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Biophysical Insights into Coronavirus Cell Membrane Interactions: The Role of Nonequilibrium Binding Energy

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Abstract

Scientists are interested in the free energy associated with the binding of viral particles to cell membranes. However, the intrinsic force associated with virus-cell membrane interactions has yet to be investigated. While the kinetic energy may be a state function, it is not the type associated with the thermodynamic equilibrium constant. Thus, the main objective of this study was to derive an equation for non-equilibrium binding energy (NEBE) that depends on an intrinsic force capable of opposing kinetic energy. Some data in the literature were used to evaluate the derived equations. Though diffusivities increased with temperature, they were substantially lower than those far from the binding region for the D and G variants of SARS-CoV-2. With breath emission equal to $1.29 \text{ exp. (+)}/\text{m}^3$, the NEBE value for the Omicron variant was higher than that

for the Delta variant. A similar trend with lower values was observed with a breath emission equal to $27.9 \text{ exp. (+)}/\text{m}^3$; the NEBE value with a breath emission rate of $9.31 \text{ exp. (+)}/\text{hour}$ is higher than with $201 \text{ exp. (+)}/\text{hour}$ for the Omicron variant only. The G variant of SARS-CoV-2 showed higher NEBEs (952-671 kcal/mol) than the Delta variant (834-588 kcal/mol), corresponding to 293.15-318.15 K. The virus's binding affinity may diminish at higher temperatures due to the decrease in maximal NEBE as temperature increases. Therefore, medication at temperatures higher than the normal body temperature is advised. Future research should be to definitively determine the size and molar mass of SARS-CoV-2 variants. **PACS Number:** 87.17.Jj; 05.70Ln.

Keywords: Coronavirus, Non-Equilibrium Binding Energy (NEBE), Diffusivities, Viscosity, Path Function

1. Introduction

“Sanity is needed in the application of science; but is it sane to teach using a black marker on a black board anywhere in the World?”

It is well known that the medium of transit determines how quickly a solute—whether a medicine, its metabolites, or a pathogen like SARS-CoV-2, which imprisons humanity and is dreaded but not more lethal than Ebola—reaches its targets. The medium provides viscosity, or resistance to motion caused by thermal energy. The biological medium's composition exacerbates viscosity's retardative effect. Since the model for nonequilibrium binding energy (NEBE) is connected to diffusivity, it is crucial to evaluate current occurrences that are close to diffusion and solute diffusivity in a heterogeneous medium. Referring to their unseen supplementary record (§3.1.), Marbach & Holmes–Cerfon (2020) ^[1] were of the opinion that momentum relaxation (typically, loss of momentum due to obstacles, narrow inter-mucin fiber space, *etc.*) for micron-scale particles occurs over a timescale of $t_m \approx \mu\text{s}$, while on much longer timescales, the equilibrium motion is diffusive with a diffusion coefficient independent of mass for large enough particles ^[2-4]. Inertia can only affect the short-time mobility of a particle ^[3, 5, 6]. However, the dependency of the diffusion coefficient (or diffusion rate) on either mass or density is known to be inversely proportional to the square root and to the cube root of either the density or the mass of the particle for the small and large molecules, respectively; this is in line with Graham's law of diffusion. It would appear, therefore, that the claim in the literature is conditional (this should not preclude new thinking).

Popovic (2022) ^[7] explained that nonequilibrium thermodynamics links the rate of a biochemical process to its driving force, the Gibbs energy change. This bridges chemical kinetics and Gibbs free energy of binding. Mechanical kinetics, which plays a

supporting role in catalyzed reactions, is not as well studied as (bio) chemical kinetics, which investigates rate constants for activation energies. This study examined the nonequilibrium binding energy (NEBE) that can technically control, if not halt, random motion occasioned by the mechanical kinetic energy (which seems in principle to be a state function); NEBE is required for virus or ligand binding on cell membranes. The Gibbs free energy of binding, which relies on the thermodynamic equilibrium constant, is a state function. Recall too that mechanical kinetic energy is not a function of a thermodynamic equilibrium constant. Meanwhile, a number of experimental techniques like fiber-optic-based fluorescence correlation spectroscopy, FB-FCS [8]; segmented fluorescence correlation spectroscopy, SFCS [9]; single-particle tracking, SPT [10]; and fluorescence recovery after photobleaching, FRAP [11], have been used to determine the diffusivity and corresponding viscosity known to reduce the former.

So far, experimental approaches have been explored for the determination of binding energy with its roots in electrostatic interaction energy, reported somehow as total energies [12]. That the binding of the novel and old (SARS-CoV) viruses to ACE2 is driven by the electrostatic interaction [12] is not without evidence to the effect that at pH 7.4, used in an earlier work, the total charges of SARS-CoV-2-RBD and SARS-CoV-RBD are +3 and +2e, respectively [12]. It has been demonstrated using steered molecular dynamics simulations (SMDSs) that a higher rupture force and extra pulling work are required to unbind SARS-CoV-2-RBD from ACE2-PD than to unbind SARS-CoV-RBD [12], similar to earlier findings [13]. Regarding the equilibrium approach as a superior method for calculating absolute binding free energy (ABFE) implies a preference for it [13]. This is despite exploring the force, characteristics of a path function inherent in mechanical energy yielding process to determine binding free energy [14]. Despite externally forcing an equilibrated system out of equilibrium *via* nonequilibrium methods, such as nonequilibrium simulation [15], binding energy is reported as a kind of free energy. Scientists seem not to have explored the driving force, a path function characteristic of any particle that must be opposed by a NEBE originating from mechanical kinetic energy that has no bearing on the thermodynamic equilibrium constant or, as often referred to, the equilibrium dissociation constant, even if it is of electrostatic or thermal origin. This study is predicated on the notion that state and path functions are separate. Such driving force is explored for the derivation of the NEBE.

The goal of this study was to show that, in contrast to the chemical kinetics-based energy that merely describes the thermodynamic stability or feasibility of fixing the ligand (such as viral spike protein) to the receptor (such as ACE2 in lipid rafts) before any further transformation, there is a minimally sufficient NEBE to counteract the mechanical kinetic energy. To achieve the desired outcome, the following objectives were pursued: The generated equation is used to determine the theoretically optimal viral number density for infection, symptom results, and progression. A more useful or empirical way to determine viral number density is to use the multiplicity of infection (*MOI*), which is mathematically related to both the breath emission rate and the viral number density in free or restricted environments. In addition to a review of the research on the impact of the medium's composition and biophysical nature on viral

infection and treatment advice, a brief explanation of the binding processes prior to infection is given. Finally, the NEBE is computed.

2. Brief Theoretical Background

2.1 Biophysical parameters-diffusivity, mean square displacement, and temperature

Given the molar mass of a solute, its translational diffusion coefficient at known temperature, the mean square displacement (MSD, $\langle \ell^2 \rangle$) (or the root MSD (RMSD), ℓ) can be computed. Earlier computation [16] explored the following equation:

$$\ell = (4m_i D_v^2 / 3k_B T)^{1/2} (m_1 / \rho_1)^{1/9} \quad (1)$$

Where m_i , D_v , k_B , T , m_1 , and ρ_1 are the mass of the solute (which can be viral particle), diffusivity, Boltzmann constant, thermodynamic temperature, mass of a molecule of water, and density of water respectively; $(m_1 / \rho_1)^{1/9} = L^6$. Solve for D_v , to give:

$$D_v = \left(\frac{3k_B T \ell^3}{4m_i L} \right)^{1/2} \quad (2)$$

The time taken to cover such displacement is also computed with the following equation:

$$\tau = \left(\frac{m_1}{\rho_1} \right)^{1/6} \left(\frac{\ell m_i}{3k_B T} \right)^{1/2} \quad (3a)$$

Equation is simplified to give:

$$\tau = \left(\frac{L \ell m_i}{3k_B T} \right)^{1/2} \quad (3b)$$

The approach in this study gives an insight into what may be expected in a practical experience. The advantage of the two equations is that information about the viscosity coefficient of the solution or media, such as cytoplasm, cell membrane, nucleoplasm, *etc.*, is not required as long as the value of D_v is known. Yet the value of D_v should be for a known medium that has a verifiable viscosity.

The determination of the viscosity of the mucus at temperatures lower and higher than 25 °C (293.15 k) is based on the equation as follows:

$$\eta_{mu}(T_{II}) = \frac{\eta_{mu}(T_I) \eta_w(T_{II})}{\eta_w(T_I)} \quad (4)$$

The viscosity coefficient based on Eqs (5) and (6):

$$\eta = \frac{L \ell m_v}{18\pi r_i^2 v^2 D_v} \quad (5)$$

$$\eta = \left(\frac{k_B T m_v L}{27\pi^2 r_i^2 \ell^3} \right)^{1/2} \quad (6)$$

$$\ell = \left(\frac{k_B T m_v L}{27\pi^2 \eta^2 R_p^2} \right)^{1/2} \quad (7)$$

$$\tau = \frac{1}{3} \left(\frac{m_p^2 L^2}{k_B T \pi \eta \bar{R}} \right)^{1/2} \tag{8}$$

2.2 Viral Number Densities, Short, and Long Intermolecular Distances

$$C_v = \frac{1}{8\pi \bar{R}_v^3} \tag{9}$$

Where C_v and \bar{R}_v are the theoretical maximum viral number density specific for a given virus and its hydrodynamic radius respectively.

$$R_0 = 2(\pi \bar{R}_v C_v)^{1/2} (R_{int} - \bar{R}_v) R_{int} \tag{10}$$

Where R_0 and R_{int} are the minimum interparticle distances wherein electrostatic attraction or repulsion commences and the maximum average intermolecular distance greater than R_0 .

$$R_{int} = \frac{\bar{R}_v + \left(\bar{R}_v^2 + \frac{1}{\pi \bar{R}_v C_v} \right)^{1/2}}{2} \tag{11}$$

2.3 Small and large nonequilibrium binding energy

$$\xi_{elect}/\epsilon_0 \epsilon_r = 12\eta \frac{\bar{R}_v D_v (\pi^3 (R_{int} - R_0) C_v)^{1/2} R_{int}^2}{R_0} \tag{12}$$

$$\xi_{elect}/\epsilon_0 = 5.241465317 \bar{R}_v \left(R_0 + \frac{R_{int}^2}{R_0} \right) \left(\frac{k_B^2 T^2 \eta^2}{m_v L} \right)^{1/6} \left(\frac{(R_0 - \bar{R}_v) \pi^3 R_{int} C_v}{\bar{R}_v} \right)^{1/6} \tag{13}$$

2.4 Multiplicity of Infection, Initial Number of Viral Particles per Cell, the Corresponding Viral Number Density

Leveraging on the idea that the multiplicity of infection (*MOI*) could be used to define the average number of viral particles that infect a cell [17], an equation derived in a manuscript under preparation is adopted in this study. This may obviate the misgiving of the term *MOI* by Shabram & Aguilar-Cordova (2000) [17].

$$m = \frac{BER * V_r * \tau_i^2}{V_m N_c} \tag{14a}$$

Where *BER*, V_r , τ_i , V_m , N_c , and m are the breath emission rate per hour, ventilation rate per minute, period of time (taken to be one minute and one hour, for the purpose of this study) under consideration, molar gas volume, number of nasal cells, and multiplicity of infection (*MOI*). If on the other hand, the viral density (B) in viral copies per cubic meter in the surrounding is known, the equation of m is given as:

$$m = \frac{B * V_r * \tau_i^2}{V_m N_c} \tag{14b}$$

In this case the viral number density ($C_{v \rightarrow B}$) per cell is given as:

$$C_{v \rightarrow B} = \frac{BER * V_r * \tau_i^2}{V_c V_m N_c (1 - e^{-m})} \tag{15}$$

Where V_c is the arbitrarily chosen volume of a cell as to imply that such, may be < or > the average volume of a mammalian cell. If the number density (or B) in the air (which may be in the room, toilet, concert hall, etc.) rather than *BER* is given, then, the number density ($C_{v \rightarrow B}$) of the virus per cell is given as:

$$C_{v \rightarrow B} = \frac{B * V_r * \tau_i}{N_{IC} V_c} \tag{16}$$

Where $N_{IC} = N_c (1 - e^{-m})$. All these with detailed basis are again in a text book in preparation.

Exploring different periods of time (one hour (a); one minute (b)) generates different values of N_{IC} and the corresponding $C_{v \rightarrow B}$ values, initial interparticle distance ($R_{int(B)}$), initial interparticle distance ($R_{0(B)}$) wherein mutual attraction or repulsion may not occur because the number density is very low, narrower interparticle distance ($R_{int(\tau \rightarrow \infty)}$), and interparticle distance ($R_{0(\tau \rightarrow \infty)}$) wherein mutual attraction or repulsion may occur because the number density is very high.

2.5 Viral Number Density ($C_{v(\tau \rightarrow \infty)}$) as Time Tends to Infinity Given Known Number Density of Viral Copies or Breathe Emission Rate in the Surrounding

$$C_{v(\tau \rightarrow \infty)} = \frac{1}{2\pi \bar{R}_v R_0} \left(\frac{4k_B T}{L m_v D_v^2} \right)^{1/3} \tag{17}$$

Where L and D_v are the average intermolecular distance in the molar volume of water and the viscosity-dependent diffusivity at ambient temperature respectively.

2.6 Proposition that Compositional Factor Determine Diffusivity and Ultimately, Nonequilibrium Binding Energy, Inferences, and Therapeutic Significance

Cross-linked, bundled, and entangled mucin fibers, which are released by goblet cells and the seromucinous glands of the lamina propria at the apical epithelium, are characteristics that can increase the viscosity of the mucus [18, 19]. The mucin fibers, 3–10 nm in diameter [20], are proteins glycosylated *via* proline, threonine, and/or serine residues by O-linked N-acetyl galactosamine as well as N-linked sulfate-bearing glycans [18]. Glycan coverage of mucin is dense, with 25–30 carbohydrate chains per 100 amino acid residues [21], and make up the larger part of the dry mass [22]. Most mucin glycoproteins have a high sialic acid and sulfate content, which leads to a strongly negative surface that increases the rigidity of the polymer *via* charge repulsion [20].

Proteins, specifically mucin fibres, increase mucus's viscous and elastic moduli [23, 24]. This is supported by reduced transport rates for IgM and small IgA aggregates in mucus, slowed by low-affinity bonds with mucin [25, 26]. This suggests that SARS-CoV-2 mobility in mucus must be several-fold lower than in aqueous solution or pure water. Engineered polymeric nanoparticles with 200 and 500 nm diameters exhibited a 6-fold and 4-fold decrease in effective diffusivities compared to water, suggesting that larger molecular mass particles, like SARS-CoV-2, may show lower diffusivity in crowded viscoelastic environments [27].

Amine-modified particles have been observed to undergo more rapid transport in cystic fibrosis sputum than carboxylated particles, an effect that may be caused by the reduced adhesivity of the neutrally charged amine-modified particles. The significance of this finding is that drugs, directly or indirectly as LNP-encapsulated drug can be made more mobile by being amine-modified so that the pathogens can be exposed to higher frequency of collision. “Agents that bear positive charge (amine-modified) may enhance viral mobility on ward to the cell membrane (this may be due to reduction of viral interaction with mucus fibers [28] while those bearing negative charge may sink the virus into the mucus and retard their mobility towards the cell membrane”. Secondary bonds that form between interacting particles and mucus affect mostly the short time-scale transport of carboxylated particles, not the long-time scale transport, which was mostly diffusional ($\langle \Delta r^2 \rangle / 2D_v \sim \Delta t$ at high, albeit with a much diminished diffusion coefficient compared with diffusion in buffer.

As noted in the literature [28] the average particle transport rates of 100-, 200-, and 500-nm particles decreased with increasing time, a small but significant fraction of the smaller particles (100 and 200 nm) exhibited more diffusive transport rates *i.e.* ($\langle \Delta r^2(\Delta t) \rangle \sim \Delta t$; *this seems to be an error, otherwise it is appropriate to state that $\langle \Delta r^2 \rangle / 2D_v \sim \Delta t$ where D_v and $\langle \Delta r^2 \rangle$ are the diffusivity and mean square displacement respectively). At long time scales ($\Delta t > 1$ s), the ensemble average MSD of 100- and 200-nm particles scales with time, $\langle \Delta r^2 \rangle / 2D_v \sim \Delta t$ suggesting that particles eventually escape their cages and move more freely [28]. This study focuses on initial and final diffusivity values, similar to the state function, end states of diffusion, and binding period to achieve a non-state function. The study found that particles move in pores too small for 500-nm particles, supporting the claim that supramolecules like SARS-CoV-2 can only show diffusivity in mucus several times less than in water [28]. Examples of this are the G and D versions of SARS-CoV-2, which have, respectively, viscosity values equal to 2.5 and 2.7 exp. (–15) m²/s. According to the literature cited thus far, medium heterogeneity—with varying microviscosities at different points—is responsible for subdiffusive events in SARS-CoV-2 variants.*

2.7 Binding Processes and Cleavage of the S Protein S1–S2 Boundary by Furin

The transmembrane S2 domain of SARS-CoV-2 mediates the fusion of viral and cellular membranes, with a polybasic cleavage site (PRRAR) required for efficient proteolytic cleavage. This site is essential for cell-cell fusion and viral entry into human lung cells. The infection begins with the binding of the spike protein to receptor-angiotensin-converting enzyme 2 (ACE2), resulting in viral RNA genome release [29–33].

3. Methodology

All of the equations used in this section were taken from the literature [16]. Furthermore, since the arithmetic means (where appropriate) of the data in the literature were explored, there was no need for materials and equipment. Given different volumes of cells in the literature, a representative nasal mucus cell volume (similar to a median value) used in this study is 1125 μm^3 . Type I cells which are large squamous cells have a volume of $\sim 2,000$ to $3,000 \mu\text{m}^3$ while Type II cells which are cuboidal cells have a volume

of ~ 450 to $900 \mu\text{m}^3$ [34]. The mean volume of the epithelial cells equal to 1256 ± 240 [35], 630 ± 180 (for 15 different tissues) and $2330 \pm 650 \mu\text{m}^3$ (for 12 types of isolated epithelial cells) [36] are known. The number of cells in nasal mucosa explored is exp. (9) [37, 38]. The root RMSDs of the SARS-CoV-2 S protein explored at 0, 20, 40, and 60°C were 1.53, 2.51, 3.26, and 2.23 nm, respectively [39]. These are molecular dynamics simulation-based (MDS-based) results.

3.1 Methods

The Methods section describes a theoretical and computational study, as no new experiments were performed. Arithmetic means or otherwise of values in the literature were explored in this study.

3.1.1 Testing the Proposition that RMSD or MSD Cannot be Arbitrarily Chosen

Equations (2), (3b), (7), and (8) were explored to test the proposition that RMSD or MSD cannot be arbitrarily chosen.

3.1.2 Viscosities at Temperatures Lower and Higher than 25 °C (298.15 K)

Equation (4) was explored for the theoretical determination of viscosities at temperatures lower and higher than 298.15 K.

3.1.3 Multiplicity of Infection

When the breath emission rate in viral copies per hour and the number density in viral copies per cubic meter in the surrounding air, whether in a room, outside, etc., are known, the multiplicity of infection (*MOI*, *m*) is computed using Eqs (14a) and (14b), respectively.

3.1.4 The Viscosity Coefficient

The viscosity of the medium in which the diffusivity of any solute at a specified thermodynamic temperature is known or given is computed using Eqs. (5) and (6).

3.1.5 Longer and Shorter Average Interparticle Distances Wherein Mutual Attraction is either Unlikely or Likely

Longer and shorter average interparticle distances wherein mutual attraction is either unlikely or likely at low and high viral number density, respectively, are determined using the same model equation, such as Eq. (10).

3.1.6 Maximum Interparticle Distance Given Specific Viral Number Density

The maximum interparticle distance given any viral number density is computed using Eq. (11).

3.1.7 Viral Number Densities per Cell

3.1.7.1 Optimum Viral Number Density per Cell

The viral number density that can give the shortest minimum interparticle distance for achieving a high-frequency collision of viral particles with the target cell membrane is computed using Eq. (9).

3.1.7.2 Low Viral Number Density per Cell Given *BER* and the Number of Viral Copies per Cubic Meter in the Surrounding

The low viral density, given the *BER* and the number of viral copies per cubic meter in the surrounding confined or open area, is computed based on Eqs (15) and (16), respectively.

3.1.7.3 Higher Viral Number Density Given *BER* and the Number of Viral Copies per Cubic Meter in the Surrounding

Given either *BER* or viral copies per cubic meter in the surrounding, confined, or open environment, the higher viral number density per cell is calculated using Eq. (17).

3.2 Determination of the Nonequilibrium Binding Energy with and without the Influence of Aqueous Relative Permittivity

Equations (12) and (13), respectively, were explored for the computation of the nonequilibrium binding energy before and after the viral particle's effective collision with the membrane following the release of hydration water.

4. Results and Discussion

All data recorded in the tables were computed by substituting literature values into derived equations. Some of these parameters include viscosity, breath emission, and the molar mass of the virus. The trends in diffusivities and NEBE with increasing thermodynamic temperatures were reported; the values of those parameters were compared between different variants of SARS-CoV-2. Pertinent issues in the literature were also discussed. The rate at which cytoplasmic proteins and other solutes (this includes prescribed drugs), as mentioned above, reach their various locations is usually limited by passive translational diffusion and the related diffusivity [40]. Given any arbitrarily or intentionally selected time, bits of diffusivity information can improve the calculation of root mean square displacement (RMSD); in contrast to time, RMSD cannot be selected arbitrarily. The importance of drugs is best appreciated, first, from the perspective of pharmacokinetics, which is primarily dependent on diffusivity, without which the intended effect of such drugs cannot be achieved; second, from the perspective of pharmacodynamics, which can be unrealizable if the drug, directly or indirectly through its metabolite, does not bind to the target object—cell membrane, protein, receptor, etc. It appears, therefore, that diffusivity is more or less “a bridge” between pharmacokinetics and pharmacodynamics; “destroy the bridge” and be faced with no distribution and binding to target macromolecules, cells, tissues, etc.

Models or equations can only become useful after their confirmation following a series of evaluations to verify their consistency and possible generalizability. In order to achieve this, RMSD (ℓ) and the time (τ) spent traveling such a displacement were calculated using Eqs. (1) and (3a/3b), respectively. Let's begin: Using FRAP, the diffusion coefficient of bovine serum albumin in normal tissue grown in a thin transparent window in the ear of a rabbit had been determined (this is an example of direct experimental determination). The average albumin diffusion coefficients (in normal tissue), molar mass, and Stokes–Einstein radius were 5.8 +/- 1.3 *exp.* (-11) m²/s, 67 kg/mol, and 3.55 nm respectively [41]. Assuming a Kelvin temperature of 310.15 K and substituting it into Eq. (1) to obtain:

$$\ell = 1.450872474 \text{ nm}$$

In order to determine the value of the average time taken to travel the computed RMSD, ℓ , the value above is substituted into Eq. (3a/3b) to give:

$$\tau = 18.14590157 \text{ ns}$$

Next, these values should be able to reproduce the literature [41] value of diffusion coefficient if the original Einstein equation ($D_i = \ell^2/2\tau$) is fitted to the data to give: 5.800293052 *exp.* (-11) m²/s.

The 2021 CODATA [42] values of Boltzmann constant and Avogadro's number were explored. If the common values such as 6.02252 *exp.* (+23)/mol. and 1.380485245 *exp.* (-23) J/mol./K for Avogadro's number and Boltzmann constant respectively, were explored the value of D_i is equal to 5.799999997 *exp.* (-11) m²/s. In all these computations, the viscosity coefficient was not explored. This important parameter is determined from two equivalent equations as follows: First, the viscosity coefficient is solved in both Eqs (5) and (6) as follows:

From Eq. (5):

$$1.11867796 \text{ exp. } (-3) \text{ kg/m/s } (\sim 1.62 \eta \text{ of pure water at } 310.15 \text{ K})$$

From Eq. (6):

$$1.11862144 \text{ exp. } (-3) \text{ kg/m/s } (\sim 1.62 \eta \text{ of pure water at } 310.15 \text{ K})$$

Both results show that the tissue (the type is not specified in the literature or probably not seen) is more viscous than pure water. With nothing else except RMSD, the diffusivity and time expended travelling the displacement computed based on Eqs (2) and (3b) respectively are shown in Table 1a. The values ranged from 3.633 to 12.099 *exp.* (-14) m²/s, with the peak value at 313.15 K following similar trend for RMSD reported elsewhere [39]. The time spent traveling the RMSD ranged from 32.215 to 43.919 μ s. The corresponding viscosity coefficients ranged from 0.54650284 (the lowest at 313.15 k) to 1.587349262 kg/m/s (the highest at 273.15 k). Cholesterol affects the initial lipid phase, causing a decrease in viscosity in gel phases due to gradual gel phase transition into liquid-ordered (Lo) phase. This results in a fluidized stem cell plasma membrane during differentiation [43-47]. This may be applicable to other kind of cells. The RMSD and the corresponding time may be determined using, respectively, Eqs (7) and (8) given that the membrane and cytoplasm have viscosities of 416 cP [48] and 50 cP [49], respectively. Thus as Table 1b, shows, the media presenting different viscosities expectedly showed RMSD, duration (τ) of traveling RMSD, diffusivity (D), translational velocity, terminal velocity, and the driving force, the thermochemical potential gradient (TCPG) that are different; the latter implies that chemical potential gradient is not enough to drive motion even at very low temperatures in which thermal energy is very low. There is a need to note inconsistencies in the viscosities in the literature.

Table 1a: Diffusivity and time of accomplishing predetermined RMSD (ℓ) at different temperatures: A test of non-arbitrariness of RMSD

T/k	273.15	293.15	313.15	333.15
* ℓ /nm	1.53	2.51	3.26	2.23
D_v (Eq. (2))/e. (-14) m ² /s	3.633	7.909	12.099	7.060
τ (Eq. (3b))/ μ s	32.215	39.830	43.919	35.218
$\ell^2/2\tau$ /e. (-14) m ² /s	3.633	7.909	12.099	7.060
η (Eq.(5))/kg/m/s	1.583	0.781	0.547	1.002
η (Eq.(6))/kg/m/s	1.583	0.781	0.547	1.002
RMSD (Eq. (7))/nm	1.53	2.51	3.26	2.23
τ (Eq. (8))/ μ s	32.215	39.830	43.919	35.218
$\ell^2/2\tau$ / e. (-14) m ² /s	3.633	7.909	12.099	7.060

*Represents data adopted from the literature [39]; *e* means exponent (*exp.*). It should be noted that the same diffusivities calculated using the original Einstein's equation ($D_v = \ell^2/2\tau$) and Eq. (2) prove the correctness of the former; this is applicable to Eqs (3b) and (8) which, though look different, yield the same values. Consequently, there is no intentional duplication.

Table 1b: Biophysical parameters associated with CM and CYT

Medium	RMSD in the medium (Å)	τ/e (-7) (s)	D/e (-12) (m ² /s)	tv/e (-3) (m/s)	tmv (Å/s)	TCPG/ <i>e</i> . (-19) (N)
CM	2.398	1.485	0.194	1.614	0.222	4.901
CYT	9.845	3.009	1.610	3.271	1.843	4.901

The values are recorded to three decimal places; RMSD, *D*, *tv* (u_{trans}), *tmv* (u_{term}), and TCPG are respectively, the root mean square displacement, diffusion coefficient, translational velocity, terminal velocity, and thermochemical potential gradient; CM and CYT are the cell membrane and cytoplasm respectively. The diffusing solute is the green fluorescent protein (GFP). The TCPG is about, 3.06 eV/m. The equations for the translational velocity and terminal velocity are derived from $E_k = [m_i(6k_B T D_i / \ell)^2]^{1/2}$ [16] and given as: $u_{trans} = (6k_B T D_i \rho_i^{1/3} / m_i^{2/3})^{1/2}$ and $m_i u_{trans} / \ell = k_B T u_{term} / D_i$ respectively. The thermodynamic temperature is 310.15 K.

Notable examples include stem cells billed for different tissues, such as osteogenic and chondrogenic tissues; Kashirina *et al.* (2020) [47] reported, at day 14, 458.2 ± 37.02 cP and 438.04 ± 36.67 cP vs. 510.47 ± 40.27 cP in control for osteogenic and chondrogenic mesenchymal stem cells (MSCs), respectively, given that advanced fetuses (or neonates) are also infested by SARS-CoV-2 or any other virus, even though adults, particularly the elderly, are most susceptible to viral infection. The drug, oxaliplatin, changes the viscosity of the plasma membrane of cancer cells that have been grown [48]. If generalizable (or with any other formulated drug), it should be useful in controlling membrane viscosity. Other examples of different viscosities are 36.3 ± 11.2 Pa.s ($\eta_{MCF-10A}$); 65.9 ± 11.4 Pa.s (η_{MCF-7}); 12.0 ± 57 Pa.s ($\eta_{MDA-MB-231}$) [50] for different cell lines and 1-50 cP [51] probably at 22 °C were also reported.

SARS-CoV-2 begins its attractive interaction with the target cell membrane within the interparticle distance (R_0) shown in Table 2. Table 2 also shows the average interparticle distance (R_{int}) outside the region of mutual electrostatic effect for the D- and G-variants of SARS-CoV-2, as well as the ideal numerical density of the virus that is adequate (as opposed to the "dilute state") to cause frequent collision with cell membranes.

A common observation is that there is always an increase in diffusivities due to decreasing viscosity with increasing temperature. This is what Tables (3) and (4) illustrate. The mobility of both beneficial and detrimental biomolecules can be affected by this. For example, germs are more likely to move through a medium like nasal mucus that has a higher viscosity than water as the temperature rises. Although cholesterol plays a dual role in stiffening the cell membrane (CM) at melting temperatures and fluidizing it at lower temperatures, the platform for the virus's binding is likely to be thermodynamically impossible when the CM is reached at temperatures higher than body temperature.

When particles of any size—macromolecules,

supramolecules, and micro-molecules—interact with the cell membrane or with any other, two likely diffusivities are present. The first happens at the beginning of an attractive or repulsive interaction, mostly because of the influence of thermal energy. However, attractive interaction is of interest for the purpose of binding. The second is because the binding interaction slows down the molecule's motion. Table 4 illustrates the increasing trend caused by rising temperature as stated earlier, even if values near binding are lower than those before binding at the start of the attractive interaction because of long-range forces. Compared to the G variant, the D variant of SARS-CoV-2 is faster.

Table 2: Physical parameters, interparticle distance and viral number density pertinent to cell-virus interaction

Average distance (R_{int}) between SARS-CoV-2 and target cell membrane	
SARS-CoV-2 (D-variant)	101.054 nm
SARS-CoV-2 (G-variant)	~ 109.138
Optimum viral number density (C_v)	
SARS-CoV-2 (D-variant)	~ 3.083 <i>exp.</i> (+20)/m ³
SARS-CoV-2 (G-variant)	~ 2.448 <i>exp.</i> (+20)/m ³
Interparticle distance (R_0) wherein mutual attraction begins	
SARS-CoV-2 (D-variant)	~ 71.456 nm
SARS-CoV-2 (G-variant)	~ 77.172 nm

* (the data for SARS-CoV-2 D and G variants are from the works of Moreno *et al.* (2022) [52]; Eqs (10) and (11) were used to compute R_{int} and R_0 respectively; the hydrodynamic radii for the G-and D-variants used are 58.499 and 54.427 nm respectively; the viral number density is according to Eq. (9).

Table 3: Translational diffusion coefficient for D- and G- variants of SARS-CoV-2 ($D_v \cdot 10^{15} / m^2/s$) due to heat energy only

T/k	$\eta/kg/m s$	D-variant	G-variant
293.15	1.801	2.398667407	2.183742183
298.15	1.600*	2.7*	2.5*
303.15	1.433	3.065210576	2.838157940
308.15	1.292	3.455799930	3.199814750
313.15	1.173	3.868150269	3.581620619
318.15	1.066	4.324378068	4.004053766

* The data for SARS-CoV-2 D and G variants are from the works of Moreno *et al.* (2022) [52].

Table 4: Translational diffusion coefficient (D_v) for D- and G- variants of SARS-CoV-2 at the beginning of electrostatic interaction and upon close to binding at different temperatures (Table 3.)

The beginning $D_{V(\epsilon_0\epsilon_r)} \cdot 10^{15} / m^2/s$		Close to binding $D_{V(\epsilon_0)} \cdot 10^{15} / m^2/s$	
D-variant	G-variant	D-variant	G-variant
4.360	4.151	1.519	1.405
4.989	4.752	1.738	1.609
5.664	5.395	1.973	1.827
6.386	6.082	2.225	2.059
7.147	6.808	2.490	2.305
7.990	7.611	2.784	2.577

D- and G-variants of SARS-CoV-2 are those studied by Moreno *et al.* (2022) [52]; this also includes reference to viscosity coefficient (1.6 Pa.s) of nasal mucus in the literature [53].

Entry into host cell marks the first step of viral infection. The S protein first undergoes proteolysis into S1 and S2 [54]. This may be mediated either by host cell furin, by serine proteases such as the transmembrane protease, serine 2 (TMPRSS2) [55], or by cathepsin proteases in the late endosome/endolysosomes [56]. But regardless of the pathway—early, late, or delayed—the coronavirus's inability to attach to the host cell membrane for whatever reason is a surefire means to disinfect the cell. The ultimate objective must always be to determine how to prevent or postpone the effective viral-membrane interaction that occurs before any type of binding. If such binding occurred, it must be made unstable, making it impossible for the coronavirus–membrane complex to be thermodynamically stable; it is therefore clear that Gibbs free energy and nonequilibrium binding energy are different parameters. A proper understanding of nonequilibrium binding energy and its formation could be helpful in designing a means of circumventing it.

Two aspects of the non-state function are examined based on the following premises: the first is the nonequilibrium binding energy, which starts at a point in space and time where the pathogens in general (but specifically SARS-CoV-2 for the purposes of this study) and the host cell membrane begin to interact attractively; the second is the point at which a vacuum-like space forms between the invading pathogen and the susceptible host cell as a result of the displacement of aqueous solvent from the cell membrane surface into the extracellular fluid.

Recall that in strictly thermodynamic terms, a "non-state function," otherwise known as a "path function," is a property that depends on the path or process taken to reach a particular state, unlike state functions, which only depend on the initial and final states. This is despite the fact that, as stated earlier, one may be interested only in what transpires at the beginning and at the end when binding with higher stability occurs, similar to the view elsewhere [57]. The findings of this study demonstrate that the nonequilibrium binding energy (the lowest and maximum) for the D- and G-variants of SARS-CoV-2 is several times more than the thermal energy denoted by R_gT , where T is between 293.15 and 318.15 K and R_g is the gas constant (Table 5). The omicron versions showed similar findings (Table 6).

Table 5: Minimum and maximum nonequilibrium binding energy (NEBE) of D- and G- variants of SARS-CoV-2

Min NEBE /cal/mol.		Max NEBE /kcal/mol.	
D-variant	G-variant	D-variant	G-variant
891.284	891.284	833.520	951.628
906.486	906.486	810.868	879.437
921.687	921.687	715.711	817.126
936.889	936.889	667.957	762.605
952.091	952.091	626.286	715.029
967.292	967.293	587.596	670.856

Min and Max represent minimum and maximum values, respectively; it is important to note that the Min NEBE values are identical for both the D- and G-variants of the virus, based on the principle that the thermal energy dominating far from the binding site should remain constant. Calculations are based on Eqs (12) and (13) for Min NEBE and max NEBE respectively.

Table 6: Minimum and maximum nonequilibrium binding energy of Omicron & delta variants of SARS-CoV-2

Min NEBE /cal/mol.		Max NEBE /kcal/mol.	
Omicron-variant	Delta-variant	Omicron-variant	Delta-variant
906.486	906.486	732.584	603.776

Min and Max stand for minimum and maximum respectively. The R_0 , R_{int} , and C_v values are 53.03300859 nm, 75 nm, and 7.542087542 *exp.* (+20) respectively for delta variant; they are 64.34671709 nm, 91 nm, and 4.222320368 *exp.* (+20) respectively for omicron variant. $2R_v$ (diameter) for delta variant is 75 nm [58]; diameter for omicron as a representative average for all variants is 91 nm [59].

There is a need to opine that the lack of definite values for the molar masses and the diameters of coronaviruses of the kind explored in this study is a major challenge. For instance, Delta and Gamma variants of SARS-CoV-2 have, respectively, 75 nm and 79 nm diameters [58]; the average SARS-CoV-2 size is 91 +/- 11 nm [59]. These independent parameters have roles in the computation of dependent variables such as those indicated by Eqs (13), (17), etc. It is the nonequilibrium binding energy that is ultimately affected.

The respiratory tract is a key part of an elaborated line of defense based on a unique cellular ecosystem. In this regard, the mucus in whatever location in the respiratory system plays a key role. Thus, secretory and multiciliated cells form a self-clearing mechanism that efficiently removes inhaled particles from the upper airways, impeding their transfer to deeper lung zones. This can be overwhelmed with time. Although the nose and bronchus share many cellular properties, which has led to the definition of a pathophysiological continuum in allergic respiratory diseases [60, 61], they differ by features such as host defense against viruses, oxidative stress [62], or antibacterial mechanisms [63, 64]. Be it as it may, individuals who are heavily infested with or without symptoms are liable to releasing viral particles in viral copies/m³ or viral copies/hr. to the surrounding. In order to determine adequate viral number density after a long time exposure to the environment, this study made an effort for the first time to link breathing rate and breath emission to the question of coronavirus infection preceded by the binding interaction of viral particles with the membrane.

The multiplicity of infection (MOI, or m for convenience) was calculated by examining the breath emission quantified in viral number density (number of viral particles per cubic meter). Next, the MOI was used to calculate the initial number of infected cells (NIC). The initial viral number density ($C_{v(\tau \rightarrow 0)}$) in its dilute state was then determined and displaced in Table 7. The maximum ($R_{int(\tau \rightarrow 0)}$) and minimum ($R_{0(\tau \rightarrow 0)}$) inter viral-membrane distance under dilute condition and the same parameters under higher number density are also shown in Table 7. With the aforementioned parameters, the minimum (influenced by the relative permittivity (ϵ_r) of the medium) and the maximum (free from ϵ_r) nonequilibrium binding energies ($\xi_{select/\epsilon_0\tau}$ and ξ_{select/ϵ_0}) were computed for breath emission (\mathcal{B}) of 1.29 and 27.9 *exp.* (7) per cubic meter [65]. The delta variant has greater values of NEBE than omicron variant (Table 7).

Table 8a provides all physicochemical parameters for all computations based on breath emission rates in virus particles per hour, which vary from 9.31 to 201 exp. (6) /hr [65]. Table 8b displays findings for the Omicron variant only that are comparable to those in Table 7. These enables indirect comparison with literature values as follows: Omicron has the highest binding affinity at -128.35 ± 10.91 kcal/mol, followed by Delta at -82.78 ± 8.84 kcal/mol and the wild type at -73.26 ± 7.46 kcal/mol, according to the results of a simulation [66]. As long as the values correspond to the Gibbs free energy of binding to ACE2, this study's findings support the idea that the Omicron variant has a lower energy barrier for binding than other variants due to its higher nonequilibrium binding energy (Table 7 & 8b), which is kinetic in nature and related to activation energy. This means that the omicron variant is likely to be more stable than other variants in its binding to the cell membrane.

Table 7: Physical parameters and cognate nonequilibrium binding energies of delta and omicron variants of SARS-CoV-2 given viral emission in viral copies per unit volume

$B/exp. (+7)/m^3$	1.29*	27.9*
$m/exp. (-5)$	7.740	167.40
$N_{IC}/exp. (+4)$	7.7397	167.25996
$C_{V(\tau \rightarrow \infty)}/exp. (+14)/m^3$	4.535323	4.538944
1.29* exp. (+7)/ m³		
Virus	Delta variant	Omicron variant
$R_{int(\tau \rightarrow 0)}/exp. (-5) m$	6.840802	6.210935
$R_{0(\tau \rightarrow 0)}/exp. (-5) m$	6.838927	6.208660
$C_{V(\tau \rightarrow \infty)}/exp. (+20)/m^3$	5.815028	5.266537
$R_{int(\tau \rightarrow \infty)}/exp. (-8) m$	8.199060	8.469441
$R_{0(\tau \rightarrow \infty)}/exp. (-8) m$	6.039711	5.761551
$\xi_{elect}/\epsilon_0 \epsilon_r /cal./mol$	1188.70900	987.38918
$\xi_{elect}/\epsilon_0 /kcal./mol$	656.020	663.212
27.9*/ exp. (+7)/ m³		
Virus	Delta variant	Omicron variant
$R_{int(\tau \rightarrow 0)}/exp. (-5) m$	6.838073	6.208458
$R_{0(\tau \rightarrow 0)}/exp. (-5) m$	6.836198	6.206183
$C_{V(\tau \rightarrow \infty)}/exp. (+20)/m^3$	5.818737	5.268640
$R_{int(\tau \rightarrow \infty)}/exp. (-8) m$	8.197222	8.468371
$R_{0(\tau \rightarrow \infty)}/exp. (-8) m$	6.037786	5.760401
$\xi_{elect}/\epsilon_0 \epsilon_r /cal./mol$	1188.688156	987.351544
$\xi_{elect}/\epsilon_0 /kcal./mol$	568.921	633.001

The symbol B signifies breath emission in number of viral particles per cubic meter. The values* of B are as in the literature [65].

Table 8a: Physical parameters and cognate nonequilibrium binding energies of omicron variant of SARS-CoV-2 given breathe emission rate per hour

BER/exp. (+6)/hr.	9.31*	201*
$m(a)$	0.149625	3.230357
$m(b)/exp. (-4)$	0.415625	8.973221
$N_{IC}(a)/exp. (+6)$	138.969198	960.456626
$N_{IC}(b)/exp. (+6)$	0.041562	0.896919
$C_{V \rightarrow B}(a)/exp. (+14)/m^3$	4.882891	15.253313
$C_{V \rightarrow B}(b)/exp. (+14)/m^3$	4.535242	4.537182
$R_{int(B)}(a)/exp. (-5) m$	5.985888	3.387679
$R_{0(B)}(a)/exp. (-5) m$	5.983613	3.385330
$R_{int(B)}(b)/exp. (-5) m$	6.210991	6.209663
$R_{0(B)}(b)/exp. (-5) m$	6.208716	6.207388

$C_{V(\tau \rightarrow \infty)}(a)/exp. (+20)/m^3$	5.464615	9.658776
$C_{V(\tau \rightarrow \infty)}(b)/exp. (+20)/m^3$	5.266490	5.267617
$R_{int(\tau \rightarrow \infty)}(a)/exp. (-8) m$	8.371544	7.099578
$R_{0(\tau \rightarrow \infty)}(a)/exp. (-8) m$	5.656167	4.747660
$R_{int(\tau \rightarrow \infty)}(b)/exp. (-8) m$	8.469465	8.468892
$R_{0(\tau \rightarrow \infty)}(b)/exp. (-8) m$	5.761577	5.760961

The alphabets, 'a' and 'b', signify computations at breath emission rate equivalent to number of viral particles emitted per hour and per minute respectively. BER values* are as in the literature [65].

Table 8b: Minimum and maximum nonequilibrium energies

Parameters	9.31 exp. (6)/hr.	201 exp. (6)/hr.
$\xi_{elect}/\epsilon_0 \epsilon_r /cal./mol$	522.390979	962.988964
$\xi_{elect}/\epsilon_0(a)/kcal./mol$	651.703	444.157
$\xi_{elect}/\epsilon_0 \epsilon_r (b)/cal./mol$	987.188601	987.273963
$\xi_{elect}/\epsilon_0(b)/kcal./mol$	683.353	663.212

The alphabets, 'a' and 'b' are as previously defined

It is necessary to comment that different approaches give different Gibbs free binding energies going by the following: Using unfamiliar method, involving a homogeneous glycan setup, the binding free energy (ΔG_{bind}) for Omicron was -30.21 ± 5.48 kcal/mol lower than -18.32 ± 1.62 kcal/mol for the WT [67]; this inference based on number theory needs to be seen as one in which higher negative magnitude of the mixed decimal signifies greater thermodynamic stability or feasibility of ligand-receptor complex. Besides they are much lower than Ju *et al.* (2024) [66] report. The binding of SARS-CoV-RBD and SARS-CoV-2-RBD to ACE2-PD with time, is driven by electrostatic interactions [12]. This is significant because it reminds researchers in science and medicine that it is possible that there may be other existing variants with stronger binding affinity that should thus, require stronger drugs or medications that can hinder binding processes. These findings are based on coarse-grained and all-atom steered (MD) simulations experiments [12] which showed that SARS-CoV-2 (-875.89 kcal/mol.) had greater binding affinity to its receptor than SARS-CoV (-695.95 kcal/mol.). It is not certain whether those values reported as electrostatic energies are free energies for binding. If they are total energies according to convention, then the kinetic components should be equal in magnitude to the former but opposite in sign. In this way, they compare very well with the results in this study. Some examples are as follows: 670.6–951.6 and 587.6–833.5 kJ/mol for G and D variants of SARS-CoV-2, respectively (Table 5). However, according to the Nudged Elastic Band (NEB) binding energy profiles, Delta has the lowest transition state barrier at 9.21kcal/mol, whereas the wild type has the highest at 17.87kcal/mol [66]. Although the Omicron form is said to have a little greater energy barrier than Delta variants, no precise figure was provided [66]. These values are completely out of line with nonequilibrium binding energies like 632.7–719.9 kcal/mol (Table 7), which span the Delta and Omicron variants and indicate the amount of work required to overcome mechanical kinetic energy in order to hold the ligand to the receptor.

4.1 Viscosity as a Measure of Resistance of the Medium on Mobility of Biomolecules and its Variation

The nature of the medium should not be the only factor considered when evaluating viscosity as a measure of resistance to any particle motion in the medium; a medium that produces high viscosity for one solute may do so at a lower viscosity for another solute with distinct physicochemical properties. In contrast to solely polar solutes lacking lipophilic functional groups, polar solutes with lipophilic properties can diffuse more quickly through lipid bilayers that contain peripheral and integral proteins, glycosylated and unglycosylated proteins, etc. Water (kinetic size: 2.68Å) and ethanol (kinetic size: 4.5Å) have diffusivities over the chitosan membrane of 2.8 and 3.32 exp. (-11) m²/s, respectively [68] which correspond to viscosities of 0.0303 and 0.01524 kg/m/s calculated in this study. The conditions in the cytoplasm and mucus are pertinent even if this is about the membrane. The scientific and medical (therapeutic) implications are that one SARS-CoV-2 variant diffuses more quickly than the other, making it more likely to strike its target membrane. If binding is stable greater stability makes such virus more contagious. Therefore, such a situation necessitates the use of more potent, highly effective medications as well as alternative therapeutic approaches.

Viscosity has a major impact on the velocity of translational diffusion in cells, which in turn affects cellular reactivity by influencing the frequency and manner of reactant collisions with enzyme active sites [57, 69]. Diffusion coefficients for macromolecules [70] and tiny solutes [57-59] are 5–50 times lower in mammalian cells than in pure water, according to research. Meanwhile, the mucus consists of two layers, with the upper layer being a gel with a 3% mucin network and the remaining 90-95% water with electrolytes, serum proteins, immunoglobulins, and lipids. However, experimental studies show mucus viscosity can be as high as 10,000 times that of water [71] due to overlapping adhesive mucin fibers. It seems unimaginable that the diffusivity of SARS-CoV-2 in mucus could be similar to that in water, and in particular, if normal body temperature remains consistent without variation. As initially cited by Adamczyk *et al.* (2021) [72], this and the study's results (Table 3.) also support the theory that the virion diffusion coefficient is substantially lower in media with a higher viscosity, like mucus or saliva, which adds to the overall virion transfer rate [73]. The cellular domains containing dimer 1 (a fluorescent molecular rotor and an effective PDT photosensitizer) had a viscosity of (300±50) cP after photodynamic treatment (PDT). This number is higher than the 50 cP value that was achieved before PDT [74]. The regulation of mucus and cytoplasmic viscosity may benefit from this discovery. Viral, medication, and Ig diffusivities can be controlled by increasing or decreasing the mucus's viscosity.

4.2 General Matters Arising

The nonequilibrium binding energy is not just about the binding of a substrate and ligand to an enzyme and a receptor, respectively, but it also includes biological porters, *i.e.*, carriers such as carrier proteins, albumin, lipid nanoparticles (LNP), etc. In order to deliver, these carriers must make contact with the target surface, usually the cell or receptor membrane. Examples of lipid-based nanoparticles as potential carriers of medications are liposomes,

nanoemulsions, solid lipid nanoparticles (LNPs), nanostructured lipid carriers, and lipid polymer hybrid nanoparticles [75]. The importance and usefulness of LNPs lie in their capacity to safeguard drugs from *in vivo* degradation, boost their solubility and efficacy, enable targeted drug delivery to the disease site, regulate drug release, and alter drug bio-distribution [76]. The most crucial element is targeted delivery, which cannot be accomplished by "saltatory mechanisms" or *American James Bond flyover action movies, frequently to the delight of teenagers*. In this case, the RMSD is crucial because it allows investigators to forecast the distance that free drugs or LNPs can travel in space before being completely distributed at equilibrium and outside of the equilibrium state as they approach the target.

In addition to the viscosity (either known or unknown) of the biological medium, there are other factors that may affect the upward trajectory of the rate of diffusion of the nanoparticles. The effectiveness of such nanoparticles in therapeutic applications is based on their size, shape, and composition, which are morphological characteristics in addition to the surface chemistry (net charge in particular) that slow down the transport of bioactive cargo, which adversely alters therapeutic efficacy [77].

Using Line-FRAP measurements, Brownian dynamics simulations, and molecular docking, researchers found that the diffusion rates of the small molecules are highly affected by self-aggregation, interactions with the proteins, and surface adsorption [78]. Some drugs (e.g., quinacrine) have their diffusion unaffected by protein crowders [78]. Based on these findings, it might be useful to create neutral carriers for medicines (like nanoparticles) that can move easily in mucus, while others could be designed to move less freely because of their interactions with mucus components, including the coronavirus.

Nonequilibrium binding energy benefits both mucosa and cytoplasm, with fluid-like and gel-like compartments. Mucus also has gel-like consistency, suggesting two intramucus compartments. The effects of crowding arise from two phenomena, hard-core repulsions and nonspecific chemical (soft) interactions [79-85]. Hard-core repulsions limit the spatial volume of biological macromolecules, favoring compact forms over expanded ones, resulting in increased viscosity. Additionally, the presence of crowding molecules not only reduces available volume or volume exclusion effects [86], but also engages in chemical interactions. Increasing medium viscosity slows viral particle diffusion, ensuring drugs, antibodies, and carriers (LNP) maintain their mobility and affinity for pathogens. However, attractive interactions may destabilize essential enzymes, potentially hindering viral replication, as seen with chymotrypsin inhibitor 2 [84, 87] in reconstituted *E. coli* cytosol [83].

5. Conclusion

A more practical method for determining the viral number density in the order of exp. (+20) was determined using the successfully derived equations for the multiplicity of infection (MOI) associated with breath emission (viral number per hour or per cubic meter) and ventilation rate. Since nonequilibrium binding energy (NEBE) is a function of these factors, the virus's size and molar mass (perhaps one of these limitations in this study aside from inconsistent viscosities) must be consistent to allow for reliable comparison of NEBE among variants. If there are no computation errors, the values of minimum NEBE ($\xi_{select}/\epsilon_0 \epsilon_r$)

remain constant because temperature largely controls the minimum NEBE. As is typical, diffusivities increase with temperature; however, near binding, the diffusivities are lower than when they are far from binding. The decreasing trend in maximum NEBE (ξ_{select/z_0}) with rising temperature implies that the binding affinity of the virus may be attenuated at higher temperatures. It is, therefore, medically plausible to administer airborne, parenteral, oral, etc., drugs at temperatures above body temperatures that are tolerable and in a controlled fashion. As long as variants of SARS-CoV-2 exhibit different values of ξ_{select/z_0} , the logical response should be the continuation of searches for better drugs and vaccines. However, future research must specify the exact values of the molar masses of the coronavirus variants.

6. Dedication

The burning issue of equal opportunity for all without divisive and derisive policies was the main concern of Professor Ambrose Folorunsho Alli, the former governor of the now-defunct Bendel State, and his deputy, Chief Demas Onolobakpovba Akpore. They made textbooks and even writing materials available for all regardless of social status; they made the state look like a one-party state not by manipulation or coercion, but by being statesmen for all in line with constitutional order; they separated the state from personality cult; hence, the governor never customized supplies, educational material, medical material, etc. after his name; rather, such supplies were labeled, "These are properties of the state and not for sale." This is an expression of selfless service to humanity.

7. Disclaimer (Artificial Intelligence)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

8. Disclaimer

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10. Competing Interests

Author(s) have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

11. Author's Contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

12. References

1. Marbach S, Holmes-Cerfon, M. Mass changes the diffusion coefficient of particles with ligand-receptor contacts in the over damped limit. *Phys. Rev. Lett.* 2020; 129:048003. Doi: 10.1103/physrevlett.129.048003
2. Schmidt J, Skinner J. Hydrodynamic boundary conditions, the Stokes-Einstein law, and long-time tails in the Brownian limit. *J Chem Phys.* 2003; 119:8062. Doi: 10.1063/1.1610442
3. Usabiaga FB, Xie X, Delgado-Buscalioni R, Donev A. The Stokes-Einstein relation at moderate Schmidt number. *J Chem Phys.* 2013; 139:214113. Doi: 10.1063/1.4834696
4. Fushimi K, Verkman AS. Low viscosity in the aqueous domain of cell cytoplasm measured by picosecond polarization microfluorimetry. *J. Cell Biol.* 1991; 112(4):719-725. Doi: 10.1083/jcb.112.4.719
5. Bian X, Kim C, Karniadakis GE. 111 years of Brownian motion. *Soft Matter.* 2016; 12(30):6331-6346. Doi: 10.1039/c6sm01153e
6. Kestin J, Sokolov M, Wakeham WA. Viscosity of liquid water in the range -8 °C to 150 °C. *J. Phys. Chem. ref. Data.* 1978; 7:941. Doi: 10.1063/1.555581
7. Popovic M. Strain wars 2: Binding constants, enthalpies, entropies, Gibbs energies and rates of binding of SARS-CoV-2 variants. *Virol.* 2022; 570:35-44.
8. Yamamoto J, Sasaki A. Fluorescence correlation spectroscopy measurement based on fiber optics for biological materials. *Appl. Sci.* 2021; 11(15):6744. Doi: 10.3390/app11156744
9. Longo E, Scalisi S, Lanzaò L. Segmented fluorescence correlation spectroscopy (FCS) on a commercial laser scanning microscope. *Sci. Rep.* 2024; 14:17555. Doi: 10.1038/s41598-024-68317-7
10. Kapanidis AN, Uphoff S, Stracy M. Understanding protein mobility in bacteria by tracking single molecules *J. Mol. Biol.* 2018; 430:4443-4455. Doi: 10.1016/j.jmb.2018.05.002
11. Loren N, Hagman J, Jonasson JK, Deschout H, Bernin D, Cella-Zanacchi F. Fluorescence recovery after photobleaching in material and life sciences: Putting theory into practice. *Q. Rev. Biophys.* 323-387. Doi: 10.1017/S0033583515000013
12. Nguyen HL, Lan PD, Thai NQ, Nissley DA, O'Brien EP, Li MS. Does SARS-CoV-2 bind to human ACE2 more strongly than does SARS-CoV? *J. Phys. Chem. B.* 2020; 124(34):7336-7347. Doi: 10.1021/acs.jpcc.0c04511
13. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science.* 2020; 367(6483):1260-1263. Doi: 10.1126/science.abb2507
14. Bhati AP, Wan S, Coveney PV. Equilibrium and nonequilibrium ensemble methods for accurate, precise and reproducible absolute binding free energy calculations. *J. Chem. Theory Comput.* 2025; 21(1):440-462. Doi: 10.1021/acs.jctc.4c01389
15. Serra E, Ghidini A, Decherchi S, Cavalli A. Nonequilibrium binding free energy simulations: Minimizing dissipation. *J. Chem. Theory Comput.* 2025; 4:2079-2094. Doi: 10.1021/acs.jctc.4c01453
16. Udemá II. Coronavirus-orchestrated pathophysiological state: Biophysical aspects of coronavirus infection. UK. LAP Lambert Academic Publishing, 2025, 22-31
17. Shabram P, Aguilar-Cordova E. Multiplicity of infection/multiplicity of confusion. *Molecular Therapy.* 2000; 2(5):420-421. Doi: 10.1006/mthe.2000.0212

18. Carlstedt I, Sheehan JK. Macromolecular properties and polymeric structure of mucus glycoproteins. *Ciba Found. Symp.* 1984; 109:157-172. Doi: 10.1002/9780470720905.ch11
19. Thornton DJ, Sheehan JK. From mucins to mucus: Toward a more coherent understanding of this essential barrier. *Proc Am Thorac Soc.* 2004; 1(1):54-61. Doi: 10.1513/pats.2306016
20. Shogren R, Gerken TA, Jentoft N. Role of glycosylation on the conformation and chain dimensions of O-linked glycoproteins: Light-scattering studies of ovine submaxillary mucin. *Biochemistry* 1989; 28: 5525-5536. Doi: 10.1021/bi00439a029
21. Lamblin G, Lhermitte M, Klein A, Houdret N, Scharfman A, Ramphal R, Roussel P. The carbohydrate diversity of human respiratory mucins: A protection of the underlying mucosa? *Am. Rev. Respir. Dis.* 1991; 144(3 Pt 2):S19-S24. Doi: 10.1164/ajrcm/144.3_pt_2.S19
22. Masson PL, Heremans JF. Sputum proteins. In M. F. Dulfano (Ed.), *Fundamentals and clinical pathology*, 1973, 412-474.
23. Harbitz O, Jenssen AO, Smidsrød O. Lysozyme and lactoferrin in sputum from patients with chronic obstructive lung disease. *Eur J Respir Dis.* 1984; 65(7):512-520.
24. Girod S, Zahm JM, Plotkowski C, Beck G, Puchelle E. Role of the physicochemical properties of mucus in the protection of the respiratory epithelium. *Eur Respir J.* 1992; 5(4):477-487.
25. Saltzman WM, Radomsky ML, Whaley KJ, Cone RA. Antibody diffusion in human cervical mucus. *Biophys J.* 1994; 66(2 Pt 1):508-515. Doi: 10.1016/S0006-3495(94)80802-1
26. Olmsted SS, Padgett JL, Yudin AI, Whaley KJ, Moench TR, Cone RA. Diffusion of macromolecules and virus-like particles in human cervical mucus. *Biophys. J.* 2001; 81(4):1930-1937. Doi: 10.1016/S0006-3495(01)75844-4
27. Lai SK, O'Hanlon DE, Harrold S, Man ST, Wang YY, Cone R, Hanes J. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104(5):1482-1487. Doi: 10.1073/pnas.0608611104
28. Dawson M, Wirtz D, Hanes J. Enhanced viscoelasticity of human cystic fibrotic sputum correlates with increasing micro-heterogeneity in particle transport. *J. Biol. Chem.* 2003; 278(50):50393-50401. Doi: 10.1074/jbc.M309026200
29. Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol. Cell.* 2020; 78(4):779-784.e5. Doi: 10.1016/j.molcel.2020.04.022
30. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, *et al.* Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat. Commun.* 2020; 11(1):1620. Doi: 10.1038/s41467-020-15562-9
31. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell.* 2020; 181(2):281-292.e6. Doi: 10.1016/j.cell.2020.02.058
32. Xia S, Lan Q, Su S, Wang X, Xu W, Liu Z, *et al.* The role of furin cleavage site in SARS-CoV-2 spike protein-mediated membrane fusion in the presence or absence of trypsin. *Signal Transduct Target Ther.* 2020; 5(1):92. Doi: 10.1038/s41392-020-0184-0
33. Li X, Yuan H, Li X, Wang H. Spike protein-mediated membrane fusion during SARS-CoV-2 infection. *J. Med. Virol.* 2023; 95(1):e28212. Doi: 10.1002/jmv.28212
34. Crandall ED, Matthay MA. Alveolar epithelial transport. *Basic science to clinical medicine. Am J Respir Crit Care Med.* 2001; 163(4):1021-1029. Doi: 10.1164/ajrcm.163.4.2006116
35. Uribe A, Gundersen HJ. Three-dimensional estimation of the glandular volume, and of the number and volume of epithelial cells in two glands from the antral mucosa of five healthy volunteers. *APMIS.* 1997; 105(7):571-574. Doi: 10.1111/j.1699-0463.1997.tb05055.x
36. Devany J, Falk MJ, Holt LJ, Murugan A, Gardel ML. Epithelial tissue confinement inhibits cell growth and leads to volume-reducing divisions. *Dev. Cell.* 2023; 58(16):1462-1476.e8. Doi: 10.1016/j.devcel.2023.05.018
37. Crapo JD, Barry BE, Gehr P, Bachofen M, Weibel ER. Cell number and cell characteristics of the normal human lung. *Am. Rev. Respir. Dis.* 1982; 126(2):332-337. Doi: 10.1164/arrd.1982.126.2.332
38. Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. *Am. J. Respir. Cell Mol. Biol.* 1992; 6(2):235-243. Doi: 10.1165/ajrcmb/6.2.235
39. Khan FI, Lobb KA, Lai D. The molecular basis of the effect of temperature on the structure and function of SARS-CoV-2 spike protein. *Front. Mol. Biosci.* 2022; 9:794960. Doi: 10.3389/fmolb.2022.794960
40. Bellotto N, Agudo-Canalejo J, Colin R, Golestanian R, Malengo G, Sourjik V. Dependence of diffusion in *Escherichia coli* cytoplasm on protein size, environmental conditions, and cell growth. *ELife.* 2022; 11:e82654. Doi: 10.7554/eLife.82654
41. Chary SR, Jain RK. Direct measurement of interstitial convection and diffusion of albumin in normal and neoplastic tissues by fluorescence photobleaching. *Proc. Natl. Acad. Sci. U.S.A.* 1989; 86(14):5385-5389. Doi: 10.1073/pnas.86.14.5385
42. Tiesinga E, Mohr PJ, Newell DB, Taylor BN. CODATA recommended values of the fundamental physical constants: 2018. *Reviews of Modern Physics.* 2021; 93(2):033105. Doi:10.1063/5.0064853
43. Wu Y, Stefl M, Olyzysnska A, Hof M, Yahioglu G, Yip P, *et al.* Molecular rheometry: Direct determination of viscosity in Lo and Ld lipid phases via fluorescence lifetime imaging. *Phys. Chem. Chem. Phys.* 2013; 15:14986-14993. Doi: 10.1039/c3cp51953h
44. Dent MR, López-Duarte I, Dickson CT, Geoghegan ND, Cooper JM, Gould IR, *et al.* Imaging phase separation in model lipid membranes through the use of BODIPY-based molecular rotors. *Phys. Chem. Chem. Phys.* 2015; 17(28):18393-18402. Doi: 10.1039/c5cp01937k
45. Meleshina AV, Kasirina AS, Dudenkova VV, Udovina NV, Zagaynova EV. Intracellular pH monitoring in stem cells during differentiation using fluorescence microscopy and pH-sensor SypHer-2. *Med. Tech.* in

- Medicine. 2018; 10(1):93-102. Doi: 10.17691/stm2018.10.1.12
46. Matsuzaki T, Matsumoto S, Kasai T, Yoshizawa E, Okamoto S, Yoshikawa HY, *et al.* Defining lineage-specific membrane fluidity signatures that regulate adhesion kinetics. *Stem Cell Rep.* 2018; 11(4):852-860. Doi: 10.1016/j.stemcr.2018.08.010
 47. Kashirina AS, López-Duarte I, Kubánková M, Gulin AA, Dudenkova VV, Rodimova SA, *et al.* Monitoring membrane viscosity in differentiating stem cells using BODIPY-based molecular rotors and FLIM. *Sci. Rep.* 2020; 10(1):14063. Doi: 10.1038/s41598-020-70972-5
 48. Shimolina L, Gulin A, Ignatova N, Druzhkova I, Gubina M, Lukina M, *et al.* The role of plasma membrane viscosity in the response and resistance of cancer cells to oxaliplatin. *Cancers.* 2021; 13(24):6165. Doi: 10.3390/cancers13246165
 49. Khismatullin DB, Truskey GA. Leukocyte rolling on P-selectin: A three-dimensional numerical study of the effect of cytoplasmic viscosity. *Biophys J.* 2012; 102(8):1757-1766. Doi: 10.1016/j.bpj.2012.03.018
 50. Dessard M, Manneville JB, Berret JF. Cytoplasmic viscosity is a potential biomarker for metastatic breast cancer cells. *Nanoscale Adv.* 2024; 6(6):1727-1738. Doi: 10.1039/d4na00003j
 51. Molines AT, Lemièrre J, Gazzola M, Steinmark IE, Edrington CH, Hsu CT, *et al.* Physical properties of the cytoplasm modulate the rates of microtubule polymerization and depolymerization. *Dev. Cell.* 2022; 57(4):466-479.e6. Doi: 10.1016/j.devcel.2022.02.001
 52. Moreno N, Moreno-Chaparro D, Usabiaga FB, Ellero M. Hydrodynamics of spike proteins dictate a transport-affinity competition for SARS-CoV-2 and other enveloped viruses. *Scientific Reports.* 2022; 12(1):11080. Doi: 10.1038/s41598-022-14884-6
 53. Leal J, Smyth HD, Ghosh D. Physicochemical properties of mucus and their impact on transmucosal drug delivery. *Int. J. Pharm.* 2017; 532:555-572. Doi: 10.1016/J.IJPHARM.2017.09.018
 54. Heald-Sargent T, Gallagher T. Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence. *Viruses.* 2012; 4(4):557-580. Doi: 10.3390/v4040557
 55. Cheng YW, Chao TL, Li CL, Chiu MF, Kao HC, Wang SH, *et al.* Furin inhibitors block SARS-CoV-2 spike protein cleavage to suppress virus production and cytopathic effects. *Cell Rep.* 2020; 33(2):108254. Doi: 10.1016/j.celrep.2020.108254
 56. Peacock TP, Goldhill DH, Zhou J, Baillon L, Frise R, Swann OC, *et al.* The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets. *Nat. Microbiol.* 2021; 6(7):899-909. Doi: 10.1038/s41564-021-00908-w
 57. Kao HP, Abney JR, Verkman AS. Determinants of the translational mobility of a small solute in cell cytoplasm. *J. Cell Biol.* 1993; 120(1):175-184. Doi: 10.1083/jcb.120.1.175
 58. Vieira DFB, Bandeira DM, Da Silva MA N, De Almeida ALT, Araújo M, Machado AB, *et al.* Comparative analysis of SARS-CoV-2 variants Alpha (B.1.1.7), Gamma (P.1), Zeta (P.2) and Delta (B.1.617.2) in Vero-E6 cells. *Braz. J. Infect. Dis.* 2024; 28(1):103706. Doi: 10.1016/j.bjid.2023.103706
 59. Ke Z, Oton J, Qu K, Cortese M, Zila V, McKeane L, *et al.* Structures and distributions of SARS-CoV-2 spike proteins on intact virions. *Nat.* 2020; 588:498-502. Doi: 10.1038/s41586-020-2665-2
 60. McDougall CM, Blaylock MG, Douglas JG, Brooker RJ, Helms PJ, Walsh GM. Nasal epithelial cells as surrogates for bronchial epithelial cells in airway inflammation studies. *Am J Respir Cell Mol Biol.* 2008; 39(5):560-568. Doi: 10.1165/rcmb.2007-0325OC
 61. Samitas K, Carter A, Kariyawasam HH, Xanthou G. Upper and lower airway remodelling mechanisms in asthma, allergic rhinitis and chronic rhinosinusitis: The one airway concept revisited. *ALGY.* 2018; 73(5):993-1002. Doi: 10.1111/all.13373
 62. Mihaylova VT, Kong Y, Fedorova O, Sharma L, Dela Cruz CS, Pyle AM, *et al.* Regional differences in airway epithelial cells reveal tradeoff between defense against oxidative stress and defense against rhinovirus. *Cell Rep.* 2018; 24(11):3000-3007.e3. Doi: 10.1016/j.celrep.2018.08.033
 63. Roberts N, Al Mubarak R, Francisco D, Kraft M, Chu HW. Comparison of paired human nasal and bronchial airway epithelial cell responses to rhinovirus infection and IL-13 treatment. *Clin. Transl. Med.* 2018; 1(13). Doi: 10.1186/s40169-018-0189-2
 64. Giovannini-Chami L, Paquet A, Sanfiorenzo C, Pons N, Cazareth J, Magnone V, *et al.* The “one airway, one disease” concept in light of Th2 inflammation. *Eur Respir J.* 2018; 52(4):1800437. Doi: 10.1183/13993003.00437-2018
 65. Zheng J, Wang Z, Li J, Zhang Y, Jiang L, Fu Y, *et al.* High amounts of SARS-CoV-2 in aerosols exhaled by patients with Omicron variant infection. *J Infect.* 2022; 84(6):e126-e128. Doi: 10.1016/j.jinf.2022.02.015
 66. Ju SP, Yang YC, Chen HY. Unraveling the binding mechanisms of SARS-CoV-2 variants through molecular simulations. *Heliyon.* 2024; 10(5):e27193. Doi: 10.1016/j.heliyon.2024.e27193
 67. Nguyen H, Thai NQ, Nguyen PH, Li MS. SARS-CoV-2 Omicron variant binds to human cells more strongly than the wild type: Evidence from molecular dynamics simulation. *J. Phys. Chem. B.* 2022; 126(25):4669-4678. Doi: 10.1021/acs.jpcc.2c01048
 68. Dudek G, Borys P. A simple methodology to estimate the diffusion coefficient in pervaporation-based purification experiments. *Polymers.* 2019; 11(2):343. Doi: 10.3390/polym11020343
 69. O’Loughlin MA, Whillans DW, Hunt JW. A fluorescence approach to testing the diffusion of oxygen into mammalian cells. *Radiat. Res.* 1980; 84(3):477-495.
 70. Wojcieszyn JW, Schlegel RA, Wu ES, Jacobson KA. Diffusion of injected macromolecules within the cytoplasm of living cells. *Proc. Natl. Acad. Sci. U.S.A.* 1981; 78(7):4407-4410. Doi: 10.1073/pnas.78.7.4407
 71. Mestecky J, Bienenstock J, McGhee J, Lamm ME, Strober W, Cebra JJ, *et al.* Historical aspects of mucosal immunology. *Mucosal Immunol.* 2005; 23-43. Doi: 10.1016/B978-0-12-415847-4.02001-2
 72. Adamczyk Z, Batys P, Barbasz J. SARS-CoV-2 virion physicochemical characteristics pertinent to abiotic substrate attachment. *Curr. Opin. Colloid Interface Sci.* 2021; 55:101466-101466. Doi: 10.1016/j.cocis.2021.101466

73. Roselli RJ, Diller KR. Biotransport: Principles and applications. 2021; Springer. Doi: 10.1007/978-1-4419-8119-6
74. Kuimova MK. Lasers for science facility (LSF) programme I: Biological intracellular viscosity increases during photo-induced cell death, 2008-2009, 152-154.
75. Mehta M, Bui TA, Yang X, Aksoy Y, Goldys EM, Deng W. Lipid-based nanoparticles for drug/gene delivery: An overview of the production techniques and difficulties encountered in their industrial development. ACS Mater. Au. 2023; 3(6):600-619. Doi: 10.1021/acsmaterialsau.3c00032
76. Shah S, Dhawan V, Holm R, Nagarsenker MS, Perrie Y. Liposomes: Advancements and innovation in the manufacturing process Adv. Drug Deliv. Rev, 2020, 154-155, 102-122. Doi: 10.1016/j.addr.2020.07.002
77. Ridolfo R, Tavakoli S, Junnuthula V, Williams DS, Urtti A, Van Hest JCM. Exploring the impact of morphology on the properties of biodegradable nanoparticles and their diffusion in complex biological medium. Biomacromolecules. 2021; 22(1):126-133. Doi: 10.1021/acs.biomac.0c00726
78. Dey D, Nunes-Alves A, Wade RC, Schreiber G. Diffusion of small molecule drugs is affected by surface interactions and crowder proteins. iScience. 2022; 25(10):105088. Doi: 10.1016/j.isci.2022.105088
79. Fodeke AA, Minton AP. Quantitative characterization of temperature-independent and temperature-dependent protein-protein interactions in highly nonideal solutions. J. Phys. Chem. B. 2011; 115(38):11261-11268. Doi: 10.1021/jp2049266
80. Knowles DB, LaCroix AS, Deines NF, Shkel I, Record MT (Jr). Separation of preferential interaction and excluded volume effects on DNA duplex and hairpin stability. Proc. Natl. Acad. Sci. U.S.A. 2011; 108(31):12699-12704. Doi: 10.1073/pnas.1103382108
81. Miklos AC, Sarkar M, Wang Y, Pielak GJ. Protein crowding tunes protein stability. J Am. Chem. Soc. 2011; 133(18):7116-7120. Doi: 10.1021/ja200067p
82. Phillip Y, Schreiber G. Formation of protein complexes in crowded environments-from *in vitro* to *in vivo*. FEBS Lett. 2013; 587(8):1046-1052. Doi: 10.1016/j.febslet.2013.01.007
83. Sarkar M, Li C, Pielak GJ. Soft interactions and crowding Biophys. Rev. 2013; 5(2):187-194. Doi: 10.1007/s12551-013-0104-4
84. Wang Y, Sarkar M, Smith AE, Krois AS, Pielak GJ. Macromolecular crowding and protein stability. J. Am. Chem. Soc. 2012; 134(40):16614-16618. Doi: 10.1021/ja305300m
85. Zhou HX. Influence of crowded cellular environments on protein folding, binding, and oligomerization: Biological consequences and potentials of atomistic modeling. FEBS Lett. 2013; 587(8):1053-1061. Doi: 10.1016/j.febslet.2013.01.064
86. Aumiller WM (Jr.), Davis BW, Hatzakis E, Keating CD. Interactions of macromolecular crowding agents and cosolutes with small-molecule substrates: Effect on horseradish peroxidase activity with two different substrates. J. Phys. Chem. B. 2014; 118(36):10624-10632. Doi: 10.1021/jp506594f
87. Minton AP. Quantitative assessment of the relative contributions of steric repulsion and chemical interactions to macromolecular crowding. Biopolymers. 2013; 99(4):239-244. Doi: 10.1002/bip.22163