

An equilibrium assembly model applied to Murine Polyomavirus

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In Keef *et al.*, Assembly Models for Papovaviridae based on Tiling Theory (submitted to *J. Phys. Biol.*), 2005 [1] we extended an equilibrium assembly model to the (pseudo-) $T = 7$ viral capsids in the family of Papovaviridae providing assembly pathways for the most likely or primary intermediates and computing their concentrations. Here this model is applied to Murine Polyomavirus based on the association energies provided by the VIPER web page Reddy *et al.* “Virus particle explorer (VIPER), a website for virus capsid structures and their computational analyses”, *J. Virol.*, 75, pp. 11943–11947, 2001.

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1. Introduction

Papovaviridae are of particular interest to mathematical biologists because they do not fit strictly into the theory of quasi-equivalence [2] because they comprise structures composed of 72 pentamers instead of a combination of pentamers and hexamers. For examples see [3–5], and for an example of packing of pentagons on the sphere see [6]. It has been shown that the surface structure of the capsids can be described by tiling theory, using kites and rhombs to tile the surface instead of quasi-equivalent triangles [7,8]. Since these tiles also provide information about intersubunit bonds, and in particular about the local environment around individual subunits, they are biologically significant and can be used as building blocks for assembly models of viral capsids.

Various models for capsid assembly have been considered, including models based on local rules [9], an equilibrium model [10], models based on molecular dynamics [11], a thermodynamical approach [12] and combinatorial approaches [13]. Our model combines local information provided by the tiles with the equilibrium model pioneered by Zlotnick. We have extended the model to the larger capsids in the family of Papovaviridae, that need to be represented via a more involved assembly tree with multiple possible assembly pathways. A short review of this approach is provided and applied to Murine Polyomavirus.

2. Equilibrium assembly models

In 1994, Zlotnick provided an assembly model for a small plant virus formed from twelve pentamers [10]. This model assumes that all 30 edge to edge contacts between the pentamers are identical and that the final capsid has dodecahedral symmetry. Incoming pentamers are added one at a time and only the most stable intermediate is considered at each iteration step during assembly. From a set of rate equations it is possible to predict concentrations of the assembly intermediates at equilibrium based on the concentration of the pentameric building blocks.

In our extended model we include the possibility of multiple incoming subunits with different association energies between the contacts [1]. We again derive the concentrations of assembly intermediates along similar lines, but also include the possibilities for branching in the assembly pathway.

First we redefine the association constant, a ratio of concentrations of assembly intermediates, to include the possibility for multiple incoming subunits:

$$K_n = \frac{[n]}{[n-1] \sum_{i=1}^a [1_i] \delta_{i,x(n)}} = S_{x(n)} S_n K'_n, \quad (1)$$

where $x(n)$ corresponds to one of the $i = 1, \dots, a$ possible different subunits added in iteration step n , i.e. $\delta_{i,x(n)} = 1$ if $x(n) = i$ and zero otherwise. S_i is defined to be the geometric degeneracy of subunit $x(n)$, for instance,

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a pentagon has five-fold rotational symmetry, hence in Zlotnick's model all incoming subunits have $S_i = 5$. S_n is defined as the ratio of the orders of discrete rotational symmetries of the intermediate at steps $n - 1$ and n , i.e. of $O_{\text{sym}}(n - 1)$ and $O_{\text{sym}}(n)$:

$$S_n = \frac{O_{\text{sym}}(n - 1)}{O_{\text{sym}}(n)}. \quad (2)$$

K'_n is the non-statistical association constant and is a function of the number $\alpha_j(n)$ of contacts formed with association energy ΔG_j^o ($j = 1, \dots, k$):

$$K'_n = e^{-\frac{\sum_{j=1}^k \alpha_j(n) \Delta G_j^o}{RT}}, \quad (3)$$

where R is the gas constant ($1.987 \text{ cal deg}^{-1} \text{ mol}^{-1}$), and T is the temperature in Kelvin, set to room temperature (298 K).

Equation (1) can be rearranged to give an equation for the concentration of the assembly intermediate at iteration step n in terms of the intermediate at iteration step $n - 1$:

$$[n] = S_i S_n K'_n [n - 1] \sum_{i=1}^a [1_i]^{\delta_{i,x(n)}} = \Omega(n) [n - 1], \quad (4)$$

where

$$\Omega(n) = \sum_{i=1}^a S_i [1_i]^{\delta_{i,x(n)}} S_n e^{-\frac{\sum_{j=1}^k \alpha_j(n) \Delta G_j^o}{RT}}. \quad (5)$$

In order to determine the concentration $[n]$ in terms of the concentration of the basic subunits $[1_i]$, equation (4) needs to be applied recursively. For this, information is required on the assembly pathway that connects different assembly intermediates.

For larger viral capsids, assembly does not always follow a strict path of stable intermediates, and at some steps of an assembly pathway there may be multiple choices of combining incoming subunits with the assembly intermediate at the previous step based on the association energies of the bonds, which leads to branching of the assembly pathways. Subsequent steps could involve further branches, and this information is encoded in the assembly tree. In [1], we show that it is possible to reduce the factor $\Omega(n)$ to a simple formula, if n and $n - 1$ are primary intermediates, i.e. intermediates located on all paths in the assembly tree. One hence obtains a recurrence relation for the concentration of the n -th primary intermediate in terms of the $(n - s)$ th primary intermediate as:

$$\begin{aligned} [n] &= \Omega(n - s + 1) \Omega(n - s + 2) \dots \Omega(n - 1) \Omega(n) [n - s] \\ &= \left(\prod_{i=1}^{s-1} \Omega(n - i) \right) [n - s], \end{aligned} \quad (6)$$

with $\Omega(n)$ as in equation (5).

3. Application to Murine Polyomavirus

In this section this set-up is applied to Papovaviridae, and in particular to Murine Polyomavirus. (Pseudo-) $T = 7$

capsids in this family are composed of 360 protein subunits arranged into 72 pentamers with 12 pentamers at the points of global five-fold symmetry. The locations and orientation of the pentamers are predicted by tiling theory, and the locations of the inter-subunit trimer and dimer bonds are described by kites and rhombs as explained in R. Twarock, "The architecture of viral capsids based on tiling theory", pp. 91–93, same volume.

We assume that all pentamers assemble first in solution and then come together to form the final capsid. When assembled there are two possible configurations for pentamers (called vertex stars in tiling theory), one at the global five-fold axes of symmetry, and one for pentamers elsewhere. These are shown in figure 1, together with the inter-subunit bonds. The building blocks are created by cutting all inter-subunit bonds perpendicularly.

We assume that in solution all pentamers are identical and choose one of the two vertex stars on contact with the assembling capsid.

For our model we need the association energies of each of these edges, and we define them as follows:

- We denote the association constant corresponding to a single C-terminal arm in a trimer (represented by a kite in the tiling model as shown in figure 1) between the two different types of pentamer as a . Correspondingly, all five edges of the pentagonal building blocks at the five-fold axes have an association constant of $2a$.
- b labels the C-terminal arm in a trimer between the secondary pentamers (those not located on five-fold axes). This is indicated by the bond between the purple and green subunits in the figure.
- c labels the association constants related to quasi-dimer bonds. They are located around the three-fold axes of the tiling and are shown as rhombs with red and blue decorations in the figure.
- d labels the association constants corresponding to strict dimer bonds along the global two-fold axes of the tiling. They correspond to the rhombs with yellow decorations in the figure.

For most viruses, all association energies within the trimer are the same and in these cases we set $a = b$. In [1] the case of SV40 has been discussed in detail. Here Murine Polyomavirus is considered.

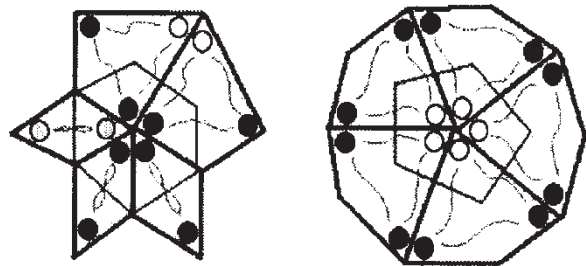


Figure 1. The building blocks for assembly of vertex-star models.

Table 1. Primary assembly intermediates for murine polyomavirus along with symmetries, incoming bonds and concentrations at equilibrium based on a pseudo critical concentration of 1.072×10^{-385} mol.

n	$O_{\text{sym}}(n)$	Bonds formed	$[n]$
1	5	–	1.072×10^{-385}
2	2	d	5.597×10^{-609}
3	1	$b + c$	7.600×10^{-765}
4	1	$2c$	1.811×10^{-845}
5	1	$4a$	2.326×10^{-936}
9	1	$6a + 2b + 3c + d$	1.879×10^{-1265}
10	2	$2a + b + d$	3.652×10^{-1267}
11	1	$b + d$	5.798×10^{-1415}
12	1	$2a + b + c$	1.927×10^{-1424}
13	1	$2c$	4.592×10^{-1505}
14	1	$2a + 2b$	4.349×10^{-1590}
15	1	$b + c + d$	2.302×10^{-1586}
16	1	$c + d$	6.415×10^{-1659}
18	1	$2b + 4c$	2.630×10^{-1668}
38	1	$36a + 15b + 15c + 8d$	1.246×10^{-2012}
39	3	$2a + b + d$	1.615×10^{-2014}
40	1	$2a + b$	4.588×10^{-2099}
41	1	$b + c + d$	2.429×10^{-2095}
42	1	$6a$	1.528×10^{-2039}
43	1	$2a + b + c$	5.079×10^{-2049}
44	1	$c + d$	1.415×10^{-2121}
50	2	$12a + 7b + 5c + 2d$	1.654×10^{-1934}
51	1	$c + d$	9.216×10^{-2007}
62	2	$20a + 10b + 11c + 5d$	7.820×10^{-1535}
63	1	$b + 2c$	7.081×10^{-1539}
64	1	$2a + 2b + d$	5.229×10^{-1464}
65	1	$6a$	3.290×10^{-1408}
66	1	$b + c + d$	1.741×10^{-1404}
67	1	$2a + b + 2c$	3.860×10^{-1262}
68	1	$2a + 2b + d$	2.851×10^{-1187}
69	1	$2a + 2b + c$	1.801×10^{-1120}
70	1	$b + 2c + d$	6.356×10^{-965}
71	5	$2b + 2c + d$	8.527×10^{-734}
72	60	$10a$	1.071×10^{-385}

The association energies for the bonds can be found on the VIPER website [14]: $a = -100 \text{ kcal mol}^{-1}$, $b = -104 \text{ kcal mol}^{-1}$, $c = -207 \text{ kcal mol}^{-1}$, and $d = -218 \text{ kcal mol}^{-1}$. Based on these values, one obtains the pathway of primary assembly intermediates for MPV. Table 1 shows where in the assembly pathway primary nodes occur, and indicates information on the symmetries, new bonds formed and concentrations for each. The concentrations are based on the critical concentration

where the concentration of subunits in solution is equal to that of the complete capsid. It shows that the concentration of intermediates is very small as with the Zlotnick case.

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