as the input of our sampling method.

Rigidity of the 3-5 fold interfaces For the 3 and 5 folds interface system, we only consider its sub-system that consists of two neighboring monomers. Despite the symmetry of the multi-fold system, the rigidity property of the complete system is different from the sub-system of only two monomers. We expect better prediction base on the analysis of the complete multi-fold system.

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