A mechanical-thermodynamic model for understanding endocytosis of COVID-19 virus SARS-CoV-2

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Abstract
We analyze the endocytosis process of COVID-19 (coronavirus disease 2019) virus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) using a mechanical-thermodynamic model. The virus particle is designed to interface with the cell membrane as a hard sphere. The role of cytoplasmic BAR (Bin/Amphiphysin/RVs) proteins is considered in the endocytosis. Interestingly, the Endophilin N-BAR cytoplasmic proteins show resistance in participating endocytosis, whereas F-BAR, Arfaptin BAR, Amphiphysin N-BAR, and PX-BAR proteins participate in endocytosis. The increase in membrane tension, concentrated force between the cell membrane receptor, and spike glycoprotein present on the surface of virus particle promote the endocytosis. Also, the increase in the bending modulus of membrane leads to the two-phase solution of BAR protein concentration on the interior of cell membrane surface. We observe an unstable region of protein concentration, which may help one to retard the endocytosis process and thus the viral infection. Though the present study is focused on SARS-CoV-2, it can be extended to understand any other viral infections, involving endocytosis process.

Keywords
mechanical-thermodynamic model, endocytosis, SARS-CoV-2, COVID-19, BAR protein

Introduction
The effect of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) or COVID-19 (coronavirus disease 2019) on humankind is one of the pandemics with high infection rate facing the globe. The genome of SARS-CoV-2 is similar to the SARS-CoV.1–3 The COVID-19 mainly causes respiratory problems while damaging cells in the respiratory system. The SARS-CoV-2 has a spherical structure with spike proteins on its surface. When a SARS-CoV-2 particle approaches cell, the spike glycoproteins on the CoV-2 are attracted by the receptors present on the cell membrane’s outer surface (see Figure 1(a)). When a glycoprotein binds with the receptor, the cell membrane deforms locally. It helps to attain the curvature of the membrane equal to the CoV-2 as shown in Figure 1(a). The modified curvature of the cell membrane is sensed by BAR proteins (like F-BAR, PX-BAR, Amphiphysin and Endophilin N-BAR9–11) present in the cytoplasm (an aqueous solution inside a cell containing salts and proteins) and they bind on the membrane’s interior surface. Other cytoplasmic proteins like clathrin and caveolae may also sense and generate the membrane curvature in endocytosis.12,13 In this work, we analyze the role of BAR proteins as they help in endocytosis by forming vesicle and tubule of the cell membrane.9,10,14–16 The membrane curvature is increased by the local crowding of BAR proteins on the membrane’s interior surface. The increase in membrane curvature increases the accessibility of spikes to the receptors and further increases the membrane curvature as shown in Figure 1(b). It helps the virus to enter into the cell gradually.

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Finally, the combined efforts made by the receptors on the membrane’s outer surface and cytoplasmic BAR proteins in the cell interior help the CoV-2 to enter into the cell as a coated vesicle as shown in Figure 1(c).

In the earlier works of Inoue et al.,17 Burkard et al.,18 and Wang et al.,19 they have done experiments and reported the endocytosis of SARS-CoV. They discovered that SARS-CoV enters target cells via clathrin-mediated endocytosis. However, their findings may not accurately reflect the endocytosis of the new coronavirus SARS-CoV-2, which caused the COVID-19 pandemic. Shang et al.20 used biochemical and pseudovirus entry tests to study the receptor binding and protease activation of SARS-CoV-2 spike glycoprotein.

They discover that SARS-CoV-2 has more affinity for binding to the hACE2 protein than the SARS-CoV. This is one of the most important mechanisms for SARS-CoV-2 endocytosis that scientists have discovered so far.

Glebov21 analyzed the process of SARS-CoV-2 endocytosis in a recent paper and identified that the clinically authorized medications as the prospective candidates for repurposing as blockers of various potential routes.

Yang and Shen22 focus their review on the role of the endocytic pathway and autophagy process in viral infection, as well as the development of therapeutic medicines that target these processes. All these reports are available on probable mechanism of infection of CoV-2 in human body and possible mode of action for its prevention. Mukherjee and Mahata23 have recently explored the endocytosis of SARS-CoV-2 using a finite element model that they proposed. The virus is modeled as hard sphere, while the cell membrane is modeled as an anisotropic elastic material. The role of BAR proteins is also examined in their study using a linear curvature-coupling model with the BAR protein concentration. But the appropriate mathematical model is missing in the literature for understanding SARS-CoV-2 endocytosis. Although, several other studies have been performed for understanding endocytosis behavior of nanoparticles. For example, Vácha et al.24 have studied the receptor-mediated endocytosis of nanoparticles of different shapes with the help of a molecular dynamics (MD) simulation. They have found that the efficiency of endocytosis is higher for spherocylindrical particles. Later on the effects of orientations and rotations of nanoparticles are considered by Dasgupta et al.25 and Tang et al.26 It is found that the endocytosis follows different wrapping pathway due to the competition between stretching and bending of membrane.26 This motivates the rotation of the nanoparticles during endocytosis. The high-aspect ratio or slender particle with round tips, participate in the endocytosis process while orienting the long axis of particle parallel to membrane, whereas the small aspect ratio particles with flat tips enter while orienting the long axis of particle perpendicular to membrane surface.25

The effect of nanoparticle geometry, stiffness, and biomechanical properties of membrane on the endocytosis process discussed in refs. 27, 28. The endocytosis process while considering the adhesion between the nanoparticle and membrane has been studied by Agudo-Canalejo and Lipowsky.29,30 As the SARS-CoV-2 enters into human body, different types of serum proteins have tendency to settle on the surface of SARS-CoV-2 called protein corona. This might affect the infection rate of virus particle.31 In the latest work of Yin et al.,32 using all-atom molecular dynamic simulations and molecular docking, they analyzed the effect of corona proteins on the infection rate of virus particle.

In this work, we analyze the endocytosis of SARS-CoV-2 using a simple mechanical-thermodynamic model. Here, using a proposed mathematical model, we analyze the effect of cytoplasmic BAR protein concentration on the endocytosis. The virus is modelled as hard sphere.

The cell membrane deformation due to the insertion of virus is modeled by Helfrich model.33,34 The adsorbed phase of proteins on interior surface of the membrane is modeled using Mahata and Das.35 For different virus particle’s depth of insertion, the concentrations of BAR proteins on the membrane surface are evaluated from equilibrium configuration of membrane cap (deformed region on the membrane surface adjacent to virus particle) generated by the virus during endocytosis. Here, we analyze parameters like bending modulus and tension of cell membrane on protein concentration.
The paper is organized as follows. In Mathematical model, a mathematical model for SARS-CoV-2 endocytosis is established.

Non-dimensionalization discusses the normalized form of the system’s total free energy.

Equilibrium configuration of spherical membrane cap generated by the virus during endocytosis is analyzed in Equilibrium of membrane cap. We discuss different model parameters in Model parameters. The results of the equilibrium configuration of the membrane cap are discussed in Results and discussion. We conclude in Conclusions.

Mathematical model

We propose a mathematical model to study the endocytosis behavior of SARS-CoV-2 as shown in Figure 2. The deformation of virus particle is negligible as compared to the deformation of cell membrane and also the molecular structure of SARS-CoV-2 retains its spherical shape during the endocytosis process. Thus, in this work we treat the virus particle as a rigid spherical particle. In general, the BAR protein will undergo small deformation during the endocytosis process. But, in order to understand the membrane deformation, we use a simplified model while assuming both BAR protein and viral particles to be rigid and analyze the endocytosis process of virus particle.

Due to the applied force \( f \), the particle as well as the cell membrane are displaced by \( h \) and it follows the curvature of spherical particle as shown in Figure 2. In recent works of Dasgupta et al.\(^2^5\) and Yi and Gao,\(^2^6\) the specific receptor-ligand interaction has been considered to generate initial curvature on the cell membrane, so that the intrinsic curvature of the cytoplasmic BAR protein sense the membrane curvature and bind on it. In the current work, we use an equivalent simplified model\(^2^4,3^7,3^8,3^9,4^0,4^1\) with a concentrated force \( f \) applied on the virus particle to initiate the non-specific adsorption of cytoplasmic BAR proteins by generating the cell membrane curvature.

The total free energy of the system can be expressed as

\[
F = F_b + F_s + F_m - fh
\]

where \( F_b \) is the bending energy of the cell membrane, \( F_s \) is the stretching energy due to lateral tension in the membrane, \( F_m \) is the mixing free energy due to adsorption of cytoplasmic proteins on the interior of the membrane surface, and \( fh \) represents the work done by virus particle, which will be equivalent to the specific interaction energy. In the following, we determine the contributions coming from all these free energies.

Bending and stretching energy

The cell membrane behaves like a fluid for in-plane shear, but it resists the out-of-plane bending.\(^4^2,4^3,4^4\) Due to this nature and small thickness of the membrane, one can consider curvature dependent bending energy obtained from Helfrich model.\(^3^3,3^4\) The bending energy is expressed as

\[
F_b = \frac{1}{2} \kappa A_m (2H_m - C_s)^2,
\]

where \( \kappa \) is the bending modulus of the membrane, \( A_m \) is the surface area of membrane cap generated by the virus particle during endocytosis, \( H_m \) is the mean curvature of the membrane cap, and \( C_s \) is the spontaneous curvature of the membrane, respectively. For a membrane cap of height \( h \) and radius \( R, A_m = 2\pi R h \) and \( H_m = 1/R \). We assume that the virus generates membrane cap of shape similar to the virus particle during the endocytosis as the cytoplasmic proteins adsorbed on the membrane surface.

The protein concentration is assumed to be linear in the spontaneous curvature of the membrane cap,\(^3^9,3^7,3^8\) so that \( C_s = C_p \theta \), where \( C_p \) is the intrinsic curvature of BAR protein, and \( \theta \) is adsorbed BAR protein concentration on the membrane cap surface. It is assumed that the intrinsic curvature of the protein does not depend on the curvature of membrane cap.

In general, the cell membrane is not much stretchable. Once the lateral tension exceeds few units, the cell membrane suddenly fails without much premonition.\(^5^0\) We consider the situation that the membrane cap generated by the virus particle is coupled to a membrane area reservoir at constant membrane tension. This is typical for the biological cells\(^5^1,5^2\) and mimicked in a situation, when a lipid membrane tether is pulled by the use of micropipette aspiration from a lipid membrane reservoir.\(^3^4,3^7\) If \( \sigma \) be the constant stretching energy per unit area due to membrane tension, then the total stretching energy can be written as

\[
F_s = \sigma A_m.
\]

Mixing free energy

The contribution of mixing free energy in equation (1) comes due to the adsorption of cytoplasmic proteins onto the surface of membrane cap generated by the virus during endocytosis. It includes the interaction free energy due to protein–protein interactions and the pressure energy due to binding of proteins on membrane surface. Before starting endocytosis of the virus particle, the chemical potential of the proteins in the cytoplasm and binding on cell membrane’s inside surface should be equal in equilibrium. Also, we can assume that the cell membrane is locally flat in this configuration. When a virus particle tries to enter into cell, the cytoplasmic proteins bounded on the inside surface of the flat membrane will also come onto the surface of the membrane cap. We should
neglect the effects of all these proteins as they are bound to the cell membrane surface before starting the endocytosis. So, there are Legendre transformation\(^5\) of mixing free energy from the flat membrane surface to the membrane cap surface. The Legendre transformed mixing free energy is expressed as \(^{38,37}\)

\[
F_m(\theta) = A_m \left[ f_m(\theta) - \frac{\mu_m}{b} \theta + P_m \right], \quad (4)
\]

where \(f_m(\theta)\) is the free energy per unit area of the adsorbed proteins on the membrane cap surface due to mixing and protein-protein interactions, \(\mu_m\) is the chemical potential of adsorbed proteins on the flat membrane surface, \(b\) is the area of an individual protein molecule, and \(P_m\) is the energy per unit area applied due to pressure of adsorbed proteins onto the membrane surface.

As noted in Introduction, we take into account the binding of cytoplasmic BAR proteins to the membrane surface. The projected shape of a BAR protein can be represented by an elliptical particle approximately. Also, the soft interactions between the adsorbed protein molecules are expected on the membrane particle. To consider shape aspect of the protein molecules and soft interactions between them, we have chosen \(f_m(\theta)\) in equation (4) form Mahata and Das\(^5\), so that

\[
f_m(\theta) = k_BT \left[ \frac{1}{b} \theta (\ln \theta - 1) - \frac{a_4}{b^2} \theta^2 \right. \quad \quad \left. + \frac{1}{\alpha + 1} \frac{a_r}{b^2} \left( \alpha \frac{\theta}{1 - \theta} - \ln(1 - \theta) \right) \right], \quad (5)
\]

where \(k_B\) is Boltzmann’s constant, \(T\) is absolute temperature, \(a_4\) is attraction parameter, \(a_r\) repulsion parameter, and \(\alpha\) represents shape parameter of the protein molecule. For elliptical particle, \(\alpha > 1\). For more details about different parameters in equation (5) can refer Mahata and Das\(^5\).

Substituting equations (2), (3), and (4) into equation (1) with \(A_m = 2\pi R h\) and \(H_m = 1/R\), we have

\[
F = 2\pi R \left[ \frac{1}{2} \left( \frac{2}{R} - C_p \theta \right)^2 + \sigma + f_m(\theta) - \frac{\mu_m}{b} \theta + P_m \right] - f h, \quad (6)
\]

where \(f_m(\theta)\) is obtained from equation (5), and \(\mu_m\) and \(P_m\) are derived in the next section. In the following, we non-dimensionalize equation (6).

**Non-dimensionalization**

We use \(h\) (the displacement of virus), \(\kappa\) (bending modulus of membrane), and \(\kappa/h\) to non-dimensionalize length, energy and force, respectively. We obtain the following non-dimensional parameters

\[
\bar{F} = \frac{F}{\kappa}, \quad \bar{R} = \frac{R}{R}, \quad \bar{C}_p = C_p h, \quad \bar{\sigma} = \frac{\sigma h^2}{\kappa}, \quad \bar{f}_m(\theta) = \frac{f_m(\theta) h^2}{\kappa}, \quad \bar{\mu}_m = \frac{\mu_m}{\kappa}, \quad \bar{b}_m = \frac{b}{h^2}, \quad \bar{P}_m = \frac{P_m h^2}{\kappa}, \quad \bar{f} = \frac{f h}{\kappa}. \quad (7)
\]

Using the above non-dimensional quantities in equation (6), we have

\[
\bar{F} = 2\pi \bar{R} \left[ \frac{1}{2} \left( \frac{2}{\bar{R}} - \bar{C}_p \theta \right)^2 + \bar{\sigma} + \bar{f}_m(\theta) - \frac{\bar{\mu}_m}{\bar{b}_m} \theta + \bar{P}_m \right] - \bar{f}. \quad (8)
\]

In equation (8), the non-dimensional free energy per unit area due to protein-protein interactions

\[
\bar{f}_m(\theta) = \frac{1}{\bar{R}} \theta (\ln \theta - 1) - \frac{\bar{\sigma}}{\bar{b}_m} \theta^2 + \frac{1}{\alpha + 1} \frac{\bar{\sigma}}{\bar{b}_m} \left( \alpha \frac{\theta}{1 - \theta} - \ln(1 - \theta) \right), \quad (9)
\]

where \(\bar{R} = \frac{R}{h^2}, \quad \bar{\sigma} = \frac{\sigma}{h^2}, \quad \bar{\mu}_m = \frac{\mu_m}{h^2}, \quad \bar{b}_m = \frac{b}{h^2}, \quad \bar{P}_m = \frac{P_m h^2}{\kappa}, \quad \bar{f} = \frac{f h}{\kappa}\) are non-dimensional area, attraction, and repulsion parameters of protein molecules, respectively. We can rewrite the equation (6) as

\[
\bar{F} = 2\pi \bar{R} \left[ \frac{1}{\bar{R}} + \bar{\sigma} + \bar{f}_m(\theta) - \frac{\bar{\mu}_m}{\bar{b}_m} \theta + \bar{P}_m \right] - \bar{f}, \quad (10)
\]

with \(\bar{f}_m(\theta) = \bar{f}_m(\theta) - \frac{\bar{\sigma}}{\bar{b}_m} \theta^2 + \frac{\bar{\sigma}}{\bar{b}_m} \left( \alpha \frac{\theta}{1 - \theta} - \ln(1 - \theta) \right)\). Now, introducing a function \(\bar{C}_m(\theta) = \bar{C}_m(\theta) - \frac{\bar{\sigma}}{\bar{b}_m} \theta^2 + \frac{\bar{\sigma}}{\bar{b}_m} \left( \alpha \frac{\theta}{1 - \theta} - \ln(1 - \theta) \right)\), with \(\bar{C}_m\) is the dimensionless surface area of membrane cap, one can obtain

\[
\bar{P}_m = \frac{\partial \bar{C}_m}{\partial \theta}, \quad \bar{f} = \frac{\partial \bar{C}_m}{\partial \theta} - \frac{\bar{\sigma}}{\bar{b}_m} \theta + \frac{\bar{\sigma}}{\bar{b}_m} \left( \alpha \frac{\theta}{1 - \theta} - \ln(1 - \theta) \right), \quad (11)
\]

and

\[
\bar{f}_m = \frac{\partial \bar{C}_m}{\partial \theta}, \quad \bar{f}_m = \frac{\partial \bar{C}_m}{\partial \theta} + \frac{\bar{\sigma}}{\bar{b}_m} \theta^2, \quad \bar{f} = \frac{\partial \bar{C}_m}{\partial \theta} + \frac{\bar{\sigma}}{\bar{b}_m} \theta^2, \quad (12)
\]

In equations (11) and (12), \(N\) is the number of protein molecules binding on the membrane cap in its reference configuration and \(\theta_m\) is the corresponding protein concentration on the interior surface of membrane. In this analysis, we assume that the size of the cell is much larger as compared to membrane cap. So, we consider the reference configuration of the membrane cap to flat locally. This leads to the radius of curvature of membrane infinity. In deformed configuration, curvature of virus particle and membrane cap are same. Also, \(\mu_m\) is associated with the flat membrane surface. Then, using \(R = \infty\) in equation (11), we have

\[
\bar{P}_m = \frac{\partial \bar{C}_m}{\partial \theta}, \quad \bar{f} = \frac{\partial \bar{C}_m}{\partial \theta} + \frac{\bar{\sigma}}{\bar{b}_m} \theta^2, \quad (13)
\]

Now, with the help of equation (9), one can evaluate \(\bar{F}_m\) and \(\bar{P}_m\) from equations (12) and (13), respectively.

**Equilibrium of membrane cap**

For the equilibrium configuration of membrane cap, the free energy of system should be minimum. To minimize free energy of the system, we differentiate the free energy with respect to \(R\) and \(\theta\) and equate them to zero, that is...
This translates into the following equations
\[
\frac{\partial^2 F}{\partial R^2} = 0 \text{ and } \frac{\partial^2 F}{\partial \theta^2} = 0.
\]

Using equation (15) in equation (14), we can write
\[
\frac{2}{R} \left( \frac{1}{R} - C_p \theta \right) = \sigma + \tilde{f}_m(\theta) - \theta \frac{\partial^2 \tilde{f}_m(\theta)}{\partial \theta^2} - \frac{C_p}{2} \theta^2 + \tilde{P}_m.
\]

We solve equation (16) for the protein concentration \( \theta \), for the specified values of \( C_p, \sigma \), and \( \overline{R} \). Here, we obtain \( \tilde{f}_m(\theta) \) and \( \tilde{P}_m \) using equations (9) and (12), respectively.

The stability criteria for the equilibrium configuration of the membrane cap are defined as
\[
\left( \frac{\partial^2 F}{\partial R^2} \right) - \left( \frac{\partial^2 F}{\partial \theta^2} \right)^2 > 0,
\]
which leads us into
\[
\frac{\partial^2 \tilde{f}_m(\theta)}{\partial \theta^2} > 0.
\]

We should choose the parameters \( \sigma, \sigma, \), and \( \overline{R} \) in equation (9), so that they will satisfy the above inequality. The details about different parameters used in this analysis will be discussed in the following section.

**Model parameters**

The approximate diameter of SARS-CoV-2 particle is 100 nm.\(^{54}\) So, in this work, we use the radius of the spherical particle \( R = 50 \text{ nm} \). The radius of cytoplasmic BAR proteins varies between 10 and 100 nm. For example, the F-BAR proteins can bind with the membrane tubule of 25-100 nm radius.\(^{55}\) The PX-BAR and Arfaptin BAR scaffold with membrane of radius of curvature 13 and 10 nm, respectively.\(^{56}\) Also, the Amphiphasin and Endophilin N-BAR proteins bind with radius of curvature 11 and 15 nm, respectively.\(^{57,58}\) Since, \( R = 50 \text{ nm} \), we cannot expect that the radius of curvature of protein greater than 50 nm can bind with the membrane. So, the BAR proteins having radius of curvature in between 10 to 50 nm can bind with the membrane cap generated during the endocytosis of virus particle. The corresponding intrinsic curvature \( C_p \) of the protein varies from 0.02 to 0.1 nm\(^{-1}\). The typical values of cell membrane tension are extensively measured for different kind of cells. It can vary from 3 pN \( \mu \text{m}^{-1} \) for epithelial cells up to about 300 pN \( \mu \text{m}^{-1} \) for keratocytes.\(^{51,52}\) So, we can choose the range \( \sigma = 3 - 300 \mu \text{m}^{-1} \) for the stretching energy per unit area due to membrane tension. Also, the typical values of bending modulus of cell membrane are taken in the range of 10 to 100 \( k_B T \).\(^{59}\)

As we pointed out in *Mixing free energy*, the projected shape of the above-mentioned BAR proteins can be approximated by an elliptical molecule as shown in Figure 3. Considering interactions between the elliptical molecules, one can determine the mixing free energy per unit area \( f_m(\theta) \) as shown in equation (5).\(^{35}\) For different BAR proteins, the axial ratio of the elliptical particles can be determined from the structural information of the proteins mentioned in Protein Data Bank (PDB). One can express the corresponding shape parameter
\[
\alpha = \frac{\pi R^2}{A_e} = \frac{4g}{\pi^2} \left[ \int_{0}^{\pi/2} \left( \frac{\sin^2 \phi}{g^2} + \cos^2 \phi \right)^{1/2} d\phi \right]^2,
\]

where \( g \) is the axial ratio of the elliptical protein molecules and \( \phi \) represents the angular position of an arbitrary point located on the boundary of the elliptical molecule.\(^{60}\) For the given major and minor axis values, one can obtain the individual area of protein molecule \( b \). From the area of protein molecules, we can determine the Lennard-Jones potential parameters of protein molecules and subsequently the attraction and repulsive parameters \( a_a \) and \( a_r \), respectively from Mahata and Das.\(^{35}\) The interaction parameters as well as curvatures of different BAR proteins are mentioned in Table 1. In this analysis, we choose the size of protein molecules at room temperature (300 K) and assume this temperature as the Boyle temperature for them. In Boyle temperature, second virial coefficient becomes zero\(^{55,61}\) and we obtain equal attraction and repulsive parameter for the protein molecules as mentioned in Table 1. Using the parameter values in equation (16), we obtain the protein concentration \( \theta \) on membrane cap for different values of \( \theta_m \). Here, \( \theta_m \) represents the equilibrium protein concentration in the interior of the cell membrane surface before starting the endocytosis. The stability condition of the membrane cap is given by equation (18). The results and corresponding discussions are mentioned in the following section.

**Results and discussion**

For different depth of insertion of virus particle, we obtain the equilibrium concentration \( \theta \) of BAR proteins...
Figure 4(a) and (b). Finally, three BAR proteins docytosis. We analyze the F-BAR, Arfaptin BAR, and Endophilin N-BAR protein may not contribute in the endocytosis process. After a critical value of membrane released into the cytoplasm, before the endocytosis. When $\theta < \theta_m$, the virus can then avoid endocytosis.

As we expect that the proteins F-BAR, PX-BAR, and Arfaptin BAR play important role in endocytosis of SARS-CoV-2, we analyze the effect of membrane tension on the concentration for these proteins as shown in Figure 5. It is observed that the concentration increases with increase in membrane tension $\sigma$. One can expect this, because the increase in membrane tension stretches the membrane to occupy more proteins on its surface. For smaller values of $\theta_m$, we observe that $\theta$ is greater than $\theta_m$. This suggests that the additional proteins can bind on the membrane surface with increase in membrane tension. After a critical $\theta_m$, $\theta$ approaches $\theta_m$ and cytoplasmic BAR proteins stop to bind on the membrane surface. This kind of behavior we observe for all the three BAR proteins considered in Figure 5.

The effect of membrane bending modulus $\kappa$ on concentration ($\theta$) for F-BAR, PX-BAR, and Arfaptin BAR shown in Figure 6. We observe a region of $\theta$, for which we do not get any stable solution of the membrane. This suggests that the proteins do not contribute in the endocytosis process. Here, we may expect two phase solution of the protein concentration on the cell membrane surface. In Figure 6(a), for F-BAR protein with $\kappa = 20 k_BT$, we observe single phase solution. As the bending modulus of cell membrane increased, the single phase transforms into a

### Table 1. The shape parameter ($\alpha$), area ($b$), attraction ($\sigma_a$), and repulsion parameter ($\sigma_r$) of different BAR proteins.

| BAR proteins          | $\alpha$ | $b$ (nm$^2$) | $\sigma_a$ (nm$^3$) | $\sigma_r$ (nm$^3$) | $C_p$ (nm$^{-1}$) |
|-----------------------|----------|--------------|---------------------|---------------------|------------------|
| F-BAR (PDB ID: 3Q0K, 2VDO, and 2X3X) | 1.864    | 78.54        | 73.264              | 73.264              | 0.02             |
| Endophilin N-BAR (PDB ID: 2ZOV and 1ZWW) | 2.236    | 35.34        | 32.966              | 32.966              | 0.067            |
| Amphiplys N-BAR (PDB ID: 1URU, 4ATM, and 3SOG) | 3.199    | 23.56        | 21.977              | 21.977              | 0.091            |
| Arfaptin BAR (PDB ID: 4DCN) | 2.618    | 29.45        | 27.471              | 27.471              | 0.1              |
| PX-BAR (PDB ID: 2RAJ, 2RAK, and 3DYT) | 1.864    | 28.27        | 26.371              | 26.371              | 0.077            |

(cytoplasmic proteins) on the surface of membrane cap against different values of BAR protein concentration $\theta_m$ in the reference configuration as shown in Figure 4. We consider the presence of different BAR proteins separately in the cytoplasm during the endocytosis of the virus particle. The Endophilin N-BAR protein does not satisfy the stability criteria of the solution of the problem as mentioned in equation (18). This suggests that the Endophilin N-BAR protein may not contribute in the endocytosis. We analyze the F-BAR, Arfaptin BAR, Amphiplys N-BAR, and PX-BAR and they participate up to the half depth of insertion of the virus as shown in Figure 4(a) and (b). Finally, three BAR proteins—F-BAR, PX-BAR, and Arfaptin BAR actively participate up to full depth of insertion of the virus as shown in Figure 4(d). If $\theta < \theta_m$, then it signifies that some of the cytoplasmic BAR proteins that have already attached on the interior surface of cell membrane released into the cytoplasm, before the endocytosis process. After a critical value of $\theta_m$, $\theta$ approaches to $\theta_m$. This suggests that no proteins release into the cytoplasm from the cell membrane surface during endocytosis. When $\theta > \theta_m$, it is expected that extra proteins have to come to bind on the cell membrane surface at the time of endocytosis.
Figure 4(a) and (b). Finally, three BAR proteins up to the half depth of insertion of the virus as shown in docytosis. We analyze the F-BAR, Arfaptin BAR, dophilin N-BAR protein may not contribute in the endocytosis process. Here, we may expect two phase solution of the problem as in the cytoplasm during the endocytosis of the virus shown in Figure 4(d). If the driving force is $\theta > \theta_{m}$, then it suggests that no proteins release into the cytoplasm from the cell membrane surface during the endocytosis. After a critical value of cell membrane released into the cytoplasm, before the proteins that have already attached on the interior surface of endocytosis.

Figure 5. The variation in the equilibrium concentration of (a) F-BAR, (b) Arfaptin BAR, and (c) PX-BAR proteins ($\theta$) on membrane surface against the protein concentration in the reference configuration ($\theta_{m}$) for different cell membrane tensions. The parameter values associated with these BAR proteins are chosen from Table 1. Here, we use $\kappa = 10 \, k_{B}T$ and $h = 2R$ (full endocytosis).

Figure 6. The variation in the equilibrium concentration of (a) F-BAR, (b) Arfaptin BAR, and (c) PX-BAR proteins ($\theta$) on membrane surface against the protein concentration in the reference configuration ($\theta_{m}$) for different bending modulus of cell membrane, $\kappa = 20 \, k_{B}T$ (black), $\kappa = 50 \, k_{B}T$ (blue), and $\kappa = 80 \, k_{B}T$ (green). The parameter values associated with these BAR proteins are chosen from Table 1. Here, we use $h = 2R$ (full endocytosis) and $\sigma = 3 \, \text{pN} \, \mu \text{m}^{-1}$.

Figure 7. The variation in the free energy of F-BAR protein against the equilibrium concentration of cytoplasmic proteins ($\theta$) for different values of concentrated force ($f$). The parameter values associated with F-BAR protein are chosen from Table 1. Here, we use $h = 2R$ (full endocytosis), $\sigma = 3 \, \text{pN} \, \mu \text{m}^{-1}$, and $\kappa = 10k_{B}T$.

two-phase solution. For Arfaptin and PX-BAR proteins, we always observe two-phase solutions for the range of $\kappa$ values considered in Figure 6(b) and (c). In Figures 4–6, for smaller values of $\theta_{m}$, the equilibrium concentration of BAR proteins $\theta$ is absent. Also, for larger values of $\theta_{m}$, $\theta$ is invariant for different BAR proteins, different values of $\sigma$ and $k_{B}T$, respectively. If we can manipulate $\theta$ and $\theta_{m}$, such that they lie in the unstable region (as observed in Figures 4–6), the virus can then avoid endocytosis.

In Figure 7, we calculate free energy $\mathcal{F}$ of the system from equation (10) against the concentration of F-BAR protein for different values of $f$. The free energy decreases as we increase the concentrated force $f$. The concentrated force is basically associated with the binding force acting between the receptor and spike glycoprotein during the endocytosis as shown in Figure 2. If the driving force is increased, then the free energy of the whole system will be
decreased. For a particular $f$, the free energy decreases as we increase the concentration of F-BAR protein. For small $f$ and $\theta$, we obtain positive free energy of the system. However, larger values of $f$ give us negative free energy of the system in the whole region of the protein concentrations as shown in Figure 7. Positive free energy signifies the absorption of energy into the system and opposite is applicable to the negative free energy. We observe that the increase in $f$ and $\theta$ decreases the free energy. This means more concentrated force adopts more F-BAR proteins on the membrane surface to achieve the endocytosis of the virus particle.

We observe that the increasing membrane tension increases the BAR protein concentration on the membrane surface as shown in Figure 5. This increasing protein concentration reduces the total free energy of the system depicted in Figure 7. Thus, increasing membrane tension enhances the endocytosis process of the virus particle. However, it contradicts with the results of Deserno. In his model, he did not consider the role of non-specific binding of BAR proteins on the membrane surface during membrane wrapping of colloidal particle. This might be the reason that Deserno’s work did not capture increase in endocytosis with increasing membrane tension. Current work can be extended to analyze endocytosis of BAR proteins while considering the kinetics of receptor-mediated endocytosis. Also, a more accurate model while considering the deformation of virus particle, a detailed interaction and corresponding binding energy between protein and cell membrane can be incorporated in the current model to analyze the endocytosis of proteins.

Conclusions

We develop a mechanical-thermodynamic model to explore the endocytosis behavior of SARS-CoV-2. With the help of present model, we observe that Endophilin N-BAR cytoplasmic protein does not participate in the endocytosis. The Amphiphysin N-BAR protein participates up to the half depth of insertion of the virus particle. However, the other cytoplasmic BAR proteins like F-BAR, PX-BAR, and Arfaptin BAR participate up to full depth of insertion and play important role in endocytosis. In general, the BAR proteins binding on cell membrane’s inside surface assist virus for endocytosis. But, if the tension of the cell membrane is increased, then the BAR proteins from the cytoplasmic solution also bind on the membrane surface to assist virus for endocytosis. As we increase the bending modulus of the cell membrane, two phase solutions of the BAR proteins are observed for full endocytosis of the virus. We obtain an unstable region of protein concentration, for which the endocytosis cannot be achieved. Increasing concentrated force associated with the binding of cell membrane receptor and spike glycoprotein of virus during the endocytosis, adopts more proteins on the membrane surface to achieve endocytosis. Although the present study is focused on endocytosis of SARS-CoV-2, but this can also help one to understand endocytosis process involved in different viral infections.

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