Density waves theory of the capsid structure of small icosahedral viruses

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We apply Landau theory of crystallization to explain and to classify the capsid structures of small viruses with spherical topology and icosahedral symmetry. We develop an explicit method which predicts the positions of centers of mass for the proteins constituting viral capsid shell. Corresponding density distribution function which generates the positions has universal form without any fitting parameter. The theory describes in a uniform way both the structures satisfying the well-known Caspar and Klug geometrical model for capsid construction and those violating it. The quasiequivalence of protein environments in viral capsid and peculiarities of the assembly thermodynamics are also discussed.

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Viruses represent rather simple biological systems which can be studied by different chemical and physical methods. Their organization and functioning show a number of universal features. The viral protein shell (capsid) encloses the genetic material (either desoxyribonucleic acid (DNA) or ribonucleic acid (RNA) [1]) responsible for the infective properties of the viruses. The capsid serves both to preserve and to transmit the genetic material to an appropriate host cell. Soon after the transmission the host cell starts the reproduction of the viral DNA (or RNA) and capsid proteins. From shell proteins and replicated genomes, new identical copies of the viruses spontaneously assemble. Though the final infective virus structure formation involves biologically specific events, some steps of the self-assembly demonstrate properties typical for ordering in passive physical systems. The host cell is not necessary for the viral capsid formation. The self-assembly does not need active local energy consumption like ATP hydrolysis and the process can be reversible [2,3]. Moreover, in many cases the viral shells assembling does not even need genomes and proceeds in vitro in purified protein solutions [1].

The problem of the capsid structure formation attracts the attention of physicists since fifty years. In their pioneer work Crick and Watson [4] stated that spherical viruses should have the symmetry (but not necessarily the structure) of one of regular polyhedra with the faces formed by identical perfect polygons. Later in 1962, Caspar and Klug (CK) argued that spherical capsids adopt icosahedral point symmetry [5]. They have seen the physical reason why the Nature prefers this type of symmetry in the fact that the icosahedron has the largest volume-to-surface ratio among the regular polyhedra. Besides, CK obtained four prominent results [5]: i) The capsid symmetry is lower than that of the regular icosahedron since the proteins are asymmetric. Identical asymmetric building blocks can compose the structures with rotational symmetry elements only, excluding inversion and mirror planes. ii) The asymmetric proteins can be located only in regular (trivial) 60-fold positions of the rotational icosahedral point group, therefore the total number of proteins in a capsid is always equal to 60N, where N is a positive integer number. iii) CK concluded, for the first time, that ‘the self-assembly is a process akin to crystallisation and is governed by the laws of statistical mechanics’. iv) They proposed a geometrical model for the viral capsid construction based on the properties of the almost regular mapping of the 2D hexagonal structure on the icosahedron surface. Specific properties of the model impose the selection rules for the value of N (and, consequently, for the total number of proteins in the shell). Only the values which satisfy the relation \( N = h^2 + k^2 + hk \), where h and k are non-negative integers are allowed by the CK selection rules. All four points and their direct consequences resulted in the principle formulated by CK and put in the basis of modern virology. Though a big number of virus capsid structures are in a good agreement with all the points of the CK scheme, there is a growing number of experimentally resolved structures which do not satisfy the CK selection rules nor their predictions about local proteins arrangement [6]. These facts show that point iv) of the principle is not universal and needs to be generalized.

In recent years the investigation of capsid structures has undergone a real burst due to the progress of the X-ray and cryoelectron microscope techniques and micromechanical experiments [7]. From the theoretical point of view the main effort was done in two directions (see [8]). On the one hand, the mean-field studies of simple model systems were performed in order to approach the thermodynamics of the self-assembly process. On the other hand, the mechanical properties of capsids and their relation to the capsid shape were investigated. Along the first line, the free energy of the viral structure has been approximated by that of a model system consisting of two types of disks located on the spherical surface [9]. The proposed pair potential of the disk interaction favors the icosahedral symmetry of the disk packing [9] provided an optimisation of several model parameters. Along the second line, the possible buckling instability of the spherical capsid structure was studied in the frame of the nonlinear physics of thin elastic shells [10]. The results of this study explain why the relatively small viruses are always spherical while the larger ones have a more
angular or faceted shape. In addition, for large viruses the use of continuum elasticity approximation can be justified. This makes the predictions of the mechanical properties [11] of viral capsids and their large-scale shape details [12] more universal. Nevertheless, the results obtained depend crucially on the model assumptions concerning the explicit form of interaction between proteins or groups of proteins (capsomers). Let us also note that all recent theoretical works on the capsid structure do not take into account the asymmetry of capsid proteins nor the restrictions on the capsid symmetry formulated in points i) and ii) of the CK principle. By contrast, the nonuniversal CK selection rules (point iv)) are taken as an ingredient in all models.

In the present work we propose to apply the Landau theory of crystallization to the problem of small capsid formation. Resulting approach to the icosahedral virus structure accounts explicitly for the protein symmetry and satisfies points i)-iii) of the CK principle but it is free of nonuniversal CK selection rules. It allows us to describe in a uniform way all experimentally observed small spherical viruses including those which can not be obtained using the CK geometrical model (e.g. L-A virus, Dengue virus, West Nile virus, Murine Polyoma virus, etc.)

Both the experimental data and the theoretical consideration [10] show that the shape of small viruses with the icosahedral symmetry is close to the spherical one. This fact gives the possibility to consider the crystallization on a spherical surface and to avoid the problems arising in the CK geometrical construction during the mapping of planar hexagonal structures upon the icosahedron surface. Like in the case of usual 3D crystal solidification [13] Landau theory of the assembly process gives simple and clear predictions in the vicinity of crystallization point. In this region the probability density $\rho$ of protein distribution in the capsid structure is presented as:

$$\rho = \rho_0 + \Delta \rho,$$

where $\rho_0$ is an isotropic density in the solution and $\Delta \rho$ corresponds to the density deviation induced by the ordering. The symmetry breaking during the crystallisation is associated with one critical order parameter which spans an irreducible representation of the symmetry group of the disordered state. In addition, in the vicinity of crystallization point, the structure of the ordered state (defined by $\Delta \rho$) is determined by the critical order parameter only, the contribution of non-critical degrees of freedom being negligible in this region. For the crystallization process the order parameter represents a critical system of density waves (CSDW) with the wave vectors of the same length and the transition free energy is an invariant function of the CSDW amplitudes [13]. The symmetry of crystals which condense from the isotropic state coincides exactly with that of the corresponding CSDW. For crystals of metals (and especially for the crystals of elements) the atomic positions in the vicinity of crystallization point can be then associated with the positions of maxima of the CSDW.

The same principles are applied here to the assembly process on a sphere. The critical part $\Delta \rho_l$ of the density is determined by a CSDW with the same wave number $l$. The spherical harmonics $Y_{lm}$ constituting CSDW on a sphere span one irreducible representation (IR) of the $SO(3)$ symmetry group of the disordered state, thus $\Delta \rho_l$ is given by:

$$\Delta \rho_l(\theta, \phi) = \sum_{m=-l}^{l} A_{lm} Y_{lm}(\theta, \phi),$$

where $l$ is the IR number, $A_{lm}$ are the amplitudes of the spherical harmonics $Y_{lm}$ and $\theta$ and $\phi$ are the conventional angular variables of the spherical coordinate system.

According to points i) and ii) of the CK principle the ordered distribution of proteins in the viral capsid has the symmetry group $I$ of the icosahedron rotations which does not contain spatial inversion nor mirror planes. This restriction is of major importance in the proposed theory. It selects the parity of the ‘active’ IR’s of the $SO(3)$ symmetry group which induce the assembly of icosahedral shells of asymmetric proteins. Thus the spherical harmonics $Y_{lm}$ with even $l$ numbers cannot form critical density (2) for viral capsids. The restriction affects also the free energy expansion of the assembly process taken in a standard for the crystallization theory form [13] $F = F_0 + F_2 + F_3 + F_4 + ...$ and containing invariant terms

$$F_2 = A(T, c) \sum_{m=-l}^{l} A_{l,m} A_{l,-m},$$

$$F_3 = B(T, c) \sum_{m_1, m_2, m_3} a_{m_1,m_2,m_3} A_{l,m_1} A_{l,m_2} A_{l,m_3} \delta(m_1 + m_2 + m_3) \equiv 0,$$

$$F_4 = \sum_k C_k(T, c) \sum_{m_1, m_2, m_3, m_4} a^k_{m_1,m_2,m_3,m_4} A_{l,m_1} A_{l,m_2} A_{l,m_3} A_{l,m_4} \delta(m_1 + m_2 + m_3 + m_4),$$

where $a_i$ are weight coefficients of the $SO(3)$ group (e.g. Clebsch-Gordan coefficients for the third order term $F_3$), $\delta(0) = 1$, $\delta(i \neq 0) = 0$, $A(T, c)$, $B(T, c)$, and $C_k(T, c)$ are temperature- and composition-dependent coefficients.
of the Landau theory. For any odd wave number \( l \) the third-order term \( F_3 \) is identically zero. This fact makes the thermodynamics of asymmetric proteins assembly quite different with respect to the thermodynamics of 3D icosahedral atomic clusters formation [14] in spite of several common points in formal description.

Next restriction on the choice of order parameters of the capsid formation comes from the fact that \( \Delta \rho_l \) function with \( I \) symmetry can be constructed not for all but for particular odd \( l \) numbers only. The analysis based upon the theory of invariants shows that any critical order parameter which drives the icosahedral assembly of asymmetric proteins has the wave number \( l \) satisfying the relation:

\[
l = 15 + 6i + 10j, \tag{4}
\]

where \( i \) and \( j \) are positive integers or zero. Eq. (4) defines the list of \( l \) numbers for which the restriction of an IR of the SO(3) group on the icosahedral group \( I \) contains at least one totally symmetric representation. The sequence of the permitted values of the wave number \( l \) is given by: \( l = (15, 21, 25, 27, 31, 33, 35...) \). As we show below this sequence determines possible capsid shell structures for small icosahedral viruses. Selection rule (4) gives the possibility to obtain the explicit form of critical density (2). Then the protein centers are associated with the positions of maxima of \( \Delta \rho_l \) function (2). Thus the density wave approach replaces nonuniversal geometrical model iv) of the CK principle.

The explicit form of the critical density function \( \Delta \rho_l(\theta, \phi) \) is given by the basis functions \( f_i^l(\theta, \phi) \) \( (i = 1, 2...n_t) \) of all \( n_t \) totally symmetric representations of the icosahedral group \( I \) in the restriction of the 'active' IR of the SO(3). The CSDW is a linear combination of these functions invariant with respect to the \( I \) group:

\[
\Delta \rho_l(\theta, \phi) = \sum_{i=1}^{n_t} B_i f_i^l(\theta, \phi), \tag{5}
\]

where \( B_i \) are arbitrary coefficients.

Their number \( n_t \) is equal to the number of integer non-negative solutions \((i, j)\) of Eq. (4) for a fixed permitted value of \( l \). Another way to calculate \( n_t \) is to use the well-known relations of characters [15]:

\[
n_t = 1/|G| \sum \xi(\hat{g}) \tag{6}
\]

where the sum runs over the elements \( \hat{g} \) of the \( I \) group, \( |G| = 60 \) is the \( I \) group order, and \( \xi(\hat{g}) \) is the character of the SO(3) group element which reads as [15]:

\[
\xi(l, \alpha) = \frac{\sin((l + 1/2)\alpha)}{\sin(\alpha/2)},
\]

where \( l \) is the IR number and the angle \( \alpha \) is determined by the element \( \hat{g} \). Then the explicit form of (6) becomes:

\[
n_t(l) = \frac{1}{60} (2l + 1 + 15\xi(l, \pi) + 20\xi(l, 2\pi/3) + 12\xi(l, 2\pi/5) + 12\xi(l, 4\pi/5)). \tag{7}
\]

For small icosahedral capsids the practical construction of the protein density distribution is simplified because the CSDW (5) contains only one function \( f_1^l(\theta, \phi) \). Indeed, according to Eq. (4) and/or Eq. (7) \( n_t = 1 \) for all \( l \leq 43 \). In this simplest case \( \Delta \rho_l(\theta, \phi) = B f_1^l(\theta, \phi) \), where \( B \) is a single arbitrary coefficient. The positions of maxima of the density function do not depend on the value of \( B \). They are generated by a single universal function \( f_1^l(\theta, \phi) \) which has no any fitting parameter. In the following consideration the functions \( f_1^l(\theta, \phi) \) possessing this properties are called irreducible icosahedral density functions and the structures generated by \( f_1^l(\theta, \phi) \) are mentioned as irreducible icosahedral structures. The explicit form of the irreducible density function \( f_1^l(\theta, \phi) \) for a given value of \( l \) is obtained by averaging of \( Y_{l,m}(\theta, \phi) \) harmonics over the \( I \) symmetry group [16].

\[
f_1^l(\theta, \phi) = \frac{1}{60} \sum_{G} Y_{l,m}(\hat{g}(\theta, \phi)). \tag{8}
\]

For any fixed value of \( m \), procedure (8) gives either the same function \( f_1^l(\theta, \phi) \) we are looking for, or zero. Functions which differ by a constant complex multiplier are considered the same.

Fig. 1 resumes the irreducible density functions \( f_1^l(\theta, \phi) \) permitted by selection rule (4) for the five smallest icosahedral capsids (Fig. 1(a-e)): the function \( f_{37}^l(\theta, \phi) \) (Fig. 1(f)) is added as an example illustrating protein density distribution with higher \( l \). The value of \( f_1^l(\theta, \phi) \) is represented using false color image: variation of colors from red to violet corresponds to the function growth. Note that all \( f_1^l(\theta, \phi) \) functions are anti-symmetric: they change their sign under the inversion of all coordinates or under the action of mirror planes of a regular icosahedron. Thus, for...
the sake of clariy, we present the positive part \( f_l(\theta, \phi) > 0 \) only. The number of maxima of the density functions is equal to \( 60N \), where \( N \) is the number of different regular 60-fold positions of the I group. In the viral capsid \( N \) corresponds to the number of different positions occupied by the proteins. Let us stress that in a sharp contrast with the CK geometrical model the crystallization theory predicts the existence of capsids with all positive integer values of \( N \) and not only for \( N = h^2 + hk + k^2 \). Functions \( f_l(\theta, \phi) \) generate in a uniform way protein distributions which can be obtained by the CK mapping of the hexagonal lattice on an icosahedron and those which can not. On the one hand, the distributions in Fig. 1(a) \( (l = 15, N = 1) \), Fig. 1(d) \( (l = 27, N = 3) \), and Fig. 1(e) \( (l = 31, N = 4) \) give classical CK structures. The positions of protein centers in a big number of viral capsids are described by these structures. Fig. 2 shows the correspondence between the maxima of \( f_{15}, f_{27}, \) and \( f_{31} \) and the protein arrangement in Satellite Tobacco Mosaic virus (Fig. 2(a)), Cowpea Chlorotic Mottle virus (Fig. 2(b)) and Sindbis virus (Fig. 2(c)), respectively. On the other hand, the distributions in Fig. 1(b) \( (l = 21, N = 2) \) and in Fig. 1(f) \( (l = 37, N = 6) \) do not satisfy the CK selection rules for \( N \) number. The distribution in Fig. 1(c) \( (l = 25, N = 3) \) shows no hexagonal arrangements of protein positions and can not be obtained by the CK geometrical model, though the number of protein positions \( N \) satisfies the CK selection rules. In addition, the comparison of distributions in Fig. 1(c) and Fig. 1(d) illustrates another striking result of the crystallization theory: there exist qualitatively different capsid structures (induced by \( f_l(\theta, \phi) \) functions with different \( l \)) but with the same number \( N \) of protein positions.

The X-ray and cryomicroscopy data show the existence of a whole series of viral capsids which violate the CK geometrical model but correspond to the distributions generated by density functions \( f_l(\theta, \phi) \). Fig 3(a) illustrates the correspondence between the positions of maxima of \( f_{21}(\theta, \phi) \) and the structure [17] of L-A virus with \( N = 2 \); Fig 3(b) relates the maxima of \( f_{25}(\theta, \phi) \) to the structure [17] of Dengue virus with \( N = 3 \) and in Fig 3(c) the maxima of \( f_{37}(\theta, \phi) \) are compared with the protein distribution [17] of Murine Polyoma virus with \( N = 6 \).

As an additional comment note, that in our opinion (contrary to the opinion of Ref. [18]) the above structures violating the CK geometrical model do not violate the CK idea about the quasiequivalence of proteins in the viral capsid. CK stated [5] that since the proteins are identical their environments in the viral structure should be similar. Initially, this idea was used by CK to justify their geometrical model. The hexagonal planar crystalline structure proposed by CK to be the first step of the model contains six proteins per unit cell. All proteins of the structure are symmetry-equivalent since they belong to the same regular orbit of the corresponding planar symmetry group. On the second step of the CK model, after the mapping of the planar structure on the icosahedron surface, the same proteins belong to different 60-fold orbits of the I-group. In any 3D icosahedral capsid structure the proteins which belong to different positions can not be symmetry-equivalent. Nevertheless, the CK geometrical construction ensures approximate structural equivalence of proteins from different orbits. This ”quasiequivalence” means that the local order around any protein (the distances between proteins, the number of nearest neighbors) is more or less the same. The latter property is intrinsic not only to the CK structures but also for the capsid structures violating the CK geometrical model including those shown in Fig 3. Indeed, each position (location of maximum) in all these structures have five or six nearest neighbors and the distances to these neighbors are approximately equal. In other words if the asymmetrical identical building blocks can be slightly deformed (it is also assumed in the original CK theory) then there is no problem to put them together in the structure in slightly different local environments.

Let us finally briefly discuss particular features of the assembly thermodynamics. Due to the absence of the cubic term in free energy (3) the icosahedral capsid assembly can be second order phase transition. Thermodynamic processes of this type have two advantages for the assembly optimization: they need no latent heat to be involved in; and they take place without nucleation process. The latter feature is confirmed experimentally for a number of small viruses [19]: at equilibrium, either intact virus shell or free proteins are dominant species while assembly intermediates (capsid germs) are found in trace concentration.

We would like to stress that irreducible icosahedral density function \( f_l(\theta, \phi) \) contains much more physical information than simple positions of proteins centers. The full density distribution generated by \( f_l(\theta, \phi) \) is very useful for understanding of biologically important properties like virus infectivity. Recent advances in virology have shown that that infectivity promoted by interaction of cell receptors with virus surface depends not only on bio-specific binding properties but also on the capsid proteins distribution. Along this line, the relation can be established between the minima of \( f_l(\theta, \phi) \) and binding sites on the capsid surface. One-to-one correspondence of the deepest minima of \( f_{25} \) (Fig. 1(c)) and the binding sites for the carbohydrate recognition domains of the dendritic cell receptors on the Dengue virus surface [Fig 2 in Ref. 20] can be taken as an illustration of the relation.

Figure captions

Fig 1. (a)-(e): The first five irreducible icosahedral density functions with the wave numbers \( l = 15, 21, 25, 27, \) and 31, respectively. Corresponding numbers of different 60-fold positions of density maxima are \( N = 1, 2, 3, 3, \) and 4. (f): Function with \( l = 37 \) and \( N = 6 \).
Fig 2. Comparison of the positions of proteins centers predicted by our model (left panel) with the experimental viral structures [17] (right panel) for the capsids satisfying selection rules of the CK geometrical model. Capsids of Satellite Tobacco Mosaic virus (a), Cowpea Chlorotic Mottle Virus (b), and Sindbis virus (c) are presented. The corresponding density functions for $l = 15$, $l = 27$, and $l = 31$, respectively, are shown in Fig. 1(a), (d), and (e).

Fig 3. Comparison of the positions of proteins centers predicted by our model (left panel) with the experimental viral structures [17] (right panel) for the capsids which can not be explained by the CK geometrical model. Capsids of L-A virus (a), Dengue virus (b), Murine Polyoma virus (c) are presented. The corresponding density functions for $l = 21$, $l = 25$, and $l = 37$, respectively are shown in Fig. 1(b), (c), and (f).

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